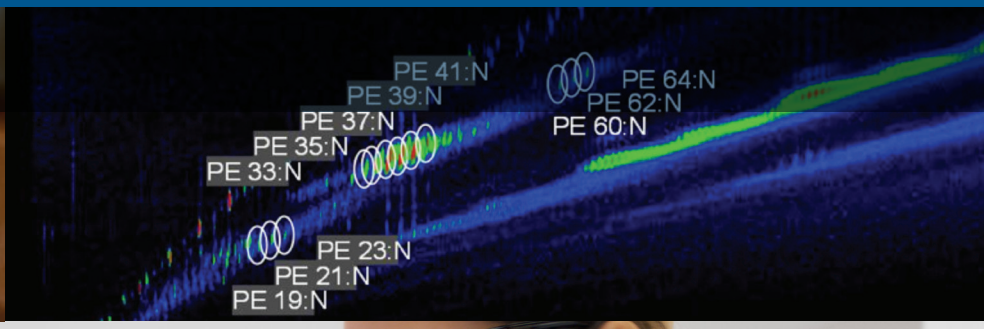
Agilent
Trusted Answers

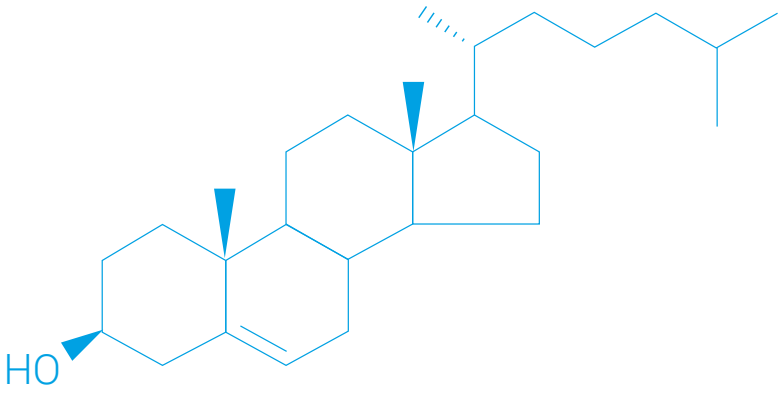
Greater Insight into Lipid Metabolism

Agilent Solutions for Lipidomics



Understanding Lipidomics

In lipidomics, the goal is to quantify and annotate all lipids in an organism in order to understand their effects on cellular processes. One powerful approach is mass spectrometry.



Lipidomics Workflows

Shotgun lipidomics 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 (TQ) 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 TQ mass spectrometers. A Q-TOF instrument offers higher sensitivity than a TQ in scan modes and higher mass accuracy but lacks the specificity of the TQ neutral loss scan.

A significant limitation of shotgun lipidomics is ion suppression caused by the chemical diversity of the lipids and their 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 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.

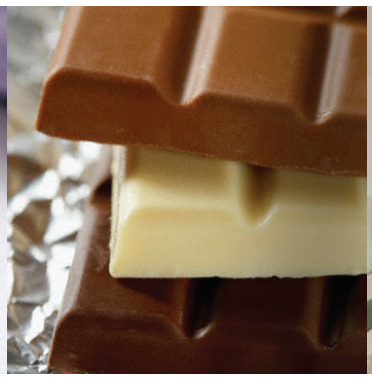
Biofuels

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 Challenge of Separating Lipids

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.

Liquid Chromatography of Lipids

Normal phase and reversed-phase LC offer different benefits for lipid separation. Normal phase LC allows separation of lipid classes (Figure 2), while reversed-phase LC provides excellent retention time reproducibility and separation of lipids by hydrophobicity, without regard to lipid 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.

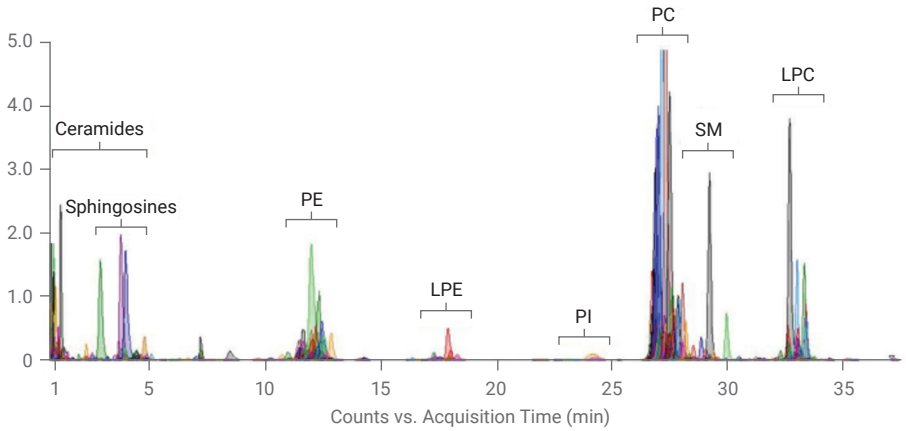


Figure 2. Normal phase LC/MS separation of liver extract demonstrating separation by lipid class. PE = phosphatidylethanolamine; LPE = lysophosphatidylethanolamine; PI = phosphatidylinositol; PC = phosphatidylcholine; LPC = lysophosphatidylcholine.

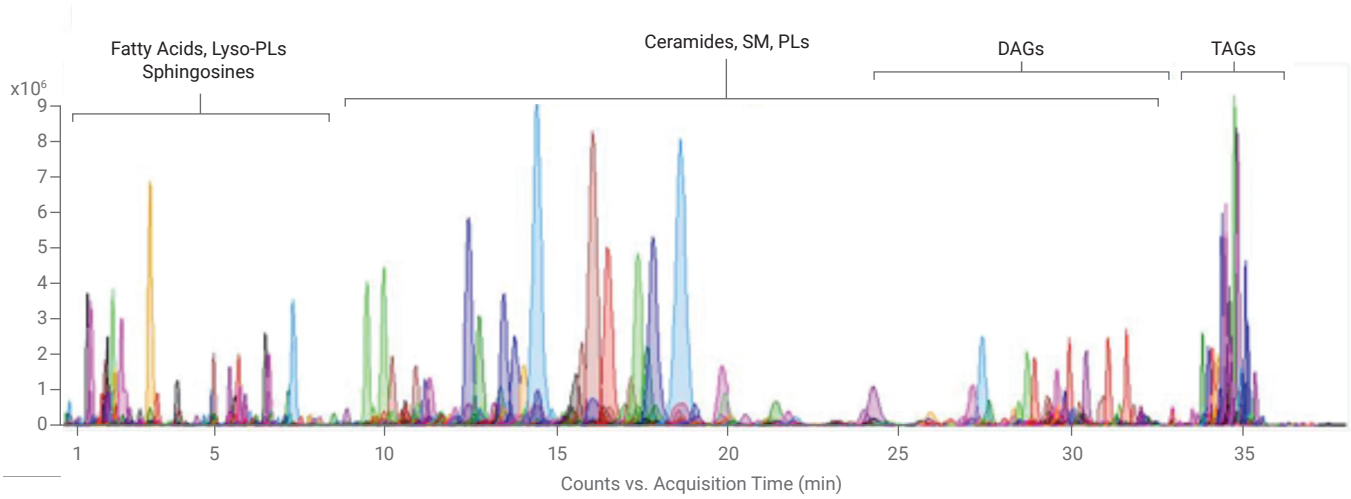


Figure 3. Reversed-phase LC/MS separation of liver extract demonstrating separation within lipid class. PL = phospholipid; SM = sphingomyelin; DAG = diacylglycerol; TAG = triacylglycerol.

Better Lipid Separation Using 2D-LC

Lipid samples are complex and interfering isobaric lipids or isotopes of lipids can lead to incorrect annotation and quantitation. In addition, some lipid classes ionize better than others, which can suppress ionization of weaker ionizing lipids, resulting in unreliable quantitation. The separation power of two-dimensional liquid chromatography (2D-LC) improves MS analysis, lipid identification, and quantitation.

The Agilent 1290 Infinity II 2D-LC system easily switches between one-dimensional UHPLC and all modes of 2D-LC, allowing the separation to be adjusted for sample complexity. Easy-to-use software enables fast method setup for heart-cutting 2D-LC, comprehensive 2D-LC, multiple heart-cutting 2D-LC, or Agilent´s unique high-resolution sampling 2D-LC.

An example of 2D-LC lipid separation (Agilent publication 5991-5532EN) with multiple heart-cutting is shown in Figure 4. The first dimension used HILIC chromatography, which elutes lipids in order of polarity from neutral lipids to more polar and charged lipids, resulting in five to six groups. The second-dimension RPLC separation, which is orthogonal to HILIC, further separated the lipids within each group.

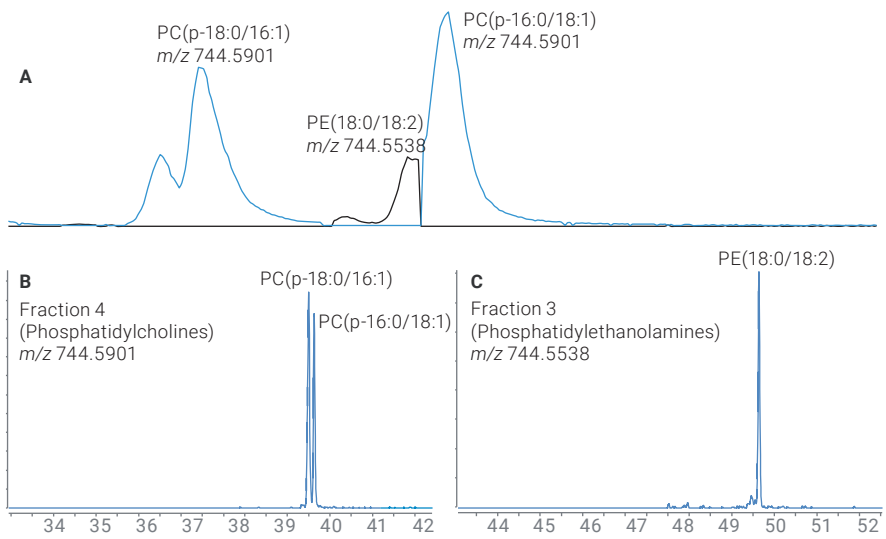


Figure 4. Extracted ion chromatogram (positive ionization) for selected PEs and PCs in a plasma sample. Top trace shows the one-dimensional reversed-phase LC separation, bottom traces show the results for the multiple heart-cutting analyses where PEs and PCs are separated in the first dimension HILIC separation.

Supercritical Fluid Chromatography of Lipids

Supercritical fluid chromatography (SFC) uses very dense carbon dioxide as the main component in its 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 5). The Agilent 1260 InfinityLab II SFC system can be coupled to any Agilent LC mass spectrometer.

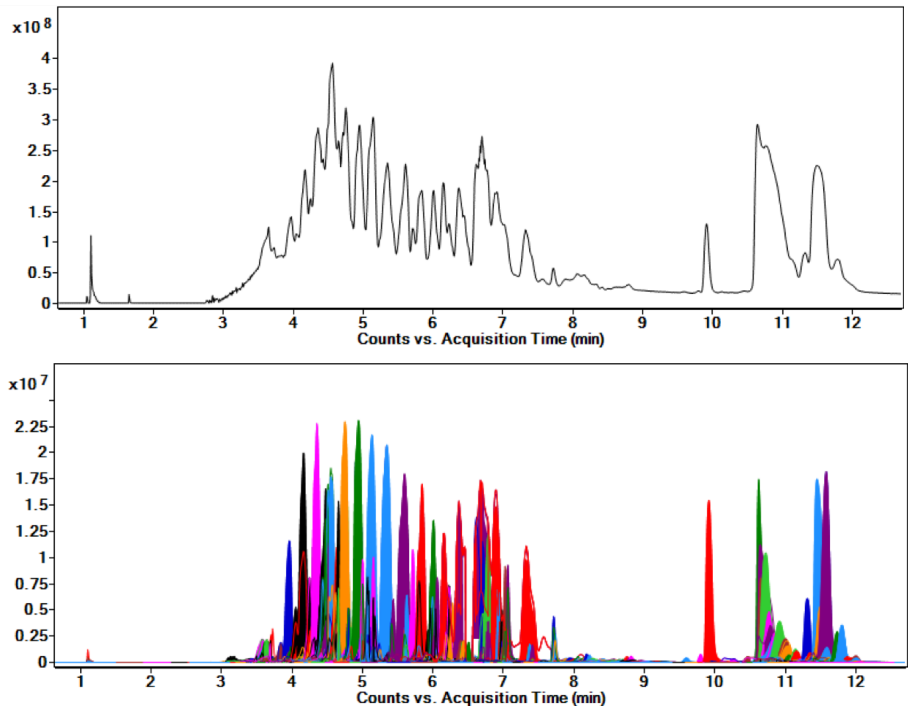
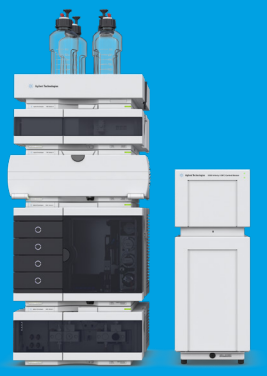


Figure 5. SFC/MS lipid profiling of an extract from liver-specific 2776 cells from 4T1 murine breast cancer cells, a highly aggressive model of triple negative breast cancer. The data shows the total ion chromatogram (top) and the compound chromatogram (bottom) found in the 2776 liver cell extract. Cells were extracted using the Bligh-Dyer protocol.

The Agilent 1260 Infinity II 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 powerful capability for lipidomics.

The Agilent 1260 Infinity II SFC system

- Integrated: Use a single software platform to control state-of-the-art SFC on all Agilent LC/MS systems
- Environmentally friendly: Quickly separates compounds that cannot easily be separated by LC methods, with limited use of organic solvents
- Powerful: Separates polar and non-polar lipids in a single run
- Versatile: Delivers high flexibility with high reliability



Ion Mobility Separation of Lipids

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 6). Ion mobility can also provide lipid class separation for complex samples. The Agilent 6560 IMS Q-TOF system (Figure 7) delivers accurate mass measurements combined with the highest resolution low-field mobility separation commercially available.

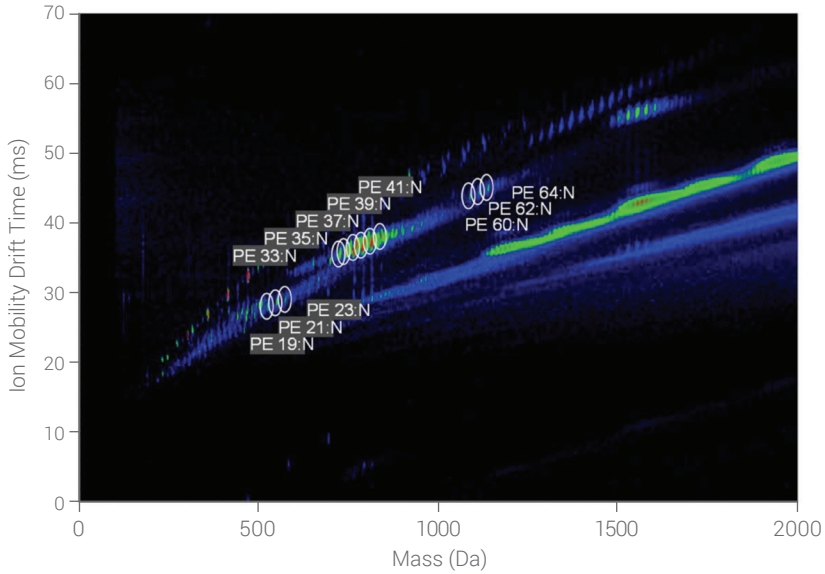


Figure 6. IMS separation of an infused mixture of phosphatidylethanolamine (PE). The increase in drift time is associated with an increase in the number of carbon atoms.

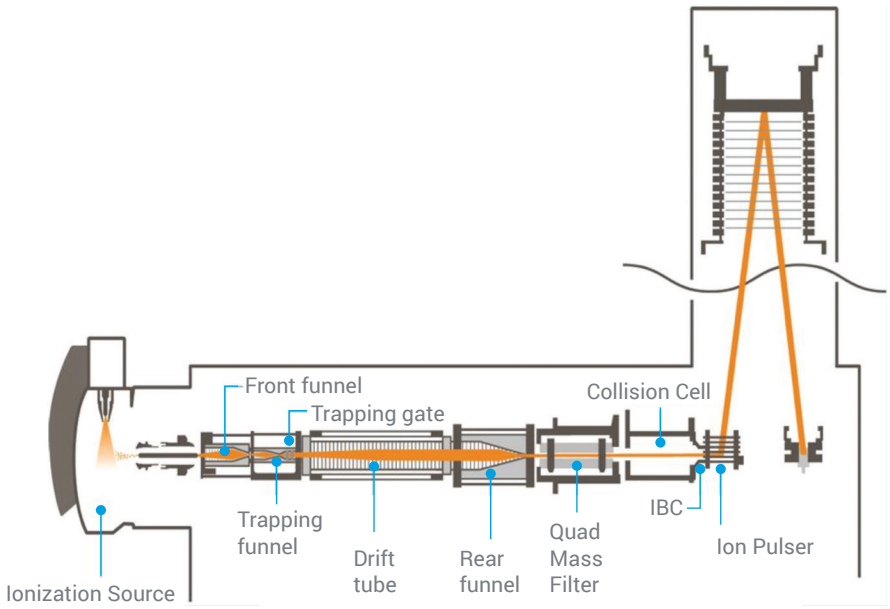


Figure 7. The Agilent 6560 IMS Q-TOF system. Each segment of the dynamic funnel assembly – which includes a front funnel for sample enrichment, trapping ion funnel, drift tube and focusing rear funnel – is carefully designed to improve ion transmission from the source to the Q-TOF high-resolution mass 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

Complete lipid identification includes class, elemental composition, R-group size and location, number and location of double bonds, and double bond orientation (cis/trans). Lipid identification is extremely challenging as there are many biologically relevant lipids and a limited number of authentic lipid standards. While there are small MS/MS spectral libraries from authentic standards for identification, the majority of lipid MS/MS are annotated using *in silico* MS/MS libraries. To facilitate lipid identification, LIPID MAPS Lipidomics Gateway was created in 2003 to support the international lipid research community with an internationally recognized classification system and curated lipid structure database. It has recently moved to the UK with support from the Wellcome Trust. The LIPID MAPS classification system comprises eight lipid categories (Figure 8), each characterized by extensive structural and functional diversity, attributable to different aliphatic chains, stereoisomerism, chirality, and head group moieties.

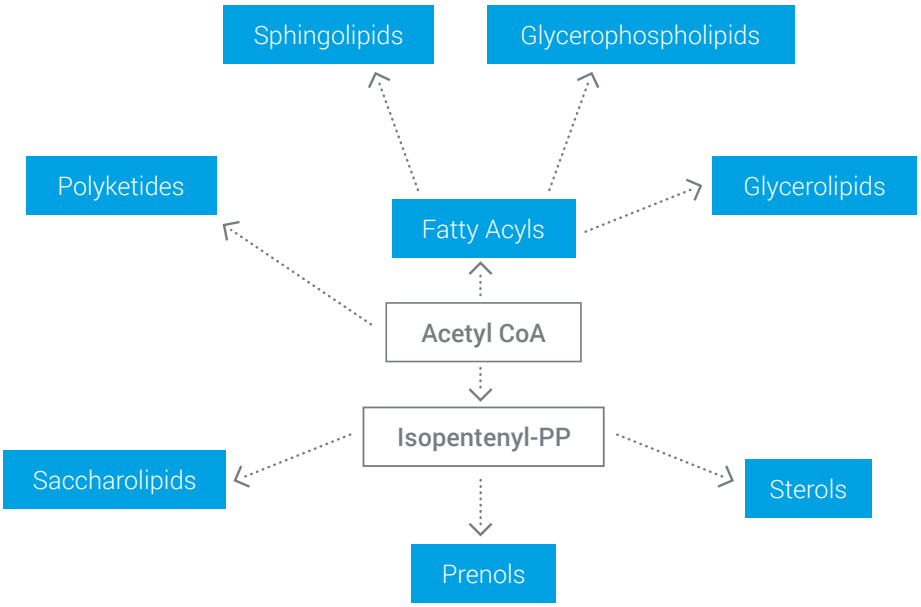


Figure 8. The eight different lipid classes as defined by the LIPID MAPS consortium.

Agilent offers solutions for identifying lipids by MS/MS spectral library matching or MS/MS *in silico* spectral matching. Spectral library matching can be performed using the Agilent-METLIN database which contains MS/MS spectra for 819 lipid standards. For *in silico* spectral matching, Agilent provides novel MassHunter Lipid Annotator software, which uses Bayesian scoring and a theoretical lipid library (LipidBlast) to annotate MS/MS spectra. The software is designed to not over-annotate lipid features by providing only the level of structural information supported by the MS/MS spectra.

Software Created for Lipidomics

In lipid profiling, lipid annotation is the biggest challenge. Agilent Lipid Annotator software is designed to rapidly and accurately annotate lipid MS/MS data and convert those results into an accurate mass, retention-time database. That lipid database is then used to easily extract annotated lipids from MS1 data using the same chromatography as used for database creation. The database can be used routinely to annotate MS1 lipid profiling data. As shown in Figure 9, Agilent's lipidomics software workflow supports Agilent Q-TOF and IM Q-TOF data. For large lipidomics projects, this targeted data mining approach provides more efficient and consistent results.

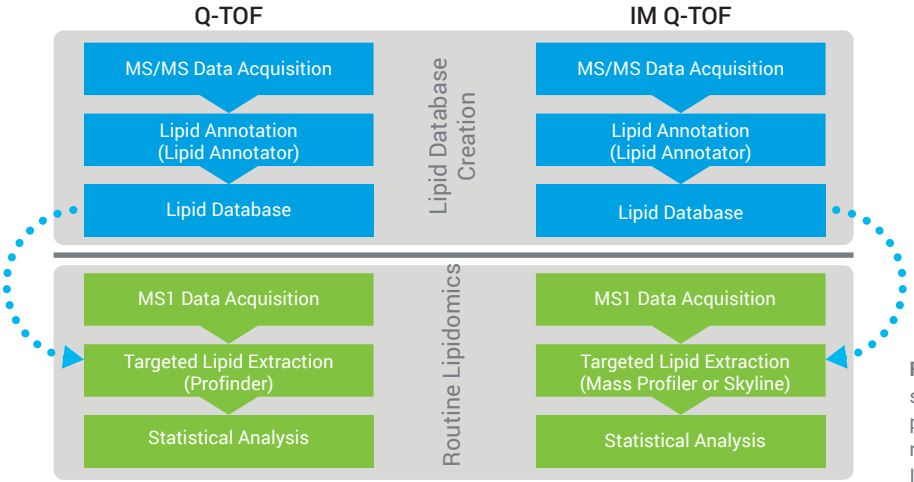


Figure 9. Agilent MassHunter Lipid Annotator software powers a lipidomics workflow by producing an annotated lipid database for routine analysis on either the Agilent Q-TOF or IM Q-TOF platforms.

Lipid Annotator provides a high-level review of annotation results, including a pie chart view of lipid classes and a configurable scatterplot of the features (Figure 10). The lipid match detail tab allows an in-depth review of the annotation results from a lipid sum composition table. Selecting an entry in the table interactively displays possible lipid constituents with associated spectral match results, including a mirror plot of the MS/MS spectral match.

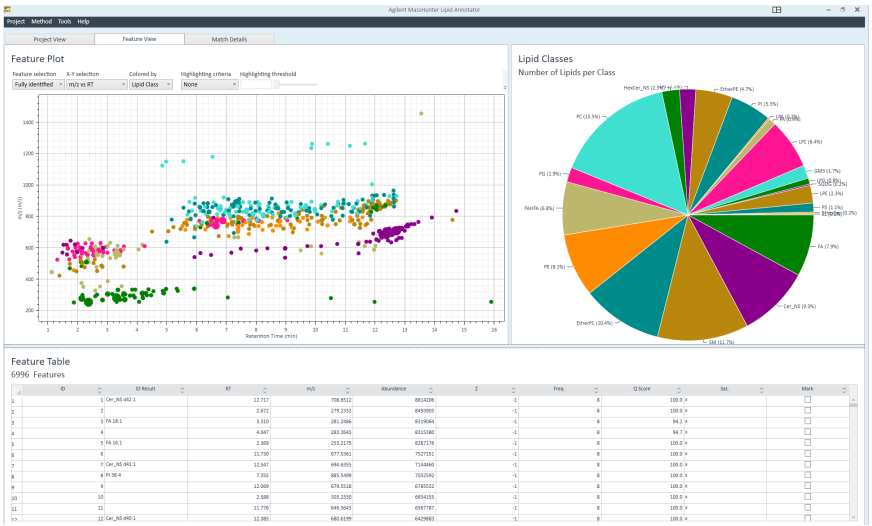


Figure 10. Agilent MassHunter Lipid Annotator software provides both high-level and detailed view of the lipid annotation results for a batch of MS/MS data files.

Software Tailored to Your Lipidomics Research

In lipidomics, as with metabolomics, multiple samples are analyzed with the intent to compare and discover differences between sample groups using multivariate statistics. Agilent offers advanced analysis software for processing and interpreting complex lipidomics data. MassHunter Mass Profiler Professional (MPP) software 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.

Lipidomics has some unique challenges compared to metabolomics, and MPP has a lipidomics experiment type to support lipidomics analysis. The workflow is designed to import annotated targeted lipid results from either Agilent Q-TOF or IM Q-TOF data. MPP supports lipid class-based internal standard normalization for greater relative quantitative accuracy. It also has several visualizations to support lipid analysis, including a lipid matrix plot and a Kendrick mass defect plot. The Kendrick plot enables the detection of subtle differences in lipid structures such as the presence of a double bond.

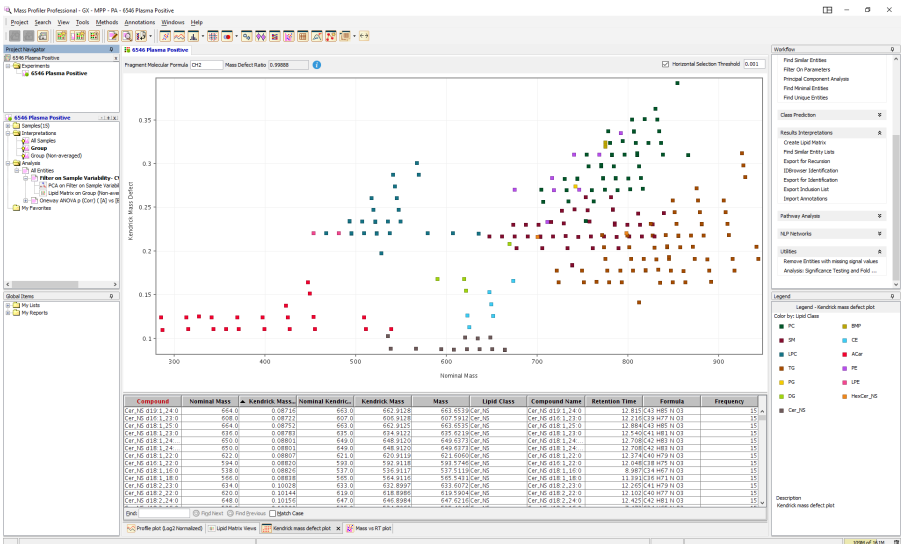


Figure 11. Agilent MassHunter Mass Profiler Professional software showing the Kendrick mass defect plot for a lipidomics experiment.

Agilent GC/MS and LC/MS Solutions for Lipidomics

GC/MS Instruments

5977B Series GC/MSD System

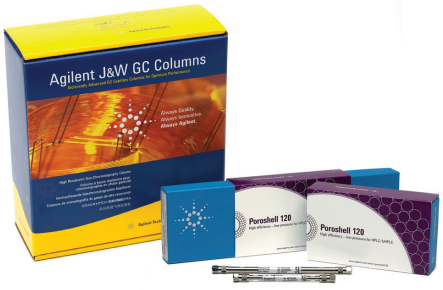
The Agilent 5977B high-efficiency source GC/MSD system incorporates an ultra-efficient electron ionization source to maximize the number of ions created and transferred into the analyzer, revolutionizing single quadrupole performance.

7000 and 7010 Triple Quadrupole GC/MS Systems

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.

7250 GC/MS Q-TOF System

The Agilent 7250 quadrupole time-of-flight GC/MS system delivers high sensitivity and MS/MS capability with the added value of high-resolution, accurate-mass data with a wide dynamic range for structural confirmation, unknown compound identification, and superior untargeted profiling capabilities.



Agilent has a wide selection 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.

LC/MS Instruments

1260 Infinity II SFC system

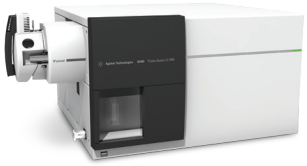
The Agilent 1260 Infinity II SFC system enables fast, high resolution separations of lipids that cannot easily be separated by other methods. Polar and nonpolar lipids can be separated in a single run, with high flexibility, high precision, and excellent reliability.

1290 Infinity II LC

The Agilent 1290 Infinity II LC system 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 superior throughput.

6500 Series Accurate-Mass Q-TOF

The Agilent 6500 Series accurate-mass Q-TOF LC/MS delivers the power of accurate mass MS/MS to identify, screen, profile, or quantitate metabolites in complex samples. The system's mass accuracy increases confidence in lipid annotations.

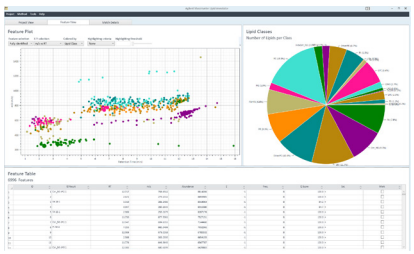


6400 Series Triple Quadrupole LC/MS

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

Agilent Bravo Automated Liquid Handling Platform

The compact Agilent Bravo Liquid Handling Platform is designed to automate sample preparation. Bravo delivers higher accuracy and reproducibility compared to manual sample preparation, making it ideal for lipidomics studies.



MassHunter Lipid Annotator software

Agilent MassHunter Lipid Annotator software provides fast and accurate *in silico* spectral matching, using the LipidBlast theoretical lipid library to annotate MS/MS spectra.

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