Comprehensive metabolite profiling of SHR0302 in human urine and feces using LC-HRMS and an advanced data processing workflow: Comparison with radiochromatographic analysis

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XenoFinder

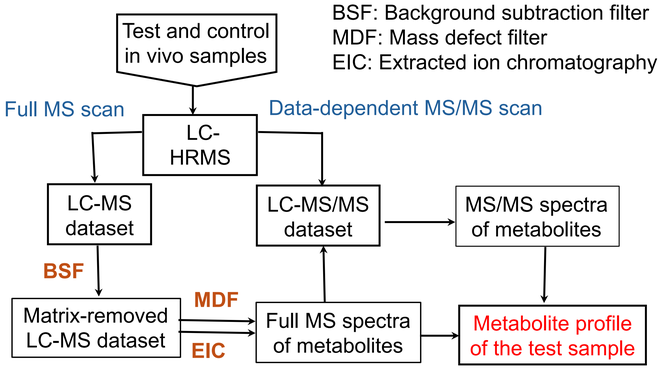
Introduction

- LC-HRMS is the single dominant analytical platform for drug metabolite profiling and identification (MetID). In the analysis, accurate LC-MS and LC-MS/MS datasets of a test sample and a control sample are required followed by targeted data processing methods such as mass defect filter (MDF) and extracted ion chromatography (EIC).
- In vivo metabolite profiling using commercially available MetID software tools often lead to many false positives or miss uncommon or low abundance metabolites due to interferences by huge amounts of endogenous biological components in urine or feces.
- The main objective of the study was to develop and apply an advanced LC-HRMS data processing workflow (Xeno-Discovery), which was based on sequential use an improved background subtraction filter (BSF) and MDF, for comprehensive profiling of low level of drug metabolites in human urine and feces. Results were compared with those generated by radiochromatographic analysis in a previously published radiolabeled ADME in human[1].

Experimental

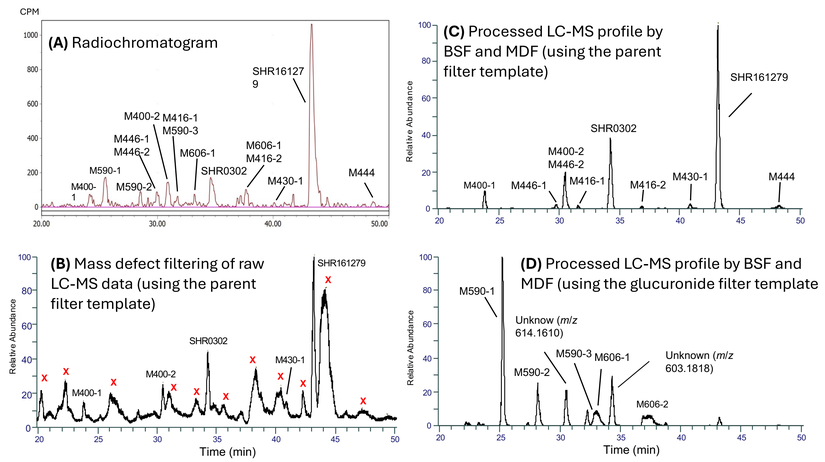
- LC-HRMS datasets of human urine and feces samples were obtained form a radiolabeled human ADME study (a single oral dose of 8 mg (80 μCi) [^{14}C]SHR0302, which has been previously published[1].
- Accurate LC-MS and LC-MS/MS data sets of a predose and pooled urine (0–24 h) and feces (0–72 h) samples were processed by the Xeno-Discovery workflow (Fig. 12 that consisted of mass defect filter (MDF) and in-house developed background subtraction filter (BSF). Structures of detected metabolites were characterized based on spectral interpretation and biotransformation knowledge.
- Metabolite profiles of the pooled urine and feces samples determined using the Xeno-Discovery workflow in this study were compared with the radiochromatograms of the same samples previously published[1]

Fig 1. Xeno-Discovery workflow developed for drug metabolite profiling of in vivo samples using LC-HRMS and sequential BSF and MDF data processing



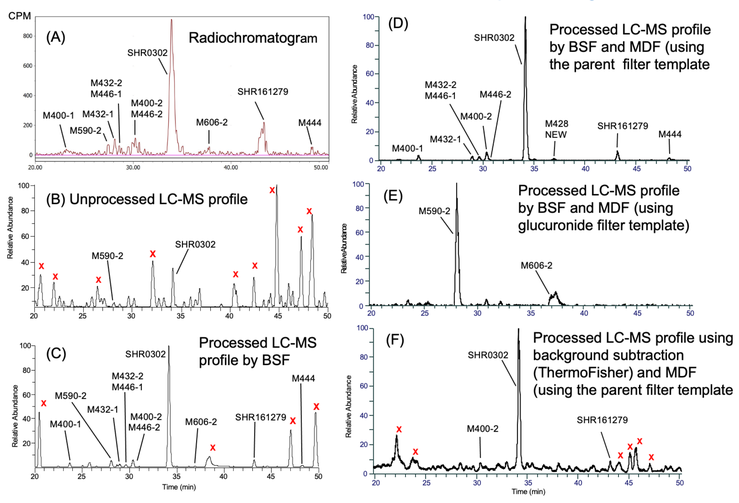
Results

Fig 2. Metabolite profiling of a pooled human urine sample (0-24 h) by LC-radiodetection and various LC-HRMS data processing methods



Xeno-Discovery processed LC-MS profiles (C, D) reveled all metabolites shown in the radiochromatogram (A) with minimal false positives, which were significantly better than MDF processing (B).

Fig 3. Metabolite profiling of a pooled human feces sample (0-72 h) by LC-radiodetection and LC-HRMS data processing methods



Xeno-Discovery processed LC-MS profiles (D, E) reveled all metabolites shown in the radiochromatogram (A) with minimal false positives, which were significantly better than using BSF along or a combination of background subtraction of Thermofisher and MDF (F).

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Fig 4. Extracted ion chromatograms for m/z 417.1452 of the fecal sample with or without data preprocessing by BSF

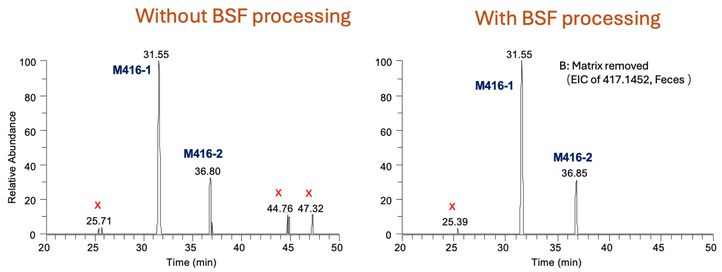
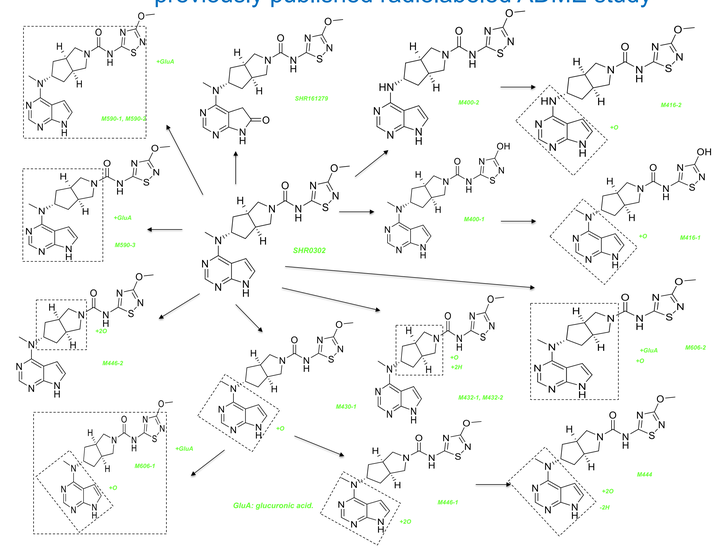


Fig 5. Metabolic pathways of SHR0302 in human determined in the previously published radiolabeled ADME study[1]



[1] Xinyu Ge, Sheng Ma et al. Mass balance study of [^{14}C]SHR0302, a selective and potent JAK1 inhibitor in humans. Xenobiotica (2023) 53:69 – 83

Conclusions

- An advanced LC-HRMS data processing workflow (Xeno-Discovery) that used a sequential BSF and MDF was developed for metabolite profiling of urine and feces samples.
- Results demonstrate that Xeno-Discovery significantly improved the sensitivity and selectivity of in vivo metabolite profiling, which were comparable to those from radiochromatographic analysis[1].
- A combination of LC-HRMS sample analysis and Xeno-Discovery data processing provided a very powerful tool in support of MIST evaluation, ADME studies of non-radiolabeled drugs in animals, and metabolite profiling of samples from Tox and pharmacological studies.