



Agilent 6400 Series Triple Quad LC/MS System

Concepts Guide

The Big Picture



Agilent Technologies

Notices

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Software Revision

This guide applies to the Agilent MassHunter Workstation Software -- Data Acquisition for 6400 Series Triple Quadrupole program version B.02.01 or higher until superseded.

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In This Guide...

The Concepts Guide presents “The Big Picture” behind the operation of the Agilent 6400 Series Triple Quadrupole LC/MS System by helping you understand how the hardware and software work.

1 Overview

Learn how the Agilent 6400 Series Triple Quadrupole helps you do your job.

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

Learn the concepts you need to understand how the Agilent triple quadrupole mass spectrometer works.

3 Agilent Triple Quadrupole MS and Sensitivity

Learn how the Agilent triple quadrupole mass spectrometer achieves high sensitivity.

4 Agilent MassHunter Workstation Software - Data Acquisition for 6400 Series Triple Quadrupole

Learn concepts behind the design of the Agilent MassHunter Workstation Software - Data Acquisition for Triple Quadrupole program.

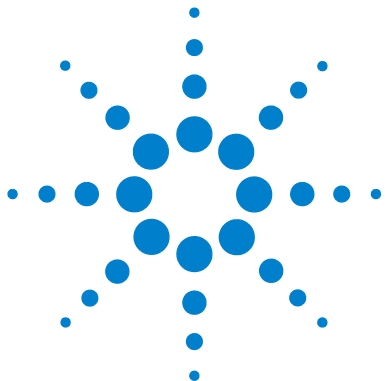
Contents

1	Overview	7
	What kind of system do you have?	8
	Help for applications	9
	Help for acquisition	10
	Help for data analysis	11
2	Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS	13
	Single quadrupole MS operation	14
	Design for a single quadrupole mass spectrometer	14
	How a single quadrupole mass spectrometer works	15
	Triple quadrupole MS operation	21
	Design of the Agilent Triple Quadrupole MS	21
	Innovative Enhancements in the 6460 Triple Quadrupole	22
	Enhancements in the 6430 Triple Quadrupole	23
	How a triple quadrupole mass spectrometer works	24
	How Dynamic MRM works	26
3	Agilent Triple Quadrupole MS and Sensitivity	29
	How the Agilent Triple Quadrupole MS improves sensitivity	30
	Noise reduction	30
	Example of chemical noise reduction	32
	Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS	34
	How each component works to improve sensitivity	40
	Agilent Jet Stream Technology	40
	LC/MS ion sources	41

Front-end ion optics	47
Collision cell	48
Detector	53
Pumping system	54

4 Agilent MassHunter Workstation Software - Data Acquisition for 6400 Series Triple Quadrupole 57

Tuning	59
Acquisition	61
Reporting	62



1 Overview

What kind of system do you have? 8
Help for applications 9
Help for acquisition 10
Help for data analysis 11

This chapter provides an overview of the Agilent 6400 Series Triple Quadrupole LC/MS components and how they help get the job done.



1 Overview

What kind of system do you have?

What kind of system do you have?

ESI – Electrospray Ionization
APCI – Atmospheric Pressure
Chemical Ionization
APPI - Atmospheric Pressure
Photo Ionization
HPLC-Chip/MS – High Performance
Liquid Chromatography on a Chip
MMI - Multimode Ionization

You can set up an Agilent 6400 Series Triple Quadrupole LC/MS in several configurations:

- For normal flow LC/MS with a binary pump, quaternary pump, well-plate sampler (or autosampler or CTC PAL autosampler). The supported ion sources are ESI, APCI, APPI, and MMI.
- For microflow LC/MS with a capillary pump, micro well-plate sampler (or CTC PAL micro-plate autosampler) and ESI, APCI or MMI ion sources
- For nanoflow LC/MS with a nanopump, micro well-plate sampler and HPLC-Chip/MS interface (used in place of standard or dual nanospray source) to increase reliability and boost performance with narrow peak dispersion and lower dead volumes.

Each Agilent combination has advantages for different applications. Each uses the same Data Acquisition program, Quantitative Analysis program and Qualitative Analysis program to enable these advantages.

The Agilent 6460 Triple Quadrupole LC/MS system is the only Triple Quadrupole that can use the Agilent Jet Stream Technology. This Technology utilizes a super-heated sheath gas to collimate the nebulizer spray which dramatically increases the number of ions that enter the mass spectrometer.

Help for applications

You can use one or more of the Agilent 6400 Triple Quadrupole LC/MS combinations to quantitate trace organic compounds in complex matrices:

- Food safety studies
- Environmental studies
- Drug discovery
- Toxicology
- Forensics

Paired with Agilent's 1200 Series LCs, the 6400 Series Triple Quadrupole MS delivers sensitive, reproducible analyses of target compounds in complex matrices.

- Femtogram-level limits of detection and quantitation
- Minimized memory effects even at very short dwell times
- Simplified operation with Agilent's newly developed data analysis software

The *dwell time* is the amount of time allotted for analyzing each ion during a scan.

Help for acquisition

To help you use the Agilent Triple Quadrupole LC/MS for these applications, the software lets you do these tasks in a single window with the Data Acquisition Program:

Prepare the instrument

To learn how to install the Agilent Triple Quadrupole LC/MS, see the Installation Guide.

To learn how to get started with the Agilent Triple Quadrupole LC/MS, see the Quick Start Guide.

To learn more about how to use the Agilent Triple Quadrupole LC/MS with real samples and data, see the Familiarization Guide

To learn how to do individual tasks with the LC/MS, see the online Help.

To learn more about an Agilent 1100LC module or 1200LC module, see the Agilent 1100LC or 1200LC User's Guide for the module.

- Start and stop the instruments from the software
- Download settings to the Agilent 1100 LC or 1200 LC and the Agilent 6400 Series Triple Quadrupole mass spectrometer in real time to control the instrument
- Evaluate if the MS parameters are within the limits to produce the specified mass accuracy and resolution with a Checktune report
- Optimize MS parameters automatically (Autotune) or manually through Agilent tuning programs and print an Autotune report
- Monitor the actual conditions of the instrument
- View the real-time plot for chromatograms and instrument parameters (both UV/Vis and MS) and print a real-time plot report
- View the centroided line spectrum of a peak or the mass range profile spectrum of a peak in real time

Set up acquisition methods

- Enter and save parameter values for all LC modules and the MS to an acquisition method
- Select and label the total ion chromatograms or extracted ion chromatograms that you want to appear in the real-time plot
- Set up time segments for each scan type and analysis where parameters change with the time segment or with the scans within the time segment
- Print an acquisition method report

A *worklist* is a list of individual samples and batches (sequences) that you enter and run automatically with the Data Acquisition program.

Acquire data

- Enter sample information and pre- or post- analysis programs (scripts) and run single samples interactively
- Enter and automatically run both individual samples and samples organized in a *worklist* (sequence of samples)
- Set up pre- and post- analysis scripts to run between samples in a worklist
- Set up and run a worklist to optimize MS acquisition parameters
- Print a worklist report
- View system events, including start and stop times, run events and errors and print an event log report

Help for data analysis

Quantitative Analysis Program

Agilent has designed the quantitative analysis program to help quantitate very low amounts of material with the following unique features:

- Imports information directly from the acquisition method
- Provides a curve-fit assistant to test all fits and statistics on curve quality
- Integrates with an automated, parameter-free integrator that uses a novel algorithm, optimized for triple quadrupole data
- Presents a Batch-at-a-Glance results window to help you review and operate on an entire batch of data at once
- Automatically detects outliers
- Provides preconfigured templates for basic reporting and enables the capability to create custom reports in Microsoft Excel

Please refer to the *Agilent MassHunter Workstation Software - Quantitative Analysis Familiarization Guide* or the online Help for the Quantitative Analysis program.

Qualitative Analysis Program

For fast method development, this software is used to quickly review the qualitative aspects of the data, such as the optimum precursor to product ion transitions.

Agilent designed the Qualitative Analysis program to present large amounts of data for review in one central location. With the program you can do these operations for any type of mass spectrometer data that you open:

- Extract chromatograms
- View and extract peak spectra
- Subtract background
- Integrate the chromatogram
- Find compounds

You can also set up methods to automatically do the tasks in the list, as well as others, when you open the data files.

Please refer to the *Agilent MassHunter Workstation Software - Qualitative Analysis Familiarization Guide* or the online Help for the Qualitative Analysis program.



2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

Single quadrupole MS operation	14
Design for a single quadrupole mass spectrometer	14
How a single quadrupole mass spectrometer works	15
Triple quadrupole MS operation	21
Design of the Agilent Triple Quadrupole MS	21
How a triple quadrupole mass spectrometer works	24
How Dynamic MRM works	26

In this chapter you learn about concepts to help you understand the inner workings of the Agilent 6400 Series Triple Quadrupole LC/MS.

The foundation for understanding the operation of a triple quadrupole mass spectrometer is the operation of a single quadrupole mass spectrometer. Therefore, an explanation of the workings of a single quadrupole mass spectrometer is presented first.



Single quadrupole MS operation

To better understand the specific hardware features of the Agilent 6400 Series Triple Quadrupole Mass Spectrometer, this section first reviews the fundamental aspects of the single quadrupole mass spectrometer.

Design for a single quadrupole mass spectrometer

Mass spectrometry is based on the analysis of ions moving through a vacuum.

The ionization of a sample occurs in the ion source that is shown, schematically, on the left. The ions are analyzed by a mass analyzer (mass filter) that controls the motion of the ions as they travel to the detector to be converted into actual signals.

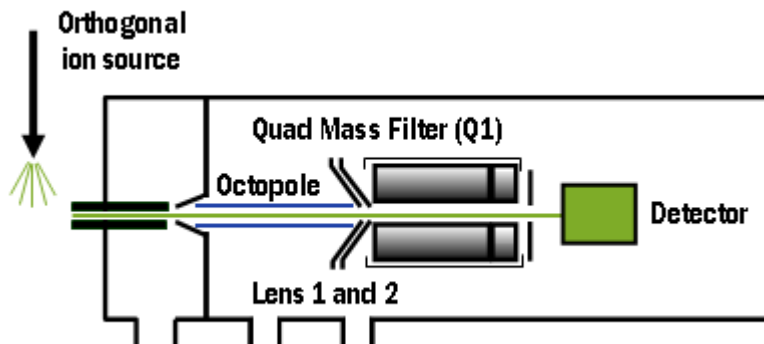


Figure 1 Schematic for single quadrupole mass spectrometer

m/z – mass/charge ratio

The quadrupole mass analyzer consists of four parallel rods to which specific DC and RF voltages are applied. These rods filter out all ions except those of one or more particular *m/z* values as determined by the voltages applied.

The RF is applied to all four rods, but the negative (–) rods are 180 degrees out of phase with the positive (+) rods. The rods are labeled + and – in reference to the DC voltages applied to them.

All ions that comprise the sample are generated at the source. However, when a specific set of voltages is applied, only ions of the corresponding m/z value may pass through the quadrupole to reach the detector. As the voltages are increased to other values, ions with other m/z values are allowed to pass through. A full MS scan is obtained by increasing the DC and RF voltages applied to the four rods over an expanded range of values.

How a single quadrupole mass spectrometer works

A diagrammatic model can be used to illustrate the concept of how a single quadrupole instrument works. See [Figure 2](#).

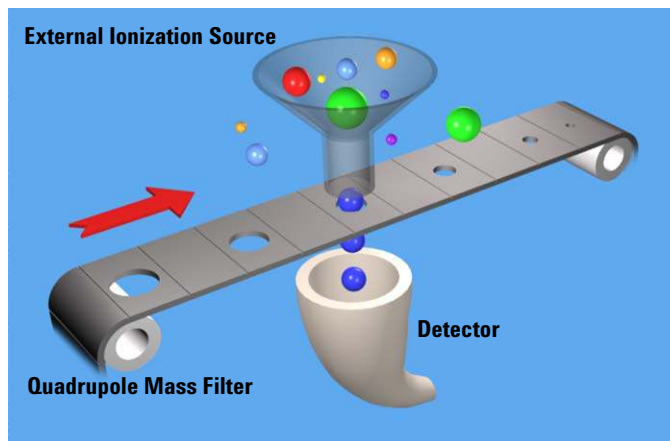


Figure 2 Conceptual model of a single quadrupole mass spectrometer

In the model,

- All of the ions contained in a sample are formed in the external ionization source and collected in a funnel. The balls of different colors and sizes represent different ions having different m/z values.

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

How a single quadrupole mass spectrometer works

- The quadrupole mass analyzer is represented by a moving belt that serves to filter the ions as they pass through openings of various sizes. The ions pass from the funnel, through the filter, to the detector.
- The detector is represented by the collecting funnel below the filtering belt.

As the belt (the analyzer) moves, or the voltages on the rods are changed, ions with different m/z values are filtered through the mass spectrometer.

As the analyzer moves from a small m/z value to increasingly larger values, a full MS scan is created.

SIM – Selected Ion Monitoring

If the belt does not move, the detector continues to monitor the same single m/z value over the entire scan period. This type of analysis is known as SIM. It is the most sensitive operating mode for a single quadrupole mass spectrometer.

The scan period is selected (fixed) by the user. The user may set the dwell time to scan a specific mass range (e.g. m/z 50 to 1000) *or* to remain on one selected ion (SIM) *or* to move to several selected ions during the scan period. The quadrupole mass filter is not scanned in this mode. The required RF/DC voltages are often set to filter a single mass at one time.

For comparison, see [“How a triple quadrupole mass spectrometer works”](#) on page 24.

Single quadrupole: SIM

To obtain the best sensitivity or quantitation, the single quadrupole is operated in SIM mode ([Figure 3](#)). The duty cycle is the measure of the instrument’s time actually devoted to measuring signals. In SIM mode, the single quadrupole analyzes the signal of a specific m/z ion almost all of the time. This results in nearly 100% acquisition during the duty cycle.

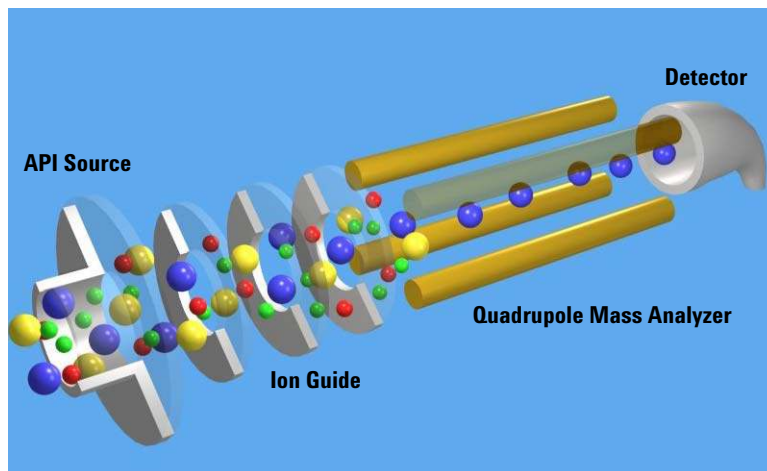


Figure 3 Single quadrupole: SIM

In this example,

- 1 All of the ions (+, -, and neutrals) are formed in the API source.
- 2 Ion optics guide the ions to the quadrupole mass analyzer.
The Agilent Ion Guide is an octopole filter of eight equally spaced rods.
- 3 In the analyzer, only ions of a particular m/z value, represented by blue balls, are allowed to pass through to the detector.
- 4 The detector completes the analysis.

This system has several advantages:

- provides the best sensitivity for quantitation
- increases selectivity
- improves chromatographic specificity
- provides no structural information

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

How a single quadrupole mass spectrometer works

Single quadrupole: Full Scan MS

In a full MS scan, the quadrupole serves as a mass filter over time, and a scan is carried out by stepping through increasing DC and RF voltages. This provides filtering through the corresponding m/z values across a mass spectrum. See Figure 4.

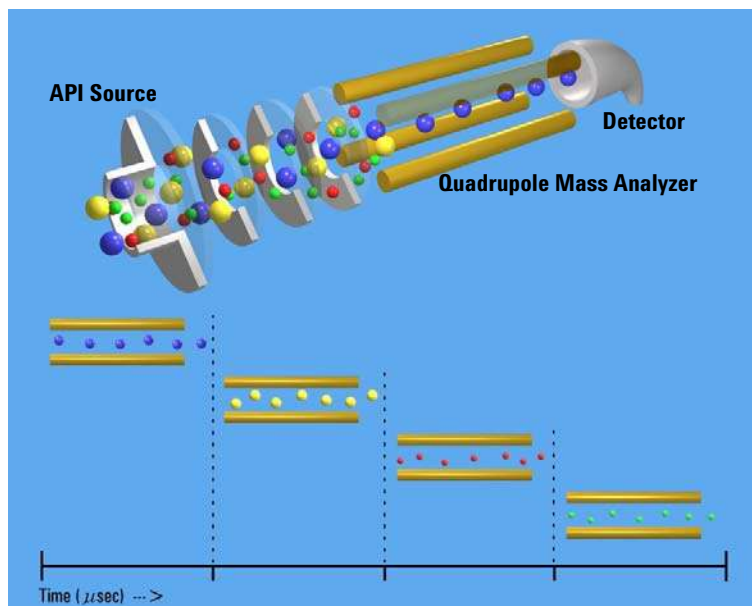


Figure 4 Single quadrupole: Full scan MS

The full scan MS mode is less sensitive because the duty cycle for each m/z is considerably less than 100%. The quadrupole mass analyzer scans sequentially, passing each m/z in the selected mass range to the detector.

A full scan MS is still a useful mode of operation because it shows all of the ions that are being formed in the ion source. This is useful for developing SIM acquisitions but also alerts analysts to other compounds co-eluting with compounds of interest.

What about fragment ions?

Full scans with a single quadrupole instrument can also be used to study fragment ions. See [Figure 5](#).

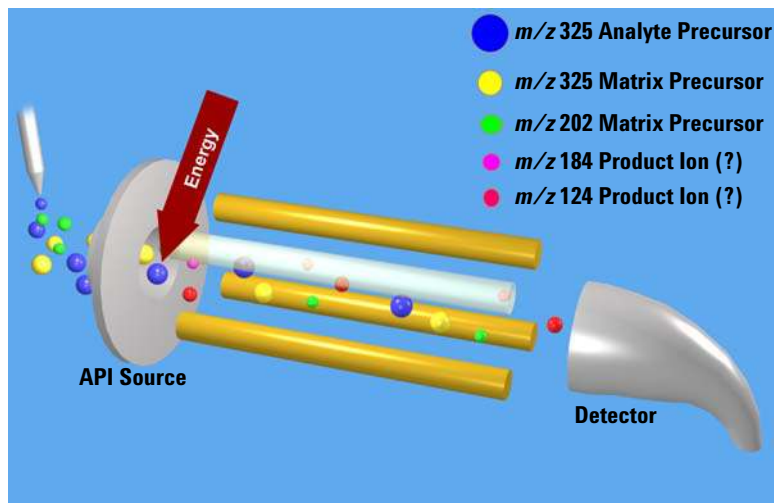


Figure 5 Fragment ions with single quadrupole MS

The diagram shows that fragment ions, also known as product ions, are formed by fragmenting or breaking apart precursor ions. Precursor ions formed in the ion source travel through the mass analyzer without change, unless extra energy is applied to their motion in a region where fragmentation can occur.

This fragmentation or collisionally induced dissociation (CID) can be carried out in a low pressure region between the ion source and the mass analyzer. The ion source is under atmospheric pressure, while the mass analyzer is at a much lower pressure because it has been evacuated of gas with a vacuum pump.

On the Agilent single quadrupole mass spectrometer, this region is between the capillary exit and the skimmer, where the gas pressure is about 2 Torr, or about three orders of magnitude below atmosphere pressure (760 Torr). Under normal operation, a voltage is applied across this region to keep the

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

How a single quadrupole mass spectrometer works

ions passing through to continue on to the mass analyzer. Even if these ions collide with the gas molecules in this region, they usually do not have enough energy to fragment.

CID – Collisionally Induced Dissociation

However, as the voltage is increased, the ions have more translational energy. Then, if the ions run into gas molecules, the collisions convert the translational energy into molecular vibrations that can cause the ions to fragment (Figure 6). This is collisionally induced dissociation (CID). Even though this fragmentation does not occur where the ions are formed at atmospheric pressure, it's a tradition to call this type of fragmentation “In-source CID.”

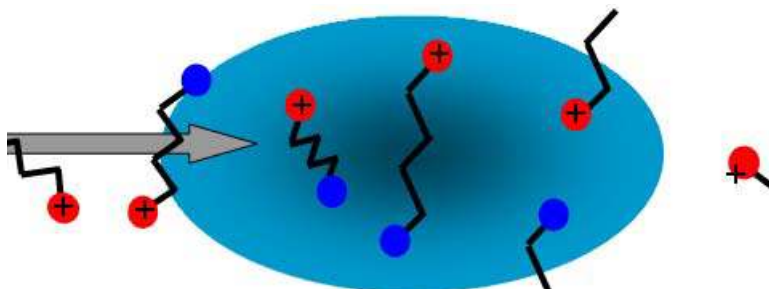


Figure 6 Ion fragmentation caused by collision-induced dissociation

A single quadrupole mass spectrometer cannot be used to do MS/MS because all of the ions formed in the ion source are transferred to the quadrupole whether fragmented or not. At the end when the mass analyzer filters the ions, it is not possible to identify which product ions came from which precursor ions.

A triple quadrupole mass spectrometer can do MS/MS, with fragmentation within its collision cell as described in the next section.

Triple quadrupole MS operation

Be sure to read the previous section on the concepts behind the operation of a single quadrupole mass spectrometer. Understanding these concepts helps you understand the operation of the triple quadrupole mass spectrometer.

Design of the Agilent Triple Quadrupole MS

The triple quadrupole mass spectrometer consists of an ion source, enhanced desolvation technology, followed by ion optics that transfer the ions to the first quadrupole positioned to the right of it. A diagram of the current two Triple Quadrupole LC/MS products is shown in Figure 7.

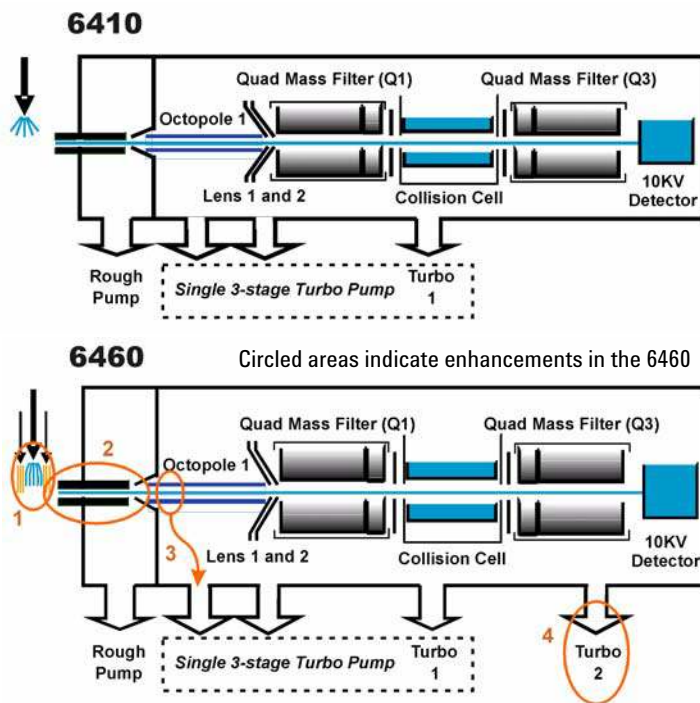


Figure 7 Innovative Enhancements in the 6460

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

Innovative Enhancements in the 6460 Triple Quadrupole

The quadrupole consists of four parallel hyperbolic rods through which selected ions are filtered before reaching a collision cell where they are fragmented. The collision cell is typically called the second quadrupole, but in this case, geometrically it is actually a hexapole filled with nitrogen, the same gas used in the ion source.

The fragment ions formed in the collision cell are then sent to the third quadrupole for a second filtering stage to enable a user to isolate and examine multiple precursor to product ion transitions (MRMs).

Innovative Enhancements in the 6460 Triple Quadrupole

Ions are generated using an electrospray ion source where the analyte is simultaneously ionized and desolvated from the liquid matrix. The first of five (5) innovative Agilent enhancements is found in the application of Agilent Jet Stream Technology (denoted as 1 in [Figure 7](#)) which improves sensitivity via thermal gradient focusing and enhanced desolvation.

The desolvated ions then enter the mass spectrometer via an innovative resistive and highly inert capillary transfer tube (denoted as 2 in [Figure 7](#)) that improves ion transmission and allows virtually instantaneous polarity switching.

Further improving the sensitivity is improved pumping in vacuum stage 2 that allows more pumping speed behind the skimmer and improved ion capturing by first octopole (denoted as 3 in [Figure 7](#)). The ions next pass through optics and into the first quadrupole analyzer. The quadrupole analyzer consists of four parallel hyperbolic rods through which selected ions based on their mass to charge ratio are filtered.

The ions passing through the first quadrupole analyzer are then directed through an improved collision cell where they are fragmented. The collision cell is typically called the second quadrupole, but in this case, geometrically it is actually a hexapole filled with nitrogen, the same gas that is used as the drying gas. Agilent innovation has led to the design of a

collision cell that has axial acceleration for high speed MS/MS analysis (denoted as 4 in Figure 7). Fragment ions formed in the collision cell are then sent to the third quadrupole for a second filtering stage to enable a user to isolate and examine product ions with respect to precursor ions.

Finally, the ions that pass through the third quadrupole are detected using a high energy detector. A second turbo pump has been added (denoted as 5 in Figure 7) to increase pumping speed and improve the vacuum which will further improve the signal to noise and enhance the limit of detection of the triple quadrupole.

Enhancements in the 6430 Triple Quadrupole

The 6430 Triple Quadrupole has many of the innovative enhancements that are included in the Agilent 6460 Triple Quadrupole.

- The same inert capillary transfer tube (denoted as 1 in Figure 8)
- Improved pumping in vacuum stage 2 (denoted as 2 in Figure 8)
- A second turbo pump has been added (denoted as 3 in Figure 8)

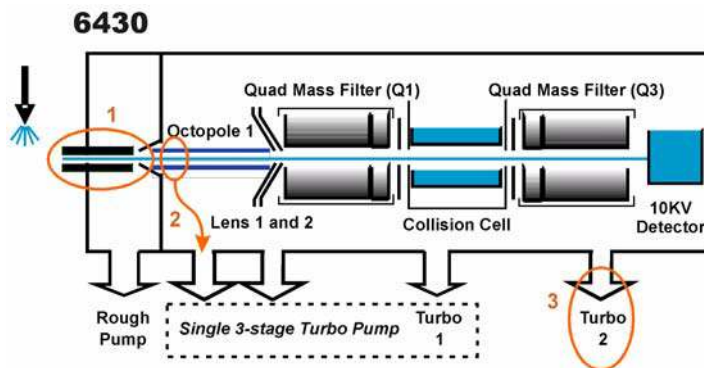


Figure 8 Enhancements in the 6430

How a triple quadrupole mass spectrometer works

Quadrupoles provide a user with the capability to do MS/MS in several ways (see Figure 9).

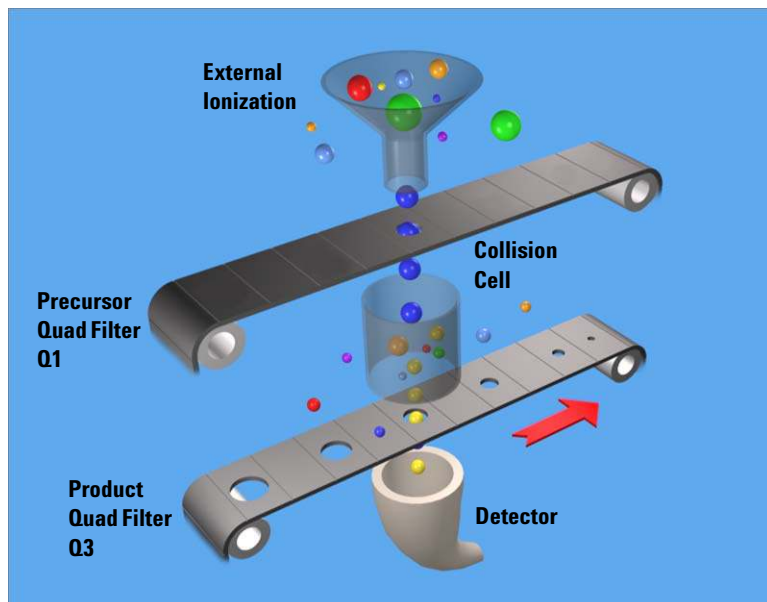


Figure 9 Conceptual model of a triple quadrupole mass spectrometer: With SIM, MS1 (Q1) and MS2 (Q3) are set at a single m/z , while with Full Scan MS/MS, MS1 (Q1) is set at a single m/z and MS2 (Q3) is scanned.

Representing the quadrupole mass analyzers as moving belts, a collision cell can be placed between the belts to fragment the ions. The first belt can be fixed to select which precursor ion travels to the collision cell. Different types of collision cells can be used.

The cell can be another quadrupole, a hexapole (six rods like the one used in the Agilent 6400 Series Triple Quadrupole LC/MS), an octopole (eight rods), or even a transverse wave guide.

Whichever geometry is used, a collision gas is required—an inert, non-reactive gas such as nitrogen or argon. Nitrogen is used here. In addition, the voltages applied to the collision cell must be different from those applied to the quadrupoles to enhance the movement of all of the product ions toward the third quadrupole.

In this example, a precursor ion is selected using the first quadrupole and is sent to the collision cell for fragmentation. The fragments are scanned through the third quadrupole resulting in a product-ion scan MS/MS. Since the fragment ions are pieces of the precursor, they represent portions of the overall structure of the precursor molecule. A triple quadrupole instrument can be used in this way to identify a compound's fingerprint.

A full scan MS/MS using a triple quadrupole MS is also not the most sensitive mode for the same reason that a full scan MS using a single quadrupole is not the most sensitive mode of operation possible (first belt remains steady; second belt moves). The most sensitive mode of operation for the triple quadrupole MS instrument is to fix both belts and only monitor a specific precursor ion and a specific product ion. This mode is called *selected reaction monitoring or SRM*.

In normal operation, a triple quadrupole MS instrument involves running multiple SRMs for the same precursor ions. This is called *multiple reaction monitoring or MRM*.

How Dynamic MRM works

Dynamic MRM is a scan type that has a single continuous Time Segment and up to 4000 transitions in the Scan Segments table.

At run time, these transitions are automatically separated into multiple “MRM Tables” according to the retention time window for each transition. These MRM tables consist of the transitions that are overlapping in retention time and can contain up to 200 transitions each. These tables are not shown in the user interface.

Dynamic MRM includes the columns Ret (Retention) Time (min) and Delta Ret Time. Ret Time is the transition retention time. Each transition is acquired from Ret Time - $1/2 * (\text{Delta Ret Time})$ to Ret Time + $1/2 * (\text{Delta Ret Time})$.

Abundance data is acquired starting at time “t” for duration “delta t”. The first MRM table in the example below acquires transitions “abcdef”. The second MRM table acquires transitions from “defghi”, and so on.

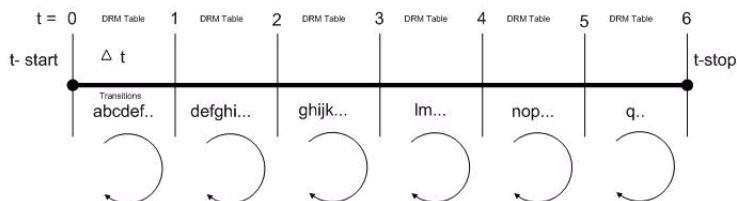


Figure 10 Automatically determining dynamic MRM tables.

The benefit of Dynamic MRM is to allow longer dwell times by performing MRM transitions around the elution time of the compound and not continuously throughout the chromatogram.

The MassHunter Data Acquisition software, the SmartCard 4 firmware, the Digital Signal Processor and the MS Hardware all are involved in the dynamic MRM algorithm.

1 MassHunter Data Acquisition Software

A list of transitions/parameters (up to 4000) are entered by the user. Based on delta retention time, retention time, dwell time and cycle time, the Data Acquisition software creates a lookup recipe that will group transitions in the digital signal processor into small MRM tables (up to 1000+ tables). Each table has the same cycle time. MRM tables are similar to “Time Segments” but have fewer transitions enabling the data file to have more data points per peak.

A transition peak may contain data points from more than one MRM table. A peak will look like a peak because the abundance value at each data point is normalized by “dwell” time.

2 SmartCard 4 Firmware

The SmartCard 4 Firmware sends the transition list to the Digital Signal Processor (DSP) memory. It also sends the lookup recipe to the DSP memory.

Peak abundance data returning from the DSP is Burst/Time filtered in SmartCard 4 firmware. The data is sent back to the Data Acquisition software which stores the data in an MRM data file which both the Qualitative Analysis program and the Quantitative Analysis program can open.

3 Digital Signal Processor (DSP)

A dynamic MRM run is controlled entirely by the Digital Signal Processor firmware.

When a Dynamic MRM Run starts, the lookup recipe starts creating MRM tables by selecting transitions from the list and then executing them. When the stop time of the MRM table is reached, the next table is created and started. There is minimal delay between changing MRM tables in the DSP and no data is lost. This process continues until all MRM tables have been run. At the end of the run, background scan continues in MRM mode.

2 Inner Workings – Triple Quadrupole MS versus Single Quadrupole MS

How Dynamic MRM works

For each individual transition, the DSP sends MS parameters to the hardware in the form of address/data pairs.

4 MS Hardware

For each transition, the DSP address/data pair sets the hardware quadrupoles and other parameters.

After the MS hardware parameters are set for each transition, the MS takes an integrated abundance measurement at the selected ion and sends the unfiltered abundance data back to SmartCard 4 firmware in the form of a structure containing header and abundance information.



3 Agilent Triple Quadrupole MS and Sensitivity

How the Agilent Triple Quadrupole MS improves sensitivity 30

Noise reduction 30

Example of chemical noise reduction 32

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole
MS 34

How each component works to improve sensitivity 40

LC/MS ion sources 41

Front-end ion optics 47

Collision cell 48

Detector 53

Pumping system 54

This chapter shows how the Agilent triple quadrupole mass spectrometer reduces chemical and electronic noise and how each component contributes to enhanced instrument sensitivity.



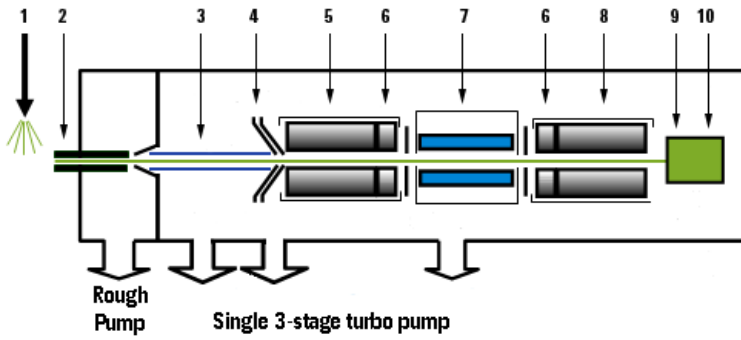
How the Agilent Triple Quadrupole MS improves sensitivity

Triple quadrupole mass spectrometers exhibit multiple sources of noise, including noise from all chemical and cluster backgrounds and electronic noise (Figure 11).

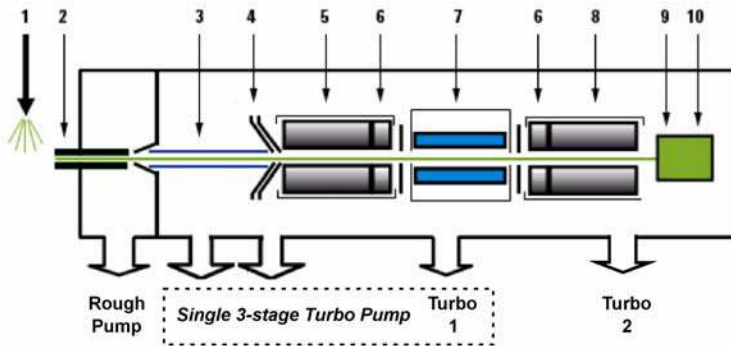
Noise reduction

The problem of noise must be addressed at several stages of the instrumentation from the ion source (1) to the detector (10) in Figure 11.

Agilent 6410



Agilent 6430



Agilent 6460 with
 Agilent Jet Stream
 Technology

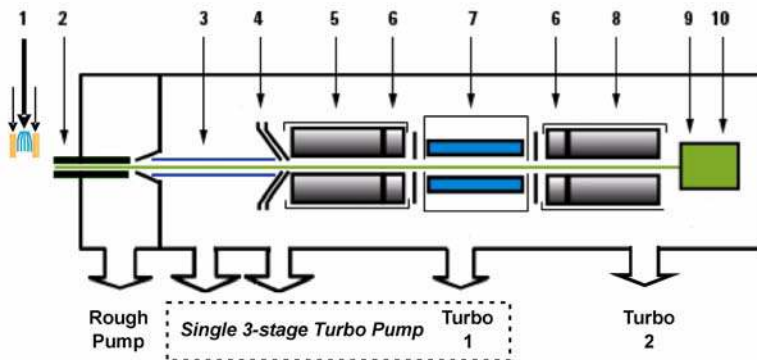


Figure 11 Multiple sources of noise

How the Agilent 6400 Series Triple Quadrupole instrument minimizes noise

- 1** Agilent's patented orthogonal spray sources maximize ionization while minimizing solvent and matrix noise.
- 2** This combination of a heated counter-current drying gas, dielectric capillary and skimmer enhances desolvation while minimizing chemical noise.
- 3** RF Octopole ion guide provides high efficiency ion capture while optimizing wide mass bandwidth ion transmission.
- 4** L2 RF enhances high mass ion transmission (Agilent patent).
- 5** Quadrupole 1 uses hyperbolic quadrupoles to optimize ion transmission and spectral resolution.
- 6** RF quadrupole segment enhances ion transmission into and out of the collision cell.
- 7** High pressure collision cell with linear acceleration optimizes MS/MS fragmentation while eliminating crosstalk, even at very low dwell times. A small diameter high frequency hexapole assembly assists with capturing and focusing fragmented ions.
- 8** Quadrupole 2 uses hyperbolic quadrupoles to optimize ion transmission and spectral resolution.
- 9** The off-axis matching dual high energy dynode detector with log amp signal compression permits a high gain with rapid polarity switching, a long life and low noise. The off-axis design allows neutrals to pass without hitting the detector.
- 10** The multiplier has a long life since only electrons impact its surface, never ions.

Crosstalk is the interference caused when two signals become partially superimposed on each other. In this case residual product ions can interfere with the product ion spectrum of a subsequent MRM experiment.

Example of chemical noise reduction

The Agilent 6400 Series Triple Quadrupole mass spectrometer passes through four transitional steps in translating a signal in the MRM process (Figure 12).

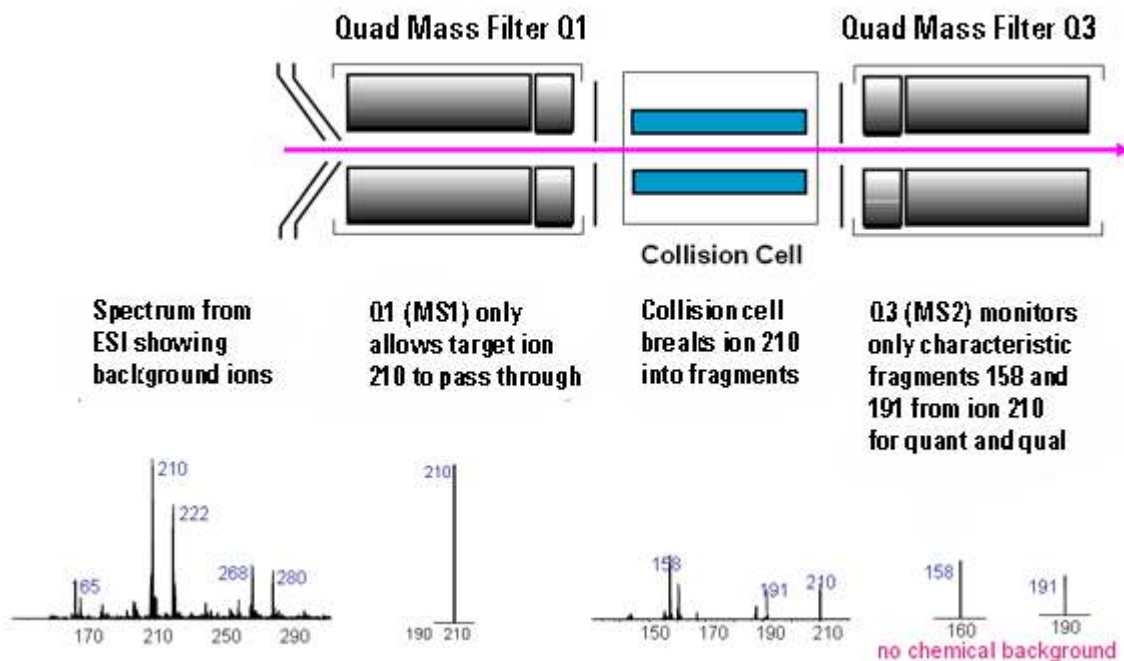


Figure 12 Multiple reaction monitoring (MRM)

Step 1 The spectrum at the far left represents everything that is being ionized at the ion source. This example shows the ESI spectrum of a phenylurea pesticide. A triple quadrupole mass spectrometer reduces chemical noise for low-level quantitation in a dirty matrix more than a single quadrupole LC/MS does.

Step 2 This step is accomplished by first selecting the pesticide of interest at m/z 210 from the co-eluting interferences seen in the rest of the spectrum. The second spectrum shows the result after passing through the first quadrupole, or MS1 (Q1).

Step 3 After MS1 (Q1), fragment ions are generated in the collision cell. The corresponding MS/MS spectrum is shown below the collision cell.

Step 4 Particular fragment ions can be selected to pass through the MS2 (Q3) quadrupole. These are selected for quantitation and confirmation. For example, the product ion at m/z 158 is more intense than the product ion at m/z 191. Therefore, the MRM transition 210 to 158 would be used for quantitation and the 210 to 191 transition would be used for confirmation, where the m/z 191 ion is considered a qualifier ion.

The second stage of selectivity using the MS2 (Q3) quadrupole removes much of the chemical background. Typically, the chance of an isobaric interference at the same exact mass as the fragmentation ion is remote.

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS

Sensitivity based on reserpine

The reserpine sensitivity specification for the Agilent 6460 Triple Quadrupole MS with the Agilent Jet Stream Technology is:

- 1 pg reserpine on-column, with
- a signal-to-noise ratio of 750:1 or greater (RMS noise)

The reserpine sensitivity specification for the Agilent 6430 Triple Quadrupole MS is:

- 1 pg reserpine on-column, with
- a signal-to-noise ratio of 300:1 or greater (RMS noise)

The reserpine sensitivity specification for the Agilent 6410 Triple Quadrupole MS is:

- 1 pg reserpine on-column, with
- a signal-to-noise ratio of 50:1 or greater (3 x RMS noise)

Three concurrent MRM transitions are monitored, but only the 609.3 to 195.1 transition is used for the quantitation. Reserpine's precursor ion mass is 609.3.

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS

The other two transitions are used to simulate the acquisition of qualifier ions, which represent real-world applications for confirmation. The instrument can detect femtogram levels of material (Figure 13 and Figure 14).



Figure 13 Reserpine Sensitivity for 6410 Triple Quadrupole (Specifications: 1 pg with S/N 50:1 or greater (3 x RMS noise))

NOTE

Currently, the sensitivity for reserpine on the 6410 is based on the ESI source.

3 Agilent Triple Quadrupole MS and Sensitivity

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS

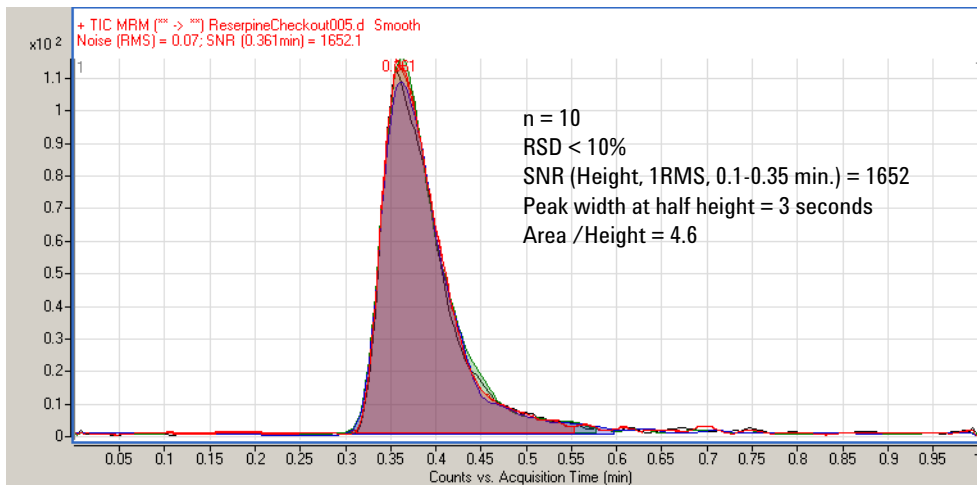


Figure 14 Reserpine Sensitivity for 6460 Triple Quadrupole with Agilent Jet Stream Technology (Specifications: 1 pg with S/N 1000:1 (RMS noise))

Linearity

Reserpine can also be analyzed with repeated injections of the sample, showing a linearity of $r^2 = 0.987$ on the Agilent 6410 (Figure 15).

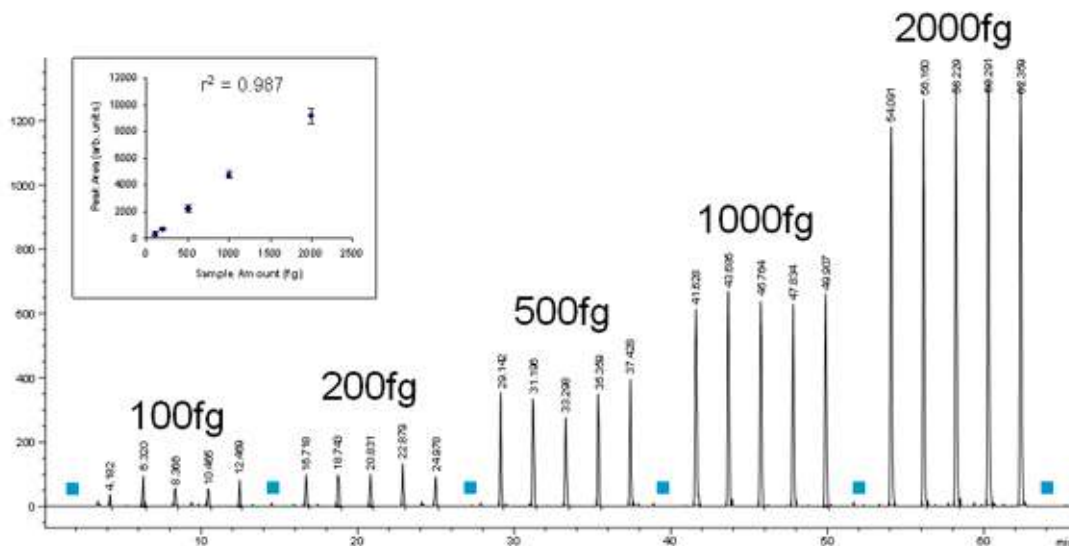


Figure 15 Demonstrating linearity in a Reserpine dilution series, in mobile phase (Reserpine, 609>195 EIC with three transitions) acquired on an Agilent 6410 Triple Quadrupole

3 Agilent Triple Quadrupole MS and Sensitivity

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS

A comparable analysis of alprazolam shows the following linearity:

- $r^2 = 0.9974$ for over four orders of magnitude (Figure 16) on the Agilent 6410
- $r^2 = 0.9998$ or $r^2 = 0.9991$ for over five orders of magnitude (Figure 17) on the Agilent 6460 with Agilent Jet Stream Technology

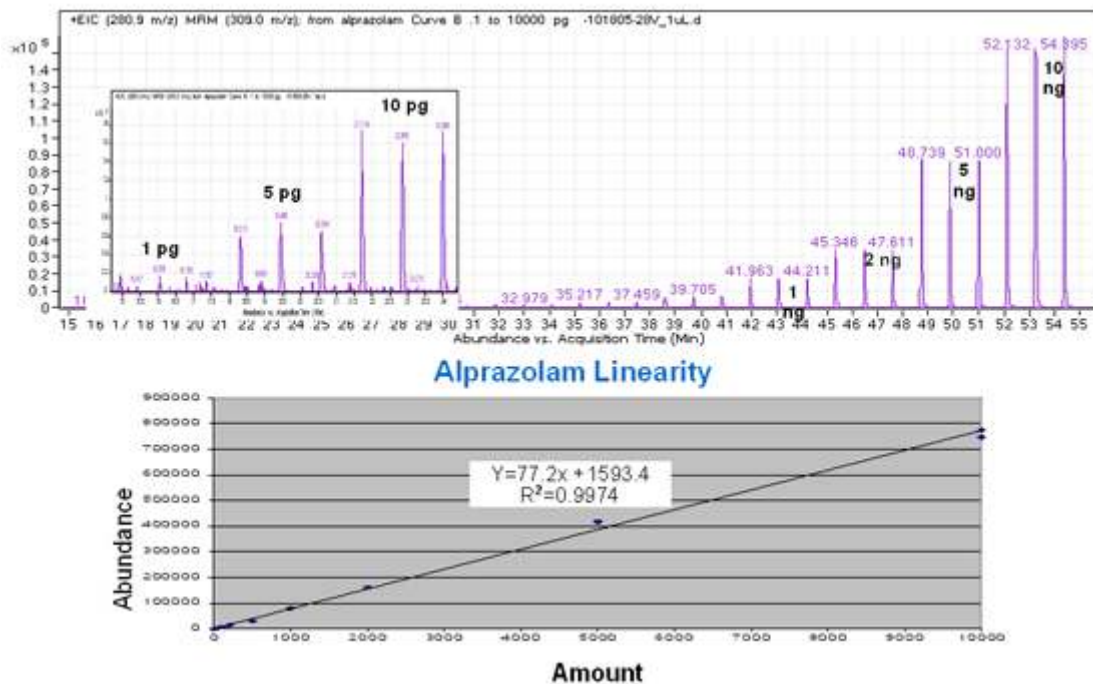
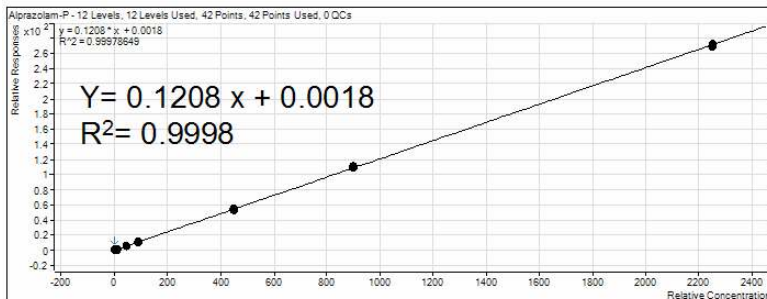
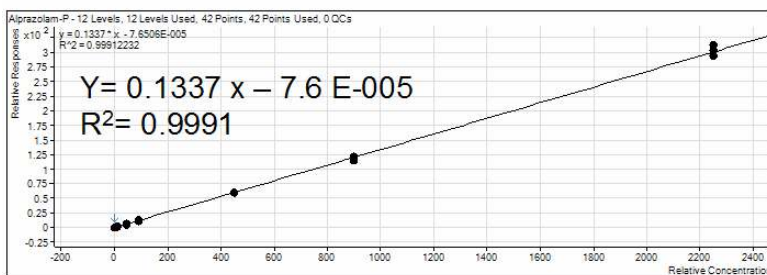


Figure 16 Alprazolam results (LP5) with 1 pg–10 ng positive ESI mode acquired with Agilent 6410 Triple Quadrupole

Sensitivity and linearity of the Agilent 6400 Series Triple Quadrupole MS



Delta EMV= 0V
 5 orders of magnitude



Delta EMV= 200V
 5 orders of magnitude

Figure 17 Alprazolam results - Calibration Curve 22.5 fg to 2.25 ng on column, linear fit, origin none, weight 1/x; acquired with Agilent 6460 Triple Quadrupole with Agilent Jet Stream Technology

How each component works to improve sensitivity

This section describes in more detail how each of the components of the Agilent 6400 Series Triple Quadrupole MS contributes to reducing noise (Figure 11).

Agilent Jet Stream Technology

Agilent Jet Stream Technology enhances analyte desolvation by collimating the nebulizer spray and creating a dramatically “brighter signal.” The addition of a collinear, concentric, super-heated nitrogen sheath gas (Figure 18) to the inlet assembly significantly improves ion drying from the electrospray plume and leads to increased mass spectrometer signal to noise allowing the triple quadrupole to surpass the femtogram limit of detection. The Agilent Jet Stream Technology is patent pending.

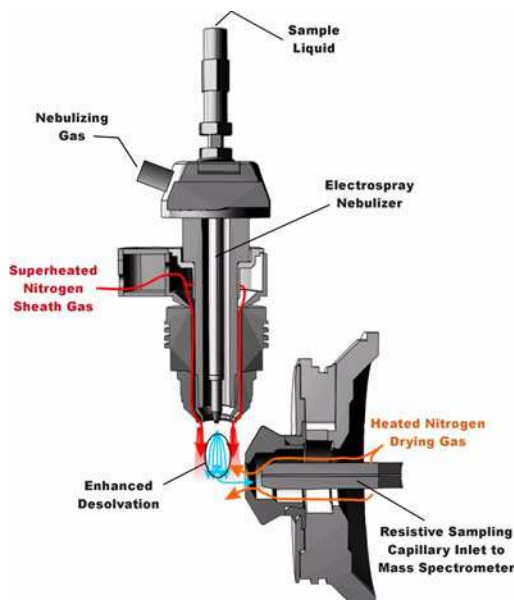


Figure 18 Electro spray Ion Source with Agilent Jet Stream Technology

Agilent Jet Stream thermal gradient focusing consists of a superheated nitrogen sheath gas that is introduced collinear and concentric to the pneumatically assisted electrospray. Thermal energy from the superheated nitrogen sheath gas is focused to the nebulizer spray producing the most efficient desolvation and ion generation possible. The enhanced molecular ion desolvation results in more ions entering the sampling capillary as shown in [Figure 18](#) and concomitant improved signal to noise. Parameters for the Agilent Jet Stream Technology are the superheated nitrogen sheath gas temperature and flow rate and the nozzle voltage.

The capillary in the 6460A is a resistive capillary that improves ion transmission and allows virtually instantaneous polarity switching. It is the same, proven capillary that is used in the fast polarity switching version of Agilent's single quadrupole product.

LC/MS ion sources

Agilent provides a choice of four ion sources to use with its triple quadrupole mass spectrometer: ESI, APCI, APPI and MMI. You can also use the HPLC-Chip.

This section describes how the different ion sources affect sensitivity.

ESI ion source design

The patented orthogonal source reduces the introduction of unwanted sample components that interfere with analysis. The advanced nebulizer design produces a uniform droplet size, which ensures maximum sensitivity. Since the source is at ground, the source is safe to use and has the advantage of reducing solvent cluster background ([Figure 19](#)).

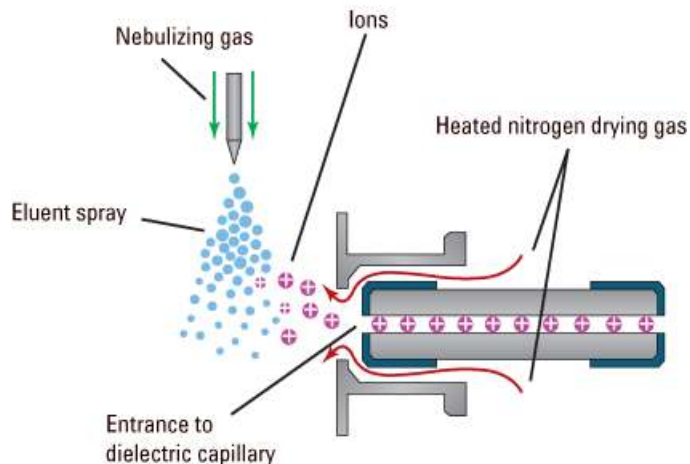


Figure 19 Orthogonal introduction and electrospray ionization

The capillary in the 6460A is a resistive capillary that improves ion transmission and allows virtually instantaneous polarity switching. It is the same, proven capillary that is used in the fast polarity switching version of Agilent's single quadrupole product.

For the Agilent 6410 Triple Quadrupole, the capillary is glass, dielectric “cold” capillary that enhances desolvation and improves the focusing of high mass ions while minimizing chemical noise. Some desolvation occurs in the capillary. This appears to work better with glass capillaries rather than stainless steel capillaries and reduces cluster noise. Ion focusing is also improved.

Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric Pressure Chemical Ionization (APCI) is a popular complement to electrospray. Because APCI does not generate multiply charged ions, and operates at higher temperatures, it is commonly used to analyze smaller, thermally stable polar and non-polar compounds. Agilent's APCI source is sensitive, yet extremely robust thanks to orthogonal spray and counterflow

drying gas. Like the ESI source, it can generate both positive and negative ions, and ion polarity can be switched on a spectrum-to-spectrum basis.

Agilent multimode source

The most versatile ion source for the Agilent 6400 Series Triple Quadrupole MS is Agilent's G1978A Multimode Source shown diagrammatically in Figure 20.

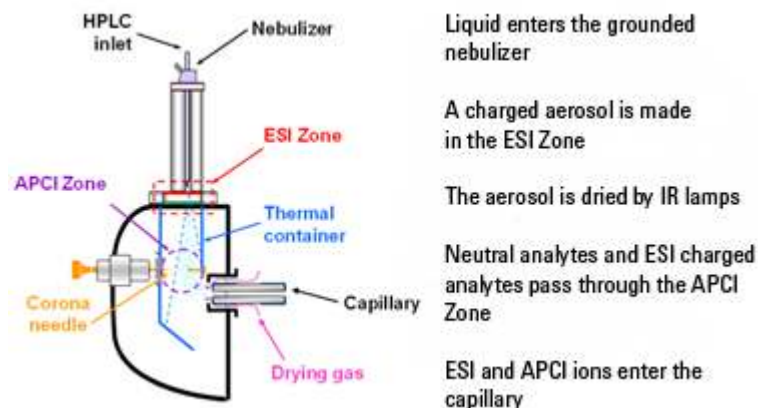


Figure 20 Agilent G1978A Multimode Source

Neutral analytes and ESI charged analytes pass through the divided chamber entering either the APCI Zone or adjacent zone.

NOTE

Analytes are distributed spatially between the two sections.

ESI and APCI are essentially incompatible processes because each needs its own conditions for aerosol drying and electrical fields. However, it is possible to form ions simultaneously from ESI and APCI if the two ionization regions are separated in space.

The HPLC effluent is nebulized using the same sprayer that is used for a dedicated ESI source. The droplets are emitted into the “ESI zone,” where a high voltage electrode charges the droplets and induces ion formation. The ions formed in this region pass through the source and enter the capillary. Residual droplets are dried using two infrared lamps (not shown) that emit at the absorption frequency of water. The vapor and analyte(s) enter the APCI zone where they are ionized. Ions are then drawn into the capillary the same way as they would be with dedicated ESI and APCI sources.

**Expected
sensitivity**

When the Multimode source is operated as an ESI or APCI source only, no loss in sensitivity is found for the compounds studied. For many compounds run in mixed mode, an equal signal response (compared to single mode operation) or sensitivity gain can be achieved.

However, studies also show that when operating the Multimode source in ESI and APCI simultaneously, there can be a loss of sensitivity up to a factor of two for some compounds.

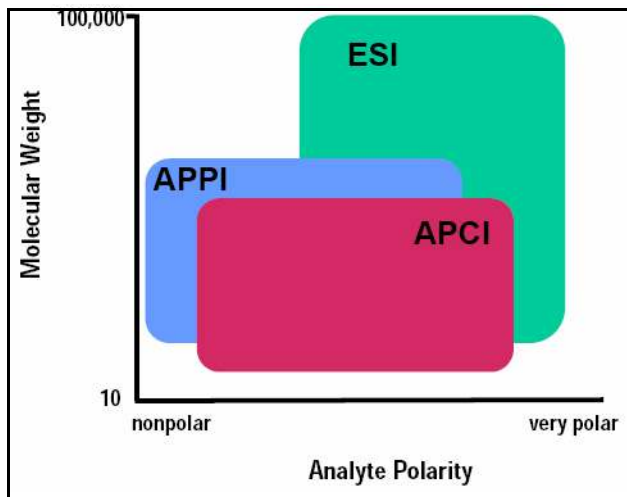
Therefore, weighing the benefits of running analyses in both modes simultaneously versus a potential loss in sensitivity is important. For most applications, a loss in sensitivity of less than 2 is negligible.

APPI (Atmospheric Pressure Photo Ionization)

For analysis of compounds that ionize poorly by ESI and APCI, the atmospheric pressure photoionization (APPI) source provides a useful alternative. It combines Agilent’s proven orthogonal spray nebulization and counterflow drying gas with innovative photoionization from Syagen Technology. The long-lasting krypton lamp emits photons at energy levels high enough to ionize many large 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.

APPI may ionize compounds that do not ionize well by ESI or APCI, such as Polyaromatic Hydrocarbons (PAHs). Also, APPI may provide better overall sensitivity for some compounds than

either ESI or APCI. Some examples of these compounds are Tetrahydrocannabinol (THC), Benzoic acid, and fat soluble vitamins. APPI may provide better sensitivity at lower flow rates than APCI. Reproducibility data indicates APPI is robust and highly reproducible.

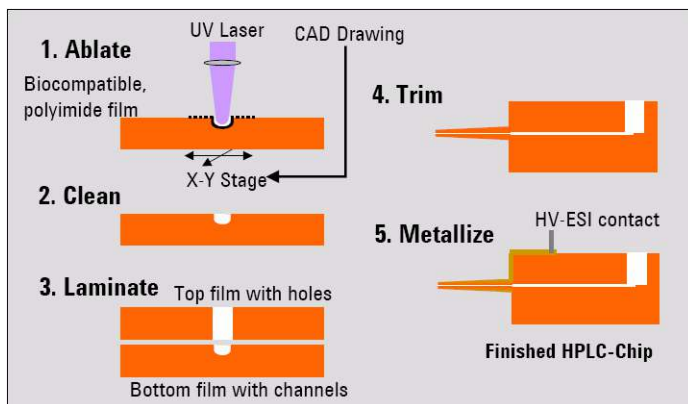


HPLC-Chip

Traditional nanospray mass spectrometry has proven its potential as a cost-effective, sensitive and reproducible technique for the identification of peptides at femtomol to attomol levels. However, connecting nano capillaries, columns and valves frequently is a tedious procedure and requires user skills and routine. When handled incorrectly, nano flow connections are prone to leakage which are difficult to detect and result in poor system performance and extended downtime of the complete system. Quartz nanospray needles are prone to blockages and require frequent replacement.

With the invention of HPLC-Chip technology, Agilent is significantly reducing the need for user interaction and making nanospray a rugged state-of-the-art technology.

The cornerstone of chip technology is the HPLC-Chip, a 3-dimensional structure made of sandwiched polyimide films. Grooves of specific dimensions are laser-ablated into a layer of polyimide film. The lamination of a top and bottom layer then forms channels of trapezoidal or triangular shape inside the chip which can either be used as capillaries or, if filled with packing material, as nanocolumns. One end of the chip tapers off into a polyimide nanospray emitter.

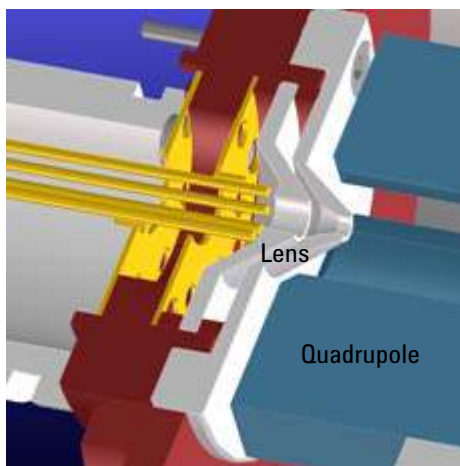
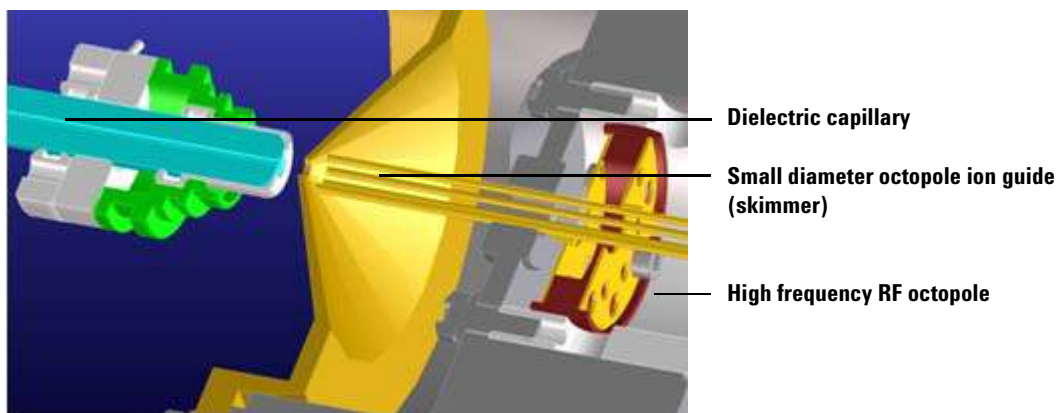


By ablating small holes into the top and bottom layers, the chip can be used as a self-connecting and sealing microvalve. The unique rotor-in-rotor microvalve in the Chip Cube moves two concentric valve rotors from one side towards the chip while a 6-port stainless steel stator face closes in from the other side, forming a pressure tight connection without any user interaction.

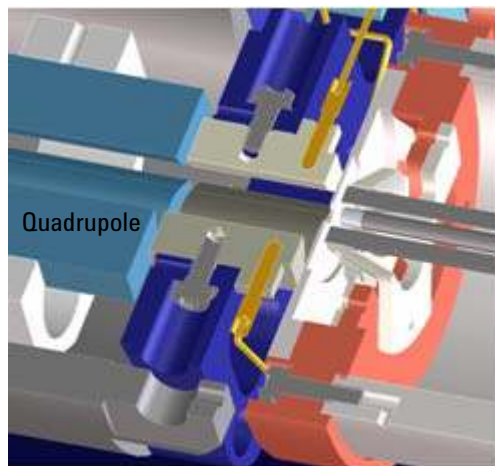
The very flexible HPLC-Chip concept can combine multiple columns, the valve plumbing and the nanospray emitter on one chip. A rugged chip holder protects the chip and retracts the fragile tip whenever the HPLC-Chip is removed from the Chip Cube.

Front-end ion optics

The key components are shown in [Figure 21](#). The triple quadrupole leverages the same front end optics as the single quadrupole mass spectrometer. An additional improvement was made for the 6460A in the vacuum region right behind the skimmer. This improvement allowed for increased pumping throughput in this region which leads to a modest increase in signal.



Lens 2 RF (transmission of higher masses)



Hyperbolic quadrupole and post-filter

Figure 21 Front-end optics

Skimmer	Agilent uses a small diameter skimmer orifice with very short distances from the capillary to the skimmer to the octopole. Consequently, more of the ions exiting the capillary are captured by the ion octopole guide, thereby keeping the ion beam very tightly focused.
Octopole 1	Higher multipoles provide better ion transmission over a wider mass range. The depth of the potential well is steeper for the higher multipoles (at like voltages), especially those close to the rods resulting in the loss of fewer ions. This explains the predominant use of octopoles as ion guides in mass spectrometers where the main objective is to transmit rather than filter the ions.
Lens 2 RF	The phase of lens 2 is matched to that of the subsequent quadrupole, MS1 (Q1), resulting in a significantly increased sensitivity. This is an Agilent patented technology.
Quad mass filters	The quadrupoles consist of hyperbolic rods that optimize ion transmission and spectral resolution. There tends to be more ion loss with circular rods.
Pre-filter	The end section of the MS1 (Q1) quadrupole assembly also consists of short hyperbolic rods, but their RF voltages are only high enough to guide ions into the collision cell. A similar set of rods on the exit side of the collision cell are part of the MS2 (Q3) quadrupole. These short RF-only rods act as pre- and post-filters to the collision cell to ensure optimum ion transmission.

Collision cell

What is the collision cell?

The collision cell is another innovation. The collision cell is a high pressure hexapole assembly with its linear acceleration adjusted to optimize MS/MS fragmentation while eliminating crosstalk even at very low dwell times (Figure 22).

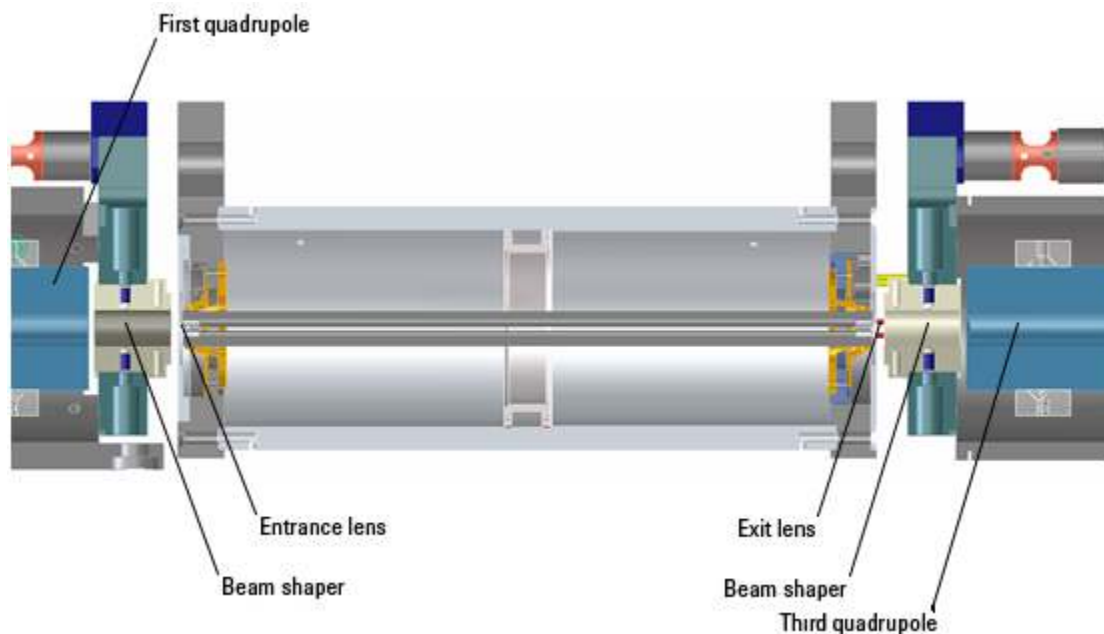


Figure 22 Collision cell technology produces higher sensitivity and faster responses without memory or cross-talk effects

The components that contribute to this higher sensitivity and faster response are

- Small diameter hexapole collision cell
- High frequency hexapole collision cell
- Linear axial acceleration
- High pressure collision cell
- High speed digital electronics

The collision cell contains nitrogen, the same gas that is used as the drying gas. The small diameter of the hexapole assembly assists in capturing fragmented ions. The addition of gas (8 mTorr) assists in the ion focusing as well.

Why a hexapole?

The geometry of a hexapole provides advantages in two domains: ion focusing and ion transmission (Figure 23).

- The first advantage is in ion focusing where a quadrupole is better than a hexapole, which is better than an octopole, that is, quadrupole > hexapole > octopole.
- The second advantage involves ion transmission across a wide mass range, or m/z bandwidth. In this case, the octopole is better than the hexapole, which is better than the quadrupole.

The hexapole is chosen because, overall, it is the best for both ion focusing and ion transmission.

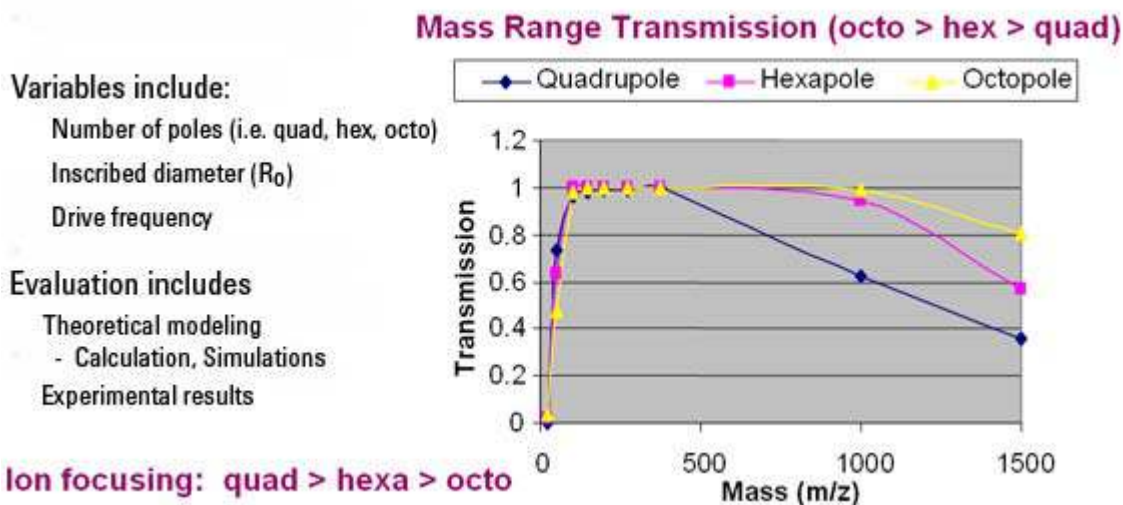


Figure 23 Broad mass range transmission and improved transmission efficiency using a hexapole

Collision cell design

The collision cell hexapole consists of six resistively coated rods used to generate a potential difference across the length of the collision cell (Figure 24).

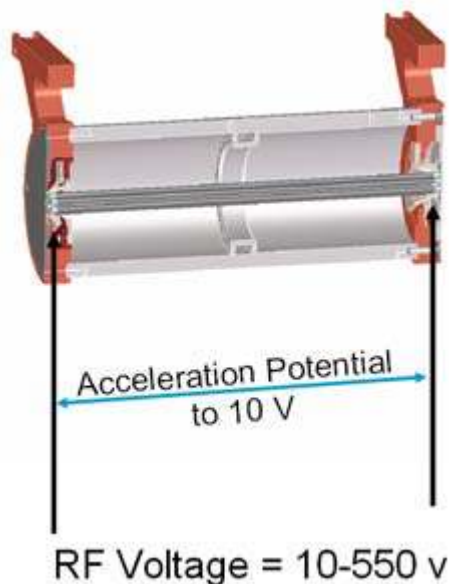


Figure 24 Collision cell design

A potential difference is always present. This ensures that the precursor ions coming from MS1 (Q1), or fragment ions generated in the collision cell, are transmitted and not allowed to drift around at random.

Sweeping out the ions in this manner avoids the issue of crosstalk where residual product ions from a previous MRM experiment can interfere with the product ion spectrum of a subsequent MRM experiment (see [Figure 25](#)). A collision energy voltage is applied over the accelerating linear voltage to generate fragments or product ions.

Length of time for collision cell flushing

The low degree of crosstalk can be demonstrated by examining how long it takes to evacuate ions from the collision cell ([Figure 25](#)).

3 Agilent Triple Quadrupole MS and Sensitivity Collision cell

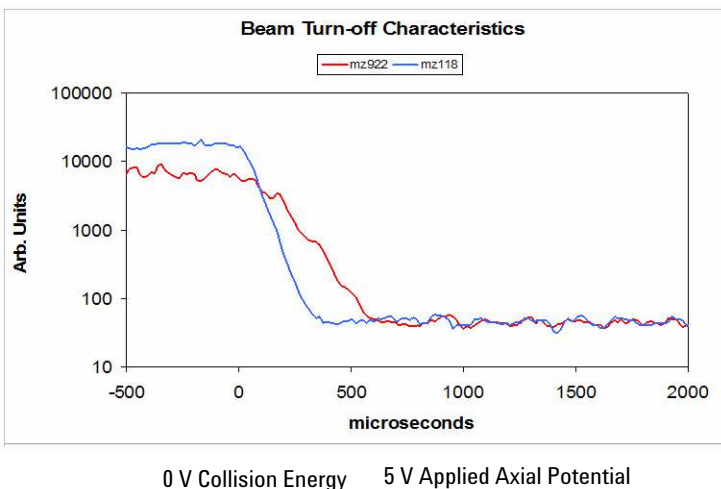


Figure 25 Collision cell clearing profile (500 pg Alprazolam, 20 ms dwell time)

The figure shows that the higher the mass the longer it takes to evacuate the collision cell. For example, m/z 922 takes about 600 μsec to evacuate the collision cell using the linear potential, while m/z 118 only takes 350 μsec . This also demonstrates the low degree of crosstalk since the Y axis is logarithmic, showing complete clearance of the cell. This means that an inter-scan delay of 5 msec will be more than adequate to flush the collision cell of all ions.

Detector

The High Energy Dynode detector assembly is unique to Agilent (Figure 26).

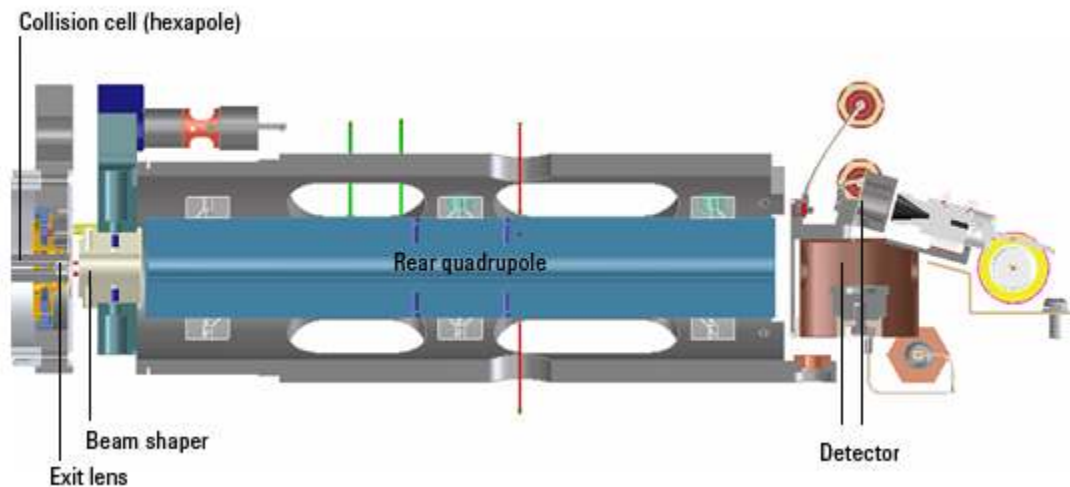


Figure 26 Detector components

The two dynodes are orthogonal to the ion beam and neutrals. This orientation reduces the possibility of neutral molecules impacting the detector while at the same time attracting the ions with high voltages. The dynodes serve to convert the ions to electrons before they impact the multiplier. The off-axis design also allows neutrals to pass through without hitting the detector.

The multiplier has a long lifetime since only electrons are allowed to impact it. Ions never impact its surface.

Pumping system

6460 and 6430 Triple Quadrupole

A single roughing pump and three-stage turbo pump are used for the first four vacuum stages. This is achieved by partitioning the turbo pump to create the multiple vacuum stages. The second turbo pump is added to the fourth vacuum stage to help pump out the gas load coming from the enhanced collision cell. The turbo pumps are backed by a single roughing (mechanical) pump (Figure 27).

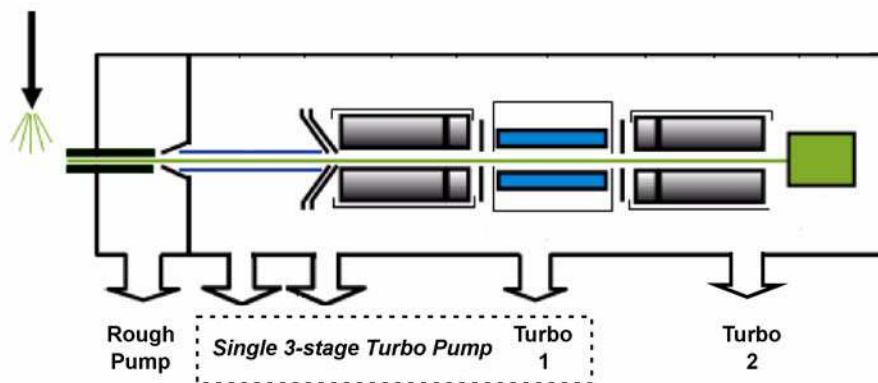


Figure 27 6460 Triple Quadrupole pumping system

6410 Triple Quadrupole

A single three-stage turbo pump is used for the entire vacuum system. This is achieved by partitioning the turbo pump to create the multiple vacuum stages. A second turbo pump is not needed for the 6410. This pump is backed by a single roughing (mechanical) pump (Figure 28).

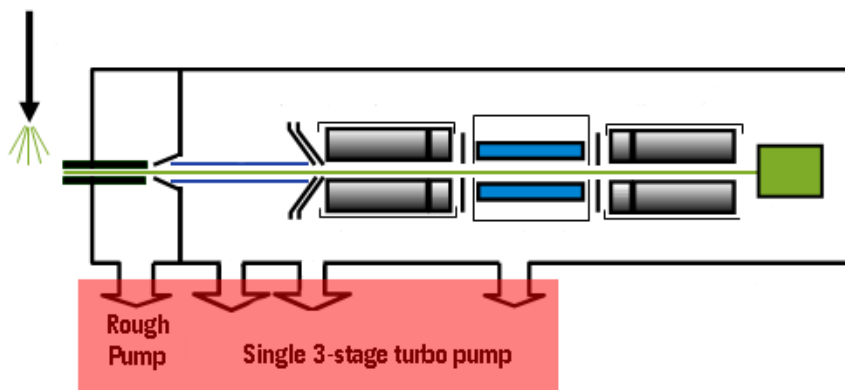
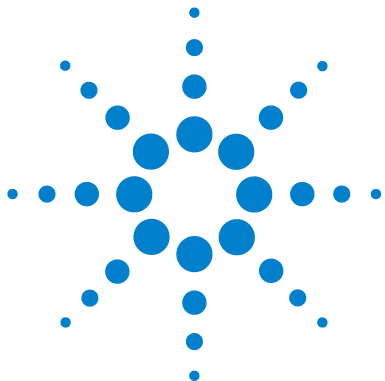


Figure 28 6410 Triple Quadrupole pumping system

3 Agilent Triple Quadrupole MS and Sensitivity Pumping system



4 Agilent MassHunter Workstation Software - Data Acquisition for 6400 Series Triple Quadrupole

Tuning 59

Acquisition 61

Reporting 62

Learn the concepts to help you understand the design and operation of the Agilent MassHunter Workstation Software - LC/MS Data Acquisition for 6400 Series program.

The Data Acquisition program ([Figure 29](#)) has the following features:

- All LC and MS parameters are immediately visible.
- Real-time plots show the instrument at work.
- Running multiple samples is easily handled through a worklist—a spreadsheet-like interface.



4 Agilent MassHunter Workstation Software - Data Acquisition for 6400 Series Triple Quadrupole

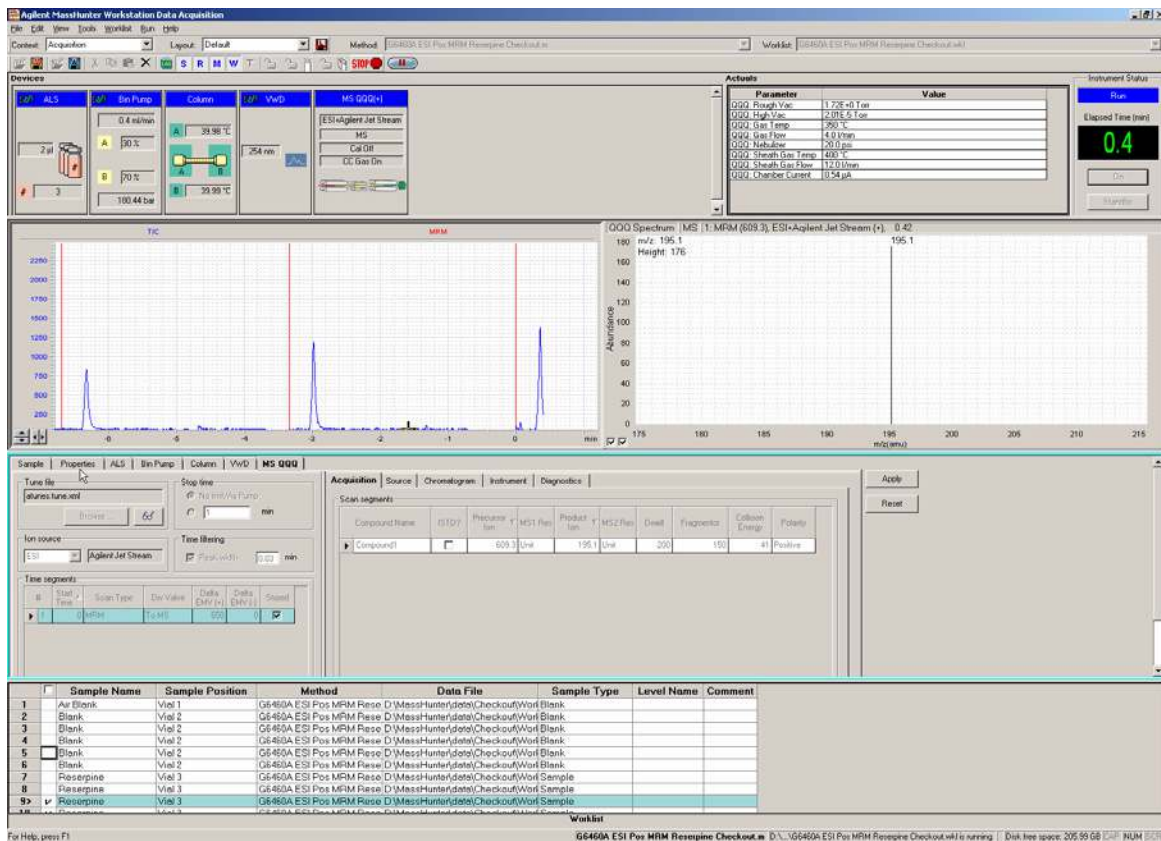


Figure 29 Acquisition component

With this window you can do these operations:

- Control and monitor instrument settings
- Tune the instrument
- Set up acquisition parameters for the LC and the Triple Quadrupole
- Monitor the chromatogram and mass spectra as samples are analyzed
- Set up worklists for sequences of samples

Tuning

Autotune and Checktune

A Checktune can be used to determine if the tuning mix ion masses are properly assigned and if the response or sensitivity of these ions is within expectations. This check tune will only take a few minutes. An Autotune taking 18–25 minutes for each polarity can be used if a more extensive tune is recommended (Figure 30).

Everything is automatic since the tuning mix is delivered by the calibrant delivery system (CDS), which is switched on automatically during the tune.

Manual Tune

A manual tune of user-defined ion masses with six corresponding profile masses is available.

4 Agilent MassHunter Workstation Software - Data Acquisition for 6400 Series Triple Quadrupole Tuning

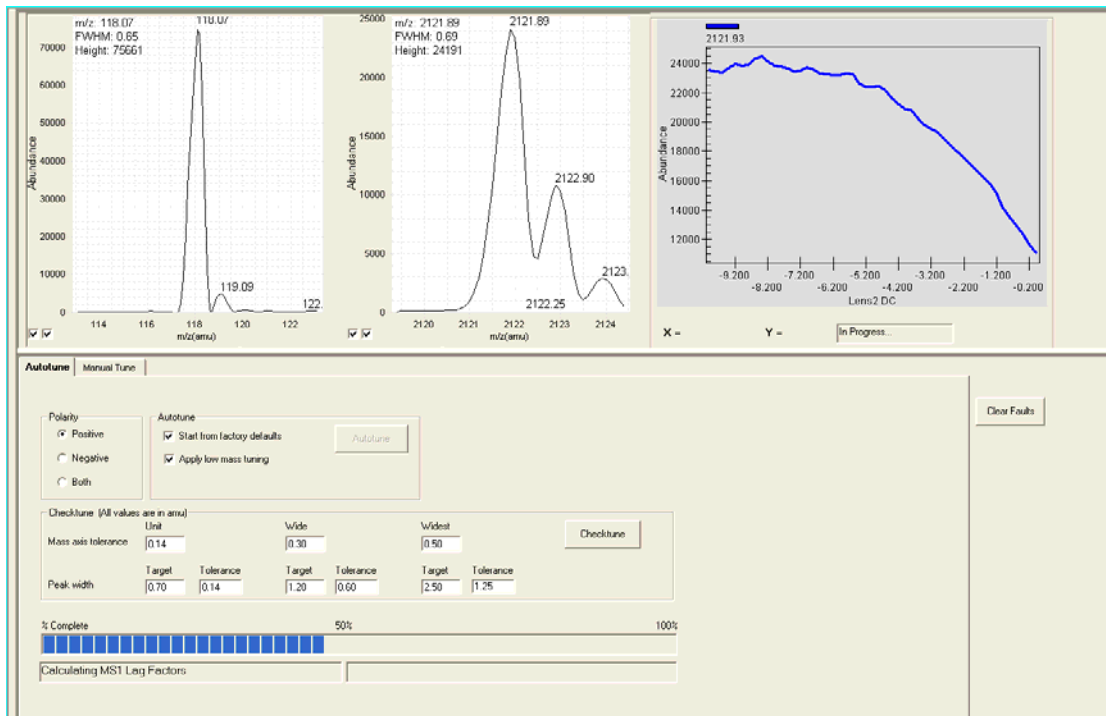


Figure 30 Autotune in progress

A Lens 2 DC voltage ramp with RF Off is shown at the top right. An automated version of this is also available for the Tuning Mix with Autotune.

Tune Report

Tune reports are also available in Microsoft Excel. See "Reporting" on page 62.

Acquisition

Many Agilent LC modules and the 6400 Series Triple Quadrupole MS can be controlled and monitored (Instrument Status pane) from the same window used for entering acquisition settings and setting up sequence lists (Method Editor pane)(Figure 29).

The Real-time Plot pane also can show the MS and UV results in real time.

Because of the large amount of information available, any of these panes can be closed for easier viewing, if necessary. However, one of the panes must always be open.

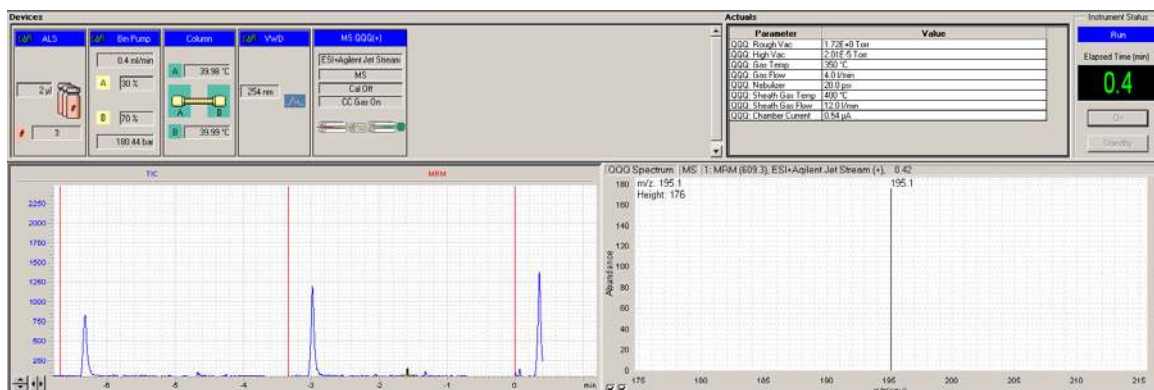



Figure 31 Instrument status and real-time plot

Reporting

Reports from the Agilent 6400 Series Triple Quadrupole LC/MS are in Microsoft Excel format to increase the flexibility for design and compatibility with other software programs. A set of example reports is also available. These sample reports can be modified to suit your own purposes. For example, you may want to insert the company's logo into the report.

In addition to customizing any report layout included with the Agilent MassHunter Workstation Software - Data Acquisition program, you can create a report by moving additional fields from a list of available fields to a desired location in the redesigned report.

To find out more about the Report Designer Add-in, please refer to the Reporting Training DVD or the online Help for the Report Designer add-in. You click the  button in the Report Designer toolbar to access the help.

- You can add tables and graphics using buttons on the toolbar.
- You can add columns by dragging them from the XML source.
- You can add formula columns to reports.
- You can change the font and format numbers in Excel.
- You can access Print Preview in Excel.

For more information, please refer to either the Reporting Training DVD or the online Help for the Report Designer Add-in.

www.agilent.com

In This Book

The *Concepts Guide* presents “The Big Picture” behind the Agilent 6400 Series Triple Quadrupole LC/MS to help you to understand how the hardware and software work.

This guide includes concepts for:

- Overview
- Inner Workings
- MS and Sensitivity
- Data Acquisition program for 6400 Series Triple Quadrupole

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