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Method of Test for Synthetic Cathinones in Urine (3)

1. Scope

This method is applicable to the determination of 73 cathinones and their metabolites (cathinone etc. listed as the attached table) in urine.

2. Method

After dilution, analytes are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization (ESI).

2.1.1.2. Column: Phenomenex Kinetex® Biphenyl, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Vortex mixer.

2.1.3. Ultrasonicator.

2.2. Chemicals

Methanol, formic acid, and ammonium acetate, HPLC grade;

Artificial urine (UTAK 88121-CDF(L) or an equivalent product), reagent grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

Cathinone etc. listed in the attached tables, reference standards;

Methcathinone-d_3 and other isotope-labeled internal standards (listed in the attached table).

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL.

2.3.2. Membrane filter: 0.22 μm, PVDF.

2.4. 50% Methanol:

Mix methanol and deionized water at the ratio of 1:1 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.4 g of ammonium acetate with deionized water to 1000 mL, add 1 mL of formic acid, and filter with a membrane filter.

2.5.2. Solvent B:

Dilute 1 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer 1 mg of each individual internal standard accurately weighed into

each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the internal standard stock solutions. Store at -20℃ in the dark. Prior to use, mix appropriate volume of the internal standard stock solutions, and dilute with 50% methanol to 100 ng/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer 1 mg of reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solutions. Store at -20℃ in the dark. Prior to use, mix appropriate volume of the standard stock solutions, and dilute with 50% methanol to 100 ng/mL as the standard solution.

2.8. Sample solution preparation

Transfer 50 μL of the homogenized sample and 50 μL of the internal standard solution into a 1-mL volumetric flask, and dilute to volume with 50% methanol. Filter with a membrane filter, and take the filtrate as the sample solution.

2.9. Calibration curve

Use the artificial urine as the blank sample. Separately take 50 μL of the artificial urine, and 5-100 μL of the standard solution and 50 μL of the internal standard solution, dilute with 50% methanol to 1 mL, and filter with a membrane filter. Take the filtrates as the calibration standard solutions. Operate LC-MS/MS according to the following conditions. Establish the calibration curve of each cathinone by the ratios of the peak area of each cathinone to that of the respective internal standard vs. the added concentrations (2.5-50 ng/mL).

LC-MS/MS operating conditions(note):

Column: Phenomenex Kinetex® Biphenyl, 1.7 μm, 2.1 mm i.d. × 10 cm.

Column temperature: 40℃.

Injection volume: 3 μL.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 0.5	98 → 80	2 → 20
0.5 → 3.0	80 → 62	20 → 38
3.0 → 3.2	62 → 62	38 → 38
3.2 → 5.0	62 → 41	38 → 59
5.0 → 5.4	41 → 41	59 → 59
5.4 → 6.6	41 → 33	59 → 67

6.7 → 7.0	33 → 10	67 → 90
7.0 → 8.0	10 → 0	90 → 100
8.0 → 8.5	0 → 0	100 → 100
8.5 → 8.6	0 → 98	100 → 2
8.6 → 11.0	98 → 98	2 → 2

Flow rate: 0.5 mL/min.

Ion spray voltage: 5.5 kV.

Ionization mode: ESI^+.

Turbo heater temperature: 550℃.

Nebulizer gas, GS1: 50 psi.

Heated gas, GS2, 60 psi.

Curtain gas: 30 psi.

Collision gas: Medium.

Detection mode: multiple reaction monitoring (MRM). Detection of ion pair, declustering potential and collision energy are shown in the attached table.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.10. Identification and quantification

Accurately inject 3 μL of the sample solution and the calibration standard solutions into LC-MS/MS separately. Operate according to the conditions in section 2.9. Identify each cathinone based on the retention time and the relative ion intensities(note). Calculate the amount of each cathinone in the sample by the following formula:

$$\text{The amount of each cathinone in the sample (ng/mL)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each cathinone in the sample solution calculated by the calibration curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the volume of the sample (mL)

Note: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities are as the following:

Relative ion intensity (%)	Tolerance (%)

> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Remark

1. Limit of quantification (LOQ) for each cathinone is 50 ng/mL.
2. Further validation should be performed when interference compounds appear in the samples.

Reference

Al-Saffar, Y., Stephanson, N. N. and Beck, O. 2013. Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-experience from the Swedish population. J. Chromatogr. B 930: 112-120.

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Reference chromatogram

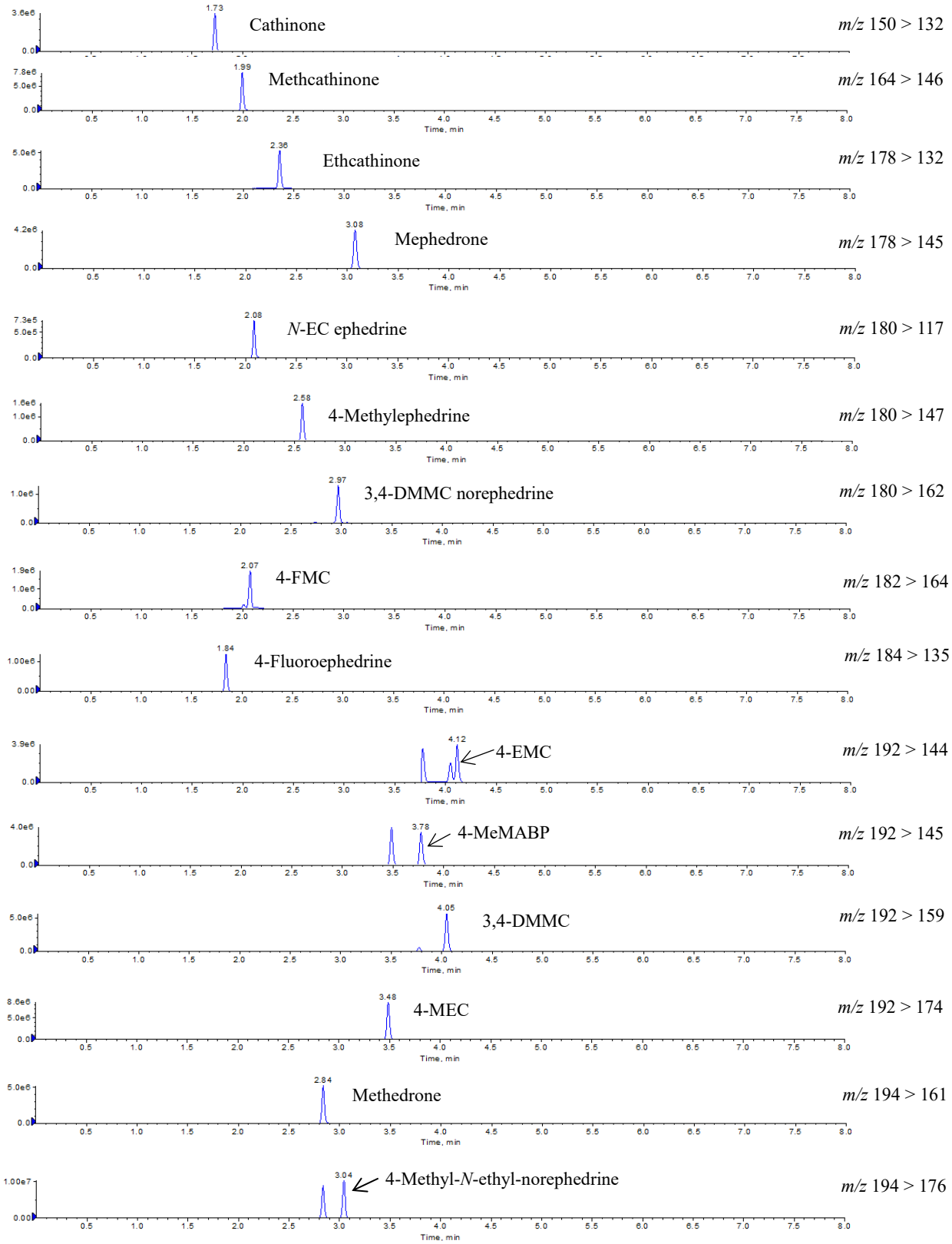

Figure. The MRM chromatograms of 73 cathinones and 14 isotope-labeled internal standards analyzed by LC/MS/MS.

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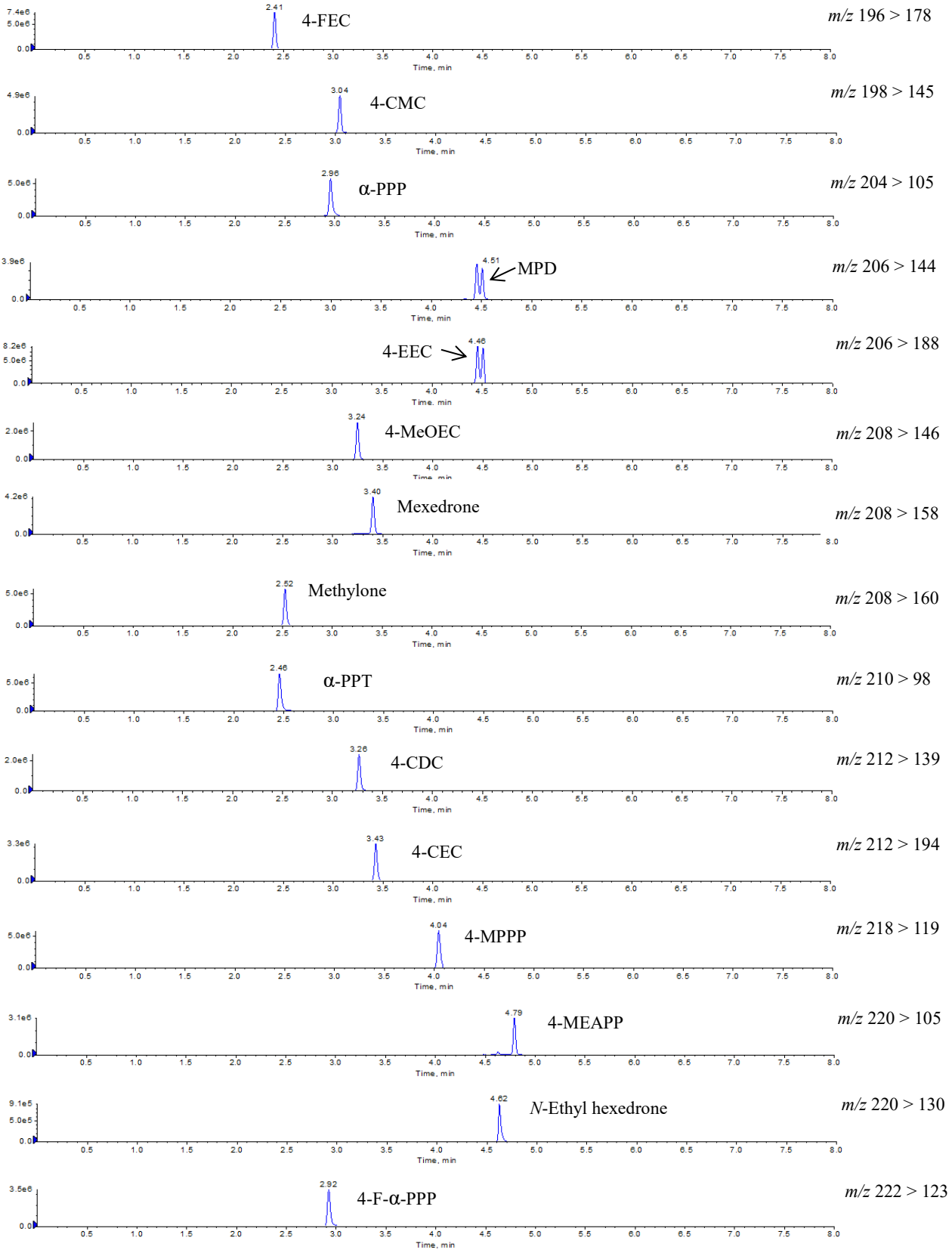

Figure. The MRM chromatograms of 73 cathinones and 14 isotope-labeled internal standards analyzed by LC/MS/MS (continued).

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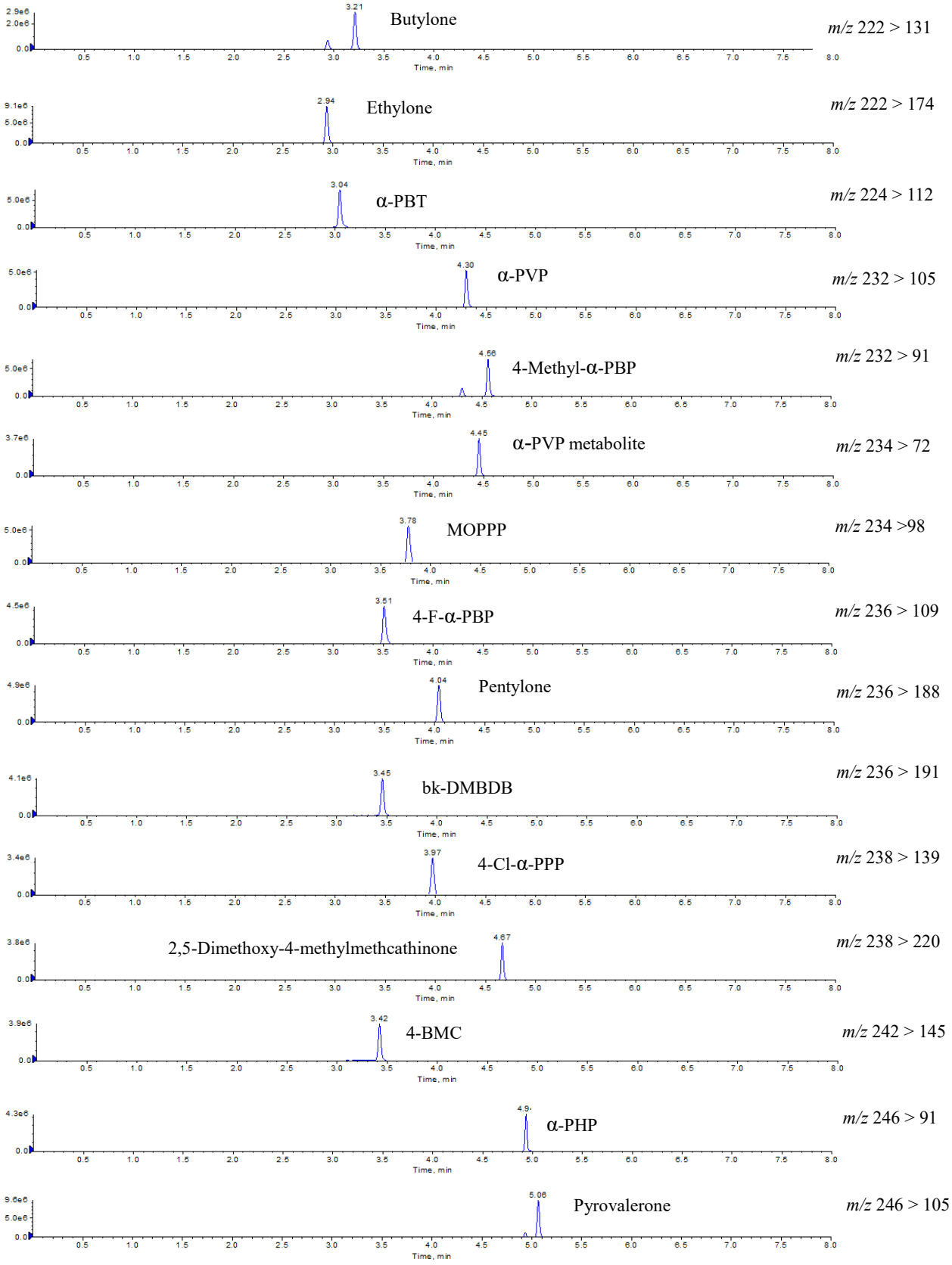

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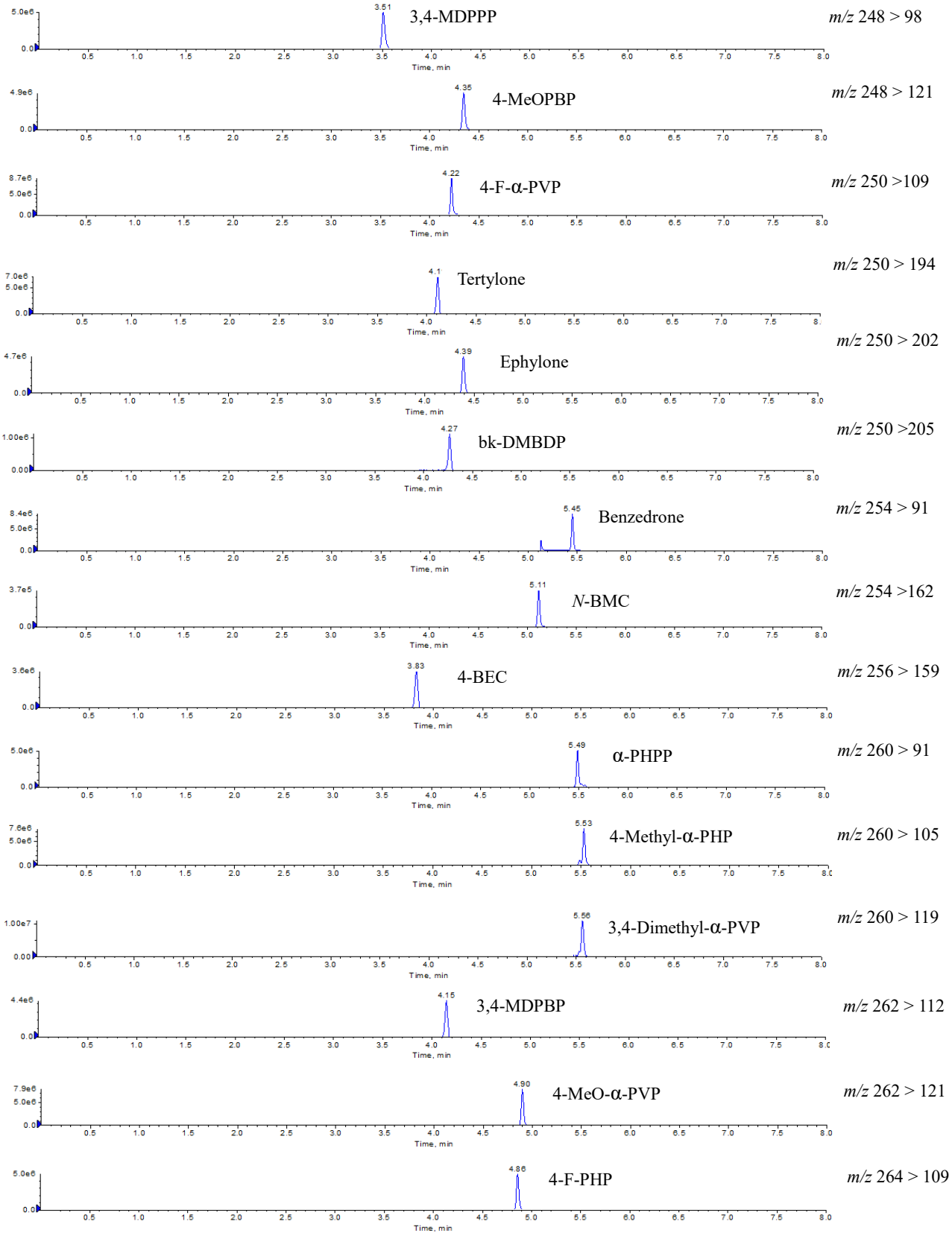

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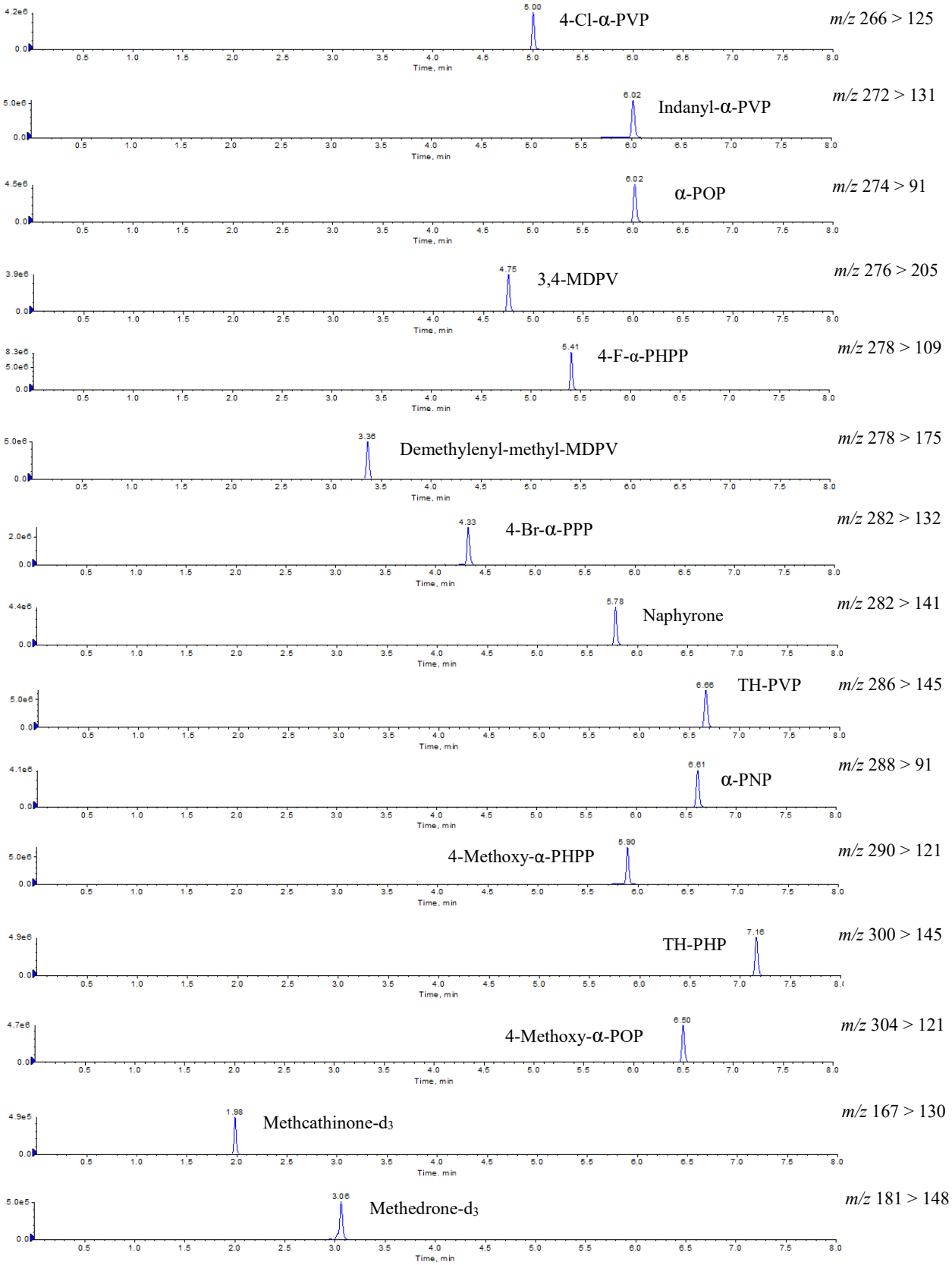

Figure. The MRM chromatograms of 73 cathinones and 14 isotope-labeled internal standards analyzed by LC/MS/MS (continued).

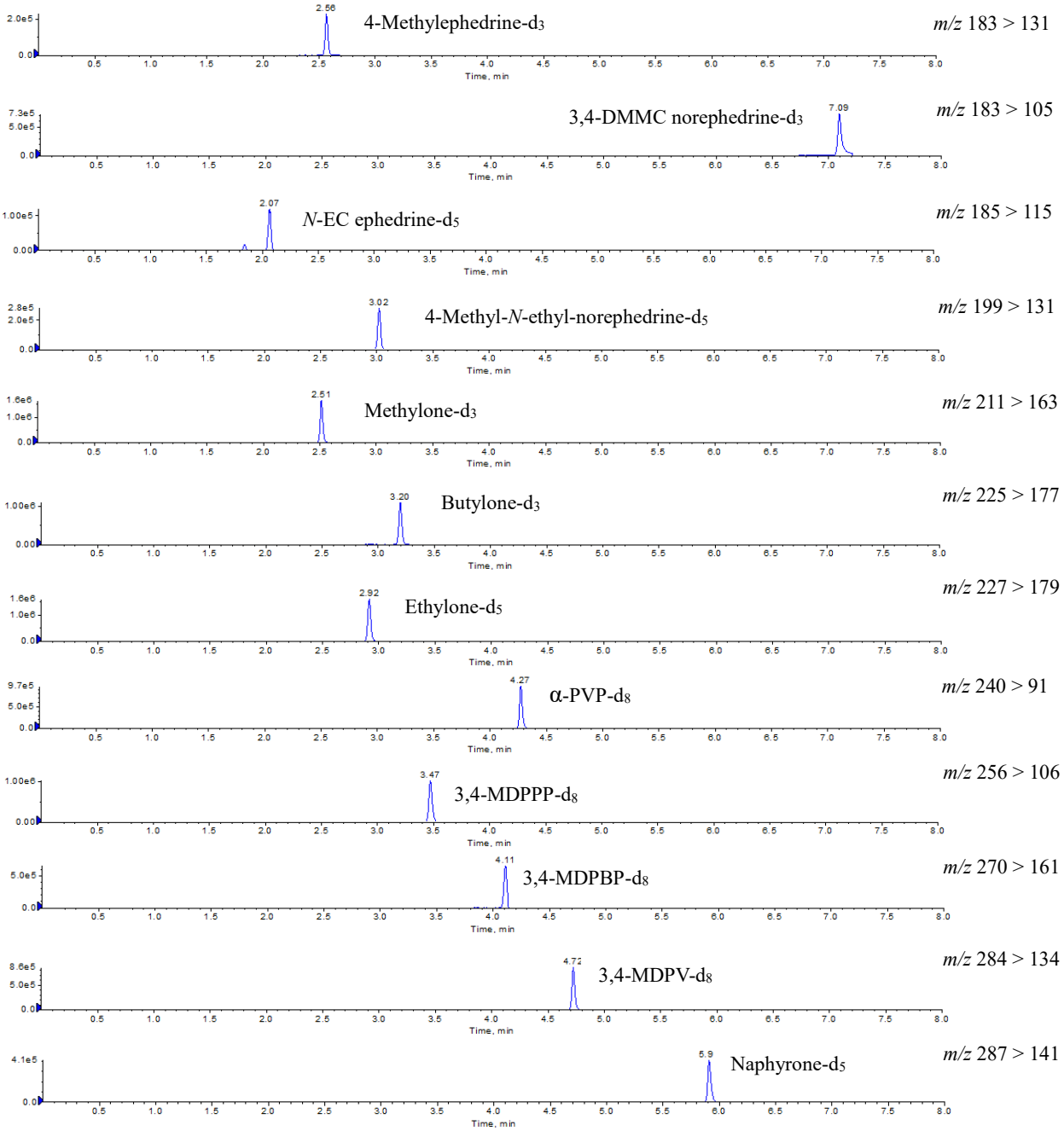

Figure. The MRM chromatograms of 73 cathinones and 14 isotope-labeled internal standards analyzed by LC/MS/MS (continued).

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Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards

Analyte	Ion pair Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
Cathinone	150 > 132*	19	16	Methylone-d_3
	150 > 117	19	30	
Methcathinone	164 > 146*	33	17	Methcathinone-d_3
	164 > 131	33	28	
Ethcathinone	178 > 132*	48	24	α-PVP-d_8
	178 > 130	48	40	
4-Methylmethcathinone (Mephedrone)	178 > 145*	37	28	Methedrone-d_3
	178 > 144	37	39	
N-Ethylcathinone ephedrine (*N*-EC ephedrine)	180 > 117*	19	29	*N*-EC ephedrine-d_5
	180 > 115	19	39	
4-Methylephedrine	180 > 147*	24	29	4-Methylephedrine-d_3
	180 > 91	24	35	
3,4-Dimethylmethcathinone norephedrine (3,4-DMMC norephedrine)	180 > 162*	22	15	3,4-DMMC norephedrine-d_3
	180 > 130	22	33	
4-Fluoromethcathinone (4-FMC)	182 > 164*	25	18	*N*-EC ephedrine-d_5
	182 > 149	25	28	
4-Fluoroephedrine	184 > 135*	17	27	3,4-DMMC norephedrine-d_3
	184 > 151	17	29	
4-Ethylmethcathinone (4-EMC)	192 > 144*	54	40	α-PVP-d_8
	192 > 77	54	67	
4-Methylbuphedrone (4-MeMABP)	192 > 145*	28	29	Methylone-d_3
	192 > 161	28	16	
3,4-Dimethylmethcathinone (3,4-DMMC)	192 > 159*	63	30	Methedrone-d_3
	192 > 158	63	41	
4-Methylethcathinone (4-MEC)	192 > 174*	41	17	Methedrone-d_3
	192 > 130	41	48	
4-Methoxymethcathinone (Methedrone)	194 > 161*	38	27	Butylone-d_3
	194 > 118	38	50	
4-Methyl-*N*-ethyl-norephedrine	194 > 176*	33	17	4-Methyl-*N*-ethyl-norephedrine-d_5
	194 > 131	33	28	

*Quantitative ion pair.

Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards (continued)

Analyte	Ion pair Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
4-Fluoroethcathinone	196 > 178*	44	17	Methylone-d_3
(4-FEC)	196 > 150	44	26	
4-Chloromethcathinone	198 > 145*	34	26	3,4-MDPBP-d_8
(4-CMC)	198 > 144	34	40	
α-Pyrrolidinopropiophenone	204 > 105*	70	29	α-PVP-d_8
(α-PPP)	204 > 98	70	33	
4-Methylpentedrone	206 > 144*	62	44	Methylone-d_3
(MPD)	206 > 105	62	27	
4-Ethylethcathinone	206 > 188*	44	18	α-PVP-d_8
(4-EEC)	206 > 159	44	27	
4-Methoxyethcathinone	208 > 146*	55	40	Methedrone-d_3
(4-MeOEC)	208 > 175	55	26	
Mexedrone	208 > 158*	41	19	Methylone-d_3
	208 > 176	41	17	
Methylone	208 > 160*	30	24	Methylone-d_3
	208 > 132	30	37	
α-Pyrrolidinopropiothiophenone	210 > 98*	68	29	3,4-MDPPP-d_8
(α-PPT)	210 > 111	68	33	
4-Chlorodimethylcathinone	212 > 139*	43	28	Methylone-d_3
(4-CDC)	212 > 167	43	22	
4-Chloroethcathinone	212 > 194*	49	19	Methylone-d_3
(4-CEC)	212 > 159	49	25	
4-Methyl-α-pyrrolidinopropiophenone (4-MPPP)	218 > 119*	30	34	Methylone-d_3
	218 > 147	30	25	
4-Methyl-α-ethylaminopentiophenone (4-MEAPP)	220 > 105*	54	30	Methcathinone-d_3
	220 > 160	54	26	
α-Ethylaminohexanophenone	220 > 130*	59	48	Methylone-d_3
(*N*-Ethyl hexedrone)	220 > 146	59	25	
4-Fluoro-α-pyrrolidinopropiophenone (4-F-α-PPP)	222 > 123*	34	32	3,4-MDPBP-d_8
	222 > 98	34	34	

* Quantitative ion pair.

Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards (continued)

Analyte	Ion pair Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
Butylone	222 > 131*	35	48	Butylone-d_3
	222 > 91	35	17	
Ethylone	222 > 174*	33	25	Ethylone-d_5
	222 > 146	33	35	
α-Pyrrolidinobutiothiophenone (α-PBT)	224 > 112*	66	29	3,4-MDPBP-d_8
	224 > 153	66	22	
α-Pyrrolidinovalerophenone (α-PVP)	232 > 91*	55	31	α-PVP-d_8
	232 > 123	55	35	
4-Methyl-α-pyrrolidinobutiophenone (4-Methyl-α-PBP)	232 > 105*	78	35	3,4-MDPBP-d_8
	232 > 161	78	24	
α-Pyrrolidinovalerophenone metabolite (α-PVP metabolite)	234 > 72*	63	25	α-PVP-d_8
	234 > 91	63	39	
4-Methoxy-α-pyrrolidinopropiophenone (MOPPP)	234 > 98*	78	28	Naphyrone-d_5
	234 > 135	78	32	
4-Fluoro-α-pyrrolidinobutiophenone (4-F-α-PBP)	236 > 109*	43	36	α-PVP-d_8
	236 > 165	43	24	
Pentylone	236 > 188*	32	24	Methylone-d_3
	236 > 218	32	18	
Dibutylone (bk-DMBDB)	236 > 191*	50	20	Methylone-d_3
	236 > 149	50	32	
4-Chloro-α-pyrrolidinopropiophenone (4-Cl-α-PPP)	238 > 139*	66	34	Ethylone-d_5
	238 > 98	66	39	
2,5-Dimethoxy-4-methylmethcathinone (2,5-Dimethoxy mephedrone)	238 > 220*	26	17	Methedrone-d_3
	238 > 189	26	28	
4-Bromomethcathinone (4-BMC)	242 > 145*	37	23	Methedrone-d_3
	242 > 128	37	61	
α-Pyrrolidinohexanophenone (α-PHP)	246 > 91*	81	32	3,4-MDPBP-d_8
	246 > 140	81	35	
Pyrovalerone	246 > 105*	81	32	3,4-MDPBP-d_8
	246 > 126	81	33	

* Quantitative ion pair.

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Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards (continued)

Analyte	Ion pair: Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
3,4-Methylenedioxy-α-pyrrolidinopropiophenone (3,4-MDPPP)	248 > 98*	73	30	Methylone-d_3
	248 > 149	73	34	
4-Methoxy-α-pyrrolidinobutiophenone (4-MeOPBP)	248 > 121*	58	38	Ethylone-d_5
	248 > 135	58	36	
4-Fluoro-α-pyrrolidinovalerophenone (4-F-α-PVP)	250 > 109*	64	32	Methylone-d_3
	250 > 126	64	35	
3,4-Methylenedioxy-*N*-tert-butylcathinone (Tertylone)	250 > 194*	18	18	Methylone-d_3
	250 > 146	18	29	
N-Ethylpentylone (Ephylone)	250 > 202*	40	26	3,4-MDPBP-d_8
	250 > 232	40	21	
N,N-Dimethylpentylone (bk-DMBDP)	250 > 205*	59	22	Methylone-d_3
	250 > 175	59	28	
Benzedrone	254 > 91*	36	45	3,4-MDPBP-d_8
	254 > 65	36	73	
N-Benzylmethcathinone (*N*-BMC)	254 > 162*	42	21	Methcathinone-d_3
	254 > 146	42	22	
4-Bromoethcathinone (4-BEC)	256 > 159*	50	24	Methcathinone-d_3
	256 > 144	50	39	
α-Pyrrolidinoheptiophenone (α-PHPP)	260 > 91*	85	32	3,4-MDPBP-d_8
	260 > 154	85	38	
4-Methyl-α-pyrrolidinohexanophenone (4-Methyl-α-PHP)	260 > 105*	93	31	3,4-MDPBP-d_8
	260 > 140	93	37	
3,4-Dimethyl-α-pyrrolidinovalerophenone (3,4-Dimethyl-α-PVP)	260 > 119*	45	31	3,4-MDPBP-d_8
	260 > 126	45	34	
3,4-Methylenedioxy-α-pyrrolidinobutiophenone (3,4-MDPBP)	262 > 112*	60	32	Methylone-d_3
	262 > 161	60	31	
4-Methoxy-α-pyrrolidinovalerophenone (4-MeO-α-PVP)	262 > 121*	75	34	3,4-MDPBP-d_8
	262 > 126	75	30	
4-Fluoro-α-pyrrolidinohexanophenone (4-F-PHP)	264 > 109*	80	33	3,4-MDPV-d_8
	264 > 140	80	37	

* Quantitative ion pair.

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Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards (continued)

Analyte	Ion pair Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
4-Chloro-α-pyrrolidinovalerophenone (4-Cl-α-PVP)	266 > 125*	69	34	3,4-MDPBP-d_8
	266 > 195	69	25	
3,4-Trimethylene-α-pyrrolidinovalerophenone (Indanyl-α-PVP)	272 > 131*	74	34	3,4-MDPBP-d_8
	272 > 201	74	26	
α-Pyrrolidinooctanophenone (α-POP)	274 > 91*	97	33	3,4-MDPBP-d_8
	274 > 168	97	36	
3,4-Methylenedioxypyrovalerone (3,4-MDPV)	276 > 205*	79	25	3,4-MDPV-d_8
	276 > 126	79	35	
4-Fluoro-α-pyrrolidinoheptiophenone (4-F-α-PHPP)	278 > 109*	44	33	3,4-MDPBP-d_8
	278 > 154	44	38	
3,4-Methylenedioxypyrovalerone metabolite (Demethylenyl-methyl-MDPV)	278 > 175*	80	27	3,4-MDPV-d_8
	278 > 126	80	34	
4-Bromo-α-pyrrolidinopropiophenone (4-Br-α-PPP)	282 > 132*	72	32	α-PVP-d_8
	282 > 98	72	34	
Naphyrone	282 > 141*	100	36	Naphyrone-d_5
	282 > 211	100	26	
3,4-Tetramethylene-α-Pyrrolidinovalerophenone (TH-PVP)	286 > 145*	82	35	Naphyrone-d_5
	286 > 215	82	28	
α-Pyrrolidinononanophenone (α-PNP)	288 > 91*	40	35	3,4-MDPBP-d_8
	288 > 182	40	39	
4-Methoxy-α-pyrrolidinoheptiophenone (4-Methoxy-α-PHPP)	290 > 121*	87	33	Naphyrone-d_5
	290 > 219	87	25	
3,4-Tetramethylene-α-pyrrolidinohexanophenone (TH-PHP)	300 > 145*	76	36	Naphyrone-d_5
	300 > 140	76	39	
4-Methoxy-α-pyrrolidinooctanophenone (4-Methoxy-α-POP)	304 > 121*	73	34	Naphyrone-d_5
	304 > 233	73	26	

* Quantitative ion pair.

Table. MRM parameters of 73 cathinones and 14 isotope-labeled internal standards (continued)

Analyte	Ion pair Precursor ion(*m/z*) >product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal standard
Methcathinone-d_3 (I.S.)	167 > 130	26	40	-
4-Methylmethcathinone-d_3 (Methedrone-d_3) (I.S.)	181 > 148	31	31	-
4-Methylephedrine-d_3 (I.S.)	183 > 131	22	27	-
3,4-Dimethylmethcathinone norephedrine-d_3 (3,4-DMMC norephedrine-d_3) (I.S.)	183 > 105	27	24	-
N-Ethylcathinone ephedrine-d_5 (*N*-EC ephedrine-d_5) (I.S.)	185 > 115	24	41	-
4-Methyl-*N*-ethyl-norephedrine-d_5 (I.S.)	199 > 131	33	28	-
Methylone-d_3 (I.S.)	211 > 163	29	25	-
Butylone-d_3 (I.S.)	225 > 177	35	26	-
Ethylone-d_5 (I.S.)	227 > 179	28	26	-
α-Pyrrolidinovalerophenone-d_8 (α-PVP-d_8) (I.S.)	240 > 91	85	32	-
3,4-Methylenedioxy-α-pyrrolidinopropiophenone-d_8 (3,4-MDPPP-d_8) (I.S.)	256 > 106	80	31	-
3,4-Methylenedioxy-α-pyrrolidinobutiophenone-d_8 (3,4-MDPBP-d_8) (I.S.)	270 > 161	82	33	-
3,4-Methylenedioxypyrovalerone-d_8 (3,4-MDPV-d_8) (I.S.)	284 > 134	91	36	-
Naphyrone-d_5 (I.S.)	287 > 141	80	34	-