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

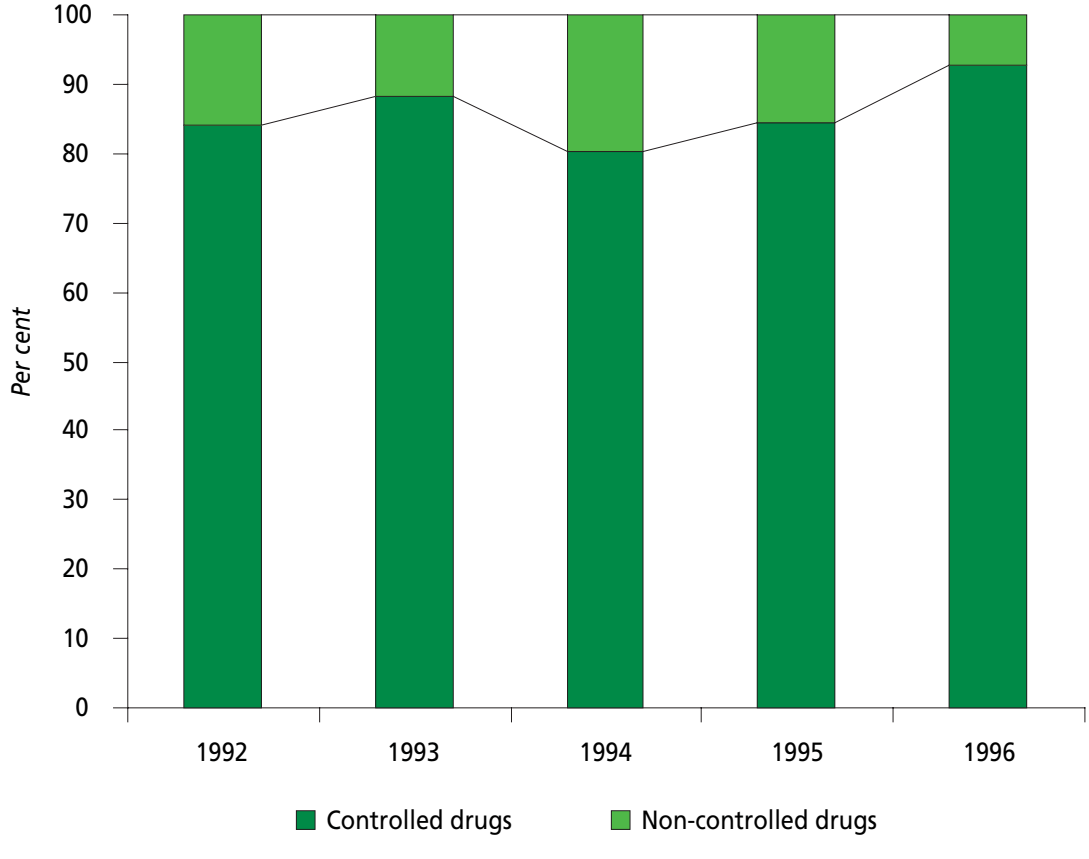
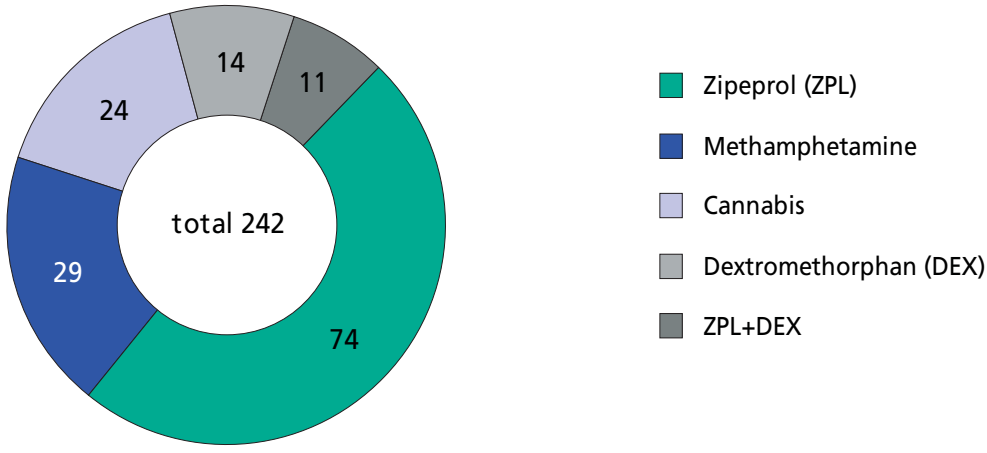


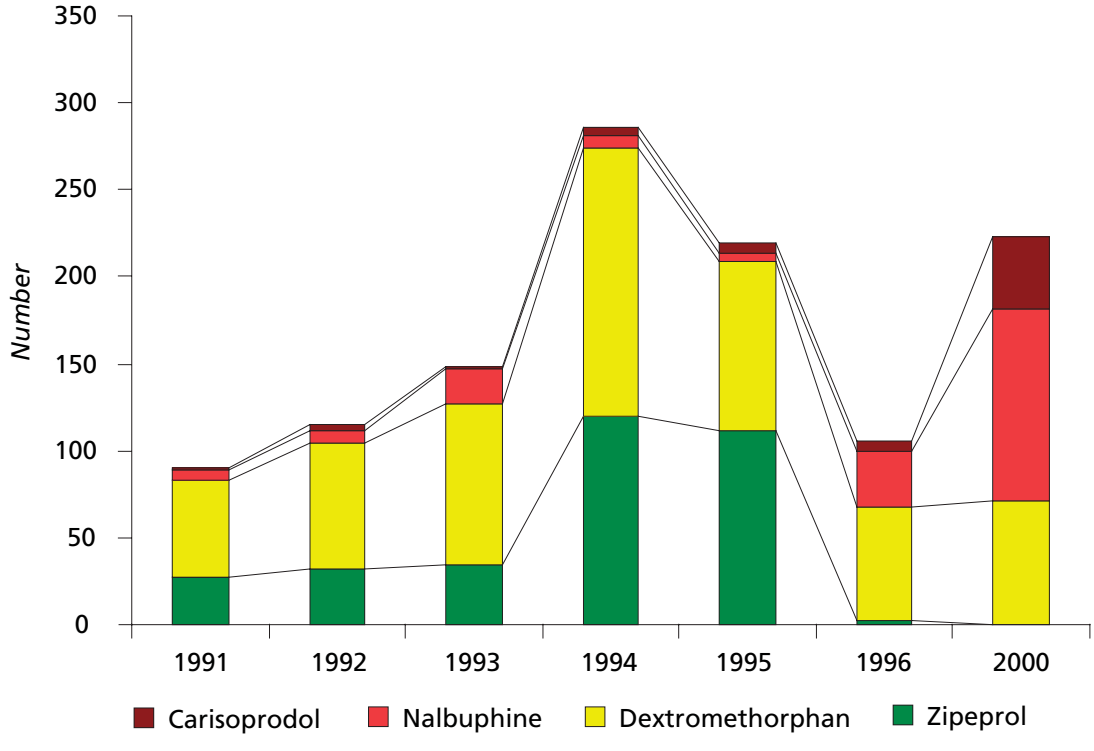
Figure II. Drugs detected in urine samples during the crackdown on suspected drug abusers in the Republic of Korea in 1995



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.

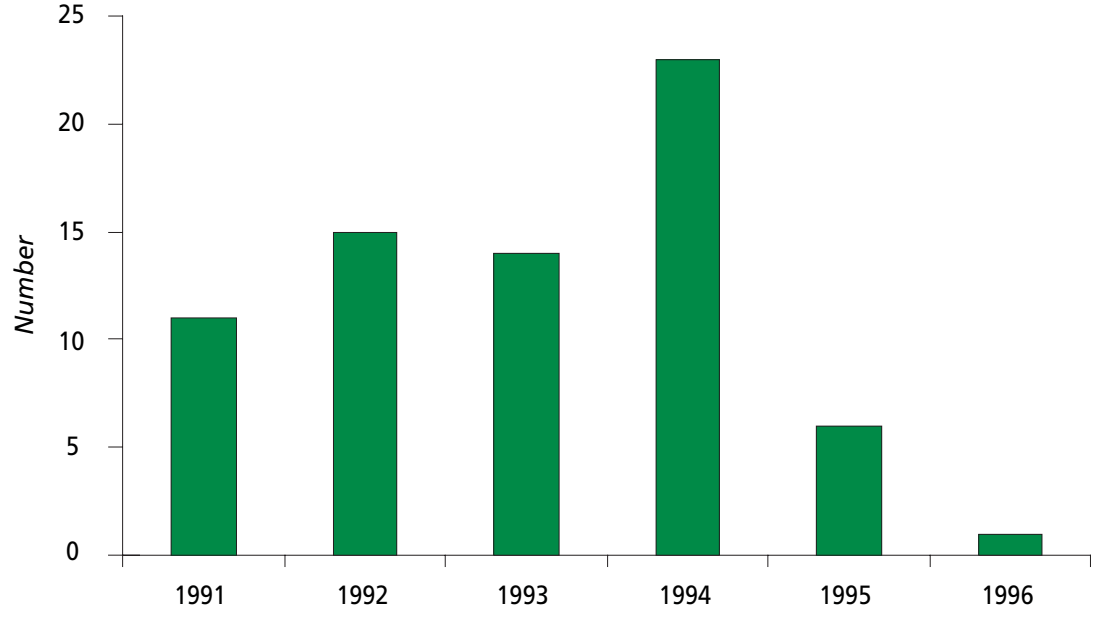
Epidemiology and testing of non-controlled drugs

Abuse of zipeprol

Epidemiology

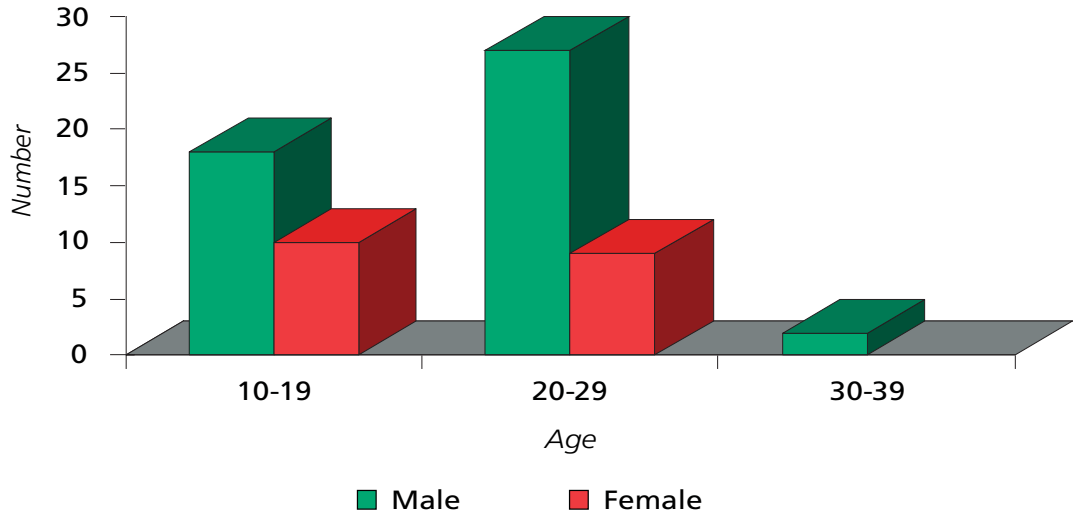
Zipeprol is a non-opiate, anti-tussive agent without the side effects of opiates. In the Republic of Korea, zipeprol dihydrochloride is marketed in capsule or tablet form under nine different trade names [7]. It is known to be a safe drug when taken as prescribed and does not lead to any physical dependence, 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



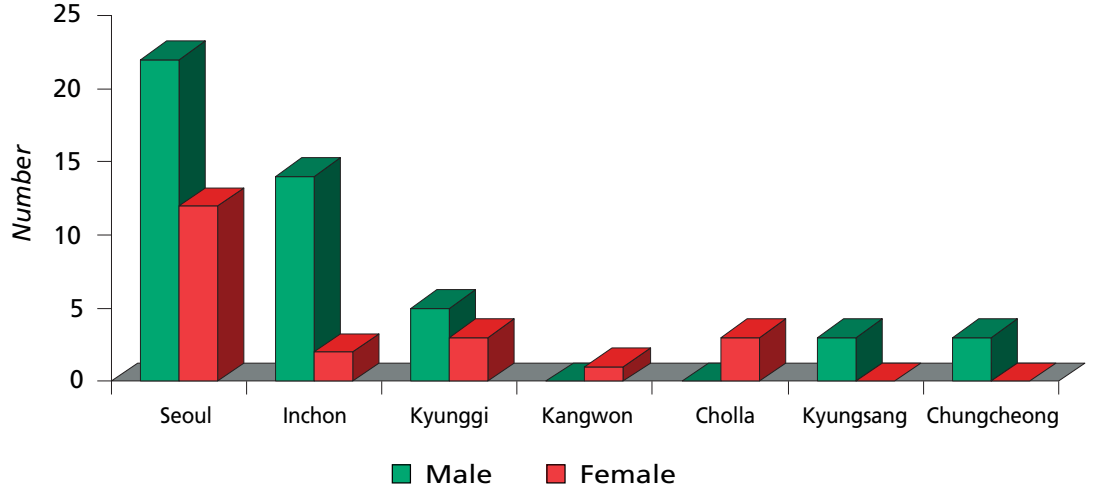
throughout the nation [10]. These fatalities are shown as a function of year in figure IV. In 1991, there were 11-zipeprol related deaths, with 15 such cases in 1992, 14 cases in 1993, 23 cases in 1994, 6 cases in 1995 and 1 case in 1996. The drop in cases from 1994 to 1995 is related to the introduction 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.

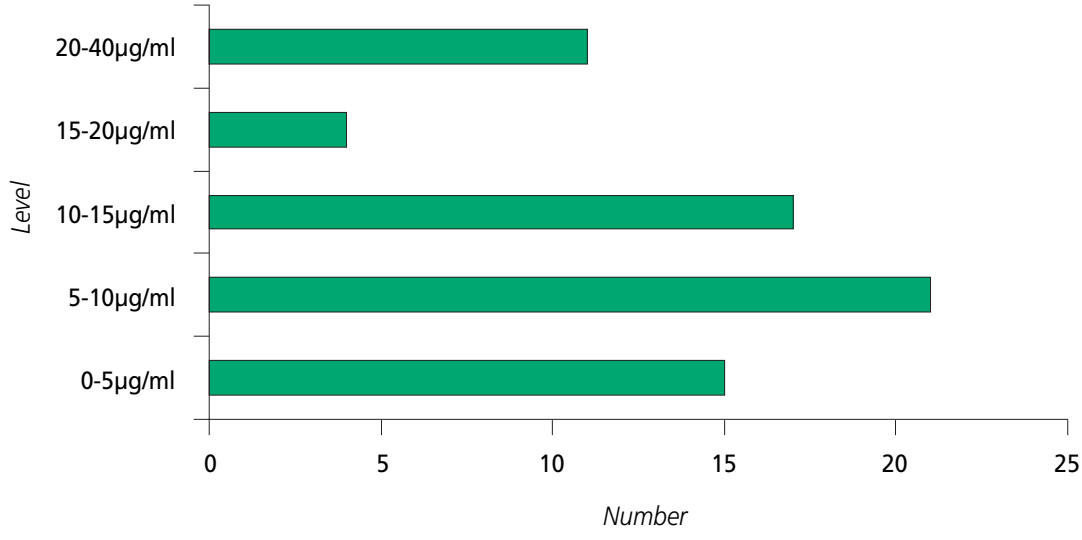
Figure VI. Distribution of zipeprol-related deaths by gender and location



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.

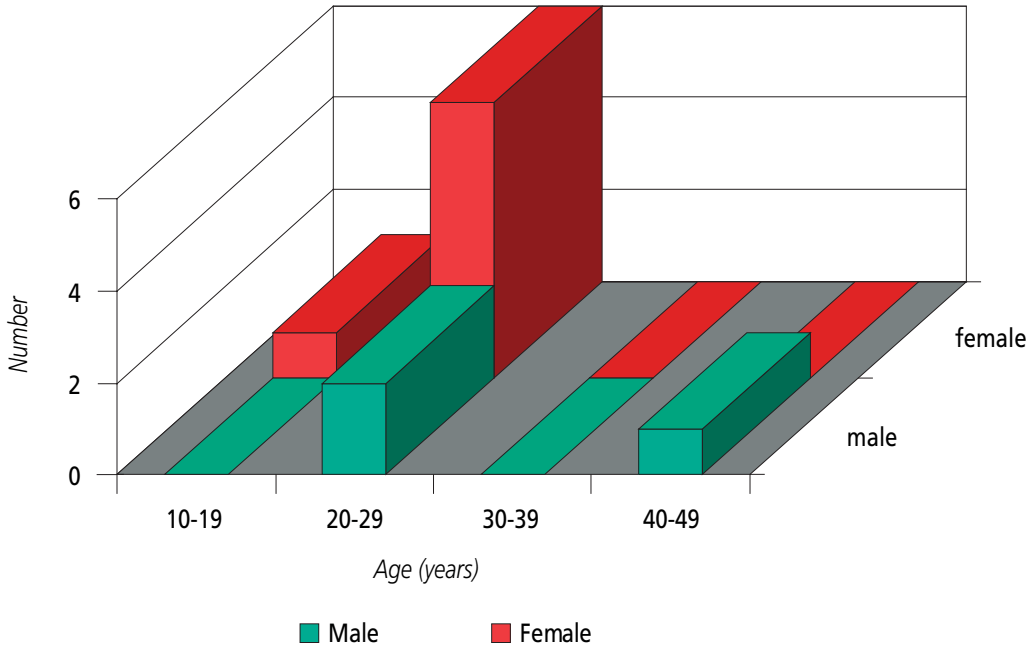
A Finnigan MAT GCQ was used to identify the drugs and metabolites. A fused-silica capillary SE-54 column (15 m × 0.32 m) was used. The column temperature was programmed from 150° C to 250° C at 10° C/min, the ionization energy was 70eV, the transfer-line temperature was 270° C and the electron multiplier (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

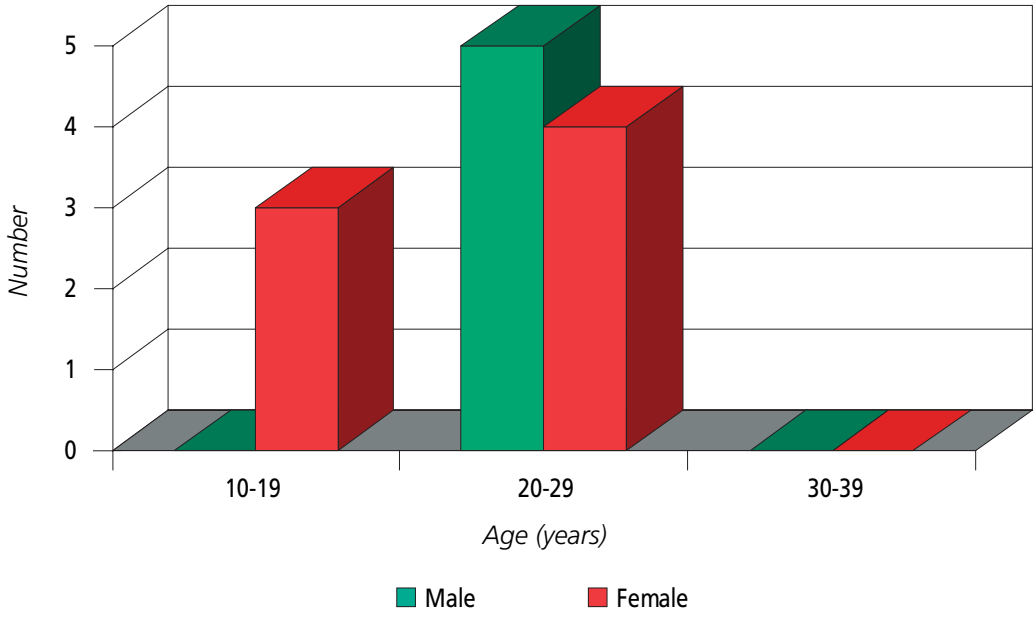


Figure X shows the distribution of the deaths involving the zipeprol and dextromethorphan combination by gender and region. As with zipeprol alone, the larger cities had a higher percentage of zipeprol/dextromethorphan-associated deaths. However, in the case of this combination, it was the city of Inchon 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

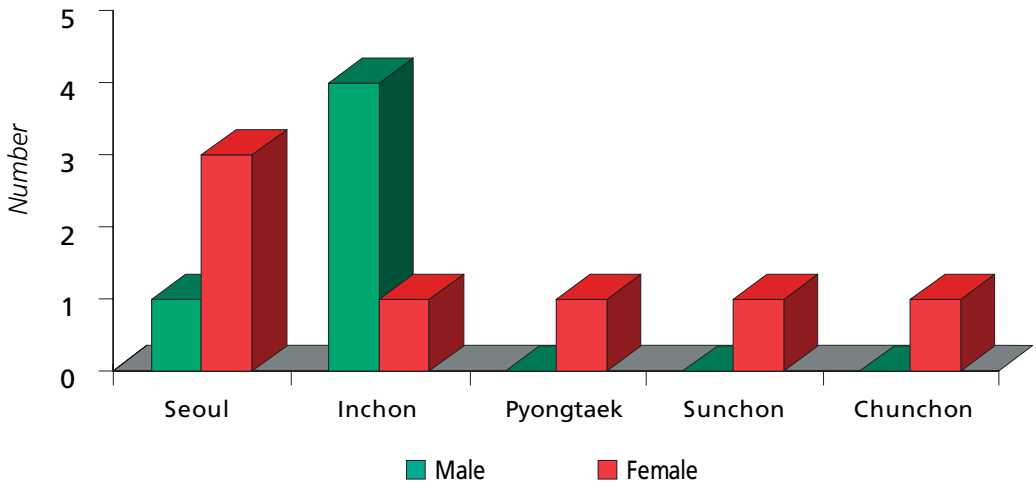
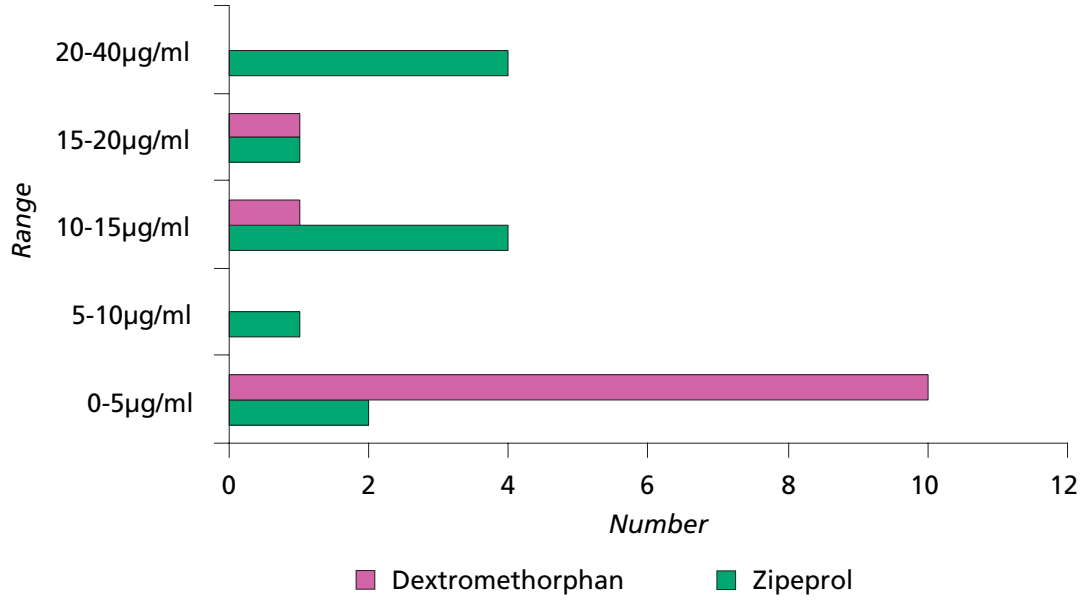


Figure XI shows the frequency distribution of zipeprol and dextromethorphan concentrations in postmortem blood samples. The blood concentration of dextromethorphan ranged from 1.1 to 18.3 μg/ml, while the zipeprol concentration in this population varied from 0.1 to 35.3 μg/ml.

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.

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