ACD/Labs

Application Note

Automation and Effective Data Sharing for Metabolite Identification

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Introduction

Identifying metabolites is a major challenge in the study of drug metabolism. Scientists want to identify structures accurately but also quickly, in a streamlined process that shortens time-to-results. Adding more challenges, laboratories often use several vendor instruments, each with their own data-processing software. This heterogeneous environment complicates data handling, analysis, and reporting. In this paper, we present a new unified approach for metabolite identification.

MetaSense®, on the ACD/Spectrus Platform, is an informatics solution designed for the workflows of scientists in metabolite analysis. It handles data from most major instrument vendors and follows a human expert's approach to spectral-data analysis. Its processing, review, and reporting tools help scientists connect parent compounds and metabolites with analytical data. Metabolites can be analyzed through four different workflows: the detect-and-identify workflow using LC/MS/MS, the radiotrace workflow, the UV-trace workflow, and the isotopic-scan workflow.

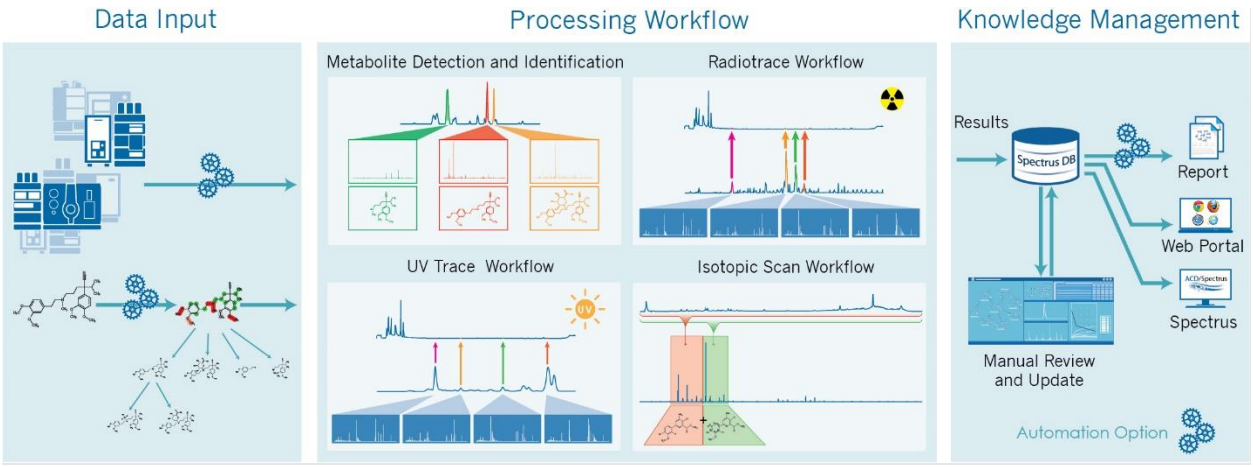

Figure 1. *MetaSense offers four different workflows for the automated analysis of metabolites. It accepts data from most major instrument vendors and metabolite predictions from Meteor Nexus (Lhasa Ltd.) and MetaSite (Molecular Discovery). The results and accompanying data are stored in a searchable database, easily accessible through a web browser, and can be automatically formatted into customizable reports.*

Compared to other metabolite-characterization approaches, MetaSense offers several benefits:

- Algorithms incorporate chemical intelligence to help scientists map biotransformations
- Analytical data is combined with computational prediction to comprehensively identify metabolites

- A single platform, purpose-built for metabolite identification, replaces a fragmented software environment
- Interpreted results and supporting data are linked and stored in a central, searchable database

Unifying Analytical Data in One Software Environment

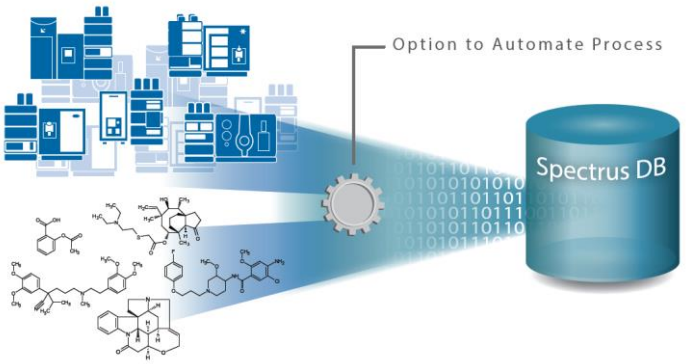

Figure 2. *MetaSense takes analytical data from different instruments, associates it to chemical structures, and unifies all information in a single environment and central repository (Spectrus DB).*

MetaSense handles over 150 different data formats. It provides a uniform environment for processing, analyzing, and databasing, so all steps, from file capture through analysis to reporting, can be streamlined and automated if desired. Whether users opt to fully automate, partially supervise, or process manually, they can review all results and revise processing and analysis routines.

The unified workflow starts with importing experimental data files and corresponding structures into the processing environment. Then, metabolites are identified and verified using both prediction and experimental-data–driven analysis. Depending on the experiments, scientists can choose between LC/MS/MS, radiotrace, UV-trace, and isotopic-scan workflows. After processing, interpreted spectra are uploaded to a central database, and the biotransformation map is automatically created. Experts can review the full project on one platform and add missing metabolites based on their knowledge.

Predicting Metabolites

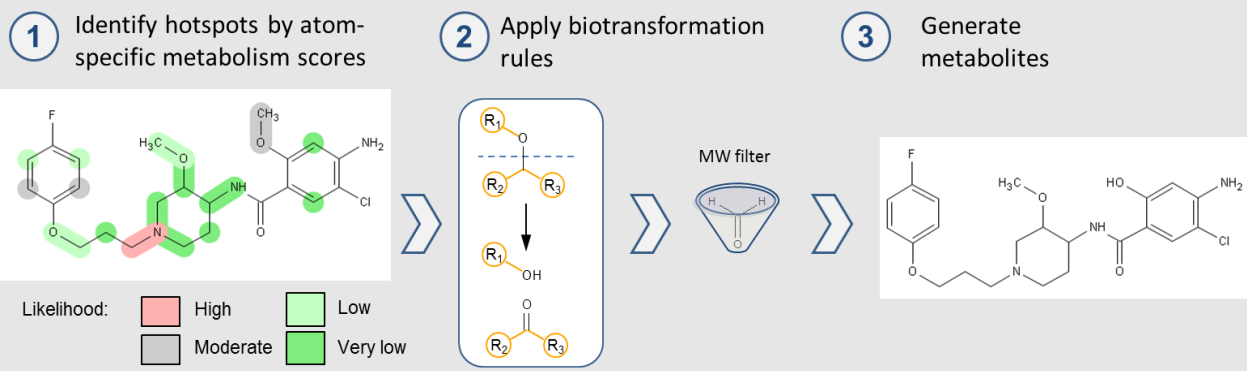

Figure 3. *MetaSense predicts both Phase I and II metabolites to expedite metabolite identification.*

To target expected metabolites, MetaSense uses structure-based prediction. For every potential transformation site on a compound, a statistical model estimates the likelihood of a metabolic reaction. Predictions can run iteratively: downstream metabolites are themselves subjected to analysis, and the software produces a full tree of Phase I and II metabolites. Scientists can also import metabolite predictions from third-party software (Meteor Nexus and MetaSite) and unique metabolic reactions that they define themselves.

Detecting and Identifying Metabolites

LC/MS traces are separated into extracted ion chromatograms (XIC). Predicted metabolites are matched to peaks by mass and isotopic pattern. Since each XIC may contain peaks from several isomeric metabolites, the biotransformation site is located with MS/MS spectra, by applying fragmentation rules and fragment-ion mass shifts. When the reaction site remains ambiguous, metabolite structures are represented using Markush notation. The software supports data-dependent acquisition, all-ion fragmentation, and MS^E.

Unexpected metabolites are identified by control-sample comparison and fractional mass difference. Since all data and interpretations are linked and stored together in MetaSense, users can always review the original chromatograms and spectra and send them to other ACD/Labs tools for structure elucidation.

Biotransformation maps and kinetic plots are automatically generated. The software uses chemical intelligence to refine the map: chemically unfeasible biotransformation steps are excluded. Together with mass spectra, chromatograms, peak areas, and study metadata, maps and plots are stored in a searchable database.

Figure 4 shows the overview screen in MetaSense. This dashboard provides quick access to all key information for each metabolite, including metabolic pathways, kinetics, spectra, chromatograms, and structures. From this panel, the scientist can check the accuracy of automated metabolite assignment, see the metabolite within its biotransformation context, and monitor compound stability and metabolite formation.

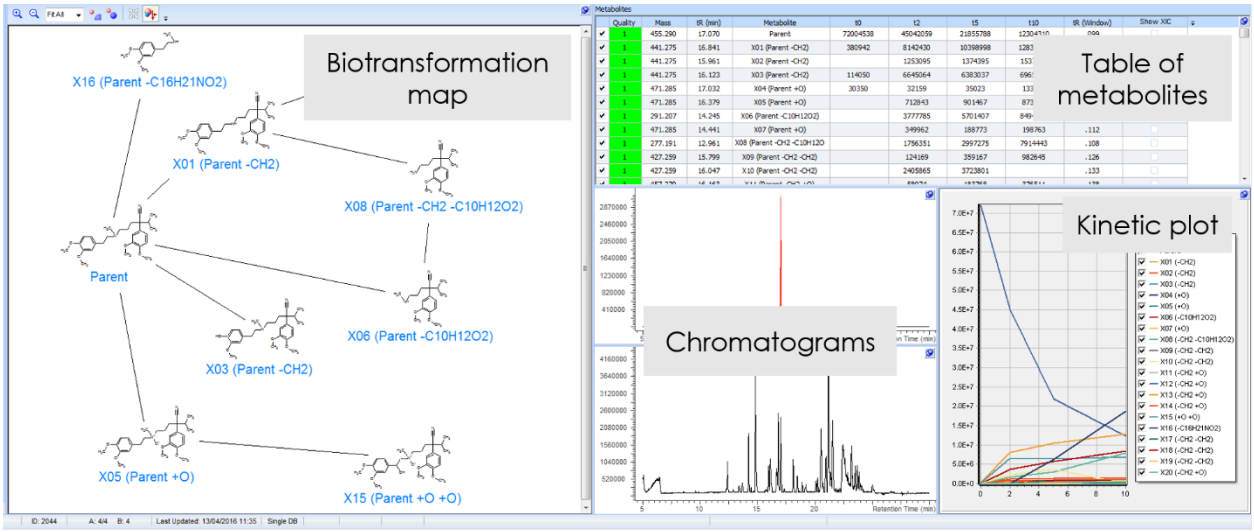

Figure 4. *The overview screen shows a metabolite-analysis dashboard for verapamil. A biotransformation map and pharmacokinetic plot are displayed alongside live spectral and chromatographic data for each analyte. The table of metabolites includes peak areas for each time point in the incubation study.*

To review identified biotransformation sites, the user can check the mirror-plot view (**Figure 5**), where the MS/MS spectra of the parent and metabolite are compared. A score is calculated for each putative metabolite based upon this comparison. Reaction sites are identified by fragment-ion mass shifts and fragmentation rules.

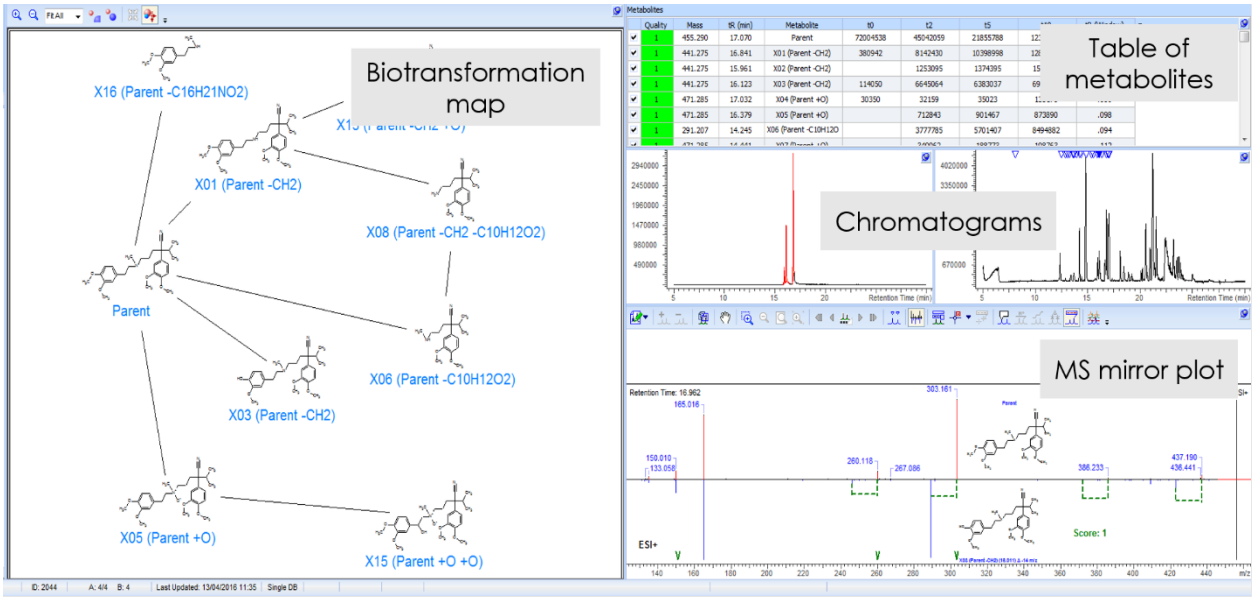

Figure 5. *The structure-oriented view allows scientists to assess identified biotransformation sites by reviewing the MS/MS spectra of the parent compound and putative metabolites.*

Conclusion

MetaSense provides a single, purpose-built platform for metabolism studies. By collecting information across instrument vendors and from all stages—raw data to final interpretation—the software helps scientists get high-quality results more quickly. With automation and batch processing, they can analyze entire studies with minimal work. And they can efficiently handle studies for which data analysis might otherwise be too laborious—including large-scale quantitative studies of intrinsic clearance and comprehensive metabolite-identification studies—increasing their understanding of target-compound biotransformation.