A Comprehensive, Fast, and Sensitive Method for the Quantitation of Synthetic Cannabinoids using the Elute UHPLC Coupled to an EVOQ LC-TQ MS

Rapid and Sensitive Quantitation of Synthetic Cannabinoids in Serum by UHPLC-Triple Quadrupole Mass Spectrometry

Abstract

This study demonstrates a sensitive, rapid and reliable method for the simultaneous quantitation of 99 synthetic cannabinoids in serum using the Bruker Elute™ UHPLC coupled to the EVOQ Elite™ triple quadrupole MS. Sample preparation was performed with liquid-liquid extraction. The method was fully validated.

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Introduction

Synthetic cannabinoids first appeared on the recreational drug scene in 2004. The term synthetic cannabinoid refers to an increasing number of man-made mind-altering chemicals that are either sprayed on dried, shredded plant material so they can be smoked, or sold as liquids to be vaporized and inhaled in e-cigarettes and other devices. Many synthetic cannabinoids are full agonists of the cannabinoid receptors CB_1 and CB_2, and therefore their psychoactive effects are similar to those of cannabis. They include a lot of different chemical structures such as e.g. naphthoyl-, phenylacetyl-, benzoyl- and cyclopropyl-indoles, cyclohexylphenoles, and various other indazole and indole derivatives (Figure 1).

In an attempt to circumvent current legislation synthetic cannabinoids are misleadingly marketed via the internet or in headshops as "bath salts, plant food or research chemicals" and are often sold in colorful small bags with creative names, for example, "Unicorn Magic Dust", "Be Happy", or "Aliens. They are then judged by consumers to be safe and legal, despite the fact that their effects can lead to high blood pressure, nausea, hallucination, psychosis, physical addiction and even life-threatening conditions. In order to keep ahead of evolving drug prohibition laws and modern detection methods, new or slightly modified variants of synthetic cannabinoids are regularly synthesized and rapidly marketed to the recreational drug user market.

This means that analytical methods need to be regularly updated to also cover the newly emergent analytes. Unlike UHPLC-triple quadrupole mass spectrometry, traditional detection methods such as immunoassay and LC-UV do not have the required
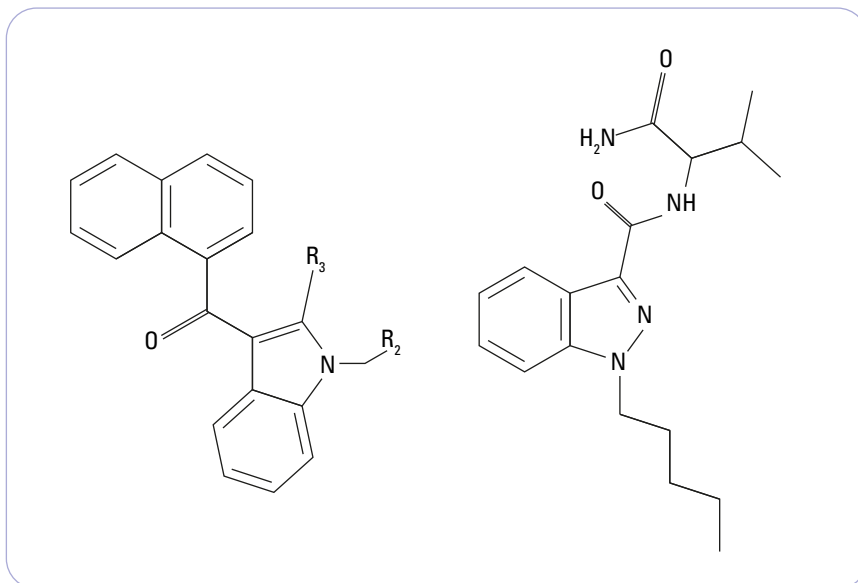

Fig. 1: Representative structures of synthetic cannabinoids; left: naphtoylindoles, right: indazole derivatives

specificity, sensitivity or flexibility to cope with this ever-growing analytical challenge. This manuscript describes a UHPLC-triple quadrupole mass spectrometry method to rapidly and reliably quantify synthetic cannabinoids in human serum at the sub ng/mL level.

Experimental

Sample Preparation

0.5 mL buffer (pH 10), 1.5 mL hexane:ethylacetate (99:1) and 10 µL internal standard mix were added to 1 mL serum. After mixing and centrifugation

Liquid chromatography	
Instrument	Bruker Elute™ UHPLC
Column	Kinetex® C18 100A, 2.6 µm (100 x 2.1 mm)
Mobile phase A	1 % acetonitrile, 0.1 % formic acid with 2 mM ammonium formate
Mobile phase B	99 % acetonitrile, 0.1 % formic acid with 2 mM ammonium formate
Gradient	0.0 – 1.0 min 20 % B 1.0 – 2.5 min to 60 % B 2.5 – 4.0 min to 65 % B 4.0 – 5.5 min 65 % B 5.5 – 8.0 min to 90 % B 8.0 – 10.0 min 90 % B 10.0 – 10.1 min to 20 % B 10.1 – 12.0 min 20 % B
Flow rate	500 µL/min
Injection volume	10 µL
Column oven	40°C

Mass Spectrometry

Instrument	EVOQ Elite™ triple quadrupole mass spectrometer
Ion source	VIP H-ESI positive, 4700 V
Probe gas	50 units at 400°C
Cone gas	25 units at 350°C
Nebulizing gas	50 units
Active exhaust	on
Collision gas	Argon, 1.5 mTorr
MRM transitions	2 per analyte, 1 per IS

1 mL supernatant was transferred to an HPLC vial. The residue was mixed with 1.5 mL hexane:ethylacetate (80:20) and 1 mL supernatant was transferred to the first HPLC vial after centrifugation. The combined supernatants were evaporated and the residue reconstituted in 100 µL eluent A:B (50:50).

Instrumentation

Method validation
The method was validated according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh). Validation parameters included selectivity, matrix effects and recovery, limits of detection and quantitation (LOD and LOQ), linearity, precision and accuracy.

To determine selectivity, blank serum from 10 individual donors was analyzed without the addition of analytes or internal standards and two blank serum samples with the addition of internal standards.

To examine matrix effects and recovery, five different serum samples from five different individuals spiked before extraction, five additional serum samples spiked after extraction and five neat standards were measured at a low (0.1 ng/ml) and a high concentration (1 ng/ml) and compared. The matrix effects and the recovery were then calculated according to Matuszewski et al[1].

LOD and LOQ were evaluated from calibration curves constructed with equidistant calibrators in the range of

Fig. 2: MRM builder; top: define compound name, precursor and number of product ions desired; bottom: results of optimization with optional export to method and/or user library

the expected LOD (5 to 50 pg/ml) and calculated according to DIN 32645[2].

To demonstrate linearity, six calibration curves with seven calibrators each were constructed, ranging from 50 pg/mL up to 1.25 ng/ml.

Precision and accuracy were determined by the analysis of two replicates of low (0.05 ng/ml), medium (0.25 ng/ml) and high (1 ng/ml) QC samples on eight consecutive days.

Results and Discussion

For the development of the MRM method, the MRM builder tool was used (Figure 2). Optimization was performed by infusing mixtures of standard solutions of the analytes.
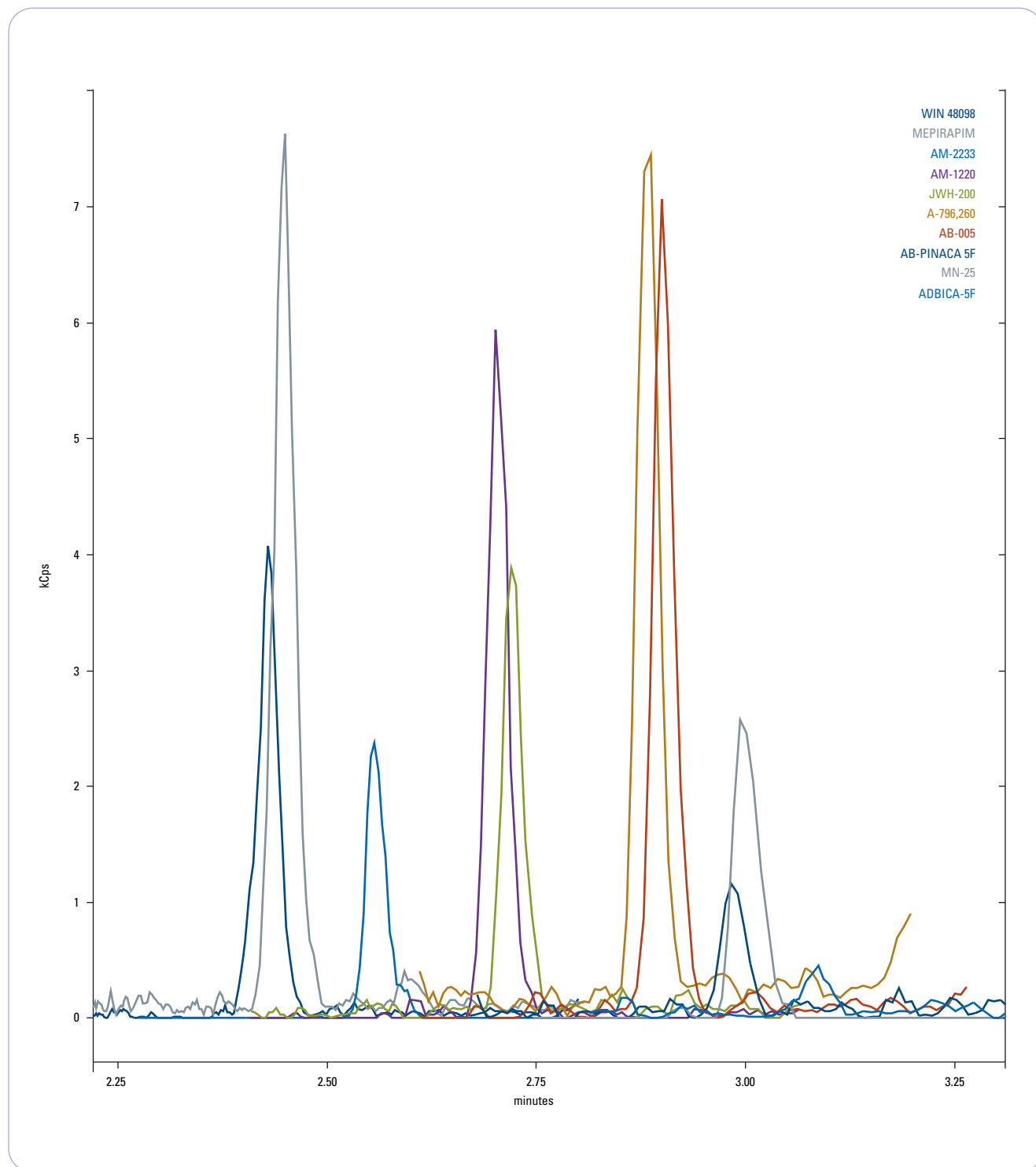

Fig. 3: Chromatogram of the first ten eluting compounds at 5 pg/ml

Curve Fit: Linear, Ignore, 1/X2

y =   +2.296994x   -0.002876

Resp. Fact. RSD: 3.905%

Coeff. Det.(r2):0.998365
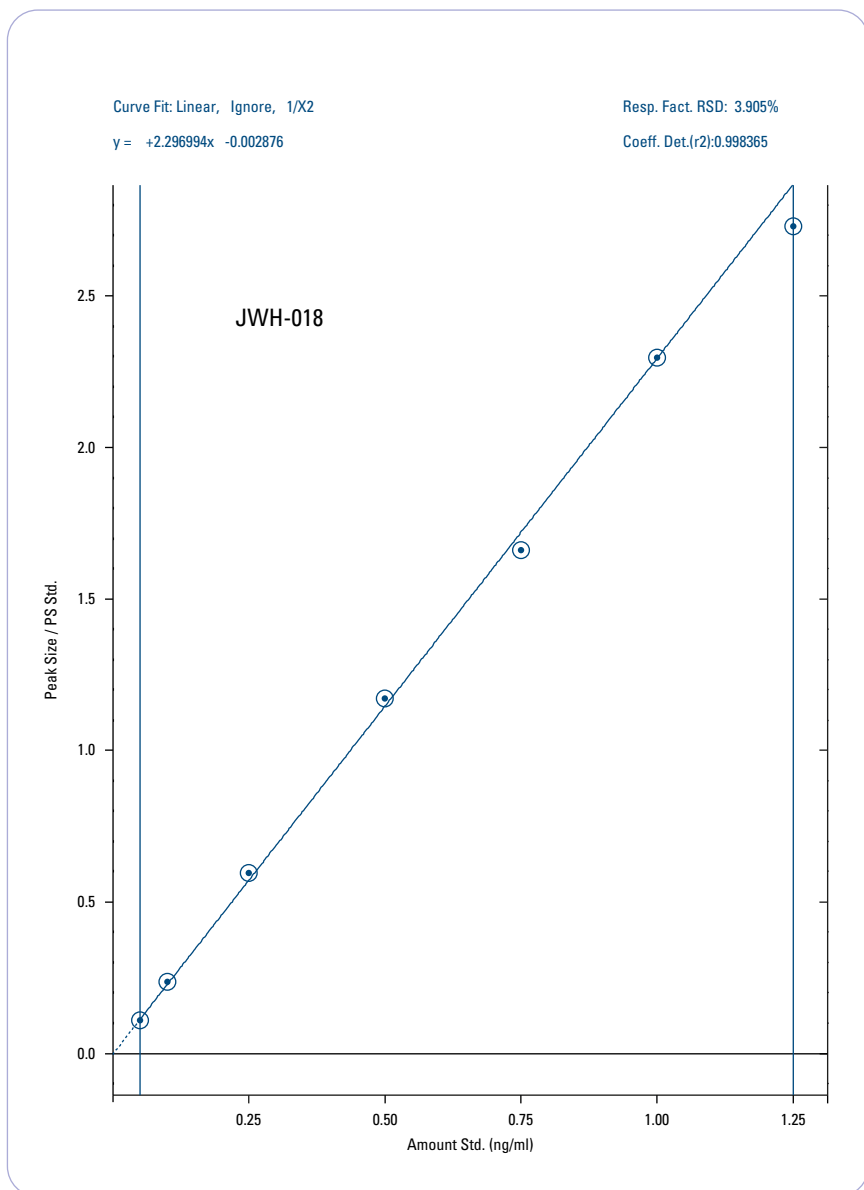
JWH-018

Peak Size / PS Std.

Amount Std. (ng/ml)

Fig. 4: Calibration curve of JWH-018

The MRM builder identified the optimal transitions and collision energies for each analyte which were then directly exported to the method and also added to a user library for future reference. Similarly, 99 synthetic cannabinoids and 17 deuterated internal standards were easily added to the method.

Scan times for each analyte were automatically calculated after defining the retention times, and the retention time windows, as well as the average peak width and required number of data points per peak (generally referred to as Compound Based Scanning).

The chromatographic separation of the 99 analytes was performed within 8.5 minutes with a total runtime of 12 minutes by the new Elute UHPLC system.

Method validation

As there were no interfering signals at the ion transitions of the analytes, the method was deemed to be selective. Matrix effects were within ± 25% for 89 of the 99 analytes. Higher matrix effects can be compensated by the use of an appropriate internal standard. Recovery was >50% for 79 analytes.

The LOD was <5 – 10 pg/mL for 89 analytes with the highest LOD being 50 pg/ml. Figure 3 shows a chromatogram of representative analytes at the concentration of 5 pg/ml. The calculated LOQ was ≤30 pg/ml for 94 of the 99 analytes and was finally defined as the lowest calibrator concentration of 50 pg/ml for all compounds.

For calibration curves a weighting factor of $1/x^2$ was used. All analytes followed a linear calibration model (Figure 4), with a bias (accuracy) within ±15% being obtained. Though for six analytes the average bias met the validation criteria, single values exceeded the ±15% range, so findings for these six analytes are given as semi-quantitative results only. For interday and intraday precision all analytes fulfilled the validation criteria with RSD's of <15%.

Conclusions

A method for the sensitive quantitation and/or the semi-quantitative determination of 99 synthetic cannabinoids including recently emerged substances has been developed using the Bruker EVOQ LC Triple Quad system. The method is easily customizable using the MRM builder to add new compounds to the user library. The low limits of detection obtained provide the necessary evidence to show recent use of synthetic cannabinoids in forensic cases such as driving under the influence of drugs or in post-mortem cases.

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