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Research Article

Gas chromatography-mass spectrometric method for the screening and quantification of illicit drugs and their metabolites in human urine using solid-phase extraction and trimethylsilyl derivatization

A simple and rapid GC-MS method has been developed for the screening and quantification of many illicit drugs and their metabolites in human urine by using automatic SPE and trimethylsilylation. Sixty illicit drugs, including parent drugs and their metabolites that are possibly abused in Korea, can be monitored by this method. Among them, 24 popularly abused illicit drugs were selected for quantification. Very delicate optimizations were carried out in SPE, trimethylsilylation derivatization, and GC/MS to enable such remarkable achievements. Trimethylsilylated analytes were well separated within 21 min by GC-MS. In the validation results, the LOD of all the analytes were in the range of 2–75 ng/mL. The LOQ of the quantified analytes were in the range of 5–98 ng/mL. The linearity (r^2) of the quantified analytes ranged 0.990–1.000 in each concentration range between 10 and 1000 ng/mL. The mean recoveries ranged from 62 to 126% at three different concentrations of each analyte. The inter-day and inter-person accuracies were within −13.3∼14.9%, and −10.1∼13.0%, respectively, and the inter-day and inter-person precisions were less than 12.9%. The method was reliable and efficient for the screening and quantification of abused illicit drugs in routine urine analysis.

Keywords: Automatic SPE / GC-MS / Illicit drugs in urine / Screening and quantification / Trimethylsilylation
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1 Introduction

Methamphetamine (MA) and cannabinoids have been the most frequently abused illicit drugs in Korea. 3,4-Methylenedioxymethamphetamine (MDMA), opiates, cocaine (COC), nalbuphine (NBP), dextromethorphan (DMP), carisoprodol (CSP), ketamine (KET), diazepam (DZ), and phentermine (PT) have also been abused, and all of these drugs have become regulated under the Controlled Substances Act. Especially NBP, DMP, CSP, and KET are the new or alternative drugs of choice in Korea [1–2]. Despite being common prescription medicines, CSP, NBP, and KET act as a central acting musculoskeletal relaxant and central nervous system depressant with analgesic properties when overdosed [3–6]. DMP, widely used as an antitussive ingredient in over-the-counter cough medicine, has hallucinogenic effects and can be fatal when overdosed [2, 7]. The presence of these drugs is monitored routinely in urine analysis. However, such routine analysis may fail to confirm these drugs due to the low level of abuse. Furthermore, the present analytical method is incapable of simultaneously screening and confirming all these drugs including MA and cannabinoids metabolite (11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, THCCOOH) in routine urine analysis.

Urine tests for amphetamine-type stimulants (ATSs), cannabinoids, opiates, COC and its metabolites, benzodiazepines, and barbiturates are usually preformed by immunological methods for screening followed by GC-MS for confirmation in forensic toxicology [8–10]. Screening analysis

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Abbreviations: AP, amphetamine; **ATS,** amphetamine-type stimulants; **BE,** benzoylecgonine; **COC,** cocaine; **CSP,** carisoprodol; **DMP,** dextromethorphan; **DRP,** dextrorphan; **DZ,** diazepam; **EME,** ecgonine methylester; **FFA,** fenfluramine; **IS,** internal standard; **KET,** ketamine; **LZP,** lorazepam; **MA,** methamphetamine; **6MAM,** 6-monoacetylmorphine; **MDA,** 3,4-methylenedioxyamphetamine; **MDEA,** 3,4-methylenedioxyethylamphetamine; **MDMA,** 3,4-methylenedioxymethamphetamine; **MOR,** morphine; **MSTFA,** *N*-methyl-*N*-trimethylsilyltrifluoroacetamide; **NBP,** nalbuphine; **NDZP,** nordiazepam; **NKET,** norketamine; **OHLSD,** 2-oxo-3-hydroxy-LSD; **OXZP,** oxamzepam; **PDMT,** phendimetrazine bitartrate; **PMA,** *p*-methoxyamphetamine; **PMMA,** *p*-methoxymethamphetamine; **PML,** pemoline; **PMT,** phenmetrazine; **PT,** phentermine; **PTB,** pentobarbital; **PTHD,** pethidine; **PTZC,** pentazocine; **QC,** quality control; **THCCOOH,** 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; **TMS,** trimethylsilylation

by immunological methods can give results in a few minutes, but suffers from the disadvantages of high costs of reagents, limitation of applicable drugs and low reliability. Analytical methods have been published for screening or quantification by GC-MS, LC-MS or LC-MS-MS for ATSs, cannabinoids, opiates, COC and its metabolites, benzodiazepines, and ephedrines in urine, blood, oral fluid, and hair [9–21]. Although LC-MS is becoming a better analytical tool than GC-MS because of its ability to support non-derivatization analysis of polar or hydrophilic compounds, GC-MS analysis remains preferred in toxicological analyses because of its low analysis cost and greater applicability in routine analyses [11]. Most GC-MS analyses for multiple drugs in urine samples include an extraction procedure by liquid–liquid extraction or SPE, and a derivatization procedure [9, 10, 16, 22]. However, liquid–liquid extraction or manual SPE is not easy to conduct for the routine urine analysis of illicit drugs due to its multiple extraction procedures and low efficiency in the treatment of sample preparation.

Derivatization is done by proper reagents such as *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), pentafluoropropionic anhydride and pentafluoro-1-propanol, or acetic anhydride for multiple drugs. However, no simultaneous derivatization of all the analytes, especially including ATSs, cannabinoids metabolite, opiates, COC and its metabolites, benzodiazepines, and barbiturates, has been presented for the screening or confirmation analysis.

The purpose of this study is to develop a more reliable and efficient method for the simultaneous screening and quantification of many illicit drugs and some of their metabolites in human urine by automatic SPE, trimethylsilylation (TMS) with MSTFA, and GC-MS. The target analytes (60 compounds) are illicit drugs already being abused in Korea, other related drugs with the potential for future abuse, and their metabolites (Fig. 1). Among these compounds, the following 24 popularly abused illicit drugs were selected for quantification: ten amphetamine-type drugs, cannabinoids metabolite (THCCOOH), six opiate-like drugs, COC and two of its metabolites, as well as two KETs, nordiazepam (NDZP) and pentazocine (PTZC). Certain other drugs such as benzodiazepines and barbiturates were also well screened.

We believe that this study makes a significant contribution towards simultaneous screening and confirming analysis for abused drugs in forensic toxicology since very delicate optimizations were carried out in SPE, TMS derivatization, and GC/MS operation to enable simultaneous screening of 60 drug components and quantitative determination of 24 components.

2 Materials and methods

2.1 Chemicals, reagents, and materials

Alprazolam, amphetamine (AP), benzoylecgonine (BE), cathinone, COC, codeine (COD), DZ, ecgonine methylester (EME), fenfluramine (FFA), KET, lorazepam (LZP), lysergic acid diethylamide, meprobamate, MA, methylphenidate (ritalin), morphine (MOR), NDZP, norfentanyl, norketamine (NKET), norpethidine (normepridine), pethidine (PTHD), phencyclidine, temazepam, zolpidem hemitartrate, THCCOOH, 2-oxo-3-hydroxy-LSD (OHLSD), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), MDMA, 4-bromo-2,5-dimethoxyphenethylamine hydrochloride, 6-monoacetylmorphine (6MAM), 7-aminoflunitrazepam, α-hydroxyalprazolam, α-hydroxytriazolam, and the deuterated internal standards (IS) AP-d_5, BE-d_3, MA-d_5, MDA-d_5, MDEA-d_5, MDMA-d_5, MOR-d_3, oxazepam-d_5, and THCCOOH-d_3 were all purchased from Cerilliant (Austin, TX, USA). Amobarbital sodium, CSP, DMP, dextrorphan (DRP), EP, methoxyphenamine, papaverine and PT hydrochlorides, mescaline sulfate, methadone, methaqualone, methylephedrine (MEP), NBP, oxamzepam (OXZP), pemoline (PML), PTZC, pentobarbital (PTB), phendimetrazine bitartrate (PDMT), phenobarbital, *p*-methoxyamphetamine (PMA), *p*-methoxymethamphetamine (PMMA), secobarbital, selegiline, ritalinic acid (methylphenidate metabolite), and MSTFA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mazindol and phenmetrazine (PMT) hydrochlorides were purchased from USP (Rockville, MO, USA). Methanol was supplied by J.T. Baker (Phillipsburg, NJ, USA).

2.2. Standard solutions

The stock standard solutions were prepared in methanol for individual analytes in predetermined concentrations as follows: 1000 ng/μL for amobarbital, CSP, DMP, DRP, EP, MEP, mescaline, methoxyphenamine, methadone, mazindol, NBP, phenobarbital, PDMT, PMA, PML, PMT, PT, PTB, PTZC, secobarbital, and selegiline; 500 ng/μL for ritalinic acid; 100 ng/μL for 4-bromo-2,5-dimethoxyphenethylamine hydrochloride, 6MAM, AP, APLZ, BE, COC, COD, DZ, EME, FFA, KET, cathinone, LSD, LZP, MA, MDA, MDEA, MDMA, MOR, meprobamate, methylphenidate, methaqualone, NDZP, norfentanyl, NKET, OXZP, papaverine, phencyclidine, PMMA, PTHD, temazepam, and zolpidem hemitartrate; 20 ng/μL for 7-aminoflunitrazepam, OHLSD, and α-hydroxytriazolam; 10 ng/μL for α-hydroxyalprazolam, norpethidine, and THCCOOH. The working standard mix for method validation was finally prepared to contain each analyte at a concentration of 5 ng/μL except 6MAM and THCCOOH (0.5 ng/μL). The deuterated IS was prepared in methanol to contain 10 ng/μL for AP-d_5, BE-d_3, MA-d_5, MDA-d_5, MDEA-d_5, MDMA-d_5, MOR-d_3, oxazepam-d_5 and 5 ng/μL for THCCOOH-d_3. The mixture of IS was finally adjusted to 2 ng/μL (THCCOOH-d_3; 1 ng/μL).

2.3 Control and calibration urine samples and real urine specimens

Drug-free urine samples were taken from five healthy volunteers to prepare the matrix for control and calibration

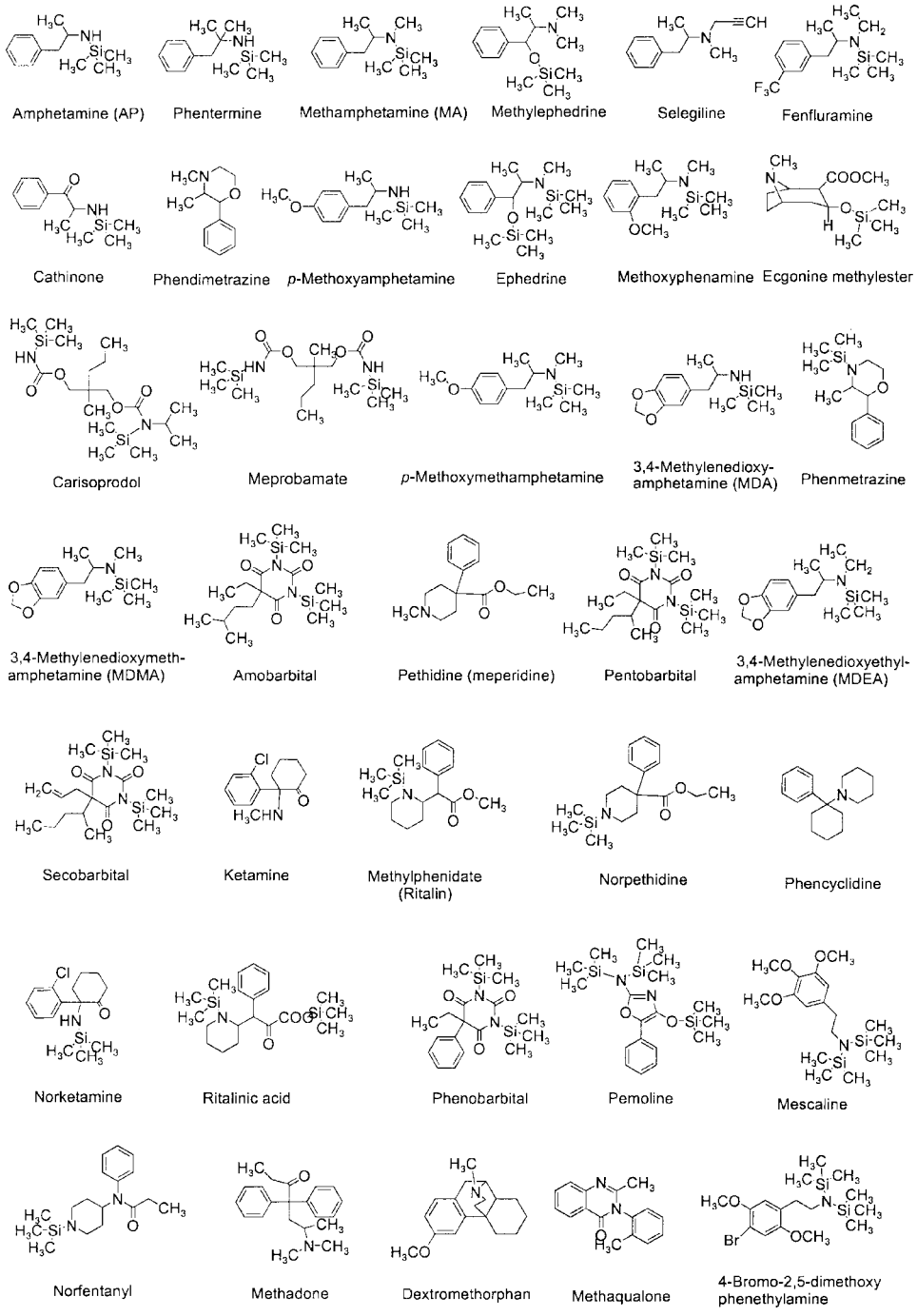

Figure 1. Molecular structures of trimethylsilylated analytes.

samples. The control and calibration samples were prepared by spiking a given amount of the working standard mix to 1 mL drug-free urine. Urine specimens were received from the Narcotics Departments at the District Prosecutors' Offices in Seoul. Eighty-two urine samples were collected from suspected drug abusers, including samples tested positive by GC-MS. Urine samples were stored in a Hetofrig CL 410 Deep Freezer (Heto, Denmark) at −60°C until analysis.

2.4 Automatic SPE and derivatization procedure

Into 10 mL glass test-tubes were added 1.0 mL of drug-free urine spiked with the working standard mix, 1.0 mL of 0.1 M phosphate buffer (pH 6.0), and 50 μL of IS mixture (containing each IS of 2 ng/μL). The samples were mixed for 10 s. Extraction was preformed by SPE using a RapidTrace SPE workstation (Caliper Life Science, Hopkinton, MA, USA). A SPE cartridge with a hydrophi-

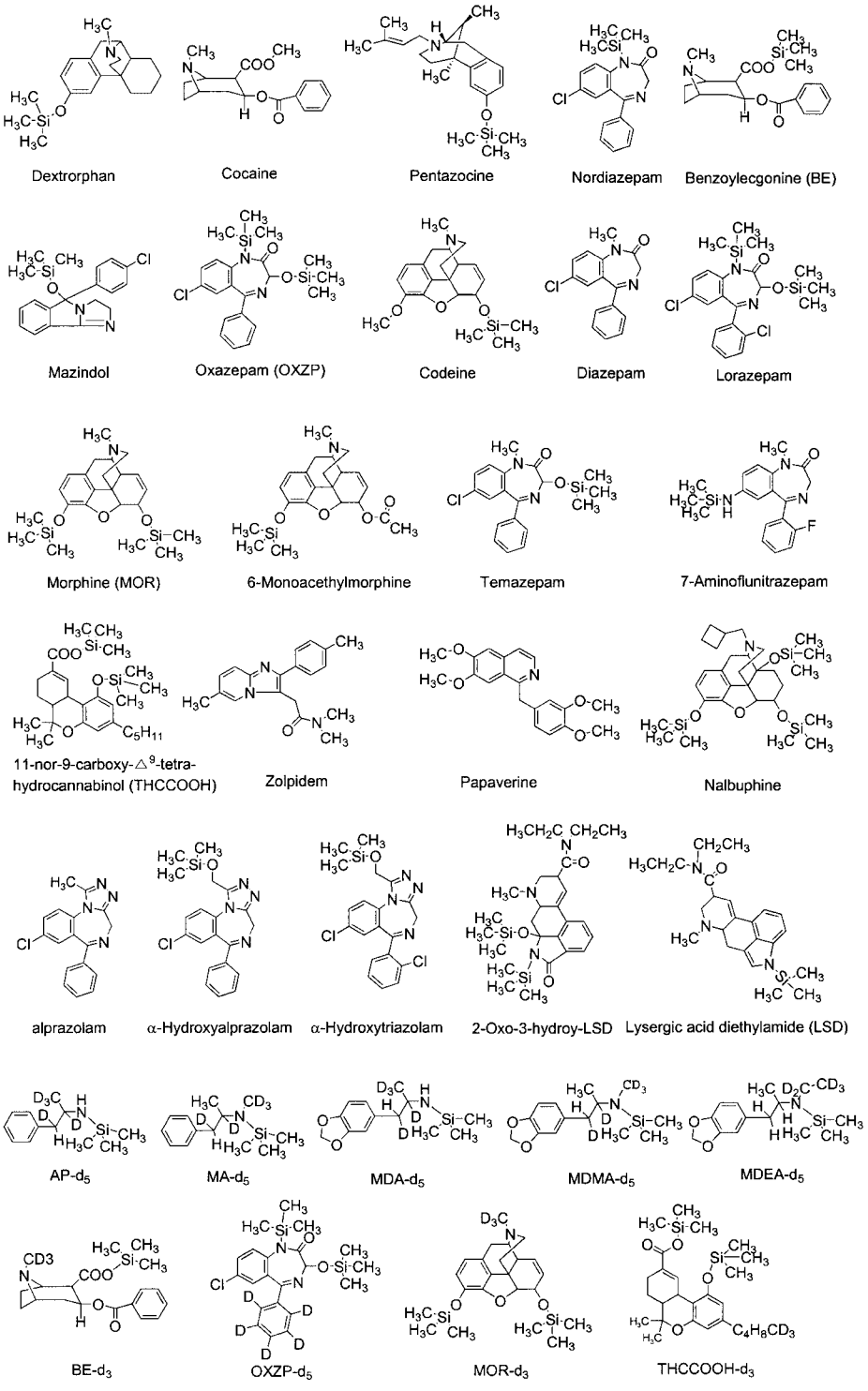

Figure 1. Continued.

lic–lipophilic balanced copolymer (60 mg, 3 mL, Waters, Milford, MA, USA) was used. The cartridge was conditioned sequentially with 2 mL MeOH and 2 mL ultra-pure water. The sample was loaded onto the cartridge, and rinsed with 1 mL 30% MeOH. The cartridge was dried under vacuum for 3 min, and the analytes were eluted with 3 mL MeOH/0.1 M HCl (98:2, v/v). The eluent was dried under a nitrogen stream at 45°C and 30 kPa. The residue was dissolved in 50 μL of MSTFA, after which every analyte was derivatized in a heating block at 70°C for 15 min. One microliter of the derivatized sample was directly injected into the GC-MS without reconstitution of the solvent.

Table 1. Retention time (RT), molecular weight (MW), ions monitored, and ion ratios for each analyte

Peak number	Analyte	Abbreviation	RT (min)	MW	Ions (*m/z*) Q1 Q2 Q3	Ion ratio (%)[a]
IS1	Amphetamine-d_5	AP-d_5-TMS	4.49	212	**120**[b]	
1	Amphetamine	AP-TMS	4.50	207	**116** 192 91	100:8.8:7.7
2	Phentermine	PT-TMS	5.27	221	**130** 114 206	100:15.0:4.2
IS2	Methamphetamine-d_5	MA-d_5-TMS	5.33	226	**134**	
3	Methamphetamine	MA-TMS	5.36	221	130 91 206	100:5.3:7.2
4	Methylephedrine	MEP-TMS	5.67	251	72 236 62	
5	Selegiline	SEL	5.85	(187)[c]	96 91 95	
6	Fenfluramine	FFA-TMS	6.02	303	144 159 288	
7	Cathinone	Khat-TMS	6.18	221	116 206 191	
8	Phendimetrazine	PDMT	6.36	(191)	**85** 117 105	
9	*p*-Methoxyamphtamine	PMA-TMS	7.01	237	**116** 121 100	100:7.5:3.5
10	Ephedrine	EP-2TMS	7.08	309	130 147 294	
11	Methoxypheamine	MPA-TMS	7.16	251	130 91 146	
12	Ecgonine methylester	EME-TMS	7.36	271	96 **82** 182	100:82.2:8.0
13	Carisoprodol	CSP-2TMS	7.50	404	160 143 176	
14	Meprobamate	MPB-2TMS	7.74	362	190 143 206	
15	*p*-Methoxymethamphetamine	PMMA-TMS	7.85	251	**130** 236 121	100:6.1:4.8
IS3	3,4-Methylenedioxyamphetamine-d_5	MDA-d_5-TMS	7.94	256	**120**	
16	3,4-Methylenedioxyamphetamine	MDA-TMS	7.97	251	**116** 236 135	100:4.8:6.2
17	Phenmetrazine	PMT-TMS	8.20	249	143 **100** 115	100:39.3:62.4
IS4	3,4-Methylenedioxymethamphetamine-d_5	MDMA-d_5-TMS	8.71	270	**134**	
18	3,4-Methylenedioxymethamphetamine	MDMA-TMS	8.74	265	130 250 135	100:5.8:3.1
19	Amobarbital	AMB-2TMS	9.13	370	355 300 285	
20	Pethidine	PTHD	9.19	(247)	247 172 218	
21	Pentobarbital	PTB-2TMS	9.46	370	285 300 355	
IS5	3,4-Methylenedioxyethylamphetamine-d_5	MDEA-d_5-TMS	9.52	284	**149**	
22	3,4-Methylenedioxyethylamphetamine	MDEA-TMS	9.55	279	**144** 264 135	100:5.4:3.4
23	Secobarbital	SCB-2TMS	9.79	382	367 297 339	
24	Ketamine	KET	10.14	(237)	**180** 138 152	100:9.9:13.5
25	Methylphenidate (ritalin)	MPD-TMS	10.16	305	156 157 118	
26	Normeperidine (norpethidine)	NPTHD-TMS	10.38	305	305 290 276	
27	Phencyclidine	PCP	10.43	(243)	200 242 186	
28	Norketamine	NKET-TMS	10.47	295	**238** 225 210	100:37.8:33.3
29	Ritalinic acid	RTA-2TMS	10.47	391	156 157 265	
30	Phenobarbital	PNB-2TMS	10.74	376	361 261 146	
31	Pemoline	PML-3TMS	10.76	392	178 163 392	
32	Mescaline	MES-2TMS	11.44	355	174 340 181	
33	Norfentanyl	NFT-TMS	11.46	304	247 289 155	
34	Methadone	MTHD	11.90	(309)[c]	72 165 91	
35	Dextromethorphan	DMP	12.02	(271)	271[b] 150 214	100:61.8:41.5
36	Mathaqualone	MTQ	12.13	(250)	235 250 233	
37	4-Bromo-2,5-dimethoxyphenethylamine	2CB-2TMS	12.17	404	174 390 175	
38	Dextrorphan	DRP-TMS	12.32	329	**329** 150 272	100:82.0:58.3
39	Cocaine	COC	12.37	(303)	**182** 303 272	100:25.7:9.0
40	Pentazocine	PTZC-TMS	12.63	357	**289** 342 357	100:37.1:26.9
41	Nordiazepam	NDZP-TMS	12.70	342	**341** 342 327	100:58.1:18.1
IS6	Benzoylecgonine-d_3	BE-d_3-TMS	12.73	364	**243**	
42	Benzoylecgonine	BE-TMS	12.74	361	**240** 361 256	100:34.3:17.8
43	Mazindol	MZ-TMS	13.07	356	245 327 267	
IS7	Oxazepam-d_5	OXZP-d_5-2TMS	13.28	436	**434**	
44	Oxazepam	OXZP-2TMS	13.29	431	429 313 415	
45	Codeine	COD-TMS	13.89	371	**371** 313 343	100:20.9:19.0
46	Diazepam	DZP	13.93	(284)	256 283 221	
47	Lorazepam	LZP-2TMS	13.96	465	429 347 449	
IS8	Morphine-d_3	MOR-d_3-2TMS	14.16	432	**432**	
48	Morphine	MOR-2TMS	14.17	429	**429** 414 401	100:51.7:32.8
49	6-Monoacetylmorphine	6MAM-TMS	14.62	399	**399** 340 287	100:68.2:44.6

Table 1. Continued

Peak number	Analyte	Abbreviation	RT (min)	MW	Ions (*m/z*) Q1 Q2 Q3	Ion ratio (%)[a)]
50	Temazepam	TMZP-TMS	14.82	372	343 372 357	
51	7-Aminoflunitrazepam	7AFTZP-TMS	15.49	355	355 327 336	
IS9	THCCOOH-d_3	THCCOOH-d_3-2TMS	15.61	491	**374**	
52	THCCOOH[d)]	THCCOOH-2TMS	15.62	488	**371** 473 488	100:26.2:16.5
53	Zolpidem	ZPD	16.34	(307)	235 307 219	
54	Papaverine	PAP	16.52	(339)	338 324 308	
55	Nalbuphine	NBP-3TMS	17.16	574	**573** 518 428	100:97.5:65.2
56	Alprazolam	APZ	17. 63	(308)	308 279 204	
57	α-Hydroxyalprazolam	OHALPZ-TMS	18.20	396	381 396 190	
58	α-Hydroxytriazolam	OHTZ-TMS	19.45	431	415 417 430	
59	2-oxo-3-hydroxy-LSD	OHLSD-2TMS	19.55	499	309 499 235	
60	Lysergic acid diethylamide	LSD-TMS	20.72	395	395 293 253	

IS: Internal standard.
a) Q1/Q1:Q2/Q1:Q3/Q1, expressed as the percentage (%).
b) Bold type: quantifier ions.
c) The MW of the underivatized analytes.
d) THCCOOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

Table 2. Calibrations, LOD and LOQ for the quantified analytes

Peak No.	Analyte	IS used	Calibration range (ng/mL)	Linearity (r^2)	LOD (ng/mL)	LOQ (ng/mL)
1	AP-TMS	IS1	25–1000	1.0000	3	10
2	PT-TMS	IS2	25–1000	0.9999	3	11
3	MA-TMS	IS2	25–1000	1.0000	6	21
8	PDMT	IS2	25–1000	0.9997	4	12
9	PMA-TMS	IS2	50–1000	0.9996	14	46
12	EME-TMS	IS6	100–1000	0.9976	22	73
15	PMMA-TMS	IS2	50–1000	0.9994	9	31
16	MDA-TMS	IS3	50–1000	1.0000	15	49
17	PMT-TMS	IS2	25–1000	0.9992	6	21
18	MDMA-TMS	IS4	100–1000	0.9999	17	56
22	MDEA-TMS	IS5	100–1000	0.9995	19	63
24	KET	IS2	25–1000	0.9996	5	18
28	NKET-TMS	IS2	25–1000	0.9997	7	23
35	DMP	IS8	25–1000	0.9966	5	16
38	DRP-TMS	IS8	25–1000	0.9908	2	5
39	COC	IS6	100–1000	0.9996	26	88
40	PTZC-TMS	IS6	25–1000	0.9996	5	16
41	NDZP-TMS	IS7	25–1000	0.9995	6	21
42	BE-TMS	IS6	100–1000	0.9996	29	98
45	COD-TMS	IS8	50–1000	0.9967	9	28
48	MOR-2TMS	IS8	50–1000	0.9961	12	39
49	6MAM-TMS	IS8	20–100	0.9979	4	15
52	THCCOOH-2TMS	IS9	10–100	0.9996	4	10
55	NBP-3TMS	IS8	50–1000	0.9974	8	27

IS1: AP-d_5, IS2: MA-d_5, IS3: MDA-d_5, IS4: MDMA-d_5, IS5: MDEA-d_5, IS6: BE-d_3, IS7: OXZP-d_5, IS8: MOR-d_3, IS9: THCCOOH-d_3.

2.5 GC-MS analysis

GC-MS analysis was performed with a 5973N mass spectrometer (Agilent Technologies, Foster City, CA, USA) equipped with a 6890N gas chromatograph and a 7683 autosampler. Data acquisition and analysis were performed using the standard software supplied by the manufacturer (Agilent Technologies, MSD ChemStation Version D.00.00). Separation was achieved on a capillary column (DB-5MS, 30 m × 0.25 mm id, 0.25 μm; J&W Scientific, Folsom, CA, USA) with helium as the carrier gas at a flow rate of 1.0 mL/min. The GC temperature program was as follows: the initial temperature of 120°C was held for 0.5 min, increased to 180°C at a rate of 10°C/min, increased to 220°C at a rate of

15°C/min, held for 0.4 min, increased to 255°C at a rate of 35°C/min, held for 0.5 min, increased to 290°C at a rate of 10°C/min, held for 0.5 min, increased to 300°C at a rate of 30°C/min, held for 5.5 min, finally increased to 310°C at a rate of 35°C/min, and held for 2.7 min. The splitless injection mode was used with a purge-on time of 0.1 min. The injector and the GC interface temperatures were 260 and 280°C, respectively. The mass spectrometer was operated at 70 eV (electron ionization) in the selected ion monitoring (SIM) mode for screening and quantification. The SIM scan time of each scan cycle was set 1.0–4.0 s, and the dwell time of each ion was set 30–80 ms. All ions considered are listed in the order of the retention times in Table 1.

2.6 Validation procedure for the quantified analytes

The method was validated with selectivity, linearity, LOD, LOQ, recovery, accuracy, and precision for the quantified analytes (24 compounds). The LODs for the analytes other than the quantified analytes were also determined. In addition, the ion ratios of fragment ions relative to a reference ion were determined for each quantified analyte to provide additional information for confirming the identification of each analyte [23].

A given amount of the working standard mix was spiked to 1 mL drug-free urine to prepare each quality control (QC) or calibration sample. The quantified analytes were analyzed at the three different QC concentrations of 150, 350, and 750 ng/mL for AP, BE, COC, COD, DMP, DRP, EME, KET, MA, MDA, MDEA, MDMA, MOR, NBP, NDZP, NKET, PDMT, PMA, PMMA, PMT, PT, and PTZC, 15, 35, and 75 ng/mL for THCCOOH, and 35 and 75 ng/mL for 6MAM, as shown in Table 2. The IS used were AP-d_5 (IS1 for AP), MA-d_5 (IS2 for KET, MA, NKET, PDMT, PMA, PMMA, PMT, and PT), MDA-d_5 (IS3 for MDA), MDMA-d_5 (IS4 for MDMA), MDEA-d_5 (IS5 for MDEA), BE-d_3 (IS6 for BE, COC, EME, and PTZC), OXZP-d_5 (IS7 for NDZP), MOR-d_3 (IS8 for COD, 6MAM, DMP, DRP, NBP, and MOR), and THCCOOH-d_3 (IS9 for THCCOOH).

2.7 Selectivity and calibrators

Drug-free and spiked urine samples were analyzed according to the described procedure to evaluate the level of interference from the urine matrix. Drug-free urine was selected among urine samples, recently collected from five individuals containing no abused drugs, in order to compare any variation in the selectivity arising from the different matrix effect. The calibration samples were prepared to evaluate the signal linearity of each analyte. The calibration curves were established with three replicates at each concentration. The concentrations were as follows: the concentration points for AP, PT, MA, PDMT, PMT, KET, NKET, DMP, DRP, PTZC, NDZP, and NBP were 25, 50, 100, 200, 300, 500, and 1000 ng/mL; for PMA, PMMA, MDA, COD, and MOR, 50, 100, 200, 300, 500, and 1000 ng/mL; for EME, MDMA, MDEA, COC, and BE, 100, 200, 300, 500, and 1000 ng/mL; for THCCOOH, 10, 20, 30, 50, and 100 ng/mL; and for 6MAM, 20, 30, 50, and 100 ng/mL. The IS mixture of 50 μL was added to each sample.

2.8 LOD and LOQ

LODs and LOQs were estimated by the relative peak height of a urine sample spiked with each analyte at a low

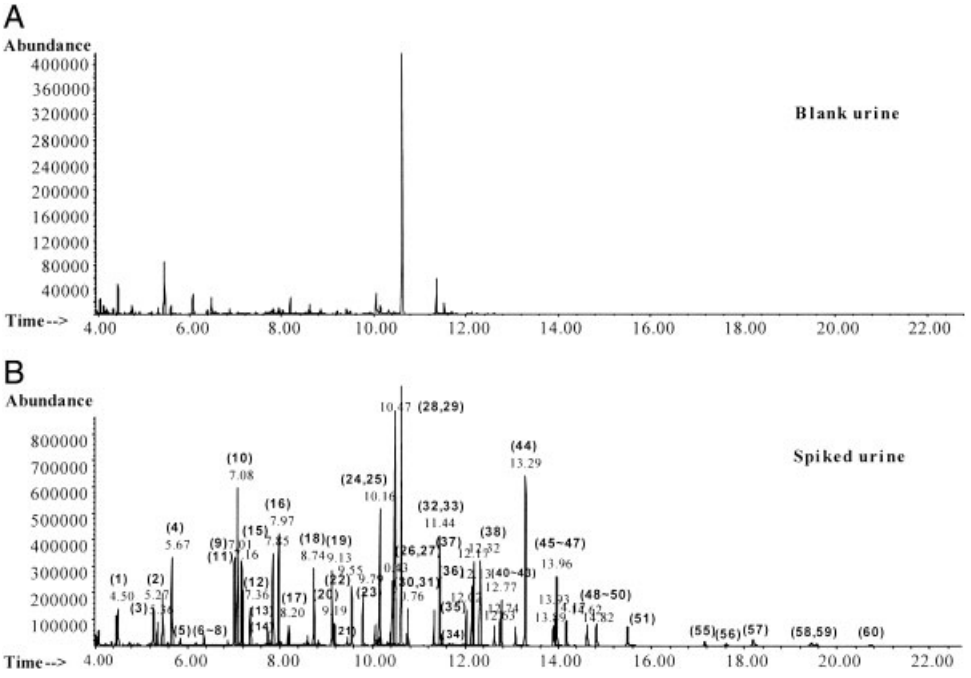

Figure 2. GC-MS chromatograms (A) in the drug-free urine sample and (B) in the urine samples spiked with 0.3 μg/mL of each analyte. Numbers in parentheses are the peak number of all the analytes listed by the retention time (as specified in Table 1).

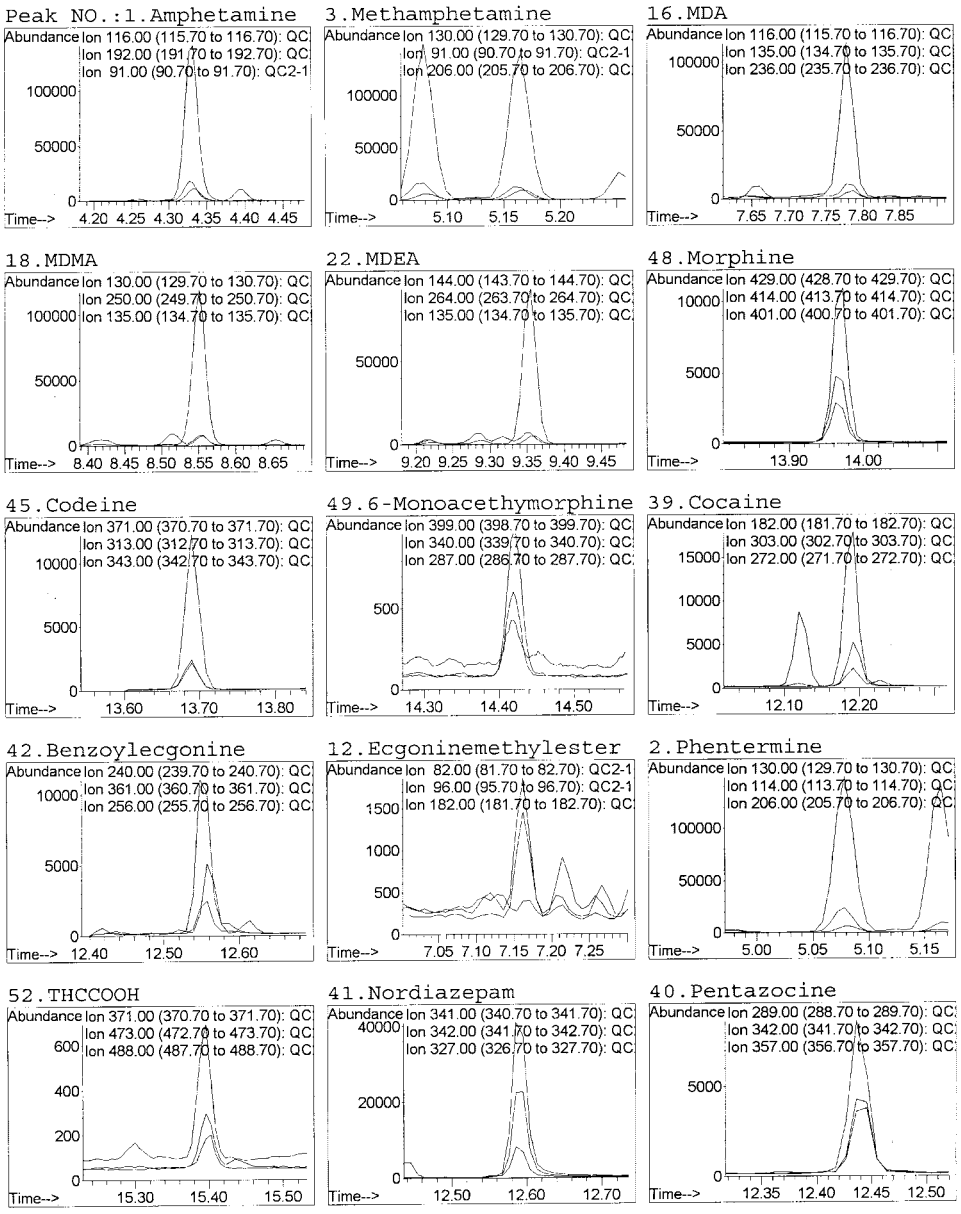

Figure 3. SIM chromatograms of the spiked urine samples at 0.15 μg/mL (THCCOOH, 6MAM: 0.015 μg/mL) of the following quantified analytes: AP (peak number; 1), MA (3), MDA (16), MDMA (18), MDEA (22), MOR (48), COD (45), 6MAM (49), COC (39), BE (42), EME (12), PT (2), THCCOOH (52), NDZP (41), PTZC (40), PDMT (peak number; 8), PMT (17), PMA (15), PMMA (9), KET (24), NKET (25), DMP (35), DRP (38), and NBP (55).

concentration based on the mean of five replicates. The concentration ranged 5–100 ng/mL, depending upon analytes. LOD was determined at a S/N ratio equal to 3 ($S/N = 3$) and LOQ, equal to 10 ($S/N = 10$).

2.9 Recovery, accuracy and precision

The relative recovery of SPE was determined by comparing the area ratios of the following two sets of runs. The first set was performed with a urine sample to which the analyte had been added after the SPE elution, while the second set was performed with a urine sample to which the analyte had been added before SPE. Recovery was computed by dividing the area ratio (the analyte to the IS) of the latter by the area ratio of the former. The IS mixture was added after SPE for the two sets of runs, of course. Recovery was determined based on the average of five replicates according to the procedure described. The inter-day accuracy and precision were determined for three independent experimental days, and the inter-person accuracy and precision were determined for three different operators using the same method described. Precision was expressed as the coefficient of variations (% CV) for the replicate measurements at each QC concentration. Accuracy

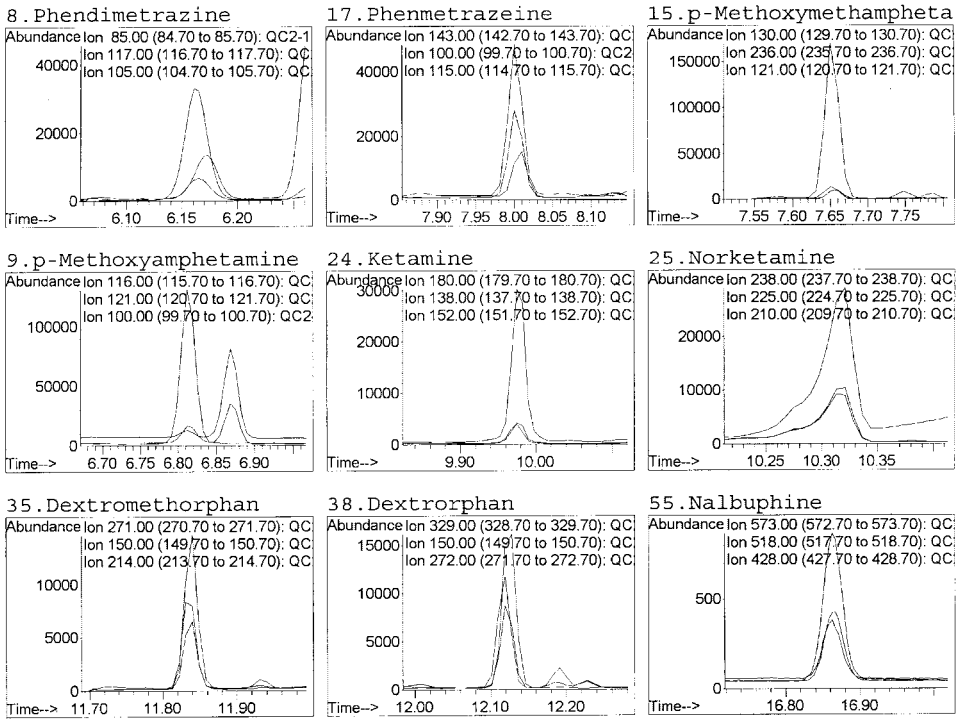

Figure 3. Continued.

(% bias) was calculated by the difference between the measured and nominal concentrations of the fortified samples.

3 Results and discussion

3.1 Derivatization and molecular structures of trimethylsilylated analytes

MSTFA was used as the derivatizing reagent for the simultaneous derivatization of the structurally different analytes. silylation has been known to yield simple and rapid derivatization for most compounds containing amine (–NH, $–NH_2$), hydroxyl (–OH), or carboxylic (–COOH) groups. In our previous experience, MSTFA was the most effective derivatization reagent for determining ATS (AP, MA, MDA, MDMA) and THCCOOH [24]. Silylation using *N,O*-bis(trimethylsilyl)fluoroacetamide with 1% trimethylchlorosilane was not effective because of the low sensitivity due to the existing secondary amine (MA or MDMA). Thus, MSTFA was chosen as the reagent in this study. The optimum temperature and time were found 70°C and 15 min after many trials at various temperatures and different reaction times. This condition was then applied throughout. MSTFA was capable of derivatizing all the analytes with hydroxyl, phenolic, carboxylic, amine, or amide groups in this condition, and the trimethylsilylated analytes were well separated by GC-MS. The structures of all the silylated analytes are shown in Fig. 1. The ability of functional groups to form TMS decreased in the following order: alcohols > phenols > carboxylic acids > amines > amides [11]. The primary amine type analytes were generally derivatized to mono-TMS. EP and OHLSD with an amine ($–NH_2$) group and a hydroxyl (–OH) group were derivatized to di-TMS.

3.2 GC temperature program and SIM

All the analytes (60 compounds) were well analyzed within 21 min by GC-MS using SIM owing to the different characteristic ions and retention times, as shown in Fig. 2. The temperature program was rather complex. The GC temperature program was tested many times in order to present well shaped ion peaks and achieve maximum separation of the analytes. The chromatograms of Fig. 3 were obtained from the urine samples to which had been added the standard solution of the analytes at the concentration of 150 ng/mL (THCCOOH, 6MAM; 15 ng/mL). The most intensive ions were chosen for each analyte, excluding ions with small molecular mass below m/z 70 and the trimethylsilylated base peak (m/z 73). NKET peaks did not show good shapes. The qualifier ions for PML were also difficult to determine. Different qualifier ions (m/z 392, 178, 171) for PML were presented by Strano-Rossi *et al.* [9].

3.3 Selection of internal standards

In this method, several kinds of IS were used for the popularly abused illicit drugs, such as ATS and their metabolites (AP, MA, MDA, MDMA), cannabinoids metabolite (THCCOOH), opiates, heroin, and their metabolites (MOR, COD, 6MAM), and COC and its metabolites (BE, EME), as shown in Table 2. AP-d_5 was used for AP for the quantification, MA-d_5 for MA, THCCOOH-d_3 for THCCOOH, MOR-d_3 for opiate-like drugs (COD, MOR, DMP, DRP, 6MAM, NBP), and BE-d_3 for COC and its metabolites. The basic rule of IS selection was structural similarity between the commercially available IS and the analyte.

Table 3. LODs of some other analytes, excluding the quantified analytes

LOD (ng/mL)	Analytes
5	LZP, MZ, OXZP, PCP, TMZP
10	DZP, EP, FFA, MEP, MPA, MTHD, MTQ, NFT, RTA, SCB
15	AMB, LSD, OHTZ, 2CB, SEL, PAP
20	OHLSD, CSP, MPB, 7AFTZP
25	MES, PML, ZPD, NPTHD
40	PNB
45	ALPZ, OHALPZ
60	MPHD, Khat
75	PTB

3.4 Validation results

The method was performed by using a small volume of urine (1 mL) for a reduced matrix effect. The SPE procedure was extensively tested by changing the values of major parameters such as sample volume, kind of rinse and extraction solvent, and solvent volume. The presented extraction procedure was found to be suitable for decreasing the matrix effect of the blank urine sample. From the analysis of five blank urine samples, the selectivity exhibited no significant variation for each analyte. When an overlapping was observed for an ion between the analyte and the blank urine, the ion was removed from the list of qualified ions, and only the analyte ions without matrix interferences were chosen for SIM analysis.

The calibration range, linearity, LOD, and LOQ of the quantified analytes (24 compounds) are listed in Table 2. The LODs of the other analytes are presented in Table 3. The quantified analytes were selected as most frequently abused drugs among the 60 ingredients that can be monitored by

Table 4. Recovery, inter-day/inter-person accuracy and precision

Peak number	Analyte	Recovery (%, $n = 5$)	Inter-day ($n = 3$)		Inter-person ($n = 3$)	
			Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)	Precision (% CV)
		150/350/750[a)]	150/350/750	150/350/750	150/350/750	150/350/750
1	AP-TMS	92/89/87	0.2/1.1/0.7	0.9/1.3/1.5	−0.1/1.9/0.8	0.8/1.1/1.6
2	PT-TMS	97/99/92	6.0/3.7/3.9	3.1/3.0/2.8	4.0/5.1/3.6	5.4/3.1/4.3
3	MA-TMS	95/94/94	1.1/0.3/0.7	0.9/1.2/1.1	−10.0/1.9/0.8	1.0/1.7/1.8
8	PDMT	80/100/90	4.7/6.1/−6.6	6.9/4.4/4.3	5.3/8.7/−9.3	8.7/6.9/7.4
9	PMA-TMS	89/97/90	4.9/9.8/4.7	4.8/4.6/3.5	2.6/3.9/3.1	9.9/8.8/6.9
12	EME-TMS	85/81/81	14.0/4.4/4.6	4.8/6.1/5.7	9.8/12.0/11.5	6.9/9.2/5.2
15	PMMA-TMS	96/96/87	5.4/4.6/8.1	6.5/5.3/4.9	3.2/7.4/6.8	7.2/4.7/6.5
16	MDA-TMS	92/91/90	−2.7/−1.5/−2.1	1.2/2.8/4.1	−1.3/0.9/1.3	1.0/1.3/2.5
17	PMT-TMS	83/94/92	6.1/10.4/5.7	3.8/7.1/5.2	−2.4/9.1/5.2	8.7/5.0/6.2
18	MDMA-TMS	97/95/95	3.6/−0.6/0.9	1.4/1.6/1.5	1.6/0.4/0.8	1.3/1.9/1.4
22	MDEA-TMS	95/89/93	3.6/3.1/0.7	3.1/3.5/4.6	2.4/2.3/0.5	2.2/2.2/2.9
24	KET	96/101/96	−5.5/13.4/10.4	4.6/4.0/12.9	−0.9/7.1/4.7	10.2/8.3/12.6
28	NKET-TMS	126/100/117	2.0/5.4/−3.9	7.3/7.0/5.3	3.2/5.6/4.6	8.6/8.4/6.5
35	DMP	93/96/100	8.8/12.4/3.2	4.0/3.2/3.6	6.1/11.2/3.0	5.0/2.8/4.5
38	DRP-TMS	95/97/100	11.0/14.9 /2.5	3.4/1.3/6.2	4.9/13.0/3.4	4.6/2.5/4.5
39	COC	98/100/105	−3.4/−4.0/−6.7	2.8/4.0/3.0	−3.5/−2.2/2.5	2.1/2.8/4.2
40	PTZC-TMS	96/98/107	−1.3/4.8/3.0	3.8/5.2/7.1	−0.3/2.4/−0.9	3.7/3.1/4.1
41	NDZP-TMS	90/96/96	−6.5/−4.0/0.7	2.4/3.4/3.9	−3.6/6.0/8.6	3.4/3.2/4.7
42	BE-TMS	76/84/78	0.6/0.8/1.3	2.2/1.5/1.9	−2.8/0.9/2.4	4.0/4.1/2.5
45	COD-TMS	94/98/97	−1.9/8.2/1.5	1.8/1.5/1.1	−2.7/9.0/2.8	1.9/1.3/1.7
48	MOR-2TMS	90/94/92	−0.6/7.2/2.2	1.3/0.8/0.9	−0.5/4.6/3.5	2.9/1.7/2.0
49	6MAM-TMS	−/98/97	−/6.4/1.1	−/1.8/1.6	−/7.0/2.6	−/2.3/1.7
52	THCCOOH-2TMS	80/84/84	−5.9/2.6/7.9	5.4/3.5/7.0	−3.2/4.0/−1.4	5.4/4.2/6.7
55	NBP-3TMS	62/72/68	−13.3/−8.6/11.7	7.5/11.1/9.0	−1.10/−7.1/8.8	3.5/9.9/6.5

a) Quality control (QC) concentrations of 150, 350, and 750 ng/mL (THCCOOH-2TMS: 15, 35, 75 ng/mL; 6MAM-TMS: 35, 75 ng/mL).

this method. The method showed a good linearity and low LODs despite a variety of different chemical structures of the analytes. The lowest concentration of the calibration range was set above the LOQ for each analyte. The square of the correlation coefficient (r^2) was from 0.990 to 1.000 for the quantified analytes. Especially it was very good for AM, MA, and MDA ($r^2 = 1.000$) in the concentration range from 25 to 1000 ng/mL. DRP, the major metabolite of DMP, showed a low LOD of 2 ng/mL and a LOQ of 5 ng/mL, but COC and COC metabolites (EME, BE) showed relatively high LODs (22–29 ng/mL) and LOQs (73–98 ng/mL).

The extraction recoveries of the quantified analytes at three different concentrations were in the range of 62–126%, as shown in Table 4. The lowest limit was 62–72% for NBP, and the highest limit, 100–126% for NKET. The recovery over 100% was observed only for NKET, and it is suspected that there is an unknown sticky urine component that is captured in the SPE cartridge and released slowly to cause interferences in detection of NKET yielding such an anomalous recovery. The reproducibility of replicate measurements for SPE recovery was better than 11.6%.

The results of the inter-day accuracy and precision, and the inter-person accuracy and precision are shown in Table 4. The inter-day accuracies (% bias) were within −13.3 to 14.9% and the inter-person accuracies were within −10.1 to 13.0%. The inter-day and inter-person accuracies of EME, PMT, KET, DMP, DRP, and NBP were within the range of −10.0∼10.0%. The inter-day precisions (% CV) were less than 7.3%, excluding KET (4.6–12.9%) and NBP (7.5–11.1%). The inter-person precisions were mostly less than 9.9%, excluding KET (8.3–12.6%).

3.5 Ion ratios for each analyte

The ion ratios of the quantified analytes (24 compounds) in human urine are shown in Table 1. The relative ion intensities were calculated as Q1/Q1, Q2/Q1, and Q3/Q1, and expressed as the percentage of the mean of the three different QC concentrations. The criteria of the US FDA for the accepted range of relative intensity fluctuation are within ±10% in the three-ion and SIM mode, and within ±20% in the three-ion and full-scan mode. However, EU exhibited a different rule for the accepted variation range of relative intensity (%). When the relative intensity is above 50% or below 10%, the ion ratio fluctuation should be within ±10% or ±50%, respectively [23]. Table 5 presents the ion ratios and variations of each analyte in real samples within the calibration ranges. The relative variations of MA, MDMA, and MOR were within ±10%. The relative variations of all the analytes were within ±20%.

3.6 Application to real urine samples

The method was applied to the urine samples ($n = 82$) collected from the suspected drug abusers. The results are shown in Table 6. DMP, MA, MDMA, NBP, PT, KET, and opiates as well as their metabolites (AP, BE, COD, DRP, MDA, MOR, NKET, THCCOOH) were detected and quantified in the urine samples. Interestingly, not COC or its metabolite (EME) but BE was detected in a suspected COC abuser's sample. FFA, LZP, PMT, PTB, and PTHD were also detected in some of the samples.

Table 5. Ion ratios range of each analyte in urine samples

Aanalyte	Sample	Q2/Q1[a)]	Q3/Q1
AP-TMS	QC[b)]	7.9–9.7	6.9–8.5
	samples ($n = 8$)	7.8–11.7	7.1–8.4
MA-TMS	QC	4.8–5.8	6.5–7.9
	samples ($n = 8$)	5.1–5.4	6.9–7.6
MDA-TMS	QC	4.3–5.3	5.5–6.7
	samples ($n = 2$)	4.9–5.1	5.1–6.1
MDMA-TMS	QC	5.2–6.4	2.8–3.4
	sample ($n = 1$)	5.7	3.4
COD-TMS	QC	19.2–23.4	17.1–20.9
	samples ($n = 8$)	19.8–22.3	16.9–19.6
MOR-2TMS	QC	47.5–58.1	29.8–36.4
	samples ($n = 14$)	50.4–56.6	31.0–34.7

a) Range of ion ratios (Q2/Q1, Q3/Q1), expressed as the percentage (%).
b) Mean of ion ratio at each different concentration (QC).

Table 6. Analyzed results of real samples from the suspected drug abusers

Analytes	Samples (n)	Detected samples[a)]	Concentration range (ng/mL)
MA/AP	9	9/9	54.7–705.7/13.2–343.4
MDMA/MDA	14	10/10	328.2-HI[b)]/75.2-HI
THCCOOH	4	3	12.2
MOR/COD	25	15/9	62.9–945.3/79.8–431.1
NBP	4	4	35.8-HI
DMP/DRP	15	13/13	43.4-HI/100.8-HI
BE[c)]	1	1	HI
PT	4	4	305.2-HI
KET/NKET	1	1/1	HI/HI

a) The samples are above the LOD.
b) The results of quantification are above the calibration range of each analyte.
c) No detection the cocaine or its metabolite (EME); only BE detected.

4 Concluding remarks

A method was presented for the simultaneous screening and quantification of multiple illicit drugs and their metabolites in urine samples. Simple and rapid procedures of SPE, TMS, and GC-MS were incorporated. This method can be applied for screening 60 target analytes with LOD of 2–75 ng/mL. The method validation was also successfully

performed in quantitative analysis for 24 highly abused components. Thus the urine samples from the Narcotics Departments at the District Prosecutors' Offices were reliably screened and quantified.

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The authors have declared no conflicts of interest.

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