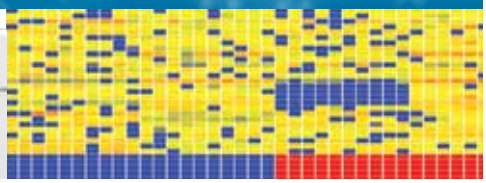
Agilent Solutions for Metabolomics

EMERGING INSIGHTS

The Measure of Confidence



Agilent Technologies

UNDERSTANDING METABOLIC FINGERPRINTS

A Powerful Way to Investigate Biology

What is Metabolomics?

Metabolomics is the study of the metabolite content of a cell or whole organism. Metabolomics studies allow researchers to view biological systems in a way that is different from but complementary to genomics, transcriptomics, and proteomics studies. It is a powerful, emerging discipline with a broad range of applications, including basic research, clinical research, drug development, environmental toxicology, crop optimization, and food science.

What are Metabolites?

Metabolites—small organic molecules—are important modulators, substrates, byproducts, and building blocks of many different biological processes. Because of this, the presence or absence of specific metabolites in a cell or sample provides important information about the physiological and functional status of the biological system or test sample. The accumulation of a specific metabolite could signal a defect in a pathway, activation of a signal response pathway, or optimization of a biosynthetic pathway.

At Agilent, we are collaborating with leading metabolomics researchers—the scientists who are developing the next generation of metabolomics research techniques and tools—and incorporating their developments into robust solutions that simplify your metabolomics research. We offer a comprehensive line of high-performance GC/MS and LC/MS instruments, providing the renowned Agilent performance and reliability that is required for making rapid, high-quality metabolomics measurements. Each instrument uses the same data analysis software, creating a common user experience for experiments that require multiple mass spectrometry configurations. Furthermore, the processed data can be analyzed using Agilent Mass Profiler Professional (MPP) software, a powerful statistical analysis and data visualization tool that quickly allows you to find and extract meaningful results from your data. You can then project your data onto curated pathways using the powerful Pathway Architect software (an optional module for MPP).

Agilent Enables Metabolomics Research for a Variety of Applications

Agilent offers researchers a comprehensive portfolio of metabolomics reagents, columns, instruments, and software to support metabolomics experiments with a number of applications including:

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/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
Identify metabolite profiles to optimize fermentation processes and biofuel production

"...we can measure, in metabolomics, the entire small molecule component of a biological sample. And what this does is it gives us a picture at the biochemical level of what the physiological status of the organism or sample was at the time that it was collected. And this gives us a window into the actual functional state of the cell in some ways that is very different from either the proteomic, or the transcriptomic, or the genomic points of view."

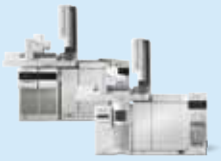
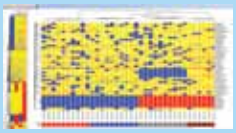
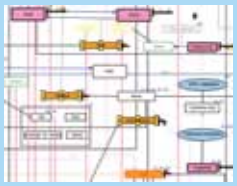
- **CHRIS BEECHER, PH.D.,**
CHIEF SCIENTIFIC OFFICER
NEXTGEN METABOLOMICS

USING THE POWER OF METABOLOMICS

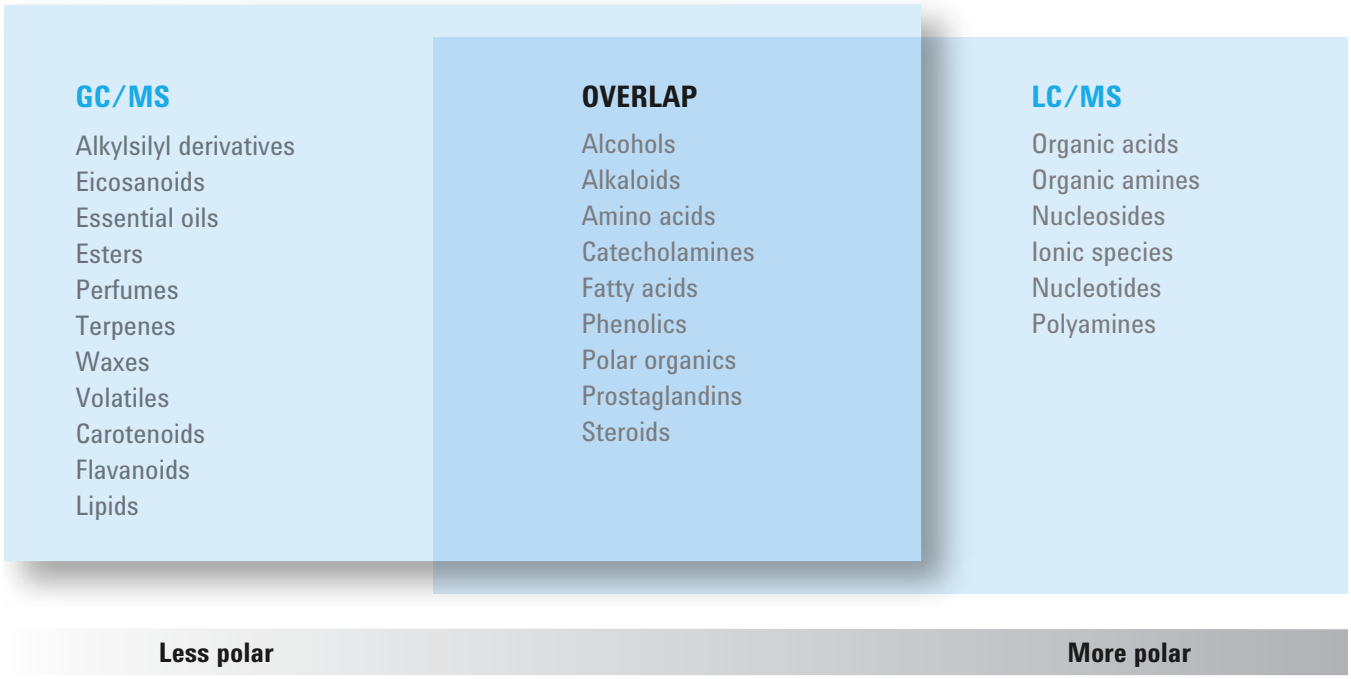
Whether you are conducting discovery metabolomics studies or targeted metabolomics studies, Agilent's metabolomics portfolio and knowledgeable scientists can help you quickly and reliably obtain and analyze the data you need.

Discovery Metabolomics

Discovery metabolomics experiments involve examining an untargeted suite of metabolites, finding the ones with statistically significant variations in abundance within a set of experimental versus control samples, and determining their chemical structure. Pathway analysis allows the researcher to connect the metabolite with the biological process or condition.

	Separation and detection	Feature finding and quantitation	Alignment and statistical analysis	Identification	Pathway analysis
		MassHunter software	MPP software		
GC/MS			 Analysis and visualization	 Compound identification	 Pathway Architect
LC/MS					

Agilent solutions for discovery metabolomics



Chemical classes suitable for GC/MS versus LC/MS

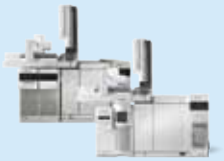
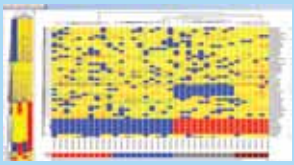
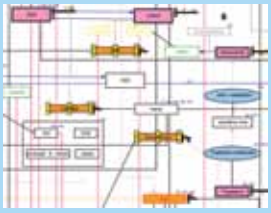
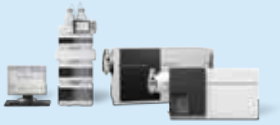
Agilent helps you overcome challenges in performing discovery metabolomics research:

- Reproducibly track unknowns from sample to sample with MS systems that provide highly reproducible measurement of mass and retention time.
- Accurately measure abundances in each sample to enable reliable comparison of metabolite amounts when standards cannot be used.
- Find features of interest with tools that enable you to review and edit your results: AMDIS (GC/MS) or MassHunter Molecular Feature Extractor (LC/MS).
- Identify unknown compounds using chemical databases integrated with Agilent MassHunter: Agilent Fiehn GC/MS RTL Metabolomics Library and METLIN Personal compound database and MS/MS spectral library.
- Effectively analyze and compare multiple samples with simple or advanced statistical analysis and visualization tools available in MPP. MPP analyzes both GC/MS and LC/MS data.
- Evaluate MS data in a biological context using the optional MPP Pathway Architect module.

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.

Targeted Metabolomics

Targeted metabolomics experiments focus on validation, and use a large number of samples to accurately measure the abundance of previously identified metabolites. It is highly quantitative and usually requires the use of analytical standards.

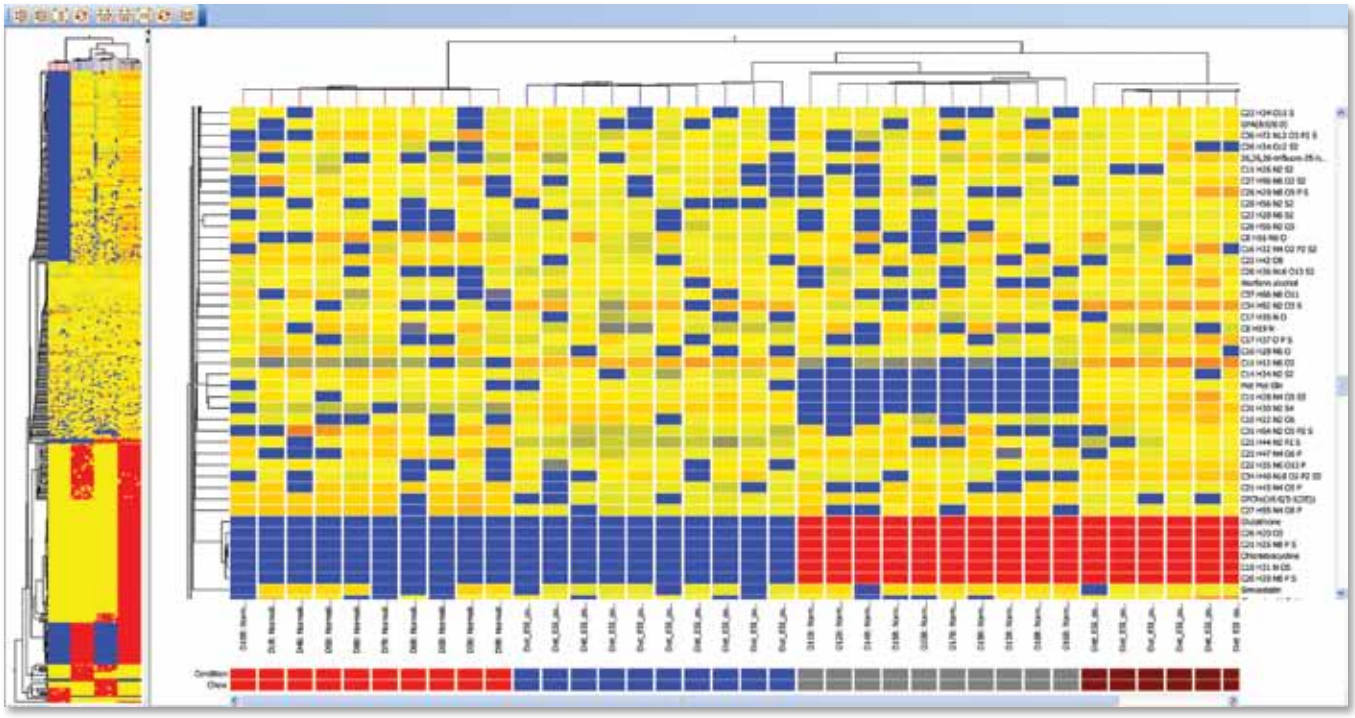
	Separation and detection	MRM quantitation	Statistical analysis	Pathway analysis
		MassHunter software	MPP software	
GC/MS		 Compound identification	 Analysis and visualization	 Pathway Architect
LC/MS				

Agilent solutions for targeted metabolomics research

Agilent helps you overcome challenges in performing targeted metabolomics research:

- Detect low-abundance targets in the presence of high-abundance metabolites with Agilent's MS systems that offer high sensitivity over a wide dynamic range.
- Maintain low coefficients of variation (CV), enabling reliable quantitation of large sample sets.
- Rapidly and efficiently analyze large sample sets with MS systems that provide robust performance and fast data acquisition speeds to keep up with fast chromatography.
- Easily review methods and data to enable batch processing of large sample sets.
- Effectively analyze and compare multiple samples with simple or advanced statistical analysis and visualization tools available in MPP software, and revalidate pathways proposed by discovery metabolomics studies.

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 and MPP analysis and visualization tools, Agilent's targeted metabolomics solutions offer unprecedented productivity for large, multi-compound batches.



MPP software allows you to identify correlations in your data through a number of clustering methods including hierarchical clustering (shown here). Hierarchical clustering connects similar abundance profiles together in a group within a tree structure. The dendrogram view reveals the relationships between mass entities in one dimension and between samples in the other dimension. In this example, rat chow diets with differing bean supplements show similarities in abundance profiles for the replicate samples.

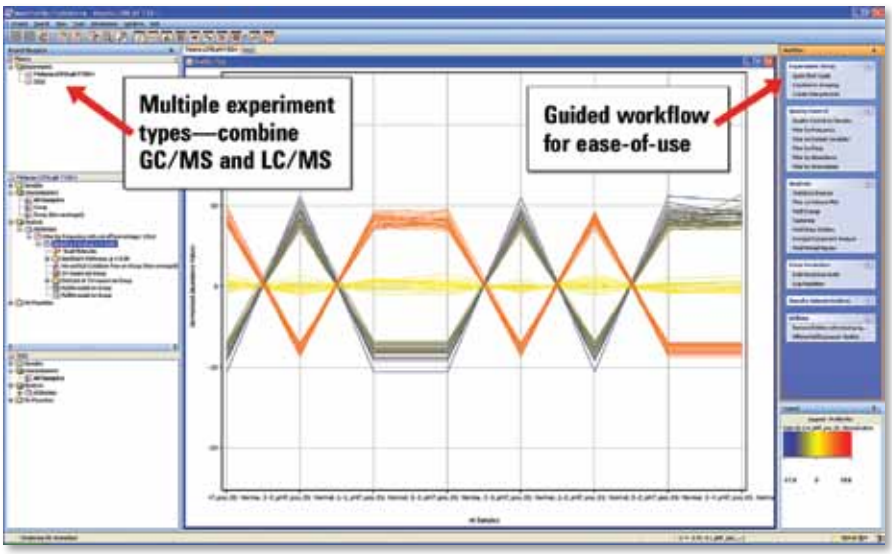
Software Tailored for Your Metabolomics Research Needs

Whether you are conducting discovery or targeted metabolomics research, you can analyze your data using the same, powerful software package, Mass Profiler Professional (MPP).

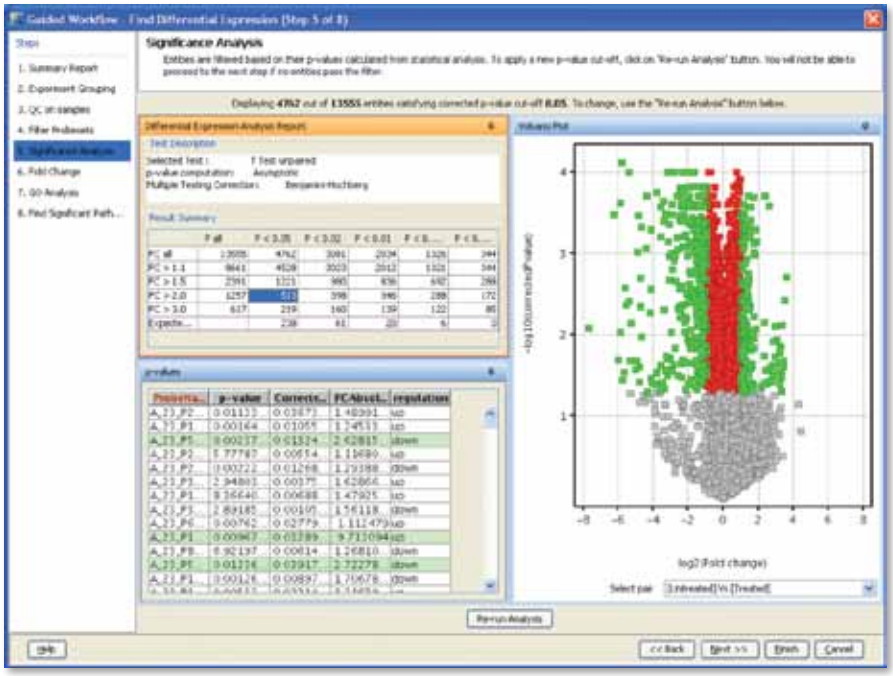
With MPP, you can compare and visualize data from large sample sets. Meta data can be added to the analysis to help you find relationships in complex experimental designs. You can also normalize, align, and compare data taken from multiple instruments, a critical feature for laboratories running multiple metabolomics experiments.

MPP makes it easy to identify important differences in the type and relative amount of metabolites present in different samples, facilitating your ability to place the data into a biological context. You can identify statistically significant differences using ANOVA, PCA, clustering, volcano plot, hierarchical clustering, class predictions, or customize analysis by writing your own R scripts.

Within the MPP environment, you can also determine the chemical identity of metabolites by searching their retention time and spectra against the METLIN Personal compound database and library or the Agilent Fiehn GC/MS RTL Metabolomics Library. In addition, for accurate-mass data, the ID Browser can calculate empirical formulas using spectral data. Empirical formulas can be combined with accurate-mass databases to increase confidence in compound identification.



Powerful, easy-to-use workflow-guided MPP software allows you to combine multiple experiment types using a single software platform.



MPP software helps you elucidate differences in your GC/MS and LC/MS 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."

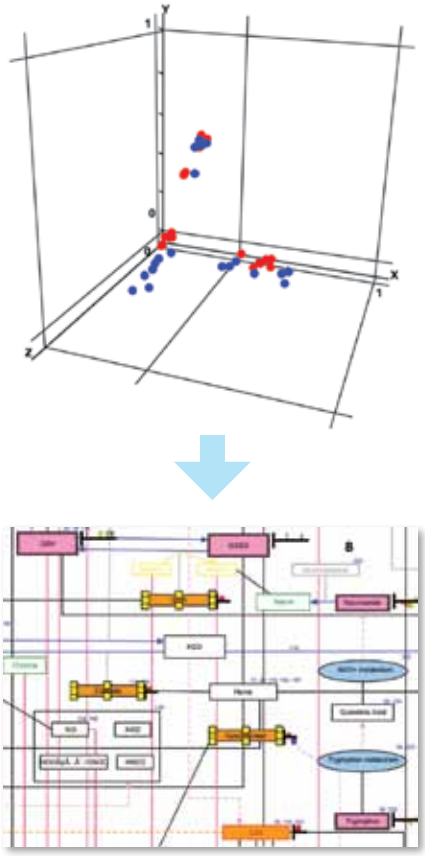
- GARY SIUZDAK, PH.D.,
SENIOR DIRECTOR, SCRIPPS CENTER FOR MASS SPECTROMETRY

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. The optional Pathway Architect module of MPP interactively filters and visualizes data on 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 in BioPax or GPML format. The experimental data is projected onto these pathways where the user can filter, zoom, or select data on the pathway. A pathway(s) can be selected and a list of metabolites, proteins, transcripts and genes can be exported for use 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 increasing your ability to find reliable answers more quickly.



MPP software allows comparison of multiple samples and/or multiple MS analysis platforms in a single project (top). The software finds relationships beyond pair-wise comparison in complex experimental designs and enables biological interpretation through Pathway Architect (bottom).

AGILENT'S METABOLOMICS INSTRUMENTS AND SOFTWARE

GC/MS Instruments and Software

5975 Series GC/MS

Best-in-class, single-quadrupole sensitivity to detect low-abundance metabolites, a chemically inert ion source to eliminate metabolite degradation, and superior reproducibility for maximum confidence in your results.

7200 GC/MS Q-TOF

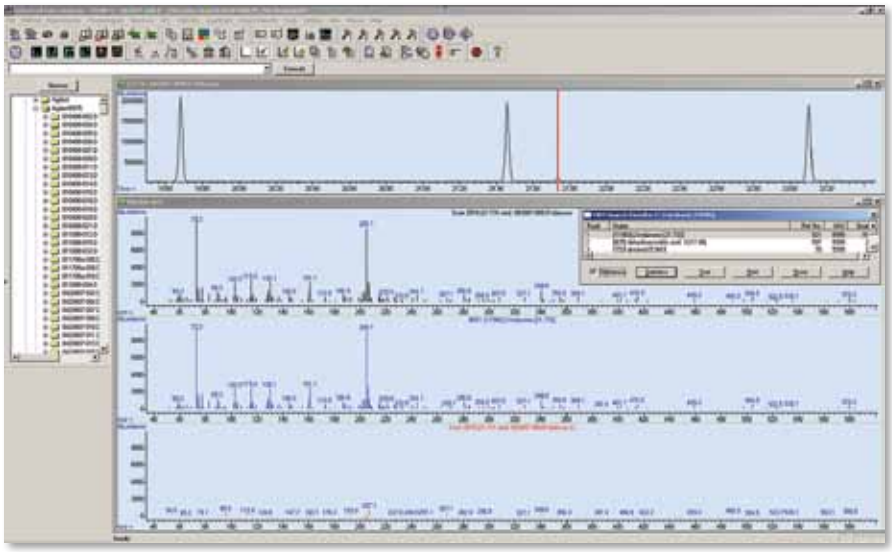
The first commercially available accurate mass Q-TOF designed specifically for classical electron impact (EI) and chemical ionization (CI) mass spectrometry. The instrument delivers high sensitivity and accurate mass MS/MS data to facilitate molecular characterization and structural identification.

7000 Series Triple Quadrupole GC/MS

Ideal for quantitation/targeted metabolomics, with superior selectivity that ensures routine femtogram sensitivity enabling the lowest limits of detection and quantitation available.

Agilent Fiehn GC/MS Metabolomics Library

Developed with Dr. Oliver Fiehn, this growing metabolomics-specific library contains searchable GC/MS EI spectra and retention-time indexes from approximately 700 common metabolites. The library comes with complete, preprogrammed GC/MS methods, and documents GC/MS metabolomic analysis to maximize research success.



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 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

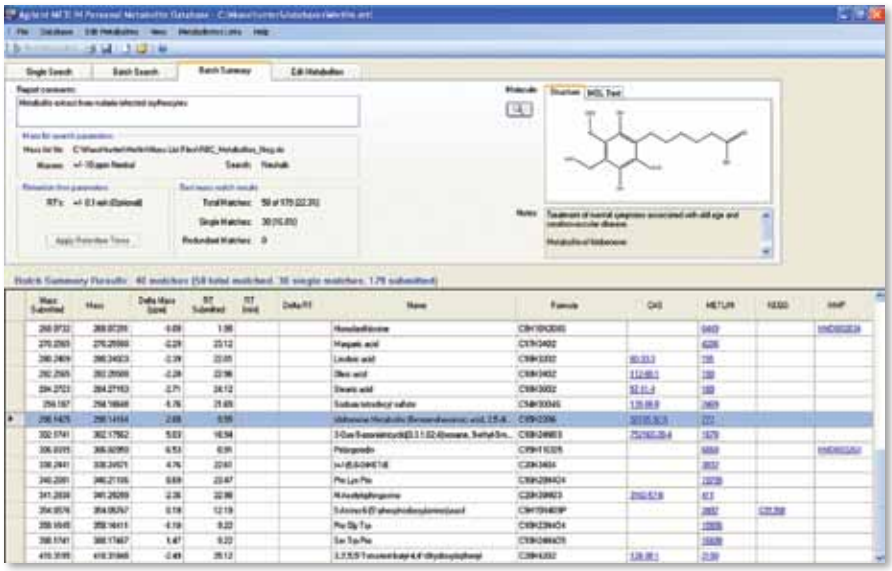
Same capabilities as the 6200 Series TOF LC/MS, but with MS/MS functionality. The typical mass accuracy (2–4 ppm MS/MS) increases confidence in small molecule identification and reduces false positives in database searches. Spectral acquisition rates of up to 50 MS/MS spectra per second take advantage 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 25,000 compounds, including 8,000 lipids with retention times for about 700 standards. 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 2,200 compounds enables more confident identification.



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