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Method of Test for Synthetic Cannabinoids in Urine (2)

1. Scope

This method is applicable to the determination of 34 synthetic cannabinoids (AB-001 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: Sunshell® RP-AQUA, 2.6 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Vortex mixer

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Formic acid, reagent grade;
Artificial urine (UTAK 88121-CDF(L)) or an equivalent product;
Deionized water, resistivity ≥ 18 MΩ · cm (at 25℃);
AB-001 and other 33 synthetic cannabinoids listed in the attached table, reference standards.
AB-CHMINACA-d_4 and 12 other internal standards listed in the attached table.

2.3. Apparatus

2.3.1. Volumetric flask: 1 mL and 10 mL.

2.3.2. Membrane filter: 0.22 μm, PVDF.

2.4. Reagent solution preparation

2.4.1. 50% Methanol

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

2.4.2. 50% Acetonitrile in Methanol

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

2.5. Mobile phase

2.5.1. Solvent A

Dilute 1 mL of formic acid with deionized water to 1000 mL, mix well and filter with a membrane filter.

2.5.2. Solvent B

Dilute 1 mL of formic acid with 50% acetonitrile in methanol to 1000 mL, mix well and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer 1 mg of the 13 internal standards 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. Upon use, mix appropriate volume of the internal standard stock solutions, and dilute with 50% methanol to 1 μg/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer 1 mg of the 34 reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solution. Store at -20℃ in the dark. Upon use, mix appropriate volume of the standard stock solutions, and dilute with 50% methanol to 500 ng/mL as the standard solution.

2.8. Sample solution preparation

Transfer 100 μL of the homogenized sample and 20 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. Transfer 100 μL of the artificial urine, 10-200 μL of the standard solution, and 20 μL of the internal standard solution to a 1-mL volumetric flask and dilute with 50% methanol to volume. Filter with a membrane filter, and take the filtrates as the calibration standard solutions. Operate LC-MS/MS according to the following conditions. Establish the calibration curve of each synthetic cannabinoid by the ratios of the peak area of each synthetic cannabinoid to that of the respective internal standard vs. the added concentrations (5-100 ng/mL).

LC-MS/MS operating conditions(note):

Column: SunShell® RP-AQUA, 2.6 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	60 → 60	40 → 40
0.5 → 12.0	60 → 10	40 → 90
12.0 → 12.5	10 → 10	90 → 90
12.5 → 12.6	10 → 60	90 → 40
12.6 → 14.5	60 → 60	40 → 40

Flow rate: 0.4 mL/min

Ion spray voltage:

5.5 kV for ESI^+ Ionization mode;

4.5 kV for ESI^- Ionization mode.

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). Selected 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 synthetic cannabinoid based on the retention time and the relative ion intensities(note). Calculate the amount of each synthetic cannabinoid in the sample by the following formula:

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

Where,

C: the concentration of each synthetic cannabinoid 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 synthetic cannabinoid is 50 ng/mL.
2. Further validation should be performed when interference compounds appear in the samples.

Reference

Freijo Jr., T. D., Harris, S. E. and Kala, S. V. 2014. A rapid quantitative method for the analysis of synthetic cannabinoids by liquid chromatography–tandem mass spectrometry. J. Anal. Toxicol. 38: 466-478.

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Reference chromatograms

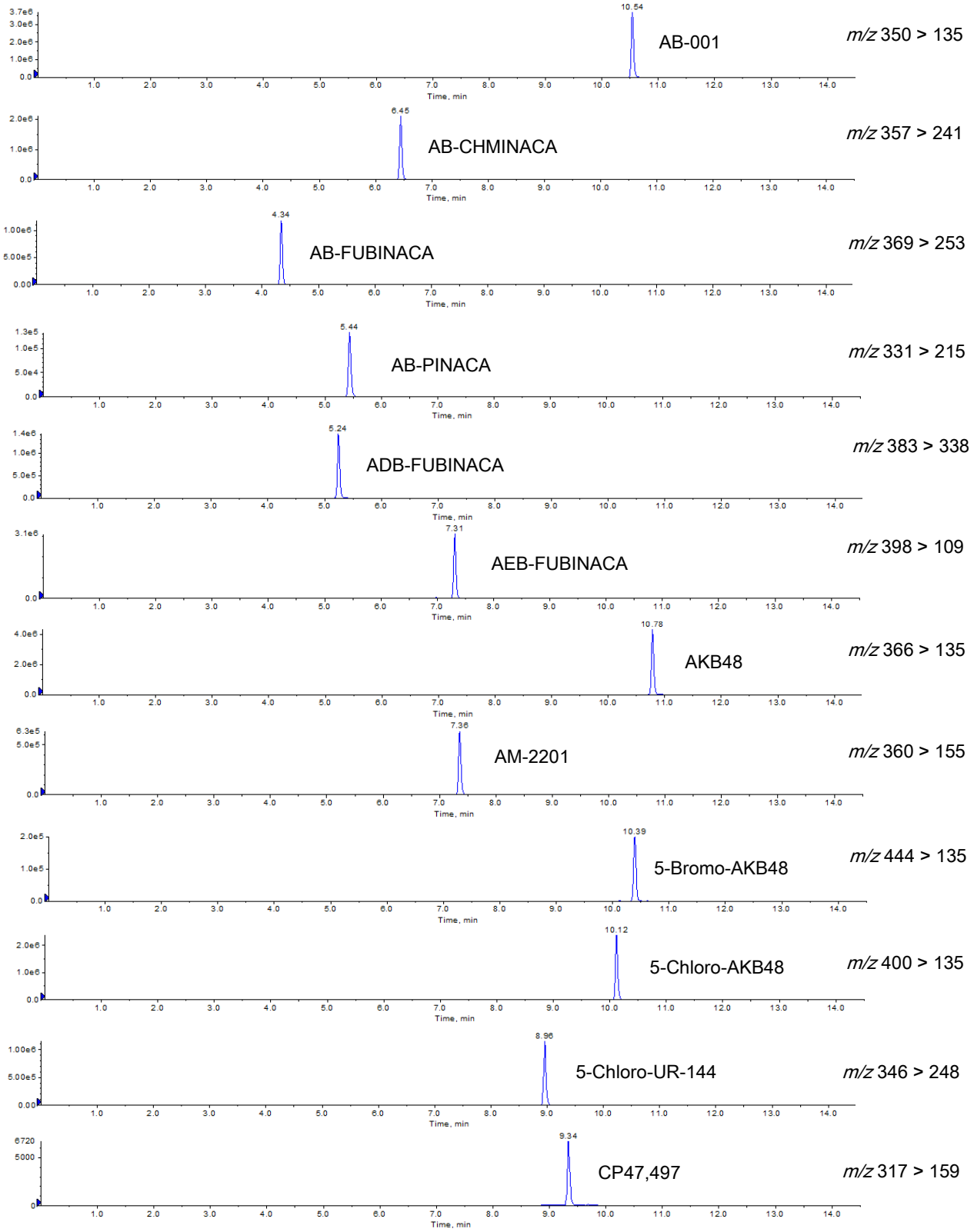

Figure. The MRM chromatograms of 34 synthetic cannabinoids and 13 internal standards analyzed by LC-MS/MS.

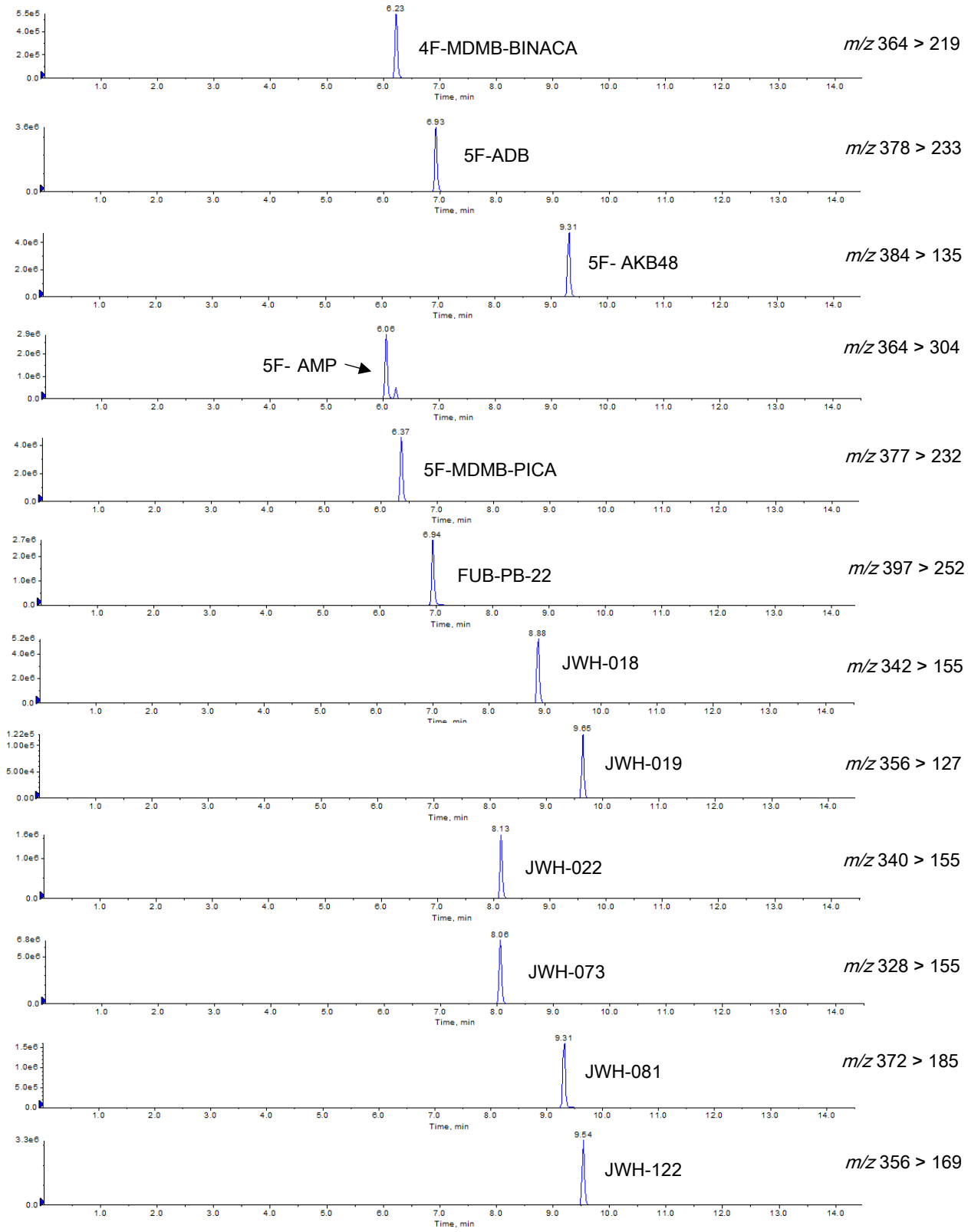

Figure. The MRM chromatograms of 34 synthetic cannabinoids and 13 internal standards analyzed by LC-MS/MS (Continued).

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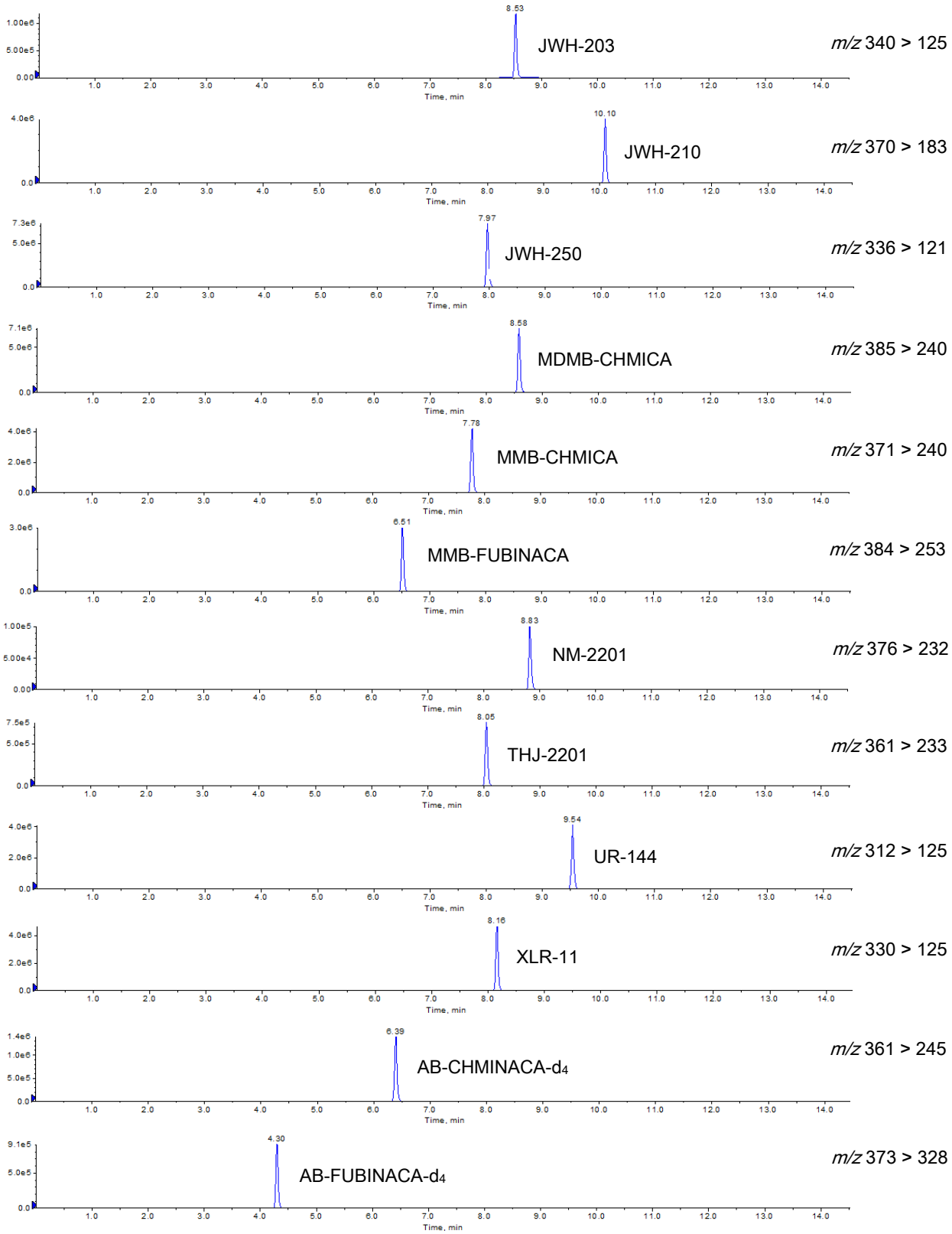

Figure. The MRM chromatograms of 34 synthetic cannabinoids and 13 internal standards analyzed by LC-MS/MS (Continued).

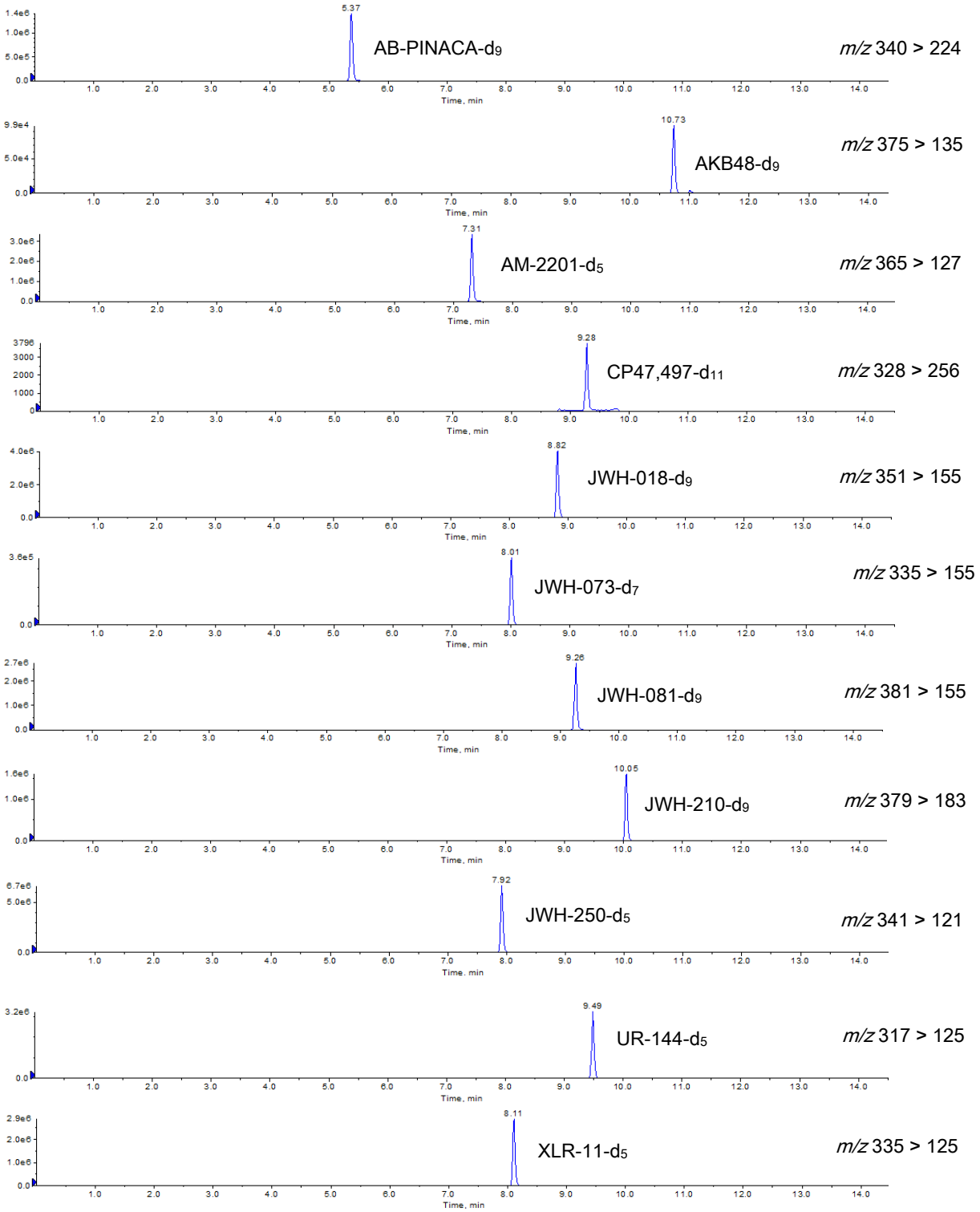

Figure. The MRM chromatograms of 34 synthetic cannabinoids and 13 internal standards analyzed by LC-MS/MS (Continued).

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Table. Parameters of selective ions for 34 synthetic cannabinoids and 13 internal standards.

Analyte	Ionization mode	Ion pair	Declustering potential (V)	Collision energy (eV)	Internal Standard
		Precursor ion (*m/z*) > product ion (*m/z*)			
1-Adamantyl-(1-pentylindol-3-yl)methanone (AB-001)	ESI^+	350 > 135* 350 > 77	154 154	39 111	JWH-210-d_9
N-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1*H*-indazole-3-carboxamide (AB-CHMINACA)	ESI^+	357 > 241* 357 > 312	59 59	35 23	AB-CHMINACA-d_4
N-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-1*H*-indazole-3-carboxamide (AB-FUBINACA)	ESI^+	369 > 253* 369 > 324	83 83	32 21	AB-FUBINACA-d_4
N-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-pentyl-1*H*-indazole-3-carboxamide (AB-PINACA)	ESI^+	331 > 215* 331 > 286	40 40	36 20	AB-PINACA-d_9
N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-[(4-fluorophenyl)methyl]indazole-3-carboxamide (ADB-FUBINACA)	ESI^+	383 > 338* 383 > 253	72 72	21 36	AB-FUBINACA-d_4
N-[[1-[(4-Fluorophenyl)methyl]-1*H*-indazol-3-yl]carbonyl]-L-valine, ethyl ester (AEB-FUBINACA)	ESI^+	398 > 109* 398 > 253	124 124	59 33	AB-FUBINACA-d_4
N-(1-Adamantyl)-1-pentylindazole-3-carboxamide (AKB48)	ESI^+	366 > 135* 366 > 93	76 76	26 66	AKB48-d_9
[1-(5-Fluoropentyl)indol-3-yl]-naphthalen-1-ylmethanone (AM-2201)	ESI^+	360 > 155* 360 > 127	117 117	37 70	AM-2201-d_5
N-(1-Adamantyl)-1-(5-bromopentyl)indazole-3-carboxamide (5-Bromo-AKB48)	ESI^+	444 > 135* 444 > 79	36 36	30 96	AKB48-d_9
N-(1-Adamantyl)-1-(5-chloropentyl)indazole-3-carboxamide (5-Chloro-AKB48)	ESI^+	400 > 135* 400 > 93	39 39	26 71	AKB48-d_9
(1-(5-Chloropentyl)-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (5-Chloro-UR-144)	ESI^+	346 > 248* 346 > 144	100 100	37 51	UR-144-d_5

*Quantitative ion

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Table. Parameters of selective ions for 34 synthetic cannabinoids and 13 internal standards (Continued).

Analyte	Ionization mode	Ion pair	Declustering potential (V)	Collision energy (eV)	Internal Standard
		Precursor ion (*m/z*) > product ion (*m/z*)			
rel-5-(1,1-Dimethylheptyl)-2-[(1*R*,3*S*)-3-hydroxycyclohexyl]-phenol (CP47,497)	ESI^-	317 > 159*	140	71	CP47,497-d_{11}
		317 > 245	140	45	
Methyl (2*S*)-2-[[1-(4-fluorobutyl)indazole-3-carbonyl]amino]-3,3-dimethylbutanoate (4F-MDMB-BINACA)	ESI^+	364 > 219*	60	33	AB-CHMINACA-d_4
		364 > 304	60	22	
Methyl 2-[[1-(5-fluoropentyl)indazole-3-carbonyl]amino]-3,3-dimethylbutanoate (5F-ADB)	ESI^+	378 > 233*	97	33	AM-2201-d_5
		378 > 318	97	23	
N-(1-Adamantyl)-1-(5-fluoropentyl)-1*H*-Indazole-3-Carboxamide (5F-AKB48)	ESI^+	384 > 135*	72	26	AKB48-d_9
		384 > 93	72	68	
Methyl (2*S*)-2-[[1-(5-fluoropentyl)indazole-3-carbonyl]amino]-3-methylbutanoate (5F-AMP)	ESI^+	364 > 304*	108	22	AB-FUBINACA-d_4
		364 > 233	108	31	
Methyl (2*S*)-2-[[1-(5-fluoropentyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate (5F-MDMB-PICA)	ESI^+	377 > 232*	89	32	XLR-11-d_5
		377 > 144	89	48	
Quinolin-8-yl 1-[(4-fluorophenyl)methyl]indole-3-carboxylate (FUB-PB-22)	ESI^+	397 > 252*	78	24	AM-2201-d_5
		397 > 109	78	49	
Naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018)	ESI^+	342 > 155*	110	32	JWH-018-d_9
		342 > 127	110	72	
(1-Hexylindol-3-yl)-naphthalen-1-ylmethanone (JWH-019)	ESI^+	356 > 127*	78	33	JWH-210-d_9
		356 > 155	78	64	
Naphthalen-1-yl-(1-pent-4-enylindol-3-yl)methanone (JWH-022)	ESI^+	340 > 155*	142	32	JWH-250-d_5
		340 > 127	142	60	
(1-Butylindol-3-yl)-naphthalen-1-ylmethanone (JWH-073)	ESI^+	328 > 155*	151	32	JWH-073-d_7
		328 > 127	151	65	
(4-Methoxynaphthalen-1-yl)-(1-pentylindol-3-yl)methanone (JWH-081)	ESI^+	372 > 185*	102	35	JWH-081-d_5
		372 > 114	102	103	
(4-Methylnaphthalen-1-yl)-(1-pentylindol-3-yl)methanone (JWH-122)	ESI^+	356 > 169*	57	34	JWH-210-d_9
		356 > 115	57	86	

*Quantitative ion

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Table. Parameters of selective ions for 34 synthetic cannabinoids and 13 internal standards (Continued).

Analyte	Ionization mode	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal Standard
2-(2-Chlorophenyl)-1-(1-pentylindol-3-yl)ethenone (JWH-203)	ESI^+	340 > 125* 340 > 214	136 136	36 35	JWH-018-d_9
(4-Ethylnaphthalen-1-yl)-(1-pentylindol-3-yl)methanone (JWH-210)	ESI^+	370 > 183* 370 > 214	138 138	33 34	JWH-210-d_9
2-(2-Methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone (JWH-250)	ESI^+	336 > 121* 336 > 91	129 129	27 65	JWH-250-d_5
Methyl (2*S*)-2-[[1-(cyclohexylmethyl)indole-3-carbonyl]amino]-3,3-dimethylbutanoate (MDMB-CHMICA)	ESI^+	385 > 240* 385 > 144	80 80	25 55	XLR-11-d_5
Methyl (2*S*)-2-[[1-(cyclohexylmethyl)indole-3-carbonyl]amino]-3-methylbutanoate (MMB-CHMICA)	ESI^+	371 > 240* 371 > 144	32 32	23 50	AB-CHMINACA-d_4
Methyl 2-[[1-[(4-fluorophenyl)methyl]indazole-3-carbonyl]amino]-3-methylbutanoate (MMB-FUBINACA)	ESI^+	384 > 253* 384 > 109	106 106	32 66	AB-FUBINACA-d_4
Naphthalen-1-yl 1-(5-fluoropentyl)indole-3-carboxylate (NM-2201)	ESI^+	376 > 232* 376 > 144	55 55	27 52	JWH-210-d_9
[1-(5-Fluoropentyl)indazol-3-yl]-naphthalen-1-ylmethanone (THJ-2201)	ESI^+	361 > 233* 361 > 145	126 126	24 48	JWH-210-d_9
(1-Pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone (UR-144)	ESI^+	312 > 125* 312 > 214	56 56	30 31	UR-144-d_5
[1-(5-Fluoropentyl)indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl)methanone (XLR-11)	ESI^+	330 > 125* 330 > 232	126 126	30 33	XLR-11-d_5
N-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-(cyclohexylmethyl)indazole-3-carboxamide-d_4 (AB-CHMINACA-d_4) (I.S.)	ESI^+	361 > 245	114	35	-

*Quantitative ion

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Table. Parameters of selective ions for 34 synthetic cannabinoids and 13 internal standards (Continued).

Analyte	Ionization mode	Ion pair	Declustering potential (V)	Collision energy (eV)	Internal Standard
		Precursor ion (*m/z*) > product ion (*m/z*)			
N-[(2*S*)-1-Amino-3-methyl-1-oxobutan-2-yl]-1-[(4-fluorophenyl)methyl]indazole-3-carboxamide-d_4 (AB-FUBINACA-d_4) (I.S.)	ESI^+	373 > 328	68	21	-
N-[(1*S*)-1-(Aminocarbonyl)-2-methylpropyl]-1-pentyl-1*H*-indazole-3-carboxamide-d_9 (AB-PINACA-d_9) (I.S.)	ESI^+	340 > 224	40	36	-
N-(1-Adamantyl)-1-pentylindazole-3-carboxamide-d_9 (AKB48-d_9) (I.S.)	ESI^+	375 > 135	69	15	-
[1-(5-Fluoropentyl)indol-3-yl]-naphthalen-1-ylmethanone-d_5 (AM-2201-d_5) (I.S.)	ESI^+	365 > 127	31	73	-
rel-5-(1,1-Dimethylheptyl)-2-[(1*R*,3*S*)-3-hydroxycyclohexyl]-phenol-d_{11} (CP47,497-d_{11}) (I.S.)	ESI^-	328 > 256	72	47	-
Naphthalen-1-yl-(1-pentylindol-3-yl)methanone-d_9 (JWH-018-d_9) (I.S.)	ESI^+	351 > 155	36	33	-
(1-Butylindol-3-yl)-naphthalen-1-ylmethanone-d_7 (JWH-073-d_7) (I.S.)	ESI^+	335 > 155	76	33	-
(4-Methoxynaphthalen-1-yl)-(1-pentylindol-3-yl)methanone-d_9 (JWH-081-d_9) (I.S.)	ESI^+	381 > 185	51	35	-
(4-Ethylnaphthalen-1-yl)-(1-pentylindol-3-yl)methanone-d_9 (JWH-210-d_9) (I.S.)	ESI^+	379 > 183	36	33	-
2-(2-Methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone-d_5 (JWH-250-d_5) (I.S.)	ESI^+	341 > 121	46	27	-
(1-Pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone-d_5 (UR-144-d_5) (I.S.)	ESI^+	317 > 125	161	31	-
[1-(5-fluoropentyl)indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl)methanone-d_5 (XLR-11-d_5) (I.S.)	ESI^+	335 > 125	61	31	-

*Quantitative ion