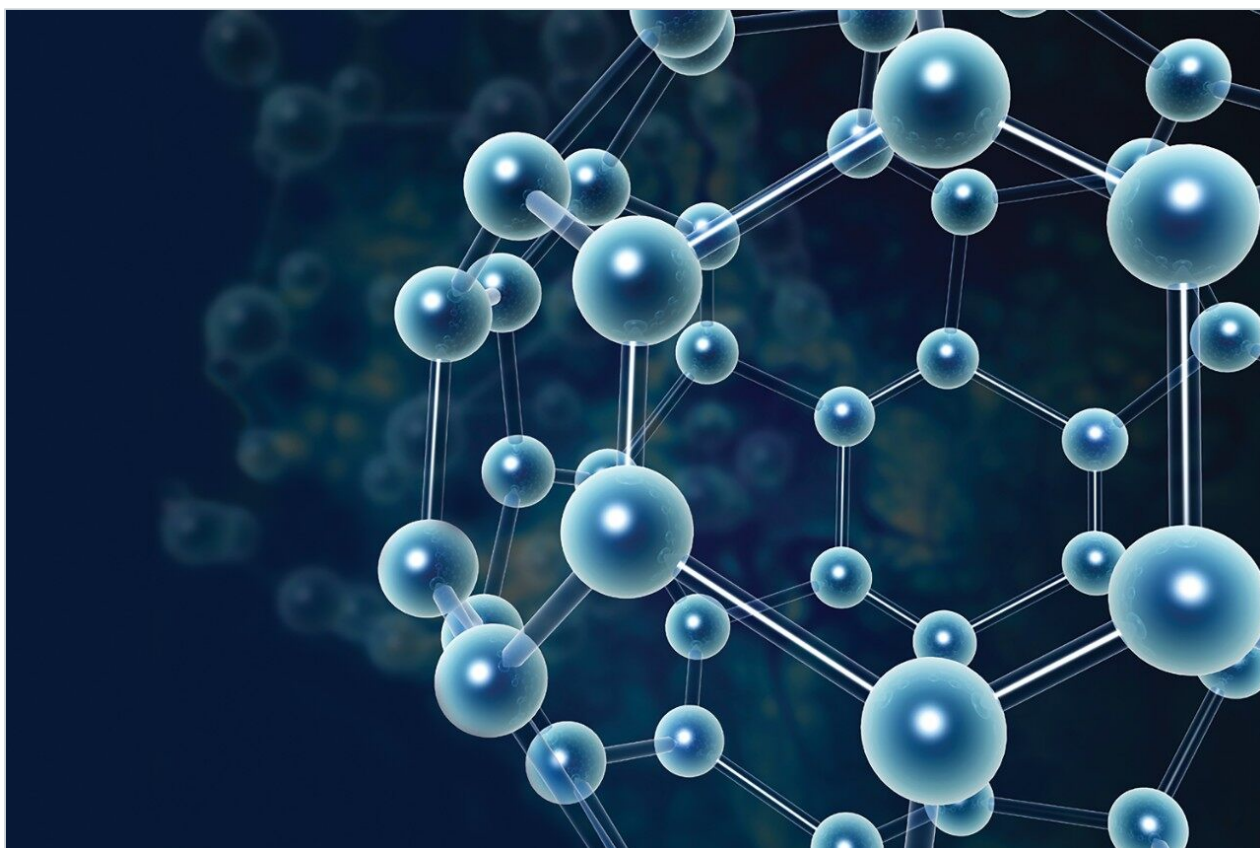


Nota applicativa

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.

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.

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^E 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.

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^E 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.

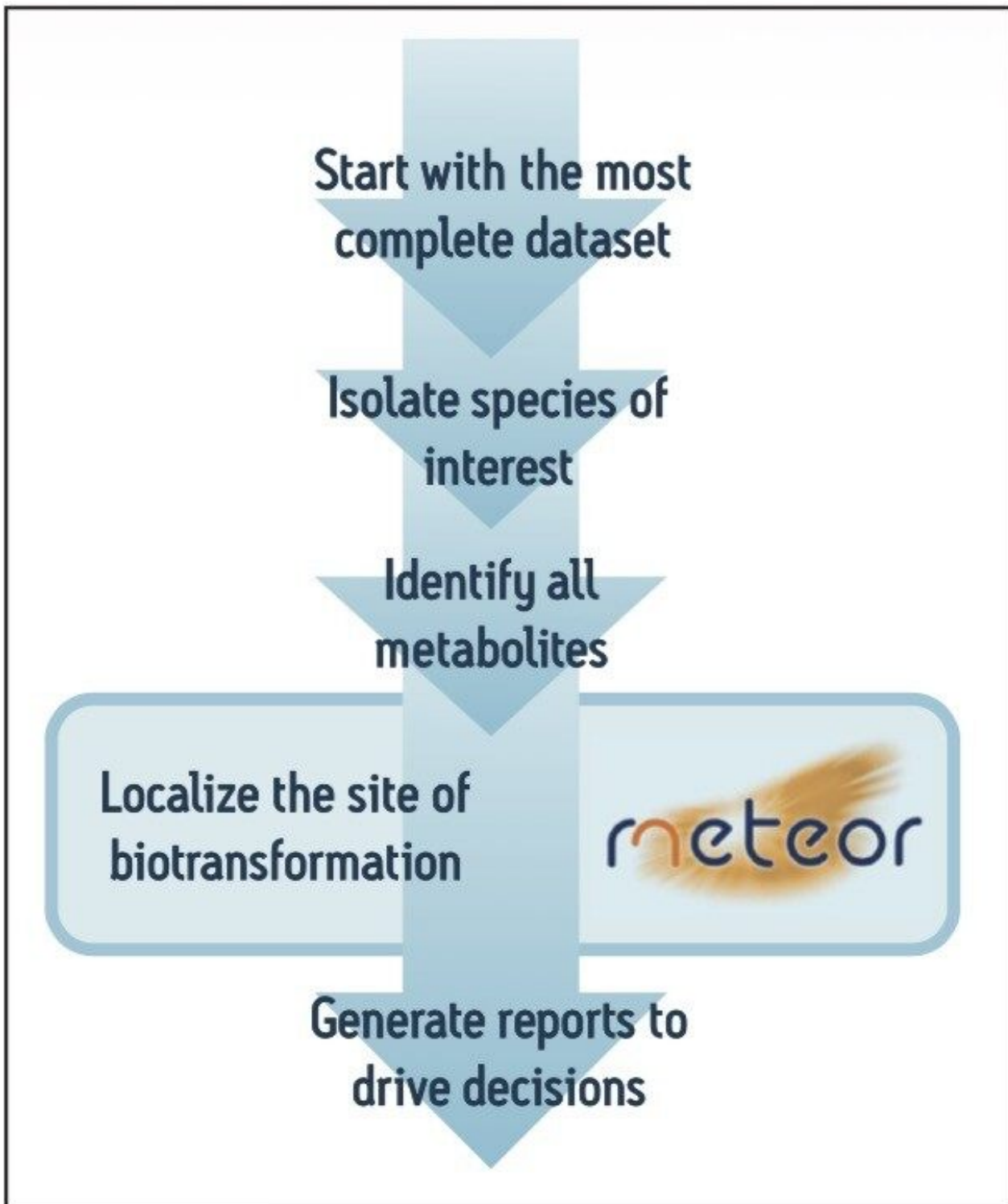


Figure 1. Incorporation of Meteor Software into UPLC-MS^F MetaboLynx XS workflow.

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 metabolic tree with a central parent node and several child nodes representing different biotransformation pathways. Below the tree is a table of 13/15 metabolites with columns for Parent, Intermid, Met#, Likelihood, Biotra, Biotransformation Name, Phase, Enzyme, Formula, Relative M, Exact M, Mass Diff, and Log P. The table lists various metabolites such as 14o,15a, 16o, 17o,18a, 19o,110a, 111o, 112o, and MR, each with associated likelihood and biotransformation details.

Parent	Intermid	Met#	Likelihood	Biotra	Biotransformation Name	Phase	Enzyme	Formula	Relative M	Exact M	Mass Diff	Log P
Q	14o,15a	M3	PROBABLE	243	Oxidative N-Dealkylation	Phase I	CYP450	C19H21N3O3	291.251	291.15029	178.06516	2.078
Q	16o	M4	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C9H9O	94.113	94.04186	375.10259	1.475
Q	17o,18a	M5	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C19H28CN5O2	393.919	393.19315	-76.03130	3.137
Q	19o,110a	M6	PROBABLE	253	Oxidative O-Dealkylation	Phase I	CYP450	C19H26CN5O3	407.902	407.17242	-62.05203	1.3
Q	111o	M7	PLAUSIBLE	67	Lactams from Aza-Alc	Phase I	CYP450	C25H30CN5O3	484	483.20372	13.97927	5.535
Q	112o	M8	PLAUSIBLE	67	Lactams from Aza-Alc	Phase I	CYP450	C25H30CN5O3	484	483.20372	13.97927	5.775
Q	MR	IRALISIRIF	73	Hydrolysis of Tera	Phase I	CYP450	C25H30CN5O3	486	016	485.21937	15.93492	3.888

The right-hand side of the interface features a 'Biotransformation Description' panel for 'Oxidative N-Dealkylation' (Biotransformation Number: 243). It includes a detailed text description of the reaction mechanism, involving hydrogen abstraction and oxidation addition (hydroxylation) at a carbon atom alpha to the nitrogen atom. Below the description are fields for 'Number of Examples' (3) and 'Number of Custom Examples' (0). An 'Examples' panel at the bottom right shows a chemical reaction scheme for the oxidative N-dealkylation of diethylpropion, with the example name 'diethylpropion: oxidative N-dealkylation' and CAS number '90-94-6'.

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.

Conclusion

Incorporation of Meteor Software in Metabolyx 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 Metabolyx 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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[MetaboLynx XS <https://www.waters.com/513803>](https://www.waters.com/513803)

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