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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OVERVIEW

- Comprehensive screening for synthethic cannabinoids in serum
- LC-MSn coupling for screening and automated data evaluation
- Successful evaluation of a new ion source type
- LODs around the LOQ of current triple quadrupole MS methods

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. ‘Spice’ or ‘K2’ and its successor products are promoted and consumed as ‘legal’ alternatives to traditional cannabis products to circumvent current legislation and drug testing. 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. Intoxications with synthetic cannabinoids requiring intensive care are on the increase - even ‘Spice’-related fatalities have been reported recently [1, 2].

This results in a high demand for comprehensive screening methods, especially for clinical and forensic toxicology. Hyphenated mass spectrometry 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.

METHODS I

Sample Preparation

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

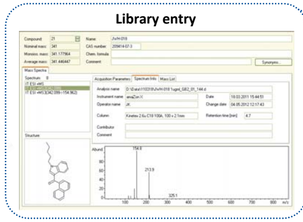
Eluents: A: 0.1 % HCOOH + 2 mmol/L NH_4^+ $HCOO^-$
B: ACN + 2 mmol/L NH_4^+ $HCOO^-$

Gradient: 8 min gradient elution / 12 min total runtime

Total flow: 500 µl/min

Oven: 40 °C

Injection vol.: 2 µl



Spectra recording and library building

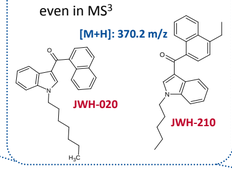
LC method development and optimization

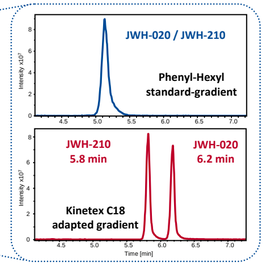
MS Evaluation ESI vs. IB

Evaluation ionBooster

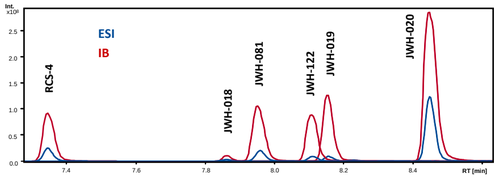
Evaluation of LOD in serum samples

Problem: Isobaric analytes
→ mixed spectra !
→ potential similar fragments even in MS^3





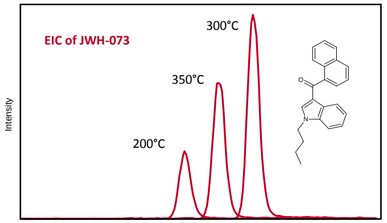
Evaluation of ionization efficiency of selected synthetic cannabinoids in eluent



The comparison of ESI vs. IB revealed significantly higher signal intensities when using IB. The observed signals were at least 2-fold higher. Within the initial comparison (concentrations: 0.1 to 1.0 ng/ml), it could be shown that the IB allows the detection of substances not found when using an ESI-source.

Compound	LOD ESI ng/ml	LOD IB ng/ml	Compound	LOD ESI ng/ml	LOD IB ng/ml
AM-694	0.25	0.1	JWH-122	0.5	0.25
JWH-007	1.0	0.1	JWH-200	0.1	0.1
JWH-015	0.1	0.1	JWH-203	1.0	0.25
JWH-018	0.25	0.1	JWH-210	1.0	0.1
JWH-019	1.0	0.1	JWH-250	1.0	0.1
JWH-073	0.1	0.1	JWH-251	0.25	0.1
JWH-081	0.5	0.1	RCS-4	0.1	0.1

Evaluation of different vaporizer gas temperatures



IonBooster evaluation was carried out in spiked eluent for all compounds listed above. The vaporizer gas temperature showed a significant effect on the ionization efficiency and should be investigated for every compound. For multi-analyte methods a reasonable compromise has to be found.

The used search algorithm matches retention times, MS and MS^2/MS^3 spectral information, in order to calculate a purity score. Findings with a score > 700 indicate positive identifications an were included in the report-file.

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 investigate selectivity. 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.

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 so far.

The method was successfully applied to authentic serum samples. Quantitative MRM results [4] of samples with analyte concentrations above the determined LOD where confirmed as positive findings by the developed screening method.

Evaluation of the limit of automated identification in spiked serum samples (banned by german narcotics law)

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	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	✓	✓	✓	JWH-019	⊗	✓	✓	JWH-251	✓	✓	✓	WIN-55.212-2	⊗	✓	✓
AM-1248	✓	✓	✓	JWH-020	✓	✓	✓	JWH-307	⊗	✓	✓	RCS-4 butyl	⊗	✓	✓
AM-2201	✓	✓	✓	JWH-073	✓	✓	✓	JWH-370	⊗	✓	✓	RCS-4 ortho isomer	✓	✓	✓
AM-2232	⊗	⊗	✓	JWH-081	⊗	✓	✓	JWH-387	⊗	⊗	✓	AM-1220-azepan	⊗	⊗	⊗
AM-2233	✓	✓	✓	JWH-122	✓	✓	✓	JWH-389	✓	✓	✓	JWH-018-adamantyl	✓	✓	✓
AM-694	✓	✓	✓	JWH-182	✓	✓	✓	JWH-412	✓	✓	✓	JWH-122-fluoropentyl	⊗	⊗	✓
CRA-13	⊗	⊗	⊗	JWH-200	✓	✓	✓	RCS-4	✓	✓	✓				
JWH-007	✓	✓	✓	JWH-203	⊗	⊗	✓	RCS-8	⊗	✓	✓	JWH-250 methyl piperidine	✓	✓	✓
JWH-015	✓	✓	✓	JWH-210	✓	✓	✓	UR-144	✓	✓	✓				
JWH-018	✓	✓	✓	JWH-250	⊗	✓	✓	WIN-48.098	⊗	✓	✓	Methanandamide	⊗	⊗	⊗

METHODS II

MS - Settings

Bruker amaZon speed™ ion trap
- UltraScan: 70 - 600 Da (32.500 Da/s)

ionBooster source / positive mode
- Vaporizer Temperature: 300°C
- Dry Gas Temperature: 200°
- Sheath Gas Flow: 150 L/h

Scheduled Precursor List (SPL) 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 [3].

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

CONCLUSIONS

We present a liquid chromatography-mass spectrometry 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 [4].

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 MS^1-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] Remane et al - Oral Presentation, 91st. Meeting of the German Society of Legal Medicine 2012

[2] Saito et al - Forensic Toxicol. 10.1007/s11419-013-0190-9

[3] Meyer et.al - ASMS 2013, Poster 546

[4] Kneisel et. al - J. Mass Spectrom. 2012, *47*, 825-835