Teacher: Marian Twohig

Waters

Quattro micro Training Course

MICROMASS
MS TECHNOLOGIES

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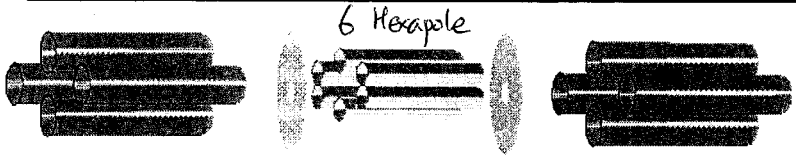
Quattro micro Overview

MICROMASS
MS TECHNOLOGIES

Quattro micro™ Course Outline

	Morning	Afternoon
Monday	Introduction: QM Overview, API & Quad Theory, MS Acquisition modes.	Instrument Tuning: Tune page settings & acquisitions
Tuesday	MassLynx Overview: File structure and the Editors	MassLynx: Chromatograms, spectra & SIR/MRM. LC/MS basics
Wednesday	Mass Calibration: Software and calibrate our Quattro micro™.	Hardware Maintenance: Routine Maintenance
Thursday	Quantification: Software	Quantification: Practical example Course Evaluation

Triple Quadrupole Instruments

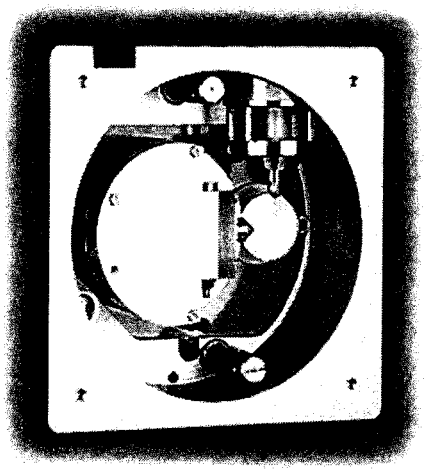


MS1 Collision Cell MS2

In a triple quadrupole or tandem mass spectrometer, MS1 and MS2 are mass analyzers that filter ions.

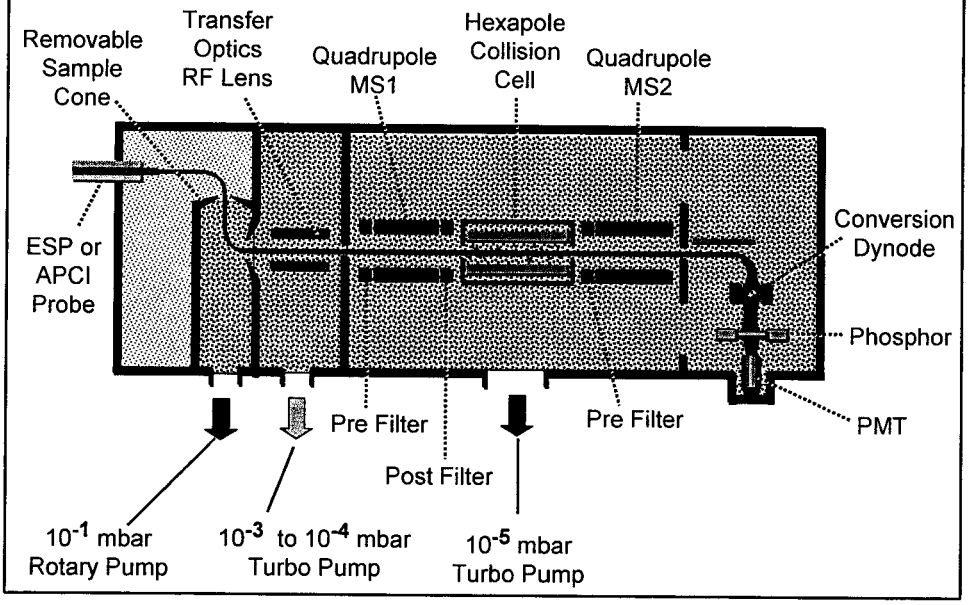
The Collision Cell is filled with Argon and potential is applied to fragment ions.

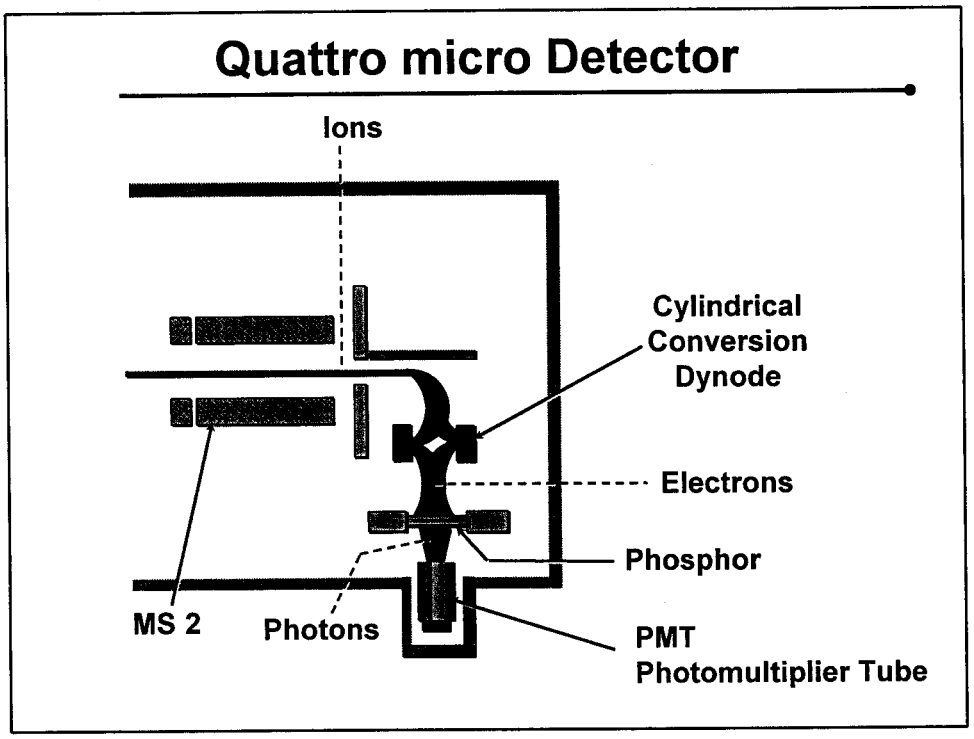
Quattro micro Z-Spray™ Ion Source

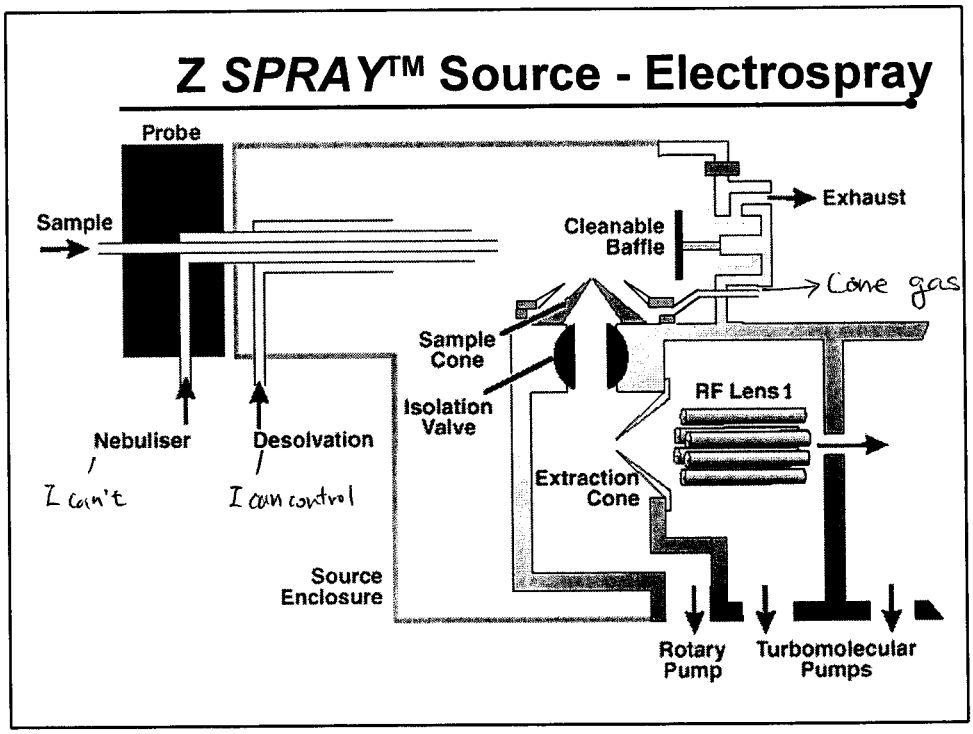


daily maintanance : cone.

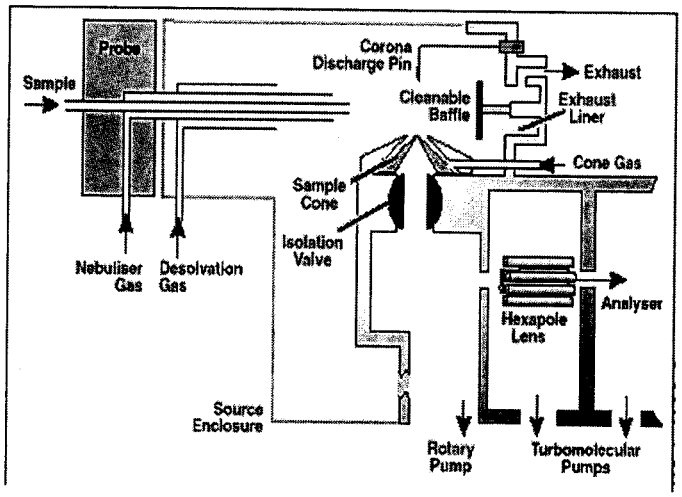
Schematic Overview of the Quattro micro




Quattro micro Detector
Ions
Cylindrical
Conversion
Dynode
Electrons
Phosphor
MS 2
Photons
PMT
Photomultiplier Tube


Z SPRAY™ Source - Electrospray
Probe
Sample
Exhaust
Cleanable
Baffle
Cone gas (on/off)
depense of solvent
Sample
Cone
Isolation
Valve
RF Lens 1
Nebuliser
Desolvation
I can't
I can control
Extraction
Cone
Source
Enclosure
Rotary
Pump
Turbomolecular
Pumps

APcI Source



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Quadrupole Theory

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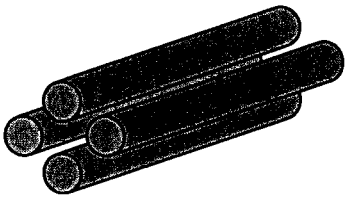
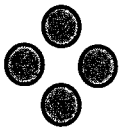
Quadrupole Theory

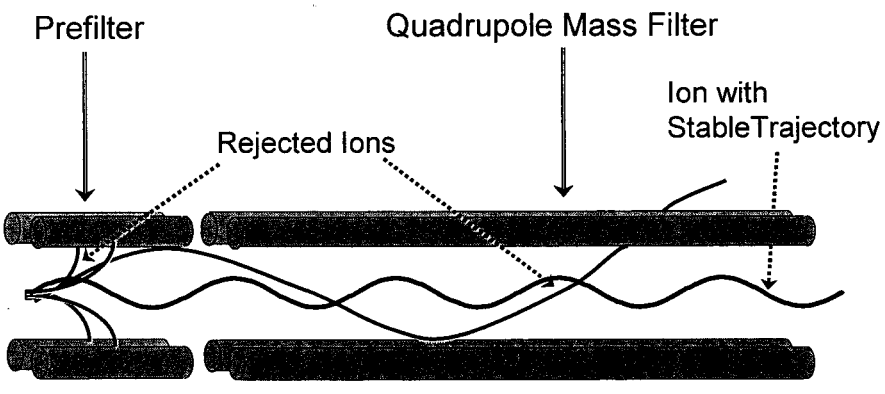
- A quadrupole is an assembly of four parallel rods arranged equidistantly from a central axis.
- Through the application of RF and DC voltages, ions can be filtered along the central axis and their mass measured to yield a mass spectrum.
- Depending upon the exact potential applied to the quadrupole, ions with masses too large or too small will not pass through the quadrupole. These ions will strike the rods and be lost.



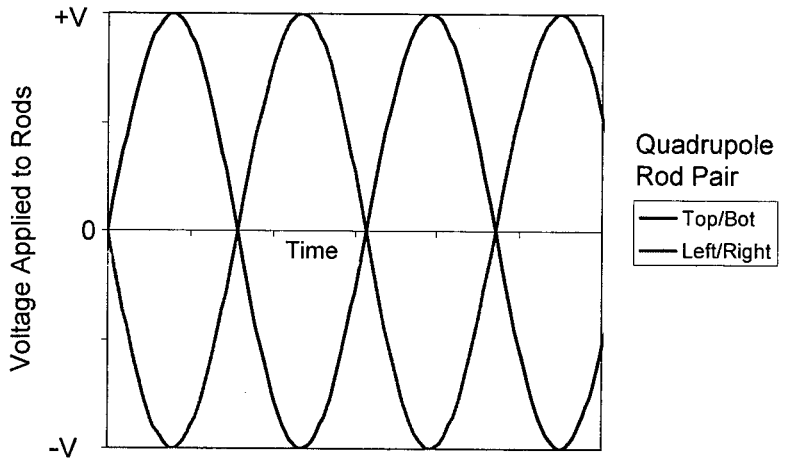


End View

Quadrupole Analyzer



RF Voltage Applied to Top/Bottom or Left/Right Pairs of Quadrupole Rods



Applied Potential to Quadrupole Rods

The voltage applied to an opposing pair of rods is given by:

$$\phi = U - V \cos \omega T$$

DC Voltage — RF Voltage

The voltage applied to the other pair of opposing rods is:

$$\phi = -U + V \cos \omega T$$

Typically: DC Voltages (U) are in the range of 1000 V
RF Voltages (V) range from 1000 to 6000 V
RF frequencies (ω) are around 1 mHz

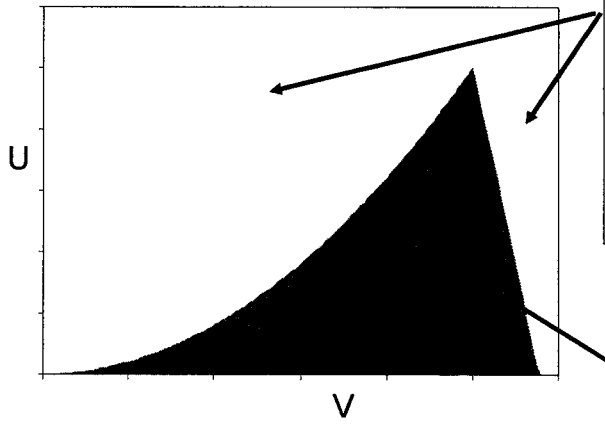
Applied Potential to Quadrupole Rods

Ions are pushed through the quadrupole by using an applied potential difference between the entrance and exit to quadrupole assembly.

The trajectories of ions as they pass through the quadrupole assembly can be calculated.

This calculation is very complicated so a qualitative description of the calculation will be given.

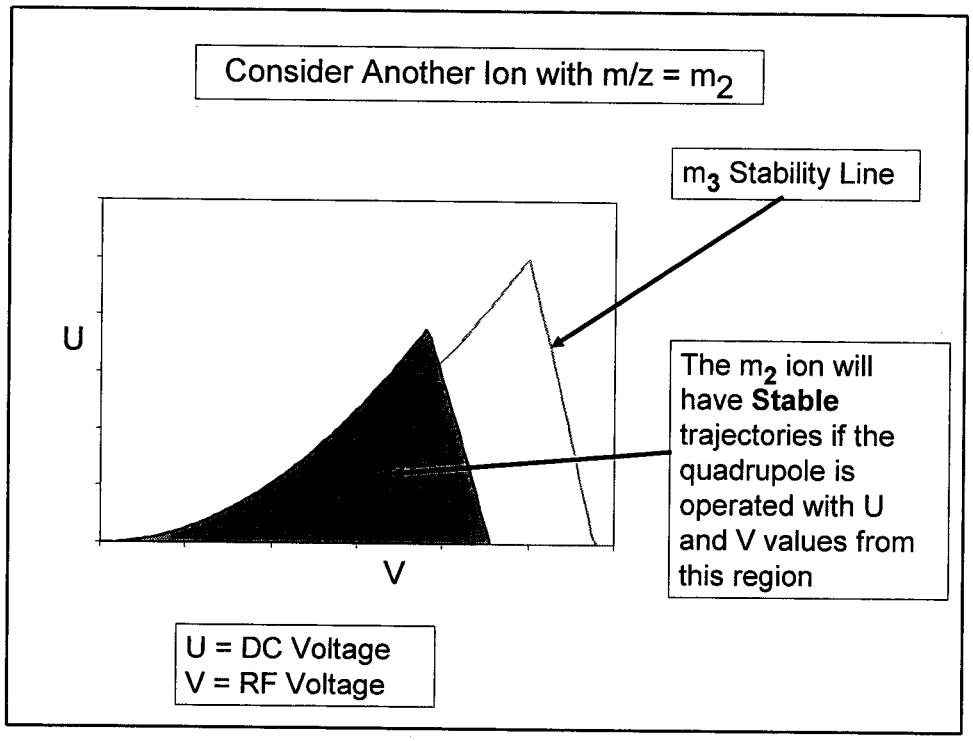
Quadrupole Theory: Settings for DC and RF Voltages When a Quadrupole is Used as a Mass Filter.
For an Ion with m/z = m_3

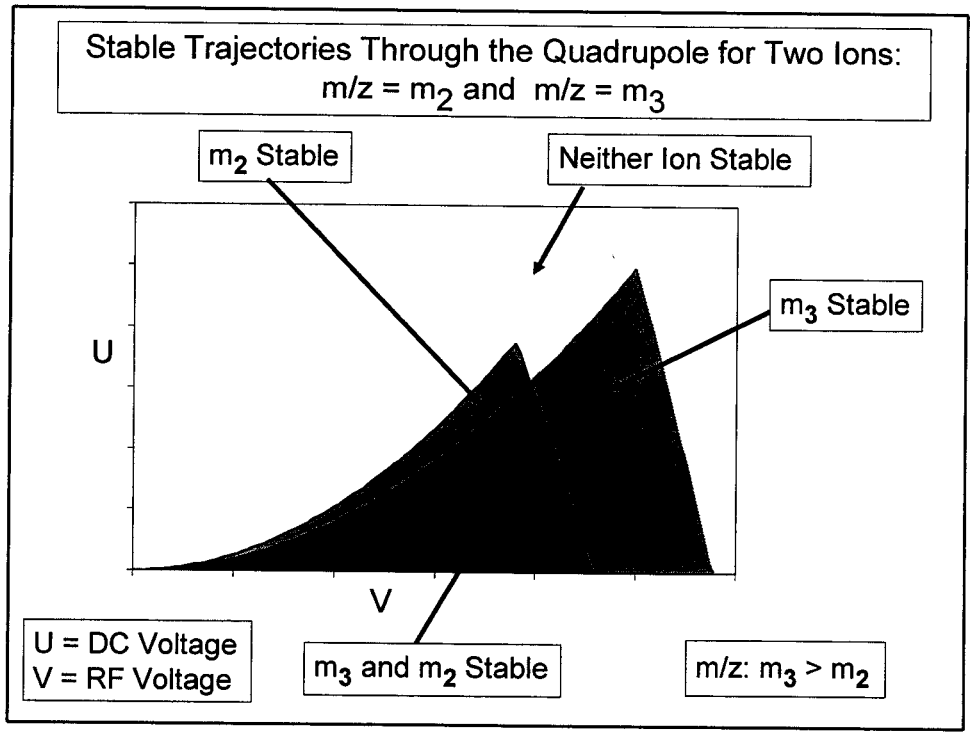


The m_3 ion will have **Unstable** trajectories if the quadrupole is operated with U and V values from this region

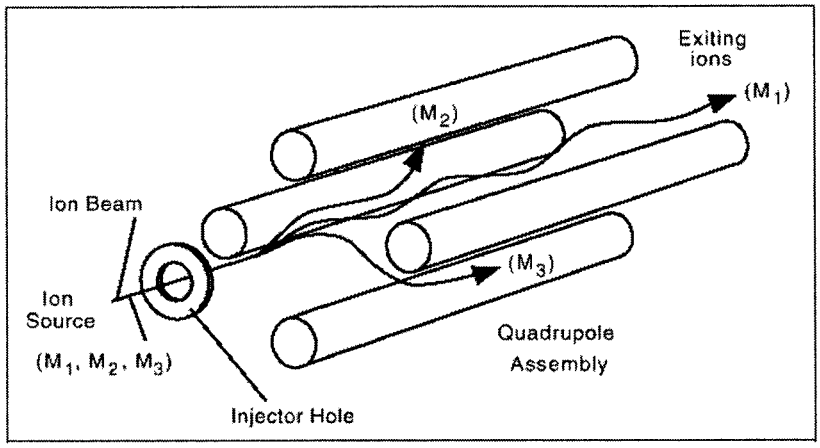
The m_3 ion will have **Stable** trajectories if the quadrupole is operated with U and V values from this region

U = DC Voltage
V = RF Voltage


Consider Another Ion with m/z = m_2
m_3 Stability Line
U
V
The m_2 ion will have **Stable** trajectories if the quadrupole is operated with U and V values from this region
U = DC Voltage
V = RF Voltage

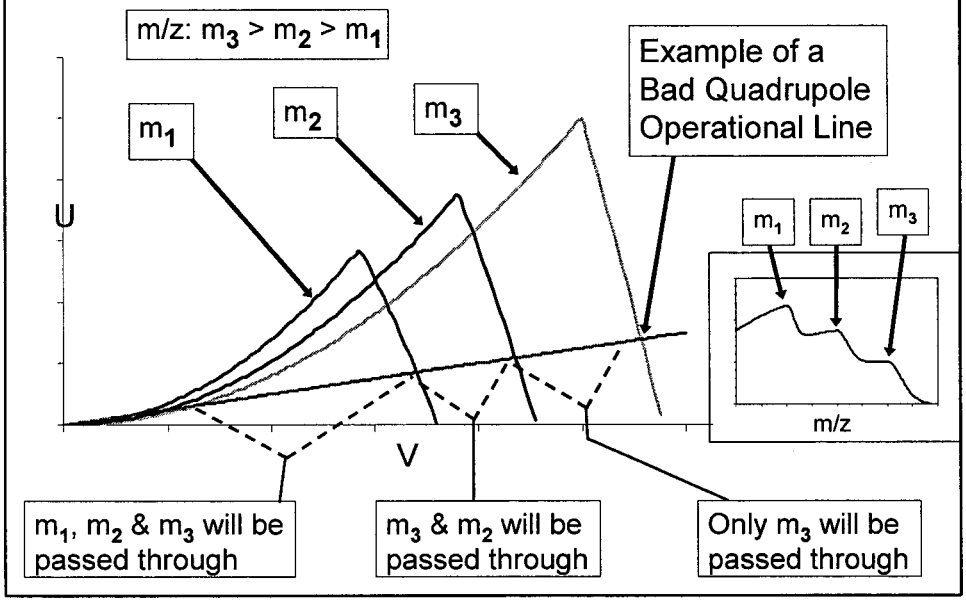

Stable Trajectories Through the Quadrupole for Two Ions:
m/z = m_2 and m/z = m_3
m_2 Stable
Neither Ion Stable
m_3 Stable
U
V
U = DC Voltage
V = RF Voltage
m_3 and m_2 Stable
m/z: m_3 > m_2

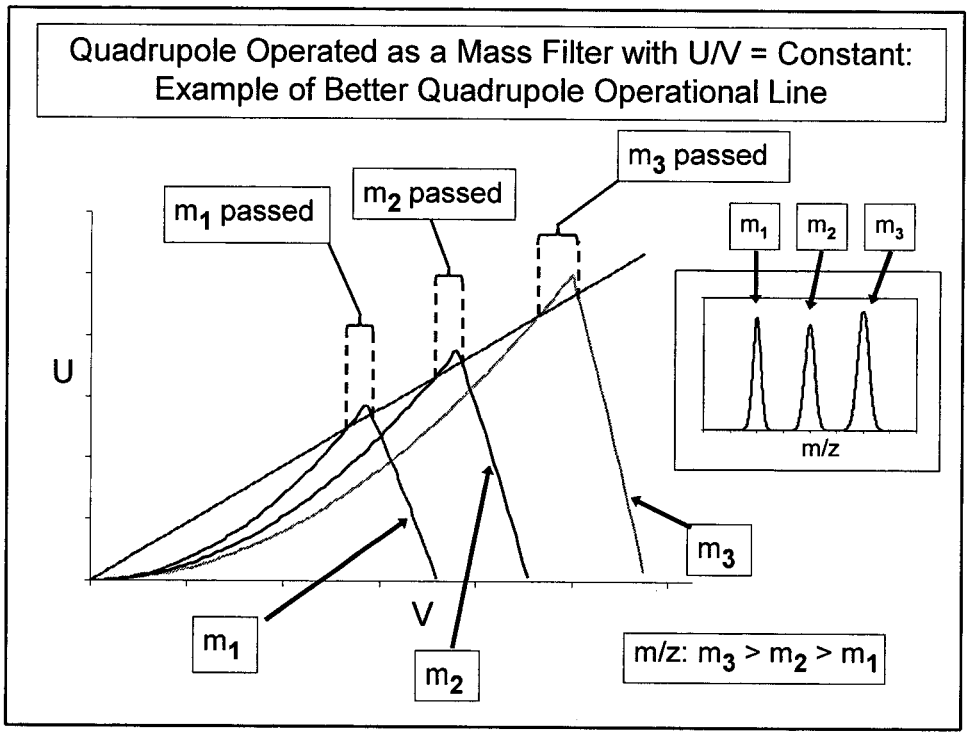
$M_1, M_2, M_3 = 1$ Operational line.

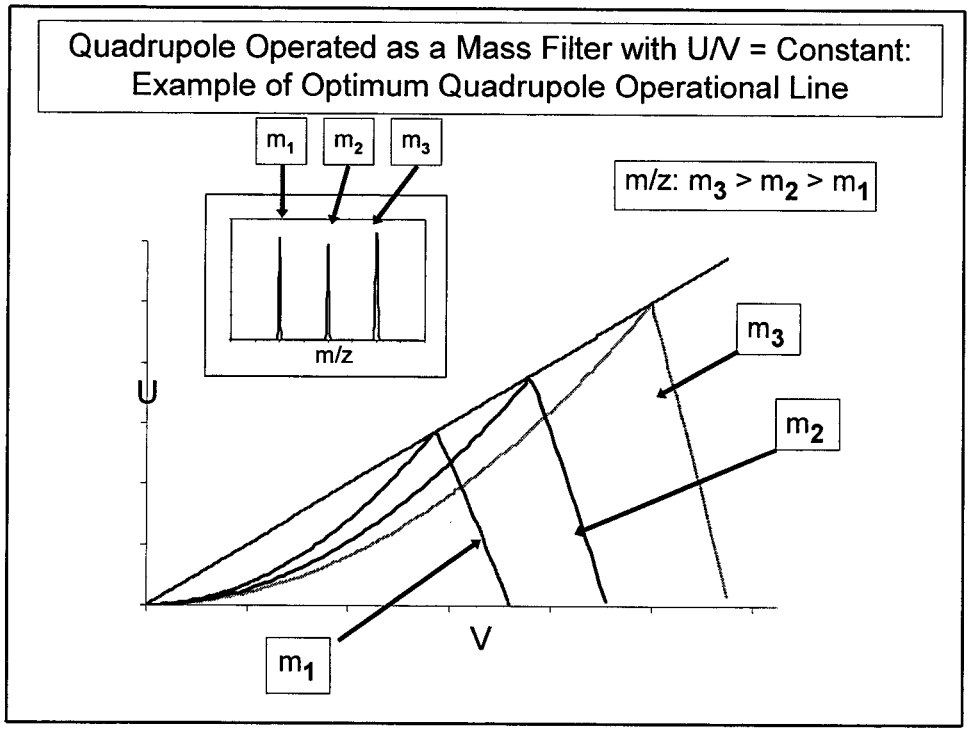


Quadrople setup so for three ions passing through the quadrupole, m_1 has a stable trajectory and m_2 & m_3 have unstable trajectories.

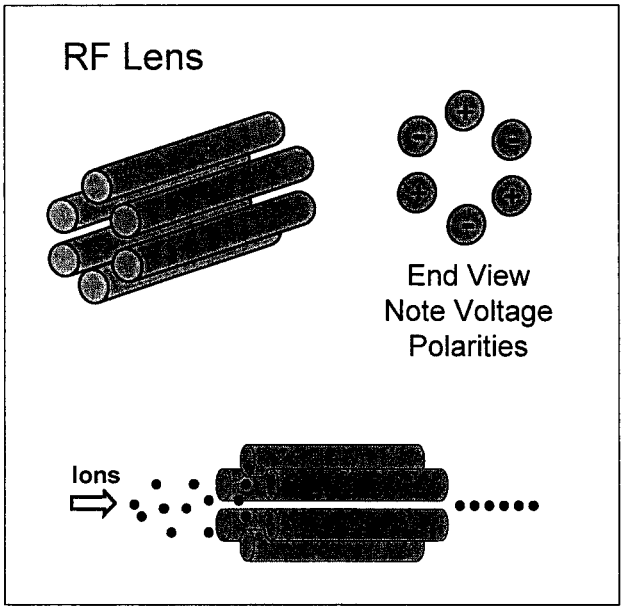
When the a Quadrupole is Operated as a Mass Filter:
U and V are Varied Linerarly (Ratio of U/V is a Constant)




Quadrupole Operated as a Mass Filter with U/V = Constant:
Example of Better Quadrupole Operational Line
m3 passed
m2 passed
m1 passed
m1
m2
m3
m/z
U
V
m1
m2
m3
m/z: m3 > m2 > m1


Quadrupole Operated as a Mass Filter with U/V = Constant:
Example of Optimum Quadrupole Operational Line
m1
m2
m3
m/z
m/z: m3 > m2 > m1
m3
m2
U
V
m1

RF Lens - Hexapole



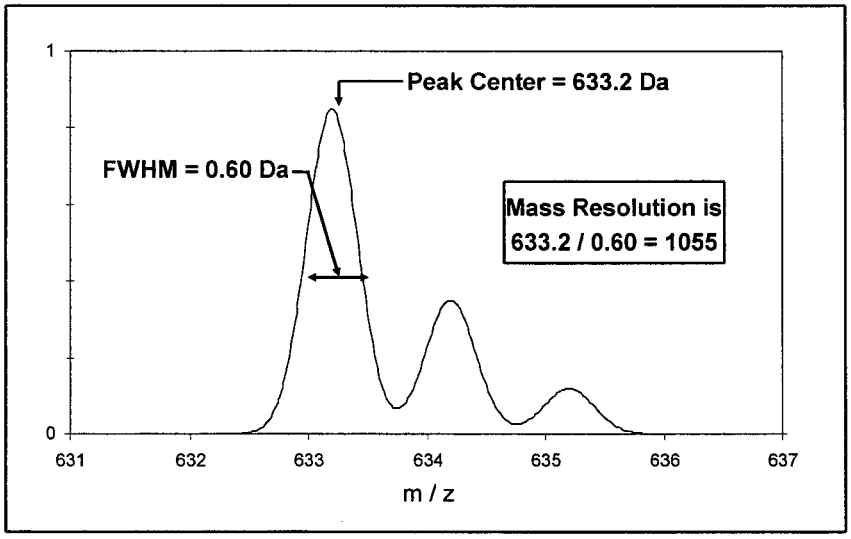
Hexapole Assembly

Radio frequency plus a small bias voltage transports all masses.

Designed to insure ion focussing in a relatively poor vacuum.

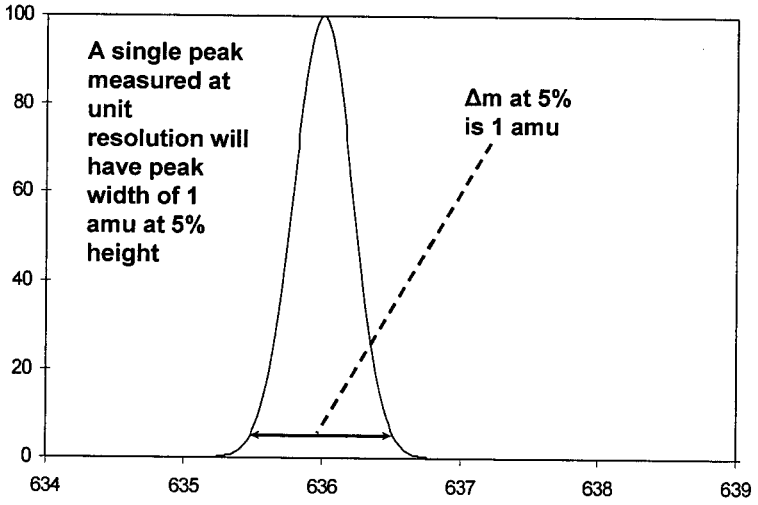
Delivers the ions in a tightly focussed beam to the quadrupole where they can be analyzed.

Mass Resolution is defined as m / Δm



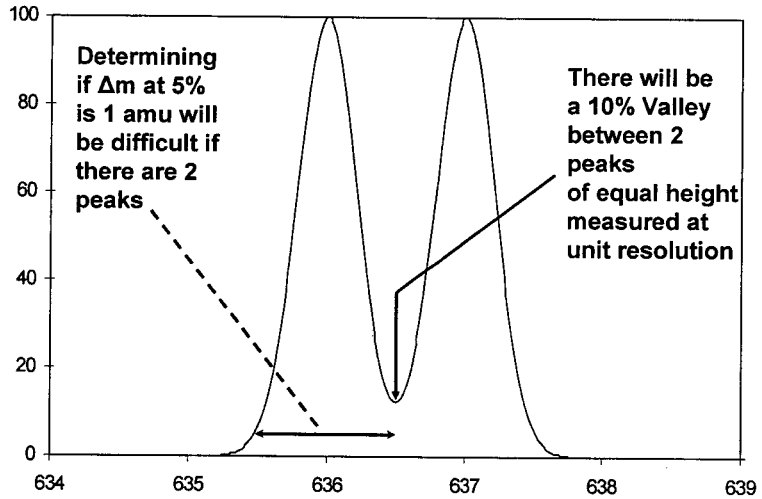
Unit Resolution

If you have a single peak that has Unit Resolution:



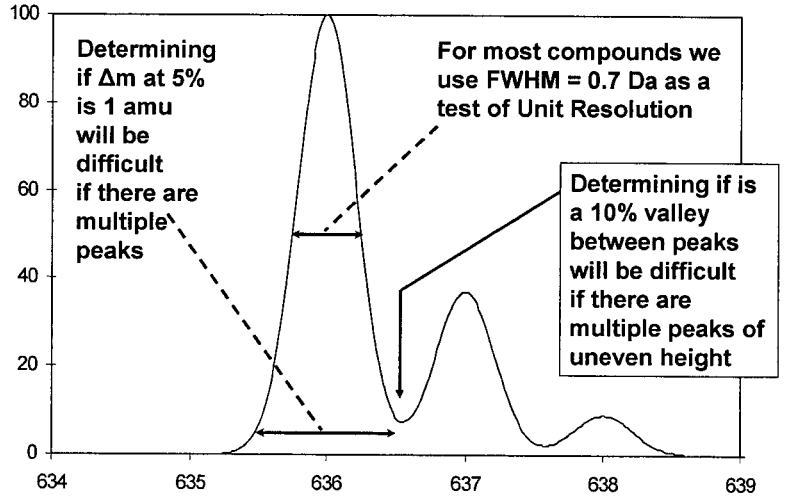
Unit Resolution

~~If you do not have a single peak, but have 2 peaks of equal height~~ at Unit Resolution:



Unit Resolution

For most compounds, you do not get a single peak or 2 peaks of equal height.



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Atmospheric Pressure Ionization (API)

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Atmospheric Pressure Ionization (API)

Electrospray -

Liquid is sprayed out of a capillary tube to which a high voltage is applied to form a spray of charged droplets.

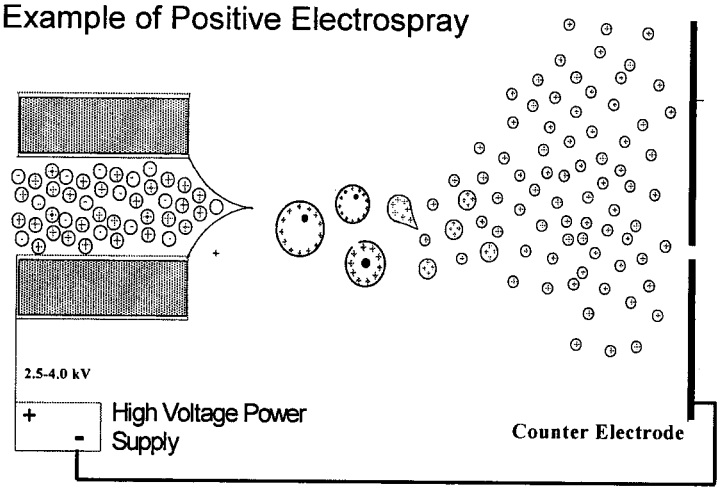
Atmospheric Pressure Chemical Ionization (APcI) -

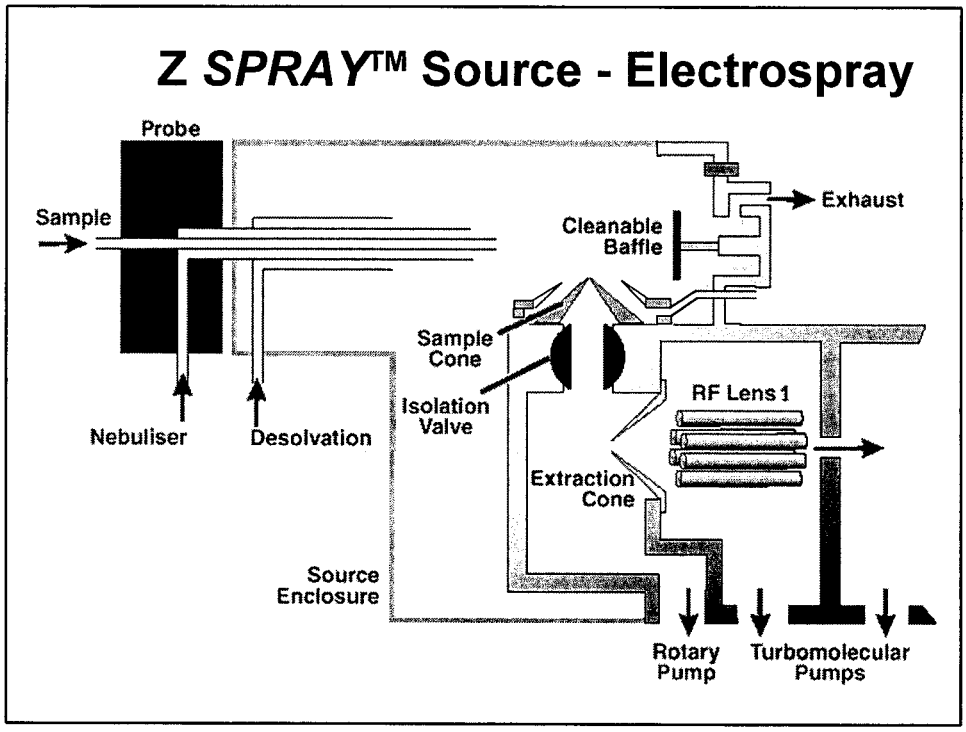
Liquid is passed through a heated tube (fused silica capillary). The liquid is evaporated to produce gas phase molecules.

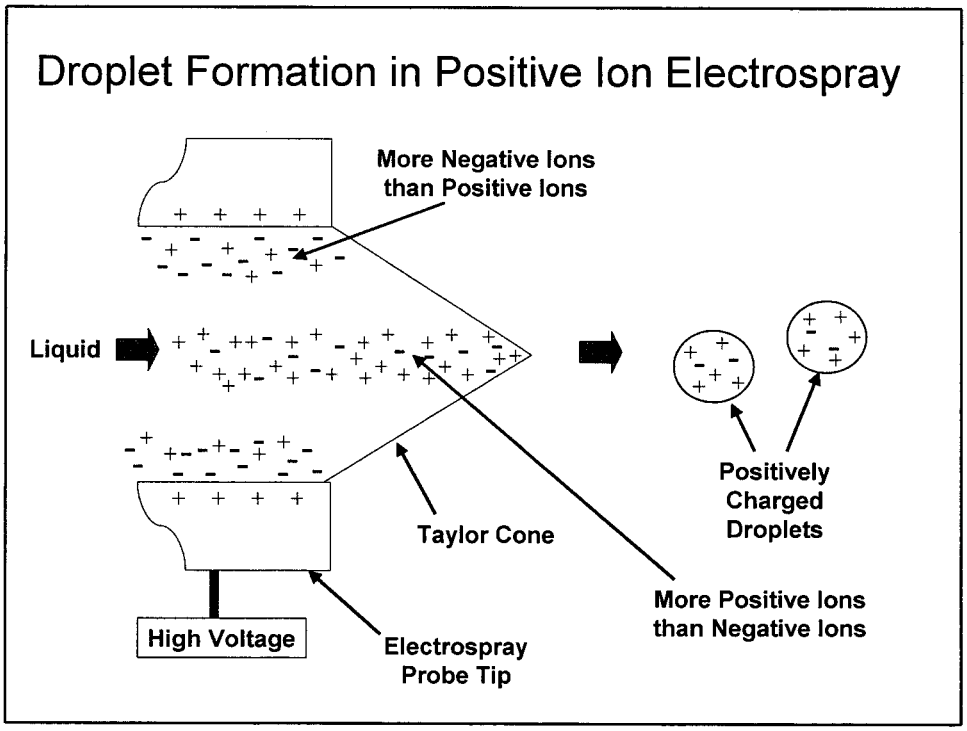
A high voltage is applied to a corona pin near the exit of the tube and the molecules are ionized when they pass through a cloud of ionized nitrogen atoms produced by the corona pin.

Electrospray Ionisation

Example of Positive Electrospray




Z SPRAY™ Source - Electrospray
Probe
Sample
Exhaust
Cleanable Baffle
Sample Cone
Isolation Valve
RF Lens 1
Nebuliser
Desolvation
Extraction Cone
Source Enclosure
Rotary Pump
Turbomolecular Pumps


Droplet Formation in Positive Ion Electrospray
More Negative Ions than Positive Ions
Liquid
Positively Charged Droplets
Taylor Cone
More Positive Ions than Negative Ions
High Voltage
Electrospray Probe Tip

The electrospray droplets carry positive charges away from the capillary tube.

To balance this flow of positive charges, electrons flow out of the capillary tube.

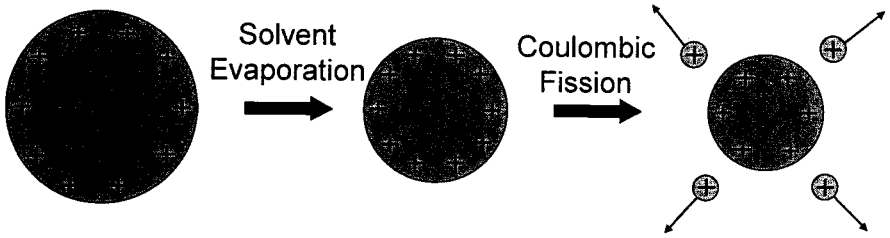
These electrons come from negative ions close to the surface of the capillary wall via an electrochemical oxidation reaction.

Electrospray can be thought of as an electrochemical process.

Example of reaction that can occur at the capillary wall:

$$2\,OH^- \longrightarrow H_2O + O + 2e^-$$

Electrospray Droplet Undergoing Fission



Charge resides on the surface of the droplet.

Solvent evaporates from the droplet and the droplet shrinks until the charge density on the surface reaches a point where the repulsive force between charges exceeds the liquid surface tension that holds the drop together.

At that point, the drop fissions and a set of small droplets are expelled from the main droplet.

Actual Droplet Undergoing Coulumbic Fission

Rough Sketch of Photo from
P. Kebarle and L. Tang, Analytical Chemistry, 64, 972 A (1993)

It is estimated, that in the fissioning process a charged droplet will lose on the order of 15% of its charge but as little as 2% of its mass.

Electrospray tends to work best with solutions that have a high percentage of organic solvents such as acetonitrile or methanol, though the solution cannot be totally organic.

The solution must have some aqueous content.

Solutions must have some ions in it for electrospray to work.

Fortunately most solutions that have an aqueous component will have some ionic species such as hydronium/hydroxyl ions and sodium ions.

Models for formation of Gas Phase Ions from Droplets

Ion Evaporation Model

Through evaporation and fissioning, droplets reduce in size to 10-20 nM in Diameter

Ions then 'evaporate' from the droplet's surface.

The more 'Surface Active' a molecule is, the more readily it will form ions in electrospray.

Charged Residue Model

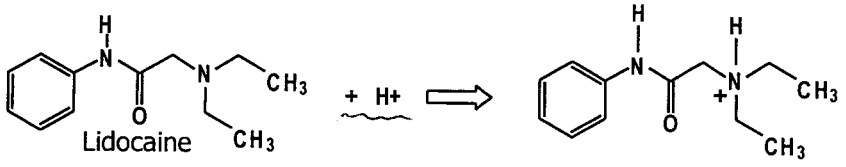
Droplets continue to lose solvent molecules through evaporation till a charged residue remains.

For an analyte of the form MX, the charged residue will be of the form:

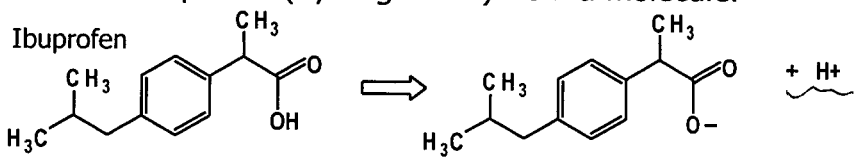
$$(M^+)_n(MX)_m$$

Electrospray Ions

Positive Electrospray Ions are produced by the addition to a molecule of a positively ion (e.g H+, NH4+, Na+). These positively charged ions that are added are often referred to as 'adducts'.



Negative Electrospray Ions are most often produced by the removal of a proton (hydrogen ion) from a molecule.



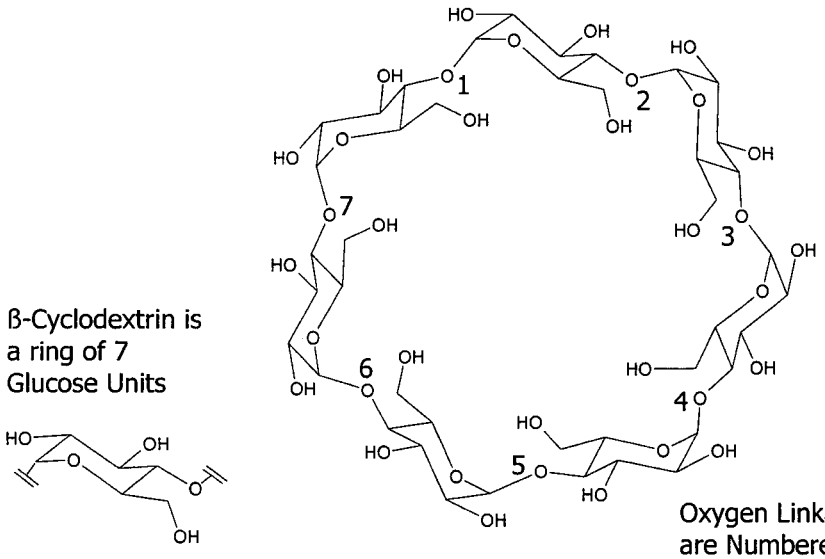
Electrospray and Ions in Solution

Electrospray is a solution process. Molecules that have a greater tendency to ionize in solution will tend to have stronger electrospray signals.

This is why certain additives to mobile phases in LC/MS analyses can enhance electrospray signals.

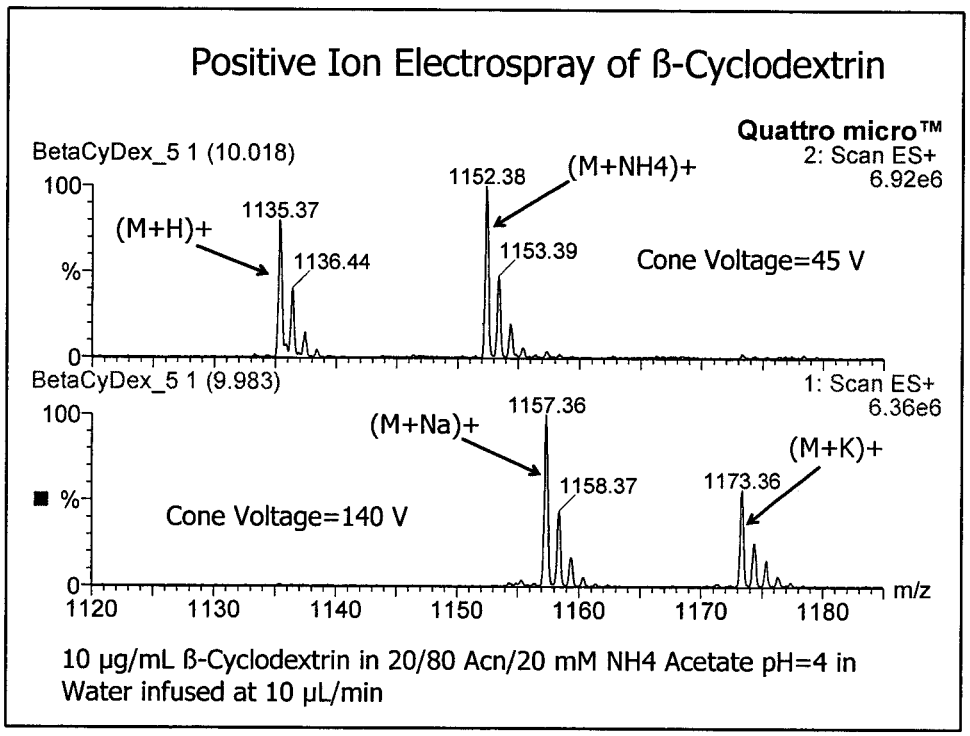
An example of this is addition of an acid (e.g. formic acid) to the mobile phase in positive electrospray LC/MS analyses. This can often result in a stronger electrospray signal by aiding in the protonation of analytes in solution.

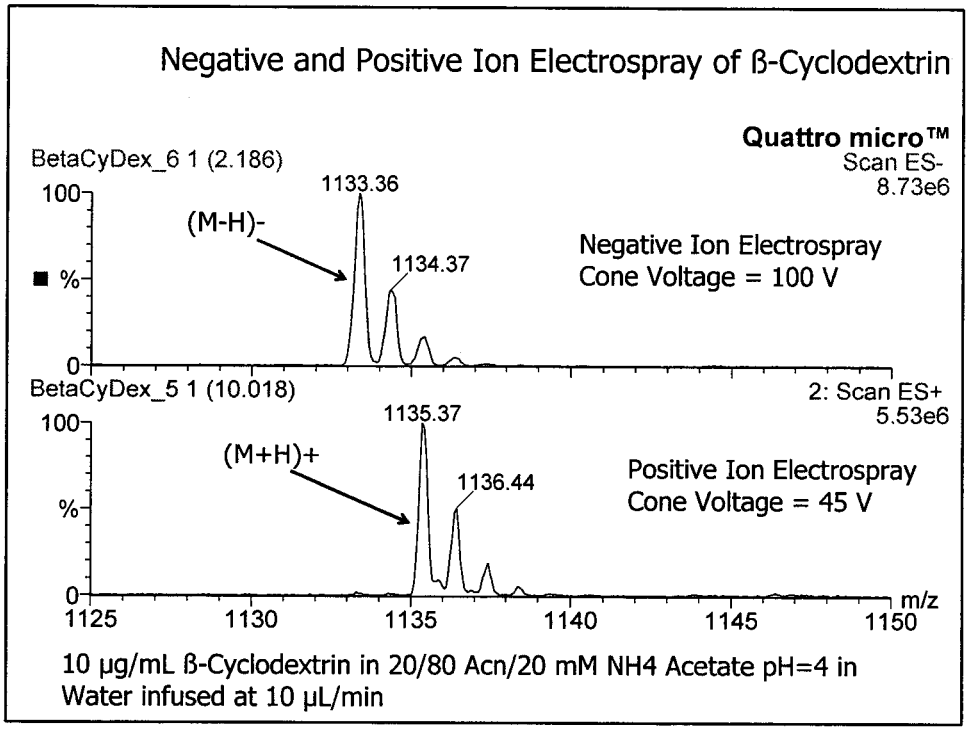
ß-Cyclodextrin - Electrospray Example



ß-Cyclodextrin is a ring of 7 Glucose Units

Oxygen Linkages are Numbered


Positive Ion Electrospray of ß-Cyclodextrin
Quattro micro™
BetaCyDex_5 1 (10.018)
2: Scan ES+
6.92e6
100
%
0
(M+H)+
1135.37
1136.44
1152.38
(M+NH4)+
1153.39
Cone Voltage=45 V
BetaCyDex_5 1 (9.983)
1: Scan ES+
6.36e6
100
%
0
(M+Na)+
1157.36
1158.37
(M+K)+
1173.36
Cone Voltage=140 V
1120
1130
1140
1150
1160
1170
1180
m/z
10 µg/mL ß-Cyclodextrin in 20/80 Acn/20 mM NH4 Acetate pH=4 in Water infused at 10 µL/min


Negative and Positive Ion Electrospray of ß-Cyclodextrin
Quattro micro™
BetaCyDex_6 1 (2.186)
Scan ES-
8.73e6
100
%
0
(M-H)-
1133.36
1134.37
Negative Ion Electrospray
Cone Voltage = 100 V
BetaCyDex_5 1 (10.018)
2: Scan ES+
5.53e6
100
%
0
(M+H)+
1135.37
1136.44
Positive Ion Electrospray
Cone Voltage = 45 V
1125
1130
1135
1140
1145
1150
m/z
10 µg/mL ß-Cyclodextrin in 20/80 Acn/20 mM NH4 Acetate pH=4 in Water infused at 10 µL/min

Samples Analyzed in ES mode

Typical ES Positive Ion Samples

- Peptides and proteins
- Small polar molecules
- Drugs and their metabolites
- Environmental contaminants
- Dye compounds
- Some organometallics
- Small saccharides

Typical ES Negative Ion Samples

- Some proteins
- Some drug metabolites (e.g. conjugates)
- Oligonucleotides
- Some saccharides and polysaccharides

Atmospheric Pressure Chemical Ionization (APcI)

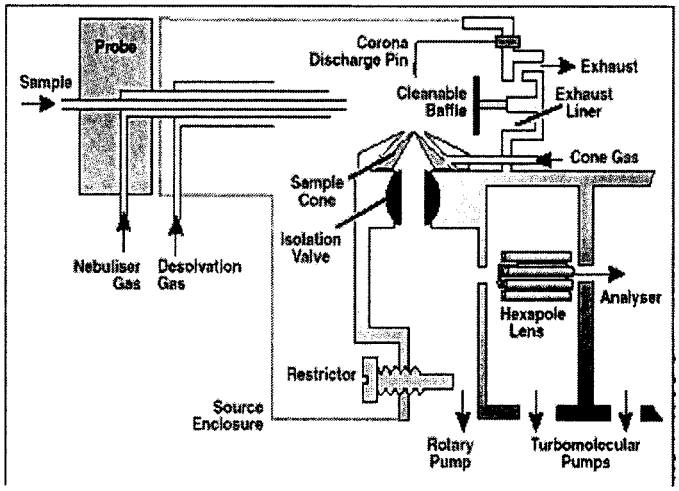
Low molecular weight (<1000 Da)

Singly charged species

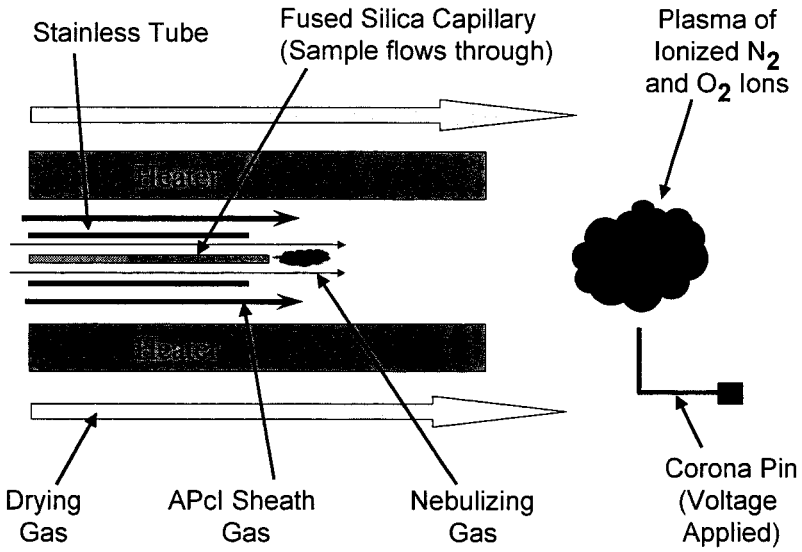
Fragmentation, even at low cone voltages

Mobile phase can be non-polar
(normal-phase chromatography)

APcI Source



APCI Probe Design



APCI Ionization

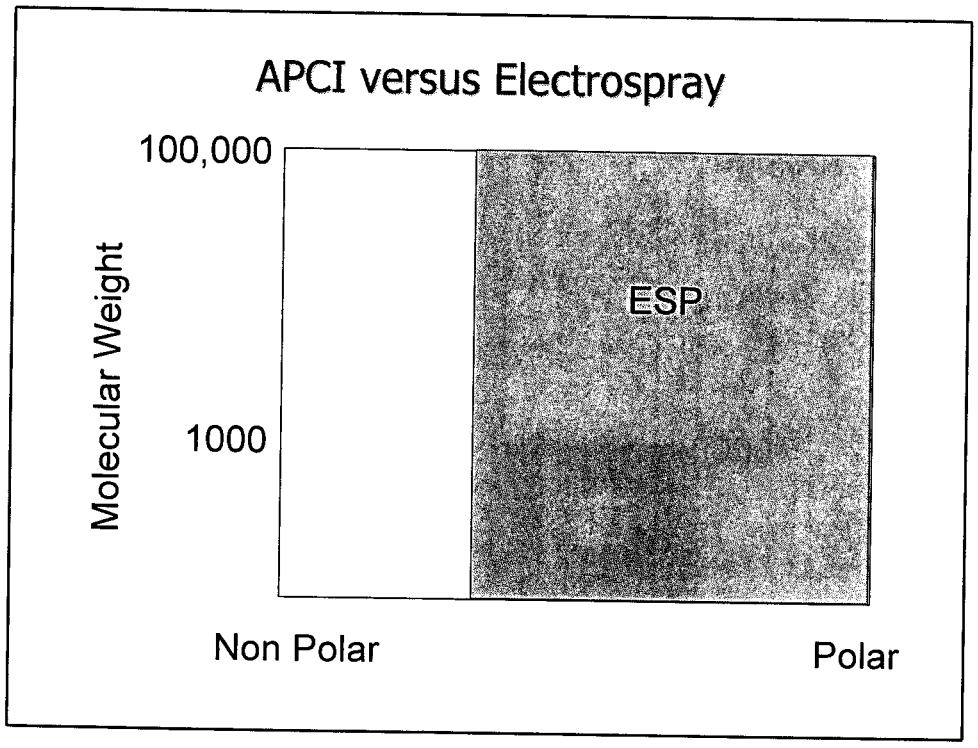
- Higher temperature, more aggressive ionization.
- Solvent molecules are in the gas phase.
- Ionization takes place in the plasma.
- Goal of the nitrogen is to evaporate solvent expelled from fused silica.
- May be more sensitive than electrospray with some non-polar molecules

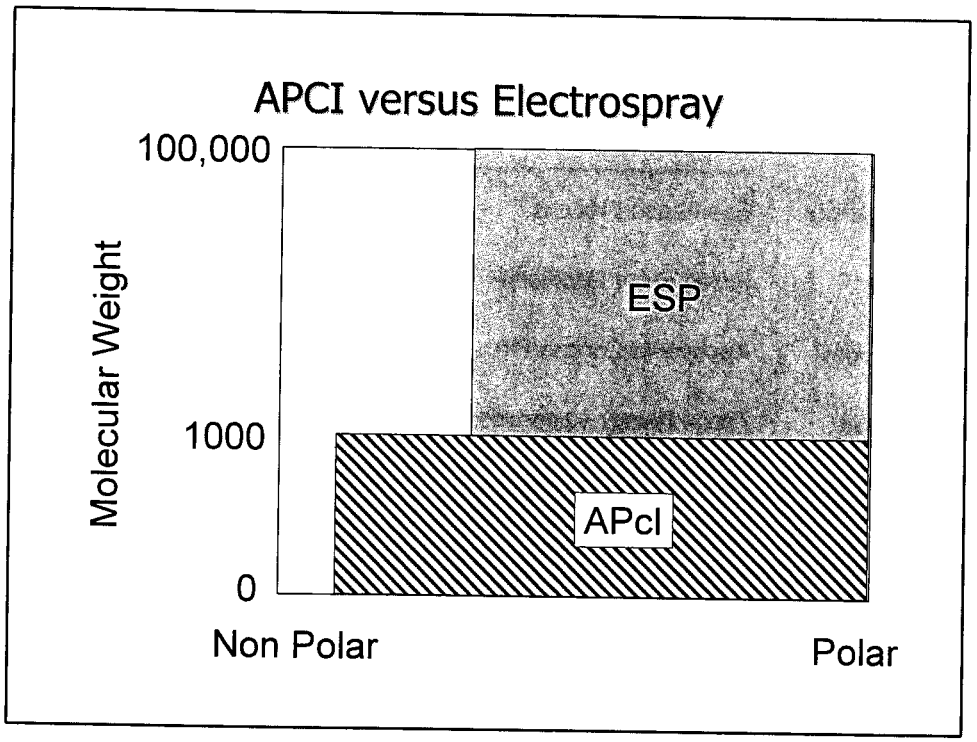
APcI Ions

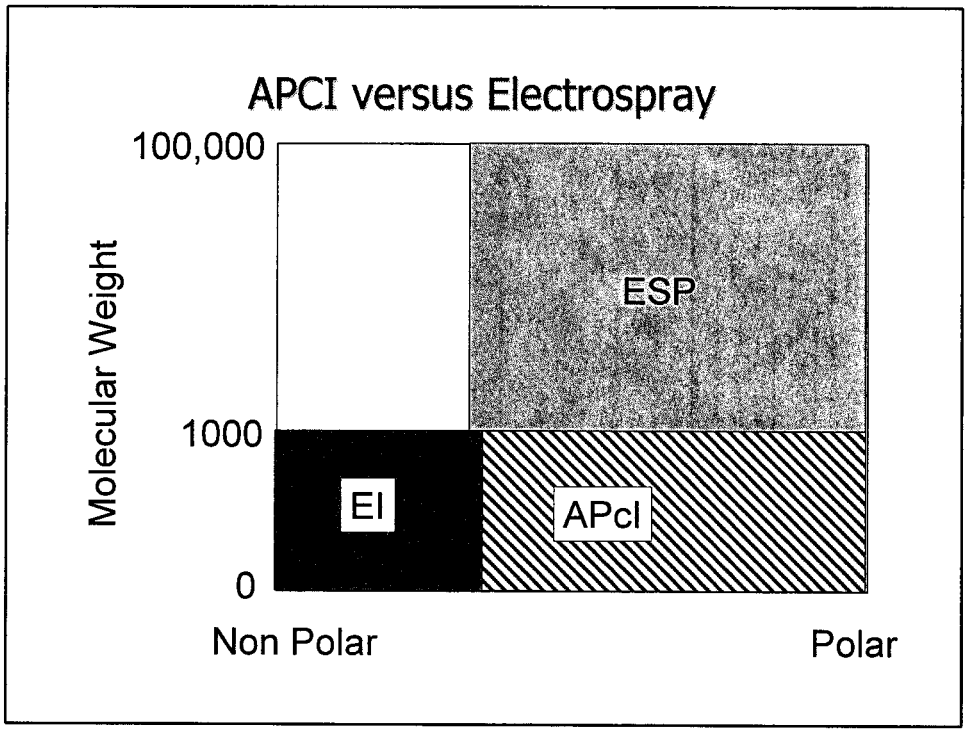
In positive ion APcI, ions similar to those formed in positive ion electrospray are formed.
For example: (M+H)+ or (M+Na)+

In negative ion APcI, the (M-H)- ion formed in negative ion electrospray is also produced.

Also in negative ion APcI, free electrons are formed. Certain types of molecules can pick up one of the free electrons produced by the corona pin and become negatively charged without a change in mass.
This process is sometimes referred to as "M+•" or "M plus dot".


APCI versus Electrospray
100,000
1000
Molecular Weight
ESP
Non Polar
Polar


APCI versus Electrospray
100,000
1000
0
Molecular Weight
ESP
APcI
Non Polar
Polar



APCI versus Electrospray

	APcI	Electrospray
Ionization	Gas Phase Process	Solution Phase Process
Probe	Fused Silica Capillary	Stainless Steel Capillary
Potential	Applied to Corona Pin	Applied to Capillary
Process	Probe heater vaporizes the liquid.	Spray of charged droplets produced.
	All molecules are now in the gas phase.	Liquid is evaporated from the droplets.
	Corona pin produces nitrogen ions.	Then droplets split into smaller droplets.
	Molecules are ionized when they collide with the nitrogen ions.	When the droplets get small enough, ions enter the gas phase.

APCI versus Electrospray (continued)

	APcI	Electrospray
Fragments	More vigorous ionization. More fragments produced.	'Gentler' ionization. Less fragments produced.
Sample Types	Low MW<1000 Can be less polar.	Small & Large Molecules Tend to be more polar.
Charges	Usually Singly Charged.	May be Multiplied Charged.
Flow Rates	0.2 - 2 mL/min	0.001 - 1 mL/min
Temperatures	Source ~ 120-140 °C Probe ~ 450-550 °C	Infusion:Source ~ 80 °C Desolvation ~ 120°C HPLC: Source ~ 120 °C Desolvation ~ 350 °C

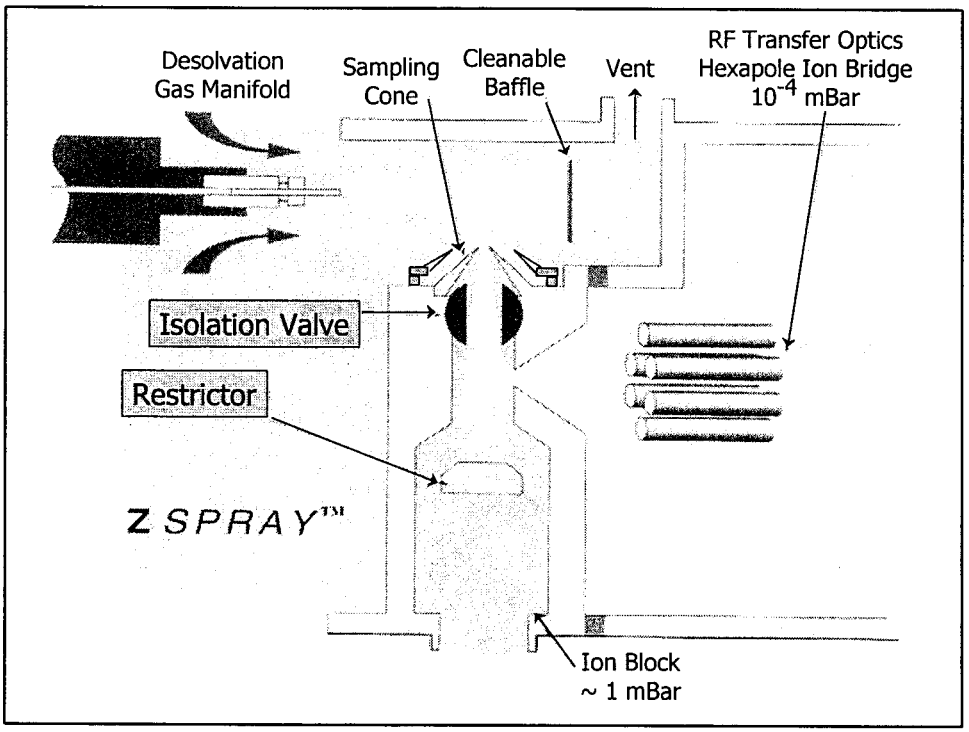
Positive or Negative?

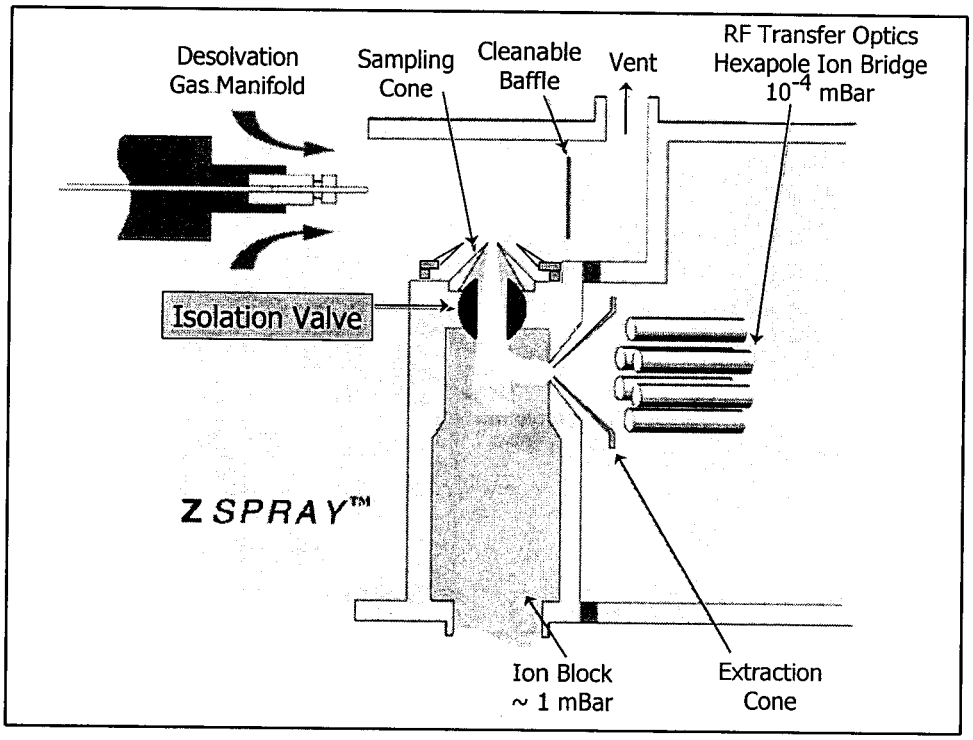
Compound Type	Easiest Formed Ions	
Basic Compounds ($-NH_2$)	**$(M+H)^+$**	**Pos Ions**
Acidic Compounds ($-CO_2H$, -OH)	**$(M-H)^-$**	**Neg Ions**

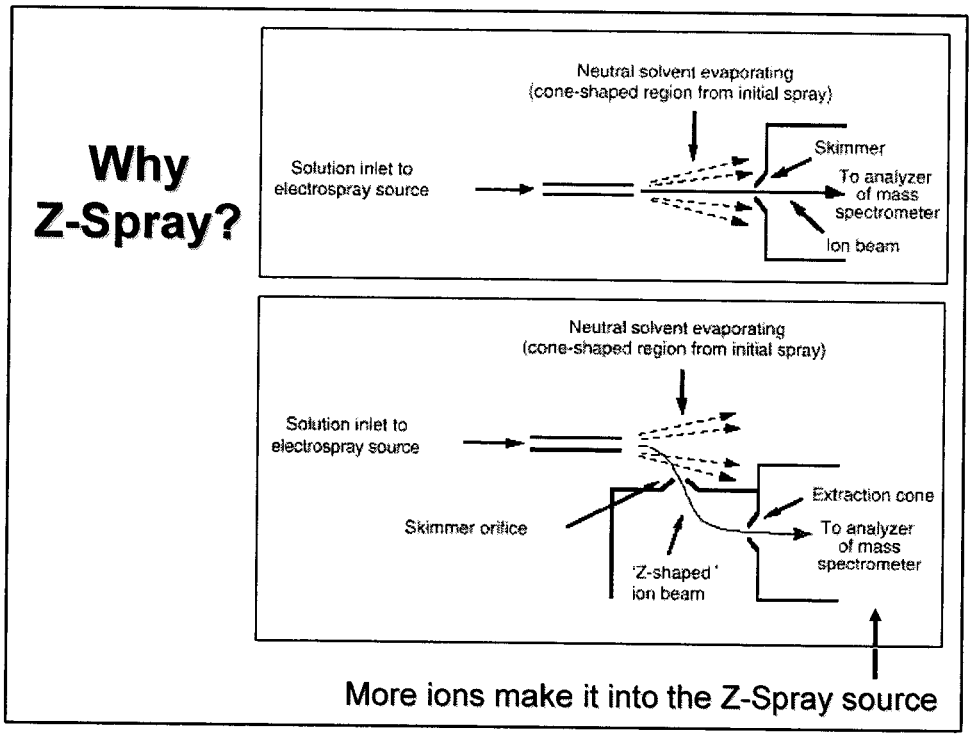
Waters

Z-Spray Source

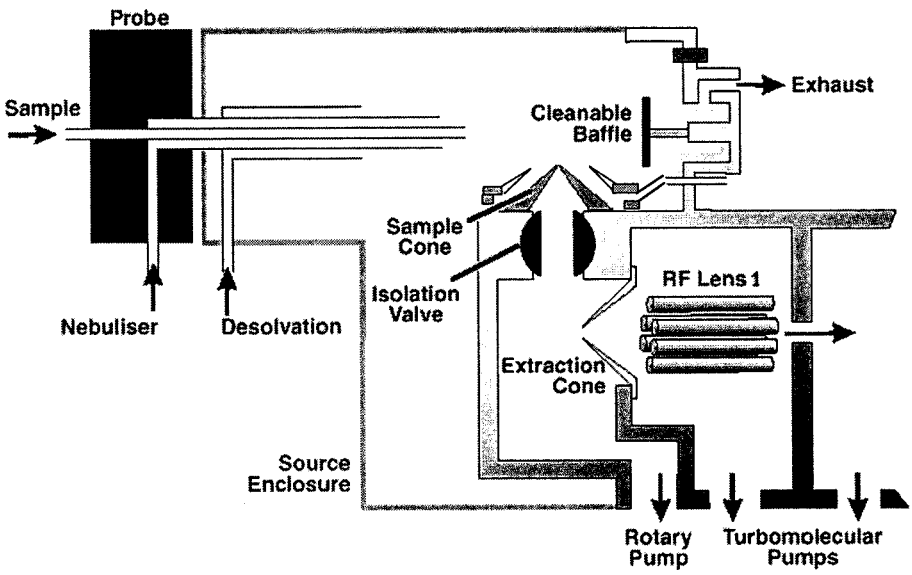
MICROMASS
MS TECHNOLOGIES



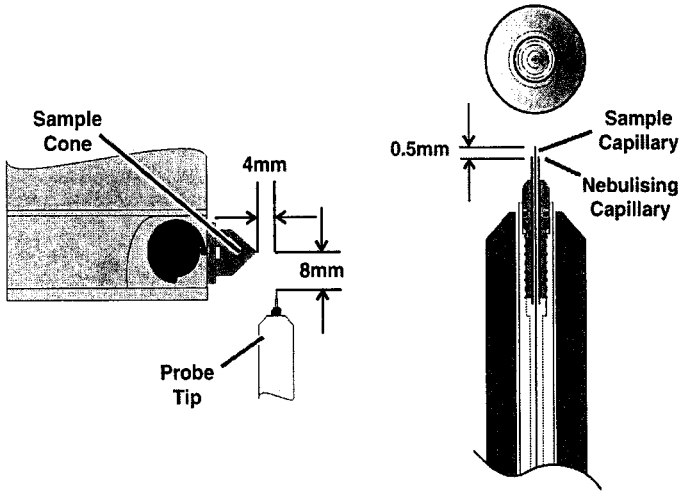

Desolvation
Gas Manifold
Sampling
Cone
Cleanable
Baffle
Vent
RF Transfer Optics
Hexapole Ion Bridge
10^{-4} mBar
Isolation Valve
Z SPRAY™
Ion Block
~ 1 mBar
Extraction
Cone

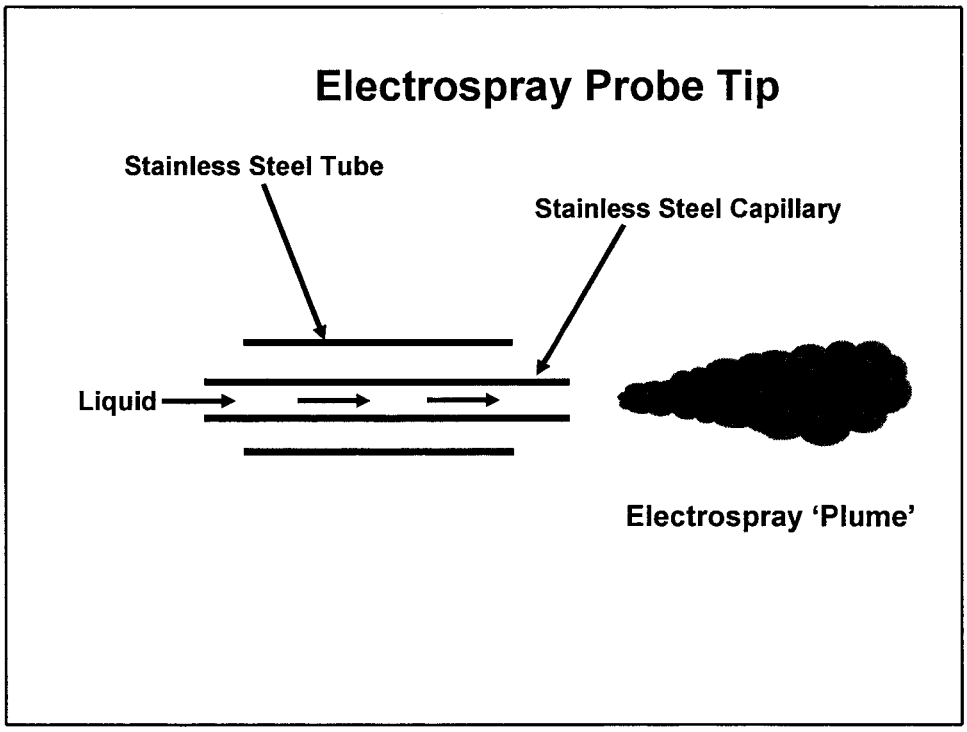

Why
Z-Spray?
Neutral solvent evaporating
(cone-shaped region from initial spray)
Solution inlet to
electrospray source
Skimmer
To analyzer
of mass
spectrometer
Ion beam
Neutral solvent evaporating
(cone-shaped region from initial spray)
Solution inlet to
electrospray source
Extraction cone
Skimmer orifice
To analyzer
of mass
spectrometer
'Z-shaped'
ion beam
More ions make it into the Z-Spray source

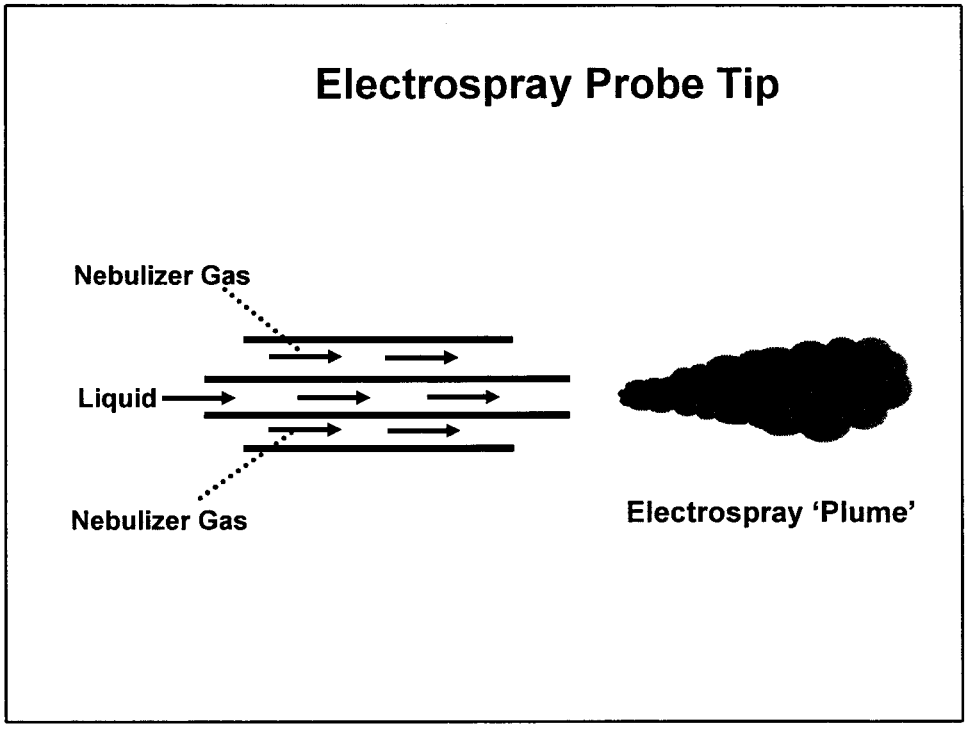
Z *SPRAY*™ Source - Electrospray



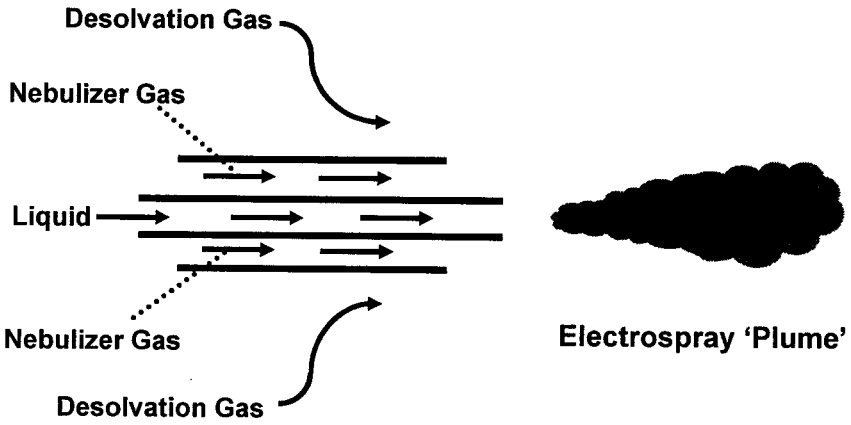
Probe Position



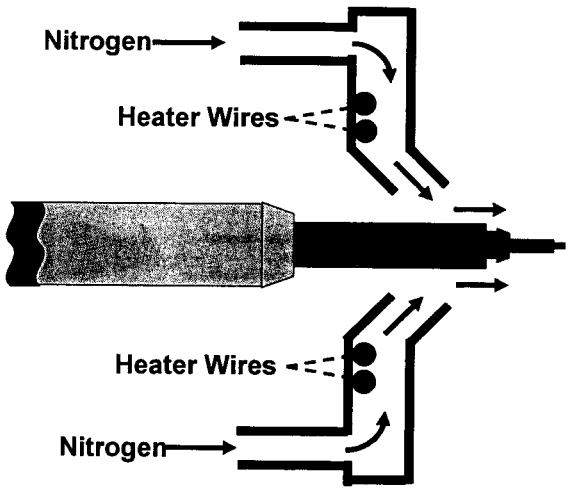

Electrospray Probe Tip
Stainless Steel Tube
Stainless Steel Capillary
Liquid
Electrospray 'Plume'


Electrospray Probe Tip
Nebulizer Gas
Liquid
Nebulizer Gas
Electrospray 'Plume'

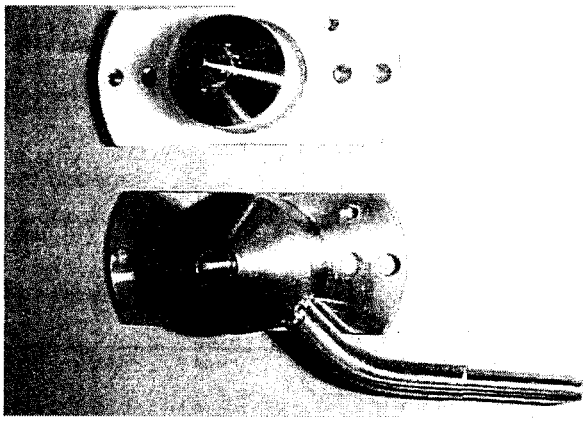
Electrospray Probe Tip

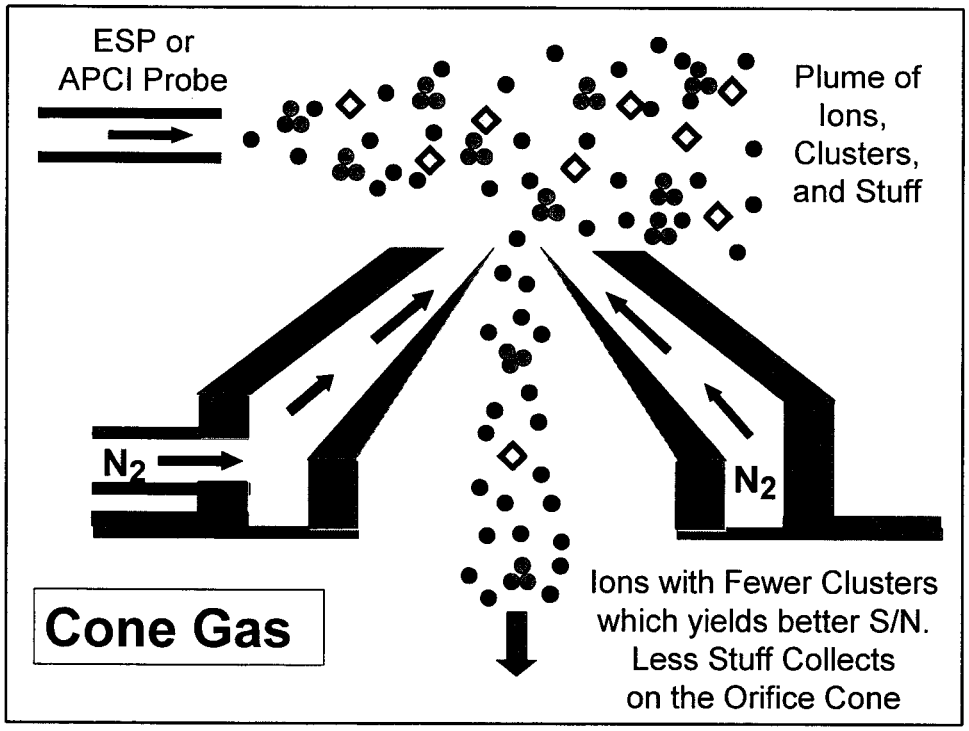


Desolvation Gas Flow



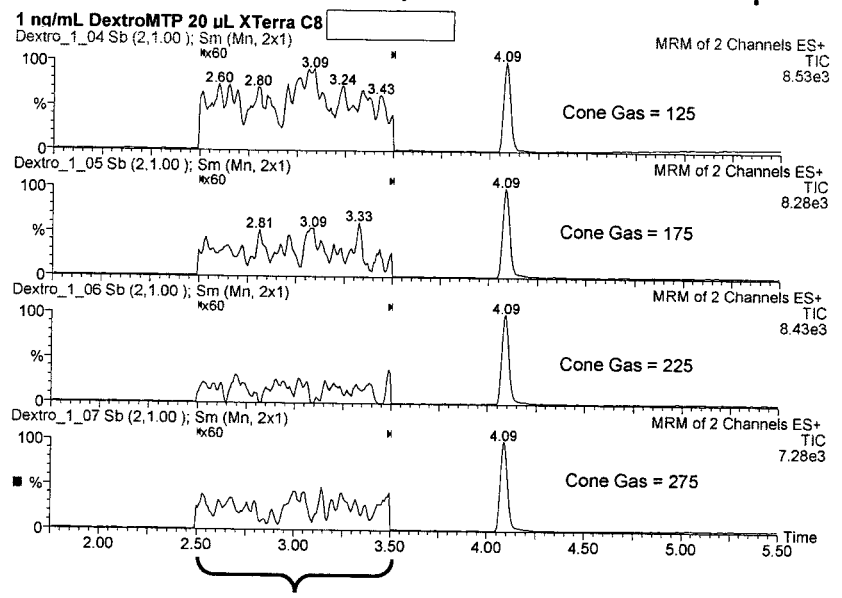
Quattro Ultima and Quattro LC: Sample Cone and Cone Gas Nozzle





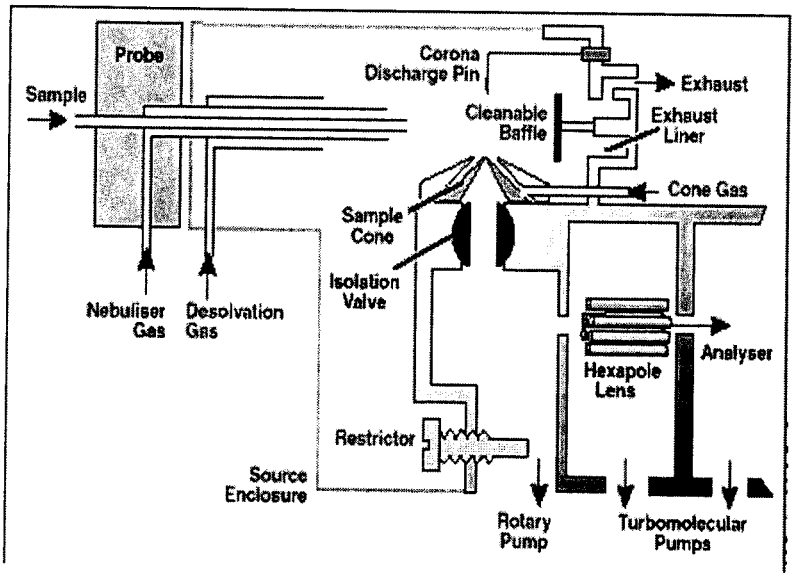
noise remove

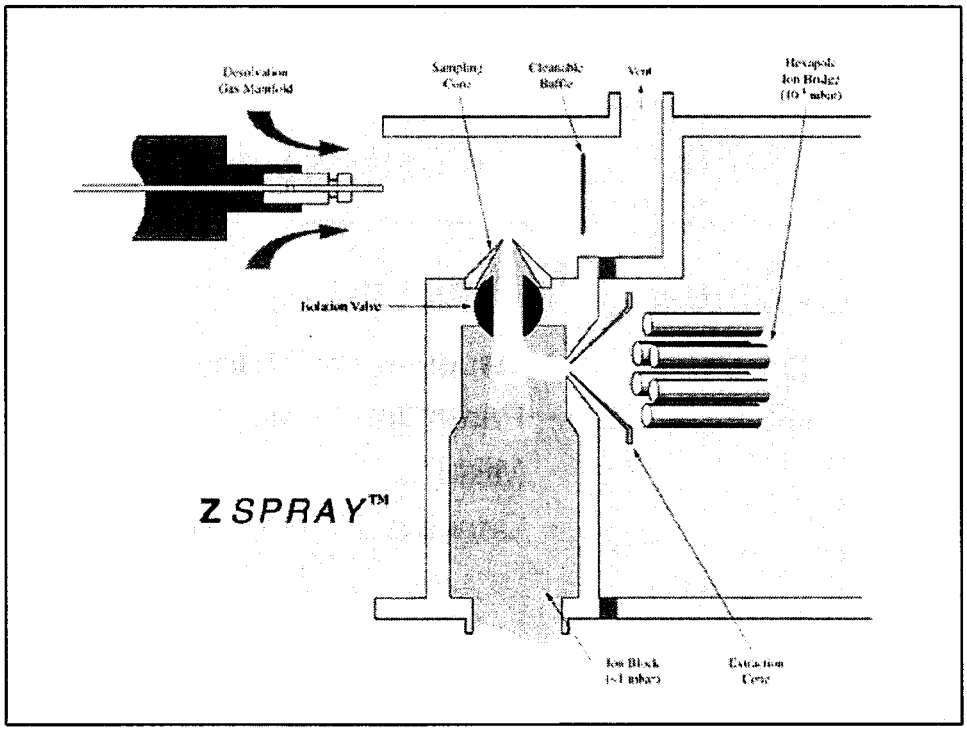
Cone Gas Example - Dextromethorphan



Baseline Noise Magnified by a Factor of 60

APcI Source





Waters

Mass Spectra

and

MS Data Acquisition Modes

MICROMASS
MS TECHNOLOGIES

Mass Spectrometer Data Acquisition Modes

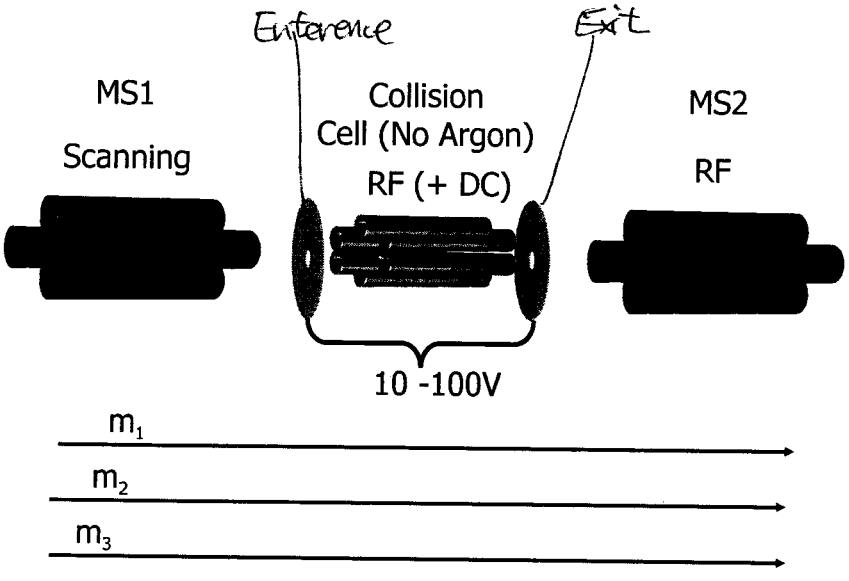
MS Modes

- MS1 Scan
- SIR

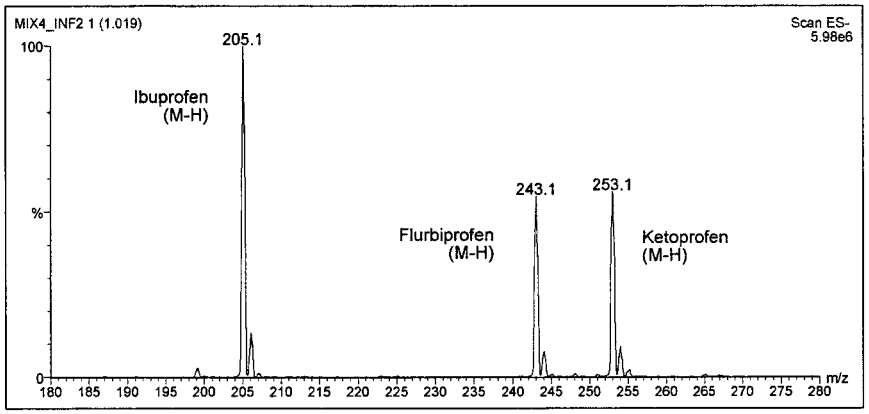
MS/MS Modes

- Daughter Ion Scan
- Parent Ion Scan
- MRM
- Constant Neutral Loss(Gain)

MS1 Scan



MS Spectra of an Infused Spectra of 'fens



Monoisotopic versus Average Molecular Weight

Monoisotopic Molecular Weight is calculated using the atomic weight of the most abundant isotope of each element.

Average Molecular Weight is calculated using the average atomic weight of each element taking into account the relative abundance of its isotopes.

For example: Naturally occurring carbon is 98.93% Carbon-12 (At Wt=12.0000) and 1.07% Carbon-13 (At Wt=13.0034).

In calculating a monoisotopic molecular weight, each carbon would add 12.0000 to the molecular weight.

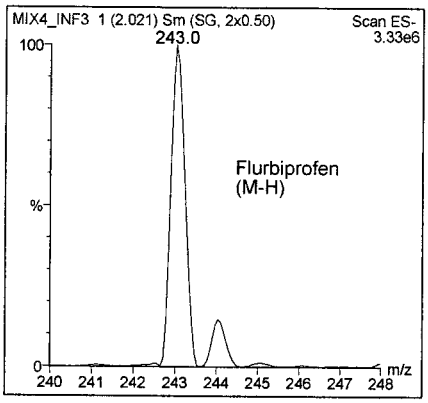
The average atomic weight of carbon is:

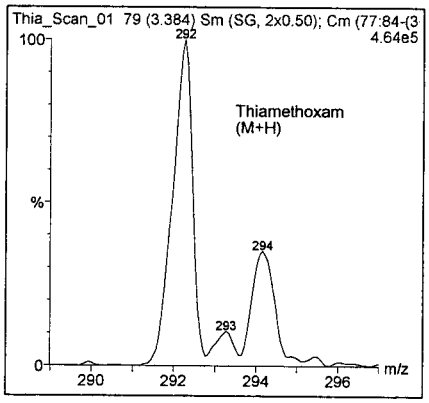
$$(0.9893)(12.0000) + (0.0107)(13.0034) = 12.0107$$

So in calculating an average molecular weight, each carbon would add 12.0107 to the molecular weight.

The molecular weight listed on reagent bottles is an average molecular weight.

Mass Spectra - Different Isotopic Forms of an Analyte



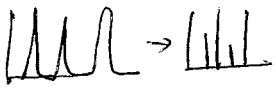


$C_{15}H_{13}O_2F$ $C_8H_{10}N_5O_3SCl$

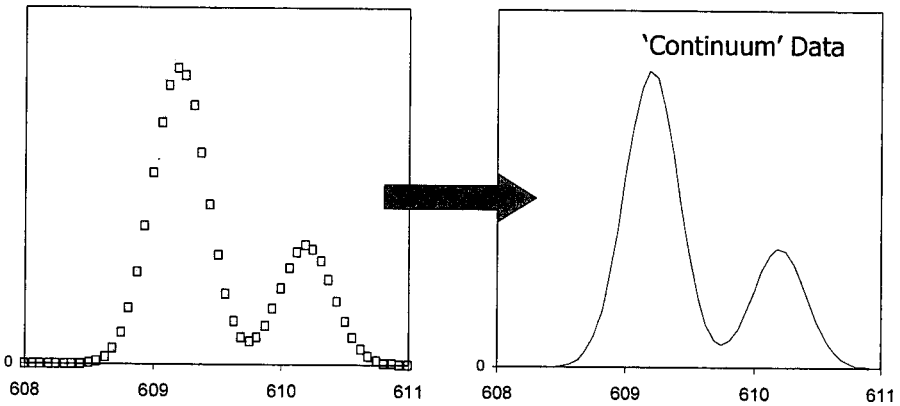
tune page
→ Optron
→ set Instrument threshold

Continuum : collection sperate spectrum

Centroid



Mass Spectrometer Acquires Spectra in the 'Profile' or 'Continuum' Mode



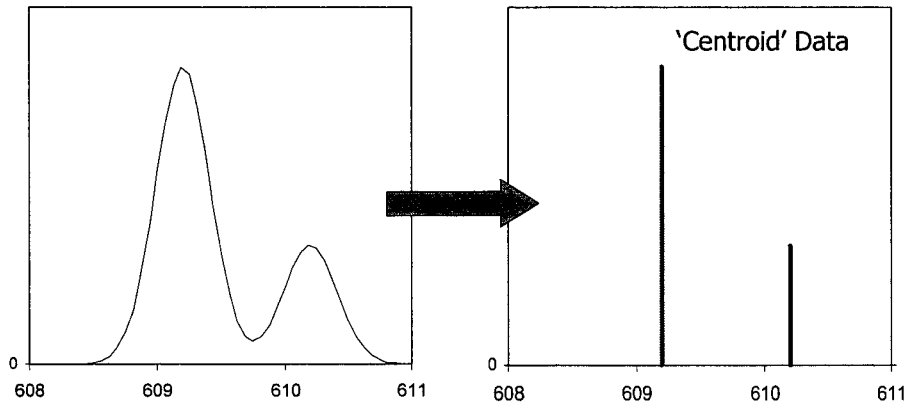
Example of Profile Data acquired at 16 points per Dalton

If directly stored by MassLynx, this is 'Continuum' Data

Example: Suppose you are acquiring spectra from 300 to 700 amu and that you are taking one complete spectrum every 1 sec. Then 60 separate continuum spectra with a range of 400 amu will be stored each minute of acquired data.

Converting Continuum Data to Centroid Data

The mass spec can also take each profile or continuum spectrum it has acquired and convert it 'on the fly' to centroid data. In this process, the center of each spectral peak is determined and only information on the center of each peak is transmitted to MassLynx.



tune page
→ Optron
→ set Instrument thresh

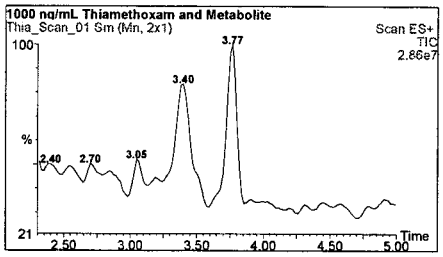
Data Acquisition Modes

- ***Continuum*** - also known as "Profile" data
  - series of spectra are acquired are stored individually
  - largest data file size
  - shows peak shape
  - can handle signals that vary with time (e.g. LC Peaks)
- ***Centroid*** - also known as "Stick" data
  - sees profile data but instantly converts it to stick data,
  - smaller data file size than continuum data
  - gives no peak shape information.
- ***MCA*** - Multi-channel Acquisition
  - is continuum data summed into one scan
  - smaller data file size than continuum but preserves the peak profile.
  - assumes there is a constant signal (infused sample)

Which Data Acquisition Mode?

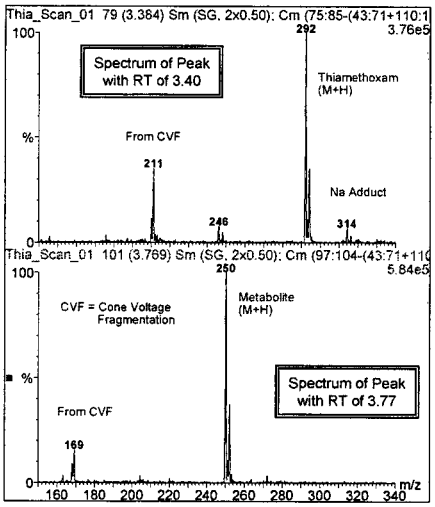
Mode	Maximum Scan Speed At 16pts/Da	Typical Use
Centroid	1000 amu/sec	GC, OpenLynx, FractionLynx, high concentration samples
MCA	1000 amu/sec (EPCAS) 400 amu/sec (TDAT)	Multiply charged species, non-time resolved data (*e.g.* Infusion)
Continuum	1000 amu/sec (EPCAS) 500 amu/sec (TDAT)	Multiply charged species, time resolved data (*e.g.* HPLC)

Mass Spectra of LC Peaks

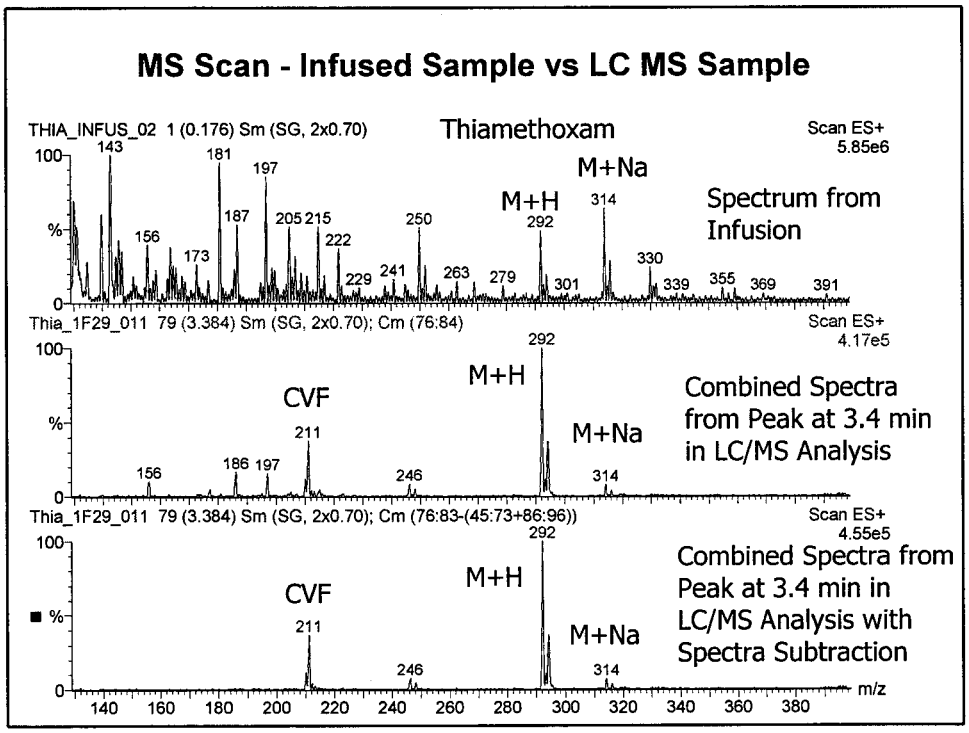


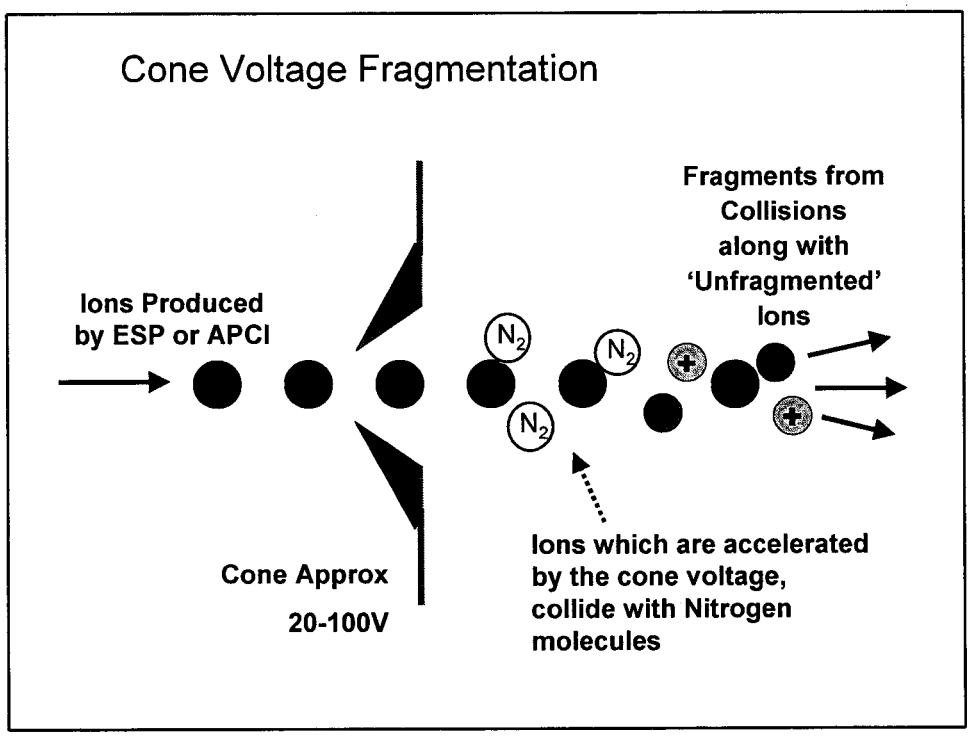
Full Scan Spectra of LC Analysis of a Standard Solution of Thiamethoxam and one of its Metabolites.

Spectra taken using 1 second scans from 100 to 400 Da.



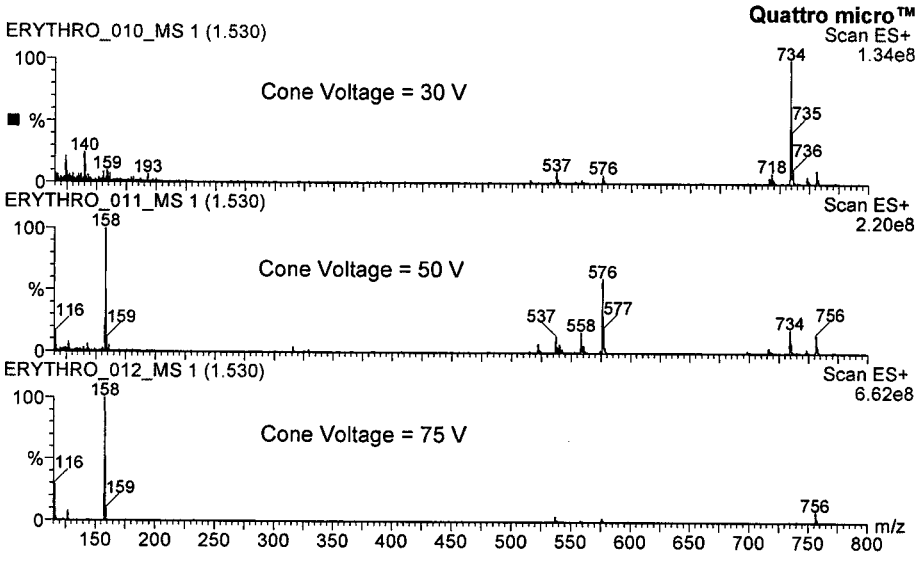
Spectra of two LC Peaks.

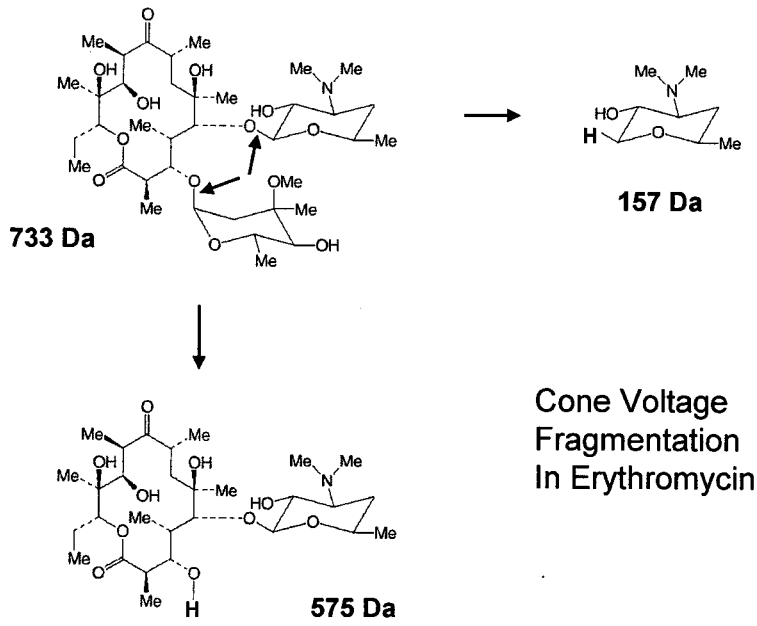

MS Scan - Infused Sample vs LC MS Sample
THIA_INFUS_02 1 (0.176) Sm (SG, 2x0.70)
Thiamethoxam
Scan ES+
5.85e6
M+Na
M+H
Spectrum from Infusion
Thia_1F29_011 79 (3.384) Sm (SG, 2x0.70); Cm (76:84)
Scan ES+
4.17e5
CVF
M+H
M+Na
Combined Spectra from Peak at 3.4 min in LC/MS Analysis
Thia_1F29_011 79 (3.384) Sm (SG, 2x0.70); Cm (76:83-(45:73+86:96))
Scan ES+
4.55e5
Combined Spectra from Peak at 3.4 min in LC/MS Analysis with Spectra Subtraction
m/z


Cone Voltage Fragmentation
Ions Produced by ESP or APCI
Fragments from Collisions along with 'Unfragmented' Ions
Cone Approx 20-100V
Ions which are accelerated by the cone voltage, collide with Nitrogen molecules

Example of Cone Voltage Fragmentation

Erythromycin : MW = 733 Da





Cone Voltage Fragmentation In Erythromycin

Recognizing Multiply Charged Ions

Mass spectrometers operate on the basis of mass-to-charge ratio (m/z).

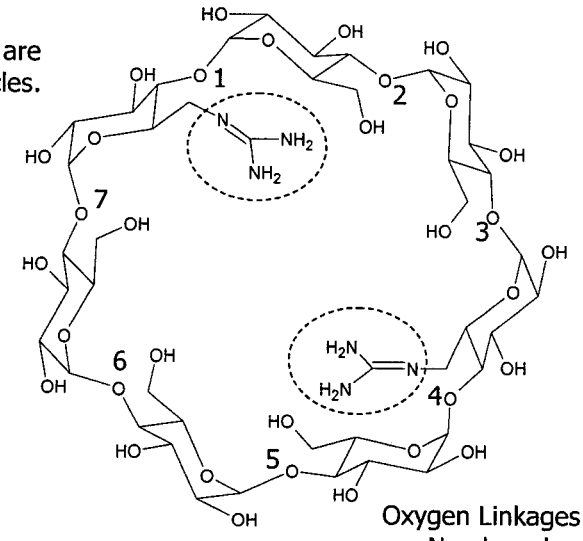
Mass assignments are normally made assuming a single charge per ion (e.g. z = 1 so m/z = m)

Single charge	m/z = (M+H)
Double charge	m/z = (M+2H) / 2
n charge	m/z = (M+nH) / n

Modified ß-Cyclodextrin - Multiplied Charged Example

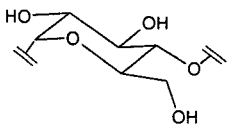
Modified ß-Cyclodextrin. Added functional groups are shown in the dashed circles.

Sample from Prof. Paul Smith, U of Maryland, Baltimore County

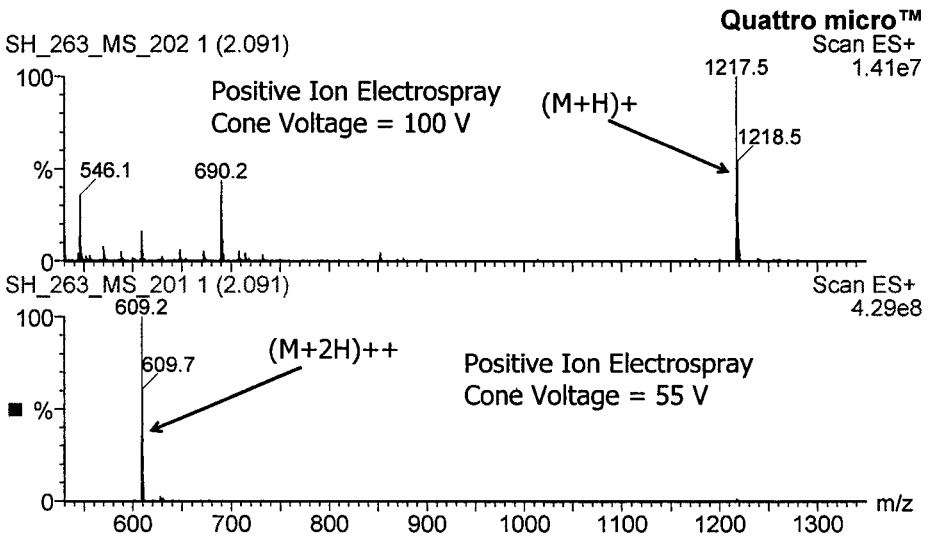


Oxygen Linkages are Numbered

ß-Cyclodextrin is a Ring of 7 Glucose Units



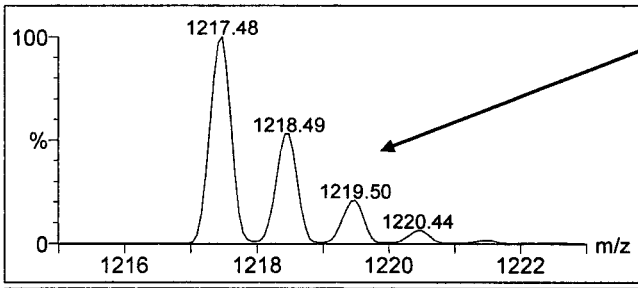
Modified ß-Cyclodextrin - Multiplied Charged Example



10 µg/mL Modified ß-Cyclodextrin in 20/80 Methanol/0.1% Formic Acid in Water infused at 10 µL/min

Modified ß-Cyclodextrin - Multiplied Charged Example

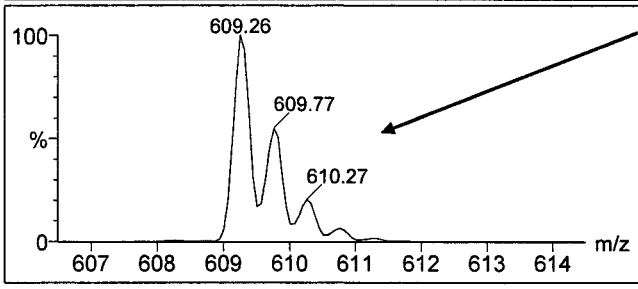
Assume Monoisotopic Mass of Analyte is M.
Mass of Isotopic Forms (mostly from C13's) = M, M+1, M+2, , ,



Top Spectra

For Singly Charged Ions (assume addition of H+) m/z of Isotopic Forms= M+1, M+2, M+3, , ,

(Monoiso MW=1216.45)

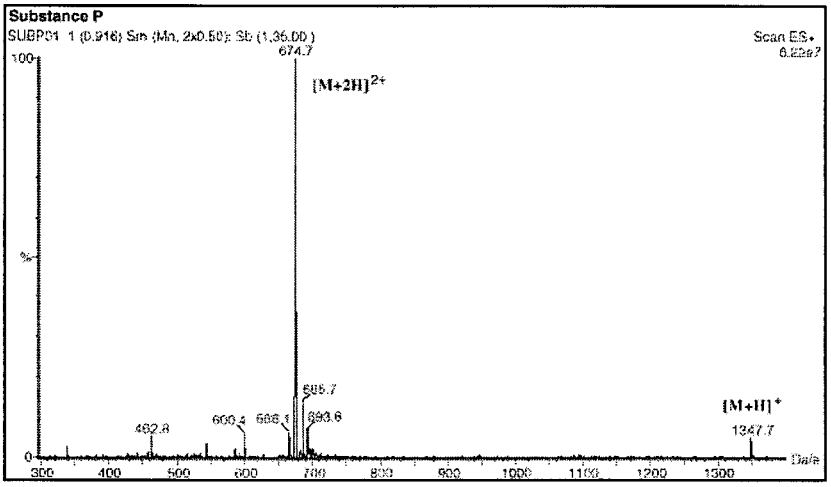


Bottom Spectra

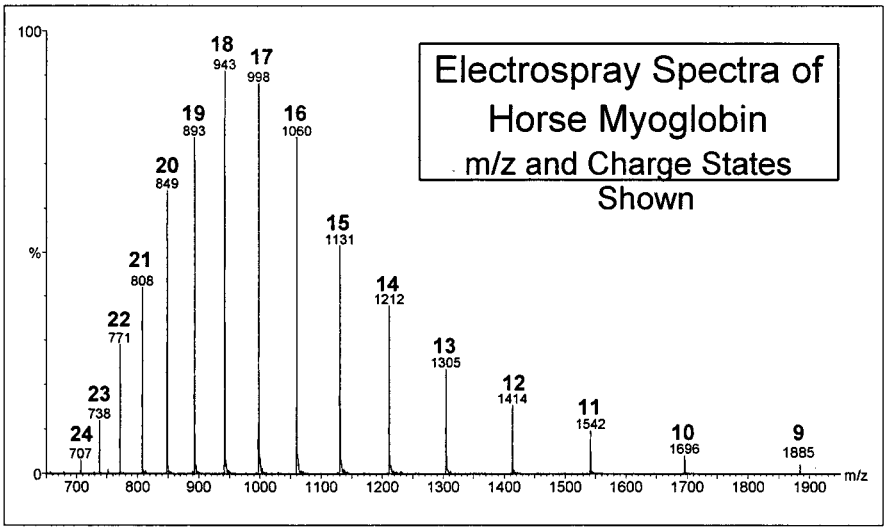
For doubly charged ions, (assume addition of 2H+) m/z of Isotopic Forms = (M+2) / 2 = M/2 + 1, (M+3) / 2 = M/2 + 1.5, (M+4) / 2 = M/2 + 2, , , ,

Multiply Charged Ions

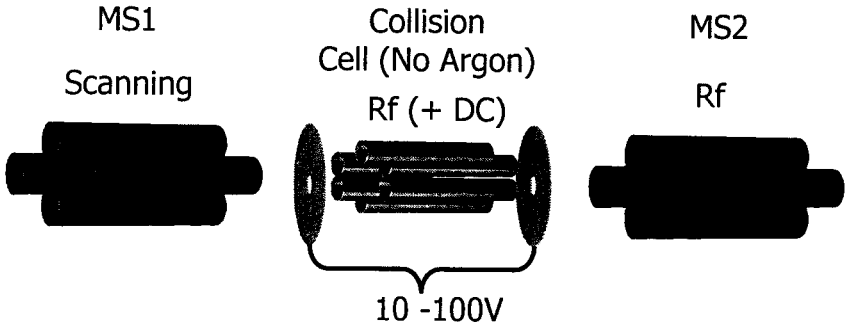
Substance P : Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met - NH2



Example of Multiply Charged Ion



MS1 Scan (Review)



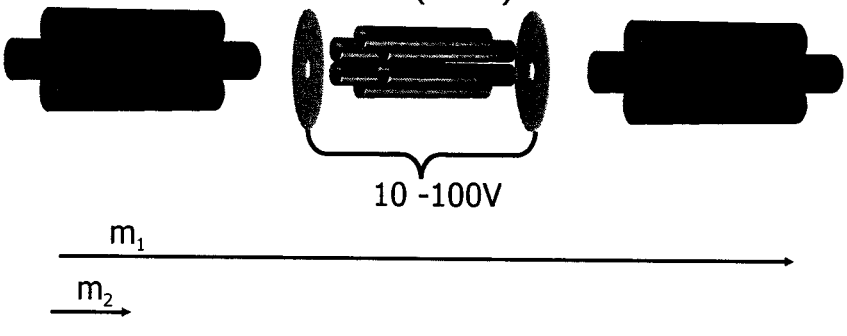
m_1

m_2

m_3

MS1 Scans are used to obtain Mass Spectra

SIR (Selected Ion Recording)



m_1

m_2

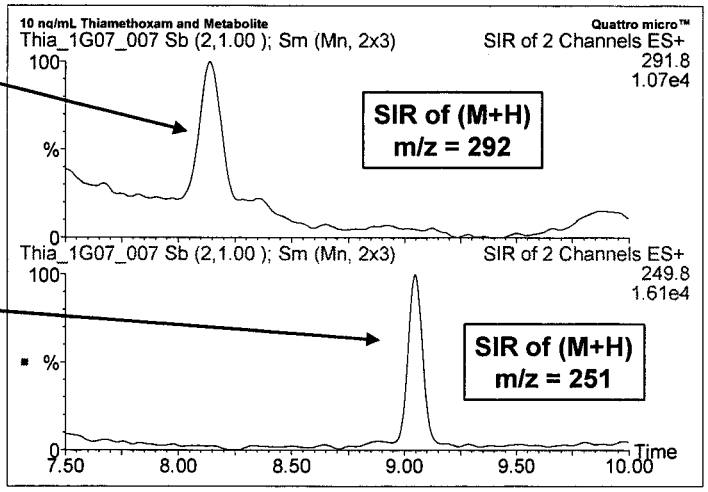
m_3

SIR's are used to monitor selected analyte(s)

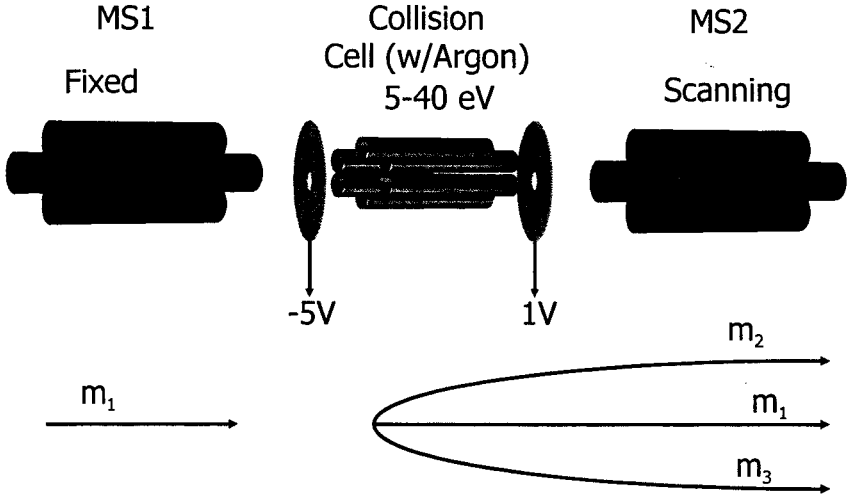
SIR Example

Thiamethoxam
MW = 291

Metabolite
MW = 250

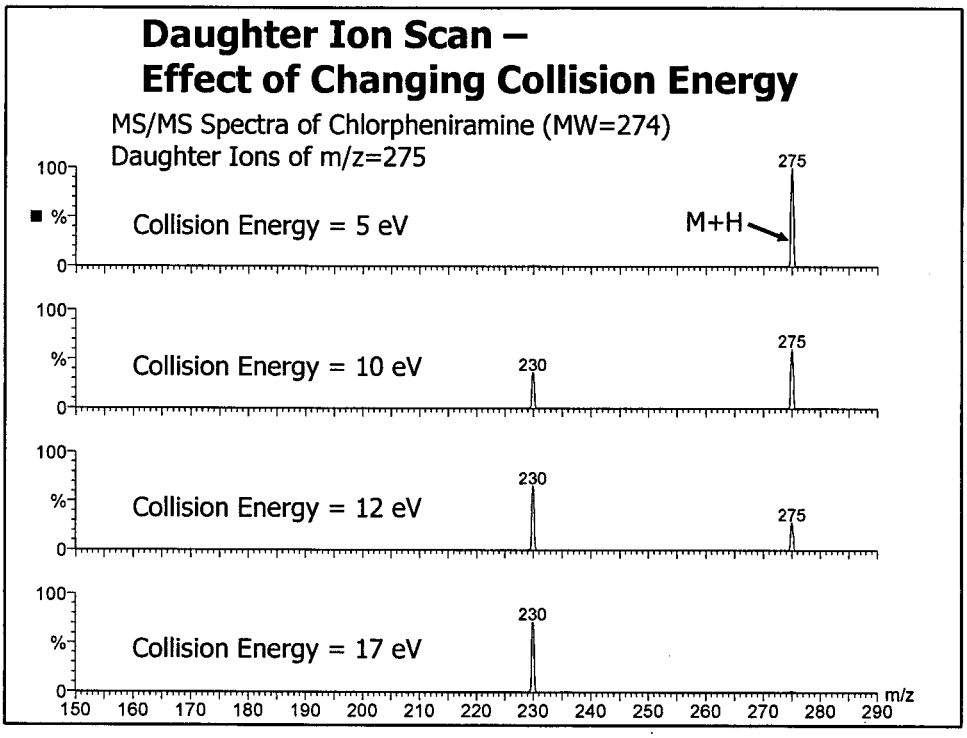


Daughter Ion Scan

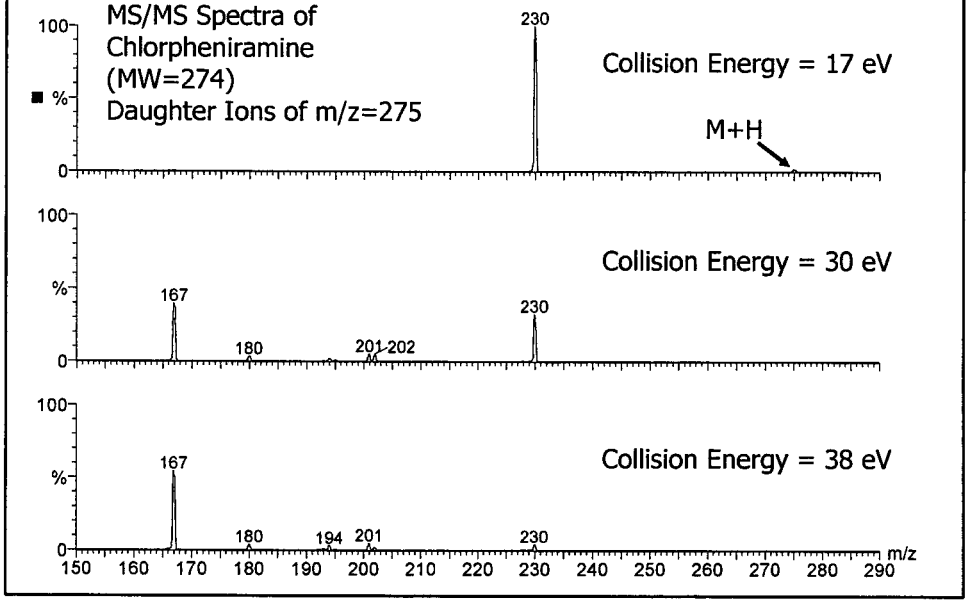


Determines Collision Induced Dissociation (CID) produced daughter ions of a particular parent ion

Daughter Ion Scan – Effect of Changing Collision Energy

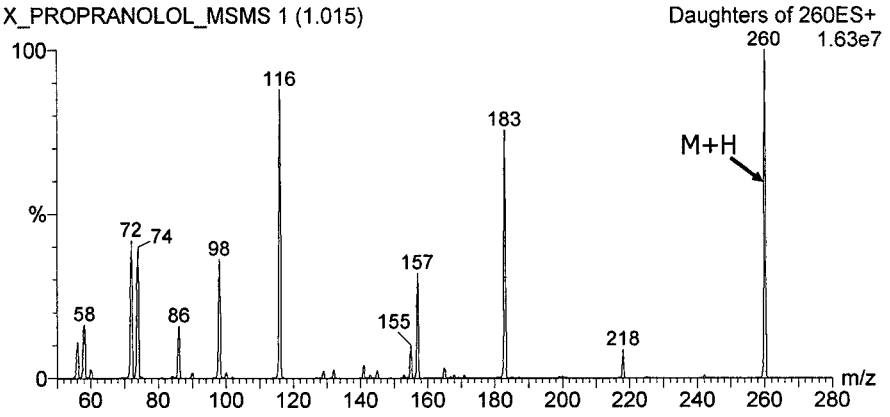


Daughter Ion Scan – Effect of Changing Collision Energy (Cont.)



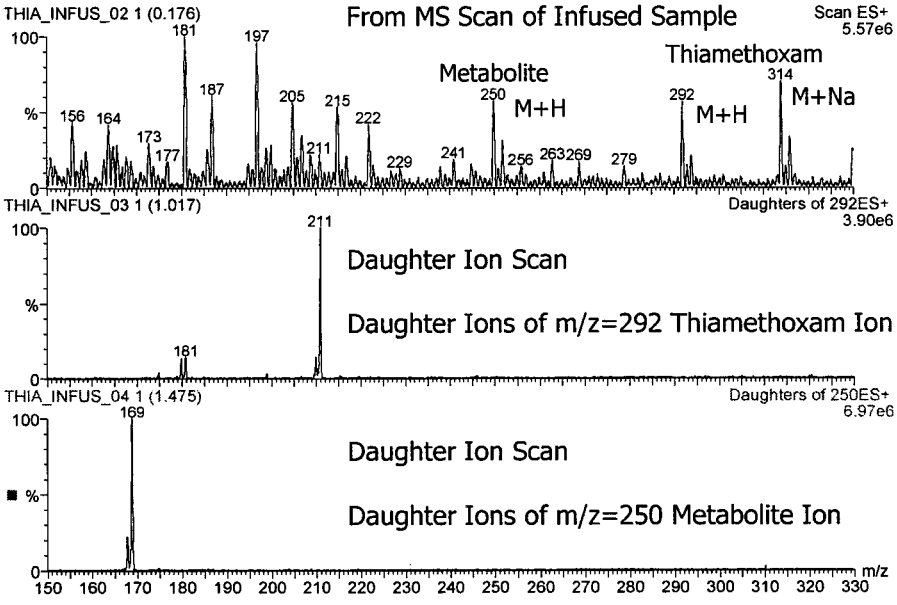
Daughter Ion Scans – Propranolol Example

MS/MS Spectra of Propranolol (MW=259) Daughter Ions of m/z=260



MS/MS spectra can often be very complicated. Maximum daughter ion signal obtained using a collision energy of 18 eV. Increasing the collision energy beyond this level, led to more CID of the parent M+H ion, but also led to more CID of the daughter ions resulting is lower daughter ion signals.

Daughter Ion Scans – Thiamethoxam

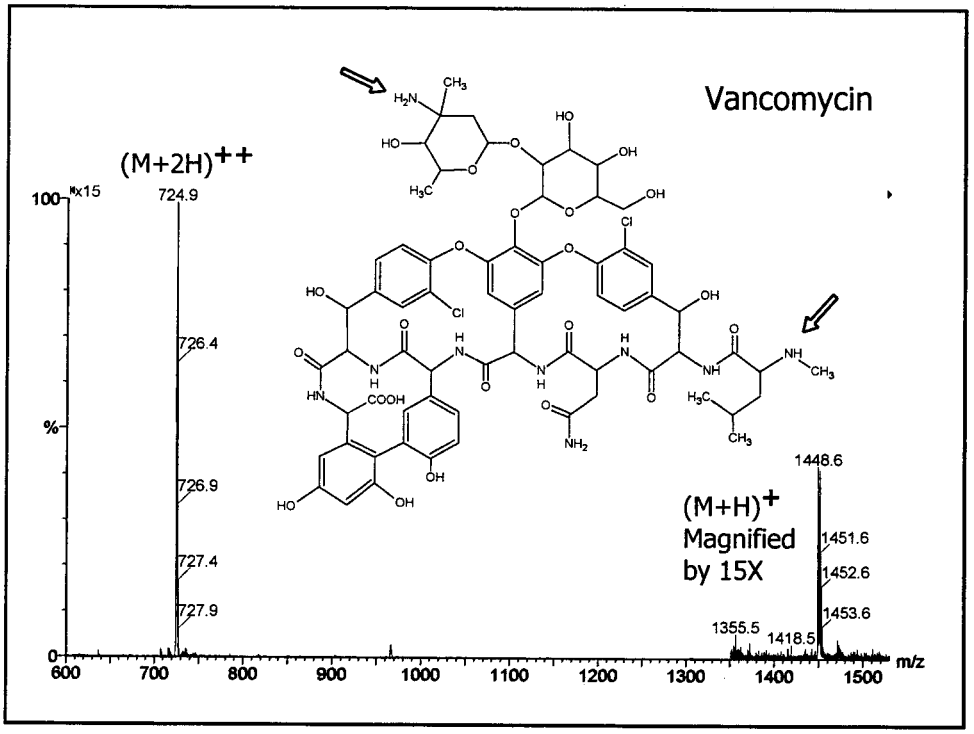


MS-MS of Multiply Charged Ions

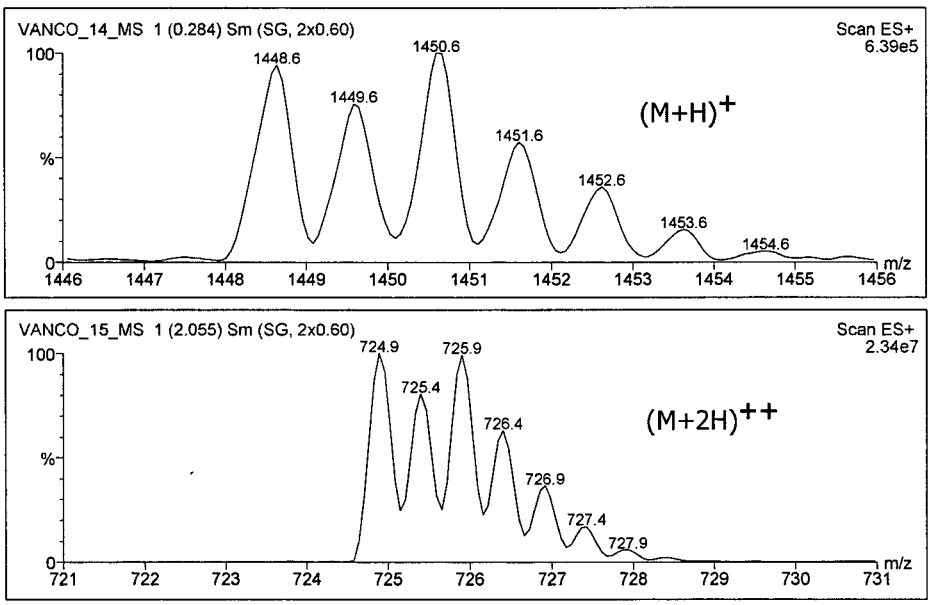
The most intense ions are normally used for MS-MS even if they are multiply charged

Multiply charged ions may require higher collision gas pressures than singly charged ions

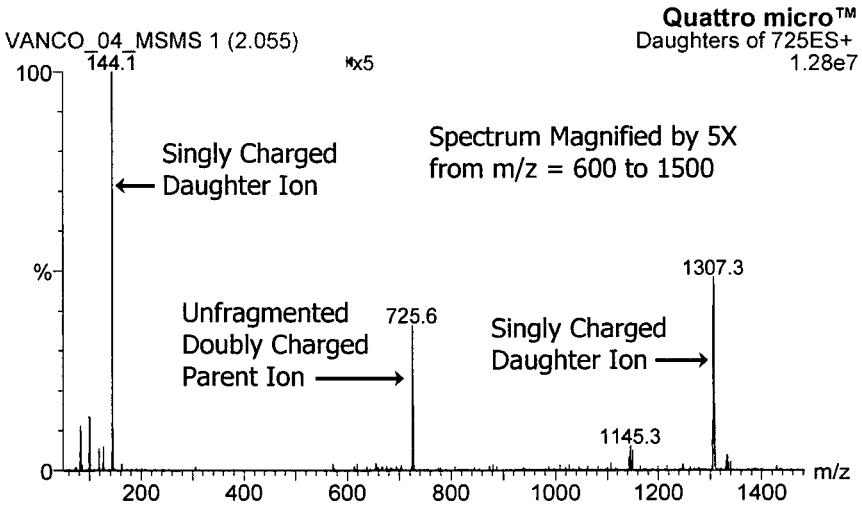
Fragment ions can be larger in apparent mass than the multiply charged precursor ions



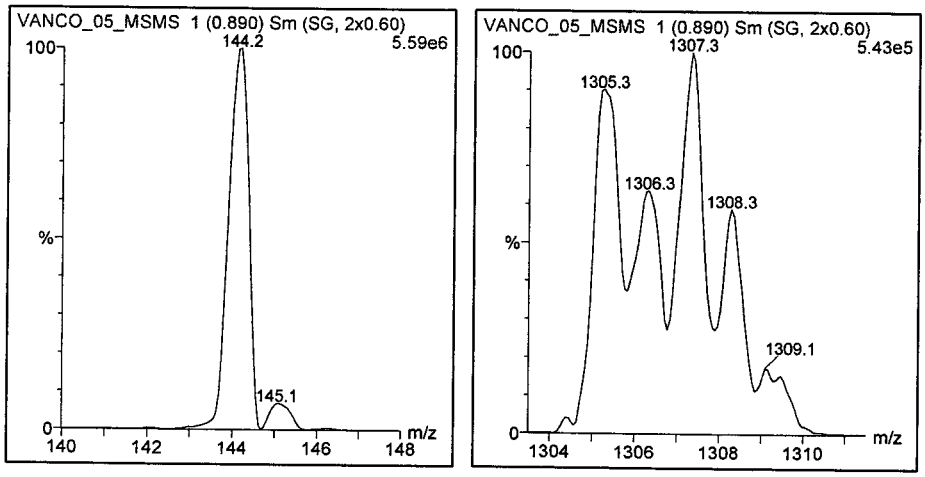
MS Spectra of Vancomycin



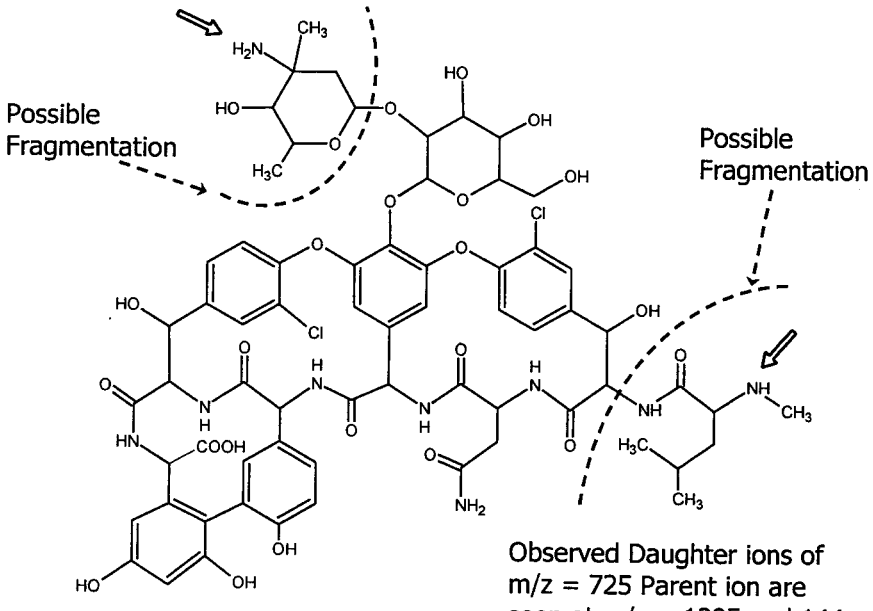
Vancomycin : Daughter Ions of $(M+2H)^{++}$ Ion (m/z=725)



Closer Look at Daughter Ions of $(M+2H)^{++}$ Ion (m/z=725)

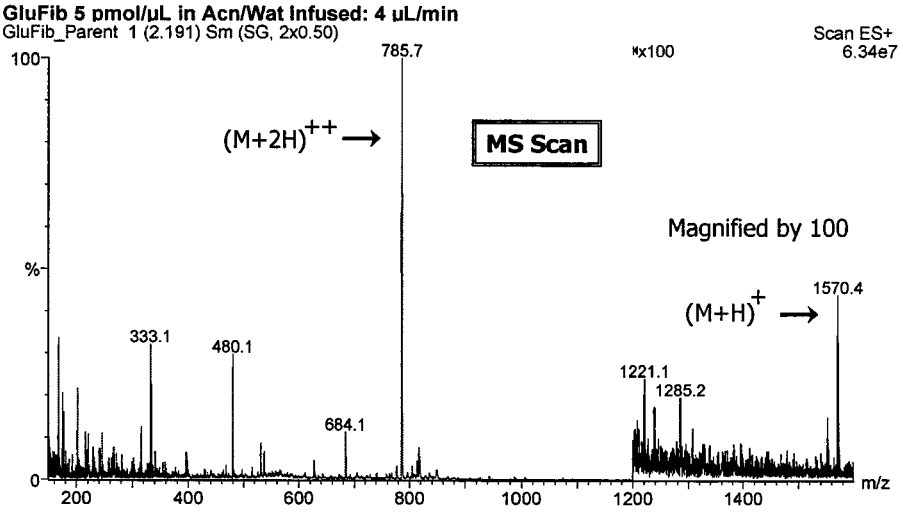


Note: LM & HM of MS1 were opened up to pass all isotopic forms of the m/z=725 ion into the collision cell. 'Isotope Peaks' are 1 Da apart.



Example of MS/MS of a Doubly Charge Ion

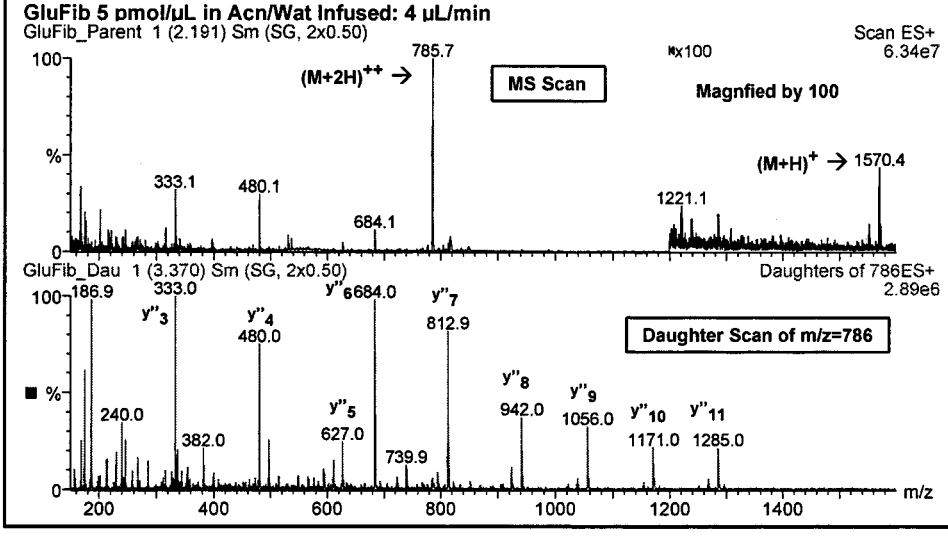
[Glu1]-Fibrinopeptide B Glu-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg



Example of Daughter Ions From a Doubly Charge Ion

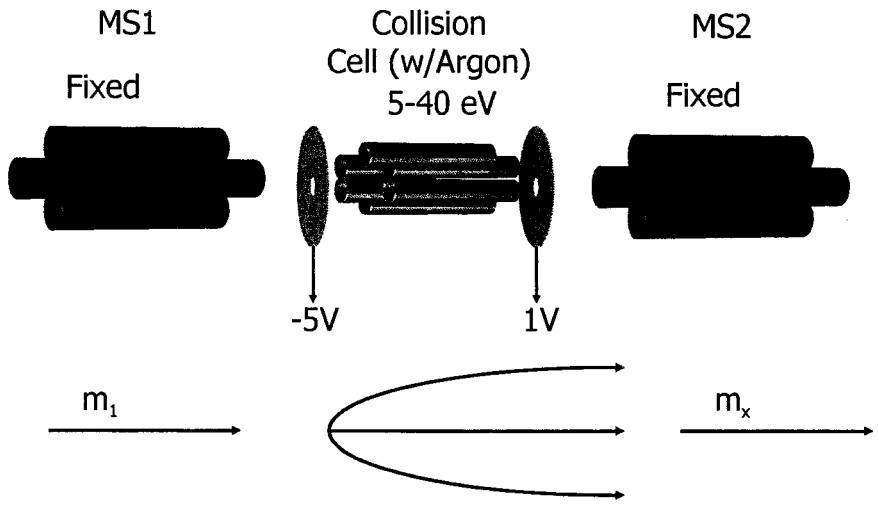
[Glu1]-Fibrinopeptide B Glu-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg

11 10 9



target product

MRM (Multiple Reaction Monitoring)

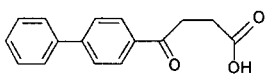


MRM's are used to monitor selected analyte(s) via their daughter ions

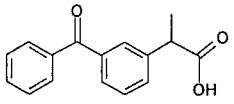
Comparing MRM and SIR - Example 1

Ion Chromatograms from SIR's of m/z=255

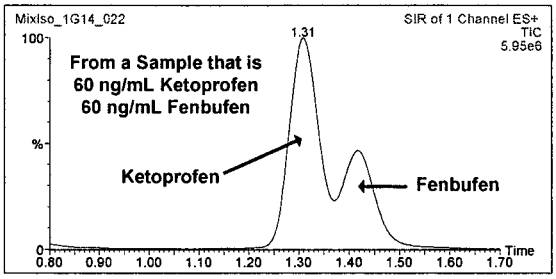
Fenbufen

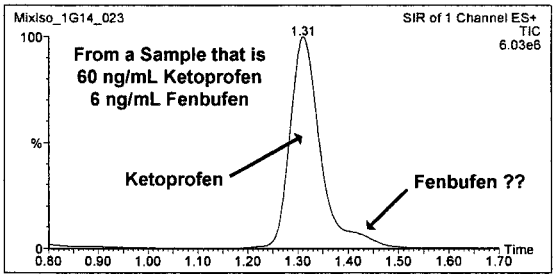


Ketoprofen



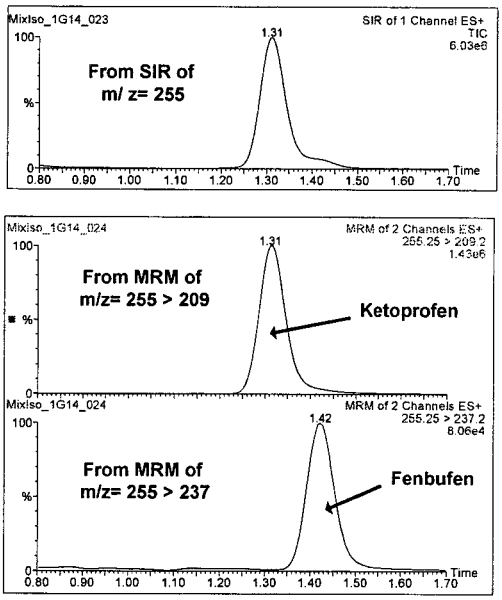
Both have a MW of 254





Comparing MRM and SIR - Example 1 (cont.)

Ion Chromatograms from SIR and MRM Analyses of a Sample that is 60 ng/mL Ketoprofen 6 ng/mL Fenbufen

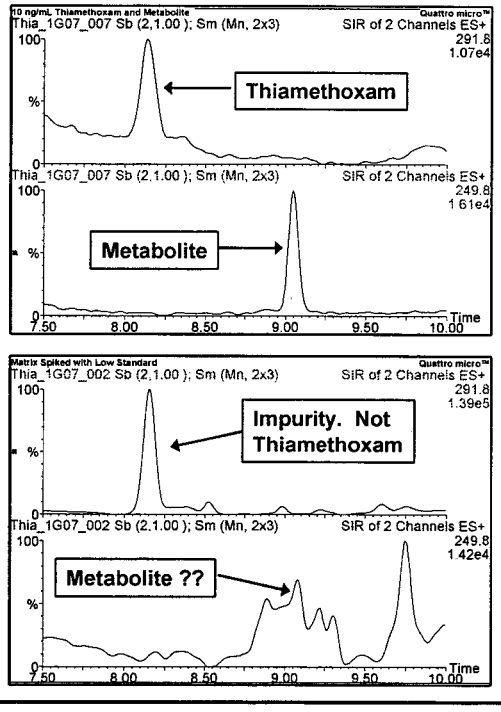


Comparing MRM and SIR Example 2

SIR's of a 10 ng/mL Standard Solution of Thiamethoxam & Metabolite

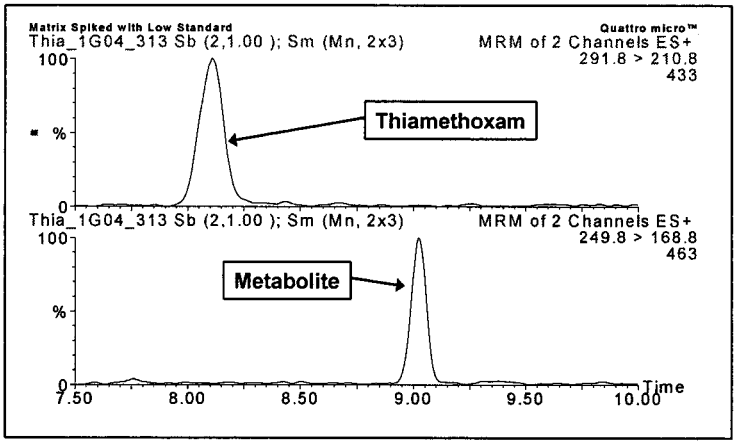
SIR's of Sample of 10 ng/mL Thiamethoxam & Metabolite in a Fruit Matrix

Peak Labeled an impurity in the fruit matrix sample is also present in blank matrix samples.



Comparing MRM and SIR Example 2 (Continued)

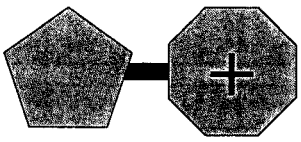
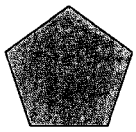
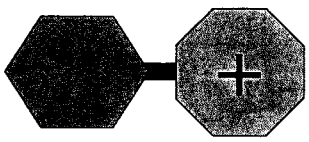
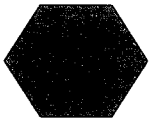
MRM's of Sample of 10 ng/mL Thiamethoxam & Metabolite in a Fruit Matrix



Peaks Labeled as Thiamethoxam and Metabolite are not present in blank matrix samples.

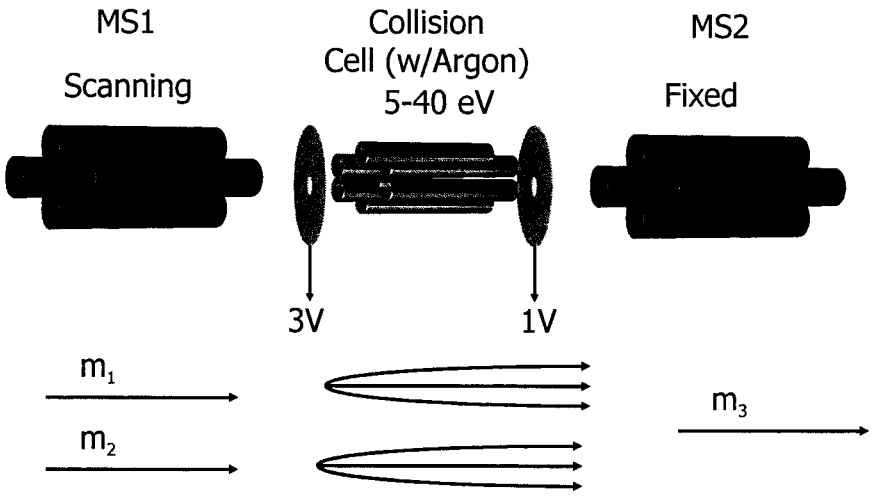
Parent Ion Scan

Consider a class of compounds that are similar in structure:

Different Compounds That Are Somewhat Similar In Structure		Different Neutral Fragments	Same Charged Fragment
	CID →	 +	
	CID →	 +	

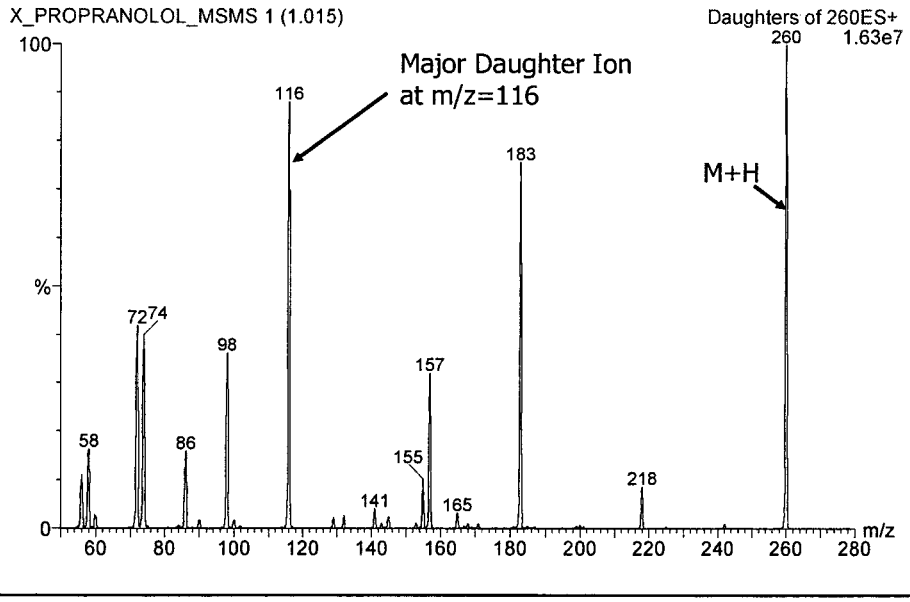
Parent Ion Scans can be used to detect those compounds whose molecular ions produce the same charge fragment.

PAR (Parent Ion Scan)



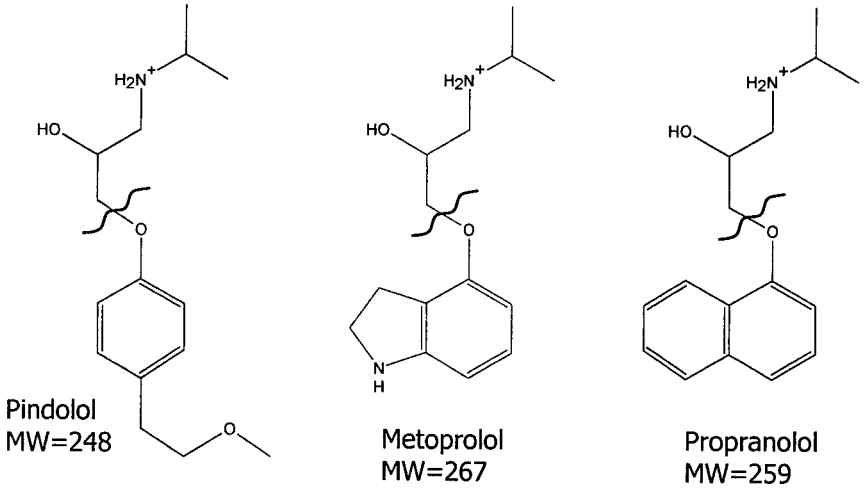
Find ions that will produce via CID, daughter ions with a particular m/z

Parent Ion Scan Example: Lets look at the MS/MS Daughter Ion Scan of a Beta Blocker, Propranolol



Example of Beta Blockers whose M+H Ion All Produce a m/z=116 Daughter Ion

Daughter Ion of m/z=116 produced by CID at spot indicated by ~~~ line.

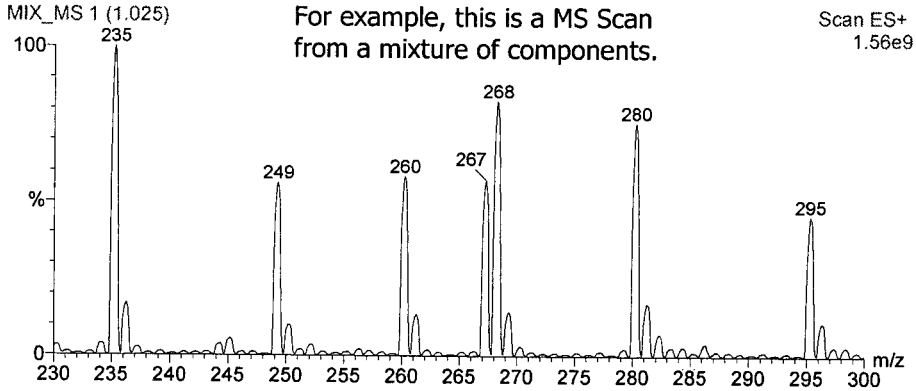


Pindolol
MW=248

Metoprolol
MW=267

Propranolol
MW=259

Parent Ion Scan Example: From a mixture, determine which components are from this class of beta blockers

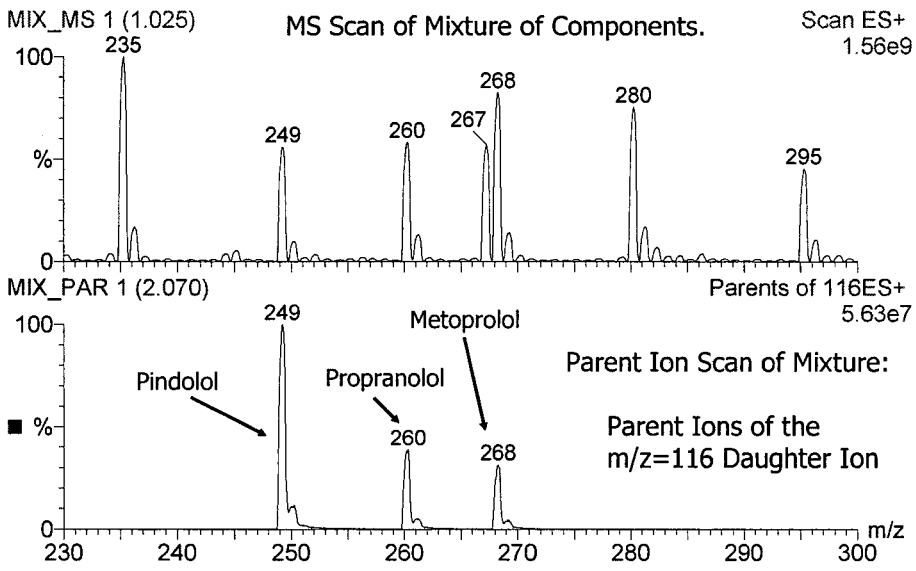


For example, this is a MS Scan from a mixture of components.

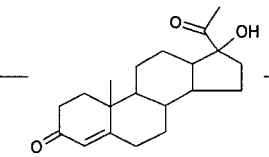
To determine which components belong to this class of beta blockers, you could perform a daughter ion scan for daughter ions of the m/z=235 parent ion and see if the m/z=235 ion produces a m/z=116 daughter ion, then another daughter ion scan on m/z=249, then 260, etc.

Alternatively, you could do one parent ion scan for parents of the m/z=116 daughter ion.

Parent Ion Scan Example:



Parent Ion Scan

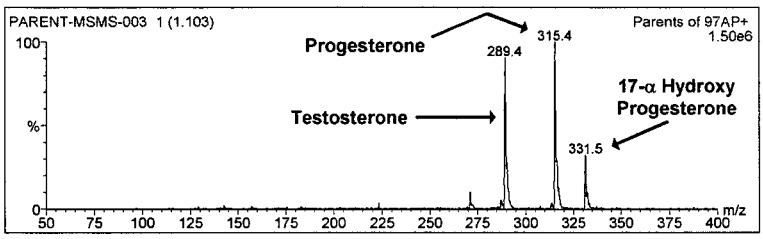


17-α Hydroxyprogesterone
MW = 330

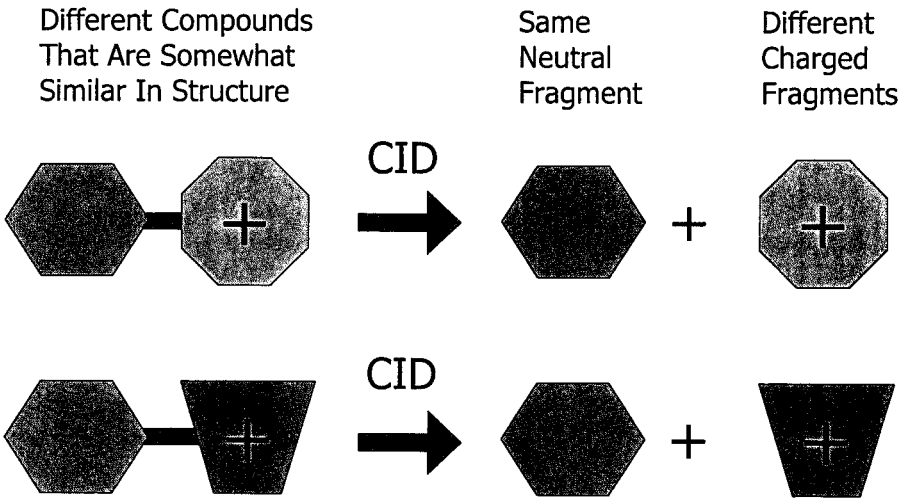
Daughter Scan for Products of m/z = 331 Ions from a 17-α Hydroxy Progesterone Sample

There is a class of steriods whose molecular ions (M+H) produce the m/z=97 and m/z=109 daughter ions when they undergo CID

Parent Scan for Parents of m/z = 97 Ions from a Mixture of Steroids

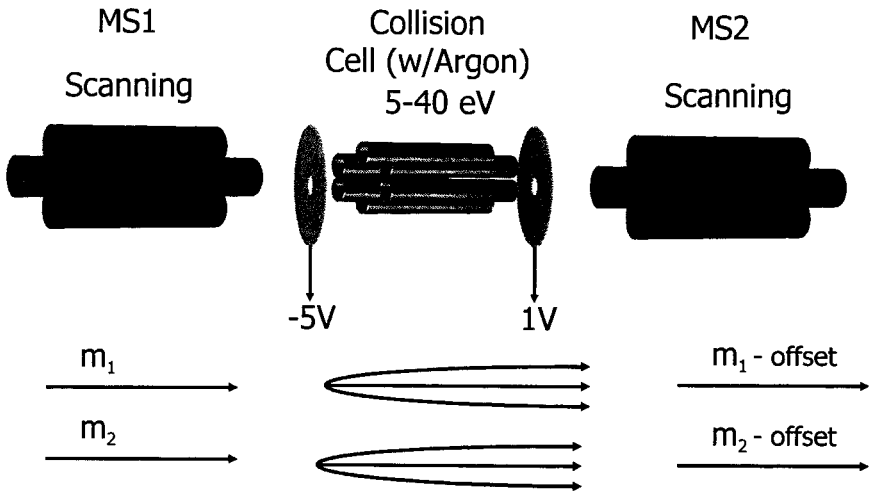


Constant Neutral Loss



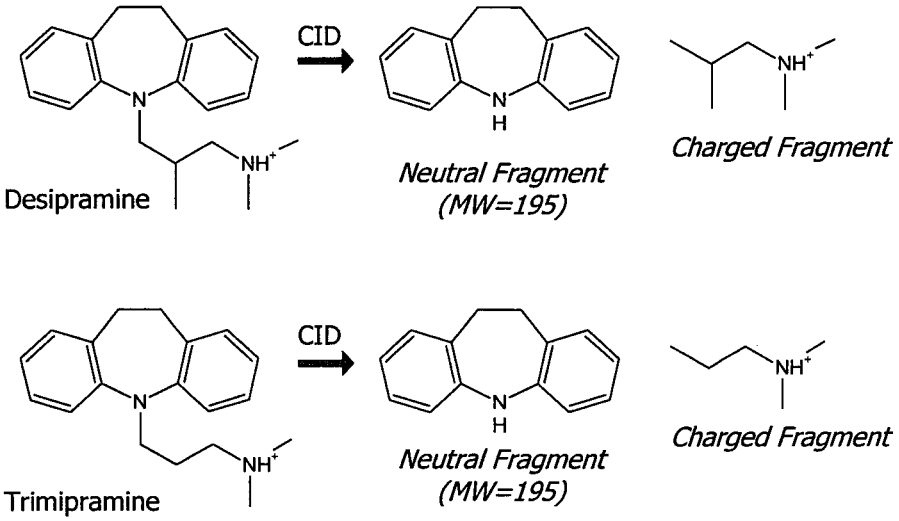
Constant Neutral Loss Scans can be used to detect those compounds whose molecular ions produce the same neutral fragment.

Constant Neutral Loss (CNL) Scan

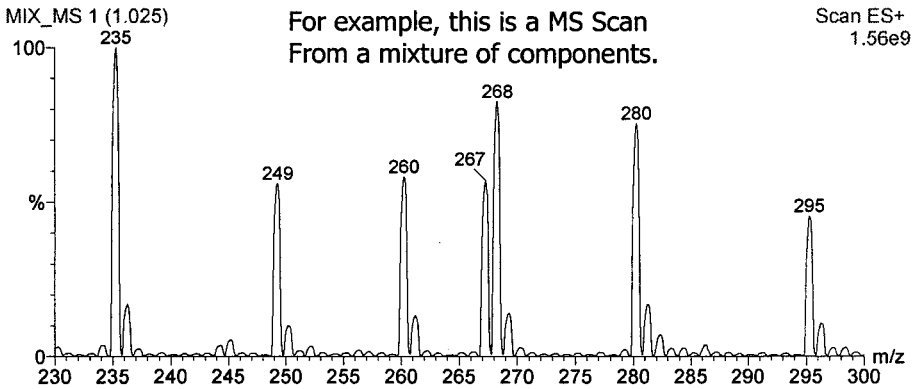


Q1 and Q2 scan together. m/z of Q2 is m/z of Q1 minus an offset.

Tricyclic Antidepressants that All Produce a Daughter Ion After a CID Loss of m/z=195



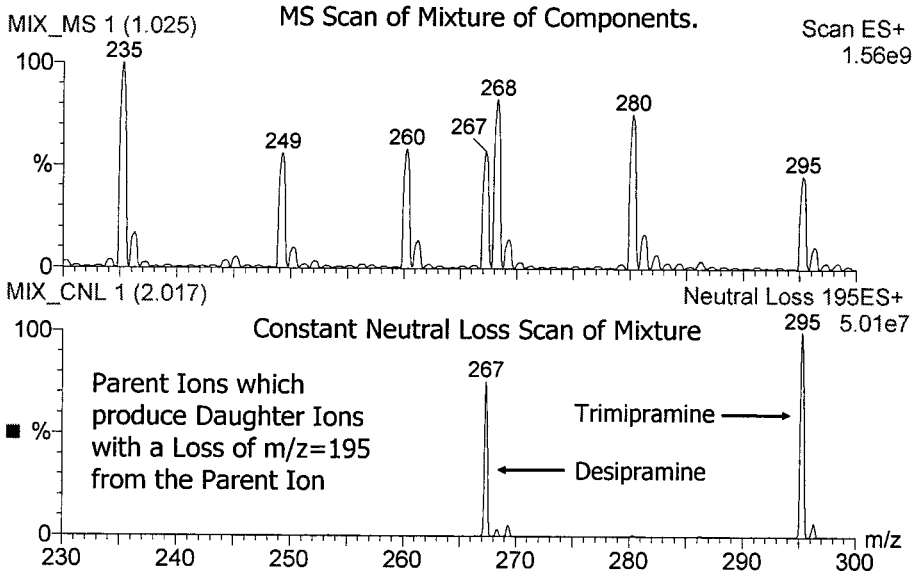
CNL Example: From a mixture, determine which components are from this class of antidepressants



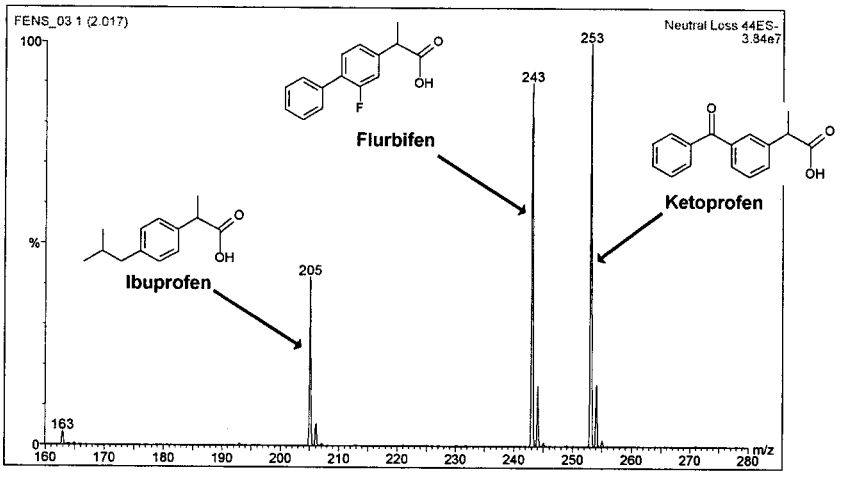
To determine which components belong to this class of tricyclic anti-depressants, you could perform a daughter ion scan for daughter ions of the m/z=235 parent ion and see if the m/z=235 produces a daughter ion that is lighter by m/z=195 (235-195=40), then repeat this with daughter ion scans of m/z=249, m/z=260, etc.

Alternatively, you could do one Constant Neutral Loss scan for losses of 195.

Constant Neutral Loss Scan Example

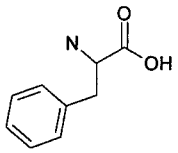


Constant Neutral Loss (CNL) Scan

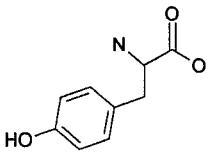


Negative Electrospray Neutral Loss Scan for loss of m/z = 44 Da from a Mixture of Compounds

Analysis of Amino Acids by MS/MS – CNL

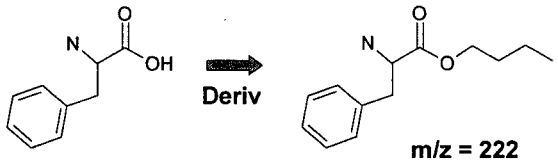


Phenylalanine

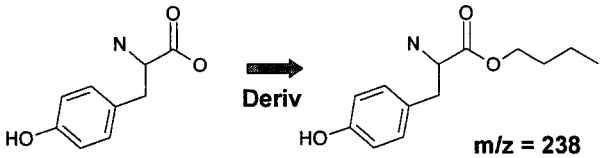


Tyrosine

Analysis of Amino Acids by MS/MS – CNL

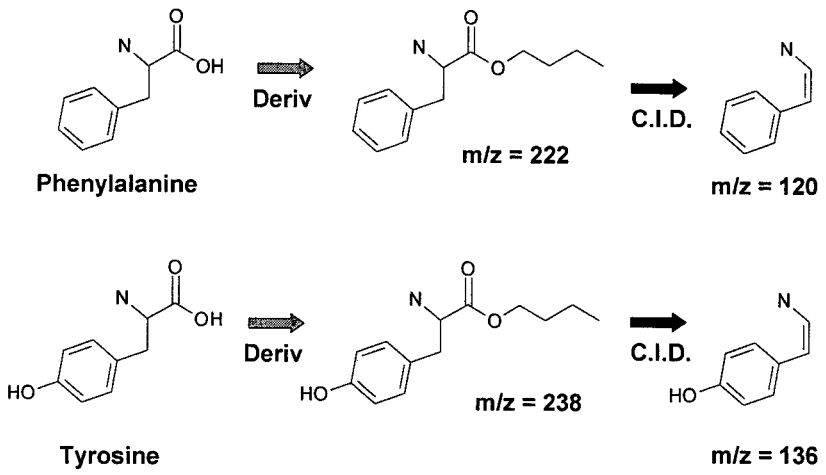


Phenylalanine



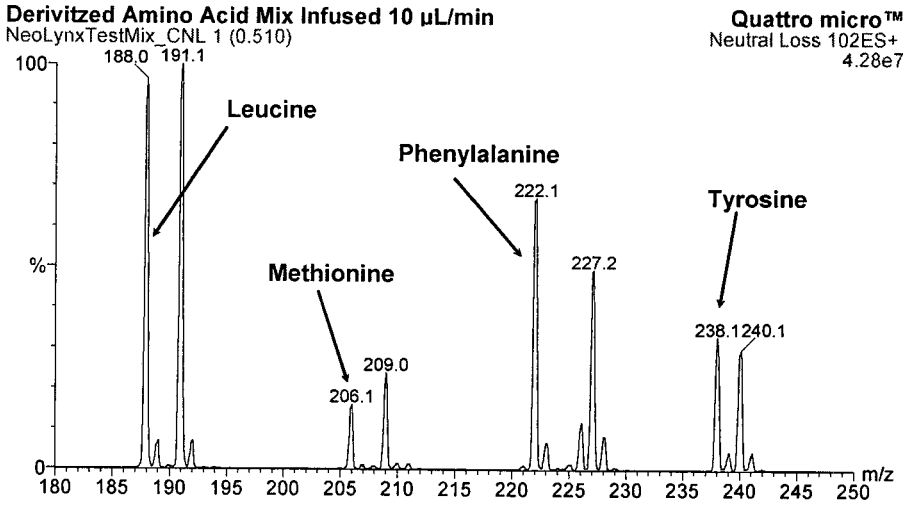
Tyrosine

Analysis of Amino Acids by MS/MS – CNL



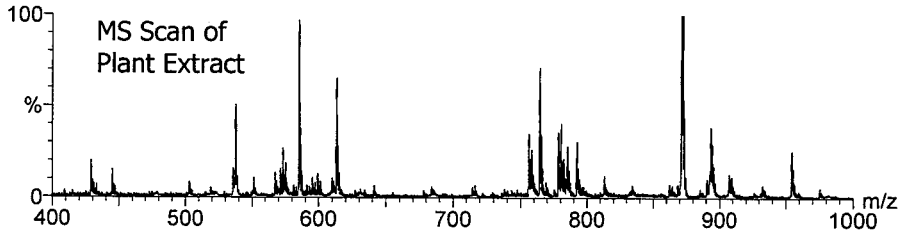
CID Results in the Loss of 102 Da

Analysis of Amino Acids by MS/MS – CNL



Other peaks are from deuterated forms of these amino acids

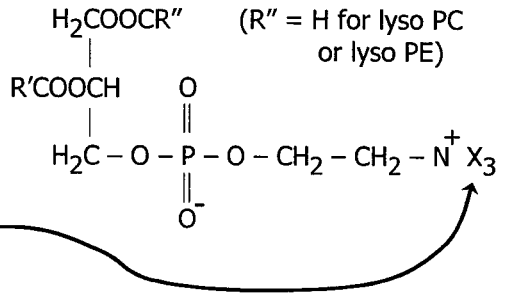
Phospholipids – Plant Extracts



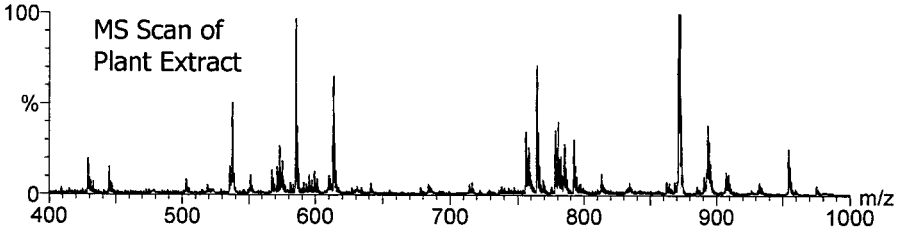
L-Phosphatidylcholine
(PC and lyso PC)

L-Phosphatidylethanolamine
(PE and lyso PE)

X = CH_3 for PC & lyso PC
X = H for PE and lyso PE

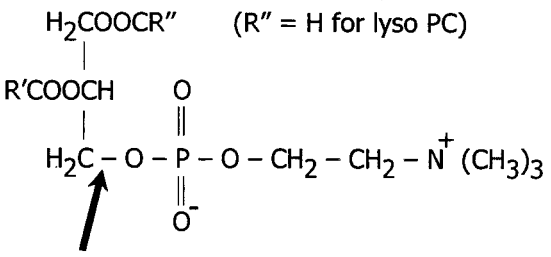


Phospholipids – Precursor Ion Scan Example

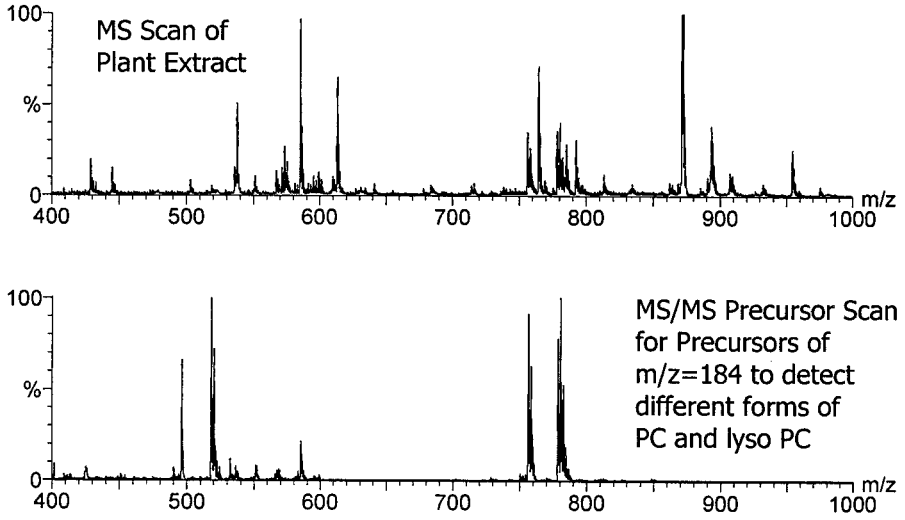


L-Phosphatidylcholine
(PC and lyso PC)

Positive ion electrospray:
When PC and lysoPC ionize and fragment at the red arrow, the bottom right fragment leaves as a charged ion with m/z=184.

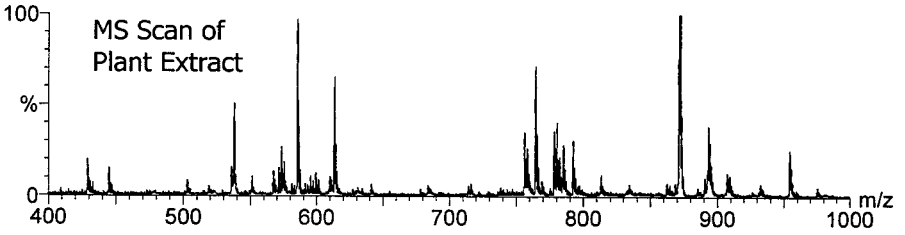


Phospholipids – Precursor Ion Scan Example



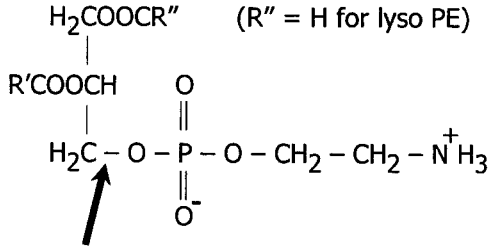
Samples provided by Dr. Ruth Welti, Kansas State University, Manhattan, KS

Phospholipids – Constant Neutral Loss Example

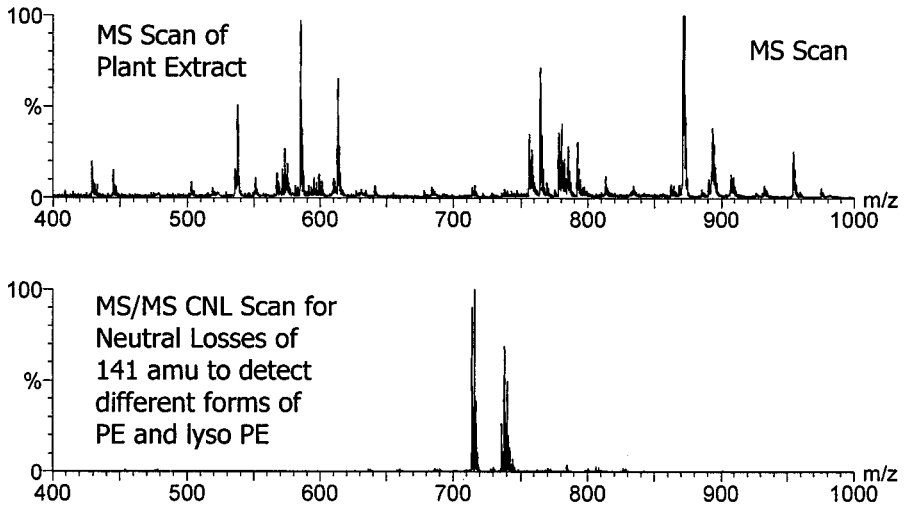


L-Phosphatidylethanolamine
(PE and lyso PE)

Positive ion electrospray:
When PE and lyso PE ionize and fragment at the red arrow, the bottom right fragment leaves as a neutral fragment of mass 141.

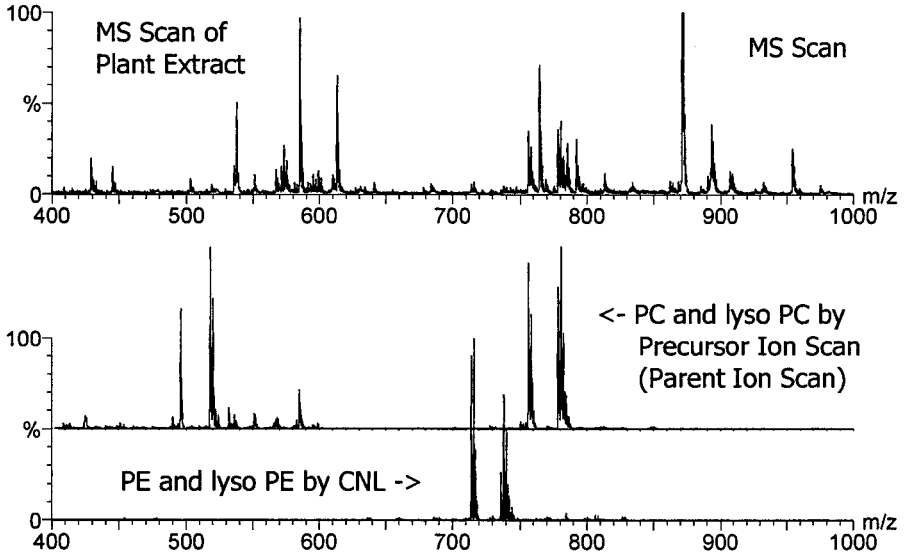


Phospholipids – Constant Neutral Loss Example

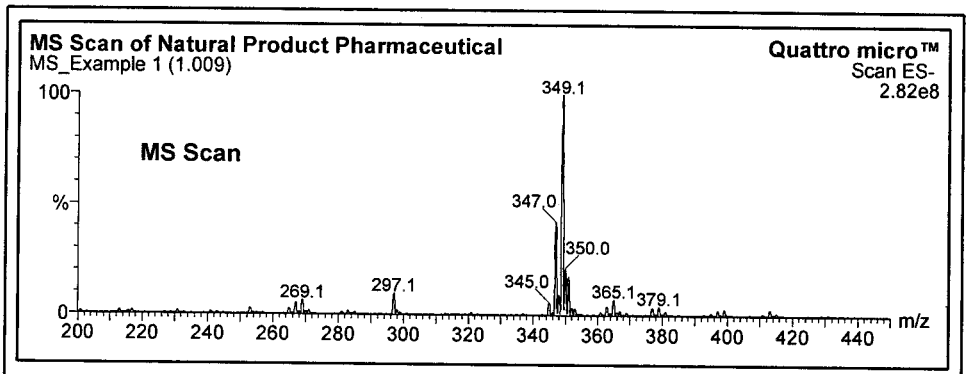


Samples provided by Dr. Ruth Welti, Kansas State University, Manhattan, KS

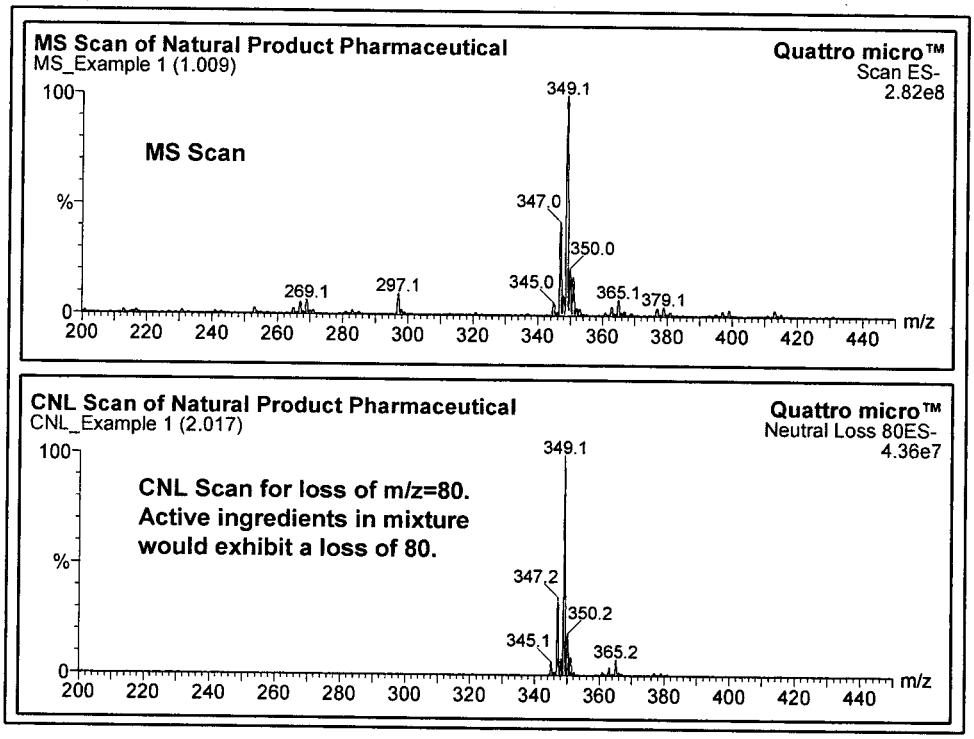
Phospholipids – Neutral Loss and Precursor Ion

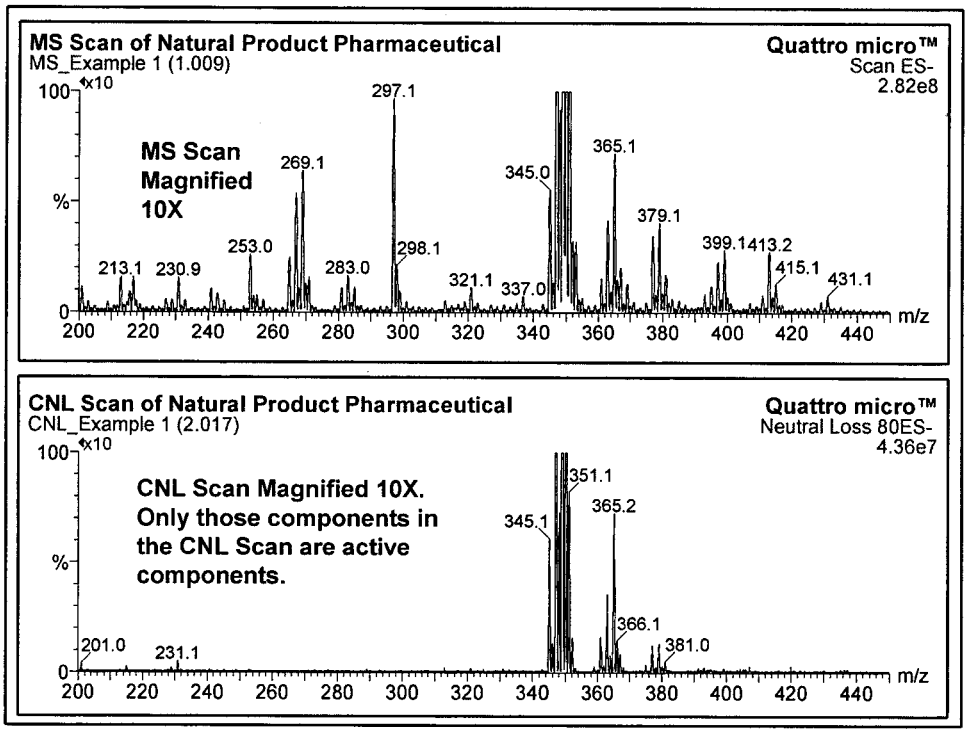


Samples provided by Dr. Ruth Welti, Kansas State University, Manhattan, KS



Example of a MS Scan of a natural product pharmaceutical.





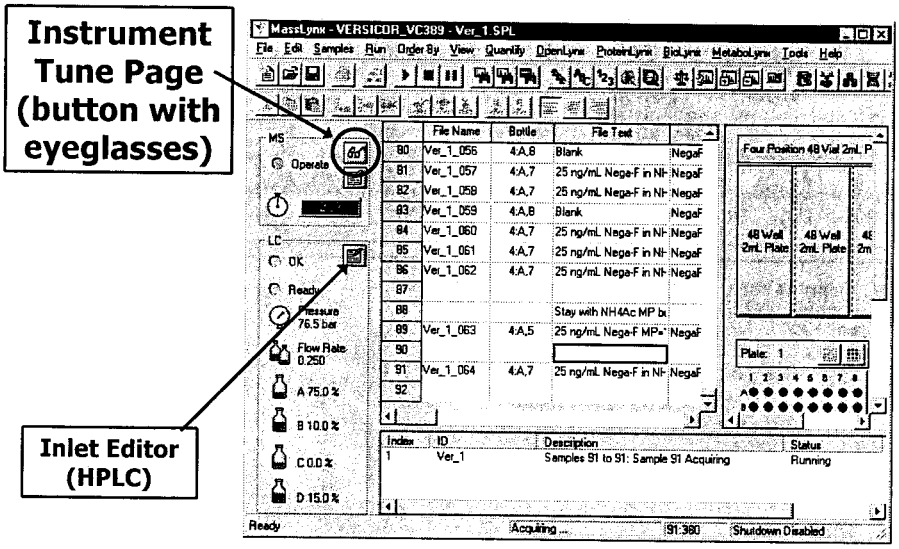
Waters

Quattro Instrument Tune Page

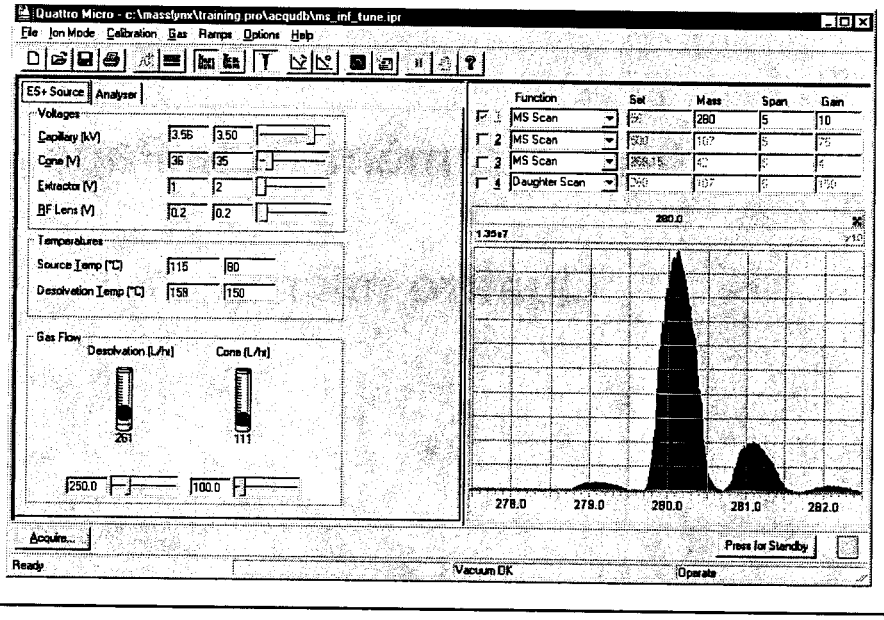
Quattro micro

MICROMASS
MS TECHNOLOGIES

Access the Instrument Tune Page from the Main MassLynx Window



Example of Quattro *micro* Instrument Tune Page

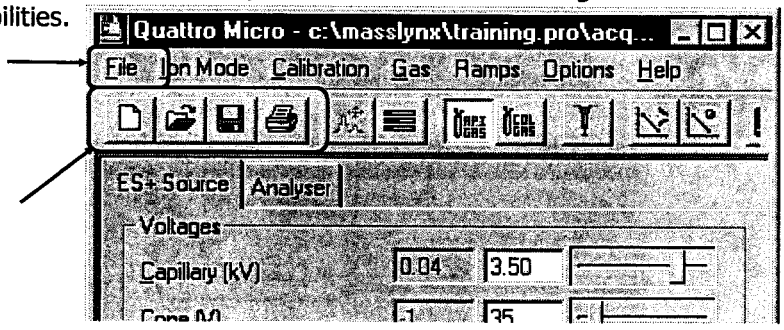


Tune Page - *the brain of the mass spec*

- Ion mode is set
- Instrument parameters are set.
- API and Collision gases are controlled.
- Parent compounds are identified and optimized
- Daughter ions are determined and optimized
- Mass Resolution is set
- Collision Energies are determined
- Acquisitions performed
- PMT is adjusted when necessary

Instrument Parameters Can Be 'Saved' in a File.

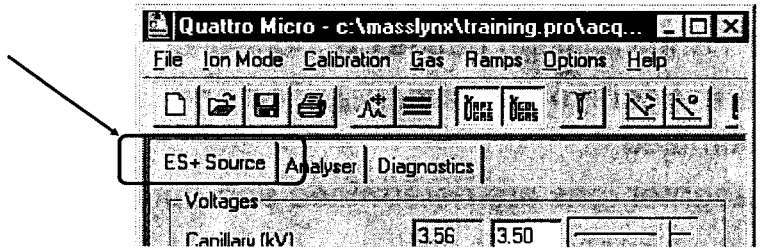
Instrument tune page parameters can be stored in a file for future use. As with most MS Windows programs that save data in a file, the 'Open' (to load previously saved parameters back into the mass spectrometer), 'Save' (store current instrument parameters) and 'Save As' (save the parameters under a different file name) features are available along with 'Print' capabilities.

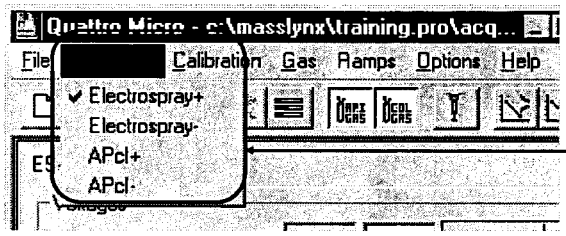


Use the 'New', 'Open', 'Save' or 'Print' buttons or use the 'File' menu for these operations.

Current Ion Mode of the Mass Spectrometer is Operating is Shown on the Front Tab

In the example shown below, the mass spectrometer is operating in the positive ion electrospray mode.

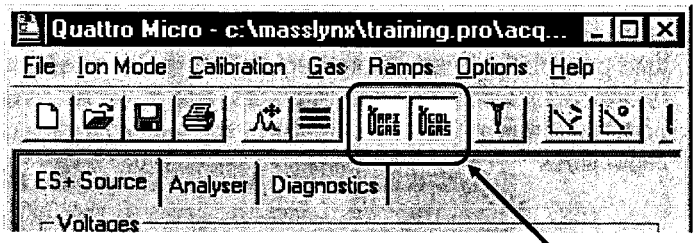


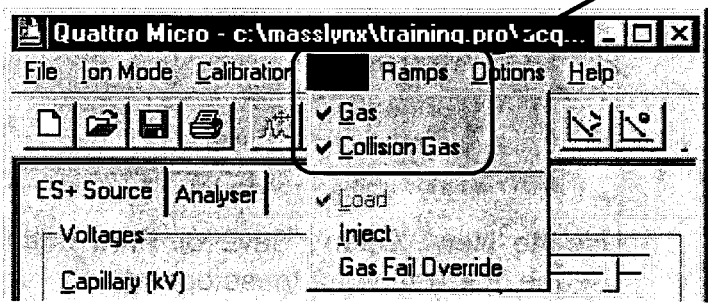


The Ion Mode Can be Changed Using the 'Ion Mode' Menu

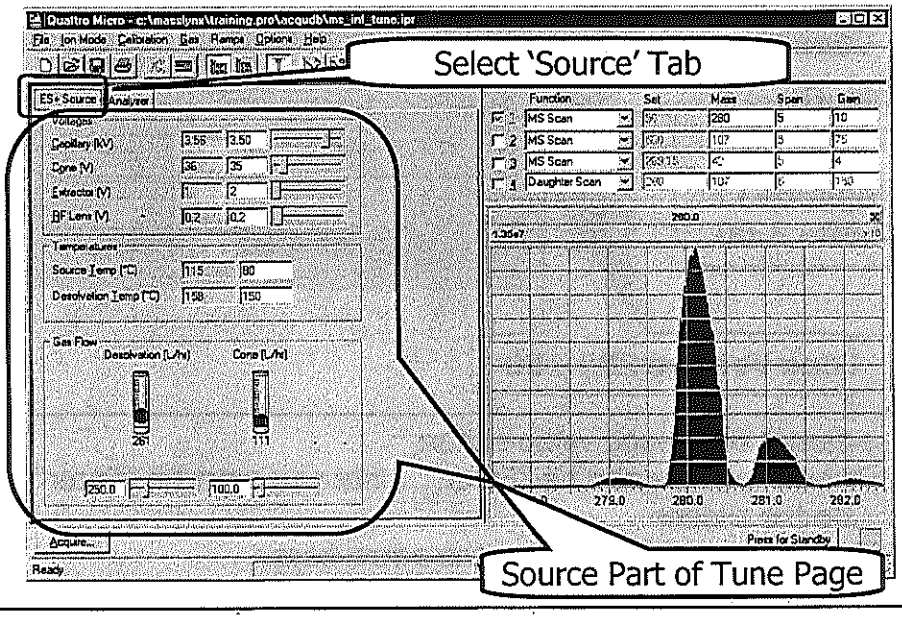
Nitrogen Gas and Collision Gas (Ar)

These Gases Can be Toggled On and Off Using Buttons or Tune Page Menu Items

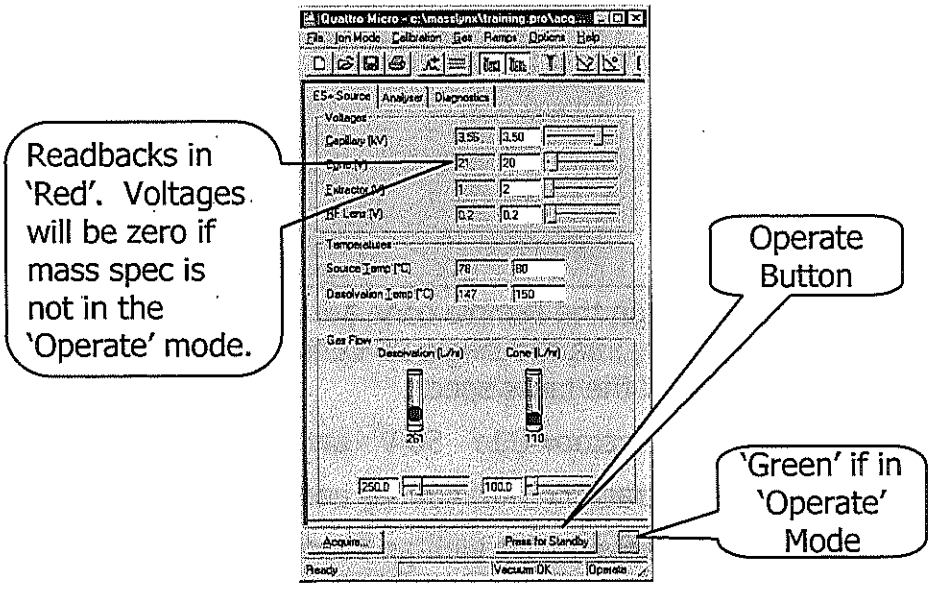




Example of Tune Page – Source Controls



Example of Electrospray Ion Source Controls

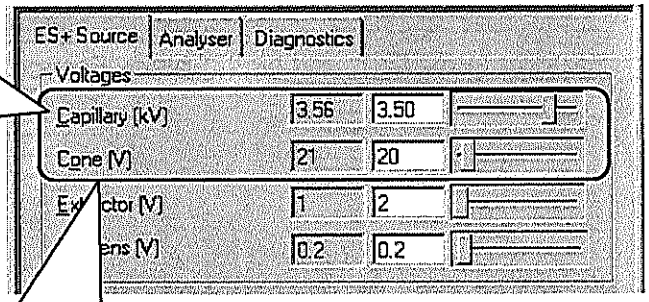


Setting Capillary and Cone Voltages

Capillary

Positive ES
2.50 - 3.50

Negative ES
2.00 - 3.00

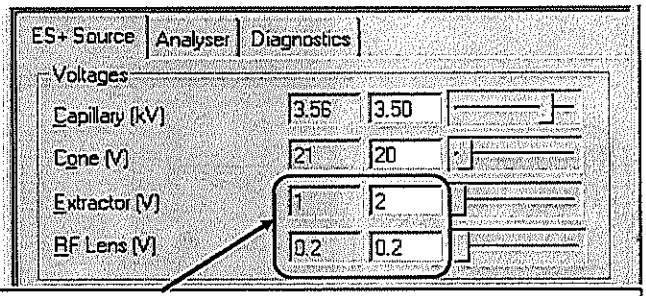


Cone Voltage 20 - 100

To adjust a tune page value either:
1) Type in a value and hit "Enter". Note you must hit "Enter".
2) Use the slider
3) Click on the slider and use the arrow keys.

Source Settings: Extractor and RF Lens Voltages

Quattro micro and Quattro LC Extractor and RF Lens Settings



Ions flowing through the sample cone are pulled through the 'Extractor' opening, into the single RF Lens and then enter the analyzer section of the mass spec.

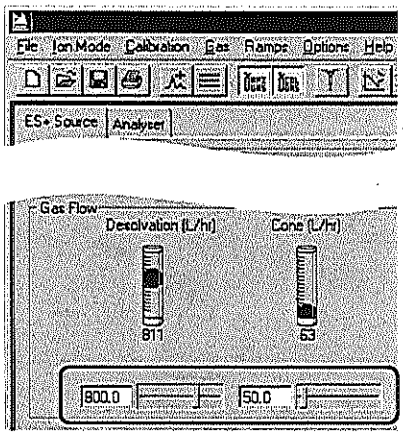
Quattro LC: Extractor= 0 to 50 V and RF Lens= 0 to 1 V

Quattro micro: Extractor= 0 to 400 V and RF Lens= 0 to 5 V

It is suggested that you start with an Extractor Voltage of 2 to 3 volts and a RF Lens Voltage of 0.2 to 0.5 volts.

Quattro micro™ Tune Page: Nitrogen Gas Flows

N2 Gas Flows Are Controlled From the Source Page



Use the sliders or enter values for the Desolvation & Cone gas flows.

Electrospray - Typical Source Temperatures and Gas Flows

Infused Sample
(e.g. Flow = 10 µL/min)

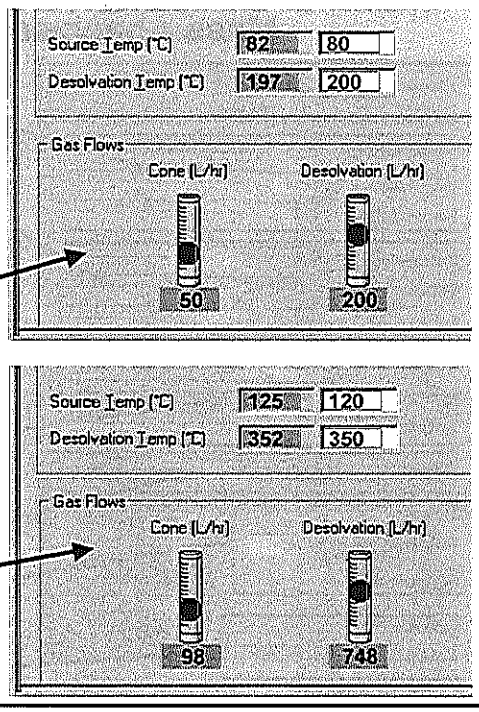
Source= 70-100 C
Desolvation = 150-200 C

Cone = 50-100 L/hr
Desolvation = 200-300 L/hr

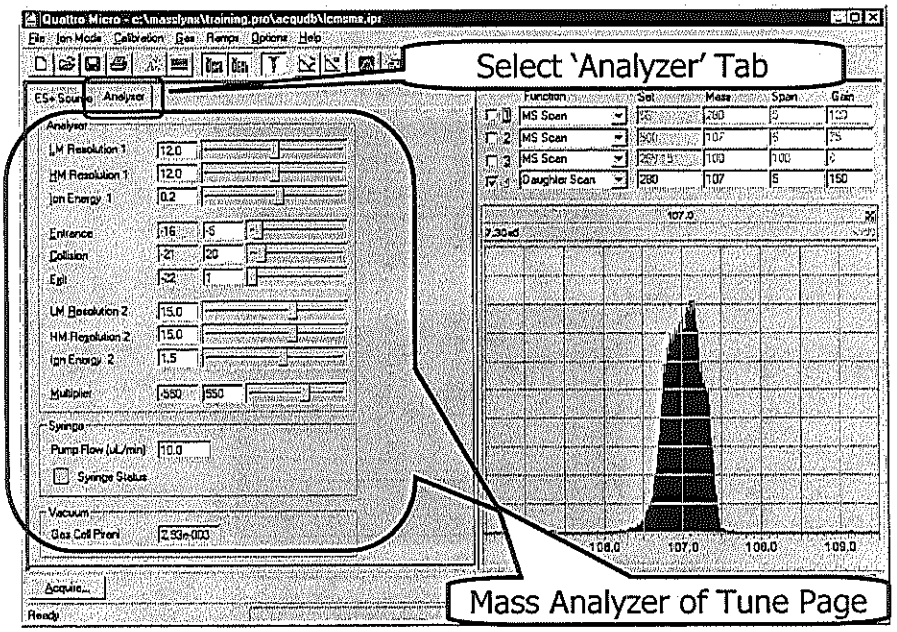
LC/MS Sample
(e.g. Flow = 0.2 mL/min)

Source= 120-130 C
Desolvation = 350-450 C

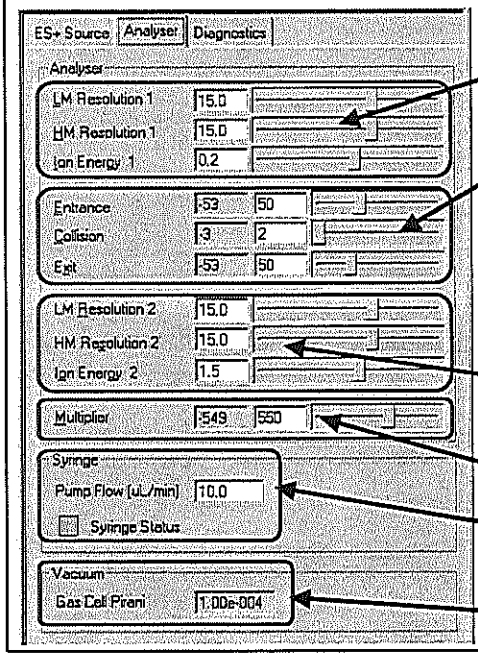
Cone = 75-150 L/hr
Desolvation = 650-850 L/hr



Mass Analyzer Part of Tune Page



Mass Analyzer Part of Tune Page



MS 1 Settings: Mass filters ions entering the analyzer section.

Collision Cell: In MS: Set to optimize transmission of ions selected by MS1 and through the rest of the analyser to the detector. In MS/MS: Set to fragment ions by CID and pass them to MS2.

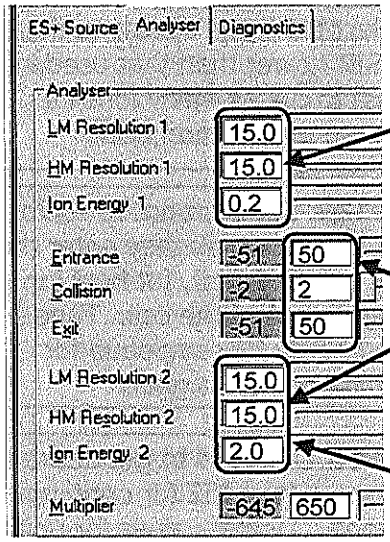
MS2 Settings: In MS/MS mass filters ions coming out of the collision cell.

Detector Multiplier Voltage

Infusion Syringe Speed

Pressure in Collision Cell (mBar)

Typical Analyzer Settings - MS Scan
(PMT Multiplier typically run between 500 to 650 V)



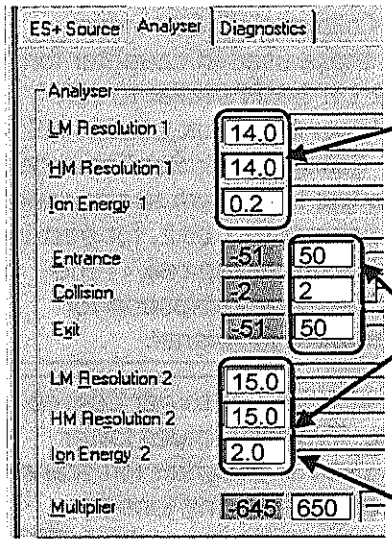
MS Scan

MS1: Select LM & HM to get desired resolution. Adjust IE1 for optimum peak shape. Typical values for unit resolution are shown

Collision Cell and MS2: Settings are chosen to optimize transmission of ions selected by MS1 through the rest of the analyser to the detector.

Use IE2 of 2-4, Adjust for Maximum Signal

Typical Analyzer Settings - SIR



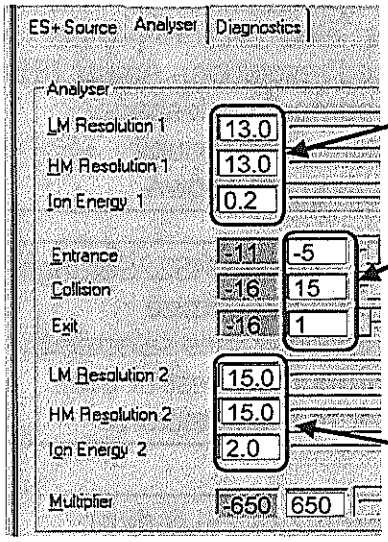
SIR

MS1: Select LM & HM to get desired resolution. Adjust IE1 for optimum peak shape. Typically lower LM & HM settings are used than those used in MS Scans.

Collsion Cell and MS2: Same as for MS Scans: Settings are chosen to optimize transmission of ions selected by MS1 through the rest of the analyser to the detector.

Use IE2 of 2-4, Adjust for Maximum Signal

Typical Analyzer Settings - Daughter Scan (Note: Collision Energy is Compound Dependent)



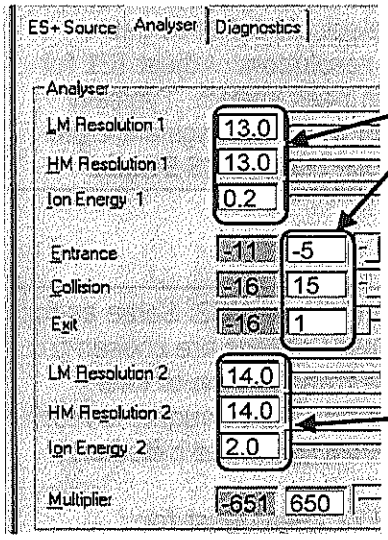
Daughter Scan

MS1: Select LM & HM similar to that used in SIR (lower than that used in MS Scans) to get as many ions as possible into the collision cell.

Collision Cell: Set Entrance to –5 to slow the ions down as they enter the cell. Set exit to 1 or 2, for best ion transmission out of the cell. Tune Collision Energy by compound for largest daughter ion signal.

MS2: Select LM & HM to get desired resolution. Adjust IE2 for optimum peak shape. Typical values for unit resolution are shown.

Typical Analyzer Settings - MRM (Note: Collision Energy is Compound Dependent)

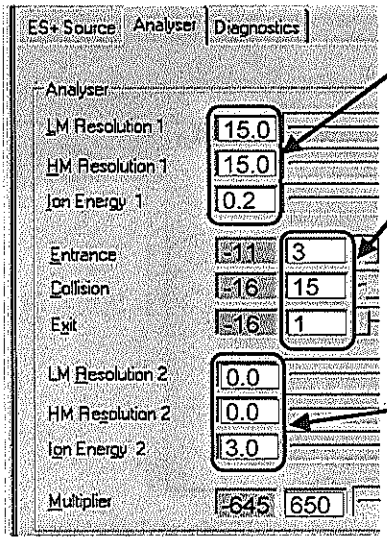


MRM

MS1 and Collsion Cell: Use values similar to those used in daughter ion scans. Set LM & HM similar to SIR settings (lower than that used in MS Scans). Collision Cell: Set Entrance to –5 to slow the ions down as they enter the cell. Set exit to 1 or 2, for best ion transmission out of the cell. Tune Collision Energy by compound for largest daughter ion signal.

MS2: Select LM & HM to get desired resolution. Typically values lower than that used in daughter ion scans are used to get as many ions as possible to the detector. Adjust IE2 for optimum peak shape.

Typical Analyzer Settings - Parent Scan (Note: Collision Energy is Compound Dependent)



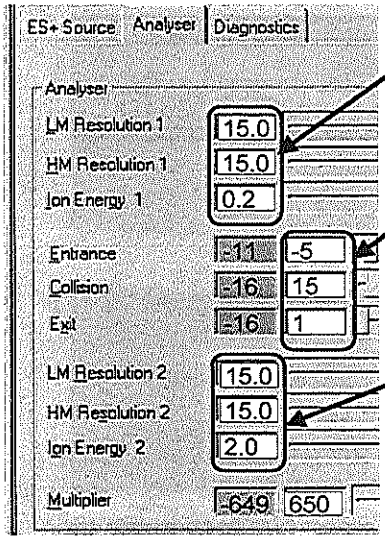
Parent Scan

MS1: Use settings similar to those used in MS Scans.

Collision Cell: Set Entrance to 3 and Exit to 1. Use a collision energy appropriate for the compound. Sometimes a slightly higher energy and a lower collision gas pressure will help get better results.

MS2:
LM and HM should be in the 0-5 range and IE2 to approximately 3.

Typical Analyzer Settings – Constant Neutral Loss (CNL&CNG) Note: Coll. Eng. is Compound Dependent)



CNL

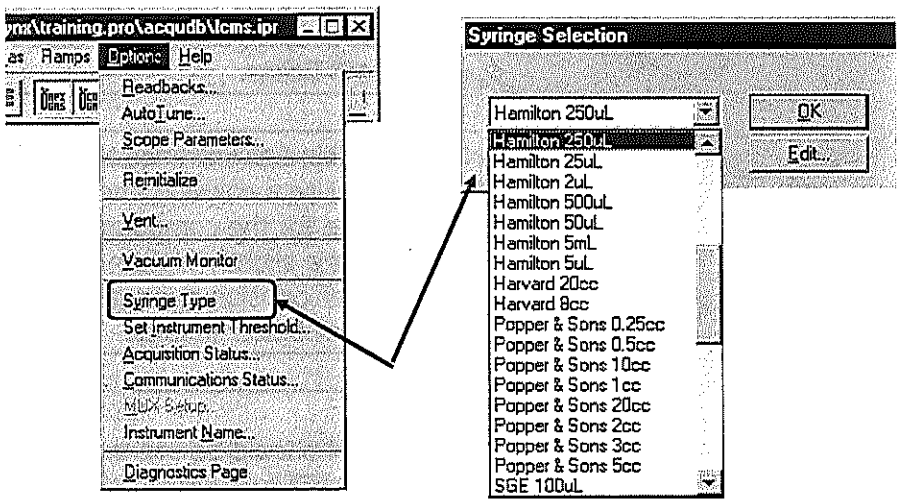
MS1: Use settings similar to those used in MS Scans.

Collision Cell: Use values similar to those used in daughter ion scans and MRM's.

MS2: Use settings similar to those used in daughter ion scans.

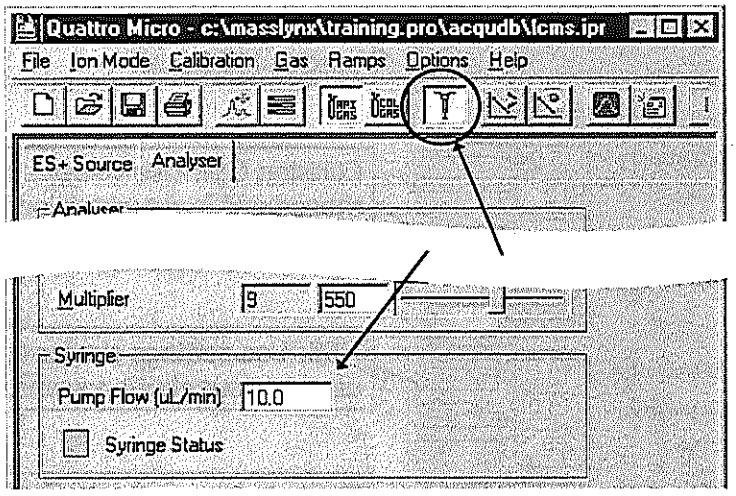
Best results are obtained using slower scan rates (~100 amu/sec)

Quattro micro™ Tune Page: Syringe Operation



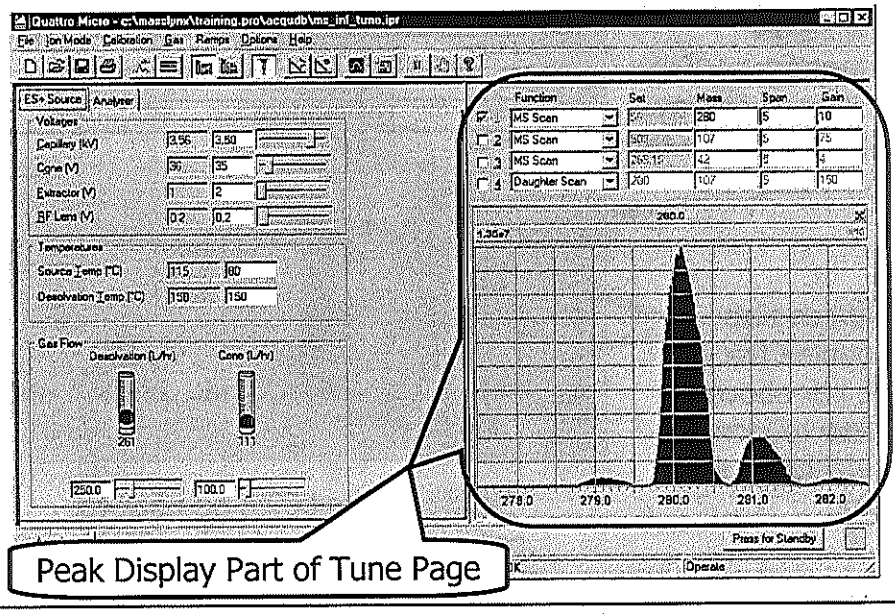
From the 'Options' menu on the tune page, select the 'Syringe Type' menu item to display a dialog box from which to select your type of syringe.

Quattro micro™ Tune Page: Syringe Operation



Enter the desired flow rate and use the 'Syringe' button to toggle the syringe pump on and off.

Peak Display on Instrument Tune Page



Example of Peak Display Setup for a MS Scan

Display # 1 set for MS Scan

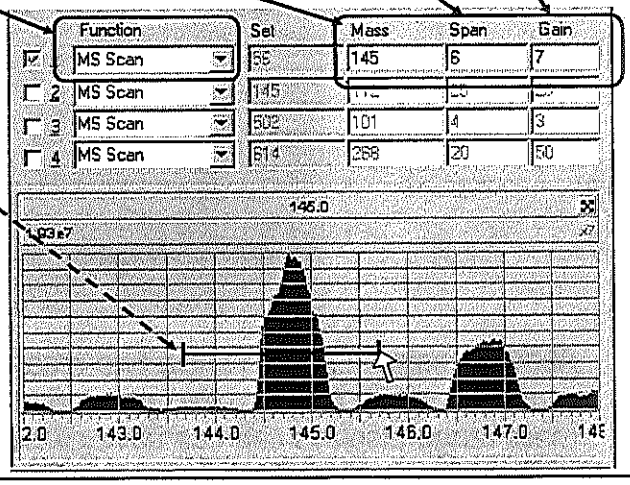
Mass of the Center of the Display

Span or Width of the display in amu (Da)

Signal Gain of the Display

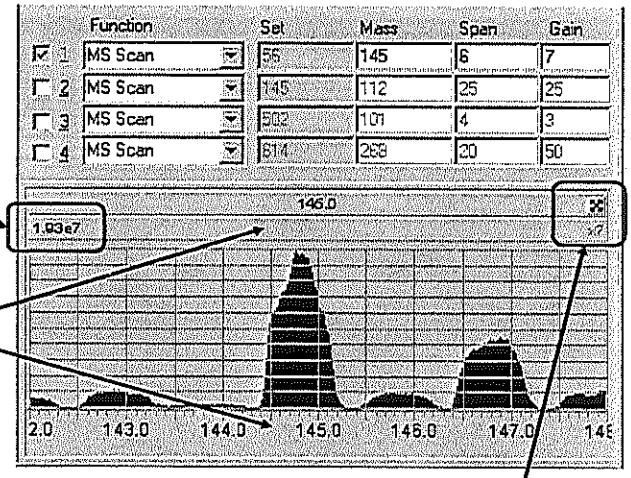
You can 'Left Click' and 'Drag' over a mass range to 'zoom in' on a peak.

Note the Signal Gain controls only how the data is displayed. It does not effect the mass spectrometer



More on Example of Peak Display Setup for a MS Scan

Ion Signal strength at 100% (Y axis of display goes from zero to this value)

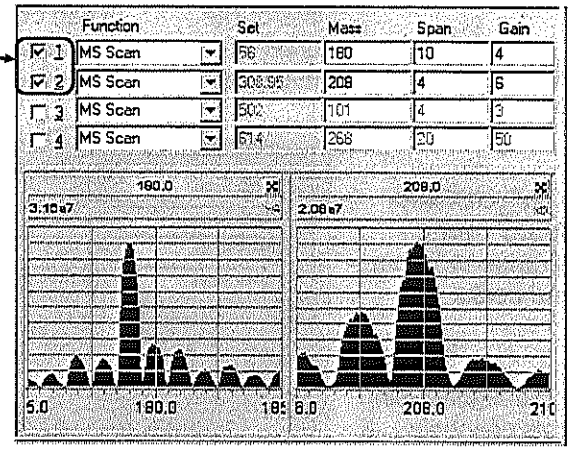


You can also 'Double Click' in the bar above or below to increase or decrease the gain.

'Click' once on 'X" to go back to previous range and gain, 'Click' again to go back to default settings.

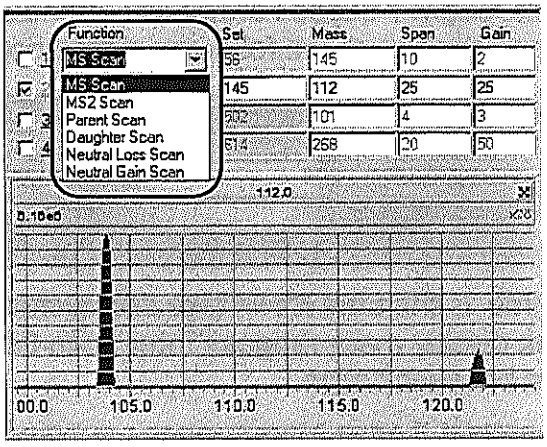
Multiple Spectra Can Be Displayed

Click on Displays you want to show



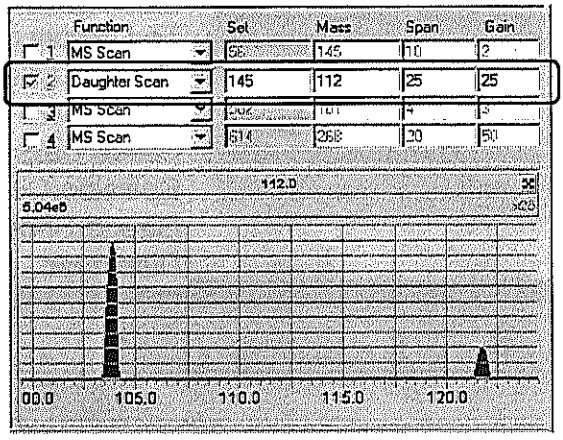
Above example is set to show two mass ranges, one centered at 180 Da and one centered at 208. Note that mass range displays can can have different spans and gains.

Other Types of Spectra Can Be Displayed



The peak display can be set to MS1, MS2, Parent, Daughter, Neutral Loss or Neutral Gain Scans using the drop down menu for each display.

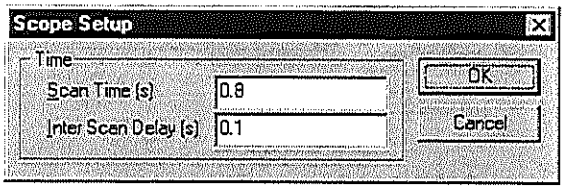
Example of Peak Display Setup for a Daughter Ion Scan



In the above example, the second window has been set up to show the Daughters of the Parent with m/z=145. The Daughter ion scan is centered on 112 Da. The span is 25 so the scan is from 99.5 to 124.5.

Scope Parameters - Can be set from the Tune Page

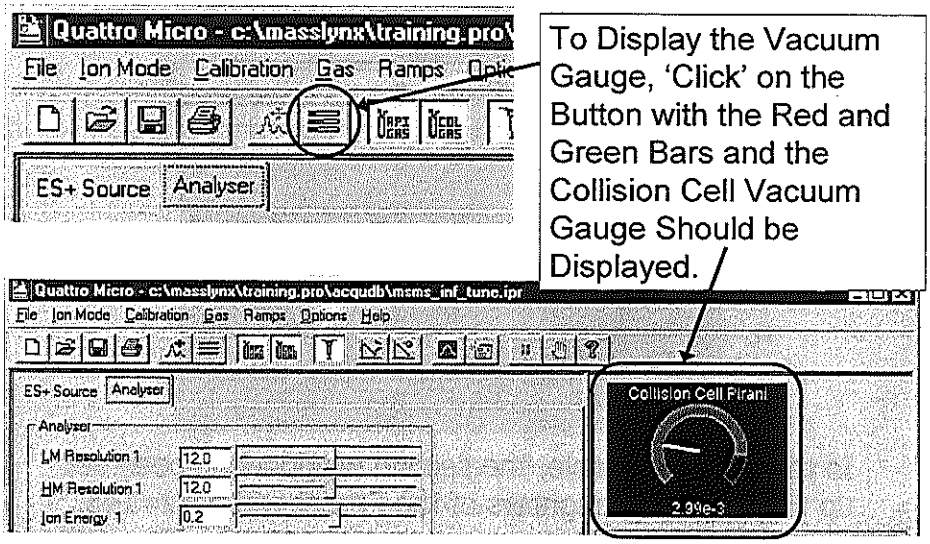
Use the 'Options / Scope Parameters' menu Item on the Tune Page to get the following dialog box.



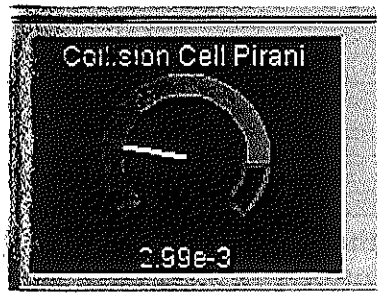
'Scan Time' is the time it will take scan across one of the peak display mass ranges. 'Inter Scan Delay' is the time between scans.

For example if 3 peak mass ranges are being displayed, it will take: 3 x (0.8 + 0.1) = 2.7 seconds to display all three mass ranges, so the scope will be updated every 2.7 seconds.

Checking the Vacuum in the Mass Spectrometer



Checking Vacuum in the Mass Spectrometer



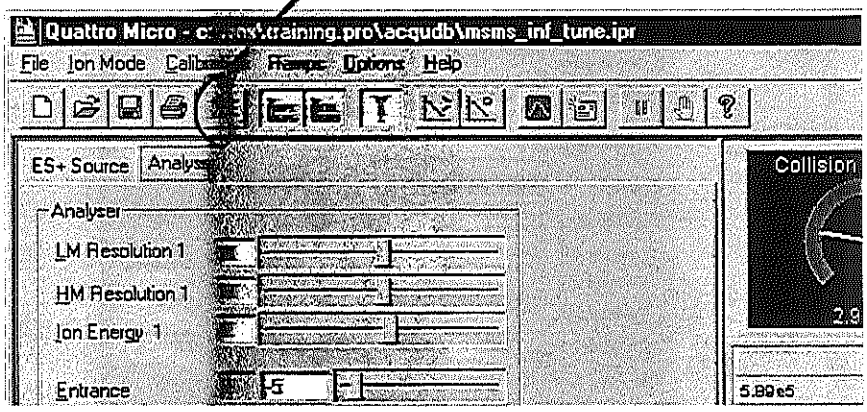
Typical Vacuum Ranges

For MS	For MS/MS (w/Collision Gas)
Collision = 1x1	Collision ~ $1x10^{-3}$ to $4x10^{-3}$

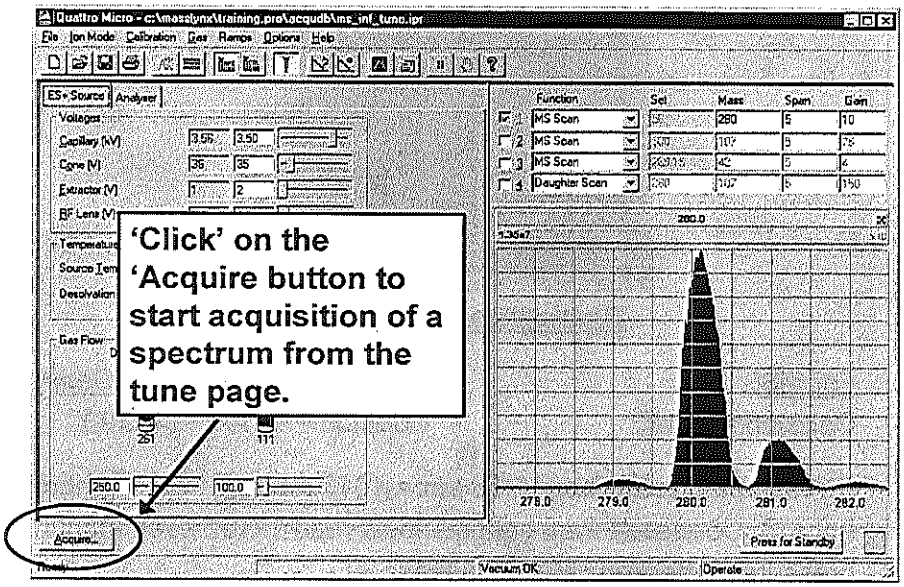
(Pressures give mbar) (760 torr = 1013 mbar = 1 atmosphere)
For MS, Collision Pirani gauge should read $1x10^{-4}$ even though pressure in the cell will be less.

To Back to Peak Masses Table:

To Return Tune Page Display so that the Peak Masses Table is Shown instead of the Vacuum Gauge, 'Click' on the with the 'Peaks'.



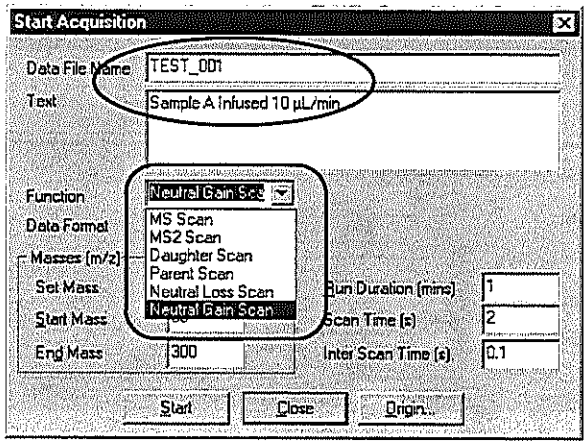
Acquiring Spectra from the Tune Page



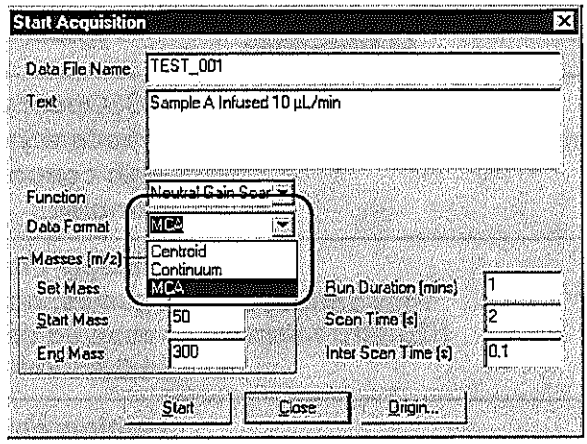
Acquiring Spectra via Tune Page -

After 'Clicking' on the 'Acquire' Button on the Tune Page, the following dialog box should appear.

Enter Run Name, Sample Description and Type of Acquisition



Acquiring Spectra via Tune Page - Select the Data Acquisition Mode



Spectra can be acquired in the Centroid, Continuum, or MCA modes

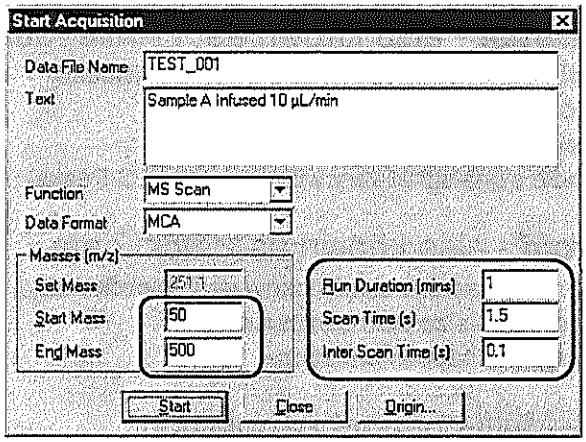
Data Acquisition Modes

- ***Continuum*** - also known as "Profile" data
  - series of spectra are acquired are stored individually
  - largest data file size
  - shows peak shape
  - can handle signals that vary with time (e.g. LC Peaks)
- ***Centroid*** - also known as "Stick" data
  - sees profile data but instantly converts it to stick data,
  - smaller data file size than continuum data
  - gives no peak shape information.
- ***MCA*** - Multi-channel Acquisition
  - is continuum data summed into one scan
  - smaller data file size than continuum but preserves the peak profile.
  - assumes there is a constant signal (infused sample)

Which Data Acquisition Mode?

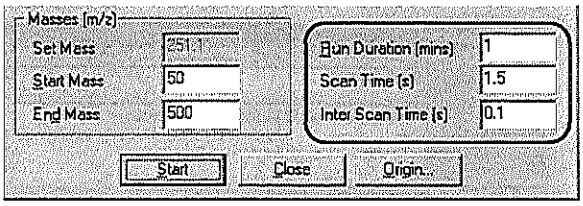
Mode	Maximum Scan Speed At 16pts/Da	Typical Use
Centroid	1000 amu/sec	GC, OpenLynx, FractionLynx, high concentration samples
MCA	1000 amu/sec (Quattro *micro*)	Multiply charged species, non-time resolved data (*e.g.* Infusion)
Continuum	1000 amu/sec (Quattro *micro*)	Multiply charged species, time resolved data (*e.g.* HPLC)

Acquiring Spectra via Tune Page - Example of Mass Range and Run Times for a MS Scan]



In the above example, spectra will be acquired from a m/z of 50 to 500. Total acquisition time will be 1 min. A spectra will be acquired in 1.5 seconds, the system will take 0.1 seconds to reset, then another spectra will be acquired.

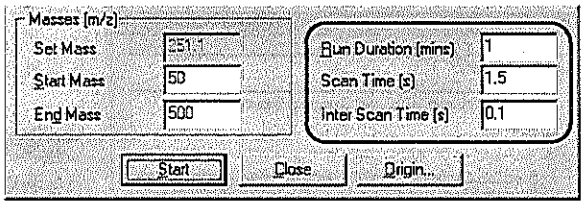
Acquiring Spectra via Tune Page - More on Run, Scan, and Inter Scan Times for a MS Scan



Run Duration: Total time data will be taken. In this example spectra will be acquired for 1 minute.

Scan Time: Time for each scan. The system is typically calibrated to scan between 100 and 1000 amu/sec. Choose a scan time that results in a scan rate within your system's calibrated range. For the above example on a system calibrated from 100 to 1000 amu/sec, a scan time of 0.45 to 4.5 seconds should be used.

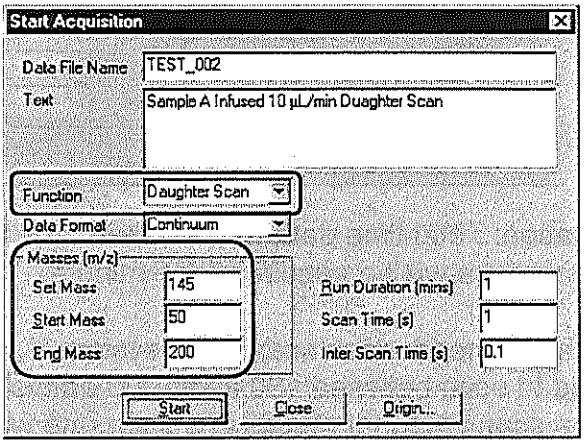
Acquiring Spectra via Tune Page - More on Run, Scan, and Inter Scan Times for a MS Scan (cont.)



Interscan Scan Time: Time between scans. At the end of each scan after a spectrum has been acquired, the system needs time to reset the electronics for the next scan. Typically a value of 0.1 second is used here.

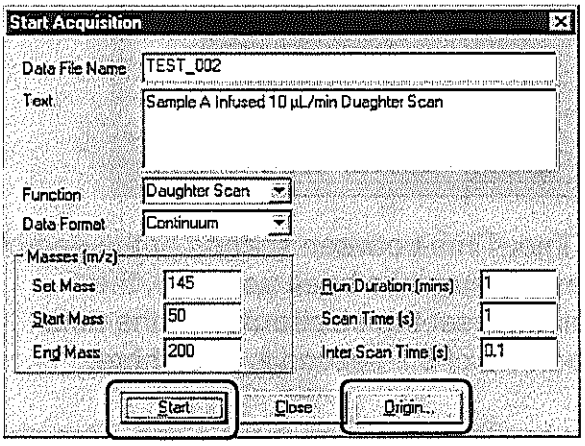
The total time to acquire a spectrum is the Scan Time plus the Inter Scan Time. In this example it is 1.5 + 0.1 = 1.6 seconds. Since the run is 60 seconds long, 60 / 1.6 = 37.5 or 38 scans will be acquired in this example.

Acquiring Spectra via Tune Page - Example of Parent Mass and Mass Range for a Daughter Scan



In the above example, spectra will be acquired of the daughter ions from a parent ion with m/z = 145. Daughter ions from a m/z of 50 to 200 will be scanned.

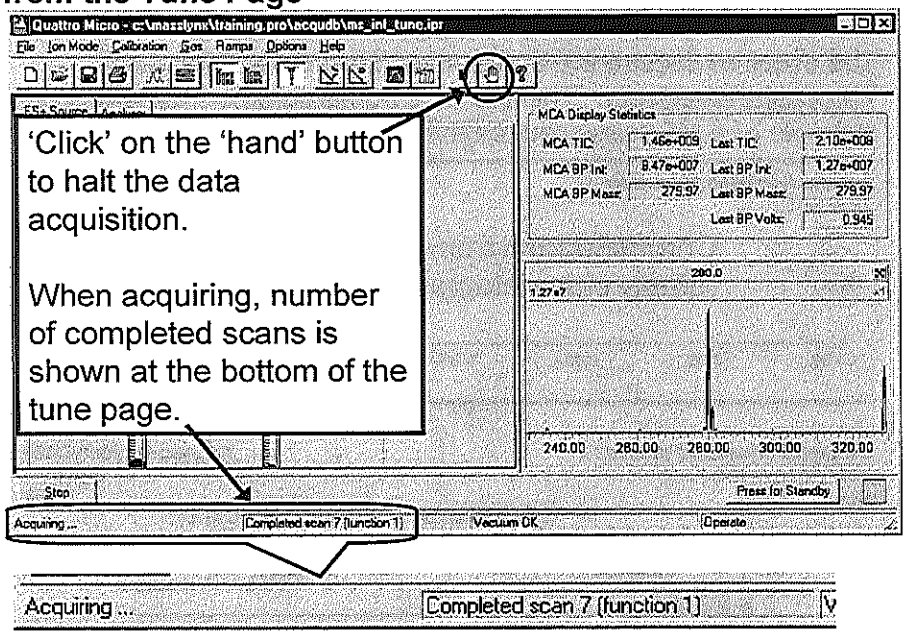
Acquiring Spectra via Tune Page – Starting the Acquisition



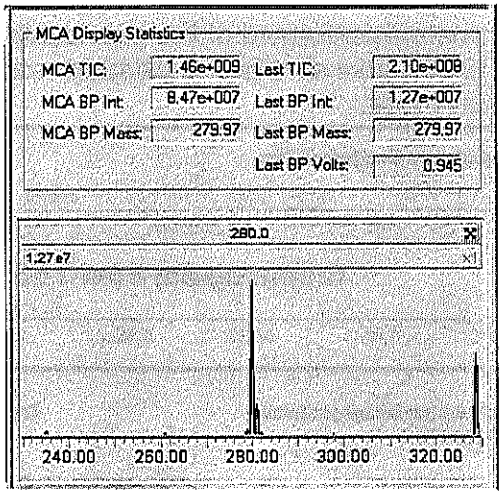
'Click' on 'Origin' to enter info on the acquisition run (e.g. Sample ID.)

'Click' on 'Start' button to begin the acquisition.
DO NOT hit the 'Enter' key as this will just 'close' the dialog box and NOT start the acquisition.

Example of MCA Spectrum Being Acquired from the Tune Page



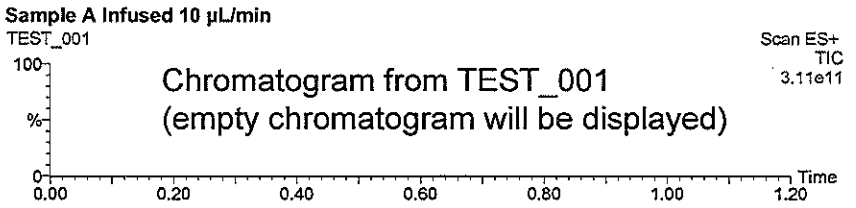
Example of MCA Spectrum Being Acquired from the Tune Page

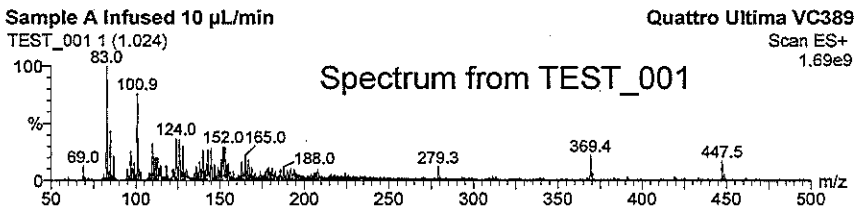


When an MCA spectrum is acquired, the spectrum will be displayed on the tune page as it is being acquired.

For Spectra Acquired by MCA

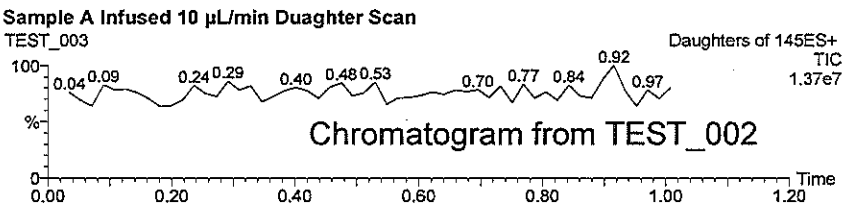
The acquired spectra are all summed together to form one 'composite' spectrum. There is no chromatogram. Test_001 is an example of a MS scan acquired in the MCA mode.

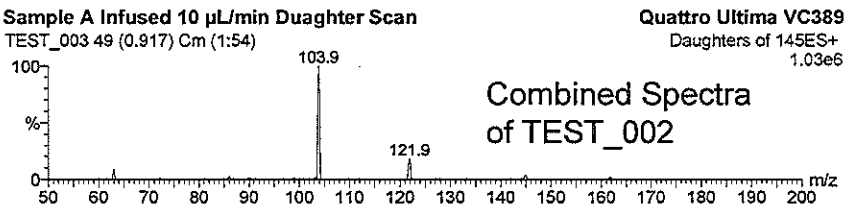




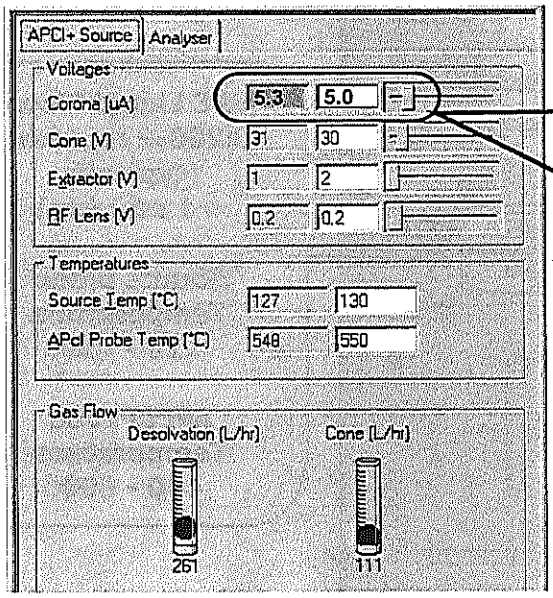
For Centroid or Continuum Acquired Spectra

There is a chromatogram formed of the individually stored spectra. Test_002 is an example of a daughter scan acquired in the continuum mode.





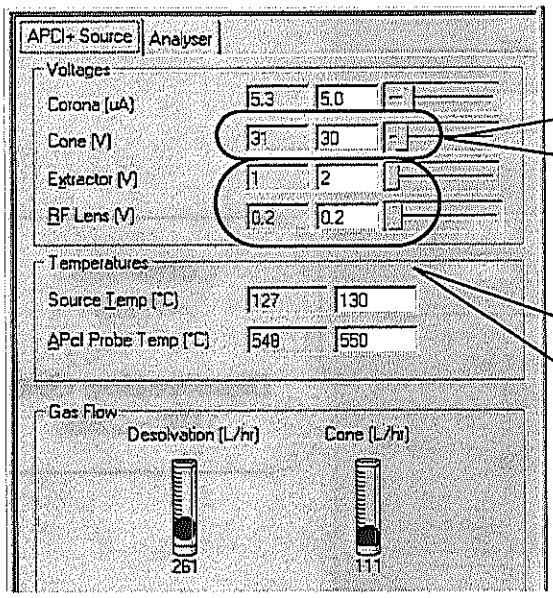
APcI Tune Page Settings - Corona Pin Current



APcI Corona Current Range 0.5-30 µA

Typically 5-10 µA

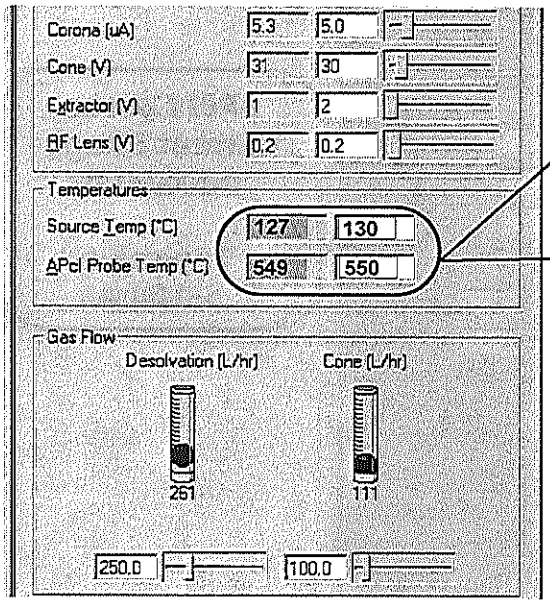
APcI Tune Page Settings – Cone and RF Lens Voltages



Set Cone Voltage to values similar to those use in Electrospray

Set Extractor and RF Lens to values similar to those use in Electrospray

APcI Tune Page Settings – Source and Probe Temperatures

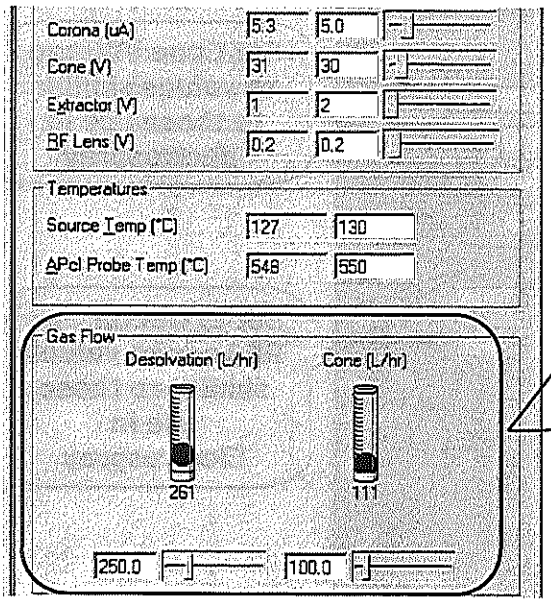


HPLC Liquid Flow= 0.200-2 mL/min (typically ~ 1 mL/min)

Source Block Temperature 120 - 150

APcI Probe Temperature 400 - 600

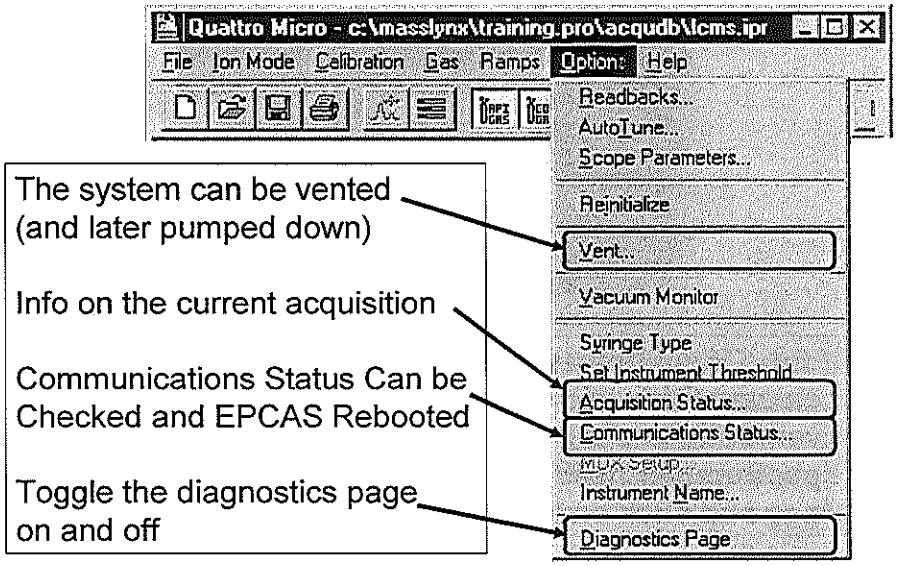
APcI Tune Page Settings - Gas Flows



Cone Gas ~ 100

Desolvation Gas 100 - 250

Tune Page Options



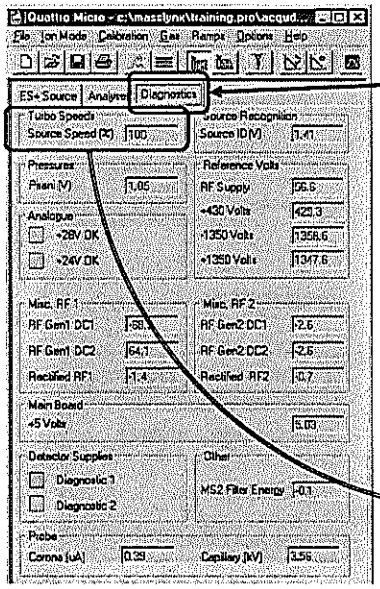
The system can be vented (and later pumped down)

Info on the current acquisition

Communications Status Can be Checked and EPCAS Rebooted

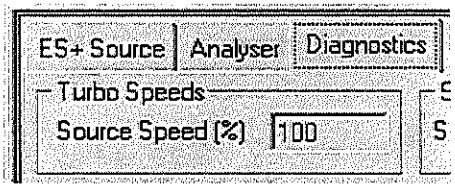
Toggle the diagnostics page on and off

Tune Page: Diagnostics Tab

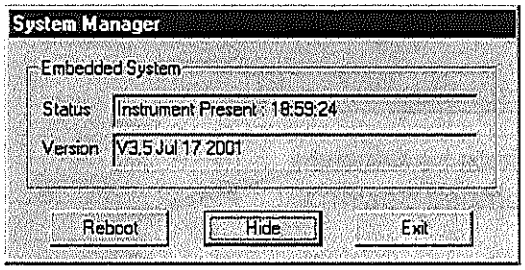


If the Diagnostics are turned on from the 'Options' menu, you can select the 'Diagnostics' Tab

Most of the readings are for use by the service engineers, but you can check your turbo pump. Under normal operations it you run in the 98-100% range.

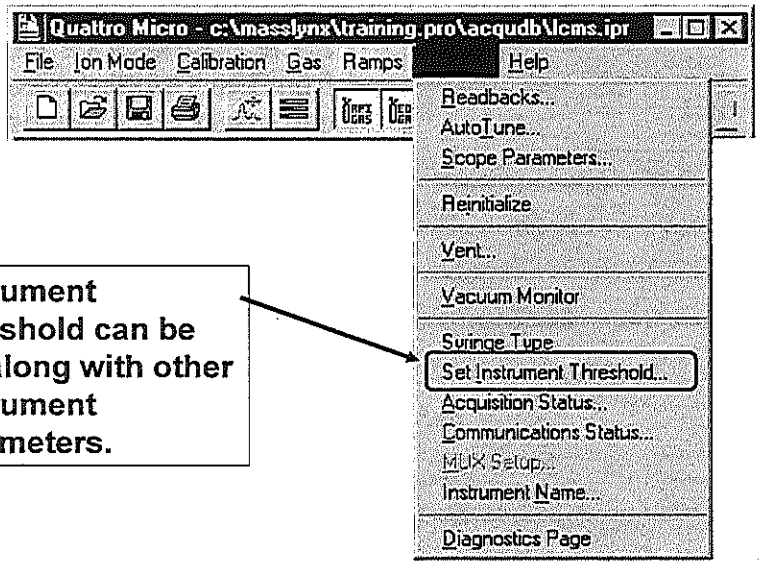


Communications Status Can be Checked and EPCAS Rebooted



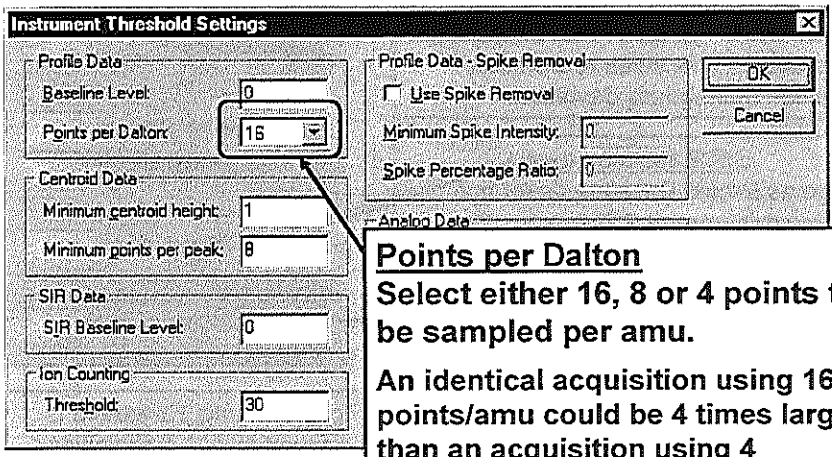
If the 'Communications Status' menu item is selected this dialog box should appear. If everything is okay, just click on 'Exit'. If the mass spectrometer needs to be rebooted, close the 'Tune' page and click on 'Reboot'.

Instrument Thresholds – Noise Rejection



Instrument Threshold can be set along with other instrument parameters.

Setting Instrument Threshold Parameters



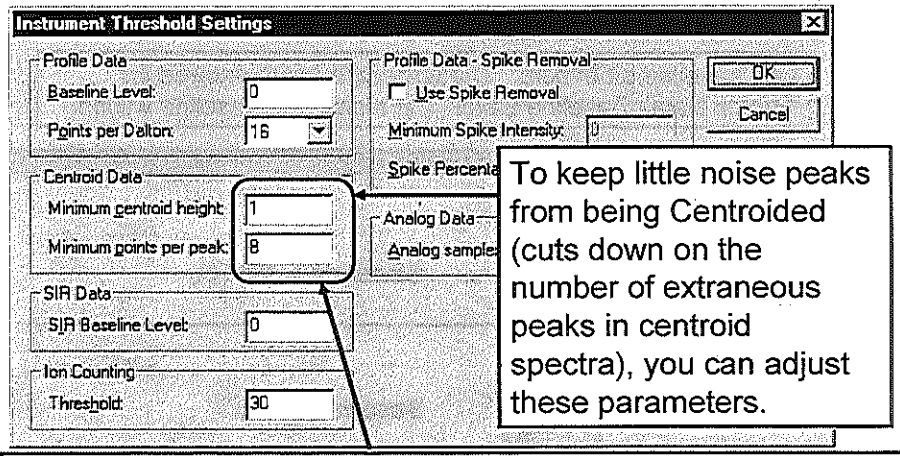
Points per Dalton

Select either 16, 8 or 4 points to be sampled per amu.

An identical acquisition using 16 points/amu could be 4 times larger than an acquisition using 4 points/amu.

The number of points/amu also effects maximum acquisition speeds.

Setting Instrument Threshold Parameters



To keep little noise peaks from being Centroided (cuts down on the number of extraneous peaks in centroid spectra), you can adjust these parameters.

Minimum Centroid Height: The height below which peaks will be ignored.

Minimum Points per Peak: The minimum number of points a continuum peak must have to be centroided.

Profile Baseline Level

Increasing the baseline level has the effect of lowering the baseline to see more noise. Typically, values less than 5 are used. This value represents multiples of standard deviation.

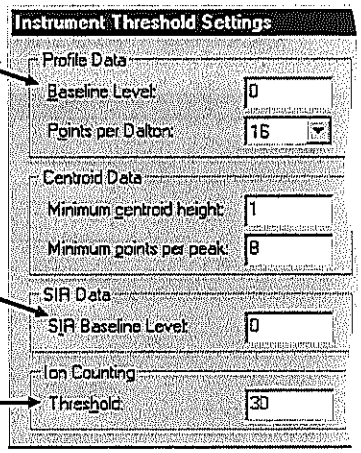
SIR Baseline Level

Same as Profile Baseline Level but applied to summed SIR points. No ability to apply negative values.

Ion Counting

This is the most significant thresholding parameter as it is applied first. Recommended values are given below.

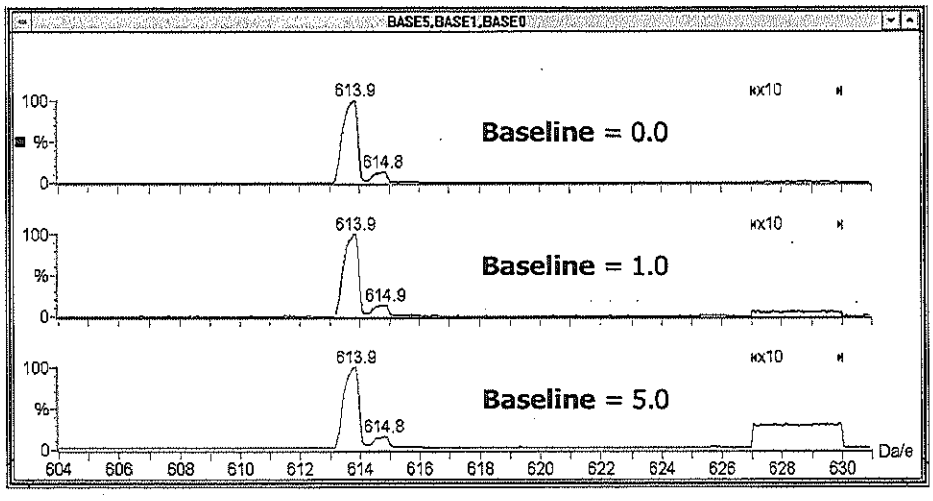
Quattro *Ultima*	30 (in range of 20 - 30)
Quattro *micro*	25 (in range of 20 - 30)
Quattro LC	15 (in range of 10 - 20)
Quattro II	4 - 10



Ion Counting Threshold is applied to ALL acquisition modes. If and Ion Counting Threshold is used, the Profile and SIR Baseline Levels should be set to zero.

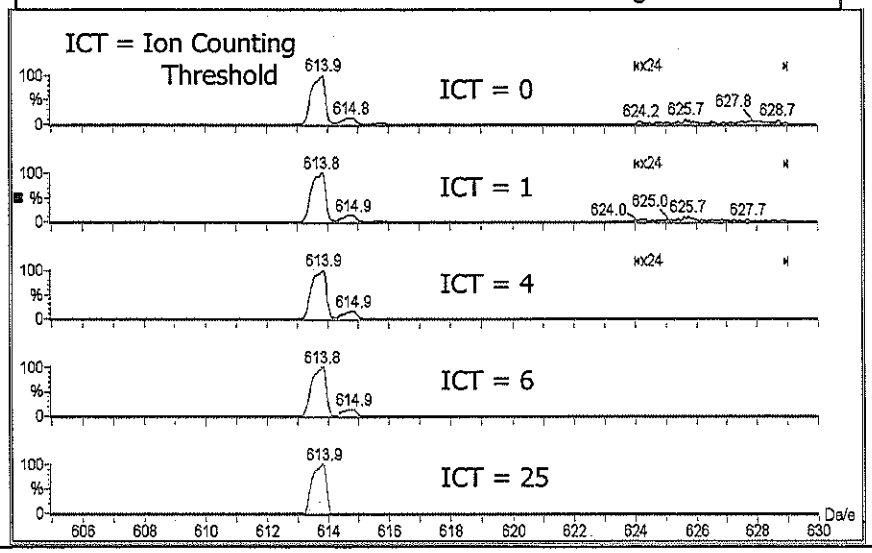
Baseline Thresholding : for Continuum Spectra

Lifts baseline off of zero. The larger the value entered for "Profile Data : Baseline Level", the higher the baseline will be.

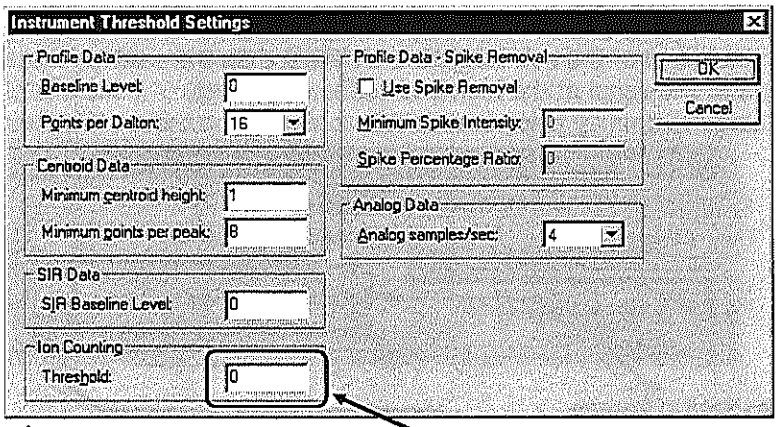


Ion Counting Thresholding – Suppress Noise

The larger the ICT value, the larger the ion signal must be before it is recorded. Use the ICT to eliminate 'little noise' signals.

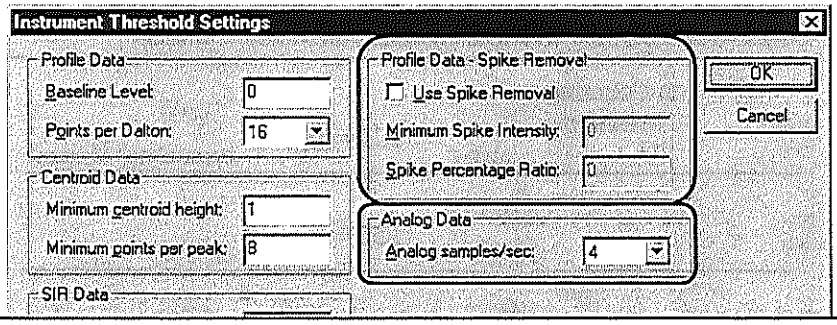


Warning for MaxEnt Users



Note : The MaxEnt algorithm needs to accurately measure noise within a data file. For this reason the Ion Counting Threshold should be set to zero when acquiring data which will be analysed using MaxEnt.

Setting Instrument Threshold Parameters

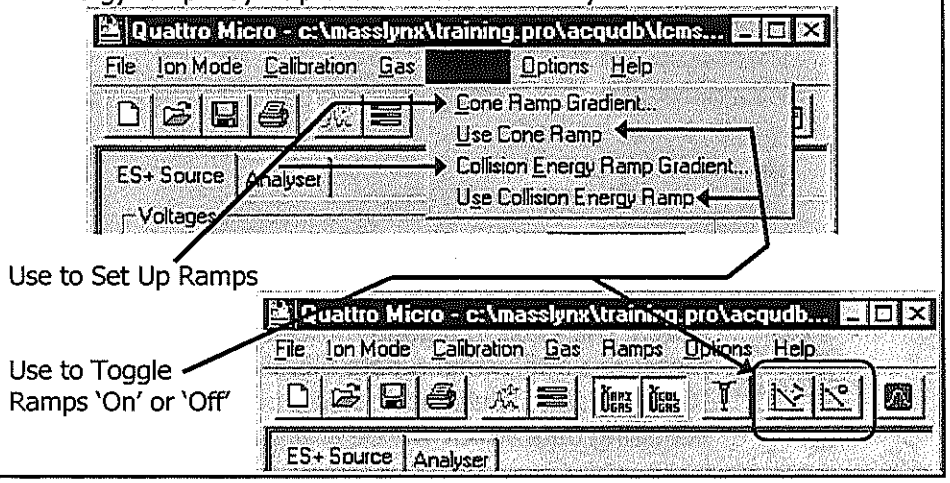


'Spike Removal on continuum spectra. For a data point not to be considered a 'Noise Spike', the data points taken around it must exceed the Spike Percentage Ratio. For example: if 'Spike % Ratio=10' and a data point of 100,000 is measured, then the point before it and after it must be at least 10,000 or it is considered a spike and is not recorded. To determine the 'Min Spike Intens', use values from the tune page.

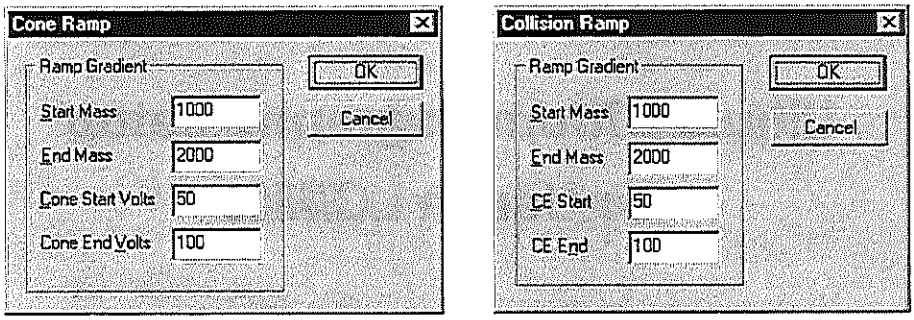
The sampling rate for analog data (e.g. UV detector) can be set here.

Cone Ramp and Collision Energy Ramp

Sometimes when spectra are taken over a wide m/z range, it turns out that the Cone (and/or Collision) settings that yield a strong ion signal at the low end of the m/z range are different than the settings that yield a strong ion signal at the high end of the m/z range. A Cone or Collision Energy ramp may help obtain better sensitivity in these situations.



Cone Ramp and Collision Energy Ramp Settings

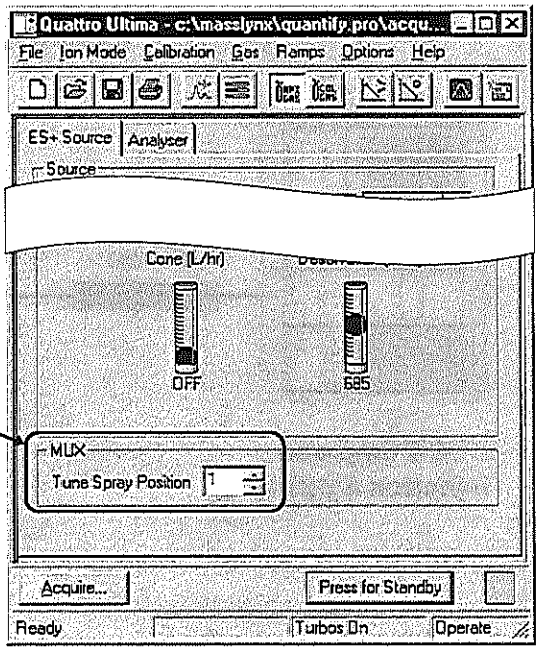


Examples of dialog boxes that can be used to set up a Cone Voltage ramp and Collision Energy ramp.

For example: The Cone Voltage ramp shown can be used to take spectra from 1000 to 2000 amu with the Cone Voltage starting at 50 V when the quadrupole is set to pass m/z=1000. As the quadrupole scans towards higher m/z, the Cone Voltage increases till at m/z=2000, the Cone Voltage is now 100 V.

Quattro MUX

The position of the MUX system can be set from the tune page

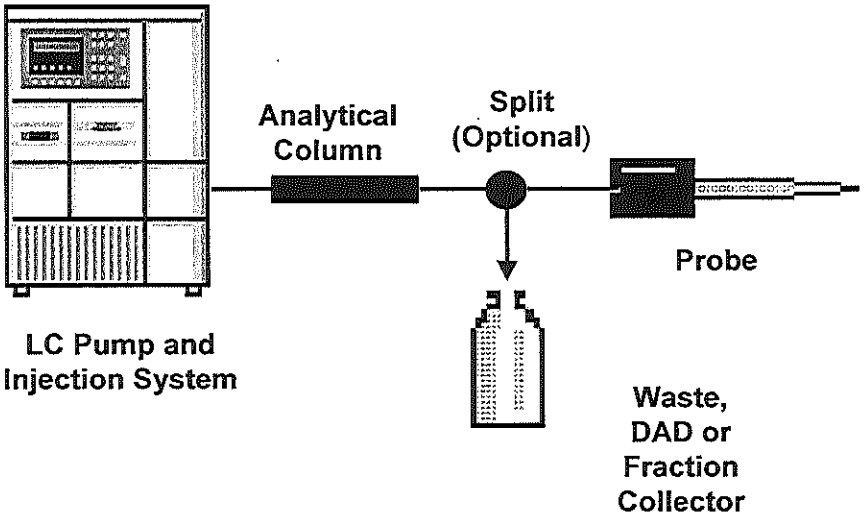


Waters

Liquid Chromatography Setup for Use in LC/MS

MICROMASS MS TECHNOLOGIES

LC-MS



Factors to Consider in LC/MS Method Development

- Ionization mode.
- Column.
  - **Diameter and Flow Rate**
  - **Plumbing**
- Mobile phase. Can effect both the chromatography and ionization process.

LC-flow into the MS

- **APCI** : 0.2 - 2 mL/min -
  No need to split the LC-flow.
- **ESI** : 1 - 300 µL/min routinely -
  Up to 1 mL/min possible. Flow is usually split at the higher LC flow rates so that approximately 250 µL/min is sent to the mass spectrometer.

LC Flow Rate

The internal diameter of the analytical column used depends on the LC flow rate.

Flow Rate	Column I.D.
0.5 - 2 mL/min	3.9 - 4.6 mm I.D.
0.1 - 0.3 mL/min	2 - 2.1 mm I.D.
40 - 50 µL/min	1 mm I.D.
< 10 µL/min	Capillary column

Column Selection

- A Wide Range of Columns are Available from a Variety of Vendors. Factors to consider are:
  - Separation of Analytes from other Analytes and Interferring Matrix Components
  - Resolution of Peaks
- For ESI, if possible use a column that does not require flow splitting.

Mobile Phase Considerations

- pH
- Solvents and additives
- Composition may be a compromise between what gives the best ionization and what gives the best LC separation.

pH Considerations

Positive ion mode -

Analysis of basic compounds

Lower pH with an acid

e.g., Formic or acetic acid

Negative ion mode -

Analysis of acidic compounds

Raise pH with a base

e.g., Ammonium hydroxide/Ammonia solution.

Commonly Used Solvents and Additives

Solvents	Additives
Water	Acetic Acid
Acetonitrile	Formic Acid
Methanol	Ammonium Hydroxide
Isopropanol	Ammonium Acetate*
	Ammonium Formate*

(* = Can be used as a buffer. Salt concentrations should be kept to 10 mM or less.)

Other Solvents and Additives

IPA, ethanol, 2-methoxyethanol, etc.

Trifluoroacetic acid (TFA) - Proteins and Peptides

Triethylamine (TEA) - Can enhance -ve ion mode,

Tetrahydrofuran (THF)

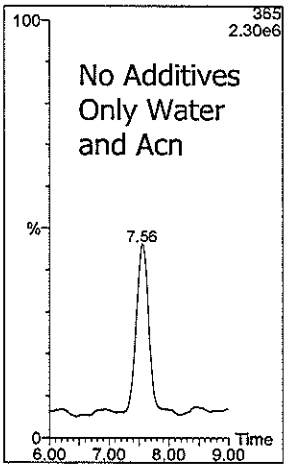
Normal phase chromatography

Column Cleanup Solvents (MeCl2, Acetone)

Solvents & Additives to be Used with Discretion

- TFA - Used with Proteins and Peptides
  - Will suppress (to some extent) positive ion electrospray at concentration over 0.1%.
  - Will greatly suppress negative ion electrospray.
- TEA
  - May enhance negative ion electrospray of less basic compounds.
  - Readily ionized to give an intense $(M + H)^+$ ion at m/z 102.
  - Will suppress positive ion electrospray of less basic compounds.
- THF
  - 100% THF is highly flammable.
  - Should not be used with APCI if air is being used as the nebulizer gas.

Effect of Formic Acid and TFA



Ketoester Example–

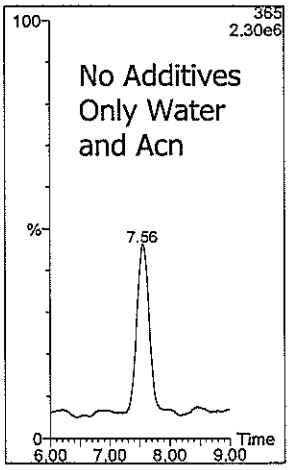
Positive Ion Electrospray

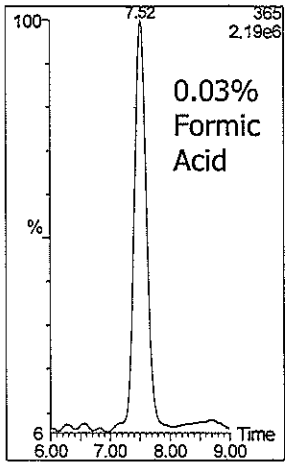
Extracted Ion Chromatogram for m/z = 365 from MS Full Scan data

Mobile Phase = 30/70 Water/Acn

Since Positive Ion ES, lowering the pH might help --->

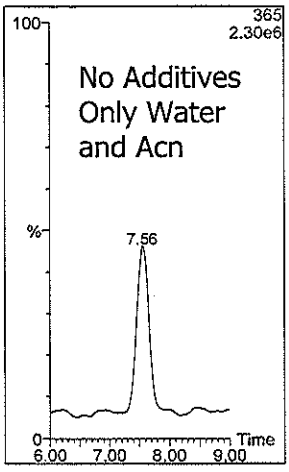
Effect of Formic Acid and TFA

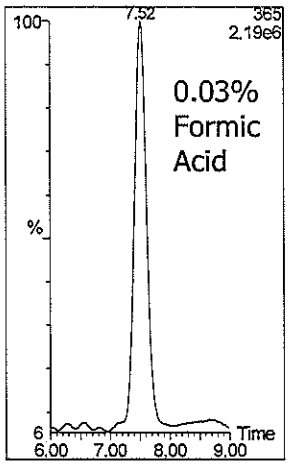


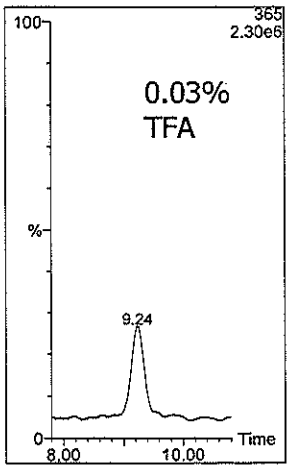


Addition of formic acid to the mobile phase decreased the pH of the mobile phase and increased the M+H signal in positive ion ES.

However Addition of TFA, which Results in Mobile Phase with a Lower pH, does not Yield a Stronger M+H Signal







Unsuitable Solvents and Buffers

Non-volatile salts (phosphates, borates, citrates, etc.)

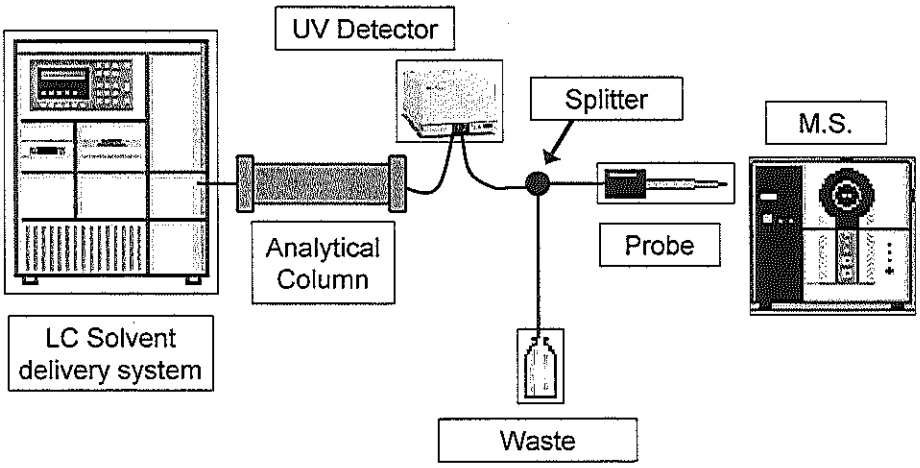
Surface active agents/detergents (suppresses ionization)

Inorganic acids (sulfuric acid, phosphoric acid, etc.)

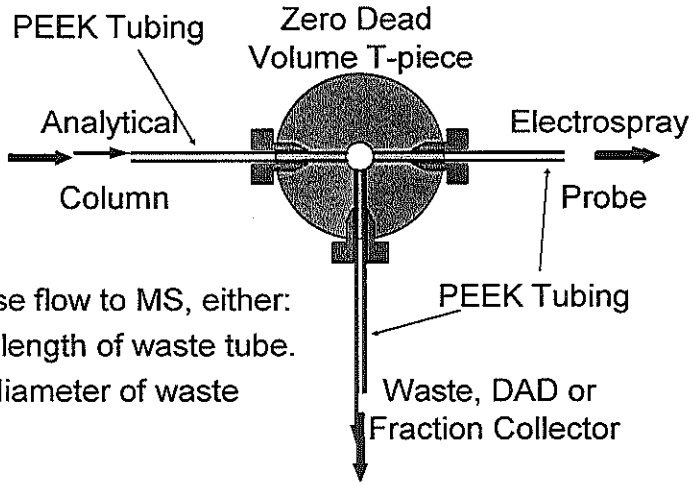
Solvents to Avoid with PEEK Tubing

Attacks PEEK	Swells PEEK
Conc. HNO_3	DMSO
Conc. H_2SO_4	THF
	CH_2Cl_2

Splitting the LC Flow



Building a Splitter



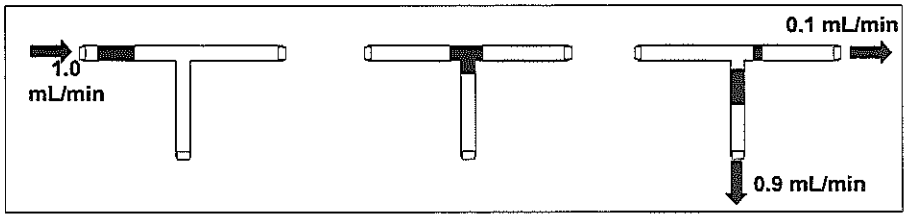
To Decrease flow to MS, either:

Decrease length of waste tube.

Increase diameter of waste tube.

Post-Column Splitting?

Concentration dependence of ESI allows post-column splitting without reduction in signal intensity.



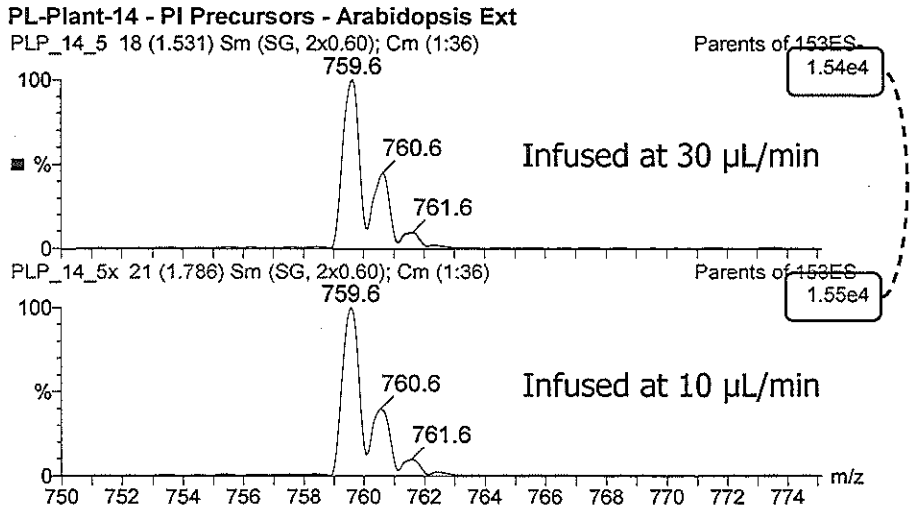
The major reasons for choosing a split are:

- **Reduces source cleaning requirements**
- **Optimizes flow rate conditions**
- **Saves valuable samples**

Column Diameter and Signal

- Loading 20 µL onto a 2 mm I.D. column will give 4x the the analyte concentration in the eluent, than loading 20 µL onto a 4 mm I.D. column.
- As ESI is concentration dependent there will be an increased response for the 2 mm column over the 4 mm column.

Effect of Infusion Flow Rate on Electrospray Signal

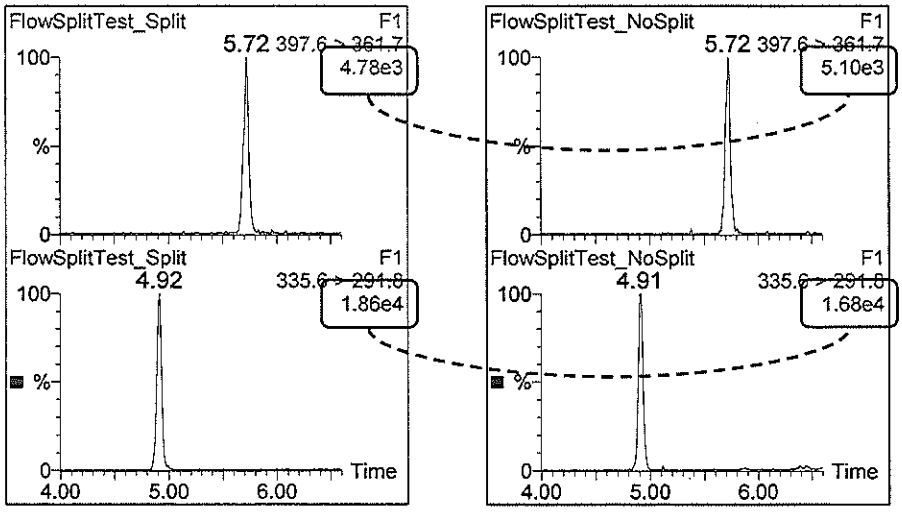


Plant Extract Sample from Dr. Ruth Welti, Kansas State University, Manhattan, KS. Parent Scan for Phospholipids.

Effect of LC Flow Rate on Electrospray Signal (MRM Analysis of Two Components)

LC Flow Rate = 0.6 mL/min
Split 1:2 (0.3 mL/min to MS)

LC Flow Rate = 0.6 mL/min
No Split (0.6 mL/min to MS)



Ion Suppression

- Caused by Interfering Components in the LC Flow that elute at the same time as the analyte of interest. These Interfering Components can
  - Interfere with Ionization Process
  - Be Preferentially Ionized.
- These Interfering Components are often present in matrix samples and can cause problems in analyses of biological and environmental samples. Matrix standards are used sometimes to account for ion suppression.
- Changes in LC Methods and Sample Preparation are often used to minimize ion suppression problems.

LC Mobile Phase Gradients

- Mobile Phase Gradients can be used to separate analytes from interfering matrix components that might cause ion suppression.
- The LC is setup with both organic and aqueous mobile phases.
- The percentage of organic solvent in the mobile phase is varied.

LC Mobile Phase Gradients

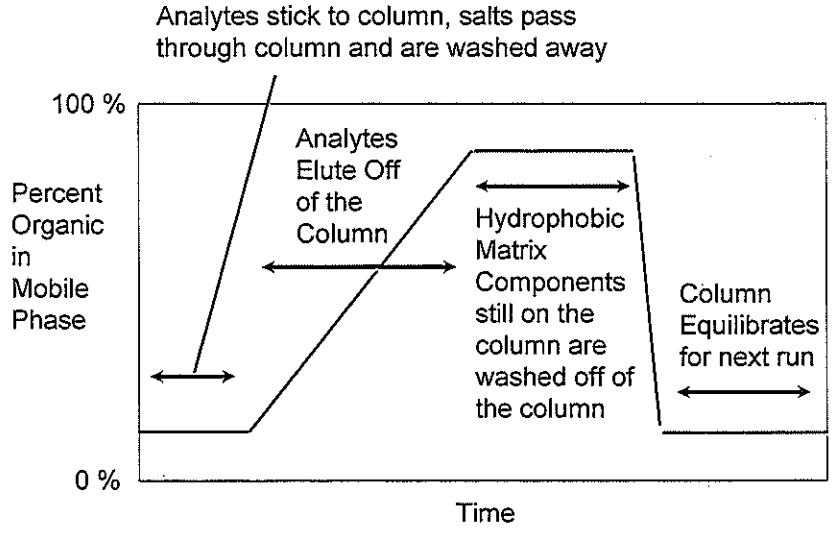
Typically, at gradient has 3 parts.

1) Start of a run: Mobile phase has a low organic content (high aqueous). The analytes are retained on the column and salts flow through the column.

2) Middle of run: Mobile phase composition is ramped from low organic (high aqueous) to high organic content (low aqueous) during which the analytes elute off of the column and go the MS where they are analyzed.

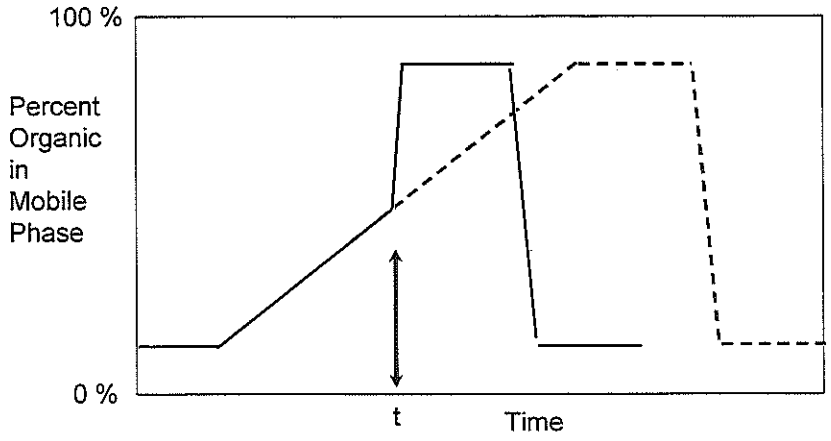
3) End of the run: Mobile phase has a high organic content. This washes any hydrophobic matrix components still on the column off of the column.

LC Mobile Phase Gradients

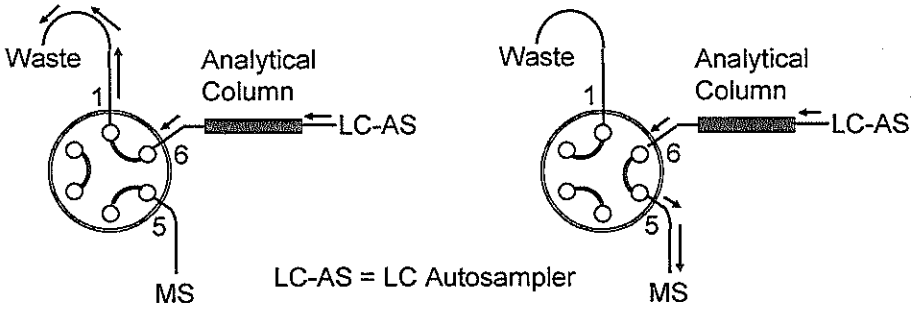


LC Mobile Phase Gradients

If the analytes of interest elute off the column by time 't', then the organic content of the mobile phase can be 'stepped' to a high level at time 't' to save time.



Divert Valve Example



'Load' Position

'Inject' Position

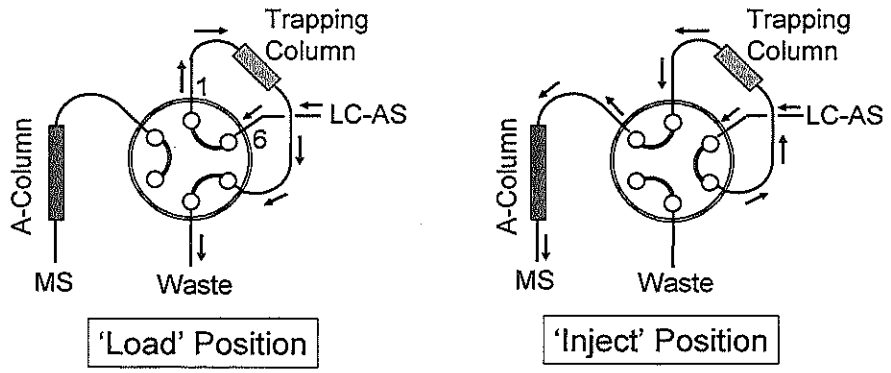
Note: 2=Waste
3=Column
4=MS
will also work

In your MS Method set the solvent delay for the time periods you want the valve to be in the 'Load' position.

LC/MS : Sample Prep Considerations

- There may be cases in which samples must be 'cleaned' up before analysis.
- Interfering components in the sample might be 'plugging' the column or may elute at same time as the analytes and through ion suppression are reducing sensitivity.
- Liquid-Liquid extraction, SPE, or other sample prep methods may be needed.
- Derivitization may be needed.
- Filters and Guard Columns may help.

Online 'SPE' Cleanup of Sample



LC-AS = LC Autosampler
A-Column = Analytical Column

In your MS Method set the solvent delay for the time you want the valve to be in the 'Load' position. Sample is usually loaded onto the Trapping Column using a high aqueous MP and eluted off using a MP with a higher organic composition.

LC/MS : More on Samples & Injections

- The solubility of the analytes in the samples can effect the choice of the composition of the liquid in which the samples are made up in.

  The composition of the liquid should be chosen with the chromatography in mind.

  Example: For reverse phase chromatography, it might help if the sample liquid has a low organic content, otherwise the sample may not be retained on the column.

- Injection volumes need to be matched to the flow rate and column. With large injection volumes and a small column, the sample injection may not evenly mix with the mobile phase when it hits the column.

Effect of Sample Solution on Chromatography