CCS-enabled drug metabolite profiling and characterization workflow for distinguishing drug metabolites

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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-PASEF non-targeted LC-timsTOF Pro metabolomics workflow was conducted to profile and characterize drug metabolites. 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 (Promega) and drugs of codeine, MDMA, fentanyl and tramadol (Sigma) into a pre-incubated NADPH regeneration system at 37^{0}C, and aliquoting 100 µL of reaction solution at 0, 5, 15, 30, 45, 60, 90 and 120 min. 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 insert sample 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 2022 (Bruker).

Results and Discussions

Fast PASEF MS/MS data acquisition

The parallel accumulation serial fragmentation (PASEF) capability in timsTOF Pro provides very fast MS/MS acquisition speed at full sensitivity following ion mobility separation to deeply profile low abundant metabolites using data dependent acquisition. Figure 1 demonstrates significant increase in number of MS/MS acquired for sample analysis under LC-timsTOF PASEF MS/MS experimental conditions.

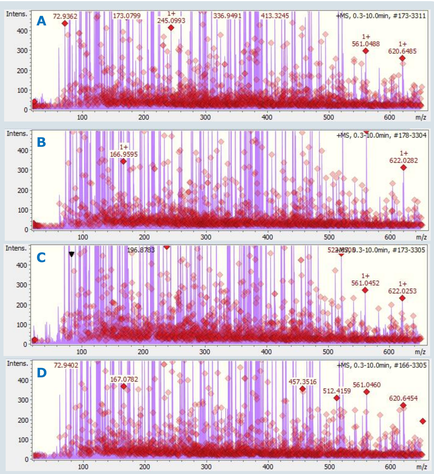

Figure 1. PASEF MS/MS data acquisition
A – Codeine, B – MDMA, C – Fentanyl, D – Tramadol
Zoom into an averaged MS spectrum along entire LC run with selected precursors highlighted in diamond. TIMS ON

EIC, EIM and CCS

The extracted ion chromatogram (EIC) and extracted ion mobilogram (EIM) of drugs and its measured CCS were processed and displayed in Figure 2 and Table 1.

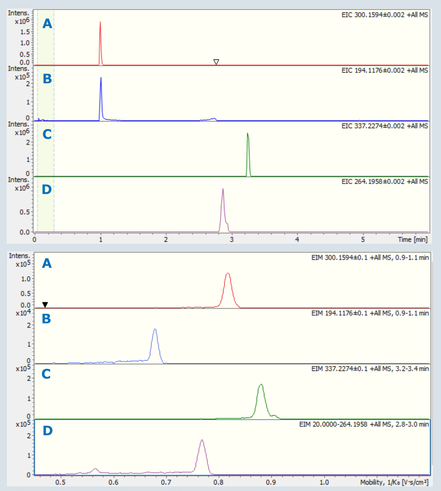

Figure 2. EIC and EIM of drugs
A – Codeine, B – MDMA, C – Fentanyl, D – Tramadol

Name	RT (min)	Trace [M+H]+	Mobility, 1/K0	Measured CCS [Å^2]	CCS [M+H]$^+$, PubChem (DT)	ΔCCS (%) vs. PubChem (DT)	CCS by CCS-Predict Pro	ΔCCS (%) vs. CCS-Predict Pro
Codeine	1.0	EIM 300.1594±0.11 + All MS	0.819	171.4	168.5	-1.71	170.2	-0.71
MAMA	1.0	EIM 194.1176±0.11 + All MS	0.680	144.7	145.8	0.73	143.2	-1.05
Fantanyl	3.3	EIM 337.2274±0.11 + All MS	0.882	183.8	184.1	0.16	184.6	0.43
Tramadol	2.9	EIM 264.1958±0.11 + All MS	0.769	168.5	160.4	-5.05	162.6	0.90

Table 1. Results of drugs' CCS

Data processing in MetaboScape and BioTransformer

Data analysis and peak findings of the investigated drugs and their metabolites were performed in MetaboScape with the T-ReX®4D algorithm applied for automatic feature extraction and alignment. The ion mobility (1/K0) and collisional cross sections (CCS) of each feature were calculated and listed in the generated feature table. 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, N-dealkylation, N-oxidation, O-dealkylation, O-Aryl demethylation and epoxidation (Figure 3). Generated chemical structures for the metabolites with different metabolism locations enabled their assignment in the acquired raw data. The postulated metabolites were verified based on mass accuracy, isotope pattern matching, and further confirmed by *in-silico* MS/MS fragment matching as well as comparison to predicted (using the CCS-Predict Pro model) and reference CCS values (Table 1).

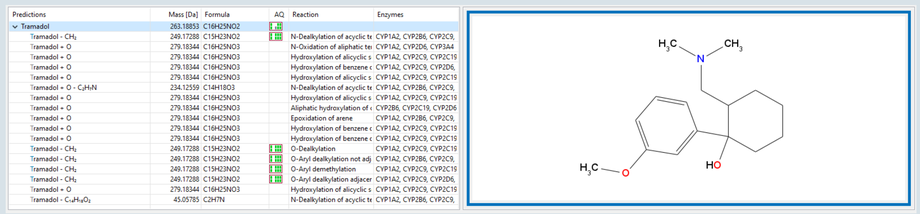

Figure 3. Tramadol drug metabolites predicted by BioTransfomer in MetaboScape

Drug metabolites profiling and characterization

The time course of the formation of Tramadol metabolites was used as example for drug profiling and structure characterization. Tramadol contains aromatic methoxy group and an aliphatic dimethyl aminomethyl group, undergoes hepatic metabolism via CYP 2D6 and CYP 3A4 and generates major active metabolite O-desmethylation tramadol (M1) and N-desmethylation tramadol (nortramadol, M2), respectively (Figure 4), which were annotated and displayed in Figure 5 to visualize the changes in abundance with respect to time. The time course visualization feature not only allows to assess drug metabolic stability at a single time point, but also to determine changes like intrinsic clearance over time.

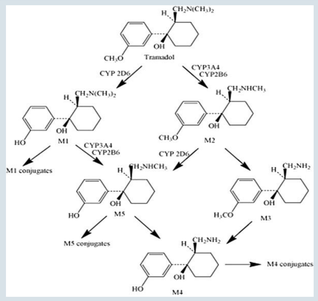

Figure 4. Metabolic pathway of Tramadol [2]

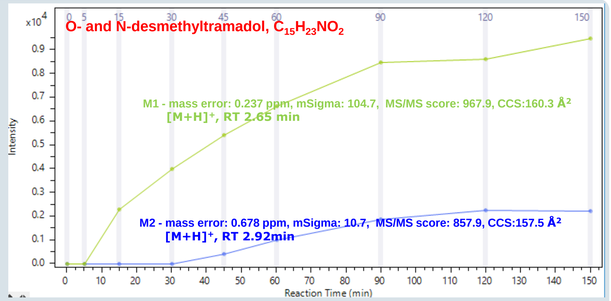

Figure 5. Tramadol metabolites peak intensity vs. incubation time

These two metabolites O- and N- demethylation tramadol show excellent mass accuracy, isotopic pattern and MS/MS score. The O-and N-demethylation metabolites [M+H]$^+$ have measured CCS of 160.3 Å^2 and 157.5 Å^2, respectively or 1.1% and -0.6% ΔCCS error when comparing to predicted CCS values from MetaboScape 2022. Further MS/MS spectral library annotation and *in-silico* MetFrag verification confirmed the two metabolites (Figure 6).

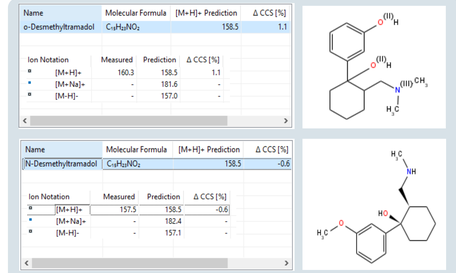

Figure 6. Comparison of measured CCS vs. predicted CCS for O- and N-desmethyltramadol metabolites

In summary, MetaboScape 2022 enabled CCS-enabled drug metabolite profiling and identification of LC-PASEF data acquired on a timsTOF Pro for this proof of concept in vitro human liver microsomal incubation experiment. Additionally, MetaboScape 2022 allows for investigating the semi-quantitative response in time course studies, and the prediction of drug metabolites for targeted or non-targeted drug discovery.

References

(1) Djoumbou-Feunang *et al.*; Journal of Cheminform, 2019:11:2

(2) Rouini M. *et al.*; DARU Journal of Pharmaceutical Sciences, 2013, 21:17.

Conclusions

- Local metabolite prediction by BioTransformer enabled secure drug metabolite annotation in MetaboScape®
- Multifactorial time-course experiments in MetaboScape supports semi-quantitative description of metabolic pathways in drug metabolism for pharma research
- **CCS-Predict Pro** allows automatic small molecule CCS prediction with assigned structure (InChI)
- Integrated software addresses common needs for advancing pharma, metabolomics, lipidomics, non-target screening and exposome research

PASEF and MetaboScape for Drug Metabolism

*BioTransformer is not a Bruker product and restrictions to utilize this software tool might apply or might change without notice from Bruker, please refer to the BioTransformer page for details - https://bitbucket.org/djoumbou/biotransformer)