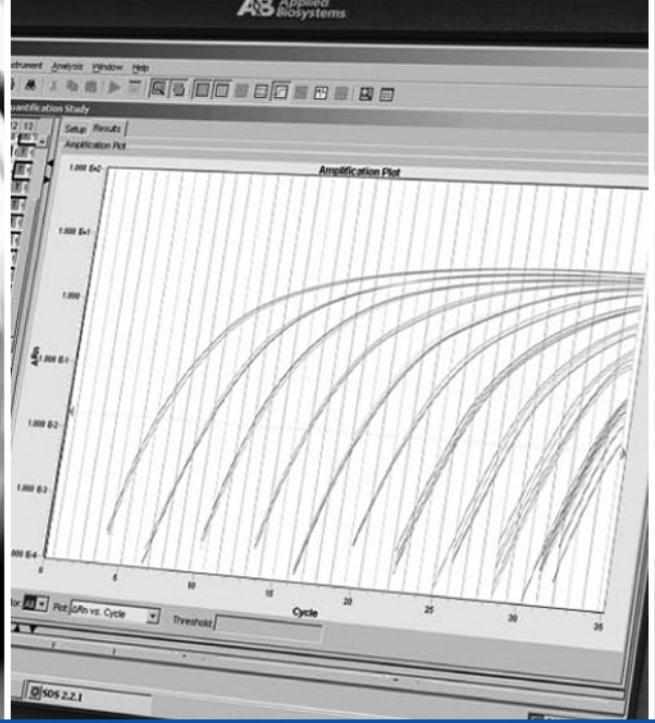

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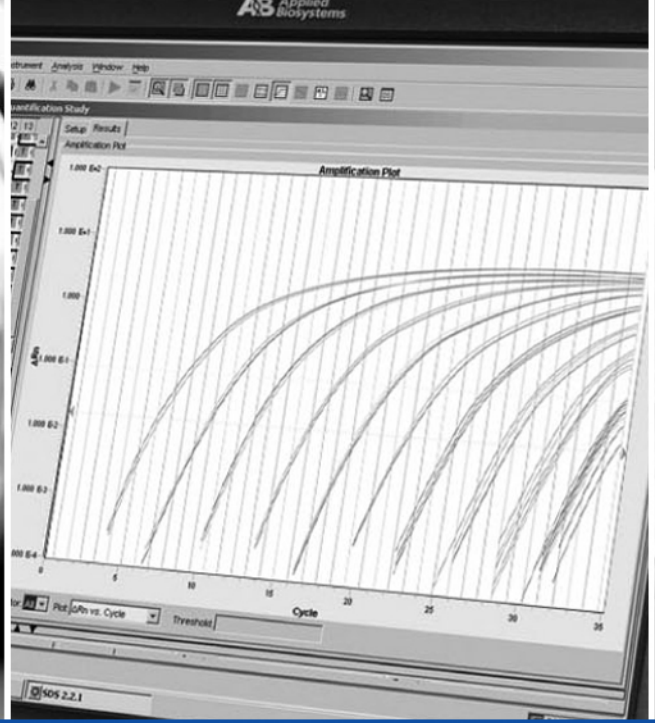
Introduction to LC-MS

Dr. Michael Baynham
Senior Specialist, Mass Spectrometry Applications
Clinical Research Network Coordinator

Overview

- Comparison of LC-MS with more traditional techniques such as LC with DAD detection, GC-MS & immunoassay analysis
- What is the difference between LC-MS and LC-MS/MS?
- LC-MS/MS system technology
- LC-MS/MS source technology
- What effects the way a compound forms an ion?
- Summary



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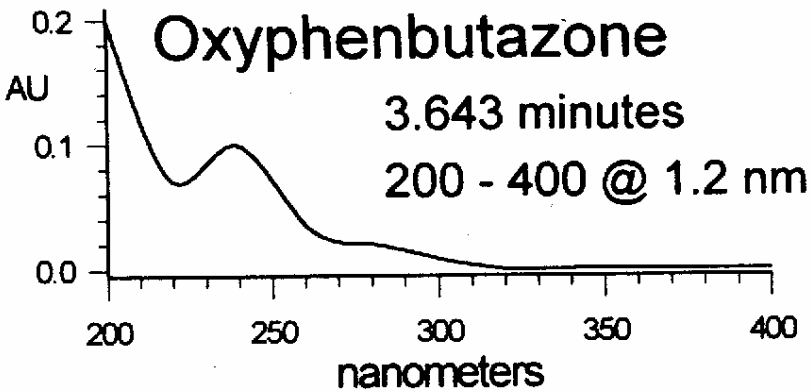
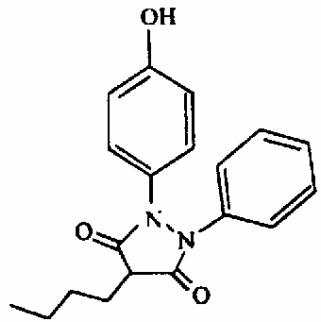
Comparison of LC-MS with Traditional Techniques

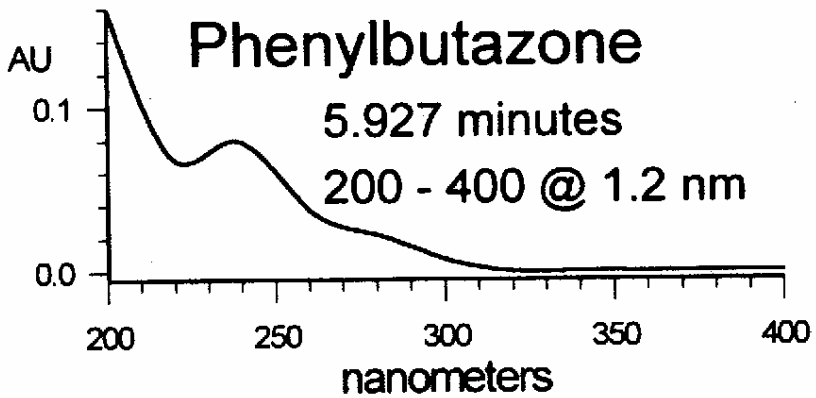
Diode Array Detection

- not specific enough for differentiation

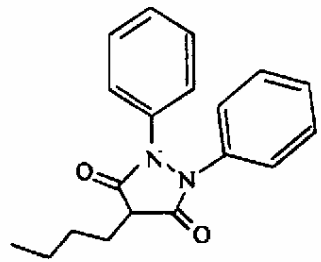


$C_{19}H_{20}N_2O_3$ FW 324.38

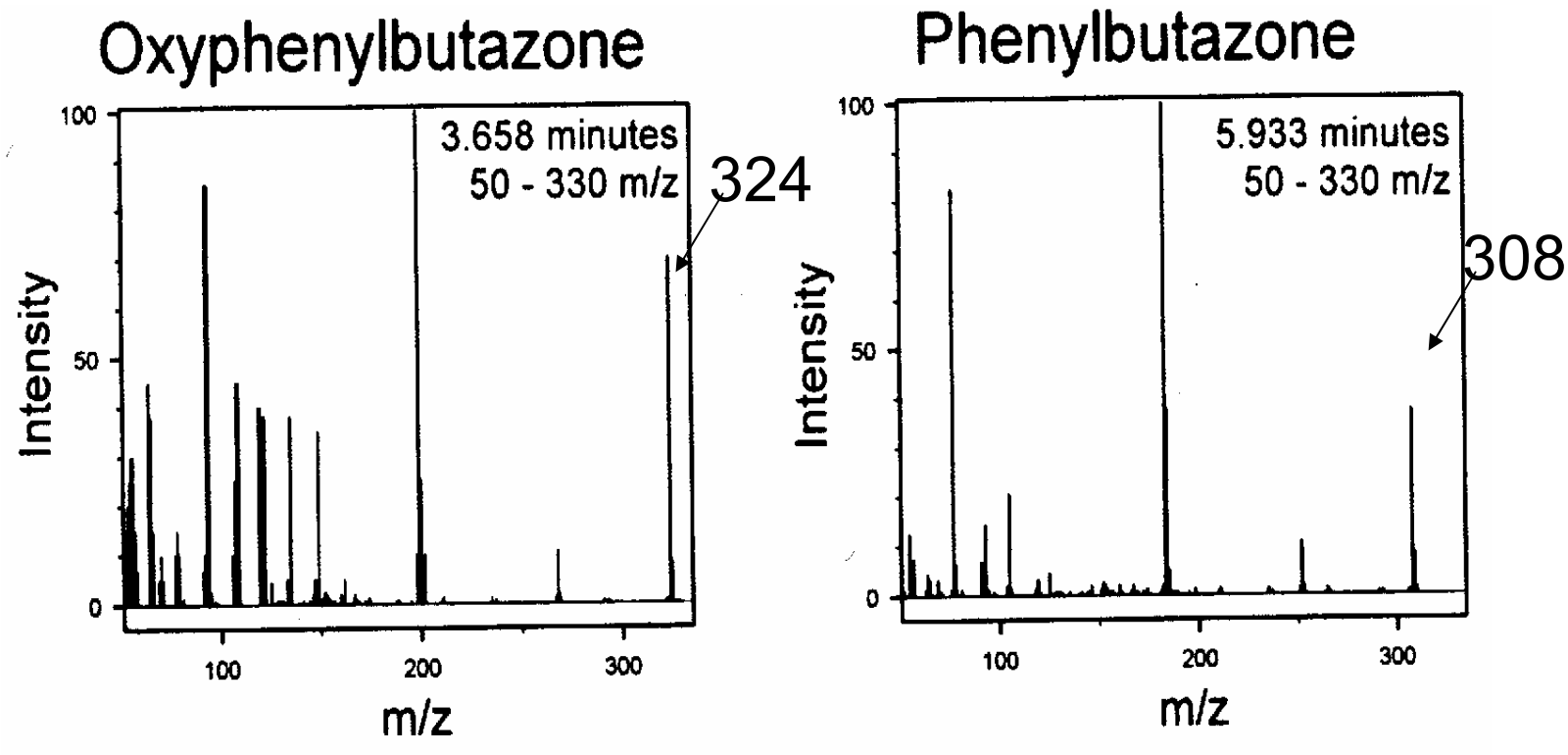




$C_{19}H_{20}N_2O_2$ FW 308.38



Mass Spectrometry Specificity



Comparison of LC-MS with GC-MS

- GC-MS requires compounds to be volatile to be ionised
  - traditionally electron impact source is used.
- LC-MS can be used to detect compounds from poly-aromatic (non-polar) to peptide and proteins.
- GC-MS is still able to detect long chain aliphatic compounds (petroleum based anlaytes) and very low mass volatile material better than LC-MS.

Comparison of LC-MS/MS with Immunoassay

- LC-MS/MS is more specific than immunoassay
  - LC-MS/MS detection is based on column retention time, parent mass and structure. Therefore it is compound specific.

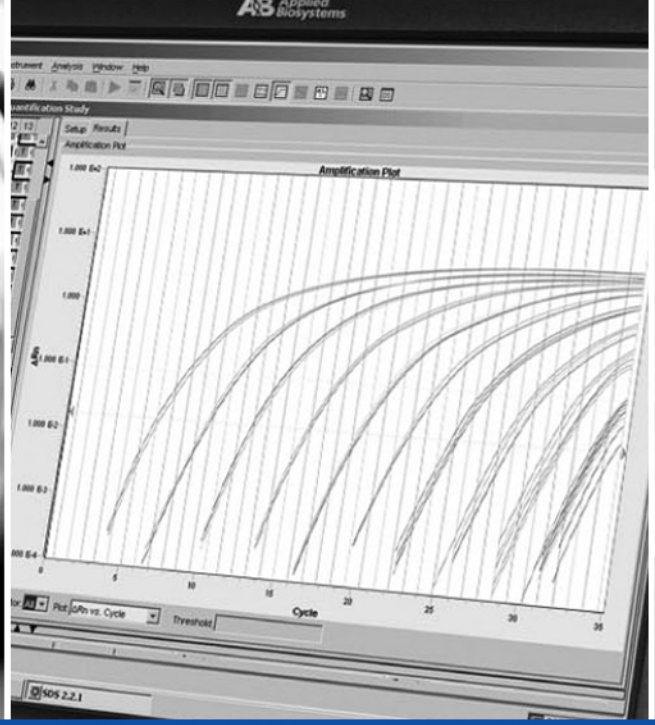
- Furthermore, derivitisation is not normally needed to detect the target compound, saving analysis time and money.
  - Immunoassay is normally based on compound class and will not be able to determine which compound in a group is detected.

Comparison of LC-MS/MS with Immunoassay

- LC-MS/MS is generally more sensitive than immunoassay
- LC-MS/MS can screen for more than one compound class with one injection
- Immunoassay measures one compound class per assay and therefore needs more sample when compound class screening
- Immunoassay is generally more rapid than LC-MS/MS

General reasons to look at LC-MS(/MS)

- Multi-component analysis
  - LC-MS(/MS) can detect over 300 compounds of different classes in just one run using often a low volume of sample
  - Reduces sample analysis time and allows for multiple analyses to be done on low volumes of samples with minimal sample pre-treatment



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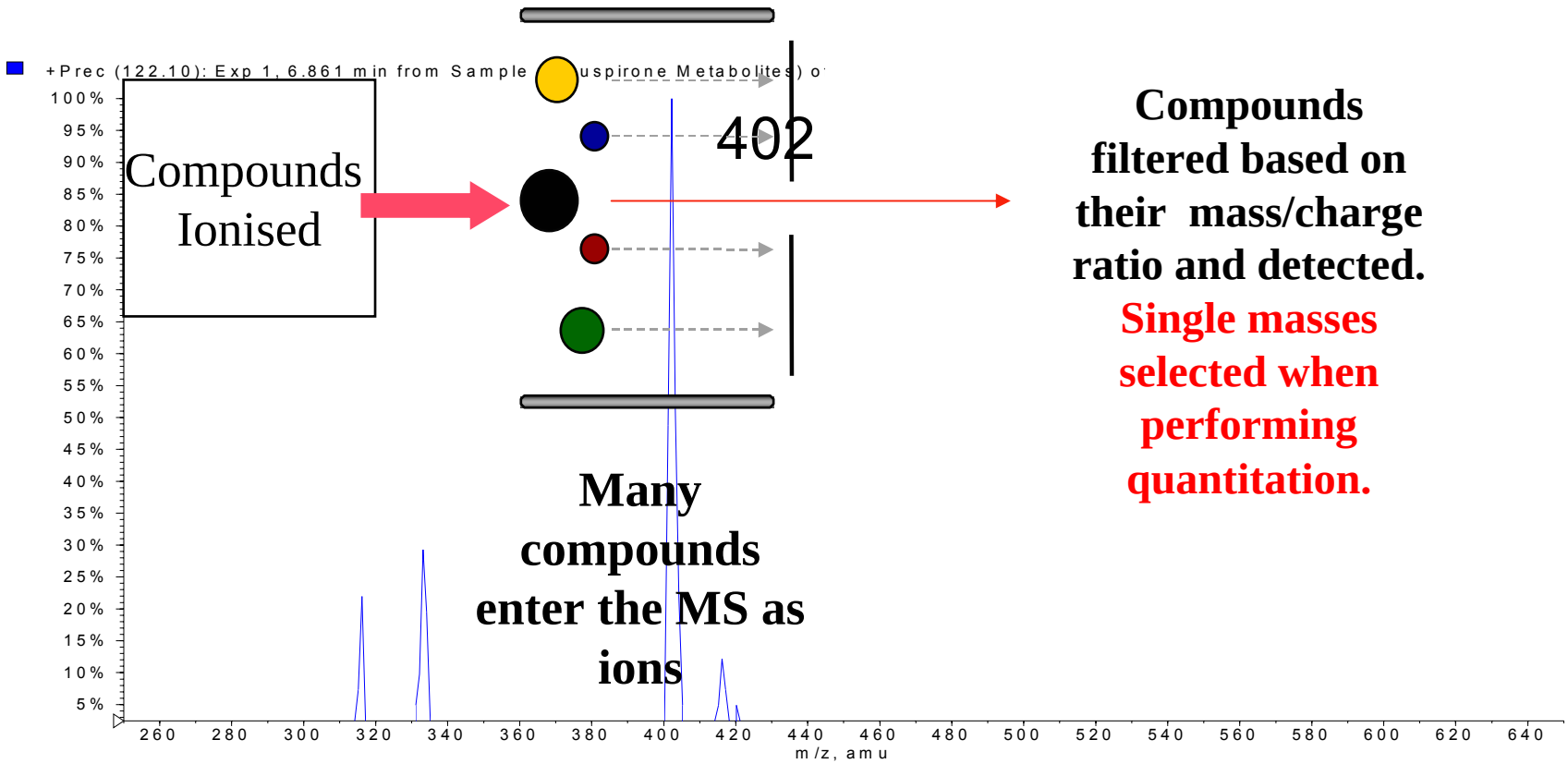
What is the difference between LC-MS and LC-MS/MS?

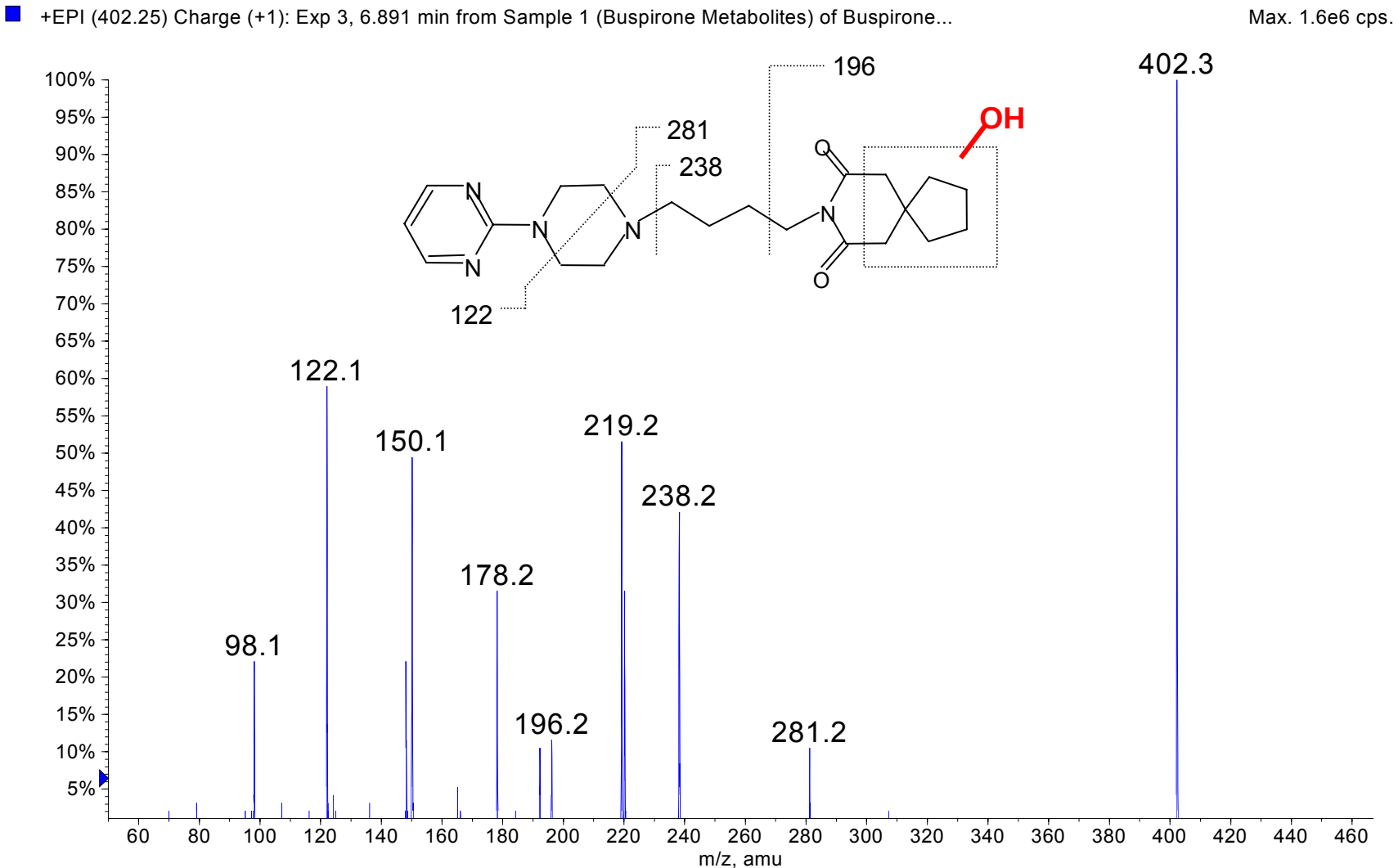
LC-MS (first stage of MS/MS)



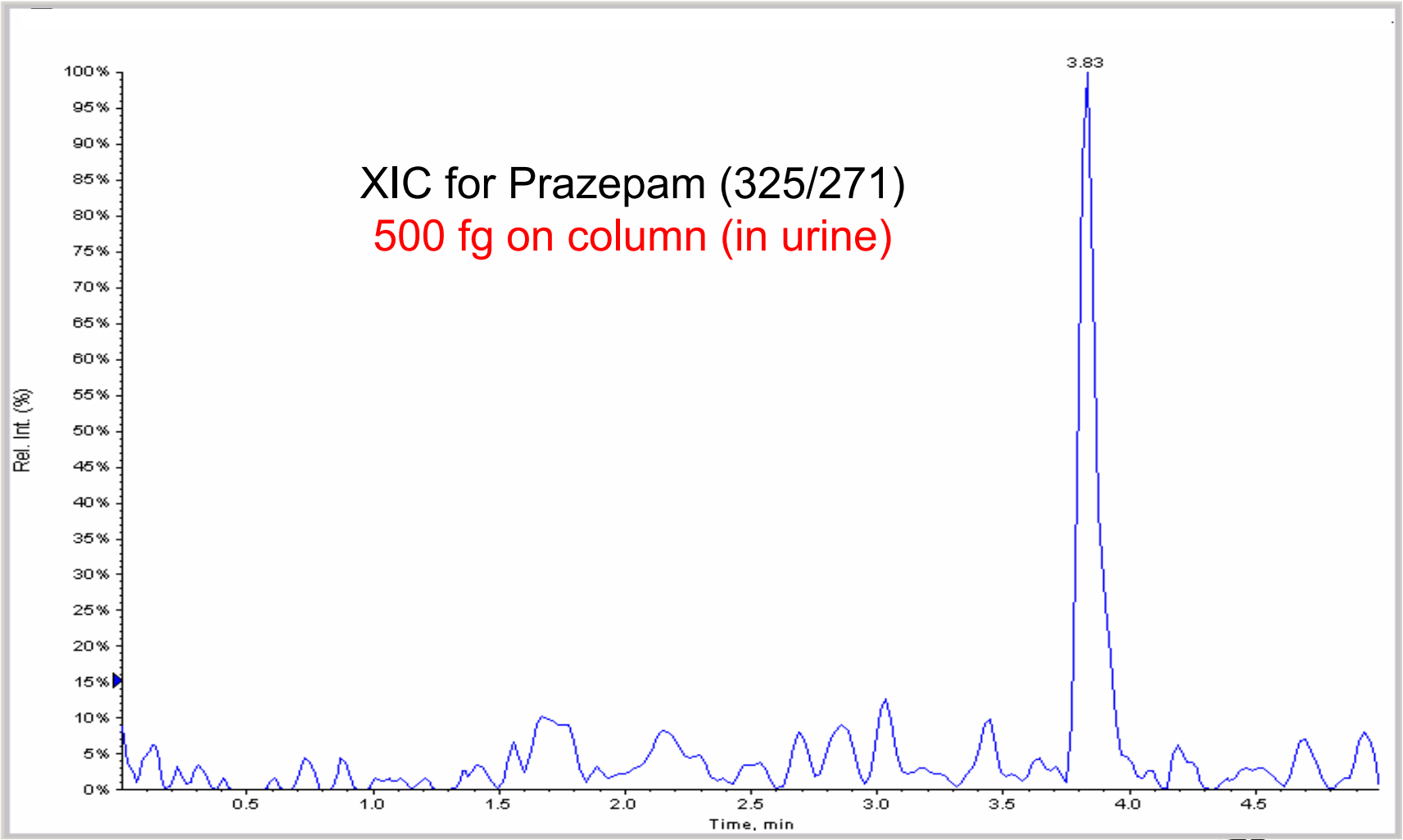
Compounds filtered based on their mass/charge ratio and detected.

Single masses selected when performing quantitation.

LC-MS/MS - Product Ion Scan



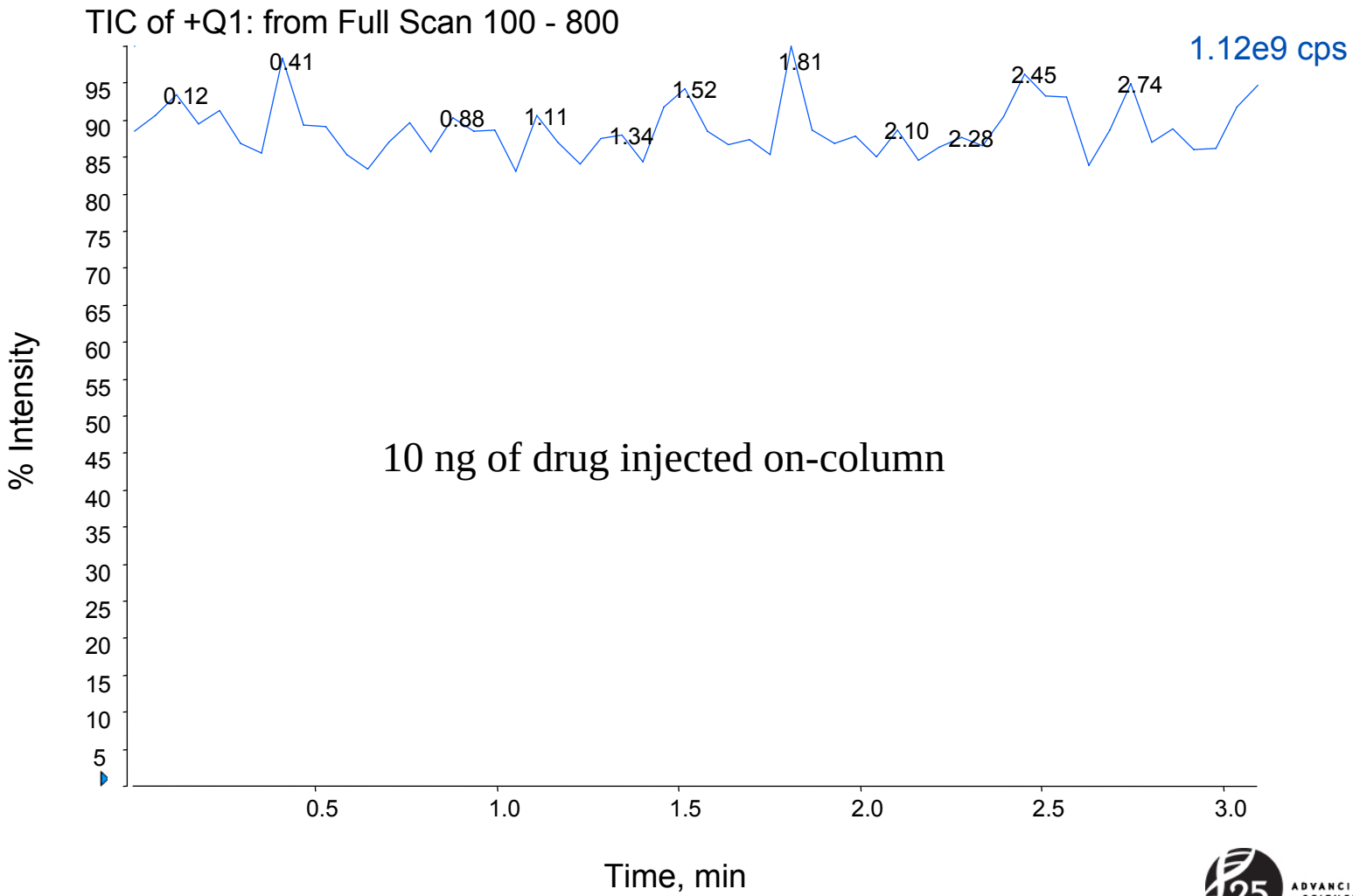
LC-MS/MS - Multiple Reaction Monitoring (MRM)



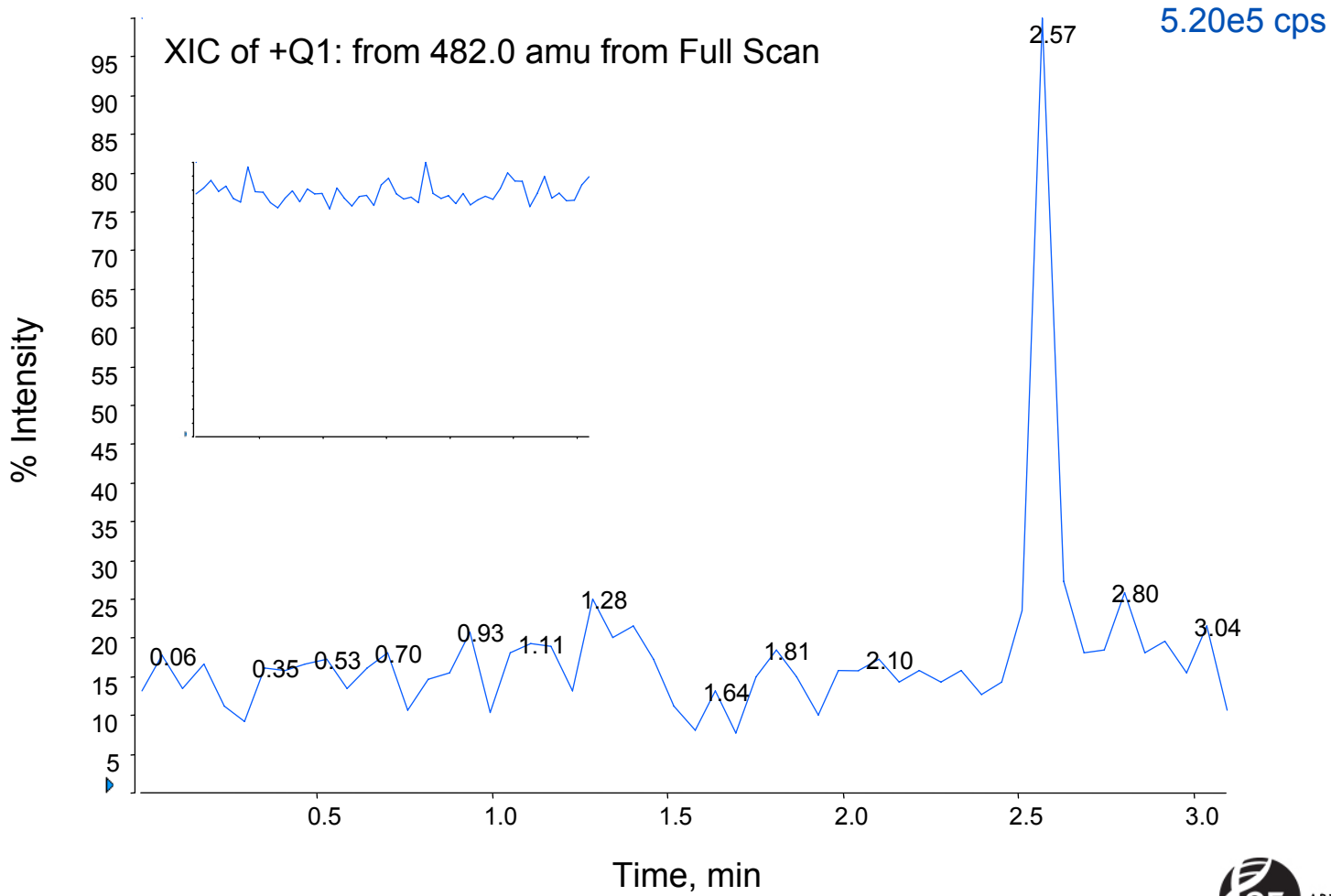
Why use LC-MS/MS rather than LC-MS

- Specificity and Sensitivity
  - LC-MS/MS is often 20 -100 times more sensitive compared to LC-MS in a multi-targeted screening approach.
  - LC-MS/MS is much more specific than LC-MS as there is a second filtering process.
    - Can use shorter columns allowing quicker run times as there is less background interference from the sample.
  - Allows the generation of library searchable spectra

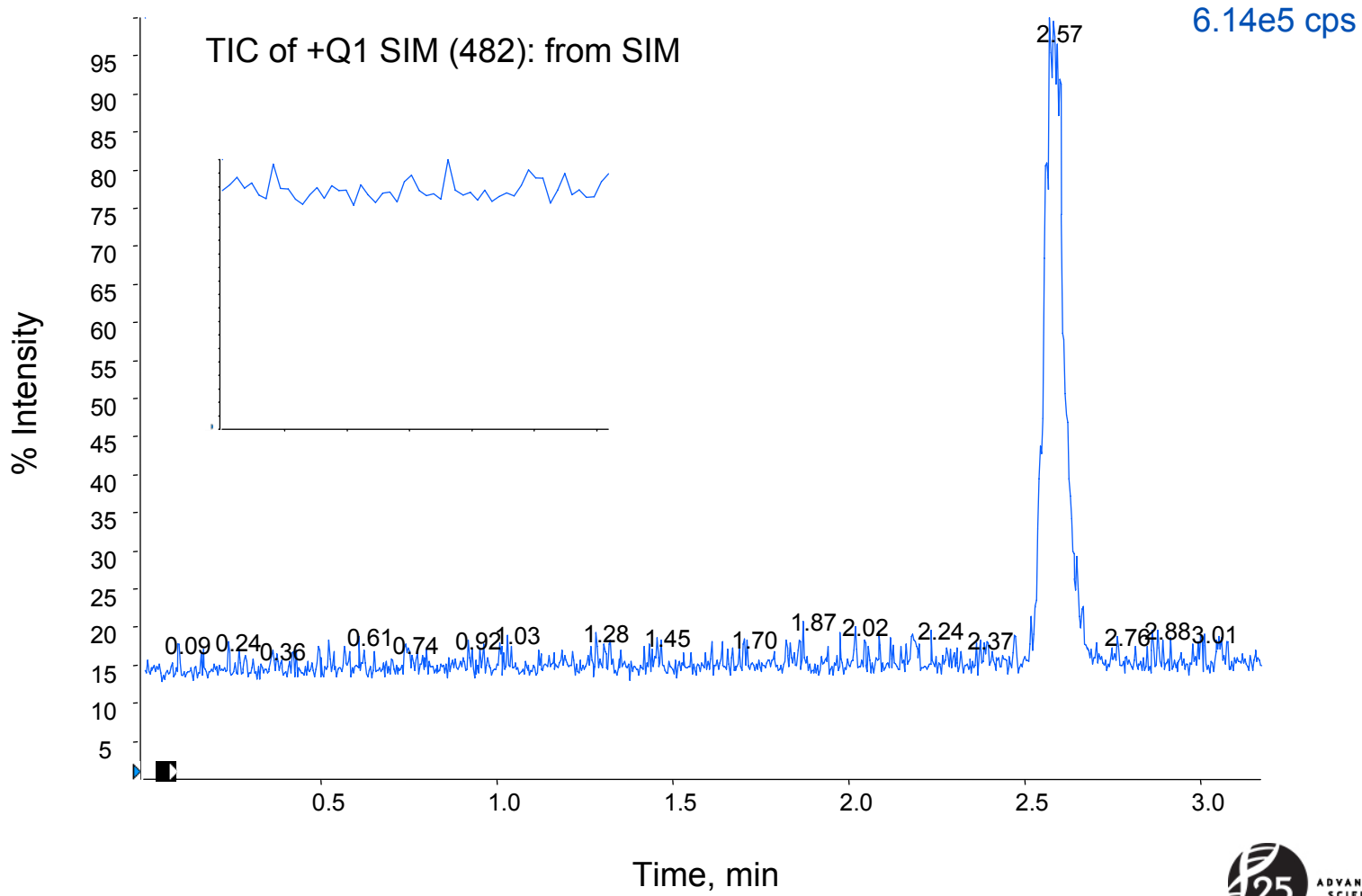
FROM MS TO MS/MS



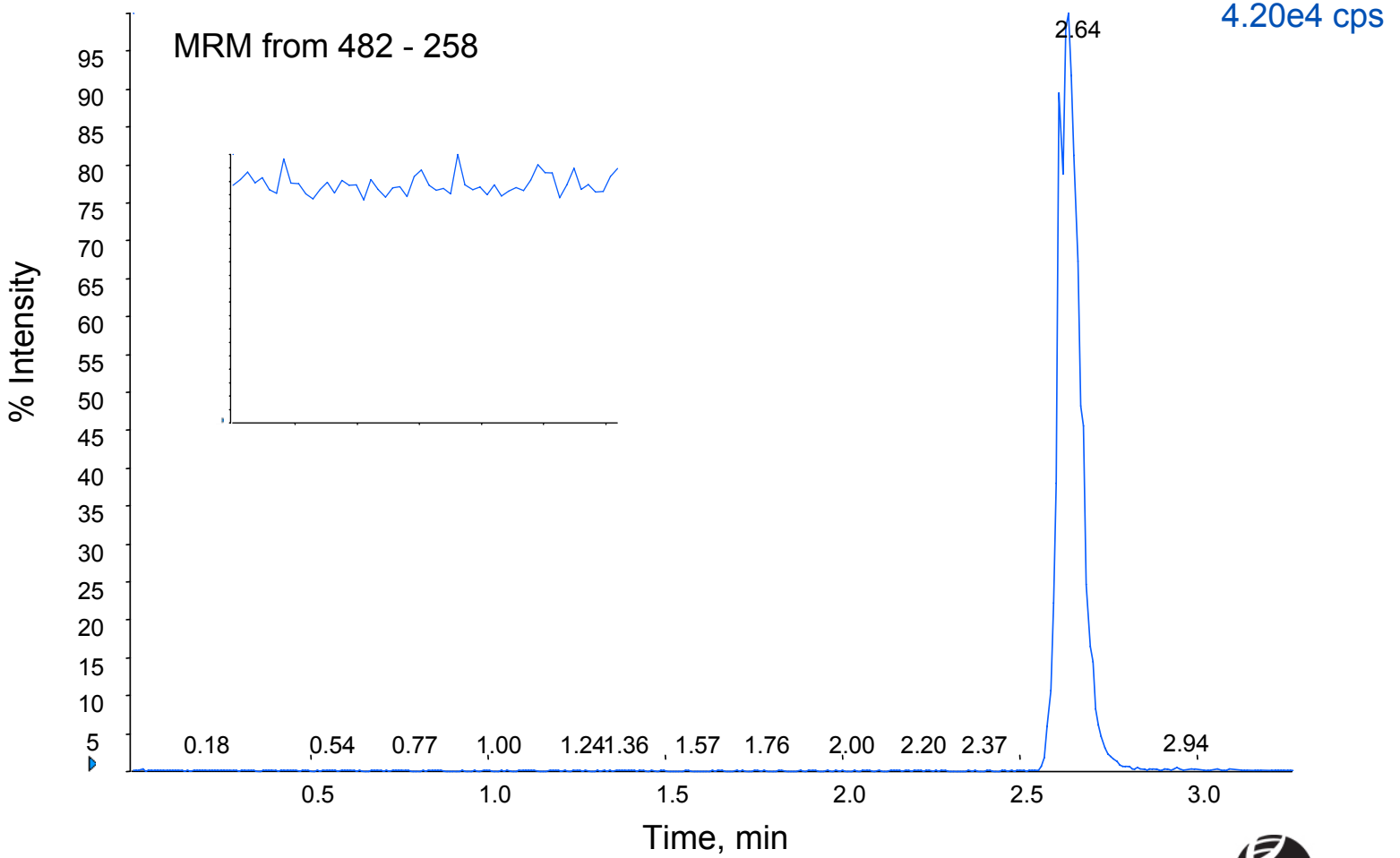
FROM MS TO MS/MS



FROM MS TO MS/MS



FROM MS TO MS/MS

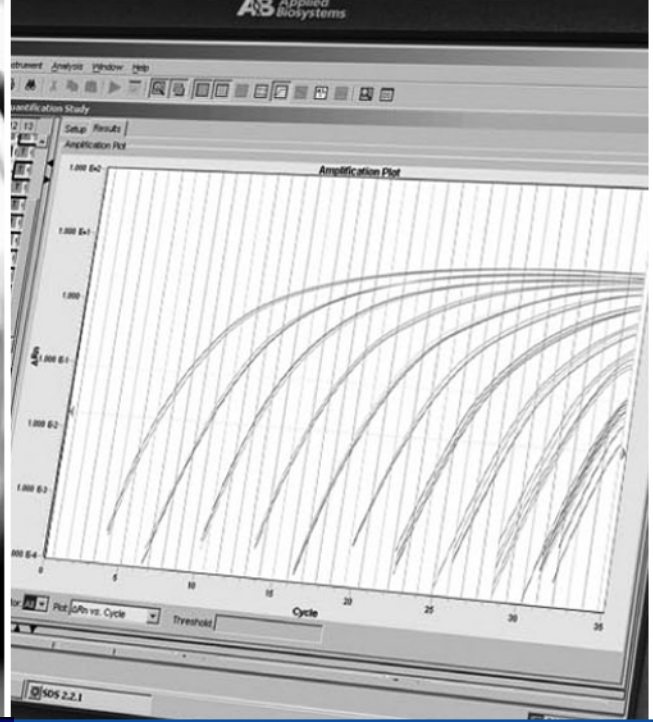


LC-MS/MS Systems for Small Molecule Analysis

- Iontrap (traditional spherical traps)
- Quadrupole Time of Flight (QqTOF) systems
- Triple quadrupole (QQQ) systems.
- Q TRAP® systems.

Similarities between these systems?

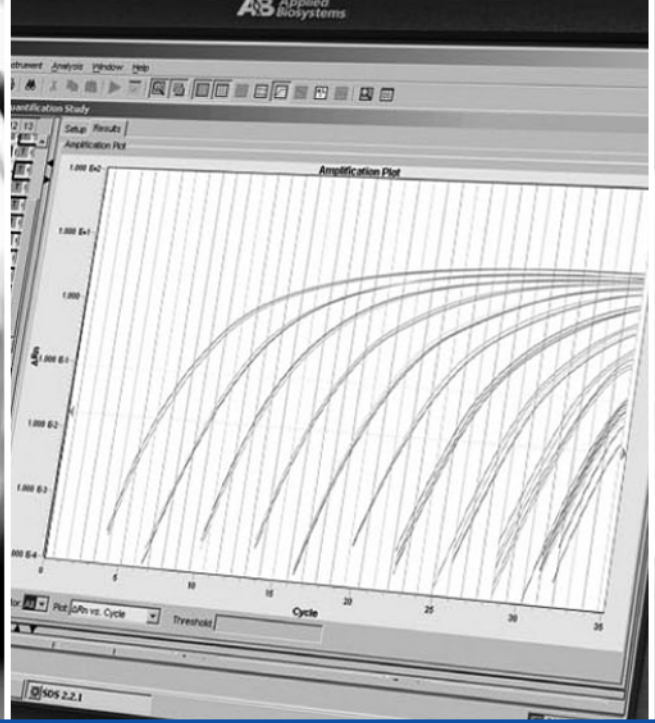
- They all separate ions based on their mass/charge (m/z) ratio
- All are capable of LC-MS/MS analysis
- In clinical samples the charge (z) is normally 1



Triple Quadrupole (QQQ) Systems

QQQ systems

- Qualitative analysis (spectral data acquisition)
  - Relative low sensitivity in scan mode due to lower duty cycle.
  - Low mass accuracy and resolution versus QqTOF systems.
- Quantitative analysis (multi-targeted applications)
  - MRM possible
  - High sensitivity and linearity
  - Simultaneous multi component analysis possible.



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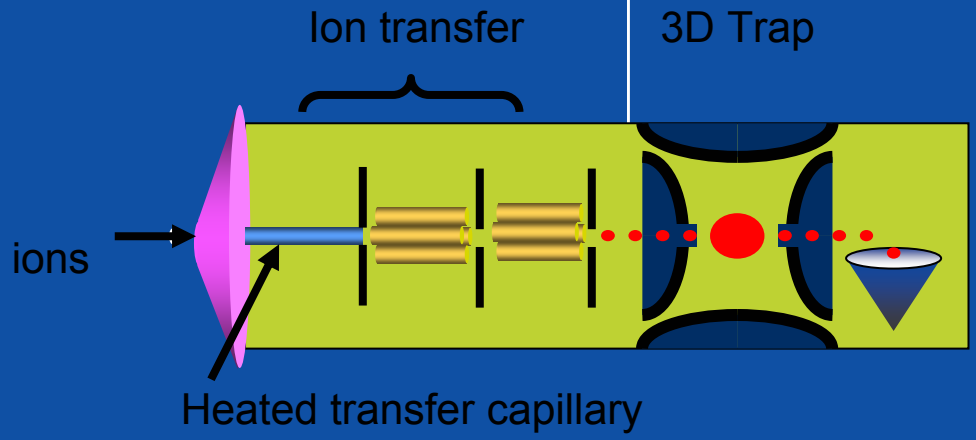
Spherical (3-dimensional) Ion Traps

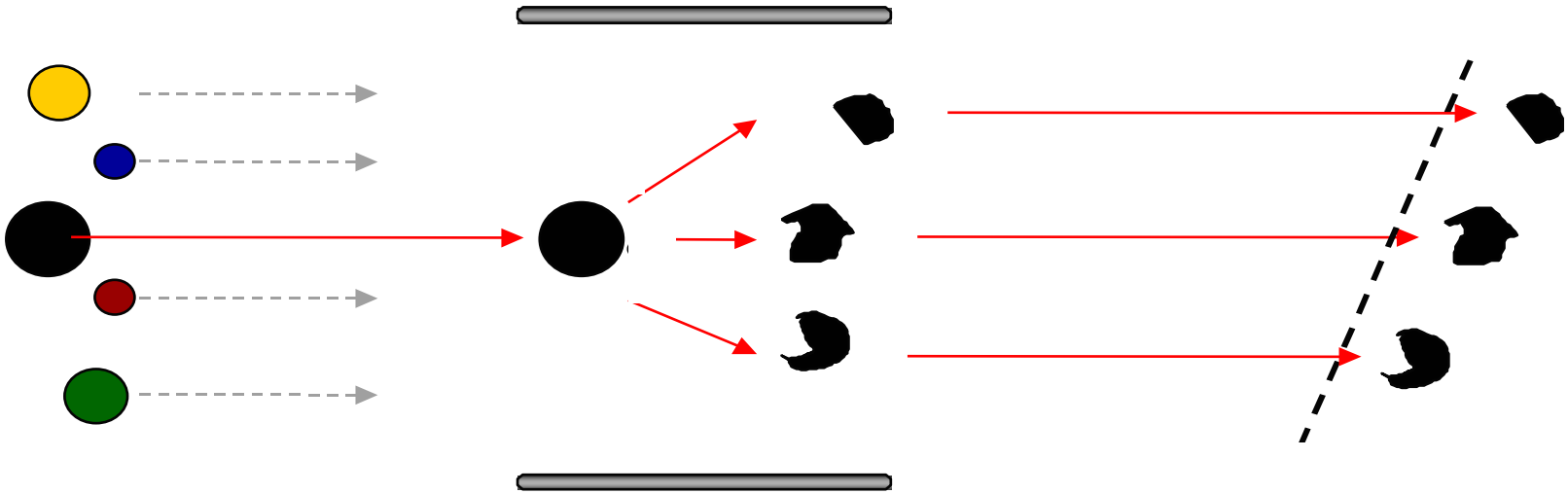
3D Trap



Spherical Ion Traps Product Ion Operation

- MS/MS in time, not in space
  - Ion processing (trapping, isolating, excitation (fragmentation) and scanning take place at the same location (inside the trap cavity).
- Can do MS^n

Spherical Ion Trap Product Ion Scan



Many analytes enter the MS

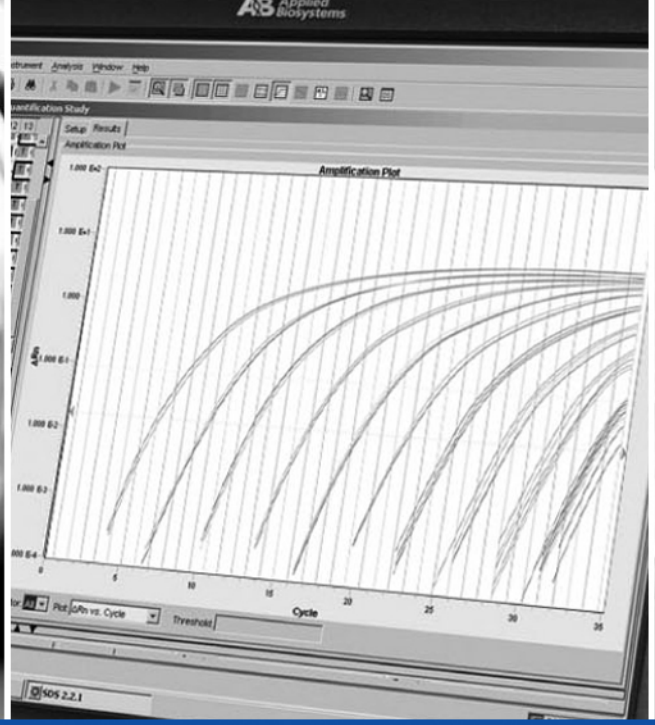
One mass isolated and then fragmented by excitation.

All the fragment ions trapped in the spherical trap are then scanned out based on their mass/charge

Spherical Ion Traps

- Qualitative analysis (spectral data acquisition)
  - High sensitivity in full scan and product ion scan mode
  - Low mass accuracy and resolution versus QqTOF systems.
  - Space charge effects possible (Resolution loss, mass shifts, isotope ratio shift, loss of dynamic range and accuracy).
  - Low mass cut off.

- Quantitative analysis (multi-targeted applications)
  - Lower sensitivity and linearity.
  - No real MRM possible.
  - No simultaneous multi component analysis (relative low scan speed compared to MRM on triple).



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QqTOF Technology

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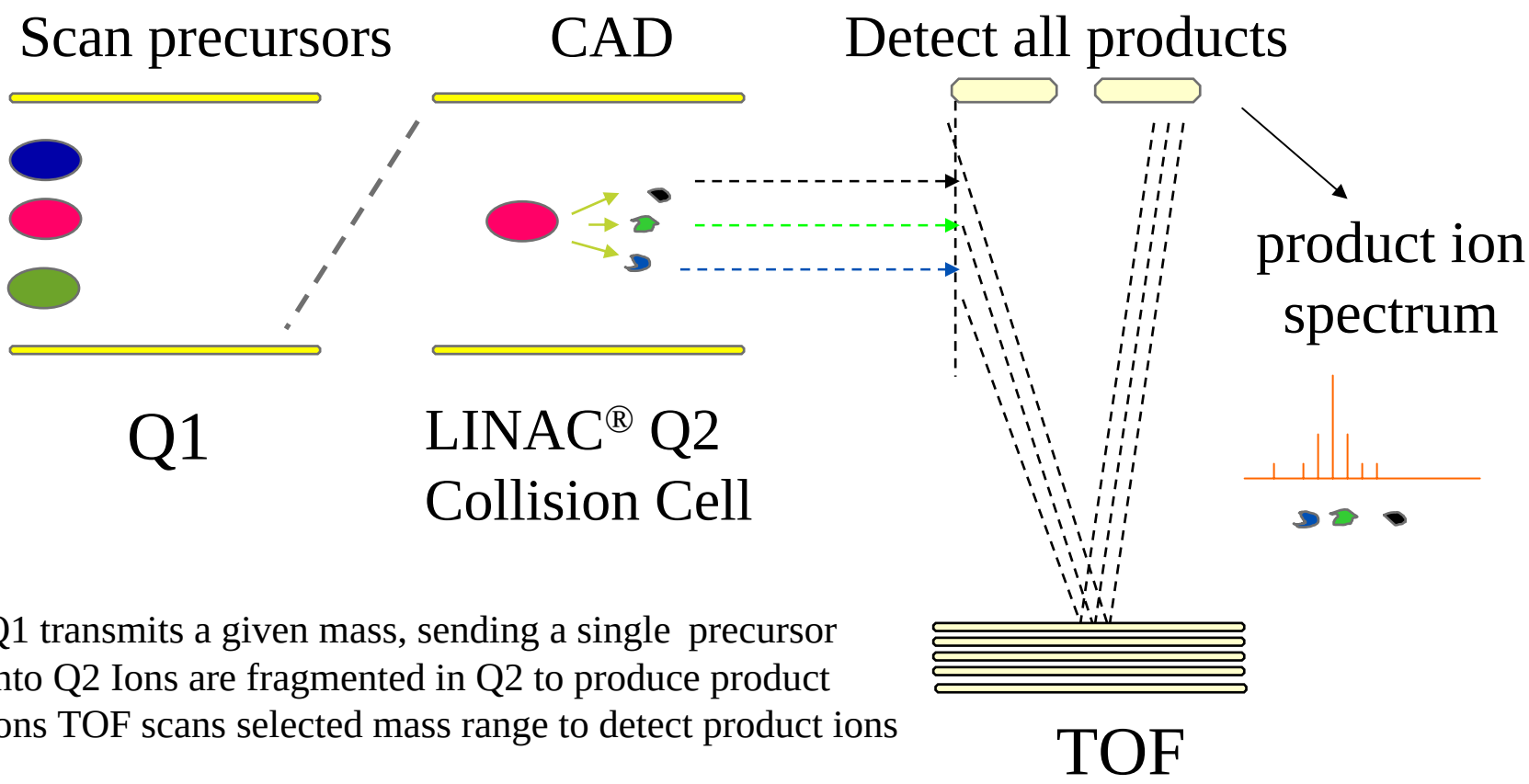
QqTOF Product Ion MS/MS Operation

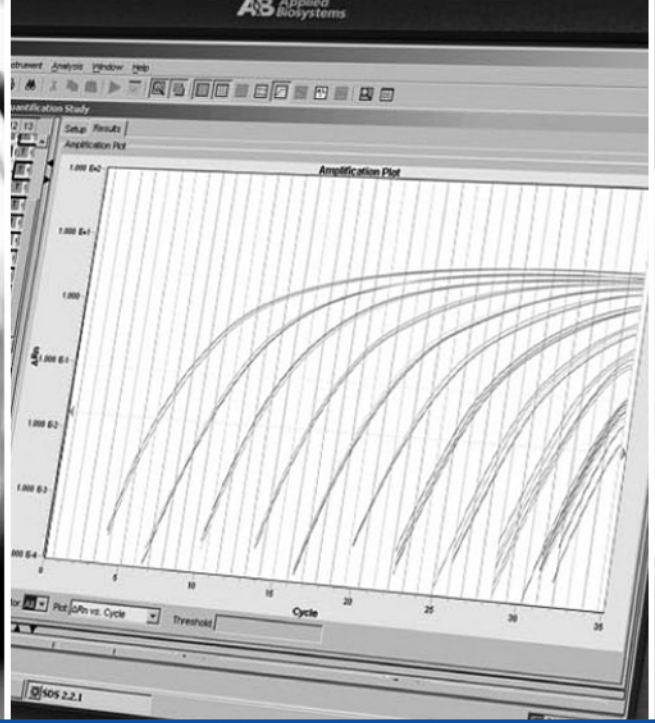


Q1 transmits a given mass, sending a single precursor into Q2 Ions are fragmented in Q2 to produce product ions TOF scans selected mass range to detect product ions

QqTOF systems

- Qualitative analysis (spectral data acquisition)
  - High sensitivity in full scan and product ion scan mode
  - High mass accuracy and resolution versus iontraps and QQQ systems (but mass accuracy does not help distinguish samples where they have have the same molecular formulae but different structures).

- Quantitative analysis (multi-targeted applications)
  - Lower sensitivity and linearity
  - No real MRM possible
  - No simultaneous multi component analysis (relative low scan speed compared to MRM on triple)



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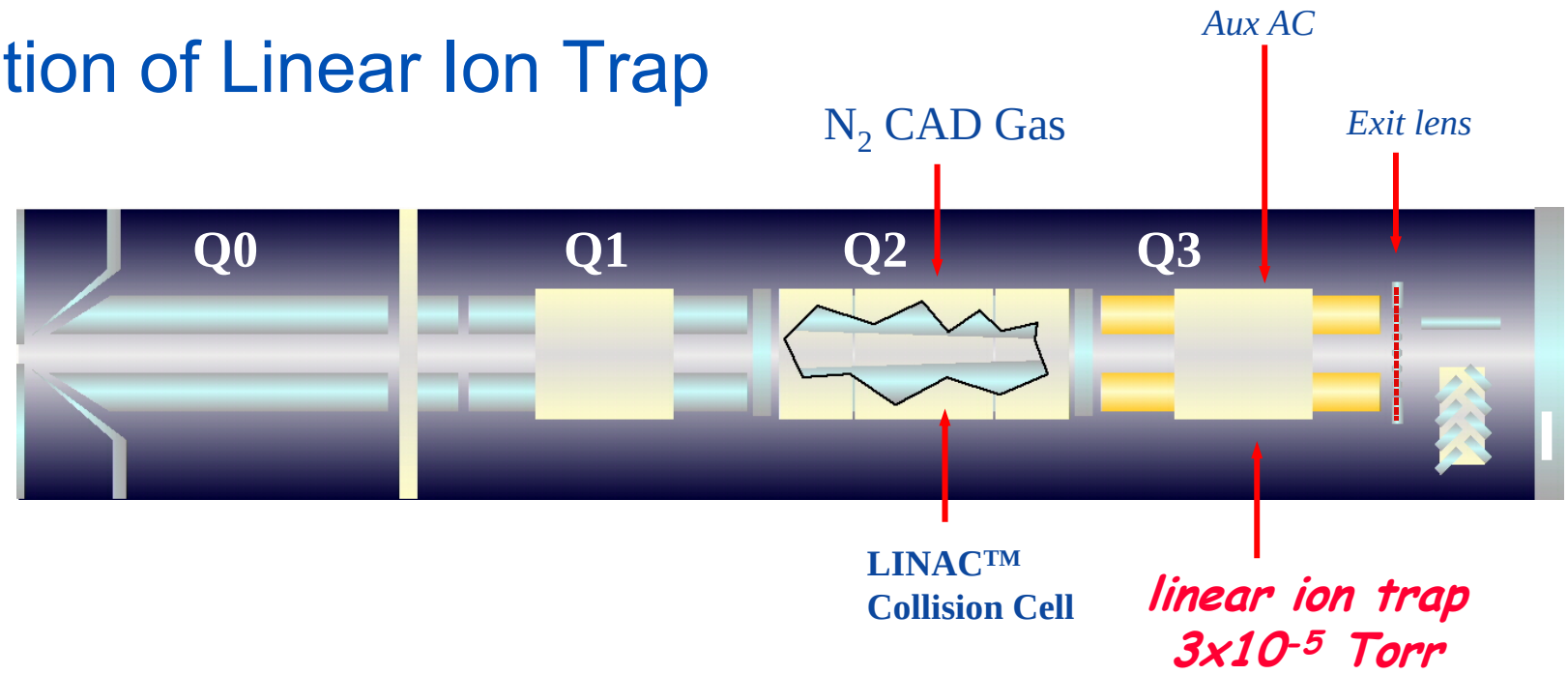
Hybrid Linear Ion Trap Technology (Q TRAP® System)

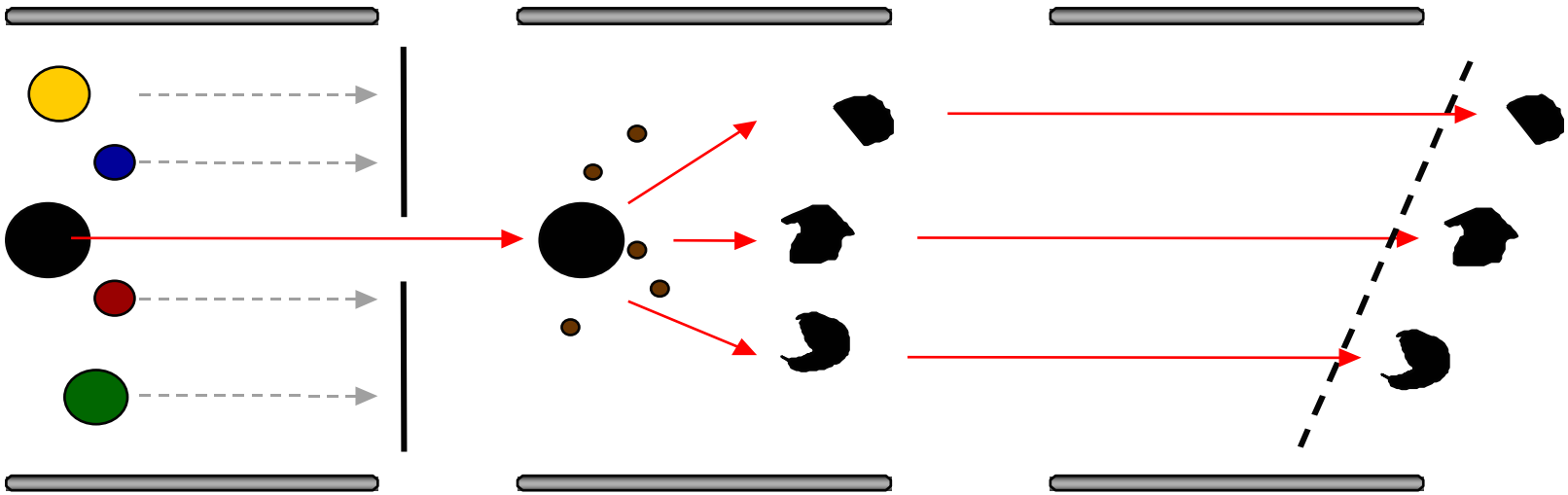
Location of Linear Ion Trap



The ion trap is in the Q3 region of a triple quadrupole.

Because the trap is now a quadrupole it can hold more ions than a conventional 3D trap which reduces space charge effects. This leads to better spectra, giving you more confidence in the results.

Q TRAP® System - Enhanced Product Ion Scan



Many analytes enter the MS and are separated by a quadrupole mass filter

One analyte selected and broken up by collisions with gas

All the fragment ions trapped in the Linear trap and then scanned out based on their mass/charge

Q TRAP® Systems

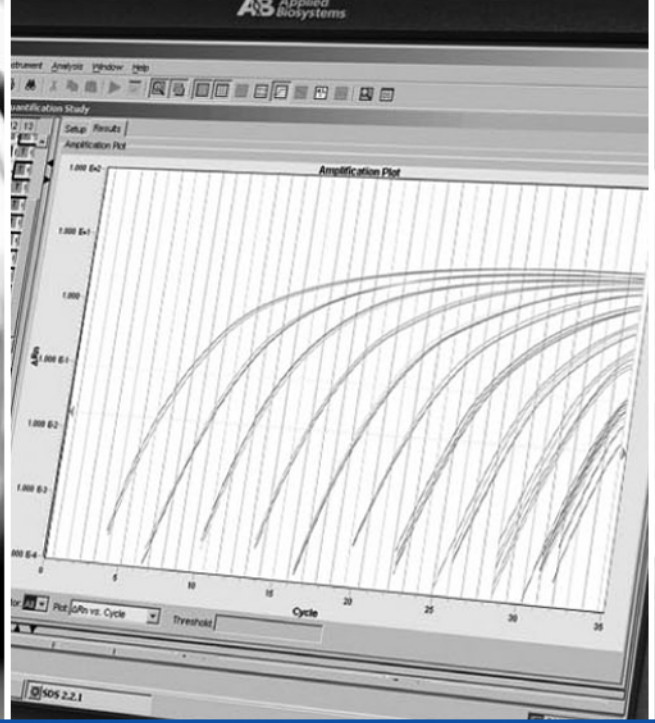
- Qualitative analysis (spectral data acquisition)
  - High sensitivity in full scan and product ion scan mode
  - Higher mass accuracy and resolution versus QQQ systems but less than QqTOF systems.
  - Reduced potential of space charge effects.
  - No low mass cut off.

- Quantitative analysis (multi-targeted applications)
  - High sensitivity and linearity.
  - MRM possible.
  - Simultaneous multi component analysis possible.

LC-MS/MS Source Technology

Ionization techniques for small molecule applications

- Electrospray Ionisation
- Atmospheric Pressure Chemical Ionisation



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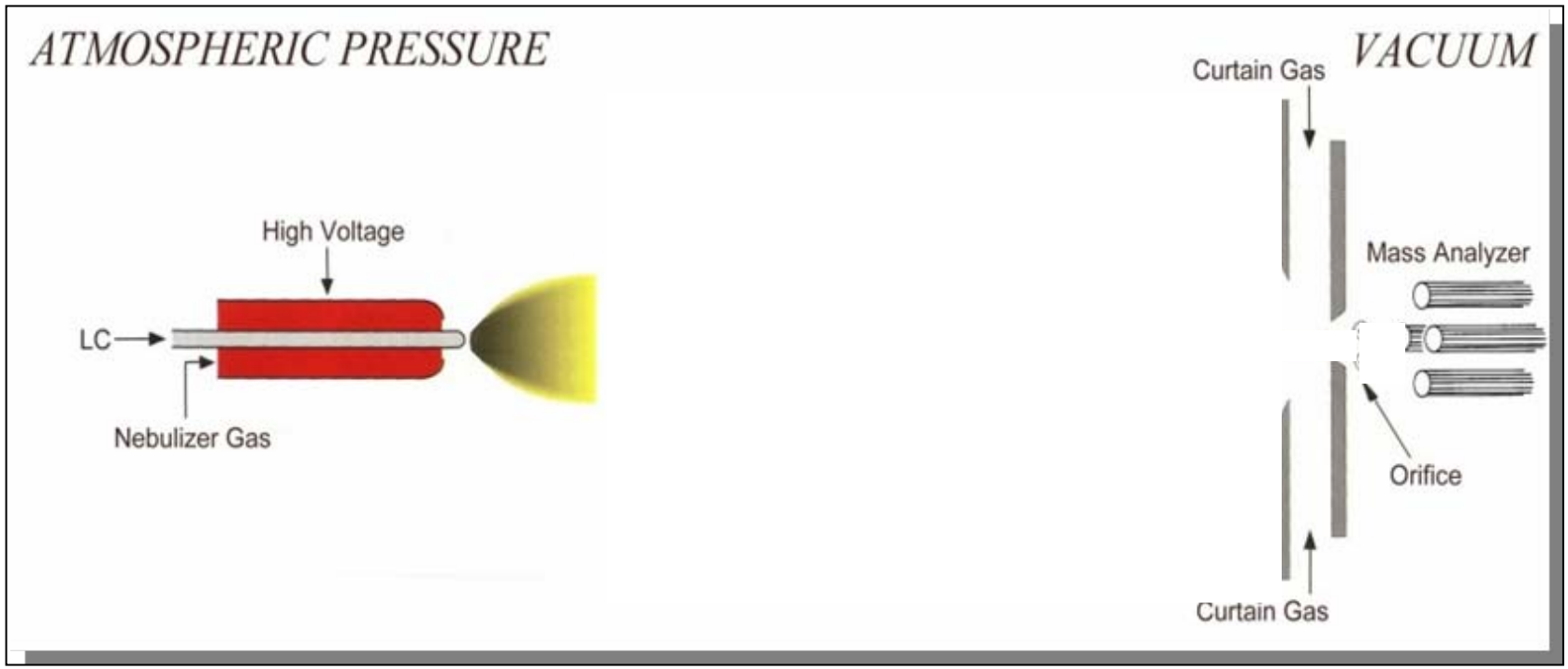
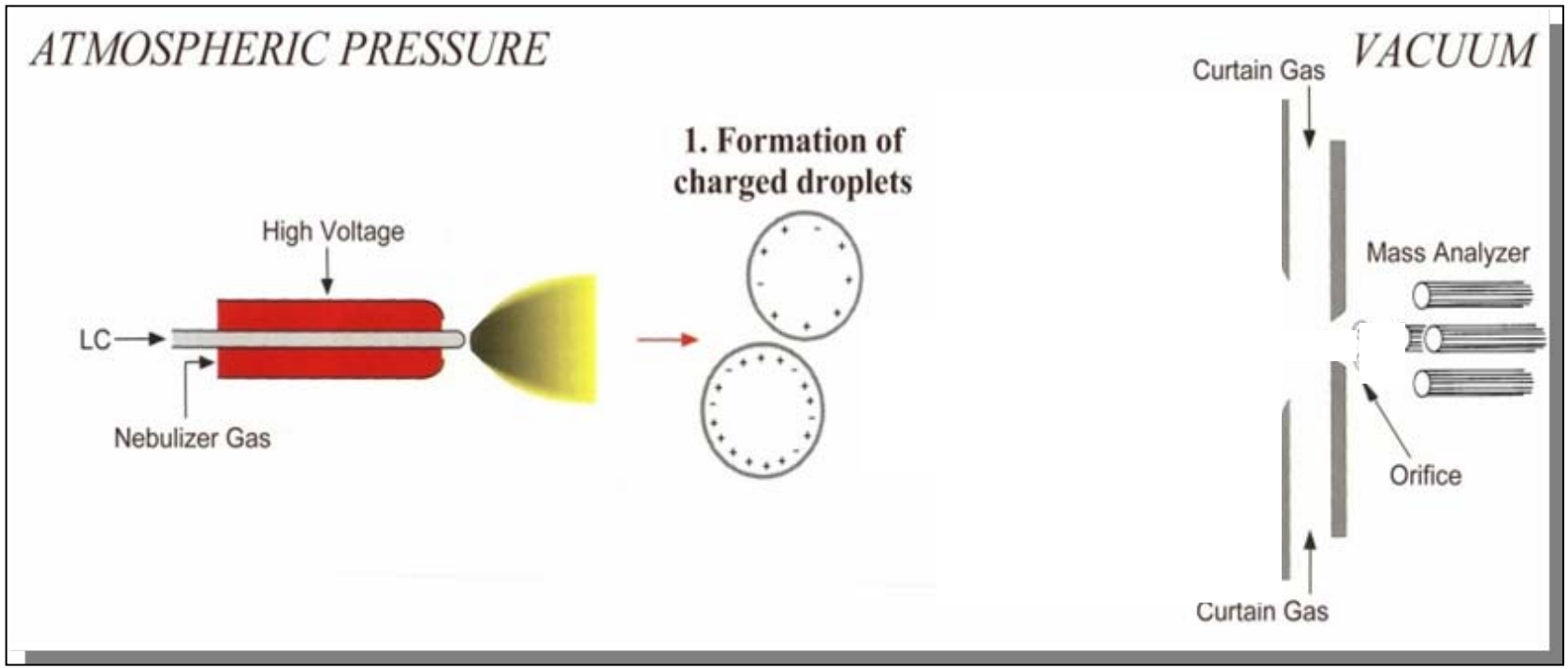
Electrospray Ionisation

Electrospray Source Principle



Ion Spray: Electrospray Ionisation (ESI)
softest ionisation technique
polar to ionic substances

Electrospray Source Principle



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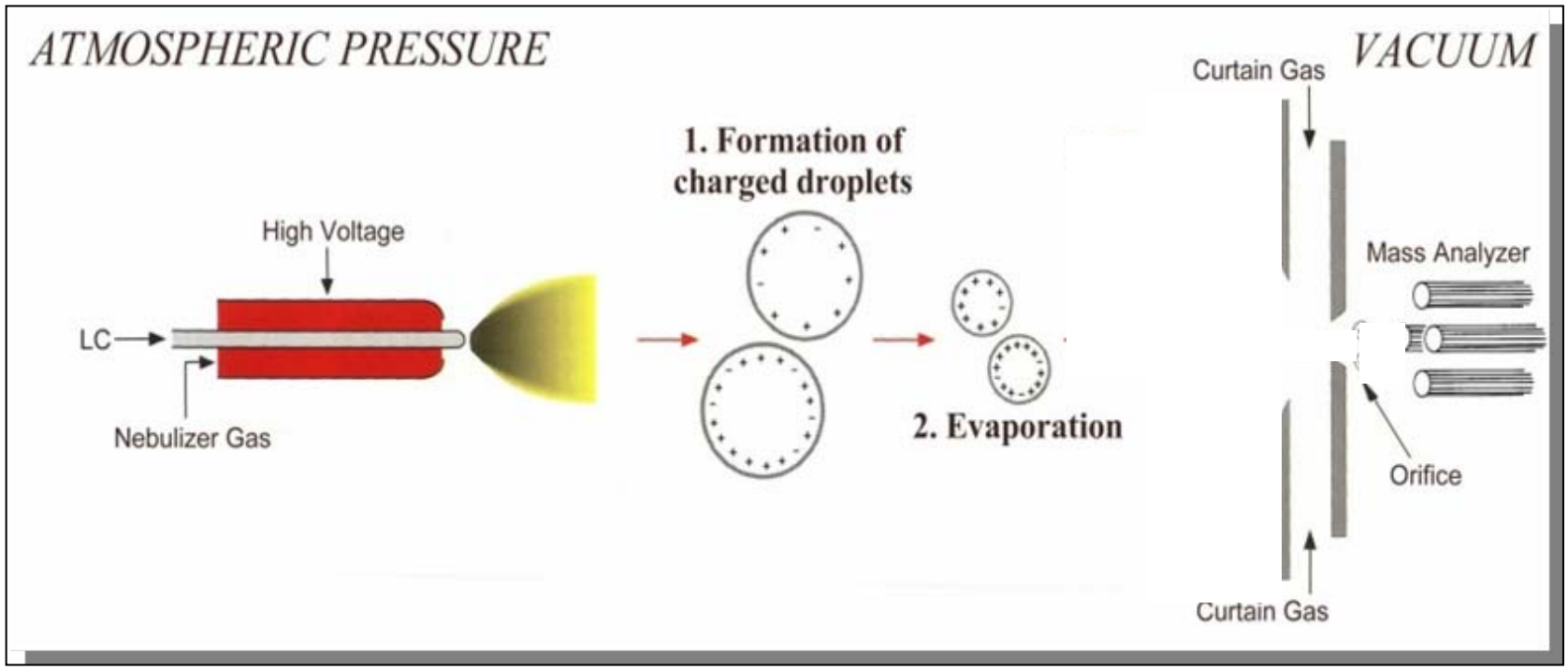

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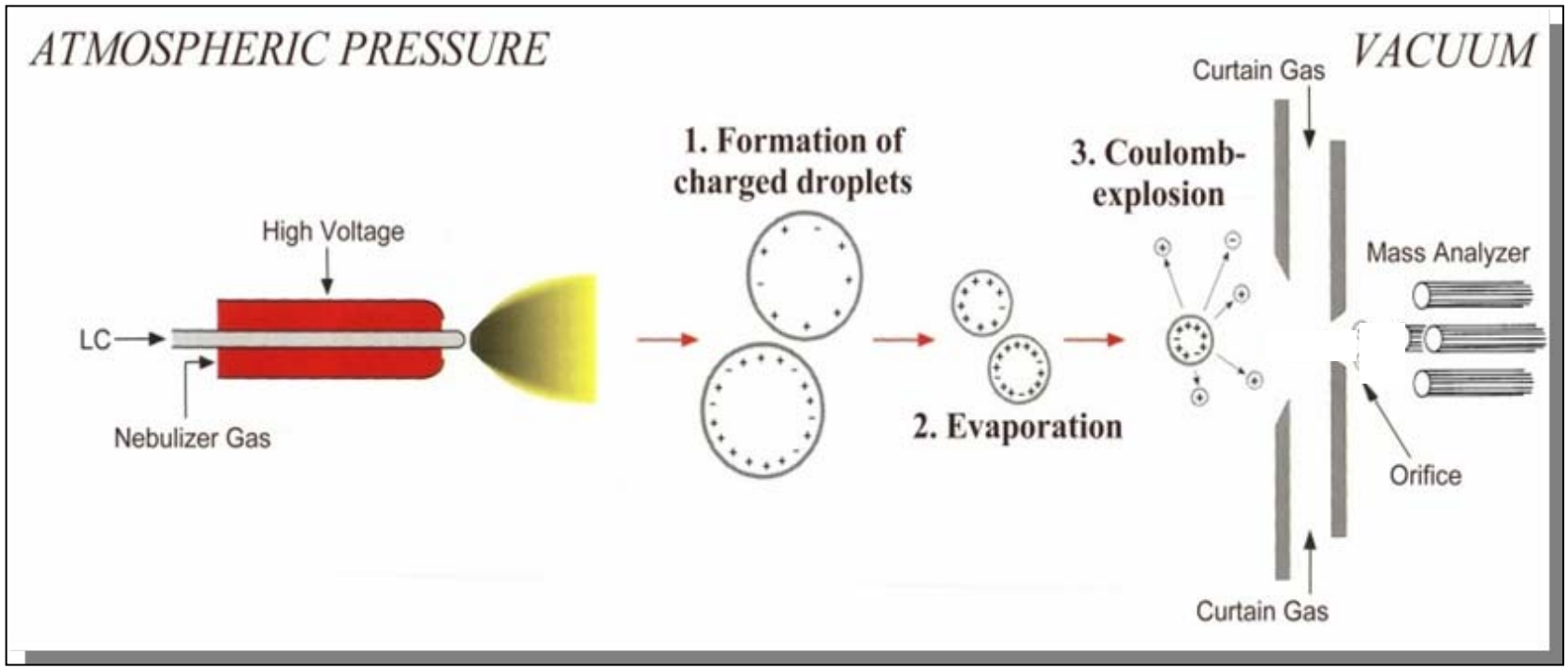

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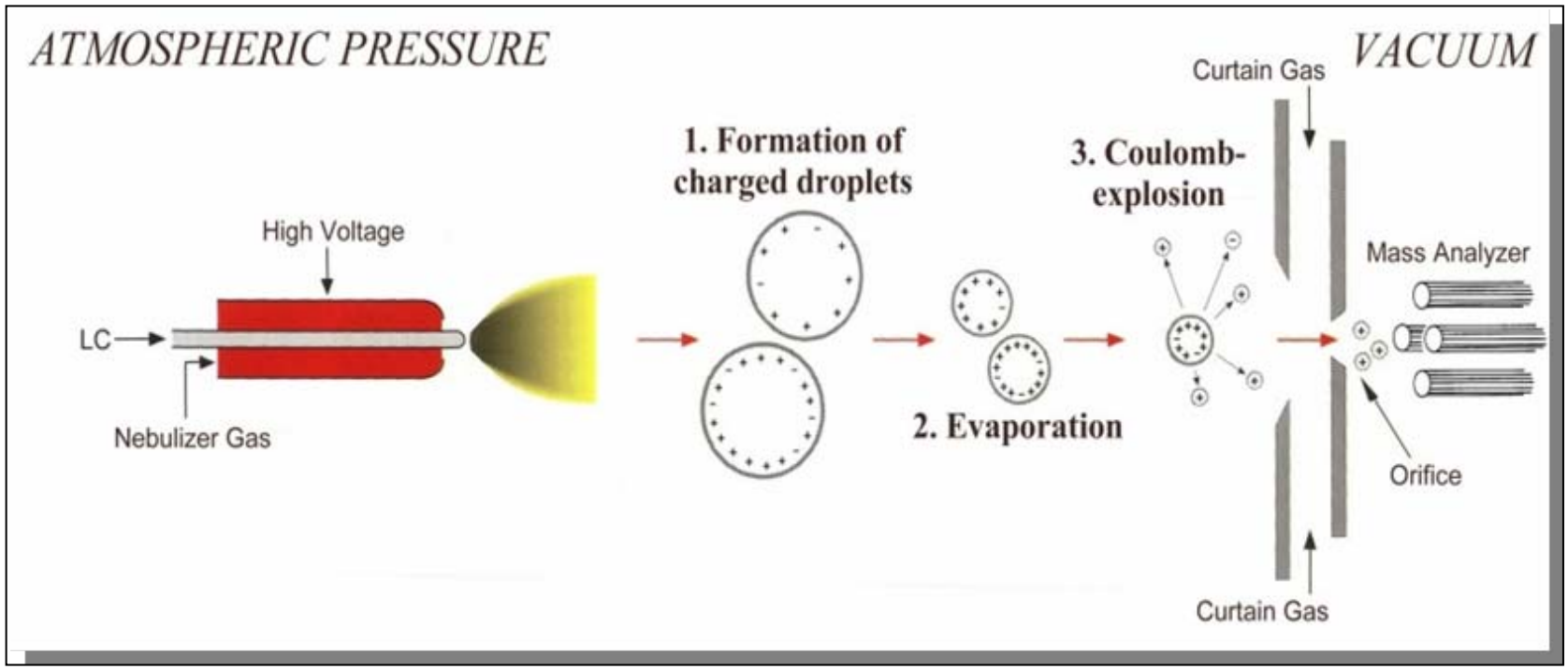

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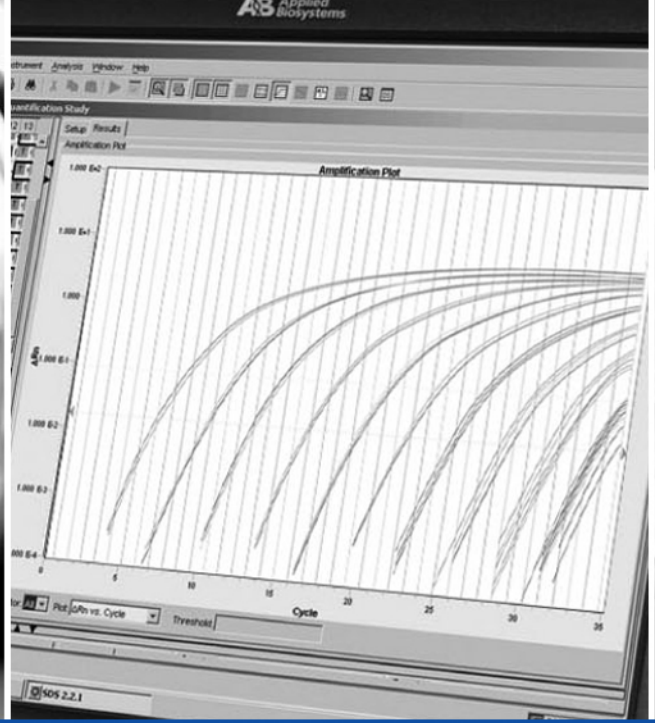

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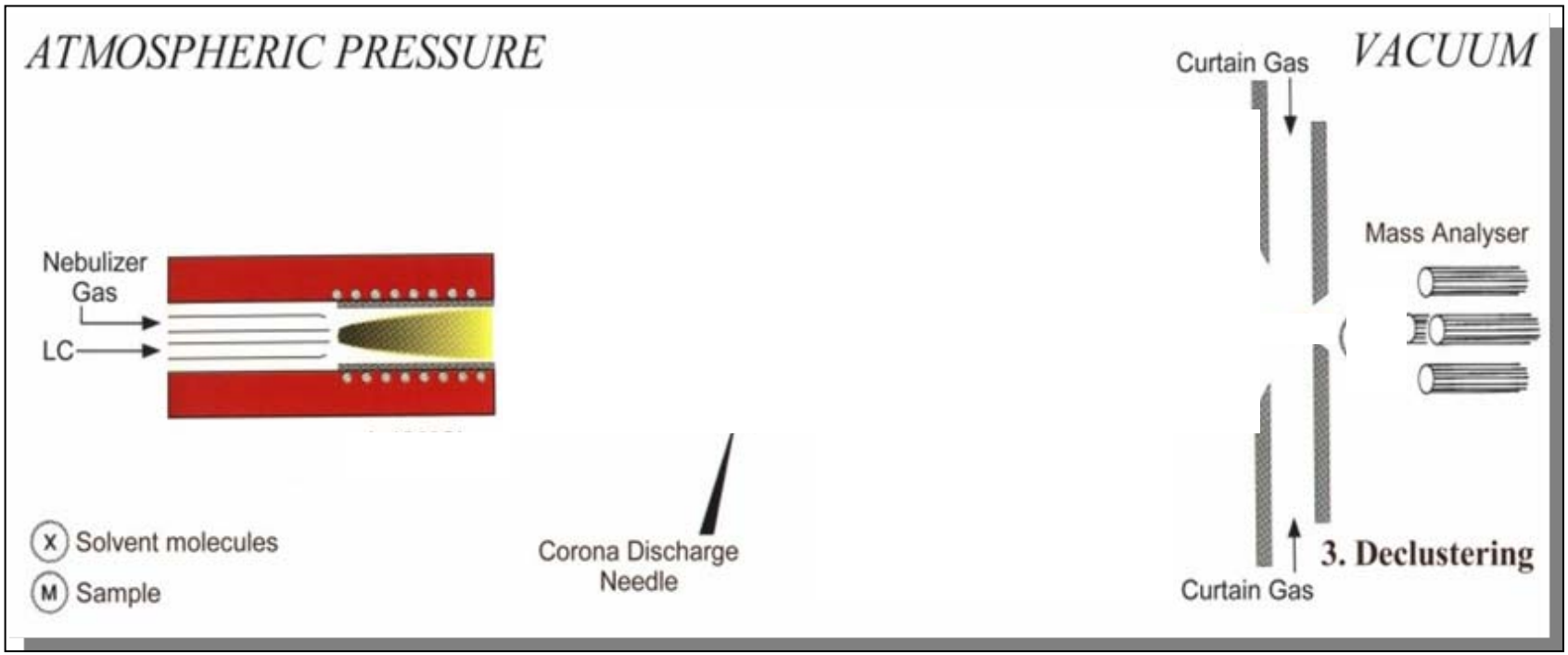
Atmospheric Pressure Chemical Ionization

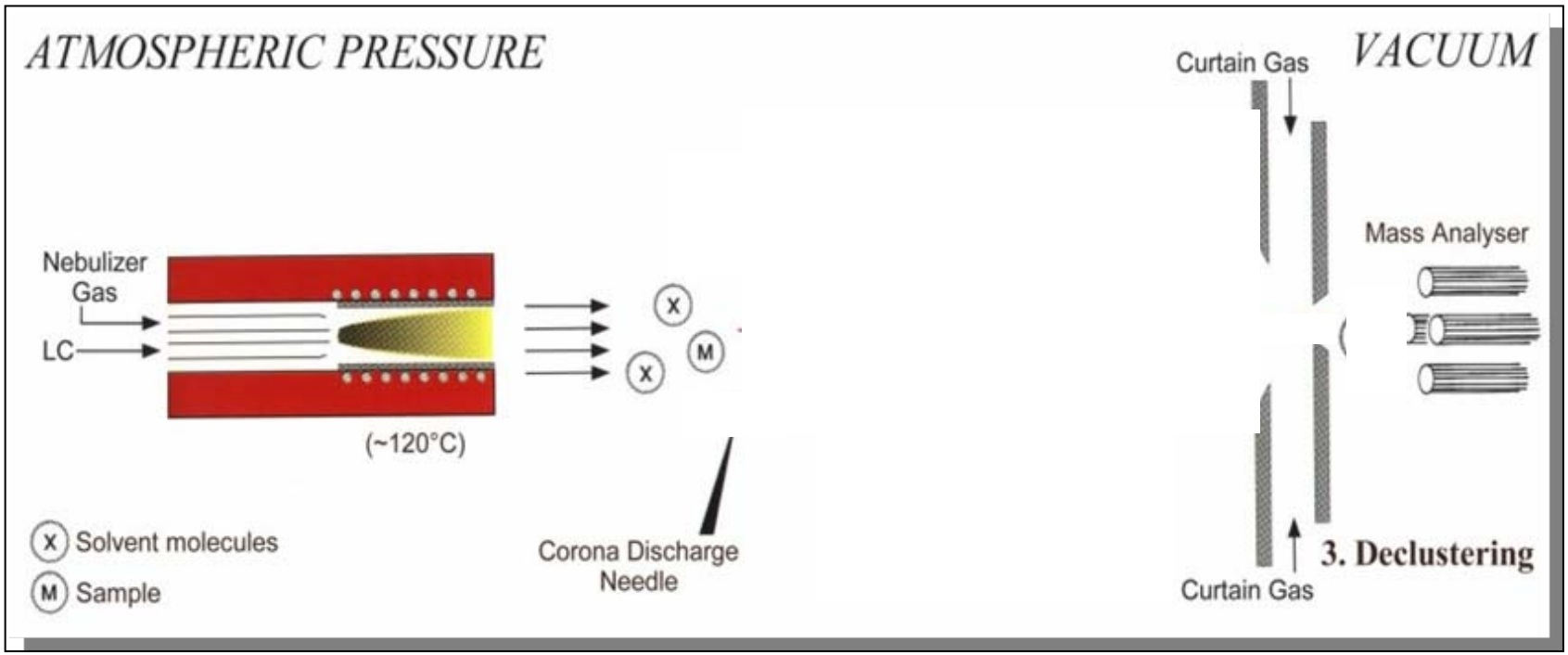
Heated Nebulizer (APCI)



Heated Nebulizer:

Atmospheric Pressure Chemical Ionisation (APCI)
corona discharge needle
polar to unpolar and thermally stable compounds

Heated Nebulizer (APCI)



Heated Nebulizer:

Atmospheric Pressure Chemical Ionisation (APCI)
corona discharge needle
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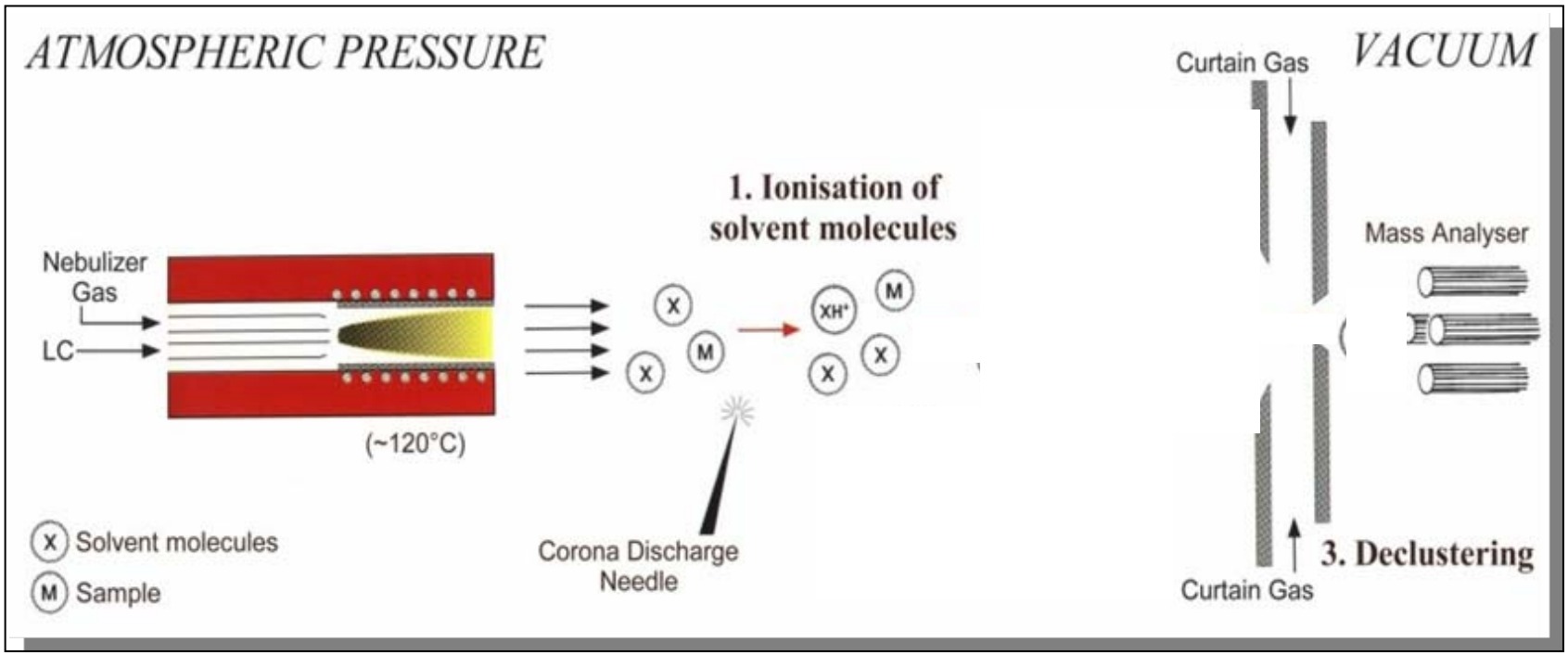
Heated Nebulizer (APCI)



Heated Nebulizer:

Atmospheric Pressure Chemical Ionisation (APCI)
corona discharge needle
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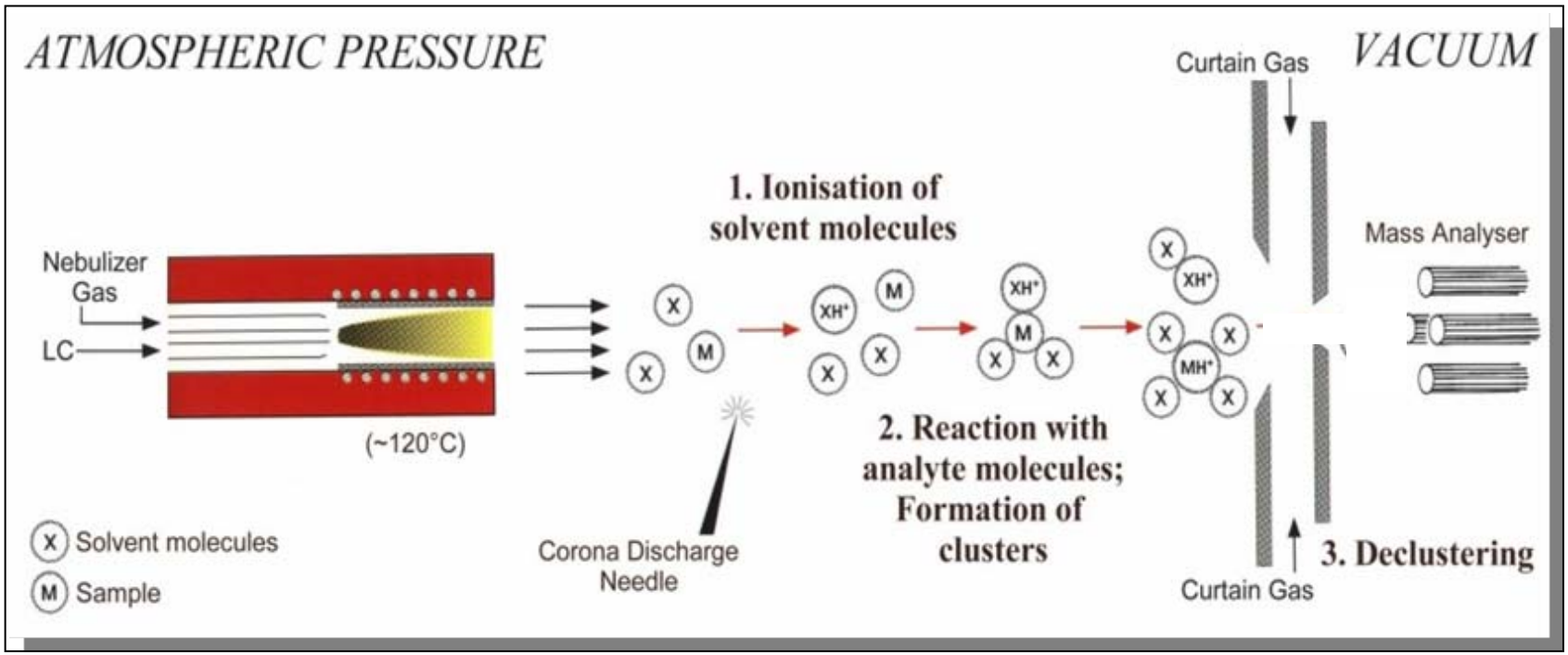
Heated Nebulizer (APCI)



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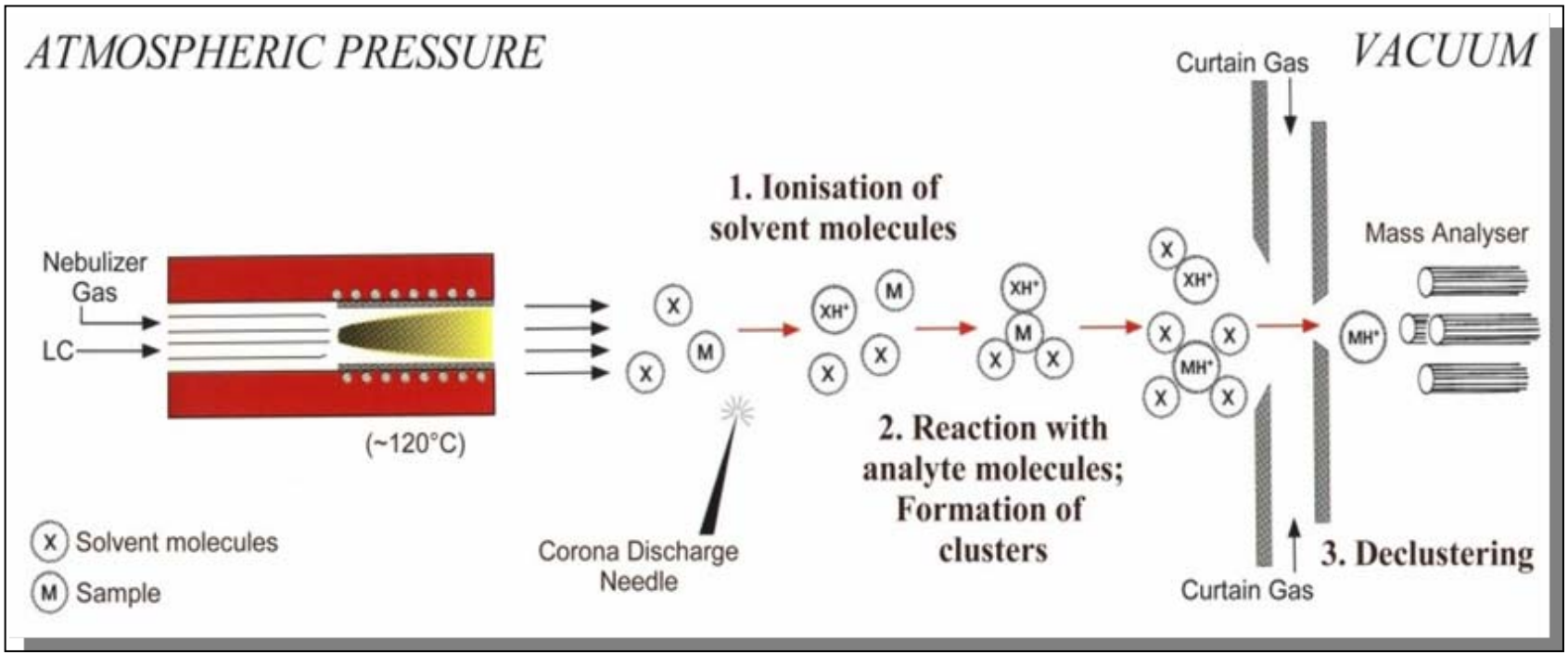
Atmospheric Pressure Chemical Ionisation (APCI)

corona discharge needle

polar to unpolar and thermally stable compounds

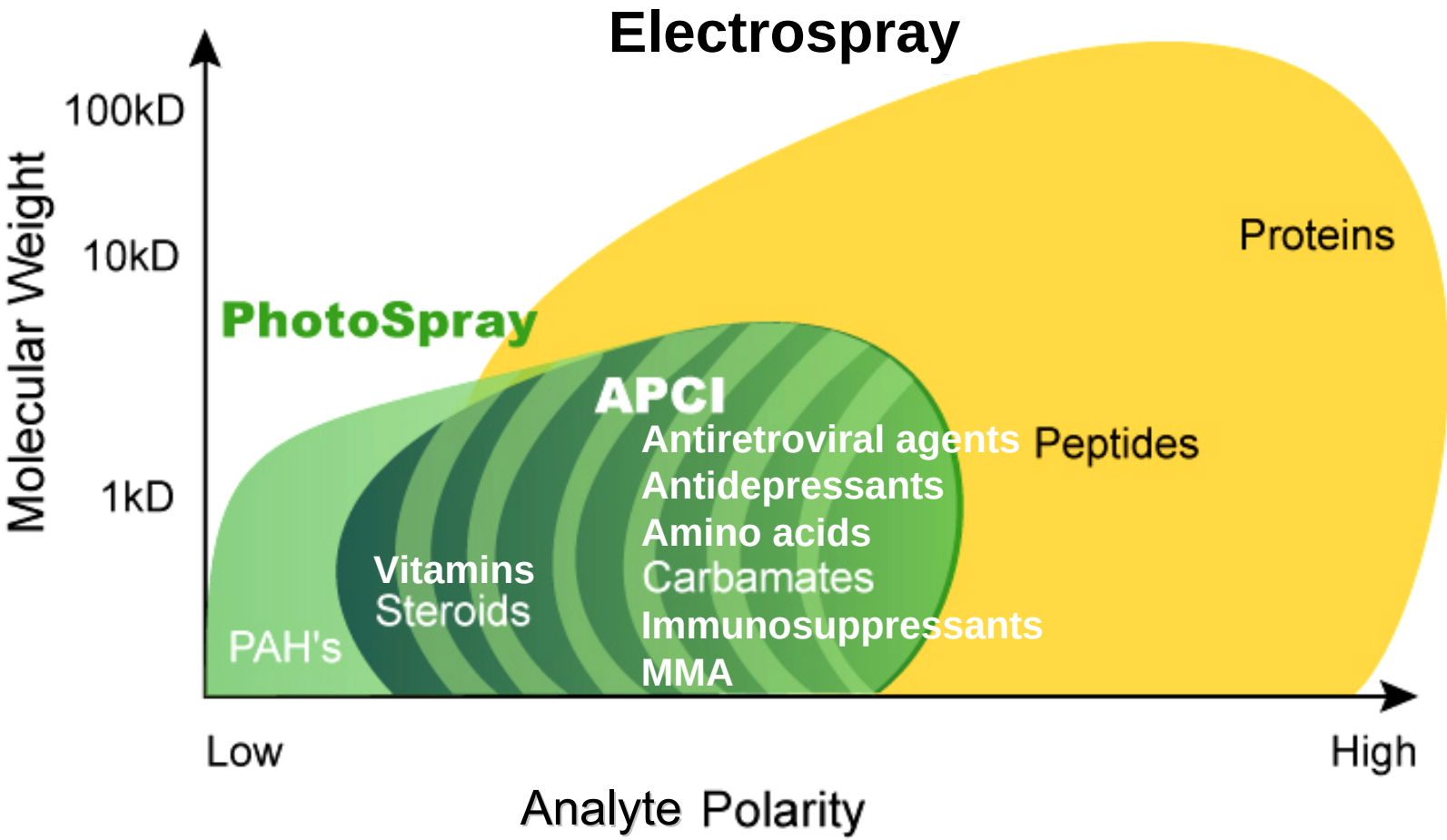
Heated Nebulizer (APCI)

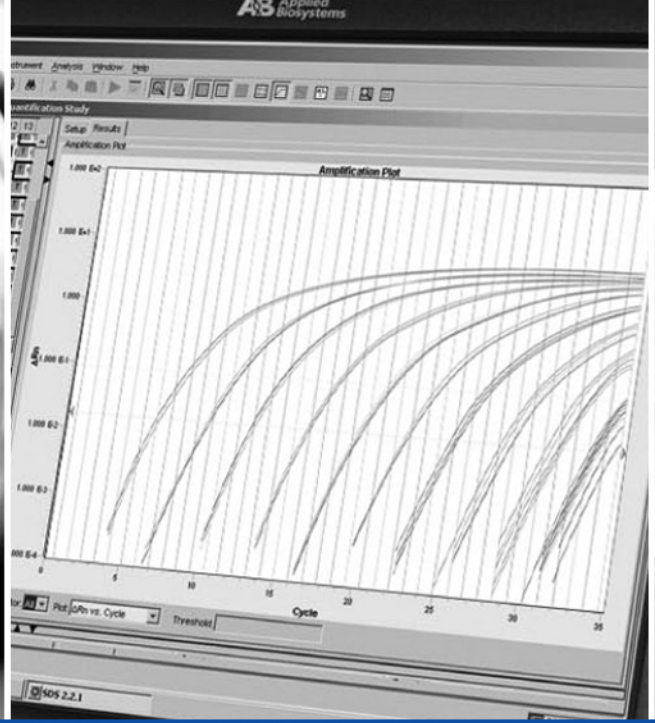


Heated Nebulizer:

Atmospheric Pressure Chemical Ionisation (APCI)
corona discharge needle
polar to unpolar and thermally stable compounds

Ionisation Methods of Choice





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What effects the way compounds form an ions?

Acid or base (pH)?

- For compounds containing Nitrogen, a low pH or high concentration of H+ ions will increase the amount of compound forming the ions (typically add formic or acetic acid to the LC system).

- For compounds containing an acid group, a higher pH or lower concentration of H+ ions will increase the amount of compound forming the ions (typically add ammonia to the LC system).

Can Standard LC conditions be Used?

- Yes, but some LC Buffers may cause problems.
- Typically methanol and acetonitrile systems buffered with ammonia acetate or formate are used in LC-MS.
- Flow rates for nL/min to mL/min
- One example of a troublesome LC-MS buffer is Phosphate buffer and the use of a non-volatile ion pairing agent (e.g. SDS) which can can cause severe suppression and complex spectra.

LC/MS/MS Summary

- LC-MS/MS offers several advantages over more traditional analytical techniques including higher sensitivity and selectivity.
- There are many different types of LC-MS/MS systems which vary functionality. It is important to understand the type of analyses intended the technology.
- There are different types of source available. Having an idea of the polarity of the compound will guide you to the most appropriate source, but generally Electrospray is the most flexible.
- Take care when transferring methods to LC-MS from LC with UV detection as the original buffer may not be ideal.

Tandem Mass Spectrometry Systems For Clinical Research

Increased Sensitivity

API 2000™ system

Entry level system

API 3200™ System

Economic high performance system Ideal for TDM / IEM / Steroids / etc.

API 4000™ system

High performance, high sensitivity system for applications such as targeted steroid analysis

API 5000™ System

Highest performance, ultimate sensitivity system

Use of TMS in Clinical Research Applications

TDM	NBS	Biomarker	Clin Tox
Immunosuppressant Antiretroviral Antipsychotics Anticonvulsive Antiviral Benzodiazepine	30-40 Diseases Fatty Acid Oxidation Disorder Amino Acid Disorders Organic Acid Disorders Other Diseases	Steroids T3/T4 Vitamin D Homocysteine Orotic Acid VLCFA Bile Acid Cancer Etc.	General unknown Screening (GUS) Drugs Of Abuse (DOA)
3200	3200	3200 to 5000	Triple / QTRAP

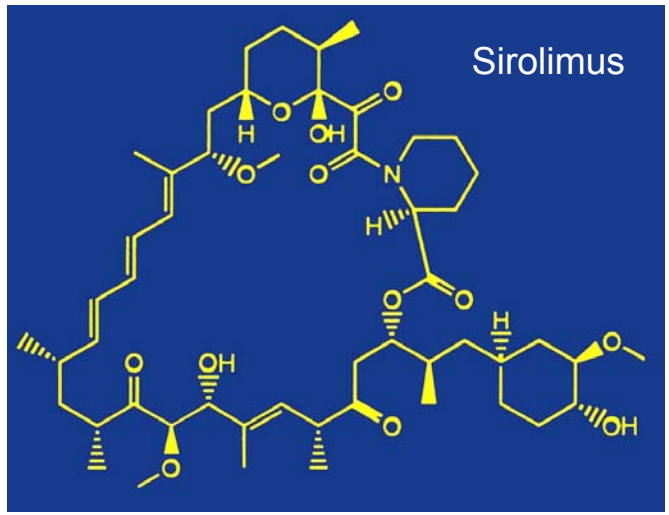
LC/MS/MS for Research on Therapeutic Drug Monitoring

Why adopt Tandem Mass Spectrometry for TDM?

- Cost advantage vs Immunoassays
- Time To Result – Increased speed and throughput
- Flexibility adding new drugs to the panel
- Simultaneous detection of all drugs
- More accurate quantitation due to less cross reactivity with metabolites – Increased selectivity and specificity
- LC/MS/MS shows excellent reproducibility and dynamic range
- Very robust assay

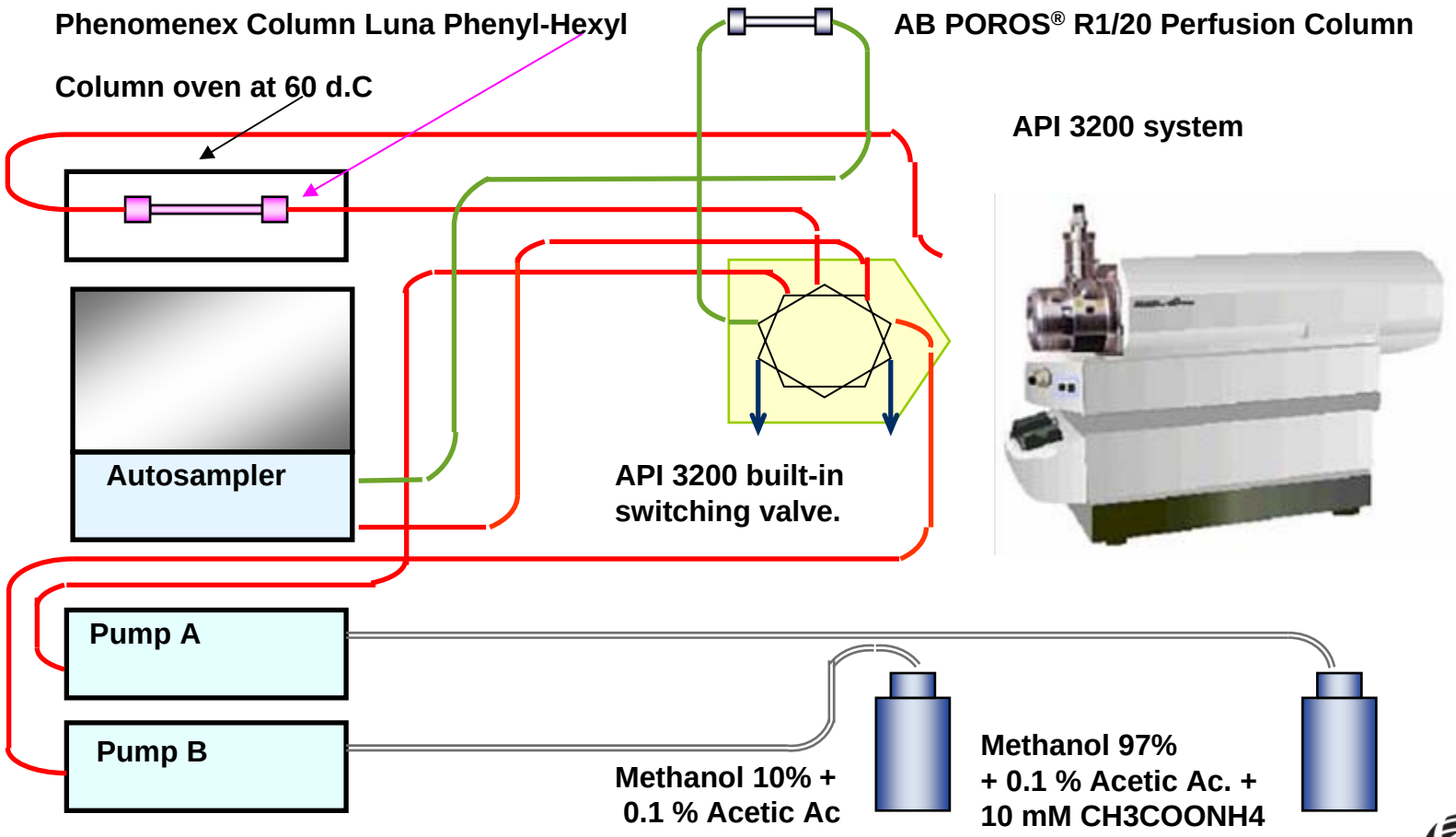
Clinical Research: Therapeutic Drug Monitoring

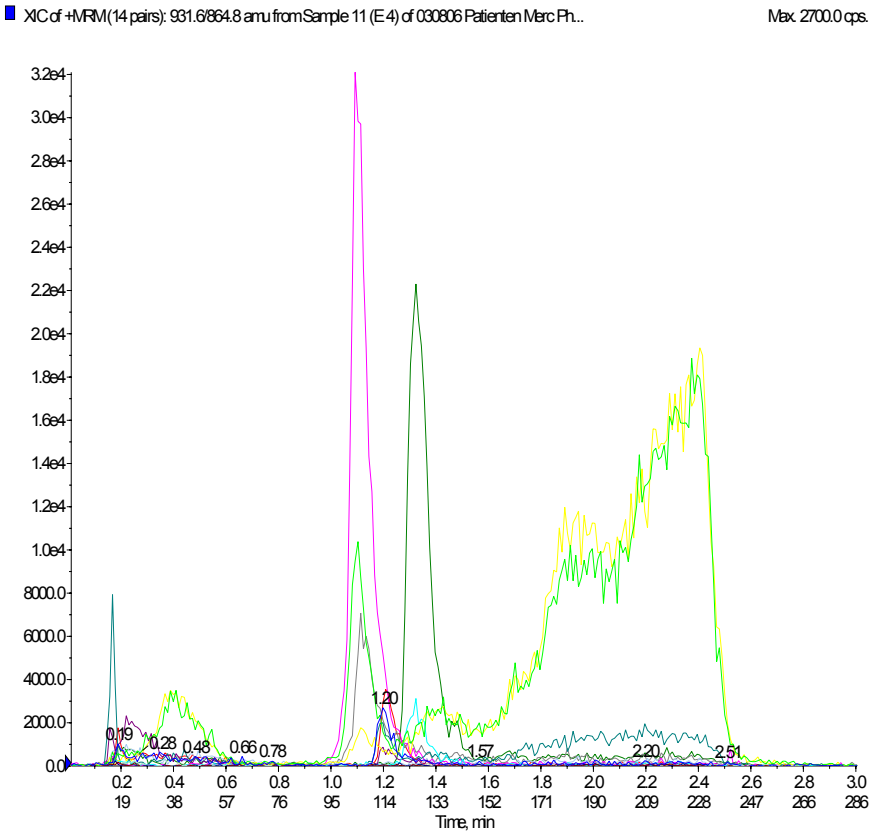
- Immunosuppressants
  - Cyclosporin A
  - Tacrolimus
  - Sirolimus
  - Everolimus
  - Mycophenolic Acid (MPA)



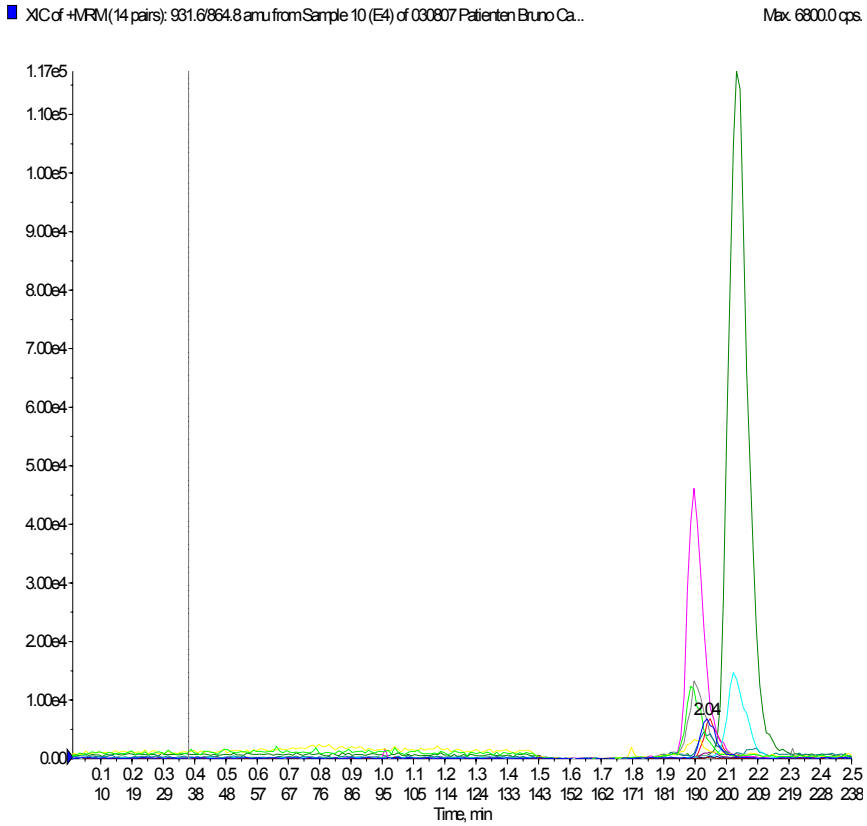
- *LC/MS/MS, the ideal alternative for immunoassays*
- *Simultaneous detection and quantitation*
- *Excellent sensitivity and very good signal-to-noise ratio*

Instrument configuration: modified setting to include MPA

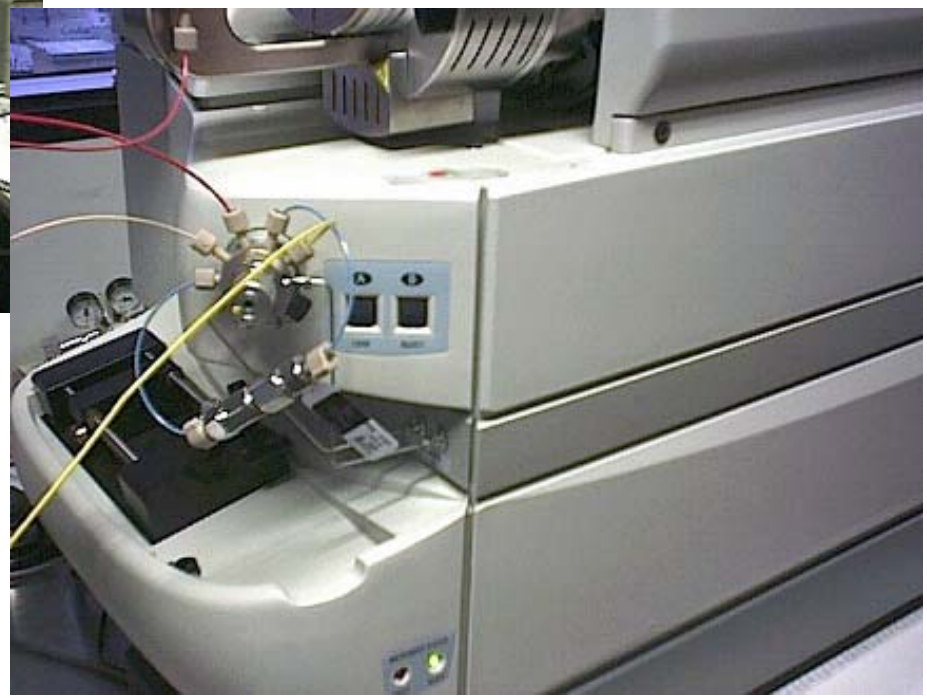




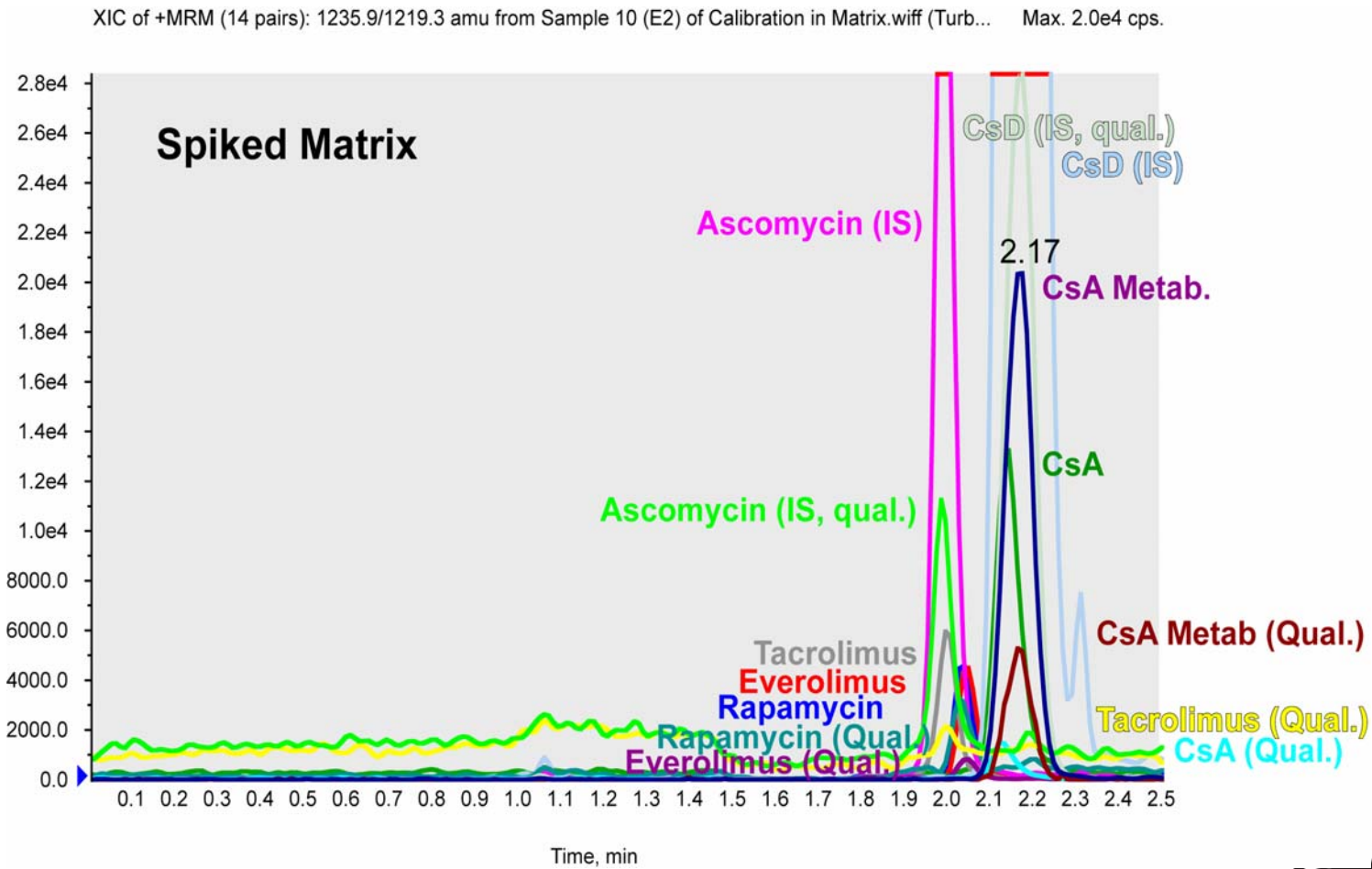
< Previous LC-MS/MS methodology



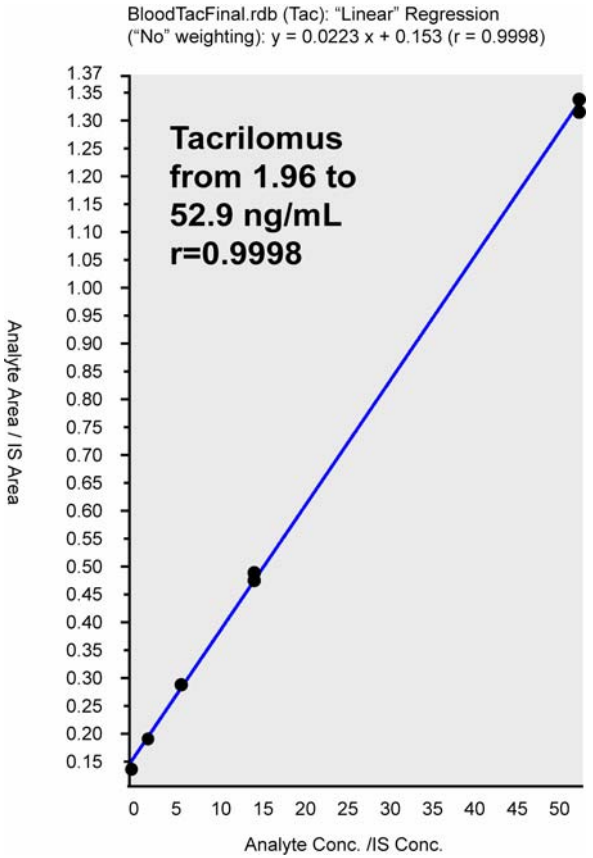
New 2 D LC-MS/MS methodology >

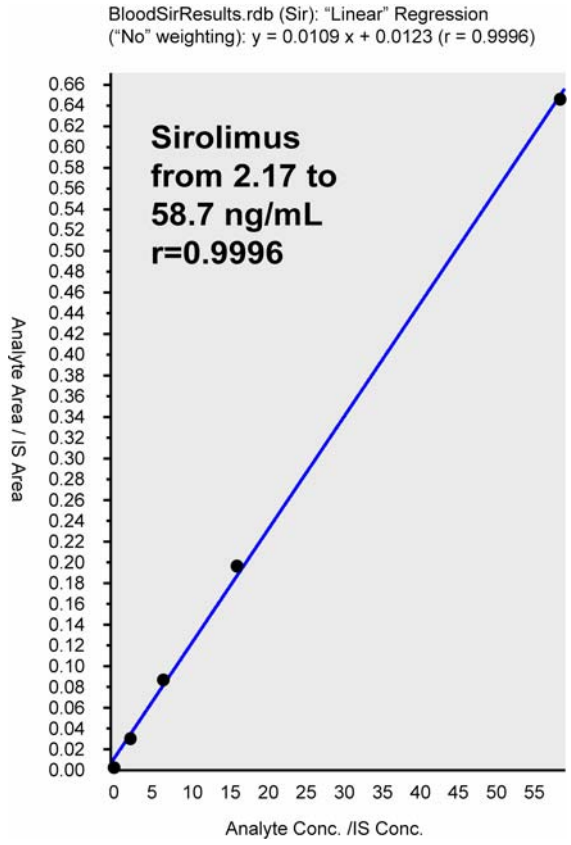


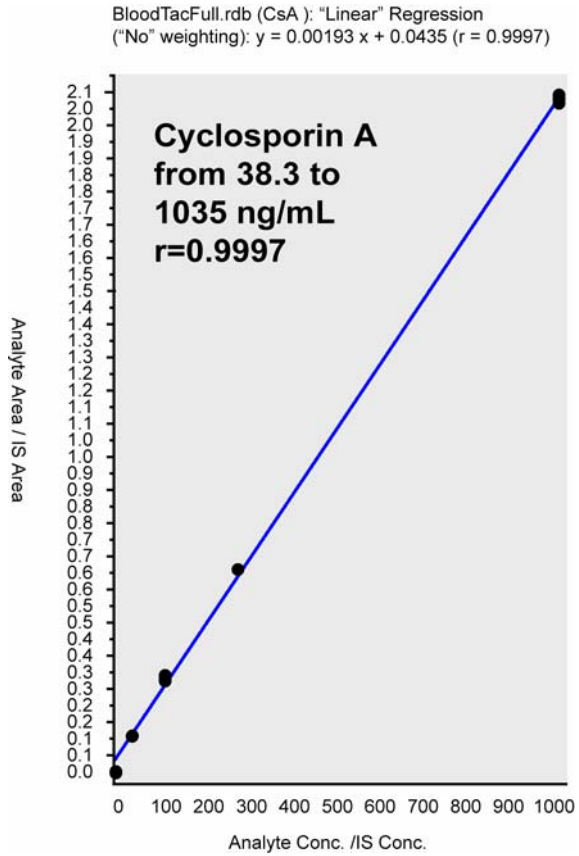
Fast Separation and Detection of Multiple Immunosuppressants and Internal Standards



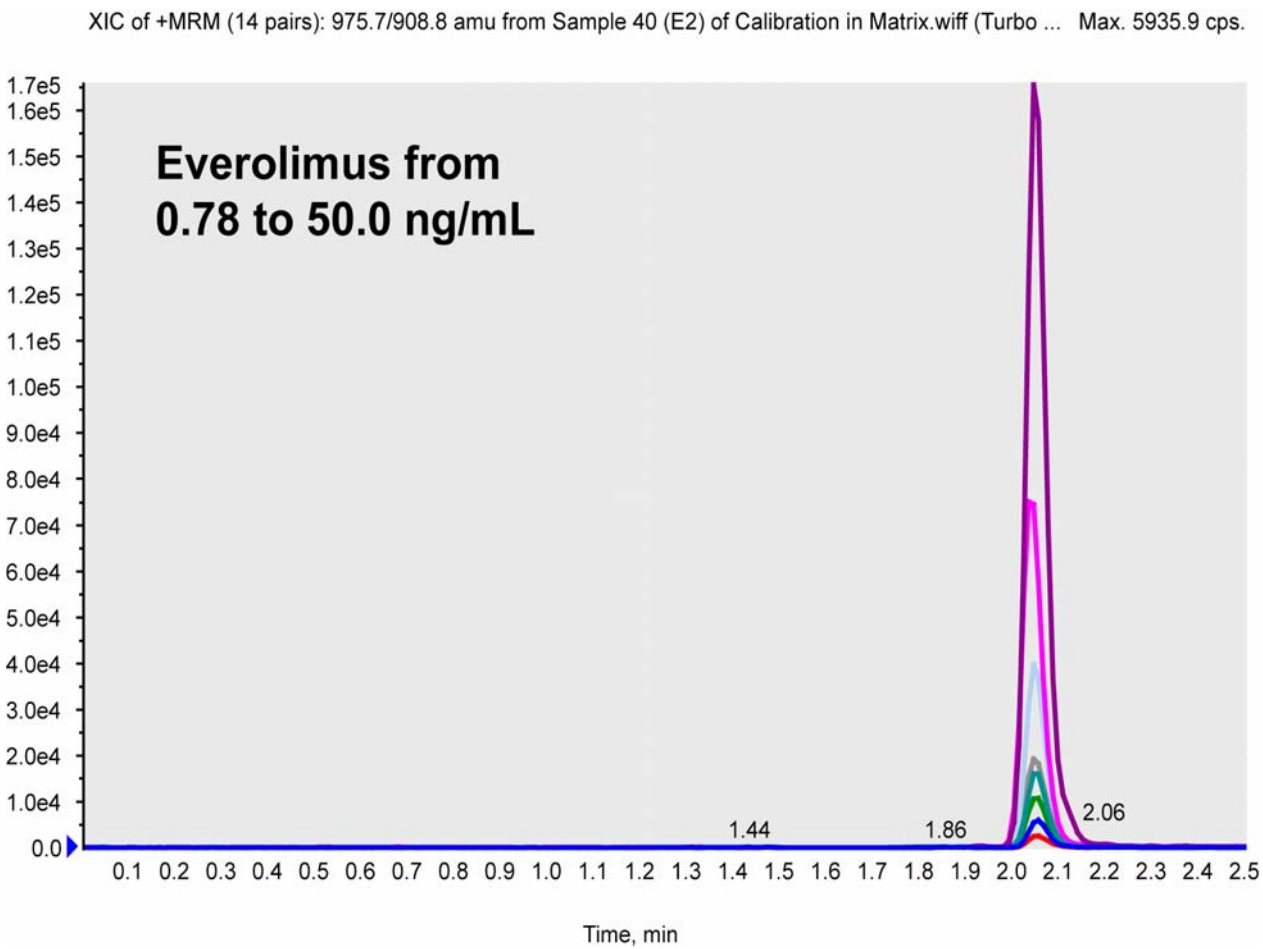
Calibration Curves with Blood Calibrators



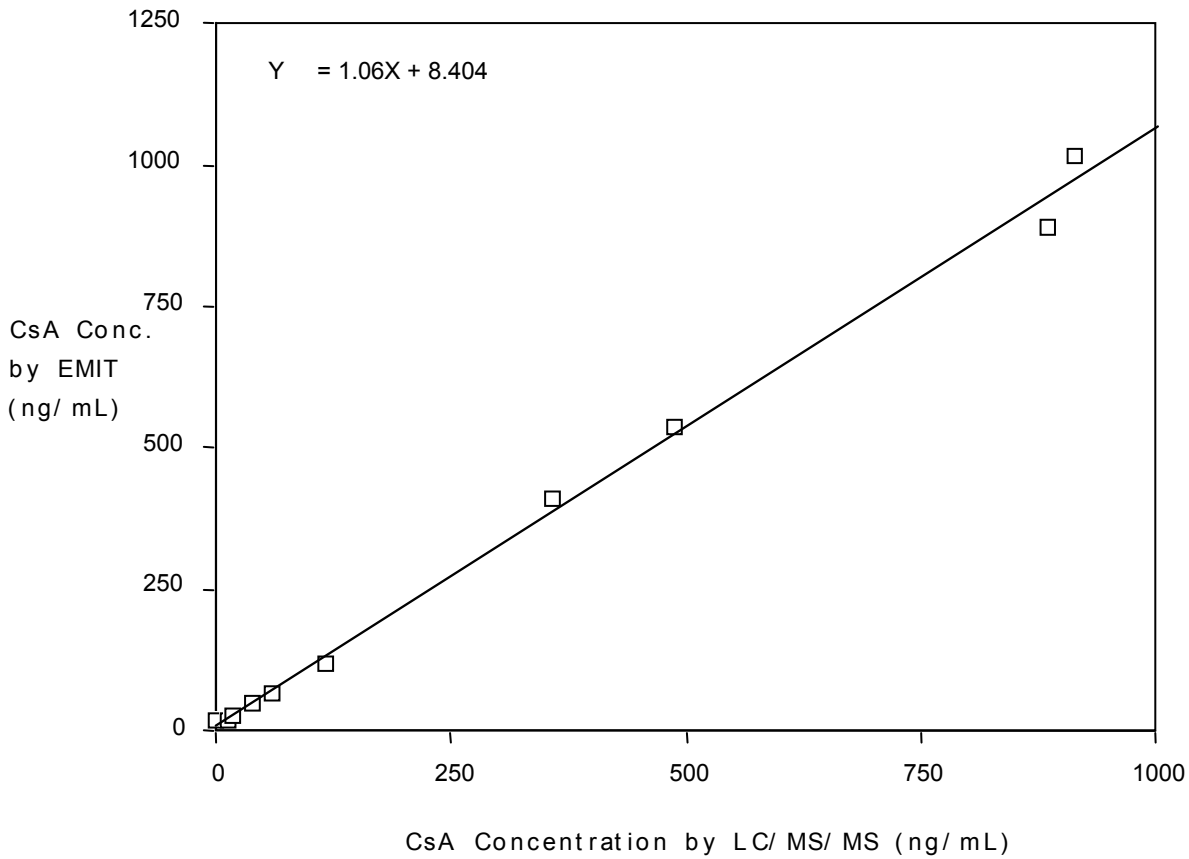




Dynamic Range and Linearity for EVEROLIMUS



CyclosporinA: EMIT Immunoassay vs. LC/MS/MS

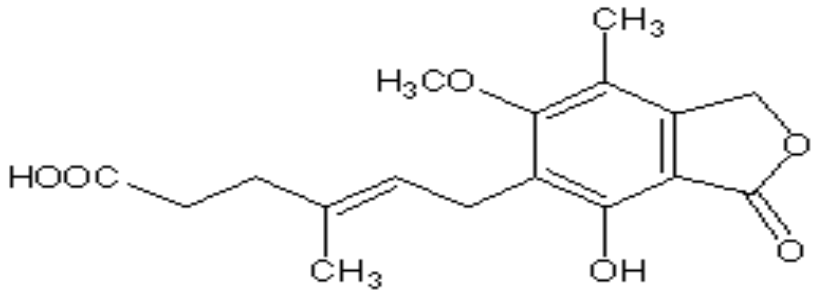


Method performance parameters determined for 25 µl spiked blood sample

	Cyclosporin A (CyA)	Tacrolimus (TRL)	Sirolimus (SRL)	Everolimus (RAD)
LOD [ng ml^{-1}]	1.3	0.1	0.1	0.1
Linearity [R^2]	0.9999	0.9997	0.9998	0.9998
Recovery [%]				
concentration 1	88.3	114.4	116.9	118.8
concentration 2	103.7	101.9	114.5	102.8
RSD [%] Intra-day (n=100)	4.6	10.2	9.3	10.3
RSD [%] Inter-day (n=40)	12.3	13.5	12.3	10.9
Accuracy [%] (n=40)	87.7 – 115.8 (Mean: 99.2)	90.3 – 110.8 (Mean: 96.9)	91.0 - 107.5 (Mean: 98.9)	91.2 – 112.1 (Mean: 103.1)

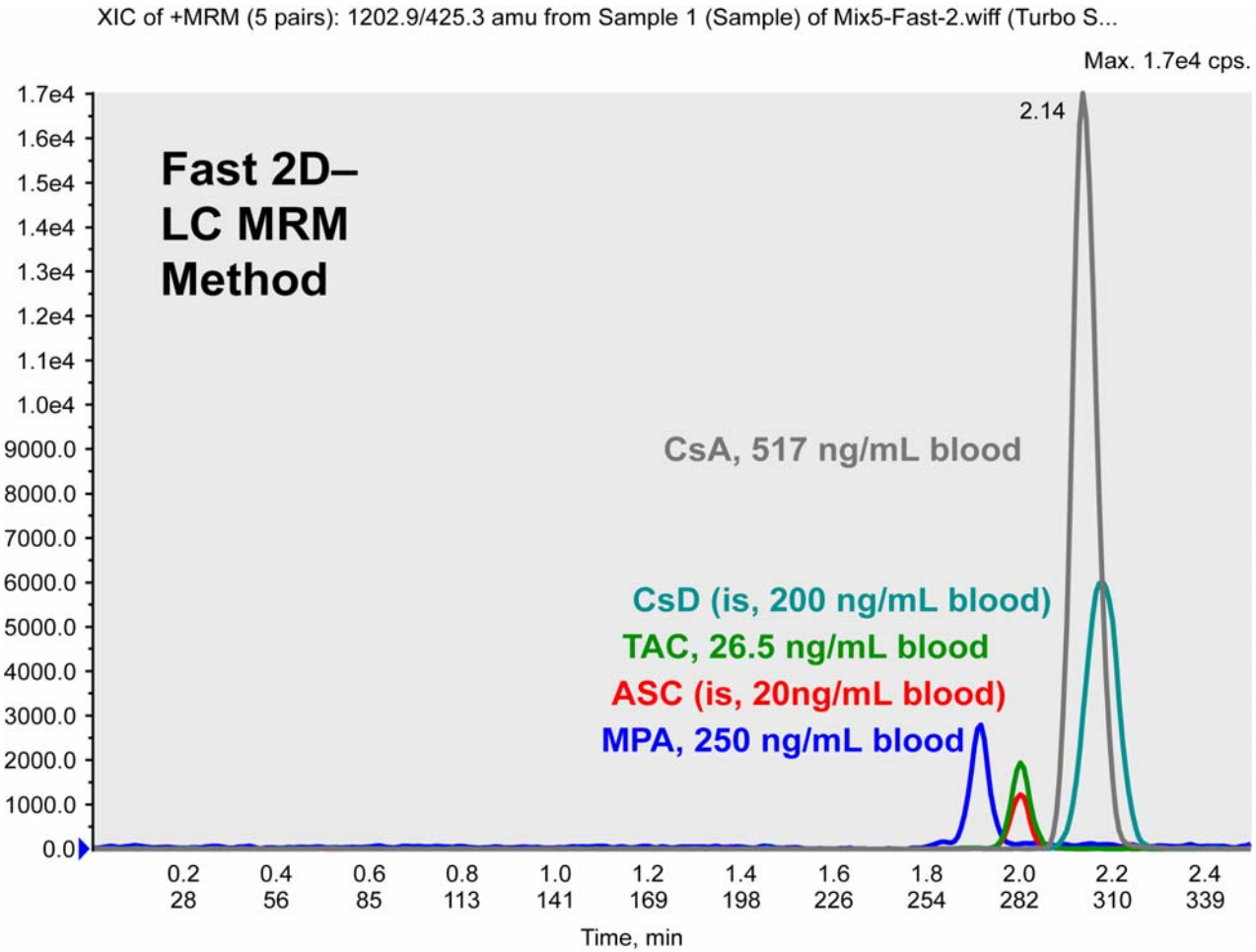
Koal et al.: J.Chromatogr. B 805 (2004) 215-222

MPA features



- MPA has a free acidic moiety which should make it more sensitive in Negative Ion Mode.
- MPA is quite labile in the ionization process.
- Reported to be metabolized at a significant extent (mainly to the Glucuronide conjugate).
- MPA prone to stick on surfaces.

Fast 2D-LC MS/MS Method including MPA



Conclusions

- LC/MS/MS technology and principles
- LC/MS/MS is superior to conventional methods with regard to specificity, accuracy, dynamic range and cost per assay
- LC/MS/MS is easy to use
- Wide range of potential uses for Therapeutic Drug Monitoring, Inborn Errors of Metabolism, Clinical Biomarkers – growing research into these areas focused on mass spectrometry.

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