Qualitative and quantitative analysis of synthetic cannabinoids in smoking mixtures of the "Spice" type using LC-MS/MS

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Abstract

Smoking mixtures of the "Spice" type generally contain highly potent synthetic cannabinoids. Therefore, a convenient and reliable method was developed, allowing for the detection of aroylindole (JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, and JWH-250) and hydroxycyclohexylphenol (CP 47,497) based cannabinoids in smoking mixtures in one measurement. In addition, quantitative analysis was employed for JWH-018, -073, and -250. Sample preparation is simple, and analysis is performed via LC-MS/MS in ESI^+ (JWH compounds) and simultaneously ESI^- (CP 47,497) mode using internal standards.
The method was applied for quantitative analysis in several authentic cases, the results were reported in forensic expertises. The validation parameters are given below, along with typical cannabinoid contents for several smoking mixtures analysed so far.

1. Introduction

Within only a few years, smoking mixtures of the "Spice" type (i.e. "Smoke", "King B", "Bonzai", "Jamaican Gold", see Fig. 1) containing illegal, highly potent synthetic cannabinoids have become a severe problem in parts of Germany. [1], [2] About 20,000 packages of these products were submitted for analysis to the BLKA until December 2010, but a decrease has not been seen yet. Consequently, German courts demanded that the content of the psychoactive ingredients be assayed for legal prosecution, particularly in regard of the quantity exceeding personal use according to the German Narcotics Law (§ 29a BtMG).

Fig. 1. Smoking mixtures of the "Spice" type.

2. Material and Methods

With the method described, cannabinoids of the aroylindole (JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, and JWH-250) and hydroxycyclohexylphenol (CP 47,497) family can be detected in smoking mixtures in one measurement. Moreover, quantitative analysis is feasible, as demonstrated for JWH-018, -073, and -250. Sample preparation is simple, consisting of homogenisation (electric grinder or deep-frozen in a mortar), ultrasonic-assisted ethanol extraction, filtration, and dilution. Analysis is performed via LC-MS/MS in ESI^+ (JWH compounds) and simultaneously ESI^- (CP 47,497) mode using internal standards (diphenylamine for ESI^+ and trichlorophenol for ESI^-).

2.1. LC conditions

Waters Alliance 2695 separation module with Waters XTerra MS C18 analytical column and guard column (100 and 10 mm × 2.1 mm, 3.5 µm), 0.1% formic acid/water/methanol gradient elution at 0.2 ml/min: initial 10/70/20, then linear to 10/5/85 within 10 min, 10 min isocratic, back to initial conditions within 1 min, finally 4 min equilibration (representative chromatogram shown in Fig. 2).

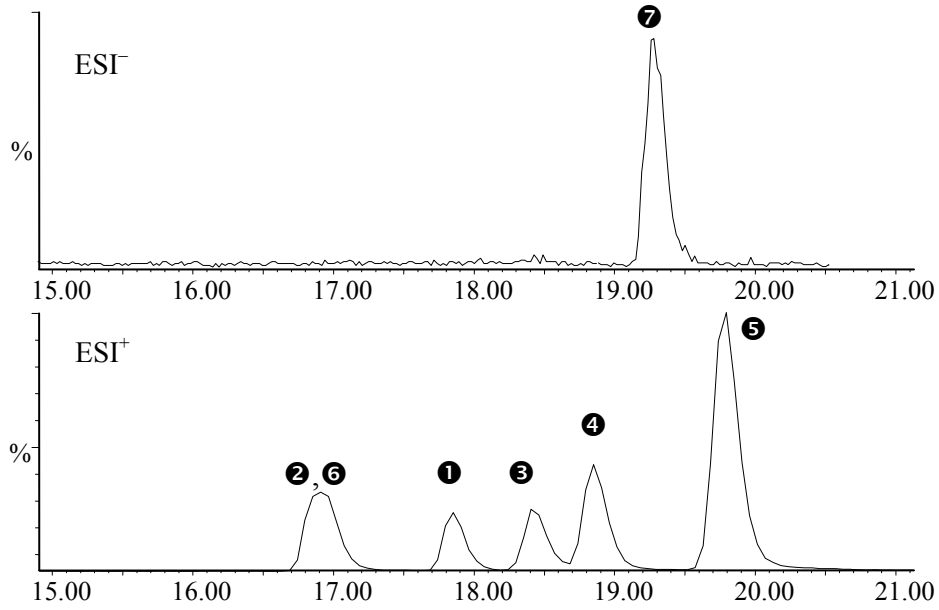

Fig. 2. Chromatogram of blank matrix (cannabinoid-free smoking mixture "2 spicy") spiked with JWH-018 (❶), -073 (❷), -081 (❸), -122 (❹), -210 (❺), -250 (❻), and CP 47,497 (❼) at 20 ng/ml (concentration estimated for JWH-122 and -210).

2.2. MS/MS conditions

Quattro Micro tandem MS (Waters) with simultaneous ESI^+ and ESI^- ionisation in MRM (multiple reaction monitoring) mode, capillary voltage 3.5 kV, source temperature 120 °C, desolvation temperature 350 °C, cone gas (nitrogen) flow 60 l/h, desolvation gas (nitrogen) flow 650 l/h, collision gas argon. Further details are given in Tab. 1.

Tab. 1. Mass spectroscopic data for seven cannabinoids.

Compound	Ionization mode	Precursor ion (m/z)	Product ions (m/z)	Cone voltage (V)	Collision energy (eV)
JWH-018	ESI+	342.2	154.99	30	25
		$[M+H]^+$	145.07		42
JWH-073	ESI+	328.1	155.12	33	22
		$[M+H]^+$	126.85		50
JWH-081	ESI+	372.1	185.25	33	25
		$[M+H]^+$	214.29		25
JWH-122	ESI+	356.35	169.43	29	25
		$[M+H]^+$	214.21		25
JWH-210	ESI+	370.25	183.46	33	26
		$[M+H]^+$	214.40		26
JWH-250	ESI+	336.2	120.95	25	20
		$[M+H]^+$	188.19		16
CP 47,497	ESI^-	317.2	299.08	45	26
		$[M-H]^-$	159.59		55

3. Results and Discussion

The method reported was optimised and validated thoroughly for JWH-018, JWH-073 and JWH-250. It was possible to obtain a linear calibration curve at 0.3, 1, 3, 10, 30 and 100 ng/ml with a 1/x weighted regression model. Validation included tests for sample preparation, workup and stability, selectivity, influence of matrix, linearity, LOD, LOQ, precision and accuracy. All calibrators and QC-samples were obtained by spiking blank matrix extracts ("2 spicy") with cannabinoid stock solutions. The results are summarised in Tab. 2.

Tab. 2. Validation data for three cannabinoids.

Compound	LOD (ng/ml)	LOQ (ng/ml)	Interassay Precision		
			QC-level (ng/ml)	RSD (%)	Bias (%)
JWH-018	0.7	2.5	5	6.6	-1.4
			50	4.4	-6.0
JWH-073	0.9	3	5	6.1	-4.3
			50	3.1	-10
JWH-250	1.6	6	5	4.8	-0.1
			50	4.3	-8.0

CP 47,497 was not quantitated, as it has not occurred in authentic samples in Bavaria yet. However, it was used as a model compound for its more relevant C8-homologue, which has not been available as certified standard at that time. As visualised in Fig. 2, a qualitative higher LOD is observed for CP 47,497 compared to aroylindoles.

After validation, the method was used to determine the cannabinoid content of some authentic samples (see Tab. 3). Only intact packages containing one single cannabinoid were used. The cannabinoid content showed the highest relative variation for JWH-018 (more than factor 10!) but still a significant variation for JWH-073 and -250. The average contents of JWH-018 and -073 were similar, that of JWH-250 was much higher. This finding is rather unexpected, as JWH-250 and JWH-073 have similar affinities to the cannabinoid receptors CB_1 and CB_2. Only JWH-018 has a higher affinity to CB_2. [3] [4] It must be noted that only a limited number of packages was analysed for most products. Only for "Dream", samples from two independent cases were assayed. Therefore, little information can be given about the batch-to-batch variation of smoking mixtures.

Tab. 3. Cannabinoid contents of typical smoking mixtures, number of packages analysed.

JWH-018:		
Product	Amount	Content (%)
Spice Gold	1	0.35
Spice Tropical Synergy	1	1.6
Spice Arctic Synergy	1	1.7
Genie	1	1.9
Smoke	1	4.4
Jah Rugh	1	3.7
Dream	1	1.4
Dream	26	1.5
69	131	2.4
Average / Sum	160	2.7

JWH-073:		
Product	Amount	Content (%)
Bombay Blue	5	1.8
King B	3	2.1
Clover Summer Breeze	3	2.5
SenCation Blackberry	3	1.8
Forrest Humus	3	3.1
Ikarus (golden Pck)	3	2.4
Ikarus (silver Pck)	3	1.8
Average / Sum	23	2.2

JWH-250:		
Product	Amount	Content (%)
WWW	3	13
Jamaican Spirit	3	16
Blaze	3	16
Bonzai Summer Boost	3	12
Bloom	3	9.5
Average / Sum	15	13

4. Conclusion

The method reported features convenient identification and quantitation of synthetic cannabinoids from smoking mixtures. It was applied for quantitative analysis in several authentic cases, the results of which were reported in forensic expertises and accepted in court (LG Ulm, March 2011).

With respect to legal requirements and commercial relevance, the number of compounds featured is still small. However, it is assumed that additional cannabinoids of the aroylindole family (i.e. JWH-xxx, AMxxx and RCS-4, WIN-xx,xxx) and the hydroxycyclohexylphenol family (i.e. CP 47,497 homologues, HU-xxx) can be included into the testing method if this should become necessary.

5. References

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