



## Application Note # LCMS-99

# Comprehensive detection and identification of synthetic cannabinoids using the Toxtyper platform

### Introduction

For nearly a decade synthetic cannabinoids are among the most prevalent new emerging psychoactive substances. Herbal mixtures containing synthetic cannabinoids are readily available over the internet. These products are marketed as incense, plant-fertilizer or car-perfume and are sold as an alleged legal alternative to cannabis products to circumvent narcotics laws and drug-testing. Accordingly, these products are of special interest to consumers who do not have easy access to cannabis as well as those that are subject to drug testing (e.g. workplace drug testing candidates, patients in drug rehabilitation centres or forensic clinics).

The active ingredients, pharmacological potencies and concentrations of synthetic cannabinoids compared to  $\Delta^9$ -tetrahydrocannabinol (THC) are generally unknown to consumers. This poses a high risk of severe and sometimes life-threatening side effects. After (unintended) uptake of high doses of these substances, tachycardia, hypertension, agitation, hallucinations, psychosis, emesis, coma and seizures are among the adverse effects known and even consumption related fatalities have been reported.

### Authors

Laura M. Huppertz, Jürgen Kempf, Volker Auwärter  
Medical Center – University of Freiburg, Institute of Forensic Medicine, Forensic Toxicology, Freiburg, Germany

Markus Meyer, Tony Drury, Andrea Kiehne  
Bruker Daltonik, Bremen, Germany

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The variety of synthetic cannabinoids is high, with new or slightly modified compounds constantly emerging and to replace those which have been legally prohibited. Due to the constant emergence of new derivatives and the large structural diversity among synthetic cannabinoids law enforcement authorities and forensic toxicology labs require comprehensive screening methodologies.

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 either biological matrices or seized material. We present the Toxtyper™: an LC-MS<sup>n</sup> library-based solution that is conveniently applied to address these analytical challenges.

## Experimental

### Sample preparation

Serum samples were prepared according to the following liquid-liquid extraction (LLE) protocol:

Internal standards (ISTD mixture, see Ref. 1.), 0.5 mL carbonate buffer (pH 10) and 1.5 mL extraction mixture (n-hexane: ethyl acetate, 99:1, v/v) were added to 1 mL of serum. After 3 min of mixing in an overhead-shaker, the solution was centrifuged at 4000 × g for 5 min. The organic phase was separated, and evaporated at 40°C with N<sub>2</sub>. The residue was dissolved in 25 µL solvent A/B (50:50; v/v).

### Herbal mixtures

For qualitative analysis 25 mg of the sample were weighed, and after addition of deuterated internal standards extracted with methanol under ultra-sonication for 5 - 10 minutes.

The extract was filtered using a 0.45 µm syringe filter. An aliquot was diluted 1:1000 using eluents A/B (50:50; v/v).

### LC-MS<sup>n</sup> conditions

Two microliters of the sample were injected and separated using the LC settings described in Table 1. A Toxtyper system (based on an amaZon speed ion trap) was used for the generation of MS, MS<sup>2</sup> and MS<sup>3</sup> spectra in autoMS<sup>n</sup> mode with positive electrospray ionization (for detailed MS settings refer to Table 2). For the analysis of serum samples a data-dependant scheduled precursor list (SPL) and an ionBooster (iB) source were used.

### ionBooster

The ionBooster (iB) source enhances sensitivity and lowers detection limits by delivering more efficient ionization across a wide range of applications. This is achieved by maintaining a controlled vaporizing temperature inside the source to enhance desolvation of analyte ions, even when using high eluent flow rates typical for UHPLC separations. The ionBooster has simple operating parameters and nitrogen consumption requirements that are similar to those of conventional ion sources (refer to Tab. 3 for a comparison of iB vs. ESI settings).

Table 1: HPLC conditions for the synthetic cannabinoid method

LC settings	
LC system	Ultimate 3000 RSLC
Eluent A	H <sub>2</sub> O, 0.1% formic acid, 2 mM ammonium formate, 1% acetonitrile
Eluent B	Acetonitrile, 0.1% formic acid, 2 mM ammonium formate, 1% H <sub>2</sub> O
Analytical column	Kinetex® 2.6 µm, C18 100 Å, 2.1 x 100 mm (Phenomenex)
Flow rate	500 µl/min
Gradient	0.0 to 1.0 min: 1% 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 99% B, linear 8.0 to 10 min: 99% B 10 to 10.2 min: 99% B to 20% B, linear 10.2 to 12 min: 20% B

Table 2: General MS settings for the synthetic cannabinoid methods

Toxtyper MS <sup>n</sup> settings	
Scan mode	UltraScan 32.500 m/z sec <sup>-1</sup>
Scan range	70 - 600 m/z
Source	Apollo II ESI/ionBooster
Polarity	positive
MS <sup>n</sup> Acquisition	AutoMS <sup>n</sup> or data dependent using a Scheduled Precursor List Active exclusion after 1 spectrum, reconsider if intensity increases by factor 5
Target mass	300 m/z
ICC	200.000

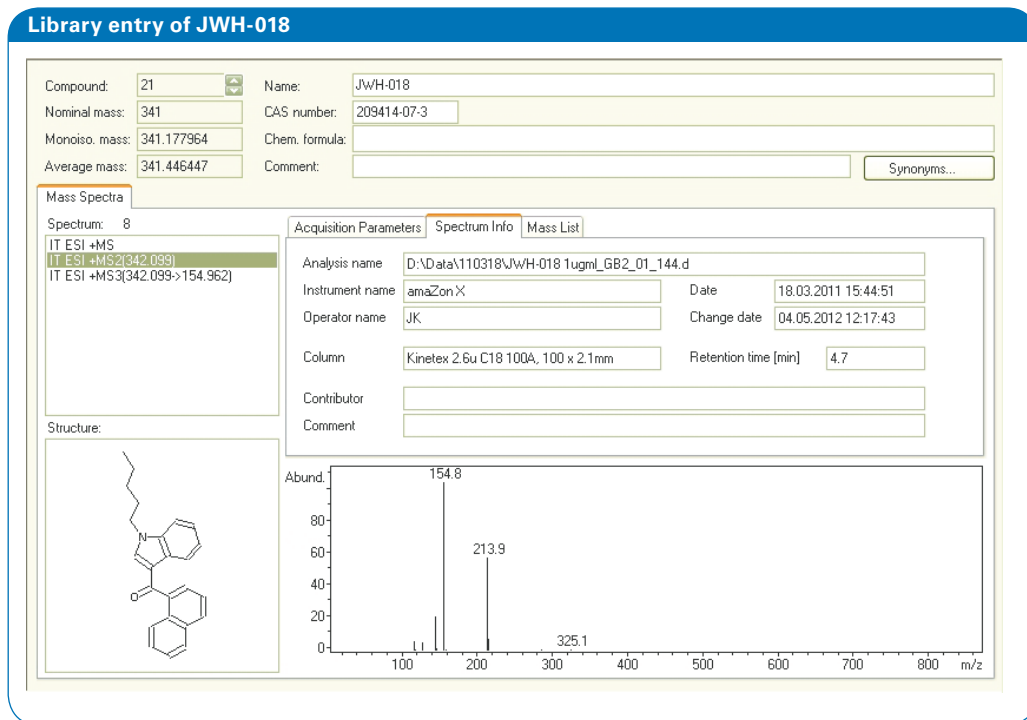


Figure 1: Library entry of JWH-018; here the MS<sup>2</sup> spectrum is shown

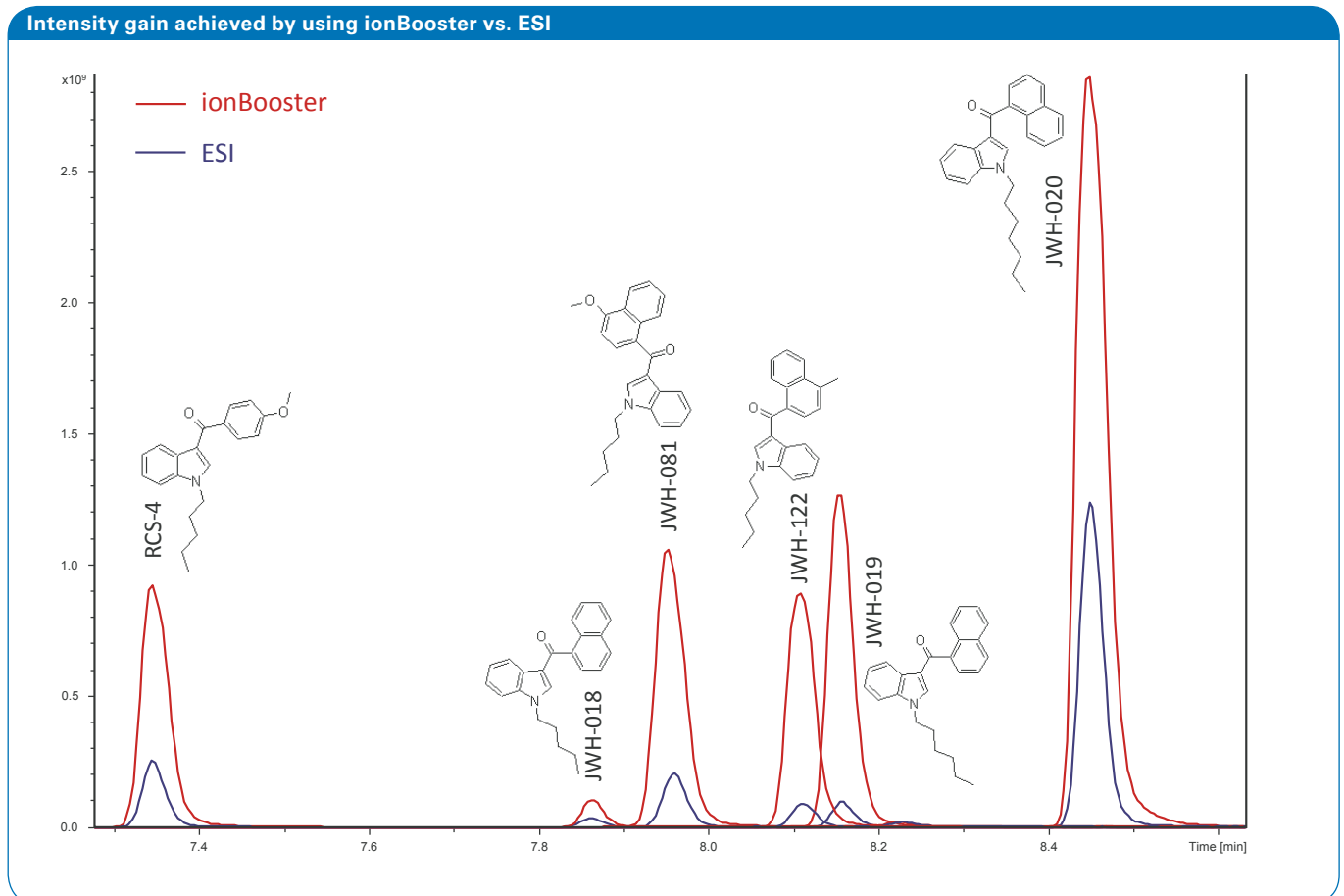


Figure 2: Intensity gain achieved by using ionBooster in comparison to ESI. Depicted are overlaid Extracted Ion Chromatograms (EICs) of different synthetic cannabinoids at a concentration level of 10 ng/ml in eluent A

Table 3: MS settings for the different methods used in this study.

	ionBooster settings	Apollo II ESI settings
Capillary	1,000 V	4,500 V
End Plate Offset	400 V	500 V
Charging Voltage	300 V	n.a.
Nebulizer	60 psi	30 psi
Dry Gas	3.0 l/min	9.0 l/min
Dry Temperature	200°C	320°C
Vaporizer Temperature	300°C	n.a.
Sheath Gas Flow	150 l/h	n.a.
ICC	On	On

### Library search and reporting

The data sets were processed using DataAnalysis (DA) 4.1 and the processed spectra were submitted to the DA 4.1 library search module. The whole process, up to final report generation and visualization of results in the web-based Compass OpenAccess interface, was driven by a predefined Toxtyper automation script. The automatically generated reports were evaluated and used for generation of the final result tables.

## Results

A spectra library, containing MS, MS<sup>2</sup> and MS<sup>3</sup> spectra of altogether 46 synthetic cannabinoids and 9 isotope-labeled analogs was set up using certified standard solutions. A typical library entry is shown in Figure 1, a fast LC method, sufficiently separating isobaric compounds and a scheduled precursor list including retention times and precursor *m/z* of the synthetic cannabinoids in the library have been developed.

Synthetic cannabinoids dissolved in LC eluent A were analyzed with LC separation using the Apollo II ESI source and the ionBooster to determine the potential benefit of the latter. Apart their structural diversity, all synthetic cannabinoids investigated proved to be highly thermostable compounds benefiting from the ionBooster source. The implementation of the ionBooster lead to a significant gain of intensity resulting in mainly lower detection limits in serum samples. Typical results illustrating the intensity gain of the ionBooster vs. conventional ESI are shown in Fig. 2. The achieved detection limits in serum samples correspond to concentrations of synthetic cannabinoids considered as reasonable cut-off values in methods published using triple-quadrupole instrumentation with multiple reaction monitoring (MRM) [see Ref. 2. Kneisel et al.].

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 sample and serum samples only containing internal standards were processed similarly to evaluate potential matrix effects.

Compared to spiked eluent deviant detection limits in serum were obtained for JWH-122, JWH-387 and UR-144 due to ion enhancement, and for AM-1220, AM-2232, CRA-13, JWH-019, JWH-081, MAM 2201, JWH-203, JWH-250, JWH-307, JWH-370, methanandamide, RCS-4 C4-analogue, RCS-8, WIN-48,098 (pravadoline), and WIN-55,12-2 caused by signal suppression.

The search algorithm matches retention times, MS, and MS<sup>2</sup>/MS<sup>3</sup> spectral information to calculate a purity score. A compound is considered as definitely identified when achieving a purity score higher than 800. Table 4 illustrates the detection limits in spiked serum samples obtained by the automated library search and result reporting tool adapted from the Toxtyper workflow (see Ref. 3). All synthetic cannabinoids included in the library were sufficiently detected and identified (assuming a purity score of >800) at a concentration of 0.5 ng/mL or lower in spiked serum samples (see Table 4).

Table 4: Limits of detection (LOD) of the synthetic drugs analyzed in this study. LODs were obtained from spiked human serum samples of different origins.

Compound	c [ng/mL]	Compound	c [ng/mL]
5F-AKB-48	0.25	JWH-203	0.5
AB-001	0.1	JWH-210	0.1
AKB-48	0.25	JWH-250	0.25
AM-1220	0.1	JWH-251	0.1
AM-1248	0.25	JWH-307	0.25
AM-2201	0.25	JWH-370	0.25
AM-2232	0.5	JWH-387	0.5
AM-2233	0.25	JWH-398	0.25
AM-694	0.1	JWH-412	0.1
APICA	0.25	MAM-2201	0.25
Cannabipiperidiethanone	0.5	RCS-4	0.1
JWH-007	0.1	RCS-4 C4	0.1
JWH-015	0.1	RCS-4 ortho isomer	0.1
JWH-018	0.1	RCS-8	0.25
JWH-019	0.25	STS-135	0.1
JWH-020	0.1	UR-144	0.1
JWH-022	0.25	UR-144 isomer	0.1
JWH-073	0.1	WIN-48.098	0.25
JWH-081	0.25	WIN-55.212-2	0.25
JWH-122	0.1	XLR-11	0.1
JWH-182	0.1	XLR-11 isomer	0.1
JWH-200	0.1		

In contrast to serum samples sensitivity is not an issue when analyzing herbal mixture extracts. Two different workflows (see Fig. 3) are used for either high sensitive targeted serum analysis or untargeted screening of seized/liquid mixtures. The latter allows retrospective analysis and identification of new drugs. The ion trap is used in an untargeted autoMS<sup>n</sup> mode without SPL triggered spectral acquisition as seized material may contain new psychoactive substance analogues that are not yet included within the previously described library and SPL. This approach leads to a comprehensive MS, MS<sup>2</sup> and MS<sup>3</sup> spectral acquisitions and opens up the possibility to retrospectively perform library searches after update of the library with new drugs or to use other external libraries.

### Summary and Conclusion

We present an LC-MS<sup>n</sup> approach offering a fast, reliable, and easy-to-use research based screening solution for the identification of synthetic cannabinoids with a high degree of automation, whilst still keeping the possibility for manual data evaluation. The combination of MS<sup>2</sup>/MS<sup>3</sup>-spectra and retention time meets common criteria for the identification and confirmation of drugs according to forensic guidelines. A significant gain in sensitivity (down to 0.1-0.5 ng/ml in serum) has been achieved by using the ionBooster source.

Due to the rather low concentrations and significant matrix background in biological samples, the targeted SPL approach is required for the analysis of serum samples.

As an alternative autoMS<sup>n</sup> spectral acquisition without SPL offers the possibility for retrospective data evaluation. Hence, when analyzing solid matter (research chemicals) or herbal mixture extracts, autoMS<sup>n</sup> data can be manually reviewed and if non-identified peaks do occur they may be reprocessed with an updated library version later.

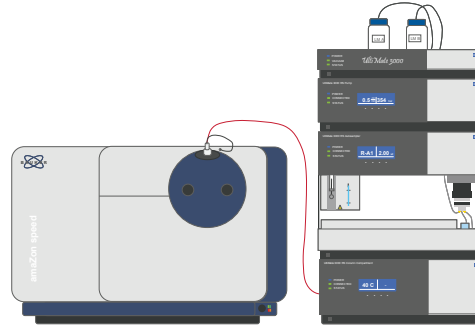
This approach can be easily customized by individually adding (new emerging) compounds to the library, thus keeping it up-to-date to meet the future demands of the rapidly expanding designer drug testing arena.



12 min LC run, AutoMS<sup>n</sup>, Spectral Library

Targeted method  
using a scheduled  
precursor list (SPL)

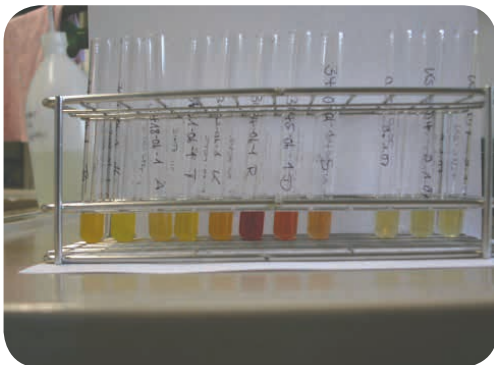
ionBooster



Untargeted method  
using AutoMS<sup>n</sup>  
(no SPL),  
retrospective analysis

ionBooster optional

High sensitivity identification in  
biological samples (e.g. serum)



Huppertz et al. JMS 2014, 49, 117-127

Seized Materials (herbal  
mixtures, liquids)



Figure 3: Illustration of two workflows for the identification of synthetic drugs in biological samples and seized material.

## References

- [1] Huppertz et al. A comprehensive library-based, automated screening procedure for 46 synthetic cannabinoids in serum employing liquid chromatography-quadrupole ion trap mass spectrometry with high-temperature electrospray ionization. *JMS*, 2014, 49(2), 117-127
- [2] Kneisel et al. Analysis of 30 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry after liquid-liquid extraction. *JMS*, 2012, 47, 825-835
- [3] Bruker Daltonics Application Note #LCMS-91: Toxtyper – a Comprehensive LS-MSn application for Clinical Research and Forensic Toxicology

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### ● Bruker Daltonik GmbH

Bremen · Germany  
Phone +49 (0)421-2205-0  
Fax +49 (0)421-2205-103

### Bruker Daltonics Inc.

Billerica, MA · USA  
Phone +1 (978) 663-3660  
Fax +1 (978) 667-5993

[ms.sales.bdal@bruker.com](mailto:ms.sales.bdal@bruker.com) - [www.bruker.com](http://www.bruker.com)