Unique Users-Initiated Biotransformation Reactions for Analysis of Xenobiotic Metabolism

Richard Lee, Rytis Kubilius, Vitaly Lashin, Alexandr Sakharov, Anne Marie Smith
Advanced Chemistry Development, Inc. Toronto, ON, Canada

Introduction

A challenge for metabolite analysis solutions are their ability to process data and to efficiently report the results. Some software solutions incorporate the use of prediction algorithms to generate structures of possible metabolites of a given parent compound, which can then be utilized further downstream during data processing.

Although these software packages have matured, there are inherent limitations to these solutions: they are only as reliable as their prediction algorithms for generating possible metabolites, they have limited prediction databases, and they are typically for mammalian species.

Here, we describe a user-created biotransformation database to augment a pre-existing prediction algorithm as part of the structure generation process. This new interface is integrated into the MetaSense™ software solution.

Metabolite Solution

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

- Workflow integration with UV, RAD, and isotope labeled processing
- 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
- An interactive searchable database to review metabolite data, alongside associated chromatographic and spectral data
- A web portal offering customer access to your results

Automated Workflow

High resolution LC/MS data is used as the data input for batch processing. Parent structure files, along with the datasets, are automatically processed within the new software routine (Figure 1). The data processing can be configured to be automated or as a user-initiated system.

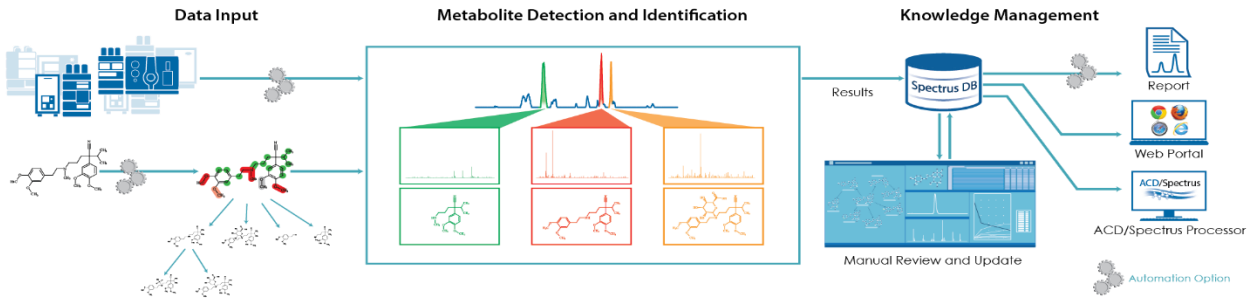


Figure 1. An overview of the MetaSense workflow.

Metabolite Prediction Engine

The predicted metabolites can be restricted to phase 1 and/or 2. A region-selective model is used to predict expected metabolite target lists in three steps (Figure 2):

1) A probabilistic statistical model is 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.

2) Once potential metabolic hotspots are established, they are checked against a database of biotransformation rules to assess the types of metabolic reactions that are defined for the respective site of metabolism

3) Then, the selected biotransformation rules are applied to generate an exhaustive list of metabolite structures.

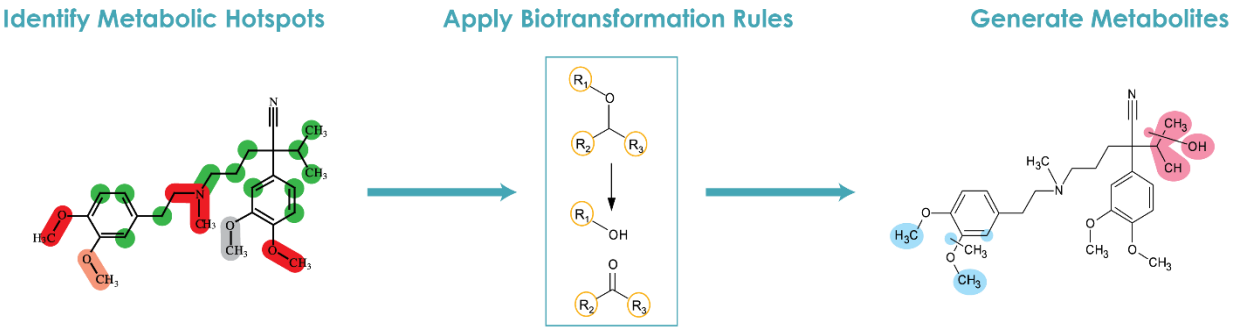
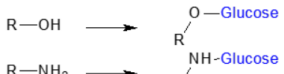
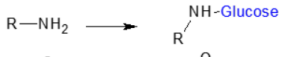
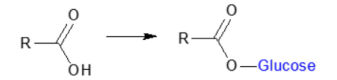
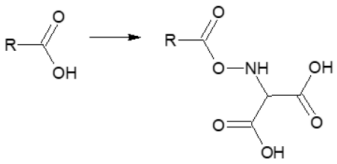
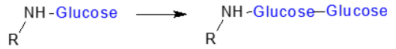
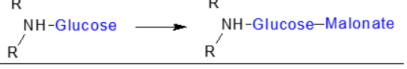


Figure 2. An overview of the region-selective model used to predict expected metabolites

Uncommon Biotransformations

Phase 1 and 2 metabolism often overlap between mammals and plants, however, conjugations found in plants can be quite different. Moreover, the environmental fate of agrochemicals are of high interest and these reactions are not well documented.[1-3]

Table 1 represents some reactions which are described to be unique to plant metabolism for pesticides, herbicides, and insecticides.

Plant Reaction	molecule conjugate
O-glucoside	
N-Glucoside	
Glucose Ester	
Amino Acid	
GSH	
Glucose	
Malonate	

User Reaction Input

Reactions are drawn and atoms are mapped between the left side of the reaction and the product side within the chemical drawing application found in MetaSense (Figure 3). The system recognizes cleavages and conjugations, including various oxidations, reductions, and multiple products. Generic reactions are updated to a SpectrusDB database where several user reactions can be contained and multiple databases can be created (Figure 4). During analysis of metabolite datasets, MetaSense can use its native prediction engine in addition to several user created reaction databases

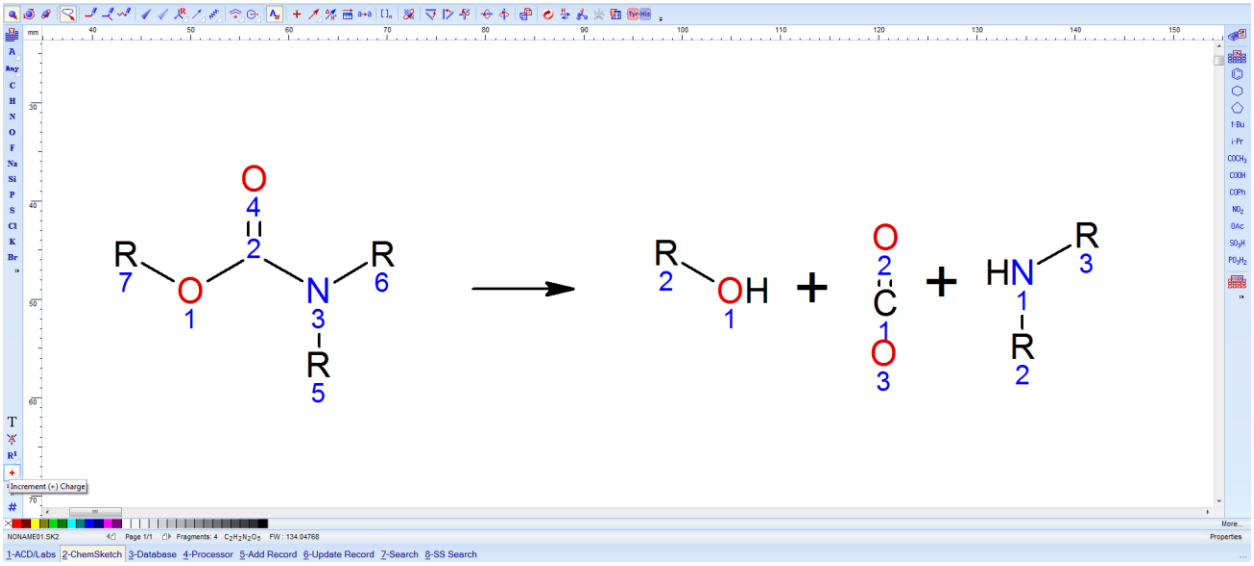


Figure 3. Atom mapping of generic metabolic reactions is input as in this example of a cleavage reaction with multiple products.

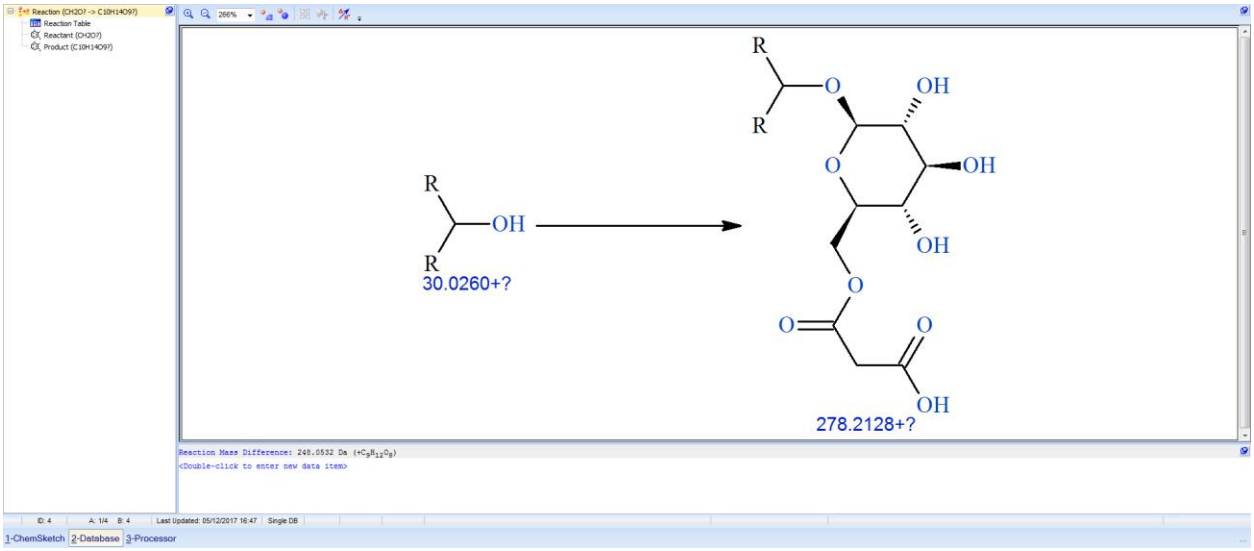


Figure 4. SpectrusDB is used to curate the various user-created metabolic reactions, and multiple reactions can be stored in one database.

Summary

ACD/Labs has developed an interface for user-defined metabolic reactions to address the lack of well represented biotransformations, where user reactions can be drawn in a generic biotransformation scheme and are uploaded to a SpectrusDB database. User-created metabolic reactions can be seamlessly integrated to work with the pre-existing prediction engine, and integration of user created metabolic reactions allows for increased chemical coverage during analysis of xenobiotic metabolism studies with the MetaSense solution.

References

1. Hoagland RE, Zablotowicz RM, Hall JC. 2000. Pesticide Biotransformation in Plants and Microorganisms. pp 2-7.
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3. Hatzios KK. 1991. Biotransformations of herbicides in higher plants. Grover R, Cessna AJ, eds. Environmental Chemistry of Herbicides. CRC Press, Boca Raton, FL. pp 141-185.