Agilent UltivoTriple Quadrupole LC/MS System

Concepts Guide

The Big Picture

Notices

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Manual Part Number

G5351-90005

Edition

Revision A, November 2017

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051

Software Revision

This guide is valid for the C.01.00 revision or higher of the Agilent MassHunter Acquisition for Ultivo LC/TQ program and compatible Qualitative Analysis and Quantitative Analysis programs, until superseded.

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In This Guide…

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

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Learn how the Ultivo LC/TQ helps you do your job.

2 Inner Workings – Ultivo LC/TQ

Learn the concepts you need to understand how the Ultivo LC/TQ mass spectrometer works.

3 Ultivo LC/TQ and Sensitivity

Learn how the Ultivo LC/TQ mass spectrometer achieves high sensitivity.

4 Dynamic MRM and Triggered MRM

Learn how Dynamic MRM and Triggered MRM work.

5 MassHunter Acquisition for Ultivo LC/TQ

Learn concepts behind the design of the Agilent MassHunter Acquisition for Ultivo LC/TQ program.

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1
Overview

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This chapter provides an overview of the Ultivo LC/TQ components and how they help get the job done.

What kind of system do you have?

ESI – Electrospray Ionization
APCI – Atmospheric Pressure Chemical Ionization
AJS – Agilent Jet Stream

You can set up an Ultivo LC/TQ 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, and AJS ESI.

The Ultivo LC/TQ can be used with a 1260 Infinity II, 1260 Infinity Prime, or 1290 Infinity II LC stack.

Help for applications

You can use one or more of the Ultivo LC/TQ combinations for quantitative and qualitative analysis of trace organic compounds in complex matrices:

- Food safety studies
- Environmental studies
- Drug discovery
- Toxicology
- Forensics
- Bioanalysis
- Protein quantitation
- Clinical
- Academia
- Pharmaceutical
- Veterinary drug

Paired with the Agilent 1260 and 1290 Infinity Series LCs, the Ultivo LC/TQ delivers sensitive, reproducible analyses of target compounds in complex matrices.

- Femtogram-level limits of detection and quantitation for the Ultivo LC/TQ
- Minimized memory effects even at very short dwell times
- Simplified operation with Agilent 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 Ultivo LC/TQ for these applications, the software lets you do these tasks in a single window with the MassHunter Acquisition Program:

Prepare the instrument

- Start and stop the instruments from the software.
- Download settings to the Agilent 1260 Infinity II, Agilent 1260 Infinity II Prime, or 1290 Infinity II LC and the Ultivo LC/TQ 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) through MassHunter tuning and print an Autotune report.
- Monitor the actual conditions of the instrument
- Viw the real-time plot for chromatograms and instrument parameters and print a real-time plot report.
- View the centroid or profile mass spectrum of a chromatographic 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 chromatogram or extracted ion chromatograms that you want to appear in the real-time plot.
- Set up time segments for each scan type where parameters change with the time segment or with the scans within the time segment.
- Print an acquisition method report.
- Optimizer software enables automated determination of compound MRM parameters, including fragmentor voltage and collision energy.
- Source Optimizer enables the automated determination of source parameters for your unique analytical method.

To learn how to install the Ultivo LC/TQ, see the Installation Guide.

To learn how to get started with the Ultivo LC/TQ, see the Quick Start Guide.

To learn more about how to use the Ultivo LC/TQ with real samples and data, see the Familiarization Guide

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

To learn more about an Agilent 1260, 1260 Prime, or 1290 Infinity II LC module, see the Agilent 1260 or 1290 Infinity II LC User's Guide for the module.

Acquire data

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

- Enter sample information and pre- or post-analysis programs (scripts) and run single samples interactively.
- Enter and run individual samples or 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 LC/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.

Study Manager program

- Create a study to group a collection of samples and operations.
- Run one or more studies automatically.
- Run Quantitative Analysis on the results automatically.
- Review the studies that have already been run.
- Create a study from an existing worklist.
- Create a study from a text file which can be in several different formats.
- Create a study from a spreadsheet to run Drug Discovery Screening.
- Create a study to optimize MS parameters (such as Fragmentor Voltage or Collision Energy).
- Run a study to optimize source parameters. This study is started from the Source Optimizer program.

Help for data analysis

Quantitative Analysis Software

Agilent designed the quantitative analysis program to help quantitate trace levels of analytes with the following unique features:

- Imports information directly from the acquisition method.
- Quant-My-Way software allows you to create your own streamlined version of MassHunter Quantitative Analysis software. You can customize the Ribbon with just the actions you need.
- Provides a curve-fit assistant to test all fits and statistics on curve quality.
- Integrates with an automated, parameter-free integrator (Agile 2) 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 PDF templates in the Report Builder program.

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

Qualitative Analysis Software

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.

Qualitative Analysis has two main programs.

Qualitative Analysis Navigator

You use this program to examine chromatograms and spectra and identify mass spectral peaks. It is especially well suited for manual, ad-hoc examination of your data.

In this program, you can use the Data Navigator window to interactively select different spectra and chromatograms. You can generate formulas or search a library/database for these spectra.

If you are looking at spectra that you have manually extracted or that are extracted by the Integrate and Extract Peak Spectra algorithm, then you want to use this program.

Qualitative Analysis Workflows

You use this program's compound mining algorithms to find evidence for compounds in your data. You can also use its identification algorithms to identify unknown compounds based on that evidence.

This view provides a compound centric view of one or more data files. You can look at information on a single compound in different windows. You change the selected compound in the Compound List window. You switch between different data files in the Sample Table.

If you want to use any of the Compound Mining algorithms, you use this program.

Please refer to the *Agilent MassHunter Workstation Software - Qualitative Analysis Familiarization Guide*, the *Agilent MassHunter Workstation eFamiliarization Guide for TQ*, or the online Help for the Qualitative Analysis programs.

2
Inner Workings – Ultivo LC/TQ

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In this chapter you learn about concepts to help you understand the inner workings of the Ultivo LC/TQ.

How a triple quadrupole mass spectrometer works

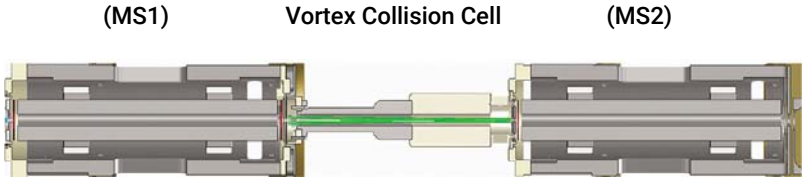


Figure 1 Ultivo LC/TQ Mass Analyzer

The versatility and benefits of tandem quadrupole MS is demonstrated by its six modes of operation. A tandem quadrupole can be operated in the following modes:

- **"Full Scan Mode"** on page 14
- **"Selected Ion Monitoring Mode (SIM)"** on page 15
- **"Multiple Reaction Monitoring Mode (MRM)"** on page 16
- **"Product Ion Scan Mode"** on page 17
- **"Precursor Ion Scan Mode"** on page 18
- **"Neutral Loss Scan Mode"** on page 19
- **"Mixed Mode"** on page 20

Full Scan Mode

This mode is full spectrum acquisition with universal detection of known or unknown compounds. The mass-analyzing quadrupole (MS2) is scanned from the first mass to the last mass without interruption in a given scan time. Full scan experiments are used to determine the identity of unknown compounds or the identity of each component in a mixture of unknown compounds (generally, a full mass spectrum is needed to determine the identity of an unknown compound). For example, you would use a full scan to determine the molecular weight of each component of the tryptic digest of a protein, because you would not know what masses to expect in the digest mixture. The full scan type gives you more information about an analyte than the selected ion monitoring (SIM) and selected reaction monitoring (SRM) scan types, but full scanning does not yield the sensitivity that can be achieved by the other two scan types.

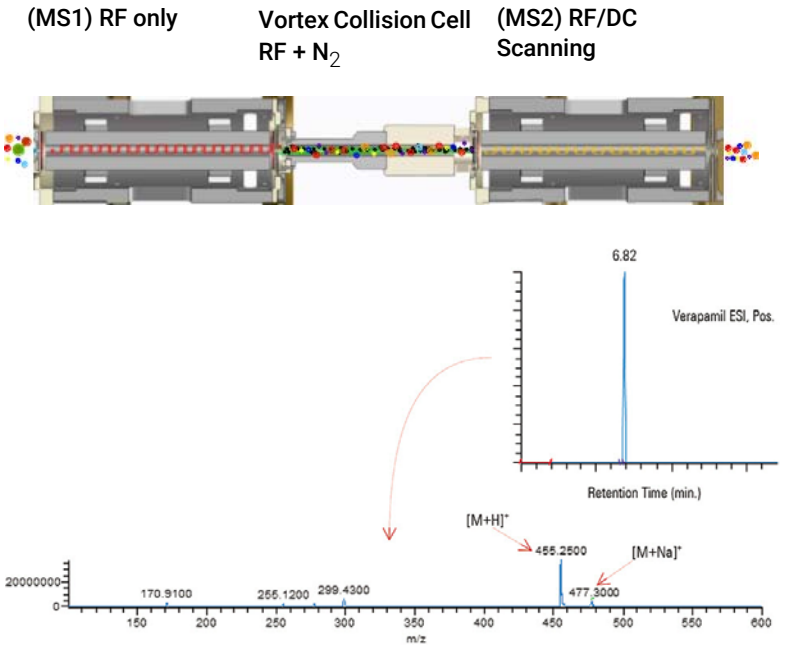


Figure 2 Full Scan Mode (MS2): Survey of a chromatographic peak

Selected Ion Monitoring Mode (SIM)

This mode is used for target detection of known compounds. The mass spectrometer acquires and records ion current at only one or a few selected mass-to-charge ratio values. Selected ion monitoring generally provides higher sensitivity than does a full scan mass spectrum since the data acquisition is done in particular mass-to-charge ratio windows, not over a broad mass range. The signal-to-noise ratio improves at the cost of the amount of structural information returned. SIM experiments are useful in detecting small quantities of a target compound in a complex mixture when the mass spectrum of the target compound is known. So, SIM is useful in trace analysis and in the rapid screening of a large number of samples for a target analyte.

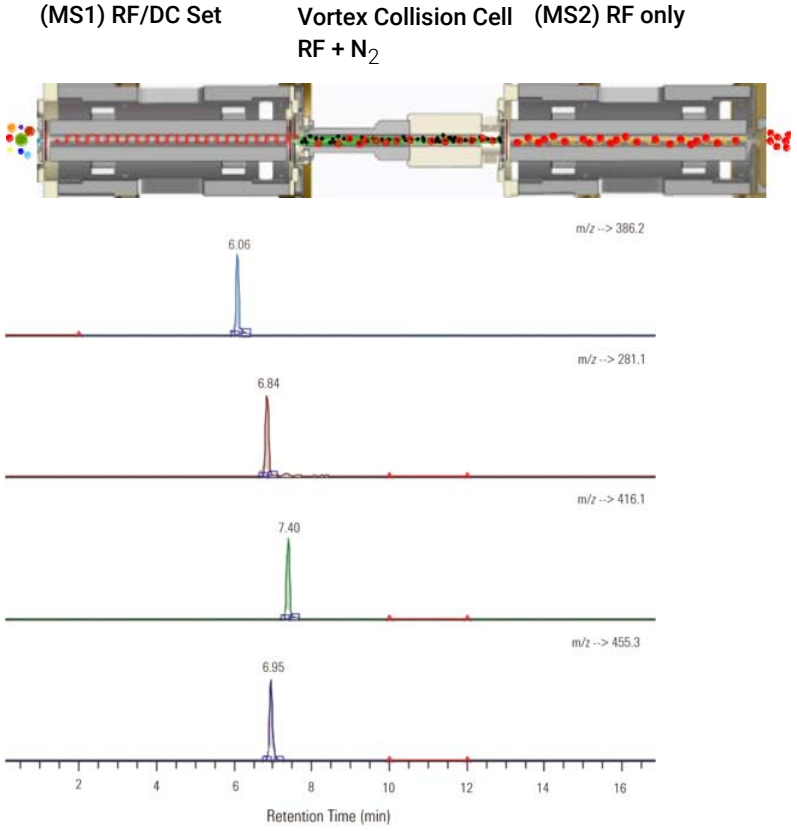


Figure 3 Selected Ion Monitoring (SIM): Quantitation on a specific ion

Multiple Reaction Monitoring Mode (MRM)

MRM is a mode of analysis where both quadrupole mass analyzers operate in SIM mode. In this mode, limited number of parent-ion / product-ion pairs are monitored. A parent ion is selected as usual; however, the entire mass spectrum of its product ions is not obtained. In this case, only one or a few selected product ions are monitored. MRM mode delivers excellent specificity (minimizes interferences) and great reduction in background chemical noise (yields higher signal-to-noise). MRM mode is considered ideal for quantitative analysis of target compounds in complex matrices where many compounds co-elute.

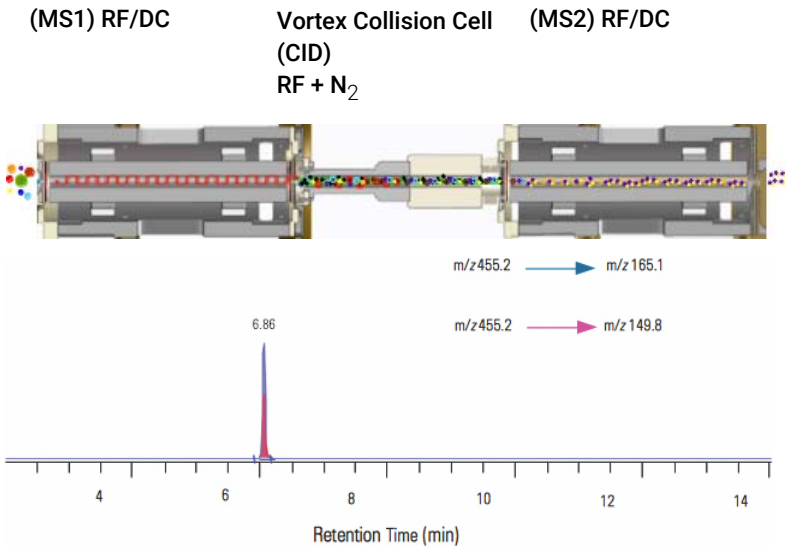
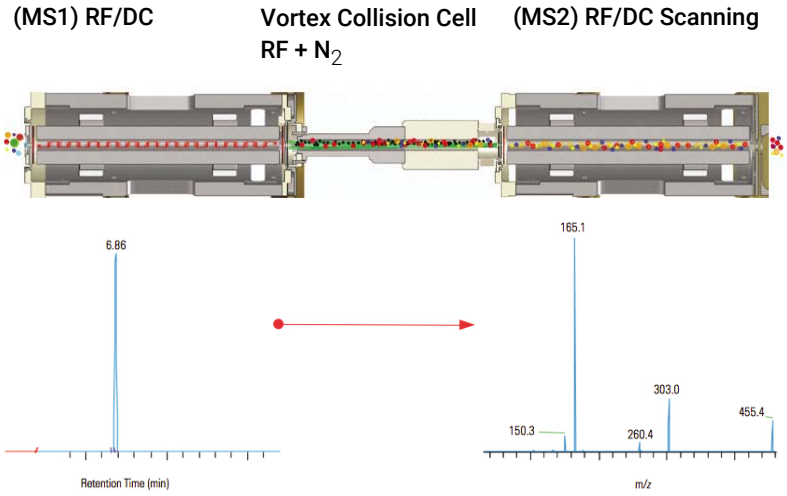


Figure 4 Multiple Reaction Monitoring (MRM): Quantitation on a single or multiple product ions - “monitor a transition for quantitation”

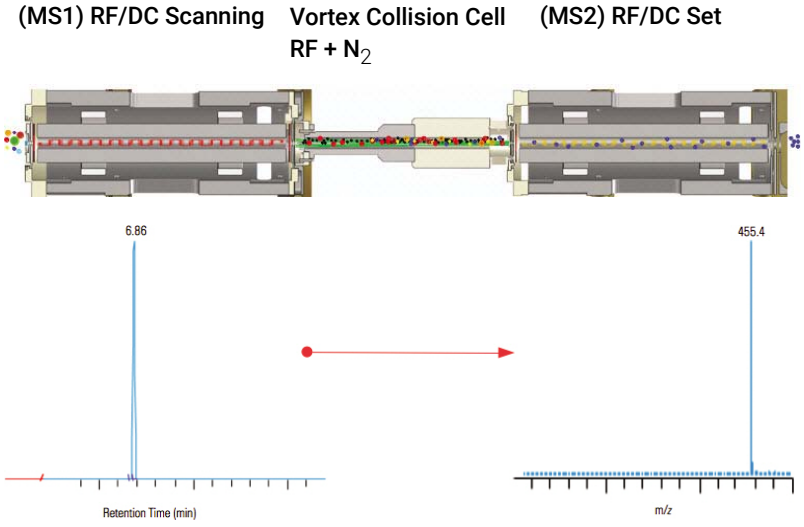
Product Ion Scan Mode

In the first stage of analysis, ions formed in the ion source enter MS1, which is set to transmit ions of a single mass-to-charge ratio (equal to the Parent set mass). The selected parent ion then enters the collision cell. In the collision cell, the parent ion fragments to yield product ions by meta-stable ion decomposition or by collision-induced dissociation. Ions formed in the collision cell (q2) enter MS2 for the second stage of mass analysis. MS2 is scanned to obtain a mass spectrum of the product ions produced from the fragmentation of the selected parent ion. A mass spectrum obtained with this mode shows all the product ions formed from the fragmentation of a selected parent ions.



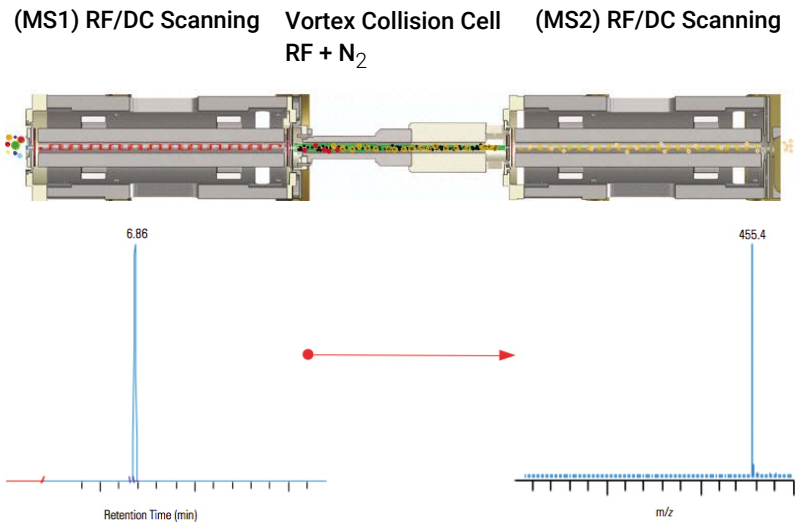
Precursor Ion Scan Mode

During the first stage of mass analysis, parent ions formed in the ion source are introduced into MS1. MS1 is scanned to transmit these ions sequentially into the collision cell (q2). In the second stage of mass analysis, the parent ions fragment in the collision cell to produce product ions by meta-stable ion decomposition or by collision-induced dissociation. The product ions formed in the collision cell enter MS2. MS2 transmits product ions of a single mass-to-charge ratio (equal to the Product set mass). A mass spectrum obtained in the Precursor scan mode shows all the precursor ions that fragment to produce a selected product ion.



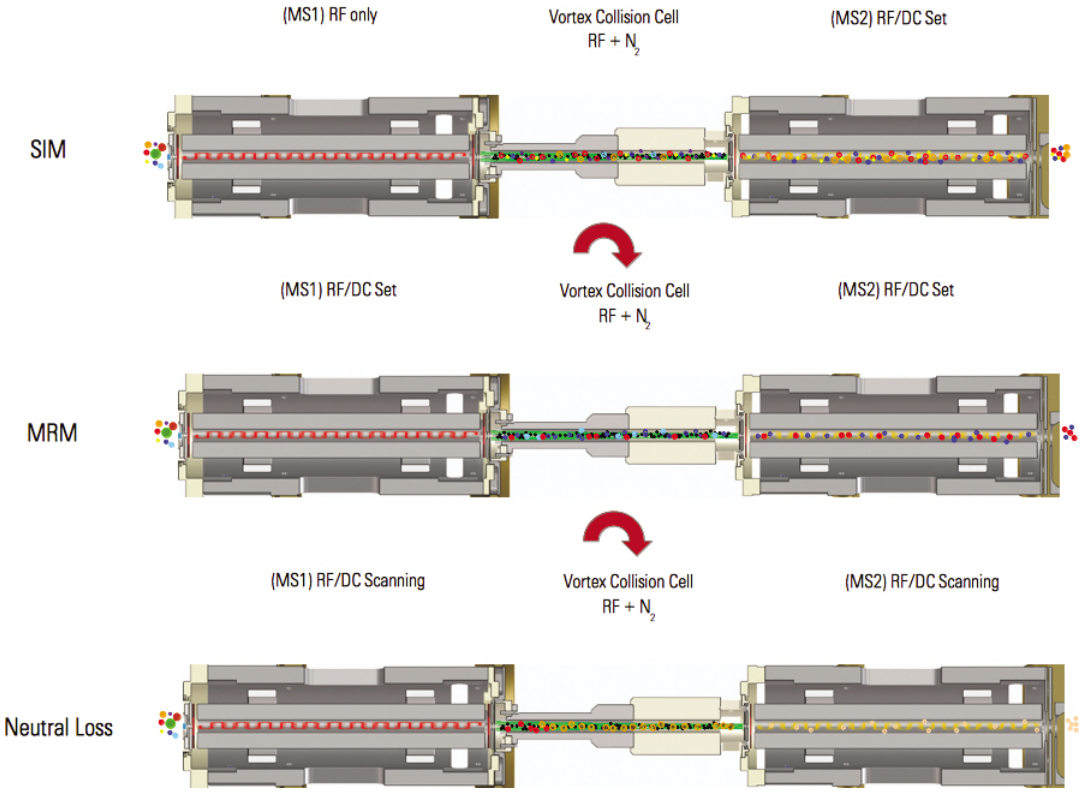
Neutral Loss Scan Mode

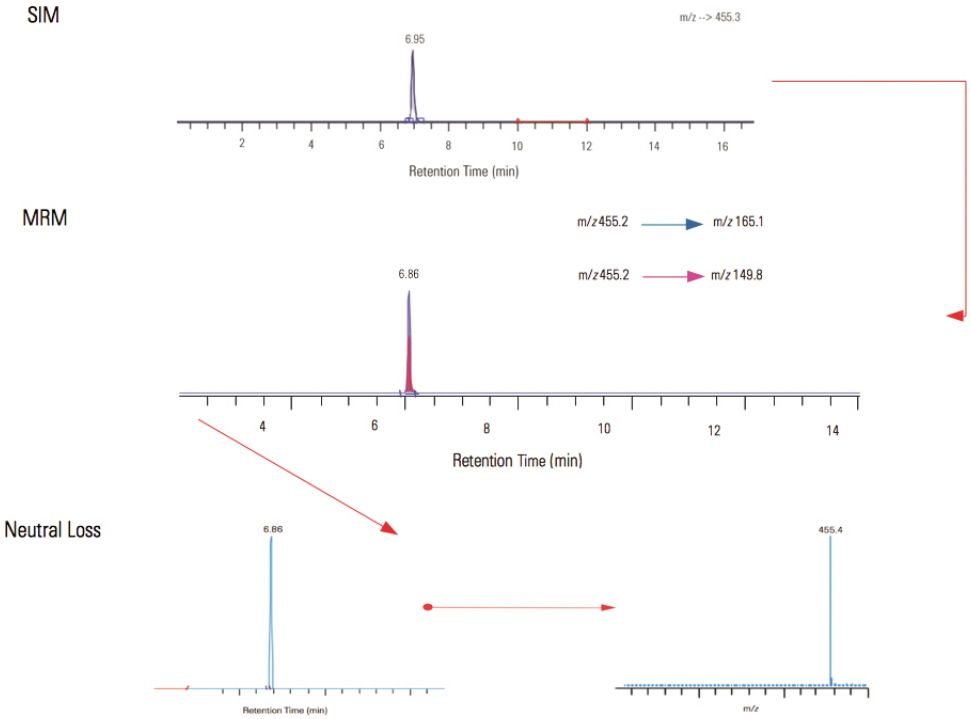
In this acquisition mode, the two mass analyzers (MS1 and MS2) are linked together so that they are scanned at the same rate over mass ranges of the same width. The respective mass ranges, however, are offset by a selected mass, such that the Product mass analyzer scans a selected number of mass units lower than the Precursor mass analyzer. For an ion to be detected, between the time the ion leaves MS1 and enters MS2, it must lose a neutral moiety whose mass (the Neutral Loss mass) is equal to the difference in the mass ranges being scanned by the two mass analyzers. Thus, a spectrum is obtained (a Neutral Loss mass spectrum) that shows all the parent ions that lose a neutral species of a selected mass. For a Neutral Loss mass spectrum, as for a Precursor mass spectrum, data for the mass-to-charge ratio axis's are obtained from Q1 (the precursor ion), whereas data for the ion intensity axis are obtained from Q3 (the product ion being monitored). Experiments in which the Neutral Loss scan mode is used (Neutral Loss experiments) are useful when a large number of compounds are surveyed for common functionality. Neutral moieties are frequently lost from substituent functional groups (for example, CO2 from carboxylic acids, CO from aldehydes, HX from halides, and H_2O from alcohols).



Mixed Mode

This mode allows for the sequential use of different acquisition modes. Here, the initial acquisition mode (SIM, MRM, MS/MS, Precursor Ion or Neutral Loss Scan Mode) selected will trigger one or more targeted acquisition modes. This mode of operation is advantageous when qualitative information is desired during a quantitative analysis, or when desiring to maximize the amount of qualitative information for an analyte with a single injection. To maximize performance with Mixed mode acquisition, conservation of the duty-cycle is imperative. If too many distinct acquisition functions are stitched together, the resulting data may be poor.




SIM
m/z --> 455.3
6.95
2
4
6
8
10
12
14
16
Retention Time (min)
MRM
m/z 455.2
m/z 165.1
m/z 455.2
m/z 149.8
6.86
4
6
8
10
12
14
Retention Time (min)
Neutral Loss
6.86
Retention Time (min)
455.4
m/z

Design of the Ultivo LC/TQ

The Ultivo LC/TQ mass spectrometer consists of an ion source with enhanced desolvation technology, followed by ion optics that transfer the ions to the first quadrupole.

- Supports ESI or AJS ion sources
- Includes Q1(MS1) and Q3 (MS2) quadrupoles that can scan up to *m/z* 1400
- Includes the Vortex collision cell that is used for CID processes and ion transmission
- Includes one 4-stage turbomolecular pump and one mechanical rough pump

Triple Quad LC/MS consists of two stages of mass analysis. The mass analyzer of a “triple quadrupole” instrument consists of two resolving quadrupoles, separated by a collision cell. This configuration is often referred to as a “tandem in space” instrument, since precursor and product ions are created and analyzed in different physical spaces. Ions must be moved from the API source to the mass analyzer, where mass analysis occurs.

Ionization of the sample occurs in the API source, and the ion products are mass analyzed by the first quadrupole assembly. In this case, mass-selected ions exiting the first quadrupole assembly (precursor ions) collide with an inert gas in the second assembly to fragment and produce a set of ions known as product ions. A chamber called the collision cell surrounds this assembly and is typically pressurized with an inert gas. The product ions undergo further mass analysis in the second quadrupole assembly to detect selected product ions. The probability for two different chemical structures with a common precursor ion producing identical product ion fragments from that precursor ion is very low. As a result, two stages of mass analysis yield far greater chemical specificity than a single-stage can achieve, because of the system’s ability to select and determine two discrete, but directly related set of masses.

Innovative Enhancements in the Ultivo LC/TQ

The Ultivo LC/TQ has these enhancements:

VacShield VacShield allows you to change the capillary without venting the system.

Figure 5 VacShield increases throughput and decreases down-time

Cyclone Ion Guide The ion guide has been improved to have a smoother transition between vacuum zones, and higher transmission efficiencies (compression of the ion beam).

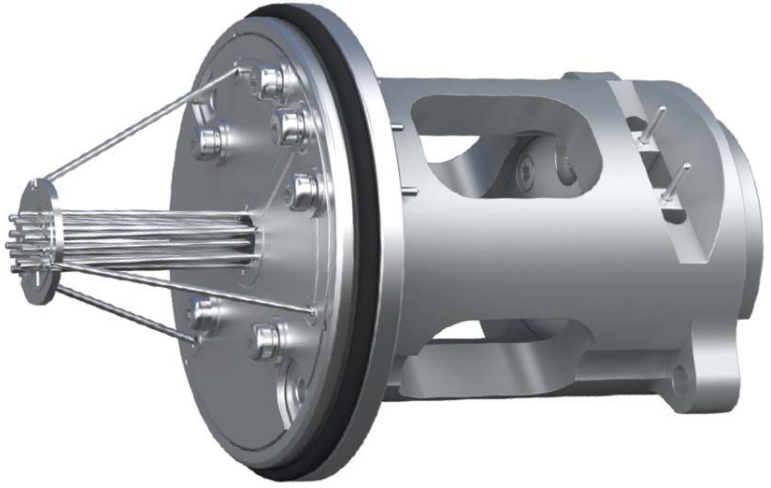


Figure 6 Cyclone Ion Guide

Vortex Collision Cell This new collision cell provides consistent mass fragmentation, transmission (compression of the ion beam), and improved clearance which allows more MRMs/sec.

Figure 7 Vortex Collision Cell

Footprint The footprint of the Ultivo LC/TQ is much smaller than traditional high performance mass spectrometers, and the Ultivo can be included at the bottom of the LC stack. Many hardware maintenance routines can be performed while the Ultivo LC/TQ is still stacked. The electron multiplier, hexapole driver boards, main boards, AC board, and QCard can be removed from the side while the ion optics, Q1 assembly, and turbo can be removed from the front.

DC only pre-/post filters The entrance and exit regions of a quadrupole DC prefilters are precisely designed to cancel out the electronic fringe fields.

3
Ultivo LC/TQ and Sensitivity

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- Detector 41
- Pumping system 42

This chapter shows how the Ultivo LC/TQ reduces chemical and electronic noise and how each component contributes to enhanced instrument sensitivity.

How the Ultivo LC/TQ improves sensitivity

Triple quadrupole mass spectrometers exhibit multiple sources of noise, including noise from all chemical or cluster backgrounds and electronic noise (**Figure 8**).

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 8**.

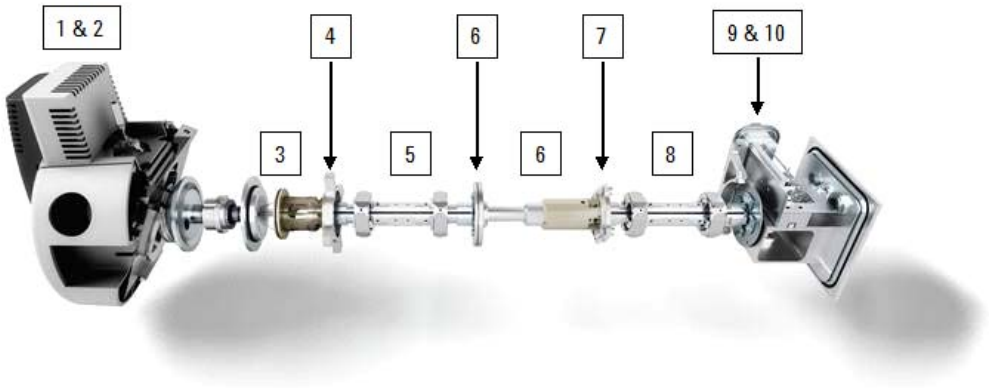


Figure 8 Multiple sources of noise on Ultivo LC/TQ

How the Ultivo LC/TQ minimizes noise

See **Figure 8**.

1. Agilent's orthogonal spray sources maximize ionization while minimizing solvent and matrix noise.
2. A combination of a heated counter-current drying gas, dielectric capillary and skimmer enhances desolvation while minimizing chemical noise.
3. The Cyclone ion guide provides high efficiency ion capture while optimizing wide mass bandwidth ion transmission.
4. A DC-only prefilter enhances high mass ion transmission
5. Quadrupole 1 (MS1) uses hyperbolic quadrupoles to optimize ion transmission and spectral resolution.
6. A DC-only postfilter enhances ion transmission into the collision cell.

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.

7. The Vortex collision cell with linear acceleration optimizes MS/MS fragmentation while eliminating crosstalk, even at very low dwell times. The increased pressure provides collisional cooling of hot fragment ions while the converging geometry of Vortex collision cell assists with focusing ions for transmission into Q3.
8. Quadrupole 3 (MS2) uses hyperbolic quadrupoles to optimize ion transmission and spectral resolution.
9. The off-axis single 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. Increased surface area of the detector horn collects ions over a wide energy spread, allowing for maximum sensitivity of the detection system resulting in greater signal.

Example of chemical noise reduction

The Ultivo LC/TQ passes through four transitional steps in translating a signal in the MRM process (**Figure 9**).

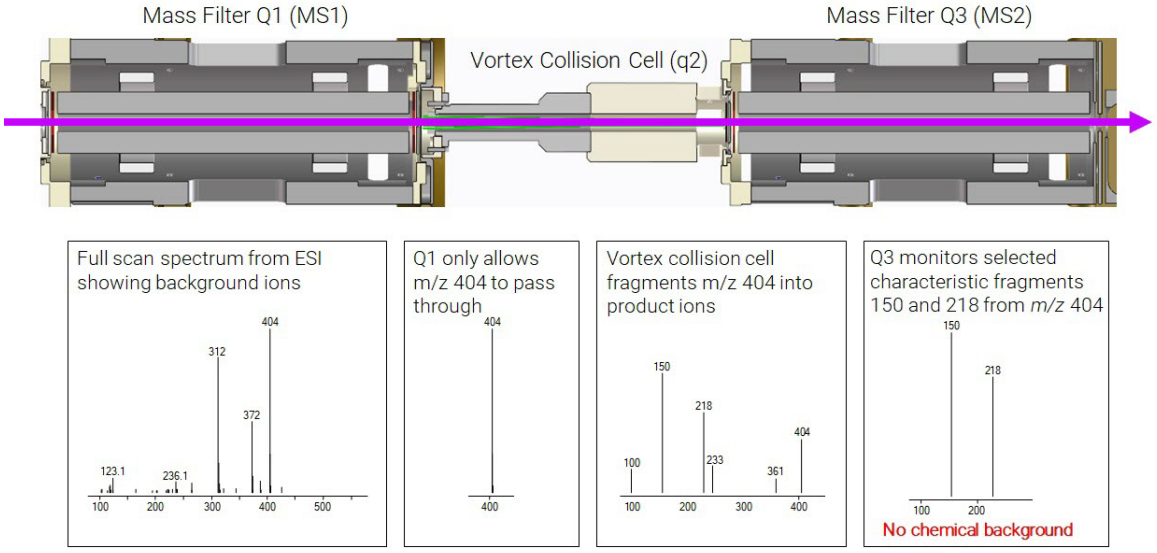


Figure 9 Multiple reaction monitoring (MRM)

Step 1 The spectrum at the far left represents everything that is being ionized at the ion source. This example shows the full scan spectrum of a pesticide. A triple quadrupole LC/MS 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* 404 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 product ion 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* 150 is more intense than the product ion at *m/z* 218. Therefore, the MRM transition 404 -> 150 would be used for quantitation and the 404 -> 218 transition would be used for confirmation, where *m/z* 218 is considered a qualifier ion.

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

Linearity of the Ultivo LC/TQ

Analyses of aminocarb demonstrate the sensitivity and linearity of the Ultivo LC/TQ. It spans from 50 fg/µL to 1 ng/µL (or 0.05 pg/µL to 1000 pg/µL).

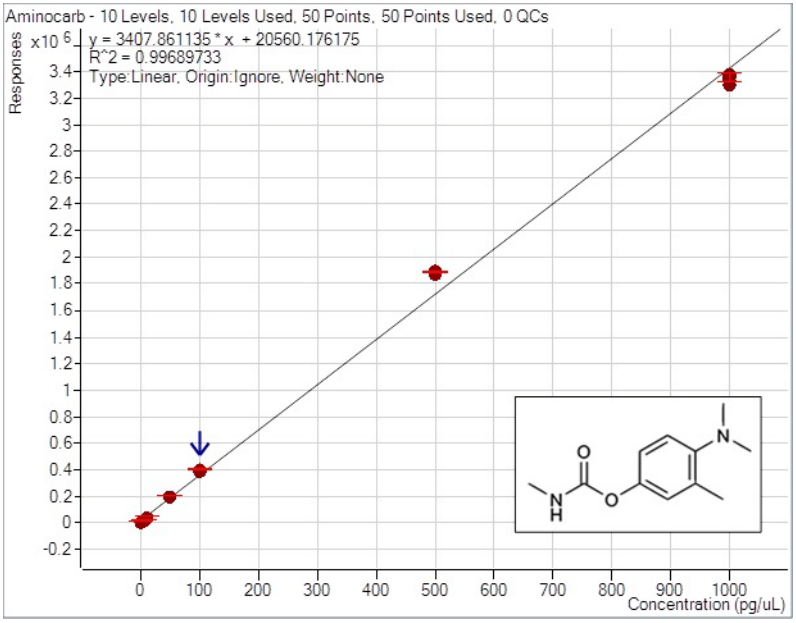


Figure 10 Quantitation Results for Aminocarb

How each component works to improve sensitivity

This section describes in more detail how each of the components of the Ultivo LC/TQ contributes to the reduction of noise and improvements in sensitivity (**Figure 8** on page 26).

Mass Spectrometer Ion Sources

Agilent provides a choice of ion sources to use with its Ultivo LC/TQ: ESI, AJS, and APCI.

This section describes how the different ion sources affect sensitivity.

Electrospray Ion Source (ESI) design

The 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 has the advantage of reducing solvent cluster background (**Figure 11**). In addition, the heated counter-current drying gas facilitates droplet desolvation.

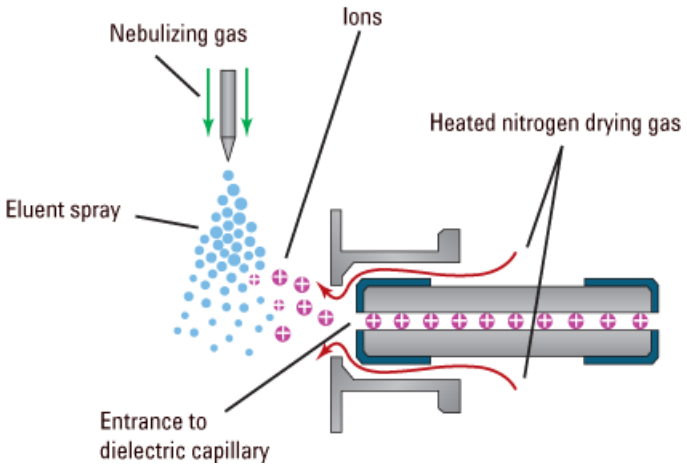


Figure 11 Orthogonal introduction and electrospray ionization

The capillary in the Ultivo LC/TQ 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 the Agilent single quadrupole product.

Agilent Jet Stream (AJS) source

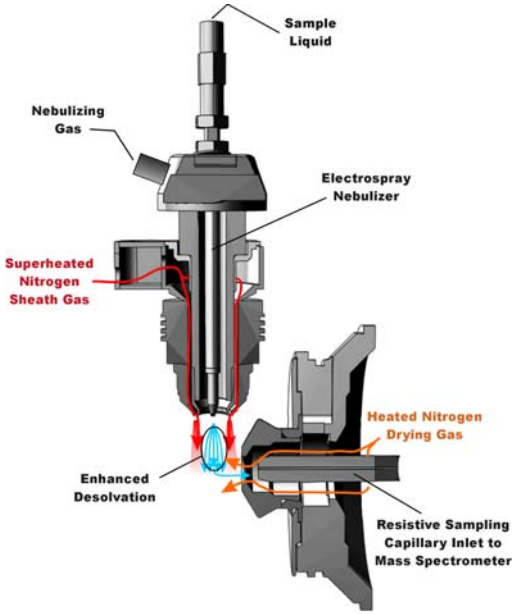


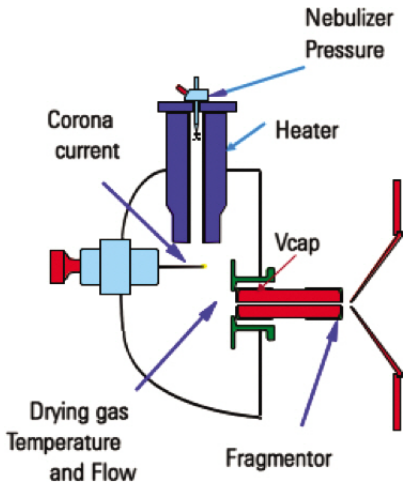
Figure 12 Agilent Jet Stream source

The AJS 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 12** and concomitant improved signal to noise. Parameters for the AJS source are the superheated nitrogen sheath gas temperature, sheath gas flow rate, and the nozzle voltage.

The capillary in the Ultivo LC/TQ 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 the Agilent single quadrupole product.

Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric Pressure Chemical Ionization (APCI) is a popular complement to electrospray. In general, 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. The Agilent 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.



Cyclone Ion Guide

The cyclone ion guide is a unique assembly comprised of six inner rods and six outer rods, shown in **Figure 13**. The ion guide is widest at the entrance which enables maximum collection of ions after the skimmer. The outer set of rods improves high mass transmission efficiency by providing additional confinement. An acceleration voltage is applied to the resistive rods to pull the ions through the guide.

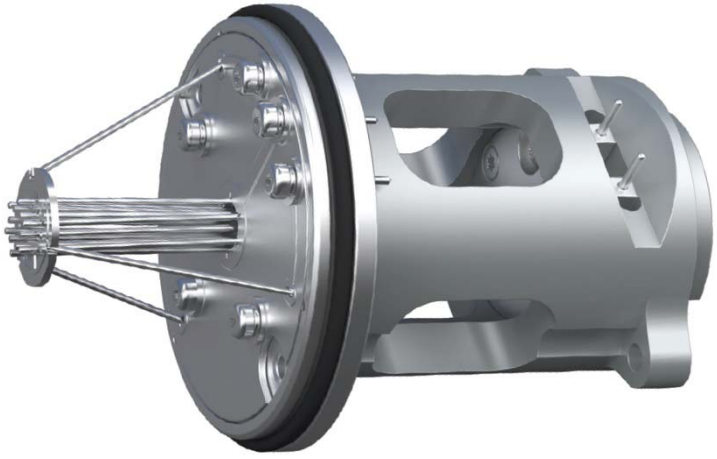


Figure 13 Cyclone Ion Guide

Skimmer The Ultivo LC/TQ uses a skimmer with a small diameter orifice to transfer ions exiting the capillary into the cyclone ion guide. The capillary and skimmer are separated by very short distances to ensure that ions exiting the capillary are captured by the cyclone ion guide.

Cyclone ion guide The cyclone ion guide is a hexadodecapole that transmits ions through multiple vacuum stages between the skimmer and MS1 quadrupole. It is made of two superimposed rod structures: an inner twisted and tapered hexapole and six shorter outer rods that extend only through the high pressure region of the device. The taper of the inner hexapole compresses the ion beam to ensure efficient acceptance into MS1 quadrupole. The twist of the inner hexapole allows a larger pressure drop because the size of the openings between the vacuum stages can be reduced. The outer rods improve

the transmission efficiency of high m/z ions by creating a second, superimposed RF field. A lower frequency RF voltage is applied to the outer six rods which interact with the inner rods to produce a dodecapolar field at the lower RF frequency. The resulting dodecapolar field improves sensitivity for high m/z ions.

Why a hexadodecapole?

The geometry of the cyclone ion guide provides advantages in multiple domains (**Figure 14**).

- Ion transmission across a wide mass range (*m/z* bandwidth) is traditionally best handled by an octopole (Octopole > Hexapole > Quadrupole). However, the fields of the six inner rods and six outer rods superimpose to create a dodecapolar field, greatly improving high-mass transmission.
- Ion beam focusing is traditionally best handled by a quadrupole (Quadrupole > Hexapole > Octopole). However, the six inner hexapole rods feature a twist and taper, efficiently focusing and compressing the ion beam.

The unique geometry of the cyclone ion guide was chosen because it is optimized for ion focusing and ion transmission.

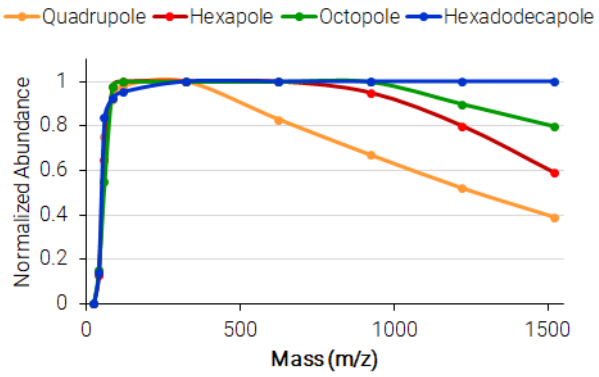


Figure 14 Ion transmission qualities of various multipoles

Quadrupole Assembly

DC-only prefilter

Prefilters enhance sensitivity by improving quadrupole transmission efficiency. Traditional Brubaker prefilters use RF applied to short quadrupole rods placed before the filtering quadrupole to suppress ion instability as ions enter the mass filter in the quadrupole fringe field region. Alternatively, ion instability in the fringe field region can be suppressed by applying a canceling DC voltage to a thin quadrupole lens before the quadrupole resulting in a larger quadrupole acceptance and higher sensitivity.

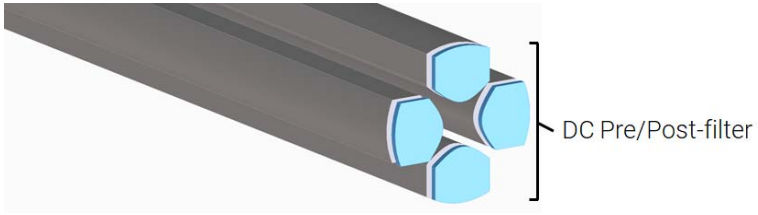


Figure 15 DC Pre/Post filter

Quad mass filters

The quadrupoles have hyperbolic rods that optimize ion transmission. There tends to be more ion loss with circular rods.

DC-only post-filter

DC-only postfilters enhance sensitivity by suppressing ion instability in the quadrupole fringe field region as ions exit the MS1 quadrupole. Like the DC prefilter, the postfilter uses a thin quadrupole lens to cancel the DC field in the fringe field region and thus improves sensitivity.

Vortex Collision cell

What is the collision cell?

The vortex 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 16**).

Figure 16 Collision cell technology for the Ultivo LC/TQ produces higher sensitivity and faster responses without memory or cross-talk effects

The components that contribute to higher sensitivity and faster response are

- Converging and twisted hexapole assembly
- High frequency RF fields
- Linear axial acceleration (cell acceleration voltage)
- Elevated pressure using nitrogen as a collision gas
- High speed digital electronics for fast switching and cell clearance

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

Collision cell design

The collision cell is widest in the front and twisted and tapered towards the back, consisting of six resistively coated rods used to generate a potential difference across the length of the collision cell

(**Figure 7** on page 24). The tapered geometry allows for additional confinement of fragmented ions, acting as an ion beam compressor for transfer into MS2 (Q3)

A potential difference known as Cell Acceleration Voltage (CAV) is always present. This ensures that the precursor ions coming from MS1 (Q1), or fragment ions generated in the collision cell, are transmitted towards MS2 (Q3) 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 17**). A collision energy voltage is applied over the cell acceleration 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 17**).

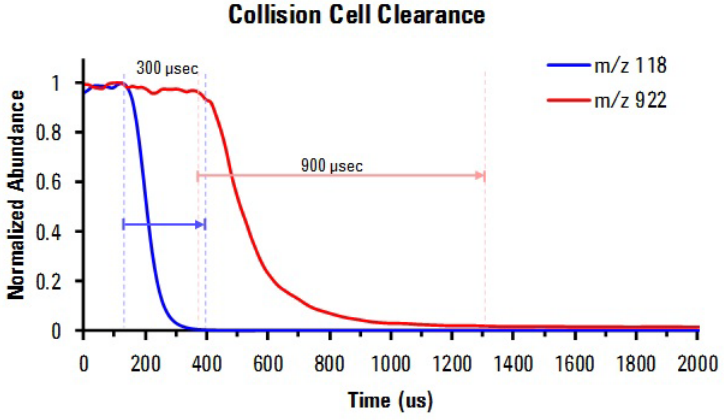


Figure 17 Collision cell clearing profile (Tune ions Betaine and HP-0921, 10 ms dwell time)

The figure shows that the higher mass ion takes longer to evacuate the collision cell. For example, *m/z* 922 takes about 900 µsec to evacuate the collision cell, while *m/z* 118 only takes 300 µsec. 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 18**).

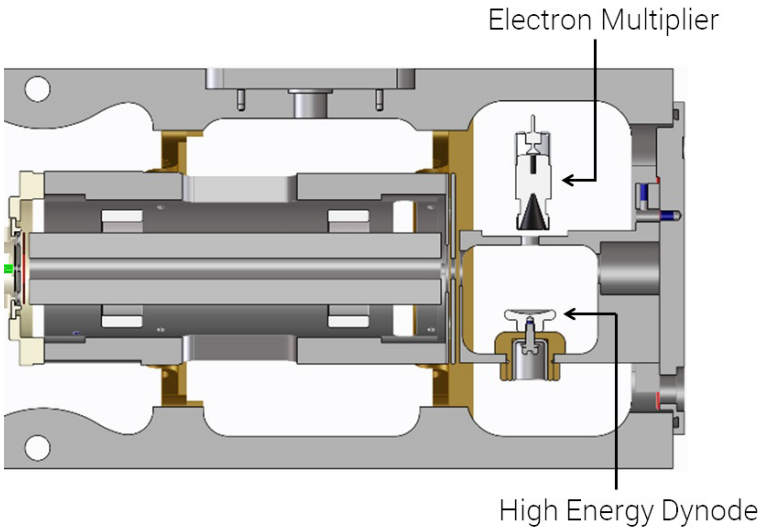


Figure 18 Detector components

The high energy dynode has a high voltage applied and is placed orthogonal to the ion beam and neutrals. The off-axis design allows neutrals to pass through without hitting the detector while attracting only ions. Ions impacting the dynode produce electrons which activate the electron multiplier.

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

Pumping system

A single roughing pump and a four-inlet turbo pump are used to provide five vacuum states (P1 through P5). This is achieved by providing proper conductance limits and partitioning the turbo pump and manifold into four inlet stages. P1 is evacuated primarily using the roughing pump while P2 through P5 are evacuated using the four turbo inlets (**Figure 19**).

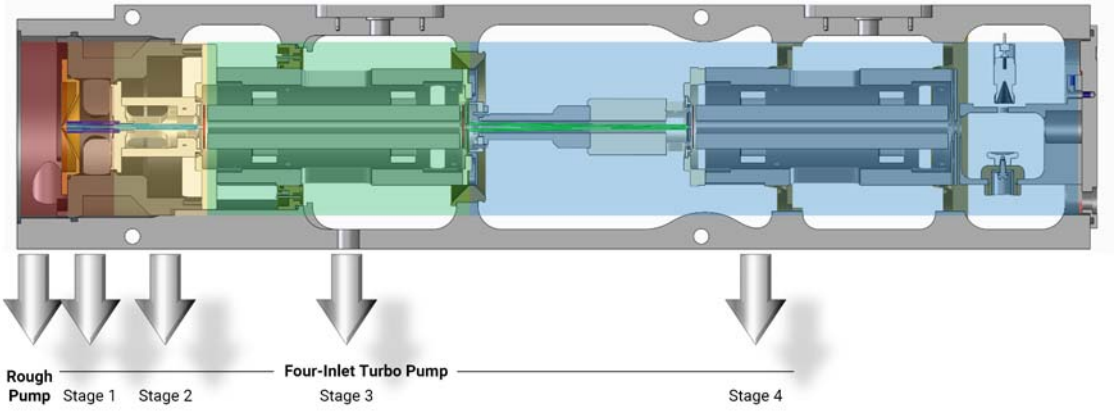


Figure 19 Ultivo LC/TQ pumping system

4
Dynamic MRM and Triggered MRM

How Dynamic MRM works 44
How Triggered MRM works 47

In this chapter you learn about Dynamic MRM and Triggered MRM.

How Dynamic MRM works

Dynamic MRM is a scan type that executes an MRM transition around the elution time of a compound. Using this mode maximizes the dwell time for a transition, thereby improving sensitivity. This scan type has a single continuous Time Segment and up to 4000 transitions in the Scan segments table. You can add a Time Segment that sets the divert valve to waste.

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 Time** (Retention Time) and **Delta Ret Time** (Delta Retention Time). **Ret Time** is the analyte's expected retention time while **Delta Ret Time** is the time window to acquire the transition. Each transition is acquired in the following range: **Ret Time** ± 1/2*(**Delta Ret Time**)

Ret Time and **Delta Ret Time** are entered in minutes.

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.

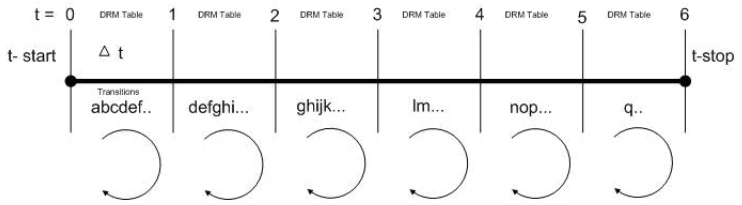


Figure 20 Automatically determining dynamic MRM tables.

The MassHunter Acquisition software, the QCard embedded software, the Digital Signal Processor and the MS Hardware all are involved in the dynamic MRM algorithm.

1 **MassHunter 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 MassHunter 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 chromatographic peak.

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

2 **QCard Embedded Software**

The QCard Embedded Software 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 the QCard Embedded Software. The data is sent back to the MassHunter Acquisition software, which stores the data in an MRM data file which can be opened in the Qualitative Analysis program or the Quantitative Analysis program.

3 **Digital Signal Processor (DSP)**

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

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.

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 QCard embedded software in the form of a structure containing header and abundance information.

How Triggered MRM works

Triggered MRM occurs when criteria for primary MRM transitions trigger confirmatory (secondary) MRMs to be acquired for a compound. If the abundances of the Primary MRMs are higher than the set thresholds and other criteria are met, then the confirmatory (or secondary) MRMs are acquired. You can have multiple primary MRMs per compound, and you can specify up to two of these as Trigger MRMs for each compound. You can also have multiple secondary transitions for each compound. All transitions with the same Compound Name belong to the same compound.

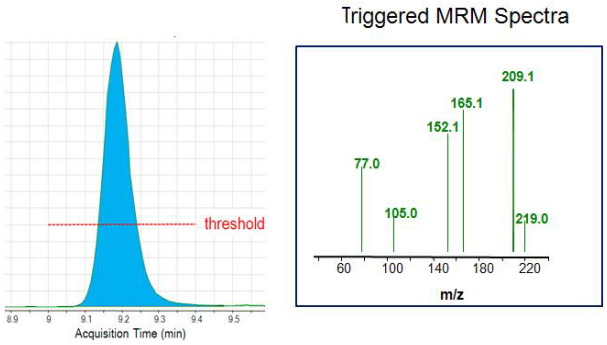


Figure 21 Explanation of threshold for Triggered MRM

In **Figure 21**, only the Trigger MRMs are acquired until the abundance of each of the Trigger MRMs is higher than the thresholds you entered. After the abundances for each Trigger MRM is higher than the threshold, then the secondary transitions may be acquired, depending on the **Trigger Entrance Delay**, **Trigger Delay** and **Trigger Window**. These additional criteria are discussed in the next section.

In the Scan Segments table, you specify which transitions are **Primary** transitions by marking the check box in the Primary column. These transitions are monitored within the peak retention time window specified for the compound. You also can specify one or two of these primary transitions as Trigger MRMs by marking the check box in the **Trigger** column. Any transition that is not marked as a Primary transition but that has the same compound name as a **Primary** transition is a secondary transition for the compound.

You specify a threshold for each Trigger MRM. If the abundances for the Trigger MRM transitions are greater than the specified thresholds and the other triggering conditions are met, then the secondary transitions are acquired. If you have two Trigger MRM transitions for a compound, then the abundances for both of these transitions must be greater than or equal to their thresholds for the secondary transitions to be acquired.

These secondary transitions are acquired for the **Number of Repeats** specified. If the trigger transition drops below the threshold, and rises again above the threshold within the peak retention time window, the secondary ions are triggered again. If the retention time window ends, the software stops acquiring these secondary transitions even if they have not been acquired for the Number of Repeats specified. The software also stops acquiring the Primary MRMs when the peak retention time window ends.

Triggers may happen at different time/abundance

Examination of the abundance of the primary transition(s) and the decision to sample the additional secondary transitions happens in real time, on a cycle-to-cycle basis, using unfiltered data. However, in general, the data stored to disk is the result of using time filtering (data for a given cycle is smoothed using data from cycles before and after the given cycle). Therefore, because of this difference, triggering may appear to start a cycle or two late, or may appear to trigger at an abundance significantly different from the trigger threshold set in the program. Usually, this is not a concern as long as triggering occurs somewhere during peak elution.

The sample matrix may also affect where triggering occurs. If triggering is set using a standard made in solvent, the triggering thresholds may be set to low abundance values. If a sample is run in matrix where there's a significant response at the trigger transition due to the matrix, triggering will happen prematurely. It is preferable to use matrix-matched standards for calibration and update of the triggering parameters.

Other triggering conditions for each compound

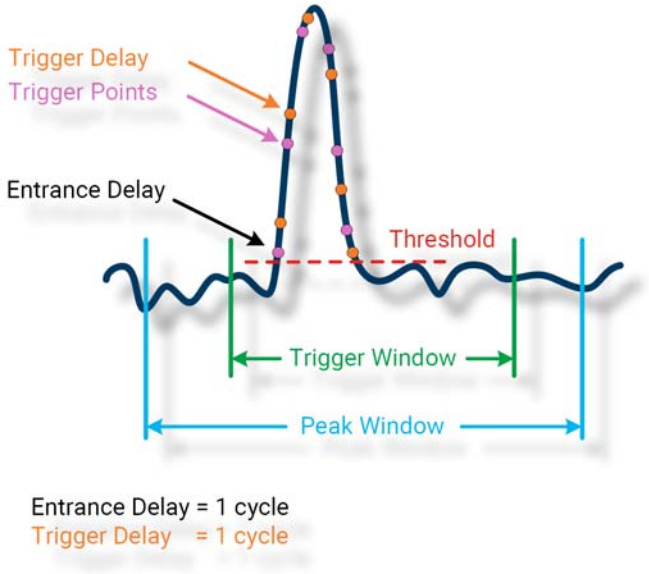


Figure 22 Example of Trigger Window

Trigger Entrance Delay

The **Trigger Entrance Delay** is the number of scans to skip after the thresholds for the Trigger transitions have been met within the **Trigger Window.** If the **Trigger Entrance Delay** is 2 and the other trigger conditions are met at scan 200, then only the primaries are acquired at scans 201 and 202 (the next 2 scans). Primary and secondary transitions are acquired starting at scan 203.

Trigger Delay

The **Trigger Delay** is the number of scans to skip between acquiring each of the secondary transitions. If the **Trigger Entrance Delay** is 0, the **Trigger Delay** is 1 and the **Repeats** is set to 3 and the other trigger conditions are met at scan 200, then the secondary transitions are acquired at scan 201, scan 203 and scan 205. Only the primary transitions are acquired at scans 202 and 204. If the **Trigger Delay** is set to 2 in the example above, then the secondary transitions are acquired at scan 201, scan 204, and scan 207. Only the primary transition are acquired at scans 202, 203, 205 and 206.

Trigger Window

The **Trigger Window** can be a narrower window within the Peak Retention Time window. The thresholds for the trigger transitions are only monitored within the Trigger Window. By default, the

Trigger Window is set to 0 which means the **Trigger Window** is the same time as the Peak Retention Time window. The value you enter for the Trigger Window is the full width of the window. **Ret Time** and **Trigger Window** are entered in minutes. The Trigger Window is **Ret Time** ± 1/2*(**Trigger Window**).

Example of Triggered MRM with four compounds

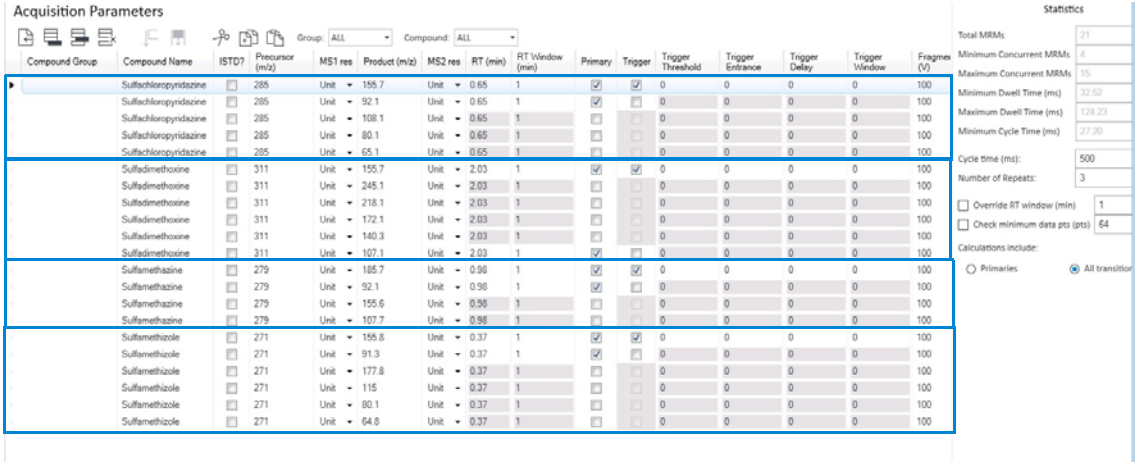


Figure 23 Triggered MRM in the Acquisition section (blue boxes added as a visual aid)

- The Scan type is set to **Dynamic MRM** and the **Triggered** check box is marked. **Repeats** is set to 3.
- This **Scan segments** table has four different compounds.
- Each of these compounds has at least one Trigger transition. You do not need to specify a Trigger transition for each compound. If you do not, no secondary transitions are triggered.
- *Sulfachloropyridazine* has two primary transitions and one of these is the trigger transition.
- *Sulfamethazine* has two primary transitions and both of these are trigger transitions.

- A compound does not have to have secondary transitions.
- If a scan is outside of the **Trigger Window**, then the secondary transitions are not acquired.
- All of these compounds do have secondary transitions. The secondary transitions for *sulfadimethoxine* are 311.1 *m/z* - > 156 *m/z* and 311.1 *m/z* -> 108 *m/z*.
- If a scan is outside of the **Peak Retention Time** window, then the primary and the secondary transitions are not acquired.
- For *sulfachloropyridazine*, if the abundance of the primary trigger transition (285 *m/z* -> 197 *m/z)* is greater than 800 at scan 80, then because the **Trigger Entrance Delay** is 2, secondary transitions are acquired starting at scan 83. Only the primary transitions are acquired at scan 81 and scan 82.
- For *sulfadimethoxine*, if the abundance of the primary trigger transition (311.1 *m/z* -> 245.1 *m/z)* is greater than 1000 at scan 200, then because the **Trigger Delay** is 1, the secondary transitions are acquired at scan 201, scan 203, and scan 205. Only the primary transitions are acquired at scan 202 and scan 204.
- For *sulfamethazine*, if the abundance of the first primary trigger transition (279.1 *m/z* -> 186 *m/z)* is greater than 900 counts and the abundance of the second primary trigger transition (279.1 *m/z* -> 155.9 *m/z)* is greater than 1000 counts and the retention time is between 0.6 minutes and 1.0 minutes (the **Trigger Window**), then the secondary transitions are acquired. The **Trigger Window** is set to a narrower range than the **Peak Retention Time** window.
- For *sulfamethizole*, all three trigger conditions are set. So, if the abundance of the primary trigger transition (285 *m/z* -> 197 *m/z*) is greater than 1100 (the threshold) at scan 60 and the retention time for scan 60 is within the **Trigger Window**, then because of the **Trigger Entrance Delay** is 2, the secondary transitions are not acquired for the next two scans (scan 61 and scan 62). Because of the **Trigger Delay** is 1, the secondary ions are acquired at scan 63, scan 65 and scan 67. One scan is skipped after each time you acquire the secondary ion; only the primary transitions are acquired at scan 64 and scan 66. If any of these scans are outside of the **Trigger Window**, then the secondary transitions are not acquired for those scans.

5
MassHunter Acquisition for Ultivo LC/TQ

Tuning 55
Acquisition 57

Learn the concepts to help you understand the design and operation of the Agilent MassHunter Acquisition for Ultivo LC/TQ program.

The MassHunter Acquisition program (**Figure 24** on page 54) has the following features:

- All LC and customer-editable 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.

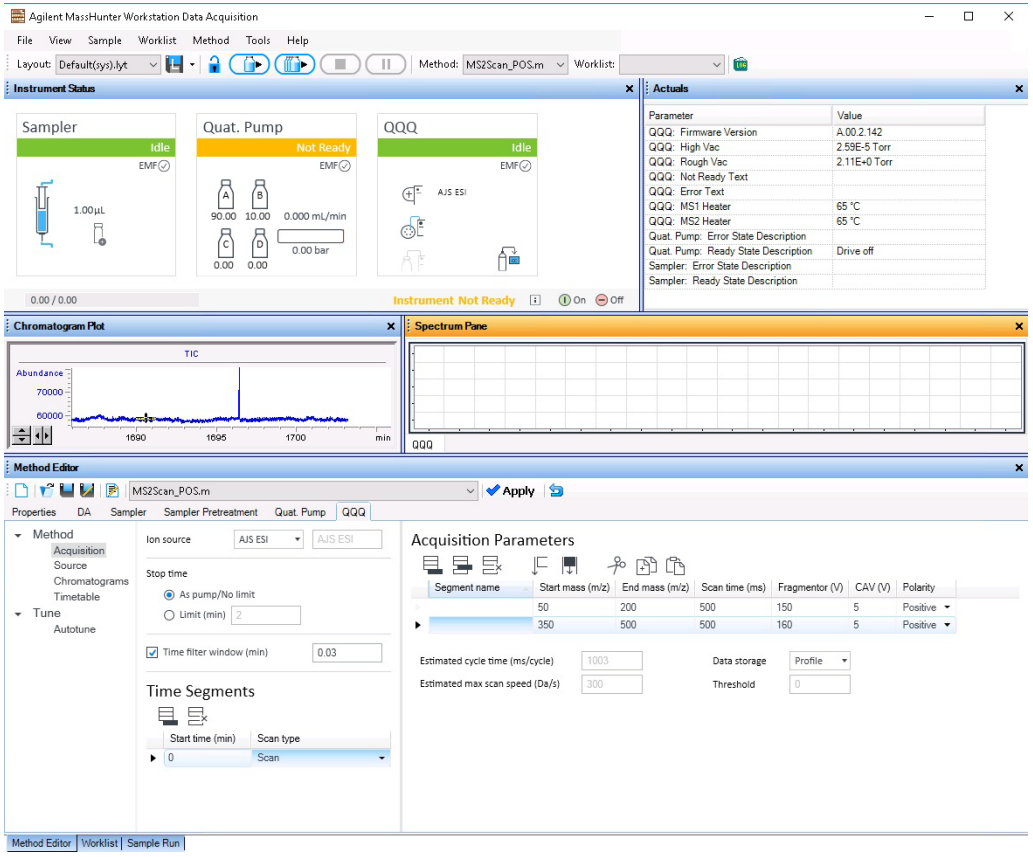


Figure 24 MassHunter Acquisition program

With these windows 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 acquired
- Set up worklists for sequences of samples

Tuning

Autotune and Checktune

A Checktune can be used to determine if the *m/z* values for tune mix ions are properly assigned and if the response or sensitivity of these ions is within expectations. In other words, a Checktune verifies peak width and mass axis to make sure they are within tolerance before you start your acquisition. This checktune takes approximately 3 to 5 minutes to run both polarities. Both polarities are run automatically.

Autotune only needs to run after preventative maintenance or if Checktune reports “Out of Tolerance”. (**Figure 25** on page 56) An Autotune can take approximately 10 minutes to finish both polarities. Everything is automatic since the tuning mix is delivered by the calibrant delivery system (CDS), which is switched on and off automatically.

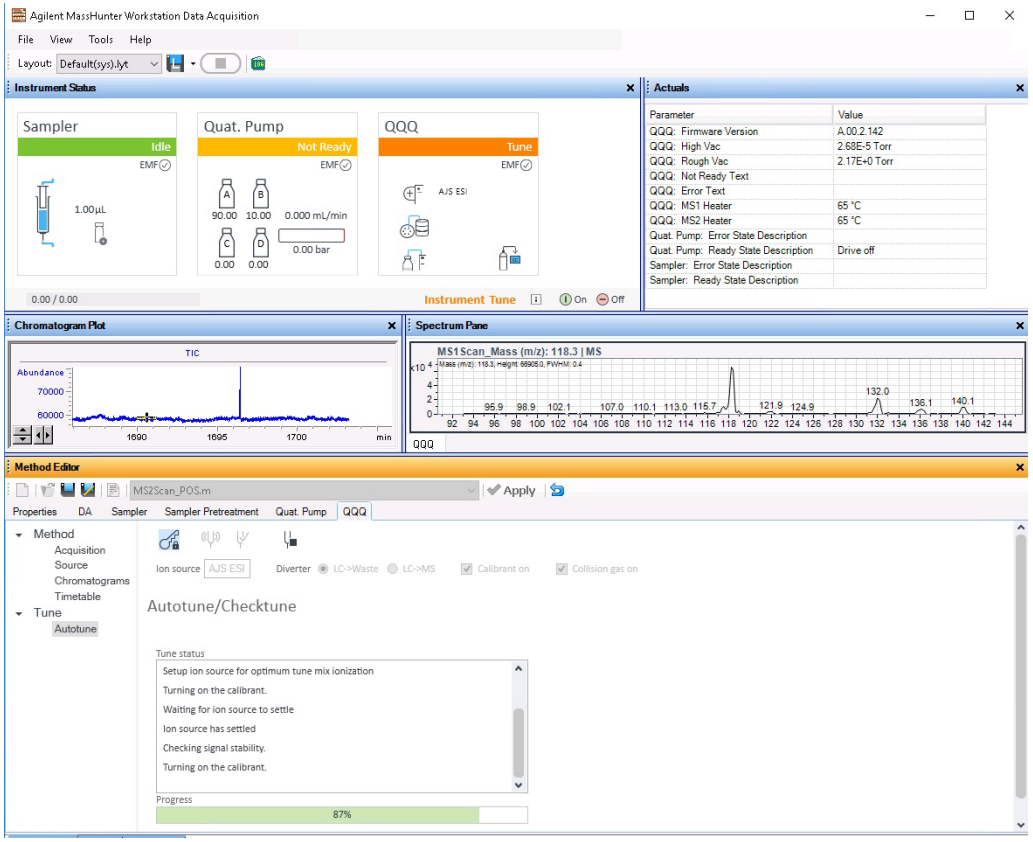


Figure 25 Checktune in progress

Acquisition

Many Agilent LC modules and the Ultivo LC/TQ can be controlled and monitored (Instrument Status window) from the same program used for entering acquisition settings (Method Editor window) and setting up lists of samples to run (Worklist window)(**Figure 24** on page 54).

The Chromatogram Plot window also can show the MS and UV chromatograms in real time.

Because of the large amount of information available, any of these windows can be closed for easier viewing, if necessary.

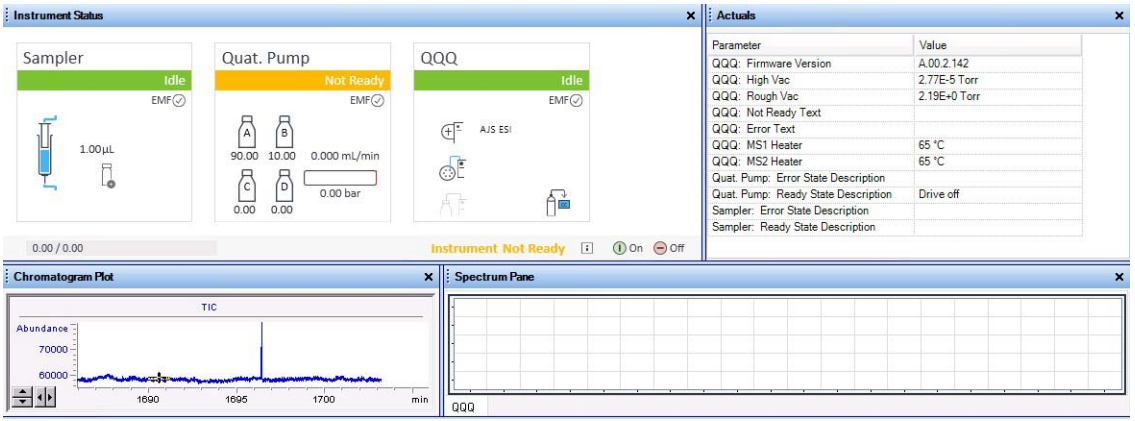


Figure 26 Instrument Status, Actuals, Chromatogram Plot, and Spectrum Pane windows

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In This Book

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

This guide includes concepts for:

- Overview
- Inner Workings
- MS and Sensitivity
- MassHunter Acquisition

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Revision A, November 2017

G5351-90005

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