BEAT DRUGS FUND FINAL REPORT

Project: Surveillance of emerging drugs of abuse in substance abusers

在濫葯社群中監測新興毒品的出現

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Summary

There has been an increasing usage of novel psychoactive substances (NPS) in recent years worldwide. Some of these drugs have been reported to cause considerable harm and even fatalities. However, currently many of these NPS cannot be detected by routine drugs of abuse testing in most clinical laboratories. The aim of this study was to establish a chromatography-based analytical system for the detection of both conventional drugs of abuse (DOAs) and NPS in urine and hair; and subsequently, to apply this system in drug abusers and high-risk individuals as surveillance of DOAs, in particular NPS. In total, 2000 urine and hair samples were collected from susceptible populations, including subjects recruited form A&E departments, substance abuse clinics, various rehabilitation centres as well as youth outreach facilities. Sample preparation entails enzyme digestion and solid phase extraction for urine; and simultaneous micropulverization/ extraction for hair. Analytes were detected by liquid-chromatography tandem mass spectrometry (LC-MS/MS). Forty-seven conventional DOA analytes (28 parent drugs and 19 metabolites) and 47 NPS analytes (45 parent drugs and two metabolites) are covered by the established method, which has been validated according to international guidelines. Analysis of the 2000 urine and hair samples revealed the presence of three NPS in five samples - PMMA was detected in three hair specimens; TFMPP and methcathinone were detected in separate urine specimens. Of the conventional DOAs, codeine, methadone, heroin and ketamine were the most frequently detected analytes. To conclude, incorporation of novel psychoactive substances into the routine drugs of abuse testing service can allow surveillance of these continually emerging drugs. Early identification of NPS can help improve the clinical management of patients, and on a wider scale the legislation, education and preventive policies of the society.

Introduction

The last decade has seen a rapid and continual growth of “emerging” drugs of abuse (DOA). These substances bear chemical and/or pharmacological resemblance to conventional drugs of abuse and pose a threat to public health, but are often (initially) not controlled by law. In 2008, 13 emerging DOA’s were reported for the first time to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA); by 2013, 73 new drugs were reported within a year [1]. These novel substances are often sold in disguise as “bath salts”, plant food, chemical standards, spice mix, herbal incense etc, whilst being explicitly labeled as “Not for human consumption”. They are readily available in head shops, from street dealers and over the internet.

As new drugs emerge continually, some disappear whilst some stay with us. MDMA and ketamine were once "emerging DOAs" in Hong Kong in the late 1990’s. They have remained and become a tremendous burden to society. When such emerging DOAs first appear, the parties concerned - healthcare professionals, social workers, teachers, law enforcement officials and legislators etc - know very little about them. It often takes a couple of years for the society to understand the nature and learn the impact of such novel DOAs. During this period, the clinical and harmful effects are poorly understood; there is no analytical tool to detect and confirm their presence; and there is a lack of specific legislation to control these substances. This period of poor preparedness has to be shortened for the society to fight the battle more effectively.

Current anti-drug-abuse strategies commonly focus on the traditional drugs of abuse. The foremost challenge in combating emerging DOAs is that such drugs are not covered by current routine DOA testing. As a result, they may have penetrated deeply into society by the time they are recognised. If penetration of an emerging DOA into society is recognized early, subsequent actions on education, clinical management and legislation may be initiated in a much timelier manner before the damage becomes permanent. To achieve this, a system to monitor the emergence of novel DOAs is mandatory. The current study aims to establish a chromatography-based analytical system for the detection of both conventional and emerging drugs of abuse in urine and hair; and subsequently, to apply this system in drug abusers and high-risk individuals as surveillance of DOAs, in particular emerging drugs.

Methods

Sample collection

This project was initiated by the Hospital Authority Toxicology Reference Laboratory, the Hong Kong Poison Information Centre and the Hong Kong Lutheran Social Service. Subsequently, 18 more clinical units within the Hospital Authority and 6 social service units were invited to collaborate in the study. In total, 2000 urine and hair specimens were collected for analysis. The target subjects include patients/ clients of the following units: (i) substance abuse clinics (SAC) within the Hospital Authority (Castle Peak Hospital, Kowloon Hospital, Kwai Chung Hospital, Pamela Youde Nethersole Eastern Hospital, Prince of Wales Hospital, Queen Mary Hospital); (ii) A&E departments within the Hospital Authority (Pamela Youde Nethersole Eastern Hospital, Pok Oi Hospital, Princess Margaret Hospital, Queen Mary Hospital, Tuen Mun Hospital, United Christian Hospital, Yan Chai Hospital); (iii) the Hong Kong Poison Information Centre (HKPIC) toxicology clinic; (iv) counselling centres for psychotropic substance abusers (CCPSA) (Evergreen Lutheran Centre, Rainbow Lutheran Centre, Cheer Lutheran Centre; (v) various rehabilitation centres including the Society of Rehabilitation and Crime Prevention (SRACP), Operation Dawn and Caritas Wong Yiu Nam (WYN) Centre; and (vi) Youth Outreach.

Subjects donate samples on a volunteer basis and informed consent was obtained. Ethics approval was obtained from the Hospital Authority Kowloon West Cluster Research Ethics Committee (KW/FR-11-011 (41-05)). Each subject may donate either urine or hair, or both, in each donation episode. They may repeatedly donate samples given that each donation is at least 8 weeks apart. Urine (~20 mL) was collected in a plastic bottle and stored frozen until analysis. For hair, a lock of hair 1–2 mm in diameter is collected from the back of the head and stored inside a desiccator cabinet at room temperature in the dark prior to analysis. The hair root end was identified to facilitate segmental analysis.

Urine analysis

The urine sample was first subjected to glucuronidase digestion. To 1.0 mL urine was added 40 μL of β-glucuronidase enzyme (from *Hallotis Rufescens*, 100,000 U/mL), 25 μL of sodium acetate buffer (1.7 M, pH 5.2) and 100 μL of internal standard mix (containing 50 ng/mL of MDMA-D5, 100 ng/mL each of ketamine-D4 and diazepam-D5, 200 ng/mL of JWH-018-D11, 500 ng/mL each of morphine-D6 and cocaine-D3, 1 μg/mL of THC-COOH-D3 and 3 μg/mL of secobarbital-D5). The sample was incubated at 55°C in the water bath for 1 hour. It was then cleaned up by solid-phase extraction (SPE) on an

automatic RapidTrace SPE Workstation (Caliper LifeSciences, MA, USA). To 1.0 mL digested urine was added 1 mL of 100 mM phosphate buffer, pH 6. SPE cartridges (Waters Oasis MCX, 3cc, 60 mg) were conditioned with 2 mL methanol, 2 mL distilled water and 1 mL 100 mM phosphate buffer, pH 6. The buffered urine sample was loaded onto the cartridge, which was then washed with 2 mL distilled water and 2 mL phosphate buffer. The acidic fraction was eluted with 1 mL 10% methanol in ethyl acetate. The cartridge was washed further with 1 mL methanol, and the basic fraction was eluted with ethyl acetate/isopropanol/ammonium hydroxide (84:14:2 $^{v}/_{v}$). The combined eluate fractions were evaporated to dryness under a stream of nitrogen at 40°C and reconstituted in 0.5 mL of distilled water/methanol (70:30 $^{v}/_{v}$). For positive and negative ionization, 10 µL and 20 µL respectively were injected for LC-MS/MS analysis.

Hair analysis

The hair specimen was first cut into 3-cm segments; the 0-3 cm segment was used for analysis. The hair was decontaminated by sequential washes with 1 mL dichloromethane, 1 mL distilled water and 1 mL acetone (1 min each on a roller mixer) and then air dried. A 20-mg fraction was weighed out, to which was added 1 mL of acetonitrile and 40 µL of the internal standard mix. The sample was then micropulverized using the protocol 6200 rpm, 50 s x3 cycles, 15 s break on a homogenizer (Precellys 24-DUAL Bead Beating Tissue Homogenizer, Bertin Technologies, France), sonicated at room temperature for 30 min and centrifuged at 13,000 rpm for 10 min. The supernatant (0.8 mL) was filtered using a 2 mL centrifugal filter tube (PVDF-HB, 0.45um, Vertical Chromatography Co., Thailand), was then blow dried under a stream of nitrogen at 30°C and reconstituted in 0.2 mL of distilled water/methanol (70:30 $^{v}/_{v}$). For positive and negative ionization, 10 µL and 20 µL respectively were injected for LC-MS/MS analysis.

LC-MS/MS analysis

LC-MS/MS analysis was performed on an Agilent 6430 triple-quadruple mass spectrometer (Agilent Technologies, Singapore) coupled with Agilent 1290 Infinity liquid chromatography (LC) system. For positive ionization, chromatographic separation was performed with an Eclipse Plus C8 (solvent saver HT) column, 1.8 µm, 3.0x100 mm (Agilent Technologies) and gradient elution comprising 5 mM ammonium formate, 0.1% formic acid in water (mobile phase A, MPA) and 100% methanol (mobile phase B, MPB). The gradient program started with 2% MPB at 0 - 0.2 min, increasing to 25% MPB at 1 min and held until 5 min. The MPB content was further increased to 35% by 7.5 min, and 99% by 14

min which was held until 20 min. Subsequently, the MPB content was reverted to 2%. The total run time was 23 min and the flow rate was 0.4 mL/min. For negative ionization, chromatographic separation was performed with an Extend C18 column, 1.8 µm, 2.1x50 mm (Agilent Technologies) and gradient elution comprising 0.025% NH_4OH in water (mobile phase A, MPA) and 100% methanol (mobile phase B, MPB). The gradient program started with 10% MPB at 0 - 0.2 min, increasing to 99% MPB at 5 min and held until 7 min. Subsequently, the MPB content was reverted to 10%. The total run time was 10 min and the flow rate was 0.3 mL/min.

Analytes were detected by mass spectrometry using multiple reaction monitoring (MRM) in either positive or negative electrospray ionization modes. **Table 1** shows the analytes detected by the method and the ionization polarity employed. The fragmentor voltage and collision energies were first optimized for each compound using a 1 µg/mL standard solution. For each analyte, 3 MRM transitions were monitored and identification was achieved by comparison of the retention time (RT) and MRM ratio against the reference standard. The MRM ratio acceptance criteria was established according to the maximum permitted tolerance for relative ion intensities published by the European Communities and the Clinical and Laboratory Standards Institute (CLSI) [2,3].

Table 1. Detection of traditional and emerging drugs of abuse by positive or negative ionization polarity

**Denotes emerging drug of abuse*

Class		Analyte	Polarity
Amphetamines	1.	4-Fluoroamphetamine*	Positive
	2.	4-Methylthioamphetamine*	Positive
	3.	Amphetamine	Positive
	4.	Bromo-dragonfly*	Positive
	5.	Chloroamphetamine*	Positive
	6.	DOB*	Positive
	7.	DOET*	Positive
	8.	DOM*	Positive
	9.	MBDB*	Positive
	10.	MDA	Positive
	11.	MDEA*	Positive
	12.	MDMA	Positive
	13.	HMMA	Positive
	14.	Methamphetamine	Positive
	15.	N-ethylamphetamine*	Positive
	16.	PMA*	Positive
	17.	PMMA*	Positive

Opiates	18.	Codeine	Positive
	19.	Codeine metabolite (nor)	Positive
	20.	Heroin	Positive
	21.	Heroin metabolite (6-MAM)	Positive
	22.	Morphine	Positive
	23.	Morphine metabolite (nor)	Positive
Cocaine	24.	Cocaine	Positive
	25.	Cocaine metabolite (Benzoylecgonine)	Positive
	26.	Cocaine metabolite (Cocaethylene)	Positive
	27.	Cocaine metabolite (nor)	Positive
Ketamine	28.	Ketamine	Positive
	29.	Ketamine metabolite (nor)	Positive
	30.	Methoxetamine*	Positive
	31.	Tiletamine*	Positive
Benzodiazepines	32.	Chlordiazepoxide	Positive
	33.	Diazepam	Positive
	34.	Diazepam metabolite (nor)	Positive
	35.	Estazolam	Positive
	36.	Flunitrazepam	Positive
	37.	Flunitrazepam metabolite (7-amino)	Positive
	38.	Midazolam	Positive
	39.	Midazolam metabolite (1-OH)	Positive
	40.	Midazolam metabolite (4-OH)	Positive
	41.	Nimetazepam	Positive
	42.	Oxazepam	Positive
	43.	Temazepam	Positive
	44.	Triazolam	Positive
	45.	Triazolam metabolite (1-OH)	Positive
Cannabinoids	46.	Cannabis metabolite (carboxy-THC)	Positive
	47.	Cannabis metabolite (THC-OH)	Positive
	48.	JWH-018*	Positive
	49.	JWH-018 metabolite (4-OH-indole)	Positive
	50.	JWH-018 metabolite (N-5-OH-pentyl)	Positive
	51.	JWH-073*	Positive
	52.	CP-47,497*	Negative
	53.	C8 hom of CP-47,497*	Negative
Miscellaneous (traditional DOA)	54.	Dextromethorphan	Positive
	55.	Dextromethorphan metabolite (dextrorphan)	Positive
	56.	LSD	Positive
	57.	LSD metabolite (nor)	Positive
	58.	LSD metabolite (OH)	Positive
	59.	Methadone	Positive
	60.	Methadone metabolite (EDDP)	Positive
	61.	Methaqualone	Positive
	62.	Phentermine	Positive
	63.	Zolpidem	Positive

	64.	Zopiclone	Positive
	65.	Amobarbital	Negative
	66.	Butabarbital	Negative
	67.	Secobarbital	Negative
Phenethylamines	68.	2C-B*	Positive
	69.	2C-H*	Positive
	70.	2C-I*	Positive
	71.	2C-T-2*	Positive
	72.	2C-T-4*	Positive
	73.	2C-T-7*	Positive
	74.	Mescaline*	Positive
Piperazines	75.	BZP*	Positive
	76.	mCPP*	Positive
	77.	MDBP*	Positive
	78.	pFPP*	Positive
	79.	pMeOPP*	Positive
	80.	TFMPP*	Positive
Beta-keto-amphetamines	81.	Cathinone*	Positive
	82.	Ethylone*	Positive
	83.	Mephedrone*	Positive
	84.	Methcathinone*	Positive
	85.	Methedrone*	Positive
	86.	Methylone*	Positive
Tryptamines	87.	5-MeO-DIPT*	Positive
	88.	Alpha-methyltryptamine*	Positive
	89.	Dimethyltryptamine*	Positive
	90.	Psilocin*	Positive
Miscellaneous (emerging DOA)	91.	MDPV*	Positive
	92.	Mitragynine*	Positive
	93.	Salvinorin A*	Positive
Internal standards (I.S.)	1.	Cocaine-D3	Positive
	2.	Diazepam-D5	Positive
	3.	JWH-018-D11	Positive
	4.	Ketamine-D4	Positive
	5.	MDMA-D5	Positive
	6.	Morphine-D6	Positive
	7.	THC-COOH-D3	Positive
	8.	Secobarbital-D5	Negative

Assay validation

The analytical method was validated according to international guidelines [3-6]. The accuracy of the method was assessed using 14 urine samples from the Royal College of Pathologists of Australasia (RCPA) External Quality Assurance Program (EQAP). The results obtained from the method were compared against the reference results issued by the EQAP. The limit of detection (LOD) was

established by spiking 10 different urine blank matrices with the analytes at a range of concentration levels (0.02 - 2500 ng/mL); the LOD was defined as the concentration where the analyte was positive identified in 10 out of 10 matrices. The false positive rate (%) was determined by the formula (false positive/total known negatives x 100%) after analyzing 20 urines that do not contain the analytes. The false negative rate (%) was determined by the formula (false negative/total known positives x 100%) after analyzing 20 urines spiked with analytes at LOD plus 20% level. The precision of the analyte retention time and MRM ratios was assessed in terms of the relative standard deviation (RSD) by monitoring the parameters over the validation period.

Results and Discussion

Sample collection

In total, 972 individuals took part in the study. There were 1224 donation episodes and 2000 specimens collected in total. The subject demographics are summarized in Table 2. Of the 1224 donation episodes, the subjects were recruited from various sites as shown in Table 3.

Table 2. Subject demographics and the number of urine and hair samples collected

Participants & samples		**Gender**		**Age**	
No. of subjects	972	**Male**	720	**18-20**	101
No. of donation episodes	1224	**Female**	252	**21-30**	282
No. of urine samples	964			**31-40**	329
No. of hair samples	1036			**41-50**	142
Total no. of samples	2000			**51-60**	90
				61-70	27
				71-80	1

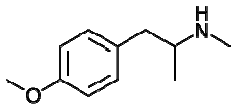
Table 3. The number of donation episodes at each of the collection sites

Site	**No. of donation episodes**
Substance Abuse Clinics	822
SRACP	130
Operation Dawn	70
CCPSA	62
Caritas WYN Centre	58
Youth Outreach	41
HKPIC toxicology clinic	28
A&E departments	13
Total:	1224

Emerging Drugs of Abuse

In the 2000 specimens analyzed, 3 emerging DOAs were detected in 5 specimens. These emerging DOAs, which will be discussed in detail below, are: PMMA (para-methoxymethamphetamine), TFMPP [1-(3-Trifluoromethylphenyl)piperazine] and methcathinone.

PMMA

Chemical structure	
Full chemical name	*N*-methyl-1-4-(methoxyphenyl)-2-aminopropane
Street names	"Happy 粉", "Death", "red Mitsubishi", "另類搖頭丸"

PMMA was detected in 3 hair specimens in the current study. These specimens were donated by 3 males, aged between 28-45, under different settings: (i) A&E; (ii) SAC; and (iii) Caritas WYN Centre. The analytical results of the 3 cases are tabulated below:

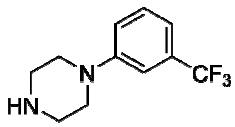
Case	Gender/Age	Setting	Drugs detected in hair	Drugs detected in urine
1	Male/ 28	A&E	PMMA Cocaine & metabolite Ketamine & metabolite	Cocaine metabolite Ketamine & metabolite
2	Male/ 45	SAC	PMMA Amphetamine Methamphetamine Cocaine & metabolites Ketamine & metabolite Zolpidem	Methamphetamine Cocaine & metabolite Ketamine & metabolite Phentermine
3	Male/ 28	Caritas WYN Centre	PMMA Cocaine & metabolites Ketamine & metabolite	Ketamine & metabolite Cannabis metabolite

PMMA is a highly toxic amphetamine derivative that had been sold on the drug market as MDMA substitute. It has been reported to cause up to 90 fatalities worldwide over the years, including 8 fatalities in Taiwan [7,8]. In particular, there have also been PMMA-associated fatalities in Hong Kong recently (not included in this study), both of which were analytically confirmed by Hospital Authority [9].

PMMA is commonly sold as MDMA substitutes and are often present in drug tablets/pills in combination with MDMA or other amphetamine-type stimulants. Indeed, previous studies have reported the concurrent detection of amphetamine, methamphetamine or MDMA in the urine of PMMA users [8,10]. In the current study, only one sample showed the simultaneous detection of amphetamine and methamphetamine. Interestingly, all 3 samples in which PMMA was detected also contained cocaine and ketamine. This is also the pattern observed in the two recent local PMMA-associated fatal cases [9].

Urine and hair specimens have different "detection windows" i.e. they reflect different time frames of drug intake. Detection in hair indicates drug ingestion in the past weeks/months; whereas detection in urine indicates recent intake (within hours/days). Although PMMA has been associated with numerous fatalities worldwide, it was unlikely to be the cause of acute toxicity in the current A&E subject, as the drug was detected only in hair and not in the urine sample of the subject. Nevertheless, PMMA has been associated with 2 fatalities recently in Hong Kong [9]. Together with the results of the current study, PMMA is likely to have arrived in the local drug scene and, given its highly toxic nature, prompt actions are warranted in the control and management of this drug.

TFMPP

Chemical structure	
Full chemical name	1-(3-Trifluoromethylphenyl)piperazine
Street names	"X4", "Molly", "PEP", "Twisted", "Flying Angel", "Wicked High"

TFMPP was detected in a urine specimen in the current study. This specimen was donated by a 26-year-old female who attended the HKPIC toxicology clinic. The analytical results of this case are as follows:

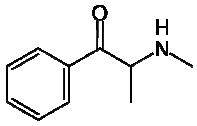
Case	Gender/Age	Setting	Drugs detected in hair	Drugs detected in urine
1	Female/ 26	HKPIC toxicology clinic	Cocaine Ketamine	TFMPP Cocaine metabolite Ketamine & metabolite

TFMPP is a piperazine derivative with mild hallucinogenic effects and, when taken with another piperazine derivative benzylpiperazine (BZP), causes ecstasy-like effects. This compound was first reported in Hong Kong in 2010, when it was detected in the urine of a known substance abuser [11]. Piperazine derivatives are known to cause dissociative and sympathomimetic toxicity [12].

TFMPP and BZP have been commonly detected in "legal herbal highs" labeled as "ecstasy" (MDMA). While TFMPP was detected in the current sample, BZP was not. On the other hand, similar to PMMA, TFMPP was detected concurrently with cocaine and ketamine in the urine specimen.

TFMPP was first reported in Hong Kong by our institution in 2010, when it was identified in the urine of a 28-year-old male who was a known substance abuser and presented to hospital with tachycardia and chest discomfort. In this patient's urine, cocaine metabolites and ketamine were also detected [11]. Between 2009 and 2012, there have been at least 7 cases of hospital admission in which TFMPP was detected in the urine of the patients.

Methcathinone

Chemical structure	
Full chemical name	α-(Methyloamino)-1-phenyl-propan-1-one
Street names	"Cat", "M-CAT", "Ephedrone", "Jeff"

Methcathinone, also known as ephedrone, was detected in the urine of a 27-year-old female who attended a substance abuse clinic (SAC). The analytical results of this case are as follows:

Case	Gender/Age	Setting	Drugs detected in hair	Drugs detected in urine
1	Female/ 27	SAC	(hair not collected)	Methcathinone Amphetamine Methamphetamine HMMA (metabolite of amphetamine-like substances) Cocaine metabolite

Methcathinone is an amphetamine-like stimulant and is among a group of synthetic cathinone compounds, commonly known as "bath salts", which have been associated with numerous fatalities worldwide [13]. Other highly toxic cathinone derivatives include mephedrone ("meow meow") and MPDV, both of which are also covered in the current analytical system but thus far have not been detected locally.

Methcathinone gained popularity in Russia and USA in the 1970s to 1990s, and was recently reported as an emerging DOA in Sweden [14]. It was detected in a urine sample in the current study, together with amphetamine, methamphetamine and cocaine. Although methcathinone does not appear to be as widely used and as toxic as its cathinone counterparts mephedrone ("Meow Meow") and MDPV (bath salt), its presence nevertheless indicates that the continual surveillance for synthetic cathinones is warranted.

Traditional drugs of abuse

Due to the versatility of the analytical method, traditional DOAs can be detected at the same time as emerging DOAs. Hence, the 2000 specimens collected had also been analysed for the usage pattern of traditional DOAs.

Urine analysis

In total, 19 types of traditional DOAs were detected in the 964 urine specimens. Fig. 1 shows the drugs detected as a percentage of the total number of urine samples. Codeine was the most commonly seen DOA, being detected in 47% of the urine samples; followed by methadone that was seen in 35% of the samples. Other frequently detected drugs include: heroin (22%), methamphetamine (21%), ketamine (20%), zopiclone (20%), amphetamine (17%), midazolam (17%) and dextromethorphan (14%). Cocaine and cannabis were detected in 6% and 3% of the urine samples respectively.

Although temazepam and oxazepam were detected in the urine samples, it should be noted that they are likely present as metabolites of diazepam. Morphine is also the metabolite of codeine and heroin; to avoid contamination of the results, morphine was only reported here as a drug in the absence of either codeine or heroin in the same sample.

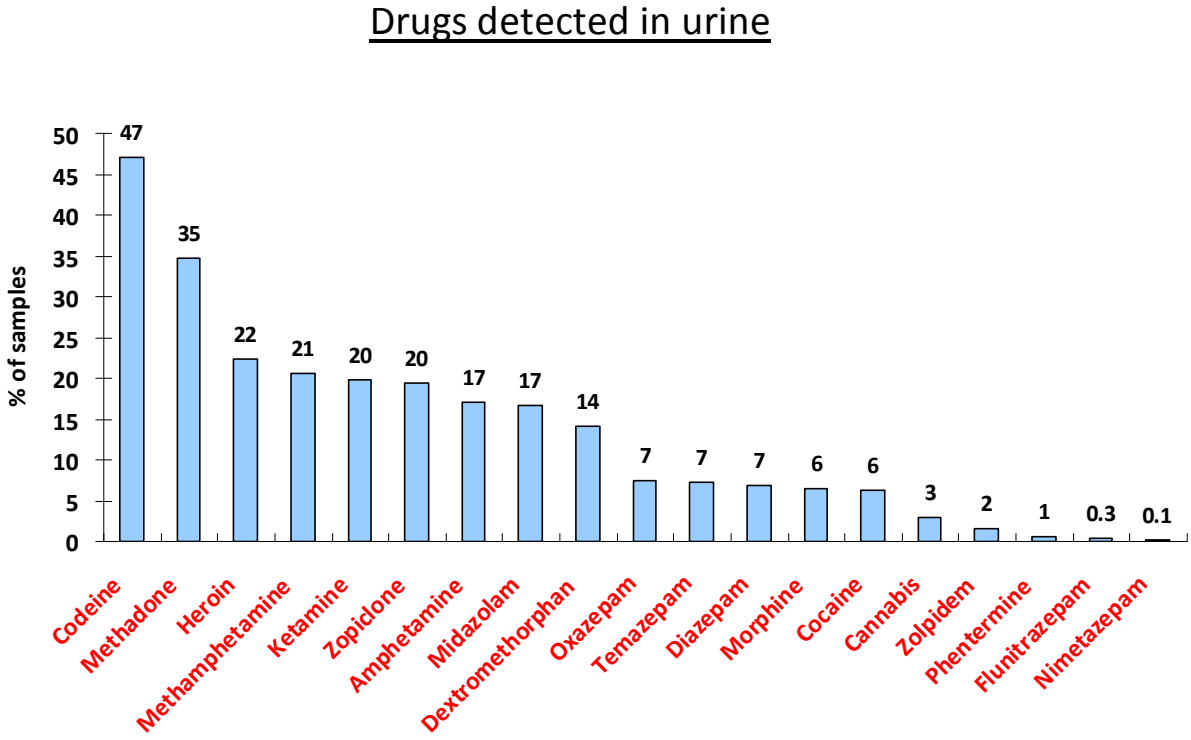


Fig. 1. Traditional drugs of abuse that were detected in urine specimens (964 in total).

Hair analysis

In total, 14 types of traditional DOAs were detected in the 1036 hair specimens. Fig. 2 shows the drugs detected as a percentage of the total number of hair samples. A similar pattern to urine was observed in hair. Codeine (36%) and methadone (35%) were the most prevalent drugs detected, closely followed by ketamine (34%), heroin (33%), methamphetamine (29%), dextromethorphan (28%) and zopiclone (26%). Cocaine (12%) and zolpidem (7%) were detected at higher rates in hair compared with urine, which may indicate that the drugs deposit relatively efficiently in hair matrix. It should be noted, however, that the metabolites of zolpidem are not included in the current assay, which may decrease the chance of its detection in urine.

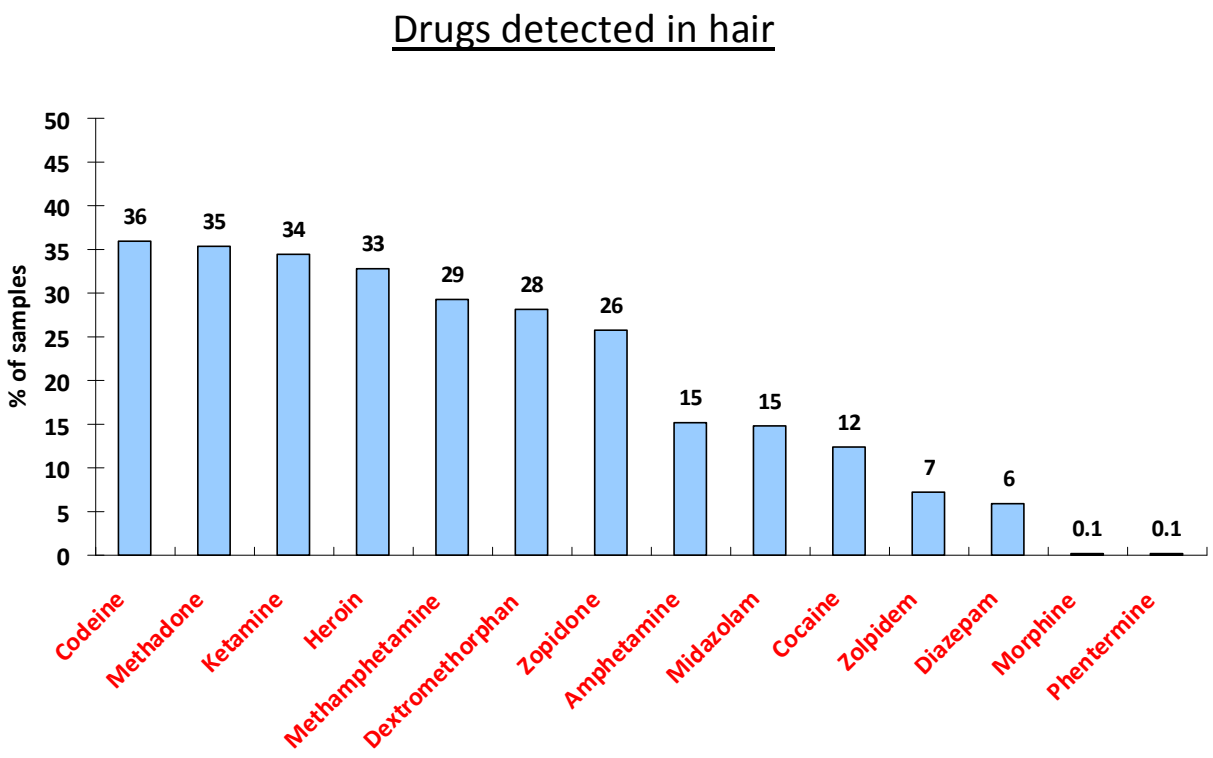


Fig. 2. Traditional drugs of abuse that were detected in hair specimens (1036 in total).

Ethnic minority (South Asian)

An interesting finding of the current study is the difference in drug use pattern observed between Chinese and South Asians (南亞裔人士). As the samples from SRACP were collected from South Asians, and the vast majority of the remaining samples were from Chinese, it was possible to compare the pattern of drug usage between the two races.

Fig. 3 shows the rates of detection of different drugs in hair collected from Chinese and South Asians. It is interesting to note that South Asians appear to have a higher tendency to consume opiates such as heroin and methadone, as well as cough medicines like codeine and dextromethorphan. On the other hand, they are less likely to consume ketamine, cocaine and tranquillizers such as zopiclone, zolpidem and diazepam. The results of urine analysis show a similar pattern (Fig. 4).

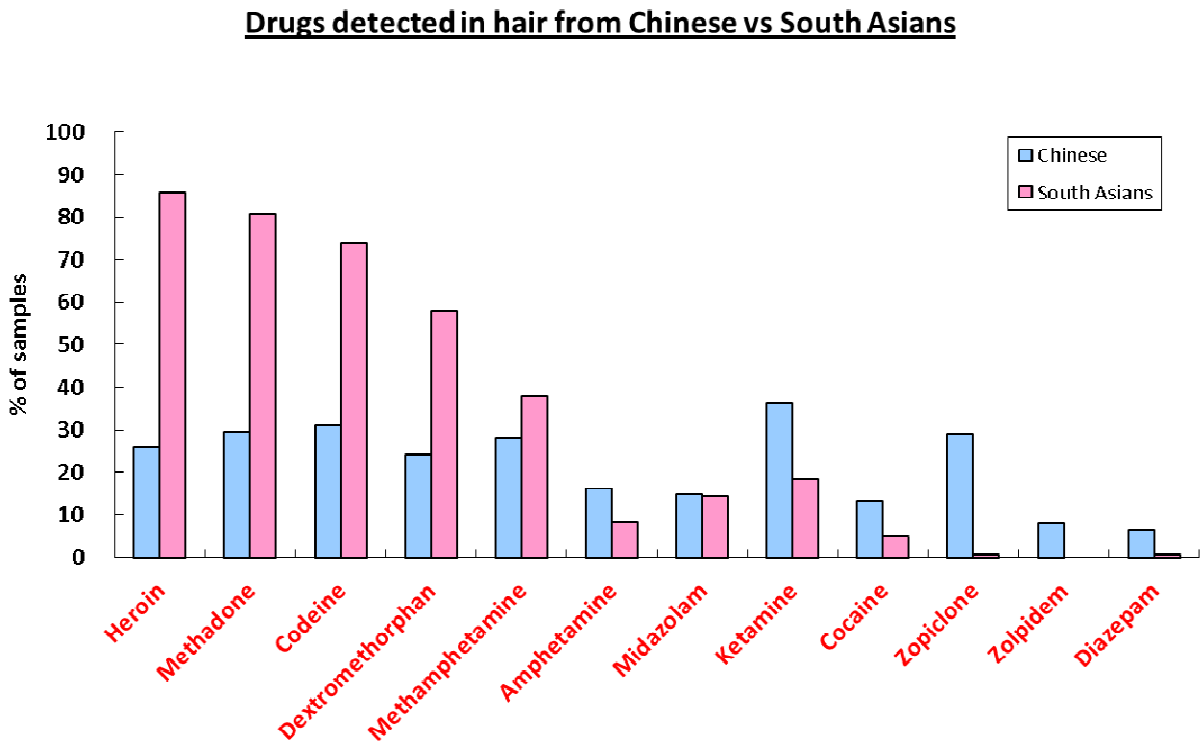


Fig. 3. Drugs of abuse that were detected in hair specimens collected from SRACP (South Asian; n=119) and non-SRACP (Chinese; n=917) sites.

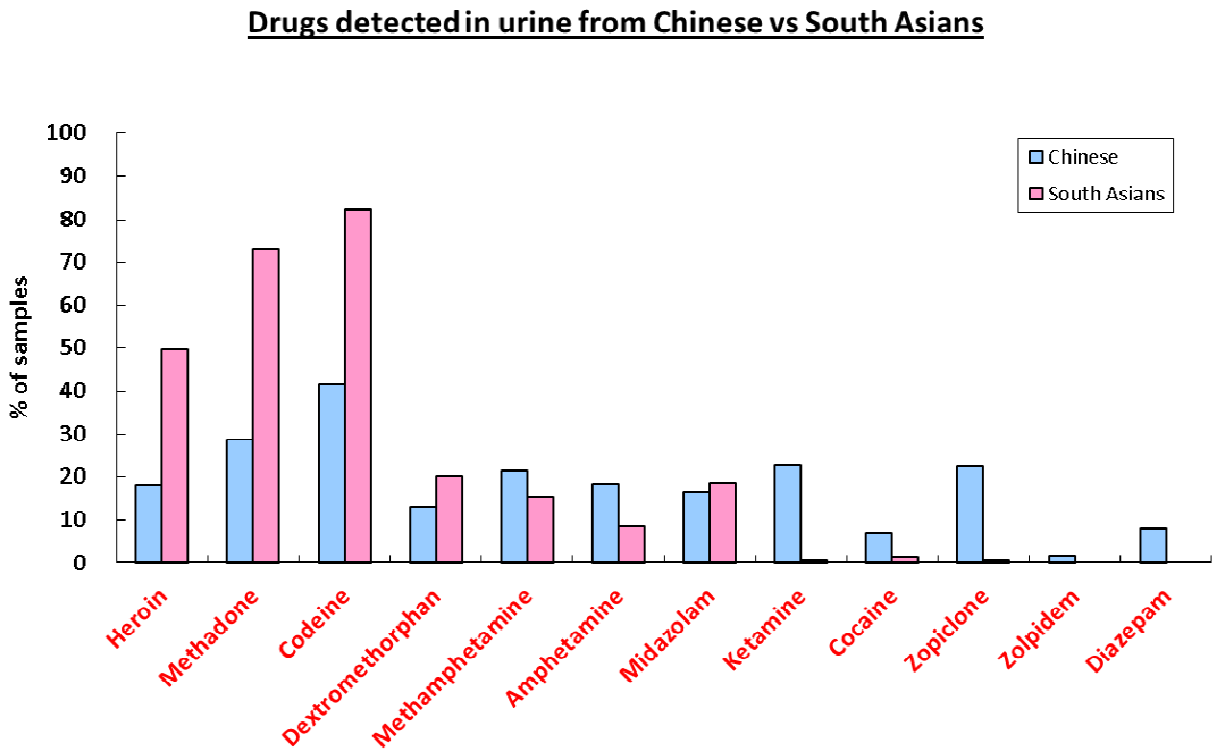


Fig. 4. Drugs of abuse that were detected in urine specimens collected from SRACP (South Asian; n=119) and non-SRACP (Chinese; n=917) sites.

Collection site setting

The samples from the current study were collected from various sites, which facilitates the study of drug use pattern in different settings according to the collection sites: (i) acute setting; (ii) drug rehabilitation setting; and (iii) high-risk population. The categorization of each setting is as follows:

Setting	Collection site(s)	No. of urine samples	No. of hair samples	Total no. of samples
Acute	A&E departments HKPIC toxicology clinic	38	40	78
Drug rehabilitation	SAC CCPSA SRACP Operation Dawn Caritas WYN Centre	885	958	1843
High-risk population	Youth Outreach	41	38	79

The analysis of urine reveals drugs that were consumed recently, hence is especially valuable in the acute setting to identify the cause of intoxication. Fig. 5 shows the drugs of abuse that were detected in urine samples collected under different settings. Of note is the particularly high percentage of ketamine and cocaine detected in samples collected at A&E departments and toxicology clinic compared with the other settings, which may indicate that these drugs carry a more acute and severe toxicity profile relative to the other drugs, resulting in the need of hospitalization. On the other hand, drugs such as codeine, methadone, heroin, zopiclone and dextromethorphan are detected in samples collected under the rehabilitation setting more than the others.

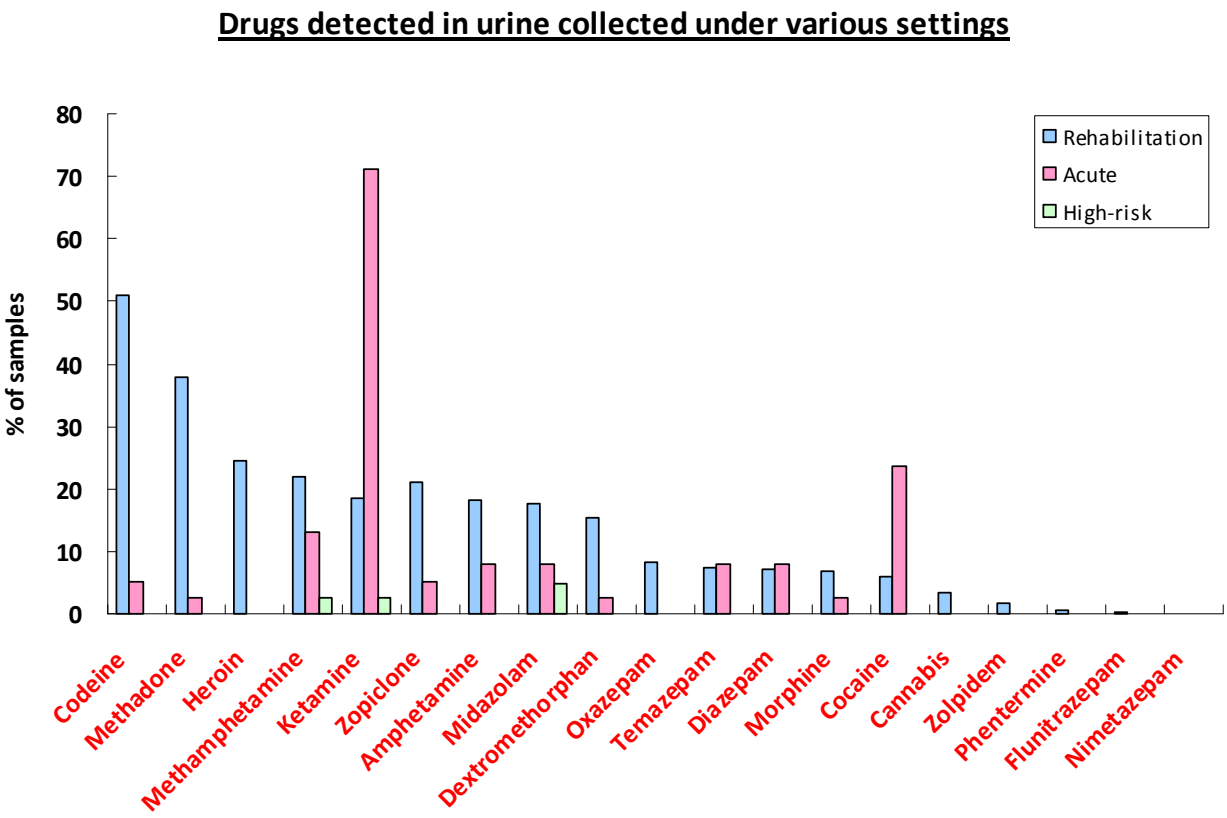


Fig. 5 Drugs of abuse detected in urine samples collected under rehabilitation, acute and high-risk population settings

When interpreting the results of the current study, it should be noted that some of the drugs may be consumed by the subjects for therapeutic purpose rather than for abuse, for example codeine, methadone, zopiclone, zolpidem, phentermine and the benzodiazepines. It was not possible to clarify the drug prescription history for each individual in the current study, and the results pertaining to the prevalence of abuse of each drug should be interpreted with care.

Assay Validation

The methods for urine and hair analysis were validated according to international guidelines. In brief, both methods were found to be fit for purpose. Details of the validation results are given below.

Urine analysis

(i) Accuracy

Fourteen external quality assurance program (EQAP) urine samples from the RCPA (Royal College of Pathologists of Australasia) Urine Toxicology program were assayed. No major discrepancy was observed in terms of the drugs of abuse covered in this assay.

Sample	**Intended results**	**Test results**
1.	Not detected	Not detected
2.	Oxazepam	Oxazepam
3.	Codeine Morphine	Codeine Morphine
4.	MDA MDMA MDEA	MDA MDMA MDEA
5.	Carboxy-THC 6-Monoacetylmorphine Morphine Diacetylmorphine (Heroin)	Carboxy-THC 6-MAM Morphine Heroin
6.	Cocaine Benzoylecgonine	Cocaine Benzoylecgonine
7.	Not detected	Not detected
8.	Morphine Midazolam and metabolite	Morphine Midazolam metabolite
9.	Methadone & Methadone metabolite Carboxy-THC	Methadone & Methadone metabolite Carboxy-THC
10.	Amphetamine Methamphetamine Benzoylecgonine	Amphetamine Methamphetamine Benzoylecgonine

	Oxazepam Morphine Cannabis metabolite (Carboxy-THC)	Oxazepam Morphine Carboxy-THC
11.	Canabis metabolite (Carboxy-THC)	Carboxy-THC
12.	PMA PMMA	PMA PMMA
13.	Codeine Temazepam Oxazepam Nordiazepam Morphine	Codeine Temazepam Oxazepam Nordiazepam Morphine
14.	Morphine Codeine 6-Monoacetylmorphine	Morphine Codeine 6-Monoacetylmorphine

(ii) Limit of detection

Ten sample blanks were independently spiked with the analytes at a range of concentration levels (from 0.02 - 2500 ng/mL). LOD was defined as the concentration where 10 out of 10 matrices were detected positive. The LOD of the analytes are summarized as follows:

Analyte	**LOD (ng/mL)**
POSITIVE IONIZATION	
4-Fluoroamphetamine	10
4-Methylthioamphetamine	20
Amphetamine	5
Bromo-dragonfly	10
Chloroamphetamine	200
DOB	10
DOET	10
DOM	2
HMMA	2
MBDB	2
MDA	50
MDEA	5
MDMA	5
Methamphetamine	5

N-ethylamphetamine	5
PMA	5
PMMA	5
Codeine	50
Codeine met (nor)	100
Heroin	50
Heroin met (6-MAM)	10
Morphine	20
Morphine met (nor)	100
Cocaine	2
Cocaine met (Benzoylecgonine)	5
Cocaine met (Cocaethylene)	2
Cocaine met (nor)	2
Ketamine	5
Ketamine met (nor)	10
Methoxetamine	5
Tiletamine	5
Chlordiazepoxide	50
Diazepam	2
Diazepam met (nor)	20
Estazolam	5
Flunitrazepam	5
Flunitrazepam met (7-amino)	5
Midazolam	5
Midazolam met (1-OH)	5
Midazolam met (4-OH)	5
Nimetazepam	5
Oxazepam	100
Temazepam	50
Triazolam	5
Triazolam met (1-OH)	20
Cannabis met (carboxy-THC)	10
Cannabis met (THC-OH)	50
JWH-018	10
JWH-018 met (4-OH-indole)	100
JWH-018 met (N-5-OH-pentyl)	2
JWH-073	10
Dextromethorphan	20
Dextromethorphan met (dextrorphan)	20
LSD	5
LSD met (nor)	2
LSD met (OH)	20
Methadone	1
Methadone met (EDDP)	10
Methaqualone	2
Phentermine	5

Zolpidem	5
Zopiclone	20
2C-B	10
2C-H	5
2C-I	20
2C-T-2	20
2C-T-4	20
2C-T-7	20
Mescaline	20
BZP	10
mCPP	10
MDBP	10
pFPP	10
pMeOPP	20
TFMPP	10
Cathinone	50
Ethylone	100
Mephedrone	10
Methcathinone	50
Methedrone	50
Methylone	10
5-MeO-DIPT	5
Alpha-methyltryptamine	10
Dimethyltryptamine	10
Psilocin	5
MDPV	10
Mitragynine	5
Salvinorin A	20
NEGATIVE IONIZATION	
Amobarbital	100
Butabarbital	200
Secobarbital	250
CP-47,497	20
CP-47,497-C8 hom	200

(iii) False negative rate at approximate LOD level

Fifteen blank urines that were spiked with analytes at LOD plus 20% level were analyzed. Three analytes (HMMA, hydroxy-THC and butabarbital) had false negative rates of 7% at approximate LOD level; whereas all other analytes had 0% false negative rate.

(iv) False positive rate

Twenty urines that do not contain the analytes were analyzed. The false positive rate was 0% for all analytes.

(v) RT imprecision

The within and between day RT imprecision was found to be below 4% RSD for all analytes. The precision data fulfill the pre-set requirement (relative standard deviation RSD < 10%).

Hair analysis

(i) Accuracy

Three hair samples from the GTFCh (Gesellschaft für Toxikologische und Forensische Chemie; or Society of Toxicological and Forensic Chemistry) Arvecon Drugs in Hair Proficiency Test program were assayed. No major discrepancy was observed in terms of the drugs of abuse covered in this test.

Sample	**Intended results**	**Test results**
1.	Cocaine Benzoylecgonine Morphine 6-MAM	Cocaine Benzoylecgonine Morphine 6-MAM
2.	Amphetamine MDMA MDA	Amphetamine MDMA MDA
3.	Diazepam Nordiazepam	Diazepam Nordiazepam

(ii) Limit of detection

Ten sample blanks were independently spiked with the analytes at a range of concentration levels (from 2 – 50,000 pg/mg). LOD was defined as the concentration where 10 out of 10 matrices were detected positive. The LOD of the analytes are summarized as follows:

Analyte	LOD (pg/mg)
POSITIVE IONIZATION	
4-Fluoroamphetamine	100
4-Methylthioamphetamine	250
Amphetamine	100
Bromo-dragonfly	100
Chloroamphetamine	250
DOB	50
DOET	50
DOM	25
HMMA	50
MBDB	25
MDA	250
MDEA	50
MDMA	50
Methamphetamine	50
N-ethylamphetamine	25
PMA	100
PMMA	25
Codeine	250
Codeine met (nor)	500
Heroin	50
Heroin met (6-MAM)	50
Morphine	250
Morphine met (nor)	1000
Cocaine	25
Cocaine met (Benzoylecgonine)	25
Cocaine met (Cocaethylene)	25
Cocaine met (nor)	25
Ketamine	25
Ketamine met (nor)	50
Methoxetamine	50
Tiletamine	50
Chlordiazepoxide	100
Diazepam	25
Diazepam met (nor)	50
Estazolam	50
Flunitrazepam	50
Flunitrazepam met (7-amino)	50
Midazolam	25
Midazolam met (1-OH)	25
Midazolam met (4-OH)	25

Nimetazepam	50
Oxazepam	500
Temazepam	100
Triazolam	50
Triazolam met (1-OH)	1000
JWH-018	25
JWH-018 met (4-OH-indole)	100
JWH-018 met (N-5-OH-pentyl)	50
JWH-073	25
Dextromethorphan	25
Dextromethorphan met (dextrorphan)	25
LSD	25
LSD met (nor)	25
LSD met (OH)	100
Methadone	10
Methadone met (EDDP)	25
Methaqualone	25
Phentermine	50
Zolpidem	10
Zopiclone	50
2C-B	100
2C-H	50
2C-I	50
2C-T-2	50
2C-T-4	50
2C-T-7	500
Mescaline	500
BZP	250
mCPP	500
MDBP	250
pFPP	250
pMeOPP	500
TFMPP	500
Cathinone	50
Ethylone	500
Mephedrone	50
Methcathinone	50
Methedrone	50
Methylone	25
_5-MeO-DIPT	10
Alpha-methyltryptamine	500
Bufotenine	250
Dimethyltryptamine	50
Psilocin	10000
MDPV	25
Mitragynine	10

Salvinorin A	10000
NEGATIVE IONIZATION	
Amobarbital	1000
Butabarbital	1000
Secobarbital	10000
CP-47,497	500
CP-47,497-C8 hom	500

(iii) False negative rate at approximate LOD level

Fifteen blank samples that were spiked with analytes at LOD plus 20% level were analyzed. The false negative rate was found to range from 0-13% for all analytes at approximate LOD level, as follows:

Analyte	**False negative rate**
2C-T-7	7%
Codeine	7%
DOET	7%
Heroin	7%
Mitragynine	7%
Morphine	7%
CP-47,497-C8 hom	13%
Norcodeine	13%
Other analytes	0%

(iv) False positive rate

Twenty samples that do not contain the analytes were analyzed. The false positive rate was 0% for all analytes.

(v) RT imprecision

The within and between day RT imprecision was found to be below 4% RSD for all analytes. The precision data fulfill the pre-set requirement (relative standard deviation RSD < 10%).

Concluding remarks

In conclusion, the present study has successfully established a chromatography-based analytical system that is capable of detecting both traditional and emerging DOAs in urine and hair. This method is now applied as a routine drug of abuse screening within our laboratory and serves as a continual surveillance of new drugs in the population. Indeed, following its implementation, the method has been successfully applied leading to the identification of a case of fatal poisoning involving PMMA [9]. Moreover, the method developed has the flexibility to increase the analytical coverage. Drugs of abuse are notorious for their protean nature; slight chemical modifications are constantly being introduced to bypass the current legislation. As such, an analytical system for emerging DOAs must be flexible for expansion to cover "newer" drugs.

The analysis of 2000 urine and hair specimens has revealed the presence of 3 types of emerging DOA within the local drug scene – PMMA, TFMPP and methcathinone. Importantly, such drugs are known to cause severe toxicity and even fatalities. The results of the current study and the continual effort in surveillance of emerging drugs of abuse may guide future directions in terms of legislation, social and clinical management of novel psychoactive substances.

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