UNITED NATIONS
Office on Drugs and Crime

BULLETIN ON NARCOTICS

Volume LVII, Nos. 1 and 2, 2005

Science in drug control: the role of laboratory and scientific expertise

UNITED NATIONS OFFICE ON DRUGS AND CRIME
Vienna

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UNITED NATIONS
New York, 2007

UNITED NATIONS PUBLICATION
Sales No. E.07.XI.3
ISBN 978-92-1-148221-8
ISSN 0007-523X

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The *Bulletin on Narcotics* is available on the World Wide Web at http://www.unodc.org/unodc/en/ bulletin_on_narcotics.html.

It should be noted that, although the year 2005 appears on its cover, the present issue was published only in 2007.

Views expressed in signed articles published in the *Bulletin* are those of the authors and do not necessarily reflect those of the United Nations Secretariat.

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PREFACE

The *Bulletin on Narcotics* is a United Nations journal that has been in continuous publication since 1949. It is printed in all six official languages of the United Nations: Arabic, Chinese, English, French, Russian and Spanish.

The *Bulletin* provides information on developments in drug control at the local, national, regional and international levels that can be of benefit to the international community.

The present double issue of the *Bulletin* (vol. LVII, Nos. 1 and 2) is the first to provide a comprehensive overview of the crucial role of scientific and laboratory expertise in drug control. It should be noted that for the sake of continuity, 2005 is given as the year of publication of this issue. Thanks go to Barbara Remberg of the Laboratory and Scientific Section of the United Nations Office on Drugs and Crime for the preparation of this issue.

EDITORIAL POLICY AND GUIDELINES FOR PUBLICATION

Individuals and organizations are invited by the Editor to contribute articles to the *Bulletin* dealing with policies, approaches, measures and developments (theoretical and/or practical) relating to various aspects of the drug control effort. Of particular interest are the results of research, studies and practical experience that would provide useful information for policymakers, practitioners and experts, as well as the public at large.

All manuscripts submitted for publication in the *Bulletin* should constitute original and scholarly work that has not been published elsewhere or is not being submitted simultaneously for publication elsewhere. The work should be of relatively high professional calibre in order to meet the requirements of a United Nations technical publication. Contributors are kindly asked to exercise discretion in the content of manuscripts so as to exclude any critical judgement of a particular national or regional situation.

The preferred mode of transmission of manuscripts is Word format. Each manuscript submitted should consist of an original hard copy and an electronic version (in Word for the text and in Excel for tables and figures) in any of the six official languages of the United Nations (Arabic, Chinese, English, French, Russian and Spanish). The manuscript should be accompanied by an abstract of approximately 200 words, a complete set of references numbered in the order of their appearance in the text and a list of key words. The manuscript should be between 10 and 20 double-spaced typewritten pages, including tables, figures and references. Tables should be self-explanatory and should supplement, not duplicate, information provided in the text.

Manuscripts, together with brief curricula vitae of their authors, should be addressed to the Editor, *Bulletin on Narcotics*, United Nations Office on Drugs and Crime, Vienna International Centre, P.O. Box 500, 1400 Vienna, Austria. A transmittal letter should designate one author as correspondent and include his or her complete address, telephone number, fax number and e-mail address. Unpublished manuscripts will be returned to the authors; however, the United Nations cannot be held responsible for loss.

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The following special issues of the *Bulletin* are also available as United Nations publications:

1993

Policy issues relating to drug abuse and the human immunodeficiency virus (HIV) (vol. XLV, No. 1)

Drug testing in the workplace (vol. XLV, No. 2)

1994

The family and drug abuse (vol. XLVI, No. 1)

General issue on drug abuse (vol. XLVI, No. 2)

1995

Special issue on gender and drug abuse (vol. XLVII, Nos. 1 and 2)

1996

Special issue on rapid assessment of drug abuse (vol. XLVIII, Nos. 1 and 2)

1997 and 1998

Double issue on cannabis: recent developments (vol. XLIX, Nos. 1 and 2, and vol. L, Nos. 1 and 2)

1999

Occasional papers (vol. LI, Nos. 1 and 2)

2000

Economic and social costs of substance abuse (vol. LII, Nos. 1 and 2)

2001

Dynamic drug policy: understanding and controlling drug epidemics (vol. LIII, Nos. 1 and 2)

2002

The science of drug abuse epidemiology (vol. LIV, Nos. 1 and 2)

2003

The practice of drug abuse epidemiology (vol. LV, Nos. 1 and 2)

2004

Illicit drug markets (vol. LVI, Nos. 1 and 2)

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CONTENTS

III. Drug abuse

IV. Quality management in laboratories

Editorial: science in drug control

B. Remberg and A. H. Stead

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United Nations Office on Drugs and Crime

Science is not obvious, nor is it a "fact factory" producing comprehensive information. It is a systematic approach to knowledge about the material world that progresses by ruling out what is not true. Contrary to the way most people think of science, its purpose is not to amass evidence in support of every critical point in an argument in order to prove truth. In some ways, the accumulation of specific facts is a by-product of science. The true objective of the scientific enterprise is understanding, which comes as much from the intellect as from experience.

Tee L. Guidotti, *Science on the Witness Stand*

Fifty-five years after the first publication was issued as part of a United Nations-led international collaborative scientific research programme in the field of drug control,* the present special issue of the *Bulletin on Narcotics* compiles 13 articles from scientists from around the world, on priority subjects of a scientific and technical nature. It is also the first issue for more than 20 years devoted specifically to the work of drug-testing laboratories. However, unlike the previous issues, published in 1984 and 1985, the present one is not limited to laboratory techniques and analytical results per se, but aims at illustrating the role of drug-testing laboratories and the contribution of science and scientific support to drug control as a whole.

In inviting scientists to contribute to this special issue, a balance has been struck between the various subjects where scientific support and laboratories contribute to drug control and the geographical spread of authors. The present issue of the *Bulletin* reflects those efforts, presenting a unique compilation of articles, written by scientists for an international non-scientific audience of policymakers and other individuals who may, for various reasons, rely on the work of scientists and laboratory findings—often without fully appreciating their

*The breadth of research carried out in a collaborative approach under the United Nations opium research programme by scientists from around the world is reflected in the United Nations document series ST/SOA/SER.K, entitled "The assay, characterization, composition and origin of opium". Over a period of 17 years, a total of 148 research papers were published, the first one in 1951; that first paper, referred to here, was entitled "The determination of morphine in opium by extraction: a new method".

value and limitations. The main aim of this issue is therefore to raise forensic awareness outside the laboratory, bridging the gap between the highly technical work of scientists and their results and the needs and expectations of the users of laboratory data and information.

Science is not a "fact factory" producing comprehensive information. Laboratories produce results, and they need to have access to, and apply, quality standards to produce them, but those results also have to be turned into information and knowledge. Despite all technological advances, and contrary to popular belief, this does not happen automatically, and the interpretation and integration of results, and their communication, in the form of findings and information relevant to the end-user, remain key areas of the work of a scientist.

Several articles in this issue of the *Bulletin* present pure research findings, while others provide summarized data or information on specific aspects (on-site testing, drugs and driving and so on). Articles are organized in the areas of drug production, drug trafficking and interdiction, and drug abuse, illustrating the critical role of the scientist and the laboratory as a resource for the entire range of drug control efforts. They include, for example, scientific contributions for law enforcement operational work (i.e. in the context of intelligence-led law enforcement), prevention (e.g. early warning systems), regulatory and monitoring purposes and policy development.

The result is that this special issue contains papers covering a wide range of subjects and considerable variation with regard to the type of scientific support, substances discussed and analytical methods employed. Articles are exemplary for the work of thousands of scientists around the world, who, day by day, carry out their work, making a critical contribution to reducing drug abuse worldwide.

Despite their central role in drug control, forensic laboratories are usually seen and used as tools and servants rather than as resources and partners. At best, the value of individual laboratory results to answer a specific question, to save a life, to help in treatment or to identify or confirm a crime is recognized. However, there is much less recognition of the fact that collectively, as a body of information, laboratory results also constitute a valuable commodity in their own right by helping to identify new potential threats and health hazards, especially those related to new drugs and manufacturing methods, new sources of drugs and drug availability, new purities and cutting agents, and new products and drug combinations.

Further, forensic drug-testing work is rarely considered revolutionary (assessed in lay terms) and is often without direct visible successes, because it is the law enforcement officers who celebrate the multi-kilogram seizure of a given drug, confirmed in identity by a laboratory; or it is the policymakers who introduce restrictions on the availability of a dependence-producing medicine or place another drug or precursor under regulatory control, only after the prevalence of the substance has been identified with the help of a laboratory.

Nevertheless, there are exceptions. A recent example from the international scientific literature, which has created significant interest among policymakers,

is an Italian study on the use of cocaine concentrations in surface waters as a new evidence-based tool to monitor community drug abuse.* Although this approach, which has since been repeated in other European countries, may currently be limited to technologically advanced countries (considering the required instrumentation, expertise and costs), it has the potential to become a standardized, objective and independent tool for monitoring drug abuse.

In general, there is a need for wider recognition of the added value of an integrated national scientific support service as an equal partner with law enforcement, judicial, regulatory and health authorities. Laboratories need to be provided with the resources they require to sustain high-quality services, and they and their scientists need to be given the opportunity to participate actively in relevant regional and global networks of forensic scientists to exchange experience and analytical findings at an early stage. Most importantly, national institutions and government agencies need to be made more aware of the range of work and possibilities of scientific support to ensure better use of available laboratory resources and greater recognition of the potential value of scientific information, beyond the use of laboratory results as evidence in court.

The present special issue is a small contribution towards that goal, and the United Nations Office on Drugs and Crime (UNODC) hopes that it will contribute to increasing appreciation for the excellent work carried out by countless scientists around the world, thriving to deliver quality findings, often with only basic equipment, but with vast experience, good scientific judgement, and great affection for their job. These scientists deliver excellent, reliable data, thus countering the belief frequently held by non-scientists (policymakers and other users of laboratory results) that the level of sophistication of a laboratory is a direct measure of the quality of the scientific support delivered. That this belief is a severe misconception can be easily demonstrated, and there are numerous examples highlighting the importance of experience and good scientific judgement (i.e. the human input), especially in the interpretation and communication of results.

It is hoped that this special issue of the *Bulletin* will also contribute to renewing the spirit of earlier collaborative scientific research programmes coordinated by the United Nations,** which proved that scientific and technical problems in the field of drug control can most effectively be dealt with at the international level. The list of subjects that would benefit from a collective, international, scientific effort is long; it includes the production of more systematic information on the purity of drugs (or: the potency of cannabis products); new drug products and combinations and their potential health hazards; the yield of drugs such as cocaine, opium and heroin from illicit drug operations;

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*Ettore Zuccato and others, "Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse", *Environmental Health: a Global Access Science Source,* vol. 4, No. 14 (2005).

**In addition to the opium research programme, there also were scientific research programmes on cannabis (1959-1979), on Papaver bracteatum research to increase codeine production (1972-1983) and on the chemical composition of khat (1971-1979). More recently, activities have focused on drug characterization/impurity profiling as a scientific tool in support of drug control activities (since 1996).

trafficking routes and drug sources; the effects and implications for different environments of chemical waste from illicit drug processing and manufacture; and the extent and implications of the abuse of traditional and herbal drugs—to name but a few.

Finally, UNODC wishes to acknowledge that many authors contributed their articles in addition to a heavy, routine workload and that several of them had to write in a language that is not their mother tongue. That added difficulty is specifically acknowledged.

Overview: contents

The 13 articles submitted for this special issue of the *Bulletin* are grouped into four sections: drug production; drug trafficking and interdiction; drug abuse; and a general section illustrating the role of, and efforts to maintain, quality assurance systems as an overarching element and integral part of modern laboratory management. What all articles have in common is that they illustrate the important role of the scientist, his or her informed judgement and expertise in turning analytical results into information and knowledge into a commodity useful to their "customers", who include personnel in law enforcement, health or regulatory authorities and the judicial system.

Section I. Drug production

Articles in section I illustrate the role and potential of laboratories (and science in general) in assessing the extent of drug production and identifying trends in clandestine processing or manufacturing methods and chemicals used. They range from the documentation of an "experimental" heroin manufacturing process in Afghanistan and the presentation of scientific tools that help assess methods used for the manufacture of illicit synthetic drugs, link precursors to end products and identify sources of starting materials, to studies of tetrahydrocannabinol (THC) content and other technical aspects related to the illicit cultivation of cannabis plants in Morocco.

The first article, by Zerell and others ("Documentation of a heroin manufacturing process in Afghanistan"), provides first-hand information on a heroin manufacturing process carried out under controlled conditions in Afghanistan. A number of policy-relevant findings emerged, namely with regard to the type and quality of the resulting heroin product, the simplicity of the process employed and the quantity of solvents used. The article thus represents a valuable source of information, in particular for regulatory and operational law enforcement personnel, on the quality and quantity of heroin that may be illicitly manufactured in Afghanistan and the required chemical input.

The article by Krawczyk and others ("Impurity profiling/comparative analyses of samples of 1-phenyl-2-propanone") is the result of the joint efforts of forensic scientists and their colleagues in law enforcement. It describes the development of a system for the impurity profiling of 1-phenyl-2-propanone (P-2-P)

(also known as benzyl methyl ketone (BMK)), a key precursor in clandestine amphetamine manufacture. The authors highlight the usefulness of the system in determining P-2-P manufacturing routes and identify samples from the same illicit source. Of particular note is the view expressed by the authors of the importance of a good working relationship and interaction between the law enforcement and forensic sectors as part of effective interdiction.

In another article, Makino and others present an approach to discriminating the manufacturing origin of ephedrine, the key precursor used in the clandestine manufacture of methamphetamine ("Investigation of the origin of ephedrine and methamphetamine by stable isotope ratio mass spectrometry: a Japanese experience"). The beauty of the approach is that the origin of the starting material, ephedrine, might be deduced from the analysis of the end product, methamphetamine. While the authors acknowledge that further refinement of the approach is required, including the examination of authentic sample material from a larger number of known manufacturing origins, the technique has already attracted interest from regulatory authorities in Japan and elsewhere, with regard to providing an independent means of determining origin, especially of precursors.

Finally, Stambouli and others analysed samples of fresh, dry and powdered cannabis herb obtained as part of a survey on the cultivation of cannabis plants in Morocco ("Cultivation of *Cannabis sativa* L. in northern Morocco"). Their work constitutes an important contribution to overall estimates of cannabis production by making available for the first time analytical data on the THC content (potency) of different cannabis products from Morocco obtained under controlled conditions. This contribution is particularly important in the context of ongoing discussions about the increasing THC content of cannabis samples.

Section II. Drug trafficking and interdiction

The common trait of articles in section II is the use of the presence of impurities in illicit drug samples—be they manufacturing by-products, residual solvents from the manufacturing process or trace metals—to establish possible links between samples and identify their sources. Such types of comparative analysis, which are not limited to drug trafficking and interdiction cases, are typically known as drug characterization/impurity profiling studies. Several of the articles in this issue of the *Bulletin* discuss details of that approach for a variety of different purposes and in their respective national contexts (see also the article by Krawczyk and others referred to above). An important conclusion drawn by most of the authors is the need for an integrated, operational programme that ensures two-way communication between the laboratory and law enforcement personnel and the timely follow-up of analytical results.

The first article in this section, by Ioset and others ("Establishment of an operational system for drug profiling: a Swiss experience"), presents a comprehensive outline of the establishment of an operational system for heroin profiling. Among the many purposes for which impurity profiling programmes might

be established, the focus by Ioset and others is on improving knowledge of the illicit heroin market and drug distribution patterns, with the operational goal of devising appropriate medium- and long-term intervention strategies. Technical aspects of analytical methods and statistical and chemometric methods are presented. The framework within which such comparative analyses are carried out is also discussed, highlighting the importance of following an integrated and standardized approach at the national level, as well as at the regional and international levels.

Hung and others describe the evolution of comparative analyses and profiling activities in Viet Nam ("Drug profiling: a new scientific contribution to law enforcement operations in Viet Nam"), based on a need to understand the pattern of heroin and methamphetamine manufacture and distribution in the region. While the establishment of a comprehensive profiling programme is still in progress, the authors present some findings from the analysis of seized heroin and methamphetamine and discuss the results from an operational point of view. This article should be seen as encouragement for all those scientific services around the world which may be faced with resource limitations, because it shows that operationally useful results can be obtained using a simple combination of a few physical characteristics of samples, such as colour and packaging material, and chemical analysis of key components.

The article by Visser and others ("Residual solvents in methylenedioxymethamphetamine tablets as a source of strategic information and as a tool for comparative analysis: the development and application of a static headspace gas chromatography/mass spectrometry method") outlines the development of a specific sub-type of profiling method targeting solvent residues trapped in the drug during the final crystallization step. While the authors conclude that this method, after further refinement, might become part of a strategy for comparative analysis, its immediate value lies in the identification of solvents used in the illicit manufacture of methylenedioxymethamphetamine, thus contributing to decisions on the monitoring and control of such substances.

The last article in section II, by Çopur and others ("Determination of inorganic elements in poppy straw by scanning electron microscopy with energy dispersive spectrometry as a means of ascertaining origin"), explores the potential of using inorganic element profiles of poppy straw for monitoring licit opium poppy cultivation and poppy straw production in Turkey and for distinguishing them from illicitly produced poppy straw or poppy straw trafficked from abroad. Again, while the analytical approach is not new, its novel application provides comparative data that could be of interest to law enforcement and regulatory authorities alike.

Section III. Drug abuse

Articles in section III address the role and challenges of drug testing when biological materials (urine, blood etc.) are involved and especially of on-site testing, for example, in connection with cases involving drugs and driving.

Toxicological analysis is also an important element in epidemiological research, as part of early warning programmes by means of which results can provide important evidence of new drug trends to be acted on by health authorities and in support of scheduling decisions.

The opening article by Drummer ("On-site drug testing") provides an overview of the concepts, applications and quality and cost considerations of on-site drug testing. The review is important because applications for non-laboratory-based techniques are increasing in number and range, for example, as part of workplace and roadside testing programmes, for the monitoring of compliance in drug courts and other treatment programmes or for testing inmates in prisons and other correctional institutions. Recognizing and appreciating not only the advantages but also the limitations of available on-site testing devices guarantees their responsible use in an ever-broadening field of applications, affecting the lives of an increasing number of individuals.

Lillsunde and Gunnar ("Drugs and driving: the Finnish perspective") describe the approach to handling roadside testing and cases involving drugs and driving in Finland. Importantly, they outline the implications of the prevailing types of legislation ("zero-tolerance" and "impairment" laws) for successful prosecution of cases involving drugs and driving, either by proving the presence of a zero-tolerance drug (or its metabolite) in the driver's blood, or by proving "impairment" of driving ability in court. This issue is still being debated in many countries.

Chung ("Role of drug testing as an early warning programme: the experience of the Republic of Korea") describes the use of drug testing as part of a national programme to monitor the abuse of common medicines. While the article focuses on the specifics of the situation in the Republic of Korea, it exemplifies the challenges faced by drug-testing laboratories that provide analytical services for early warning programmes, namely, the need to identify what could potentially be any one of a huge number of drugs of abuse or poisons and to develop suitable, reliable and specific methods of detection.

The article by Chan and others ("Psychoactive plant abuse: the identification of mitragynine in ketum and in ketum preparations") outlines the central role of drug testing in an approach to controlling the abuse of a traditional drug in Malaysia. It reminds the reader that drug-testing laboratories in different parts of the world must be able to analyse a wide range of drugs, which are frequently not listed in the international drug control conventions and for which they may need to develop suitable analytical methods.

Section IV. Quality management in laboratories

The article by Salas and others ("Quality management systems and the admissibility of scientific evidence: the Costa Rican experience"), the last in this special issue, gives some insight into the meticulous and responsible work of laboratories, which underpins the contribution of drug testing in general and the quality of its results specifically. While the article describes the Costa Rican

experience, it is exemplary regarding the establishment of quality systems, outlining the resource requirements and the range of activities carried out and maintained continuously "behind the scenes". As noted above in connection with the analytical work of scientists and forensic laboratories in general, those systems constitute another key aspect of forensic laboratory work that is without any visible output but is essential to the delivery of quality results and services.

Drug-testing laboratories exist around the world. They often function under difficult conditions and with limited resources. Through the publication of this set of 13 thematically related but wide-ranging articles on science in drug control, it is hoped that this special issue of the *Bulletin* will contribute to improving those conditions by raising forensic awareness and stimulating the increased use and enhanced integration of drug-testing laboratories at the national level in all relevant drug control efforts, including policy development.

Section I. Drug production

Documentation of a heroin manufacturing process in Afghanistan

U. Zerell, B. Ahrens and P. Gerz*

Federal Criminal Police Office, Wiesbaden, Germany

ABSTRACT

The present article documents an authentic process of heroin manufacturing in Afghanistan: white heroin hydrochloride produced using simple equipment and a small quantity of chemicals. The quantities of chemicals actually used corresponded to the minimum needed for manufacturing heroin. The only organic solvent used was acetone, and only a very small quantity of it was used.

Because the chemicals used in the demonstration were from actual seizures in Afghanistan, some of the chemicals had been disguised or repackaged by smugglers. Others had been put into labelled containers that proved to be counterfeit, and some glass containers used were not the original containers of the manufacturer displayed on the label.

The brown heroin base prepared as an intermediate step in the process shares some of the characteristics of the South-West Asia type of heroin preparations often seized in Germany. The final product of the documented heroin manufacturing process was white heroin hydrochloride, which shares the key characteristics of the white heroin occasionally seized in Germany and other countries in Western Europe since 2000. The present article demonstrates that this kind of heroin can be produced in Afghanistan.

Introduction

The United Nations Office on Drugs and Crime (UNODC) has estimated that Afghanistan is home to the world's largest area of opium poppy cultivation, supplying opium, the basic material used for the illicit manufacturing of heroin. Up to 90 per cent of the heroin seized in Western Europe is considered to be of Afghan origin. While, until 1999, most of the cases of opium smuggling were recorded in States bordering Afghanistan, in recent years there have been more and more indications that illicit heroin manufacturing is increasing in Afghanistan itself. In addition, in Western Europe, there has been a growing

*The authors would like to thank the Central Analysis Laboratories I and II and the Organic Materials section of the Forensic Science Institute of the Federal Criminal Police Office for their support in analysing the samples.

number of seizures of heroin of particularly high quality, referred to as "white heroin", whose origin is believed to be Afghanistan.

There are few descriptions of the methods used in illicit heroin manufacturing in publicly available literature. And almost all share a common flaw: they are based on either purely theoretical calculations or mere "evidence by inspection", in the form of an observer's description of the synthesis of heroin. Much of the information available lacks authenticity; so, estimates of the quantity of heroin manufactured, based on the total area known to be under opium poppy cultivation and the potential opium yield of that area, have, until now, relied on data that have not been scientifically substantiated. Analyses of illicit heroin manufacturing processes, including forensic sampling and sample examination, are rarely found in available literature.

Against that backdrop, the offer extended to German authorities by the central office of the Counter-Narcotics Police of Afghanistan (CNPA) in Kabul to observe an authentic heroin manufacturing process in Afghanistan presented a unique opportunity. In addition to gathering information and documenting the process, the task would be to collect for analysis authentic samples of the basic material (opium), the chemicals used and the heroin itself. The information obtained would be used in the German heroin analysis programme.

The Federal Criminal Police Office (BKA) of Germany seconded to Afghanistan two forensic chemists and a police officer responsible for criminal investigations in order to carry out the task. The method used to process the heroin was demonstrated by two male Afghan nationals from Nangarhar province, who described themselves as illiterate farmers. The laboratory equipment (chemicals, devices and aids) and the opium needed had been obtained in the course of seizures, and were provided by CNPA in Kabul. Samples of the substances and the chemicals used were taken at all stages of the manufacturing process.

Origin and selection of the opium

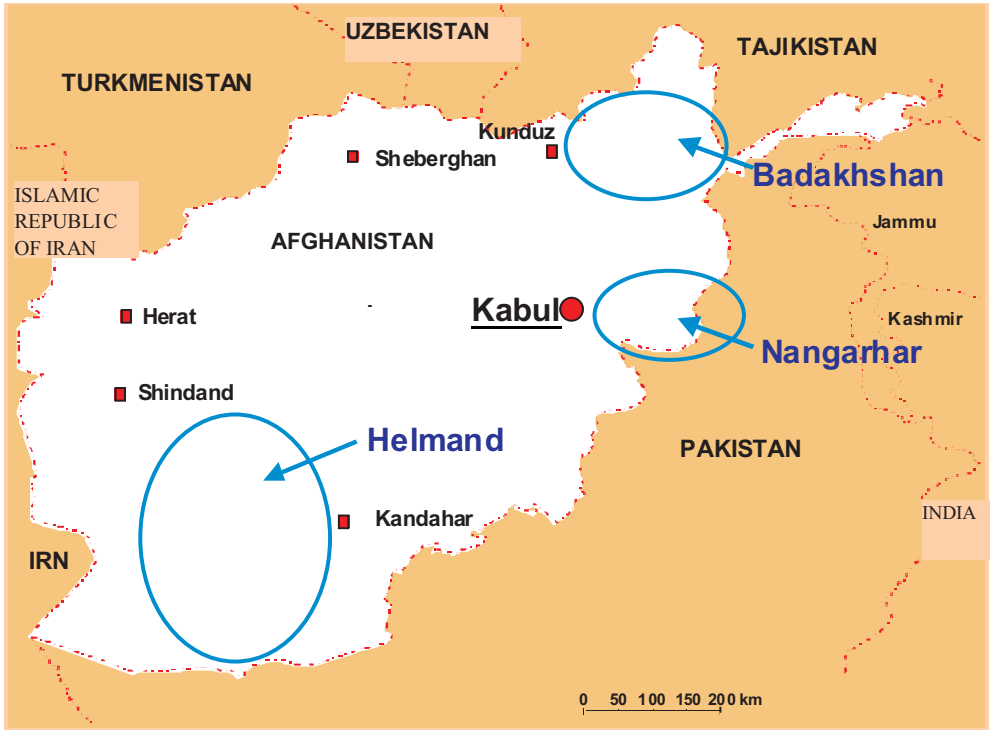
In Afghanistan, opium poppy is cultivated mainly in Badakhshan, Nangarhar and Helmand provinces (see the map). The raw opium used as the basic material to manufacture heroin is the air-dried milky latex from lanced opium poppy capsules.

Selection of the raw opium

At the CNPA facilities, the persons involved in processing the heroin assessed several batches of seized raw opium on the basis of appearance, odour and consistency. They selected opium of varying quality, with a total weight of 70 kilograms, as the base material for the heroin production.

According to CNPA, the raw opium was seized in Nangarhar, but because opium dealers frequently transport their goods from one province to another for processing, it could not be ruled out that all or part of the selected raw opium originated in another province.

Principle areas of opium poppy cultivation in Afghanistan: Badakhshan, Nangarhar and Helmand provinces



Note: The boundaries shown do not imply official endorsement or acceptance by the United Nations.

Documentation of the heroin manufacturing process

The present article includes a description of all stages of the heroin manufacturing process, followed by a flow chart summarizing those steps and listing the intermediate products and the chemicals needed. Explanations given and the names of substances used were confirmed by means of forensic analysis and expert knowledge. Finally, the process is discussed with reference to published accounts of manufacturing methods.

Extracting the morphine from raw opium

The raw opium was unwrapped, crushed and divided into two portions. The wrapping material was not entirely removed. The crushed opium was poured into two barrels and hot water was added.

The composition was stirred until it became a homogeneous suspension. The pH value was 8. The remaining plastic wrapping floated to the surface of the liquid and was scooped out. Then calcium oxide (anhydrous lime) was added, together with more hot water. The suspension was stirred well from time to time, for a period that lasted about an hour. During that period, sometimes hot water and sometimes a solution of calcium oxide (anhydrous lime) and hot water were used to rinse off any opium still stuck to the wrapping material. The rinsing solution was poured into the barrels containing the main substance. The barrels were then filled with hot water and left to stand overnight. By the next morning, a brownish foam residue and an oily film had appeared on the surface of the morphine solution. The pH value was measured at between 10 and 12. In the course of the extraction process, other water-soluble substances were co-extracted with the morphine.

Separating the morphine solution from the water-insoluble opium components

A hose was used to siphon the clear, dark brown morphine solution into two tubs.

After that, the solution was divided into four empty barrels. The sediment was stirred up, ladled out of the barrels with buckets and filtered through sacks that had been soaked in water. The entire filtrate was then poured back into the four barrels containing the morphine solution.

Treatment of the water-insoluble opium constituents

The sacks containing the opium residue were placed in a pressing device, and the liquid was squeezed out of them.

The liquid pressed from the sacks was added to the barrels containing the morphine solution. Then the press cake was removed from the sacks, divided in two parts, put in two barrels and treated with hot water to dissolve out more morphine. After being filtered and pressed, the additional liquid extracted from the sacks was also added to the main morphine solution.

Precipitation, isolation and drying of the morphine

Then ammonium chloride was added to each barrel while stirring continuously. The morphine base precipitated. The barrels were covered and left to stand overnight.

The next morning, the morphine base was filtered using two filtering baskets lined with cloth that had been soaked in warm water. The solution had a pH value of 9. The main morphine base substance, which was in the sediment, was stirred using some of the remaining liquid, thus producing a suspension. The suspension was then filtered out, and the filtrate was discarded. The moist morphine base remained in the cloth-lined filtering baskets.

The morphine base was wrapped in the filtering cloths and stamped out. Finally, the morphine base was spread out on a cloth to dry. Then, the air-dried morphine base was weighed.

Conversion of morphine to heroin

The amount of acetic anhydride needed for heroin synthesis was weighed out. (For the quantities of the chemicals used, see the section entitled "Laboratory equipment and chemicals" below.)

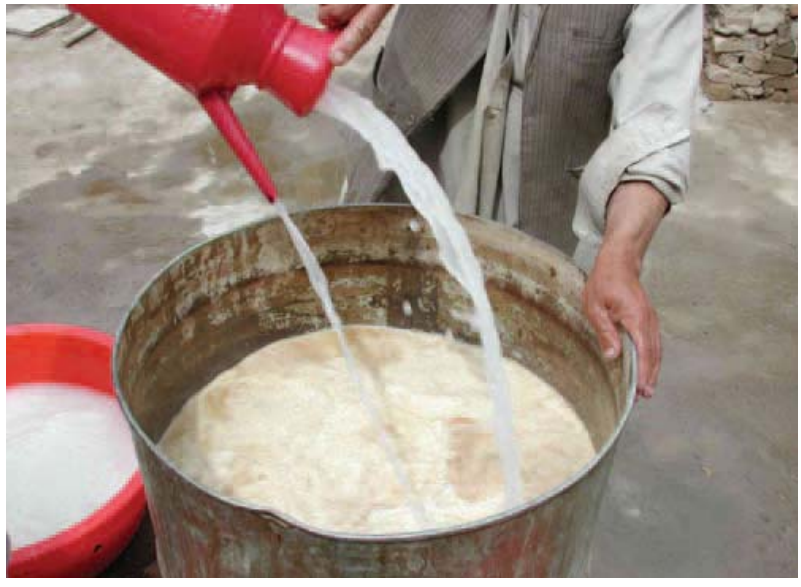
Then the acetic anhydride was added to the morphine base, which had been placed in an aluminium pot. A small excess of the chemical was added.

The pot was stirred until the morphine base had dissolved. The pot was covered, and the reaction solution was allowed to stand for 45 minutes. Then the pot was placed on a fire, and the reaction solution was heated for another 30 minutes.

After that, the reaction mixture was poured into a bowl that had been filled with hot water. Then the solution was filtered through a cloth, and the filtered solution was poured into an empty barrel.

Precipitation and isolation of the brown heroin base

Portions of sodium carbonate solution were poured into the barrel until gas was no longer released and the heroin base precipitated out.



The precipitated heroin base was immediately filtered out. The pH value of the solution was 10. The heroin base was then stirred up in hot water and filtered again. The washing process was repeated once more. Then the brown heroin base was poured into a bowl.

Purification of the brown heroin base

The brown heroin base was dissolved in diluted hydrochloric acid. The solution had a pH value of 7-8. Because not all of the heroin base had dissolved, the solution was filtered through a cloth. Activated carbon was then stirred into the

solution, and the liquid was allowed to stand for 30 minutes. Then the activated carbon was filtered out using a cloth. Because the solution was not yet clear, it was filtered a second time, using a paper filter.

Precipitation and isolation of the white heroin base

Then, the heroin base was precipitated using a diluted ammonia solution. The pH value was 12.

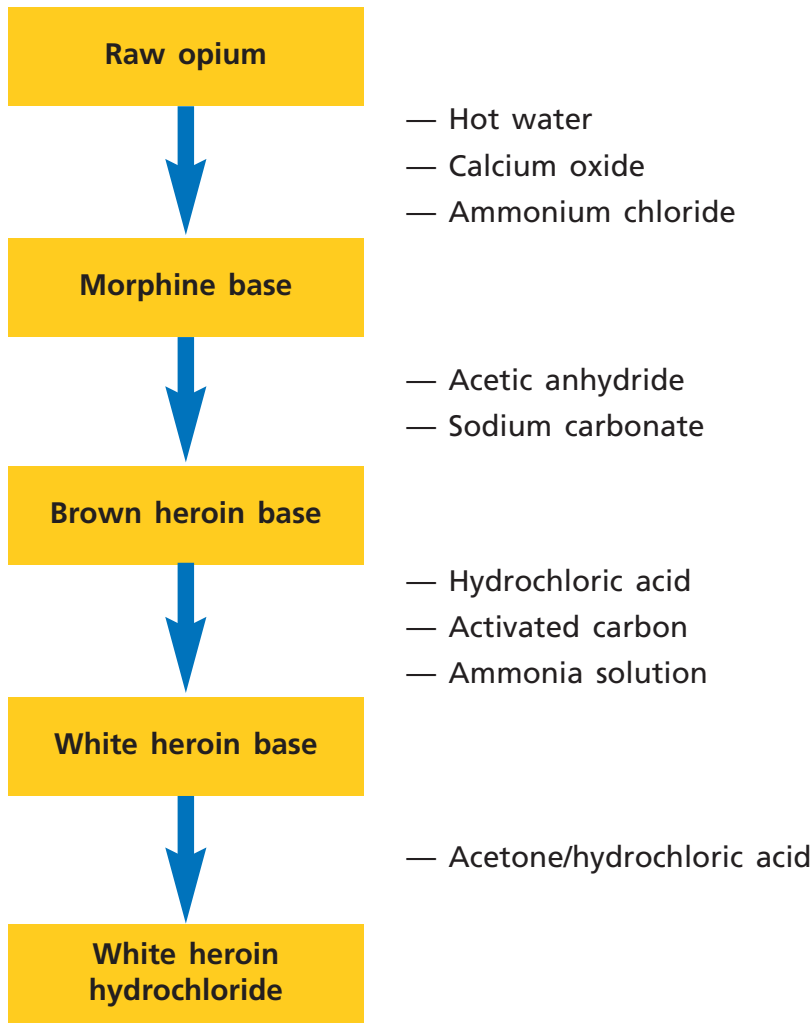
The white heroin base was filtered through a cloth.

Conversion of the heroin base to heroin hydrochloride

The white heroin base was dissolved in a mixture containing hydrochloric acid and a small amount of acetone. The heroin solution was then filtered through a paper filter into a metal bowl and evaporated on a water bath. The white heroin hydrochloride precipitated.

A flow chart showing the basic steps in the heroin manufacture process is presented in figure I.

Figure I. Flow chart of the heroin manufacture process



Discussion

Currently, there are few publicly available descriptions of the processes used to make illicit heroin. The production processes, for which only very general descriptions are provided, use the Thiboumery and Mohr method, also known as the lime method ([1], p. 6) for the first step of extracting morphine from opium.

For example, Cooper [2] reported on the illicit production of heroin based on the extraction of morphine base using hot water and adding calcium oxide, followed by precipitation with ammonium chloride. The conversion to heroin base occurs by adding a large excess of acetic anhydride to the dried morphine base and heating it for 30 minutes. A further conversion to heroin hydrochloride is not described.

Recent publications of the United Nations Office on Drugs and Crime provide flow charts [3] and schematic presentations [4] of the illicit manufacturing of heroin preparations and refer to the main features of the Thiboumery and Mohr method as well as the use of organic solvents in an optional purification step for morphine isolation and the conversion of morphine into heroin hydrochloride. A report of the International Narcotics Control Board (INCB) presents a similar, but greatly simplified, flow chart for illicit manufacture of heroin hydrochloride [5].

The extraction of morphine base during the process observed by the authors was based, for the most part, on the Thiboumery and Mohr method. Unlike in the production process mentioned above, the morphine base was not purified with charcoal. That first purification step was carried out at the stage of the heroin base, that is, after the morphine had been converted to heroin. In this process, only a very small quantity of organic solvent was used, when the purified heroin base was transformed into heroin hydrochloride.

Laboratory equipment and chemicals

CNPA in Kabul provided the equipment (devices and aids) (see table 1) and chemicals (see table 2) used to manufacture the heroin.

The persons processing the heroin identified the chemicals by their external characteristics such as odour and appearance. Sparing use was made of all chemicals required for the production process, with the exception of water. Only a minute quantity of an organic solvent was used. Hot water was used as a solvent throughout the production process. Only a small quantity of the substance referred to as the "key chemical", acetic anhydride, was used. That amount was so small that it was at the bottom of the range of quantities of acetic anhydride reported to have been used in the process elsewhere. According to the persons processing the heroin, in this case, the fact that such a small quantity was used was not the consequence of a lack of availability of the chemical; they considered the amount sufficient for the traditional method that they used to make heroin.

Table 1. Equipment used to manufacture heroin

Device or aid	*Purpose*
Fireplace	To heat water
Firewood	To be used as heating material for the fireplace
Eight empty 200-litre barrels	To heat water, extract the morphine and precipitate the morphine base, etc.
Four plastic tubs	To collect pressed-out juice and filtrates, dissolve the heroin base etc.
Three or four tubs with punched holes	To be used as filtering baskets
Two or three laundry baskets	To hold the textile cloths used for filtering
Two small baskets	To hold the paper filters
Sacks and cloth	To filter solutions
Paper filters	To filter solutions
Press device	To press the water-insoluble opium residues
Large aluminium pot	To convert the morphine base into heroin
Watering can	To rinse the vessels and add the soda solution
Two cups with handles	To add ammonium chloride and activated carbon and mixing solutions
Two evaporators, consisting of: four portable stoves two stands two aluminium pots two metal bowls	To evaporate the heroin hydrochloride solution on a water bath
pH test paper	To test the pH value
Beam balance	To weigh raw opium, acetic anhydride, morphine base and heroin hydrochloride

Table 2. Chemicals used to make white heroin hydrochloride from 70 kilograms of raw opium

Chemical	*Estimated quantity*	*Estimated quantity per kilogram of opium*	*Estimated quantity per kilogram of heroin hydrochloride*
		kilograms	
Calcium oxide (CaO)	7	0.1	1.8
Ammonium chloride (NH_4Cl)	20	0.29	5.1
Acetic anhydride ($C_4H_6O_3$)	8	0.11	2.1
Sodium carbonate (Na_2CO_3 x 10 H_2O)	20	0.29	5.1
Activated carbon	6	0.09	1.5
		litres	
Water (H2O)	2,000	28.6	512.8
Concentrated hydrochloric acid	1.5	0.02	0.38
Concentrated ammonia solution	1	0.02	0.26
Acetone (C_3H_6O)	0.15	0.002	0.04

According to CNPA, clandestine heroin laboratories are generally located in places inaccessible by motor vehicle. The chemicals and the equipment required must be carried by mule to the production site. For that reason, transport capacities can be very limited. Given those conditions, the minimum quantities of the chemicals required were used.

Some of the chemicals provided by CNPA for the demonstration came from actual seizures made by the authorities in Afghanistan. In some cases, the smugglers had repackaged the chemicals in an attempt to keep them from being discovered. For example, the sodium carbonate used in the demonstration was in a plastic bag originally intended for sugar. The acetic anhydride had been hidden in plastic canisters labelled "Hydrogen peroxide", which had been packed into 200-litre steel barrels, and the remaining space filled with petroleum jelly. Further, several chemical containers proved to be clearly counterfeit. The glass vessels used were not the original containers of the manufacturer displayed on the label. The trading unit labels, which at first sight appeared to be originals, on closer inspection turned out to be fake.

Analysis of the documented process

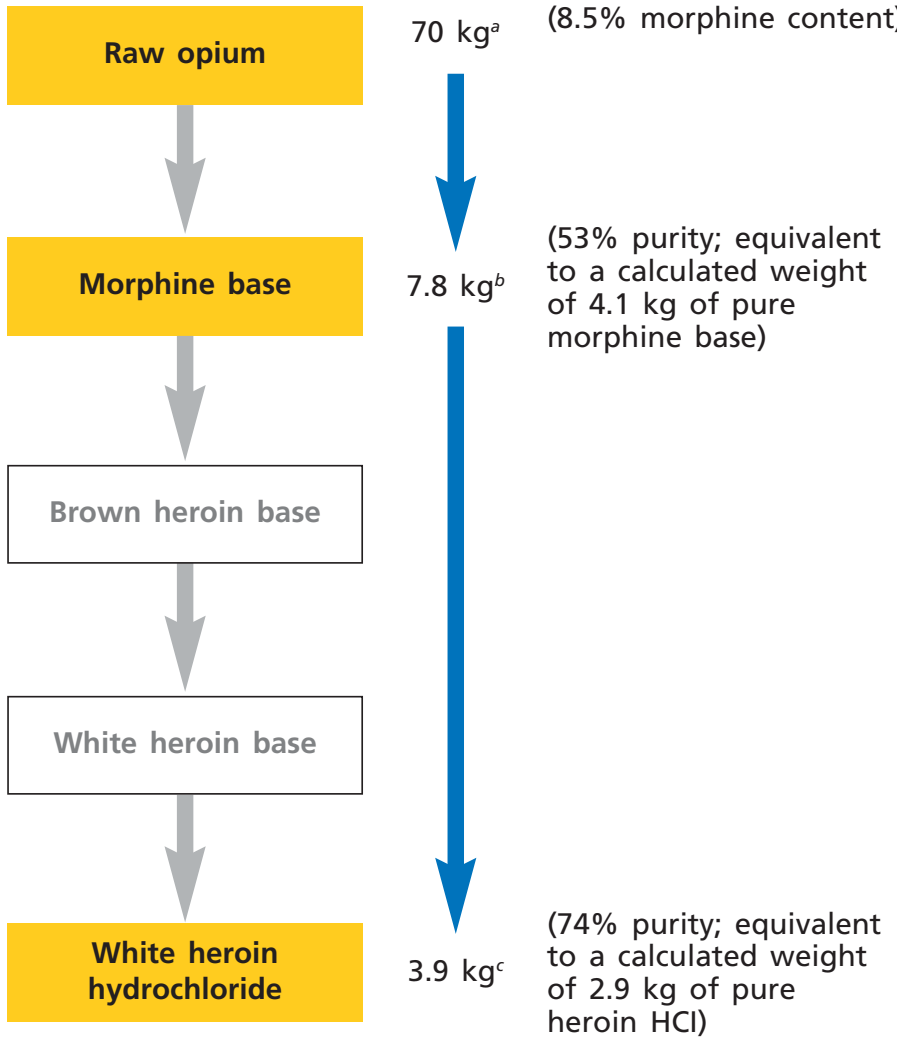
Yield

Some 7.8 kg of morphine base were obtained from 70 kg of raw opium, which is a yield of 11 per cent in terms of the weight of the raw opium. The yield of the final product, white heroin hydrochloride of 74 per cent purity, was 3.9 kg, that is, 6 per cent of the weight of the raw opium (see figure II). It was not possible to weigh the brown and the white heroin bases during the production process, because they were not dried but directly processed while wet.

In the literature, there are few instances in which the yield obtained at each step of the illicit heroin manufacture process is specified. According to UNODC [1], 10 kg of opium yields about 1 kg of morphine base—a yield of 10 per cent—which is considered to produce, in turn, about 1 kg of heroin base. The quantity of the morphine base produced using the method documented in this article is almost identical to that published quantity. However, the more recent UNODC data indicate a higher morphine content in raw opium from Afghanistan. Because of this and/or increased laboratory efficiency, a higher yield reportedly can be achieved, with a conversion ratio of between 7:1 and 6:1 [6]. For the above reasons, in the manufacture process documented, no measurement of the yield of heroin base could be made to compare with the data provided by UNODC.

The materials were weighed on location using a beam balance and weights that CNPA had borrowed from a local trader. Because the lightest weight was 500 grams, it was not possible to establish the exact weight of the materials. Thus, weights had to be estimated using expert knowledge. However, the quantity of acetic anhydride used could be precisely determined, because the weight of the acetic anhydride was established on the basis of the weight of the morphine base used. Due to its sticky consistency, the raw opium was weighed together with its wrapping material.

Figure II. Observed process for manufacture of heroin hydrochloride, stating the quantities of the raw opium, the intermediate morphine base and the final product



[a]The weight given for the opium used is that of the raw material at the place of manufacture.
[b]The weight given for the morphine base is that of the air-dried substance weighed on site.
[c]The weight of the white heroin hydrochloride is that of the substance dried on the water bath.

Analysis of active ingredients

The samples of the raw opium, the morphine base, the press cake, the brown heroin base, the white heroin base and the white heroin hydrochloride were transferred to polyethylene containers at the production site and stored at room temperature until they were analysed in Germany, at the Forensic Science Institute of BKA. Before the samples were analysed, they were dried over phosphorus pentoxide until their weight remained constant, except for the raw opium samples and the morphine base, which were analysed immediately.

The raw opium, the morphine base and the press cake were analysed to determine their active ingredients (see table 3).

Table 3. Analysis of active ingredients of the raw opium, the morphine base and the press cake, in free base form
(Percentage by weight)

Sample	*Morphine*	*Codeine*	*Papaverine*	*Narcotine*
Raw opium[a]	8.5	2.7	1.4	8.6
(minimum-maximum)	(6.1-11.1)	(1.8-3.6)	(0.6-2.2)	(6.8-9.8)
Morphine base	53.1	3.8	2.4	20.3
Press cake	0.2	<0.1	1.2	3.7

[a]Average content calculated from the four visually distinguishable quantities.

The raw opium used was made up of four visually distinguishable quantities. The persons preparing the heroin examined the raw opium and said that they were not satisfied with it because it was of poor quality. Dry weights were used, except for the alkaloid content of the raw opium, for which the undried weight was used. The additional drying of the raw opium at 110 °C to a constant weight led to no significant change in the alkaloid content.

The average opium alkaloid content in dried, raw opium [1] is: morphine, 11.4 per cent (range: 3.1-19.2 per cent); codeine, 3.5 per cent (range: 0.7-6.6 per cent); papaverine, 3.2 per cent (range: <0.1-9.0 per cent); and narcotine, 8.1 per cent (range:1.4-15.8 per cent). A more recent report from UNODC states that the morphine content of raw opium from Afghanistan ranges from 8.4 to 23.5 per cent [6]. The average morphine content of the raw opium used in the process documented is somewhat lower than those published values; that confirms the assessment made by the persons demonstrating this method of processing heroin.

The very lower residual morphine content of the press cake (0.2 per cent) indicates that almost all the alkaloid content was extracted from the raw opium used.

The brown heroin base, the white heroin base and the white heroin hydrochloride were analysed to determine their alkaloid content (see table 4).

Table 4. Analysis of the alkaloid content of the brown heroin base, the white heroin base and the white heroin hydrochloride, in free-base form
(Percentage by weight)

Sample	*Diacetyl-morphine*	*Monoacetyl-morphine*	*Morphine*	*Acetylcodeine*	*Papaverine*	*Narcotine*
Brown heroin base	68.1	7.8	1.8	5.0	1.1	6.0
White heroin base	78.5	6.8	2.0	4.7	—	—
White heroin hydrochloride	74.0	5.4	0.3	4.4	—	—

A relatively high monoacetylmorphine content (5.4-7.8 per cent) was found in the samples of the brown heroin base, the white heroin base and the white heroin hydrochloride. That could be a result of hydrolysis: because of the cool, rainy weather at the time of synthesis, it was not possible to completely dry the samples. According to the persons who demonstrated this method of heroin processing, intermediate products are usually directly processed or, as in the case of the morphine base, laid out and air-dried.

Heroin analysis programme

Within the framework of the German heroin analysis programme, seized heroin preparations are analysed to identify the quality of the material, the area of origin and links between individual cases. To that end, the *Länder* (federal states) submit an analysis report on the main components of seized heroin and/or an actual sample if the quantity seized is more than 100 grams.

The heroin analysis programme evaluates analyses of the main alkaloids of seized samples and, if the actual samples are available, also analyses trace components by comparing chromatographic patterns. The results obtained by that process are stored in a database. Thus, the database of the heroin analysis programme contains both data taken from reports on analysis results and data obtained from the direct analysis of samples.

The heroin analysis programme distinguishes three main types of heroin, according to area of origin: South-West Asian heroin, found in samples from Afghanistan and other countries in that subregion; South-East Asian heroin, found in samples from the Golden Triangle (the Lao People's Democratic Republic, Myanmar and Thailand) and other countries in that subregion; and South American heroin. At present, 7,100 sets of data, based on 4,050 data sheets and 3,050 actual samples, are stored in the database of the heroin analysis programme, which began in 1981.

Comparison with heroin analysis programme data

The results of the chemical analysis of the composition of main and trace substances contained in the samples taken from the documented manufacture process are compared with data from the heroin analysis programme and discussed below.

Morphine base

The morphine base of the process observed in Kabul had a purity of 53.1 per cent (see table 3). Morphine base has been seized in only a few cases in Germany. A total of 10 sets of data on samples of this type were stored in the database of the heroin analysis programme. Those samples had an average purity of 59.0 per cent, with a range of 38.4-83.6 per cent. The sample from Kabul has a similar average purity. The same is true for the content of the opium alkaloids codeine and papaverine. The 10 database samples had an average codeine

content of 5.0 per cent (range: 2.3-6.8 per cent) and an average papaverine content of 2.2 per cent (range: 0.6-3.8 per cent). The narcotine content of the 10 database samples varied widely, from 0.3 to 60.3 per cent. However, the average narcotine content, 21.4 per cent, was almost identical to that of the sample from Kabul.

Brown heroin base

The database of the heroin analysis programme contained 925 sets of data for undiluted and unadulterated brown heroin base from South-West Asia. There is no analytical information or intelligence on heroin of this type coming from South-East Asia.

The brown heroin base from Kabul has a diacetylmorphine content of 68.1 per cent (see table 4), which is higher than the average value of 53.7 per cent (range: 12.2-89.0 per cent) of the 925 database sets. In contrast, the narcotine content of the Kabul sample is relatively low: 6.0 per cent. The narcotine content of the database samples ranges from not detectable to 66.8 per cent. This shows that the narcotine must have been lost directly after acetylation (the reaction with acetic anhydride), either during the hydrolysis of the excess acetic anhydride (when adding the reaction solution to water) or during the precipitation of the heroin base. The brown heroin base from Kabul corresponds to the pattern of South-West Asian heroin, which predominates in the heroin preparations seized in Germany. A singular characteristic of the Kabul sample is its low narcotine content: 6.0 per cent (see table 4). The sets of data on samples attributed to South-West Asia have an average narcotine content of 21.8 per cent. Of the 925 sets of data from the database, only seven had similar ratios of total morphine (sum of the percentages of diacetylmorphine, monoacetylmorphine and morphine) to acetylcodeine, total morphine to papaverine, total morphine to narcotine and papaverine to narcotine, which made those samples suitable for comparison on the basis of the composition of the main ingredients. Five of the seven data sets came from data sheets; so, in those cases there were no actual samples available for additional examination (the comparison of the trace profiles). The trace profiles of the two remaining samples with a similar composition of main components, however, differed greatly from the trace profile of the brown heroin base from Kabul. Thus, it was not possible to find a comparable heroin preparation in the collection of BKA. The brown heroin base from Kabul is a variation of the South-West Asian type of heroin that had not been detected in Germany before.

White heroin base

The white heroin base has a clearly higher diacetylmorphine content than the brown heroin base: 78.5 per cent. In the process observed, the purification of the heroin preparation with the help of activated carbon was effective: an additional separation of papaverine and narcotine was achieved in the course of that processing stage.

The database of the heroin analysis programme does not contain data on white heroin base samples. It is not known whether such preparations have appeared yet in the drug scene in Germany. Probably, white heroin base is an intermediate product of white heroin hydrochloride manufacture that does not usually appear on the illicit market. The persons demonstrating how heroin was prepared confirmed that assumption when they said that they had never heard of anyone ordering that product.

White heroin hydrochloride

The conversion of the white heroin base to the final product of white heroin hydrochloride resulted in a slight decrease in the diacetylmorphine content, from 78.5 to 74.0 per cent. That could easily be explained by the additional dissolving and filtering process.

The heroin analysis programme collection contains 11 preparations of white heroin hydrochloride from 2002 and 2003 that have a similar diacetylmorphine content. None of those samples have a composition equivalent to the white hydrochloride from Kabul. The white heroin hydrochloride manufactured in the observed process is not identical to the type of heroin that is usually seized in Germany. As a rule, what is found in Germany is brown heroin base, which, in the manufacture process demonstrated, is no more than an intermediate product.

The so-called "white heroin" seized occasionally in Germany since 2000 is white or off-white heroin hydrochloride with a purity greater than 60 per cent calculated as base, believed to come from the South-West Asian region. Until now, it was unknown in which country that heroin preparation was produced. As the final product in Kabul shares key features of that "white heroin", it is clear that this kind of heroin can be produced in Afghanistan.

Additional findings

Upon questioning, the two Afghans who demonstrated the heroin manufacturing process provided valuable forensic information on the set-up and organization of a clandestine heroin laboratory in Afghanistan. They explained that they themselves did not own a clandestine heroin laboratory but had been hired by a person operating a laboratory and that that person also provided the equipment and the chemicals. They gave no information about the usual size of such laboratories or their production capacity. They said that the person ordering the heroin manufacture would provide the person running the laboratory with the base material, raw opium, in plastic bags in the form of "opium bread" weighing approximately 0.5-1 kilogram, which would be bundled and put into larger plastic bags weighing one "khaltar" (approximately 7 kg). Several "khaltar" would then be put in a sack for transport. The conclusion to be drawn from that is that the raw opium that is converted to heroin can consist of multiple batches of varying quality coming from different areas of production. The person ordering the narcotic drug also decides which product is to be manufactured and monitors the manufacture process. The preparation of the morphine

base and its subsequent conversion to heroin do not have to take place at the same laboratory. The manufacture itself takes place around the clock, without interruption, with a typical manufacture time of about 2-3 days. The process demonstrated by the two Afghans took about 50 hours.

It became clear from talking with the two Afghans and observing them that their work was the result of acquired skills communicated orally. They carried out all steps with great care and skill. It can be assumed, however, that they did not have any scientific training. They did not reveal whether they were able to use other methods of processing heroin.

Conclusion

An authentic process of heroin production in Afghanistan was documented. White heroin hydrochloride was manufactured using simple and widely available equipment and a small quantity of chemicals. The quantities of chemicals actually used corresponded to the minimum required for processing heroin. The only organic solvent used was acetone, and only a very small quantity of it was used. The brown heroin base prepared as an intermediate product during the manufacture process shares some characteristics with the South-West Asian-type of heroin preparations usually seized in Germany.

Previously, it had not been possible to confirm the hypothesis that heroin with a high purity level ("white heroin") seized in countries in Western Europe, including Germany, could be from Afghanistan, as suggested by police investigations, because samples of heroin seized in Afghanistan had not been available for forensic analysis. The final product of the heroin manufacture process documented in this article was white heroin hydrochloride, which, forensic analysis has revealed, shares the key features of the "white heroin" occasionally seized in Germany since 2000. Thus, it has been proved that this type of heroin can be produced in Afghanistan. The question remains whether white heroin is manufactured in other countries as well.

The authors were unable to determine whether the documented manufacture process is typical of Afghanistan because it is the only authentic heroin manufacture process that BKA of Germany has so far documented. The way in which the two Afghans prepared the heroin suggested that it was a commonly used method. The question remains whether other methods of processing heroin exist and, if so, how many. Nevertheless, the information gained provides numerous clues about the amount of heroin that can be produced from opium and the quantities of chemicals required.

The documentation of the heroin manufacture process has provided useful insight into the operations of clandestine heroin laboratories. That information will be used for training forensic scientists and drug law enforcement officers.

The information obtained in the course of the demonstration with regard to the chemicals used (their origin, type, amount, utilization, disguise and counterfeiting) supports operational drug enforcement measures. For example, as stated above in this article, smuggled chemicals are deceptively labelled or put

in containers intended for other chemicals. It is hoped that making use of all this information in law enforcement operations will help reduce heroin manufacture in Afghanistan, although such a reduction clearly depends, first and foremost, on social conditions in Afghanistan itself.

References

1. *Recommended Methods for Testing Opium, Morphine and Heroin: Manual for Use by National Drug Testing Laboratories* (ST/NAR/29/Rev.1).
2. D. A. Cooper, "Clandestine production processes for cocaine and heroin in clandestinely produced drugs, analogues and precursors: problems and solutions", M. Klein and others, eds., *Proceedings of the International Conference on Assessment of Drug Control Issues of Controlled Substance Analogues, Rabat, 8-11 September 1987* (Washington, D.C., United States Department of Justice, Drug Enforcement Administration, 1989), pp. 95-116.
3. *Clandestine Manufacture of Substances under International Control: Manual for Use by National Law Enforcement and Narcotics Laboratory Personnel* (ST/NAR/10/Rev.2), p. 167.
4. *The Opium Economy in Afghanistan: an International Problem* (United Nations publication, Sales No. E.03.XI.6), p. 133.
5. *Precursors and Chemicals Frequently Used in the Illicit Manufacture of Narcotic Drugs and Psychotropic Substances: Report of the International Narcotics Control Board for 2004 on the Implementation of Article 12 of the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988* (United Nations publication, Sales No. E.05.XI.6), p. 77.
6. United Nations Office on Drugs and Crime, *Limited Opium Yield Assessment Surveys—Technical Report: Observations and Findings; Guidance for Future Activities* (2003).

Impurity profiling/comparative analyses of samples of 1-phenyl-2-propanone

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ABSTRACT

1-Phenyl-2-propanone (P-2-P), also known as benzyl methyl ketone (BMK), is the main precursor used in amphetamine synthesis. In recent years, the number of seizures of P-2-P from both licit and illicit drug manufacture has increased. The present article comprises a discussion of some of the largest seizures of P-2-P diverted from regular production to the illicit market. It also presents the methods used in clandestine laboratories to synthesize P-2-P and a forensic approach to identify and differentiate between these methods.

To that end, and to facilitate the monitoring of the P-2-P market, a method of P-2-P impurity profiling was designed for comparative purposes and for the identification of the synthesis route. P-2-P samples were analysed by means of gas chromatography/mass spectrometry (GC/MS). Out of 36 identified impurities, 14 were selected as markers for sample comparison. On the basis of the GC peak areas of those 14 markers, a cluster analysis was carried out, resulting in three clusters, each corresponding to a given P-2-P synthesis route.

The results of P-2-P impurity profiling are stored in both a forensic database and a police database. The forensic database comprises chemical data, such as those on P-2-P purity, additives and specific impurities, as well as information on seized P-2-P samples having a similar impurity profile. Data stored in the police database, which is linked with the forensic database by case identification number, cover the circumstances of seizures and personal details of offenders. The databases enable the full use of forensic data in intelligence work and police investigative activities.

Keywords: 1-phenyl-2-propanone; profiling; cluster analysis; synthetic methods; mass spectrum; intelligence; law enforcement; clandestine laboratories

Introduction

Poland, together with Belgium and the Netherlands, is one of the main countries in which amphetamine is manufactured for the illicit market. In Poland, several

clandestine laboratories manufacturing amphetamine by means of Leuckart synthesis are closed down every year. In order to suppress this branch of illicit drug manufacture, law enforcement authorities strive to reach illicit producers and "retailers" of the main precursor: 1-phenyl-2-propanone (P-2-P), also known as benzyl methyl ketone (BMK).

In the last several years, there have been several seizures of P-2-P and dismantlings of clandestine P-2-P laboratories in Poland. The appearance on the illicit market of P-2-P originating from both legitimate (through diversion) and illicit manufacture called for the development of an analytical method that would make it possible to differentiate production batches of precursors. Furthermore, the police demanded answers to questions such as which P-2-P samples might come from the same source and what their method of synthesis was.

In order to meet those expectations, a method of P-2-P impurity profiling was elaborated. The main purpose of the method is to assist the police in their efforts to combat the illicit manufacture of and trafficking in P-2-P. The identification of the synthesis method allows the police to focus their site investigations on specific chemicals, while the identification of links between P-2-P samples facilitates the monitoring of trafficking in that precursor, as well as the identification of the sources and smuggling routes used.

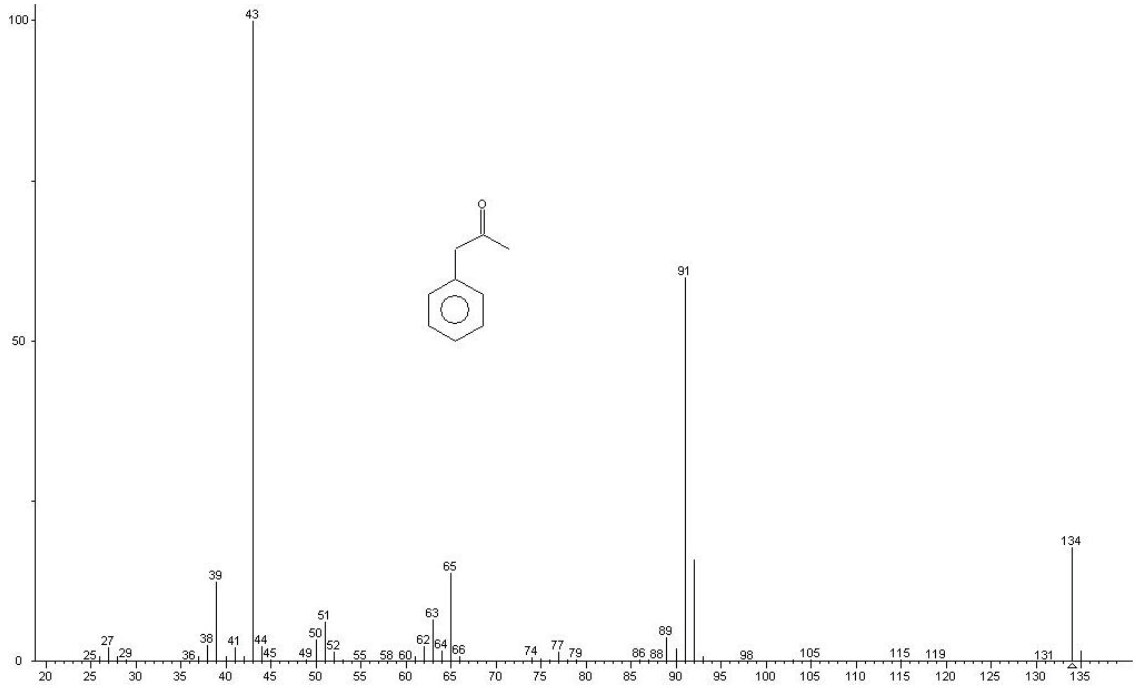
1-Phenyl-2-propanone

Characteristics of 1-phenyl-2-propanone

P-2-P is a colourless or slightly yellowish liquid. The colour of illicitly manufactured P-2-P may vary from yellow to dark brown. It has a density similar to that of water and a pleasant scent. P-2-P is most frequently used as a precursor for the manufacture of amphetamine. The mass spectrum and molecular formula of P-2-P are shown in figure I; physical and chemical data for P-2-P are as follows:

Name used in Table I of the 1988 Convention:	1-phenyl-2-propanone
Other names:	P-2-P; benzyl methyl ketone (BMK); phenylacetone
Chemical Abstracts Service number:	103-79-7
Molar mass:	134.18
Molecular formula:	$C_9H_{10}O$
Melting point:	−15 °C
Boiling point:	214 °C
Density:	1.0157 g/cm^3

Figure I. Mass spectrum and molecular formula of 1-phenyl-2-propanone



Manufacture and legitimate and illegal uses

At the moment, countries where P-2-P is licitly manufactured are difficult to name because relevant data vary, depending on the source of the data. For example, the European Anti-Fraud Office (OLAF) mentions China, France and Japan among licit producers, while the International Narcotics Control Board (INCB) would also add India and the United States of America to the list. Up-to-date information on the total annual volume of world P-2-P manufacture is still unavailable.

Despite the lack of official data, the analysis of the situation in the area of illicit manufacture of synthetic drugs allows speculation that licit manufacture of P-2-P takes place also in the Russian Federation and Ukraine.

The legitimate use of P-2-P in the chemical and pharmaceutical industries is practically limited to the manufacture of amphetamine and methamphetamine and their derivatives. Another legitimate use of P-2-P is to produce, through photolysis, benzyl radicals, which in turn are used for the production of propylhexedrine in the process of organic synthesis. In Turkey and the United States, P-2-P is also found in cleaning agents and stain removers. In Poland, P-2-P has no legitimate use other than in scientific research.

In addition, P-2-P is used as a precursor for the illicit manufacture of amphetamine and methamphetamine. It is estimated that P-2-P is used to manufacture nearly 100 per cent of amphetamine from illicit sources and approximately 10 per cent of methamphetamine from illicit sources.

The official price of P-2-P is about €100 per kilogram, while the mean European black market price is approximately €900 per kilogram.

Methods of synthesis of 1-phenyl-2-propanone, with particular emphasis on those employed in clandestine laboratories

P-2-P is a substance listed in Table I of the United Nations Convention against Illicit Trafficking in Narcotic Drugs and Psychotropic Substances of 1988 [1] and, because its legitimate uses are limited, its availability on the licit market is also limited. As a result, one apparent advance has been observed in methods of illicit P-2-P synthesis. Among several dozen known synthesis routes, the ones most frequently encountered in clandestine laboratories are those starting from phenylacetic acid, benzyl cyanide and benzaldehyde (nitrostyrene method).

Synthesis from phenylacetic acid

In recent years, synthesis of P-2-P from phenylacetic acid has become one of the most popular methods used in clandestine laboratories. Although phenylacetic acid is a controlled substance, it is readily available because of its widespread industrial use. In some cases, clandestine laboratories replace the controlled phenylacetic acid with phenylacetic acid chloride, which is subsequently transformed into phenylacetic acid by mixing with water. P-2-P can be generated in a reaction of phenylacetic acid with acetic acid or acetic anhydride, and both acetic acid or acetic anhydride are cheap and easy to procure. Two significant difficulties in the synthesis of P-2-P from phenylacetic acid are the stringent reaction conditions and poor product yield. Despite those drawbacks, illicit manufacture of P-2-P from phenylacetic acid has proved to be cost-effective and constitutes a notable contribution to the illicit precursor market.

Synthesis from phenylacetic acid and acetic acid

Synthesis of P-2-P from phenylacetic and acetic acid is a straightforward, one-step process.

$$\text{Phenylacetic acid} + \text{acetic acid} \rightarrow \text{P-2-P}$$

However, it requires sophisticated equipment, high temperatures and high pressure.

The yield of this reaction is approximately 50 per cent; that is, 500 ml of P-2-P are synthesized from 1 kg of phenylacetic acid.

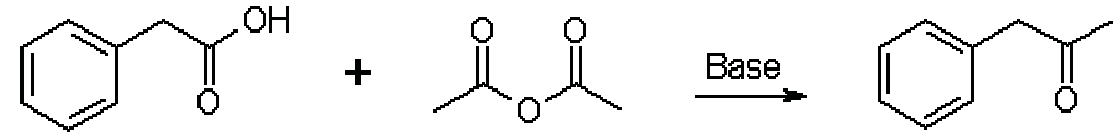
Indicators of synthesis of 1-phenyl-2-propanone from phenylacetic acid and acetic acid

- Phenylacetic acid and acetic acid have a characteristic sharp odour
- Sophisticated equipment and/or stringent reaction conditions:
  - A steel reaction vessel, as well as heating units and equipment used for sustaining temperatures in the range of 300°-400 °C
  - Bottles with nitrogen for regeneration of catalyst
- High consumption of electricity on the premises to sustain high temperatures
- Phenylacetic acid chloride (a non-scheduled substance) can be used instead of phenylacetic acid.

Synthesis from phenylacetic acid and acetic anhydride

Unlike the synthesis route using acetic acid, the reaction of phenylacetic acid with acetic anhydride (see figure II) does not require extreme conditions. The reaction occurs at the boiling point of the reaction mixture, about 150 °C, and requires only basic glassware.

Figure II. Synthesis of 1-phenyl-2-propanone from phenylacetic acid and acetic anhydride



Phenylacetic acid + acetic anhydride ➔ P-2-P

The yield ranges from 50 to 70 per cent; that is 500-700 ml of P-2-P can be obtained from 1 kg of phenylacetic acid.

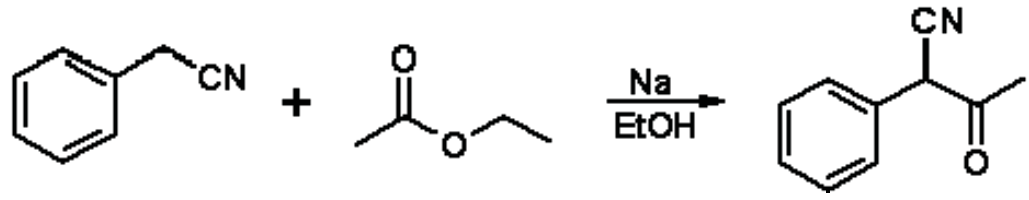
Indicators of synthesis of 1-phenyl-2-propanone from phenylacetic acid and acetic anhydride

- Phenylacetic acid and acetic anhydride have a characteristic sharp odour.
- Considerable amounts of acetic acid salt are required (sodium acetate, potassium acetate or lead acetate).
- During the synthesis, large quantities of carbon dioxide gas are released.

Synthesis from benzyl cyanide

Benzyl cyanide is commonly used in the pharmaceutical industry. It is not classified as a controlled substance under European Parliament and Council Regulation No. 273/2004 and therefore is a precursor of choice among illicit manufacturers of P-2-P. The synthesis of P-2-P from benzyl cyanide occurs in two stages. During the first stage (see figure III) benzyl cyanide is condensed with ethyl acetate in the presence of sodium ethylate.

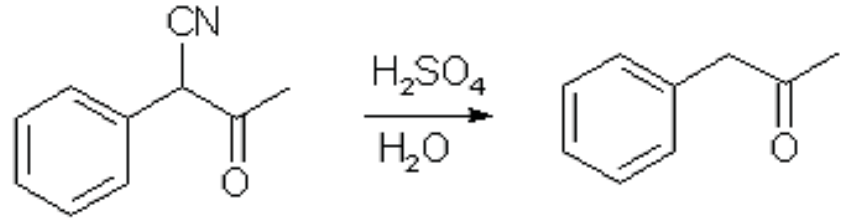
Figure III. Synthesis of 1-phenyl-2-propanone from benzyl cyanide, first stage



Benzyl cyanide + ethyl acetate ➔ α-phenylacetoacetonitrile

The second stage (see figure IV) involves hydrolysis of the nitrile group and subsequent decarboxylation of the resulting acid. The overall yield of P-2-P after both steps amounts to approximately 80 per cent; that is, 900 ml of P-2-P can be obtained from 1 kg of benzyl cyanide.*

Figure IV. Synthesis of 1-phenyl-2-propanone from benzyl cyanide, second stage



α-phenylacetoacetonitrile → P-2-P

Indicators of synthesis of 1-phenyl-2-propanone from benzyl cyanide

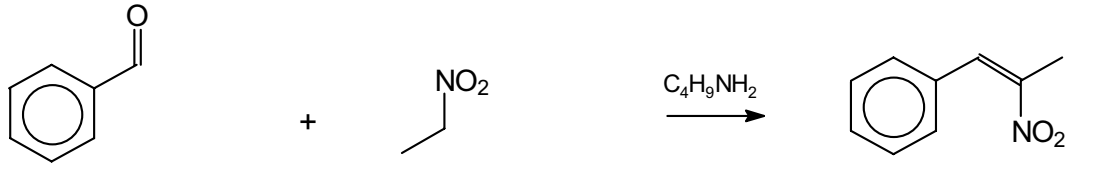
- Metallic sodium used in the first stage ignites upon contact with water (and reacts violently with ethanol), which entails a serious fire risk.
- Phosphoric acid, which has no other application in clandestine laboratories, is used at the stage of hydrolysis and decarboxylation.
- Cooling equipment (ice or dry ice) is vital.
- Due to the need to operate in an anhydrous environment, drying agents, such as calcium oxide or phosphorous pentoxide, are necessary.
- The process has to be conducted by a chemist or a person with vast experience in working in and coping with an anhydrous environment.

*This calculation takes the stoichiometric ratio (i.e. the quantitative relationship) of the molecular masses of the starting material and end product into account.

Synthesis from benzaldehyde

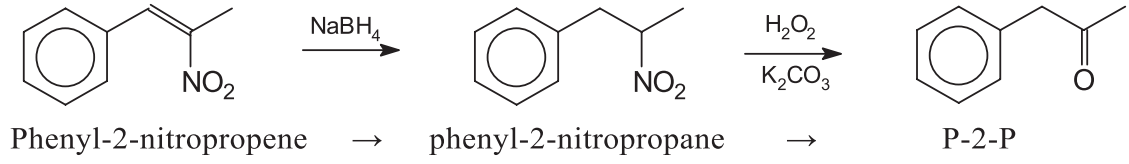
The synthesis of P-2-P from benzaldehyde also occurs in two stages. However, the interval between those stages can be of any desired length, because the intermediate product is chemically stable and can be isolated. In the first stage, an ethanolic solution of benzaldehyde is made to react with nitroethane, using butylamine as a catalyst (see figure V). A crystalline yellow precipitate of phenyl-2-nitropropene is formed at the end of the stage.

Figure V. Synthesis of 1-phenyl-2-propanone from benzaldehyde, first stage: production of phenyl-2-nitropropene



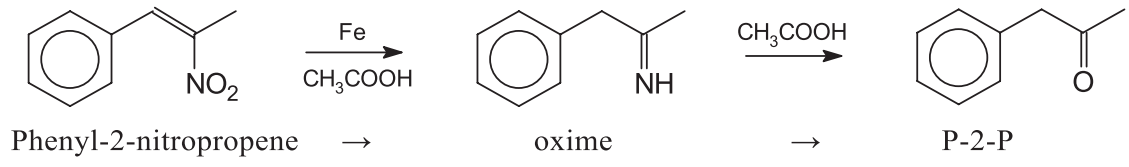
In the second stage of the synthesis, phenyl-2-nitropropene is reduced to phenyl-2-nitropropane, which is subsequently transformed into P-2-P by one of two routes. The first route (see figure VI) results in an overall yield of final product of about 70 per cent; that is, approximately 900 ml of P-2-P are obtained from 1 kg of benzaldehyde.*

Figure VI. Synthesis of 1-phenyl-2-propanone from benzaldehyde, second stage, first route: reduction of phenyl-2-nitropropene to 1-phenyl-2-propanone by means of sodium borohydride



The second route (see figure VII) results in an overall yield of approximately 75 per cent; that is, 960 ml of P-2-P are produced from 1 kg of benzaldehyde.*

Figure VII. Synthesis of 1-phenyl-2-propanone from benzaldehyde, second stage, second route: reduction of phenyl-2-nitropropene to 1-phenyl-2-propanone with ferrous powder in acidic environment



*This calculation takes the stoichiometric ratio (i.e. the quantitative relationship) of the molecular masses of the starting material and end product into account.

Indicators of synthesis of 1-phenyl-2-propanone from benzaldehyde

- Uncommon chemicals, nitroethane and butylamine are used.
- Phenyl-2-nitropropene (intermediate product) is a solid, crystalline substance with a characteristic yellow colour.
- Amphetamine could be produced directly from the intermediate product (phenyl-2-nitropropene), but the use of a strong reducing agent, such as lithium aluminium hydride, would be required.
- Execution of the second stage requires considerable experience on the part of the chemist, and the process must be continuously controlled.

Substantial seizures of 1-phenyl-2-propanone

The International Narcotics Control Board (INCB) reported that in 2001 a total of 23 tons of P-2-P was seized worldwide, the largest total volume ever seized in a single year. The majority of the seizures, totalling 18.2 tons, were effected in the Netherlands, in the port of Rotterdam harbour. The illegal consignments had been shipped from China ([2], paras. 105-106).

Considering the usual modus operandi of criminals on the P-2-P trafficking route leading to the Netherlands (illicit manufacture, or diversion from the legitimate market, in China, followed by smuggling by sea to the Netherlands), the position of Poland is quite interesting. There are reports of P-2-P being smuggled into Poland both by sea from China and overland from the east (Belarus and Ukraine) and the south (Czech Republic). In some years from 1989 to 2000, seizures of P-2-P effected in Poland from land transport were the world's largest: 1.135 tons in 1994 and 710 kg in 1995.

P-2-P seizures in Poland are also associated with detecting, closing down and dismantling clandestine amphetamine laboratories. For example, of the total of 255 kg of P-2-P seized in Poland in 2003, 90 per cent was seized in illicit laboratories raided by the police. According to police investigative findings, in the majority of those cases P-2-P had originated in Belarus or Ukraine.
The following seizures from land transport deserve mention:

(a) On 2 February 1993, at the Medyka border crossing between Poland and Ukraine, 290 l of P-2-P were discovered in a truck driven by a Bulgarian citizen. The precursor was concealed in wood-impregnated barrels. The smuggling route from Bulgaria to Poland led through Romania and Ukraine. The shipment was to be delivered to a Warsaw company specially set up by an organized criminal group in order to facilitate criminal activities. The initial source of the P-2-P shipment from Bulgaria was not identified;

(b) On 14 February 1994, at the Cieszyn border crossing between the Czech Republic and Poland, 700 kg of P-2-P was found hidden in a truck with a Bulgarian registration plate, driven by a Bulgarian citizen. The route from Bulgaria to Poland went through Hungary and the Czech Republic. As in the

previous case, the consignee was a company specifically set up by an organized criminal group and the source of the P-2-P shipment remained unknown;

(c) On 26 December 1994, at the Hrebenne border crossing with Ukraine, 435 l of P-2-P was being smuggled from Ukraine to Poland in the petrol tank of a truck registered in Ukraine and driven by a Ukrainian citizen. Ukraine was identified as the country of origin of the P-2-P consignment;

(d) Two subsequent confiscations of considerable amounts of P-2-P shipped from Belarus (100 l in 2001 and 57 l in 2002) persuaded the criminals to change their smuggling method; the transporting of huge quantities of the precursor was given up. Nowadays, "retail smuggling" predominates, whereby smaller quantities of P-2-P are carried by couriers in, for example, hot water bottles (capacity: 1 l);

(e) The largest attempted illegal import of P-2-P into Poland took place in 2004 and resulted in the world's largest confiscated amount of P-2-P that year: 4,680 l were seized in Gdynia harbour. The Central Bureau of Investigation of the General Police Headquarters in Warsaw conducted the investigation and intelligence proceedings in cooperation with the Belgian police. The case involved an international criminal group specializing in facilitating illegal immigration, trafficking in human beings and money-laundering, as well as drug trafficking. The activities of the group took place in the territories of Belgium, Germany, the Netherlands, Poland and Sweden;

(f) On 29 March 2004, in Shanghai harbour, a container was loaded onto a trans-oceanic freight vessel. According to customs documentation, the container was carrying sesame oil destined for a company specifically set up by an organized criminal group and owned by a person involved in criminal activity. On 30 April 2004, the container, supposedly containing 15,600 l of sesame oil (in 260 60-litre barrels) arrived in Gdynia harbour. On 5 May 2004, officers of the Central Bureau of Investigation and customs officers confiscated the delivery. Following preliminary tests on selected barrels, the substance was identified as P-2-P. Subsequently, the entire consignment was analysed to confirm that P-2-P had been smuggled in 78 60-litre barrels. The total amount of precursor, 4,680 l, would have been sufficient to manufacture approximately 3,300 kilograms of amphetamine using the Leuckart method;

(g) After the detention of the persons responsible for organizing delivery in Belgium and Poland, direct cooperation with INCB was initiated in order to carry out a so-called backtracking investigation. At the same time, direct collaboration with the relevant authorities in China was initiated, leading to the arrest of the detained persons, for diversion of P-2-P from the licit market.

Licit manufacture of 1-phenyl-2-propanone and routes of diversion

According to INCB, 27 cases of diversion and attempted diversion of P-2-P from the licit market were reported between 1995 and 2001. Most of them occurred in India, followed by Germany, Belgium and China. The destination countries

identified included Belgium, Bulgaria, Germany, Hungary, the Netherlands, Romania, Ukraine and the countries that once were part of the former Yugoslavia.

In order to mislead law enforcement authorities, criminals often organize illicit shipments via countries not normally associated with illicit drug production. Quantities of P-2-P smuggled in a single consignment range from a few kilograms or litres to several tons. As regards the modus operandi, there have been instances in which criminal organizations used names of existing companies that were completely unaware that they were named as end-recipients in the customs documents or letters of conveyance. In the majority of cases, however, criminals employed the names of companies regularly importing goods from the countries producing P-2-P, and the goods declared in the customs documents were consistent with the companies' business profiles. Obviously, there have also been instances in which discrepancies between the goods for delivery and a company's profile were spotted, such as when a construction company supposedly imported peaches, and the peaches had in fact been replaced by P-2-P.

Usually, the methods used for the diversion of P-2-P are similar to those observed for other controlled substances:

(a) Attempts by criminal organizations to purchase P-2-P by using forged import licences obtained in the name of companies specifically set up for that purpose ("pillars");

(b) Falsifying customs documentation (declaring fake goods);

(c) Smuggling.

In 13 out of the 27 cases reported to INCB between 1995 and 2001, criminals designed complicated transport routes leading through three or more countries in order to make the verification of the legal status of the consignment more difficult. In six cases, orders were placed in the name of fake companies set up specifically for that purpose ("pillars"), and in two cases false import licences were presented.

It should be emphasized that precursors used for the manufacture of synthetic drugs in Europe are procured mainly on the illicit market, through either smuggling or illicit manufacture. Case studies of attempts at smuggling P-2-P during the past 15 years have highlighted several common features of this category of crime:

(a) In more than 90 per cent of smuggling attempts, the countries of destination were in Europe;

(b) In the majority of cases analysed, the P-2-P was intended to be used as a precursor for the illicit manufacture of amphetamine; only in the United States was it also used for methamphetamine synthesis (some attempts to manufacture methamphetamine from P-2-P in Asia are known as well);

(c) As regards the largest consignments analysed, diversions or illicit manufacture occurred in China, the shipments were dispatched by sea and the destination countries were in Europe;

(d) In recent years, there have been more and more reports of smaller consignments of P-2-P being transported overland across the eastern border of member States of the European Union (that is, from Belarus, the Russian Federation and Ukraine). However, the role of those countries in P-2-P trafficking is not yet known; it has not been determined whether P-2-P is licitly manufactured in those countries and diverted from legitimate trade or whether those countries are merely used as transit countries.*

These trends are also reflected in the fact that more than three quarters of amphetamine seizures worldwide take place in Europe, and two thirds of those take place in Western European countries (Germany, the Netherlands, Sweden, the United Kingdom of Great Britain and Northern Ireland) [3].

Utilization of results of 1-phenyl-2-propanone impurity profiling in police intelligence work

Combining and complementing police intelligence work with information from forensic laboratories dealing with the examination of drugs contribute to building the most desirable system in support of the process of effective suppression of drug-related crime. Several countries and international organizations have joined efforts to enhance this partnership by undertaking various international activities aimed at counteracting cross-border drug-related crime. One of them is Project Prism, launched by INCB in 2002. Its objectives include the strengthening and improvement of cross-border cooperation between law enforcement authorities through the use of specifically tailored, modern strategies and intelligence tools for suppressing the illicit manufacture, smuggling and diversion of precursors.

Project Prism targets five fundamental precursors used in the illicit manufacture of amphetamine-type stimulants, a group of amphetamine derivatives that includes methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA, commonly known as Ecstasy). The precursors of amphetamine-type stimulants are:

(a) Ephedrine and pseudoephedrine, used in the manufacture of methamphetamine;

(b) P-2-P, the main precursor used for the manufacture of amphetamine;

*However, on the basis of numerous instances of reported smuggling of P-2-P into Poland from Belarus and Ukraine, revealed by the Polish investigative services in cooperation with their counterparts in other countries, and from intelligence, it can be concluded that P-2-P is very likely to be licitly manufactured in the Russian Federation and Ukraine and then diverted to illicit markets and then exported. That hypothesis is supported by the analysis of illicit amphetamine manufacture in Poland. Data on the number and types of clandestine laboratories detected and closed down in Poland during the past 10 years show that in the mid-1990s many more laboratories manufactured P-2-P and not amphetamine. The situation has changed recently. For example, in 2000, only 2 out of 14 clandestine laboratories that were shut down specialized in P-2-P manufacture; and in 2004, the figure was only 2 out of 20. Investigations have shown that the reason for the decrease in illicit P-2-P manufacture in Poland was the easy availability of less costly P-2-P, possibly illegally exported from the manufacturing country, Ukraine.

(c) 3,4-methylenedioxyphenyl-2-propane (3,4-MDP-2P) also known as piperonyl methyl ketone (PMK), and safrole used for the manufacture of MDMA (Ecstasy).

Project Prism is based on two parallel approaches:

(a) Tackling the diversion of precursors from licit markets;

(b) Investigative measures aimed at launching backtracking investigations to identify:

(i) Means, methods and routes of diversion, attempted diversion or smuggling;

(ii) Sources and precursor trafficking networks;

(iii) Equipment used in illicit manufacture.

The main aims of investigations in drug-related crime are to identify persons who act as key coordinators of criminal activities and to provide support for future investigations and prevention efforts. Therefore, in the context of backtracking investigations, seizures of illicit precursor consignments should be considered the beginning of the investigative process rather than its conclusion.

A number of regional and international organizations such as INCB, OLAF, the European Police Office (Europol) and the Customs Cooperation Council (also called the World Customs Organization), emphasize the immense importance of providing support for those investigations by means of fast and reliable exchanges of information on licit markets for chemicals, seizures of precursor consignments and results of requested forensic examinations, known as precursor profiling studies.

In order to comply with those requirements in the light of illicit manufacture of synthetic drugs, particularly amphetamine, in Poland the authorities investigating drug-related cases and the forensic services responsible for specialized examinations have been operating in close partnership for several years. A significant outcome of that partnership is Poland's participation in the Eastern Baltic Sea Amphetamine Project, implemented by the Task Force on Organized Crime in the Baltic Sea Region. Thanks to the multilevel cooperation of law enforcement authorities with forensic laboratories in participating countries,* intelligence-led investigations resulted in the identification of several clandestine amphetamine laboratories.

Contribution of impurity profiling of 1-phenyl-2-propanone to law enforcement investigations

In order to propagate and make the best use of the experiences in regional cooperation, the Central Forensic Laboratory of the Police in Poland has proposed

*Cooperation comprises the setting up of computerized forensic and intelligence databanks, exchange of information, the submission of samples of amphetamine seized by all countries participating in the project, comparing amphetamine profiles and their exchange between, for example, the National Forensic Institute in Sweden and the Central Forensic Laboratory of the Police in Poland.

the launching of another profiling project. The subject of the new undertaking is the profiling of P-2-P samples seized, for example, in clandestine laboratories on the territories of participating countries.

The impurity profiling of seized P-2-P samples is aimed at identifying common features of and discrepancies between those samples in order to address the following questions:

(a) Are two or more evidential samples linked with each other?

(b) If links exist, are they of significance, for example, to help to trace a P-2-P sample back to a dealer or a producer?

(c) Can linked samples be traced back to the same local, regional or international network of illicit retailing and distribution?

(d) What is the source of a P-2-P sample (licit or illicit manufacture)?

Data to answer the above questions can be obtained from chemical analyses. They will be of help in the investigative process and may guide further intelligence work by, for example:

(a) Providing information on methods of P-2-P synthesis;

(b) Classifying a sample as part of a group of samples that can be traced back to one source;

(c) Providing supportive evidence on the structure of national, regional and international networks of retailing and distribution;

(d) Determining whether P-2-P samples are from licit or illicit manufacture;

(e) Identifying in a timely manner newly emerging sources of P-2-P.

At the moment, the project is in the implementation phase. Its framework was presented in June 2005 during a meeting of the Expert Group on Narcotics of the Task Force on Organized Crime in the Baltic Sea Region. The project was received with general approval by representatives of all 10 participating countries. Lithuania and Sweden have already expressed their intention to participate, and the decisions of other countries are expected soon.

Also in 2005, representatives of the Polish Police held an official meeting with representatives of the Ukrainian militia to discuss partnership in the area of combating international drug-related crime. On that occasion, the Polish representation presented the P-2-P profiling project and appealed to the Ukrainian militia to support the project by contributing data on the licit market of P-2-P in Ukraine (e.g. the number of manufacturers, the total production volume). Another important issue was the provision of samples from all companies licitly manufacturing P-2-P, to be used as authenticated reference samples in comparative analyses.

Forensic examination of 1-phenyl-2-propanone

From an analytical point of view, the identification of P-2-P is very straightforward and does not pose any problems. The best and quickest results are

obtained by means of gas chromatography/mass spectrometry (GC/MS), by which P-2-P can be identified on the basis of two parameters: retention time and mass spectrum (see figure I). For investigative purposes, in addition to identifying P-2-P, linking samples with a source or a given synthesis batch and identifying the synthesis route are important issues. In order to address those points, the following multi-stage methodology of P-2-P impurity profiling has been elaborated in the Central Forensic Laboratory of the Police:

(a) Sample preparation;

(b) Analysis of samples by means of GC/MS;

(c) Identification of impurities;

(d) Statistical analysis of results;

(e) Introducing the results into the database.

Analytical procedure

Preparation of 1-phenyl-2-propanone samples for analysis

The methodology of P-2-P impurity profiling was elaborated using 80 samples from seized consignments, which investigative information showed to have been produced by different synthesis routes. Every sample was prepared for analysis by mixing 100 ml of P-2-P and 1 ml of chloroform with an internal standard (diphenylamine at a concentration of 0.3 mg/ml); 1 ml of that solution was used for analysis. A typical impurity profile of P-2-P is presented in figure VIII.

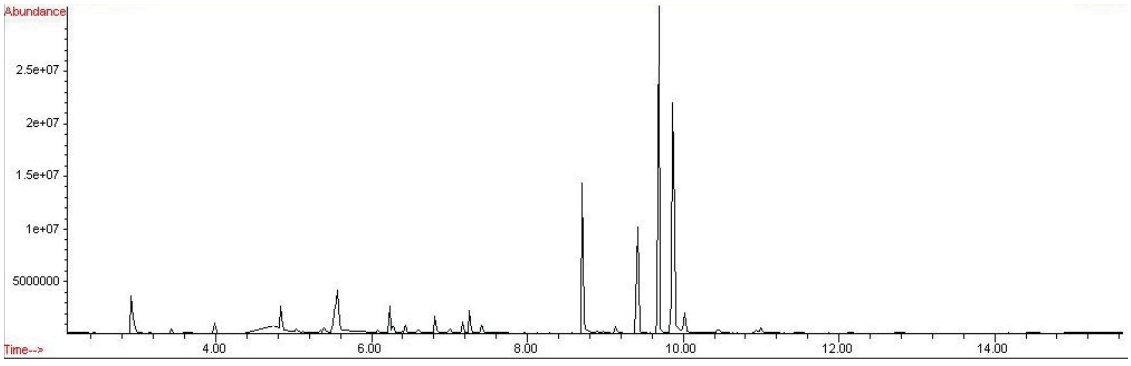
Parameters of analysis by gas chromatography/mass spectrometry

Operation conditions

Instrument:	GC HP-6890/MSD HP 5973 (Hewlett Packard)
Column:	HP5 MS 30 m x 250 μm x 0.25 μm capillary column
Carrier gas:	helium, continuous flow at 1.6 ml/min, split: 20:1
Temperature programme:	70 °C for 0.5 min, 70°-290 °C with ramp of 15 °C/min, 290 °C for 0.5 min (total analysis time: 15.67 min)
Transfer line temperature:	290 °C
Detector:	MSD (TIC 40.5-550 amu), detector turned off in interval from 4.35 to 4.65 min*

*In this process the detector was turned off between 4.35 and 4.65 min; that is, at the expected retention time of the P-2-P peak. A high concentration of P-2-P in the prepared solution would have caused saturation of the detector and would have interfered with the identification of the remaining peaks in the chromatogram.

Figure VIII. Impurity profile of a 1-phenyl-2-propanone sample, obtained through gas chromatography/mass spectrometry



The repeatability of the method was assessed on the basis of 10 analyses of one randomly selected P-2-P sample. All tests were performed on the same day. The standard deviation and relative standard deviation were calculated for retention times and peak areas of two selected peaks, the internal standard (diphenylamine) and phenylacetic acid. The results obtained are presented in table 1.

Table 1. Analyses of one randomly selected sample of 1-phenyl-2-propanone: retention times and peak areas of diphenylamine and phenylacetic acid

	Diphenylamine		*Phenylacetic acid*	
Parameter	*Retention time (min)*	*Peak area (counts)*	*Retention time (min)*	*Peak area (counts)*
Average	8.7179	207 165 059	5.5688	203 856 669
Standard deviation	0.001853	10 983 337	0.001549	4 653 705.3
Relative standard deviation (percentage)	0.02	5.30	0.03	2.28

The reproducibility of the method was determined in 30 analyses of various randomly selected P-2-P samples. The analyses were performed on different days during one month. Standard deviation and relative standard deviation were calculated for the internal standard (diphenylamine). The results obtained are presented in table 2.

Table 2. Analyses of various randomly selected samples of 1-phenyl-2-propanone: retention times and peak areas of diphenylamine

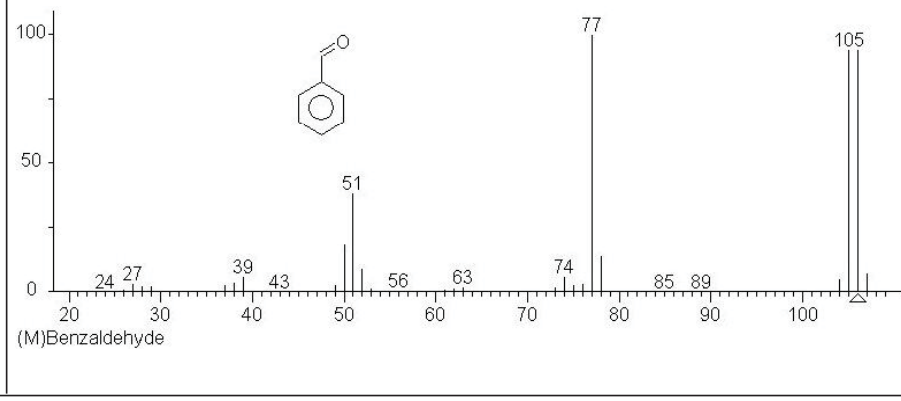
	Diphenylamine	
Parameter	*Retention time (min)*	*Peak area (counts)*
Average	8.717867	224 402 574
Standard deviation	0.001737	21 425 609
Relative standard deviation (percentage)	0.02	9.55

The above findings confirm that the method is repeatable and reproducible.

Identification of impurities

In the 80 P-2-P samples analysed, 36 impurities were identified on the basis of their mass spectra and a comparison of predicted and actual retention time. Fourteen marker impurities were selected for sample comparisons; that is, to make profiles for the entire population of samples (see figure IX).

Figure IX. Impurities identified in samples of 1-phenyl-2-propanone

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
2.93		

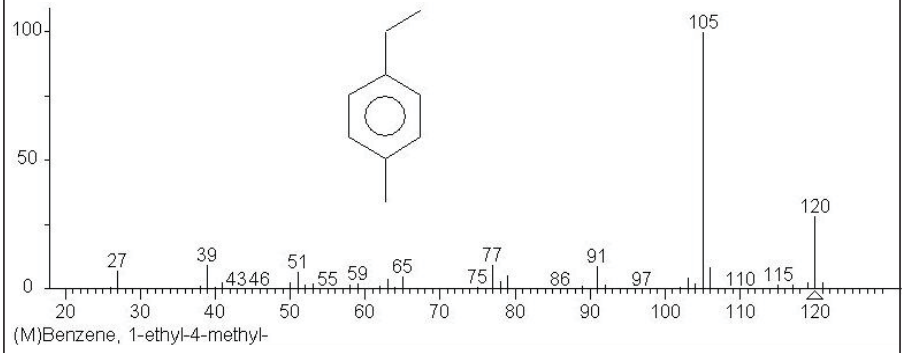
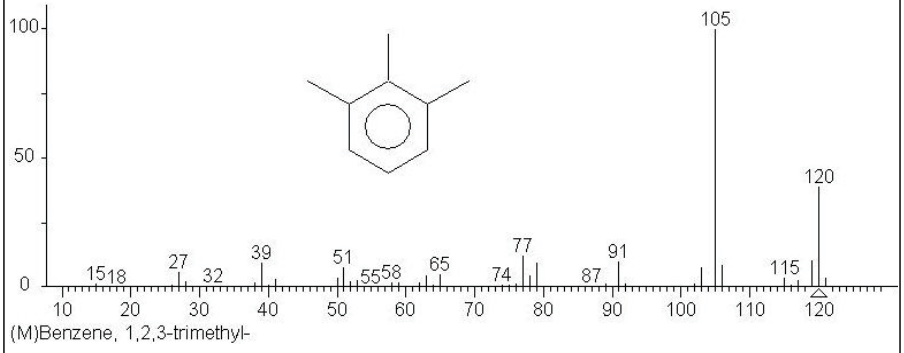
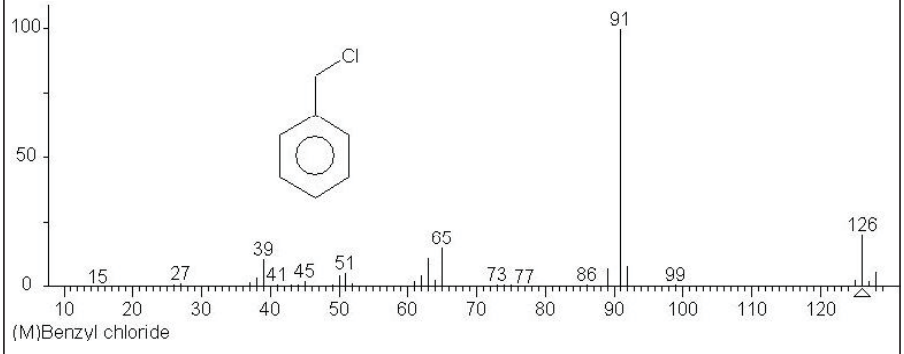
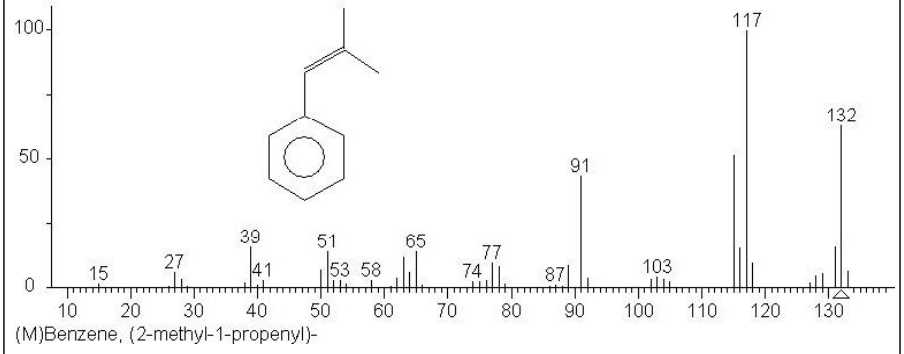
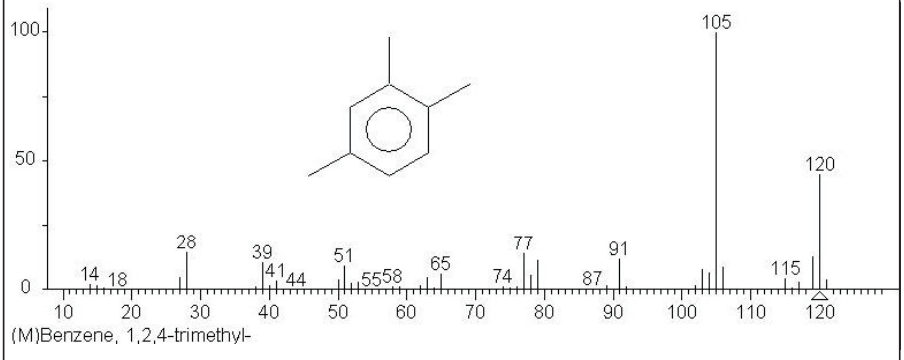
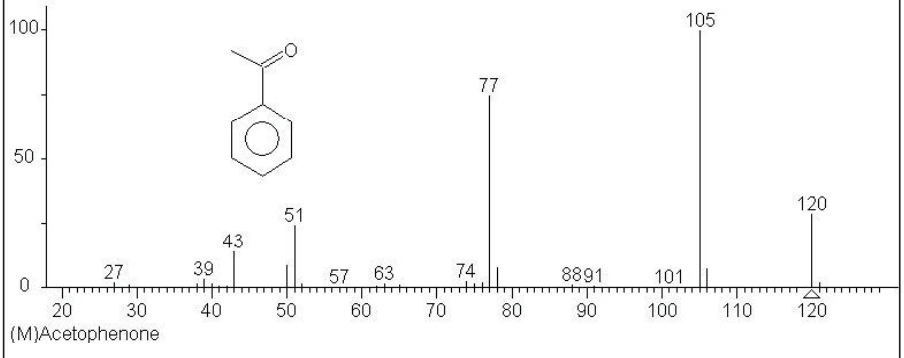
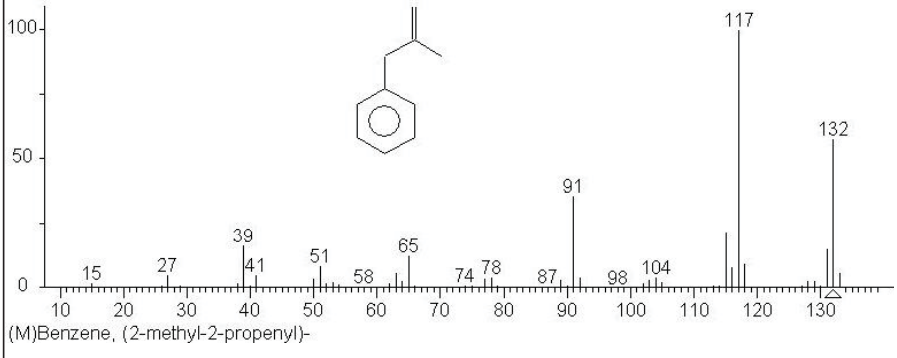
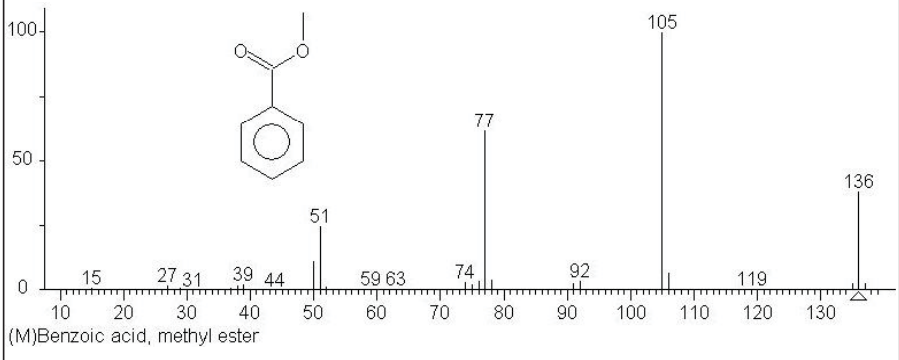
Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
3.08	 (M)Benzene, 1-ethyl-4-methyl-	
3.20	 (M)Benzene, 1,2,3-trimethyl-	
3.38	 (M)Benzyl chloride	
3.44	 (M)Benzene, (2-methyl-1-propenyl)-	1

Figure IX. ***(continued)***

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
3.46		
3.85		2
4.00		3
4.12		

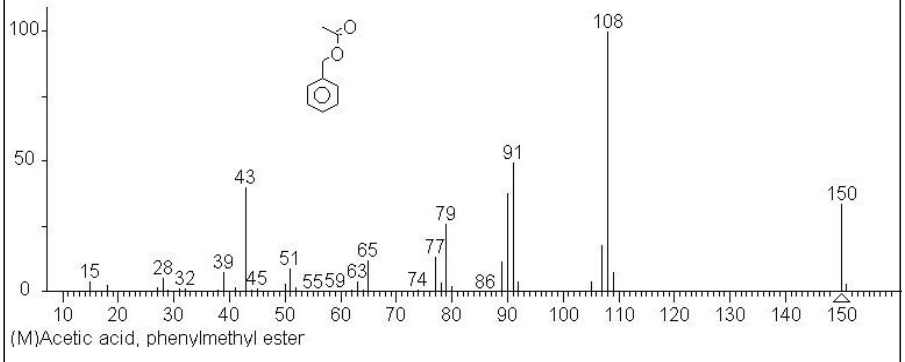
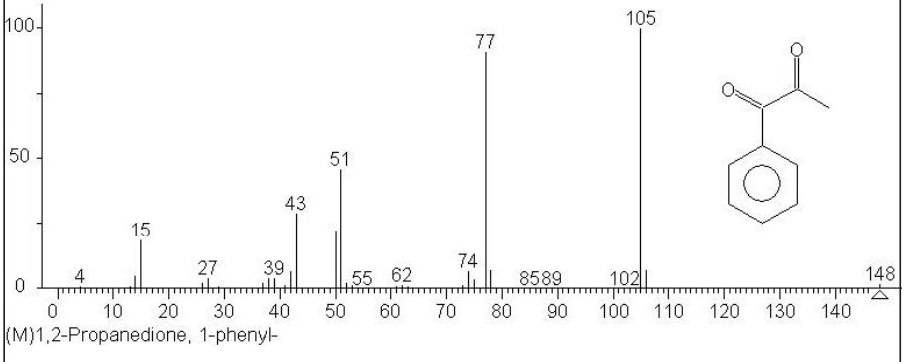
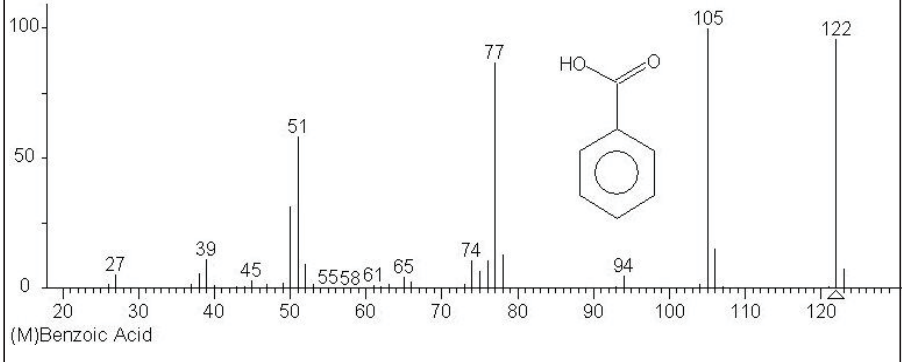
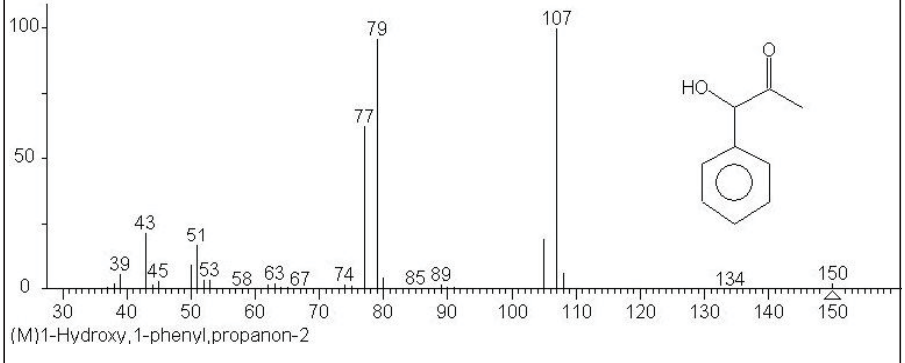
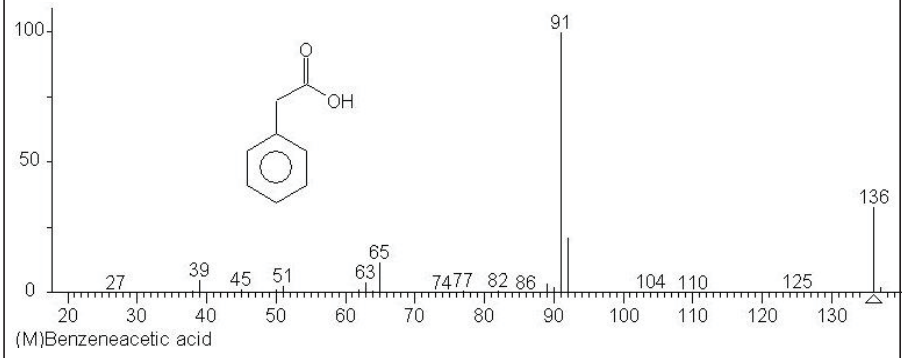
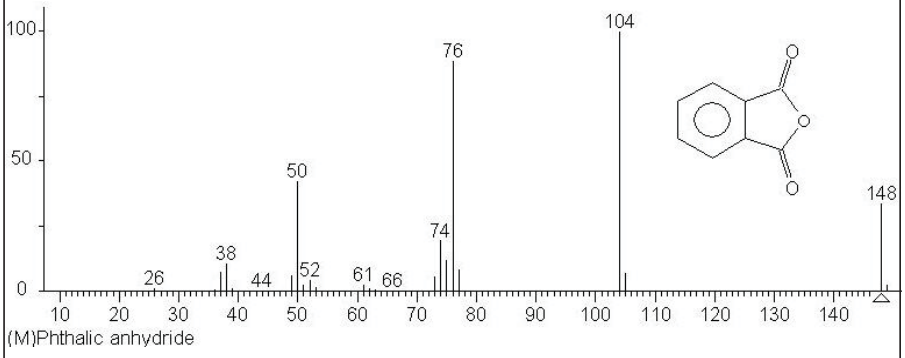
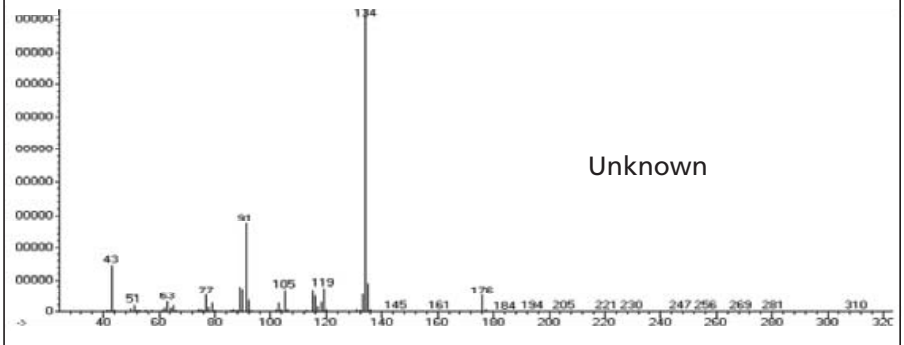
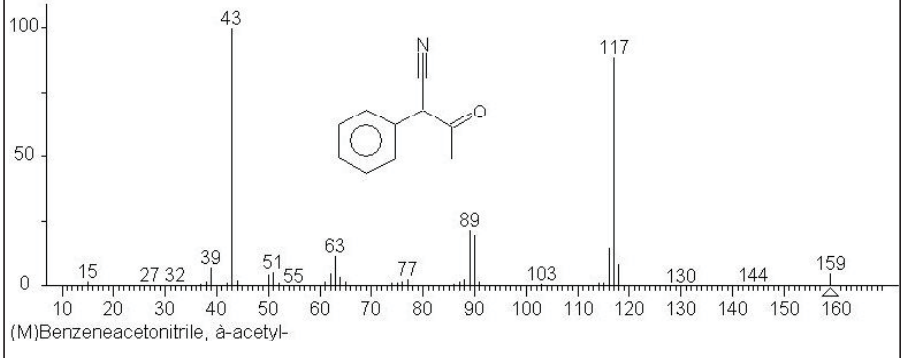
Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
4.77		
4.84		
4.98		4
5.40		5

Figure IX. ***(continued)***

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
5.57		6
6.18		
6.25	 Unknown	7
6.29		8

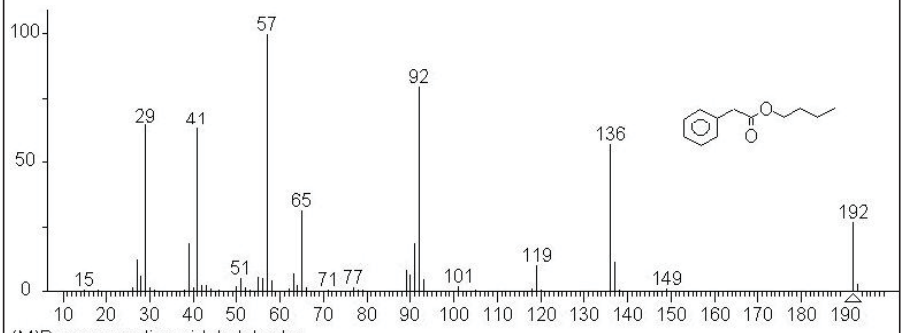
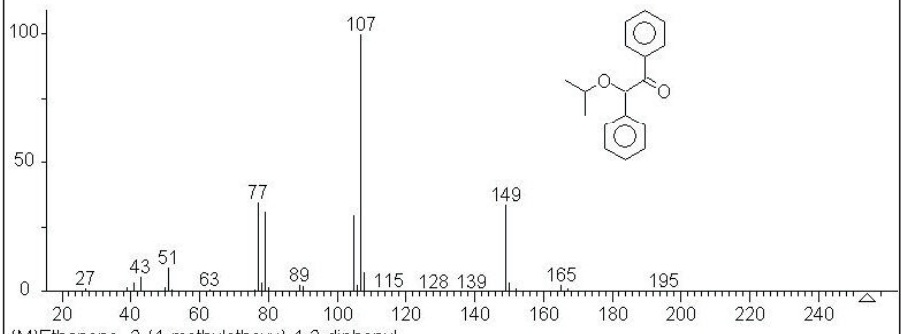
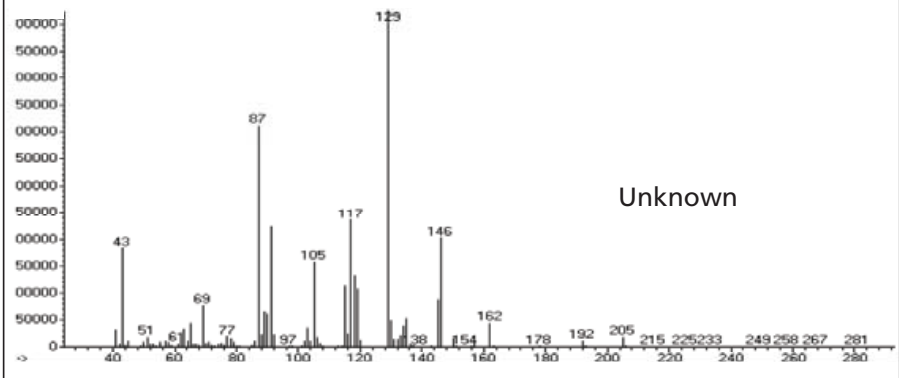
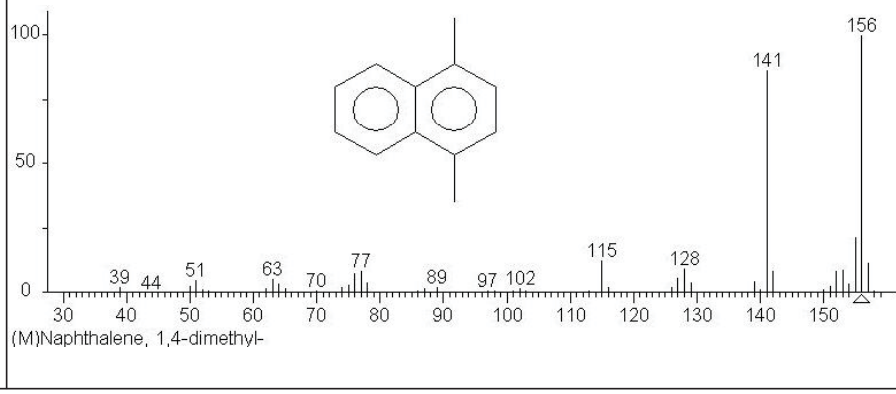
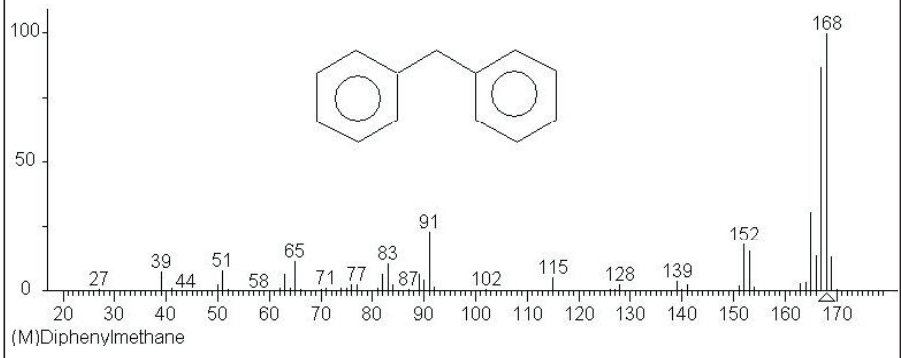
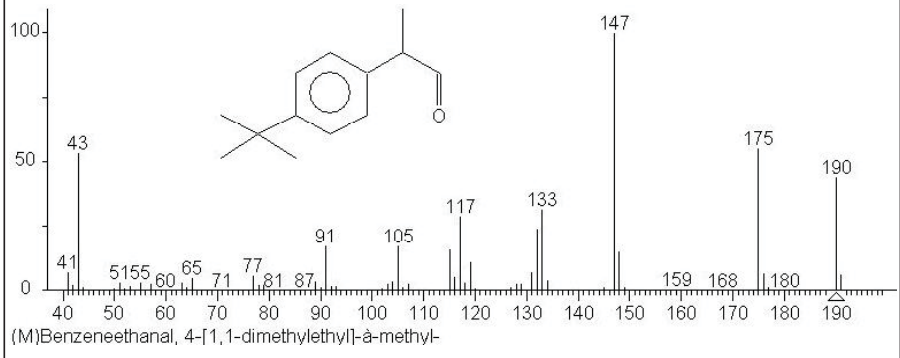
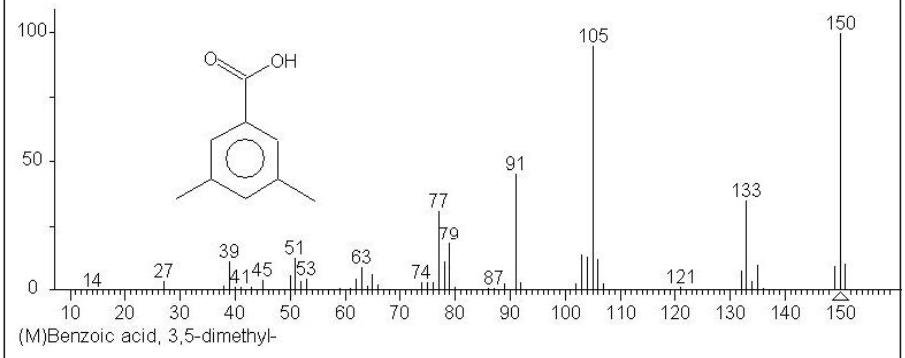
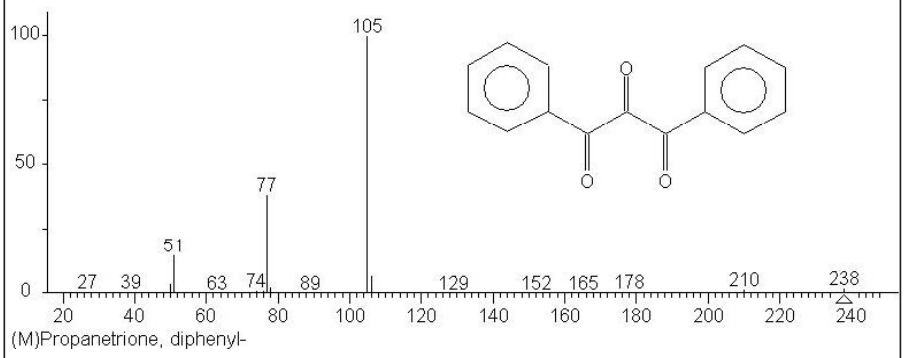
Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
6.80		9
6.87		
7.00	 Unknown	10
7.11		

Figure IX. ***(continued)***

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
7.20		
7.31		11
7.78		
7.89		

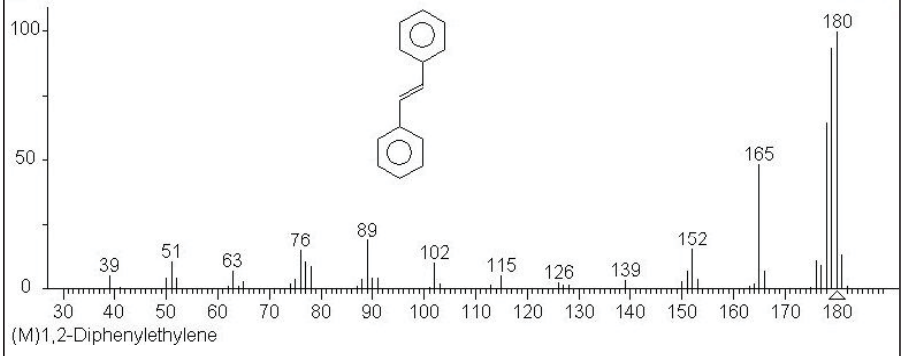
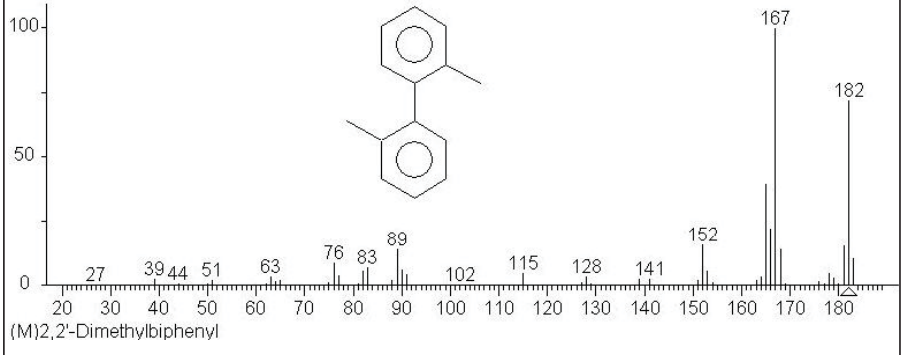
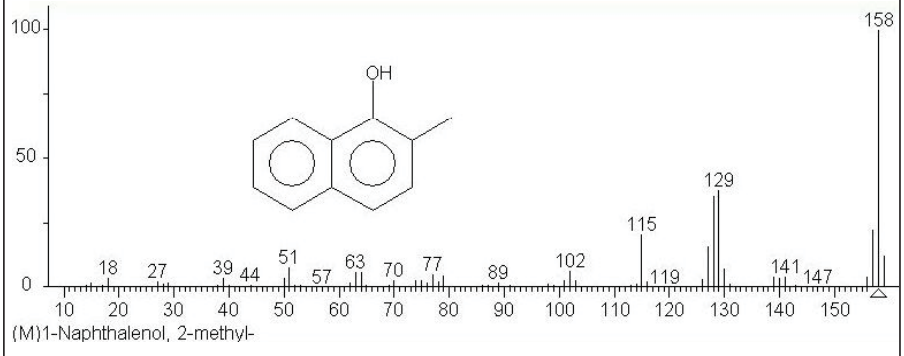
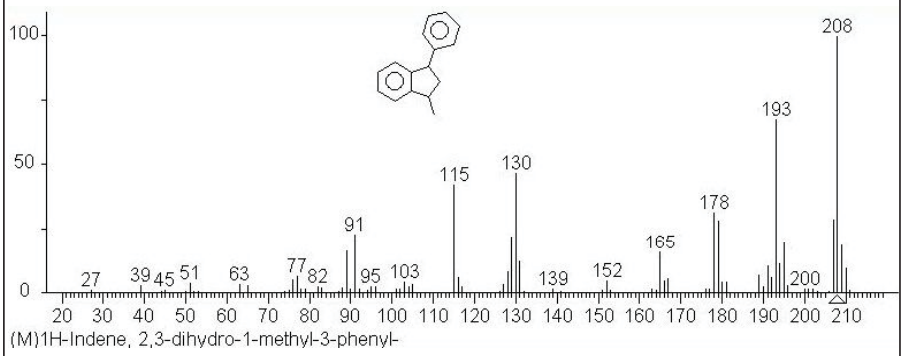
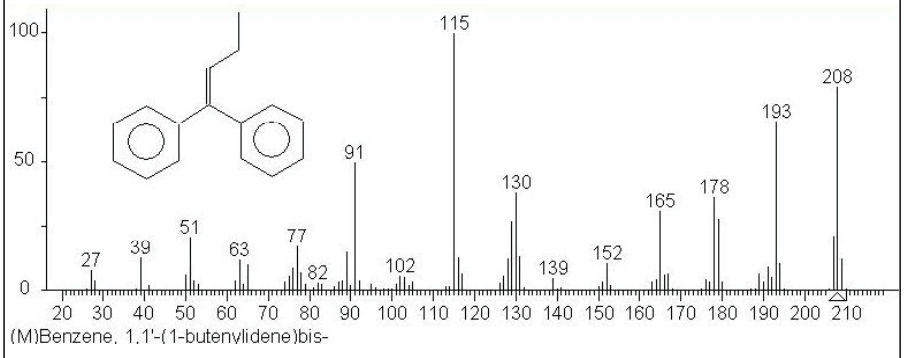
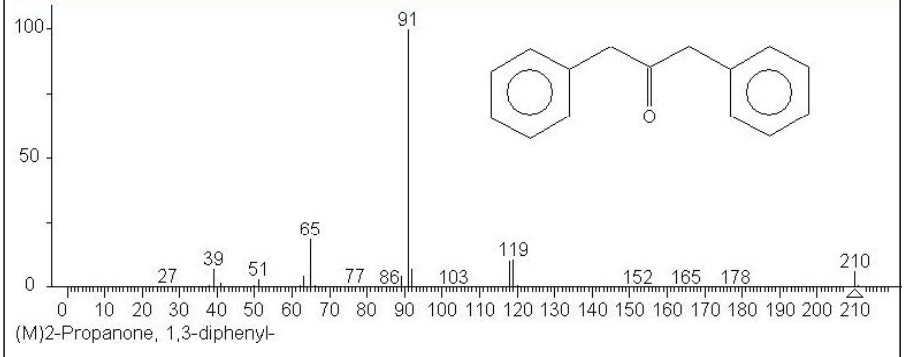
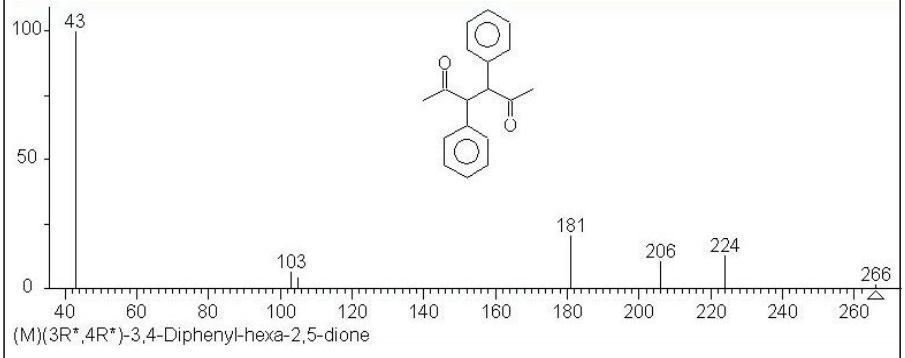
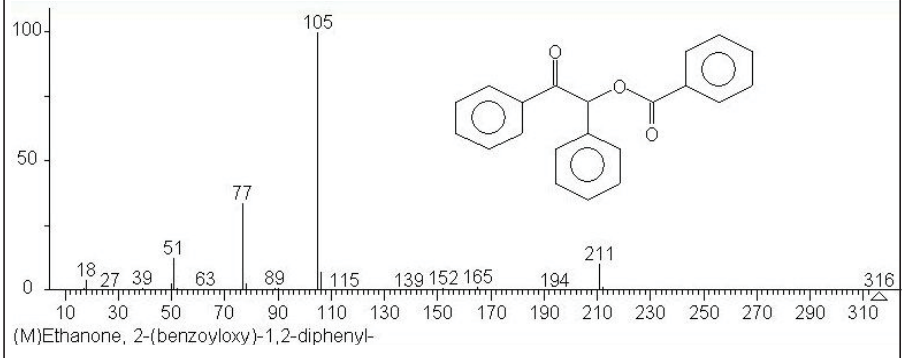
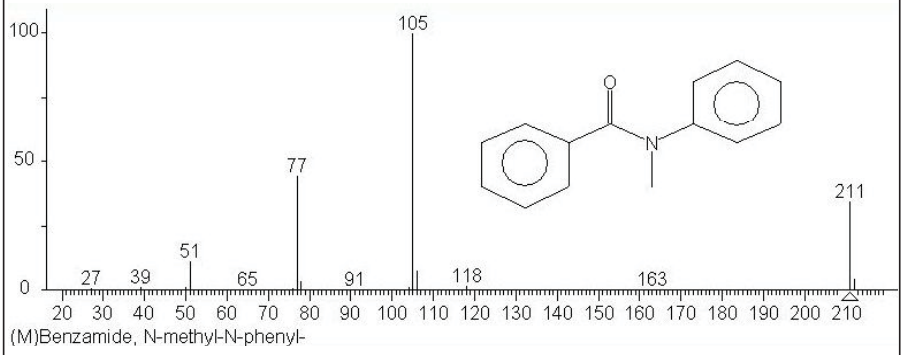
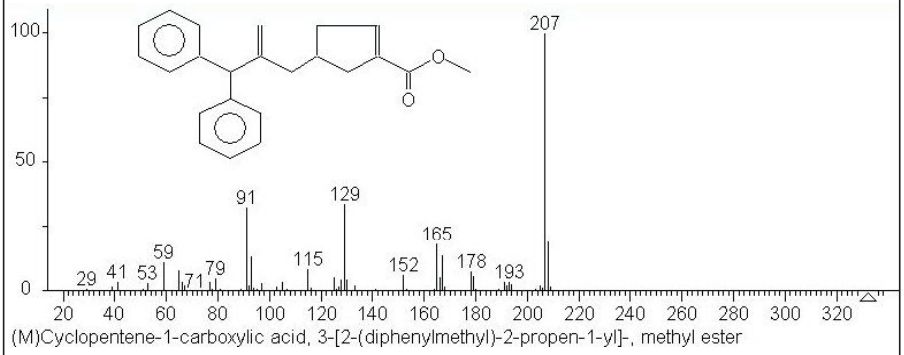
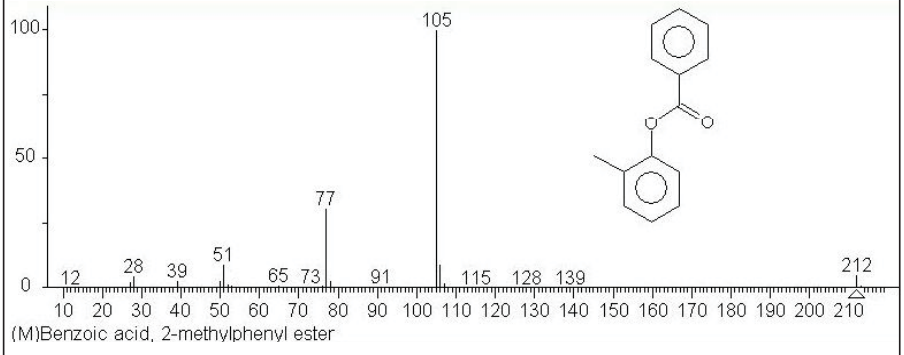
Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
7.95	 (M)1,2-Diphenylethylene	
8.07	 (M)2,2'-Dimethylbiphenyl	
9.14	 (M)1-Naphthalenol, 2-methyl-	
9.42	 (M)1H-Indene, 2,3-dihydro-1-methyl-3-phenyl-	

Figure IX. ***(continued)***

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
9.69		
9.87		12
10.46		13
11.39		14

Retention time (min)	*Name, structural formula, mass spectrum*	*Number of marker*
11.41		
11.52		
12.23		

Peak areas of the 14 compounds chosen as markers were included in statistical calculations. The selection of the 14 marker impurities was as follows:

(a) Twelve compounds were selected as general markers (numbers 1-5, 7 and 9-14) on the basis of frequency of occurrence in various P-2-P samples analysed. They are independent of the P-2-P synthesis route;

(b) Two markers were selected as route-specific markers, indicative of a given P-2-P synthesis route (number 6 for P-2-P synthesized from phenylacetic acid and number 8 for P-2-P synthesized from benzyl cyanide).

The route-specific marker characteristic for P-2-P synthesized from phenylacetic acid is the remainder of unreacted starting material, phenylacetic acid (marker number 6). By contrast, the impurity found in profiles of P-2-P synthesized from benzyl cyanide is α-phenylacetoacetonitrile (marker number 8), the intermediate product generated in the first stage of synthesis.

In some cases, batches of P-2-P are diluted with acetophenone (marker number 2) to increase the volume (bulk) of the P-2-P precursor. Contamination with that substance thus becomes a characteristic feature, which is why it was included in the profile. From a forensic point of view, it is important to recognize that amphetamine made from P-2-P diluted with acetophenone is characterized by specific impurities. It is therefore possible, on the basis of the impurity profile of the end-product amphetamine, to determine that a mixture of P-2-P and acetophenone was used as the starting material. It is also possible to establish links between amphetamine and the P-2-P samples diluted by acetophenone.

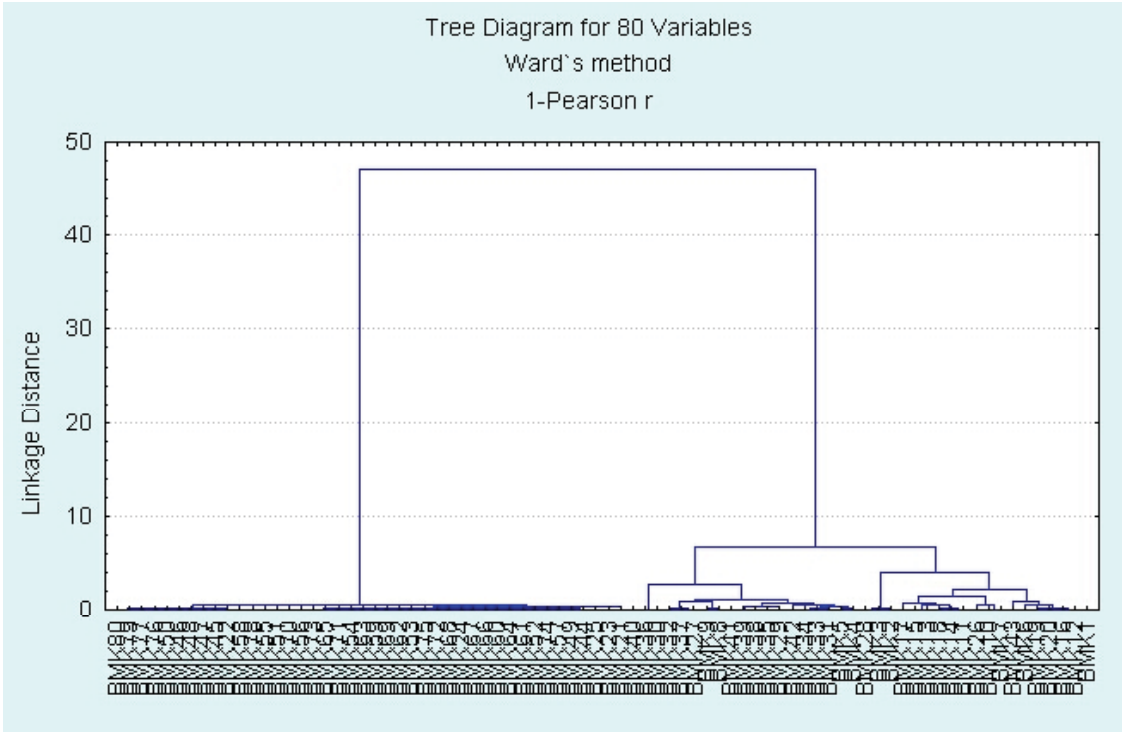
As a result of the impurity analysis, it was possible to classify the 80 P-2-P samples into four groups, characterized by key marker impurities: *(a)* phenylacetic acid group; *(b)* *a*-phenylacetoacetonitrile group; *(c)* acetophenone group; and *(d)* unknown method group, when none of the aforementioned substances was detected. Hence, the selected markers of the P-2-P profiles accurately reflect similarities and discrepancies between individual P-2-P samples.

Statistical analysis of results

A statistical analysis was carried out on the impurity profiles of 80 P-2-P samples from various seizures. A combination of different methods of cluster analyses (complete linkage, single linkage and Ward's methods) and various distance measurements (Euclidean distance, square of Euclidean distance, city-block (Manhattan) distance, Chebychev's distance, 1-Pearson r, power distance, percentage of disagreement) between individual P-2-P samples was applied. Calculations were performed with the peak areas of the 14 selected markers, standardized according to the formula $SV = (V - \mu V)/S_v$, where SV is the standard value, V is the original value, μV is the mean of original values and S_v is the standard deviation of original values.

The application of most of the methods resulted in a division of the 80 samples into three clusters. The separation of the clusters was most pronounced for the combination of 1-Pearson r distance with Ward's method (a method of cluster analysis based on minimalization of the variance in the clusters) (see figure X). The 1-Pearson r distance is defined according to the formula $d_{ik} = [1- r_{ik}^2]^{1/2}$, where d_{ik} is the distance between samples i and k and r_{ik} is the Pearson correlation coefficient between samples i and k. For similar samples the correlation coefficient is close to one and the distance between samples is close to zero.

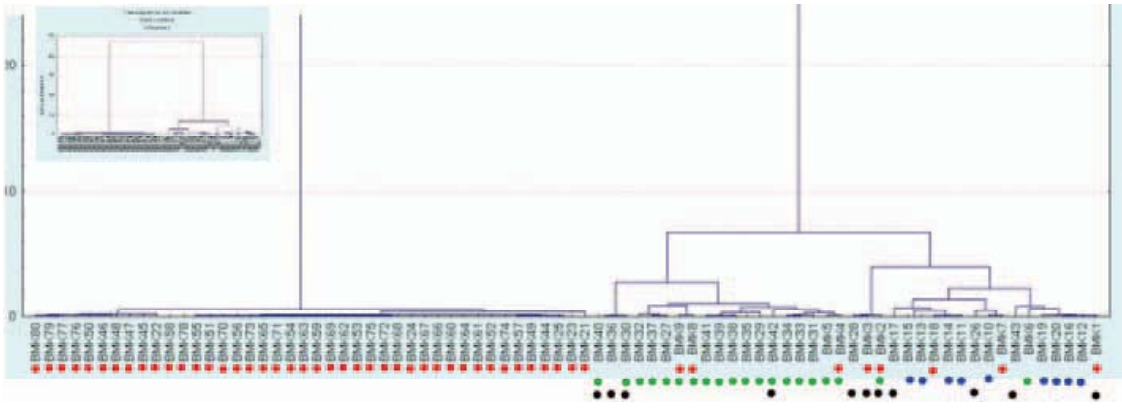
Figure X. Result of cluster analysis of 80 samples of 1-phenyl-2-propanone



Interpretation of statistical data

The P-2-P samples analysed were produced by three synthesis methods: using phenylacetic acid, using benzyl cyanide and by an unknown method. Those three synthesis routes are reflected in three clusters resulting from the cluster analysis (indicated by coloured dots in figure XI).

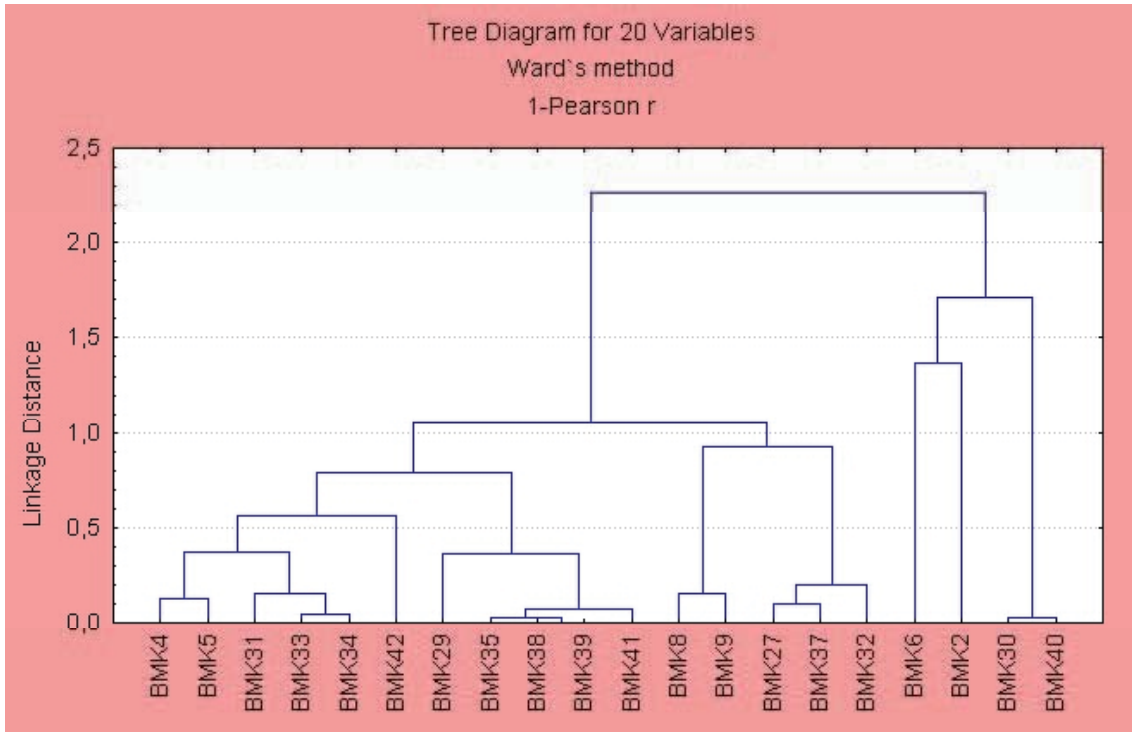
Figure XI. Results of cluster analysis



- P-2-P from phenylacetic acid (red dot)
- P-2-P from benzyl cyanide (green dot)
- P-2-P obtained through unknown method (blue dot)
- P-2-P containing acetophenone (black dot)

Each of the four groups can be further divided into profile classes, or subgroups. Thus, cluster analysis can be used for the differentiation of impurity profiles according to the synthesis method, but also according to batches or sources. For example, the benzyl cyanide group is further split into several clusters (see figure XII).

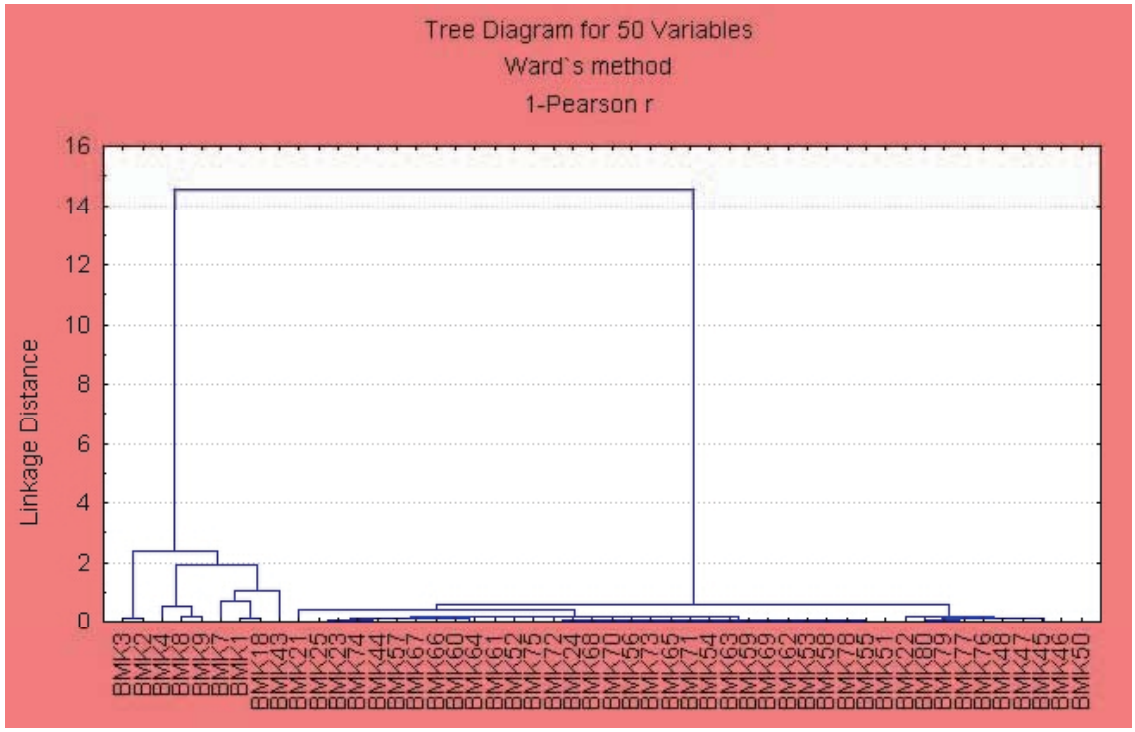
Figure XII. Tree diagram for the benzyl cyanide group



For the phenylacetic acid group, there are several subgroups, indicating differences among samples (see figure XIII). However, the significance of those differences is not yet clear. The objective of further examinations will be to determine the threshold values of the linkage distances classifying P-2-P samples as belonging to the same batch (i.e. same synthesis run) or as coming from the same laboratory but different synthesis runs.

The results of the P-2-P analyses are stored in both a forensic and a police database. The forensic database is maintained by the Central Forensic Laboratory of the Police in Warsaw and comprises P-2-P impurity profiles, results of analytical tests (purity, additives, main impurities), police and laboratory case identification numbers, place and date of seizure, case circumstances, volume of seizure and information on similarities to previously examined P-2-P samples. The police database comprises detailed data on confiscations, such as names, telephone numbers and addresses of persons involved, modus operandi and forensic and law enforcement links to other seizures. In addition, the database

Figure XIII. Tree diagram for the phenylacetic acid group



contains information from the forensic database (on purity, additives, forensic links to other samples) with relevant police case identification numbers that can be used for cross-referencing the databases. The police database is maintained by the Central Bureau of Investigation of the General Police Headquarters.

The databases facilitate the monitoring of the P-2-P market in Poland, the identification of sources of P-2-P (manufactured licitly or illicitly) and the linking of police cases based on the origin of the precursor.

Conclusions

Amphetamine is one of the most popular synthetic drugs in Europe. Its illicit manufacture usually requires the use of the precursor P-2-P. The source of precursor may be licit or illicit manufacture. In Poland, the system of P-2-P profiling elaborated by the Central Forensic Laboratory of the Police enables the determination of the route of synthesis and the identification of samples from the same source. Results of analyses and information on circumstances of seizures are entered into the forensic and police databases, which are used in intelligence and investigative police work.

All aspects discussed above accentuate the crucial importance of the interaction of police investigative and intelligence services with the forensic sector in the process of effective detection and suppression of drug-related crime.

References

1. United Nations, *Treaty Series*, vol. 1582, No. 27627.

2. *Report of the International Narcotics Control Board for 2002 on the Implementation of Article 12 of the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988* (United Nations publication, Sales No. E.03.XI.4).

3. *Global Illicit Drug Trends 2003* (United Nations publication, Sales No. E.03.XI.5).

Investigation of the origin of ephedrine and methamphetamine by stable isotope ratio mass spectrometry: a Japanese experience*

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ABSTRACT

Illicit drug abuse is a serious global problem that can only be solved through international cooperation. In Asian countries, the abuse of methamphetamine is one of the most pressing problems. To assist in the control of methamphetamine, the authors investigated in detail the character of ephedrine, which is a key precursor for the illicit manufacture of methamphetamine.

Commercial ephedrine is produced by one of three methods: (a) *extraction from* Ephedra *plants,* (b) *full chemical synthesis or* (c) *via a semi-synthetic process involving the fermentation of sugar, followed by amination. Although chemically there is no difference between ephedrine samples from different origins (natural, synthetic or semi-synthetic), scientific and analytical tools such as drug-characterization and impurity-profiling programmes may provide valuable information for law enforcement and regulatory activities as part of precursor control strategies.*

During the research under discussion in the present article, in addition to classical impurity profiling of manufacturing by-products, the use of stable isotope ratio mass spectrometry was investigated for determining the origin of the ephedrine that had been used as a precursor in seized methamphetamine samples. The results of carbon and nitrogen stable isotope ratio ($\delta^{13}C$ and $\delta^{15}N$) analysis of samples of crystalline methamphetamine seized in Japan suggested that the drug had been synthesized from either natural or semi-synthetic ephedrine and not from synthetic ephedrine.

Stable isotope ratio analysis is expected to be a useful tool for tracing the origins of seized methamphetamine. It has attracted much interest from precursor control authorities in Japan and the East Asian region and may prove useful in the international control of precursors.

Keywords: drug profiling; IRMS; methamphetamine; ephedrine; precursor; origin.

*The authors wish to express their gratitude to Naoki Kurashima of the Central Customs Laboratory, Ministry of Finance, Japan, and Rie Satou of the Shoko company of Japan for the measurement of the stable isotope ratios of samples. The research was supported by a health sciences research grant from the Ministry of Health, Labour and Welfare of Japan.

Introduction

Illicit drug abuse is a serious global problem that can only be solved through international cooperation. The abuse of methamphetamine is one of the most pressing drug problems in Asian countries. Methamphetamine is illicitly produced across the region, as demonstrated by the dismantling of clandestine laboratories in China, Indonesia, Malaysia, Myanmar, the Philippines, Taiwan Province of China and Viet Nam. At many such laboratories, precursors and chemicals for methamphetamine production have been seized together with crystalline methamphetamine.

The prevention of production is one of the most effective drug control measures. In the case of synthetic drugs, precursor control is an important component of the strategy for preventing production. Many government agencies have cooperated in international activities to prevent the diversion of precursors and chemical substances, such as Operation Topaz to counter the illicit production of heroin, Operation Purple against cocaine and Project Prism against amphetamine-type stimulants (ATS). International efforts to monitor the distribution of precursor chemicals and to promote the rapid exchange of information about suspicious imports and exports have met with some success. For example, chemical information from drug impurity profiling programmes is increasingly recognized as a valuable supplement to precursor control strategies. Information about precursors and synthetic routes of illicit manufacture may help drug law enforcement authorities trace the source of precursors or obtain other information of strategic relevance.

In view of the extent of the problem of methamphetamine abuse in Japan and the East and South-East Asian regions, Japan has taken a significant interest in this field. At two expert meetings held in Japan, recognition was accorded to the role of drug experts and scientists in facilitating the sharing of chemical information on illicit drugs and the development of analytical methods under the coordination of the United Nations Office on Drugs and Crime (UNODC). The first of the two meetings was a consultative one on profiling and the characterization of methamphetamine and other ATS, organized by UNODC and held in Tokyo in 1998, and the second was a Group of Eight (G8) ad hoc meeting of drug experts, held in Miyazaki in 2000. In addition, as part of the overall drug control approach with a focus on ATS in the Japanese five-year plan, Japan also hosted two international forums on the control of precursors for ATS; these were held in 2004 and 2005.

In terms of chemical analytical research, Japan has been involved for some years in developing methods for the impurity profiling of methamphetamine [1-3]. More recently, the research group responsible for the research under discussion in the present article has also investigated carbon and nitrogen stable isotope ratio analysis as a promising new tool for the characterization of methamphetamine [4].

This technique already has an established place in the fields of biochemistry and food chemistry, where it is used to identify the geographical

origin of natural products, such as tea, wine and honey. It is based on the fact that most elements exist in several different isotopic forms (that is, forms of the same element that differ slightly in their atomic masses) and that the abundances of the different isotopes vary according to environmental conditions, thus allowing a differentiation according to origin.

Since ephedrine, the main precursor for the synthesis of methamphetamine, may be of natural, semi-synthetic or synthetic origin, the research team investigated whether the stable isotope ratio values for carbon and nitrogen enabled the discrimination of ephedrine according to its origin. It was further investigated whether the carbon and nitrogen stable isotope ratio values of ephedrine used as a precursor were correlated with the corresponding values in the end product, methamphetamine.

The present article describes the approaches to sample comparison with the overall aim of identifying the sources and synthesis routes of illicitly manufactured methamphetamine and its precursors, in particular ephedrine. Preliminary research is presented on the potential of a promising new technique, carbon and nitrogen stable isotope ratio analysis by isotope ratio mass spectrometry (IRMS) [4], together with an application example for some methamphetamine samples. The usefulness and limitations of chemical information in drug precursor control are discussed.

Comparative analyses

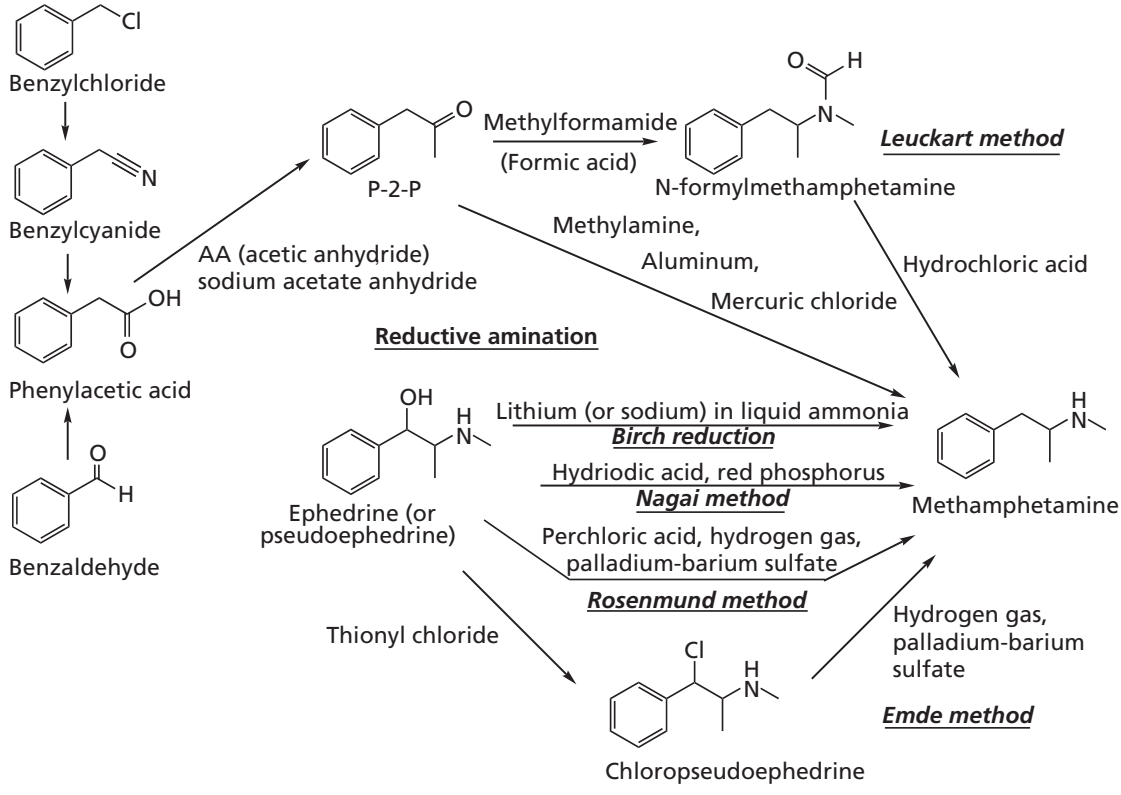
Determination of the synthetic route of methamphetamine by impurity profiling

Methamphetamine is synthesized in clandestine laboratories by a variety of routes, as shown in figure I [5]. The two main precursors used for clandestine methamphetamine synthesis are ephedrine (or pseudoephedrine), and 1-phenyl-2-propanone (P-2-P). Clandestine methamphetamine often contains impurities arising from incomplete reaction.

The traditional means of comparative sample analysis of illicitly manufactured drugs is a technique known as "drug characterization/impurity profiling". It is based on the analysis of impurities and by-products from the manufacturing process, that is, organic compounds that are present in the illicit drug end product due to clandestine manufacturing conditions.

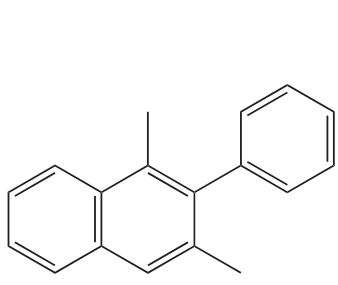
Many methods have been reported for the isolation and identification of the characteristic impurities of the various synthetic pathways of methamphetamine [1, 6-9]. In a study funded by a health sciences research grant from the Ministry of Health, Labour and Welfare of Japan, the impurity profiling of methamphetamine was investigated, focusing on the synthetic route and the precursor [3, 10-12]. As part of the research activities, methamphetamine was synthesized in the laboratory by the four main synthetic methods, that is, the Nagai, Emde and Leuckart methods and reductive amination (see figure I), and

Figure I. Interrelationship of methamphetamine and main precursor substances

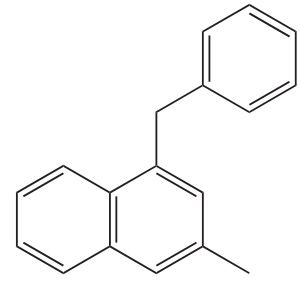


the impurities specific to each method were identified from among the many impurities generated. The following compounds were identified as key route-specific impurities:

1. Naphthalenes (1,3-dimethyl-2-phenylnaphthalene and/or 1-benzyl-3-methylnaphthalene)

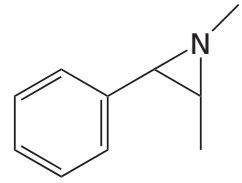


1,3-dimethyl-2-phenylnaphthalene



1-benzyl-3-methylnaphthalene

2. Aziridines (*cis*- and/or *trans*-1,2-dimethyl-3-phenylaziridine)

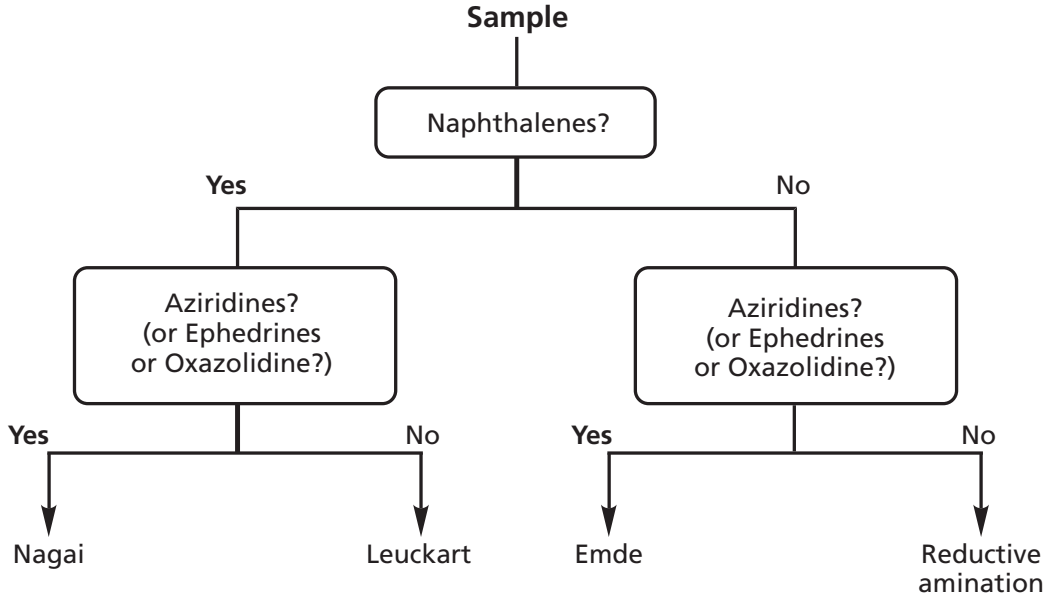


cis/*trans*-1,2-dimethyl-3-phenylaziridine

Specifically, it was found that the naphthalenes were generated only in methamphetamine synthesized via the Nagai and Leuckart methods and were not formed in the Emde method or during reductive amination. Aziridines were detected only in the cases of the Nagai and Emde methods.

The impurities could be detected by gas chromatography-mass spectrometry (GC-MS). In some cases, the aziridines were difficult to detect in methamphetamine prepared by the Nagai or Emde methods. In those cases, confirmation by GC-MS or high pressure liquid chromatography of the presence of trace amounts of ephedrine or a compound related to ephedrine, that is, erythro-3,4-dimethyl-5-phenyloxazolidine (oxazolidine), may be required [3, 10, 13]. The results are summarized in the flow chart shown in figure II, which presents a decision tree based on the presence in crystalline methamphetamine samples of a limited set of route-specific impurities.

Figure II. Flow chart for identifying the synthetic route used in the preparation of methamphetamine samples



As a result of this work it has become possible to infer the synthetic route by looking for route-specific impurities [11, 12]. Using this approach, it was possible to confirm that most methamphetamine samples seized in Japan were manufactured from ephedrine. Given the relevance of this precursor, it deserves further investigation, as detailed below.

Determination of the origin of ephedrine from the stable isotope ratios of carbon and nitrogen

The basis for stable isotope ratio analysis lies in the fact that most elements exist in several different isotopic forms (that is, forms of the same element that differ slightly in their atomic masses), and that the abundances of the different

isotopes vary according to environmental conditions. For example, the natural abundance of nitrogen-14 (^{14}N) is 99.635 per cent and that of nitrogen-15 (^{15}N) is 0.365 per cent. Isotopes have slightly different chemical and physical properties because of their mass differences. Heavy isotopes undergo the same chemical reactions as light isotopes, but react more slowly. Such slight differences in reaction rate mean that products have isotope ratios that differ from those of the source materials.

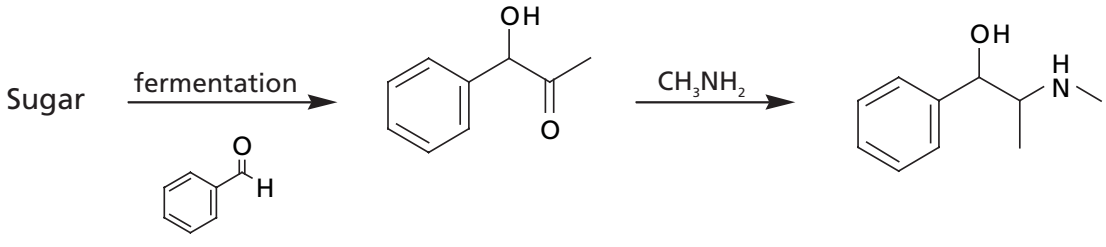
Stable isotope ratios are determined as per mille (‰) differences, that is, in units of parts per thousand, relative to international standard materials and are expressed, for example, for carbon and nitrogen as $\delta^{13}C$ and $\delta^{15}N$ respectively. In principle, precise analysis of stable isotope ratios enables investigation of the circulation of materials on a global scale and the estimation of the contribution of physical or biological factors.

Commercial ephedrine, one of the key precursors of methamphetamine, is produced by one of three processes, as shown in figure III. Natural ephedrine is prepared by extraction from *Ephedra* plants. This process is typically employed for ephedrine manufactured in China. Semi-synthetic ephedrine is prepared by fermentation of sugar followed by amination, a process known to be used in India. Fully chemically synthesized ephedrine is produced elsewhere.

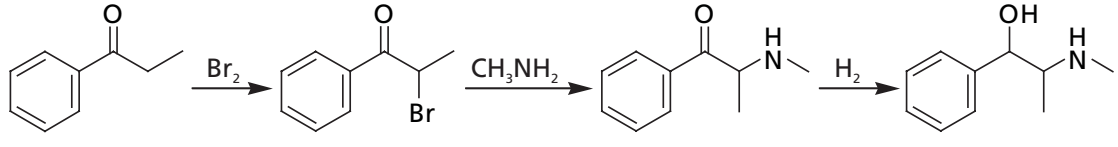
Figure III. Production schemes of ephedrine

(1) Extraction from *Ephedra* plants

(2) Fermentation of sugar followed by amination



(3) Chemical synthesis: bromination of propiophenone followed by amination



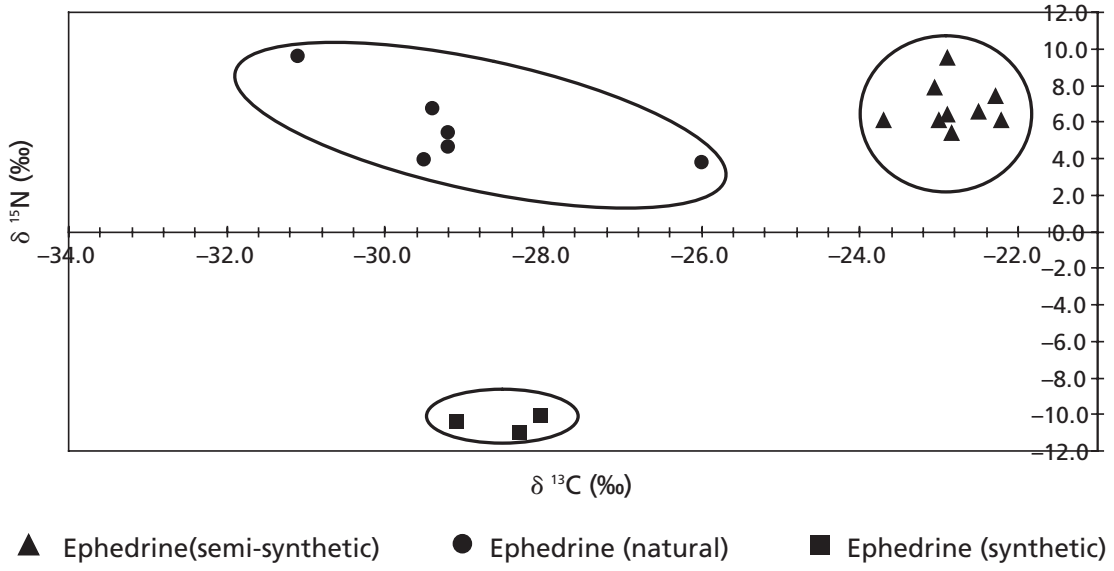
As can be seen in figure III, in the case of natural ephedrine (1), the entire molecule is isolated from plant material and is therefore of natural origin. In cases (2) and (3), different parts of the molecule may be from different sources, with the pre-precursors possibly also being from different natural, semi-synthetic or synthetic origins. In cases (2) and (3), the nitrogen source is methylamine (CH_3NH_2), which is added in step two of the manufacturing process.

In the research, it was investigated whether the stable isotope ratio values for carbon and nitrogen allowed the discrimination of ephedrine according to its origin (natural, semi-synthetic or synthetic). In a second step, it was further investigated whether the carbon and nitrogen stable isotope ratio values of ephedrine used as a precursor were correlated with the corresponding values in the end product, methamphetamine.

When the use of ephedrine as a precursor has been confirmed by classical drug impurity profiling as described above, the stable isotope ratio analysis of carbon and nitrogen may give further useful information to discriminate the origin of ephedrine.

Figure IV shows the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of ephedrine samples of different origins, determined using IRMS.

Figure IV. Ephedrine: carbon and nitrogen isotope ratios



Note: The experimental conditions were as follows [4]:

Instrument: stable isotope ratio mass spectrometer Delta^{Plus} (ThermoFinnigan, United States of America), equipped with an elemental analyser flash EA1112 (ThermoFinnigan).

Sample size: 250 μg.

Stable isotope ratios (average of five analyses) are expressed relative to the conventional standards: Peedee Belemite for carbon and atmospheric N_2 for nitrogen.

Precision: 0.1‰ or less for ^{13}C, and 0.2‰ or less for ^{15}N.

Focusing on the $\delta^{15}N$ values of the samples examined (y-axis), figure IV shows that there are indeed remarkable differences between synthetic ephedrine (■) on the one hand, and natural (●) and semi-synthetic (▲) ephedrine on the other. $\delta^{15}N$ values are smaller (more negative) in the samples of fully chemically synthesized ephedrine than in those of natural or semi-synthetic ephedrine.

With regard to $\delta^{13}C$ values (x-axis), figure IV shows that the values of the samples of natural and synthetic ephedrine examined were lower (more negative) than those of semi-synthetic ephedrine. Figure IV also shows that the $\delta^{13}C$ values of the natural ephedrine samples examined were widely dispersed, from −31.1 to −26.0 units of parts per thousand.

Sources of nitrogen

The nitrogen in an ephedrine molecule may be of natural origin in the case of ephedrine of natural origin or it may be introduced as part of a synthesis step in synthetic and semi-synthetic ephedrine (see figure III). The source of nitrogen in the cases of synthetic and semi-synthetic ephedrine is methylamine, which may itself be of natural origin or prepared by the chemical reaction of ammonia and methanol, then purified by distillation. With regard to the latter, synthetic methylamine, it was shown in earlier work [4] that the nitrogen-15 isotope ratio of methylamine changed to more negative values with successive distillations. It is assumed that this is the result of stable nitrogen isotope fractionation in the distillation step.

The low $\delta^{15}N$ values of the samples of fully chemically synthesized ephedrine in figure IV suggest the use of synthetic, purified methylamine for the manufacture of those samples. If synthetic, purified methylamine had also been used for manufacture of the semi-synthetic ephedrine samples in figure IV, then the $\delta^{15}N$ value would be expected to be similar to that of chemically synthesized ephedrine. However, figure IV shows that the $\delta^{15}N$ value of semi-synthetic ephedrine is similar to that of natural ephedrine. This suggests that methylamine extracted from biological sources (plants) may have been used for the semi-synthetic ephedrine samples examined.

Sources of carbon

In plants, there are two main routes of photosynthesis, C3 and C4. C3-photosynthesis is characterized by the formation of a three carbon-atom molecule during the first steps of carbon dioxide assimilation. It occurs in plants of temperate origin, such as sugar beets, tobacco, clover and soybeans, so-called C3-plants. All major plant families, or about 90 per cent of all plant species on Earth, are C3-plants. The C4-photosynthesis initially produces four carbon-atom molecules and occurs in plants of tropical origin, such as sugar cane, cotton and corn. It is reported that C4-plants contain more carbon-13 than C3-plants [14-15].

Sugar from sugar cane (a C4-plant) is the typical starting material for semi-synthetic ephedrine. *Ephedra*, by contrast, the raw material for natural ephedrine, is a C3-plant. It was anticipated that the characteristics of the starting material would be reflected in the $\delta^{13}C$ values of the ephedrine produced.

As shown in figure IV and noted above, the $\delta^{13}C$ values of natural ephedrine are lower (more negative) than those of semi-synthetic ephedrine and are dispersed widely from −31.1 to −26.0 units of parts per thousand. It was presumed that the wide variations of $\delta^{13}C$ values of natural ephedrine reflected differences in humidity and other conditions in the growing areas. Further work using authenticated ephedrine samples from *Ephedra* plants of known geographical origin will be needed to test this hypothesis.

d-Pseudoephedrine, another key precursor of methamphetamine, is usually manufactured from *l*-ephedrine by acid isomerization. Figure V shows the results of carbon and nitrogen stable isotope ratio analysis of samples of pseudoephedrine obtained from different manufacturers (A-F), together with the corresponding results of ephedrine samples given in figure IV.

It can be seen that the $\delta^{15}N$ values of *d*-pseudoephedrine from Manufacturer A (Δ) are lower than that of semi-synthetic ephedrine (▲), but significantly higher than those of synthetic ephedrine (■), while $\delta^{13}C$ values of *d*-pseudoephedrine are comparable with those of semi-synthetic ephedrine. Background information from Manufacturer A indeed confirms that the samples were manufactured from semi-synthetic ephedrine and that both the ephedrine and pseudoephedrine samples had the same country of origin. It is presumed that the observed lower $\delta^{15}N$ value of *d*-pseudoephedrine is a result of nitrogen isotope fractionation during the manufacturing (isomerization) process, similar to the impact of successive distillations on nitrogen-15 values in methylamine.

Figure V. Ephedrine and pseudoephedrine: carbon and nitrogen isotope ratios

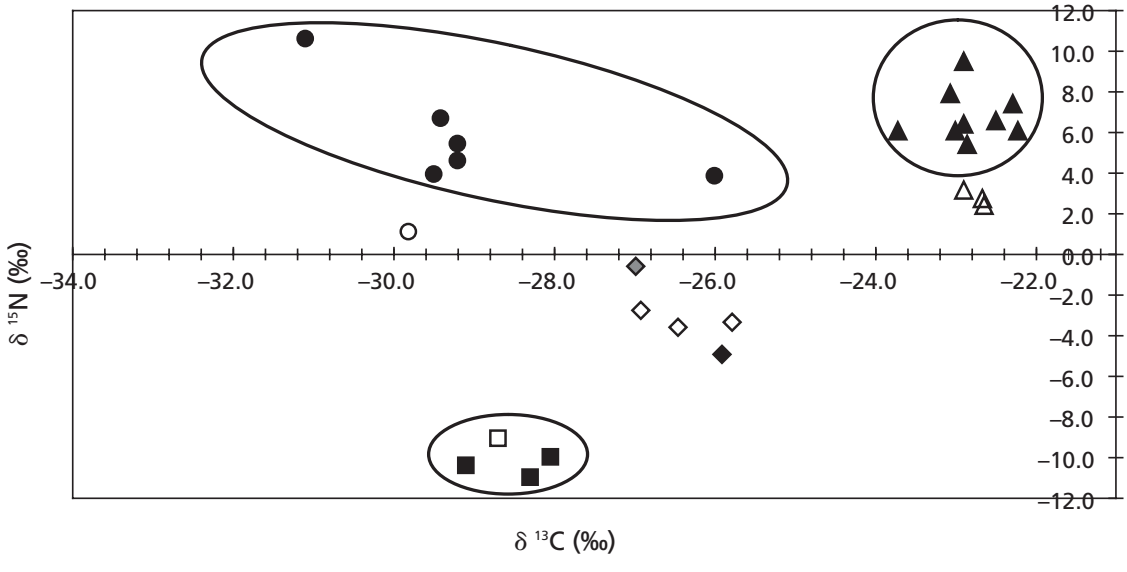


Figure V also shows $\delta^{15}N$ and $\delta^{13}C$ values of *d*-pseudoephedrine samples from five other manufacturers (B, C, D, E and F). For samples of Manufacturer C (◇), not only $\delta^{15}N$, but also $\delta^{13}C$ values were lower than the corresponding values of semi-synthetic ephedrine (▲). Background information from Manufacturer C suggests that the samples were imported from Europe. Assuming that semi-synthetic ephedrine in Europe would be manufactured from sugar beets instead of sugar cane as starting material, it is presumed that the lower $\delta^{13}C$ value is a reflection of the differences between a C3-plant (sugar beet) and a C4-plant (sugar cane). The lower $\delta^{15}N$ value is, again, presumed to be a result of nitrogen fractionation during the isomerization process.

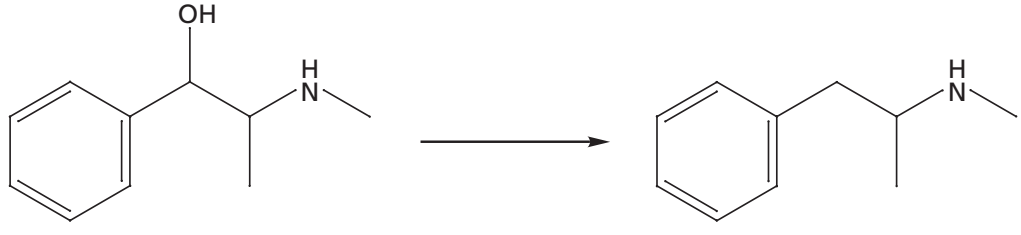
The team was informed that the sample from Manufacturer B (○) was prepared from natural ephedrine and the sample from Manufacturer F (□) was synthesized chemically, thus confirming the IRMS results. Samples from Manufacturers D and E were reagent-grade pseudoephedrine available on the chemicals market. Their close proximity to pseudoephedrine from Manufacturer C suggests that these samples, too, were manufactured from European semi-synthetic ephedrine.

Carbon and nitrogen stable IRMS thus has the potential to discriminate between ephedrine of natural, semi-synthetic and synthetic origin. In order to make full operational use of the results obtained, comprehensive knowledge of the manufacturing processes employed by legitimate manufacturers and the nature and origins of the raw materials used are required. Further, if authentic sample material from many pharmaceutical companies was available, and if it was possible to differentiate ephedrine samples from different manufacturers, this technique may enable the source (manufacturer) of ephedrine and pseudoephedrine to be identified.

Relationship between ephedrine (precursor) and methamphetamine (end product) based on the stable isotope ratios of carbon and nitrogen

The clandestine manufacture of methamphetamine from ephedrine only consists of the elimination of a hydroxyl group from the ephedrine molecule, with all other parts of the molecule remaining unchanged (figure VI). As a result, the synthesized methamphetamine has the same carbon, hydrogen and nitrogen atoms as the precursor ephedrine and it can be expected that the carbon and nitrogen stable isotope ratios of both precursor and end product are closely related.

Figure VI. Schematic presentation of the manufacture of methamphetamine (right) from ephedrine (left)



To investigate whether the carbon and nitrogen stable isotope ratios of ephedrine are carried through to the end product, methamphetamine was synthesized in the laboratory from ephedrine of three different origins by means of the Nagai method. The results, shown in table 1, show that $\delta^{13}C$ and $\delta^{15}N$ values for the precursor were indeed well correlated with those for the end product. This suggests that IRMS may be a useful analytical tool to link precursor and end product.

Table 1. Comparison of $\delta^{13}C$ and $\delta^{15}N$ values of ephedrine precursor and methamphetamine end product

Sample	*$\delta^{13}C$ (‰)*	*$\delta^{15}N$ (‰)*
Natural ephedrine	–29.2	+4.2
Methamphetamine from the above natural ephedrine	–29.5	+3.9
Semi-synthetic ephedrine	–23.1	+6.2
Methamphetamine from the above semi-synthetic ephedrine	–23.1	+5.8
Synthetic ephedrine	–29.2	–10.5
Methamphetamine from the above synthetic ephedrine	–29.2	–11.1

Application example: profiling of seized crystalline methamphetamine by means of key impurity analysis and carbon and nitrogen stable isotope ratio analysis

A total of 15 samples of crystalline methamphetamine seized in Japan, with law enforcement information as to the presumed source countries, were investigated by impurity profiling and stable isotope ratio analysis. Brief information on each sample and the synthetic route identified by impurity profiling, using the flow scheme in figure II, are listed in table 2. Figure VII shows the $\delta^{13}C$ and $\delta^{15}N$ values of the different samples as a two-dimensional plot.

Table 2. List of methamphetamine samples used for impurity profiling and stable isotope mass spectrometry analysis

	Information on methamphetamine	*Estimated synthetic pathway*
1.	Crystals seized in the Sea of Japan near Ishikawa Prefecture in 1999	Emde method
2.	Crystals smuggled into Japan from Hong Kong Special Administrative Region of China via Incheon airport, Republic of Korea, by five Koreans in 2002	Nagai method
3.	Crystals smuggled into Japan from Malaysia	Emde method
4.	Crystalline methamphetamine from Dainippon Pharmaceutical (renamed Dainippon Sumitomo Pharma on 1 October 2005) (Japan)	Emde method

Table 2 *(continued)*

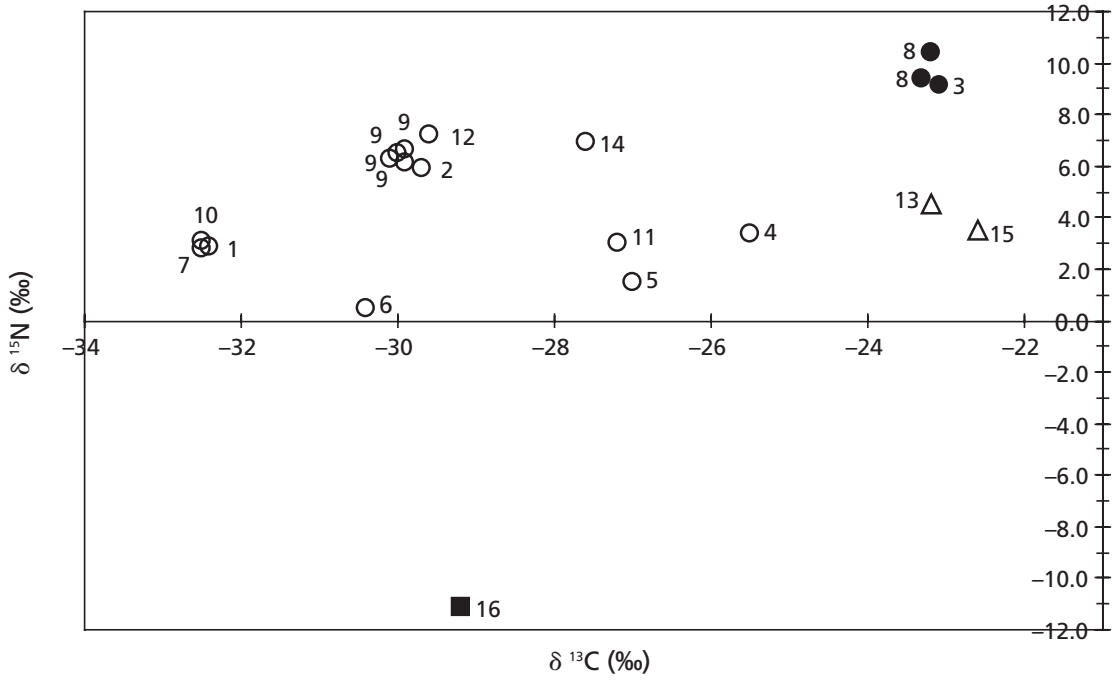
	Information on methamphetamine	*Estimated synthetic pathway*
5.	Crystals seized on a ship registered in China at the port of Sakai, Shimane Prefecture, smuggled into Japan from a port of origin in the Democratic People's Republic of Korea in 1999	Emde method
6.	Crystals seized on a ship registered in the Democratic People's Republic of Korea at the port of Hamada, Tottori Prefecture, in 1999	Emde method
7.	Crystals smuggled from the Philippines	Emde method
8.	Crystals from Canada seized at Narita airport in 2003	Nagai method
9.	Crystals from Canada seized at Narita airport in 2003	Emde method
10.	Crystals seized in the East China Sea near Kagoshima Prefecture in 1999	Emde method
11.	Crystals seized at the port of Yokohama in 2004	Nagai method
12.	Crystals seized at the port of Hosojima, Miyazaki Prefecture, in 1997	Emde method
13.	Crystals seized in Australia	Nagai method
14.	Crystals seized in the Republic of Korea	Emde method
15.	Crystals seized in the United States of America	Nagai method

Using stable carbon and nitrogen isotope ratios as indicators, the results suggest that the precursor of all seized methamphetamine samples investigated was natural or semi-synthetic ephedrine, not synthetic ephedrine. This is consistent with information from drug law enforcement authorities. More specifically, for the samples investigated, the following conclusions about the relationship of precursor and product can be drawn:

- The $\delta^{13}C$ and $\delta^{15}N$ values of samples from Canada (8) and Malaysia (3) agree very closely with those of semi-synthetic ephedrine;
- The values of samples seized in Australia (13) and in the United States (15) agree very closely with those of semi-synthetic pseudoephedrine;
- The values of a few samples seized in Japan (1 and 10) and one sample smuggled from the Philippines (7) agree closely with those of natural ephedrine;
- Considering the observed wide dispersion of $\delta^{13}C$ values of natural ephedrine shown in figure IV, the values of several other methamphetamine samples, such as samples 2 and 9 with law enforcement links to the Republic of Korea and Canada respectively, and possibly also samples 5, 11 and 14, can be assumed to have been manufactured from natural ephedrine.

The findings for sample 8 from Canada are supported by reports suggesting that large amounts of medical ephedrine or pseudoephedrine are imported into Canada from India, where the major product is semi-synthetic ephedrine [16].

Figure VII. Methamphetamine: carbon and nitrogen isotope ratios



Legend:

1 Crystals seized in the Sea of Japan near Ishikawa Prefecture in 1999.

2 Crystals smuggled into Japan from Hong Kong Special Administrative Region of China via Incheon airport, Republic of Korea, by five Koreans in 2002.

3 Crystals smuggled into Japan from Malaysia.

4 Crystalline methamphetamine from Dainippon Pharmaceutical (renamed Dainippon Sumitomo Pharma on 1 October 2005) (Japan).

5 Crystals seized on a ship registered in China at the port of Sakai, Shimane Prefecture, smuggled into Japan from a port of origin in the Democratic People's Republic of Korea in 1999.

6 Crystals seized on a ship registered in the Democratic People's Republic of Korea at the port of Hamada, Tottori Prefecture, in 1999.

7 Crystals smuggled from the Philippines.

8 Crystals from Canada seized at Narita airport in 2003.

9 Crystals from Canada seized at Narita airport in 2003.

10 Crystals seized in the East China Sea near Kagoshima Prefecture in 1999.

11 Crystals seized at the port of Yokohama in 2004.

12 Crystals seized at the port of Hosojima, Miyazaki Prefecture, in 1997.

13 Crystals seized in Australia.

14 Crystals seized in the Republic of Korea.

15 Crystals seized in the United States of America.

16 Methamphetamine synthesized from synthetic ephedrine (shown for reference purposes).

Where numbers occur more than once, as in the case of 8 and 9, they indicate that those samples were seized on the same occasion.

Conclusion

The chemical characterization of drug samples can provide useful information for drug law enforcement, such as information regarding drug supply and distribution networks at the local, national, regional and international levels, and the methods and precursors used in clandestine drug production [17]. The similarities or differences among seized methamphetamine samples can give information on the links between suppliers and users for evidential purposes, and information about synthetic methods would be helpful in finding clandestine laboratories by monitoring trade not only in precursors, but also in key chemicals such as thionyl chloride and Pd-black, both of which are used as key chemicals when methamphetamine is produced by the Emde method.

In addition to the classical impurity profiling of methamphetamine by chromatographic methods, the use of carbon and nitrogen stable IRMS was investigated as a means of sample characterization. It was successfully shown that the origin of ephedrine and pseudoephedrine can be discriminated by IRMS and that this discrimination of the origin of the precursor is even possible from analysis of the end product, methamphetamine. Using these results, based on $\delta^{13}C$ and $\delta^{15}N$ values, it is clear that natural ephedrine was the main precursor for the crystalline methamphetamine seized in Japan.

The authors believe that stable isotope ratio analysis should prove particularly useful in cases where classical impurity profiling is of limited value, such as those of high purity samples, where the number and amount of manufacturing by-products is insufficient to draw operationally useful conclusions.

Indeed, in recent years, very pure samples of crystalline methamphetamine, suspected to have been produced by the Birch reduction method, mentioned in figure I, were seized in Canada and the United States. Some of those methamphetamine samples did not show marked differences in their impurity profiles and did not contain the two key impurities that would allow identification of the synthetic route (see figure II). The information on the values of $\delta^{13}C$ and $\delta^{15}N$ should be useful for the detailed discrimination of such methamphetamine samples.

Other potential targets for IRMS include norephedrine (phenylpropanolamine) and P-2-P. Recently, medical use of norephedrine has been discontinued because of serious side-effects. The increase in availability of *d*-pseudoephedrine, which is now widely used as a substitute for norephedrine for medical purposes, may also result in an increasing use of that substance in illicit methamphetamine synthesis.

As controls of ephedrine and pseudoephedrine are tightened and/or their availability becomes more limited, another precursor that may gain importance as a starting material for methamphetamine is P-2-P. When used for the illicit synthesis of methamphetamine, P-2-P will result in the racemic (50:50) mixture of *d*- and *l*-methamphetamine, which will have to be treated by chiral separation. Although this is a difficult procedure, the $\delta^{13}C$ and $\delta^{15}N$ ratios of methamphetamine synthesized from P-2-P should be investigated in the near future.

Another area of research relates to the identification of the geographical origin of *Ephedra* plants used for the manufacture of natural ephedrine. While the present article has shown that the origin of ephedrine and pseudoephedrine can be discriminated by carbon and nitrogen stable isotope ratio analysis, it is not yet possible to identify the growing area of *Ephedra* plants by means of $\delta^{13}C$ and $\delta^{15}N$ isotope ratio analysis. Hydrogen stable isotope analysis, which has proven useful for estimating the natal or breeding latitudes of migrating birds [18], may be an option. It may therefore become possible in the future to determine the origin of *Ephedra* plants used for the production of ephedrine and pseudoephedrine if a suitable database of the relationship between the growing area of *Ephedra* plants and the hydrogen stable isotope ratio becomes available.

The authors are seeking to obtain samples from pharmaceutical and other relevant companies worldwide for IRMS analysis and to collect information on manufacturing methods of current precursors. Comparison of the $\delta^{13}C$ and $\delta^{15}N$ values of an illicit sample with a database of values of legitimately manufactured precursors should then be useful to confirm the origin of precursors used in the manufacture of seized methamphetamine. It would also be helpful if the major exporting countries of ephedrine and pseudoephedrine provided data on licit trade and manufacturing methods.

Finally, since the value of stable isotope ratios is expressed as deviations from international standards (as δ notation expressed in units of parts per thousand), considerable care is necessary to obtain accurate results. Nevertheless, the authors hope that stable isotope ratio analysis will become a general analytical technique that can be applied throughout the world.

References

1. Ken Tanaka, Takeshi Ohmori and Takako Inoue, "Analysis of impurities in illicit methamphetamine", *Forensic Science International*, vol. 56, 1992, pp. 157-165.
2. Yoshiteru Marumo, Takako Inoue and Shigeki Seta, "Analysis of inorganic impurities in seized methamphetamine samples", *Forensic Science International*, vol. 69, 1994, pp. 89-95.
3. Yukiko Makino, Yasuteru Urano and Tetsuo Nagano, "Impurity profiling of ephedrines in methamphetamine by high-performance liquid chromatography", *Journal of Chromatography A*, vol. 947, 2002, pp. 151-154.
4. Naoki Kurashima and others, "Determination of origin of ephedrine used as precursor for illicit methamphetamine by carbon and nitrogen stable isotope ratio analysis", *Analytical Chemistry*, vol. 76, No. 14 (2004), pp. 4233-4236.
5. B. Remberg and A. H. Stead, "Drug characterization/impurity profiling, with special focus on methamphetamine: recent work of the United Nations International Drug Control Programme", *Bulletin on Narcotics*, vol. LI, Nos. 1 and 2 (1999).
6. M. Lambrechts and K. E. Rasmussen, "Leuckart-specific impurities in amphetamine and methamphetamine seized in Norway", *Bulletin on Narcotics*, vol. XXXVI, No. 1, 1984, pp. 47-57.
7. T. S. Cantrell and others, "A study of impurities found in methamphetamine synthesized from ephedrine", *Forensic Science International*, vol. 39, 1988, pp. 39-54.

8. H. F. Skinner, "Methamphetamine synthesis via hydriodic acid/red phosphorus reduction of ephedrine", *Forensic Science International*, vol. 48, 1990, pp. 123-134.
9. K. L. Windahl and others, "Investigation of the impurities found in methamphetamine synthesised from pseudoephedrine by reduction with hydriodic acid and red phosphorus", *Forensic Science International*, vol. 76, 1995, pp. 97-114.
10. Tetsuo Nagano and others, "The basic research on methamphetamine profiling", report funded by a health sciences research grant from the Ministry of Health, Labour and Welfare of Japan, 1999, pp. 60-62.
11. Tetsuo Nagano and others, "The basic research on methamphetamine profiling", report funded by a health sciences research grant from the Ministry of Health, Labour and Welfare of Japan, 2000, pp. 53-57.
12. Tetsuo Nagano and others, "The basic research on methamphetamine profiling", report funded by a health sciences research grant from the Ministry of Health, Labour and Welfare of Japan, 2001, pp. 61-64.
13. A. H. Beckett, G. R. Jones and D. A. Hollingsbee, "Degradation of (-)-ephedrine in solution and during extraction with diethyl ether", *Journal of Pharmacy and Pharmacology*, vol. 30, 1978, pp. 15-19.
14. M. H. O'Leary, "Carbon isotope fractionation in plants", *Phytochemistry*, vol. 20, 1981, pp. 553-567.
15. G. D. Farquhar, J. R. Ehleringer and K. T. Hubick, "Carbon isotope discrimination and photosynthesis", *Annual Review Plant Physiology and Plant Molecular Biology*, vol. 40, 1989, pp. 503-537.
16. International Narcotics Control Board, "Precursors and chemicals frequently used in the illicit manufacture of narcotic drugs and psychotropic substances" (United Nations publication, Sales No. E.05.XI.6) (2005), pp. 14-17.
17. United Nations Office for Drug Control and Crime Prevention (Scientific Section), *Drug Characterization/Impurity Profiling: Background and Concepts* (United Nations publication, Sales No. E.01.XI.10) (2001).
18. T. D. Meehan and others, "Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's hawks migrating through the Florida Keys", *Condor*, vol. 103, 2001, pp. 11-20.

Cultivation of *Cannabis sativa* L. in northern Morocco

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ABSTRACT

Field studies on cannabis cultivation have provided socio-economic data relating to, inter alia, production, yield and income. But only laboratory analyses of cannabis plants can provide information on their chemical composition and their levels of psychoactive constituents, thus enabling them to be classed as a drug type or a fibre type.

The present study, which covers cannabis in its fresh, dried and powdered forms, drew on fresh samples, obtained on the day they were harvested or immediately after preparation; that was done in order to prevent any alteration in the Δ-9-tetrahydrocannabinol (THC) caused by the oxidation that takes place as the product ages. The purpose of this study is to determine the THC level in 245 specimens obtained from 30 cannabis plots in three provinces of northern Morocco: Al Hoceima and Chefchaouen, where cannabis cultivation has a long tradition, and Larache, where cannabis cultivation has started only recently.

Qualitative analysis using high performance liquid chromatography with diode array detection revealed the presence of both the acid and the decarboxylated form of the main cannabinoids, cannabidiol, THC and cannabinol, and gas chromatography/mass spectrometry was used for the characterization of minor cannibinoids.

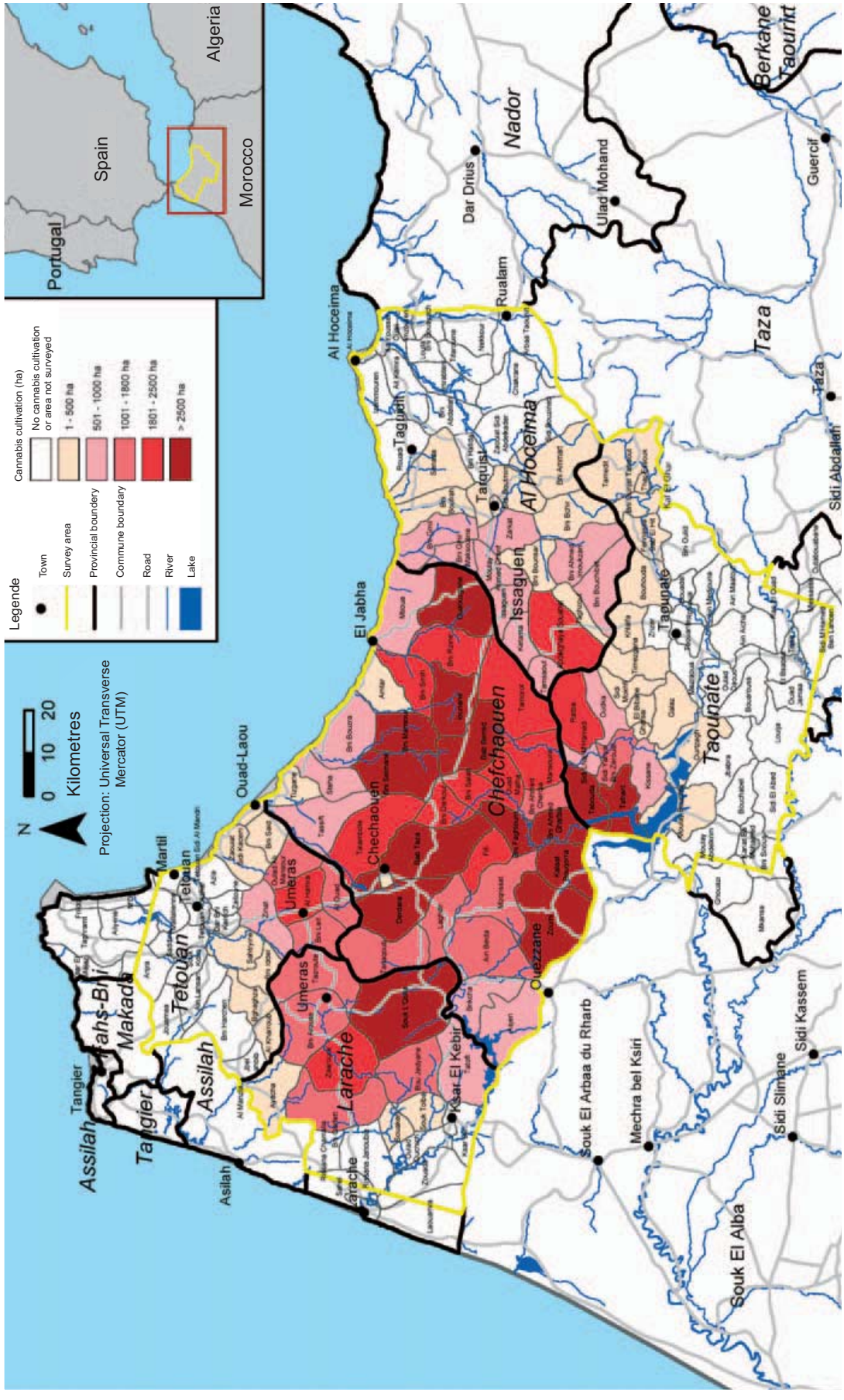
Quantitative analysis using gas chromatography coupled with mass spectrometry made it possible to determine the average Δ-9-THC content of cannabis in its fresh form (0.5 per cent), its dry form (2.21 per cent) and its powdered form (8.3 per cent). The results show that the traditional areas of cannabis cultivation—Al Hoceima and Chefchaouen—produce cannabis with a higher Δ-9-THC content than the Larache region.

In addition, the present study establishes that male plants, often considered deficient in Δ-9-THC, contain levels of the same order as those recorded for female plants, both in the leaves and in the tops.

Keywords: *Cannabis sativa* L; Δ-9-tetrahydrocannabinol; gas chromatography/mass spectrometry; high performance liquid chromatography with diode array detection

[*]The authors would like to thank the Agency for the Promotion and the Economic and Social Development of the Northern Prefectures and Provinces of Morocco for having taken the Forensic Science Laboratory of the Gendarmerie Royale into partnership for this study.

Figure I. Northern provinces of Morocco: cannabis cultivation, 2004



Source: United Nations Office on Drugs and Crime, *Morocco: Cannabis Survey 2003* (December 2003).

Note: The boundaries shown do not imply official endorsement or acceptance by the United Nations.

Introduction

The plant *Cannabis sativa* L. is grown widely throughout the world, in temperate and tropical countries. According to the *World Drug Report 2005* [1] of the United Nations Office on Drugs and Crime (UNODC), cannabis cultivation is widespread in Africa, the Americas, Asia and Europe. Identifying a total of 86 countries where the cannabis plant is grown, the *World Drug Report 2005* states that world cannabis production in 2004 was 47,000 tons, compared with 687 tons of cocaine and 565 tons of heroin. A total of 7,206 tons of cannabis products were seized in 2003, which is 15 times the total of cocaine seized and about 65 times the total of heroin seized.

Cannabis cultivation in Morocco, particularly in the central Rif, dates to the seventh century. Originally confined to a largely mountainous area, cannabis cultivation now takes place in the traditional growing areas of Chefchaouen and Al Hoceima – in the central Rif – and in recently designated extension areas north-west of Tetouan and Larache and south-east of Al Hoceima (figure I).

To evaluate the levels of THC of cannabis grown in Morocco, a study was conducted in three northern areas that together accounted for more than 80 per cent of the country's cannabis production in 2004 (figure II). The first, the Al Hoceima area of the central Rif, is characterized by small plots of land on hilly

Figure II. Distribution of cannabis production in the northern provinces of Morocco, 2004

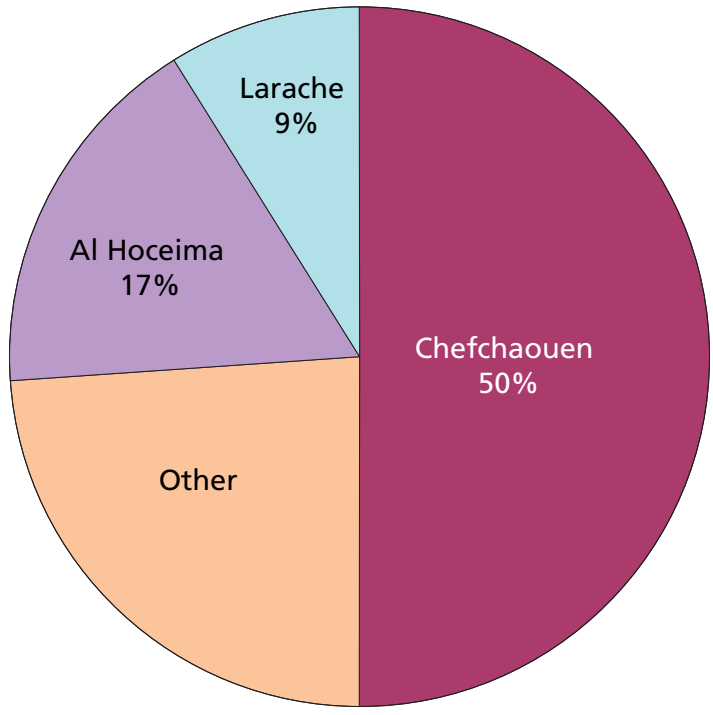


Figure III. Widespread use of traditional agricultural methods

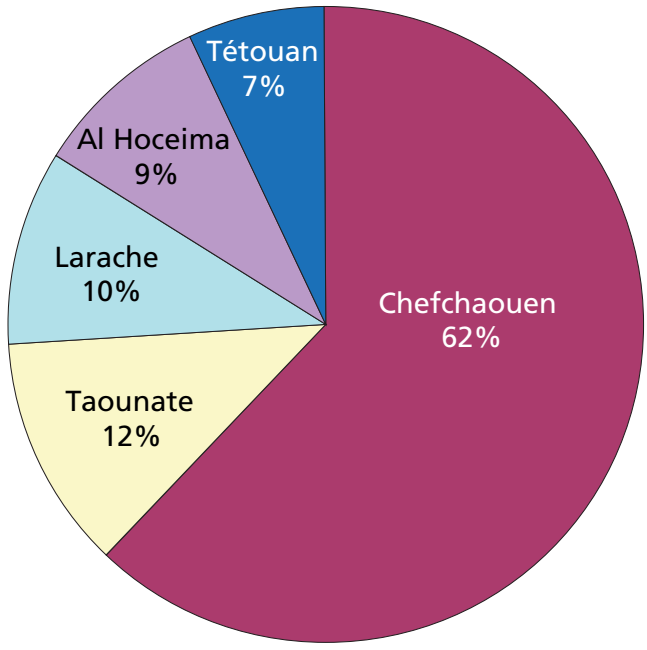
terrain where rudimentary agricultural techniques are still used (figure III). The second area is Chefchaouen, which was extended with the encouragement of the Protectorate in 1912 to pacify the rebel tribes of Ketama. The third area, situated in the Larache plain, was designated an extension area for cannabis cultivation 20 years ago, and modern production methods are used there.

The aim of this study, which was conducted in the framework of a partnership between the Agency for the Promotion and the Economic and Social Development of the Northern Prefectures and Provinces of Morocco (APDN) and the Forensic Science Laboratory of the Gendarmerie Royale, was to assess the quality of the cannabis produced in northern Morocco and determine the levels of the psychoactive constituent Δ-9-tetrahydrocannabinol (THC) for the different growing areas. The study was carried out pursuant to a cooperation agreement concluded with UNODC in February 2004, complementing a study carried out in the northern areas of Morocco in 2003 that focused on socio-economic data related to cannabis cultivation in the country.

Synthesis of social-economic data

The territories where most cannabis cultivation is located [2] total about 20,000 square kilometres, or 2.7 per cent of the total surface area of Morocco (figure I). It is estimated that in 2004, cannabis crops were grown on a total of 120,500 hectares (ha), with the largest cultivation area (figure IV) found in Chefchaouen (75,195 ha, or 62 per cent of the total cultivation area), followed

Figure IV. Distribution of total land area under cannabis cultivation in Morocco, by province, 2004

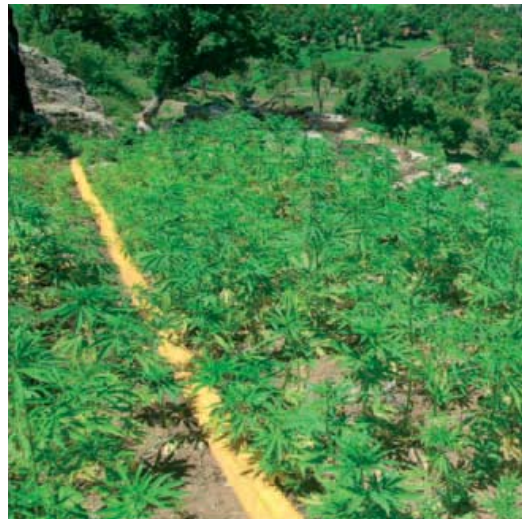


by Taounate (14,718 ha, or 12 per cent), Larache (11,892 ha, or 10 per cent), Al Hoceima (10,524 ha, or 9 per cent) and Tetouan (8,225 ha, or 7 per cent).

Most agricultural land in Morocco (88 per cent) is not irrigated but rain-fed (bour), and the yield of cannabis herb is, on average, 750 kg/ha, depending greatly on rainfall, soil quality, the number of successive years of cultivation, the use of chemical fertilizers and climatic conditions. (Figures V and VI show non-irrigated and irrigated cannabis cultivation.)

Figure V. Non-irrigated (*bour*) cultivation

Figure VI. Irrigated cultivation



The crop, once it has been dried in the sun, is called kif. Kif is either sold (66 per cent) or converted into cannabis resin at the production site (34 per cent). About 100 kg of kif is required to obtain 1-3 kg of resin by pounding and shaking, sifting it through fine nylon netting and pressing at either ambient or an elevated temperature. The final product is a slab wrapped in cellophane. Pounding the dried plant produces three qualities of powder:

Average share of cannabis converted into powder (percentage)			*Overall average share of cannabis converted into powder (percentage)*
Quality 1	Quality 2	Quality 3	
1.04	0.94	0.84	2.82

The first-quality powder, which is called *sigirma*, is golden beige in colour, is produced through the reduction of the flowering tops and the inflorescences and is reputed to have a THC content of up to 20 per cent. The second quality, which is called *hamda*, also contains plant waste, giving it a greenish colour; more or less intensive sifting of this powder yields products of varying quality, with a THC content of 2-10 per cent.

The population of the areas under cannabis cultivation in Morocco accounts for 2.7 per cent of the country's total population; the population density of 124 inhabitants per square kilometre is high compared with the national average of only 34 inhabitants per square kilometre. The number of rural families engaged in cannabis cultivation is estimated to be 96,600, which translates into a total of about 800,000 people.

The average annual family income from the sale of cannabis products is about $2,200, while the annual sale value of cannabis resin from Morocco on

the international market is estimated to be $13 billion. The income from cannabis received by farmers of Chefchaouen and Al Hoceima provinces, where cannabis has long been cultivated, accounts for 62 per cent of their total income. In the province of Larache, by contrast, where cannabis cultivation is a recent phenomenon, only 15 per cent of the income of farmers is estimated to be from cannabis.

Literature on cannabis

Botany

Cannabis is a member of the Cannabinaceae family. It is a dicotyledon, herbaceous (a non-woody plant whose aerial part dies after fruiting), annual, apetalous (the flower has no corolla) and most often dioecious (the male plants are distinct from the female plants). The height of the plant varies between 60 cm for the smallest varieties and 7 m for the largest. Under optimum conditions, the average height is about 3 m. The leaves on the lower part and the middle of the stalk are palmate, that is to say, consisting of 5-7 unequal, elliptical segments with dentate margins. The plants are a fairly dark shade of green.

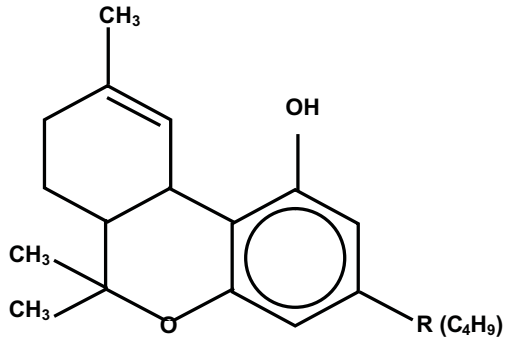
Cannabis is anemophilous, being pollinated only by the wind, but the male plants are often lifted young to prevent pollination of the female plants, in order to produce the sinsemilla variety, which is the only one used for the commercial production of cannabis herb, powder and resin.

The morphological, biological and pharmaco-chemical characteristics of cannabis depend on the growing conditions – altitude, temperature, humidity and light conditions – and the type of fertilizer used. As a general rule, crops grown in countries with a temperate climate contain only a small quantity of resin and thus have a low THC level. Indoor cultivation of cannabis plants can produce specimens with a high Δ-9-THC content.

Chemical composition

Several hundred different compounds have been isolated from cannabis [3], including terpene-based essential oils, flavonoids, sugars, fatty acids, phenolic spiro-indanes, dihydrostilbenes and nitrogenous compounds. The most interesting constituents, however, are the cannabinoids, found in the leaves and concentrated in the bracts and the resin. These are terpenophenols, classified in several groups according to their structure, the main ones being Δ-9-THC and its acid, cannabidiol (CBD) and cannabinol (CBN). These compounds are accompanied by homologues with shorter side chains (propyl and methyl cannabinoids), precursors (cannabigerol (CBG)) and chromane derivatives (cannabicyclol and cannabichromene), among others. In addition, R. Smith [4] has noted the existence of homologues with the butyl side chain C4H9 (figure VII), but at a concentration barely 1 per cent higher of that of pentyl homologues. The structures of those homologues (butyl-THC, butyl-CBD and butyl-CBN) were

Figure VII. Inferior homologues of Δ-9-tetrahydrocannabinol



determined by means of gas chromatography/mass spectrometry, using cannabis fractions concentrated by preparative thin-layer chromatography.

Active constituents

In addition to the usual constituents of a great number of plants, such as flavonoids and terpenes, more than 60 cannabinoids have been found to be present in cannabis. The main cannabinoids (figure VIII) having pharmacological effects on humans [5] include:

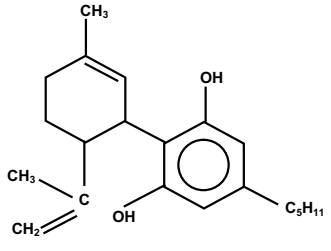
- Δ-9-THC, the product with the strongest psychoactive effect on humans;
- Δ-8-THC, which is less psychoactive than Δ-9-THC;
- CBD;
- CBN, which is not psychoactive but may have an anti-inflammatory effect;
- Δ-8-THC acid and Δ-9-THC acid (the latter is not active, but it is converted into Δ-9-THC when heated);
- CBG, which is not psychoactive but may have a bacteriological effect;
- Cannabichromene, cannabicyclol and their acids;

Cannabis varieties or chemotypes

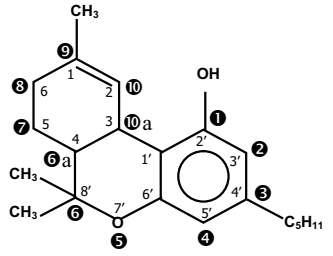
The varieties, or chemotypes, of cannabis depend on the biosynthesis of the cannabinoid constituents. The first stage in that process [6], shown in figure IX, is the condensation of geranyl pyrophosphate (I) with olivetol (II) to form CBG (III), the precursor of cannabichromene (IV), CBD (V) and Δ-9-THC (VI). Each stage is controlled by a specific enzymatic action [7-9] linked to the biogenetic factor that has an influence on the biosynthesis of the cannabinoids and on their abundance in the plant. Thus, there are different cannabis chemotypes: the drug type, the fibre type and the intermediate type. In practice, it is possible to distinguish between those chemotypes simply by determining the Δ-9-THC level [10].

Drug type, with a high Δ-9-THC content (>2 per cent). This type of composition may be observed in all cannabis plants that grow in hot climatic zones and produce a great deal of resin. There are many types of these plants, whose names differ from country to country.

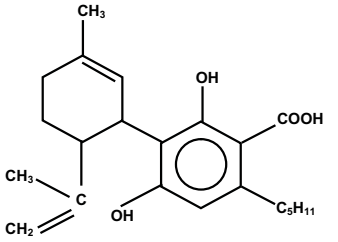
Figure VIII. Chemical structures of the principal cannabinoids characteristic of cannabis



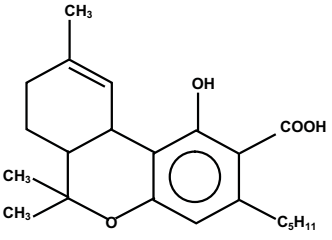
Cannabidiol
Synonym: CBD
$C_{21}H_{30}O_2$ = 314.5



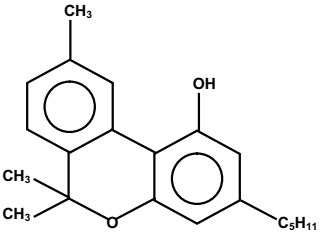
$\triangle^9$-Tetrahydrocannabinol
Synonym: $\triangle^1$-THC ; $\triangle^9$-THC
(-)-trans-$\triangle^9$-Tetrahydrocannabinol
$C_{21}H_{30}O_2$ = 314.5



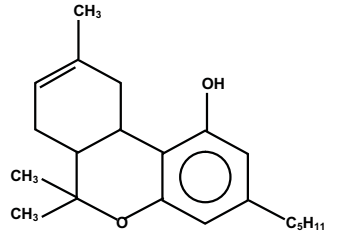
Cannabidiolic acid
Synonym: CBDA
$C_{22}H_{30}O_4$ = 358



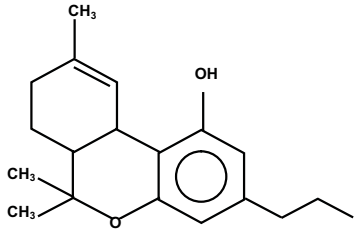
Tetrahydrocannabinol acid
Synonym: CBNA
$C_{22}H_{30}O_4$ = 358



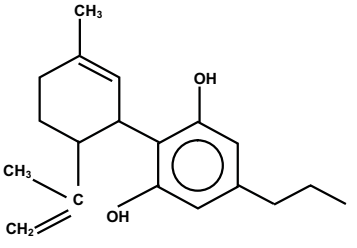
Cannabinol
Synonym: CBN
$C_{21}H_{26}O_2$ = 310.4



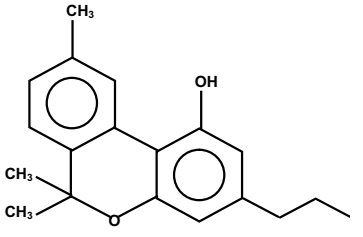
$\triangle^8$-Tetrahydrocannabinol
Synonym: $\triangle^{1\text{-}6}$–THC
(-)-trans-$\triangle^8$-Tetrahydrocannabinol
$C_{21}H_{30}O_2$ = 314.5



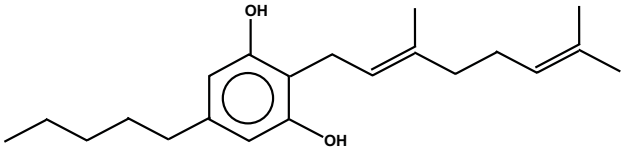
$\triangle^9$–Tetrahydrocannabivarin
Synonym: Cannabivarol
$C_{19}H_{26}O_2$ = 286



Cannabidivarin
$C_{19}H_{26}O_2$ = 286



Cannabivarin
Synonym: CBV
$C_{19}H_{22}O_2$ = 282



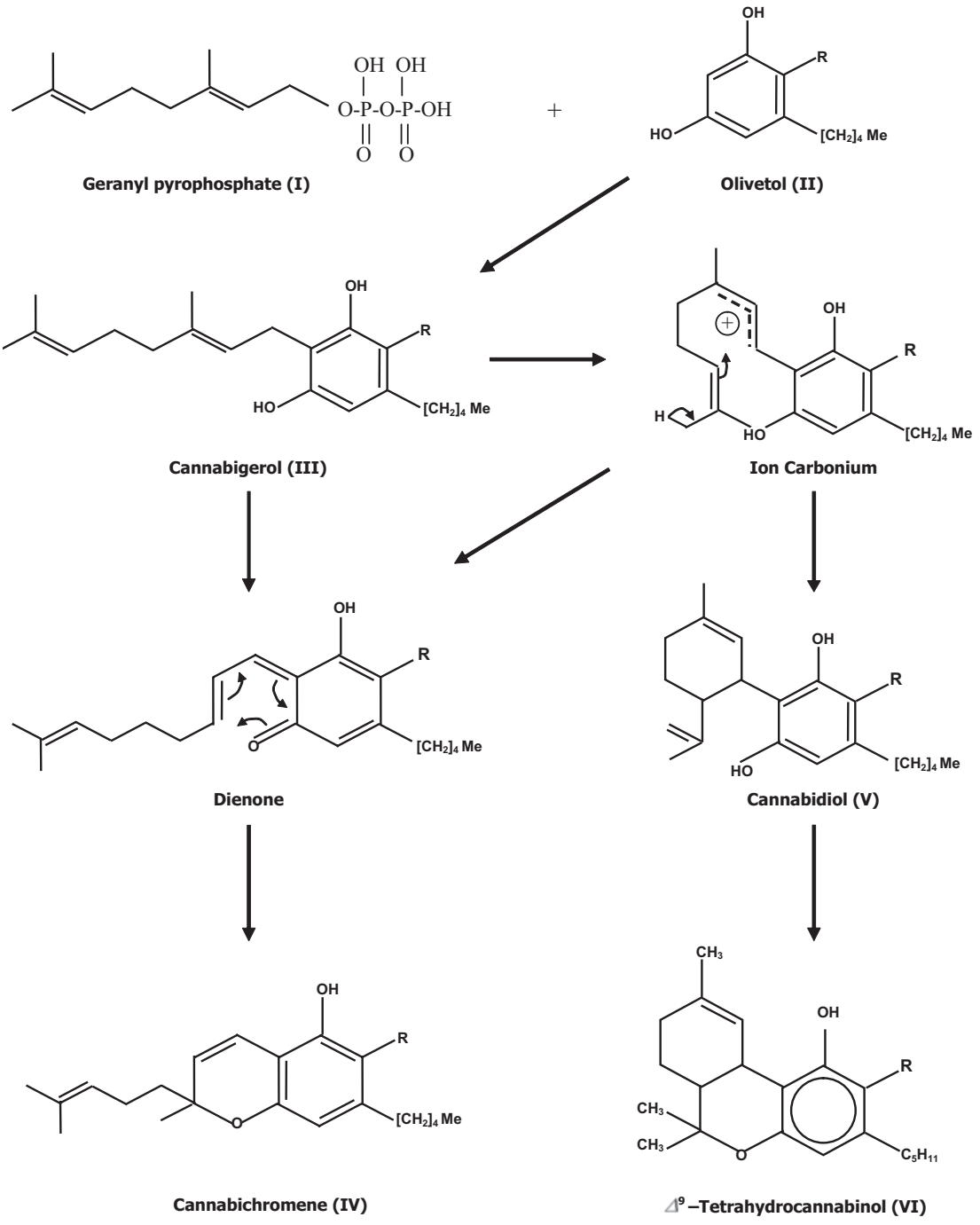
Cannabigerol
Synonym: CBG
$C_{21}H_{32}O_2$ = 316

Fibre type, with a very low Δ-9-THC content (<0.3 per cent) and a high CBD content. The plant is grown for the manufacture of special kinds of paper, non-woven textiles and animal litter. The Δ-9-THC content of most varieties grown in northern temperate zones for the manufacture of textiles does not exceed 0.03 per cent.

Intermediate type, high in Δ-9-THC (>0.5 per cent) and CBD (>0.5 per cent).

In the three chemotypes described above, the biosynthesis of the cannabinoids reaches completion. Recently, however, Fournier [11], has described a cannabis chemotype, the Santhica 23 and 27 varieties, in which biosynthesis stops

Figure IX. Biosynthesis of the principal cannabinoids



R = H, cannabinoid form
R = COOH, acid form of cannabinoid

at the CBG stage. The chemical composition of these varieties includes barely more than 0.1 per cent CBD, and they lack THC (both the acid and neutral forms of Δ-9-THC and Δ-8-THC). For that reason, they do not have any psychotropic properties. It is proposed that they be considered "second-generation fibre varieties". The chemical content of the three chemotypes [7] is summarized in table 1.

Table 1. Cannabinoid content of cannabis chemotypes [7]

	Compound content by chemotype (percentage)			
Cannabinoids	*Drug*	*Intermediate*	*Fibre*	
Δ-9-Tetrahydrocannabinol	>2	>0.5	<0.3	<0.1
Cannabidiol	—	>0.5	>0.5	<0.1
Cannabigerol	—	—	<0.1	>0.5

Different forms of cannabis

Stockley [12] describes several kinds of preparations based on the drug-type cannabis plant, whose shape, colour, consistency and other characteristics differ according to the country of origin. In particular, he describes a cannabis preparation derived from the compressed herb (marijuana) and one derived from the resin (hashish). The first takes the form of blocks of pulverized vegetable matter, including the various parts of the plant: the inflorescences, the leaves, the stalk and the seeds. When the males plants are lifted and the female plants are not pollinated, the resulting product, known as sinsemilla, has a high Δ-9-THC content. The second preparation, known as cannabis resin (or hashish), is, according to Stockley, made up of sticky, oily layers derived from the flowering tops of the plant, which are collected and compressed into blocks that can be malleable or hard, dry and powdery.

The slabs of cannabis produced in Morocco, known locally as *chira* or hashish and in Europe as cannabis resin, are produced by compressing the powder obtained by drying, pounding or sifting the dry female plant. They are stamped with a variety of marks (see figure X).

According to Mura and Piriou [13], kif (as it is called in Morocco), marijuana (in Canada and the United States of America) or takrouri (in Tunisia) is a mixture of flowering tops and leaves, dried and powdered, whereas, cannabis resin, also called hashish, is a compact brownish or yellowish powder that is obtained by pounding and sifting the dry leaves and flowering tops (see figure XI) and compressed into blocks (see figure XII).

Cannabis oil is a viscous liquid, greenish-brown to black in colour, with a characteristic smell. It is derived by extraction using 90-per-cent alcohol, followed by exposure to the sun to evaporate the alcohol. The liquid thus obtained is heated to solidify it, making it a marketable product. The oil has a Δ-9-THC content of 30-60 per cent.

Variations in the level of Δ-9-tetrahydrocannabinol in cannabis products

The differences in the level of Δ-9-THC found in various cannabis products can undoubtedly be attributed in large part to climatic and growing conditions. Factors such as hours of sunshine, temperature, humidity, altitude, maturity of the plant and the genetics of the sown seeds are particularly significant [14-20].

Figure X. Sample marks stamped on slabs of chira

The dried leaves of fibre hemp contain less than 0.5 per cent Δ-9-THC, whereas drug-type cannabis has a Δ-9-THC content of about 5 per cent, even 7-8 per cent. In the United States, a variety containing 15 per cent Δ-9-THC is produced in California, while cannabis grown indoors in the Netherlands

Figure XI. Pounding and sifting the dried cannabis plant

Figure XII. Cannabis resin packaged in various-sized slabs, stamped with a mark and wrapped in cellophane

produces cannabis resin containing up to 30 per cent Δ-9-THC [21]. However, lack of standardization of analytical laboratory procedures also results in data that may not be directly comparable. An overview of recent scientific studies on the subject is presented below.

A retrospective study of the Δ-9-THC content of cannabis confiscated in the United States between 1980 and 1997 [22], covering 35,213 samples of cannabis and its derivatives, taken from a total of 7,717 tons of confiscated products, showed that the average level of Δ-9-THC in samples of cannabis rose from 1.5 per cent in 1980 to 3.3 per cent in the period 1983-1984, staying at about the 3 per cent mark until 1992. After that, there was an upward trend, with the average level of Δ-9-THC rising from 3.1 per cent in 1992 to 4.2 per cent in 1997. The average Δ-9-THC in all cannabis products followed the same trend, rising from 3 per cent in 1991 to 4.47 per cent in 1997. In contrast, the average level of Δ-9-THC in cannabis oil did not follow any particular trend.

A study by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [23] provides statistics on the levels of Δ-9-THC in cannabis herb and resin declared by European countries. According to the study, the most recent data, collected in 2001 and 2002, indicate a Δ-9-THC concentration of 1.6-15.2 per cent in cannabis herb and 2-20.6 per cent in cannabis resin.

A study in France [24] of 5,152 results of analyses conducted between 1993 and 2000 on cannabis-based products confiscated by customs officials, the police and the gendarmerie revealed wide variations in the concentration of Δ-9-THC in both cannabis herb and cannabis resin. In particular, 18 per cent of the samples analysed had a Δ-9-THC level below 2 per cent; until 1995, 75 per cent of the samples of cannabis herb had a Δ-9-THC level below 5.5 per cent; and 47 per cent of the samples of cannabis resin had a Δ-9-THC content of 5-10 per cent. Although that general trend continued after 1996, there was an exponential increase in products with an extremely high Δ-9-THC concentration. For example, it was noted that 3 per cent of the samples of cannabis herb and 18 per cent of the samples of cannabis resin in 2000 had a Δ-9-THC concentration greater than 15 per cent.

A study carried out in Greece [25] on 36 samples of cannabis herb seized during 1996 in the northern and southern parts of the country revealed a Δ-9-THC level ranging from 0.24 to 4.41 per cent in the north and 0.08 per cent and 3.41 per cent in the south. The study also drew attention to the difficulty of differentiating between the drug and fibre chemotypes of 20 per cent of the 36 samples analysed on the basis of the following ratios:

$$\frac{\%\ \Delta\text{-9-THC} + \%\ \text{CBN}}{\%\ \text{CBD}} \quad \text{or} \quad \frac{\%\ \Delta\text{-9-THC}}{\%\ \text{CBD}} \quad \text{and} \quad \frac{\%\ \text{CBN}}{\%\ \text{CBD}}$$

A study of the Δ-9-THC level in 220 cannabis products seized on entry into the United Kingdom of Great Britain and Northern Ireland between 1979 and 1981 [26] found that samples of cannabis herb had an average Δ-9-THC concentration of 1.0-8.5 per cent. The level for the cannabis resin seized was between 3.8 per cent and 21 per cent, the average value being in the range 5.8-12.5 per cent. The Δ-9-THC concentrations in three samples of cannabis resin probably of Moroccan origin were estimated in the study to be 6.8 per cent, 7.1 per cent and 8.2 per cent. Fairly similar concentrations were found in samples of cannabis resin that came from Lebanon, Pakistan and Turkey.

Lastly, the Forensic Science Laboratory of the Gendarmerie Royale determined that 30 samples of cannabis resin seized in Morocco in 2004 had an average Δ-9-THC content of approximately 6 per cent. The Δ-9-THC concentration of those samples varied within a range of 0.4-16.0 per cent, with a confidence interval of 4.5-7.5 per cent. Thus, there was wide variation in the content of Δ-9-THC on the market.

Study of cannabis in Morocco

Presentation of the study

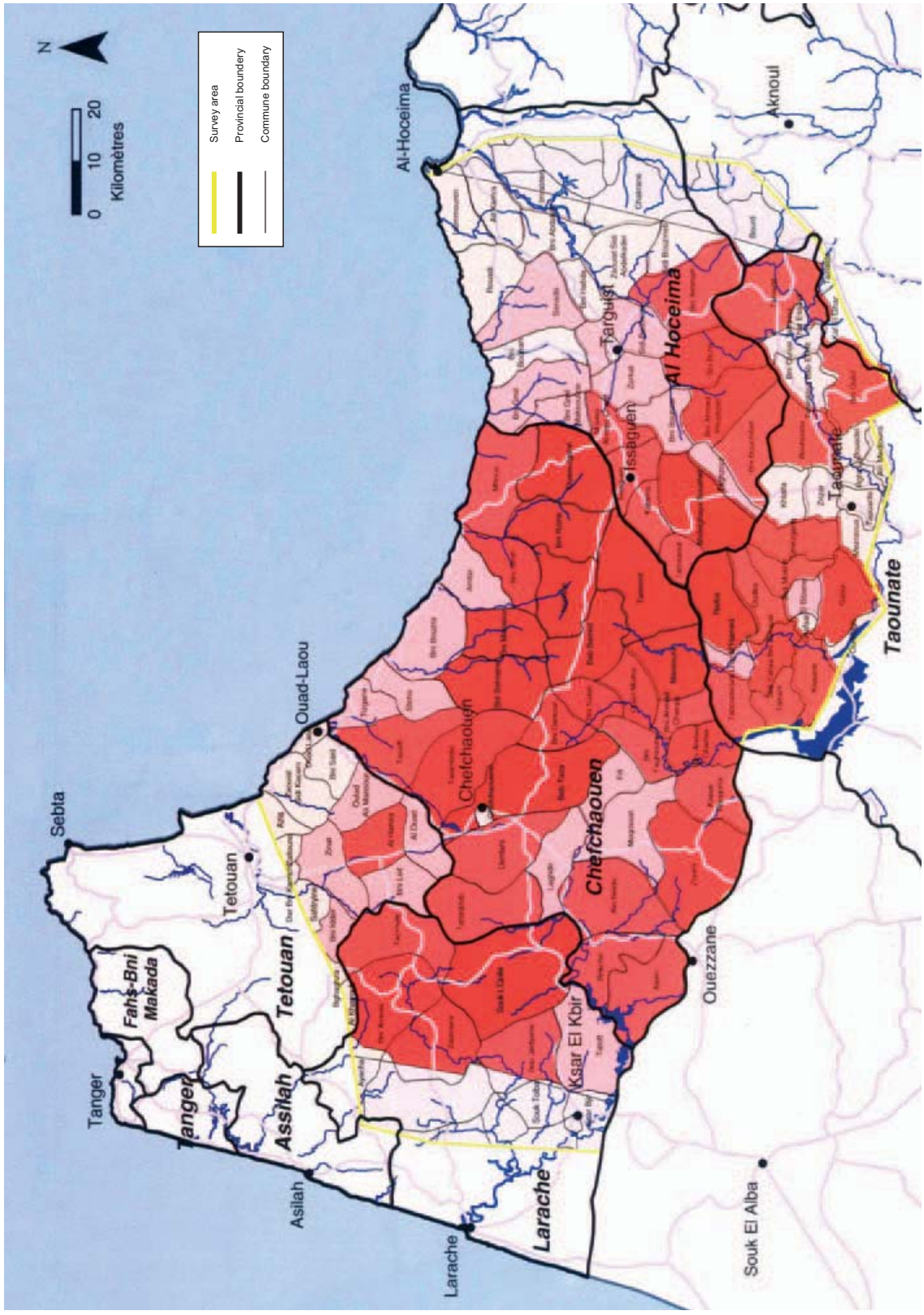
The purpose of the present study was to determine the chemical composition of various cannabis crops grown in northern Morocco and assess the levels of the psychoactive constituent Δ-9-THC found in them. The approach adopted was to subject Moroccan cannabis to qualitative analyses using high performance liquid chromatography with diode array detection (HPLC-DAD) and gas chromatography/mass spectrometry (GC/MS) and to determine, by means of GC/MS, the levels of the psychoactive constituent Δ-9-THC. The analyses were conducted on the growing (fresh) plant, the dry, mature plant and the powdered form obtained by drying, pounding and sifting, taking into account the contribution of the flowering tops and the leaves. In total, 245 samples of leaves and inflorescences were analysed: 180 samples of fresh male and female plants (inflorescences and leaves), 52 samples of dry female plants (inflorescences and leaves) and 13 samples of powdered plants. The THC concentrations in the male plants, which are usually removed early to prevent pollination, were determined and compared with those in female plants at the same stage of growth.

The study covered the areas of Chefchaouen, Al Hoceima and Larache (see figure XIII). The choice of plots took account of the traditional agricultural methods used in the Chefchaouen and Al Hoceima areas, where cannabis cultivation is a long-established practice, in contrast to the modern methods used in the Larache area, which was established two decades ago. The cannabis plants sampled came from both irrigated land, accounting for 12 per cent of the total area of cultivation, and unirrigated (*bour*) fields, which make up the remaining 88 per cent. In 2004, average raw cannabis production for those two forms of cultivation was 1,270 kg/ha and 750 kg/ha, respectively.

From an analytical point of view, it has been established that, while the qualitative analysis of cannabis poses no real difficulty, quantitative analyses aimed at determining the Δ-9-THC level in cannabis often entail the problem of the reproducibility of the results. That factor, which is liable to affect the accuracy of the values obtained, is due principally to the plant's heterogeneity: the flowering tops normally have a higher Δ-9-THC concentration than the leaves, while the stalks and the seeds do not contain Δ-9-THC. The lifting and sampling stages are therefore of great importance, and care was taken in the study not to neglect those stages but to try to assess their impact on the reliability of the concentration determinations. The study also took into account another influential factor: the drying process. Although several authors [11, 24 and 27] recommend the systematic drying of samples before analysis, at a temperature below 70° C for 6-8 hours, until a constant weight is achieved, the risk of losing Δ-9-THC due to the transformation process remains. One source [25] reports a loss of Δ-9-THC when cannabis is stored at temperatures above room temperature (37°-50° C).

Another difficulty lies in choosing the analytical technique that is most appropriate for determining the Δ-9-THC concentration: liquid or gas chromatography.

Figure XIII. Geographic distribution of plots studied



This study was conducted using GC/MS with autoinjector. That technique has the advantage of permitting the determination of the total Δ-9-THC, because the two forms, the psychoactive (Δ-9-THC) and the acid (THCA), are measured

simultaneously after decarboxylation of the acid [26] as a result of the high temperatures in the injection part of the gas chromatograph. Decarboxylation may continue even during elution of the analytical column, which is also heated to high temperatures (Tf = 280° C).

Materials and methods

Samples were taken from plots located in areas of Morocco where cannabis is traditionally grown (Chefchaouen and Al Hoceima) and the recently established cultivation area (Larache). The sowing of those plots took place over a period extending from February to May 2004, after which the Δ-9-THC levels in the crops of the three regions were monitored. Sampling was optimized by lifting a large number of plants from each plot and by taking material from the upper and lower thirds of each plant.

The study covered growing plants, mature plants dried in the sun and plants converted to powder form. A total of 245 samples of leaves and inflorescences from male and female plants were collected in the three regions:

- Samples of green, growing plants (a male and two female plants about 10 metres apart) were collected from the middle of 30 plots (13 in Chefchaouen, 8 in Al Hoceima and 9 in Larache).
- Bunches of dry plants were collected from 26 plots (10 in Chefchaouen, 8 in Al Hoceima and 8 in Larache).
- Several grams of powdered cannabis derived by drying, pounding and sifting the leaves and flowering tops were obtained from 13 plots (5 in Chefchaouen and 8 in Al Hoceima). No samples of powdered cannabis were available in the Larache area, where the conversion of dry plants into powder is believed to be still uncommon.

Table 2. Summary of cannabis samples, 2004 growing season

Province	*Green plant (sampling date: 21 July 2004)*	*Dry plant (sampling date: 10 Sept. 2004)*	*Powdered plant (sampling date: 10 Sept. 2004)*
Chefchaouen	13	10	5
Al Hoceima	8	8	8
Larache	9	8	—
Total	30	26	13

Analytical procedure

For the extraction of the samples, use was made of organic solvents and standards of analytical quality. Extractions were carried out in a 9:1 methanol/chloroform solution with a 0.05 g/l nonadecane internal standard.

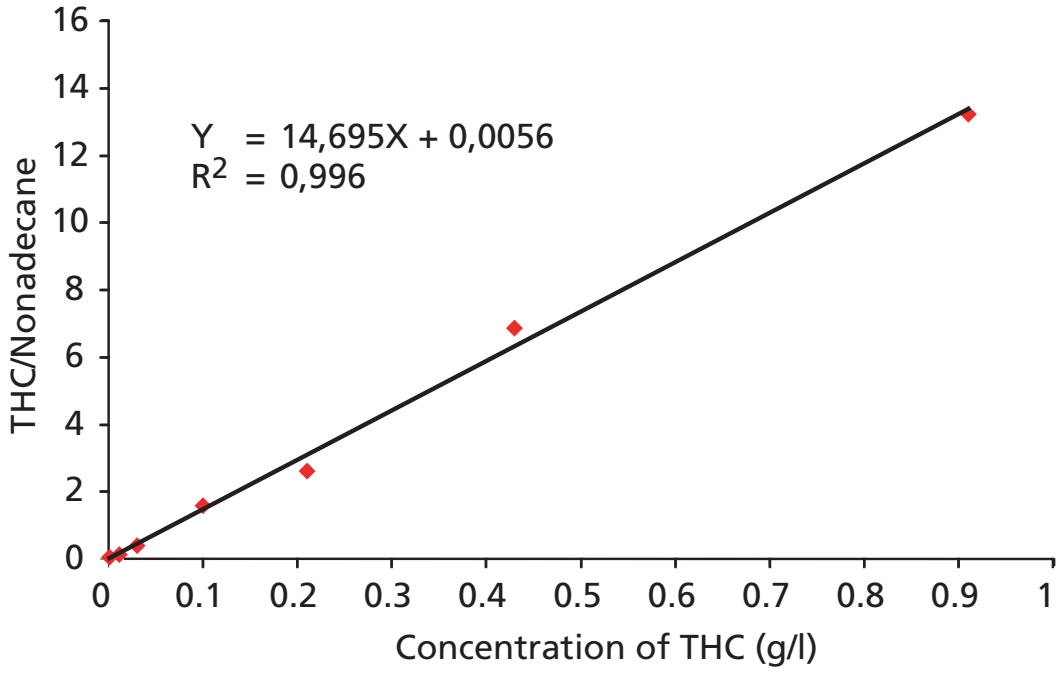
In the case of the fresh plant, two distinct types of samples were taken and analysed separately. The first type consisted entirely of flowering tops, and the

second of a one-to-one mixture of leaves taken from the lower and upper extremities of the plant. The samples, wrapped in aluminium paper, were immersed in liquid nitrogen. They were then crushed, and a test specimen of 100 mg was soaked in 3 ml of extraction solution. In the case of the dry plant, leaves from the lower and upper extremities and flowering tops were removed separately. They were ground, and a test specimen of 15 mg of each powder thus obtained was soaked in 3 ml of extraction solution. For powdered cannabis, a test specimen of 10 mg was taken directly after homogenization and soaked in 6 ml of extraction solution. The extractions were made by sonication for 30 minutes. The resulting solutions were dried over magnesium sulfate and filtered, and 1 μl of each solution was injected into the GC/MS system, or 20 μl was injected into the HPLC system.

Internal standard

The Δ-9-THC level in the cannabis plant and powder was estimated using the internal standard method. The calibration curve was obtained by injecting into the GC/MS system 1 μl of seven standard solutions of Δ-9-THC in concentrations of 0.65-0.91 g/l, again with a 0.05 g/l nonadecane internal standard. The correlation coefficient of the curve (see figure XIV) is 0.996.

Figure XIV. Calibration curve of Δ-9-tetrahydrocannabinol



Instrumentation

The analyses by means of liquid chromatography (HPLC-DAD) were carried out using a Merck L-5025 injection system, a Hypersil column ODS (100 mm × 4 mm × 3 μm), a Merck Hitachi L-3000 diode array detector and a Merck L-6200 A

pump. The mobile phase used was a 0.02 M acetonitrile/water/0.02 M sulphuric acid mixture, in the proportions 70:20:10, with a flow rate of 1 ml/min.

The GC/MS analyses were carried out using a Varian CP-3800 gas chromatograph coupled with a Saturn 2200 ion trap mass spectrometer, equipped with a CTC Analytics CombiPAL automatic sampler and a PTV 1079 injector.

Separation was carried out using a 5 per cent phenyl methyl siloxane capillary column (HP-5) (25 m × 0.2 mm × 0.11 μm), with helium as the carrier gas. A 22-minute oven temperature programme was adopted: Ti 60° C (2 min), temperature ramp 15° C/min, Tf 280° C (5 min). The injector, operating in the splitless mode, was set at an isothermal temperature of 270° C.

Mass spectrometry was carried out using 70 eV electron impact over a mass range of 35-500 amu. The trap temperature was 180° C and the transfer line temperature was 280° C.

Results and discussion

Qualitative analysis

A qualitative analysis was carried out on each of the three forms of the plant, fresh, dry and powdered, using GC/MS and HPLC-DAD. GC/MS is suitable for dealing with the plant's thermally stable compounds, while HPLC-DAD, being more sensitive, registers even the thermally labile acid forms and thereby gives a better idea of the real cannabinoid composition of the plant (acid forms and decarboxylated forms).

The GC/MS-type chromatographic profiles did not indicate any dissimilarities between the products of the regions studied in any of the three plant forms. GC/MS revealed (see figure XV) a terpenic fraction eluting before the nonadecane internal standard and a fraction of cannabinoids, the most characteristic of which were Δ-9-THC, CBD and CBN in trace amounts. Their retention times were 14.920 min, 14.380 min and 15.293 min, respectively.

A series of other cannabinoids was revealed by reconstitution of the specific ions from the total ion current. The presence of inferior homologues of the plant's active constituent (methyl-, ethyl-, propyl- and butyl-THC), along with its natural precursors (cannabigerol, cannabichromene, cannabivarin and others), was noted (figure XVII). In addition, as reported in the literature [18, 28-31], three natural THC isomers were present: trans-Δ-8-THC, cis-Δ-9-THC and trans-Δ-9-THC (figures XVI and XVII). Although the trans-Δ-8-THC form was the most thermodynamically stable, the trans-Δ-9-THC form was the most common. Those three isomers, having similar mass spectra, were identified by their respective retention times (14.17 min, 14.61 min and 14.920 min). That sequence in the order of elution has been partially described by R. Smith [4].

Lastly, GC/MS analysis makes it possible to trace the natural development of Δ-9-THC from cannabidiol from the time that the green plant is growing to the dry plant stage (see figure XVIII, which shows a variation in opposite directions of the intensity of peaks 2 and 3 on chromatograms A and B) and also

Figure XV. GC/MS-profile of the organic extract from the dried cannabis plant and mass spectra of the principal cannabinoids

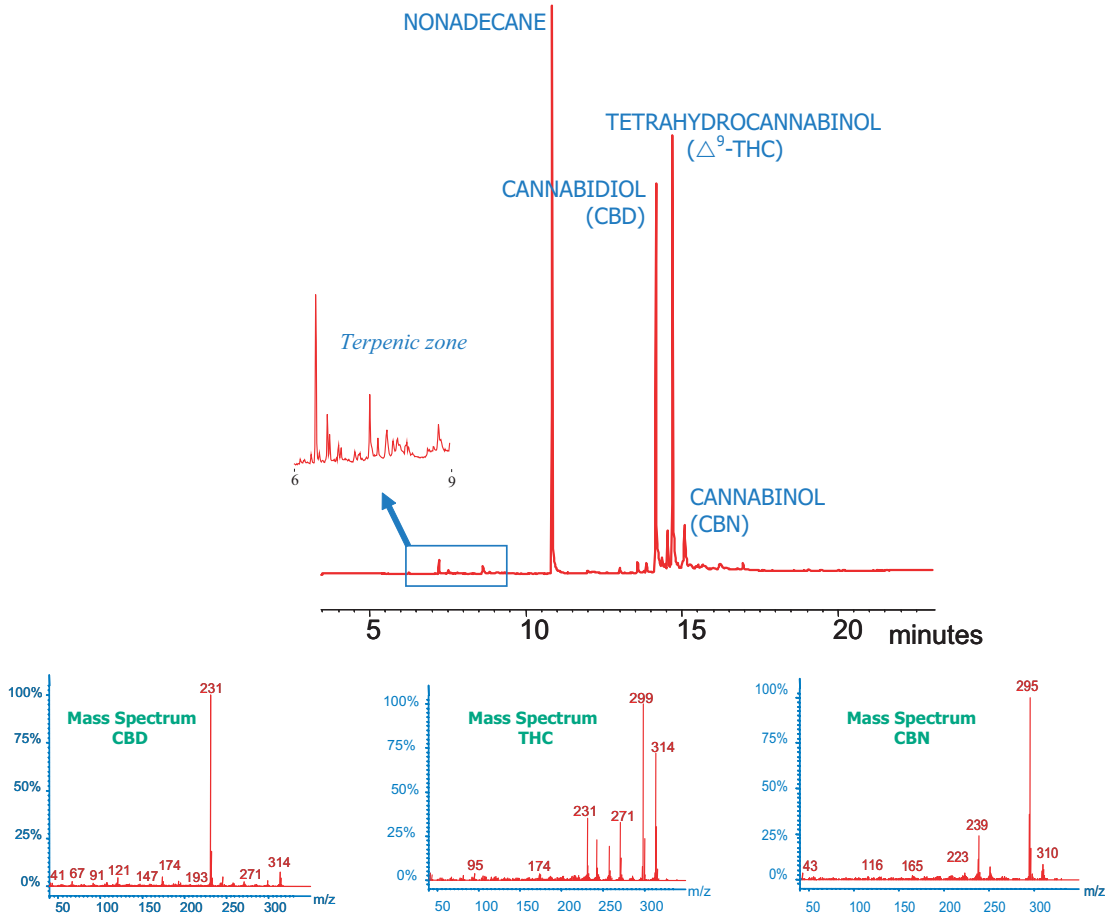
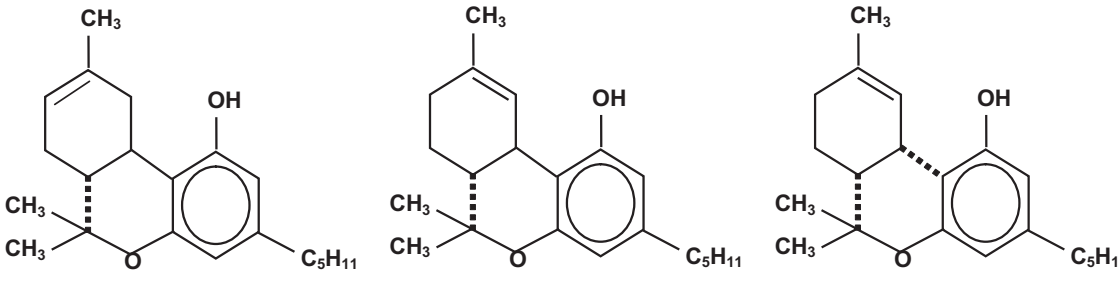
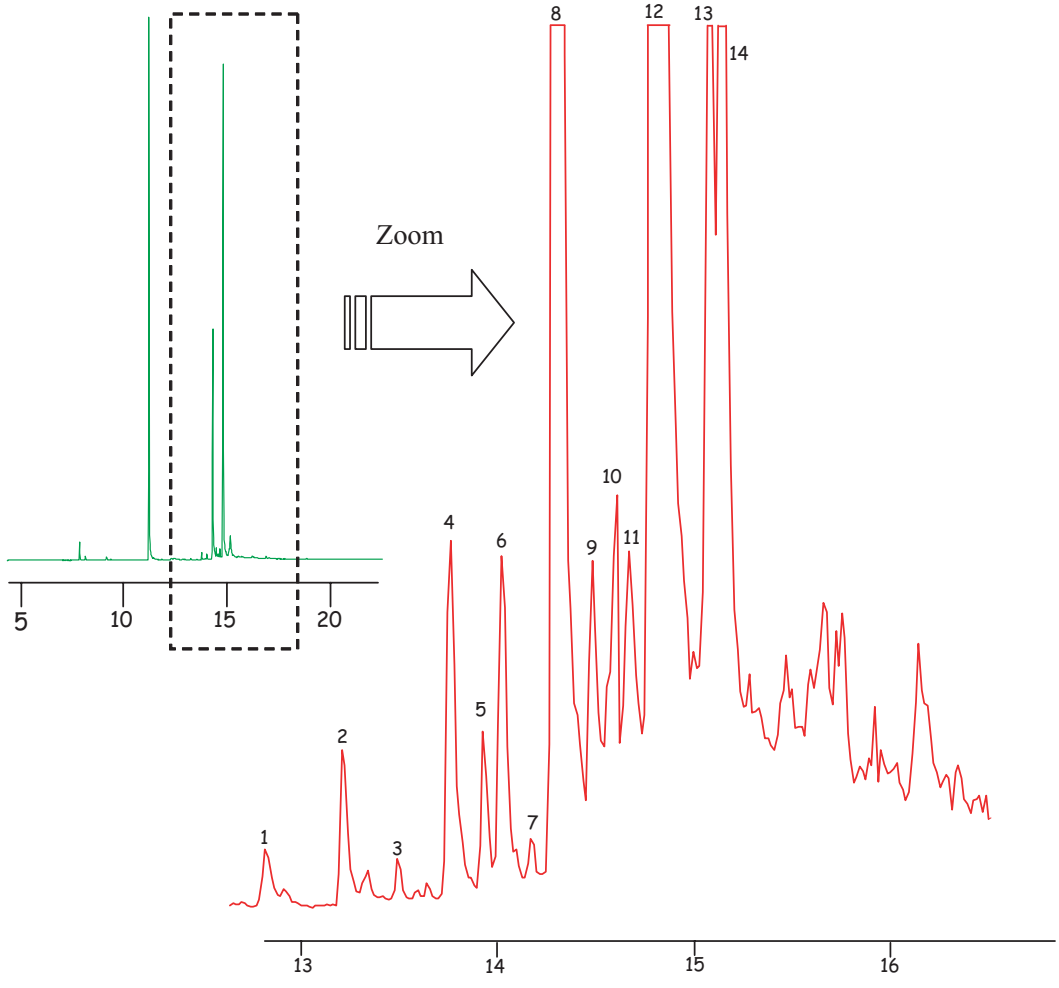


Figure XVI. Chemical structures of the natural isomers of tetrahydrocannabinol present in Moroccan cannabis



Trans-Δ^8-Tetrahydrocannabinol Trans-Δ^9-Tetrahydrocannabinol Cis-Δ^9-Tetrahydrocannabinol

Figure XVII. Determination by gas chromatography/mass spectrometry of the cannabinoids present in *Cannabis sativa* L. in powdered form

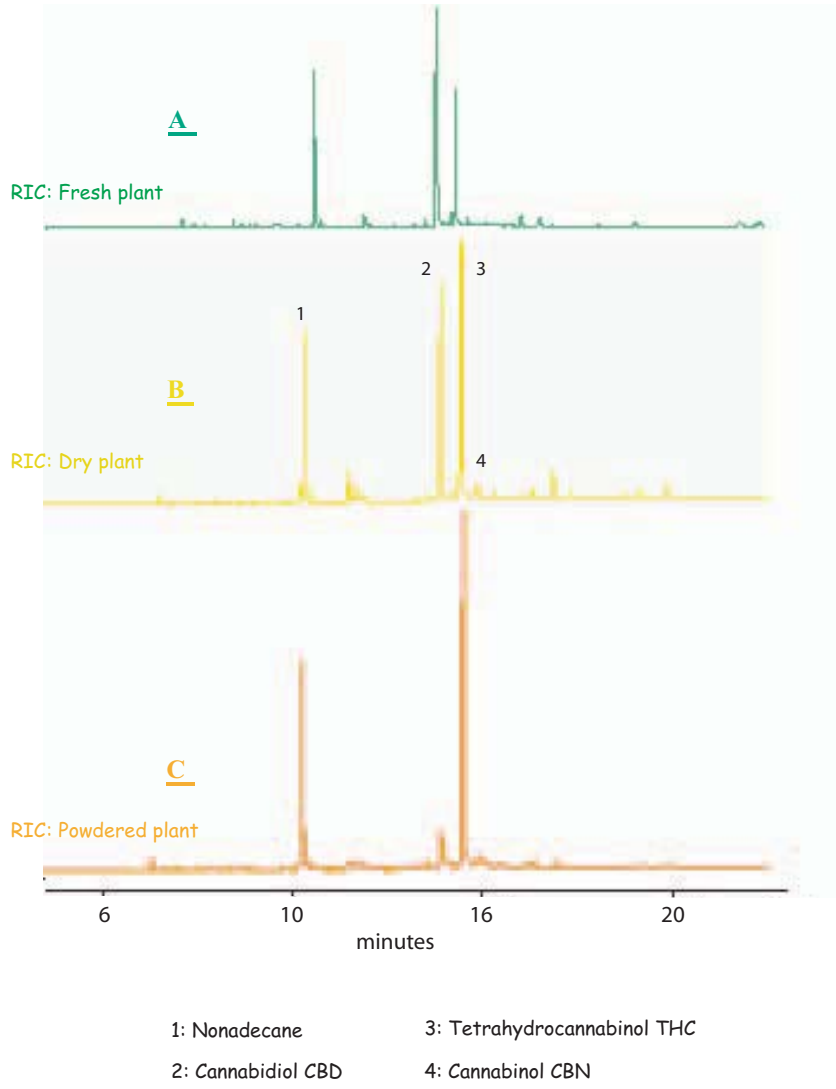


1 : Methyl-tetrahydrocannabinol m/z = 358
2 : Cannabivarol m/z = 386
3 : isomer CBD m/z = 314
4 : Tetrahydrocannabivarin m/z = 386
5 : Butyl-tetrahydrocannabinol m/z = 300
6 : Cannabichromene m/z = 314
7 : Trans Δ^8 -THC m/z = 314
8 : Cannabidiol m/z = 314
9 : Cannabicoumaronone m/z = 328
10 : Cis Δ^9 -THC m/z = 314
11 : Hydroxy-tetrahydrocannabinol m/z = 330
12 : Trans Δ^9 -THC m/z = 314
13 : Cannabigerol m/z = 316
14 : Cannabinol m/z = 310

the increase in Δ-9-THC in cannabis powder as a result of the preparation process (see figure XVIII, which shows the increase in the relative intensity of peak 3 as between chromatograms B and C).

HPLC-DAD analysis was carried out to determine the levels of the major cannabinoids contained in the cannabis and to trace their development from the growing plant stage to the stage of maturity and following the plant's conversion into powder. The presence of the two principal cannabinoids, THC and CBD, with traces of CBN, was observed, as expected; most notable, however, was the clear dominance, at various stages of the plant's growth, of the acid forms cannabidiolic acid (CBDA), cannabinolic acid (CBNA) and

Figure XVIII. Evolution of the GC/MS profile of the organic extract from cannabis in fresh, dry and powdered form

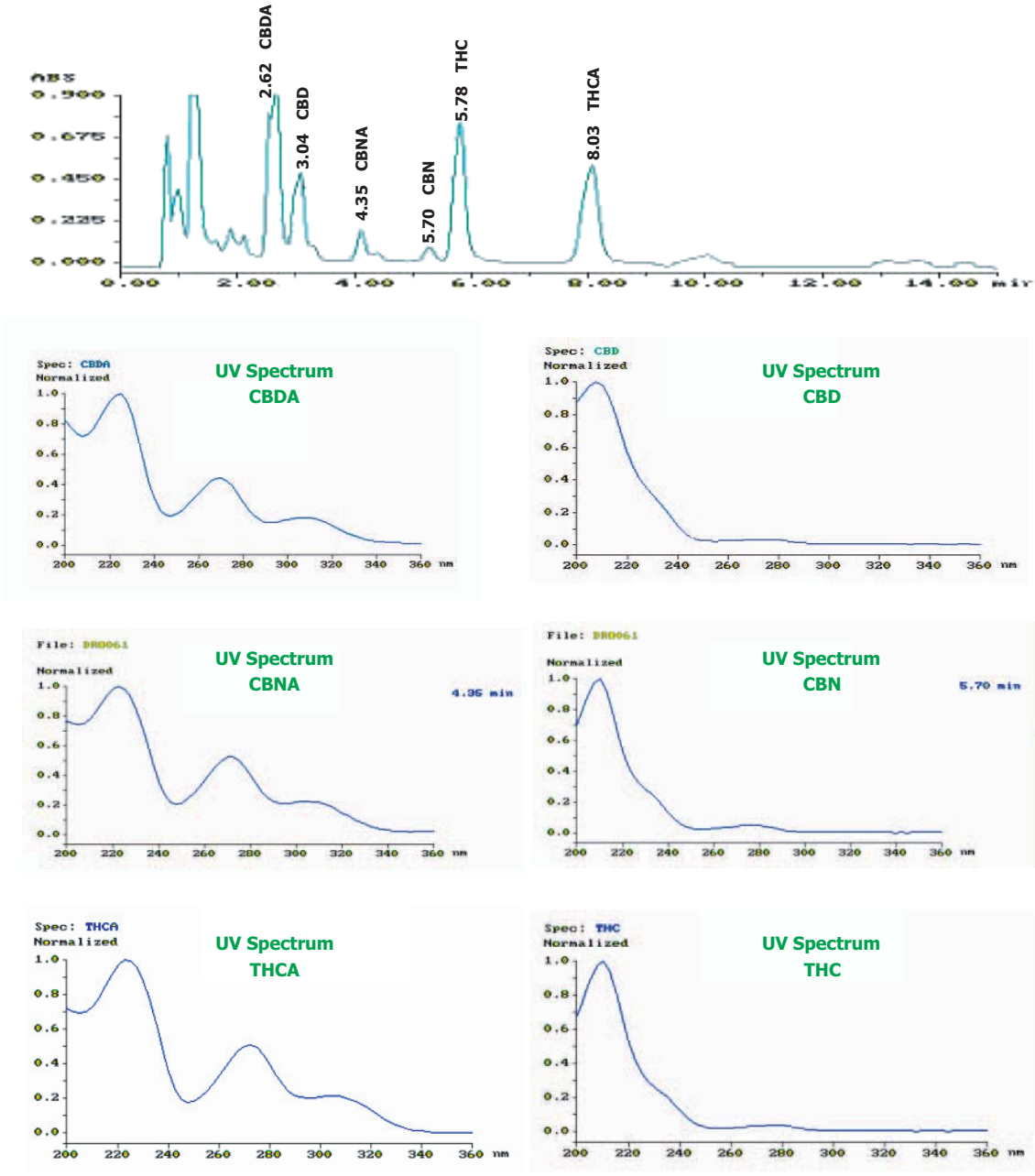


Δ-9-tetrahydrocannabinolic acid (THCA), which were not detectable by GC/MS (see figure XIX). The same results were obtained for all samples from three areas.

Quantitative analysis

The GC/MS analysis of organic extracts from the cannabis plant was used in determining the thermally stable components THC, CBD and CBN and their respective acid forms THCA, CBDA and CBNA, which are decarboxylated under the effect of heat (injector and oven), giving the forms THC, CBD and CBN. The Δ-9-THC levels in the three sample types – the green plant, the dry plant and the powdered plant – were determined by applying the peak area ratio Δ-9-THC chromatographic peak area/internal standard area to the previously established calibration curve.

Figure XIX. Chromatographic profile of powdered cannabis obtained using high performance liquid chromatography-diode array detection and UV spectra of key cannabinoids and their acid forms



(CBD = cannabidiol; CBDA = cannabidiolic acid; CBN = cannabinol; CBNA = cannabinolic acid; THC = tetrahydrocannabinol; THCA = tetrahydrocannabinolic acid)

Determination of Δ-9-tetrahydrocannabinol levels in fresh cannabis plants

Female plants

The Δ-9-THC levels of the leaves of the fresh female plants varied from region to region (figure XX). The average levels were of the same order (0.4 per cent) in the three regions.

In the case of the flowering tops of the fresh plants, which had average levels of the order of 0.6 per cent (see figure XXI), the highest average concentrations were found in the samples from Al Hoceima (0.7 per cent) and Chefchaouen (0.6 per cent), compared with the samples from Larache (0.4 per cent).

More generally, a comparison between the average Δ-9-THC concentrations in the inflorescences and the leaves revealed, as expected, high concentrations in the inflorescences (figure XXII).

Figure XX. Average Δ-9-tetrahydrocannabinol content in the leaves of fresh female cannabis plants from three areas in Morocco

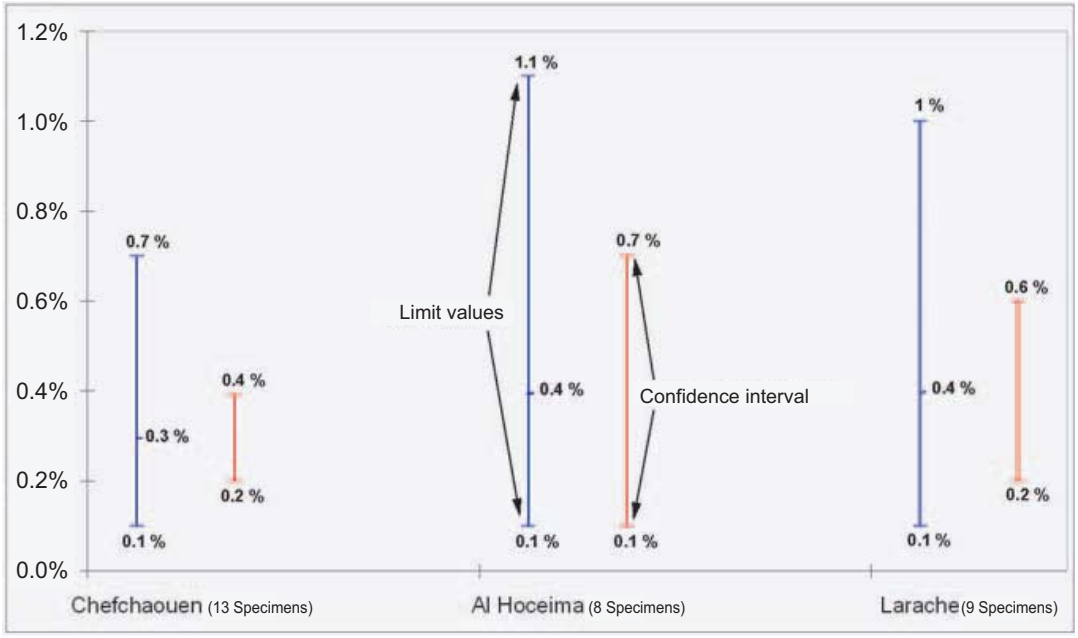


Figure XXI. Average Δ-9-tetrahydrocannabinol levels in the flowering tops of fresh female cannabis plants from three areas in Morocco

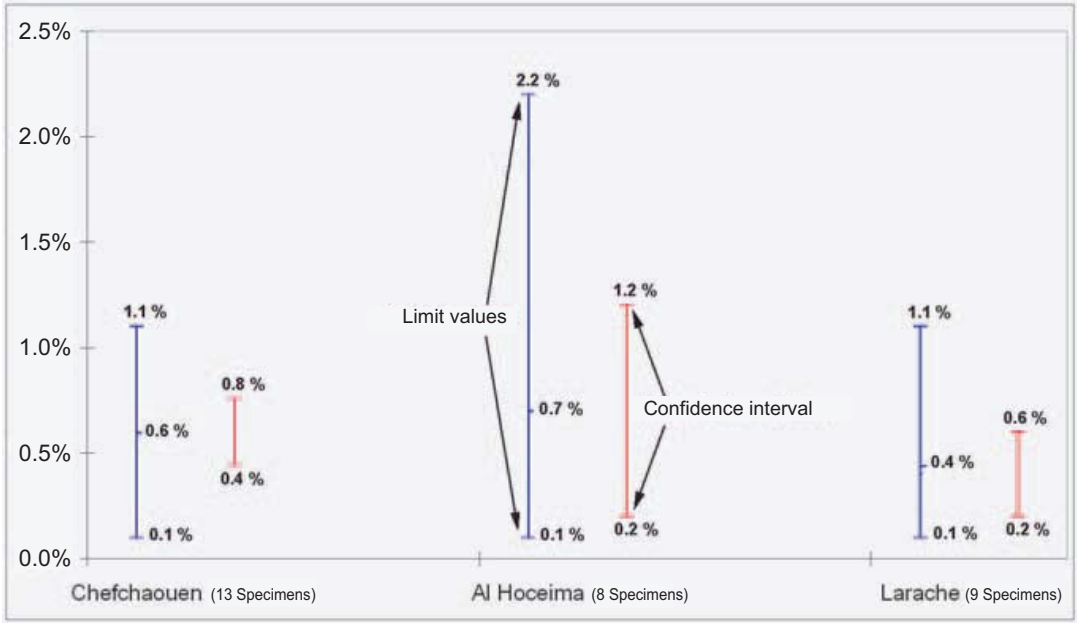
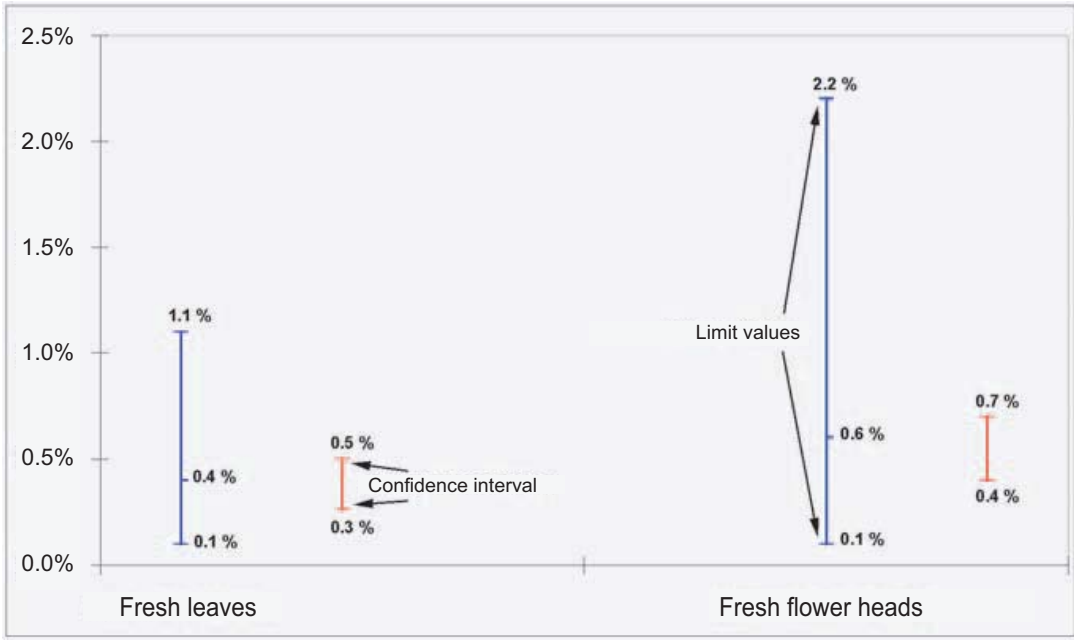
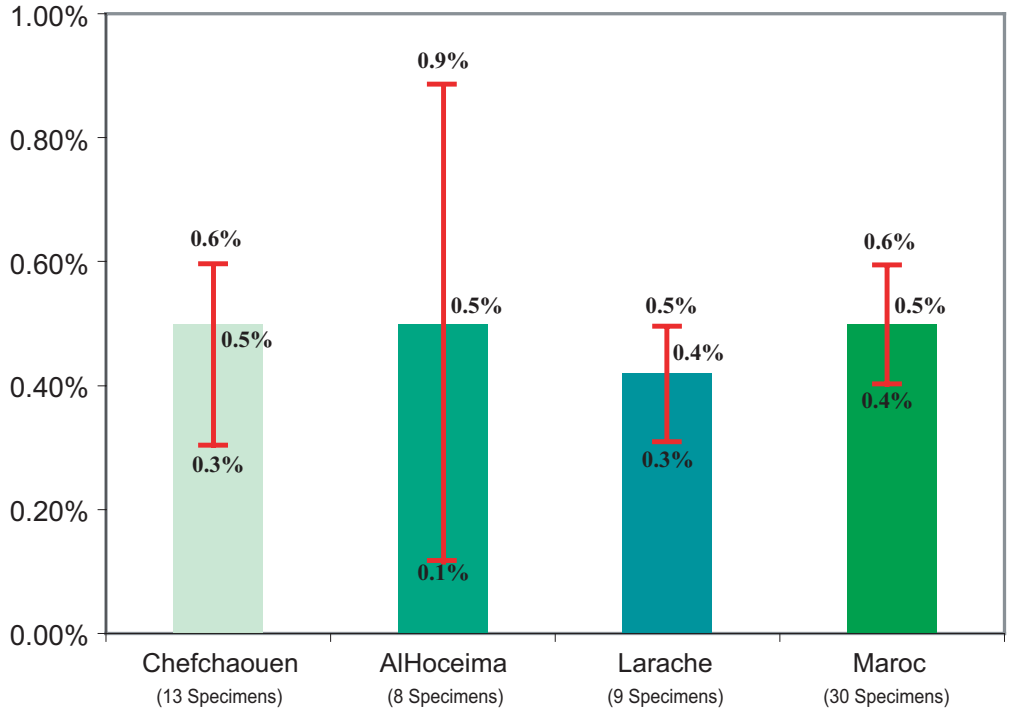


Figure XXII. Average Δ-9-tetrahydrocannabinol content in the leaves and tops of fresh female cannabis plants



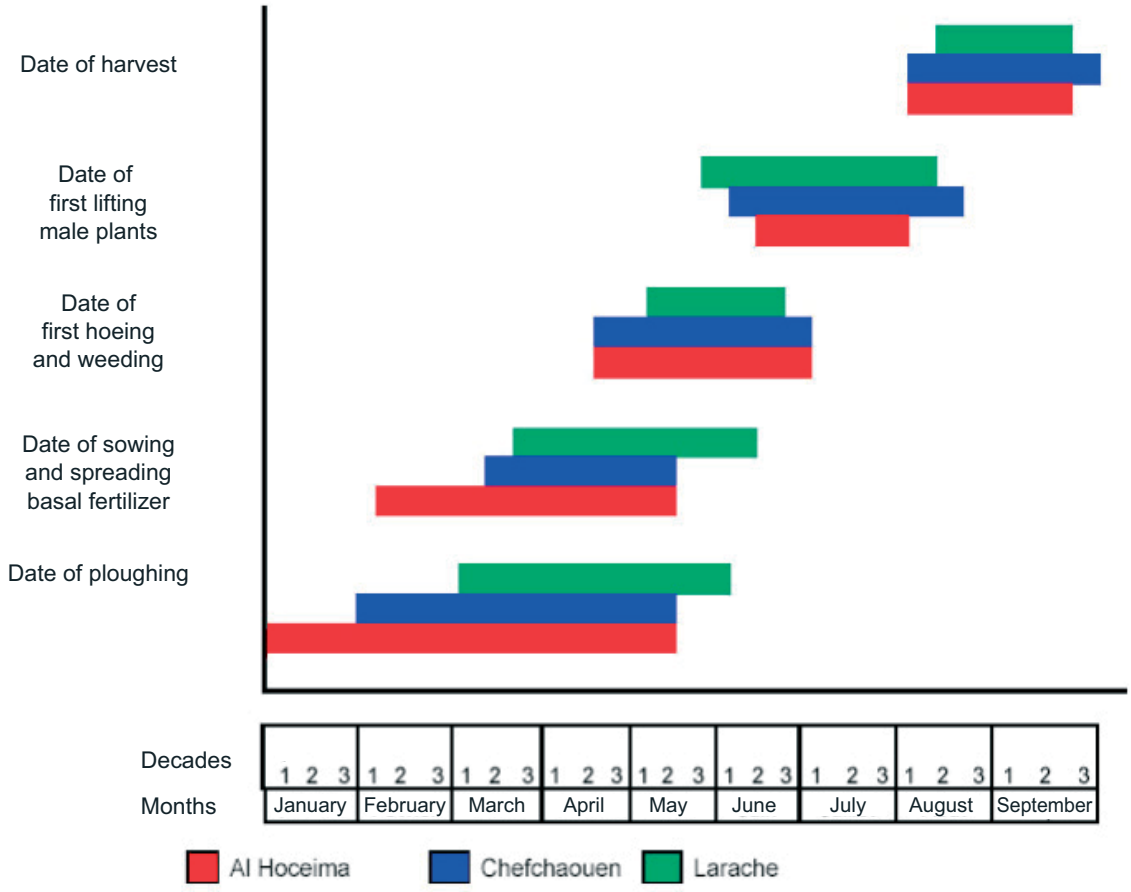
Lastly, a comparison of the average Δ-9-THC levels, including both flowering tops and leaves, and the respective confidence intervals for the three areas (see figure XXIII) show that, at this stage of growth, the cannabis from Chefchaouen and Al Hoceima had slightly higher levels of Δ-9-THC than that from Larache.

Figure XXIII. Differences in the Δ-9-tetrahydrocannabinol content of fresh female cannabis plants from Chefchaouen, Al Hoceima and Larache, with their respective average



It should be emphasized that the comparison of cannabis plants that are still growing is of a merely indicative nature, owing to the fact that sowing dates differed from one plot to the next. In the 2004 season in Larache, Al Hoceima and Chefchaouen, there were differences with respect to the time of ploughing, sowing, weeding, the removal of male cannabis plants and harvesting (see figure XXIV).

Figure XXIV. Management and harvest periods in cannabis cultivation, 2004



Male plants

The present study has demonstrated that, contrary to the widespread belief that male cannabis plants do not secrete the active constituent Δ-9-THC, the compound was, in fact, present in the leaves and tops of male plants.

The leaves of the male plants contained appreciable Δ-9-THC levels, the regional variations of which are shown in figure XXV. The average levels were similar, at about 0.4 per cent.

The values recorded for flowering tops of fresh male plants (figure XXVI) indicated average concentrations of 0.2 per cent for Chefchaouen, 0.3 per cent for Al Hoceima and 0.5 per cent for Larache.

Figure XXV. Average Δ-9-tetrahydrocannabinol content in the leaves of fresh male cannabis plants from three areas in Morocco

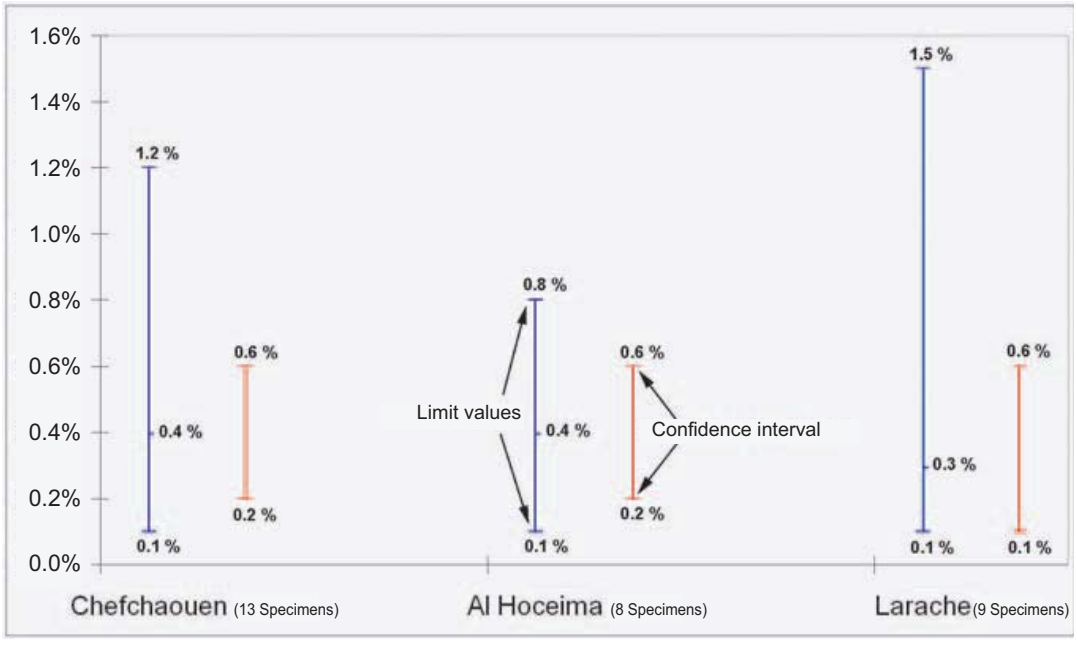
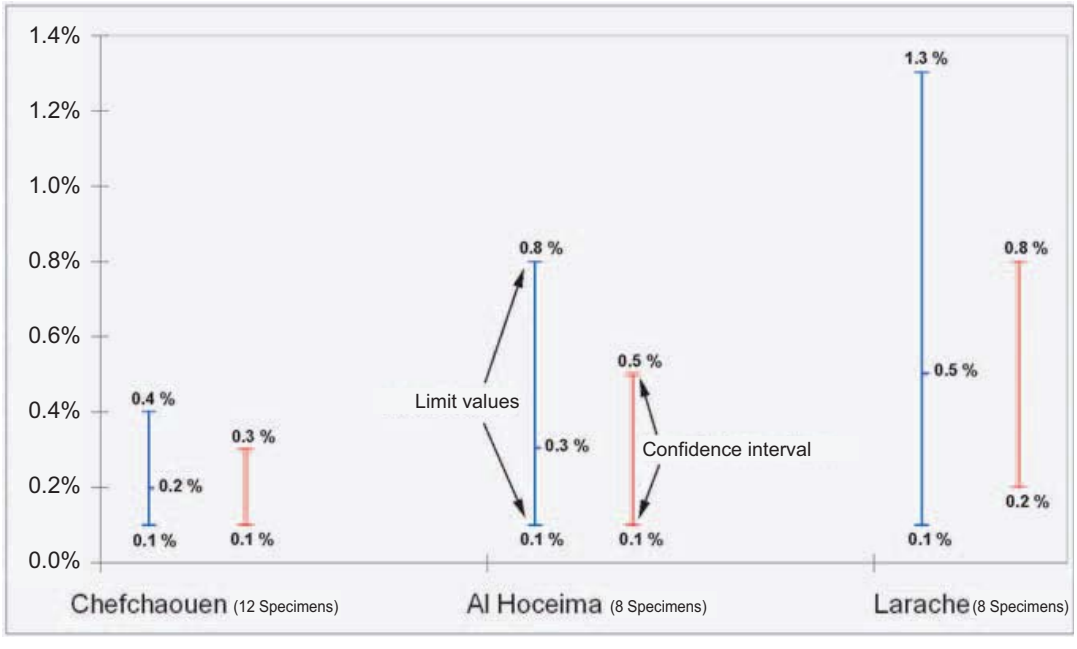
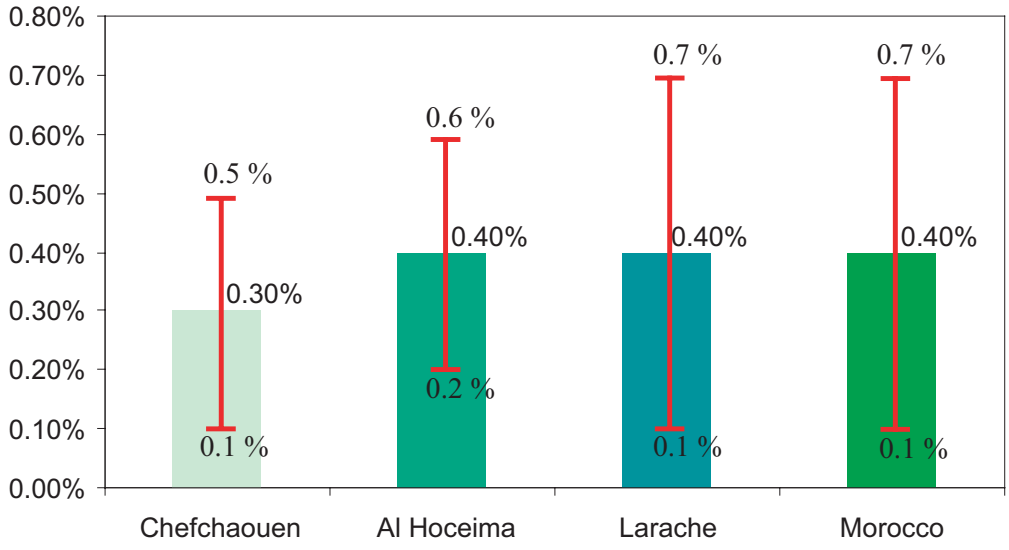


Figure XXVI. Average Δ-9-tetrahydrocannabinol content in the flowering heads of fresh male cannabis plants from Chefchaouen, Al Hoceima and Larache in Morocco



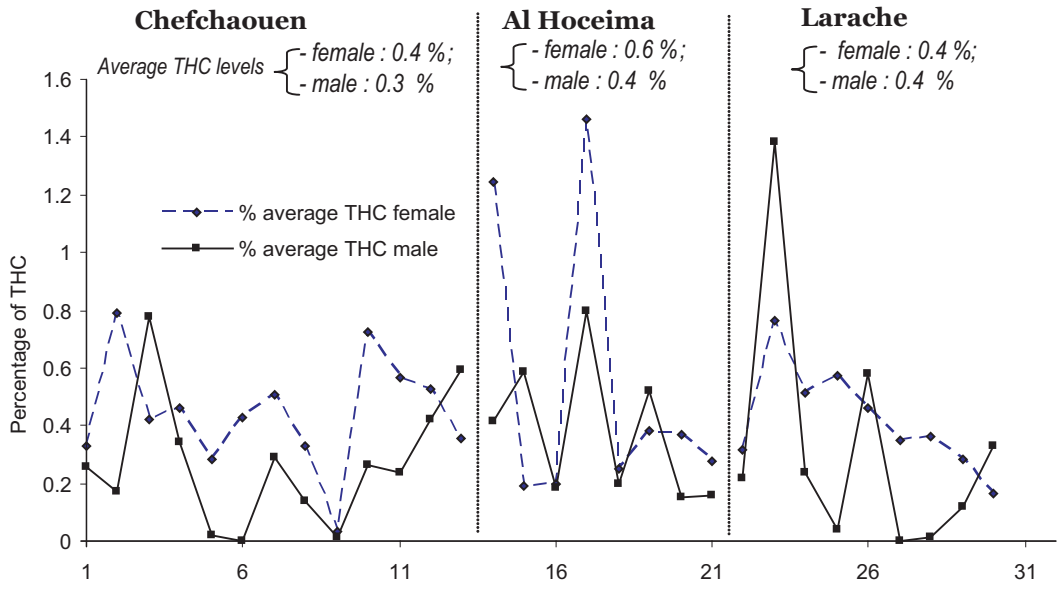
Those results confirm studies [32, 33] that have reported that Δ-9-THC levels are similar in male and female cannabis plants grown under the same conditions. The average general Δ-9-THC level in male cannabis plants has been estimated at 0.4 per cent, and the average levels for tops and leaves were very similar in Chefchaouen, Al Hoceima and Larache (see figure XXVII).

Figure XXVII. Average Δ-9-tetrahydrocannabinol content of fresh male cannabis plants from Chefchaouen, Al Hoceima and Larache in Morocco



Those values, while substantial, were slightly lower than those found in female plants. This is due to the fact that the vegetative cycle of the male plant is longer than that of the female. Moreover, the farmers' practice of removing male plants to prevent pollination of the female plants [21] tends to promote the formation of a variety that is similar to sinsemilla and richer in Δ-9-THC. The two curves in figure XXVIII represent the variations in the average Δ-9-THC level in green male and female cannabis plants from the 30 plots studied.

Figure XXVIII. Average Δ-9-tetrahydrocannabinol content of male and female cannabis plants from Chefchaouen, Al Hoceima and Larache in Morocco



On the other hand, given the random variations recorded in the Δ-9-THC levels in plants from different plots, it was not possible to establish any correlation with bioclimatic factors or cultivation conditions. Analysis showed that neither the leaves nor the inflorescences of two female plants growing 10 metres apart on the same plot consistently presented the same Δ-9-THC level. For that reason, this study gives the average Δ-9-THC concentration in the leaves and flowering tops obtained from two female plants that were analysed separately.

Determination of Δ-9-tetrahydrocannabinol levels in dry cannabis plants

In presenting the analysis results for the dry cannabis plants, it is worth considering the problems resulting from the methods of lifting and sampling such plants. As mentioned already, the dry plants from the 30 plots studied were lifted and randomly combined into bunches, each containing about 30 plants. Whereas the average Δ-9-THC level of the flowering tops were not significantly affected by the height on the plant at which samples were taken, in the case of the leaves, there were non-negligible variations according to sampling height. With respect to the Δ-9-THC content of leaves from the lower third of the dry plants and leaves from the upper third, there was a general tendency towards higher concentrations of Δ-9-THC in the leaves from the upper part (see table 4). Thus, in this study, Δ-9-THC levels in dry cannabis plants were determined based on samples from both the top and the base of the plant.

Table 4. Comparison of the Δ-9-tetrahydrocannabinol content of leaves taken from the lower and upper thirds of the cannabis plant

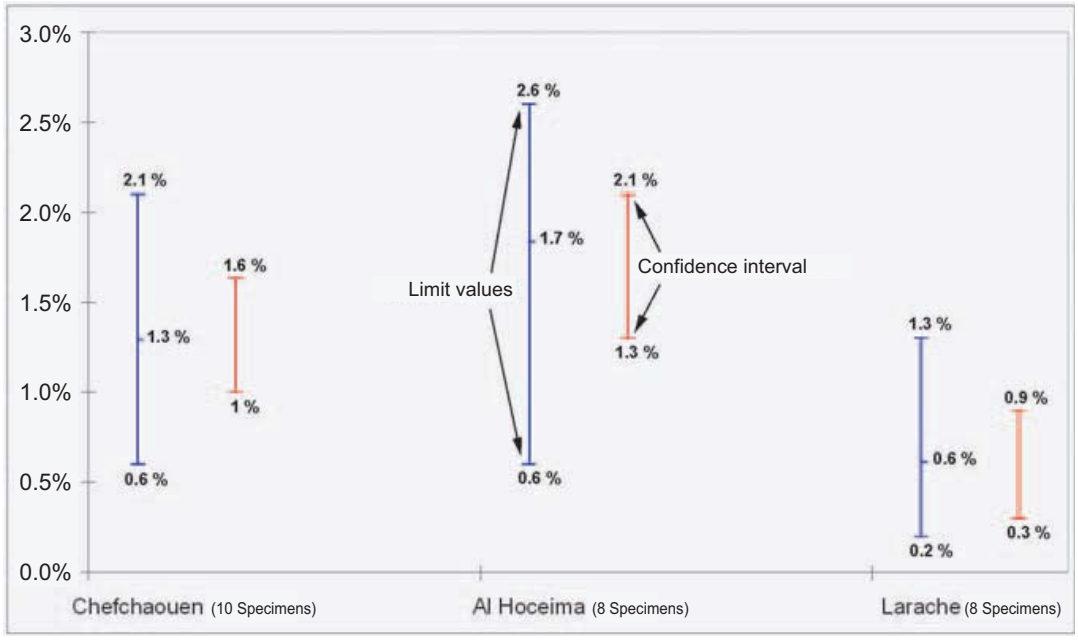
	Plant 1		*Plant 2*	
Sample	*Lower third*	*Upper third*	*Lower third*	*Upper third*
1	0.01	0.09	0.21	0.33
2	0.30	0.39	0.16	0.15
3	0.10	0.11	0.07	0.87
4	0.07	0.21	0.77	1.76

Δ-9-Tetrahydrocannabinol levels in dry cannabis plants

The leaves of dry, mature plants contain Δ-9-THC levels that differ noticeably from one region to another (see figure XXIX). The Al Hoceima area stands out as having the highest concentration, 1.7 per cent on average; it is followed by the Chefchaouen area at 1.2 per cent and the Larache area at 0.6 per cent.

Also in the case of the inflorescences of dry, mature plants (see figure XXX), the highest Δ-9-THC levels were recorded in samples from Al Hoceima (4.1 per cent on average). This confirms the tendency noted in the case of leaves from

Figure XXIX. Average Δ-9-tetrahydrocannabinol content of dried cannabis leaves from three areas in Morocco



that area. The Chefchaouen area, with an average level of 2.1 per cent, is in second position; it is followed by Larache, with an average level of 1.8 per cent.

As in the case of fresh plants, a comparison between the Δ-9-THC levels of the inflorescences and leaves of dry plants (see figure XXXI) revealed that the inflorescences contained levels that were higher by a factor of 2-3. That predictable result was corroborated by a study [34] showing that the Δ-9-THC

Figure XXX. Average Δ-9-tetrahydrocannabinol content of dry cannabis plant tops from three areas in Morocco

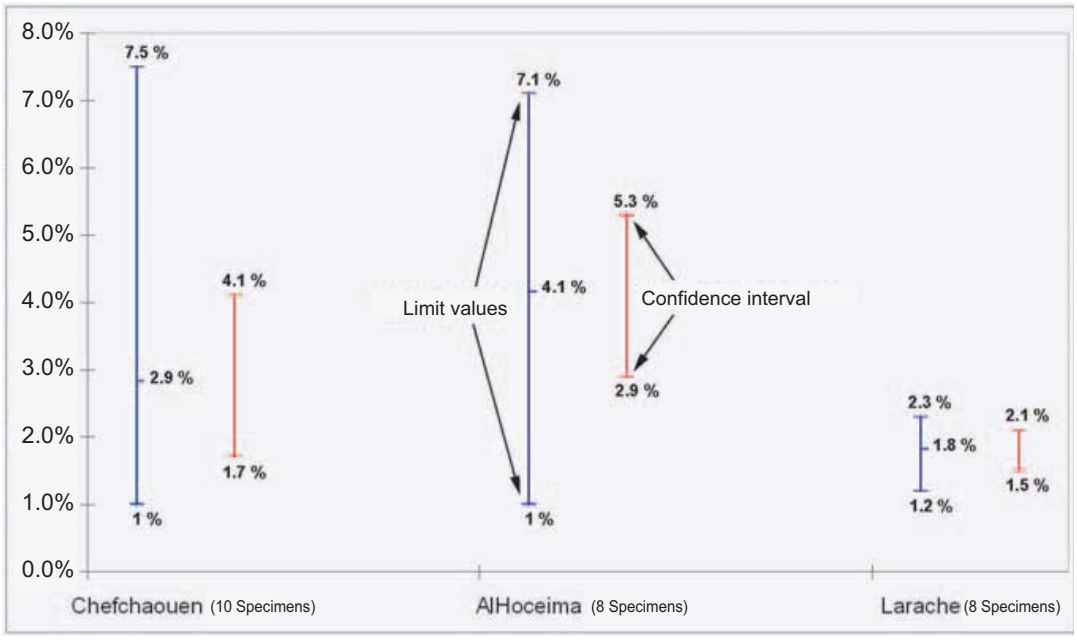
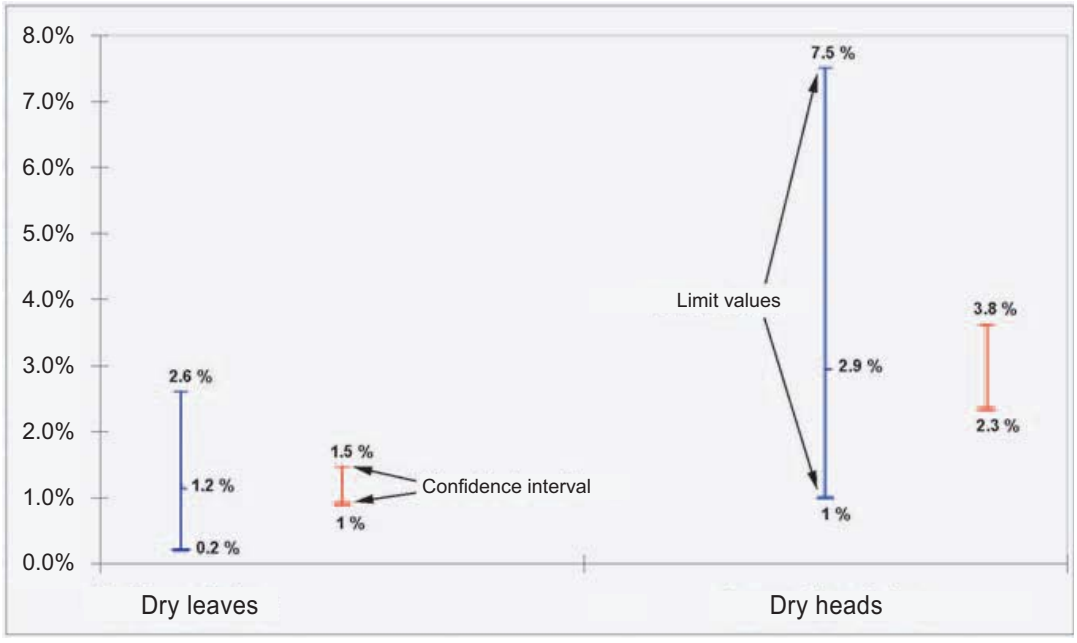


Figure XXXI. Average Δ-9-tetrahydrocannabinol content of leaves and heads of dry cannabis plants (total specimens: 26)



levels of a plant's parts decrease in the following order: bracts, flowers, leaves, stalks, roots and seeds.

More generally, the average Δ-9-THC level in the inflorescences and leaves of the dry plants analysed varied within a range of 0.7-4.8 per cent, and most of the plants had a Δ-9-THC level higher than 1 per cent. Larache was notable for the fact that three of the plots produced cannabis low in Δ-9-THC (<1 per cent), while in Al Hoceima, a relatively high concentration (>3 per cent) was recorded at four plots. Cannabis plants grown in Chefchaouen were characterized by intermediate levels of Δ-9-THC (1-3 per cent). Only one plot in Chefchaouen had cannabis with a fairly high Δ-9-THC level (4.8 per cent).

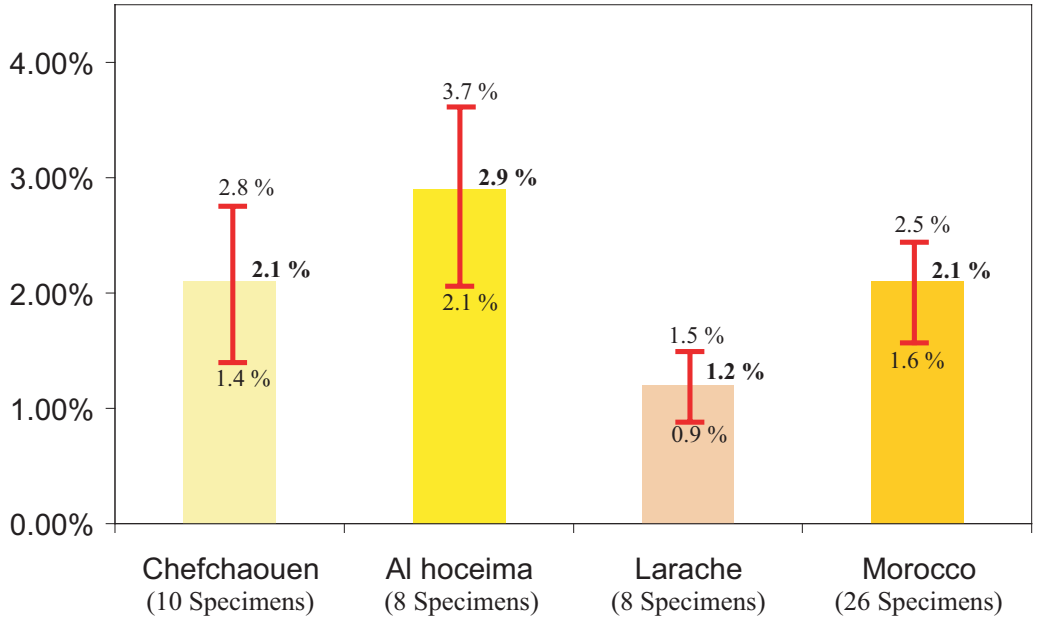
It is clear from figure XXXII, which shows the Δ-9-THC levels for the three areas studied, that there is a marked difference between the different crops. Calculating the average Δ-9-THC level in each area, and taking into account the respective confidence interval, a ranking can be established headed by Al Hoceima and Chefchaouen, the two areas where the practice of cannabis cultivation is long-standing with average Δ-9-THC levels of 2.9 per cent and 2.1 per cent, respectively, followed by Larache, where the average Δ-9-THC level is below 1.2 per cent. The overall average Δ-9-THC level was 2.1 per cent.

The trend in the Δ-9-THC levels noted in dry female plants confirms the above-mentioned results for fresh female plants.

Influence of irrigation on Δ-9-tetrahydrocannabinol levels

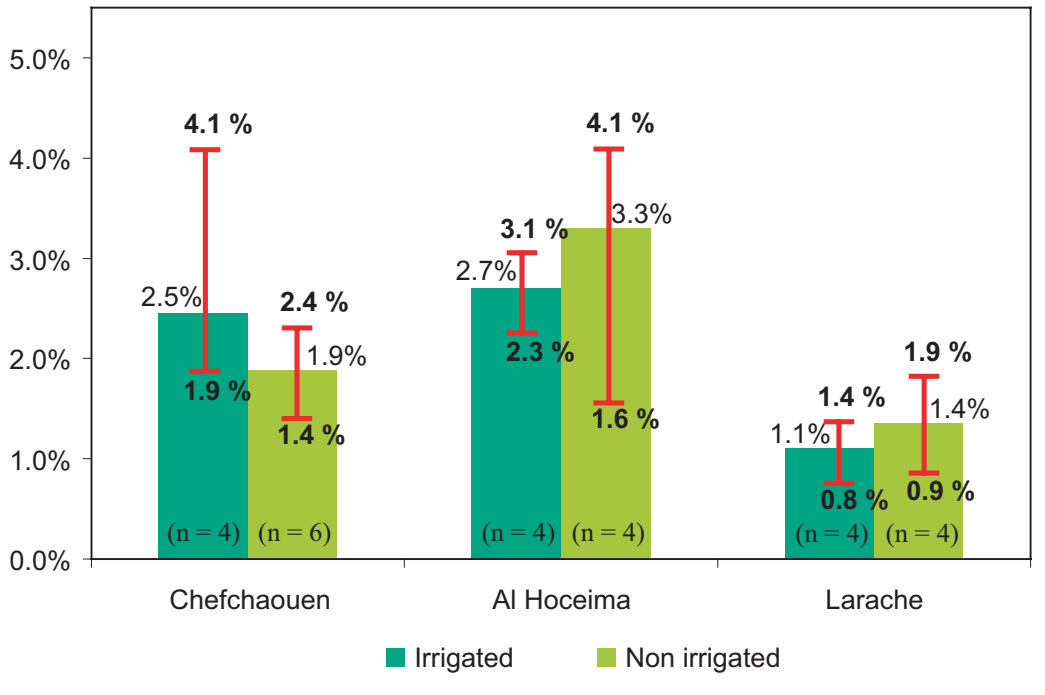
It proved difficult to establish a relationship between the results obtained and the state of irrigation of the land under cannabis cultivation. The average Δ-9-THC levels for dry cannabis leaves and tops in the three regions under

Figure XXXII. Differences in Δ-9-tetrahydrocannabinol in dry cannabis plants from three regions in Morocco



consideration varied according to whether irrigation was used, but in an inconsistent manner (see figure XXXIII). In Al Hoceima and Larache, the Δ-9-THC levels were higher in the unirrigated areas than in the irrigated ones. The average Δ-9-THC levels ranged from 2.7 per cent to 3.3 per cent in Al Hoceima and from 1.1 per cent to 1.4 per cent in Larache. In Chefchaouen, however, the average levels did not conform to that pattern; they were higher in the irrigated areas (2.5 per cent) than in the unirrigated ones (1.9 per cent).

Figure XXXIII. Influence of irrigation on Δ-9-tetrahydrocannabinol levels



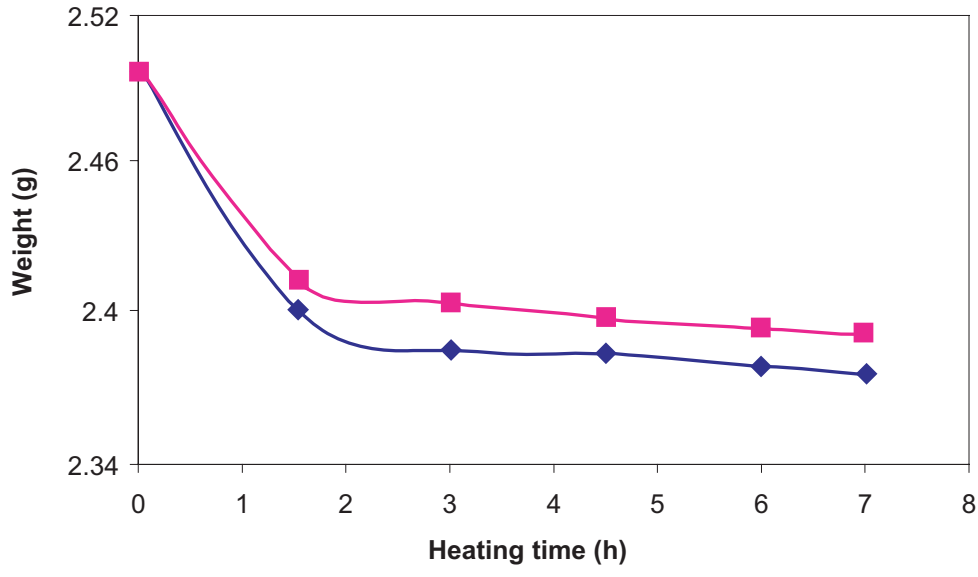
In order to better establish a correlation between irrigation and average Δ-9-THC content, it would be necessary to study a much larger number of samples covering the three areas in their entirety and to take into account factors such as rainfall, sowing periods, bioclimatic stages, the use of phytosanitary products and fertilizers and the genotype of the sown seeds.

Determination of Δ-9-tetrahydrocannabinol levels in powdered cannabis

(a) Effect of drying on the assessment of Δ-9-tetrahydrocannabinol levels of cannabis

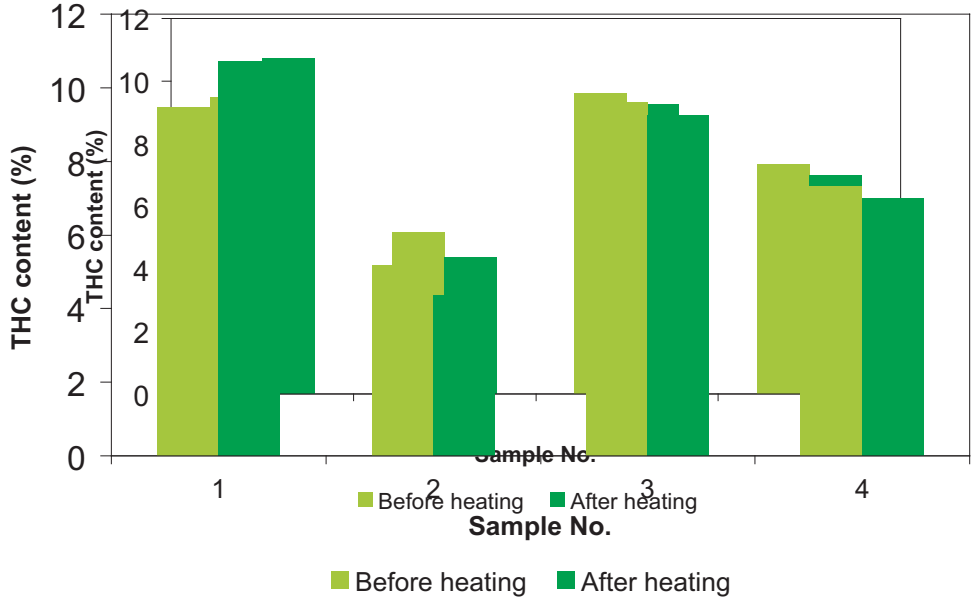
The drying of cannabis samples before determination of their Δ-9-THC content has been described by several authors [11, 24 and 27]. The purpose of the process, which consists of heating at a temperature below 70° C until a constant sample weight is achieved, is complete dehydration in order to achieve greater accuracy. However, heating always entails the risk of denaturing the product through the conversion of Δ-9-THC into CBN. The effect of drying cannabis at 70° C on the behaviour of Δ-9-THC and thus on the accuracy of the concentration calculations was examined in this study. Two samples of powder with an initial weight of 2.5 g were heated at a temperature of 70° C for seven hours, and the loss of weight over time was checked every 90 minutes (see figure XXXIV). The loss of weight was 4 per cent after three hours of drying, and the mass stabilized around that level during the next four hours.

Figure XXXIV. Variations in the mass of two samples of powdered cannabis when heated at 70° C



The effects of this reduction in weight on the calculations of the Δ-9-THC level were then assessed. The tests carried out for that purpose consisted of determining the Δ-9-THC concentrations in the powder before and after drying for seven hours at 70° C. The results of some of those tests (see figure XXXV)

Figure XXXV. Influence of drying on the Δ-9-tetrahydrocannabinol content of powdered cannabis

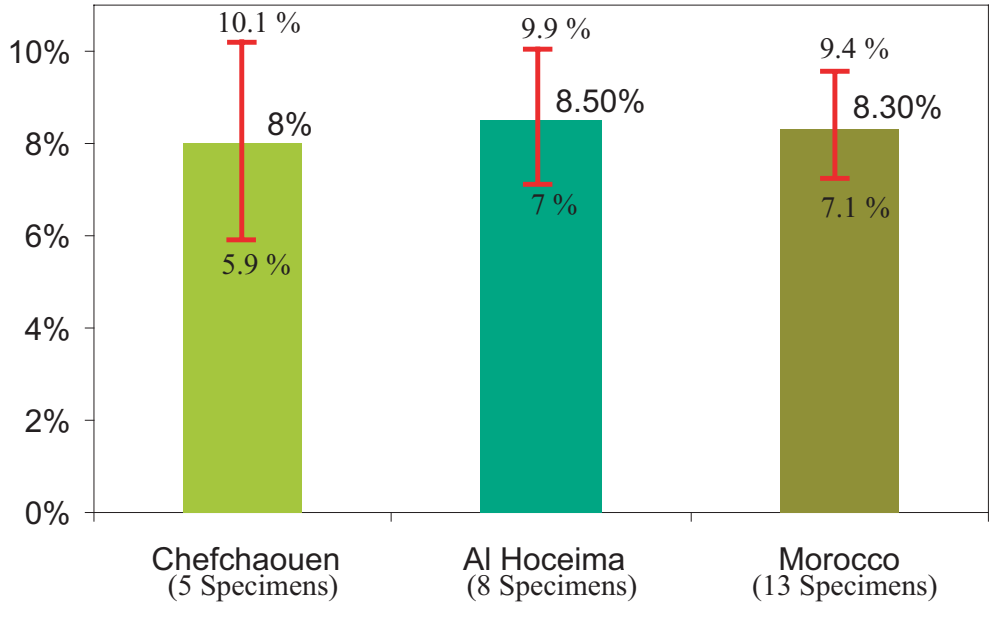


show that drying had little effect on the Δ-9-THC concentrations in the powder. That was probably due to the fact that the powder had just been prepared and its humidity level was very low: slightly more than 4 per cent.

(b) Δ-9-Tetrahydrocannabinol levels in powdered cannabis

The cannabis powder samples studied came exclusively from Al Hoceima and Chefchaouen, where the conversion of dry cannabis into powder form is a long-established practice. The Δ-9-THC levels in the cannabis powder analysed

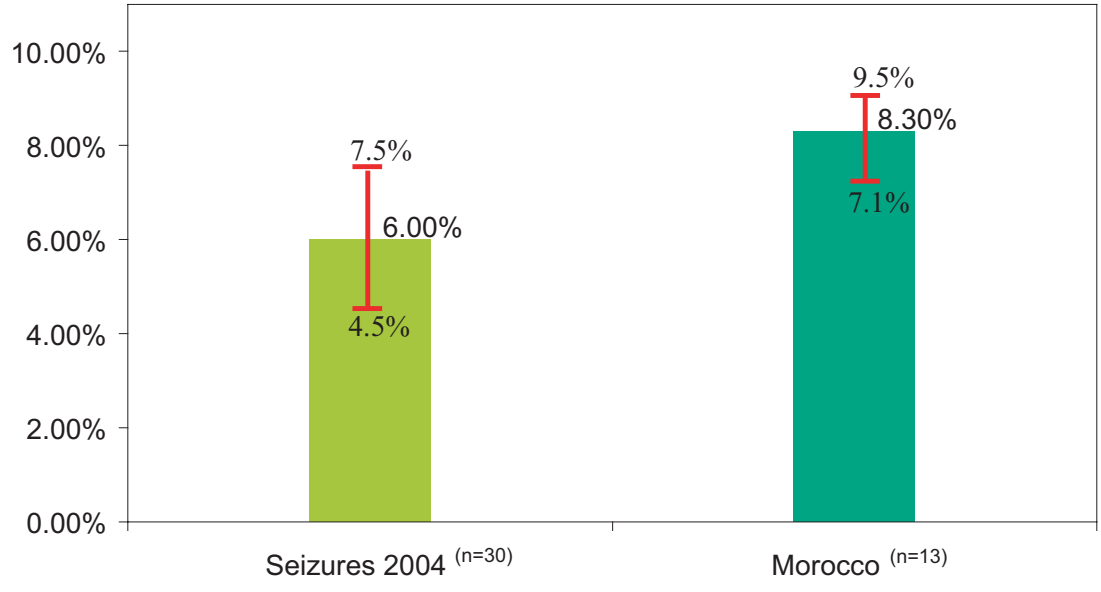
Figure XXXVI. Δ-9-Tetrahydrocannabinol levels in powdered cannabis: range and average



were found to be between 5.5 per cent and 11.3 per cent, with an overall average estimated at 8.3 per cent. The powders from plots in Al Hoceima had an average Δ-9-THC level of 8.5 per cent, slightly higher than those from Chefchaouen, the average Δ-9-THC level of which was 8 per cent. Figure XXXVI shows the variations in the Δ-9-THC levels in powdered cannabis from Chefchaouen and Al Hoceima, together with the overall average Δ-9-THC level.

It should be noted that this average level of Δ-9-THC in freshly prepared powdered cannabis, at 8.3 per cent, was higher than the estimated Δ-9-THC levels (of 6 per cent) in samples from 30 consignments of cannabis resin seized during 2004 by the Gendarmerie Royale (see figure XXXVII). This difference may be due to the effects of the methods used in preparing the powder, adulteration prior to seizure and/or the conditions under which the plants and the resin blocks had been stored for various periods of time.

Figure XXXVII. Average Δ-9-tetrahydrocannabinol level of the powdered cannabis analysed in the present study and that of samples of cannabis resin seized in 2004



Evolution of Δ-9-tetrahydrocannabinol levels through the various stages

Figure XXXVIII illustrates the development of Δ-9-THC in the crops grown on the 13 plots which supplied the three specimen types: fresh cannabis plants, dry cannabis plants and powdered cannabis. It shows that, in each region, the Δ-9-THC levels increased markedly as the plant grew and was then converted into powder.

The estimated average Δ-9-THC level in cannabis was 0.5 per cent in its fresh plant state and 2.1 per cent in its dry plant state. Conversion of the plant to powdered form was accompanied by a marked increase in its Δ-9-THC level, to 8.3 per cent, probably because of the substantial contribution made by the inflorescences and the resin of the plant (see figure XXXIX).

Figure XXXVIII. Evolution of Δ-9-tetrahydrocannabinol levels in cannabis crops from two areas in northern Morocco

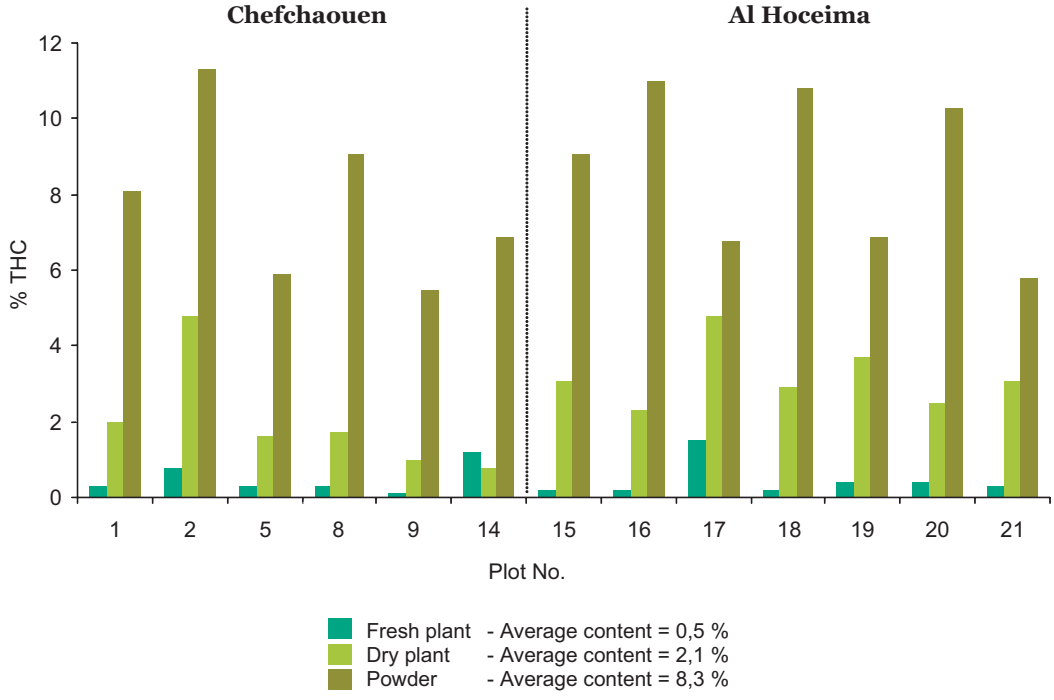
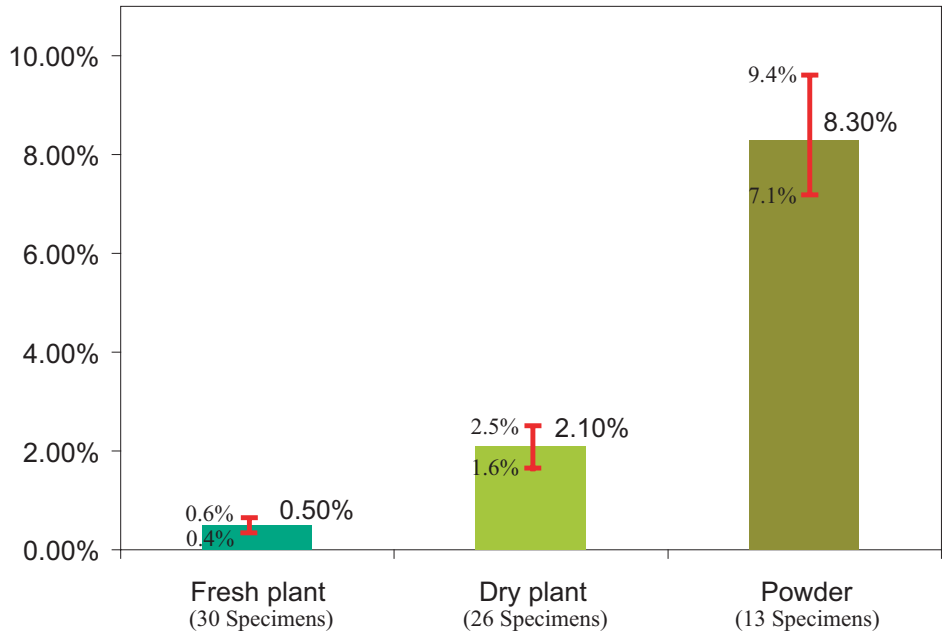


Figure XXXIX. Evolution of Δ-9-tetrahydrocannabinol levels at the fresh plant, dry plant and powdered cannabis stages



Conclusions

The first survey conducted in northern Morocco, in 2003, yielded socio-economic data about the territories where cannabis cultivation has been prevalent for many years and about the recently established cultivation areas. The second survey,

conducted in the Moroccan Rif during 2004, had a different purpose: to assess the quality of local cannabis crops. Three areas, accounting for more than 75 per cent of Morocco's cannabis production in 2004, were selected for the study: Chefchaouen and Al Hoceima, where cannabis cultivation had been a long-standing practice, and Larache, where cannabis had only been cultivated for two decades.

Field studies of cannabis cultivation provide socio-economic data on production, yields and income, among other things, but only the laboratory analysis of cannabis crops can provide the information on chemical composition and levels of psychoactive constituents making it possible to classify them as drug type or fibre type. The analytical work carried out on fresh plants, dry plants and powdered plants benefited from the use of fresh specimens, obtained on the day of harvesting or immediately after preparation, in order to minimize any Δ-9-THC transformations as a result of oxidation due to ageing.

Qualitative analyses of cannabis grown in Morocco using HPLC-DAD provided chromatographic profiles giving a clearer picture of the cannabinoid composition of the plant, dominated by the acid forms (CBDA, THCA and CBNA) along with the corresponding decarboxylated forms (CBD, THC and CBN). Qualitative analyses using GC/MS revealed the principal cannabinoids present in trace amounts in Moroccan cannabis. Tetrahydrocannabinol is present as three natural isomers: cis-Δ-9-THC, trans-Δ-9-THC and trans-Δ-8-THC. Its inferior homologues butyl-Δ-9-THC, methyl-Δ-9-THC and propyl-Δ-9-THC were also found. The qualitative study did not, however, reveal any difference in chemical composition between the cannabis crops grown in the three areas in northern Morocco.

The quantitative analysis of the cannabis crops grown in the three areas in northern Morocco was carried out using GC/MS. It focused exclusively on determining the levels of the psychoactive constituent Δ-9-THC in the growing plant, at the stage of maturation and after its reduction to powder, which is the last stage before it is turned into blocks of chira.

The Δ-9-THC levels found were 0.1-1.5 per cent for the growing plant, 0.7-4.8 per cent for the dry plant and 5.5-11.3 per cent for powdered cannabis. Thus, it is clear that the plants progressively gain in Δ-9-THC. Average levels were calculated for each stage: 0.5 per cent for the growing plant, 2.1 per cent for the dry plant and 8.3 per cent for the powder.

It is worth placing those values in a wider context, comparing them with the Δ-9-THC levels found in cannabis seized in various parts of the world. A retrospective study of Δ-9-THC levels in cannabis seized in the United States between 1980 and 1997 [22] pointed to average Δ-9-THC concentrations in samples of cannabis herb within the range 3-4.47 per cent. A study by the European Monitoring Centre for Drugs and Drug Addiction [23], which presents data reported by European countries on Δ-9-THC levels in cannabis herb and resin, should also be noted. According to that study, the most recent information, compiled in 2001 and 2002, points to Δ-9-THC concentrations of 1.6-15.2 per cent in the plant and 2.0-20.6 per cent in the resin.

Analysis of the flowering tops and leaves of male plants confirmed the secretion of Δ-9-THC at different stages of plant growth. Although the values are

slightly lower than those obtained for female plants, they are very significant; they are due to the fact that the vegetative cycle of the male plant is longer than that of the female plant. In addition, a comparison of Δ-9-THC levels in the inflorescences and leaves of dry plants shows that the inflorescences contain higher concentrations of Δ-9-THC by a factor of 2-3.

Lastly, the study shows that in Larache, where cannabis cultivation is relatively recent, the cannabis crop has Δ-9-THC levels lower than those recorded in Al Hoceima and Chefchaouen, where such cultivation is a longer-established practice. In addition to the know-how accumulated by the farmers in the latter two areas over the years, other factors should be taken into consideration when attempting to explain this fact, for example, growing conditions, rainfall, altitude, hours of sunshine, nature of the soil, irrigation, phytosanitary treatment and even the genotype of the seeds sown.

References

1. *World Drug Report 2005* (United Nations publication, Sales No. E.05.XI.10).
2. United Nations Office on Drugs and Crime, *Morocco: Cannabis Survey 2003* (December 2003).
3. J. Bruneton, *Pharmacognosie: phytochimie, plantes médicinales* (Cachan, France, Lavoisier, Editions Technique et Documentation, 1999).
4. R. Smith, "Identification of butyl cannabinoids in marijuana", *Journal of Forensic Sciences*, vol. 42, No. 4 (1997), pp. 610-618.
5. P. Mura and others, "*Cannabis sativa* var. *indica:* une plante complexe aux effets pervers", *Annales de Toxicologie Analytique*, vol. 16, No. 1 (2004), pp. 7-17.
6. E. de Meijer and others, "The inheritance of chemical phenotype in *Cannabis sativa* L.", *Genetics*, vol. 163, No. 1 (2003), pp. 335-346.
7. F. Taura, S. Morimoto and Y. Shoyama, "Purification and characterization of cannabidiolic acid synthase from *Cannabis sativa* L.", *Journal of Biological Chemistry*, vol. 271, No. 29 (1996), pp. 17411-17416.
8. S. Morimoto and others, "Purification and characterization of cannabichromenic acid synthase from *Cannabis sativa*", *Phytochemistry*, vol. 49, No. 6 (1998), pp. 1525-1529.
9. M. Fellermeier and M. Zenk, "Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol", *Federation of European Biochemical Societies (FEBS) Letters*, vol. 427, No. 2 (1998), pp. 283-285.
10. G. Fournier and others, "Identification of a new chemotype in *Cannabis sativa*: cannabigerol-dominant plants, biogenetic and agronomic prospects", *Planta Medica*, vol. 53, No. 3 (1987), pp. 277-280.
11. G. Fournier, O. Beherec and S. Bertucelli, "Santhica 23 et 27: deux variétés de chanvre (*Cannabis sativa* L.) sans Δ-9-THC", *Annales de Toxicologie Analytique*, vol. 16, No. 2 (2004).
12. D. Stockley, *Drug Warning* (London, Optima Books, 1992).

13. P. Mura and A. Piriou, "Le Cannabis", *Toxicologie et pharmacologie médicolégales*, P. Kintz, ed. (Paris, Elsevier, 1998), pp. 543-554.

14. R. J. Bouquet, "Cannabis", *Bulletin on Narcotics* (United Nations publication), vol. 2, No. 4 (1950), pp. 14-30.

15. L. Grlic, "Study of some chemical characteristics of the resin from experimentally grown Cannabis from different origins" (ST/SOA/SER.S/10).

16. B. J. Taylor, J. D. Neal and T. A. Gough, "The physical and chemical features of *Cannabis* plants grown in the United Kingdom of Great Britain and Northern Ireland from seeds of known origin, part III: third and fourth generation studies", *Bulletin on Narcotics* (United Nations publication), vol. 37, No. 4 (1985), pp. 75-81.

17. J. E. Pitts, J. D. Neal and T. A. Gough, "Some features of Cannabis plants grown in the United Kingdom from seeds of known origin", *Journal of Pharmacy and Pharmacology*, vol. 44, No. 12 (1992), pp. 947-951.

18. R. Mechoulam, ed., *Marijuana, Chemistry, Pharmacology, Metabolism and Clinical Effects* (New York, Academic Press, 1973).

19. G. Nahas, "Symposium on marijuana: Reims, France, 22-23 July 1978", *Bulletin on Narcotics* (United Nations publication), vol. 30, No. 3 (1978), pp. 23-32.

20. P. B. Baker and others, "Determination of the distribution of cannabinoids in cannabis resin using high performance liquid chromatography", *Journal of Analytical Toxicology*, vol. 4, No. 3 (1980), pp. 145-152.

21. P. Mura and A. Piriou, *Le cannabis: alcool, médicaments, stupéfiants et conduite automobile* (Paris, Elsevier, 1999), p. 61.

22. M. A. ElSohly and others, "Potency trends of Δ9-THC and other cannabinoids in confiscated marijuana from 1980-1997", *Journal of Forensic Sciences*, vol. 45, No. 1 (2000), pp. 24-30.

23. L. King, C. Carpentier and P. Griffiths, *European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) Insights No. 6: an overview of cannabis potency in Europe* (Luxembourg, Office for Official Publications of the European Communities, 2004), pp. 30-31.

24. P. Mura and others, "L'augmentation des teneurs en delta-9 tétrahydrocannabinol dans les produits à base de cannabis en France: mythe ou réalité?", *Annales de Toxicologie Analytique*, vol. 13, No. 2 (2001).

25. C. Cristofol and others, "The cannabinoid content of marijuana samples seized in Greece and its forensic application", *Forensic Science International*, vol. 95, No. 2 (1998), pp. 153-162.

26. P. B. Baker and others, "Variation in the THC content in illicitly imported Cannabis products − Part II", *Bulletin on Narcotics* (United Nations publication), vol. 34, Nos. 3-4 (1982), pp. 101-108.

27. S. A. Ross and M. A. ElSohly, "CBN and Δ9-THC concentration ratio as an indicator of the age of stored marijuana samples", *Bulletin on Narcotics* (United Nations publication), vol. 49, No. 1, pp. 139-147.

28. R. Razdan, contributor, *Progress in Organic Chemistry*, W. Carruthers and J. K. Sutherland, eds., vol. 8 (London, Butterworths, 1973).

29. R. Mechoulam, N. K. McCallum and S. Burstein, "Recent advances in the chemistry and biochemistry of cannabis", *Chemical Reviews*, vol. 76, No. 1 (1976), pp. 75-112.

30. R. Mechoulam, P. Braun and Y. Gaoni, "A stereospecific synthesis of (-)-Δ^{1}- and (-)-$\Delta^{1(6)}$-tetrahydrocannabinols", *Journal of the American Chemical Society*, vol. 89, No. 17 (1967), pp. 4552-4554.

31. R. Mechoulam, P. Braun and Y. Gaoni, "Syntheses of Δ^{1}- tetrahydrocannabinol and related cannabinoids", *Journal of the American Chemical Society*, vol. 94, No. 17 (1972), pp. 6159-6165.

32. U. Avico, R. Pacifici and P. Zuccaro, "Variations of tetrahydrocannabinol content in cannabis plants to distinguish the fibre-type from drug-type plants", *Bulletin on Narcotics* (United Nations publication), vol. 37, No. 4 (1985), pp. 61-65.

33. A. Ohlsson and others, "Cannabinoid constituents of male and female *Cannabis sativa*", *Bulletin on Narcotics* (United Nations publication), vol. 23, No. 1 (1971), pp. 29-32.

34. P. S. Fetterman and others, "Mississippi-grown *Cannabis sativa* L.: preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex and plant part", *Journal of Pharmaceutical Sciences*, vol. 60, No. 8 (1971), pp. 1246-1249.

Section II. Drug trafficking and interdiction

Establishment of an operational system for drug profiling: a Swiss experience

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ABSTRACT

The present article describes the profiling process developed at the Institute of Forensic Science of the School of Crime Sciences of the Faculty of Law at the University of Lausanne. The technique is oriented towards an operational approach that can be applied directly by drug units of local law enforcement authorities.

The background of the development of that technique and issues relating to data sources are outlined. Analytical, statistical and computerized methods for detecting, managing and visualizing linkages are examined in the context of drug profiling. Harmonization of methods and operational use of links are discussed and explained using examples. Finally, adequate communication of forensic information/intelligence is explored as an area of development.

This endeavour has helped demonstrate the enormous potential that linking seizures made in different regional markets has for police investigations.

The next stage is to focus on implementing this model in a more systematic manner and, if possible, at the national level and even the international level. That harmonization of methods should be pursued in order to maximize the potential of the detected linkages.

In conclusion, links established through profiling, combined with traditional information, can be utilized to better understand the market's structure and implement medium- and long-term investigation strategies.

Keywords: drug profiling; forensic intelligence; harmonization; contextualization; intelligence-led approach; police data integration

Background

There are many possible strategies to combat drug trafficking, focusing on preventive or enforcement action against trafficking networks at every stage in the process, from drug production to drug distribution and consumption in local markets. For law enforcement authorities, that means selecting the most effective operational methods and strategies given available resources. An

intelligence-led approach bases action choices on sound knowledge of the criminal mechanisms involved, obtained through the structured and systematic processing of available data.

That approach requires the design and implementation of information processing methods to provide intelligence. The first stage involves gathering and organizing relevant data from human and electronic sources. Once that information has been collated in databases, it is interpreted to produce useful hypotheses and formulate recommendations that aid decision-making [1].

The considerable quantities of drugs seized from traffickers yield data collections that can form a useful basis for such procedures. However, one key preliminary issue remains largely unresolved: what intelligence can be derived from those illicit substances?

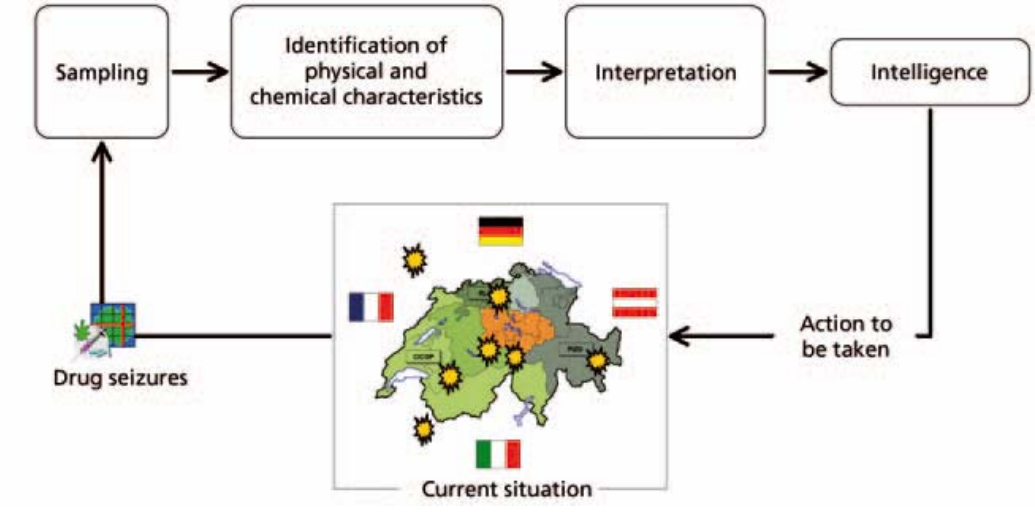
Swiss profiling experiences seek to answer that question by systematically recording the physical and chemical characteristics of various seized drugs. The resulting data sets are interpreted to produce useful intelligence on drug trafficking from both the operational and the strategic perspectives.

Typical legal procedures require the analysis of qualitative and quantitative aspects of the banned substances. Such analysis is generally interpreted as the evidence on which the justice system bases itself in formulating its decisions according to the laws in force. An intelligence-led approach is more ambitious and expands the possible ways of exploiting data sets on seized drugs.

Process description

A general representation of an intelligence process based on drug seizures is shown in figure I. Several organizations cooperate in carrying out the process according to a division of tasks integrating each participant's responsibilities. For example, police officers and border guards seize drugs, transfer them to sometimes distant laboratories, which analyse the samples and interpret the results. All those operations take place within a legal and economic context that can be very limiting and which often varies from jurisdiction to jurisdiction.

Figure I. Integration of intelligence



To be useful, intelligence has to be transmitted in real time, that is, within a time period appropriate to the pace of developments of the crime phenomena being dealt with. Strategic analysis, which is used to evaluate the extent, patterns and impact of trafficking, accommodates delays in information availability more easily than does operational analysis, where intelligence can be used to guide an investigation in progress. Thus, in dispatching seized drugs to laboratories for identification of physical and chemical characteristics, speed is crucial. Once the drugs are in the possession of the laboratory or laboratories, physical and chemical analyses can be carried out to isolate the characteristics of the seized substances. However, the entire seizure cannot be systematically analysed, since such an operation would be far too costly and time-consuming. Thus, only small quantities (samples) are selected for analysis. The measured quantities (the profile) will then be assumed to be representative of the characteristics of the entire seizure. That operation, called "sampling", follows strategies based on statistical considerations.

It would not be feasible to centralize all processing activities in a single laboratory with the aim of creating an international databank. Thus, it is necessary to ensure that all entities involved use the same methods in the same manner, with similar facilities so that the results obtained will be comparable. The quest for harmonization does not stop there, given the many parameters to be taken into account and the sensitivity of the methods applied.

The data are then arranged in a memory, usually in an electronic format. Seizures are not stored individually but are collated and grouped in classes according to similarities of the profiles identified. How are the classes defined and interpreted? The links between seizures can be of different types and of different degrees: do those links mean that the drugs are from the same batch, are distributed through the same networks or come from the same region?

The question of the representativity of collected data warrants particular attention. Are such data more indicative of police activity or of the real characteristics of drug trafficking? A clear description of the conditions in which seizures were made can provide details to be incorporated into interpretative analysis.

Once the memory is established, it can be used to generate intelligence. For that purpose, computerized techniques for recognizing patterns in large quantities of data can be applied. Such patterns should draw attention to specific data sets of special interest. For example, a compound may appear in an unusual quantity during a particular period. Those patterns, which may be concealed by the large quantity of recorded data, can provide valuable intelligence on trafficking trends. The potential offered by such techniques is now being systematically studied.*

*Swiss National Science Foundation, Recognition of Patterns in Forensic Case data: the Use of Chemical/Physical Signature of Illicit Drug Seizures in an Intellience Perspective, project No. 105211-107862.

The potential of compiling information in such databases is still not entirely appreciated. Combining intelligence with information from police investigations and strategic analyses gives an indication of the possible applications of those data sets.

Data sources: the Swiss context

Switzerland is divided into 26 cantons, each with a law enforcement authority that is largely independent in the field of drug control. In addition, two municipal police authorities (Zurich and Lausanne), with extensive legal authority, conduct investigations in their jurisdiction. The Federal Criminal Police, which in theory has sole responsibility for drug trafficking as part of organized crime, constitutes a further entity within this highly fragmented system.

Since 1998, the Institute of Forensic Science (IPS) of the School of Crime Sciences of the Faculty of Law at the University of Lausanne, through its specialist drug analysis team, has been raising police awareness of the advantages of physical and chemical profiling. Operational intelligence for investigators and strategic intelligence for decision makers are provided, in addition to qualitative and quantitative data required by judicial officers to determine the crimes committed. Police quickly realized the value to investigations offered by this new form of intelligence.

Drugs seized in the cantons of Geneva, Vaud, Neuchâtel, Jura and Tessin by the Federal Criminal Police and the Lausanne city police are analysed in the IPS laboratories. An initiative carried out in coordination with the Scientific Forensic Service of the Zurich City Police resulted in a representative analysis of seizures of amphetamines and derivatives over a large area (see figure II).

The data thus relate to western and southern Switzerland, as well as to the Zurich area for amphetamine-type stimulants and derivatives. The lack of a centralized structure or network of laboratories using harmonized methods is regrettable. The lack of coordination partially conceals linkages, making a nationwide picture impossible—a problem known as linkage blindness. However, the data are sufficiently representative that they can be used in an operational system for the systematic analysis of seized drugs in order to provide law enforcement officials with concrete operational intelligence within a limited area. Based on acquired experience a more ambitious process, covering a wider area can be envisaged.

Each year, 1,600 analyses are carried out for some 260 cases on average. The drugs most analysed are heroin, followed by cocaine, amphetamine derivatives and cannabis. Various other drugs, such as amphetamine, khat, psilocybine and lysergic acid diethylamide (LSD) are seized only occasionally in the areas concerned.

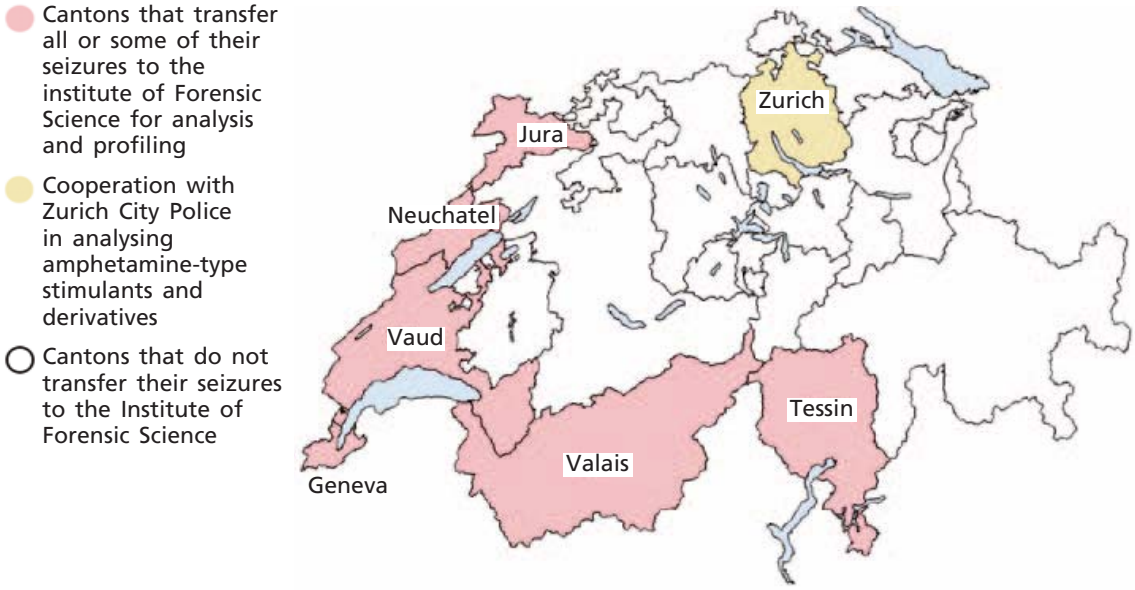
Submission of seizures

The standard procedure for the submission of seizures (see figure III) generally begins with the forensic department (investigating technical officers) of the

canton concerned. The department photographs the containers, collects relevant trace evidence such as DNA and fingerprints, notes the gross and net weights of the seizure and takes samples using an ad hoc protocol.

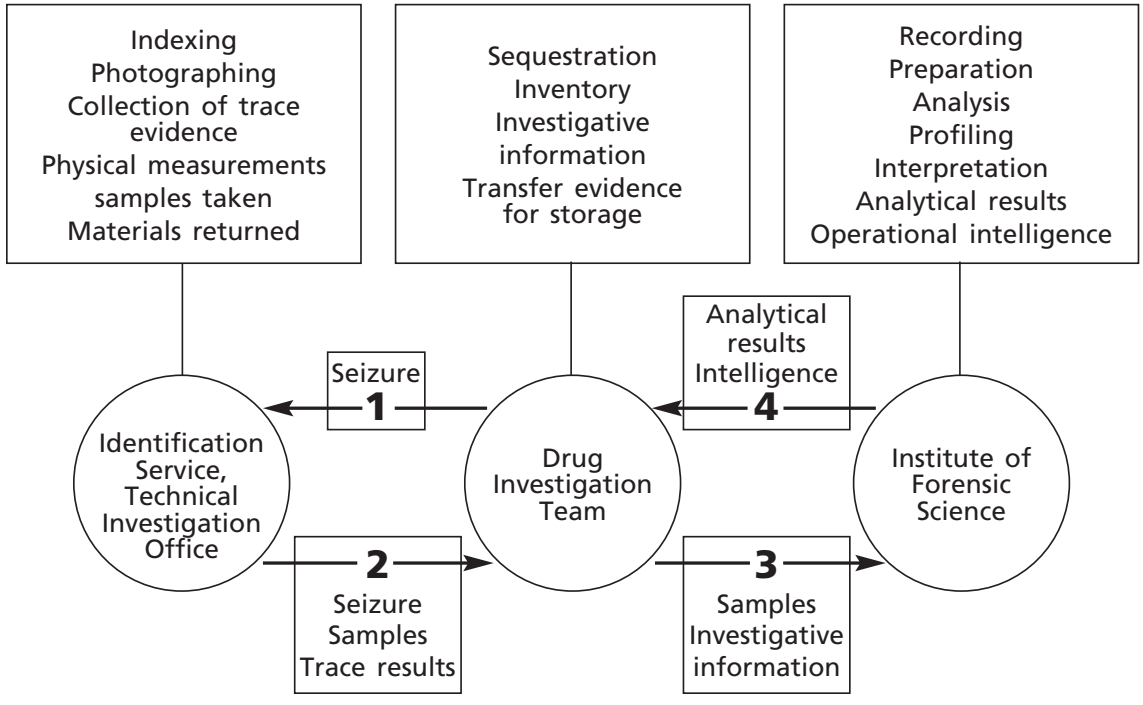
The samples and their transfer record are then forwarded by the inspectors in charge of the case. This procedure makes it possible to obtain investigative information, such as dates and place of seizure, which are stored in the memory and subsequently incorporated into the process of interpretation.

Figure II. Cantons submitting seized drugs to the Institute of Forensic Science



Note: The boundaries shown do not imply official endorsement or acceptance by the United Nations.

Figure III. Standard procedure for the submission of samples



Handling and analysis of seizures by the laboratory

Sampling

Crime investigation officers and forensic scientists are often faced with very large quantities of seized drugs. Only a limited quantity—ideally, the most representative sample of the seizure—is selected for analysis. However, if the sampling does not reflect variations that exist in the seizure, it is highly unlikely that the information on the composition of the seizure will be representative. Thus, the role of sampling should in no way be underestimated.

Under Swiss law and in the procedure described, sampling and the ensuing analysis must meet two requirements. First, from a legal viewpoint, the drug has to be identified and quantified, because those two parameters are important for determining the gravity of the offence committed, pursuant to Swiss federal law on narcotic drugs and psychotropic substances.

Secondly, from an intelligence viewpoint, it is important to examine the homogeneity of the seizure with respect to its constituent chemical and physical profiles. Mathematical tools available for this purpose can be divided into two main categories. The so-called frequentist approach gives an estimate of the quantity of drug based solely on the result of the analysis. The Bayesian approach is more comprehensive. It incorporates a set of information on the samples, including a priori homogeneity and the results of analyses, that makes it possible to assess the probability that the remainder of the seizure will contain a certain proportion of drug [2].

Economic factors can play an important role in the sampling process, because analyses are not free. A compromise has to be found between the information required and the expenses incurred in acquiring that information in collaboration with judicial and investigating officers.

The importance of the sampling stage will be discussed below, taking into account that in practice, choices are often made on the basis of the specific situation faced.

Two examples, which have been simplified and rendered anonymous, illustrate how sampling during the investigation affects laboratory analysis.

In case A, 12 kilograms of heroin were seized at the Swiss border. An investigator of the Federal Criminal Police suspected that the person arrested at the time of the seizure belonged to a particular criminal network. The investigator wished to compare the chemical profile of the 12 kilograms of heroin with the profiles of other seizures previously forwarded by him to IPS for analysis. In this case, a member of the laboratory went to the site of the seizure to collect a small sample quantity, the canton authorities being required to safeguard the evidence. The seizure consisted of 24 blocks of about 500 grams each. The optimum method would have been to drill into the blocks at several places chosen at random (core sampling). However, in view of the number of blocks and the restrictive conditions for taking samples—time was limited and the operation was carried out in a basement—it was decided to break each block in two and remove a piece across the entire width of the block. All pieces were homogenized by crushing and then analysed.

In another case a seizure of approximately 600 tablets took place. In this case, the criminal identification department of the canton carried out the sampling itself and forwarded 60 tablets, that is, about 10 per cent of the total quantity. From that first sample, only 10 tablets were selected for analysis owing to cost considerations. In such cases, if the physical and chemical characteristics of the 10 samples proves similar, the analysis ends there. If, however, several profiles are identified, the opportunity remains of increasing the number of samples analysed. That permits a greater representativity of the composition of the heterogeneous seizures.

The above-mentioned examples show that constraints influence the sampling process and that choices are made on a case-by-case basis. The analysis laboratory has little or no direct control over such law enforcement, practical, time and cost constraints. However, contact and communication between the laboratory and the relevant authorities can raise awareness among those who collect samples.

Determining variables for profiling purposes: criteria for selecting a method

Definition of a variable

Drugs contain not only their illicit compound, such as cocaine or 3,4 methylenedioxymethamphetamine. When a sample is analysed, it is possible to detect several other types of compounds, referred to as variables, which are mixed with the illicit drug in the course of its history, from production to seizure. Such compounds come from the living plant, synthesis (such as precursors and solvents) and the addition of cutting agents. In addition to those chemical variables, there are the physical variables, such as logos and the physical appearance of samples and their packaging.

The profile of a sample consists of the ensemble of those variables. Thus, there are several profile types (chemical, physical or mixed), resulting in the complexity, as well as the potential, of intelligence derived from drug seizures.

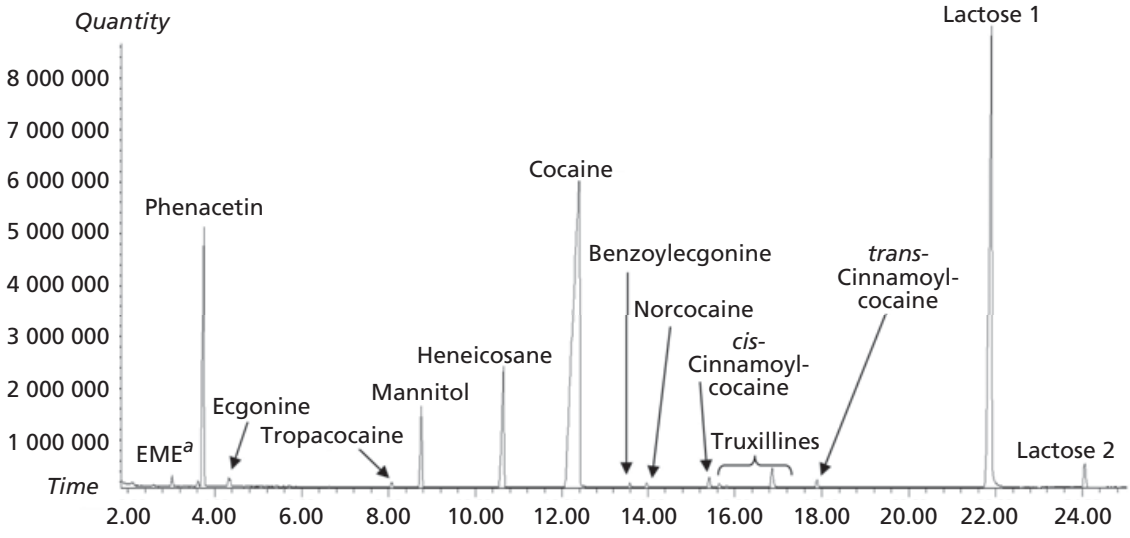
Selection criteria

Development efforts to date have focused primarily on chemical variables, which are presumed to have good intelligence potential. Also, the traditional function of commissioned laboratories has been to determine the active ingredient content. That is one of the chemical variables to be obtained from samples of heroin (diacetylmorphine) and cocaine (methylbenzoylecgonine). As a result, the analysis of such variables is an integral part of established expertise.

Laboratories have several analytical methods at their disposal to identify those variables. The aim, obviously, is to find the analytical method that produces the desired information as simply, as quickly and as economically as possible.

Gas chromatography has the advantage of producing maximum information from a single analysis, whereas other methods require a series of laborious and more costly operations and yield little relevant additional information. The analytical result of gas chromatography takes the form of a chromatogram, such as that in figure IV.

Figure IV. Typical chromatogram of a cocaine sample



[a]Ecgonine methylester.

Selection of variables

The selection of useful variables is based on several criteria:

- The identified variables must be present in each sample.
- Their concentration must be sufficient to allow chromatography and differentiation from background noise.
- Their measurement must be reproducible and repeatable.
- They must be sufficiently numerous and of variable quantities in order to group samples possessing similar characteristics, while differentiating uncorrelated samples.

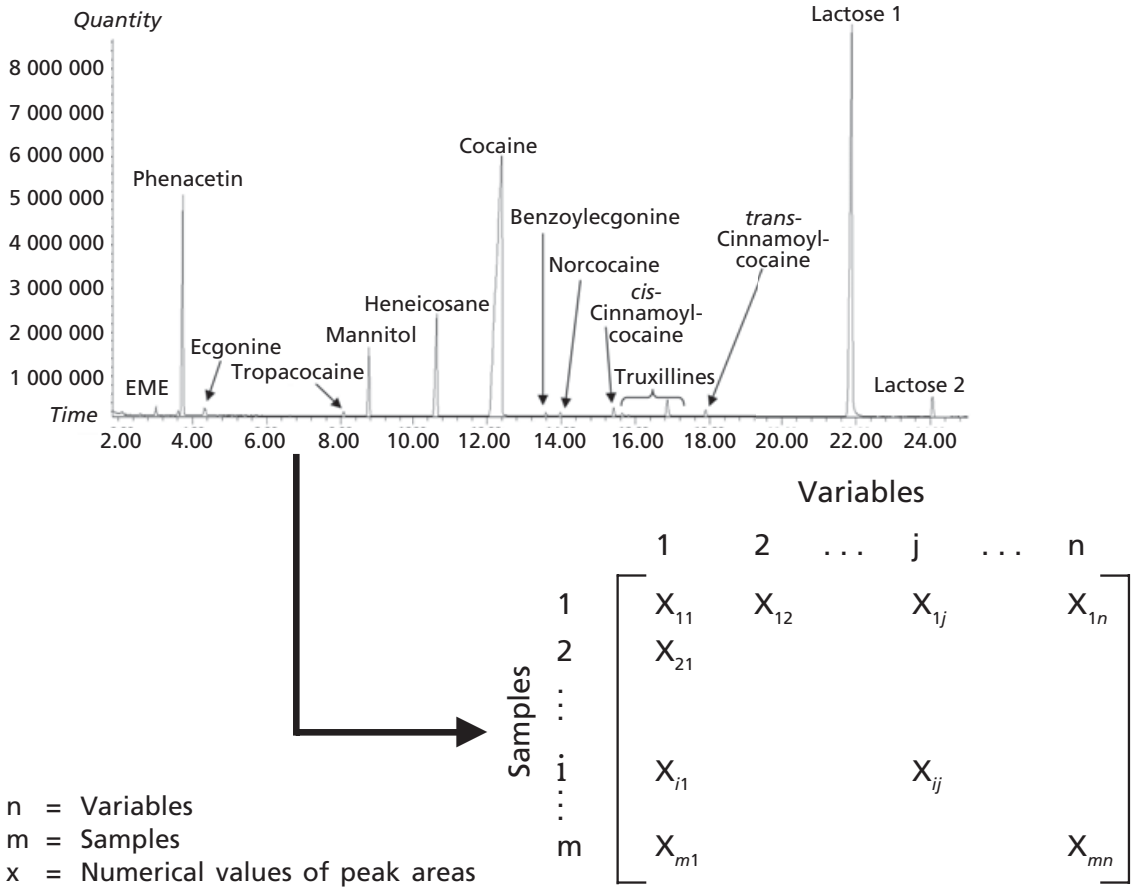
The relative quantity of variables is measured for each sample. In this case, as seen in figure V, the structure of the data can be represented as a matrix whose columns represent the peak area values of the selected variables and whose rows represent the different samples in the database. That matrix of numbers will be used for the mathematical operations described below.

Quality of results

Controlling the quality of results obtained is essential to be able to have comparable results in a single laboratory and among various laboratories. It is

essential to ensure that results are reproducible. A result obtained for a sample must be the same as one obtained one or two months later. The testing procedure also monitors the analytical quality of equipment used for analysis, as well as the comparison methodology used, including analyses of controls, test samples and standard solutions. Any defect potentially causing discrepancies in the profiling process is thus detected, bringing an immediate response from the analyst. That procedure ensures the reproducibility of the results entered in the memory of the system. The same monitoring procedures must also be implemented at all laboratories involved in order to harmonize their results.

Figure V. Extraction of data on profiling variables



Harmonization of the analytical method

Comparability of results among laboratories

As stated above, the system's current weakness lies chiefly in the fact that a drug seizure analysed by laboratory X cannot be compared with a seizure analysed in laboratory Y unless the profiling methodology used by laboratories X and Y is identical. However, various initiatives have been introduced in recent years to harmonize the collation of information with linked physical and chemical characteristics. For example, the Fifth European Union Framework Programme

project for the development of a harmonized method for the profiling of amphetamines, [3, 4] supported by the Standards, Measurement and Testing Programme of the Directorate-General for Research of the European Commission, and project No. 97.0487 of the State Secretariat for Education and Research, brought together seven countries seeking to harmonize the profiling of amphetamines. Other initiatives include the Collaborative Harmonization of Methods for the Profiling of Amphetamine-type Stimulants (CHAMP) project for the period 2004-2006 on the profiling of Ecstasy and methamphetamines, with seven participating countries; the CASTEL project (2003-2005)* on the profiling of cocaine seized in the French-Swiss cross-border area; and the International Drug Profiling Conference, organized under the auspices of the Drug Enforcement Administration of the United States of America which dealt with heroin and Ecstasy.

Such projects form an indispensable basis for method harmonization, which is essential for the effective application of profiling in combating drug trafficking. IPS is involved in those projects and intends to use the experience gained to undertake a similar initiative encompassing the entire territory of Switzerland.

To return to the example of case A, cited above, the heroin blocks had been analysed by a laboratory in the canton of seizure. The lack of an operational programme for method harmonization led the investigator to have the substance analysed a second time in order to compare it with previously selected samples, that is, samples taken from other seizures made within the same criminal network.

In case B, a cocaine seizure took place in a canton that transfers all its samples to IPS. A person from the suspect's family living in the south of France was arrested by the French police for possession of cocaine. When that information reached the Swiss investigators, they asked for the two seizures to be compared. The cocaine seized in France was forwarded so that a comparison could be carried out.

Conceptual interpretation model

Concept of linkage and chemical and physical links

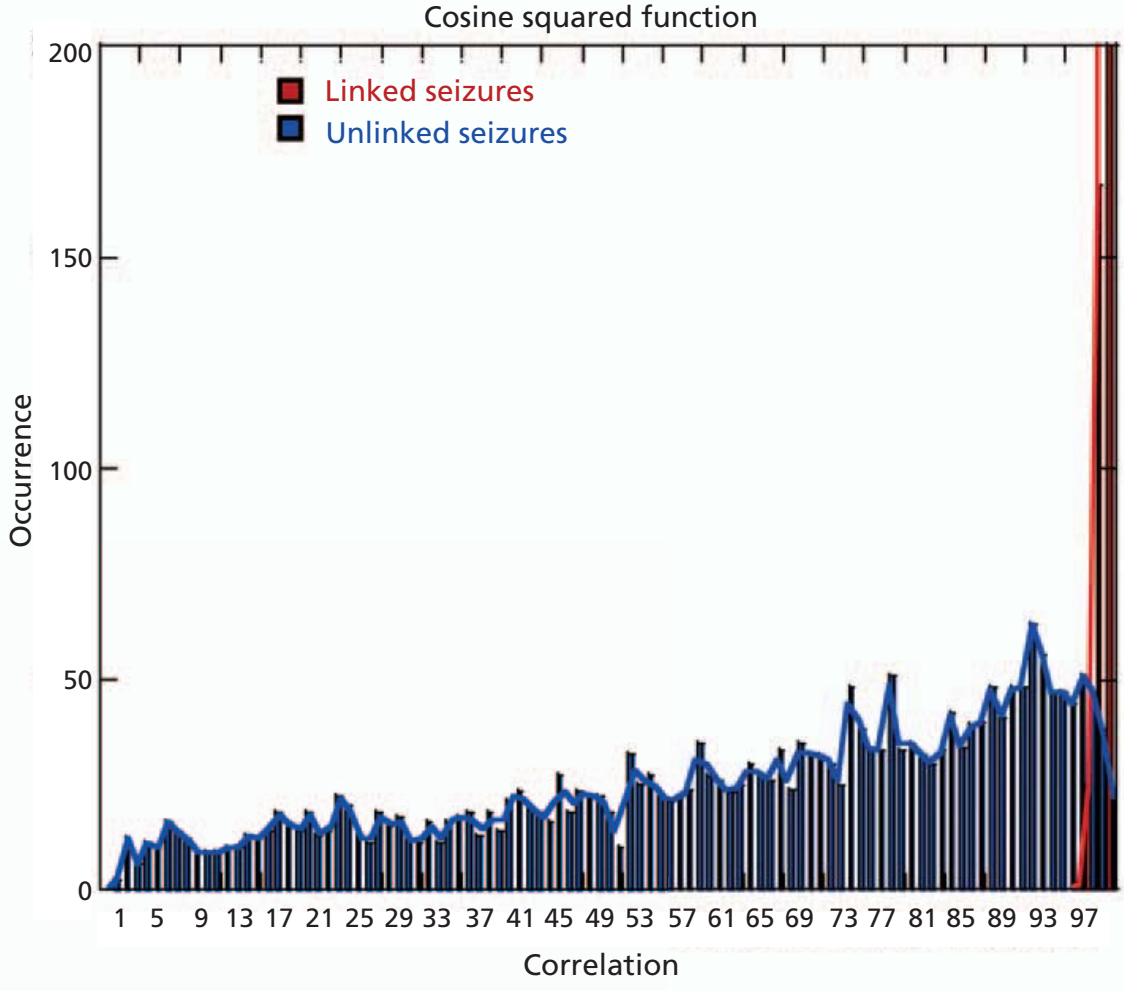
Various types of links can be established between different drug seizures. [5-7] Linkages can be made through chemical and physical profiling or through circumstantial information from police investigations. A chemical link is established when two samples are judged to have similar profiles according to previously defined criteria, as described below. Under the methodology currently used at IPS, a chemical link is defined as the most elementary level indicating a single production batch. Because seizures taken directly from clandestine laboratories are not available (the laboratory's technique is aimed primarily at heroin- and cocaine-type drugs), the intra- and inter-variability study was carried out using large-scale seizures.

*French-Swiss project, entitled "Apport scientifique à la lutte contre le phénomène transfrontalier de stupéfiants: mise en réseau de l'information", a part of the INTERREG III project.

To operationalize the concept of linkage, various mathematical distance-measuring and correlation methods were assessed. That research indicated that the correlation measurements (Pearson correlation and the cosine correlation) had the best potential for differentiating samples belonging to the same seizure from samples taken from seizures with different chemical profiles.

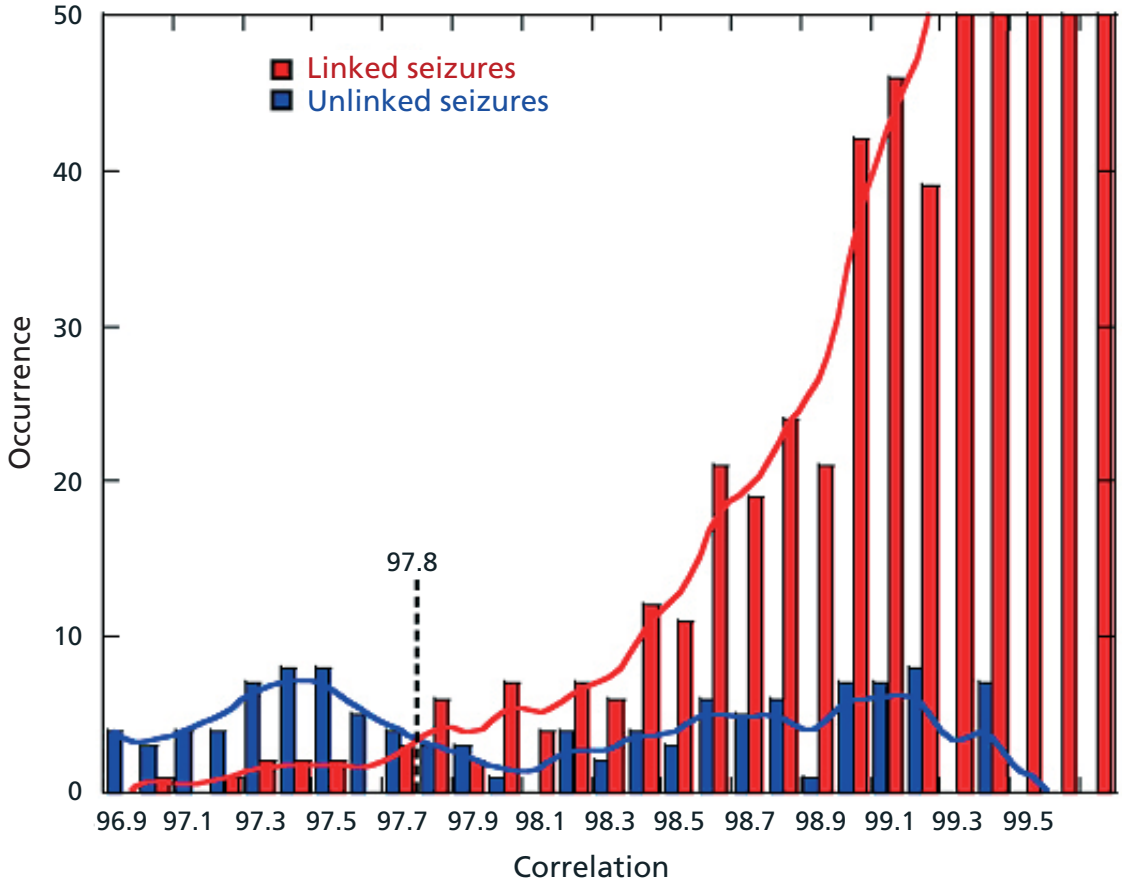
The strategy for detecting linkages was defined as follows [8, 9]. Samples taken from a common source were selected and compared with each other. The common source (the production batch) was defined as samples taken from a single seizure and having similar chemical profiles. That procedural step makes it possible to evaluate the intra-variability of a production batch. Inter-variability is evaluated by selecting samples that have been taken from different seizures and that have different chemical profiles and then carrying out the same comparison procedure that was used for the first group. Figure VI shows the results of those comparisons.

Figure VI. Correlation values of inter-variability between unlinked seizures and correlation values of intra-variability



Based on those observations, a threshold value can be extracted, giving an indication of whether two samples are from the same production batch.

Figure VII. Expansion of curves of correlation values obtained for linked and unlinked samples using cosine squared function



Grouping according to chemical class is the most effective way of recording samples with similar chemical profiles. That requires criteria for measuring similarity in order to determine whether two drug seizures possess a degree of similarity meeting the threshold fixed by the scientist according to his or her initial hypotheses. In the case being examined, the initial hypothesis stated that analysing a large drug seizure makes it possible to establish the statistical criteria for determining whether a sample belongs to a given production batch. The chemical class can provide information on whether seizures belong to the same production batch or the same distribution network. Such an approach can be applied to any type of organic or inorganic variable used for profiling analysis. Samples with similar logos, for example, can be grouped in the same physical class, based on their physical profile. However, without any samples of known origin, the level of linkage cannot be located without certainty.

Combining those classes with related police data makes it possible to corroborate intelligence derived from detected linkages. That stage is described below.

Database

The technique adopted is based on the systematic profiling of all drug samples entering the Institute [10] (focus on intelligence purposes). This

procedure is preferred to case-to-case comparison (focus on court purposes), which is the method most frequently adopted by laboratories conducting drug profiling work.

The case-to-case comparison process directly responds to judicial requirements, in cases where comparison assignments are clearly defined by the judge or investigators. In those cases, seizures to be compared are pre-selected, and no comparison with a comprehensive database is performed, unlike the technique adopted. The existence of a comprehensive database makes it possible to search for links between samples stored in the database memory in order to reveal unsuspected connections. The memory groups together cases that have been previously analysed and organized according to predefined physical and chemical classes.

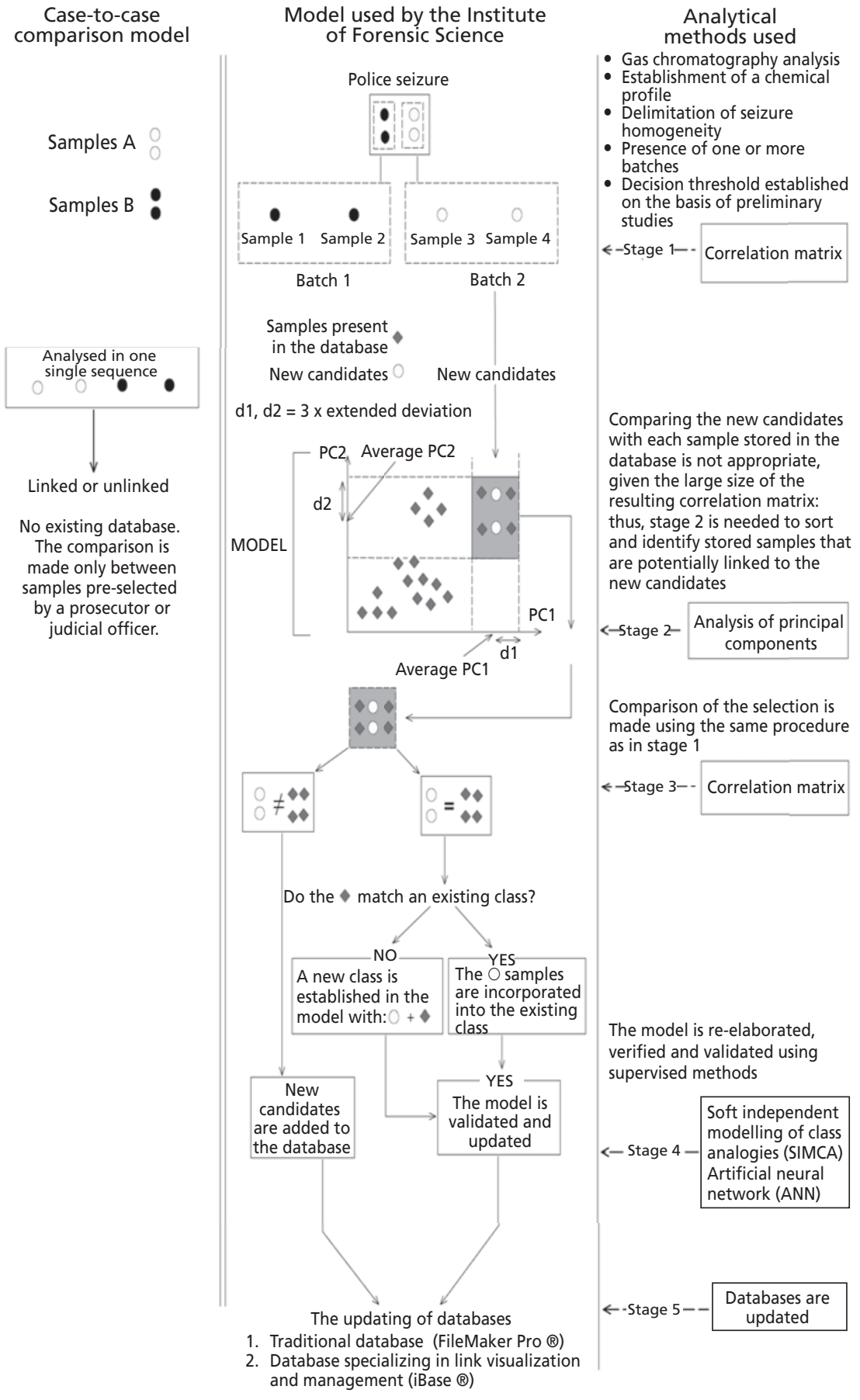
Figure VIII below, represents the comparison procedure carried out for each new entry. First, the characteristic variables of the drug's profile are entered in the system's memory, whose structure is provided by a database developed using FileMaker Pro®. The first step is to determine whether the seizure is homogenous (belonging to a single batch), or inhomogeneous (made up of several separate batches). In the second step, samples from each batch are compared with the database memory in order to select the samples contained in the memory that have chemical or physical characteristics similar to the new candidates for comparison. Principal component analysis is used to make a preselection. The pre-selected samples are then compared in detail using correlation measuring methods to assess similarity. The third step is sample-by-sample comparison enabling the experts to determine whether a link among the target samples can be established using the method described above [11].

The analysis carried out in the third step has three possible outcomes. In the first, new candidates have no link with samples in the memory; in that case, the new candidates are added to the memory, which is updated. In the second, samples compared are linked to samples in the memory that already belong to a chemical class, the new samples are classified as part of that specific chemical class. In the third outcome, if new candidate samples show links with samples that are in the memory but do not belong to a chemical class, a new chemical class is established.

Classification of samples in an existing chemical class entails a validation phase, the fourth step, which is carried out using supervised statistical methods such as soft independent modelling of class analogies or artificial neural networks. Those methods enable the various chemical and physical classes to be modelled. That way, new candidates can be compared with the models to confirm whether they belong to an existing chemical class. If a candidate is validated, it is assigned to the corresponding class, and the model is redeveloped. The same process for updating is carried out when a new class is identified.

Finally, in the fifth step the different database types, that is, the databases for data storage and specific databases used to manage and visually represent physical and chemical links, are updated to take into account the classifications made in the above-mentioned steps.

Figure VIII. Procedure for establishing chemical links: two different models



To return to the example of case A, cited above, the samples of the heroin seized at the Swiss border were analysed a second time, at IPS. They could then be compared with the samples from that case that had already been forwarded, analysed and stored in the memory. Those steps are not performed under the case-to-case comparison procedure used in other laboratories. Where analysis is to be performed, samples selected by the investigator should all be analysed at the same time.

Management, visualization and application of linkages

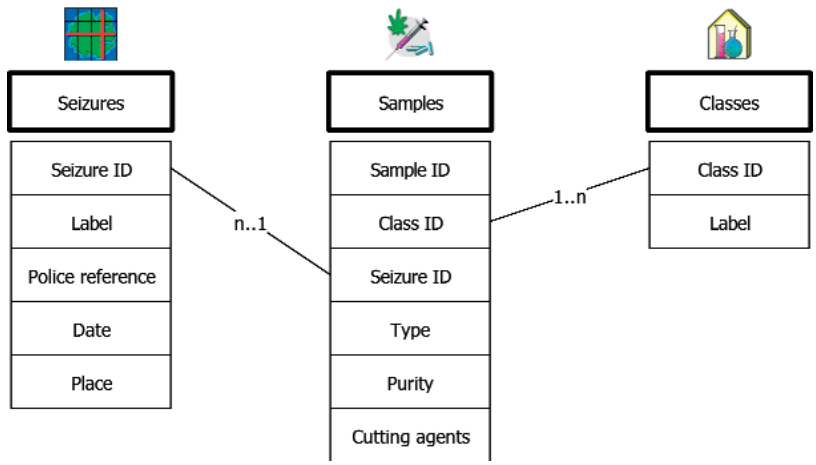
Tools for managing and visualizing links

Disseminating information on chemical links established by the procedure described is crucial and requires the rational and consistent management of such linkages [5]. The test is well performed using software specially designed to manage this type of data (iBase®) and display it (Analyst's Notebook®).

Database structure

Each sample is entered in the database, including details of the type of substance found, its purity, cutting agents and its chemical class, which consists of samples with the same chemical profile (see figure IX).

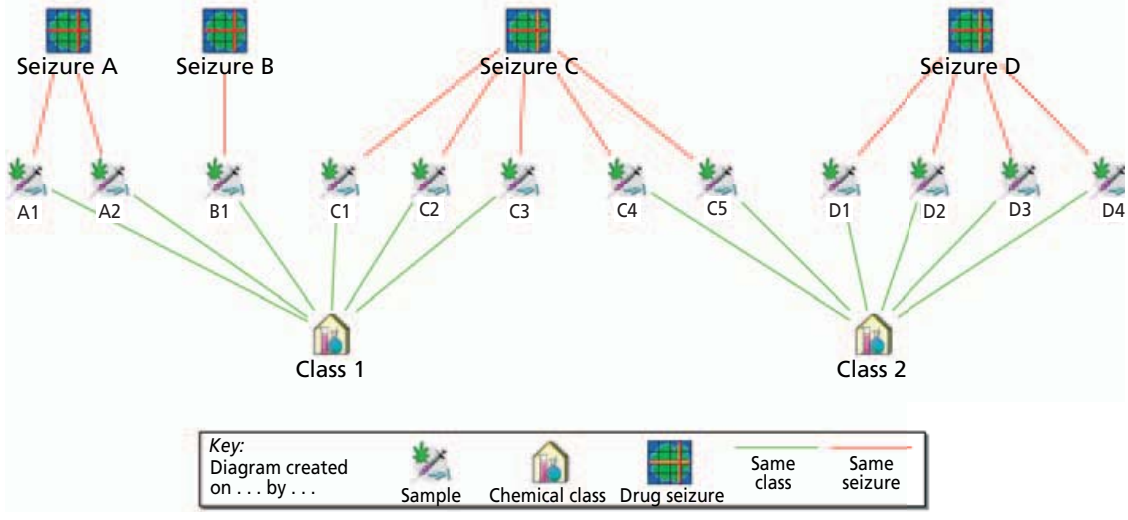
Figure IX. Database structure



The sample is the basic unit in the management of links. That basic level provides the basis for the general level which defines the sample's relationship to a seizure (circumstantial data) and to a chemical class (analytical and chemical data). Chemical classes are linked directly to the samples taken from a seizure and not to the seizure itself. A seizure can yield samples with different chemical profiles. In that case, samples are grouped accordingly and are linked to different chemical classes. Figure X shows how such relationships can be visualized using Analyst's Notebook, a software programme that has a dynamic,

bi-directional connection to the database. In addition to visualizing entities and their connections, the software automatically updates the database according to any modification made to the visual representation.

Figure X. Representation of linkages



Note: Seizure C forms two groups. Samples C1/C2/C3 and C4/C5 have different chemical profiles. The first group belongs to chemical class 1, and the second group to chemical class 2.

Seizures constitute another recorded entity. The information entered on seizures comprises, inter alia, the label given to the seizure, its police reference and the date and place of seizure.

Exploitation of linkages: inference structures

Clearly, grouping chemical and physical links according to class has great potential for describing phenomenon and series. It is, however, important to bear in mind that, because of the complexity associated with their entities, such databases cannot be used in the same way that automated fingerprint identification systems or DNA databases can [12]. Databases of the latter type are effective in identifying the source of trace evidence: for example, matching a suspect's DNA to the trace evidence found at the crime scene. In contrast, in a database for drug profiling, two samples with a similar chemical profile can be linked at any point from the production stage to the distribution stage.

Using links to corroborate police information

In the course of a police investigation of a drug distribution network, investigators obtain large amounts of circumstantial information. On the basis of those data, they infer and define links that may exist between different persons active within a distribution network. Linkages revealed using such traditional methods of investigation can be corroborated and even substantiated by the detection

of physical or chemical links, which can, in turn, be used to reveal previously undetected investigative links, as described below.

The example given in figure XI illustrates those observations. In case X, the investigator concluded that seizures A, B, C, D and E were part of the same criminal network. Those five seizures and the related investigative details constituted the police view of the trafficking network. Linkages detected through chemical profiling then revealed that seizures B and D were connected through seizure C, whose samples belonged to the same chemical classes as seizures B and D (see box 1 of figure XI). The investigator's view of the links between seizures B, C and D was thus corroborated by profiling.

That example demonstrates the importance of sampling. Seizure C produces samples belonging to three separate chemical classes. It is unlikely that a chemical class would have been identified if sampling had not been carried out. That omission would have resulted in a loss of information. For example, the chemical class in the middle of box 1 was shown to be linked to another seizure, thus providing a broader view of the trafficking network. If seizure C had not been sampled, that information would have been lost.

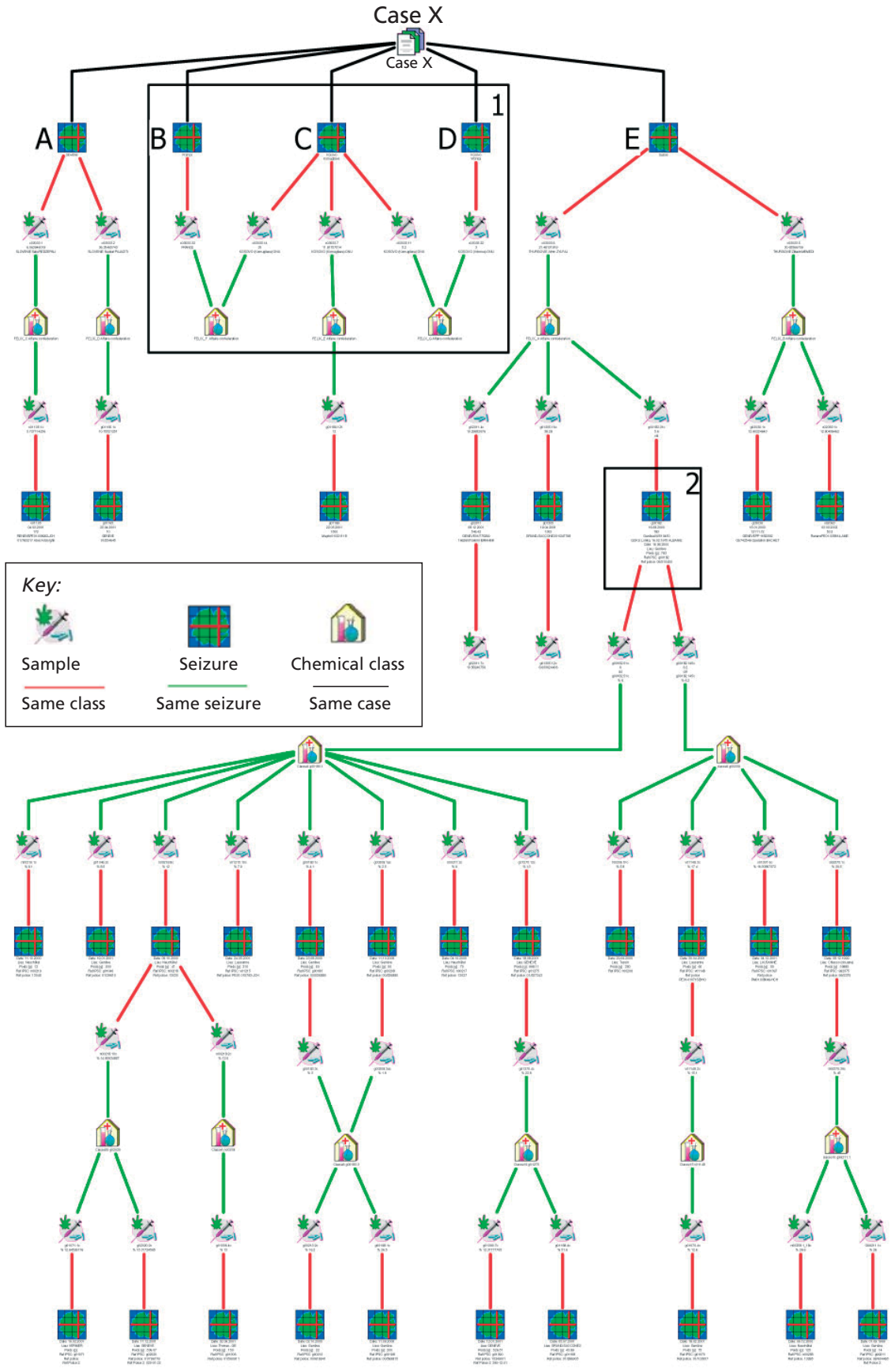
An example of the interaction between links obtained from chemical profiles and police data is represented in figure XII. Figure XII shows the links established by monitoring telephone calls through call billing data. An informer has notified police that the two suspects contact each other indirectly using telephone booths located at the buying and selling places frequented by them. Figure XII shows the number of calls and the call direction. The analysis of the telephone monitoring clearly corroborates the information given by the informer (see box 1 of figure XII), and the drug seizure analyses confirm that hypothesis, because they reveal that the investigated individuals were in possession of samples belonging to the same chemical class (see box 2).

The converse order of corroboration is also possible. Data from police investigations can link two chemical classes that were established to be different by the laboratory's analytical methods and criteria. Thus, the two sources of information complement one another in building a view of a network and its operation. The interaction of police investigation and drug profiling data enables the database memory, and the inferences that can be drawn from it, to be kept up to date. Interpreting the results obtained by investigators in the light of physical and chemical links is key to advancing the reasoning that produces valuable intelligence.

Example

In case 1, of Figure XIII, cocaine was found inside fans at the time of seizure A. It emerged that all the samples taken from that seizure belonged to the same chemical class, as did the samples from another seizure of fans, seizure B, which took place a few days later. The two sources of information, the packaging and the chemical class, matched. Six months later, a further seizure, seizure C, was made at a dealer's home. Samples from that seizure were shown to belong to

Figure XI. Investigation of a complex drug network



Note: Box 1 illustrates an example of corroborating investigative data. Box 2 illustrates how an important link provided by drug analysis leads to the prioritization of police investigation.

the same chemical class as the samples taken from the earlier seizures A and B. In the new case, because of its uniqueness, the packaging could be used as a marker of a drug distribution network. The fact that all samples from seizure C belonged to the same chemical class alerted the investigator to the fact that the same distribution network was probably active once again.

Figure XII. Integration of police data

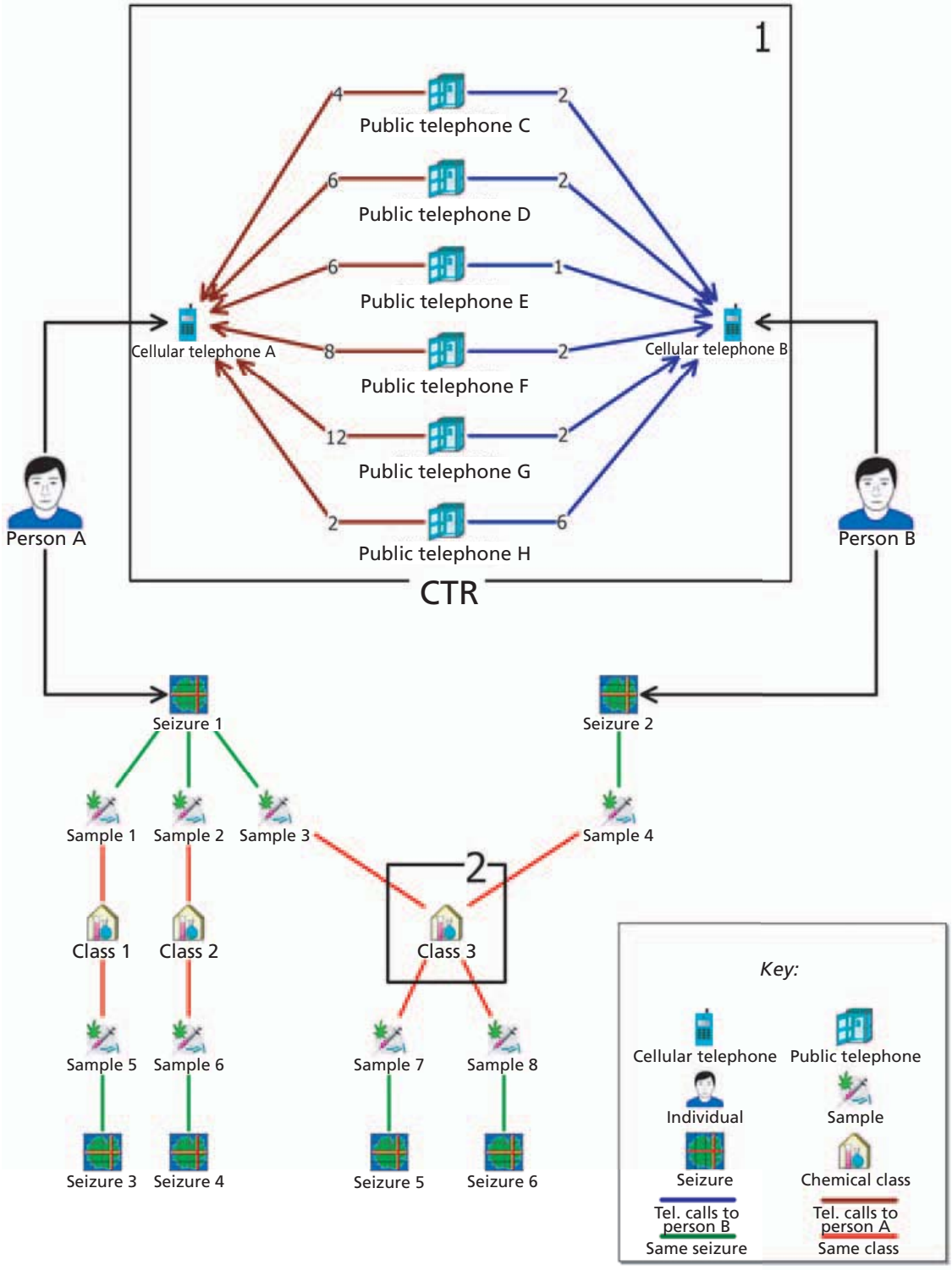
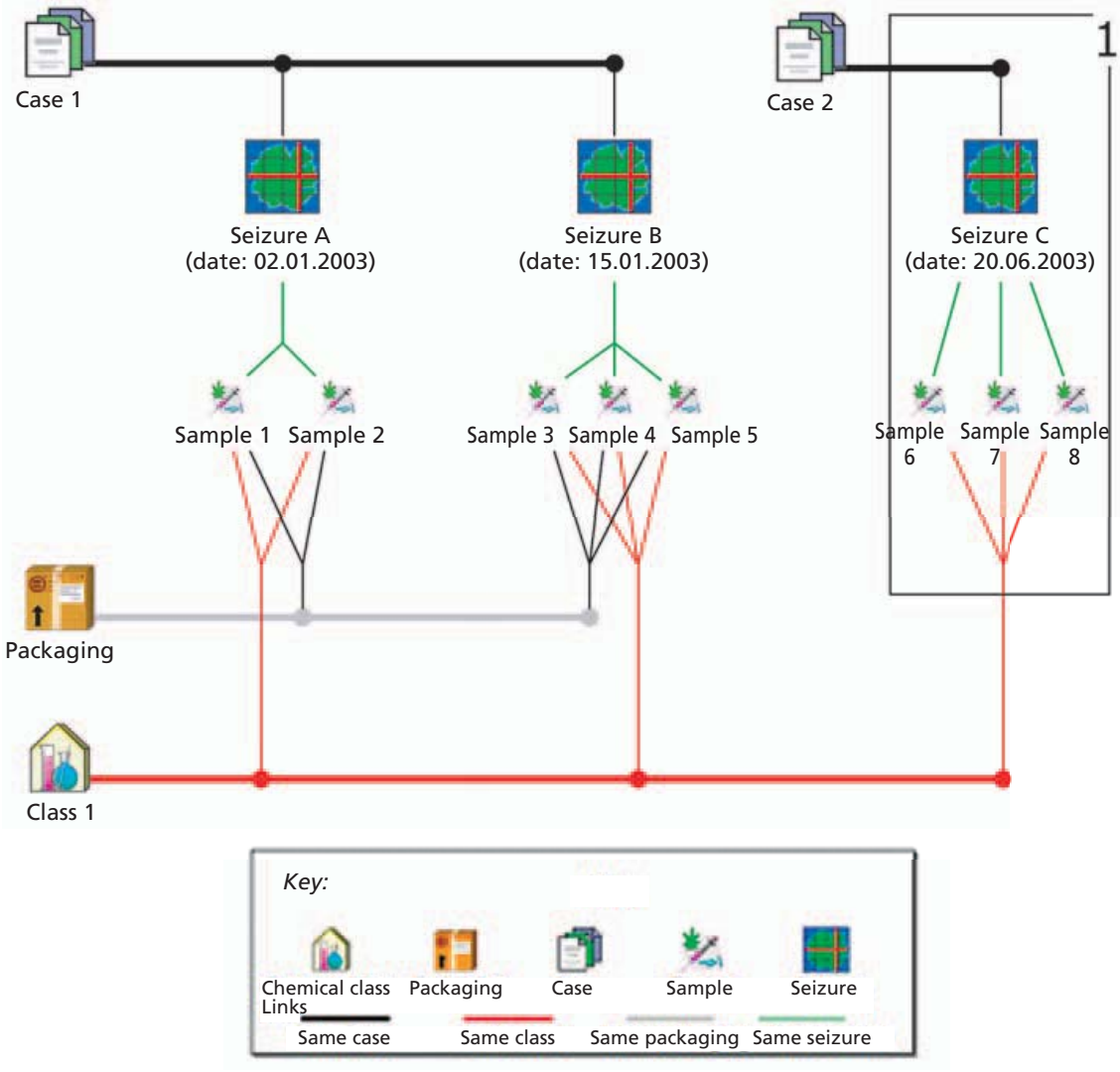


Figure XIII. Importance of investigative data for linking seizures over a six-month period



Using links to guide investigations and suggest priorities

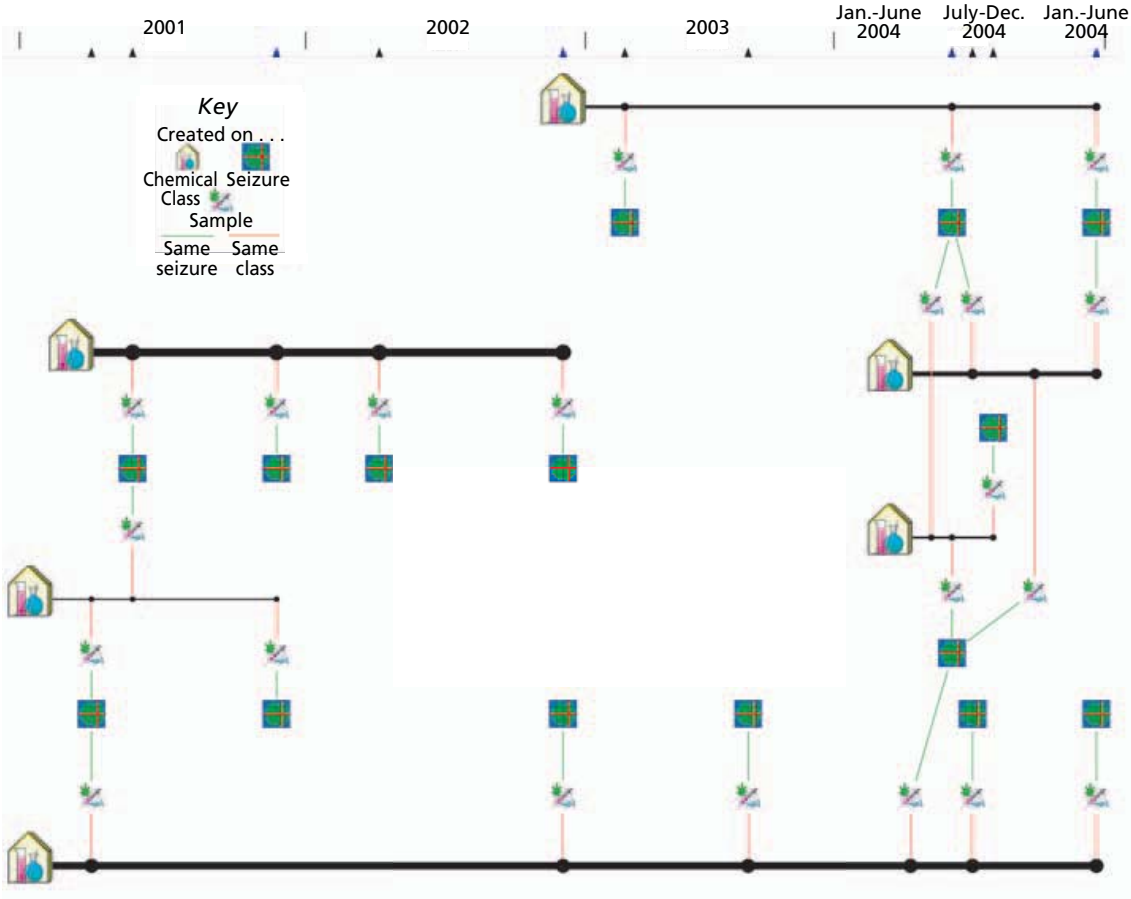
The visualization of links between several seizures can take an investigation in a previously unexplored direction. Establishing links can also lead to new investigations or decide the priorities for action when an individual is found to be linked to other individuals and plays a key role in a specific market. Links can uncover associations that are not detected by regular investigative methods owing to concealment efforts of users and traffickers.

Box 2 of figure XI highlights a seizure yielding several samples with various chemical profiles. The seizure is the only entity in that case that links the top and the bottom of the chart. Thus, the individuals connected to that seizure are of greatest interest, because they appear to play a key role in the overall case.

Estimating the extent of trafficking

Data on chemical profiles, including cutting agents, and physical profiles, including information specific to tablets, can be used to estimate the length of time a batch was distributed, its total size and its geographical distribution. Figure XIV shows the lifespan of different batches. The length of the horizontal lines is proportional to the time period during which the chemical class was observed, while the thickness of the horizontal lines is proportional to the quantity of the drug seized.

Figure XIV. Estimated lifespan and total marketed quantity for six chemical classes



Cutting agents

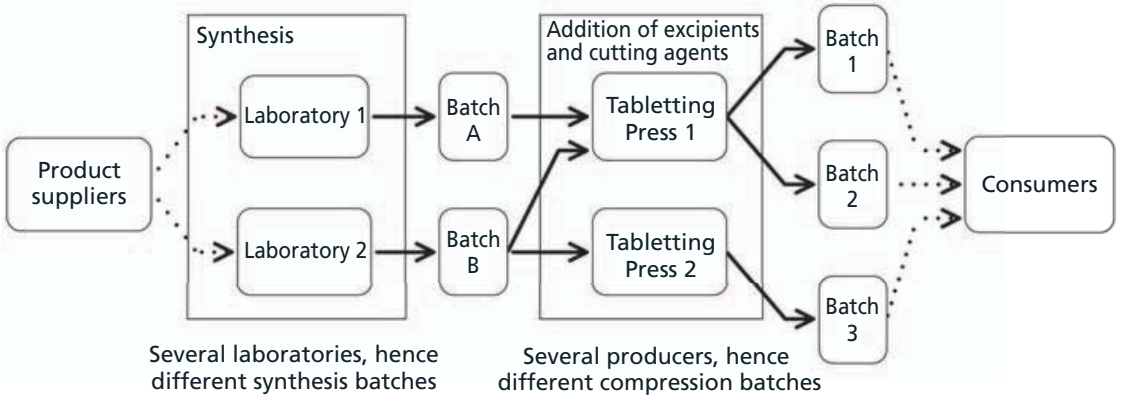
Most drugs are sold in powder form, which makes it possible to add cutting agents at any point in the drug distribution process. With the resulting increase in volume, more doses can be sold, and revenue can be increased. The profile of cutting agents can thus change at each stage in the chain leading from the producer to the dealer, via the wholesaler. The methodology used at IPS can identify production batches by analysing the principal component, heroin or

cocaine. Batch purity and the combination of cutting agents provide information allowing hypotheses to be made about the stage in trafficking to which the seizure belongs.

In the case of tablets, the process of adding substances ends at the tabletting phase. Once pressed, tablets can no longer be altered. They contain excipients or cutting agents, which are used to facilitate tabletting or to increase the drug's weight for reasons of profit. Hypothesized links for such substances are thus different from those made for drugs in powder form: linkage stops at the compression stage, as shown in figure XV. Thus, when a pill contains a sufficiently differentiated chemical mixture, it is possible to trace a line back to the tablet-making laboratory, which may be either the synthesis laboratory, if it is equipped with a press, or, as shown in figure XV, a different laboratory specializing in pressing tablets.

It should be noted, however, that the presence of different mixtures does not mean that the tabletting stage took place at a different laboratory. It could simply mean that the producer obtained supplies elsewhere.

Figure XV. Stages in the manufacture of Ecstasy tablets



Note: Batch 1 and batch 2 have the same logo but not the same chemical profile. Batch 2 and batch 3 have the same chemical profile but not the same logo.

Communication of information

Visualization: optimizing data usage

The combination of iBase® and Analyst's Notebook® makes for an excellent tool for visualization and interpretation. Given the different entities it can record, such as seizures, samples and physical and chemical classes, iBase® allows all linkages related to a specific element to be developed. Sample results are presented in figure XI and figure XII.

Training

The main difficulty in implementing such a process is finding suitable operators to manage and interpret the new data. Although the analysis of the data is intended for use by drug team investigators, it would be unrealistic to ask them to manage such data, for two main reasons. First, from a purely practical standpoint, they already have a heavy workload. Secondly, the information is highly complex. That complexity calls for specific training and time to realize the full potential of this new tool.

Ideally, a crime analysis unit would be established to centralize, analyse and redistribute information. Such a unit could put forward hypotheses concerning the structure of criminal associations and explain their connections. The unit could make the most rational use of such data, adopting a deductive approach to formulate working hypotheses, which would be evaluated using collected information and data from law enforcement investigations.

Linkage management and interpretation may be represented in diagram form as shown in figure XVI.

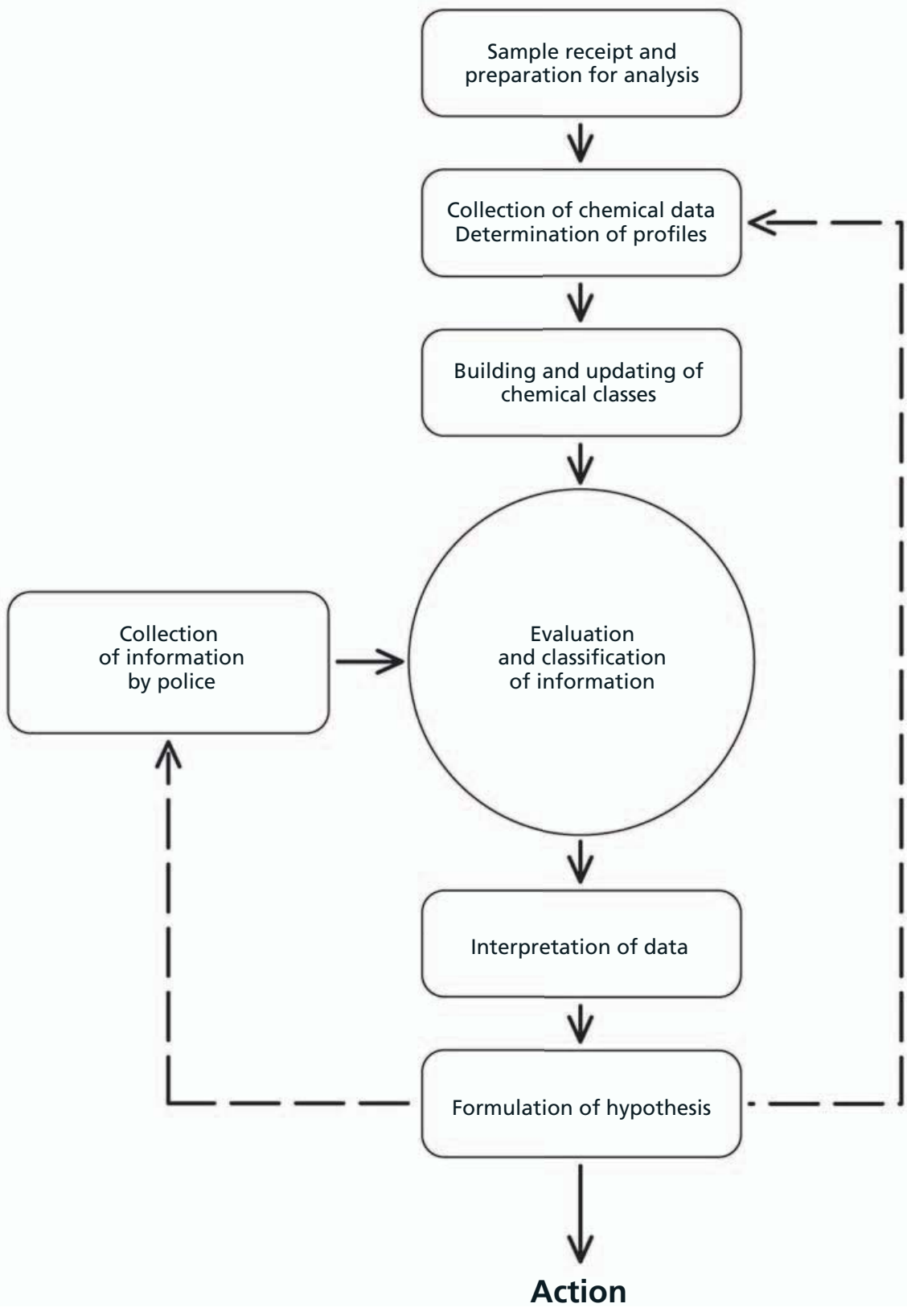
To summarize, the different linkages managed in iBase® provide an up-to-date view of trends in the distribution market for drugs targeted by the police. When a distribution network is detected and a related sample drug class can be established, links can be made between different seizures. Drug seizures can then be connected with the persons involved, and a picture of the actual situation that is as accurate and as complete as possible can be obtained. The process of managing linkages between drug seizures enables police to set priorities for action and have consolidated information on the scope of a trafficking network at the time of an arrest. All that can be done provided that the chemical linkage data are obtained quickly.

Prospects

The profiling technique described in the present article is currently used in the IPS laboratories at Lausanne on a regular basis. It combines analytical methods for establishing physical and chemical profiles with management and visualization tools. In addition, detected chemical links are systematically transmitted to law enforcement authorities. Each year, a three-person team analyses, on average, 250 seizures involving some 2,000 drug samples. In approximately 20 per cent of cases, a chemical link (a relationship to another sample of the same chemical class) is detected and communicated to the investigators dealing with the case. Specific profiling procedures have also been applied to particular cases and are combined with the material evidence and the type of information more traditionally used in judicial investigations. Experiences using the linkage process described illustrate the rich intelligence potential offered by chemical links. However, aside from routinely reported chemical links, mechanisms for sharing information with law enforcement authorities have not been adequately established and are activated only sporadically. That lack of formal mechanisms

results in a loss of information. Accordingly, development efforts now focus on ways of achieving a more systematic integration of profiling intelligence from both the operational and strategic perspectives.

Figure XVI. Management and interpretation of chemical links



In order to better define the cooperation needed between law enforcement officials and scientists in global problem-solving, a number of specific cases are currently being handled in collaboration with law enforcement authorities. Each of those experiences is analysed in conjunction with the relevant chemical and physical information in order to identify patterns that can be used in a more systematical fashion.

One development has been the addition to the central law enforcement database of a dedicated field showing links detected through drug analysis. A judicial investigator who finds items of information relative to his case will automatically benefit from profiling intelligence. However, to complement such innovations, law enforcement officials should also be familiarized with those new sources of information, because transmitting profiling data to them from the laboratory directly without further commentary would achieve only limited results.

The field of policing is undergoing fundamental transformations, essentially driven by the development of new technologies, economic constraints and a general awareness of the clear links that exist between organized crime and terrorism. Intelligence is becoming the key element of operations. Intelligence-led strategies, in which information derived from physical and chemical links plays a crucial role, have not yet been widely implemented in the combat of illicit drugs in Switzerland. However, it appears that several entities for drug analysis in the country are starting to endorse this proposed approach. That trend is an encouraging sign for the development of drug profiling in the national context.

Conclusions

The present paper provides a description of the profiling process developed at IPS. The originality of the process is its focus on an operational approach of direct use to the drug investigation teams of local law enforcement authorities. The method of drug profiling, including analytical, statistical and computerized methods, was tested and developed with the aim of rapidly identifying linkages between drug samples. The challenge was to manage those chemical links in a consistent manner and to provide an optimal visual representation of those links, which is essential to interpreting and disseminating that data. That work has helped demonstrate that the identification of linkages between seizures in different regional markets has enormous potential for police investigations.

The methodology and the prototype introduced have proved effective, enabling large numbers of samples, some 3,000 heroin samples and some 2,800 cocaine samples, to be processed. The promising results have been confirmed and utilized by judicial authorities. The next step is to focus on implementing the model on a more systematic basis and, if possible, at the national level and even the international level. This will be necessary to gain the maximum potential of intelligence from detected linkages. Also, the linkages established cut across the various cantons whose samples are analysed by IPS.

Large-scale heroin seizures carried out in the canton of Tessin have been shown to be linked to samples from seizures made in the cantons of Geneva, Vaud and Neuchâtel. It has been shown that, through the Swiss distribution network, drugs entering via Tessin supply the Berne and Zurich areas, which, together with the Basel area, constitute the major centres for drug storage.

Thus, it seems that the current view must be broadened in order to better assess the size and the mechanics of the Swiss market. In the opinion of the authors, that requires the establishment of a unit responsible for gathering such information and forming a more comprehensive picture of possible connections between the various cases.

The need for the harmonization of data transmission and accessibility was taken into account in creating the new JANUS database of the Federal Criminal Police. That computerized system, which is used by all Swiss police authorities, pools intelligence on organized crime, including counterfeiting, traffic in human beings, economic crime, money-laundering and drug trafficking. In 2005, drug profiling was integrated into that system. That development constitutes a significant advance in the use of chemical links derived from drug analysis. It is believed to be the first database to combine traditional police information and profiling data in a single structure.

That initiative makes it possible to study connections between those different sources of information and formulate hypotheses on the structure of markets or trafficking networks (validation, significance and phenomena).

In conclusion, links established through profiling, combined with traditional information, can be used to better understand the market's structure and implement medium- and long-term investigation strategies. An investigator will thus have at his or her disposition a diagram showing all seizures chemically linked to a seizure. That approach, which combines investigative data with data from physical and chemical analysis, requires the establishment within police services of an entity capable of managing such information.

References

1. O. Ribaux and others, "Forensic intelligence and crime analysis", *Law, Probability and Risk*, vol. 2, No. 1 (2003), pp. 47-60.
2. European Network of Forensic Science Institutes, Drugs Working Group, *Guidelines on Representative Drug Sampling*, 2004.
3. L. Aalberg and others, "Development of a harmonized method for the profiling of amphetamines: I. Synthesis of standards and compilation of analytical data", *Forensic Science International*, vol. 149, Nos. 2-3 (2005), pp. 219-229
4. L. Aalberg and others, "Development of a harmonized method for the profiling of amphetamines: II. Stability of impurities in organic solvents", *Forensic Science International*, vol. 149, Nos. 2-3 (2005), pp. 231-241.
5. O. Guéniat and P. Esseiva, *Le profilage de l'héroïne et de la cocaïne: une méthodologie moderne de lutte contre le trafic illicite* (Lausanne, Switzerland, Presses polytechniques et universitaires romandes, 2005).

6. P. Esseiva, "Le profilage de l'héroïne et de la cocaïne: mise en place d'une systématique permettant une utilisation opérationnelle des liens chimiques", doctoral thesis, University of Lausanne, Switzerland, 2004.
7. O. Guéniat, "Le profilage de l'héroïne et de la cocaïne: les méthodes d'analyse, la modélisation du concept de profilage, la gestion et l'exploitation des liens", doctoral thesis, University of Lausanne, Switzerland, 2001.
8. P. Esseiva and others, "A methodology for illicit heroin seizures comparison in a drug intelligence perspective using large databases", *Forensic Science International*, vol. 132, No. 2 (2003), pp. 139-152.
9. L. Dujourdy and others, "Evaluation of links in heroin seizures", *Forensic Science International*, vol. 131, No. 2 (2003), pp. 171-183.
10. P. Esseiva and others, "Les signatures chimiques à partir des saisies de produits stupéfiants: utilité et gestion de l'information", *Revue internationale de criminologie et de police technique et scientifique*, vol. 55, No. 1 (2002), pp. 104-111.
11. P. Esseiva and others, "Chemical profiling and classification of illicit heroin by principal component analysis, calculation of inter sample correlation and artificial neural networks", *Talanta*, vol. 67, No. 2 (2005), pp. 360-367.
12. O. Ribaux and P. Margot, "Inference structures for crime analysis and intelligence: the example of burglary using forensic science data", *Forensic Science International*, vol. 100, No. 3 (1999), pp. 193-310.

Drug profiling: a new scientific contribution to law enforcement operations in Viet Nam

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ABSTRACT

Since 1995 heroin sample comparisons have been carried out in Viet Nam to establish links between wholesalers and retailers. To that end, the physical and chemical characteristics of samples are analysed: their colour, the packaging material, including fingerprints, diacetylmorphine (heroin) content and the composition of some main alkaloids.

At the beginning of 2002, having acquired expertise on impurity profiling and with the support of new instruments, the Institute of Forensic Sciences of Viet Nam introduced the routine impurity profiling of seized heroin and methamphetamine and later undertook to explain that process to national law enforcement bodies.

Since then, 375 heroin and 29 methamphetamine samples have been analysed for major and minor impurities. Substances detected in the analysis of illicit heroin include diacetylmorphine, morphine, codeine, O^6-monoacetylmorphine and acetylcodeine as well as adulterants such as paracetamol and caffeine. Since methamphetamine impurity profiling began, 29 samples have so far been analysed, and some samples have been grouped through the application of cluster analysis.

In the case of heroin, impurity profiling has established a link between two major trafficking groups suspected of obtaining heroin from the same source of production. Analysis has also revealed a link between one wholesaler and several retailers in one region. In addition, impurity profiling provides new information on the preparation and production of some methamphetamine and fake Ecstasy tablets.

Keywords: forensic science; heroin; methamphetamine; gas chromatography (GC); gas chromatography/mass spectrometry (GC/MS); linking samples; methods of production

*The authors would like to express their gratitude to K. Tanaka for his knowledge of impurity profiling, to H. Stead and B. Remberg for their encouragement and assistance in preparing the present article, to the Japan International Cooperation Agency and the United Nations Office on Drugs and Crime for their support in establishing impurity profiling in Viet Nam and to the Asia Research Center and the Korea Foundation for Advanced Studies for their financial support.

Introduction

Worldwide, the impurity profiling of drugs such as heroin and methamphetamine has a long history [1-7]. In Viet Nam, from 1993 until 2002, the Institute of Forensic Science had only one gas chromatograph (GC) for drug analysis.

In Viet Nam, heroin sample comparison is done to answer the questions of law enforcement bodies, for example, whether there is a link between a wholesaler and specific retailers in a given region. Before 2002, heroin sample comparisons were based simply on the sample's physical characteristics; colour (typically yellow, brown, pale white or white), the characteristics of the paper or plastic bag used as packaging, fingerprints on the packaging material, and the qualitative and quantitative analysis of diacetylmorphine content and the main alkaloidal impurities, such as O^6-monoacetylmorphine (O6MAM) and acetylcodeine.

The Institute incorporated impurity profiling into its work at the end of 2002. At that time, it had received sophisticated instrumentation, including a gas chromatograph (GC), a gas chromatograph-mass spectrometer (GC-MS), a Fourier Transform infrared (FT-IR) spectrometer, a high-performance liquid chromatography mass spectrometer (HPLC-MS) and an inductively coupled plasma mass spectrometer (ICP-MS).

Impurity profiling analysis began with heroin and methamphetamine samples alone. Going beyond the process used for heroin sample comparison prior to 2002, which consisted of determining physical characteristics such as colour, logos (imprint), hardness, weight and fingerprints on the packaging material, the new method included the analysis of chemical characteristics of the packaging material, such as paper, cellophane and polyethylene bags, using FT-IR. Since 2004, the Institute has analysed some impurities of heroin samples, including adulterants and diluents. Further, in 2006 and 2007, minor compounds, by-products and traces of solvents in heroin samples will be analysed with the help of GC-MS, headspace-GC and HPLC.

This article presents the first results of the impurity profiling analysis of heroin and methamphetamine samples using GC and GC-MS. Those results have provided a great deal of valuable information for law enforcement authorities. Some operational findings are discussed.

Impurity profiling of heroin

Heroin is the drug most frequently encountered in Viet Nam, accounting for 60-70 per cent of all seized samples of drugs. Heroin is a semi-synthetic product derived from morphine, which, in turn, is extracted from the opium poppy, *Papaver somniferum* L. Differences in cultivation and manufacturing procedures produce the different concentrations of opium alkaloids present in the heroin samples. Those processing by-products, as well as adulterants added, can provide information on origin and processing, thus enabling analysts to establish links between groups trafficking in illicit heroin. All these parameters constitute a profile to be used in comparative analysis.

Analytical aspects

The present article is based on the analysis of 375 heroin samples selected from single-item and multiple-item seizures, in other words, seizures of one bag or multiple bags. Prior to analysis, all samples were dissolved in methanol at a concentration of 1 mg/ml to determine the quantity of heroin, acetylcodeine, O6MAM, caffeine, paracetamol and phenobarbital, and at a concentration of 10 mg/ml to determine the quantity of codeine, morphine, papaverine and noscapine. N-Octacosane ($C_{28}H_{58}$) is used as the internal standard.

Instrumentation: Agilent 6890N and Thermo-Finnigan GC-MS

Column: Ultra II fused silica capillary column, 5% phenyl GC 95% dimethyl polysiloxane, crosslinked, 25 m x 0.2 mm x 0.33 μm

Injector: 280° C (splitless, with purge flow: 1.2 min)

Carrier gas: nitrogen at flow rate of 1.0 ml/min

Temperature programme: 160° C at a rate of 8° C/min to 250° C, then at 10° C/min to 300° C, 8-min hold

Flame ionization detector:

Hydrogen flow: 35 ml/min

Air flow: 400 ml/min

Make-up flow (nitrogen): 30 ml/min

Temperature: 300° C

Mass spectrometer:

70 eV

50-650 m/z

Ion source temperature: 250° C

Transfer-line temperature: 280° C

Results and discussion

The first step of heroin sample characterization is performed by means of visual inspection. The 375 selected heroin samples can be classified in four colour groups: white, pale white, brown and yellow.

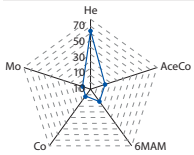
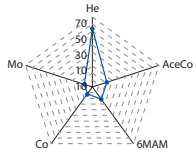
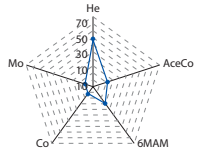
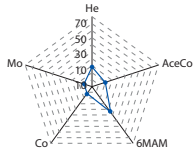
After visual inspection, the next step is the qualitative and quantitative analysis of diacetylmorphine, of major and minor impurities such as morphine, codeine, acetylcodeine, O^6-monoacetylmorphine, noscapine and papaverine, and of adulterants such as paracetamol, caffeine and phenobarbital.

Street samples obtained in Viet Nam often contain adulterants such as chloramphenicol, vitamin C, ephedrine, aspirin, paracetamol, caffeine, sulfonamide and diluents such as calcium carbonate, glucose and starch, which have been added by drug vendors. Table 1 shows the types of heroin found among the 375 samples investigated.

Table 1. Heroin colour types and corresponding adulterants

Colour of heroin sample	Frequency of occurrence in samples		Year of occurence
	Samples (number)	Proportion (percentage)	
White	337	89.9	1995-2005
Pale white	23	6.1	1995-2005
Brown	13	3.5	1996-2003
Yellow	2	0.5	1995 and 2002

Table 2. Average content of heroin and alkaloid impurities, by colour type *(Percentage)*

Colour type of heroin sample[a]	Heroin		Acetylcodeine	
	Average concentration	Range	Average concentration	Range
 White	68.4	60-89	13.2	—
 Pale white	61.4	50-70	12.4	—
 Brown	46.6	20-67	11.0	—
 Yellow	17.8	6.6-29	9.1	4.5-13.6

[a]He = heroin
AceCo = acetylcodeine
6MAM = O^6-monoacetylmorphine
Co = codeine
Mo = morphine

[b]Not detected in 19.3 per cent of white heroin samples.

[c]Not detected in 73.6 per cent of white heroin samples.

[d]Not detected in 53.9 per cent of brown heroin samples.

[e]Not detected in yellow heroin samples.

Adulterant					
Paracetamol		*Caffeine*		*Phenobarbital*	
Samples (number)	*Proportion (percentage)*	*Samples (number)*	*Proportion (percentage)*	*Samples (number)*	*Proportion (percentage)*
3	0.9	25	7.4	1	0.3
3	1.3	—	—	—	—
6	36.1	1	0.8	—	—
—	—	—	—	—	—

O^6-monoacetylmorphine		*Codeine*		*Morphine*	
Average concentration	*Range*	*Average concentration*	*Range*	*Average concentration*	*Range*
4.5	—	0.02[b]	—	0.30[c]	—
10.4	—	0.08	—	0.27	—
17.9	—	0.19	—	0.5[d]	—
32.5	28-37	0.21	0.2-0.22	—[e]	—[e]

As can be seen in table 1, the number of adulterated heroin samples is small: 39 of 375 samples. The most common adulterants are caffeine and paracetamol, which are easy to obtain. A single sample contained phenobarbital. Adulterants were found in only three heroin colour types: white, pale white and brown.

The colour of the heroin samples is a product of the quality of the processing methods used. White and pale white heroin samples are the most common colour types found in Viet Nam, accounting for 96 per cent of seized heroin. Brown heroin samples were seized in Viet Nam between 1996 and 2003, but no such seizures have been reported since then. Yellow heroin was discovered once in 1995. It surfaced again in 2002, although in small amounts.

In addition to the analysis of colour and adulterants, the 375 heroin samples were analysed for their diacetylmorphine (heroin) content and for selected alkaloid impurities. Heroin samples seized in Viet Nam typically have a high concentration of diacetylmorphine and very low concentrations of morphine and codeine. That may be the result of skilled production processes that use good quality morphine as starting material.

Low morphine levels were detected in the heroin samples analysed. Table 2 shows that morphine levels in white and pale white heroin samples were almost the same—roughly half the average amount present in brown heroin. Thus, the colour of the samples may serve as a rough indicator of the quality of the heroin, given that the concentration of diacetylmorphine is high in white heroin samples and lower in brown and yellow heroin samples.

It should also be noted that morphine was present in only 26.4 per cent of white heroin samples and in 46.1 per cent of yellow heroin samples. Codeine was found to be present in 80.7 per cent of all white heroin samples.

Noscapine and papaverine were not detected in the raw heroin samples analysed using the GC method. They might be found with the help of capillary electrophoresis (CE), or HPLC, and there are plans to investigate heroin samples using those techniques in the future. However, it is also possible that those substances were not detected because the heroin samples were produced from morphine extracted from opium from the region containing very low noscapine and papaverine levels.

In one case in 1996, an analysis of brown heroin samples revealed a high average acetylcodeine content of approximately 20 per cent. That unusual finding (compared with typical acetylcodeine levels of approximately 10 per cent, as seen in table 2 above) was communicated to Vietnamese law enforcement officials. A small illicit laboratory producing heroin from terpine codeine cough tablets was subsequently dismantled in 1996. On the other hand, other analytical results of that same year could not be fully used in law enforcement operations at the time. For example, some samples of pale white heroin seized in 1996 showed an unusual average diacetylmorphine content, and some white heroin samples were characterized by the presence of unknown, red impurities, which made those samples visibly different from other white heroin samples. Only later, in 2005, with the statements of offenders belonging to a large dismantled network responsible for trafficking 1,000 kg of heroin over the

previous years, it was confirmed that the larger amounts of white and pale white heroin were imported and that the smaller amounts, typically of brown heroin, were produced in Viet Nam.

Table 2 also shows that, of the four types of heroin samples found in Viet Nam, yellow heroin has the lowest heroin content, a high acetylcodeine content and the highest O6MAM content. Those alkaloid levels translate into relatively high acetylcodeine:heroin and O6MAM:heroin ratios, a fact which suggests that another, distinct production/preparation method for yellow heroin might exist or that those samples are particularly prone to hydrolysis.

It is observed that, during sample storage heroin concentration decreases as O6MAM concentration increases, with the result that the total concentration of the two alkaloids remains constant. Consequently, the ratio of acetylcodeine:heroin + O6MAM) changes very little during the storage of heroin samples. The acetylcodeine concentration also remains stable.* Provided that short-term storage conditions are acceptable, thus preventing degradation, the ratios of acetylcodeine:heroin and of acetylcodeine:O6MAM can be used to determine whether two samples come from the same source.

Figure I illustrates the relationships between samples from three heroin cases investigated in Viet Nam. To establish links between samples, scatter diagrams were prepared using the acetylcodeine:heroin ratio as the vertical axis and the ratio acetylcodeine:6-MAM as the horizontal axis.

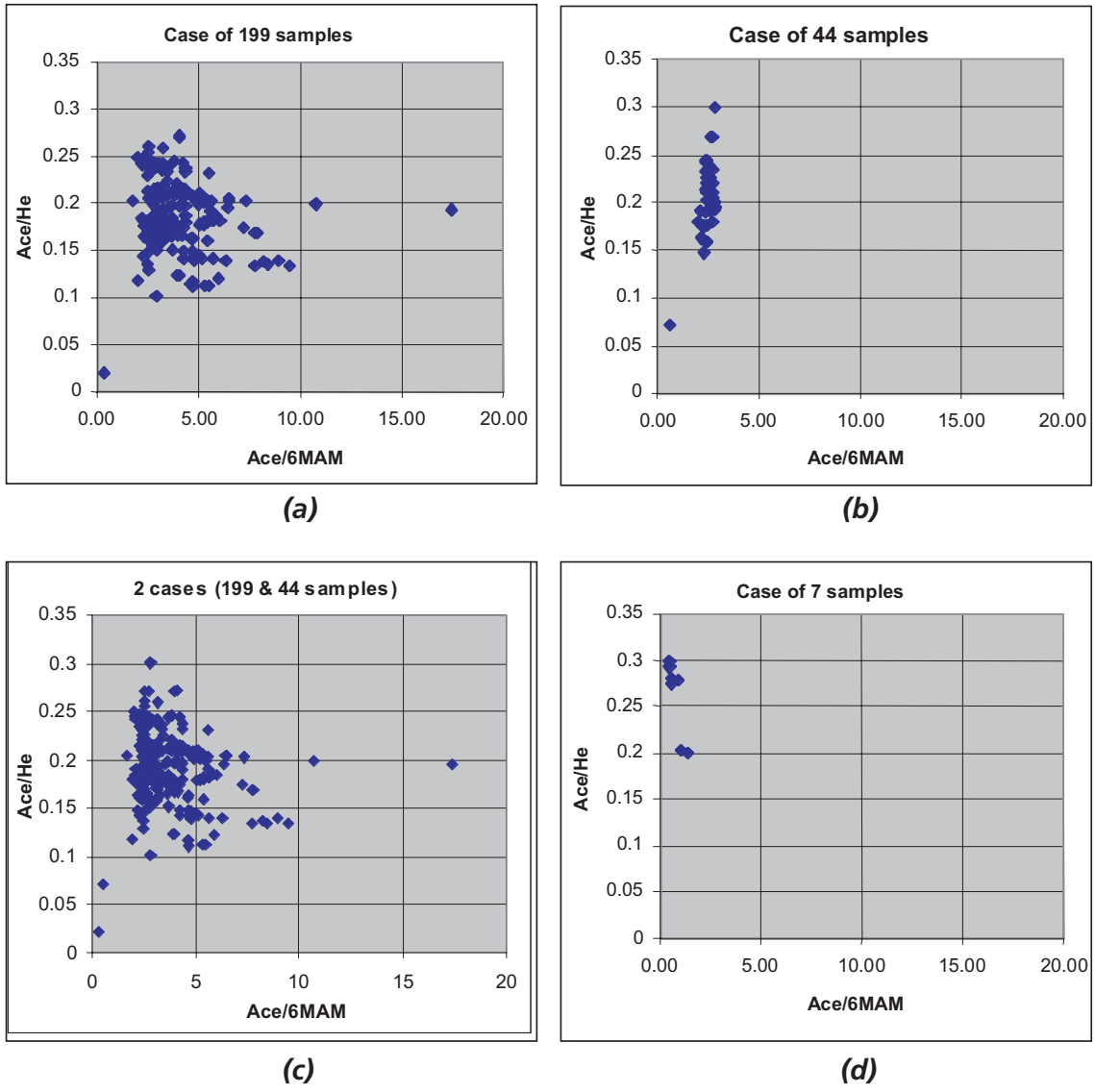
Case 1 involved the analysis of 199 heroin samples taken from 199 separate bags seized in a single operation. Those bags had been found hidden in several cartons in two different cars. Figure I *(a)* shows that most of the 197 heroin samples form one large group. As can be seen, two samples are clearly different from the other 197 samples and from each other. In figure I *(a)* sample 1 is located close to the point of origin. And sample 2 is located close to the coordinates 17.5, 0.2. Sample 1 is characterized by a low acetylcodeine content of 1.7 per cent, which suggests that it may have been produced from good quality morphine. Sample 2 exhibits a low, 0.7 per cent O6MAM content, which suggests that it may have been produced under good reaction conditions, resulting in a stable product.

Case 2 involved the analysis of 44 heroin bags taken from a car that had been brought from the western border to the centre of Viet Nam. Analysis indicated that 41 of the 44 bags form a single group (see figure I *(b)*).

Analysing samples seized from different trafficking groups enables us to establish links between those groups. Figure I *(c)* combines the results of the samples from case 1 and case 2. It is shown that the samples can be classified in three groups. The largest group comprises samples from the larger groups of the two cases, that is 197 of 199 samples from case 1 and 41 of 44 bags from case 2. The second group comprises one sample of the 44-bags case and one

*Because the codeine concentration was at the detection limit of the technique, the ratio of codeine:acetylcodeine is not a useful indicator to determine the origin of a sample. In the future, heroin samples will be derivatized in order to quantify codeine and morphine, or they will be analysed by other methods such as CE or HPLC.

Figure I. Comparison of three heroin cases (199, 44 and 7 bags of seized heroin, based on two alkaloid ratios)



sample from the 199-bags case (the two points located close to the origin of the axes). And the third group comprises a single sample of the 199-bags case, located at the coordinates 17.5, 0.2.

It is important to note that there are both adulterated and non-adulterated heroin samples in the main group of the 41 of 44 heroin bags. The fact that those samples form one group based on their acetylcodeine:heroin and acetylcodeine:O6MAM ratios suggests that the heroin seized in both cases was produced by the same production process and that adulteration occurred later along the trafficking chain, during distribution.

In the case involving 199 bags, the wholesaler appears to have added caffeine and paracetamol to some samples in order to increase bulk weight. Despite that post-processing alteration to increase weight, impurity profiling analysis made it possible to determine that the adulterated heroin samples came from the same source as those not containing adulterants. Law enforcement

authorities were able to link the two cases with the help of that forensic information.

Finally, the final scatter diagram in figure I (d) shows the analytical results of case 3, which involves seven heroin samples from a single case. The samples were seized at five locations from three individuals. Three samples were found in the house and kitchen of woman A. One sample was found in the garden of her neighbour B, and another in an abandoned car near the house of neighbour B. Finally, two samples were found in the house of offender C.

Analysis confirmed that six of the seven samples contained the same concentrations of heroin and caffeine, while one of the samples seized in the house of offender C did not contain any adulterants. The ratios of key alkaloids of all seven samples was similar, which suggests that all samples came from a single source. The existence of a chemical link between the samples led to the suspicion that there might also be a link between individuals A, B and C, although all three offenders denied any such relation existed. When the results of that analysis were combined with evidence from law enforcement officials, it was concluded that C sold drugs to B, and B sold drugs to A.

Figure II. Some logos of samples of heroin seized in Viet Nam

"globe and lion"

"one ruby with crown"

"dragon"

"999/AAA"

"rose flower/AAA"

The print most frequently encountered on packaging material of seized heroin is the "globe and lion" logo (see figure II), which is also found in other Asian countries. The "one ruby with crown" logo was encountered for the first time in Viet Nam in the 199-bag case of 2003. Heroin cakes with other logos, such as the "AAA", "999" and "rose flower" logos, are usually trafficked in standard weights of 360 grams, but occasionally small cakes of 60 grams are encountered. It is typical of heroin trafficking in Viet Nam that most offenders carry only one cake at a time. That may be a result of the 2001 narcotic drug law, which established 100 grams of product, regardless of purity, as the threshold for capital punishment. However, the courts can adjust punishment in accordance with the diacetylmorphine content of the seized drugs.

Impurity profiling of methamphetamine

From 2000 to 2004, seizures of amphetamine-type stimulants (ATS) tablets in Viet Nam increased steadily: 17,000 tablets were seized in 2000, 43,160 in 2001, 44,428 in 2002, 27,218 in 2003 and 61,000 in 2004. Most were methamphetamine tablets. ATS accounts for approximately 20 per cent of all seized drugs. The amount of ATS seized in Viet Nam is expected to increase further in the future, reflecting a worldwide trend.

Recently, an increasing variety of logos has been observed on tablets sold as Ecstasy—with the same weight and size as real Ecstasy tablets—on the illicit markets in Viet Nam, especially in discotheques. Analysis has shown that some of those tablets did not contain methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA) or other Ecstasy-type substances but only methamphetamine. In response, our laboratory introduced the impurity profiling of ATS in 2002, extending its range of analytical tools beyond qualitative and quantitative analyses, which characterized the laboratory's work on methamphetamine prior to 2002. The impurity profiling of that drug now makes it possible to establish links between samples, determine whether new types of crystalline methamphetamine have appeared on the market, give the courts evidence on chemical links and determine whether there are new production and preparation processes for methamphetamine tablets.

Analytical aspects

Prior to analysis, tablets are ground into powder. One hundred milligrams of the powder are dissolved in 1 ml of 0.1M phosphate buffer. The solution is made basic with 0.25 ml of 10 per cent Na_2CO_3 solution, and 0.4 ml of ethylacetate containing an internal standard ($C_{30}H_{62}$) are added. The solution is shaken vigorously for 5 minutes, centrifuged, and the organic layer is transferred to an insert of a microvial; 1 μl of the solution is injected into the GC.

Instrumentation 1: Thermo-Finnigan GC-MS

Column: Rtx1-ms (100% dimethylpolysiloxane, crosslinked, 30 m x 0.25 mm x 0.25 μm

Injector temperature: 275° C; mode: splitless

Carrier gas: Helium, constant flow at 1.0 ml/min

Temperature programme: 100° C, hold for 1 min, at 10° C/min to 270° C, 20 min hold

Transfer line 275° C; ion source: 200° C

MS mode: full-scan 50-650 m/z

Instrumentation 2: Agilent 6890N GC

Column: Ultra II: 5% phenyl 95% dimethylpolysiloxane, crosslinked, 25 m x 0.2 mm x 0.33 μm

Injector and detector temperature: 280° C

Carrier gas: nitrogen at flow rate of 1 ml/min; splitless

Temperature programme: 50° C, hold for 1 min, at 10° C/min to 300° C, 9 min hold

Results and discussion

In 1995, an illicit methamphetamine laboratory in Ho Chi Minh City operated by a person who was not a national of Viet Nam was dismantled. Authorities seized 234 kg of crystalline methamphetamine that had been packed in plastic green tea bags one kilogram in weight ready for export. Ten years later, in 2005, crystalline methamphetamine was seized once again. The question of law enforcement authorities was whether there was a link between the two seizures of crystalline methamphetamine, in particular whether they were manufactured using the same synthesis route.

Figure III. Chromatogram of crystalline methamphetamine seized in 1995 (1=1,2-dimethyl-3-phenylaziridine; 2=methamphetamine; 3=ephedrine; 4=unknown; 5="naphthalene"; IS=internal standard)

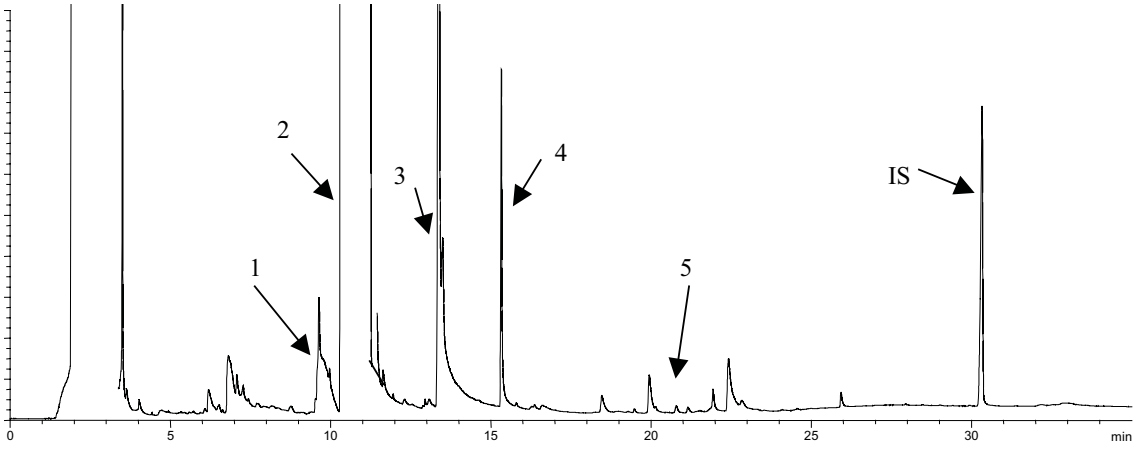
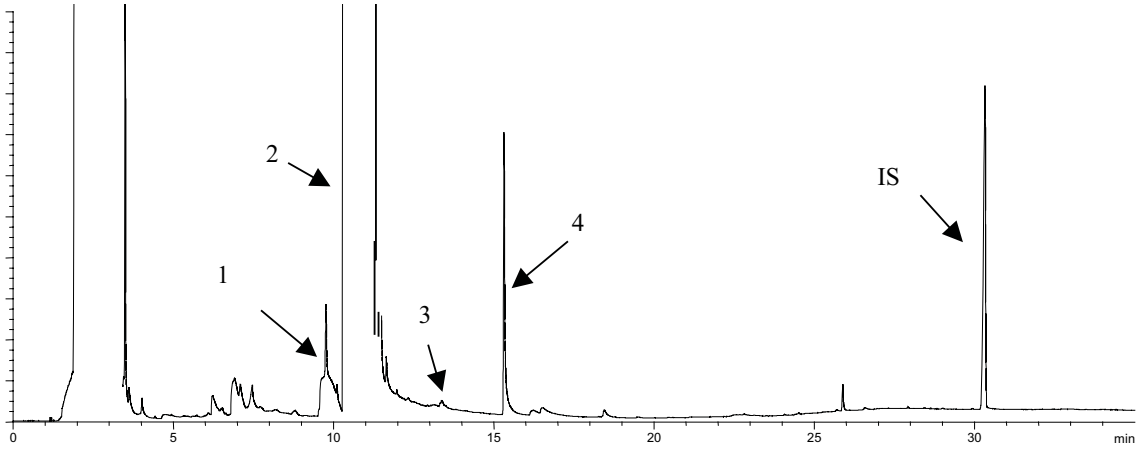


Figure IV. Chromatogram of crystalline methamphetamine seized in 2005 (1=1,2-dimethyl-3-phenylaziridine; 2=methamphetamine; 3=ephedrine; 4=unknown; IS=internal standard)



As can be seen from figures III and IV, there are significant differences in the impurity profiles of the two methamphetamine samples, which suggests that the methamphetamine seized in 2005 had a production process different from that seized in 1995. The main difference between the two chromatograms is the presence of peak No. 3 (at Rt=13.5 min.) in the impurity profile of the sample seized in 1995 (figure III). That peak, which was identified to be ephedrine, is absent in the profile of the sample sized in 2005 (figure IV). Because of the crystalline nature of the two samples, it is unlikely that ephedrine was added as a cutting agent. That suggests that the two samples were produced using different production processes. That finding contradicted the view of law enforcement authorities that there was only one method of methamphetamine production. However, the presence of traces of ephedrine in both samples suggests that both batches of crystalline methamphetamine were synthesized using that precursor.

Unlike the crystalline methamphetamine discussed in the foregoing case, most methamphetamine found in Viet Nam is in tablet form. "Red tablets", as methamphetamine tablets are commonly called in Viet Nam because of their red colour, have been imported into the country since 2000 and have become popular.

Tablets with the "WY" logo found in Viet Nam resemble those from the Golden Triangle; they have the same logo (see figure V), the same size (0.6 x 0.25 cm), the same weight (80-90 mg) and the same colour (such as red, orange and green [8]). The tablets have a 10-24 per cent methamphetamine content and contain adulterants such as caffeine, ethylvanillin and ketamine.

Figure V. Some types of the "WY" logo of methamphetamine tablets

In May and June 2005, Ecstasy tablets were seized in more than 20 discotheques in the country's two biggest cities, Hanoi and Ho Chi Minh City. The tablets physically resembled real Ecstasy tablets with the same weight, nearly 0.25 and 0.34 g, and the same size, 0.8 x 0.5 cm. However, analysis of the tablets showed that they were very different from real Ecstasy, in both qualitative and quantitative terms. Some tablets contained MDMA, some contained methamphetamine and some contained a combination of both. Ketamine, which in Viet Nam is controlled by Government decree No. 133 of 6 November 2003, was also detected in most tablets, and was sometimes the main active component. Paracetamol and caffeine were frequently present. Table 3 shows selected results of the analysis of the seized tablets being sold as Ecstasy.

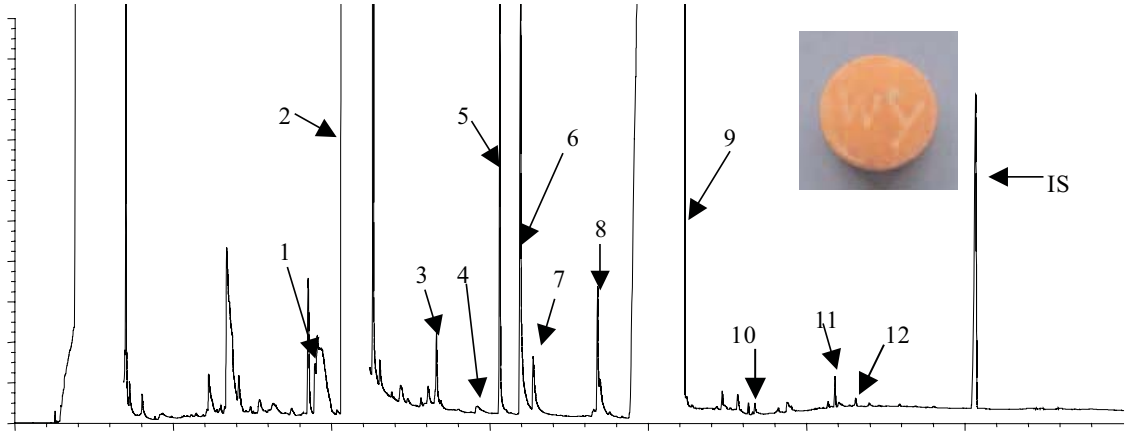
Table 3. Physical characteristics and chemical composition of different types of tablets sold as Ecstasy

Tablet	*Size (cm)*	*Weight (g)*	*Methamphetamine content (percentage)*	*MDMA[a] content (percentage)*	*Adulterants*
	0.8 x 0.5	0.358	0.52	0	Paracetamol, caffeine and ketamine
	0.9 x 0.5	0.325	0.62	0	Paracetamol, caffeine and ketamine
	0.9 x 0.4	0.320	0.87	0	Paracetamol, caffeine and ketamine
	0.8 x 0.4	0.342	33.18	0	Ketamine
		0.283	0.03	21.15	Ketamine
	0.7 x 0.4	0.299	0.05	25.18	Ketamine
	0.7 x 0.4	0.263	1.68	66.86	Ketamine
	0.8 x 0.4	0.365	0	36.69	Ketamine
	0.6 x 0.5	0.191	0	81.96	Ketamine

[a]3,4-Methylenedioxymethamphetamine.

Figure VI shows the impurity profiles of two traditional "WY" logo methamphetamine tablets—orange and green—and a fake Ecstasy tablet carrying a spider logo. Other logos seen on fake Ecstasy tablets include the "E" (euro), "leaf", "ox head", "crocodile", "butterfly" and "XO" logos. A variety can be seen in figure VII.

Figure VI. Impurity profiles of two traditional methamphetamine tablets carrying the "WY" logo

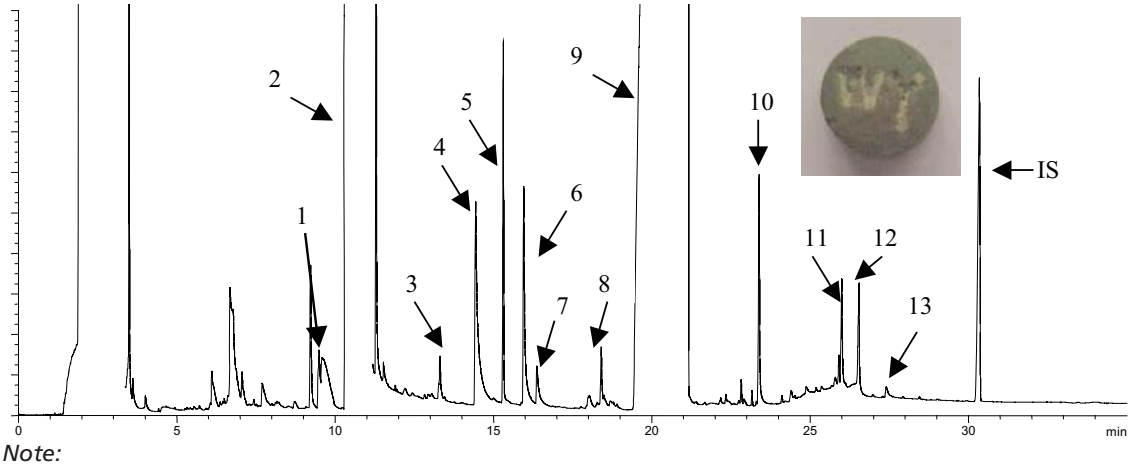


Note:

1. 1,2-Dimethyl-3-phenylaziridine
2. Methamphetamine
3. Ephedrine
4. Ethylvanillin
5. Unknown-1
6. *N*-Formyl methamphetamine
7. Acetylmethamphetamine
8. Acetylephedrine
9. Caffeine
10. Unknown-2
11. Unknown-3
12. O^6-Monoacetylmorphine

IS Internal standard

Figure VIa. Impurity profiles of two traditional methamphetamine tablets carrying the "WY" logo

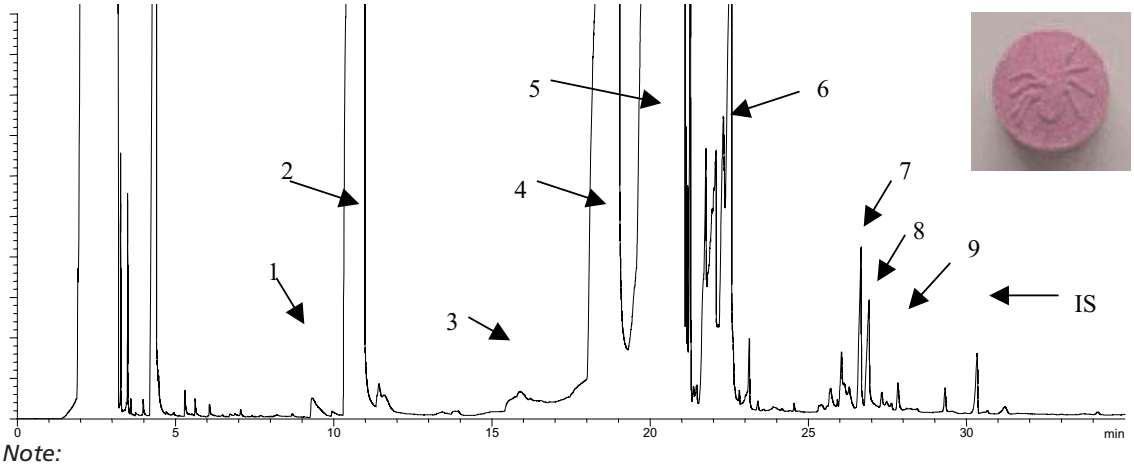


Note:

1. 1,2-Dimethyl-3-phenylaziridine
2. Methamphetamine
3. Ephedrine
4. Ethylvanillin
5. Unknown-1
6. *N*-Formyl methamphetamine
7. Acetylmethamphetamine
8. Acetylephedrine
9. Caffeine
10. Unknown-2
11. Unknown-3
12. O^6-Monoacetylmorphine
13. Heroin

IS Internal standard

Figure VII. Impurity profile of fake pink Ecstasy tablet with a spider logo



Note:

1. 1,2-Dimethyl-3-phenylaziridine
2. Methamphetamine
3. Methylenedioxymethamphetamine
4. Caffeine
5. Ketamine
6. Phenobarbital
7. Acetylcodeine
8. O^6-Monoacetylmorphine
9. Heroin

IS Internal standard

Figure VIII. Logos of fake Ecstasy tablets seized in 2005

Fake Ecstasy tablets containing sassafras oil, a pre-precursor of MDMA, and adulterants such as diazepam and other substances of the benzodiazepine group have also been encountered. The profile analysis of those tablets point to the existence of illicit sites that pressed the fake Ecstasy tablets that have appeared in recent times. In fact, law enforcement bodies have eradicated an illicit site containing a tablet press and a variety of punches.

Conclusion

Impurity profiling in the strict sense was not done in Viet Nam before 2002. Since then, with the help of the Japan International Cooperation Agency (JICA) and the United Nations Office on Drugs and Crime, the forensic chemists of the Institute of Forensic Sciences have acquired a great deal of knowledge in the field.

In the case of heroin, combating the results of physical characteristics and impurity profiling, as done in table 2, can provide information about the sources of heroin that may subsequently help law enforcement agencies stop the

supply of heroin imported into Viet Nam. Moreover, because there are potential domestic sources of raw materials for the manufacture of illicit heroin, impurity profiling can help identify those starting materials and provide valuable information for suitable regulatory control measures. Such potential domestic sources include small-scale illicit opium production, medicinal morphine available for legitimate use against cancer and codeine, which is available in low concentrations in cough medicine from pharmacies.

The impurity profiling of ATS can provide law enforcement authorities with information to determine how many different production processes are being used to produce seized methamphetamine tablets with the "WY" logo. The profiling of two seizures of crystalline methamphetamine revealed that although both were manufactured from the same precursor, ephedrine, different chemicals must have been used in their respective production processes. Analysis of new forms of both real and fake Ecstasy tablets reveals the subtle and devious skills of the manufacturers. They are able not only to make great profits but also to make things more difficult for law enforcement authorities and forensic teams. The findings on fake Ecstasy tablets also demonstrate the value of profiling analysis in monitoring actual drug availability, as opposed to perceived or reported availability based on the physical appearance or presentation and/or marketing of drug samples on illicit markets.

In order to make full use of the potential of drug characterization and impurity profiling analyses, law enforcement authorities must be trained to understand the necessity and the usefulness of impurity profiling and to combine investigative and forensic information. Stopping big trafficking groups may be one of the main duties of law enforcement agencies in the future. Impurity profiling gives law enforcement authorities supplementary information for the detection of heroin and methamphetamine trafficking and production.

Building adequate databases is critical to impurity profiling. In addition to the work on heroin and methamphetamine, law enforcement authorities will have to provide the Institute of Forensic Sciences with sufficient MDMA samples so that it can create a database on the impurity profiling of that drug too. Hopefully, the impurity profiling of major drugs such as heroin and methamphetamine—and MDMA by 2007—will become routine work in drug law enforcement laboratories.

References

1. P. J. O'Neil, P. B. Baker and T. A. Gough, "Illicitly imported heroin products: some physical and chemical features indicative of their origin", *Journal of Forensic Sciences*, part 1 in vol. 29, No. 3 (1984), pp. 889-902; part 2 in vol. 30, No. 3 (1985), pp. 681-691.
2. H. Neumann, "Analysis of opium and crude morphine samples by capillary gas chromatography", *Journal of Chromatography*, vol. 315, 1984, pp. 404-411.
3. E. Kaa, "Impurities, adulterants and diluents of illicit heroin: changes during a 12-year period", *Forensic Science International*, vol. 64, 1994, pp. 171-179.

4. C. Barnfield and others, "The routine profiling of forensic heroin samples", *Forensic Science International*, vol. 39, 1988, pp. 107-117.
5. M. Chiarotti, N. Fucci and C. Furnari, "Comparative analysis of illicit heroin samples", *Forensic Science International*, vol. 50, 1991, pp. 47-55.
6. J. Robertson, "Drug profiling in Australia", report presented at the 17th Meeting of the International Association of Forensic Sciences, 21-26 August 2005, Hong Kong Special Administrative Region of China.
7. J. H. Comparin, report presented at the drug profiling workshop held during the 17th Meeting of the International Association of Forensic Sciences, 21-26 August 2005, Hong Kong Special Administrative Region of China.
8. "DrugNet Asia", a regional newsletter for participating laboratories at the UNDCP Consultative Meeting of Heads of Drug Testing Laboratories in South-East Asia, No. 4, 2005.

Residual solvents in methylenedioxymethamphetamine tablets as a source of strategic information and as a tool for comparative analysis: the development and application of a static headspace gas chromatography/mass spectrometry method*

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ABSTRACT

Various solvents can be used in the synthesis of the illicit synthetic drug methylenedioxymethamphetamine (MDMA, commonly known as Ecstasy). In the crystallization process, traces of those solvents can be trapped inside crystals; during the following tabletting process, the solvent traces remain present in the tablets. The forensic investigation of tablets for solvents may increase knowledge of production methods and contribute to a possible choice of monitoring or regulating certain organic solvents. Further, the identification and quantification of solvents in MDMA tablets may contribute to the chemical characterization of illicit tablets for comparative examination.

The methods of analysis of volatile components in illicit MDMA tablets described so far are often based on solid-phase micro extraction (SPME). To avoid several disadvantages of SPME, a quantitative static headspace method was developed using gas chromatography/mass spectrometry (GC/MS); for quantification, the standard addition method appeared to be advantageous. The residual solvents in 155 MDMA tablets were analysed and 150 of them were quantified.

Keywords: MDMA; Ecstasy; static headspace; residual solvents; volatiles; illicit drugs; tablets

Introduction

The analysis of synthetic drugs in a forensic laboratory can be performed on various levels, the number and depth of which usually depend on the aim of the analysis, as follows:

*The authors wish to thank C. Koper for her assistance in preparing this manuscript and E.R.A. Lock for his valuable comments.

(a) Physical description of the material: for powders, often limited to colour and type of material; in the case of tablets and capsules, usually including more descriptors such as shape, dimension, weight and/or imprint (logo) [1];

(b) Qualitative analysis to determine the presence of one or more controlled drugs;

(c) Qualitative analysis of other compounds added to the main drug as so-called "cutting agents" or, in the case of tablets, as "excipients";

(d) Quantitative analysis of the controlled drug;

(e) Quantitative analysis of other controlled drugs, if present, and/or one or more non-controlled drugs;

(f) More in-depth analysis, which may consist of qualitative and quantitative analysis of organic by-products that may be present as a result of the natural origin of compounds or of chemical synthesis or a combination of both. Depending on the type of drug, this will be done on a percentage level or on a trace level [2-12];

(g) "Other" chemical or physical analysis characterizing the material, such as the determination of (trace) elements [13] or isotope-ratio-mass spectroscopy [14].

The number of analyses carried out on a specific item is usually determined by the aim of the analysis. Most often analysis will be related to criminal cases, where the assessment of the identity of the main drug is the main target for court purposes. In many jurisdictions a quantitative analysis is also required, although that requirement may be related to certain (weight) limits being surpassed or to the type of violence involved. Such analyses are not costly, mainly because of their relatively "routine" character; the laboratory time needed for a full qualitative and quantitative analysis of the main drug can be estimated at between 1 and 2 hours per item. More time will be needed if pictures have to be taken or if database registrations are required.

In many forensic laboratories, the types of analysis mentioned under points *(c)-(g)* are not "routine" procedures since they are time-consuming and costly, and not strictly necessary in the majority of cases. However, many laboratories perform (some) comparative analysis in response to requests from police or prosecutors in specific cases. Here, the aim is to link specific samples, cases or suspects to each other.

In some laboratories, extensive analysis is performed with the aim of obtaining strategic information, that is, information that may not be directly used in specific cases but that may be useful in gaining insights into type and scale of production and may reveal possible links between certain seizures that were not previously expected.

In a comparative analysis for casework, a number of characteristics of item A are determined and compared with those of item B. If they match, the samples may have a link. There are no strict rules about how many

characteristics should be compared, while the degree of similarity may also be a point of discussion. Nevertheless, there is a logical approach from which a reasonable strategy can be derived. If item A is very close to item B as regards the determined characteristics, that assessment is of limited value if the characteristics are very common and shared by half of all the samples of that type of drug. On the other hand, if samples A and B are very similar and it can be demonstrated that both are different from many other, non-related samples, then the similarity is of some significance. Since the analysis of a large number of "other" samples is not done at the same time as that of samples A and B, that comparison is done by comparing the data of such samples as have been collected in a database. This is known as "retrospective" analysis [6, 15].

In practice, the casework comparative analysis of tablets of methylenedioxymethamphetamine (MDMA, commonly known as Ecstasy) is usually done by comparing the external characteristics, the MDMA content and the identity of the excipients and often by making an organic impurity profile, that is, a spectrum of the by-products (impurities) that may be present in the MDMA as a result of impurities from the base material and of side reactions during its synthesis.

In the present article, the focus is on traces from solvents that may be present in tablets. The first aim is of a strategic character. By determining the solvents present, more may be learned from the production process, more information may indicate what solvents were used and a more reliable basis may be gained for decisions as regards possible control or monitoring of those solvents. In that respect, the important role of precursor chemicals in the manufacture of illicit drugs is mentioned, as also the role of the United Nations in trying to limit their misuse for "illicit" purposes.* A second aim is in casework comparative analysis, where the identity—and amount—of a trace solvent can be useful characteristics.

Synthesis of MDMA can be performed via various routes [17], but "reductive amination" is most common. Several organic solvents can be involved in the process. In the first step, MDMA base is formed from piperonyl methyl ketone (PMK), methylamine and a reductive agent; the reaction takes place in an alcoholic solvent such as methanol, ethanol or 2-propanol (IPA). Next, the alcohol and excess of methylamine are removed by distillation. The remaining raw MDMA base is a liquid that is converted into the corresponding hydrochloride salt by dissolving it into an organic solvent, followed by the addition of hydrochloric acid to form a powder. In the illicit drug manufacturing sites found in the Netherlands, the solvent most often used was acetone, but other solvents are occasionally used. During the crystallization process, solvent molecules may be trapped in the MDMA hydrochloride crystals. After its collection, the MDMA hydrochloride is dried, either at room temperature or by heating it in an oven or in some other way. However, the occluded solvent residues are

*See, for example, the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988 [16].

usually not removed in the drying process and stay present, also when the MDMA powder is later (sometimes elsewhere) pressed into a tablet.

Since the residual solvents in MDMA tablets are at a very low concentration level, a sensitive analysis method is required to identify and quantify them. For the analysis of such solvent residues, various methods can be used [18, 19], including the recently developed solid-phase micro extraction (SPME) [20]. SPME is known as an easy and rapid technique with minimal sample handling, small sample volume requirements and high detection sensitivity [21, 22]. However, the application of SPME in the author's laboratory revealed some disadvantages [23]. For instance, the high sensitivity of the SPME fibre resulted in interfering environmental contamination. Although there are many types of fibre material available [24], fibres often fail to absorb all the polar and non-polar components of interest at the same time. Additionally, fibre saturation can affect the linearity of compounds already at the 200 parts per billion (ppb) level [23, 25], which will affect the quantitative determination. In order to obtain proper peak shapes for good quantification, additional cryo-cooling of the injector is advised. The short lifetime of a fibre (50-100 injections) makes the technique rather expensive and less convenient than it first appeared [23]. Static headspace is a well-established technique for the analysis of volatiles and is based on pre-concentration of volatiles in a closed system in an equilibrium between the liquid and the gas phase [26].

In the present research, the latter technique was investigated and validated for the qualitative and quantitative analysis of residual solvents in MDMA tablets. The qualitative results of 155 MDMA tablets are reported, together with the quantitative results of 150 of them.

Analytical procedure

Chemicals

Acetone, toluene and ethanol were purchased from Merck (Darmstadt, Germany). Tris(hydroxymethyl)aminomethane (99+ per cent), isopropanol and sodium chloride (p.a.) were purchased from Acros (Geel, Belgium). 2-butanone (p.a.) was purchased from Fluka (Buchs, Switzerland). Methanol (HPLC grade) and ethanol (glass- distilled grade) were purchased from Rathburn Chemicals Ltd. (Walkerburn, United Kingdom of Great Britain and Northern Ireland). Water was of ultra pure quality, filtered by a MilliQ System (Millipore Corporation, United States of America).

Instrumentation

Gas chromatography/mass spectrometry (GC/MS) analyses were run using Agilent 6890N GC and Agilent 5973 mass spectrometer detector. The ion source temperature was 230 °C, the quadrupole temperature 150 °C and the MS interface temperature 280 °C. The total ion current (TIC) mode was used and the atomic mass unit (amu) range was set at mass 29-200. Helium was used as the carrier gas at a constant flow of 1 millilitre per minute (ml/min). The column

was a ValcoBond VB-1, 30 metre (m), 0.25 mm, 1 μm. Oven temperature programming: 40 °C (held for 1 minute), 10 °C/min to 130 °C, then 40 °C/min to 250 °C. All injections were in split mode (20:1). A straight liner of 1.5 mm diameter was used (Agilent Technologies, Palo Alto, USA). The injector temperature was 275 °C. For the headspace sampling and injections, a Gerstel Multi Purpose Sampler MPS2 was used, equipped with a 2.5 ml gas-tight syringe and an agitator (Gerstel, Mülheim an der Ruhr, Germany). The syringe was kept at 70 °C and the agitator at 60 °C. The agitator speed was set at 500 rpm and the sample was stirred for 60 minutes. The injection volume was 1,200 μl with an injection speed of 370 μl/s. The injection penetration was set at 40 mm. After the injection, the needle was flushed with nitrogen gas for 5 minutes. Alltech (Deerfield, United States) 20 ml headspace vials and magnetic crimp caps were used.

Buffer solution preparation

The Tris buffer was prepared by dissolving 121.1 grams (g) of Tris(hydroxymethyl)aminomethane in 800 ml ultra pure water. Concentrated hydrochloric acid was added up to pH 8.1 ± 0.05. Then the solution was diluted to 1 l. The shelf life of the buffer solution was set at one month at 5 °C.

Reference standard solution preparation

A reference standard solution of ethanol, acetone, IPA, diethyl ether, methyl ethyl ketone (MEK) and toluene (each 7 g/l) was prepared in methanol by accurate weighing. The solvents were added by pipette into the methanol, and concentrations (weight to weight (w/w)) were calculated from the weights using their densities.

For safest storage, the solution was then poured into a 50 ml glass bottle with a screw cap, filled to the rim to prevent evaporation of the volatiles as much as possible. The solution was freshly made before analysis.

Control sample and samples

The control sample was prepared by homogenizing a seizure of MDMA tablets, containing 25.0 per cent MDMA hydrochloride, 61.0 per cent lactose, talc and magnesium stearate.

The test samples consisted of 155 mostly different MDMA tablets, selected from 140 cases.

Preparation of samples

To a vial was added 3.0 g sodium chloride, 5.0 ml Tris buffer (pH 8.1) and one whole MDMA tablet (not ground). The vial was immediately capped. To another vial was added 3.0 g sodium chloride, 5.0 ml Tris buffer (pH 8.1), one of the same MDMA tablets (not ground) and 3.0 μl of the reference standard solution (at room temperature). The reference standard solution was accurately added using a 10 μl syringe. The vial was immediately capped.

Results and discussion

Method development

Choice of solvent

A buffer was considered necessary to ensure that all tablets released the volatiles under the same conditions. Different buffers and pHs were tested; a Tris buffer at pH 8.1 gave the most consistent results.

Salt addition

The addition of a salt can be used to lower detection limits of the volatiles of interest [27]. The "salting out" effect is responsible for the greater partitioning of the occluded volatiles into the headspace [26]. Since the salt concentration has a pronounced effect on the volatiles in the headspace, the salt was accurately weighed into the vial in order to ensure a saturated solution.

Incubation of the sample

Various headspace parameters were optimized such as incubation temperature, incubation time and mixing speed. The agitator of the autosampler stirs the sample at a maximum speed at 60 °C for 1 hour. Under the chosen conditions, 150 of the 155 (97 per cent) of the tablets disintegrated completely in the buffer releasing the trapped volatiles. The other five tablets (3 per cent) did not fully disintegrate, but a sufficient amount of the organic solvent was released for a qualitative determination. The long stirring time (1 hour) is not a restrictive time factor, since the samples within a sequence are automatically transferred into the agitator during analysis of previous samples.

The "one-point" standard addition method

Initial experiments showed that quantification with an internal standard gave irreproducible results. This is attributed to the fact that each tablet type creates a specific type of matrix, which releases the trapped volatiles to a different extent. Therefore, the use of a standard addition method was considered essential. Thus two MDMA tablets are required for quantification. In the first measurement, the peak area of the volatile in the first tablet is measured. In the second measurement, a standard mixture of volatiles is added and the total peak area is determined. The increment of the peak area of the volatile varies, depending on the type of matrix. The real concentration of the volatile in the sample can be calculated according to formula 1, where W_o is the original concentration (parts per million (ppm)) in the sample, W_a is the added amount of volatile (ppm), A_o is the original peak area and $A(_{o+a})$ is the total peak area.

Formula 1:

$$W_o = \frac{W_a * A_o}{A_{(o+a)} - A_o}$$

In practice, tablets from the same batch show only a minimum variation in weight, so weight correction in comparative cases was seldom necessary. However, between different types of tablet, the amount of organic volatile was expressed per 100 mg of tablet; no normalization on the amount of MDMA hydrochloride was applied.

Method validation

General features of the method

For the release of volatiles from MDMA tablets, the tablets were disintegrated in a solution; for better control, a buffer solution was preferred. The repeatability of the concentrations of acetone and other residual solvents was determined in spiked buffer solutions. Subsequently, a buffer solution was used to determine detection and quantification limits.

Repeatability in buffer solutions

The repeatability of the method was tested by successively analysing 10 sample solutions. The results are summarized in table 1.

Table 1. Repeatability data for residual solvents, obtained with 10 sample preparations in buffer solutions

Solvent	*Repeatability in percentage relative standard deviation (n=10)*
Acetone	1.6
2-Propanol (IPA)	3.9
Diethyl ether	1.5
Toluene	1.4
Methyl ethyl ketone (MEK)	1.7
Ethanol	11.3[a]

[a]The high relative standard deviation percentage value of ethanol is a result of low peak area and poor peak shape, due to the polarity of ethanol.

Detection and quantification limits determined in buffer solutions

For this method, the detection and quantification limits for residual solvents in the spiked buffer solutions were determined. The results are summarized in table 2. The detection limit is determined by the smallest concentration measured with GC/MS. The values of the quantification limits fulfil the condition of S/N > 3 and are all within the linear concentration range.

Application to tablet matrices

Repeatability studies on the control sample and on three different types of MDMA tablet were performed and are reported in table 3. The repeatability of

acetone in the control sample was very close to that of the spiked buffer solutions. The somewhat higher relative standard deviation (RSD) values in the tablets compared with the homogeneous control sample may indicate an intra-batch variation. In the same table, the results of the eight-month reproducibility study are reported.

Table 2. Limits of detection and quantification (concentrations in vials), for residual solvents determined in buffer solutions

	Detection limit		*Quantification limit*	
Solvent	*Parts per billion*	*Microgram per vial*	*Parts per billion*	*Microgram per vial*
Acetone	50	0.25	100	0.5
2-Propanol (IPA)	100	0.5	200	1
Diethyl ether	1	0.005	5	0.025
Toluene	0.5	0.003	5	0.025
Methyl ethyl ketone (MEK)	10	0.05	20	0.1
Ethanol	500	2.5	1 000	5
Methanol	nd[a]	nd	5 000	25

[a]nd: not determined.

Table 3. Repeatability and reproducibility data for the acetone concentration in tablet matrices

Sample	*Tablet logo*	*Repeatability acetone (percentage relative standard deviation)*	*Reproducibility acetone (percentage relative standard deviation)*
Control sample		1.7[a]	7.9[b]
Type 1 of MDMA tablets	@	5.2[c]	7.9[d]
Type 2 of MDMA tablets	Mitsubishi	5.8[c]	8.5[e]
Type 3 of MDMA tablets	FF (2nd F upside-down)	3.9[c]	4.9[e]
Type 4 of MDMA tablets	Wooden shoe	nd[f]	10.1[g]
Type 5 of MDMA tablets	Play station/square	nd	3.5[e]

[a]n: 5.
[b]n: 21 in 8 months.
[c]n: 10.
[d]n: 14 in 8 months.
[e]n: 4 in 8 months.
[f]nd: not determined.
[g]n: 6 in 8 months.

Linearity in tablet matrices

The linearity was tested by taking four tablets from one type and adding four different volumes of a reference standard solution (including acetone, IPA, diethyl ether and toluene) resulting in vial concentrations of 5, 10, 15 and 22 ppm

respectively. Since each tablet type creates its own specific type of matrix, the linearity was further tested by analysing three other types of tablet. The resulting buffer solutions thus varied from a kind of gel matrix to a solution with a clear upper layer after sedimentation.

Although all matrices trapped the volatiles to a different extent, linear results in all four matrices were obtained for all compounds (see table 4); in table 4, the linearity maximum is given as the lowest value out of the four types of tablet. The quantification limit in matrices could not be determined, since almost all tablets already contained a certain amount of acetone.

Table 4. Linearity data (concentrations in vials), for residual solvents in methylenedioxymethamphetamine tablets, determined in four different types of tablet (four different matrices)

	Linearity maximum[a]		
Solvent	*Parts per billion*	*Microgram per vial*	*Correlation coefficient*[b]
Acetone	16	80	0.997 to 1.000
2-Propanol (IPA)	15	75	0.996 to 0.999
Diethyl ether	7	35	0.997 to 1.000
Toluene	10	50	0.992 to 0.999

[a]Lowest value of four linearity curves, applicable to all four tablet matrices.
[b]Lowest and highest correlation coefficient of four linearity curves.

It should be noted that when a standard addition of 25 μg acetone per vial is used, with a linearity maximum of 80 μg acetone per vial, a maximum concentration of 55 μg acetone per vial in the sample can be measured. Overall a "one-point" standard addition method can be used in a sufficient linearity range of acetone, IPA, diethyl ether and toluene. Methanol was used as the solvent for the standard addition solution and therefore not included in the linearity tests. Ethanol was not included in this study because of bad peak shapes; MEK was not included in the quantitative study because of its absence in tablets so far.

Analyses results

Application of the method

From different cases in the period 2002-2004, 155 tablets were analysed for solvents. Since five of them did not dissolve well in the buffer, 150 tablets were quantified.

Qualitative results

The qualitative results are summarized in table 5. Acetone was by far the most frequently encountered solvent, which is consistent with the findings in illicit

Table 5. Solvents detected in 155 methylenedioxymethamphetamine tablets

Solvent	*Detected in n tablets*[a]	*Samples (percentage)*
Acetone	146	94
Toluene	46	30
Diethyl ether	16	10
2-Propanol (IPA)	10	6
Dichloromethane	6	4
Ethanol	7	5
Chloroform	1	1
Trichloroethane	1	1
Methyl ethyl ketone (MEK)	0	0
Methanol	0	0
Specification of acetone and one other solvent		
Acetone (only)	78	50
Acetone + toluene	42	27
Acetone + diethyl ether	10	6
Acetone + IPA	9	6
Acetone + ethanol	7	5
Solvent combinations without acetone		
Ethanol + IPA	1	1
Diethyl ether + toluene	4	3
No solvents detected	2	2

[a]The total number is more than 155, because combinations of two or more solvents in some tablets were observed. The number of tablets containing two solvents are specified in the lower section of the table.

laboratories seized in the Netherlands. Thirty per cent of the samples contained toluene. This was not consistent with the author's experience in illicit laboratories, where toluene was almost never encountered. The explanation was found in the extreme sensitivity of the system to toluene; the concentrations found do not point to its use in the process, but to other, so far unidentified, sources. Contamination within the forensic laboratory was excluded by the analysis of a large number of blanks, control samples and excipients, none showing any toluene. In future investigations in illicit laboratories, an attempt will be made to find the source of toluene, which may be the solvents and precursor chemicals used, such as PMK, in which toluene has already been found.

Diethyl ether and IPA were known to be used in the illicit production. MEK was not detected in any of the MDMA tablets analysed. Methanol has a high detection limit because of its polarity and was not detected in any of the tablets analysed. In 48 per cent of the tablets, combinations of two solvents were seen; six tablets had a combination of three solvents and one tablet contained five solvents. Besides the solvents listed in table 5, the aldol-condensation product of two molecules acetone 4-methyl-3-penten-2-one and some acetic acid alkyl ester-like components were observed in the chromatograms. Examples of chromatograms are given in figures I and II.

Figure I. Residual solvents in a methylenedioxymethamphetamine tablet (I)

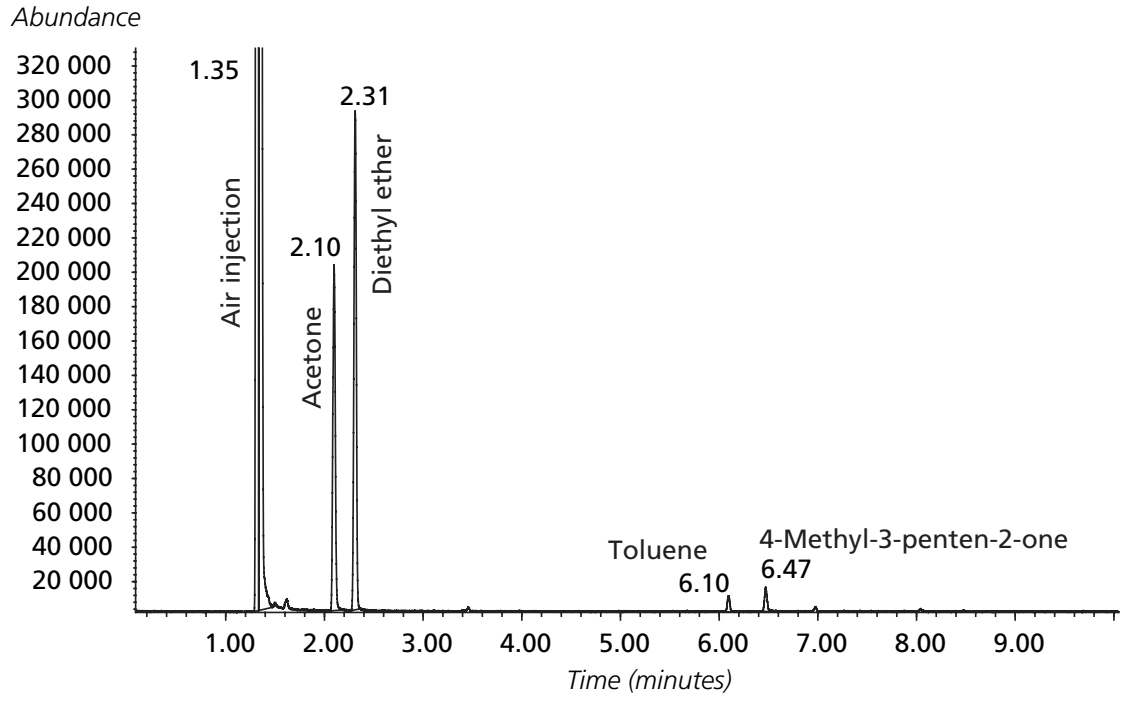
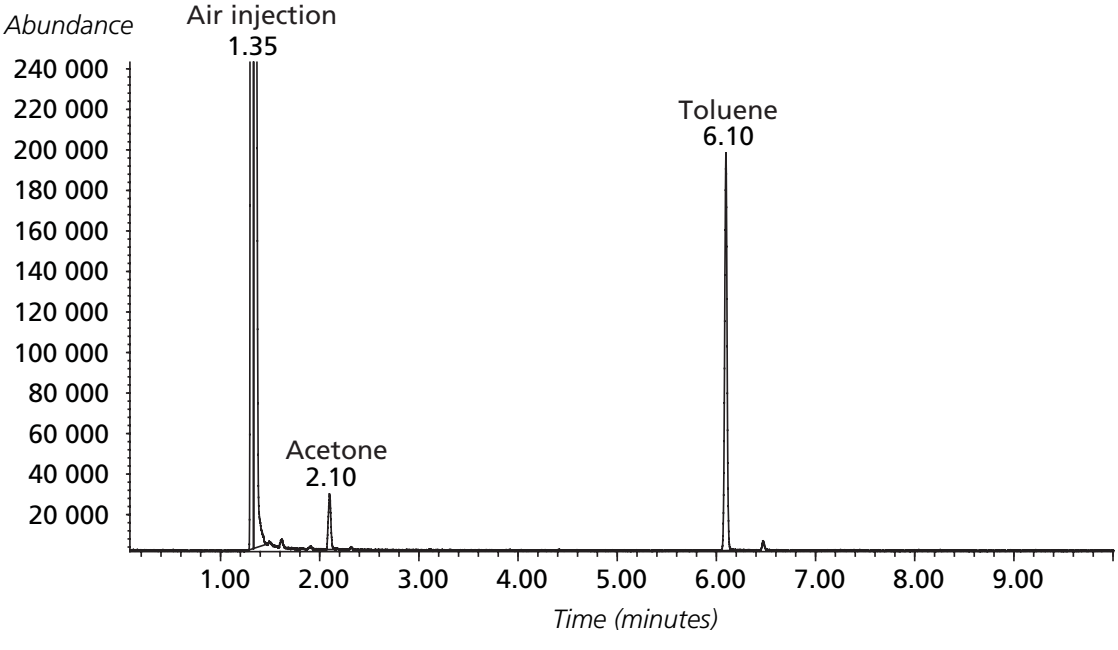


Figure II. Residual solvents in a methylenedioxymethamphetamine tablet (II)



Quantitative results

Figure III shows a chromatogram with the components of the reference standard solution, used for quantification. Figure IV depicts an overview of the acetone concentrations found in the 150 tablets. The highest measured concentration of acetone was 9.4 μg/100 mg tablet. The average concentration of acetone was 2.4 μg/100 mg tablet and the median 2.1 μg/100 mg tablet. The

Figure III. Chromatogram of a headspace analysis showing the components of the reference standard solution

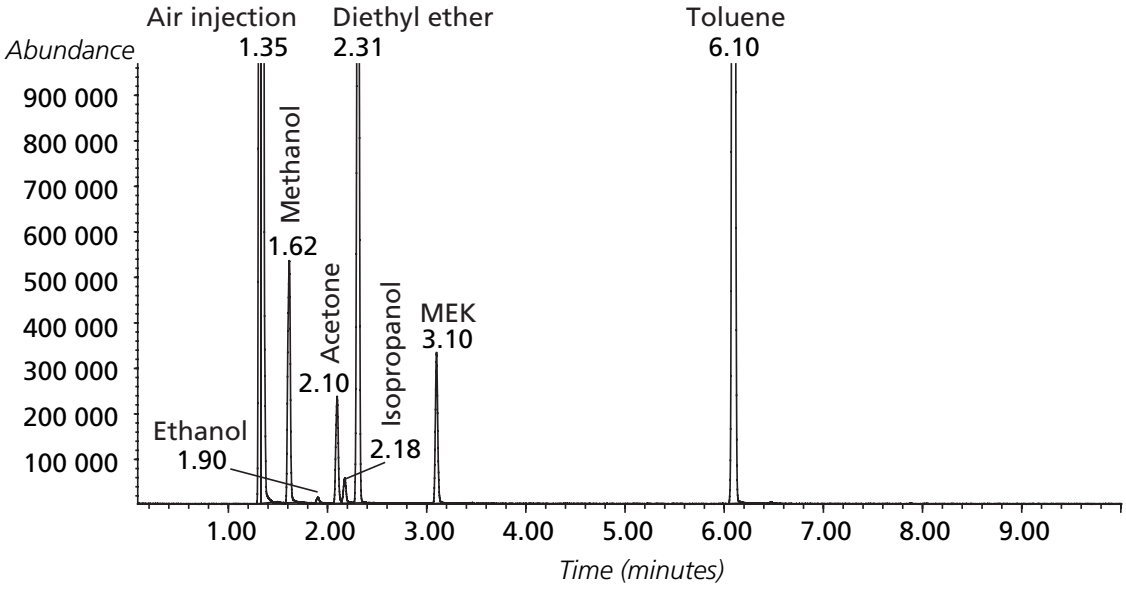
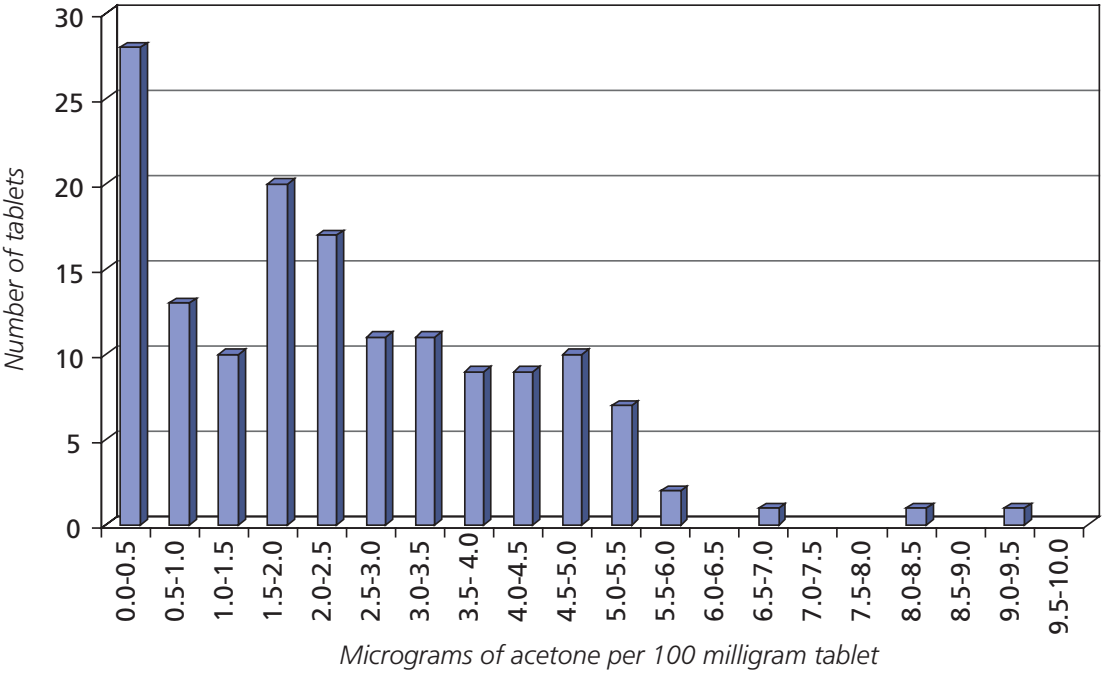


Figure IV. Acetone concentration in 150 quantified methylenedioxymethamphetamine tablets



variation in the acetone concentration makes this analytical method interesting for comparative examinations.

The highest concentration of toluene measured was 7.6 μg/100 mg tablet. In the majority of the toluene-containing tablets, it was present in a concentration of between 0.002 and 0.05 μg/100 mg tablet. Diethyl ether was quantified with a highest measured concentration of 1.9 μg/100 mg tablet. In the majority

of the diethyl ether-containing tablets, concentrations were between 0.01 and 0.2 μg diethyl ether/100 mg tablet. The highest measured concentration of IPA was 5.4 μg/100 mg tablet. The highest measured concentration of ethanol was 4.3 μg/100 mg tablet.[2] Other volatiles were not quantified.

Excipients

In order to exclude solvents originating from sources other than MDMA hydrochloride, some frequently used excipients were tested, involving various batches of lactose, glucose, starch, talc, microcellulose, caffeine and magnesium stearate, originating from illicit production sites. No residual solvents were detected.

Stability test

The stability of the acetone concentration in the control sample powder and five types of (whole) MDMA tablet were investigated by drying the samples for three hours in an oven at 60 °C. The results are presented in table 6. The control sample and two of the five tablets (types 4 and 5) did not lose acetone; the other tablets (types 1, 2 and 3) lost 60, 80 and 15 per cent, respectively, of their acetone. This supports the general opinion that the occluded acetone may be very stable in the crystals. However, it also suggests that additional acetone in the tablet can—in contrast to the trapped acetone—be removed by heating. At this point, further research needs to be done. The control sample appears to be stable over a period of eight months (see table 3). The preliminary conclusion is that the laboratory must be very careful with the interpretation of quantitative results, especially when there are indications of different sample treatment or long periods of time between the seizures.

Table 6. Stability test: acetone concentration before and after heating for three hours at 60 °C

Sample[a]	*Humidity (percentage)*	*Micrograms of acetone per 100-milligram tablet in original sample*	*Micrograms of acetone per 100-milligram tablet in heated sample*[b]
Control sample (powder)	0.2	2.11	2.18[c]
Type 1 of MDMA tablets	4.3	5.4	2.09
Type 2 of MDMA tablets	2.4	2.72	0.54
Type 3 of MDMA tablets	2.7	2.35	2.00
Type 4 of MDMA tablets	0.3	4.22	4.54[c]
Type 5 of MDMA tablets	2.8	1.65	1.64[c]

[a]See table 3 for the corresponding tablet logos.

[b]Not corrected for humidity.

[c]Values do not mean an increase since they are within the reproducibility of the method.

[2]It should be taken into account that the repeatability of ethanol is high (RSD 11 per cent) because of its polarity, resulting in low peak area and poor peak shape.

Application in casework

The method was applied three times for cases where a comparison between two tablets was requested (see table 7). Both the organic impurities and the volatiles were analysed. In two of the three cases, both the analysis of the volatiles and the organic impurity profile indicated links. In the third case, different acetone concentrations for the "cherry" tablets suggested no batch relation between the tablets. This was consistent with the results of the organic profiling: the conclusion was "not linked".

Table 7. Tablet comparisons

Comparison	*Tablet logo*	*Micrograms of acetone per 100-milligram tablet*	*Conclusion of solvent determination*	*Conclusion of organic profiling*
A1	Crown	2.8	Match	Match
A2	Crown	2.8		
B1	Alien	0.5	Match	Match
B2	Alien	0.5		
C1	Cherry	0.3	No match	No match
C2	Cherry	0.9		

Conclusions

A method was developed for the detection and quantification of solvents in MDMA tablets based on static headspace GC/MS. The different tablet matrices required the use of a standard addition method. From different cases received at the Netherlands Forensic Institute, 155 MDMA tablets were analysed for residual solvents, which were detected in all but two of the tablets. Acetone was found in 94 per cent of the tablets. This is consistent with the findings for illicit production laboratories of MDMA where acetone is widely used at the crystallization stage. The highest measured concentration of acetone was 9.4 μg/100 mg tablet. Toluene was present in 30 per cent of the tablets. In 48 per cent of the tablets, two organic solvents were detected, and in only 5 per cent, three or more solvents.

Regarding toluene, the result was not consistent with the author's experience with illicit laboratories, where toluene was almost never encountered. Since contamination in the Forensic Science Laboratory was excluded, other sources in the illicit production laboratory are suspected to contain traces of toluene, such as the solvents or precursors used. Preliminary tests showed toluene to be present in several samples of PMK, a precursor.

Another crystallization solvent is diethyl ether, which was detected in 10 per cent of the tablets, with a highest measured concentration of 1.9 μg/100 mg tablet.

Alcoholic solvents, which are used in the first synthetic stage in MDMA synthesis, were only occasionally detected in MDMA tablets, most probably because of their high detection limit, a result of their polar character.

The method developed can be used for strategic purposes. The resulting data can be useful since they give insights into the production process and the role of certain solvents. That information can be used as an intelligence tool or as an input for investigations, or as a basis for monitoring and control of precursor chemicals.

Further, it can be used in comparative analysis. It gives information on the solvents used in the synthesis of MDMA, especially on the crystallization stage. The wide variety in the concentration of the solvents may be of value in a comparison. However, it is advisable to be circumspect in drawing conclusions on the quantitative data, since at the present time there is insufficient insight into the stability of those concentrations over time.

References

1. C. Zingg, "The analysis of ecstasy tablets in a forensic drug intelligence perspective", doctoral thesis (Lausanne, Université de Lausanne, 2005).
2. T. Lukaszewski, "Spectroscopic and chromatographic identification of precursors, intermediates and impurities of 3,4-methylenedioxyamphetamine synthesis", *Journal of the Association of Official Analytical Chemists*, vol. 61, No. 4 (1978), pp. 951-967.
3. A. M. A. Verweij, "Impurities in illicit drug preparations: 3,4-(methylenedioxy) amphetamine and 3,4-(methylenedioxy)methyl-amphetamine", *Forensic Science Review*, vol. 4, No. 2 (1992), pp. 138-146.
4. R. J. Renton, J. S. Cowie and M.C.H. Oon, "A study of the precursors, intermediates and reaction by-products in the synthesis of 3,4-methylenedioxymethylamphetamine and its application to forensic drug analysis", *Forensic Science International*, vol. 60 (1993), pp. 189-202.
5. M. Bohn, G. Bohn and G. Blaschke, "Synthesis markers in illegally manufactured 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine", *International Journal of Legal Medicine*, vol. 106 (1993), pp. 19-23.
6. H. Huizer and others, "Heroin impurity profiling: a harmonization study for retrospective comparisons", *Forensic Science International*, vol. 114, 2000, pp. 67-88.
7. P. Gimeno, F. Besacier, H. Chaudron-Thozet, J. Girard and A. Lamotte, "A contribution to the chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets", *Forensic Science International*, vol. 127, 2002, pp. 1-44.
8. F. Palhol, S. Boyer, N. Naulet and M. Chabrillat, "Impurity profiling of seized MDMA tablets by capillary gas chromatography", *Analytical and Bioanalytical Chemistry*, vol. 374, 2002, pp. 274-281.
9. *Drug Characterization/Impurity Profiling: Backgrounds and Concepts* (United Nations publication, Sales No. E.01.XI.10).
10. M. Swist, J. Wilamoski, D. Zuba, J. Kochana and A. Parczewski, "Determination of synthesis route of 1-(3,4-methylenedioxyphenyl)-2-propanone (MDP-2-P) based on impurity profiles of MDMA", *Forensic Science International*, vol. 149, Nos. 2-3 (2005), pp. 181-192.

11. P. Esseiva, "Le profilage de l'héroïne et de la cocaïne : mise en place d'une systématique permettant une utilisation opérationnelle des liens chimiques", doctoral thesis, Université de Lausanne, 2004.

12. European Commission, "Development of a harmonised method for the profiling of amphetamines", final report, project SMT-CT98-2277 (Brussels, European Commission, 2003).

13. S. Comment, E. Lock, C. Zingg and A. Jakob, "The analysis of ecstasy tablets by ICP-MS and ICP-AES", *Problems of Forensic Sciences*, vol. 46, 2001, pp. 131-146.

14. F. Palhol, C. Lamoureux, M. Chabrillat and N. Naulet, "15N/14N isotopic ratio and statistical analysis: an efficient way of linking seized Ecstasy tablets", *Analytica Chimica Acta*, vol. 510, 2004, pp. 1-8.

15. H. Huizer, "A contribution to comparison", *Forensic Science International*, vol. 69, 1994, pp. 17-22.

16. United Nations, *Treaty Series*, vol. 1582, No. 27627.

17. T. A. Dal Cason, "An evaluation of the potential for clandestine manufacture of 3,4-methylenedioxyamphetamine (MDA) analogs and homologs", *Journal of Forensic Sciences*, vol. 35, No. 3 (May 1990), pp. 675-697.

18. J. Cartier, O. Guéniat and M. Cole, "Headspace analysis of solvents in cocaine and heroin samples", *Science and Justice*, vol. 37, No. 3 (1997), pp. 175-181.

19. M. D. Cole, "Occluded solvent analysis as a basis for heroin and cocaine sample differentiation", *Forensic Science Review*, vol. 10, 1998, pp. 113-120.

20. M. Chiarotti, R. Marsili and A. Moreda-Piòeiro, "Gas chromatographic-mass spectrometric analysis of residual solvent trapped into illicit cocaine exhibits using headspace solid-phase microextraction", *Journal of Chromatography B*, vol. 772, 2002, pp. 249-256.

21. K. G. Furton, Jing Wang, Ya-Li Hsu, J. Walton, J. R. Almirall, "The use of solid-phase microextraction-gas chromatography in forensic analysis", *Journal of Chromatographic Science*, vol. 38, July 2000, pp. 297-306.

22. H. Lord, J. Pawliszyn, "Evolution of solid-phase microextraction technology", *Journal of Chromatography A*, vol. 885, 2000, pp. 153-193.

23. Personal communication of M. Visser-van Leeuwen, Methode ontwikkeling voor de analyse van vluchtige organische componenten in tabletten met synthetische drugs met behulp van SPME-GC/MS, HLO afstudeerverslag NFI, June 2003.

24. C. W. Huck and G. K. Bonn, "Recent developments in polymer-based sorbents for solid-phase extraction", *Journal of Chromatography A*, vol. 885, 2000, pp. 51-72.

25. Z. Penton, "Comparison of static headspace and headspace SPME for determining EPA 5021 volatiles in water", *Varian Chromatography Systems*, www.varianinc.com, SPME Application Note 19.

26. D. R. Morello, B. S. and P. Meyers, "Qualitative and quantitative determination of residual solvents in illicit cocaine HCl and heroin HCl", *Journal of Forensic Sciences*, vol. 40, No. 6, 1995, pp. 957-963.

27. J. Pawliszyn, *Solid Phase Microextraction: Theory and Practice* (Wiley-VCH, Inc., 1997), p. 50.

Determination of inorganic elements in poppy straw by scanning electron microscopy with energy dispersive spectrometry as a means of ascertaining origin

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ABSTRACT

Cultivation of poppy as a source of opium alkaloids for legitimate medical purposes has a long tradition in Turkey. The main products are poppy straw and concentrate of poppy straw, obtained from dried poppy capsules.

The aims of the study reported in the present article were to establish inorganic element profiles for the poppy-growing provinces of Turkey by means of X-ray analysis by scanning electron microscopy with energy dispersive spectrometry (SEM/EDS) and to explore the potential of the technique for determination of origin. Ten elements (sodium, magnesium, silicon, phosphorus, sulphur, chlorine, potassium, calcium, copper and zinc) were analysed in poppy straw samples from 67 towns in nine provinces.

As regards the determination of origin, the most significant finding was the presence of copper and zinc in the poppy straw samples from 8 of the 15 towns in Afyon Province. Since those elements are not normally found in soil, it is assumed that their presence is the result of environmental (industrial) contamination.

Differences in the samples from the other eight provinces were less significant, possibly a result of their geographical proximity. Nevertheless, differences in the samples were apparent. Because the findings are relative rather than absolute in terms of presence or absence of individual inorganic elements, further research is required to convert them into operationally usable results.

The inorganic element profiles generated in the study have been used to form the basis for the development of a comprehensive database on poppy straw samples, which may be used in comparing samples and determining their origin.

Keywords: Inorganic elemental analysis; scanning electron microscope; poppy straw

Introduction

Opium poppy, which was cultivated in Anatolia during the Hatti empire, around 3000 B.C., continues to be one of the most significant economic and industrial elements in Turkey. The plant, which is grown mainly in west and central Anatolia, where the climate and ecological conditions are conducive to high alkaloid content, is cultivated as a source of opium alkaloids used for legitimate medical purposes. Because of its long tradition of cultivating the opium poppy, Turkey, like India, is officially recognized as a traditional supplier of international markets.

The production of opium alkaloids in Turkey involves the use of dried poppy capsules (poppy straw) and the concentrate of poppy straw (CPS) method, so as to prevent the diversion of raw poppy straw into illicit channels. Thus, the ancient technique of lancing unripe poppy capsules to obtain opium has been replaced by extraction and concentration of dry material by means of the CPS technique. Processing of poppy straw is centralized in one specialized factory to maintain systematic overall control. The Afyon-Bolvadin Alkaloids Factory has the capacity to process 20,000 tons of poppy straw per year.

Because of the potential for diversion from legitimate into illicit channels, determination of the origin of the various opium poppy products—opium and poppy straw—has always been of prime interest. Since the relative content of the five main opium alkaloids (morphine, codeine, noscapine/narcotine, thebaine and papaverine) varies according to country and region, traditionally, for opium, the percentage of major and minor alkaloids has been used to determine origin. However, that technique allows only for satisfactory classification of samples by region of origin (e.g. South-West Asia or South-East Asia), not by country or by area within a country. It therefore does not suffice to use only one analytical method for comparison and determination of origin.

For the samples from Turkey used in the study under review, the provinces of origin were located in a limited area close to each other (west and central Anatolia). The climatic conditions were thus similar, a fact reflected in the absence of characteristic, distinct alkaloid profiles of the samples. In view of this, alternative means of sample comparison might be advantageous, such as methods to identify inorganic elements. The content and the relative concentration of such elements in plant materials depend on soil characteristics, environmental contamination and climatic conditions.

Many studies have been made of determination of inorganic element contents of samples of opium poppy, heroin and other drugs. For example, Chiarotti and others [1] determined iron and zinc levels in heroin samples by atomic absorption spectrometry (AAS). Sanger and others [2] used AAS and the neutron activation method to evaluate inorganic compounds in illicit drugs. AAS was also used by Hernandez and others [3] for the determination of lead in heroin samples. In studies by Bermejo and others [4], electrothermal AAS was used for the analysis of metal in cocaine samples. Bermejo and others [5-11] also used electrothermal AAS and flameless AAS methods for analysis of

inorganic elements in cocaine and heroin. Infante and others [12] analysed 198 illicit heroin samples by AAS. Bora and others [13] used electrothermal AAS and inductively coupled plasma-atomic emission spectrometry (ICP-AES) for the analysis of levels of trace and major elements in illicit heroin. Those techniques might also be useful for determinating the the origin of poppy capsules and poppy straw.

In Turkey, inorganic elements have traditionally been analysed by colorimetry, AAS and AES. In the present study, 10 inorganic elements (sodium (Na), magnesium (Mg), silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), copper (Cu) and zinc (Zn)) were determined by X-ray analysis using scanning electron microscopy with energy dispersive spectrometry (SEM/EDS).

In many fields of chemistry, material science, geology and biology, detailed knowledge of the physical nature and chemical composition of the surfaces of solids on a submicrometer scale is gaining importance. Since SEM provides morphological and topographical information about the surfaces of solids, it is often the first step in the study of the surface properties of a solid. In addition, SEM furnishes qualitative and quantitative information about the elemental composition of various areas of a surface. Compared with other techniques, such as colorimetry, AAS and AES, SEM/EDS enables the simultaneous determination of all inorganic elements in a non-destructive way. In addition, interference effects, which can cause difficulties in other methods, are not a problem if SEM/EDS techniques are used.

The purpose of the study was therefore to characterize poppy straw samples originating in provinces within a closely defined area of Turkey, based on their inorganic elements. The resulting profiles reflect the relationship between elemental composition and province of origin of the particular poppy straw sample and may thus contribute to studies of determination of origin. Another important aspect of the study was the provision of comparative data in the form of inorganic element profiles for the provinces in Turkey where opium poppy is grown. Those data may support the poppy straw industry in its efforts to introduce better production procedures, since inorganic element profiles reflect soil characteristics, including environmental contamination.

The inorganic element profiles generated in the study, together with data from other studies (e.g. alkaloid profiles), have been used to form the basis for the development of a comprehensive database on poppy straw samples. The database may be used for comparisons of samples and determination of origin, as also for comparison of unknown poppy straw samples to determine whether they came from licit or illicit opium poppy cultivation. Finally, the study was also aimed at helping to identify illicit trafficking from overseas, because poppy straw from different geographical regions is expected to differ not only in its alkaloid profiles, but also in its inorganic element profiles.

By using SEM/EDS, the present study aimed at developing a robust, fast and practical method, based on modern technology, which is convenient for routine use.

Analytical procedure

Samples

Poppy straw samples were collected in the third quarter of the year from 67 towns in nine Turkish provinces: Afyon, Amasya, Burdur, Denizli, Isparta, Konya, Kütahya, Manisa and Uşak. Tables 1-9 list the towns sampled in each province.

Method

Sample preparation prior to SEM/EDS analysis consisted of two steps:

1. *Homogenization*: The poppy straw samples were powdered, homogenized and sieved using a 100-mesh sieve.
2. *Introducing surface conductivity*: The surfaces of the samples were made conductive by coating with carbon, using a carbon coater tool under the following conditions:

   Vacuum pressure: 10^{-4} torr
   Arc temperature: 2,700°C
   Pressure: 6Pa/$6x10^{-2}$ millibars
   Applied voltage: 5-6 volts
   Analysis period: 30 seconds
   Flow: 20 amperes

Each sample was analysed five times and the average results recorded. These are expressed as the percentage content of individual elements relative to the total inorganic element content.

Instrumentation

A SEM (JEOL 5410) EDS (NORAN) was used, under the following conditions:

Voltage: 20 kV accelerated voltage, tailored to the elements of interest
Working distance: 25 millimetres
Take-off angle: 25°
Counting time: 100 seconds
Magnification: 100
Maximum energy: 20 kilo-electronvolts
Detector: lithium-added silicon detector on SEM/EDS

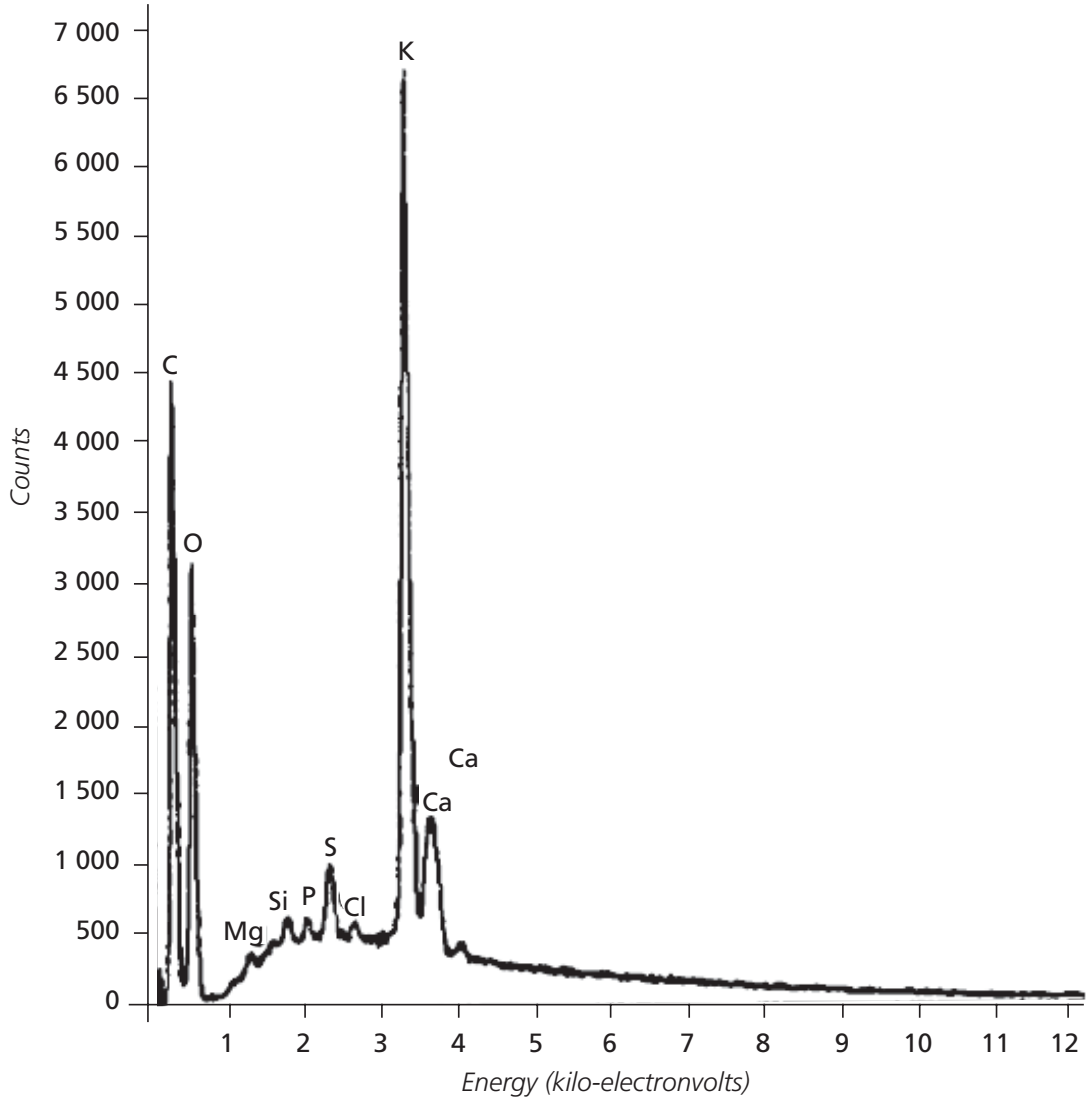
An instrument with carbon-coated evaporation power supply unit (POLARON CA 508) was used.

Results and discussion

The concentrations of 10 inorganic elements (Na, Mg, Si, P, S, Cl, K, Ca, Cu and Zn) in poppy straw samples from 67 towns in nine Turkish provinces (see

above) were determined by SEM/EDS. An SEM/EDS spectrum of poppy straw is shown in figure I. The energy levels of the peaks in these spectra provide qualitative data on the elements present in the sample. Peak amplitudes also give quantitative data in terms of percentage concentration of elements.

Figure I. Example of a SEM/EDS spectrum of a sample of poppy straw



Mg = Magnesium
Si = Silicon
P = Phosphorus
S = Sulphur
Cl = Chlorine
K = Potassium
Ca = Calcium

Tables 1-9 summarize the SEM/EDS results for poppy straw samples from individual towns, grouped by province. Inorganic element profiles of samples from selected towns (one per province) are also presented graphically in figures II-X. Table 10 summarizes the ranges of the relative concentrations of the 10 elements detected in the samples from each of the nine provinces and table 11 lists the towns with the lowest and highest concentration percentages of individual elements.

Table 1. Average[a] content of inorganic elements in poppy straw from Manisa Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Kula	—	4.60	0.91	2.91	6.99	—	63.47	21.13	—	—
Selendi	—	2.01	0.76	1.41	4.00	—	74.76	17.06	—	—

[a]N=5.

Table 2. Average[a] content of inorganic elements in poppy straw from Isparta Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Şarkikara-ağaç	—	1.30	0.41	0.82	3.71	1.91	60.29	31.55	—	—
Gönen	—	1.41	0.34	0.33	4.10	—	73.15	20.67	—	—
Keçiborlu	—	0.94	0.80	1.23	5.57	—	76.54	13.50	—	—
Yalvaç	—	1.13	0.39	0.14	4.27	1.19	55.96	36.92	—	—
Gelendost	1.10	1.31	1.33	0.52	5.50	1.15	66.59	22.55	—	—-
Merkez	—	0.74	0.61	0.52	3.31	2.64	76.42	15.75	—	—

[a]N=5.

Table 3. Average[a] content of inorganic elements in poppy straw from Amasya Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Göynücek	—	0.71	1.19	1.82	3.28	—	82.28	10.72	—	—
Gümüş-hacıköy	1.95	1.12	2.04	1.01	9.39	—	50.96	33.53	—	—
Merkez	—	3.26	1.19	2.12	4.56	1.27	69.99	16.80	—	—
Merzifon	—	1.59	0.69	2.14	4.37	—	68.44	22.78	—	—

[a]N=5.

Table 4. Average[a] content of inorganic elements in poppy straw from Konya Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Doğanhisar	—	0.96	0.51	1.52	5.38	3.65	50.11	37.77	—	—
Altınova	2.21	2.17	5.49	3.59	8.21	2.47	45.69	30.16	—	—
Derbent	—	0.50	0.61	1.22	3.10	1.41	71.49	21.67	—	—
Tuzlukçu	—	4.74	0.30	1.00	4.91	2.38	70.48	16.19	—	—
Ilgın	—	2.09	0.67	2.72	3.86	1.59	72.37	16.70	—	—
Seydişehir	1.28	1.23	0.48	0.50	4.52	0.78	63.36	27.86	—	—
Höyük	—	0.53	0.21	1.07	4.46	1.23	70.63	21.88	—	—
Selçuklu	—	1.75	0.95	2.45	4.11	1.90	56.58	32.27	—	—

[a]N=5.

Table 5. Average[a] content of inorganic elements in poppy straw from Kütahya Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Pazarlar	—	0.88	0.57	1.33	5.07	0.94	69.79	21.43	—	—
Tavşanlı	—	1.61	0.21	0.57	3.77	0.68	77.16	15.99	—	—
Merkez	1.66	1.71	0.59	2.21	4.60	0.88	68.23	20.12	—	—
Şaphane	2.83	0.84	5.82	2.09	9.87	2.64	52.12	23.79	—	—
Dumlupınar	1.46	0.88	0.38	1.26	5.60	0.48	62.90	27.05	—	—
Hisarcık	0.89	1.47	0.36	0.69	4.63	1.55	73.46	16.95	—	—

[a]N=5.

Table 6. Average[a] content of inorganic elements in poppy straw from Afyon Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Evciler	—	3.10	1.96	0.52	4.61	4.98	68.03	16.80	—	—
İscehisar	—	0.64	0.70	1.90	4.62	1.22	60.23	10.96	10.70	8.96
Dazkırı	—	1.63	0.39	3.22	4.32	2.55	59.71	11.56	9.85	6.67
Çay	1.85	2.40	0.97	0.81	5.97	1.53	59.82	26.66	—	—
Çobanlar	—	1.19	0.66	2.29	3.91	2.07	57.73	32.16	—	—
Bolvadin	—	1.64	0.66	3.54	4.88	2.80	39.42	22.62	13.96	10.49
Sincanlı	—	0.65	0.76	1.67	5.53	3.58	50.02	37.79	—	—
Emirdağ	—	0.66	0.61	2.06	5.19	3.06	67.65	20.77	—	—
Hocalar	—	0.76	0.80	0.57	4.25	0.61	48.53	19.48	14.65	10.36
Merkez	1.75	0.92	1.77	1.08	9.48	—	66.85	18.14	—	—
Bayat	—	1.05	0.94	2.15	3.48	0.95	45.76	24.95	11.68	9.04
Sultandağı-Doğancık Köyü	0.85	1.81	0.42	1.24	4.44	0.70	44.17	21.50	14.95	9.90
Şuhut	—	0.68	0.30	1.29	4.34	1.10	59.00	10.10	12.29	10.90
Sandıklı	—	1.76	0.65	3.35	3.98	2.75	42.14	23.16	11.46	10.76
Sultandağı-Yakasenek Köyü	—	1.59	0.43	0.59	4.14	—	67.55	25.71	—	—

[a]N=5.

Table 7. Average[a] content of inorganic elements in poppy straw from Uşak Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Sivaslı	2.86	0.83	1.01	1.23	10.14	2.66	61.46	19.82	—	—
Ulubey	3.23	1.78	2.59	0.56	5.35	—	51.35	35.15	—	—
Banaz	—	0.64	2.40	1.73	5.70	—	51.88	37.66	—	—
Karahanlı	—	0.55	0.53	0.47	4.32	1.08	61.23	31.82	—	—
Eşme	—	1.40	0.26	0.41	5.03	1.64	66.12	25.14	—	—
Merkez	—	1.68	0.66	1.62	5.55	1.17	70.83	18.48	—	—

[a]N=5.

Table 8. Average[a] content of inorganic elements in poppy straw from Burdur Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Karamanlı	—	2.55	0.46	1.77	4.38	—	63.35	27.50	—	—
Tefenni	—	1.13	2.02	1.63	6.40	—	63.75	25.08	—	—
Bucak	—	0.55	0.93	1.44	5.84	—	69.21	22.02	—	—
Merkez	—	2.02	2.31	4.25	8.08	—	62.87	20.47	—	—
Çavdır	—	1.13	0.79	1.54	3.93	—	71.48	21.13	—	—
Yeşilova	—	2.27	0.74	2.05	4.46	—	73.97	16.51	—	—
Kemer	—	2.86	1.35	1.17	4.79	2.87	62.95	24.02	—	—
Çeltikçi	—	1.38	1.22	0.93	4.01	1.92	64.78	25.76	—	—
Ağlasun	—	0.64	0.61	1.94	2.30	—	77.21	17.30	—	—

[a]N=5.

Table 9. Average[a] content of inorganic elements in poppy straw from Denizli Province of Turkey
(Percentage)

Towns	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Çivril	1.86	2.93	0.49	1.42	6.92	—	69.53	16.86	—	—
Acıpayam	1.56	5.26	12.69	2.84	7.17	4.06	47.61	18.81	—	—
Çardak	—	3.75	4.31	0.95	5.61	2.91	62.21	20.24	—	—
Çal	1.64	3.33	0.37	0.59	5.38	1.16	73.53	14.01	—	—
Serinhisar	2.16	5.02	1.67	2.04	7.64	1.06	59.41	21.00	—	—
Buldan	1.49	0.99	1.28	0.81	6.48	1.55	59.46	27.95	—	—
Güney	2.88	1.22	0.63	1.43	9.17	1.38	63.00	20.30	—	—
Merkez	1.18	0.77	0.82	1.14	5.93	—	69.17	17.64	—	—
Tavas	—	0.79	2.15	1.24	5.30	3.20	58.70	28.62	—	—
Honaz	2.95	2.71	1.13	2.05	9.44	1.34	60.90	19.48	—	—
Bekilli	—	1.05	0.30	0.53	3.92	2.30	70.03	21.87	—	—

[a]N=5.

Table 10. Lowest and highest concentration, and mean, of inorganic elements in poppy straw samples from selected provinces of Turkey
(Percentage)

Provinces	*Sodium*	*Magnesium*	*Silicon*	*Phosphorous*	*Sulphur*	*Chlorine*	*Potassium*	*Calcium*	*Copper*	*Zinc*
Afyon										
Range	0.85-1.85	0.64-3.10	0.30-1.96	0.52-3.54	3.48-9.48	0.61-4.98	39.42-68.03	10.10-37.79	9.85-14.95	6.67-10.90
Mean	1.48	1.36	0.80	1.75	4.88	2.15	55.77	21.49	12.44	9.64
Amasya										
Range	1.95	0.71-3.26	0.69-2.04	1.01-2.14	3.28-9.39	1.27	50.96-82.28	10.72-33.53	—	—
Mean		1.67	1.28	1.77	5.40		67.92	20.96		
Burdur										
Range	—	0.55-286	0.46-2.31	0.93-4.25	2.30-8.08	1.92-2.87	62.87-77.21	16.51-27.50	—	—
Mean		1.61	1.16	1.86	4.91	2.40	67.73	22.20		
Denizli										
Range	1.49-2.95	0.77-5.02	0.30-12.69	0.53-2.84	3.92-9.44	1.06-4.06	47.61-73.53	14.01-28.62	—	—
Mean	1.97	2.53	2.35	1.38	6.63	2.11	63.05	20.62		
Isparta										
Range	1.06	0.74-1.41	0.34-1.33	0.33-1.23	3.31-5.57	1.15-2.64	55.96-76.54	15.75-36.92	—	—
Mean		1.14	0.65	0.59	4.41	1.72	68.16	23.49		
Konya										
Range	1.28-2.21	0.50-4.74	0.21-5.49	0.50-3.59	3.10-8.21	0.78-3.65	45.69-72.37	16.19-37.77	—	—
Mean	1.75	1.75	1.15	1.76	4.82	1.93	62.59	25.56		
Kütahya										
Range	0.89-2.83	0.85-1.71	0.21-5.82	0.57-2.21	3.77-9.87	0.48-2.64	52.12-77.16	15.99-27.05	—	—
Mean	1.71	1.23	1.32	1.36	5.59	1.20	67.28	20.89		
Manisa										
Range	—	2.01-4.6	0.76-0.91	1.41-2.91	4.00-6.99	—	63.47-74.76	17.06-21.13	—	—
Mean		3.31	0.84	2.16	5.50		69.12	19.10		
Usak										
Range	2.86-3.23	0.55-1.78	0.26-2.59	0.41-1.73	4.32-10.14	1.08-266	51.35-70.83	18.48-37.66	—	—
Mean	3.05	1.47	1.24	1.00	6.01	1.64	60.48	29.01		

Table 11. Towns and provinces in Turkey with the highest and lowest concentration of inorganic elements[a] *(Percentage)*

Element	*Highest concentration*	*Town (and province)*	*Lowest concentration*	*Town (and province)*
Sodium	3.23	Ulubey (Uşak)	0.85	Sultandağı-Doğancik (Afyon)
Magnesium	5.26	Acipayam (Denizli)	0.50	Derbent (Konya)
Silicon	12.69	Acipayam (Denizli)	0.21	Höyük (Konya)
			0.21	Tavşanli (Kütahya)
Phosphorus	4.25	Merkez (Burdur)	0.14	Yalvaç (Isparta)
Sulphur	10.14	Sivasli (Uşak)	2.30	Ağlasun (Burdur)
Chlorine	4.98	Evciler (Afyon)	0.48	Dumlupinar (Kütahya)
Potassium	82.28	Göynücek (Amasya)	39.42	Bolvadin (Afyon)
Calcium	37.79	Sincanli (Afyon)	10.10	Şuhut (Afyon)
Copper	14.95	Sultandağı-Doğancik (Afyon)	9.85	Dazkırı (Afyon)
Zinc	10.90	Şuhut (Afyon)	6.67	Dazkırı (Afyon))

[a]The results represent the average of five determinations.

Figure II. Inorganic element profile of poppy straw from Bolvadin, in Afyon Province of Turkey

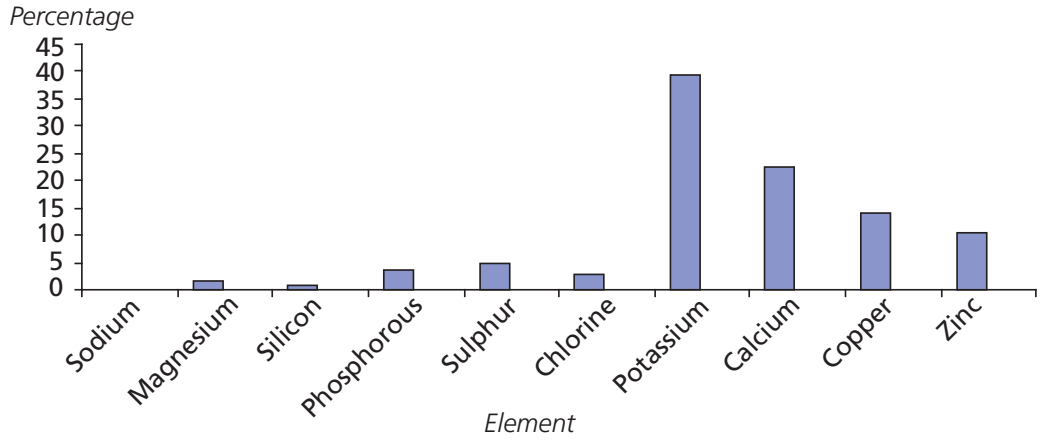


Figure III. Inorganic element profile of poppy straw from Altinova, in Konya Province of Turkey

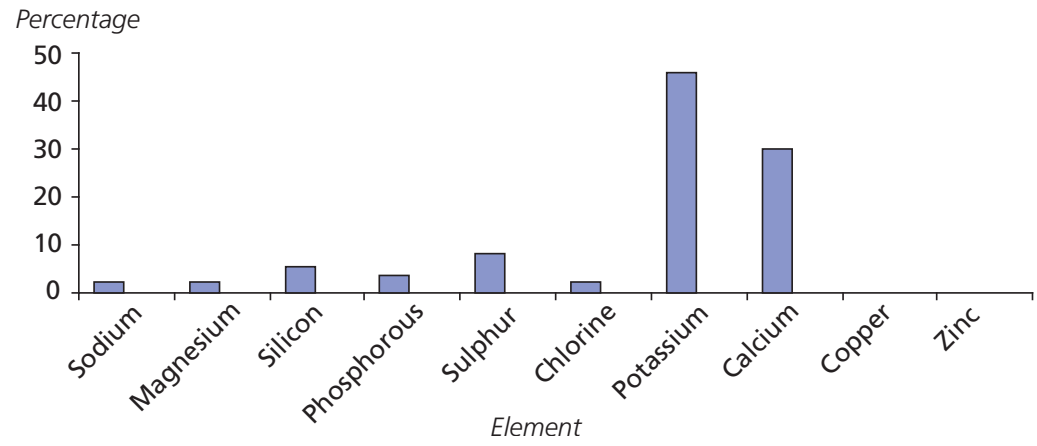


Figure IV. Inorganic element profile of poppy straw from Şaphane, in Kütahya Province of Turkey

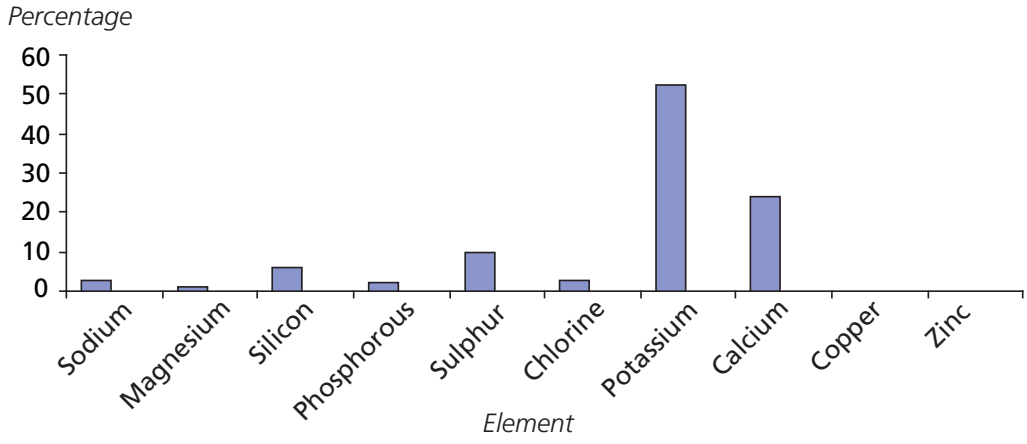


Figure V. Inorganic element profile of poppy straw from Tefenni, in Burdur Province of Turkey

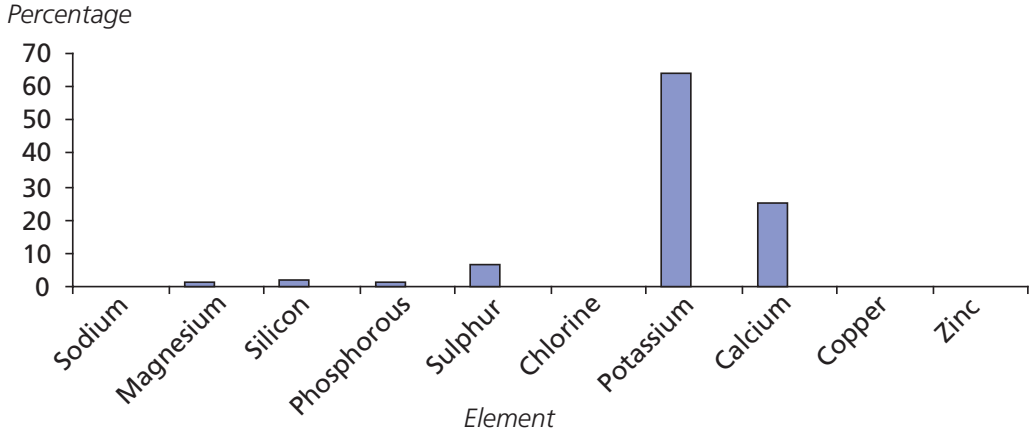


Figure VI. Inorganic element profile of poppy straw from Sivaslı, in Uşak Province of Turkey

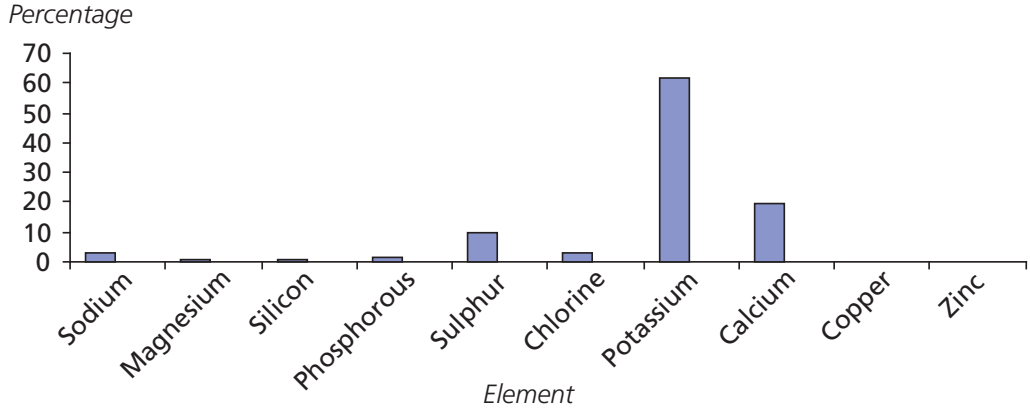


Figure VII. Inorganic element profile of poppy straw from Acıpayam, in Denizli Province of Turkey

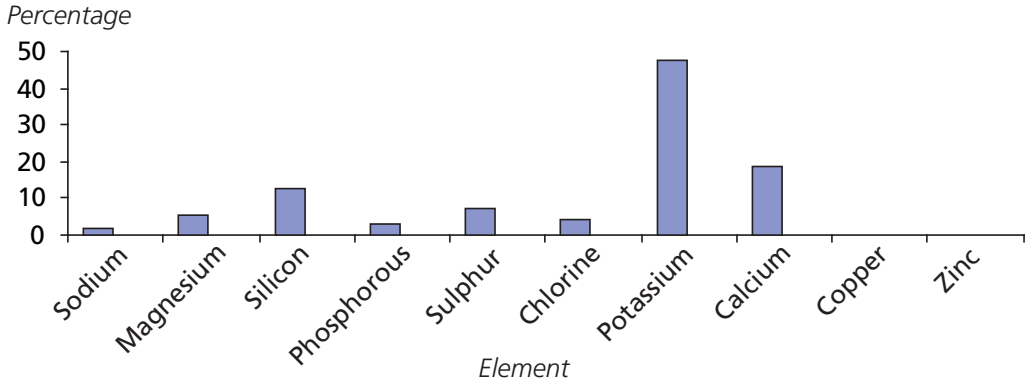


Figure VIII. Inorganic element profile of poppy straw from Göynücek, in Amasya Province of Turkey

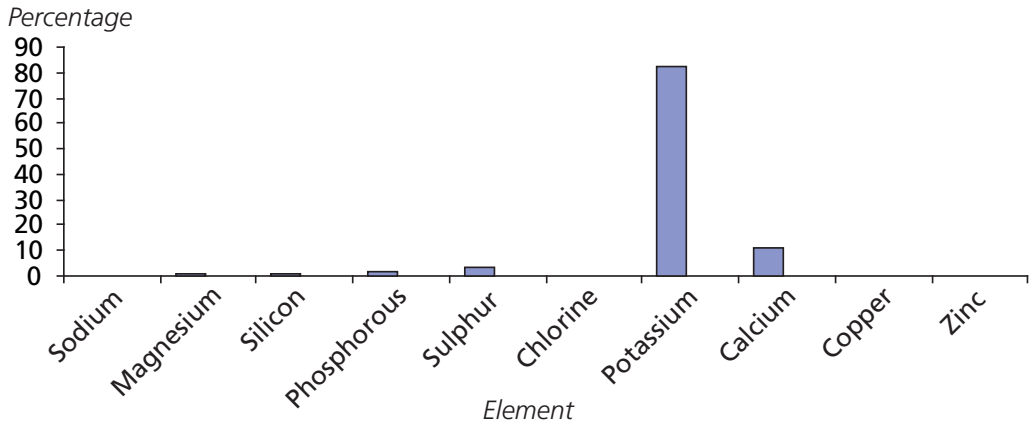


Figure IX. Inorganic element profile of poppy straw from Kula, in Manisa Province of Turkey

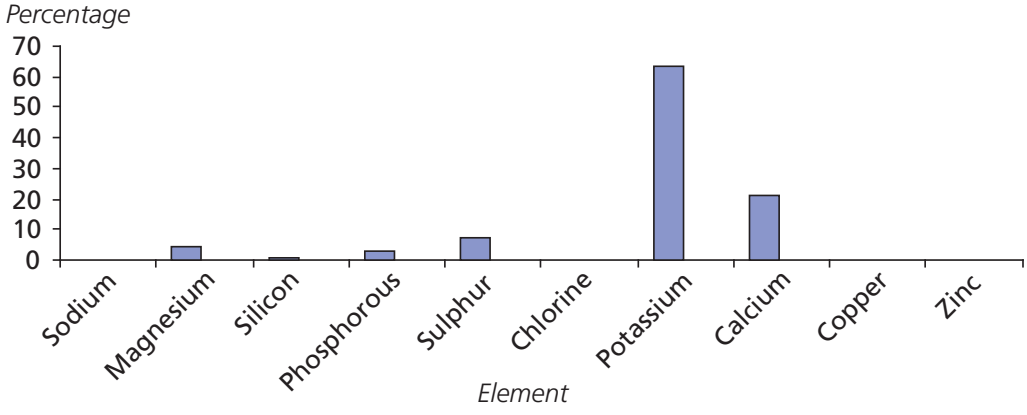
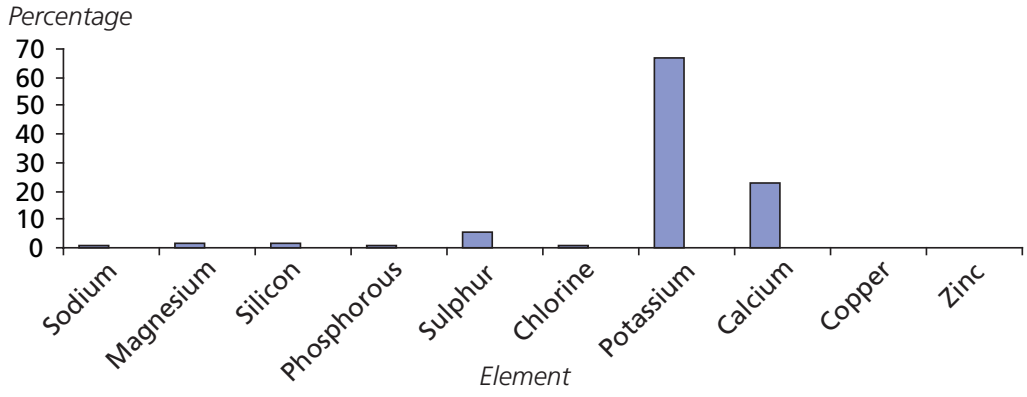


Figure X. Inorganic element profile of poppy straw from Gelendost, in Isparta Province of Turkey



Because it is used as a fertilizer, it is not surprising that potassium was found to be the element with the highest concentration in all the poppy straw samples. The second highest amount of inorganic element in poppy straw is calcium. Calcium infiltrates into poppy straw from water and calcareous soil. Sodium, magnesium, silicon and chlorine are naturally present in soil or as salts dissolved in water. The relative percentages of those elements therefore depend on the nature of the soil in which the opium poppy is grown. Phosphorous, similar to potassium, is usually added to soil by manuring. Sulphur is found mostly in land with clayey soil. However, opium poppy is not typically grown in overly clayey soils. Copper and zinc are not naturally present in soil. High concentrations of those elements suggest therefore that the soil had been exposed to environmental contamination, for example, in industrial areas.

The results presented in tables 1-9 show that, of the 10 elements, 6 (calcium, magnesium, phosphorous, potassium, silicon and sulphur) were present in straw samples from all 67 towns. Copper and zinc were only detected in samples from Afyon Province and only in samples from 8 out of the 15 towns in that province. The other two elements, sodium and chlorine, were present in some samples and absent in others, with similarities between towns of the same province. For example, sodium was not detected in any sample from Burdur or Manisa Province and only in a few samples from the five other provinces. By contrast, it was detected in almost all samples from Denizli Province and in most of those from Kütahya Province. In total, sodium was found in 21 samples. Chlorine was not detected in any sample from Manisa Province and only in a few samples from Amasya and Burdur provinces. It was detected in all samples from Konya and Kütahya provinces and in most of the samples from the remaining four provinces. In total, chlorine was detected in samples from 47 towns.

With the exception of samples from Afyon Province, which are characterized by the unique presence of copper and zinc, possibly from industrial contamination, differences in elemental profiles between other provinces were small. This may have been the result of the other provinces being close to each other geographically and very alike in soil and climatic conditions. In the absence of other characteristic environmental conditions, therefore, qualitatively similar

element profiles can be expected. Figures XI-XX show for the 10 inorganic elements the average concentration percentages of the nine provinces. Quantitative differences are evident, reflected also in more detail in tables 1-9, and show the need for further research and refined statistical analysis to turn the findings into operationally usable results.

Figure XI. Average concentration of sodium in poppy straw samples from selected provinces of Turkey

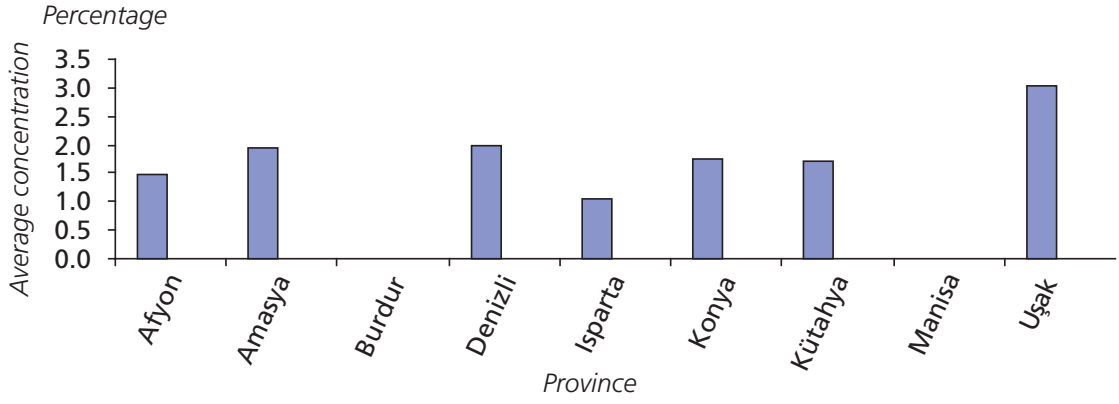


Figure XII. Average concentration of magnesium in poppy straw samples from selected provinces of Turkey

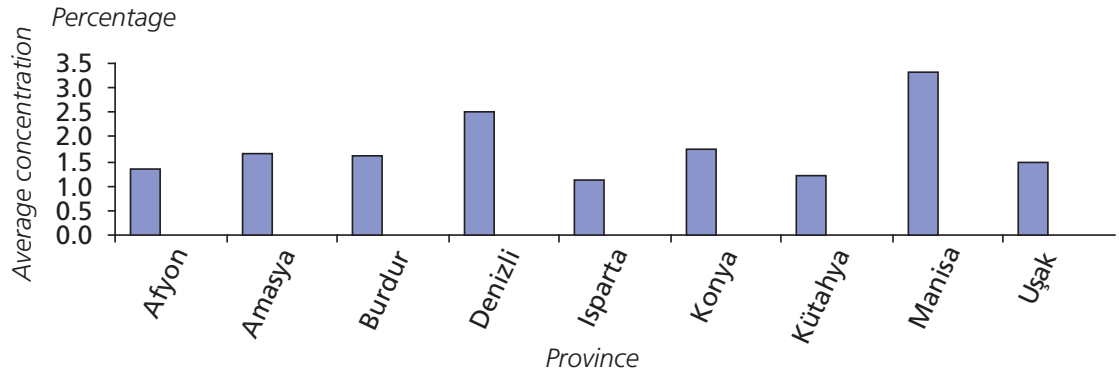


Figure XIII. Average concentration of silicon in poppy straw samples from selected provinces of Turkey

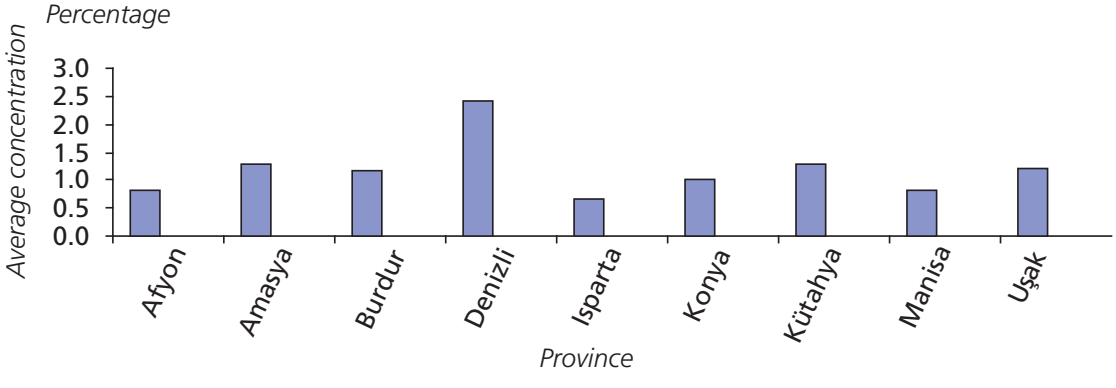


Figure XIV. Average concentration of phosphorous in poppy straw samples from selected provinces of Turkey

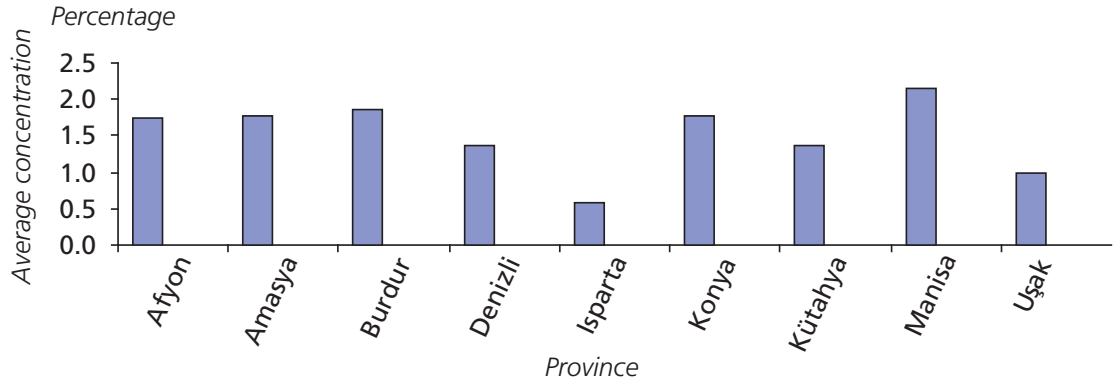


Figure XV. Average concentration of sulphurs in poppy straw samples from selected provinces of Turkey

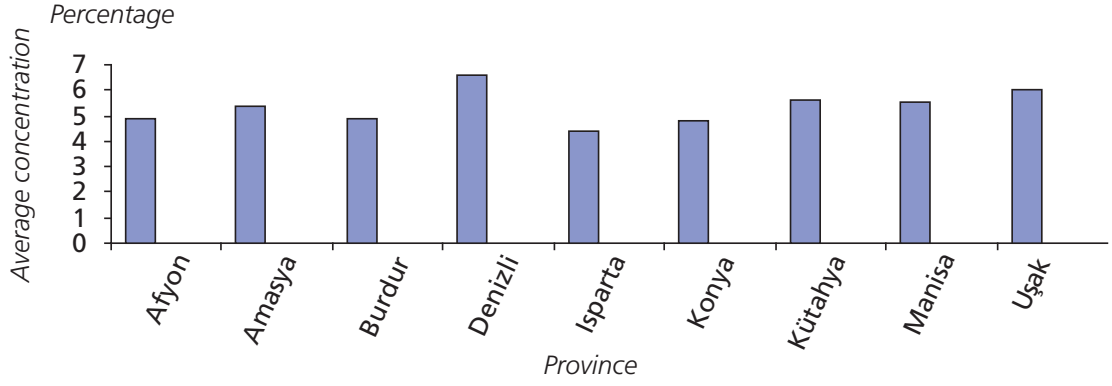


Figure XVI. Average concentration of chlorine in poppy straw samples from selected provinces of Turkey

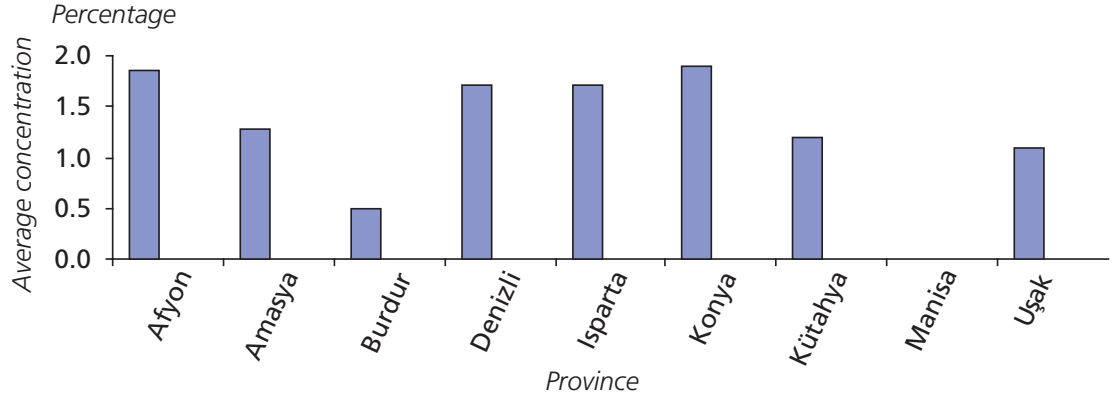


Figure XVII. Average concentration of potassium in poppy straw samples from selected provinces of Turkey

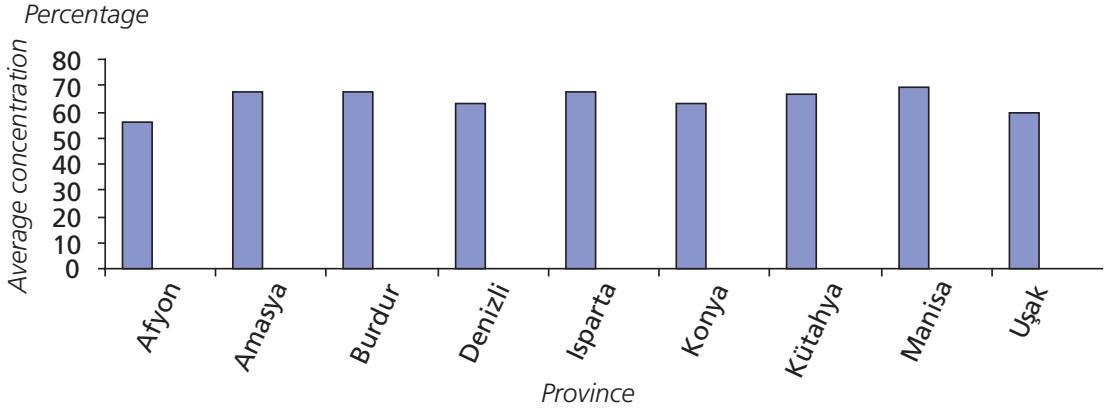


Figure XVIII. Average concentration of calcium in poppy straw samples from selected provinces of Turkey

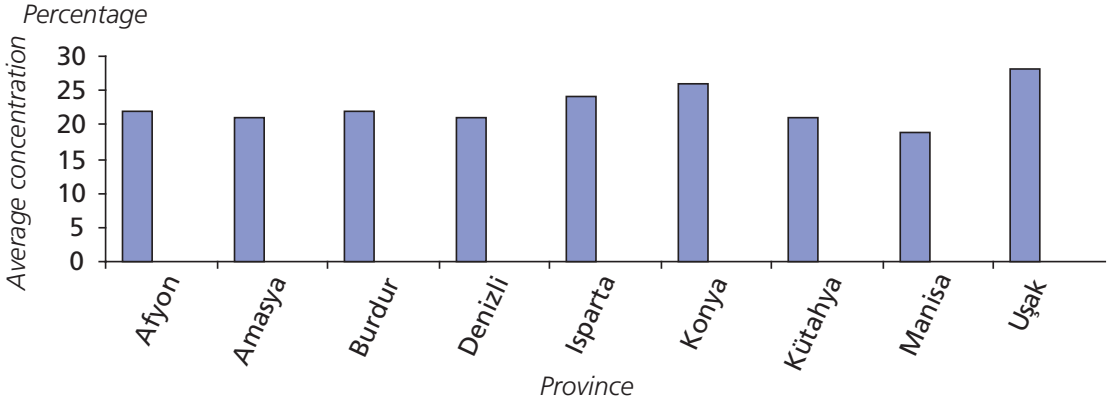


Figure XIX. Average concentration of copper in poppy straw samples from selected provinces of Turkey

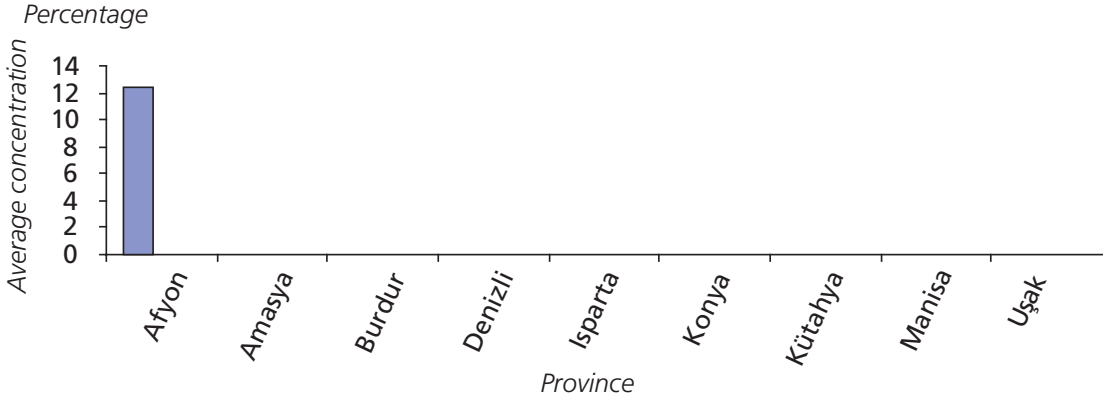
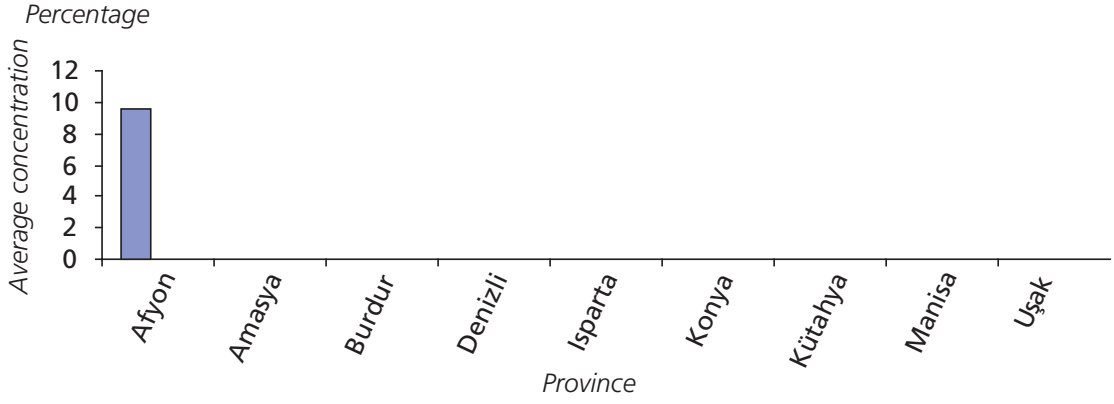


Figure XX. Average concentration of zinc in poppy straw samples from selected provinces of Turkey



With regard to the second objective of the study, to provide comparative data for poppy straw samples from the nine provinces in Turkey where opium poppy is grown, figures II-X show inorganic element profiles of samples from selected towns (one per province). From the figures, and despite the dominance of potassium and calcium in most profiles, it is clear that minor differences in element profiles do exist. However, those differences are relative rather than absolute in terms of presence or absence of individual inorganic elements.

For example, the straw sample from the town of Acıpayam, in Denizli Province showed a significantly higher silicon concentration (12.69 per cent, see table 9) than all the other samples analysed (see table 10). This is assumed to be the result of a higher silicon content of the soil at the high altitude of Acıpayam. The straw sample from the town of Göynücek, in Amasya Province is characterized by a slightly higher than average potassium content (82.28 per cent, see tables 3 and 10). The potassium content is also higher than average in four other provinces (Burdur, Isparta, Kütahya and Manisa, see table 10). Although potassium is also present in soil naturally, manuring practices are thought to be responsible for the high potassium content. Differences in manuring practices are also presumed to be responsible for the low phosphorous concentration (0.14 per cent) found in the sample from Yalvaç, in Isparta Province (see table 2). By contrast, calcareous soil or water could be responsible for the high concentration of calcium in samples from Uşak Province (see table 7). Soil characteristics are also likely to explain the higher than average magnesium content of samples from Manisa Province (see table 10).

The reasons for these differences, and their implications, are not yet fully understood, but are the subject of further investigation.

Conclusion

In Turkey, inorganic elements have traditionally been analysed by colorimetry, AAS and AES. The study reported here explored the potential of SEM/EDS as a means of establishing inorganic element profiles for the opium-poppy-growing

provinces of Turkey for comparative analysis and determination of origin. Ten inorganic elements (Na, Mg, Si, P, S, Cl, K, Ca, Cu and Zn) were identified in an initial set of 67 poppy straw samples from 67 towns in nine provinces in Turkey. The validity of SEM/EDS results has been confirmed by comparison with results gained using other basic methods for the measurement of inorganic elements.

For purposes of determination of origin, the most significant finding was the presence of copper and zinc in the poppy straw samples from Afyon Province. Differences in the samples from the other eight provinces were less significant, possibly as a result of their geographical proximity and the similarity of their soil and climatic conditions. Further studies will examine poppy straw samples from a wider area, including from different countries. In addition, the relationship between different climatic conditions and soil characteristics, on the one hand, and the inorganic element profiles of poppy straw samples, on the other, will be investigated further to determine more precisely the source of the inorganic elements.

The inorganic element profiles generated in the study have been used to form the basis for the development of a database, which will provide comprehensive sets of data on poppy straw samples, including inorganic element profiles, soil characteristics and traditional data, such as alkaloid profiles. These data may be useful not only in comparing samples and determining origin, but also for the poppy straw industry. More specifically, the detection of similarities or differences in inorganic element and/or alkaloid profiles of unknown samples in relation to straw samples of Turkish origin may contribute to controlling licit opium poppy cultivation and to the identification of illicit opium poppy cultivation or trafficking from overseas.

Ultimately, with a large enough set of samples analysed, it is hoped that the technique used here will also help to establish links between poppy straw and the seeds from which the poppies were grown.

In terms of analytical technique, SEM/EDS has proved to be a robust, fast and practical technique, which is convenient for routine use. Interference effects, which may be problematic in other techniques, are minimized. Additional advantages are that all inorganic elements can be determined simultaneously, in a non-destructive way, that is, SEM/EDS samples can be re-analysed using other methods (for example, AAS or AES).

References

1. M. Chiarotti, N. Fucci, C. Furnari, "Comparative analysis of illicit heroin samples", *Forensic Science International*, vol. 50, No. 1 (1991), pp. 47-55.

2. D. G. Sanger, I. J. Humphreys and J. R. Joyce, "A review of analytical techniques for the comparison and characterization of illicit drugs", *Journal of the Forensic Sciences Society*, vol. 19, No. 1 (1979), pp. 65-71.

3. C. Hernandez-Cueto, A. Luna and E. Villanueva, "Niveles de plomo en muestras de heroína; método para la identificación de las diferentes muestras", *Revista de Toxicología*, vol. 2, No. 2 (1985), pp. 115-120.

4. P. Bermejo-Barrera and others, "A study of illicit cocaine seizure classification by pattern recognition techniques applied to metal data", *Journal of Forensic Sciences*, vol. 44, No. 2 (1999), pp. 270-274.

5. P. Bermejo-Barrera and others, "Determination of lead in illicit drugs by electrothermal atomic absorption spectrometry using palladium as a chemical modifier", *Analytica Chimica Acta*, vol. 310, No. 2 (1995), pp. 355-367.

6. P. Bermejo-Barrera and others, "Effectiveness of palladium as a chemical modifier for direct silver and manganese determination in cocaine and heroin by electrothermal atomic absorption spectrometry", *Talanta*, vol. 43, No. 10 (1996), pp. 1783-1792.

7. P. Bermejo-Barrera and others, "Dosage d'aluminium et strontium dans les stupéfiants par spectrométrie d'absorption atomique électrothermique", *Analusis*, vol. 24, No. 6 (1996), pp. 263-266.

8. P. Bermejo-Barrera and others, "Determination of traces of chromium in cocaine and heroin by flameless atomic absorption spectrometry", *Talanta*, vol. 43, No. 1 (1996), pp. 77-87

9. P. Bermejo-Barrera and others, "Direct determination of nickel in heroin and cocaine by electrothermal atomic absorption spectrometry using deuterium arc background correction combined with chemical modification", *Journal of Analytical Atomic Spectrometry*, vol. 10, No. 11 (1995), pp. 1011-1017.

10. P. Bermejo-Barrera and others, "Copper determination in cocaine and heroin by electrothermal atomic absorption spectrometry using palladium-magnesium nitrate and nitric acid as chemical modifiers", *Química Analítica*, vol. 14, 1995, pp. 201-209.

11. P. Bermejo-Barrera and others, "Determination of traces of cadmium in cocaine and heroin by electrothermal atomic absorption spectrometry", *Química Analítica*, vol. 16, 1997, pp. 35-40

12. F. Infante and others, "Metal contamination in illicit samples of heroin", *Journal of Forensic Sciences*, vol. 44, No. 1 (1999), pp. 110-113.

13. T. Bora, M. Merdivan and C. Hamamcı, "Levels of trace and major elements in illicit heroin", *Journal of Forensic Sciences*, vol. 47, No. 5 (2002), pp. 959-963.

Section III. Drug abuse

On-site drug testing

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ABSTRACT

Drug testing outside the laboratory environment has become widespread and provides presumptive results within minutes of collection of the specimen. This has become particularly useful for testing of urine and oral fluid. Applications include workplaces where drug use has safety implications, drivers of vehicles at the roadside and situations where drug impairment is suspected. The present article explores the relative advantages of this form of testing for the specimens that can be collected and discusses issues such as cut-offs, the need for laboratory confirmation and safeguards to ensure legal defensibility.

Keywords: on-site drug testing; cut-offs; initial tests; confirmatory tests; quality management

Introduction

In recent years drug testing has undergone a technological revolution. Drug testing has become more sensitive and more flexible. New confirmation techniques have also become available for laboratory-based testing. In addition, the number of kits for initial testing (often called "initial screening"*) has increased and these are now widely used. In particular, kits are now designed for on-site drug detection without the need for sophisticated laboratory screening equipment and are able to provide a presumptive (unconfirmed) drug result within minutes. However, such devices do have their limitations, which should be recognized before a decision is made to replace laboratory-based screening with on-site testing (also known as "point-of-care" testing).

The present article outlines the roles and applications of on-site testing for drugs of abuse and describes the relative advantages and disadvantages of this form of testing compared with laboratory testing. The focus is on testing for drugs controlled under the international drug control conventions. The article does not cover clinical applications of on-site drug testing for therapeutic substances, but includes a review of the specimens that can be used with this application.

*The process of testing a specimen for the presence of drug or metabolite using non-specific class tests.

Applications

On-site testing spans a range of applications that are similar to laboratory-based testing. Primarily it provides a rapid presumptive drug result at the site where drug use is not desired. This may be a workplace where testing is conducted on a random basis or following an incident or it may be at the roadside and involve the driver of a motor vehicle. Other applications include testing of inmates of prisons and other correctional institutions and monitoring by drug courts of efforts of former drug abusers to abstain from abusing drugs.

One of the most frequently conducted on-site drug tests is for alcohol (ethanol). Breath tests are commonplace throughout the world and, depending on the device used, are considered a reliable indication of the presence of alcohol in the body.

A result that can be obtained in this way for abused drugs can assist the employer, medical practitioner or other authority in deciding if drug use may have adversely affected a person or indicate that the person is a drug abuser. It must be stressed that the on-site test result may not be confirmed by subsequent laboratory analysis and is by definition an unconfirmed positive. While this does not occur too often, users of on-site testing should ensure that they have performed a thorough risk analysis on the application of this form of testing, as opposed to laboratory-based testing, which may take a day or more to produce a result.

Specimens

The specimens primarily used for on-site testing are oral fluid (saliva) and urine. The relative advantages of these types of specimen are listed in table 1. They have also been described elsewhere [1-3].

Table 1. Relative advantages and disadvantages of on-site specimens

Urine	*Oral fluid*
Large volume (up to 50 ml)	Limited volume (up to 1 ml); sometimes not collectable in a reasonable time frame
Privacy issues for collection; requires a toilet	Few (if any) privacy issues
Significant potential for adulteration or substitution	Low potential for adulteration and substitution, although can be affected by pH changes, food debris and rinsing with fluids
Metabolites predominately detected	Parent drugs predominately detected
Drug data reflect only past use	Drug data usually reflect recent use

Urine has been the specimen taken most frequently for on-site drug analysis. Its main application is detection of past use of abused drugs. For most drugs, this means that a result reflects use, or abstinence, for 1-3 days, although in the case of cannabis, excretion of cannabinoids can occur for a period ranging from many days to weeks after last use, depending on the amount and frequency of exposure. Table 2 summarizes the approximate detection times for commonly abused drugs in urine and oral fluid.

Table 2. Approximate detection times of selected drugs in urine and oral fluid

	Detection time (days)[b]	
Drug[a]	*Urine*	*Oral fluid*
Amphetamine	1-3	1
Methamphetamine	1-3	1
MDMA[c]	1-3	1
Cocaine	1-2	<1
Morphine	1-3	<1
Cannabis	3-4	<1
Diazepam	7-21	1-3

[a]Data refers to the main type found in the respective specimen.
[b]Detection times will vary depending on amount used and duration of exposure.
[c]Methylenedioxymethamphetamine, commonly known as Ecstasy.

Urine output and subsequently drug concentration is subject to considerable variation. Of most importance is the degree of hydration and overall kidney function of a person. Persons who are mildly or substantially dehydrated will have much more concentrated urine than persons who are much more hydrated. This will be reflected in the concentration of the drug in the urine at any part of the excretion profile of a substance. People who load themselves with large amounts of water (>1,000 millilitres (ml)) shortly before a urine test for drugs can reduce the concentration of drug to below the cut-off* levels applied for detection of drugs in urine. The measurement of urinary creatinine and/or specific gravity (SG) can be used to monitor for this possibility. For example, creatinine concentrations of less than 200 milligrams (mg) suggest diluted urine, as does a low SG (less than 1.0030). For this reason it is usual to conduct validity tests on urine to ensure that no adulteration has occurred. Such validity tests can also include colour of urine, pH of urine and tests for substances added to urine to potentially affect the drug screening process, including oxidizing agents such as glutaraldehyde, chromium, nitrite and halogens. Most of these factors can now be screened for on-site using multifunctional test strips.

*A cut-off is a threshold concentration above which drug presence is reported.

Sometimes pathological changes will contribute to unusual drug concentrations in urine. All results should therefore be reviewed by a medical officer or other suitably qualified person before a result leads to an action against a person.

Oral fluid (saliva) is excreted primarily by three glands, the parotid, the submaxillary and the sublingual, and also by other smaller glands. Oral fluid has a low protein content (5 per cent of plasma) and can vary in flow rate from zero to several ml/minute (min) depending on various factors, including emotional state and hunger. A review on the physiology of oral fluid is available [4].

Oral fluid can be collected less invasively than urine, in that privacy issues are not involved. The subject can either take a specimen him/herself or allow a collector to take the fluid without regard to privacy issues. The collection of oral fluid can occur through use of an absorbent sponge or wad placed in the mouth for a short time, usually one or several minutes, depending on the amount of fluid in the oral cavity. In some cases a simple "swipe" on the surface of the tongue or inside the cheek can provide an adequate sample for on-site analysis. Since the specimen can be viewed by a second person while it is collected, issues such as potential adulteration are less of an issue than for urine, although it is still possible that the oral cavity can be treated with some substance prior to collection in order to affect the amount or composition of the oral fluid itself.

It may happen that oral fluid cannot be collected within a few minutes because of a dry mouth. People can experience a dry mouth if they are dehydrated or nervous. Alternatively, drugs such as cannabis and the amphetamines can also cause a dry mouth.

Cut-off and threshold concentrations

Cut-off concentrations are applied to immunoassay initial testing methods. For example, a positive result for cannabinoids (i.e. >50 ng/ml cut-off) in urine will require confirmation by gas chromatography/mass spectrometry (GC/MS). Any result below this cut-off is reported as "not detected". A "not detected" result implies only that no drug was detected at or above the cut-off value chosen. The choice of a lower cut-off value or the use of a more sensitive assay that has a lower threshold concentration may subsequently detect the presence of drug.

Any programme designed to detect drugs will need to take into account the concentrations of drugs that can be detected with reasonable reliability. On-site devices are no different from laboratory-based initial tests in that the detectability of drugs in a particular specimen will vary from drug to drug and also between commercial products. This is further compounded by the lack of international harmonization of cut-offs [5]. For example, the initial screening cut-offs for opiates may vary from 300 ng/ml (Australia, Europe) to 2,000 ng/ml (United States of America). The choice of a higher cut-off for opiates (i.e. 2,000 ng/ml) has the advantage of reducing the detection of codeine users that are ordinarily not an interest group as distinct from persons using heroin. For heroin users, 6-acetylmorphine can be monitored in urine as a specific marker for heroin use.

For class tests* not all members of the family will have similar detection limits for all drugs within the class. For example, for amphetamines, opiates and benzodiazepines classes, the sensitivities of the initial test will be different for the various drugs. These can vary considerably, so it is important that the selection of on-site testing device best reflects the needs of the testing authority. For example, in countries in Western Europe amphetamine is much more common than methamphetamine, whereas in Australia, the United States and countries in South-East Asia methamphetamine is much more frequent. A monoclonal test kit for amphetamine will not detect methamphetamine and vice versa. Consequently, the appropriate immunoassay needs to be selected to target a specific type of amphetamine.

Confirmatory or final testing

Whatever type of initial screening test is conducted, the specimen must be subject to confirmatory testing** in a properly certified laboratory. This should occur as soon as feasible after the initial test, using techniques that have been appropriately validated on the specimen (or specimens) of interest. The preferred technique should involve mass spectrometry (MS), since this is far more specific and sensitive than most other techniques and is universally accepted as the most reliable and specific technique for final testing.

MS can be linked to a gas chromatograph (GC-MS), a liquid chromatograph (LC-MS) or even to a capillary electrophoresis instrument (CE-MS). Some laboratories will be able to use tandem mass spectrometry (MS-MS) or high-resolution MS such as time-of-flight MS (TOF-MS). Whatever the technique, it is important that the detection limits or cut-offs applied to confirmation testing are the same, or preferably lower, than the initial testing threshold concentration. This avoids not being able to confirm an initial on-site positive because of insufficient sensitivity. Most drugs are metabolized and metabolites will often cross-react with antibodies used in most on-site testing devices, thus giving a higher apparent concentration of the drug than is actually present in the specimen, since one substance is being targeted in the final testing. For example, in urine cannabinoids (all cannabis metabolites) are often screened with a cut-off of 50 ng/ml, but the metabolite carboxy-tetrahydrocannabinol (THC-COOH) is confirmed with a cut-off of 15 ng/ml.

Quality considerations

Laboratories performing any form of drug testing are expected to conduct their procedures using standardized and validated methods by appropriately trained staff. The laboratory staff and the analysts should also take part in proficiency testing programmes to ensure that satisfactory results are produced. Moreover,

*Tests that detect more than one member of a class of drugs (amphetamines, opiates etc.).

**Final testing that determines unequivocally the presence of a drug or drug metabolite.

in many parts of the world, laboratories require accreditation or another form of certification to ensure that all aspects of the testing meet current scientific and quality system standards. For example, in many parts of the world the International Organization for Standardization ISO 17025 quality standard is applied. Specific technical standards are also applied in different parts of the world.

This means that laboratories testing batches of specimens should also employ blank samples, samples with known concentrations (calibrators) and quality controls to ensure that the results of each batch of specimens meet appropriate laboratory performance criteria. Only results from those batches where performance criteria are satisfactorily met should be accepted. All other results are rejected and the analysis repeated.

These principles of good laboratory practice should also be considered in on-site testing. In practice this may be more difficult, given that in on-site testing, the environmental conditions and location are much less controlled than in a laboratory. Nevertheless, it is imperative that the collection and testing process is as controlled as reasonably feasible and the staff performing the collection of specimens and the testing are properly trained; otherwise it is likely that initial on-site results will be less reliable, and that may produce a higher rate of false negatives* and false positives.**

Furthermore, it is recommended that on-site testing facilities incorporate a checking process for each batch of cases, or once daily, with known blank (drug-free) and drug-positive (control specimens or solutions) cases. Periodic testing of externally submitted proficiency material is also highly recommended. All of these quality steps provide an assurance to the client and any authority that the on-site testing is reliable and has been properly conducted.

Cost considerations

Depending on the nature of an on-site testing programme, it can lead to reduced costs compared with laboratory-based initial testing. Cost savings may be associated with reduced transportation costs of specimens for initial testing at laboratories. Larger savings are associated with the ability to act on a result at the time of collection rather than waiting for a laboratory test result. The risk of drug users at a workplace causing an accident can be significant and in the case of post-accident testing can lead to improved care management practices. There is often little difference in unit cost per initial test between laboratory and on-site device costs, but there can be significant differences depending on the local arrangements and the type of kit used [6].

*Specimens containing a drug above the cut-off that is not detected as being drug-positive.

**Positive initial test results that are not confirmed by subsequent testing.

References

1. S. George and R. A. Braithwaite, "Use of on-site testing for drugs of abuse", *Clinical Chemistry*, vol. 48, No. 10 (2002), pp. 1639-1646.
2. M. Grönholm and P. A. Lillsunde, "A comparison between on-site immunoassay drug-testing devices and laboratory results", *Forensic Science International*, vol. 121, Nos. 1-2 (2001), pp. 37-46.
3. G. A. Bennett, E. Davies and P. Thomas, "Is oral fluid analysis as accurate as urinalysis in detecting drug use in a treatment setting?", *Drug and Alcohol Dependence*, vol. 72, No. 3 (2003), pp. 265-269.
4. J. K. M. Aps and L. C. Martens, "Review: the physiology of saliva and transfer of drugs into saliva", *Forensic Science International*, vol. 150, Nos. 2-3 (2005), pp. 119-131.
5. A. G. Verstraete and A. Pierce, "Workplace drug testing in Europe", *Forensic Science International*, vol. 121, Nos. 1-2 (2001), pp. 2-6.
6. R. J. Ozminkowski and others, "The cost of on-site versus off-site workplace urinalysis testing for illicit drug use", *Health Care Manager*, vol. 20, No. 1 (2001), pp. 59-69.

Drugs and driving: the Finnish perspective

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ABSTRACT

Drugs can cause behavioural impairment of the driver's ability to operate safely. That impairment of driving ability can be documented, and biological fluids can be tested for drugs. Most countries have legislation that covers driving under the influence of alcohol and/or drugs. Some countries have introduced zero-tolerance laws (per se laws), which prohibit the operation of a motor vehicle while an illicit drug or its metabolite is present in the body, whether or not impairment is manifested. There is growing interest in using saliva (oral fluid) in preliminary roadside testing. Legislation in the state of Victoria, Australia, already allows the use of oral fluid for evidentiary testing in the case of cannabis and methamphetamine. Nevertheless, blood testing will probably remain the most common form of evidentiary testing.

It has been estimated that the prevalence of illicit drug use among the general driving population in Europe is in the range of 1-5 per cent, while the prevalence of licit drugs, such as benzodiazepines, affecting driving performance is higher: 5-10 per cent. Epidemiological research is often carried out on offenders and drivers involved in collisions. Among drivers suspected of driving under the influence of drugs, there is a high percentage of licit and/or illicit drug use, as the statistics for Finland in the present article show. The drugs of most concern are amphetamine and amphetamine-type substances, cocaine, cannabis, opiates and benzodiazepines and other sedative-hypnotics. The handling of drugs and driving cases are presented, and a summary of areas for further study are provided.

Keywords: drugs and driving; driving under the influence; behavioural tests of impairment; epidemiology

Background

While it is well known that driving performance is impaired by alcohol even in low dosages [1], many other drugs are linked to impairment. The effects of drugs other than alcohol on driving ability are more complex, and there are a number of substances with potential effects. Further, drug/drug, drug/alcohol and drug/subject interactions are to be considered. Drugs of special concern are benzodiazepines and related drugs, opioids, amphetamine, cocaine and other stimulant drugs, cannabis, antidepressants and antihistamines [2, 3].

The number of fatal traffic accidents and traffic accidents involving alcohol has decreased very markedly in Western countries over the past decades. Although the number of cars in the traffic flow has increased, significant progress has been made in reducing the number of impaired driving accidents in the industrialized world [4]. Alcohol has been a major traffic safety problem worldwide. However, alarmingly, the problem of drugs and driving is rapidly growing. In Finland, the number of road traffic accidents involving intoxicants other than alcohol has risen sharply [5]. Thus, more effective countermeasures are needed. Most countries have introduced legislation to prohibit driving under the influence of alcohol and/or drugs.

The legislative basis for drugs and driving cases

In the past 10 years, many countries have changed their legislation and procedures in order to address the problem of driving under the influence of drugs. In most European countries, drugs and driving regulations are part of general legislation on drunken driving or impaired driving.

Zero-tolerance law ("per se" law) and impairment law

Countries use two types of legislation: zero-tolerance law and impairment law, or a combination of both. The presence of drugs in the blood while driving is prohibited by zero-tolerance law. Following the analytical approach, the detection of drugs through chemical testing is sufficient for prosecution, whereas the impairment approach requires documenting the impairment of the behaviour of the driver. Under impairment law, it is the impairment because of drug use that is prohibited. Impairment law too involves testing biological fluids to determine whether a driver is under the influence of drugs. By documenting behavioural impairment and drug concentrations in the blood, the driver's ability to safely operate the vehicle can be estimated.

Belgium, Finland, France, Germany and Sweden apply zero-tolerance law for drugs and driving. The legislation varies from country to country. In Belgium and Germany, the analytical thresholds for specific drugs are set out in the law.* Only a few drugs—namely, amphetamine, methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), tetrahydro- cannabinol, cocaine and its metabolite benzoylecgonine, and morphine—are included in the zero-tolerance legislation of those countries. In Finland and Sweden, all controlled substances, including medicinal drugs such as benzodiazepines, fall within the scope of zero-tolerance drug laws if the driver does not have prescription for them.

*These cut-off, or threshold, levels are introduced for practical purposes, because any analytical procedure will always cause background "noise". In interpreting the results from blood testing in drugs and driving cases under a zero-tolerance law, measured drug concentrations below those cut-off levels are disregarded, and the results are considered negative.

A third type of legislation, establishing legal limits for blood drug concentrations, similar to existing laws on blood alcohol levels, is not currently feasible, because establishing such limits cannot easily be done, given current knowledge.

The situation in Finland

In February 2003, zero-tolerance legislation on illicit drugs and driving was introduced in Finland. The law contains a schedule of drugs including the drugs listed in the United Nations conventions on narcotic drugs and psychotropic substances (Finland's Narcotics Act (No. 1289/93) of 17 December 1993 and chapter 50 of the Penal Code of Finland). Drugs that have a potentially harmful effect on driving ability have warning labels on their package (Circular No. 1758/81 of the National Board of Health).

The zero-tolerance law is applied if controlled drugs or their active metabolites are found in the blood; it is not applied if the driver has a right to use the controlled substance (for example, if he or she has a prescription).

Before the implementation of the zero-tolerance law, the police had difficulty proving in the court impairment of driving ability. Thus, a significant portion of drugs and driving cases may have previously gone undetected in Finland. That was the main reason for the Government's introduction of zero-tolerance legislation for drugs that are hazardous or potentially hazardous to traffic safety.

However, at the same time, the impairment law remains in the background of legislation. A driver will be convicted for driving while intoxicated if it can be proved that his or her driving ability was impaired by the use of drugs. That applies to any substance. A driver will be convicted for consuming any drug, including medicinal drugs, if it can be proved that he or she was intoxicated to the point of being a threat to traffic safety (Penal Code, chapter 23).

Impairment must be proved in court. Symptoms of drug use must be documented by a police officer and by means of a clinical sobriety test, also known as a clinical performance test, conducted by a physician. Impairment must be proved also when prosecuting a driver for severe drunken driving due to drugs, that is, when the driver was so intoxicated that he or she presented a serious threat to traffic safety. To obtain a conviction for severe drunken driving attributable to the use of zero-tolerance drugs, there must be proof of impairment in addition to the detection of drugs in the blood. The statutory limit for a drinking and driving offence in Finland is 0.50 per thousand (w/w). The limit for severe drunken driving is 1.2 per thousand. The corresponding breath alcohol control limits are 0.22 mg/l and 0.44 mg/l (Law No. 655/1994 amending chapter 23 of the Penal Code).

Handling of drugs and driving cases in Finland

Police

In order to identify persons driving under the influence of drugs or alcohol, Finnish police are authorized by law to submit drivers to a preliminary test, a

breath test or an oral fluid drug test on site, even where no suspicion exists. Devices for on-site testing for alcohol and drugs have the same position under national law. The main reasons for using screening tests are random checks, impaired or dangerous driving, road traffic accidents or information from a bystander.

The police officer who arrests the driver also provides evidence of impairment. To demonstrate impairment caused by drugs, the police use a standardized field sobriety observation sheet (see annex I). All external symptoms of drug use are documented.

Physician and health-care unit

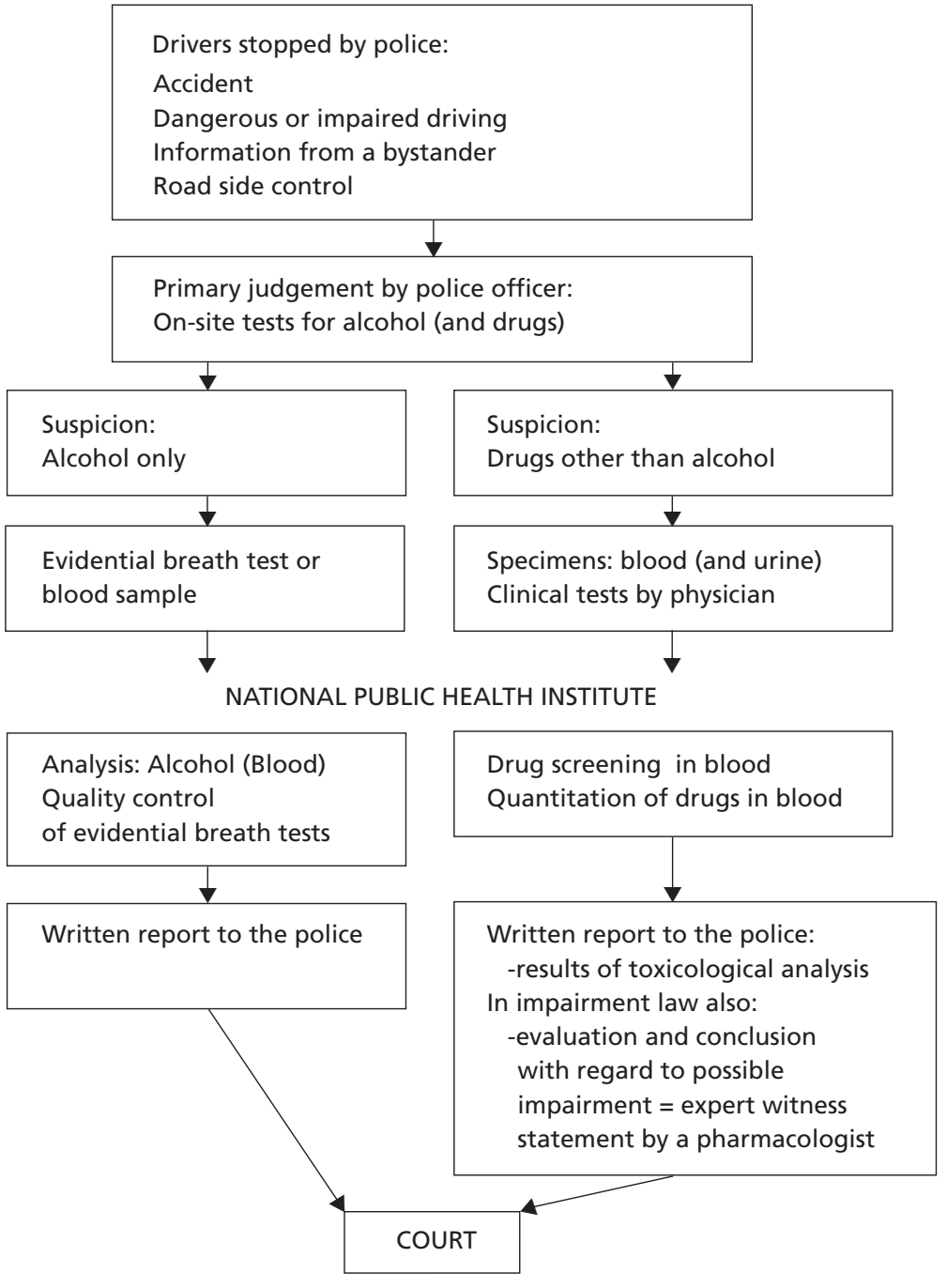
A clinical field sobriety test (see annex II) is performed by a physician at the request of the police. When the screening test is positive or when drug-induced impairment of driving-related skills is suspected, samples are taken as evidence that drugs were present in the body fluids at the time of driving. If necessary, a blood sample can be taken, even against the driver's will (according to the law on coercive means). It is recommended that both blood and urine samples be taken in cases where driving under the influence of drugs is suspected.

Laboratory

In cases where a person is suspected of driving under the influence of alcohol and/or drugs, the alcohol and drug analysis is carried out by the National Public Health Institute (KTL) of Finland. Drug analysis is performed at the request of the police. Qualitative drug screening of blood and urine is carried out, and the concentrations of substances found in the blood are measured in order to assess their possible effects on driving ability.

The written laboratory report to the police includes the results of the toxicological analysis. When zero-tolerance drugs are detected, only the test report of the toxicological analysis (qualitative and quantitative) is needed. Under impairment legislation, a pharmacological evaluation and conclusion with regard to possible impairment is also required (see figure I). The evaluation is done individually, taking into account the general characteristics of the drug, the purpose of its use, the concentration of the drug in the blood and whether drug use was acute or chronic, whenever that information can be objectively assessed, that is, using the concentration ratio of the parent drug to the metabolite.

Figure I. Handling of cases involving persons suspected of driving under the influence of drugs and/or alcohol in Finland



Prosecution

In practice, a driver under the influence of drugs is liable to prosecution if the presence of a zero-tolerance drug in his or her blood can be measured or if a significant amount of a prescribed drug or other substance can be measured, impairment of performance has been demonstrated and the drug is considered to have a possible causative role.

Courts

For illicit drugs and controlled medicines, zero-tolerance law is applied. For other substances, driver impairment must be proved in court. That proof is based on *(a)* the documentation by the police officer of external signs of drug use; *(b)* a clinical sobriety test performed by a physician; and *(c)* the laboratory report, including a pharmacological evaluation based on the test results.

External symptoms of drug use

Traditionally, police have apprehended impaired drivers by watching for signs of erratic driving. Signs of impairment are grounds for the police officer's initial suspicion (see figure I). In some countries, physicians perform the clinical tests for impairment. The observation and the documentation of external signs of drug use are an important step in the prosecution process, especially under impairment legislation. The observation sheet, in addition to the laboratory report, the physician's clinical performance test report and other possible police reports, is presented in court to show that driving ability was impaired.

Drug use may be difficult to detect. When there is no smell of alcohol, or when the preliminary on-site alcohol screening is negative, the police officer might not suspect other substances are the cause of erratic driving. In the United States of America, a drug recognition expert (DRE) system was developed for use by police officers performing traffic control. The DRE programme consists of 12 steps [6], including the opinion of a DRE on the drug class present. Several evaluations show that the decisions of the DRE are quite consistent with toxicological test findings [7, 8]. In 1997, the Homburg/Saar University and the Bundesanstalt für Straßenwesen in Germany modified the United States DRE system, adapting it to European requirements. The German police officer training programme and observation sheet were further modified for use in Finland.

On-site tests for preliminary roadside screening

The conclusion of the European Roadside Testing Assessment (ROSITA 1) project [2] was that there was a need for roadside tests, that is, preliminary tests allowing police officers to take immediate on-site measures. However, biological specimens are needed for confirmation from laboratory analysis. Roadside drug tests increase police confidence when preventing persons from driving under the influence of drugs, withdrawing driving licences and ordering drivers to give blood samples for laboratory tests. Roadside tests can thus save time and facilitate the law enforcement process. Those involved in the ROSITA project noted that drivers suspected of using drugs were impressed by on-site test results and often confessed when confronted with a positive test result, sometimes in the wake of their denial of any drug use before finding out the test result. In addition, roadside tests and public awareness of the use of such tests may have a preventive effect, because the tests increase the risk that persons who use drugs and drive will be caught [2].

On-site tests are preliminary tests based mostly, if not exclusively, on immunological methods and can give false positive results. Thus, laboratory confirmation of on-site test results, preferably using gas chromatographic–mass spectrometric methods (GC-MS), is therefore required.

While blood is considered to be the best body fluid for confirmation analysis because the presence of drugs in blood corresponds best with recent drug use and impairment, oral fluid is considered to be the best specimen for on-site drug testing. Advantages include ease of sample collection, minimal opportunity for sample adulteration or substitution and possible indications of recent drug use by the person tested. Furthermore, saliva sampling on the road is generally well accepted by the subjects, much better than urine sampling. In some countries, urine is used as a specimen for roadside testing, but adequate facilities, such as a sanitary van, are needed. In some countries participating in the ROSITA 1 project, urine sampling at the roadside was considered unacceptable [2].

However, the reliability, the sensitivity and the user-friendliness of oral fluid devices still need to be improved [9]. Projects are under way to develop devices that are more suitable to that purpose. Roadside tests are useful under both impairment and zero-tolerance legislation.

An ongoing collaborative study (ROSITA 2), which involves partners from Europe and the United States, tests commercially available oral fluid devices and evaluates their suitability and their reliability for on-site drug testing. There are plans to continue collaborative studies in this field because of the importance of the issue of drugs and driving.

Laboratory methods for detecting drugs in urine, blood and oral fluid in Finland

In the daily laboratory praxis, substances hazardous or potentially hazardous to traffic safety are screened by analysing whole blood samples using immunological methods as well as GC-MS and are further confirmed by means of separate GC-MS methods. Finnish legislation stipulates that the drugs must be detected in the blood.

Immunological screening includes illicit drugs (cocaine, cannabis, amphetamine, methamphetamine and opioids) and benzodiazepines. Immunological screening devices and methods for urine specimens are developed by commercial companies. When those devices are used with blood specimens, the sample preparation step must be modified [10, 11]. The analytical protocol used in Finland involves two comprehensive semi-quantitative/quantitative screenings: a method combining GC-MS and gas chromatography-electron capture detection [12] screens for a total of 51 compounds including, for example, 12 benzodiazepines, 3 cannabinoids, 8 opioids, cocaine, 13 anti-depressants, 5 anti-psychotics and 2 anti-epileptics, as well as carisoprodol, meprobamate, orphenadrine, tizanidine, zaleplon, zolpidem and zopiclone. Another GC-MS method screens for amphetamine, methamphetamine and various designer drugs, such as MDMA, MDEA, methylenedioxyamphetamine (MDA), MBDB and

3,4-methylenedioxyphenyl-2-butanamine (BDB) [13, 14]. Furthermore, all samples are tested for buprenorphine, which is increasingly abused in Finland.

Oral fluid: comparison of on-site test results with results of gas chromatography-mass spectrometry

There are about 10 oral fluid drug testing devices available on the market. They were submitted for preliminarily evaluation as part of the ROSITA 2 project. Noticeable differences were found in the reliability of the various testing devices. The European ROSITA project evaluations of the on-site oral fluid testing devices were compared with the results of GC-MS analysis of oral fluid. The results of the ROSITA 2 project are expected to be available in 2006. The preliminary results of one device, tested in Finland, are presented in the table.

Preliminary test results of an on-site testing device tested in Finland as part of the ROSITA 2 project

Analyte	*Cannabis*	*Amphetamines*	*Cocaine*	*Opiates*	*Benzodiazepines*
True positive (TP)	18	125	2	4	28
False positive (FP)	7	2	2	-	1
False negative (FN)	8	4	1	1	9
True negative (TN)	115	18	144	144	30
Total[a]	**148**	**149**	**149**	**149**	**68**
		Percentage			
Sensitivity: TP/(TP+FN)	69.2	96.9	66.7	80.0	75.7
Specificity: TN/(TN+FP)	94.3	90.0	98.6	100.0	96.8
Accuracy: (TP+TN)/(TP+TN+FN+FP)	89.9	96.0	98.0	99.3	85.3

Source: The data were presented at the meeting on the ROSITA 2 project held in Santiago de Compostela, Spain, on 24 and 25 May 2004. The project is ongoing.

[a]One tetrahydrocannabinol positive screening test could not be confirmed because of the limited sample amount of whole blood (no saliva was available). In addition, the test was opiate- and amphetamine-positive, both confirmed positive by gas chromatography–mass spectrometry.

Comparison of laboratory confirmation results for oral fluid versus those for blood and urine

Drug concentration in oral fluid reflects free, unbound drug concentration in blood plasma. High saliva-to-plasma (S/P) ratios are advantageous for saliva testing, increasing the reliability of the tests. The S/P ratio has been noted to be high for basic drugs such as opiates (6-MAM S/P=6 and codeine S/P = 1 3) [15, 16], as well as for amphetamines (S/P=2.8) [17]. Also, cocaine concentrations in oral fluid are at easily measurable levels. In contrast, the S/P ratio of benzodiazepines (S/P = 0.3) and cannabis are not so favourable. Therefore, the

recommended cut-offs for confirmation of benzodiazepines in oral fluid are low—generally lower than existing on-site testing devices can reach.

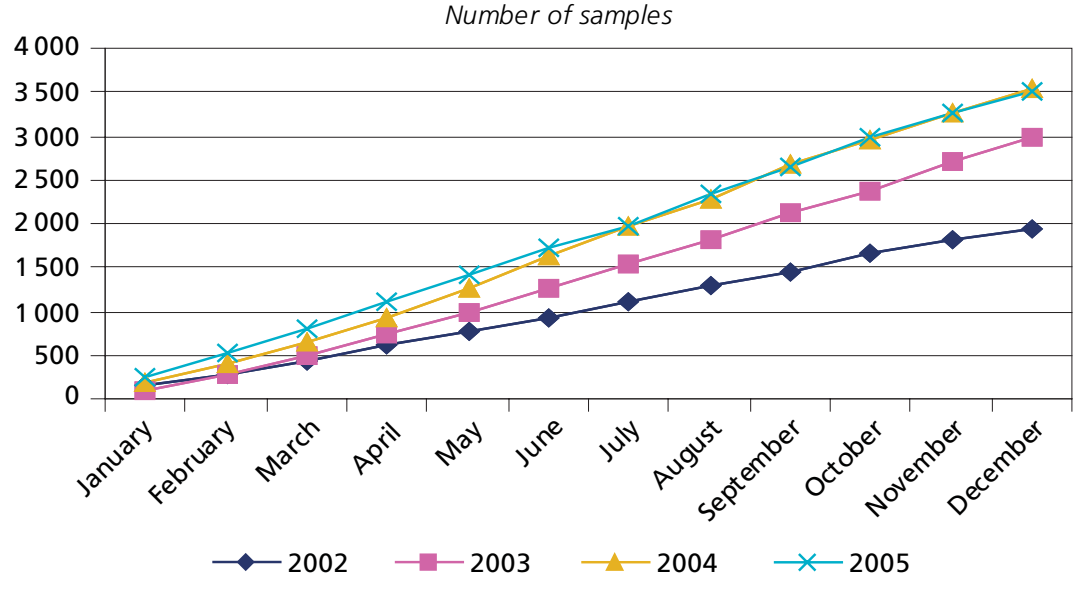
The ROSITA 1 project, which compared drug concentrations in various body fluids, found that, for amphetamines and cocaine, there is a close correlation among the measurable concentrations in urine, oral fluid and blood [2]. Opiate concentrations tend to be higher in oral fluids than in blood. After heroin use, the metabolite 6-acetylmorphine was often detected in oral fluid, although it was not detectable in blood. For benzodiazepines, rapid urine tests gave moderately good results, but the sensitivity of oral fluid tests needs to be improved. For cannabinoid on-site tests, oral fluid devices were not sensitive enough, and urine could test positive several weeks after use. Cannabis use is detected in oral fluid mainly because of oral cavity residues after smoking [18].

Thus, in practice, amphetamine can be easily detected in oral fluid using on-site tests, but there are difficulties in detecting cannabis and benzodiazepines.

Drugs and driving: trends in Finland

The number of blood samples sent to laboratories for analysis increased by about 60 per cent in the year following the introduction in February 2003 of the zero-tolerance law in Finland (see figure II). Based on the noticeable increase in the number of samples and on comments from police officers, it is clear that the police are satisfied with the zero-tolerance law. That is mainly a result of the fact that, when illicit drugs are found in the blood, the police do not need to prove in court that drug-induced driving impairment occurred; confirmation of the presence of an illicit drug in the blood is enough for prosecution for drugs and driving under current drunk driving legislation [19].

Figure II. Blood samples sent to laboratories for analysis in Finland, 2002-2005



Note: The zero-tolerance law was introduced in Finland in February 2003.

The drugs most commonly found are benzodiazepines, which are often taken together with illicit drugs. Since the late 1990s, in about 70-80 per cent of benzodiazepine cases, other illicit drugs, mainly amphetamine and/or cannabis, are simultaneously found. Also since the late 1990s, benzodiazepines have been found in about 70 per cent of investigated cases and illicit drugs, mainly amphetamine and/or cannabis, in 60 per cent (see figure III). In 2004, illicit drugs were simultaneously found in 79 per cent of benzodiazepine cases (see figure IV).

Figure III. Proportion of total investigated cases of drugs and driving in Finland in which any drugs, benzodiazepines and illicit drugs other than benzodiazepines were found, 1992-2004

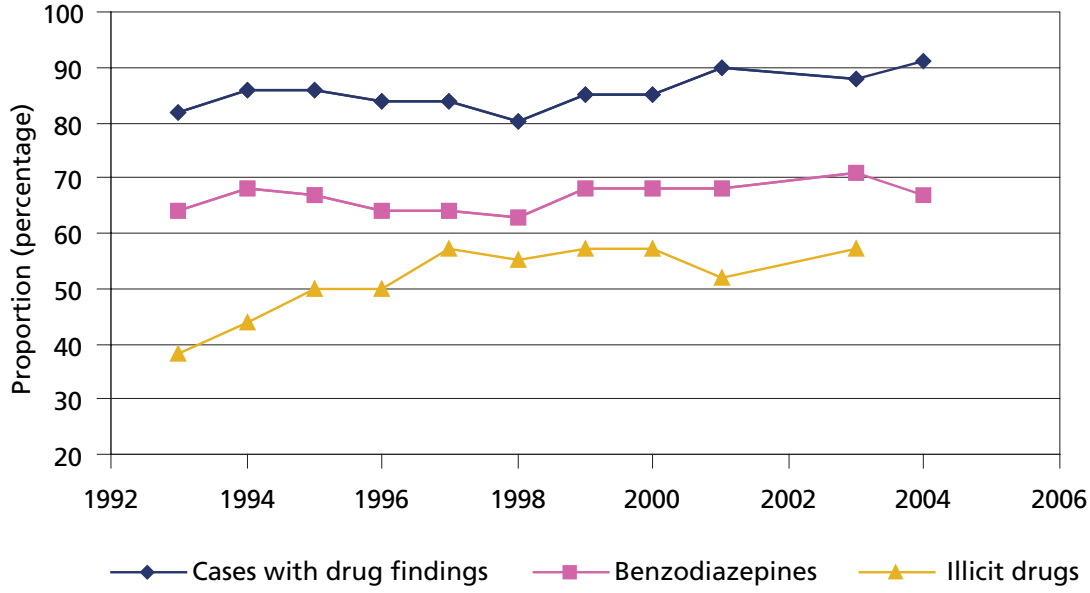
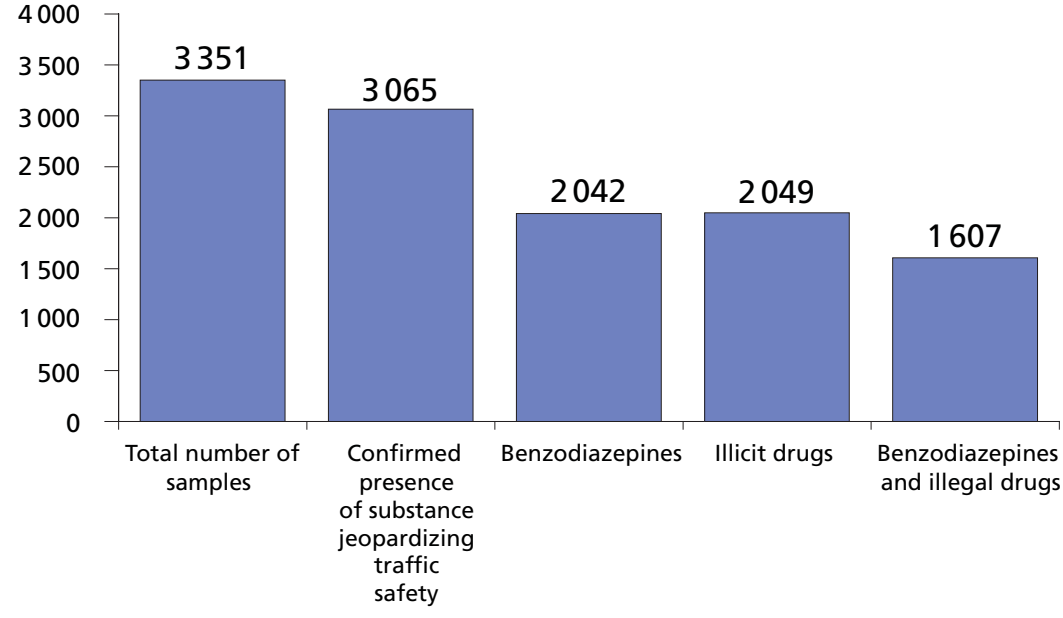


Figure IV. Investigated cases of drugs and driving in Finland in which benzodiazepines, illicit drugs and a combination of both were found, 2004



Diazepam, oxazepam and alprazolam were the benzodiazepines most often found (see figure V). Phenazepam is a new benzodiazepine derivative found on illicit drug markets in Finland. It is not found in the schedules of the international drug control conventions, nor is it registered for use as a medicinal drug in Finland. Therefore, the use and the sale of phenazepam in Finland cannot be controlled. In a neighbouring country, the Russian Federation, phenazepam is therapeutically used as a benzodiazepine for the treatment of anxiety and insomnia. It is comparable with lorazepam in terms of the strength of its action. In 2003, there were 20 positive phenazepam findings in suspected drugs and driving cases in Finland. The impairment law is the only legislative means by which driving under the influence of drugs such as phenazepam, which are not controlled at the national or international level, can be tackled.

Flunitrazepam, which is commonly abused and which is encountered in drugs and driving cases in other countries, is not available for medical use in Finland. However, as flunitrazepam is under international control, any drugs and driving cases in which that substance is involved would be dealt with under the zero-tolerance law.

Figure V. Relative occurrence of benzodiazepines and non-benzodiazepine sedative-hypnotics, 2003

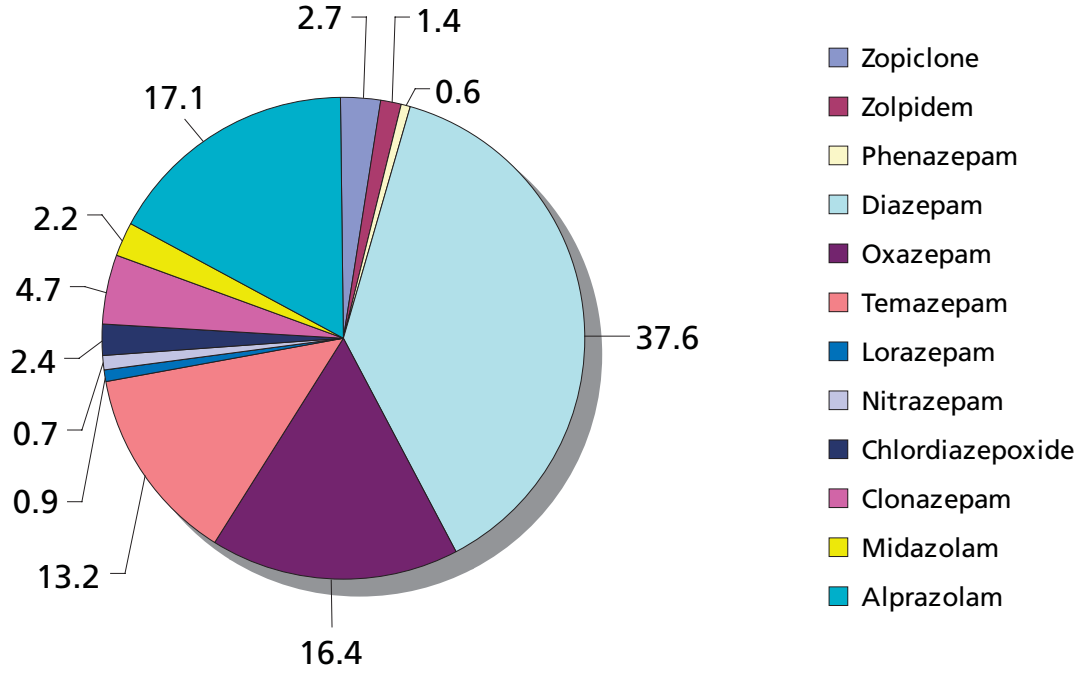
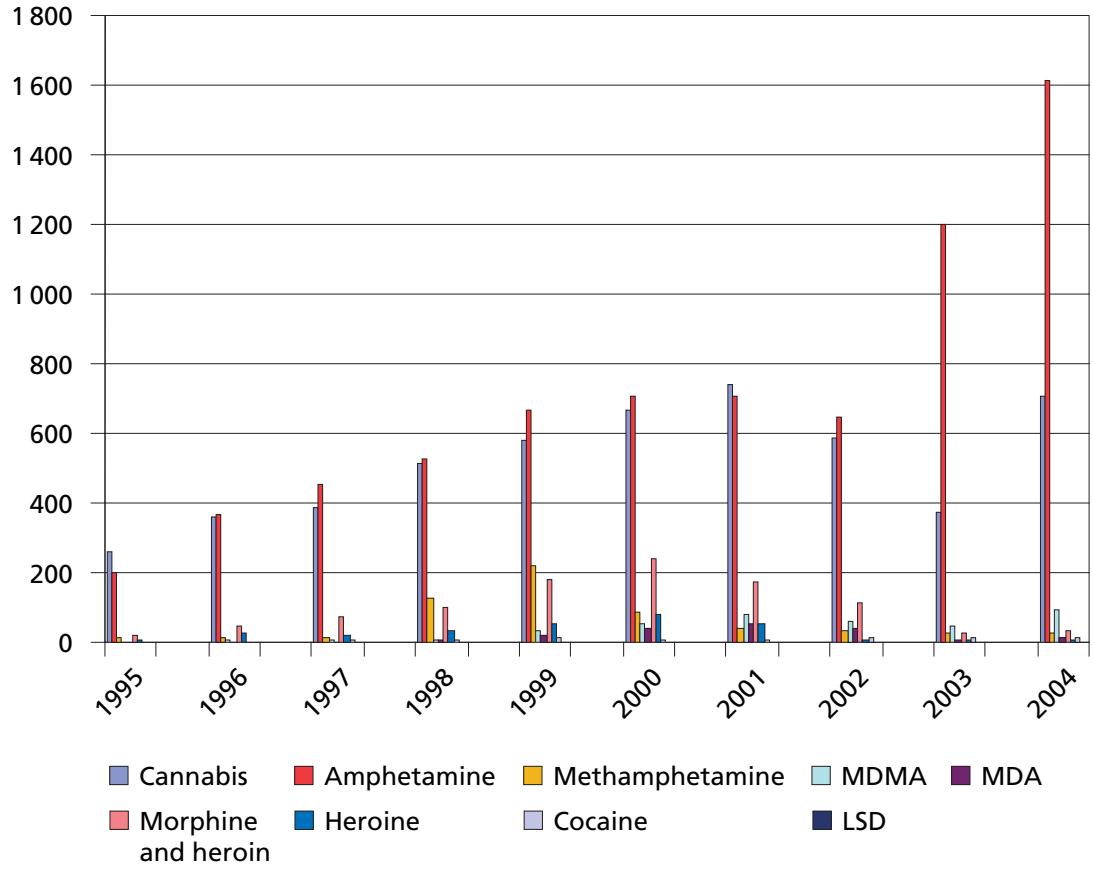


Figure VI summarizes the detection of non-medicinal illicit drugs over the 10-year period 1995-2004 in cases involving driving under the influence of drugs. The most common non-medicinal illicit drugs found were amphetamine and cannabis. Methamphetamine was often found around 1999. After that, it almost disappeared. A marked increase could be seen in the number of amphetamine cases after the introduction of the zero-tolerance law (in 2003). The simultaneous

use of alcohol, benzodiazepines and illicit drugs has been common in Finland for several years [20].

Figure VI. Occurrence of illicit drugs in drugs and driving cases in Finland, 1995-2004



Conclusions

About 20,000 drivers are convicted annually for driving under the influence of alcohol in Finland. The zero-tolerance law for driving under the influence of drugs has sharply increased the number of such cases that have been prosecuted. Under that new legislation, the medicinal use of a drug under the supervision of a physician has been put into a category separate from illicit drug use. After introducing the zero-tolerance law, the authorities have had better means with which to prosecute a person who has driven while under the influence of drugs.

Drugs and driving is included in the European Union Action Plan to Combat Drugs. It has also become an important issue for United States drug policy. There are a number of planned or ongoing international collaborative studies in the field of drugs and driving involving partners from several countries and sites.

To enhance the impact of those studies and to enable best practices to be identified in the field of police control and training, there must be an exchange of information at the international level. Also, the focus of those studies might

need to be extended from illicit drugs to a range of medicines whose use by drivers might increase the risk of accidents. Because medicines are used by a larger percentage of the population than are illicit drugs, their impact might be great. To better understand the problem of drugs and driving, experiments and epidemiological studies are needed. Diverse and unpredictable patterns of drug use increase the difficulty of assessing the problem. The combined use of illicit drugs, medicines and alcohol often results in significant impairment.

Although the effects on performance of drivers in cases involving both illicit drug use and medicinal drug use are very similar, the user groups are different, and more attention needs to be given to that aspect when developing countermeasures.

Another problem is that statistics collected in different countries on the prevalence of drug use in road accidents are too fragmentary and not comparable. Statistics do not, at present, give a sufficiently detailed picture of the situation and do not permit the identification and the evaluation of the most effective countermeasures.

Finally, in contrast to the case with alcohol, establishing a concentration-effect relationship for drugs is much more complex. Thus, concentration levels above which driving should be prohibited are still difficult to establish. At present, legislators are presented with two options: the zero-tolerance option, as applied in a number of countries, such as Belgium, Finland, Germany and Sweden; or the evaluation of the deterioration of driving ability under the influence of drugs (impairment) by specially trained police officers, or by medical doctors where required. Once reliable on-site devices have been developed, a zero-tolerance law can make effective roadside control possible.

Research efforts to develop practical and reliable detection equipment for the roadside testing of drugs and medicines should therefore continue. That equipment is needed for carrying out daily control and epidemiological surveys on the road. The use of oral fluid offers potential for those types of study.

The issue of driving under the influence of drugs is a complex one, cutting across a number of fields and areas of expertise. In addition to concrete scientific-technical aspects such as the further development of reliable on-site testing devices and protocols, the research agenda extends to other areas such as epidemiological studies and studies on the impact of various types of legislation, in order to identify best practices, standardize procedures and develop and introduce countermeasures.

References

1. E. J. D. Ogden and H. Moskowitz, “Effects of alcohol and other drugs on driver performance”, *Traffic Injury Prevention*, vol. 5, No. 3 (2004), pp. 185-198.
2. A. G. Verstraete, “Roadside Testing Assessment (ROSITA): a European Union project on roadside drug testing”, paper presented at the International Conference on Alcohol, Drugs and Traffic Safety, Stockholm, 21-26 May 2000.

3. J. M. Walsh and others, "Drugs and driving", *Traffic Injury Prevention*, vol. 5, No. 3 (2004), pp. 241-253.
4. K. Stewart, "Introduction to the special issue on alcohol and drug impaired driving", *Traffic Injury Prevention*, vol. 5, No. 3 (2004), pp. 173-174.
5. A. Penttilä and others, "Trends in drink driving in Finland in 1990-2000", *Proceedings of the Sixteenth International Conference on Alcohol, Drugs and Traffic Safety*, D. Mayhew and C. Dussault, eds. (Montreal, Canada, Société de l'assurance automobile du Québec, 2002).
6. T. E. Page, "The drug recognition expert officer: signs of drug impairment at roadside", *Proceedings of the Sixteenth International Conference on Alcohol, Drugs and Traffic Safety*, D. Mayhew and C. Dussault, eds. (Montreal, Canada, Société de l'assurance automobile du Québec, 2002).
7. S. J. Heishman, E. G. Singleton and D. J. Crouch, "Laboratory validation study of drug evaluation and classification program: ethanol, cocaine, and marijuana", *Journal of Analytical Toxicology*, vol. 20, No. 6 (1996), pp. 468-483.
8. S. J. Heishman, E. G. Singleton and D. J. Crouch, "Laboratory validation study of drug evaluation and classification program: alprazolam, *d*-amphetamine, codeine, and marijuana", *Journal of Analytical Toxicology*, vol. 22, No. 6 (1998), pp. 503-514.
9. A. G. Verstraete, "Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges", Forensic Science International, vol. 150, Nos. 2-3 (2005), pp. 143-150.
10. P. Lillsunde and T. Korte, "Comprehensive drug screening in urine using solid-phase extraction and combined TLC and GC/MS identification", *Journal of Analytical Toxicology*, vol. 15, No. 2 (1991), pp. 71-81.
11. P. Lillsunde and others, "Comprehensive drug screening in blood for detecting abused drugs or drugs potentially hazardous for traffic safety", *Forensic Science International*, vol. 77, No. 3 (1996), pp. 191-210.
12. T. Gunnar and others, "Validated semiquantitative/quantitative screening of 51 drugs in whole blood as silylated derivatives by gas chromatography-selected ion monitoring mass spectrometry and gas chromatography electron capture detection", *Journal of Chromatography B*, vol. 806, No. 2 (2004), pp. 205-219.
13. A. Kankaanpää and others, "Single-step procedure for gas chromatography-mass spectrometry screening and quantitative determination of amphetamine-type stimulants and related drugs in blood, serum, oral fluid and urine samples", *Journal of Chromatography B,* vol. 810, No. 1 (2004), pp. 57-68.
14. P. Lillsunde and T. Korte, "Determination of ring- and *N*-substituted amphetamines as heptafluorobutyryl derivatives", *Forensic Science International*, vol. 49, No. 2 (1991), pp. 205-213.
15. W. Schramm and others, "Drugs of abuse in saliva: a review", *Journal of Analytical Toxicology*, vol. 16, 1992, pp. 1-9.
16. V. Spiehler, D. Baldwin and C. Hand, "Cut-off concentrations for drugs of abuse in saliva for DUI, DWI or other driving-related crimes", report presented at the Thirty-seventh Meeting of the International Association of Forensic Toxicologists, 1999.
17. S. Wan, S. Matin and D. Azarnoff, "Kinetics, salivary excretion of amphetamine isomers, and effect of urinary pH", *Clinical Pharmacology and Therapeutics*, vol. 23, 1974, pp. 585-590.

18. M. Grönholm and P. Lillsunde, "A comparison between on-site immunoassay drug-testing devices and laboratory results", *Forensic Science International*, vol. 121, Nos. 1-2 (2001), pp. 37-46.
19. P. Lillsunde and others, "Zero-tolerance law for drugs and driving in Finland: the effects on annual statistics", *Proceedings of the Sixteenth International Conference on Alcohol, Drugs and Traffic Safety*, D. Mayhew and C. Dussault, eds. (Montreal, Canada, Société de l'assurance automobile du Québec, 2002).
20. P. Lillsunde and others, "Drugs usage of drivers suspected of driving under the influence of alcohol and/or drugs: a study of one week's samples in 1979 and 1993 in Finland", *Forensic Science International*, vol. 77, Nos. 1-2 (1996), pp. 119-129.

Annex I

STANDARDIZED FIELD SOBRIETY OBSERVATION SHEET

Date

Concerns R-report nr.	Laboratory sample nr (filled out by KTL)
Surname and initial names	Social security nr.

OBSERVATIONS REGARDING WAY OF DRIVING, WEATHER AND ROADWAY

Way of driving

☐ No own observations ☐ Secure ☐ Unsteady ☐ Inappropriate speed ☐ Violation of way of priority

☐ Winding, deviation from straight line up to _____ meters.

Number of deviations: on a _____ meters of observation

☐ Other attentions

Control of devices of vehicle

☐ Driving with low revolutions ☐ Insecure use of gears ☐ Roaring of motor

☐ Other

Fault and defects of vehicle

☐ No ☐ Yes, what?

Weather and lighting

☐ Rain ☐ Hard wind / storm ☐ Snow / sleet ☐ Fog ☐ Daylight ☐ Dusk ☐ Dark

Roadway

☐ Good ☐ Poor ☐ Construction on way ☐ Good lighting ☐ Poor lighting ☐ Dry ☐ Wet ☐ Icy / snowy

OBSERVATIONS DURING STOPPING AND CONFRONTING

Reactivity

☐ Normal ☐ Slow ☐ Very slow

Physical deviations

☐ None ☐ Sweating ☐ Tremor ☐ Womiting ☐ Restlessness

Appearance

☐ Neat ☐ Shabby ☐ Filthy

Speaks Finnish or Swedish

☐ Yes ☐ No ☐ Faltering

Speech

☐ Clear ☐ Sputtering ☐ Thick ☐ Lisping

Communication, sense of time and place

☐ Clear sense of time ang place ☐ Drowsy ☐ Wakes up ☐ Deep sleep / unconscious ☐ Altered

Behaviour

☐ At ease, behaved ☐ Agitated ☐ Aggressive ☐ Matey ☐ Frivolous ☐ Uninterested ☐ Defiant ☐ Weepy

Rising out of vechicle

☐ Normal ☐ Balance disturbed ☐ Has to lean on vehicle

Walking

☐ Secure ☐ Dragging ☐ Wobbly ☐ Balance disturbed

Smell of alcohol

☐ Yes ☐ No

Alcometer test

☐ Yes: time ‰ ☐ No ☐ Cannot be done ☐ Refused

Positive on site tests

Cozart	Time	☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC
Drugwipe	Time	☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC
Other, what?		☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC

Eyes

☐ Nothing abnormal ☐ Conjunctivas reddish ☐ Watery / gleaming ☐ Restless

Pupils

☐ Normal ☐ Dilated ☐ Contracted **Reaction to light** ☐ Slow ☐ Fast

Right about mm Left about mm

Nystagmus

☐ Jerky movement ☐ No jerking observed

Lighting conditions on test site

☐ Daylight ☐ Dusk ☐ Night, streetlights ☐ Night, indoor

☐ Other, what?

Conspicuous behaviour

☐ Did not change during evaluation ☐ Increased during evaluation ☐ Decreased during evaluation

Test started: time	Test ended	**The ability of the driver**
		☐ Is not impaired ☐ Is impaired ☐ Is considerably impaired

Further information: like other observations, confiscated substances, pills, paraphernalia etc.

Time and place	Signature and name of observer

Annex II

Draft translation of physician's impairment evaluation — Table 2

Inquirer of the examination.……

Examination place ……

Name of the subject …… Social security number ……

Proving of identity ☐ proved by the police ☐ other ……

The reason for examination ☐ drunken driving ☐ other felony ☐ other

Wanted examination ☐ blood sample ☐ urine sample ☐ clinical examination

Diseases

According to the subject ☐ none ☐ yes What? ☐ no answer

Blood pressure ……/…… mmHg Pulse ……/min

Observed symptoms ☐ none ☐ yes What?

Observed injuries ☐ none ☐ yes What?

Liquid treatment of the injured ☐ no ☐ yes What, how much?

Drugs and medication. Has the subject used these before or after the incident? What, when, how much?

Injection marks ☐ no ☐ yes

According to The subject ☐ none ☐ yes regularly ☐ yes occasionally ☐ no answer

On-site test ☐ saliva ☐ urine ☐ neg. ☐ pos. What?

Alcohol breath test ……..o/oo

Clinical examination

Weight ……. kg ☐ weighed ☐ given

Height ……. cm ☐ measured ☐ given

Body structure ☐ normal ☐ slim ☐ obese

Examinations, observations	normal	slightly deviating	clearly deviating (underline the observation)
Consciousness	☐		☐ numb, sleepy, almost unconscious
Aware of the date and time, memory	☐	☐	☐
Walking straight forward	☐	☐	☐
Full turn while walking	☐	☐	☐
Romberg's test with eyes closed	☐	☐	☐
Finger to finger test	☐	☐	☐
Pulling oneself together			☐ observed
Behaviour	☐		☐ uninhibited, aggressive, angry, talkative, arrogant, unresponsive, limp, absentminded
Speech	☐		☐ inarticulate, spluttering, thick, faltering
Train of thought	☐		☐ illogical, jumpy, muddled
Mood	☐		☐ euphoric, irritated, distressed, varying, restless, upset, bored
Size of the pupils	☐		☐ strongly dilated, pointed
Pupils' reaction to light	☐		☐ slowed down, non-reacting
Nystagmus			☐ strong ☐ after following the object ☐ spinning induced
Other unusual findings		☐	☐ sweating, spasms, chills, dry mouth, running nose, tremor, watering or bloodshot eyes

Other observation:

Samples* The skin was cleaned with ☐ water ☐ other. What? ……

Blood sample ……/…… at …….. ☐ 2 tubes according to the instructions ☐ other ……

Urine sample ……/…… at …….. under supervision ☐ yes ☐ no quality of urine ☐ normal ☐ unusual How?

Urine test slip glucose ☐ no ☐ yes keto compounds ☐ no ☐ yes

Signature** …… Clarification of name and job position ……

Evaluation of the degree of the functional disorder (the total degree of errors)

1. Functional disorders ☐ were not observed ☐ were observed ☐ examinations were not carried out, because……
2. The degree of the functional disorder ☐ is in the limits of normal variation
   ☐ deviates from the normal state and is at least ☐ mild ☐ of medium strength
3. To my knowledge these functional disorders/errors have been caused by
   ☐ drugs and/or ☐ medication and/or ☐ alcohol ☐ decease ☐ injury ☐ I can't evaluate

This I affirm by my honour and conscience

Date …… Signature ……

Clarification of name and job position……

*Personal data and sampling time is written on the sample tubes. **The signature of the person who took the samples, if not the same as the signature of this form.

Role of drug testing as an early warning programme: the experience of the Republic of Korea

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ABSTRACT

Drug testing plays an important role in the provision of information to health authorities on trends in drug abuse. In the Republic of Korea, the testing of urine and postmortem specimens has been used as part of a programme to monitor and control the abuse of non-controlled drugs, i.e. substances that were not originally included in the lists of controlled substances in that country. Zipeprol, dextromethorphan, carisoprodol and nalbuphine are examples of such drugs, which are widely used as medicines. Increasing levels of abuse of these drugs, including abuse that resulted in fatalities, were confirmed in the Republic of Korea by the results of drug testing. Based on the accumulated data from postmortem specimens, the health authorities in the Republic of Korea subsequently introduced controls on these drugs. A significant drop in fatalities related to the abuse of these non-controlled drugs underlined the importance of timely action for improving community health.

In the context of drug testing, the analysis of non-controlled and new drugs always presents a scientific challenge, because specific analytical methods for testing for those drugs are not available. In the Republic of Korea, as part of the drug abuse warning programme, it was necessary to establish methods for the detection and quantification in biological fluids of all four non-controlled drugs and their metabolites in order to monitor the trends in drug abuse.

The present paper puts forward epidemiological and clinical data on abuse and fatalities associated with zipeprol, dextromethorphan, carisoprodol and nalbuphine, as well as details of the analytical methods developed.

Keywords: drug testing; health; warning system; zipeprol; dextromethorphan; carisoprodol; nalbuphine

Introduction

Drug abuse was not a social problem in the Republic of Korea until the early 1980s [1]. Even more recently, the level of drug abuse is not a matter of serious concern when viewed in terms of the drug offender rate. The number of

drug offenders per 100,000 of the population recorded in the Republic of Korea was 10 in 1994, 12 in 1995, 14 in 1996, 15 in 1997 and 18 in 1998, numbers that are quite small compared with 400 in the United States of America, 250 in Australia or several dozen in the countries of Europe [2]. However, the number of drug offenders had been on the increase, from 5,418 in 1995 to 10,304 in 2000, which is a twofold increase over a period of 5 years. The numbers were similar in 2001 and 2002, at 10,102 and 10,673, respectively. However, the numbers dropped to 7,546 in 2003 and 7,747 in 2004, indicating an effective crackdown on drug smuggling by law enforcement authorities.

Methamphetamine is the drug that is most abused in the Republic of Korea, followed by cannabis and opiates. From the mid-1980s, there was a sharp increase in trafficking and abuse of methamphetamine and a large number of abusers were apprehended in 1988. Epidemiological study regarding methamphetamine drug offenders revealed that those in their thirties represented the largest group, accounting for around 40 per cent of the total, followed by those in their forties at about 26 per cent, those in their twenties at 16 per cent, those over 50 at 10 per cent and those under 20 years old at less than 0.3 per cent. The breakdown of individuals arrested for methamphetamine drug offences by gender showed that males accounted for the majority, at over 80 per cent, while females accounted for 11.7 to 17.2 per cent. By type of violation, the number of people arrested for consumption ranked highest, accounting for 76.4 per cent of the total, followed by trafficking, possession and smuggling, comprising 13.4 per cent, 6.5 per cent and 1 per cent, respectively [2].

The abuse of methamphetamine is so serious that fatalities from overdose of this drug have occurred. Since 1985, 40 fatalities have been reported as being associated with overdose of methamphetamine [3].

Cannabis is the second most abused drug after methamphetamine and was the main drug used for recreational purposes. The majority of cannabis consumed in the Republic of Korea is domestically grown. Cannabis has traditionally been cultivated for the manufacture of a fabric for special clothes in the Republic of Korea. In recent years, hashish resin smuggled from abroad has been encountered.

Opium poppy has been cultivated on a very small scale, but there has been no case of cultivating poppy for illicit manufacture of heroin and opiates. Recently, there have been many cases of smuggling of raw opium, mainly from China. Most of this raw opium contains a low content of morphine and codeine [4].

Recent characteristics of trends in drug abuse include increased drug smuggling from abroad, involvement of organized groups in drug trafficking, a spread in drug abuse over greater areas of the country and engagement of foreigners in the Republic of Korea in drug smuggling. Since the lifting of restrictions on immigration in 1995, methylenedioxymethamphetamine (commonly known as Ecstasy), methamphetamine tablets (Yaba) and lysergic acid diethylamide (LSD), which were relatively new in the Republic of Korea, have taken up an increased proportion of overall seizures, revealing diversification in the types of smuggled drugs [5].

In addition, there has been a growing tendency for abuse of common medicines among young people. This is a result of the easy availability of common medicines. Even though these non-controlled drugs represented only less than 20 per cent of the total abuse picture, the seriousness of this abuse is related to how easily these medicines can be obtained [6]. As shown in figure I, the proportion of abuse of these non-controlled drugs as a factor of the total level of drug abuse represented 18 per cent in 1992, 13 per cent in 1993, 25 per cent in 1994, 18 per cent in 1995 and 8 per cent in 1996, according to the statistics based on drug testing conducted by the National Institute of Scientific Investigation (NISI) in the Republic of Korea. In March 1995, when there was a crackdown on the abuse of illegal drugs among gang members in the western port city of Inchon, 242 urine samples were submitted for drug testing. The results revealed that zipeprol was detected in 74 samples, methamphetamine in 29 samples, cannabis in 24 samples, dextromethorphan in 14 samples and both dextromethorphan and zipeprol in 11 urine samples (see figure II), showing the extent of the abuse of these commonly available medicines [6]. Among the drugs detected in this case, only methamphetamine and cannabis were controlled at that time, while zipeprol and dextromethorphan were sold freely as common medicines at pharmacies.

Figure I. Proportion of abusers of non-controlled drugs to abusers of controlled drugs in the Republic of Korea, 1992-1996

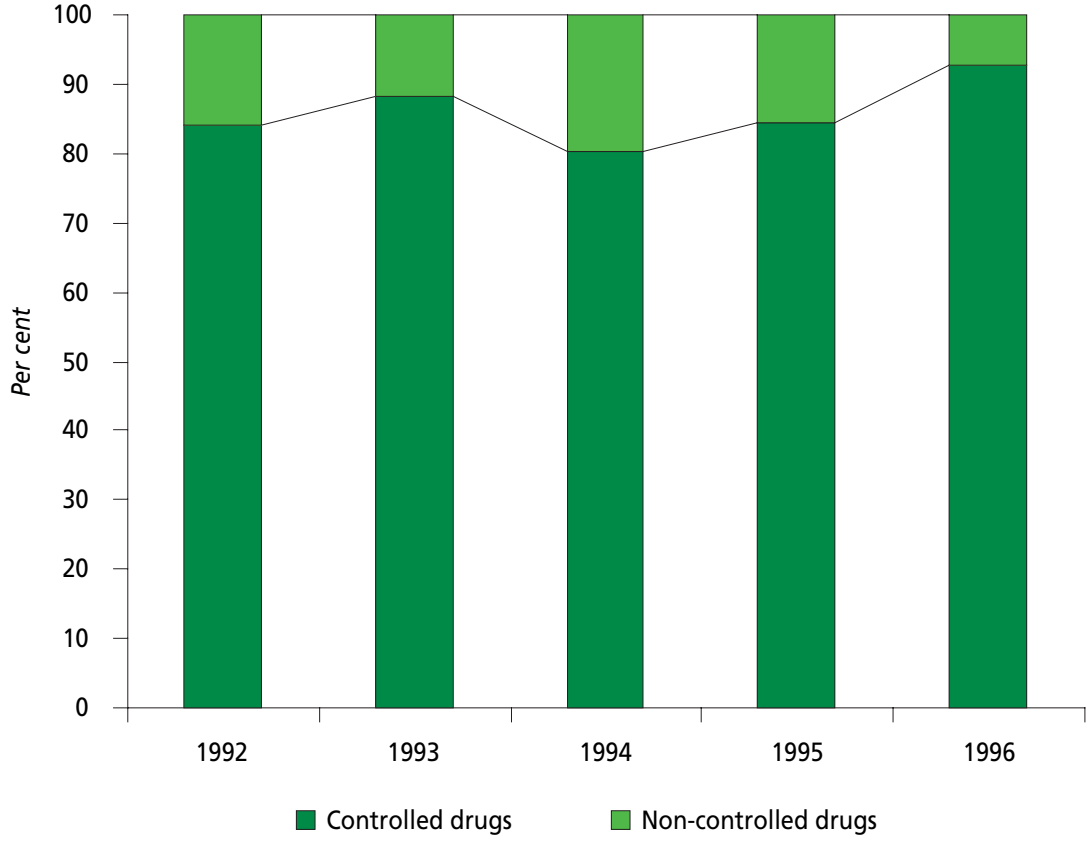
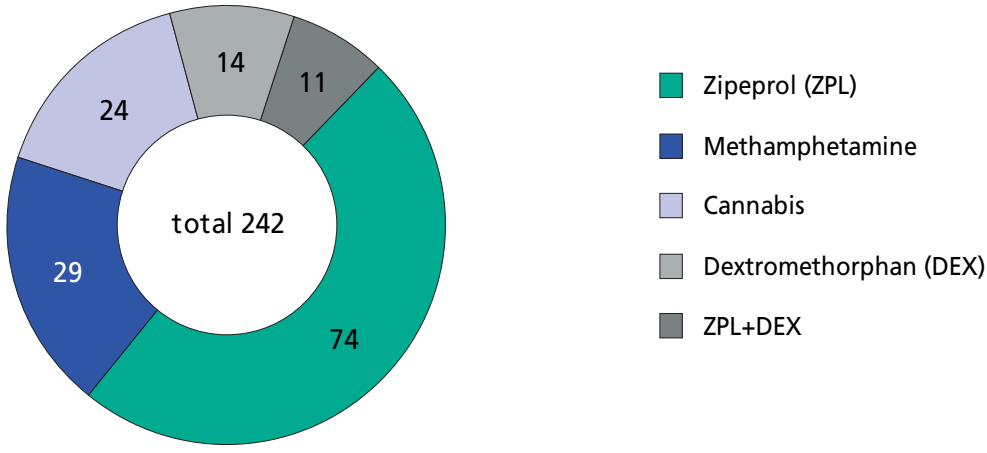


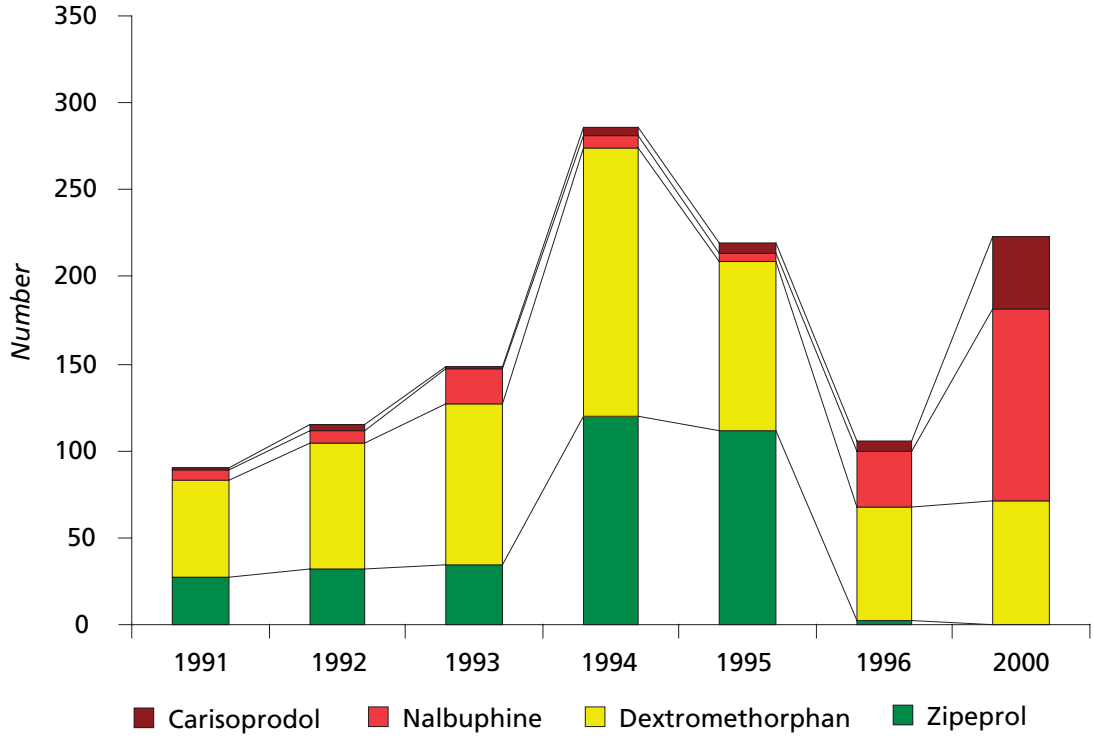
Figure II. Drugs detected in urine samples during the crackdown on suspected drug abusers in the Republic of Korea in 1995



Note: 90 urine samples tested were drug-free.

The number of cases over a 10-year period of abuse of the most commonly abused non-controlled drugs (zipeprol, dextromethorphan, nalbuphine and carisoprodol) are shown in figure III. The statistics revealed by drug testing by NISI showed that zipeprol was detected in 27 cases in 1991, but the number of cases had soared to 120 in 1994 and 112 in 1995. In the case of dextromethorphan, 56 urine samples tested positive in 1991, with positive results in 73 cases in 1992, 93 cases in 1993, 154 cases in 1994, 97 cases in 1995, 66 cases in

Figure III. Trends in the number of abusers of non-controlled drugs in the Republic of Korea (urine drug detections), 1991-2000



1996 and 71 cases in 2000, indicating a steady growth in prevalence. While there were only four nalbuphine cases in 1995, this figure shot up to 32 in 1996 and then 110 in 2000. The NISI statistics also showed that there were only between 5 and 6 positive results for abuse of carisoprodol each year from 1994 to 1996, but that that number rocketed to its highest level of 42 cases in 2000.

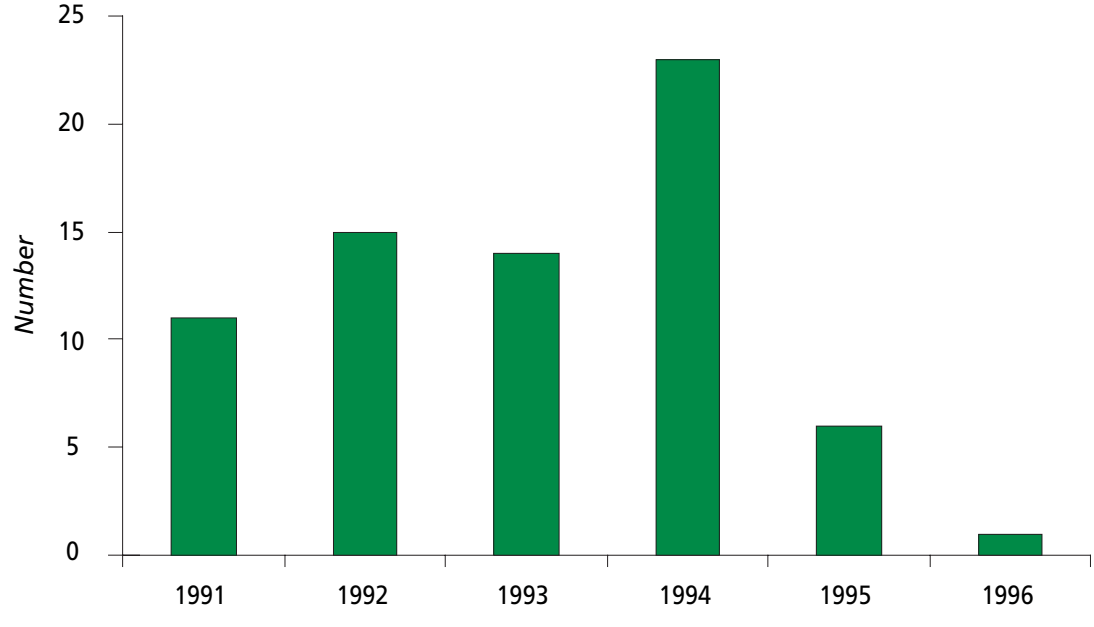
Epidemiology and testing of non-controlled drugs

Abuse of zipeprol

Epidemiology

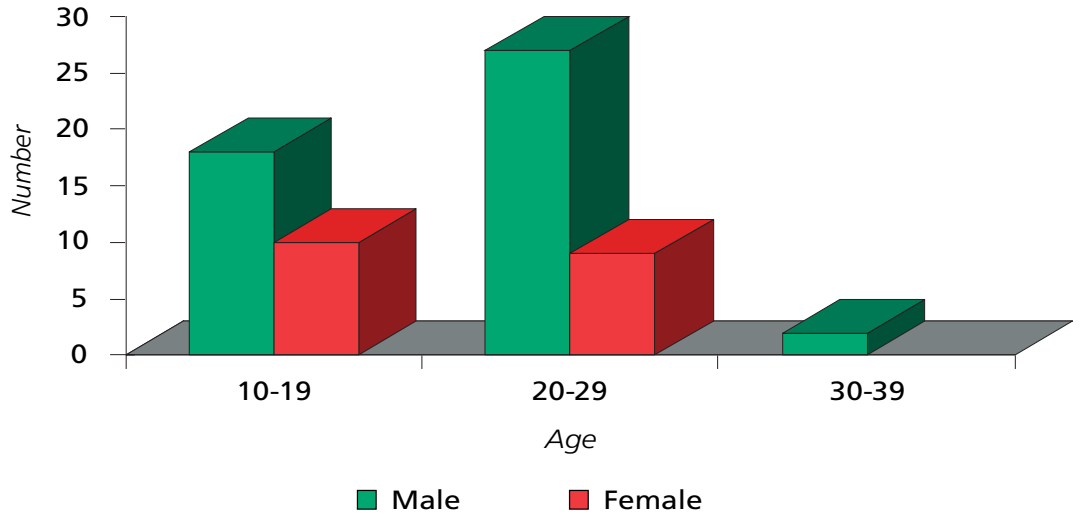
Zipeprol is a non-opiate, anti-tussive agent without the side effects of opiates. In the Republic of Korea, zipeprol dihydrochloride is marketed in capsule or tablet form under nine different trade names [7]. It is known to be a safe drug when taken as prescribed and does not lead to any physical dependence, but it produces opiate-like euphoria if taken in large quantities. At the time when zipeprol abuse surfaced in the Republic of Korea, there were only a few reports of the abuse of that substance worldwide. In the Republic of Korea, abuse of zipeprol was prevalent in particular among young people, especially addicts, who used doses 10 to 15 times higher than the recommended 75 mg single dose in order to achieve the opiate-like effect. Because abusers took large doses of the drug for its hallucinogenic effects, reports of fatalities from zipeprol overdose have started to rise in the period since 1991. Yoo and others [8] reported postmortem distribution of zipeprol in 10 fatal cases and postmortem zipeprol blood concentration in 23 cases during 1991 to 1993 [9]. However, during the 5-year period between 1991 and 1995, a total of 69 zipeprol-related deaths occurred

Figure IV. Annual number of zipeprol-related deaths in the Republic of Korea, 1991-1996



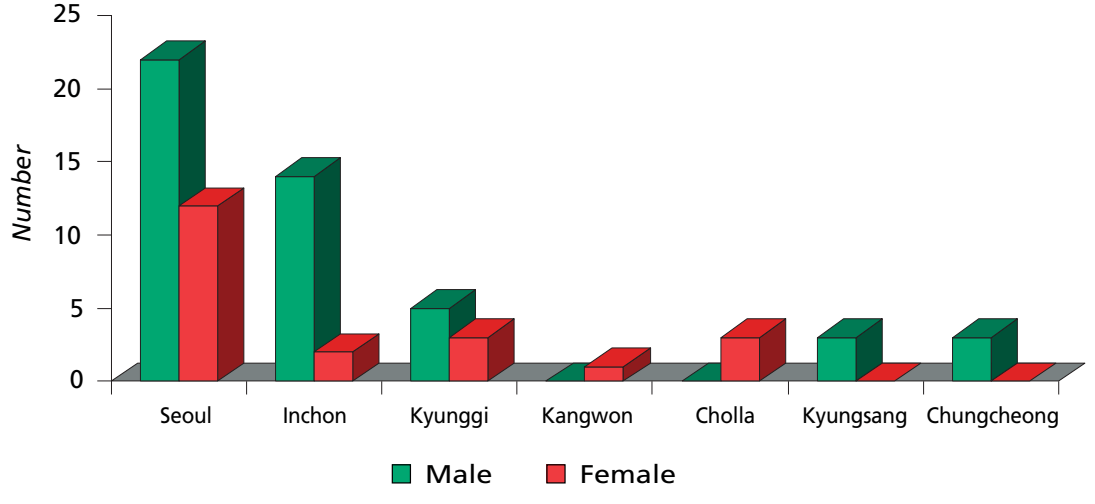
throughout the nation [10]. These fatalities are shown as a function of year in figure IV. In 1991, there were 11-zipeprol related deaths, with 15 such cases in 1992, 14 cases in 1993, 23 cases in 1994, 6 cases in 1995 and 1 case in 1996. The drop in cases from 1994 to 1995 is related to the introduction by the Government of measures to control the trade and possession of zipeprol. The controls were put into place in September 1995.

Figure V. Distribution of zipeprol-related deaths by age and gender



It was also possible to break out geographic demographics from the data obtained from these cases. As shown in figure VI, the majority of zipeprol-associated deaths occurred in the larger cities of the Republic of Korea, Seoul and Inchon (34 and 16 deaths, respectively). Seoul and Inchon together thus demonstrated an 80 per cent incidence of zipeprol-associated deaths, with the remaining 20 per cent reported from smaller cities. Seoul alone accounted for 50 per cent of the total.

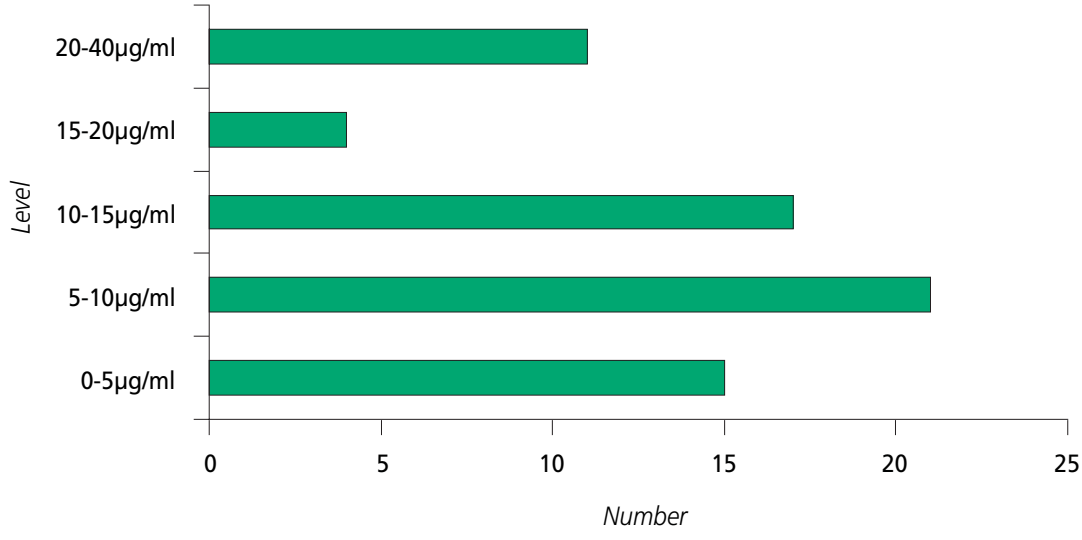
Figure VI. Distribution of zipeprol-related deaths by gender and location



In terms of gender, similar to the overall trend, males accounted for a share of 72 per cent of all deaths in both Seoul and Inchon. Interestingly, however, and although smaller total numbers were involved, the opposite trend was observed in Kangwon and Cholla provinces, where the distribution was skewed towards a higher female incidence rate.

In figure VII, the frequency distribution of zipeprol postmortem blood concentrations is presented. Overall, the concentration of zipeprol in blood samples ranged from 0.1 to 38.3 μg/ml. One third (31 per cent) of cases demonstrated a zipeprol blood level of from 5 to 10 μg/ml, a quarter (25 per cent) of cases demonstrated levels ranging from 10 to 15 μg/ml and in 11 cases (16 per cent) a concentration range of 20 to 40 μg/ml was noted.

Figure VII. Range of concentration of zipeprol in postmortem blood samples (μg/ml) in drug-involved deaths in the Republic of Korea



As the statistics of drug testing showed in figure III, the number of detections of zipeprol in urine plummeted from 1995 to 1996, from 112 to 2, when the health authorities started to control this drug as a psychotropic agent beginning in September 1995. This was a sign of timely action against the abuse of zipeprol.

Development of the detection method

From an analytical, drug testing point of view, the analysis of zipeprol, like any new drug, presented a scientific challenge. Typically, drug samples are first analysed using a general method that allows the rapid screening of large numbers of samples to get an indication of the presence or absence of one or more specific drugs. Then, in a second step, another method is used to confirm the results of the screening method.

For controlled drugs, standard operating procedures are available for screening in biological fluids, typically by immunoassay followed by confirmation using gas chromatography/mass spectrometry. However, since there was no specific

method for zipeprol, both a rapid and sensitive gas chromatography method for screening and a gas chromatography/mass spectrometry for the confirmation of the drug and its metabolites in blood were developed.

Analytical aspects [8, 9]

Analyses were performed on 1 ml of blood. Samples were adjusted to pH 11-12 with 6N sodium hydroxide (NaOH) and extracted three times with 5 ml ethyl acetate. The pooled ethyl acetate was evaporated under vacuum and the residue was dissolved in 100 μl of internal standard containing ethyl acetate. One microlitre of this solution was then injected into the gas chromatograph. The ratio of the peak area of drugs to that of the chromatographic standard was used to calculate the concentration of each analyte. A calibration curve for zipeprol over the range of 1, 5 and 20 μg/ml was established.

The zipeprol standard was purchased from Sigma Co. and all other chemicals and solvents were of analytical reagent grade. The standard stock solution of zipeprol was 1 mg/ml in ethanol. Working standards were prepared by dilution with ethanol. Cinnarizine was used as the internal standard for quantification.

A Varian model 4600 gas chromatograph equipped with a thermionic specific detector (TSD) and a Star data system was used for the isolation of the drugs. A DB-5 megabore column (15 m × 0.53 mm) was used. The temperature was programmed from 150° C to 250° C at 10° C/min, the injection port temperature was 270° C and the detector temperature was 280° C. The carrier gas (helium) had a flow rate of 7 ml/min.

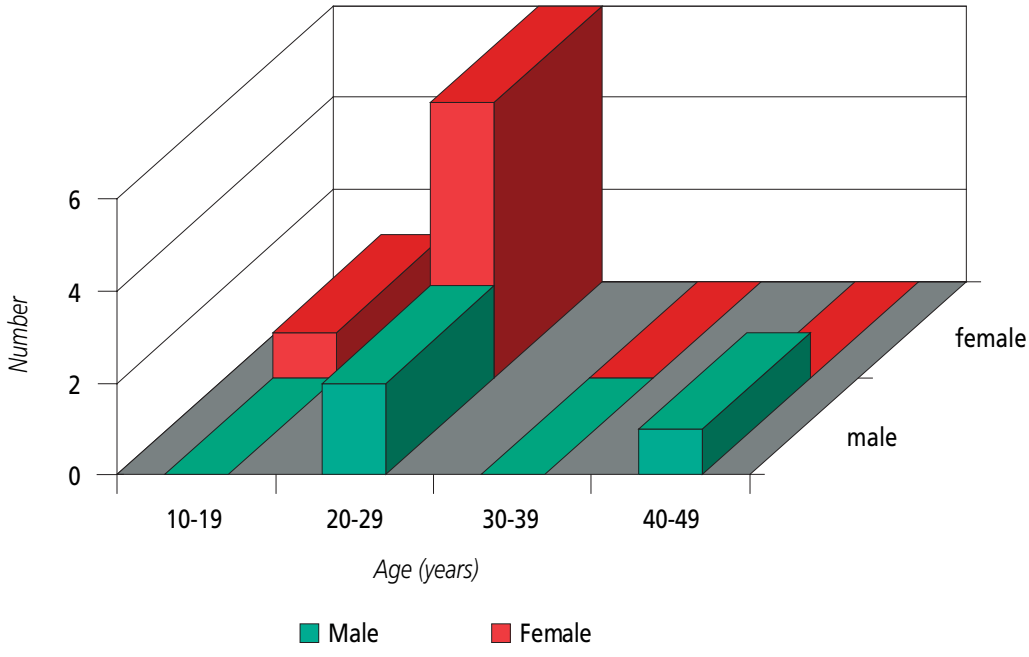
A Finnigan MAT GCQ was used to identify the drugs and metabolites. A fused-silica capillary SE-54 column (15 m × 0.32 m) was used. The column temperature was programmed from 150° C to 250° C at 10° C/min, the ionization energy was 70eV, the transfer-line temperature was 270° C and the electron multiplier (EM) voltage was 1,600 V.

Abuse of dextromethorphan

Epidemiology

The abuse of dextromethorphan has a long history [11]. Although the drug is known to be non-addictive and produces little or no central nervous system depression, the manifestations of acute overdose are known to include hallucination, insomnia and toxic psychosis [12-14]. The hallucinogenic effect is also the reason for abuse of dextromethorphan by young people for recreational purposes [15]. Taking large amounts of this drug to obtain a hallucinogenic effect resulted in 10 fatalities from the overdose of this drug being reported in the Republic of Korea [6]. The distribution by gender of dextromethorphan-related deaths in figure VIII shows that more females than males died from abuse of this drug. This finding appears to be the result of a higher prevalence of abuse among females. Specifically, there was a speculation that many women who worked in the red-light district abused this medicine.

Figure VIII. Distribution of dextromethorphan-related deaths in the Republic of Korea by age and gender



The age of those deceased from dextromethorphan abuse ranged from 19 to 42 years, with an average age of 24. The postmortem blood concentrations in these cases ranged from 3.18 to 33.6 μg/ml. From the obtained history it was determined that individuals take this drug for suicidal or recreational purposes. The relationship between employment status and dextromethorphan-involved deaths shows that a relatively high percentage of the deceased had been employed prior to their death.

The number of dextromethorphan cases during the 10-year period from 1991 to 2000 ranged from 56 in 1991 to 154 in 1994 and 71 in 2000 (figure III). However, in 2003, the number soared to 422 indicating the rapidly escalating number of abusers.

Against the background of abuse and the death toll from overdose of this drug, the Government of the Republic of Korea introduced the control of trade and possession of dextromethorphan in October 2003. Since then, the number of cases of abuse of this drug has dropped to 163 in 2004, which was just 40 per cent of the number in 2003 [16].

Development of the detection method

A method for the gas chromatographic analysis of dextromethorphan and its metabolites in biological fluids was established. In addition, the enantiomeric separation of dextromethorphan from levomethorphan was studied, because in the Republic of Korea these two substances are controlled under different control regimes (as psychotropic and narcotic agents, respectively). Using chiral high-performance liquid chromatography, stereochemical identification of these two substances was established to differentiate them for forensic purpose.

Analytical aspects [11]

1 ml of blood was adjusted to pH 11-12 with 6N NaOH and extracted three times with 5 ml ethyl acetate. The pooled ethyl acetate was then evaporated under vacuum. The residues were then dissolved in 100 μl of internal standard containing ethyl acetate. One microlitre of this solution was then injected into the gas chromatograph. The integrated peak area ratio of the drug analytes to that of the external standard was used to calculate the concentration of each analyte.

The standard stock solution of dextromethorphan was 1 mg/ml in ethanol. Working standards were prepared by dilution with ethanol. Cinnarizine was used as an internal standard for the quantification of dextromethorphan.

A Varian model 4600 gas chromatograph equipped with a TSD and a Star data system was used for the determination of drug concentrations. A DB-5 megabore column (15 m × 0.53 mm) was programmed from 150° C to 250° C at 10° C/min, the injection port temperature was 270° C and the detector temperature was 280° C. The carrier gas (helium) flow rate was 7 ml/min. A Finnigan MAT GCQ was used to identify the drugs and metabolites. A fused-silica capillary SE-54 column (15 m × 0.32 m) was utilized in this instrument. The column temperature was programmed from 150° C to 250° C at 10° C/min, the ionization energy was 70eV, the transfer-line temperature was 270° C and the EM voltage was 1,600 V.

Calibration curves for dextromethorphan over the range of 1-20 μg/ml were established.

Combined abuse of zipeprol and dextromethorphan

Epidemiology

In order to obtain a stronger hallucinogenic effect, a commonly observed pattern of abuse was that of combining zipeprol and dextromethorphan [17]. Abusers deliberately take these two drugs together. Dextromethorphan, which is also an anti-tussive agent, produces little or no central nervous system depression [12], but manifestations of an acute overdose are known to include hallucinations and toxic psychosis [13, 14]. As a result of the combined abuse of large amounts of zipeprol and dextromethophan for recreational purposes, 12 fatal poisonings have been reported since 1991.

Similar to zipeprol alone, deaths related to the combined abuse of zipeprol and dextromethorphan were also broken down by age, gender and geographic places of origin of each of the 12 decedents. Figure IX illustrates this information in graphic form. The age range of 5 men and 7 women in this population was from 19 to 29 years, with an average age of 21.6 years. More females than males died from this overdose combination, with a female/male ratio of 1.4:1 being observed. The majority of these overdose victims (75 per cent) were in their twenties, with the remaining 25 per cent of the population in their teenage years.

Figure IX. Distribution of combined zipeprol- and dextromethorphan-related deaths in the Republic of Korea by age and gender

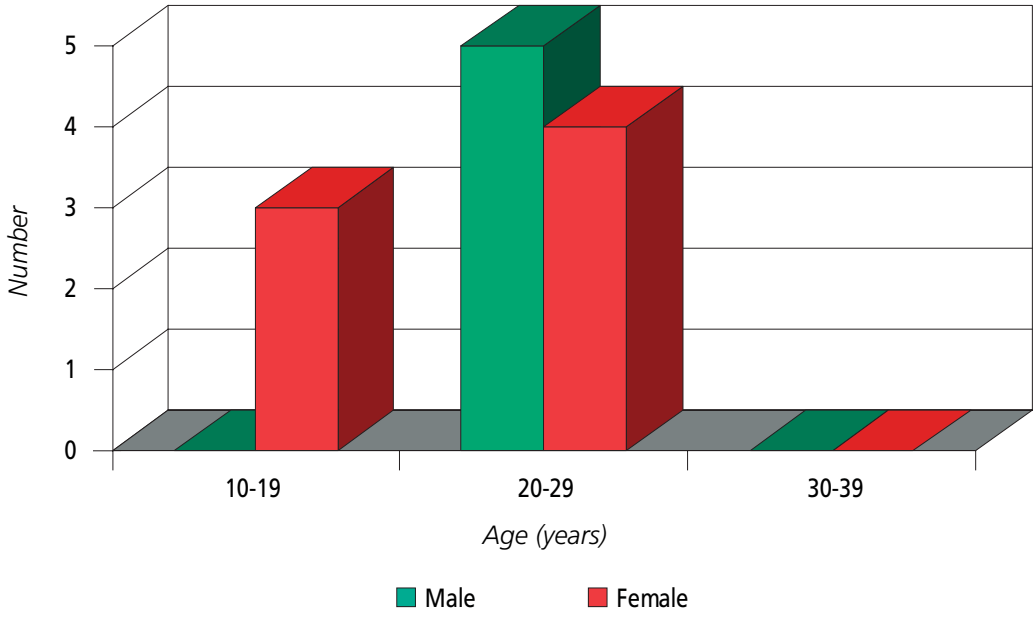


Figure X shows the distribution of the deaths involving the zipeprol and dextromethorphan combination by gender and region. As with zipeprol alone, the larger cities had a higher percentage of zipeprol/dextromethorphan-associated deaths. However, in the case of this combination, it was the city of Inchon that had the larger number of cases. Of the total combined-drug death cases, 41.7 per cent of the deaths occurred in Inchon and 33.3 per cent in Seoul. Interestingly, Seoul, again, showed a greater number of deaths in the female population. In smaller cities such as Pyongtaek, Sunchon and Chunchon, all reported deaths were female. This is consistent with the speculation that many women who work in the red-light district had abused dextromethorphan.

Figure X. Distribution of zipeprol- and dextromethorphan-related deaths in the Republic of Korea by gender and location

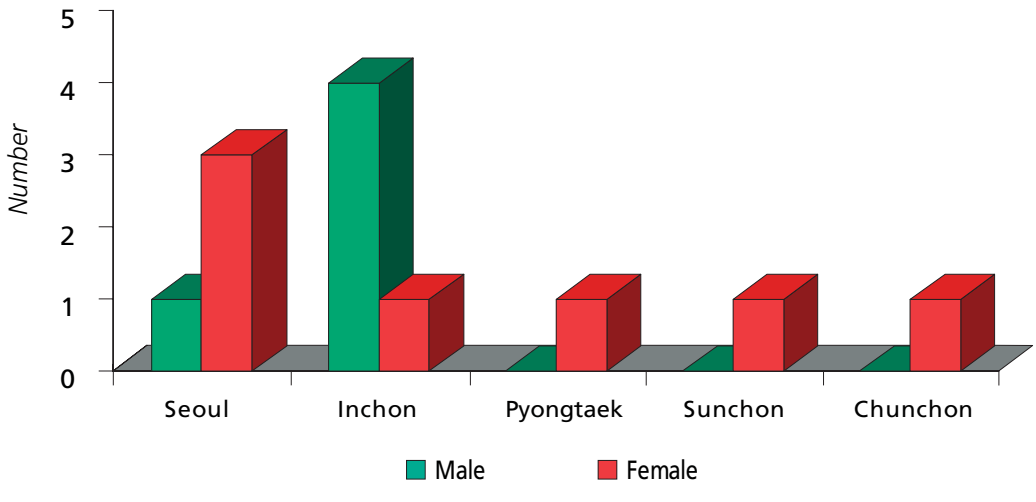
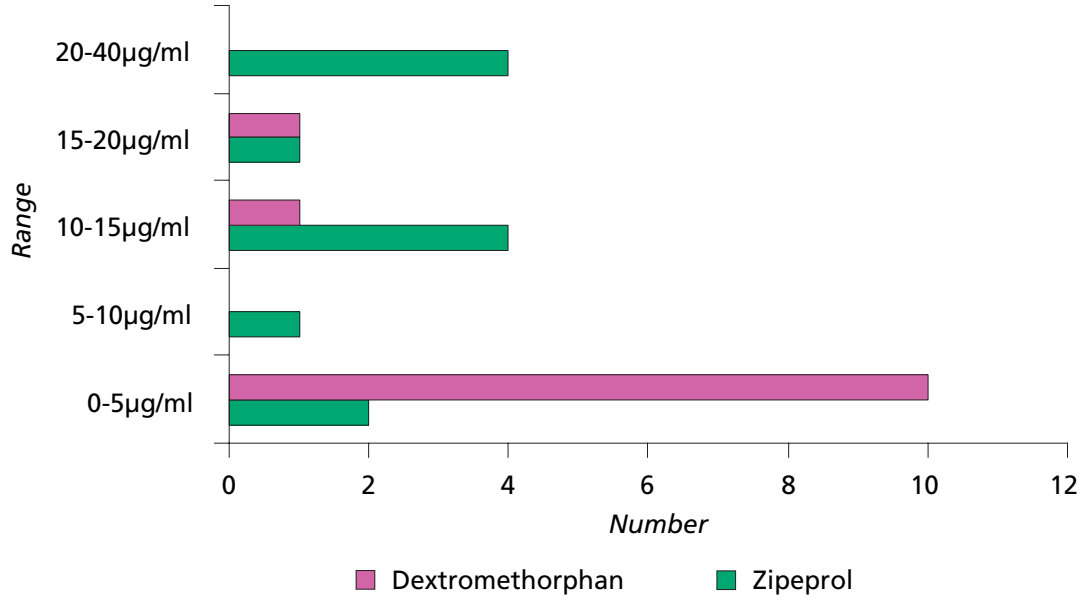


Figure XI shows the frequency distribution of zipeprol and dextromethorphan concentrations in postmortem blood samples. The blood concentration of dextromethorphan ranged from 1.1 to 18.3 μg/ml, while the zipeprol concentration in this population varied from 0.1 to 35.3 μg/ml.

Figure XI. Range of concentration of zipeprol and dextromethorphan in postmortem blood samples (μg/ml) in drug-involved deaths in the Republic of Korea



For zipeprol, the variation in concentration demonstrated the following ranges: 16.6 per cent of the case group were within the 0-5 μg/ml range, 8.3 per cent were in the 5-10 μg/ml range, 33.3 per cent in the 10-15 μg/ml range and 33.3 per cent were in the range of over 20 μg/ml. For dextromethorphan, in 83.3 per cent of these same cases, the blood concentration was in the 0-5 μg/ml range and 8.3 per cent were each in the range of 10-15 μg/ml and 15-20 μg/ml.

Development of the detection method

Similar to zipeprol and dextromethorphan alone, gas chromatography and gas chromatography/mass spectrometry methods for the simultaneous detection of zipeprol and dextromethorphan in biological fluids (blood and gastric content) had to be developed for both screening and confirmation [17]. Appropriate dilution factors were applied to the gastric content samples for calculating their concentrations, because of their varied drug concentrations.

Analytical aspects [17]

Analyses were performed on 1 ml of blood and 1 g of gastric contents. Samples were adjusted to pH 11-12 with 6N NaOH and extracted three times with 5 ml ethyl acetate. The combined organic extracts were re-extracted with 2 ml 0.25 N sulphuric acid (H_2SO_4). After discarding the organic layer, the pH was adjusted to pH 11-l2 by adding 6N NaOH to the aqueous phase and this solution was extracted twice with 5 ml ethyl acetate. The pooled ethyl acetate was evaporated under vacuum and the residue was dissolved in 100 μl of the 10 μg/ml cinnarizine. One microlitre of this solution was then injected into the gas chromatograph. The ratio of the peak area of zipeprol and dextromethorphan to that of the chromatographic standard was used to calculate the concentration of each analyte. Appropriate dilution factors were applied to the gastric content samples for calculating their concentrations because of their varied drug concentrations. Gas chromatography/mass spectrometry was used for the identification of the drug. Calibration curves for zipeprol and dextromethorphan over the range of 1, 5, 10 and 20 μg/ml were established using cinnarizine as the chromatographic standard. The recoveries of drugs from 1 ml of drug-free whole blood spiked with drugs were calculated from these curves. The standard stock solutions of zipeprol and dextromethorphan were 1 mg/ml in ethanol. Working standards (1, 5, 10 and 20 μg/ml) were prepared by dilution with ethanol.

A Varian model 4600 gas chromatograph equipped with a TSD and a DS 654 data system was used for the screening and quantitation of zipeprol and dextromethorphan. A DB-5 megabore column (15 m × 0.53 mm) was programmed from 150° C (1 min) to 250° C (10 min) at 10° C/min. A Finnigan MAT ITD 800 was used to identify the drugs. The mass spectrometry conditions were as follows. A fused-silica capillary SE-54 column (15 m × 0.25 mm) was used. The column temperature was programmed from 150° C to 250° C at 10° C/min; the ionization energy was 70 eV; the transfer-line temperature was 270° C and the EM voltage was 1,600 V.

Abuse of Carisoprodol

Carisoprodol was freely sold (without prescription) as a skeletal muscle relaxant and used for the relief of pain and muscle spasm [6]. The major metabolite of carisoprodol, meprobamate [18], is itself used as a sedative, anti-anxiety drug and a muscle relaxant [13]. While meprobamate has long been controlled as a psychotropic agent, the parent compound, carisoprodol, was not controlled.

Carisoprodol is believed to induce hallucination if large amounts are ingested [19]. It is thought to act by causing sedation rather than by direct skeletal relaxation and to produce a withdrawal syndrome characterized, for example, by agitation, hallucinations and seizures from large doses [20].

The abuse of carisoprodol for recreational purposes was prevalent among young people of the Republic of Korea [21]. There were less than 6 abuse cases reported every year from 1991 to 1996 (figure III), but the number soared to 185 in 2003 according to NISI data. There have also been fatalities due to the

overdose of this medicine [21]. In seven suicide cases, carisoprodol was detected in the biological fluids after autopsy. The table below shows the age, gender and blood levels of carisoprodol and its metabolite, meprobamate, as well as other drugs detected in the seven fatal cases. The age of the deceased ranged from 27 to 43 years, with an average age of 35. Fatalities involved five males and two females. The blood concentrations of carisoprodol ranged from 22.9 to 124.4 μg/ml, while the concentrations of meprobamate were from 26.8 to 144.5 μg/ml. The ratio of meprobamate to carisoprodol ranged from 0.3 to 6.2, that is, there was no correlation between the concentrations of the parent drug and its metabolite. In two cases, dextromethorphan was also present and in one case an alcohol concentration of more than 0.05 per cent was detected.

Carisoprodol postmortem blood levels in 7 carisoprodol-related deaths

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*
CSP* (μg/ml)	61.7	22.9	124.4	58.3	33.1	48.7	123.6
MPB** (μg/ml)	144.5	143.1	35.5	81.4	26.8	39.9	128.1
Combination	—	—	—	—	Alc 0.05%	DEX*** 8.5	DEX 11.6
MPB/CSP ratio	2.3	6.2	0.3	1.4	0.8	0.8	1.0
Gender/age	M/36	F/43	M/32	M/27	F/41	F/-	M/29

*Carisoprodol.
**Meprobamate.
***Dextromethorphan.

Against the background of carisoprodol abuse and related fatalities, the Government of the Republic of Korea introduced controls of this drug in October 2003. Since then, carisoprodol has not been detected either in urine or other biological fluids.

Development of the detection method

Since there was no method available for the determination of carisprodol in biological fluids, a gas chromatograhy method was developed [21] and then applied to the analysis of carisoprodol in biological fluids (blood, bile juice, urine or homogenized tissues).

Analytical aspects [21]

To 1 ml of blood, bile juice and urine or 1 g of homogenized tissues, 2 ml of 0.1 M phosphate buffer (pH 6.0) and 10 μl of brompheniramine (1,000 μg/ml) as an internal standard were added, vortex mixed and then centrifuged at 2,500 rpm for 10 minutes. The supernatant was extracted with a Bond Elute SPE column. Columns were preconditioned with 2 ml methanol, 2 ml of 0.1 M phosphate buffer (pH 6.0). Then the supernatant was applied onto the column at the rate of 1-2 ml/min. The columns were washed with 1 ml deionized water and 0.5 ml of 1.0 M acetic acid. Then the columns were dried with 0.5 ml of methanol. To each column, 3 ml of $CHCl_3$:acetone (50:50) was added and eluted completely. The eluates were evaporated under a nitrogen stream. The residue was reconstituted with ethanol and injected into the gas chromatograph/mass spectrometer.

Gas chromatography/mass spectrometry (Agilent 6890/5973) was used with the selected ion monitoring mode for identification. An HP-5 MS capillary column (30 m x 0.25 mm x 0.25 μm) was applied from 120° C to 270° C with helium as a carrier gas to measure the contents of carisoprodol in the biological fluids.

Abuse of nalbuphine

Epidemiology

The abuse of the nalbuphine was noticed for the first time in late 1991, when it was abused as an alternative for methamphetamine because of limited availability of that drug [22].

Nalbuphine is a synthetic partial opiate agonist. It is used as an analgesic for the treatment of moderate to severe pain as well as a supplement in balanced surgical anaesthesia. Nalbuphine is also used to provide preoperative and postoperative sedation [23]. Its preparations are varied, including oral, subcutaneous, intramuscular and intravenous applications [7]. Clinical study showed that the compound has low abuse liability, although it produces sedation, central nervous system depression, hallucination and euphoria when abused. The development of physical and psychological dependence of this drug is so quick and conspicuous that even after just one week users reveal symptoms such as reduced appetite, weight loss, goose flesh, sweat and tremor [24].

The abuse of nalbuphine proliferated from 4 in 1995 to 32 in 1996 and 110 in 2000 (figure III). Because its abuse had been widespread with the number of cases rising to 1,520 in 2001, the Government of the Republic of Korea started to control nalbuphine as a psychotropic agent in 2001. As a result, there were only two urine specimens that tested positive for the drug in 2002.

Development of the detection method

To respond to the widespread prevalence of abuse of nalbuphine, there was a need to develop an analytical method for the identification and quantitation of nalbuphine and its three metabolites in urine. The development of gas chromatography-based methods (gas chromatography and gas chromatography/mass spectrometry) was complicated by the chemistry of nalbuphine, which carries three hydroxyl moieties [25]. To reduce the polarity of nalbuphine, a derivational method was developed, using a combination of silylating agents (N-methyl-trimethylsilyltrifluoroacetamide (MSTFA), trimethylsilylimidazole (TMSI) and trimethylchlorosilane (TMCS) to produce tri-trimethylsilyl nalbuphine [22].

Analytical aspects [22]

The extraction of nalbuphine was performed with Clean Screen DAU columns that were installed on a vacuum manifold. The column was preconditioned with 2 ml methanol and 1 ml 0.1 M phosphate buffer (pH 3.3). A 1 ml urine sample was vortex mixed for 30 seconds and passed through the column at a rate of 1.5 ml/min. The column was then washed with 1 ml 0.1 M phosphate buffer (pH 3.3), 0.5 ml acetic acid (0.01 M, pH 3.3) and 3 ml methanol. After drying under vacuum for 2 minutes, the drug was eluted by passing through the column 2 ml of 2 per cent ammoniated methanol at a flow rate of 0.5 ml/min. The eluate was evaporated under vacuum. Recovery was performed after adding 0.5, 1 and 5 μg/ml nalbuphine in urine.

To the dried extract, 50 µl of MSTFA-TMSI-TMCS (100:2:5) was added and incubated at 70° C for 20 minutes. One microlitre of this was injected onto the gas chromatograph and the gas chromatograph/mass spectrometer.

A Varian model 4600 chromatograph equipped with a flame-ionization detector and a fused silica wide bore DB-5 capillary column (15 m×0.53 μm i.d., 1.0 μm film thickness) was utilized for the screening and quantitation of nalbuphine. Column temperature was programmed from 200° C (1 min) to 280° C (10 min) at 10° C/min. The injection port and detector temperatures were 270° C and 290° C, respectively. A Finnigan gas chromatograph/mass spectrometer Model 4021 connected to a Nova 4 system was used to identify nalbuphine and its metabolites. Mass spectrometer conditions were as follows: column, a fused-silica capillary column SE-54 (15 m×0.25 mm i.d.); ionization energy, 70 eV; ion source temperature, 240° C; transfer-line temperature, 270° C and EM voltage, 1,400 V.

Conclusions

Drug testing plays an important role in the provision of information on trends in drug abuse to health authorities. In the Republic of Korea, drug testing has been used as an element of a programme to monitor, control and prevent the spread of abuse of non-controlled drugs. These drugs are typically available as medicines, but are frequently abused, in particular among young people. There

have been several cases of non-controlled drugs, including zipeprol, dextromethorphan, carisoprodol and nalbuphine, in which drug testing and identification was used as part of a monitoring programme for drug abuse. This information subsequently became the basis for controlling those drugs.

Since none of these substances were on the list of controlled drugs, it was necessary to develop methods for their detection in biological fluids to monitor the drug abuse trends. Specific gas chromatograph and gas chromatograph/mass spectrometry methods were developed to identify and quantitate these drugs and/or their metabolites in biological fluids. Analytical data obtained from postmortem specimens were accumulated for providing the fundamental information to create a drug abuse warning system.

Based on the results of drug testing, the health authorities in the Republic of Korea introduced controls on four common medicines: zipeprol was controlled in 1995, nalbuphine in 2001 and dextromethorphan and carisoprodol in 2003. After the introduction of the control measures, the abuse of these substances and the frequency of their detection in abuse cases decreased significantly, underlining the importance of timely action as a means for improving community health.

References

1. H. S. Chung, "Drug abuse trends and analysis of abused drugs in Korea", *Korean Criminological Review*, vol. 8, No. 1, pp. 109-152 (1997).
2. Supreme Prosecutor's Office, *Drug Crimes Yearbook 1994: Drug Control in Korea* (Seoul, Daejong Press, 1994) pp. 341-378.
3. Y. C. Yoo, H. S. Chung and H. K. Choi, "Determination of methamphetamine and its metabolite amphetamine in biological fluids from 11 fatal cases", *Archives of Pharmacal Research*, vol. 16, No. 3, pp. 175-179 (1993).
4. J. S. Lee and others, "Analysis of opiate alkaloids in seized Chinese analgesics, 'bokbanggamchopyeon'", Proceedings of the Convention of the Pharmaceutical Society of Korea (2003).
5. D. P. Jung, "The movements and situation of drug offences in Korea", Proceedings of the Anti-Drug Liaison Officials' Meeting for International Cooperation (Seoul, 1999).
6. H. S. Chung and others, "Recent trends of drug abuse and drug-associated deaths in Korea", *Annals of the New York Academy of Sciences*, vol. 1025, pp. 458-464 (2004).
7. B. S. Kim, *Medical Index*, 6th ed. (Seoul, Korean Medical Index Co., 1991).
8. Y. C. Yoo, H. S. Chung and E. M. Kim, "Gas chromatographic analysis of zipeprol in blood", *Yakhak Hoeji*, vol. 36, No. 3, pp. 191-198 (1992).
9. Y. C. Yoo and others, "Postmortem distribution of zipeprol", *Journal of Analytical Toxicology*, vol. 18, pp. 213-216 (1994).
10. H. S. Chung and others, "Demographic characteristics of zipeprol-associated deaths in Korea", *Archives of Pharmacal Research*, vol. 21, No. 3, pp. 286-290 (1998).

11. Y. C. Yoo and others, "Blood concentration of dextromethorphan in fatal overdose", Annual report, National Institute of Scientific Investigation, 20, p. 160 (1988).
12. J. L. Bem and R. Peck, "Dextromethorphan: an overview of safety issues", *Drug Safety*, vol. 7, No. 3, p. 190 (1992).
13. R. Baselt and R. Cravey, *Disposition of toxic drugs and chemicals in man*, 5th ed. (Foster City, California, Chemical Toxicology Institute, 2000).
14. M. N. Darboe, G. R. Keenan and T. K. Richards, "The abuse of dextromethorphan-based cough syrup: a pilot study of the community of Waynesboro, Pennsylvania", *Adolescence*, vol. 31, p. 633 (1996).
15. H. S. Chung and others, "Metabolic phenotyping and genotype of dextromethorphan in Korea", *Yakkak Hoeji*, vol. 46, No. 3, pp. 179-184 (2002).
16. S. C. Kim and others, "Sterochemical analysis of dl-3-methoxy-17-methylmorphinan by High Pressure Liquid Chromatography equipped with PDA", 43rd International Meeting of the International Association of Forensic Toxicologists (Seoul, 2005).
17. Y. C. Yoo and others, "Fatal zipeprol and dextromethorphan poisonings in Korea", *Journal of Analytical Toxicology*, vol. 20, pp. 155-158 (1996).
18. P. Kintz and others, "A rapid and sensitive gas chromatographic analysis of meprobamate or carisoprodol in urine and plasma", *Journal of Analytical Toxicology*, vol. 12, pp. 73-74 (1988).
19. B. K. Logan, G. A. Case and A. M. Gordon, "Carisoprodol, meprobamate, and driving impairment, *Journal of Forensic Sciences*, vol. 45, No. 3, pp. 619-623 (2000).
20. R. C. Backer, R. Zumwalt and P. Mafeeley, "Carisoprodol concentrations from different anatomical sites: three overdose cases", *Journal of Analytical Toxicology*, vol. 14, No. 5, pp. 332-334 (1990).
21. H. Y. Choi and others, "Postmortem blood and tissue concentration of carisoprodol and meprobamate", *Journal of the Korean Society of Clinical Toxicology*, vol. 1, No. 1, pp. 34-39 (2003).
22. Y. C. Yoo and others, "Determination of nalbuphine in drug abusers' urine", *Journal of Analytical Toxicology*, vol. 19, pp. 120-123 (1995).
23. M. W. Lo, W. L. Schary and C. C. Whitney, "The disposition and bioavailability of intravenous and oral nalbuphine in healthy volunteers", *Journal of Clinical Pharmacology*, vol. 27, pp. 866-873 (1987).
24. D. R. Jasinski and P. A. Mansky, "Evaluation of nalbuphine for abuse potential", *Clinical Pharmacology and Therapeutics*, vol. 13, pp. 78-90 (1972).
25. C. L. Lake and others, "High-performance liquid chromatographic analysis of plasma levels of nalbuphine in cardiac surgical patients", *Journal of Chromatography*, vol. 233, pp. 410-416 (1982).

Psychoactive plant abuse: the identification of mitragynine in ketum and in ketum preparations

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ABSTRACT

Recently, the abuse of ketum, an indigenous psychoactive plant, has received a lot of attention in Malaysia. To help national law enforcement agencies control its abuse, the laboratory of the Forensic Division has developed a procedure for its positive identification. Botanical identification may not be practical or conclusive, owing to the wide range of ketum materials available on the market, including dry macerated leaves, powdered leaves and drinks. In order to confirm that a substance is, in fact, ketum or that a preparation is derived from ketum, gas chromatography–mass spectrometry is used to definitively identify the presence of the psychoactive principle mitragynine.

Keywords: ketum; mitragynine; identification by gas chromatography–mass spectrometry

Introduction

Mitragyna speciosa Korth. (Rubiaceae) is a tropical plant indigenous to Thailand and peninsular Malaysia. In Thailand, the leaves of the plant are known as "kratom", while in Malaysia, they are commonly called "ketum" or "biak". Traditionally, ketum leaves have been used by local populations for their opium-like effect and their coca-like stimulative ability to combat fatigue and enhance tolerance to hard work under the scorching sun. In 1907, Wray [1] described how the leaves were processed and used as a substitute for opium in peninsular Malaysia. It is reported in the local media that traditional healers use ketum to wean addicts off heroin addiction, to deworm, to cure diarrhoea, to improve blood circulation and even to treat diabetes. However, a study conducted in Thailand in 1975 [2] showed that ketum users became addicted. Typical withdrawal symptoms include hostility, aggression, excessive tearing, inability to

*The authors would like to thank the Pharmaceutical Services Division and the Institute of Medical Research of the Ministry of Health of Malaysia for providing the mitragynine reference standard. The authors would also like to thank Ikram M. Said of the National University of Malaysia for providing some of the bibliographical references.

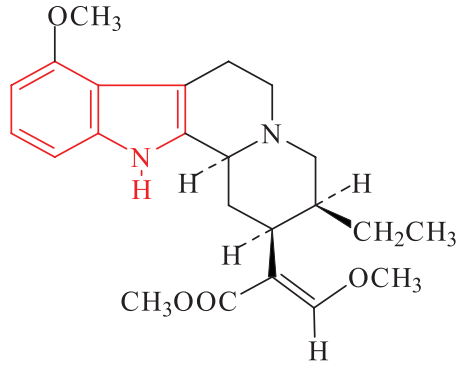
work, aching of muscles and bones and the jerky movement of limbs. It was also reported in the study that anorexia, weight loss and insomnia were common among long-term ketum addicts. The ketum plant has also been banned in Thailand since 1943 and has also been banned in Australia and Myanmar.

In Malaysia, the use of most indigenous plant-based drugs (with the exception of cannabis) is not commonplace. From 1978 to 2003 there were only a handful of cases involving the use of *Datura stramonium*—which contains the tropane alkaloids scopolamine and hyoscyamine—and ketum leaves. Recently, there has been a growing trend among drug addicts to use the bitter-tasting ketum leaves to get high when they are unable to get their regular supply of cannabis or heroin. In early 2004, stalls selling ketum drinks and teas had mushroomed in several towns around the country, and youths were reported to be drawn to the concoctions. That upsurge in ketum abuse has caused considerable concern among the public and law enforcement authorities, and there is a perception that the consumption of ketum leads to the abuse of other drugs such as cannabis and heroin. The ready availability and very low price of ketum compared with other controlled drugs has contributed to its popularity. Fresh and powdered leaves for making drinks are available for 4 ringgit (RM) (about 1 United States dollar) and RM 25 per kilogram, respectively, while small packet drinks are sold at RM 1, according to information from the media and law enforcement authorities.

To curb and control ketum abuse in Malaysia, its major alkaloidal constituent, mitragynine (see figure I), was listed in the First Schedule and the Third Schedule (psychotropic substances) of the Poisons Act 1952 of January 2003. Under the Act, the planting of the tree is not an offence, and enforcement agencies have no authority to fell the trees. The maximum penalty for possessing or selling ketum leaves or other ketum preparations such as drinks and teas containing mitragynine is a fine of RM 10,000, a four-year jail sentence or both. Since the alkaloid is exclusive to *Mitragyna speciosa*, the Act has had the effect of controlling ketum abuse without making the trees illegal or requiring them to be cut down. In general, law enforcement agencies in the country are calling for all ketum trees nationwide to be cut down, while ketum proponents point to its potential medicinal value and warn of the irreparable loss to the country's biodiversity should the indigenous tree be eradicated.

The present article provides information on the identification of mitragynine in ketum and ketum preparations seized by law enforcement agencies for prosecution purposes. The samples submitted to the laboratory for analysis ranged from small packet drinks to several kilograms of fresh leaves, dry leaves and finely ground powder. It would be quite impossible to identify ketum herbal products and preparations on the basis of general morphological features, especially when the materials examined are in the form of drinks, powdered leaves, macerated dry leaves or decaying leaves. But identification can be made on the basis of the presence of mitragynine. Mitragynine is identified by comparing samples against a reference standard using a gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC/MS).

Figure I. Chemical structure of mitragynine



Mitragynine $C_{23}H_{30}N_2O_4$
MW : 398.5

Analytical procedure

Reagents

Mitragynine reference standard was obtained from the Institute of Medical Research of the Ministry of Health of Malaysia. Methanol and chloroform were purchased from Fisher Scientific.

Extraction

Leaves and powdered leaves

Two grams of powder or crushed leaves were ultrasonicated in 25 ml of $CHCl_3$/methanol (1:4) for 10 minutes. The solution was allowed to settle, and an aliquot was taken for analysis.

Liquid samples (drinks and teas)

Twenty ml of the liquid sample were acidified with a few drops of concentrated HCl and extracted with 20 ml of diethyl ether. The ether layer was discarded, and the aqueous portion was basified with an NaOH solution and checked with pH paper. The solution was extracted with 20 ml of chloroform twice, and the combined extracts were washed with distilled water. The chloroform extract was filtered through anhydrous sodium sulphate and left to dry in the fume cupboard. The dry extract was taken up in 1 ml of methanol for analysis.

Gas chromatography-flame ionization detector

A Shimadzu gas chromatograph GC-17A with a flame ionization detector and fitted with a 30 m × 0.25 mm id, 0.25 μm film thickness HP-5 capillary column was utilized. The temperature programme was 200° C, held for 2 minutes, then increased at 10° C per minute to 300° C and held for 20 minutes. The injector

and detector temperature were set at 280° C. One μl of the standard (approximately 0.10 mg/ml in methanol) and each sample solution were injected using an auto-sampler.

Gas chromatography-mass spectrometry

A Shimadzu GCMS-QP5050 mass spectrometer interfaced with a Shimadzu GC-17 gas chromatograph and equipped with a 30 m × 0.25 mm id, 0.1 μm film thickness DB-5 capillary column was utilized. The temperature programme described above was used. One μl of the standard (approximately 0.10 mg/ml in methanol) and each sample solution were injected using an auto-sampler.

Results and discussion

The GC/FID spectra of mitragynine reference standard and leaf extract are shown in figure II and III, respectively. The peak at 16.5 minutes in figure III was tentatively identified as mitragynine. The mitragynine peak in the various ketum preparations was definitively identified by comparing its retention time and mass spectrum with the mitragynine reference standard (see figures IV-VII).

Figure II. Gas chromatogram of mitragynine reference standard

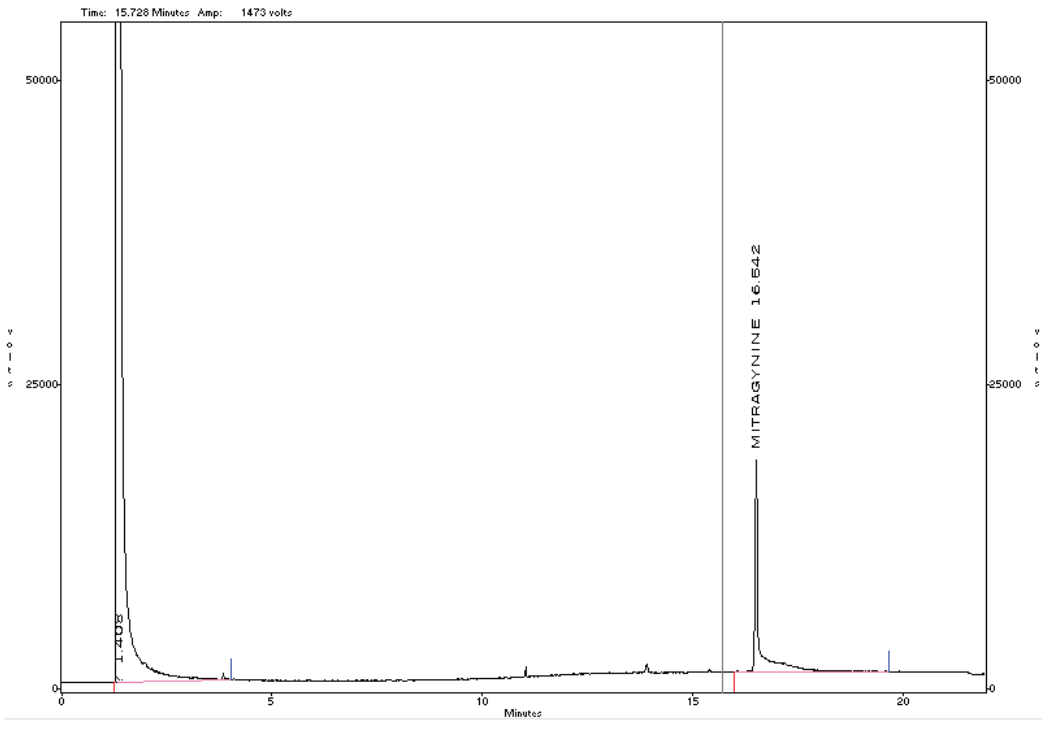


Figure III. Gas chromatogram of leaf extract

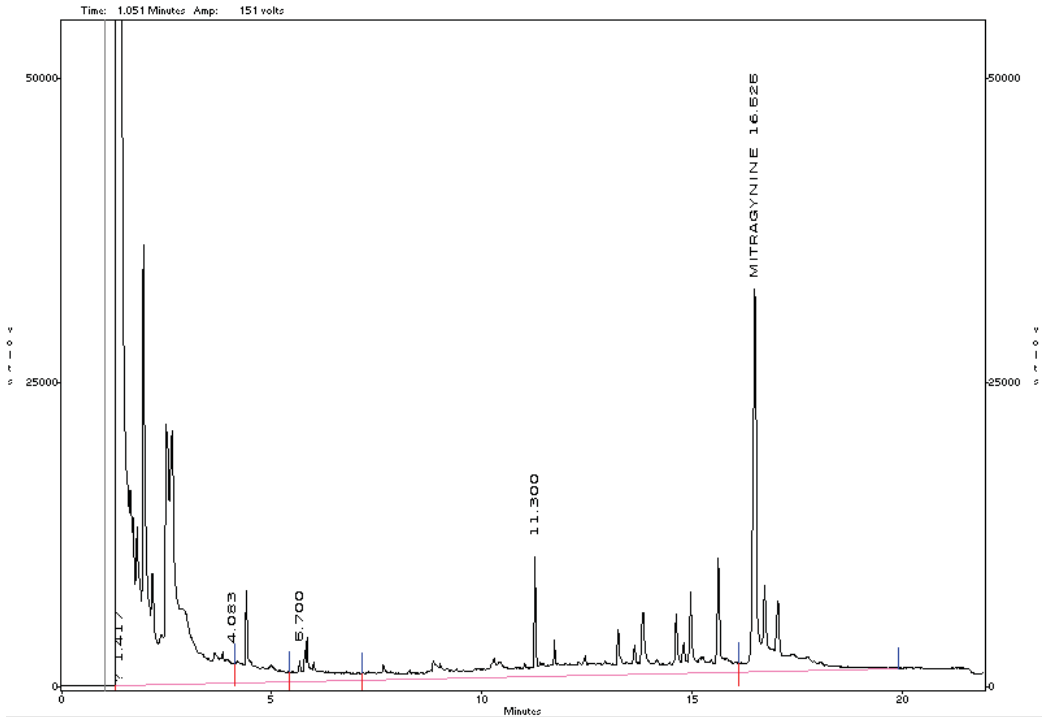


Figure IV. Total ion chromatogram of mitragynine reference standard (ca. 0.10 mg/ml)

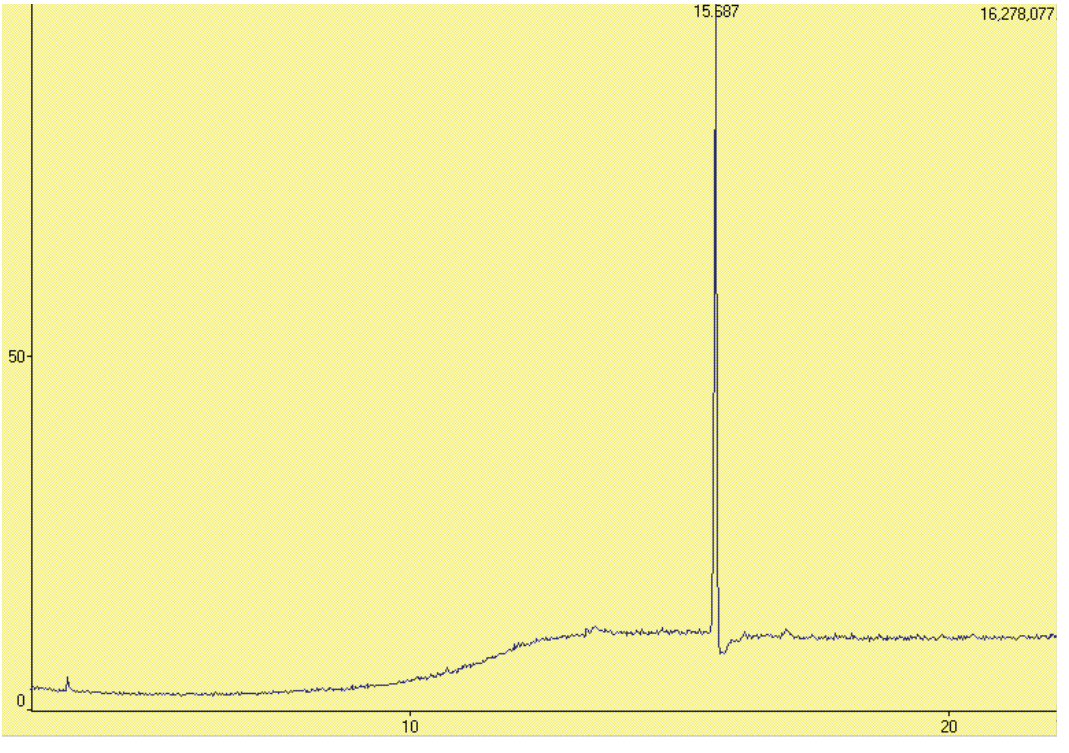


Figure V. Mass spectrum of mitragynine reference standard

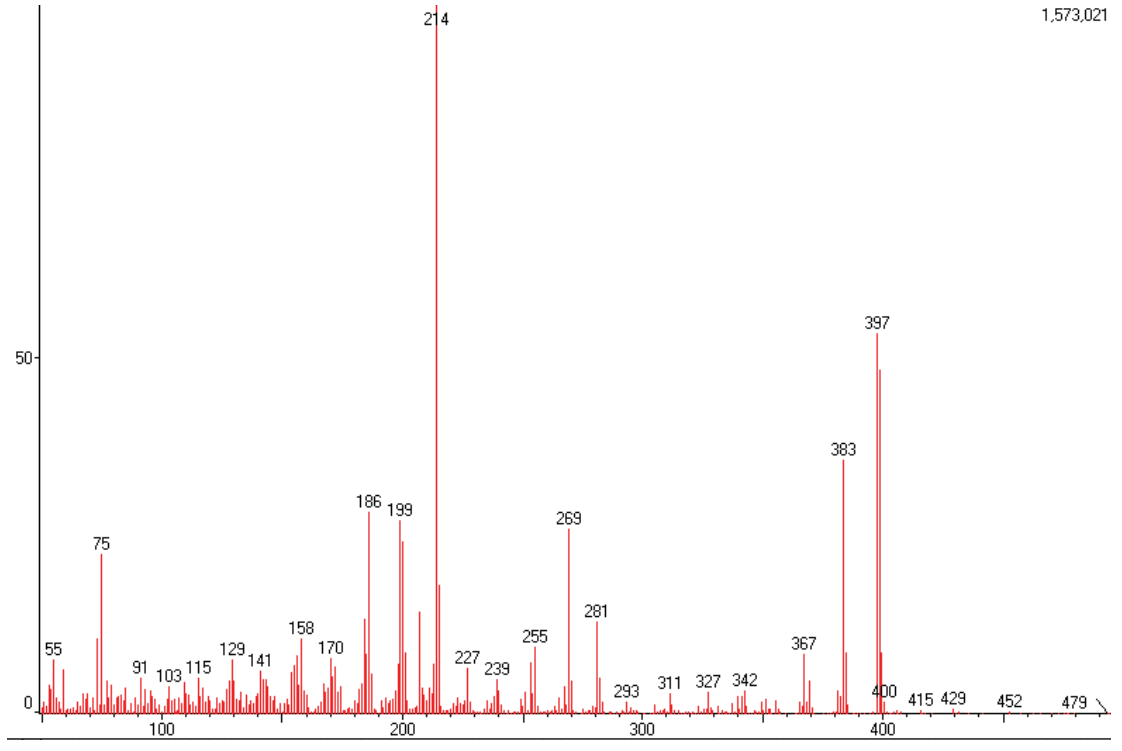


Figure VI. Total ion chromatogram of leaves extract

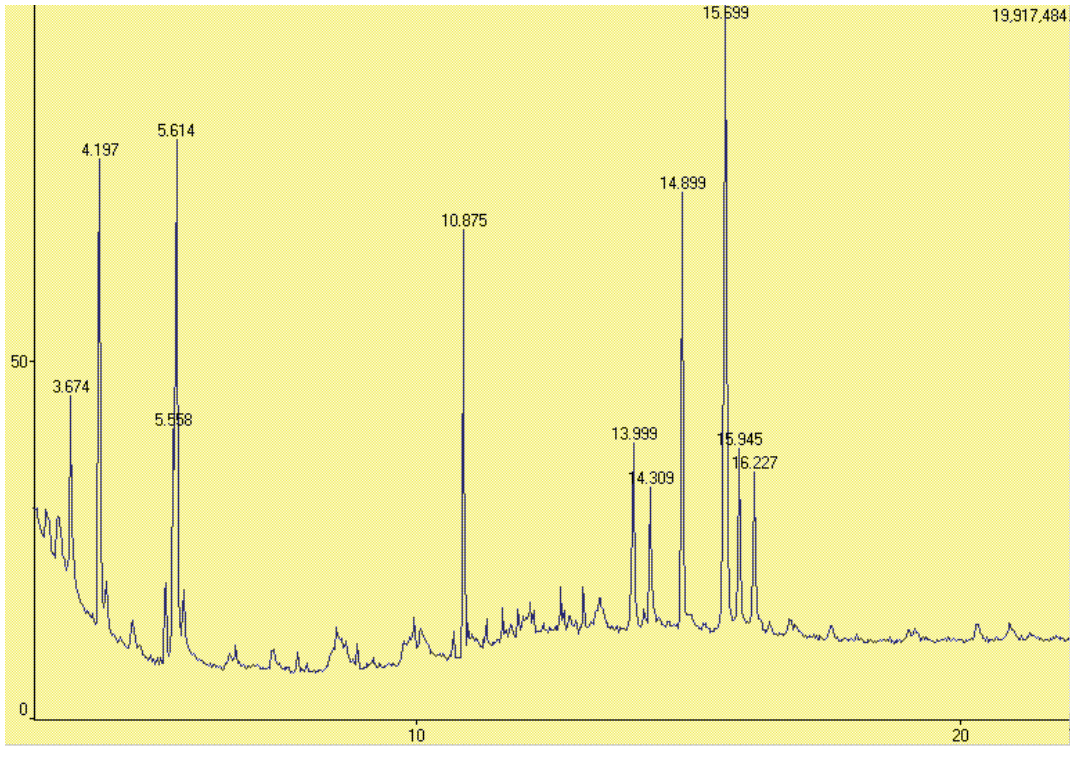
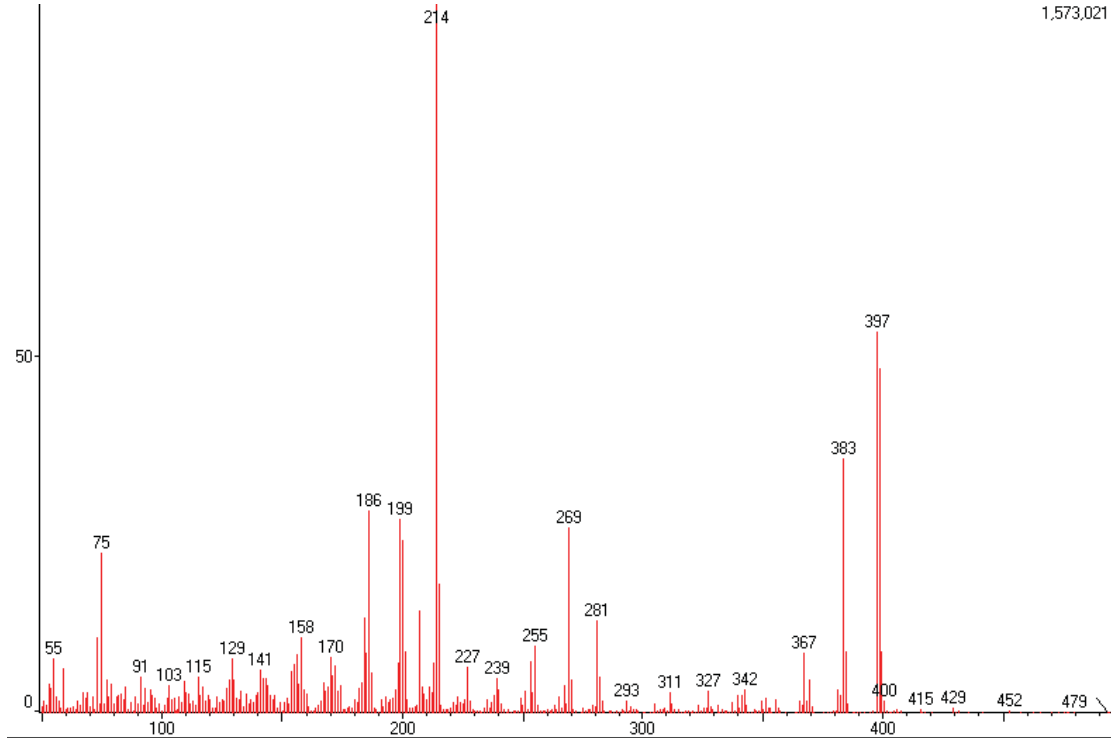


Figure VII. Total ion chromatogram of drink extract



Methanolic extracts of the leaves and powder were studied initially, but the resulting gas chromatograms were not satisfactory. Extraction with a mixture of methanol and chloroform gave much better results.

Owing to the limitation of the thin-layer chromatography technique, which is not a definitive technique, and the scarcity of the mitragynine reference standard, identification by that technique was not attempted. The screening of dry macerated leaves and ketum powdered leaves using the Duquenois-Levine test for cannabis produced a dull blackish green colour that was not extractable into the chloroform layer. Beyond the differentiation from cannabis herb, other colour tests, for example, the van Urk, Ehrlich and Wasicky reagents, which probably react with mitragynine's indole moiety, were not performed as part of the present work.

The laboratory has reprised its main function of providing crucial drug-testing services to the national law enforcement agencies in their effort to control the abuse of an indigenous psychoactive plant and preparations derived from it. With the provision of that testing, the relevant agencies can now confidently monitor, control and prevent the abuse of the plant in all its forms.

References

1. H. Burkill, *A Dictionary of the Economic Products of the Malay Peninsula*, vol. II (London, Crown Agents for the Colonies), 1935.
2. S. Suwanlert, "A study of kratom eaters in Thailand", *Bulletin on Narcotics*, vol. 27, No. 3 (1975) (United Nations publication), pp. 21-28.

Bibliography

Alkaloids from *Mitragyna speciosa*. *By* P. J. Houghton *and others. Phytochemistry* 30:1:347-350, 1991.

Burkill, H. A dictionary of the economic products of the Malay Peninsula, vol. II. London, Crown Agents for the Colonies, 1935. 1481 p.

The Merck index. 13. ed. New York, John Wiley and Sons, 2001. 1110 p.

New indole alkaloids from the leaves of Malaysian *Mitragyna speciosa*. *By* H. Takayama *and others. Tetrahedron* 54:8433-8440, 1998.

Shellard, E. I. The alkaloids of *Mitragyna* with special reference to those of *Mitragyna speciosa* Korth. *Bulletin on Narcotics* 26:2:41-55, 1974.

United Nations publication.

Suwanlert, S. A study of kratom eaters in Thailand. *Bulletin on Narcotics* 27:3:21-28, 1975.

United Nations publication.

Takayama, H. Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, *Mitragyna speciosa. Chemical and Pharmaceutical Bulletin* 52:8:916-928, 2004.

U Win Pe. A short note on the identification of mitragynine. *Forensic Science International* 15:2:149-151, 1980.

Section IV. Quality management in laboratories

Quality management systems and the admissibility of scientific evidence: the Costa Rican experience

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ABSTRACT

Certainty and truth are, by definition, objectives of science. There is a tendency among people to believe that anything produced by a scientist is science and is therefore certain. On the contrary, scientific findings are not free of error. In fact, science evolves, among other things, by questioning and verifying the ideas and theories that are held to be scientifically valid and by continuously searching for new knowledge.

As judicial systems in several countries have evolved over time, they have established minimum criteria for the admissibility of scientific evidence in order to ensure accuracy as far as possible. Forensic laboratories in countries with such requirements have established quality systems as a tool for verifying the standards of the scientific information they provide to courts as evidence. The International Standard ISO/IEC 17025 has been chosen in testing laboratories, including forensic laboratories, to provide uniform technical criteria for developing a quality management system.

There is agreement between the ISO standard and admissibility requirements for courts. Therefore, the application of international quality standards to forensic laboratories is of interest to, and must be understood by, not only scientists but also judicial authorities. The present article describes the Costa Rican experience.

Keywords: physical evidence; scientific evidence; system of justice; crime scene; forensic scientist; forensic laboratory; quality; quality management system; ISO/IEC 17025; standard operating procedure; inter-laboratory studies; occupational health

Importance of quality for a forensic laboratory

Requirements for the operation of a forensic laboratory vary in accordance with the level of experience and development of the justice system that the

laboratory serves, the importance that the system attaches to the quality of scientific evidence and the demands imposed by the judicial system in that respect.

That becomes clear when considering the potential consequences of ignorance on the part of the parties involved, including the judiciary, of the strengths and weaknesses of the scientific tools used to evaluate the physical evidence as part of the overall body of evidence and, even more so, when trying to understand the impact of those tools on how a given conclusion is reached. Today, it is generally accepted that physical evidence provides invaluable support in the conduct of criminal investigations, because it yields information that is equal in value to testimonial evidence, and more objective.

The ideal of science is to seek truth or certainty through the use of the scientific method. Experience over the centuries has shown that, generally speaking, scientific theories and ideas must be continually reviewed by the scientific community in order to reconfirm their validity or further develop them, by applying the appropriate scientific methods and principles to substantiate the results obtained.

Between the time it is collected at the crime scene and the moment when it is submitted to a court, physical evidence is given to a forensic scientist, who must examine and analyse it in order to obtain scientific results that provide useful information on the case under investigation, thus supporting or rejecting any theory formulated by the police or the investigating group. Those scientific results are communicated in writing (as an expert report) and orally (during an appearance in court). It is the forensic scientist's interpretation of the physical evidence that transforms it into scientific evidence.

Understanding the impact of scientific evidence on legal proceedings can significantly enhance a justice system, depending on that system's evidentiary requirements. It should be kept in mind, however, that, given its experimental nature, scientific evidence is not immune to error and must therefore meet minimum requirements before it can be deemed admissible. Lack of awareness of its limitations can give rise to complacent systems that consider anything labelled "scientific" to be true without first subjecting such evidence to a critical assessment.

Thus, there are general standards governing the methods used, the results obtained and the interpretation of those results by the forensic scientist that serve as guidelines for determining the admissibility of evidence. The courts of the United States of America widely use the following criteria, which have been adopted by other countries, as a guide for admitting scientific evidence:

(a) The scientific technique or theory can be or has been tested;

(b) The technique or theory has been submitted to prior review and publication;

(c) The potential margin of error of the technique is known;

(d) Standards for monitoring the application of the technique exist and are maintained;

(e) The scientific theory has gained wide acceptance among the relevant sections of the scientific community;

(f) The courts and the legal community accept the scientific findings concerned and use them as relevant evidence.

It is clear that the foregoing criteria broadly relate to the universal concept of quality. Today, no forensic laboratory concerned with quality could fail to incorporate a quality management system into its expert procedures because such systems are closely related to the basic, routine laboratory procedures that are so crucial to the justice system.

In Costa Rica, the Department of Forensic Science of the Judicial Investigation Bureau of the Judicial Power has adopted a strategic development plan to promote productivity and efficiency policies and improve the value of available expertise as scientific evidence, with the aim of obtaining conclusions of greater certainty, using due procedures with the help of a quality management system. The aims are spelled out in the vision and mission statement of the Department:

> "Mission: to serve the administration of justice efficiently and effectively by integrating technical and scientific knowledge into analysis procedures, thus ensuring that evidence is collected in a lawful, useful and truthful manner.
>
> "Vision: by constantly keeping up to date with the most recent advances in technical and scientific knowledge, to establish an organizational model for forensic science that serves as an example across the continent and, at the same time, to optimize analysis quality levels and production in order to respond to the needs and requirements of society."

In short, the primary focus is on providing a better service to users without departing from the rules and standards for the admissibility and attainment of scientific results, which should be an integral part of both the concept of quality that experts are trained to respect and the development of an organizational culture.

The task of establishing and developing quality management systems in forensic institutions and laboratories, as an aspect of scientific and administrative development, has been undertaken not only in Costa Rica, by the Department of Forensic Science; it is a global trend that has been growing in recent years. As is happening in most countries, in developing a quality management system, the Department of Forensic Science has adopted ISO/IEC 17025 of the International Organization for Standardization (ISO), which provides the general requirements for the competence of testing and calibration laboratories, divided into two categories: management requirements and technical requirements. Given that the standard is of a generic nature, various institutions and groups have produced manuals and guides for applying the standard to forensics. Those have been used by the Department of Forensic Science for guidance on the implementation and application of ISO/IEC 17025.

ISO/IEC 17025 provides tools for ensuring compliance with the above-mentioned criteria determining the admissibility of scientific evidence, such as documentation control, development of standard operating procedures, validation of analytical methods and introduction of measures to ensure that the quality of analytical results is regulated and consistent. That helps to ensure that physical evidence can be used to provide scientific evidence by applying forensic scientific knowledge and skill in the analysis of evidence.

The Department of Forensic Science started to use ISO/IEC 17025 in implementing its quality management system during the first quarter of 2000. The strategic approach adopted was to provide training so that each of the Department's eight sections could achieve a basic level of quality assurance. During the initial phase, all staff members received training in the requirements of ISO/IEC 17025 and the applicable procedures. Department staff compiled lists of all the activities and expert tasks carried out in the various sections in order to learn the details of the individual procedures being used. As part of that process, a distinguished professional in the area of natural sciences with extensive experience in metrology was brought in to coordinate and promote quality assurance measures. At the same time, an induction process on the forensic science work carried out in the Department, lasting more than a year, was introduced.

The Department of Forensic Science also introduced training courses and lectures, given by both in-house staff and outside experts, on the subject of quality and forensics so that uniform standards could be achieved throughout the Department in matters relating to quality assurance and scientific development, with a view to fostering a culture of excellence in tandem with the move to improve quality, develop new scientific methods and modernize the administration of the Department. In other words, the strategic objectives proposed for the period 2000-2005 encompassed scientific and administrative development as well as the implementation of a quality management system.

In the context of that vision of development, one aspect of which is the establishment of a quality management system, there have been several major achievements.

Quality management system

As part of the Department's quality management system, a quality manual to be used in every area covered by the system will be drafted. The manual will contain cross references to manuals on general and specific standard operating procedures, always taking into account the admissibility and the chain of custody of evidence. The general standard operating procedures include administrative processes such as procurement and documentation control, with the aim of achieving standardization in the various sections of the Department.

Documentation control

The control of documentation generated both outside and within the laboratory is an essential part of the quality management system because it ensures that

documentation is safe and accessible at all times, as required by chain-of-custody principles and regulations of Costa Rica.

The Department of Forensic Science now has lists of standard operating procedures, worksheets for experts, equipment operation manuals and bibliographic material. In addition, a single uniform system of physical and electronic archiving has been introduced to facilitate the storage and retrieval of the information generated.

Within the framework of a major project initiated by the judicial authorities in Costa Rica, the Department of Forensic Science is in the process of computerizing all its offices to provide an essential means of communication in areas such as the dispatch of advisory opinions and reports to various judicial offices. That computerized infrastructure is being developed on the basis of experience acquired during visits to forensic laboratories in Europe and will be used for information management within the quality management system, especially with regard to standard operating procedures. One of the recommendations made by laboratories consulted by the Department was that controls to ensure information security should be implemented as part of the computerization process, in order to show that information is safe from unauthorized modifications. One option is to install specialized software already available on the market. Alternatively, software could be built to suit the specific needs of the system.

Purchasing services and supplies

The purchasing of services and supplies was considered a critical factor at meetings held under the auspices of the United Nations to promote regional integration in forensics, attended by the directors of eight forensic laboratories in the region.

One practical recommendation made in the course of the meetings was to draw up lists of possible suppliers of goods and services and establish systems for the technical assessment of those suppliers, in cooperation with the personnel in each country's procurement departments responsible for implementing and ensuring compliance with the administrative procedures involved in contracting.

Further, a computer system has been installed in the Department of Forensic Science to monitor, line by line, contracted materials, delivery dates and inventory flows, including the registration of data from the relevant suppliers.

The above-mentioned measures have enabled the Department of Forensic Science to maintain a uniform methodological standard in assessing the availability, accessibility and cost of materials for any contingency. Action has also been taken to establish control over possible sources of error caused by changes in reagent lots, for example. All this has had an impact on the proper functioning of the Department and on the efficiency and quality of its service.

Corrective actions

A range of possible corrective actions has been incorporated into all standard operating procedures developed by the Department of Forensic Science, to deal with situations in which the criteria for the validity of results are not fulfilled.

Control of records

One crucial aspect of a quality management system for forensic work is documenting the results of the work carried out. Thus, the Department of Forensic Science has set itself the task of rationalizing the recording of general information from expert worksheets and standardizing their formats. The Department now has lists and a general format for the preparation of expert worksheets, each of which has a code and a unique number to prevent mistaken identification.

Record control is considered to be vital to meeting the criteria for the admissibility of scientific evidence. In meeting those criteria, it becomes possible to maintain a manual or electronic record of the results of the various activities and tasks required by the standard operating procedures. Thus, results can be assessed and monitored by the person who generated them and by external experts wishing to check the reliability and the quality of the data underlying the conclusions of a given report.

Human resources

The Department of Forensic Science has set up a prototype database to register all required staff information, such as academic training, courses and participation in inter-laboratory tests, on which each employee's initial assessment can be based.

It should be noted that in order to maintain the high level of competence that is established by the standards and the criteria for the admissibility of scientific evidence, forensic scientific knowledge must be constantly updated. That is a major obstacle for Cost Rica, because the country lacks formal or vocational university courses in forensics, which would enable the Department of Forensic Science to offer training and refresher courses to staff and thus avoid hiring external experts, which is a much more expensive and limited option.

The assistance provided through the United Nations has been important for the Department of Forensic Science, which has benefited from study tours, meetings of the directors of forensic laboratories in the region, regional internships and other activities generating feedback valuable for guiding the development of the Department in the area of forensic science.

The staff of the Department of Forensic Science have been an invaluable resource in the development activities carried out to date, because the limited nature of their international contacts and of their access to first-hand sources of information and to courses in specialized fields has been compensated by their determination in their constant search for information and in their experimental work.

Environmental conditions and security of custody

One of the achievements of the Department of Forensic Science is the establishment of temperature- and humidity-controlled areas to carry out various expert tasks. They include special drying rooms for items of evidence that need to be dried, such as bloodstained clothes, and separate areas for processing those items of evidence that come from victims and those from the accused. Most storage areas have video cameras and digital access systems. Those are among the most crucial aspects of ensuring the authenticity and the integrity of the physical evidence required by the chain of custody, regardless of the system used.

Test and calibration methods and method validation

Given the need for clearly described written procedures, the Department of Forensic Science has developed a master guide for the development of further standard operating procedures. It requires specific factors to be detailed, such as objective, scope, theoretical basis, symbols, equipment, reagents and reference materials, management of primary and secondary evidence, validation parameters, criteria for the acceptance or rejection of results, corrective action, uncertainty calculation and assessment, analysis and result reports and bibliography. Those procedures are based on methods and techniques accepted by the international scientific community—a crucial factor for the admissibility of scientific evidence—or on internally developed methods and techniques combined with appropriate checks and adjustments to ensure their correct use.

A basic requirement in this area is the validation of analytical methods and uncertainty calculations. To that end, procedures can draw on international publications such as *The Fitness for Purpose of Analytical Methods: Laboratory Guide to Method Validation and Related Topics from Eurachem* and the *Guide to the Expression of Uncertainty in Measurement* by ISO and the Bureau international des poids et mesures. One of the six criteria for the admissibility of scientific evidence is knowing the potential margin of error of a technique. The process of validation and uncertainty calculations are two tools that can be of assistance in that connection; they also provide an opportunity for verification by any of the parties involved in legal proceedings.

Equipment

The maintenance and the calibration and/or verification of equipment are of crucial importance for the values or results obtained, which have a significant impact on the final outcome of an analysis.

Since the end of 2002, having succeeded in making its administrative bodies aware of the importance of maintaining and calibrating the instruments used to obtain analytical data, the Department of Forensic Science was able gradually to implement, as part of its management plan, a preliminary programme involving contracting out the relevant services. In addition, drafts of the

standard operating procedures for various items of equipment were drawn up, establishing the basic steps to be taken for the daily or periodic verification of the condition of that equipment.

Unfortunately, over the past year, the costs of maintenance, calibration and/or verification services have unexpectedly increased by up to 70 per cent. As a result, it has become necessary to consider options such as transferring responsibility for some services, especially verification and/or calibration, to Department staff. If that course of action is followed, the Department will require service manuals, which are not traditionally included in procurement contracts, for each item of equipment. In addition, staff would need to be trained to carry out the work.

Measurement traceability

Of particular importance is the use of reference materials, which are indispensable both for validating analytical methods and for checking day-to-day measurements and how the technical procedures are carried out. Those actions are essential to determining the admissibility of scientific evidence. In that regard, control charts play a crucial role in monitoring the variability of processes over time.

In recent years, the Department of Forensic Science has made efforts to obtain reference materials, including certified reference materials, both through the United Nations International Collaborative Exercises programme and by purchasing them from private companies. Laboratories should invest in the acquisition of such materials, since they are indispensable for those carrying out specific analyses and tests.

Sometimes, however, it is extremely difficult to find certain materials, owing to the nature of the samples analysed by forensic laboratories. Further, the situation can be particularly serious in the case of certified reference materials, which are prohibitively expensive.

Handling of test and calibration items

With regard to the handling of test and calibration items, since 2000, the Department of Forensic Science has been promoting a project on the use of bar codes and a digital system to register and generate labels for identifying and locating items of evidence, request forms and samples. The project is already at the implementation stage, especially in the area of DNA, thanks to the support of the Computer Science Department of the Judicial Power. It is hoped that the project will be fully implemented throughout the Department of Forensic Science within the next two years. Such controls are commonly used in many forensic laboratories around the world, enabling evidence and documentation to be handled more expeditiously and objectively.

Assuring the quality of test and calibration results

Forensic institutions have widely adopted inter-laboratory study programmes and proficiency tests as a means of assuring the quality of results, because those

two verification procedures are considered to be among the most objective mechanisms for monitoring the proper operation of the techniques used, which is a requirement for determining the admissibility of scientific evidence.

Accordingly, over the past two years, the Department of Forensic Science has participated in 12 different types of inter-laboratory studies—on DNA, paints, fibres, accelerants, tool marks, ballistics, seized drugs, drugs in biological specimens, alcohol in blood, and clinical chemical analyses such as pregnancy tests and urinalyses—and obtained satisfactory results. The Department has, however, encountered difficulties in arranging inter-laboratory studies relating to all disciplines or types of analysis, perhaps owing to its geographical location and the lack of a regional forensic organization to promote such studies. In a recent report to the United Nations, Costa Rica proposed that support be provided for a body similar to the European Network of Forensic Science Institutes (ENFSI) and the American Society of Crime Laboratory Directors (ASCLD). With significant support from the Guardia Civil of Spain, the Latin American Academy of Criminalistics and Forensic Studies (AICEF) was established last year, in Bogotá, Colombia. The Academy's membership includes almost all official forensic laboratory directors from Latin America, Spain and Portugal. Like ENFSI, it works to develop forensic science and the standardization of procedures and quality assurance in all member countries; those standards include a code of conduct and collaboration agreements. The organization is expected to conduct inter-laboratory tests in the near future.

It should be mentioned that the United Nations International Collaborative Exercises, which cover seized drugs and drugs in biological specimens, have given Costa Rica invaluable experience in monitoring the technical competence of the laboratory of the Department of Forensic Science in those areas and in finding more selective and accurate analytical methods.

Scientific development

Scientific development is an extremely important aspect of an integrated quality management system. For that reason, modernizing the skills and improving the service quality and the response times of the various sections have been central issues for the Department of Forensic Science.

In that connection, several initiatives have been adopted in the laboratory during the transition phase under way:

(a) The Department of Forensic Science is promoting the automation of the drug analysis equipment used in analytical chemistry and toxicology and the use of analytical techniques such as solid-phase extraction and microextraction;

(b) The Department now uses instrumental techniques such as gas chromatography, gas chromatography/mass spectrometry, liquid chromatography, liquid chromatography/mass spectrometry, Fourier transform infrared spectroscopy and plasma emission spectroscopy to confirm analytical results for various kinds of matrix;

(c) The Gunpowder and Explosives Unit now issues reports on specialized subjects such as firing distances and gunpowder residues on hands, compiled using instrumental methods for corroboration. Also, the Central Evidence Inspection Unit has been established in response to the need for a central area in the Department of Forensic Science where packages of trace evidence can be opened, the trace evidence described and preserved and, as part of the same process, samples can be taken using, among other things, CrimeScope alternative light sources, for dispatch to the various sections;

(d) One major advance in recent years has been the automation of DNA analysis, using ABI PRISM 310 and 3100-Avant sequencers;

(e) The analysis of mitochondrial DNA has been introduced, resulting in far fewer tentative results than did the morphological tests formerly carried out on hair samples, and use of the technique has been extended to other kinds of samples, such as body fluids, blood, fingernails and bone;

(f) A more efficient method of extracting DNA from bone remains has been introduced. Faced with the impossibility of obtaining amplifiable DNA from some bone remains that had been exhumed or which had been discovered after lengthy exposure to the elements, the DNA Unit of the Department of Forensic Science decided that it needed an alternative method specifically for such cases. The method adopted was that used by the DNA Analysis Unit of the Federal Bureau of Investigation. That has made it possible to obtain DNA profiles in some cases where the previously used routine method failed. The new method has become the method of choice, with the additional advantages of taking less time and costing less;

(g) Progress has been made in the conduct of analyses using forensic digital photography and in the use of three-dimensional computer animation for accident reconstruction and crime scenes, among other things;

(h) In the field of toxicology, work has continued on implementing new methods of instrumental analysis. That has enabled the Department of Forensic Science to corroborate many results that, in the past, had to be reported in a tentative fashion owing to the lack of a method with the specificity needed to meet international standards. Foremost among those new methods is blood-alcohol analysis.

It is important to note that a quality management system may be established in any laboratory, regardless of the analytical instruments at its disposal. The most important factors in establishing such a system are the commitment of staff, their knowledge of the scope and the implications of the work involved, their mastery of the methods used and their strengths and weaknesses in terms of providing satisfactory advice to the courts about the significance of the results and conclusions. The laboratory of the Department of Forensic Science is a good example of that because it has evolved in a sustained manner over recent years, though many problems have yet to be overcome, such as that of tentative analyses that need to be replaced or supplemented by corroborative analyses. Many laboratories worldwide have already transcended those limitations. This

situation has not, in any case, proved to be an obstacle in implementing a quality management system while gradually improving the level of performance.

Occupational health

In addition to the above-mentioned achievements, there has been an improvement in the measures for ensuring the safety and occupational health of staff of the Department of Forensic Science. One objective in that regard has been to improve the management of solid waste, especially biological waste. To that end, the Department has purchased materials and equipment such as containers designated for specific types of waste, masks, safety goggles, needle disposal units, sterilization bags and protective clothing for the management of dangerous substances. In addition, all staff have been trained in areas such as the management and disposal of infectious waste.

The level of development achieved by the Department of Forensic Science has been surpassed by many forensic laboratories in the world; however, since the establishment of the Department in 1974 and, above all, in the past five years, efforts have been directed towards constant further development, in a quest to achieve a level considered average by international standards. In addition to strengthening the justice system in Costa Rica, that would allow expert results produced by the Department to be accepted by courts elsewhere, in the context of the globalization of crime. Another aim of these efforts is to create greater opportunities for cooperating and sharing information and experience with forensic laboratories worldwide.

Bibliography

P. de Bievre and others, *The Fitness for Purpose of Analytical Methods: a Laboratory Guide to Method Validation and Related Topics* (Eurachem, 1998).

K. Inman and N. Rudin, *Principles and Practice of Criminalistics: the Profession of Forensic Science* (New York, CRC Press, 2000).

R. Saferstein, *Criminalistics: an Introduction to Forensic Science,* 8th ed. (Pearson Prentice Hall, 2004).

J. Siegel, P. Saukko and G. Knupfer, eds., *Encyclopedia of Forensic Sciences* (London, Academic Press, 2000).

P. White, ed., *Crime Scene to Court: the Essentials of Forensic Science* (Cambridge, Royal Society of Chemistry, 1998).

ISO 9000:2005: Quality Management Systems; Fundamentals and Vocabulary (International Organization for Standardization, 2005).

ISO/IEC 17025:2005: General Requirements for the Competence of Testing and Calibration Laboratories (International Organization for Standardization and the International Electrotechnical Commission, 2005).

Guide to the Expression of Uncertainty in Measurement (International Organization for Standardization, 1995).

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United Nations publication

ISBN: 978-92-1-148221-8
ISSN 0007-523X
Sales No. E.07.XI.3

V.06-58844—April 2007—2.015

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