Comprehensive metabolite characterization using orthogonal MS/MS data

Eva Duchoslav; Disha Thakkar; Yves Le Blanc; Jason Causon
SCIEX, Concord, Canada

ABSTRACT

This presentation reports on the utility of "orthogonal" MS/MS fragmentations of complementary TOF MS precursors for a more comprehensive characterization of metabolites. Based on the LC/MS data from the extracts of model drugs incubated in rat hepatocytes that covered two fragmentation mechanisms, we found that unique peaks that pinpoint the structure details could be found in either data set. Integration of the complementary MS/MS information aided in confident structure characterization of studied drug metabolites.

INTRODUCTION

Qualitative applications of high-resolution mass spectrometry (HRMS), such as automated LC-MS/MS workflows using collision induced dissociation (CID), have been crucial for pharmaceutical drug development to investigate the metabolism of candidate modalities at the early stages of the development.

Recent HRMS technology advancements, including improvements in the duty cycle, enabled the application of the electron activated dissociation (EAD) on LC timescales and the integration of this complementary MS/MS fragmentation mechanism into LC-MS/MS workflows, providing a more confident characterization of the compounds of interest.

MATERIALS AND METHODS

Sample preparation:
Verapamil, buspirone and nefazodone were incubated in rat hepatocytes at a 1 µM starting concentration. Samples were removed from incubation and quenched with acetonitrile at 0-, 30- and 120-minute time points.

HPLC conditions:
LC separation was performed on a Phenomenex Luna Omega Polar C18, 150 mm column, using a 5 µL injection volume. Gradient separation using 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) was performed over 4.75 minutes, from 5%B to 95%B with a total runtime of 6.5 minutes.

MS/MS conditions:
The samples were analyzed in the information-dependent mode using Zeno CID IDA and Zeno EAD IDA on a ZenoTOF 7600 system.

Survey TOF MS experiment with the accumulation time of 50ms and the mass range from 100 to 1000 was followed by 10 dependent TOF MS/MS experiments; the TOF MS/MS precursors were selected after a dynamic background subtraction and, optionally, a mass defect filter were applied to the survey TOF MS scan. For the CID MS/MS data collection, the accumulation time was 20ms, the mass range 40 to 1000 and the collision energy 45±15eV; the EAD data were acquired with the accumulation time of 90ms, the mass range from 60 to 1000 and the electron kinetic energy of 10eV.

Data processing:
LC HRMS data were processed with a prototype version of Molecule Profiler software. This tool provided determination of the precursor ion types, grouping the TOF MS features, tailored CID and EAD MS/MS processing, as well as the integration of and interaction with multiple annotated MS/MS spectra for each metabolite.

Name	*IDA CID MS/MS coverage*	*IDA EAD MS/MS coverage*	*phase I oxidative metabolites*	*cleavage metabolites*	*glucuronide conjugates*
Buspirone	91%	100%	7	2	
Nefazodone	83%	92%	11	3	
Verapamil	94%	92%	5	5	5

Table 1. Summary of studied model drugs and their metabolites

RESULTS AND DISCUSSION

The IDA data provided excellent MS/MS coverage for the TOF MS peaks of interest in both CID and EAD runs; on average, 92% of predicted and unexpected metabolites had the MS/MS data collected within the same IDA run.

As the TOF MS peaks were detected within the Molecule Profiler software, their charge agents (ion types) were determined as well. This allowed grouping of multiple TOF MS features for a compound and collation of their MS/MS spectra (Figure 1).

Within the qualitative processing in Molecule Profiler software, the peaks in the MS/MS spectrum of the parent drug are fully annotated with respect to the subparts of the parent drug structure; this annotation is used in the proposal and ranking of structures of candidate metabolites[1]. Alternately, for the metabolites that have structures sufficiently different from the parent drug or were obtained by different fragmentation mechanism and thus yield significantly different fragmentation fingerprints, the annotation of fragments with respect to a hypothetical structure is used without relying on the annotation of the MS/MS spectrum of parent drug[2].

The CID and EAD MS/MS spectra were found to contain different features; therefore, different settings were required for each fragmentation type to successfully propose and rank potential metabolite structures. For example, the EAD spectra annotation used a larger fragment set and, also, it was more open to the appearance of radical fragments.

Reconciling the evidence for a site of modification from the Zeno CID and Zeno EAD MS/MS data reduced the ambiguity in the structure proposal and enabled confident elucidation of positional isomers (Figure 2). We found that for the glucuronide conjugates, the EAD data provided more specific MS/MS fragments than CID and thus was instrumental in correctly pinpointing the site of modification. With the improvement of TOF MS/MS sensitivity due to Zeno trap activation, we found that the low abundance, accurate MS/MS fragments of $[M+Na]^+$ could be used to confirm and complement the evidence supporting the structure characterization of drug metabolites (not shown).

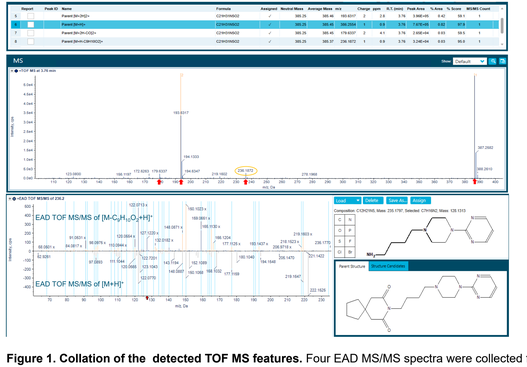


Figure 1. Collation of the detected TOF MS features. Four EAD MS/MS spectra were collected for different precursor peaks (red arrows) corresponding to Buspirone. The MS/MS of internal fragment $[M-C9H10O2+H]^+$ reveals new fragments, such as m/z 127.12, that can aid in the annotation of respective cleavage metabolites.

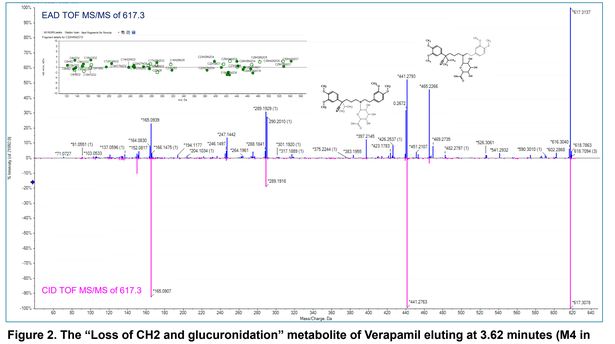


Figure 2. The "Loss of CH2 and glucuronidation" metabolite of Verapamil eluting at 3.62 minutes (M4 in Figure 3). The alignment of CID and EAD MS/MS spectra highlights the additional fragment peaks that often provide definite diagnostic information regarding the structure of investigated compound, such as fragments 397 and 465 that support the N-glucuronide conjugation. EAD TOF MS/MS fragment mass accuracy was within 2mDa.

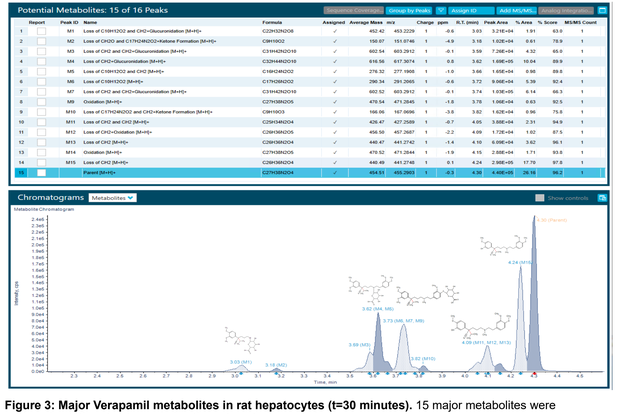
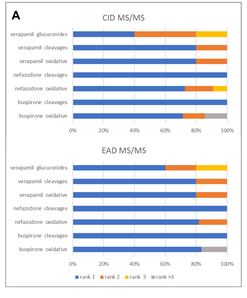


Figure 3: Major Verapamil metabolites in rat hepatocytes (t=30 minutes). 15 major metabolites were assigned putative structures during the automated processing.



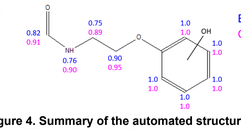


Figure 4. Summary of the automated structure assignment using CID and EAD MS/MS data.
A: Metabolites listed in Table 1 were investigated. One Buspirone oxidative metabolite did not have its structure generated automatically due to a core structure rearrangement.. 80% of the studied metabolites had the structures correctly assigned.

B: Normalized structure assignment (SOM) scores for the candidate hydroxylation positions on a Nefazodone metabolite. EAD MS/MS spectrum allowed to distinguish the site of modification to a greater detail than the CID MS/MS, because of more extensive set of fragments.

CONCLUSIONS

- The ZenoTOF 7600 system generated accurate comprehensive HR MS/MS data with two different fragmentation mechanisms under fast LC gradient methods.
- The improved sensitivity in MS/MS while maintaining the mass accuracy gives a comprehensive fragment set for compound characterization.
- The orthogonal CID and EAD MS/MS data were found to improve the confidence and outcome in the metabolite structure assignment.
- The research-grade Molecule Profiler SW offered streamlined CID and EAD data processing and interaction allowing fast navigation through the qualitative results.

REFERENCES

1. Dindyal-Popescu, A., Shek, I., Jooyhandeh, M., Moore, I., Duchoslav, E., ASMS 2017 conference proceedings, WP 106
2. Bruderer, T., Varesio, E., Hidasi, A., Duchoslav, E., Burton, L., Bonner, R., Hopfgartner, G. (2018). Analytical and Bioanalytical Chemistry. 410. 10.1007/s00216-018-0860-x.
3. Narayanaswami, P., Duchoslav E., ASMS conference 2022, TP 386
4. Molecule Profiler Software Brochure (sciex.com)

ACKNOWLEDGMENT

We would like to thank Kevin Bateman and Chris Kochansky of Merck for providing the rat hepatocyte incubates used in this study.

TRADEMARKS/LICENSING

To receive a copy of this poster:
- Scan the code with your phone camera
- Complete the form