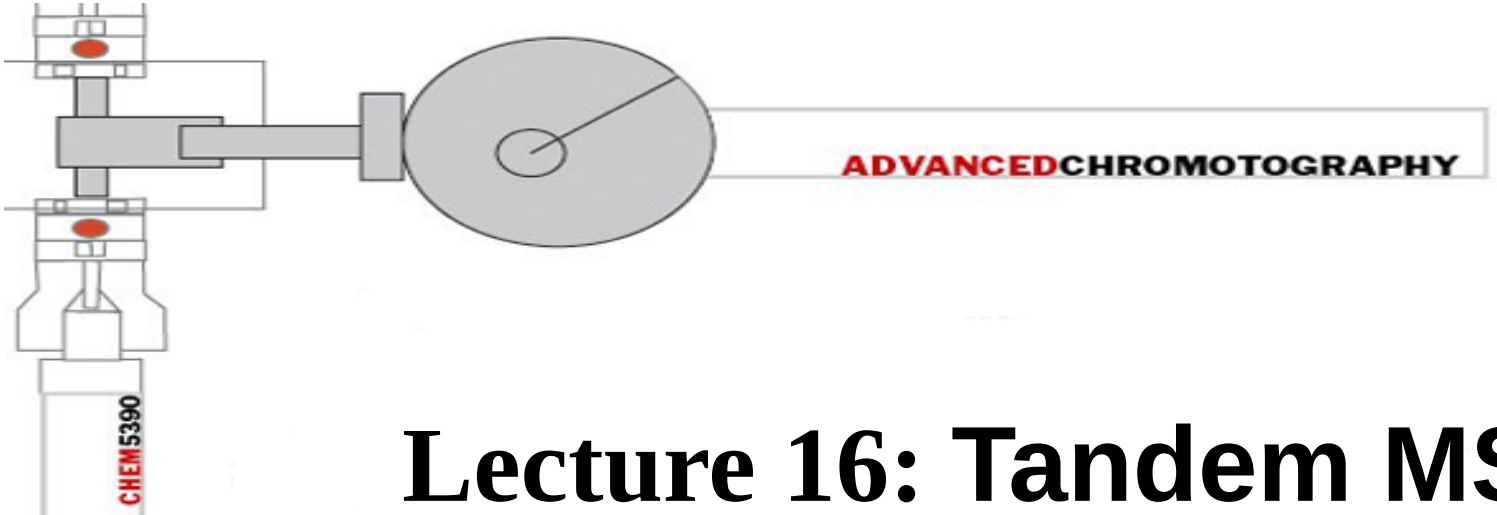
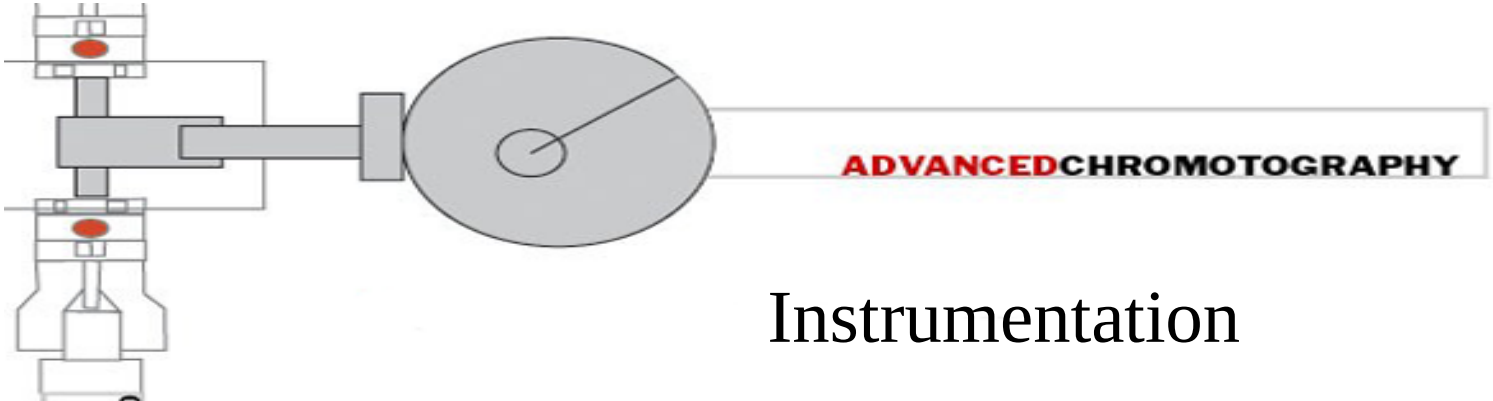


Lecture 16: Tandem MS



Instrumentation

Tandem Mass Spectrometry (MS–MS)

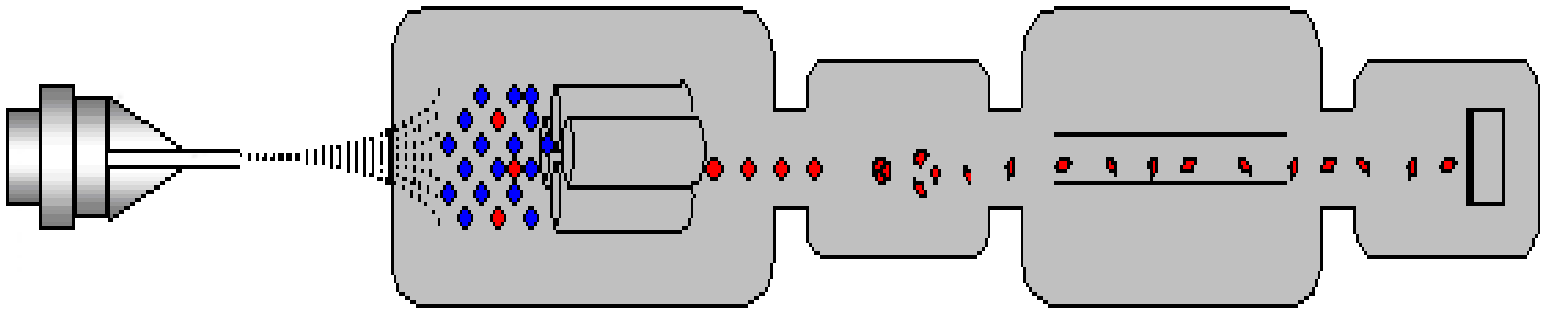
Tandem mass spectrometry (MS–MS) is a term which covers a number of techniques where

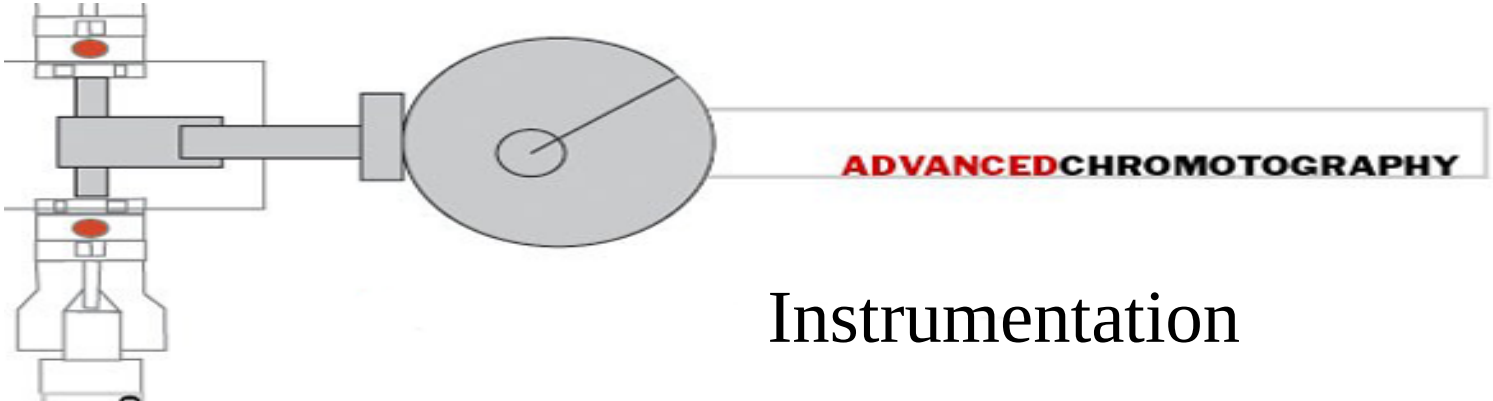
one stage of mass spectrometry (not necessarily the first) is used to isolate an ion of interest and a

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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)





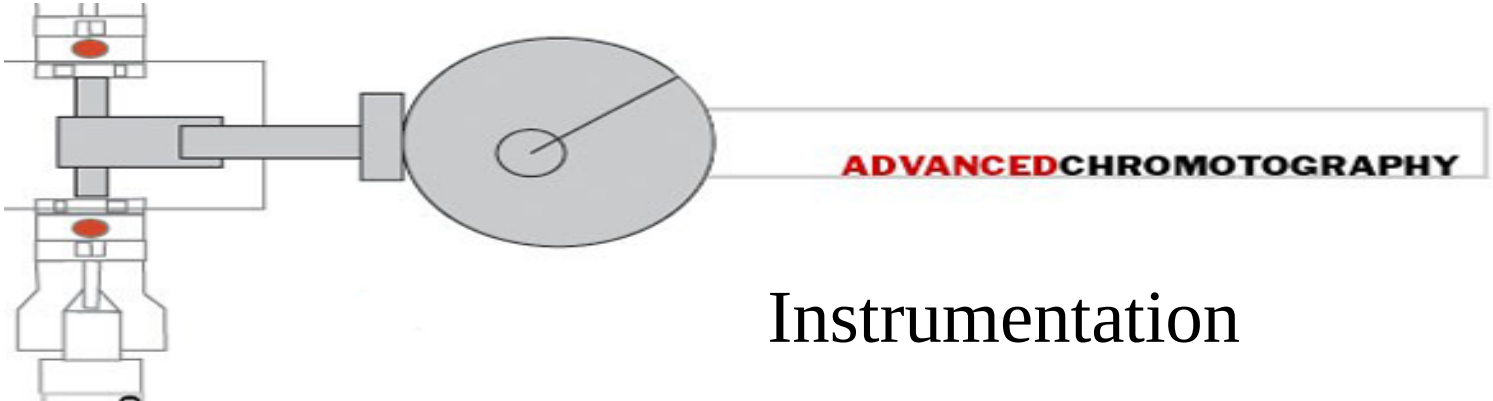
Instrumentation

Tandem Mass Spectrometry (MS–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.



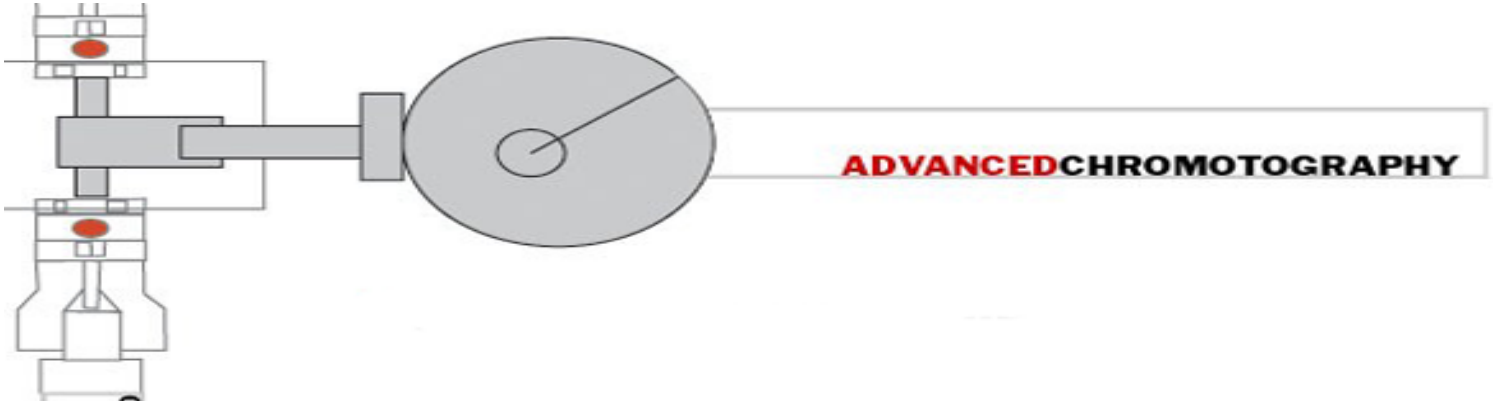
Instrumentation

Tandem Mass Spectrometry (MS–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.



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.

TIC - Total ion current or total ion chromatogram

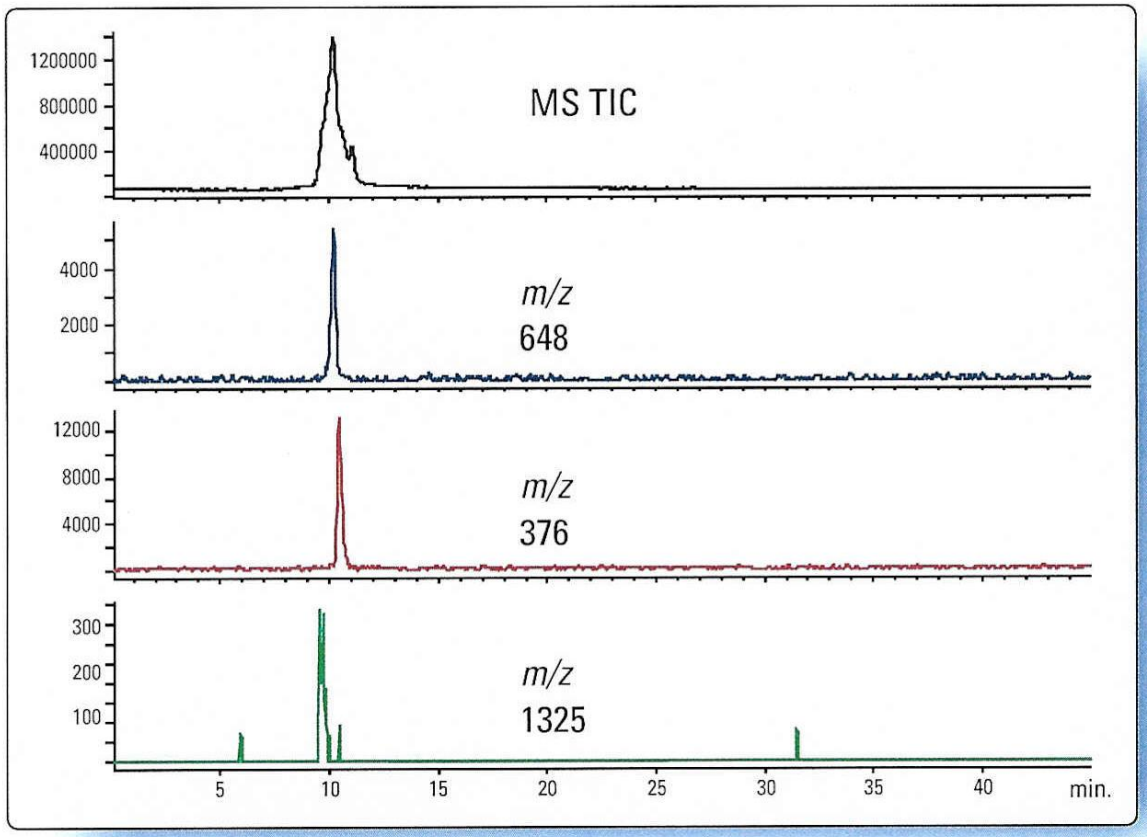
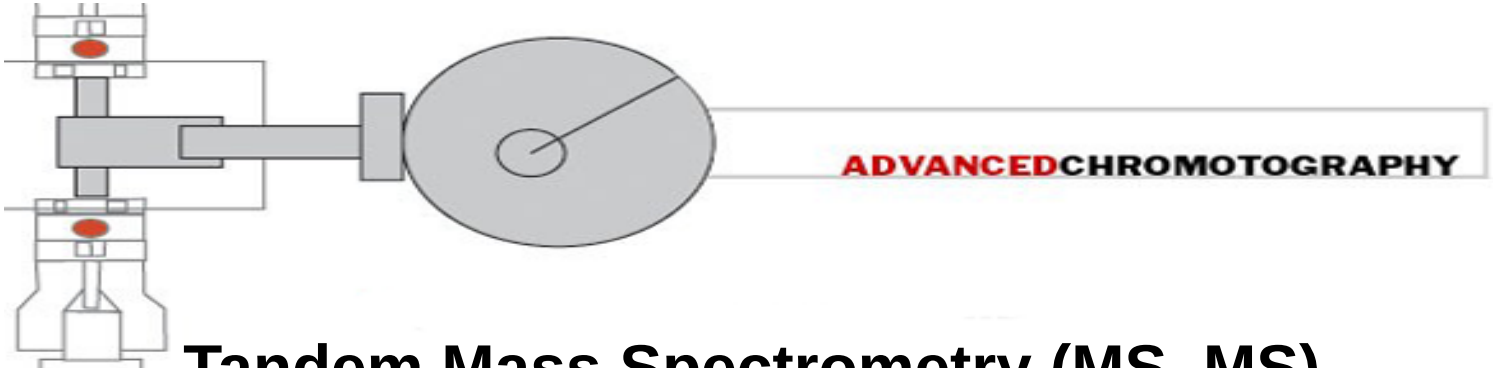


Figure 2. Identification of three components in a chromatographically unresolved peak



Tandem Mass Spectrometry (MS–MS)

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.

Collision-Induced Dissociation (CID)

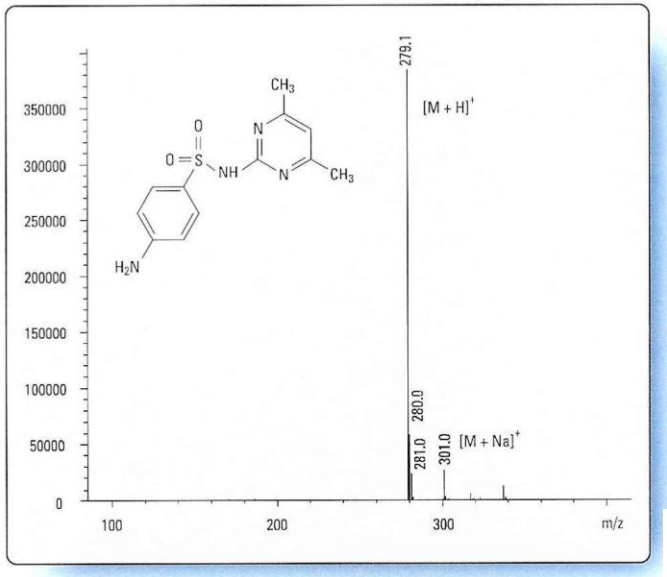


Figure 13. Mass spectrum of sulfamethazine acquired without collision-induced dissociation exhibits little fragmentation

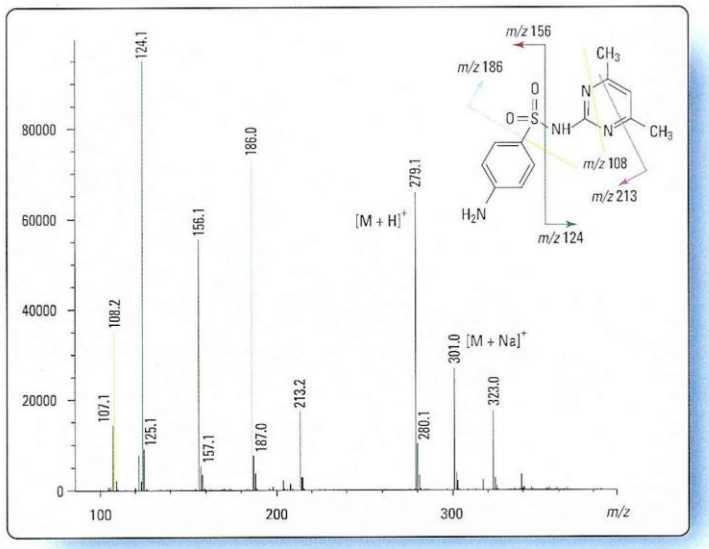


Figure 14. Mass spectrum of sulfamethazine acquired with collision-induced dissociation exhibits more fragmentation and thus more structural information

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,

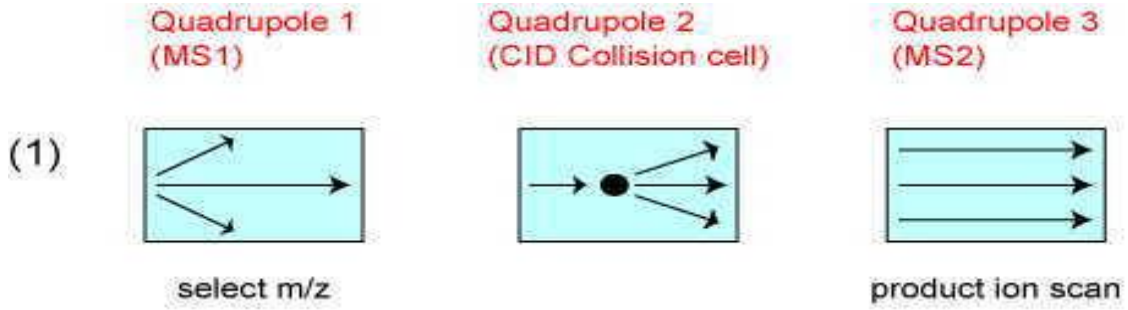
(iii) the constant-neutral-loss scan, and

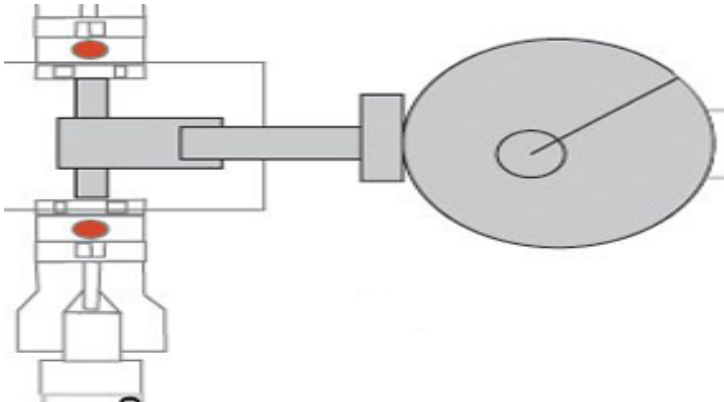
(iv) selected decomposition monitoring.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

Product Ion Scan





Instrumentation

Tandem Mass Spectrometry (MS–MS)

The 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.**

Tandem Mass Spectrometry (MS–MS)

The 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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

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.**

Instrumentation

Tandem Mass Spectrometry (MS–MS)

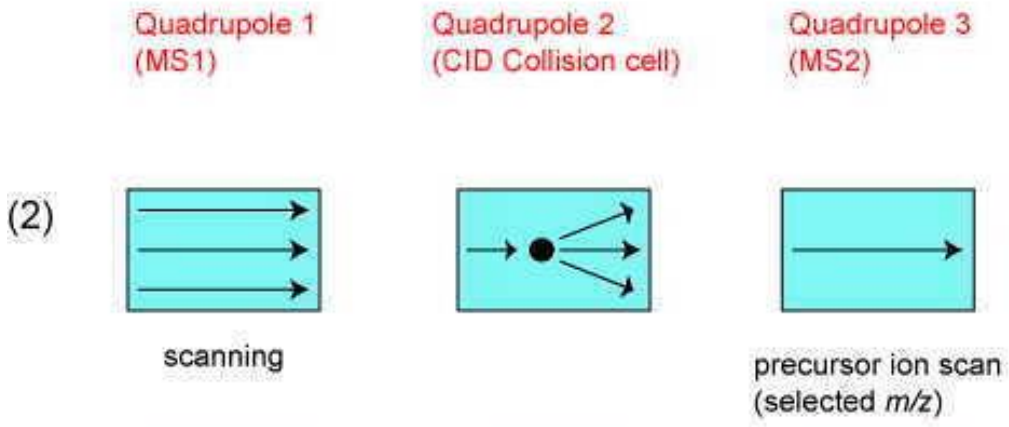
The Product-Ion Scan Applications

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

Instrumentation

Tandem Mass Spectrometry (MS–MS)

Precursor Ion Scan



Instrumentation

Tandem Mass Spectrometry (MS–MS)

The 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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

The Precursor-Ion Scan

•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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

The Precursor-Ion Scan Applications

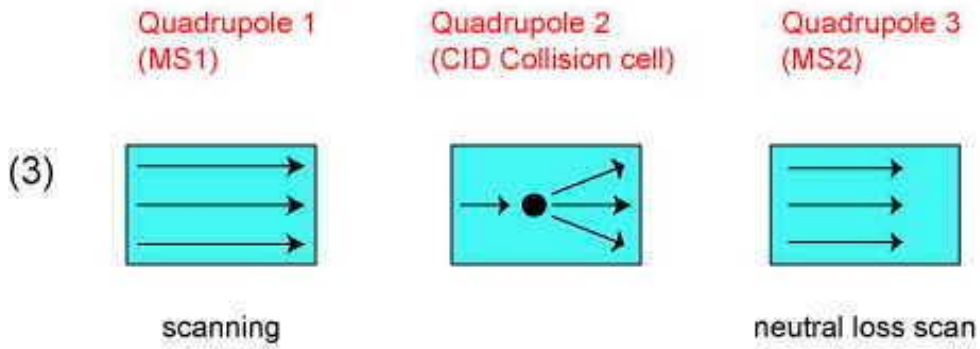
Ion-traps and Q–ToF instruments are not capable of carrying out this type of scan.

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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

Constant Neutral Loss Scan

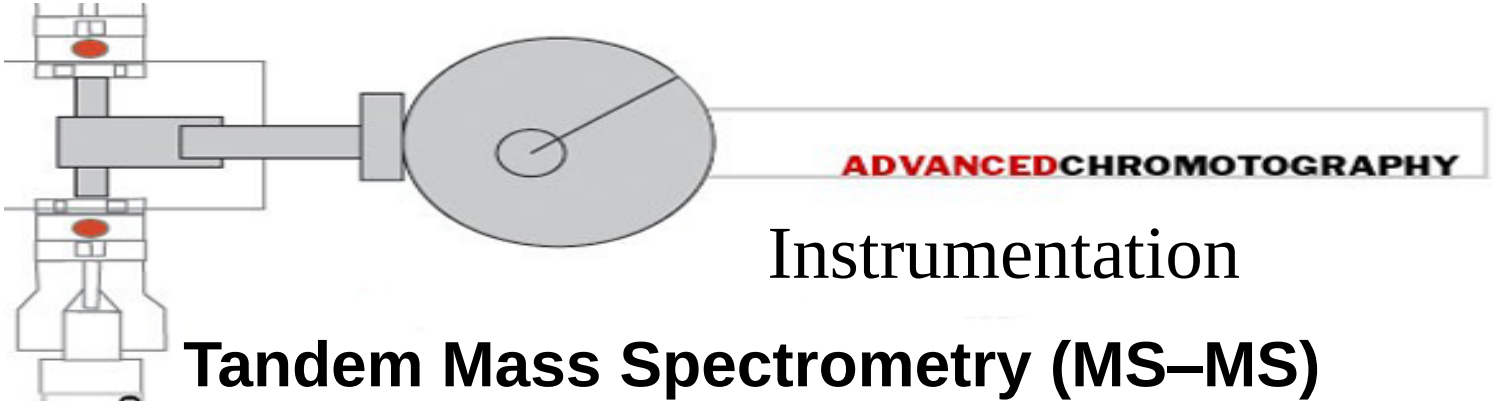


Tandem Mass Spectrometry (MS–MS)

The 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.

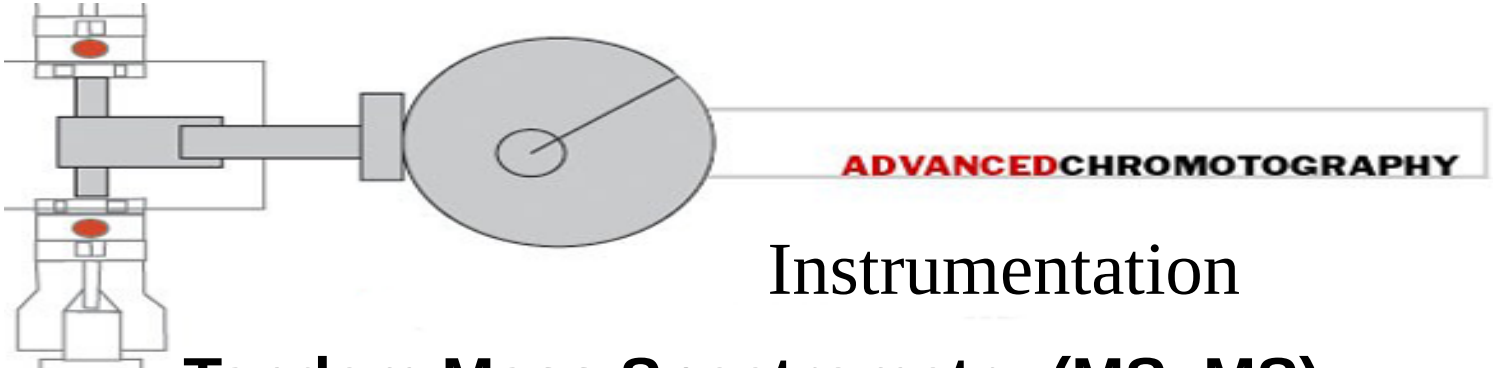


Tandem Mass Spectrometry (MS–MS)

The 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.



Tandem Mass Spectrometry (MS–MS)

The 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.

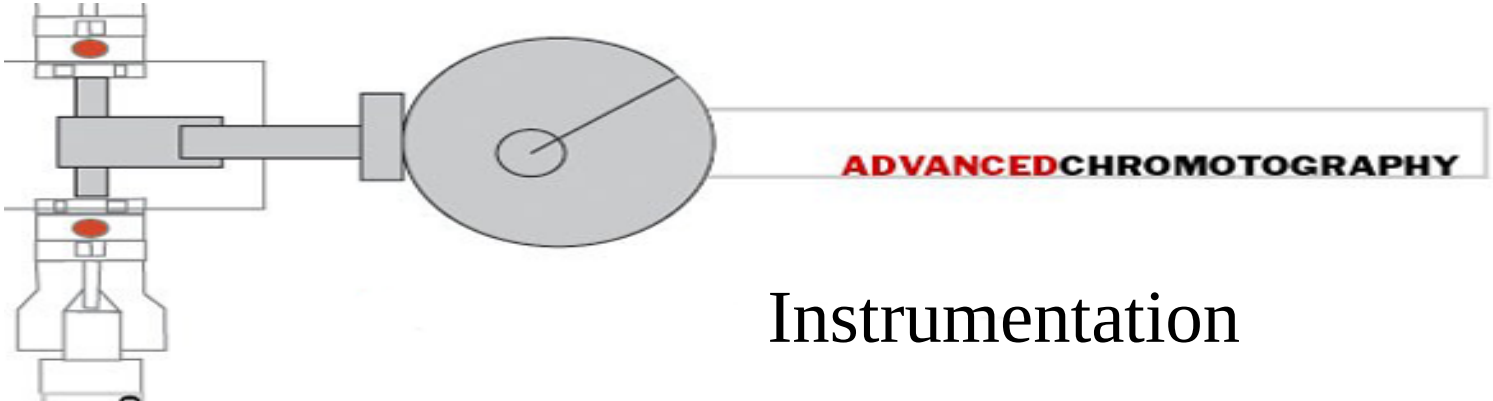
Instrumentation

Tandem Mass Spectrometry (MS–MS)

The 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, CO2, 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.



Instrumentation

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.

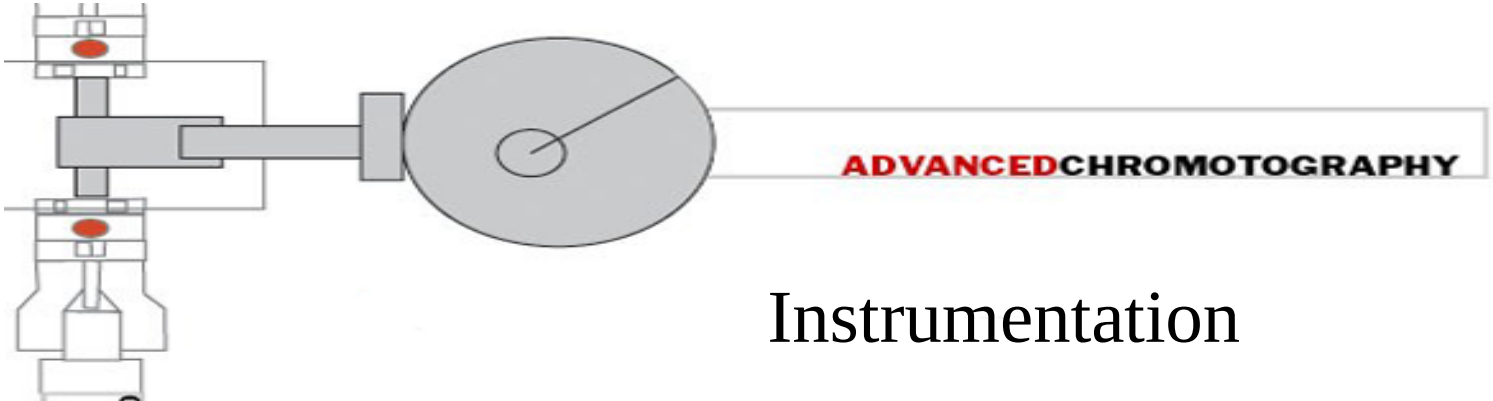
Instrumentation

Tandem Mass Spectrometry (MS–MS)

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.



Instrumentation

Tandem Mass Spectrometry (MS–MS)

Selected-Decomposition Monitoring Applications

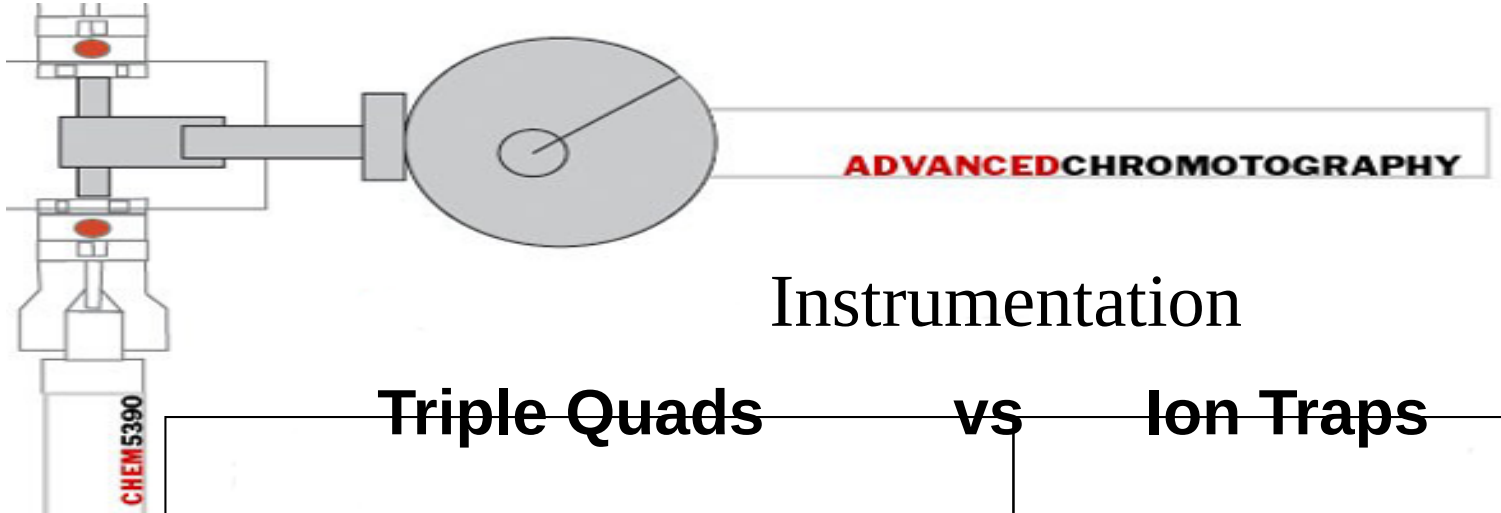
The compound under scrutiny must be known and have been well-characterized previously before this type of experiment is undertaken.

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.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

- Triple Quadrupole (QqQ)
  - Two mass filtering quadrupoles bracket an Rf only collision cell.
- Ion Trap (IT)
  - A single ion trap serves as mass analyzer and collision cell.
- Hybrids (e.g. LIT)
  - Instrument is in the QqQ geometry, but one quadrupole can also trap and store ions.

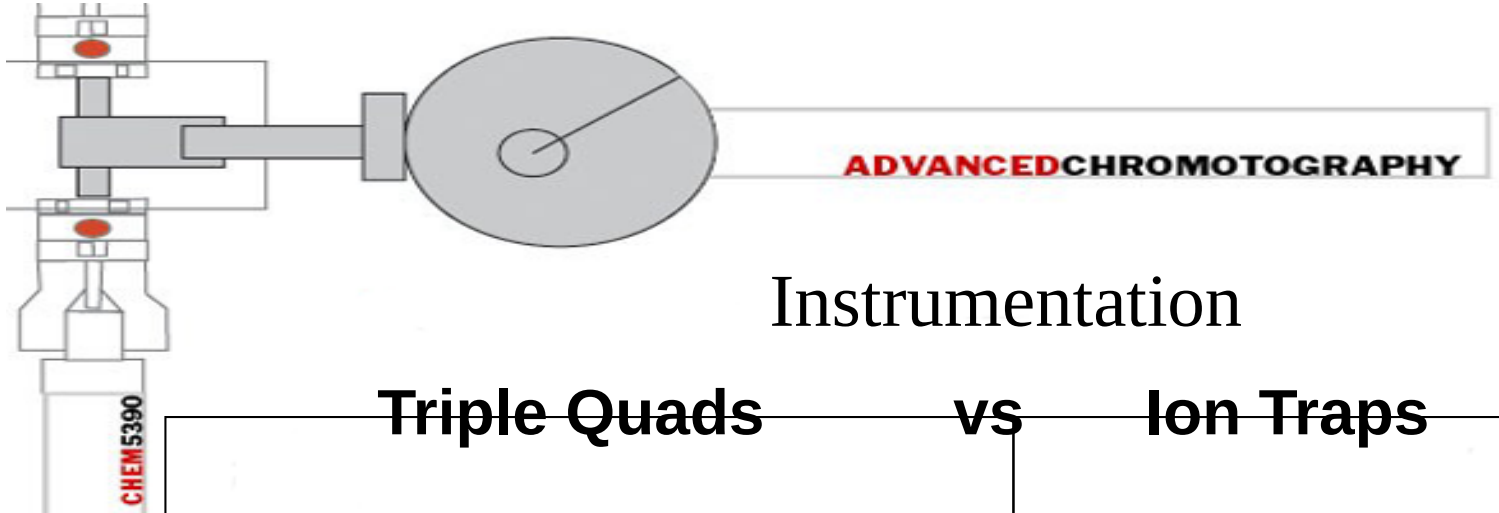


Instrumentation

Triple Quads vs Ion Traps

- Advantages
  - Very sensitive. (SIM)
  - Good for quantitation
  - Some useful MS scanning modes
- Limitations
  - No MS^n
  - Expensive
  - Limited to unit mass resolution.
  - Less sensitive in full scan mode.

- Advantages
  - Higher full scan sensitivity
  - Higher mass resolution
  - MS^n
- Limitations
  - Not as good for quantitations.
  - Space Charge Effects
  - 1/3 cut-off rule*.
  - Cannot perform certain MS experiments.



Instrumentation

Triple Quads vs Ion Traps

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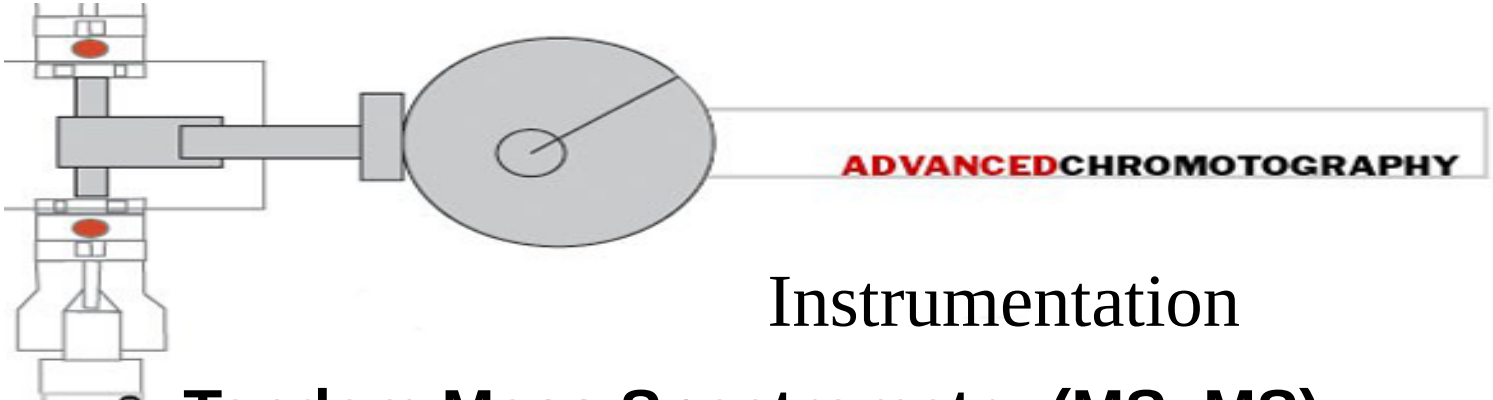
  - Very sensitive. (SIM)
  - Good for quantitation
  - Some useful MS scanning modes
- Limitations
  - No MS^n
  - Expensive
  - Limited to unit mass resolution.
  - Less sensitive in full scan mode.

- 1/3 cut-off rule*.

The low mass cut-off, or so-called “1/3 Rule”, is where the IT fails to trap the ions at the lower end of the m/z range (below ~1/3 the m/z value of the precursor ion) during resonance excitation CID tandem MS.



Instrumentation

Tandem Mass Spectrometry (MS–MS)

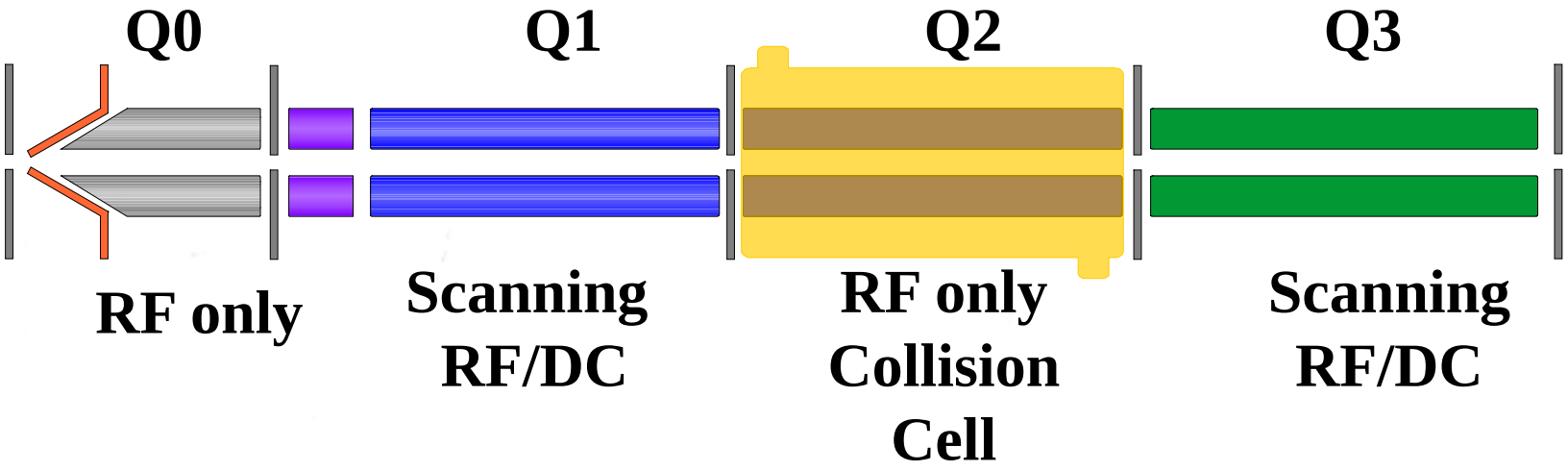
The Triple Quadrupole

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.

Instrumentation

Triple Quad



Q1 and Q3 are standard mass filter quadrupoles.

The can scan masses sequentially (e.g. 50 to 500 amu)

The can be used to select a single mass.

Q2 is an RF only quadrupole that is in a gas filled chamber.

Q2 is the “collision cell” where mass fragmentation occurs.

Q2 does not filter ions. It accepts all ion sent to it by Q1 and passes all ions formed by collision to Q3 to be sorted.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

The Triple Quadrupole

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.

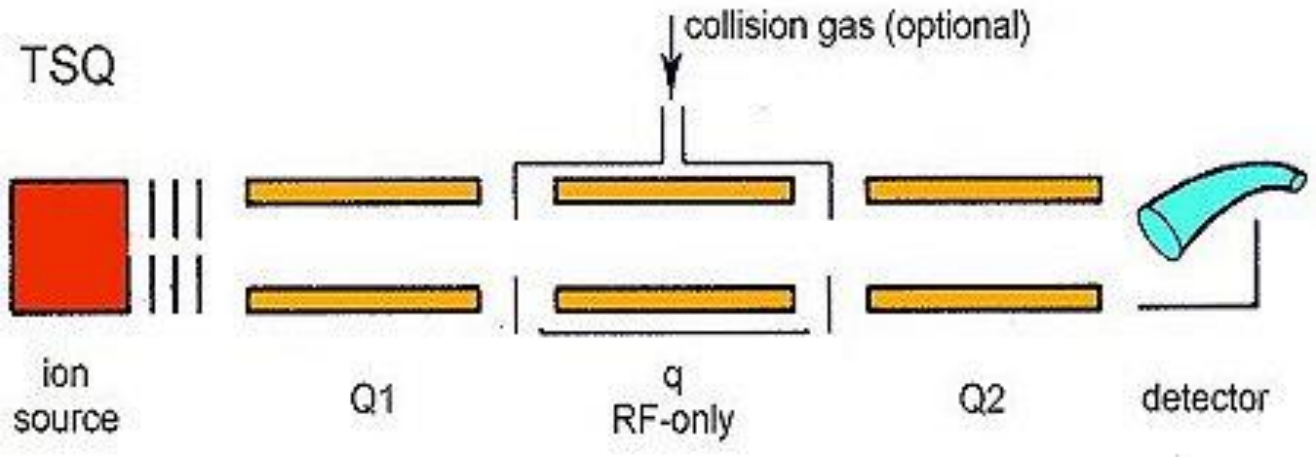
Both sets of rods may be controlled to allow the transmission of ions of a single m/z ratio or a range of m/z values to give the desired analytical information.

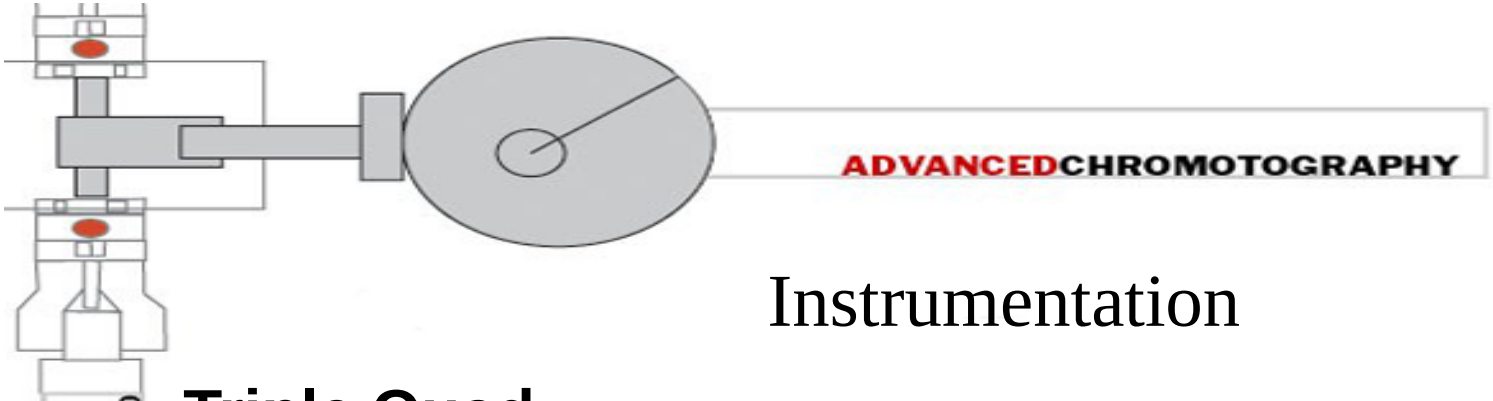
Instrumentation

Tandem Mass Spectrometry (MS–MS)

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.





Instrumentation

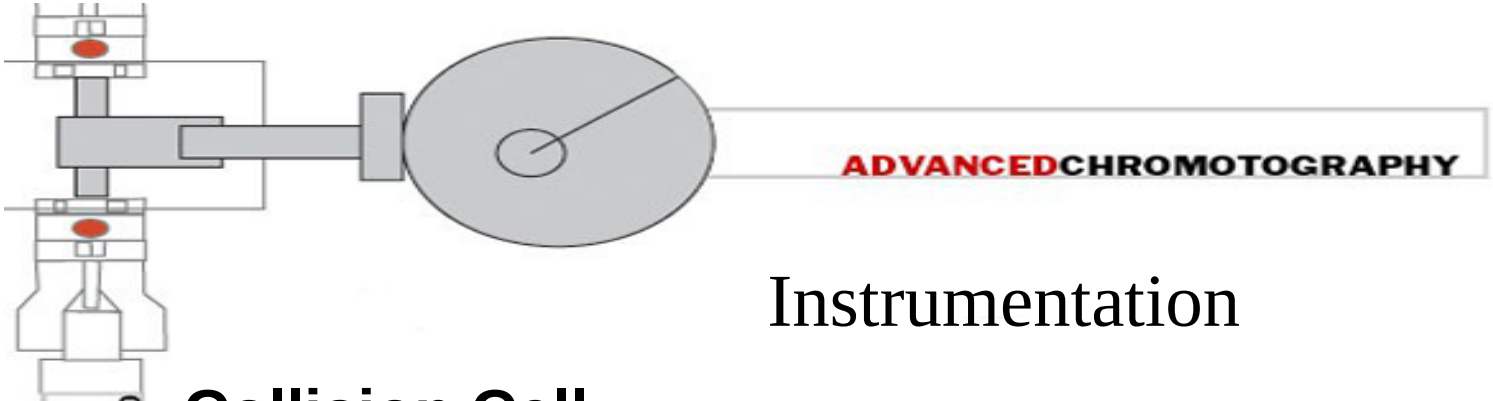
Triple Quad

In scanning mode 99% ions lost between the rods.
Poorer full scan sensitivity

In SIM (selective ion monitoring) mode 100% of selected ion reaches detector.
Makes it highly sensitive and great for quantitation

Mass resolution typically limited to “unit” (+/- 0.2 amu)

Fragmentation is controlled by the energy ions have when they enter the collision cell.
Higher energy >> greater fragmentation.



Instrumentation

Collision Cell

LINAC (linear accelerator) Collision Cell

Filled with N_2 gas at roughly $3x10^{-5}$ torr.

Drives ions out, reducing "cross-talk"

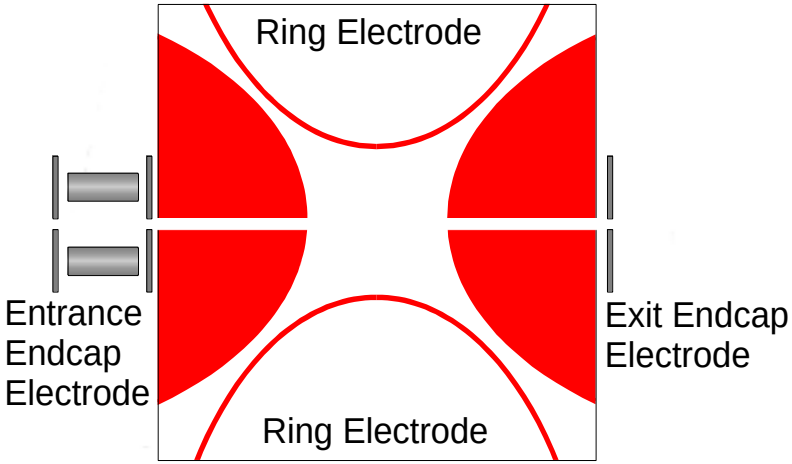
The analyte molecules undergo collision activated disassociation by energetic collision with the N_2 molecules.

The N_2 also acts to "cool" fragments, facilitating transport to the detector.

Instrumentation

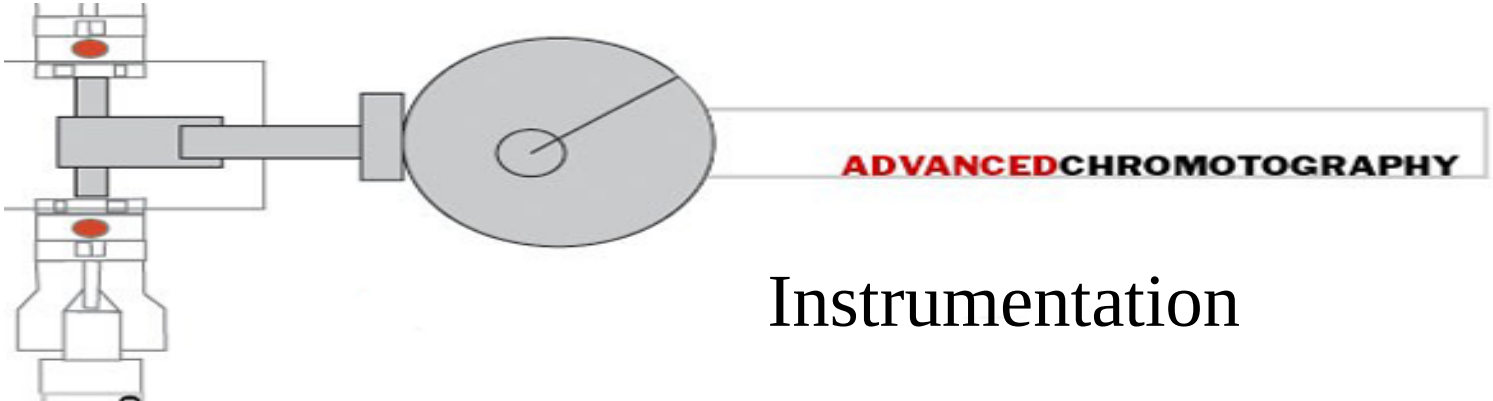
Tandem Mass Spectrometry (MS–MS)

Ion Traps



Ions fill the space between a ring electrode and a pair of end-cap electrodes.

Mass analysis and fragmentation occur in the same space.



Instrumentation

Ion Traps

In full scan mode: Ions fill and are trapped in space then masses are scanned out of the trap sequentially.

Ions are not lost, so full scan sensitivity is better, but filling/closing cycles make them poorer at quantitation.

Mass resolution is controlled by the “speed” at which masses are scanned out of the trap.

slower scanning = better mass resolution.

Instrumentation

Tandem Mass Spectrometry (MS–MS)

The Hybrid Mass Spectrometer

When the first quadrupole of a triple quadrupole is replaced by a another mass analyzer, the instrument is termed a hybrid.

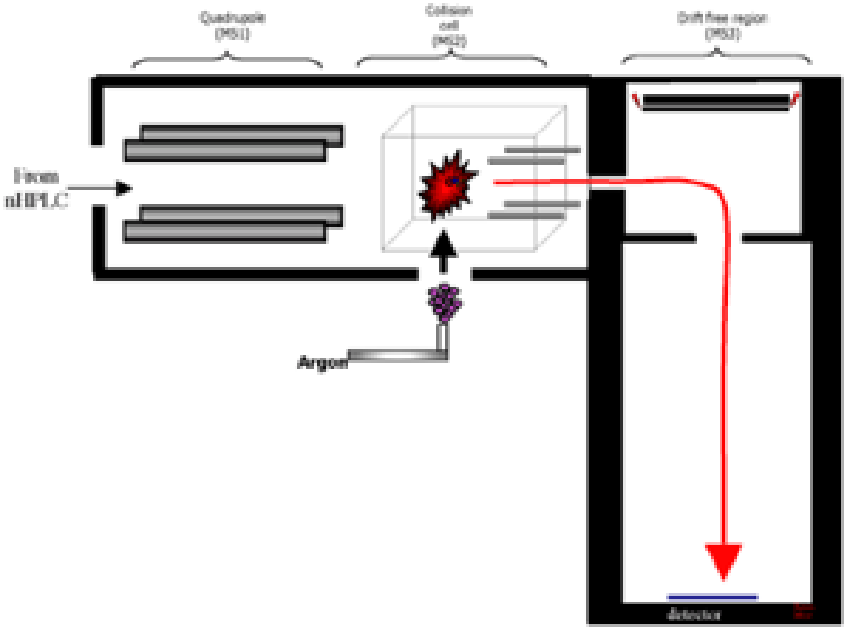
The advantage of this configuration is that the instrument can be used under high-resolution conditions to select the ion of interest.

Instrumentation

The Hybrid Mass Spectrometer

QTOF (Quadrupole Time-of-flight)

In the QTOF, precursor ions are selected in the Quadrupole and sent to the Collision Cell for fragmentation.
The generated product ions are detected by time-of-flight (TOF) mass spectrometry.



Instrumentation

The Hybrid Mass Spectrometer

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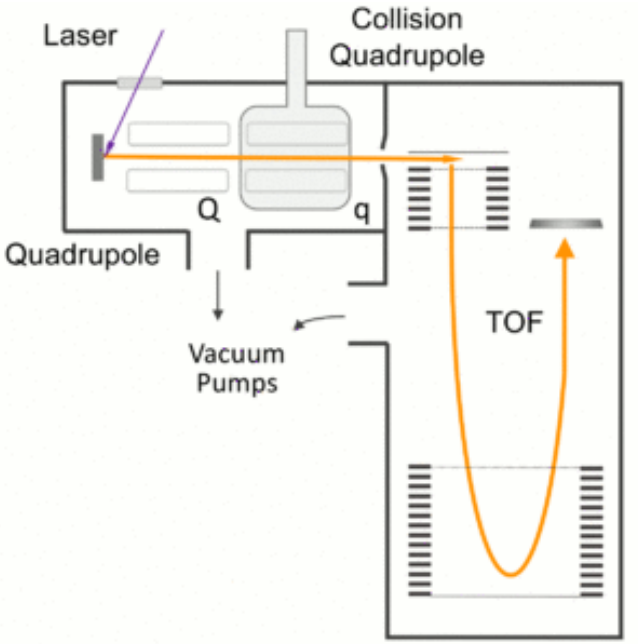
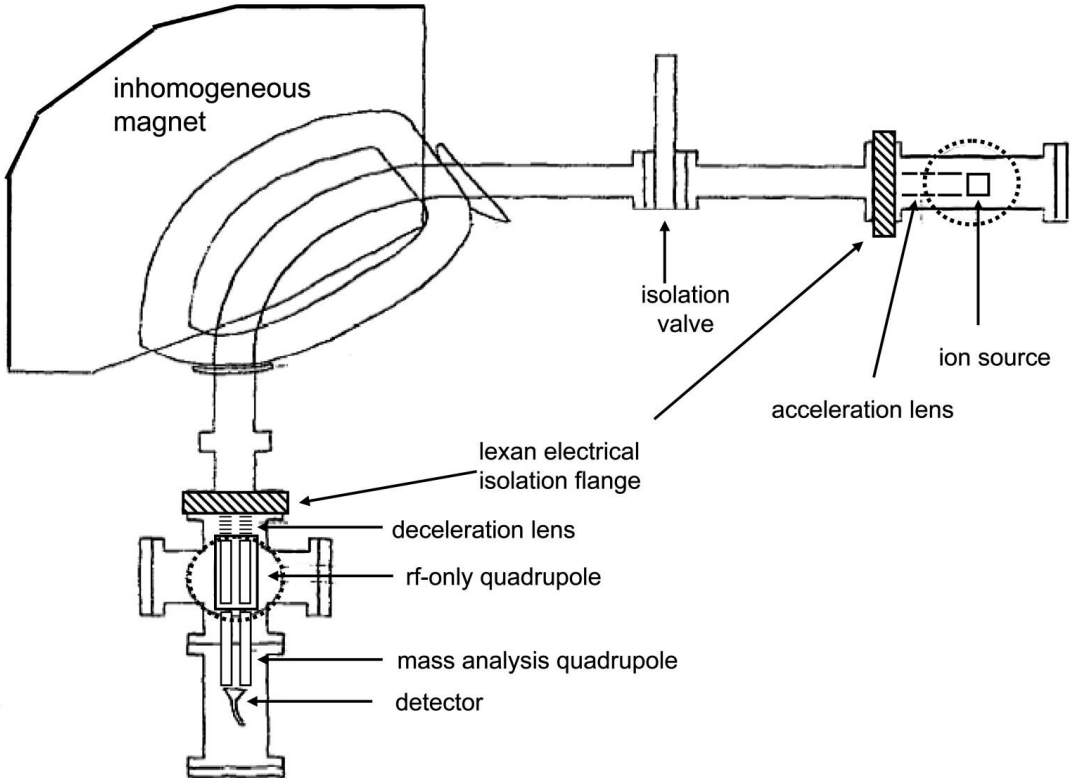


Instrumentation

The Hybrid Mass Spectrometer

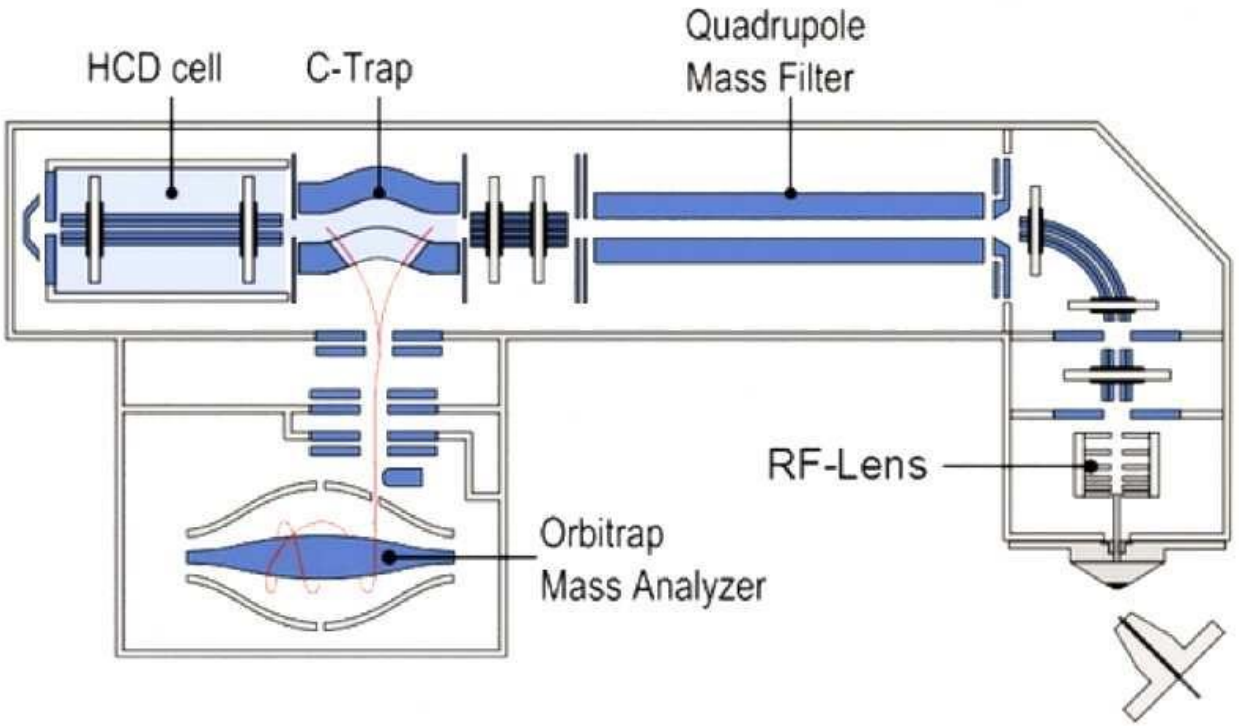
Double focusing Quad



Instrumentation

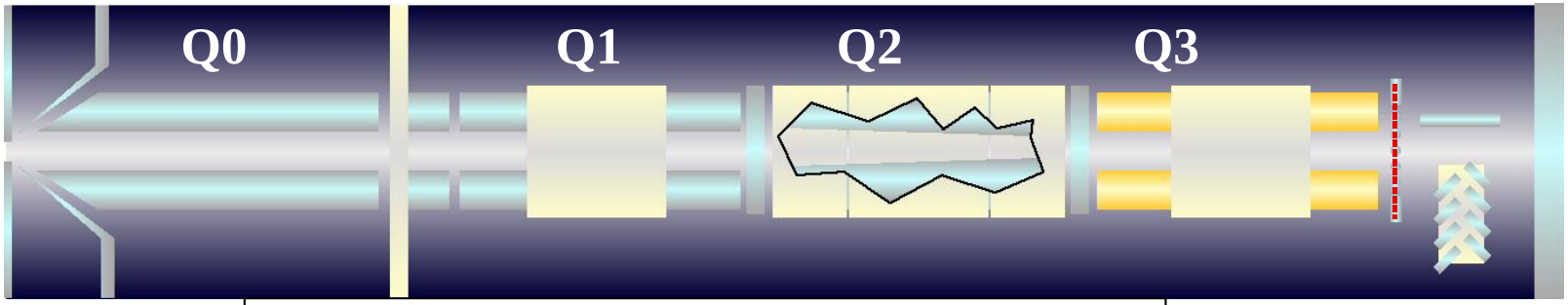
The Hybrid Mass Spectrometer

Ion Trap/Quad



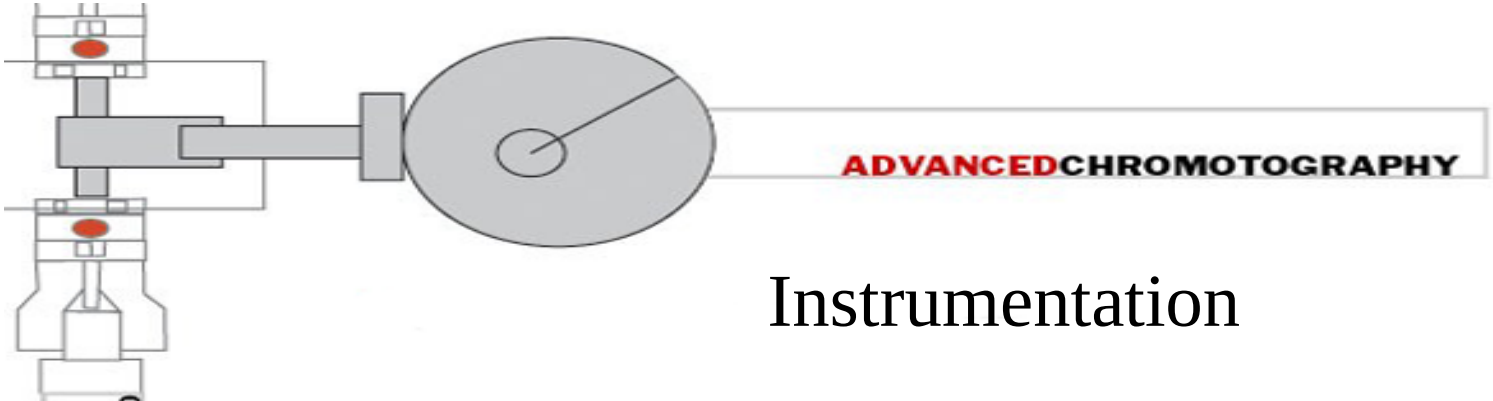
Instrumentation

Ion Traps – Hybrid



Applied Biosystems 3200 Qtrap System

Typically in the same configuration as a triple quadrupole instrument.
On the Qtrap Q3 is the hybrid quadrupole dubbed a “linear ion trap” or LIT.
Q3 can function as a quadrupole OR an LIT.



Instrumentation

Advantages of LIT vs. IT

Has a larger "volume" so it can be filled with more ions before exhibiting space charge effects.

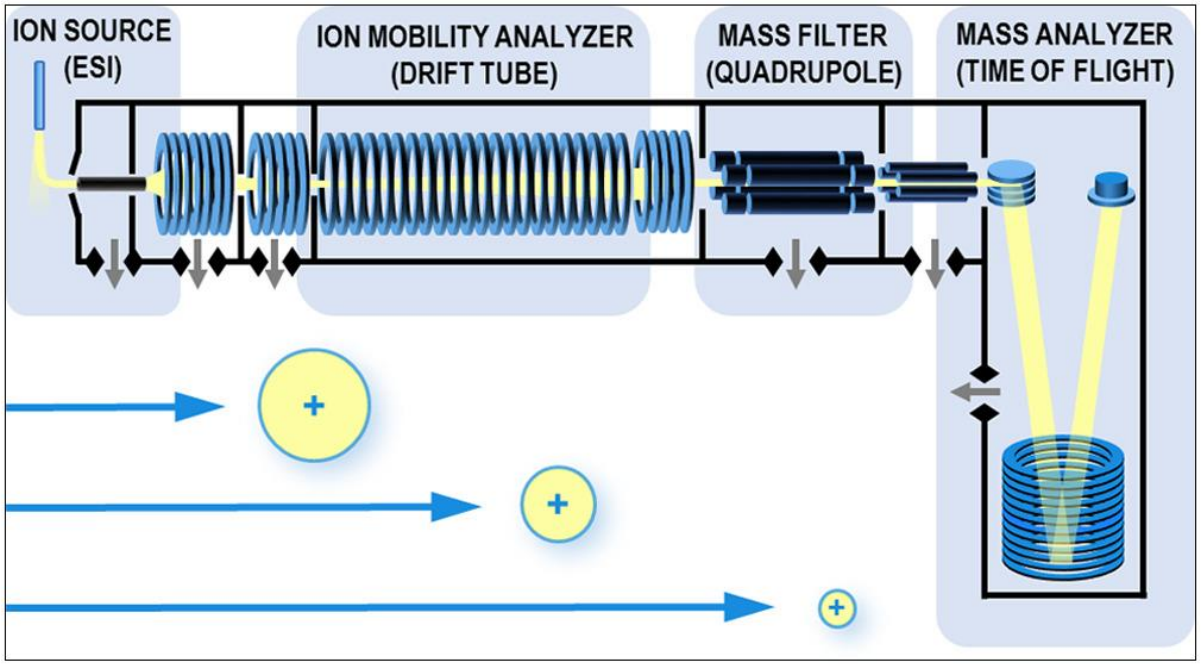
Ions are formed outside the trap, so it is not limited by the 1/3 rule.

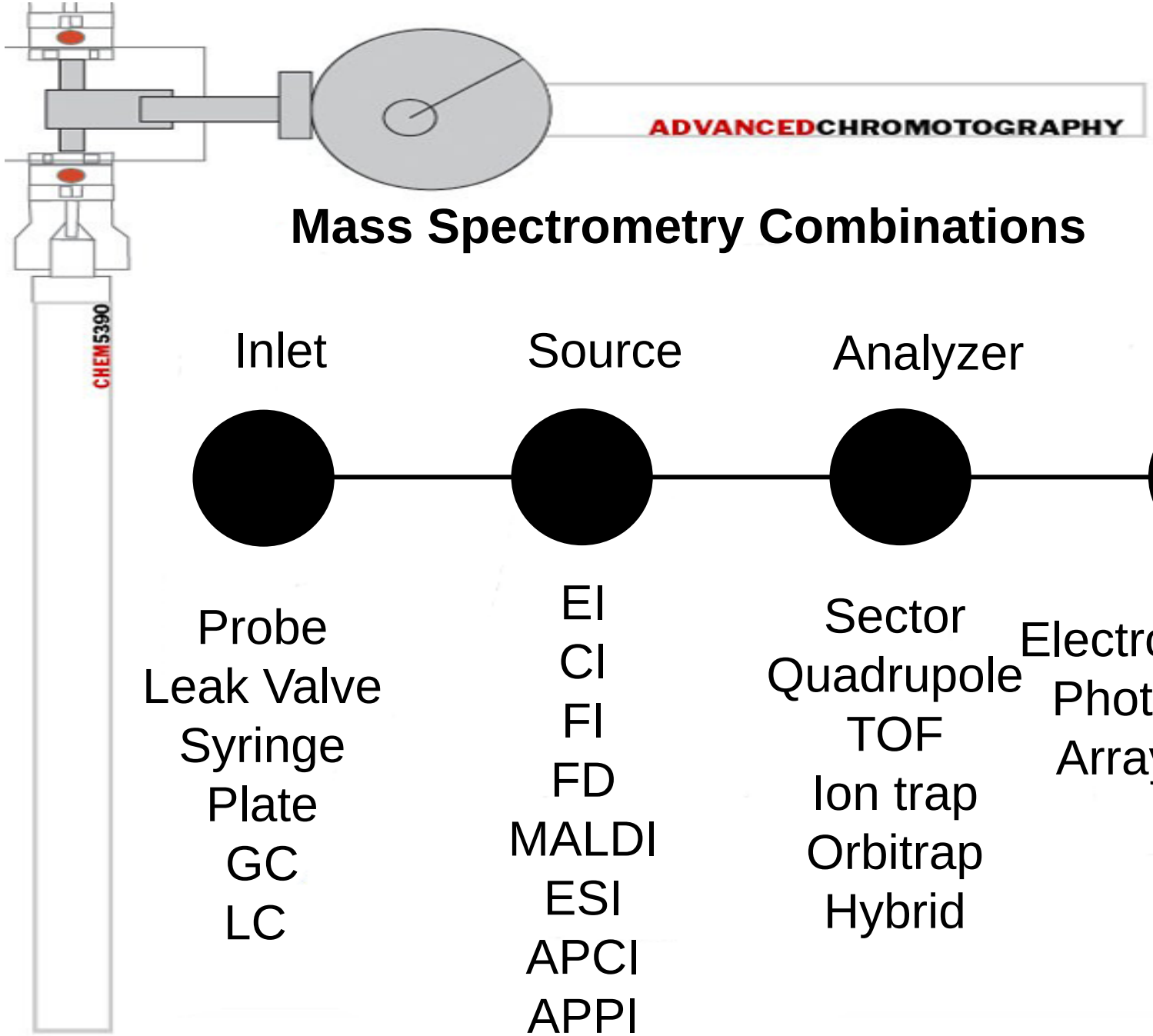
Can perform MS/MS/MS experiments by selecting an ion and fragmenting it using the spillover collision gas. (1/3 rule applies here…)

Instrumentation

The Hybrid Mass Spectrometer

Ion Mobility/Quad/TOF





Mass Spectrometry Combinations

Inlet	Source	Analyzer	Detector
Probe	EI	Sector	Electron Multiplier
Leak Valve	CI	Quadrupole	Photomultiplier
Syringe	FI	TOF	Array Detector
Plate	FD	Ion trap	MCP
GC	MALDI	Orbitrap	
LC	ESI	Hybrid	
	APCI		
	APPI		

Tandem Mass Spectrometry (MS–MS) Applications

Structural Determination

Ginseng root contains more than a dozen biologically active saponins which contain multiple oligosaccharide chains. Structural elucidation of these compounds can be complicated.

MS-MS allows multiple stages of precursor ion isolation and fragmentation. This stepwise fragmentation permits a great deal of structural information.

Tandem Mass Spectrometry (MS–MS) Applications

Structural Determination

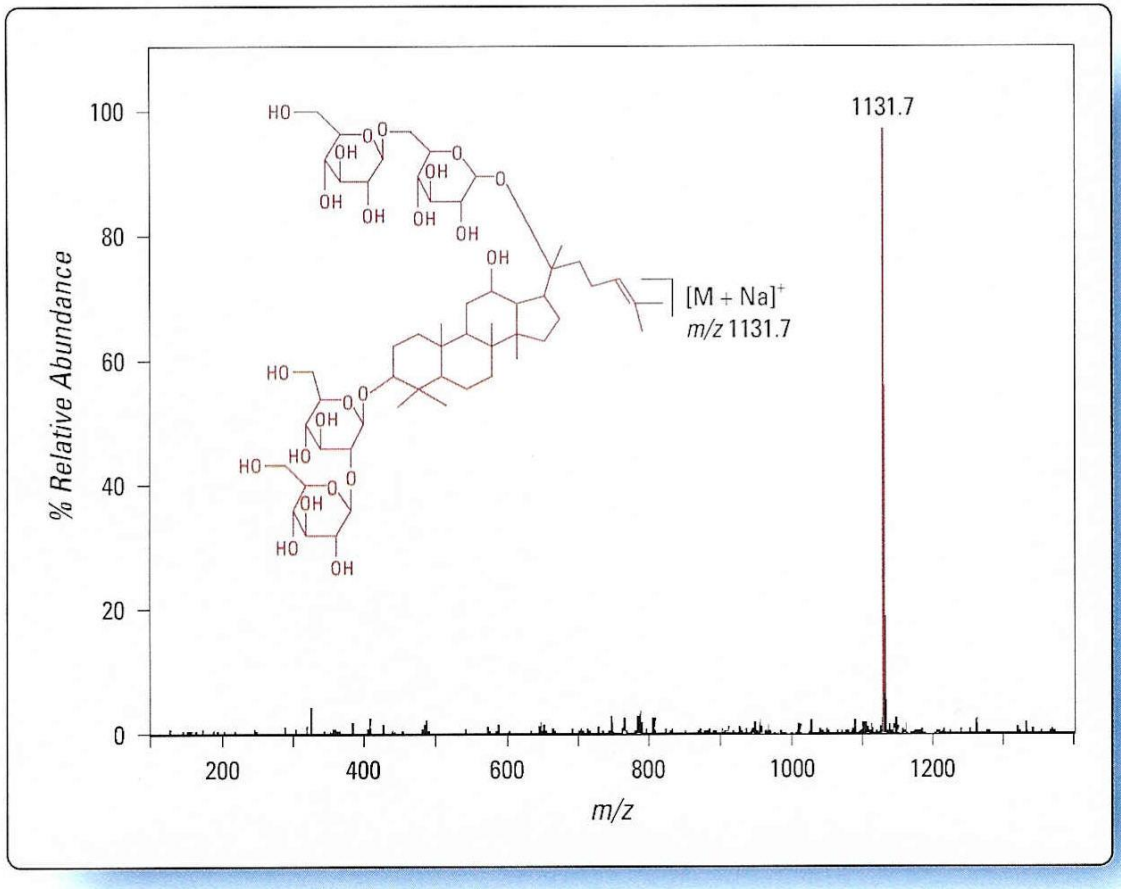


Figure 24. Full scan mass spectrum of ginsenoside Rb1 showing primarily sodium adduct ions

Tandem Mass Spectrometry (MS–MS) Applications

Structural Determination

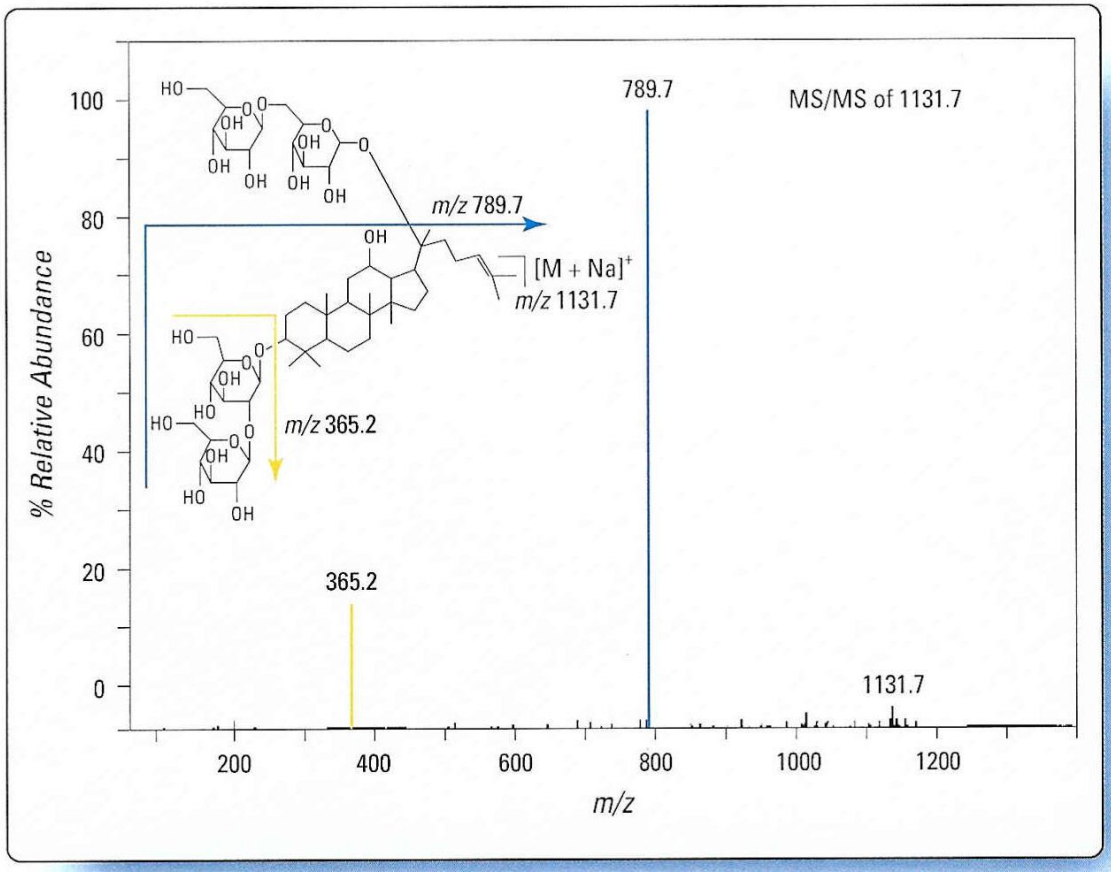
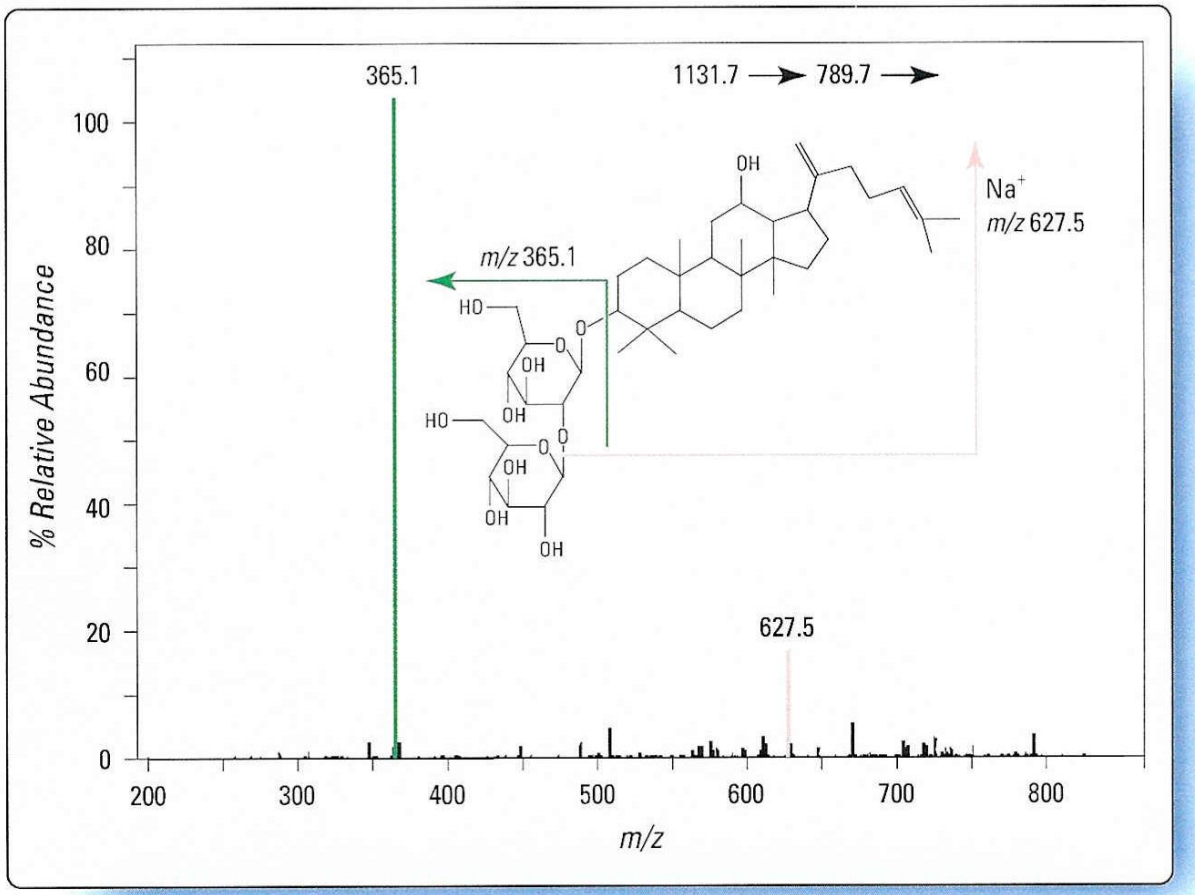


Figure 25. Full scan product ion (MS/MS) spectrum from the sodium adduct at *m/z* 1131.7

Tandem Mass Spectrometry (MS–MS) Applications

Structural Determination

Figure 26. Subsequent full scan product ion spectrum (MS^3) from the ion at *m/z* 789.7



Tandem Mass Spectrometry (MS–MS) Applications

Pharmaceutical Applications

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

Pharmaceutical Applications

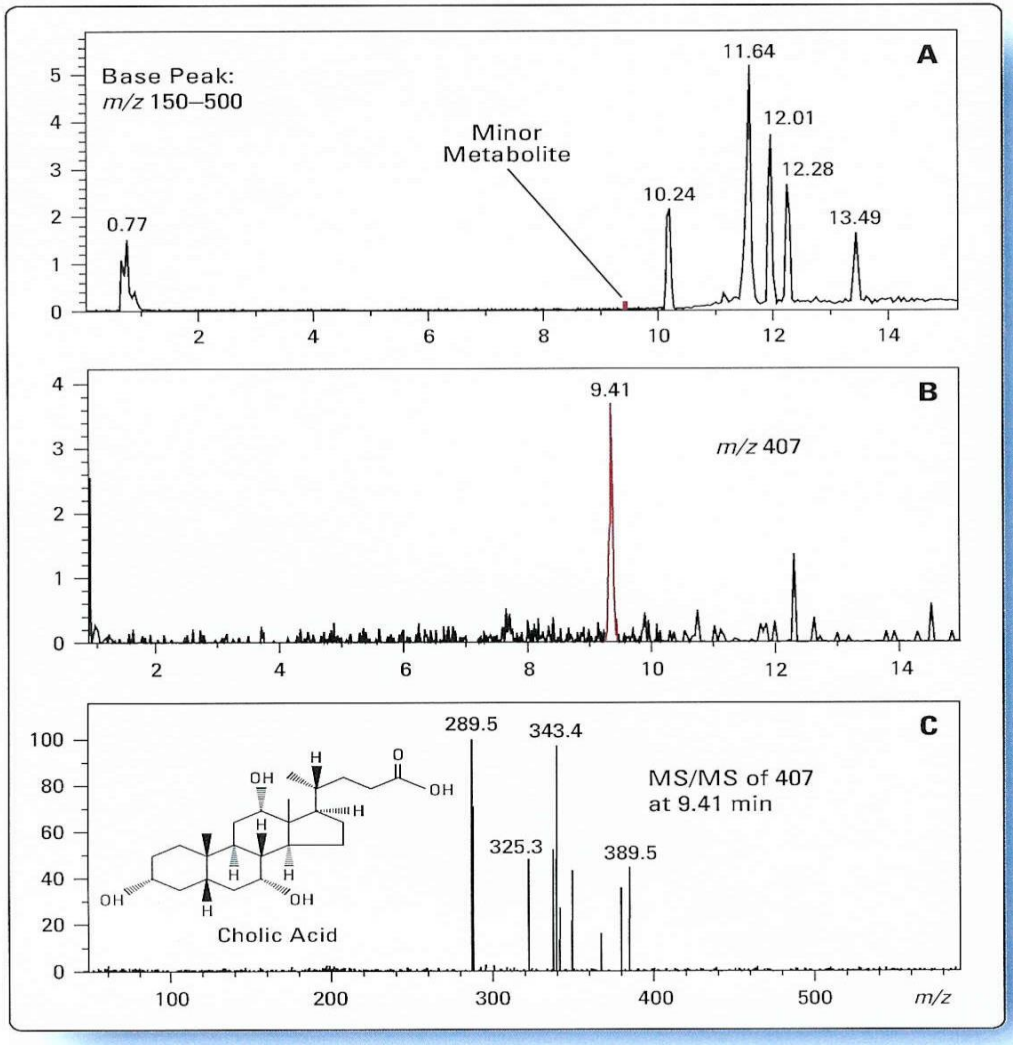


Figure 28. Identification of a minor metabolite of deoxycholic acid through MS/MS

Tandem Mass Spectrometry (MS–MS) Applications

Food Applications

Vitamin D is essential in nutrition. Traditional GC/MS analysis methods for vitamin D3 in feed extracts require extensive and time-consuming sample preparation.

MS-MS greatly speeds analysis time without the need of extensive preparation.

Tandem Mass Spectrometry (MS–MS) Applications

Food Applications

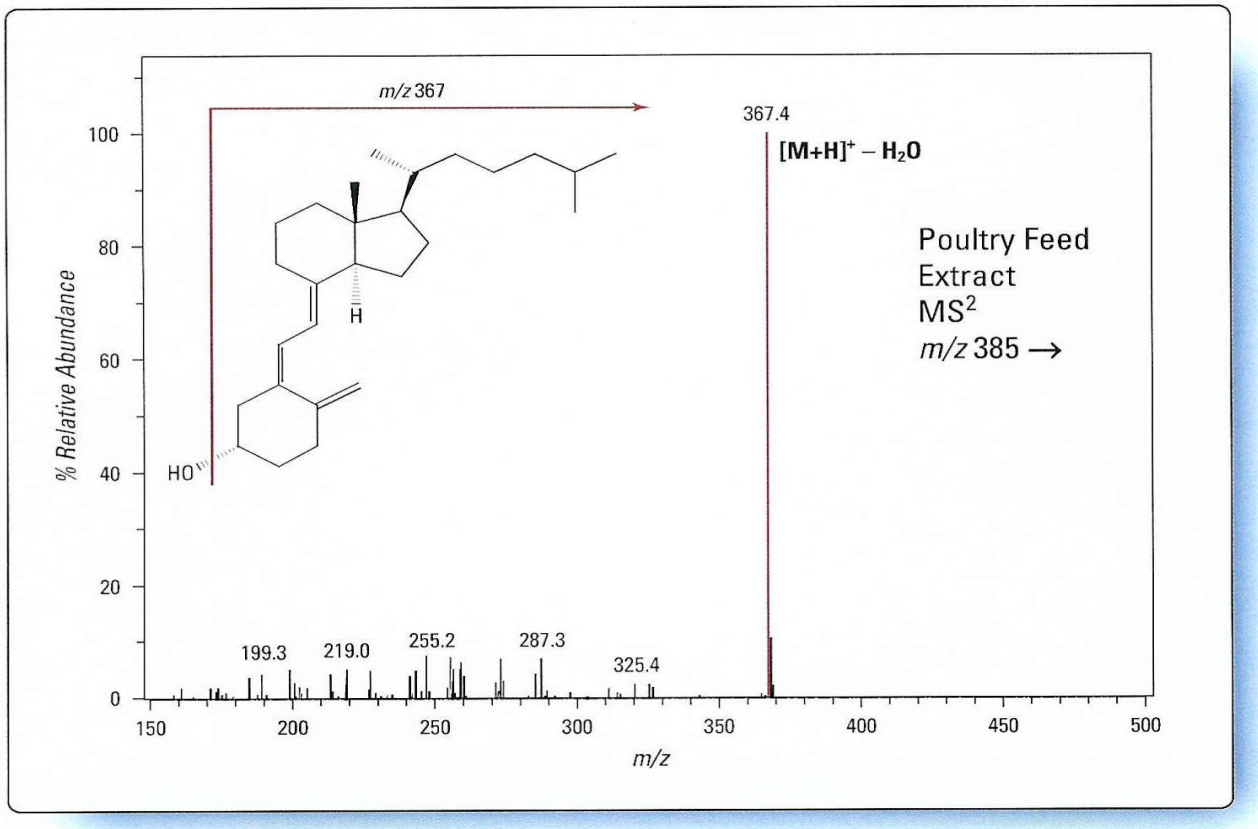


Figure 34. Full scan MS/MS product ion spectrum from the precursor ion at *m/z* 385 showing primarily the nonspecific loss of a water molecule

Food Applications

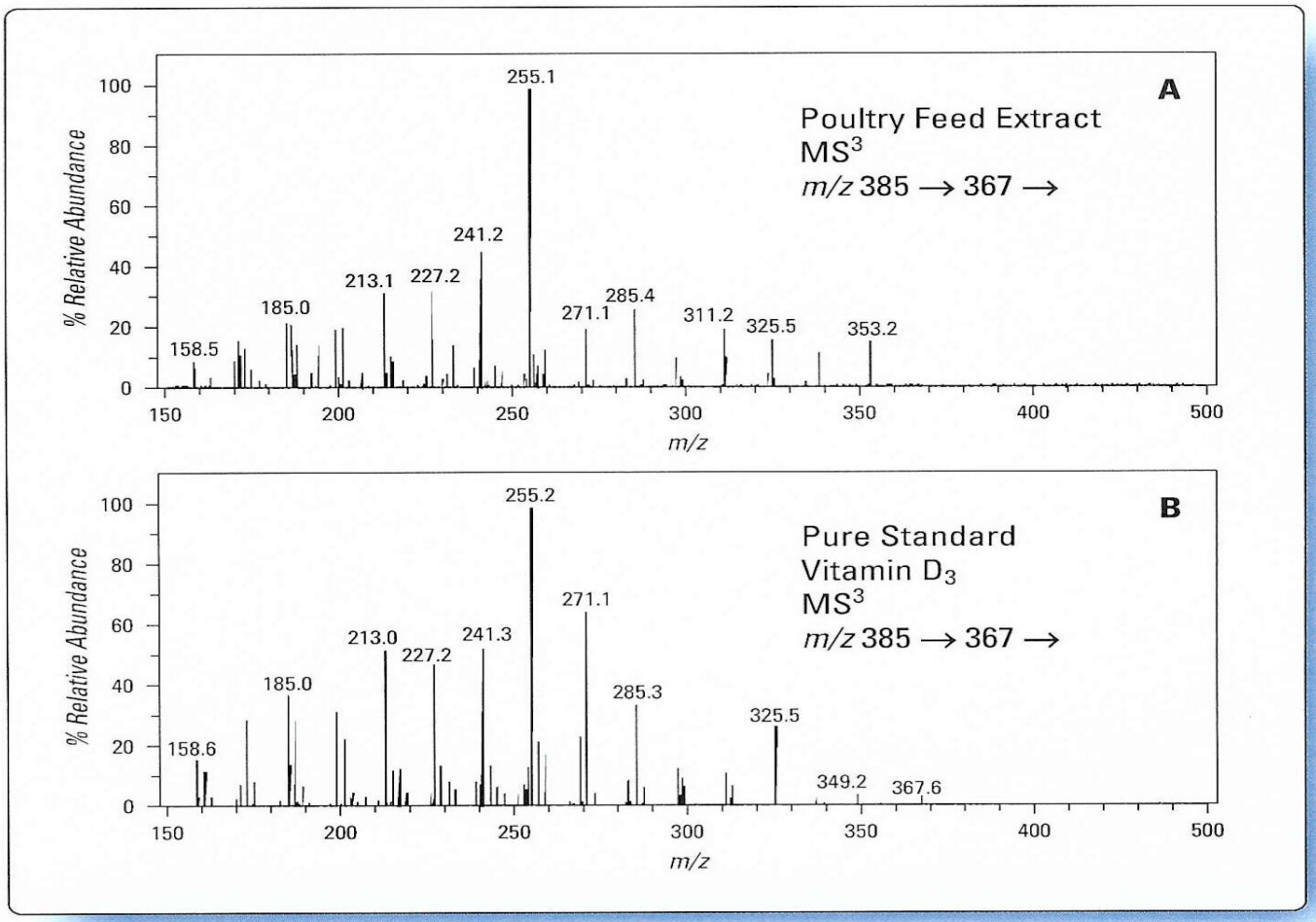
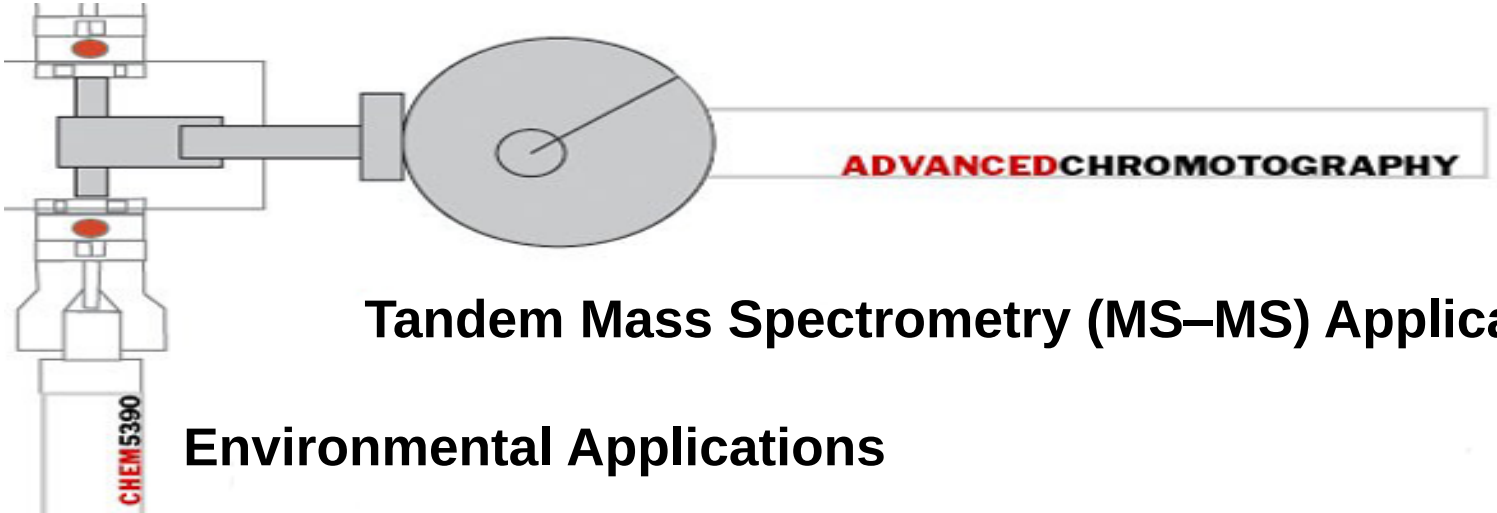


Figure 35. Full scan MS^3 product ion spectra show much more structural information



Tandem Mass Spectrometry (MS–MS) Applications

Environmental Applications

Pesticides in foods and beverages can be a significant route to human exposure.

Analysis of the carbamate pesticide carbaryl in extracts of whole food by ion trap LC/MS/MS proved more specific than previous analysis by HPLC/Fluorescence/MS because coeluting compounds were a problem.

Environmental Applications

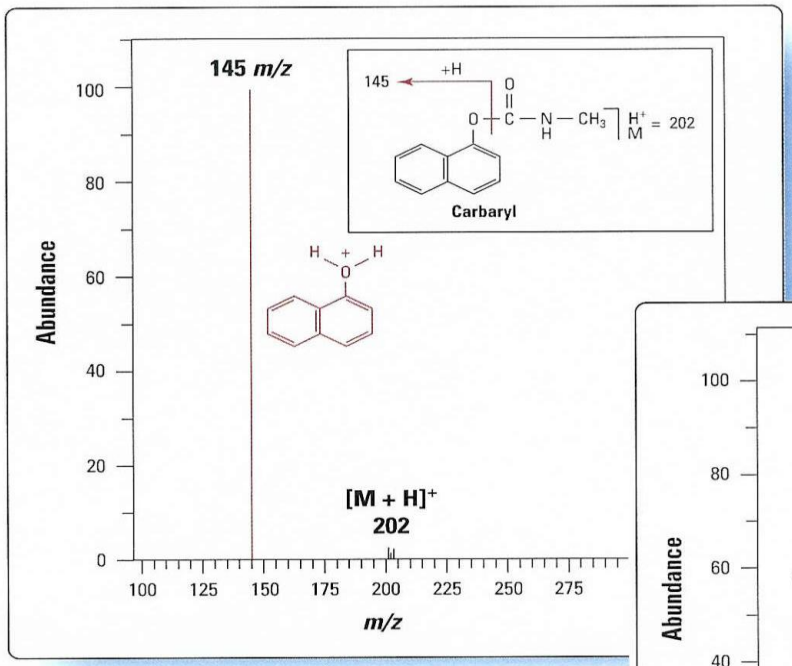


Figure 37. Full scan product ion spectrum generated by collision-induced dissociation of the $[M + H]^+$ carbaryl ion at *m/z* 202

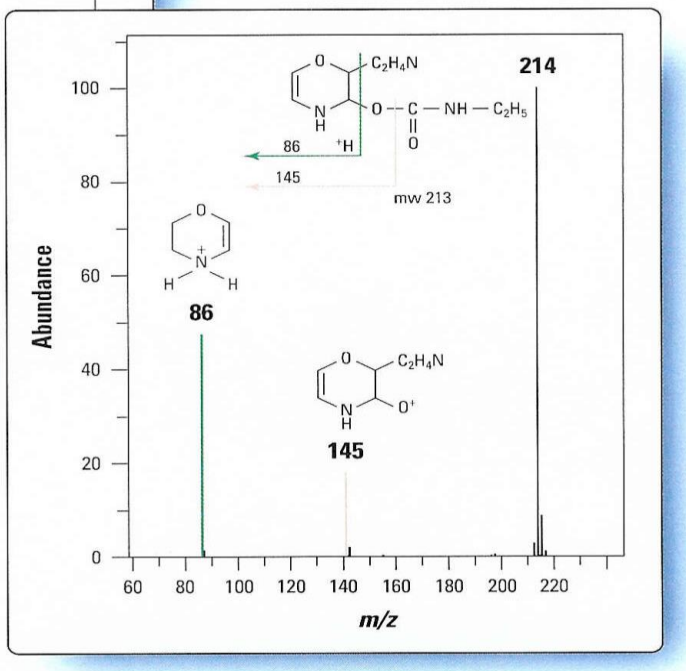
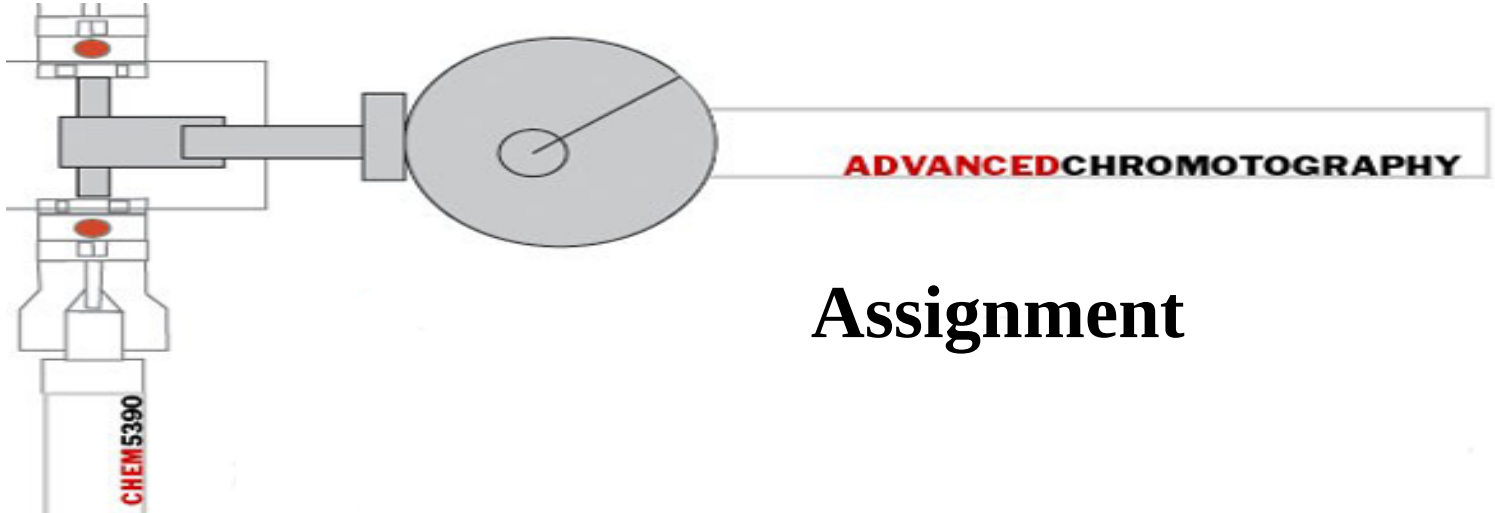


Figure 38. Full scan product ion spectrum of coeluting compound that produced false positives in HPLC fluorescence analysis



Assignment

- Read Chapter 6: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.