Rapid update of screening methods for the detection of synthetic cannabinoid use in human urine by software assisted metabolite identification

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Overview

- **LC-Q-ToF-MS screening for metabolites of synthetic cannabinoids in urine samples**
- **Rapid workflow for updating the screening method**

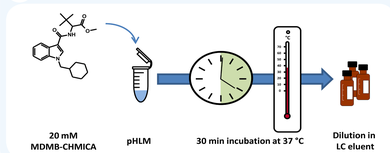
Introduction

Cannabinoid receptor agonists, commonly referred to as synthetic cannabinoids (SC), are one of the most predominant classes within the group of new psychoactive substances (NPS) and pose a great challenge to the forensic field. Over the last seven years, synthetic cannabinoids offered for purchase have undergone significant structural changes making immunochemical testing unsuitable. Consequently, mass spectrometric methods are the gold standard but have to be adapted frequently to include newly emerged compounds. Offering a non-invasive sample collection with a relatively wide window of detection, urine analysis is usually the method of choice for abstinence control. However, for urine analysis metabolite identification of the respective SC is inevitable since most of these compounds are metabolized extensively prior to renal excretion. After conjugate cleavage with β-glucuronidase, 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 inexpensive and fast alternative to gain preliminary data on phase I metabolites that may be relevant for analysis of human urine samples. Furthermore, the pHLM extracts can be used for liquid chromatography mass spectrometry method development.

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

Workflow

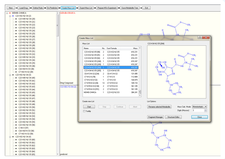
1. Pooled human liver microsomes (pHLM) incubation



2a. Metabolism prediction

Manual: Accurate masses of potential phase I metabolites. Anticipated phase I biotransformations: Mono- and dihydroxylation, carboxylation, ester hydroylsis, amide hydrolysis, dihydrodiol formation, reduction as well as combinations of these reactions, following known metabolism patterns of related synthetic cannabinoids.

Automatic:
Generation of precursor mass lists via e.g. MetabolitePredict software.



Generation of preferred mass list:

2b. LC-Q-ToF-analysis

UltiMate 3000RS HPLC
Column: Kinetex C_{18} 2.1x100 mm, 2.6 µm
Eluents: A: 1% ACN + 0.1% HCOOH + 2 mM $NH_4^+COO^-$
B: ACN + 0.1% HCOOH + 2 mM $NH_4^+COO^-$
Gradient elution: 20 min total runtime
Total flow: 0.5 mL/min
Oven: 40 °C
Injection vol.: 2 µL

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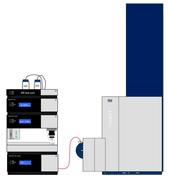
Bruker impact II™ QTOF

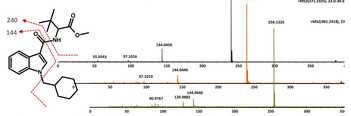
Analysis was performed in positive ESI mode using data-dependent MS/MS fragmentation and bbCID mode.



3. Data evaluation

Manual

1. Extracted Ion Chromatograms

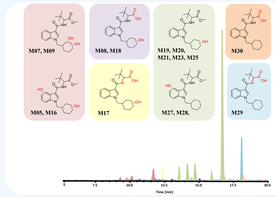


2. Evaluation of the bbCID data based on characteristic fragments
3. Mass defect filtering

Automatic

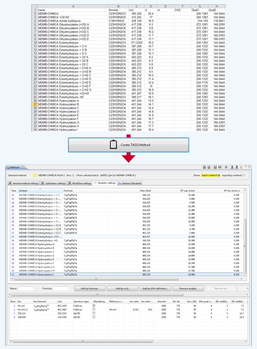
MassMetaSite software was used to analyze the LC-MS/MS datasets of the incubations, revealing 10 metabolites with at least two fragment masses each.

4. Tentative identification of the main *in vitro* phase I metabolites



5. Generation / update of TASQ method

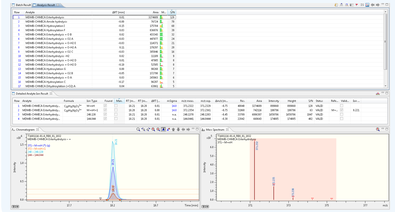
The name, formula, retention time and the accurate mass of the fragment ions are added into a .csv file.
Afterwards the .csv file is converted into a TASQ method.



6. Analysis of routine case work samples

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

7. Processing in TASQ software



Contact

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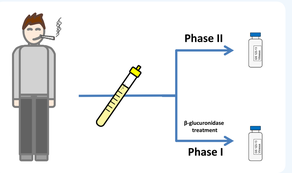
8. Reporting

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).

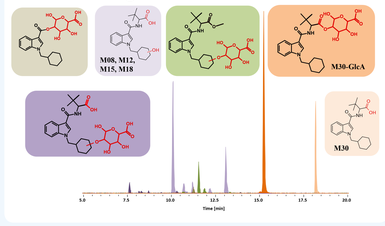
9. Further screening of the positive authentic sample



The identified positive urine sample is screened after conjugate cleavage in analogy to the pHLM sample to identify the predominant *in vivo* phase I metabolites and to screen for additional metabolites.

Additionally, the sample is screened after protein precipitation for phase II metabolites to allow an update of methods omitting conjugate cleavage in the sample preparation step.

Without glucuronidase treatment



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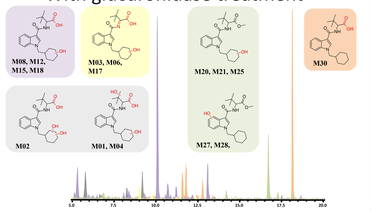
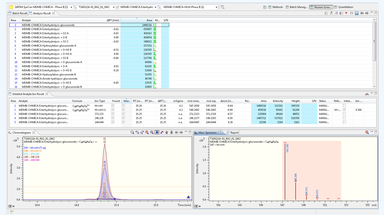
With glucuronidase treatment



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.

10. Final TASQ method



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

Conclusion

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.

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