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

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Introduction
Cannabinoid receptor agonists, commonly referred to as synthetic cannabinoids (SC), are one of the most predicament 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 immunochromatographic testing unsuitable. Consequently, mass spectrometric methodologies 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 absolute identification. However, the metabolism of new 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 possible target analytes. Consequently, the metabolism of new SC needs to be known prior to updating analytical methods. In cases where no authentic sample material with confirmed uptake of the particular compound is available, postulated 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 metabolite identification. Rapid update of screening methods for the detection of synthetic cannabinoid use in human urine

Workflow

1. Posed human liver microsomes (pHLM) incubation
   - Analysis was performed in positive ESI mode using data-dependent MS/MS fragmentation and QToF mode.

2a. Metabolite prediction
   - Manual: Accurate masses of potential phase I metabolites. Anticipated phase I biotransformations: Mono- and dihydroxylation, conjugation, ester hydrolysis, amide hydrolysis, aldehyde formation, reduction as well as combinations of these reactions. MetabolitePred software was used to analyze the LC-MS/MS data lists via e.g. MassMetaSite software.

2b. LC-Q-ToF analysis
   - Column: XBridge C-18, 2.6 µm, 100 mm x 2 mm, 2.6 µm
   - Flow: 0.5 mL/min
   - Injection volume: 2 µL
   - MassMetaSite software was used to analyze the LC-MS/MS data of the incubation, revealing 10 metabolites with at least two fragment masses each.

3. Data evaluation
   - 1. Extracted Ion Chromatograms
   - 2. Generation of preferred mass list
   - 3. Analysis of supernatant in bbCID mode

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. glauconidase treatment
   - 2. Manual prediction and generation of preferred mass list
   - 3. Analysis of supernatant in bbCID mode
   - Processing in TASQ software

7. Without glucuronidase treatment

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

9. Further screening of the authentic sample

10. Final TASQ method

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 intake.

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

For proof of concept of the presented workflow the highly potent synthetic cannabinoid MDMB-CHMICA (method A: N-[2-(cyclohexylmethyl)-2-hydroxyethyl]-5-carboxy-3-methylulval) was chosen as a model compound, being one of the most prevalent SC in Germany and the cause for numerous intoxications worldwide.

References

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1. Overview
   - Introduction
   - Workflow
   - Conclusion

2. Rationale
   - Cannabinoid receptor agonists, commonly referred to as synthetic cannabinoids (SC), are one of the most predicament 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 immunochromatographic testing unsuitable. Consequently, mass spectrometric methodologies 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 absolute identification. However, the metabolism of new 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 possible target analytes. Consequently, the metabolism of new SC needs to be known prior to updating analytical methods. In cases where no authentic sample material with confirmed uptake of the particular compound is available, postulated 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 metabolite identification.

3. Methodology
   - Rapid update of screening methods for the detection of synthetic cannabinoid use in human urine by software assisted metabolite identification
   - Workflow
     - 1. Posed human liver microsomes (pHLM) incubation
     - 2a. Metabolite prediction
     - 2b. LC-Q-ToF analysis
     - 3. Data evaluation
     - 4. Tentative identification of the main in vitro phase I metabolites
     - 5. Generation / update of TASQ method
     - 6. Analysis of routine case work samples
     - 7. Without glucuronidase treatment
     - 8. Reporting
     - 9. Further screening of the authentic sample
     - 10. Final TASQ method

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

5. 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 intake.