DEPARTMENT OF CHEMISTRY
MASS SPECTROMETRY RESEARCH FACILITY

UNIVERSITY OF OXFORD

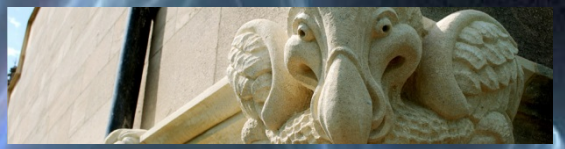
GRADUATE COURSE IN MASS SPECTROMETRY

LECTURE 1

COURSE INTRODUCTION AND THE FORMATION OF IONS

PROFESSOR JAMES MCCULLAGH

Course overview



Lecture 1: Introduction: Formation of Ions

- Introduction to the course
- Introduction to Mass Spectrometry
- Interpretation of the mass spectrum
- Methods of ionisation: EI, CI, ESI and MALDI


James McCullagh

Lecture 2: Mass Analysers: Quadrupoles, Time of Flight, Orbitraps and hybrid instruments.

- Principals of mass analysis
- Time of Flight MS and common configurations
- Quadrupoles, ion traps Orbitrap and hybrid instruments


Shabaz Mohammed

Lecture 3: Collisions in vacuum: cooling, activating, and sizing ions.

- Mean free path and collisional cross sections, cooling and focussing of ions
- Collisions activation
- Ion-mobility

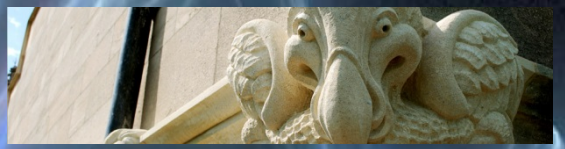

Justin Benesch

Lecture 4: Ion Chemistry: Inside the mass spectrometer.

- Analyte to gas-phase ion: Chemical processes involved in ion creation and extraction
- (EI, CI, ES, MALDI and others).
- Processing Gas phase ions: The chemistry of MS/MS (CID, etc.)


James Wickens

Course overview



Lecture 5: Separation methods (chromatography) coupled to mass spectrometry.

- Theory of chromatography and other separations methods
- Reversed Phase, ion-exchange, HILIC and others
- Coupling chromatography to mass spectrometry


James Wickens

Lecture 6: Proteomics

- Challenges in proteomics: Complexity, sensitivity and dynamic range
- High resolution mass spectrometry
- Enzymatic digestion, tandem nano-LC/MS
- Protein identification by database seaching


Shabaz Mohammed

Lecture 7: Mass spectrometry for biophysics and structural biology

- Native electrospray mass spectrometry
- Native ion-mobility mass spectra of protein assemblies
- Activation of protein assemblies


James McCullagh

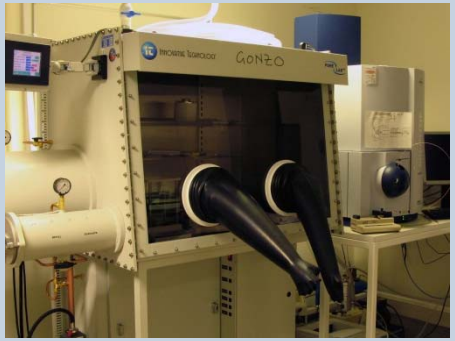
Lecture 8: Metabolomics

- Introduction to metabolomics and metabolomes and analytical challenges
- Workflows and instrumentation
- Case study: Targeted and untargeted analysis of cancer cell metabolism.
- The use of metabolomics as a hypothesis generating tool


Justin Benesch

The Principles of Mass Spectrometry










"All mass spectrometers measure the mass to charge ratio of ions in the gas phase."

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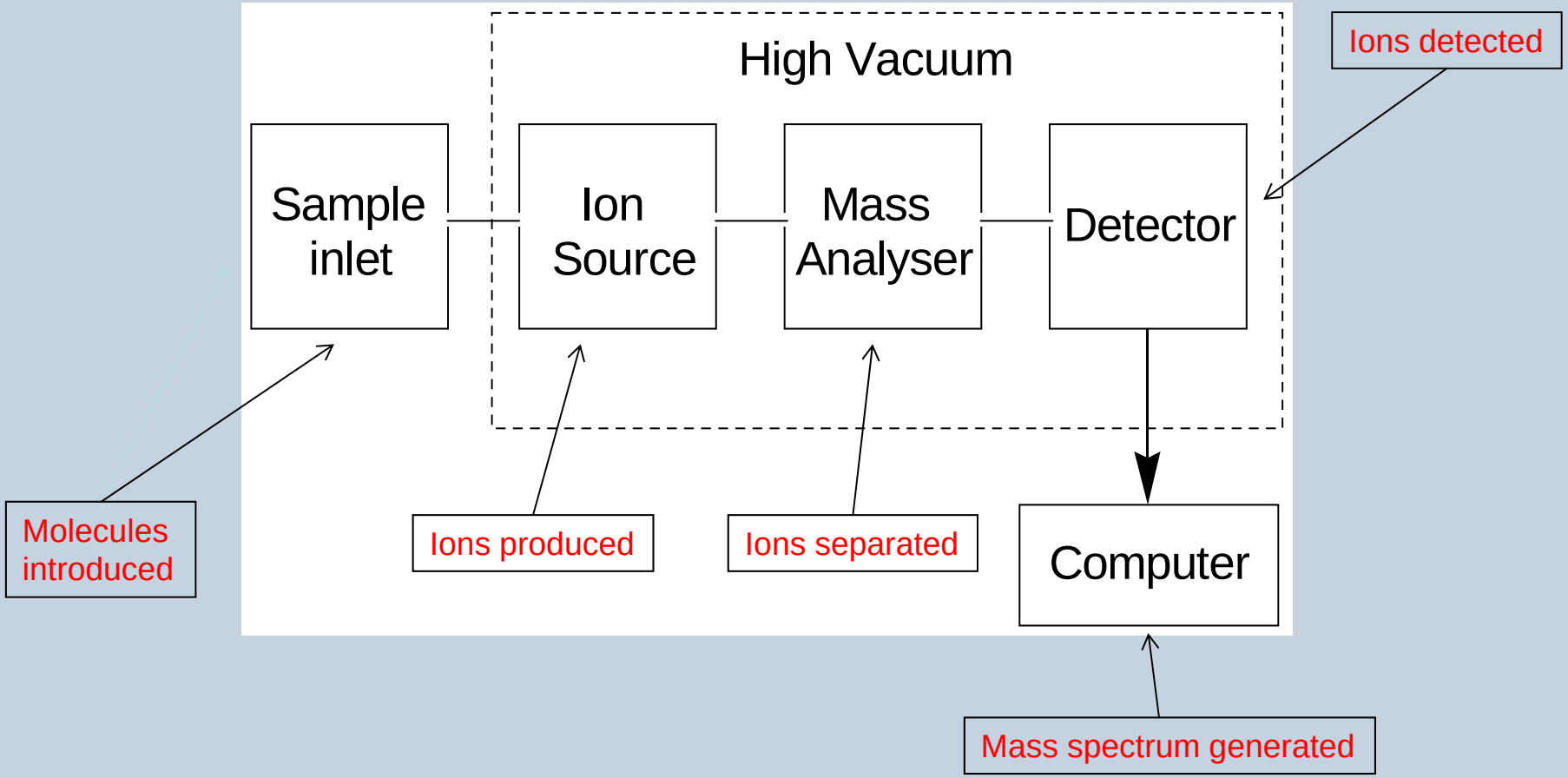
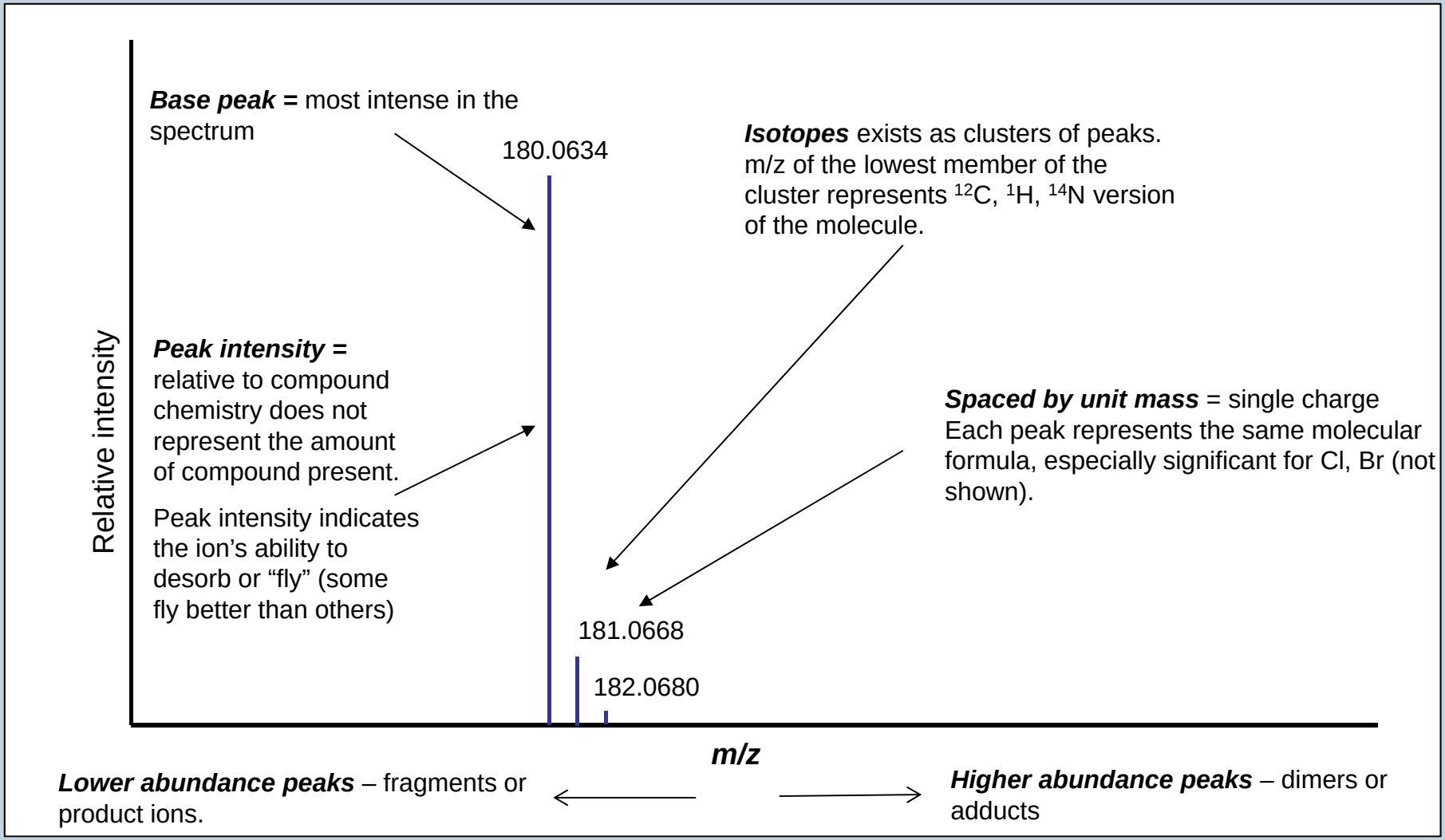
The mass spectrometer

All mass spectrometers involve four basic processes



The Mass Spectrum of a single compound



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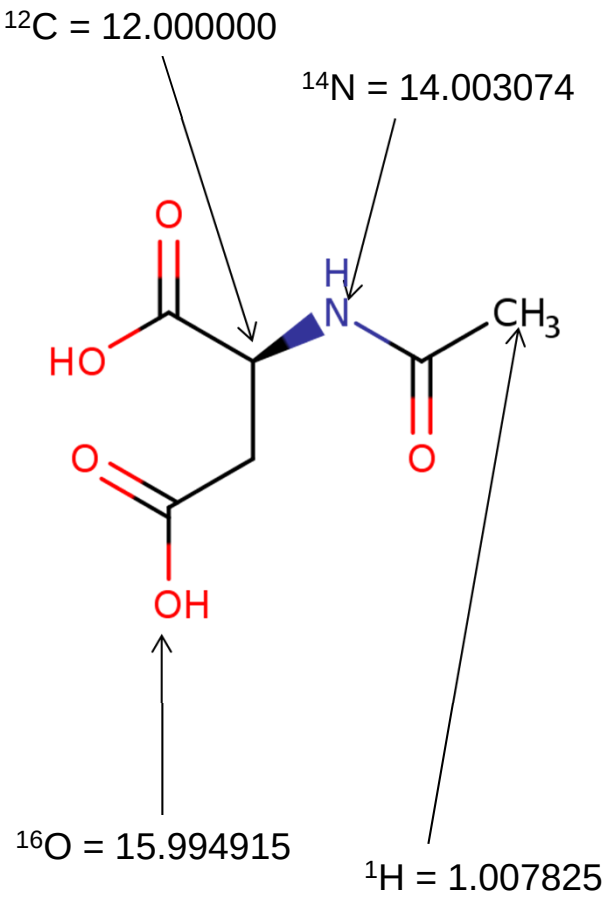
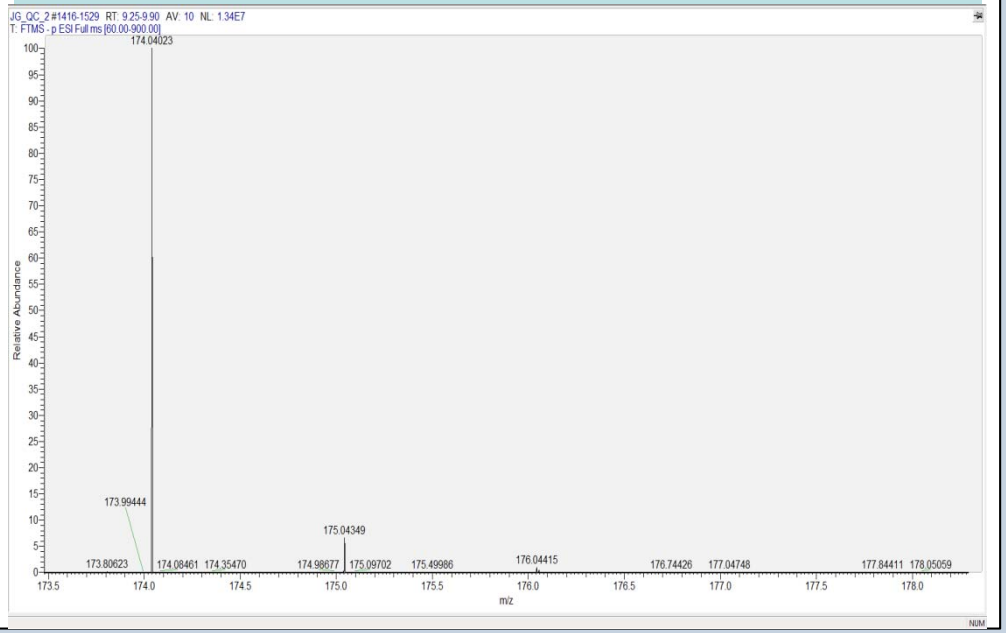
What masses are measured?

N-acetyl-L-aspartic acid ($C_6H_9NO_5$)



6x ^{12}C = 12.000000 = 72.0000
9x ^{1}H = 1.007825 = 9.070425
1x ^{14}N = 14.003074 = 14.003074
5x ^{16}O = 15.994915 = 79.974575

Monoisotopic mass **= 175.04807**
-H = 174.04023
= $C_8H_9N_4O_2$



Proton = 1.00728 ; Neutron = 1.0087; Electron = 0.00055

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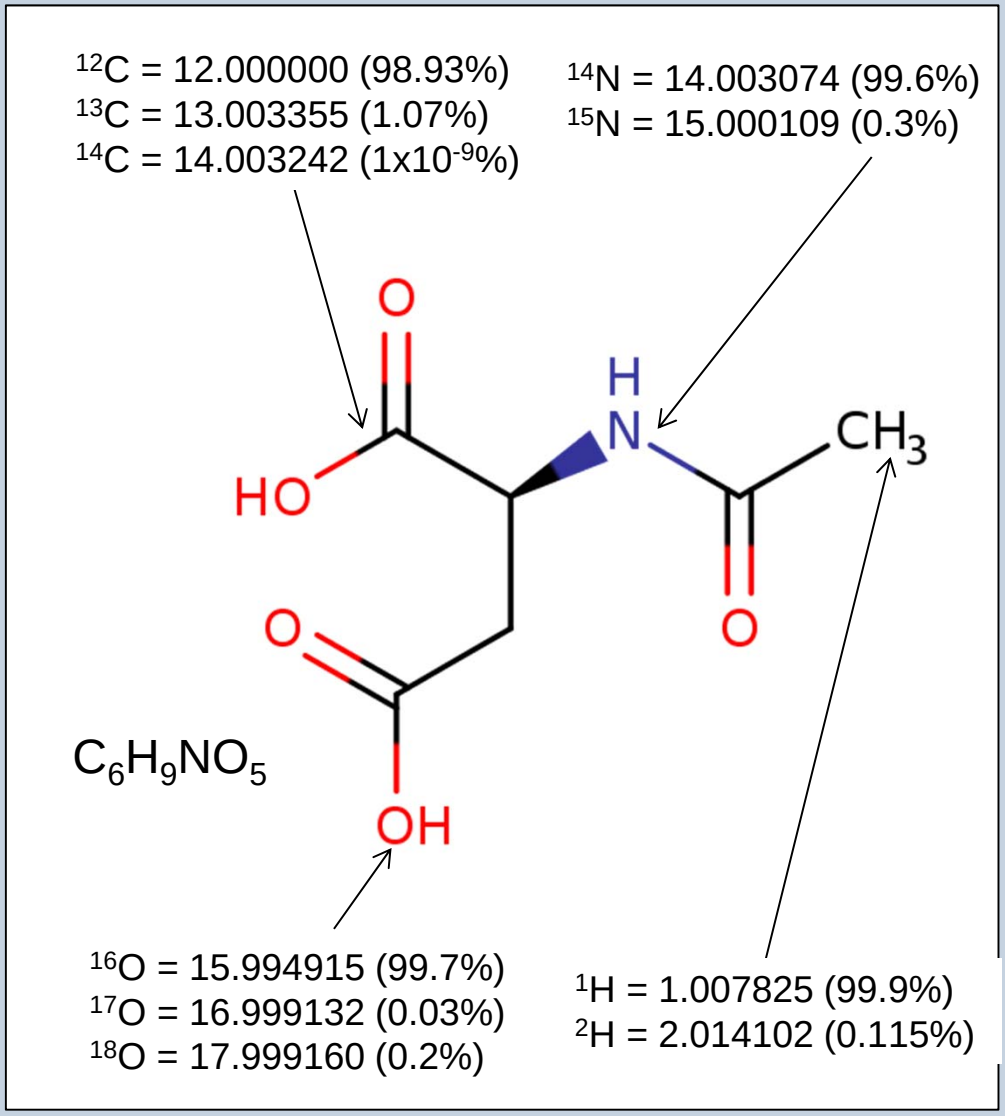
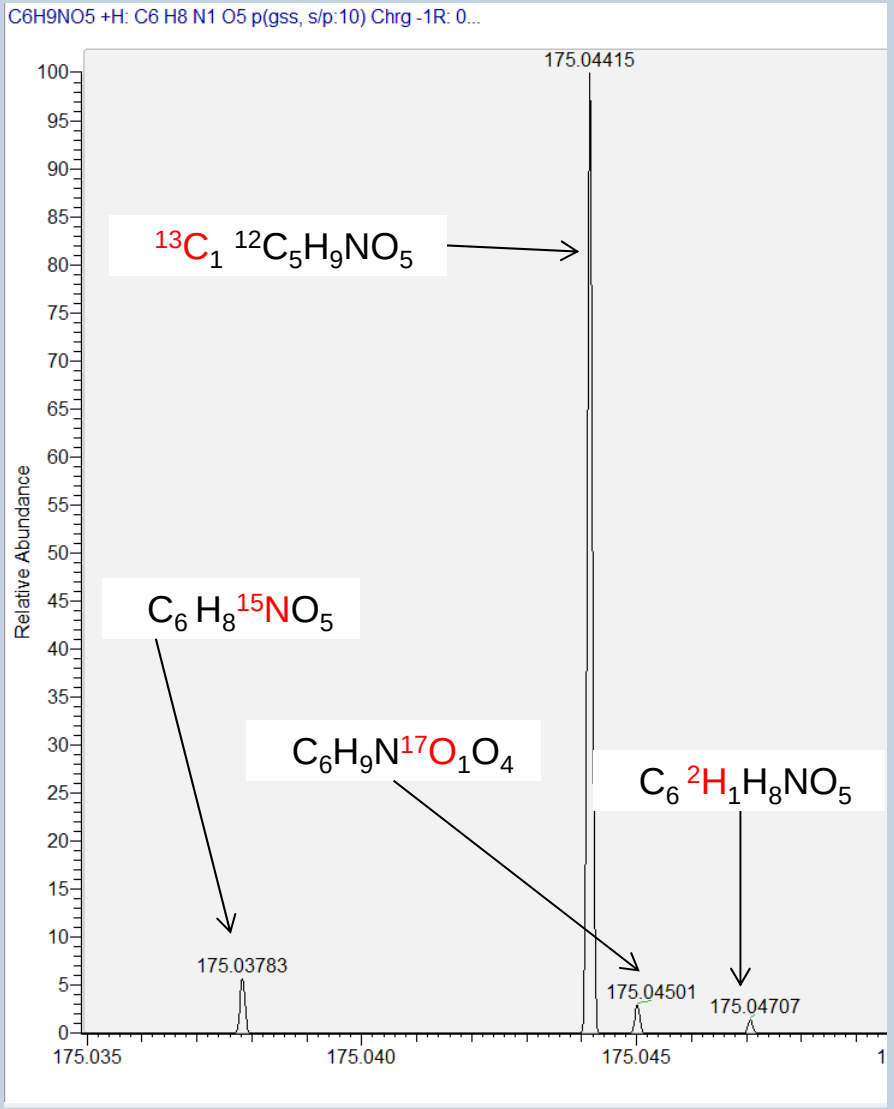
Individual isotopomers distinguished at high resolution?





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What is meant by mass?

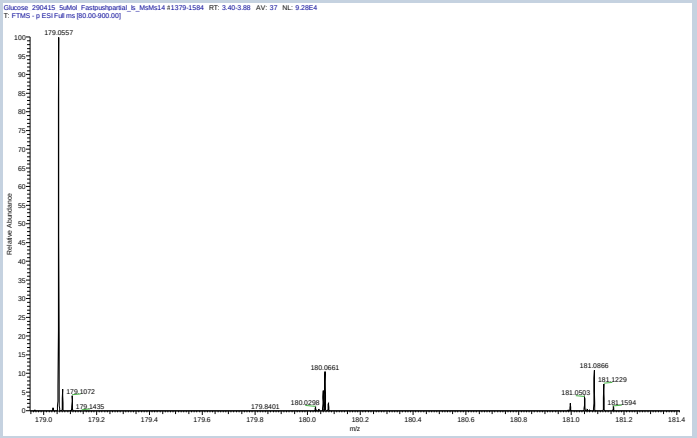
Mass spectrometers measure mono-isotopic masses. There are a number of online calculators available for example here:

Relative atomic mass

Weighted average of isotopes present

Glucose = **180.15588**

Mono-isotopic mass
(mass spectrometers measure)

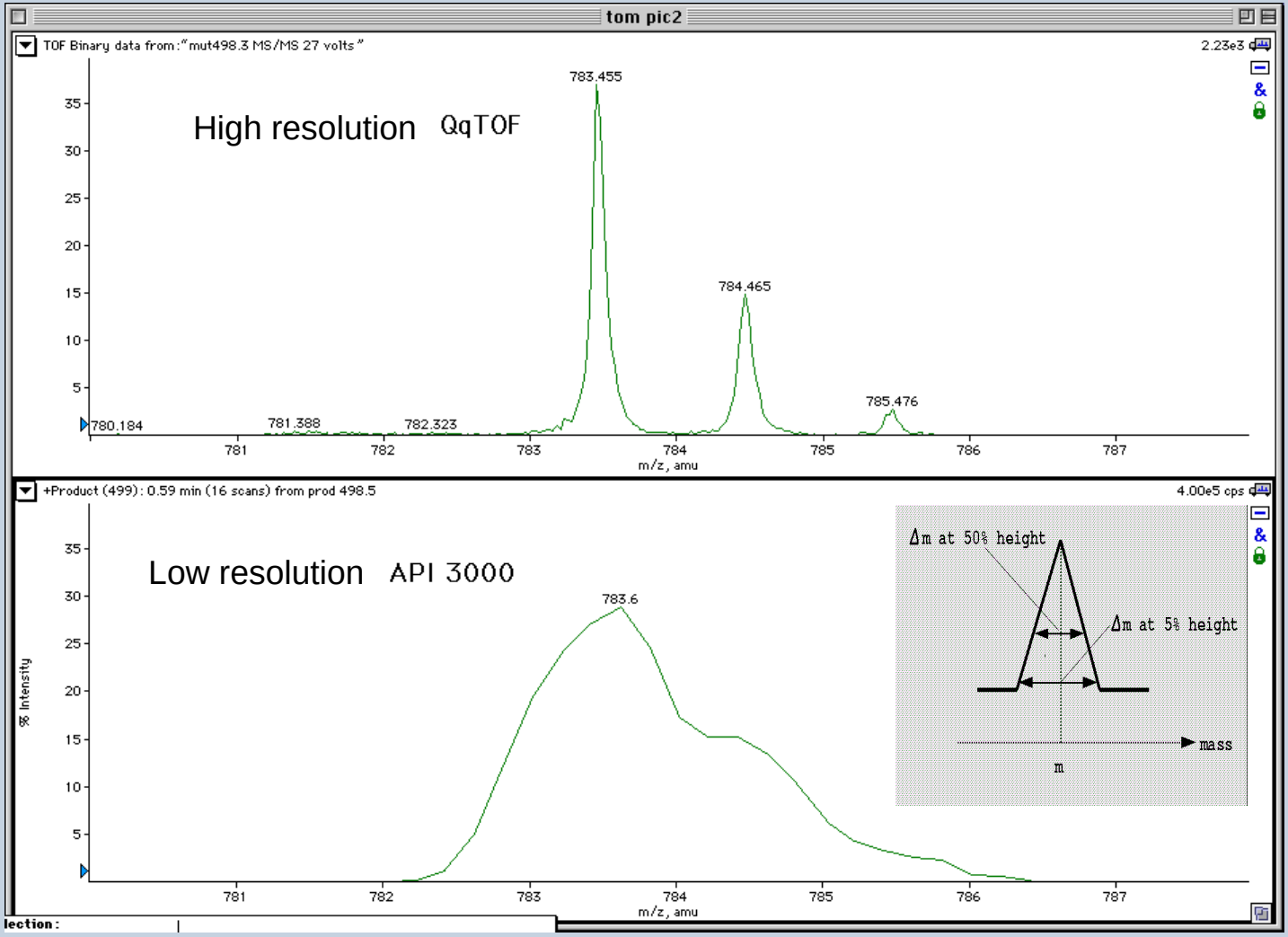


The **monoisotopic mass** is the sum of the most abundant isotope for each element.

Glucose = **180.0634**

Mass Spectrometry deals with measurement of mono-isotopic masses not relative atomic masses (average masses).

Resolution of mass spectral peaks



Width of peak indicates the **resolution** of the MS instrument.

The better the resolution or resolving power, the **better the mass accuracy**.

Peak width at half height is used to compared peak resolution between mass analysers.

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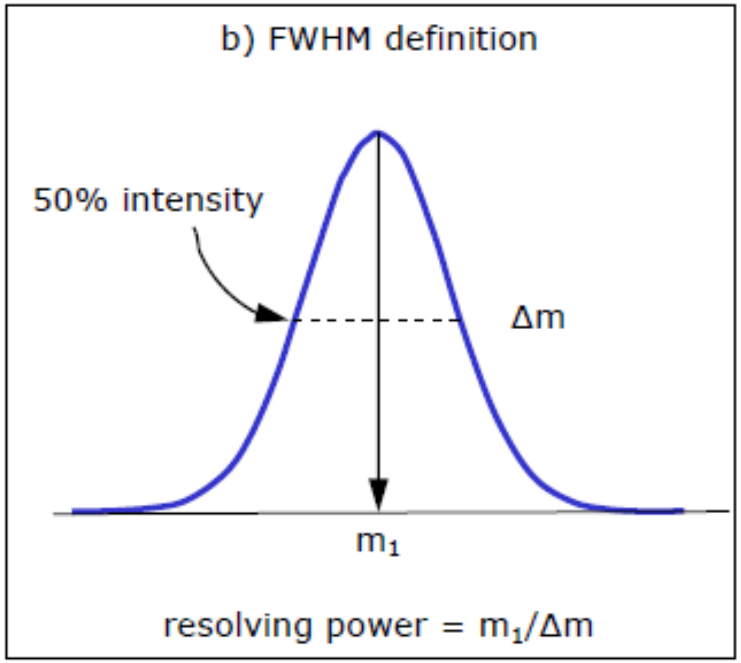
Mass accuracy determined by resolution

Resolution is defined by the ability of the mass spectrometer to differentiate between two similar masses. An instrument's *resolving power* is given by the equation: **Resolving power = measured mass/peak width (R = m/Δm)**

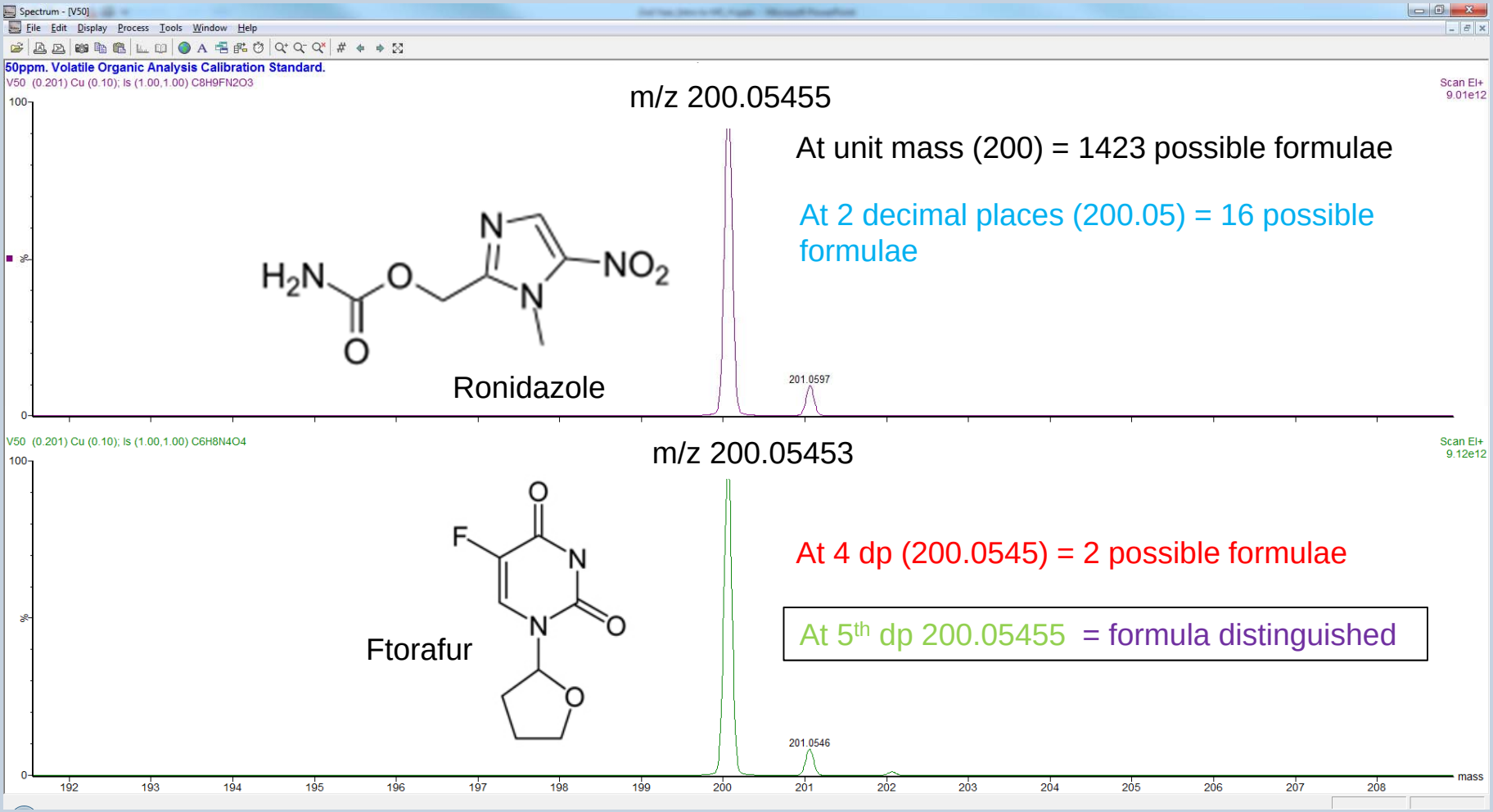
Where **m** is the measured mass and **Δm** = resolution of a peak. Resolution **(Δm)** is the width of the peak measured at a specified fraction of the peak height. Usually 50% and this is known as FWHM (full width at half maximum).



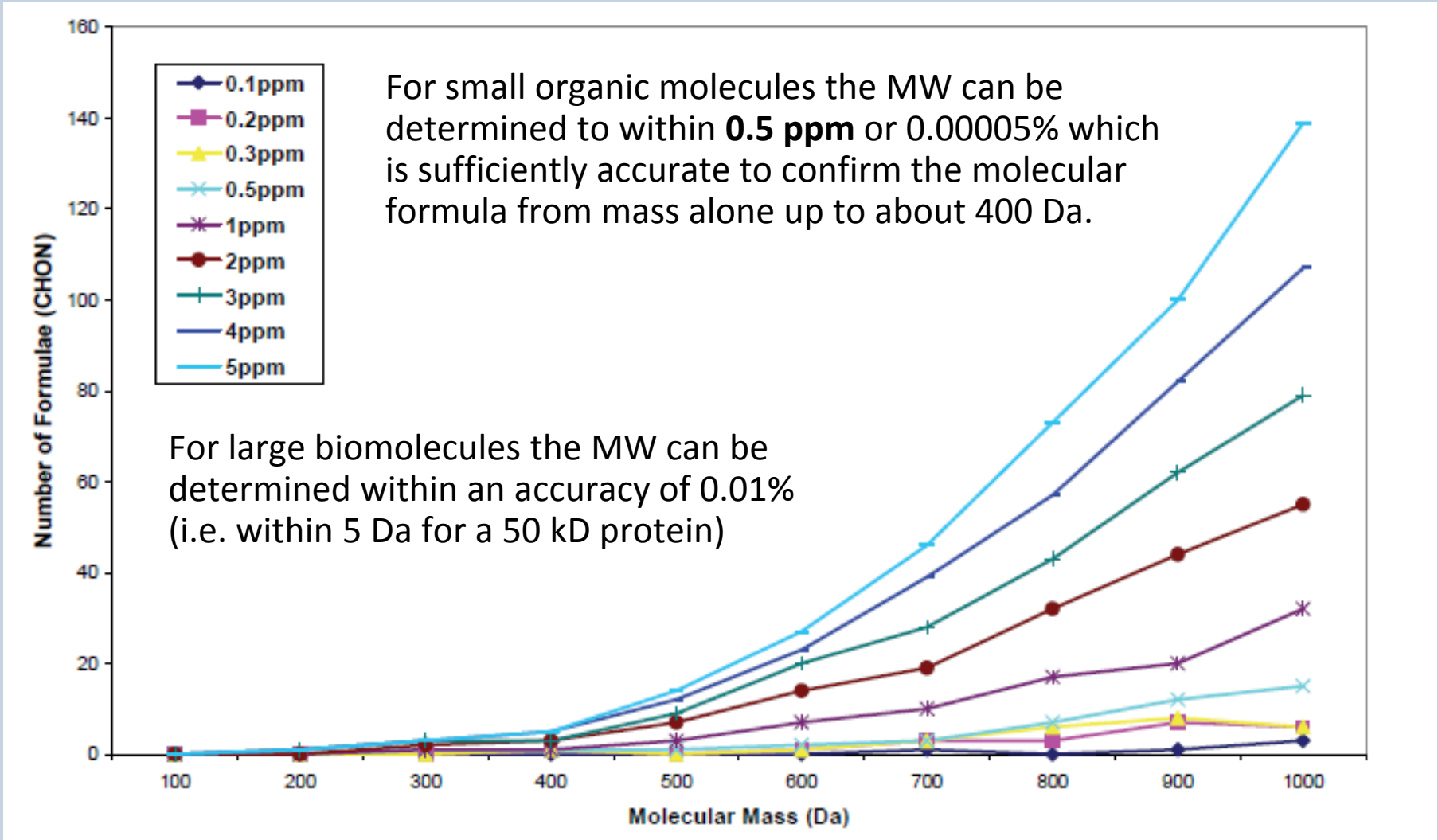
Determined mainly by the type of mass analyser being used.

The better the resolution or resolving power, the **better the mass accuracy**.

How mass accuracy affects identification



Relationship between mass measurement accuracy and number of theoretical chemical formulae (ppm)



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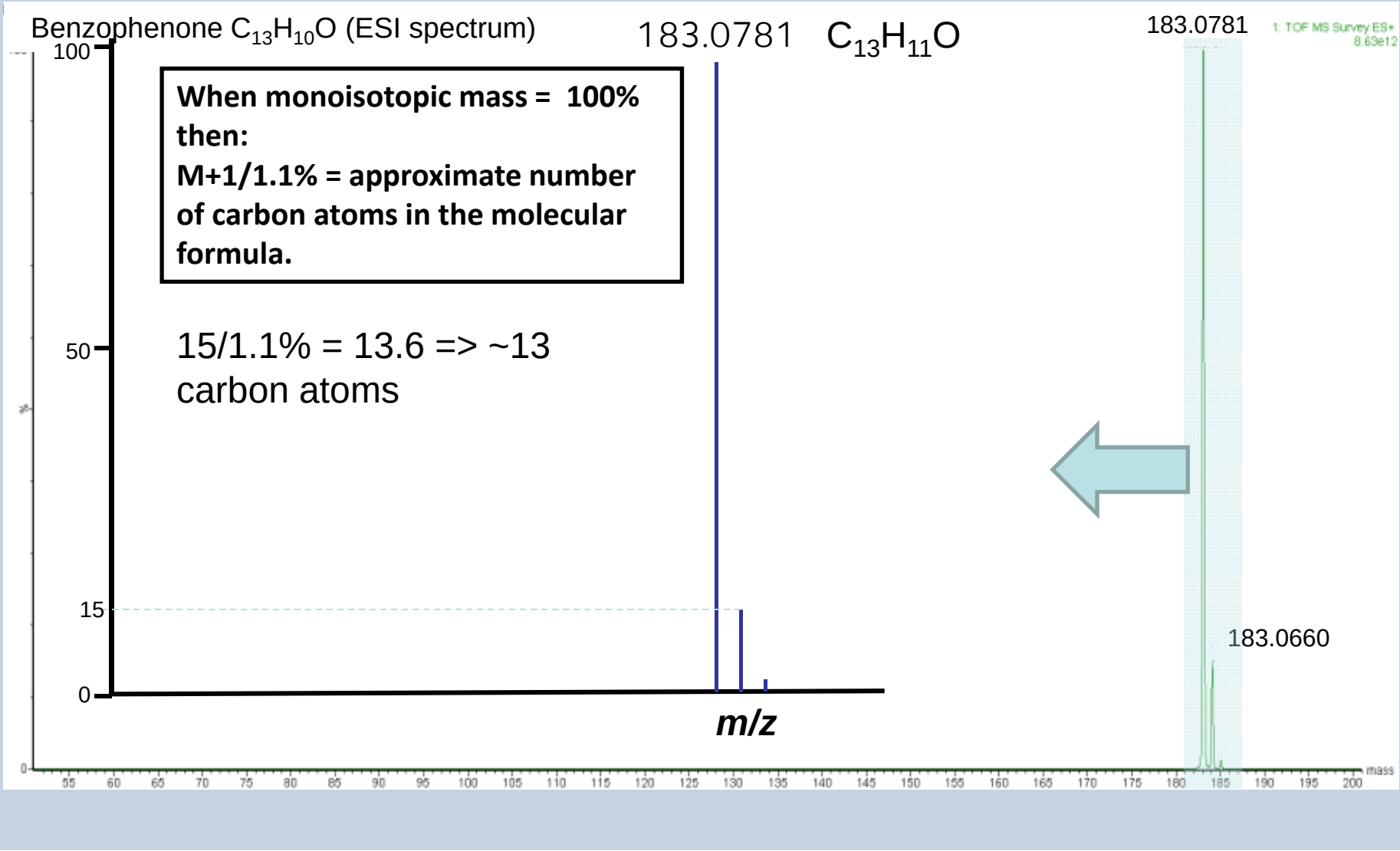
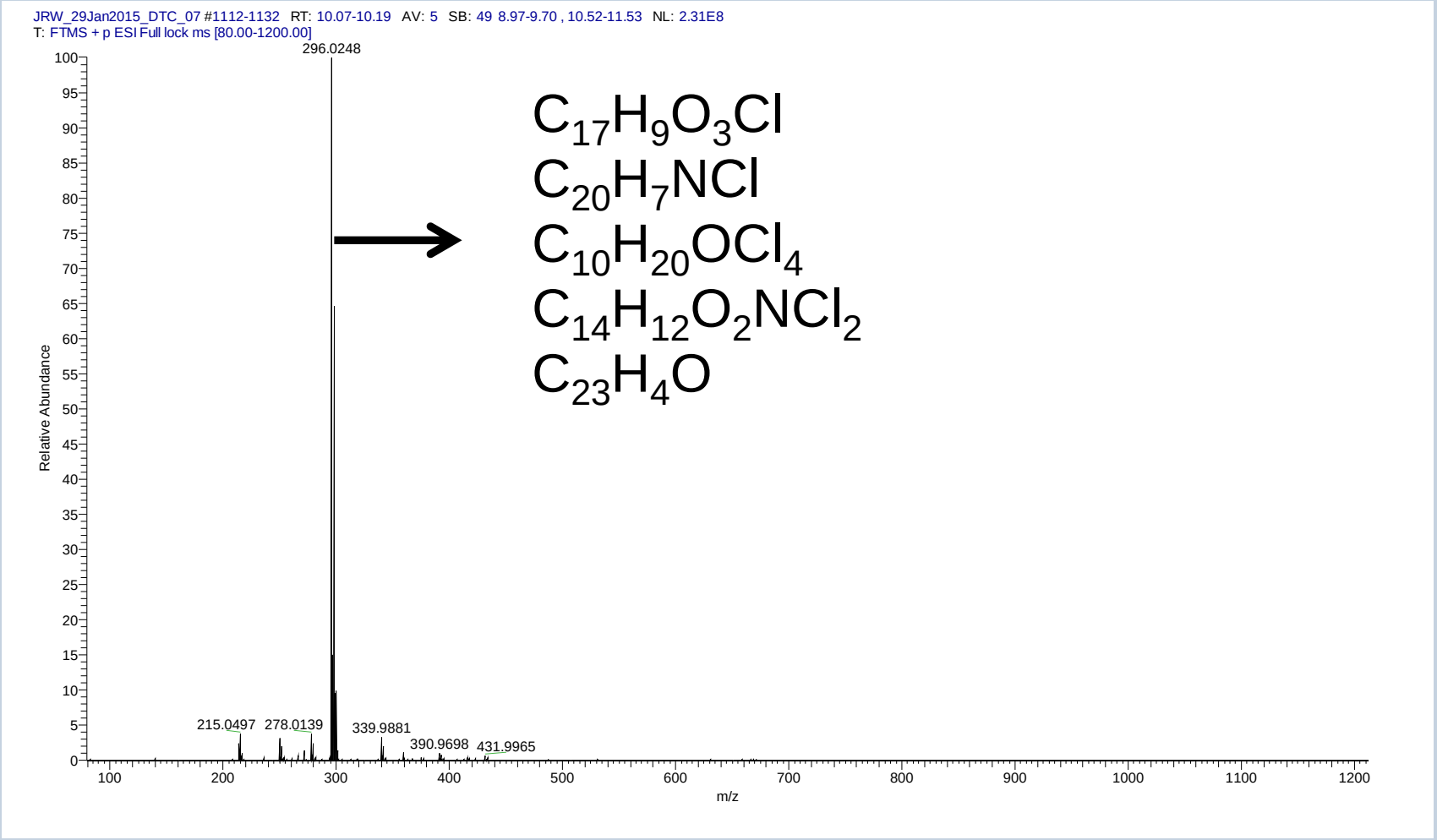
Predicting number of carbon atoms



Identification of and unknown by accurate mass



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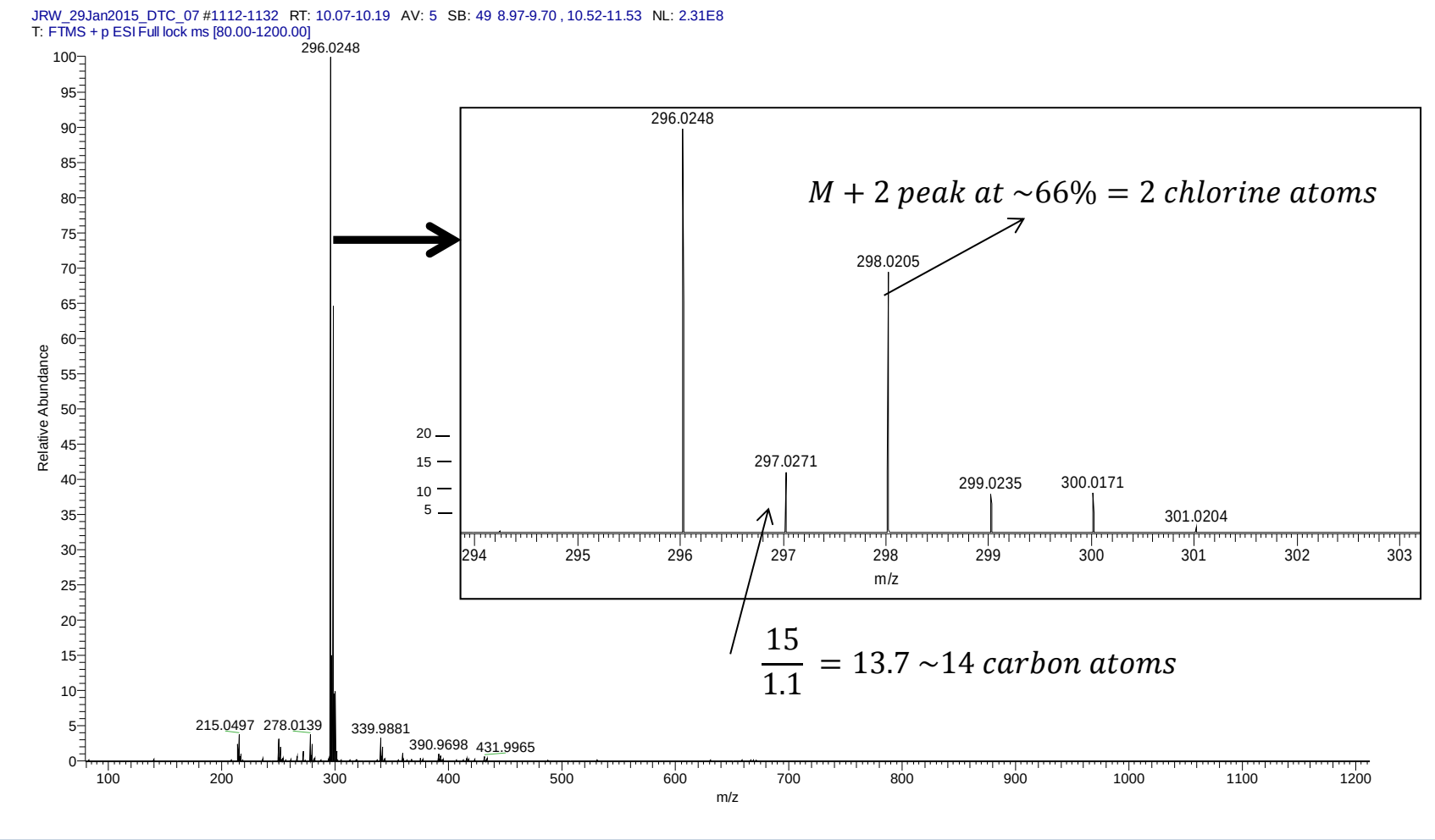
Identification of and unknown by accurate mass



Identification of and unknown by accurate mass

Formula predictions:
(5ppm error tolerance)

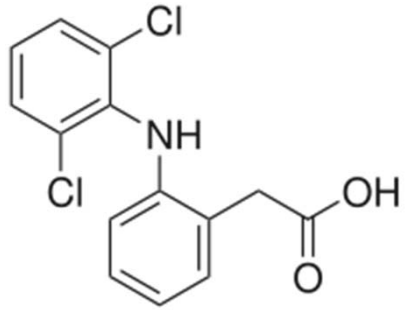
$C_{17}H_9O_3Cl$
$C_{20}H_7NCl$
$C_{10}H_{20}OCl_4$
$C_{14}H_{12}O_2NCl_2$
$C_{23}H_4O$

+ Information from mass spectrum

- Number of C atoms: 14
- M+2 peak shows Cl_2 present.

Identification: $C_{14}H_{11}Cl_2NO_2$

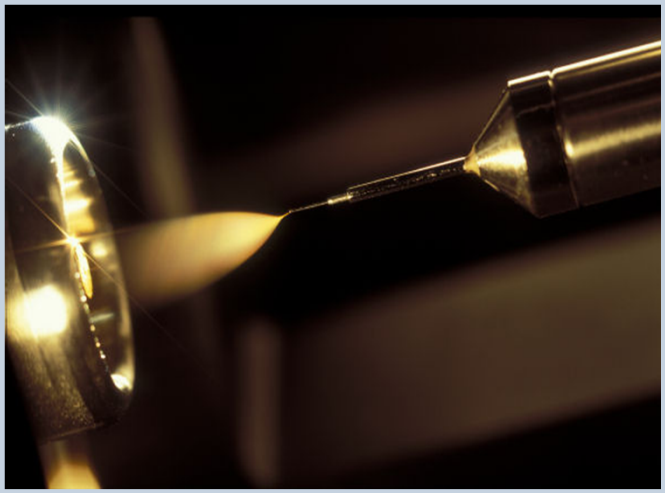
…but not the structure. We will discuss identifying the structure of compounds in future lectures.



Diclofenac

Section 3: Ion sources

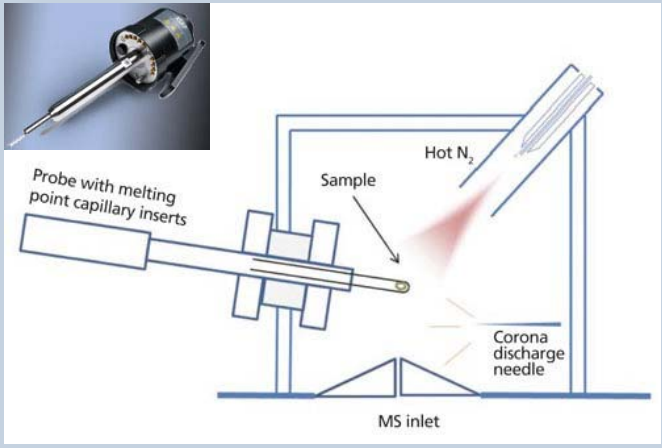
EI/CI ion source



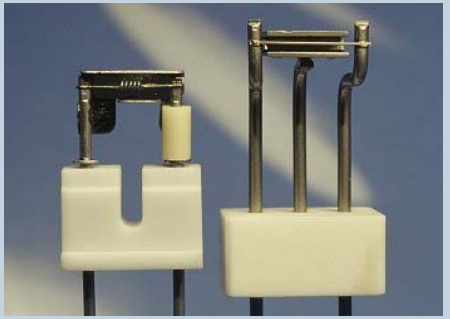
Electrospray ion source

Solid-liquid based ambient ionisation

Matrix assisted laser desorption ionisation

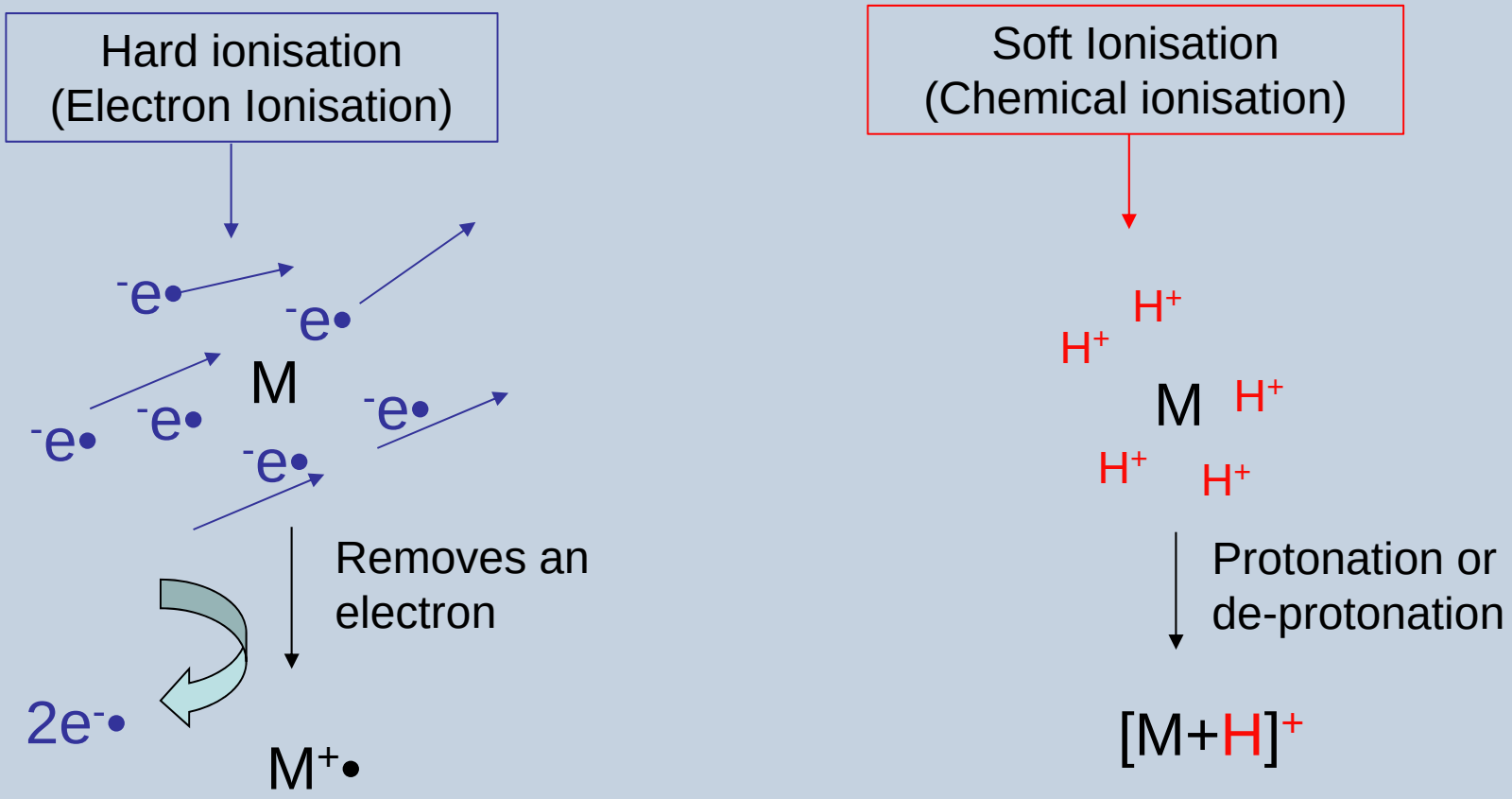


ASAP probe for solids

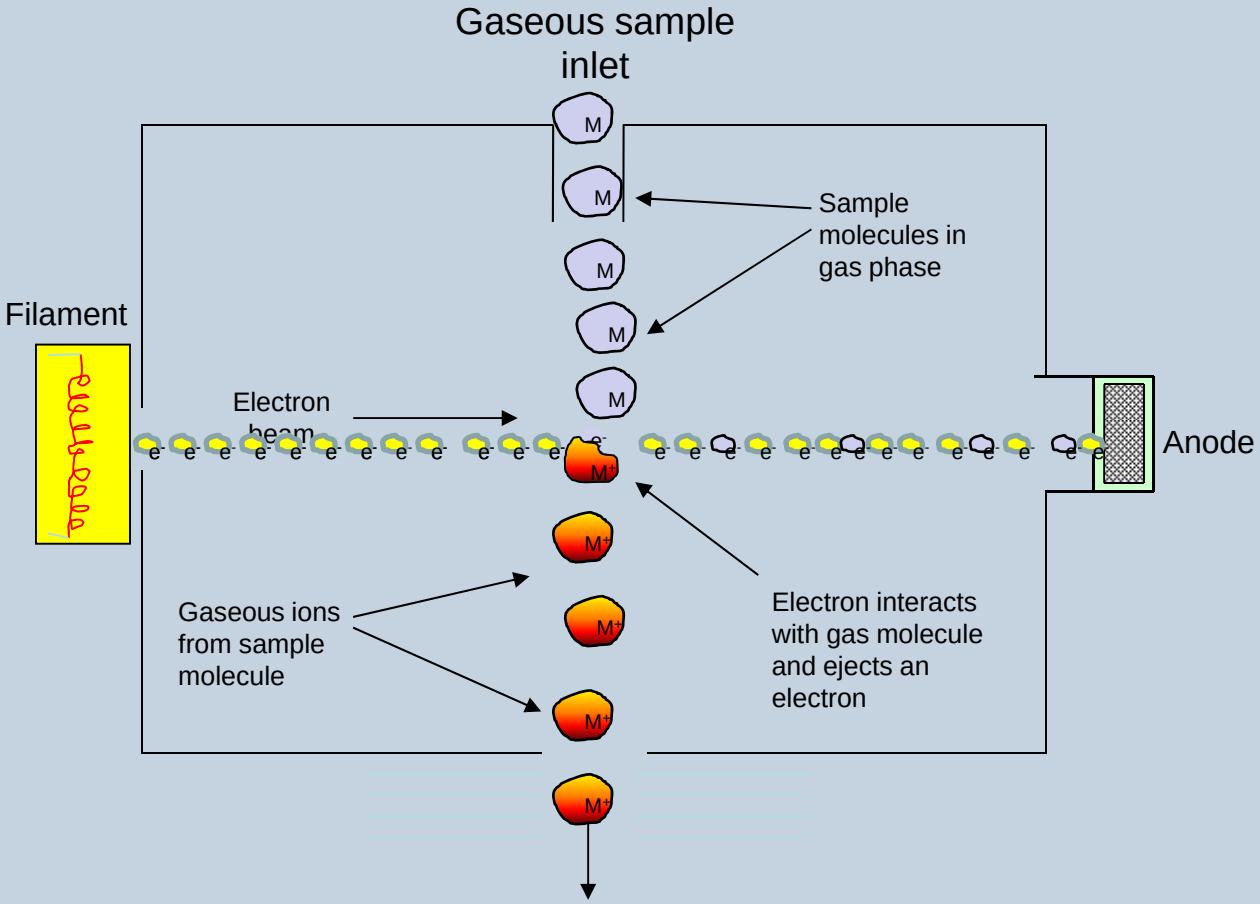


EI/CI electron filament

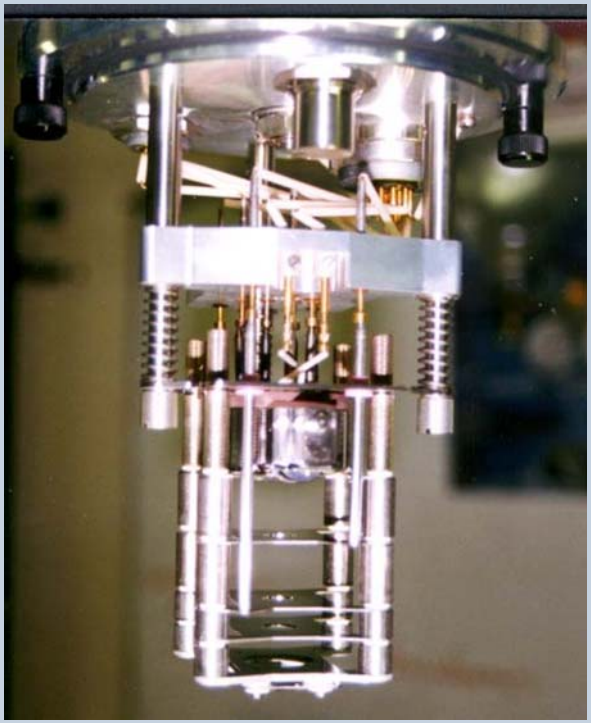
Two basic approaches to ionisation



Two basic approaches to ionisation



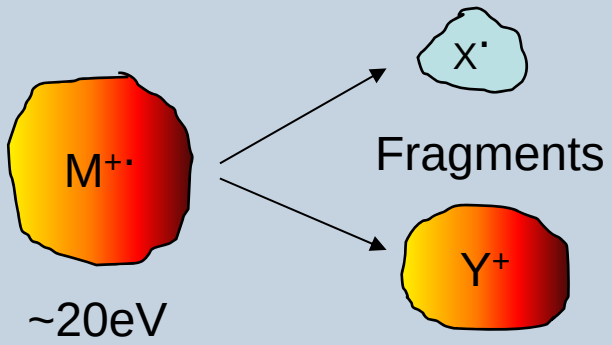
Analytes must be volatile

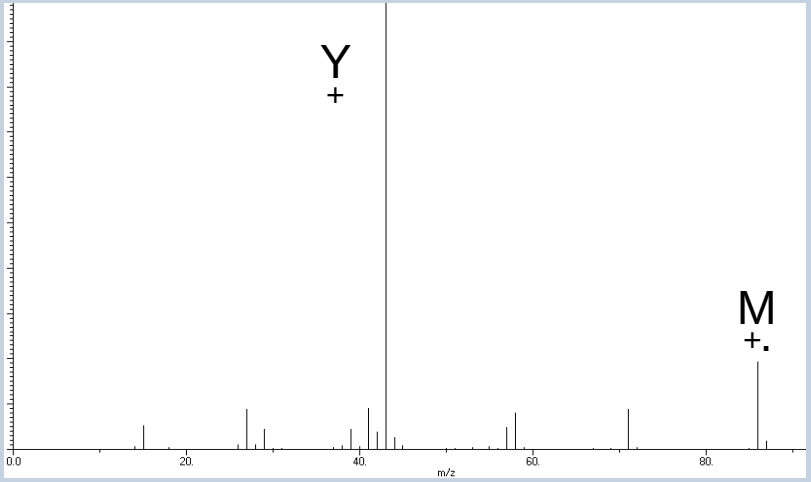


- Sample introduced into instrument by heating it until it evaporates
- Bombarded by high energy electrons
- Fragmentation often occurs
- Fragment ions enter the

Fragmentation

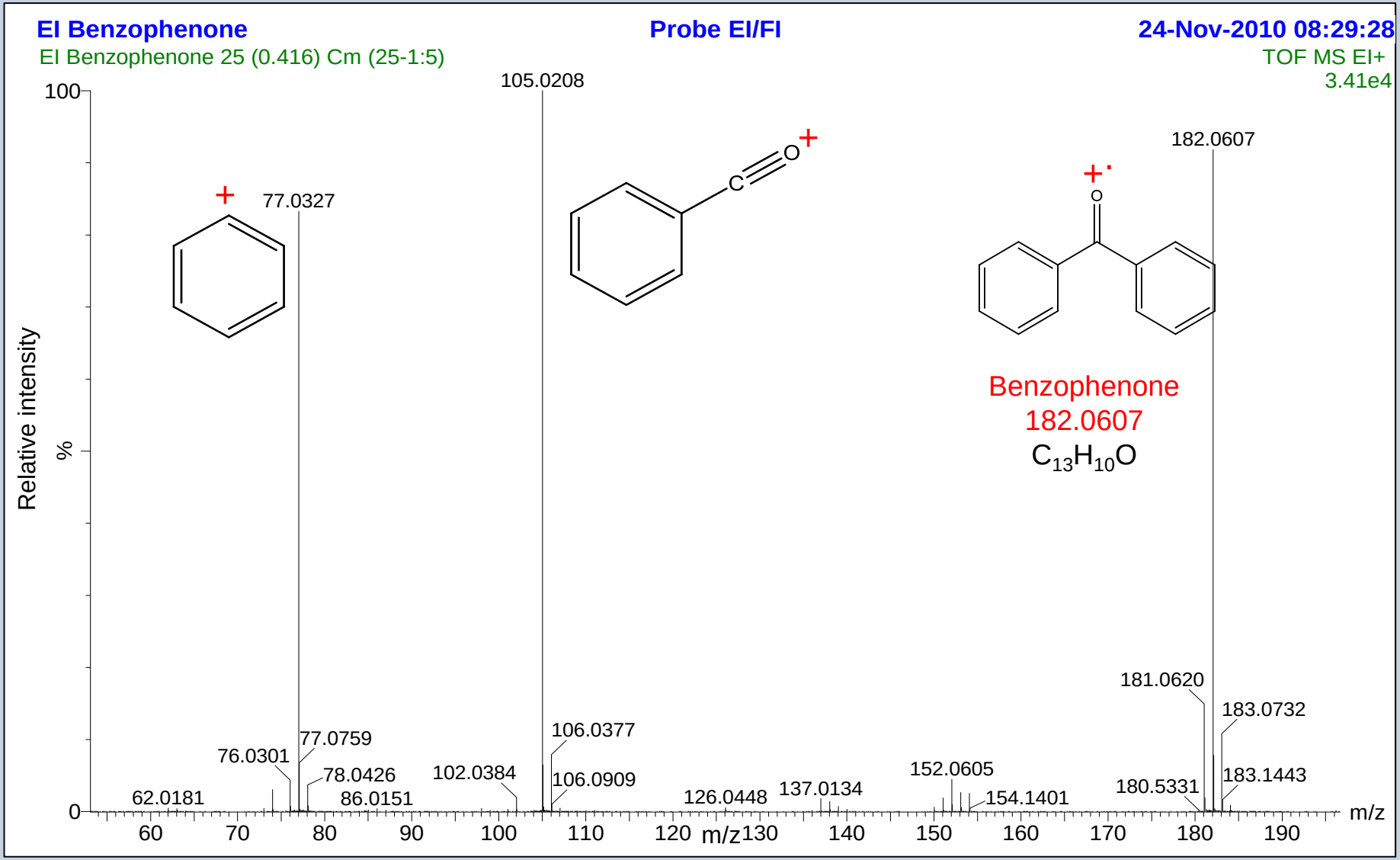
Interaction with electrons at **70eV** confers enough energy to create a **radical cation** and often raises its internal energy beyond some of its internal bond energies (~10eV). This often leads to fragmentation of the cation.





EI fragmentation provides a **unique mass fingerprint** for molecular identification. Highly reproducable has lef to development of large libraries of EI fragmentary spectra (NIST & others) enable identification of EI spectra

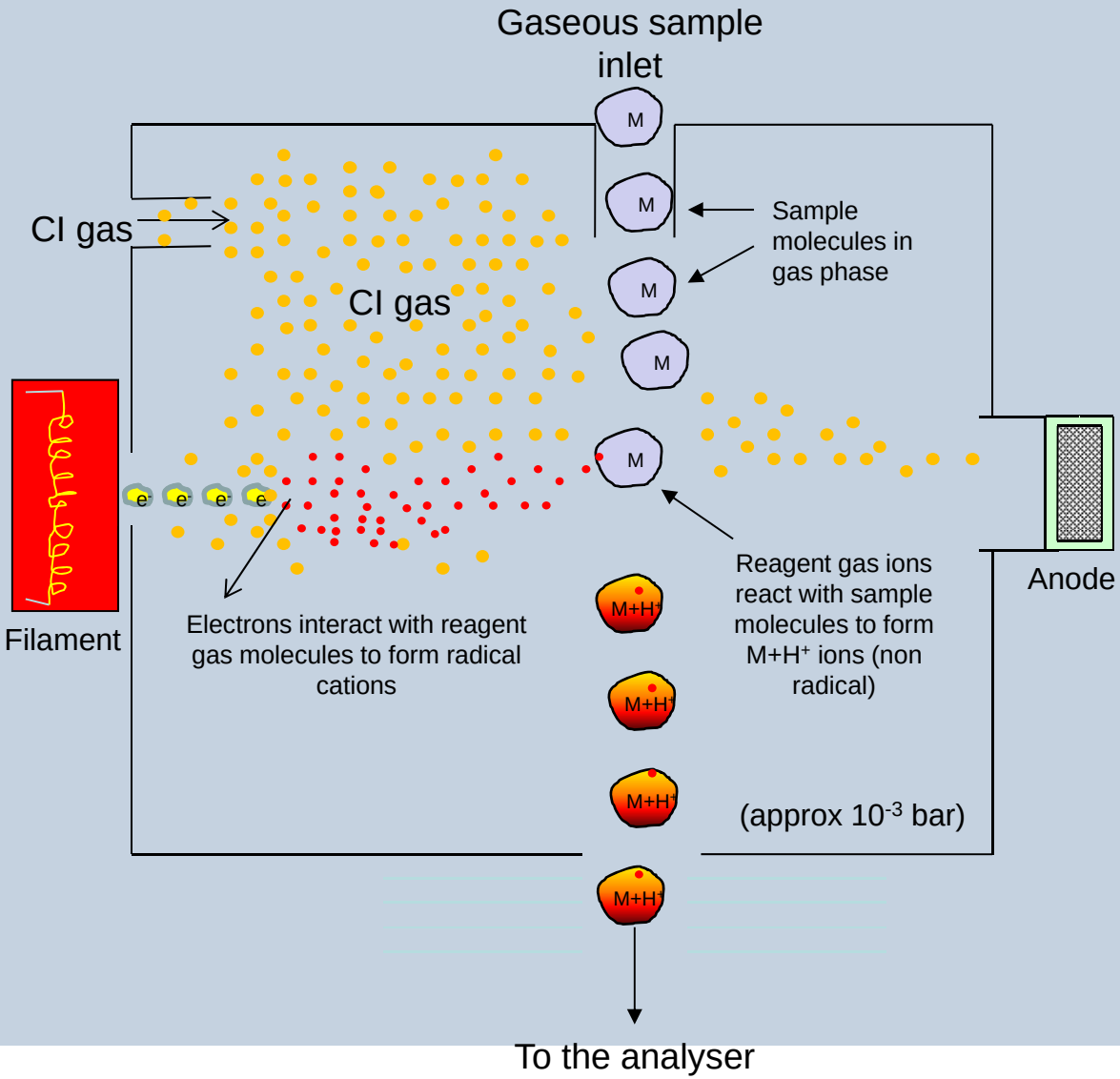
Electron ionisation spectrum



Characteristics of electron ionisation (EI)

- Used to be the only ionisation source available for MS.
- Found very commonly on GC/MS systems due to computability with volatile compounds.
- Used for structural analysis.
- 1 in 1000 gas molecules ionised.
- Unsuitable for thermally labile compounds.
- Sample pressure is directly proportional to ion current hence EI. is a rather uniquely quantitative ionisation source (unlike soft approaches).

Chemical ionisation



CI ionises a reagent gas in a similar process to EI. **Reagent radical cations then react with analyte molecules (at higher pressure) leading to lower energy proton transfer.** This leads to significantly less fragmentation than EI with the formation of predominantly protonated molecular ions.

Typical Chemical Ionisation charge transfer process

Chemical ionisation is a soft ionisation technique which uses a similar apparatus and process to Electron Ionisation but at a higher pressure in the presence of a reagent gas (~10^{-3} bar) such as methane, ammonia, H_2 and water.

Reagent gas: Methane

(a) $CH_4 + e^{-*} \rightarrow CH_4^{+\bullet} + 2e^-$ methane molecular ion formation

(b) $CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet$ carbocation formation

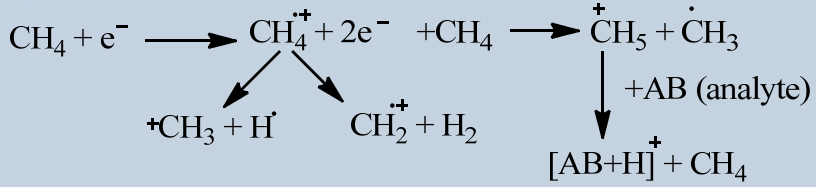
(c) $CH_5^+ + AB \rightarrow CH_4 + [AB+H]^+$ protonated analyte formation

(d) $CH_4^{+\bullet} \rightarrow CH_3^+ + H_2$ alternative carbocation formation

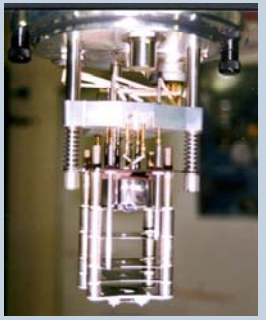
(e) $CH_3^+ + M \rightarrow CH_4 + [AB-H]^+$ alternative analyte ion formation

(f) $CH_5^+ + CH_4 \rightarrow C_2H_5^+ + H_2$ side reaction carbocation formation

(g) $C_2H_5^+ + AB \rightarrow [AB+C_2H_5]^+$ analyte adduct ion formation



Chemical Ionisation Summary



- Less energy transferred and hence less fragmentation than EI.

- The ion formed is a protonated molecule and hence strictly should not be referred to as the ‘molecular ion’. Some texts refer to it as a ‘pseudo-molecular’ ion.

- Spectra usually free of fragmentation ions – however they are not as reproducible as EI spectra.

- No libraries!

- Gas phase ionisation: As for EI analytes must be thermally stable and volatile.

CRL: GC/MS: Both EI and CI ion sources

Agilent GC/MS

GC/MS: Useful for high resolution, high sensitivity separation of compounds in the gas-phase with identification based on IE mass spectral libraries.

- Complex mixture analysis
- volatiles
- SPME
- Headspace analysis
- Metabolomics

Mass Spectrometry website: http://www.chem.ox.ac.uk/spectroscopy/mass-spec/

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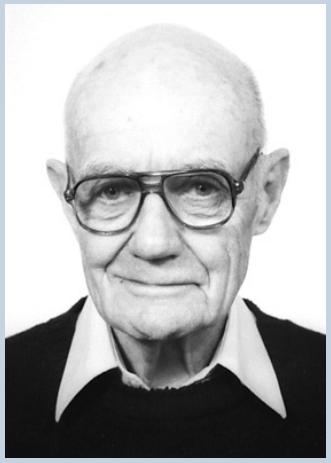
Characteristics of electron ionisation (EI)

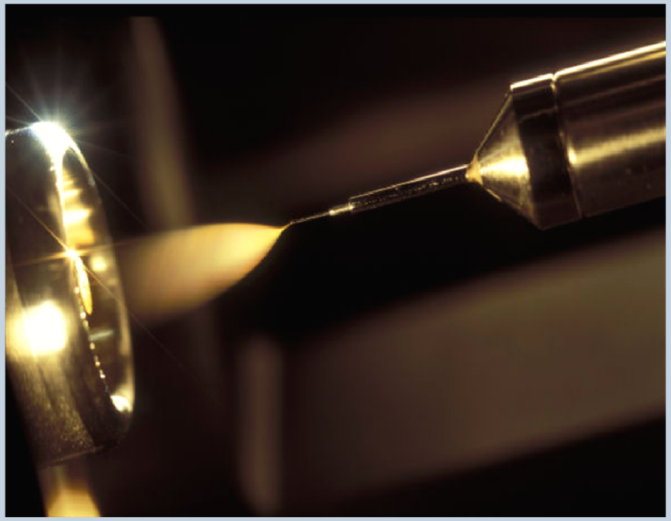


John B. Fenn

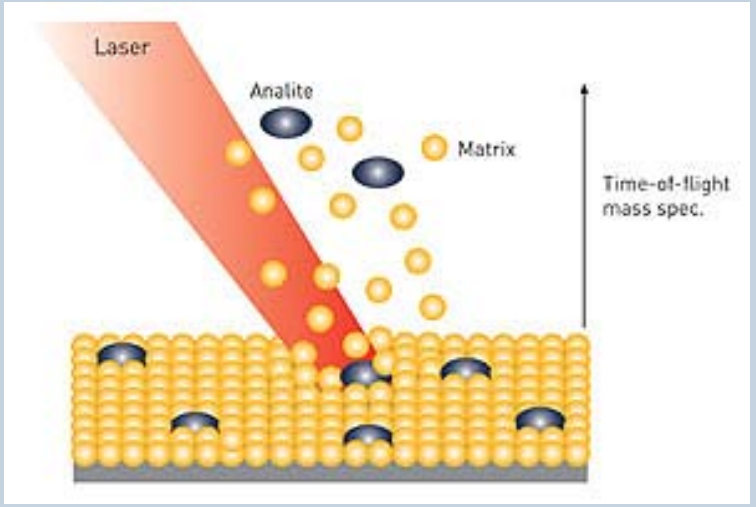
The Nobel Prize in Chemistry 2002

The 2002 Nobel Prize for Chemistry was awarded *"for the development of methods for identification and structure analyses of biological macromolecules"*

Koichi Tanaka



Electrospray Ionisation



MALDI

"To give wings to molecular elephants" – John Fenn

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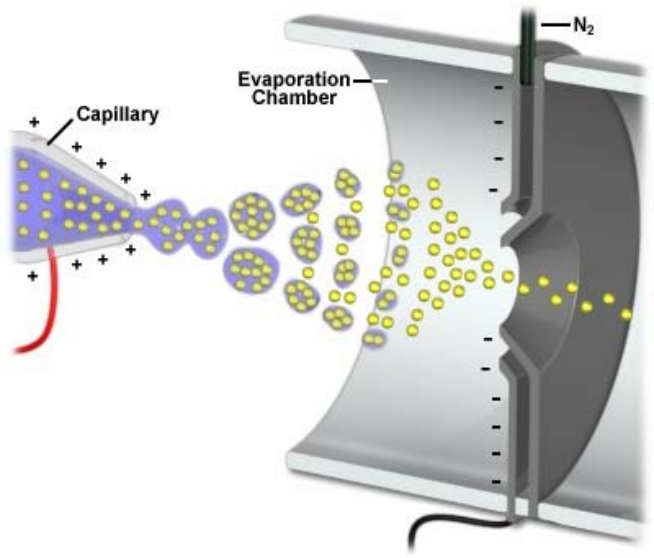
Electrospray Ionisation (ESI)

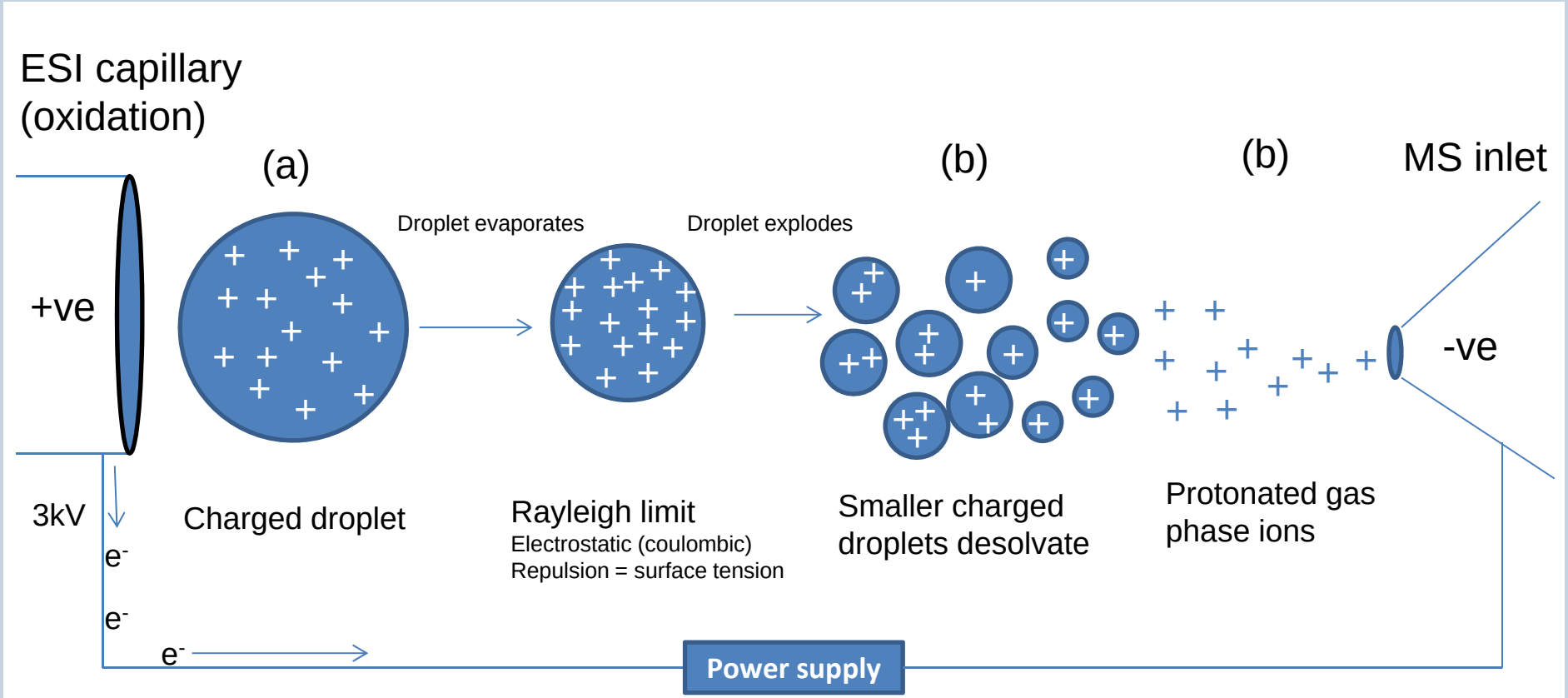
Problem: How to introduce compounds in solution into the mass spectrometer at atmospheric pressure?



- Sample dissolved in polar, volatile buffer and pumped through a stainless steel capillary.
- Strong voltage (3-4 kV) applied at tip along with flow of nebulizing gas causes the sample to “nebulize” or aerosolize
- The aerosol evaporates quickly to near atomic size (still carrying charges) and enters the MS as a psuedo-molecular ion.

Liquid containing analyte(s) is forced through a steel capillary at high voltage to electrostatically disperse droplets. Charge is imparted across the rapidly evaporating liquid which leads to protonation or deprotonation upon full evaporation of the solvent.

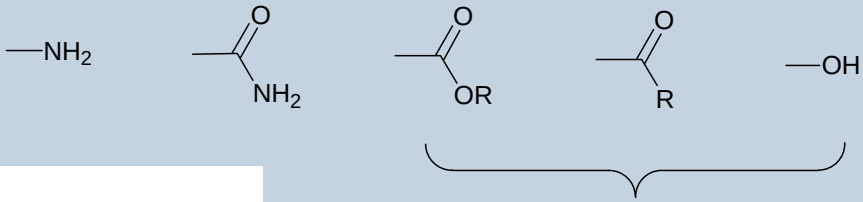
The Electrospray Ionisation Process



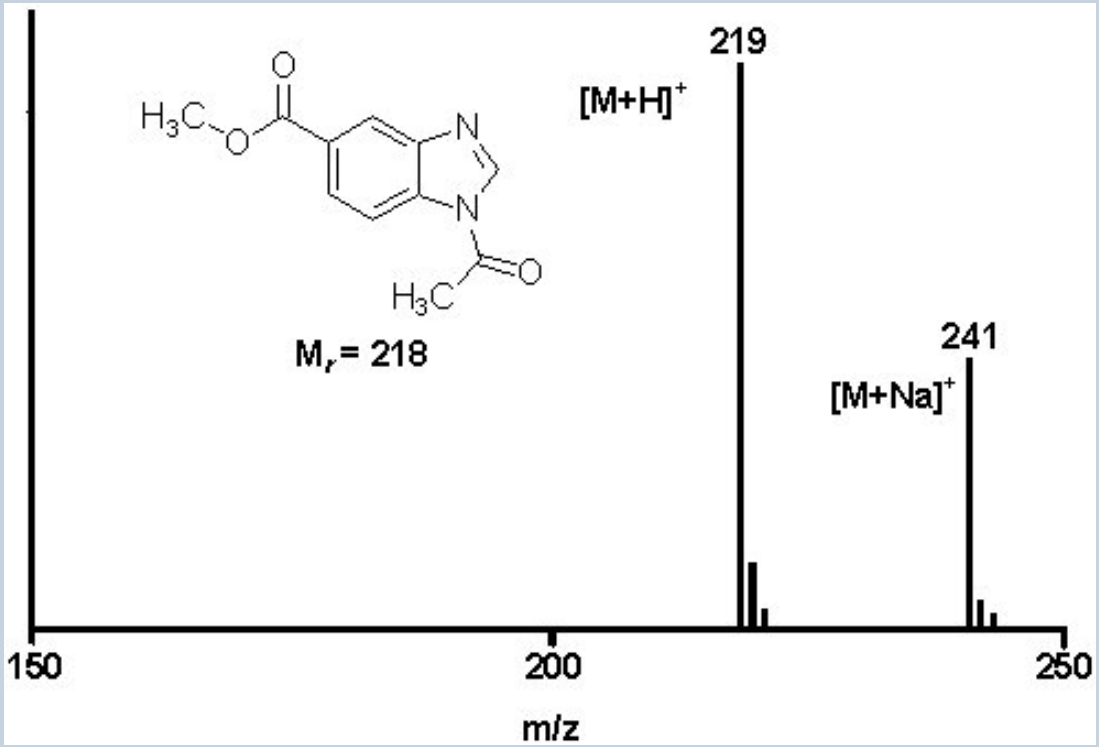
Electrospray ionisation is an atmospheric pressure soft ionisation process.
Good description in: Introduction to Mass Spectrometry, Watson and Sparkman, Wiley

Positive ions by ESI

Positive ion (most basic atoms)
e.g. $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$

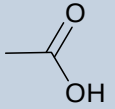


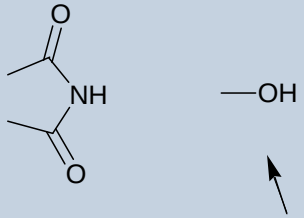
Often more than 1 group required



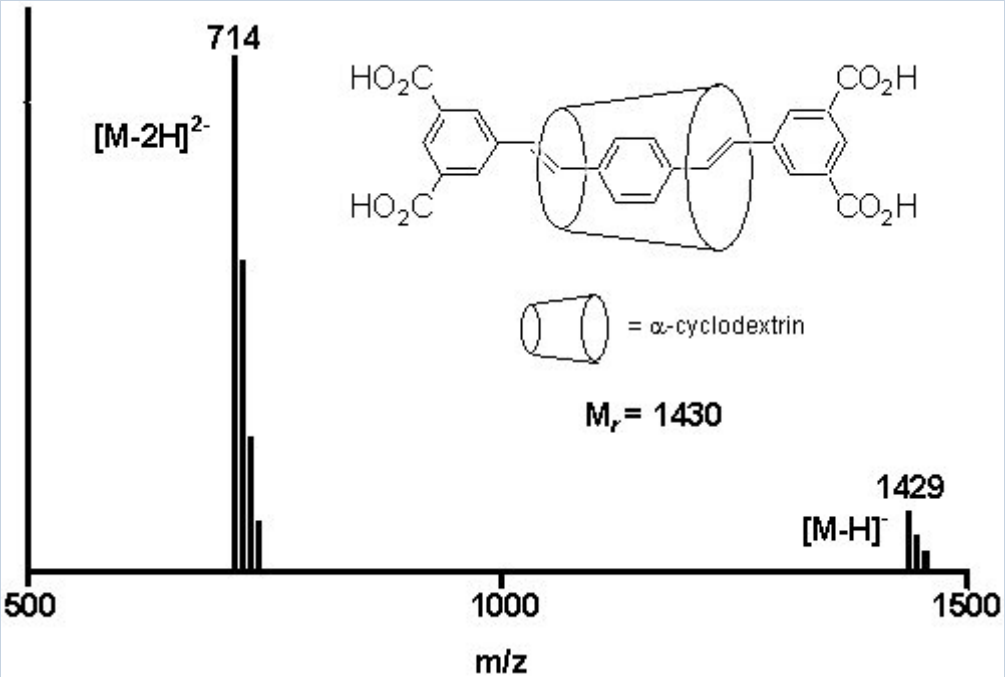
Negative ions by ESI

Negative ions (most acid atoms)
e.g. $[M-H]^-$, $[M+Cl]^-$, $[M+HCO_2]^-$



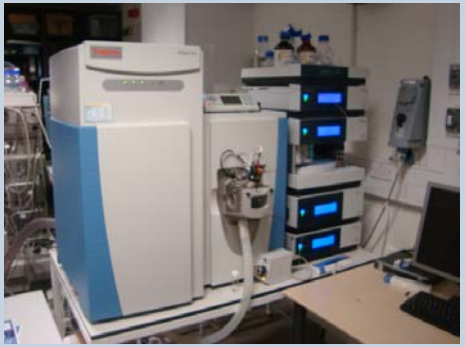


Often more than 1 group required



Electrospray ionisation characteristics

- Can be modified to “nanospray” system with flow <1 µL/min.
- Very sensitive technique, requires less than a picomole of material.
- Strongly affected by salts & detergents.
- Electrospray Ionization can be easily interfaced to LC.
- Absolute signals from Electrospray are reproducible. good for quantitation.
- Multiply charge ions tend to fragment easier than singly charge ions.
- Resolution is better at lower m/z values, therefore, ESI helps obtain better resolution at higher m/z values.



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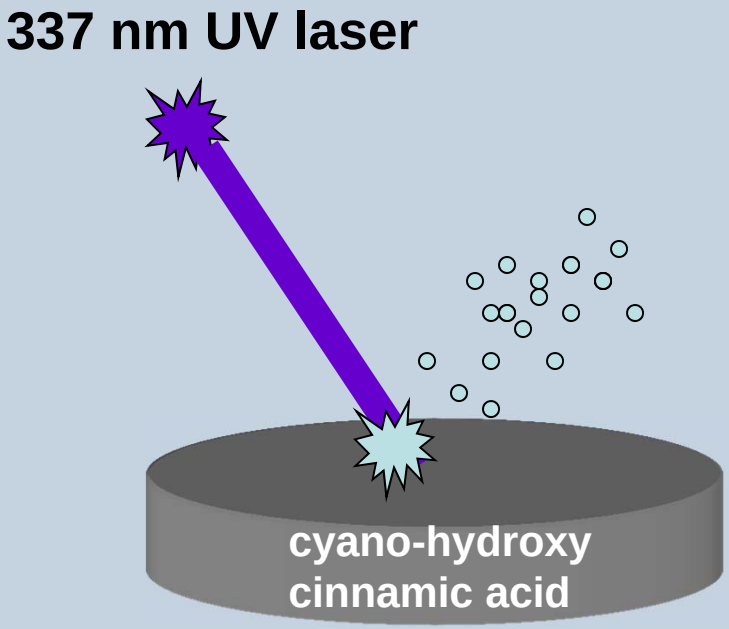
Matrix Assisted Laser Desorption Ionisation

MALDI uses laser energy to desorb and ionise sample embedded in a matrix. It is one of the most sensitive laser technique for ionisation used in mass spectrometry although the exact mechanism of ion formation is not fully understood. It usually only forms singly charged ions.



Mechanism

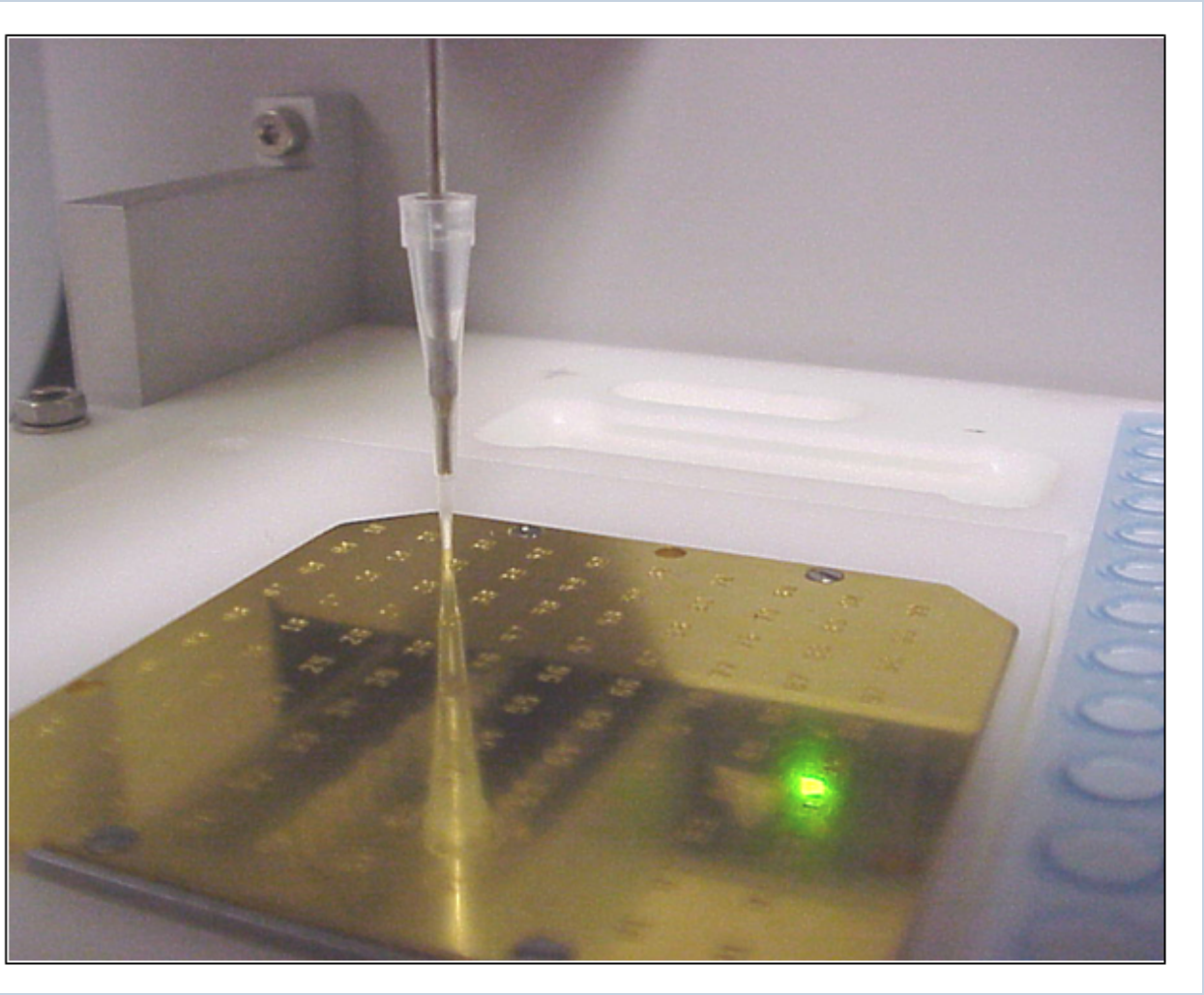
(i) *Formation of a 'Solid Solution'* using a matrix (small organic molecules).

(ii) *Matrix Excitation*: The laser beam is focussed onto the surface of the matrix-analyte solid solution. The chromophore of the matrix couples with the laser frequency causing rapid vibrational excitation.

(iii) *Analyte Ionisation*: The photo-excited matrix molecules are stabilised through proton transfer to the analyte. Cation attachment to the analyte is also encouraged during this process.

Introduction to Mass Spectrometry, Watson and Sparkman, Wiley

Matrix Spotting onto a MALDI plate



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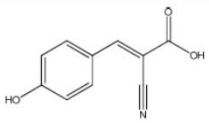
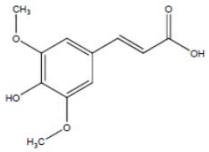
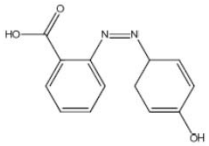
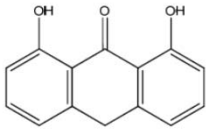
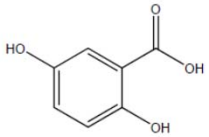
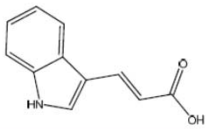
Matrices

Common MALDI matrices and substrates	Structure	m/z $[M+H]^+$	Common sample substrates	Common solvent
CHCA: ά-cyano-4-hydroxy cinnamic acid		189.04	Peptides, polymers and intact bacteria	MeOH, THF, Acetone
Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid)		224.07	Proteins, peptides and polymers	MeOH, THF, Acetone
HABA		244.08	Polar and non-polar synthetic polymers	THF
Dithranol (1,8-Dihydroxy-9(10H)-anthracenone)		226.06	Resins, unsaturated aromatic polyesters	THF, $CHCl_3$, HFIP
DHB (2,5-dihydroxybenzoic acid)		154.03	Peptides, Carbohydrates, Polymers Glycolipids	MeOH, CAN, H_2O
IAA – β-indole acrylic acid		187.06	Polymethyl methacylates	Acetone

Need to be involatile (Solids at room temp).

Need to absorb at laser wavelength in use (usually 337nm)

Common examples: sinapinic acid for proteins, 4-hydroxycinnaminic acid.

Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering).

Organic Chemist's Desk Reference. Caroline Cooper (ed), 2011, CRC press.

MALDI Summary

- Unlike ESI, MALDI generates spectra with generally singly charged ions making spectral interpretation simpler.
- Positive mode generates ions of M+H.
- Negative mode generates ions of M-H.
- Generally more robust than ESI (tolerates salts and nonvolatile components).
- Higher throughput and generally higher mass detection limits.
- Requires 1uL of 1 pmol/mL sample (generally lower than ESI).
- Mass Accuracy not as good as ESI.
- Fragmentation more ‘difficult’ particularly for larger ions than ESI.

Characteristics of electron ionisation (EI)

AB Sciex MALDI TOF-TOF

Waters MALDI micro

High sensitivity analysis of:

- Peptides/proteins
- Oligonucleotides
- Polymers.
- Small molecules above 450 Da.

- Fast and efficient (~1uL of sample soluiton required).

- Two systems in Chemistry Dept.

Mass Spectrometry website: http://www.chem.ox.ac.uk/spectroscopy/mass-spec/

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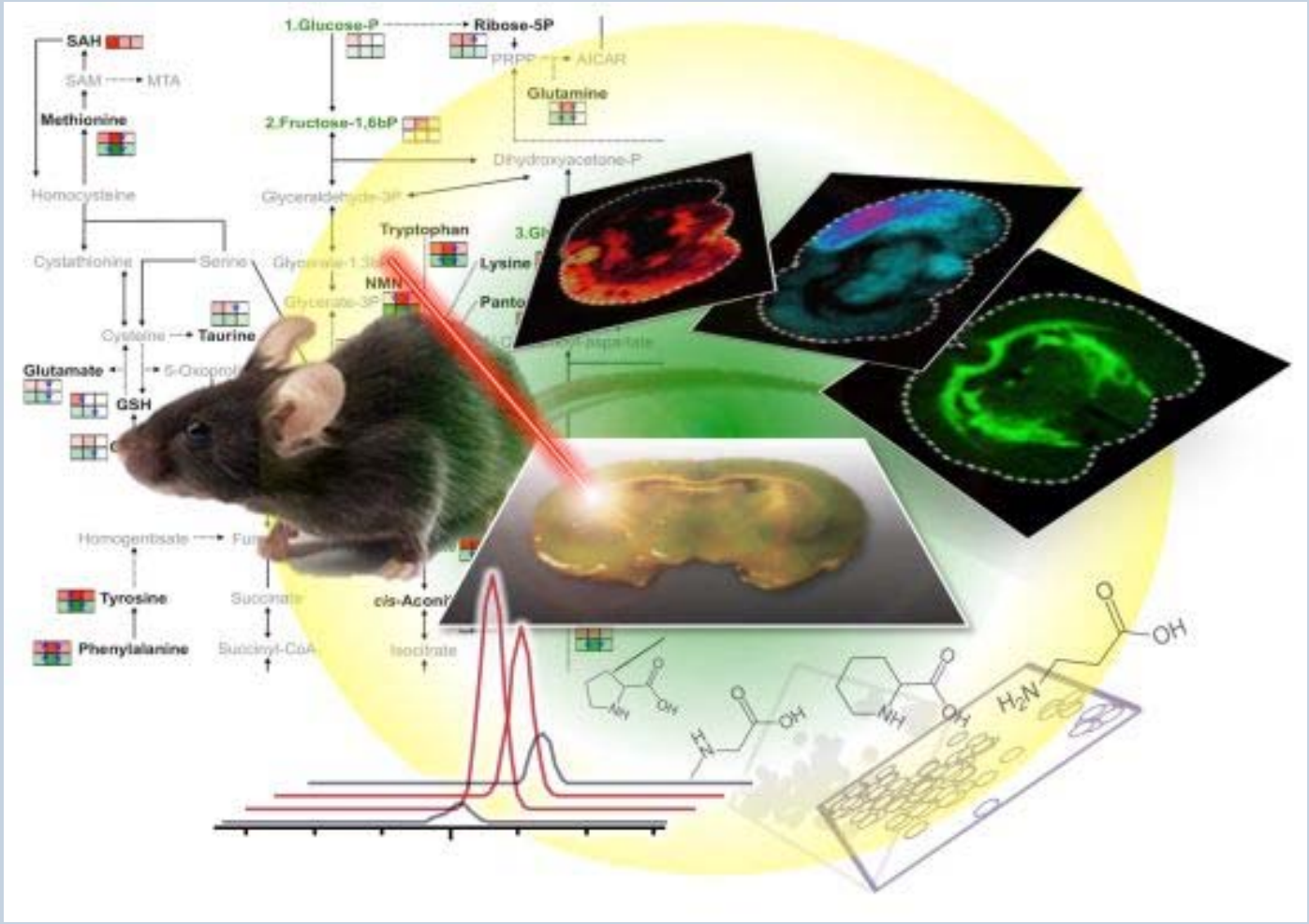
MALDI Imaging



Yoshinori Fujimura and Daisuke Miura. **MALDI Mass Spectrometry Imaging for Visualizing *In Situ* Metabolism of Endogenous Metabolites and Dietary Phytochemicals.** *Metabolites* **2014**, *4*(2), 319-346; doi:10.3390/metabo4020319.

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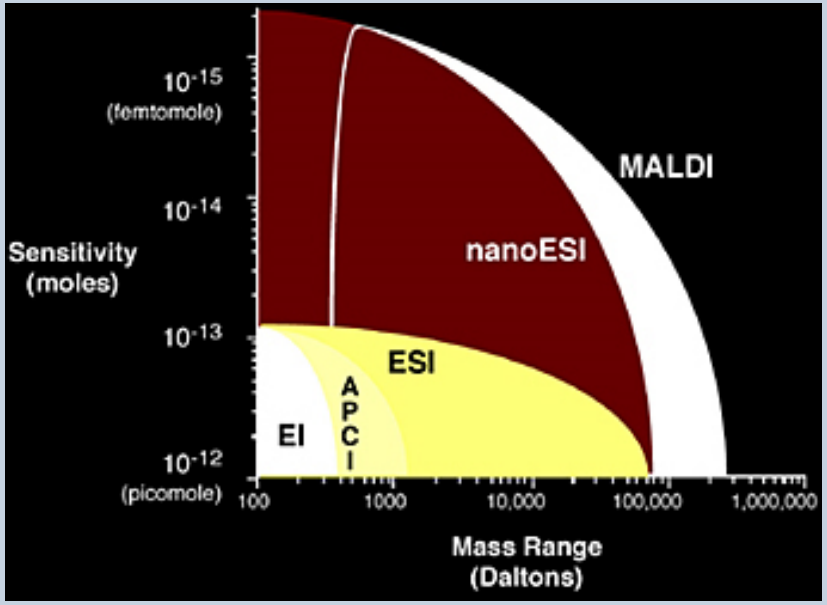
Sensitivity of the different ions sources

Mass spectrometer sensitivity is mostly determined but ionisation efficiency



Ion-source	Sensitivity	Mass range (Da)	Hard or soft
EI	Picomole	0- 750	Hard
ESI	picomole	100-100,000	Soft
Nano-ESI	Femtomole	100-100,000	Soft
MALDI	Femtomole	500-500,000	Soft
NMR	micromolar		

1 femtomole concentration is 1 quadrillionth of a mole!

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Summary of Ionisation Methods

Table 1: Summary of ionisation modes

	Ions formed in a vacuum		Ions formed at atmospheric pressure			Ions formed in vacuum or atmospheric pressure
Ionisation mode	**Electron Ionisation (EI)**	**Chemical Ionisation (CI)**	**Electrospray Ionisation (ESI)**	**Atmospheric Pressure CI (APCI)**	**Atmospheric Pressure Photoionisation (APPI)**	**Matrix-Assisted Laser Desorption/Ionisation (MALDI)**
Types of compound	Non-polar, and moderately polar species, *e.g.* hydrocarbons, aromatics *etc.* Molecule must be volatile and thermally stable.	As for EI. Increased chance of detecting a molecular ion. Appropriate choice of reagent gas is required.	Any compound sufficiently basic (in gas phase) to accept a proton or other cation (positive mode), or sufficiently acidic to lose a proton (negative mode).	Many compounds which will not ionise by ESI will be protonated by APCI as stronger gas phase acids are present in source.	Optimised for non-polar compounds. New technique – range of applications being evaluated.	Wide range, from non-polar to ionic, can be analysed. Good for large molecules.
Nature of ionising mechanisms	Loss of electron leads to radical cation. Excess internal energy may result in significant fragmentation.	+ve ion: reaction with ionised reagent gas (*e.g.* ammonia or methane). Ionisation mostly by cation attachment. -ve ion: electron capture or anion attachment.	+ve ion: addition of cation (*e.g.* H^+, Na^+, NH_4^+). -ve ion: loss of proton or anion attachment. Molecular clusters are common.	+ve ion: addition of proton most common. -ve ion: electron capture.	+ve ion: addition of proton. -ve ion: electron capture.	+ve ion: radical cation or addition of proton. Molecular clusters also formed. -ve ion: electron capture or loss of proton.
Typical ions observed	+ve ion: $M^{+\cdot}$, $[M - H]^+$ -ve ion: EI not effective in negative mode	+ve ion: $[M + H]^+$, $[M + NH_4]^+$ -ve ion: $M^{-\cdot}$	+ve ion: $[M + H]^+$, $[M + Na]^+$, $[M + nH]^{n+}$ -ve ion: $[M - H]^-$, $[M + X]^-$	+ve ion: $[M + H]^+$ -ve ion: $M^{-\cdot}$, $[M - H]^-$	+ve ion: $M^{+\cdot}$, $[M + H]^+$ -ve ion: $M^{-\cdot}$	+ve ion: $M^{+\cdot}$, $[M + H]^+$, $[M_2 + H]^+$, $[M + 2H]^{2+}$ -ve ion: M^-,$\cdot[M - H]^-$
Fragmentation	Significant fragmentation. Very informative about structure of molecule.	Much less fragmentation than with EI; more likely to observe an ion closely related to the original molecule.	Low energy process; few fragments. Greater fragmentation by MS-MS or increased source voltages.	As for ESI.	As for ESI.	Little or no fragmentation. Greater fragmentation requires MS-MS.
Sample introduction	Directly from a temperature controlled probe or *via* a GC column.	As for EI.	Sample must be dissolved in an appropriate solvent.	As for ESI.	As for ESI.	Sample needs to be applied in an appropriate matrix.
Typical solvent	GCMS requires volatile non-polar solvent. GC injection temperature can dictate choice. Insoluble samples can be introduced as a solid if compound is sufficiently volatile and thermally stable.	As for EI.	Mixture of water/organic solvent with optional addition of electrolyte (*e.g.* formic acid or ammonium acetate); frequently typical reversed phase HPLC gradient mixtures. Solvent choice may be critical.	As for ESI. Also, hydrocarbon/alcohol mixtures as for normal phase HPLC.	As for APCI.	Solvent from which sample will form crystalline mixture with matrix. Choice of matrix and sample preparation can be critical.

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