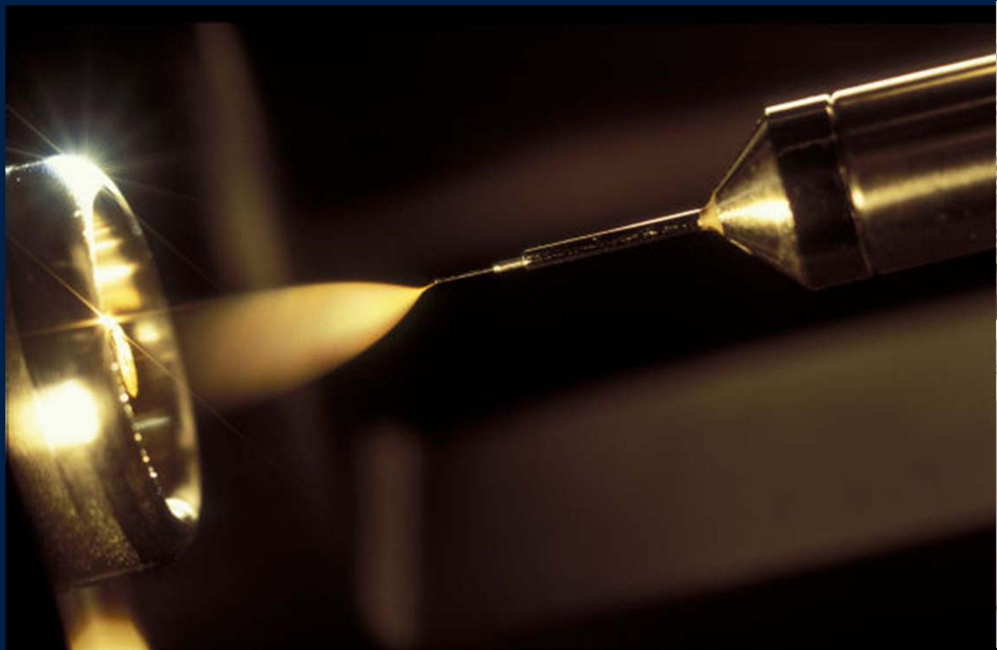
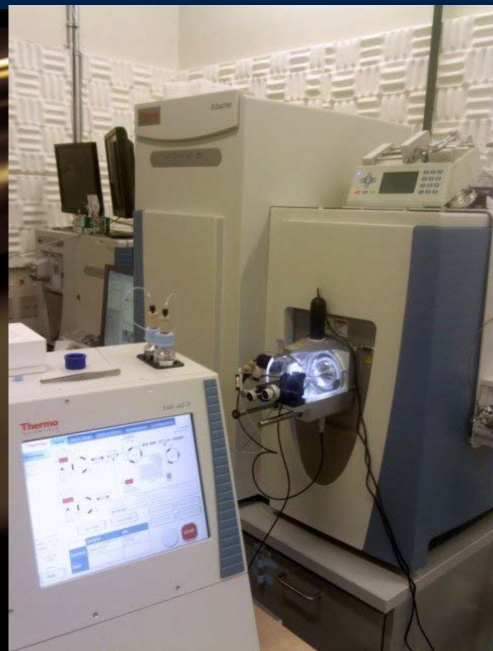
DEPARTMENT OF CHEMISTRY

UNIVERSITY OF OXFORD

GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 4

Ion Chemistry



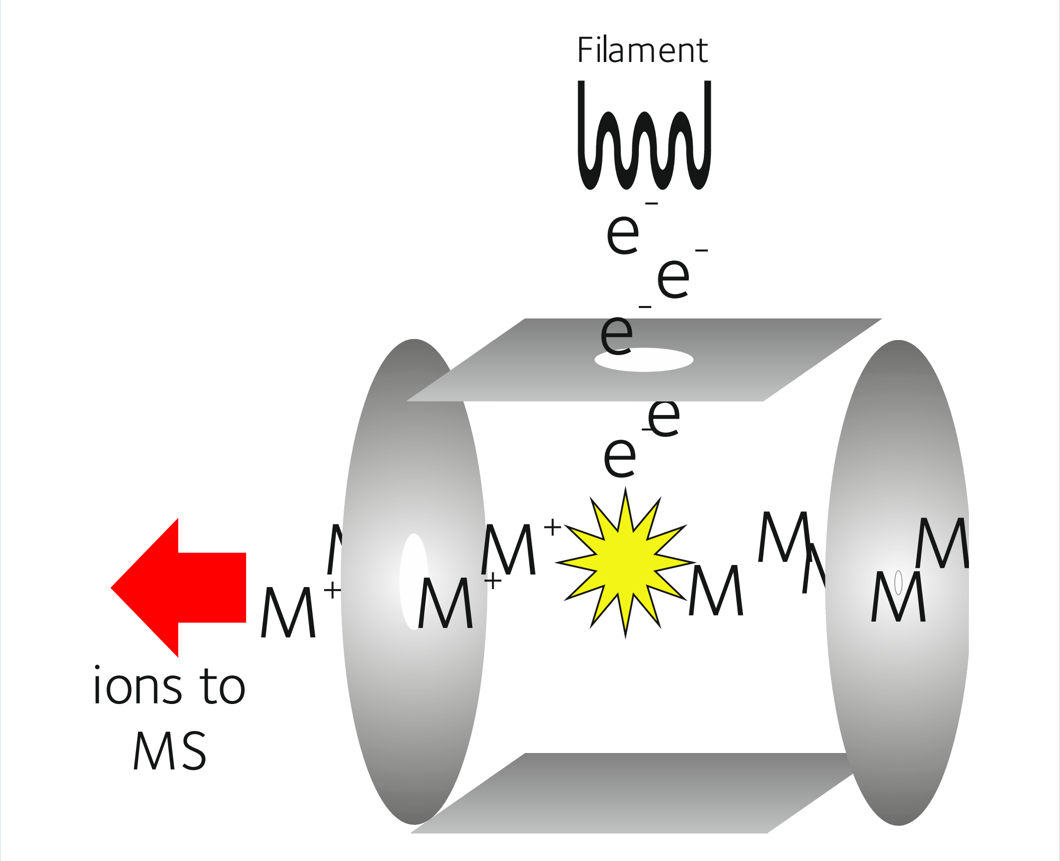


James Wickens, 4th November 2015

Ion Chemistry
Lecture overview

- Chemistry occurring in common mass spectrometry processes
  - EI
  - CI
  - MALDI
  - APCI
  - Electrospray
  - MS/MS
- Examples
  - Peptide modification
  - Salbutamol

Electron ionisation



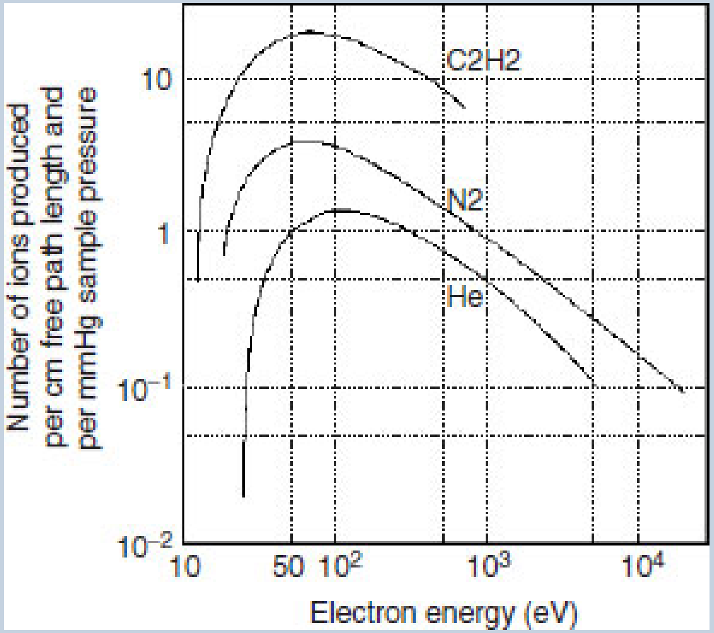
Electron ionisation – what's happening?

Various explanations

- Simple:
  - 70eV electron, <20eV ionisation potential

$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

- Increasingly complex:
  - 70eV → de Broglie λ 1.4Å
  - "when the wavelength is close to the bond length, the wave is disturbed and becomes complex"*
    - Low eV; below ionisation energy
    - High eV; molecules become transparent



Issues

- How does this apply to monoatomic ions
- Recent developments in low eV instruments

*Hoffmann & Stroobant: Mass Spectrometry: Principles and Applications.

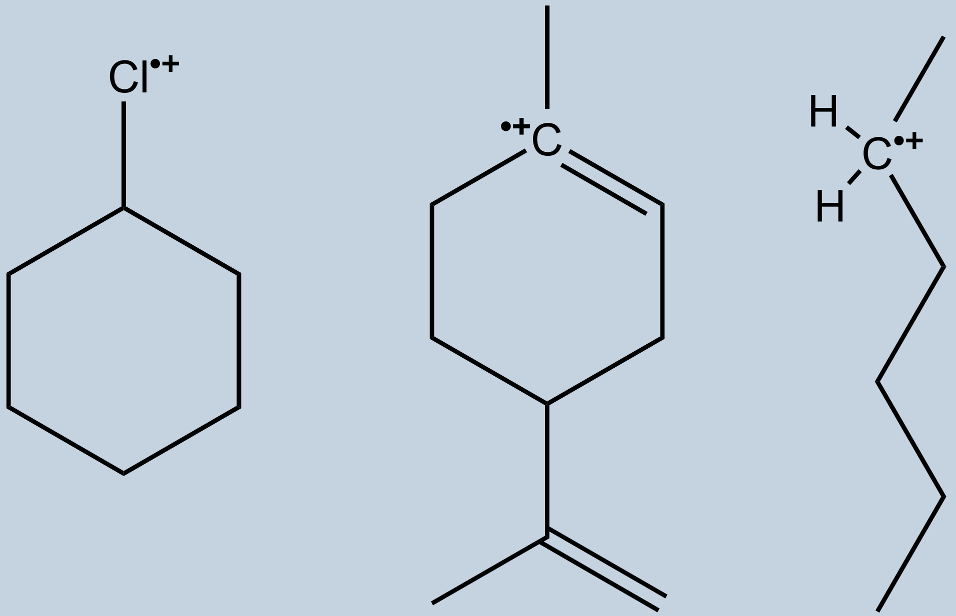
Electron ionisation chemistry

- Extensively studied and well understood
- Less studied over recent years
- Provides a good window into MS ‘chemistry’
    - Some of the ‘rules’ of EI

EI 'chemistry' 1: Which electron?

Highest
Occupied
Molecular
Orbital

n > π > σ

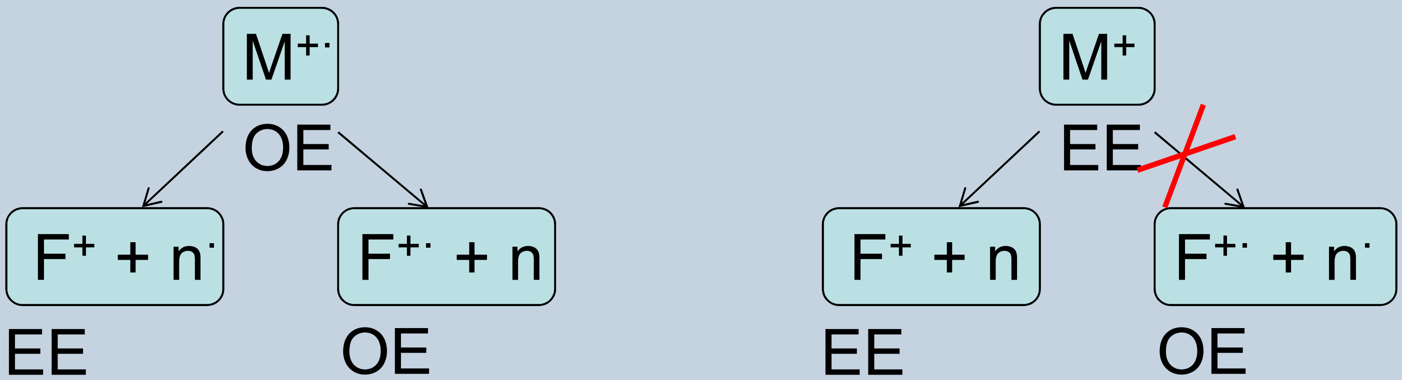


Site of the weakest ionisation potential

EI 'chemistry' 2: Count your electrons

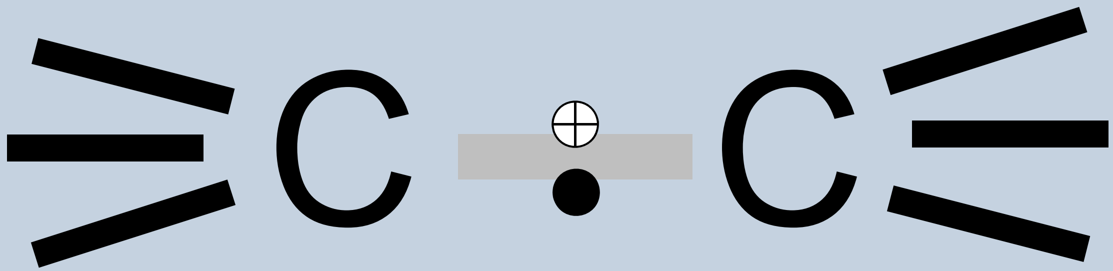
- $M^{+\cdot}$ is a *radical* cation
  - Therefore it has an odd number of electrons
  - An ODD ELECTRON species (OE)
    – Un-stabilised charge AND unpaired electron

- Fragmentation: High energy → low energy



EI Chemistry 3: σ-bond cleavage

- Also know as direct dissociation



EI Chemistry 3: σ-bond cleavage

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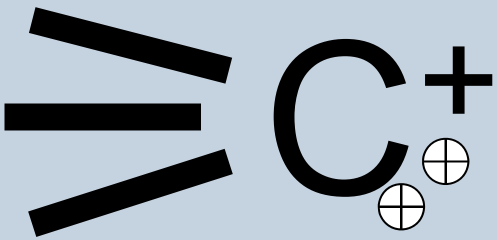
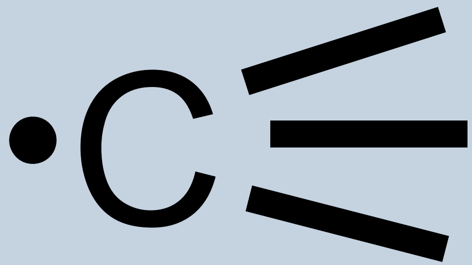
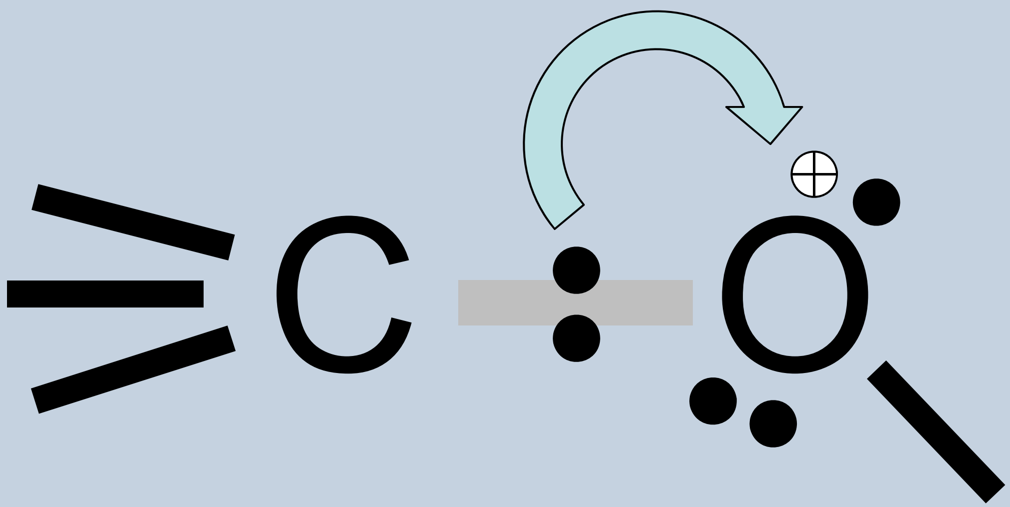


Even
electron
ion

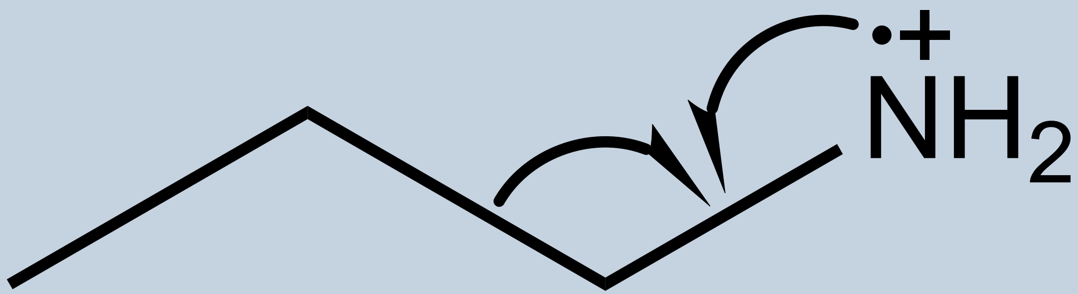


The radical formed is the one that would has the highest ionisation potential

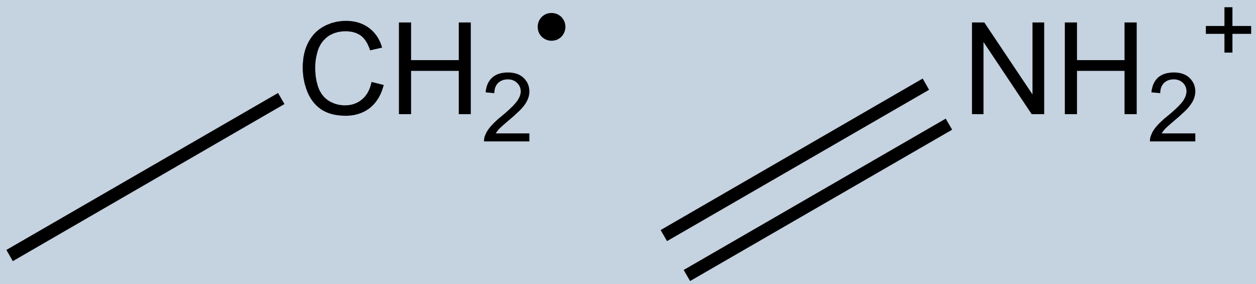
EI Chemistry 3: σ-bond cleavage



EI Chemistry 4: α-bond cleavage



EI Chemistry 4: α-bond cleavage

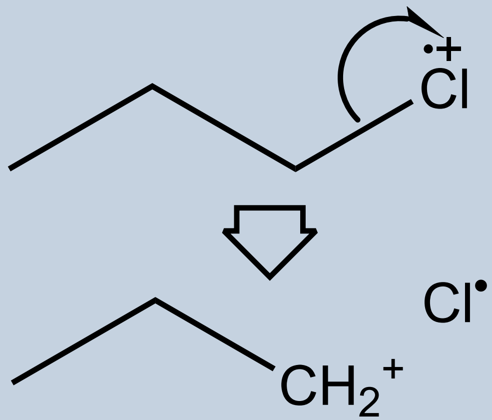


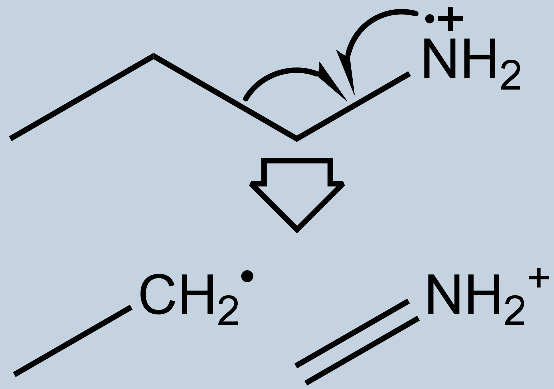
Even electron ion

EI Chemistry 3/4: α-bond vs adjacent bond

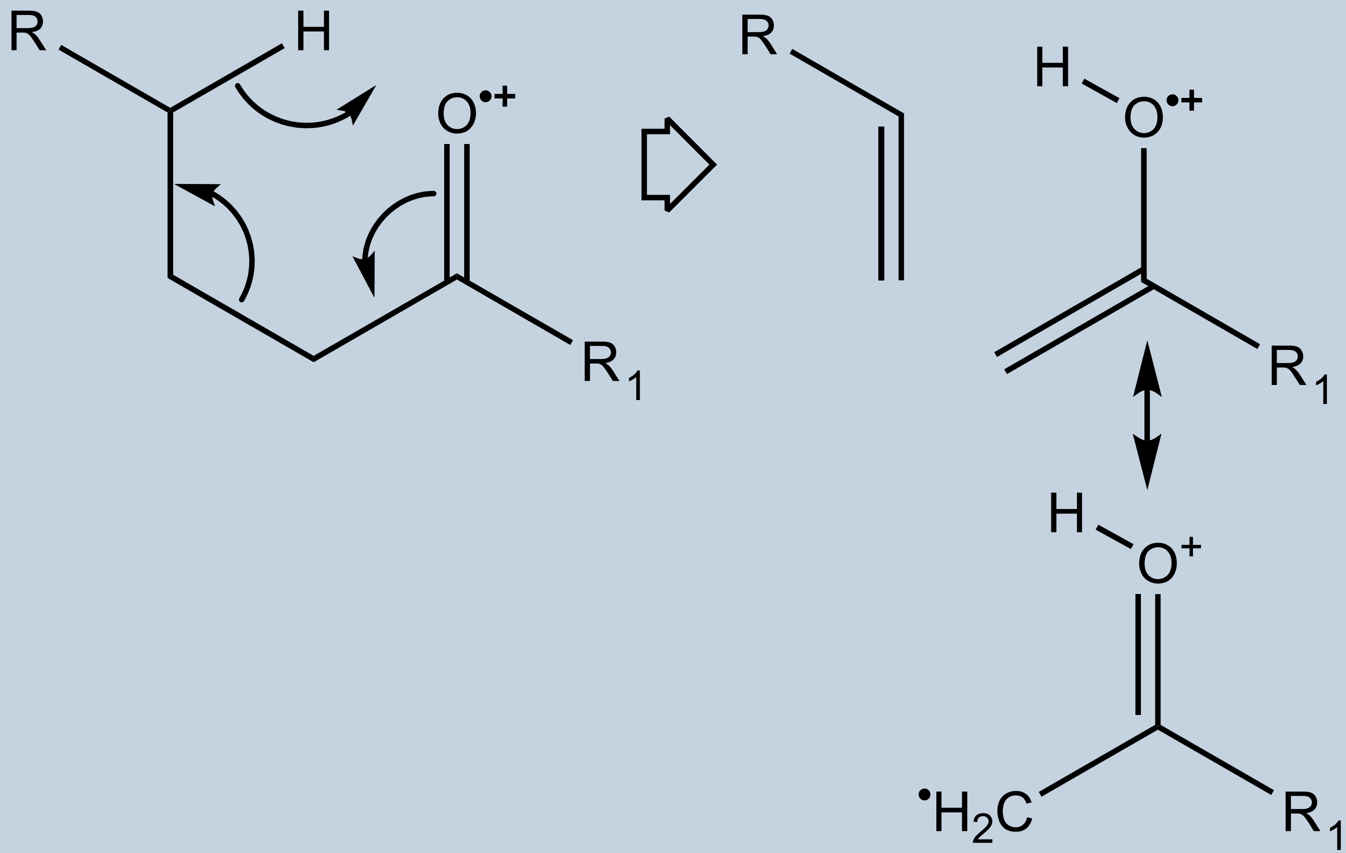
- The more electronegative the hetero atom, the more easily the adjacent bond will break

[σ-bond cleavage] Halogen > π, S, O > N [α-bond cleavage]



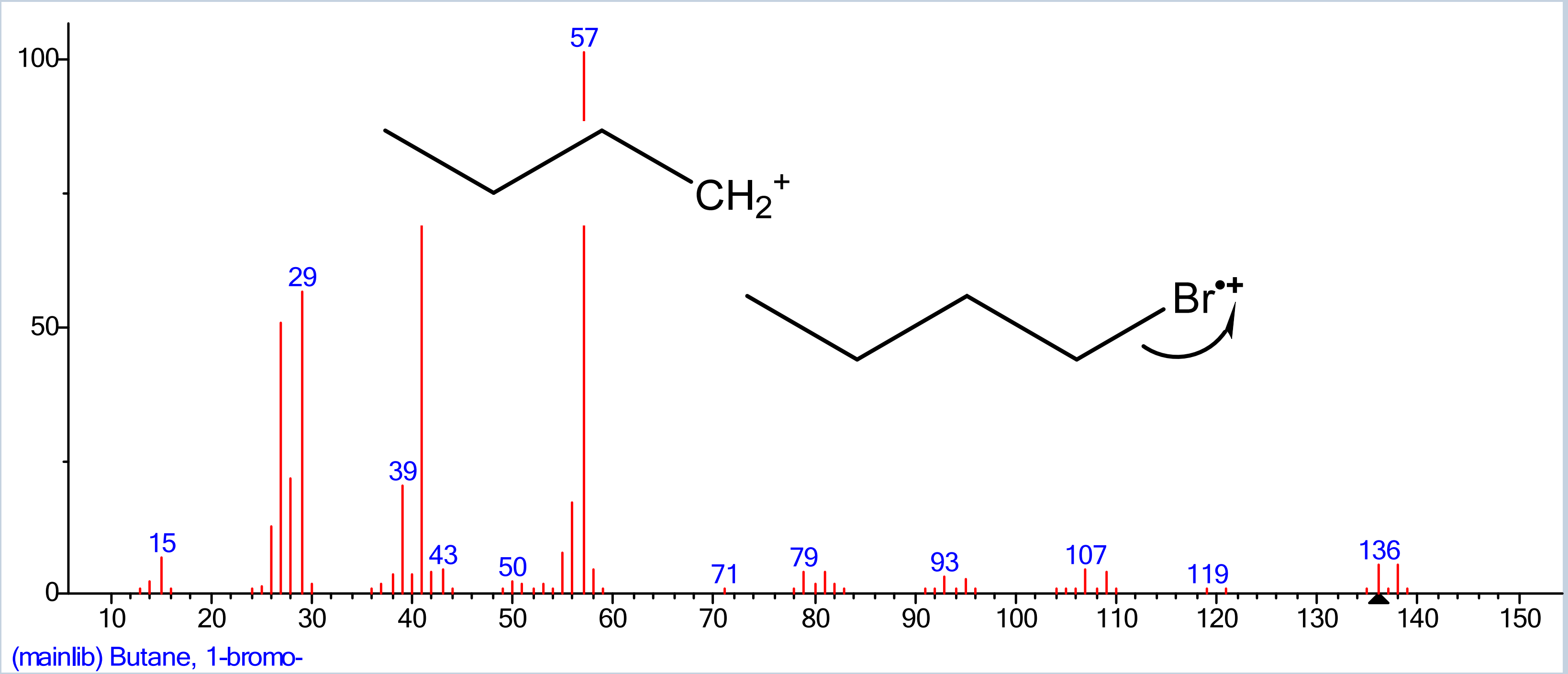


EI Chemistry 5: rearrangement (example)

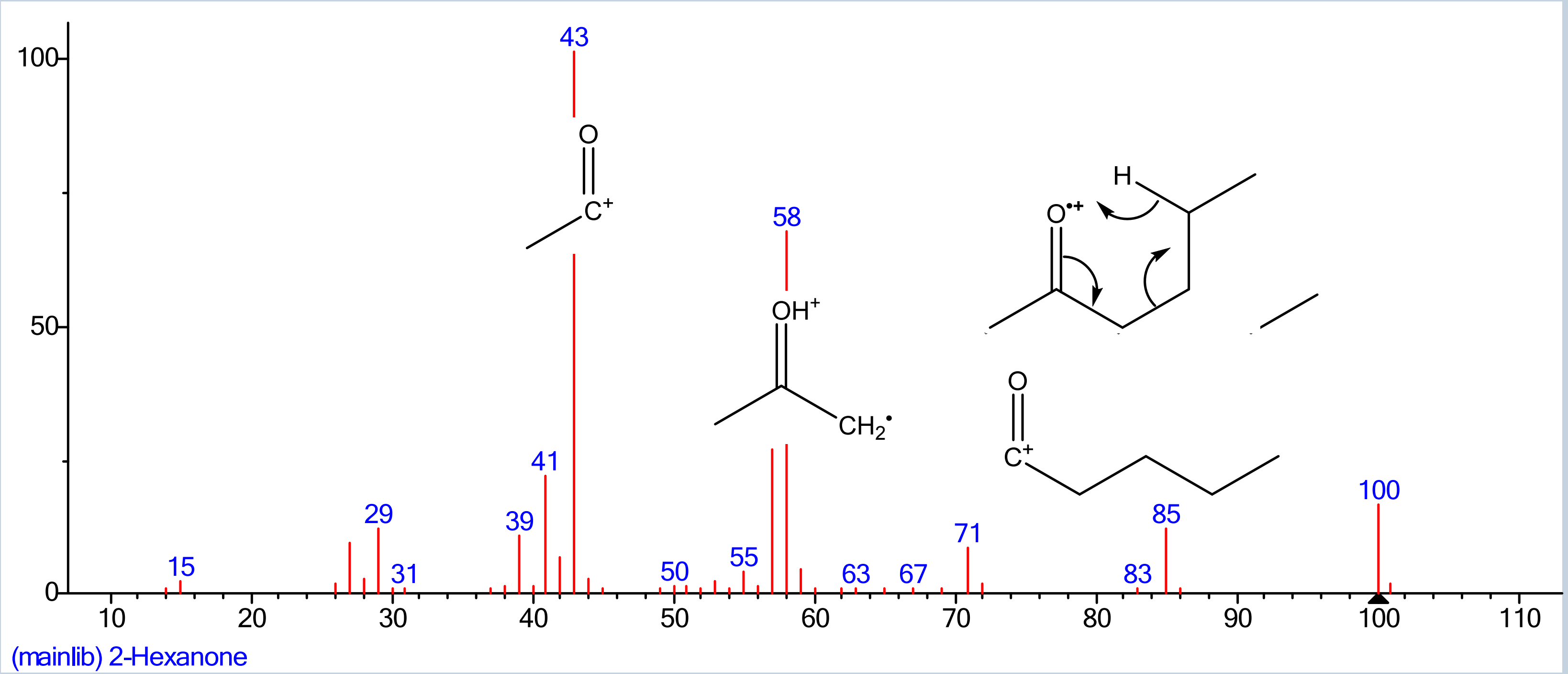


F. W. McLafferty. Anal. Chem., 1959, 31 (1), pp 82–87

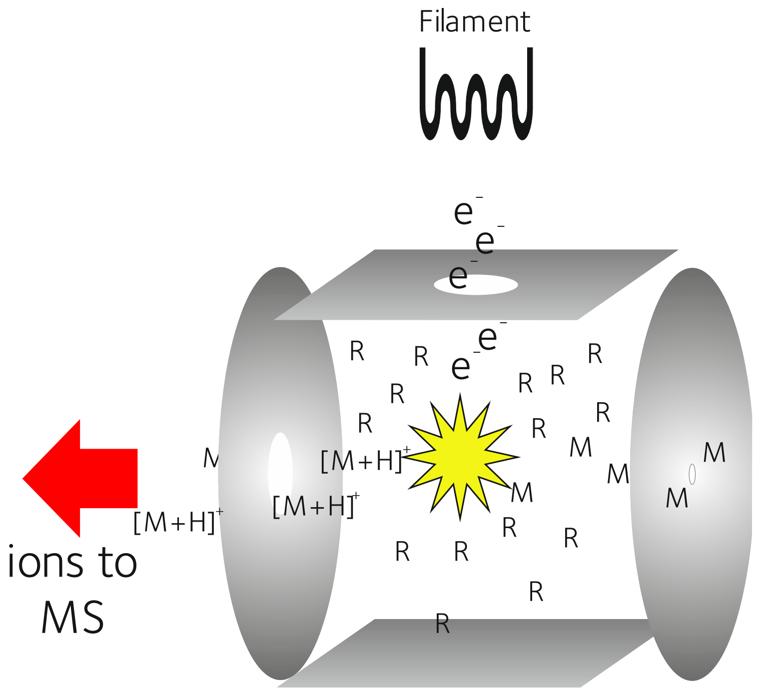
EI Chemistry: Spectra
1-bromobutane



EI Chemistry: Spectra
Hexanone



Chemical ionisation



Chemical ionisation

- Introduce a moderate pressure of reagent gas into the source
  - Common reagent gasses: Methane, isobutane, ammonia
  - Typically less than 1torr, massive excess in comparison to sample pressures

- Reagent molecules are ionised and react with other reagent molecules in a cascade of reactions:
  - eg. $CH_4^{+\cdot} + CH_4 \rightarrow CH_5^{+} + CH_3^{\cdot}$

- These product then typically ionise analyte molecules *via* proton transfer reaction
  - $CH_5^{+} + M \rightarrow CH_4 + [M+H]^{+}$

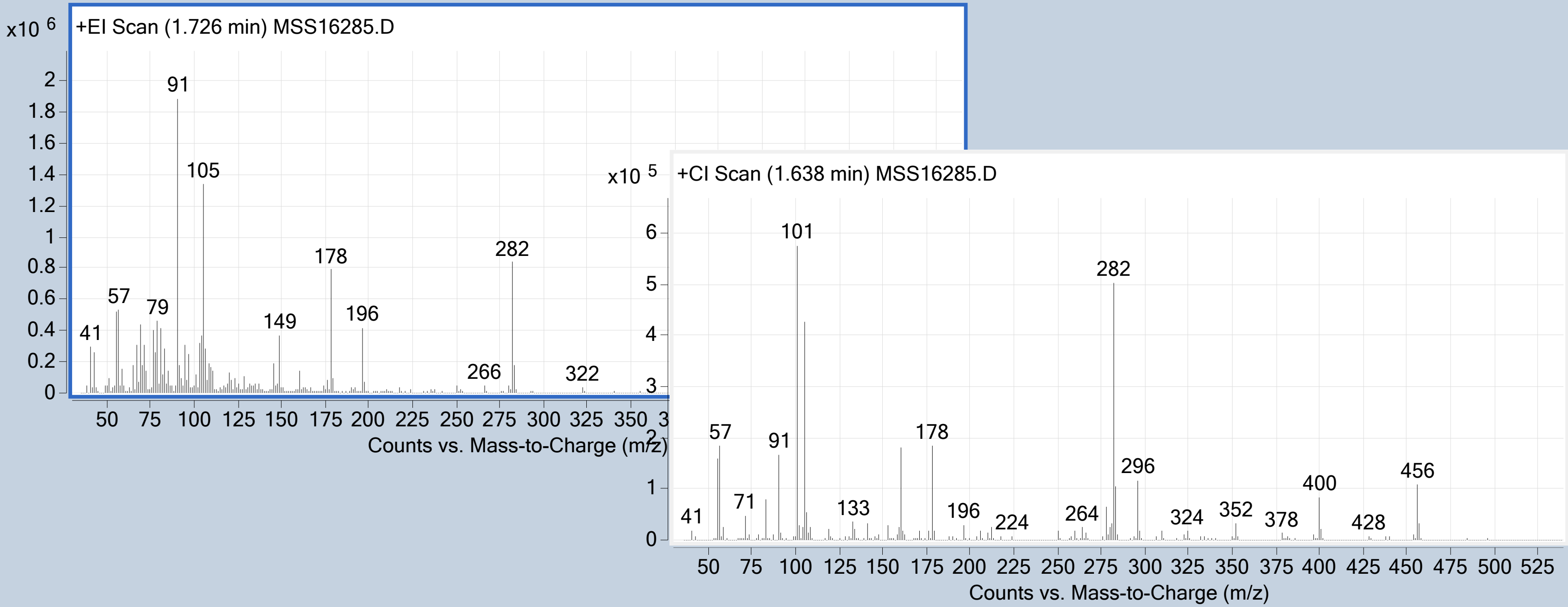
- The dominant factor is the proton affinity of the analyte

Nomenclature interlude

- The term ‘Molecular ion’ has a specific definition.
  → radical ions
- $[M+H]^+$, $[M-H]^-$, $[M+Na]^+$, $[M+NH_4]^+$, $[M^+]$ ions are not molecular ions
  - → more appropriate terms include:
  - Pseudomolecular ion
  - Protonated ion $[M+H]^+$
  - Deprotonated ion $[M-H]^-$
  - Ion of the molecular species

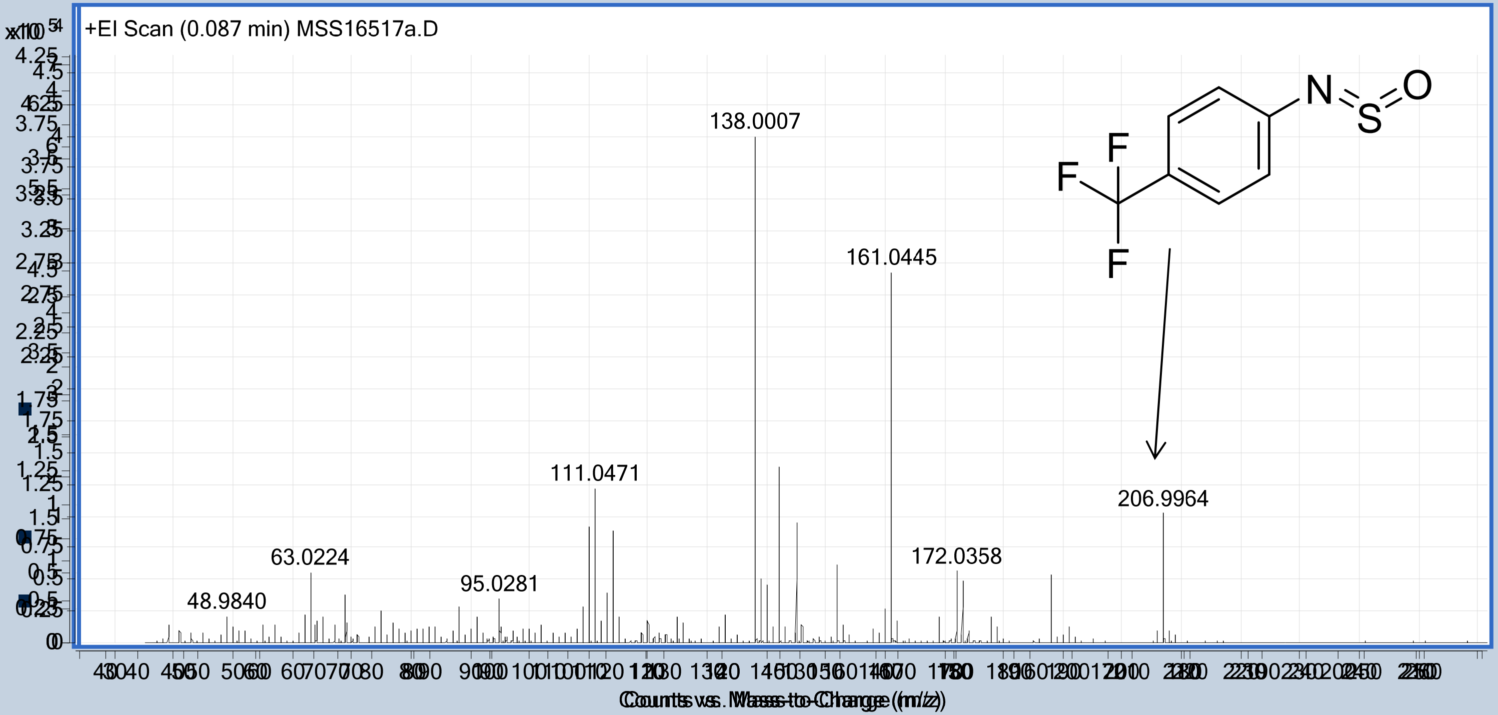
Chemical ionisation
What are the benefits

- Energy transfer in CI is far lower than in EI.
- Higher chance of generating an 'ion of the molecular species'

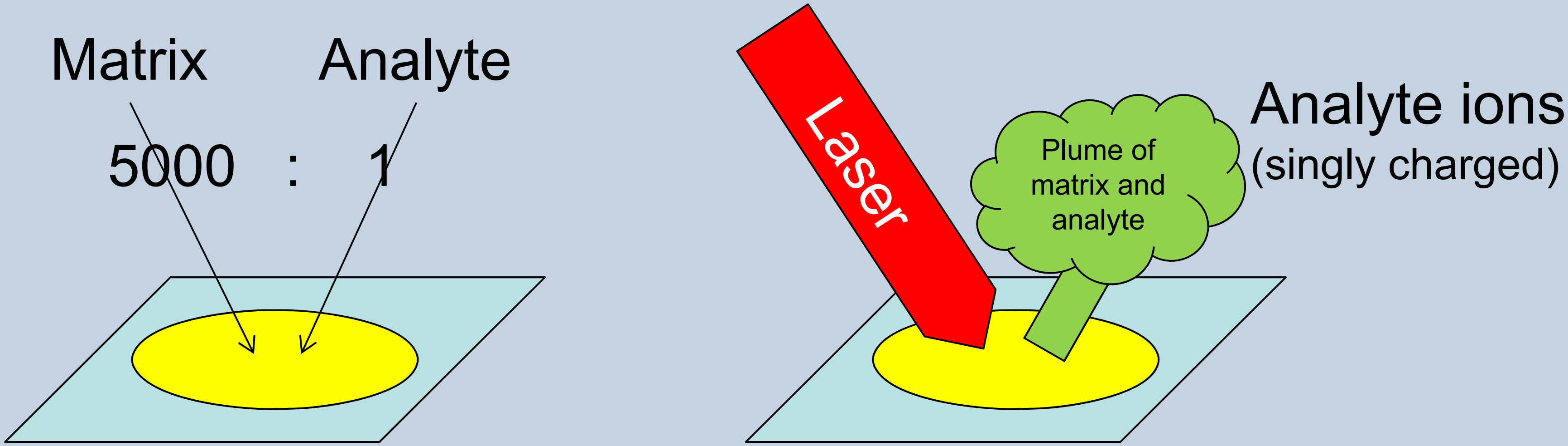


Chemical ionisation
Caveat!

- Chemical ionisation is chemistry!

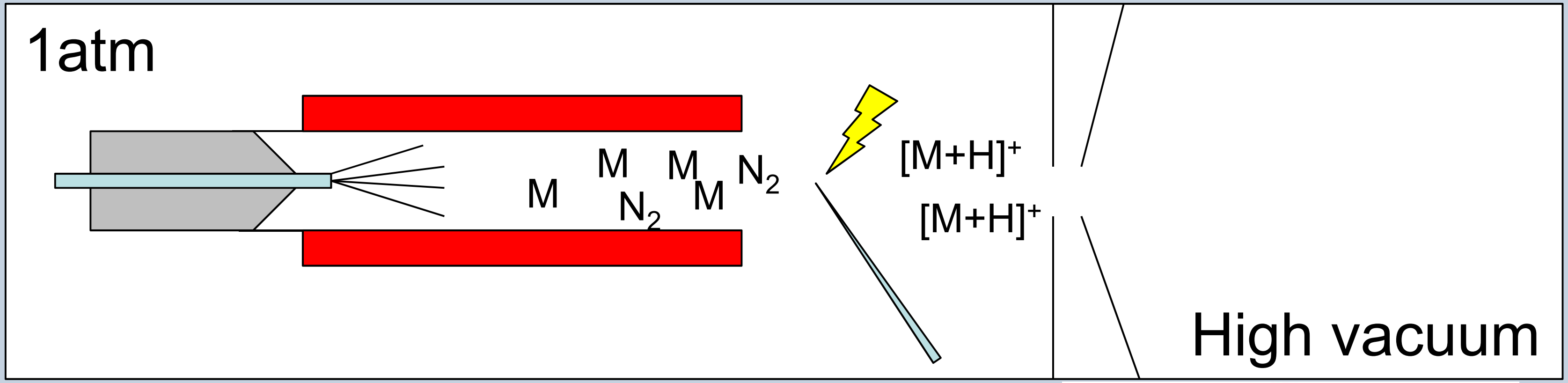


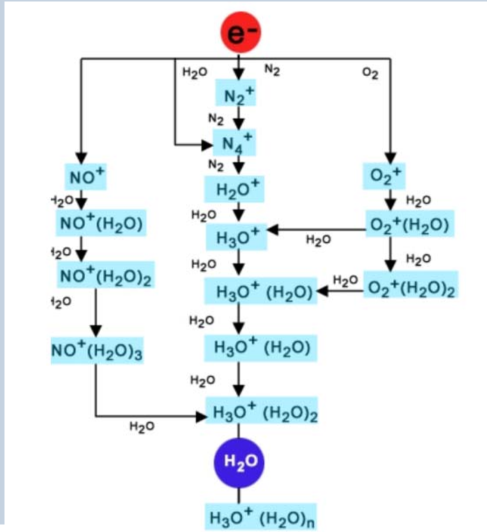
MALDI



Matrix
- UV absorbing
- Proton donor/acceptor (TFA/amine)
- Charge transfer electron acceptors (napthene)

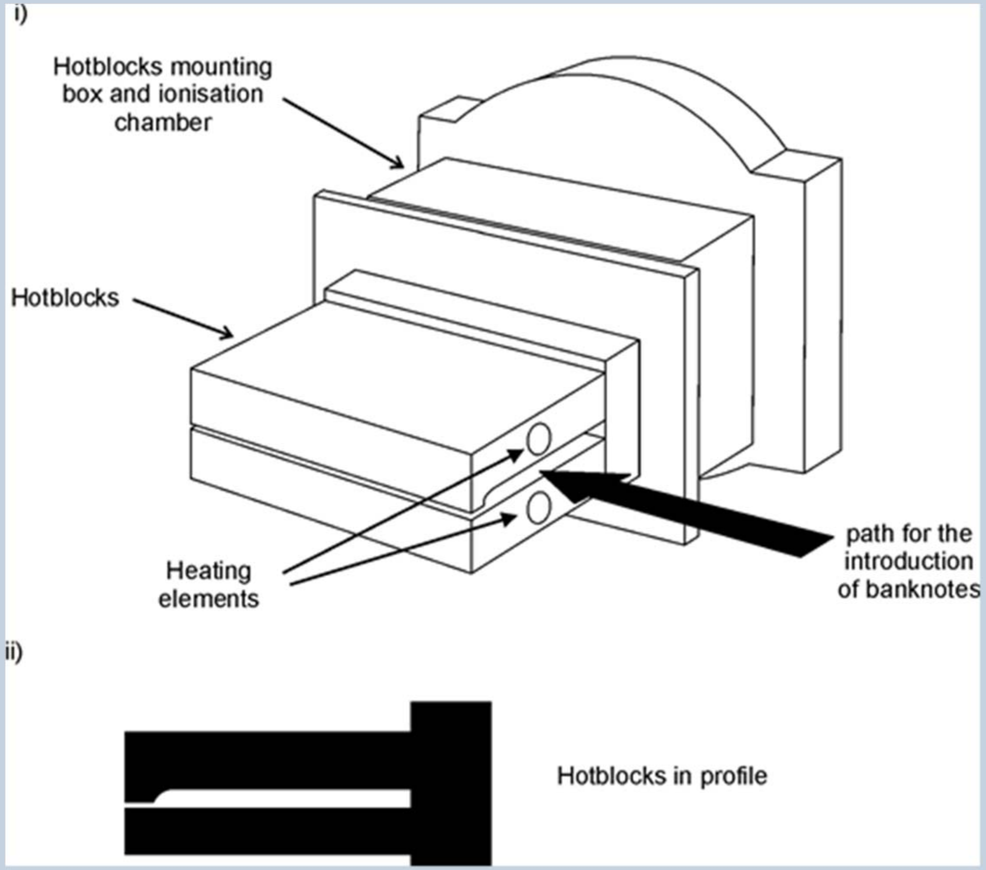
APCI



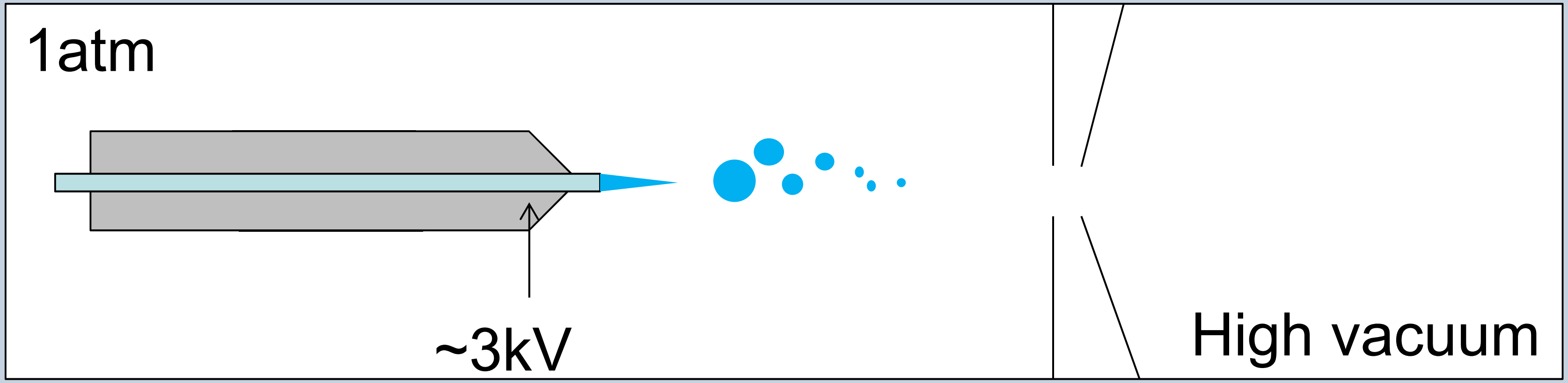


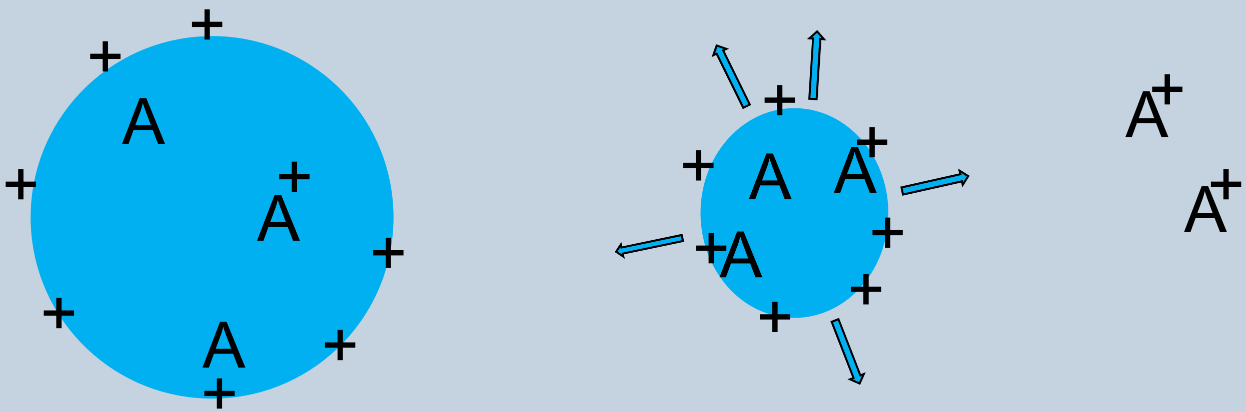
- Very analogous to CI but the reagent gas is an mixture of N_2 and solvent
  - Typically $[M+H]^+$ ions in positive mode
  - Negative mode, $[M-H]^-$ and adducts eg. $[M+Cl]^-$

APCI
"solvent free"

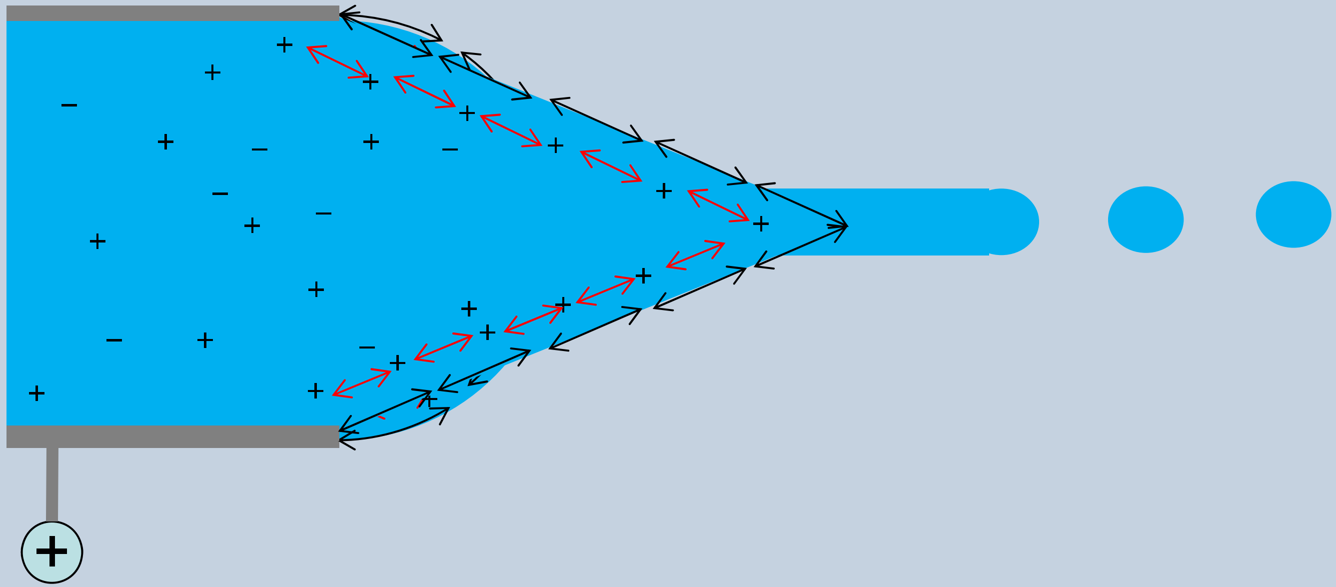


Electrospray

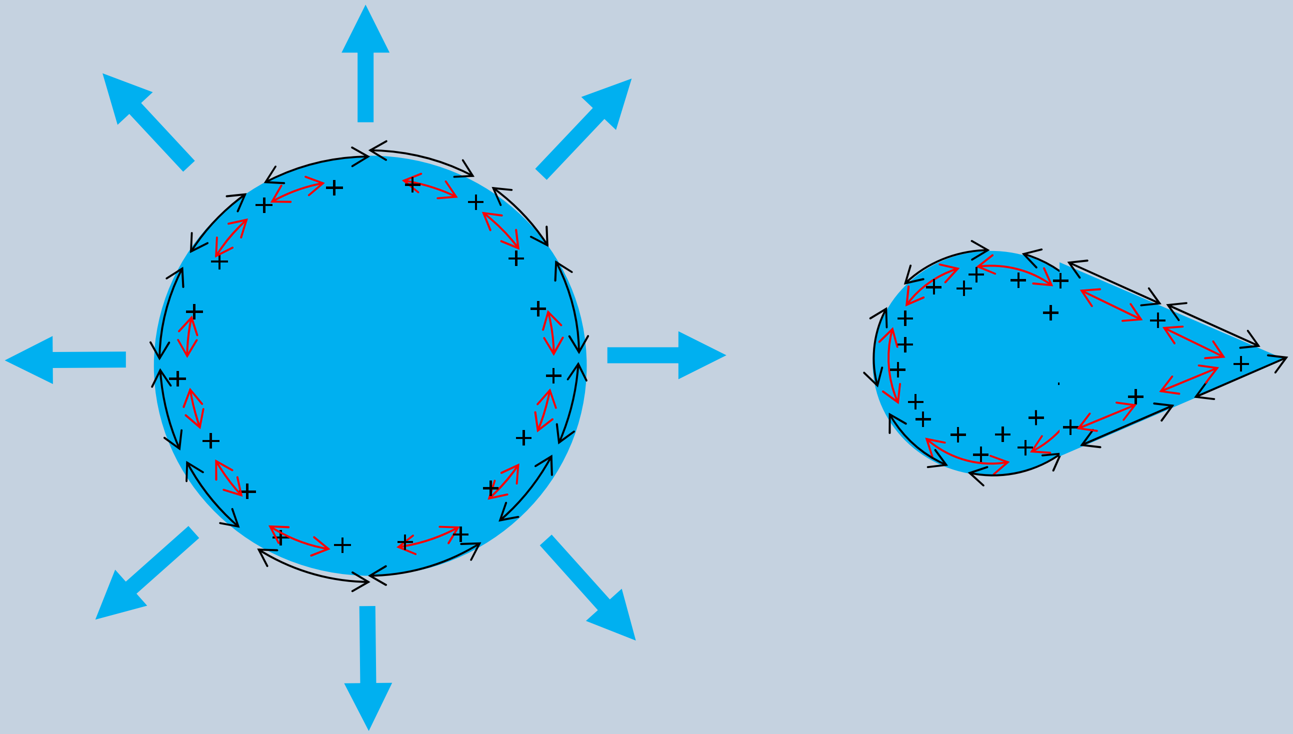




Electrospray



electrospray

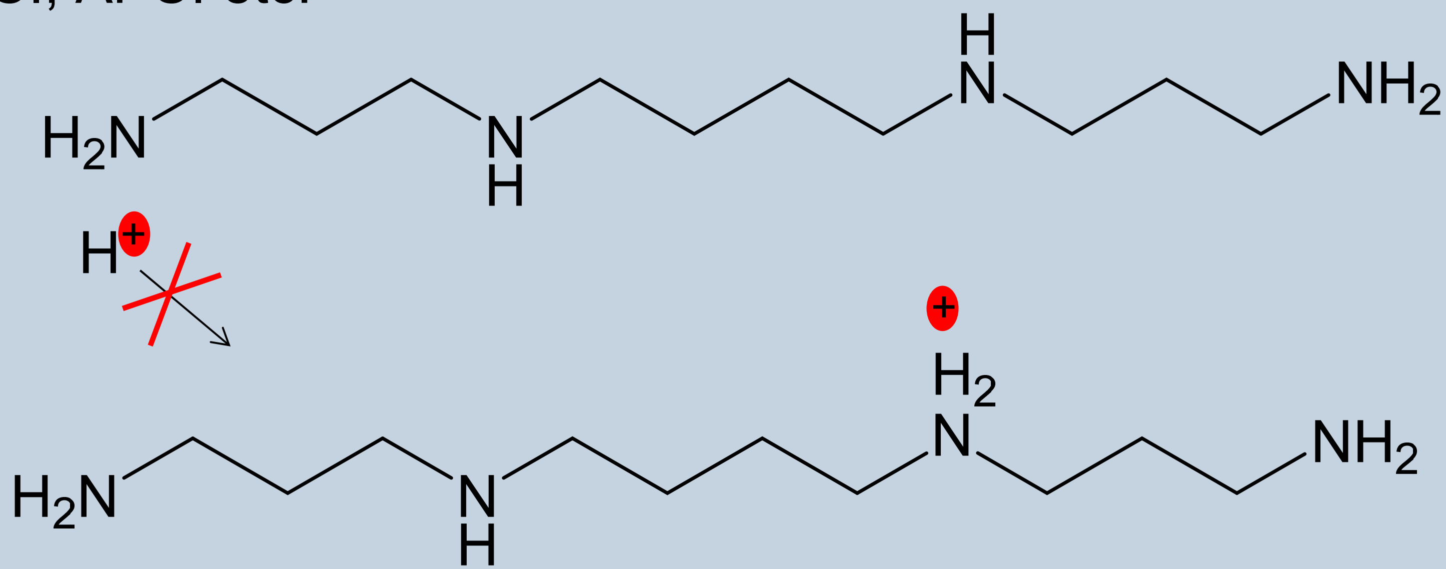


Electrospray Mechanisms

- Charged residue model (CRM)
  - Extremely small droplets
    - One analyte
    - Surface charge(s)
  - Solvent evaporates to leave charged residue
- Ion evaporation model (IEM)
  - Droplets get to a certain size
  - Ions are ejected directly from the droplet surface
- Additional processes in the gas phase
  - cf. CI and APCI

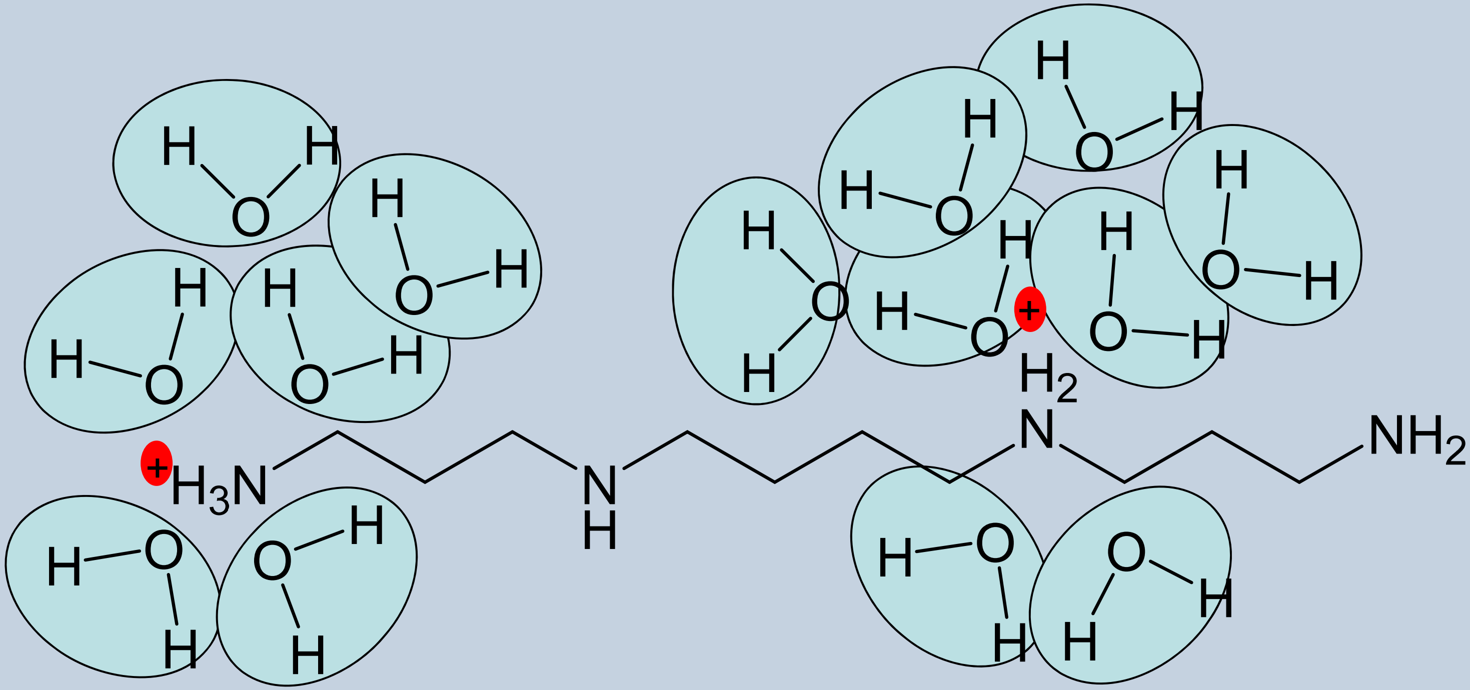
Electrospray
ES, not ESI?

- Multiple charging is possible in electrospray but not gas phase ionisation processes
- CI, APCI etc:



Electrospray
ES, not ESI?

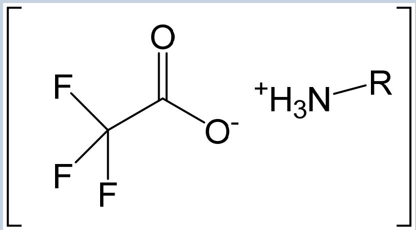
- Electrospray:



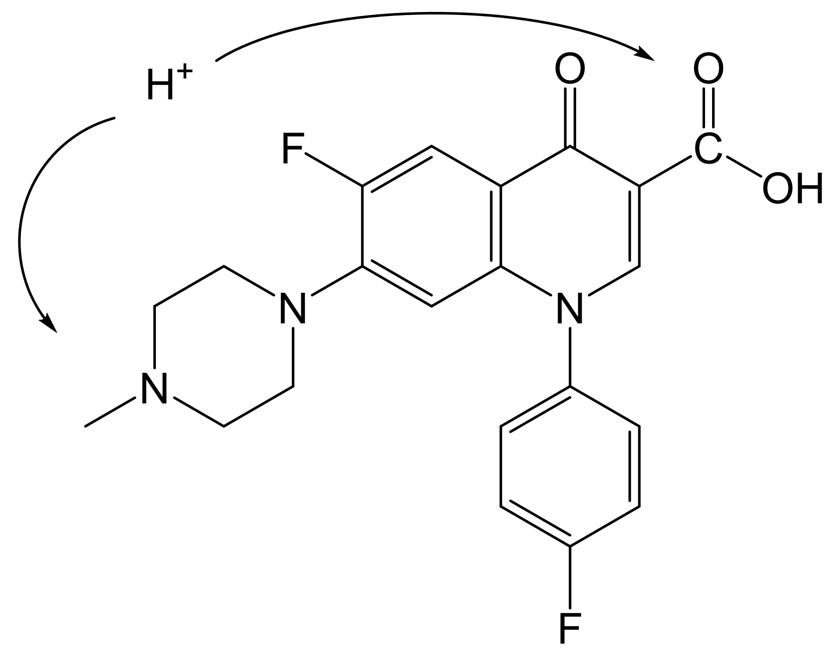
Electrospray additives

- Salt concentration and pH can have powerful effects on electrospray mass spectra
  - Adding ammonia can enhance deprotonation of weak acids
  - Weak acids can provide a ready source of protons boosting $[M+nH]^{n+}$ abundance

- BUT BEWARE – incorrect additives can cause serious problems in electrospray
  - Trifluoroacetic acid, TFA
    - Negative mode - deprotonates in preference to almost everything
    - Positive mode it forms ion pairs with cations



Notation interlude
Where do you put a proton?



- Difloxacin

A. Kaufmann, P. Butcher, K. Maden, M. Widmer, K. Giles, D. Uría. Rapid Commun. Mass Spectrom. 2009, 23, 985.
Cris Lapthorn, Trevor J. Dines, Babur Z. Chowdhr1, George L. Perkins, Frank S. Pullen*. Rapid Commun. Mass Spectrom. 2013, 27, 2399–2410

MS/MS

- Many ways of performing MS/MS:

CID

SID

BIRD

IRMPD

ECD

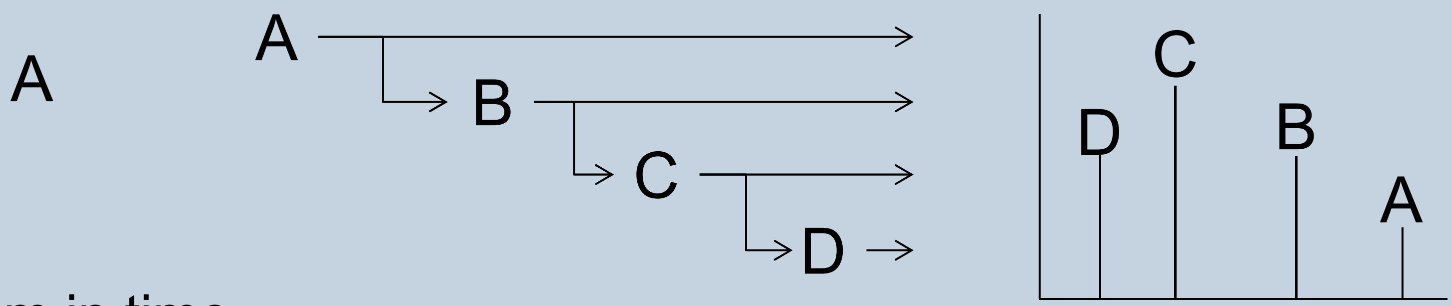
ETD

High energy CID

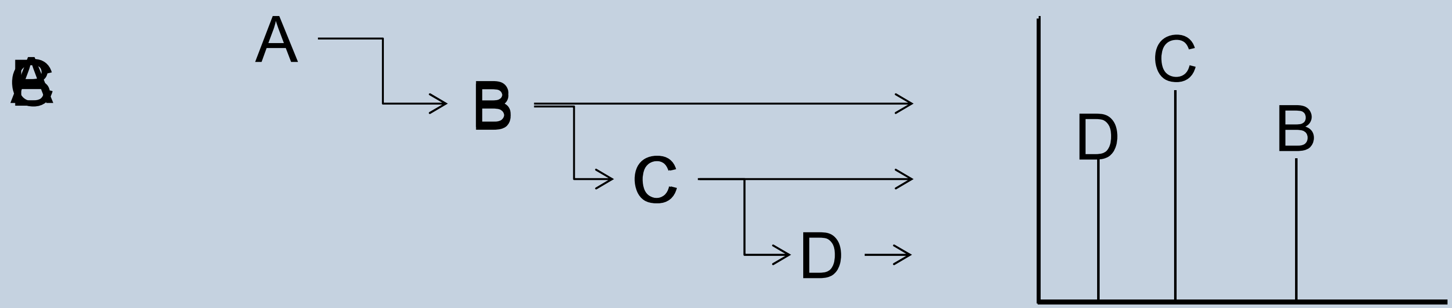
- We are going to focus on low energy CAD/CID

Tandem in time *vs.* tandem in space

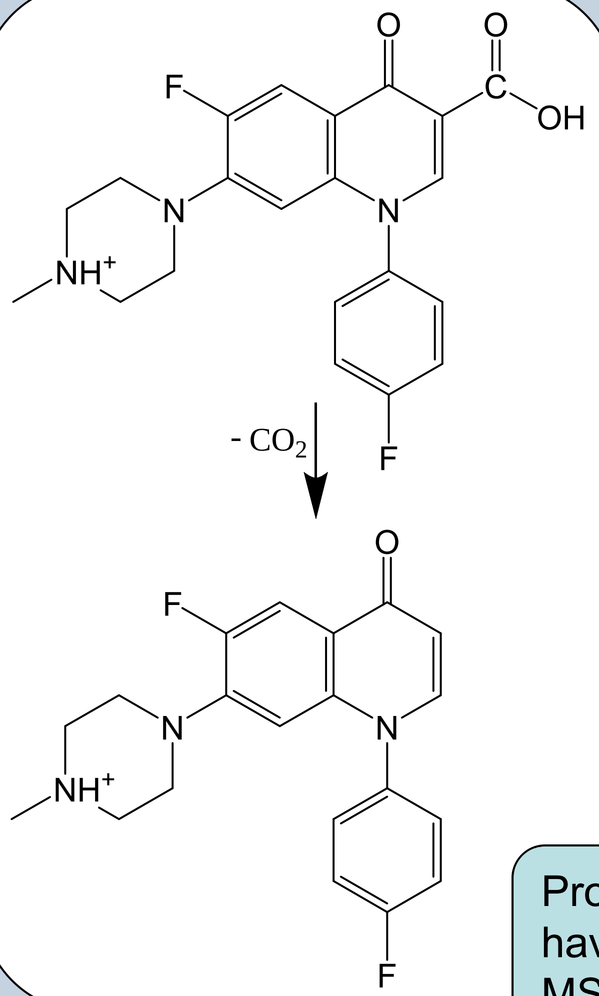
- Tandem in space

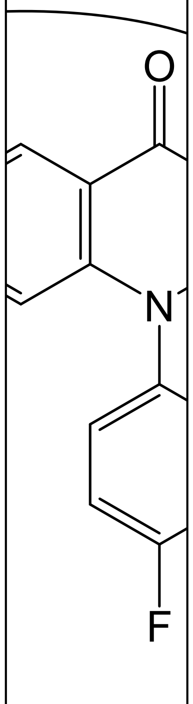


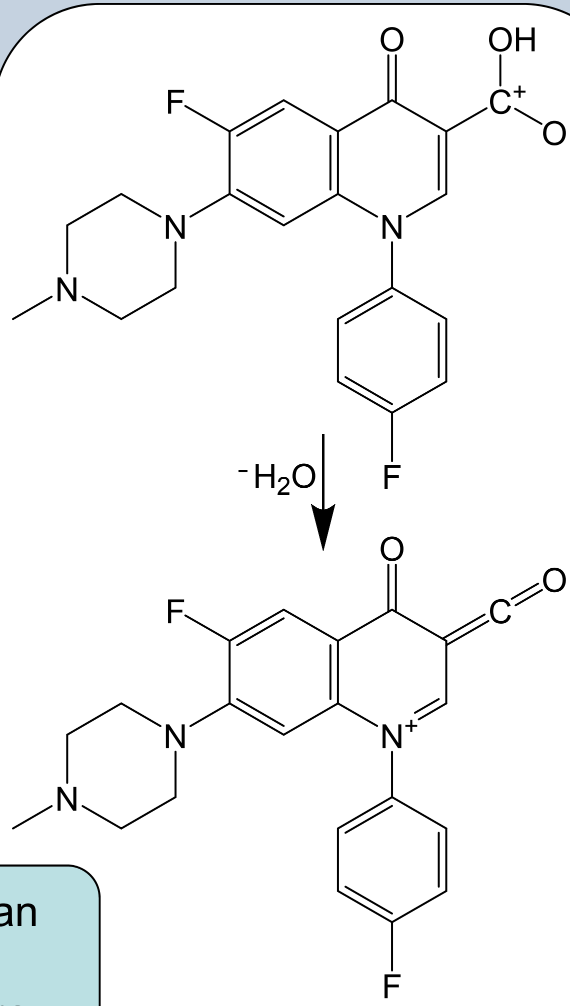
- Tandem in time



Where did the proton go?







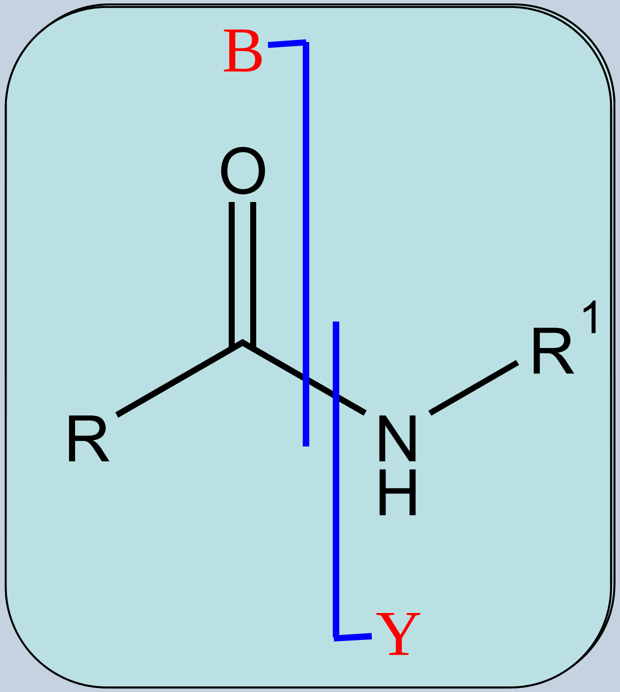
Proteomers can have different MS/MS spectra

A. Kaufmann, P. Butcher, K. Maden, M. Widmer, K. Giles, D. Uría. Rapid Commun. Mass Spectrom. 2009, 23, 985.
Cris Lapthorn, Trevor J. Dines, Babur Z. Chowdhr1, George L. Perkins, Frank S. Pullen*. Rapid Commun. Mass Spectrom. 2013, 27, 2399–2410

Examples

Example1
Peptide chemistry

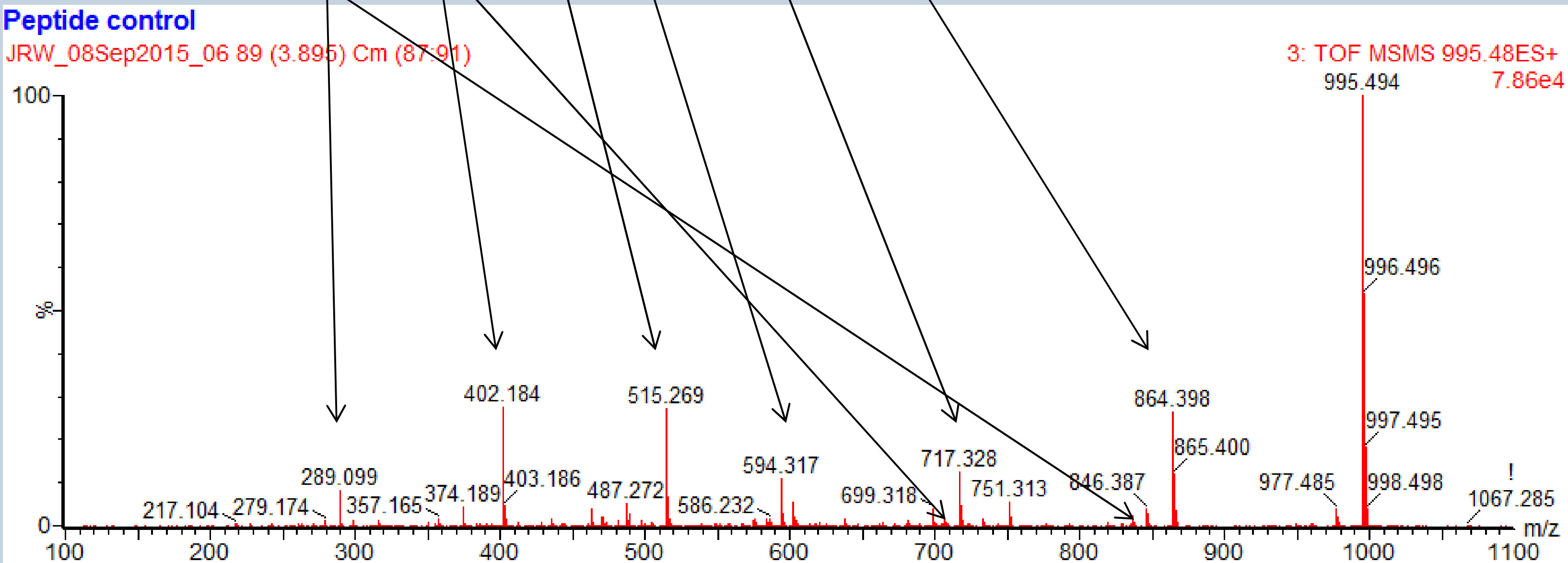
- Peptide bonds can break in a number of ways:



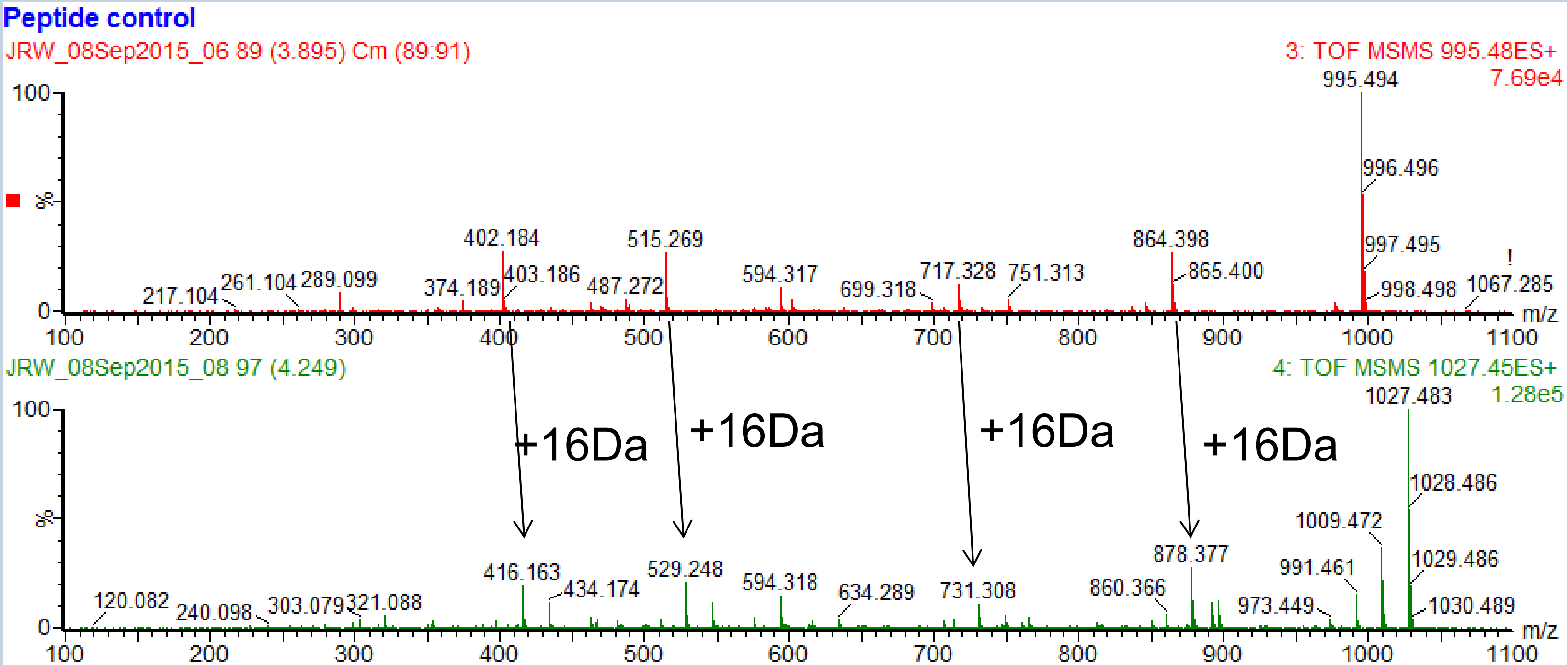
- But in (low energy) CID, the b and y ions dominate

Example1
Peptide chemistry

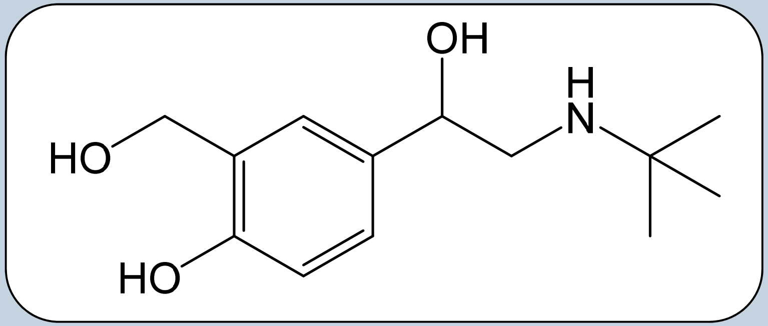
b	104.0	161.0	218.1	289.1	402.2	515.3	602.3	717.3	864.4	-
	1	2	3	4	5	6	7	8	9	10
	Cys	Gly	Gly	Ala	Ile	Ile	Ser	Asp	Phe	Ile
	10	9	8	7	6	5	4	3	2	1
y	-	890.5	833.4	776.4	705.4	592.3	479.2	392.2	277.2	130.1



Example1
Peptide chemistry



Example 2
Salbutamol



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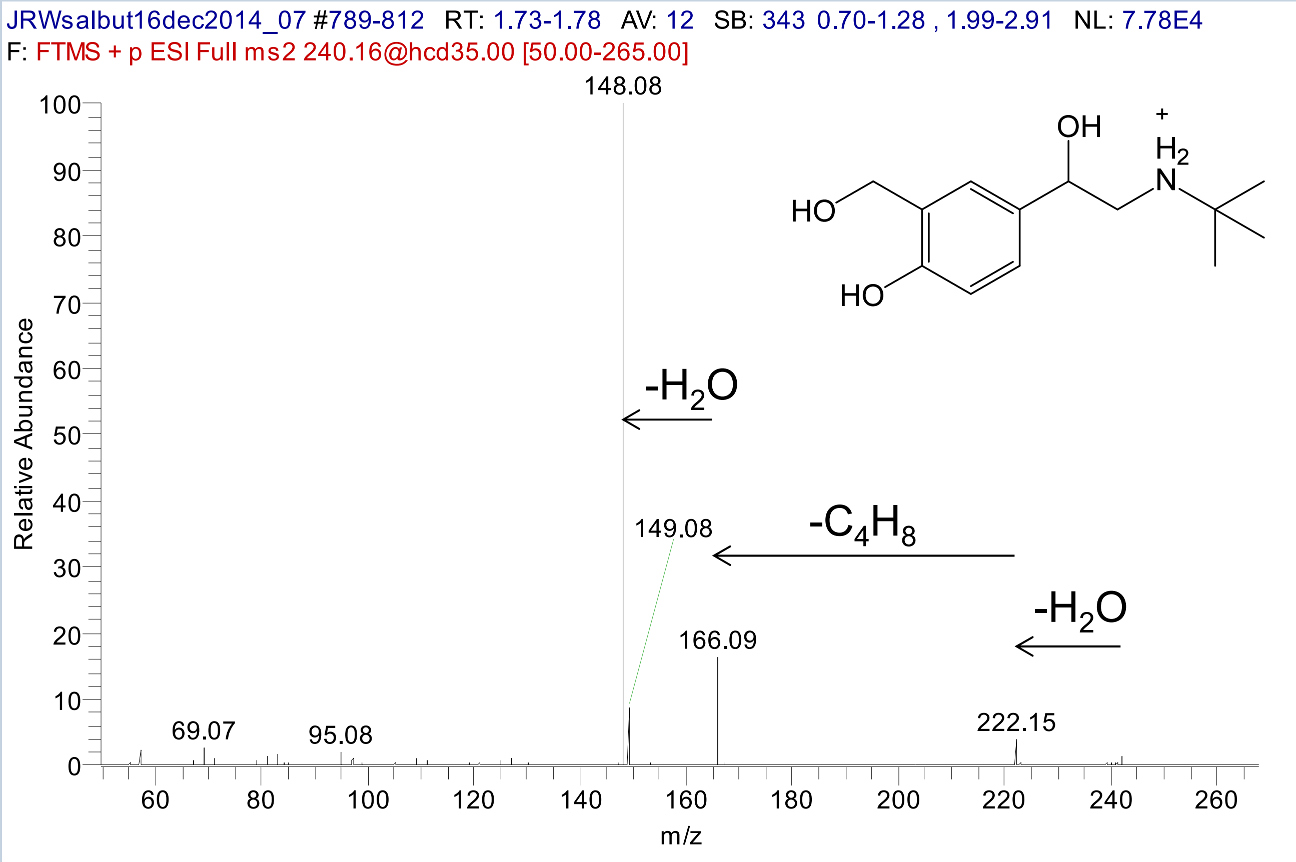
Salbutamol



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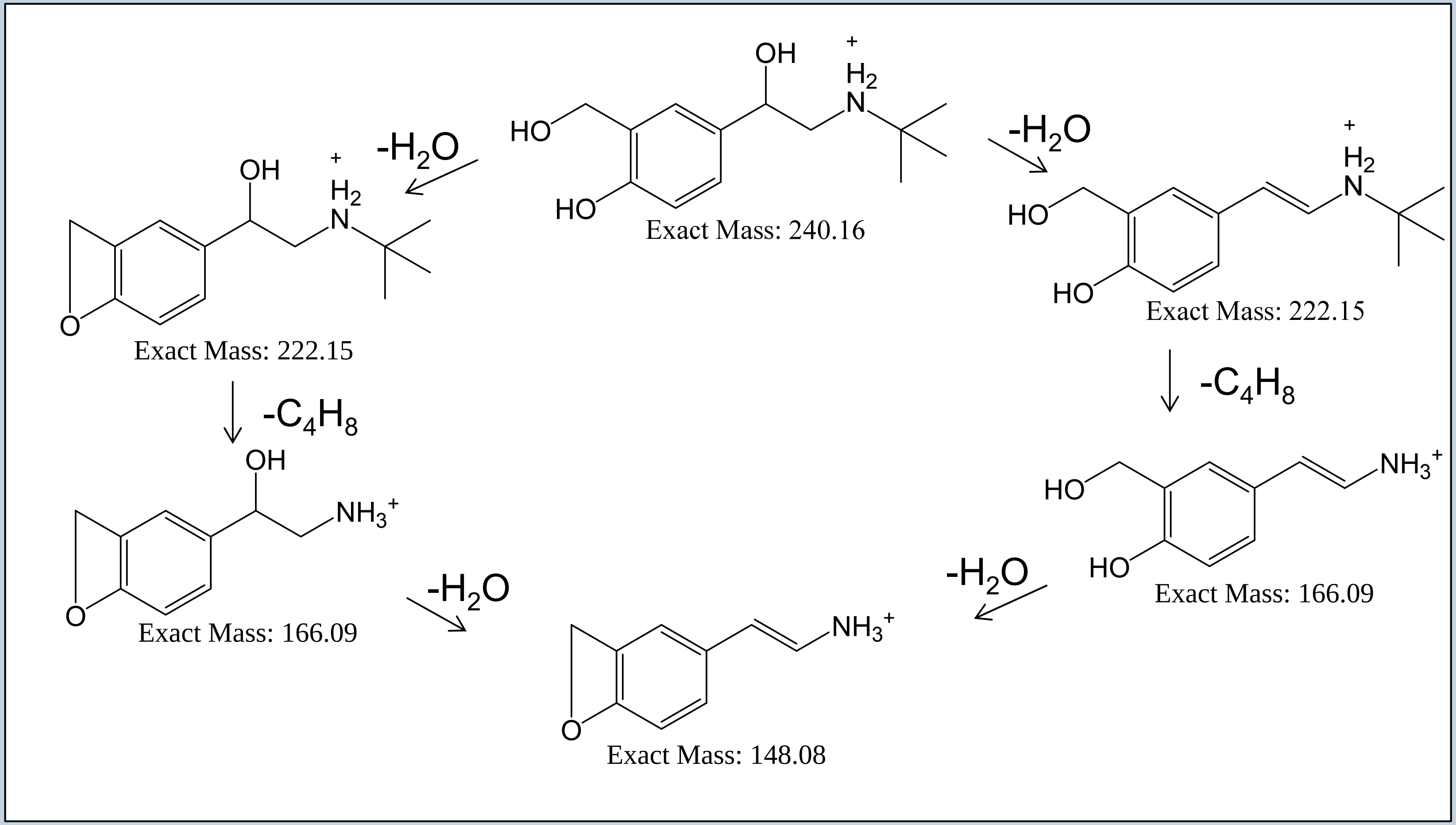
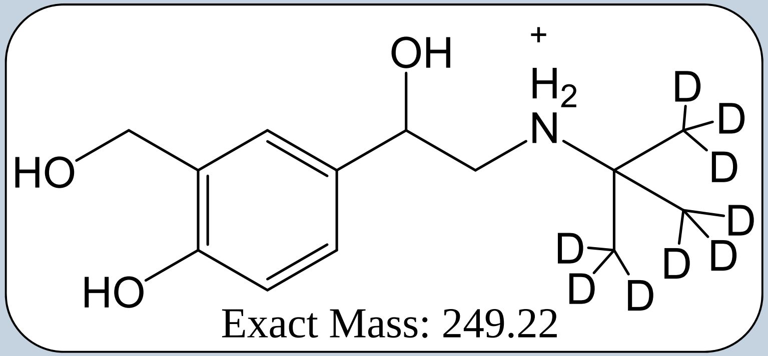


Example 2
Salbutamol, Isotopic Labels



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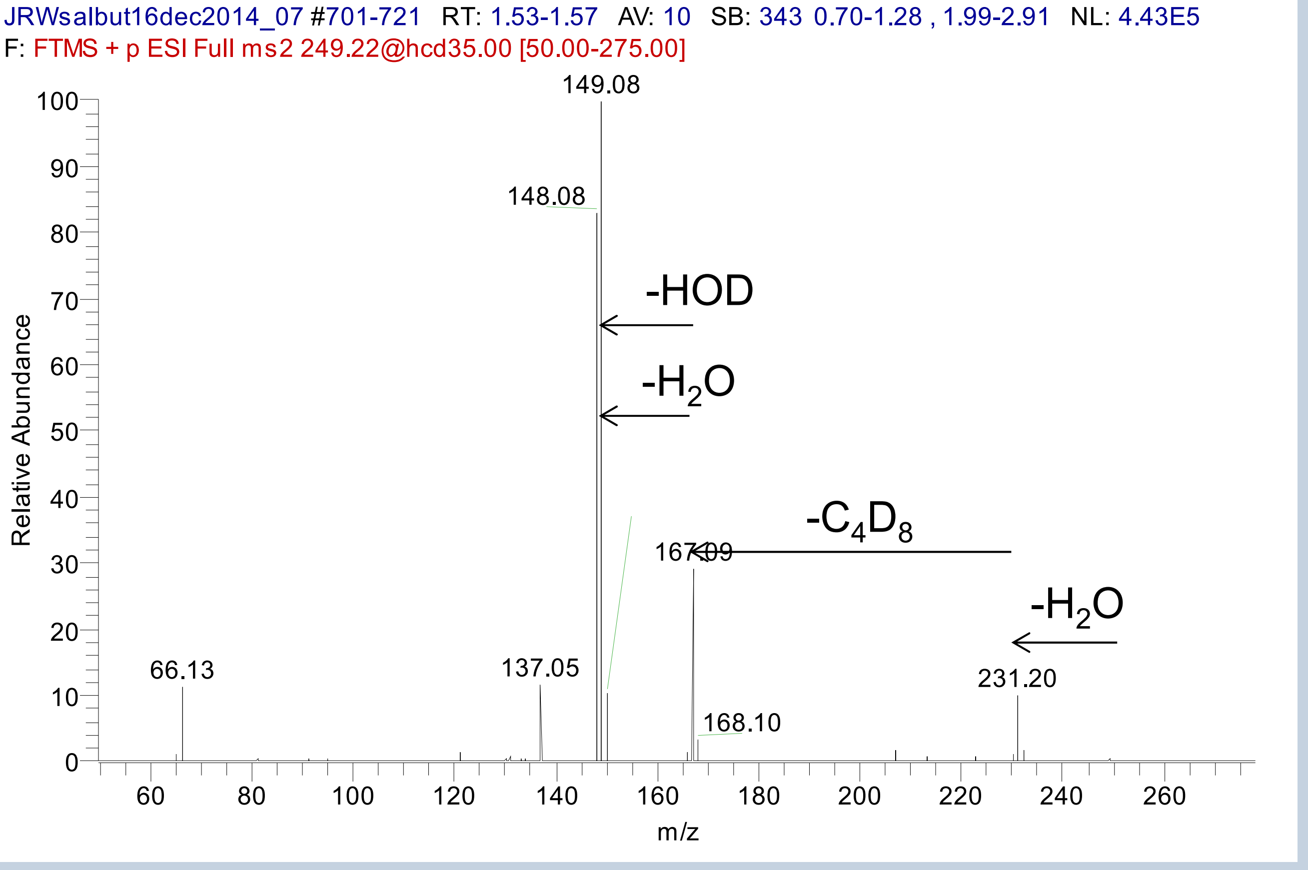

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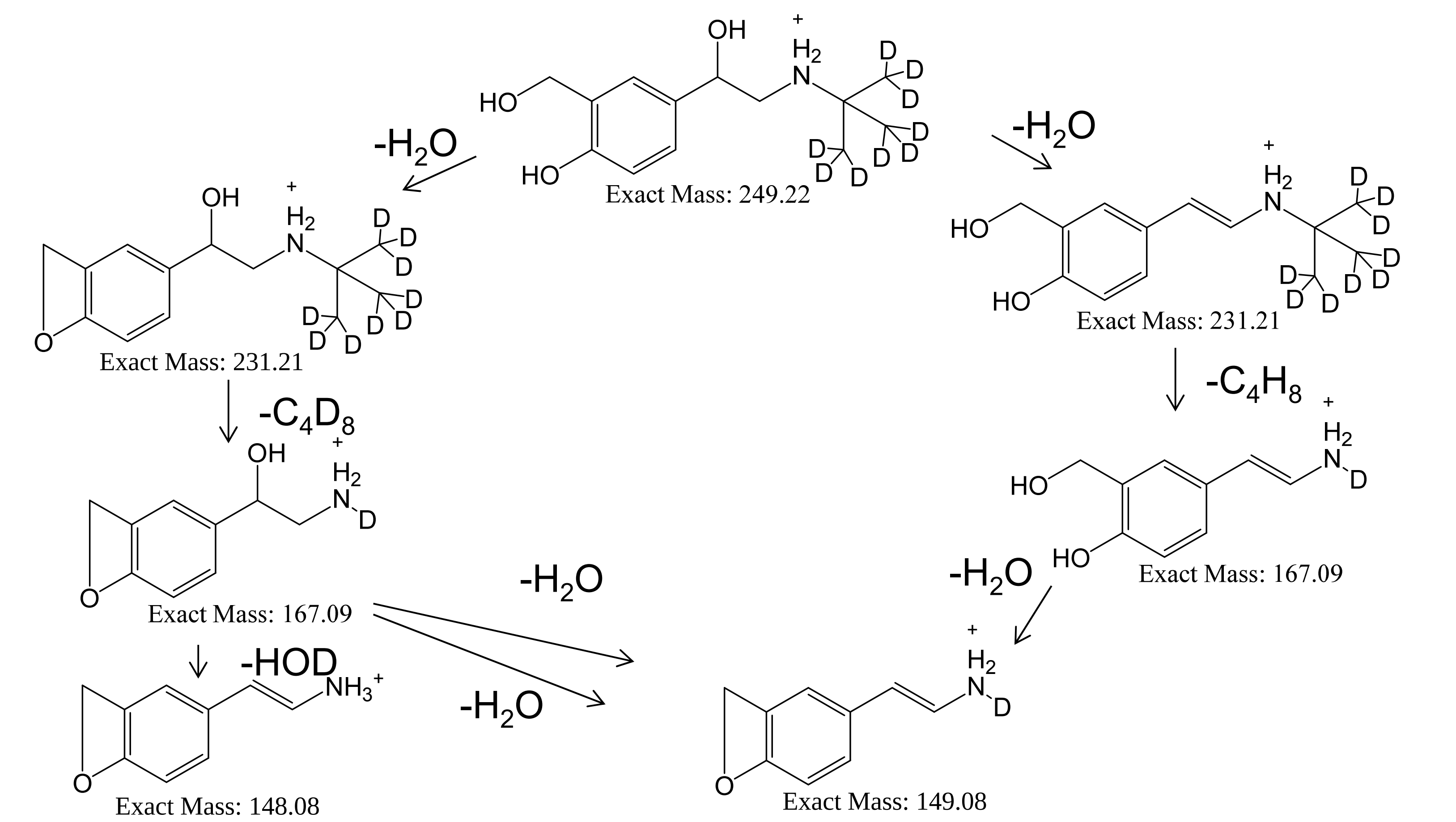

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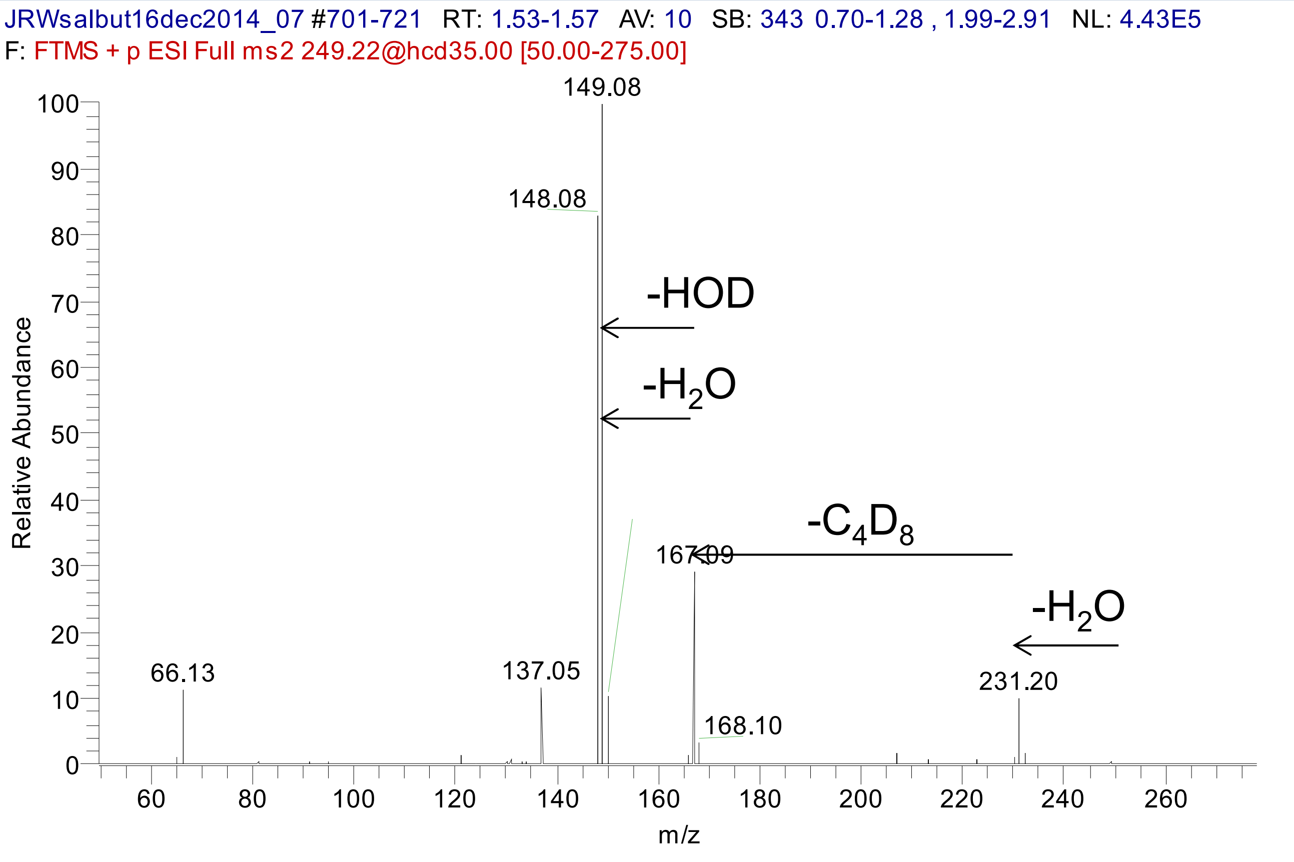

Review

- The ‘chemistry’ of mass spectrometry
  - Broad subject
  - Some very well understood
  - Still active area of research