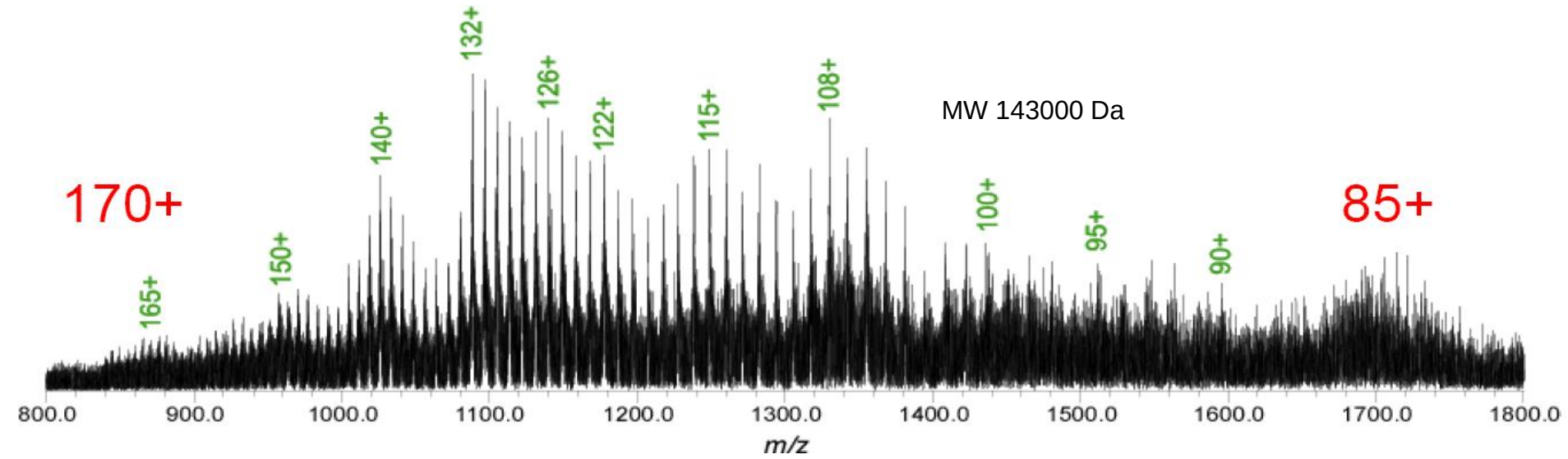
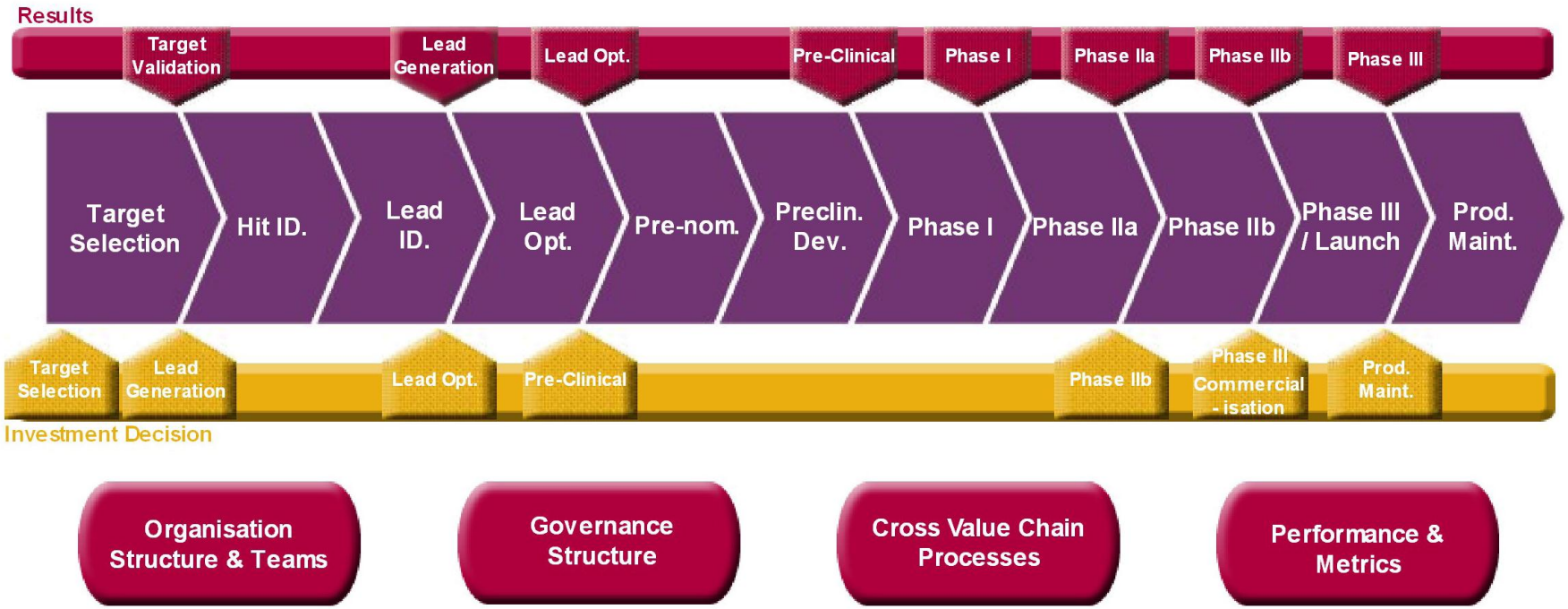
Masspektrometri-tillämpningar i läkemedelsindustrin

Gunnar Stenhagen

Stenhagen Analyslab AB



Drug Project Operating Model Processes

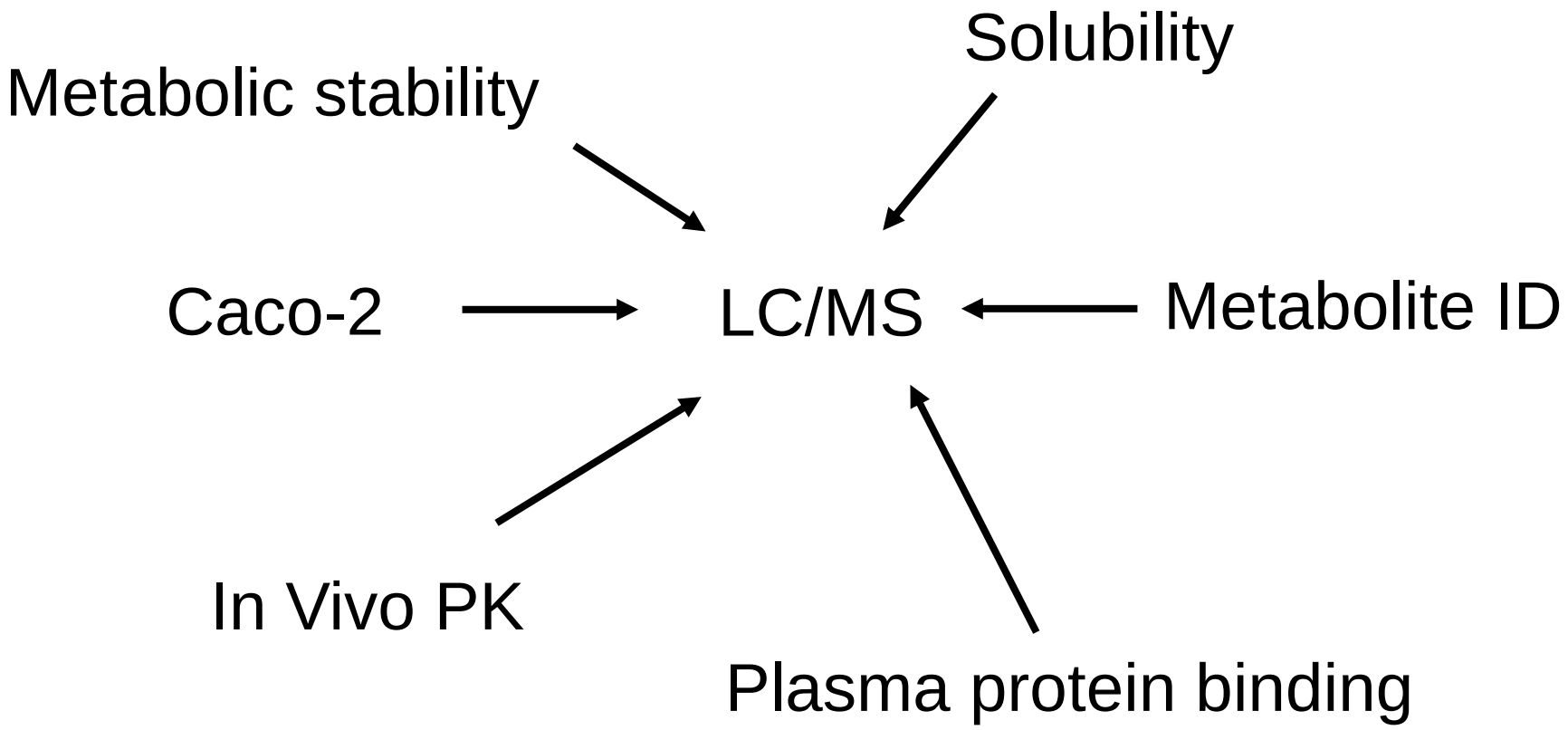


Drug Metabolism and Pharmacokinetics (DMPK) both *in vitro** and *in vivo** studies

- **A**dsorption
- **D**istribution
- **M**etabolism
- **E**xcretion

* Term originate from Latin and *vivo* translate to “life” and *vitro* to “glass”

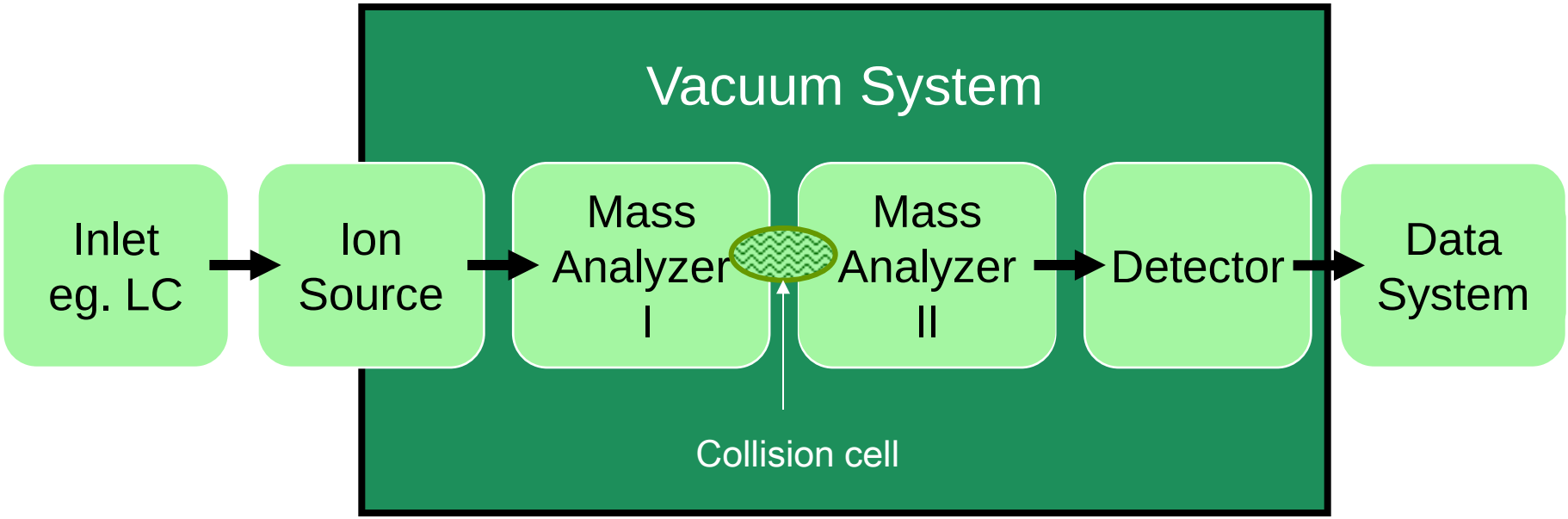
LC/MS in drug metabolism/disposition studies



Mass spectrometers separate ions according to their mass to charge (m/z) ratios

1. Quantitative information about samples
2. Qualitative structural information
3. Sensitive for a wide range of compounds
4. Extremely selective

Components of a Mass Spectrometer



Ion Sources

- **High vacuum sources**
  - **Electron Ionization (EI)**
  - Chemical Ionization (CI)
  - Field Desorption (FD, FI)
  - Fast atom bombardment (FAB. LSIMS)
  - **Matrix-Assisted Laser Desorption (MALDI)**
- **Atmospheric Pressure Ionization (API)**
  - **Electro spray Ionization (ESI)**
  - **Atmospheric Pressure Chemical Ionization (APCI)**
  - Atmospheric Pressure Photo ionization (APPI)
  - Atmospheric Matrix-Assisted Laser Desorption

ELECTRON IONISATION (EI)

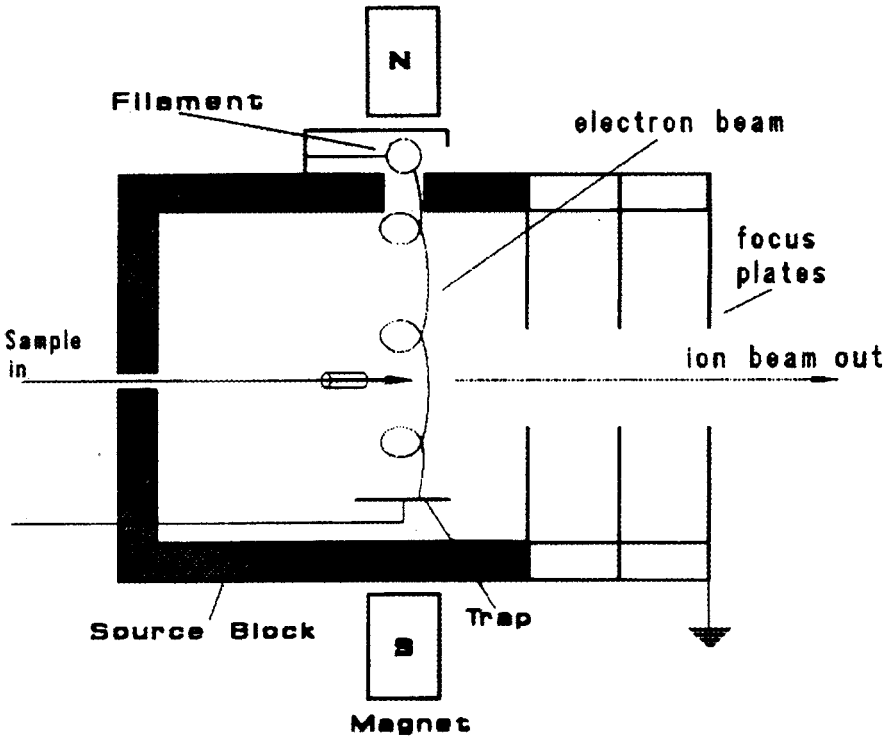
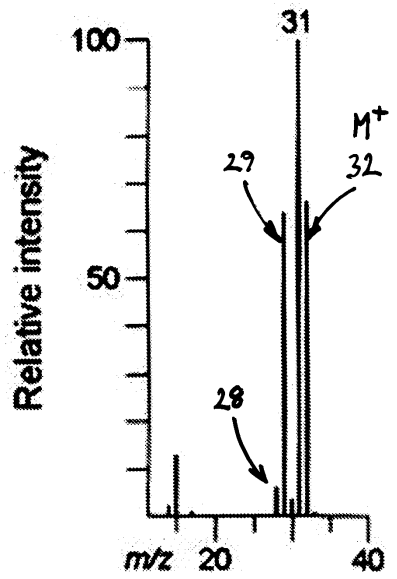
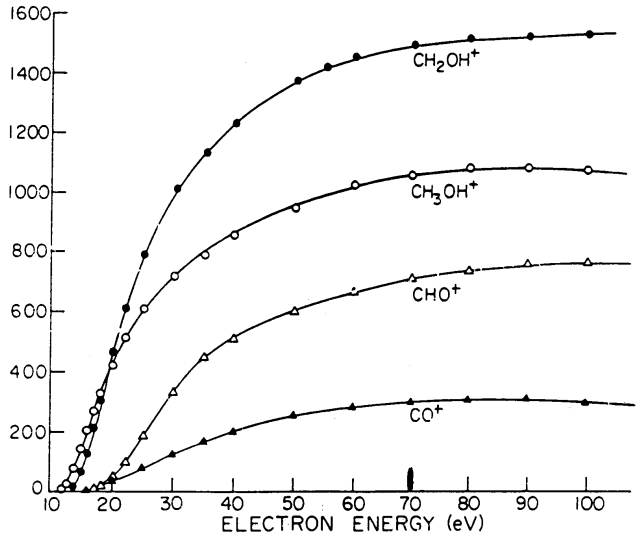


Figure 3. Simple Ion Source, showing the housing (block) with electron beam for EI .

EI mass spectrum of methanol



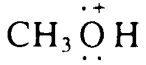


1. Ionization efficiency curves for major ions in the mass spectrum of methanol.

Mass spectra (EI) are routinely obtained at an electron beam energy of 70 eV. The simplest event that occurs is the removal of a single electron from the molecule in the gas phase by an electron of the electrom beam to form the molecular ion, which is a radical cation ($M^{\cdot +}$). For example, methanol forms a molecular ion.

$$CH_3OH + e \longrightarrow \underset{m/z\ 32}{CH_3OH^{\cdot +}} + 2e$$

When the charge can be localized on one particular atom, the charge is shown on that atom.



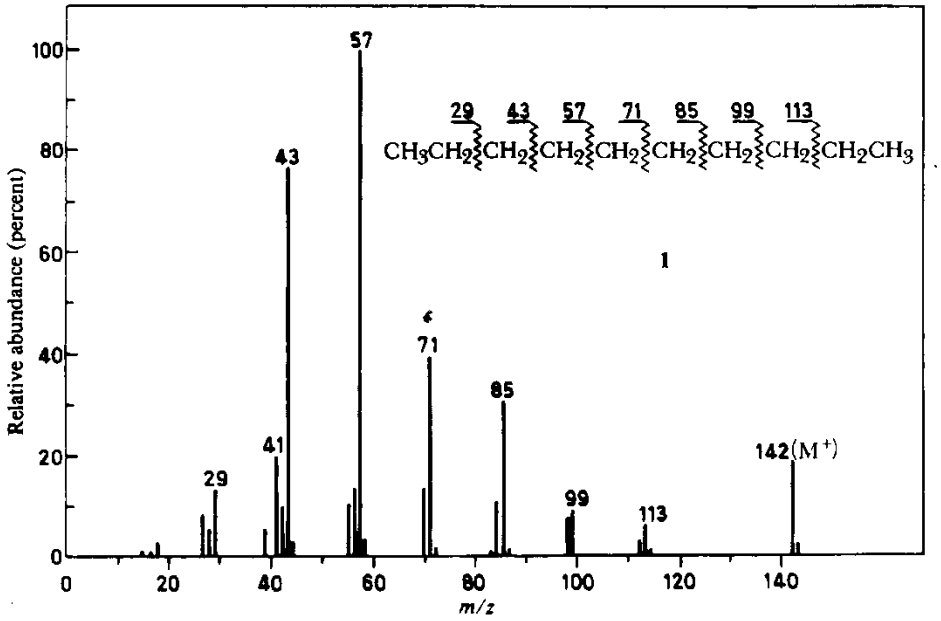
The single dot represents the odd electron. Many of these molecular ions disintegrate in 10^{-10}–10^{-3} s to give, in the simplest case, a positively charged fragment and a radical. A number of fragment ions are thus formed, and each of these can cleave to yield smaller fragments. Again, illustrating with methanol

$$CH_3OH^{\cdot +} \longrightarrow CH_2OH^+ \ (m/z\ 31) + H^{\cdot}$$

$$CH_3OH^+ \longrightarrow CH_3^+ \ (m/z\ 15) + {\cdot}OH$$

$$CH_2OH^+ \longrightarrow CHO^+ \ (m/z\ 29) + H_2$$

EI mass spectrum of decane ($C_{10}H_{22}$) Mw 142



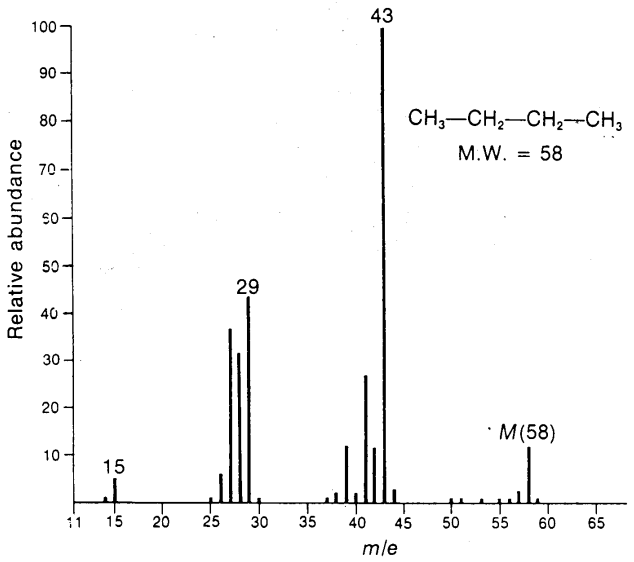
$C_4H_9-CH_2:CH_2-C_4H_9 \xrightarrow{-e^-} C_4H_9-CH_2^{+\bullet}-CH_2-C_4H_9$

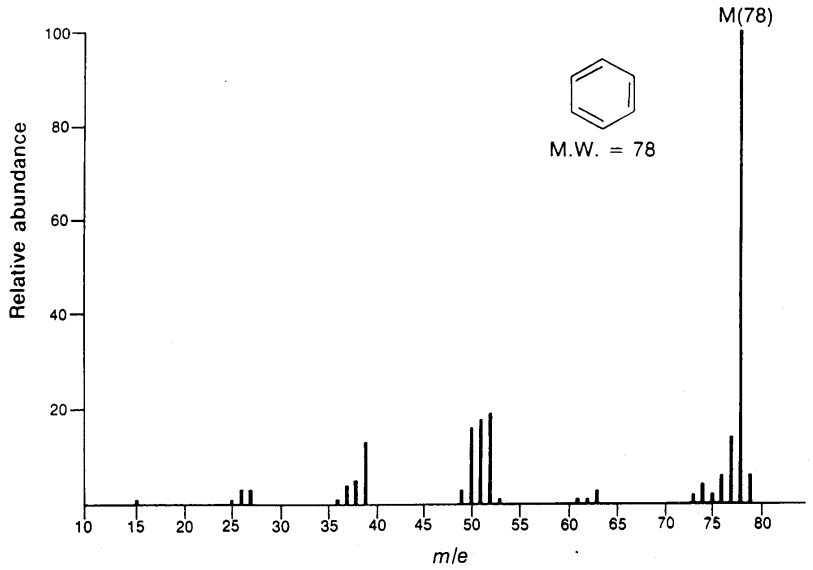
Parent ion 142 m/z

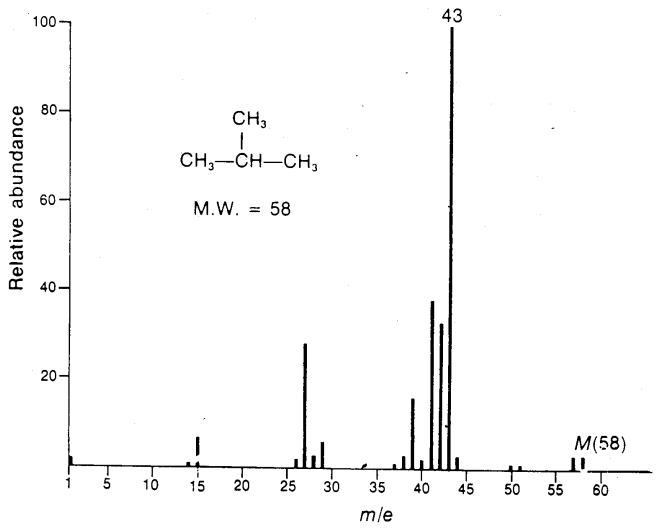
Fragmentering:

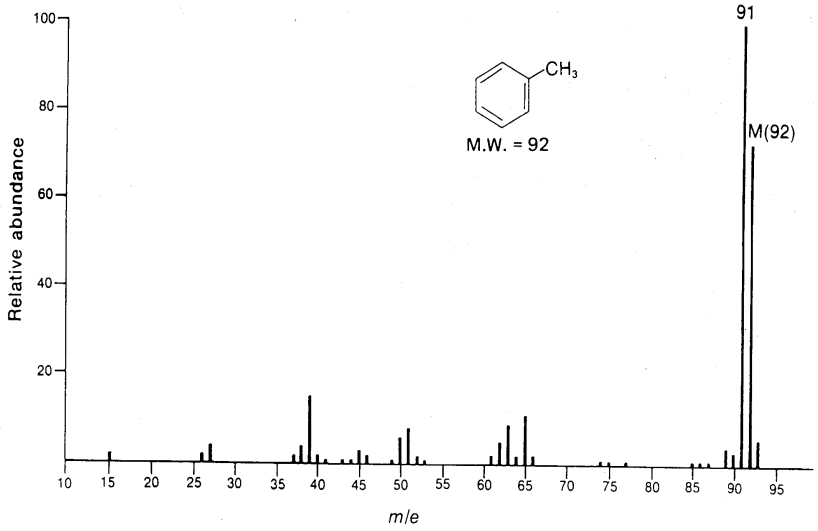
ex. $C_4H_9-CH_2^{+\bullet}-CH_2-C_4H_9 \longrightarrow C_4H_9-CH_2^{+} + \bullet CH_2-C_4H_9$

71 m/z







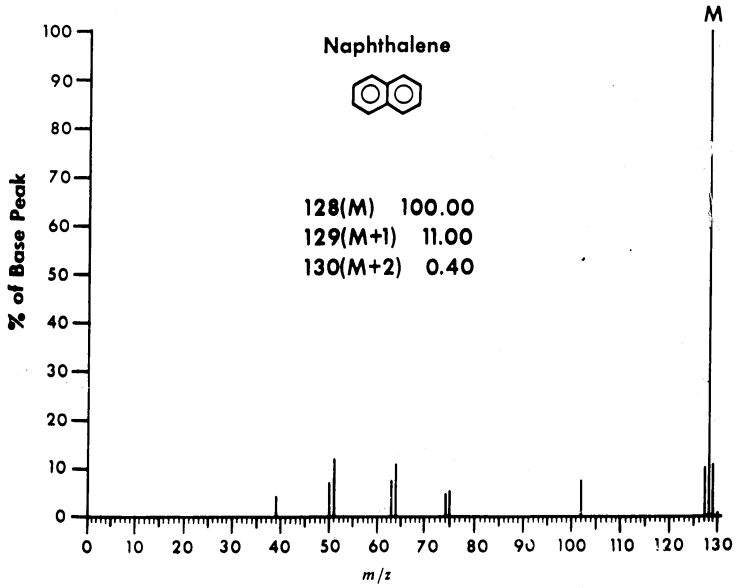


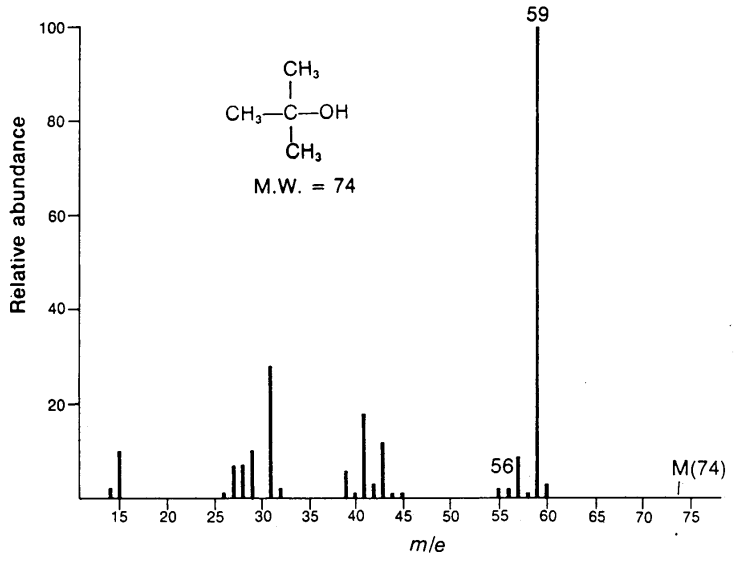
ORDER FOR MOLECULAR ION ABUNDANCE TO CHEMICAL CLASSES

AROMATICS
HETEROAROMATICS
⌄
CYCLOALKANES
SULPHIDES
THIOLS
CONJUGATED ALKENES
⌄
ALDEHYDES
KETONES
ALKENES
CARBOXYLIC ACIDS
AMIDES
ETHERS
AMINES
N-ALKANES
⌄
BRANCHED ALKANES
HALIDES
NITRILES
ALCOHOLS
ACETALS

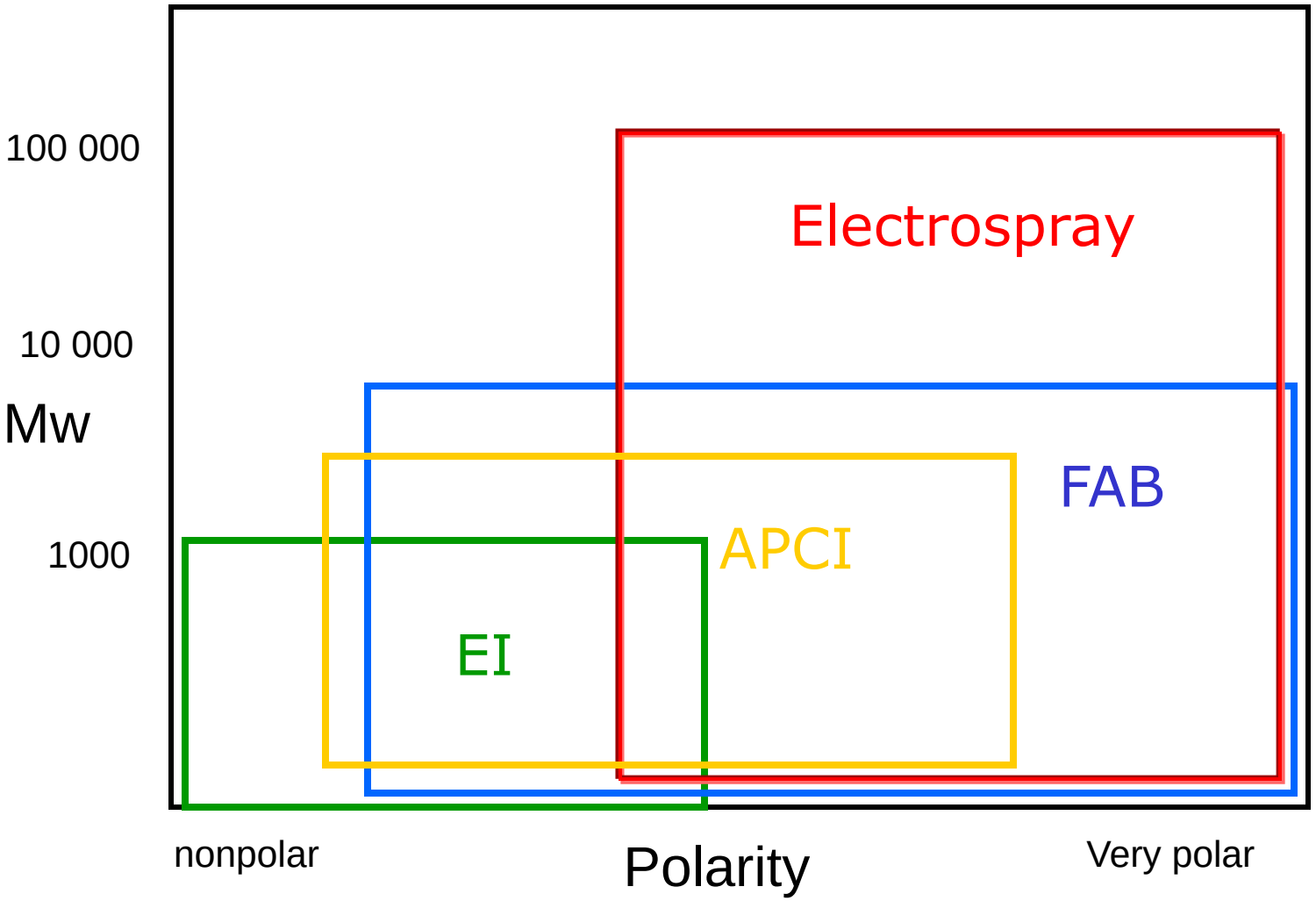
Real molecular-ion peaks have **even** m/z ratio , unless an **odd** number of nitrogen atoms is present in the parent molecule.

This rule covers all organic molecules containing the common elements: carbon, hydrogen, oxygen, nitrogen, phosphorus, suphur, silicon and the halogens.





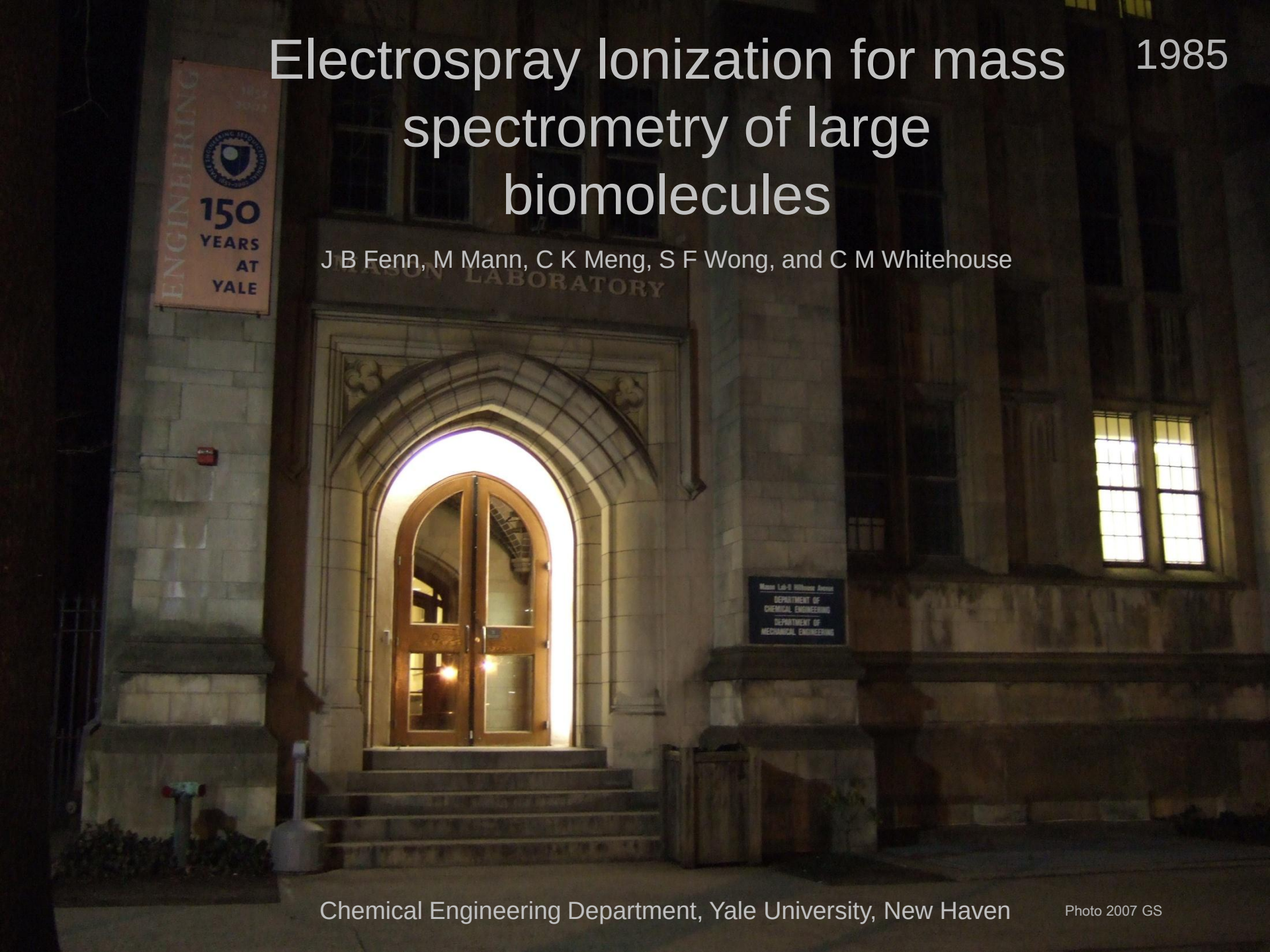
Application range of various ion sources



Electrospray Ionization for mass spectrometry of large biomolecules

1985

J B Fenn, M Mann, C K Meng, S F Wong, and C M Whitehouse



Chemical Engineering Department, Yale University, New Haven

Photo 2007 GS

Electrospray (ESI)

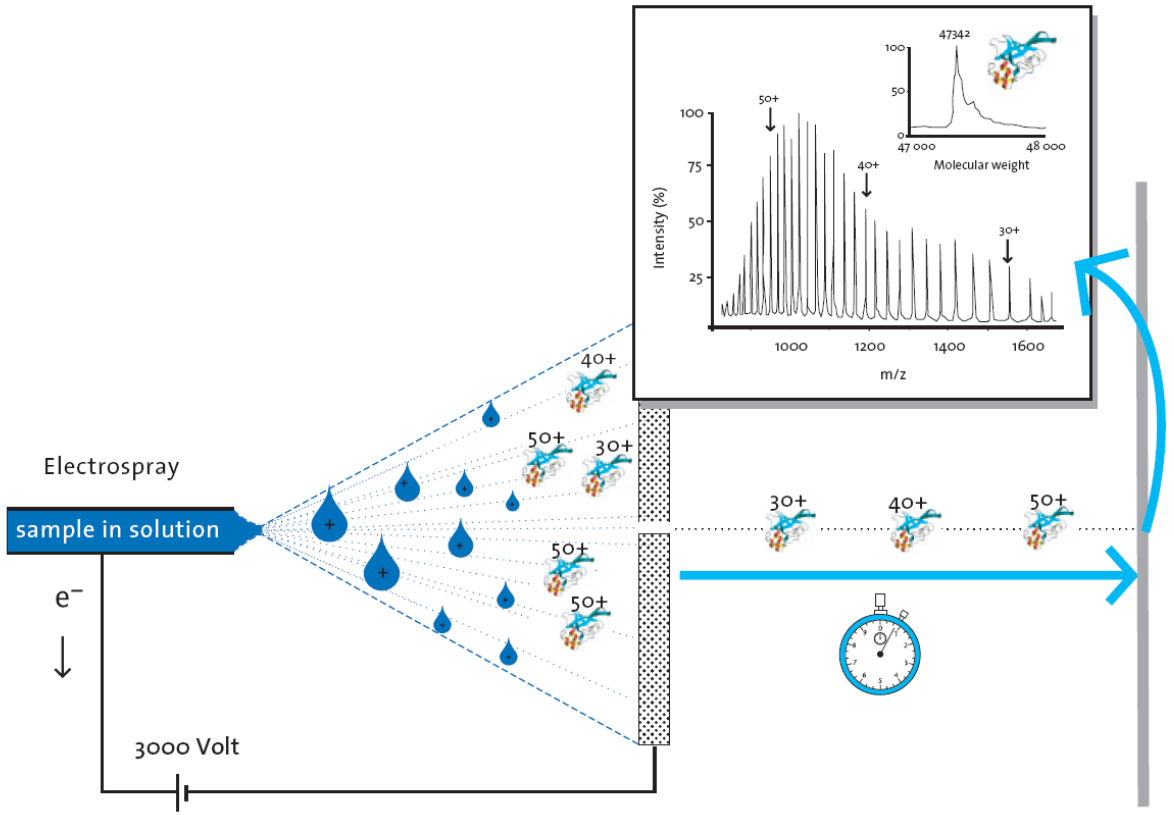
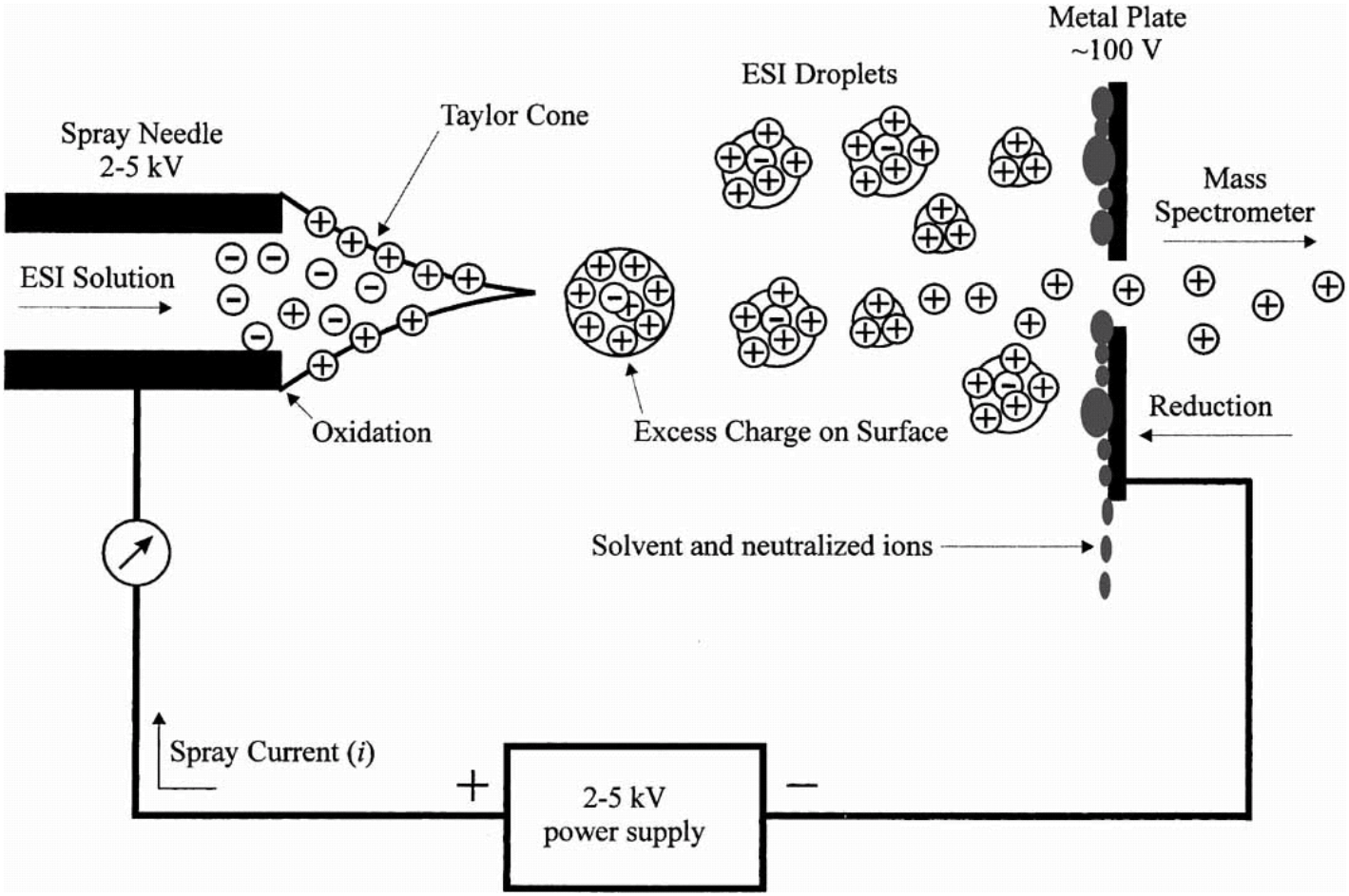


Figure 1. The electrospray process.

- Suitable to a wide range of polar non-volatile compounds
- Sensitive (low pg amounts)
- Soft ionization technique (gives molecular weight)
- Robust
- Interface LC-MS

Nobel Prize 2002 to John B. Fenn for application of ESI-MS to biological macromolecules

Schematic of electrospray ionization process



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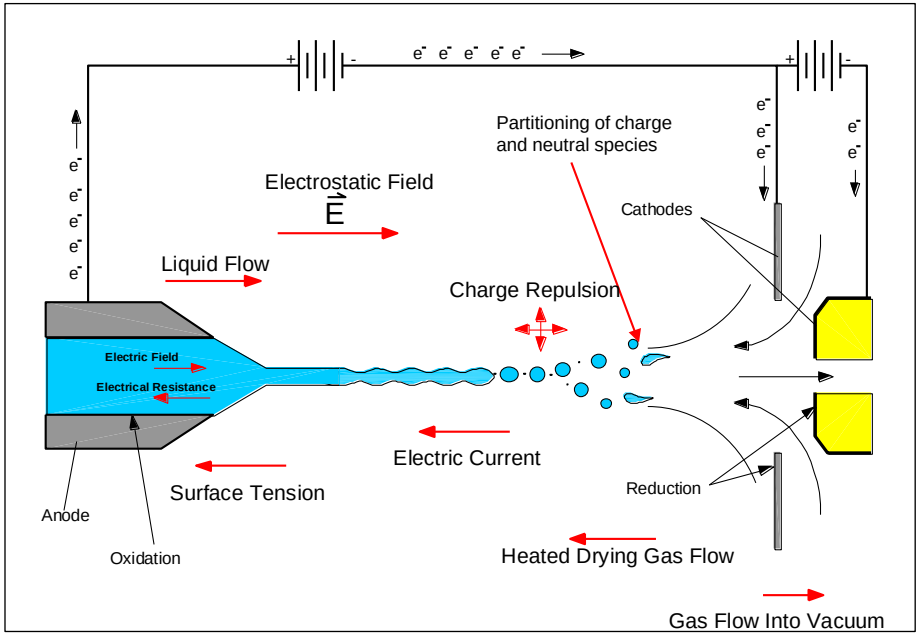
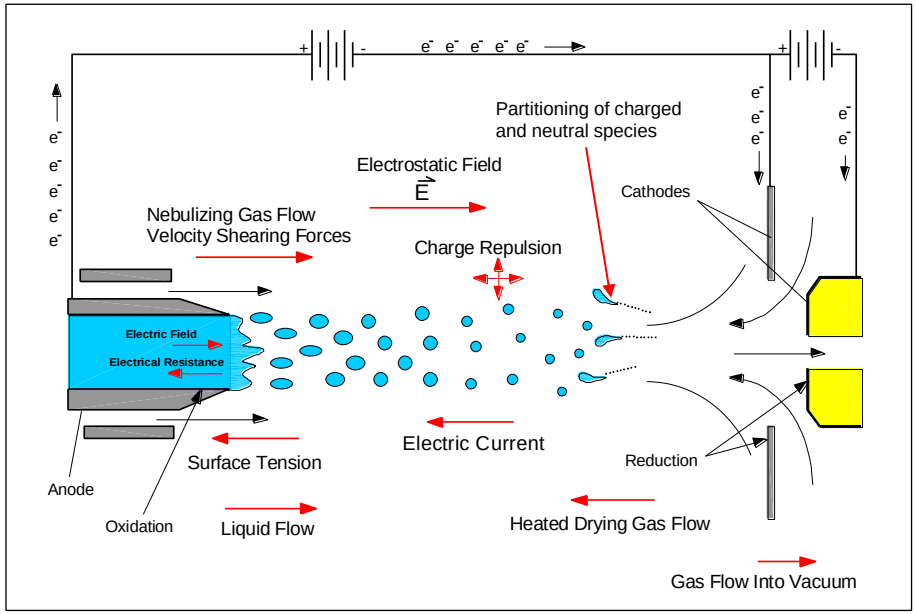
N B Cech & C G Enke Mass Spectrom Reviews 2001, 20, 362

Electrospray – Positive Ion Polarity



Electrospray with Pneumatic Nebulization Assist – Positive Ion Polarity



The major steps in ESI

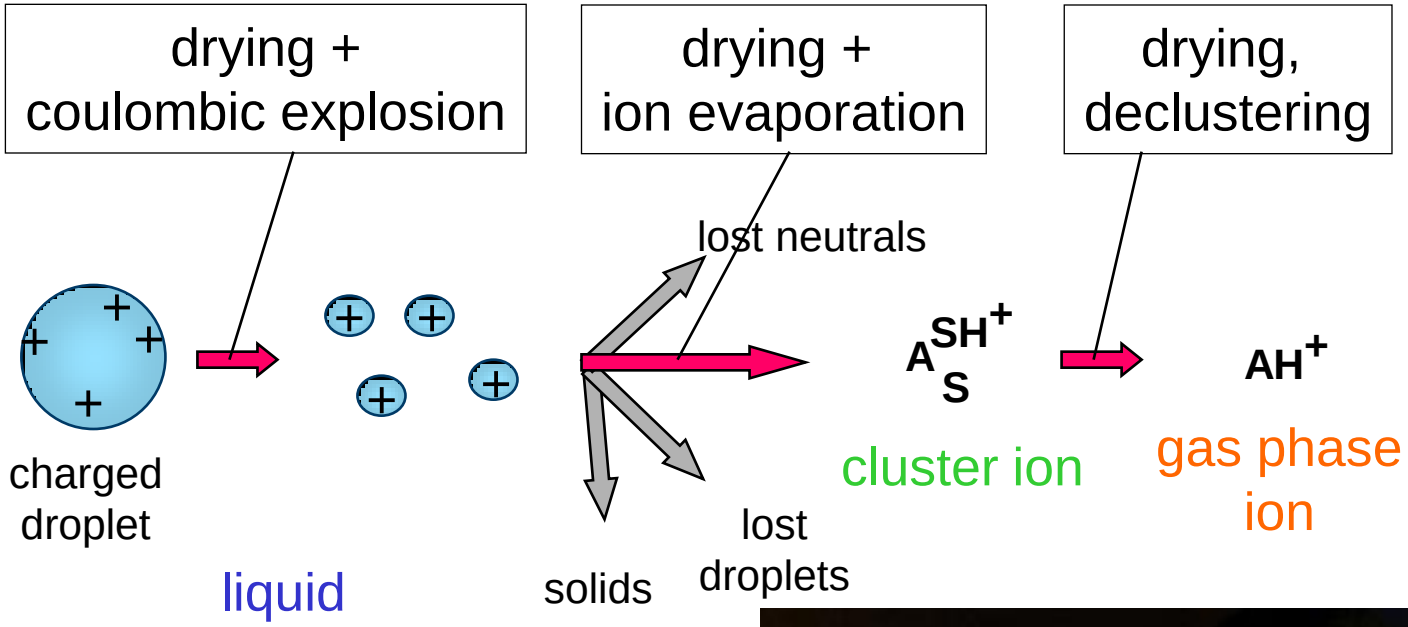
- Formation of a Taylor cone with excess of positive charge on its surface
- Formation of charged droplets
- Shrinkage of droplets through evaporation and coulombic fission (droplet disintegration into smaller droplets due to increased charge density)
- Ionization takes place in gas-phase ions produced by ion evaporation from small highly charged droplets

N B Cech & C G Enke Mass Spectrom Reviews 2001, 20, 362

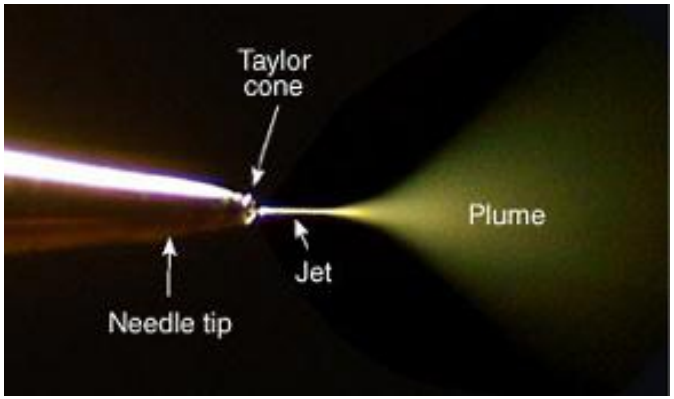
E de Hoffmann & V Stroobant Mass Spectrom: Principles and Applications, 2nd ed, J Wiley & Sons, 1999

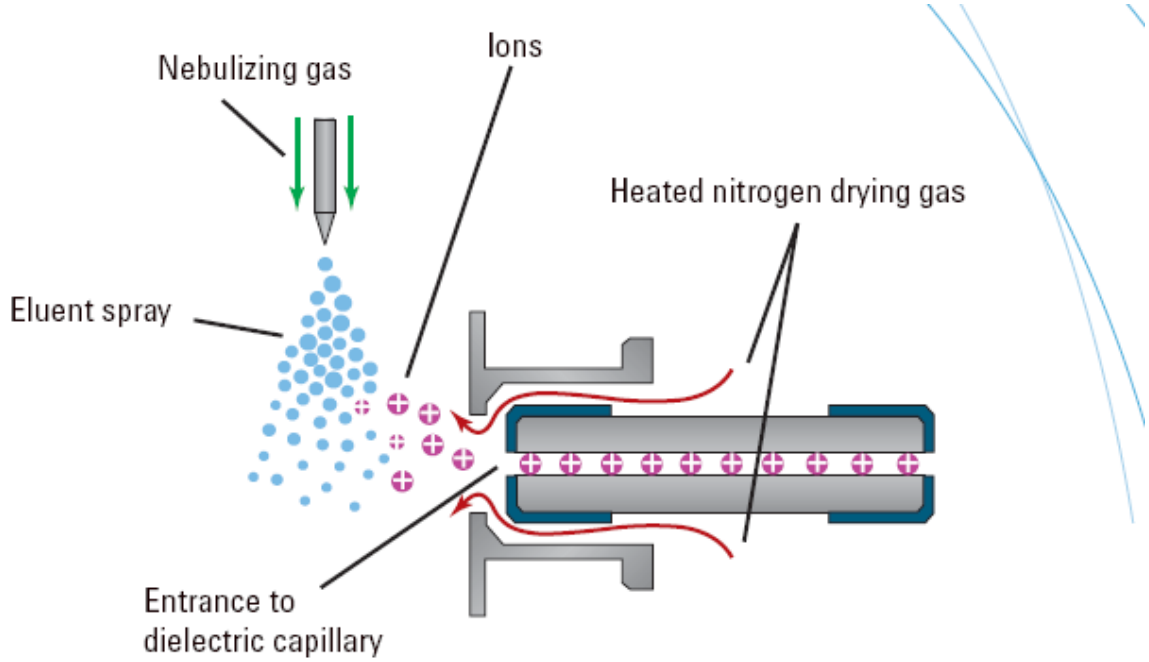
Electrospray ionization process

Analyte: A
Solvent: S



Suppression: Interacts with evaporation or with gas phase ion formation

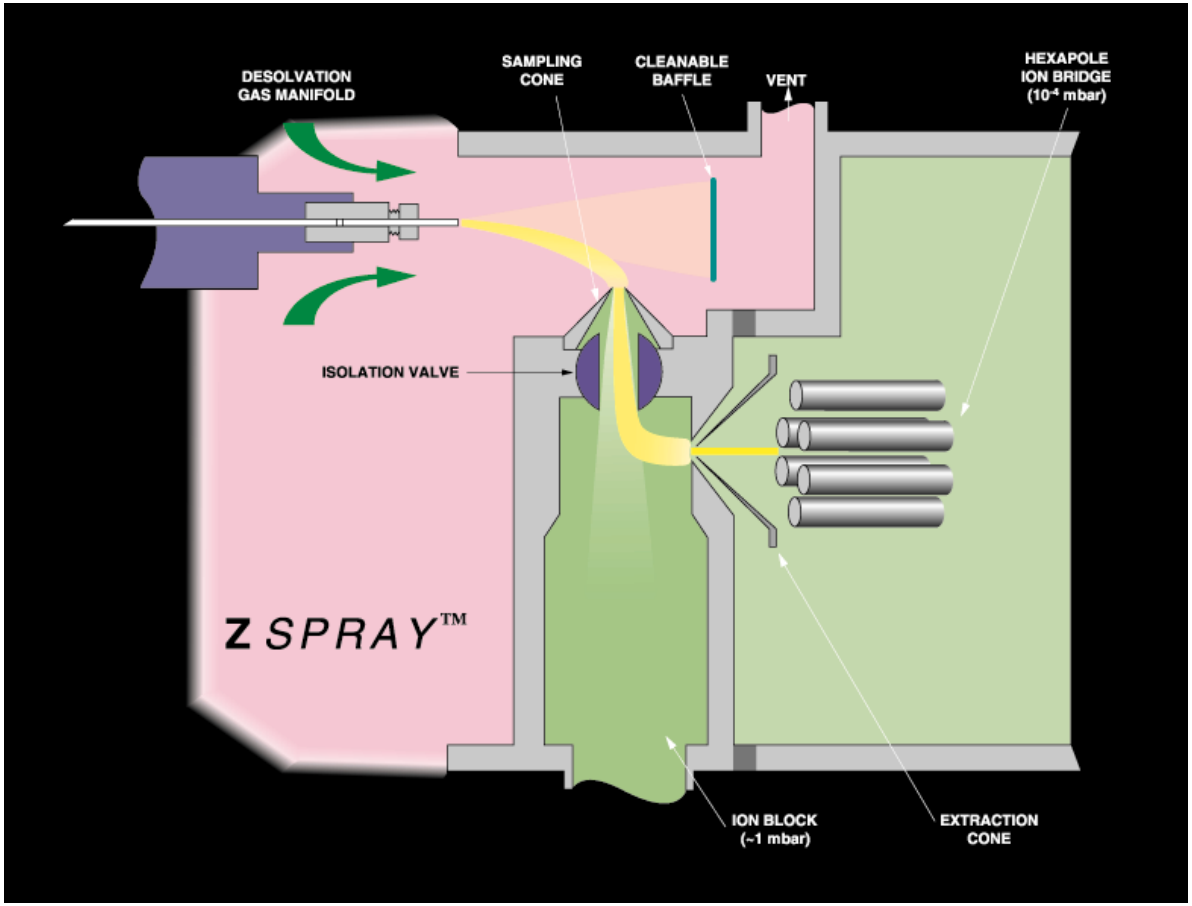




Orthogonal nebulization and high drying capacity reduce droplet noise for better sensitivity, and also reduce maintenance requirements

Orthogonal Spraying

The concept used by most manufactures today



Factors affecting electrospray ion formation

System variables

- ES-capillary diameter
- ES-capillary voltage
- Distance to counter electrode
- Heat capacity of ambient gas
- Solvent saturation level of ambient gas

Compound variables

- Surface activity
- Proton affinity
- pKa
- Solvation energy

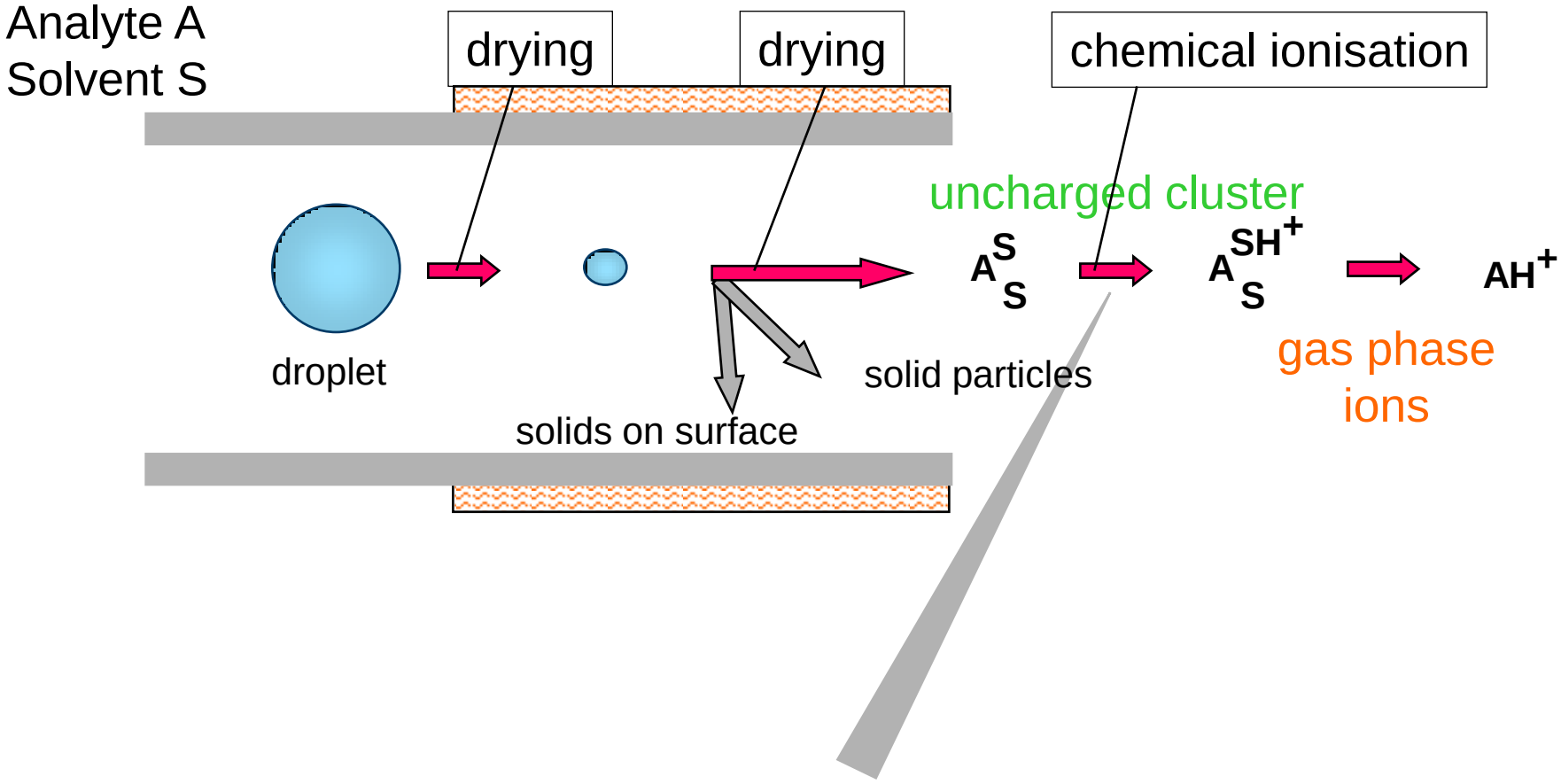
Method variables

- Flow rate
- pH
- Solvent properties (boiling point, surface tension etc.)

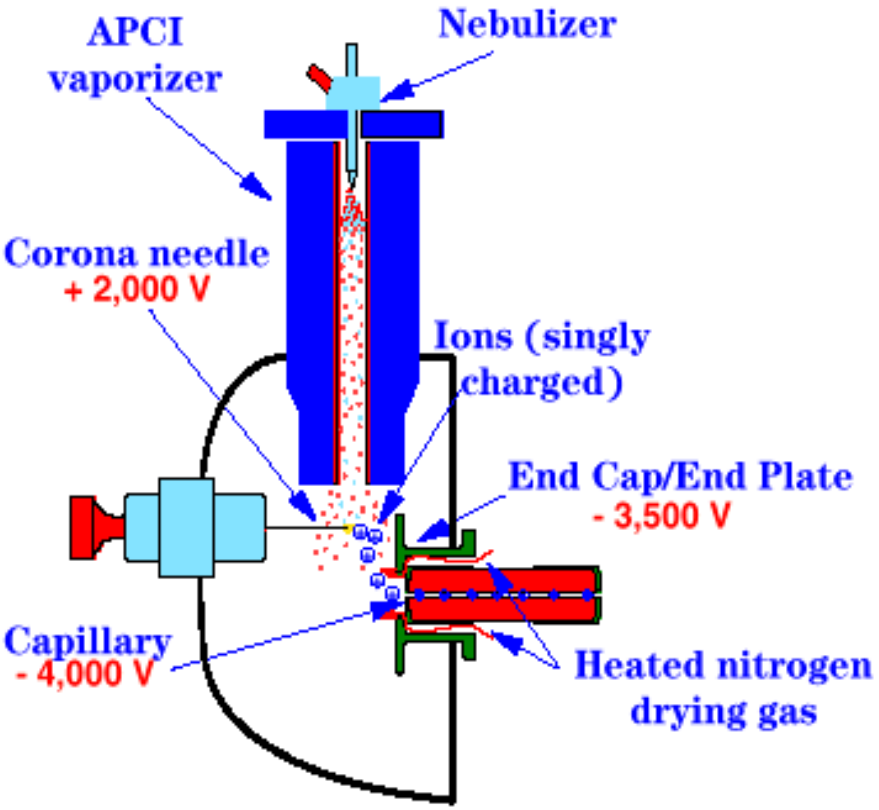
Atmospheric Pressure Chemical Ionization APCI

- The mobile phase and analyte are nebulized
- The droplets are vaporized
- The mobile phase molecules are ionized by electrons from the corona discharge
- The analyte molecules are ionized by the mobile phase ions
- Ionization takes place in the gas phase

APCI ionisation process



APCI Ionization (positive mode)



HEWLETT PACKARD

ESI vs APCI

APCI

+ No flow splitting
+ Can handle polar and non-polar substances
+ Large linear range
- Can not be used with thermally labile substances
- No multicharging

ESI

+ Few parameters to optimise
+ Excellent for proteins
+ Can handle unstable molecules
+ Works with nanoLC
- Low sensitivity with non-polar substances
- Limited linear range

Compatability of API-MS with Chromatography

TABLE 2. Compatability of API–MS with Various Chromatographic Modes

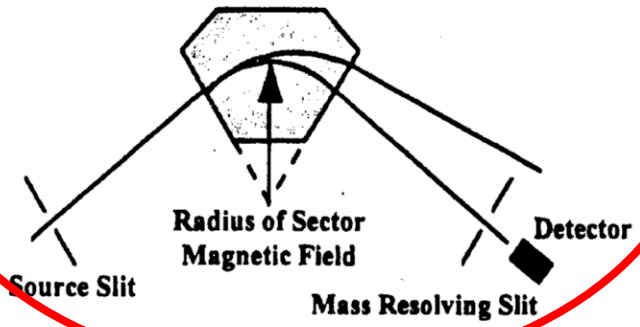
Mode	ES	APCI	Comments
Reversed phase	+++	++	Formation of ions in solution is possible; usually limited sample volatility
Normal phase	+	+++	Ions in solution (nonaqueous miscibility) may be a problem; usually sample is volatile
Size exclusion	+++	+	Buffers to suppress nonexclusion mechanisms may cause problems; most likely sample is not volatile and is a high molecular weight
Ion pair	++	++	Reagent ions may compete for ion-evaporation process; volatility of mobile-phase additive
Ion exchange	+	++	High ionic strength may be a problem; limited volatility of mobile-phase additives
Hydrophobic interaction	+	+	Uses salt gradients to elute biomolecules; salt is not compatible with API–MS
Immunoaffinity	+++	+	Mobile phase often compatible with API–MS, usually nonvolatile sample

[a] A greater number of plus signs indicates a larger degree of compatibility.

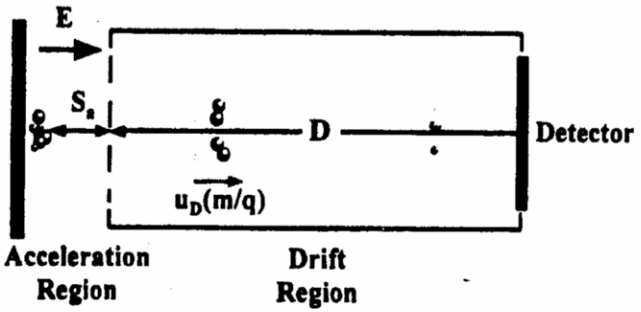
Mass Analysers

- Magnetic Sector
- Quadrupole
- Time-of-flight (TOF)
- Ion Trap
- Orbitrap
- Fourier Transform Ion-Cyclotron Resonance (FTICR)

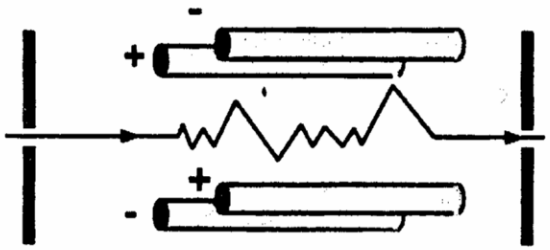
(a) MAGNETIC SECTOR



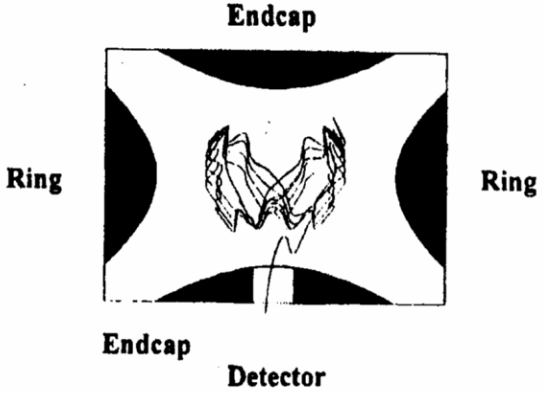
(b) TIME OF FLIGHT



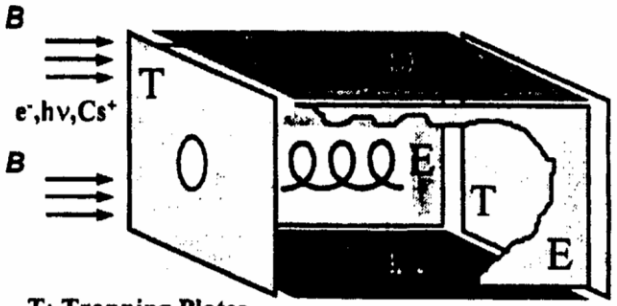
(c) QUADRUPOLE FILTER



(d) ION TRAP



(e) ION CYCLOTRON RESONANCE



T: Trapping Plates
E: Excitation Plates
D: Detection Plates

Magnetic sector mass spectrometer

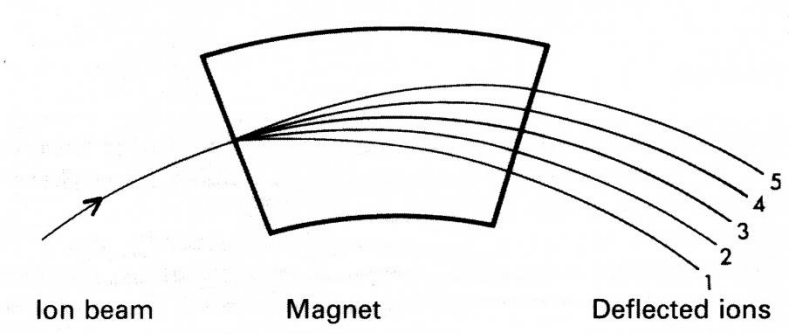
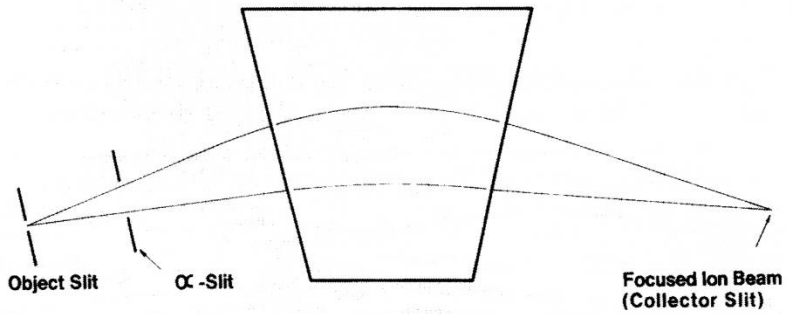


Figure 2. Deflection in a magnetic field of an ion beam consisting of increasing mass-to-charge ratios, $m_1/z....m_5/z$ and split into different trajectories (1-5) respectively



gure 3. Directional (or angular) focusing of a magnet.

Double-focusing mass spectrometer

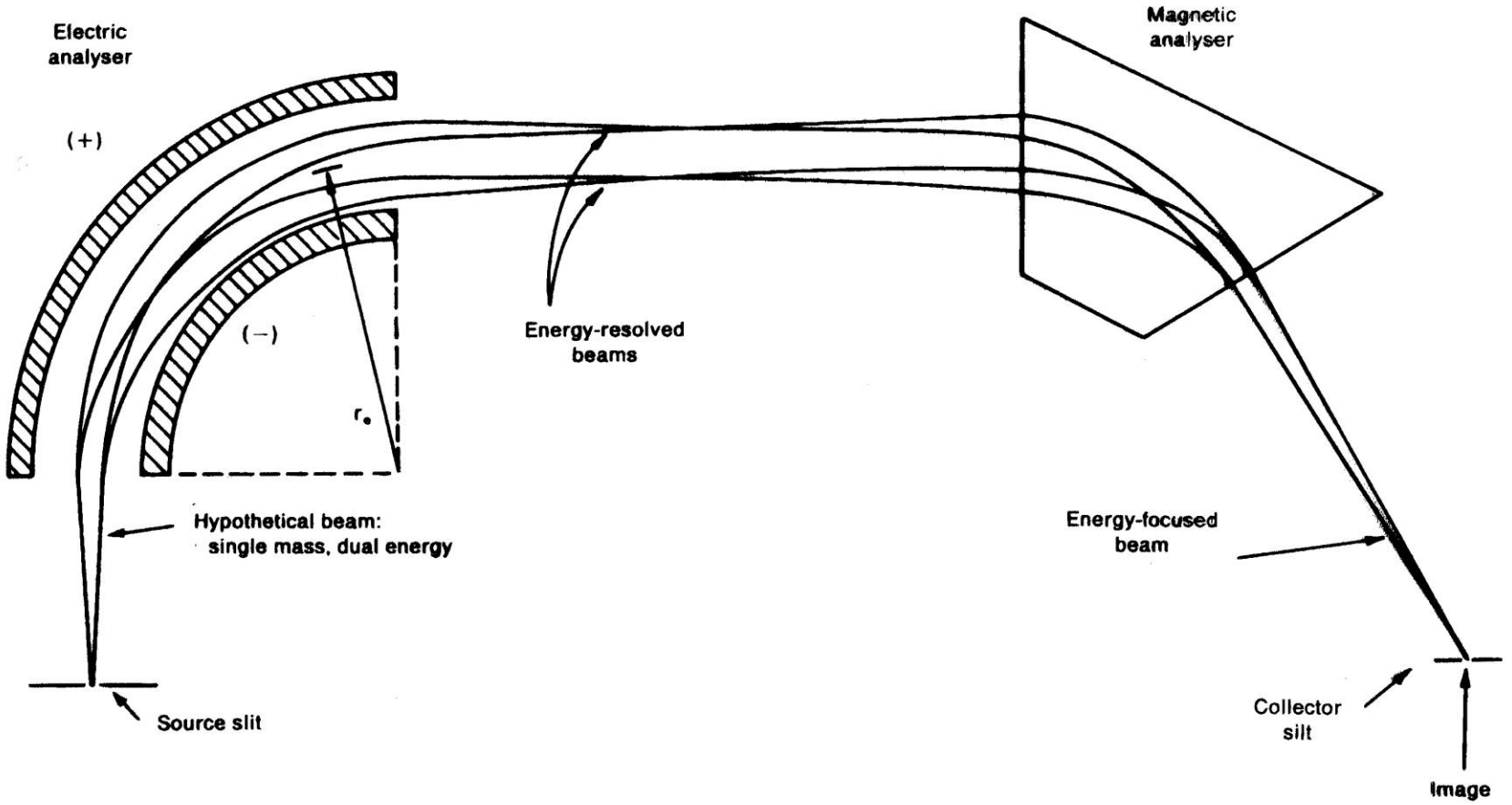


Figure 6.2. Nier-Johnson double-focusing mass spectrometer. The two sets of ion paths illustrate focusing for dispersion and for ion kinetic energy (shown in exaggerated form; Ligon 1979). Modern commercial instruments give a resolving power of 10^4–10^5 and mass measuring accuracy of 2–5 ppm.

Definition of Resolution

1.7 The Definitions and Measurement of Resolution

The resolution of a mass spectrometer may be defined in terms of its capacity to separate ions of adjacent mass number. A standard definition of separation has not, however, been adopted. The resolution necessary to separate two ions of mass m and $(m + \Delta m)$ respectively is given by

$$R = \frac{m}{\Delta m} \tag{1.19}$$

Resolution is sometimes expressed in terms of 'parts per million':

$$R(ppm) = 10^6 \frac{\Delta m}{m} \tag{1.20}$$

Resolution may be measured by reference to one or two peaks. For example, consider the 10% valley definition of resolution in which two peaks of equal intensity are considered to be resolved when they are separated by a valley which is just 10% of the height of either peak and which is made up from a 5% contribution from each component (Fig. 1.17). From the figure it can be seen that Δm can be measured from the separation of the two peaks or from the width of a single peak at 5% height. If a single peak is used, then the operator relies on a previous calibration of the peak display to measure resolution. If, on the other hand, a doublet of known separation is used, it is relatively easy to confirm that resolution has been achieved by measurement of the valley height between the peaks.

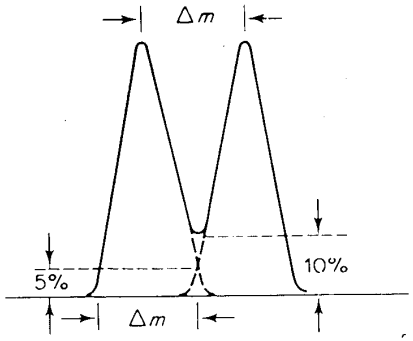
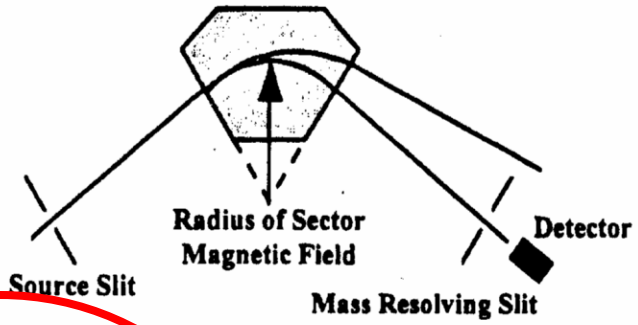
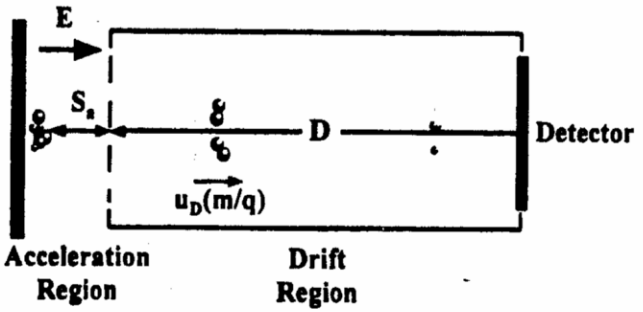


Fig. 1.17 Definitions of resolution

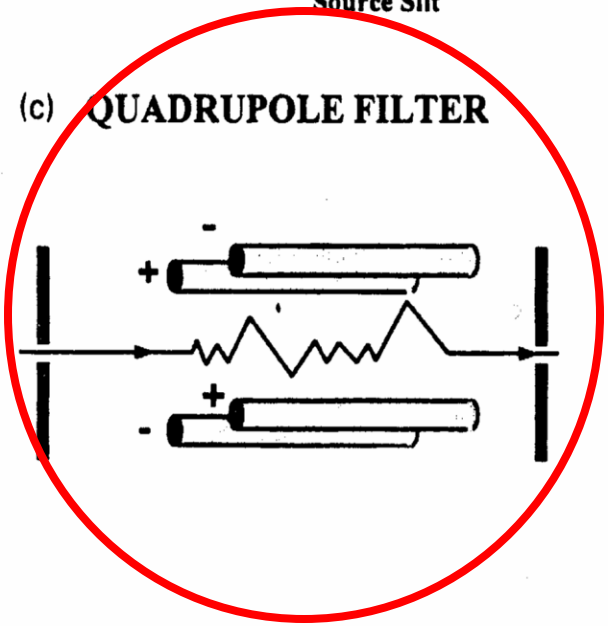
(a) **MAGNETIC SECTOR**



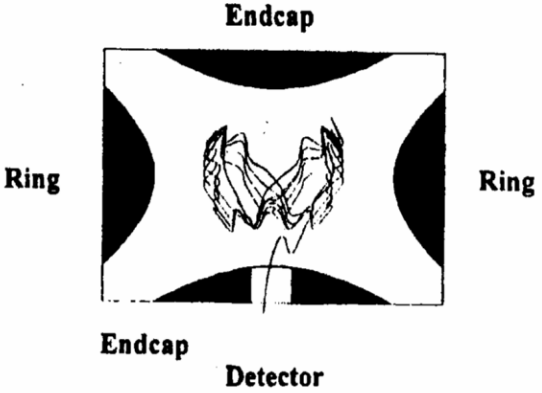
(b) **TIME OF FLIGHT**



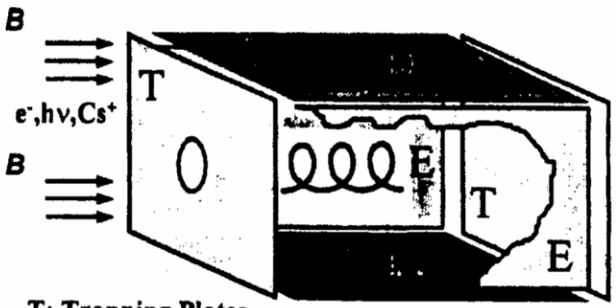
(c) **QUADRUPOLE FILTER**



(d) **ION TRAP**



(e) **ION CYCLOTRON RESONANCE**



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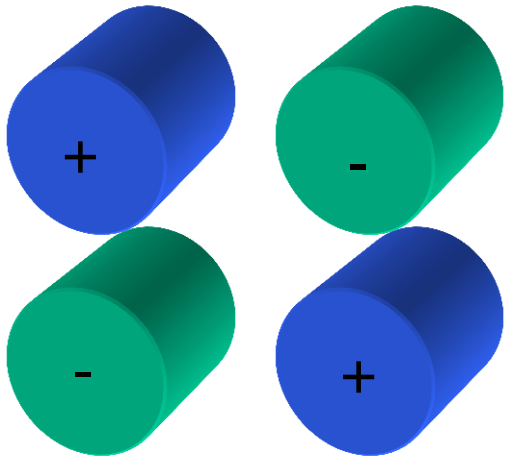
E: Excitation Plates
D: Detection Plates

How does the quadrupole work?

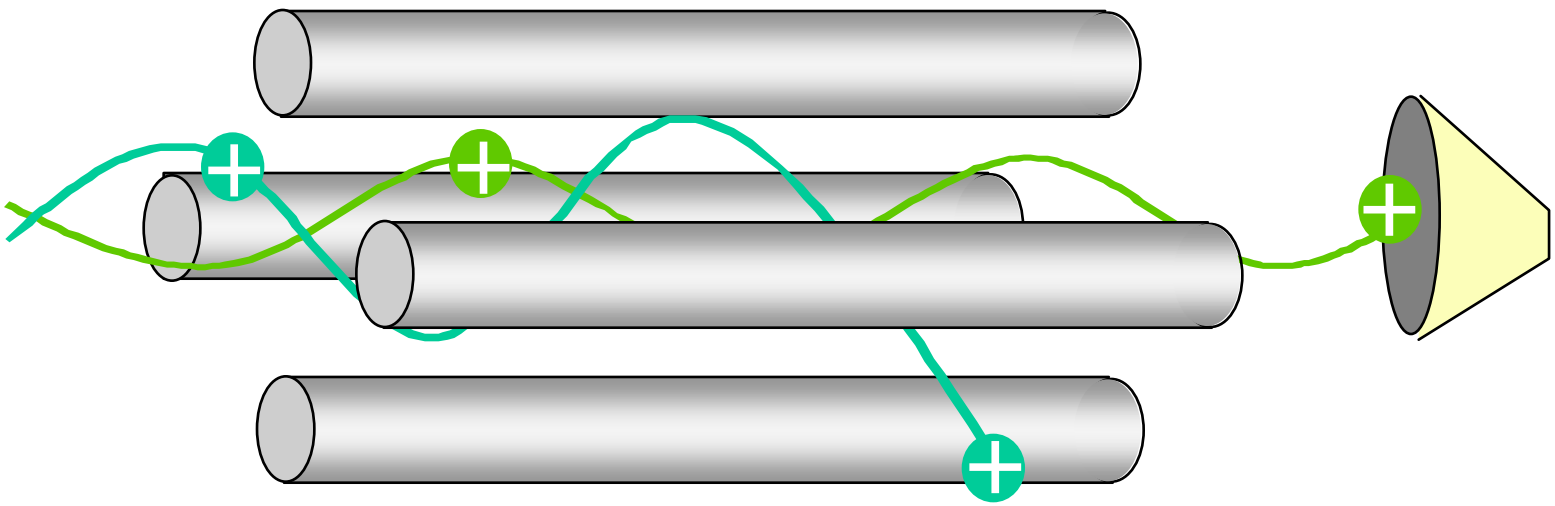
The quadrupole consists of four parallel rods. The opposing rods have the same polarity while adjacent rods have opposite polarity.



An electrical field is created by applying a direct current (DC) and radio frequency (RF) voltage to each rod.

Ions are scanned by varying the DC/RF quadrupole voltages. Only ions with the selected mass to charge ratio will have the correct oscillatory pathway in the RF field.

All other ions are filtered out of the ion beam.



The ● ion is transmitted along the quadrupole in a stable trajectory Rf field. The ● ion does not have a stable trajectory and is ejected from the quadrupole.

The quadrupole scans by ramping the DC/RF voltage in time across the rods.

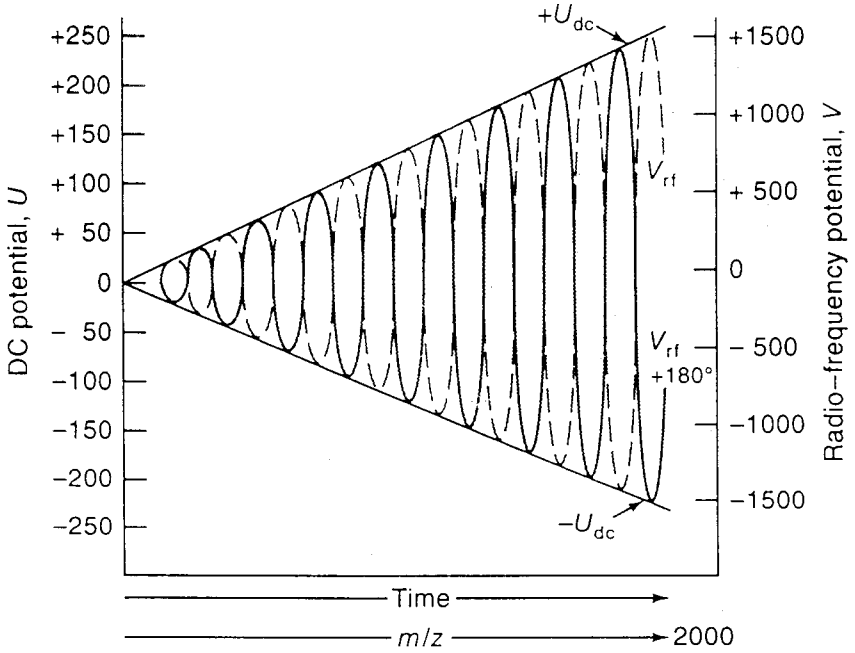


FIGURE 18–10 Voltage relationships during a mass scan with a quadrupole analyzer.

At any given time ions of only one m/z are allowed to pass through to the detector. All other ions are rejected.

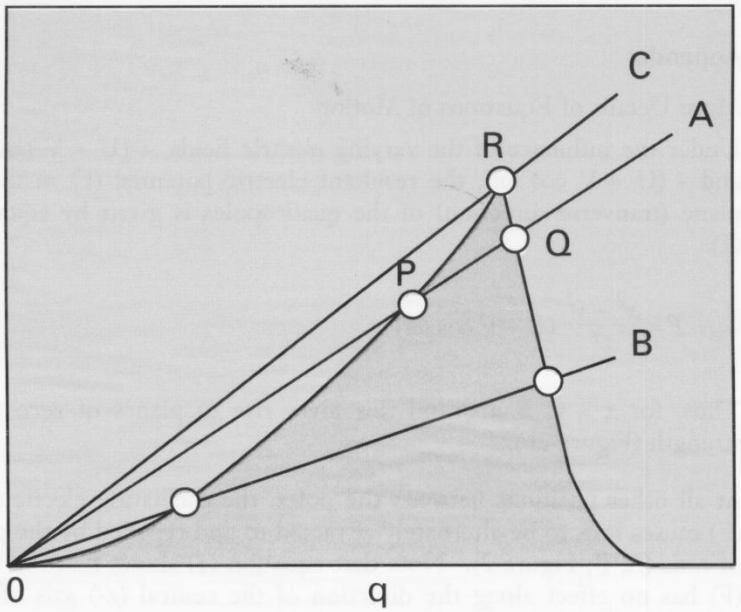


Figure 4. Relationship between a and q. The shaded area indicates regions of stable ion motion through the quadrupolar field

Passage through the quadrupole assembly is described as stable motion, whilst those trajectories which lead to ions striking the poles is called unstable motion.

From mathematical solutions to the equations of motion for the ions, based on equation (1) two factors (a,q; equation 2) emerge as being important in defining regions of stable ion trajectory.

$$a = \frac{8zU}{mr^2\omega^2} \quad ; \quad q = \frac{4zV}{mr^2\omega^2} \quad ; \quad \frac{a}{q} = \frac{2U}{V} \qquad (2)$$

QUADRUPOLE ION OPTICS

For small values of a and q, the shaded area in Figure (4) indicates an area of stable ion motion, v.i.z., it shows all values for a,q for which ions can be transmitted through the quadrupole assembly.

To gain some idea of the meaning of this shaded area, consider the straight line OA of slope (a/q) shown in Figure (4). The line enters the region of stable motion at P and leaves it at Q. For typical values of U(1000 volts), V(6000 volts), ω (1.5 MHz) and r (1.0), equations (2) predict that point P corresponds to an ion of m/z 451 and Q to m/z 392. Therefore, with these parameter values, all ions having m/z between 392 and 451 will be transmitted through the quadrupole.

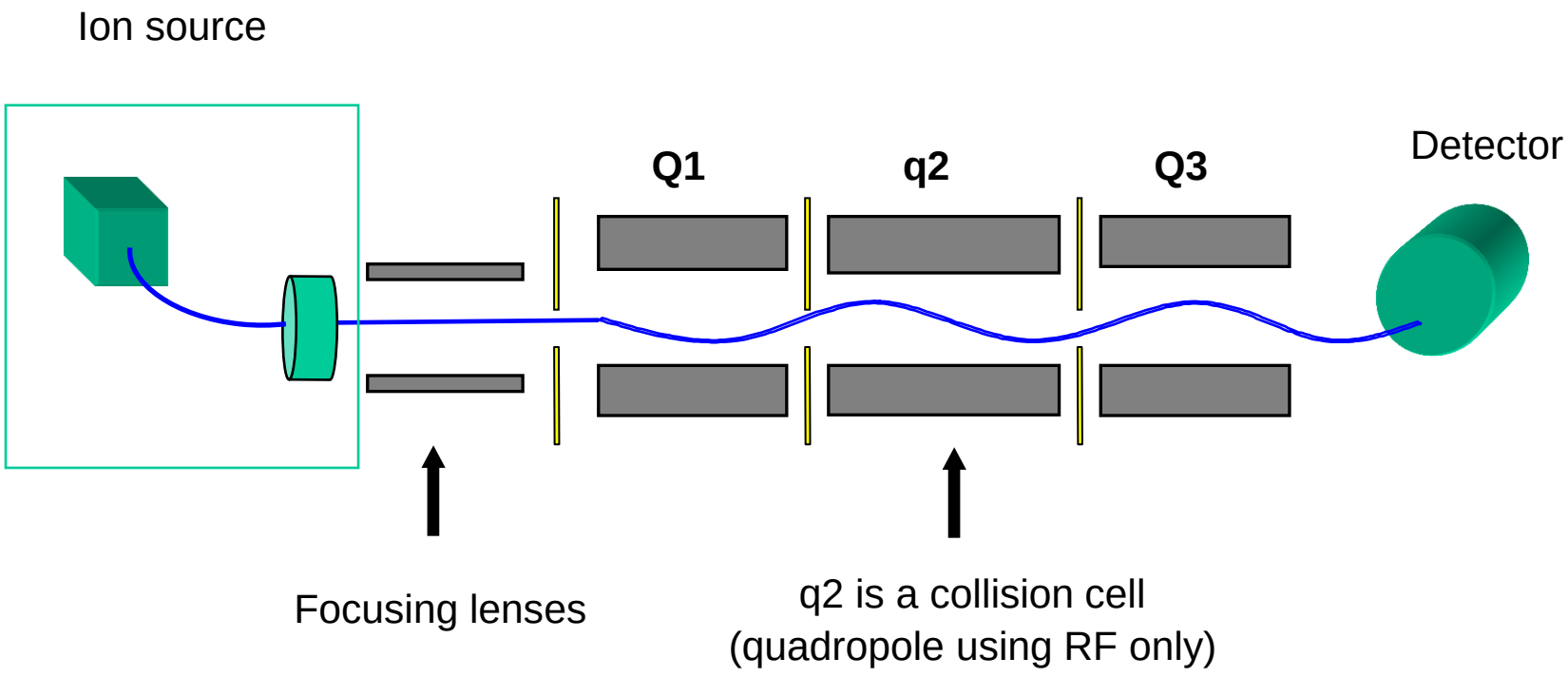
For a line (OB; Figure 4) of smaller slope (smaller a/q or smaller U/V), an even greater range of m/z values will be transmitted through the quadrupole assembly. Conversely, for a line OC which passes through the apex (R) of the region of stability, no ions of any m/z valve are transmitted.

To ensure that only ions of any one selected m/z are transmitted (maximum resolution), the parametres (U,V,ω) must be chosen such that a/q (or 2U/V) fits a line which passes close to R but which still lies within the region of stability. This will give maximum resolution for the instrument. For example, with U (1000 volts), V (6000 volts), ω (2MHz) and r(1.0 cm), only ions of m/z 862 would be transmitted. To transmit ions of other m/z, the parameters U, V, ω and r have to be changed.

For a given assembly, r is fixed and electronically, it is easier to change voltages (U, V) than frequencies (ω). Therefore, to transmit ions of other m/z values, the frequency is kept constant but the voltages (U,V) are varied in such a way that U/V (or a/q) remains constant. By continuously increasing or decreasing U and V whilst keeping U/V constant, ions of increasing or decreasing m/z successively traverse the quadrupole assembly to give a mass spectrum.

For convenience, different frequencies may be used for different mass ranges, e.g. the RF may be 1.5 MHz for 0 - 1000 amu and 0.8 MHz for 1000 - 4000 amu.

Schematic of a Triple Quadrupole Mass Spectrometer



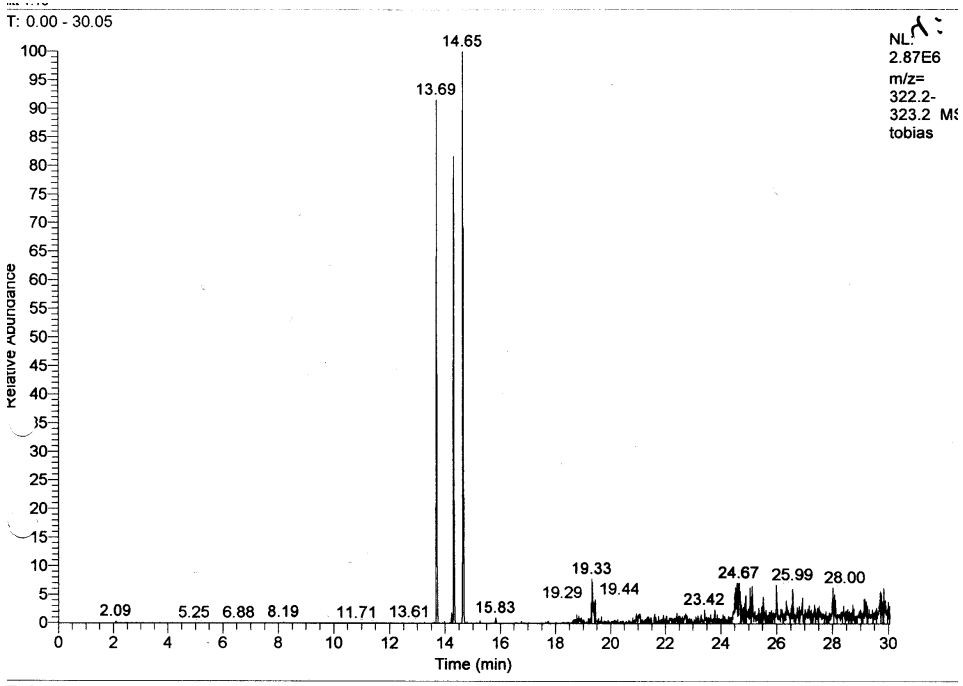
MS/MS-scan functions

- **Daughter ion scan** – MS1 is set on parent ion mass and MS2 is scanned
- **Parent ion scan** – MS2 is set on daughter ion mass and MS1 is scanned
- **Neutral loss scan** – MS1 and MS2 are linked with an offset of the lost mass and both are scanned synchronized

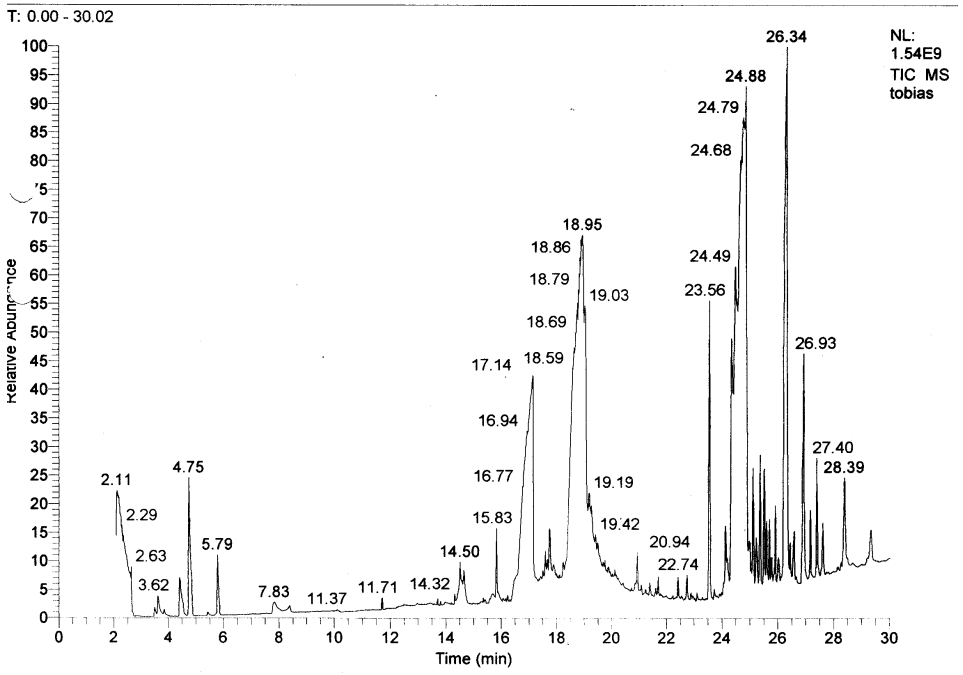
Quantitation

To increase the sensitvity of the system we can use the high selectivity of the mass spectrometer.

- **Total-ion chromatogram** (TIC) by summing the intensities of all the peaks in each spectrum vs the spectrum number, *i.e*. time
- **Mass chromatogram** by summing intensities of peaks in a narrow mass window
- **Selected Ion Monitoring/Recordning** (SIM, SIR)
- **Selected/Multipel Reaction Monitoring** (SRM, MRM)

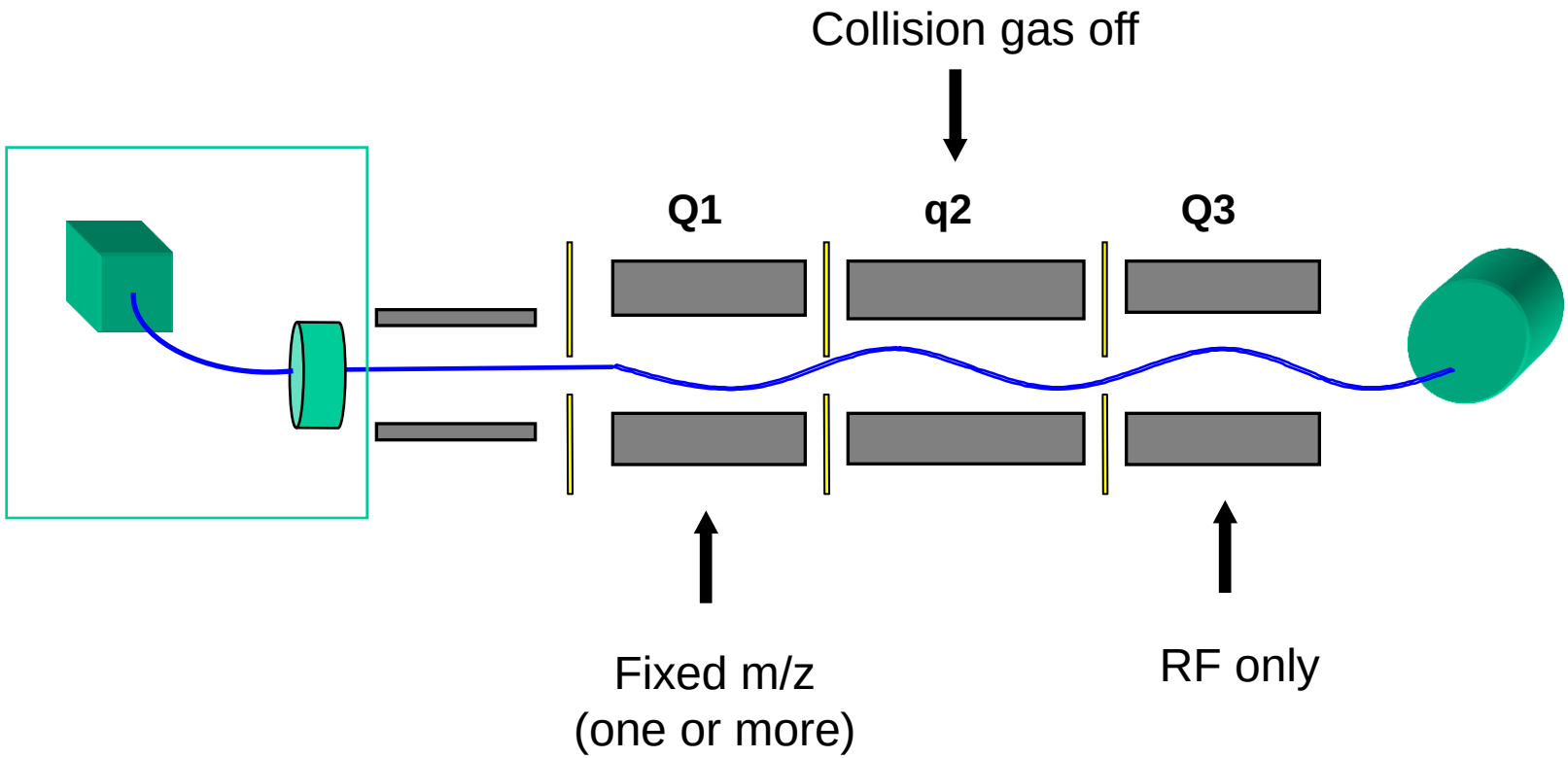


Mass Chromatogram



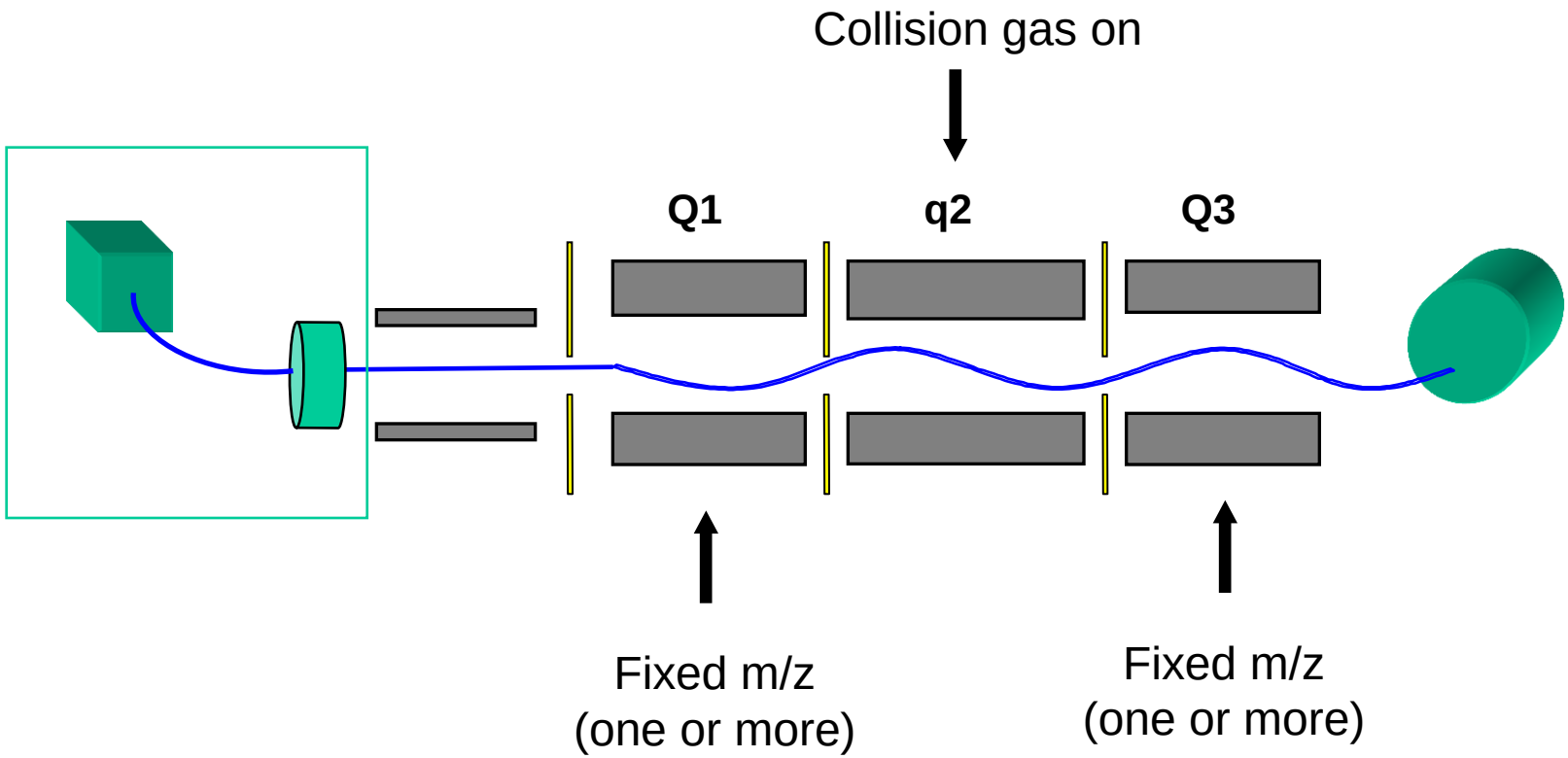
TIC (Total Ion Current)

Selected Ion Monitoring (SIM)



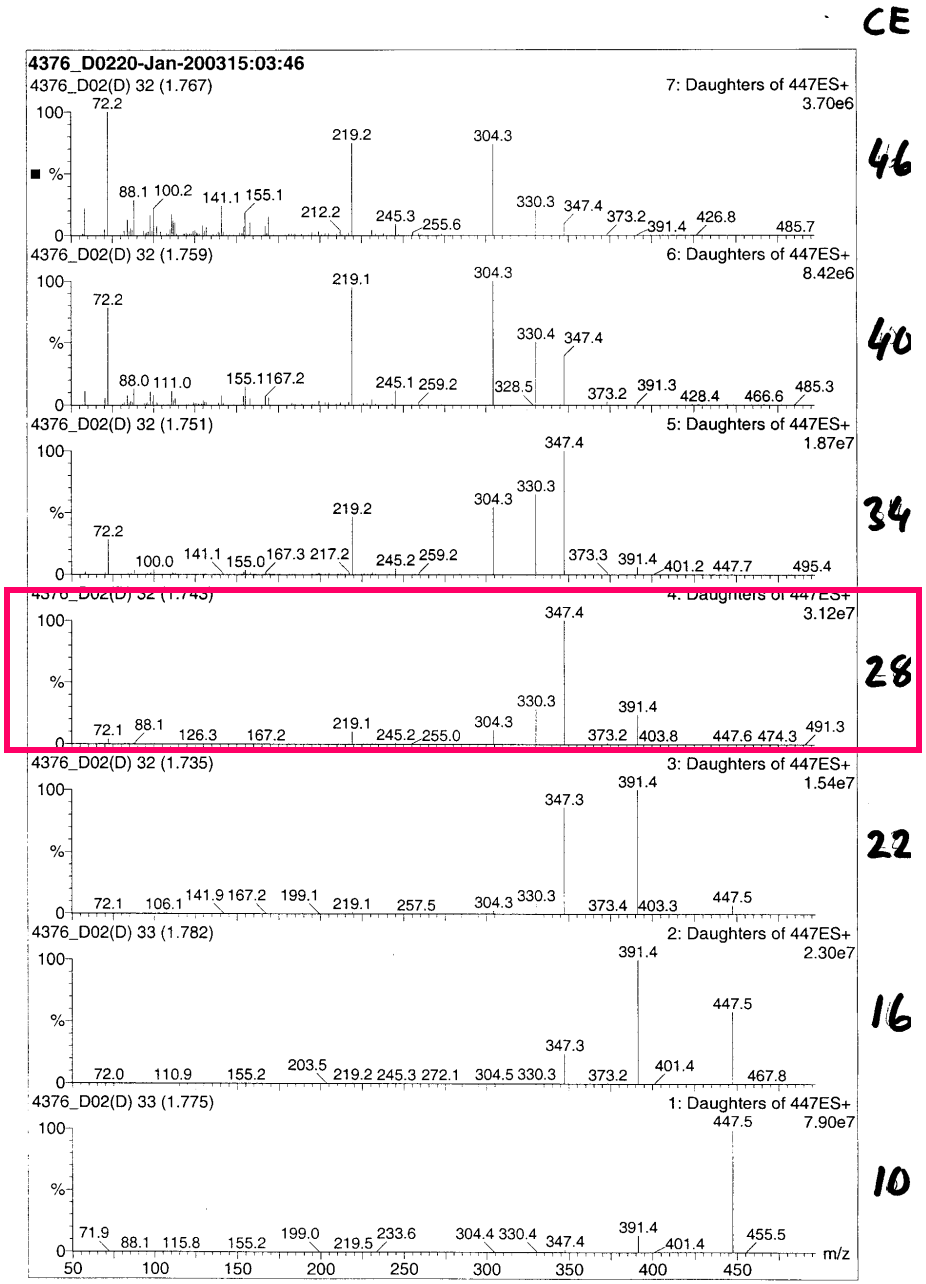
We could also do this on a single quadrupole

Selected Reaction Monitoring (SRM)

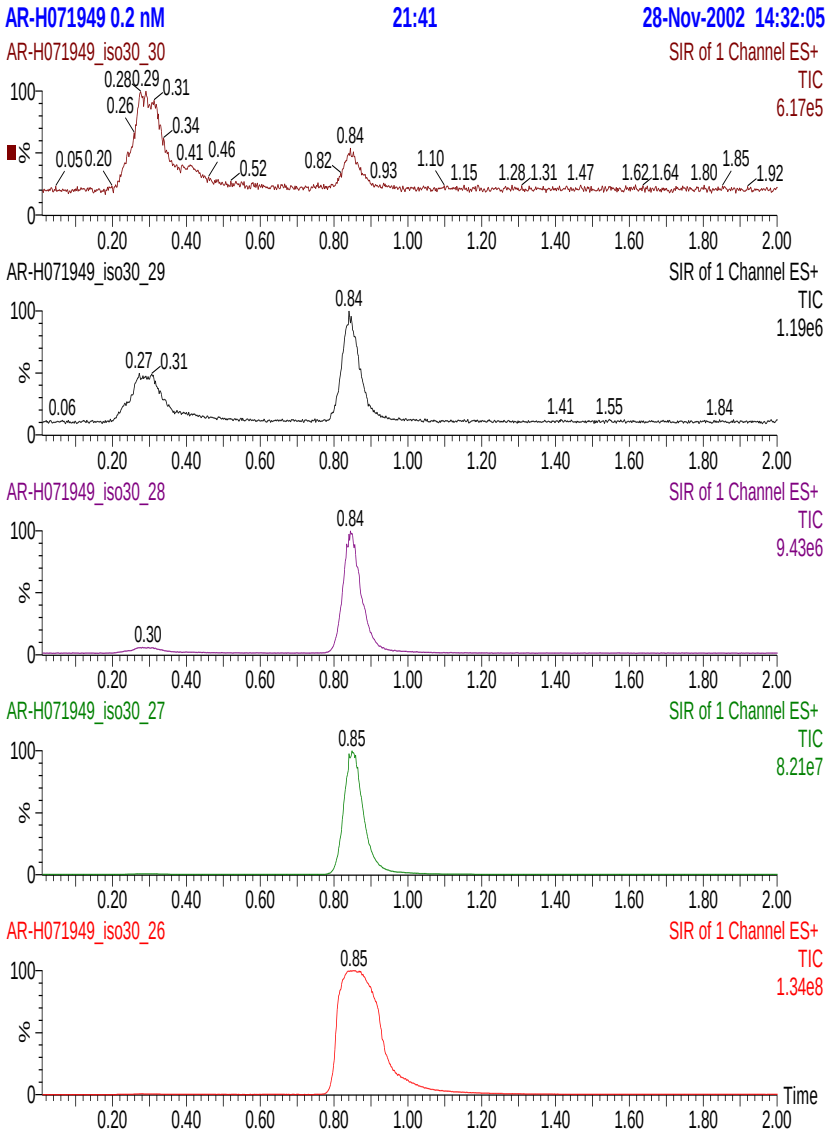


We need a triple quadrupole to do this

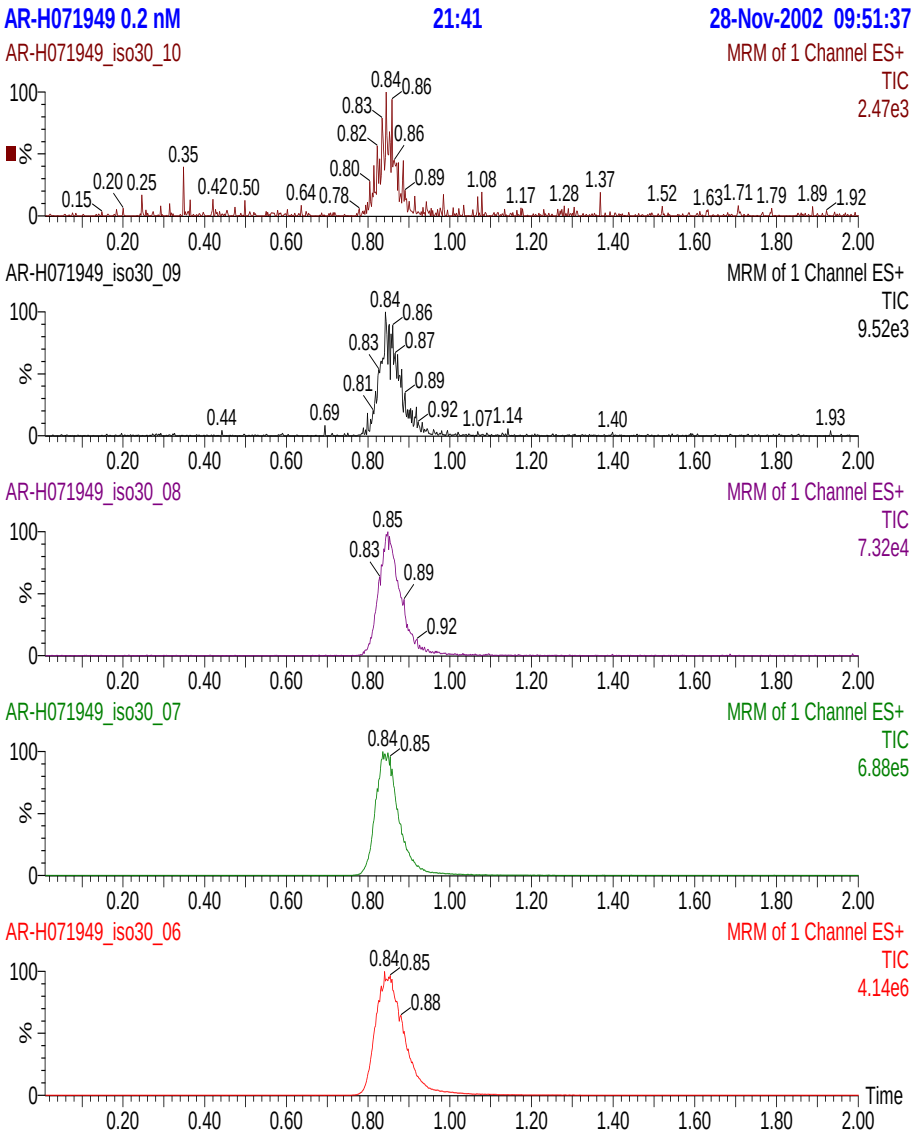
Optimization using daugter mass spectra at different collision energy



SIR



MRM

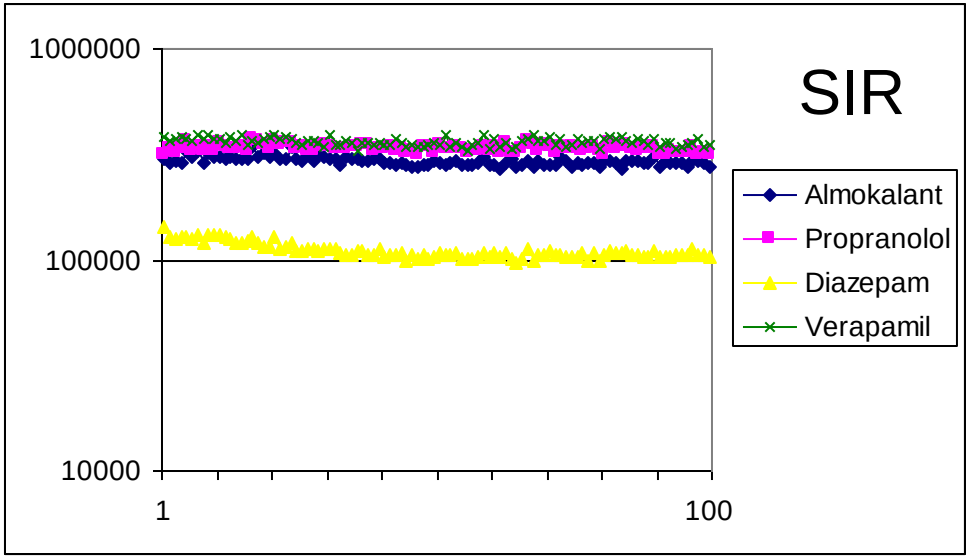


Stenhagen Analyslab AB

Comparison SIR/MRM on control substances

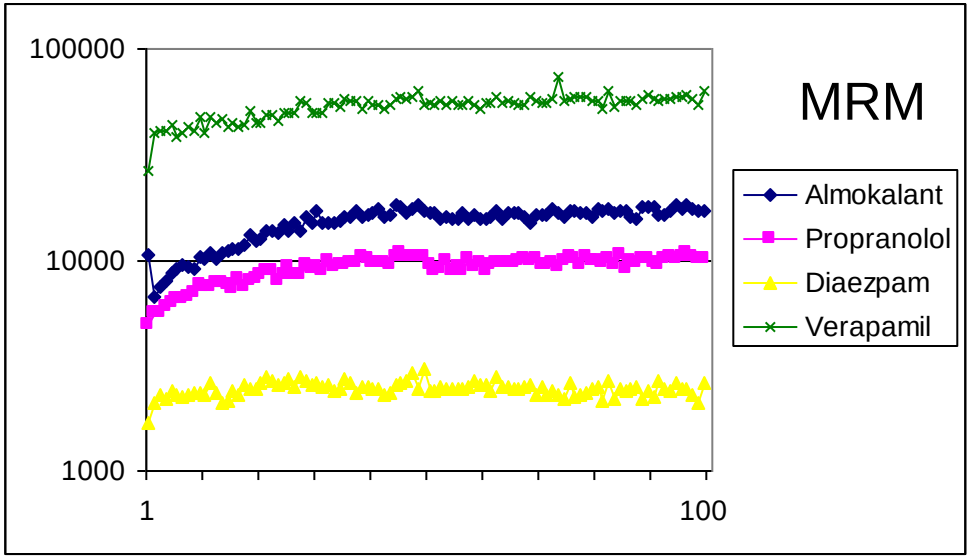
Advantage SIR

- No need for optimisation
- Sensitivity similar for many compounds
- High signal level
- Simpler set up



Advantage MRM

- Very selective
- Easy to integrate peaks
- High signal to noise



Sample preparation

Protein Precipitation (PPT)

1. **Aliquot of sample**
2. **Spike with internal standard**
3. **Add cold acetonitrile**
4. **Vortex**
5. **Centrifuge**
6. **Remove supernatant**
7. **Reconstitute**
8. **Transfer to plate**
9. **Inject onto LC column**

Chromatographic method

Autosampler: CTC/PAL

LC gradient system: Shimadzu

LC columns: **1) Waters Xbridges IS C18 2.5µm 2.1x20mm**

2) AMT Halo C18 2.7µm 2.1x30mm

Mobile phase flow: 0.7ml/min

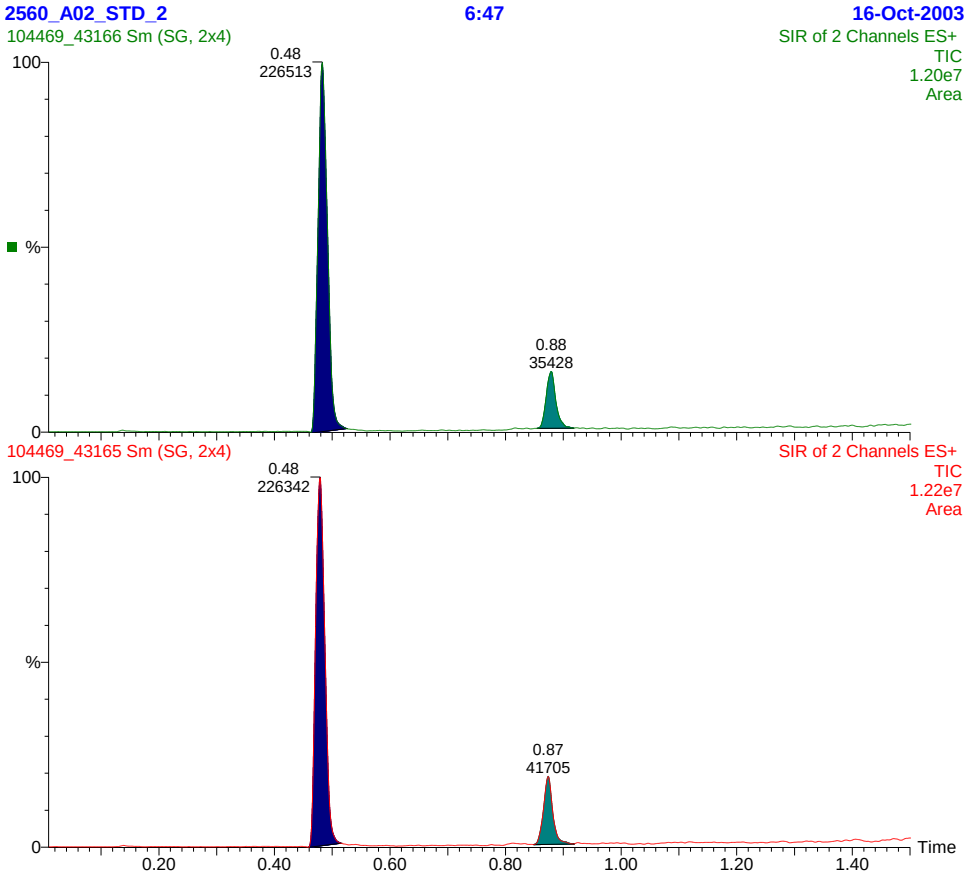
Gradient:5-95% Acetonitrile/H2O in 1min + 95% for 0.5 min

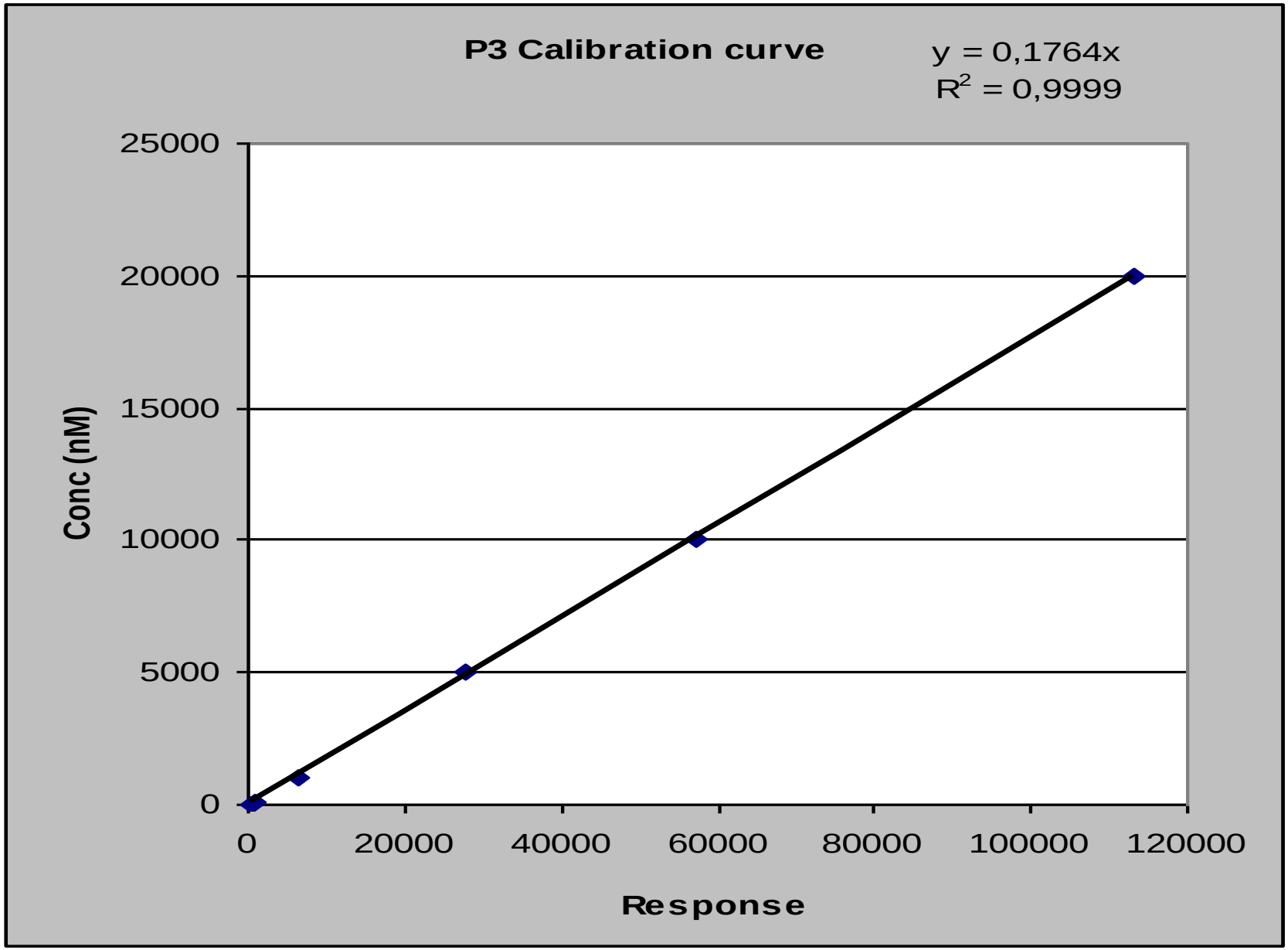
Injection volumn: 5µl

Liquid Chromatogram

Column: 30mm x 2.1mm ID, C18
Flow: 0.6ml/min, 5% - 95% ACN:H2O 1.5min

Cycle time: 2.2 min
A CaCo-2 assay with 640 samples can be analysed in less 24 hours



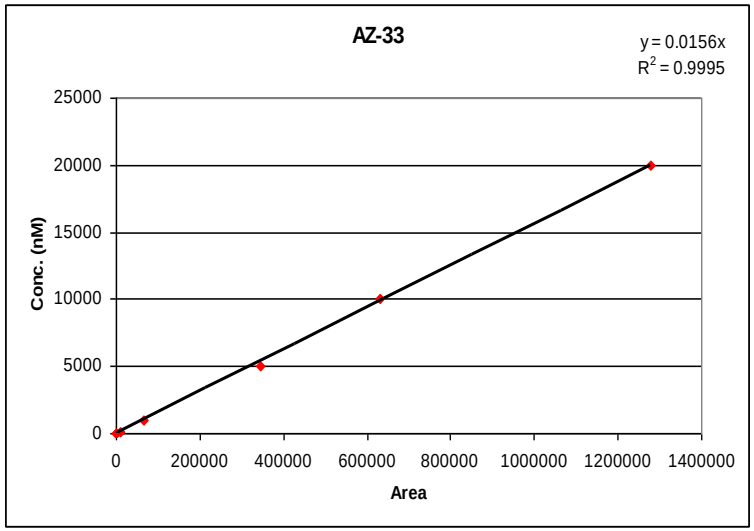

P3 Calibration curve
y = 0,1764x
R2 = 0,9999
25000
20000
15000
10000
5000
0
Conc (nM)
0
20000
40000
60000
80000
100000
120000
Response

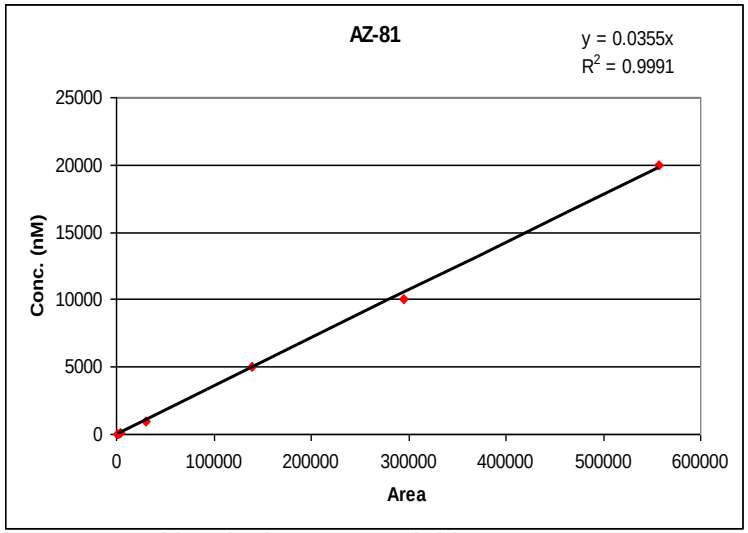
FDA guidelines:

- **The calibration curve should cover the entire anticipated range.**
- **The simplest model that adequately describes to concentration-response relationship should be used.**
- **Selection of weighting and use of a complex regression equation should be justified.**

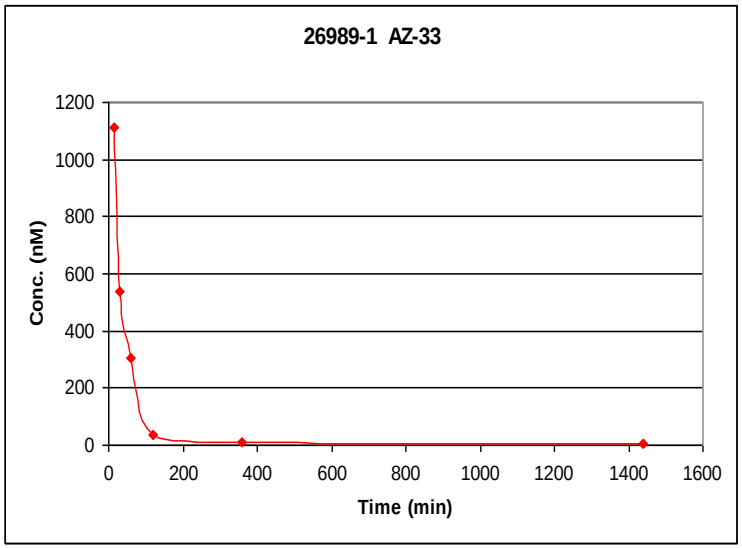
Calibration curve

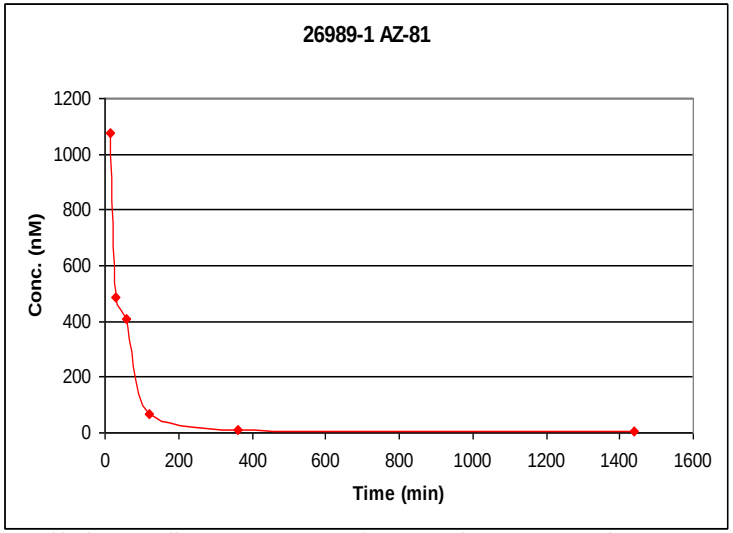
Mice plasma





Time exp.



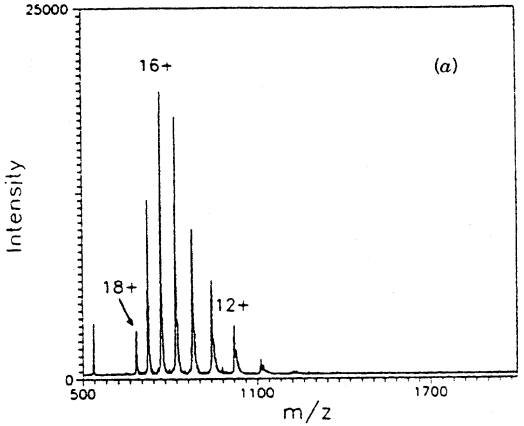


LC Shimadzu/CTC PAL, Xbridges C18 2.5µm 2.1x20mm, flow 0.7ml/min, gradient 5-95%ACN/H_2O 1min 95% 0.5min

Bovine Cytochrome C

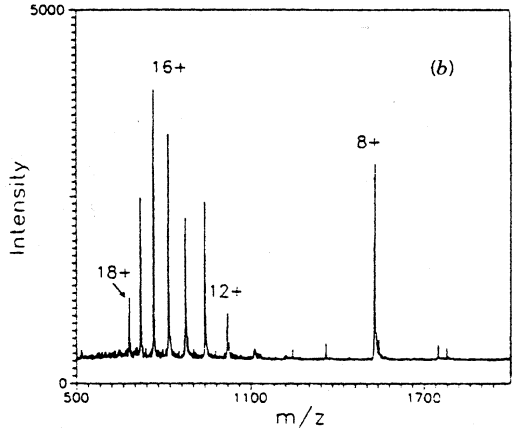
Mw 12 240 Da

Positive ion ESI mass spectra

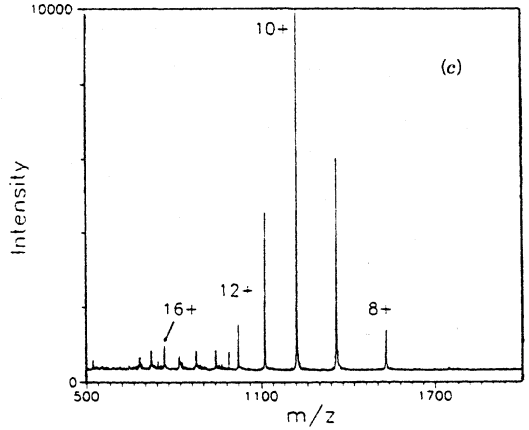


pH 2.6

unfolded

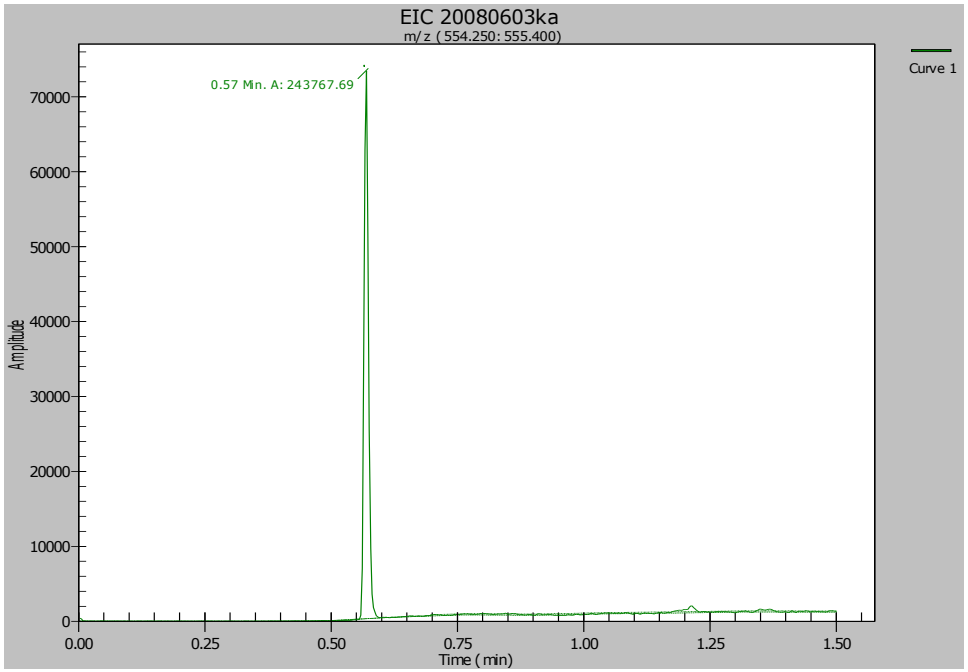


pH 3.0



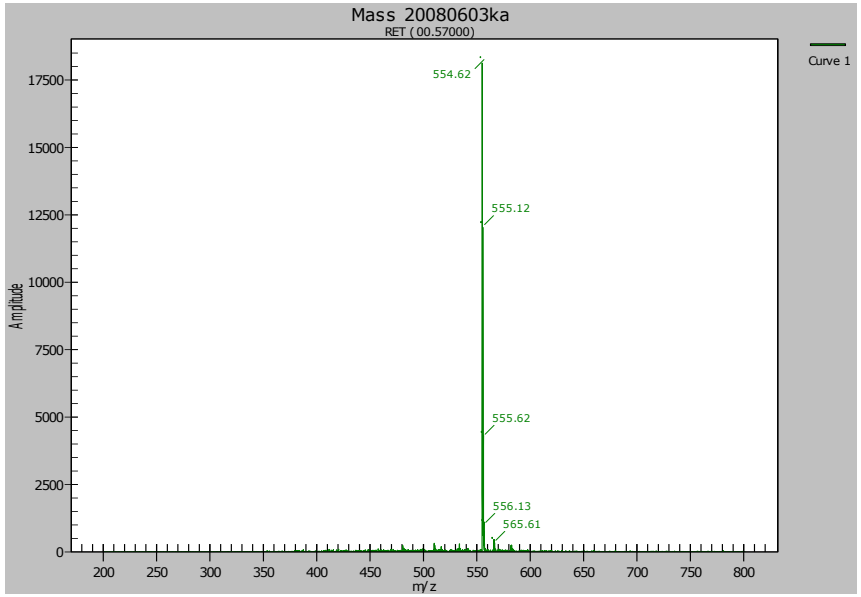
pH 5.2

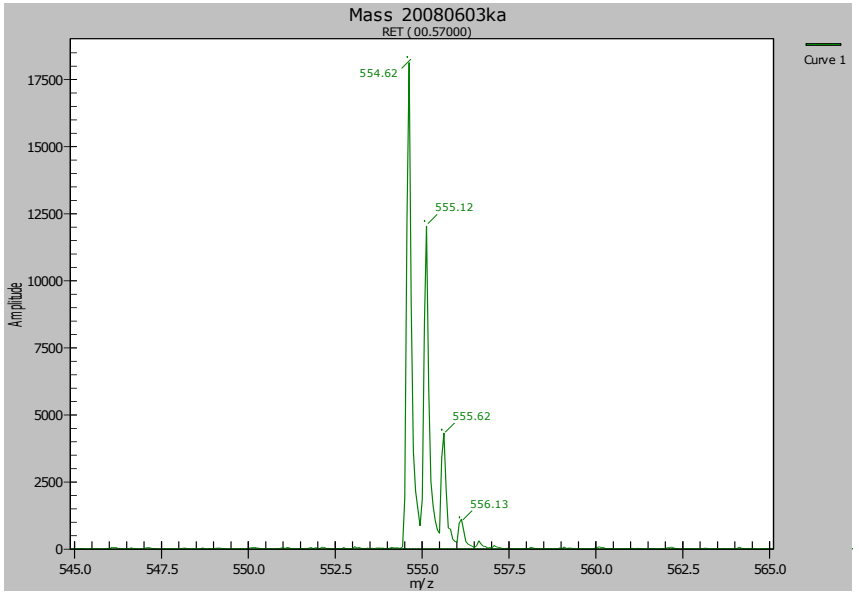
folded



PL33

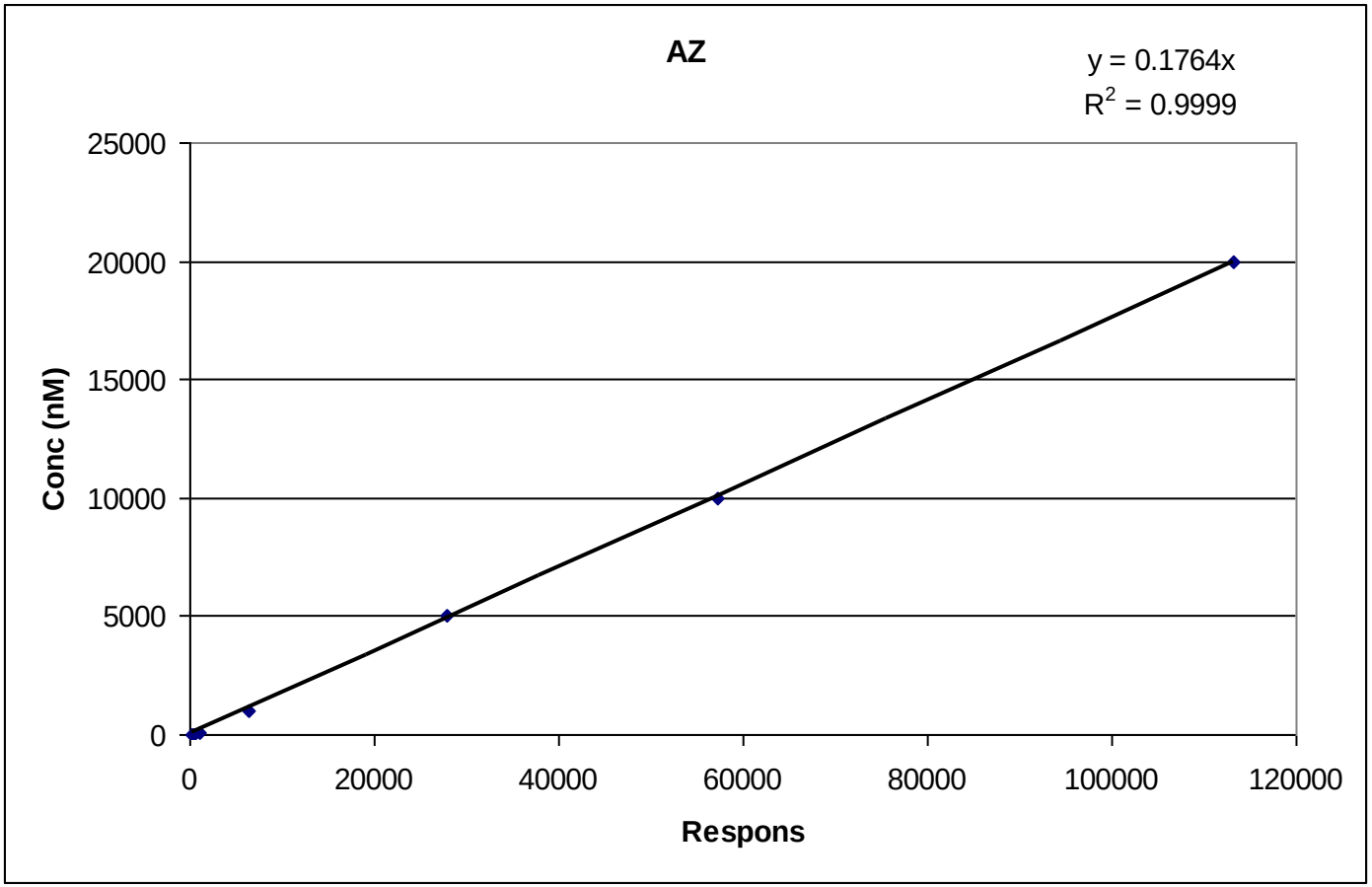
LC Shimadzu/CTC PAL, Xbridge IS C18 2.5 µm 2.1x20mm, flow 0.7ml/min, gradient 5-95%ACN/H_2O 1min 95% 0.5min





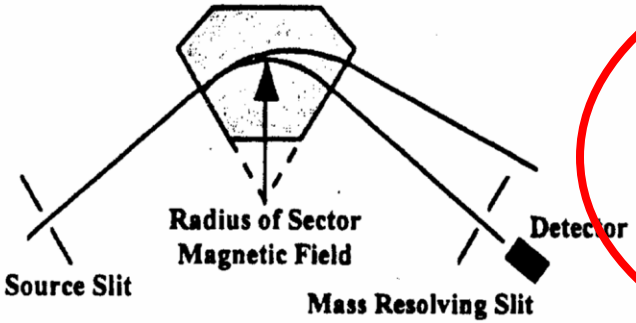
Calibration curve PL 33

1 – 20 000 nM

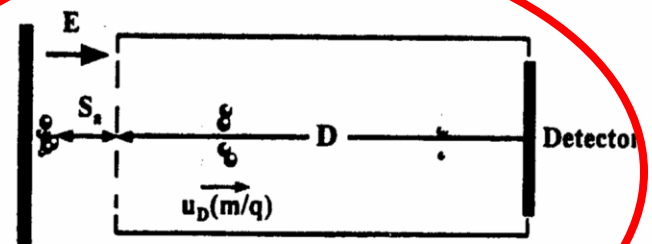


1, 10, 100, 1000, 5000, 10000, 20000 nM standard points

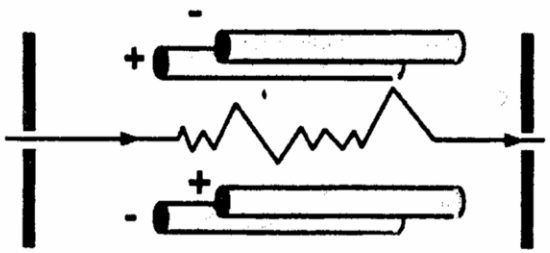
(a) **MAGNETIC SECTOR**



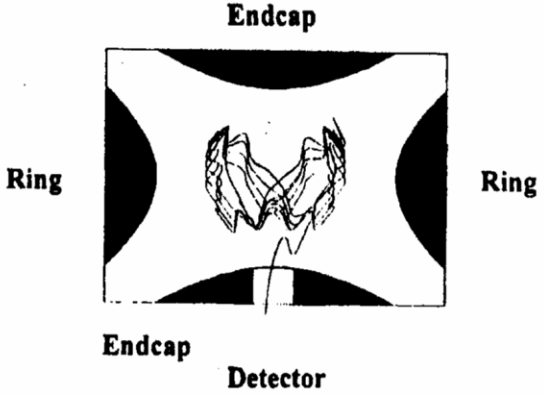
(b) **TIME OF FLIGHT**



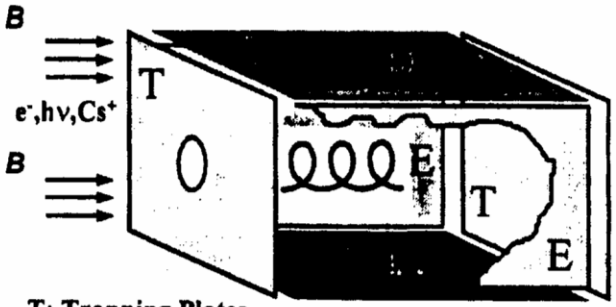
(c) **QUADRUPOLE FILTER**



(d) **ION TRAP**

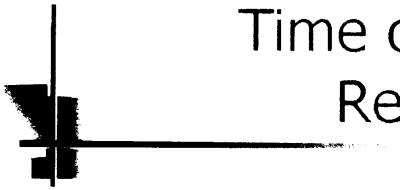


(e) **ION CYCLOTRON RESONANCE**



T: Trapping Plates
E: Excitation Plates
D: Detection Plates

Time-of-flight mass spectrometry (TOF)



Time of flight and Resolution

- Time of flight
  - Newtonian physics:
    $E = \frac{1}{2} m v^2 = \frac{1}{2} m (s/t)^2$
  - Energy electric field:
    $E = qV$

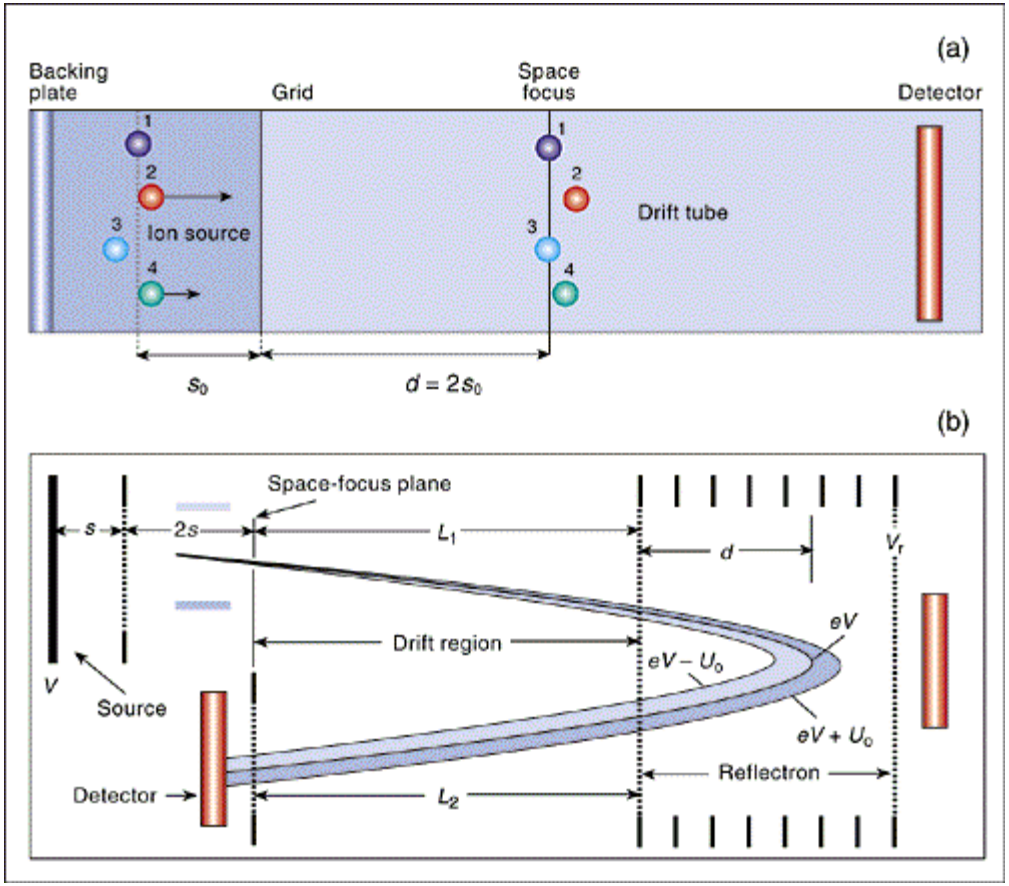
$$TOF = K\sqrt{m}$$

- Resolution

$$Resolution = \frac{m}{\Delta m} = \frac{t}{2\Delta t}$$

(Δm: FWHM)

Time-of-flight mass spectrometry (TOF)



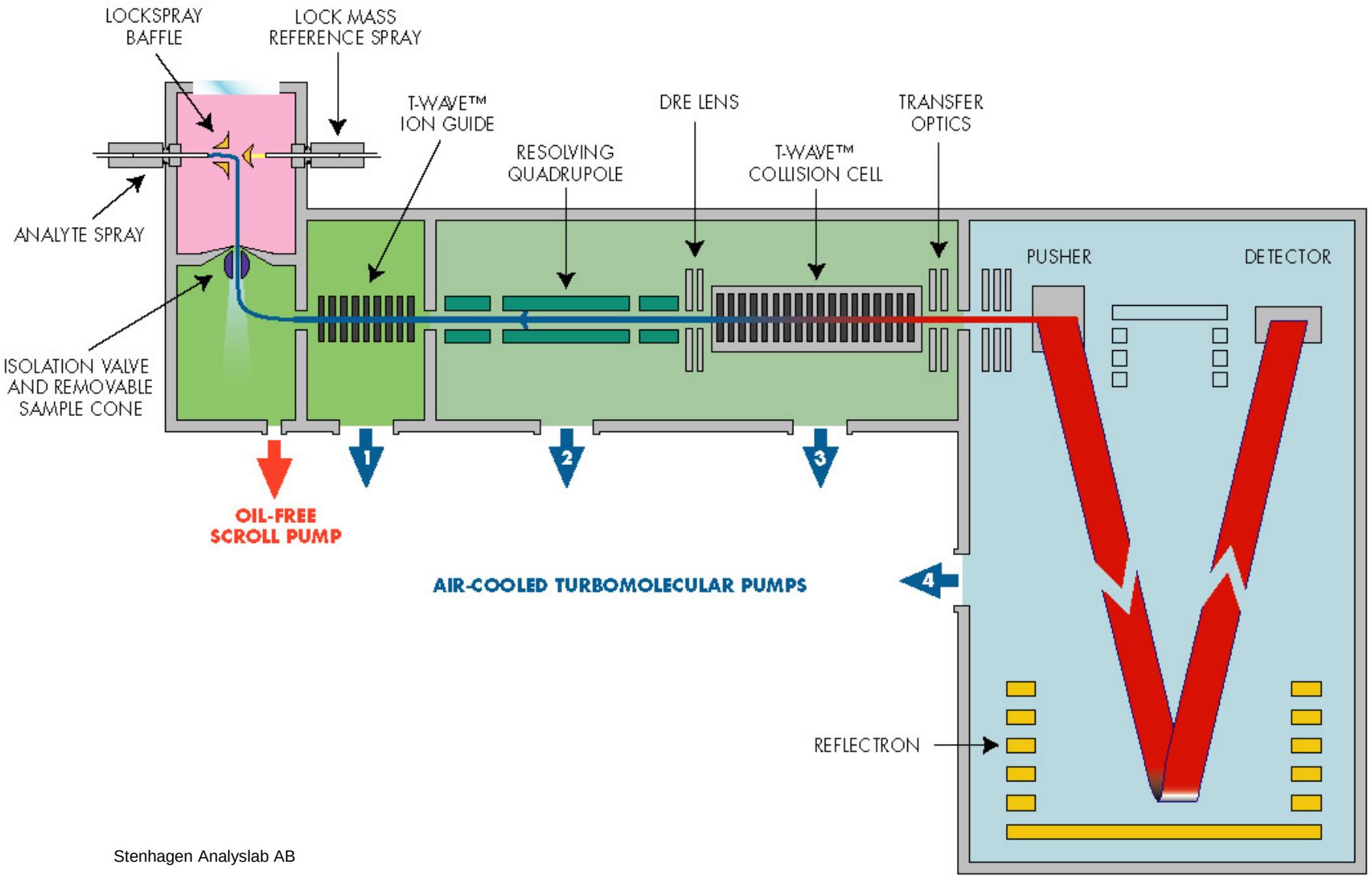
Linear TOF

1. Bundles of ions are pulsed down the flight tube.
2. Ions have a velocity relative to their mass.
3. Their arrival time at the detector will be relative to their m/z.

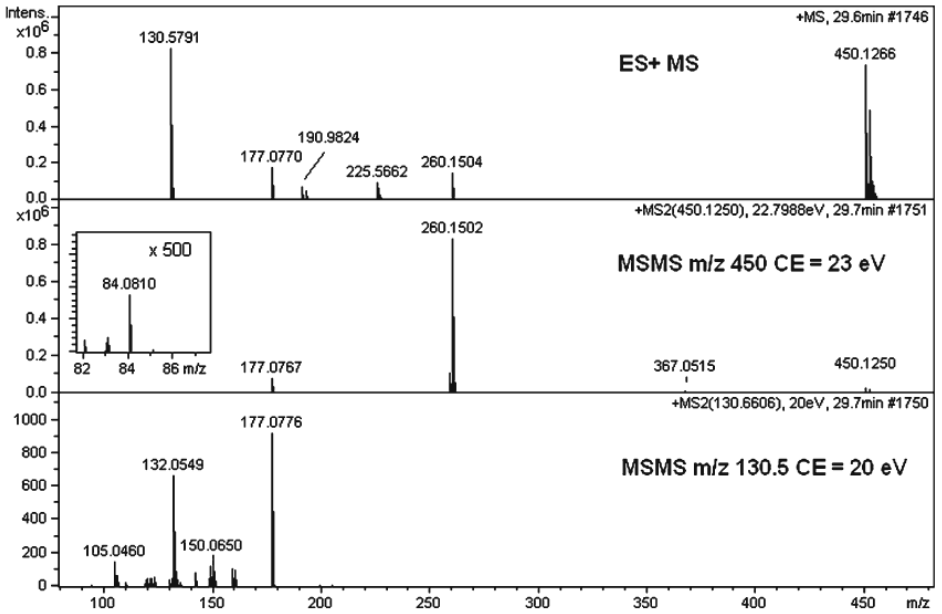
Single-stage reflectron TOF

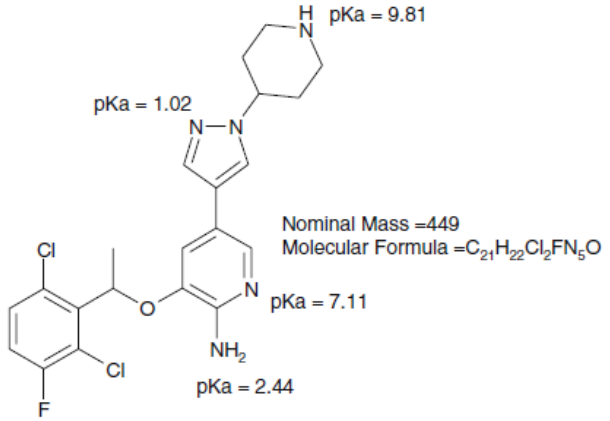
R J Cotter Anal Chem 1999, 445A

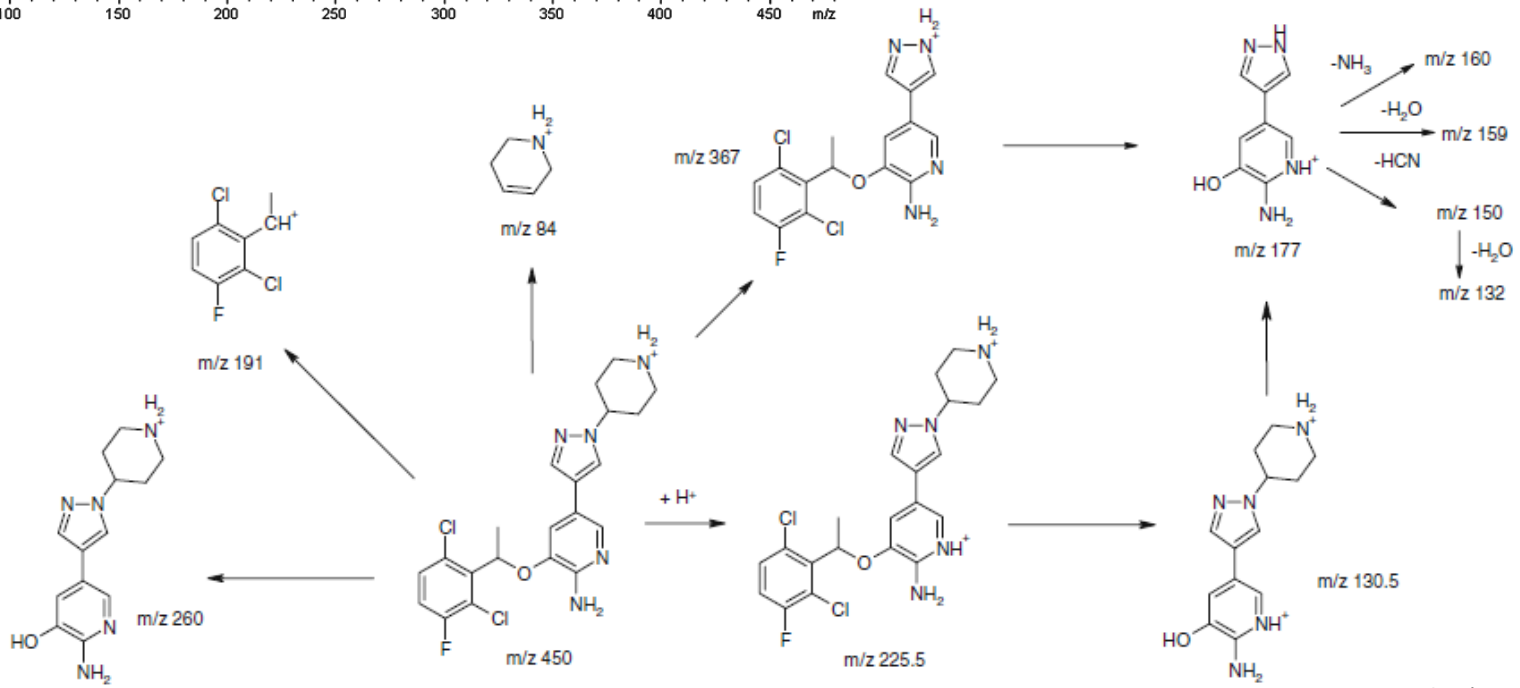
Q-Tof


LOCKSPRAY
BAFFLE
LOCK MASS
REFERENCE SPRAY
T-WAVE™
ION GUIDE
RESOLVING
QUADRUPOLE
DRE LENS
T-WAVE™
COLLISION CELL
TRANSFER
OPTICS
ANALYTE SPRAY
ISOLATION VALVE
AND REMOVABLE
SAMPLE CONE
PUSHER
DETECTOR
1
2
3
OIL-FREE
SCROLL PUMP
AIR-COOLED TURBOMOLECULAR PUMPS
4
REFLECTRON

Crizotinib (Pfizer)







Scheme 1. Crizotinib–MS and CID MS/MS fragmentation

Drug Metabolism: phase 1 reactions

Oxidation

Dealkylation

Reduction

Hydrolyse

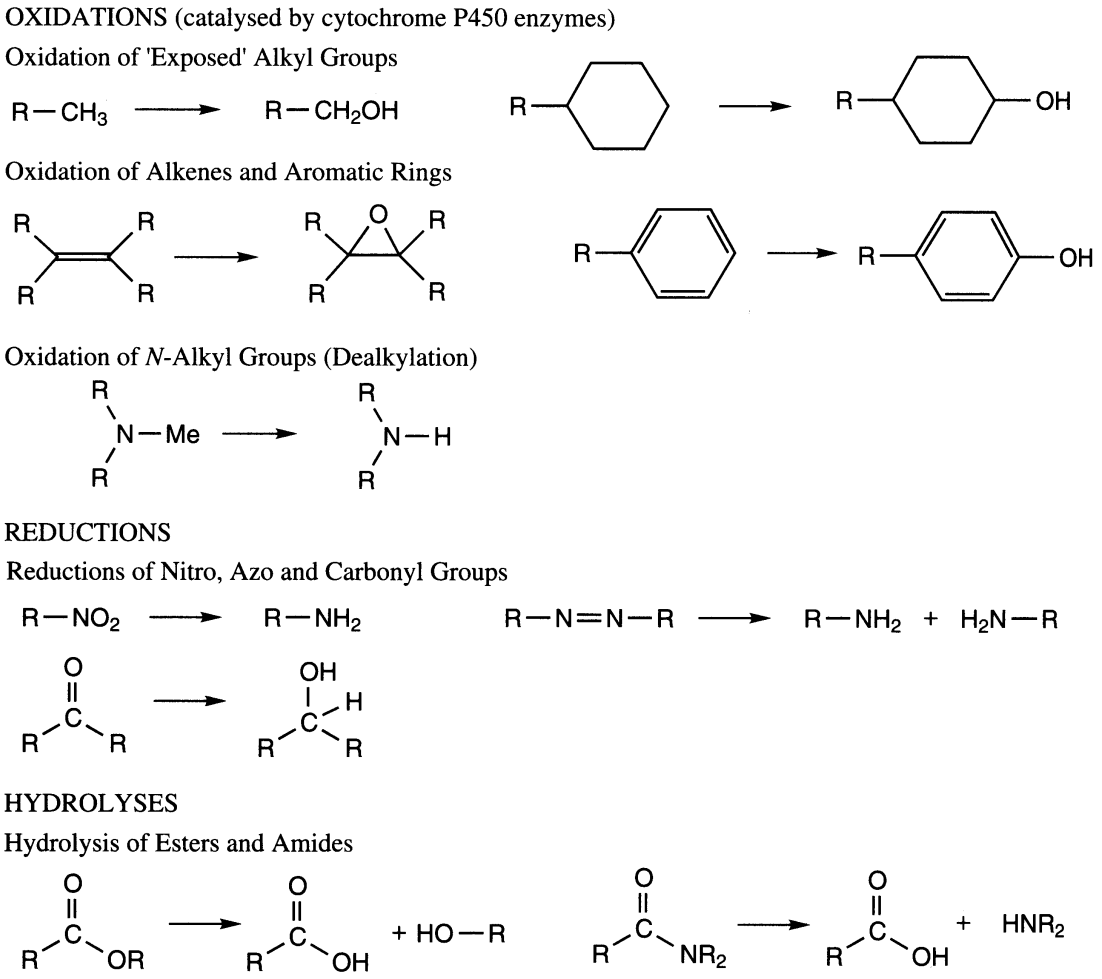
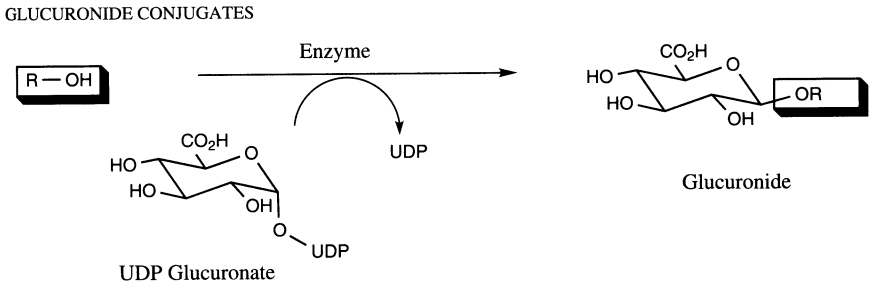


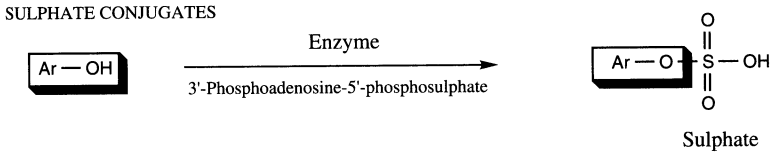
Fig. 8.39 Drug metabolism: phase I reactions.

Drug Metabolism: phase 2 reactions

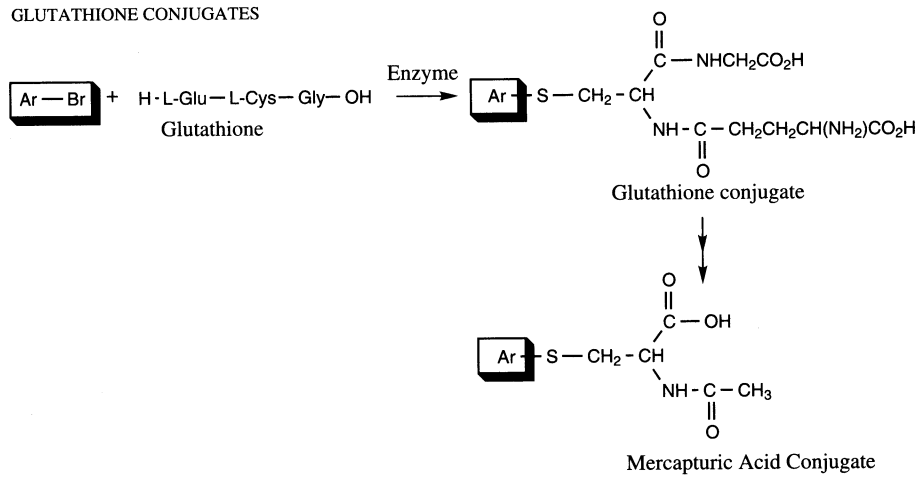
Glucuronide



Sulphate



Glutathione



Methylation, acetylation

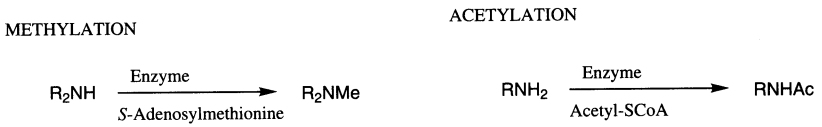
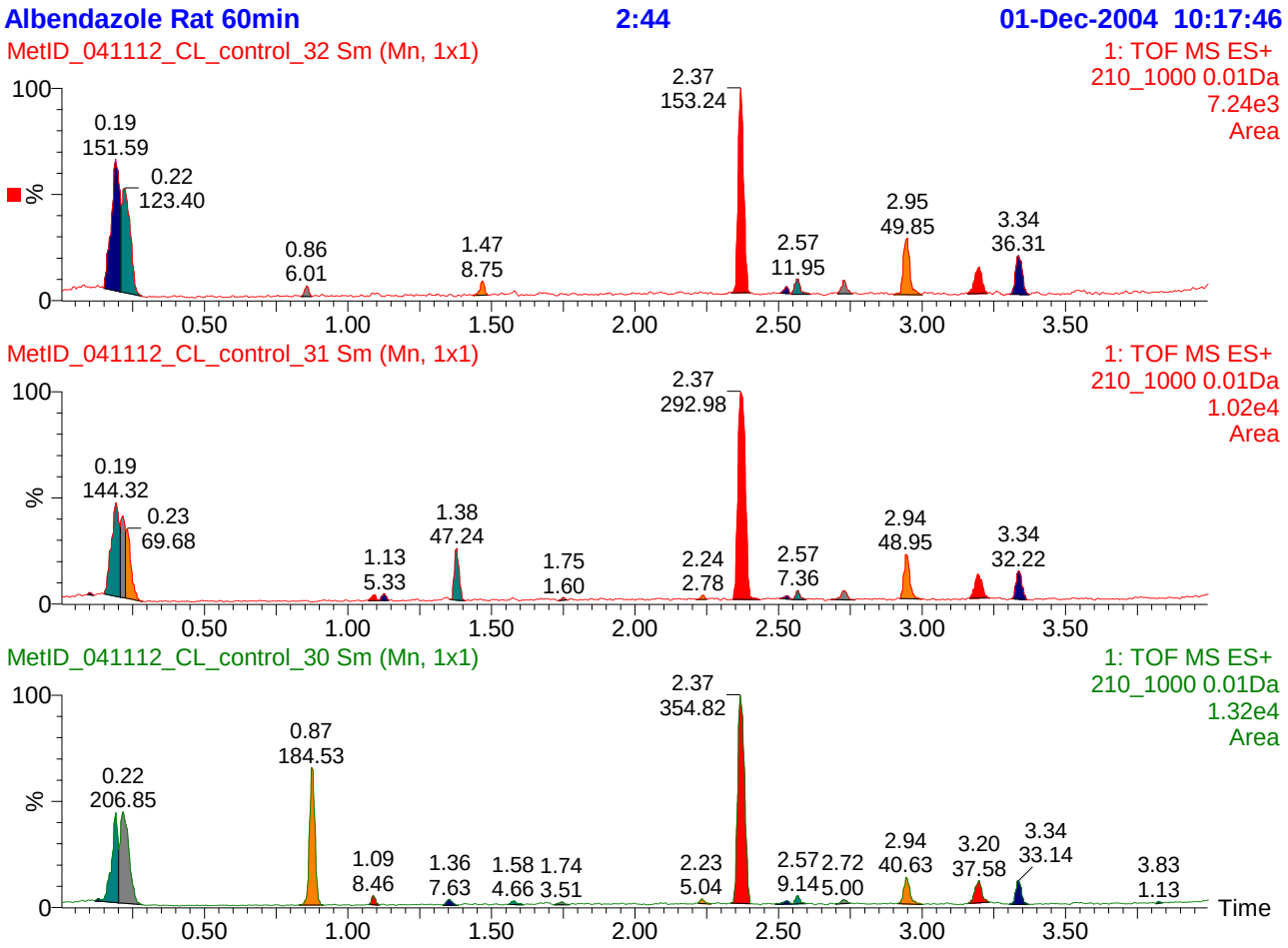


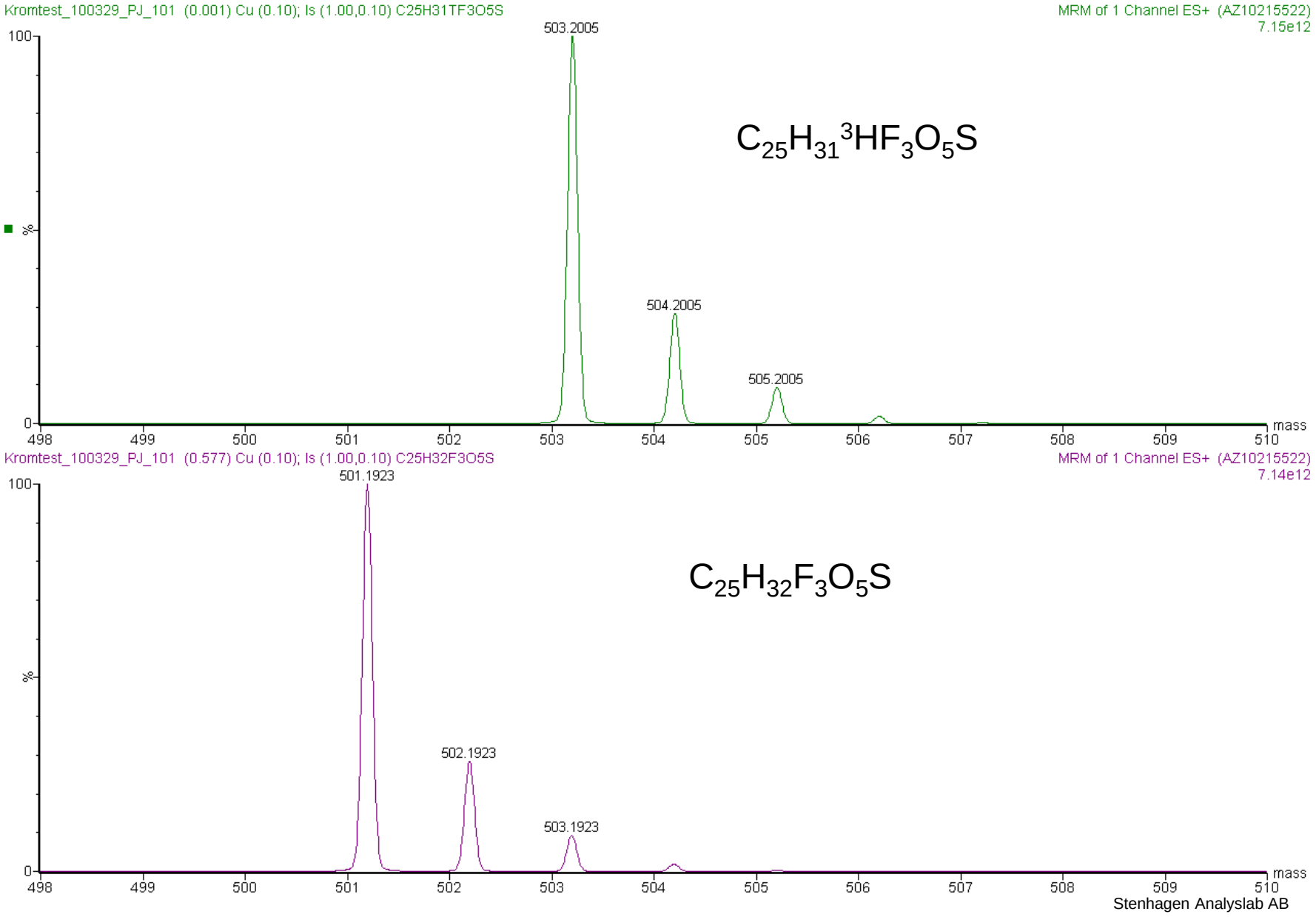
Fig. 8.40 Drug metabolism: phase II reactions (conjugation).

Met ID using 1.7µm 50 x 2.1mm C18 column

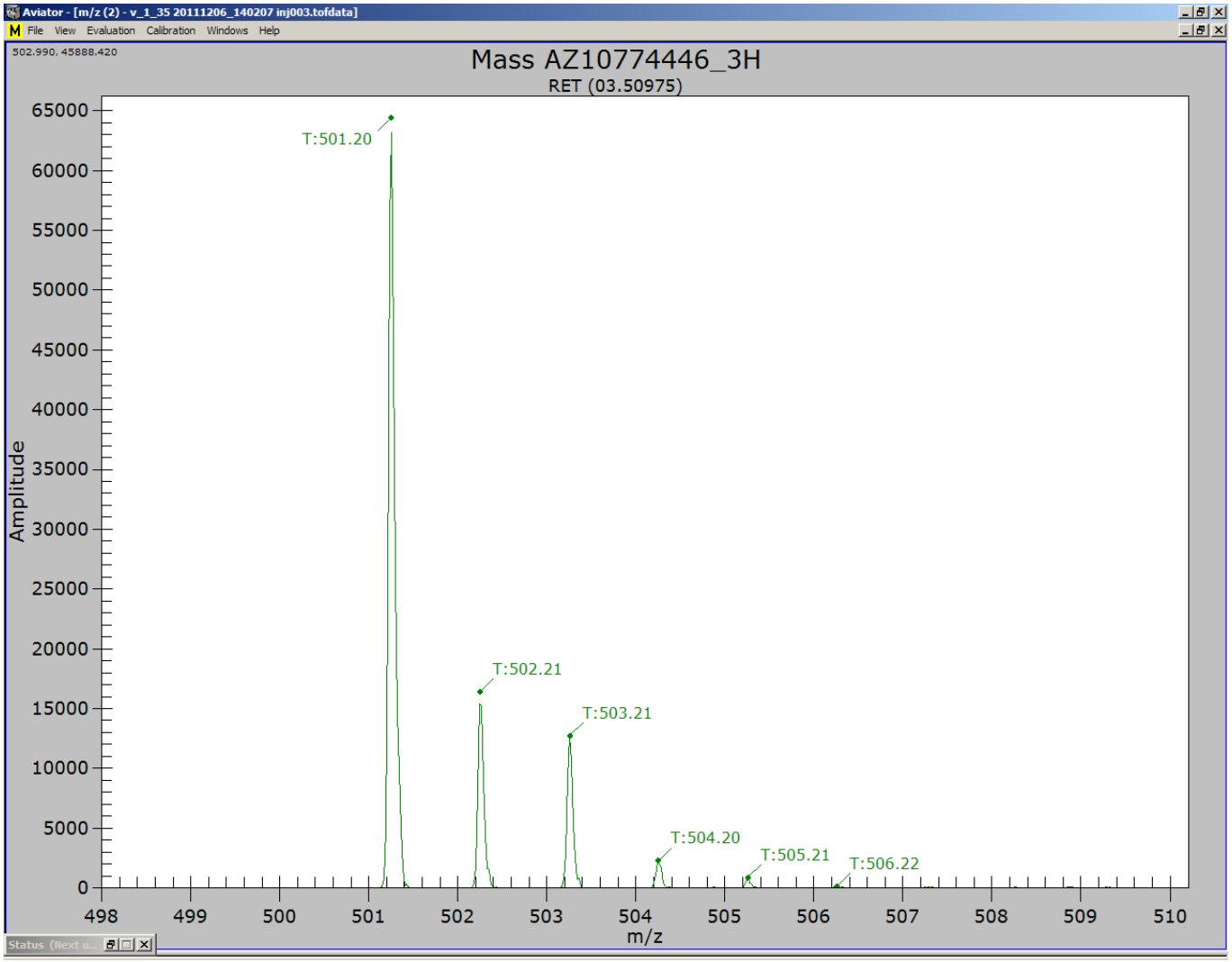
Flow rate 0.7 ml/min, gradient 5-95% ACN:H2O in 4 min



Isotope modelling



Stenhagen Analyslab AB


Aviator - [m/z (2) - v_1_35 20111206_140207 inj003.tofdata]
File View Evaluation Calibration Windows Help
502.990, 45888.420
Mass AZ10774446_3H
RET (03.50975)
T:501.20
T:502.21
T:503.21
T:504.20
T:505.21
T:506.22
Amplitude
m/z
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
498
499
500
501
502
503
504
505
506
507
508
509
510

Isotope Labelling

	AZ10774446
SN	
AZ	AZ10774446
Submitter	Jonas
Formula, non labeled,not charged	C25H31F3O5S
charge state (tex -1 eller 1)	1
label atom	T
Maximal number of label positions	2
Purity in % (UV 210nm)	
Project name	
Exact mass for labelled compound	**502.1927**
Exact mass for non-labelled compound	**501.1917058**
Average molecular mass	**500.707**
Specifik Radioaktivitet (kBq/nmol):	**92.21**

Results in percent

m/z	Sorted data	
501	249990.08	501.2013
502	57909.83	502.2064
503	45649.01	503.2098
504	7925.51	504.2036
505	2638.84	505.21
506	596.93	506.2154

iterative	**serial**	**number**
91.240	90.518	0
8.854	9.339	1
-0.094	0.188	2
0.000	-0.046	3
0.000	0.002	4
0.000	0.000	5
0.000	0.000	6
0.000	0.000	7
0.000	0.000	8
0.000	0.000	9
0.000	0.000	10
0.000	0.000	11
0.000	0.000	12
0.000	0.000	13
0.000	0.000	14
0.000	0.000	15

High Resolution Accurate Mass Measurement Elemental Compositions

Atomic and Molecular Mass: Fragment Ion Mass

The actual mass of an atom is very small indeed. For example, a hydrogen atom weighs something like 10^{-24}g. Instead of using such an absolute scale, a relative integer mass scale is easier to handle. On this relative scale, all atomic masses have values near to integers. Table 1 gives integer masses for some of the commoner elements.

Although this relative integer scale is acceptable in many circumstances where only approximate values are needed, for some applications, accurate relative masses are needed. By definition, on this accurate mass scale, carbon (^{12}C) is given the value of exactly twelve, ie, 12.00000. Table 1 indicates the accurate masses for some elements compared with their integer values.

Table 1. Relative Integer and Accurate Atomic Masses for some commoner Elements

Element	Symbol	Isotope	Integer Mass	Accurate Mass
Hydrogen	H	^{1}H	1	1.00783
(Deuterium)	(D)	^{2}H	2	2.01410
Carbon	C	^{12}C	12	12.00000
		^{13}C	13	13.00335
Nitrogen	N	^{14}N	14	14.00307
Oxygen	O	^{16}O	16	15.99491
Chlorine	Cl	^{35}Cl	35	34.96885
		^{37}Cl	37	36.96590
Silicon	Si	^{28}Si	28	27.97693

Accurate Mass Measurements

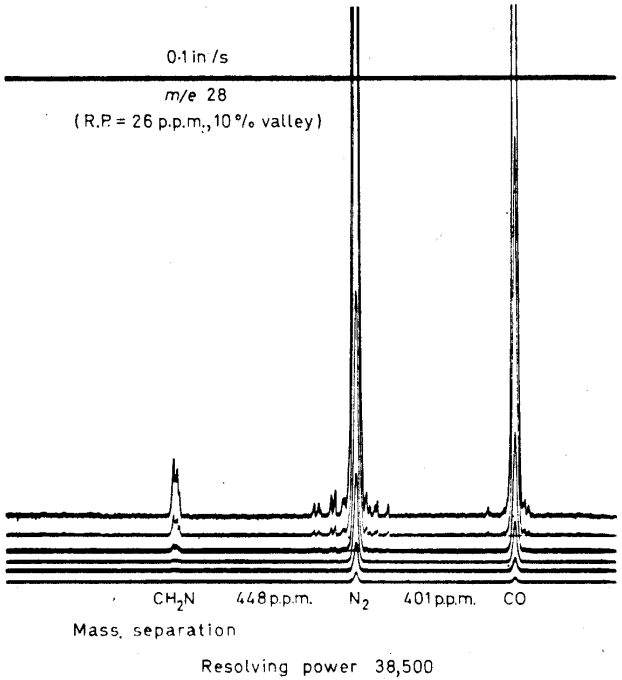


Figure 1.2.2. Multiplet peak at mass 28 at 38,500 resolution

ACCURATE MASS MEASUREMENTS

Chemical name	Formula	Integer Mass	Accurate Mass
Carbon monoxide	CO	28	27.99491
Ethene	C_2H_4	28	28.03132
Nitrogen	N_2	28	28.00614

Figure 3. Integer and accurate masses for three different gases, each having the same integer relative molecular mass (RMM = 28).

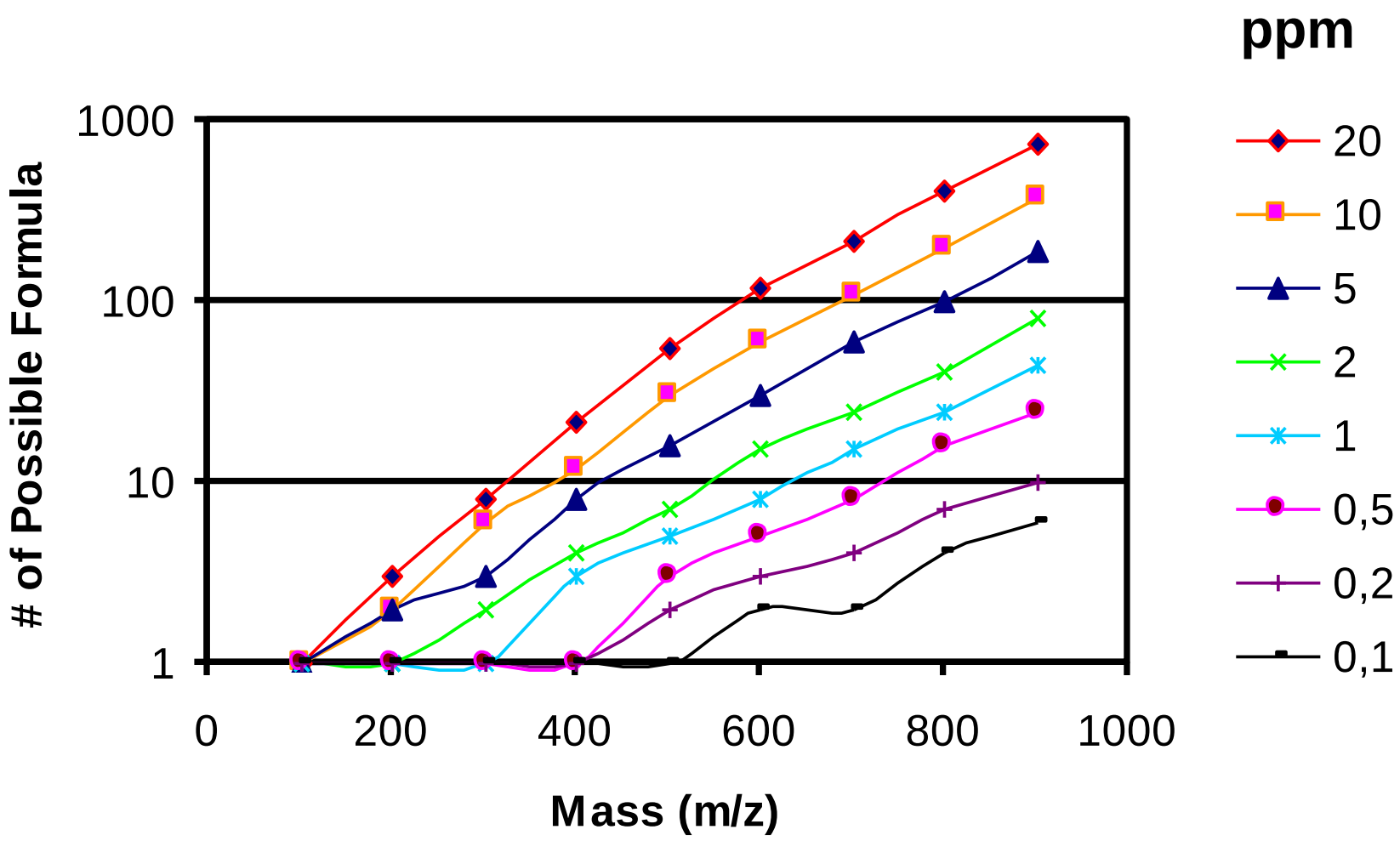
Accurate Mass at Nominal Integer Mass 58

Table 2. A Listing of Elemental Compositions versus Accurate Mass at Nominal Integer Mass of 58.

Integer Mass = 58				Accurate Mass
* C	H	N	O	
1	-	1	2	57.992902
1	2	2	1	58.016711
1	4	3	-	58.040520
2	2	-	2	58.005478
2	4	1	1	58.053096
2	6	2	-	58.053096
3	6	-	1	58.041862
3	8	1	-	58.065671
4	10	-	-	58.078247

* Compositions are read from left to right. Thus the fifth entry would be C_2H_4NO, of accurate mass 58.053096.

How many ppm Accuracy is needed?



Elemental composition

Elemental Composition

File Edit View Process Help

Single Mass Analysis

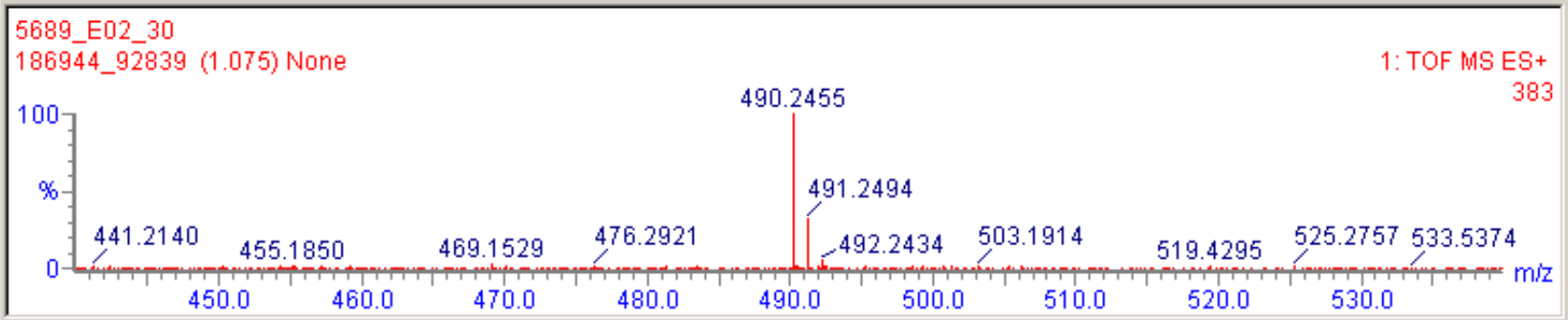
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

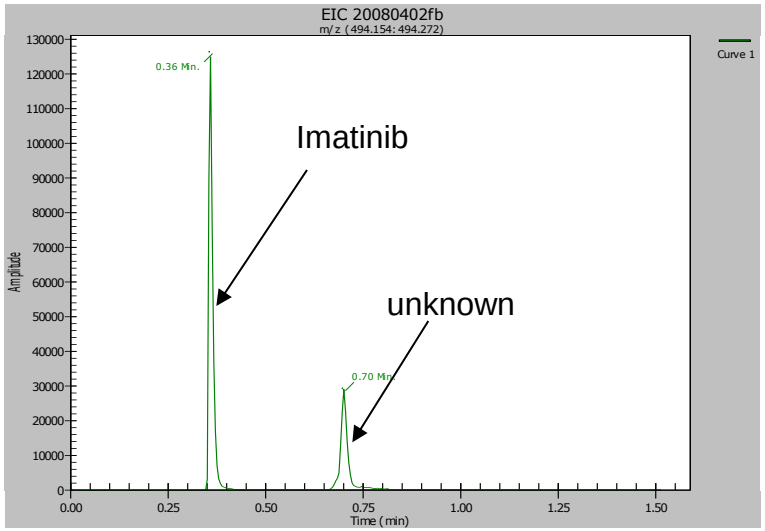
461 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	N	O
490.2455	490.2454	0.1	0.1	14.5	C27 H32 N5 O4	1	27	32	5	4
	490.2468	-1.3	-2.6	14.0	C29 H34 N2 O5	3	29	34	2	5
	490.2441	1.4	2.9	9.5	C26 H36 N O8	4	26	36	1	8
	490.2441	1.4	2.9	15.0	C25 H30 N8 O3	2	25	30	8	3
	490.2481	-2.6	-5.3	19.0	C30 H30 N6 O	5	30	30	6	1
	490.2427	2.8	5.6	10.0	C24 H34 N4 O7	6	24	34	4	7
	490.2495	-4.0	-8.1	18.5	C32 H32 N3 O2	7	32	32	3	2
	490.2414	4.1	8.3	10.5	C22 H32 N7 O6	8	22	32	7	6
	490.2500	-4.5	-9.1	6.0	C18 H34 N8 O8	10	18	34	8	8
	490.2409	4.6	9.4	23.0	C36 H30 N2	9	36	30	2	



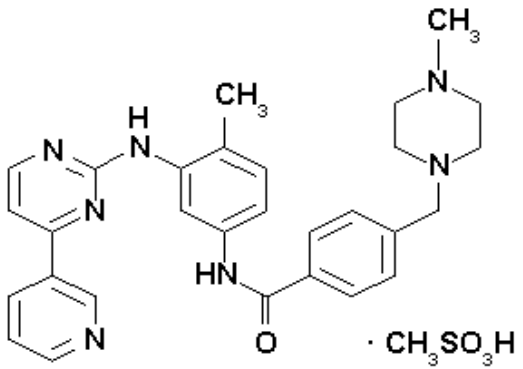
For Help, press F1

Unknown mass chromatographic peak

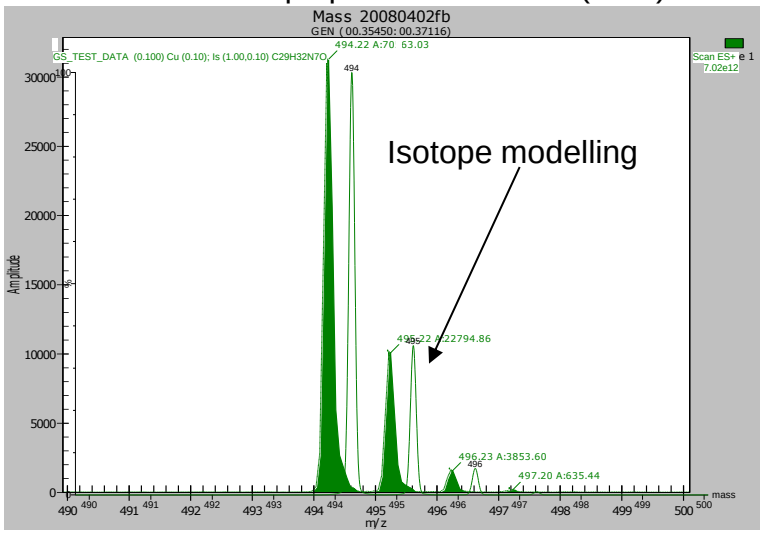


Mass chromatogram of m/z 494

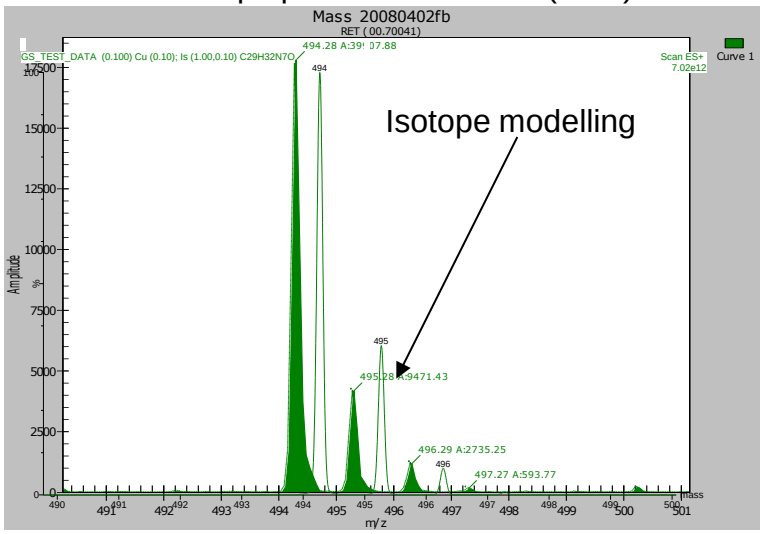
Imatinib



Isotope peaks of Imatinib (filled)

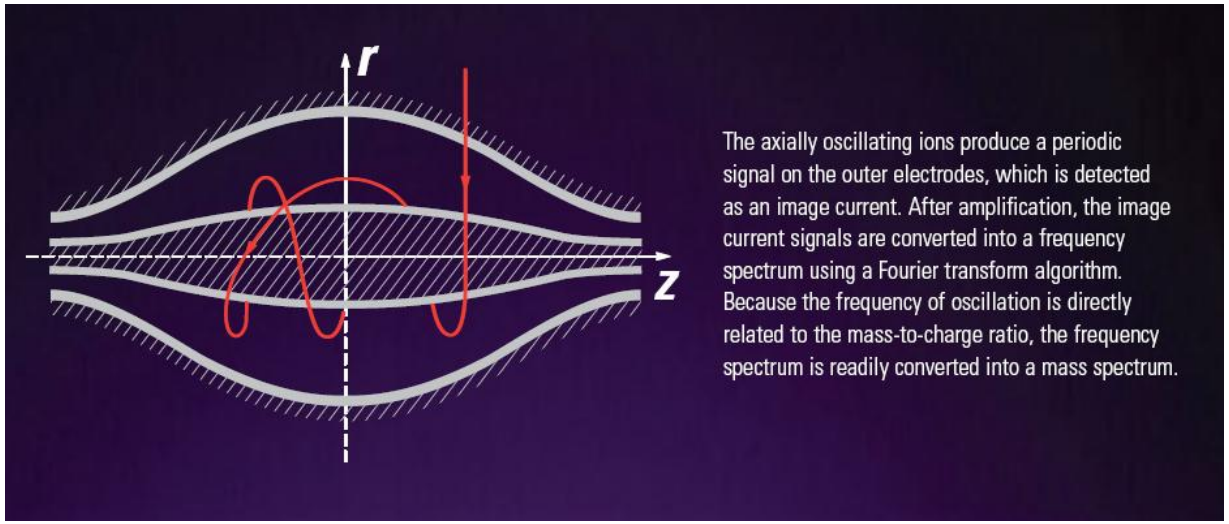


Isotope peaks of unknown (filled)



LC Shimadzu/CTC PAL, Halo C18 2.7µm 2.1x30mm, flow 0.7ml/min, gradient 5-95%ACN/H_2O 1min 95% 0.5min

Orbitrap



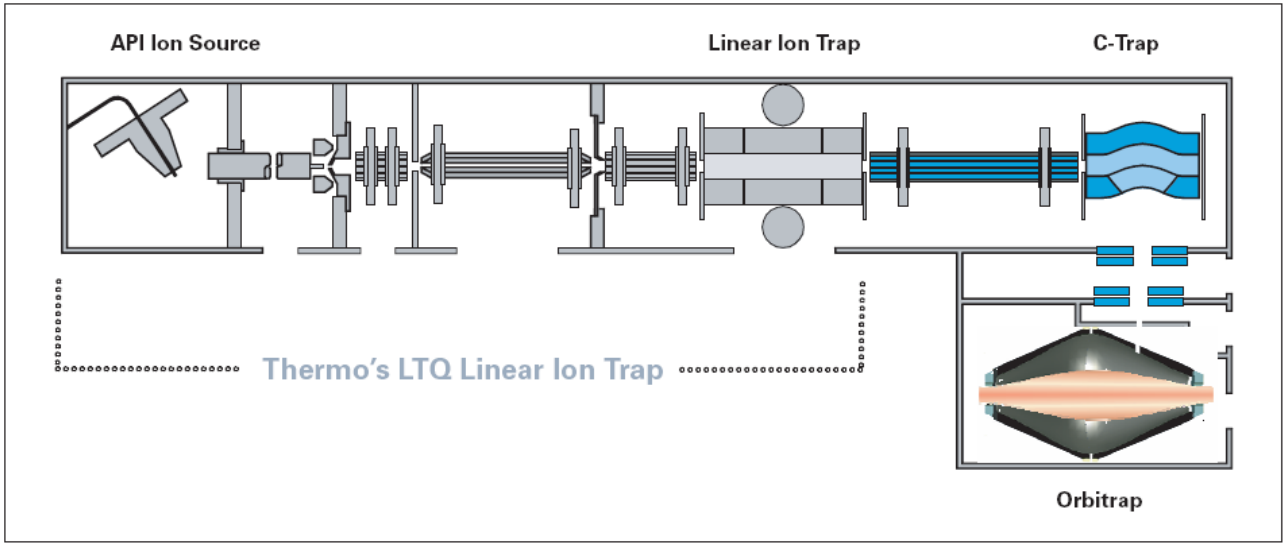
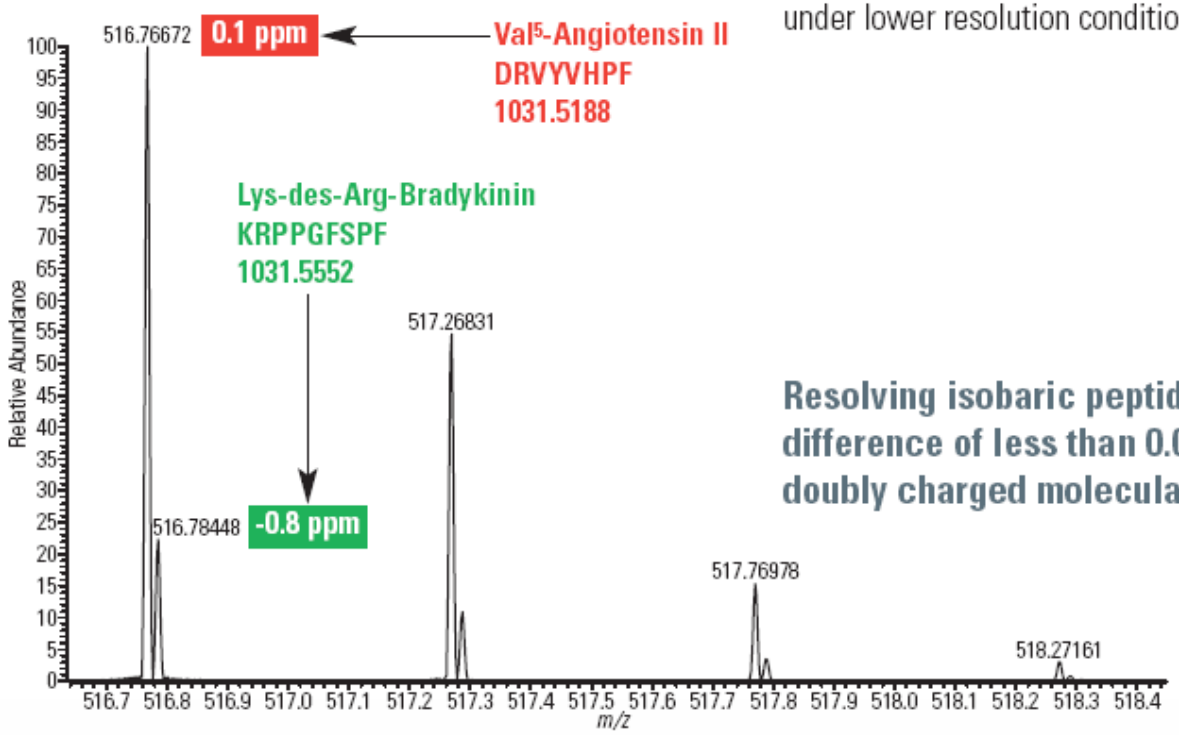


Figure 1: Diagram of the LTQ Orbitrap

Analysis of unknown samples requires both high resolution and high mass accuracy.

High resolution enables the detection and differentiation of isobaric signals, not discernable under lower resolution conditions.

Not only can these ions be detected, but their exact mass can be determined for unequivocal compositional and structural elucidation. Resolutions of 7,500, 15,000, 30,000, 60,000 and 100,000 (at *m/z* 400) are available on the LTQ Orbitrap.



Resolving isobaric peptides with *m/z* difference of less than 0.02 Da on the doubly charged molecular ion

LTQ Orbitrap Full Scan (R = 60,000 at *m/z* 400)

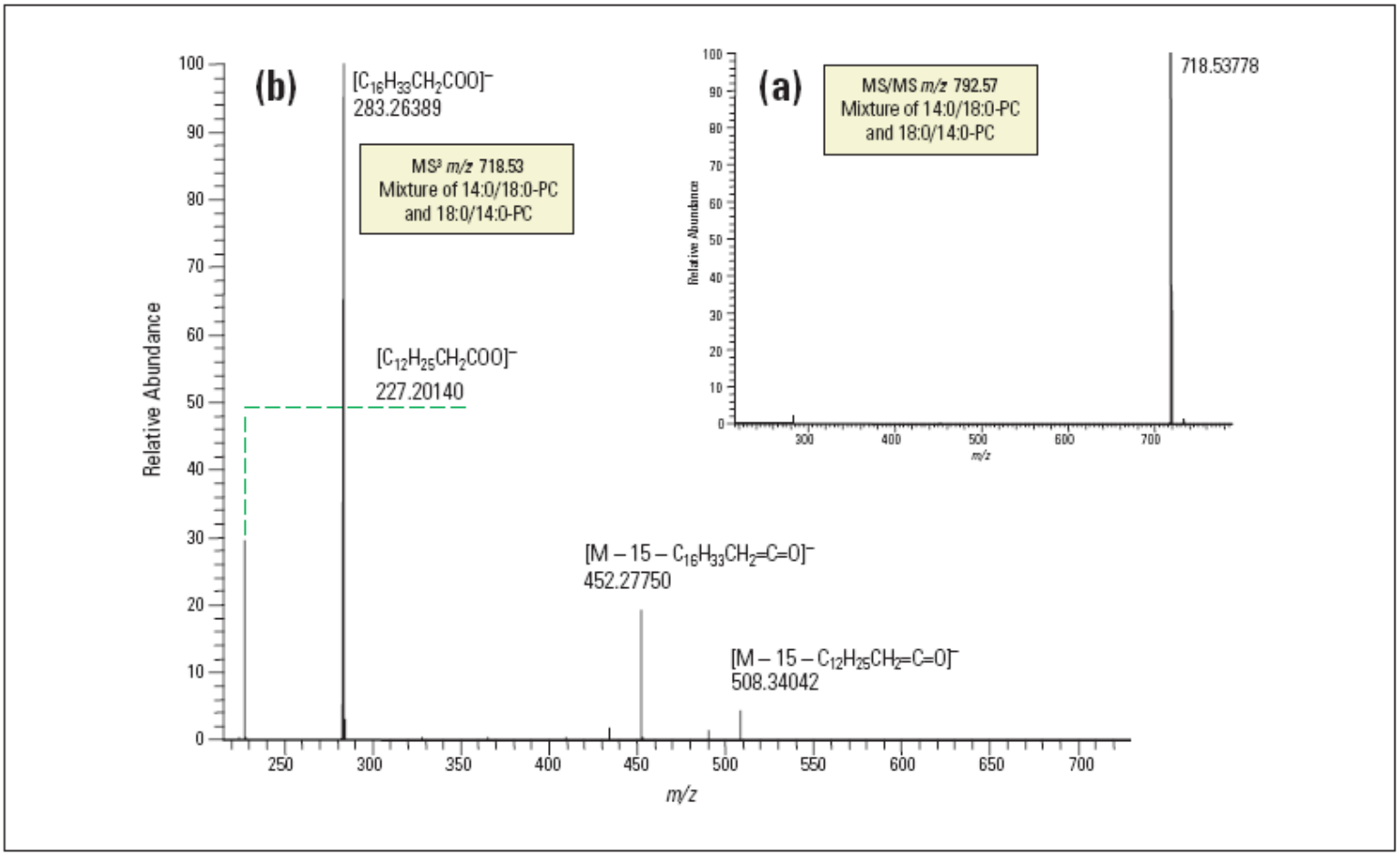
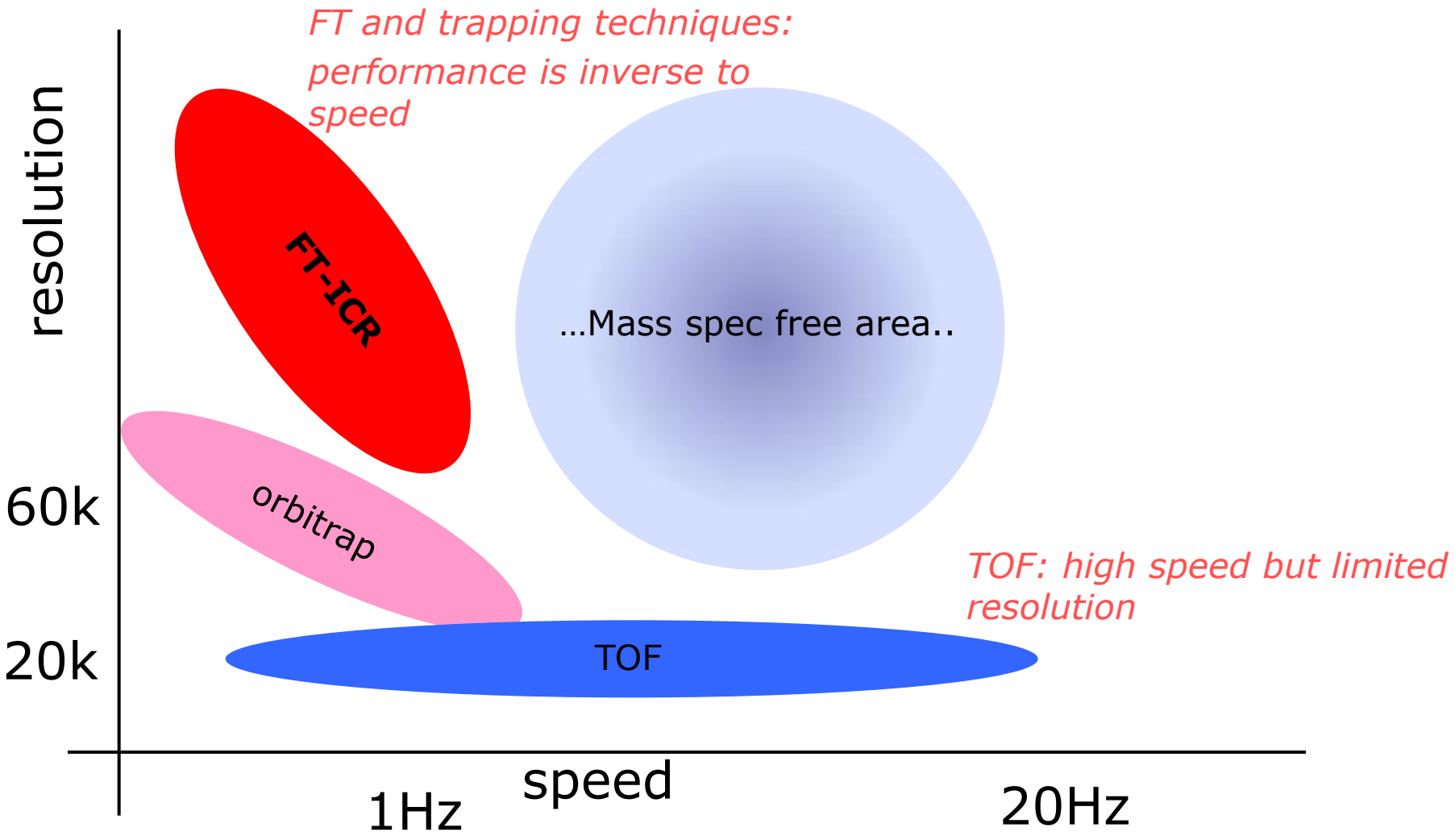
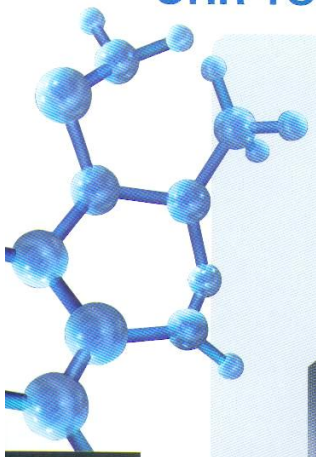


Figure 2: Relative quantitation of two positional isomers, 14:0/18:0-PC and 18:0/14:0-PC, in a mixture. **a**: MS/MS spectrum of *m/z* 792.57 (acetate adduct). Abundant fragment at *m/z* 718.54 corresponds to demethylated PC $[M–15]^-$. **b**: MS^3 spectrum of *m/z* 718.54. The $[M-15]^-$ ions undergo futher fragmentation by neutral loss of ketenes giving $[M–15–C_{12}H_{25}CH_2=C=O]^-$ (*m/z* 508.34) and $[M–15–C_{16}H_{33}CH_2=C=O]^-$ (*m/z* 452.28), and by yielding acyl anions of stearic (*m/z* 283.26; C18:0) and myristic (*m/z* 227.20; C14:0) acids directly.

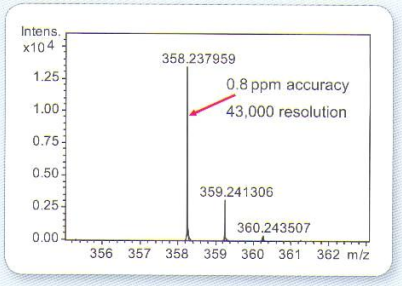
Extreme MS Performance: The Speed Compromise

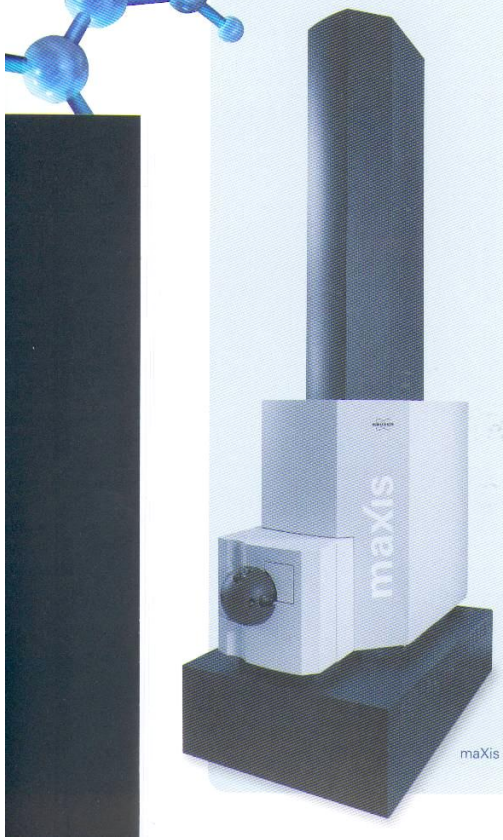


UHR-TOF



Both, mass accuracy and resolution are maintained, even at an acquisition rate of 10 spectra/s.





maXis

The latest development from Bruker, the novel UHR-TOF ultra high resolution technology, again proves Bruker Daltonics leadership in the design of cutting-edge mass spectrometry.

Maximum information @ maximum speed

maXis™ is a high-resolution tandem mass spectrometer offering a no-compromise solution for exceptional accurate mass, high resolution and high sensitivity analysis at a speed able to take full advantage of ultra-high performance chromatography.

With resolution in excess of 40,000 FWHM and MS and MS/MS mass accuracy typically between 600 – 800 ppb at speeds of up to 20 full spectra per second simultaneously, no other mass spectrometer is better equipped to deliver definitive data on complex samples in proteomics, metabolomics and small molecule identification challenges.

Distance-of-Flight MS Instrument

Alexander W. G. Graham, Steven J. Ray, Christie G. Enke, Charles J. Barinaga,
David W. Koppenaal, Gary M. Hieftje

J. Am. Soc. Mass Spectrom. (2011) 22:110-117

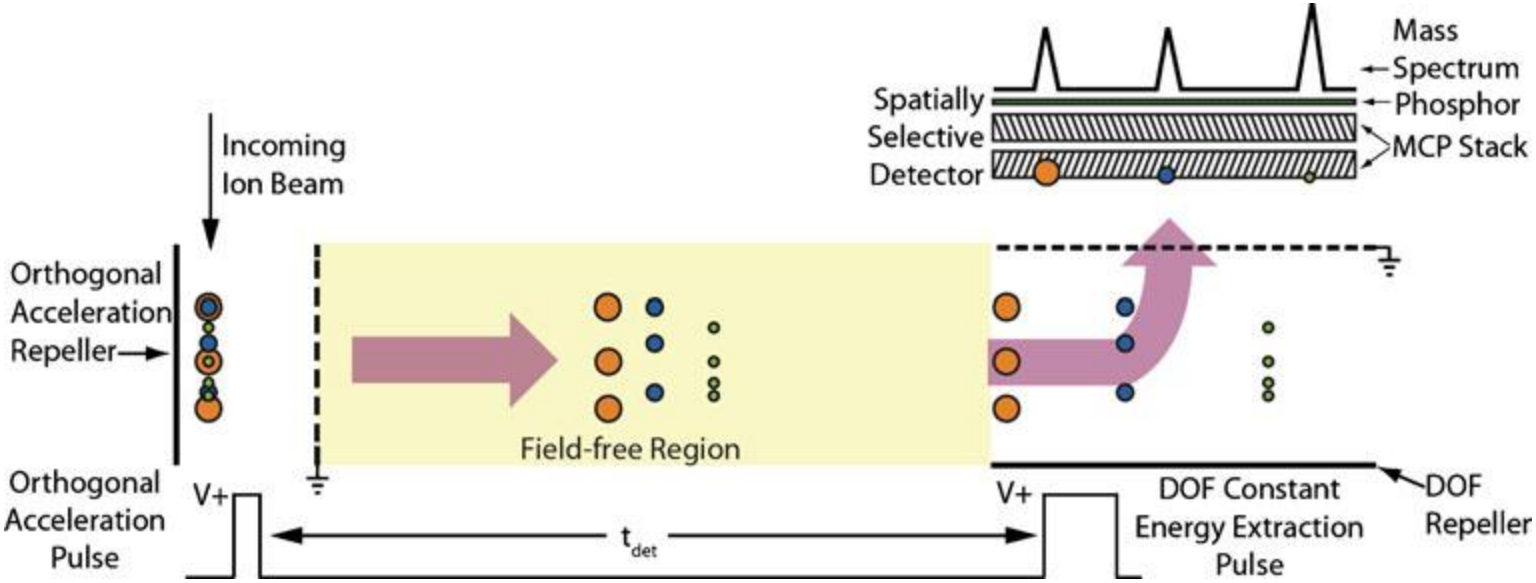
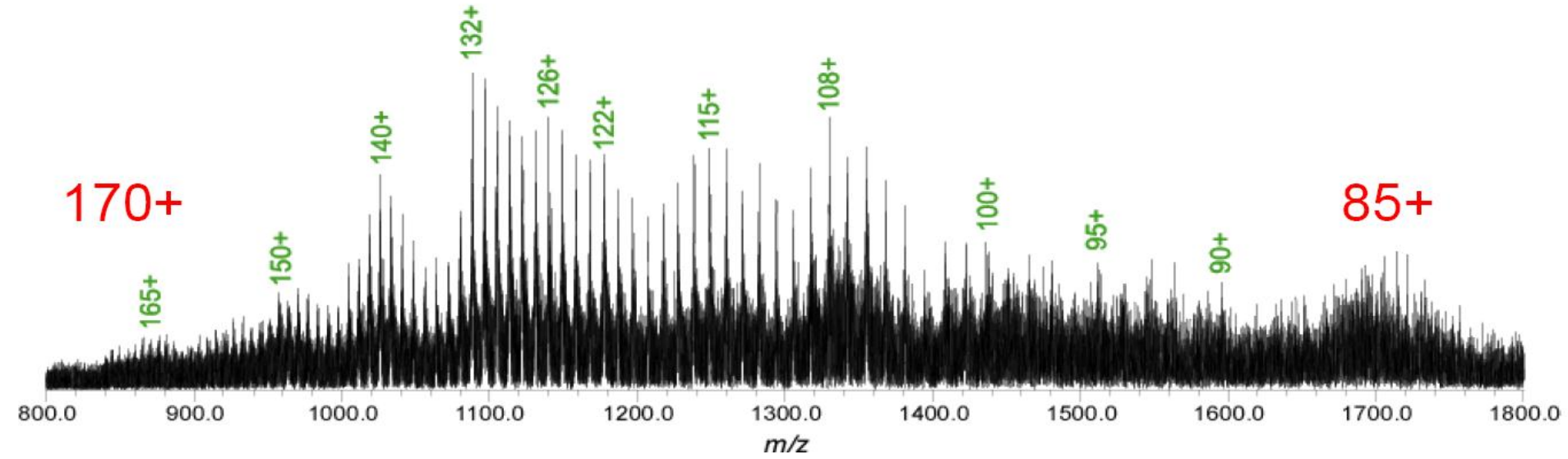


Figure 1. Schematic diagram illustrating the operating principle of DOFMS. Ions are electrostatically pushed to a constant momentum along a direction orthogonal to the incoming ion beam. Once within the field-free region, ions separate according to their mass-dependent velocities (ions of larger m/z are represented by bigger circles). At a given detection time (t_{det}), ions are collectively accelerated onto a position-selective detector via a constant-energy voltage pulse applied to the DOF repeller electrode. In our current instrument, a microchannel plate (MCP)–phosphor detector is employed to determine ion position at t_{det}.

Electrospray Ionization of PurL

(Formylglycinamide Ribonucleotide Amidotransferase)

Observed MW: 143 500 +/- 23
Theoretical MW: 143 635



Fred W. McLafferty et. al, ASMS 2008

Top Down Identification and Characterization of Biomolecules

Protein mixture

↓

Accurate mass of each molecular ion

↓

Dissociation* (MS/MS) of each directly give its sequence

*)
- Collisionally activated dissociation, CAD
- Infrared multiphoton dissociation. IRMPD
- Electron-capture dissociation. ECD
- Electron-transfer dissociation. ETP
- Prefoldin dissociation. PFD
- Activated ion electron-capture dissociation. AI-ECD
- High energy dissociation HCD

↓

Further dissociation (MS^3) give location of modification etc.

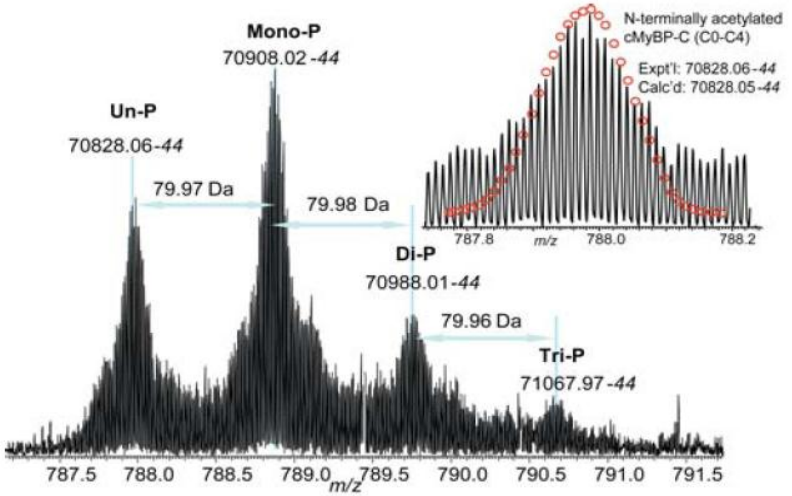


Fig.2. ESI/FTMS analysis of one truncated cMyBP-C overexpressed in *Baculovirus* (C0-C4, 71 kDa).

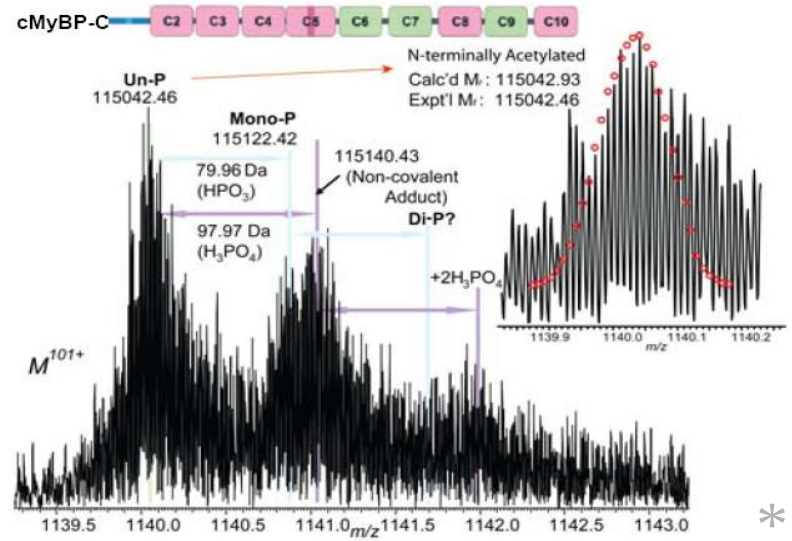


Fig.3. ESI/FTMS analysis of the second truncated cMyBP-C overexpressed in *Baculovirus* (C2-C10, 115 kDa).

Goals for Top Down Disease Proteomics:

Finding the meaning of protein modifications occurred *in vivo*

•Solve its molecular complexity and for quantification of positional isomers even with labile modifications

•Allowing to establishing the relevance of such modifications to physiological functions and disease status

* to date the largest protein resolved isotopically

Top Down Disease Proteomics: Deciphering Protein Modifications for Understanding and Diagnosis of Human Diseases

Ying Ge, Lisa Xu, Inna Rybakova, Vlad Zabrouskov, Richard L. Moss, Jeffery W. Walker

Characteristics of Mass Analysers

Method	Measured	Mass to Charge Range	Resolution	Mass Accuracy	Dynamic Range	Operating Pressure (torr)
Sector Magnet	Momentum	10^5	10^5	<3 ppm	10^7	10^{-6}
Time of flight	Flight time	10^6	10^3-10^5	<2 ppm	10^4	10^{-6}
Ion Trap Electrostatic IT	Frequency	10^4-10^5	10^3-10^4 10^5	0.1% <2 ppm	10^4	10^{-3} 10^{-7}
Quadrupole	Filters for m/z	10^3-10^4	10^3-10^4	<10ppm	10^5	10^{-5}
Ion Cyclotron Resonance	Frequency	10^5	10^6	<1 ppm	10^4	10^{-9}
Orbitrap	Frequency	10^5	10^5	<2 ppm	10^4	10^{-9}

Uses of Mass Spectrometry in Organic and Biological Chemistry

Application	Samples	Methods	Comment
Molecular weight determination	Pure compounds, mixtures	Recognize intact molecular ion in spectrum	Several ionisation methods can be used for confirmation
Molecular formula determination	Usually pure compounds but also mixtures by LC-MS or GC-MS	High accuracy mass measurement on molecular ion	High accuracy alone seldom gives a unique molecular formula
Molecular structure determination	Pure compounds or mixtures by LC-MS, GC-MS, and MS-MS	Spectrum-structure correlations; library comparisons	Confirmation of suspected structures is usual; de novo interpretations rare
Sequence determination	Proteins, other biopolymers	Tandem mass spectrometry (MS-MS)	Sensitive, very rapid and increasingly useful
Isotopic incorporation and fractionation	Naturally and artificially labelled compounds (^{13}C, ^{2}H, ^{18}O, etc.)	Ion abundance measurements	Precise isotope ratio measurements require special instrument
Quantification	Mixtures by LC-MS or GC-MS	Selected ion detection (SIR) or multiple reaction monitoring (MRM)	Sensitive and very selective

Summary of what we can do with mass spectrometry

Molecular weight

Molecular formula

Molecular structure

Sequence

Isotopic incorporation

Quantification

End