Metabolite profiling and characterization by ion mobility LC-timsTOF Pro PASEF

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Introduction

Rapid and accurate identification and characterization of drug metabolites play a critical role in preclinical and clinical development stages to assist lead compound structure optimization, screening drug candidates, and finding active or potentially toxic metabolites. In this work, a DDA non-targeted LC-timsTOF Pro PASEF (the parallel accumulation serial fragmentation) metabolomics workflow was conducted to profile and characterize drug metabolites (Figure 1). Metabolites were postulated by utilizing BioTransformer[1], a knowledge and machine learning based approach to predict small molecules metabolism. Metabolite structures were elucidated by *in silico* fragmentation, MS/MS spectral library, comparison of acquired to reference or predicted CCS values using a CCS prediction algorithm. Together, each of these steps forms a fully integrated workflow that utilizes the four-dimensional data to ensure low level drug metabolites can be annotated.

Methods

A time-series experiment was conducted by spiking pooled human liver microsomes (HLM, Sigma) and Canadine (TRC) into a pre-incubated NADPH regeneration system at 37^{0}C; 100 µL of reaction solution at 0, 5, 15, 30, 45, 60, 90 and 120 min was aliquoted; the reactions were stopped by adding cold acetonitrile; all samples were centrifuged at 12,000 rpm at 4^{0}C for 10 min; the supernatant was transferred into sample insert vial and 5 µL was injected (n=3) for each of the two biological replicates. Analysis was performed by LC-trapped ion mobility (TIMS) using an Elute UHPLC connected to a timsTOF Pro system (Bruker) with PASEF data acquisition and ESI positive mode. The resulting four-dimensional data (m/z, RT, mobility, and MS/MS) was processed using DataAnalysis 6.1 and MetaboScape 2023 (Bruker), where raw data was automatically recalibrated for mass and mobility.

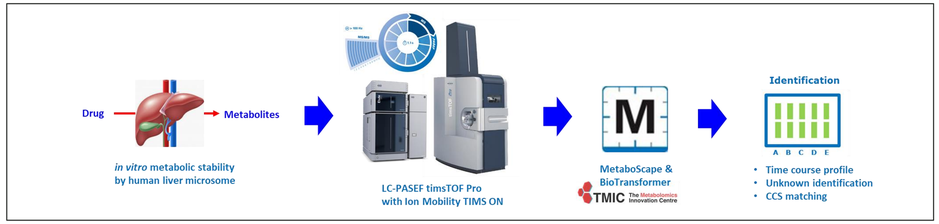

Figure 1. In vitro HLM-Drug metabolism by LC-timsTOF Pro PASEF

Results and Discussion

PASEF in timsTOF Pro provides very fast MS/MS acquisition speed at full sensitivity following ion mobility separation and enables very low abundant metabolites could be picked up for fragmentation. About 81 transformations of the predicted Canadine metabolites were listed in Figure 2 based on Cytochrome P450 Phase I biotransformation metabolism by BioTransformer.

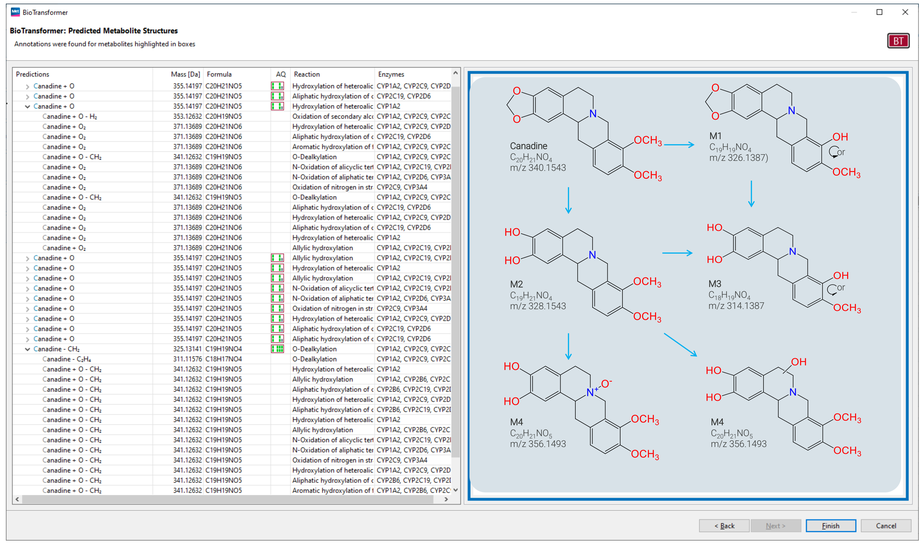

Figure 2. Canadine biotransformation and metabolic pathway in human liver microsomes

Peak finding of Canadine drug metabolism was performed in MetaboScape with the T-ReX ® 4D algorithm, which automatically extracts and aligns features based on mass accuracy, isotope pattern, MS/MS and CCS information for each feature enabled confident annotation by using SmartFormula, Analyte List, Spectral Library etc. Based on Canadine metabolic pathway, a "Target List" was generated to annotate the data which matches well with the Biotransformer annotation (see Figure 3). The extracted ion chromatogram confirms the O-demethylation Canadine M1 (m/z 326.1387) and demethylation Canadine M3 (m/z 314.1387), and the O,O'-demethyl Canadine metabolite M2 (m/z 328.1543) and oxidated Canadine M4 (m/z 356.1493) which end two isomers at different retention time. The extracted ion mobilogram displays different ion mobilities of Canadine and its metabolites where the oxidated Canadine M4 ends multiple isomers.

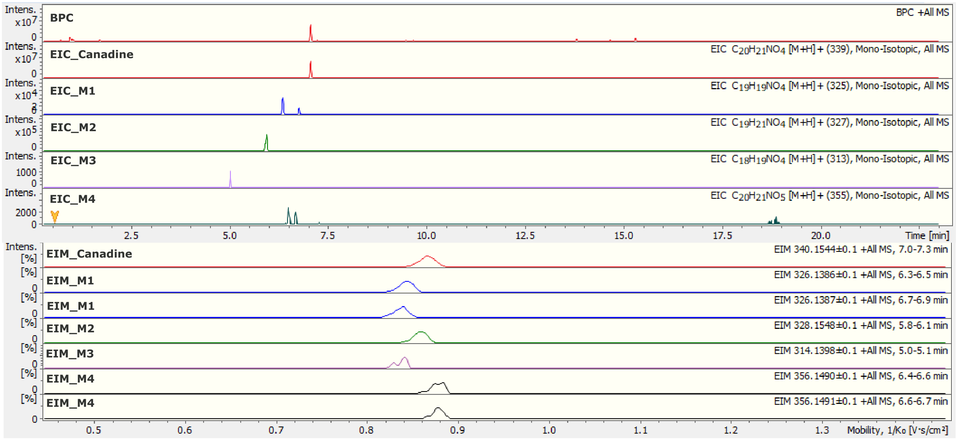

Figure 3. EIC and EIM of Canadine and its metabolites

References

[1] Djoumbou-Feunang et al.; J. Cheminform, 2019:11:2

Conclusion

- *in vitro* HLM drug metabolism sample analysis workflow by LC-timsTOF Pro PASEF was presented.
- Predicted metabolites could be annotated by BioTransformer together other tools in MetaboScape.
- Ion mobility data provides further confident in metabolite fast profiling and characterization.

LC-timsTOF for drug metabolism