Using TargetScreener with Software Assisted Data Mining Strategies to Identify Metabolites of New Psychoactive Substances

Introduction

As one of the largest and most predominant substance classes amongst the group of new psychoactive substances (NPS), synthetic cannabinoids have challenged the forensic field since first appearing a decade ago. Over the last seven years, synthetic cannabinoids offered for purchase have undergone significant chemical modifications making immunochemical testing unsuitable. Mass spectrometric methods for the detection and identification in biological and non-biological samples have become the gold standard. However, due to frequent chemical modifications to the product portfolio resulting in several new compounds, emerging analytical methods have to be adapted frequently.

Keywords: Synthetic Cannabinoids, MDMB-CHMICA, Identification of Metabolites, Mass-MetaSite™, QTOF

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Offering a non-invasive sample collection with a relatively wide window of detection, urine analysis is usually the method of choice for abstinence control. Yet, metabolite identification is inevitable in urine analysis since most synthetic cannabinoids are metabolized extensively prior to renal excretion. After conjugate cleavage with β-glucuronidase, the main phase I metabolites represent suitable target analytes.

Therefore, the metabolism of new synthetic cannabinoids needs to be known prior to updating analytical methods. In cases where no authentic human sample material with confirmed uptake of the particular compound is available, pooled human liver microsomes (pHLM) offer an inexpensive and fast alternative to gain preliminary data on phase I metabolites that may be relevant for analysis of human urine samples.

Table 1: HPLC conditions used in the TargetScreener workflow.

LC-MS settings	
Mass spectrometer	impact II Full scan with Auto MS/MS mode, 50-600 m/z @ 4 Hz Full scan with bbCID mode, 50-600 m/z @ 2 Hz ESI, Positive ion mode
UHPLC	UltiMate 3000RS HPLC
Column	Kinetex C18 2.1x100 mm, 2.6 µm
Eluents	A: 1% ACN + 0.1% HCOOH + 2 mM NH4+COO- B: ACN + 0.1% HCOOH + 2 mM NH4+COO-
Gradient	14 min gradient elution, 20 min total runtime
Total flow	0.5 mL/min
Oven	40 °C
Injection vol.	2 µL

For proof of concept of the presented workflow the highly potent synthetic cannabinoid MDMB-CHMICA (methyl N-{[1-(cyclohexylmethyl)-1H-indol-3-yl]carbonyl}-3-methylvalinate) was chosen as a model compound, being one of the most prevalent synthetic cannabinoids in Germany and the cause for numerous intoxications worldwide.

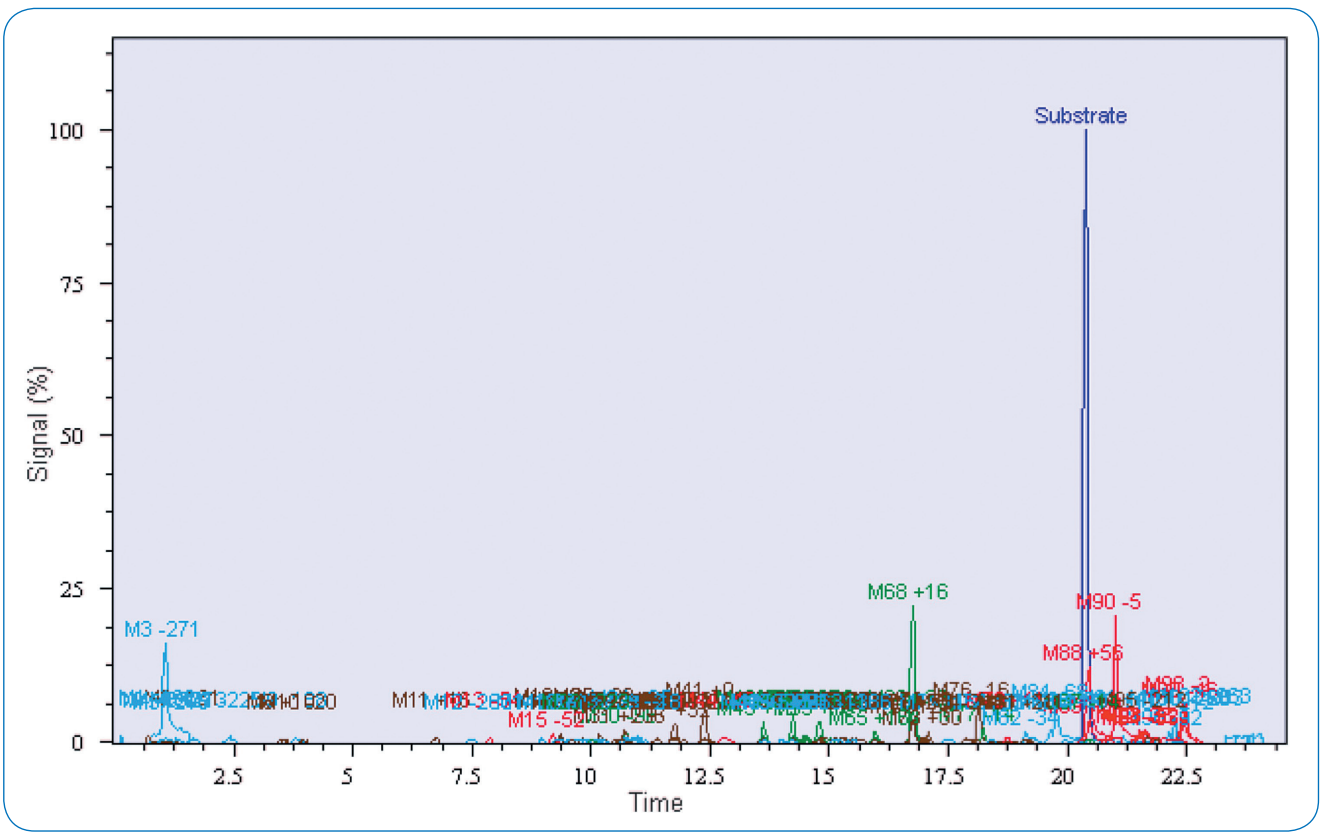


Fig. 1: Overview of extracted ion chromatograms of possible metabolites in Mass-MetaSite software

Name	RT	z	m/z obs.	m/z shift	Area %	Area Abs
M40 +32	11.96	1	417.2420	+32	0.08	2.3e+03
M21 +32	9.84	1	417.2412	+32	0.15	4.4e+03
M43 +32	12.39	1	417.2388	+32	0.11	3.3e+03
M16 +32	9.37	1	417.2384	+32	0.29	8.5e+03
M25 +32	10.17	1	417.2377	+32	0.28	8.3e+03
M37 +32	11.78	1	417.2376	+32	1.02	3e+04
M73 +30	17.08	1	415.2204	+30	0.30	8.9e+03
M27 +22	10.28	1	407.1940	+22	0.09	2.5e+03
M35 +18	11.16	1	403.0722	+18	0.08	2.4e+03
M20 +18	9.70	1	403.0721	+18	0.32	9.6e+03
M22 +18	9.90	1	403.0714	+18	0.12	3.6e+03
M24 +18	10.03	1	403.0711	+18	0.20	6e+03
M18 +18	9.42	1	403.0708	+18	0.19	5.7e+03
M45 +16	13.61	1	401.2445	+16	0.76	2.2e+04
M54 +16	14.48	1	401.2445	+16	0.25	7.3e+03
M68 +16	16.77	1	401.2436	+16	7.07	2.1e+05
M51 +16	14.25	1	401.2435	+16	1.09	3.2e+04
M55 +16	14.81	1	401.2430	+16	1.14	3.4e+04
M65 +16	15.95	1	401.2428	+16	0.42	1.2e+04
M33 +16	10.91	1	401.2081	+16	0.10	3e+03
M11 +16	6.75	1	401.2072	+16	0.15	4.3e+03

Fig. 2: Excerpt of a mass list giving possible metabolites. Mass differences stated result from the respective metabolic changes of the parent compound MDMB-CHMICA.

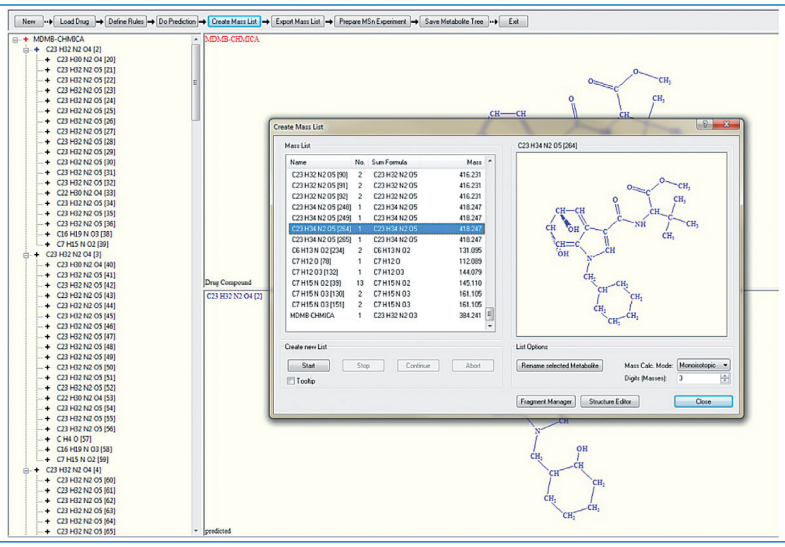


Fig. 3: For MS^n experiments a scheduled precursor list was generated with Metabolite Predict out of the Metabolite tools software.

Method and Material

The pHLM assay was performed according to a standard protocol. LC-QTOF-MS analysis was performed on an UltiMate™ 3000 RSLC coupled to an impact II instrument in positive ESI mode using data-dependent MS/MS fragmentation and bbCID. Mass-MetaSite™ software (Molecular Discovery) was used to analyze LC-MS/MS datasets of the incubations sampled at time points zero and one hour.

Anticipated phase I biotransformations were: Mono- and dihydroxylation, carboxylation, ester hydroylsis, amide hydrolysis, dihydrodiol formation, reduction and potential combinations of these reactions. These suggested biotransformations follow known metabolism patterns of related synthetic cannabinoids. In Figure 1 an excerpt of the generated mass list giving possible metabolites is shown. Furthermore a precursor mass list was generated using MetabolitePredict software for targeted MS/MS experiments (Figure 2).

Precursor and fragment information of the identified metabolites were used to create a screening method using TASQ™ software.

Results and Discussion

As stated above, the highly potent and prevalent synthetic cannabinoid MDMB-CHMICA was chosen as a model compound. Analysis of the pHLM incubations of MDMB-CHMICA with Mass-MetaSite™ software revealed 10 metabolites with at least two fragment masses each. Figure 3 shows the extracted ion chromatograms of possible metabolites in Mass-MetaSite™ Software. The major in vitro phase I metabolites previously

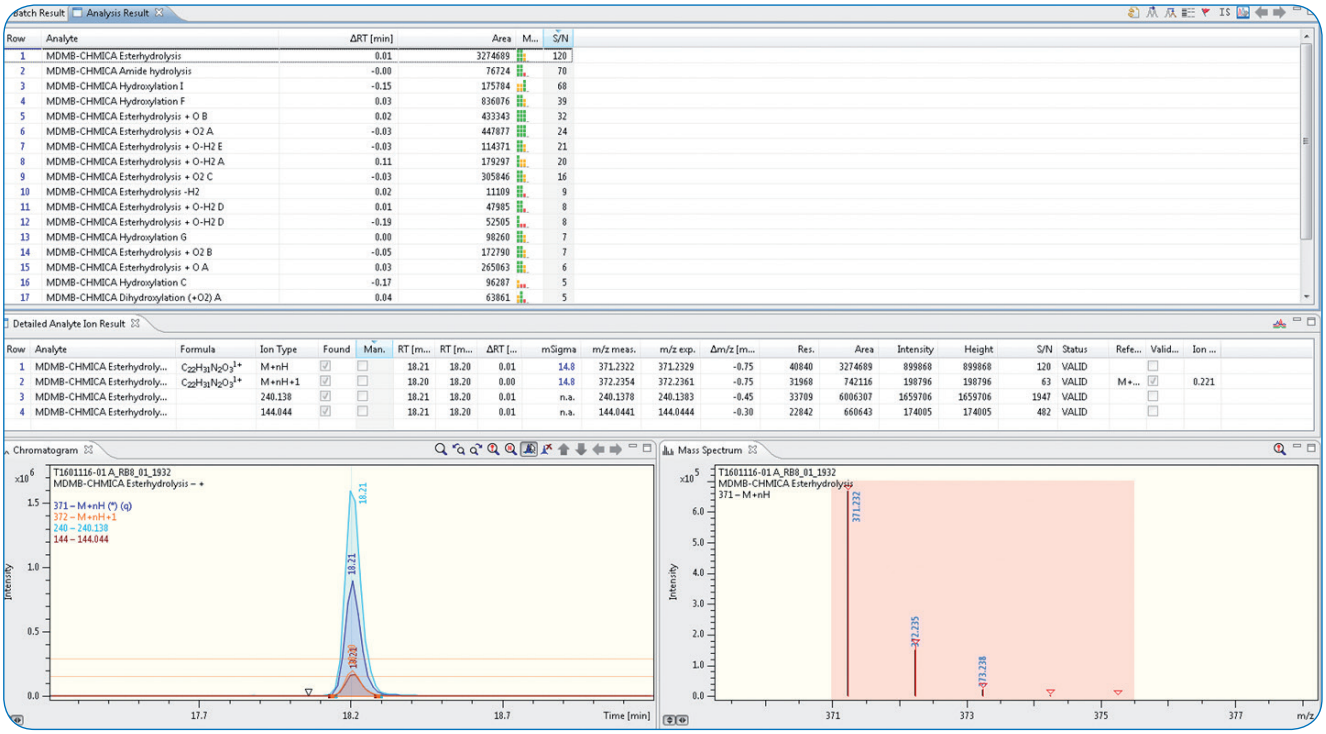


Fig. 4: The identified metabolites were used together with their fragment information (qualifiers) and retention time to set up a screening method in TASQ software. Below the screening results of a urine sample positive for MDMB-CHMICA are shown.

described in different studies[1,2] were detected with the software. The metabolite and fragment information was used to generate a screening method in Bruker TASQ™ software. Additionally, the retention times were adapted and added to the database. Authentic forensic case samples were screened and processed with the updated TASQ™ method (Figure 4 and 5). Despite varying relative abundances of the detected metabolites, the in vitro and in vivo data showed good agreement with respect to the chosen MDMB-CHMICA metabolites.

Conclusion

- Mass-MetaSite™ software and the described workflow proved to be a suitable, less laborious and time consuming procedure compared to manual data evaluation.
- The described approach can be helpful for updating screening methods with metabolite information.
- Having metabolites included in screening methods is necessary when dealing with analytes that are extensively metabolized such as synthetic cannabinoids.
- Identification of metabolites along with parent compounds can serve as a plausibility check and may help in estimating the time of the last drug uptake.

Batch Screening Results 160502 SynCan MDMB-CHMICA

Analysis: T1601116-01 A_RB8_01_1932

Creation Date	2016-05-03 09:04	Sample Type	Sample
Method	MDMB-CHMICA-HLM-1	Mass Calib. Date	2016-05-02 15:21
Station Name	Default	Operator	LH
Instrument	impact II	Instrument SN	1825265.10039
Data Path	D:\Data\2016\160502 SynCan MDMB-CHMICA\T1601116-01 A_RB8_01_1932.d		

Screening Results

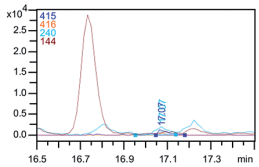
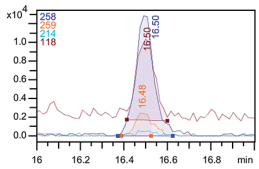
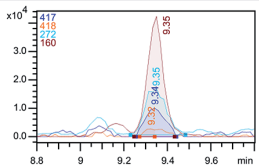
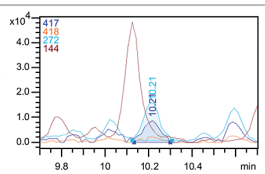
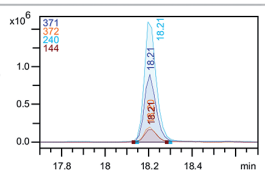
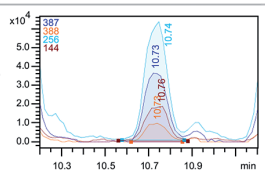
AnalyteName	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	Chromatogram
MDMB-CHMICA +O2-H2	415.2227	0.299	0.07	99	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	17.00	●●●	●●●	●	-	
MDMB-CHMICA Amide hydrolysis	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	
	258.1489	-2.608	-0.00	214	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	16.50	●●●	●●●	●	-	
MDMB-CHMICA Dihydroxylation (+O2) A	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	
	417.2384	-2.463	0.04	233	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	9.30	●●	●●●	●	-	
MDMB-CHMICA Dihydroxylation (+O2) C	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	
	417.2384	-1.609	0.01	84	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	10.20	●●●	●●●	●	-	
MDMB-CHMICA Esterhydrolysis	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	
	371.2329	-2.009	0.01	15	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	18.20	●●●	●●●	●●	-	
MDMB-CHMICA Esterhydrolysis + O A	m/z theo.	Δ m/z [ppm]	Δ RT [min]	mSigma	mand. Ions	
	387.2278	-1.813	0.03	17	0/0	
	RT theo.	m/z Score	RT Score	σ Score	Ions Score	
	10.70	●●●	●●●	●●	-	

Fig. 5: Example of an pdf. report generated in TASQ SW. These reports can be obtained per batch or for each sample individually.

Learn More

You are looking for further Information? Check out the Link or scan the QR Code.

www.bruker.com/TargetScreener

References

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2. **Franz et al. Drug Test Anal. 2016 Aug 9. doi: 10.1002/a.2049. [Epub ahead of print]**

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