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RESEARCH ARTICLE

Rapid analysis of drugs of abuse and their metabolites in human urine using dilute and shoot liquid chromatography–tandem mass spectrometry

Tae Yeon Kong[1] · Ju Hyun Kim[1] · Jin Young Kim[2] · Moon Kyo In[2] · Kyung Ho Choi[3] · Hee Seung Kim[2] · Hye Suk Lee[1]

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Abstract Liquid chromatography-tandem mass spectrometric method for analysis of 113 abuse drugs and their metabolites in human urine was developed and validated. A simple sample clean-up procedure using the "dilute and shoot" approach, followed by reversed phase separation, provided a fast and reliable method for routine analysis. Drugs were separated in a Capcell Pak MG-III C_{18} column using a gradient elution of 1 mM ammonium formate with 0.1% formic acid in water and acetonitrile. The total time for analysis was 32 min. The multiple reaction monitoring mode using two transitions (e.g., quantifier and qualifier) was optimized for both identification and determination. The calibration curves for each analyte were linear over the concentration ranges of 1–100, 5–100, or 10–100 ng/mL using 400 μL of human urine sample with the coefficient of determination above 0.9921. The coefficient of variation and accuracy for the intra- and inter-assays of the tested drugs at three QC levels were 1.1–14.6 and 86.7–106.8%, respectively. The present method was successfully applied to the analysis of forensic urine samples obtained from 17 drug abusers. This method is useful for the rapid and accurate determination of multiple drug abuse with a small amount of urine in forensic and clinical toxicology.

Keywords Drugs of abuse · Psychoactive substances · Human urine · LC–MS/MS · Forensic toxicology

✉ Hee Seung Kim
hskjay@spo.go.kr

✉ Hye Suk Lee
sianalee@catholic.ac.kr

[1] Drug Metabolism and Bioanalysis Laboratory, College of Pharmacy, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon, Gyeonggi 14662, Republic of Korea

[2] Forensic Chemistry Laboratory, Forensic Science Division, Supreme Prosecutor's Office, 157 Banpo-daero, Seocho-gu, Seoul 06590, Republic of Korea

[3] Department of Emergency Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul 11765, Republic of Korea

Introduction

Classical illicit drugs such as amphetamine/methamphetamine, cannabis, cocaine, and opioid related substances have been frequently used as psychoactive drugs and have been the main focus of forensic toxicological laboratories. In recent years, new psychoactive substances, also known as 'legal highs', 'designer drugs', or 'bath salts', with the same biological targets as the classically illicit drugs, have become a global issue via their continual emergence on the recreational and illicit drug markets (UNODC 2013). Psychoactive substances including synthetic cannabinoids, synthetic cathinones, substituted phenethylamines, piperazines, tryptamines, piperidines, benzodiazepines, zolipdem, ketamine, and related substances, in addition to classical substances such as ethanol, cannabis, cocaine, and amphetamines, have been detected in both criminal and postmortem caseworks (Hill and Thomas 2011; Elliott and Evans 2014; Helander et al. 2014).

There has been a steady increase in new psychoactive substances and nonmedical uses of psychoactive substances in the population. As a result, there has been a rapid development of analytical methods for the screening and simultaneous determination of chemically different psychoactive substances in both human blood and urine samples. Of particular note, there have been advances in analytical instrumentation, such as changing to liquid chromatography-tandem mass spectrometry (LC–MS/MS) and high

resolution mass spectrometry (LC-HRMS) systems (Dresen et al. 2010; Favretto et al. 2013; Li et al. 2013; Tsai et al. 2013; Paul et al. 2014; Remane et al. 2014; Sempio et al. 2014; Shin et al. 2014; Tang et al. 2014; Concheiro et al. 2015; Adamowicz and Tokarczyk 2016) from traditional gas chromatography-mass spectrometry (GC–MS) systems. In addition, sample clean-up procedures such as solid-phase extraction (SPE) (Tang et al. 2014; Concheiro et al. 2015) are now used over protein precipitation (PPT) (Sempio et al. 2014; Adamowicz and Tokarczyk 2016) and liquid–liquid extraction (LLE) (Paul et al. 2014).

There are several considerations in selecting sample clean-up procedures for screening and confirmatory analysis for drugs of abuse. These include drug characteristics such as pH and hydrophobicity, as well as sample handling cost and time, and analytical purpose (i.e., identification vs. quantitation). Traditionally, an immunoassay followed by GC–MS analysis constituted the analytical methods used for screening and confirmatory analysis of drugs of abuse (Lachenmeier et al. 2006; von Mach et al. 2007). There is no doubt that LC–MS/MS is the most common method of confirmatory analysis (Kintz et al. 2005; Deveaux et al. 2008) and is increasingly used for screening applications in drugs of abuse.

For screening and quantitative applications of drugs of abuse, there are a few methods that are widely accepted. First, there is the targeted analysis, which uses a triple quadrupole mass analyzer in multiple reaction-monitoring (MRM) mode (i.e., multi-target screening). Second, there is a non-targeted analysis (i.e., general unknown screening), which is based on time-of-flight. Finally, there is the use of an orbitrap mass analyzer operated in high resolution accurate MS/MS (HRAM) mode (Mueller et al. 2005). There are pros and cons in using each mode. For example, MRM-based methodology is very fast, simple, and highly sensitive for the quantitative identification of targeted compounds, but is limited to only known compounds. On the other hand, HRAM mode, which is used in non-targeted analysis is highly attractive for investigating new or unknown drugs, but is generally more complicated, time consuming, and less sensitive for detecting compounds at levels as low as those that can be detected using MRM mode.

The purpose of the present study was to provide a general strategy or guidance for a fast, simple, selective, sensitive, and simultaneous screening and confirmatory analysis of 113 illicit drugs and their metabolites using LC–MS/MS operated in MRM mode. These drugs include 20 benzodiazepines and their 6 metabolites, 24 synthetic cannabinoids, 31 phenethylamines, cocaine and its two metabolites, 12 synthetic opioids, 7 piperazines, and 10 others. Human urine was chosen as the sample matrix in this study because these samples may contain both parent drug and metabolites, and can be collected non-invasively (Brown and Melton 2011). In addition, urine is widely accepted as the primary choice for forensic purposes (Humbert et al. 2014).

Materials and methods

Chemicals and reagents

Reference standards of 113 analytes and 13 internal standards (Table 1) were obtained from Cayman Chemical Company (Ann Arbor, MI, USA), Cerilliant (Austin, TX, USA), and Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol (HPLC-grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Deionized water was prepared using the Direct-Q water purification system (Millipore, Gedford, MA, USA). Ammonium formate and formic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Drug-free urine samples were obtained from members of our laboratory staff.

Preparation of calibration standards and quality control samples

Stock solutions (1 mg/mL) of each analyte were prepared in methanol. Mixed stock solution (5 μg/mL) was prepared by adding 5 μL of 113 stock solutions to 435 μL methanol. Working standard mixture solutions of 10, 20, 30, 50, 100, 150, 200, 300, 400, 600, 700, 800, and 1000 ng/mL were prepared in solution by serial dilution of the mixed stock solution (5 μg/mL) with methanol. Internal standard (IS) stock solutions (10–100 μg/mL) were prepared in methanol and a working IS mixture solution (0.1–2 μg/mL) was prepared in methanol by adding individual IS. All solutions were stored at −20 °C.

Human urine calibration standards at concentrations of 1, 2, 5, 10, 20, 40, 60, 80, and 100 ng/mL were prepared by adding 40 μL of the working standard solutions (10, 20, 50, 100, 200, 400, 600, 800 and 1000 ng/mL) to 360 μL of drug-free human urine. The quality control (QC) samples were prepared at 3 (low 1 QC), 15 (low 2 QC), 30 (low 3 QC), and 70 (high QC) ng/mL by combining 40 μL of the appropriate working standard mixture solutions (30, 150, 300, and 700 ng/mL) with 360 μL of drug-free human urine. QC samples were stored at −80 °C until further analysis.

Sample preparation

Four hundred microliter of human blank urine, calibration standards, and QC samples were vortex-mixed with 40 μL of IS mixture solution for 3 min at high speed. After centrifugation at 50,000*g* and 4 °C for 5 min, 5 μL of the supernatant was injected into LC–MS/MS.

Table 1 Retention times, MRM transitions, and MS parameters of 113 drugs of abuse and 13 internal standards

Compounds	t_R	Precursor ($[M+H]^+$)				Quantifier		Qualifier		Figure 1 no.	I.S.
	(min)	(*m*/*z*)	DP	EP	CEP	(*m*/*z*)	CE	(*m*/*z*)	CE		no.
Benzodiazepines											
Alprazolam	13.9	309.0	70	10	22	281.0	36	205.3	60	81	10
7- Aminoclonazepam	8.7	286.0	63	5	16	121.1	40	222.0	13	44	7
7-Aminonitrazepam	4.3	252.1	56	9	14	121.1	36	94.1	53	7	10
Bromazepam	12.0	316.0	60	10	22	182.1	43	209.1	36	70	1
Chlordiazepoxide	9.9	300.2	40	5	16	282.2	32	227.0	34	58	5
Clobazam	15.1	301.1	40	10	18	224.4	44	259.2	28	90	10
Clonazepam	14.0	316.1	70	4	23	270.1	36	214.1	52	82	11
Delorazepam	15.0	305.0	65	4	19	140.2	43	206.2	50	89	2
Diazepam	16.0	285.1	63	6	16	193.1	42	154.1	37	92	10
Estazolam	13.6	295.2	60	10	16	267.1	28	205.1	51	76	5
Flunitrazepam	14.8	314.1	60	4	24	268.1	34	239.1	46	87	7
Flurazepam	10.5	388.1	55	6	18	315.1	32	134.3	74	65	10
α-Hydroxyalprazolam	13.0	325.1	64	4	24	297.0	34	216.1	57	73	7
α-Hydroxymidazolam	10.7	342.0	70	4	24	324.1	34	203.1	35	66	10
α-Hydroxytriazolam	13.0	359.2	60	4	18	331.0	34	239.2	59	74	1
Lorazepam	13.8	321.1	50	4	20	275.2	28	229.1	38	79	1
Lormetazepam	15.2	335.1	48	4	21	289.0	29	177.0	59	91	10
Midazolam	10.5	326.1	70	3	20	291.2	38	249.0	50	64	10
N-desmethylclobazam	13.8	287.0	40	10	14	245.2	28	210.3	41	80	10
N-desmethyl-flunitrazepam	13.6	300.1	46	5	20	254.1	33	198.1	43	77	8
Nitrazepam	13.6	282.0	60	10	15	236.1	30	180.1	50	78	10
Nordiazepam	14.5	271.1	60	6	17	140.1	37	165.3	40	85	10
Oxazepam	13.5	287.0	45	4	16	241.1	32	104.2	50	75	10
Prazepam	17.7	325.2	60	4	20	271.2	34	140.3	54	100	12
Temazepam	14.8	301.1	45	4	20	177.1	52	193.1	46	88	1
Triazolam	14.1	343.1	70	4	23	239.1	53	308.1	35	83	10
Synthetic cannabinoids											
AB-005	12.3	353.1	64	4	22	98.1	53	112.1	33	71	12
AKB-48 *N*-pentanoic acid	18.2	396.1	56	6	28	135.1	33	93.1	78	102	2
AKB-48 *N*-(5-fluoropentyl)	20.7	384.2	56	6	20	93.3	70	135.1	32	113	2
AM-2201	18.8	360.2	66	7	24	127.3	70	155.2	35	106	2
AM-2201 *N*-(4-hydroxypentyl)	17.0	376.2	66	7	24	127.2	75	155.2	36	95	2
AM-694	18.3	436.0	71	9	28	231.0	37	203.2	59	104	2
5-F-AKB48 *N*-(4-hydroxypentyl)	18.4	400.2	51	6	22	135.1	31	93.1	75	105	2
5-F-SDB-006	17.2	339.1	64	4	24	91.1	63	232.1	33	96	2
JWH-018 *N*-pentanoic acid	16.8	372.2	69	6	24	155.1	33	127.0	72	94	12
JWH-019 *N*-(6-hydroxyhexyl)	17.6	372.1	54	7	23	155.1	31	127.1	71	99	12
JWH-200	12.4	385.1	49	7	20	155.1	30	144.1	37	72	13
JWH-200 5-hydroxy-indole	10.4	401.0	66	7	24	155.2	32	114.3	42	63	10
JWH-250	19.2	336.1	51	6	24	65.2	110	91.2	66	109	2
JWH-250 *N*-(5-hydroxypentyl)	16.2	352.1	51	6	18	121.2	30	65.2	105	93	10
MAM-2201	19.2	374.2	76	10	24	141.2	98	115.3	33	108	2
MAM-2201 *N*-(4-hydroxypentyl)	17.5	390.2	76	7	24	169.2	35	141.2	63	98	2
RCS-4	19.1	322.1	66	4	22	135.1	32	77.3	75	107	2
SDB-006	18.2	321.1	66	5	22	91.1	58	214.1	31	103	2
STS-135	19.4	383.1	81	7	26	135.1	43	93.1	74	111	2

Table 1 continued

Compounds	t_R	Precursor ($[M+H]^+$)				Quantifier		Qualifier		Figure 1 no.	I.S.
	(min)	(*m*/*z*)	DP	EP	CEP	(*m*/*z*)	CE	(*m*/*z*)	CE		no.
THJ-2201	19.3	361.1	61	8	26	233.1	26	145.1	49	110	2
UR-144 *N*-(5-hydroxypentyl)	17.9	328.1	61	5	20	125.1	23	230.1	34	101	12
URB-937	14.3	355.1	61	6	22	213.1	32	230.1	20	84	5
XLR-11 *N*-(4-hydroxypentyl)	17.3	346.1	61	5	26	248.1	33	144.1	50	97	2
XLR-12	19.6	352.1	61	5	22	254.1	36	125.1	33	112	2
Phenethylamines											
Amphetamine	4.9	136.1	26	4	10	91.1	23	119.1	12	11	1
6-APB	7.7	176.1	26	6	10	131.1	25	159.1	11	36	1
Benzphetamine	10.1	240.1	46	4	16	91.2	39	119.3	22	60	7
Buphedrone	6.2	178.1	36	6	10	131.1	31	160.1	15	18	9
Butylone	6.9	222.1	36	4	14	174.1	23	204.1	18	25	5
Cathinone	3.4	150.1	31	4	10	132.1	16	117.1	31	4	9
Ephedrine	3.7	166.1	31	5	10	148.2	15	91.2	46	5	5
4-Ethylmethcathinone	8.7	192.1	31	6	12	144.1	43	105.1	35	43	3
Ethylone	5.8	222.1	36	4	12	174.1	26	204.1	14	16	1
4-Fluoroamphetamine	6.3	154.1	26	5	10	109.1	24	137.1	11	20	1
Fenfluramine	9.9	232.2	46	4	16	159.1	32	109.1	57	57	10
MDA	5.6	180.0	21	5	10	163.1	14	105.2	28	14	1
MDEA	7.3	208.1	31	4	12	163.2	15	105.3	34	30	7
MDMA	6.4	194.1	36	5	12	163.1	17	105.1	36	21	1
MDPV	9.0	276.1	56	7	16	135.1	35	126.1	35	47	10
Mephedrone	7.1	178.1	31	5	10	145.1	24	160.1	16	28	3
Mescaline	4.8	212.2	31	4	12	195.2	13	180.1	22	12	1
Methamphetamine	6.4	150.1	31	4	10	91.2	28	119.2	15	17	8
Methoxetamine	8.2	248.2	36	4	12	121.1	40	91.2	62	40	5
Methoxyphenamine	7.6	180.1	31	5	10	149.1	15	121.1	24	34	5
4-Methylephedrine	6.9	180.1	36	4	10	162.1	16	91.2	37	24	7
Methylone	4.5	208.1	31	4	12	160.1	26	132.1	36	8	5
Methylphenidate	8.4	234.2	14	4	14	84.2	35	56.1	68	41	9
N-ethylcathinone	4.7	178.1	36	5	10	160.1	16	130.1	39	9	3
Norephedrine	3.1	152.1	21	4	10	134.1	11	117.1	23	3	1
Pentedrone	7.9	192.1	36	8	12	132.1	23	174.1	15	38	8
Phendimetrazine	5.7	192.1	51	5	12	146.1	33	91.1	50	15	8
Phenmetrazine	5.5	178.1	51	6	10	115.1	38	117.1	28	13	1
Phentermine	6.7	150.1	21	4	12	91.1	32	133.1	13	22	11
PMA	6.2	166.1	16	6	10	149.1	13	121.1	24	19	1
PMMA	6.8	180.1	31	5	10	121.1	26	149.1	15	23	1
Piperazines											
2C-B-BZP	7.1	317.1	41	4	20	231.1	24	201.1	40	27	10
1-Benzylpiperazine	2.3	177.1	46	6	10	91.2	31	65.2	66	1	10
DBZP	8.9	267.1	51	8	16	175.1	23	91.2	46	45	7
1-(4-Fluorobenzyl)-piperazine	2.6	195.1	46	5	12	109.1	32	83.1	61	2	10
mCPP	8.5	197.1	56	10	12	154.1	28	118.1	46	42	5
Phenylpiperazine	4.8	163.1	51	5	10	120.1	26	77.1	56	10	1
TFMPP	9.4	231.2	56	4	14	188.1	29	118.1	55	53	1
Synthetic opioids											
Acetylfentanyl	9.5	323.1	56	4	20	105.1	51	188.1	30	54	5

Table 1 continued

Compounds	t_R	Precursor ($[M+H]^+$)				Quantifier		Qualifier		Figure 1 no.	I.S.
	(min)	(*m/z*)	DP	EP	CEP	(*m/z*)	CE	(*m/z*)	CE		no.
Butyrylfentanyl	11.0	351.1	51	4	22	105.4	58	188.3	33	67	10
Dihydrocodeine	3.8	302.1	66	5	18	199.2	44	128.2	86	6	4
EDDP	11.0	278.1	66	10	20	234.1	42	249.1	36	68	8
Fentanyl	10.4	337.2	61	4	22	188.2	32	105.1	51	62	6
Meperidine	9.0	248.2	51	7	14	174.2	29	220.2	29	48	10
Methadone	11.7	310.1	36	4	20	265.3	21	77.3	78	69	8
Methaqualone	14.5	251.1	71	10	16	91.1	59	132.1	37	86	8
Nalbuphine	7.1	358.2	56	6	24	340.2	33	185.2	50	29	2
Norfentanyl	7.6	233.1	46	4	14	84.2	26	55.3	56	35	1
Norsufentanyl	9.0	277.1	36	6	18	96.2	33	128.1	19	49	10
Pentazocine	9.7	286.1	51	8	16	218.2	325	69.1	43	55	7
Others											
Cocaine	8.9	304.2	46	4	18	105.1	48	182.2	26	46	3
Benzoylecgonine	8.1	290.1	46	4	20	168.2	28	105.2	41	39	13
Cocaethylene	9.8	318.1	51	3	22	196.1	28	82.1	45	56	10
Dextromethorphan	10.2	272.2	66	8	18	171.1	53	128.2	88	61	10
Ketamine	7.6	238.1	36	5	12	125.1	34	207.1	17	33	10
LSD	9.3	324.2	51	4	20	223.2	33	208.2	36	52	8
Norketamine	7.3	224.1	36	4	12	125.1	33	207.1	16	31	7
5-MeO-DALT	9.3	271.1	31	6	16	174.1	25	110.1	20	50	2
2-Oxo-3-hydroxy-LSD	7.0	356.1	51	5	20	237.2	31	222.2	40	26	7
Pemoline	7.8	177.2	36	5	12	106.2	18	79.2	37	37	1
Phencyclidine	9.9	244.2	26	5	14	91.3	45	86.3	20	59	11
Zolpidem	9.3	308.1	70	10	18	263.1	29	325.0	44	51	13
Zolpidem phenyl-4-carboxylic acid	7.5	338.1	70	4	20	265.1	45	219.1	74	32	7
Internal standards											
Amphetamine-d_5	4.8	141.1	26	6	10	93.1	25	124.1	11	IS-1	
AM-2201-d_5	18.9	364.1	71	8	18	155.1	40	127.1	84	IS-2	
Cocaine-d_3	9.0	307.1	46	5	18	185.1	28	105.1	48	IS-3	
Dihydrocodeine-d_6	3.8	308.1	71	10	18	202.1	45	128.1	83	IS-4	
Ephedrine-d_3	3.7	169.1	26	7	10	151.1	15	117.1	27	IS-5	
Fentanyl-d_5	10.4	342.1	56	9	18	105.1	56	188.1	33	IS-6	
MDEA-d_5	7.3	213.1	36	4	12	163.1	21	105.1	39	IS-7	
Methamphetamine-d_8	5.8	158.1	26	8	10	93.1	31	124.1	14	IS-8	
Methylphenidate-d_9	8.4	243.1	41	7	14	93.1	35	61.1	71	IS-9	
Nordiazepam-d_5	14.5	276.1	66	9	20	140.1	43	165.1	40	IS-10	
Phentermine-d_5	6.7	155.1	21	6	10	96.1	35	138.1	16	IS-11	
XLR-11-d_5	19.6	335.1	71	9	16	125.1	33	55.1	60	IS-12	
Zolpidem-d_6	9.3	314.1	76	4	22	235.1	47	65.1	106	IS-13	

t_R retention time, *DP* declustering potential (V), *EP* entrance potential (V), *CEP* collision cell entrance potential (V), *CE* collision energy (V)

LC–MS analysis

The LC–MS/MS system consisted of an Agilent 1200 series (Agilent Technologies, Santa Clara, USA) system coupled with an API 3200 Q Trap triple-quadrupole mass spectrometer (ABSCIEX, Foster city, CA, USA) equipped with a Turbo V Ion Spray source. Separation was performed on a Capcell Pak MG-III C18 (5 μm, 2.0 mm i.d. × 150 mm, Shiseido, Japan) using a gradient elution of 1 mM ammonium formate with 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate of 0.2 mL/min, 10% mobile phase B for 1 min, 10–60% mobile phase B

for 11 min, 60–90% mobile phase B for 4 min, 90% mobile phase B for 9 min, 90–10% mobile phase B for 1 min, and 10% mobile phase B for 6 min. The column and autosampler temperatures were 40 and 6 °C, respectively. The analytical run time was 32.0 min. The positive electrospray ionization (ESI) settings for analysis of the analytes and IS were as follows: ion source gas 1 (nebulizer gas), 60 (arbitrary units); ion source gas 2 (turbo heater gas), 55 (arbitrary units); curtain gas, 20 (arbitrary units); turbo-gas temperature, 500 °C; ion spray voltage, 5500 V. MRM mode was used for quantification (Table 1). Analyst 1.5.1 software was used for the LC–MS/MS system control and data processing. Similarly, a Q-Exactive orbitrap mass spectrometer equipped with an Accela UPLC system (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used. The ESI source settings were as follows: sheath gas flow rate, 35 (arbitrary units); auxiliary gas flow rate, 15 (arbitrary units); spray voltage, 4 kV; capillary voltage, 90 V; tube lens voltage, 125 V; skimmer voltage, 28 V; heater temperature, 350 °C. Full MS scan data were obtained from *m/z* 100 to 500 at a resolution of 70,000, while data-dependent MS/MS spectra data were acquired at a resolution of 35,000 using a normalized collision energy of 45 eV. Data were acquired using Xcalibur software (Thermo Fisher Scientific Inc.).

Method validation

To complete the method validation, the batches consisting of triplicate calibration standards from the range of 1–100 ng/mL and six replicates of low (3, 15, or 30 ng/mL) and high (70 ng/mL) QC samples were analyzed on three consecutive days. Precision was indicated by the coefficient of variation (CV) and accuracy by the percentage of the measured mean from the nominal values. For the evaluation of carryover effect, blank urine samples were injected between high QC samples.

The matrix effect was studied by post-column infusion of each analyte using a Harvard syringe pump at a flow rate of 10 µL/min after the pre-treated urine sample was injected. The most intense MRM transition for each analyte was monitored.

For the evaluation of long-term storage stability and short-term storage stability, six replicates of the QC samples at low (3, 15, or 30 ng/mL) and high concentrations (70 ng/mL) were stored at −80 °C for 28 days or 4 °C for 24 h before processing, respectively. Post-extraction batch integrity was determined by batch reinjection after 36 h of storage in the auto-sampler.

Method application

The validated method was applied to urine samples ($n = 17$) from suspicious drug abusers apprehended by the Narcotics Departments at the District Prosecutors' Offices in the Seoul metropolitan area. All urine samples were processed according to described sample preparation and were analyzed by LC–MS/MS.

Results

Liquid chromatography

The optimization of LC separation involving multiple analytes generally leads to time consuming attempts at testing different sets of LC columns and mobile phases. Several LC columns were tested using common LC–MS/MS mobile phase combinations. These included water and organic solutions (methanol or acetonitrile) with formic acid (0.1 or 0.5%) and/or ammonium formate (10 or 50 mM) as additives. A reversed phase type column was initially tested and substances including benzodiazepines, anoretics, synthetic cannabinoids, opioids, and cocaines and their metabolites showed adequate retention and peak shapes on a Capcell Pak MG-III C18 column (5 µm, 2.0 mm i.d. × 150 mm, Shiseido, Japan) using a gradient elution of 1 mM ammonium formate—0.1% formic acid and acetonitrile. This was adequate compared to Poroshell C_{18} (2.7 µm, 2.1 mm i.d. × 100 mm, Agilent, USA), Scherzo C_{18} (3 µm, 2.0 mm i.d. × 75 mm, Imtakt, USA), Atlantis dC_{18} (3 µm, 4.6 mm i.d. × 100 mm, Waters, USA) and X-bridge C_8 (3 µm, 3.5 mm i.d. × 150 mm, Waters, USA). Phenyl, HILIC, and PFP columns showed little or no retention, excessive equilibration time, deteriorating peak shape, as well as severe peak tailing for some compounds under the optimized mobile phase composition. No further optimization was investigated. The retention times of the analytes are shown in Table 1 and Fig. 1. Total analysis time was 32 min, which included a 6 min equilibrium cycle before the subsequent injection.

Mass spectrometry

The final item that should be considered for developing an MS-based analytical method is selecting an adequate mass analyzer for analytical requirements. To select an adequate mass analyzer for screening and confirmatory analysis, triple quadrupole-based MRM and orbitrap-based HRAM scan modes were evaluated in this study. Based on instrumental capabilities, both mass analyzers were comparable on the limit of quantitation, linear dynamic range, and matrix interference effects (data not shown). The orbitrap analyzer has distinct advantages with respect to accuracy and retrospective data analysis. However, the triple quadrupole mass analyzer demonstrated better data processing time, daily maintenance such as mass

calibration, instrumental robustness, and operational cost. Further investigation was performed on analytes in the urine with the triple quadrupole analyzer and MRM mode using two characteristic transitions for quantification (quantifier in Table 1) and confirmation (qualifier in Table 1). The MS/MS parameters for each analyte were optimized by the flow-injection method to achieve maximum sensitivity (Table 1).

Method validation

Calibration curves for the analytes were linear in the range of the tested concentrations of 1–100, 5–100, 10–100, or 1–40 ng/mL with the coefficient of determination (r^2) $\geq$ 0.9921 (Table 2). The lower limit of quantification (LLOQ) for the analytes was set at 1, 5, or 10 ng/mL using 400 µL of human urine with a signal-to-noise ratio higher than 10.

The intra- and inter-day precision and accuracy data at low and high QC levels containing the 113 analytes are summarized in Table 2. Both intra- and inter-day CV values for the 113 analytes ranged from 1.1 to 14.6%. Intra- and inter-assay accuracy values ranged from 86.7 to 106.8%. These results indicated that the accuracy and precision of the present method were acceptable.

In the analysis of the blank urine samples obtained from ten volunteers, there were no significant interference peaks observed at the retention times of the analytes, indicating the selectivity of the present method (Fig. 1a). Sample carryover effects were not observed.

Significant ion suppression or enhancement were not observed at the retention time of each analyte. This was evaluated during post-column infusion of each analyte following the injection of pretreated urine samples (Fig. 2). This result indicated that the matrix effects of the analytes have little effect on the determination of each analyte. High-speed centrifugation at 50,000*g*, following the addition of a small amount of deuterated internal standard mixture solution, was used to make the sample preparation. This process decreased both sample preparation time and sample loss.

The stability of processing (long-term storage at −80 °C and short-term storage at 4 °C) and chromatography (re-injection) were evaluated and shown to be of insignificant effect (Table 2). Specifically, long-term storage at −80 °C for 28 days and short-term storage of QC samples at 4 °C for 24 h, at both low and high concentrations prior to analysis, had little effect on quantification. Re-analysis of the pretreated urine samples stored for 36 h at 6 °C showed acceptable accuracy and precision of the QC samples.

Some compounds failed to meet validation acceptance criteria. These including 6-acetylmorphine, cannabidiol, cannabinol, codeine, HU-210, morphine, *N*-hydroxyphentermine, norcodeine, delta-9-tetrahydrocannabinol, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, zopiclone, zopiclone *N*-oxide, 4-fluoromethcatninone, 5-fluoro-THJ, ADB-PINACA *N*-(5-hydroxypentyl), JWH-018, JWH-019, methcathinone, SER-601, PB-22, and psilocin. It is possible to detect these compounds using this method, but a separate approach should be made for their quantitation.

Real sample analysis

The validated method was applied successfully to analyze forensic urine samples obtained from 17 suspicious drug abusers. Figure 1f shows representative MRM chromatograms of urine samples from a drug abuser. The identification and quantification results of the analytes in urine samples are summarized in Table 3. Drug abuse was frequently related to multi-drug consumptions with different chemical groups and, therefore, this LC–MS/MS method seemed to be appropriate for the simultaneous identification and quantification of various drugs of abuse.

Discussion

When developing a method for forensic analysis, there are some items that should first be considered. Specifically, the analytical requirement should be defined and it should be confirmed that the method under consideration is suitable for purposes such as screening or quantitation. Depending on the analytical requirement, a sample cleanup screen should be carefully selected, which is particularly important for LC separation. There are hundreds of different types of interference that can occur with the use of biological matrices such as urine. As such, it is difficult to identify the target analyte using the resulting complex chromatograms. SPE and LLE generally provide cleaner sample extracts than a dilute and shoot method based on a PPT scheme. However, small charged hydrophilic drugs often lead to poor recovery in SPE or LLE. This is particularly the case for the amphetamine and cathinone-type substances investigated in this study. The dilute and shoot scheme used in this study is relatively simple and generates clean sample extracts, which are suitable for rapid analysis. Matrix effects were not observed and easily minimized by employing stable isotope-labeled internal standards. It is safe to conclude that a PPT-based sample cleanup procedure, such as the dilute and shoot or QuEChERS type schemes, should be considered for multiple drug analysis involving different classes of substances.

If the analytical requirement for a particular study is to characterize or structurally elucidate new drugs of abuse, an HRAM analyzer must be employed. Forensic analysts should be aware that there is no concept that pertains to screening a new compound, since characterizing a new

Table 2 Linearity range, intra- and inter-day precision and accuracy, and sample stability of 113 drugs of abuse and their metabolites

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
2C-B-BZP	10–100	30	4.4	5.3	91.7	95.8	3.0	97.8	3.0	99.8	2.5	93.5
		70	3.4	3.5	92.8	96.6	5.4	96.9	3.9	103.2	2.4	95.5
AB-005	5–100	15	2.4	2.6	92.6	91.3	3.0	97.0	2.2	96.9	2.3	94.9
		70	2.1	4.2	92.4	96.6	1.9	96.0	2.7	100.4	3.2	99.7
Acetylfentanyl	1–100	3	7.4	6.3	94.3	96.8	4.2	94.7	6.9	98.3	3.7	96.8
		70	3.4	3.5	96.2	94.8	1.9	101.4	2.3	92.7	4.6	96.7
AKB-48 *N*-pentanoic acid	5–100	15	2.2	3.8	93.2	106.8	5.0	98.6	3.5	111.6	3.5	122.5
		70	1.6	3.0	90.1	95.8	4.1	97.0	8.3	104.9	3.0	112.3
AKB48 *N*-(5-fluoropentyl)	5–100	15	2.2	3.4	96.5	97.9	5.1	99.4	3.2	107.3	3.9	104.3
		70	2.7	5.0	95.5	98.8	2.4	98.3	4.1	102.2	3.3	108.0
Alprazolam	5–100	15	1.3	4.7	96.9	99.8	4.9	101.3	2.7	109.5	3.7	106.1
		70	3.0	1.8	90.9	99.0	4.0	100.0	1.8	108.0	2.0	101.6
AM-2201	1–100	3	2.9	3.0	92.0	99.0	2.8	94.7	3.2	107.3	1.9	98.0
		70	1.3	3.2	96.2	94.8	3.2	106.4	2.6	100.7	1.5	104.6
AM-2201 *N*-(4-hydroxy pentyl)	1–100	3	2.9	4.1	97.9	100.9	3.9	98.6	4.4	109.5	3.6	101.3
		70	1.6	6.9	90.4	95.9	3.0	102.7	7.5	105.3	2.8	110.9
AM-694	5–100	15	2.6	2.3	98.8	98.4	3.7	99.5	2.9	103.4	3.1	105.6
		70	1.5	4.7	96.6	95.2	4.2	98.8	5.7	98.6	2.5	103.8
7-Aminoclonazepam	5–100	15	1.4	4.5	97.1	97.0	3.9	99.7	7.2	100.7	5.0	100.4
		70	2.3	3.9	96.8	97.7	5.3	97.0	3.5	99.3	5.1	102.5
7-Aminonitrazepam	5–100	15	4.1	4.4	89.3	95.0	4.3	93.3	1.8	111.0	3.0	96.5
		70	3.5	2.9	94.5	97.2	4.3	96.7	2.0	109.8	1.4	95.2
Amphetamine	5–100	15	3.9	5.5	96.9	97.8	2.3	103.3	3.3	108.5	2.9	98.7
		70	3.1	2.6	87.7	97.7	0.7	102.1	1.5	105.8	3.2	99.4
6-APB	5–100	15	5.2	4.0	99.2	98.6	5.1	96.5	1.8	106.6	4.2	102.7
		70	5.0	3.5	96.7	97.6	4.7	95.0	1.7	96.3	3.7	98.2
Benzoylecgonine	5–100	15	4.8	4.7	99.1	94.9	5.1	109.0	3.9	92.0	3.5	116.4
		70	3.3	2.8	95.1	99.2	3.1	101.4	3.6	95.2	3.0	112.5
Benzphetamine	5–100	15	3.1	3.6	97.7	93.4	3.1	98.2	6.5	97.8	4.5	91.2
		70	2.2	5.5	97.2	95.9	3.3	94.3	2.4	96.0	5.0	98.5
1-Benzylpiperazine	10–100	30	1.3	2.4	87.5	91.7	4.2	91.9	2.4	98.6	2.0	94.0
		70	8.8	5.2	98.4	96.8	4.9	95.4	2.7	102.2	1.5	94.9
Bromazepam	10–100	30	4.7	3.1	94.6	96.7	2.6	95.7	1.9	96.7	3.5	100.1
		70	6.0	2.7	92.2	96.8	2.5	92.7	1.9	94.9	4.5	101.3
Buphedrone	5–100	15	3.9	3.4	99.9	95.8	5.8	94.1	3.7	95.9	3.5	93.3

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	2.8	3.5	102.2	97.8	2.7	98.6	3.1	96.0	3.8	91.2
Butylone	5–100	15	4.3	2.8	98.3	100.6	3.7	99.2	3.4	105.4	6.1	94.9
		70	3.2	3.4	94.4	98.0	4.6	95.9	1.9	100.9	4.2	92.5
Butyrylfentanyl	5–100	15	1.7	6.5	101.2	98.0	2.5	105.3	2.6	114.9	3.7	100.2
		70	4.8	2.8	97.5	96.0	3.5	97.4	3.1	103.4	2.1	93.4
Cathinone	10–100	30	3.0	4.4	99.2	96.0	3.0	105.7	2.2	101.2	2.5	87.3
		70	3.3	4.0	94.6	96.4	2.1	104.7	2.5	100.1	5.2	89.5
Chlordiazepoxide	5–100	15	2.5	4.4	97.1	96.5	5.8	97.1	5.8	90.9	4.1	100.7
		70	3.8	4.0	98.1	96.5	2.3	100.5	1.7	90.3	5.1	100.5
Clobazam	5–100	15	2.0	2.9	100.5	95.1	4.2	99.3	2.7	103.1	2.6	99.8
		70	3.7	2.7	93.1	96.2	3.4	94.4	1.5	101.0	2.3	98.5
Clonazepam	10–100	30	6.1	6.0	105.8	99.0	1.8	108.5	5.5	103.8	5.1	95.6
		70	3.9	4.5	96.0	98.2	4.5	99.7	3.5	103.2	5.9	97.1
Cocaethylene	5–100	15	3.2	4.9	96.7	99.7	5.4	94.5	5.0	106.4	4.4	75.7
		70	2.1	4.9	86.8	95.9	4.7	89.9	2.8	104.1	4.2	80.3
Cocaine	5–100	15	7.8	5.7	100.5	95.0	6.9	94.8	3.2	92.1	2.1	94.4
		70	3.3	3.9	101.6	97.1	4.4	93.2	2.2	89.9	5.1	89.4
DBZP	5–100	15	6.1	5.1	102.0	97.7	6.9	101.5	5.8	86.4	4.6	100.3
		70	4.4	4.8	101.0	97.7	4.7	94.8	4.3	87.1	5.3	101.1
Delorazepam	5–100	15	4.6	5.5	94.9	97.6	4.2	99.5	3.9	104.2	2.8	103.3
		70	1.9	6.5	88.6	94.1	2.6	99.1	7.3	101.5	1.5	106.7
Dextromethorphan	5–100	15	1.3	3.3	96.3	91.2	5.6	97.2	1.7	99.6	2.2	95.0
		70	2.0	3.0	95.9	98.1	3.7	95.7	2.5	103.1	0.4	98.0
Diazepam	5–100	15	1.7	3.3	104.2	101.3	3.6	106.4	1.5	109.9	2.5	107.6
		70	1.7	2.4	94.0	95.3	2.9	97.7	1.5	101.9	1.8	98.2
Dihydrocodeine	10–100	30	5.7	4.6	99.3	95.8	2.1	98.5	4.2	100.5	4.1	97.3
		70	4.9	2.4	95.5	96.0	4.4	96.7	3.4	98.9	3.8	96.8
EDDP	1–100	3	2.4	5.6	96.7	102.2	2.4	97.7	4.8	106.1	3.1	81.8
		70	3.2	2.7	96.3	98.3	2.7	97.4	1.7	103.3	2.2	99.1
Ephedrine	5–100	15	4.3	3.1	98.5	97.5	3.6	99.2	2.2	101.9	4.3	100.9
		70	3.3	2.7	95.4	98.3	1.7	98.9	1.8	98.5	3.5	102.3
Estazolam	5–100	15	3.6	3.9	100.8	99.7	5.2	97.8	3.5	98.9	6.7	109.9
		70	4.2	3.1	97.7	99.2	3.4	95.7	1.9	94.5	4.3	107.0
4-Ethylmethcathinone	5–100	15	3.7	4.8	95.5	92.6	5.6	93.5	3.8	97.5	2.8	84.8

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	2.4	4.6	100.0	97.0	3.2	92.0	2.3	99.6	3.6	87.8
Ethylone	5–100	15	4.9	5.3	95.3	98.7	5.9	93.7	4.9	105.4	4.8	84.3
		70	3.6	4.1	88.5	96.3	3.2	89.5	2.6	103.9	3.0	88.7
Fenfluramine	1–100	3	3.7	5.5	91.0	92.8	6.7	95.0	5.3	95.9	5.5	89.2
		70	2.9	3.7	95.3	95.7	4.2	97.7	2.5	103.6	1.9	95.1
Fentanyl	5–100	15	4.1	4.1	100.2	97.1	1.8	97.6	4.4	98.3	3.4	96.9
		70	3.6	2.9	94.5	96.4	3.7	96.5	2.8	96.6	4.4	100.0
Flunitrazepam	5–100	15	4.6	4.0	101.7	97.4	4.9	103.6	5.8	99.5	3.8	100.8
		70	4.2	5.9	92.5	98.5	3.2	101.6	3.3	100.6	2.0	105.0
5-Fluoro-AKB48 *N*-(4-hydroxypentyl)	5–100	15	2.9	2.9	95.3	99.8	4.1	100.2	4.6	102.8	3.6	111.1
		70	1.6	7.8	92.5	97.2	4.8	99.6	7.4	99.9	3.8	109.3
4-Fluoroamphetamine	5–100	15	3.9	3.2	94.8	95.5	4.5	96.8	2.7	101.5	2.9	98.3
		70	3.5	3.1	93.9	96.6	2.6	97.5	2.1	100.4	3.0	97.8
1-(4-Fluorobenzyl)-piperazine	10–100	30	2.9	2.9	86.7	93.2	3.1	96.7	2.5	104.5	2.0	92.3
		70	2.6	3.8	89.1	96.5	3.2	96.6	2.9	107.8	1.5	94.2
5-Fluoro-SDB-006	1–100	3	3.9	4.9	93.3	97.5	2.7	97.0	3.7	105.7	5.8	96.1
		70	1.9	6.2	90.5	96.0	4.1	102.8	7.8	106.5	4.5	103.3
Flurazepam	5–100	15	2.3	4.7	99.5	98.5	4.3	103.3	3.9	106.2	1.8	101.6
		70	3.4	2.6	93.4	97.8	2.8	99.2	3.2	106.1	2.5	102.5
α-Hydroxyalprazolam	10–100	30	5.6	3.1	101.3	99.4	3.0	99.8	6.0	99.1	2.5	103.2
		70	8.2	4.2	92.4	98.2	3.9	94.6	3.4	97.6	3.4	106.1
α-Hydroxymidazolam	5–100	15	1.2	5.1	96.9	94.8	4.3	101.7	3.9	103.7	2.4	98.5
		70	2.7	4.2	92.0	96.2	4.1	100.0	2.7	107.9	0.9	96.7
α-Hydroxytriazolam	10–100	30	2.0	4.1	107.1	102.2	2.6	104.2	1.0	97.9	2.5	108.5
		70	4.6	3.0	92.0	98.6	2.1	92.6	3.0	94.2	3.2	105.3
JWH-018 *N*-pentanoic acid	5–100	15	3.4	7.2	97.7	103.1	4.7	92.7	3.4	97.6	4.0	116.5
		70	3.1	6.7	95.1	98.7	2.8	88.8	3.2	92.7	3.0	114.0
JWH-019 *N*-(6-hydroxy hexyl)	5–100	15	4.7	3.1	102.5	99.4	2.2	97.6	1.9	105.6	3.5	106.2
		70	2.6	5.4	95.9	95.1	1.3	91.4	4.4	99.8	3.1	105.3
JWH-200	1–100	3	5.2	7.6	96.0	93.7	3.8	88.3	2.8	99.9	4.3	92.1
		70	2.7	4.6	99.9	96.7	3.5	90.1	2.7	95.6	3.1	107.5
JWH-200 5-hydroxyindole	5–100	15	3.1	4.6	97.3	93.8	4.4	99.3	1.4	106.2	4.9	97.8
		70	2.9	3.7	96.3	98.3	4.5	98.4	2.7	106.6	1.5	97.0
JWH-250	5–100	15	4.1	3.9	97.0	97.9	5.9	106.8	2.0	108.4	4.4	100.0

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	3.1	3.4	94.7	97.6	3.7	103.0	3.5	106.5	83.0	101.2
JWH-250 *N*-(5-hydroxy pentyl)	1–100	3	4.3	3.9	92.7	98.6	2.9	92.7	3.6	103.5	2.6	95.5
		70	2.1	5.7	91.8	95.8	3.0	99.8	7.5	102.2	3.2	111.7
Ketamine	5–100	15	4.7	6.7	97.1	93.5	4.3	101.3	6.4	98.2	4.2	97.2
		70	2.0	3.7	90.7	97.5	2.4	97.9	3.5	102.3	3.6	97.1
Lorazepam	10–100	30	5.9	4.0	106.8	99.9	1.5	96.1	4.0	94.5	1.9	102.8
		70	5.2	3.6	99.3	97.9	2.0	89.7	1.6	92.8	2.3	100.8
Lormetazepam	5–100	15	2.6	3.9	100.4	103.0	3.7	108.5	1.4	110.8	2.1	110.7
		70	3.1	2.1	90.4	94.6	2.7	100.5	1.6	104.8	2.1	97.3
LSD	5–100	15	3.2	3.0	96.3	100.6	3.3	104.6	5.6	108.8	4.4	103.6
		70	3.5	3.7	92.7	94.8	3.7	100.2	2.7	98.0	2.1	99.4
MAM-2201	1–40	3	4.7	5.1	93.0	98.8	5.5	91.3	6.2	106.5	6.4	93.7
		30	3.0	4.2	98.4	92.7	4.0	101.9	2.5	101.9	3.4	94.6
MAM-2201 *N*-(4-hydroxypentyl)	5–100	15	2.9	3.2	97.3	101.3	2.7	99.1	1.8	106.3	2.9	111.4
		70	1.6	5.8	95.2	96.1	3.8	99.0	6.2	100.2	3.5	107.3
mCPP	5–100	15	4.2	5.1	96.3	92.5	6.5	92.0	5.4	94.9	4.5	90.7
		70	4.7	5.1	97.9	97.8	3.1	95.5	1.3	92.9	5.5	91.3
MDA	5–100	15	2.3	4.7	97.0	95.1	2.7	97.9	4.2	100.6	3.1	98.4
		70	1.1	3.6	91.8	97.2	2.3	96.3	1.3	101.1	3.0	99.4
MDEA	5–100	15	4.0	4.9	101.7	97.9	2.8	100.7	8.3	101.2	6.0	98.5
		70	2.9	4.8	94.0	98.7	5.3	98.5	2.9	103.4	5.1	102.7
MDMA	1–100	3	5.2	5.5	89.0	95.5	2.5	93.0	1.9	101.7	3.4	86.6
		70	3.9	3.1	95.7	97.7	2.7	96.6	2.5	99.1	3.6	100.9
MDPV	5–100	15	3.4	5.1	97.1	97.2	4.8	102.9	2.9	109.5	4.4	101.8
		70	2.4	3.0	96.1	96.8	4.8	99.2	3.0	102.3	2.6	98.8
5-MeO-DALT	1–100	3	6.2	5.9	91.9	97.9	5.4	95.4	6.2	102.6	6.2	93.1
		70	4.3	6.1	89.2	94.8	3.3	99.3	6.6	104.2	2.4	102.9
Meperidine	5–100	15	2.2	4.5	103.9	98.7	3.3	102.1	2.3	98.8	4.8	101.6
		70	3.4	6.4	97.1	98.3	4.0	93.3	2.4	94.9	3.3	102.2
Mephedrone	5–100	15	4.5	5.0	91.9	92.1	3.9	94.1	3.8	97.1	2.7	85.9
		70	2.0	4.1	98.5	96.6	5.8	94.0	4.4	100.5	5.2	87.1
Mescaline	10–100	30	2.7	3.8	92.2	95.9	3.9	97.5	2.7	99.1	2.2	98.6
		70	2.3	3.0	91.9	97.0	2.5	98.5	2.7	99.6	4.6	99.5
Methadone	1–100	3	1.1	3.0	93.7	94.0	3.9	94.0	3.2	96.2	0.9	91.0

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	4.5	2.3	93.1	98.3	2.7	98.0	2.2	100.8	3.2	97.6
Methamphetamine	5–100	15	14.6	3.3	95.9	97.4	1.2	103.3	1.9	101.6	2.1	102.0
		70	3.8	2.7	87.7	97.9	2.7	100.0	1.5	103.6	2.9	99.1
Methaqualone	1–100	3	3.3	3.3	91.0	94.5	2.2	90.7	3.7	102.7	2.3	92.6
		70	3.6	2.3	95.4	97.8	2.5	95.5	0.3	99.7	3.1	99.5
Methoxetamine	5–100	15	4.1	4.1	98.8	102.6	5.2	102.7	3.7	103.0	5.4	104.0
		70	3.5	3.4	96.5	97.2	2.6	100.1	1.4	100.1	4.9	103.6
Methoxyphenamine	5–100	15	4.2	3.3	94.7	100.6	1.9	99.2	3.7	105.3	6.3	105.2
		70	2.5	3.9	97.1	97.8	3.3	101.9	2.0	101.4	4.0	102.7
4-Methylephedrine	5–100	15	3.9	3.7	101.9	99.7	6.0	101.3	3.7	104.4	3.7	100.6
		70	3.7	5.2	94.9	95.0	3.1	94.9	3.0	100.6	4.3	99.0
Methylone	5–100	15	4.5	3.6	100.3	97.7	3.2	89.5	4.7	105.4	3.8	80.4
		70	4.2	4.7	94.4	96.2	4.6	88.7	2.9	101.8	3.1	84.2
Methylphenidate	5–100	15	5.0	4.8	104.4	97.8	3.4	97.2	5.9	98.6	4.4	88.0
		70	1.6	3.7	102.5	96.3	4.2	95.8	4.7	91.9	5.4	90.2
Midazolam	5–100	15	4.9	5.9	96.5	95.8	4.7	103.3	4.0	111.9	3.5	101.4
		70	3.2	4.4	92.9	97.1	2.5	101.9	2.9	108.4	1.1	97.8
Nalbuphine	5–100	15	3.7	4.9	92.7	92.4	7.1	92.7	4.8	100.8	2.2	99.6
		70	2.6	6.8	91.0	93.1	5.1	94.9	8.4	103.3	3.1	102.2
N-desmethylclobazam	5–100	15	4.1	4.5	98.6	92.8	2.7	99.4	2.3	102.4	2.1	98.6
		70	3.6	3.6	93.8	96.0	2.2	95.3	2.1	103.2	1.1	96.8
N-desmethylflunitrazepam	5–100	15	7.5	6.4	100.3	99.1	2.6	104.5	4.4	103.5	5.3	100.6
		70	5.0	3.3	88.8	98.0	3.2	100.7	2.5	104.1	3.0	100.7
N-ethylcathinone	5–100	15	1.9	4.5	97.1	92.2	3.5	84.1	4.4	95.6	4.2	72.3
		70	2.5	5.0	96.8	95.7	4.6	82.1	3.4	100.7	3.3	73.6
Nitrazepam	5–100	15	4.3	5.5	101.7	96.5	4.0	104.4	2.1	108.4	3.9	100.2
		70	3.0	3.6	91.2	96.5	3.4	99.7	2.9	107.7	1.7	98.8
Nordiazepam	10–100	30	3.6	3.3	105.2	100.6	4.3	106.5	2.5	109.5	2.6	105.3
		70	1.6	2.9	96.2	97.9	2.8	96.4	2.0	101.1	2.1	100.3
Norephedrine	10–100	30	3.6	2.9	92.3	97.5	1.3	100.2	1.7	105.1	2.9	98.4
		70	5.1	2.4	87.8	97.5	1.6	102.0	2.4	103.1	4.5	99.4
Norfentanyl	10–100	30	6.6	5.8	89.7	102.7	3.2	98.9	2.6	114.5	5.9	102.5
		70	4.0	3.6	92.1	96.4	2.2	99.0	4.2	108.3	3.6	98.2
Norketamine	5–100	15	6.8	5.1	100.7	97.6	5.8	99.2	7.4	100.9	6.3	100.7

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	4.6	5.9	95.6	96.6	5.9	92.5	3.1	101.3	3.7	102.3
Norsufentanyl	5–100	15	3.2	4.6	95.9	92.4	4.3	95.3	5.0	101.3	4.9	94.7
		70	6.9	4.6	98.7	96.2	4.1	90.6	1.4	107.6	3.8	92.7
Oxazepam	5–100	15	1.1	3.7	99.0	97.0	5.2	104.2	7.7	104.6	4.6	100.0
		70	2.0	4.4	90.8	95.1	4.8	98.2	2.9	98.6	4.5	101.9
2-Oxo-3-hydroxy-LSD	5–100	15	2.0	3.9	101.4	99.7	6.9	103.5	5.1	106.5	2.5	96.8
		70	2.7	6.7	98.3	94.5	3.3	95.8	3.4	98.4	5.4	95.0
Pemoline	5–100	15	6.3	5.1	97.7	96.2	6.5	93.7	5.1	99.0	3.8	97.9
		70	4.1	4.1	95.2	96.9	2.6	91.3	1.8	95.4	5.3	98.9
Pentazocine	5–100	15	3.7	4.8	101.9	101.3	5.4	102.0	6.2	110.0	5.1	105.1
		70	3.7	4.5	98.3	97.2	3.5	94.0	2.3	98.8	4.2	103.6
Pentedrone	5–100	15	4.8	4.3	98.9	98.4	2.3	87.7	3.9	102.0	3.7	84.1
		70	2.6	3.3	100.3	98.5	3.0	91.4	3.5	98.3	4.1	84.1
Phencyclidine	10–100	30	3.5	5.2	104.8	102.3	2.0	99.0	5.7	95.7	3.1	105.6
		70	2.7	5.7	99.9	102.2	2.3	94.5	4.6	93.8	3.8	104.8
Phendimetrazine	10–100	30	3.6	2.6	95.0	95.4	2.6	98.1	2.9	102.5	3.1	96.3
		70	3.4	2.2	98.0	98.2	3.8	98.3	2.5	101.7	2.7	98.5
Phenmetrazine	5–100	15	2.5	3.3	96.8	96.1	2.3	95.9	2.9	103.4	2.5	99.7
		70	4.0	2.7	95.2	97.0	2.8	94.0	0.9	99.6	3.3	97.2
Phentermine	5–100	15	4.2	4.6	94.4	99.5	2.8	106.1	1.9	105.3	3.2	103.7
		70	4.9	4.7	92.5	96.8	2.3	98.4	2.6	99.9	3.4	98.8
Phenylpiperazine	5–100	15	3.2	3.5	90.3	94.4	5.4	92.9	2.9	97.0	3.6	92.9
		70	4.8	3.0	92.3	97.7	2.7	95.0	1.8	101.0	3.3	97.3
PMA	5–100	15	3.9	4.7	99.3	95.7	3.6	95.7	3.6	104.8	2.8	103.4
		70	3.3	4.3	94.8	97.2	1.8	93.8	1.2	100.0	4.2	101.2
PMMA	5–100	15	1.8	3.2	95.0	98.7	3.1	99.0	1.1	104.3	4.4	102.2
		70	2.6	2.7	92.9	98.0	2.2	96.4	2.3	100.3	4.1	100.3
Prazepam	5–100	15	3.8	4.5	103.8	103.8	5.7	98.1	3.6	103.4	4.1	117.8
		70	2.9	5.5	97.0	97.0	2.7	88.5	2.7	93.9	3.5	111.9
RCS-4	1–100	3	5.6	3.4	89.0	98.3	3.2	95.0	3.5	108.6	1.5	98.6
		70	1.1	2.8	92.1	94.1	2.5	104.3	4.1	103.3	3.2	98.7
SDB-006	5–100	15	3.0	5.6	94.2	98.9	4.1	105.2	1.6	107.1	3.2	103.2
		70	3.4	6.4	91.2	98.8	2.7	105.5	7.2	106.9	3.0	101.6
STS-135	1–100	3	3.8	3.1	88.3	95.0	3.8	96.0	3.3	108.3	4.4	94.8

Table 2 continued

Compounds	Linearity (ng/mL)[a]	QC (ng/mL)	Precision (% CV)		Accuracy (%)		Auto-sampler stability[b]		Short-term stability[b]		Long-term stability[b]	
			Intraday[b]	Interday[c]	Intraday	Interday	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
		70	3.4	3.1	93.3	97.0	2.8	103.3	4.8	109.1	4.4	104.1
Temazepam	10–100	30	5.0	4.1	105.0	101.6	4.4	100.4	4.8	100.1	3.4	109.9
		70	5.4	4.4	95.7	98.4	2.9	94.8	1.7	96.1	3.2	104.4
TFMPP	10–100	30	2.5	3.4	93.3	94.9	3.0	97.3	3.0	95.8	1.9	95.1
		70	5.2	3.7	92.3	96.3	1.7	94.8	2.1	95.3	3.2	96.3
THJ-2201	5–100	15	3.3	2.9	100.1	96.8	3.1	99.0	1.3	106.1	5.0	101.3
		70	2.9	3.6	97.7	97.2	2.6	98.8	2.8	103.3	2.4	99.9
Triazolam	5–100	15	4.1	4.5	99.7	100.3	2.4	109.7	3.0	100.2	5.2	105.8
		70	2.3	4.1	91.8	96.2	3.0	98.9	3.6	96.7	1.6	105.2
UR-144 *N*-(5-hydroxy pentyl)	5–100	15	2.6	3.7	95.3	102.2	4.8	102.4	2.3	104.4	4.5	111.7
		70	2.5	5.0	89.9	98.3	2.1	97.6	2.6	102.1	4.0	110.9
URB-937	5–100	15	4.6	3.1	100.3	101.8	5.0	97.9	3.3	95.8	3.5	106.5
		70	6.3	3.9	96.7	98.8	2.8	99.7	2.8	93.1	5.4	104.7
XLR-11 *N*-(4-hydroxy pentyl)	5–100	15	3.3	3.6	93.0	97.4	5.4	102.3	3.1	104.2	2.8	108.2
		70	1.5	6.1	86.9	95.8	4.5	103.4	8.6	108.0	4.7	104.9
XLR-12	5–100	15	2.0	4.4	90.7	91.2	4.2	95.9	4.1	105.2	5.9	92.0
		70	4.3	3.6	89.7	95.4	5.2	102.8	8.4	110.9	3.4	93.0
Zolpidem	5–100	15	5.2	3.9	98.1	97.7	3.2	98.9	5.3	104.5	3.5	102.6
		70	4.4	3.4	93.5	98.9	4.0	92.3	1.3	99.2	2.5	104.1
Zolpidem phenyl-4-carboxylic acid	5–100	15	6.1	5.2	100.7	98.7	6.9	103.6	4.2	107.1	7.3	101.4
		70	4.7	7.0	94.9	96.9	4.7	94.0	1.8	101.7	7.5	97.7

[a] n = 8 (n = 2/day)

[b] n = 6

[c] n = 18 (n = 6/day)

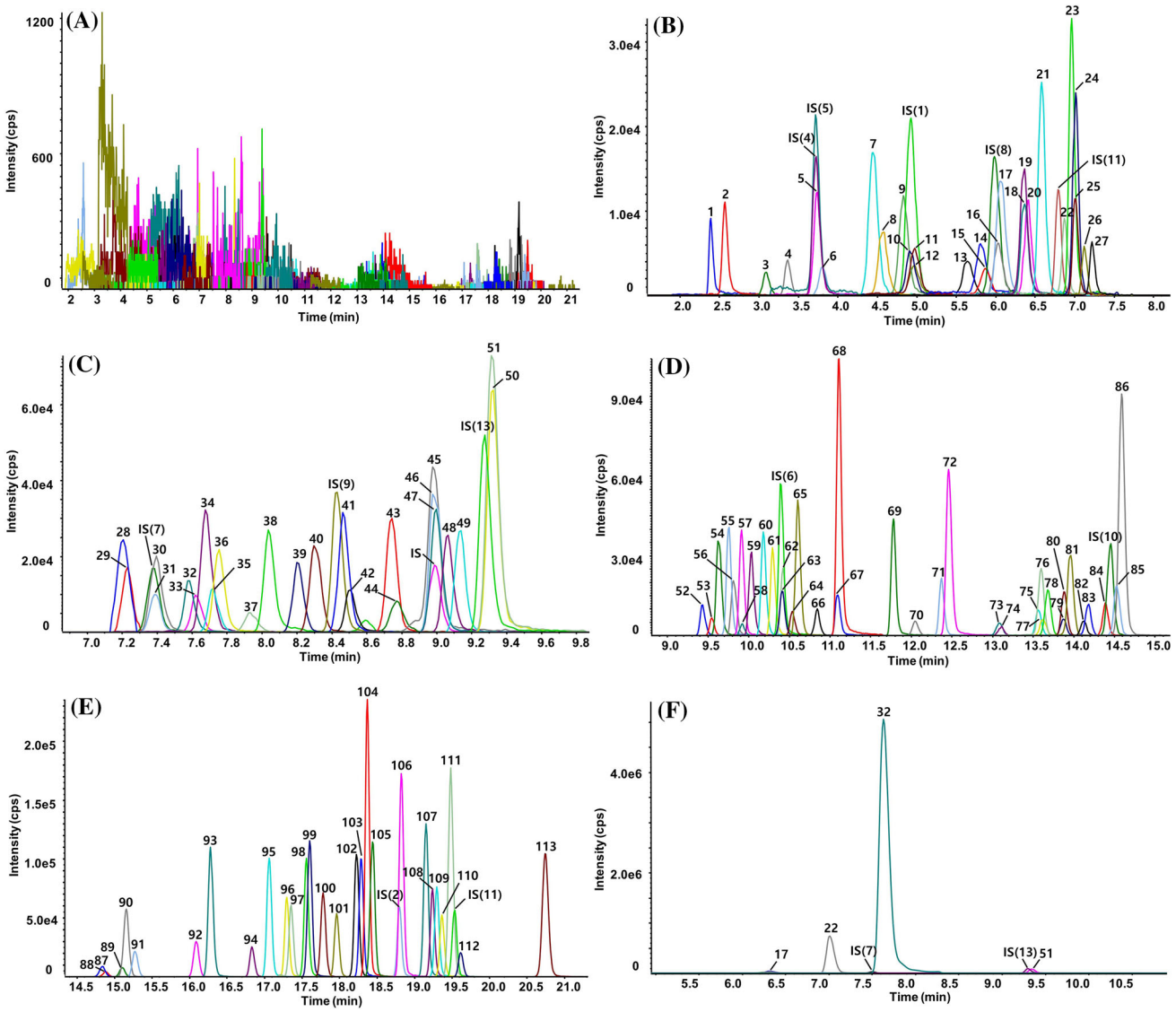

Fig. 1 Representative MRM chromatograms of **a** blank human urine at 126 MRM transitions and spiked human urine samples containing 20 ng/mL of compounds **b** 1–27, **c** 28–51, **d** 52–86, and **e** 87–113, and **f** urine samples of a drug abuser. Compound numbers are shown in Table 1

compound is a completely different area and requires more than a simple screening approach. Both triple quadrupole and HRAM analyzers are powerful analytical tools for general screening or confirming purposes, despite the many tradeoffs between these two mass analyzers. For screening and confirming approximately 300 or fewer compounds, the triple quadrupole analyzer would be the better fit for performing routine analysis.

Based on this study, a simple guideline for selecting a rapid screening method for drugs of abuse can be proposed. First, the sample clean-up process should be a universal method and "dilute and shoot" approach would be an adequate starting point. Generalized HPLC column selection should be considered and a reversed phase type column, especially C_{18} column would serve the analytical purpose well. Finally, a targeted screening method employing MRM transition mode would be a good choice for screening and confirmation purpose.

In conclusion, a rapid and simple LC–MS/MS method for the simultaneous determination of 113 drugs and their metabolites used for drug-facilitated crimes and toxicities in human urine was developed. This method was created using high-speed centrifugation at 50,000*g* after the addition of a small amount of deuterated internal standard mixture solution as sample preparation. This study demonstrated the simplicity, selectivity, reproducibility, stability, and applicability of the present method in the analysis of 17 forensic urine samples. This method is useful

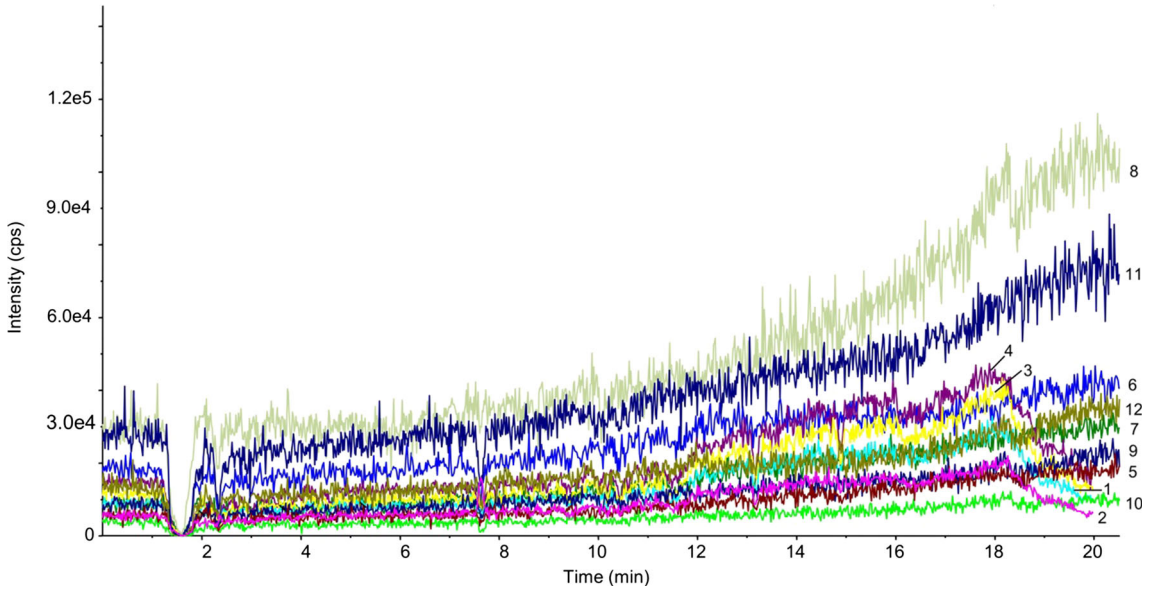

Fig. 2 Representative chromatograms for demonstrating the matrix effect of the urine on MRM signals of *1* ephedrine (3.7 min), *2* amphetamine (4.9 min), *3* MDMA (6.4 min), *4* phentermine (6.7 min), *5* 2C-B-BZP (7.1 min), *6* benzoylecgonine (8.1 min), *7* cocaine (8.9 min), *8* zolpidem (9.3 min), *9* diazepam (16.0 min), *10* temazepam (14.8 min), *11* JWH-122 *N*-(5-hydroxypentyl) (16.2 min), and *12* XLR-11 *N*-(4-hydroxypentyl) (17.3 min) using post-column infusion

Table 3 Quantitative results of the analytes in urine samples obtained from drug abusers

Compounds	Quantitation of analyte in urine samples of drug abusers																
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17
Amphetamine	–	○	–	○	–	–	–	–	–	–	–	–	–	–	–	–	–
Cocaine	–	○	–	○	–	–	○	○	○	○	○	–	–	–	–	–	–
Benzoylecgonine	–	–	–	–	–	–	–	○	○	–	–	–	–	–	–	–	–
Cocaethylene	–	–	–	–	–	–	–	○	–	–	–	–	–	–	–	–	–
Diazepam	–	–	–	–	–	–	○	–	–	–	–	–	–	–	–	–	–
Nordiazepam	–	–	–		–	–	○	–	–	–	–	–	–	–	–	–	–
Ephedrine	○	○	○	○	○	–	–	–	–	–	○	○	○	○	○	–	○
Norephedrine	○	○	○	○	○	–		–	–	–	○	○	○	○	○	–	○
MDMA	–	–	–		–	–	–	–	–	○	–	–	–	–	–	–	–
Methamphetamine	–	○	–	○	–	○	–	–	–	–	–	–	–	–	–	–	–
Oxazepam	–	–	–	–	–	–	○	–	–	–	–		–	–	–	–	–
Phenmetrazine	○	–	–	–	○	–	–	–	–	–	–	○	–	–	–	–	–
Phendimetrazine	–	–	–	–	○	–	–	–	–	–	–	○	–	–	–	–	–
Phentermine	–	–	○	○	○	○	–	–	–	–	–	○	–	○	○	○	○
Temazepam	–	–	–	–	–	–	○	–	–	–	–	–	–	–	–	–	–
Zolpidem	–	–	–	○	–	○	–	–	–	–	–	–	–	–	–	–	–
Zolpidem phenyl-4-carboxylic acid	–	–		○	–	○	○	–	–	–	–	–	–	–	–	–	–

– Not detected, ○ determined

for rapid and accurate determination of multiple drug abuse using a small amount of urine in forensic and clinical toxicology.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

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