Software Assisted Metabolite Identification – A Tool for the Rapid Updating of Screening Methods for Synthetic Cannabinoids in Human Urine by LC-QToF-MS

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Introduction

Synthetic cannabinoids (SC) pose a great challenge to practitioners in the forensic field. Over the last years, SC available on the market have undergone significant structural changes making immunochemical testing unsuitable. Accordingly, MS methods have become the gold standard for analysis of SC in biological specimens but require frequent adaption to include newly emerged SC.

Since most SC are metabolized extensively prior to renal excretion, metabolite identification is inevitable for urine analysis. After conjugate cleavage, the main phase I metabolites are suitable target analytes. Consequently, the metabolism of new SC 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 alternative to gain preliminary data on phase I metabolites relevant for analysis of human urine samples. Also, pHLM extracts can be used for LC-MS/MS method development and optimization.

Software (SW) assisted metabolite identification (MID) has been reported as faster though equally efficient as manual data evaluation in various areas of research.

The highly potent synthetic cannabinoid MDMB-CHMICA (methyl *N*-{[1-(cyclohexylmethyl)-1*H*-indol-3-yl]carbonyl}-3-methylvalinate) - one of the most prevalent SC in Germany and the cause of numerous intoxications worldwide - was chosen as model compound to proof the concept of SW assisted MID for forensic analysis and to develop a workflow to rapidly update screening methods with LC-QToF-MS.

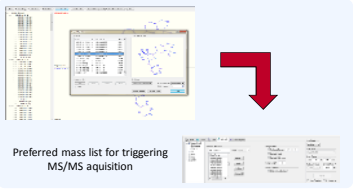
I. Analytical Workflow

1.) Metabolite Prediction

Accurate masses of potential phase I metabolites

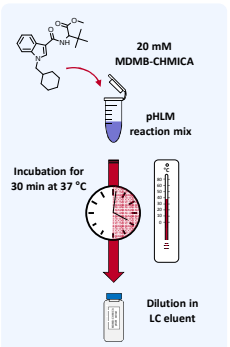
Manual anticipation of potential phase I biotransformations, e.g. mono- and dihydroxylation, carboxylation, ester hydrolysis, reduction, and combinations of these reactions, following known metabolism patterns of synthetic cannabinoids.

Automatic generation of precursor mass lists via suitable software e.g. MetabolitePredict.



Preferred mass list for triggering MS/MS aquisition

2.) pHLM Incubation



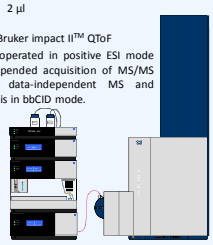
3.) LC-QToF Analysis

LC-Settings: Dionex UltiMate 3000RS HPLC

Column:	Kinetex C18 2.1x100 mm, 2.6 µm
Gradient:	20 min total runtime
Eluents A:	1% ACN + 0.1% HCOOH + 2 mM $NH_4^+COO^-$
Eluent B:	ACN + 0.1% HCOOH + 2 mM $NH_4^+COO^-$
Total flow:	0.5 ml/min
Oven:	40 °C
Injection vol.:	2 µl

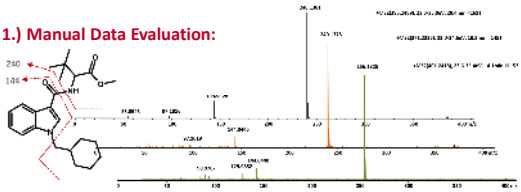
MS-Settings: Bruker impact II™ QToF

The MS was operated in positive ESI mode using data-depended acquisition of MS/MS spectra and data-independent MS and MS/MS analysis in bbCID mode.



II. Data Evaluation

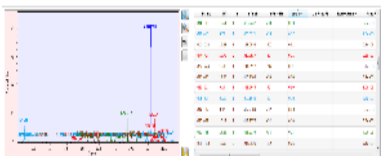
1.) Manual Data Evaluation:



1.) Evaluation of Extracted Ion Chromatograms (EIC)
2.) Evaluation of bbCID data based on characteristic fragments
3.) Mass defect filtering
With manual data evaluation 15 possible metabolites could be identified.

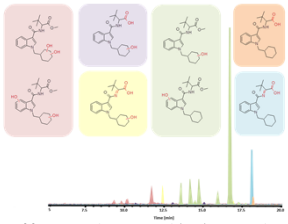
vs.

2.) Automatic Data Evaluation:



MassMetaSite software was used to analyze the LC-MS/MS datasets of the incubation, revealing 10 metabolites with at least two fragment ions each.

3.) Identification of the main *in vitro* phase I metabolites

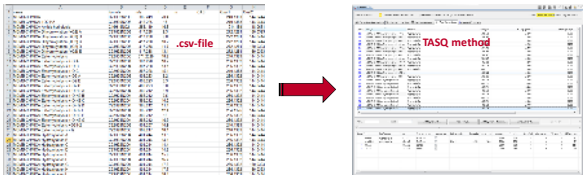


By manual data evaluation 5 additional metabolites at low intensities could be identified. However, the main *in vitro* metabolites described in the literature[1,2] were identified by the software.

[1] Grigoryev et al. Forensic Toxicol. (2016) 34:316-328 doi: 10.1007/s11419-016-0319-8
[2] Franz et al. Drug Test Anal. doi: 10.1002/dta.2049. [Epub ahead of print]

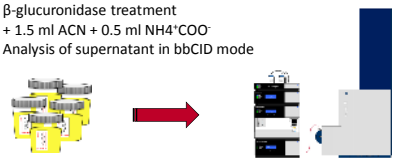
III. Generation / Update of the Screening Method

The name, formula, retention time and the accurate mass of the precursor and fragment ions of potential phase I metabolites are added to a .csv-file. Subsequently, a TASQ method for screening authentic samples is generated from this .csv-file.

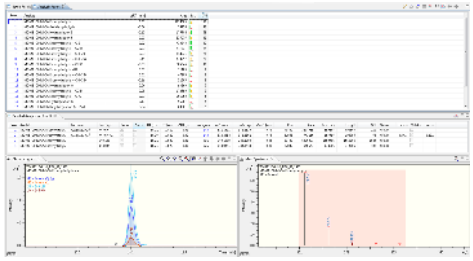


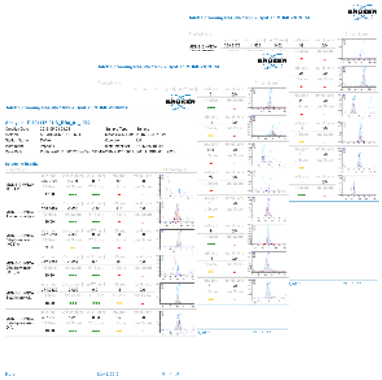
IV. Analysis of Routine Case Work Samples

1.) β-glucuronidase treatment
2.) + 1.5 ml ACN + 0.5 ml $NH_4^+COO^-$
3.) Analysis of supernatant in bbCID mode

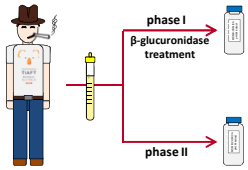


Reports are generated by TASQ software for each sample listing every hit. The hits are rated based on their mass and retention time error, mSigma value and the presence of qualifier ions (MRSQ-Score).





V. Additional Screening of Positive Authentic Samples

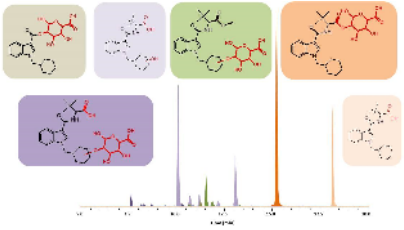


Positive urine samples are screened after conjugate cleavage in analogy to the pHLM sample to identify the predominant *in vivo* phase I metabolites and to screen for further metabolites.

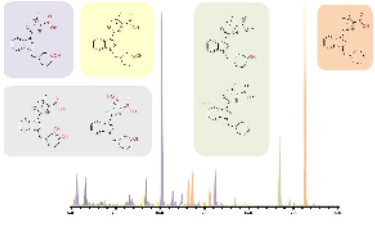
Additionally, samples are screened for phase II metabolites after protein precipitation allowing to update methods omitting conjugate cleavage in the sample preparation process.

VI. Additional Screening Results

1.) Without Cleavage of Glucuronides

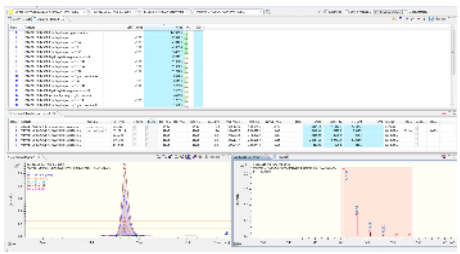


2.) After Cleavage of Glucuronides



Despite varying relative abundances of the detected metabolites, the *in vitro* and *in vivo* data showed good agreement with respect to the MDMB-CHMICA metabolites chosen and subsequently included in the final screening method. Additional metabolites could be identified in the *in vivo* samples so a total of 42 metabolites of MDMB-CHMICA (phase I and II) could be added to the database.

VII. Final TASQ Method



Based on the additional *in vivo* metabolism data, the TASQ method could be updated now allowing for identification of phase I and phase II metabolites of MDMB-CHMICA.

Conclusions

Using MassMetaSite software and the described workflow proved to be a suitable, less laborious and time consuming procedure compared to manual data evaluation. The here described approach can be helpful for updating screening methods with metabolite information. This is necessary whenever dealing with analytes that are extensively metabolized such as SC. In other cases identification of metabolites along with the parent compound can serve as a plausibility check and may help in estimating the time of the last drug uptake.

Acknowledgement

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