Technical Overview

Optimizing the Agilent Multimode Source

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Abstract

The Agilent multimode source is a highly versatile, high-performance LC/MS ion source. Because the multimode source incorporates both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) into a single ion source, it requires slightly different settings than single-mode ion sources. This technical note will help users set up, get the best possible performance from, and maintain, their Agilent multimode LC/MS ion source. It covers:

- **Which applications are and are not good matches for the multimode source**
- **How to determine which operating mode (ESI-only, APCI-only, or mixed) to use**
- **How to choose the optimum LC solvent flow rate, solvent composition, modifiers, and column**
- **How to set up the multimode source for operation in all three operating modes**
- **How to check the performance of the multimode source**
- **How to modify ESI and APCI methods for use on the multimode source**
- **Using the multimode source on a TOF-based LC/MS instrument**
- **Cleaning and maintaining the multimode source**

Introduction

The Agilent multimode source (Figures 1, 2) is an ionization source for Agilent liquid chromatograph/mass spectrometer (LC/MS) systems. It is unique in that it can *simultaneously* generate ions by both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). It can also operate in ESI-only and APCI-only modes with sensitivity the same as, or very close to, that of dedicated ESI and APCI sources. The primary benefits of the multimode source are:

- Eliminates the need to run samples twice to ensure all components are ionized and identified
- Eliminates the time required to switch ion sources on an instrument
- Allows each mass spectrometer to run more types of analyses
- Makes sample scheduling much easier
- Reduces the nitrogen use of the mass spectrometer by 50%
- Improves lab productivity

Figure 1. Agilent multimode source

Overview of the Multimode Source

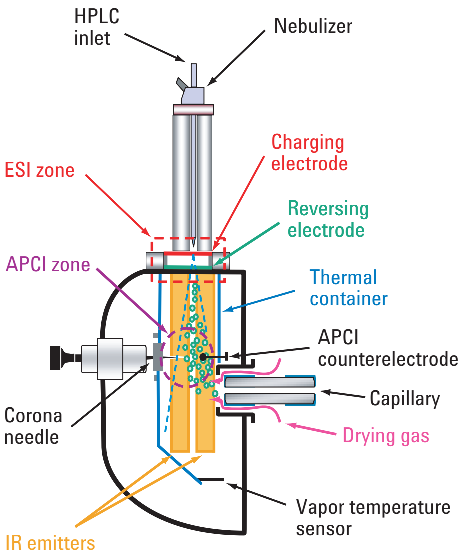

Figure 2. The Agilent multimode source generates ions by ESI and APCI in separate zones within the ion source

1. LC eluent and nebulizing gas enter the grounded nebulizer
2. A charged aerosol is generated in the ESI zone; spray direction is orthogonal to capillary axis
3. The aerosol is dried by infrared emitters and heated drying gas, producing ions by ESI
4. The aerosol and ions flow with the nebulizing gas from the ESI zone to the APCI zone
5. Infrared emitters completely vaporize the solvent and analyte in the APCI zone
6. A corona is produced between the corona needle and APCI counterelectrode, ionizing the solvent
7. Ionized solvent transfers charge to the analyte molecules, producing analyte ions
8. ESI ions pass behind a separator (not shown) that screens them from the APCI corona
9. Power to the infrared emitters is controlled by the vapor temperature sensor, maintaining constant temperature
10. ESI and APCI ions simultaneously enter the capillary

Applications well suited to the multimode source

The Agilent multimode source is a versatile, general purpose ion source. It works extremely well in laboratory settings where there is a wide diversity of samples and where high throughput is needed. It is also ideally suited for walk-up, shared-access situations.

High sample diversity and high throughput applications

The multimode source is ideal when sample diversity is large and the optimum mode of ionization is not known, or it is known that the sample will require both ESI and APCI to achieve full analyte coverage. It is also ideal for high-throughput applications such as library verification. It eliminates the need to run samples twice to ensure all components are identified.

Walkup applications

Its flexibility makes the multimode source ideal for walkup mass spectrometry applications where multiple researchers using multiple methods share an instrument. It increases system capacity because it can be used as both an ESI and APCI source. Switching between modes is controlled by the analytical method and requires no physical changes.

High solvent-flow applications

The multimode source works well for almost all LC/MS applications conducted at normal (roughly 100 µL/min – 2 mL/min) solvent flow rates. Thanks to the added drying capacity supplied by the infrared emitters, it is tolerant of high solvent-flow rates. Flow splitting is not required for flow rates up to 2 mL/min. This reduces liquid solvent waste compared to ion sources that require flow splitting.

Applications not recommended for the multimode source

While the multimode source can run almost every application that can be run by a dedicated ESI or APCI source, there are still applications where the multimode source is not the best choice.

Regulated or established methods

If an application uses a regulated method, then the changes required to run it on a multimode source, though minor, will probably be unacceptable. Also, if a method is well established and new data must be directly comparable to previously acquired data (e.g. relative abundances of multiple target compounds), then the multimode source is not a good choice.

Large sample amounts

The multimode source does not tolerate sample overload as well as the dedicated ESI or APCI sources. If the amount of analyte exceeds 1 µg, chromatographic peak shapes may become distorted. This should not be an issue for most analyses except possibly mass-based fraction collection.

Acetonitrile mobile phase

The multimode ion source does not produce optimum results with acetonitrile mobile phase. If sample chromatography absolutely requires an acetonitrile mobile phase, then a standard ion source should be used. Many LC/MS methods can be converted from acetonitrile to methanol with equivalent, or even better, results.

Very low solvent-flow rates

The multimode source has not been tested extensively on applications with very low solvent flows. If a solvent flow of less than 25 µL/min is required, the method should be tested for compatibility with the multimode source. A dedicated ESI source may be required instead.

Choosing an operating mode

The multimode source can be operated in ESI-only mode, APCI-only mode, or in mixed mode (simultaneous ESI and APCI). The choice of operating mode should be based on characteristics of the analyte(s) and objectives of the analysis.

ESI works well for analytes with a wide range of volatilities. ESI does not work well for analytes that are non-polar or only slightly polar; it works much better on moderately to strongly polar analytes. ESI operates at lower temperatures so it is better for thermally fragile analytes.

APCI does not work well for non-volatile analytes. On the other hand, APCI will work well for both non-polar and polar analytes. APCI operates at higher temperatures so it is less well suited for thermally fragile analytes.

For example, crystal violet is a quaternary amine with virtually no volatility, so it is best analyzed by ESI. Vitamin D, on the hand, is very non-polar and ionizes best by APCI.

If the samples are well understood or the analyses are targeted, then ESI or APCI can be chosen based on the characteristics of the analytes. If the samples are well understood, but contain a mixture of ESI- and APCI-suited analytes, or if maximum coverage of unknowns is the objective, then mixed mode is the better choice.

Choosing the LC solvent flow rate, solvent composition, and column

Getting the most information out of the multimode ion source involves optimizing ion source chemistry. Ion source chemistry is dictated primarily by the choice of solvents and mobile phase modifiers.

Solvent flow rates

The multimode ion source uses two infrared emitters for solvent drying. The emitters are controlled by a dynamic feedback loop. It measures vapor temperature directly and makes adjustments to maintain a constant temperature. This allows the operator to set the vapor (emitter) temperature based exclusively on optimum analyte response. The feedback loop compensates *automatically* for changes in solvent flow rate and composition.

The multimode ion source is optimized for solvent flow rates between 400 and 1000 µL/min, but lower and higher flow rates are supported. The high-wattage (270 W) emitters have sufficient drying capacity for aqueous flow rates up to 2000 µL/min without requiring splitting of the HPLC flow.

Organic solvents

The multimode ion source is two ion sources—ESI and APCI—in one. As such, it has the same ionization behaviors as dedicated ESI and APCI sources. For optimal performance, the choice of organic solvent is dictated by the mode of ionization. The two most common solvents are acetonitrile and methanol.

In ESI mode, the multimode source does not show significant differences in ion formation when using either acetonitrile or methanol. The exception is for very weakly basic analytes, where the use of acetonitrile results in substantially reduced sensitivity.

In APCI mode, ionization is controlled by vapor chemistry. The use of acetonitrile in APCI can prevent ion formation in both positive- and negative-ion polarities. For maximum analyte coverage in APCI, methanol is strongly preferred for the organic solvent.

In mixed mode (simultaneous ESI and APCI), methanol is the preferred organic solvent. Use of acetonitrile will suppress or prevent atmospheric pressure chemical ionization.

Use of modifiers

The choice of mobile-phase modifiers is driven by both chromatography and ion formation. ESI requires modifiers to facilitate ionization. APCI does not require modifiers for ionization, but since the modifier may also be a reagent in the gas phase, it can affect ion formation in APCI.

Choice of modifiers must also take ion polarity into account. For operation in positive ion mode, acetic and formic acid and their ammonium salts generally work well. For operation in negative ion mode, ammonia, 1- to 5-mM ammonium acetate, or low concentrations of acetic acid will work well.

When positive/negative ion switching is used, the choice of modifiers is somewhat limited because a modifier that minimizes ion suppression in both the liquid and gas phase and in both ion polarities must be found. Tests show that best results are usually achieved using 1- to 5-mM ammonium acetate or 0.2% acetic acid, or a blend of both if a blend is needed for chromatography. Formic acid should be avoided because it significantly suppresses negative ions in ESI, APCI, and mixed modes of operation.

Use of 2.1-mm vs. 3.0-mm columns

Because of its high-wattage infrared emitters, the multimode source generally is not limited by the ability of the source to vaporize the solvent. However, the rules of physics that govern individual ESI and APCI sources still apply to the multimode source.

The ESI process is concentration dependent. It produces better sensitivity with the higher sample concentration that a 2.1-mm column provides.

The APCI process is mass flow limited. It produces better sensitivity with the greater mass flow achievable with a 3.0-mm column.

Tests show that 2.1 mm columns operated at liquid flow rates between 400 and 600 µL/min provide good sensitivity for ESI, APCI, and mixed modes. Sample analysis times are shorter and the method is robust. In addition, 2.1-mm columns reduce solvent consumption compared to 3.0-mm columns, and reduce the frequency of ion source cleaning.

Setting up the multimode source

The multimode source has a number of parameters that affect ionization. Some of the parameters have settings that are nearly universal; the same set point will work for ESI, APCI and mixed modes. Other parameters require different set points depending on the ionization mode used. In almost all cases, the parameters have broad maxima so that complex, iterative, multivariable adjustments are not necessary.

In mixed mode, a few of the parameters—especially corona current and vaporizer temperature—require compromises between the setting that would produce the best ESI results and the setting that would produce the best APCI results. Minor experimentation may be required to find the best settings for a particular analyte or set of analytes.

Table 1 lists all of the multimode source parameters and typical set points for each ionization mode. The parameters are discussed in more detail in the material following the table.

Table 1. Typical and starting settings for multimode source adjustment and operation.

Parameters	ESI	APCI	Mixed Mode
Capillary voltage (Vcap)			
single ion polarity	2000 V	2000 V	2000 V
polarity switching	1000 V	1000 V	1000 V
Charging electrode	2000 V	0 V	2000 V
Corona current	0 µA	4 µA	2 µA
Drying gas flow	5 L/min	5 L/min	5 L/min
Drying gas temperature	300 °C	300 °C	300 °C
Nebulizer pressure	60 psig	30 to 60 psig	40 to 60 psig
Vaporizer temperature	150 °C	250 °C	200 °C

Capillary voltage

The capillary voltage (Vcap) on the multimode source is only used for ion guidance; it does not play a role in the ion formation process. A capillary voltage (Vcap) of 2000 V works well for ESI, APCI, and mixed modes.

If positive/negative ion polarity switching is used, Vcap should be set to 1000 V for all ionization modes. The lower voltage improves the speed of recovery, and hence data quality, after the polarity switches. However, this lower voltage on the Vcap is not recommended if masses greater than *m/z* 1000 are to be analyzed. The lower voltage will not supply sufficient ion guidance. Brief testing may reveal an optimum setting between 1000 V and 2000 V.

Charging electrode

The charging electrode is unique to multimode source. It plays a critical role in ion formation in ESI and mixed modes of operation. 2000 V is a typical charging electrode setting for these modes. The charging electrode is not used in APCI mode. The software automatically turns it off.

Corona current

Corona discharge is used in APCI. 4 µA is a good starting point for the corona current, and it works well for many analytes, but the corona current can be adjusted as necessary for particular analytes and samples. In ESI-only mode, the corona current is automatically turned off. For operation in mixed mode, a compromise setting of 2 uA is a good starting point.

Drying gas flow and temperature

A drying gas flow of 5 L/min and a drying gas temperature of 300 °C work well for most analytes in all ionization modes. Both parameters, however, can be adjusted. If analytes are, for example, very thermally fragile, reducing the drying gas temperature slightly may improve performance.

Nebulizing gas pressure

The nebulizing gas (nebulizer) pressure is dictated by the solvent flow rate. In ESI mode, the multimode source operates best with a setting of 60 psig for the nebulizer pressure.

The optimum setting for the nebulizer pressure in APCI mode is more variable. The optimum nebulizer pressure increases as the solvent flow rate increases. A good starting value for APCI is 30 psig at a flow rate of 400 µL/min. A setting of 60 psig is optimum at flow rates of 800 µL/min and above.

For mixed-mode ionization, the optimum setting will depend on the mixture of analytes. Some experimentation may be required. A good starting value for mixed mode is 40 psig at a flow rate of 400 µL/min. A setting of 60 psig is optimum at flow rates of 800 µL/min and above.

Vaporizer temperature

In the multimode source, infrared emitters provide solvent drying. The set point for the infrared emitters is called the vaporizer temperature. Vaporizer temperature is measured directly and controlled by a dynamic feedback loop, so it remains constant as the solvent composition changes.

ESI mode typically uses a vaporizer temperature of approximately 150 °C. This relatively low temperature helps protect thermally fragile molecules.

APCI mode requires a higher vaporizer temperature. 250 °C is typical.

The correct vaporizer temperature for mixed-mode ionization depends largely on the relative mixture of ESI-favoring versus APCI-favoring analytes in the samples. 200 °C is a good starting point, but minor experimentation may be required to find the correct balance between preserving thermally fragile analytes and achieving optimum APCI.

Sensitivity checks for the multimode source

The ChemStation software includes methods for testing the multimode source in ESI, APCI, and mixed modes. The test sample is reserpine. The tests must be performed on a system that is clean and has recently been tuned. Specifications for the multimode source are listed in Table 2.

Table 2. Sensitivity in selected ion monitoring (SIM) mode*

	Dedicated APCI source	**Dedicated ESI source**	**Multimode source–APCI mode**	**Multimode source–ESI mode**
6110 & 6120 Quadrupole LC/MS	10 pg, 20:1	10 pg, 20:1	10 pg, 10:1	10 pg, 20:1
6130 & 6140 Quadrupole LC/MS	1 pg, 20:1	1 pg, 20:1	1 pg, 10:1	1 pg, 20:1

*All signal-to-noise ratios listed are peak to peak

The mixed-mode check-out test verifies that all test compounds are detected in mixed-mode operation at *no less* than 20% of their pure-mode response. The nebulizer pressure and/or corona current setting can be adjusted to change the balance in the ratio of ESI and APCI response to pass the mixed mode test.

Modifying ESI and APCI methods for use on the multimode source

Methods developed for dedicated ESI and APCI sources must be modified to work on a multimode source. The modifications are generally minor, but can depend on the analyte(s).

Converting an ESI method to a multimode ESI method

When moving an ESI method from dedicated ESI source to a multimode source, the changes are fairly straight forward:

- Set the voltage for the charging electrode to 2000 V
- Reduce the capillary voltage (Vcap) to 2000 V
- Set the nebulizer gas pressure to 60 psig
- Set the vaporizer temperature to 150 °C

These are general settings; minor adjustments may be required for particular samples. For example, if excessive amounts of sodium and potassium adducts are observed, it is an indication that the multimode source is too hot. The vaporizer and drying gas temperatures would need to be lowered.

Converting an APCI method to a multimode APCI method

APCI methods for the dedicated APCI sources and the multimode sources are very similar. Changes that must be made when converting an APCI method to a multimode APCI method include:

- Set the voltage for the charging electrode to 0 V
- Reduce the vaporizer temperature to 250 °C
- Set the corona current to 4 µA regardless of ion polarity

The difference in vaporizer temperature is not as great as the numbers imply, because vaporizer temperature is measured differently in the two sources. Still, a dedicated APCI source may work slightly better for some analytes with low volatility.

Converting ESI and APCI methods to mixed-mode methods

Converting a method for a dedicated ESI or APCI source to a multimode mixed-mode method is only slightly more involved than converting it to a multimode ESI or multimode APCI method. The method should first be converted to a multimode ESI or APCI method. Then, the following parameters:

- Charging electrode voltage
- Corona needle current
- Nebulizer pressure
- Vaporizer temperature

will need to be adjusted for multimode operation as outlined in the previous section: *Setting up the multimode source.*

Use of multimode source on a TOF LC/MS system

The multimode source can be used on an Agilent 6210 Time-of-Flight LC/MS. The only additional requirement is that reference mass solution needs to be added to the LC eluent through a “T” junction. The reference mass solution added should be approximately 5–10% of the solvent flow, by volume, but some empirical adjustment will probably be necessary. Enough reference mass solution must be added that the reference masses can be detected, but not so much that they interfere with the analytes.

An LC pump is recommended over a pressurized bottle as a means of introducing the reference mass solution. A pump provides more accurate delivery. An isocratic pump with a splitter works well, and the split not used can be returned to the reservoir, eliminating waste.

When preparing the reference solution, methanol should be substituted 1:1 for the normal acetonitrile. 5-mM ammonium acetate should be used as a modifier instead of formic acid.

Maintaining the multimode source

The multimode source is more complex than either dedicated ESI or APCI sources. However, it is designed to be very robust and relatively easy to clean. Frequent cleaning is not recommended. The multimode source should be cleaned *only* when symptoms indicate it is necessary. Symptoms associated with the need for source cleaning include:

- Visible salt deposits on the inner surfaces of the source
- Increasing spectral background that is interfering with analyses
- Increasing corona voltage when other parameters remain unchanged

Cleaning the multimode source

The source ***must be cooled*** before cleaning. The interior of the chamber can be cleaned by thoroughly rinsing it (***do not submerge it***) with deionized water. See the instructions that come with your multimode source for complete details, warnings, and cautions.

If chemical background persists, the most likely culprit is the charging electrode structure. It can be disassembled and cleaned. If the charging electrode, or the multimode source in general, needs to be cleaned too frequently, the most likely cause is the nebulizer pressure being set too low. Increasing the nebulizer pressure should improve source cleanliness.

Replacing the corona needle

The corona needle slowly erodes and will need to be replaced occasionally. Also, the counter-electrode needle gets dirty and will need to be cleaned or replaced. Problems with corona current are an indication that these parts are in need of cleaning or replacement. A problem with these parts can also be detected by monitoring the corona voltage needed to produce the desired corona current. As the parts get dirty or erode, more corona voltage will be needed to achieve the same level of ionization.

Cleaning the capillary cap and field-shaping electrodes

The capillary cap and field-shaping electrodes eventually get dirty in the course of normal operation. They can be cleaned by soaking them in a solution of 20% nitric acid for 30 minutes. They should be thoroughly rinsed with deionized water after cleaning, and dried before reinstallation.

Replacing infrared emitters.

The infrared emitters last a long time (estimated to be 3 years in typical operation). They are similar to a light bulb in that they will work correctly up to the moment of their failure. Infrared emitters can be replaced by an Agilent service representative or by the user. See the user information supplied with the multimode source for more information.

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Printed in the U.S.A. July 31, 2007

5989-6463EN