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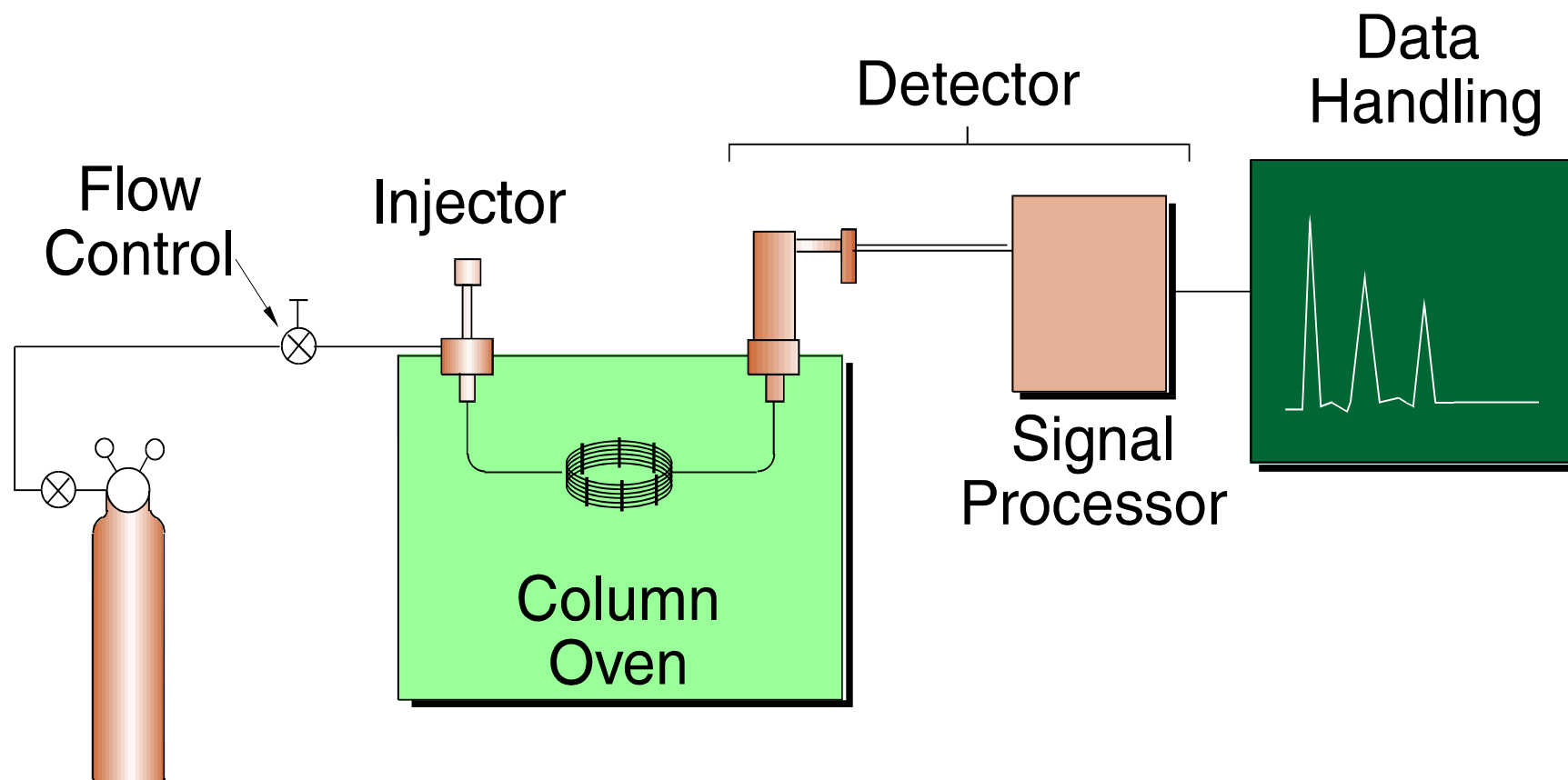
GC-MS 概論

Single Quadrupole and Ion Trap

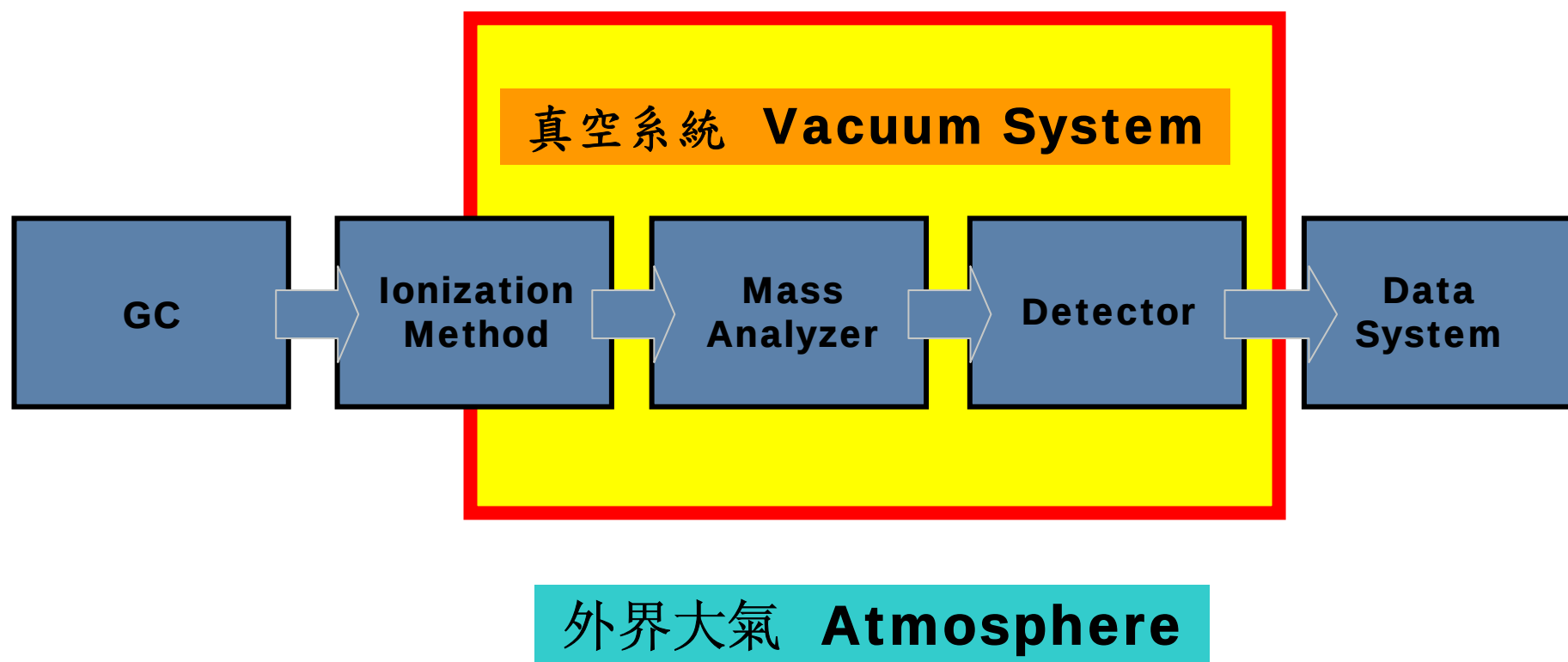
單段四極柱與離子阱

氣相層析儀之基本系統架構

Basic GC System



氣相層析儀 / 質譜儀之基本系統架構



質譜是什麼

Mass Spectrometry

- 特殊的天平：
秤量離子的質量
- 質譜學：
是一門研究氣相離子結構、性質及反應行為的科學



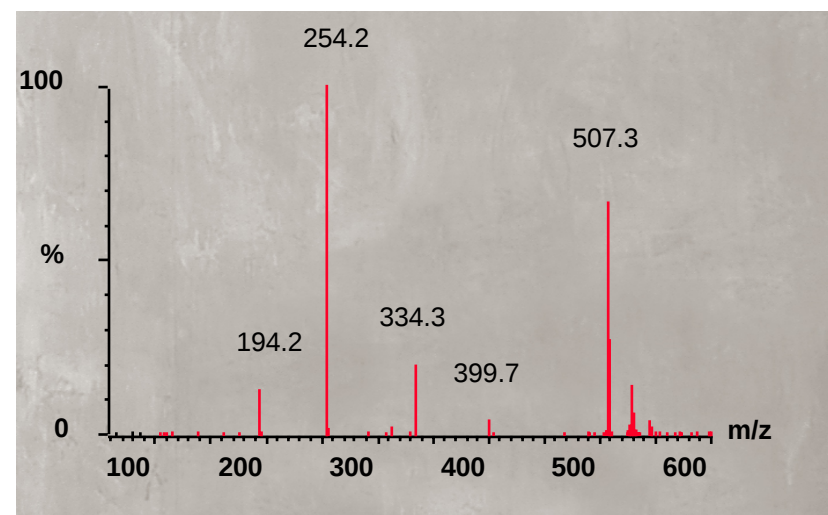
何謂質譜儀 What is Mass Spectrometry

“The basis of MS (mass spectrometry) is the production of ions that are subsequently separated or filtered according to their mass-to-charge (m/z) ratio and detected. The resulting mass spectrum is a plot of the (relative) abundance of the produced ions as a function of the m/z ratio.”

質譜儀之原理乃是將樣品所產生的離子，依據其質荷比(質量 / 電荷之比值)進行分離或是篩選，最後執行偵測之儀器。將這些離子依據質荷比的分佈狀況，與各種離子的(相對)強度，便可以得到欲分析樣品物質在離子化之後的質譜圖

質譜能做什麼

- 定性：化合物的結構
- 定量：混合物的組成含量
- 領域：化學、生物學、醫學、藥學、環境、物理、材料、能源等



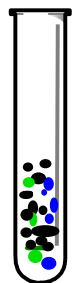
質譜能做什麼

4S 特性：

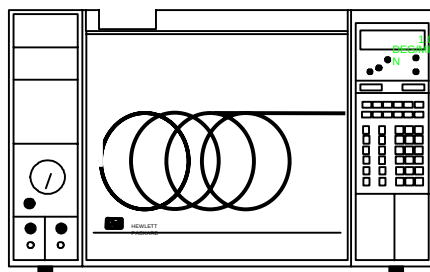
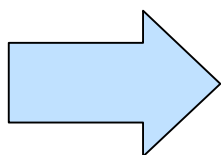
- Sensitivity (感度好)
- Speed (快速)
- Specificity (專一性佳)
- Stoichiometry (化學計量)

常用概念

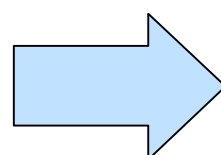
分析流程



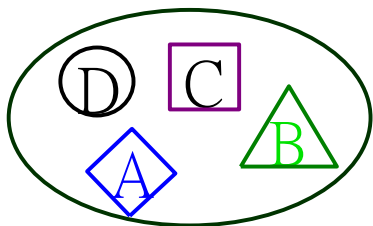
Sample



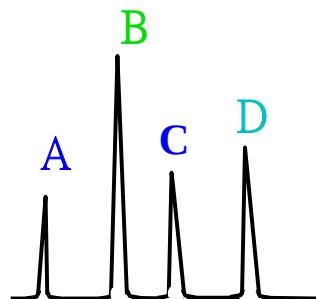
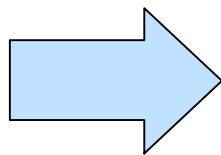
Gas Chromatograph



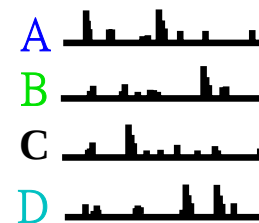
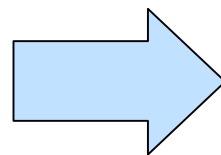
Mass Spectrometer (MS)



Sample



Separation



Identification

GC-MS v.s GC

- GC-MS方法的定性參數增加，定性可靠。
- GC-MS檢測感度高於氣相層析儀的其他檢測器。
- GC-MS可採用選擇離子模式分離氣相層析儀上所不能分離的化合物，藉降低雜訊以提高信噪比(S/N)。
- 一般經驗來說質譜儀定量不如氣相層析儀。但是採用同位素稀釋法和搭配使用內標準品等技術，GC-MS依舊可以達到較高精度的定量分析。



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Ionization Methods

離子化的方式

Electron Impact

電子撞擊法

Ionization Methods 離子化的方式

化學游離 **CI (NICI; PICI)**

電子衝擊 **EI**



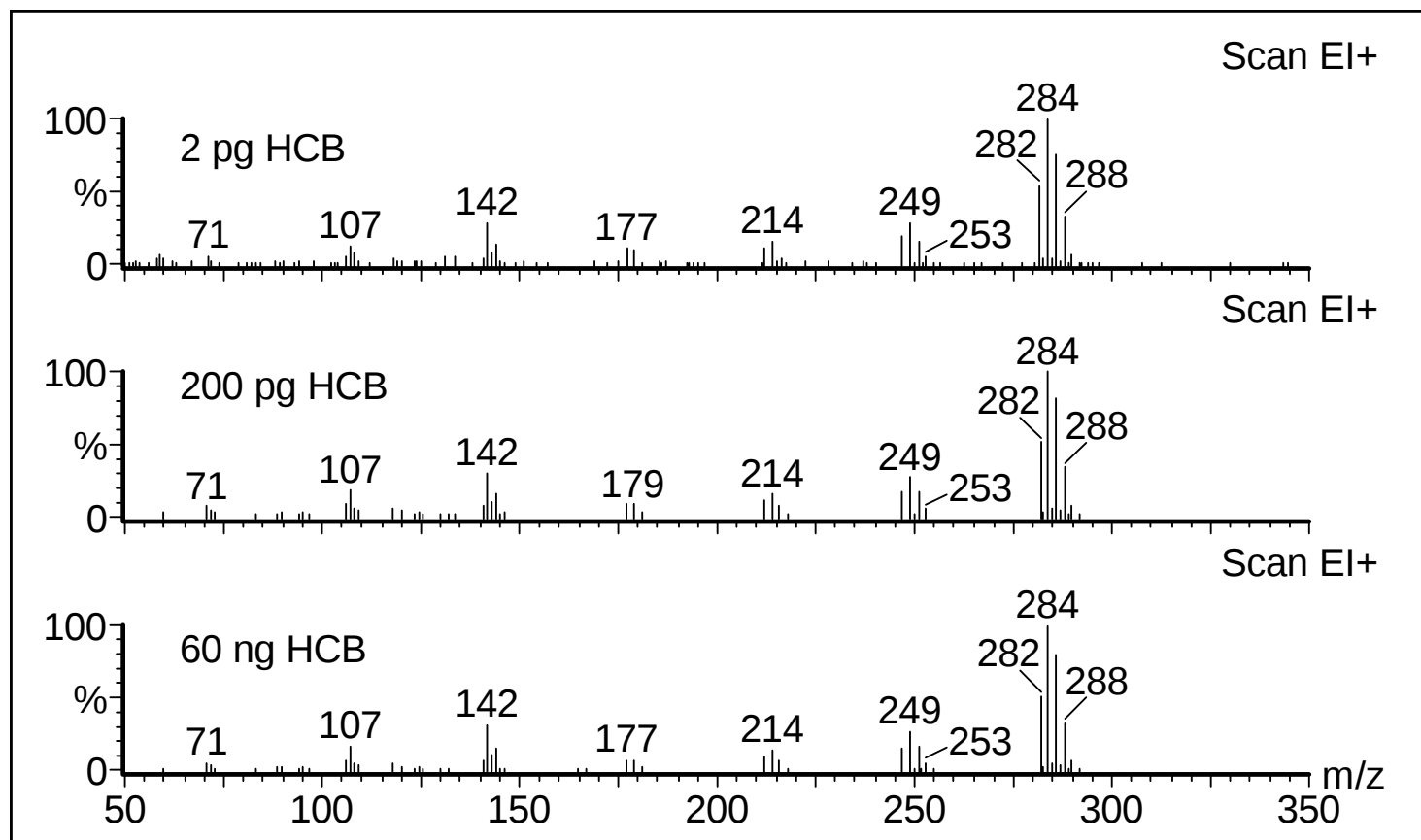
碎片少 **No Fragments**

碎片多 **Fragments**

Soft ionization gives low fragmentation
Molecular weight determination
Produces “theoretical” spectra
High sensitivity (low pg levels)

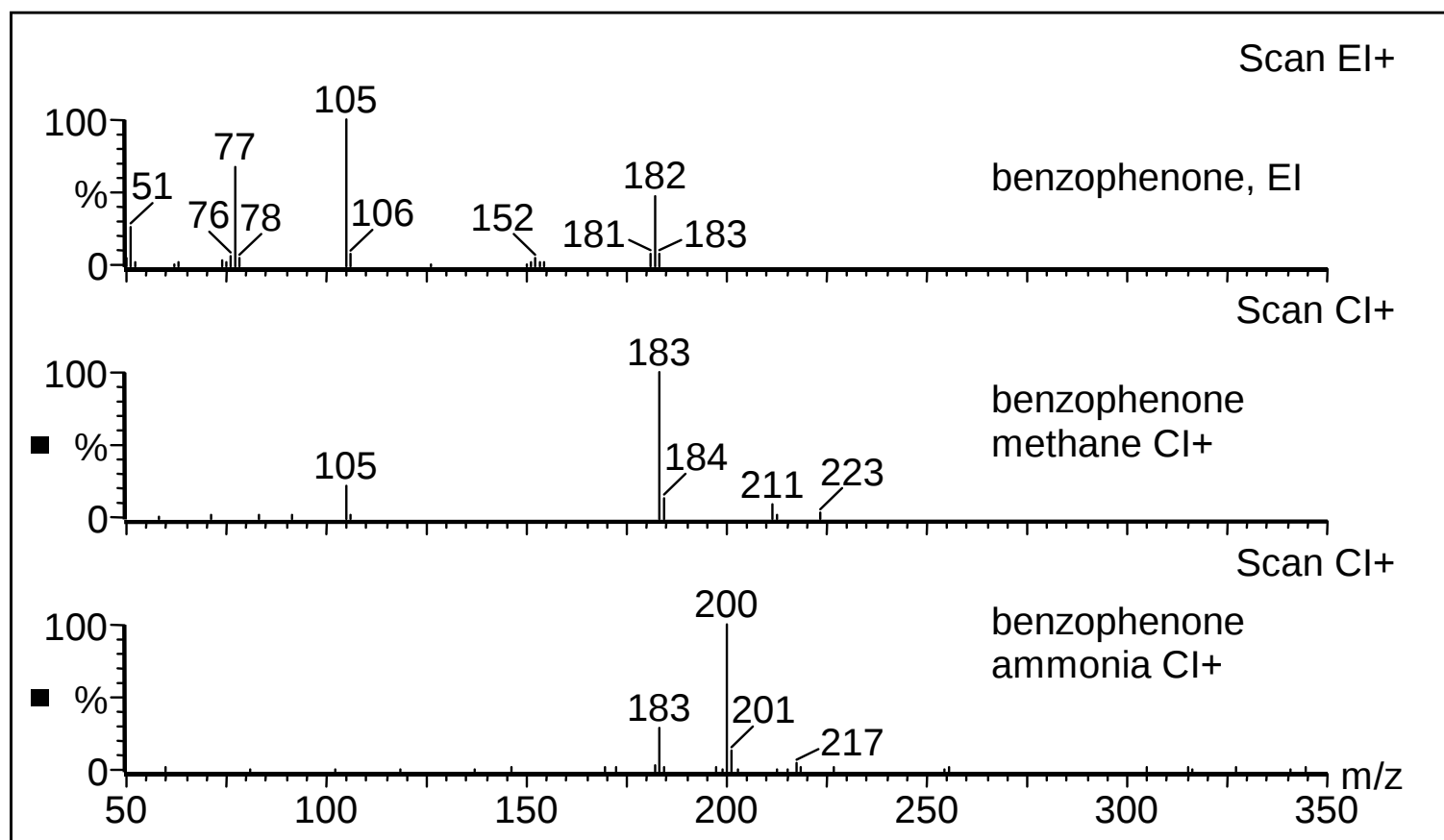
El Spectra from TRACE DSQ

- HCB spectra from 2 pg up to 60 ng



CIP Spectra

- Comparison of EI with PCI using methane and ammonia



電子游離法 Electron Ionization

- GCMS最常見的游離方式
 - 可用於定量與定性分析工作
 - 可得到樣品分子的質譜圖
 - 樣品離子碎片之指紋圖譜
 - 搭配滯留時間的比對可作為陽性結果的鑑定工作之用
 - 用單一離子來做定量計算, 用單一或多種離子來做定性鑑別工作
-
- Most common method of ionization for GC-MS
 - Used as both a qualitative and quantitative tool
 - Produces mass spectra of molecules
 - Fragmentation fingerprints
 - Combine with retention time for positive identification
 - Use single ion for quantitation with one or more ions for verification



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Ionization Methods

離子化的方式

Chemical Ionization

化學游離法



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Ionization Methods

離子化的方式

Positive Ion Chemical Ionization

正離子化學游離法

正離子化學游離法

- 試劑氣體與電子反應，形成高活性的物質
Reagent gas reacts with electrons to form reactive species
- 產生“軟性”的游離模式，可藉此得到主要為分子離子的相關訊息
Result is “softer” ionization which results in primary formation of molecular ions.
- 主要應用在於鑑定欲分析物的分子量
Main use is molecular weight confirmation

正離子化學游離法之結論 PICI Summary

- ◆ 可以得到分子量的訊息
Molecular weight information
- ◆ 對許多物質可增加分析工作上的選擇性
Increased selectivity for many compounds
- ◆ 以不同的試劑氣體可調整分析工作的選擇性
Selectivity affected by reagent gas
- ◆ 高壓力的試劑氣體可得到完全的CI圖譜，可
得到加成反應的離子訊號用以判定分子量
High pressure CI produces true CI spectra
(including adduct ions for confirmation)



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Ionization Methods

離子化的方式

Negative Ion Chemical Ionization

負離子化學游離法

負離子化學游離法 NICI

- 試劑氣體與電子反應，形成所謂電漿或是類似熱電子之類的物質

Reagent gas reacts with electrons to form “plasma” of thermal electrons

- 適用於具備高電子親和力，易於捕捉電子的欲分析物質（即所謂高電負度的欲分析樣品物質）

Ionization is favored by molecules which have a high electron affinity – electron capture

- 適用於高複雜基質環境下的選擇性分析工作，例如食品或是廢棄物樣品中的殺蟲劑分析

Useful for selective analysis in heavy matrices, i.e. pesticide in food or waste matrix.

負離子化學游離法之結論 NICI Summary

- ◆ 可以得到分子量的訊息

Molecular weight information

- ◆ 具有高靈敏度與高選擇性，適用在複雜基質狀況下的樣品分析工作

Highly sensitive and selective - ideal for analytes in complex matrices

Detector (偵測器)

- **Electron Multiplier (Most common)**
- **Faraday Cup**
- **Photomultiplier Conversion Dynode**
- **Array Detector**
- **Charge (or Inductive) Detector**



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資料採集模式

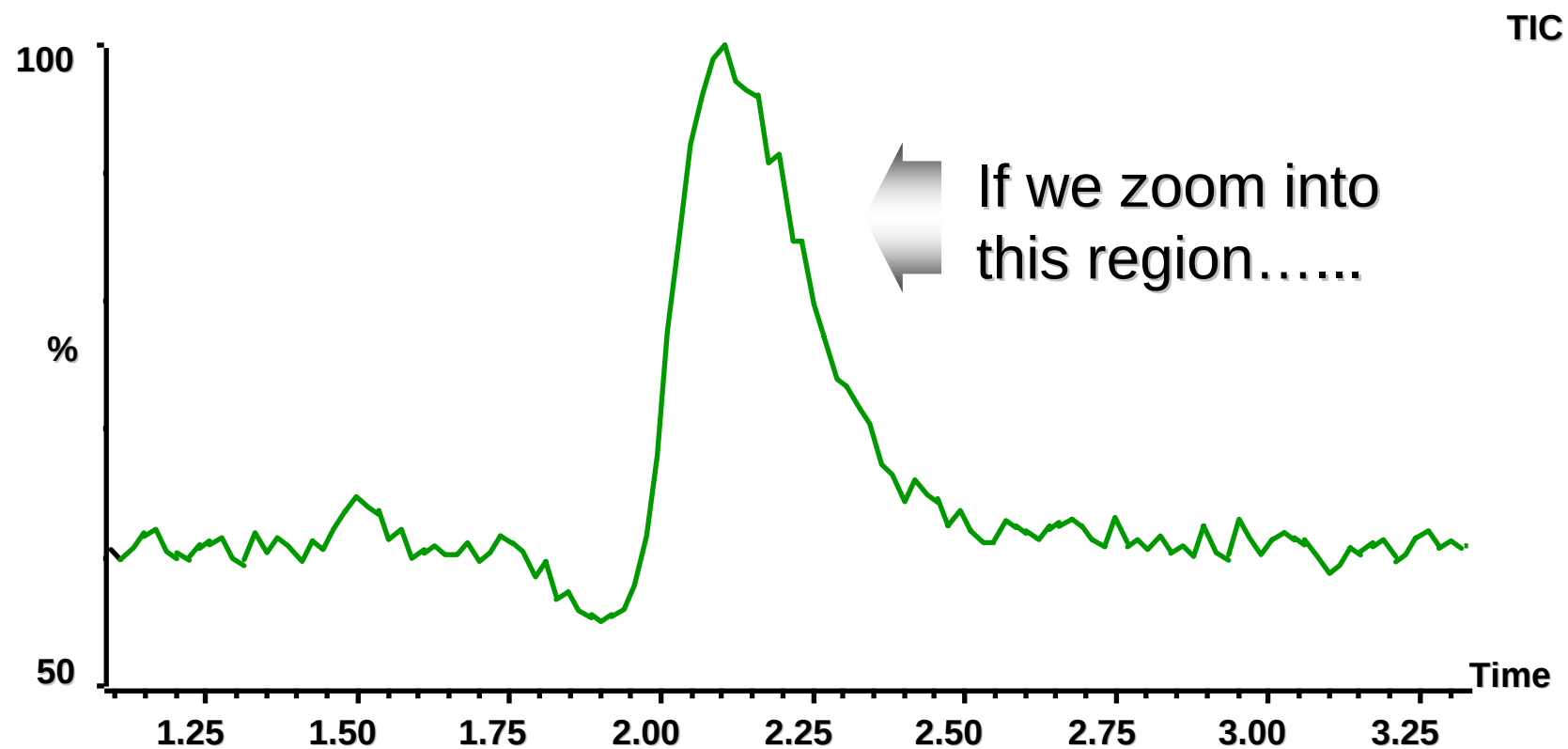
質譜偵測 MS Detection

- Answers the two most frequently asked questions in Analytical chemistry-
 - 定性 IDENTITY - What is in this sample?
 - 定量 QUANTITY - How much of it is in this sample?

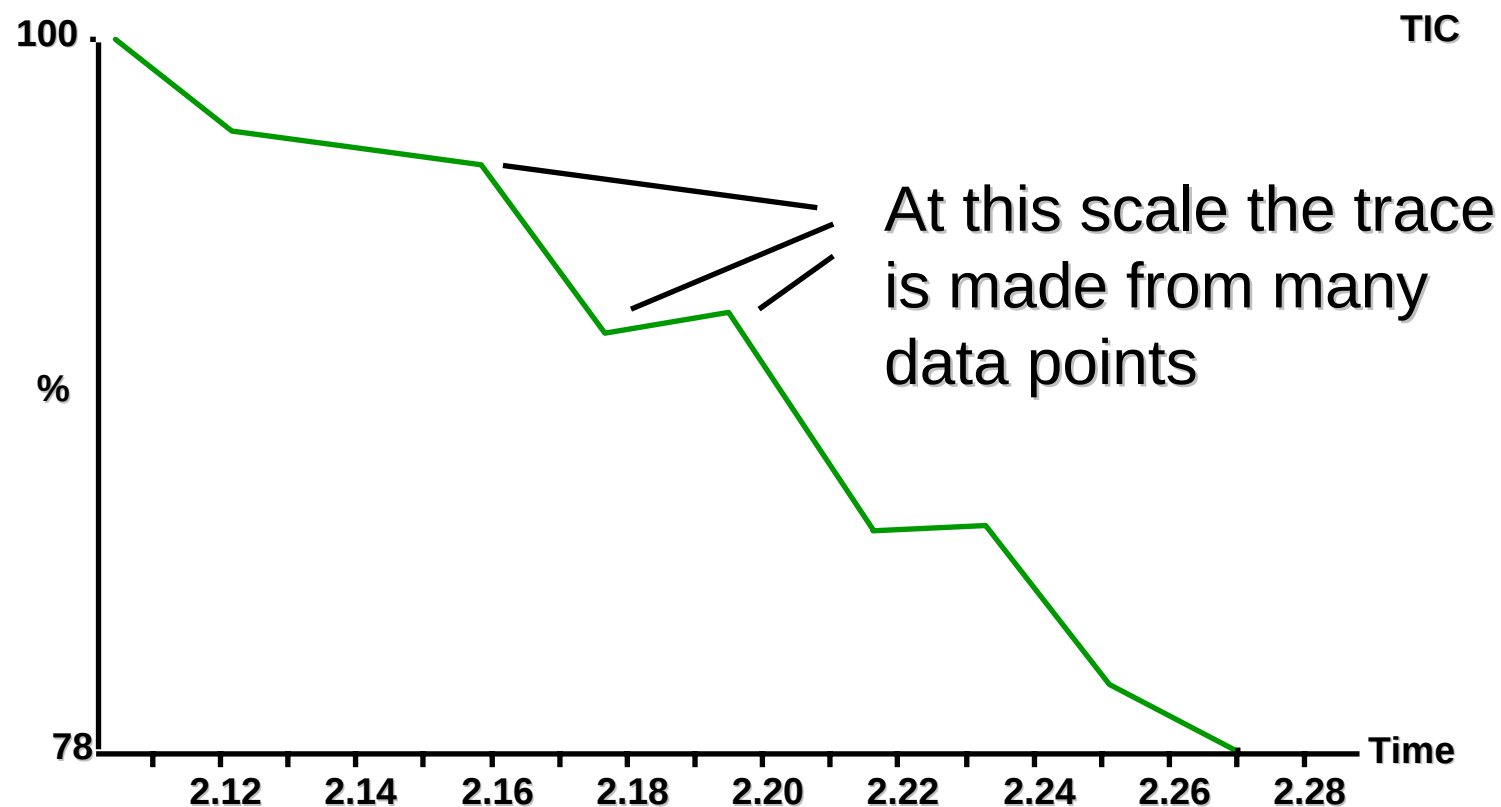
質譜儀是如何運作 How does an MS detector do this?

- MS detectors constantly acquire 質譜 **Mass Spectra**
- **1-5 spectra** are acquired per second
 - i.e. mass range 50-550 amu, scan rate 5 scan/sec,
 - ~ 2500 amu/s
- A chromatographic run is defined by thousands of mass spectra

層析圖譜 Chromatograms?

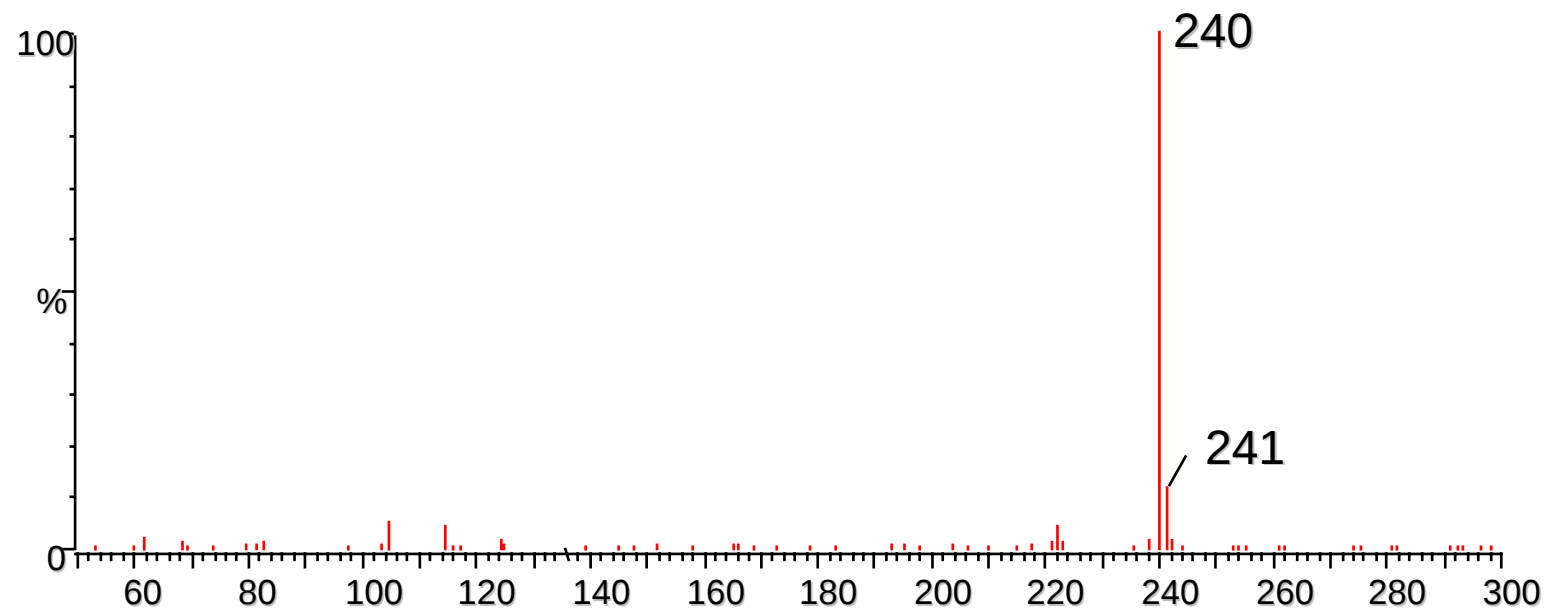


將層析圖放大觀察 Chromatograms close-up



質譜圖 Mass Spectrum

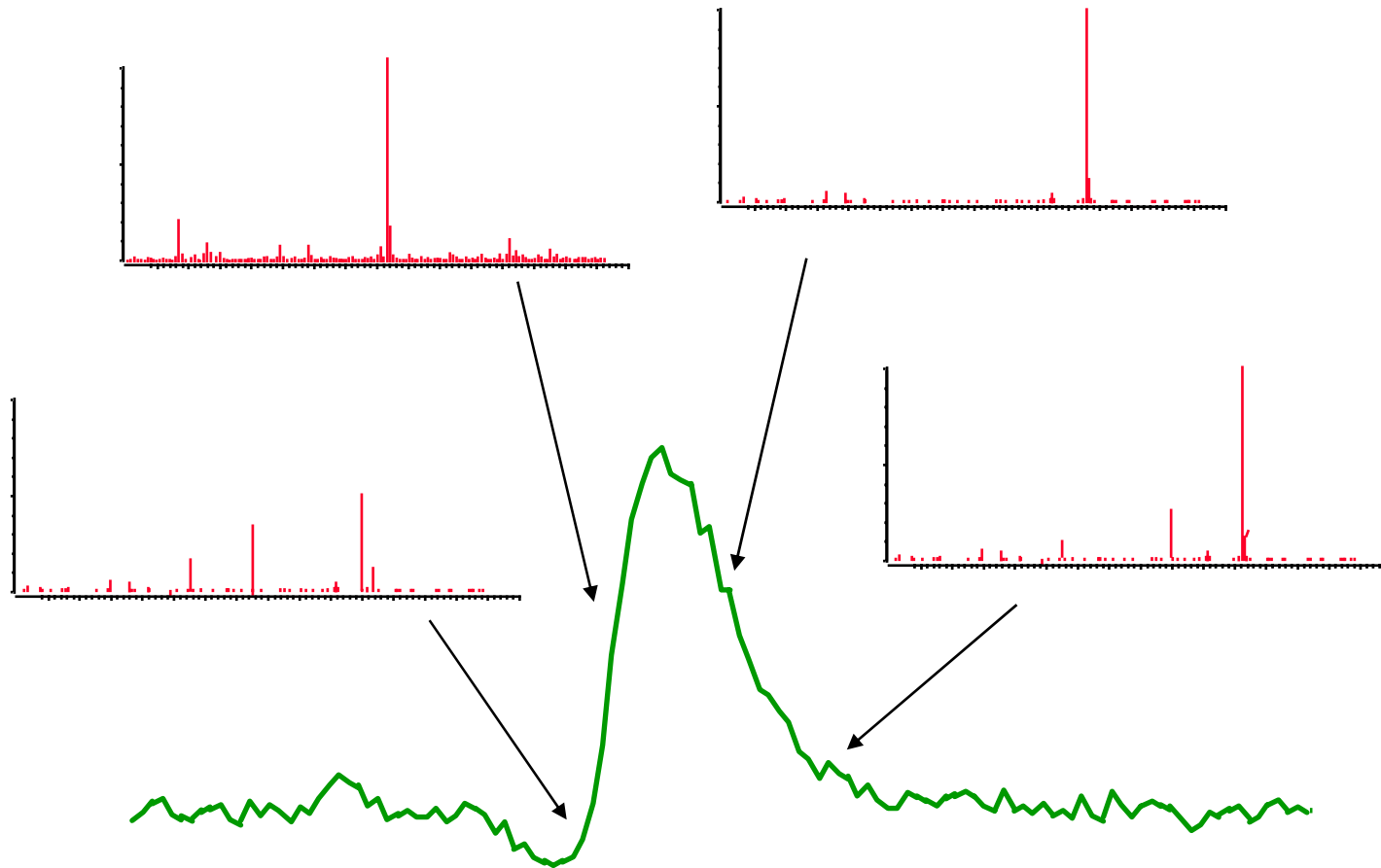
- produced by scanning the MS between 50 and 300 amu whilst compound elutes into the system



什麼是質譜圖 What is a Mass Spectrum?

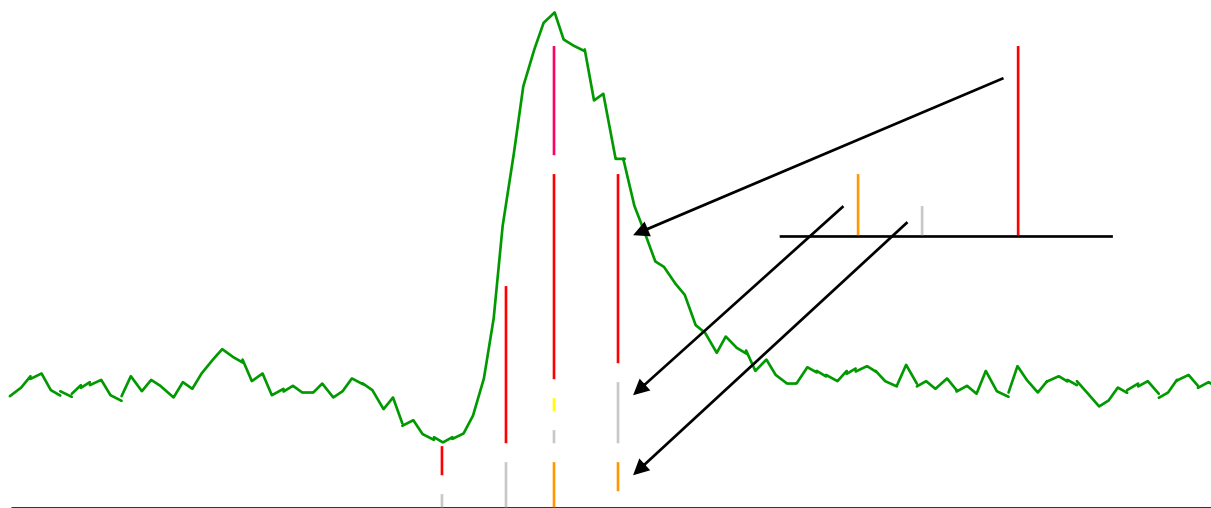
- A mass spectrum is a “化學指紋圖chemical fingerprint”
- It contains:
 - 分子量訊息 Molecular weight information
 - 結構訊息 Structural information

在每一層析圖位置都包含特定質譜圖
Each position on the chromatogram has a unique mass spectrum



Trace produced by summing all observed masses in each scan

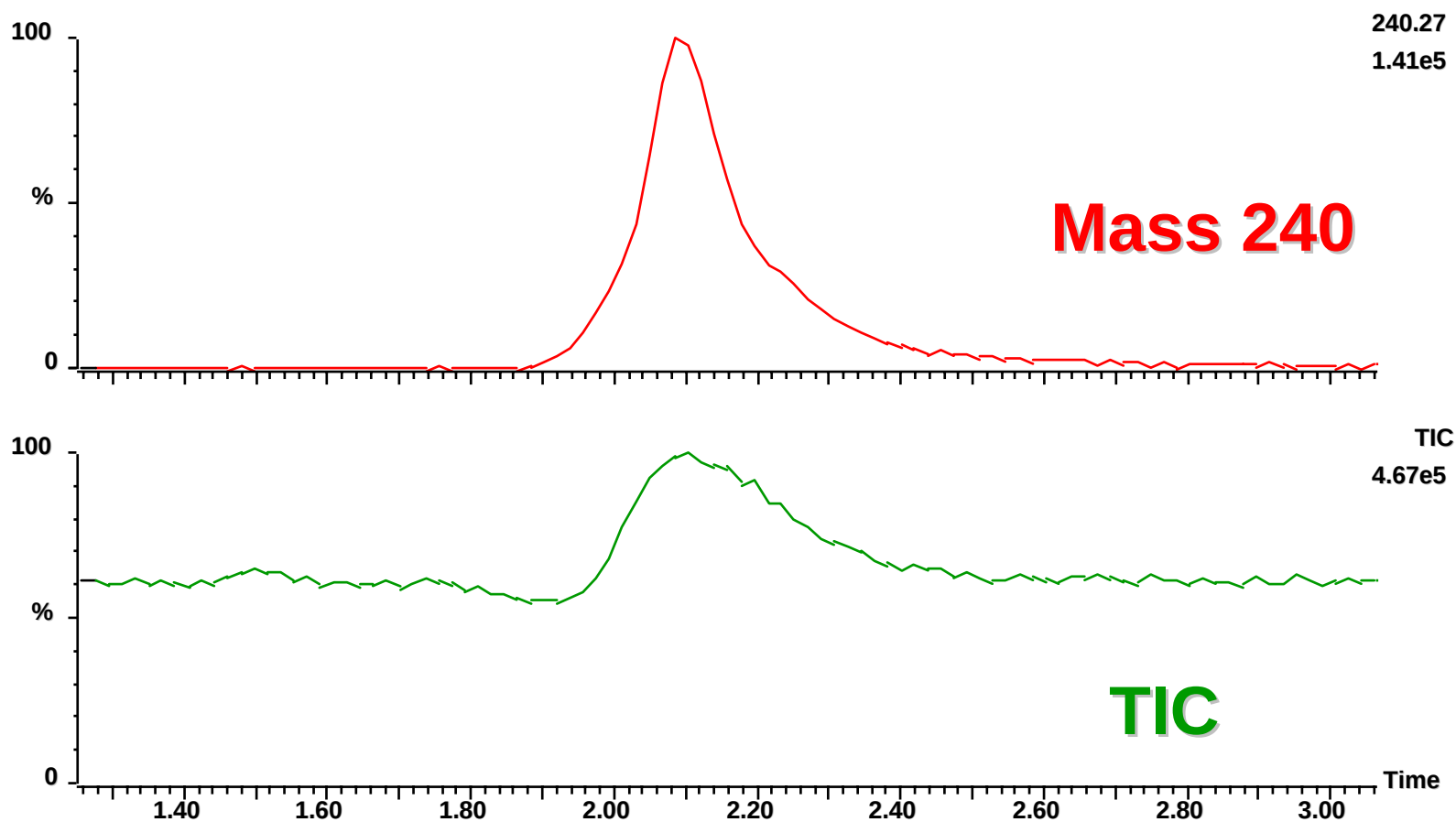
- 總離子層析圖 Total Ion Chromatogram or TIC



Data View - Chromatogram

- We can also just show the data for a specific mass
- This lets us see only the information from a single compound

質譜層析圖 Mass chromatogram

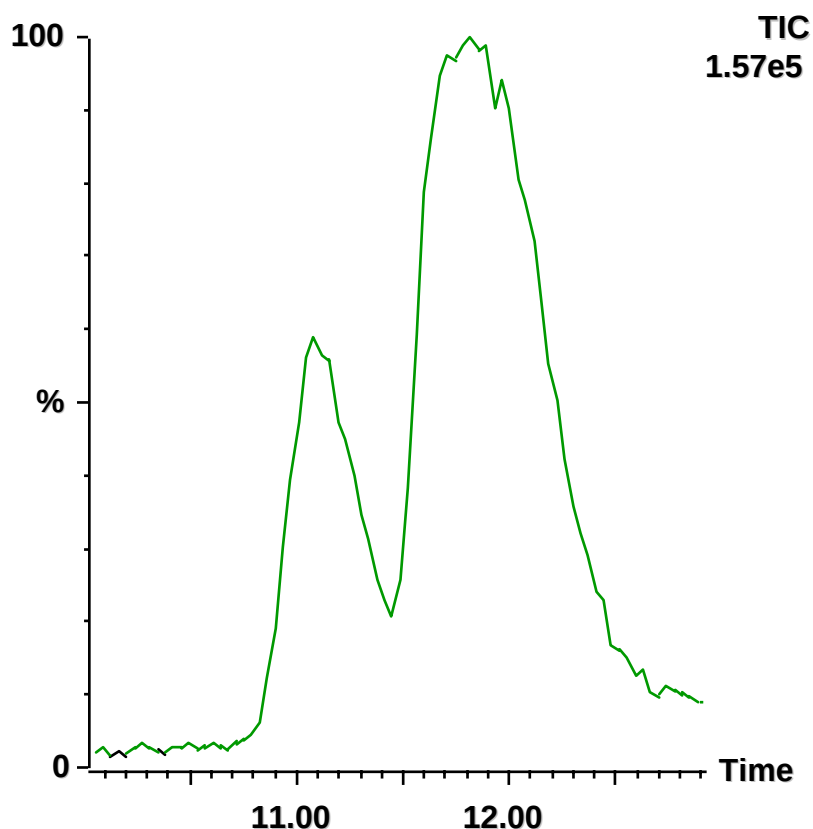


Data View - Extracted ion trace

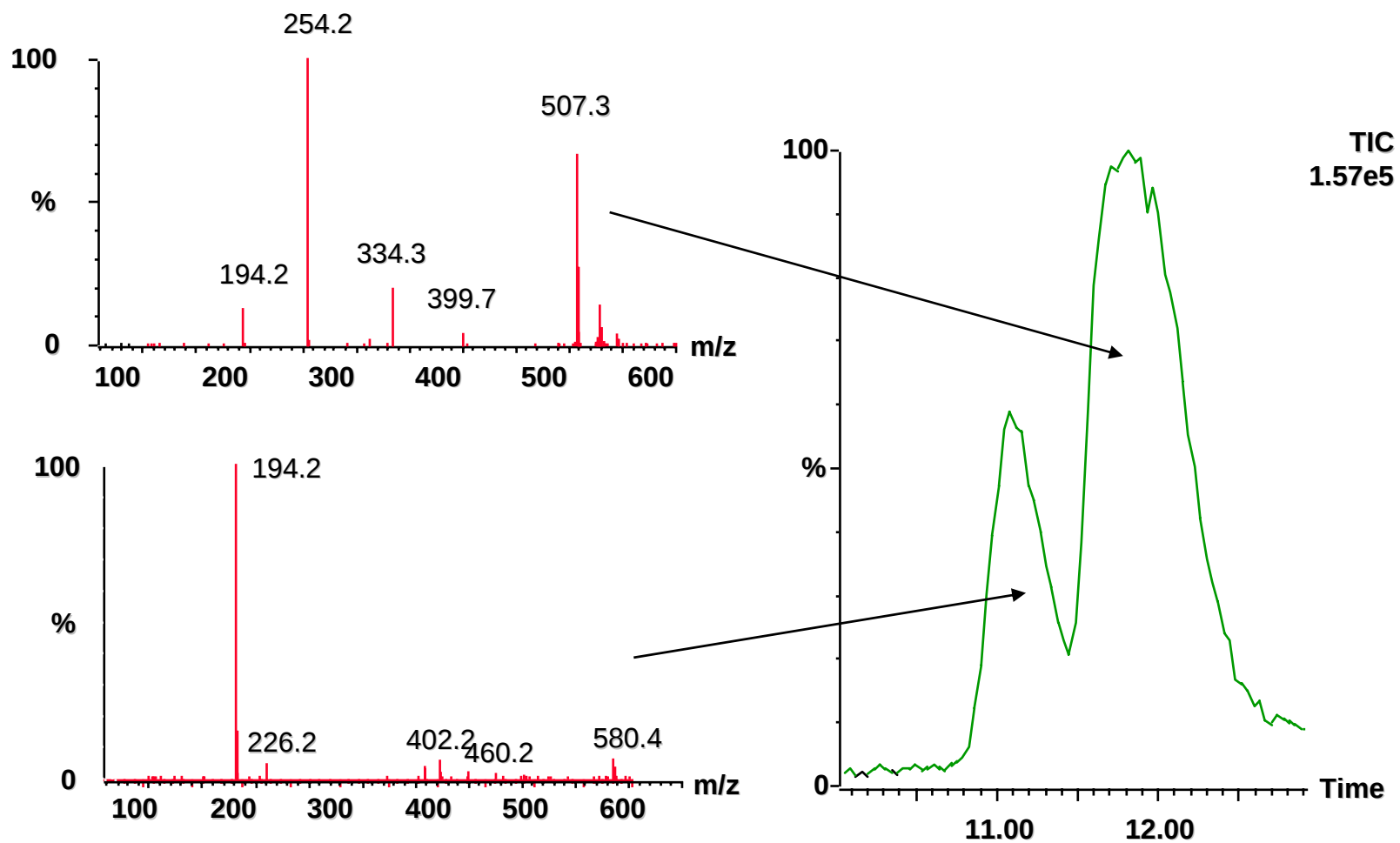
- Removes the effect of background
- Increases signal to noise
- Separates co-eluting peaks

Data View - Extracted ion trace

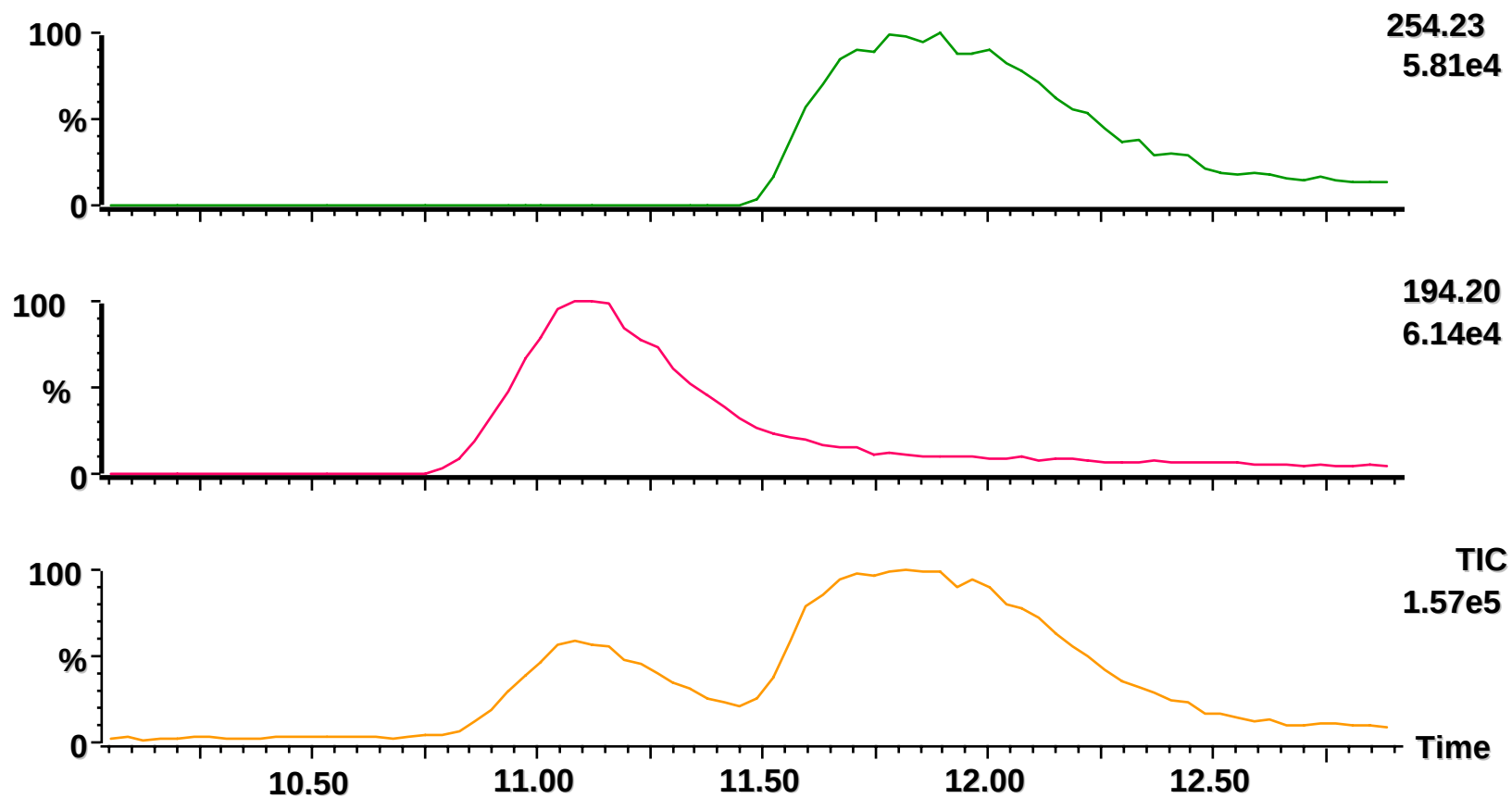
- 2 closely eluting peaks as seen on the TIC. But each peak represents a different compound with a different mass spectral fingerprint.



Data View - Extracted ion trace



Separating co-eluting peaks



Extracted ion traces

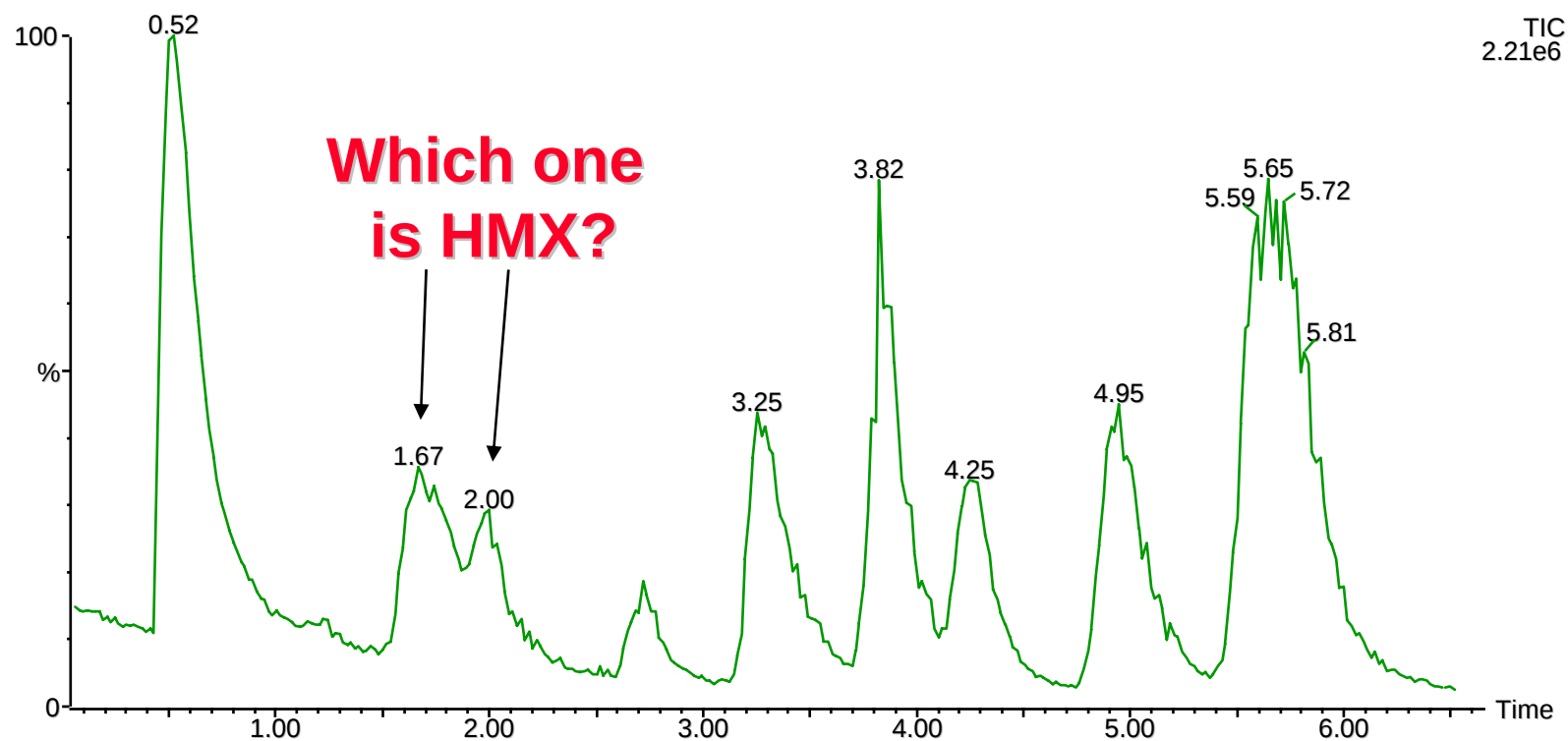
- Extracted ion traces give better S/N
 - removing background
 - Separating co-eluting peaks
- Extracted ion traces can also be used for:

PEAK TRACKING

ACCURATE QUANTITATION

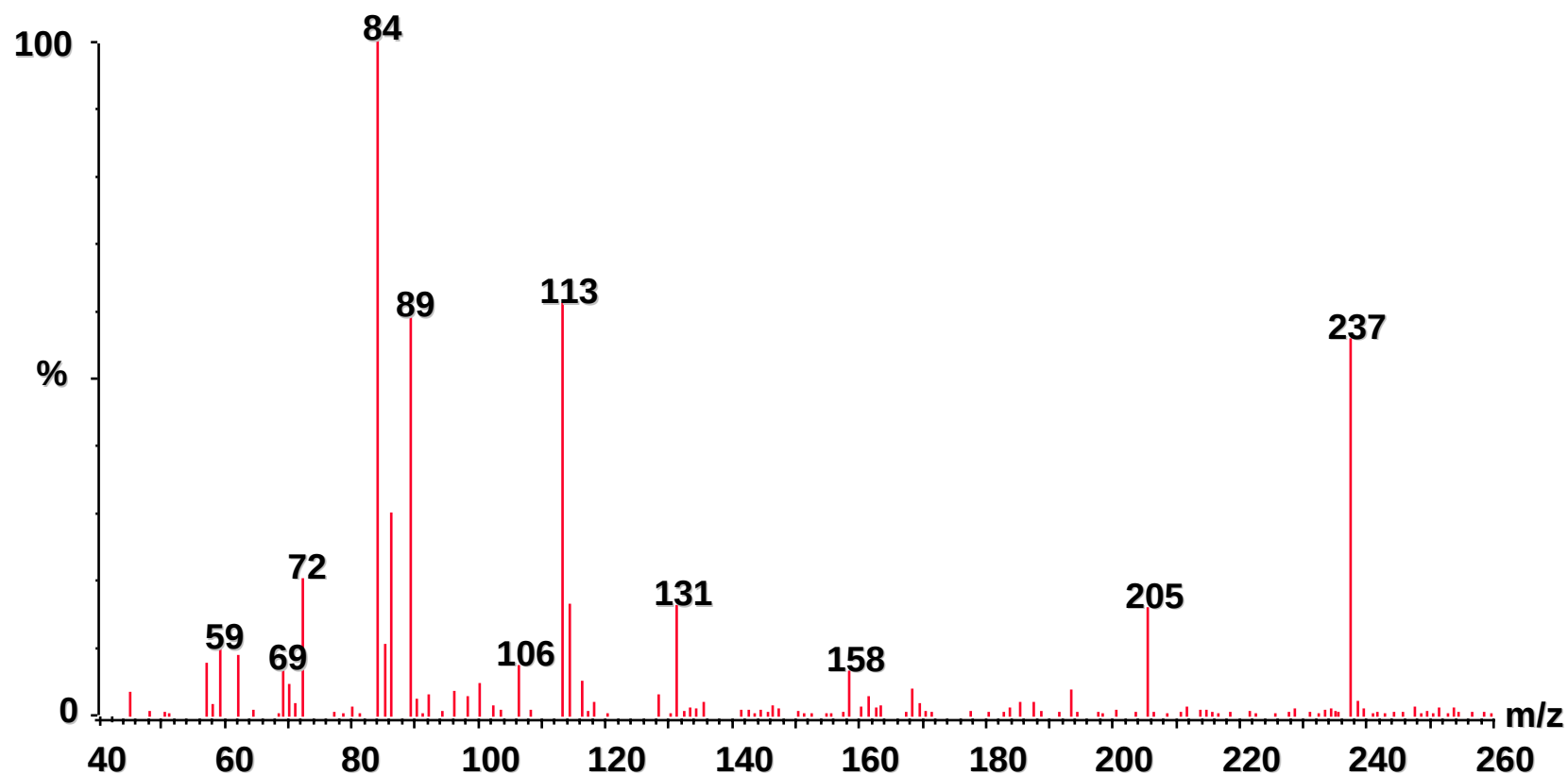
Peak Tracking

- TIC of explosives mixture

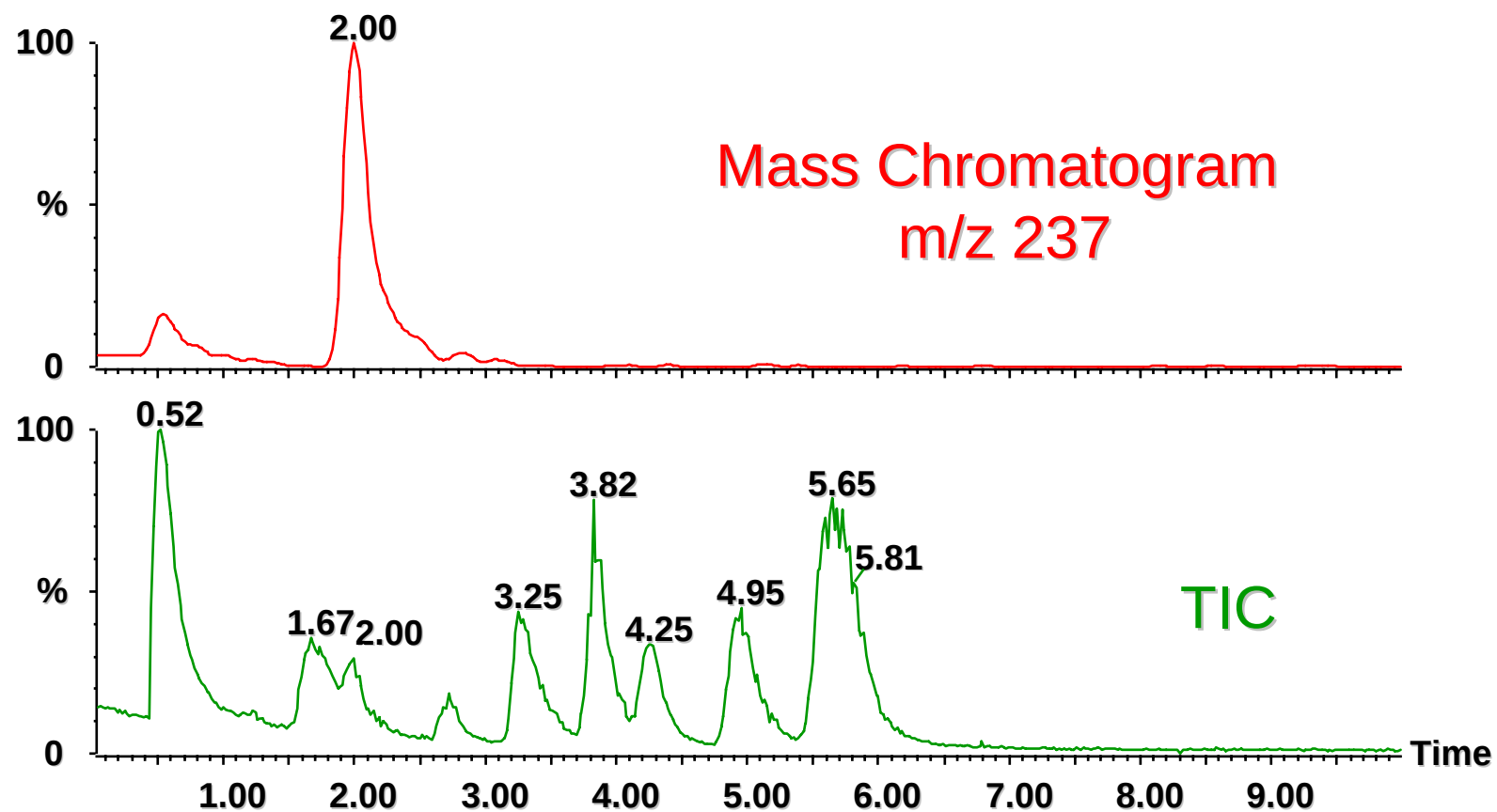


Peak Tracking

- Mass spectrum of HMX



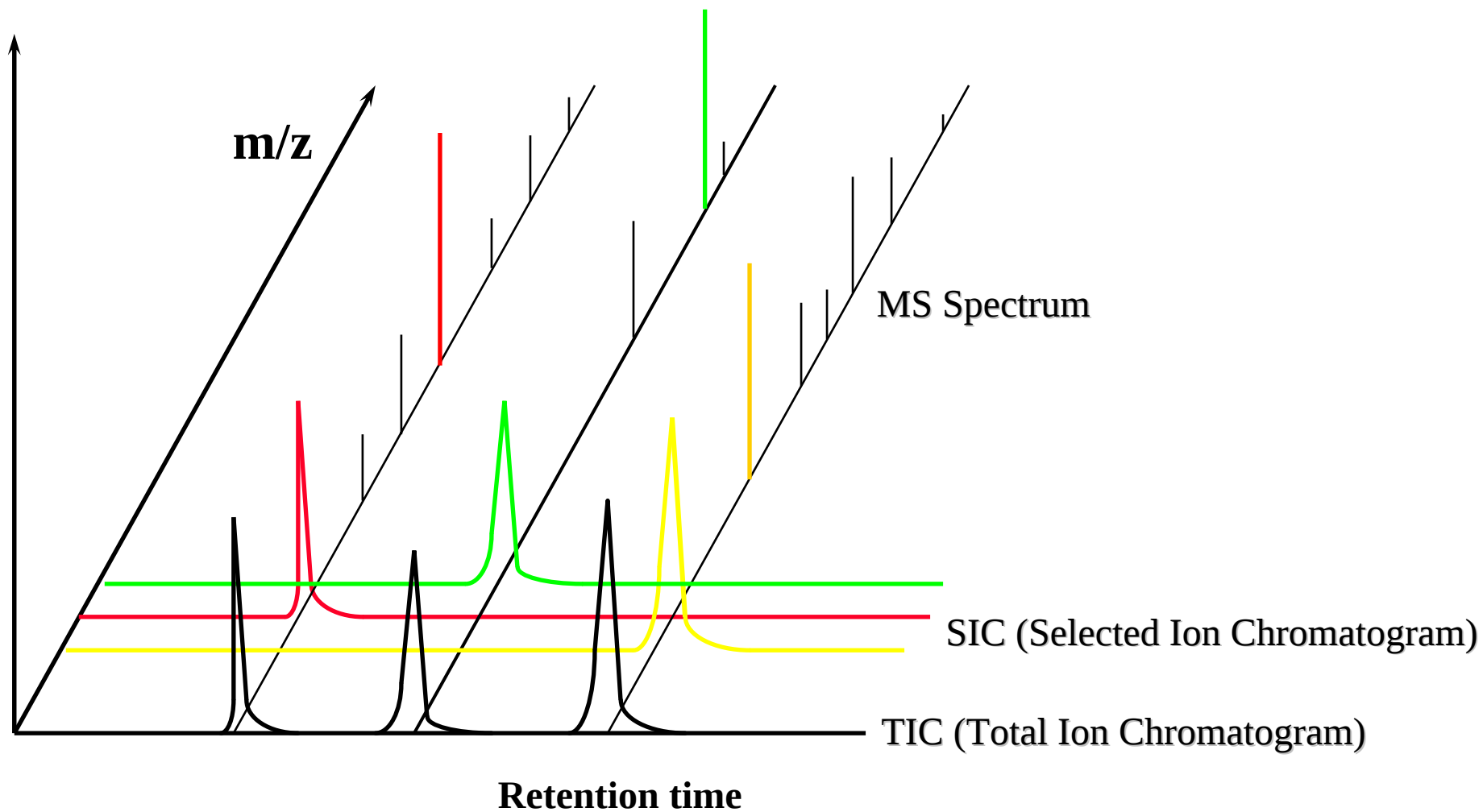
Peak Tracking



Data View - Options

- Total Ion Chromatogram
 - The best way to view the whole chromatogram and all peaks visible
- Extracted Ion Traces
 - The best way to see data from just one compound

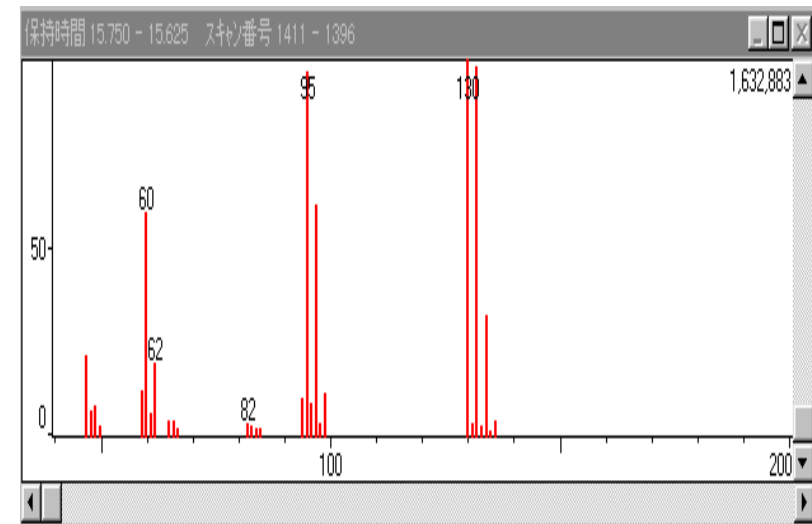
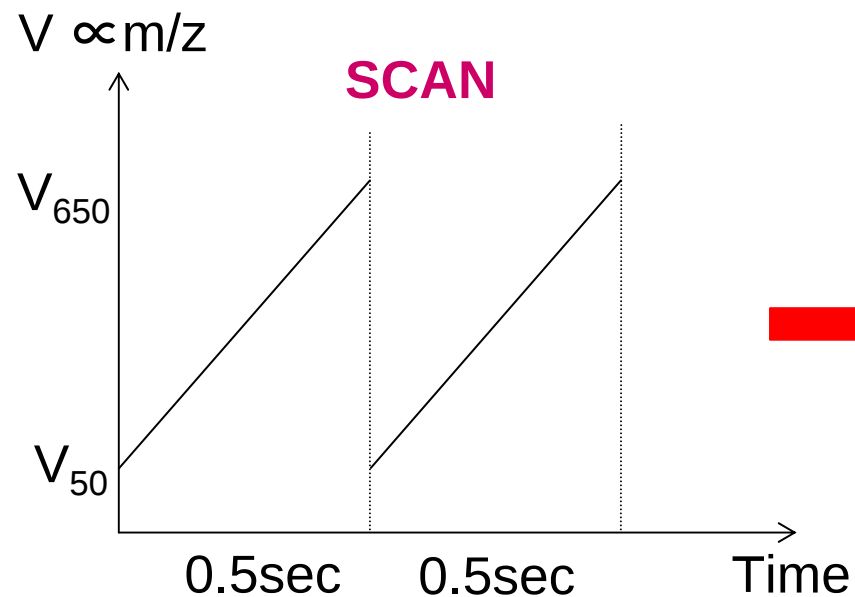
層析圖與質譜圖



資料採集模式

SCAN mode (掃描模式)

- 當電壓掃描時，離子依次進入偵測器。



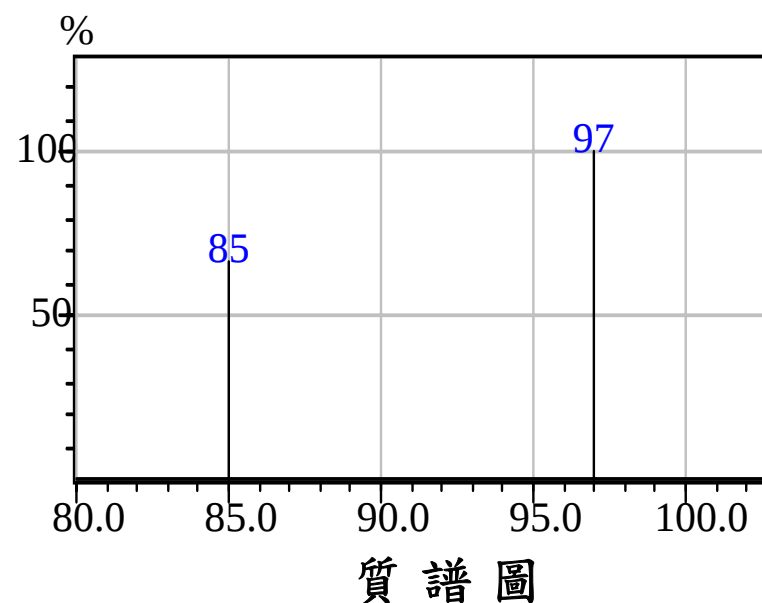
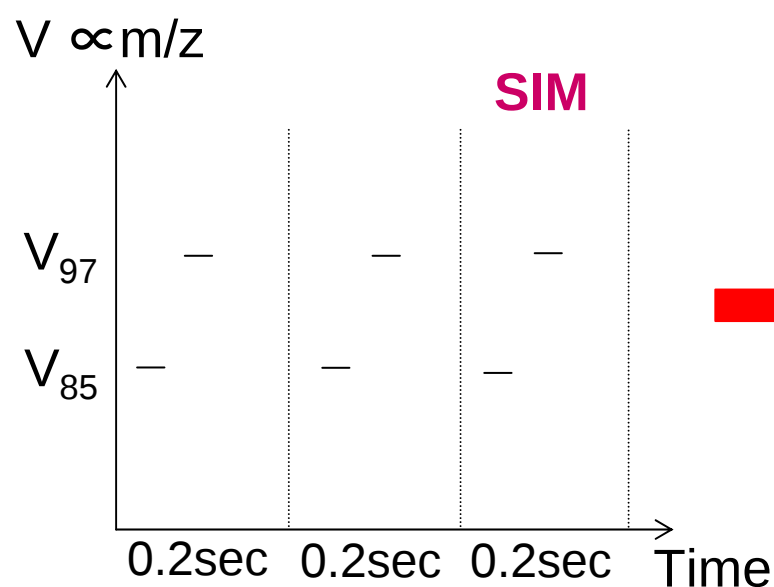
質譜圖

資料採集模式

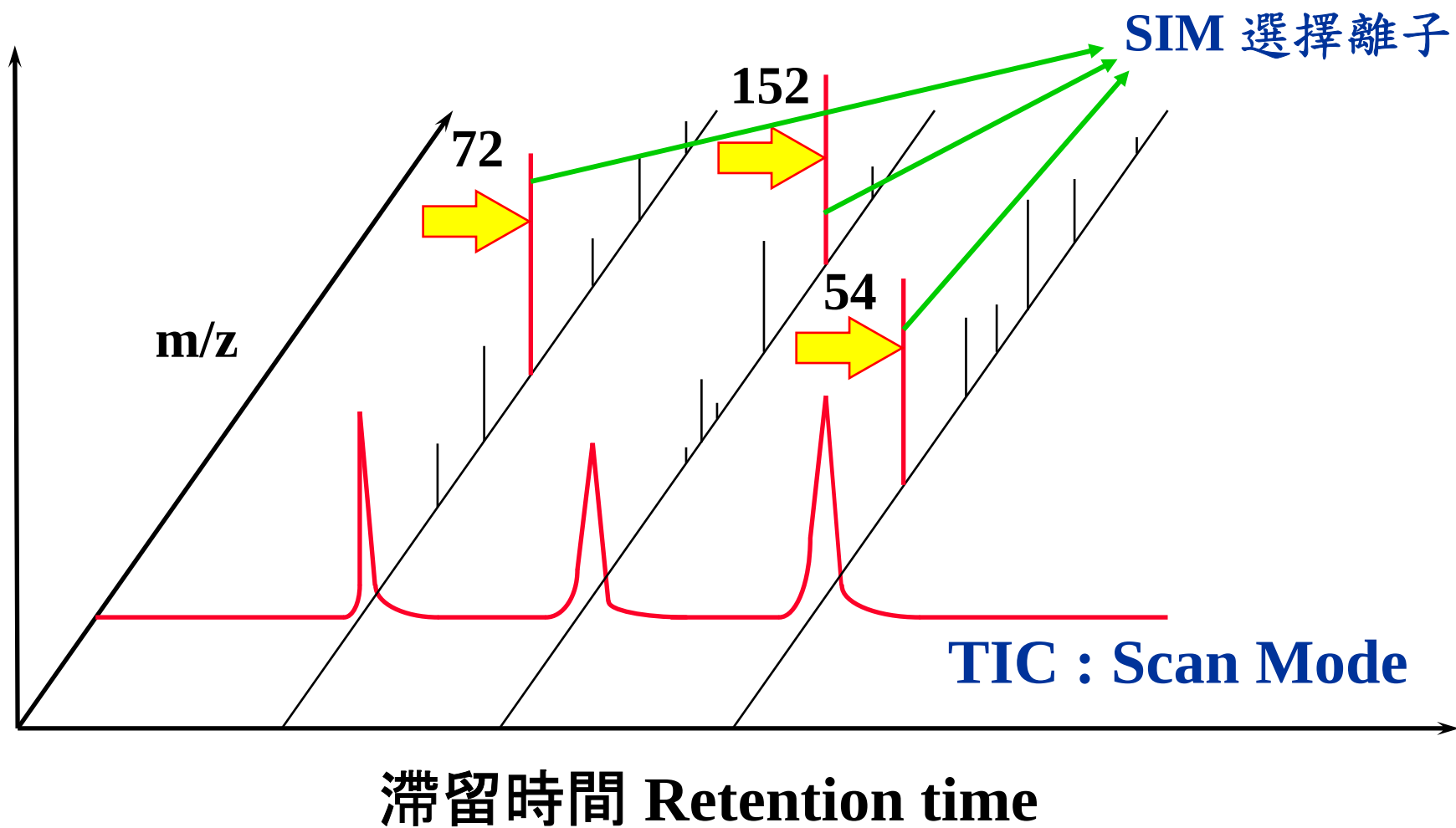
SIM(**S**electe**d** **I**on **M**onitoring) mode；選擇離子模式

只有特定質量數的離子被檢測

- 根據目標化合物選擇合適的偵測離子相當重要。
- 感度取決於所選擇的檢測離子。



Scan & SIM Mode



Summary so far...

- MS detectors acquire mass spectra which can be used for:-
 - Molecular weight determination
 - A TIC chromatogram
 - An extracted ion chromatogram
 - Improves s/n
 - Removes matrix
 - Separates co-eluting peaks

分析方法

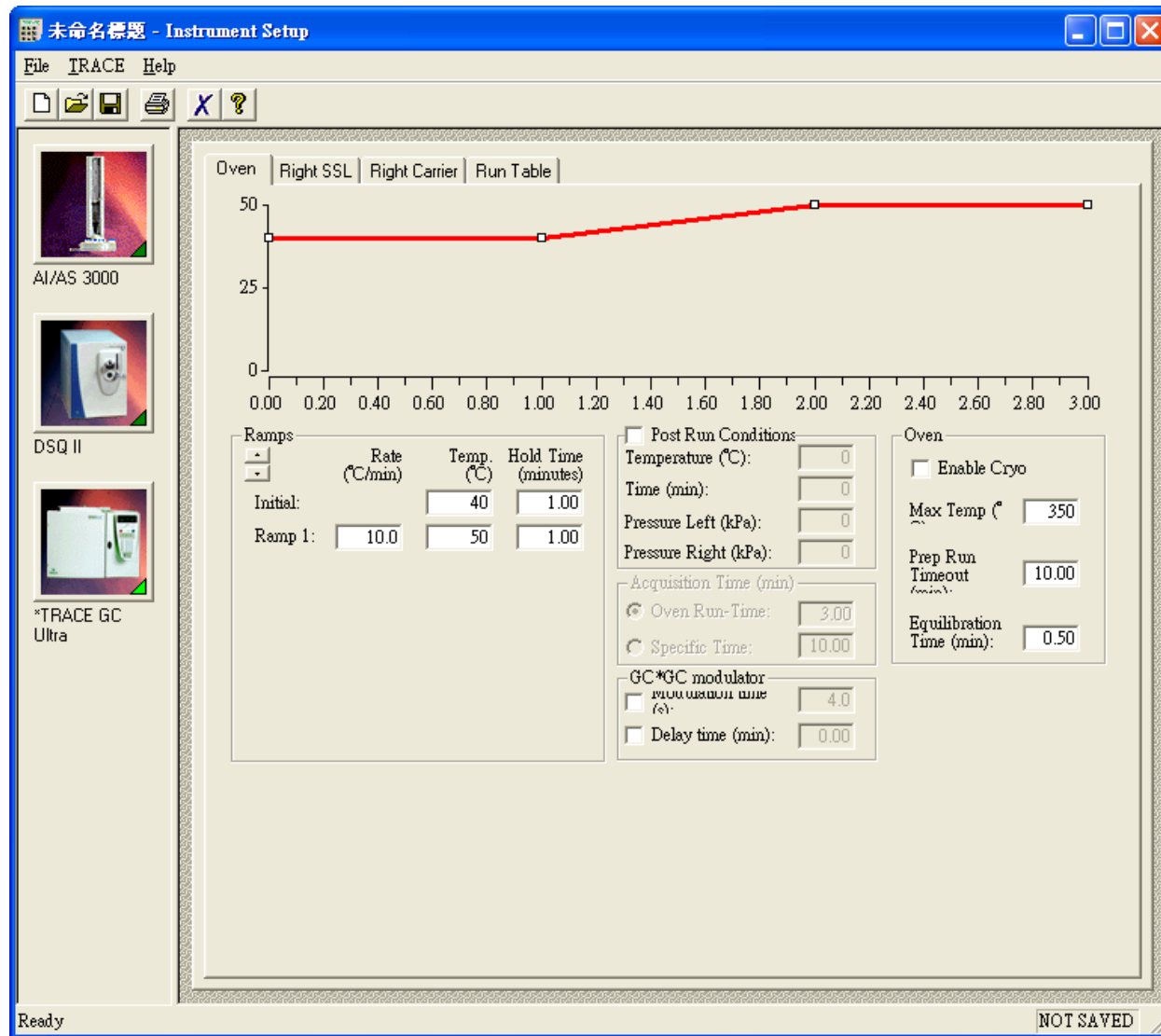
➤ 儀器參數

- GC參數：層析管柱、載流氣體流量、oven溫度等
- MS參數：溫度、偵測器電壓、掃描範圍等

➤ 資料處理參數

- 積分參數：波峰寬度、基線等
- 定性參數：資料庫搜尋條件等
- 定量參數：計算方法、校正級別等

分析方法：層析儀參數



分析方法：質譜儀參數

未命名標題 - Instrument Setup

File DSQ II Help

Icons: [New] [Open] [Save] [Print] [X] [Help]

Left Panel:

- AI/AS 3000
- DSQ II
- *TRACE GC Ultra

Main Panel:

End run:

- ☒ GC run time
- ☐ Probe run time
- ☐ After 10.00 minutes

Heated zones:

Ion source (°C): 200

Reagent gas: Methane

Acquire cal gas: EI/NICI

☐ Acquire profile data

Acquisition threshold: 0

Buttons: Tune, Auto Tune

Table:

Seg	Start	Scan events
1	0.00	MS

Buttons: Add Segment, Delete Segment

Segment 1:

Start time (min): 0.00 Reagent Gas Flow (mL/min): 0.3 ☐ Fil/Mult/Dyn off

Detector gain: 1.00 X 10⁵ (Multiplier voltage: 1000 V) ☐ Chromatographic filter (Scan event 1)

Total scan time (s): 1.22 Peak width (s): 0

Buttons: Add Event, Delete Event

Scan 1:

Scan mode: ☒ Full Scan ☐ SIM **Parameters**

Mass range: 50 - 650

Ions: ☒ Positive ☐ Negative ☒ Use for entire segment

Tune: Tune file: autotune ☒ Use for entire segment **View**

Emission Current: ☒ Use tune file ☐ Use method (µA): 0 ☒ Use for entire segment

Scan 1 Details:

Scan time (s): 1.22

Rate

Scans per second: 0.8170

Scan rate (amu/s): 500.0

Mass defect: (mmu/100 amu) 0

Ready NOT SAVED

定量參數

校正級別（檢量線計算方法）

- 單點校正（liner）
- 多點校正（兩點和兩點以上）
 - 線性迴歸
 - 點到點連線（折線）
 - 二次曲線
 - 三次曲線

單點校正

- 對每一個成分只做一個濃度的標準樣品
- 標準樣品濃度儘可能接近實際樣品中成分的濃度
- 單點校正曲線通過原點

多點校正

- 對每一成分至少作兩個不同濃度的校正點
- 標準品濃度範圍應包含實際樣品中各成分的濃度
- 多點校正有幾種方式
 - 1.波峰面積(高度)隨濃度變化不呈線性變化時，使用多線性校正（折線校正）或二次曲線、三次曲線
 - 2.波峰面積(高度)隨濃度變化呈線性變化時，使用最小二乘法，進行線性回歸校正

多點校正：計算方法

- 面積百分比法
- 校正面積百分比法
- 外標法
- 內標法

計算方法：

➤ 外標法

此法是應用最廣泛的方法之一，其誤差來源主要是進樣誤差，因此，分析前一定要做面積重複性（即進樣重複性）實驗。

➤ 內標法

在樣品中添加內標，通過成分與內標波峰的面積比，對成分進行定量。

此方法減小了進樣誤差對定量結果的影響。

內標準品的選擇

- 內標準品波峰與樣品中所有成分的波峰完全分離。
- 內標準品波峰與目標成分波峰滯留時間不應差太遠。
- 內標準品具有與分析目標成分類似的化學性質



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GC-MS 概論

The World of Mass
Analyzers

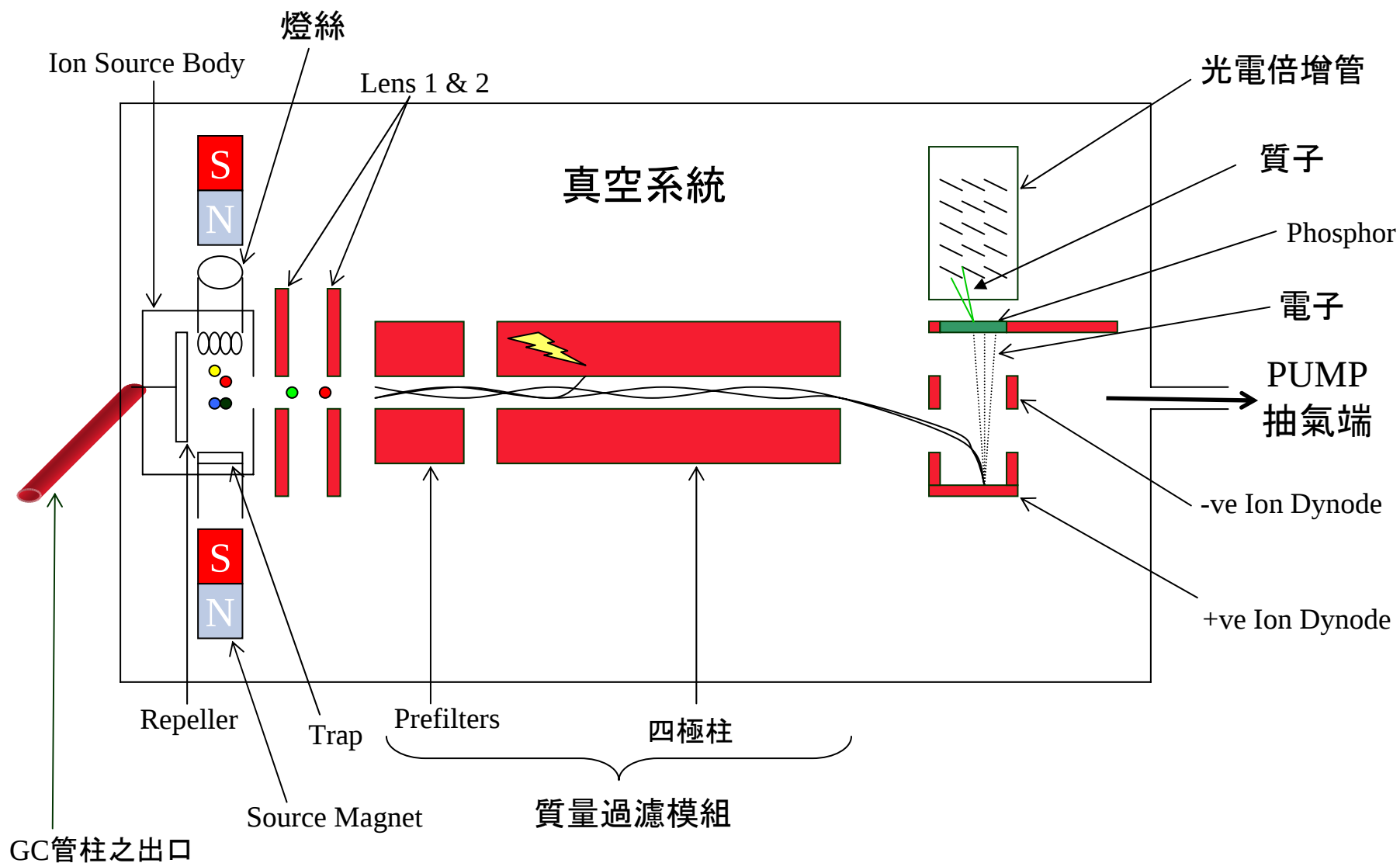
質量分析儀之種類

常見的質量分析裝置

- **四極柱 (Quadrupole)** — 由兩組相對的圓柱，此質量分析儀是藉由結合RF與DC模組來選擇離子，此種分析儀可提供單個amu的離子解析度
- **離子阱 (Ion Trap)** — 操作原理與四極柱相同，然而離子可以貯存於其中以進行後續的分析需求，以改變阱內電場的方式選擇所要捕捉的離子，以改變阱內RF的操作模式，將所捕捉的離子由低質荷比至高質荷比陸續給釋放出來
- **扇型磁場式 (Magnetic Sector)** — 結合磁場與電場來選擇離子，離子先通過電場進行聚焦，之後再經過磁場解析開來
- **飛行時間 (Time of Flight; TOF)** — 經由離子源來形成離子物質／碎片，然後直接進入飛行腔中，在已知的飛行距離中，透過各離子不同的飛行時間來達成分離的效果

四極柱質譜儀示意圖

Quadrupole





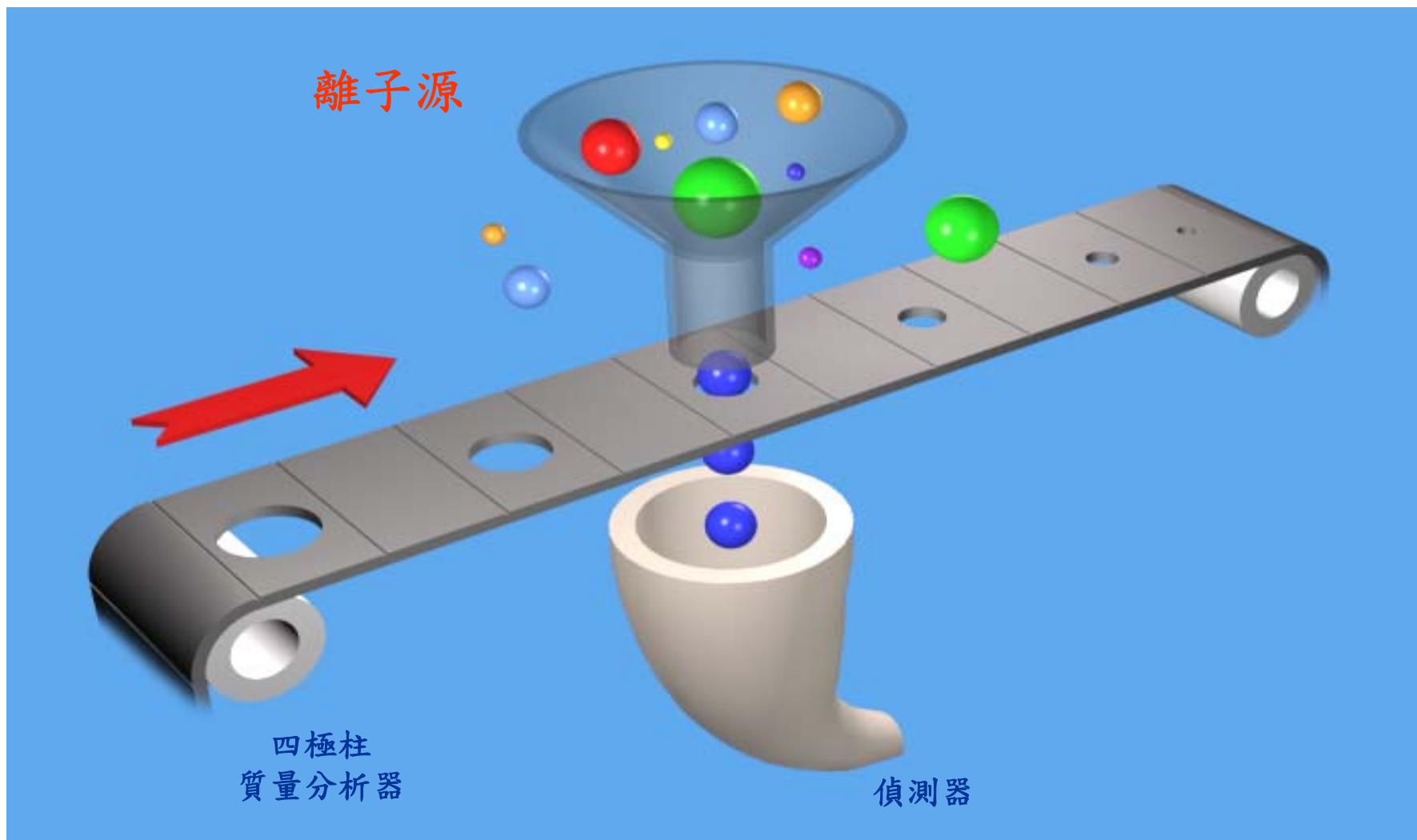
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GC-MS 概論

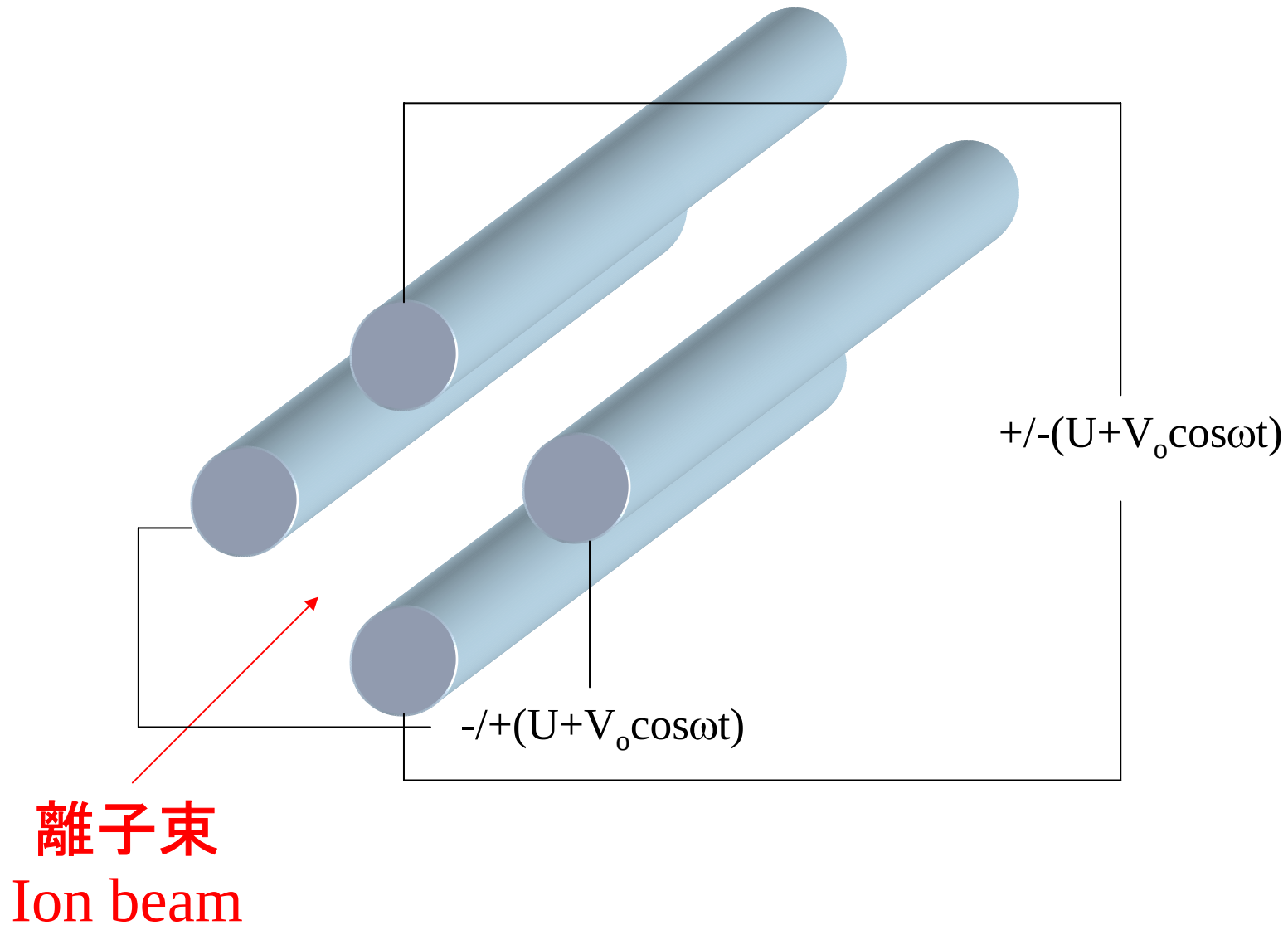
Single Quadrupole Mass Analyzers

單段四極柱式質量分析儀

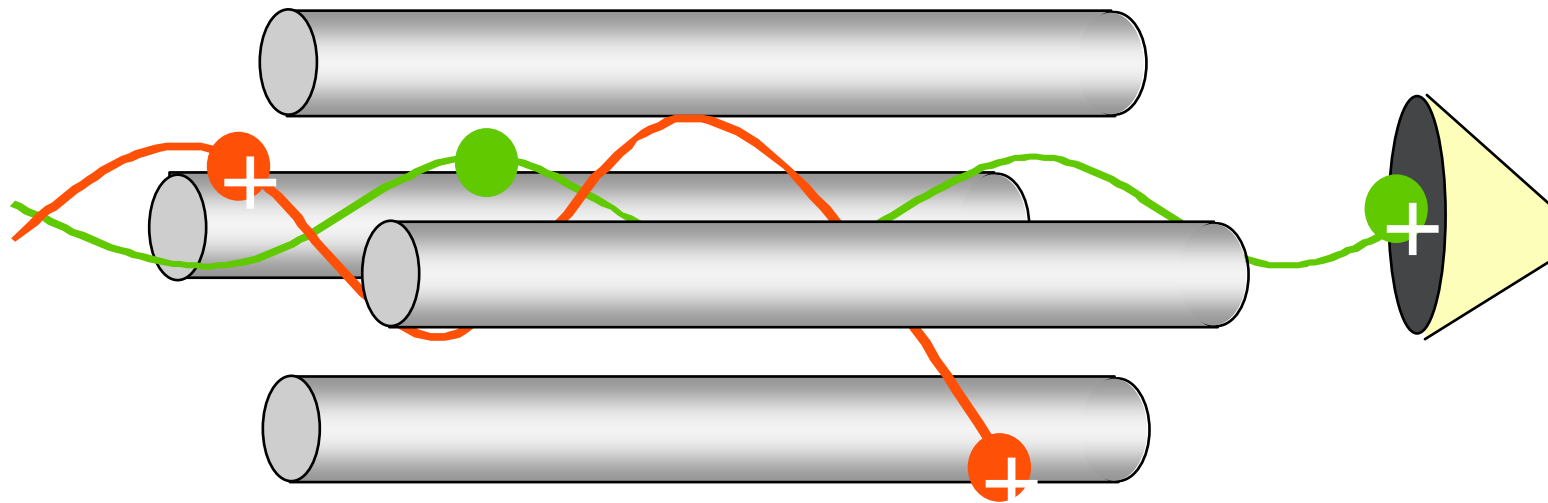
四極柱質量分析器示意圖



四極柱之位向 Quadrupole Arrangement

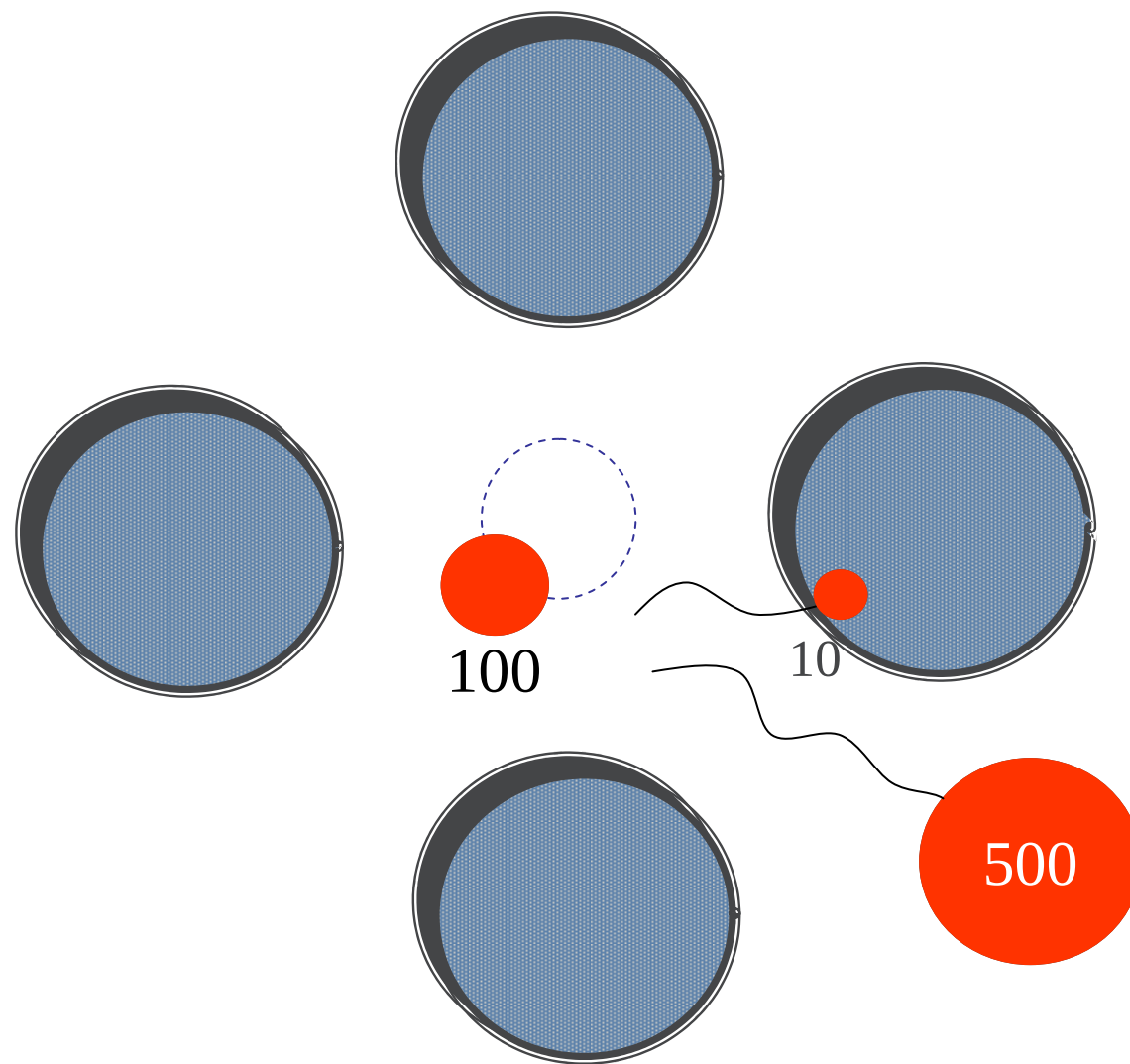


四極柱質譜儀：採空間分離模式

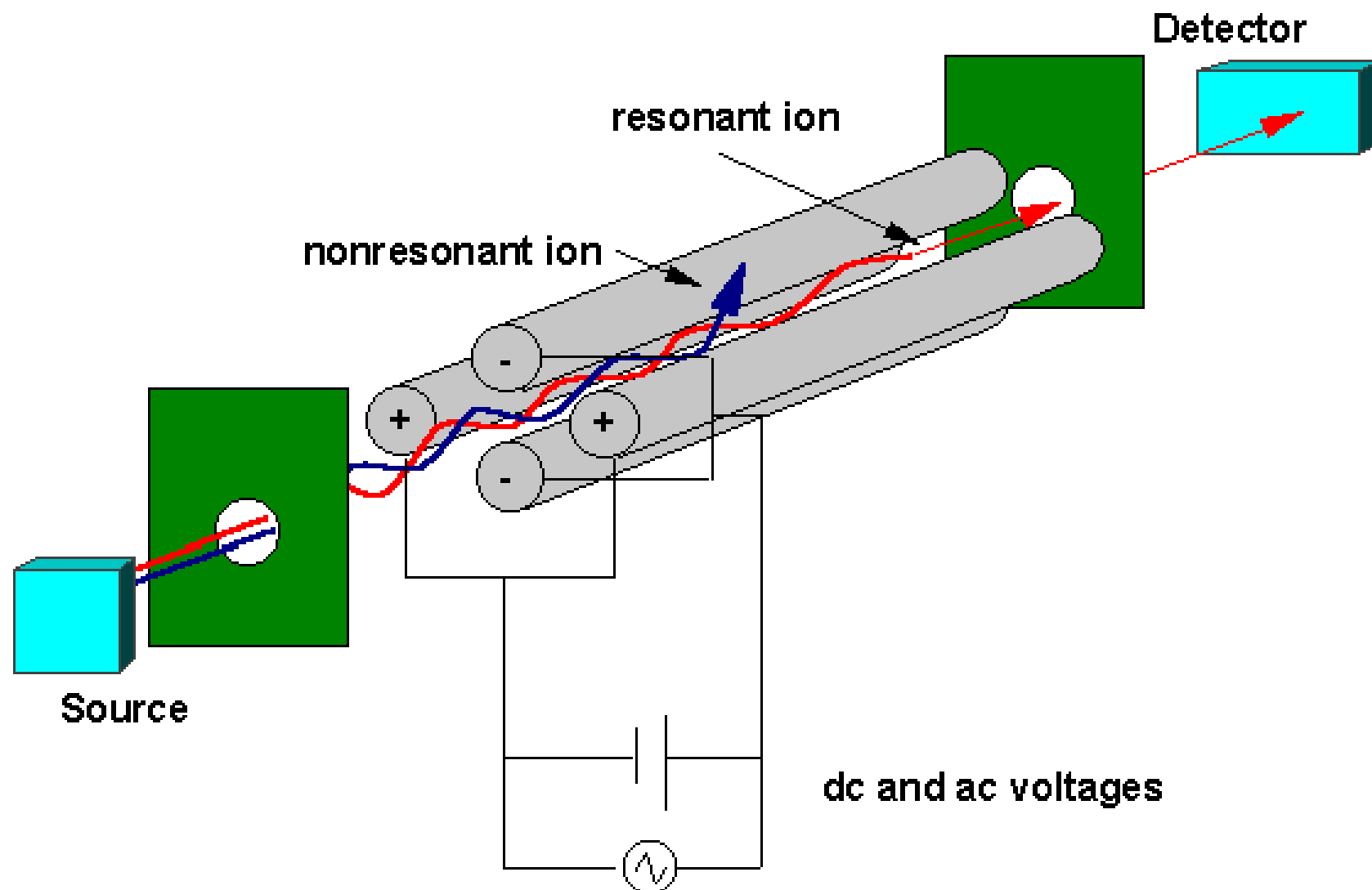


- Ions scanned by varying the DC/RF voltage across the quadrupoles
- 藉著改變四極柱之DC/RF電壓來執行離子掃描

特定電場下只有特定質量的離子可通過



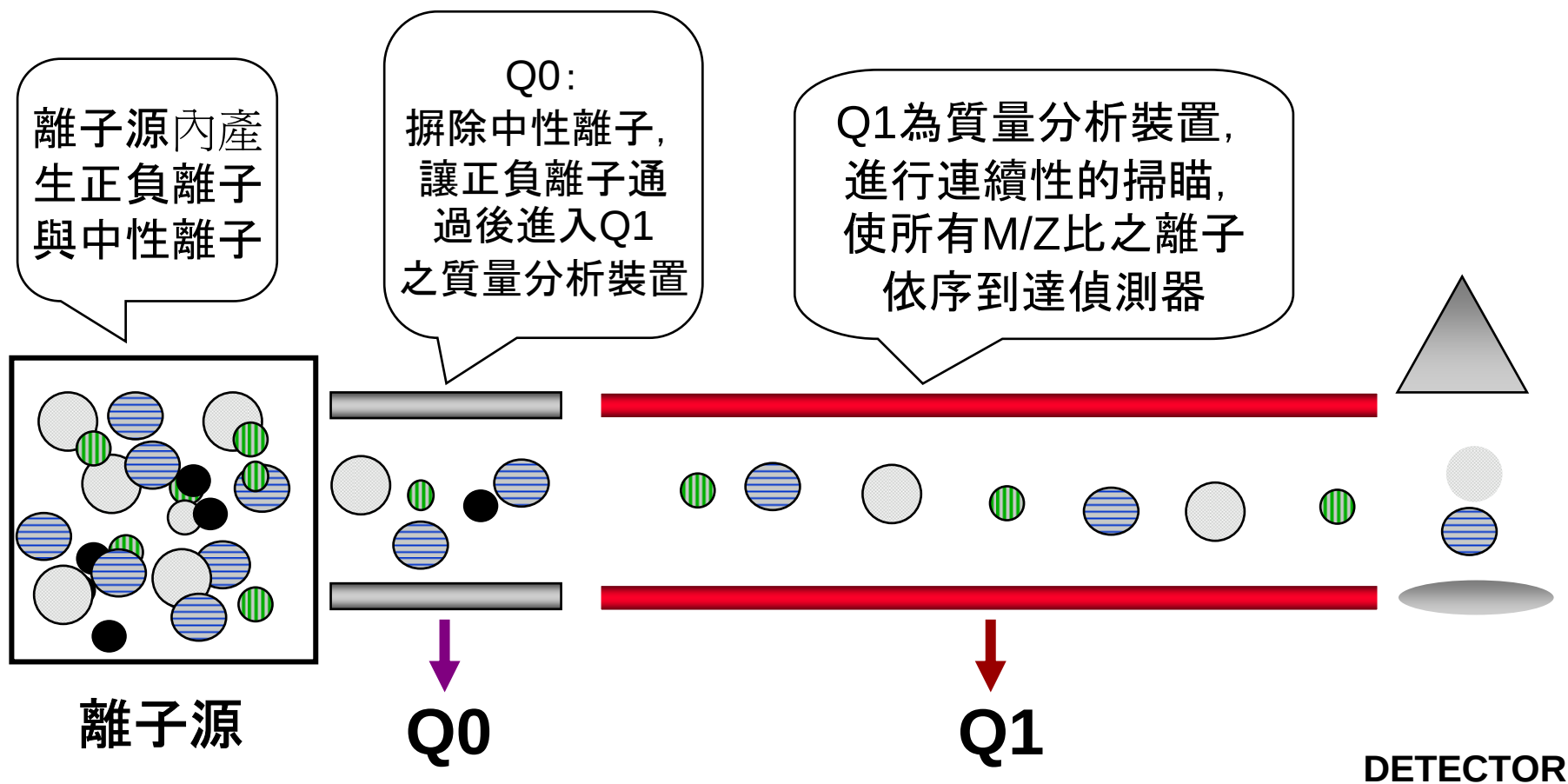
Quadrupole Illustration of Mass Separation



掃描模式 (Scan Mode)

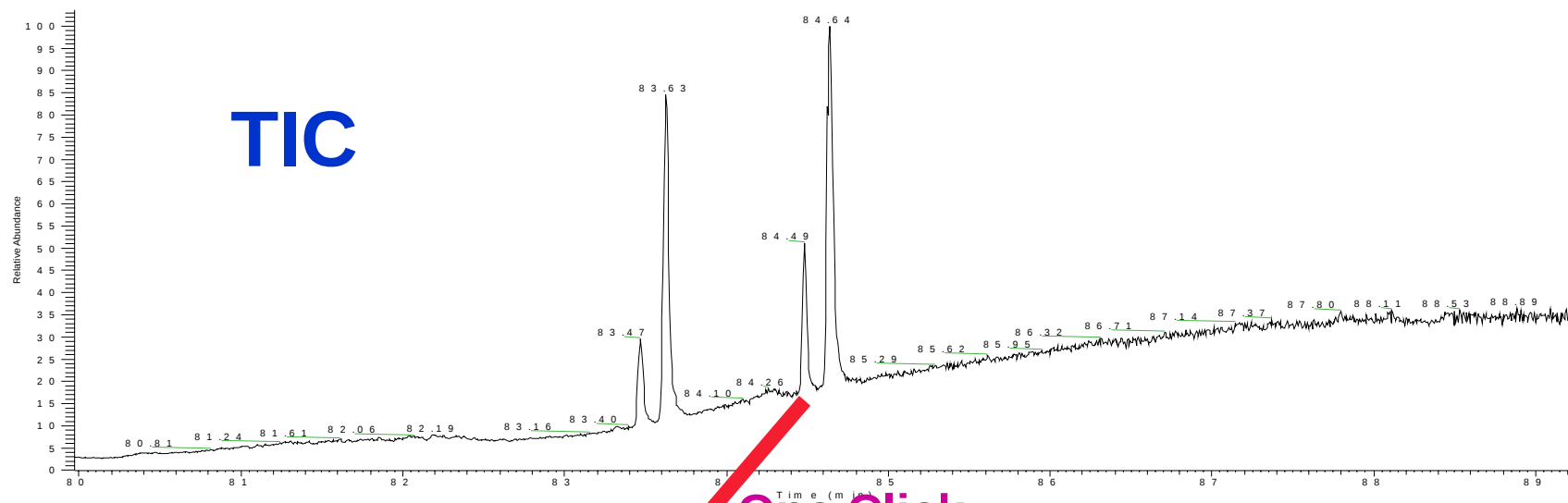
- **Full Scan :**
全質量範圍掃描一定性分析、Library Search
- **SIM (Selected Ion Monitoring) :**
選擇離子監測分析一定量分析
- **Sequential Full Scan :**
質量區間掃描一定性兼定量分析
- **SIM events :**
依據掃描次數改變 SIM 的離子

單段四極柱 全掃描質譜模式 Full Scan MS



Full Scan

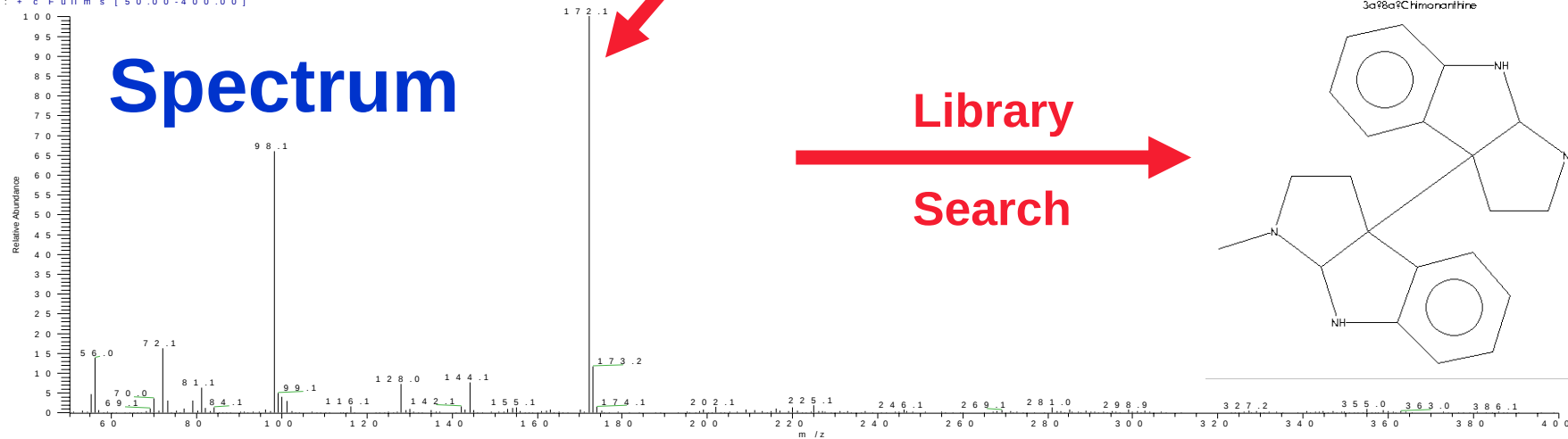
RT: 79.97 - 89.26



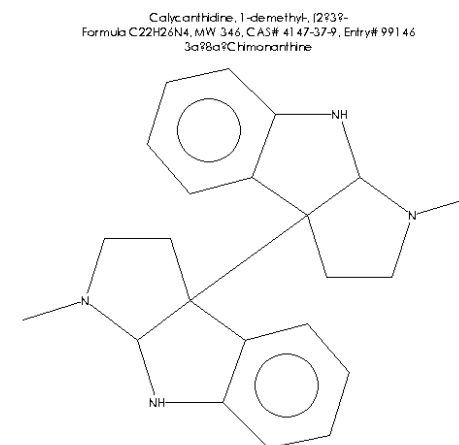
NL: 2.71E7
TIC: F: M S
93070704_040708144331
331

One Click

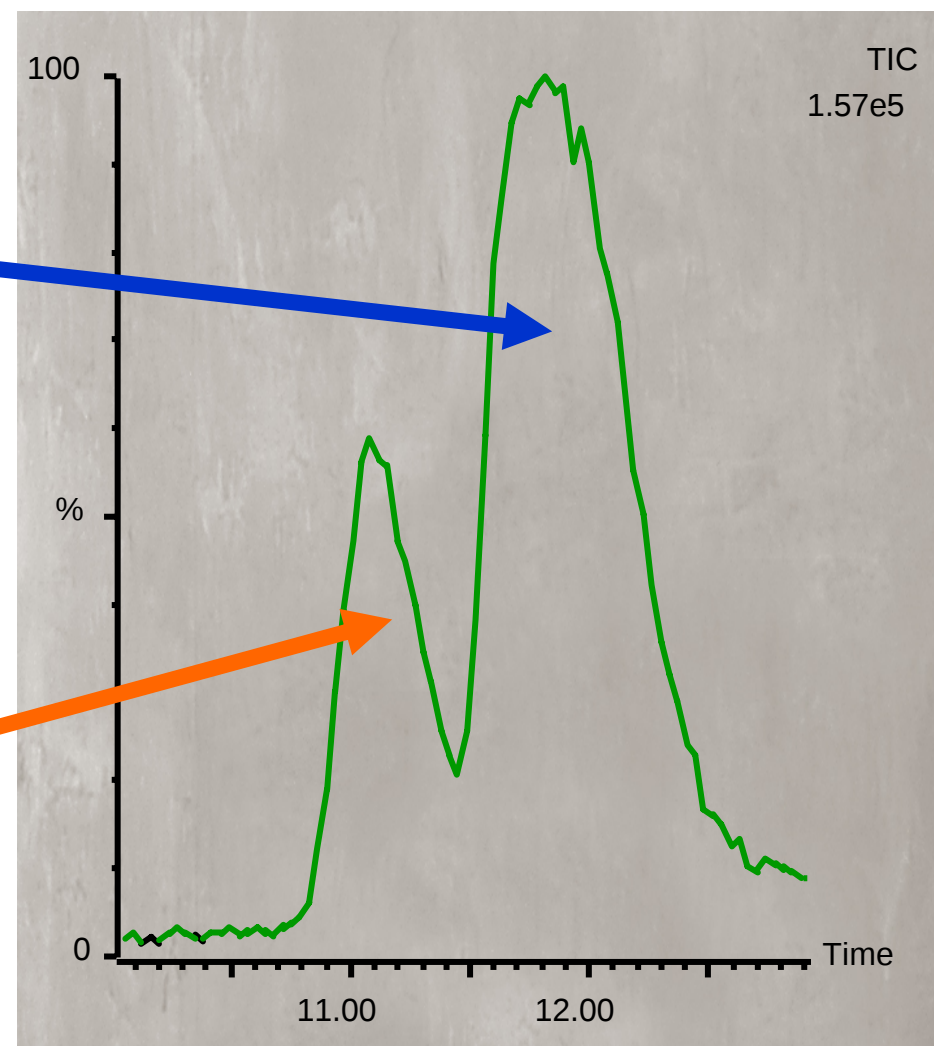
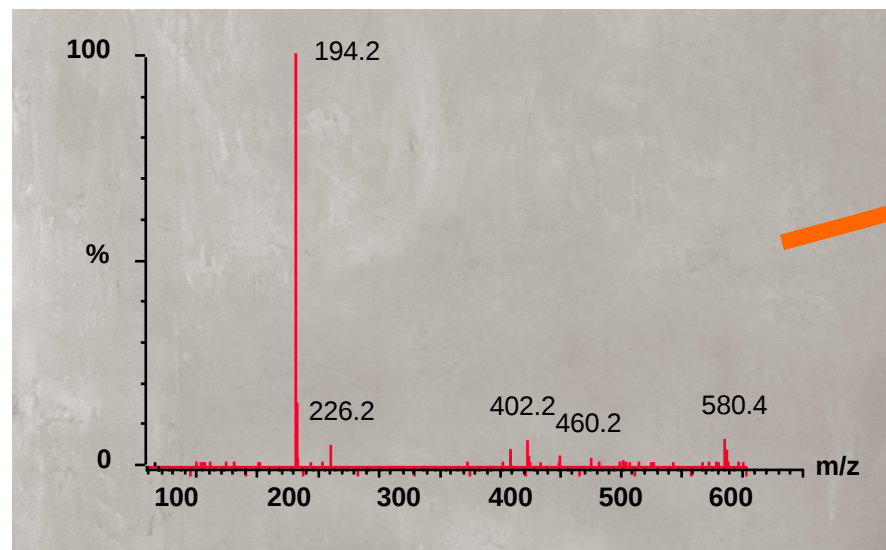
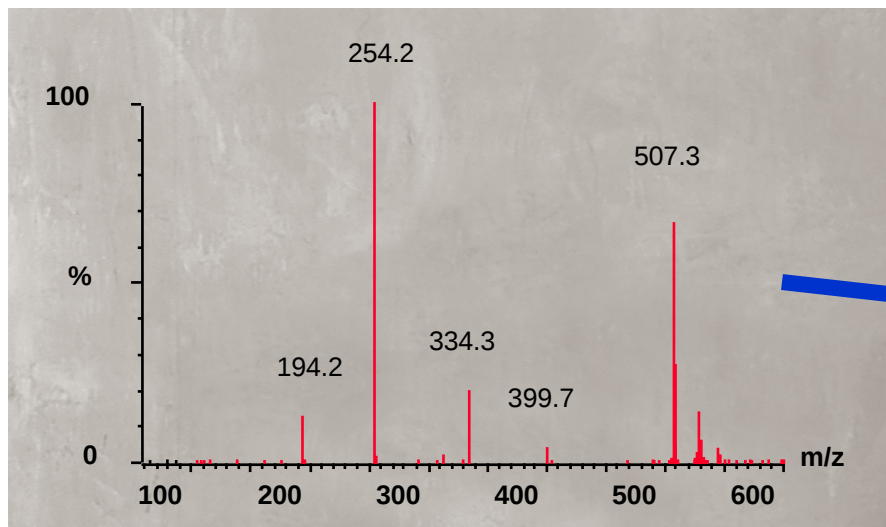
93070704_040708144331 #12992 RT: 84.65 AV: 1 NL: 5.94E6
T: + c Full MS [50.00-400.00]



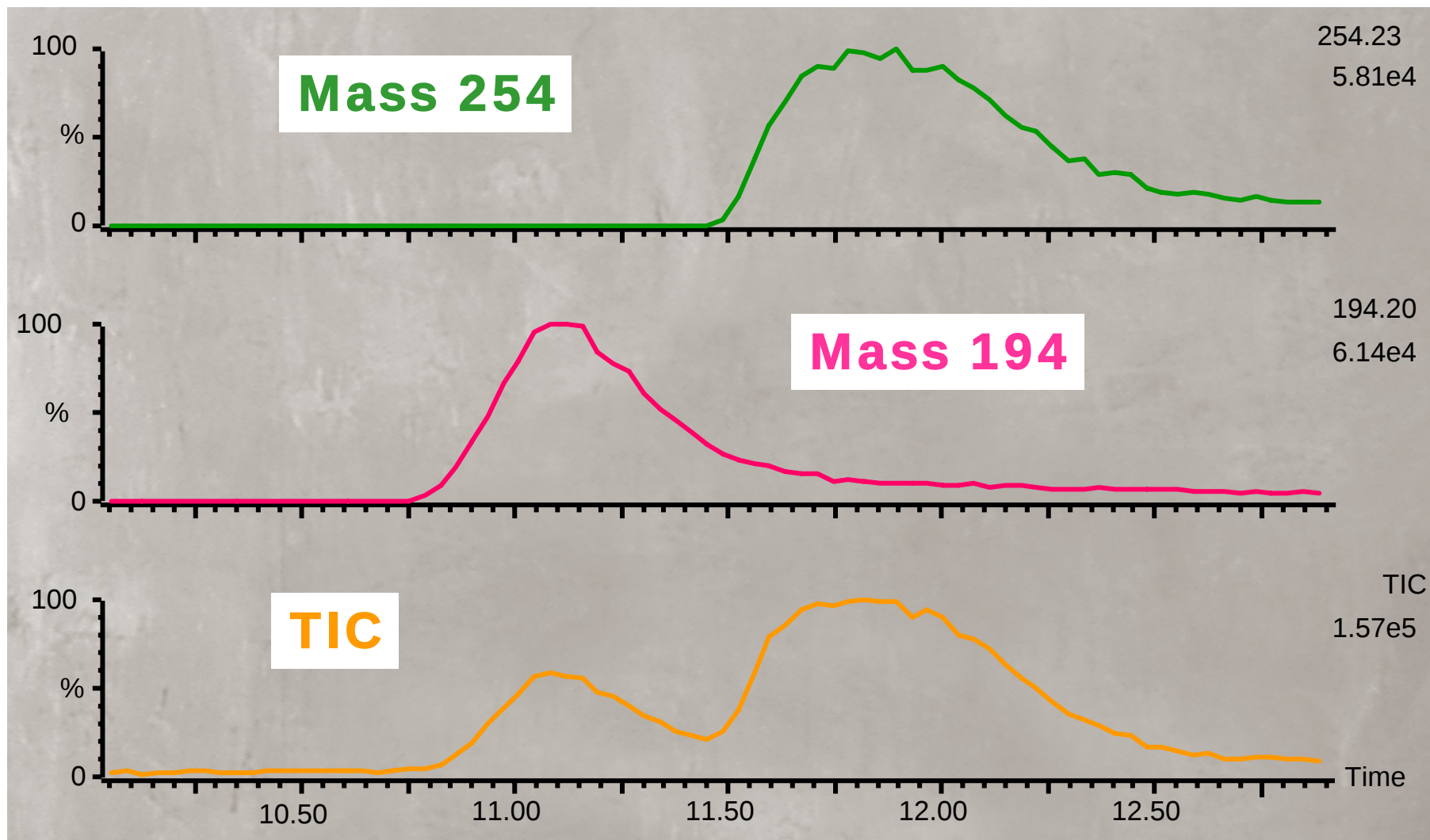
Library
Search



利用 EIC 分離 Co-eluting 成分



成功分離 co-eluting peaks



提升靈敏度 Improving Sensitivity

Single Ion Monitoring
單一離子掃描模式

與

Multiple Ion Monitoring
多重離子掃描模式

SIM 與 MIM

■ 單一離子掃描模式：SIM

- 在層析的特定滯留時間範圍內，將四極柱設定在只允許單一離子通過

Set quadrupole to pass a single characteristic ion during a retention time window in the chromatogram

- 可增加靈敏度達**10~100**倍
Increases sensitivity 10-100X

- 喪失圖譜的完整明確性
Lose spectral specificity

SIM 與 MIM

■ 多重離子掃描模式：MIM

- 監看**2~5**個特性離子

Monitor 2 to 5 characteristic ions in addition to SIM quantitation ion

- 以各離子間的比例關係，用以當作鑑別之用

Set acceptable qualifier ion “ratios” to confirm detection

- 採用越多的特性離子做定性鑑定時，其可信度與正確性均會較高，但是會損失分析的靈敏度

More qualifier ions boost confidence but reduce sensitivity gains

四極柱質譜儀

■ 優點

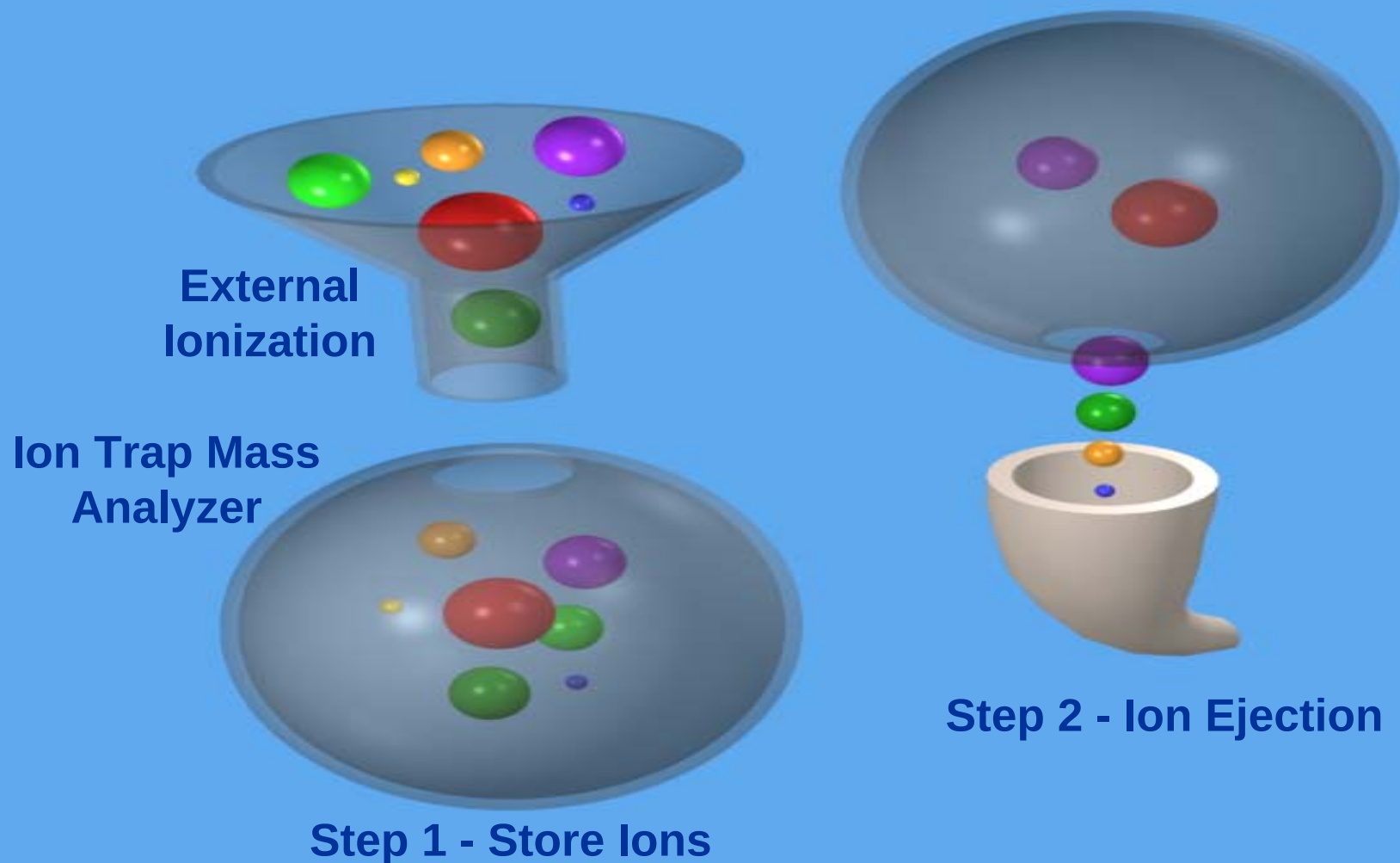
- 空間質譜儀
- 堅固耐用
- 價格低廉
- 操作簡單
- SIM 模式的感度高

■ 局限

- 缺乏 MS/MS 資訊
- 缺乏精確質量數資訊
- 掃描速率比飛行時間分析器慢

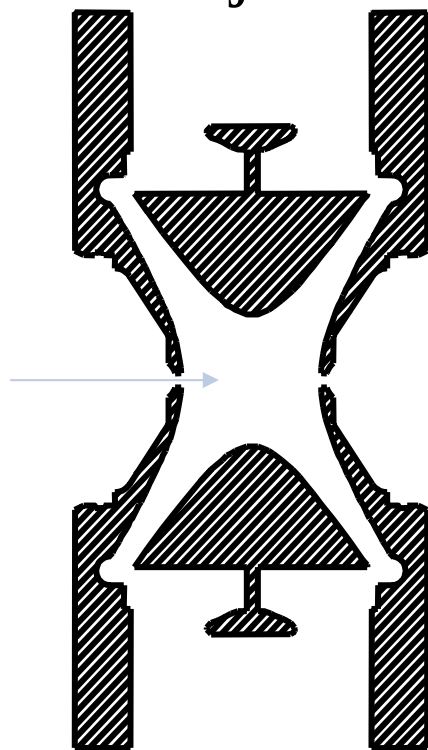
離子阱質量分析器示意圖

- Ions of all m/z stored until time to detect

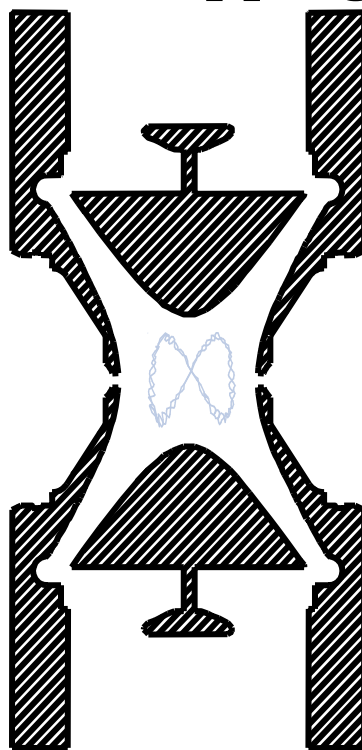


基本步驟 Basic Steps

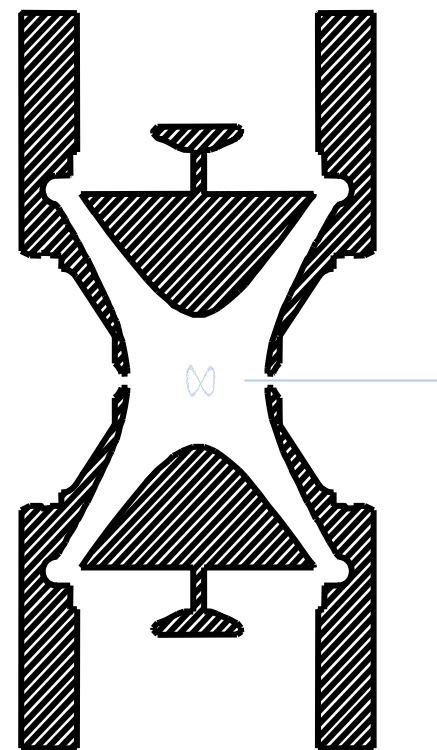
離子注入
Ion Injection



離子捕捉
Ion Trapping



質量選擇釋放
Mass-selective
Ejection



掃描功能 Scan functions

- 全離子掃描

Full Scan

- 選擇離子掃描

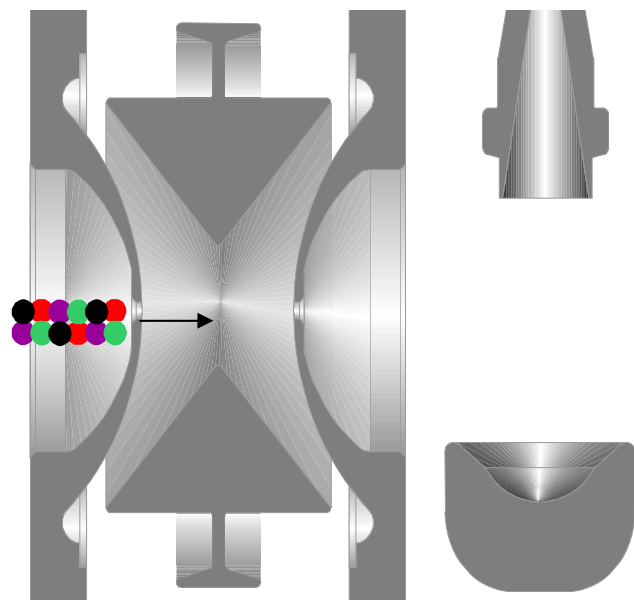
SIM (Selected Ion Monitoring)

- 多重質譜功能, 將預先以選定之母離子,
再碎裂成所謂的子離子

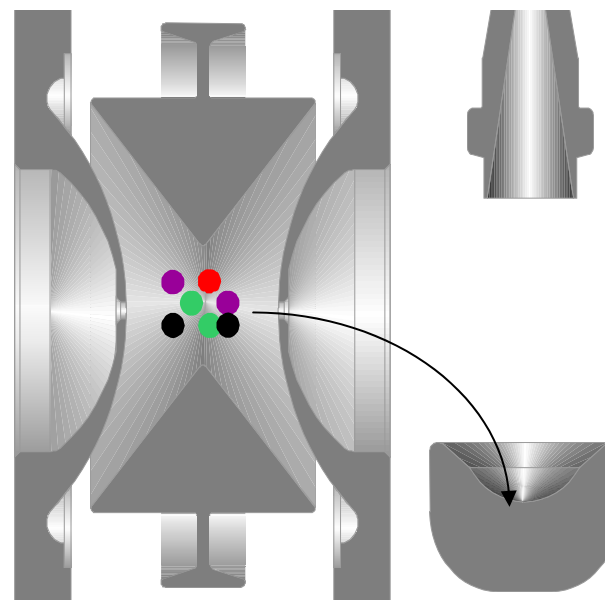
MSn Isolate a parent ion and fragment to form daughter ions.

全離子掃描模式 Full Scan MS

1. Inject

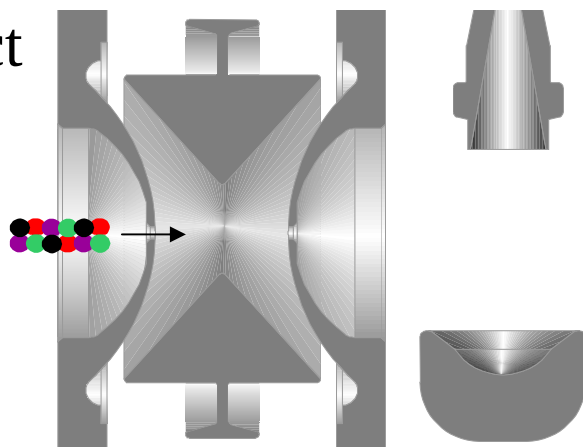


2. Detect

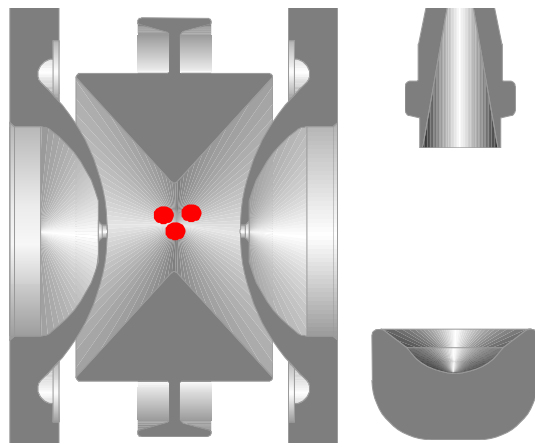


選擇離子模式 Single-Ion Monitoring (SIM)

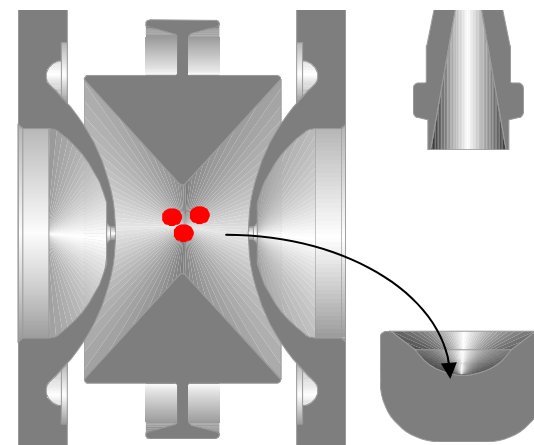
1. Inject



2. Isolate

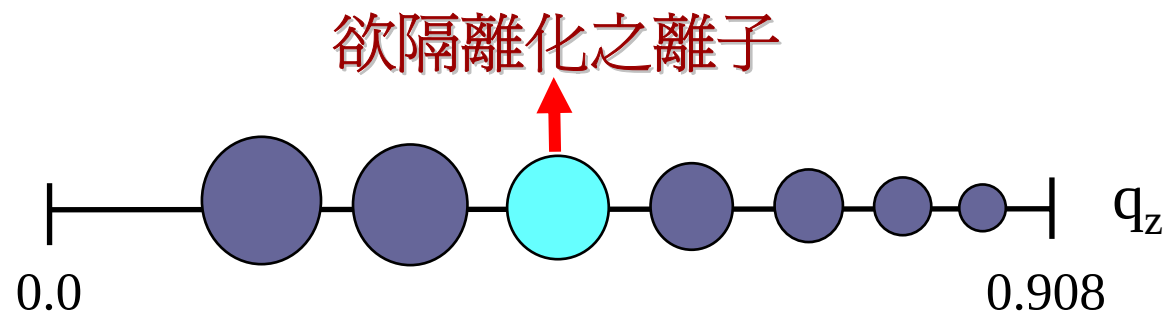
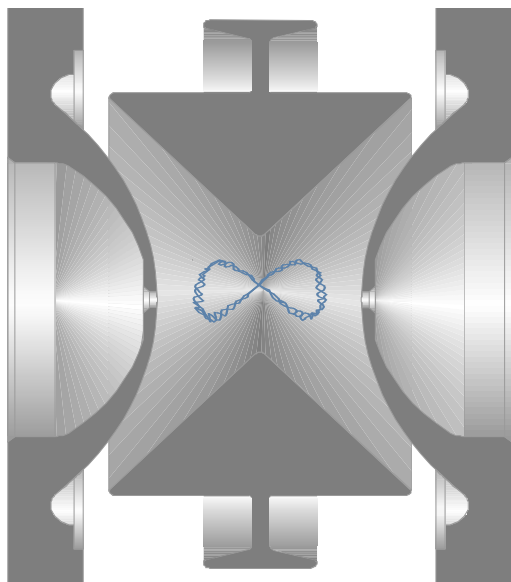


3. Detect



隔離單一離子 Isolation of Ions

- 離子在不同的 q_z 值環境下，會以不同的頻率(ω_0)進行振盪
- Ions at different q_z values oscillate at different frequencies (ω_0)



$$\omega_0 \approx \frac{q_z \Omega}{2\sqrt{2}}$$

Analytical Benefits of MS/MS

- 增加靈敏度 Increased selectivity
 - Concentration of analyte too low
 - Chemical interferences too high (complex matrix)
- 結構判定 Structural characterization
 - Provides information about chemical structure of unknown compounds
- 操作簡易 Ease of use
 - Routine GC/MS/MS is as practical as GC/MS

何謂靈敏度 What is Sensitivity?

Sensitivity is a function of the **Signal to Noise**

$$\text{Signal/Noise} = \text{Sensitivity}$$

To improve Sensitivity

Increase Signal
or
Reduce Noise

How we improve Sensitivity

靈敏度: $S/N = \text{Sensitivity}$

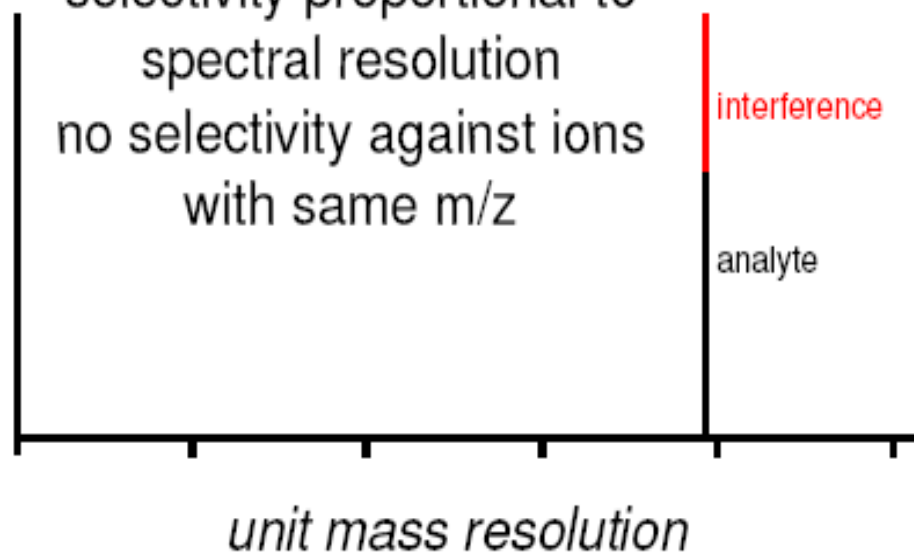
Quadrupoles use SIM to **increase S**

Ion trap uses MS/MS to **reduce N**

去除分析物干擾來提升選擇性

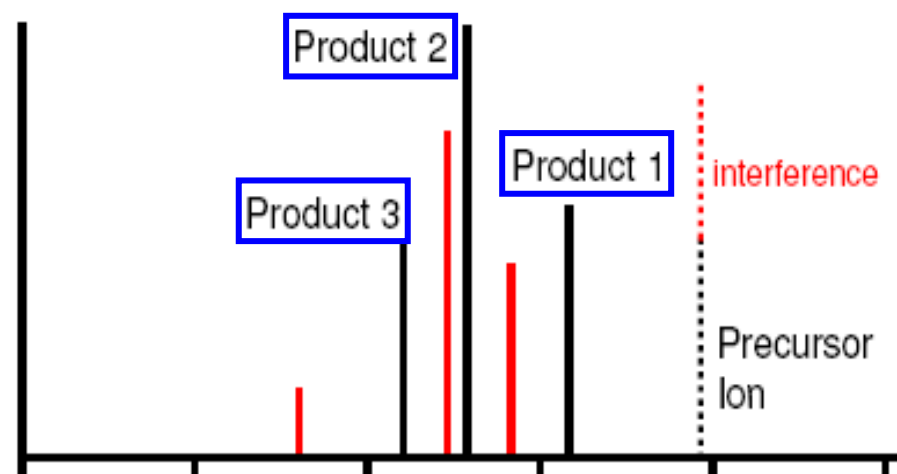
EI-SIM

selectivity proportional to
spectral resolution
no selectivity against ions
with same m/z



EI-MS/MS

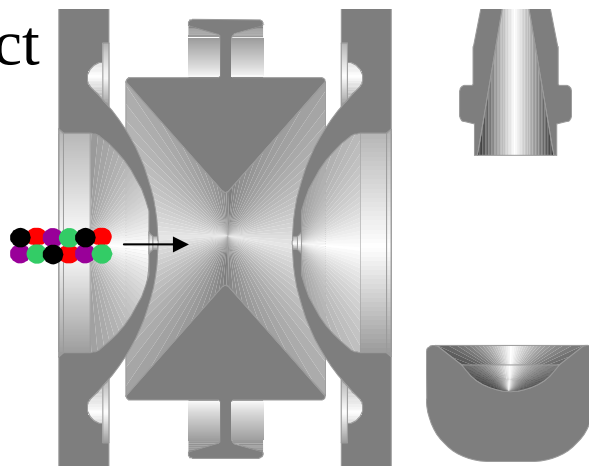
Precursor selectivity same as SIM
High probability that at least one product
ion will be a unique dissociation product
of the precursor BUT not the interference



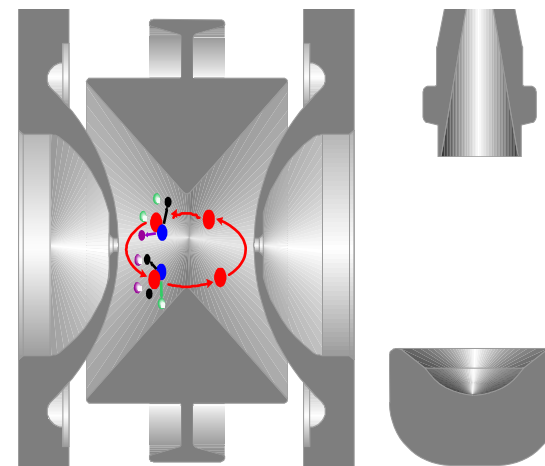
The precursor ion should **NOT** be used for ion
ratios or quantitation since the interferences will
be the same as the SIM ion

MS/MS 於離子阱內之分析模式

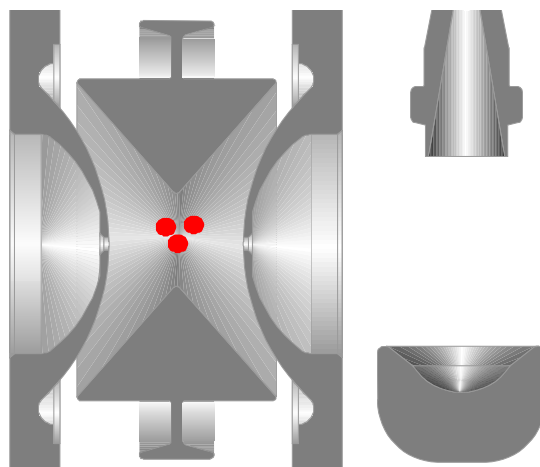
1. Inject



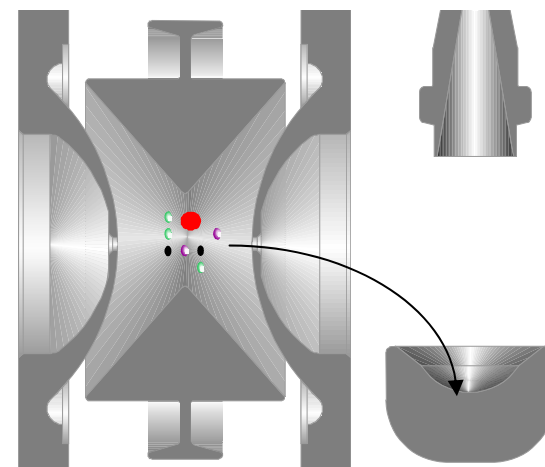
3. Fragment



2. Isolate

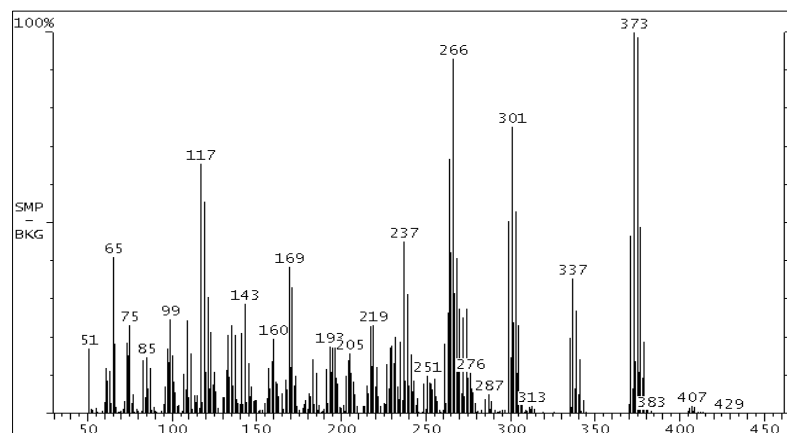


4. Detect

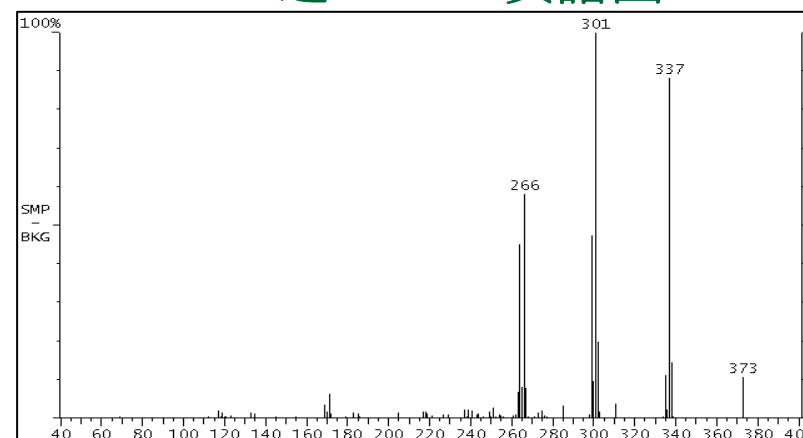


MS/MS 樣品分析範例

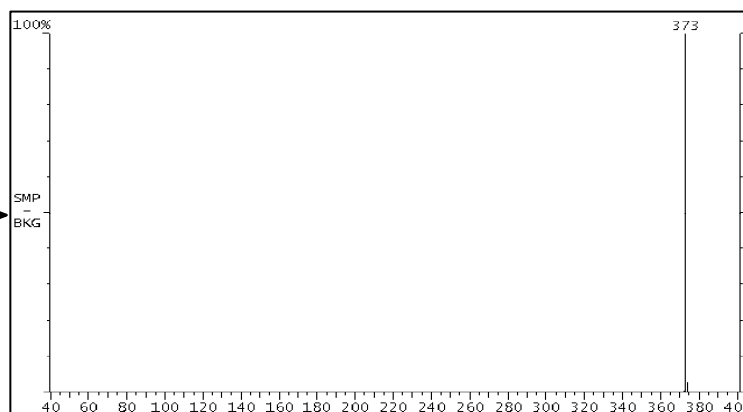
EI 之一次離子質譜圖



EI 之MS/MS質譜圖



母離子隔離階段
Isolation Event



啓動碰撞能量
Collisional
Activation Event

MS/MS於分析上的優勢 Analytical Benefits

- **增強選擇性** (Increased selectivity)
 - 適用於當欲分析物相對於其背景之化學干擾，樣品之濃度顯得偏低的狀況 Advantageous when concentration of analyte is low compared to chemical interferences (complex matrix)
- **提供結構鑑定的資訊** (Structural characterization)
 - 對於未知物種的分析工作，提供其化學結構上的訊息 (Provides information about chemical structure of unknown compounds)
- **操作簡易** (Ease of use)
 - 使用方式與一般例行性之分析方法無異 (Routine GC/MS/MS is as practical as GC/MS)

MS/MS於分析上的限制

- 靈敏度低於MS模式
(Less sensitive than MS)
- 較適用於已知目標分析物的鑑別工作上
(Can only analyze for target analytes)

GC/MS Ion Trap

■ 優點

- 時間質譜儀
- 多重質譜(MS^n)
- Scan 模式感度佳
- 可自動產生MS/MS結果
- 質譜斷裂碎片途徑，可以清楚釐清

■ 局限

- 缺乏精確質量數資訊
- 無法進行真正的SIM
模式：感度會比四極柱差
- MS/MS的低質譜區的斷裂碎片較少

Should I sell Single Quadrupole or Ion Traps?



ISQ



ITQ 1100 GC/MSⁿ

Table of Contrasts

Ion Traps

Mass Separation in time

High sensitivity Full Scan

Selectivity of **MS/MS**

Single Quadrupoles

Mass Separation in space

Lower sensitivity Full Scan

High sensitivity SIM

Library Search

Outcomes of Contrasts

Ion Traps

Mass Separation in Time

High sensitivity Full Scan
by Trapping over Time

Lower sensitivity SIM

Multiple stages of MS
For Confirmation/
And Structure Elucidation

Single Quadrupoles

Mass Separation in Space
Allows for Fast GC

Lower sensitivity Full Scan

High sensitivity SIM
By scanning a single ion
over time

No multiple stages of MS

**Thank you
for your attention**

