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Screening Method Development for 60 Synthetic Cannabinoids Including 317 Metabolites in Urine and Application on Authentic Cases

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1. Introduction

Synthetic cannabinoids (SC) still represent one of the largest groups of NPS. Changing consumption trends demand for frequent actualization of the analytical methods. Our aim was the development of an updatable LC-MS method for qualitative detection of SC and their metabolites in urine.

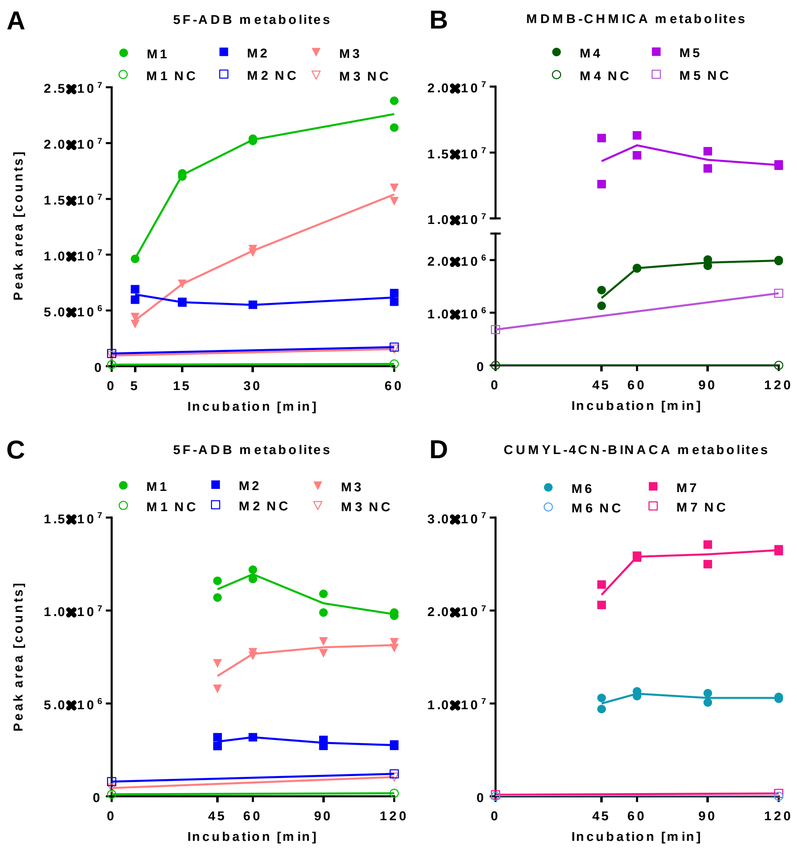

Fig.1: Synthetic cannabinoid metabolite peak areas (n=2) relative to the incubation time for the preliminary experiment covering 5-60 min incubation time for **A**) 5F-ADB-5-OH (M1), 5F-ADB-5-COOH (M2) and 5F-ADB-EH-loss of H (M3) and the experiment covering 45-120 min for **B**) 5F-ADB-5-OH (M1), 5F-ADB-5-COOH (M2) and 5F-ADB-EH-loss of H (M3); **C**) MDMB-CHMICA-OH (M4) and MDMB-CHMICA-EH (M5) and **D**) CUMYL-4CN-BINACA-4-COOH (M6) and CUMYL-4CN-BINACA-OH (M7) and the corresponding negative controls (NC).

4. Final sample preparation

Final sample preparation was defined as follows:

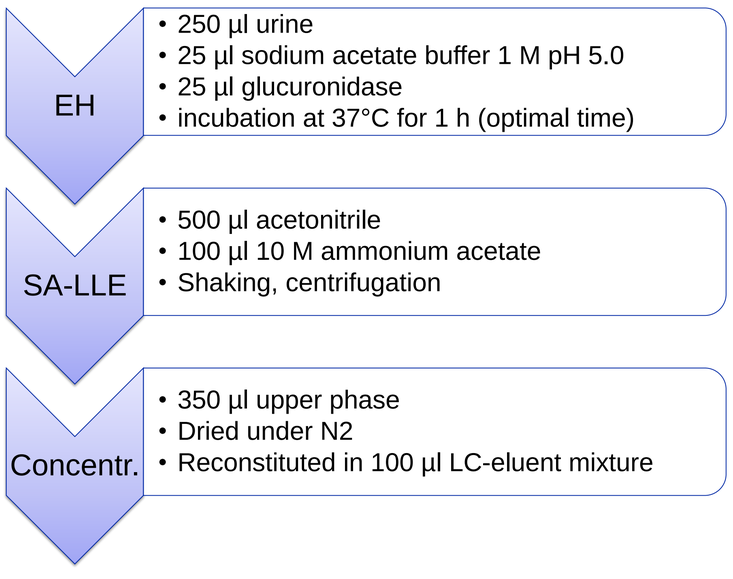

Fig.3: 250 µl urine was mixed with 25 µl of 1 M sodium acetate buffer pH 5.0, 25 µl glucuronidase, and 10 µl IS-mixture. After incubation at 37°C for 60 min (optimal incubation time), 500 µl acetonitrile and 100 µl 10 M ammonium acetate were added. 350 µl upper layer was dried and reconstituted in 100 µl eluent.

2. Method development

In vitro assays for metabolite production

- Human liver microsome assays were performed based on standard procedures for drug metabolism investigations [1].
- Analysis of the microsomal incubation mixtures was performed on a Sciex 6600 LC-QTOF System.

LC-MS/MS-EPI method development

- Based on the LC-QTOF measurements, a MRM table for a Sciex 5500 LC-Qtrap system containing relevant metabolite fragments was created.
- The assays were analysed in unscheduled MRM-EPI mode with 3 different collision energies (25, 35, 45 eV) on the LC-Qtrap system.
- For each analyte, a MRM with high sensitivity and its optimal collision energy was chosen. The MS2 EPI mass spectrum was added to an in-house library.

Optimization of enzymatic hydrolysis

- The optimal incubation time for enzymatic hydrolysis of the phase II metabolites was investigated using pooled authentic urine samples
- In a preliminary experiment, urine from three individuals containing 5F-ADB metabolites were pooled **(Fig.1A)**.
- In a second experiment, four authentic urine samples were pooled finally containing 5F-ADB metabolites, CUMYL-4CN-BINACA metabolites and MDMB-CHMICA metabolites. Incubation time was extended to 45 min, 60 min, 90 min and 120 min **(Fig.1B-D)**.

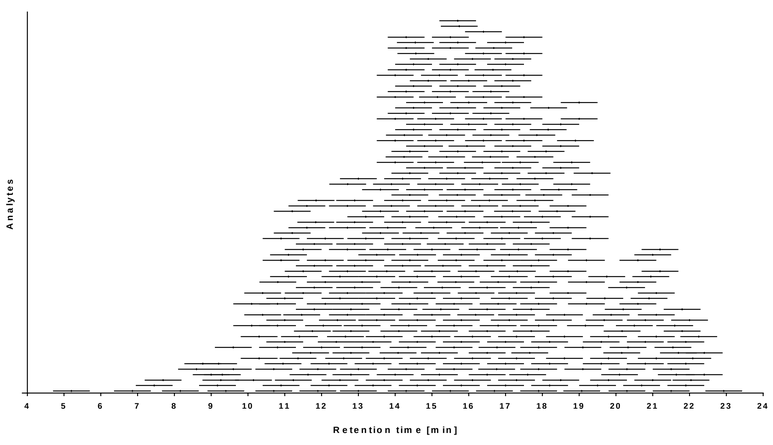

Fig.2: Schematic depiction of all MRM windows. Between 14 and 17 min a maximum of 61 MRM windows overlap.

5. Validation and Application

Method validation

- Ten blank authentic urine samples from different sources were analyzed for interfering peaks.
- Selectivity regarding other drugs was investigated injecting a mixture of drugs of abuse
- For prospective validation, authentic urine samples were analyzed and the results were compared to the results from the Institute of Forensic Medicine, Freiburg, Germany

Application on authentic cases

- Authentic urine samples from abstinence control cases that were submitted to the authors' laboratory were analyzed
- To date, metabolites of 5F-ADB, MDMB-CHMICA, AB-FUBINACA, AMB-FUBINACA, UR-144, XLR-11, MAM-2201 and 5F-Cumyl-P7AICA were identified in authentic cases
- An example of a positive case was depicted in **Fig.4.**

3. Final LC-MS/MS-EPI method

- In the final LC-MS/MS method, the MS operated in the scheduled MRM-IDA-EPI mode. For each analyte, a single transition was used with a retention time window of 60 s.
- Identification criteria were defined to include retention time ±0.2 min, signal-to-noise-ratio >3:1 and the (subjective) fit to the reference spectrum.
- For each synthetic cannabinoid at least two metabolites were included.
- To date, the method includes 444 MRM (73 Cannabinoids and 367 metabolites. Chromatographic separation is depicted in **Fig.2**.
- Currently a maximum of 61 MRM windows are overlapping. This leads to a minimal dwell time of 18 ms, which is not at the lower limit yet.

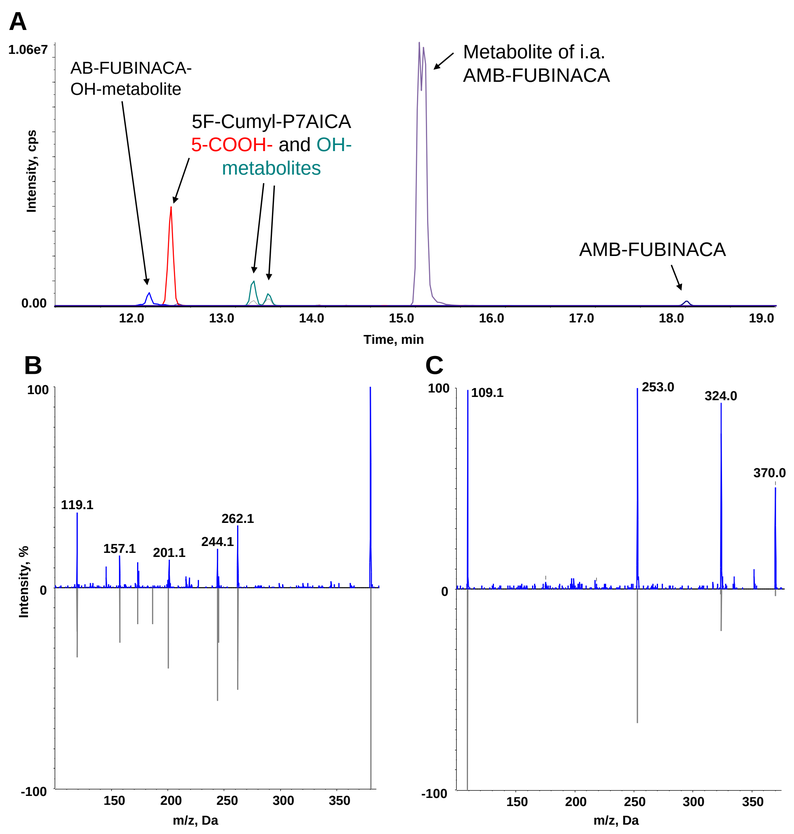

Fig.4: **A**) Chromatogram of an authentic sample containing metabolites of AB-FUBINACA, AMB-FUBINACA and 5F-Cumyl-P7AICA and the corresponding EPI mass spectra of **B**) 5f-Cumyl-P7AICA-5-COOH at 12.3 min and **C**) AB-/AMB/EMB-FUBINACA-COOH at 15.1 min. The sample spectrum in depicted in blue, the library spectrum in grey.

Conclusion

An LC-MS/MS-EPI method for the detection of numerous synthetic cannabinoid metabolites in urine was developed, optimized and successfully applied on authentic cases. Despite the rather high number of 444 MRM transitions, the method still can be updated with more cannabinoids in the future, as only 1 MRM per analyte is used.

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References

1. Peters FT, Meyer MR. In vitro approaches to studying the metabolism of new psychoactive compounds. Drug Test Anal 2011;3 (7-8):483-495. doi:10.1002/dta.295