



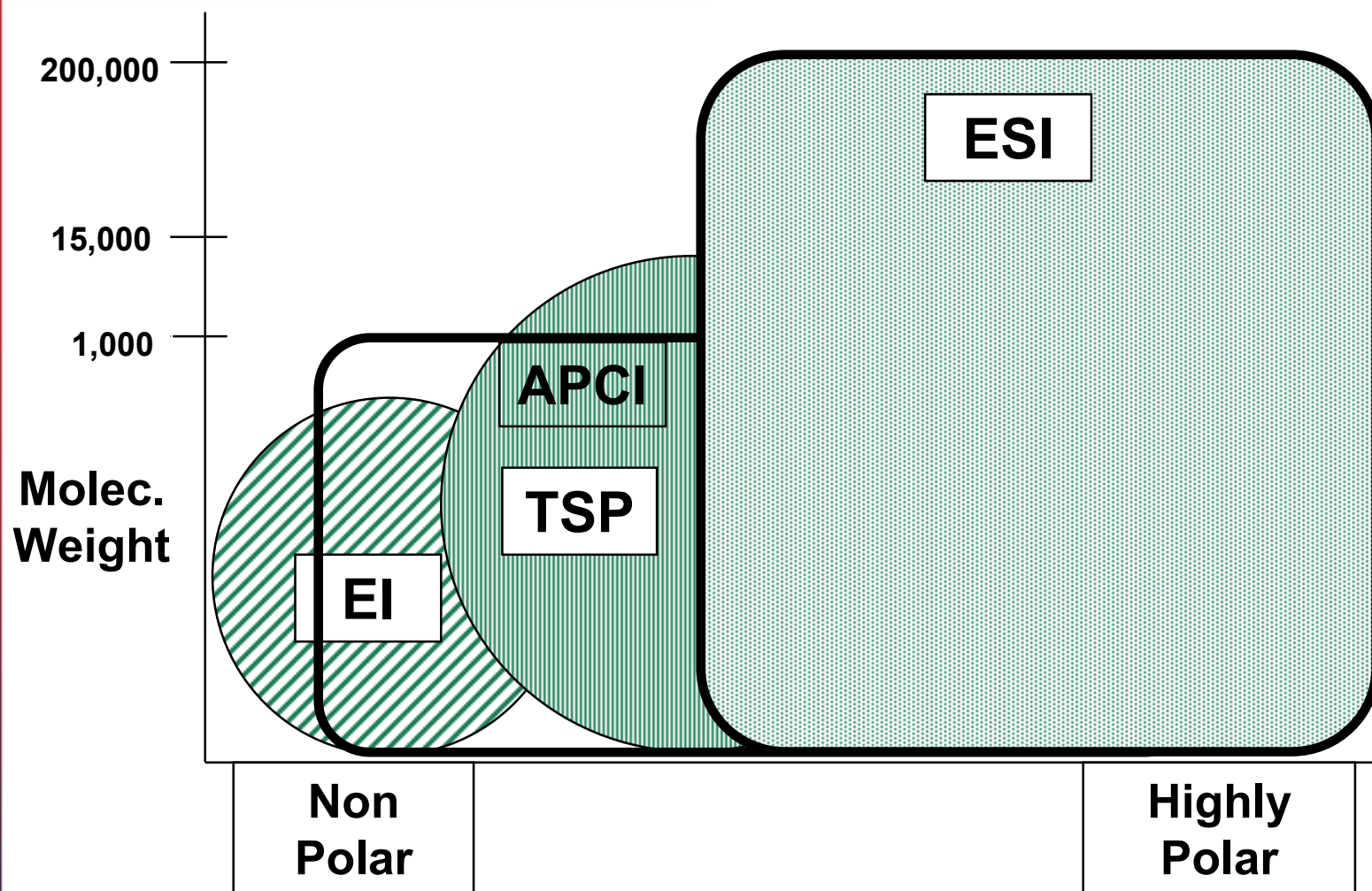
Mass Spectrometry Ionisation Techniques

How do we handle different types of sample ?

Types of ionisation techniques

- ▶ Volatile samples
 - Electron Ionisation
 - Chemical Ionisation
 - GC (and LC) inlets
- ▶ Non-volatile samples
 - Fast Atom Bombardment
 - Thermospray
 - Matrix Assisted Laser Desorption Ionisation
 - Electrospray Ionisation
 - Atmospheric Pressure Chemical Ionisation
 - LC (and GC) inlets

Comparison of Ionisation Techniques



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Electron Ionisation

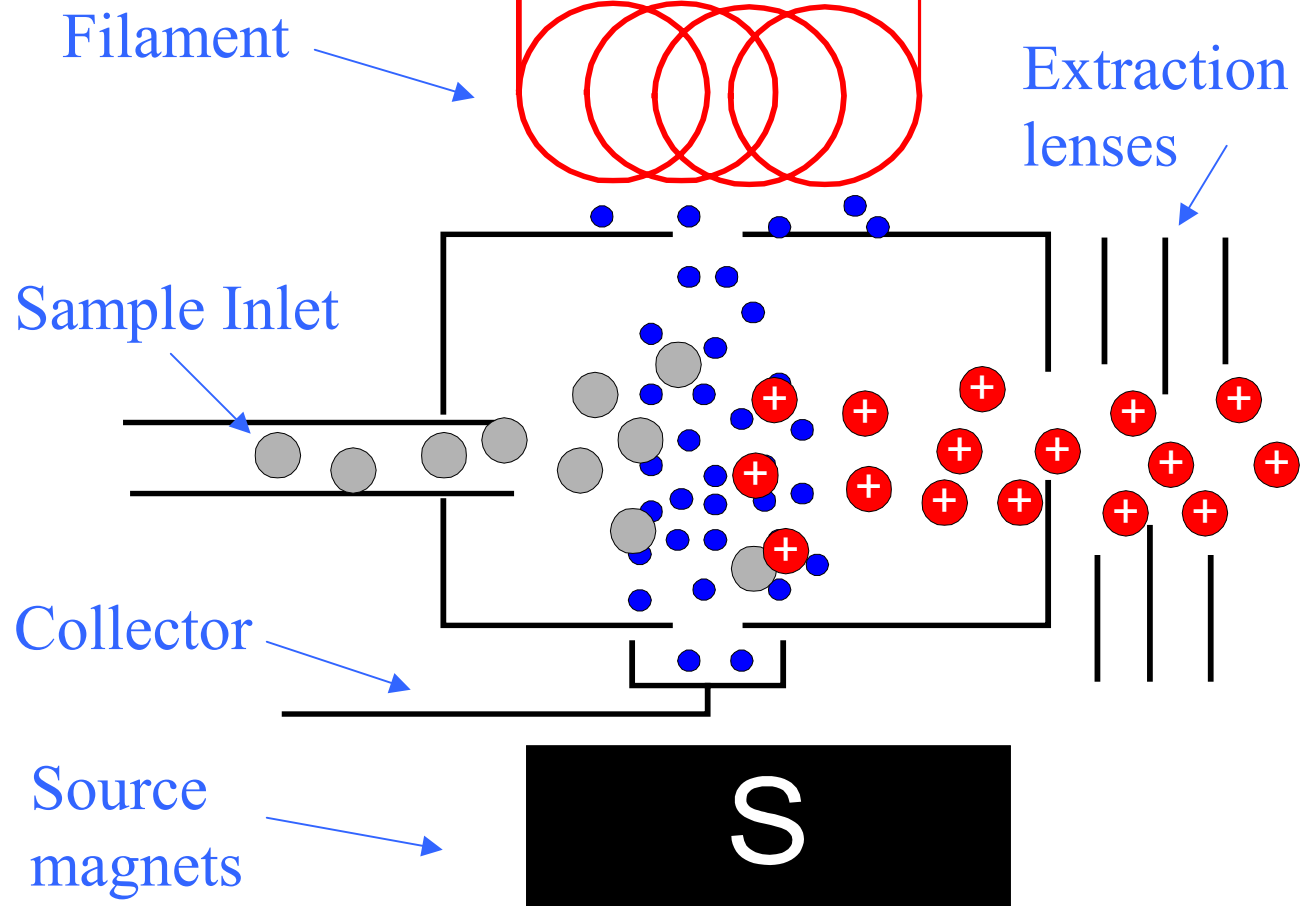
- Widely used technique when coupled to GC
- Suitable for volatile organic compounds
 - eg hydrocarbons, oils, flavours, fragrances
- Not really coupled to LC today
- Also called electron impact

Electron Ionisation

- Produces M^+ radical cation giving molecular weight
- Produces abundant fragment ions
- Library searchable spectra
- Energetic process. A heated filament emits electrons which are accelerated by a potential difference of usually 70eV into the sample chamber. Ionisation of the sample occurs by removal of an electron from the molecule thus generating a positively charged ion with one unpaired electron.

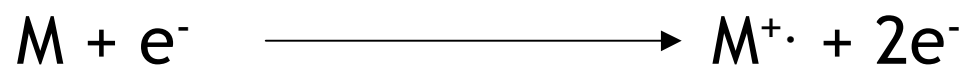
Electron Ionisation

N

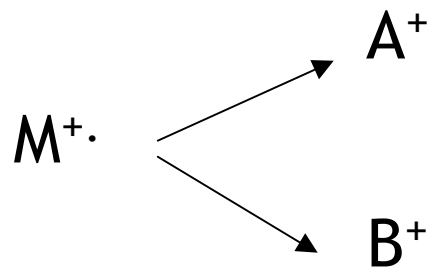


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Electron Ionisation



Fragmentation



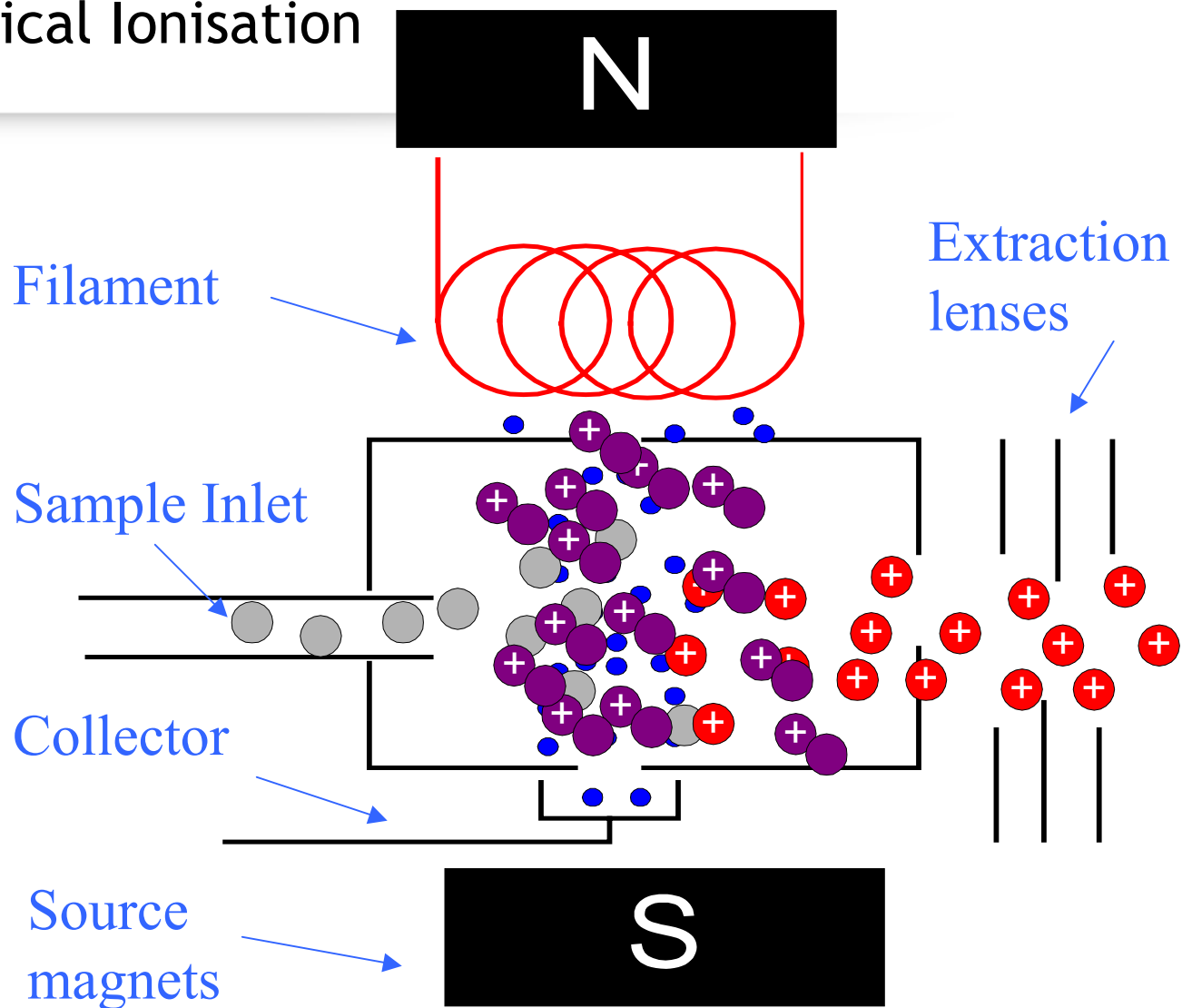
Chemical Ionisation

- Development from EI
- Same compound classes as EI
- Gives molecular weight
- Softer ionisation technique
- Produces $M+H^+$ ions or $M - H^-$ ions
- Used to produce more abundant molecular ions when the molecule under investigation fragments using EI

Chemical Ionisation

- ▶ Similar ionisation technique to EI except that a reagent gas is introduced into the chamber in excess of the sample
- ▶ Positive CI uses methane, isobutane or ammonia as reagent gases
- ▶ Negative CI uses methane reagent gas in electron capture mode
- ▶ Ionised reagent gas protonate the sample molecules leaving a neutral reagent gas species
- ▶ Not reproducible from lab to lab, hence no CI libraries.

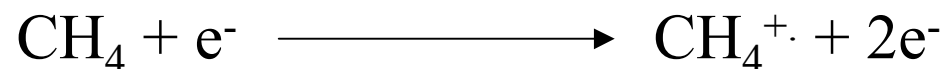
Chemical Ionisation



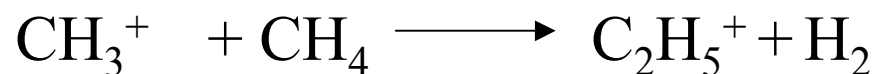
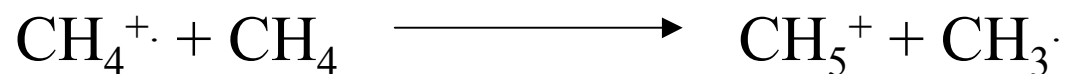
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Chemical Ionisation

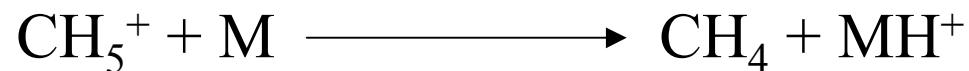
Primary ions



Secondary ions



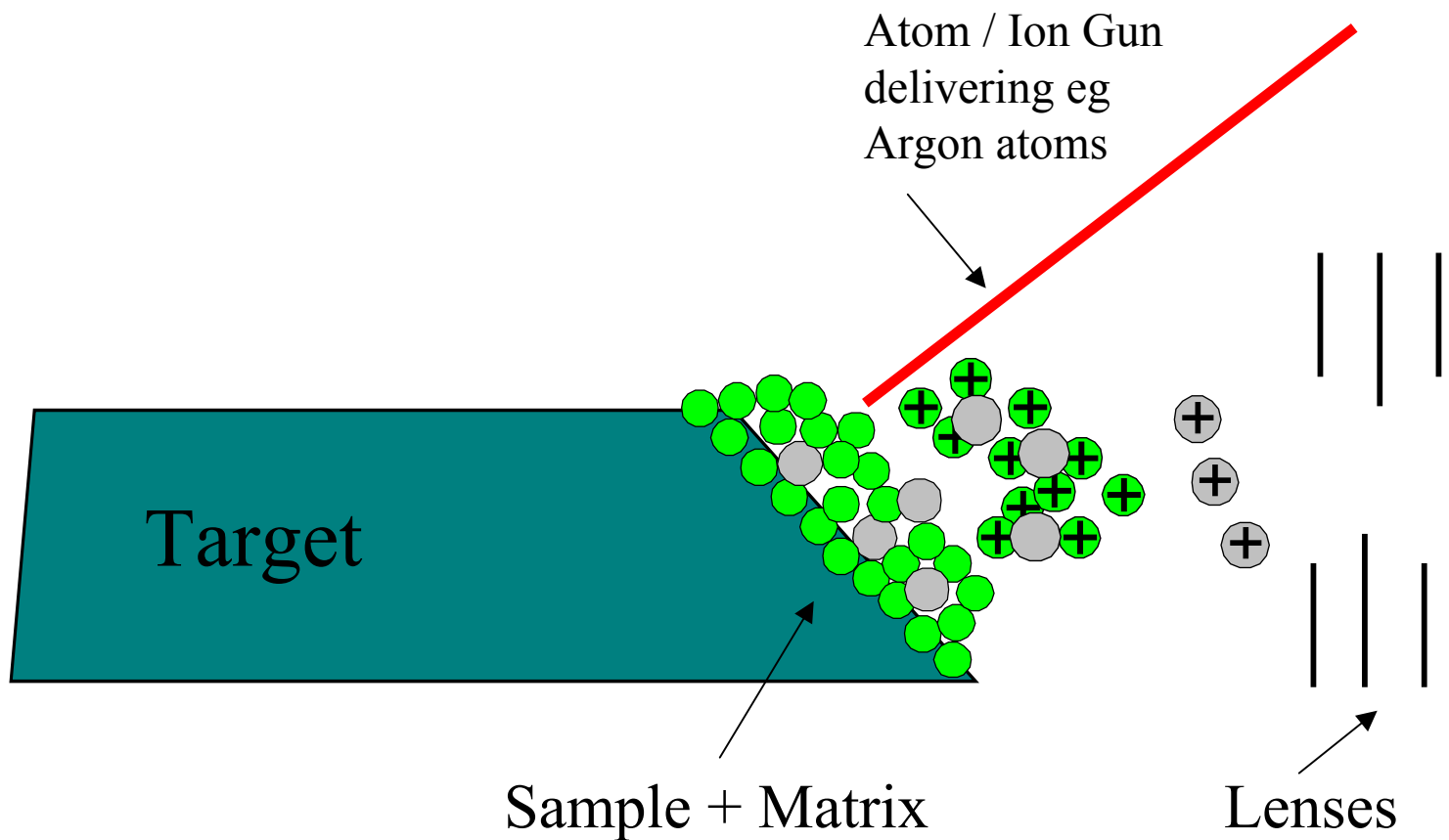
Proton donation



Fast Atom Bombardment

- Used for large compounds with low volatility (eg peptides, proteins, carbohydrates)
- Solid or liquid sample is mixed with a non-volatile matrix (eg glycerol, crown ethers, nitrobenzyl alcohol)
- Immobilised matrix is bombarded with a fast beam of Argon or Xenon atoms. Charged sample ions are ejected from the matrix and extracted into the mass analysers
- Gives $M+H^+$ or $M+Na^+$ ions
- Choosing correct matrix is difficult

FAB Source



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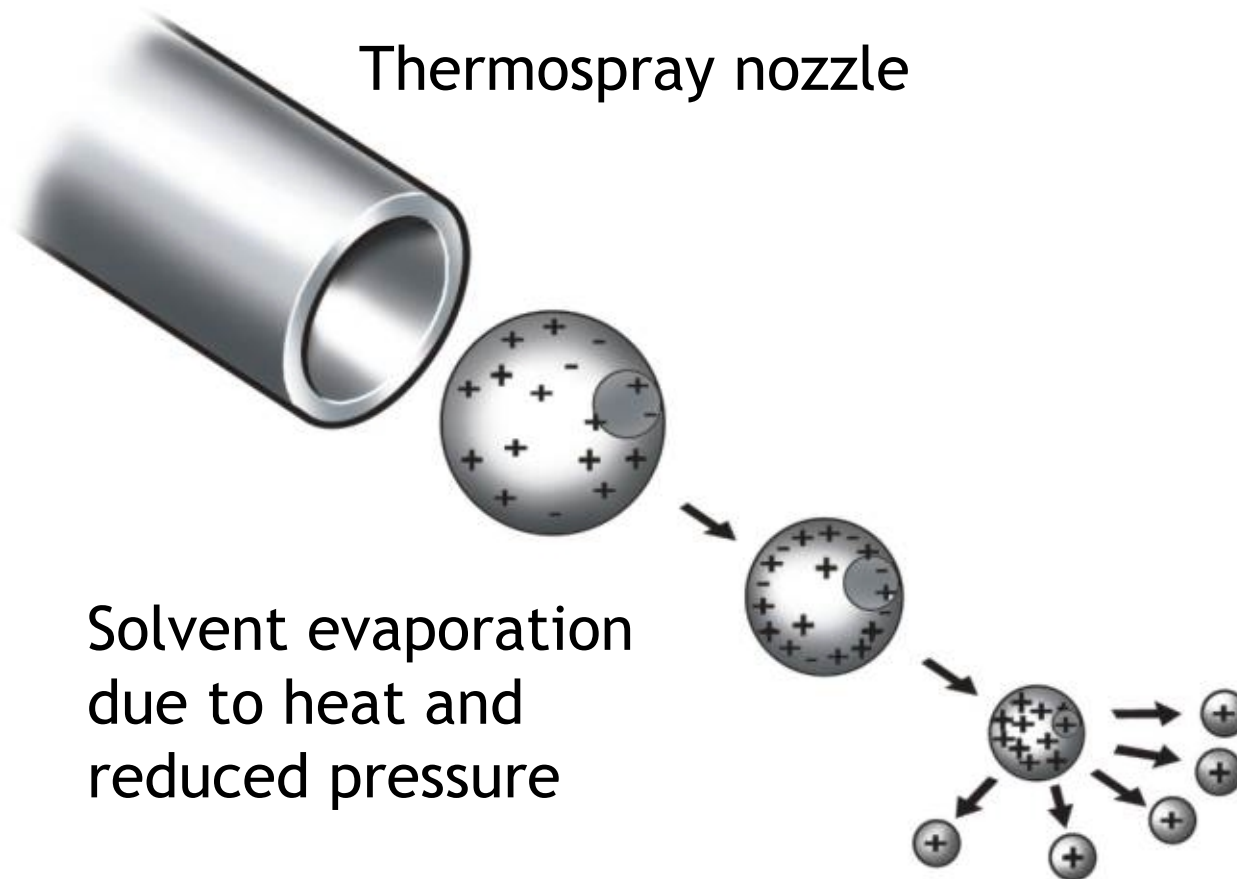
Matrix Assisted Laser Desorption Ionisation

- Similar process to FAB
- Sample is dissolved in matrix which absorbs light from a short pulse of laser of a specific wavelength. The sample becomes ionised and extracted towards the mass analysers
- Coupled to Time of Flight MS
- Not coupled to LC
- High mass range achievable
- Calibrants may be external or included in sample
- Reproducibility issues

Thermospray

- First widely used LC/MS interface
- Flow rates 0.5 - 1.5 ml/min
- Good for polar compounds
- LC eluent containing sample and ammonium acetate is pumped through a heated vaporiser. The jet of vapour contains small charged droplets which evaporate under the heat and vacuum expelling charged ions from the surface
- Produces $M+H^+$ or $M - H^-$ ions
- Not commercially available today

Thermospray Process



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Atmospheric Pressure Ionisation

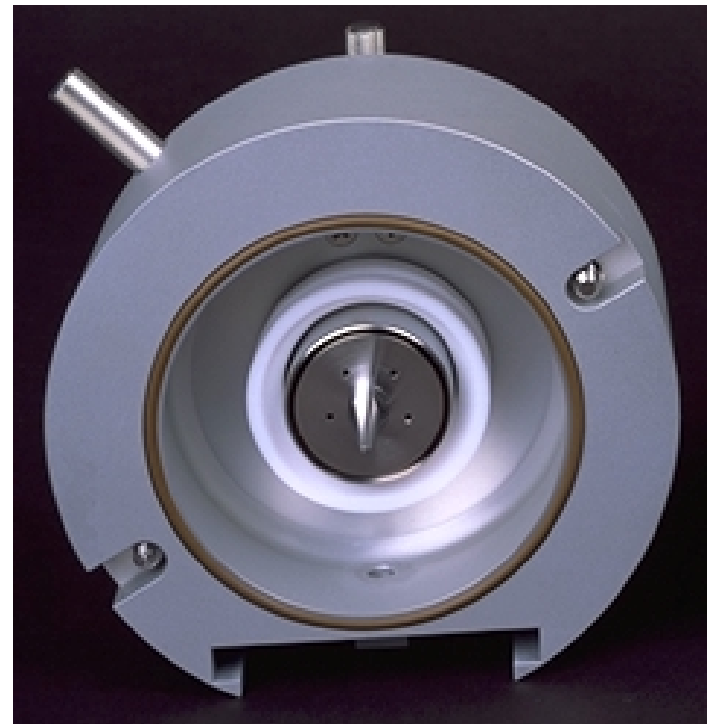
- Most important and widely used LC / MS technique
- API two types
 - Electrospray
 - Atmospheric Pressure Chemical Ionisation
- > 99% new LC/MS use API source
- Ionisation takes place outside vacuum region

Atmospheric Pressure Ionisation

- ▶ API coupled to LC or CE or Nanospray
- ▶ Handle wide range of flow rates
- ▶ Produce Intense $M+H^+$ ions
- ▶ Very little fragmentation
 - Need MS/MS for structural information
- ▶ Applicable to wide range of compounds
- ▶ Sample must be in solution

Electrospray

- ▶ Electrospray also known as :
 - Ionspray
 - Nanospray
 - Sonic Spray
 - "Pure" Electrospray
 - ESI, ES, IS

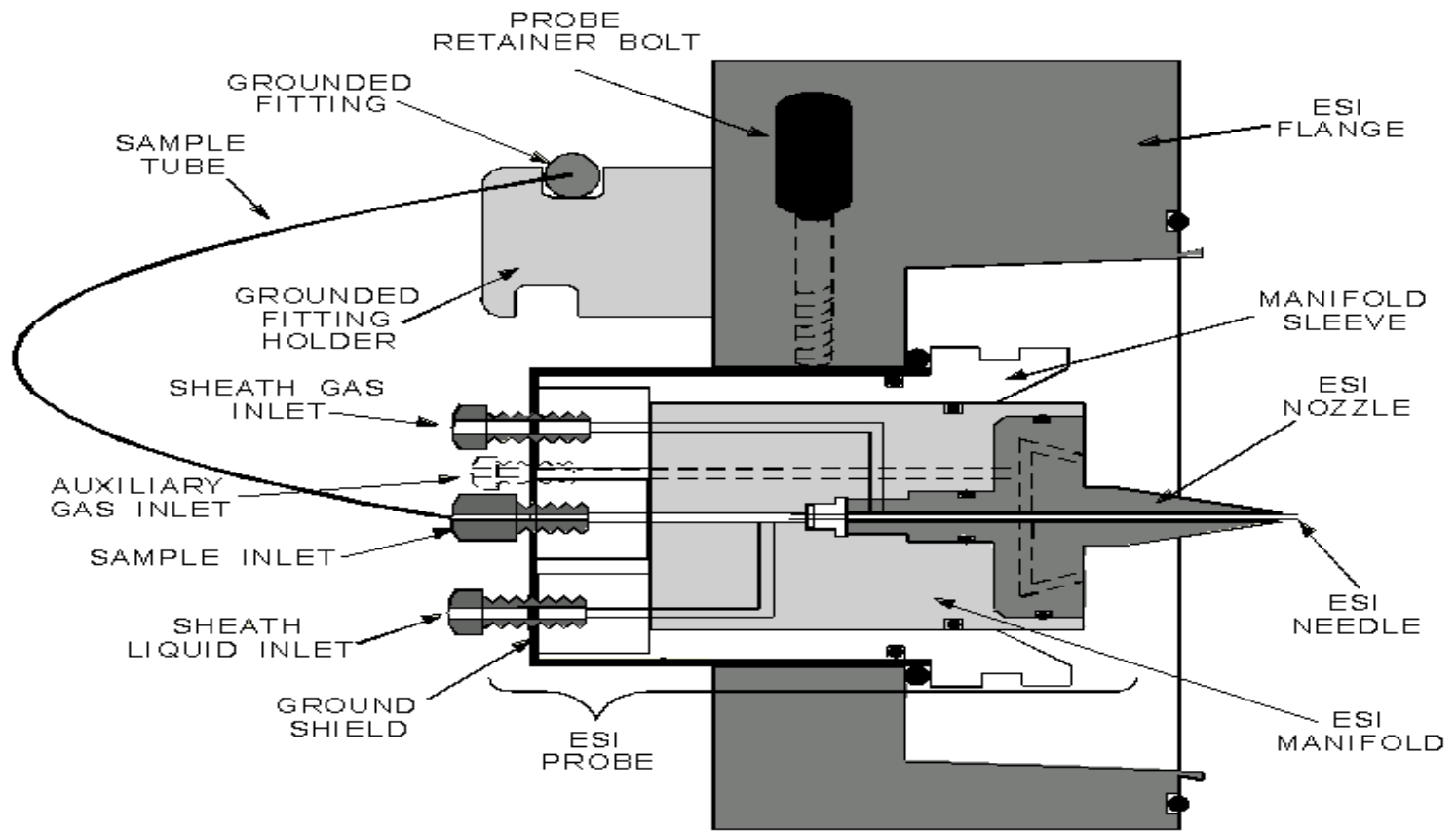


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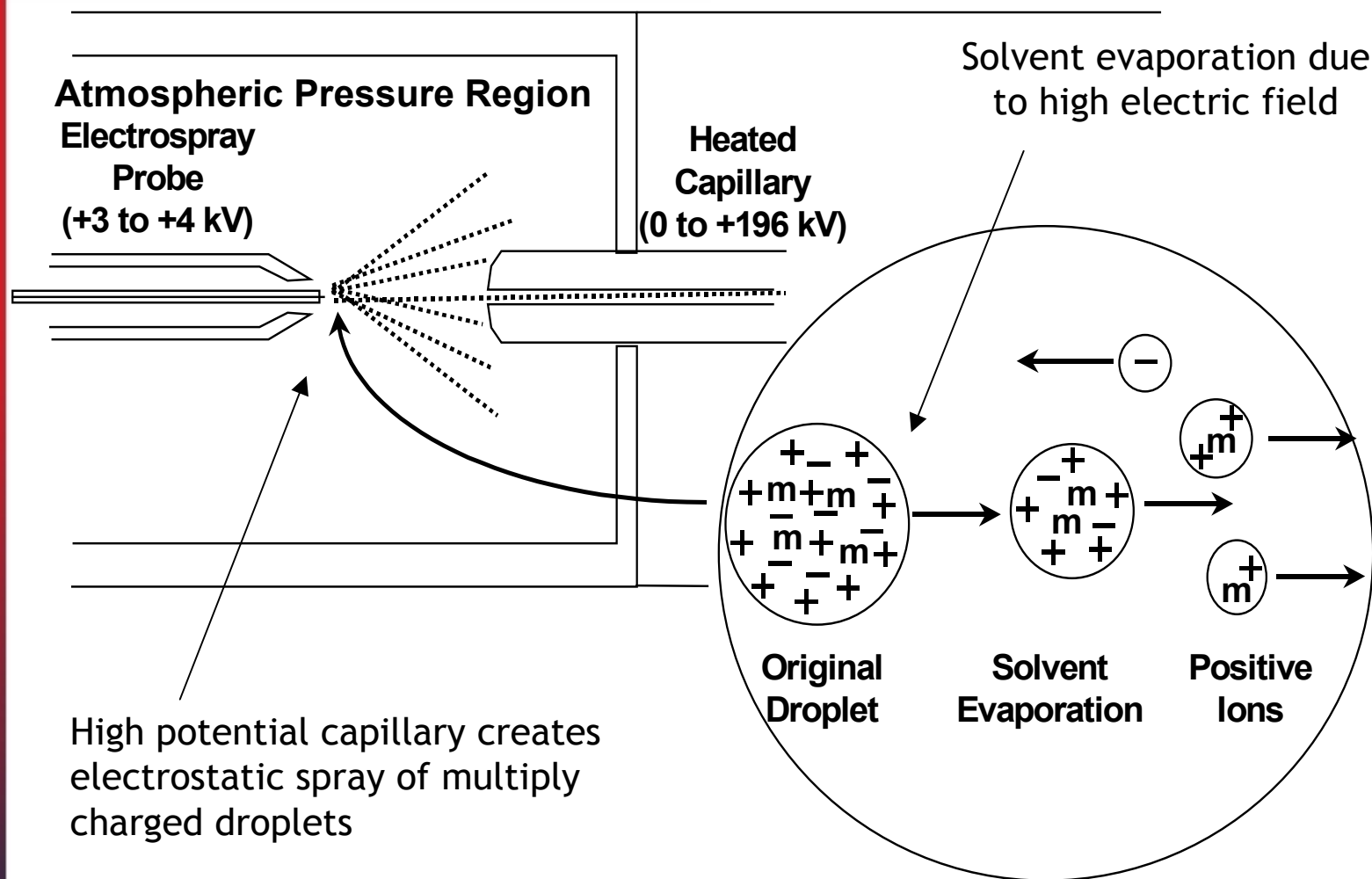
Electrospray

- Softest ionisation technique
- Best for polar non-volatile compounds (proteins, peptides, nucleic acids, Pharmaceuticals, natural products)
- Coupled to LC at a flow range of 2-1000 $\mu\text{L}/\text{min}$, nanospray (10 nL/min - 2 $\mu\text{L}/\text{min}$)
- Ions are ejected from charged vapour droplets to gas phase producing $M+H^+$ or $M - H^-$ ions
- Can produce multiply charged ions allowing determination of high molecular weight proteins
- Not very tolerant of non-volatile salts

ESI Probe

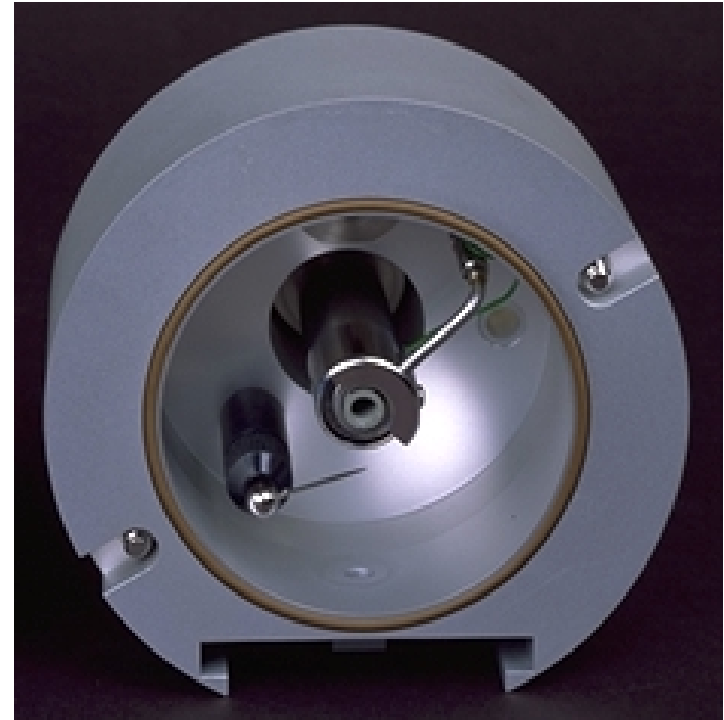


Electrospray Process



APCI

- ▶ Atmospheric Pressure Chemical Ionisation, also known as :
 - APCI
 - Heated nebuliser
 - APci

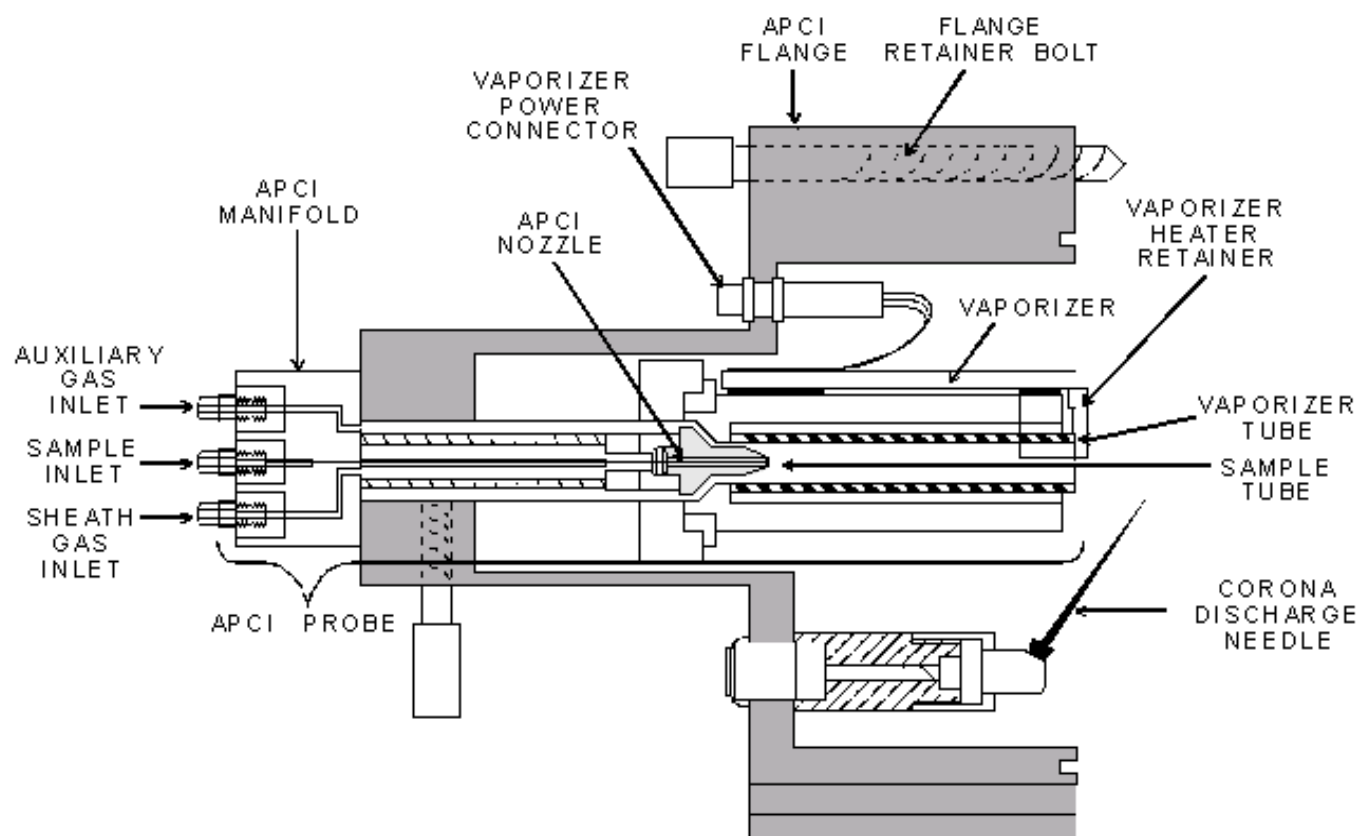


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APCI

- Used for wide range polarity of compounds
- HPLC eluent (up to 2ml/min flow rate) is vaporised at up to 600 °C
- The Corona discharge needle ionises solvent molecules. A combination of collisions and charge transfer reactions between the solvent and the analyte results in the transfer of a proton to form either $M+H^+$ or $M-H^-$ ions
- Compounds can thermally degrade
- Multiply charged ions rare
- More tolerant to salts

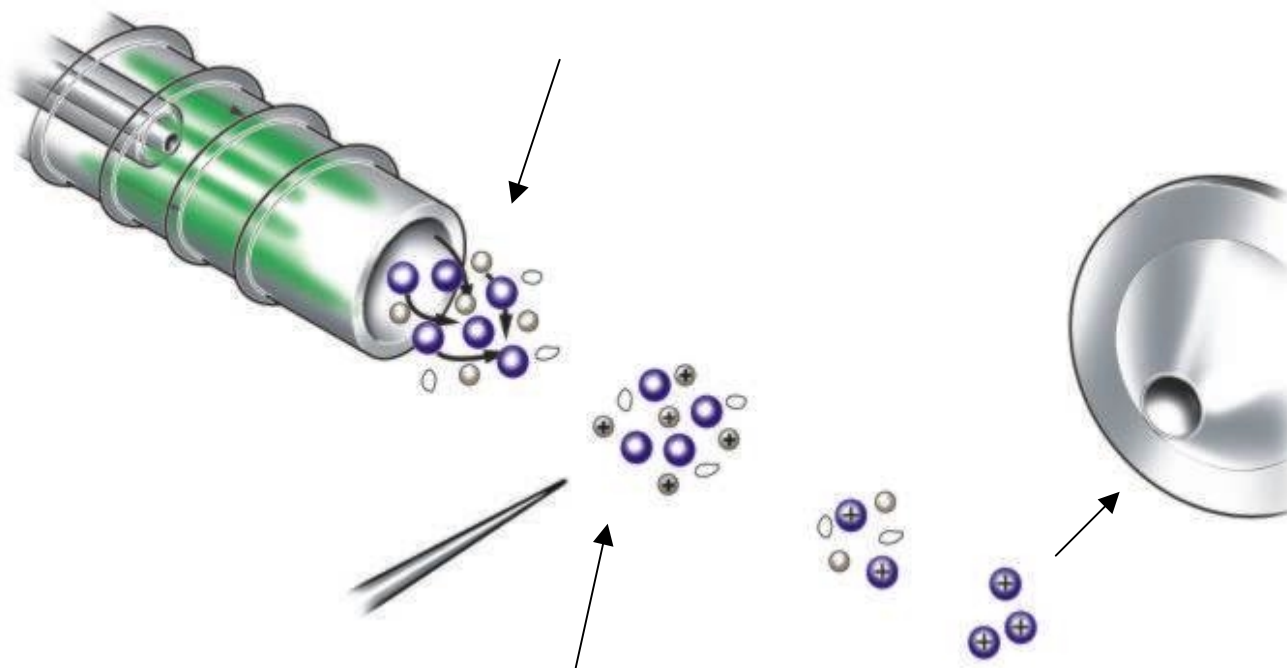
APCI Probe



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APCI Process

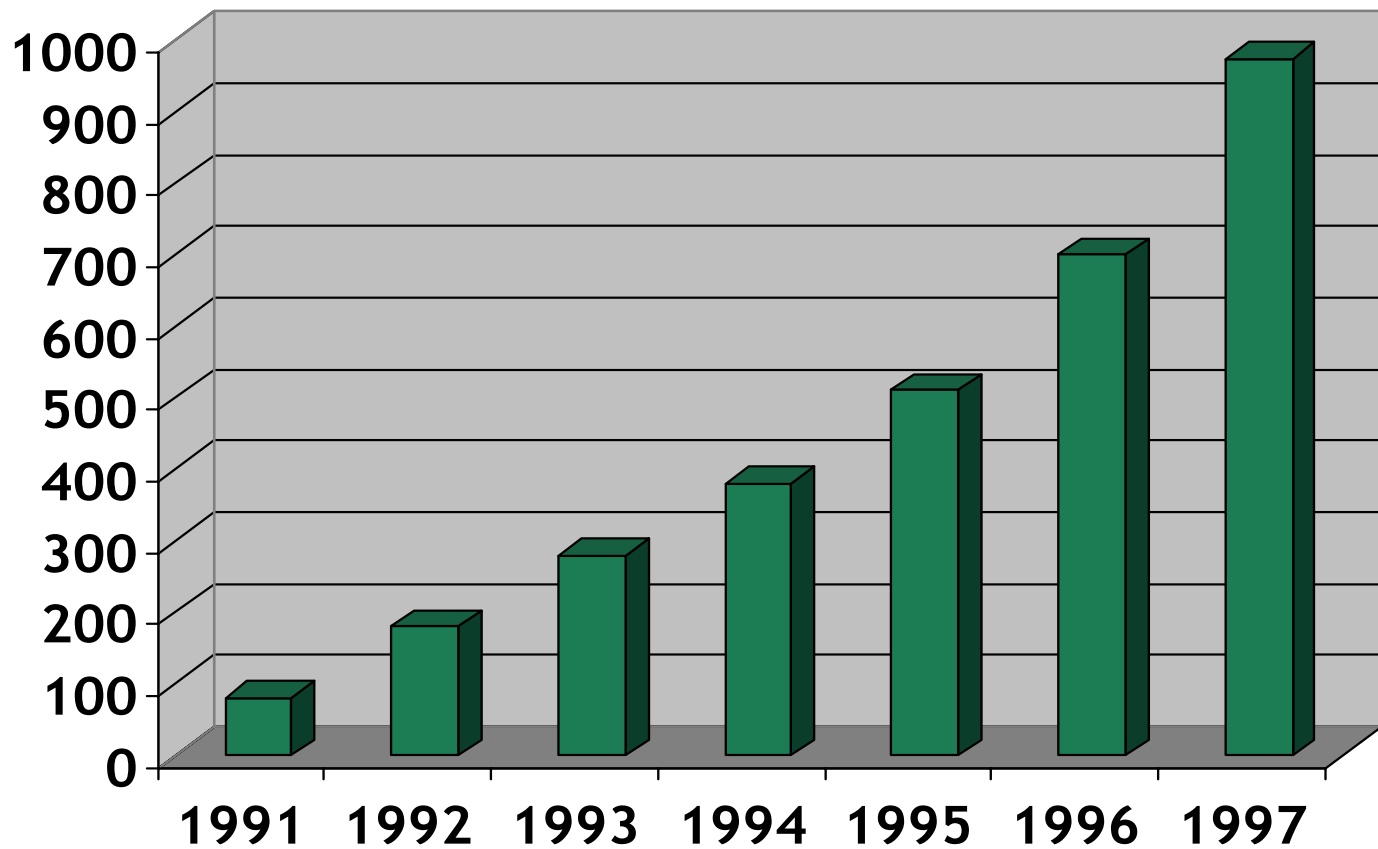
LC eluent evaporated
from heated vaporiser



Corona discharge needle ionises
solvent to generate a chemical
ionisation reagent gas plasma

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API Publications



Halket JM and Down S, LC/MS Update, HD Science, Nottingham

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Solvent suitability

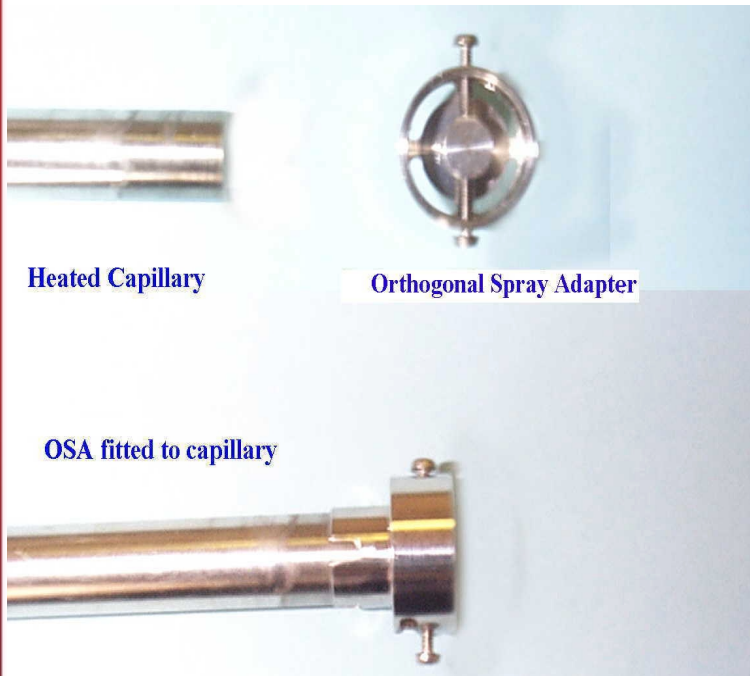
▶ HPLC buffers

- Reversed phase most often used
- MeOH, ACN, H₂O,
- TFA, formic acid, acetic acid, Ammonium formate, ammonium acetate
- Normal phase can be used

▶ Non-volatile buffers

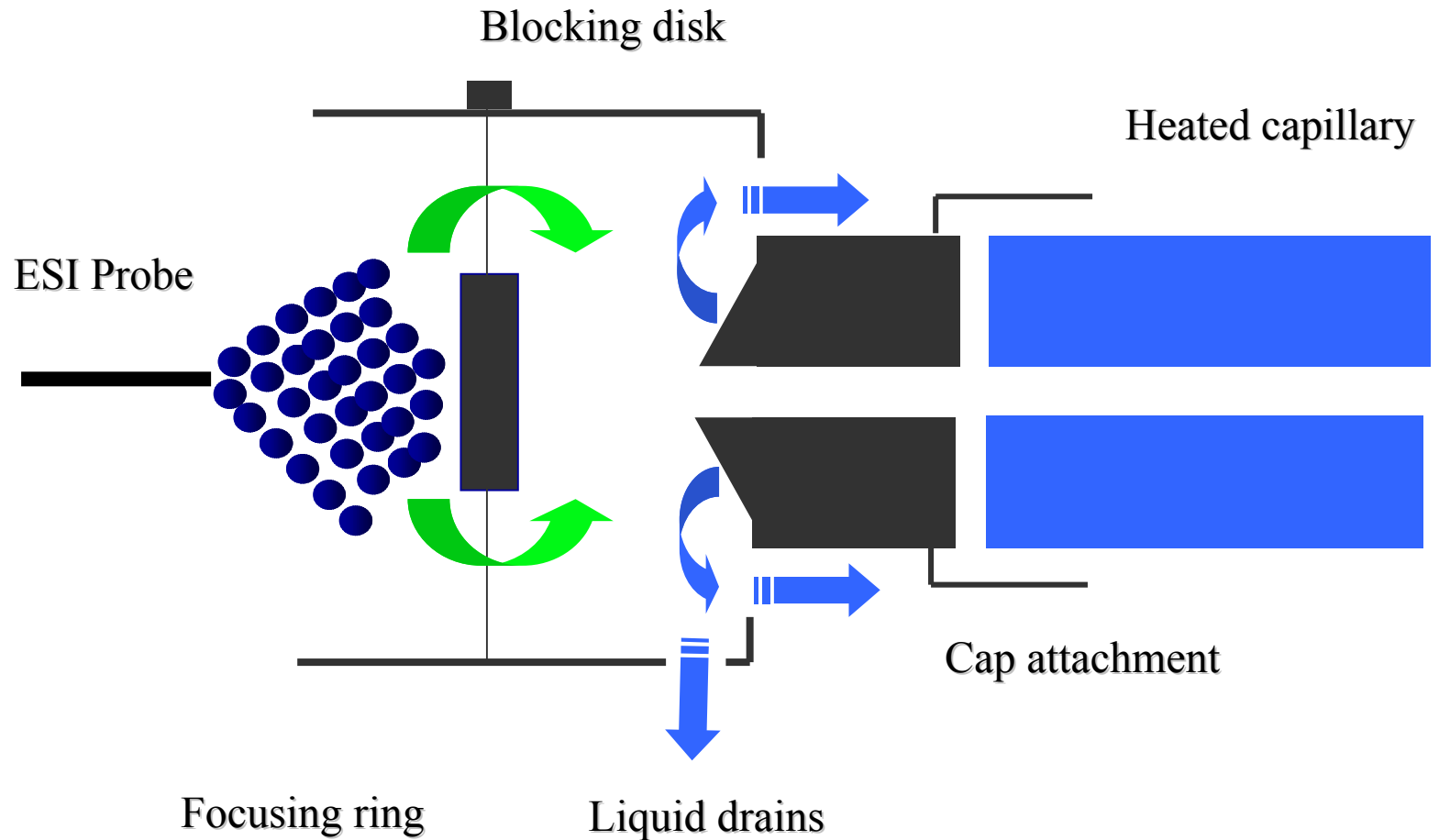
- OSA, aQa self cleaning source, off-axis probe

Orthogonal Sampling Adaptor

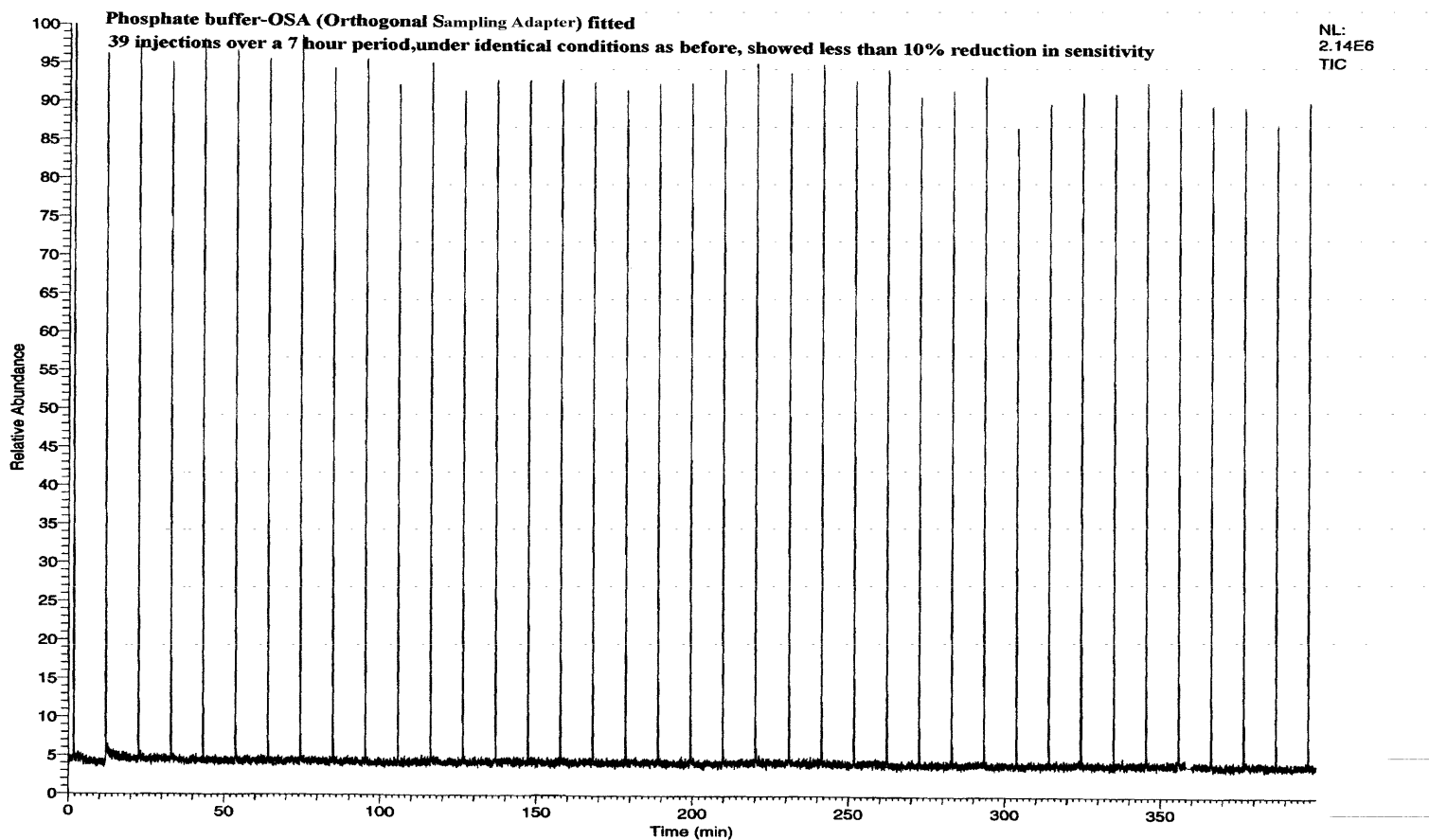


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Orthogonal Sampling Adaptor



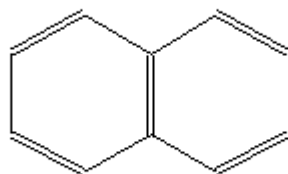
OSA fitted - 10mM Phosphate solution



Problems

How would you analyse this compound ?

Naphthalene



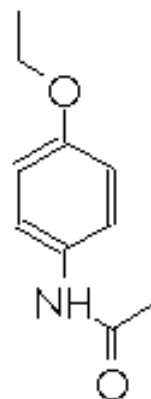
What sample introduction technique could you use ?
Which ionisation technique ?

A: EI, GC/MS

Problems

How would you analyse this compound ?

Phenacetin



What sample introduction technique could you use ?
Which ionisation technique ?

A: API (either APCI or ESI), LC/MS

Problems

How would you analyse myoglobin ?

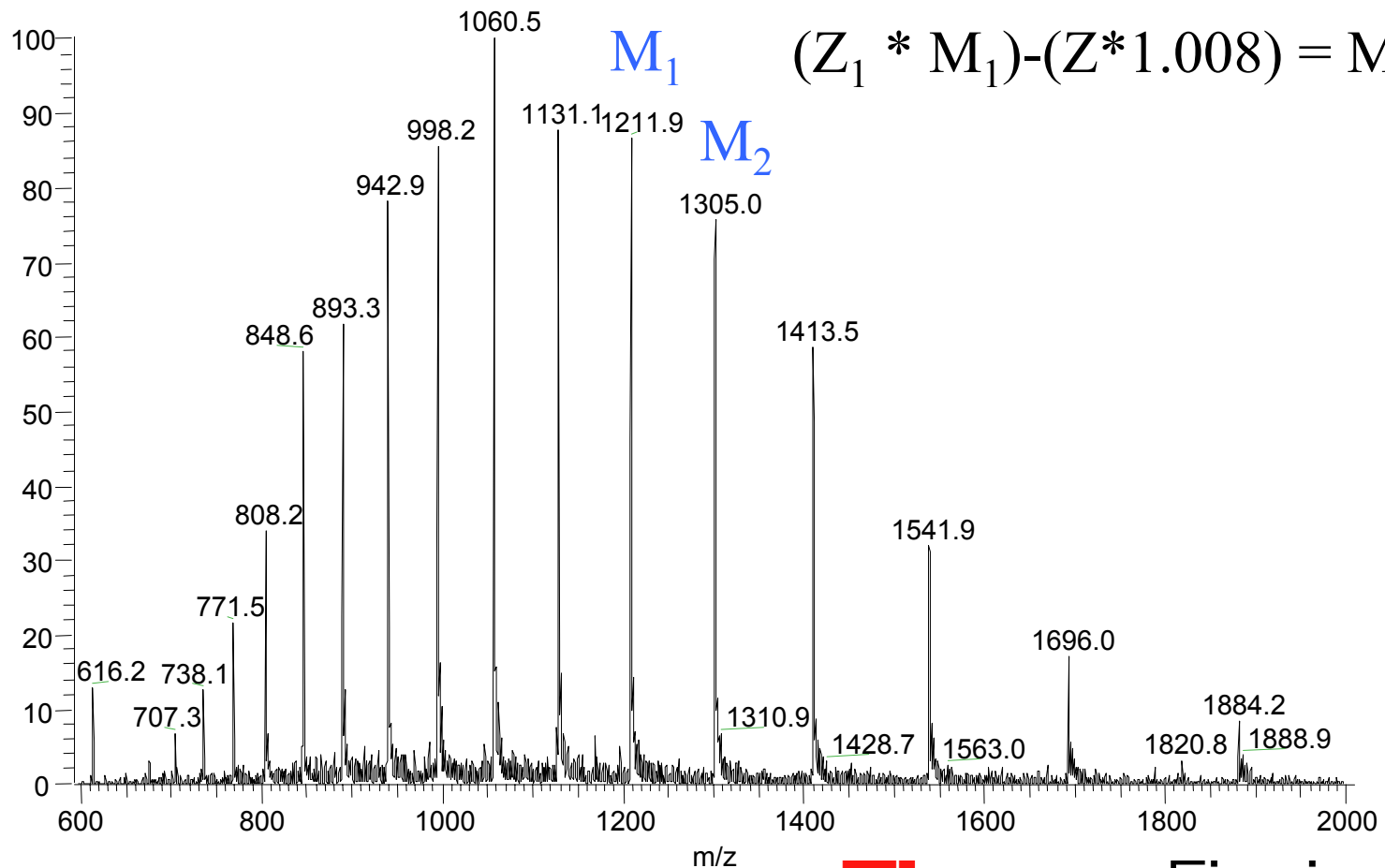
Myoglobin is a protein with a molecular weight of 16,951.

If the Mass Spectrometer has a mass range of up to 4,000, how can you analyse high molecular weight proteins ?

Multiply charged myoglobin ions from ESI

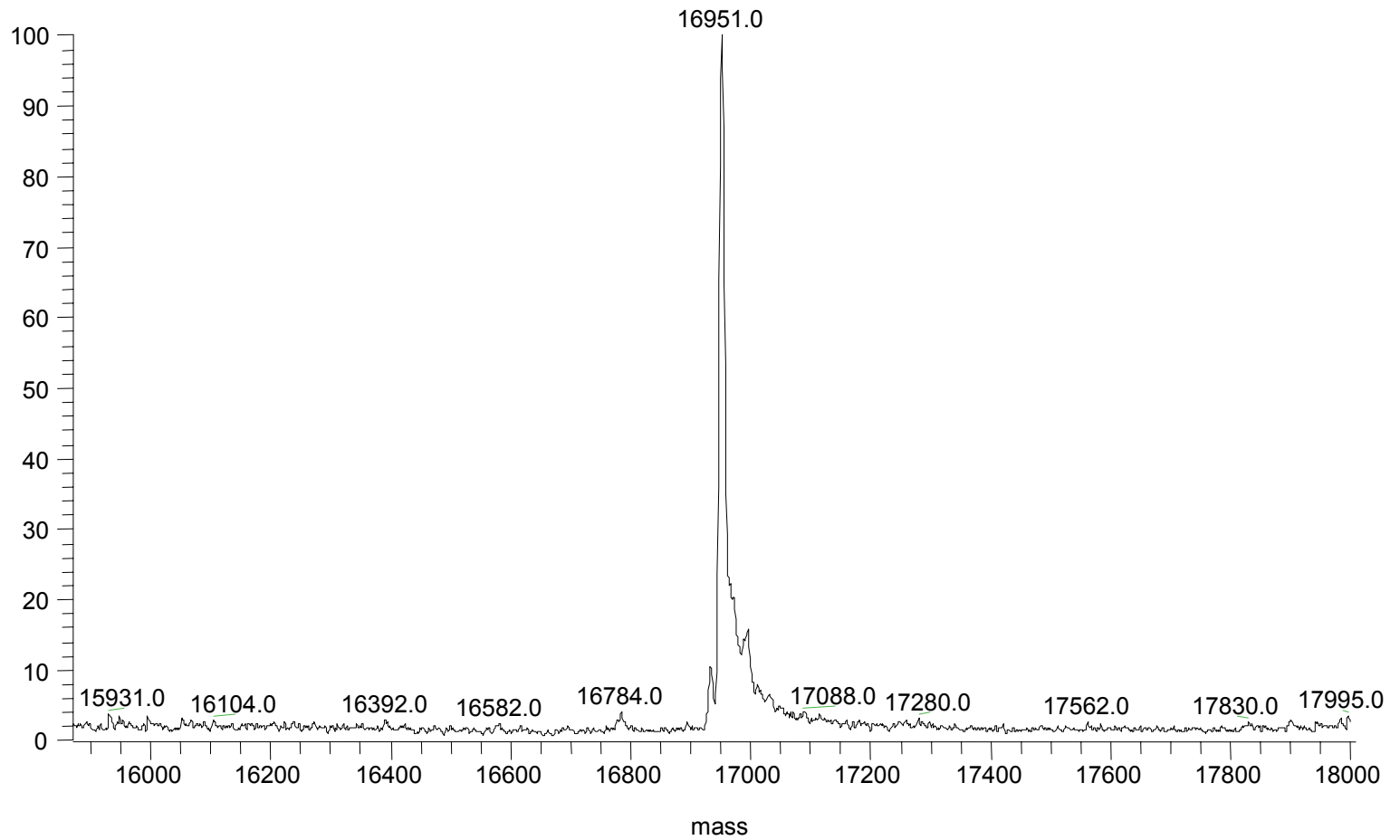
$$(M_2 - 1.008) / M_1 - M_2 = Z_1$$

$$(Z_1 * M_1) - (Z * 1.008) = M_{wt}$$



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Deconvoluted myoglobin spectrum



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