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Trusted Answers

Practical Guide to Metabolomics

Agilent Clinical Research

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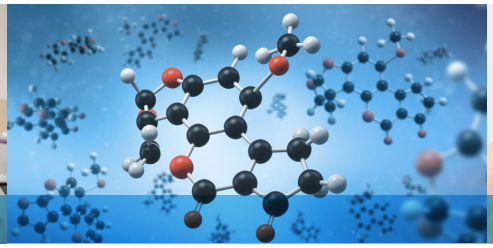


Understanding metabolomics

What is metabolomics?

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

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.

Metabolomics in clinical research

Discovery metabolomics is an unbiased comparison of the metabolites present between two or more sample groups, for example untreated versus treated, or control versus disease. The goal is to determine which metabolites and pathways are significantly changed between sample groups. This approach can be used for different purposes, such as gaining an understanding of biological processes, for biomarker discovery, or to understand drug mode of action. Targeted metabolomics measures previously defined metabolites. Newborn screening is a classic example of measuring these targeted metabolites as biomarkers for disease due to dysregulation of biosynthetic pathways caused by various genetic defects.

Agilent metabolomics solutions

Innovative metabolomics solutions from Agilent provide a powerful portfolio of sample preparation, 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.

Figure 1. Agilent LC/MS solutions.

Targeted metabolomics

Targeted metabolomics assays focus on vendor- or literature-derived methodologies with specific metabolites of interest, giving you the biological insights you desire, with a faster time to reach results. Triple quadrupole mass spectrometers are best suited for this task because of their broad dynamic range, high sensitivity, and selectivity for compound confirmation. The results are statistically analyzed, and differential features are found.

Agilent offers three highly curated, fit-for-purpose methods for targeted metabolomics. The **biocrates AbsoluteIDQ p180** and **MxP Quant 500** kits are available for general metabolite and lipid information, including over 100 small molecules and over 500 lipids. The ion-pairing Agilent **Metabolomics dMRM Database and Method** offers day-in and day-out performance for long sample runs but will require a dedicated LC system for ion-pairing use. This solution offers coverage of amino acids, tricarboxylic acid (TCA) cycle, and other energy metabolism pathways, with over 200 small molecules in the database. The HILIC-Z dMRM method offers an extended biological pathway coverage of core pathways and building blocks, with over 400 small molecules in the database. But, as with any HILIC method, special care must be taken when preparing buffers for analysis. So, whatever the best solution is, Agilent can help with your targeted metabolomic goals.

Discovery metabolomics

When you need to move beyond a targeted approach to metabolomics, discovery metabolomics will provide insights across a wider range of metabolic pathways, and offers the potential of new metabolite discovery. Discovery metabolomics is the 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 found and then identified.

Agilent has developed robust workflows for performing global metabolite profiling by gas chromatography/mass spectrometry (**GC/MS**), liquid chromatography/mass spectrometry (**LC/MS**), capillary electrophoresis/mass spectrometry (**CE/MS**), and supercritical fluid chromatography/mass spectrometry (**SFC/MS**), including metabolomics-specific software. Agilent **MassHunter Profinder** software is uniquely designed to find and visualize features in a sample batch. Results are then imported into Agilent MassHunter **Mass Profiler Professional (MPP)** software for visualization and statistical analysis. MPP processing methods can be stored and used to automate analysis. MPP contains powerful statistical algorithms, mathematical models, numerous visualizations, pathway analysis, metabolite identification, and R scripting. Critical to any discovery, metabolomics workflow is a compound identification strategy. Agilent provides the tools for compound ID using our Agilent MassHunter PCDL Manager software, allowing users to create custom databases with retention time and MS2 data. Customer-built or Agilent **METLIN** databases can be used to screen data to make first pass identification. Iterative MS/MS or targeted MS/MS studies are then generated to match to the METLIN spectra library or use the **SIRIUS/CSI:FingerID** structure prediction tools.

Sample preparation for metabolomics

Sample preparation for metabolomics (Figure 2) is also driven by an understanding of the biological processes and need for polar and nonpolar chemistries in sample preparation. A clinical research lab may want to consider the need to collect multiple fractions to achieve coverage of a broader chemical space when preparing their samples. This is especially true for untargeted metabolomics but may also be relevant in some targeted applications. Additionally, when preparing the sample, the use of preservatives and additives to the samples should be minimized to avoid unintended chemical interactions that may transform or degrade the metabolites. This is also true for sample collection and avoidance of certain tube types, such as heparin or EDTA. In addition, for untargeted metabolomics experiments, derivatization is often avoided to preserve the ability to identify the proper molecular chemistry of unannotated metabolites.

Once you have chosen your metabolites of interest and the best way to preserve and isolate them, be aware that consistent, robust, and reproducible sample preparation is one of the most crucial components to metabolomics studies, and is vital to generating quality results. The strategy moving forward must address quenching metabolism, lysing cells where required, and effectively extracting metabolites. This process must be tailored for the metabolites of interest, compatible with the analytical method, and reproducible.

The sample matrix may present challenges in the analysis, including irreproducible chromatography, system fouling, and ionization suppression. Proteins and lipids are two common classes of biomolecules that should be removed from the sample extract. Proteins are precipitated during quenching, and lipids are traditionally removed by liquid to liquid extraction (which can suffer from reproducibility issues). **Agilent Captiva EMR–Lipid** technology (Figure 3) offers a robust alternative approach that both filters the protein precipitate and performs efficient lipid removal in one simple step. The lipids are removed based on a combination of steric hinderance and hydrophobic interaction. Effective Captiva EMR–Lipid removal significantly improves method reliability and ruggedness while reducing ion suppression of target metabolites.

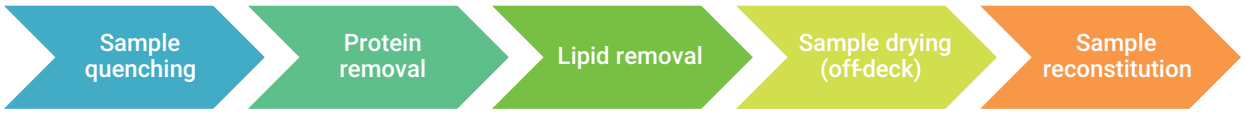


Figure 2. Metabolomics sample preparation workflow.

Figure 3. Agilent Captiva EMR–Lipid cartridges.

Automated sample preparation solutions

Manual sample preparation is time consuming and prone to error, which can impact the quality of a metabolomics study. Automation can achieve consistent and reproducible results that are operator independent. Based on the Agilent Bravo automated liquid handling platform , the **Bravo Metabolomics Sample Prep Platform** (Figure 4) is designed for extracting metabolites from plasma samples.

This standardized plasma sample preparation incorporates room-temperature quenching and Captiva EMR–Lipid removal technology, as seen in this application note: **Enabling Automated, Low-Volume Plasma Metabolite Extraction with the Agilent Bravo Platform (5994-2156EN)**. After preparation, samples are dried off deck and then reconstituted on deck prior to LC/MS analysis. This automated sample preparation workflow improves overall sample quality over manual sample preparation, resulting in less error and more confidence. By reducing risk to precious sample and the hands-on time required to process, Bravo sample preparation frees up personnel for other efforts, providing a high return of investment and overall cost savings.

Agilent Bravo Metabolomics Workbench software and Agilent **VWorks automation control software** use a form-based interface and require no complex programming, allowing any user to walk up and start the automated sample preparation. This workbench provides greater batch-to-batch consistency, more precise pipetting, and reduced variability between users, as shown in Figure 5, comparing automated and manual preparations.

Figure 4. Agilent Bravo Metabolomics Sample Prep Platform.

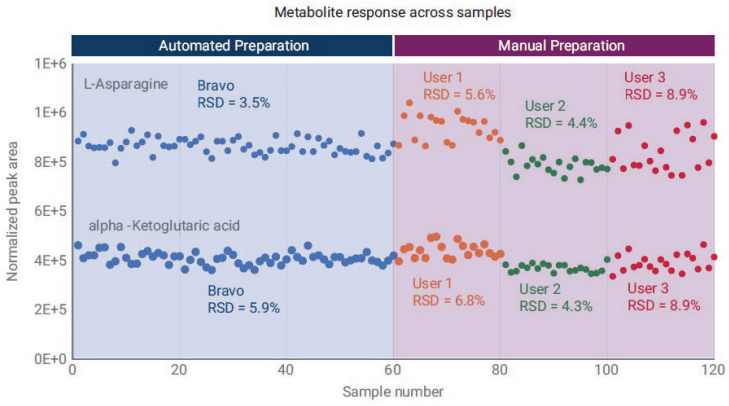


Figure 5. Normalized peak area %RSDs for L-asparagine and alpha-ketoglutaric acid (the actual injection order was randomized).

Biological challenges

Metabolomics in the clinical research laboratory is driven by understanding biological process and their relevance to understanding qualitative and quantitative clinical data and outcomes. For example, a clinical metabolite panel of catecholamines may focus on quantitation of dopamine, epinephrine, and norepinephrine: the three most common biomarkers of stress. However, in a translational laboratory, the focus would be on the larger biosynthetic pathway for catecholamines to understand the underlying production of the neurotransmitters, their interactions, and their mechanistic relationships as a marker of stress. Analytical challenges in a clinical research or translational laboratory are ultimately driven by the biological process and clinical chemistry, which need to be understood. Knowledge of not only the chemistry of the clinical panel, but a broader perspective of metabolism is required to help solve analytical challenges and maximize study return of investment.

Chromatography challenges

To begin, one of the first decisions an investigator needs to make is a selection of clinical processes or the biochemical pathways to be investigated, and whether those metabolites are more amenable to methodologies for polar or nonpolar analysis. For example, looking at cellular energetics, the focus is on glycolysis and the citric acid cycle where the polar metabolites would be compatible with HILIC chromatography methods. On the other hand, when looking at markers for inflammation, the focus is on metabolism of cytokines and oxylipins, which are nonpolar and therefore more amenable to reversed-phase analysis.

The chemical diversity of the biological extracts is so varied that no single chromatographic or MS method can achieve comprehensive coverage of the metabolome. Therefore, application of multiple methods may be required to achieve broader coverage and achieve greater biological insights. In practice, many metabolomics labs use multiple LC reverse-phase techniques such as Agilent InfinityLab Poroshell 120 columns (Figure 6), including C18 to cover semipolar compounds and C8 to cover nonpolar compounds and lipids. HILIC chromatography is often used to separate polar metabolites. Additionally, not all compounds ionize by a single MS polarity. Therefore, analysis in both positive and negative polarity is most often required.

Figure 6. Agilent InfinityLab Poroshell 120 columns.

Mass spectrometry challenges

In metabolomics, investigators need to understand that the challenges of chromatography and mass spectrometry are naturally linked. As mentioned previously, the need for volatile buffers for the mass spectrometer limits the selection of reagents that can be used for the chromatographic separation. However, mass spectrometry has its own set of unique challenges that will be discussed here. Fundamentally, basic mass spectrometry considerations include performance, data quality, versatility, productivity, and ease-of-use.

When evaluating performance in an MS system, sensitivity in matrix as well as robustness are key considerations. The ability to sensitively detect key analytes in a heavy biological matrix without the need for complicated sample preparation is essential to your productivity. The **Agilent Jet Steam technology ion source** generates up to three to five times more ions than a standard ESI source, without being concentration dependent. Instrument downtime is one of the key factors for lost productivity and opportunity in the metabolomics lab, and selection of an instrument that performs well for long sample runs is essential. Critically, the Agilent Jet Stream technology ion source has an orthogonal spray and counter current drying gas flow. This protects the system from contamination and dramatically increases robustness and sample throughput, while decreasing the service intervals of the instrument.

Understanding the dynamic range of metabolism is a critical component in mass spectrometer selection. For example, instruments not only need to have low limits of detection such as the **Agilent 6495C Triple Quadrupole LC/MS**, but also extended dynamic range in biological matrix to gather the highest quality biological insights across a range of concentrations. Caution must be taken when considering instruments based upon ion trap designs; these instruments have limited ion capacity and dynamic range can be compromised, in turn compromising your biological insights. Leveraging triple quadrupole systems for targeted metabolomics or quadrupole time-of-flight systems for untargeted or hybrid-targeted untargeted studies will provide you best performance and dynamic range, uncompromised by matrix, producing the most meaningful biological results.

One of the critical challenges in metabolomics is having high-quality data and software tools to make confident identifications and translating them into biological knowledge. Often a data analyst will have to look at hundred or even thousands of mass signals and properly identify them to generate context to the data. One potential strategy would be to deploy highly curated and analytically validated Agilent targeted metabolomic solutions, which decrease start-up time from data acquisition to insight by bypassing the need to determine new structures. For untargeted experiments, Q-TOF studies leveraging accurate mass measurement with highly accurate isotopic measurements help limit the number of possible compound IDs for any given mass, with the 6546 LC/Q-TOF demonstrating the highest performance attributes. Additionally, high-quality MS/MS fragmentation data generated from iterative MS/MS studies leverages a dynamic exclusion list to ensure that new metabolites are fragmented in each repeat injection of sample. Having software tools like Agilent MassHunter **Lipid Annotator** and the Agilent **METLIN** metabolite personal compound database and library (PCDL) will decrease time spending identifying spectra and more time understanding biology.

Methods to suit your needs

To get you started with this complex method development process, Agilent has a variety of liquid chromatography solutions for any skill level. These include: (1) the biocrates kits, (2) the ion-pairing dMRM solution, and (3) HILIC Z dMRM solution. A comparison of the different solutions is displayed in Table 1.

1. The **biocrates AbsoluteIDQ p180** (Figure 7) and **MxP Quant 500** (Figure 8) kits use the **Agilent 1290 Infinity II LC** and the Agilent **6470 triple quadrupole LC/MS**, and the **6495C triple** quadrupole LC/MS system, respectively, providing a simple, reproducible, and curated method by supplying consumables and software to get up and running quickly. These highly validated and easy-to-use kits provides basic metabolism and lipid information, including over 100 small molecules and 500 lipids.

Figure 7. The Biocrates AbsoluteIDQ p180 kit.

2. With highly stable chromatography and high sensitivity, the ion-pairing **Metabolomics dMRM Database and Method** offers day-in and day-out performance for long sample runs. Using the **1290 Infinity II LC** and 6470 triple quadrupole LC/MS or **6495C triple** quadrupole LC/MS, this method provides good coverage of amino acids, the TCA cycle, and other energy metabolism pathways, with over 200 small molecules in the database. It must be noted that when using ion-pairing reagents, a dedicated LC system must be considered, as removing the ion-pairing reagents from the system is very challenging, and residual effects from reagents may linger for the lifetime of the LC.

Figure 8. The Biocrates MxP Quant 500 kit.

3. The HILIC-Z dMRM method offers an extended coverage of core metabolic pathways and biological building blocks using the **Agilent 1290 Infinity II LC** or **1290 Infinity II bio LC system** coupled with the **6495C triple** quadrupole LC/MS. With over 400 small molecules in the database, this method offers more comprehensive biological information without the use of ion-pairing reagents. The **Agilent InfinityLab Poroshell 120 HILIC-Z** column allows for superior retention of polar metabolites using MS-compatible solvents, but also requires chromatographic expertise and the ability to follow method details exactly.

Methods to suit your needs

For metal-sensitive metabolites, when using either of the ion-pairing or HILIC methods above, the Agilent **InfinityLab deactivator additive** can be used to improve detection and peak shape by blocking active sites within the LC flow path. This product must continually be used to maintain best peak shape for metal-sensitive analytes. For best peak shape performance out of the box, the **1290 Infinity II bio LC system**, manufactured with MP35N alloy, dramatically reduces the active sites in the LC flow path. The InfinityLab deactivator additive can then be used in conjunction with the 1290 Infinity II bio LC system for blocking active sites when stainless steel LC columns are used.

When considering method development for metabolite separations, one must also strongly consider the composition and pH of the solvent system. Additives like formic acid or ammonium acetate not only influence the buffering capacity of the solution, but also the pH. The pH of the solution will influence whether the metabolites are protonated or deprotonated during the separations, influencing peak shape, degree of metal sensitivity, and retention time of the analytes. Also consider that the pH of the solvent system also helps drive ionization in the mass spectrometer. Basic pH helps drive negative ionization by deprotonation, and acidic pH helps facilitate positive ion formation by protonation. Keep in mind that volatile LC buffers must be used when coupled with mass spectrometry. For example, phosphate, borate, and sulfate buffers should never be used when coupled to a mass spectrometer.

Table 1. Solution comparison.

	Biocrates AbsoluteIDQ p180 and MxP Quant 500 Kits	Ion-Pairing dMRM Method	HILIC-Z dMRM Method
Hardware	1290 Infinity II LC and 6470 triple quadrupole LC/MS or 6495C triple quadrupole LC/MS	1290 Infinity II LC and 6470 triple quadrupole LC/MS or 6495C triple quadruple LC/MS	1290 Infinity II LC or 1290 Infinity II bio LC and 6495C triple quadrupole LC/MS
Advantage	Simple, automated, curated, reproducible	Stable chromatography, high sensitivity	Flexible without the use of ion pair
Suitable For	Prospective metabolomics researcher	Day-in/day-out performance for long sample runs	Extended biological pathway coverage
Biological Insights	General metabolites/lipids coverage, with 107 small molecules and 523 lipids in database	Great coverage of amino acids, TCA cycle, and other energy metabolism pathways, with 219 small molecules in database	Extended coverage of core metabolic pathways and biological building blocks (400+ small molecules in database)

LC/MS analysis

Agilent 6500 Series accurate-mass quadrupole time-of-flight (Q-TOF) LC/MS systems

Agilent 6546 LC/Q-TOF

Next-generation electronics make the Agilent 6546 LC/Q-TOF (Figure 9), the ideal choice for metabolomics analysis in clinical research. With the highest Agilent Q-TOF resolution, there is no need to sacrifice speed for resolution, providing the best of both worlds. This allows for fast metabolomics chromatographic separations, performed more confidently than ever before. Powerful, rugged, and reliable, the 6546 LC/Q-TOF is easy to use and allows for faster biological insights in metabolomics research.

Figure 9. **Agilent 6546 LC/Q-TOF**.

Agilent 6560 ion mobility LC/Q-TOF

Ion mobility provides even more vision to see more metabolites by providing even greater separation power. With the ability to measure collisional cross section of metabolites, even more confidence can be added to compound identification. This cutting-edge technique can help understand different conformers of lipids and other small molecules, leading to new insights in clinical research.

Figure 10. **Agilent 6560 IM LC/Q-TOF**.

Agilent 6400 Series triple quadrupole LC/MS systems

6495C triple quadrupole LC/MS

The 6495C triple quadrupole LC/MS system (Figure 11) provides next-generation sensitivity for the most demanding clinical metabolomics applications. Ultrafast electronics provide excellent performance at low dwell times, maximizing the sensitivity with a high number of transitions. This enables the use of methods that target more metabolites than ever before with sensitivity, precision, and confidence.

Figure 11. **Agilent 6495C LC/TQ**.

6470 triple quadrupole LC/MS

A backbone of many metabolomics operations is the 6470 triple quadrupole LC/MS (Figure 12). Trusted for years to generate reliable results with high-quality data, the 6470 triple quadrupole LC/MS provides fantastic value and proven results.

Figure 12. **Agilent 6470 LC/TQ**.

GC/MS analysis

The Agilent **5977B GC/MSD** (Figure 13) with high-efficiency source incorporates an ultra-efficient electron ionization source to maximize the number of ions created and transferred into the analyzer, revolutionizing single quadrupole performance.

Figure 13. **Agilent 5977B GC/MSD system**.

The Agilent **7000D** and **7010B** triple quadrupole GC/MS systems (Figure 14) provide low detection limits, robust performance, and software tools, which make it easy to optimize your methods. The new Agilent MassHunter Optimizer software creates MRM transitions for analytes from a user-provided, full-scan data file or program collection. Optimizer finds the compounds, searches the library spectra, and selects precursor ions. Product ions are then analyzed and the CE voltage is optimized.

Figure 14. **Agilent 7010B GC/TQ**.

The Agilent **7250 GC/Q-TOF** (Figure 15) 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. With a low-energy EI-capable source and compatibility with chemical ionization techniques, molecular ions can also be generated for precursor ion characterization and additional MS/MS studies.

Figure 15. **Agilent 7250 GC/Q-TOF**.

To maximize the biological interpretation of your results we have partnered with Dr. Oliver Fiehn, to develop the **Agilent Fiehn GC/MS Metabolomics Retention Time Locking Library**. This is the largest commercially available and growing metabolomics-specific library, containing searchable GC/MS EI spectra and retention time indices from approximately 1,437 common metabolites. The library comes with complete, preprogrammed GC/MS methods, and documents for GC/MS metabolomic analysis to maximize research success. Agilent also provides comprehensive options for metabolite identification in GC/MS by supporting the latest release of the **NIST 20 spectral libraries** and NIST MS program. Spectral libraries that can be searched directly through MassHunter software for ease of use.

Software tailored to your metabolomics research needs

Producing biological insights from data quickly and efficiently is made easier with fit-for-purpose software for clinical metabolomics research. Extracting, quantifying, and understanding results in a biological context has been made easier than ever before with the Agilent software portfolio. You can confidently extract metabolite signals, identify, and map onto biological pathways to understand changes in the metabolome in your research. Whether it be routine targeted analysis or complex untargeted studies requiring machine learning, Agilent provides the tools to achieve insights more quickly.

Agilent MassHunter quantitative analysis software

Agilent MassHunter quantitative analysis software provides a powerful platform to analyze your targeted metabolomics data. Create analysis methods directly from a targeted metabolite database and achieve results even more quickly. Intuitive design and dynamic data-processing capabilities make it easier to interrogate your data.

MassHunter Profinder software

Untargeted metabolomics data is highly complex and MassHunter Profinder software is ready to meet the challenge. Profinder tackles this complexity by a revolutionary recursive molecular feature-finding algorithm. Leveraging the power of batch analysis, Profinder finds even greater consistency in detection, adduct and isotope assignment. Providing the highest levels of data quality, Profinder puts your data in the best position for statistical analysis.

MassHunter METLIN metabolite personal compound database and library (PCDL)

The MassHunter METLIN metabolite PCDL contains approximately 80,000 compounds, including 39,000 lipids and 11,800 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.

Mass Profiler Professional software

Metabolomics researchers face challenges as studies become larger and more complex. Multivariate statistics are used to find differences between study groups. However, it is not enough to know what metabolites are differential; understanding the biological context is critical. Visualizing processed study results onto metabolic pathways facilitates biological understanding. Agilent offers advanced analysis software for processing and interpreting complex metabolomics data.

Software tailored to your metabolomics research needs

Mass Profiler Professional (MPP) software is a comprehensive integrated biology platform for exploiting the high information content of MS data and integrating with other data sources, including clinical metadata. MPP can be used in any MS-based differential analysis to determine relationships among two or more sample groups and large numbers of metabolites. The software provides wizard-based guidance to import, align, and normalize various kinds of data, including both GC/MS and LC/MS results.

The MPP software includes comprehensive univariate statistical analysis techniques, including parametric, nonparametric, paired, and unpaired versions of both t-tests and f-tests (ANOVA). Additionally, MPP makes powerful multivariate statistics like principal component analysis hierarchical clustering, and machine learning algorithms, such as partial least squares discriminate analysis or random forest analysis, intuitive and interactive, without the need for special programming languages. These powerful tools allow you to efficiently extract meaningful information and visualize results for large sample quickly. To enhance your ability to understand the context of your MS results, clinical or biological metadata can be added to the analysis to help find and extract new relationships in complex sample data. To assist with identification of unknown statistically relevant features, the built-in ID browser can annotate based upon matches to retention time and spectra against the highly curated METLIN LC/MS database or the Fiehn GC/MS library. These metabolomics-specific databases include compound identifiers for subsequent mapping to pathways to further contextualize and drive biological or clinical insights from your results.

SIRIUS 4 and CSI:FingerID

From the laboratory of Dr. Sebastian Böcker at the University of Jena comes a new revolutionary tool for compound annotation. When library matches are not available or database searches return multiple possibilities, SIRIUS 4 and CSI:FingerID provide an alternative method to gain compound structure insights using the power of machine learning algorithms. Using accurate mass LC/MS/MS fragmentation data, SIRIUS 4 helps determine the most likely empirical formulas by generating hypothetical fragmentation trees to rationalize the top results. The empirical formula results are then integrated with CSI:FingerID, a powerful machine learning algorithm, which predicts the structure of a small molecule from thousands of MS/MS library spectra. This third-party software is free for academic use and supports the Agilent MassHunter CEF file format, allowing you to drag and drop your results into the program for easy import and use. With Agilent, SIRIUS 4, and CSI:FingerID, you can be confident that the latest in compound annotation techniques are at your fingertips.

Agilent Technologies offers solutions for all aspects of the metabolomics workflow, from sample preparation to mass spectrometry analysis to data processing. Agilent is proud to be your partner in metabolomics for clinical research.

The Agilent End to End Metabolomics Solution

Sample Preparation

Bravo Metabolomics Sample Prep Platform
Captiva EMR-Lipid

Separation

1290 Infinity II Series
C18 & HILIC columns portfolio

Method Solutions

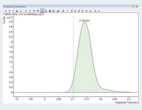
Biocrates AbsoluteIDQ p180
and MxP Quant 500 Metabolomics dMRM
Database & Method

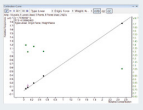
Detection

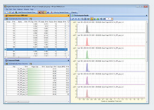
LC/TQ and LC/Q-TOF,
GC/TQ and GC/Q-TOF

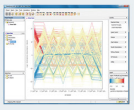
Software Suite

MassHunter Quantitative Analysis
MassHunter Profinder
Mass Profiler Professional (MPP)









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