AN AUTOMATED LC-ION TRAP MS SCREENING FOR SYNTHETIC CANNABINOIDS

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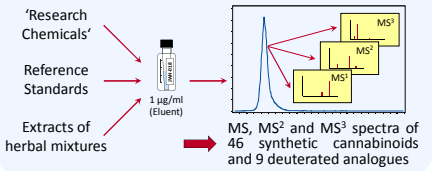
INTRODUCTION

Considering the vast variety of synthetic cannabinoids and herbal mixtures - commonly known as 'Spice' or K2' - on the market and the resulting increase of severe intoxications related to their consumption, there is a need in clinical and forensic toxicology for comprehensive up-to-date screening methods. Hyphenated mass spectrometry is the technology of choice for these applications. This project aimed at developing and implementing an automated screening procedure for the detection of synthetic cannabinoids in serum using an LC-ion trap-MS system and a spectra library-based auto-MS^n approach based on the Toxtyper^{TM} workflow (Bruker Daltonik). In the process of method development the ionBooster^{TM} (IB), a high-temperature ESI-source, and its effect on the ionization efficiency of the investigated synthetic cannabinoids was evaluated and compared to a conventional ESI-source.

METHOD DEVELOPMENT

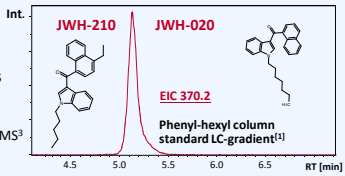
Spectra recording and library building



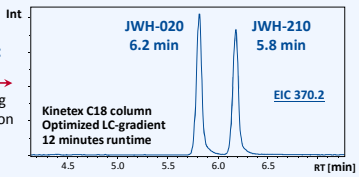
MS, MS^2 and MS^3 spectra of 46 synthetic cannabinoids and 9 deuterated analogues

LC method development and optimization

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

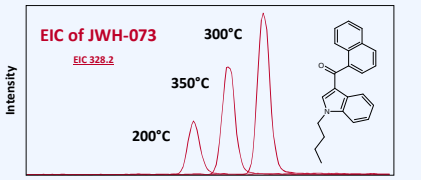


Solution:
optimizing LC separation



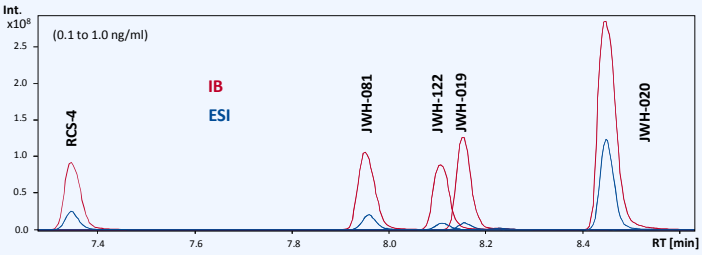
Evaluation ionBooster

Evaluation of different vaporizer gas temperatures

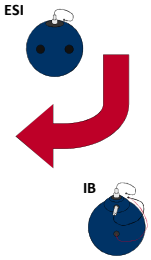


The evaluation was carried out in spiked eluent for all compounds. The vaporizer gas temperature showed a significant effect on the ionization efficiency and should be investigated for every compound. Ultimately, 300°C were found to be the best setting for all synthetic cannabinoids investigated.

MS Evaluation ESI vs. IB of selected synthetic cannabinoids in eluent



The observed signals were in average 5-fold higher using the IB (minimal intensity gain: 2-fold). Within the initial comparison it could be shown that the IB allows the detection of substances not found when using ESI.

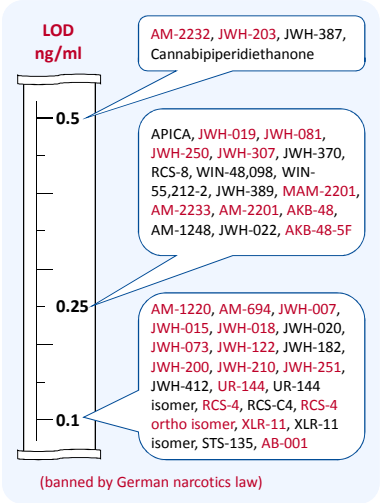


RESULTS

Evaluation of LOD in serum samples

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.

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 and were included in the report-file.



AM-1220-azepane-derivative, methanandamide and CRA-13 generally have a poor ESI-response and could not be identified at the spiked levels. Methanandamide did not yet occur as adulterant in herbal mixtures.

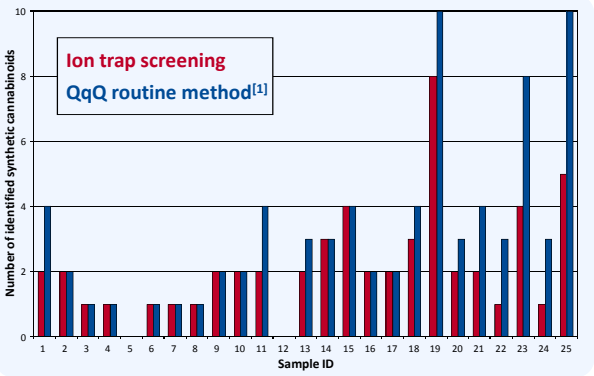
CRA-13 and AM-1220-azepane-derivative have been found in mixtures but not in serum samples analyzed in our lab so far.

Authentic serum samples were re-analyzed subsequent to routine analysis with the presented screening approach. The extracts were evaporated and dissolved in 25 µl appropriate eluent.

The figure on the right compares the results of a subset of the samples. In general the results of the screening are in good agreement with those of the routine MRM-method. In all samples in which one or more compounds were identified by the MRM-method (e.g. #24), at least one synthetic cannabinoid was found and listed in the automatically generated result report of the screening. Also, no synthetic cannabinoids were identified in samples found negative by routine analysis (#5 and #12).

Sample # 19	QqQ	Ion Trap
AM-2201	3.3 ng/ml	☺
AB-001	< 2.0 ng/ml	☺
JWH-018	< 0.1 ng/ml	☹
JWH-022	positive	☺
MAM-2201	0.17 ng/ml	☺
STS-135	positive	☺
UR-144	positive	☹
UR-144 isomer	positive	☺
XLR-11	positive	☺
XLR-11 isomer	positive	☺

Authentic samples



Sample # 14	QqQ	Ion Trap
AM-2201	0.25 ng/ml	☺
JWH-210	3.9 ng/ml	☺
JWH-018	0.2 ng/ml	☺

Sample # 24	QqQ	Ion Trap
AM-2201	0.17 ng/ml	☹
XLR-11	positive	☹
XLR-11 isomer	positive	☺

[1] 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*, 825-835 111

CONCLUSION

We present an LC-MS^n method, based on the Toxtyper 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. The combination of MS^2/MS^3-spectra and retention time meets common criteria for identification according to forensic guidelines.

The 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.

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. In contrast to targeted MRM-methods, this full-scan approach at least obtaines MS^1-information of new compounds which can be used for further investigations. The presented approach can also be applied to other specimens, such as oral fluid and herbal mixtures or solid matter suspected to contain synthetic cannabinoids.

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