Drug metabolite analysis with Trapped Ion Mobility Mass Spectrometry and Mass-MetaSite high throughput data analysis

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Introduction

An important aspect of metabolomics includes metabolic profiling studies wherein metabolites of the drug product are identified and quantified. This data is important to gain a thorough understanding of the routes of elimination, drug metabolizing enzyme pathways, drug-drug interactions and any safety or toxicity concerns in humans.[1]

Here, we describe a workflow for metabolites identification of the drug Verapamil using Trapped Ion Mobility Spectrometry (TIMS) and Mass-MetaSite (Molecular Discovery) for software processing.

Trapped Ion Mobility Spectrometry primarily is a separation technique in gas phase, which resolves sample complexity with an added dimension of separation independently and in addition to HPLC and mass spectrometry, increasing peak capacity and confidence in compound characterization.

Summary

The use of Trapped Ion Mobility Spectrometry (TIMS) and Mass-MetaSite data processing and analysis for metabolite identification allows for increased speed and confidence the result

References

(1) Drug Bank online: https://go.drugbank.com/drugs/DB00661

Methods

Verapamil (TRC, Sigma) was spiked into a pre-incubated NADPH regeneration system in two biological replicates, incubated at 37 ^{0}C for 0, 5, 15, 30, 45, 60, 90 and 120 min. The reactions were stopped by adding cold acetonitrile. Samples were centrifuged at 15,000 rpm, 4 ^{0}C for 10 min and 0.2 µm filtered. 100 µL of supernatant was transferred into insert vials and 5 µL was injected (n=3).

Metabolites were separated on a reversed-phase column (Bruker Intensity Solo 1.8 C18-2, 2.1 x 100mm), and data were acquired on a timsTOF Pro 2 (Bruker Daltonics) with Trapped Ion Mobility (TIMS) and Parallel Accumulation Serial Fragmentation (PASEF) data acquisition in ESI positive mode.

Automatically data processing in Mass-MetaSite 4.1 was performed followed by automatically loading into Oniro/WebMetabase 1.2 for collective data interpretation.

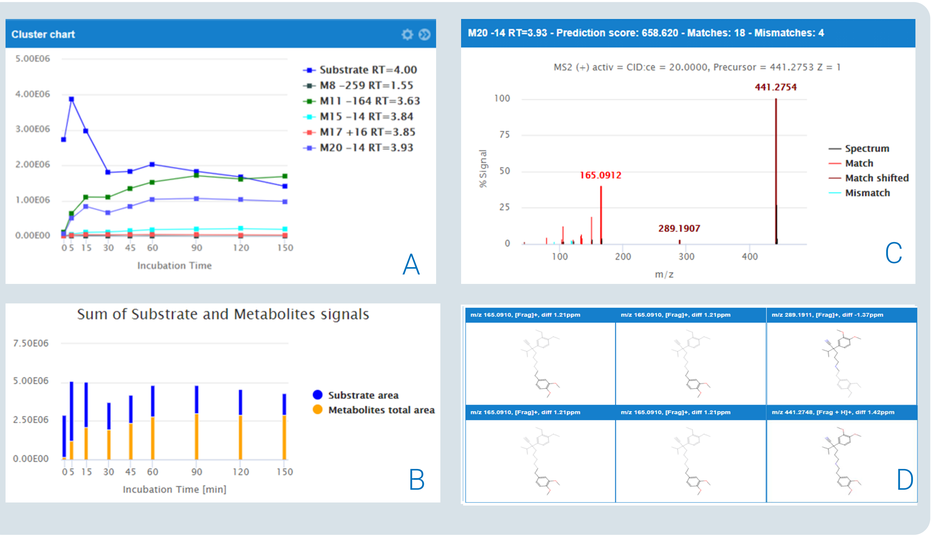

Fig. 1: Mass-MetaSite data processing results: A-B kinetic plots; C-D MS/MS data interpretation

Results

- Isomers of Verapamil are separated by Trapped Ion Mobility Mass Spectrometry (TIMS) (Fig 2)

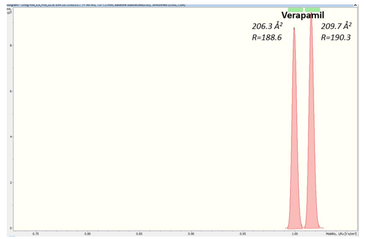

Fig. 2 Ion mobility separation of Verapamil isomers with timsTOF Pro2

- Mass-MetaSite data processing allows to identify rapidly the specific spots within the drug molecule which are labile for metabolic transformations. The soft spot in Verapamil is related to the reaction over the methyl amine as it is found in the kinetic analysis (Figure 1 A&B).
- There were 4 main metabolites identified at all conditions; 1 N-demethylation, 1 O-dealkylation, 1 N-Dealkylation and a hydroxylation which are in line with metabolites reported in literature [1].
- All found metabolites show a kinetic behaviour compatible with a metabolic transformation (Fig 1 A&B).
- The Mass-MetaSite fragmentation analysis of the obtained MSMS spectra data gave an accurate prediction of the structure of the metabolites found. (Fig 1 C&D) Usage of ion mobility allowed for faster and increased confidence in the data interpretation due to cleaner MS/MS spectra
- TIMS can be used to derive collisional cross sections (CCS) values for additional confidence. Comparison of the experimental CCS values of the found metabolites with the predicted CCS values showed an excellent correlation within 2%. (Table 1)

Compound	Exp. CCS (Å²)	Pred. CCS (Å²)	Δ(%)
Verapamil	209.7	209.4	0.1
1 N-demethylation	205.5	206.0	0.2
1 O-dealkylation	203.2	05.8	1.0
1 N-Dealkylation	173.7	170.8	1.7
Hydroxylation	217.9	214.4	1.6

Table 1: Comparison of exp. CCS values vs predicted

- The obtained data are easily reported in customized reports which allows easy data review and study approval.

Conclusions

- The use of Trapped Ion Mobility Spectrometry (TIMS) and Mass-MetaSite data processing and analysis allows for increased speed in data processing
- TIMS allows for more confidence due to cleaner spectra, better reliable data interpretation and separation of possible (metabolic) isomers present
- Mass-MetaSite data processing and analysis provides a comprehensive tool for fast and reliable metabolite identification with easy reporting.

timsTOF Pro 2