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ABI Q Trap LC/MS/MS

Introduction to the Q Trap LC/MS/MS System

The Q Trap LC/MS/MS system is a hybrid triple quadrupole linear ion trap (LIT) mass spectrometer. The Q3 region can be operated as either a standard quadrupole mass spectrometer or a linear ion trap mass spectrometer. The unique scan modes of both configurations can be linked to provide more and higher quality data than either instrument alone. For example, a precursor ion scan in Transmission mode can be used as a survey scan in order to target specific ions to be used in an enhanced product ion scan (in LIT mode). Conversion between the two modes of operation is rapid, since it involves only the addition or removal of the resolving DC voltages.

The Q Trap LC/MS/MS system retains all of the traditional triple quadrupole scan types such as:

- Q1 MS (Q1)
- Q1 Multiple Ion (Q1 MI)
- Q3 MS (Q3)
- Q3 Multiple Ion (Q3 MI)
- Multiple Reaction Monitoring (MRM)
- Precursor Ion (Prec) (This is not possible with a conventional ion trap.)
- Product Ion (MS2)
- Neutral Loss (NL)

When Q3 operates as an LIT mass spectrometer, a number of new advantages and capabilities are available:

- High sensitivity product ion scanning
- Fast scanning (4000 amu per second)
- High resolution capabilities at reduced scan speeds
- MS/MS/MS capabilities
- Reduced space charge effects

In LIT mode, a pulse of ions is introduced into the ion trap. The main RF fields trap the ions in the radial direction, while DC voltages applied to the lenses at both ends of Q3, trap the ions axially. The trapped ions are allowed to cool for several milliseconds, then the RF voltage is scanned in the presence of a low voltage auxiliary AC applied to the rods. The ions ejected axially toward the detector are counted.

If you configure the mass spectrometer with Q1 operating as a standard quadrupole mass spectrometer and Q3 operated as an LIT mass spectrometer, you can achieve the following enhanced scan types:

- Enhanced MS (EMS)
- Enhanced Resolution (ER)

- Enhanced Product Ion (EPI)
- Enhanced Multi-Charge (EMC)
- Time Delayed Fragmentation (TDF)
- MS/MS/MS (MS³)

In LIT mode, a pulse of ions passes through Q1 operated as a conventional quadrupole mass spectrometer to select the precursor ion of interest. The precursor ions are accelerated into the pressurized Q2 to promote fragmentation. The fragment and residual precursor ions are then trapped in the Q3 linear ion trap. The Q3 RF voltage is ramped and the ions ejected toward the detector are reported. For more information about these enhanced scans, see “Q Trap LC/MS/MS Enhanced Modes of Operation” on page 10.

Triple Quadrupole/Linear Ion Trap Mass Spectrometer

The Q Trap LC/MS/MS system uses a TurboIonSpray, Heated Nebulizer, or Flow Nanospray ion source to produce ions from liquid samples. The term LC/MS/MS, applied to the triple quadrupole series, is a generic label for the combined analytical processes of liquid separation and subsequent mass spectrometric analysis. The instrument is configured to perform complex MS/MS and MS/MS/MS analysis. For less rigorous analytical requirements, it can perform single MS (LC/MS) scans.

The Q Trap LC/MS/MS system allows all modes of MS/MS and MS/MS/MS operation for full characterization of biopharmaceutical compounds and the specificity needed for new drug development. For pharmaceutical and pharmacokinetic samples, MS/MS has the sensitivity and specificity required to analyze hundreds of samples per day without extensive sample preparation.

For peptides and proteins, molecular weights can be determined with accuracies better than 0.01% at 200 kDa.

The major components of the Q Trap LC/MS/MS system are shown in the figure *Q Trap LC/MS/MS system components with pump* on page 7.

Principles of MS

In Single Quadrupole mode, the Q Trap LC/MS/MS system separates ions representative of the sample molecular components based on their m/z ratio. Ions of a unique m/z ratio can be separated by the single mass filter quadrupole and counted to provide mass spectra for the sample.

The mass filter quadrupole consists of four cylindrical rods mounted in a ceramic collar surrounding the ion path. Fixing the ratio of RF to DC voltages applied to the quadrupole rods determines the mass of the ions exiting the quadrupole.

Ions of a unique m/z ratio pass unobstructed through the quadrupole as a function of the quadrupole power supply (QPS) voltages applied. Ions of different m/z ratios have unstable oscillations that increase in amplitude until they collide with the quadrupole rods and are removed from the ion stream.

As an example, a sample mixture containing three molecules, R, M, and N, is introduced into the ion source. Soft ionization in the ion source generates R⁺, M⁺, and N⁺ ions (quasi-molecular ions formed typically by attaching one or more protons in the Positive mode, or by removing one or more protons or attaching an electron in the Negative mode).

Isolation of mixture R, M, and N

Additional structural information can sometimes be obtained by fragmenting the precursor ion in a primary collision region between the orifice and the skimmer. This process is

often referred to as collision induced dissociation mass spectrometry (CID/MS).

Isolation of product ions from a sample using the orifice-skimmer technique

The ions generated in the ion source are drawn through a curtain of dry inert gas into the ion optics housed inside the vacuum chamber. The mass filter quadrupole in the vacuum chamber selectively filters the ions based on their m/z ratio. The filtered ions are focused to the detector. As ions collide with the detector, they produce a pulse of electrons. The electron pulse is collected and converted to a digital signal to provide an ion count as a function of ion mass. The acquired data is relayed to the computer where it can be displayed as either full mass spectra, intensity of single or multiple ions versus time, or total ion current versus time.

Principles of MS/MS

In Triple Quadrupole mode, the Q Trap LC/MS/MS system uses two identical mass filter quadrupoles (Q1 and Q3) separated by a collision cell, which encloses an RF-only quadrupole (Q2). The fundamental principle of MS/MS is illustrated in the figure *Isolation of product ions from a mixture of R, M and N* on page 8.

As an example, a sample mixture containing three molecules, R, M and N, is introduced into the ion source. Soft ionization in the ion source generates R^+ , M^+ , and N^+ ions (quasi-molecular ions formed typically by attaching one or more protons in the Positive mode, or by removing one or more protons or attaching an electron in the Negative mode).

Isolation of product ions from a mixture of R, M and N

In a Product Ion scan, the first mass filter, Q1, separates or filters ions according to their m/z ratio, and allows only one ion to enter the collision cell (M^+). The M^+ ion enters Q2 where it is fragmented by collision with neutral gas molecules in a process referred to as collision activated dissociation (CAD). The fragment ions generated are then passed into Q3 and filtered to provide a mass spectrum. The ions created by the source are referred to as precursor ions, the collision products are referred to as product or fragment ions.

In a Precursor Ion scan, the third quadrupole (Q3) is fixed to the fragment mass of interest and the first quadrupole (Q1) is scanned over a range. The resulting mass spectrum displays the masses of all the compounds that produced the specified fragment mass.

In a Neutral Loss scan, both quadrupoles (Q1 and Q3) are scanned with a constant mass difference between them. The resulting mass spectrum displays the mass of the compounds that have undergone the specified loss. This type of scan is useful in identifying compounds from similar functional groups.

The fragment ions are filtered in Q3 before they are collected at the detector. As ions collide with the detector, they produce a pulse of electrons. The pulse is converted to a digital signal that is counted to provide an ion count. The acquired data is relayed to the computer where it can be displayed as either full mass spectra, intensity of single or multiple ions versus time, or total ion current versus time.

The technique of MS/MS is well suited to mixture analysis because the characteristic fragment ion spectra can be obtained for each component in a mixture without interference from the other components, assuming that the ions have a unique m/z ratio. This analysis can also be used for targeted analysis by monitoring specific precursor/product ions with Q1 and Q3 respectively while the sample is eluting. This type of analysis is more specific than single MS, which only discriminates on the basis of molecular weight.

The MS/MS technique is well suited to structural elucidation studies. The same fragmentation pattern that provides identification of a compound in a complex mixture can also reveal pertinent information regarding the structure of all their precursors. Additional structural information can sometimes be obtained by fragmenting the precursor

ion in a primary collision region between the sampling orifice skimmer. The fragment ions (for example, a second generation fragment ion spectrum), provide structural information on both the original precursor ions and the first generation fragment ions.

Isolation of second generation product ions from mixture M

The triple quadrupole instruments contain the same components as the single quadrupole instruments with the addition of a second mass filter (Q3). The high-pressure region is the same, but the high vacuum region contains the Q1 prefilter (stubbies) and the Q1 and Q3 mass filter quadrupoles that are separated by the collision cell. The collision cell is a ceramic housing enclosing the Q2 RF-only quadrupole, which when pressurized with CAD gas provides a local high-pressure region for ion fragmentation.

Ions pass through the same path as in the single quadrupole instrument until they reach the Q2 RF-only quadrupole. The selected ions arrive at Q2, while those rejected eventually collide with the rods and are lost.

The Q2 RF-only quadrupole is separated from the Q1 and Q3 mass filters by the interquad lenses IQ2 and IQ3 (or ST3, depending on the triple quadrupole series). The Q2 region has no mass filtering capabilities; it operates in Total Ion mode. If no CAD gas is present to fragment the sample ions, Q2 transports the ions directly into Q3. If CAD gas is present, the ions that enter Q2 collide with the neutral CAD gas molecules. If pressurized, the voltage drop between the entrance lenses and Q2 provides the ions with the energy to induce fragmentation when the ions collide with CAD gas molecules. Through the energetic collisions, the ion translational energy is converted into internal energy that fractures bonds and causes ion fragmentation. After collision, the unfragmented precursor ions and the fragmented ions are transported to Q3 where they are again filtered.

When operating in MS/MS mode, the Q3 mass filter is physically and functionally identical to Q1. The ions, including a mixture of precursor and fragment ions, enter Q3 where they are filtered according to mass. In Single MS Operating mode (Q1 scan type), Q3 acts as an ion transporter (like a Q0 or RF-only quadrupole) with no filtering action. Terms used to describe this operation are Total Ion mode, RF-only mode, and AC-only mode.

Q Trap LC/MS/MS Enhanced Modes of Operation

The Q Trap LC/MS/MS system has a number of enhanced modes of operation. A common factor of the enhanced modes is that ions are trapped in the Q3 quadrupole region and then scanned out to produce full spectrum data. Many spectra are rapidly collected in a short period of time and are significantly more intense than spectra collected in a comparable standard quadrupole mode of operation. The widths of the peaks in the spectra are usually much narrower than peaks observed in the standard quadrupole mode.

During the collection phase, ions pass through the Q2 collision cell where CAD gas focuses the ions into the Q3 region. The Q3 quadrupole is operated with only the main RF voltage applied. Ions are prevented from passing through the Q3 quadrupole rod set and are reflected back by an exit lens to which a DC barrier voltage is applied. After the fill time elapses (a time defined by the user), a DC barrier voltage is applied to a Q3 entrance lens (IQ3). This confines the collected ions in Q3 and stops further ions from entering. The entrance and exit lens DC voltage barriers and the RF voltage applied to the quadrupole rods confine the ions within Q3.

During the scan out phase, a potential of a few volts is applied to the exit lens to repel the charged ions. An auxiliary AC frequency is applied to the Q3 quadrupole. The main RF voltage amplitude is ramped from low to high values, which sequentially brings masses into resonance with the auxiliary AC frequency. When ions are brought into resonance

with the AC frequency, they acquire enough axial velocity to overcome the exit lens barrier and are axially ejected towards the mass spectrometer ion detector. Full spectra data can be acquired from the ions collected in Q3 by rapidly scanning the main RF voltage.

The enhanced modes of operation are:

- **Enhanced MS (EMS):** Ions are transferred directly from the ion source and orifice region to the Q3 quadrupole where they are collected. These ions are scanned out of Q3 to produce enhanced single-MS type spectra. Use the EMS mode when you need a rapid enhanced sensitivity survey type scan.
- **Enhanced Resolution (ER):** This mode is similar to the Enhanced Product Ion mode except that the Q1 precursor ions pass gently through the Q2 collision cell without fragmenting. A small range about the precursor mass is scanned out of Q3 at the slowest scan rate to produce a narrow window of the best-resolved spectra.
- **Enhanced Product Ion (EPI):** Product ions are generated in the Q2 collision cell by the precursor ions from Q1 colliding with the CAD gas in Q2. These characteristic product ions are transmitted and collected in Q3. These ions are scanned out of Q3 to produce enhanced product ion spectra. Use the EPI mode if you need enhanced resolution and intensity.
- **Enhanced Multi-Charge (EMC):** This mode operates similarly to the Enhanced MS mode except, before scanning the ions out of Q3, there is a delay period in which low charge state ions (primarily singly charged ions) are allowed to preferentially escape from the Q3 quadrupole. When the retained Q3 ions are scanned out, the multiply charged ion population dominates the resulting spectra.
- **Time Delayed Fragmentation (TDF):** Product ions are generated and collected in Q3. During the first part of the collection period, the lower mass ions are not collected in Q3. During the second part of the collection period, all masses over the mass range of interest are collected. The resultant enhanced product ion spectra are simplified compared to EPI scan type spectra. The nature of the spectra aids in the interpretation of the structure and fragmentation pathways of the molecule of interest.
- **MS/MS/MS (MS3):** In MS/MS/MS mode, product ions are generated in the Q2 collision cell by the precursor ions from Q1 colliding with the CAD gas in Q2. These characteristic product ions are transmitted and collected in Q3. Applying the normal mode resolving DC voltages to the Q3 quadrupole isolates a specified mass (m/z) of ion and removes all other ions from Q3. By properly applying a second auxiliary AC frequency to Q3, the specified ion can be resonantly excited. These excited ions collide with the residual nitrogen in Q3 and may fragment, producing a characteristic spectrum of ions. These secondary product ions of the isolated product ion result in MS/MS/MS product spectra.



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