Crossing the Chasm: One integrated solution for advancing LC-PASEF based pharma, metabolomics, non-target screening and exposome research

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Introduction

Pharma, Metabolomics, Phenomics, Exposomics and non-target screening have different aims. But with that said, all these disciplines share some common needs, like rapid de-replication of compounds of interest (drug metabolites, possible live style or environmental exposure markers). Also, ID of known compounds and identification of unknown targets are another shared need. Here we will highlight a workflow, designed for non-targeted Metabolomics and Lipidomics, featuring options such as raw data extraction, statistics, automatic assignment of known compounds and pathway mapping. This workflow was extended with additional capabilities to cross the chasm for common needs in different application areas: Support for evaluating time series experiments and prediction of metabolites based on known precursor structures by a local and hence secure BioTransformer [1]*/** instance.

Methods

Two time-series experiments were conducted (a) human liver microsomes (Promega) and drugs (TRC, Sigma) were spiked into a pre-incubated NADPH regeneration system, incubated at 37 ^{0}C for 0, 5, 15, 30, 45, 60, 90 and 120 min, and the reactions were stopped by adding cold acetonitrile; (b) spent media samples (kindly provided by Just Biotherapeutics) were collected at day of 0, 6, 14 and 20 from three different cell culture media conditions. All above samples were centrifuged at 15,000 rpm for 10 min and 0.2µm filtered. 100 µL of supernatant was transferred into vial and 5µL was injected (n=5). Analysis was performed by Elute UHPLC - timsTOF Pro (Bruker) with PASEF data acquisition in ESI positive mode. Data analysis was conducted in a pre-release Version of MetaboScape® 2021 (Bruker).

Results and Discussions

Data acquisition - PASEF MS/MS speed

The parallel accumulation serial fragmentation (PASEF) capability in timsTOF Pro provides extra fast MS/MS acquisition speed at full sensitivity which makes it possibility to deeply perform unknown compounds profiling and identification. Figure 1 demonstrates significant increase in number of MS/MS acquired between PASEF MS/MS and regular autoMSMS modes using the same LC-MS experimental conditions for a spend media sample.

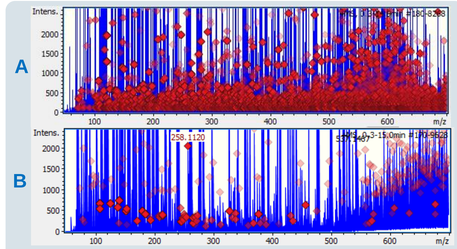

Figure 1. MS/MS acquisition speed (A – PASEF, B – autoMSMS)
Zoom into an averaged MS spectrum along entire LC run with selected precursors highlighted in red.

Processing - MetaboScape and BioTransformer

Peak finding of drug metabolism and spend media analysis data were performed in MetaboScape with the T-ReX ® 4D algorithm, which automatically extracts and aligns features in timsTOF Pro LC-PASEF data. The available mass accuracy, isotope pattern, MS/MS and CCS information for each feature enabled confident annotation by using SmartFormula, Analyte List, Spectral Library and BioTransformer [1]*/** tools.

(a) Human liver microsomal stability analysis

The open source BioTransformer tool was used to predict drug metabolites. From within MetaboScape a local BioTransformer instance was triggered for predicting Cytochrome P450 Phase I biotransformation metabolism of Chlorpromazine (Figure 2).

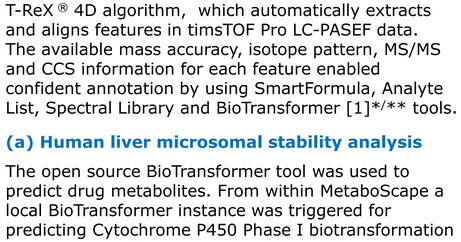

Figure 2. Chlorpromazine metabolites predicted by BioTransformer and visualized in MetaboScape

Two major metabolites were annotated (see Table 1) by MetaboScape based on the BioTransformer predictions as Chlorpromazine + O ($C_{17}H_{19}ClN_2SO$, m/z 335.0979). The metabolites show similar retention time at 2.03 min and 2.08 min. Chlorpromazine $C_{17}H_{19}ClN_2S$, m/z 319.1030) was mediated by CYPs 2D6, 1A2 and 3A4. According to literature these CYPs likely generate the Chlorpromazine +O metabolites 3 or 7-hydroxylation, N-oxidation or sulfoxidation. The extracted ion mobilograms (Figure 3) substantiate the assignment as two isobaric compounds as both have different CCS values of 171.1 and 176.5, respectively.

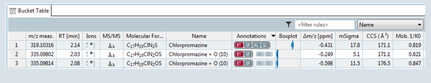

Table 1. Chlorpromazine bucket table results

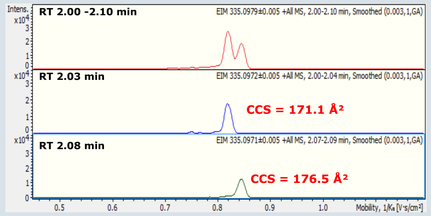

Figure 3. Extracted ion mobility of Chlorpromazine + O
Top - for the whole RT range for both close eluting compounds; Middle - for the LC peak with RT 2.03 min; Bottom - for the LC peak with RT 2.08 min

Spectral library matching (Bruker MetaboBASE® Personal Library 3.0) for the Chlorpromazine + O metabolite at RT 2.03 min returned high MS/MS scores for the hydroxy- and sulfoxide forms. The low MS/MS library score for the N-oxide indicated that this is an unlikely structure candidate for this early eluting compound. *In-silico* fragmentation by MetFrag [2] in MetaboScape assigned no fragment for the N-oxide metabolite. For this reason the N-oxide was excluded as structure candidate. Comparison of the measured CCS values for the peak at 2.03 min to predicted CCS values [3], enabled tentative assignment as Chlorpromazine sulfoxide: ΔCCS error of 1.44% for Chlorpromazine sulfoxide compared to 1.84% for hydroxychlorpromazne (1.84%).

For the peak at RT 2.08 min, the MS/MS spectral library score for N-oxide Chlorpromazine was significantly lower compared to hydroxychlorpromazine indicated the N-oxide to be an unlikely structure candidate. The peak at RT 2.08 min was tentatively assigned as 7-hydroxychlorpromazine as it matched to four MS/MS characteristic fragment ions (Figure 4).

Figure 4. *In-silico* MetFrag fragmentation for the metabolite at RT 2.08 min supports tentative ID as 7-hydroxychlorpromazine

The time course of the formation of Chlorpromazine sulfoxide and 7-hydroxychlorpromazin metabolites are displayed in Figure 5 to visualize the changes in abundance with respect to time. This new time course visualization feature in MetaboScape 2021 not only allows to assess drug metabolic stability at a single time point, but also to determine changes like intrinsic clearance over time.

In summary, MetaboScape 2021 enabled CCS-Aware drug metabolite profiling and identification of LC-PASEF LC-timsTOF Pro data for this proof of concept vitro human liver microsomal incubation experiment. Additionally, MetaboScape 2021 allows for investigating the quantitative response in time course studies for targeted or non-targeted drug discovery.

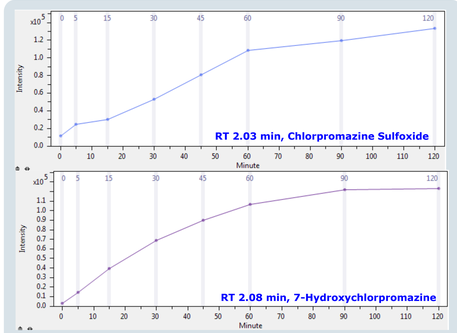

Figure 5. Metabolites peak intensity vs. incubation time

(b) Cell culture spent media analysis

There is an increasing need to profile and identify the depletion of nutritional components and the formation of new metabolites in media during cell culture for better cell growth, quality and final protein yield. This demand is addressed with the new feature for evaluating multiparametric time course experiments in MetaboScape. It enables to readily monitor changes along time courses and compare differences between culture media for automatically identified known cell culture nutrients including amino acids, hormones, growth factors, enzyme inhibitors, vitamins, antibiotics, nucleotides and lipids which are essentially for the cell to synthesize protein.

In this study we observed that most of the amino acids decreased with increasing incubation time which indicates that these are essential nutrients in protein synthesis. Figure 6 highlights the changes of histidine, serine, guanine and adenine in three different spent cell culture media from 0 to 20 days. Each amino acid shows different depletion rates for the different spend media. Both guanine and adenine could not be detected in days 6, 14 and 20, indicating that higher amounts for these compounds might be beneficial in the starting media for higher yield. In general, these time course differences could serve as a useful parameter for correlation with production quality control and quantity.

The profile of metabolites increased during the cell culture time course (Figure 7) can also serve for monitoring product quality control, and for optimizing cell culture conditions for high product yield.

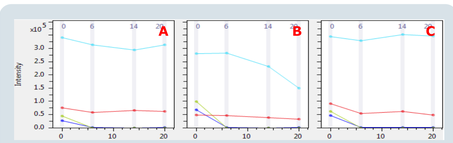

Figure 6. Change of amino acids level during cell culture
A – spent media - 23; B – spent media – 40; C – spent media – 10
Lines – Histidine (light blue); Serine (red); Guanine (green) and Adenine (blue)

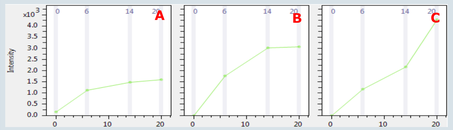

Figure 7. metabolite formation in different spent media during cell culture
A – spent media - 23; B – spent media – 40; C – spent media – 10;
Cytidine monophosphate (green)

References

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

(2) Wolf *et al.*; BMC Bioinformatics 2010, 201011:148. doi: 10.1186/1471-2105-11-148

(3) http://faculty.washington.edu/libinxu/2020/01/08/ccsbase/ and http://allccs.zhulab.cn/prediction/

Conclusions

- Local metabolite prediction by BioTransformer enabled secure drug metabolite annotation in MetaboScape®
- Support for multifactorial time-course experiments in MetaboScape supports semi-quantitative description of metabolic pathways in drug metabolism and spend media analysis for (Bio)pharma research
- Integrated software addresses common needs for advancing pharma, metabolomics, lipidomics, non-target screening and exposome research

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)