YOUR PATH TO SUCCCESS

AGILENT SOLUTIONS
FOR METABOLOMICS

Agilent Technologies

UNDERSTANDING METABOLOMICS

Agilent is the leading global solution vendor for measuring metabolism, offering researchers a broad array of cutting-edge instrumentation and innovative informatics solutions.

What is Metabolomics?

Metabolomics is the study of endogenous metabolites, called the metabolome, which is a collection of low molecular weight (50-1,500 Da) compounds with a wide range of physiochemical properties. Measuring the metabolome provides important information about the functional status of a biological system. Its close proximity to the phenotype of an organism provides complementary information to genomics and proteomics.

What is Qualitative Flux Analysis?

Metabolomics is a powerful technique for understanding biological systems by measuring the abundance of metabolites, however, understanding is often complicated by a lack of dynamic information. Significant changes in flux through a pathway may not result in altered abundance of metabolite intermediates. Changes in flux are the result of either differences in the amount of enzyme present (e.g. transcriptional levels) or activity of the enzyme (e.g. inhibitors or mutations). Qualitative flux analysis highlights the relative rate of reactions using stable isotope tracing (typically containing ^{13}C, ^{15}N, or ^{2}H) and results in changes in the natural isotopic pattern of downstream metabolites.

Agilent Metabolomics Solutions

Agilent's innovative metabolomics solutions provide a powerful portfolio of instruments and informatics tools. A common software platform combines the results from multiple analytical techniques, helping answer challenging biological questions faster. Agilent collaborates with leading metabolomics scientists to develop next-generation solutions and workflows to accelerate your research.

METABOLOMICS TOOLS FOR A VARIETY OF RESEARCH APPLICATION

A Powerful Way to Investigate Biology

Basic and Clinical Research

Identify and verify 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

Systems 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

DISCOVERY METABOLOMICS

Discovery metabolomics typically involves global profiling of metabolites by hyphenated MS techniques. Following separation and detection of compounds, features are found across all data files. The results are statistically analyzed and differential features are identified. The identified metabolites are visualized on biological pathways to aid interpretation.

Agilent workflow for discovery metabolomics

Agilent has developed robust workflows for performing global metabolite profiling for metabolites by GC/MS, LC/MS, CE/MS, and SFC/MS. Data is usually acquired in both ion polarities and a wide variety of ion sources are available. For discovery metabolomics, typically an Agilent GC/MSD, GC/Q-TOF, LC/TOF or LC/Q-TOF system is used.

Feature finding extracts data based on mass spectral and chromatographic characteristics to generate a complete list of compounds which includes molecular weight, retention time, *m/z*, and abundance. MassHunter Profinder is uniquely designed to work on a batch of data and has a recursive mode to address missing features. Profinder results are exported in a file format designed for Mass Profiler Professional (MPP), software developed for statistical analysis of mass spectrometry data

MPP uses a combination of advanced processing capabilities and powerful statistical and mathematical models to analyze complex MS data sets. Within MPP, features can be annotated using the Agilent-METLIN PCDL or the Agilent-Fiehn Library. Pathway Architect is a visualization tool in MPP that maps results (metabolites, proteins, and genes) onto curated pathways.

TARGETED METABOLOMICS

Targeted metabolomics experiments focus on measuring specific metabolites. Triple quadrupole mass spectrometers are best suited for this task because of their broad dynamic range, highest sensitivity, and selectivity for compound confirmation.

Agilent workflow for targeted metabolomics research

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.

Metabolomics dMRM Database and Method

The Agilent Metabolomics dMRM Database and Method enables straightforward implementation of an optimized LC/MS/MS analysis of >215 central carbon metabolites, including organic acids, sugars, sugar phosphates, and nucleotides. In order to generate a robust methodology that yields high quality results, the analytical method was developed with Adam Rosebrock, PhD, from the University of Toronto. The solution includes a curated database with retention times, optimized MS/MS acquisition parameters, and a data acquisition and analysis method.

The dMRM database and method are part of Agilent's comprehensive metabolomics workflows and results are supported in Agilent's leading software workflow. Exported results include metabolite names, integrated peak abundances, and chemical identifiers (CAS) for easy import into Mass Profiler Professional and Pathway Architect for multivariate sample comparisons and pathway analysis.

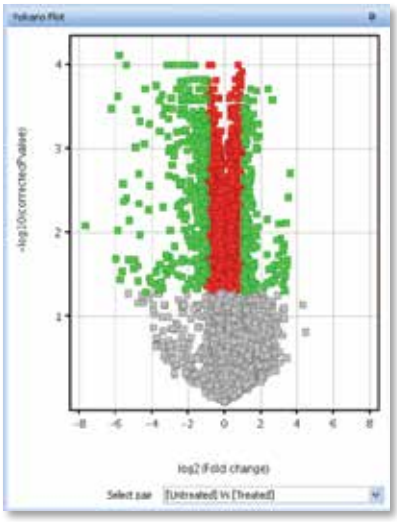
Software Tailored to Your Metabolomics Research Needs

Metabolomics researchers face a serious challenge analyzing sets of data that are becoming increasingly large and more complex. Multivariate statistics are commonly used to find differences between sample groups. However, it's not enough to know what metabolites are differential; understanding the biological context is critical. Projecting and visualizing processed data sets onto metabolic pathways utilizes existing knowledge and facilitates biological understanding. Agilent offers advanced analysis software for processing and interpreting complex metabolomics data.

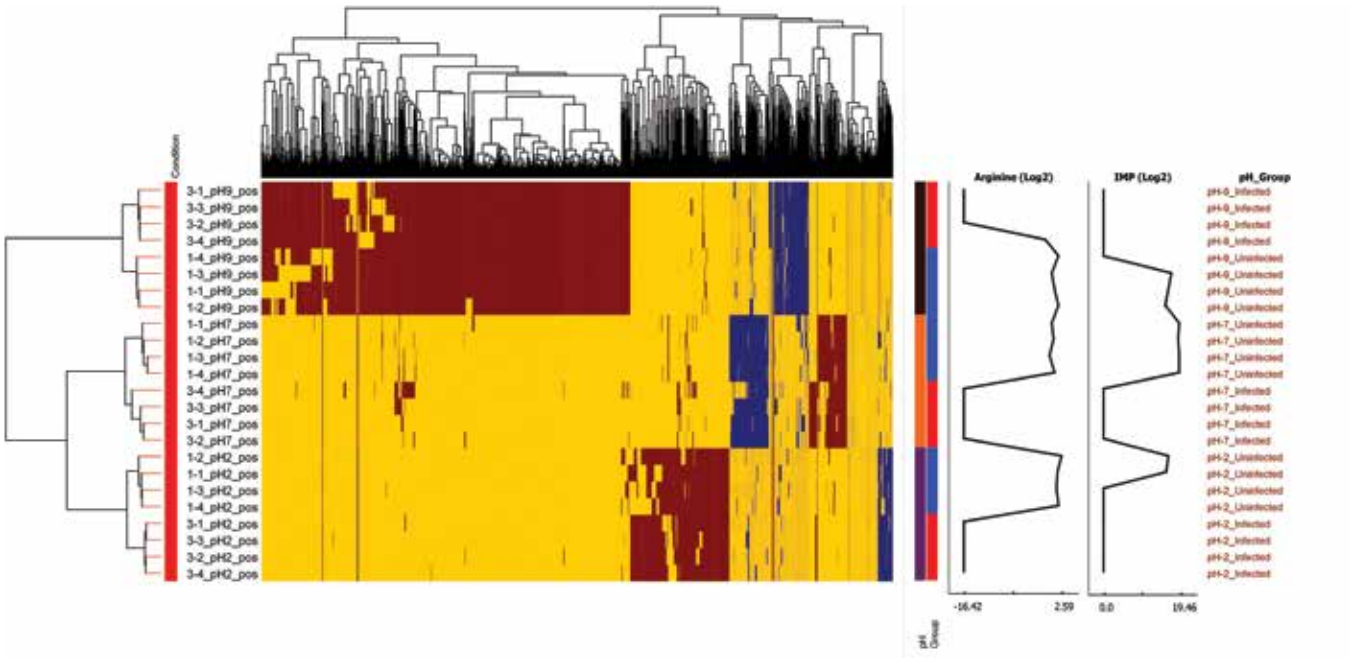
In discovery metabolomics, either untargeted or targeted data mining is the first step in the software workflow. MassHunter Profinder provides targeted and untargeted batch feature extraction for mass spectrometric data. It supports MS-only data from Agilent's broad portfolio of mass spectrometers including TOF-based instruments as well as nominal mass GC/MS systems. Profinder offers grouping of replicate data files, binning & alignment, chromatogram overlay, manual re-integration of compounds, and easy export of results for subsequent statistical analysis.

Agilent Mass Profiler Professional software provides multivariate analytical tools for discovery and targeted metabolomics. This includes principal component analysis, ANOVA, clustering algorithms, correlation analysis, and class prediction, to efficiently turn large sample sets into meaningful information. Metadata can be added to the analysis to help find relationships in complex sample data.

Within MPP, features can be annotated using the built-in ID Browser function. ID Browser matches features based on retention time and spectra against the highly-curated Agilent-METLIN LC/MS database or the Agilent-Fiehn GC/MS Library. These metabolomics specific databases include compound identifiers for subsequent mapping to pathways.



Mass Profiler Professional software includes volcano plot functionality to simultaneously calculate the fold-change in abundance for each mass entity as well as p-value significance. These settings can be changed interactively and the results can be viewed in graphical as well as table format.



Mass Profiler Professional software includes correlation analysis tools, which can measure the strength and directionality of pair-wise relationships between any two variables. This heat map shows mass spectrometry abundance data after hierarchical clustering with the correlated metabolites (Arg and IMP) on the right.

"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

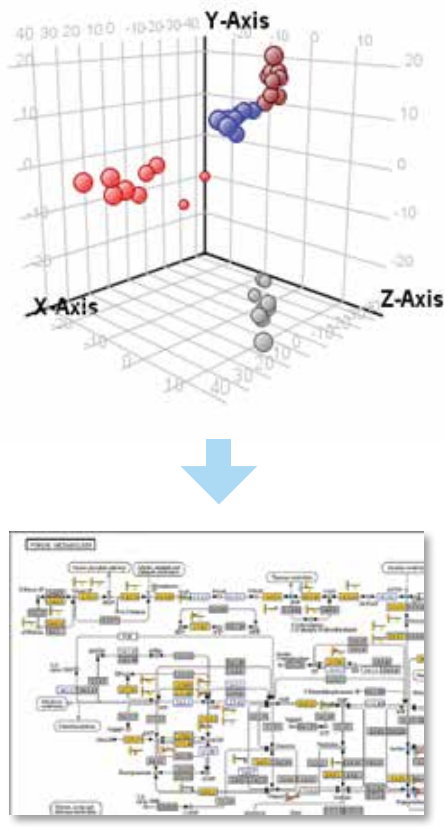
Pathway Analysis

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. MPP's Pathway Architect module interactively filters, maps, and visualizes data on biological pathways using publicly available pathway databases such as KEGG, BioCyc and Wikipathways. The software maps metabolites, proteins, and genes onto curated pathways, graphically projecting data onto pathways for interactive analysis.

The experimental results from MPP are projected onto 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 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. This pathway-centric workflow speeds the route from discovery to insight and enables efficient planning and execution of the next series of experiments.

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 provide enough constraints to greatly improve the signal-to-noise of the analysis. 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.

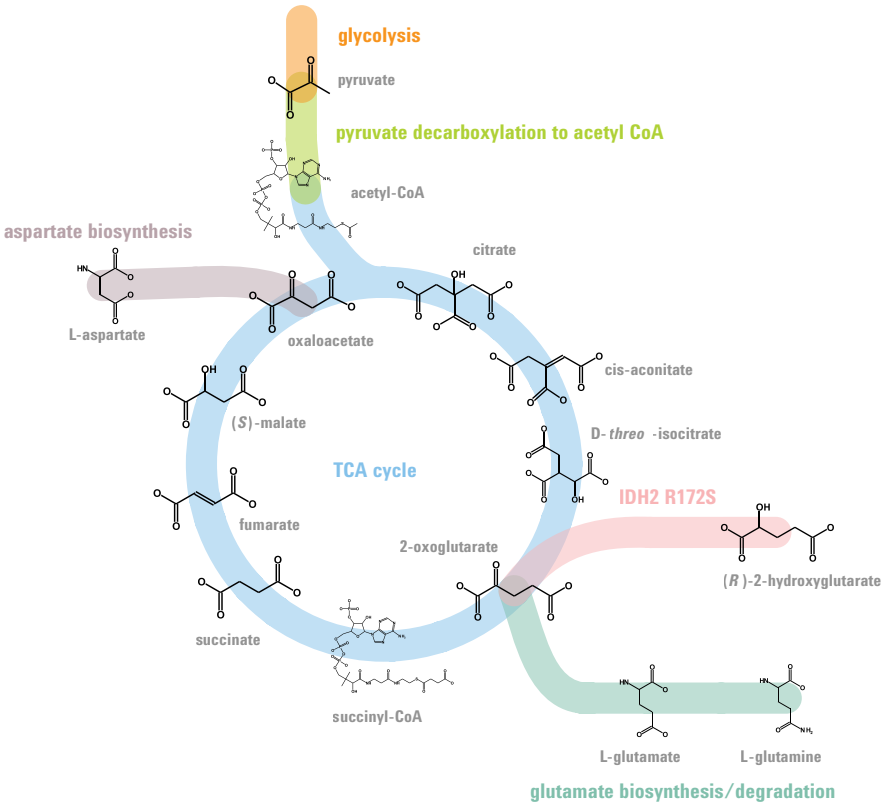


The KEGG pathway for purine metabolism shows nodes (teal) that represent metabolites; adjacent to the nodes are "HeatStrips" summarizing the average differential abundance values for the different conditions. A teal bar along the HeatStrip indicates metabolite; a yellow bar indicates a result for gene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

Software Tailored to Your Metabolomics Research Needs

Qualitative flux analysis allows researchers to quickly obtain information about selected pathways and fluxes using stable isotope label tracing with mass spectrometric analysis. Qualitative flux analysis presents multiple analytical challenges such as mining the target metabolites, accounting for isotopologues, correcting for naturally occurring isotopes, and visualizing the results in a biological context. MassHunter VistaFlux is software designed to meet these challenges and is designed as a qualitative flux analysis solution for MS-only data from Agilent TOF-based high resolution LC/MS systems.

VistaFlux is composed of 4 software packages that facilitate the workflow: create and edit a target metabolite list (Pathways to PCDL and PCDL Manager), extract metabolite isotopologue data (Profinder), and visualize results on pathways (Omix Premium).



Omix Premium visualization of the TCA cycle modified for use in a IDH2 mutant cell line study.

In qualitative flux analysis, a stable isotope labeled tracer (^{13}C, ^{15}N, or ^{2}H) is introduced into the biological system and results in changes in the natural isotopic pattern of downstream metabolites. Following analysis by LC/MS, the data is mined in Profinder using a target list derived from known metabolic pathways. Metabolites differing only in isotopic composition (isotopologues) are measured for each target compound and this information is used to track metabolic flux.

Profinder easily exports batch results (.pfa format) to Omix Premium for pathway visualization and biological interpretation. Using both static and animated pathway visualizations, experimental results are easy to communicate and it is simple to export figures for use in publication and presentations.

A Powerful Way to Investigate Biology

An Innovative Approach to Metabolism

Measuring functional metabolism in live cells and determining the metabolic phenotype provides a powerful guide for metabolomics experimental design.

Agilent Seahorse XF Analyzers provide a downstream approach to measuring cellular bioenergetics, enabling rapid assessment of metabolic function in live cells under multiple conditions.

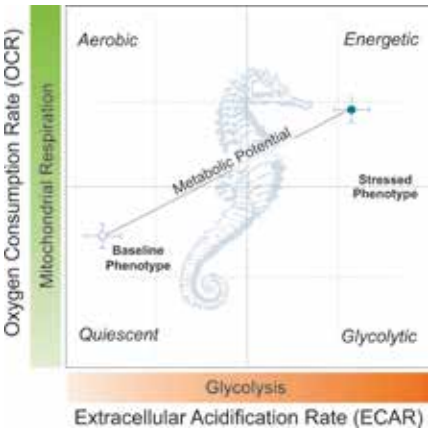
Determining the effects of chemical or genetic manipulations on cellular metabolic function quickly and easily yields complementary data that adds efficiency and direction to metabolomic research.

Metabolic Rates in Minutes

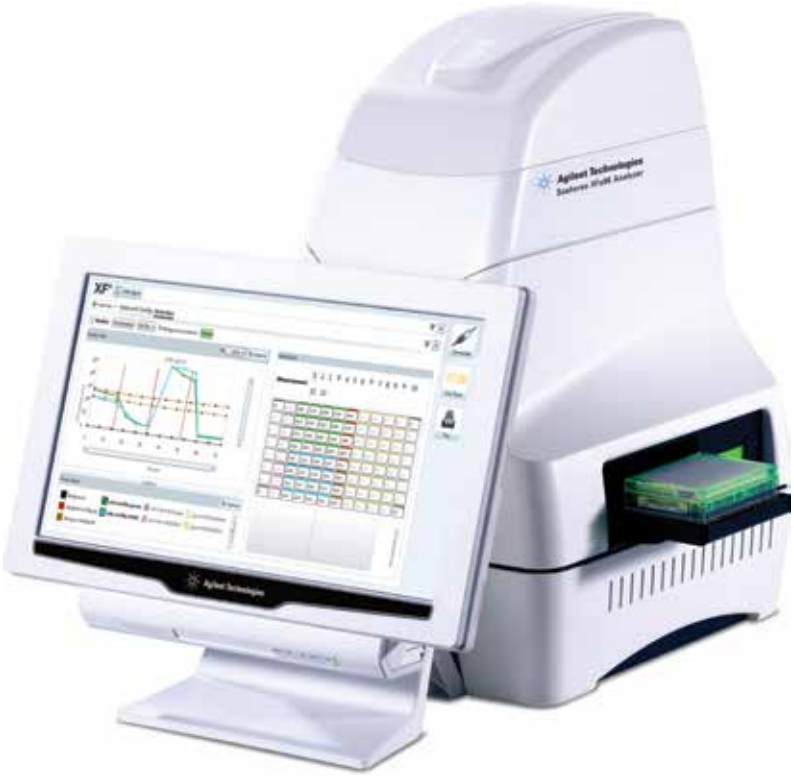
Seahorse XF Analyzers measure the activity of the two major energy-producing pathways of the cell – mitochondrial respiration and glycolysis – in live cells, in real time.

Seahorse XF Analyzers feature automated compound addition, solid-state fluorescence sensors in a disposable microplate cartridge, and software that automatically calculates and analyzes metabolic parameters.

Seahorse XF assay kits and reagents specifically developed for pathways, such as oxidative phosphorylation, glycolysis, and fatty acid oxidation, enable researchers to further interrogate and analyze cell function with same-day results.



The Seahorse XF Cell Energy Phenotype Test provides a high-level assessment of metabolic state and pathway preference, delivering results in under an hour.



Seahorse XF analyzers are available in 96, 24, and 8-well formats to accommodate a range of throughput and sample needs: adherent and suspension cells, cultured or ex vivo samples, model organisms, and isolated mitochondria.

AGILENT'S METABOLOMICS SOLUTIONS

GC/MS Instruments and Databases

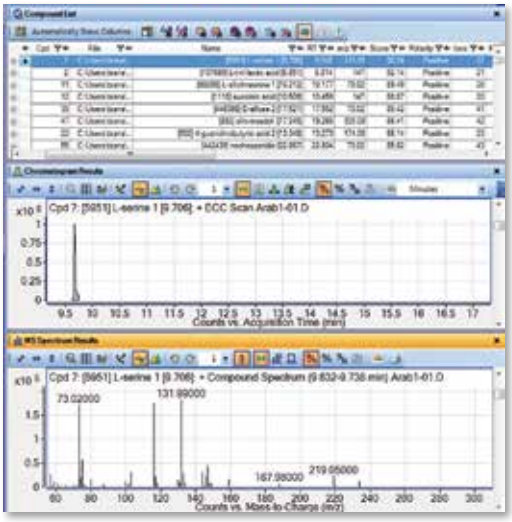
The Agilent 5977B High Efficiency Source GC/MSD system incorporates an ultra-efficient electron ionization source to maximize the number of ions created and transferred into the analyzer, revolutionizing single quadrupole performance.

The Agilent 7000 and 7010 Series Triple Quadrupole GC/MS systems provide low detection limits, robust performance, and software tools that make it easy to optimize your methods.

The Agilent 7200B GC/Q-TOF system delivers high sensitivity and selectivity with the added value of accurate mass and high resolution data for structural confirmation, unknown compound identification and superior untargeted screening capabilities.

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 1437 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 Databases

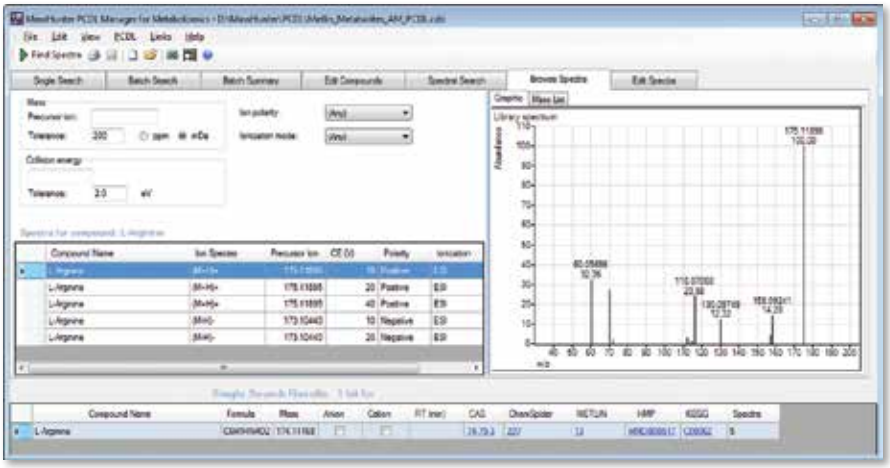
The Agilent 6200 Series Accurate-Mass TOF LC/MS systems provides the ability to identify both small molecules and large biologic compounds with high resolution, accurate mass analyses data for maximum value in qualitative assays.

The Agilent 6500 Series Accurate-Mass Q-TOF LC/MS brings the power of accurate mass MS/MS to provide strong confidence in identifying, screening, profiling, or quantitation in complex samples.

The Agilent 6400 Series Triple Quadrupole LC/MS systems provides triple quadrupole performance that gives you superior sensitivity, renowned reliability, and overall system robustness.

METLIN Personal Compound Database and Library

Contains approximately 80,000 compounds, including 39,000 lipids and 9500 metabolites with curated MS/MS spectra. Used with TOF and Q-TOF data, identification is enabled using accurate mass and/or retention time database searching. Matching MS/MS data to the spectral library provides more confident metabolite identification.



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