A new method for analyzing MS^E/All Ions Fragmentation in Xenobiotic metabolism studies

Richard Lee,[1] Vitaly Lashin,[2] Andrey Paramonov,[2] Alexandr Sakharov[2]

[1]Advanced Chemistry Development, Inc. (ACD/Labs), 8 King Street East, Toronto, ON. M5C 1B5. Canada; [2]ACD/Labs, Moscow, Russia

METID CHALLENGE

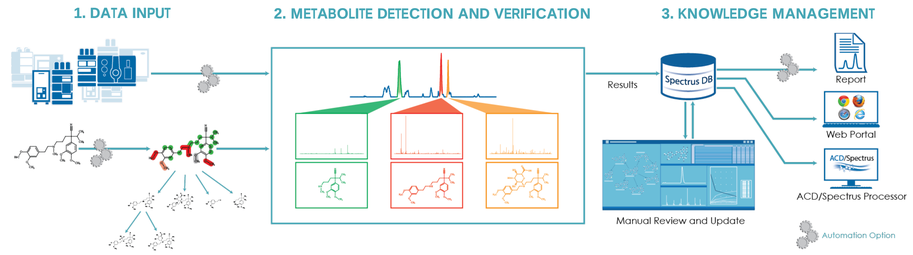
As an alternative to traditional Data Dependent Acquisition (DDA), the use of MS^E/All Ions Fragmentation (AIF) has become common in metabolite identification workflows for the analysis of metabolic hot spots. Although, MS^E/AIF has its advantages, the non-specific fragmentation of precursor ions possess a challenge for structure elucidation due to the confounding fragment ions from other species. Here we present a solution for analysis of MS^E/AIF in metID studies.

METID SOLUTION

MetaSense™ is a new solution that can efficiently process LC/MS data acquired by MS^E/AIF methods for traditional metabolite identification by:

- **AUTOMATION** through structure based prediction and MS data interpretation to detect and identify predicted and unexpected metabolites
- **AUTOGENERATION** of biotransformation maps, stability and pharmacokinetic plots, and reports
- **INTERACTIVE SEARCHABLE DATABASE** support to review metabolite data, and associated spectral and chromatographic data
- **WEB PORTAL** offering customer access to your results and reports

AUTOMATED WORKFLOW



EXPERIMENTAL

SAMPLE PREPARATION

Midzolam was inclubated in HLM and samples were extracted at 0 hr and 1 hr time intervals.

DATA ACQUISITION

- High resolution LCMS data
- MS^E mode
- Waters Synapt QTOF

1. DATA INPUT

High resolution LCMS data were used as the data input for batch processing. Parent structure files, along with the datasets, were automatically processed within the new software routine.

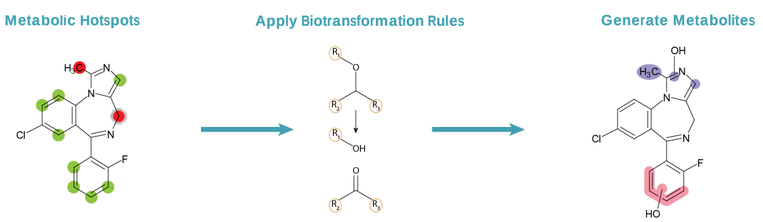
2. METABOLITE PREDICTION AND VERIFICATION

The predicted metabolites were restricted to phase 1 and 2. Metabolite target lists were generated in three parts:

i. A **regio-selective model** was used to predict expected metabolites

- A probabilistic statistical model was applied to determine the likelihood of a metabolic reaction taking place at each potential site of metabolism in the compound of interest to identify hotspots.
- Once potential **metabolic hotspots** were established, they were checked against a database of biotransformation rules to assess the types of metabolic reactions that are defined for the respective site of metabolism.
- Then, the selected **biotransformation rules** were applied to generate metabolite structures.

REGIO-SELECTIVE MODEL



ii. **Predicted metabolites** were detected based on their accurate mass and theoretical isotope pattern calculated from molecular formulae. **Unexpected metabolites** were identified using a complementary procedure employing a fractional mass filter.

iii. **Structure verification** was performed by comparing the high energy spectra of the parent and metabolite, and fragmentation analysis was performed on the parent structure to assign fragments to the high energy spectrum. Due to the high levels of background fragment ions, only those ions of greater than 25% relative intensity were used for the analysis. Once assigned, the parent and metabolite high energy spectra were compared (as shown in the Metabolite Centric View).

Visit ACD/Labs at Booth #771

RESULTS

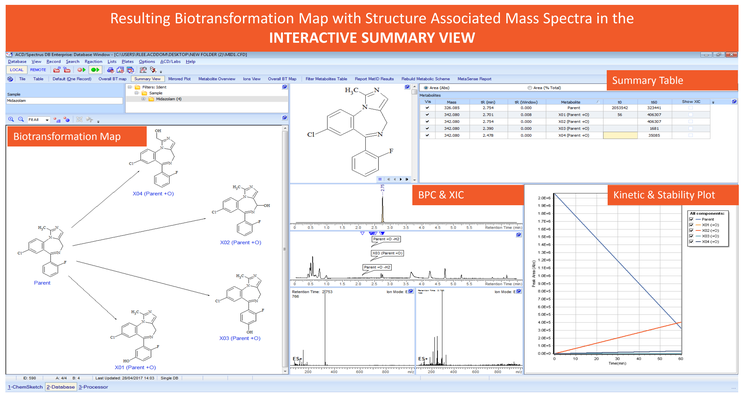
The top 4 metabolites detected were hydroxylated species at various sites around the molecular scaffold. The parent and metabolites were tracked throughout the study, where the peak areas were visualized in the stability/kinetic plot. From the study, metabolite X02 was determined to be the most prominent metabolite detected. The automated processing was determined to correspond to the manually curated results.

3. INTERACTIVE KNOWLEDGE MANAGEMENT

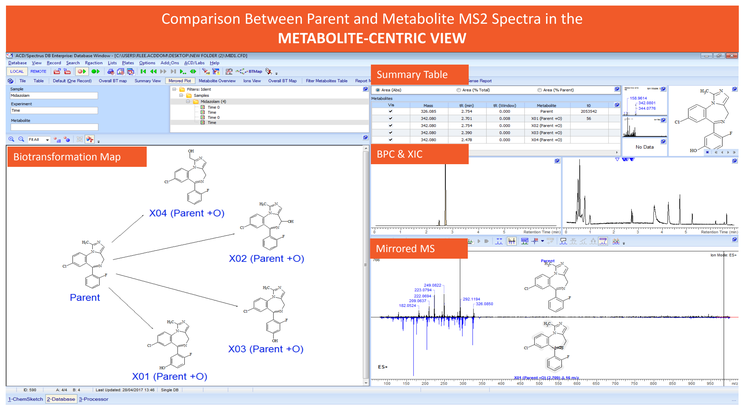
KNOWLEDGE DATABASE

A summary of the data was automatically generated and updated to an interactive knowledge database upon completion of the data processing routine. As shown in the Summary View, both the predicted and unexpected metabolites were combined into a single biotransformation map, where all mass spectra were associated to each of the structures.

Resulting Biotransformation Map with Structure Associated Mass Spectra in the **INTERACTIVE SUMMARY VIEW**



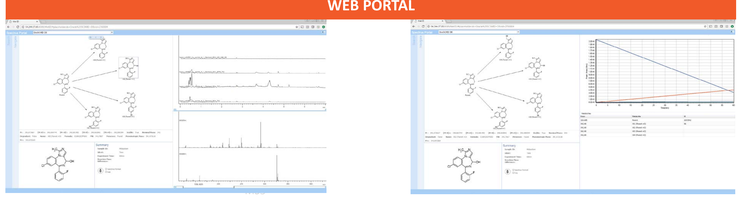
Comparison Between Parent and Metabolite MS2 Spectra in the **METABOLITE-CENTRIC VIEW**



WEB PORTAL

A java based web portal was designed to communicate the results and increase collaborative efforts. The viewer in this case includes a display of the biotransformation map as the main feature. Users can perform metadata search, and more importantly, structures can be searched by substructure, similar structure, and exact structure search via the drawing applet within the web portal.

Communicated results and performed searches in the java-based **WEB PORTAL**



MetaSense

www.acdlabs.com/metasense

metasense@acdlabs.com

1-800-304-3988

@acdlabs