TRAPPING 'SPICE'

A comprehensive, automated LC-ion trap-MS screening approach for the detection of currently 38 synthetic cannabinoids in serum

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Introduction

Since the first detection of synthetic cannabinoids as ingredient in 'Spice' in 2008, a growing number of herbal mixtures containing various, steadily changing synthetic cannabinoids have flooded the markets worldwide. These mixtures are labelled as 'incense' and are readily available over the internet. 'Spice' and its successor products are promoted and consumed as 'legal' alternatives to traditional cannabis products to circumvent current legislation and drug testing. Intoxications with synthetic cannabinoids requiring intensive care are on the increase – even 'Spice'-related fatalities have been reported recently. In the past, new or slightly modified variants of synthetic cannabinoids began to appear in the mixtures, nearly instantly after the legal status of certain compounds was changed. Currently, 14 compounds are subject to the German narcotics law (BtMG). This results in a high demand for comprehensive screening methods, especially for clinical and forensic toxicology.

Hyphenated mass spectrometry (MS) is the method of choice and state-of-the-art for developing analytical methods to detect and identify synthetic cannabinoids in biological specimens. We describe the first screening procedure for synthetic cannabinoids in serum using an ionBooster™ (IB) ion-source coupled to an ion trap MS system.

Method Development

Fig. 1: Library Entry of JWH-018 (MS^2)

A spectra library, containing MS, MS^2 and MS^3 spectra of altogether 38 synthetic cannabinoids and 9 isotope-labelled analogs, was set up using certified standard solutions (where available), solid matter samples or extracts of herbal mixtures. NMR, GC-MS and TLC were used to verify the identity and purity of substances not obtained from professional vendors. A typical library entry is shown in figure 1.

A fast LC-method, sufficiently separating isobaric compounds and a scheduled-precursor-list including the obtained retention times of the synthetic cannabinoids in the library have been developed. Synthetic cannabinoids spiked to LC-eluent were analyzed using either a conventional electrospray ionization source (ESI) or the new IB-source to determine the potential benefit of the latter. The developed LC-MS^n-screening approach was applied to human serum samples and an automated data reporting procedure using Compass OpenAccess (COA) was designed.

Analytical Method

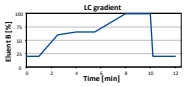
Sample Preparation

Sample preparation was performed according to an alkaline liquid-liquid extraction (LLE) as follows:

1 mL serum
+ 1 ng D7-JWH-015 (ISTD)
+ 0.5 mL carbonate buffer (pH 10)
+ 1.5 mL hexane/ethyl acetate (100:1) (v/v)
→ 5 min mixing, 20 min centrifugation at 4000 rpm
→ Evaporation of organic phase with N_2 at 40 °C
→ Residue is resolved in 25 µL solvent A/B (50:50) (v/v)

LC - Settings

LC-System:	Dionex UltiMate 3000 LC-System
Column:	Kinetex™ 2.6u C18 100 x 2.10 mm
Eluents:	A: 0.1 % HCOOH + 2 mmol/L NH_4^+ $HCOO^-$ B: acetonitrile + 2 mmol/L NH_4^+ $HCOO^-$
Gradient:	8 min gradient elution / 12 min total runtime
Total flow:	500 µL/min
Oven:	40 °C
Injection vol.:	2 µL

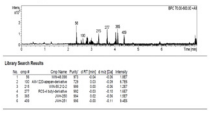


MS - Settings

Bruker amaZon speed™ ion trap

- Ultra Scan: 70 - 600 Da (32.500 Da/s)
- ionBooster source / positive mode
  - Vaporizer Temperature: 300°C
  - Dry Gas Temperature: 200°C
  - Sheath Gas Flow: 150 L/h
- Scheduled Precursor List to trigger data dependent acquisition of MS^2- and MS^3-spectra.
- 'Active exclusion' of precursor after 1 spectrum for 0.1 min but 'reconsider' if it's intensity increases by a factor of 5.

Data Evaluation and Reporting



DataAnalysis 4.1 software package for automated data processing and result-reporting according to the Toxtyper-workflow [1].

Pdf-reports can be accessed via web or automatically sent by e-mail.

Results and Discussion

The comparison of conventional ESI vs. IB revealed significantly better responses and higher signal intensities when using IB. Throughout this study the observed signals were at least 2-fold higher. The sensitivity gain for the ion trap MS achieved by the use of the new ion-source type, offers the detection and secure identification of synthetic cannabinoids at concentrations, considered as cut-off-values in methods using triple-quadrupole-instrumentation and multiple reaction monitoring (MRM) [2].

Within the initial comparison of IB and ESI (spiked concentrations: 0.1 to 1.0 ng/mL), it could be shown that the former allows the detection of substances not found when using a conventional ESI-source (Figure 2).

Detection limits were obtained by analyzing blank human serum samples of different origins spiked with synthetic cannabinoids at different concentrations down to 0.1 ng/mL. Blank serum samples and samples only containing ISTD, were processed similarly to evaluate potential matrix effects.

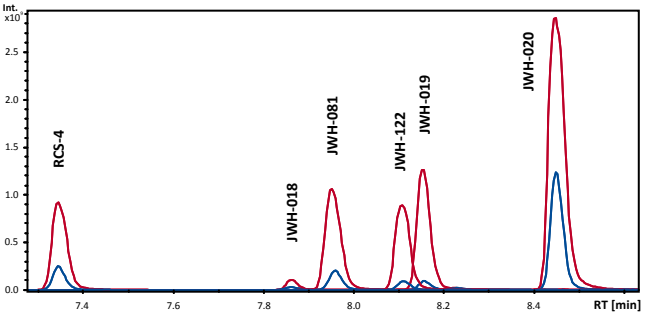


Fig. 2: Comparison of ESI and IB

Table 1: Results of serum spiked with different concentrations of synthetic cannabinoids (subject to BtMG)

Compound	0.1 ng/mL	0.25 ng/mL	0.5 ng/mL	Compound	0.1 ng/mL	0.25 ng/mL	0.5 ng/mL
AM-1220	Y	Y	Y	JWH-200	Y	Y	Y
AM-1220-azepane-derivative	N	N	N	JWH-203	N	N	Y
AM-1248	Y	Y	Y	JWH-210	Y	Y	Y
AM-2201	Y	Y	Y	JWH-250	N	Y	Y
AM-2232	N	N	Y	JWH-250 methyl-piperidine-derivative	Y	Y	Y
AM-2233	Y	Y	Y	JWH-251	Y	Y	Y
AM-694	Y	Y	Y	JWH-307	N	Y	Y
CRA-13	N	N	N	JWH-370	N	Y	Y
JWH-007	Y	Y	Y	JWH-387	N	N	Y
JWH-015	Y	Y	Y	JWH-398	Y	Y	Y
JWH-018	Y	Y	Y	JWH-412	Y	Y	Y
JWH-018-adamantyl-derivative	Y	Y	Y	Methanandamide	N	N	N
JWH-019	N	Y	Y	RCS-4	Y	Y	Y
JWH-020	Y	Y	Y	RCS-4 butyl-derivative	N	Y	Y
JWH-073	Y	Y	Y	RCS-4 ortho-isomer	Y	Y	Y
JWH-081	N	Y	Y	RCS-8	N	Y	Y
JWH-122	Y	Y	Y	UR-144	Y	Y	Y
JWH-122-5-fluoropentyl-derivative	N	N	Y	WIN-48.098	N	Y	Y
JWH-182	Y	Y	Y	WIN-55.212-2	N	Y	Y

The used search algorithm matched retention times, MS and MS2/MS3 spectral information, in order to calculate a purity score (score >700 indicate positive identifications).

Table 1 lists the obtained LODs of the 38 synthetic cannabinoids obtained from spiked serum samples after LLE by the automated library search and result reporting tool. 35 of the 38 synthetic cannabinoids currently included in the library could be identified at a concentration of 0.5 ng/mL in human serum. All compounds included in the library which are subject to the BtMG, were detected at 0.5 ng/mL. Among those, 10 synthetic cannabinoids could be detected at 0.1 ng/mL.

AM-1220-azepane-derivative, CRA-13 and methanandamide, generally have a poor response and could not be identified at the spiked levels. Methanandamide did not yet occur as adulterant in herbal mixtures. CRA-13 and AM-1220-azepane-derivative have been found in mixtures but not in serum samples analyzed in our lab.

The method was successfully applied to authentic serum samples. Quantitative MRM results of samples with analyte concentrations above the determined LOD where confirmed as positive findings by the developed screening method.

Conclusion

We present a liquid chromatography-mass spectrometry (LC-MS) approach offering a fast, reliable and easy-to-use screening solution for the detection of synthetic cannabinoids in serum with a high degree of automation - but still keeping the possibility for manual data evaluation. The combination of MS^2/MS^3-spectra and retention time meets common criteria for identification according to forensic guidelines.

This method is ideally suited for the 'pre'-screening of serum samples due to the lack of sufficient and reliable immunoassays for synthetic cannabinoids. Moreover, we offer a fast and economically priced alternative to other methods since approx. 75% of samples processed do not contain any synthetic cannabinoids at all and positive findings can be confirmed by complementary quantitative methods [2].

The use of parent compounds as analytical targets offers the possibility of instantly adding new emerging compounds to the library and immediately applying the updated method to serum samples, allowing the rapid adaptation of the screening method to ongoing forensic or clinical requirements. Continuous addition of new emerging compounds keeps the screening procedure up-to-date and since a full-scan method is used, at least MS1-information of new compounds is obtained. The presented approach can also be applied to other specimens, such as oral fluid or hair, and herbal mixtures or solid matter suspected to contain synthetic cannabinoids.

References

[1] Huppertz et. al.: An automated MS-based screening procedure for clinical and forensic toxicology. Poster presentation, XVIII. Mosbacher Symposium, April 18th - 20th, **2013**

[2] Kneisel et. al.: Analysis of 30 Synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *J. Mass Spectrom.* **2012**, *47*, 825-835