Quantitation of Synthetic Cannabinoids in Serum: A Comprehensive and Sensitive Multiplex Assay for 99 compounds by LC-MS/MS

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Overview

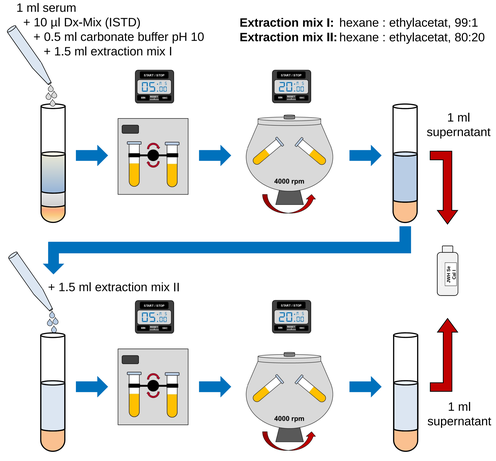
- A vast variety of synthetic cannabinoids keep on challenging forensic toxicologists
- Comprehensive LC-MS/MS method for the detection of about 100 synthetic cannabinoids
- User Library and MRM-Builder for easy customization
- Distinguished low limits of detection

Introduction

Since their first appearance in 2008, forensic toxicologists are challenged by the large numbers of synthetic cannabinoids (SC) that keep on flooding the drug markets worldwide. Sold as incense or plant fertilizer, synthetic cannabinoids are promoted as an alleged 'legal' alternative to traditional cannabis products to circumvent current legislation and/or routine drug testing methods.
Once the legal status of certain compounds was changed, new or slightly modified variants of synthetic cannabinoids emerge, replacing those being scheduled. These frequent alterations require analytical methods for the detection and quantitation of synthetic cannabinoids to be updated regularly and cover a broad range of analytes. In addition, these methods have to be sufficiently sensitive since serum concentrations are generally way below 5 ng/ml and even below 1 ng/ml for high potent substances of this class.
The presented multiplex assay allows the simultaneous quantitation of about 100 synthetic cannabinoids in serum samples. The method includes first generation SCs (e.g. JWH 018, JWH 210) as well as more recent compounds like Cumyl-PINACA-5F, MDMB-CHMICA or EG-018.

Methods

Sample Prep.: 2-step liquid-liquid extraction

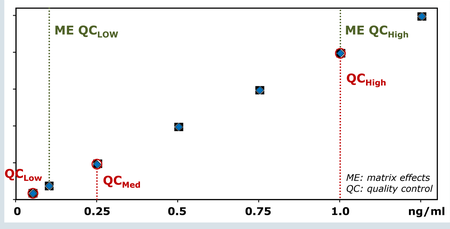


The supernatant is evaporated at 40°C and the residue is reconstituted in 50 µl eluent A:B (50:50).

LC Conditions	
LC system	Bruker Advance™ UHPLC
Eluent A	Water, 2 mM ammonium formate, 0.1% formic acid, 1% acetonitrile
Eluent B	Acetonitrile, 2 mM ammonium formate, 0.1% formic acid, 1% water
Analytical column	Kinetex® C18 100A, 2.6µm (100 x 2.1mm)
Flow rate	0.5 ml/min
Injection volume	100 µl
Gradient:	0.0 to 1.0 min: 20% B
	1.0 to 2.5 min: 20% B to 60% B, linear
	2.5 to 4.0 min: 60% B to 65% B, linear
	4.0 to 5.5 min: 65% B
	5.5 to 8.0 min: 65% B to 90% B, linear
	8.0 to 10.0 min: 90% B
	10.0 to 10.1 min: 90% B to 20% B, linear
	10.1 to 12.0 min: 20% B

MS Conditions	
Mass Spectrometer	Bruker EVOQ Elite™ Triple Quadrupol MS
Source	VIP-HESI, positive mode, 4700 V
Probe gas	50 units at 400°C
Cone gas	25 units at 350°C
Nebulizing gas	50 units
Collision gas	Argon, 1.5 mTorr
Mode	Multi Reaction Monitoring (MRM), 2 transitions per analyte, 1 transition per IS

The method was validated according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh).



Validation parameters (calibration range, QC levels) of the method.

Selectivity: Blank serum of 10 individuals was analyzed without addition of analytes and ISTDs and two blank serum samples were analyzed after being fortified with ISTD.

Linearity: For determination of linearity, six calibration curves were analyzed. Each calibration consisted of seven calibrators, made by fortifying blank serum (n=6) with ethanolic solution of the analytes.

LOD and LOQ: LOD and LOQ were determined according to DIN 32645 using five equidistant calibrators in the range of the expected LOD.

Accuray: Two replicates of a low, medium and high QC sample (pooled serum, n=5) were analyzed on eight consecutive days.

Matrix effects: Matrix effects (ME), recovery (RE) and process efficiences were examined according to Matuszewski et al. using a low and high QC sample.

Results

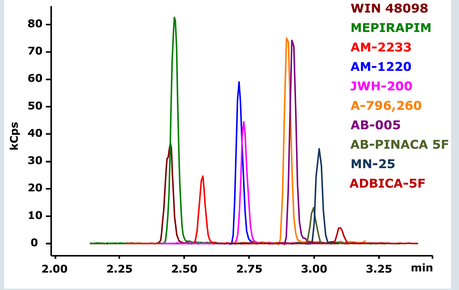
The MRM-Method was set up using the MRM-Builder tool. Ethanolic mixtures of 4-6 compounds were injected into the MS by infusion. For each analyte, the most intense MRM transitions and the corresponding ideal collision energy were determined by the software. In total, MRM information of 106 synthetic cannabinoids was stored in a user library.

The final method included 99 synthetic cannabinoids available as reference substances of sufficient quality in the authors' laboratory at the time of method validation and 17 deuterated analogs.

Blank serum samples (n=10) and blank serum samples fortified with ISTDs showed no interfering signals on the ion transitions of the analytes.

Examination of linearity showed satisfying values for all 99 compounds. Recalculation of the calibrator concentrations led to deviations less than ± 15% for all concentrations.

Limits of detection (LOD) ranged from 5 to 10 pg/ml for 89 of the investigated analytes. Highest LOD (c = 50 pg/ml) was found for AB-PINACA and FUB-AKB-48.



MRM transitions of the quantifier ions of 10 synthetic cannabinoids at their lowest calibration point (c = 50 pg/ml) in human serum.

Calculated limits of quantitation (LOQ) ranged from 10 to 30 pg/ml for the majority of compounds. So the lowest calibrator (c = 50 pg/ml = QC_{Low}) was defined as LOQ for all compounds.

Accuracy was calculated as bias for all three QC levels and found to be less than ± 15% except for PX-1 (QC_{Low}: -15%, QC_{MED}: -13.5%, QC_{High}: -7.5%) For PX2, CAF-3, FUB-AKB-48, ADB-PINACA-5F, and AB-PINACA-5Cl the average bias met the validation criteria but single values exceeded the ± 15% range. So findings of these six compounds were given as semi-quantitative results only.

Evaluation of matrix effects showed noticeable ion suppression for AB-001, AKB48, EG-018, FDU-PB-22, JWH-398 and THJ (ME: 54 - 73%) and signal enhancement for CAF-3, MDMB-CHMINACA, NPD-22, NPD-22-5F, and SDB-005-5F (ME: 130 - 158%), respectively.

	Matrix Effects	RSD	Recovery	RSD
QC_{Low}	54 - 158 %	3.4 - 34 %	4.5 - 79 %	0.3 - 11 %
QC_{High}	55 - 134 %	1.3 - 21 %	5.0 - 80 %	0.9 - 11 %

Though, the influence of matrix effects could be compensated by use of an appropriate ISTD.

Conclusions

- Sensitive quantitation of about 100 SC including recently emerged substances.
- Easily customizable method by using the user library and MRM-Builder for adding new compounds.
- Low limits of detection allow to prove recent uptake of synthetic cannabinoids.
- Suitable method for detection and quantification of synthetic cannabinoids in forensic cases (DUID, post mortem…)

LC-MS/MS