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A LC-MS/MS method for determination of 73 synthetic cathinones and related metabolites in urine

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ABSTRACT

Synthetic cathinones, which are a group of β-keto analogs of phenethylamine, have been reported as the most emerging new psychoactive substances in the past decade. The quantity and variety of synthetic cathinones have continued to increase, which poses considerable risks to public health and social security. In this study, an analytical method based on liquid chromatography-tandem mass spectrometry (LC—MS/MS) was established for the simultaneous determination of 73 synthetic cathinones and related metabolites in urine. The chromatographic analysis was performed using a Kinetex® Biphenyl column (10 cm ×2.1 mm, 1.7 μm), applying a gradient mobile phase, comprising 0.1 % formic acid aqueous solution with 5 mM ammonium acetate and 0.1 % formic acid methanolic solution; the entire run time of the analysis was within 8 min. The multiple reaction monitoring (MRM) mode was employed to collect the monitoring and quantitative ion pairs. Intra-day/inter-day precision and accuracy were less than 10 % for all the studied analytes. The limits of detection and quantification for all the analytes were 0.1–0.5 ng/mL and 0.5–1.0 ng/mL, respectively. The matrix effect was satisfactory for all the analytes, with a deviation lower than 20 %. The present method was further applied to 67 authentic urine samples in which 13 different synthetic cathinones were detected from 32 positive samples. The abuse of poly-synthetic cathinones was examined that up to seven items was detected in one case from authentic samples in this study.

1. Introduction

New psychoactive substances (NPS) have emerged as a threat in recent years. According to the UNODC World Drug Report 2018, the top five NPS in 2017 by amount were synthetic cannabinoids, ketamine, synthetic cathinones, tryptamines, and phenethylamines [1]. In Europe, more than 670 NPS were being monitored by 2017 [2]. To evade the law, increasing varieties of NPS are synthesized. Among these compounds, synthetic cathinones are notorious for their varieties and hazards.

Cathinone, a natural stimulant produced by the “khat” plant, has been used for a long history in East Africa and the Arabian Peninsula [3]; it resembles amphetamine in terms of chemical structure and physical effects, and has been controlled by UNODC listing in Schedule I of the 1971 Single Convention on Psychotropic Substances. In the 1920s, some compounds mimicking the chemical structure of cathinone, such as methcathinone and mephedrone, were synthesized and regarded as the first synthetic cathinones [4]. Since then, more and more related compounds were synthesized. Synthetic cathinones are β-keto analogs of phenethylamine inhibiting transport of monoamines, such as serotonin, norepinephrine, and dopamine, and even affect central nervous system function by increasing synaptic concentrations of monoamines [5,6].

Initially, synthetic cathinones were produced for medicinal purposes; however, the severe side effects of these compounds overrode the advantages in medical use [7]. Synthetic cathinones were labeled as “legal highs” or “bath salts” and the production and abuse have been increasing worldwide since the 2000s [8,9,10,11,12]. Fatalities are continuously reported since the first report was revealed in Europe in 2008 [13]. The poly-drug abuse related to synthetic cathinones and other substances has been observed frequently among drug users and poses a challenge in terms of identifying targets from similar analogs. The chromatography coupled with mass spectrometer (MS) have introduced the ion monitoring mode (e.g. selected ion monitoring of MS or multiple reaction monitoring of MS/MS) as an effective tool for screening NPS of same category. This technique increases selectivity and sensitivity of target analysis by means of designating ions of desired analytes and decreasing noise from non-target

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Table 1
MRM parameters of 73 target analytes and 14 IS of synthetic cathinones.

Ite m	Analyte	Retention time (min)	Ion pairs Precursor (*m/z*) > Product (*m/z*)	DP (V)	CE (eV)	Referential IS (by Item)
1	Cathinone	1.73	150 > 132*	19	16	80
			150 > 117	19	30	
2	Methcathinone	1.99	164 > 146*	33	17	74
			164 > 131	33	28	
3	Ethcathinone	2.36	178 > 132*	48	24	83
			178 > 130	48	40	
4	Mephedrone (4-methylmethcathinone)	3.08	178 > 145*	37	28	75
			178 > 144	37	39	
5	*N*-EC ephedrine (metabolite of ethylcathinone)	2.08	180 > 117*	19	29	78
			180 > 115	19	39	
6	4-Methylephedrine (metabolite of mephedrone)	2.58	180 > 147*	24	29	76
			180 > 91	24	35	
7	3, 4-DMMC norephedrine (metabolite of 3, 4-DMMC)	2.97	180 > 162*	22	15	77
			180 > 130	22	33	
8	4-FMC (4-fluoromethcathinone)	2.07	182 > 164*	25	18	78
			182 > 149	25	28	
9	4-Fluoroephedrine (metabolite of 4-FMC)	1.84	184 > 135*	17	27	77
			184 > 151	17	29	
10	4-EMC (4-ethylmethcathinone)	4.12	192 > 144*	54	40	83
			192 > 77	54	67	
11	4-MeMABP (4-methylbuphedrone)	3.78	192 > 145*	28	29	80
			192 > 161	28	16	
12	3, 4-DMMC (3, 4-dimethylmethcathinone)	4.05	192 > 159*	63	30	75
			192 > 158	63	41	
13	4-MEC (4-methylethcathinone)	3.48	192 > 174*	41	17	75
			192 > 130	41	48	
14	Methedrone (4-methoxymethcathinone)	2.84	194 > 161*	38	27	81
			194 > 118	38	50	
15	4-Methyl-*N*-ethyl-norephedrine (metabolite of 4-MEC)	3.04	194 > 176*	33	17	79
			194 > 131	33	28	
16	4-FEC (4-fluoroethcathinone)	2.41	196 > 178*	44	17	80
			196 > 150	44	26	
17	4-CMC (4-chloromethcathinone)	3.04	198 > 145*	34	26	85
			198 > 144	34	40	
18	α-PPP (alpha-pyrrolidinopropiophenone)	2.96	204 > 105*	70	29	83
			204 > 98	70	33	
19	MPD (methylpentedrone)	4.51	206 > 144*	62	44	80
			206 > 105	62	27	
20	4-EEC (4-ethylethcathinone)	4.45	206 > 188*	44	18	83
			206 > 159	44	27	
21	4-MeOEC (4-methoxyethcathinone)	3.24	208 > 146*	55	40	75
			208 > 175	55	26	
22	Mexedrone	3.40	208 > 158*	41	19	80
			208 > 176	41	17	
23	Methylone	2.52	208 > 160*	30	24	80
			208 > 132	30	37	
24	α-PPT (alpha-pyrrolidinopropiothiophenone)	2.46	210 > 98*	68	29	84
			210 > 111	68	33	
25	4-CDC (4-chlorodimethylcathinone)	3.26	212 > 139*	43	28	80
			212 > 167	43	22	
26	4-CEC (4-chloroethcathinone)	3.43	212 > 194*	49	19	80
			212 > 159	49	25	
27	4-MPPP (4-methyl-α-pyrrolidinopropiophenone)	4.04	218 > 119*	30	34	80
			218 > 147	30	25	
28	4-MEAPP (4-methyl-α-ethylaminopentiophenone)	4.79	220 > 105*	54	30	74
			220 > 160	54	26	
29	*N*-Ethyl hexedrone (alpha-ethylaminohexanophenone)	4.62	220 > 130*	59	48	80
			220 > 146	59	25	
30	4-F-α-PPP (4-fluoro-alpha-pyrrolidinopropiophenone)	2.92	222 > 123*	34	32	85
			222 > 98	34	34	
31	Butylone	3.21	222 > 131*	35	48	81
			222 > 191	35	17	
32	Ethylone	2.94	222 > 174*	33	25	82
			222 > 146	33	35	
33	α-PBT (alpha-pyrrolidinobutiothiophenone)	3.04	224 > 112*	66	29	85
			224 > 153	66	22	

Table 1 (*Continued*)

Ite m	Analyte	Retention time (min)	Ion pairs Precursor (*m/z*) > Product (*m/z*)	DP (V)	CE (eV)	Referential IS (by Item)
34	α-PVP (alpha-pyrrolidinovalerophenone)	4.30	232 > 91* 232 > 126	55 55	31 35	83
35	4-Methyl-α-PBP (4-methyl-alpha-pyrrolidinobutiophenone)	4.56	232 > 105* 232 > 161	78 78	35 24	85
36	α-PVP metabolite 1 (metabolite of α-PVP)	4.45	234 > 72* 234 > 91	63 63	25 39	83
37	MOPPP (4-methoxy-alpha-pyrrolidinopropiophenone)	3.78	234 > 98* 234 > 135	78 78	28 32	87
38	4-F-α-PBP (4-fluoro-alpha-pyrrolidinobutiophenone)	3.51	236 > 109* 236 > 165	43 43	36 24	83
39	Pentylone	4.04	236 > 188* 236 > 218	32 32	24 18	80
40	bk-DMBDB (dibutylone)	3.45	236 > 191* 236 > 149	50 50	20 32	80
41	4-Cl-α-PPP (4-chloro-alpha-pyrrolidinopropiophenone)	3.97	238 > 139* 238 > 98	66 66	34 39	82
42	2, 5-Dimethoxy mephedrone (2, 5-dimethoxy-4-methylmethcathinone)	4.67	238 > 220* 238 > 189	26 26	17 28	75
43	4-BMC (4-bromomethcathinone)	3.42	242 > 145* 242 > 128	37 37	23 61	75
44	α-PHP (alpha-pyrrolidinohexanophenone)	4.94	246 > 91* 246 > 140	81 81	32 35	85
45	Pyrovalerone	5.06	246 > 105* 246 > 126	81 81	32 33	85
46	3, 4-MDPPP (3, 4-methylenedioxy-alpha-pyrrolidinopropiophenone)	3.51	248 > 98* 248 > 149	73 73	30 34	80
47	4-MeOPBP (4-methoxy-alpha-pyrrolidinobutiophenone)	4.35	248 > 121* 248 > 135	58 58	38 36	82
48	4-F-α-PVP (4-fluoro-alpha-pyrrolidinovalerophenone)	4.22	250 > 109* 250 > 126	64 64	32 35	80
49	D-Tertylone (3, 4-methylenedioxy-N-tert-butylcathinone)	4.11	250 > 194* 250 > 146	18 18	18 29	80
50	Ephylone (*N*-ethylpentylone)	4.39	250 > 202* 250 > 232	40 40	26 21	85
51	bk-DMBDP (*N*, *N*-dimethyl pentylone)	4.27	250 > 205* 250 > 175	59 59	22 28	80
52	Benzedrone	5.45	254 > 91* 254 > 65	36 36	45 73	85
53	*N*-BMC (*N*-benzylmethcathinone)	5.11	254 > 162* 254 > 146	42 42	21 22	74
54	4-BEC (4-bromoethcathinone)	3.83	256 > 159* 256 > 144	50 50	24 39	74
55	α-PHPP (alpha-pyrrolidinoheptiophenone)	5.49	260 > 91* 260 > 154	85 85	32 38	85
56	4-Methyl-α-PHP (4-methyl-alpha-pyrrolidinohexanophenone)	5.53	260 > 105* 260 > 140	93 93	31 37	85
57	3, 4-Dimethyl-α-PVP (3, 4-dimethyl-alpha-pyrrolidinovalerophenone)	5.56	260 > 119* 260 > 126	45 45	31 34	85
58	3, 4-MDPBP (3, 4-methylenedioxy-alpha-pyrrolidinobutiophenone)	4.15	262 > 112* 262 > 161	60 60	32 31	80
59	4-MeO-α-PVP (4-methoxy-alpha-pyrrolidinovalerophenone)	4.09	262 > 121* 262 > 126	75 75	34 30	85
60	4-F-PHP (4-fluoro-alpha-pyrrolidinohexanophenone)	4.86	264 > 109* 264 > 140	80 80	33 37	86

Table 1 (*Continued*)

Ite m	Analyte	Retention time (min)	Ion pairs Precursor (*m/z*) > Product (*m/z*)	DP (V)	CE (eV)	Referential IS (by Item)
61	4-Cl-α-PVP (4-chloro-alpha-pyrrolidinovalerophenone)	5.00	266 > 125* 266 > 195	69 69	34 25	85
62	Indanyl-α-PVP (3, 4-trimethylene-alpha-pyrrolidinovalerophenone)	6.02	272 > 131* 272 > 201	74 74	34 26	85
63	α-POP (alpha-pyrrolidinooctanophenone)	6.02	274 > 91* 274 > 168	97 97	33 36	85
64	MDPV (methylenedioxypyrovalerone)	4.75	276 > 205* 276 > 126	79 79	25 35	86
65	4-F-PHPP (4-fluoro-alpha-pyrrolidinoheptiophenone)	5.41	278 > 109* 278 > 154	44 44	33 38	85
66	Demethylenyl-methyl-MDPV (metabolite of MDPV)	3.35	278 > 175* 278 > 126	80 80	27 34	86
67	4-Br-α-PPP (4-bromo-alpha-pyrrolidinopropiophenone)	4.33	282 > 132* 282 > 98	72 72	32 34	83
68	Naphyrone	5.78	282 > 141* 282 > 211	100 100	36 26	85
69	TH-PVP (3, 4-tetramethylene-alpha-pyrrolidinovalerophenone)	6.66	286 > 145* 286 > 215	82 82	35 28	87
70	α-PNP (alpha-pyrrolidinononanophenone)	6.61	288 > 91* 288 > 182	40 40	35 39	87
71	4-Methoxy PHPP (4-methoxy-alpha-pyrrolidinoheptiophenone)	5.90	290 > 121* 290 > 219	87 87	33 25	87
72	TH-PHP (3, 4-tetramethylene-alpha-pyrrolidinohexanophenone)	7.16	300 > 145* 300 > 140	79 79	36 39	87
73	4-Methoxy-α-POP (4-methoxy-alpha-pyrrolidinooctanophenone)	6.50	304 > 121* 304 > 233	73 73	34 26	87
74	Methcathinone-d_3	1.98	167 > 130*	26	40	–
75	Methedrone-d_3	3.05	181 > 148*	31	31	–
76	4-Methylephedrine-d_3	2.56	183 > 131*	22	27	–
77	3, 4-DMMC norephedrine-d_3	7.09	183 > 105*	27	24	–
78	*N*-EC ephedrine-d_5	2.07	185 > 115*	24	41	–
79	4-Methyl-*N*-ethyl-norephedrine-d_5	3.02	199 > 131*	33	28	–
80	Methylone-d_3	2.51	211 > 163*	29	25	–
81	Butylone-d_3	3.20	225 > 177*	35	26	–
82	Ethylone-d_5	2.92	227 > 179*	28	26	–
83	α-PVP-d_8	4.27	240 > 91*	85	32	–
84	3, 4-MDPPP-d_8	3.47	256 > 106*	80	31	–
85	3, 4-MDPBP-d_8	4.11	270 > 161*	82	33	–
86	3, 4-MDPV-d_8	4.72	284 > 134*	91	36	–
87	Naphyrone-d_5	5.91	287 > 141*	80	34	–

* quantifier.

compounds [14]. Therefore, chromatography techniques, such as GC–MS and LC–MS/MS, are effective tools employed frequently in forensic and clinical toxicology applications for the detection of versatile psychoactive substances and are time-saving with run times less than 40 min [15,16,17,18].

LC–MS/MS is a technique widely used in laboratories due to its superior sensitivity, selectivity, and adaptability compared to GC—MS [18–20]. LC—MS/MS methods for the determination of synthetic cathinones have been reported previously. Waters et al. consolidated a GC—MS/MS and LC—MS/MS database for the detection of psychotropic compounds comprising 29 synthetic cathinones [21]. In addition, several studies have applied LC—MS/MS for the determination of 5–11 synthetic cathinones with varying limit of detection (LOD) and limit of quantification (LOQ) [19–22]. However, more synthetic cathinones have been synthesized and abused with time in recent years. The capability of previously reported methods was limited in detecting synthetic cathinones that are more diverse. Meanwhile, most studies focused on developing methods for the analysis of multi-type drugs rather than specific group of analytes such as synthetic cathinones.

To expand the applicability and variety for detecting synthetic cathinones, this study aimed to develop a sensitive method to simultaneously determine 73 synthetic cathinones and related metabolites in urine using LC–MS/MS. The present method was further applied to analyze authentic samples to examine the synthetic cathinones abused in Taiwan.

2. Materials and methods

2.1. Reagents

Reference standards of pyrovalerone, 3, 4-methylenedioxypyrovalerone metabolite 1 (demethylenyl-methyl-MDPV, metabolite of MDPV) and α-pyrrolidinovalerophenone

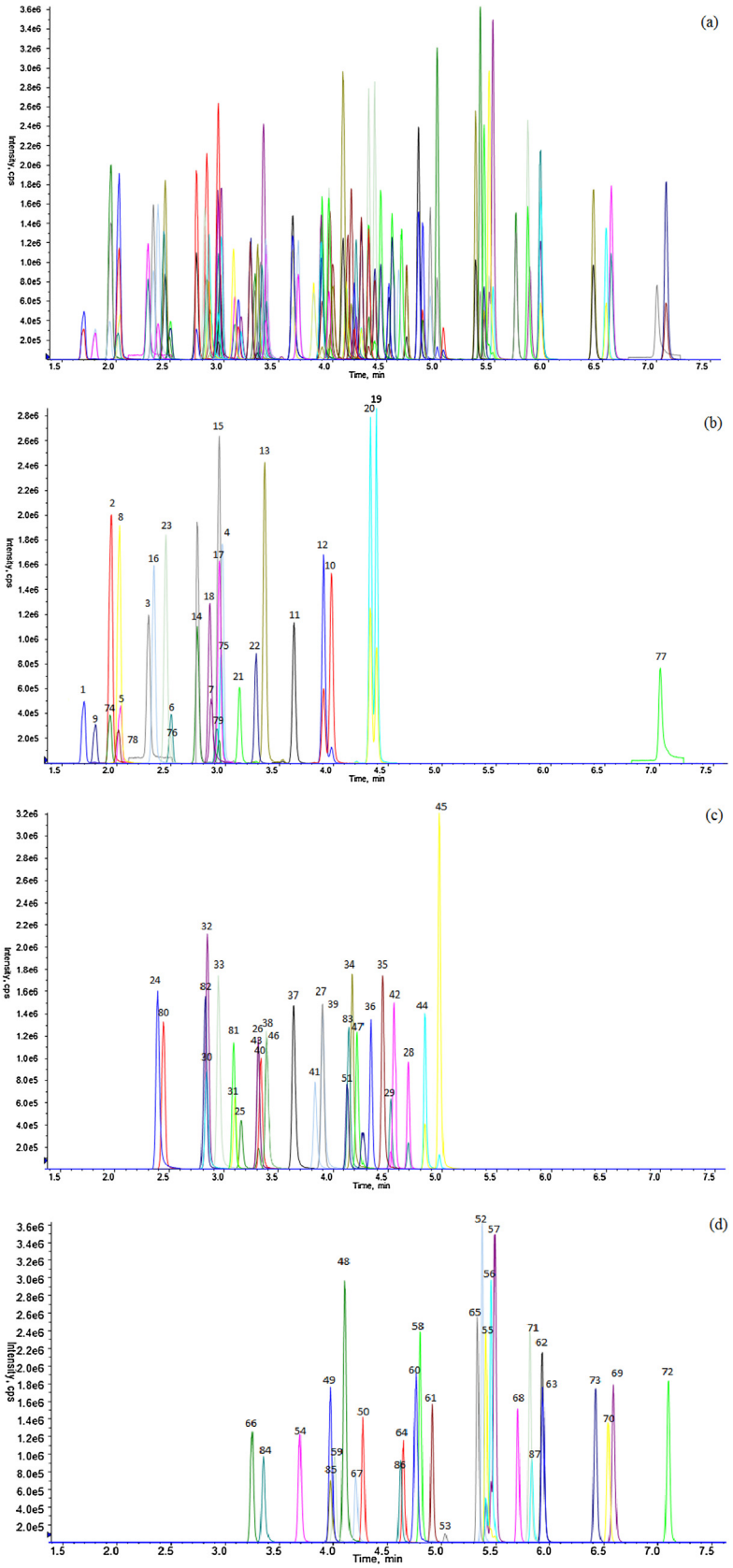

Fig. 1. Total ion chromatograms (TIC) of 73 target analytes and 14 IS of synthetic cathinones in urine, numbered by MRM order from Table 1. (a) Overall TIC; (b), (c), (d) Separate TICs.

Table 2
Linearity, LOD, and LOQ of 73 target analytes of synthetic cathinones.

Item	Analyte	Concentration range (ng/mL)	Linearity (R)	LOD (ng/mL)	LOQ (ng/mL)
1	Cathinone	0.5–50	0.9993	0.1	0.5
2	Methcathinone	0.5–50	0.9999	0.1	0.5
3	Ethcathinone	0.5–50	0.9999	0.1	0.5
4	Mephedrone	0.5–50	0.9997	0.1	0.5
5	*N*-EC ephedrine	0.5–50	0.9998	0.1	0.5
6	4-Methylephedrine	0.5–50	0.9999	0.1	0.5
7	3, 4-DMMC norephedrine	1.0–50	0.9950	0.5	1.0
8	4-FMC	1.0–50	0.9997	0.5	1.0
9	4-Fluoroephedrine	0.5–50	0.9999	0.1	0.5
10	4-EMC	1.0–50	0.9984	0.5	1.0
11	4-MeMAPB	0.5–50	0.9993	0.1	0.5
12	3, 4-DMMC	0.5–50	0.9994	0.1	0.5
13	4-MEC	0.5–50	0.9996	0.1	0.5
14	Methedrone	0.5–50	0.9998	0.1	0.5
15	4-Methyl-*N*-ethyl-norephedrine	0.5–50	0.9977	0.1	0.5
16	4-FEC	0.5–50	0.9998	0.1	0.5
17	4-CMC	0.5–50	0.9975	0.1	0.5
18	α-PPP	0.5–50	0.9981	0.1	0.5
19	MPD	0.5–50	0.9989	0.1	0.5
20	4-EEC	0.5–50	0.9971	0.1	0.5
21	4-MeOEC	0.5–50	0.9997	0.1	0.5
22	Mexedrone	0.5–50	0.9995	0.1	0.5
23	Methylone	0.5–50	0.9997	0.1	0.5
24	α-PPT	0.5–50	0.9973	0.1	0.5
25	4-CDC	1.0–50	0.9976	0.5	1.0
26	4-CEC	1.0–50	0.9982	0.5	1.0
27	4-MPPP	0.5–50	0.9991	0.1	0.5
28	4-MEAPP	0.5–50	0.9989	0.1	0.5
29	*N*-Ethyl hexedrone	1.0–50	0.9983	0.5	1.0
30	4-F-α-PPP	0.5–50	0.9980	0.1	0.5
31	Butylone	1.0–50	0.9991	0.5	1.0
32	Ethylone	0.5–50	0.9998	0.1	0.5
33	α-PBT	0.5–50	0.9964	0.1	0.5
34	α-PVP	0.5–50	0.9999	0.1	0.5
35	4-Methyl-α-PBP	0.5–50	0.9976	0.1	0.5
36	α-PVP metabolite 1	0.5–50	0.9988	0.1	0.5
37	MOPPP	0.5–50	0.9988	0.1	0.5
38	4-F-α-PBP	1.0–50	0.9988	0.5	1.0
39	Pentylone	0.5–50	0.9994	0.1	0.5
40	bk-DMBDB	0.5–50	0.9993	0.1	0.5
41	4-Cl-α-PPP	0.5–50	0.9997	0.1	0.5
42	2, 5-Dimethoxy mephedrone	0.5–50	0.9994	0.1	0.5
43	4-BMC	1.0–50	0.9986	0.5	1.0
44	α-PHP	0.5–50	0.9997	0.1	0.5
45	Pyrovalerone	0.5–50	0.9999	0.1	0.5
46	3, 4-MDPPP	1.0–50	0.9996	0.5	1.0
47	4-MeOPBP	0.5–50	0.9998	0.1	0.5
48	4-F-α-PVP	0.5–50	0.9960	0.1	0.5
49	D-Tertylone	0.5–50	0.9999	0.1	0.5
50	Ephylone	0.5–50	0.9998	0.1	0.5
51	bk-DMBDP	0.5–50	0.9955	0.1	0.5
52	Benzedrone	0.5–50	0.9996	0.1	0.5
53	*N*-BMC	1.0–50	0.9988	0.5	1.0
54	4-BEC	0.5–50	0.9999	0.1	0.5
55	α-PHPP	0.5–50	0.9981	0.1	0.5
56	4-Methyl-α-PHP	0.5–50	0.9996	0.1	0.5
57	3, 4-Dimethyl-α-PVP	0.5–50	0.9992	0.1	0.5
58	3, 4-MDPBP	0.5–50	0.9983	0.1	0.5
59	4-MeO-α-PVP	0.5–50	0.9998	0.1	0.5
60	4-F-PHP	0.5–50	0.9984	0.1	0.5
61	4-Cl-α-PVP	0.5–50	0.9996	0.1	0.5
62	Indanyl-α-PVP	0.5–50	0.9997	0.1	0.5
63	α-POP	0.5–50	0.9996	0.1	0.5
64	MDPV	0.5–50	0.9997	0.1	0.5
65	4-F-PHPP	0.5–50	0.9995	0.1	0.5
66	Demethylenyl-methyl-MDPV	1.0–50	0.9997	0.5	1.0
67	4-Br-α-PPP	1.0–50	0.9997	0.5	1.0
68	Naphyrone	0.5–50	0.9995	0.1	0.5
69	TH-PVP	0.5–50	0.9997	0.1	0.5
70	α-PNP	0.5–50	0.9995	0.1	0.5
71	4-Methoxy PHPP	0.5–50	0.9997	0.1	0.5
72	TH-PHP	0.5–50	0.9997	0.1	0.5
73	4-Methoxy-α-POP	0.5–50	0.9999	0.1	0.5

Table 3
Matrix effect, precision and accuracy for 73 target analytes of synthetic cathinones.

Item	Analyte	Spiked concentration (ng/mL)	Matrix effect		Intra-day		Inter-day	
			Value	RSD (%)	Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
1	Cathinone	5	1.13	2.98	0.43	6.53	2.97	6.12
		25	1.05	4.31	2.87	7.27	4.25	10.83
		50	0.98	2.02	2.63	2.72	1.63	3.76
2	Methcathinone	5	0.88	1.45	0.93	6.03	2.25	3.18
		25	0.91	0.59	1.07	3.20	3.81	1.08
		50	0.92	0.56	0.78	1.83	3.56	1.77
3	Ethcathinone	5	1.12	0.67	0.83	1.53	2.39	1.00
		25	1.07	2.77	2.20	1.84	3.76	0.90
		50	1.04	1.23	0.63	2.62	4.30	2.24
4	Mephedrone	5	0.94	1.12	0.21	0.45	1.46	0.18
		25	0.94	2.24	1.61	0.09	3.39	1.64
		50	0.96	2.79	1.55	7.19	6.35	5.51
5	*N*-EC ephedrine	5	0.89	1.72	1.11	2.97	1.68	4.26
		25	1.03	2.38	1.51	5.24	2.52	6.94
		50	1.02	1.87	1.40	5.01	1.44	3.72
6	4-Methylephedrine	5	0.95	0.48	0.65	2.52	1.11	1.32
		25	0.95	2.07	0.29	2.61	2.46	2.30
		50	0.96	0.99	0.27	2.97	2.39	0.18
7	3, 4-DMMC norephedrine	5	0.98	3.71	3.08	10.00	3.72	8.06
		25	1.00	1.74	1.60	10.87	3.10	10.10
		50	0.96	2.94	2.82	0.68	2.52	2.25
8	4-FMC	5	1.10	2.09	4.44	1.28	2.65	1.42
		25	0.97	4.14	0.66	1.01	3.04	0.80
		50	0.95	2.38	0.87	1.60	2.28	0.45
9	4-Fluoroephedrine	5	1.07	1.48	0.41	5.30	3.13	1.78
		25	1.07	3.08	2.01	5.19	3.38	2.54
		50	1.02	2.02	0.79	4.35	4.24	0.59
10	4-EMC	5	0.97	0.47	0.77	1.69	2.24	0.24
		25	0.98	0.68	1.05	3.52	3.58	1.56
		50	1.00	3.55	1.85	7.94	6.90	2.54
11	4-MeMAPB	5	1.00	2.81	1.12	7.75	3.69	5.94
		25	0.96	5.58	4.05	5.24	4.35	7.91
		50	0.95	3.58	2.48	0.80	1.54	1.53
12	3, 4-DMMC	5	0.95	2.38	1.35	0.34	2.87	2.92
		25	0.97	1.13	0.74	2.09	3.02	0.65
		50	0.99	3.62	2.17	5.26	6.50	0.03
13	4-MEC	5	0.98	1.29	0.23	0.71	2.36	3.52
		25	0.98	1.45	1.02	0.91	3.48	0.49
		50	0.98	1.89	1.46	5.40	6.57	0.93
14	Methedrone	5	0.96	1.96	1.02	0.36	2.66	2.60
		25	0.97	1.81	1.55	1.71	2.96	0.56
		50	0.99	2.87	2.51	8.46	6.51	3.47
15	4-Methyl-*N*-ethyl-norephedrine	5	0.97	1.55	1.12	1.46	3.71	6.60
		25	0.97	2.85	0.65	2.71	3.94	0.18
		50	0.94	0.91	0.77	1.59	3.58	3.64
16	4-FEC	5	1.14	0.62	0.57	3.49	2.67	1.00
		25	1.08	2.07	2.43	3.03	4.07	0.07
		50	1.03	1.00	0.46	2.39	4.75	2.89
17	4-CMC	5	0.81	0.62	1.58	10.97	4.60	15.24
		25	0.87	1.26	1.42	1.06	5.50	5.04
		50	0.94	3.06	3.32	2.53	7.66	8.62
18	α-PPP	5	0.93	3.26	2.15	1.58	2.01	3.08
		25	0.96	3.41	2.68	0.04	2.56	1.00
		50	0.97	1.78	1.09	0.82	3.97	3.86
19	MPD	5	1.01	3.23	0.36	3.77	3.57	5.80
		25	0.97	3.38	1.71	1.86	3.64	3.36
		50	0.97	1.09	1.47	2.25	0.98	1.95
20	4-EEC	5	0.95	2.03	2.25	2.03	2.49	0.34
		25	0.98	0.52	1.46	2.86	3.06	1.57
		50	0.96	2.80	2.10	6.71	6.26	2.43
21	4-MeOEC	5	1.00	1.75	1.22	0.39	2.73	2.16
		25	0.98	1.45	0.86	1.80	3.00	1.44
		50	0.99	4.18	2.33	9.00	6.38	5.34
22	Mexedrone	5	0.97	2.40	0.68	4.54	3.93	3.36
		25	0.97	3.63	2.03	4.01	3.72	6.19
		50	0.96	2.11	2.18	2.88	1.46	2.82
23	Methylone	5	0.99	2.62	0.26	6.43	2.60	4.56
		25	0.96	3.72	2.90	2.53	4.31	3.04
		50	0.95	2.08	1.64	1.92	1.73	0.45
24	α-PPT	5	0.94	3.54	1.38	1.57	1.70	2.94
		25	0.97	2.91	2.65	0.35	2.18	0.46
		50	0.98	3.94	2.30	0.98	4.09	3.40
25	4-CDC	5	1.01	4.00	0.83	8.35	5.11	8.28
		25	0.97	3.92	3.53	2.45	4.78	6.34

Table 3 (*Continued*)

Item	Analyte	Spiked concentration (ng/mL)	Matrix effect		Intra-day		Inter-day	
			Value	RSD (%)	Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
		50	0.96	3.58	2.35	4.22	1.49	5.36
26	4-CEC	5	0.86	2.75	1.31	5.99	2.87	5.26
		25	0.86	3.63	2.55	2.39	4.28	4.78
		50	0.92	2.72	1.98	2.01	1.45	2.28
27	4-MPPP	5	0.99	0.46	0.53	6.63	1.73	5.20
		25	0.99	2.68	1.75	3.56	2.61	3.43
		50	0.97	2.65	0.77	1.14	2.45	1.55
28	4-MEAPP	5	1.00	4.65	0.65	5.68	3.47	4.08
		25	0.95	3.07	2.95	1.62	3.97	3.86
		50	0.95	1.53	1.29	2.24	0.76	1.88
29	*N*-Ethyl hexedrone	5	0.98	4.12	2.42	7.03	3.22	4.82
		25	0.97	1.39	0.73	0.56	2.94	1.60
		50	0.98	2.11	1.25	4.60	1.70	2.49
30	4-F-α-PPP	5	0.91	3.13	3.13	1.82	2.52	3.38
		25	0.96	3.03	2.05	1.62	1.96	1.04
		50	0.97	2.85	1.33	0.76	3.84	3.45
31	Butylone	5	0.96	1.44	0.67	0.42	1.56	1.18
		25	0.97	2.26	1.15	1.58	2.85	1.45
		50	0.97	2.53	1.03	1.61	2.49	1.03
32	Ethylone	5	0.94	1.37	0.68	2.83	1.82	0.98
		25	0.96	1.79	0.97	2.97	3.11	3.11
		50	0.95	0.92	0.64	0.57	2.51	1.74
33	α-PBT	5	0.94	3.85	1.45	5.34	2.81	7.16
		25	1.00	2.82	2.01	0.27	2.83	1.58
		50	1.00	4.52	2.72	0.97	5.07	4.54
34	α-PVP	5	0.95	3.87	2.17	0.27	1.88	0.76
		25	0.98	2.38	1.58	0.31	2.03	0.31
		50	0.98	1.53	1.48	1.67	3.54	2.17
35	4-Methyl-α-PBP	5	0.95	2.73	1.19	3.32	2.27	5.36
		25	0.98	3.66	2.13	0.88	1.96	1.32
		50	0.98	1.49	1.20	1.18	4.14	3.64
36	α-PVP metabolite 1	5	0.95	3.47	1.94	1.41	2.38	0.30
		25	0.99	3.94	2.84	3.84	2.19	3.20
		50	0.99	2.55	1.48	0.43	3.93	3.92
37	MOPPP	5	0.97	0.88	0.58	3.74	1.83	1.98
		25	0.95	2.97	1.25	2.16	3.11	2.72
		50	0.95	3.25	2.77	2.16	2.82	0.36
38	4-F-α-PBP	5	0.94	3.34	2.30	0.31	2.09	1.42
		25	0.98	2.59	2.23	1.61	2.20	1.01
		50	0.98	3.22	2.14	1.19	4.52	3.71
39	Pentylone	5	1.01	2.31	1.14	7.43	1.75	5.48
		25	0.99	1.87	0.73	4.94	2.66	5.52
		50	0.98	1.47	0.64	1.51	2.41	0.68
40	bk-DMBDB	5	0.96	2.02	1.09	3.53	1.25	3.26
		25	0.99	1.97	0.73	3.18	2.35	3.42
		50	0.98	2.34	1.30	1.90	2.62	0.67
41	4-Cl-α-PPP	5	0.93	1.93	1.87	1.71	2.02	0.32
		25	0.97	2.66	2.20	1.37	1.89	1.46
		50	0.97	1.99	2.06	3.22	3.89	0.94
42	2, 5-Dimethoxy mephedrone	5	1.08	4.81	2.99	1.29	3.98	1.26
		25	0.99	8.46	5.17	1.00	6.14	1.36
		50	0.94	5.66	2.16	7.55	7.21	6.65
43	4-BMC	5	0.81	1.54	1.56	3.62	1.15	3.80
		25	0.87	0.56	0.63	3.67	4.62	3.81
		50	0.93	2.85	0.84	0.65	6.79	0.98
44	α-PHP	5	0.95	2.04	0.81	1.99	2.05	2.26
		25	0.96	0.87	1.00	0.56	2.76	0.52
		50	0.97	2.33	1.91	2.94	2.74	0.24
45	Pyrovalerone	5	0.96	0.31	0.21	2.93	1.74	1.78
		25	0.95	2.43	0.85	1.24	2.71	2.06
		50	0.95	2.03	1.96	1.53	2.50	0.97
46	3, 4-MDPPP	5	0.96	1.16	1.29	0.07	1.21	0.76
		25	0.95	2.38	1.42	0.66	3.00	1.60
		50	0.94	3.10	2.28	1.81	2.43	0.34
47	4-MeOPBP	5	0.94	1.05	0.83	1.17	1.49	1.70
		25	0.93	2.11	0.49	1.39	2.96	0.96
		50	0.94	2.89	2.28	1.60	3.03	1.46
48	4-F-α-PVP	5	0.91	3.38	2.03	5.47	5.51	7.70
		25	0.98	8.21	5.68	2.29	3.69	3.61
		50	1.03	7.25	5.34	3.61	6.60	3.46
49	D-Tertylone	5	0.98	1.70	0.42	3.68	1.41	2.96
		25	0.99	2.63	1.69	1.98	3.09	2.16
		50	0.98	1.85	1.75	2.73	3.09	0.53
50	Ephylone	5	0.96	2.16	1.29	1.54	1.82	1.86
		25	0.96	2.49	1.68	0.26	2.93	0.77

Table 3 (*Continued*)

Item	Analyte	Spiked concentration (ng/mL)	Matrix effect		Intra-day		Inter-day	
			Value	RSD (%)	Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
		50	0.96	1.77	0.66	0.30	2.01	1.55
51	bk-DMBDP	5	1.07	0.79	3.13	10.69	6.42	10.36
		25	0.99	5.70	6.39	4.15	5.62	5.15
		50	0.90	7.00	4.28	1.08	3.07	3.55
52	Benzedrone	5	0.91	2.25	1.56	1.35	2.74	1.86
		25	0.92	4.19	1.79	1.22	4.43	1.23
		50	0.94	3.33	2.35	2.25	4.85	7.07
53	*N*-BMC	5	0.92	2.54	1.33	2.02	2.70	0.01
		25	0.92	3.20	3.62	2.11	3.35	3.24
		50	0.97	3.66	3.97	7.96	6.99	2.26
54	4-BEC	5	0.82	2.09	1.10	2.08	3.19	3.16
		25	0.85	4.96	3.56	0.51	5.68	2.17
		50	0.90	3.03	2.47	2.04	2.03	0.87
55	α-PHPP	5	1.02	1.92	0.76	1.95	5.10	2.12
		25	0.97	8.64	5.03	1.32	6.49	0.19
		50	0.94	8.83	4.30	3.95	4.94	1.68
56	4-Methyl-α-PHP	5	0.99	0.91	0.16	0.87	1.13	1.76
		25	0.95	3.34	1.58	0.65	3.59	0.40
		50	0.94	3.38	2.79	0.21	2.51	1.58
57	3, 4-Dimethyl-α-PVP	5	0.95	0.22	0.61	3.52	3.84	5.56
		25	0.91	6.83	6.01	4.91	6.46	4.68
		50	0.94	7.34	3.99	3.02	5.39	6.36
58	3, 4-MDPBP	5	0.99	1.78	1.07	1.00	1.77	1.24
		25	0.97	3.59	1.90	1.29	3.12	2.30
		50	0.91	3.03	1.77	0.85	1.99	0.96
59	4-MeO-α-PVP	5	0.92	0.93	0.77	0.57	1.66	0.12
		25	0.93	1.47	1.58	2.07	2.48	2.35
		50	0.90	2.16	0.87	0.33	1.45	0.18
60	4-F-PHP	5	1.01	2.40	1.77	9.23	3.15	8.84
		25	0.96	3.48	2.42	2.76	4.34	7.05
		50	0.96	2.28	1.51	5.47	1.08	6.50
61	4-Cl-α-PVP	5	0.97	1.32	0.56	1.99	1.58	2.32
		25	0.93	3.39	1.14	0.48	3.54	1.29
		50	0.95	3.48	2.97	1.23	2.29	0.36
62	Indanyl-α-PVP	5	0.98	1.06	0.41	2.13	1.86	0.02
		25	0.95	1.33	1.17	1.61	4.15	0.83
		50	0.94	1.91	1.60	2.11	3.42	5.51
63	α-POP	5	0.94	1.90	0.73	1.61	1.75	2.20
		25	0.96	1.43	1.04	3.27	3.92	3.66
		50	0.92	1.97	0.86	1.98	1.42	1.98
64	MDPV	5	0.97	0.93	0.93	2.00	2.75	1.26
		25	0.95	1.81	1.01	1.56	3.54	1.03
		50	0.93	2.80	2.11	0.77	2.73	2.79
65	4-F-PHPP	5	0.97	1.38	0.55	1.67	1.63	2.52
		25	0.96	2.10	0.78	0.85	3.62	2.11
		50	0.96	2.90	2.52	2.09	2.72	0.28
66	Demethylenyl-methyl-MDPV	5	0.94	2.27	1.47	3.99	2.29	1.52
		25	0.94	0.97	1.34	3.41	3.20	2.81
		50	0.94	1.70	1.47	1.18	2.79	1.28
67	4-Br-α-PPP	5	0.96	2.02	0.88	1.69	1.36	1.00
		25	0.95	3.33	1.48	2.21	3.32	2.83
		50	0.94	3.31	2.97	1.69	2.24	0.17
68	Naphyrone	5	0.96	2.20	1.29	0.37	2.77	
		25	0.93	2.64	1.71	0.41	4.21	
		50	0.90	2.31	1.19	0.01	1.35	
69	TH-PVP	5	0.98	0.81	0.52	3.39	2.34	1.12
		25	0.95	2.69	1.09	0.73	4.44	0.12
		50	0.95	2.67	2.03	1.73	3.93	5.63
70	α-PNP	5	0.85	1.00	0.58	3.86	2.13	2.10
		25	0.92	1.49	0.82	2.74	5.81	1.40
		50	0.94	1.95	1.59	0.55	4.21	5.14
71	4-Methoxy PHPP	5	0.94	1.52	0.20	2.08	1.68	2.08
		25	0.96	1.21	0.88	2.14	3.87	3.89
		50	0.94	0.74	0.48	2.11	1.52	1.40
72	TH-PHP	5	0.89	1.86	1.20	4.80	3.76	4.56
		25	0.92	1.58	0.55	0.63	6.06	3.62
		50	0.92	1.79	1.12	2.15	1.72	0.89
73	4-Methoxy-α-POP	5	0.87	0.37	0.30	3.96	2.78	3.98
		25	0.96	1.10	0.84	1.95	4.71	3.22
		50	0.96	0.85	1.04	3.21	2.17	0.85

metabolite (α-PVP metabolite 1, metabolite of α-PVP) were obtained from Cayman Chemical (Ann Arbor, Michigan, USA). Standards of 4-fluoroephedrine (metabolite of 4-FMC), *N*-ethylcathinone ephedrine (*N*-EC ephedrine, metabolite of ethylcathinone), 4-methylephedrine (metabolite of mephedrone), 3, 4-dimethylmethcathinone norephedrine (3, 4-DMMC norephedrine, metabolite of 3, 4-DMMC), 4-methyl-*N*-ethyl-norephedrine (metabolite of 4-MEC) and all isotopically labelled internal standards were methanolic solutions (1 mg/mL) obtained from Cerilliant Corporation (Austin, Texas, USA). All remaining standards listed in Table 1 were synthesized by GreenChem Corporation (Taichung, Taiwan). The full names and abbreviations of all analytes are shown in Table 1. Formic acid, methanol, and ammonium acetate were purchased from Sigma-Aldrich Corporation (Saint Louis, Missouri, USA). LC–MS grade water was purchased from Scharlau (Barcelona, Spain). Artificial urine was purchased from UTAK Laboratories, Inc. (Valencia, California, USA). In total, 67 authentic urine samples were collected and provided by the local law enforcement agencies which the sampling in this study followed the regulations made by Ministry of Health and Welfare, Taiwan.

2.2. Instrumentation and chromatographic conditions

The experiments were performed on a Waters Acquity UPLC® system (Waters Assoc., Milford, Massachusetts, USA) coupled to a AB SCIEX QTRAP® 6500 triple quadrupole linear ion trap mass spectrometer equipped with an electrospray ionization (ESI) source (Applied Biosystems, MDS Sciex, Concord, Ontario, Canada) and operated in multiple reaction monitoring (MRM) mode. Chromatographic analysis was carried out on a Phenomenex Kinetex® Biphenyl column (10 cm ×2.1 mm i.d., 1.7 μm) at 40℃ with a constant flow rate of 0.5 mL/min using gradient elution of mobile phase A (0.1% formic acid aqueous solution with 5 mM ammonium acetate) and mobile phase B (0.1% formic acid methanolic solution). Each sample was analyzed with an injection volume of 3 μL. The total chromatographic run time was 8 min. Elution was performed as follows: 0–0.5 min 2%–20% B, 0.5–3.0 min 20%–38% B, 3.0–3.2 min 38% B, 3.2–5.0 min 38%–59% B, 5.0–5.4 min 59 % B, 5.4–6.6 min 59%–67% B, 6.7–7.0 min 67%–90% B, and 7.0–8.0 min 90%–100% B. After injection of each sample, the needle was rinsed alternately with methanol and water. The MS ion source was set as ESI in positive mode under the following conditions: ion spray voltage, 5.5 kV; temperature, 550℃; curtain gas pressure, 30 psi; collision gas pressure of medium level; ion source gas, 50 psi.

2.3. Preparation of standard solutions

Stock solutions of the 73 standards and 14 IS were prepared in methanol at 1 mg/mL and 0.1 mg/mL, respectively. The standard stock solution was diluted with artificial urine to 2 μg/mL to prepare a standard working solution. An adequate volume of each IS stock solution was mixed and, then, diluted with 50 % methanol aqueous solution to 1 μg/mL to prepare an IS working solution. All stock and working solutions were stored at -20℃ and acclimated to controlled room temperature prior to use.

2.4. Sample preparation

The raw urine samples were centrifuged at 3000×*g* for 5 min and then collected the supernatant. A mixture solution comprising 50 μL supernatant, 50 μL IS working solution (100 ng/mL), and 950 μL 50 % methanol aqueous solution was prepared. The mixture solution was subsequently filtered through a 0.22 μm PVDF filter and then collected the filtrate which was used for analysis. Drug-free urine (DFU) was used as the negative control sample. The samples were analyzed directly without any pretreatment or purification.

2.5. Method validation

The method was validated following the guideline of Scientific Working Group for Forensic Toxicology Standard Practices (SWGTOX) for Method Validation in Forensic Toxicology [23]. Validation was performed by evaluating the following parameters: carryover, selectivity, linearity, sensitivity, matrix effects, precision, and accuracy. The carryover was evaluated by injecting blank samples after analyzing the spiked urine samples of serial concentrations (100–1000 ng/mL) in triplicate. Selectivity was evaluated by analyzing 10 different DFU samples to ensure absence of interferential peaks for the targets. Good selectivity could be achieved only if signals from endogenous origins of the matrix did not have evident interference as characteristic ions at adjacent retention time so that the analysis could be unimpeded.

Linearity was assessed by analyzing standard solutions of the 73 target analytes of synthetic cathinones (n = 3) at 7 concentrations (0.5, 1.0, 5.0, 10.0, 20.0, 25.0, and 50.0 ng/mL) and plotting the peak area ratio of standard/IS versus the concentration of standard using the least-square method. The correlation coefficient r was determined and the acceptable value was 0.995 and above. The samples were quantified by deducing the content through calibration curve ranged in 0.5–50 ng/mL employing internal standard method.

Sensitivity was evaluated using the LOD and LOQ. The LOD is the lowest concentration of analyte that can be detected with the estimated signal-to-noise (S/N) ratio of 3. The LOQ is the lowest concentration of analyte that can be quantified with suitable precision and accuracy using an estimated S/N ratio of 10. The evaluation of LOD and LOQ for each analyte was performed in six replicates.

Matrix effects were assessed using the direct comparison method. Sets of samples covering three concentration levels, 5, 25, and 50 ng/mL, for the 73 analytes (5 ng/mL IS included) were prepared in DFU (A) and water (B). Matrix effects were evaluated with three replicates (n = 3) and calculated using the following formula:

$$\text{Matrix effects} = \frac{\left(\frac{P}{P'}\right) \text{ of } A}{\left(\frac{P}{P'}\right) \text{of } B};$$

where, *P* represents peak area of analyte and *P'* represents peak area of IS.

Precision and accuracy were evaluated by introducing quality control (QC) for the analyte-spiked urine samples. The intra-day and inter-day accuracy (% bias) and precision (% CV) of the assay were assessed at three concentration levels from low to high within the calibration curve (5.0, 25.0, and 50.0 ng/mL) in triplicate over five different runs. The acceptable bias was 20 % of each concentration.

3. Results

3.1. Method development

Pre-tests indicated that the ESI source in positive mode, i.e., monitoring protonated molecular $[M+H]^+$ for target analytes, had a stronger response than the negative mode. As this result was consistent with literature, ESI^+ was selected as the ionization source mode for method development in this study [17]. To attain better specificity, the MRM mode was applied to collect the respective monitoring and quantitative ions. The MRM parameters

and referential IS for each analyte are shown in Table 1, whereas the chromatographic analysis was shown as an overall TIC for all analytes in Fig. 1.

3.2. Method validation

For sample analysis, it is important to ensure the authenticity and credibility of chromatographic results. First, carryover and selectivity were evaluated. In the assessment of carryover, no residual peaks were detected in the chromatograph for all analytes, indicating that the preceding sample did not interfere with analysis. To avoid possible carryover that affects the results of identification and quantification, attention should be paid during sample analysis. The selectivity was evaluated and no interferential peaks, traces of IS, or cross-interference among analytes were observed during analysis, indicating that the present method was selective for all analytes.

Linearity was assessed up to 50.0 ng/mL and the correlation coefficient r values were higher than 0.995 for all analytes, indicating that all IS applied in the qualification were highly recommendable for the targets analyzed in this study. The LOD and LOQ determined for all analytes were 0.1–0.5 ng/mL and 0.5–1.0 ng/mL, respectively.

The present method revealed good performance in determining the target analytes of synthetic cathinones and allowed the determination of targets at a low limit. The linearity and sensitivity data are shown in Table 2.

The evaluation of matrix effect for biological specimen analysis involves the sensitivity, precision, accuracy, and reproducibility of the present method as well as quantification of target analytes. Thus, matrix effect becomes an index for whether further pretreatment or purification is needed for samples to obtain better performance in determining target analytes. The matrix effect was satisfactory for all analytes with a deviation lower than 20 % (i.e. 0.8–1.2). The intra-day and inter-day precision and accuracy of the assay was evaluated by analyzing triplicate QC samples at three analyte concentration levels. The intra-day and inter-day precision were 0.16–7.66, whereas the accuracy were 0.04–10.87 % for all analytes. The result was satisfactory within a value within ±20 % for all analytes. The data of matrix effect, precision and accuracy are shown in Table 3.

3.3. Application to authentic samples

The present method was further applied to analyze authentic urine samples to detect the target analytes and examine the synthetic cathinones abused in Taiwan. In total 67 urine samples were analyzed and the result of analysis is demonstrated in Table 4. The result showed that 32 samples were tested positive of 13 targets, including mephedrone, 4-methylephedrine, butylone, bk-DMBDB, methylone, 4-MEAPP, ephylone, 4-CMC, MPD, 4-CDC, 4-CEC, ethylone, and 4-EEC.

Table 4
Targets detected above LOD from authentic urine samples.

Sample No.	Target detected
1	Mephedrone, 4-Methylephedrine
4	4-Methylephedrine
6	Butylone, bk-DMBDB
8	4-Methylephedrine
10	Butylone, bk-DMBDB
12	Mephedrone, 4-Methylephedrine
22	Methylone
23	bk-DMBDB
24	4-MEAPP
26	Ephylone
28	Methylone
30	4-Methylephedrine, 4-CMC, MPD, 4-CDC, 4-MEAPP
34	4-Methylephedrine
35	Mephedrone, 4-Methylephedrine
38	Mephedrone, 4-Methylephedrine, 4-MEAPP, Ephylone
39	4-Methylephedrine
40	Mephedrone, 4-Methylephedrine
41	Mephedrone, 4-Methylephedrine
42	Ephylone
45	Mephedrone, 4-Methylephedrine, 4-CDC, 4-CEC, 4-MEAPP, Ephylone
46	4-Methylephedrine
47	4-CMC, 4-EEC, MPD, 4-CDC, 4-CEC, 4-MEAPP, Ephylone
54	Ephylone
55	4-CDC
57	Mephedrone, 4-Methylephedrine, 4-CEC, 4-MEAPP, Ethylone
58	4-CMC, 4-CEC, 4-MEAPP, Ephylone
59	Mephedrone, 4-Methylephedrine
60	4-Methylephedrine
61	Mephedrone, 4-Methylephedrine, Ephylone
64	4-EEC, MPD, 4-MEAPP
67	Mephedrone, 4-Methylephedrine

4. Discussion

This study established an inclusive and sensitive LC–MS/MS method for screening synthetic cathinones in urine. In the chromatographic analysis, signals from adjacent peaks with the same mass were observed for couple analytes, such as 4-EMC, 4-MeMABP, and 3,4-DMMC (192); methedrone and 4-methyl-*N*-ethyl-norephedrine (194); MPD and 4-EEC (206); 4-F-α-PPP and butylone (222); α-PVP and 4-methyl-α-PBP (232); α-PHP and pyrovalerone (246); as well as benzedrone and N-BMC (254). Misinterpretation was precluded by cross-comparing the respective retention time of each analyte; the subsequent method validation also confirmed that cross contributions were eliminated and the method demonstrated good specificity in quantifying analytes with the same mass.

The dilute-and-shoot procedure is advantageous in practical forensic applications by diminishing the process of sample preparation. Matrix effect was evaluated and the result indicated an ignorable influence on urinary analysis of synthetic cathinones. In the analysis of authentic samples, 10 out of the 32 positive samples were detected having two to seven synthetic cathinones in one case, indicating that the abuse of poly-synthetic cathinones was observed among drug abusers in Taiwan. According to the report from Taiwan's early warning system of drug abuse "Analytic Laboratory Urine and Drug Abuse Report System" (UDARS), 51 synthetic cathinones were monitored which the top 10 synthetic cathinones reported most frequently in Taiwan were mephedrone, 4-MEAPP, methylone, 4-CEC, CMC, ephylone, ethylone, MPD, bk-DMBDB and 4-MDMC (4-methyl-*N*, *N*-dimethylcathinone) [24]. Except for 4-MDMC as a latest item monitored that is not included in this study, all other 9 targets monitored by UDARS were detected, which indicated a same trend as that reported by UDARS. I addition, the present method has incorporated couple fatal cathinones identified in previous reports [6, 25,26,27,28,29]; some of them, including ephylone, mephedrone/4-methylephedrine, and methedrone were also detected from the authentic samples collected in Taiwan. Besides, it is particularly noteworthy that the fatal synthetic cathinone mephedrone was detected as original form and/or metabolite in the result of urinalysis. For the 18 mephedrone-positive samples, mephedrone and its metabolite 4-methylephedrine were detected synchronously in 11 samples while only 4-methylephedrine was detected in seven samples. In contrast with the literatures, the published methods included mephedrone in the list of the targets yet omitted the metabolite 4-methylephedrine which might lead to the false-negative of mephedrone [17,19,20,30]. As a result, the metabolite

4-methylephedrine is recommended to be included in the list while monitoring mephedrone.

Additionally, most studies focused on developing methods for detecting diverse drugs in one procedure; however, with the growing items of synthetic cathinones, the methods became insufficient in detecting drugs of single species. The present method demonstrated a more extensive applicability in analyzing multiple synthetic cathinones compared with previous literatures; Adamowicz and Tokarczyk established a screening method for 143 NPS including 36 synthetic cathinones which 23 items had been incorporated in the method of this study; Waters et al. developed a database applying LC-ESI-MS/MS for detection of 104 abused substances including 29 synthetic cathinones which 17 items had been incorporated in the method of this study [21,30]. Except for the comprehensiveness for detection of synthetic cathinones, the present method possessed a superior sensitivity for determination of synthetic cathinones as compared to previously reported methods. Namely, drugs of various species pose divergent characteristics upon analysis within a single method, such as intensities and sensitivities, which may result in the discrepancy of interpretation or quantification. Al-Sarffar et al. developed a screening method for detection of 27 NPS incorporating 11 synthetic cathinones, for which the LODs and LOQs were 0.8–10 ng/mL and 0.5–50, respectively; Bell et al. established a method for detection of eight NPS incorporating five synthetic cathinones with LODs of 2.0–3.4 ng/mL and LOQs of 6.5–11.3 ng/mL; Tang et al. built up a method for detection of 93 emerging drugs including 6 synthetic cathinones with LODs of 10–100 ng/mL [17,19,20]. The present method demonstrated a better sensitivity for urinary analysis of synthetic cathinones than the literatures mentioned above, which the LODs and LOQs were 0.1–0.5 ng/mL and 0.5–1.0 ng/mL respectively for all 73 targets of this study.

5. Conclusions

An inclusive LC–MS/MS method for determination of 73 synthetic cathinones and related metabolites in urine was established. The present method was further validated and provided good specificity in detecting targets. Authentic urine samples were analyzed by this method which 32 out of 67 samples were detected positive and 13 targets were identified. The abuse of poly-synthetic cathinones in Taiwan was also examined that up to seven cathinones were detected in one case. These results indicated the present method as a feasible technique for identifying multiple components of synthetic cathinones in urine.

Authorship

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