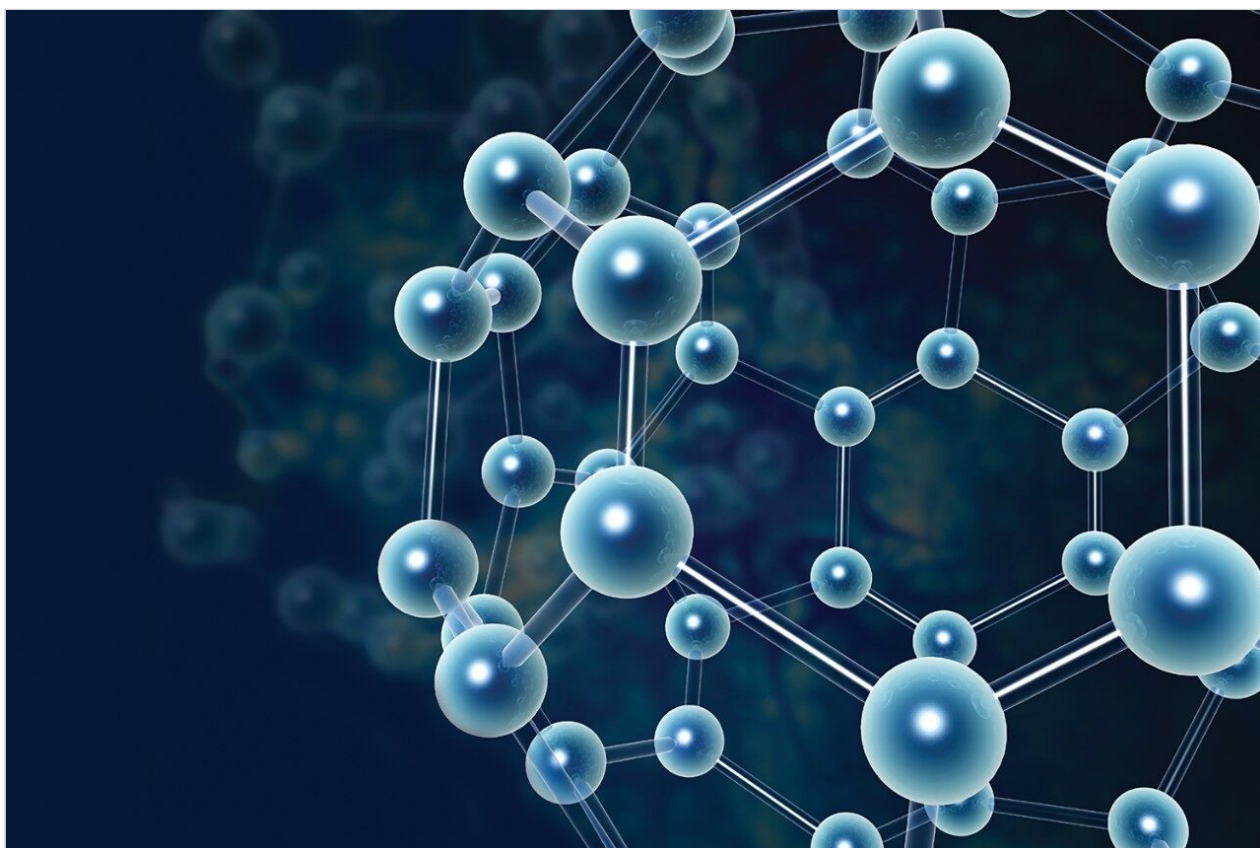


# Integration of Metabolism Prediction into the Metabolite Identification Workflow

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

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

## Benefits

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

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## Introduction

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 MS<sup>E</sup> 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](http://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.

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## Results and Discussion

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 Software.

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 MS<sup>E</sup> 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.

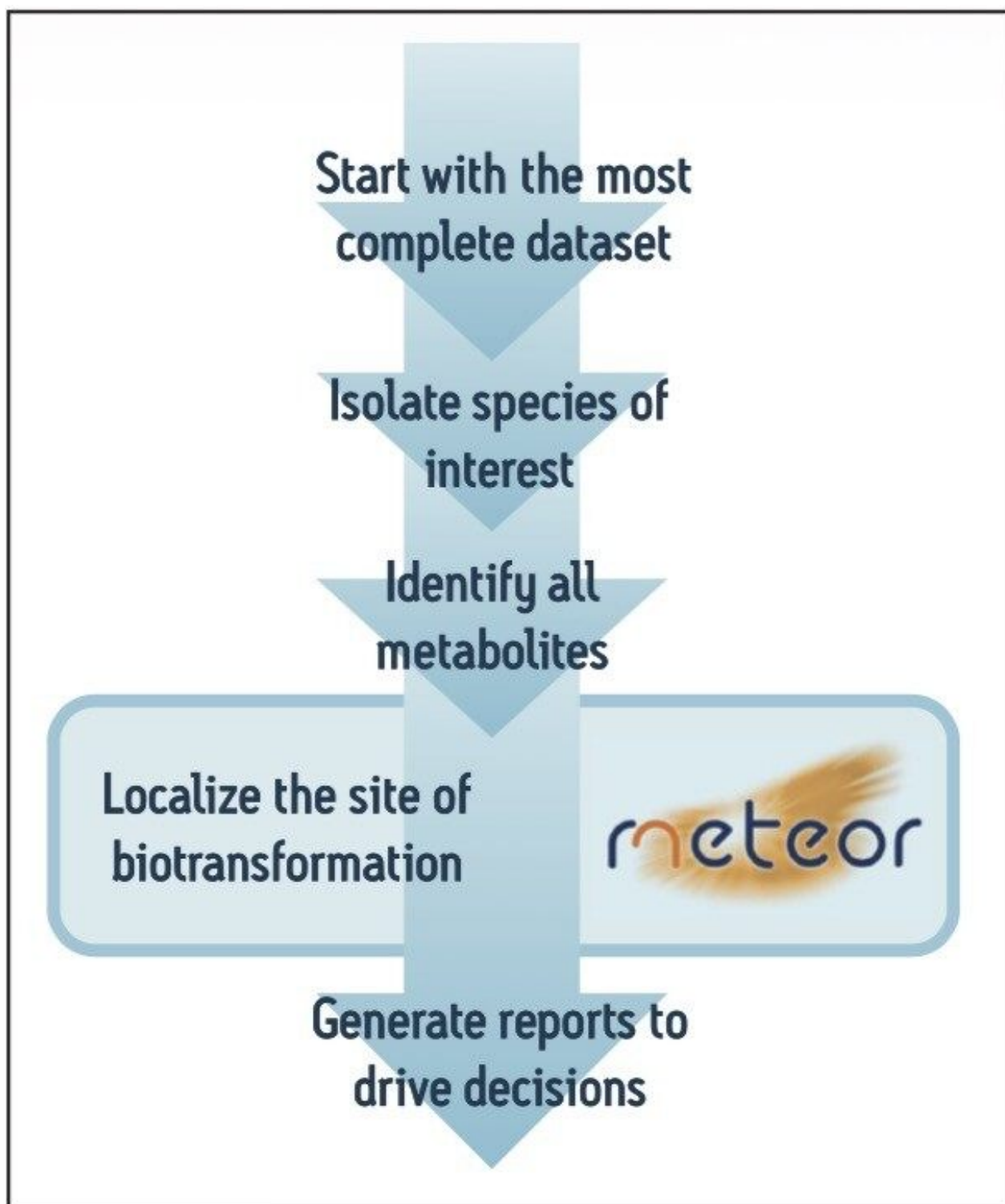


Figure 1. Incorporation of Meteor Software into UPLC-MS<sup>E</sup> MetaboLynx XS workflow.

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).

The screenshot displays the MetaboLynx XS Browser interface for the file [HLM Nefazodone.rpt]. The main window shows a tree view of metabolites, with the parent metabolite Nefazodone at the top. Below it, several predicted metabolites are listed, each with a chemical structure and a likelihood score. A table at the bottom provides detailed information for these metabolites, including their parent, intermediate, metabolite, likelihood, biotransformation name, phase, enzyme, formula, relative mass, exact mass, mass difference, and log P.

Parent	Intermedi	Met	Likelihood	Biotra	Biotransformation Name	Phase	Enzyme	Formula	Relative Mass	Exact Mass	Mass Diff	Log P
Q	I4oJ5a	M3	PROBABLE	243	Oxidative N-Dealkylation	Phase I	CYP450	C19H21N3O3	291.351	291.15829	178.06616	2.078
Q	I6o	M4	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C6H6O	94.113	94.04186	375.18259	1.475
Q	I7oJ8a	M5	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C19H26ON5O2	393.919	393.19315	-76.03130	3.137
Q	I9oJ10a	M6	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C19H26ON5O3	407.902	407.17242	-62.05203	1.3
Q	I11o	M7	PLAUSIBLE	67	Lactams from Aza-Alc	Phase I	CYP450	C29H30ON5O3	484	483.20372	13.97927	5.535
Q	I12o	M8	PLAUSIBLE	67	Lactams from Aza-Alc	Phase I	CYP450	C29H30ON5O3	484	483.20372	13.97927	5.775
Q	I13o	M9	PLAUSIBLE	73	Hydrolysis of Tertiary Amine	Phase I	CYP450	C29H30ON5O3	486.016	485.21937	15.98442	3.889

The detailed view of the biotransformation step (1) shows the conversion of Nefazodone to a metabolite. The reaction is labeled as Oxidative N-Dealkylation. The description states: "Oxidative N-dealkylation (sometimes called deamination) is an important biotransformation in mammalian xenobiotic metabolism. The reaction is of wide scope and has been demonstrated for secondary and tertiary amines - both aliphatic and aromatic. The rate of N-dealkylation seems to be directly related to the lipid solubility of the substrate. The reaction is nearly always catalysed by cytochrome P450s. The mechanism involves hydrogen abstraction and oxidation addition (hydroxylation) of a carbon atom alpha to the nitrogen atom. This first step may involve the intermediacy of an iminium cation. Bond scission results from hydrolysis of the initially formed carbinolamine intermediate. Carbinolamines are sometimes stable enough to be conjugated and detected in urine. Occasionally, oxidation of the carbinolamine to the amide is a competitive pathway."

The examples section shows the conversion of Nefazodone to a metabolite, with the example name "Nefazodone: oxidative N-dealkylation" and the CAS number "90-84-6".

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

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.

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## Conclusion

Incorporation of Meteor Software in Metabolynx XS Software 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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## Featured Products

MetaboLynx XS <<https://www.waters.com/513803>>

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