Rapid detection of drugs and metabolites in urine by Flow Injection Analysis coupled to Magnetic Resonance Mass Spectrometry (MRMS)

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Introduction

Detection of metabolites and drugs in body fluids such as plasma or urine by LCMS is a routine method in metabolomics and doping analysis. Routine UPLC-MS measurements are performed typically in 15 min. Therefore, the number of analyzed samples is highly limited. In this work, a fast method for detection of drugs and their metabolites in urine using FIA-MRMS is presented. Roughly 250 samples can be measured in 24h using this technique.

Methods

Data acquisition:
- scimaX MRMS with 7 T superconducting magnet and new dynamically harmonized analyzer cell and 2 omega detection
- mass range m/z 107 – 3000
- ionization: ESI(+) and ESI(-)
- resolving power of 1,350,000 at m/z 200
- 28 single scans were averaged for the final mass spectrum

Mass calibration:
- external calibration with NaTFA cluster
- internal recalibration with several know metabolites in urine

Sample Introduction:
- FIA with a sample loop of 20uL using UPLC Elute HT. During the FIA experiment the flow was reduced to get constant signal for roughly 1.5 min.

Data processing:
- MetaboScape 4.0

Samples:
- 6 pooled urine samples purified by SPE using Merck LiChrolut EN SPE cartridges. Samples were extracted with MeOH and diluted 1:100 with MeOH for FIA-MRMS.
- 2 Blanks (SPE purified)
- Each sample was measured in 9 replicates

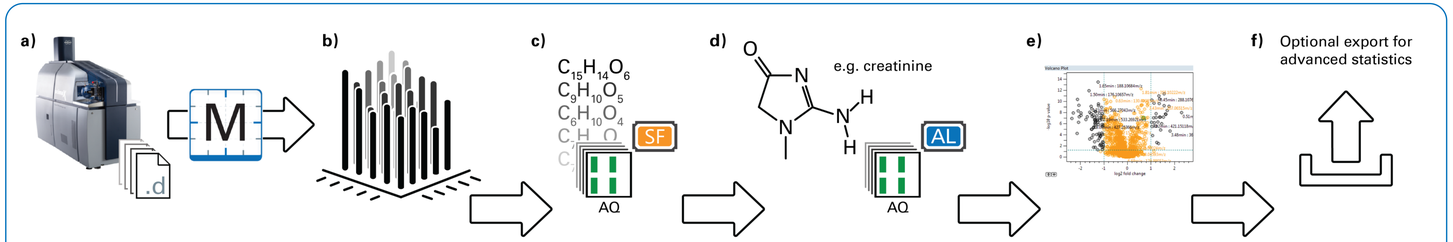

Fig. 1: Schematic (1) MRMS aXelerate workflow: a) FIA-MRMS acquisition using a scimaX MRMS, b) Data processing and evaluation using T-ReX 2D in MetaboScape 4.0, c) Generate list of molecular formula annotations including annotation qualities, d) Putative metabolite annotations using AnalyteList of known and expected compounds, e) Statistical analysis to identify features of interest, f) Optional export for advanced statistical analyses.

Results

The ESI(+) and ESI(-) data after extraction of the features in MetaboScape were combined for further analysis. More than 2100 features were found for the pooled urine samples. More than 90% of these could automatically be assigned with a molecular formula. 300 drug candidates were tentatively annotated in the urine samples using an AnalyteList derived from the HMDB urine database1 with a mass error tolerance of only 0.5 ppm. The workflow is shown in Fig. 1.

The detected drugs were compared with the medication of all patients. List of medication was known for all patients but without assignment to specific pooled samples. Based on medication of all patients several drugs have been found. Most of the drugs have been detected in one or a few pooled urine samples (Fig. 2a and b). Some specific drugs could be only found in positive or negative ion mode, respectively. By comparing the relative abundances of features across all samples, possible metabolites of drugs could be assigned (Fig. 3). As an example metabolites for Cefuroxime (an enteral second-generation cephalosporin antibiotic) could be detected only in the pooled urine sample 4. Several features had the same abundance pattern (here, only found in pooled sample 4) which could indicate that these compounds are metabolites of this antibiotic. The calculated molecular formula of these compounds agree with this assumption.

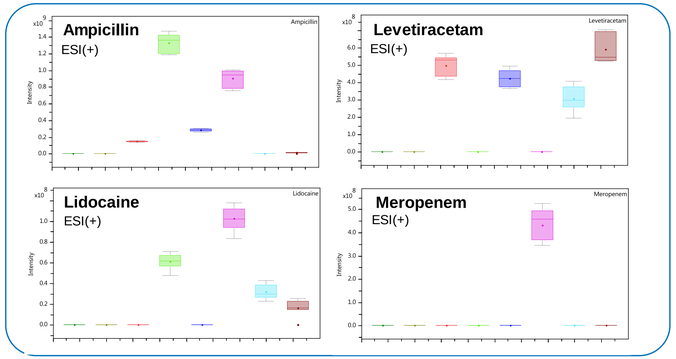

Fig. 2a: Bucket statistic (box plots) of tentatively annotated drugs in pooled urine samples using FIA-MRMS in positive ion mode.

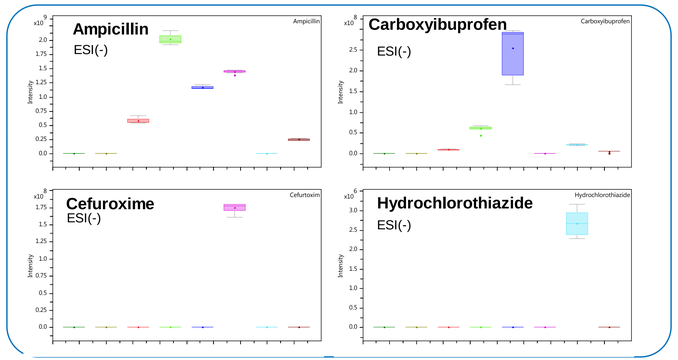

Fig. 2b: Bucket statistic (box plots) of tentatively annotated drugs in pooled urine samples using FIA-MRMS in negative ion mode.

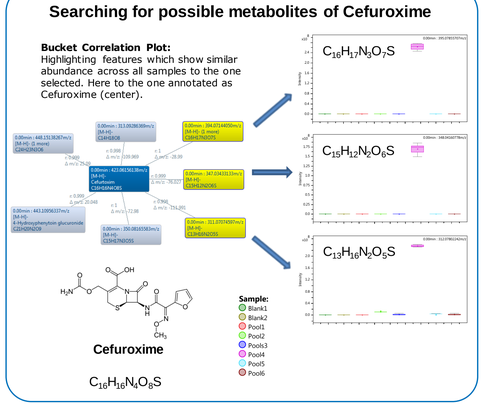

Fig. 3: Detection of possible metabolites of Cefuroxime in ESI(-) using Bucket Correlation Plot (left). Feature can be directly annotated with molecular formula due to very accurate mass detection.

Conclusions

- Drugs and their possible metabolites could be detected by FIA-MRMS very quickly.
- The presented FIA-MRMS screening workflow is much faster than a conventional workflow using UPLC-MS.
- The fast screening method could be used for quantification with the use of internal drug standards (^{13}C labeled compounds) in the sample.

MRMS Metabolomics