Agilent 6300 Ion Trap LC/MS Systems

Concepts Guide

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Notices

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In this Guide...

This guide contains background information about the Agilent 6300 Series Ion Trap System.

1 Ion Trap System Overview

This chapter gives you an overview of the Ion Trap system hardware and software.

2 How the Agilent Ion Trap Works

This chapter describes the concepts behind the operation of the Agilent ion trap.

3 How Ion Trap Methods Work

This chapter presents the organization of methods and data files so that you can set up LC/MS methods and automate analyses more easily.

Contents

1
Ion Trap System Overview

This chapter presents an overview of the Ion Trap hardware and gives a short explanation how each component works within the system to produce the spectra.

Ion Trap - an LC/MS(n) system

Trap system

The 6300 Series Ion Trap LC/MS is a complete LC/MS/MS system. The hardware includes an optional Agilent 1200 Series LC, the ion trap mass spectrometer and the data system. Additionally, the Ion Trap LC/MS comes with a syringe pump for both low-flow and high-flow direct infusion work. The software includes the TrapControl program for trap control, data acquisition, data analysis, quantitative analysis, and the Agilent ChemStation program for sample automation and LC control.

The mass spectrometer (1) with its interface (2), the Agilent 6300LC (3), monitor (4) and computer (5) are shown in Figure 1. The printer and syringe pump are not shown.

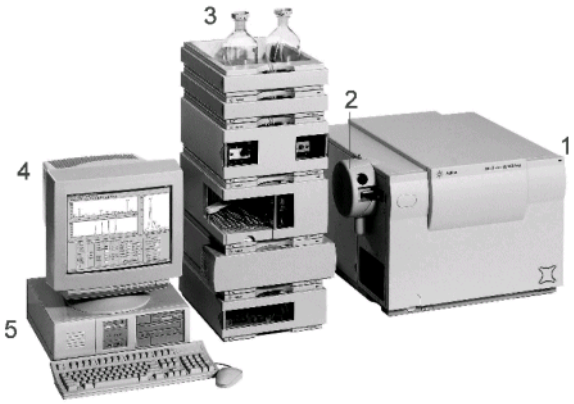


Figure 1 Ion Trap system

Trap mass spectrometer

The Agilent 6300 Series Ion Trap LC/MS consists of these components (Figure 2):

- interface to generate ions
- ion optical elements to guide the ions from the interface to the mass analyzer (ion trap)
- ion trap to collect the ions and then release them according to mass-to-charge ratio
- ion detector (and its electronics, firmware and software) to convert the ions to a mass spectrum
- vacuum pumps to keep the system at low pressure to ensure efficient ion transmission and detection

The program controls the parameters associated with generating, guiding, accumulating and analyzing the ions.

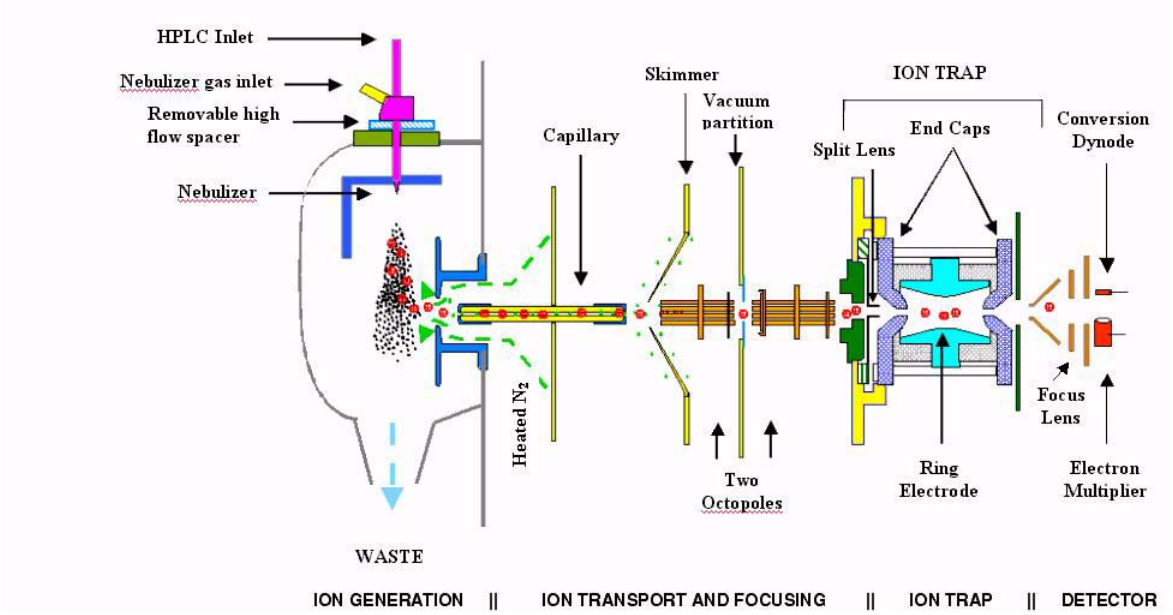


Figure 2 Trap mass spectrometer for ion transmission and detection

Electron Transfer Disassociation (ETD)

The 6340 Trap with ETD NCI Gun consists of these components (Figure 3):

- ESI ion accumulation
- Precursor ion isolation
- NCI and accumulation of fluoranthene anions
- ETD fragmentation (40-100 msec)
- Generate product ion scan

Isolated analyte cation packets are pulsed into ion optics followed by negative ion packets. All ions are collected in the ion trap and reacted via gas phase ion-ion interactions.

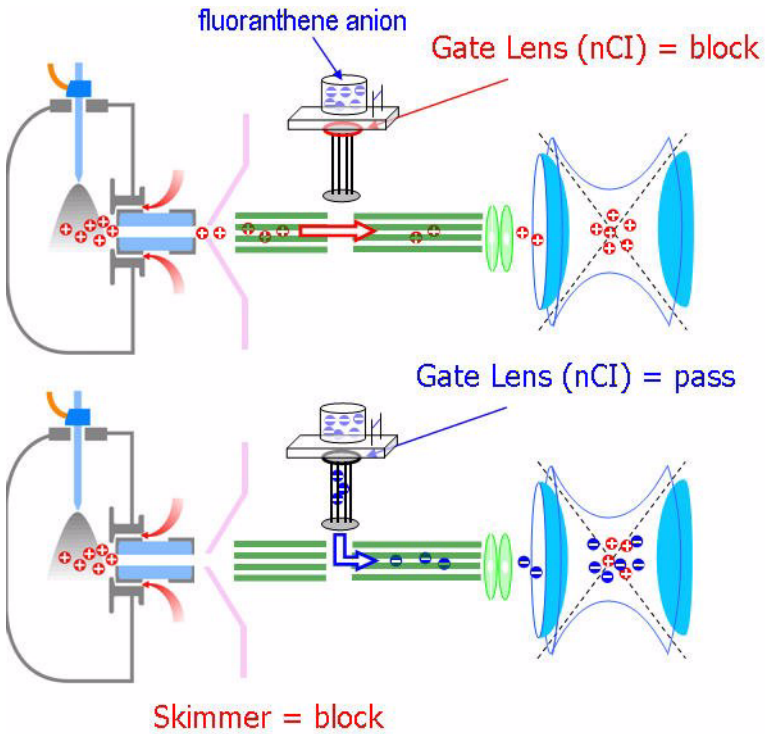


Figure 3 6340 Series trap with ETD NCI Gun

An ion trap performs several important steps to produce mass spectra:

- mass accumulation,
- selective mass isolation and excitation for collision-induced dissociation (MS/MS), and
- sequential mass ejection to produce a mass spectrum.

With such flexibility, the ion trap has some advantages over linear quadrupole systems for use in highly sensitive and selective mass measurements.

See "Ion sources" on page 11 for more information on the interfaces that you can use with the Ion Trap.

Ions are generated outside the trap by one of several possible API (Atmospheric Pressure Ionization) sources, which produces a continuous source of ions. These naturally divergent ions have to be focused from the source into the trap by a combination of electrostatic lenses and a split RF octopole ion guide (Figure 2).

See "Ion transport and focusing region" on page 21 for more details on how the Ion Trap transports and focuses the ions before entry into the trap.

During the first part of the mass analysis cycle, ions are accumulated in the trap. During later parts of the mass analysis the continuous ion beam from the source must be prevented from passing through the trap, which would result in detector noise. Appropriate voltages ("block voltages") are applied to the elements in the ion transport and focusing region to deflect the ion beam during these later stages of mass analysis.

See "Ion trap" on page 25 for background on the design of the Ion Trap.

During the "scanning" step, ions of a particular mass are ejected in increasing order of mass from the trap. The trap can also isolate a precursor ion, which is then fragmented into product ions to produce MS/MS spectra. You can also tell the trap to fragment the product ions as well, to give the trap MS(n) capability.

Sample inlets

You can introduce samples into the Trap mass spectrometer via delivery systems that differentiate themselves primarily by the liquid flow rates for which they are designed:

- Agilent 1200 Series LC with quaternary, binary or isocratic pump (10–2000 µL/min typically, maximum 5000 µL/min)
- Agilent 1200 Series LC with capillary pump (1µL-20µL/min)
- Agilent 1200 Series LC with nano pump (100–1000 nL/min typically, maximum 4000 nL/min)
- Syringe pump (0.3–10 µL/min alone and 100–1000 µL/min with LC pump, maximum 5000 µL/min)
- Capillary electrophoresis

You can also choose to use a divert valve to lead the sample from the delivery system either to the source or via bypass to waste.

HPLC system

A High Peformance Liquid Chromatography (HPLC, or simply LC) system (see Figure 4) is the most common form of sample delivery for the Ion Trap. The Ion Trap electrospray interface (ESI) accepts flow rates up to 1 mL/min. With the Atmospheric Pressure Chemical Ionization (APCI) option, flow rates up to 2 mL/min. are possible.

You can operate the LC system in several modes with the Ion Trap. Typical modes include standard LC analysis, analysis without LC separation (flow injection analysis, FIA) and combined flow with the low-flow syringe pump.

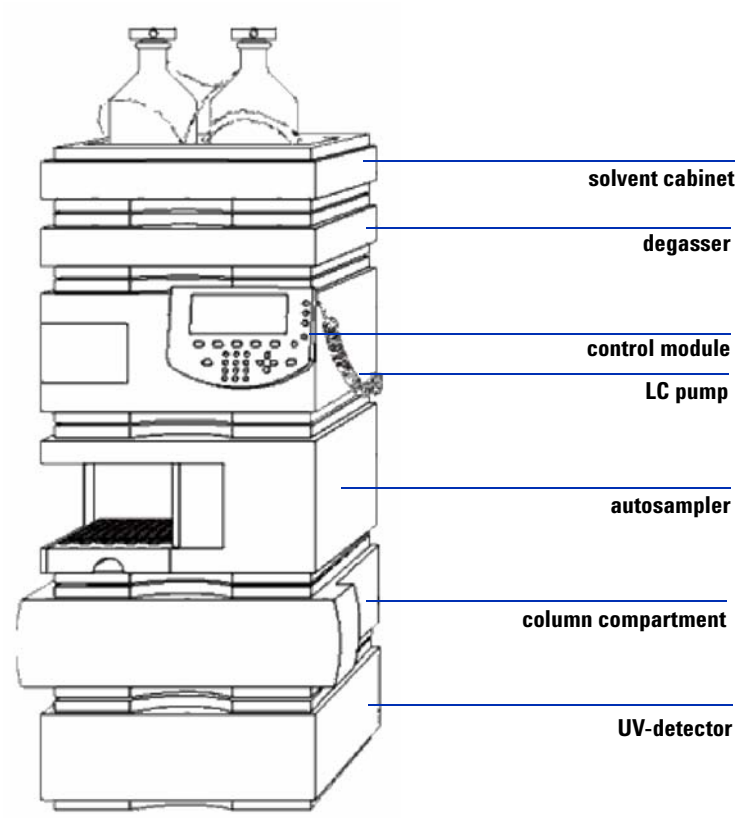


Figure 4 Representative example of the modular Agilent 1200 Series HPLC system

Syringe pump

A small syringe pump (see Figure 5) is included with the Ion Trap system to introduce samples directly to either the electrospray or APCI ion sources.

When used with the electrospray interface, two modes of operation are available.

- Delivery of the sample in solution directly to the nebulizer under low-flow conditions (typically 1–10 µL/min.), or
- Delivery of the sample in solution into the LC system main flow through a T-connector. This combined operation is particularly convenient for the optimization of instrument parameters and the development of MS/MS methods.

For APCI operation the combined operation mode is preferred, because it is not practical to operate the syringe pump at the optimal 1-2 mL/min. flow rates for APCI.

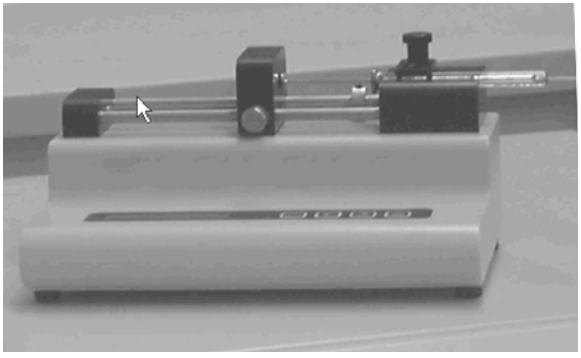


Figure 5 Syringe pump

Capillary electrophoresis (CE)

Capillary Electrophoresis (CE) is a migration of electrically charged compounds in solution under the influence of an applied electrical field (Figure 6). CE has the following special features:

- CE/MS compared to LC/MS provides different selectivity, higher separation efficiency and usually a shorter analysis time.
- Although CE/MS offers a greater mass sensitivity than LC/MS, its practical limits of detection are about 1000 times higher because of the lower mass loading capacity and dilution by the sheath liquid necessary for CE/MS operation.
- CE reduces sample preparation and analysis time for compounds in complex matrices, and MS/MS allows unambiguous identification.
- CE/MS(n) is suited for the analysis of compounds at ppm concentrations in small complex-matrix samples.

Figure 6 Capillary electrophoresis (CE) system

Divert valve

The divert valve lets the sample flowstream bypass the ionization source when analysis is not needed in order to reduce the need for cleaning.

The divert valve is located to the right of the source housing and behind the panel. The divert valve outlet is connected to the source (tan tubing at top of Figure 7) while its inlet is connected to the solvent delivery system (red tubing at bottom of the figure).

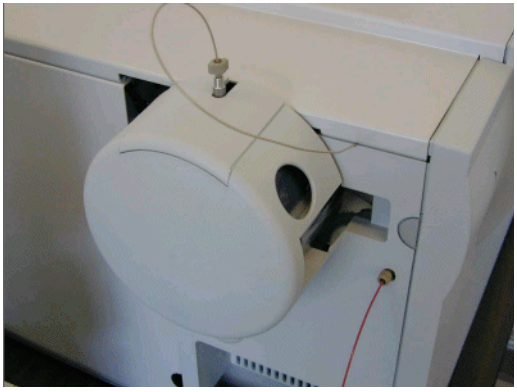


Figure 7 Divert valve connection

The divert valve may be time-programmed during the analysis to send undesired portions of the sample (e.g., salts, solvents, major components) to waste.

Ion sources

The Ion Trap can operate with API (Atmospheric Pressure Interface) sources such as:

- ESI (Electrospray ionization)
- APCI (Atmospheric pressure chemical ionization)
- APPI (Atmospheric pressure photo-ionization)
- NanoElectrospray
- AP-MALDI (see the *User's Guide* for more information)

Electrospray Ionization (ESI)

The electrospray interface generates ions in a spray chamber (Figure 8 and Figure 9). The system then transports and focuses the ions into the ion trap mass analyzer.

Figure 8 API-electrospray interface

Electrospray ionization (ESI) consists of four steps:

1. Formation of ions
2. Nebulization
3. Desolvation
4. Ion evaporation

These steps take place in the spray chamber shown below.

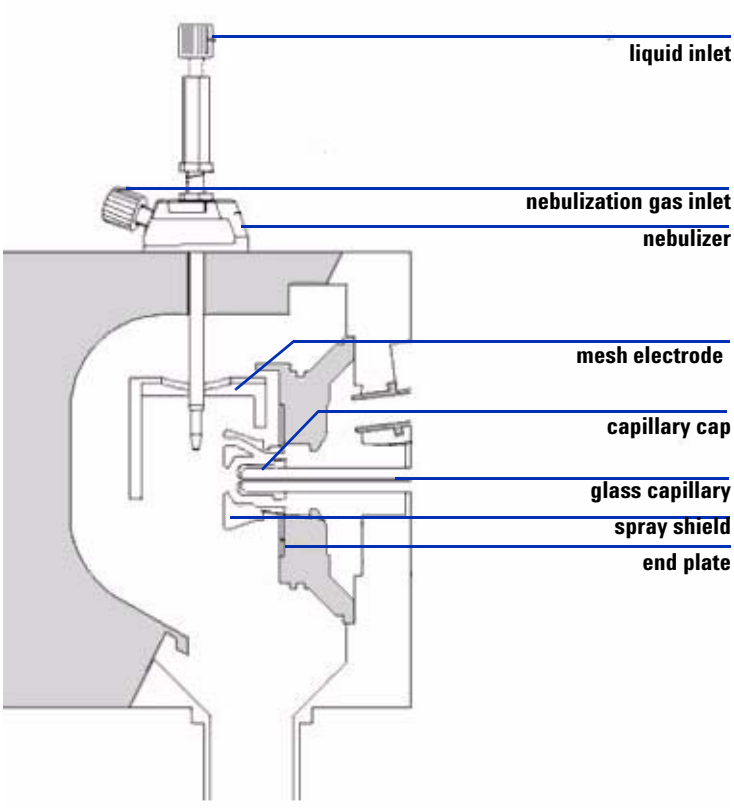


Figure 9 Electrospray chamber

Formation of ions

Ion formation in API-electrospray occurs through more than one mechanism. If the chemistry of analyte, solvents, and buffers is correct, ions can be generated in solution before nebulization. When possible, and done properly, this results in high analyte ion concentration and good API-electrospray sensitivity.

Preformed ions are not required for ESI. Analytes that do not ionize in solution can still be analyzed. The process of nebulization, desolvation, and ion evaporation creates a strong electrical charge on the surface of the spray droplets. This can induce ionization in analyte molecules at the surface of the droplets.

Nebulization

Nebulization (aerosol generation) takes the sample solution through these steps:

- **a** Sample solution enters the spray chamber through a grounded needle (*nebulizer*–see Figure 10).
- **b** For high flow electrospray, nebulizing gas enters the spray chamber concentrically through a tube that surrounds the needle.
- **c** The combination of strong shear forces generated by the nebulizing gas and the strong voltage (2–6 kV) at the *mesh electrode* and *end plate* in the spray chamber draws out the sample solution and breaks it into droplets.
- **d** As the droplets disperse, ions of one polarity preferentially migrate to the droplet surface due to electrostatic forces.
- **e** As a result, the sample is simultaneously charged and dispersed into a fine spray of charged droplets, hence the name *electrospray*.

Because the sample solution is not heated when the aerosol is created, ESI does not thermally decompose most analytes.

The droplets create noise signals if they are allowed to enter the vacuum system. Therefore, the nebulizer spray is aimed orthogonally to the vacuum entrance (glass capillary) to avoid this effect.

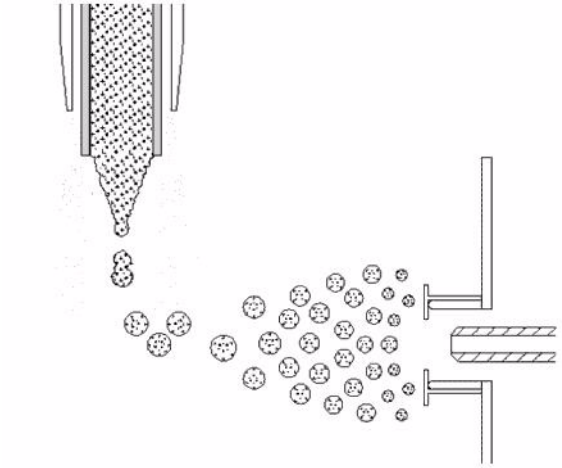


Figure 10 Droplet formation at the needle tip within the spray chamber

Desolvation

Before the ions can be mass analyzed, solvent must be removed to yield a bare ion.

A counter current of neutral, heated *drying gas*, typically nitrogen, evaporates the solvent, decreasing the droplet diameter and forcing the predominantly like surface charges closer together (see Figure 11).

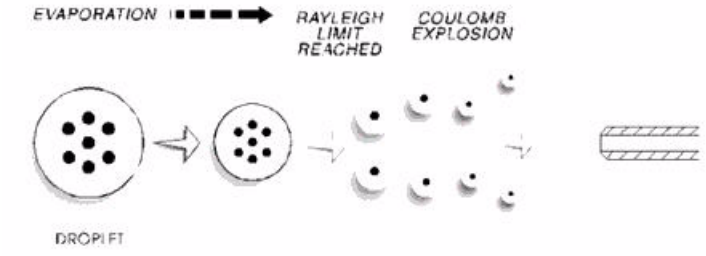


Figure 11 Coulomb explosions produce charged droplets within the spray chamber (• analyte)

When the force of the Coulomb repulsion equals that of the surface tension of the droplet, the droplet explodes, producing smaller charged droplets that are subject to further evaporation. This process repeats itself, and droplets with a high surface-charge density are formed. When charge density reaches approximately 10^8 V/cm^3, ion evaporation occurs (direct ejection of bare ions from the droplet surface).

The choice of solvents and buffers is a key to successful ionization with electrospray. Solvents like methanol that have lower heat capacity, surface tension, and dielectric constant, promote nebulization and desolvation.

Atmospheric Pressure Chemical Ionization (APCI)

APCI is a gas phase chemical ionization process. The APCI technique passes LC eluent through a nebulizing needle, creating a fine spray, which is passed through a heated ceramic tube, where the eluent droplets are fully vaporized (Figure 12).

The resulting gas/vapor mixture is then passed over a corona discharge needle, where the solvent vapor and carrier gas are ionized to create reagent gas ions. These ions in turn ionize the eluent sample molecules via a chemical ionization process. The sample ions are then introduced into a conductive capillary.

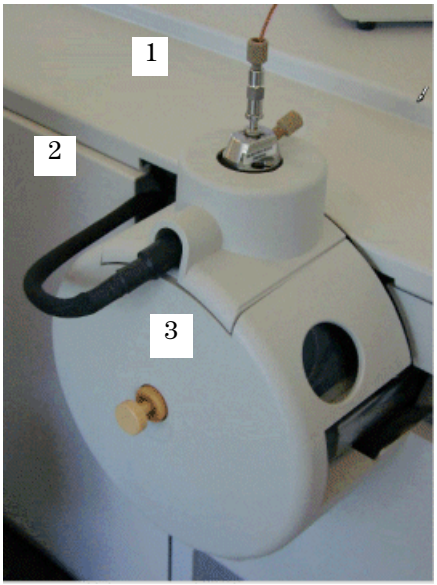


Figure 12 APCI source with APCI nebulizer (1), APCI heater cable (2), and corona needle (3)

APCI requires that the analyte must be in the gas phase for ionization to occur. To vaporize the solvent and analyte, the APCI source is typically operated at vaporizer temperatures of 400° to 500 °C.

Atmospheric Pressure Photo-Ionization (APPI)

The APPI technique involves passing LC eluent through a nebulizing needle to create a fine spray. This spray is passed through a heated ceramic tube where the eluent droplets are fully vaporized. The resulting gas/vapor mixture passes through the photon beam of a krypton lamp to ionize the eluent sample molecules (Figure 13). The sample ions pass through a conductive capillary and through a series of skimmers and lenses before being introduced into the mass spectrometer for analysis.

Thus APPI and APCI are similar, with APPI substituting a lamp for the corona needle for ionization. APPI often also uses an additional solvent or mobile phase modifier, called a “dopant”, to assist with the photo-ionization process.

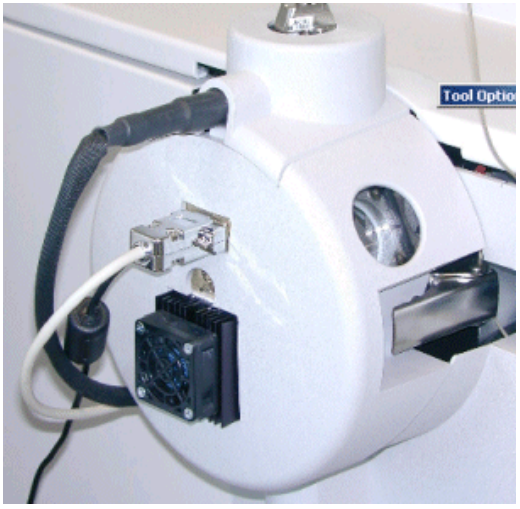


Figure 13 APPI source with lamp source instead of a corona needle

NanoElectrospray

Nanoelectrospray (Figure 14) is an atmospheric pressure electrospray ionization technique for very low flow rates of 100–500 nL/min. It is desirable to run this source with either a syringe pump at low flow rates or with a nanospray needle filled with a couple of microliters of sample solution.

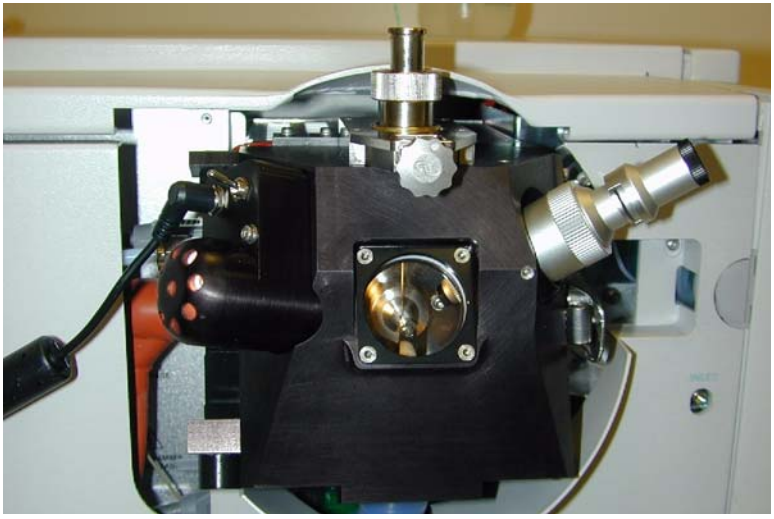


Figure 14 Nanoelectrospray ion source

Multimode Source

The multimode source is an ion source that can operate as an APCI, ESI or simultaneous APCI/ESI source. The source is intended to operate at normal chromatographic flow rates 50 to 2000 ìL /min. It is capable of fast reversing pos/neg switching. The multimode source can be run in three different modes of operation (MM-ES, MM-APCI or MM-ES+APCI).

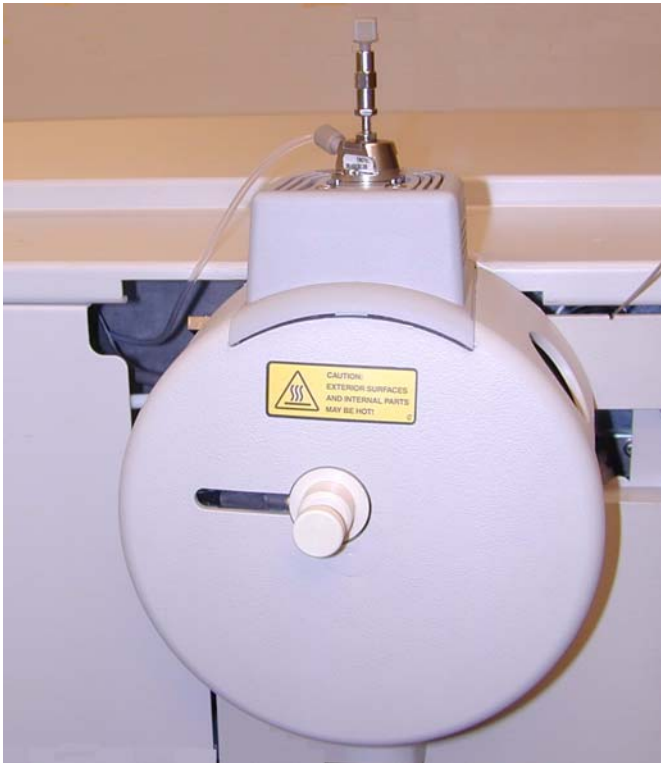


Figure 15 Multimode Source

G4240A Chip Cube

Miniaturization of ESI-MS: for ESI-MS, ionization efficiency improves at nanoflow rates and sample volume can be reduced without loss in MS signal to noise. ESI-MS behaves like a concentration sensitive detector, so decreasing the peak elution volume will increase the sensitivity.

Flow in HPLC

1000 mL/min in Standard HPLC

5 mL/min in mHPLC

0.3 mL/min in nanoflow HPLC

Figure 16 Chip Cube

Ion transport and focusing region

The ion transport and focusing region of the Ion Trap is enclosed in the vacuum manifold. Since the components of this region are not all visible when the source is assembled and installed, the diagrams in Figure 17 on page 22 and Figure 18 on page 23 may be helpful.

The ion transport and focusing region has four distinct vacuum stages established by the pumps of the Ion Trap system. They create a flow from atmospheric pressure into the mass spectrometer. In the vacuum stages, the pumps remove drying gas and solvent molecules.

The ions pass through the glass capillary into the first of the vacuum stages at the end of the capillary. The skimmer removes the bulk of the drying gas. The ions then pass into an octopole ion guide that focuses and transports the ions from a relatively high pressure position directly behind the skimmer to the focusing/exit lenses coupling the ion transport to the ion trap.

You control the voltages in the ion transport and focusing region with the data system.

Capillary

The capillary has two primary functions, namely it is:

- A sampling orifice that transfers ions from the spray chamber at atmospheric pressure to the vacuum region of the mass spectrometer.
- A barrier that separates the atmospheric pressure region of the source from the vacuum region of the mass spectrometer.

The metallized ends of the capillary carry potentials with the following functions:

- In the positive ion mode the entrance to the capillary is at –2 to –6 kV relative to the needle and approximately –500 V relative to the end cap. The electrostatic gradient created by these voltages supports the production of charged droplets and helps them to migrate towards the capillary entrance. Once near the entrance, the analyte ions are pushed through the capillary by the pressure gradient between the spray chamber and the first pumping stage.
- The exit end of the capillary (see Figure 17) is typically set in a range from 80–280 V through the instrument control program. As ions exit the capillary, they are electrostatically drawn towards the first skimmer, which is at a lower voltage. Because of the dimensions of the capillary and the pressure gradient, the ions emerge from the end of the capillary in a subsonic jet flow.

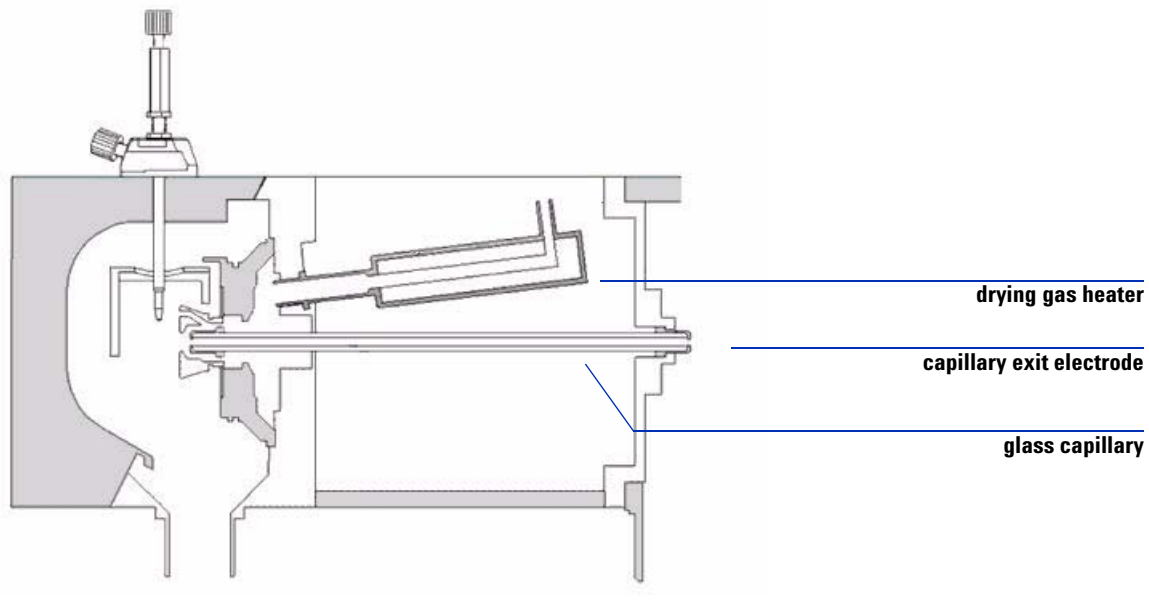


Figure 17 Electrospray source and sampling capillary diagram

Skimmer, octopoles, and lenses

The 6310 and 6320 models of the Ion Trap use one skimmer.

The skimmer(s), ion guide (octopoles), and lenses (see Figure 18) concentrate and focus the analyte ions. You can set voltages for the skimmer(s), octopole (dc and rf) and exit lenses in the TrapControl program. The voltages established for these ion-optical components determine the ion transport efficiency as well as the background level of spurious noise events.

When the ion trap is accumulating ions, the values of these components are set to maximize ion transmission. At all other times that the ion trap is being used for MS and MS(n) analysis, the voltages are set to block the ion transmission into the trap. If not blocked, ion transmission would result in unwanted background and other interference with the analysis.

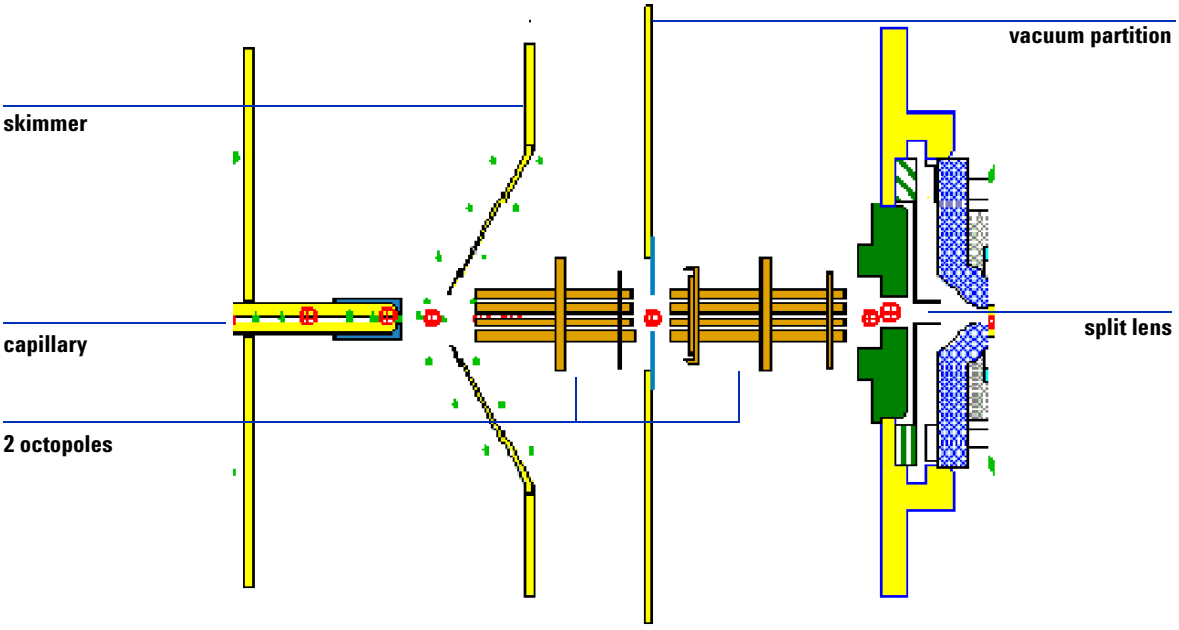


Figure 18 Detail of skimmer and ion guide sampling

Vacuum system

The function of the Ion Trap vacuum system (see Figure 19) is to evacuate regions of ion focusing and transport and keep the ion trap at low pressure. Most of the solvent is vented from the spray chamber and never reaches the inlet capillary, because of the orthogonal-flow sprayer design. Only ions, drying gas, and a small amount of solvent are transmitted through the capillary.

The Ion Trap has four vacuum stages controlled by a rough pump (18 m^3/h approximate 5 liters/s), a split-flow, drag-stage, turbomolecular pump (260 liters/s at the main inlet port), and a second turbomolecular pump (70 liters/s). Pressure gradients created by the first two vacuum stages assist in driving the transport of ions by collisions with the background gas being pumped by the system.

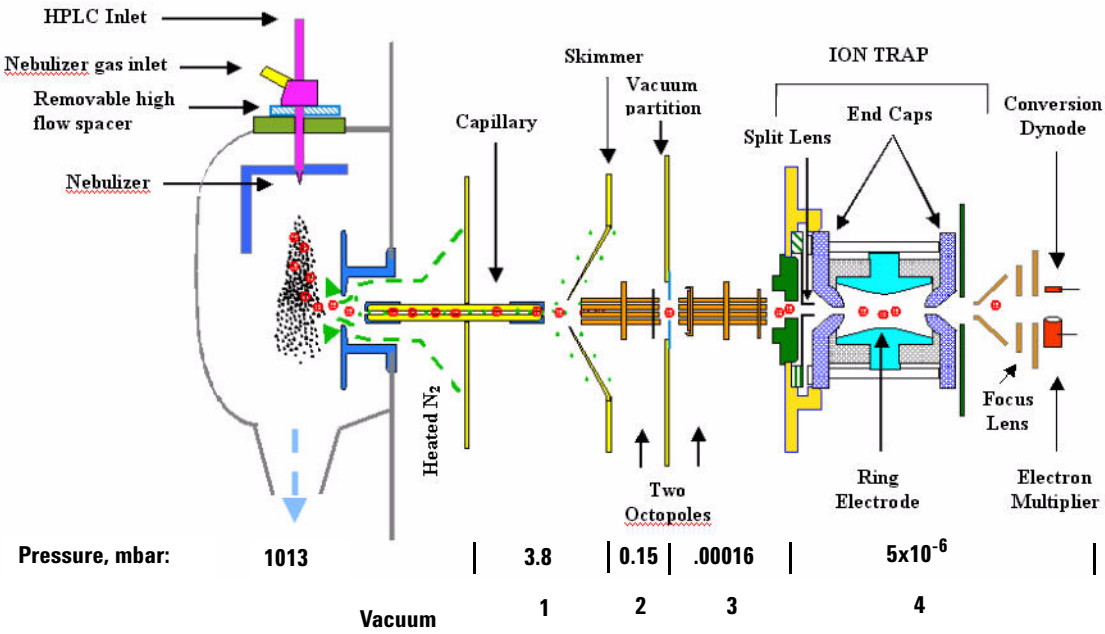


Figure 19 Vacuum system and pressures in API-electrospray LC/MS/MS system (6310 or 6320)

Ion trap

To learn more about how the Ion Trap works, see Chapter 2, "How the Agilent Ion Trap Works".

The ion trap is an ion storage and mass analysis device that consists of four main components:

- Ring electrode and end cap electrodes
- Helium
- Detector components
- Stage 4 of the vacuum system (Figure 19 on page 24)

Ring electrode and end caps

The end caps have small perforations in the center to allow the ions to enter and exit the ion trap. The end of the ion transport optics (ion guide, lenses 1 and 2) extend directly to the outside of the inlet end cap. After ions are accumulated in the ion trap mass analyzer, repelling voltages are established on some of the ion optical elements to prevent more accumulation of ions. This effect allows control of the trapped ions during a scan or MS(n) without letting more ions in to disrupt that control.

The surface of these three electrodes is manufactured to very high standards. The precise shape of the electrodes produces a non-linear field inside the ion trap. This field allows the ion trap to scan up to 26,000 mass/charge per second at unit resolution.

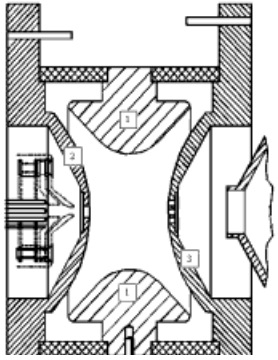


Figure 20 Ring electrode (1), end-cap electrode entrance (2), exit (3)

Helium

For efficient trapping and cooling of the ions generated by the electrospray interface, helium gas is introduced into the ion trap. This helium gas also serves as a collision gas during MS(n) operation. The helium pressure in the trap is set by a mechanical pressure regulator.

Detector components

The detector (Figure 21) used on the Ion Trap system is a conversion dynode/electron multiplier system that also includes a lens system to focus the ions toward the detector. This approach allows for highly sensitive detection of both positive and negative ions.

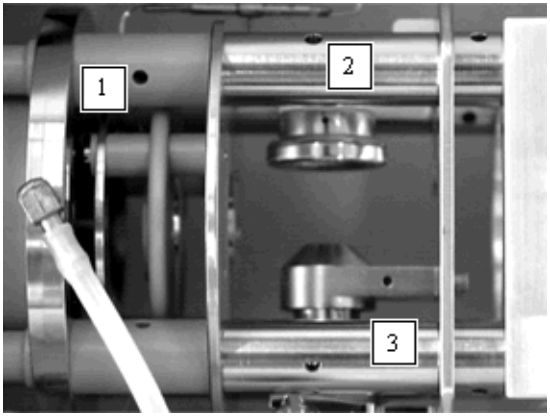


Figure 21 Ion lens (1), dynode (2), and electron multiplier (3)

2
How the Agilent Ion Trap Works

This chapter gives you a more extensive explanation of how the Agilent ion trap produces mass spectra.

How the Agilent ion trap works—an overview

The ion trap is an ion storage and mass analysis device that consists of three main components:

- Ring electrode and end cap electrodes
- Helium
- Detector components

Each of these components plays an important role in generating MS and MS/MS spectra. The Trap produces spectra through several processes:

1. Trapping and accumulating ions
   a. Trapping ions
   b. Preventing escape
   c. Accumulating ions
2. Analyzing ion masses (scanning)
   a. Keeping unwanted ions out
   b. Limiting the mass range of ejected ions
   c. Preparing to eject
   d. Ejecting ions in mass order
   e. Detecting ions
3. Generating MS/MS spectra
   a. Isolating precursor ions
   b. Fragmenting ions
   c. Repeating step 2

The trap also has the capability to switch rapidly between measuring positive ions and negative ions, described in the section “Detecting positive and negative ions" on page 44.

Trapping and accumulating ions

Trapping ions

The ion trap consists of a ring electrode between two end-cap electrodes (Figure 22). The internal surface shape of these three electrodes follows a three dimensional, nearly hyperbolic profile. Holes at the center of the end caps allow ions to pass in and out of the trap. A high voltage RF potential (Ω = 781 kHz) is applied to the ring, while the end caps are held at ground. The oscillating potential difference established between the ring and end cap electrodes forms a substantially *quadrupolar field*.

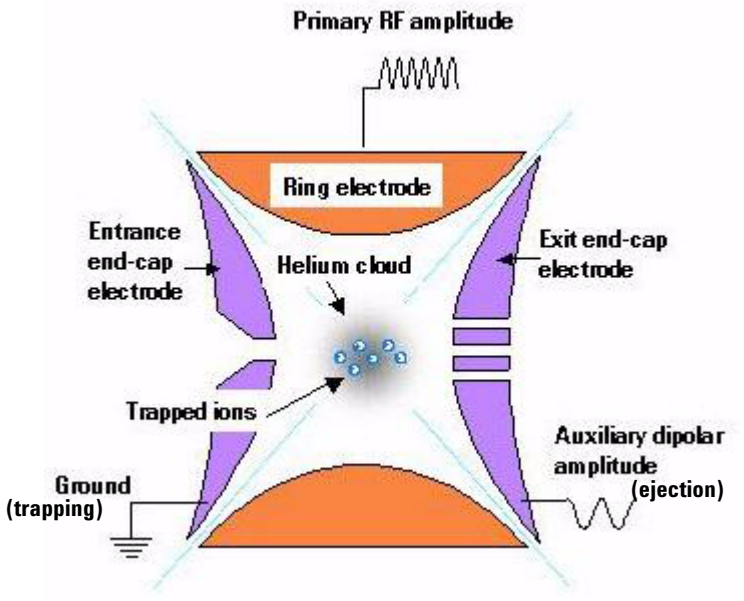


Figure 22 Ion trap geometry (cross-sectional view through the cylindrical axis)

Depending on the level of the RF voltage, the field can trap ions of a particular mass range. The quadrupolar field can be thought of as a three dimensional trough or a pseudo-potential well. The depth of this well is related to the mass of the ion and the level of the RF voltage. The quadrupolar field also induces an oscillatory harmonic motion in the ions. In practice, the range of masses that experience a trapping force from this quadrupolar field is wide enough that the ion trap can effectively produce full scan spectra at high sensitivity.

Preventing escape

Because the ion trap receives its ions from an external source, it must have a means to capture the ions in the pseudo-potential well. Without any barriers ions focused from an external source would simply pass through the first end cap, “roll down” into the pseudo-potential well created by the quadrupolar field, and continue to “roll up” and out through the other end cap. For this reason, as well as others, a *collision gas* (helium) must be present in the trap to extract energy from the ion beam and cause retention of at least a certain portion of the ions injected into the ion trap.

Additional parameters that affect trapping efficiency include the energy of the incoming ion beam, the *m/z* value and the mass of the ion, the depth of the pseudo-potential well and the actual phase of the RF voltage for any particular ion at the point of injection.

Accumulating ions

Because the ion trap is a storage device, it can accumulate very small currents of ions injected into the trap for an extended period of time, thereby improving the signal/noise dramatically. When the ion current is large, accumulation times may be as short as 10 μs but increase up to about 1 s for infusion experiments involving trace analytes. Typical accumulation times for LC/MS and LC/MS/MS experiments range from 0.01–200 ms. By varying the accumulation time, the dynamic range of the ion trap analyzer is greatly extended.

Avoiding too many trapped ions

Space charge limits are reached when too many ions are stored in the trap. As the pseudo-potential well of the ion trap begins to fill with ions, the harmonic motion of the ions begins to be affected. The resonance for ions of each specific m/z value is spread over a range of frequencies giving rise to broad peaks centered at a higher mass. For good mass accuracy and resolution the number of ions in the trap must be controlled.

During an LC analysis of a typical sample, the number of ions generated by the electrospray source varies widely. If the number of ions in the trap is not limited, the most intense chromatographic peaks exhibit spectra containing the space charge effects of poor resolution and incorrect mass assignment. The use of Ion Charge Control (ICC) prevents this problem. The accumulation time for the background signal or of low intensity peaks is established by the user at a maximum level, commensurate with the minimum sampling rate of the chromatographic signal.

As a compound of higher concentration begins to present itself to the ion trap, the resulting ion charge is measured and automatic adjustments to the accumulation time are made if the maximum allowable charge level is exceeded. Conversely, when the ion beam current begins to drop as the LC peak falls, then the length of the accumulation time is increased until the maximum time is reached.

Even for intense LC peaks, the accumulation time can be adjusted to prevent overloading the trap. The measured intensities using ICC are scaled by the accumulation time factor so that in the Total Ion Current (TIC) chromatogram and in the spectra the intensities shown are independent of the actual accumulation time.

Analyzing ion masses (scanning)

Keeping unwanted ions out

During the first part of the analysis cycle, ions are accumulated in the trap. During the mass analysis the continuous ion beam from the source must be prevented from passing through the trap, which would result in detector noise. Since this voltage creates a repelling field, the polarity is the same as the polarity of the ions.

The Ion Trap 6310 and 6320 instruments only have one skimmer. To keep ions out of the ion trap after accumulation an effect known as 'double-beam' gating is applied. The first stage of ion beam gating occurs between the capillary exit and the skimmer where a retarding voltage of 200 V (polarity opposite to that of the ions) is applied. On the skimmer itself a repelling voltage of 40V is applied with a polarity equal to that of the ions. The second stage of ion beam gating takes place between Lens 1 and Lens 2. A retarding voltage of 200 V is applied to Lens 1 while lens 2 is set to 0 volts.

Limiting the mass range of trapped ions

The major component of the oscillatory harmonic motion in the ions induced by the quadrupolar field is called the "*secular frequency*." The actual frequency of oscillation of the ions is principally determined by the *m/z* ratio of the ion and the RF drive level. A lower *m/z* value results in a higher secular frequency.

The range of ion masses that can be trapped simultaneously is described by the stability diagram. The stability diagram is a two dimensional plot that indicates under what particular potentials (both RF drive as well as any imposed DC potential between the ring and end caps) ions of a particular *m/z* value are stable or unstable in the field.

The Ion Trap works in the RF-only mode, on the line indicated in Figure 23. As the RF drive level is increased, then the corresponding point on the plot for a given mass is shifted to the right. Higher masses are found to the left of smaller masses, i.e. $m_2 > m_1$ in Figure 23. For any given point in the stability region, the ion experiences a different pseudo-potential well depth and hence a specific secular frequency. This situation is analogous to the change in the period of a pendulum oscillation, when the length is changed.

From the stability diagram, one observes an important consequence: the existence of a *cut-off mass*. The diagram shows that there is a boundary of stability along the line β_z=1 (β_z depends on the geometry of the ion trap; β_z is proportional to the RF voltage and inversely proportional to the mass of the ions). If an ion reaches the borders of the stability diagram (β_z= 0 or β_z=1), the trajectory of the ions becomes unstable and hence they leave the ion trap in the axial direction (β_z).

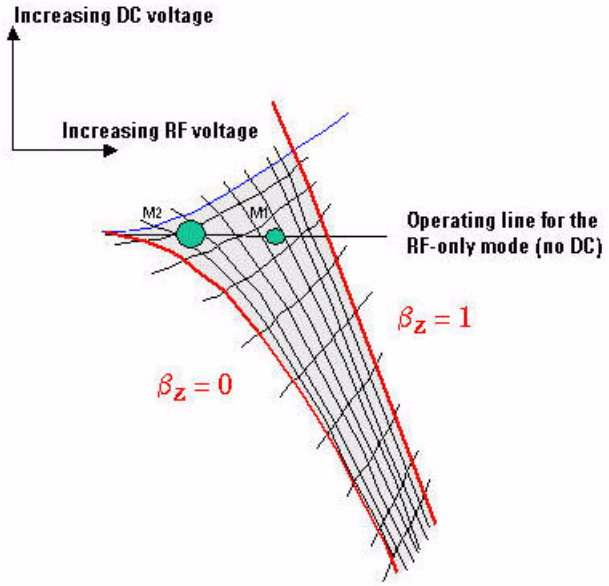


Figure 23 Mathieu stability diagram for ion trap

This means that there is always a lowest mass stable in the field, with all lower masses unstable in the field. The range of masses that can be trapped simultaneously thus has as a lower limit, the cut-off mass. The cut-off mass is determined by the RF level on the ring electrode, and it can also be found as the mass whose secular frequency is close to one half of the RF drive frequency ($\Omega/2 \sim 390.5$ kHz).

Theoretically, there is no upper limit to storable mass range. However, for practical purposes and thermal reasons, there is an upper limit as well. This upper limit is about 20–30 times the cut-off mass; ions with an m/z above this limit are not efficiently trapped by the RF field.

Preparing to eject

The Ion Trap makes use of a “multipole-superimposed” ion trap in which the mainly quadrupolar field has contributions from hexapolar, octopolar and even higher-order fields. The effect is created by a slight change to the angle of the asymptotes associated with the hyperbolic profile formed by the electrodes. A pure quadrupolar field has a linear increase in the field as the ion moves from the center of the trap towards the ring or the end caps.

The presence of the higher order poles in the design means that the field increases faster than linear away from the center. This induces certain non-linear resonances within the stability diagram that cause energy to be far more quickly taken up in the ion motion. These non-linear resonances occur if the secular frequency and the driving frequency of the ion trap have an integer relationship.

Application of an auxiliary dipole field across the end caps of the ion trap triggers the energy take-up of the ion motion (Figure 22). Since the ions are confined in the trap and experiencing periodic oscillatory motion, it is possible to couple additional energy into their motion, analogous to the pushing on a pendulum at its fundamental frequency. If the frequency of this dipolar field is the same as the secular frequency of the ions, then energy is taken up by the ions and the oscillatory motion increases in its displacement from the center of the trap.

When the resonance, between the secular frequency of the ions and the additional dipolar field, coincides with a non-linear resonance brought about by the higher order multipole contributions in the Ion Trap, the ions take up energy very quickly and leave the ion trap without becoming unstable in the field. This multiple resonance is unique to the ion trap design of the Ion Trap and allows superior combinations of scan speed and mass resolution.

The integer relationship between the secular frequency and the driving frequency ensures that in successive measurements identical field interactions are experienced between the ions and the trapping and dipolar fields. This is accomplished by phase locking the trapping and dipolar fields. Phase locking makes it possible to achieve reproducible ion excitation necessary for accurate mass assignment and precise MS/MS excitation. It is of particular significance that the additional resonance introduced by the higher order multipoles at Ω/3 satisfies this integer relationship (Figure 24).

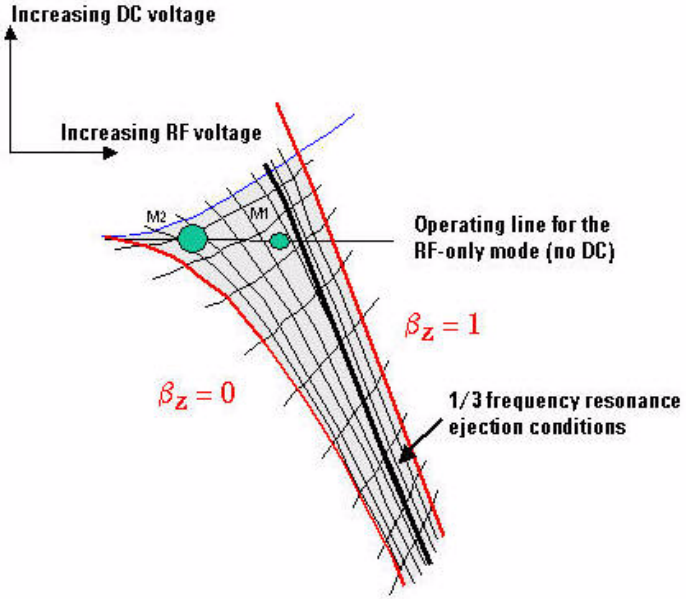


Figure 24 Mathieu Stability Diagram with Non-Linear Resonance (Ω/3)

Ejecting ions in mass order

The non-linear resonance conditions are used to generate the mass spectrum. The presence of a strong coupling between multiple charged ions and the stability which arises from phase-locking the driving and the auxiliary fields, results in precise m/z resonance and accelerated ion ejection.

When the amplitude of the RF voltage is progressively increased, ions with successively larger m/z values encounter the $\Omega/3$ point in the stability diagram. Each successive mass in turn takes up energy very quickly and is ejected from the trap.

The scan ramp generator controls the amplitude of the quadrupolar field and is digitally calibrated so that the ramp rate is as high as 1 mass unit in 38 microseconds (Ultra Scan), while still maintaining unit resolution. The frequency of this primary RF field is controlled by a frequency generator. The exit end cap opposite the detector is connected to the auxiliary RF generator which produces the dipole field. The auxiliary RF generator also has a programmed amplitude for optimized performance. The proper use of multipole field geometry results in preferential ejection of the ions through the exit end cap.

Detecting ions

The ion detector produces an electrical current recording the mass spectrum. From the ramp rate, the intensity versus time profile is converted into a mass spectrum. The conversion makes use of a calibration file to ensure good mass accuracy.

The detector, (see Figure 25) used on the Ion Trap system, is a conversion dynode/electron multiplier system that also includes a lens system to focus the ions toward the detector. This approach allows for highly sensitive detection of both positive and negative ions.

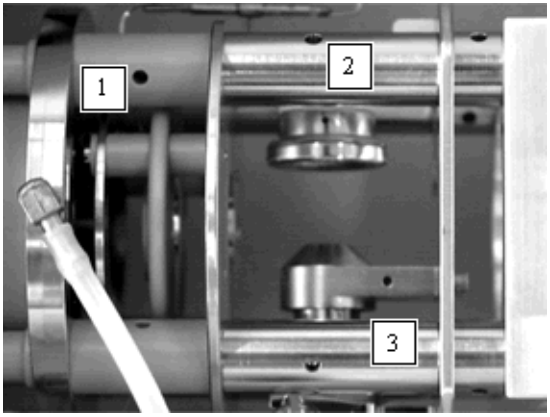


Figure 25 Ion lens (1), dynode (2), and electron multiplier(3)

Summary of an MS scan sequence

The separate events described above, of accumulation and mass analysis, when put in succession comprise the basic elements of a scan sequence. The following diagram displays the timing of

the primary and auxiliary voltages, as well as the blocking, or gating, voltage applied to keep more ions out of the trap after the accumulation time has ended.

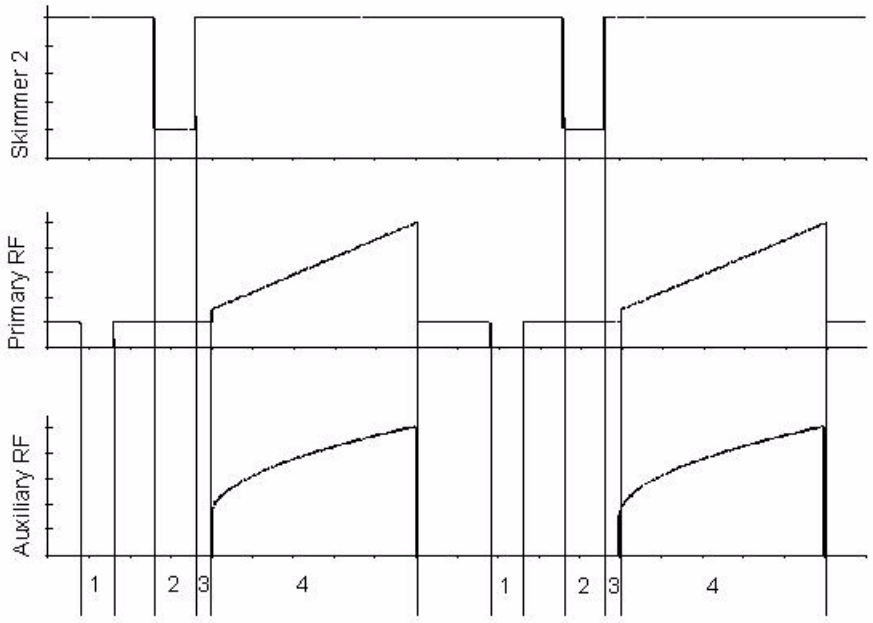


Figure 26 The important scan segments for an MS scan

1	Clear Trap	2	Accumulation Time
3	Scan Delay	4	Mass Analysis

This diagram represents the following steps:

1 Clear trap

- **a** The quadrupolar field is dropped to zero to remove any ions from the trap.
- **b** The trap is set to its initial conditions and skimmer 2 is set to block, or gate, ions from entering the mass analyzer.

2 Accumulation time

- **a** The trap is filled with ions from the ion source by dropping the repelling voltage on skimmer 2 to pass the ion beam.
- **b** Ions are trapped in the RF field using a low quadrupolar amplitude (referred to as the Trap-Drive mass in the Expert or Smart Tune page of the Trap Control program).

3 Scan delay

- **a** After a given accumulation time, the skimmer 2 voltage is increased to prevent the ions from entering the ion trap.

4 Mass analysis

- **a** The accumulated ions are “cooled” by collisions with the helium bath gas to ensure that the ion cloud is positioned in a small packet at the center of the trap.
- **b** During the scan the quadrupolar and dipolar fields are increased to progressively eject ions of ever-increasing m/z value out of the trap by passing through the exit end cap.

1 Clear trap

- **a** At the end of the scan, the quadrupolar field is dropped to zero to remove the remaining ions from the trap.
- **b** The cycle is repeated when the trap is set to its initial conditions and skimmer 2 remains set to block accumulation.

Isolating and fragmenting ions

To increase the amount of information about a compound, trap technology lets you isolate and induce dissociation of a specific ion to generate product ions. The trap ejects these product ions to produce an MS/MS spectrum (or MS(n) spectrum, if you tell the trap to continue the process, where “n-1” is the number of fragmentations), which helps you identify the compound.

Under normal conditions, before the trap excites a specific ion to induce dissociation, it removes all other ions so that only the precursor ion of interest is available for fragmentation.

Generating MS/MS spectra

Isolating precursor ions

The Ion Trap has the ability to directly eject all ions simultaneously except for the precursor ion of interest.

Because each particular mass has its own specific resonance, the trap must synthesize a wide-band composite of frequencies in order to excite and eject ions in a broad mass range. When the trap isolates a particular precursor ion, its electronics generates a broadband frequency spectrum with all resonating frequencies present, except the frequency that corresponds to the resonance of the precursor ion.

Fragmenting the ions (MS/MS)

To induce fragmentation, the energy of the ion of interest is increased by resonance excitation with the dipole field. The resonance excitation waveform, actually comprises a small frequency band above and below the precise resonance frequency. This increases the stability of the excitation and compensates for the frequency shift caused by a change in the amplitude of the ion motion,G because of the non-linear field components. The amplitude of the excitation is less than that used for ejection and is typically about 1 volt.

The resonating precursor ions quickly take up energy from the dipolar field and begin to collide with the helium background gas. The collisions cause the ions to dissociate, producing predictable and reproducible mass spectra. You can vary the time associated with MS/MS excitation and collision-induced dissociation, but the time is 40 ms by default.

Upon fragmentation of the precursor ion the product ions need to experience a trapping potential from the quadrupolar field. The primary RF drive level determines this potential during the fragmentation. If the primary RF drive level is too high, then the trap ejects low mass product ions. If the RF drive level is too low, then the pseudo-potential well will be insufficient to allow for effective MS/MS excitation. Typically, the low mass cutoff is set at slightly less than one-third the precursor m/z value. (Default setting under Cut-Off Selection in the Fragmentation options of the MS(n) tab-view of the Trap Control program.)

Ion trap versus quadrupole MS/MS

There is one major difference between the operation of MS/MS excitation on an ion trap and on a quadrupole instrument. In quadrupole MS/MS, ions are excited by accelerating them and passing them through a high pressure collision cell. Because not all momentum is necessarily lost in the first collision, subsequent collisions with the background gas can result in further MS/MS product ions. The fragmentation is not mass-selective.

In ion trap MS/MS, only the precursor ions collide with the background gas, and upon fragmentation little energy remains in the product ions that would result in subsequent fragmentation. Because the product ions are not resonated by the dipolar frequency, they do not continue to be excited. The result is that MS/MS spectra in quadrupole instruments may contain product ions that are the result of two or more stages of MS/MS excitation and fragmentation. The MS/MS spectra from ion trap instruments are generally the result of a single stage of excitation.

Optimizing MS/MS spectra

You can optimize MS/MS experiments by changing these variables:

- Width of the isolation window (Ion Trap can perform mono-isotopic isolation in the mass range from 50–2200 *m/z*)
- Depth of the pseudo-potential well during the fragmentation (referred to in the program as the cutoff)
- Fragmentation amplitude and time.

Summary of an MS/MS scan sequence

The scan cycle for MS/MS differs from that shown for just MS (Figure 26). Additional segments are used for isolation and fragmentation (Figure 27).

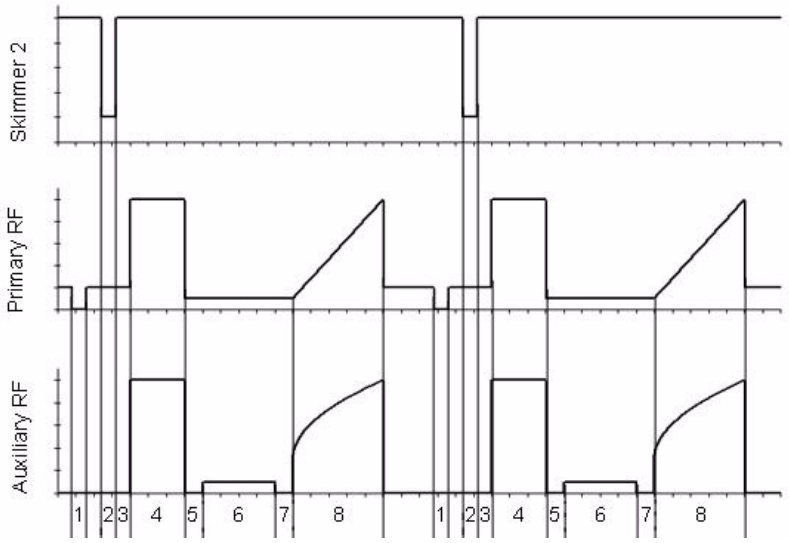


Figure 27 The important scan segments for an MS/MS scan

1	Clear Trap	2	Accumulation Time	3	Isolation Delay
4	Precursor Ion Isolation	5	Fragmentation Delay	6	Fragmentation
7	Scan Delay	8	Mass Analysis		

With the trap full of product ions, you can ramp the RF drive level, as described previously, to produce a full mass spectrum, or you can initiate an additional stage of MS/MS isolation and fragmentation.

Generating (MS(n)) spectra

Instead of mass analyzing the fragments directly, upon your instruction the trap can insert subsequent steps into the scan sequence to further isolate and fragment the initially-produced fragment ions. This isolation and fragmentation process concludes with a mass analysis and can be repeated several times. This is referred to as MS(n) capability. Because of the high efficiency associated with the quadrupolar trapping field, a very high percentage of the ions are conserved between each stage of MS(n). This important property of ion trap MS/MS makes MS(n) very practical. Ion trap MS(n) is a powerful tool for elucidating structure when coupled with “purer” MS/MS fragmentation.

Detecting positive and negative ions

By reversing the potentials in the electrospray (or APCI) interface, negative ions are generated. Reversing the potentials on the capillary, skimmers, the offset to the octopole, and other focusing elements allows for the introduction of these negative ions into the ion trap. Because the ion trap is an RF device, it treats both positive and negative ions alike. For positive as well as negative ions, the same calibrations can be used. A conversion dynode detector allows for the detection of negative ions.

Fast polarity switching

Some applications require that positive and negative ions must be recorded simultaneously by alternating polarity between scans. To this end, the Ion Trap has fast-switching power supplies for the API source, the lens system, and the detector. The Trap, an RF-only device, treats positive and negative ions alike. A polarity switchable conversion dynode allows for detection of both positive and negative ions while the voltage on the electron multiplier is kept at a constant negative value.

3
How Ion Trap Methods Work

This chapter gives you an understanding of how LC/MS methods are organized and how data processing works with the method structure in the TrapControl program. Then, you can easily develop LC/MS methods and set up to process data, either with or without your intervention.

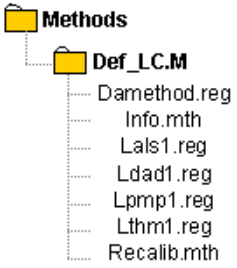
LC/MS method setup

How LC/MS methods are organized

Types of methods

The TrapControl program lets you create and modify five types of methods:

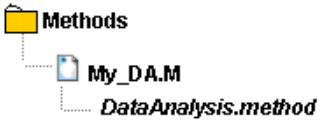
LC only method Contains only an LC method part, which consists of LC acquisition parameters that are entered and saved in the ChemStation program.



MS only method Contains a Trap Control method part, which consists of Ion Trap acquisition parameters that are entered and saved in the TrapControl program (EsquireAcquisition.method). This method may also contain a DataAnalysis (DA) method part.

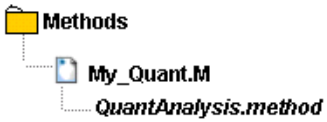
Data analysis method

Contains only a DataAnalysis method part, which consists of data analysis parameters and a VB script to automate processing tasks. DA parameters and VB scripts are entered and saved from the DataAnalysis program (DataAnalysis.method).



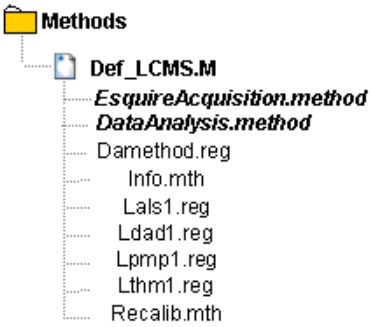
Quantitation method

Contains only a QuantAnalysis (QA) method part, which consists of calibration and quantitation parameters, which are entered and saved in the QuantAnalysis program (QuantAnalysis.method).



LC/MS method

Must contain at least the LC and Trap Control method parts and may also contain the DataAnalysis and QuantAnalysis method parts.



CAUTION **Do not copy components of any method folder to another method folder. The resulting method will not work.**

How to tell methods apart

All five methods include a .m extension. If you see methods in the Methods directory with other extensions, such as .M or .ms, these methods were created with older versions of the program.

You can distinguish between the methods when you attempt to load, open or save a method.

When you select a method, the parts contained within the method appear in the box at the bottom of the dialog box.

Also, the default Filter is set so that only the methods containing the parts associated with the selected menu item appear initially. For example:

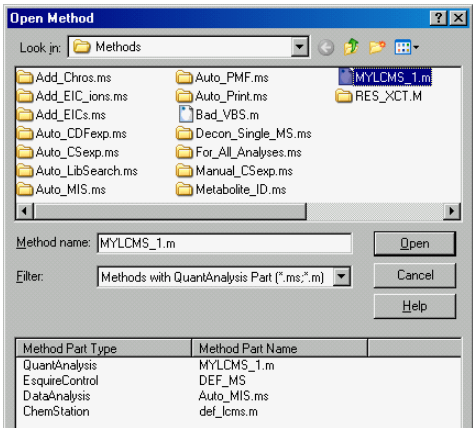
Load or Save Method As...in ChemStation – only LC and LC/MS methods appear

Load or Save As - 6300 Series TrapControl Part – only methods with MS method parts appear initially

Open or Save Method As... in DataAnalysis – only methods with DA method parts appear initially

Open or Save As... in QuantAnalysis – only methods with QA method parts appear initially

You can show all the methods in any dialog box, except for ChemStation Load or Save Method, if you set the Filter to “All”.



LC/MS method information transferred to a data file

The LC/MS method used to acquire a data file is always stored with the data file and can be retrieved from the data file, if necessary. If an LC/MS method used to acquire the data file contained a DataAnalysis method part, it is also stored with the data file.

For more information on processing data with a DataAnalysis method part inside the LC/MS method, see "Data acquisition with or without automatic processing" on page 65 and "Interactive data processing" on page 67.

If you open a raw data file containing a stored DA method part in the DataAnalysis program, the contained DA parameters and VB script automatically load and are available for processing. If you open a raw data file acquired with an LC/MS method that does not contain a DA method part, a default set of DA parameters and an empty script is added to the analysis.

If, before acquisition, you activate the VB script associated with the stored DA method part within an LC/MS method, the raw data is automatically processed after the acquisition using the contained VB script and DA parameters.

A QuantAnalysis method part within an LC/MS method is not stored with the data file. You can load the QA method part from the LC/MS method, however, and process data from the entire batch with the LC/MS method.

Creation of LC/MS methods with method parts

To find the definitions for all the menu items in the ChemStation and TrapControl program Method menus, see "Method menu items in ChemStation and the TrapControl program" on page 54.

You load all LC/MS methods, as well as LC methods, only in ChemStation. You edit an LC method only in ChemStation, and you edit the MS part of an LC/MS method only in the TrapControl program, although you can access this software from ChemStation.

Summary of ways to remove, add and edit method parts

The table below summarizes all the ways that you can remove, add, edit, combine and replace method parts within an LC/MS method in the order of importance to your work.

Table 1 LC/MS method creation/editing

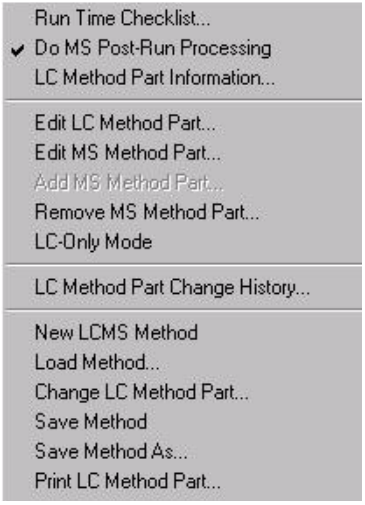
If you want to do this:	Then do this:	Notes
Edit the LC and MS acquisition parameters in an LC/MS method.	**a** From ChemStation, select **Method > Load Method...**. **b** Edit LC parameters directly or select **Method > Edit LC Method Part...**, which guides you through the LC method wizard. **c** Select **Method > Edit MS Method Part...**, which switches you to the Trap Control program. **d** From the TrapControl program, make changes to the MS acquisition parameters, and click **Apply**. **e** From ChemStation, select **File > Save Method** or **File > Save Method As...**.	• Although you can save changes to the Trap Control method part of an LC/MS method from the TrapControl program, always save these changes to an LC/MS method from ChemStation. 

Table 1 LC/MS method creation/editing

If you want to do this:	Then do this:	Notes
Create a new LC/MS method	**a** From ChemStation, select **New LCMS Method**. **b** Repeat steps b-d above. **c** From ChemStation, select **File > Save Method As...**.	• The read-only method Def_LCMS.M is loaded. Save changes under a new name or the name of an existing method.
Deactivate MS method part to run an LC/MS method in LC-only mode.	**a** From ChemStation, select **Method > LC-Only Mode**. **b** Select **Run Control > Run Method** or **Run Sequence**.	• You do not need to remove an MS method part to run in LC-only mode. • The LC-only mode flag is not stored as part of the LC/MS method.
Change the entire MS method part of an LC/MS method from ChemStation.	**a** From ChemStation, select **Method > Remove MS Method Part**. **b** Select **Method > Add MS Method Part**. **c** Select a method with the desired MS method part, and click **Add**. **d** Select **File > Save Method**.	• You cannot add an MS Method part unless you remove one from the LC/MS method or you are working with an LC only method. • Removing and adding an MS method part happens after you confirm that this is what you want to do.
Change the entire LC method part of an LC/MS method.	**a** From ChemStation, select **Method > Change LC Method Part...**. **b** Select a method with the desired LC method part, and click **Change**. **c** Select **File > Save Method**.	• Loads the entire LC method part of another LC/MS or LC-only method to the "active" LC/MS method in ChemStation without changing the "active" LC/MS method name.
Add an MS method part to an LC-only method to create an LC/MS method.	**a** From ChemStation, select **Method > Add MS Method Part...**. **b** Select **File > Save Method**.	• Adding an MS method part happens after you confirm that this is what you want to do.

Table 1 LC/MS method creation/editing

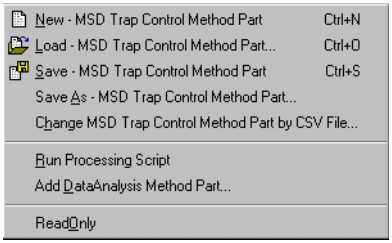
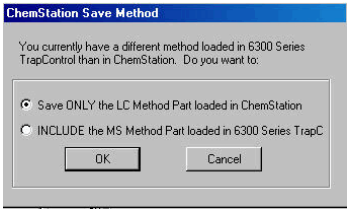
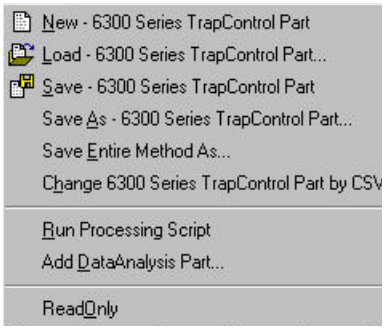
If you want to do this:	Then do this:	Notes
Change the entire MS method part of an LC/MS method from the TrapControl program.	**a** From the TrapControl program, select **Method > Load 6300 Series TrapControl Part**.  **b** Select an MS-only or other LC/MS method that contains the desired MS method part, and click **Open**. **c** From ChemStation, select **Method > Save Method** or **Method > Save Method As...**.  **d** When the ChemStation Save Method (As...) dialog box appears, select to **INCLUDE the MS Method Part loaded in 6300 Series TrapControl**.	• At this point, different methods are "active" in the TrapControl program and ChemStation. • If you select "Save ONLY the LC Method Part loaded in ChemStation" instead, only changes in the LC method part of the method "active" in ChemStation are saved.
Load a CSV file to change the MS Ion Trap method part.	**a** From Trap Control, select **Method > Change 6300 Series TrapControl Method Part by CSV File**. **b** Select the CSV file, and click **OK**.	• The MS parameters in the CSV file are loaded into the "active" Trap Control method part.

Table 1 LC/MS method creation/editing

If you want to do this:	Then do this:	Notes
Save the Trap Control method part directly into a new or existing LC/MS method. (possible only if no ChemStation is started, e.g., when the system is running in AP-Maldi mode)	• Change MS parameters, and select **Method > Save Entire Method As...**. 	• This selection appears only if no LC is present.
Add new DataAnalysis method part to MS method part or to LC/MS method.	**a** From the TrapControl program, select **Method > Add DataAnalysis Method Part...**. **b** Select a DataAnalysis method part that contains the desired DataAnalysis parameters and VB script, and click **OK**.	• If the "active" method in the TrapControl program is the same as the LC/MS method active in ChemStation, the DataAnalysis part is saved to the LC/MS method. • If the "active" Trap Control method part is different from the LC/MS method active in ChemStation, the DataAnalysis method part is saved to the method active in the TrapControl program.
Activate VB script in LC/MS method so that the software automatically processes the raw data file after acquisition.	**a** From the TrapControl program, select **Method > Run Processing Script**. **b** Select **Method > Save - 6300 Series TrapControl Part**.	• The "Run Processing Script" flag is saved as part of the LC/MS or MS-only method.
Remove the ReadOnly restriction from a method file.	• From the TrapControl program, deselect the **ReadOnly** flag (no check mark).	• Only users with Administrator privileges can remove the ReadOnly restriction from a method file. • For the DEF_MS.M and Def_LCMS.M methods, the ReadOnly flag can never be removed (greyed out).

Method menu items in ChemStation and the TrapControl program

This section describes each menu item used to work with LC and MS method parts to create or edit LC/MS methods.

ChemStation Method menu items You can work with both LC and MS method parts to create or edit LC/MS methods from ChemStation.

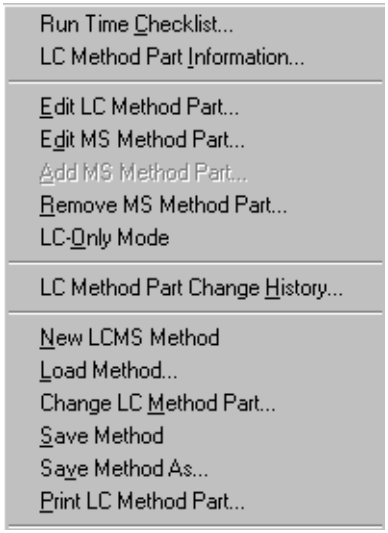


Figure 28 Method menu for LC/MS method loaded in ChemStation

Do MS Post-Run Processing Set this flag to trigger automatic post-acquisition execution of a VB Script, which can be saved in a DataAnalysis method part with an LC/MS method. You can toggle the flag On (indicated by a check mark) or Off and save the setting with the method. You can add a DA method part with a VB Script using the **Add DataAnalysis Method Part** function described below.

LC Method Part Information Enter information that describes the LC method part.

Edit LC Method Part Guides you through editing the different LC method part sections using a Wizard

Edit MS Method Part

Menu item is only active if the loaded method is an LC/MS method (Figure 28).

- Switches you to the TrapControl program window.
- If the LC/MS method loaded in ChemStation is not active in the TrapControl program, its associated MS method part is loaded.

Add MS Method Part

Copies the MS method part of another method (MS or LC/MS) to the currently loaded LC-only method to create an LC/MS method.

- The MS method part of the newly created LC/MS method will be loaded (set 'active') in the TrapControl program.
- If the selected MS method part contains a DA and/or a QA method part, those parts are also copied into the currently loaded LC/MS method.

Remove MS Method Part...

Menu item is only active if the loaded method is an LC/MS method. Removes the MS Method part and, if present, the DA and QA Method parts from the currently loaded LC/MS method.

LC-Only Mode

Runs an LC/MS method as an LC-only method, only executing the LC method part. It is not necessary to remove an MS method part in order to run in LC-only mode.

The LC-only flag is not a method parameter and is not stored with method. This flag does not appear for LC-only methods (Figure 29).

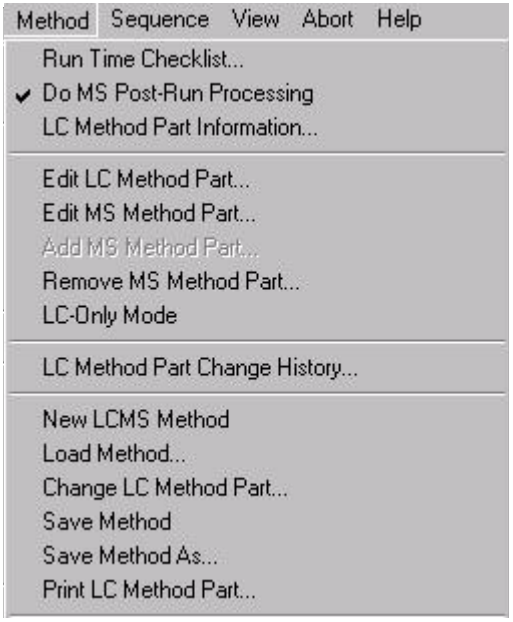


Figure 29 ChemStation Method menu for LC-only method

New LCMS Method Loads the default LC/MS method 'Def_LCMS.M' in ChemStation. The associated MS method part is loaded (set 'active') in the TrapControl program.

The Def_LCMS.M is permanently set to read-only. If you apply changes, they need to be saved using the 'Save Method As...' command, under a new name or an existing 'non read-only' method.

Load Method Loads the LC method part of the selected method, and if the selected method is an LC/MS method, the MS method part is loaded (set 'active') in the TrapControl program.

If changes have been applied to the currently loaded method, you are asked if you want to save those changes before loading a new method.

Change LC Method Part... Lets you exchange the entire LC method part with the LC method part of another method. The 'active' method in ChemStation stays the same.

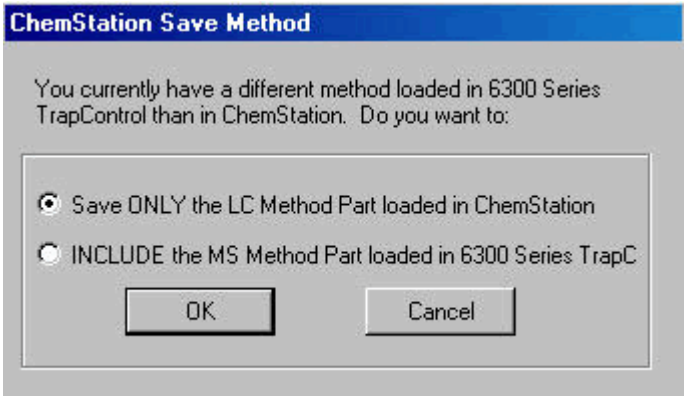
Save Method – CASE 1: **Same active LC/MS method in the TrapControl program and ChemStation**

Changes applied to the LC and MS method parts of the currently loaded method are saved to disk.

Save Method – CASE 2: **Different active method in the TrapControl program and ChemStation**

This case is more complex:

- Changes applied to the LC method part of the currently loaded method are saved to disk.
- If the method currently loaded in ChemStation is an LC/MS method, you are presented the following choice:



Case 2 occurs under two conditions:

- If you load an LC/MS method from ChemStation first, and then an MS-only method, or another LC/MS method from the TrapControl program.
- If you load an LC-only method in ChemStation.

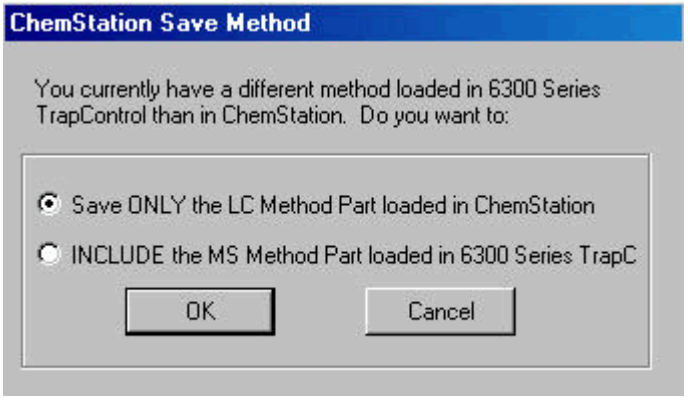
Save Method As... – CASE 1: **Same active LC/MS method in the TrapControl program and ChemStation**

Changes applied to the LC and MS method parts of the currently loaded method are saved to disk into the selected new or existing method.

Save Method As.... - CASE 2: **Different active method in the TrapControl program and ChemStation**

Again, this situation is less simple.

- Changes applied to the LC method part of the currently loaded method are saved to disk into the selected new or existing method.
- If the method currently loaded in ChemStation is LC-only, and the selected existing method is an LC/MS method, the MS method part and any existing DA method part or QA method part are removed. The newly saved method will be an LC-only method as well.
- If the method currently loaded in ChemStation is an LC/MS method, you will be presented the following choice:



Case 2 occurs under the same two conditions as Case 2 for the Save Method selection.

TrapControl program Method menu items You can work with MS method parts and DataAnalysis method parts to create MS-only methods or edit the MS method part of LC/MS methods from the TrapControl program.

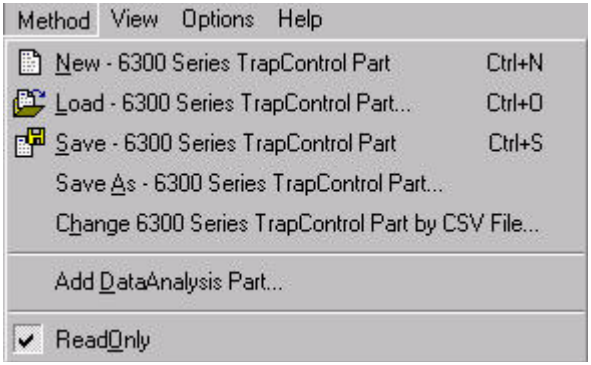


Figure 30 Method menu in the TrapControl program with ChemStation (LC/MS system)

New - 6300 Series TrapControl Part

Loads the default MS-only method 'Def_MS.M'.

- The Def_MS.M is purposely set to read-only. If you apply changes, they need to be saved using the 'Save As – 6300 Series TrapControl Part' command, under a new name or an existing 'non read-only' method.
- If you previously loaded an LC/MS method from ChemStation, you now have two different methods 'active' in the TrapControl program and ChemStation. (See "Save Method – CASE 2:" on page 57.)

Load - 6300 Series TrapControl Part

Loads the Trap Control method part from an MS-only or LC/MS method.

- By default the 'Filter' in the 'Load 6300 Series TrapControl Part' dialog is set to and display only 'Methods with 6300 Series TrapControl Part'.
- If you previously loaded an LC/MS method from ChemStation, and you select to load the Trap Control method part from a different method, you will now have two different methods active in the TrapControl program and ChemStation. (See "Save Method – CASE 2:" on page 57.)

Save - 6300 Series TrapControl Part

Changes applied to the MS method part of the MS-only or LC/MS method currently 'active' in the TrapControl program are saved to disk.

Save As - 6300 Series TrapControl Part

The Trap Control method part of the method currently 'active' in the TrapControl program, including changes you might have applied, are saved to disk into the selected new or existing method. The selected new or existing method is set 'active' in the TrapControl program, but not in ChemStation. (See "Save Method As.... - CASE 2:" on page 58.)

- No 'Filter' is in place. You can select any method type.
- If you select a new method name, the new method will be MS-only. If the currently loaded method in the TrapControl program does contain a DA or QA method part, those parts are not copied into the selected new method.
- If you select an existing LC-only method, the Trap Control method part is added into the method, to create an LC/MS method.
- If you select an existing LC/MS method, only the Trap Control method part is overwritten.

Change 6300 Series TrapControl Part by CSV...

Overwrites one or several MS method parameters in the currently loaded method from a specially formatted CSV file. Examples for those CSV files are provided on the Trap Control Installation CD. (See *Quick Start Guide*.)

This feature is not commonly used in normal operation of the instrument. It is intended that software developers use this function to customize future applications.

Save Entire Method As...

This menu item is only present if no ChemStation software is present, e.g. when doing infusion experiments after launching the TrapControl program only, or working in AP-MALDI mode.

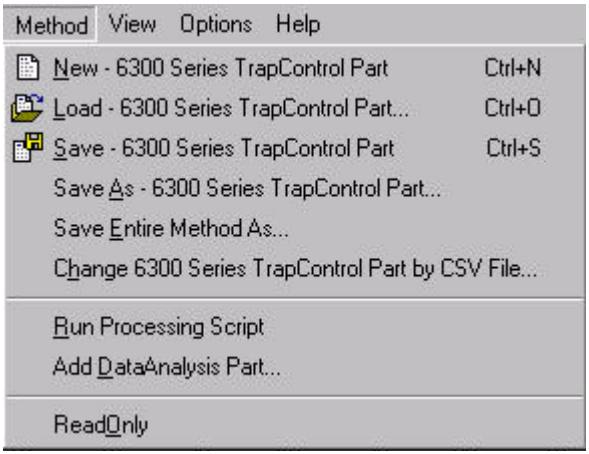


Figure 31 Method menu in the TrapControl program without ChemStation
(e.g. AP-MALDI)

- The Trap Control method part of the method currently 'active' in the TrapControl program, including changes you might have applied, is saved to disk into the selected new or existing method.
- If the currently loaded method also contains a DA or QA method part, those parts are also saved (copied) into the selected new or existing method.

Run Processing Script

Set this flag to trigger automatic post-acquisition execution of a VB Script, which can be saved in a DataAnalysis method part with an MS-only method.

You can toggle the flag On (indicated by a check mark) or Off and save the setting with the method. You can add a DA method part with a VB Script using the **Add DataAnalysis Method Part** function described below.

This option is only available when the TrapControl program is running as a stand-alone application (without ChemStation running).

Add DataAnalysis Method Part

Lets you select a DA method part, consisting of DA parameters and a VB Script, to an LC/MS- or MS-only method.

Initially, the ‘Filter’ is set to show only ‘Methods with DataAnalysis Method Part’. If the selected method also contains a QuantAnalysis method part, it will not be added to the currently loaded method. Once a DA method part has been added, you can initiate automatic post-acquisition execution of the VB Script by setting the ‘Run Processing Script’ flag.

ReadOnly A flag that can be set to designate a method as non-editable. In non-compliant mode, every user can set or unset this ReadOnly flag on all methods except the default MS method Def_MS.M and the default LCMS method Def_LCMS.M.

In compliant mode (requires the add-on Ion Trap Security 1.0 Pack), only Administrators are permitted to change the ReadOnly flags of methods.

CAUTION **DO NOT set the read-only file attribute of any file in a method directory. Doing so will cause irreversible errors during method editing.**

LC/MS data processing setup

All data processing takes place within the DataAnalysis software. You can process different types of chromatographic data stored in the raw data file:

- 3D data set from mass spectra acquired over time
- Single profile mass spectra
- 2D chromatograms from different channels in a variable wavelength detector or diode array detector (DAD)
- 2D chromatograms from a 3D data set of UV spectra acquired over time

You can access UV spectra and process LC/UV data files that do not contain MS data only from ChemStation UV Data Analysis.

All processing tasks use a set of DataAnalysis parameters. In addition, you can automate a sequence of processing tasks through an optional VB script. Both the DataAnalysis parameters and the VB script are ultimately stored with the data file when processed results are saved. The combination of DataAnalysis parameters and a VB script makes up the DataAnalysis (DA) method part.

Addition of a DataAnalysis method part to an LC/MS method

Adding a custom DA method part to the LC/MS or MS-only method used to acquire data files has two advantages for data processing.

- Because a copy of the DataAnalysis parameters and a VB script (not activated) is stored with the data file, the proper parameters and script are already available for you to start processing the data file in DataAnalysis.
- If you have activated the VB script in the LC/MS method before acquisition, you can automate the entire sample analysis so that processing occurs immediately after acquisition without your interaction.

You can add a DA method part to an LC/MS method in one of two ways:

- Save the DA parameters and VB script loaded in DataAnalysis to the LC/MS method (Figure 32).

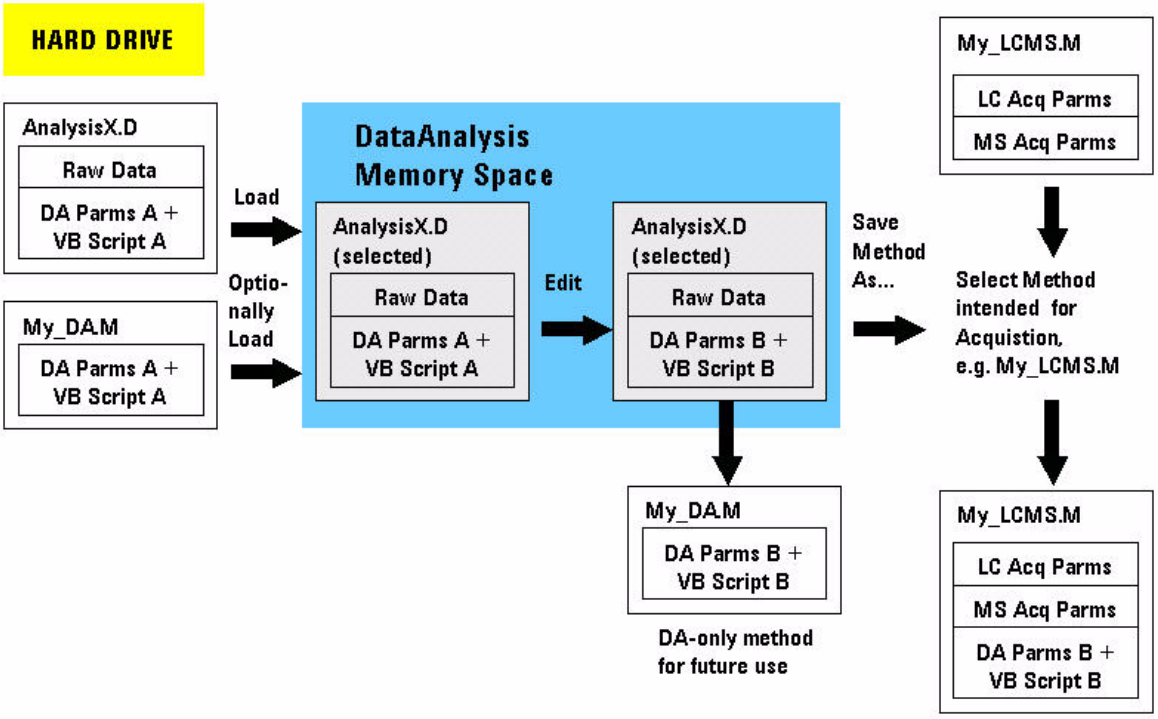


Figure 32 Add DA method part to LC/MS method through DataAnalysis

A DA method part can be loaded into DataAnalysis from any method that contains a DA method part. This method could be an LC/MS or MS-only method or typically a DataAnalysis-only method, which is used as a repository for a custom set of DataAnalysis parameters and a VB Script.

- Add a DA method part in the TrapControl program (Figure 33)

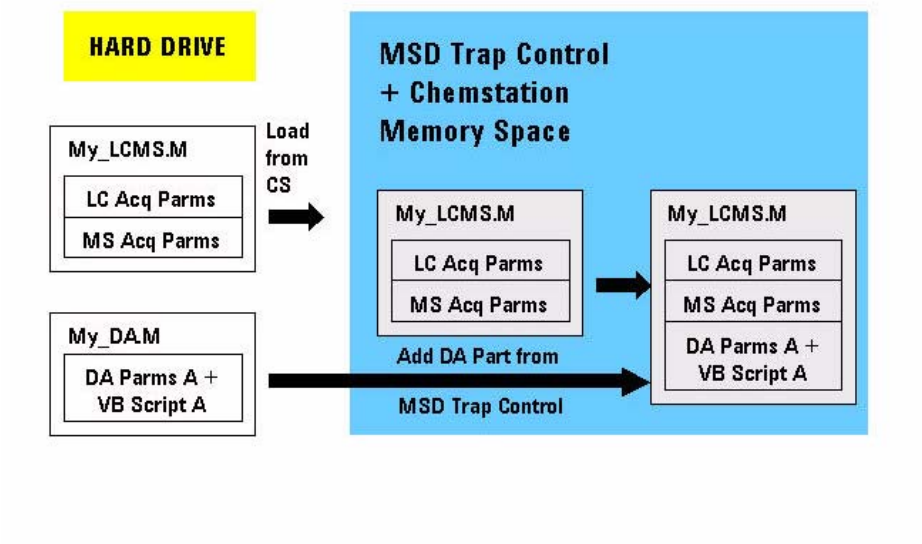


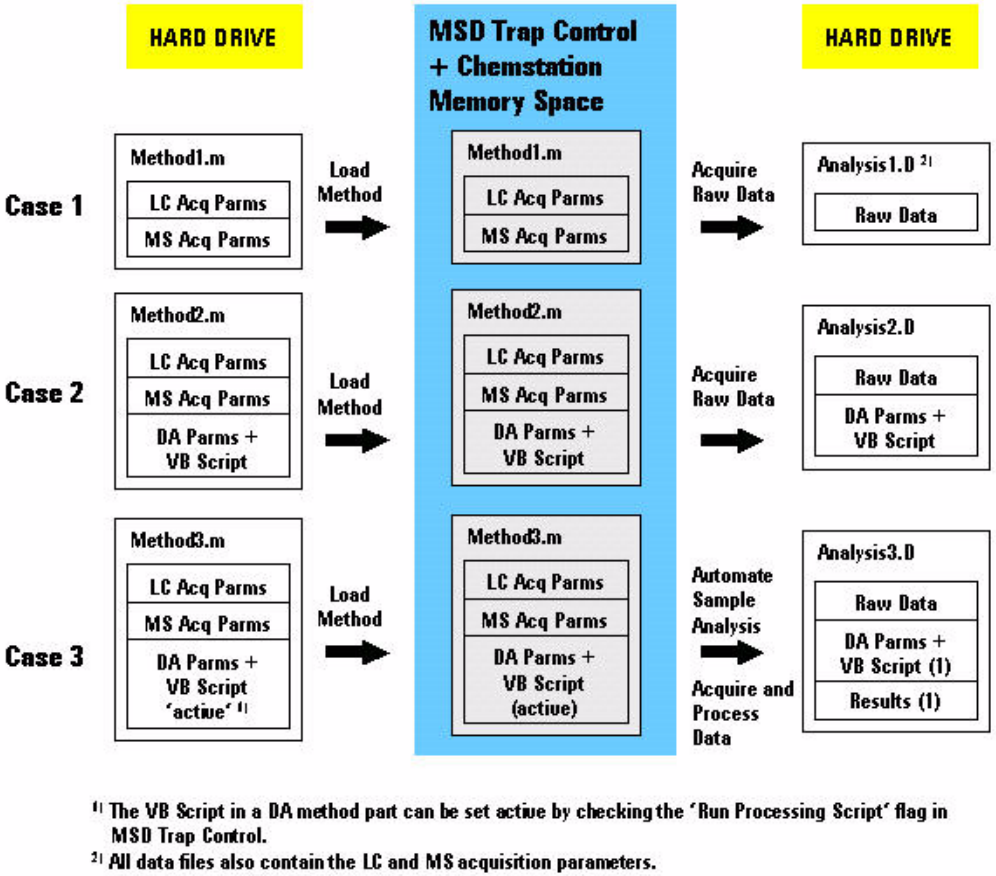
Figure 33 Add DA method part to LC/MS method through the TrapControl program

Data acquisition with or without automatic processing

You can acquire data with one of three types of LC/MS methods:

- Does not contain a DA method part
- Contains a DA method part with an “inactive” VB script
- Contains a DA method part with an “active” VB script (Flag for running the processing script is set to “on”.)

In the first two cases, only raw data is acquired. In the third case, the raw data is processed immediately after acquisition without your interaction (Figure 34).



[1] The VB Script in a DA method part can be set active by checking the 'Run Processing Script' flag in MSD Trap Control.

[2] All data files also contain the LC and MS acquisition parameters.

Figure 34 Data acquisition with three types of LC/MS methods

Interactive data processing

What happens after you load a data file into DataAnalysis

If more than one data file is loaded Each loaded data file has its own memory segment for its DA method part. Changes to the DA method parameters or the VB Script apply only to the memory segment of one selected analysis. Even when the changes applied are not saved to the analysis yet, they are stored in memory and are remembered when switching to another analysis and back.

If no DA method part is attached If no custom DA method part was added to the LC/MS or MS-only method before data acquisition, the software provides two options for associating DataAnalysis parameters and a VB script with the raw data (Figure 35 - Case 1):

- A built-in set of DataAnalysis parameters and an empty script are added from within DataAnalysis into the respective memory segment for the selected analysis. You can edit those parameters to your liking and do manual interactive processing tasks. When you save results, the used parameters and the (potentially still empty) script are stored with the results.
- Within DataAnalysis you can define a default DA method part using 'Method > Default...'. This method part automatically attaches to the data file instead of the built-in parameters and VB script when an analysis without a DataAnalysis method part is loaded.

Initial processing

After you have loaded the data file either with or without a DataAnalysis method part, you can choose to process the file in either of 2 ways (Figure 35 - Case 1 and Case 2):

- Manually process the file (e.g., select the Find Compounds or other commands) using the DA parameters but ignoring the VB script.
- Automatically process the file by running the script that contains a sequence of processing tasks (Method > Run)

Reprocessing

After saving a processed result, along with the DataAnalysis parameters and an optional VB Script used to obtain the result, you can continue to change DataAnalysis parameters, apply additional processing tasks and save further results (Figure 35 - Case 3). For each result saved, its set of DataAnalysis parameters and optional VB Script are saved with the result.

You can go back and forth between different result versions using File > Results History.... When a specific result version is loaded into DataAnalysis, the associated DataAnalysis parameters and VB Script are loaded back into the memory segment for the selected analysis.

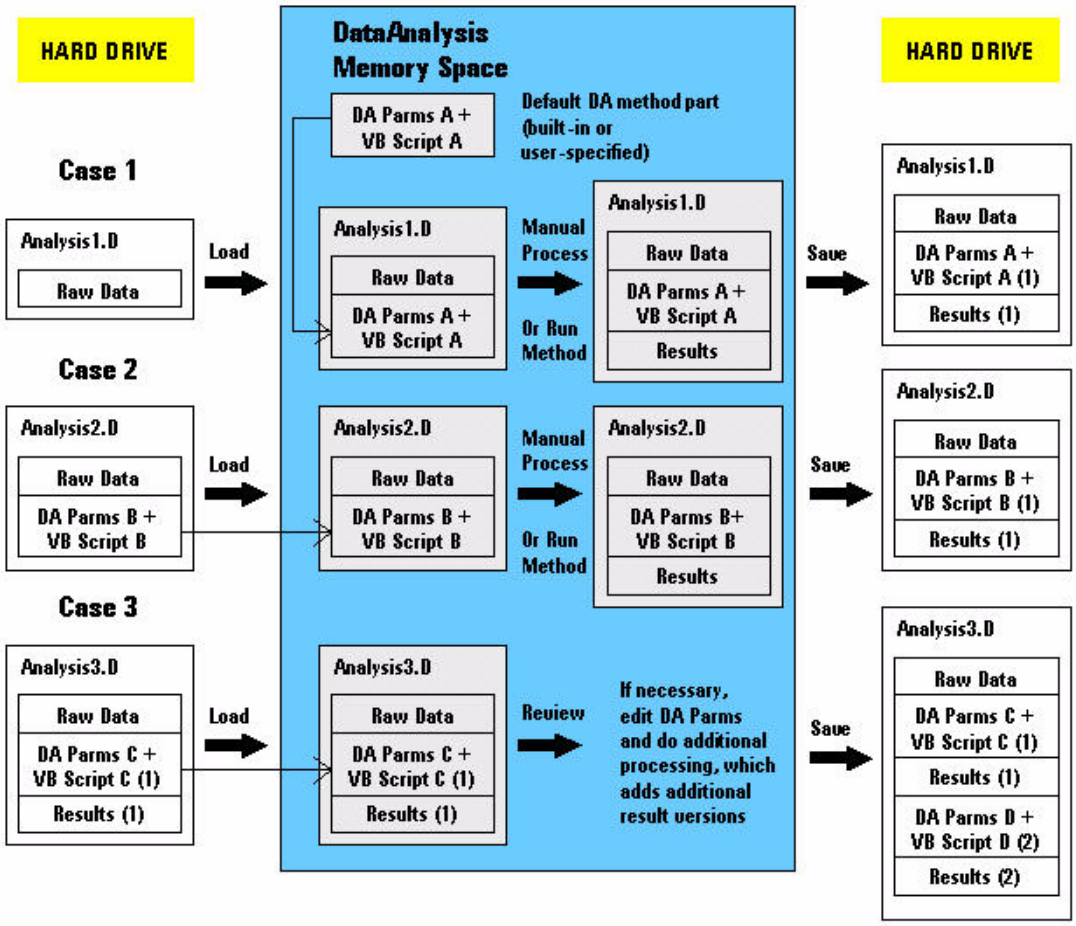


Figure 35 Interactive data processing

In summary—LC/MS method development

Given the method and data structure outlined in the previous sections, it is recommended that you use the following method development strategy to set up your methods in the Ion Trap software.

1. In ChemStation open the Def_LCMS.m LC/MS method.
2. Develop the LC part of the method.
   a. In ChemStation, make changes to LC acquisition parameters.
   b. If you prefer to develop the chromatographic conditions for the method with the UV-detector only, select **Method > LC-Only** mode before starting the run in ChemStation.
   c. If parameters are acceptable, save the method in ChemStation, first deselecting the LC-Only mode.
3. Develop the MS part of the method.
   a. In the TrapControl program, make changes to MS parameters and acquire only MS data by starting the run from within the TrapControl program. (Infuse the tuning mix.)
   b. If parameters are acceptable, save in ChemStation.
4. Acquire LC/MS data by starting the run in ChemStation.
5. Develop the DataAnalysis part of the method.
   a. Open the acquired data file in the DataAnalysis software.
   b. If you know a DA method part with suitable DA parameters and VB script to start with, select **Method > Open** and select the LC/MS-, MS-only or DA-only method to load the DA method part

      Or, start with the default DA parameters and process data manually (e.g., Find Compounds, deconvolution, export, reporting), or create and execute a VB script.
   c. If the results are not acceptable, do the following:
      - Select **Method > Open** to select a DA method part with different DataAnalysis parameters and a script.

- Select **Method > Parameters** and edit the DataAnalysis parameters.
- Select **Method > Script** and edit the VB script.

d Repeat processing, results review and editing of DA parameters and/or script until the results are optimized.

e Select **Method > Save As** to save your DataAnalysis method under a new or existing name.

6 In the TrapControl program select **Method > Add DataAnalysis Method Part...** and select the DA method from step 5e.

7 Select **Method > Run Processing Script** in the TrapControl program to implement automated sample analysis (acquisition and processing run sequentially).

You do not have to mark the **Standard Data Analysis** check box in the Runtime Checklist of ChemStation unless you want to process UV data in ChemStation Data Analysis.

8 In ChemStation re-save the LC/MS method.

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In This Book

The *Agilent 6300 Series Ion Trap LC/MS System Concepts Guide* presents information to help you understand how the Agilent series and ion trap systems work and how the method file and the data file structures are organized.

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