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Simultaneous Quantitation of Methamphetamine, Ketamine, Opiates and their Metabolites in Urine by SPE and LC–MS-MS

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Abstract

Heroin, methamphetamine and ketamine have been the most commonly abused drugs in Taiwan. The presence of these drugs and their metabolites in postmortem specimens has been routinely monitored in our laboratory mostly by gas chromatographic-mass spectrometric methods. This study aimed to evaluate a more effective approach to simultaneously quantify these analytes (i.e., amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA), morphine, codeine, 6-acetylmorphine, 6-acetylcodeine, ketamine and norketamine) in postmortem urine and blood specimens by liquid chromatography–tandem mass spectrometry (LC–MS-MS). Samples (1 mL) were extracted via solid-phase extraction, evaporated and reconstituted in the mobile phase for injection into the LC–MS-MS system. Respective deuterated analogs of these analytes were used as internal standards. Chromatographic separation was achieved by an Agilent Zorbax SB-Aq analytical column at 50°C. Mass spectrometric analysis was performed by electrospray ionization in positive-ion dynamic multiple reaction monitoring mode with optimized collision energy for respective precursor ion selected for each analyte, and the monitoring of two transition ions. Performance characteristics were assessed using drug-free samples that were fortified with 50–1,000 ng/mL of the 10 analytes. Analytical parameters evaluated and resulting data are as follows: (i) average extraction recoveries ($n = 3$) were better than 80%, except for MDMA (71%) and morphine (74%); (ii) inter-day and intra-day precision ranges (%CV) were 1.59–8.80% and 0.57–3.89%, respectively; (iii) calibration linearity (r^2), detection limit and quantitation limit for all analytes were >0.999, 1 and 5 ng/mL, respectively; (iv) matrix effects (ion suppression) were observed for three analytes, but were satisfactorily compensated for by the deuterated internal standards adopted in the analytical protocol. This method was successfully applied to the analysis of specimens collected from unknown death cases from various district prosecutors' offices in Taiwan, and was also found helpful to understanding whether the detected opiates were derived from heroin or legal morphine/codeine-containing medications.

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Introduction

Drug abuse has long been a global issue. In Taiwan, drug abuse statistics provided by Food and Drug Administration (1) and Investigation Bureau (2) pointed to heroin, methamphetamine and ketamine as the three most commonly abused and seized drugs during the last 5 years. The detection rate of these drugs and their metabolites in postmortem specimens has never been higher and the rate in finding multiple drugs has also increased (3). Therefore, developing an effective method for simultaneous analysis of these drugs (and their metabolites) is particularly helpful to toxicological investigation of forensic cases.

Gas chromatography–mass spectrometry (GC–MS) methodology has long been used for the analysis of opiates (4), methamphetamine (5, 6), ketamine (7, 8) and related drugs. However, simultaneous analysis of these drugs and their metabolites by GC–MS is challenging because it is difficult to develop a "single extraction and chemical derivatization protocol" that could work optimally for all analytes (9, 10). Under certain circumstances, e.g., the presence of certain interference substances at a high concentration level, GC–MS-based methodologies could reportedly lead to the misidentification of amphetamines (11, 12).

Recent advances in the LC–MS-MS technology have clearly demonstrated that LC–MS-MS-based approach can now be more effectively applied to simultaneous analysis of multiple drugs (13, 14). This approach can significantly simplify the sample preparation process and provide higher selectivity (15). Advantages of LC–MS-MS (over GC–MS methodologies) included higher sensitivity (allowing lower cutoffs), no need of chemical derivatization (under most circumstances), and more suitable for simultaneous analysis of multiple drugs (14, 16, 17). Specific examples included the applications of LC–MS-MS approaches to the quantifications of (i) opioids in plasma (18); (ii) basic drugs in oral fluid—using solid-phase extraction (SPE) for sample preparation (19); (iii) ketamine and norketamine—following SPE extraction from urine (20); (iv) amphetamine and related amine drugs in urine (21) and (v) amphetamine, opiates, ketamine and their metabolites in urine (14).

In our laboratory, two separate GC–MS-based protocols have long been used for the analysis of opiate and amphetamine drug categories, while a liquid chromatography–tandem mass spectrometry (LC–MS-MS) approach was used for ketamine and it metabolites. Specifically, the GC–MS-based methodology for the analysis of total codeine and total morphine included acid hydrolysis, liquid–liquid extraction (LLE) and TMS-derivation (22–24); while the quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) were conducted by LLE (with a different solvent system) and HFBA-derivatization (25–27). A single protocol capable of simultaneous analysis of these three drug categories is highly desirable. Analytes included in this study were similar (but not identical) to those reported in an earlier report on the analysis of these drugs in urine; (14) however, this current study further advances this approach to (i) the analysis of these analytes in postmortem specimens, including urine and blood; (ii) understanding multiple-drug use-patterns and (iii) potential differentiation of opiates (and their metabolites) derived from legal and illicit sources.

Materials and Methods

Sources of standards, internal standards, reagents and case specimens

Amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine, norketamine, amphetamine-d_8, methamphetamine-d_8, MDA-d_5, MDMA-d_5, morphine-d_6, 6-acetylmorphine-d_6, codeine-d_6, 6-acetylcodeine-d_3, ketamine-d_4 and norketamine-d_4 were purchased from Cerilliant (Round Rock, TX, USA); 6-acetylcodeine-d_3 was from Lipomed AG (Arlesheim, Switzerland). Methanol, ethyl acetate, ammonium hydroxide, acetonitrile were all LC grade and were obtained from J. T. Baker (Phillipsburg, NJ, USA). Deionized water was produced by a PURELAB^{TM} Ultra water purification system from ELGA LabWater VWS Ltd. (Bucks, UK). ACCUBOND EVIDEX cartridges (200 mg, 3 mL) were purchased from Agilent Technologies (Wokingham, UK). Hydrochloric acid and potassium dihydrogen phosphate were from E. Merck (Darmstadt, Germany); potassium hydroxide was from Wako Pure Chemical Industries and formic acid was from Sigma-Aldrich (St. Louis, MO, USA).

Blank urine specimens were obtained from laboratory personnel volunteers and confirmed to be free of the 10 analytes. Postmortem specimens were collected from unknown death cases from various Taiwan district prosecutors' offices.

Working standard solutions of standards and internal standards, calibrators, and controls

A mixed working standard solution was prepared (in methanol) to contain 5 μg/mL (each) of the 10 analytes (amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine and norketamine). Similarly, a mixed working internal standard solution was prepared (in methanol) to contain 5 μg/mL each of amphetamine-d_8, methamphetamine-d_8, MDA-d_5, MDMA-d_5, morphine-d_6, 6-acetylmorphine-d_6, codeine-d_6, 6-acetylcodeine-d_3, ketamine-d_4 and norketamine-d_4.

Standards and positive controls were prepared by spiking appropriate volumes of the working standard solution (containing 5 μg/mL of all analytes) to contain the targeted concentrations of all analytes. A series of five standards (50, 125, 250, 500 and 1,000 ng/mL) were prepared for linearity calibration. Positive controls (targeted at 250 ng/mL) were prepared similarly using a working standard solution prepared by a different analyst in the laboratory. When available, standard materials obtained from different sources were used for the preparation of this working standard solution designated for the preparation of controls. Negative controls were drug-free urine or blood samples.

Sample preparation procedure for standards, controls and test specimens

A typical sample preparation procedure (for standards and test specimens) was described as follows. About 50 μL of the deuterated working internal standard solution and 1-mL 0.1-M phosphate buffer (pH 6.0) were added to 1-mL aliquot of sample. After centrifuging at 4,000 rpm for 5 min, the supernatant was transferred to a SPE cartridge using an automatic Biotage/Caliper/Zymark RapidTrace SPE Workstation. ACCUBOND EVIDEX Cartridges were pre-conditioned with a mixture of 1-mL methanol and 1-mL 0.1-M phosphate buffer (pH 6.0). Following the loading of the test specimen, the SPE cartridge was washed successively with 5-mL ultra-pure water, 2-mL 0.01-N HCl and 2-mL methanol; and then dried. The elution was performed with 2-mL ethyl acetate/methanol/ammonium hydroxide (75:25:2 v/v/v). The eluent was evaporated to dryness under a stream of nitrogen at 50°C and reconstituted to 125 μL with the starting mobile phase, i.e., A:B 90:10 (v/v). After centrifuging at 13,000 rpm for 5 min, 100 μL of the supernatant was transferred to a vial, of which 5 μL was injected into the LC–MS-MS

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system. Drying at 50°C did not appear to cause the loss of analytes, such as amphetamine.

When the quantification result of an analyte in a specimen was found higher than the highest calibration standard (1,000 ng/mL), the specimen was diluted and reanalyzed.

Blood specimens were deproteinized by the addition of 1-mL acetonitrile, followed by centrifugation at 4,000 rpm for 15 min. Supernatants were then processed with the SPE extraction, following the same scheme applied to urine samples.

Instrumentation and experimental conditions

A Biotage/Caliper/Zymark RapidTrace SPE Workstation was used for the sample preparation process. LC–MS-MS analysis was performed on an Agilent 1200 infinity LC system coupled with an Agilent 6410 Triple Quadrupole spectrometer (Agilent, Palo Alto, CA, USA).

Chromatographic separation was performed on an Agilent Zorbax SB-Aq (100 mm × 2.1 mm, 1.8 μm particle) analytical column. The flow rate was 0.32 mL/min with a mobile phase consisting of 0.1% formic acid in water (solvent A) and methanol (solvent B). The gradient program was: 0–1.5 min, 90% A; 1.5–7.0 min, 90–0% A; 7.0–8.0 min, 0% A; 8.0–9.0 min, 0–90% A; 9.0–10.0 min, 90% A. Column temperature was maintained at 50°C during the analysis and the injection volume was 5 μL. The total run time was 10 min.

Ionization was achieved using an electrospray ionization source in positive mode. Operation parameters were set as follows: gas temperature, 350°C; drying gas, 10 L/min; nebulizer pressure, 40 psi; capillary voltage, 4,000 V; precursor isolation width, 4 amu; spectra acquired, MS1 and MS2; Vcap, −3,500 V; Vend, −500 V; capillary exit lens, 130.0 V; skimmer1, 40.0 V; lens1, −5.0 V; lens2, −60.0 V; cycle time, 500 ms. The acquisition mode for detection and quantitation was performed in dynamic multiple reaction monitoring mode (DMRM), with optimized collision energy for each selected precursor ion and the monitoring of two transition ions for each analyte. The transitions and MS-MS conditions for each analyte and internal standard are summarized in Table I.

Method evaluation

Deuterated analogs of the analytes, including amphetamine-d_8, methamphetamine-d_8, MDA-d_5, MDMA-d_5, morphine-d_6, codeine-

Table I. Transitions and MS-MS conditions for the 10 analytes and their internal standards

Compound	Retention time (min)	Precursor ion (m/z)	Fragment (V)	Target ion (m/z)	Collision energy (V)	Qualifier ion (m/z)	Collision energy (V)
Amphetamine	2.13	136.1	77	119	4	91	16
Amphetamine-d_8	2.08	144.2	82	127.1	4	97.1	16
Methamphetamine	2.66	150.1	87	119	8	91	16
Methamphetamine-d_8	2.61	158.2	92	124.1	8	93	20
MDA	3.09	180.1	77	163	4	105	20
MDA-d_5	3.05	185.1	82	168	8	110.1	24
MDMA	3.92	194.1	92	163	8	105	24
MDMA-d_5	3.88	199.1	97	165	8	107	24
Morphine	1.45	286.2	166	181	36	165.1	44
Morphine-d_6	1.44	292.2	166	181	36	153.1	48
Codeine	3.23	300.2	166	215	24	165	48
Codeine-d_6	3.15	306.2	171	218.1	24	165	52
6-Acetylmorphine	4.86	328.2	166	211.1	24	165.1	36
6-Acetylmorphine-d_6	4.77	334.2	171	211.1	24	165	48
6-Acetylcodeine	6.9	342.2	161	225	24	165	60
6-Acetylcodeine-d_3	6.9	345.2	162	225.1	28	165.1	60
Ketamine	6.49	238	102	125	29	115	60
Ketamine-d_4	6.47	242.1	114	224.1	12	129	28
Norketamine	6.2	224	92	207	5	125	25
Norketamine-d_4	6.16	228.1	102	211	8	129	28

Table II. SPE recovery (%) of analytes from urine ($n = 3$)

Analyte	Conc. (ng/mL)				
	50	125	250	500	1,000
Amphetamine	90.03 ± 4.01	89.35 ± 5.32	82.68 ± 1.10	84.21 ± 2.16	83.11 ± 3.76
Methamphetamine	84.20 ± 4.66	82.84 ± 6.49	79.40 ± 1.82	79.52 ± 1.99	79.31 ± 4.23
MDA	90.30 ± 4.64	84.76 ± 1.51	82.30 ± 1.80	83.62 ± 2.17	83.38 ± 2.63
MDMA	82.88 ± 4.68	80.52 ± 6.06	77.45 ± 1.49	79.09 ± 1.92	77.52 ± 4.89
Morphine	78.53 ± 5.74	74.27 ± 1.41	71.06 ± 2.09	71.91 ± 1.77	72.71 ± 5.39
6-Acetylmorphine	90.39 ± 2.81	88.40 ± 1.39	86.19 ± 2.13	85.07 ± 1.41	83.87 ± 2.67
Codeine	89.29 ± 3.26	84.48 ± 1.50	82.44 ± 2.01	83.72 ± 2.63	82.12 ± 2.55
6-Acetylcodeine	99.60 ± 1.64	89.22 ± 1.63	85.21 ± 1.90	88.76 ± 4.20	86.12 ± 2.47
Ketamine	91.61 ± 4.09	87.25 ± 2.38	86.25 ± 3.05	88.29 ± 3.66	86.98 ± 2.24
Norketamine	84.93 ± 4.36	79.51 ± 2.91	79.81 ± 3.83	81.50 ± 3.64	79.58 ± 3.76

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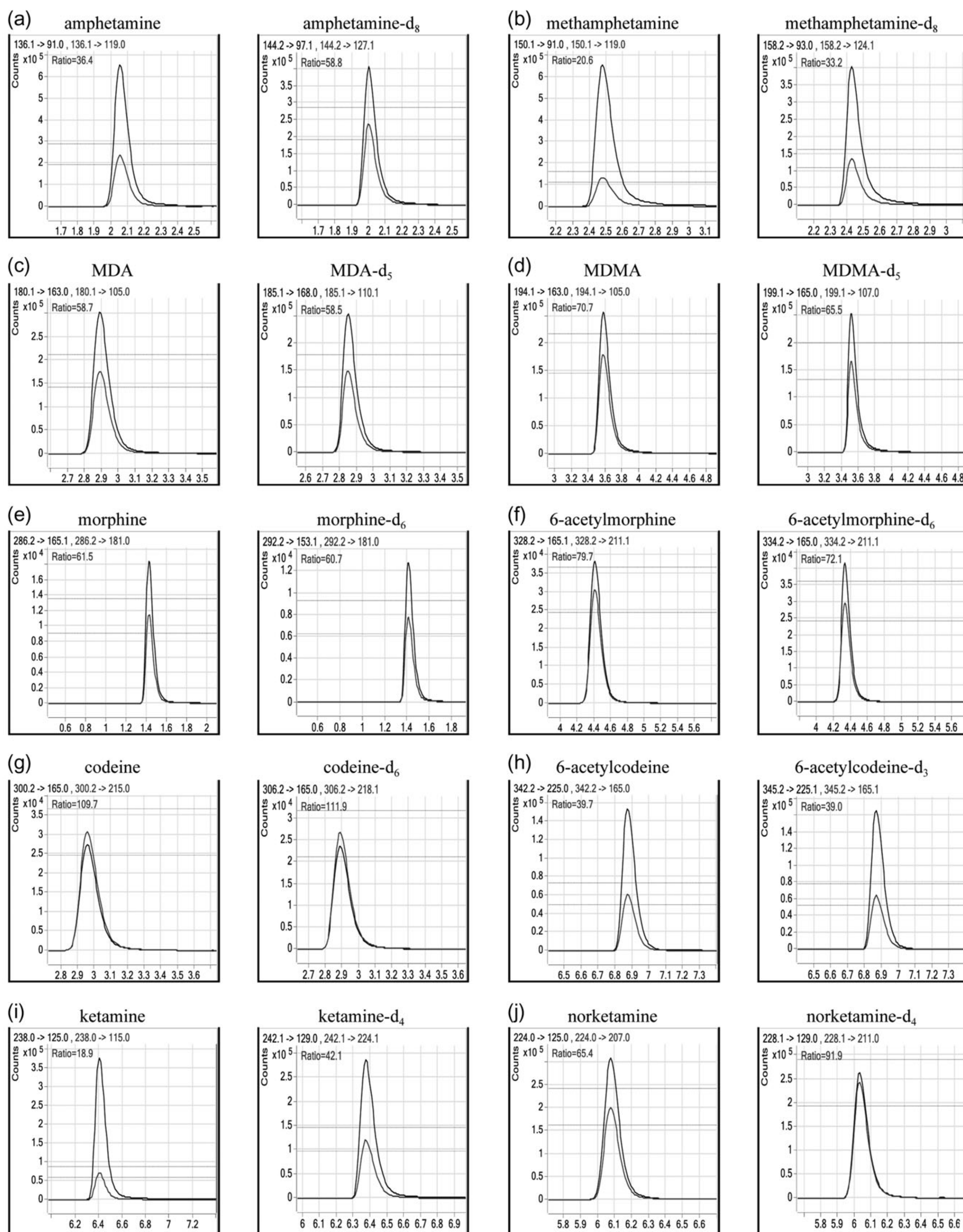

Figure 1. DMRM chromatograms of analyte/internal standard pairs containing 5,000 ng/mL of potential interference compounds and 250 ng/mL of analytes: (a) amphetamine/amphetamine-d_8, (b) methamphetamine/methamphetamine-d_8, (c) MDA/MDA-d_5, (d) MDMA/MDMA-d_5, (e) morphine/morphine-d_6, (f) 6-acetylmorphine/6-acetylmorphine-d_6, (g) codeine/codeine-d_6, (h) 6-acetylcodeine/6-acetylcodeine-d_3, (i) ketamine/ketamine-d_4 and (j) norketamine/norketamine-d_4.

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d_6, 6-acetyl-morphine-d_6, 6-acetyl-codeine-d_3, ketamine-d_4 and norketamine-d_4, were used as the internal standards for method evaluation. Analytical parameters assessed included: extraction recovery, calibration linearity (r^2), inter-day and intra-day accuracy and precision, limit of detection (LOD) and limit of quantitation (LOQ), and matrix effect. The analytical protocol was then used for the analysis of samples prepared with known analytes concentrations and case specimens.

Recovery

Recoveries of these analytes were estimated at five concentration levels (50, 125, 250, 500, 1,000 ng/mL). Recovery rates were calculated by comparing the peak areas of the analytes derived from the following two sets of experiments: (i) Samples in the first set were drug-free urine spiked with the analytes (or their ISs) prior to the sample preparation steps. (ii) Samples for the second set were prepared identically using the initial gradient LC–MS mobile phase as the solvent. Samples in this latter set were subject to LC–MS-MS analysis without going though the SPE step. Each experiment was performed with three replicates.

Precision and accuracy

Precision and accuracy were evaluated at three concentration levels (50, 250 and 1,000 ng/mL). Intra- and inter-day precisions were assessed with five determinations (for each concentration) conducted in one (for intra-day assessment) and 5 consecutive days (inter-day assessment).

Calibration, LOD and LOQ and QC

A calibration curve for each analyte was prepared by the analysis of the five calibrators containing the following concentrations of all analytes: 50, 125, 250, 500 and 1,000 ng/mL. Analyte/IS peak area ratios were calculated by using Mass Hunter software (Agilent). Data were fit to a least-square linear regression curve with a 1/× weighting factor and not forced through the origin. The LOD was determined by analyzing a serial of standard solutions with decreasing concentrations of the analytes (with 1, 5 and 10 ng/mL at the low end), and defined as the lowest concentration at which the acceptable criteria for the identification of a analyte were met, i.e., (i) the chromatographic peak shape was acceptable and (ii) quantitation/qualifier transition ion ratio was comparable to the 250 ng/mL calibration standard (within ± 20%). The LOQ was defined as the lowest concentration (of the analyte) at which (i) the LOD criteria were met and (ii) the observed analyte concentration value was within ±20% of the expected value.

Test results of a control samples were considered acceptable if: (i) the chromatographic peak shape was acceptable; (ii) quantification/qualifier transition ion ratio was comparable to the 250 ng/mL calibration standard (within ±20%); and (iii) the observed analyte concentration value was within ±20% of its expected concentration.

Evaluation of interference and matrix effect

Three categories of compounds adopted for interference studies included phentermine, pseudoephedrine, ephedrine, phenylpropanolamine, PMA, PMMA, PMEA, 4-fluoroamphetamine, 4-chloroamphetamine, methylone, butylone, pentylone, ethylone, MDPV, mephedrone, methedrone, eutylone (analogs of amphetamines); hydromorphone, hydrocodone, oxymorphone, oxycodone, normorphine, norcodeine, fentanyl, norfentanyl, buprenorphine, norbuprenorphine (isomers or analogs of opiates or opiate-like compounds); and 4-chloro-αPPP, 4-chloro-αPVP, PCP (analogs of ketamine). These compounds (at the 5,000 ng/mL level) were added to 250 ng/mL standard and studied.

Ion suppression or enhancement (expressed as percentage) was assessed by a post-column infusion system. The system used an infusion syringe pump to permanently add monitoring analyte standards to the LC column eluent via a mixing tee (28, 29). Drug-free samples from five different resources were extracted and injected into the LC–MS-MS system and infused with a calibration standard (250 ng/mL). Matrix effect was evaluated by comparing the relative chromatographic intensity before (set A) and after (set B) the infusion of the calibration standard. The peak areas (for each analyte) derived from Set A and Set B were used for calculation using the following equation (29, 30):

$$[1 - (A - B)/A] \times 100\% \quad (1)$$

Results and Discussion

Method evaluation

Extraction recovery and interference studies

Recovery data for all analytes, from the SPE protocol, at five concentration levels (50, 125, 250, 500, and 1,000 ng/mL) of urine standard solutions are summarized in Table II. The average extraction recovery (n = 3) was above 80%, but slightly lower for MDMA (77%) and morphine (71%).

Table III. Intra- and inter-day precision and accuracy data as presented in % ($n = 5$)

Analyte	Intra-day						Inter-day					
	Precision (CV)			Accuracy (bias)			Precision (CV)			Accuracy (bias)		
	50	250	1,000	50	250	1,000	50	250	1,000	50	250	1,000
Amphetamine	2.00	1.85	1.43	1.3	−1.8	−0.5	2.95	5.77	6.83	−0.2	−0.3	−1.1
Methamphetamine	2.56	1.85	1.54	5.6	−2.8	−0.2	4.88	7.68	8.80	0.1	−2.0	−0.4
MDA	3.86	2.26	2.21	3.9	−3.7	0	2.61	5.32	6.23	−0.2	−1.3	−0.8
MDMA	2.99	1.37	1.29	4.6	−3.8	0.4	3.50	7.31	8.29	1.4	−2.6	−0.3
Morphine	2.48	1.57	0.80	5.8	−4.5	0.5	3.34	7.19	7.95	2.4	−2.6	−0.3
6-Acetylmorphine	2.91	2.26	1.12	4.5	−4.1	0.3	1.59	4.15	4.60	1.2	−2.4	−0.1
Codeine	3.89	2.68	0.92	3.9	−3.2	−0.4	2.30	4.04	5.47	1.0	−1.5	−0.2
6-Acetylcodeine	2.84	2.53	1.62	5.9	−4.2	0.4	3.09	6.63	8.47	2.0	−2.5	−1.0
Ketamine	2.48	1.85	0.57	1.7	−2.6	−0.3	2.89	5.64	7.02	2.2	−2.8	0.2
Norketamine	2.14	1.25	0.73	0.9	−2.1	−0.7	3.34	5.77	6.61	3.7	−2.4	−0.1

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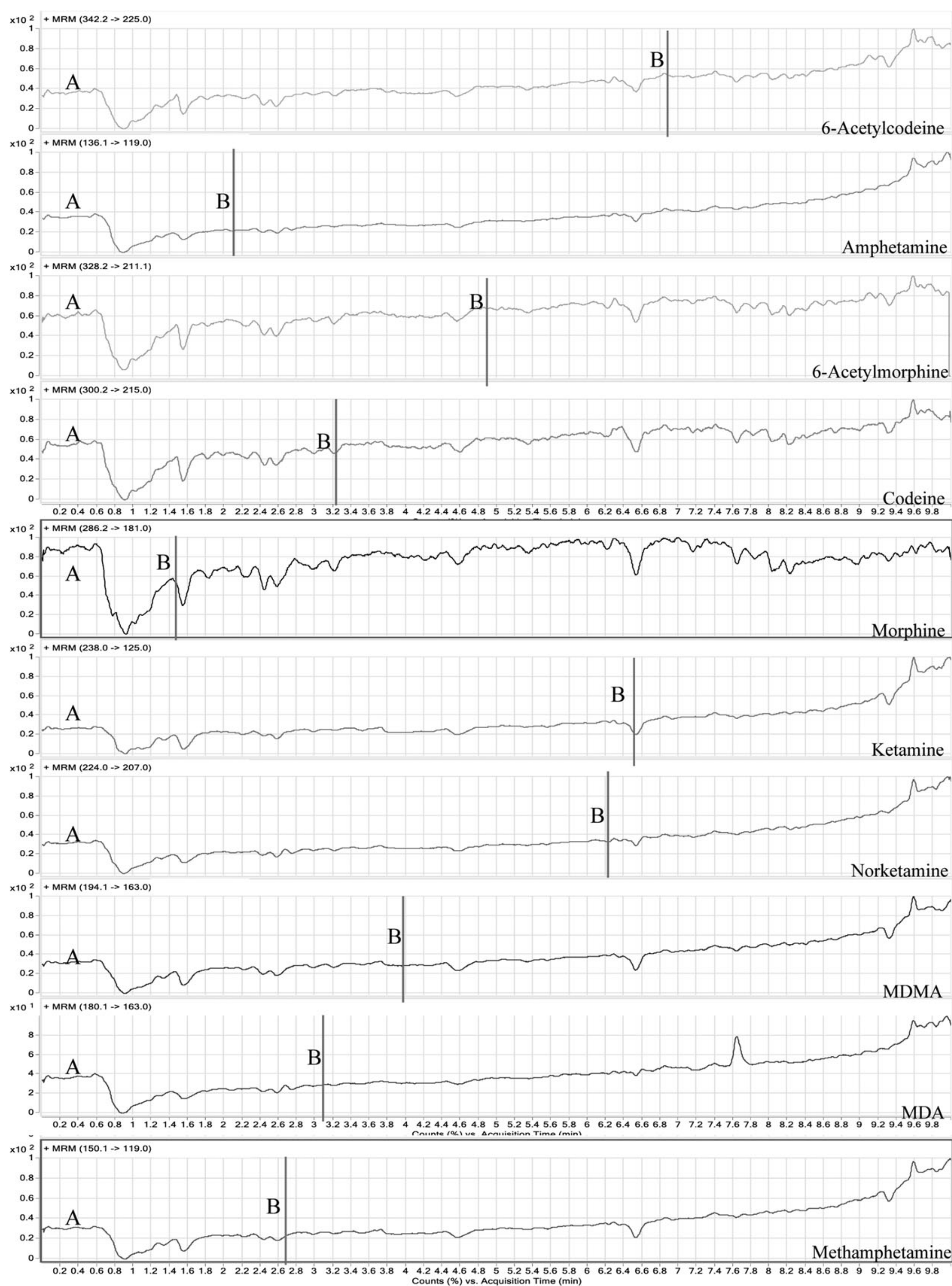

Figure 2. The liquid chromatographic relative intensity profiles of opiates, amphetamines, ketamine and norketamine in urine showing the matrix effects. The A's and B's represent the chromatographic relative intensity profiles before and after, respectively, the post-column infusion of standards (mixture of analytes) with the chromatographic eluent.

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As shown in Figure 1, the presence of these three categories of compounds (5,000 ng/mL), that may potentially cause interference, did not appear to affect the analysis of the 10 analytes using the protocols adopted by this study.

Calibration linearity (r^2), intra- and inter-day accuracy and precision and LOD and LOQ

Calibration studies were performed at the 50–1,000 ng/mL range. The resulting calibration linearity (r^2), intra- and inter-day accuracy and precision data are summarized in Tables III. These data indicate: (i) the coefficients of determination (r^2) of all analytes exceed 0.999; (ii) the intra-day and inter-day precisions are 0.57–3.89% and 1.59–8.80%, respectively; and (iii) the intra-day and inter-day accuracies are −4.5–5.9% and −2.8–3.7%, respectively. These data are considered acceptable (CV% < 15%).

The LOD and the LOQ were found to be 1 and 5 ng/mL, respectively, for the simultaneous determination of all analytes. These parameters were taken based on the criteria adopted for defining LOD and LOQ; it is debatable whether 5 ng/mL is truly a valid LOQ value, considering that the concentration of the lowest calibrator is 50 ng/mL and no control at a lower concentration level was included in each analytical batch.

Matrix effect

The black lines in Figure 2 indicate the respective retention times of the analytes. "A" components in Figure 2 are the liquid chromatographic relative intensity profiles of the post-column infusion of the mixed analytes before mixing with the chromatographic eluent. The "B" components represent the overall DMRM intensity profiles after the mixing. A > B is an indication of ion suppression; while A < B is an indication of ion enhancement (30).

Chromatograms shown in Figure 2 and data derived from Equation (1) were used to assess potential matrix effect that may impact the analysis of these analytes spiked into urine matrix from five different sources: (i) no or minimal matrix effect was found for MDA (83.2%), MDMA (90.0%), codeine (81.3%), 6-acetylmorphine (101.8%), 6-acetylcodeine (97.9%), ketamine (86.1%) and norketamine (96.5%) and (ii) ion suppression was observed for amphetamine (70.7%), methamphetamine (77.2%) and morphine (60.6%). However, matrix effect found for the latter three analytes appeared to be satisfactorily compensated for by using their respective deuterated analogs as internal standards.

Application to the analysis of standards, controls and case specimens

Urine and blood quality control (QC) samples, containing 250 ng/mL of each of the 10 analytes, were quantified using the urine calibration curve. The analytical accuracy data (Table IV) indicated better than 97% accuracies in urine and 87% in blood. However, more thorough validation parameters for blood samples are yet to be conducted.

The proposed analytical scheme was applied to the analysis of unknown death case urine specimens submitted by all district prosecutors' offices in Taiwan during a 2-month period. Out of a total of 531 specimens analyzed, 34 specimens were found to be positive for one or more of the 10 analytes. Analytical data summarized in Table V indicate morphine as the most often detected analyte (appearing in 24 specimens), while 6-acetylmorphine and 6-acetylcodeine in 15 and 16 specimens, respectively. Since these specimens have not been analyzed by the GC–MS methodologies previously used for the analysis of these compounds, analytical findings derived from GC–MS and LC–MS-MS could not be directly compared. However, one of our earlier studies (31) did present direct comparisons and demonstrated that the LC–MS-MS approach was significantly more effective in detecting the presence of drugs and their metabolites.

Analytical findings of case specimens are indicatives of multiple-drug use and combination pattern. Examination of the raw data summarized in Table V also revealed the following combination patterns and frequencies: (i) amphetamine/opiate combination in eight specimens; (ii) amphetamine/ketamine combination in two specimens; (iii) ketamine/opiate combination in two specimens and (iv) amphetamines/opiates/ketamine combination in two specimens. These multiple-drug use data will be statistically more meaningful when test results from additional case specimens are available.

In Taiwan, there has been concern on death resulting from the congestion of products derived from legal medications containing components such as morphine or codeine (24, 32). Simultaneous detection of 6-acetylmorphine and/or 6-acetylcodeine, in addition to morphine and/or codeine, in urine was often interpreted as heroin-related death—bearing in mind that 6-acetylmorphine is a metabolite of heroin while some illicit heroin contains 6-acetylcodeine as an impurity (33). On the other hand, if only morphine or/and codeine was/were found, the death would most likely be associated with the use of legal medication or products thereby derived. For opiate-positive postmortem urine specimens, our test data revealed: (i) only morphine and/or codeine were found in seven specimens; (ii) 6-acetylmorphine was found along with morphine and/or codeine in two specimens; (iii) 6-acetylcodeine was found along with morphine

Table IV. Analytical accuracies for 250 ng/mL urine and blood controls using urine calibration curve ($n = 3$)

Analyte	Urine			Blood		
	Mean ± SD	Accuracy	CV (%)	Mean ± SD	Accuracy	CV (%)
Amphetamine	248.94 ± 4.85	99.58	1.95	238.19 ± 7.21	95.28	3.03
Methamphetamine	250.77 ± 8.36	100.31	3.34	243.33 ± 22.44	97.33	9.22
MDA	246.72 ± 2.97	98.69	1.21	226.59 ± 5.20	90.63	2.29
MDMA	244.02 ± 3.49	97.61	1.43	217.41 ± 6.82	86.97	3.14
Morphine	249.16 ± 11.39	99.66	4.57	219.48 ± 16.11	87.79	7.34
Codeine	246.35 ± 5.82	98.54	2.36	218.55 ± 19.79	87.42	9.05
6-Acetylmorphine	252.50 ± 15.09	101.00	5.97	237.02 ± 29.00	94.81	12.24
6-Acetylcodeine	249.68 ± 11.08	99.87	4.44	247.39 ± 45.20	98.96	18.27
Ketamine	246.42 ± 3.89	98.57	1.58	226.27 ± 2.02	90.51	0.89
Norketamine	243.53 ± 3.67	97.41	1.51	226.40 ± 2.72	90.56	1.20

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Table V. Frequency and concentration of analytes found in 34 positive postmortem urine specimens

Analyte	Frequency	Concentration range (ng/mL)	Mean (ng/mL)
Amphetamine	13	7.71–585	198
Methamphetamine	13	5.36–10,299	1,872
MDA	1	2,438	
MDMA	2	7.16–43,834	21,921
Morphine	24	11.9–79,688	4,345
Codeine	20	5.49–803	249
6-Acetylmorphine	15	12.8–547	195
6-Acetylcodeine	16	5.00–193	36.5
Ketamine	11	5.07–23,031	2,982
Norketamine	9	5.87–8,341	1,629

and/or codeine in three specimens and (iv) both 6-acetylmorphine and 6-acetylcodeine were found along with morphine and/or codeine in 13 specimens. Quantitative data of 6-acetylmorphine, 6-acetylcodeine, morphine and codeine are helpful to potential differentiation of the source of opiates found in case specimens.

Conclusions

The LC–MS-MS approach hereby developed for the simultaneous determination of amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine and norketamine has been found effective and significantly less labor-intensive. This study also demonstrates this analytical scheme can be successfully applied to routine identifications and quantifications of the 10 commonly encountered analytes in postmortem urine specimens. Data derived from limited applications of this method to the analysis of blood specimens are encouraging; however, more thorough validation steps are needed. Simultaneous determination of morphine, codeine, 6-acetylmorphine and 6-acetylcodeine is also helpful to understanding multiple-drug use-patterns and whether the observed opiates came from heroin or legal morphine/codeine-containing medications.

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