A new biotransformation prediction engine integrated into a metabolite identification solution.

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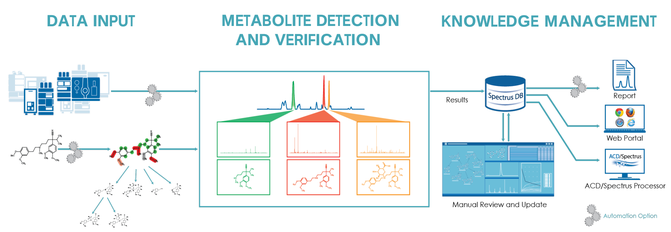
METID CHALLENGE

In the analysis of xenobiotic metabolism, there have been a number of advancements in the hardware used, as well as the software that processes LC-MS data for metabolite identification. However, bottlenecks remain in the workflow, and especially in the structure elucidation phase. Here we present a new prediction algorithm that determines the likelihood of biotransformation reactions, and subsequent metabolite identification, within an automated processing routine.

METID SOLUTION

METASENSE™ WORKFLOW



MetaSense is a new solution that can efficiently process LC/MS data from high-throughput environments by:

- **AUTOMATED** identification of metabolites, file capture, and data processing
- **VENDOR NEUTRAL** solution that allows data from major mass spectrometry vendors to be processed
- **MANUAL** capabilities to update information, for example modifying the identification of a metabolite; keeping the expert in control
- **INTERACTIVE KNOWLEDGE MANAGEMENT** support to review metabolite data and metadata

PREDICTION ENGINE

The new prediction algorithm presents two significant advantages:

1. Enhanced coverage of metabolite structures
2. The resulting increase of structures can be filtered by data regardless of study model

NEW PREDICTION ENGINE

- Allows for greater metabolite structures to be generated regardless of species
- Incorporates additional metabolic reactions representing Phase 1 and Phase 2 biotransformations
- Allows for cross combination of these reactions

PREVIOUS ENGINE

- Based on a Human Liver Microsome model
- Decreased coverage of structures when predicting metabolites from other species despite common bio-transformational pathways between HLM and other species

EXPERIMENTAL

SAMPLE PREPARATION

- Samples were collected from a rat microsomal incubation
- Test articles (10 mM in DMSO) were dispensed by an acoustic dispenser (25 nL) into to 25 µL 10 mM phosphate buffer (pH 7.4) containing 2 mM NADPH
- This solution (12.5 µL) was added to 12.5 µL rat liver microsomal protein (1 mg/mL)
- At specific time points (0, 2, 5, and 10 min), the reactions were terminated by the addition of 10 µL acetonitrile/formic acid (93:7)

DATA ACQUISITION

- Elite Hybrid Velos Pro Ion Trap/Orbitrap (ThermoFisher Scientific, CA, USA) mass spectrometer equipped with an electrospray (ESI) source operating in positive mode electrospray ionization
- Data-dependent acquisition based on a list of m/z values of potential metabolites was applied
- Resolution was set to 30,000 in full scan mode and 15,000 for high energy collision dissociation (HCD) MS2

DATA ANALYSIS

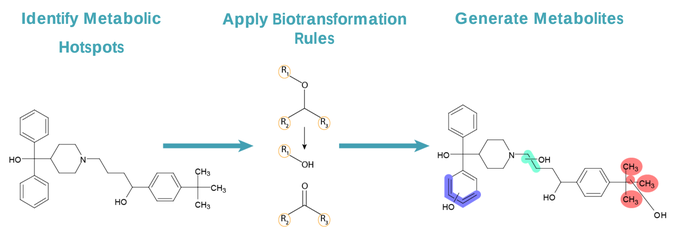
Data was processed using MetaSense with:

1. The original prediction algorithm
2. The newly extended metabolism model

As a comparison, prediction was set to only generated Phase 1 structures and all other prediction parameters were consistent between the original and extended model.

PREDICTION MODEL

REGIO-SELECTIVE MODEL



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

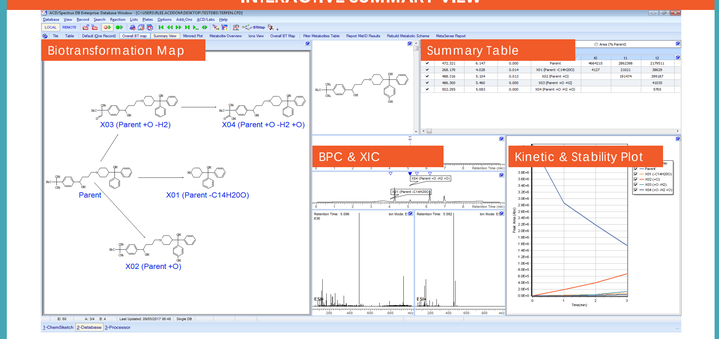
- A probabilistic statistical model was applied to determine the likelihood of a metabolic reaction taking place at each potential site of metabolism in the compound of interest to identify hotspots. Each site was afforded a probability value and each atomic environment was compared against a database.
- Once potential **metabolic hotspots** were identified, they were checked against a database of biotransformation rules to determine the types of metabolic reactions that could occur. The original metabolism prediction algorithm was designed to predict only a singular biotransformation reaction, i.e., only one reaction per site. The current extension of the model goes beyond this limitation and predicts structures for all likely metabolic reactions, i.e., multiple reactions per site resulting in a significant increase in the number of structures generated.

RESULTS

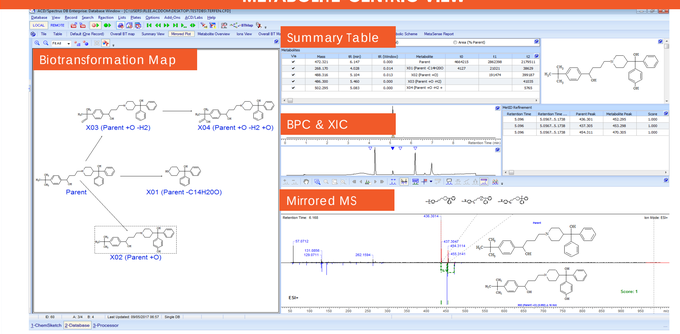
The extended model predicted 30% more phase 1 metabolite structures than the original algorithm. This revealed an additional two previously unidentified metabolites.

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**



SUMMARY

The implementation of the newly extended prediction model displays an advantage compared to the restricted original model. This expansion of prediction rules, and removing the restriction of "one reaction per site", allows for greater confidence of identifying all metabolites when processing LC/MS/MS data within the MetaSense™ solution.

MetaSense

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