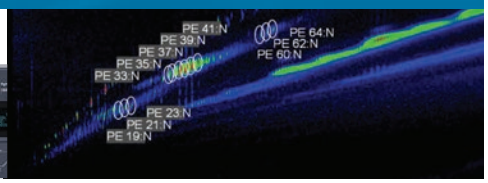
Agilent Solutions for Lipidomics

GREATER INSIGHT INTO LIPID METABOLISM

The Measure of Confidence

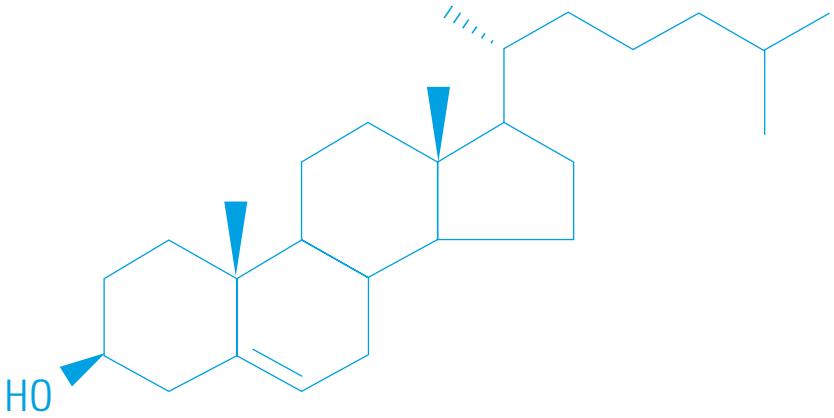


Agilent Technologies

UNDERSTANDING LIPIDOMICS

What is Lipidomics?

The term "lipidome" refers to all the lipids that exist in an organism and their effects on diverse cellular processes. To understand the lipidome, it is important to characterize and quantify lipids, both collectively and individually. Mass spectrometry has emerged as a powerful analytical detection tool supporting effective lipid profiling for lipidomics research.



Lipidomics Workflows

The shotgun lipidomics workflow is a well-established infusion-based technique designed to rapidly yield quantitative lipid class data on a small number of lipids using class internal standards. This approach yields information on lipid class, composition, and R-groups, but does not provide unambiguous identification of the lipids. Shotgun lipidomics can be performed on either a triple quadrupole (QQQ) or quadrupole time-of-flight (Q-TOF) mass spectrometer. Users can target specific lipid classes using precursor ion scan or neutral loss scan modes on QQQ mass spectrometers. A Q-TOF instrument offers higher sensitivity than a QQQ in scan modes and higher mass accuracy but lacks the specificity of the QQQ neutral loss scan.

A significant limitation of shotgun lipidomics is ion suppression caused by the chemical diversity of the lipids and their very different ionization efficiencies. Without separation of the lipids, MS and MS/MS information alone cannot resolve biologically relevant differences in structure such as double bond location, R-group position, etc. The diverse chemical nature of lipid classes presents an ongoing challenge to the development of separation methodologies to resolve and identify individual lipids.

Profiling lipidomics, a separation-based technique, has emerged as a more comprehensive approach, yielding relative quantitation and identification of hundreds of lipids in a single analysis. This development is made possible by advances in chromatography, as well as the development of ion mobility mass spectrometry (IMS) and advanced software analysis tools.

LIPIDOMICS TOOLS FOR A VARIETY OF APPLICATIONS

Agilent is a leading provider of chromatography and mass spectrometry instruments, supplies, informatics, and technical support for global lipidomics research across multiple application areas.

Basic and Clinical Research
Study lipids in complex biofluids to identify lipid biomarkers and support understanding of cellular metabolism at a level of detail not attainable with classical analytical methods. Lipidomics can be used to document lipid profiles and reveal lipid alterations that occur in metabolic disorders, and is playing a pivotal role in understanding the mechanics of atherosclerosis, stroke, hypertension, and obesity.

Agriculture
Understand the roles of lipids in agriculture through their impact on soil and plant biology.

Food and Nutrition
Identify and evaluate how lipids, independently and together with proteins, regulate cellular and sub-cellular functions, including signaling and gene expression. Comprehensive lipidomics studies are unlocking new discoveries of the links between the food we eat and our health.

Pharmaceutical
Identify lipids to improve drug discovery and provide the foundation for more effective treatments for debilitating diseases.

Biofuels
Profile lipids in fatty acids and oil-producing microalgae as important markers for determining engine compatibility and performance metrics for biodiesels. Lipidomics is playing a role in engineering new strains to produce fatty acid ethyl esters (FAEEs), a component of biodiesel.

"Working with Agilent, we have co-developed enhanced workflows for the detection of low-abundance lipids using a combination of capture, separation, and nano-fluidic chromatography followed by high-resolution tandem mass spectrometry. This approach has added critical detail to our understanding. Through our partnership with Agilent, we are also extending our research into areas related to lipidomics, including metabolomics, glycomics, and proteomics."

MARKUS WENK, PH.D.
NATIONAL UNIVERSITY OF SINGAPORE

THE LIPIDOMICS SEPARATION CHALLENGE

Multiple Approaches, Multiple Solutions

The structural diversity of lipids necessitates many separation approaches, with no single solution being suitable for all classes. Gas chromatography/mass spectrometry (GC/MS) has traditionally been used for fatty acyl characterization; this gives detailed R-group information but loses the lipid-level information due to the sample preparation (saponification). Terpenes and sterols are preferentially analyzed by GC/MS due to superior chromatographic separation and ionization.

Both liquid chromatography (LC) and supercritical fluid chromatography (SFC) are very broadly applicable techniques that preserve the lipid-level information, require no derivatization, and interface easily to atmospheric pressure mass spectrometers. The choice of chromatographic method impacts the class of lipids resolved and detected, and therefore depends on the application.

Lipid Category	GC/MS	LC/MS	SFC/MS
Fatty acids (acyls)	•••	••	••
Glycerolipids (triglycerides)	•	•••	••
Glycerophospholipids		•••	••
Sphingolipids		•••	••
Sterol lipids	•••	••	••
Prenol lipids		••	••
Saccharolipids		•••	••
Terpenes (plants)	•••	••	••
Polyketides	•	••	••

Table 1. Overview of the relative strength of the different chromatographic separations for various lipid classes. ••• indicates the best separation technique for a given class.

Agilent supports a range of lipidomics solutions (Figure 1) with a unified data analytics platform across GC/MS, LC/MS, and SFC/MS.

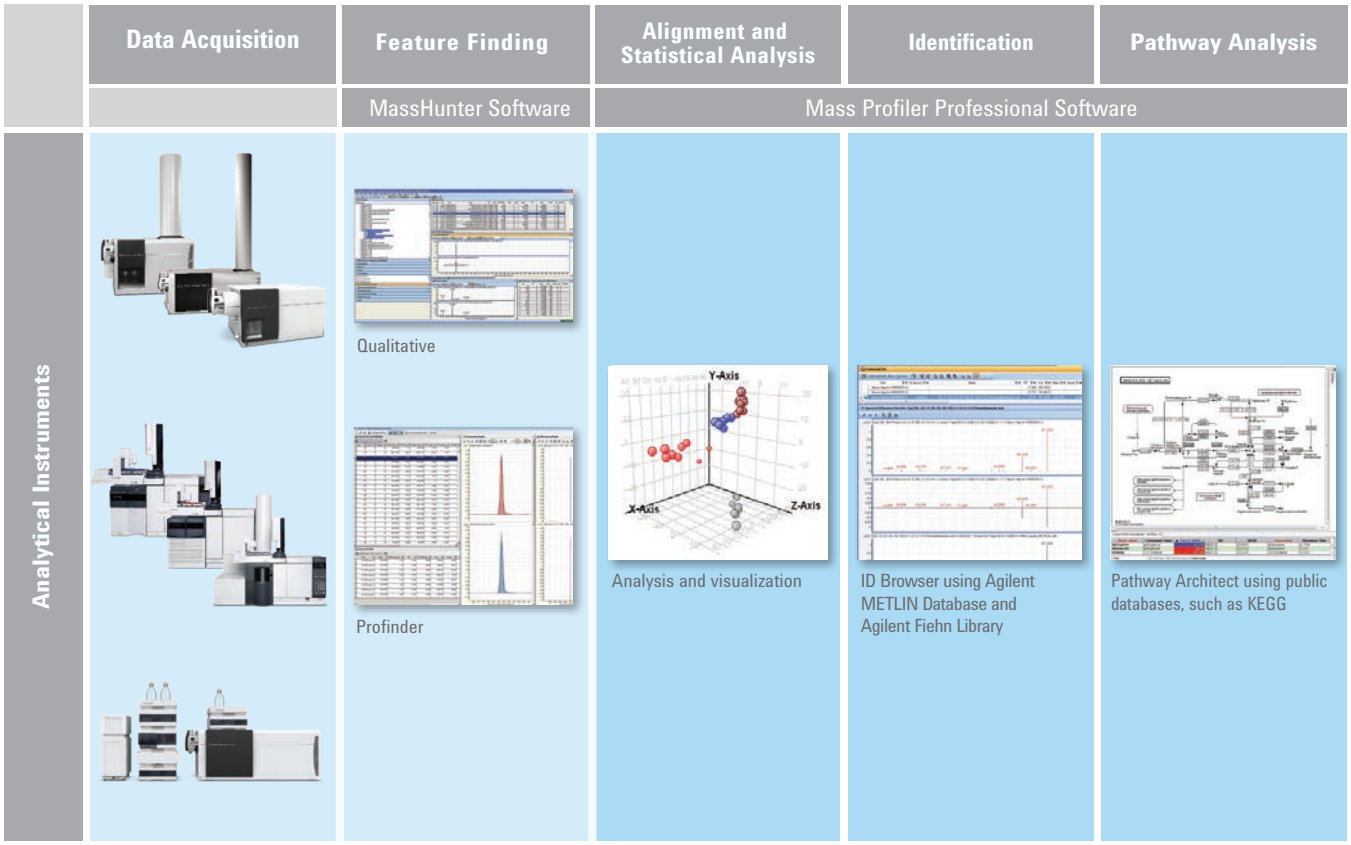


Figure 1. Agilent lipidomics workflow solutions.

POWERFUL, FLEXIBLE, AND RELIABLE TECHNOLOGY FOR LIPIDOMICS

The Chromatography of Lipids

Normal phase and reversed-phase LC offer different benefits for lipid separation. Normal phase LC allows quick assessment of lipid classes (Figure 2), while reversed-phase LC provides excellent retention time reproducibility and separation of lipids within a class (Figure 3). In comprehensive lipid analysis, normal phase LC is used for class-based fractionation, followed by reversed-phase LC of the fractions to resolve individual lipids.

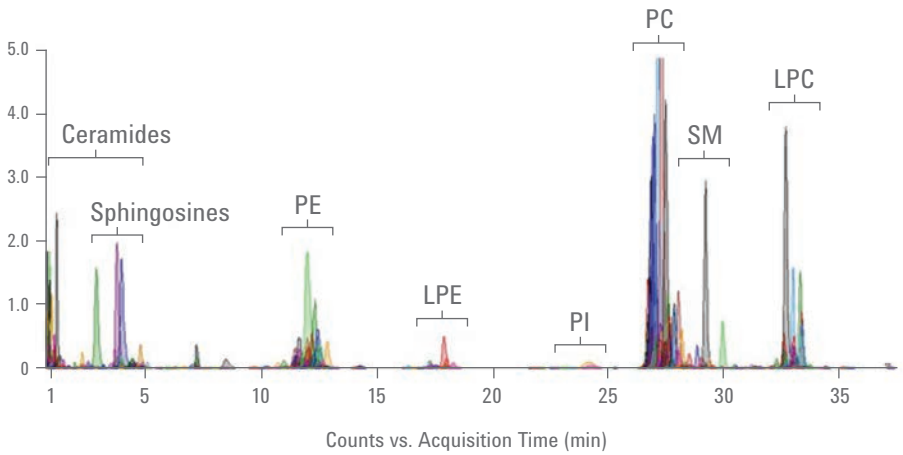


Figure 2. Normal phase LC/MS separation of liver extract demonstrating separation by lipid class. PE = phosphatidylethanolamine; LPE = lysophosphatidylethanolamine; PI = phosphatidylinositol; PC = phosphatidylcholine; LPC = lysophosphatidylcholine.

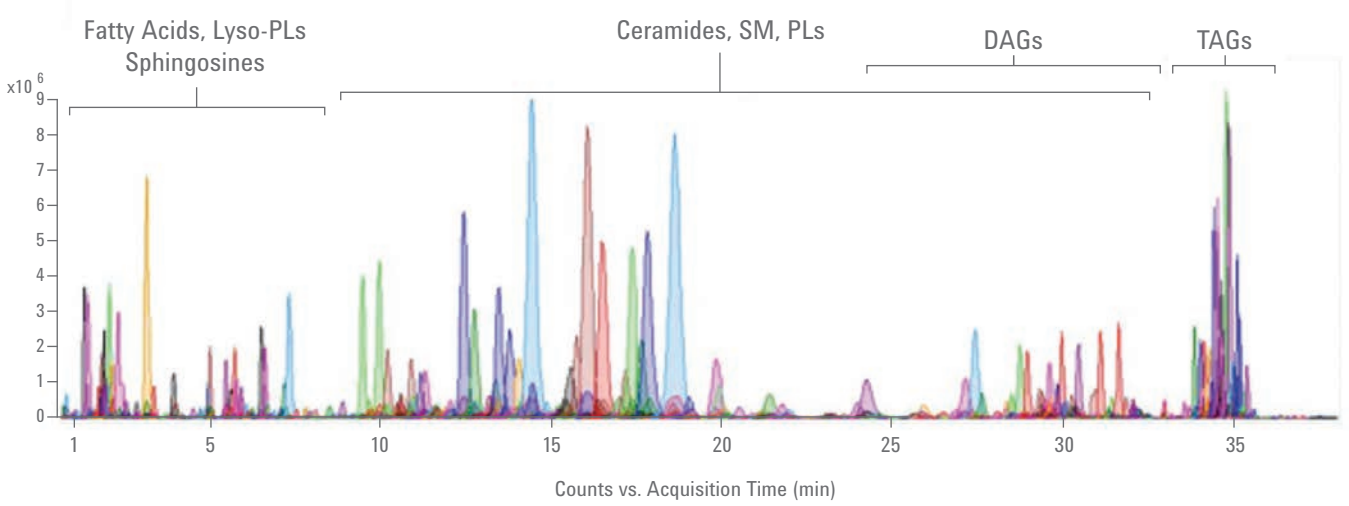


Figure 3. Reversed-phase LC/MS separation of liver extract demonstrating separation within lipid class. PL = phospholipid; SM = sphingomyelin; DAG = diacylglycerol; TAG = triacylglycerol.

Supercritical Fluid Chromatography (SFC)

SFC uses very dense carbon dioxide as the main component in the SFC mobile phase. A form of normal phase chromatography, SFC is orthogonal to reversed-phase LC, providing high-resolution separation of polar and non-polar lipids in a single analysis. SFC is remarkably effective at resolving complex lipid mixtures (Figure 4). The Agilent 1260 SFC system is designed to easily change between LC and SFC mode by means of a simple valve switch.

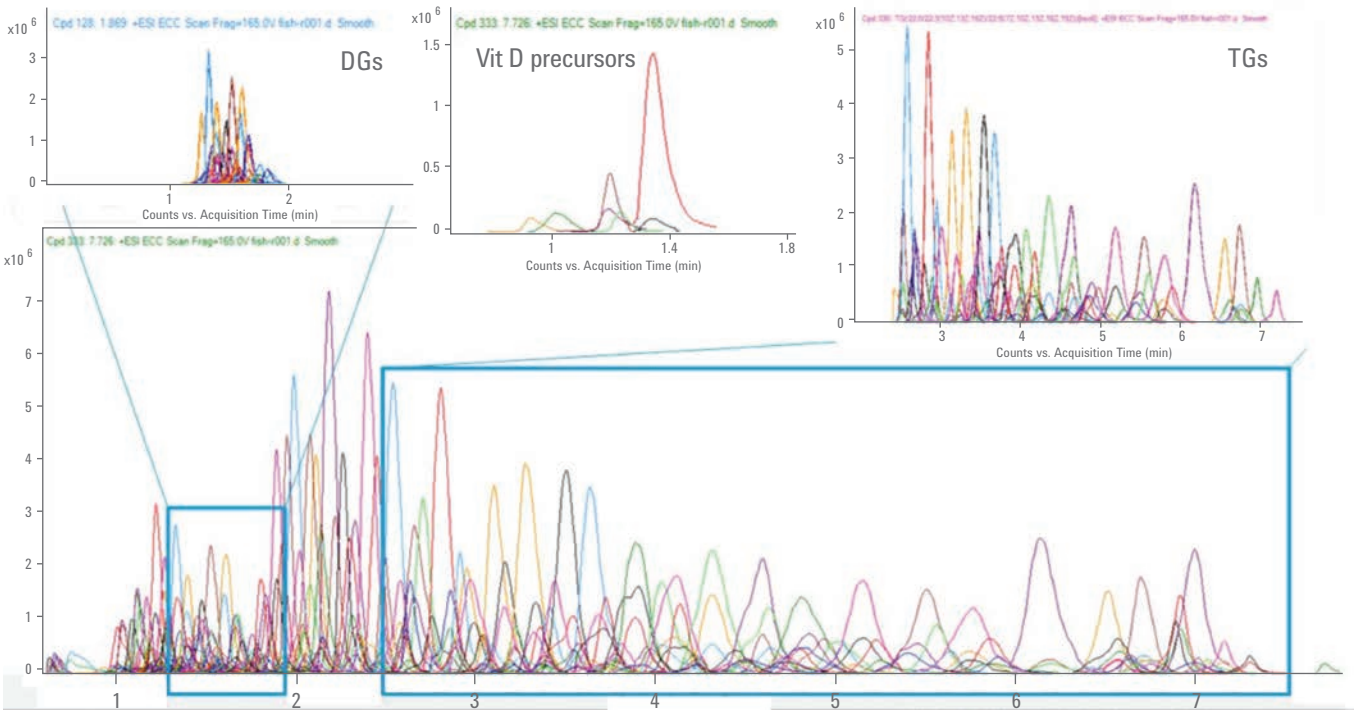


Figure 4. SFC/MS analysis of fish oil from a commercial dietary supplement. Extracted ion chromatograms demonstrate the high resolving power SFC offers for the analysis of complex lipid mixtures.

The Agilent 1260 Infinity Analytical SFC/UHPLC Hybrid System enables even greater versatility when a mass spectrometer (MS) is incorporated as an additional detector. The ability to rapidly switch back and forth between SFC/MS and HPLC/MS is a very powerful capability for lipidomics.

The Agilent 1260 SFC System

- Integrated – Single software control of state-of-the-art SFC now on all Agilent LC/MS platforms
- Environmentally friendly – Allows fast, high-resolution separation of compounds that cannot easily be separated by LC methods, with limited use of organic solvents
- Powerful – Polar and non-polar lipids can be separated in a single run
- Versatile – Delivers high flexibility with the greatest reliability

The Ion Mobility Advantage

Ion mobility technology provides an additional, orthogonal dimension of separation for complex samples such as lipids. Following chromatographic separation, lipids are further resolved in the gas phase, providing separation based on the collision cross section of the lipid ion (Figure 5). Ion mobility can also provide lipid class separation for complex samples. The Agilent 6560 IMS Q-TOF system (Figure 6) delivers accurate mass measurements combined with the highest resolution mobility separation commercially available.

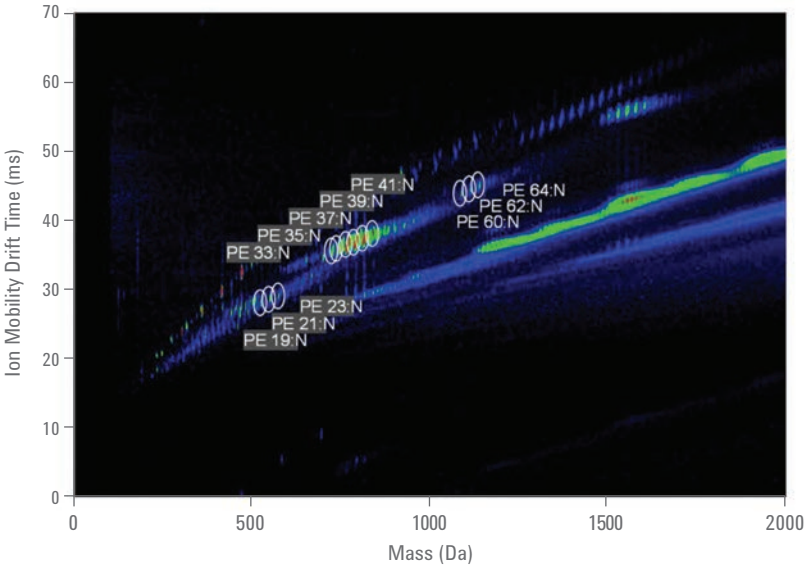


Figure 5. IMS separation of an infused mixture of phosphatidylethanolamine (PE). The increase in drift time is associated with an increase in the number of carbon atoms.

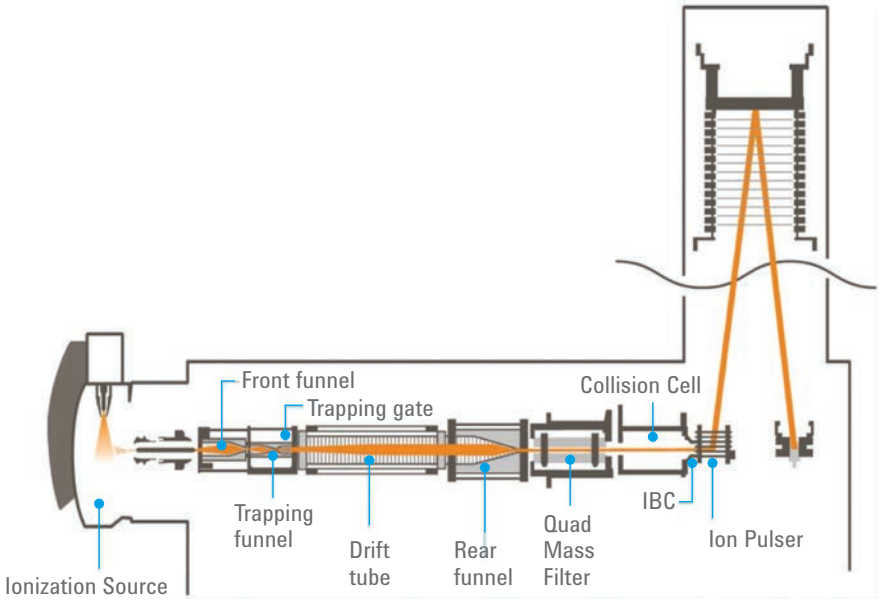


Figure 6. Each segment of the dynamic funnel assembly – which includes a front funnel for sample enrichment, trapping ion funnel, drift tube and focusing rear funnel – is carefully designed to maximize ion transmission from the source to the Q-TOF high-resolution mass analyzer. This enables resolution and characterization of complex samples using LC/IM/MS analysis while maintaining high sensitivity, providing a means to study the structural diversity of target molecules.

Lipid Identification

LIPID Metabolites and Pathways Strategy (LIPID MAPS) is a consortium of laboratories created to develop the first internationally recognized lipid classification system, including lipid nomenclature and the structural representations necessary for identifying a large number of lipids. The LIPID MAPS classification system comprises eight lipid categories (Figure 7), with each category characterized by extensive structural and functional diversity, attributable to different aliphatic chains, stereoisomerism, chirality, and head group moieties.

Complete lipid annotation and identification includes class, elemental composition, R-group size and location, number and location of double bonds, and double bond orientation (cis/trans).

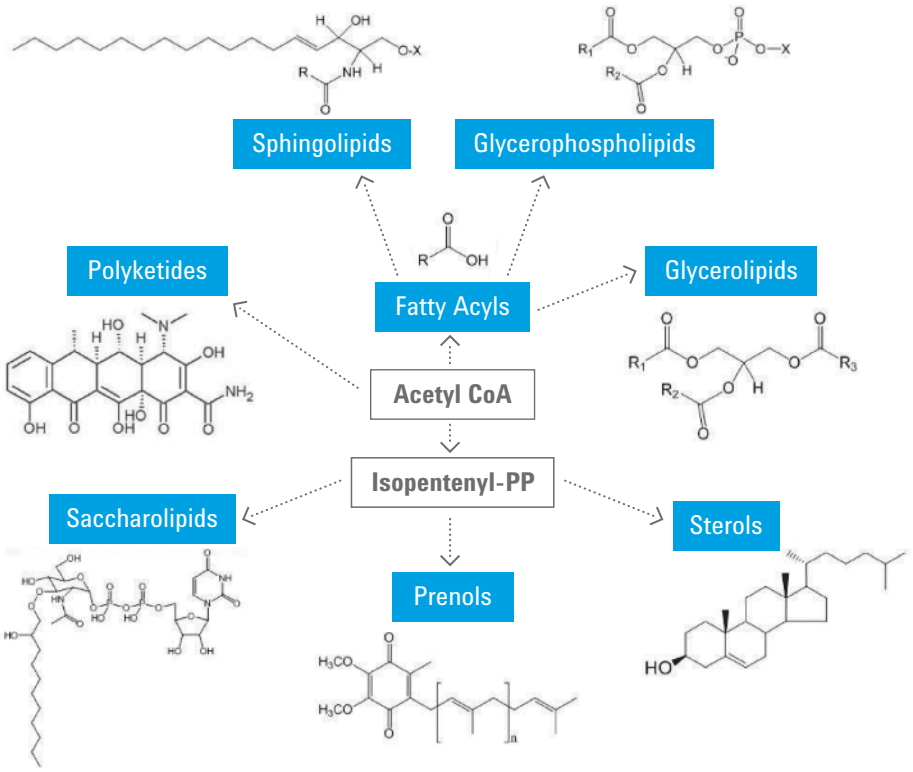


Figure 7. The eight different lipid classes as defined by the LIPID MAPS consortium.

Agilent offers different solutions for annotating or identifying lipids: database matching, MS/MS spectral library matching, or MS/MS theoretical spectral matching. Database matching can be performed using either the Agilent-METLIN database or SimLipid, a third-party software package from PREMIER BioSoft. MS/MS spectral library matching is performed only with the Agilent-METLIN MS/MS library, which is produced using chemical standards. SimLipid also supports MS/MS theoretical matching of lipid spectra.

The Agilent-METLIN database and MS/MS library can be used for annotation and identification of lipids. It contains approximately 36,600 lipid entries from LIPID MAPS with approximately 640 of these having MS/MS spectra from standards. The SimLipid database includes eight lipid categories and 36,224 lipid entries.

Software Designed for Flexibility

In lipid profiling, multiple samples are analyzed with the intent to compare and discover lipid differences between sample groups. Agilent offers MassHunter Profinder software for lipid feature extraction and alignment across samples. The MS-only results can be exported to either SimLipid for identification or Mass Profiler Professional (MPP) for statistical analysis. If SimLipid is not used for identification, lipids can be identified in MPP using the built-in ID Browser and the Agilent-METLIN database for accurate mass matching. Depending on the data analysis approach, various workflows can be employed, using a combination of Agilent software solutions (Figure 8).

If MS/MS data was acquired, analysis can be performed using MassHunter Qualitative Analysis software, with identification through either SimLipid or the Agilent METLIN Metabolite Personal Compound Database and Library (PCDL)(Figure 9).

SimLipid is available from PREMIER Biosoft International through their website **www.premierbiosoft.com/lipid/index.html**.

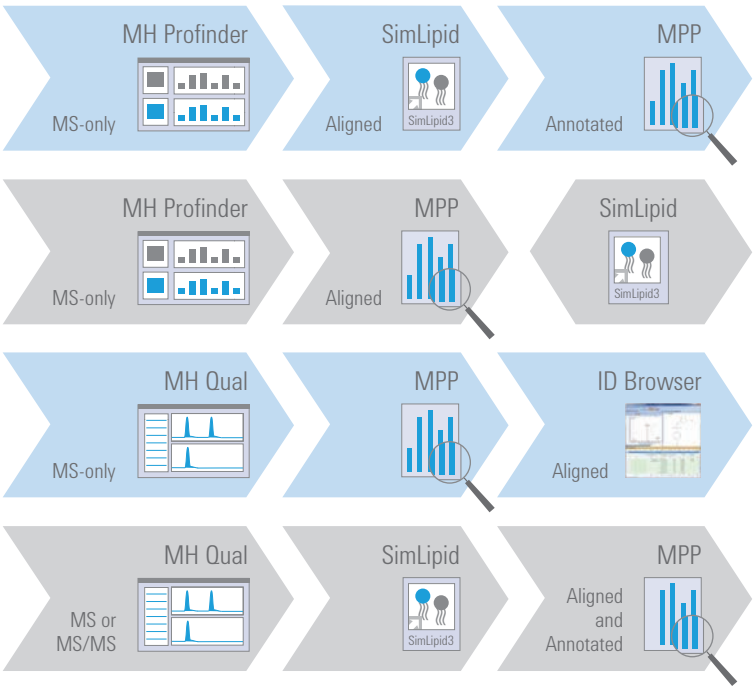


Figure 8. Lipidomics workflow integrated into MassHunter software.

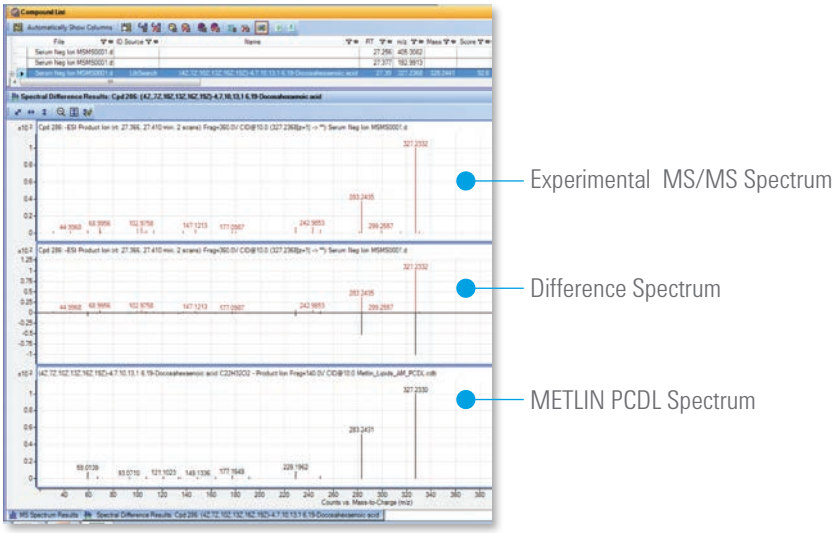


Figure 9. Results of MS/MS library searching of a human serum sample in MassHunter Qualitative Analysis against the METLIN Metabolite PCDL. The top spectrum is the MS/MS collected on an Agilent Q-TOF for one of the compounds; the bottom is the library spectrum from METLIN. The middle shows the difference spectrum. The score in the Compound List (92.6) indicates a high-quality hit.

Pathway Architect Reveals Important Biological Context

Analysis of lipid samples by LC/MS presents special challenges due to the choice of appropriate chromatography, as well as annotation and identification of compounds found. Untargeted profiling approaches will likely provide a large number of candidate lipids without a biological context.

MPP's Pathway Architect module searches, 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, while 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 and allows users to interactively filter, zoom, or select data. Pathways can be selected and a list of lipids, proteins, metabolites, transcripts, and genes can be exported and used by other programs to create new pathway-directed experiments.

In this example (Figure 10), a lipid analysis workflow begins with untargeted profiling of a sample by MS and MS/MS to find as many lipid compounds as possible. SimLipid identification software is used to annotate the results, which are then visualized in Pathway Architect on a KEGG pathway.

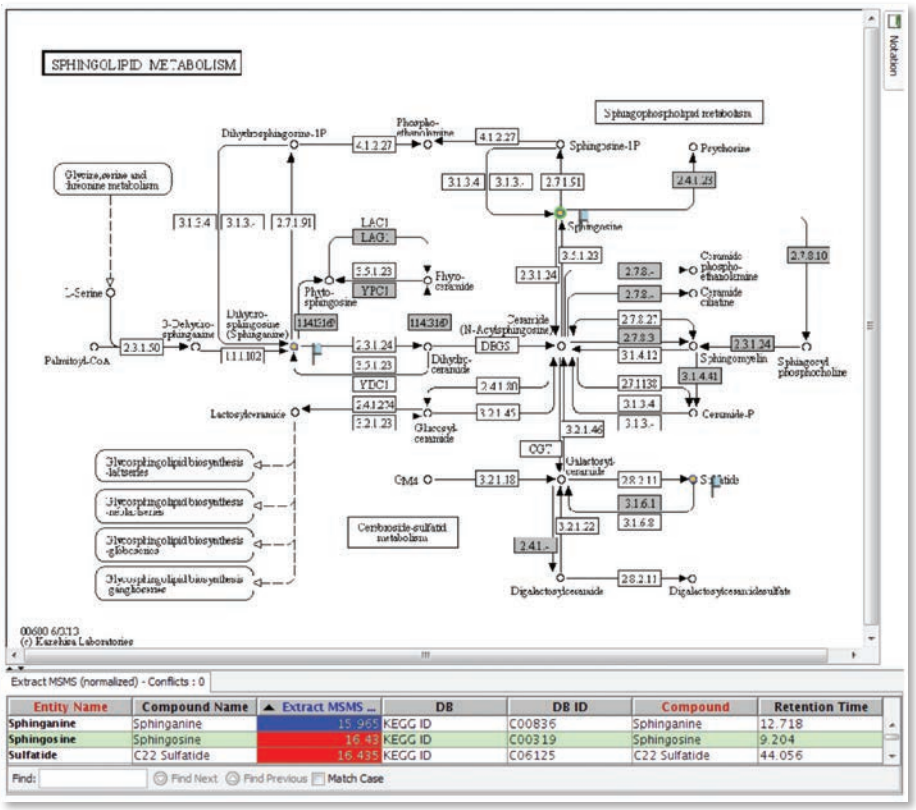


Figure 10. Results of a pathway analysis of a human serum sample in Pathway Architect using the KEGG pathway database. Three lipid compounds were identified using SimLipid and found to be present in the sphingolipid metabolism pathway. Compounds are highlighted as yellow dots on the pathway; the currently selected compound (sphingosine) is outlined in green.

AUTOMATION FOR LIPIDOMICS

Automating Sample Prep for Improved Results

Automated protocols for lipidomics sample preparation increase reproducibility and decrease the time spent preparing samples for analysis. In addition, automation frees valuable personnel for more complex tasks and minimizes the impact of staff turnover, which is especially important in long-term studies. For example, a semi-automated extraction method for phospholipids and sphingolipids from human plasma was developed using the Agilent Bravo Automated Liquid Handling Platform in the lab of Dr. Markus Wenk at SLING, the lipidomics incubator at the National University of Singapore.

Comparison of steps in manual versus semi-automated protocol

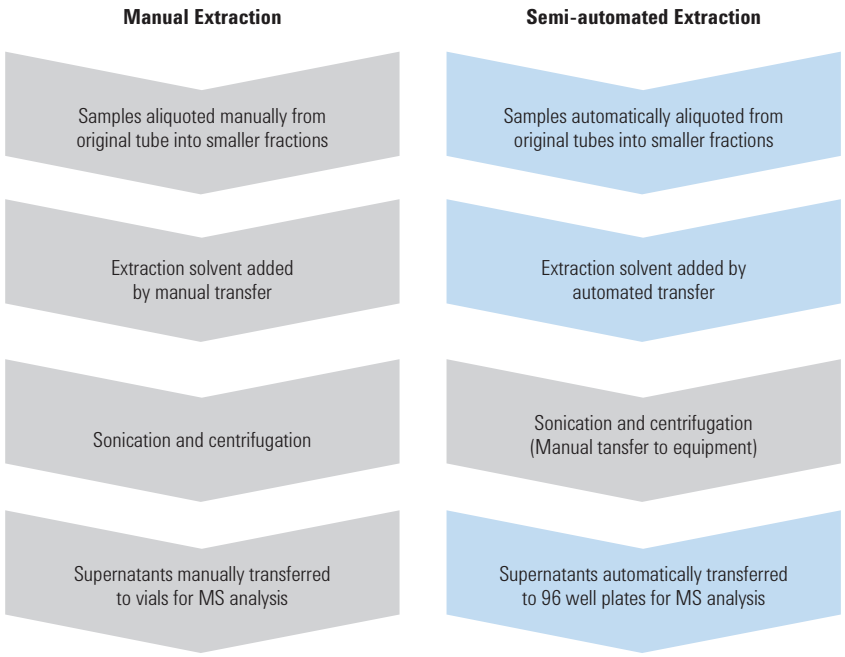
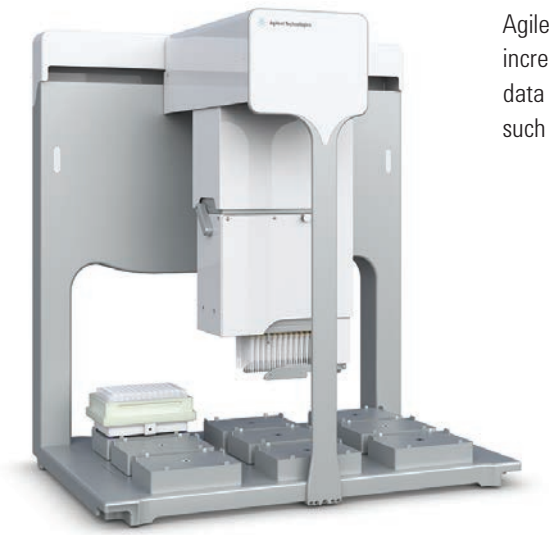
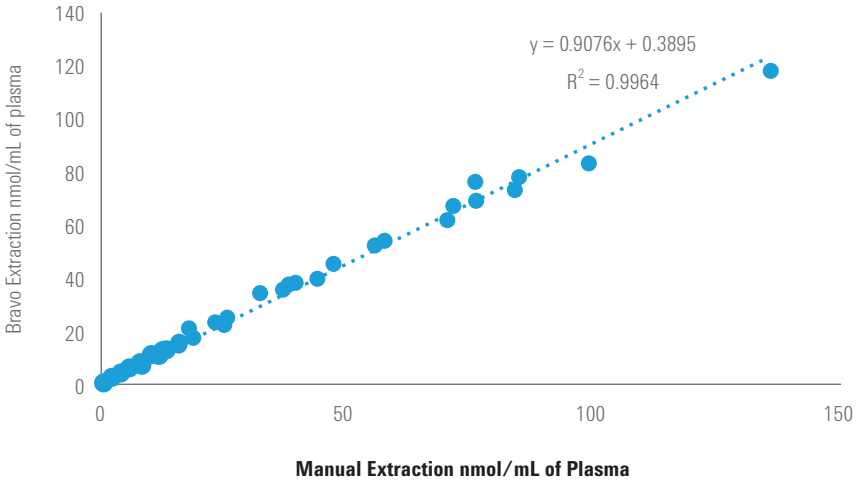


Figure 11: In the Wenk lab, an automated method using the Bravo Liquid Handling Platform replaces three of the four steps in preparing phospholipid and sphingolipid samples.



Agilent's automated liquid handling solutions increase throughput without compromising data quality by simplifying error-prone tasks such as liquid handling, labeling and sealing.



Lipid standard	% RSD Semi-automated	% RSD Manual
PE 14:0/14:0	4.80	7.13
PC 14:0/14:0	5.85	5.72
C17 Ceramide	7.32	11.97
LysoPC 20:0	5.27	5.05
SM 30:1	3.79	4.96
GluCer d18:1/8:0	6.81	6.34

Figure 12. The regression plot shows equivalent quantitation results for the semi-automated preparation method on the Bravo Liquid Handling Platform compared to the manual method. In the table to the right, you can see that the semi-automated method is better at quantifying the standard lipids.

AGILENT GC/MS AND LC/MS SOLUTIONS FOR LIPIDOMICS

GC/MS Instruments

5977A Series GC/MSD System

Ideal for routine targeted and discovery analysis. The Agilent 5977A GC/MSD provides reliability and ease of use for screening large numbers of samples.

7000C and 7010 Triple Quadrupole GC/MS Systems

For more demanding target analyses, achieve the best MS/MS quantification and the lowest detection limits with the 7010.

7200B GC/MS Q-TOF System

Identify for previously unknown lipids with high-resolution MS/MS data. The Agilent 7200B GC/MS Q-TOF complements the separation power of the 7890B GC with continuous high-resolution data.

Agilent has a full family of GC and LC columns and supplies, for all the instruments in your lab, to support lipidomics research. Easily find the best set of solutions for your lab at **www.agilent.com/chem/selectiontools**

LC/MS Instruments

1260 Infinity Analytical SFC Systems

The Agilent 1260 SFC/MS allows fast, high-resolution separations of lipids that cannot easily be separated by other methods. Polar and nonpolar lipids can be separated in a single run, with high flexibility, high precision, and excellent reliability.

6500 Series Accurate-Mass Q-TOF

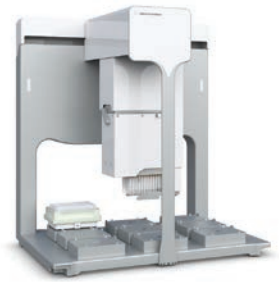
Ideal for profiling and identifying low molecular-weight compounds, the 6500 Series Q-TOF provides MS/MS functionality. The typical mass accuracy increases confidence in lipid identification and reduces false positives in database searches.

1290 Infinity II LC

Achieves unmatched separation and detection performance, delivering data of the highest quality for ultimate confidence. Unmatched sample capacity and injection cycle speed combine with new levels of usability for highest throughput.

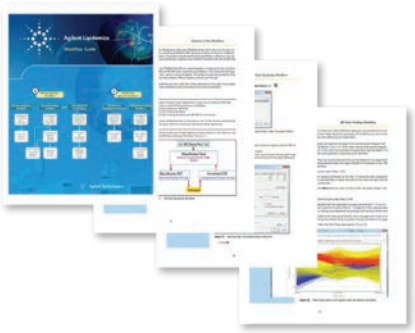
6400 Series Triple Quadrupole LC/MS

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. Sub-femtogram-level sensitivity enables detection of low-abundance lipids.



Bravo Automated Liquid Handling Platform

For lipid sample preparation, the Bravo platform is capable of transferring samples from collection tubes to wellplate format, performing liquid additions and sample extraction.



For more information, download the Lipidomics Workflow Overview and Guide showing instructions for lipidomics analyses using MassHunter Qualitative Analysis, Profinder, SimLipid, and MPP software. These guides provide step-by-step details for lipidomics workflows. Find them by searching for 5991-1643EN and 5991-1644EN, available at **www.agilent.com/chem/library**.

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