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Detection of Abused Drugs in Urine by GC-MS

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ABSTRACT

This paper reviews gas chromatography-mass spectrometry (GC-MS) methods for the analysis of amphetamines, ketamines, opioids, cocaine, and other abused drugs in urine that were developed by authors in Taiwanese institutions, and published during the 2000 to early 2008 period. Information on sample preparation, derivatization, internal standard, GC column, detection mode, and validation data for the reported methods are summarized in table format to facilitate readers' reference and adaptation.

Key words: Review, GC-MS, amphetamines, ketamine, cocaine, opioids

INTRODUCTION

The identification and quantification of abused substances and their metabolites in urine have been a major task in the forensic drug testing industry. Ideally, the adopted methodology should be robust and capable of generating analytical data of utmost accuracy. Current practice requires the use of two assays based on different analytical principles. First, immunoassays are frequently used for urine screening in order to differentiate between negative and presumptively positive samples. Positive results must be confirmed by a second independent method. Mass spectrometry, particularly in combination with chromatographic techniques, has become a preferred tool in forensic science. Today, gas chromatography–mass spectrometry (GC-MS) is the most widely used method for confirmation of positive tests[1-4], since it provides high levels of specificity and sensitivity. The mandatory Guideline for Abused Drug Urine Testing in Taiwan is also based on the GC-MS as confirmation method.

The GC-MS procedures were reviewed in many studies. In 1992 Maurer[2] has discussed the systematic toxicological analysis of drugs and their metabolites by GC-MS. Goldberger and Cone[3] have reviewed the confirmational tests for drugs in the workplace by GC-MS. Kraemer and Maurer[4] have reviewed the principal information on GC-MS procedures for the determination of amphetamine, methamphetamine and amphetamine derived designer drugs in 1998. Chen *et al.*[5] have reviewed the determination of ketamine and its metabolites in biological samples.

With the implementation of workplace urine drug testing policy in Taiwan, researchers in various Taiwanese institutions have made substantial efforts to develop analytical methods suitable for this application. We thought it would be informative to prepare a review focusing on this narrowly defined scope — Papers published by these Taiwanese authors during the 2000 to early 2008 period. Information hereby provided would complement earlier reviews[2-5] that were mainly based on papers published by American and European authors. Procedures are critically reviewed for the determination of amphetamines, ketamine, opioids and other abused drugs in human urine. Because of length limitation of the report, a descriptive comparison of different analytical procedures is not included. Likewise, animal studies are not described since sufficient human studies are available to adequately describe metabolism of the drugs in urine.

Basic information about the sample extraction method, derivatization, internal standard, GC column, detection mode and validation data of each procedure are summarized in tables. The tables are organized based on the drug class. The sample preparation is summarized in the "Extraction method, derivatization agent" column. The selection of the internal standard (I. S.) is given in the "Internal standard" column. The principal information on the GC column and on the detection mode is listed. Validation data like recovery (REC), linearity (LIN), limit of

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detection (LOD), and limit of quantification (LOQ) are summarized in the "Validation data" column. The "Validation data" column indicates whether a paper deals with a quantitative assay. Precision data was omitted since all reviewed procedures were suitable for urine analysis.

AMPHETAMINES

Amphetamine (AM, *R*, *S*-1-phenyl-2-propanamine) and methamphetamine (MA, *R*, *S*-*N*-methyl-1- phenyl-2-propanamine) are powerful stimulants in the central nervous system. These drugs are often abused and used doping agents in sports[6]. Stimulants in doping control consists of a heterogeneous group of compounds, the majority of which is structurally related to amphetamine[6]. There are many designer amphetamines, such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy methamphetamine (MDMA, Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butamine (MDB) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)[7]. In addition, amphetamine and methamphetamine are the metabolic products of other drugs such as amphetaminil, benzphetamine, clobenzorex, selegiline (deprenyl), dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorex, mefenorex, mesocarb, and prenylamine[8-10].

Most of the GC-MS procedures for the determination of amphetamines in urine followed the same principles, i.e. extraction, derivatization, separation, and detection (Table 1). Extraction was performed using liquid-liquid extraction (LLE) at an alkaline pH, at which the amphetamines are unionized[11-14] by solid-phase extraction (SPE)[15-18]. Note that the traditional LLE suffers from lengthy and complicated operation procedure, excessive use of harmful organic solvents, high background, and low level of automation whereas conventional SPE may suffer from cartridge clogging. A simultaneous supercritical fluid extraction (SFE) and chemical derivatization (CD) procedure for the determination of amphetamines in urine was described and evaluated by Wang *et al.*[19] Because the trend towards automation and miniaturization has resulted in improved techniques of sample preparation, solid-phase microextraction (SPME) has been increasingly popular. A one-step process[17] was adopted to complete the absorption/derivatization process for the analysis of amphetamines' enantiomeric compositions by adding the derivatizing reagent directly into the sample matrix in a regular sample vial.

Derivatization of the amphetamines is necessary to improve their GC properties to form more characteristic mass spectral fragment ions. Amphetamines were derivatized prior to GC analysis by trifluoroacetic anhydride (TFAA)[14,19,20], pentafluoropropionic anhydride (PFPA)[13,15,16], and acetic anhydride (AA)[18]. (*S*)-(−)-*N*-(trifluoroacetyl)-prolyl (*l*-TPC) was used to

Table 1. GC-MS procedures for the identification and quantification of amphetamines in human urine

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
MA	MA-d_5 MA-d_8 MA-d_9	LLE, TCAA, MSTFA, PFPA	HP-Ultra-1 100-300/20°C	EI, Full scan, SIM	REC: ? LIN: 50 - 20000 ng/mL LOD: ? LOQ: ?	11
MA	MA-$^2H_9$	SPE, PFPA		EI, Full scan, SIM	REC: ? LIN: 100-9600 ng/mL LOD: ? LOQ: ?	15
AM MA	AM-d_8 MA-d_8	SFE, TFAA	DB-5 100-280/10°C	isotope dilution MS, EI, SIM	REC: 89-95% LIN: 100-50000 ng/mL LOD: 19-50 ng/mL LOQ: 21-100 ng/mL	19

Table 1. Continued

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
d-AM *l*-AM *d*-MA *l*-MA	AM-d_8 MA-d_8	SPME, *l*-TPC	HP-5 60-250/25°C	EI, Full scan, SIM	REC: ? LIN: 50 - 1000 ng/mL LOD: 30 ng/mL LOQ: 50 ng/mL	17
AM MA MDA MDMA MDEA and others	AM-d_8 MA-d_8 MDA-d_5 MDMA-d_5	LLE, TFAA,	HP-5 70-250/30°C	EI, Full scan, SIM	REC: ? LIN: 0-1000 ng/mL LOD (ng/mL): 11 (AM), 19 (MA), 11(MDA), 12 (MDMA), 11 (MDEA) LOQ (ng/mL): 20 (AM), 20 (MA), 25(MDA), 25(MDMA), 25 (MDEA)	12
AM MA	AM-d_8 MA-d_8	LLE, PFPA	HP-5 60-250/25°C	EI, SIM	REC: 75% LIN: 50-1000 ng/mL LOD: 40 ng/mL LOQ: 50 ng/mL	13
AM MA MDA MDMA MDEA	AM-d_8 MA-d_8	SPE, AA	CP-Wax capillary 52 CB 130-260/15°C	Furan C I-MS/MS, Full scan, SIM	REC: 86 – 113% LIN (ng/mL): 1-1000 (AM), 2-1000 (MA) LOD (ng/mL): 1 (AM), 0.4 (MA), 0.5 (MDA), 0.5 (MDMA), 0.5 (MDEA) LOQ (ng/mL): 2 (AM), 1 (MA), 1 (MDA), 1 (MDMA), 1 (MDEA)	18
AM MA (Famprofazone)	AM-d_8 MA-d_8	LLE, TFAA, *l*-TPC	HP-5 90-240/15°C to 300/10°C Enantiomers: 150-250/20°C	EI, SIM	REC: 85- 119% LIN: 100-8000 ng/mL LOD: 100 ng/mL LOQ: 100 ng/mL	14
PMMA PMA and others	MA-d_8	LLE, HFBA	HP-1 60-300/20°C	EI, SIM	REC: ? LIN: 100-2000 ng/mL LOD: ? LOQ: ?	58

form diastereomers for chiral analysis [14,17]. Different chemical derivatization approaches were applied to meet analytical needs. Further details on derivatization for the analysis of drugs by GC-MS were discussed in the study of Lin *et al*.[21] Commonly used derivatization reagents for silylation, acylation, and alkylation were summarized along with comments on practical considerations[21]. After derivatization, the analytes were separated on capillary columns and detected in the single-ion monitoring (SIM) mode[13,14,19] or a combination of full scan and SIM mode[11,12,15,17,18].

The selection of the is essential to ensure precision of the method. Stable isotopes are the most suitable I.S., since they have the same analytical properties as the corresponding analyte. Specifically, AM-d_8, MA-d_8, MDA-d_5, and MDMA-d_5 were more favorable isotopes (Table 1). Nevertheless, ion-pairs (analyte/I.-S.) contributed to the quality of quantitation results for the analysis of methamphetamines[11,15]. Methamphetamine $^2H_9$-analog was used as a sample to study the effect of molecular abundance on the intensity ratio of an ion-pair that was designated for the analyte and I.S.[15]. Chang *et al*.[15] claimed that the intensity ratio of an ion-pair selected to designate the sample's analyte-to-^{2}H-I.S. concentration ratio changes with the following operating parameters: (a) constitution solvent volume; (b) injection volume, and (c) column temperature program rate. Molecular abundance (intensity) in ion source and different retention times between analyte and its I.S. are the two main causes of the interfering phenomenon. Lin *et al*.[11] suggested that derivatization methods (trimethylsilyl-, trichloroacetyl- and pentafluoropropionyl-), internal standards (MA-d_5, MA-d_8, MA-d_9), and ion-pairs all contribute to the quality of quantitation results for the analysis of methamphetamines. Furthermore, Chen *et al*.[22] developed an approach to assess accuracy of the cross-contribution data between the ions designating the analyte and the deuterated I.S. They concluded that the normalized ion intensity data can be reliably used for the calculation of cross-contribution values, at least for the systems studied. They further demonstrated that an ion-pair with about 5% (or higher) cross-contribution would result in a very limited linear calibration range. Chiu *et al*.[16] also stated that different retention times and intensities between the methamphetamine and its isotopic ^{2}H-analogs I.S. seemed to have caused the observed interference in the calibration curve.

Although methamphetamine is one of the two major drugs of abuse in Taiwan[23], Lua *et al*.[24] showed a high prevalence of ketamine (K) and MDMA detection in urine samples from participants in a disco clubs in Taiwan. Detailed analysis of the drugs of abuse profiles in club urine samples and Detainee's samples revealed a very different pattern. K and MDMA positive rates were extremely high in club urine samples. Therefore, simultaneous detection and determination of AM, MA, MDA, MDMA, MDEA, K, Norketamine (NK), and dehydronorketamine (DHNK) in urine samples to monitor abuse of multiple drugs was reported[20]. In this procedure, urine samples were extracted with organic solvent and derivatized with TFAA, although only a few investigators have reported the derivatization of ketamines prior to GC-MS analysis.

Famprofazone is one of the 14 amphetamine precursors and is a component of the multi-ingredient medication used for pain relief (Gewolen®)[25]. Famprofazone is included in the prohibited list of the World Anti-Doping Agency[6]. This drug has been demonstrated to metabolize to methamphetamine and amphetamine following administration and produce positives in urine drug tests[26,27]. A case report[14] showed that a urine specimen collected during a national sport competition in Taiwan tested positive for both methamphetamine and amphetamine. The athlete claimed that she had taken Gewolen®. This study has demonstrated effectiveness of using *l*-TPC as derivatization agent for separation of the *d*- and *l*-enantiomers of amphetamine and methamphetamine.

KETAMINES

Ketamine is synthesized and marketed as an anesthetic drug for human and animal use[28,29]. NK and DHNK, the main metabolites of K[30], have been abused and caused deaths[24,31]. This abuse trend has created a need for clinical laboratories to develop methods for the analysis of K and its metabolites in biological matrices. K and its major metabolite are usually determined with GC-MS.

Sample preparation involves isolation, cleavage of conjugates and/or derivatization of the K and its metabolites. Isolation was performed by LLE usually at an alkaline pH[12,32-35] or by SPE[36,37]. Cleavage of conjugates has been reported by Lin and Lua[32]. In order to determine the presence of conjugated metabolites during K metabolism, urine samples were hydrolyzed with concentrated hydrochloric acid, alkalinized and extracted with organic solvent. Electron impact mode was employed to determine K, NK, and DHNK. The acidic hydrolysis led to a significant increase of K, NK, and DHNK concentration in many samples analyzed. The median concentration ratio of hydrolyzed to unhydrolyzed K, NK, and DHNK was 1.15, 1.35, and 1.44, respectively.

While derivatization of the ketamine was not necessary[32,33,36,37], several procedures with derivatization[12,34,35] were used in this paper (Table 2). As shown in the ''Validation data'' column in Table 2, all procedures with or without derivatization led to similar results.

Nevertheless, some authors stated that their analytical procedures have specific advantages. For example, an analytical scheme using GC-isotope dilution mass spectrometry (IDMS) assisted by precedent LLE and chemical derivatization was described by Chou *et al*.[34] The

Table 2. GC-MS procedures for the identification and quantification of ketamines

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
K NK DHNK	K-d_4 NK-d_4	LLE	HP-5 110-180-270/5-45°C	EI, Full scan, SIM	REC: ? LIN: 0-500 ng/mL LOD (ng/mL): 1 (K), 5 (NK) LOQ (ng/mL): 5 (K), 10 (NK)	32
K NK	K-d_4 NK-d_4	LLE, PFBC	HP-5 100-250/25°C (EI) 70-150-250/30-25°C (PCI)	GC/isotope dilution MS, EI, PCI, Full scan, SIM	REC: 71-97.8 % LIN: 100 - 5000 ng/mL LOD (ng/mL): EI- 10 (K), 5 (NK) PCI- 75 (K), 50 (NK) LOQ (ng/mL): EI- 15 (K), 10 (NK) PCI- 100 (K), 75 (NK)	34
K NK DHNK	K-d_4 NK-d_4	LLE	HP-5 120-220-260/35-40°C	EI, SIM	REC: ? LIN (ng/mL): 1-7000 (K), 5-4000 (NK), 20-5000 (DHNK) LOD (ng/mL): 1 (K), 5 (NK), 20 (DHNK) LOQ (ng/mL): 5 (K), 10 (NK), 40 (DHNK)	33
K NK DHNK and others	K-d_4 NK-d_4	LLE, TFAA	HP-5 70-250/30°C	EI, Full scan, SIM	REC: ? LIN: 0-250 ng/mL LOD (ng/mL): 2 (K), 1 (NK), 6 (DHNK) LOQ (ng/mL): 20 (K), 20 (NK), 30 (DHNK)	12
K NK DHNK	K-d_4 NK-d_4	LLE, AA, TFAA, HFBA, PFBC and other silylation reagents	HP-5 100-280/25°C	EI, Full scan, SIM	REC: ? LIN: ? LOD (ng/mL): AA- 5 (K), 3 (NK), 20 (DHNK) TFAA- 5 (K), 3 (NK), 10 (DHNK) HFBA: 10 (K), 3 (NK), 5 (DHNK) PFBC: 5 (K), 3 (NK), 5 (DHNK) LOQ (ng/mL): AA- 5 (K), 5 (NK), 50 (DHNK) TFAA- 5 (K), 5 (NK), 10 (DHNK) HFBA: 10 (K), 5 (NK), 5 (DHNK) PFBC: 5 (K), 5 (NK), 5 (DHNK)	35

Table 2. Continued

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
K NK DHNK	K-d_4 NK-d_4	SPE	HP-5 130-170-200-280/10-5-40°C	EI, Full scan, SIM	REC: 53.4 – 77.9% LIN: 30 - 1000 ng/mL LOD (ng/mL): 10 (K), 10 (NK), 10 (DHNK) LOQ (ng/mL): 15 (K), 10 (NK), 20 (DHNK)	36
K NK and others	K-d_4 NK-d_4	SPE	HP-5 130-170-200-250/10-5-10°C	EI, Full scan, SIM	REC: 71.4 – 96.5% (K) 68.4 – 90.1% (NK) LIN: 30 - 600 ng/mL LOD (ng/mL): 15 (K), 5 (NK) LOQ (ng/mL): 15 (K), 20 (NK)	37

Table 3. GC-MS procedures for the identification and quantification of opiods

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
MO CO	MO-d_3 CO-d_3	SPE, MSTFA, BSTFA, BSA, MBTFA, TFA, HFBA, PFPA, AA	Rtx-5 100-250/20°C	Isotope dilution EI, PCI, NCI, Full scan SIM	REC: 60.7 – 83.6% (MO) 65.2 – 90.2% (CO) LIN: 50 - 1000 ng/mL LOD (ng/mL): EI- 1.1 (MO), 1.4 (CO) PCI- 6.1 (MO), 1.3 (CO) LOQ (ng/mL): EI- 3.5 (MO), 4.7 (CO) PCI- 14.2 (MO), 4.3 (CO)	41
MO CO 6-AMO and others	MO-d_3, MO-d_6, CO-d_3, CO-d_6, 6-AMO-d_3, 6-AMO-d_6, and others	LLE, 1. methoxyamine and hydroxylamine 2. TMS or propionyl group	HP-5 160-250/20°C	EI, Full scan, SIM	REC: MO-propionyl-TMS (74.1%) CO-TMS (86.5%) 6-AMO- propionyl (86.3%)	39

Table 3. Continued

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
					LIN: ? LOD and LOQ (ng/mL): MO-propionyl-TMS: 30 MO-2 propionyl: 200 CO-TMS: 50 CO- propionyl: 100 6-AMO- propionyl: 30	
BUP	BUP-d_4	Toxi-Tubes A, LLE,	HP-5	EI, Full scan, SIM	REC: ?	40
NBUP	NBUP-d_3	MBTFA, BSTFA, AA, TFAA, PFPA, HFBA, MSTFA with 1% TMCS (or BSTFA with 1% TMCS)	120-250-300/40-10°C		LIN (ng/mL):: 0 – 500 (BUP), 0-1000 (NBUP) LOD and LOQ (ng/mL): Acetyl- 5 (BUP), 2.5-10 (NBUP) TMS- 10 (BUP) , 5-50 (NBUP)	

simultaneous derivatization of the primary-amine NK and secondary-amine K with pentafluorobenzoyl chloride (PFBC) not only enhanced their instrumental responses and mass-spectrum uniqueness, but also allowed more appropriate and easier selection of qualifier and quantifier ions and hence achieved better identification and quantitation. GC-IDMS operated in the PCI (positive ion chemical ionization) mode could offer both qualitative and quantitative information complementary to those given by the EI mode. Unfortunately, the authors did not incorporate DHNK into the analyte list. Although Chou *et al*.[34] utilized PFBC as the derivatization reagent to develop the analytical procedure for K and NK, no data was shown for other derivatization approaches. A comparative study on the utilization of different derivatization groups for the analysis of K and NK was performed by Wu *et al*.[35] Their results showed that the ion intensity levels of K from various derivatization reagents were in the following order: PFBC > TFAA > acetic anhydride > BSTFA (*N,O*-bis (trimethylsilyl)trifluoroacetamide) > MSTFA (*N*-methyl-bis(trifluoroacetamide)) > HFBA (heptafluorobutyric anhydride) > MBTFA (*N*-methyl-bis(trifluoroacetamide)). The corresponding order for NK and DHNK was PFBC > HFBA > TFAA > MBTFA > BSTFA > MSTFA > acetic anhydride. Authors[35] claimed that PFBC provided the best performance characteristics. Lin and Lua[12] pointed out that an advantage of derivatization with that TFAA was that it did not produce a false-positive result for MA under a simultaneous detection of amphetamines, ketamines and a high concentration of ephedrine or pseudoephedrine in urine sample.

OPIOIDS

Both codeine and heroin are metabolized to morphine by the hepatic enzymes[38]. Codeine is biotransformed via O-demethylation to morphine under the catalysis of cytochrome P450 enzyme. Nevertheless, heroin is first deacetylated to 6-acetylmorphine via blood esterase and later hydrolyzed to morphine in the liver. Buprenorphine is a semi-synthetic organic compound with a chemical structure similar to morphine. Currently, this drug is prescribed as an alternative to methadone for the treatment of heroin addiction. Its main metabolite is desalky-BUP or norbuprenorphine (NBUP).

Sample preparation was performed by LLE[39] or Toxi-Tube A[40] or SPE[41]. Since 6-acetylmorphine was an important metabolite, the analytical protocol did not include a hydrolysis step[41]. The study data showed the concentrations of these drugs/metabolites in their free forms. Wang *et al*.[41] presented a more detailed comparison accompanied by GC-IDMS methodology on the CDs of MO and CO, with twelve CD agents. Efficiency of the CD, analyte-IS ion cross-contribution, shelf-life of the derivative, and experimental conditions of the CD were also evaluated in this study. The results demonstrated

that SPE and BSA (*N,O*-bis(trimethylsilyl)acetamide)-CD followed by GC-IDMS was a sound analytical scheme for the conclusive determination of MO and CO in urine[41]. However, 6-acetylmorphine was not included. The validation data for the MO-BSA and CO-BSA are presented in Table 3.

An ethoxyimino/propionyl/TMS (trimethylsilyl) three-step derivatization approach was developed for the simultaneous analysis of 8 opioids: morphine, codeine, 6-acetylmorphine, hydromorphone, oxymorphone, hydrocodone, oxycodone, and noroxycodone[39]. Distinct derivatization products, with good chromatographic and mass spectrometric characteristics, were generated for all analytes. Chen *et al*.[39] indicated that application of methoxyimino/propionyl/TMS groups, in the order listed, facilitated the simultaneous analysis of these 8 opiates in urine samples and led to satisfactory LOD, LOQ and linearity (Table 3).

Various chemical derivatization approaches adapted for the analysis of buprenorphine and its major metabolite (norbuprenorphine) were conducted by Wu *et al*.[40] These approaches included alkylation, acylation, and silylation resulting in the formation of methyl, acetyl, trifluoroacetyl, pentafluoropropionyl, heptafluorobutyryl, and trimethylsilyl derivatives. The criteria included reaction yields and ionization efficiency of the derivatization products, chromatographic characteristics, cross-contributions to the intensities of ions designating the analytes, and the internal standards. Among all acetyl- and TMS-derivatization approaches studied, derivatization by acetyl anhydride resulted in the best performance characteristics. Unfortunately, the authors published no recovery data.

OTHER ABUSED SUBSTANCES

Relatively limited papers concerning applications of GC-MS to cocaine[42], gamma-hydroxybutyrate (GHB) [43], pentazocine[44] and 7-aminoflunitrazepam (7-amino-FM_2)[45,46] were available to date.

Chemical derivative products of the analyte and the selected I.S. must generate ions able to indicate the analyte and the I.S. These ions should not have significant cross-contribution, such as I.S. contribution to the intensities of the ions designated for the analyte and vice versa[47-50]. However, researches have been demonstrated analyte-I.S. ion cross-contribution for amphetamines[11,15], ketamines[35], opioids[39,41], buprenorphine[40] and barbitals[51,52]. In addition, Wang *et al*.[42] further evaluated the isotopically labeled internal standards and derivatization methods for quantitative determination of cocaine and related compounds, i.e. norcocaine, benzoylecgonine, cocaethylene, ecgonine, ecgonine methyl ester, anhydroecgonine methyl ester. The isotopically labeled analogs of the analytes were used as internal standards (Table 4). In addition to the systematic presentation of full scan spectra, the cross-

Table 4. GC-MS procedures for the identification and quantification of cocaine, gamma-hydroxybutyrate, pentazocine, and 7-aminoflunitrazepam

Compound	Internal standard	Extraction method, Derivatization agent	Column	Detection mode	Validation data	Ref
Cocaine Benzolecgonine and others	Cocaine-d_3 Benzolecgonine-d_3 Benzolecgonine-d_8	LLE, TFAA, PFPA, HFBA, MSTFA with 1% TMCS, MTBSTFA with 1% t-BDMCS, and others	HP-Ultra-1, Parameters were adapted in the literature for various CD products.	EI, Full scan, SIM	REC: ? LIN: ? LOD:,? LOQ: ?	42
GHB	GHB-d_6	SPE, LLE, BSTFA	HP-5 60-100-250/15-25°C	EI, Full scan, SIM	REC: 23.7% (LLE), 60.7% (SPE) LIN: 500 - 10000 ng/mL LOD: 300 ng/mL (LLE) 100 ng/mL (SPE) LOQ: 500 ng/mL (LLE) 200 ng/mL (SPE)	43
Pentazocine	Dextromethorphan	SPE, BSTFA-TMCS	DB-5 150-280/20°C	EI, SIM	REC: 72.4-82.1% LIN: 125 - 1500 ng/mL LOD: 62.5 ng/mL, LOQ: 125 ng/mL,	44
7-amino-FM_2	7-amino-FM_2-d_7	LLE, MSTFA	HP-5 150-300/20°C	EI, SIM	REC: ? LIN: 80-200 ng/mL LOD: 2.5 ng/mL LOQ: 5.0 ng/mL	46

contribution data of these ion pairs were evaluated using data collected under the SIM mode. The data exhibited similar cross-contribution characteristics in each alkyl, acetyl or TMS series. The cross-contribution data derived from the use of I.S. labeled with more deuterium atoms were generally more favorable.

A paper was published on the determination of GHB with simultaneous extraction and BSTFA-CD followed by GC-EIMS SIM[43]. GHB as its BSTFA derivative was recovered from urine in 23.7% through the LLE-CD procedure, in contrast to 60.7% via the SPE-CD counterpart. The validation data are shown in Table 4. SPE protocol provided lower LOD and LOQ than did the LLE protocol. However, in acidic media, an average of 23.8% of γ-butyrolactone (GBL) was hydrolyzed into GHB, whereas 11.8% of GHB was converted to GBL.

Collaborative study was carried out on the determination of pentazocine in urine by GC-MS[44]. This method used SPE and BSTFA with 1% TMCS derivatization, followed by GC-MS analysis using dextromethorphan as the internal standard (Table 4). The analytical protocol was further applied to an inter-laboratory study. Nine drug-abuse urine testing laboratories in Taiwan participated in the collaborative study. All of the testing laboratories passed their own quality control and were accredited by the National Bureau of Controlled Drugs (NBCD), Department of Health, Executive Yuan, Taiwan, R.O.C. Individual analysts in each laboratory were given flexibility while following the established criteria. Specifically, minimum performance criteria with system suitability test had to be met, but analysts were encouraged to use their routine analytical system (e.g., instrument, injector and column) and to use individual judgment in adjusting the operating conditions. Moreover, a GC-MS method for the analysis of 7-amino-FM_2 in urine[45] was subjected to an inter-laboratory collaborative study[46]. LLE and MSTFA derivatization were performed and 7-amino-FM_2-d_7 was used as the internal standard (Table 4). Authors concluded that the methods[44-46] showed acceptable repeatability and reproducibility.

CONCLUSIONS AND PERSPECTIVES

Most publications on abused drug analysis with GC-MS published in the last decade by Taiwanese researchers emphasized investigating and improving the extraction and derivatization methods, internal standards and ion-pairs. These factors all contribute to the quality of quantitation results. Most of the procedures are suitable for routine clinical and forensic purposes.

In Taiwan, there are two legal precursor drugs, famprofazone and seligiline, which are metabolized by the body to AM and MA. Famprofazone is an antipyretic and analgesic multi-ingredient medication containing famprofazone, acetaminophen (paracetamol), propyphenazone and caffeine. Two legally and locally manufactured brand name medicines (Gewolen® tablet and Paisao® capsule) containing famprofazone are found on the Department of Health website in Taiwan[53]. From a forensic standpoint, it is important that the precursor drugs are controlled and dispensed by prescription. Unfortunately, famprofazone can be purchased in many drug stores without prescription. Thus, famprofazone users may lack a valid medical prescription to help the interpretation and usage. Neugebauer *et al.*[54] reported that urine from a famprofazone user contained 2831 ng/mL of MA and 567 ng/mL of AM. Results also showed that the *l*-enantiomer of MA exceeded that of the *d*-form. As a consequence of the discussion on false positive MA results, NBCD instructed its certified drug-abuse urine testing laboratories that at least 100 ng/mL of AM must be present in urines which are positive for MA$>$500 ng/mL. Note that misinterpretation of positive immunoassay and even GC-MS results is possible because the parent compound is not detectable for as long as the two metabolites AM and MA. Since the AM and MA metabolized from famprofazone contain both the *d*- and *l*-enantiomers, detection of the parent drug and its metabolites, together with the concentrations and enantiomeric composition of AM and MA, is highly valuable for the determination of the involvement of this drug.

Another precursor drug, seligiline, is a prescription agent used for the treatment of Parkinson disease and depression. It produces *l*-amphetamine and *l*-methamphetamine metabolites, which give a positive result on immunoaassays[55]. Unfortunately, routine GC-MS also does not distinguish between the 2 isomers and requires chiral chromatography to differentiate between the *d*- and *l*- forms[9].

To date only a few papers were published by Taiwanese researchers on the determination of AM- and MA-generating precursors, their metabolites and enantiomers. Excretory studies (especially in the later phase of excretion) should be performed to prove whether enantiomeric profiles for AM and/or MA metabolically formed from precursor drugs will allow the differentiation in relation to the abuse of illicit AM and/or MA.

The ring-methoxylated phenethylamine derivatived paramethoxyamphetamine (PMA) and paramethoxymethamphetamine (PMMA) are structurally related to the MDMA, MDA and MDEA compounds. These drugs exhibit all hallucinogenic properties[56]. Following the 8 recent PMM A fatalities reported by Lin *et al.* in Taiwan, biological fluid specimens including heart blood, gastric, bile, and urine were tested. Other drugs, such as MDA, MDMA, K, NK, hydroxymidazolam, MA, and pentobarbital, were also found in these cases[56]. However, PMMA were not detectable in routine analytical procedures in many laboratories. Thus, rarely occurring designer drugs such as PMA or PMMA could not be discovered by routine analytical methods.

Stimulants and narcotic analgesics have been subjects of doping control analysis since the International

Olympic Committee's drug testing program in 1967. At that time, gas-liquid chromatography and thin-layer chromatography were performed for the doping controls. Subsequently, more comprehensive screening and also specific confirmation procedures based on GC-MS were established and are still employed in sports drug testing[57-61]. The method of choice for screening procedures covered more than 200 target analytes plus new and unknown derivatives or designer drugs. This should be taken into account when amending the lists of banned substances. It is important for our researchers to continue developing analytical techniques for new, undetectable and identifiable substances.

LIST OF ABBREVIATIONS

AA	Acetyl anhydride
AM	Amphetamine
AMO	6-Acetylmorphine
t-BDMCS	*t*-butyldimethylchlorosilane
BSA	*N,O*-bis(trimethylsilyl)acetamide
BSTFA	*N,O*-bis(trimethylsilyl)trifluoroacetamide
BUP	Buprenorphine
CO	Codeine
CD	Chemical derivatization
DHNK	Dehydronorketamine
EI	Election impact ionization
GBL	Gamma-butyrolacton
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GHB	Gamma-hydroxybutyrate
HFBA	Heptafluorobutyric anhydride
IDMS	Isotope dilution mass spectrometry
I.S.	Internal standard
K	Ketamine
LIN	Linearity
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MA	Methamphetamine
MBDB	*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine
MBTFA	*N*-methyl-bis(trifluoroacetamide)
MDA	3,4-methylenedioxyamphetamine
MDB	3,4-methylenedioxyphenyl-2-butamine
MDEA	3,4-methylenedioxyethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MO	Morphine
MSTFA	*N* -methyltrimethylsilyltrifluoroacetamide
MTBSTFA	*N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide
NBCD	National Bureau of Controlled Drugs
NBUP	Norbuprenorphine
NK	Norketamine
PCI	Positive ion chemical ionization
PFBC	Pentafluorobenzoyl chloride
PFPA	Pentafluoropropionyl anhydride
PMA	Paramethoxyamphetamine
PMMA	Paramethoxymethamphetamine
REC	Recovery
SFE	Supercritical fluid extraction
SIM	Single-ion monitoring
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TCAA	Trichloroacetic anhydride
TFAA	Trifluoroacetic anhydride
TMS	Trimethylsilyl
TMCS	Trimethylchlorosilane
TPC	(*S*)-(−)-*N*-(trifluoroacetyl)-prolyl chloride

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