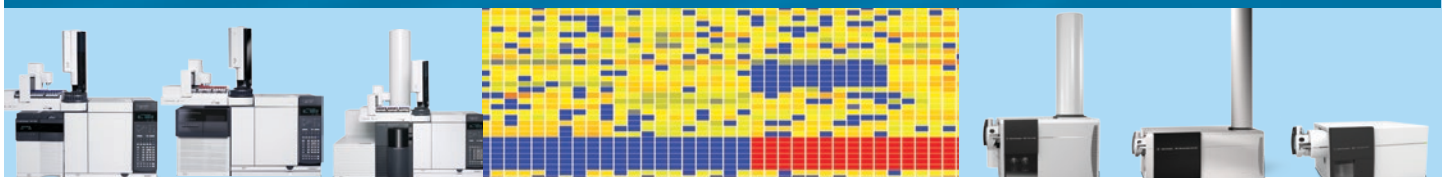
Agilent Solutions for Metabolomics

YOUR PATH TO SUCCESS

The Measure of Confidence



Agilent Technologies

UNDERSTANDING METABOLOMICS

Agilent is the leading global metabolomics vendor, offering our customers a broad array of cutting-edge instrumentation and associated informatics solutions for mass spectrometry.

What is Metabolomics?

Metabolomics is the study of endogenous metabolites at a particular point in time, and can be used to reveal the general physiological status of a cell, tissue, or organism. The collection of low molecular weight (50-1,500 Da) metabolites in a biological system is called the Metabolome. Its close proximity to the phenotype of an organism provides complementary information to genomics and proteomics, facilitating a more integrated readout of a biological system.

Metabolites are important modulators, substrates, byproducts, and building blocks for many different biological processes. The presence or absence of specific metabolites in a cell, cell culture, or biofluid provides important information about the physiological and functional status of the biological system. For example, the accumulation of specific metabolite(s) could indicate a defect in the signaling response of a biological pathway, or perhaps optimization of a biosynthetic pathway.

Agilent's innovative metabolomics solutions provide our customers with a diverse, powerful portfolio of instruments, software, and informatics tools. A common software platform allows you to combine the results from multiple analytical techniques, helping you answer challenging biological questions faster.

Agilent collaborates with leading metabolomics researchers on the development of next-generation bioanalytical assays and workflows for finding and identifying novel metabolites and metabolite signatures. These efforts ultimately improve our products and benefit our customers.

METABOLOMICS TOOLS FOR A VARIETY OF RESEARCH APPLICATIONS

A Powerful Way to Investigate Biology

Basic and Clinical Research
Identify and validate metabolite biomarkers that correlate with disease states as well as provide fundamental insights into biology

Agriculture
Identify and understand metabolic pathways to optimize crop development, yield improvement, and pesticide/herbicide resistance

Food and Nutrition
Identify the presence or absence of metabolites that correlate with major traits such as food quality, authenticity, taste, and nutritional value, and aid in the development of nutraceuticals

Pharmaceutical
Identify metabolites and markers of toxicity for drug discovery and development

Environmental
Identify metabolites that relate to the effects of chemicals and other stressors in the environment on a biological system

Biofuels and Synthetic Biology
Identify metabolite profiles to optimize fermentation processes and biofuel production

Toxicology
Find predictive signatures of toxicity in plasma and urine that can act as surrogates for evaluating the level of exposure to drugs and environmental pollutants

"Agilent's mass spectrometry systems combine the high acquisition speed and wide dynamic range necessary to identify and quantitate a diverse range of cellular metabolites. We can simultaneously find needles in a haystack and measure the haystack."

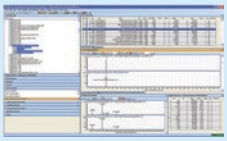
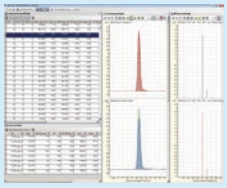
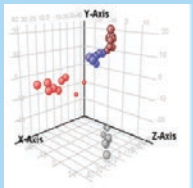
AMY A. CAUDY, PH.D.
THE DONNELLY CENTRE FOR CELLULAR AND BIOMEDICAL RESEARCH
UNIVERSITY OF TORONTO

APPLYING THE POWER OF DISCOVERY METABOLOMICS

Agilent's industry-leading metabolomics portfolio of hardware and software, combined with Agilent's renowned world-wide technical support, is available to help you quickly and reliably acquire and analyze your data.

Discovery Metabolomics

Discovery or non-targeted metabolomics experiments typically involve global profiling of metabolites by hyphenated MS techniques. Following separation and detection of compounds, subsequent peak finding is performed using Agilent MassHunter software. Statistically significant differences in abundance between two or more sample groups are then found, annotated, and identified. Pathway analysis in Pathway Architect software allows the researcher to map a list of metabolites onto biological pathways.

	Data Acquisition	Feature Finding	Alignment and Statistical Analysis	Identification	Pathway Analysis
		MassHunter Software	Mass Profiler Professional Software		
Analytical Instruments	 	 Qual  Profinder	 Analysis and visualization	 ID Browser using METLIN Database and Agilent Fiehn Library	 Pathway Architect using public databases, such as KEGG

Agilent solutions for discovery metabolomics.

"The Profinder workflow reflects the way that we, as biologists, approach experimental design. Intuitive access to both raw data and statistics-ready output enables my group to focus on forming and testing hypotheses. Profinder has enabled us to replace a half-dozen software packages that we previously used in our enzyme discovery efforts. We have been able to empower the students and scientists performing the wet lab experiments to analyze their experiments directly, eliminating the need for dedicated analysts."

ADAM ROSEBROCK, PH.D.
THE DONNELLY CENTRE FOR CELLULAR AND BIOMEDICAL RESEARCH
UNIVERSITY OF TORONTO

Untargeted Discovery

Agilent has developed robust workflows for performing global metabolite profiling for metabolites by LC/MS, GC/MS, CE/MS, and SFC/MS. Various reverse-phase and polar chromatographic methods for separation are supported. Data can be acquired in both negative and positive ion polarities for MS, MS/MS, and ALL Ions MS/MS data acquisition modes using a wide variety of ion sources.

For discovery metabolomics, Agilent GC/MS (single quadrupole and Q-TOF) and LC/MS (TOF and Q-TOF) systems are designed to deliver high-performance data with the reproducibility you need for large sample sets.

Pathway Analysis

Scientists face a serious challenge interpreting sets of data that are becoming increasingly large and more complex.

This can be addressed by projecting and visualizing processed data sets onto biological pathways, thereby taking advantage of knowledge that has accumulated over the last century and a half of research.

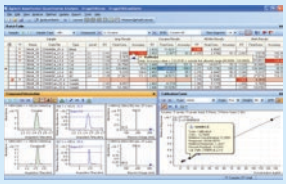
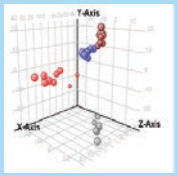
By incorporating a pathway-centric workflow into omics experiments, scientists can concurrently focus on the analysis, and potentially accelerate the process of discovery to biological insight.

Pathway Architect is a visualization tool in MPP that combines publicly available pathway database information with specialized software to deliver a better user experience in the understanding of their biological results. The software maps metabolites, proteins, and genes onto curated pathways, graphically projecting data onto pathways for interactive analysis.

Targeted Metabolomics

Targeted metabolomics experiments focus on quantitating specific metabolites, and use a larger number of samples to accurately measure the abundance of known or previously identified metabolites. It typically involves absolute quantitation and requires the use of analytical standards. However, due to their diverse chemical structures and physical properties, analyzing hundreds of endogenous metabolites can be very challenging. Therefore, it's important to establish optimized MRM methods on triple quadrupole instruments to accurately quantitate hundreds of known metabolites from complex biological samples.

Triple quadrupole instruments are best suited for this task because of their broad dynamic range, highest quantitative sensitivity, and selectivity for compound confirmation.

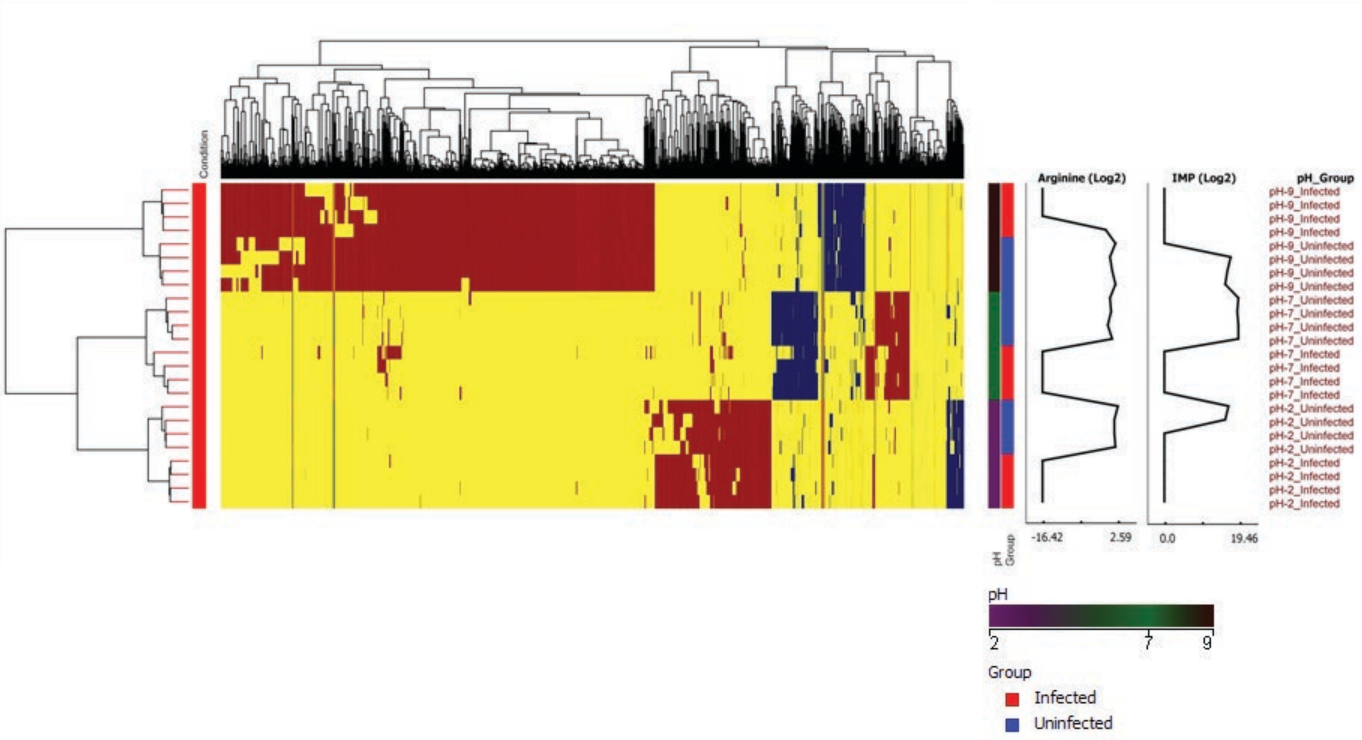
	Separation and Detection	MRM Quantitation	Statistical Analysis	Pathway Analysis
		MassHunter Software	Mass Profiler Professional Software	
Analytical Instruments		 Quant	 Analysis and visualization	 Pathway Architect using public databases, such as KEGG

Agilent solutions for targeted metabolomics research.

For targeted metabolomics, Agilent's triple quadrupole LC/MS and GC/MS systems provide sensitive detection over a large dynamic range, and offer the reproducibility and robustness needed to handle large sample sets. Together with MassHunter Quantitative Analysis reporting software and MPP analysis and visualization tools, Agilent's targeted metabolomics solutions offer unprecedented productivity for large, multi-compound batch analyses.

Agilent's MS systems help you excel in targeted metabolomics by:

- Facilitating detection of low-abundance targets in the presence of high-abundance metabolites
- Providing the user with high sensitivity measurements over a wide dynamic range
- Maintaining low variability for reliable quantitation across large sample sets
- Ensuring robust performance and fast data acquisition speeds to keep up with fast chromatography
- Allowing easy review of methods and data to enable batch processing of large sample set
- Effectively comparing multiple samples using either simple or advanced statistical analysis and visualization tools available in MPP software
- Enabling revalidation of pathways that were proposed by discovery metabolomics studies



Agilent's Mass Profiler Professional software includes Correlation Analysis tools, which can measure the strength and directionality of pair-wise relationships between any two variables. Sample metadata plots provide visual clues to the sample grouping patterns in relation to clustering analysis.

Software Tailored to Your Metabolomics Research Needs

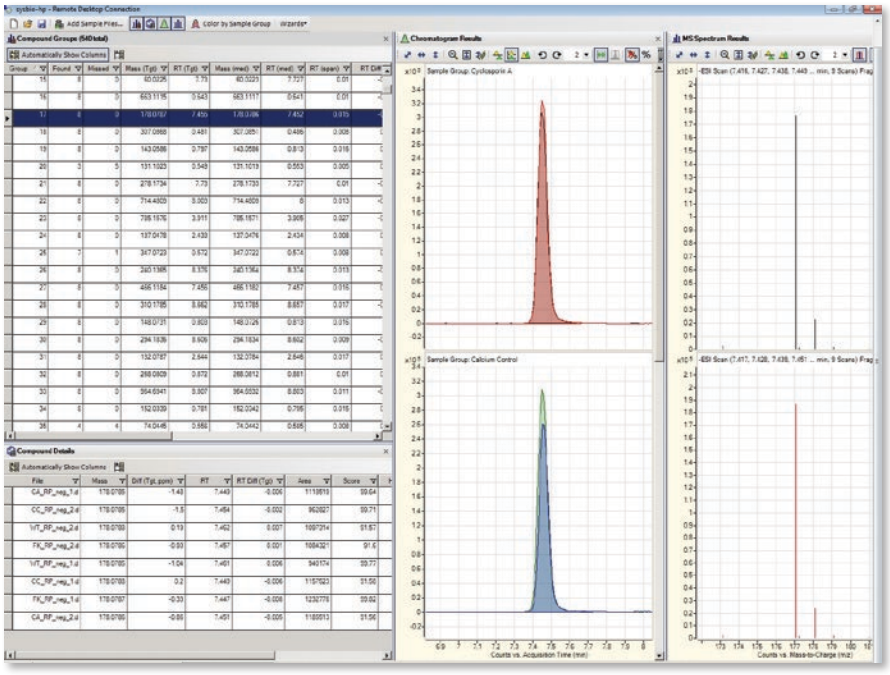
Agilent offers advanced analysis software for processing complex metabolomics data. Features are extracted using either MassHunter Qualitative Analysis or Profinder. Profinder is the most advanced feature-finding solution.

Two different data mining workflows are supported: 1. untargeted feature extraction, and 2. targeted feature extraction. Extracted features are then analyzed in MPP – a comprehensive biostatistics and visualization tool for MS data.

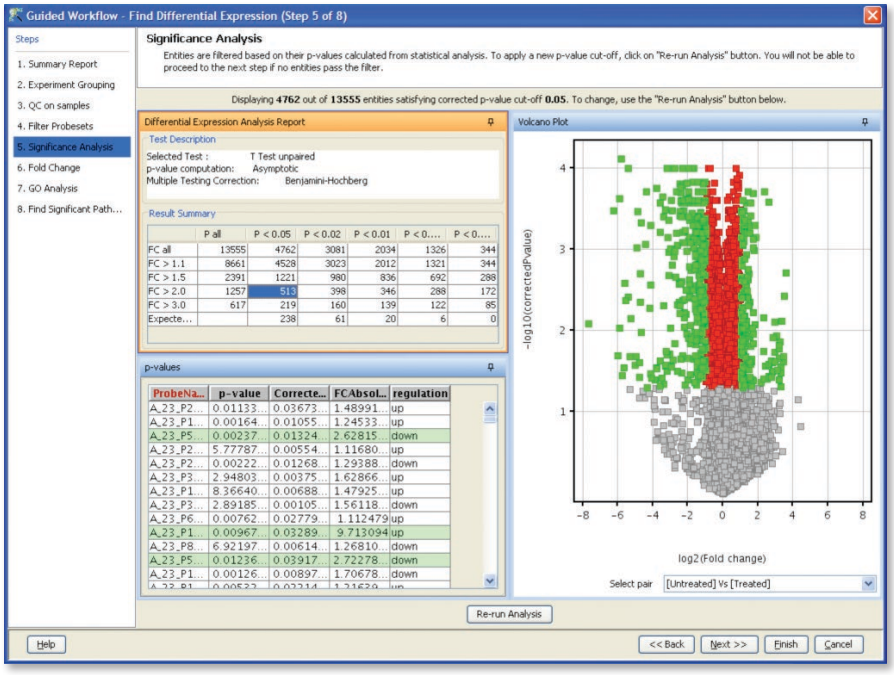
With MPP, you can align, normalize, compare, and visualize data from large sample sets. Metadata can be added to the analysis to help find relationships in complex sample data.

MPP supports finding correlations between different samples, groups, or entities. Statistically significant differences can be found using ANOVA, PCA, correlation clustering, class predictions, or with customized R scripts.

Within MPP, you can also annotate features by matching their retention time and spectra against the METLIN Personal compound database and library or the Agilent Fiehn GC/MS RTL Metabolomics Library.



MassHunter Profinder performs robust feature extraction of data acquired in untargeted mode. Specifically designed for speed, it enables analysis of a large number of data files through a simple, user-friendly GUI.



MPP software helps you elucidate differences in your metabolomics experiments. Agilent's Mass Profiler Professional software includes multivariate analytical tools, such as principal component analysis, ANOVA, clustering algorithms, and class prediction, to efficiently turn large sample sets into meaningful information.

"Compound identification is a major bottleneck in metabolomics. To address this challenge, I am pleased to collaborate with Agilent Technologies to help them develop the METLIN Personal compound database with MS/MS spectral library, and I look forward to our continued efforts with Agilent to develop new tools that benefit the metabolomics community."

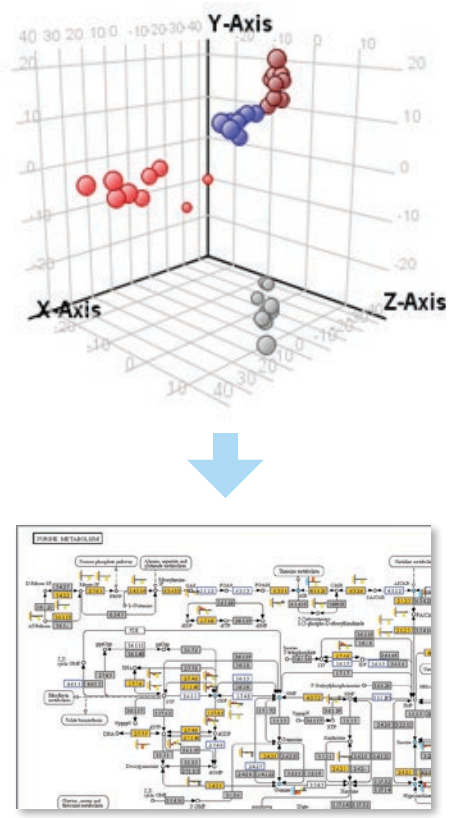
GARY SIUZDAK, PH.D.,
SENIOR DIRECTOR, SCRIPPS CENTER FOR MASS SPECTROMETRY

Pathway Analysis

It's not enough to know what metabolite or protein is responsible for the observed differences; you want to understand the biological context. MPP's Pathway Architect module interactively filters, maps, and visualizes data onto biological pathways. Two types of pathway analyses are supported: one is a Literature Derived Network analysis based on natural language processing of published literature; the other is designed to analyze publicly available curated biological pathways, such as KEGG, BioCyc and Wikipathways. The experimental data is projected onto these pathways where the user can filter, zoom, or select data on the pathway. Any pathway(s) can be selected and a list of metabolites, proteins, transcripts and genes can be exported and used by other programs to create new "Pathway Directed Experiments." For example, the protein identifiers for a given pathway can be exported to create a targeted peptide analysis.

Integrating Metabolomics with Other Omics

While genomics, transcriptomics, proteomics, and metabolomics are in wide use in both industry and academia, these experiments—performed alone—are often insufficient to uncover meaningful correlations amid the high level of noise omics experiments typically generate. Integration of data from multiple omics can, in some cases, provide enough constraints to greatly reduce the false discovery rate. The Pathway Architect module of Mass Profiler Professional allows either single omics analysis or joint analysis of multiple omics, enabling you to discover commonly affected pathways and aid in your ability to find reliable answers more quickly.



The KEGG pathway for purine metabolism shows nodes (teal) that represent metabolites; adjacent to the nodes are "HeatStrips" summarizing the average differential abundance values for the different conditions. A teal bar along the HeatStrip indicates metabolite; a yellow bar indicates a result for gene expression.

AGILENT'S METABOLOMICS SOLUTIONS

GC/MS Instruments and Software

5977A Series GC/MSD System

The Agilent 5977A Series GC/MSD is the latest addition to the industry's broadest, most feature-rich portfolio of GC and GC/MS systems and software.

7000C Triple Quadrupole GC/MS System

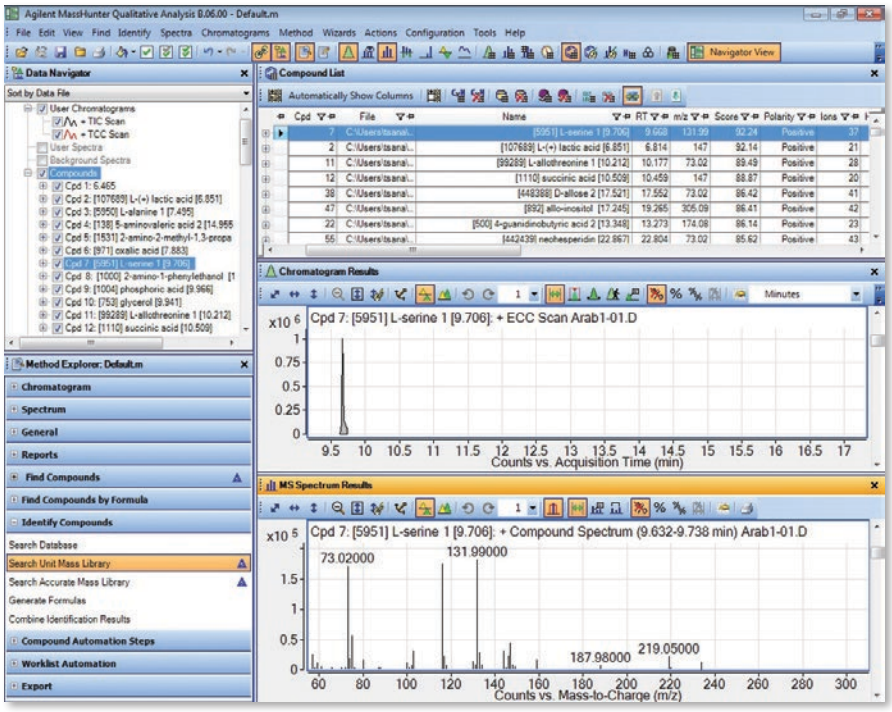
The 7000C Triple Quadrupole GC/MS was designed to deliver the most accurate quantitative results and confident identification even in the most complex matrices.

7200B GC/Q-TOF System

The 7200B Series GC/Q-TOF system expands on the proven separation power of the Agilent 7890B GC and now includes a backflush-ready configuration with every system.

Agilent Fiehn GC/MS Metabolomics Library

Developed with Dr. Oliver Fiehn, this is the largest commercially available and growing metabolomics-specific library, containing searchable GC/MS EI spectra and retention-time indices from approximately 900 common metabolites. The library comes with complete, preprogrammed GC/MS methods, and documents for GC/MS metabolomic analysis to maximize research success.



MassHunter Qual supports feature extraction of Agilent GC/MSD files using the "Find by Chromatographic Deconvolution" algorithm. Feature extracted peaks are matched to the Agilent Fiehn GC/MS Metabolomics Library for identification.

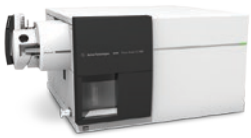
LC/MS Instruments and Software

6200 Series Accurate-Mass TOF

Ideal for profiling and identifying low molecular-weight compounds, the 6200 TOF LC/MS provides a typical mass accuracy of 1–2 ppm with attomole level sensitivity. The instrument has a linear dynamic range of five orders of magnitude and spectral acquisition rates of up to 20 spectra per second to take advantage of UHPLC separations.

6500 Series Accurate-Mass Q-TOF

Offers the same capabilities as the 6200 Series TOF LC/MS, plus MS/MS functionality. The typical mass accuracy (2–4 ppm MS/MS) increases confidence in small molecule identification and reduces false positives in database searches. Spectral acquisition rates of up to 50 MS/MS spectra per second take advantage of UHPLC separations.

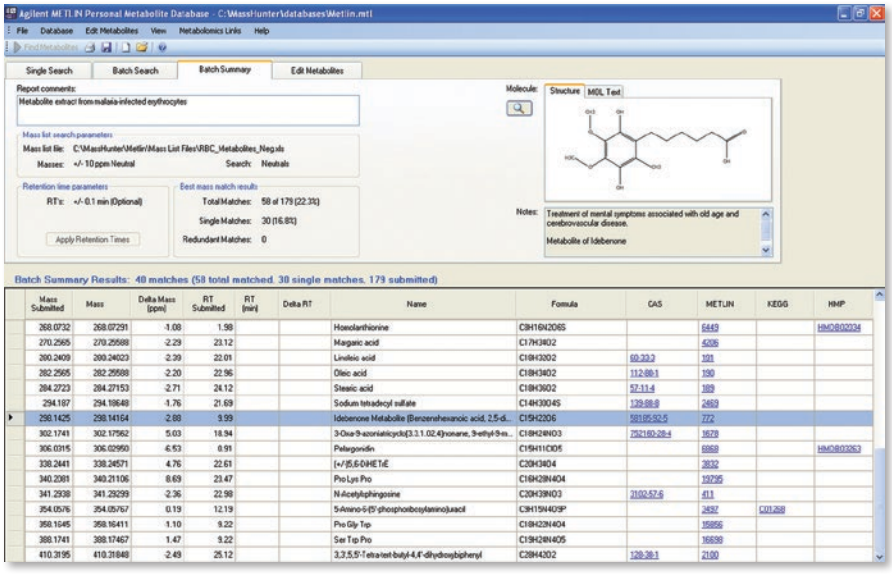


6400 Series Triple Quadrupole LC/MS

The sub-femtogram-level sensitivity enables detection of low-abundance compounds. With extremely fast MRM transitions and robust and reliable performance, the 6400 Series Triple Quadrupole LC/MS enables maximum uptime to analyze large sample sets. The common ion optics also allow easy method transfer from the Agilent Q-TOF to the triple quadrupole as you progress from discovery to validation.

METLIN Personal Compound Database and Library

METLIN Personal compound database contains approximately 80,000 compounds, including 36,500 lipids. Used with TOF and Q-TOF data, identification is enabled using accurate mass and/or retention time database searching. Searching the MS/MS spectral library with more than 9,000 compounds provides more confident metabolite identification.



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