

METABOLOMICS GENOMICS INFORMATICS PROTEOMICS
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Considerations for Selecting GC/MS or LC/MS for Metabolomics

Due to the wide variety of endogenous metabolites, truly comprehensive metabolomic research requires both GC/MS and LC/MS technologies. In practice, most metabolomic experiments are not comprehensive, and factors such as chemical nature of the metabolites, sample matrix, and cost influence the choice between GC/MS and LC/MS. This selection guide reviews some of the defining characteristics of GC/MS and LC/MS and presents factors that should be considered when choosing between them. It also provides guidance as to the major components required for GC/MS and LC/MS systems for the various steps in the metabolomics workflow.

GC/MS analysis

Gas chromatography and mass spectrometry (GC/MS) are an effective combination for the analysis of volatile chemicals. Gas chromatography uses a carrier gas to move analytes through a coated, fused silica capillary. Separation occurs based on differential partition between the gas phase and the coating inside the capillary. GC/MS requires the analyte to be vaporized in order for migration through the capillary to occur. Analytes, therefore, must be volatile or amenable to chemical derivatization to render them volatile.

Certain types of samples are particularly well suited to GC/MS analyses (see Figure 1). These include plant terpenes and essential oils, which are volatile and do not ionize well by LC/MS techniques. After derivatization, free fatty acids are also amenable to GC/MS. The high chromatographic resolution of GC permits separation of structurally similar fatty acids that would be very difficult to separate by HPLC. GC/MS provides greater sensitivity than LC/MS for free fatty acids. Other analytes generally compatible with GC/MS include

steroids, diglycerides, mono-, di- and trisaccharides, and sugar alcohols.

Electron ionization (EI) is the most commonly used GC/MS ionization technique. It is very robust and reproducible. It does not suffer from ion suppression, where a compound suppresses the ionization of a co-eluting compound. Electron ionization causes characteristic mass spectral fragmentation patterns. Thus, EI spectra from unknowns can be searched against libraries of EI spectra to achieve identification.



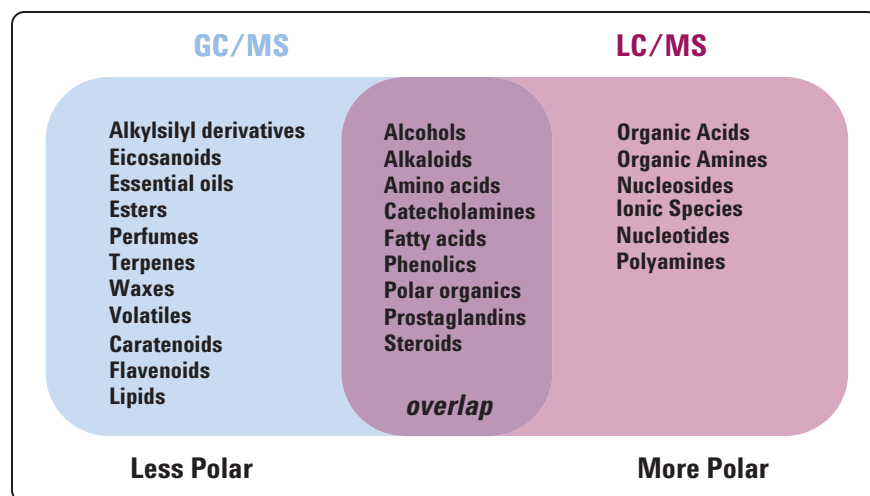


Figure 1. Classes of chemicals and the analytical techniques with which they are most compatible.

A simple identification method uses only the background-subtracted EI spectra and a search of a general-purpose EI library such as the NIST library. A more powerful identification method involves searching both chromatographic retention time and mass spectra of an analyte against an application-specific library containing expected retention times and EI spectra for compounds. Currently, there are no publicly available EI libraries devoted exclusively to endogenous metabolites.

With EI, the molecular ion is often lost. If the metabolite spectrum is not in the library being searched, the absence of mass information for the molecular ion makes it difficult to limit the number of chemical possibilities. Complementary chemical ionization (CI) can be used to preserve the molecular ion, but it comes with the loss of the structural information that EI fragmentation provides. Therefore, GC/MS is best suited for targeting known or anticipated metabolites.

The cost of GC/MS systems is substantially less than that of LC/MS systems.

LC/MS analysis

Liquid chromatography can separate metabolites that are not volatile and have not been derivatized. As a result, LC/MS can analyze a much wider range of chemical species than GC/MS. Samples commonly analyzed by LC/MS include amino acids (18 out of 20 amino acids can be derivatized, but the remaining two can not) and sugars larger than trimers.

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the two ionization techniques most commonly used in LC/MS. Unlike EI for GC/MS, ion suppression can occur with both ESI and APCI so co-eluting compounds may be underestimated or not detected at all. Therefore, for complex samples, greater separation is necessary for the reliable LC/MS results. Unlike GC/MS, LC/MS almost always produces a molecular ion that can be used to limit the possible identities of a given analyte.

There are no spectral libraries for LC/MS identification. However, because the molecular ion is usually present in LC/MS analyses, its mass can be used to search a database of metabolites such as the METLIN database. In addition, the development of accurate-mass time-of-flight mass spectrometers has enabled the calculation of an empirical formula from the molecular ion. To truly address unknown metabolites, MS/MS fragmentation on a Q-TOF with manual *de novo* interpretation is the next step in the identification process.

LC/MS is best suited for a discovery-based approach when researching unknown metabolites, or when many of the targeted metabolites are not readily amenable to GC/MS analysis due to volatility issues.

The cost of an LC/MS system is substantially more than that of a GC/MS system.

Ordering information for LC/MS metabolomics systems

LC/MS systems for metabolomics research are more varied than GC/MS systems. Different steps in the workflow

are best served by different types of LC/MS systems. TOF LC/MS systems are excellent for low-cost profiling. Q-TOF LC/MS systems can also be used for profiling, but generates both the

MS/MS data and accurate-mass data necessary for identification. Triple quadrupole LC/MS systems are unsurpassed for high-throughput targeted validation.

Recommended LC/MS systems for metabolomics

Part Number	Description	LC/MS Basic Profiling System	LC/MS Advanced Profiling System	LC/MS ID System	LC/MS Validation System
LC					
1200 Series Rapid Resolution					
G1312B	Binary SL Pump	X	X	X	X ¹
Opt 031	Solvent Selection	X	X	X	X
G1379B	Vacuum Degasser	X	X	X	X
G1367C	High Perf Autosampler SL	X	X	X	X
G1330B	Thermostat for well plate sampler	X	X	X	X
G1373A	Injector Purge kit	X	X	X	X
G1316B	Column Compartment	X	X	X	X
Opt 057	Valve for ACR				X
Opt 060	Capillary kit	X	X	X	X
G1315C	Diode Array Detector	X ²	X ²	X ²	X ²
Opt 10	Micro flow cell	X	X	X	X
Opt 18	Standard flow cell	X	X	X	X
Consult your Agilent sales representative ³	Columns and accessories				
MS					
G3250AA	6210 TOF LC/MS with ESI source, TOFChemStation software, and data system (PC)	X			
G6510AA	6510 QTOF LC/MS with ESI source, MassHunter Workstation software, and data system (PC)		X	X	
G6410AA	6410 Triple Quadrupole LC/MS with ESI source, MassHunter WorkStation software, and data system (PC)				X
G1978A	Multimode ESI/APCI source	X ⁴	X ⁴	X ⁴	X ⁴
Software					
G3297AA	MassHunter Profiling Software for Agilent TOF	X ⁵	X ⁵		
G1792AA	GeneSpring MS Workstation 1-year license	X ⁵	X ⁵		X

1. A second binary pump is recommended for high-throughput validation.

2. Optional.

3. Columns and related accessories will depend on the type of samples being run and the sample preparation you do. Your Agilent sales representative or application engineer can help you select the best column for your application.

4. Optional ion source upgrade.

5. GeneSpring MS is preferred over MassHunter Profiling Software for statistical analysis and profiling when more than a few samples are to be analyzed.

Ordering information for GC/MS metabolomics systems

The same basic GC/MS hardware and software is used for all steps in the metabolomics workflow. Adding the option of chemical ionization in the

identification system makes it possible to acquire molecular ion, as well as fragment, information. This can aid in identifying metabolites not found in spectral libraries. GeneSpring MS is used for profiling and validation, but is not needed for identification.

Recommended GC/MS systems for metabolomics

Product Number	Description	GC/MS Profiling System	GC/MS ID System	GC/MS Validation System
G3442A	7890A GC with split/splitless inlet and interface for an MSD	X	X	X
G3243A	5975C inert XL MSD with electron ionization, ChemStation software, and PC	X	X	X
G1033A	NIST 05 Library including AMDIS deconvolution software	X	X	X
G1792AA	GeneSpring MS Workstation 1-year license	X		X
G3245A	5975C inert XL MSD with EI/CI, ChemStation software, and PC		X ¹	
Consult your Agilent sales representative ²	Columns and accessories	X	X	X

1. G3245A with EI/CI can be substituted for G3243A with only EI

2. Columns and related accessories will depend on the type of samples being run and the sample preparation you do. Your Agilent sales representative or application engineer can help you select the best column for your application.

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