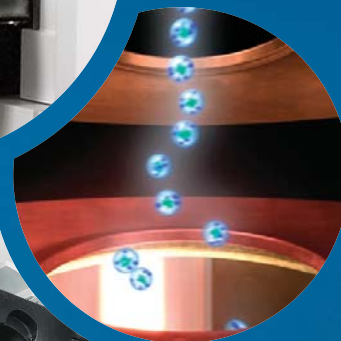


Agilent LC/MS Ion Sources
Maximizing LC/MS performance



Agilent Technologies

Better ion source technology maximizes LC/MS performance

Effective ionization of analytes is an essential step in the successful mass spectrometric analysis of any sample. Agilent Technologies is a leader in ion source technology, offering an expansive selection of LC/MS ion sources to meet the widest range of applications and analytical requirements. Agilent ion sources use highly effective technologies such as orthogonal nebulization and heated counter-flow drying gas to maximize performance, reliability, and ease of use.

Superior nebulization delivers more ions to the mass spectrometer

Agilent's patented nebulizer technology produces very uniform droplets that desolvate in a very consistent manner. The result is more ions delivered to the mass spectrometer for greater sensitivity.

Agilent nebulizers require no adjustments or repositioning to compensate for changes in flow rate or solvent composition. After the initial needle installation, no nebulizer adjustments should be required until the needle is replaced.

High drying capacity improves performance

Agilent's LC/MS ion sources include a high-volume, heated, counter-flow drying gas system. It improves spectral quality, sensitivity, and reproducibility by reducing solvent clusters and mobile-phase adducts. Drying gas flow and temperature can easily be adjusted electronically to match flow rate and solvent composition changes.

In the Agilent multimode source, drying capacity is enhanced by powerful infrared emitters tuned to the absorption frequency of water.

Orthogonal nebulization simplifies operation and reduces maintenance

Nebulization (spray) orthogonal to the axis of the sampling capillary reduces noise related to incomplete drying of solvent droplets. This allows the use of on-axis ion optics elements for greater ion transmission efficiency without increased noise. It also keeps the sampling capillary and ion optics cleaner.

Together, superior nebulization, counter-flow drying gas, and orthogonal nebulization increase mass spectrometer performance by increasing signal while simultaneously reducing noise. They also make Agilent's ion sources very tolerant of nonvolatile components in samples and mobile phases.

Dual nebulizers maximize mass accuracy

Some Agilent ion sources are available with dual nebulizers for use with TOF and Q-TOF mass spectrometers. The additional nebulizer is used to introduce a low-concentration reference mass solution, facilitating much more accurate mass axis adjustment. The result is almost effortless attainment of spectacular mass accuracy.

Orthogonal nebulization and high drying capacity increase performance and reliability. This APCI source was still generating useful data.



Dual-nebulizer ion sources help achieve the best possible mass accuracy in TOF-based instruments

Multiple ionization techniques to serve more applications

No ionization technique is suitable for all applications. Agilent offers you a wide range of choices. The Agilent ion sources are described in more detail in the following pages. Table 1 provides some general compatibility guidelines. These are only general guidelines; your Agilent customer team can help you choose the best solution for your specific applications and samples.

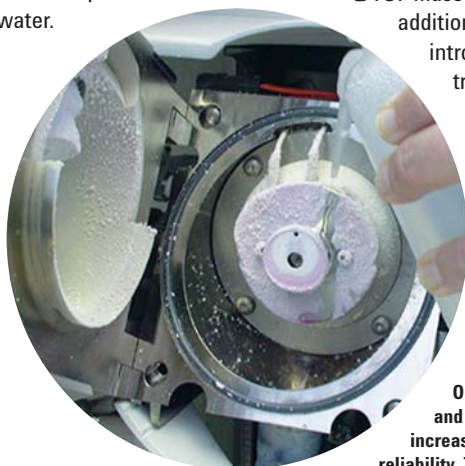


Table 1. General guidelines for applicability of ionization techniques

	Ionization Technique				
	Electrospray	APCI	Multimode	APPI	MALDI
Significant Compound Classes					
DNA	++	0	++	0	+++
Proteins	+++	0	++	0	+++
Peptides	+++	0	++	0	+++
Amino acids	++	+	++	+	+
Polymers	++	+	++	0	+++
Carbohydrates	++	+++	+++	+++	+
Triglycerides	+	+++	+++	+++	0
Aromatic hydrocarbons	0	+	+	+++	0
Aliphatic hydrocarbons	0	+	+	++	0
Functional Groups					
Acids	+++	++	+++	++	++
Alcohols	+	++	++	++	+
Aldehydes and Ketones	+	++	++	++	+
Alkanes	0	+	+	++	0
Alkynes	0	++	++	+++	0
Amines	+++	+++	+++	+++	+
Carbonyls	+	++	++	++	0
Esters	++	+++	+++	+++	0
Ethers	+	++	++	++	0
Phenols	++	+++	+++	+++	+
Chemical/Physical Properties					
Polar	+++	++	+++	++	++
Non-polar	0	++	++	+++	0
Volatile	+++	+++	+++	+++	0
Non-volatile	+++	0	+++	0	+
Thermally stable	+++	+++	+++	+++	+++
Thermally unstable	+++	0	+	0	+
Susceptible to ion suppression	0	++	++	++	++
LC Solvents					
Polar solvents	+++	+++	+++	+++	0
Non-polar solvents	0	+++	+++	+++	0
Salts and buffers	+	++	++	++	+++
Ion pairing agents	+	++	++	+	+++
Volatile modifiers	+++	+++	+++	+++	+++
LC Flow Rate					
Greater than 1 mL/min	+	+++	+++	+++	0
500 – 1000 µL/min	++	+++	+++	+++	0
100 – 500 µL/min	+++	++	+++	++	0
1 – 100 µL/min	+++	+	++	+	0
Less than 1 µL/min	+++	0	0	0	0

+++ = good match with highest chance of success

++ = generally works well or can be made to work with minor method adjustments

+ = unlikely to provide best results, but may work for some samples or with significant method adjustments

0 = poor match with little chance of success, or not applicable

Electrospray

Electrospray ionization (ESI) is a mainstay of LC/MS. Thanks to multiple charging and relatively low operating temperatures, it is particularly well suited to the analysis of biological macro-molecules such as proteins and peptides. But ESI can also be used to analyze many smaller molecules, especially more-polar molecules.



Electrospray is a concentration-sensitive technique; more-concentrated samples improve sensitivity. ESI sources designed for lower LC flows rates—combined with sample concentration—can dramatically increase LC/MS sensitivity.

Standard-flow-rate electrospray is robust, reliable, and productive

Agilent's standard electrospray ion source uses our patented orthogonal nebulization and counter-flow drying gas system to achieve excellent sensitivity and extremely robust performance. On mass spectrometers that support it, ion polarity can be switched on a spectrum-to-spectrum basis to double the information acquired from a single run.

Capillary electrospray increases sensitivity without sacrificing reliability

The standard Agilent electrospray ion source can be equipped with a capillary nebulizer optimized for low microliter-per-minute LC flow rates. Used with lower LC flows and smaller-diameter columns, it can significantly increase LC/MS sensitivity. No modifications of the source are required. You retain all the reliability benefits of orthogonal nebulization and counter-flow drying gas.

Electrospray ion source blends sensitivity and robustness

Nanoelectrospray delivers maximum sensitivity and flexibility

When you have limited sample amounts or need to analyze trace-level components in complex mixtures, nanocolumns and electrospray at nanoliter-per-minute flow rates can provide you with the ultimate in LC/MS sensitivity.

Agilent offers two sources designed specifically for maximizing performance at nanoliter-per-minute flow rates: the standard nanoESI source and the HPLC-Chip nano-electrospray source (page 7).

The nanoESI source provides attomole-level sensitivity for online LC separations. It gives you the flexibility of using conventional nanocolumns in one-dimensional or multidimensional chromatography to achieve optimum sample separation. The nanoESI source requires minimal adjustment and is sealed for increased safety when working with potentially hazardous biological samples.

Electrospray for capillary electrophoresis

CE/MS is sometimes used to analyze hydrophilic compounds and other samples that do not separate well by LC. CE offers different selectivity and outstanding separation efficiency.

A CE nebulizer is available for use with the standard electrospray source. The CE nebulizer operates at ground potential, making CE separation conditions and MS operating conditions independent. The CE nebulizer can also be used with Agilent APCI, multimode, and APPI ion sources.



NanoESI source combines extreme sensitivity with flexibility

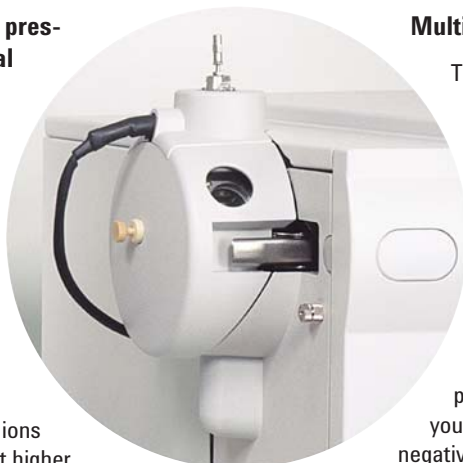
Atmospheric pressure chemical ionization

Atmospheric pressure chemical ionization (APCI) is a popular complement to electrospray. It generates primarily singly charged ions and operates at higher temperatures, so it is most commonly used to analyze small, thermally stable compounds. APCI is applicable to a wide range of less-polar and non-polar molecules.

APCI is a mass-flow-sensitive technique. The more sample molecules, the better the performance. APCI works better with larger columns, higher LC flow rates, and larger total sample amounts.

Agilent's APCI source is extremely robust, thanks to orthogonal nebulization and counter-flow drying gas. The patented corona needle can be replaced in minutes—without tools.

On mass spectrometers that support it, ion polarity can be switched on a spectrum-to-spectrum basis.



APCI source facilitates analysis of less-polar and non-polar compounds

Multimode

The Agilent multimode source is a true breakthrough in LC/MS ionization—the only ion source capable of *simultaneously* generating ions by electrospray and APCI. It is also compatible with spectrum-to-spectrum polarity switching. Thus, you can acquire positive ESI, negative ESI, positive APCI, and negative APCI spectra in a single run; maximizing productivity by ensuring that all compounds are identified regard-

less of the technique to which they best respond.

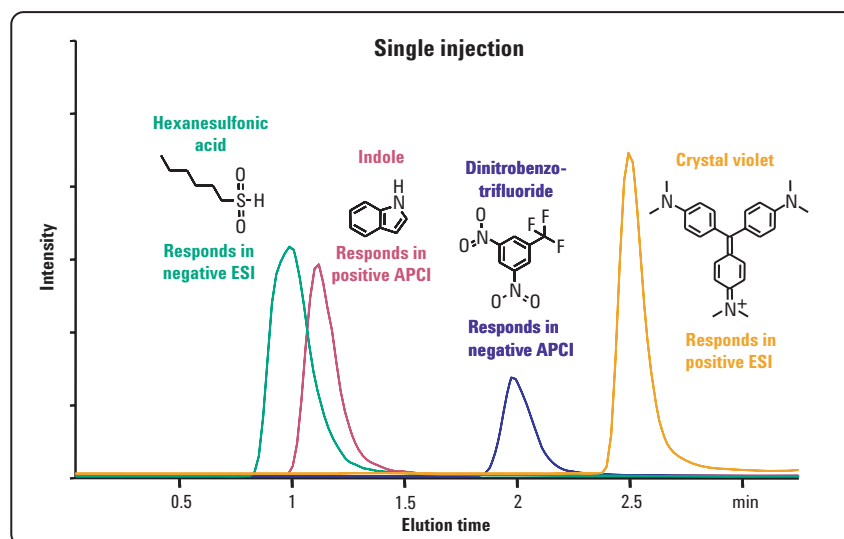
Some ion sources try to approximate simultaneous ionization by switching between ESI and APCI. The result is dead time during switching and a significant loss of data and sensitivity. With the multimode source there is no

dead time, and sensitivity in mixed (simultaneous) mode is close to that provided by Agilent's state-of-the-art individual ESI and APCI sources.

For robust reliability, the multimode source uses the same orthogonal nebulization and heated, counter-flow drying gas as other Agilent ion sources. It also includes two infrared emitters that aid in drying solvent droplets. The drying power of these emitters makes the multimode source extremely tolerant of high aqueous content and high LC flow rates.

When you are sure of the best ionization technique for your samples, you can operate the multimode source in ESI-only or APCI-only mode. If necessary, you can still switch modes on a time-segment basis during a chromatographic run.

Revolutionary multimode source provides simultaneous ESI and APCI to maximize coverage and sample throughput



With simultaneous ESI and APCI, and spectrum-to-spectrum ion polarity switching, the multimode source allows identification of very diverse compounds in a single run

Atmospheric pressure photoionization

For analysis of compounds that ionize poorly by ESI and APCI, the PhotoMate atmospheric pressure photoionization (APPI) source provides a useful alternative. It combines Agilent's proven orthogonal nebulization and counter-flow drying gas with innovative photoionization from Sygen Technology.



APPI source provides an alternative for compounds that do not respond well to ESI and APCI

The RF-resonated krypton lamp emits photons at energy levels high enough to ionize many classes of compounds, but low enough to minimize the ionization of air and common HPLC solvents. Relatively low ionization energy means the APPI source causes minimal fragmentation and generates mostly molecular ions and protonated molecules. The result is easier determination of molecular weights. Thanks to a high photon flux, the lamp also eliminates the need for dopants.

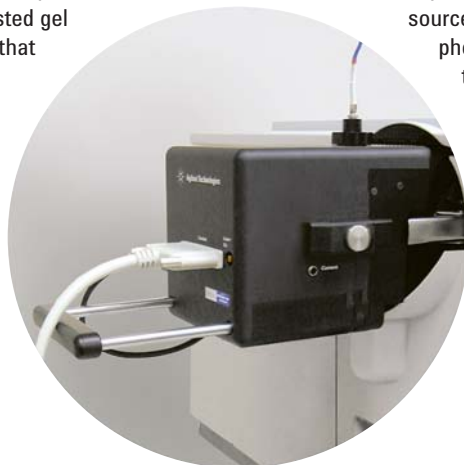
The RF-resonated krypton lamp last longer than comparable direct-discharge lamps.

PDF-MALDI

Matrix-assisted laser desorption ionization (MALDI) is one of the cornerstones of LC/MS analysis of biomolecules. The pulsed dynamic focusing MALDI (PDF-MALDI) ion source developed by MassTech uses Agilent's patented atmospheric-pressure MALDI technology to provide fast, easy, and ultra-sensitive analysis of proteolytically digested gel spots and samples that do not need online separation. It is an excellent solution for your high-throughput screening and expression profiling studies.

Pulsed dynamic focusing directs more sample ions from the MALDI target plate into the mass spectrometer, improving the sensitivity and consistency of the MS analysis. Heated drying gas directed onto the sample plate helps reduce the formation of matrix clusters, resulting in cleaner mass spectra. The PDF-MALDI

source operates at atmospheric pressure, so a target plate can be loaded in seconds—without tools.



Pulsed dynamic focusing in the PDF-MALDI source improves sensitivity and consistency

HPLC-Chip nanoelectrospray source

Agilent's HPLC-Chips represent a revolution in LC separations. The reusable HPLC-Chips seamlessly integrate the sample enrichment and separation nanocolumns, tubing, connections, and spray needle of a traditional nanoelectrospray LC/MS system into a biocompatible polymer chip. HPLC-Chips eliminate all post-column dead volumes so peak dispersion is virtually eliminated. You get narrower, better-defined peaks and greatly improved separations. Overall robustness, reliability, and ease of use are greatly improved compared to conventional nanocolumns, allowing routine automated use of nanoelectrospray. And because electrospray is a concentration-dependent technique, HPLC-Chips can dramatically improve LC/MS sensitivity.

The HPLC-Chip nanoelectrospray source and HPLC-Chip robotic interface allow you to take advantage of the spectacular performance of HPLC-Chips by interfacing them to your Agilent LC/MS system.

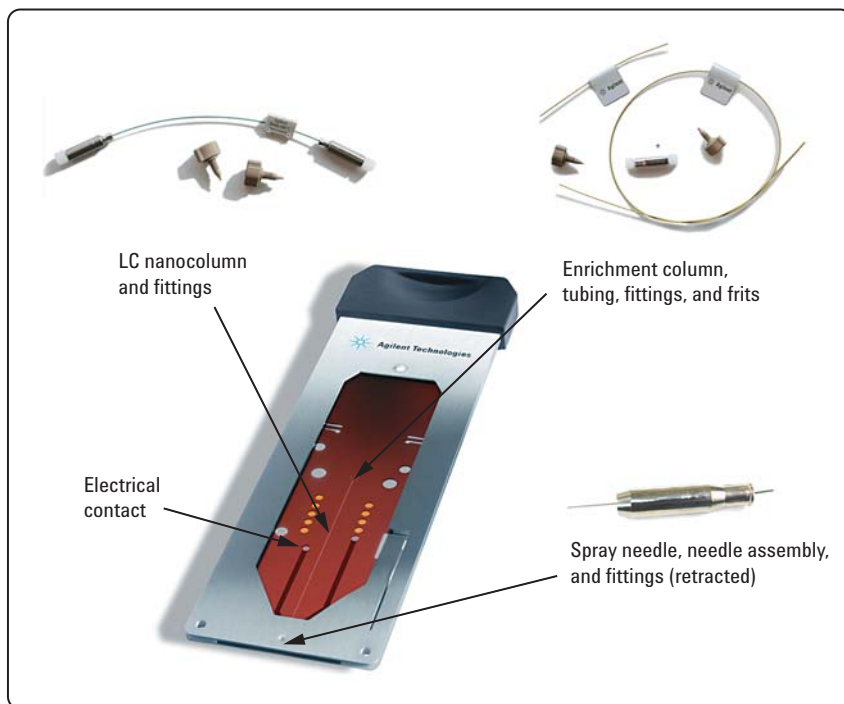
The HPLC-Chip nanoelectrospray source uses orthogonal nebulization and controlled radiant heating to maximize performance and reliability. The source is sealed so that contaminants in the lab cannot enter, and potentially dangerous samples cannot exit except through the mass spectrometer.

The HPLC-Chip nanoelectrospray source can be used with third-party spray needles for troubleshooting and used with conventional nanocolumns. However, the Agilent nanoESI source is recommended if your primary application requires conventional nanocolumns.



HPLC-Chip robotic interface provides fully automated handling of HPLC-Chips

The HPLC-Chip robotic interface provides fully automated handling of the HPLC-Chips. An HPLC-Chip inserted into the robotic interface is automatically connected to the LC solvent lines and positioned for maximum sensitivity. Replacement of an HPLC-Chip takes only seconds and requires no tools.



By incorporating many components of a traditional nanoflow LC/MS system into a biocompatible polymer chip, HPLC-Chips deliver uncompromising separations and dramatically increase LC/MS sensitivity

Compatibility of ion sources and mass spectrometers

In general, most Agilent LC/MS ion sources work with most Agilent LC/MS instruments. However, there are some combinations of ionization technique and mass spectrometer that have little analytical utility. There are also some combinations

that, for physical or software reasons, are not supported. Table 2 provides a general guide to compatibility. Your Agilent sales representative or service representative can provide more specific information about compatibility between your specific model of mass spectrometer and the various Agilent ion sources.

Table 2. Compatibility of Agilent ion sources and LC/MS systems

Ion Source	Mass Spectrometer				
	6100 Series Quadrupole	6200 Series TOF	6300 Series Ion Trap	6400 Series Triple Quad	6500 Series Q-TOF
Electrospray	X	X ¹	X	X	X ¹
Capillary electrospray nebulizer	X	X	X	X	X
Nanoelectrospray (nanoESI)		X ¹	X		X ¹
Atmospheric pressure chemical ionization	X	X ²	X	X	X ²
Atmospheric pressure photoionization	X	X ²	X	X	X ²
Multimode	X	X ²	X	X	X ²
PDF-MALDI		X	X		X
HPLC-Chip nanoelectrospray	X ³	X ²	X	X ³	X ²
Capillary electrophoresis nebulizer	X	X	X		X

1 – Dual-nebulizer version available for TOF and Q-TOF instruments

2 – Requires reference mass solution to be introduced into the LC eluent for maximum mass accuracy

3 - Available in mid-2007

For more information

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