[ TECHNOLOGY BRIEF ]

# Integration of Metabolism Prediction into the Metabolite Identification Workflow

Stephen McDonald, Andy Baker

## GOAL

To incorporate predictive technologies into a class-leading metabolite identification workflow to increase confidence and facilitate decision making in drug metabolism studies.

## BACKGROUND

Within the pharmaceutical and biotechnology industry, LC/MS is widely used for both quantitative and qualitative bioanalysis in DMPK. Continuous improvements in LC/MS instrumentation and the introduction of the MSE acquisition technique have led to increased sample throughput capability. The major obstacle is the ability to process the data in order to extract the required information about the metabolic rate and route, identify metabolic "soft spots," and identify compound or metabolic liabilities to support the "fail fast, fail cheap" paradigm in drug discovery.

Waters' metabolite identification workflow has been shown to automate the labor-intensive task of extracting potential metabolite peaks from complex chromatograms and present the results in an intelligently reduced data set. The intuitive review process then allows users to rapidly identify and differentiate true metabolites from false positives in a rapid, yet thorough, manner. Once these metabolites are identified, the use of predictive tools (literature or expert knowledge-based) to further enhance and verify the results is desired.

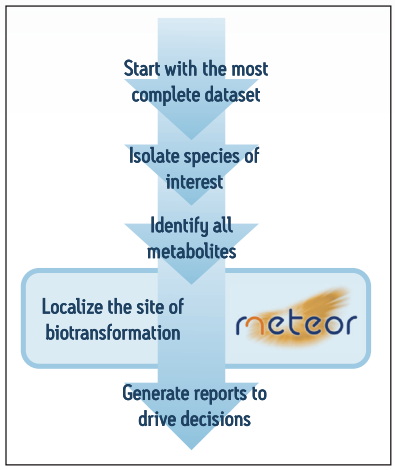
In this technical brief, we introduce an extension to this workflow that incorporates in-silico metabolite prediction using Meteor Software (Lhasa Ltd., Leeds, UK; www.lhasalimited.org/meteor). The aim of this software is to aid scientists who need information about the metabolic fate of chemicals and want to make more efficient, more effective, and more confident decisions.

**Meteor from Lhasa Limited is an integrated metabolite prediction suite that expands the capabilities of the MetaboLynx™ XS v.2.0 workflow.**

## THE SOLUTION

It is well understood that the fundamental metabolic potential of a compound is defined by its structure. Decisions made based upon this information in conjunction with expert knowledge are critical in enabling accurate interpretations to be performed by a drug metabolism scientist. For this reason, we have further extended our chemically-intelligent software algorithms into MetaboLynx™ XS Application Manager, v. 2.0, for MassLynx™ Sofware.

These algorithms use the specific structure of each substrate to direct studies, performing predictive cleavage of the molecule and generating new metabolites. The predictions are then combined with known biotransformations to produce an intelligent target list for metabolite identification. These same algorithms can be used to localize the site of biotransformation using empirical MSE fragment data.




This same structure information is automatically transferred to Meteor Software, where a database of expert knowledge rules in metabolism is used to predict the metabolic fate of the substrate. This can be seen in the workflow diagram shown in Figure 1.

*Figure 1. Incorporation of Meteor Software into UPLC®/MS$^E$ MetaboLynx XS workflow.*

The results of these predictions are subsequently filtered to show only the metabolites observed in the mass spectrometer and are presented as metabolic trees. Meteor Software also provides comments and literature citations as evidence to support its predictions. This supporting evidence is specific to the metabolic route shown and includes mechanistic rationale, literature references, and information about factors that may affect the likelihood of each biotransformation occurring (such as enzyme affinity, lipophilicity, or competing reactions). These are presented in both graphical and textual format, making the predictions easier to interpret and evaluate (Figure 2).

Meteor Software can be used to suggest the most likely molecular structure of a metabolite, or group of metabolites, when only empirical formula data is available. In some cases, Meteor may predict more than one possible molecular structure for a single empirical formula. When this happens, the program will predict the molecular structure that is more likely.

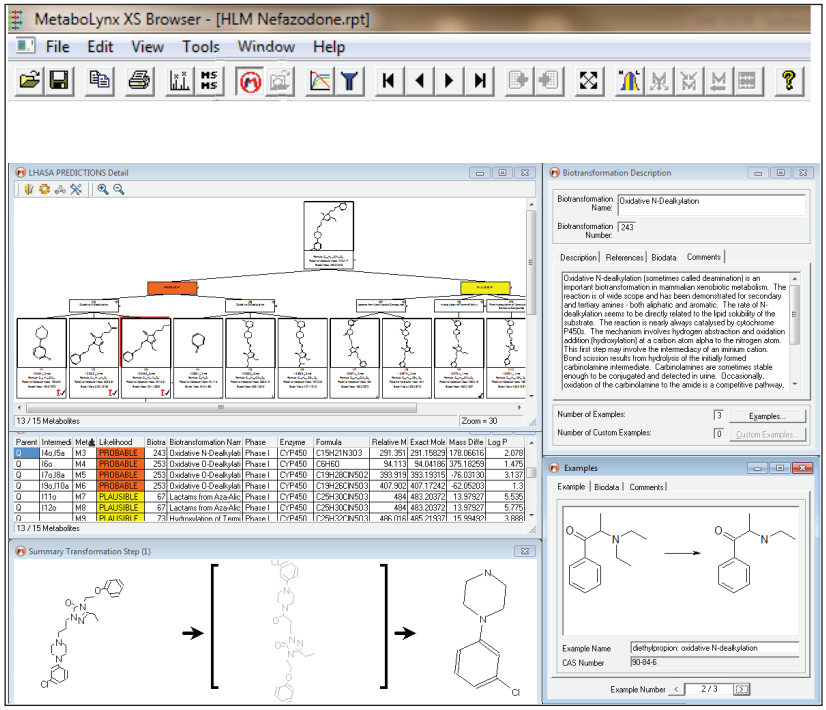



*Figure 2. Automated Meteor Software prediction, annotated with peaks found in MetaboLynx XS.*

## SUMMARY

Incorporation of Meteor Software in Metabolynx XS Sofware allows the structures of potential metabolites to be automatically predicted and easily paired using structural elucidation tools that exist in the metabolite identification workflow.

This adds valuable additional metabolism prediction and augments the chemically intelligent tools in Metabolynx XS to give scientists a more confident foundation on which to base determinations of rates and routes of metabolism.

This, in turn, can help drive key decisions made for a compound or class of compounds, boosting the efficiency of the metabolite identification process while simultaneously increasing user confidence and accuracy in the analysis.

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**Waters Corporation**
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com