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Method of Test for Synthetic Cannabinoids in Urine (1)

1. Scope

This method is applicable to the determination of 39 synthetic cannabinoids (AB-CHMINACA etc. listed as the attached table) in urine.

2. Method

After hydrolysis and purification, analytes are determined by liquid chromatography/tandem mass spectrometery (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

2.1.3. Ultrasonicator

2.1.4. Reciprocal shaking water bath

2.1.5. Nitrogen evaporator

2.1.6. Solid phase vacuum extraction manifold

2.2. Chemicals

Methanol and acetonitrile, HPLC grade;
β-glucuronidase (100,000 units/mL);
Acetic acid, analytical grade;
Ethyl acetate, sodium acetate (CH_3COONa), reagent grade;
Formic acid, mass spectrometry grade;
Artificial urine (UTAK 88121-CDF(L) or an equivalent product), reagent grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);
AB-CHMINACA etc. listed in the attached table, reference standards;
AB-CHMINACA-d_4 and other isotope-labeled internal standards (listed in the attached table).

2.3. Apparatus

2.3.1. Volumetric flask: 1 mL and 10 mL

2.3.2. Centrifuge tube: 15 mL, PP

2.3.3. Membrane filter: 0.22 μm, PVDF

2.3.4. Solid-supported liquid-liquid extraction cartridge: Novum™ SLE cartridge, 3 mL, or an equivalent product

2.4. Reagent solution preparation

2.4.1. 0.1 M sodium acetate solution

Dissolve and dilute 0.82 g of sodium acetate with deionized water to 100 mL.

2.4.2. 20% acetic acid solution

Dilute 20 mL of acetic acetate with 80 mL of deionized water to 100 mL.

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 methanol/acetonitrile solution (1:1, v/v) to 1000 mL, mix well and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer 1 mg of the 18 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 adequate volume of the internal standard stock solutions, and dilute with methanol to 1 μg/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer 1 mg of the 39 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. Upon use, mix adequate volume of the standard stock solutions, and dilute with methanol to 1 μg/mL as the standard solution.

2.8. Sample solution preparation

2.8.1. Hydrolysis

Mix 1 mL of the homogenized sample accurately with 20 μL of the internal standard solution, and 1 mL of 0.1 M sodium acetate solution in a 15 mL centrifuge tube. Add 500 μL of β-glucuronidase and mix well. Transfer the above solution into the reciprocal shaking water bath and incubate at 56 ℃ with 80 rpm for 15 min. Allow the solution to cool to room temperature and add 500 μL of 20% acetic acid solution for subsequent purification.

2.8.2. Purification

Transfer accurate 400 μL of the hydrolyzed sample solution mentioned in

section 2.8.1. into the solid-supported liquid-liquid extraction cartridge and hold for 5 min. Elute the cartridge with 0.9 mL of ethyl acetate for twice. Collect the eluent and evaporate to dryness by gently flushing with a steam of nitrogen at 40℃. Dissolve the residue with 1 mL of methanol and filter with a membrane filter as the sample solution.

2.9. Calibration curve

Use the artificial urine as the blank sample. Separately take 5-100 μL of the standard solution and mix with artificial urine to 1 mL. Prepare the calibration solutions following the procedure in section 2.8. 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 internal standard vs. the added concentrations (5-100 ng/mL).

LC-MS/MS operating conditions[(1)]:

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:

Electrospray ionization positive mode (ESI^+): 5.5 KV

Electrospray ionization negative mode (ESI^-): -4.5 KV

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 pairs, declustering potential and collision energy are shown in

the attached table.

Note 1: 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[2]. Calculate the amount (ng/mL) 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 2: 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

Scheidweiler, K. B. and Huestis, M. A. 2014. Simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semi-quantification of 12 alkyl hydroxy metabolites in human urine by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1327: 105-117.

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Reference chromatograms

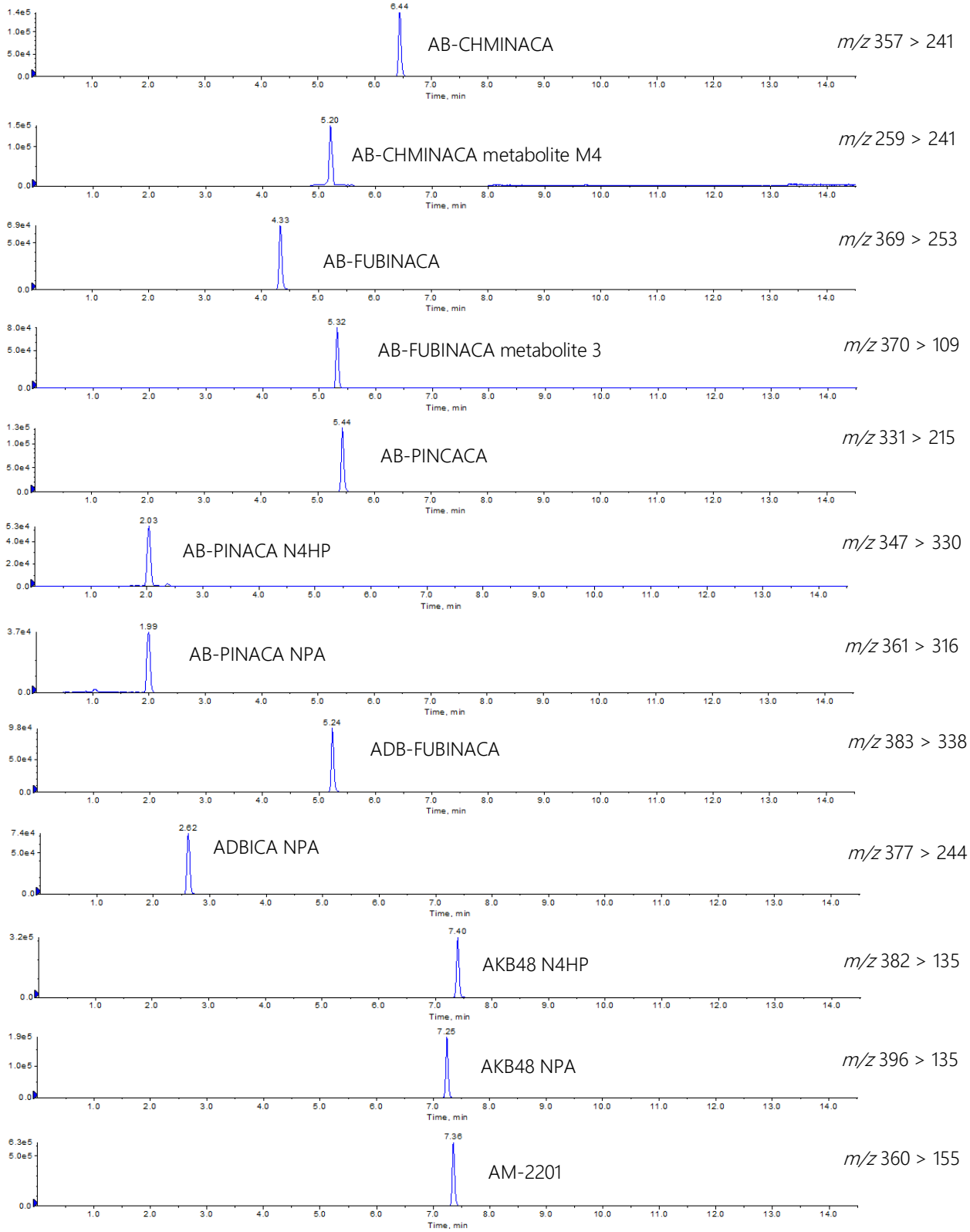

Figure. The MRM chromatograms of 39 synthetic cannabinoids and 18 internal standards analyzed by LC/MS/MS.

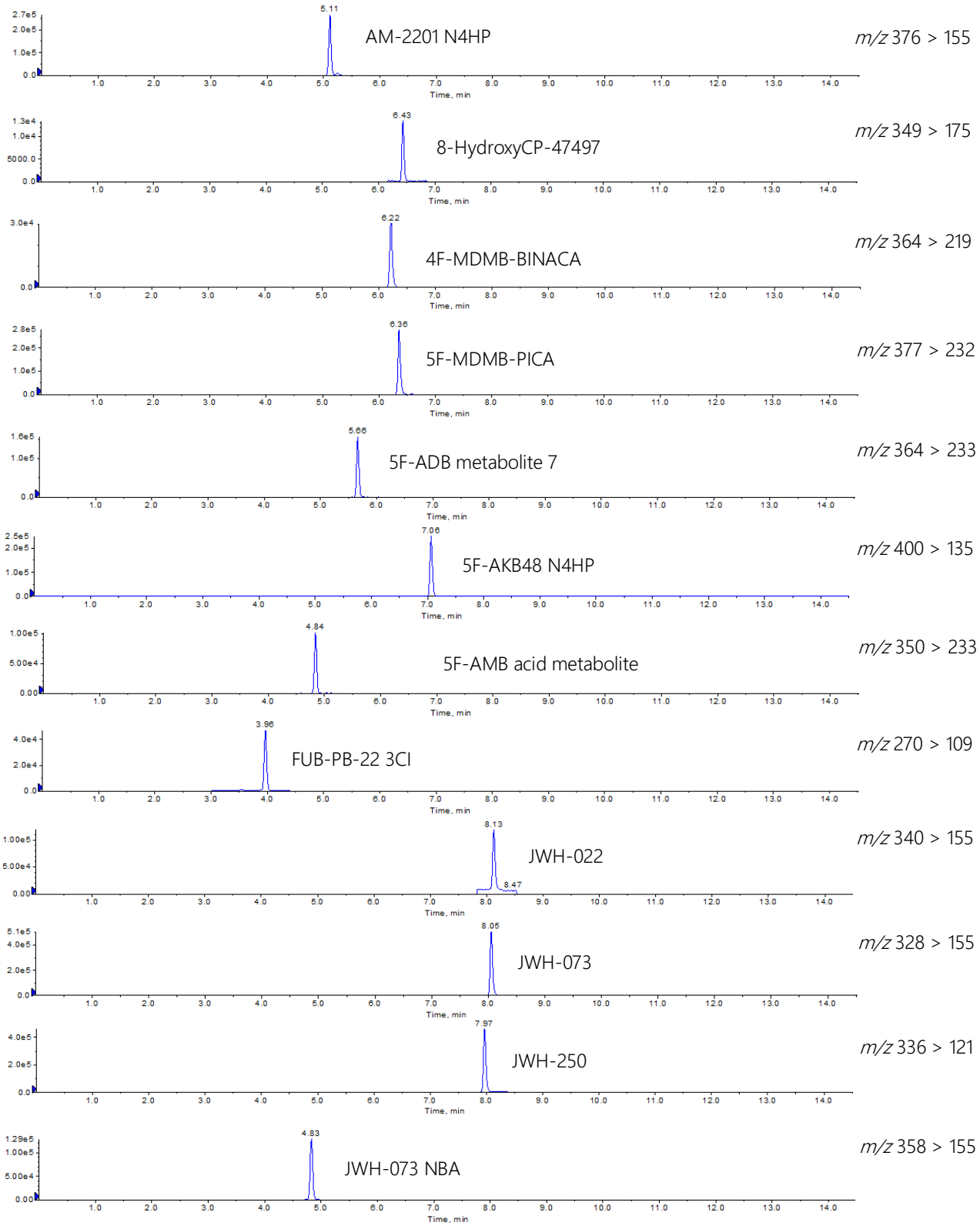

Figure. The MRM chromatograms of 39 synthetic cannabinoids and 18 internal standards analyzed by LC/MS/MS (continued).

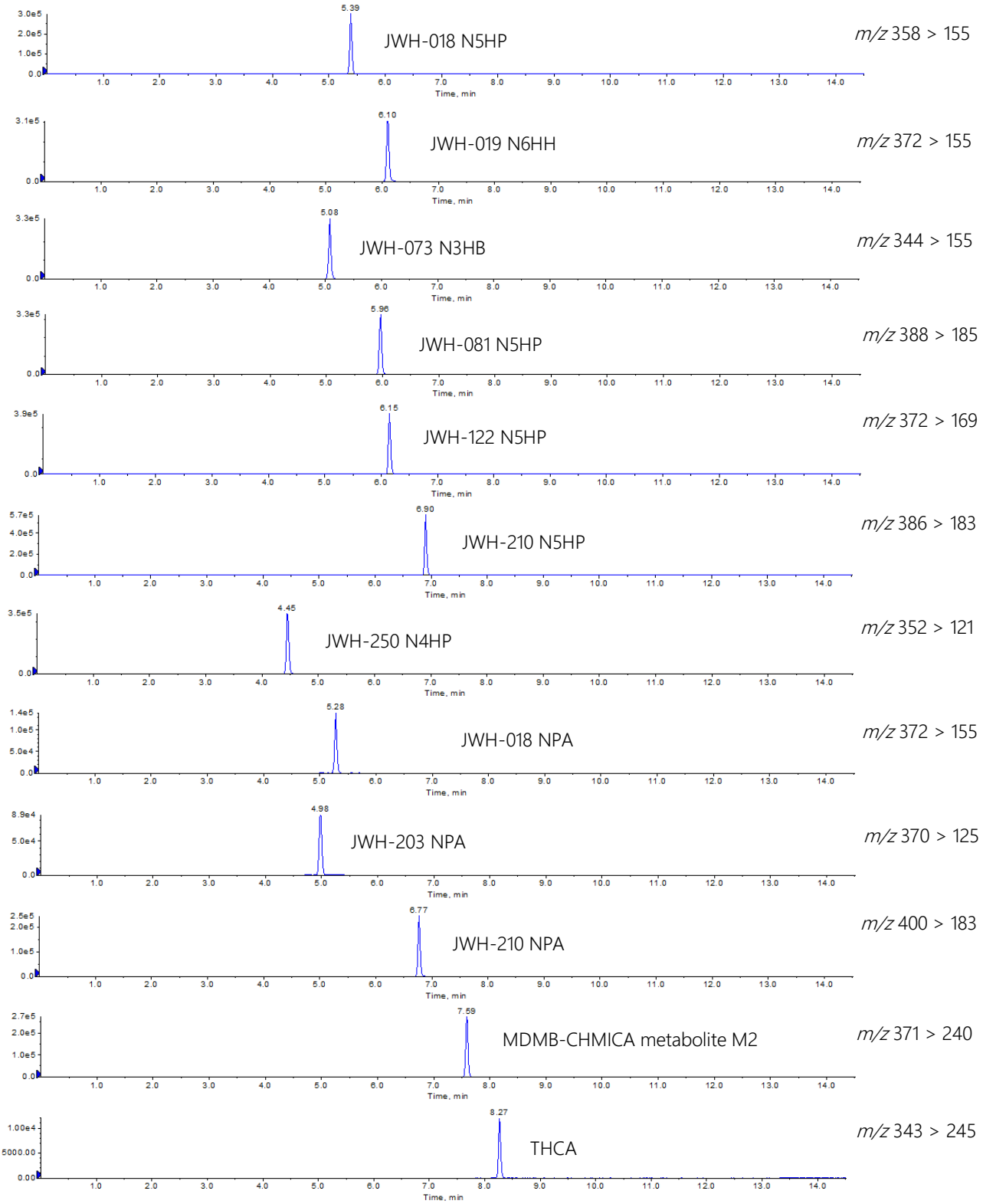

Figure. The MRM chromatograms of 39 synthetic cannabinoids and 18 internal standards analyzed by LC/MS/MS (continued).

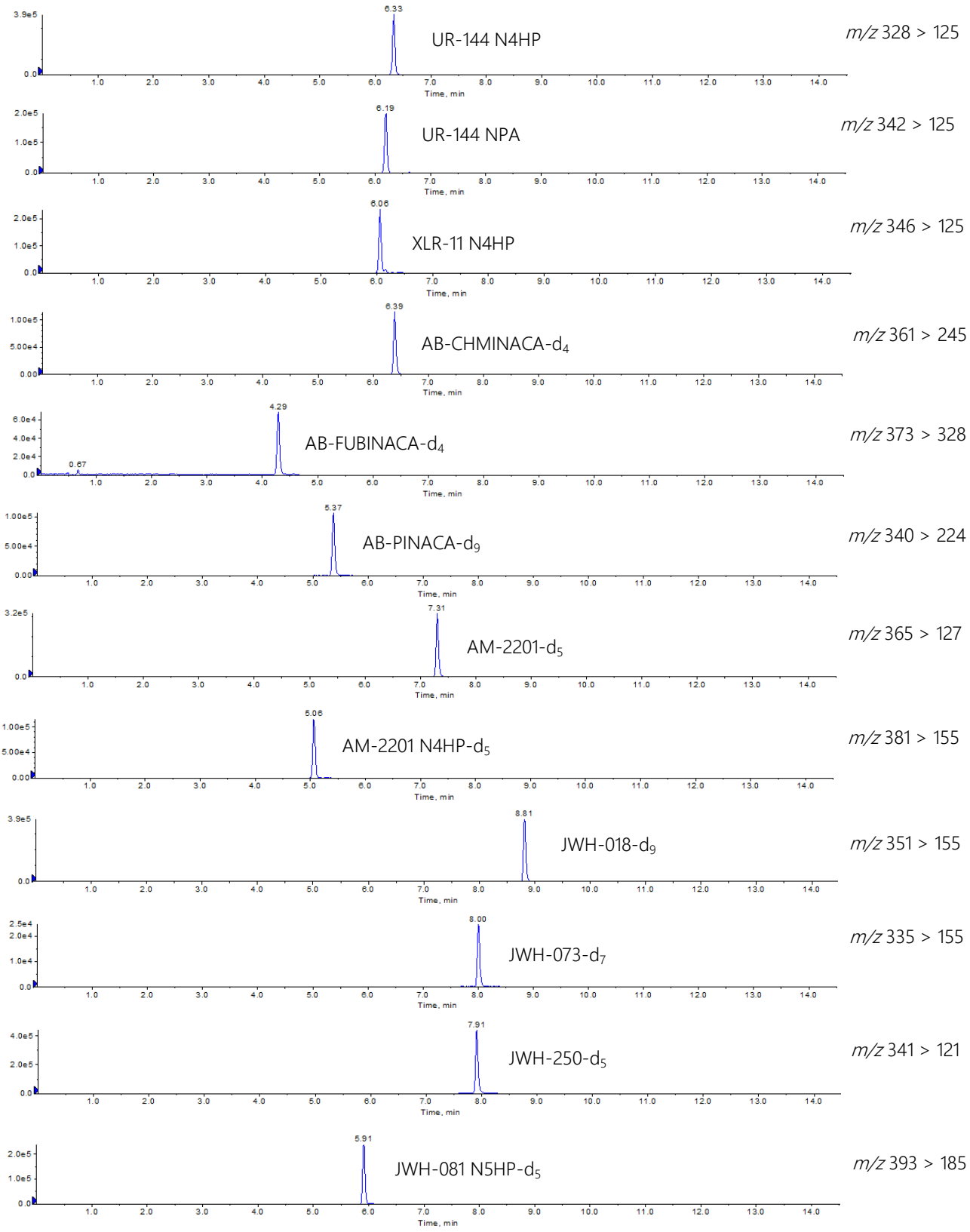

Figure. The MRM chromatograms of 39 synthetic cannabinoids and 18 internal standards analyzed by LC/MS/MS (continued).

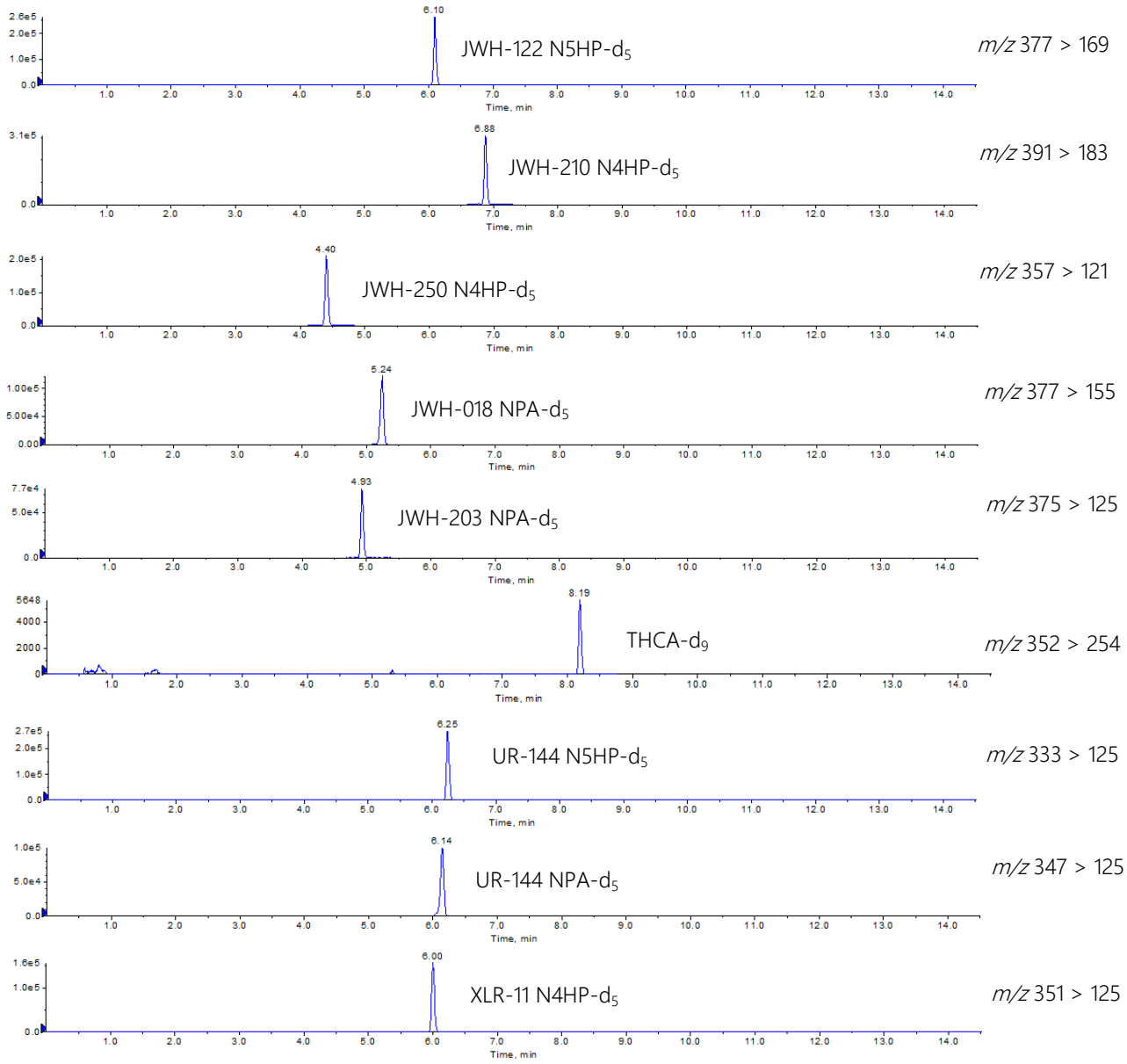

Figure. The MRM chromatograms of 39 synthetic cannabinoids and 18 internal standards analyzed by LC/MS/MS (continued).

Table. MRM parameters of 39 synthetic cannabinoids and 18 internal standards.

Analyte	Ion pairs: Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal Standard
AB-CHMINACA	357 > 241*	59	35	AB-CHMINACA-d_4
	357 > 312	59	23	
AB-CHMINACA metabolite M4	259 > 241*	53	21	JWH-018 NPA-d_5
	259 > 145	53	34	
AB-FUBINACA	369 > 253*	83	32	AB-FUBINACA-d_4
	369 > 324	83	21	
AB-FUBINACA metabolite 3	370 > 109*	76	70	UR-144 NPA-d_5
	370 > 253	76	31	
AB-PINACA	331 > 215*	40	36	AB-PINACA-d_9
	331 > 286	40	20	
AB-PINACA *N*-(4-hydroxypentyl) metabolite (AB-PINACA N4HP)	347 > 330*	65	14	UR-144 NPA-d_5
	347 > 213	65	38	
AB-PINACA 5-pentanoic acid metabolite (AB-PINACA NPA)	361 > 316*	64	22	UR-144 NPA-d_5
	361 > 217	64	42	
ADB-FUBINACA	383 > 338*	72	21	AB-FUBINACA-d_4
	383 > 253	72	36	
ADBICA *N*-pentanoic acid metabolite (ADBICA NPA)	377 > 244*	58	30	UR-144 NPA-d_5
	377 > 144	58	52	
AKB48 *N*-(4-hydroxypentyl) metabolite (AKB48 N4HP)	382 > 135*	56	30	JWH-018 NPA-d_5
	382 > 93	56	75	
AKB48 *N*-pentanoic acid metabolite (AKB48 NPA)	396 > 135*	75	29	UR-144 NPA-d_5
	396 > 93	75	73	
AM-2201	360 > 155*	117	37	AM-2201-d_5
	360 > 127	117	70	
AM-2201 *N*-(4-hydroxypentyl) metabolite (AM-2201 N4HP)	376 > 155*	76	34	AM-2201 N4HP-d_5
	376 > 127	76	61	
CP 47,497-C8-homolog C-8-hydroxy metabolite (8-HydroxyCP-47497)	349 > 175*	59	19	UR-144 NPA-d_5
	349 > 331	59	12	
4F-MDMB-BINACA	364 > 219*	60	33	AB-FUBINACA-d_4
	364 > 304	60	23	

*Quantitative ion

Table. MRM parameters of 39 synthetic cannabinoids and 18 i internal standards (continued).

Analyte	Ion pairs: Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal Standard
5F-MDMB-PICA	377 > 232*	89	32	AM-2201-d_5
	377 > 144	89	48	
5-fluoro MDMB-PINACA metabolite 7 (5F-ADB metabolite 7)	364 > 233*	89	32	UR-144 NPA-d_5
	364 > 318	89	22	
5-fluoro AKB48 *N*-(4-hydroxypentyl) metabolite (5F-AKB48 N4HP)	400 > 135*	136	28	UR-144 NPA-d_5
	400 > 93	136	74	
(S)-5F-AMB acid metabolite (5F-AMB acid metabolite)	350 > 233*	120	28	UR-144 N5HP-d_5
	350 > 145	120	53	
FUB-PB-22 3-carboxyindole metabolite (FUB-PB-22 3CI)	270 > 109*	68	25	JWH-018 NPA-d_5
	270 > 83	68	81	
JWH-022	340 > 155*	142	32	JWH-018-d_9
	340 > 127	142	60	
JWH-073	328 > 155*	151	32	JWH-073-d_7
	328 > 127	151	65	
JWH-250	336 > 121*	129	27	JWH-250-d_5
	336 > 91	129	65	
JWH-073 *N*-butanoic acid metabolite (JWH-073 NBA)	358 > 155*	55	30	JWH-203 NPA-d_5
	358 > 127	55	64	
JWH-018 *N*-(5-hydroxypentyl) metabolite (JWH-018 N5HP)	358 > 155*	75	29	JWH-018 NPA-d_5
	358 > 127	75	67	
JWH-019 *N*-(6-hydroxyhexyl) metabolite (JWH-019 N6HH)	372 > 155*	91	32	UR-144 NPA-d_5
	372 > 127	91	72	
JWH-073 *N*-(3-hydroxybutyl) metabolite (JWH-073 N3HB)	344 > 155*	40	34	JWH-203 NPA-d_5
	344 > 127	40	60	
JWH-081 *N*-(5-hydroxypentyl) metabolite (JWH-081 N5HP)	388 > 185*	152	33	JWH-081 N5HP-d_5
	388 > 157	152	58	
JWH-122 *N*-(5-hydroxypentyl) metabolite (JWH-122 N5HP)	372 > 169*	85	32	JWH-122 N5HP-d_5
	372 > 115	85	97	
JWH-210 *N*-(5-hydroxypentyl) metabolite (JWH-210 N5HP)	386 > 183*	145	34	JWH-210 N4HP-d_5
	386 > 153	145	65	

*Quantitative ion.

Table. MRM parameters of 39 synthetic cannabinoids and 18 internal standards (continued).

Analyte	Ion pairs: Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal Standard
JWH-250 *N*-(4-hydroxypentyl) metabolite (JWH-250 N4HP)	352 > 121*	86	28	JWH-250 N4HP-d_5
	352 > 91	86	67	
JWH-018 *N*-pentanoic acid metabolite (JWH-018 NPA)	372 > 155*	72	33	JWH-018 NPA-d_5
	372 > 127	72	74	
JWH-203 *N*-pentanoic acid metabolite (JWH-203 NPA)	370 > 125*	135	34	JWH-203 NPA-d_5
	370 > 200	135	27	
JWH-210 *N*-pentanoic acid metabolite (JWH-210 NPA)	400 > 183*	70	34	JWH-210 N4HP-d_5
	400 > 153	70	70	
MDMB-CHMICA metabolite M2	371 > 240*	79	28	UR-144 NPA-d_5
	371 > 144	79	48	
(±)-11-nor-9-carboxy-Δ9-THC (THCA)	343 > 245*	-140	-38	THCA-d_9
	343 > 191	-140	-42	
UR-144 *N*-(4-hydroxypentyl) metabolite (UR-144 N4HP)	328 > 125*	94	27	UR-144 N5HP-d_5
	328 > 97	94	34	
UR-144 *N*-pentanoic acid metabolite (UR-144 NPA)	342 > 125*	47	30	UR-144 NPA-d_5
	342 > 244	47	32	
XLR-11 *N*-(4-hydroxypentyl) metabolite (XLR-11 N4HP)	346 > 125*	94	28	XLR-11 N4HP-d_5
	346 > 248	94	33	
AB-CHMINACA-d_4 (I.S.)	361 > 245	114	35	-
AB-FUBINACA-d_4 (I.S.)	373 > 328	68	21	-
AB-PINACA-d_9 (I.S.)	340 > 224	40	36	-
AM-2201-d_5 (I.S.)	365 > 127	31	73	-
AM-2201 N-(4-hydroxypentyl) metabolite-d_5 (AM-2201 N4HP-d_5) (I.S.)	381 > 155	88	35	-
JWH-018-d_9 (I.S.)	351 > 155	36	33	-
JWH-073-d_7 (I.S.)	335 > 155	76	33	-
JWH-250-d_5 (I.S.)	341 > 121	46	27	-
JWH-081 *N*-(5-hydroxypentyl) metabolite-d_5 (JWH-081 N5HP-d_5) (I.S.)	393 > 185	118	32	-
JWH-122 *N*-(5-hydroxypentyl) metabolite-d_5 (JWH-122 N5HP-d_5) (I.S.)	377 > 169	91	33	-

*Quantitative ion

Table. MRM parameters of 39 synthetic cannabinoids and 18 internal standards (continued).

Analyte	Ion pairs: Precursor ion (*m/z*) > product ion (*m/z*)	Declustering potential (V)	Collision energy (eV)	Internal Standard
JWH-210 *N*-(4-hydroxypentyl) metabolite-d_5 (JWH-210 N4HP-d_5) (I.S.)	391 > 183	111	32	—
JWH-250 *N*-(4-hydroxypentyl) metabolite-d_5 (JWH-250 N4HP-d_5) (I.S.)	357 > 121	76	29	—
JWH-018 *N*-pentanoic acid metabolite-d_5 (JWH-018 NPA-d_5) (I.S.)	377 > 155	64	34	—
JWH-203 *N*-pentanoic acid metabolite-d_5 (JWH-203 NPA-d_5) (I.S.)	375 > 125	73	35	—
(±)-11-nor-9-carboxy-Δ9-THC-d_9 (THCA-d_9) (I.S.)	352 > 254	-160	-38	—
UR-144 *N*-(5-hydroxypentyl) metabolite-d_5 (UR-144 N5HP-d_5) (I.S.)	333 > 125	74	25	—
UR-144 5-pentanoic acid metabolite-d_5 (UR-144 NPA-d_5) (I.S.)	347 > 125	64	31	—
XLR-11 *N*-(4-hydroxypentyl) metabolite-d_5 (XLR-11 N4HP-d_5) (I.S.)	351 > 125	83	29	—