Overview and Intro to LC-MS and LC-MS/MS

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The State Laboratory

Types of LC-MS in use in The State Lab

- 6 LC-MS/MS Triple Quadrupole Instruments
- 1 Exactive and 1 Q-Exactive
- 1 LC-Quadrupole-Time of Flight, 1 LC-Ion Trap
- 5 QTRAP instruments
- Systems from Agilent, Sciex, Thermo and Waters
- Technique is used in nearly every Section in The State Lab

Mass Spectrometry

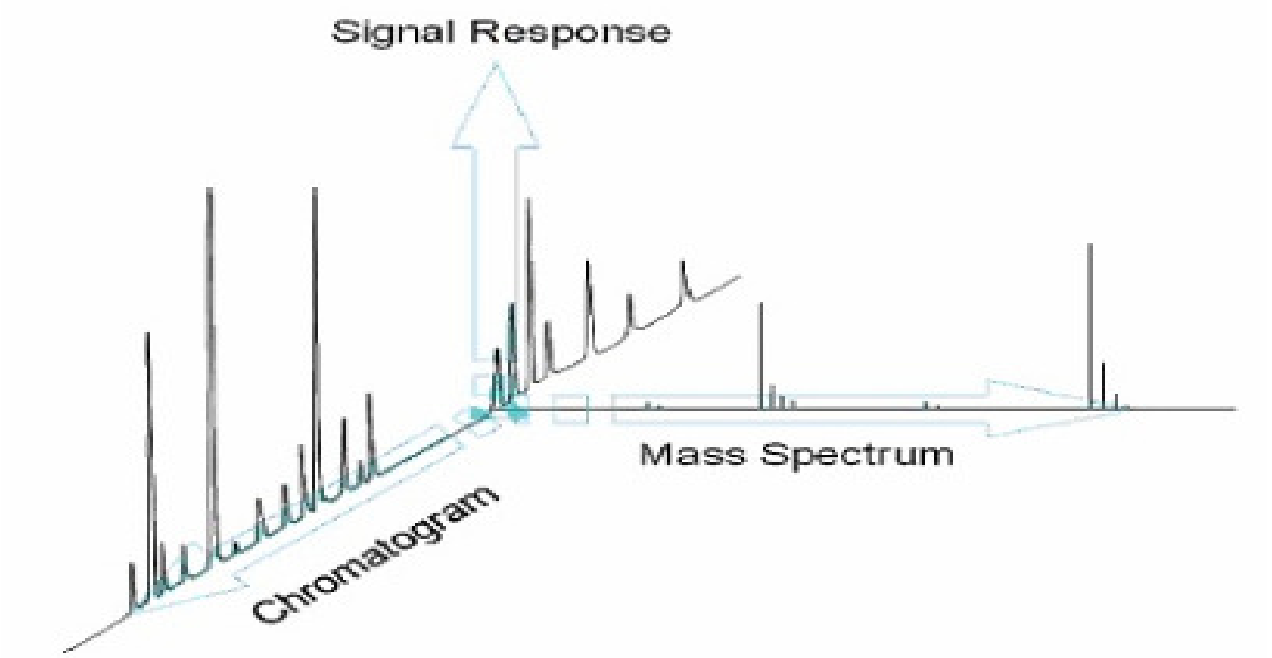
- Mass spectrometry is a very sensitive analytical technique and is widely regarded as having good selectivity.
- A Mass Spectrometer produces charged particles (ions) from chemical substances then uses electric and magnetic fields to measure the mass of the charged particles.
- Separates and measures ions based on their mass-to-charge (m/z) ratio.
- Operates under high vacuum; key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds of Mass Spectrometer exist: quadrupole, time-of-flight (TOF) and ion traps (orbital and linear) are most used.

Liquid Chromatography with Mass Spectrometry

- In many applications it is necessary to isolate the target analyte from what could be a sample containing thousands of other different molecules.
- For example, more than 1,500 compounds may have the same molecular mass at around 250 Da.
- An additional separation technique is needed before presenting the sample to the mass spectrometer.
- Liquid chromatography-mass spectrometry (LC-MS) is the combination of two selective techniques that allows the analyte(s) of interest in highly complex mixtures to be isolated and measured.
- LC differentiates compounds by their physico-chemical properties and MS differentiates compounds by mass (specifically their mass-to-charge ratio).

LC-MS Selectivity

- It is this dual selectivity that makes LC-MS such a powerful analytical tool.
- The mass spectrometer acts not only as the “LC detector” but, at least in principle, it provides the capability to identify the species corresponding to each chromatographic peak through its unique mass spectrum.



Why LC-MS

- Directly coupling LC with MS offers the following advantages:
  - Structural information
  - Speed of analysis
  - Convenience
  - Analysis of multicomponent mixtures
  - Accurate quantitation
  - Evaluation of chromatographic peak purity

Why LC-MS/MS?

- Why Liquid Chromatography?
  - Analysis of labile analytes
  - Analysis of more polar compounds without derivatization
  - Analysis of significantly higher masses
  - Reduction of lengthy clean-up
- Why MS/MS?
  - Additional structural elucidation
  - Further reduction of clean-up (?)
  - Specificity
  - Useful MS modes

Sample introduction

- Ion Souce
  - Transforms sample molecules to ions
  - Soft ionization
    - Places positive or negative charge on the analyte without significantly fragmenting the analyte
    - M+1 ion (or M-1 ion)
    - No need to volatilize
    - Down to fmol detection limits
  - Atmospheric Pressure Ionization (API)
    - Electrospray
    - MALDI
    - APCI
    - APPI

Which is Best?

- It depends on the exact application.
- Increasing polarity and molecular weight and thermal instability favors electrospray.
  - Most drugs of abuse are highly polar and are easily analyzed using electrospray.
  - High molecular weight proteins also require electrospray
- Lower polarity and molecular weight favors APCI or APPI.
  - Lower background, but compounds must be more thermally stable (some steroids).

Atmospheric Pressure Ionization (API)

- API techniques now provide highly sensitive detection using conventional and capillary flow rates on benchtop MS detector systems.
- These interfaces work with typical solvent and eluent compositions, whether the separation is achieved by isocratic or gradient elution.
- API using Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI) interfaces have proven invaluable in meeting sensitivity requirements for quantitative methods.

What is API?

- (API) is a technique for interfacing the eluent flow from an LC column to a mass spectrometer.
- The API interface must:

1) separate the analytes from the solvent

2) ionize the analyte molecules

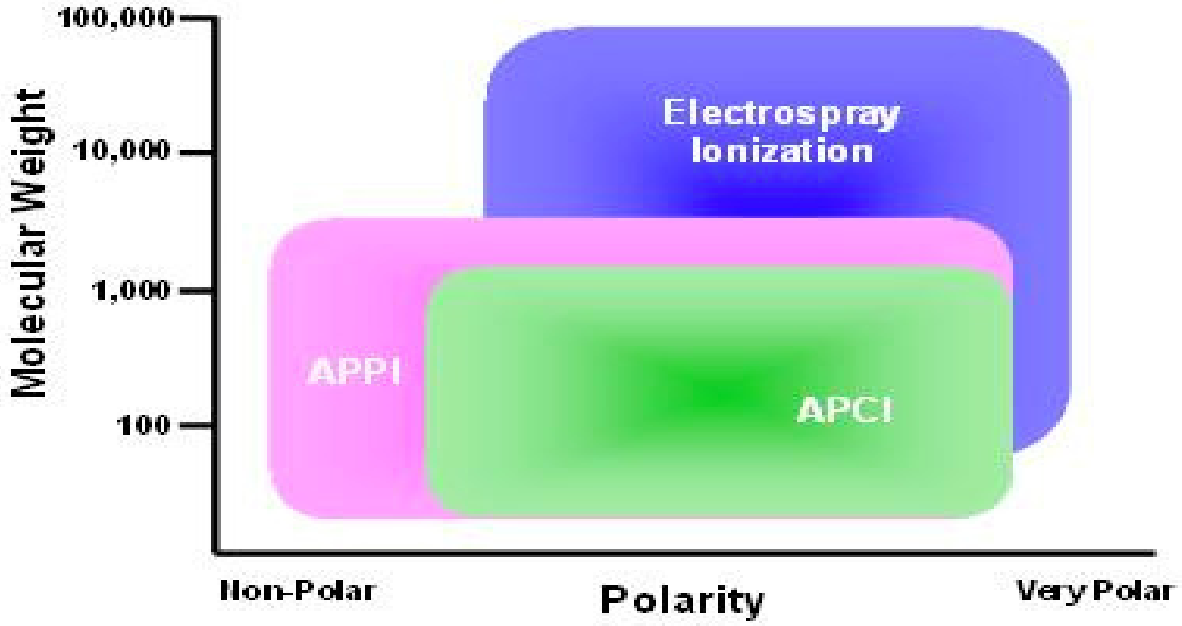
3) maintain vacuum in the mass detector.

Incompatibilities exist when combining LC and MS

- First, the eluent stream being liquid (and often aqueous) can flow at >1 mL/min, while the MS operates at a pressure of about 10–6 torr (1.3 × 10-4 Pa).
- Therefore, it is not possible to pump the eluent directly into the source of the mass spectrometer while maintaining the necessary vacuum.
- Second, the majority of analytes likely to be separated are relatively involatile and/or thermally labile and are not amenable to ionization techniques used in gas chromatography/mass spectrometry (GC/MS).

Alternative ionization and interface techniques

- To overcome the incompatibilities of LC and MS alternative ionization and interface techniques have been developed.
- Two types of API interfaces are commonly encountered. These are Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI).



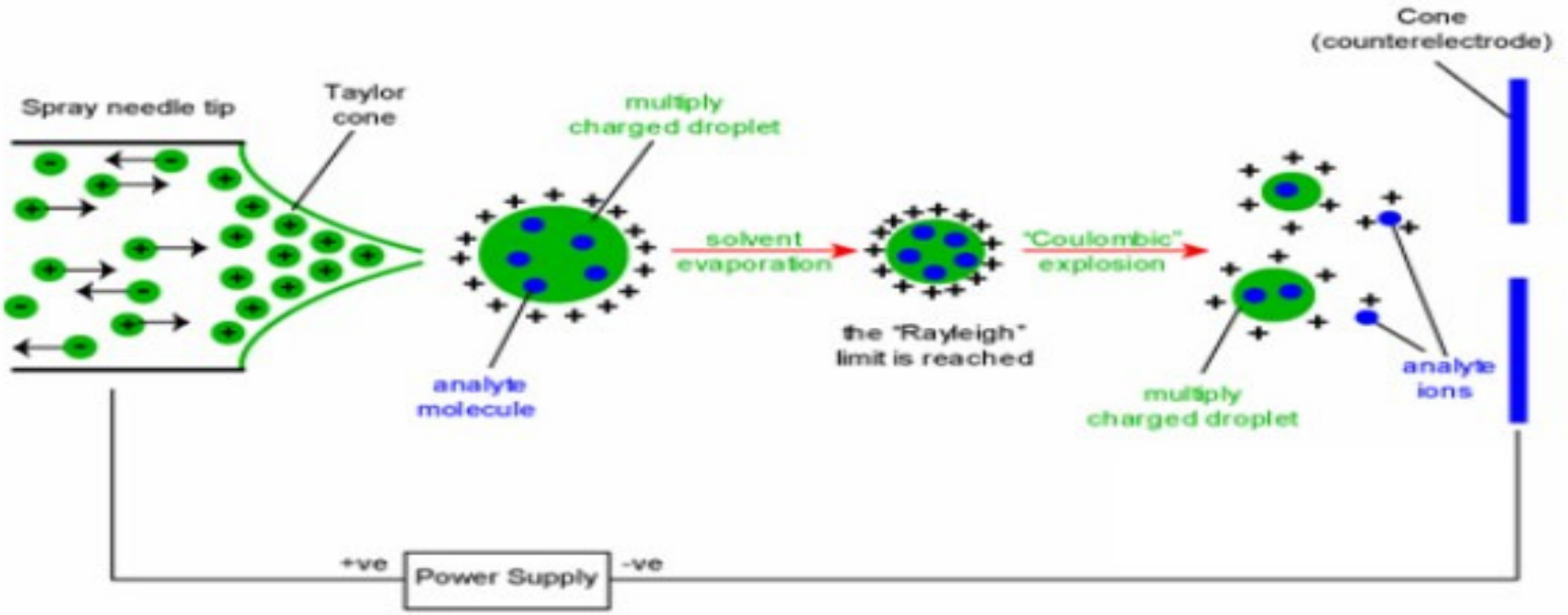
What is ESI?

- ESI is an API technique that provides a simple, real time means of analyzing a wide range of polar molecules.
- For small molecules (up to 1-2 K Daltons in molecular mass), either an [M+H]+ or [M-H]– ion is detected depending on whether positive or negative ion detection has been selected.
- This is similar to the traditional Chemical Ionization (CI) used in GC/MS, but ESI is a much softer ionization technique, producing significantly less fragmentation.
- In addition, the analyte does not have to be exposed to high temperatures (this is good for thermally labile compounds) or be volatile.
- The mass spectrometer does require the analyte to be in the gas phase and to be in a charged state (two basic requirements of all mass spectral analytes) this is accomplished without the conventional GC/MS techniques employed for vaporization and ionization.

How does ESI work?

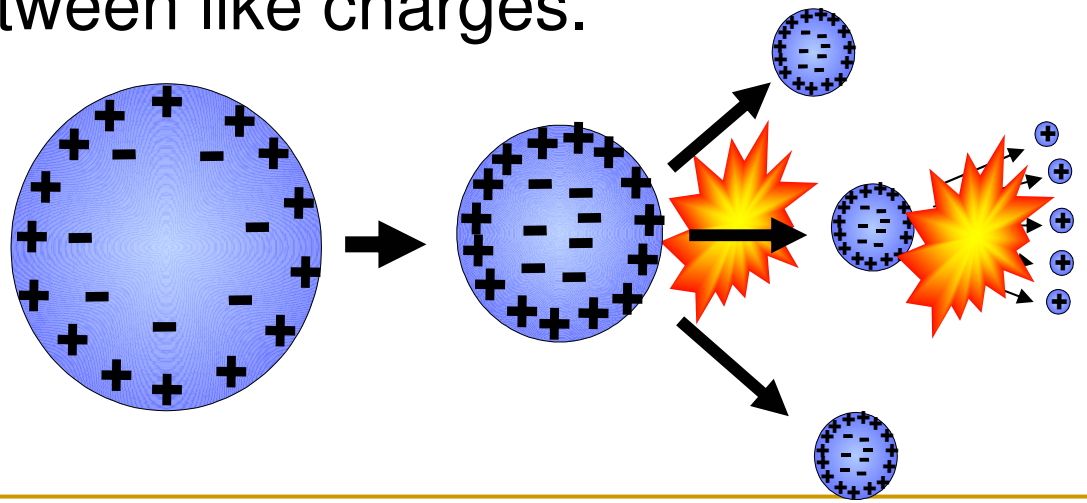
- The mechanism of electrospray ionization involves the emission of ions from a droplet into the gas phase at atmospheric pressure, a process known as ion evaporation.
- In ESI, the eluent passes through a stainless steel capillary that carries a high potential, typically 2 to 4 kV. The strong electric field generated by this potential and a concentric nebulizing nitrogen gas flow cause the formation of a fine spray of highly charged droplets at the tip of the capillary (hence ESI).
- The ion evaporation process is assisted by a flow of heated gas. This heating process, which is close to the source entrance cone, enables the routine use of a wide range of liquid flow rates in ESI mode (50 µL to >1 mL min–1).

- As a sample droplet moves through the heated sheath gas, the solvent evaporates, decreasing the size of the droplet, which increases charge-to-volume ratio.
- When this ratio reaches the Rayleigh instability limit, the droplet undergoes a coulombic explosion, producing a smaller droplet with a lower charge-to-volume ratio.
- As more solvent is eliminated, the process is repeated. The end result yields ions in the gas phase. The newly formed ions flow into the entrance cone, pulled by the strong electric field and pressure differential at the entrance orifice.

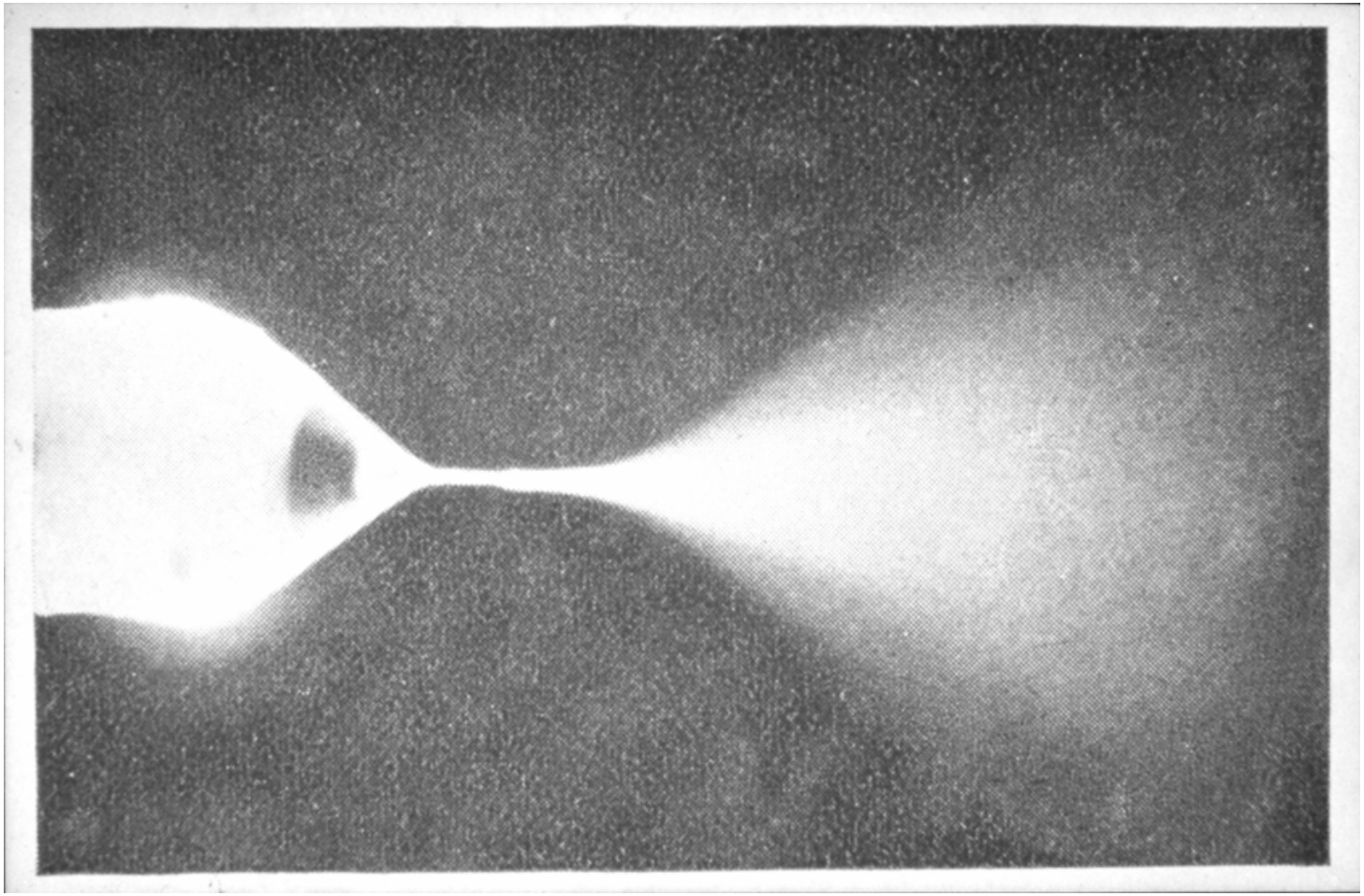


ESI: Droplet size reduction & fission

- Droplet size reduction occurs by the continual repetition of two processes:
  1. Desolvation (evaporation of neutral solvent and volatile buffers)
  2. Droplet fission caused by electric repulsion between like charges.



The Electrospray Phenomenon



Electrospray Ionisation

ESI: Ionization Efficiency

- Enhanced by the production of smaller droplets.
  - Lower mobile phase flow rate yields smaller droplets.
  - Nebulizing gas promotes droplet formation.
  - Use of volatile mobile phases promotes desolvation and droplet fission.

- Enhanced by increasing the concentration of analyte ions at the end of the capillary tip.
  - Matrix modifiers to promote solution ion formation.
  - Chromatography to produce narrow highly concentrated bands of analyte.

ESI: Pros and Cons

Pros

- Soft ionization technique, resulting in little decomposition of labile analytes.
- Generally produces only molecular ions.
- Multi charged analytes easily produced, allowing proteins to be analyzed.
- Wide range of analytes
- Efficient ion production

Restrictions of ESI

- Non-volatile salts should be avoided as signal suppression will result.
- Strong ion-pairing agents such as trifluoroacetate should similarly be avoided.
- Need polar solvents for ESI.
  - Normal phase not compatible with ESI (Use APCI)
- Additives must be compatible with ionization mode. (i.e. pH of separation must match desired ions)

ESI: Pros and Cons

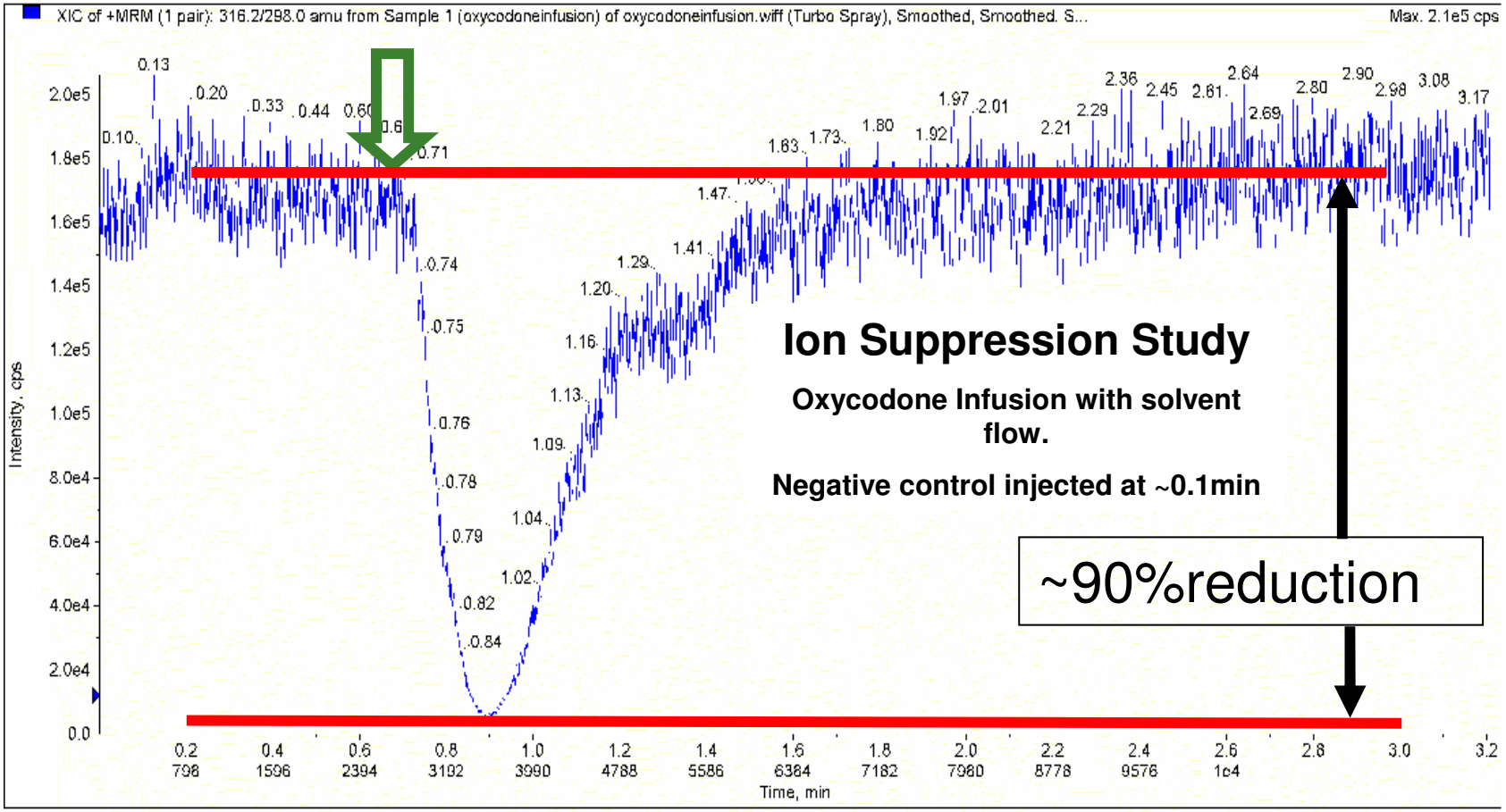
Cons

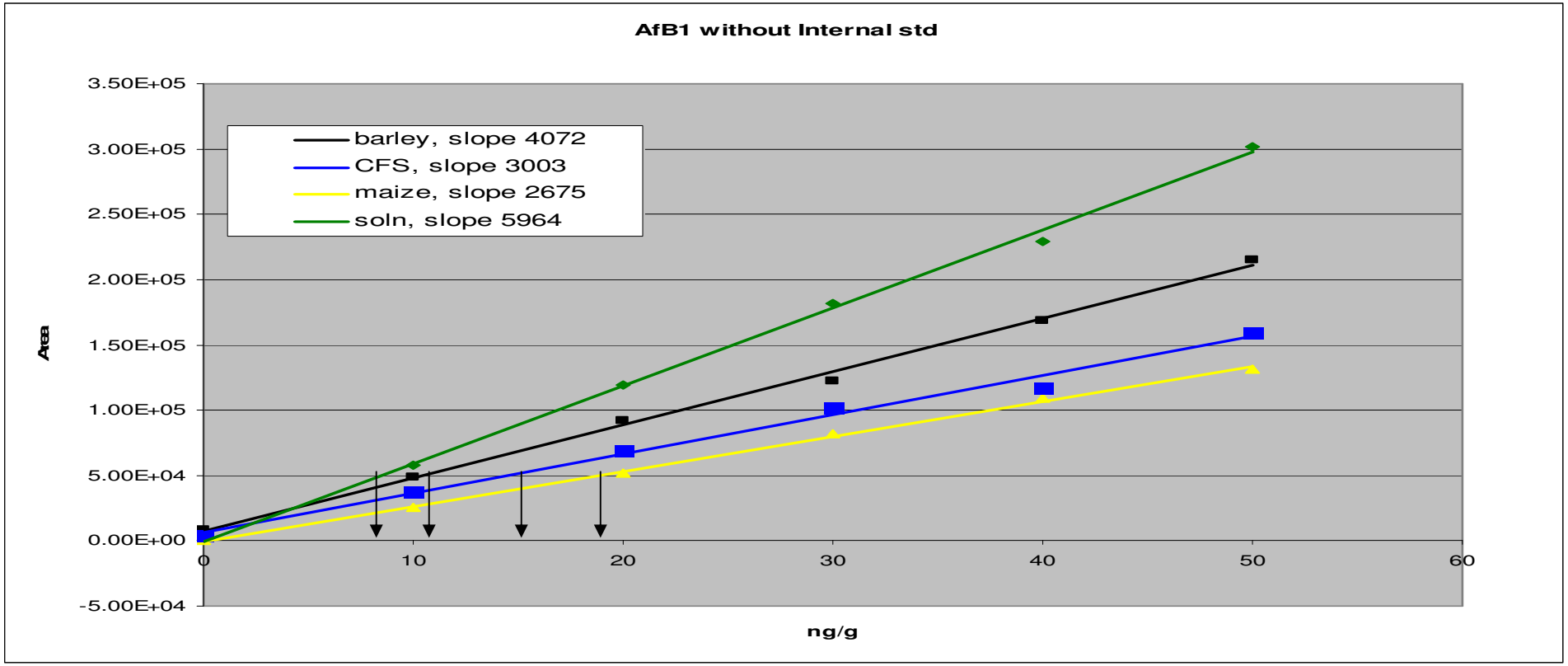
- Lower flow rates
  - concentration dependent
  - nL/min (nanospray)
- Analyte must form solution phase ion
  - HCl or Na salt good indicator of suitability
- *Ion Suppression*

ESI: Ion Suppression

- The disadvantage of electrospray.
- Often results from inefficient droplet formation.
- Causes:
  - Nonvolatile buffers or salts (phosphates)
  - Nonvolatile materials in mobile phase Ion pairing
  - Reported that higher molecular weight analyte ions can suppress smaller analytes
- Generally more prominent early in an RP-LC run, but can occur at anytime.
- Underscores the need for good chromatography.

ESI: Ion Supression





For Aflatoxin B1 without IS; Area of 50,000 counts would give concentration of

(i) 8 ng/g using solvent curve

(ii) 11 ng/g using barley matrix curve

(iii) 14 ng/g using compound feed matrix curve

(iv) 19 ng/g using maize matrix curve

For Zearalenone; Area of 200,000 counts would give concentration of

(i) 15 ng/g using solvent curve

(ii) 36 ng/g using barley matrix curve

(iii) 44 ng/g using maize matrix curve

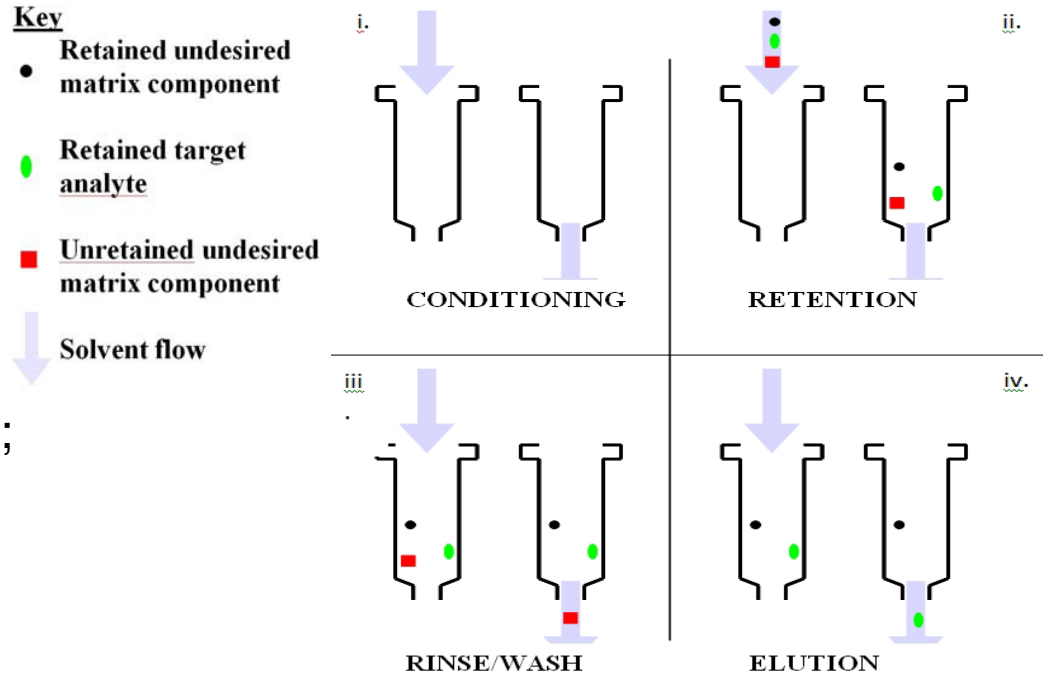
Ion Suppression;

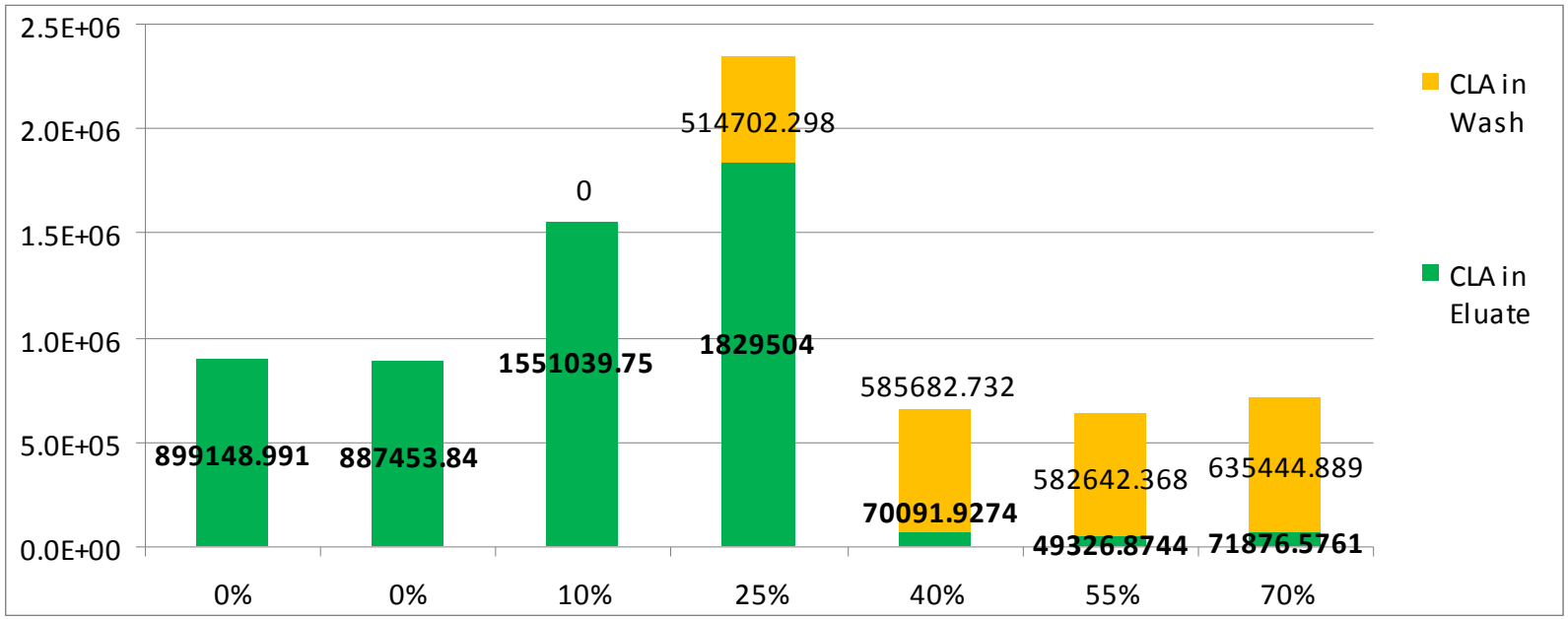
Less analyte=bigger response?

Chlormadinone Acetate in Kidney Fat;

Effect of different wash solvents on SPE Purification; Detector Response

0% Ethyl Acetate in Hexane ⟶ 70%

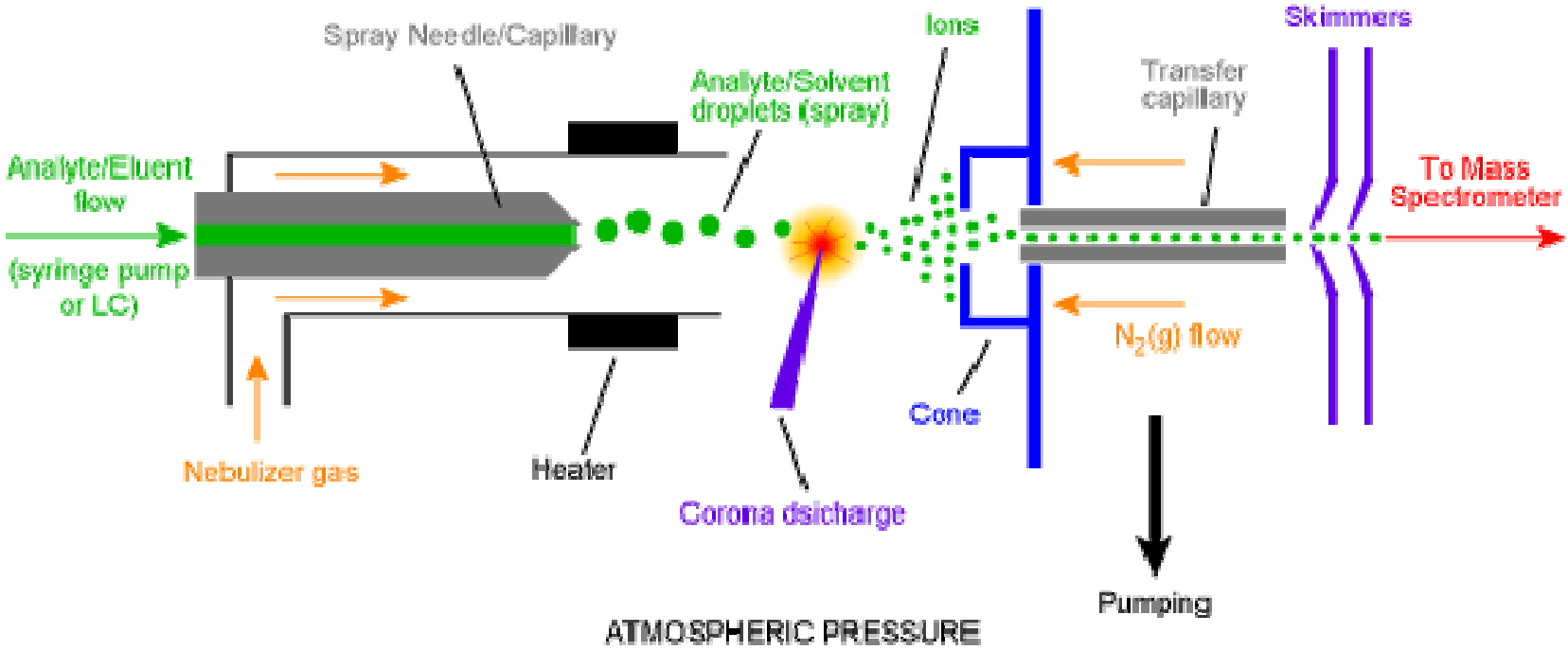




Atmospheric Pressure Chemical Ionisation (APCI)

- APCI uses analyte desolvation and charge transfer reactions in vapour phase analyte ions.
- Eluent introduced into interface using capillary similar to ESI, no potential applied to capillary.
- Liquid emerges from the capillary surrounded by a flow of inert gas into a heated region.
- Combination of gas and heat forms an aerosol that begins to rapidly evaporate.
- A pin is placed within the heated region that has a high potential applied to it and produces an electrical discharge that ionises eluent molecules via charge transfer reactions.

APCI Schematic



Electrospray Ionization (ESI)	Atmospheric Pressure Chemical Ionization (APCI)
Summary: The sample solution is sprayed from a hollow capillary needle across a high-potential difference (a few kilovolts) into an orifice in the interface. Heat and gas flows are used to desolvate the ions existing in the sample solution. Electrospray ionization can produce multiply charged ions with the number of charges tending to increase as the molecular weight increases.	**Summary:** A corona discharge is used to ionize the analyte in the atmospheric pressure region. The gas-phase ionization in APCI is more effective than ESI for analyzing less-polar species. ESI and APCI are complementary methods.
Sample Introduction • Flow injection • LC/MS • IC/MS • Typical flow rates are from 50 µL/min up to >1 mL/min	**Sample Introduction** • Flow injection • LC/MS • Typical flow rates are from 200 µL/min up to over 1 mL/min
Benefits • Good for charged, polar, or basic compounds • Excellent for IC or reversed-phase LC • Permits detection of high-mass compounds at low mass-to-charge ratios(m/z less than 2000) • Very low chemical background yields excellent detection limits • Can control presence or absence of fragmentation by varying interface potentials	**Benefits** • Good for less polar compounds • Excellent liquid chromatographic interface for normal phase separations • Complementary to ESI • Can control presence or absence of fragmentation by varying the interface potentials
Mass Range • Low-moderate. Typically less than 1000 Da (singly charged m/z) • Med-high. Typically less than 100,000 Da (multiply charged m/z)	**Mass Range** • Low-moderate. Typically less than 1000 Da (singly charged m/z only in APCI)

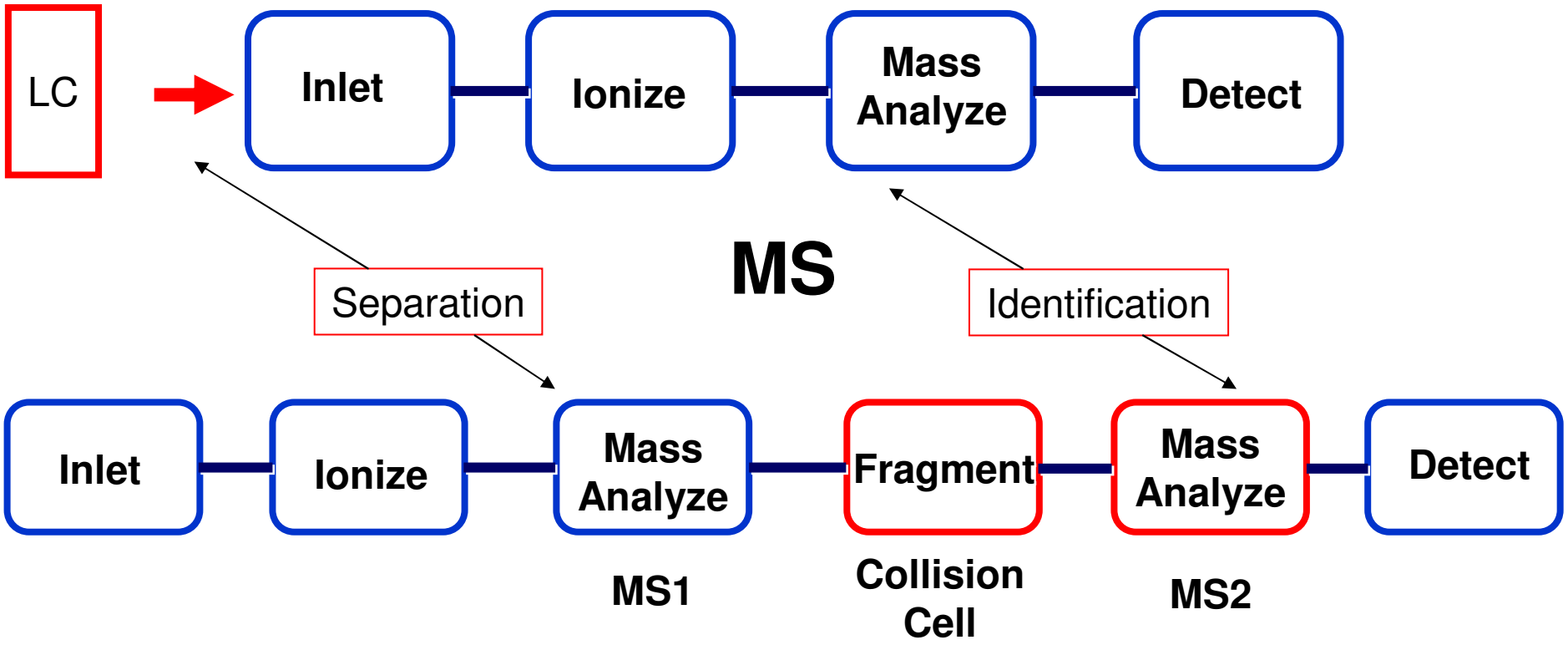
Congratulations!

You have made an ion.

Now what do you do with it?

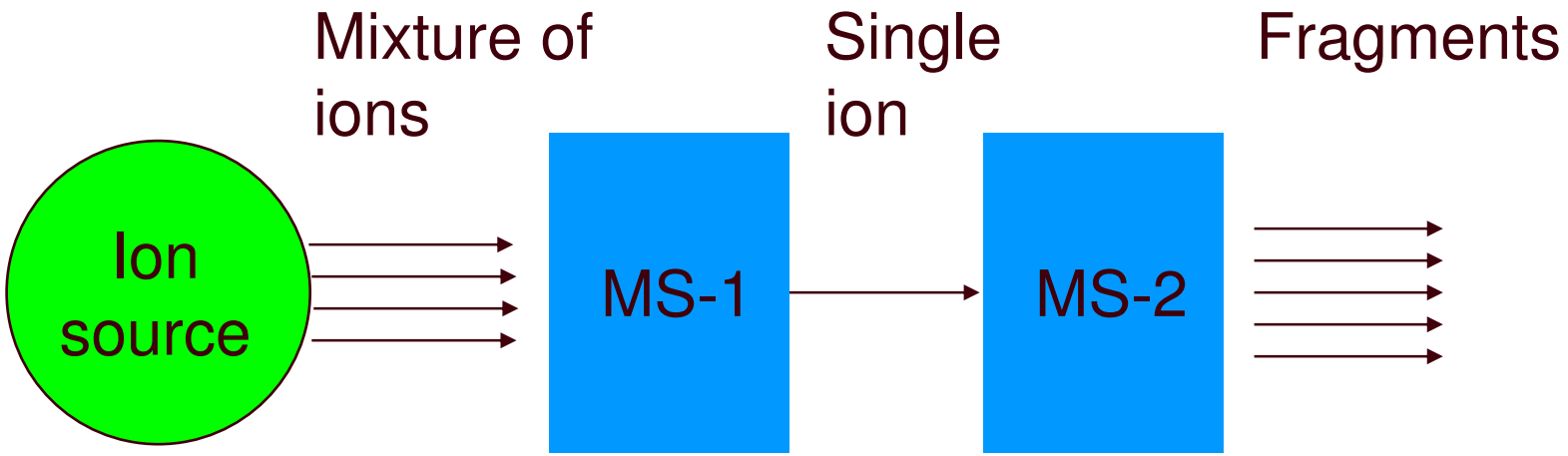
Mass spectrometer type	Features and benefits	Disadvantages
Single Quadrupole	Good scan function sensitivity Good selectivity/sensitivity via SIM scanning High duty cycle with SIM Good dynamic range (3-4 orders) Fast positive/negative ionisation	Limited mass range (generally up to 3000 *m/z*) SIM functionality can be prone to matrix interferences thus limit detection limits Low resolution (around 1500 FWHM or 0.7 Da)
Triple Quadrupole	Good scan function sensitivity Good SIM function Excellent selectivity with MRM, even with matrix Excellent duty cycle with MRM Ability to run multiple analytes simultaneously with MRM High dynamic range (4-5orders) Fast pos/neg ionisation Other scan functions available, *e.g.* neutral loss, product and precursor ion	Low resolution generally (1500 FWHM or 0.7 Da) Limited mass range (up to 3000 *m/z* generally)
Ion Trap (low resolution)	Very high full scan sensitivity Full scan MSMS and MS^n capability (ideal for structural identification) Can perform targeted quantitation with 3 orders of dynamic range using SIM scan functions. Some linear ion traps can perform simultaneous full scan and MRM experiments	Can suffer from matrix interferences (particularly 3D traps) Duty cycle is generally slower when compared to a triple quad, especially when doing simultaneous full scan and MRM acquisitions Low resolution generally (1500 FWHM/0.7 Da) but can run at higher resolution (but loses duty cycle)
Ion Trap (high resolution)	High full scan sensitivity in MS, MSMS and MS^n mode Good dynamic range (3 orders) High resolution (>100,000 FWHM) can provide good selectivity using exact mass measurement	Resolution can be affected by scan speed, *i.e.* the faster the acquisition speed, the lower the resolution Orbital trapping devices can have a limited dynamic range and be affected by matrix Limited mass range (up to 4000 *m/z* typically)
TOF (high resolution)	Good scan functionality and sensitivity High resolution (up to 40,000 FWHM) provides high selectivity through exact mass measurement Good dynamic range (with newer ADC based detection systems, typically 3-4 orders) Ability to get quantitation on multiple analytes in a single acquisition Mass range in excess of 20,000 *m/z*	No MS/MS functionality or other scan functions Generally, lower sensitivity when compared to a triple quadrupole running MRM Sensitivity can be affected by scan speed
qTOF (high resolution)	Good full scan sensitivity Good MSMS scan functions High resolution (>40,000 FWHM) providing high degree of selectivity via exact mass measurement Good dynamic range with newer ADC based detection systems (3-4 orders) Ability to get quantitation on multiple analytes during a single run Mass range in excess of 20,000 *m/z* Resolution not affected by increased scan speed	Generally, lower sensitivity when compared to a triple quadrupole running MRM Sensitivity can be affected by scan speed

MS *vs.* MS/MS


LC
Inlet
Ionize
Mass Analyze
Detect
MS
Separation
Identification
Inlet
Ionize
Mass Analyze
Fragment
Mass Analyze
Detect
MS1
Collision Cell
MS2
MS/MS

What is Tandem MS?

- Uses 2 (or more) mass analyzers in a single instrument
  - One purifies the analyte ion from a mixture using a magnetic field.
  - The other analyzes fragments of the analyte ion for identification and quantification.



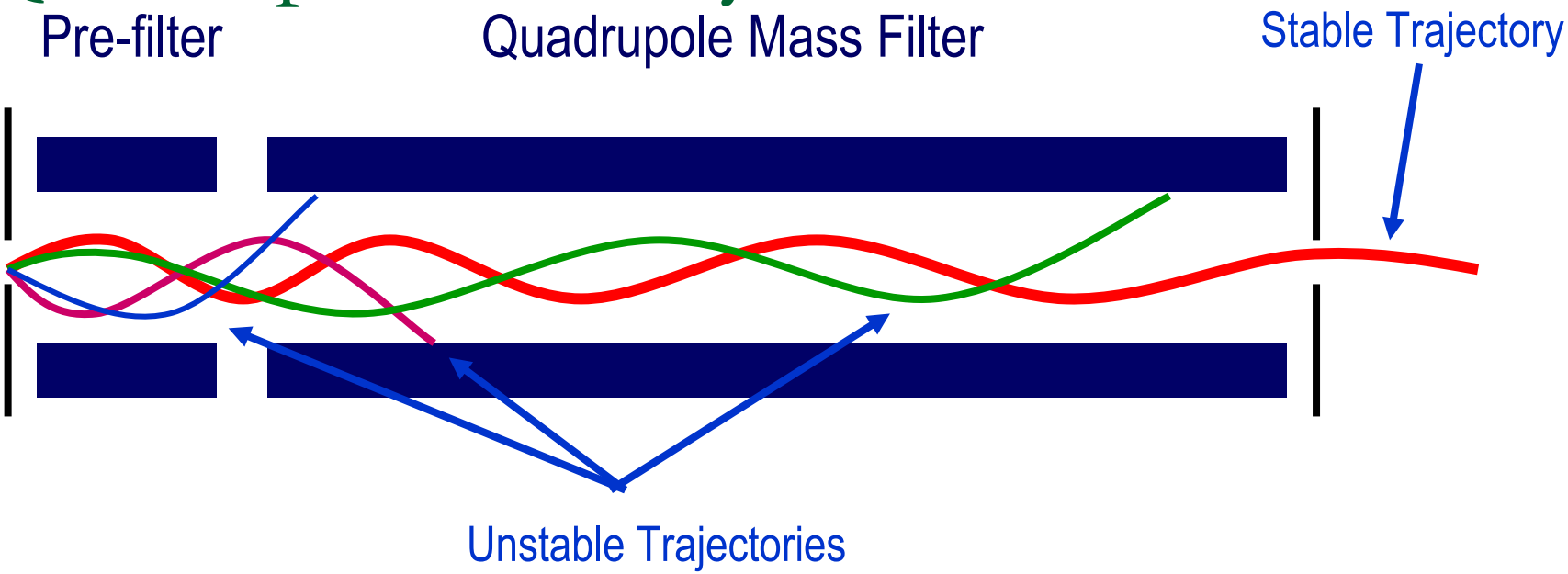
Analytical Assays used in Pharmaceutical Industry Labs for New Chemical Entities

Method	**1990**	**1998**	**2000**	**2006**
HPLC (UV &Fluorescence)	75%	50-60%	20%	2%
GC/MS	12%	3%	2%	0
LC/MS/MS	3%	40-50%	60-75%	**98%**
Immunoassay (ELISA/FPIA etc.)	10%	10%	10%	0

Applications of Tandem MS

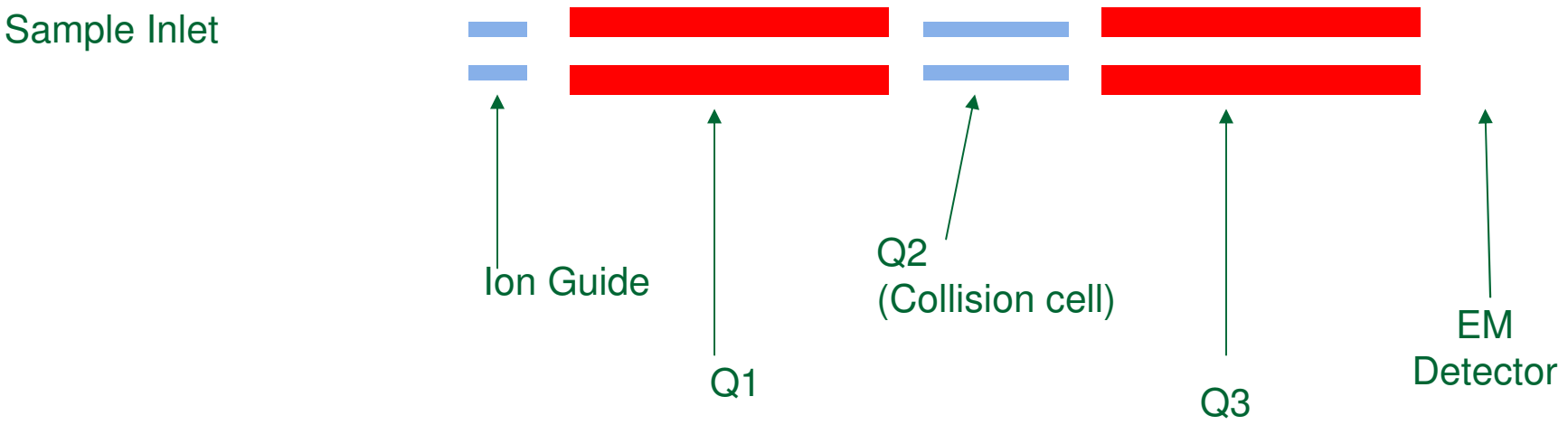
- Biotechnology & Pharmaceutical
  - To determine chemical structure of drugs and drug metabolites.
  - Detection/quantification of impurities, drugs and their metabolites in biological fluids and tissues.
  - High through-put drug screening
  - Analysis of liquid mixtures
  - Fingerprinting
    - Nutraceuticals/herbal drugs/tracing source of natural products or drugs
- Clinical testing & Toxicology
  - inborn errors of metabolism, cancer, diabetes, various poisons, drugs of abuse, etc.

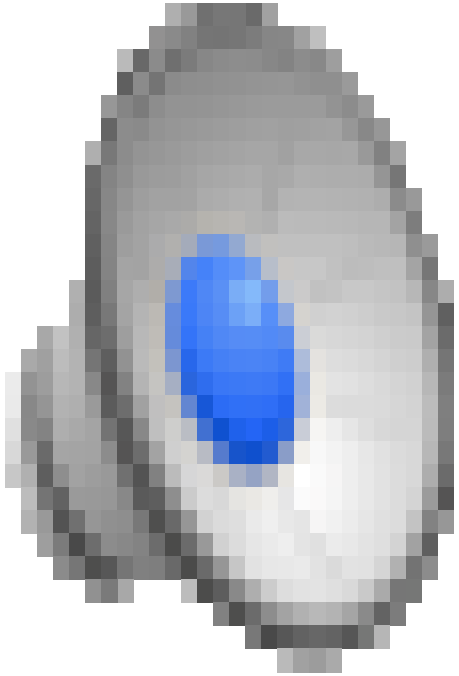
Quadrupole Theory



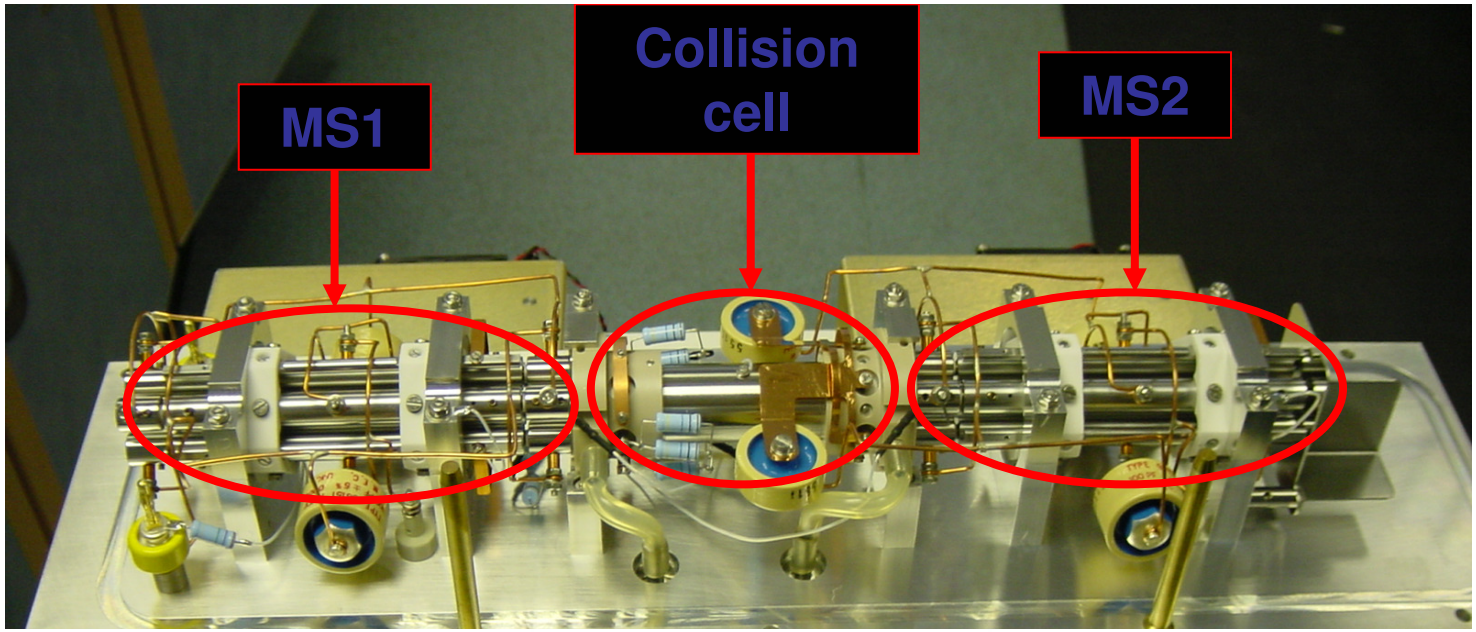
- Only ions with the correct m/z values have stable trajectories within an RF/DC quadrupole field.
- Ions with unstable trajectories collide with the rods, or the walls of the vacuum chamber, and are neutralised.

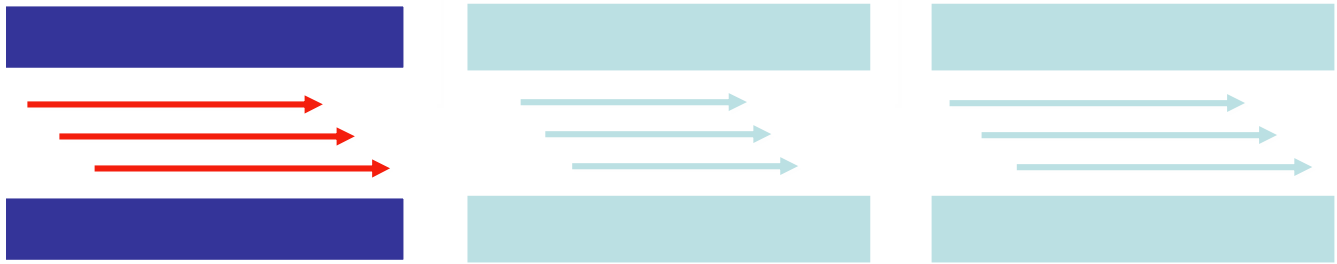
Triple Quadrupole Mass Analyzer





Full Scan Acquisition Mode



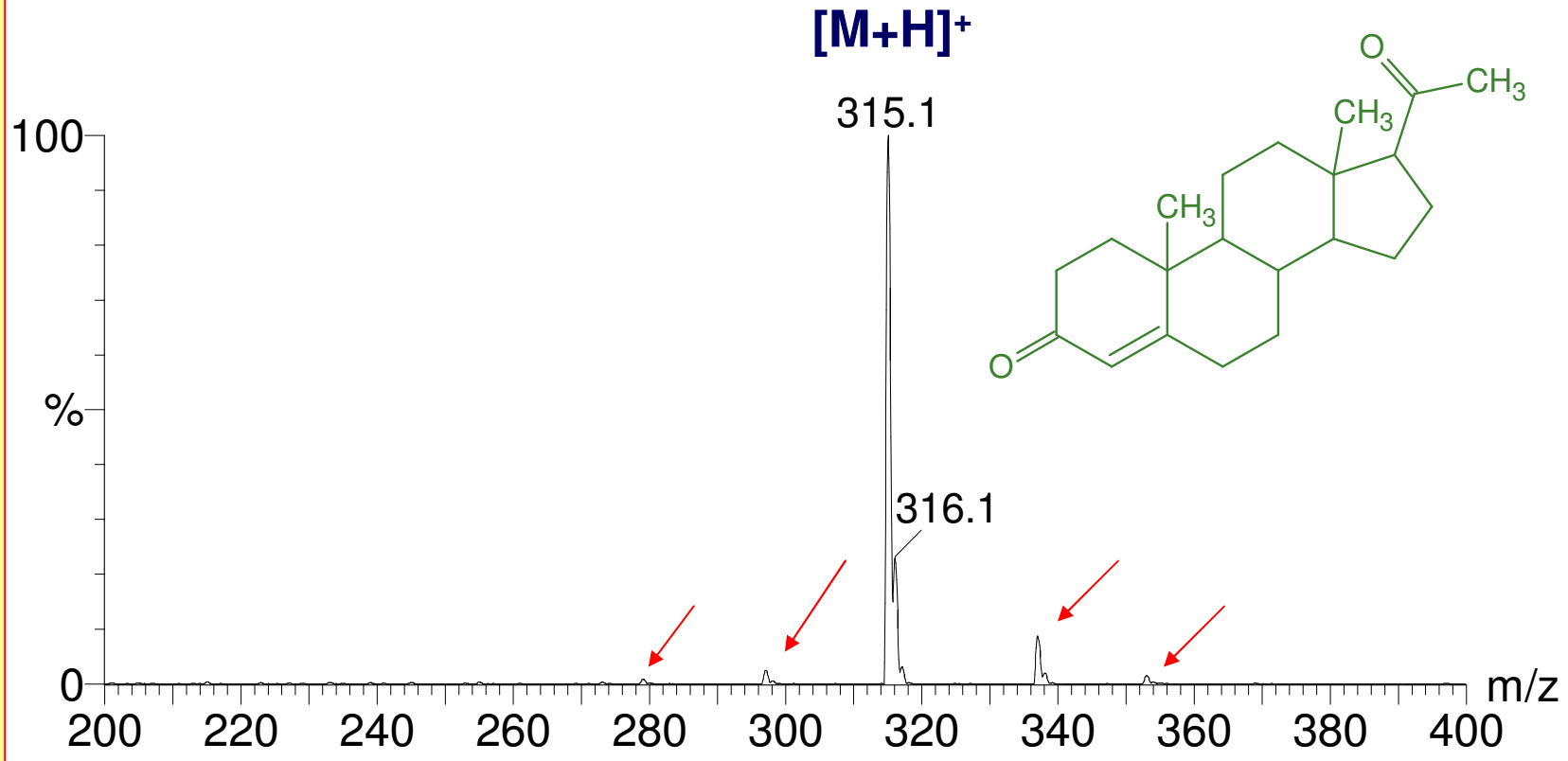


Scanning ***Rf only, pass all masses***

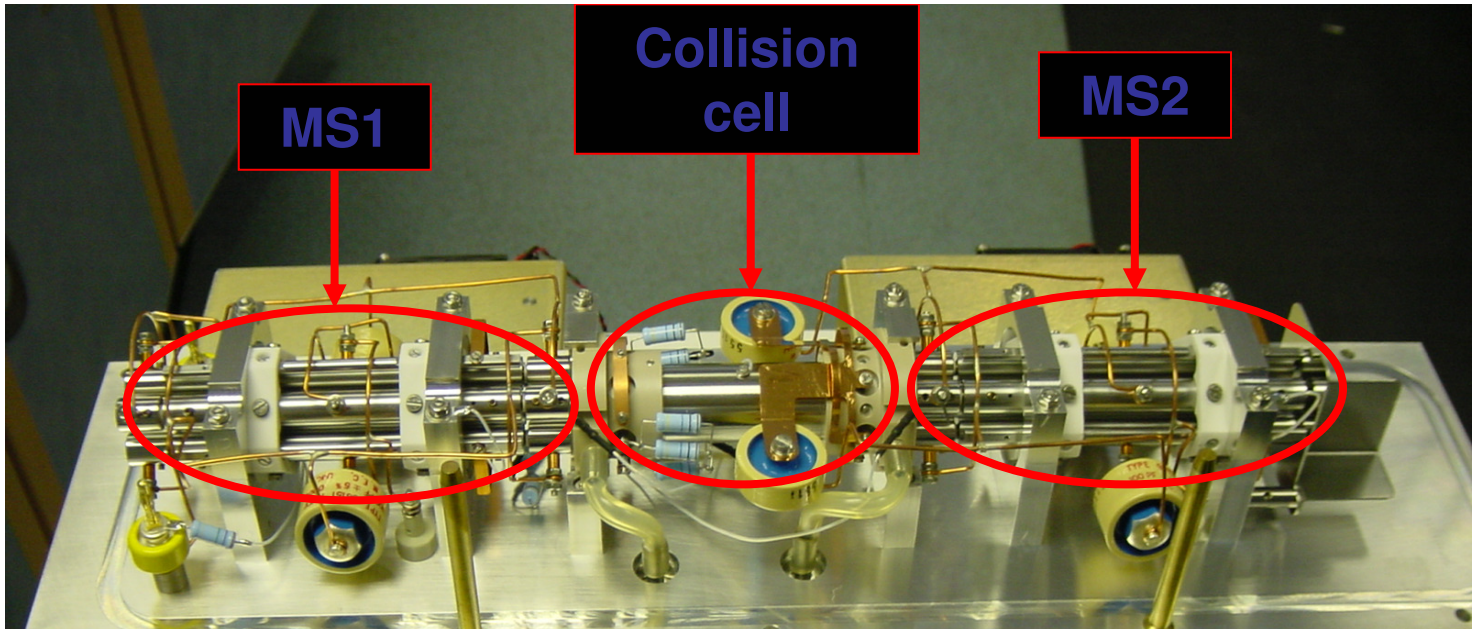
SCANNING MODE: The first quadrupole mass analyzer is ***Scanning*** over a mass range. The collision cell and the second quadrupole mass analyzer allow all ions to pass to the detector.

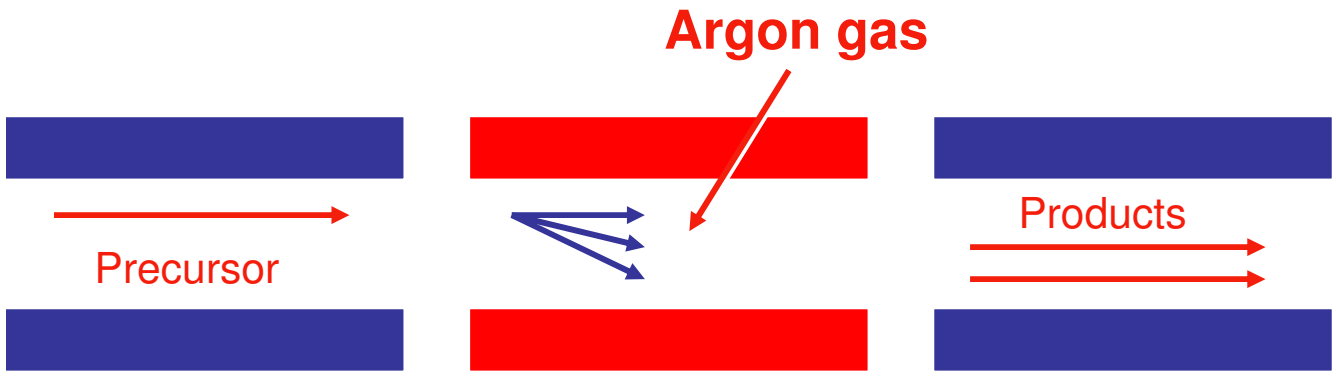
Full Scan Acquisition Mode

Mass Spectrum: Progesterone



Product ion scanning

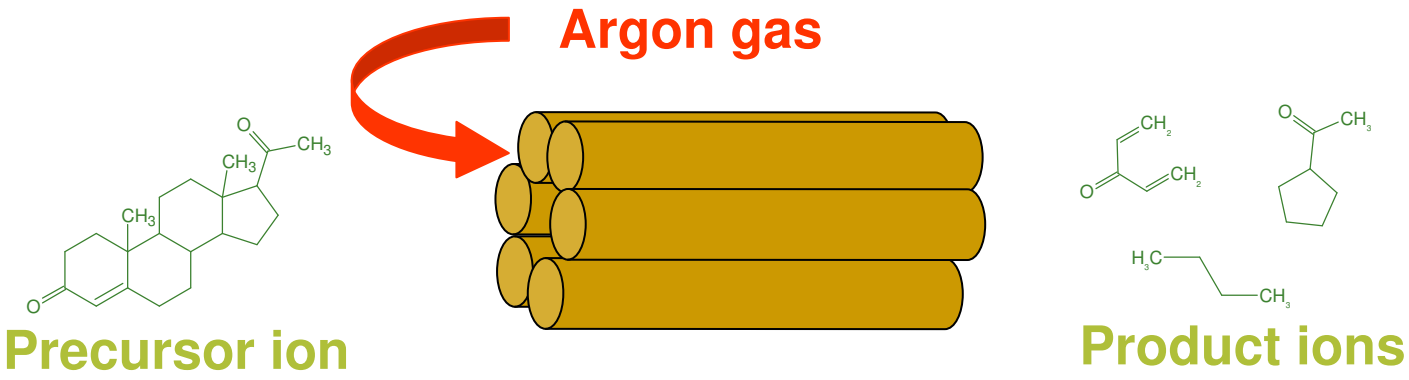




Static (m/z 315.1) ***Scanning***

The first quadrupole mass analyzer is fixed at the mass-to-charge ratio (*m/z*) of the precursor ion to be interrogated while the second quadrupole is ***Scanning*** over a user-defined mass range.

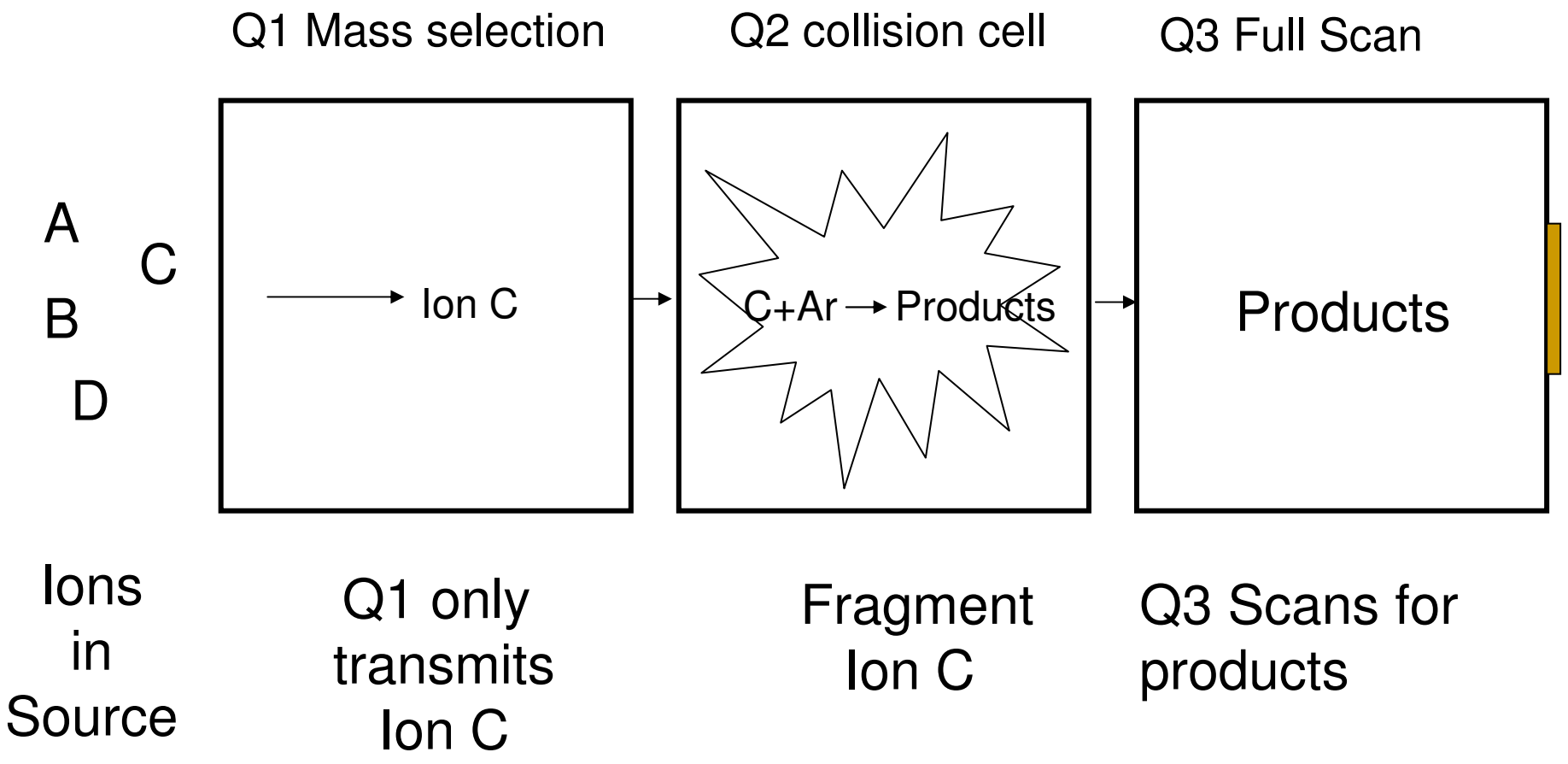
Collision induced dissociation



- In the collision cell, the **TRANSLATIONAL ENERGY** of the ions is converted to **INTERNAL ENERGY**.

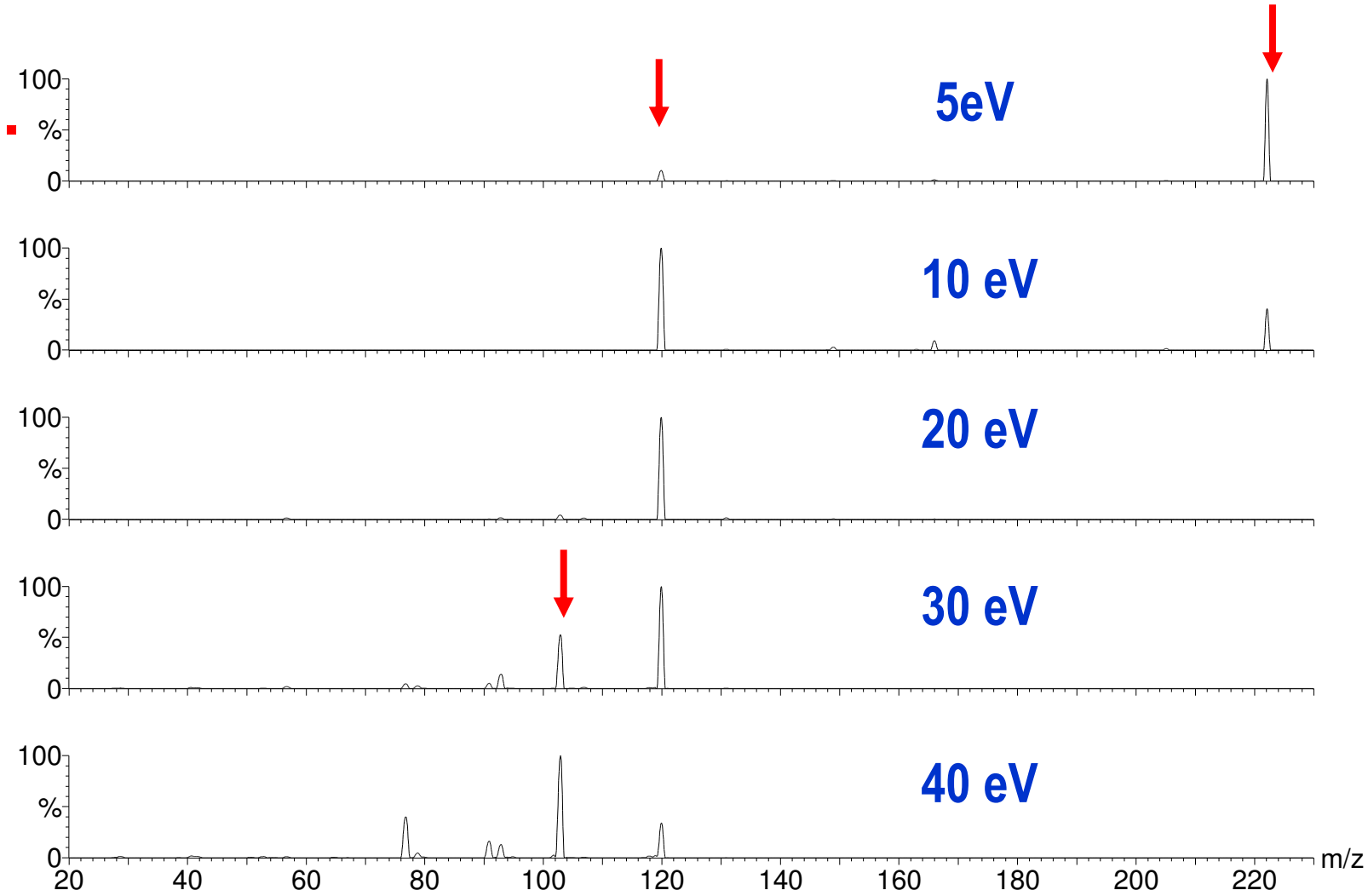
- Collision conditions **(FRAGMENTATION)** are controlled by altering:
  - The collision energy (speed of the ions as they enter the cell)
  - Number of collisions undertaken (collision gas pressure)

Product Ion Scan Mode in a Triple Quadrupole



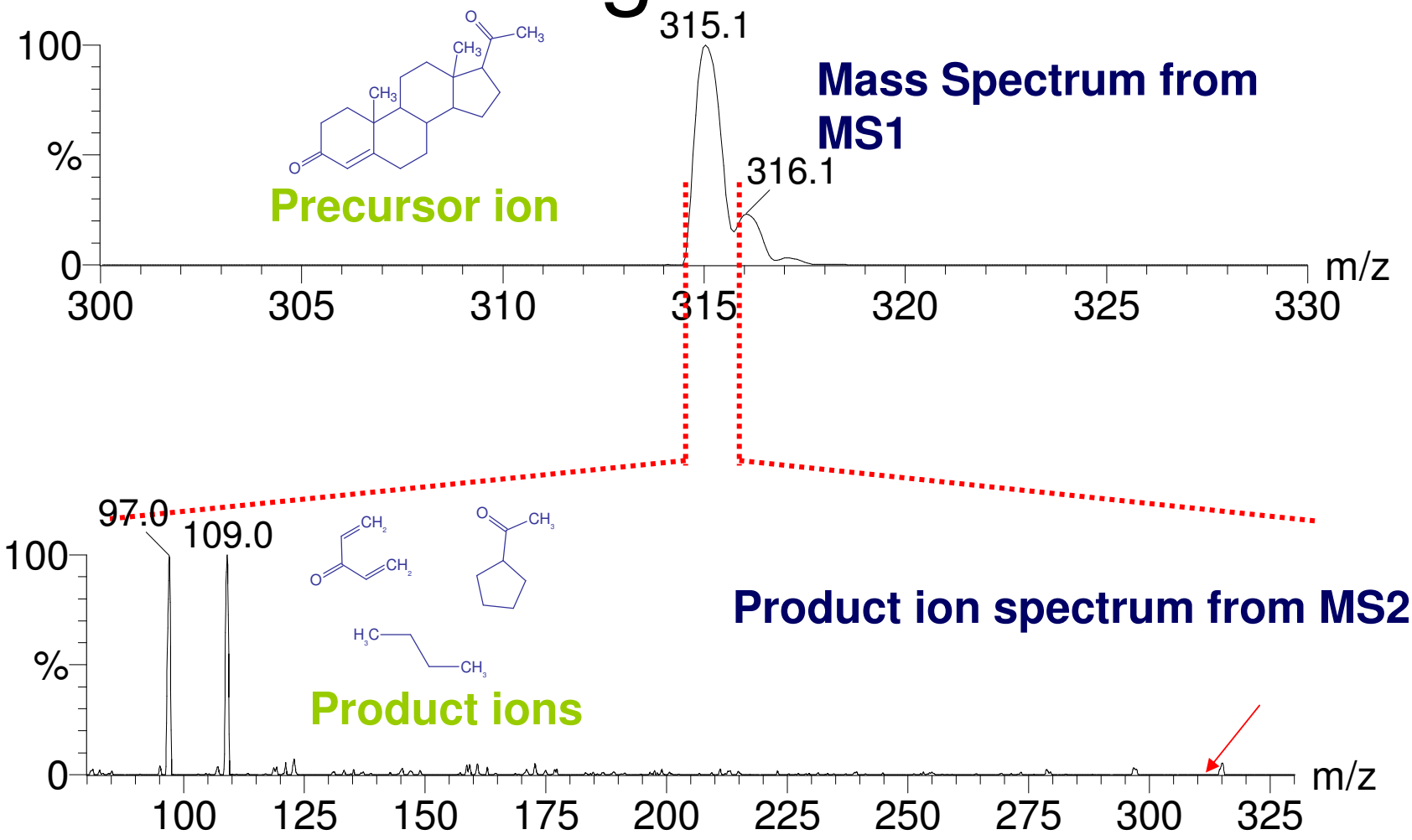
Product ion scanning

↑ collision energy > ↑ fragmentation

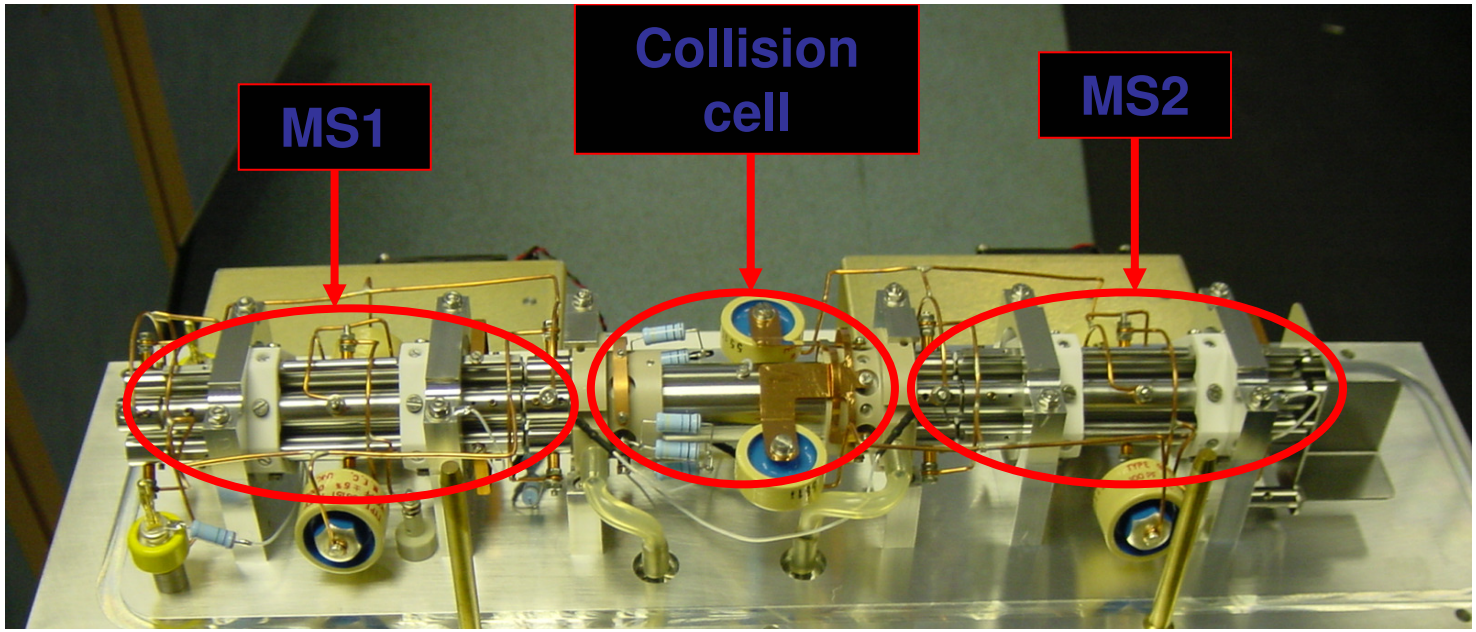


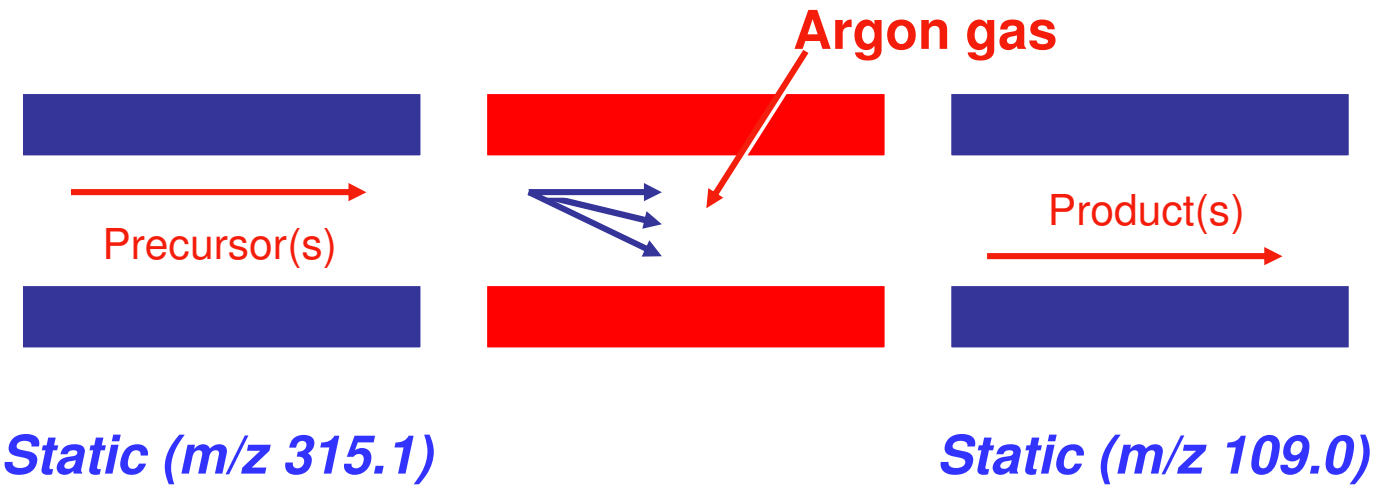
Product ion scanning

Product Ion Spectrum: Progesterone



Multiple Reaction Monitoring

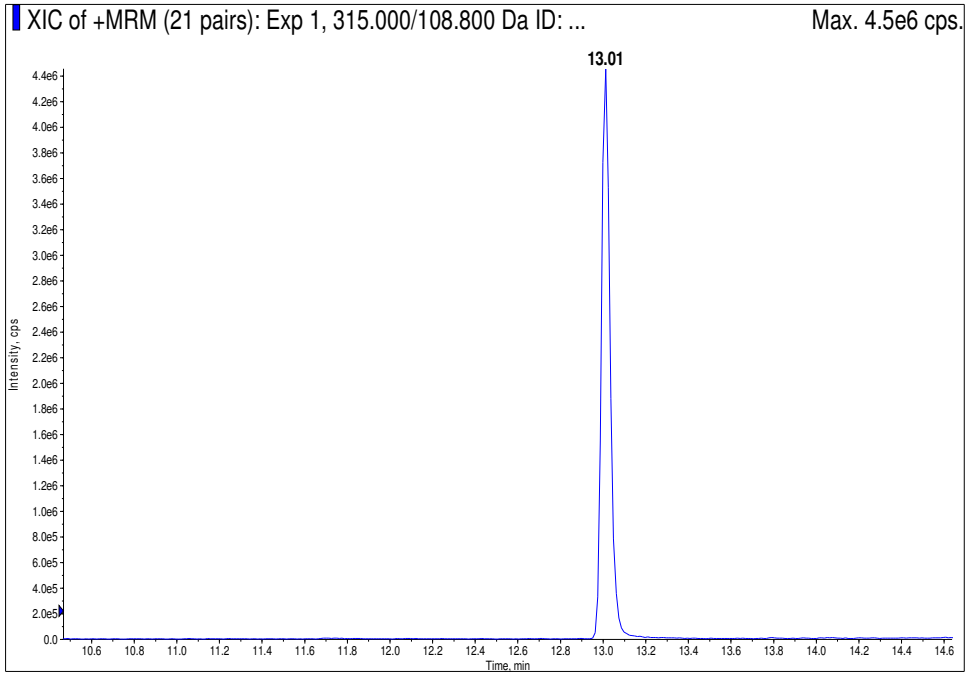


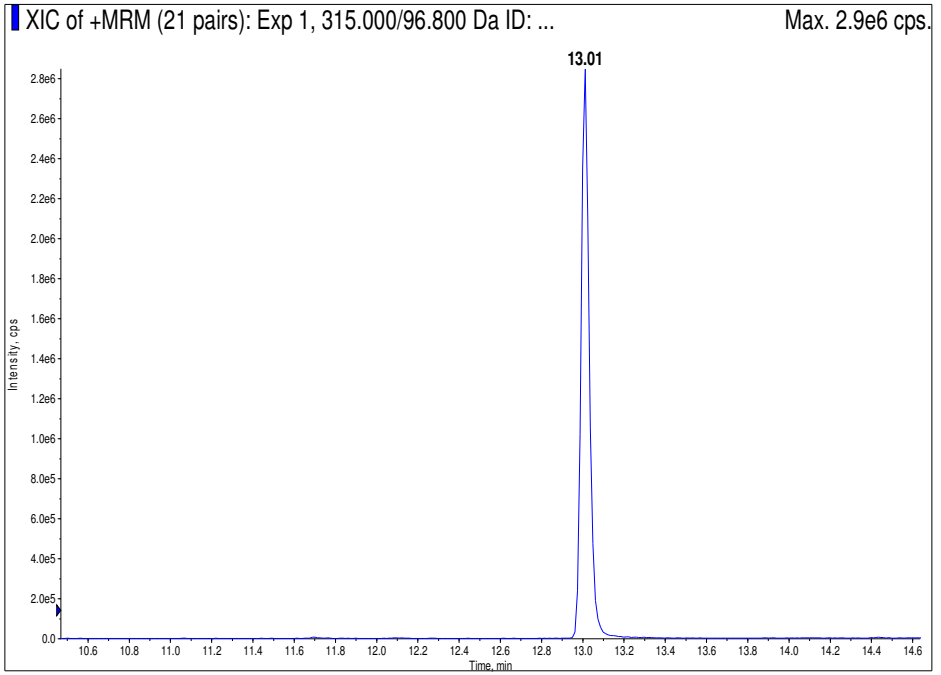


Both the first and second quadrupole mass analyzers are held ***Static*** at the mass-to-charge ratios (*m/z*) of the precursor ion and the most intense product ion, respectively.

Progesterone Analysis The State Lab

- Bovine Plasma sample fortified at 1 ppb





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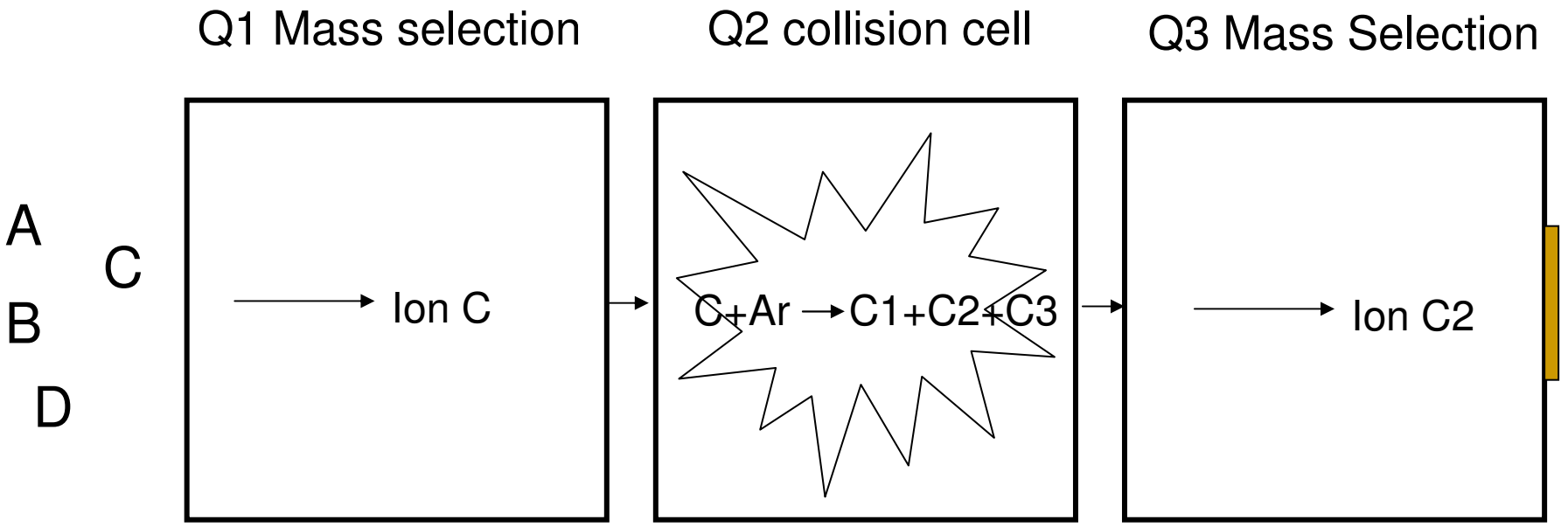
An tSaotharlann Stáit

Multiple Reaction Monitoring (MRM) in a Triple Quadrupole



Ions in Source

Q1 only transmits Ion C

Fragment Ion C

Q3 only transmits Ion C2

Analyte	Internal Standard	Precursor ion MS-MS (m/z)	Product ions	Ion ratio monitored
DMZ	DMZ-d3	142.2	96.4 (1) 81.4 (2)	96.4/81.4
RNZ	RNZ-d3	201.2	140.1 (1) 110.3 (2)	110.3/140.1
MNZ	DMZ-d3	172.0	82.5 (1) 128.2 (2)	82.5/128.2
IPZ	IPZ-d3	170.0	124.3 (1) 109.4 (2)	109.4/124.3
SDZ	SPZ	251.0	156.1(1) 110.3(2)	110.3/156.1
SMZ	SPZ	279.1	156.1(1) 186.0(2)	186.0/156.1
CAR	DMZ-d3	263.1	130.0(1) 175.0(2)	175.0/130.0
CAP	CAP-d5	321.0	152.0(1) 257.0(2)	257.0/152.0

Specificity of Detection for LC

- UV – chromophore
  - all compounds with a chromophore responding at the selected wavelength will interfere
- MS – molecular mass
  - interference from isobaric compounds
  - chemical noise
- MS/MS – molecular mass and structural information
  - interference from structural isomers only

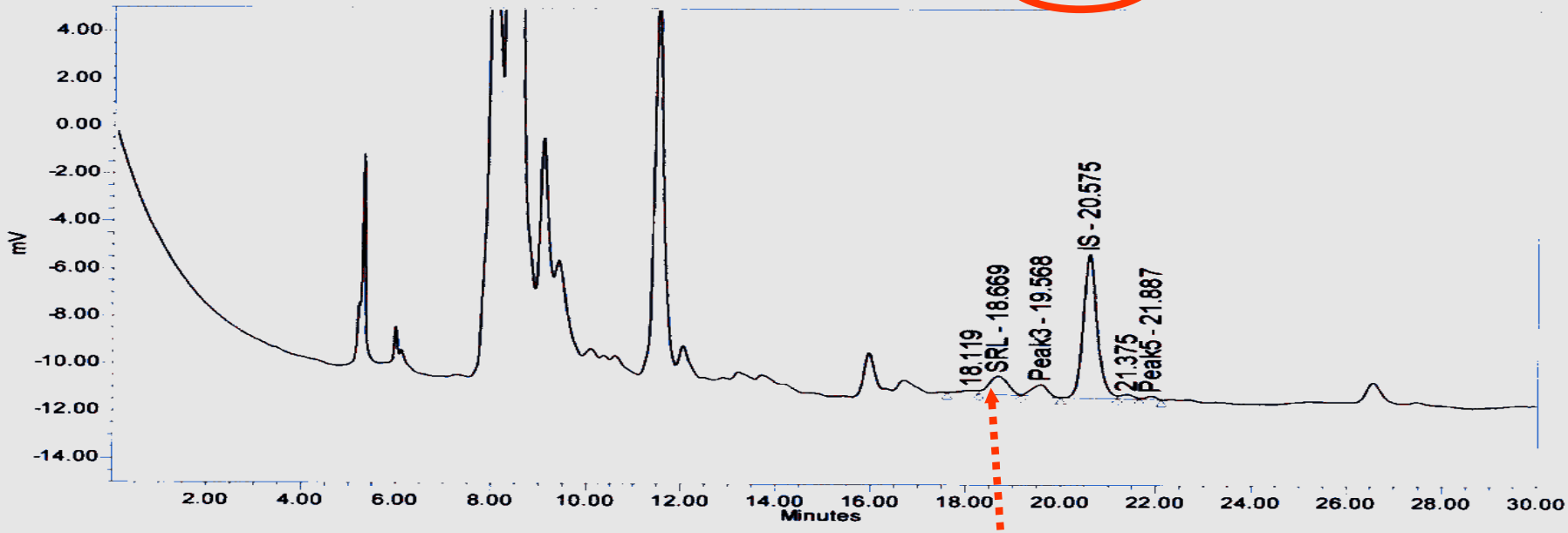
HPLC-UV Analysis of Sirolimus in Whole Blood

1. Wash all glassware in methanol x2 and tert-butyl methyl ether (TBME) x2.
2. Place 50µL of internal standard (in methanol) into each screw-cap glass tube.
3. Add 200µL Sirolimus calibrator (5x concentrated in methanol) or 200µL methanol for patient samples.
4. Add 1.0mL blank whole blood to calibrators or 1.0mL patient whole blood.
5. Add 2.0mL 0.1M ammonium carbonate buffer.
6. Mix thoroughly.
7. Add 7.0mL TBME and extract for 15min.
8. Transfer upper layer to clean tube and re-extract lower layer with 7.0mL TBME.
9. Combine TBME extracts and evaporate to dryness.
10. Redissolve residue in 5.0mL ethanol and evaporate to dryness.
11. Redissolve residue in 1.0mL ethanol, transfer to Eppendorf tube and evaporate to dryness.
12. Redissolve residue in 100µL 85% methanol.
13. Inject 80µL (equivalent to 800µL whole blood) and analyse using two 4.6mm x 250mm C18 columns connected in series (30min run time).

Sirolimus: HPLC - UV Example

SAMPLE INFORMATION

Sample Name:	Sample-2	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	1/25/02 6:10:25 PM
Vial:	7	Acq. Method Set:	Sirolimus
Injection #:	1	Date Processed:	1/25/02 6:40:27 PM
Injection Volume:	80.00 ul	Processing Methoc	Intégration Sirolimus1
Run Time:	30.0 Minutes	Channel Name:	Satin Ch 1
Sample Set Name	Run 25 janvier 2001	Proc. Chnl. Descr.	278 nm

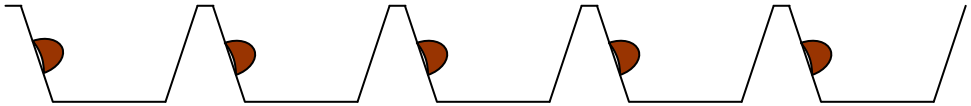


	Peak Name	RT	Area	% Area	Height	Amount	Units
1		18.119	3591	2.14	145		
2	SRL	18.669	22437	13.37	790	11.410	ng/mL
3	Peak3	19.568	12037	7.17	517		
4	IS	20.575	123777	73.78	6083	250.000	ng/mL
5		21.375	3793	2.26	188		
6	Peak5	21.887	2140	1.28	139		

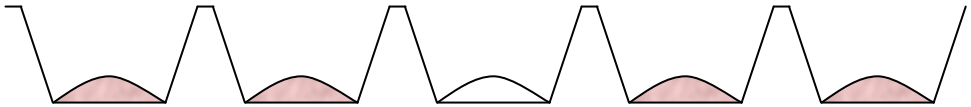
Immunosuppressant Sample Preparation

LC-MS/MS Analysis

Whole Blood
(10µL - 40µL)

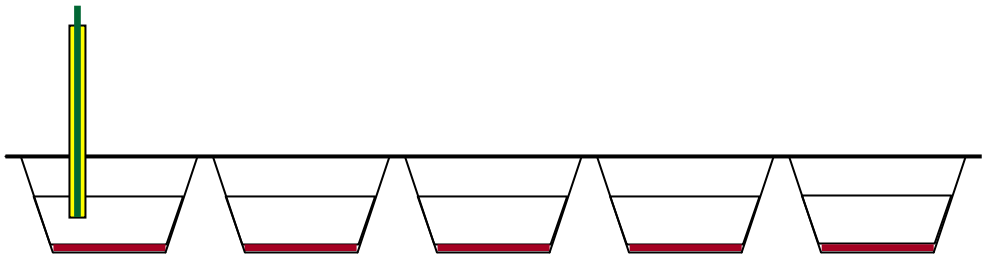


Add $ZnSO_4$ Soln.



Add 2 volumes MeCN
with IS, Seal & Vortex Mix

Centrifuge,
Inject 5 - 20µL

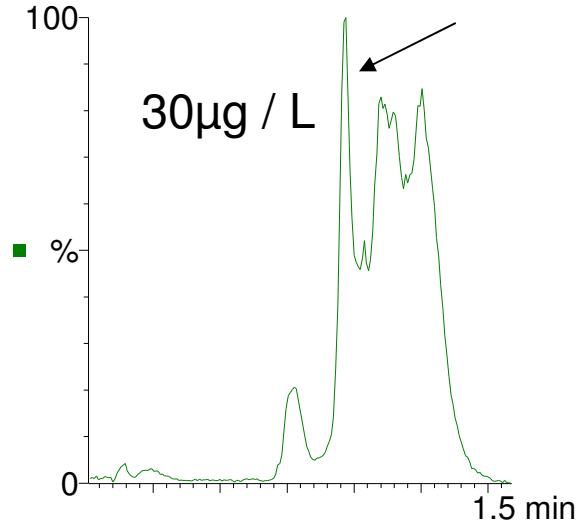


Single ion monitoring (MS)

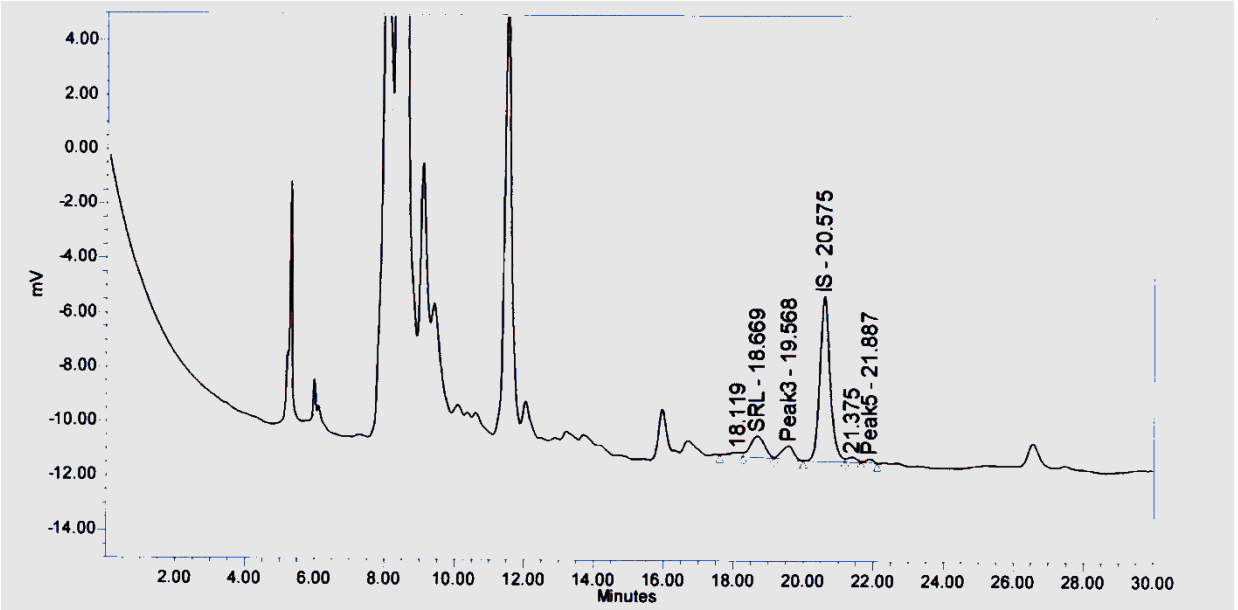
Sirolimus:
LC-MS (SIM) *vs* LC-UV

HPLC-MS

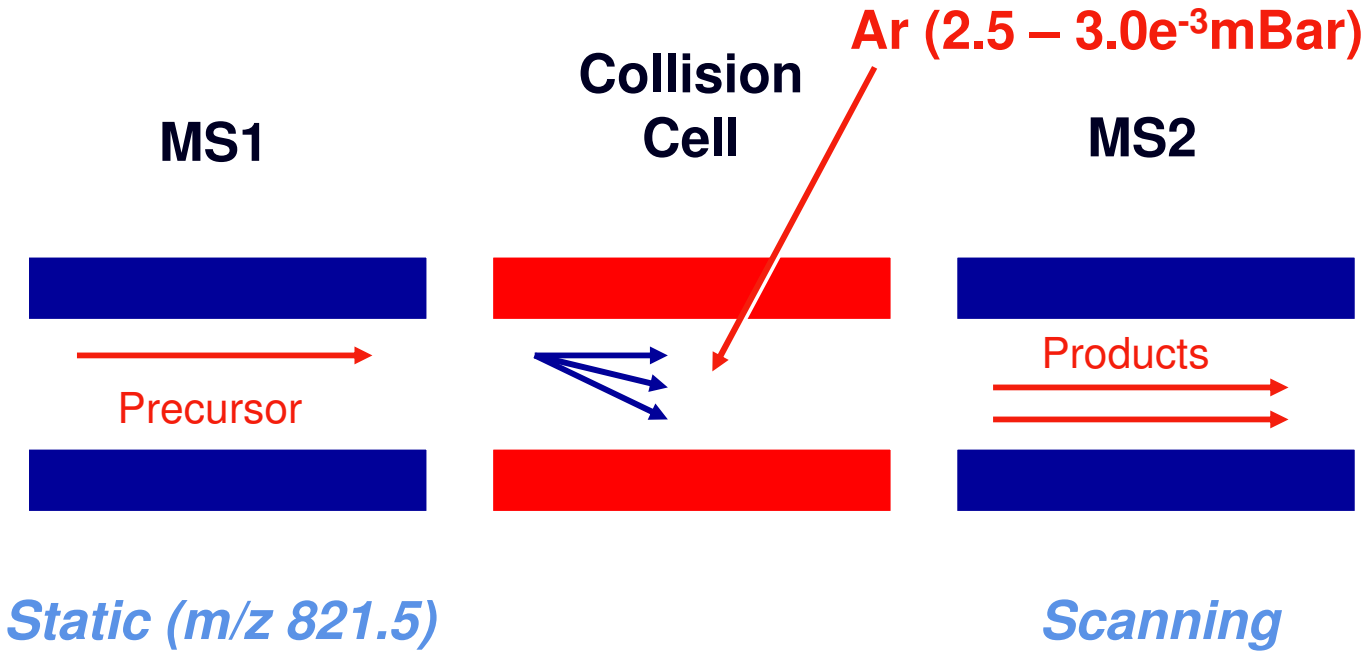
SIM *m/z* 821



HPLC-UV

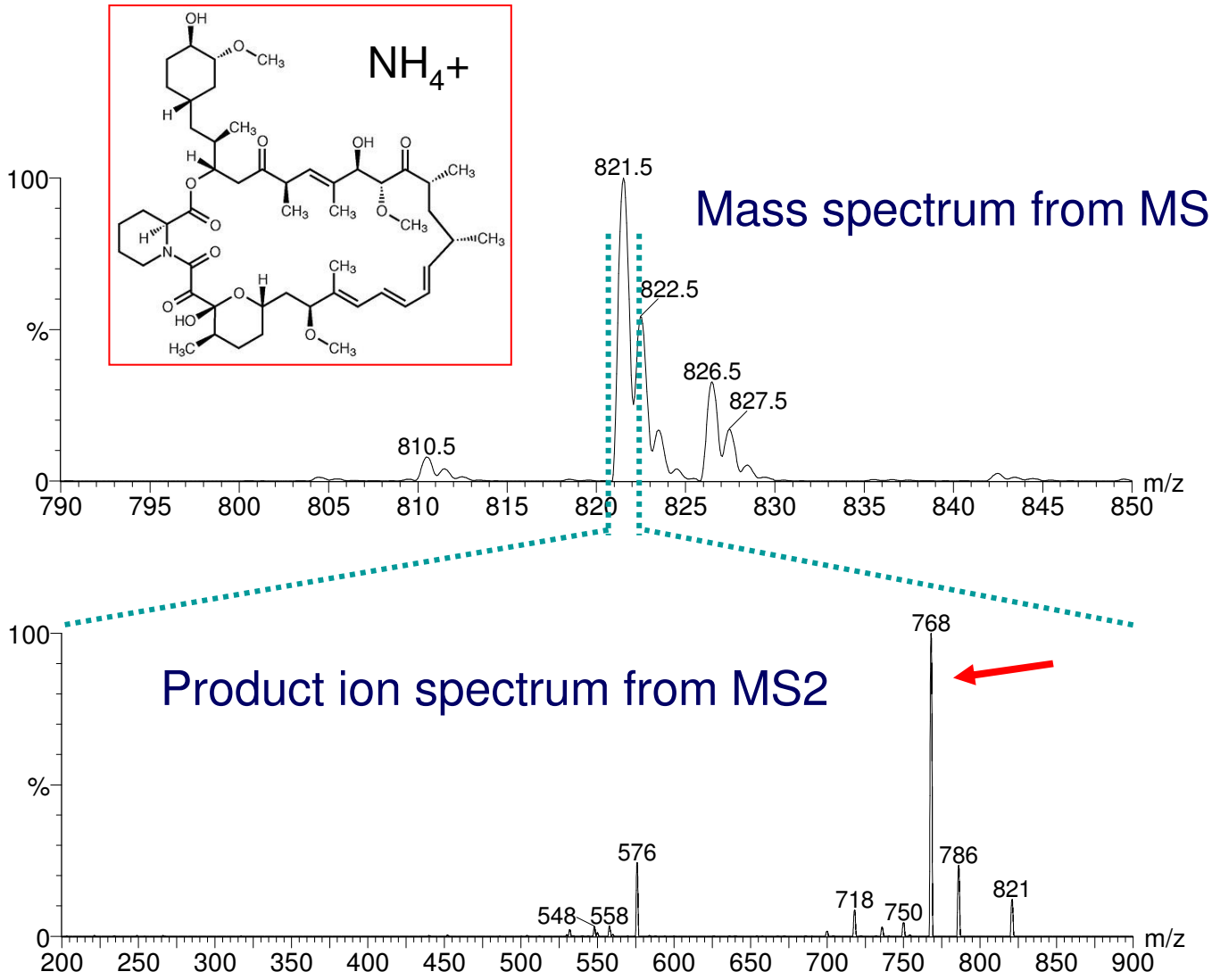


Product ion scanning

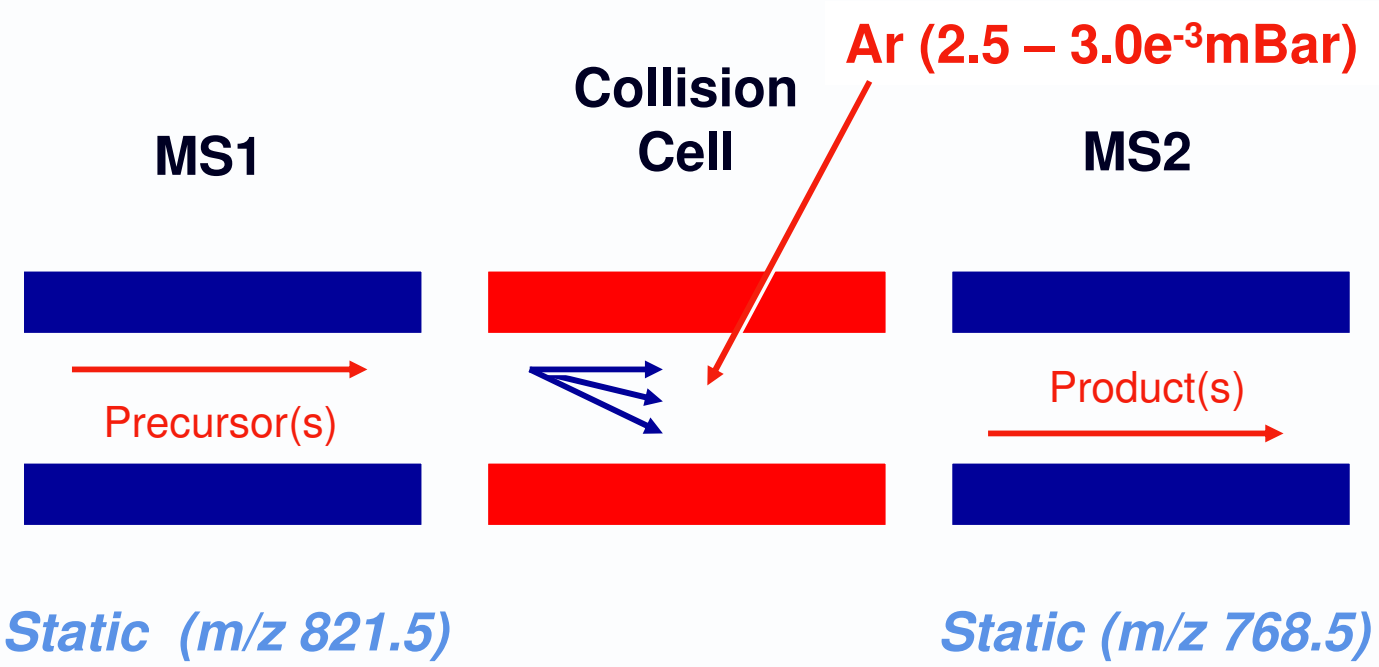


The first quadrupole mass analyzer is fixed, or ***Static***, at the mass-to-charge ratio (*m/z*) of the precursor ion to be interrogated while the second quadrupole is ***Scanning*** over a user-defined mass range.

Product ion scanning



Multiple Reaction Monitoring

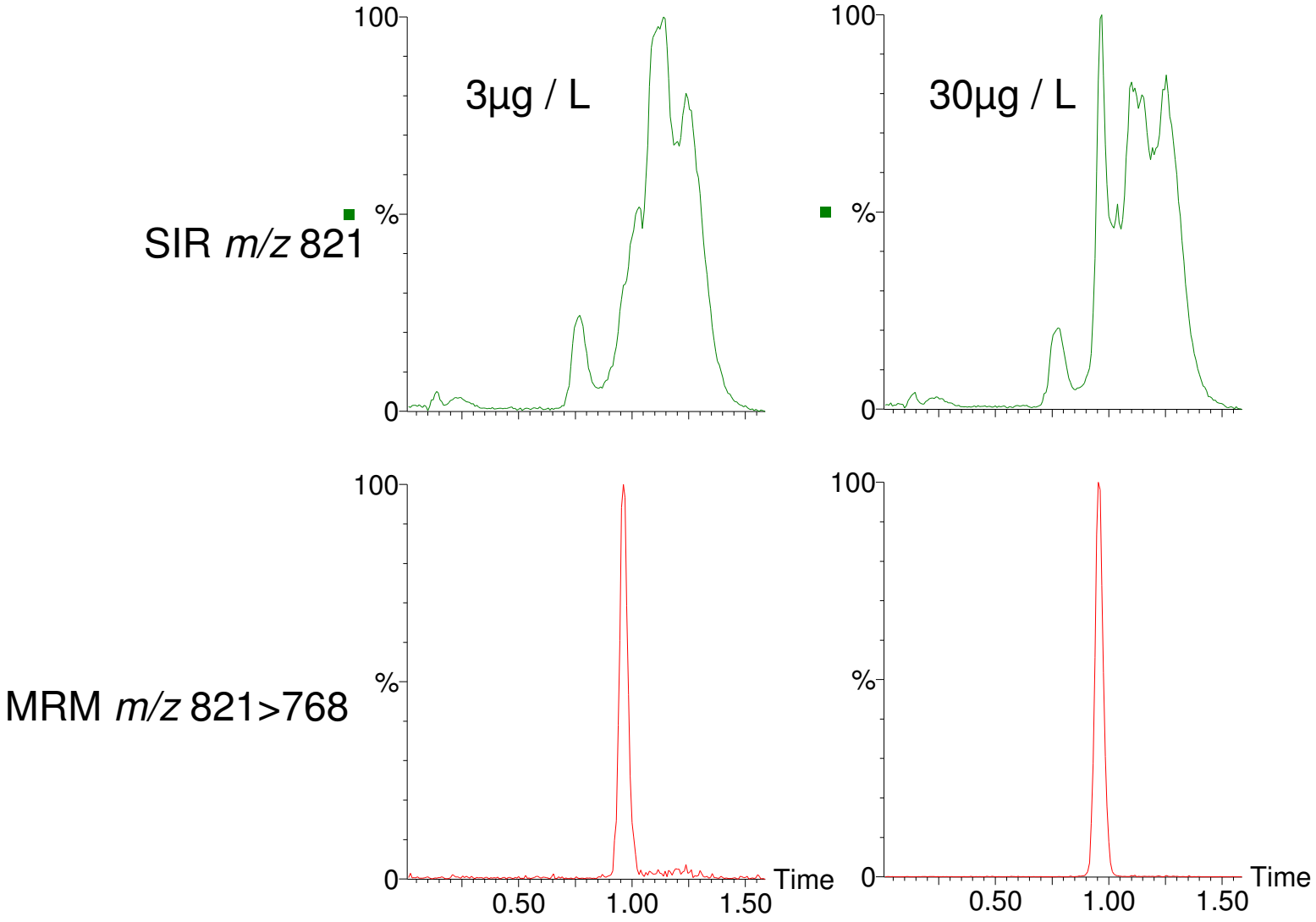


MS/MS : Compound-Specific Monitoring

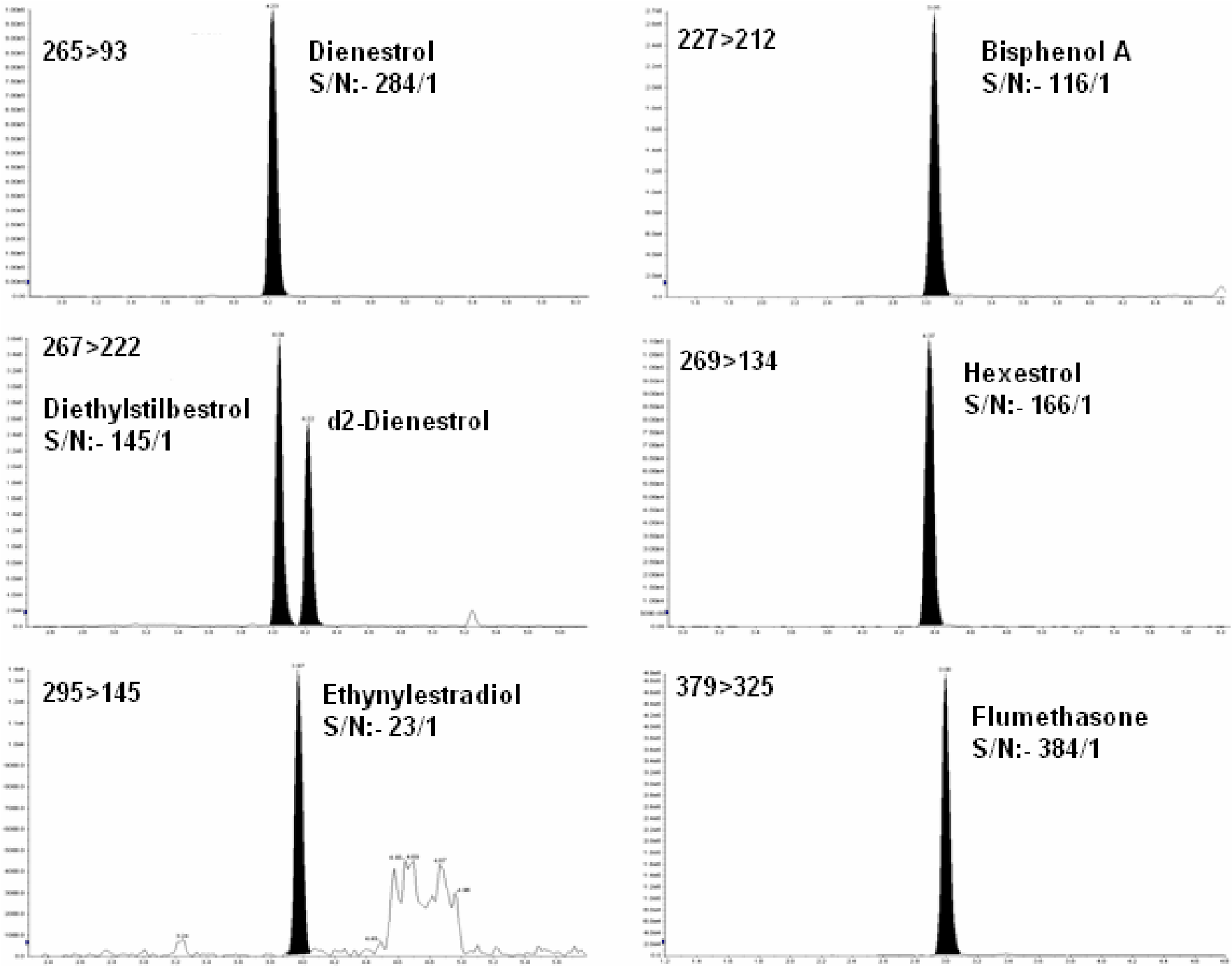
Multiple Reaction Monitoring

Sirolimus

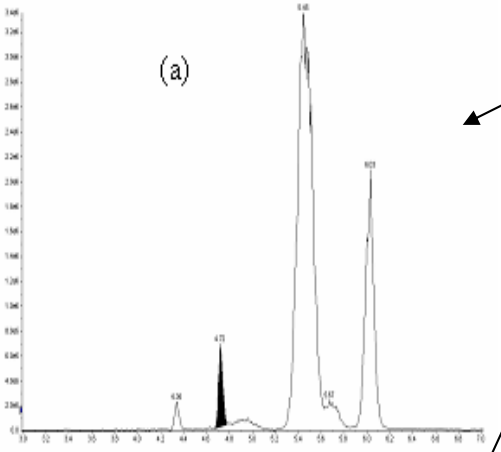
LC-MS(SIM) *vs* LC-MS/MS (MRM)



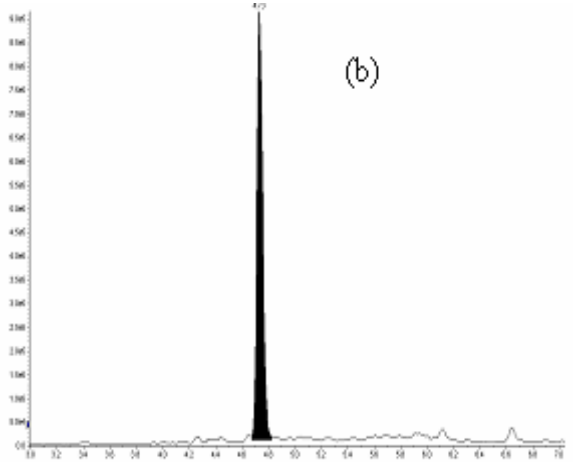
Benefit: Excellent Sensitivity; Steroids in Bovine Muscle, 1 ppb

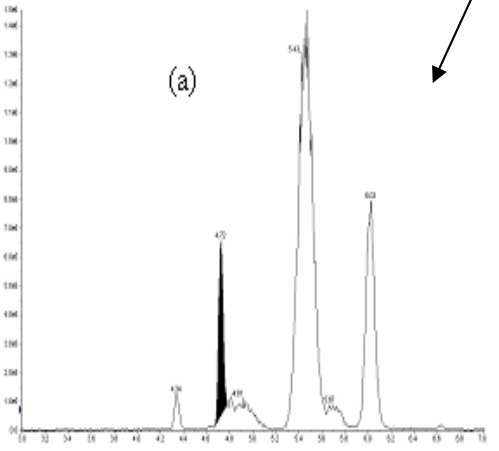

265>93
Dienestrol
S/N:- 284/1
227>212
Bisphenol A
S/N:- 116/1
267>222
Diethylstilbestrol
S/N:- 145/1
d2-Dienestrol
269>134
Hexestrol
S/N:- 166/1
295>145
Ethynylestradiol
S/N:- 23/1
379>325
Flumethasone
S/N:- 384/1

Still need for good Sample Preparation? Methyltestosterone in Milk

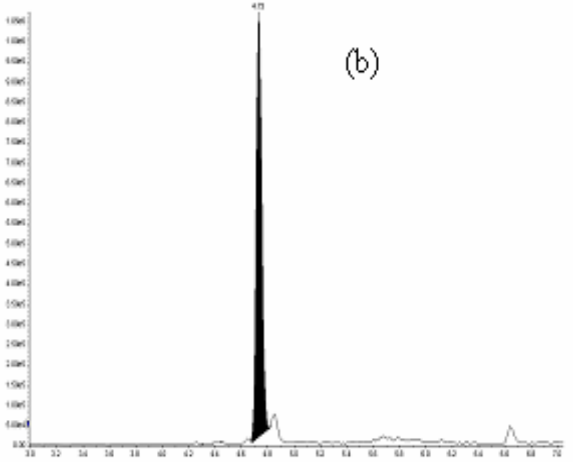


Traditional method using NaCl as only purification step





Use of additional purification by dispersive SPE using C18 sorbent material



Confirmatory Information

- In the field of Vet Residues and other fields (sports doping.)
- LC-MS/MS is seen as providing unequivocal confirmation of compound identity if certain criteria are met.
- These criteria are:
  - Relative retention time
  - Ion ratio

Relative retention Time

2.3.3. **Performance criteria and other requirements for mass spectrometric detection**

Mass spectrometric methods are suitable for consideration as confirmatory methods only following either an on-line or an off-line chromatographic separation.

2.3.3.1. *Chromatographic separation*

For GC-MS procedures, the gas chromatographic separation shall be carried out using capillary columns. For LC-MS procedures, the chromatographic separation shall be carried out using suitable LC columns. In any case, the minimum acceptable retention time for the analyte under examination is twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the test portion shall match that of the calibration standard within a specified retention time window. The retention time window shall be commensurate with the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0,5 % for GC and ± 2,5 % for LC.

	1	2	3	4	5	6
1	**RRT H0908**					
2						
3	Compound		beta-Boldenone			
4						
5	Sample ID		RT analyte	RT Int Std	RRT	Confirmed
6						
7	1 ppb Std		18.17	18.15	1.001	
8	2.5 ppb Std		18.17	18.15	1.001	
9	5 ppb Std		18.17	18.15	1.001	
10	7.5 ppb Std		18.17	18.13	1.002	
11	10 ppb Std		18.16	18.14	1.001	
12						
13	Average RRT				1.001	
14						
15	2.5% of Average RRT				0.025	
16						
17	Average RRT + 2.5%				1.026	
18	Average RRT - 2.5%				0.976	
19						
20	V08G0795		18.16	18.14	1.001	yes
21	V08G0862		18.16	18.14	1.001	yes
22						

Ion Ratio

Table 4

Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS[n] LC-MS, LC-MS[n] (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

	1	2	3	4	5	6
1	**Ion Ratio H0208pos**					
2						
3	Compound		beta Boldenone			
4						
5	Sample ID		Area Ion 1	Area Ion 2	Ion Ratio	Confirmed
6						
7	1 ppb Std		865232	432958	0.500	
8	2.5 ppb Std		2140536	1148491	0.537	
9	5 ppb Std		4544076	2252456	0.496	
10	7.5 ppb Std		7788873	3980443	0.511	
11	10 ppb Std		10373555	5204509	0.502	
12						
13	Average Ion Ratio				0.509	
14						
15	20% of Average Ion Ratio				0.102	
16						
17	Average Ion Ratio + 20%				0.611	
18	Average Ion Ratio - 20%				0.407	
19						
20	V08G0028		3262398	1523344	0.467	yes
21	V08G0022		793230	407077	0.513	yes
22	V08G0023		165698	231709	1.398	no
23	V08G0024		132261	136586	1.033	no
24	V08G0027		201919	288766	1.430	no
25						

Benefits of LC-MS/MS

- Provides confirmatory data.
- Highly sensitive technique even in complex matrices.
- Requires less stringent sample preparation methodologies.
- Allows for multi analyte analysis.
- Highly selective technique.

Benefits of LC-MS/MS QqQ

- Very good quantitative perfomance.
- Possible to detect very low levels of analyte even in complex mixtures.
- Less calibration and maintenance required than higher resolution systems.
- Allows accurate quantitation over a wide range of concentrations.
- In general cheaper to purchase than alternative high resolution systems.

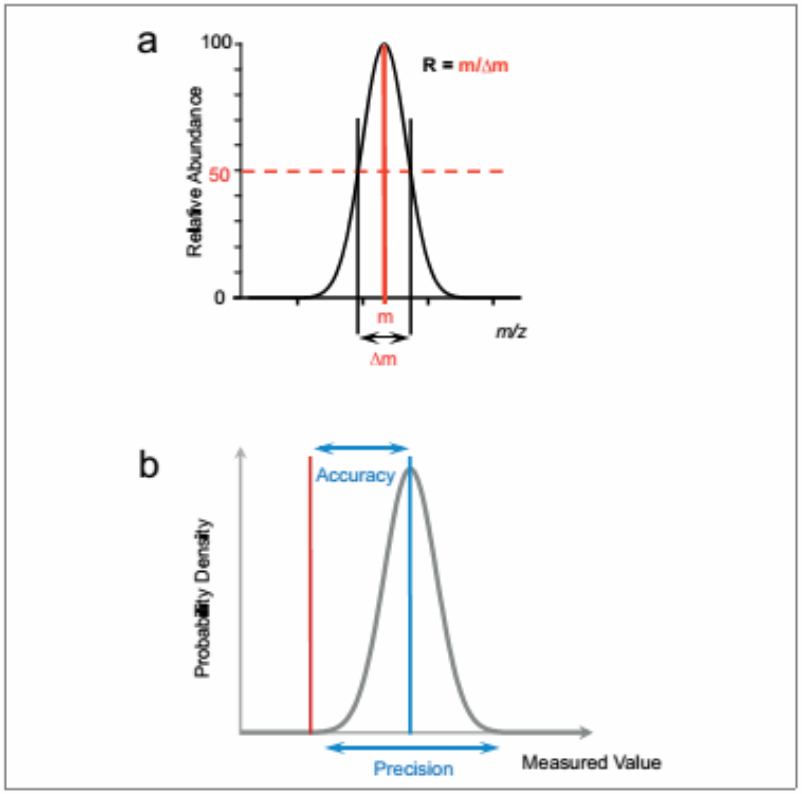
Drawbacks of LC-MS/MS QqQ

- Targeted Analysis only; you won't detect anything you have not asked the method to look for.
- Each individual transition needs to be tuned for a variety of parameters (Collision Energy, Capillary Voltage, Ion Spray Voltage). Gets quite detailed when trying to analyse for lots of compounds.
- Limit to the number of analytes it is possible to detect due to limitations of the duty cycle of the instrument.
- No possibility of retrospective analysis of data to check for presence of substances which you may have new-found suspicions about.

High Resolution and Accurate Mass LC-MS Systems

- Time of Flight and (TOF) Orbital Trapping (Orbitrap) systems offer high resolution and accurate mass capability.
- Resolution values for TOF up to 50,000 and for Orbitrap values greater than 250,000 are possible.
- Mass Accuracy is independent of resolution and values of < 2ppm are readily achievable.

Mass Resolution, Mass Accuracy and Precision



(a) Mass resolution R = m/Δm at FWHM, (b) accuracy and precision of mass determination.

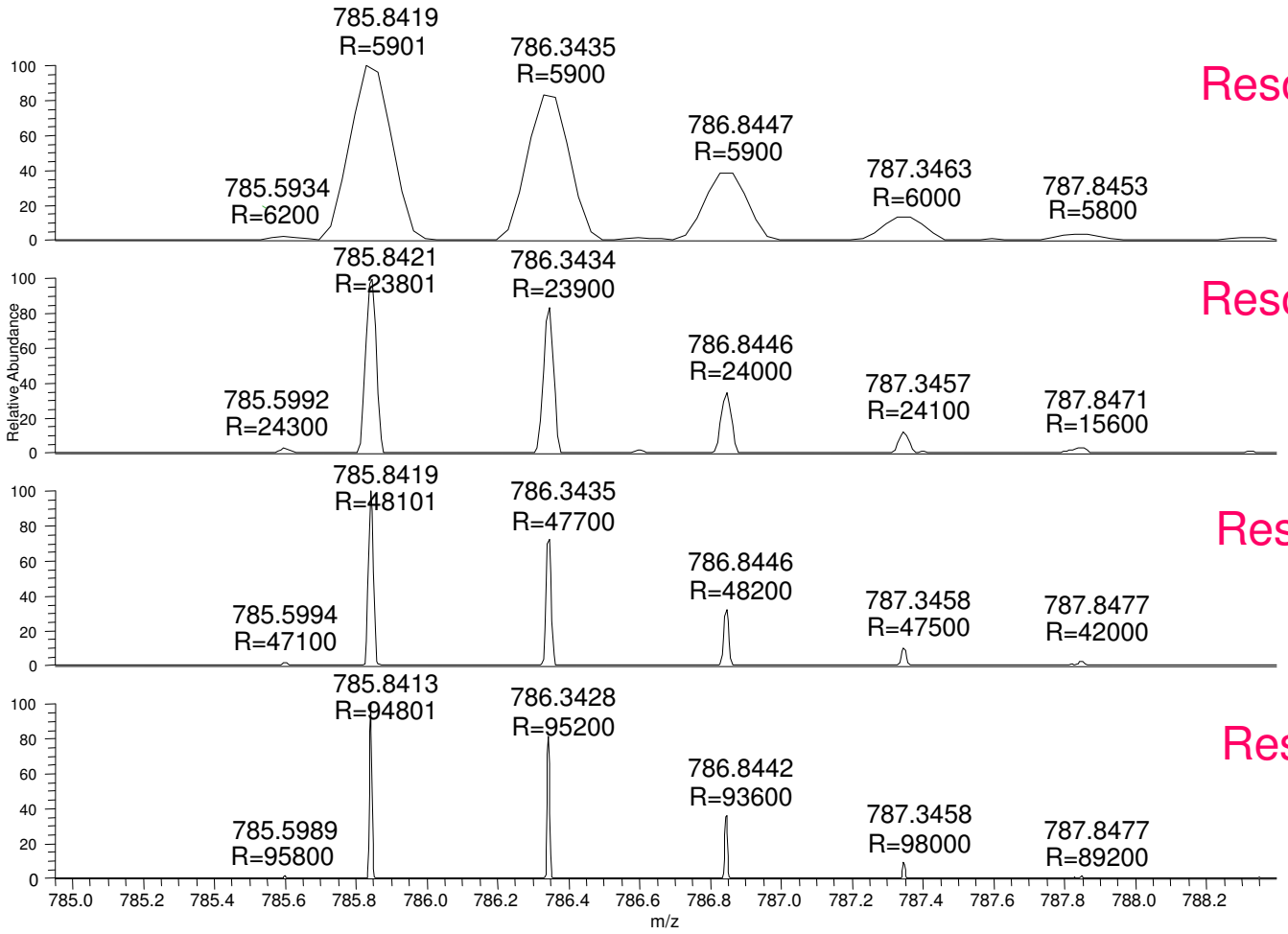
- **Resolution**: Mass resolution is typically a large number that describes the ability to distinguish between ions differing in the mass/charge (m/z) value by a small increment.
- **Mass accuracy** is the closeness of the agreement between the result of a measurement and a true value (exact mass).
- **Mass precision** is the closeness of agreement between independent mass measurement results.

Calculate Resolution and Mass Accuracy

- Mass resolution is defined as the observed m/z value divided by the smallest difference (Δ) m/z for two ions that can be separated: (m/z) / Δ (m/z).
- For Orbitrap technology an example of a peak at m/z 200.0000, with a peak width of 0.002 FWHM, the mass resolution is R = m/ Δ m = 100,000.
- For Mass Accuracy, typically reported in ppm and this is calculated as (Theoretical Mass-Measured Mass/Theoretical Mass)*(1,000,000)
- Example: Measured Mass from LC-MS: 314.22421
  Theoretical Mass of Progesterone: 314.22458

  Mass Error of 1.2 ppm

Resolving Power vs Cycle Time

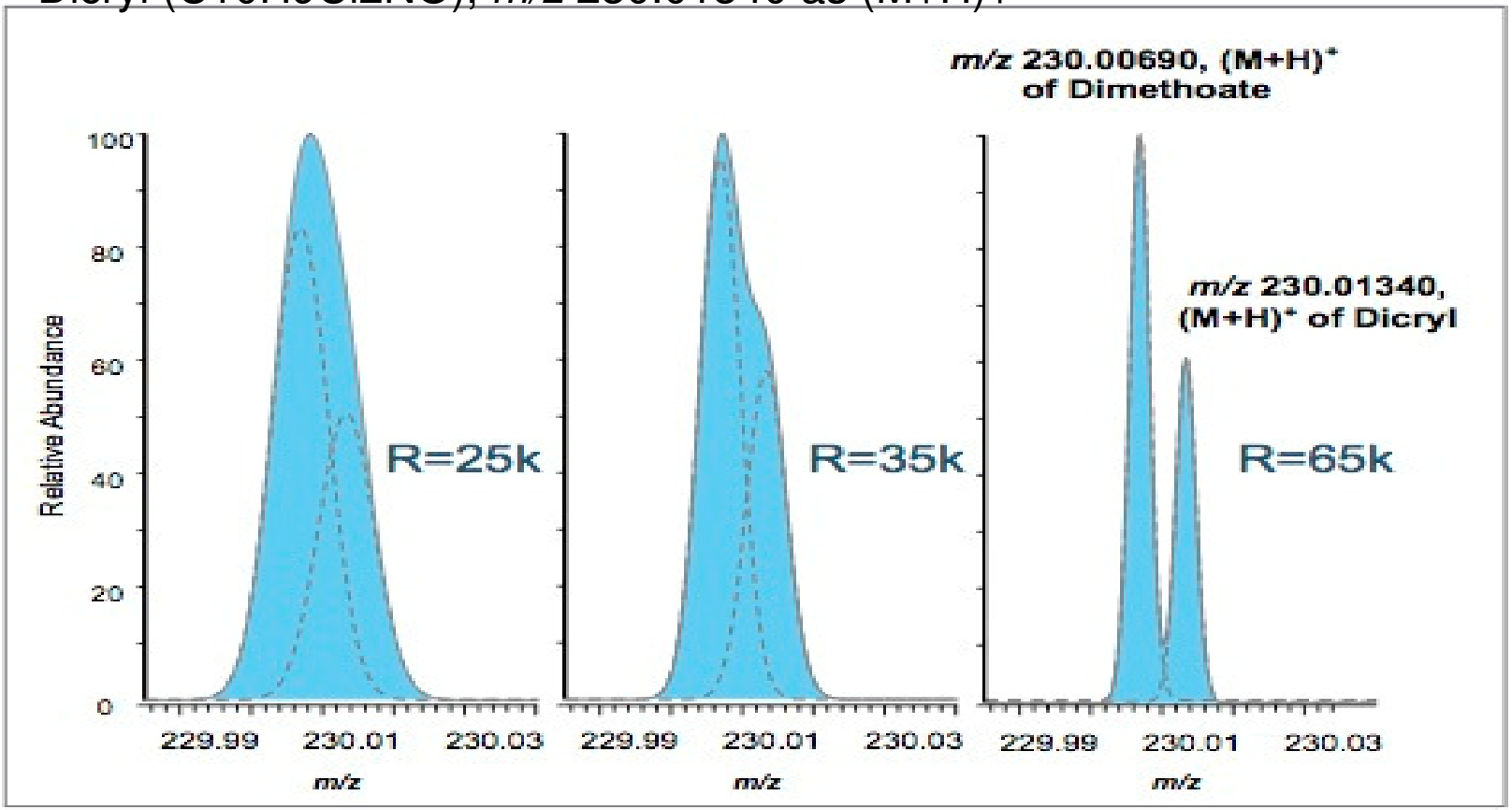


Benefit of Resolution

- Resolves analytes of interest from each other
- Two Pesticides:

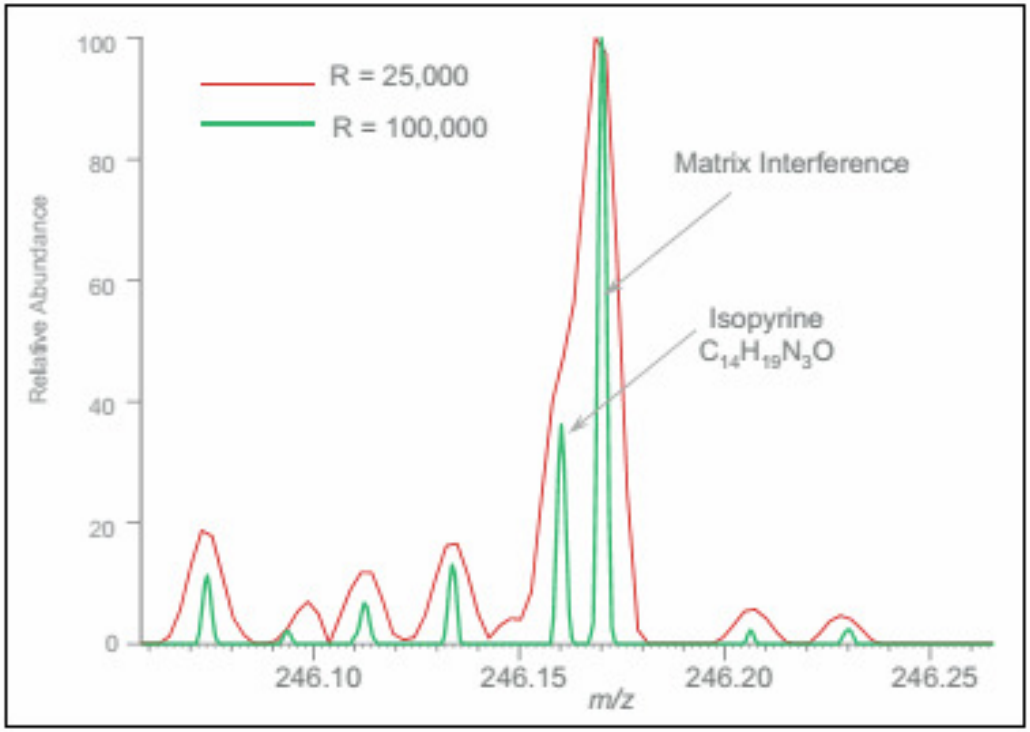
  Dimethoate ($C_5H_{12}NO_3PS_2$), *m/z* 230.00690 as $(M+H)_+$

  Dicryl (C10H9Cl2NO), *m/z* 230.01340 as (M+H)+



Benefit of Resolution

- Resolves analytes of interest from matrix components



Mass resolution of R = 25,000 at *m/z* 200 masks the pesticide isopyrine due to a non-resolved matrix interference (red); whereas, resolution setting of R = 100,000 at *m/z* 200 resolves the analyte molecule from the matrix background.

Isotopes

+Most elements have more than one stable isotope.

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

+Why do we care?

Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

Z	Name	Symbol	Mass of Atom (u)	% Abundance
1	Hydrogen	^{1}H	1.007825	99.9885
	Deuterium	^{2}H	2.014102	0.0115
	Tritium	^{3}H	3.016049	*
2	Helium	^{3}He	3.016029	0.000137
		^{4}He	4.002603	99.999863
3	Lithium	^{6}Li	6.015122	7.59
		^{7}Li	7.016004	92.41
4	Beryllium	^{9}Be	9.012182	100
5	Boron	^{10}B	10.012937	19.9
		^{11}B	11.009305	80.1
6	Carbon	^{12}C	12.000000	98.93
		^{13}C	13.003355	1.07
		^{14}C	14.003242	*
7	Nitrogen	^{14}N	14.003074	99.632
		^{15}N	15.000109	0.368
8	Oxygen	^{16}O	15.994915	99.757
		^{17}O	16.999132	0.038
		^{18}O	17.999160	0.205
9	Fluorine	^{19}F	18.998403	100
10	Neon	^{20}Ne	19.992440	90.48
		^{21}Ne	20.993847	0.27
		^{22}Ne	21.991386	9.25
11	Sodium	^{23}Na	22.989770	100
12	Magnesium	^{24}Mg	23.985042	78.99
		^{25}Mg	24.985837	10.00
		^{26}Mg	25.982593	11.01
13	Aluminum	^{27}Al	26.981538	100
14	Silicon	^{28}Si	27.976927	92.2297
		^{29}Si	28.976495	4.6832
		^{30}Si	29.973770	3.0872
15	Phosphorus	^{31}P	30.973762	100
16	Sulphur	^{32}S	31.972071	94.93
		^{33}S	32.971458	0.76
		^{34}S	33.967867	4.29
		^{36}S	35.967081	0.02
17	Chlorine	^{35}Cl	34.968853	75.78
		^{37}Cl	36.965903	24.22
18	Argon	^{36}Ar	35.967546	0.3365
		^{38}Ar	37.962732	0.0632
		^{40}Ar	39.962383	99.6003
19	Potassium	^{39}K	38.963707	93.2581
		^{40}K	39.963999	0.0117
		^{41}K	40.961826	6.7302
20	Calcium	^{40}Ca	39.962591	96.941
		^{42}Ca	41.958618	0.647
		^{43}Ca	42.958767	0.135
		^{44}Ca	43.955481	2.086
		^{46}Ca	45.953693	0.004
		^{48}Ca	47.952534	0.187
21	Scandium	^{45}Sc	44.955910	100
22	Titanium	^{46}Ti	45.952629	8.25
		^{47}Ti	46.951764	7.44
		^{48}Ti	47.947947	73.72
		^{49}Ti	48.947871	5.41
		^{50}Ti	49.944792	5.18
23	Vanadium	^{50}V	49.947163	0.250
		^{51}V	50.943964	99.750
24	Chromium	^{50}Cr	49.946050	4.345
		^{52}Cr	51.940512	83.789
		^{53}Cr	52.940654	9.501
		^{54}Cr	53.938885	2.365
25	Manganese	^{55}Mn	54.938050	100
26	Iron	^{54}Fe	53.939615	5.845
		^{56}Fe	55.934942	91.754

Accurate Mass and Elemental Composition

- Ability to directly determine the identity of the elemental composition of an analyte.
- The accurate determination of the monoisotopic mass peak restricts the pool of possible elemental composition combinations significantly.
- High mass resolution in combination with accurate-mass measurements enable the determination of isotopic patterns.
- From this information it is possible to produce a list of possible chemical formula.
- A database search (such as on ChemSpider), easily reveals the identity of the compound.

Benefit of Accurate Mass

- How much Mass Accuracy is needed for identification of molecular formula.
- For example Reserpine (C33H40N2O9) has a protonated ion at m/z 609.28066.
- Single quadrupole MS reports mass to +/- 0.1 = 165 ppm
- Number of possible formulas using only C, H, O & N, at various mass errors:
- • 165 ppm 209, • 10 ppm 13, • 5ppm 7, • 3ppm 4, • 2ppm 2
- 209 (single quad resolution); 2 possibilities accurate mass systems.
- Accurate mass limits the number of possible formulae for a given m/z measurement in this case to two possible formulas.

Benefits of High Resolution LC-MS

- Ability to detect and determine unknown substances.
- Not necessary to tune for each analyte singly but can use generic conditions specified over a mass range of interest (100-600 m/z).
- Technically no limit to the number of substances you can detect within a given mass range.
- Possible to carry out retrospective analysis of data to look for substances that you may now suspect could be present.
- Can be a very specific technique.

Drawbacks of High Resolution LC-MS

- Traditionally not as good at quantitation as triple quadrupole systems.
- More time required to calibrate systems and calibration needs to be carried out more regularly.
- Very large data files produced which can prove difficult to store.
- Requires higher user knowledge to trawl through the huge amount of data produced and requires a fast PC.
- Typically not as sensitive a technique as triple quadrupole analysis.

Thanks
and
Any Questions