CHEMISTRY 5570

Advanced Analytical Chemistry

Lecture 22

Tandem MS or MS-MS

Tandem Mass Spectrometry (MS–MS)

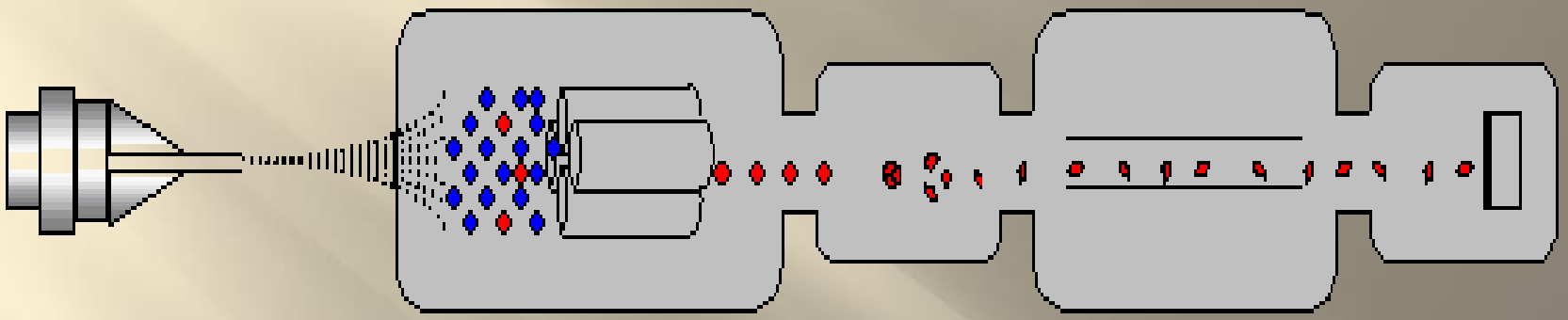
Tandem mass spectrometry (MS–MS) is a term which covers a number of techniques where

one stage of mass spectrometry (not necessarily the first) is used to isolate an ion of interest and a

second stage is then used to probe the relationship of this ion with others from which it may have been generated or which it may generate on decomposition.

Tandem MS or MS-MS

Tandem Mass Spectrometry (MS–MS)



Mass Spectrometry (MS)

The two analyzers (MS-MS) can be separated by a collision cell (can be another MS) into which an inert gas (e.g. argon, xenon) is admitted to collide with the selected sample ions and bring about their fragmentation.

Tandem MS have the ability to perform multiple steps on a single sample.

The MS selects a specific ion, fragment the ion, and generate another mass spec – able to repeat the cycle several times.

Mass Spectrometry (MS)

The analyzers can be of the same or of different types, the most common combinations being:

- **quadrupole - quadrupole**
- **magnetic sector - quadrupole**
- **magnetic sector - magnetic sector**
- **quadrupole - time-of-flight**

Fragmentation experiments can also be performed on certain single analyzer mass spectrometers such as ion trap and time-of-flight instruments, the latter type using a post-source decay experiment to effect the fragmentation of sample ions.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

TIC - Total ion current or total ion chromatogram

The TIC represents the sum of all signal intensities of a single scan spectrum. The TIC is usually calculated by the data system of the mass spectrometer and plotted against time or scan number to give a measure for evaporation/ionization of a sample over the duration of the whole measurement.

Mass Spectrometry

TIC - Total ion current or total ion chromatogram

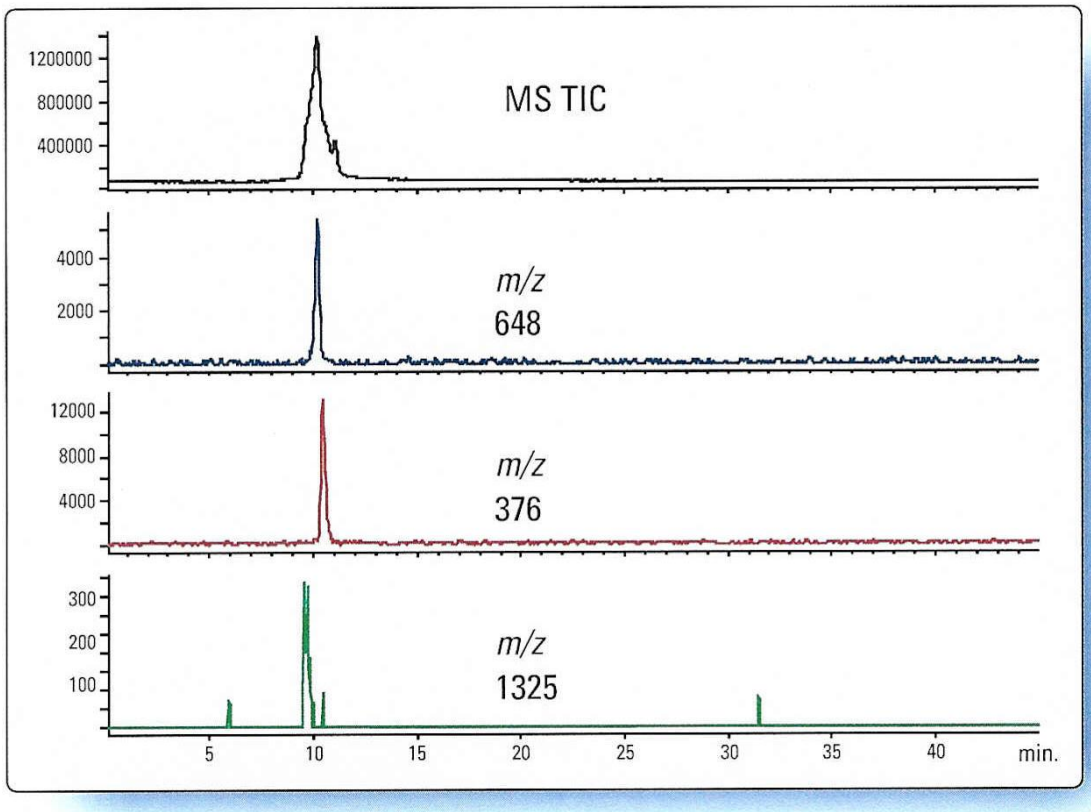


Figure 2. Identification of three components in a chromatographically unresolved peak

Mass Spectrometry

Collision-Induced Dissociation (CID)

To obtain structural information, analyte ions are fragmented by colliding them with neutral molecules (CID).

Voltages are applied to the analyte ions to add energy to the collisions and create more fragments.

Mass Spectrometry

Collision-Induced Dissociation (CID)

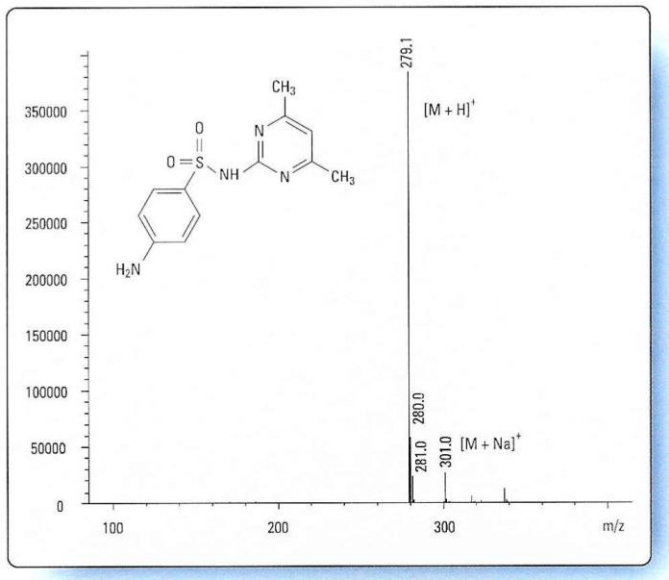


Figure 13. Mass spectrum of sulfamethazine acquired without collision-induced dissociation exhibits little fragmentation

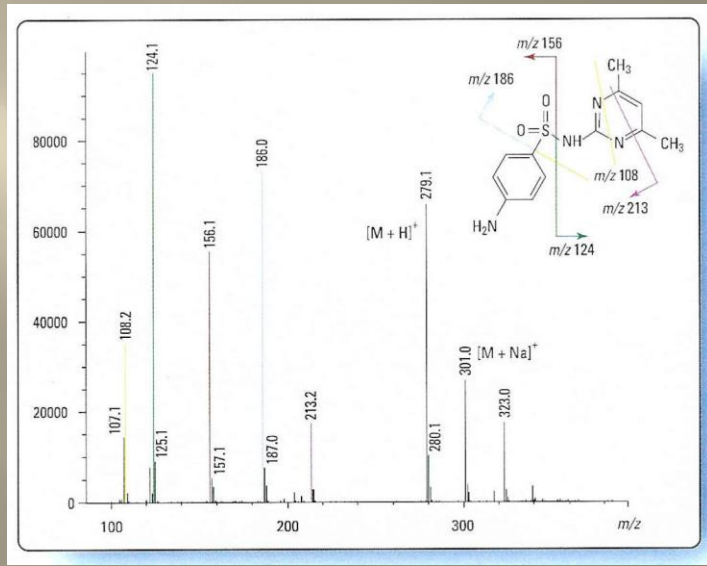


Figure 14. Mass spectrum of sulfamethazine acquired with collision-induced dissociation exhibits more fragmentation and thus more structural information

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

The two stages of mass spectrometry are related in specific ways in order to provide the desired analytical information.

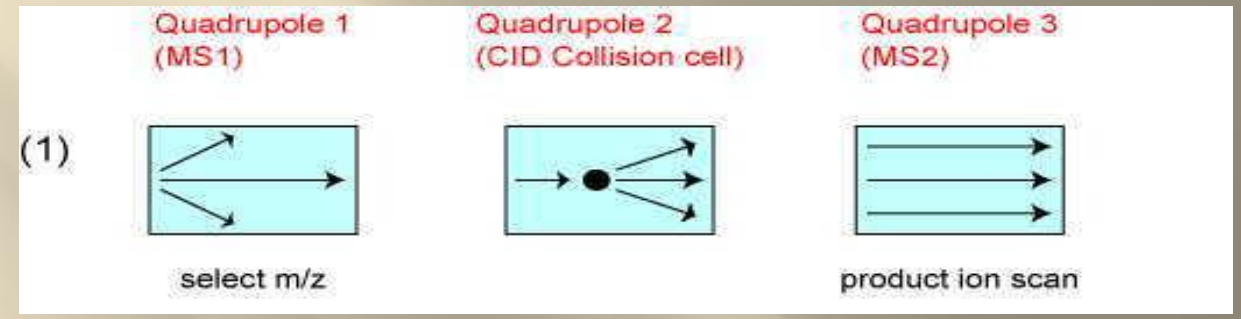
There are a large number of different collision-induced dissociation MS–MS experiments that can be carried out but the four most widely used are

(i) the product-ion scan,
(ii) the precursor-ion scan,
(iii) the constant-neutral-loss scan, and
(iv) selected decomposition monitoring.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Product Ion Scan



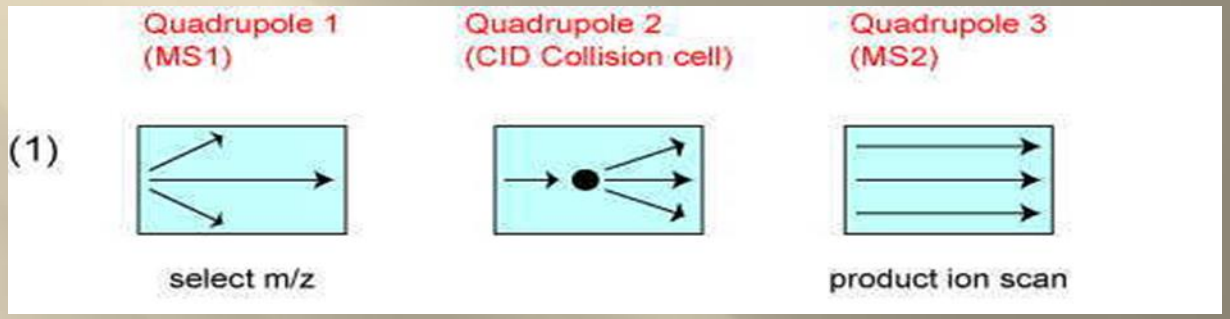
The first stage of mass spectrometry (MS1) is used to isolate an ion of interest for LC–MS (often the molecular species from the analyte.)

Fragmentation of the ion is then done by collision with gas molecules in a collision cell, i.e. MS2 in the triple quadrupole.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Product Ion Scan



The second-stage mass spectrometer (MS3) is scanned to provide a mass spectrum of the ions formed in the collision cell, i.e. the product (fragment) ions.

Interpretation of this spectrum is carried out in a similar way to the interpretation of an electron-ionization spectrum, although it must be remembered that the mechanisms occurring in MS–MS are not identical to those occurring in EI.

Mass Spectrometry

The Product-Ion Scan – Ion-trap is used instead of quadrupole

Ionization of the sample is carried out as in conventional operation and ions of all m/z ratios take up stable trajectories within the trap.

In MS–MS operation, ions of all m/z ratios, except that required for further study, are made unstable and ejected from the trap.

The ions remaining in the trap, only those of the selected m/z ratio, are now 'excited' to bring about their dissociation. The resulting product ions are then sequentially made unstable and sent to the detector to generate the product-ion spectrum.

Mass Spectrometry

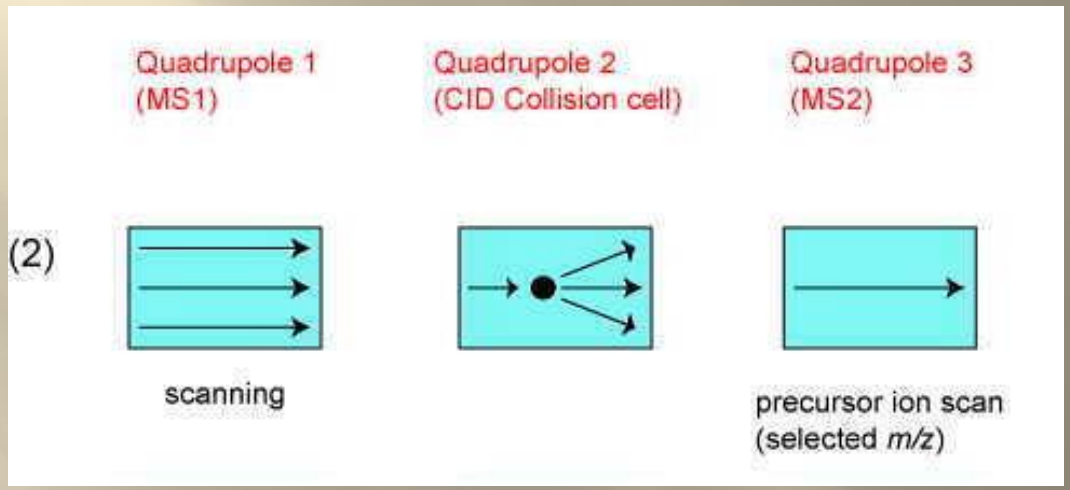
The Product-Ion Scan Applications

This type of experiment is particularly useful for providing structural information of small organic molecules and for generating peptide sequence information.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Precursor Ion Scan



The first stage (MS1) is set to scan through the mass range of interest.

The fragmentation of ions passing through MS1 being again carried out in the collision cell.

The second stage of mass spectrometry (MS2 or MS3) is set to transmit a single m/z ratio, namely that of the product (fragment) ion of interest.

A signal is seen at the detector only when ions are being transmitted by both MS1 and MS3, i.e. when an ion being transmitted by MS1 fragments to give the desired ion.

Mass Spectrometry

Precursor-Ion Scan Applications

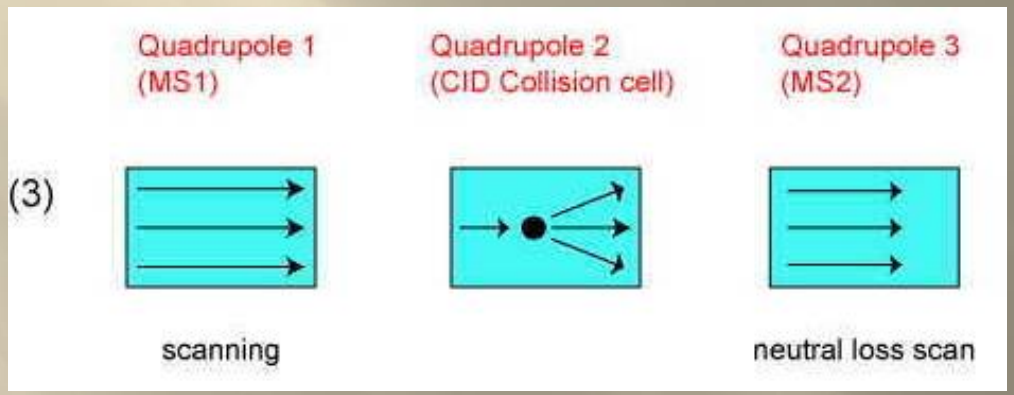
Ion-traps and Q–ToF instruments are not capable of carrying out this type of scan.

However this type of experiment is particularly useful for monitoring groups of compounds contained within a mixture which fragment to produce common fragment ions, e.g. glycosylated peptides in a tryptic digest mixture, aliphatic hydrocarbons in an oil sample, or glucuronide conjugates in urine.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Constant Neutral Loss Scan



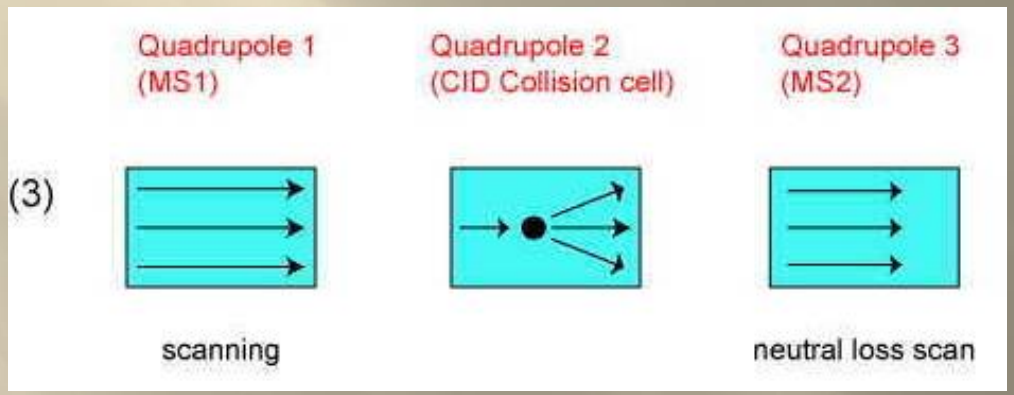
Rearrangement reactions may also occur in MS–MS instruments and the constant-neutral-loss scan enables the analyst to observe all of the ions in the mass spectrum that fragment with a particular mass loss and therefore contain a specific structural feature.

This knowledge can be of great value when attempting to interpret the mass spectrum of an unknown material.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Constant Neutral Loss Scan



Carried out by scanning both of the stages of mass spectrometry with a constant, specific, mass difference between them.

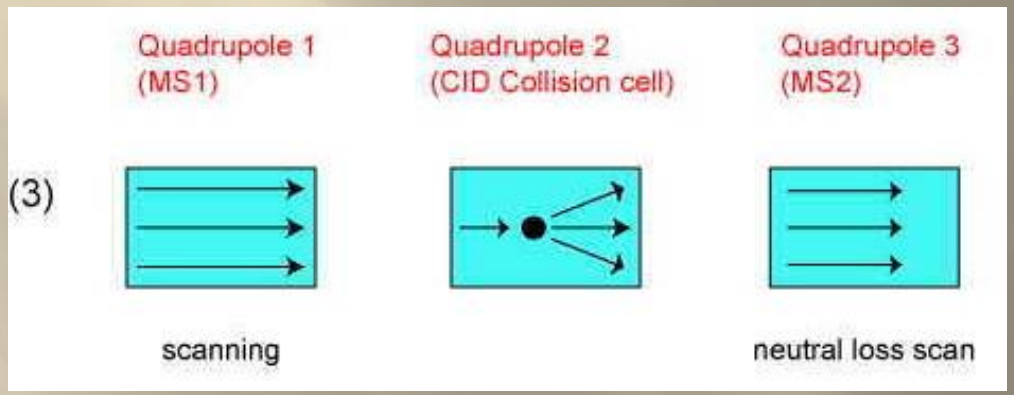
i.e. If the constant neutral loss of interest is 42 Da,

As MS1 moves to m/z 101, MS3 would move in conjunction to m/z 59, and when MS1 moves to m/z 102, MS3 would move to m/z 60, etc.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Constant Neutral Loss Scan



A signal is only obtained at the detector when both MS1 and MS3 are transmitting ions,

i.e. only when the ion being transmitted by MS1 fragments with loss of the mass of interest.

If it fragments by any other loss, the resulting product ion is not transmitted by MS3.

Mass Spectrometry

Constant-Neutral-Loss Scan Applications

Again, ion-traps and Q–ToF instruments are not capable of carrying out this type of scan.

This type of experiment could be used to monitor all of the carboxylic acids in a mixture.

Carboxylic acids tend to fragment by losing a (neutral) molecule of carbon dioxide, CO_2, which is equivalent to a loss of 44 Da or atomic mass units. All ions pass through the first analyzer into the collision cell. The ions detected from the collision cell are those from which 44 Da have been lost.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS)

Selected-Decomposition Monitoring (SDM)

The fragmentation of a selected precursor ion to a selected product ion is monitored.

This is carried out by setting each of the stages of mass spectrometry to transmit a single ion, i.e. the precursor ion by MS1 and the product ion by MS3.

In SDM, however, the product ion 'scan' is confined to only one m/z ratio, namely that of the product ion of interest.

Mass Spectrometry

Selected-Decomposition Monitoring (SDM)

In the ion-trap, the precursor is selected by the methodology described above for the product-ion scan.

In SDM, however, the product ion 'scan' is confined to only one m/z ratio, namely that of the product ion of interest.

Mass Spectrometry

Selected-Decomposition Monitoring Applications

The compound under scrutiny must be known and have been well-characterized previously before this type of experiment is undertaken.

This methodology is used to confirm unambiguously the presence of a compound in a matrix e.g. drug testing with blood or urine samples. It is not only a highly specific method but also has very high sensitivity.

Mass Spectrometry

The Triple Quadrupole

This is probably the most widely used MS–MS instrument.

The hardware, as the name suggests, consists of three sets of quadrupole rods in series.

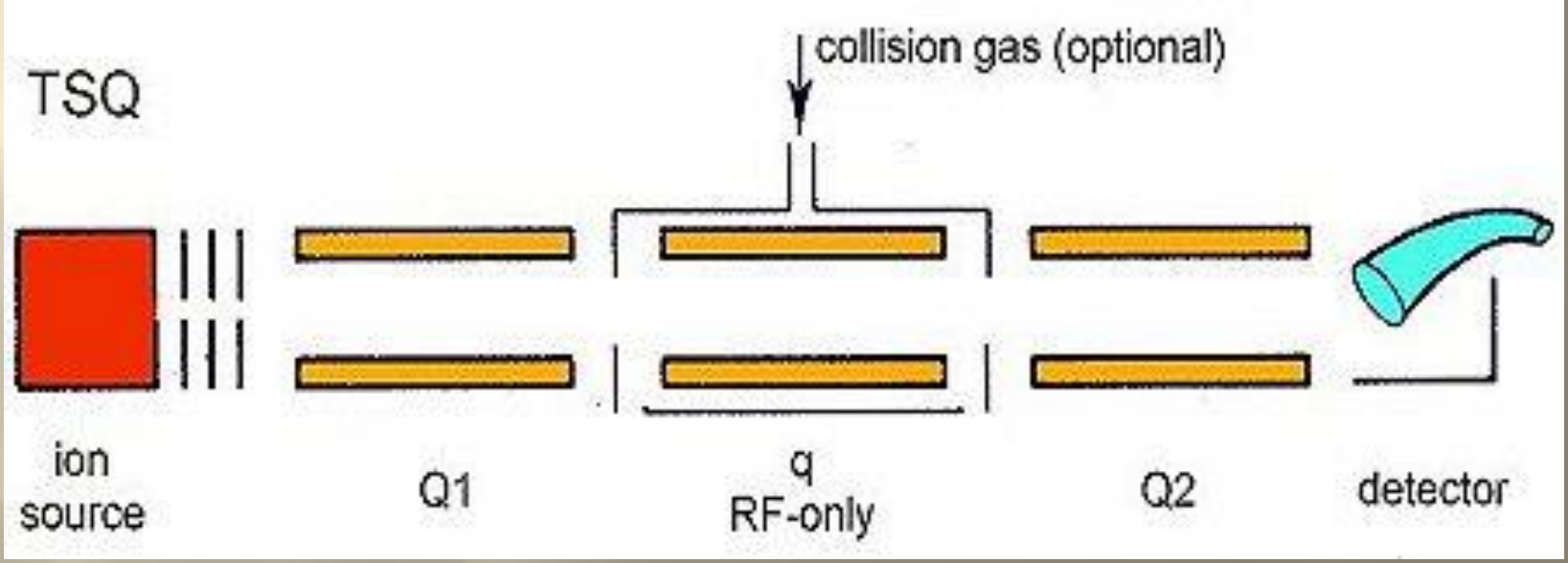
The second set of rods is not used as a mass separation device but as a collision cell, where fragmentation of ions transmitted by the first set of quadrupole rods is carried out, and as a device for focusing any product ions into the third set of quadrupole rods.

Both sets of rods may be controlled to allow the transmission of ions of a single m/z ratio or a range of m/z values to give the desired analytical information.

Mass Spectrometry

The Triple Quadrupole

Triple quadrupole instruments allow MS/MS experiments to be made with ease. For this purpose, Q1 is used for mass analysis, q for fragmentation (RF-only quadrupole) and Q2 for mass analysis of ions produced within the q region.



Mass Spectrometry

The Hybrid Mass Spectrometer

When the first quadrupole of a triple quadrupole is replaced by a double-focusing mass spectrometer, the instrument is termed a hybrid.

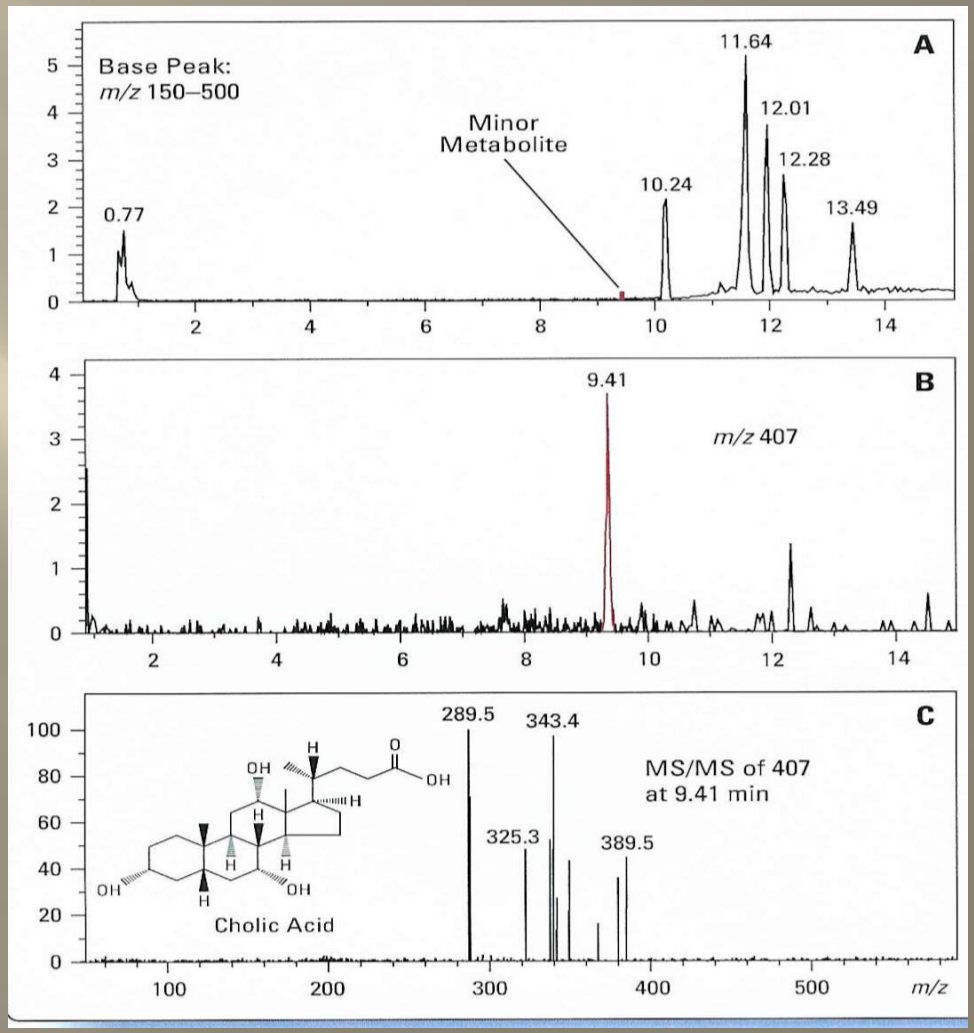
The advantage of this configuration is that the instrument can be used under high-resolution conditions to select the ion of interest.

Mass Spectrometry

Tandem Mass Spectrometry (MS–MS) Application

Pharmaceutical Applications

Identification of bile acid metabolites. MS-MS is an excellent way to identify minor metabolites at very low abundances.



Assignment

Chem 5570