LC-MS/MS based method for the detection and partial quantification of the major metabolites of 18 synthetic cannabinoids in human urine samples

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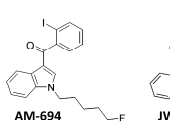
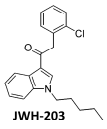
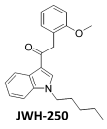
Introduction

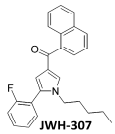
Several methods for the analysis of synthetic cannabinoids in blood, serum and urine have been published recently. However, comprehensive methods for urine screening covering all currently relevant substances are lacking. For development of methods for urine analysis the metabolism of the synthetic cannabinoids has to be investigated first, as the unchanged compounds usually are not found in urine after consumption. Aim of this study was to develop an LC-MS/MS based analytical method for detection and partial quantification of metabolites of synthetic cannabinoids in urine.

Synthetic cannabinoids covered by the method

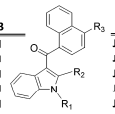




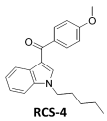


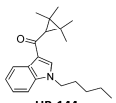


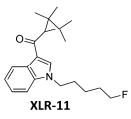
	R1	R2	R3
AM-2201	5-fluoropentyl	H	H
JWH-007	pentyl	methyl	H
JWH-018	pentyl	H	H
JWH-019	hexyl	H	H
JWH-073	butyl	H	H



	R1	R2	R3
JWH-081	pentyl	H	methoxy
JWH-122	pentyl	H	methyl
JWH-200	4-ethylmorpholino	H	H
JWH-210	pentyl	H	ethyl
JWH-398	pentyl	H	chloro
MAM-2201	5-fluoropentyl	H	methyl

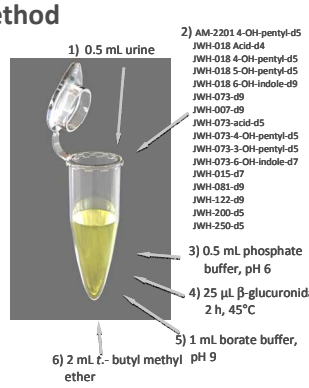


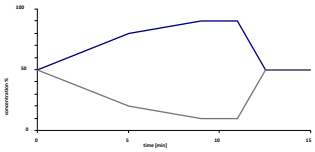




Method

At least 2 metabolites for each of the 18 different synthetic cannabinoids (AM-694, AM-2201, JWH-007, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-307, JWH-398, MAM-2201, RCS-4, UR-144 and XLR-11) were included in the sMRM (scheduled Multiple-Reaction-Monitoring) method. For sample work-up, an alkaline liquid-liquid extraction after incubation with β-glucuronidase was applied.





The LC–MS/MS system consisted of an API 5000 mass-spectrometer fitted with a TurboIonSpray interface and a Shimadzu Prominence HPLC system. Separation was achieved on a Luna C18 column (150 mm × 2 mm, 5 µm particle size) with an equivalent guard column (4 mm × 2 mm) and gradient elution using solvents A (water with 0.2 % formic acid and 2 mmol/L ammonium formate) and B (acetonitrile). A post-column addition of isopropanol at a flow rate of 0.2 mL/min enhanced sensitivity. The injection volume was 10 µL.

Results

The LC-MS/MS method was validated for all metabolites with reference material available, whilst for the remaining metabolites the method was used as a screening-method. Method validation was carried out according to the German Society of Toxicology and Forensic Chemistry (GTFCh) guidelines for method validation.

Analyte	Linearity [ng/mL]	LOD [ng/mL]	LLOQ [ng/mL]	[MH]+	Ion1 [m/z]	Ion2 [m/z]	Ion3 [m/z]	Internal standard	RT [min]
AM-2201 4-OH-pentyl	0.05-10	0.01	0.05	376.2	155.1	127.1		D5-AM-2201 4-OH-pentyl	6.0
AM-2201 6-OH-indole	0.05-10	0.025	0.05	376.2	155.1	127.1		D5-AM-2201 4-OH-pentyl	6.3
AM-694 pentanoic acid	no reference material			448.1	231.1	203.1	244.1		4.8
AM-694 defluorinated alkyl OH	no reference material			434.2	231.1	203.1	186.1		5.2
AM-694 alkyl-OH	no reference material			452.2	231.1	203.1	221.1		5.5
JWH-007 pentanoic acid	no reference material			386.2	155.1	258.1			6.2
JWH-007 alkyl-OH	no reference material			372.2	155.1	158.1			6.0
JWH-018 2-OH-pentyl	0.05-10	0.025	0.05	358.2	155.1	127.1		D7-JWH-018 6-OH-indole	7.9
JWH-018 3-OH-pentyl	0.05-10	0.010	0.05	358.2	155.1	127.1		D7-JWH-018 6-OH-indole	7.1
JWH-018 4-OH-indole	0.05-10	0.025	0.05	358.2	155.1	127.1		D9-JWH-081	10.9
JWH-018 4-OH-pentyl	0.05-10	0.025	0.05	358.2	155.1	127.1		D5-JWH-018 4-OH-pentyl	6.2
JWH-018 5-OH-indole	0.05-10	0.025	0.05	358.2	155.1	127.1		D7-JWH-018 6-OH-indole	7.9
JWH-018 5-OH-pentyl	0.05-10	0.010	0.05	358.2	155.1	127.1		D5-JWH-018 5-OH-pentyl	6.0
JWH-018 6-OH-indole	0.05-10	0.025	0.05	358.2	155.1	127.1		D7-JWH-018 6-OH-indole	7.9
JWH-018 7-OH-indole	0.05-10	0.025	0.05	358.2	155.1	127.1		D7-JWH-018 6-OH-indole	8.7
JWH-018 pentanoic acid	0.05-10	0.025	0.05	372.2	155.1	127.1		D4-JWH-018 pentanoic acid	5.6
JWH-019 6-OH-hexyl	0.05-10	0.025	0.05	372.2	155.1	127.1		D7-JWH-073 6-OH-indole	6.7
JWH-019 5-OH-indole	0.25-10	0.250	0.25	372.2	127.1	155.1		D7-JWH-015	9.0
JWH-019 hexanoic acid	no reference material			386.2	155.1	127.1			6.4
JWH-073 3-OH-butyl	0.05-10	0.025	0.05	344.2	155.1	127.1		D5-JWH-073 3-OH-butyl	6.0
JWH-073 4-OH-butyl	0.05-10	0.010	0.05	344.2	155.1	127.1		D5-JWH-073 4-OH-butyl	5.4
JWH-073 6-OH-indole	0.05-10	0.025	0.05	344.2	155.1	127.1		D7-JWH-073 6-OH-indole	7.0
JWH-073 butanoic acid	0.1-10	0.025	0.05	358.2	155.1	127.1		D5-JWH-073 butanoic acid	5.2
JWH-081 indole-OH	no reference material			388.2	157.1	230.1			8.3
JWH-081 naphthyl-OH	no reference material			388.2	201.2	214.2			9.2
JWH-081 5-OH-pentyl	0.1-10	0.025	0.05	388.2	185.1	157.1		D5-JWH-018 5-OH-pentyl	6.6
JWH-122 2-OH-pentyl	0.05-10	0.025	0.05	372.2	168.9	141.2		D7-JWH-018 6-OH-indole	8.4
JWH-122 3-OH-pentyl	0.05-10	0.025	0.05	372.2	168.9	141.1		D7-JWH-073 6-OH-indole	7.9
JWH-122 4-OH-pentyl	0.05-10	0.025	0.05	372.2	168.9	141.1		D5-JWH-122 5-OH-pentyl	6.8
JWH-122 5-OH-indole	0.05-10	0.025	0.05	372.2	168.9	141.1		D7-JWH-018 6-OH-indole	8.9
JWH-122 5-OH-pentyl	0.05-10	0.025	0.05	372.2	168.9	141.1		D5-JWH-122 5-OH-pentyl	6.8
JWH-122 6-OH-indole	0.05-10	0.025	0.05	372.2	168.9	141.1		D7-JWH-073 6-OH-indole	8.9
JWH-122 pentanoic acid	0.05-10	0.025	0.05	386.2	169.2	141.1		D5-JWH-018 4-OH-pentyl	6.4
JWH-122 naphthyl-OH	no reference material			372.2	185.1	214.1			8.3
JWH-200 4-OH-indol	0.1-10	0.100	0.10	401.2	114.1	155.1		D5-JWH-200	3.0
JWH-200 5+6-OH-indol (sum parameter)	0.05-10	0.025	0.05	401.2	155.1	114.1		D5-JWH-200	1.6
JWH-203 pentanoic acid	no reference material			370.2	125.1	218.1	244.1		5.3
JWH-203 indole-OH	no reference material			356.2	125.1	230.1			6.7
JWH-203 alkyl-OH	no reference material			356.2	125.1	186.1			5.8
JWH-210 4-OH-pentyl	0.05-10	0.025	0.05	386.2	183.1	153.1		D7-JWH-018 6-OH-indole	7.9
JWH-210 5-OH-indole	0.05-10	0.025	0.05	386.2	183.1	230.1		D5-JWH-250	9.7
JWH-210 5-OH-pentyl	0.05-10	0.010	0.05	386.2	183.1	153.1		D7-JWH-018 6-OH-indole	7.9
JWH-210 pentanoic acid	0.05-10	0.010	0.05	400.4	183.1	155.1		D7-JWH-073 6-OH-indole	7.2
JWH-210 naphthyl-OH	no reference material			386.2	214.1	144.1			8.9
JWH-250 4-OH-pentyl	0.05-10	0.025	0.05	352.2	121.1	91.1		D5-JWH-073 butanoic acid	5.0
JWH-250 5-OH-indole	0.25-10	0.250	0.25	352.2	121.1	91.1		D7-JWH-073 6-OH-indole	7.0
JWH-250 5-OH-pentyl	0.05-10	0.050	0.05	352.2	121.1	91.1		D5-JWH-073 butanoic acid	5.0
JWH-250 indole-OH	no reference material			352.2	121.1	91.1			7.7
JWH-250 phenyl-OH	no reference material			352.2	137.1	107.1			7.3
JWH-307 fluorophenylpentylpyrrol-di-OH	no reference material			418.2	155.1	246.1	228.2		2.7
JWH-307 fluorophenylpentylpyrrol + naphthyl di-OH	no reference material			418.2	171.1	230.1	248.2		5.1
JWH-307 fluorophenylpentylpyrrol-di-OH	no reference material			418.2	155.1	246.1	228.2		4.7
JWH-307 fluorophenylpentylpyrrol-OH	no reference material			402.2	155.1	230.1			7.1
JWH-307 fluorophenylpentylpyrrol-OH	no reference material			402.2	155.1	230.1			7.9
JWH-398 4-OH-pentyl	0.05-10	0.050	0.05	393.2	189.1	161.1		D7-JWH-018 6-OH-indole	7.8
JWH-398 5-OH-pentyl	0.05-10	0.050	0.05	393.2	189.1	161.1		D7-JWH-018 6-OH-indole	7.8
JWH-398 pentanoic acid	0.05-10	0.050	0.05	406.3	189.1	161.1		D7-JWH-073 6-OH-indole	7.2
MAM-2201 indole-OH	no reference material			390.2	169.1	141.1	248.1		7.1
MAM-2201 naphthyl-OH	no reference material			390.2	185.1	144.1	232.1		6.5
RCS-4 4-OH-pentyl	0.05-10	0.025	0.05	338.2	135.1	77.1		D5-JWH-073-butanoic acid	4.5
RCS-4 5-OH-pentyl	0.05-10	0.050	0.05	338.2	135.1	77.1		D5-JWH-073-butanoic acid	4.6
RCS-4 indole-OH	no reference material			338.2	135.1	230.1			4.0
RCS-4 phenyl-OH	no reference material			338.2	151.1	123.1			7.4
UR-144 4-OH-pentyl	0.05-10	0.025	0.05	328.4	125.1	97.1		D7-JWH-018 6-OH-indole	7.6
UR-144 5-OH-pentyl	0.05-10	0.050	0.05	328.4	125.1	97.0		D7-JWH-018 6-OH-indole	7.5
UR-144 pentanoic acid	0.1-10	0.100	0.10	342.4	125.1	97.1		D5-JWH-122 5-OH-pentyl	7.0
XLR-11 4-OH-pentyl	0.05-10	0.010	0.05	346.3	248.1	144.1		D5-JWH-122 5-OH-pentyl	6.6
XLR-11 cyclopropyl-OH	no reference material			346.2	232.1				6.7

Calibration model: For all compounds, a linear relationship between the response and the concentration in the range of the LLOQ (0.05-0.5 ng/mL) to the highest calibrator (10 ng/mL) was confirmed by Mandel test (99 % significance). To compensate for heteroscedasticity, a weighted least squares model with a weighting factor 1/x was applied for all compounds.

Selectivity: Blank and zero samples did not reveal any interference on the MRM transitions of the analytes or the internal standard.

Accuracy and Precision: The intra- and interday day precisions for all compounds were all below 15 % for all concentration levels (0.07, 0.7 and 7 ng/mL) with a maximum RSD of 10.6 % and a maximum bias of 8.3 %.

Analytical limits: The limit of detection ranged between 0.01 and 0.25 ng/mL, the lower limit of quantification between 0.05 and 0.25 ng/mL.

Processed sample stability: All analytes were stable in the autosampler for at least 21.5 h at both concentration levels (0.7 and 7 ng/mL).

Matrix effects: Matrix effects ranged from 88-113 % QC_{low} (0.7 ng/mL, RSD ≤ 20 %) and from 95-128 % QC_{high} (7 ng/mL, RSD ≤ 19 %).

Recoveries: Recoveries ranged from 69-121 % QC_{low} (0.7 ng/mL, RSD ≤ 31 %) and from 58-82 % QC_{high} (7 ng/mL, RSD ≤ 11 %) (except for JWH-073 6-OH-indole (12 %/48 %), JWH-073 butanoic acid (46 %/33 %)).

Process efficiencies: Process efficiencies ranged from 64-126 % QC_{low} (0.7 ng/mL, RSD ≤ 27 %) and from 52-102 % QC_{high} (7 ng/mL, RSD ≤ 18 %) (except for JWH-073 Acid (43 %/35 %)).

Conclusion

The method complements existing methods for serum or blood analysis. As urine is the preferred matrix for abstinence screening and previously published methods cover only a relatively small range of analytes, comprehensive methods are of high value for effective abstinence control.

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