CCS-enabled timsTOF Pro PASEF workflow for in vitro human liver microsome drug metabolites profiling and characterization

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Introduction

Fast 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 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 and comparison of acquired to reference or predicted CCS values using a novel 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 human liver microsomes (HLM, Promega) and fentanyl (Sigma) 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 Elute UHPLC timsTOF Pro (Bruker) with PASEF data acquisition and ESI positive mode. Data analysis was conducted in DataAnalysis 5.3 and MetaboScape 2022b (Bruker).

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

Results and discussions

The parallel accumulation serial fragmentation (PASEF) capability in timsTOF Pro provides very fast MS/MS acquisition speed at full sensitivity following ion mobility separation, which could detect very low abundant metabolites for MS/MS with data dependent acquisition. Figure 2 demonstrates significant increase in number of MS/MS acquired for sample analysis under LC-timsTOF PASEF (MS/MS).

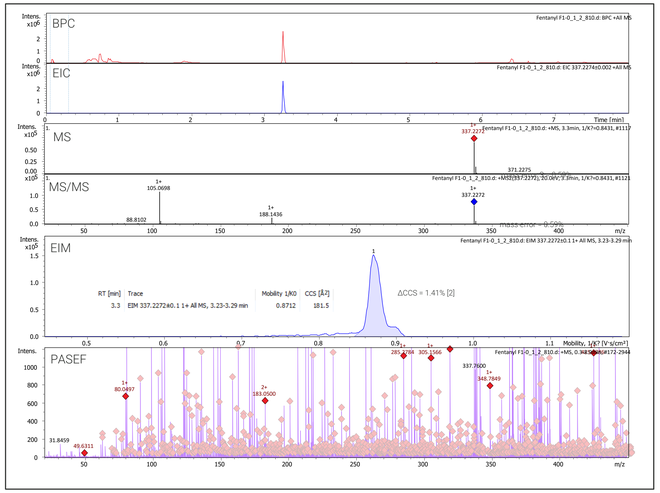

Figure 2. Fentanyl BPC, EIC, EIM, MS, PASEF (MS/MS) by LC-timsTOF Pro PASEF

Data analysis and peak picking were performed in MetaboScape with the T-ReX®4D algorithm applied for automatic feature extraction, RT alignment, mass and CCS calibration. Data was further evaluated using the BioTransformer tool to predict drug metabolites based on Cytochrome P450 Phase I biotransformation. All possible metabolites from enzymatic reactions of hydroxylation, terminal desaturation, N-dealkylation, N-oxidation and epoxidation were listed in Figure 3, and its metabolites were displayed in Figure 4 which were annotated based on mass accuracy, isotope pattern matching, and further confirmed by *in silico* MS/MS fragment and CCS predict Pro model (Figure 5).

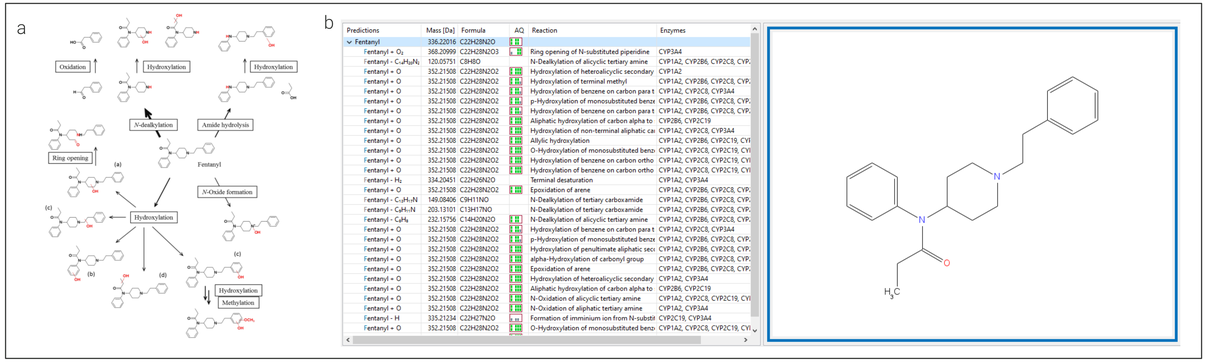

Figure 3. Fentanyl biotransformation (a) from reference [3] and (b) from BioTransformer in MetaboScape

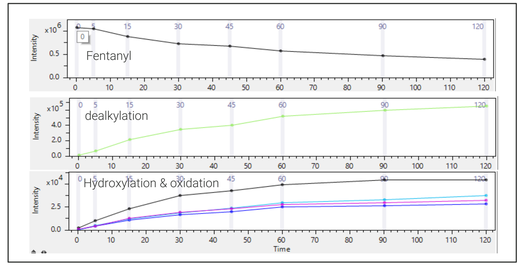

Figure 4. time profile of fentanyl and its metabolites

References

1) Djoumbou-Feunang et al.; Journal of Cheminform, 2019:11:2
2) https://pubchem.ncbi.nlm.nih.gov/compound/3345#section=Dissociation-Constants
3) Wilde M., et al.; Frontier in Pharmacology, 2019, 238 (10): 1-16

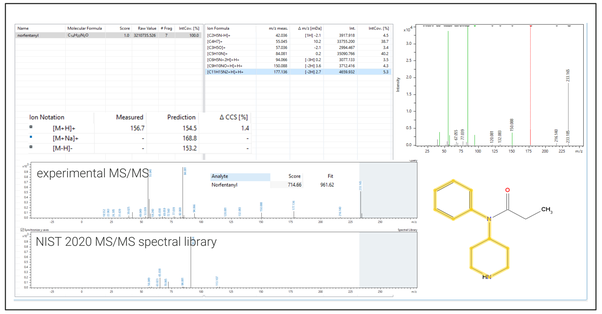

Figure 5. Fentanyl metabolite confirmation by spectral library, *in silico* fragmentation and CCS prediction

Summary

- *In vitro* HLM/fentanyl drug metabolism analysis by TIMS enabled timsTOF Pro PASEF metabolomics workflow
- Data Analysis was performed in MetaboScape 2022b on metabolite profiling and characterization
- Integrated software addresses common needs for advancing pharma, metabolomics, lipidomics, non-targeted screening and exposome research

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