Mass Spectrometry Ionisation Techniques

How do we handle different types of sample ?

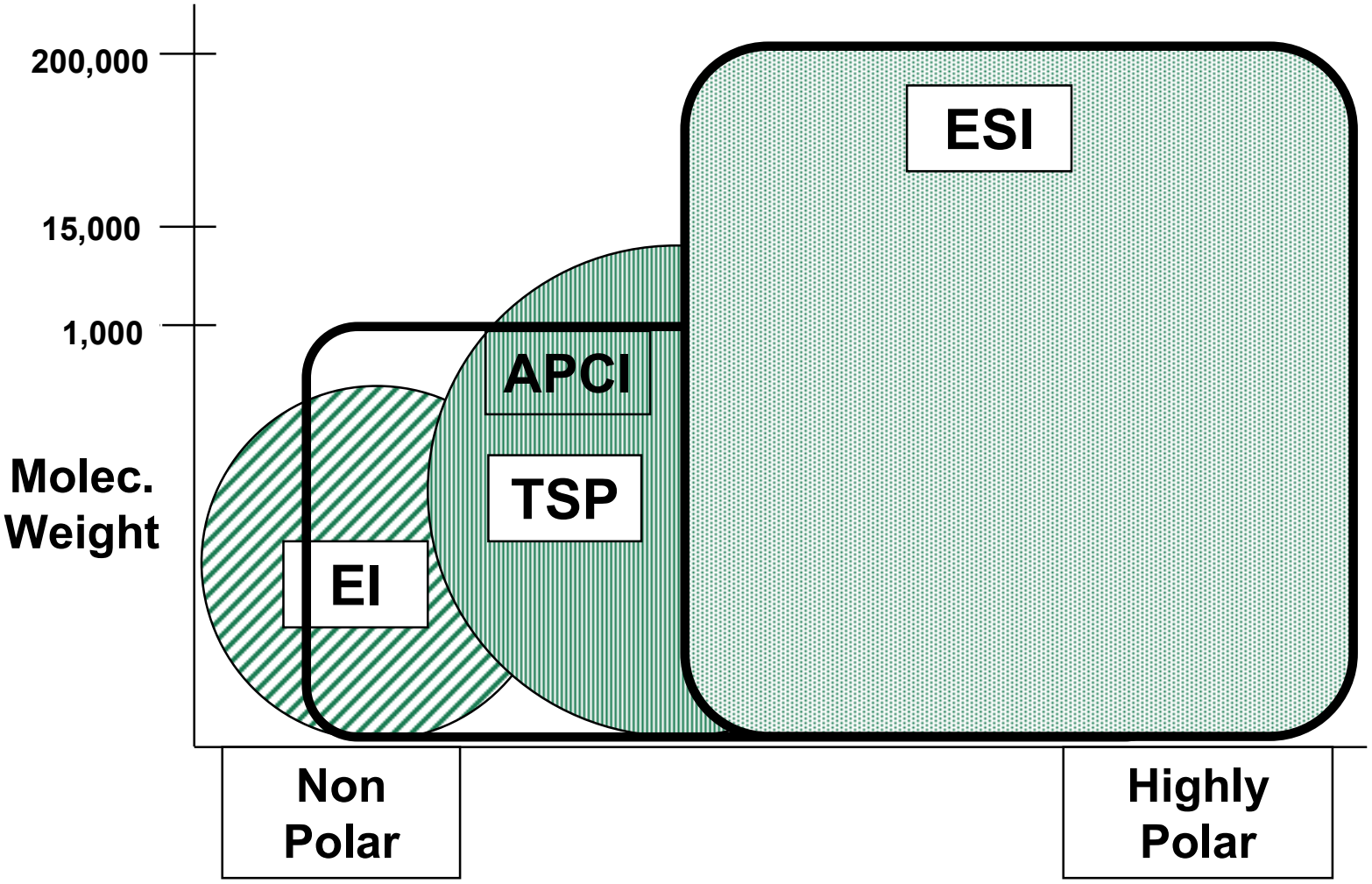
Types of ionisation techniques

- 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

Thermo Finnigan

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

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Electron Ionisation

- Produces $M^{+\cdot}$ 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.

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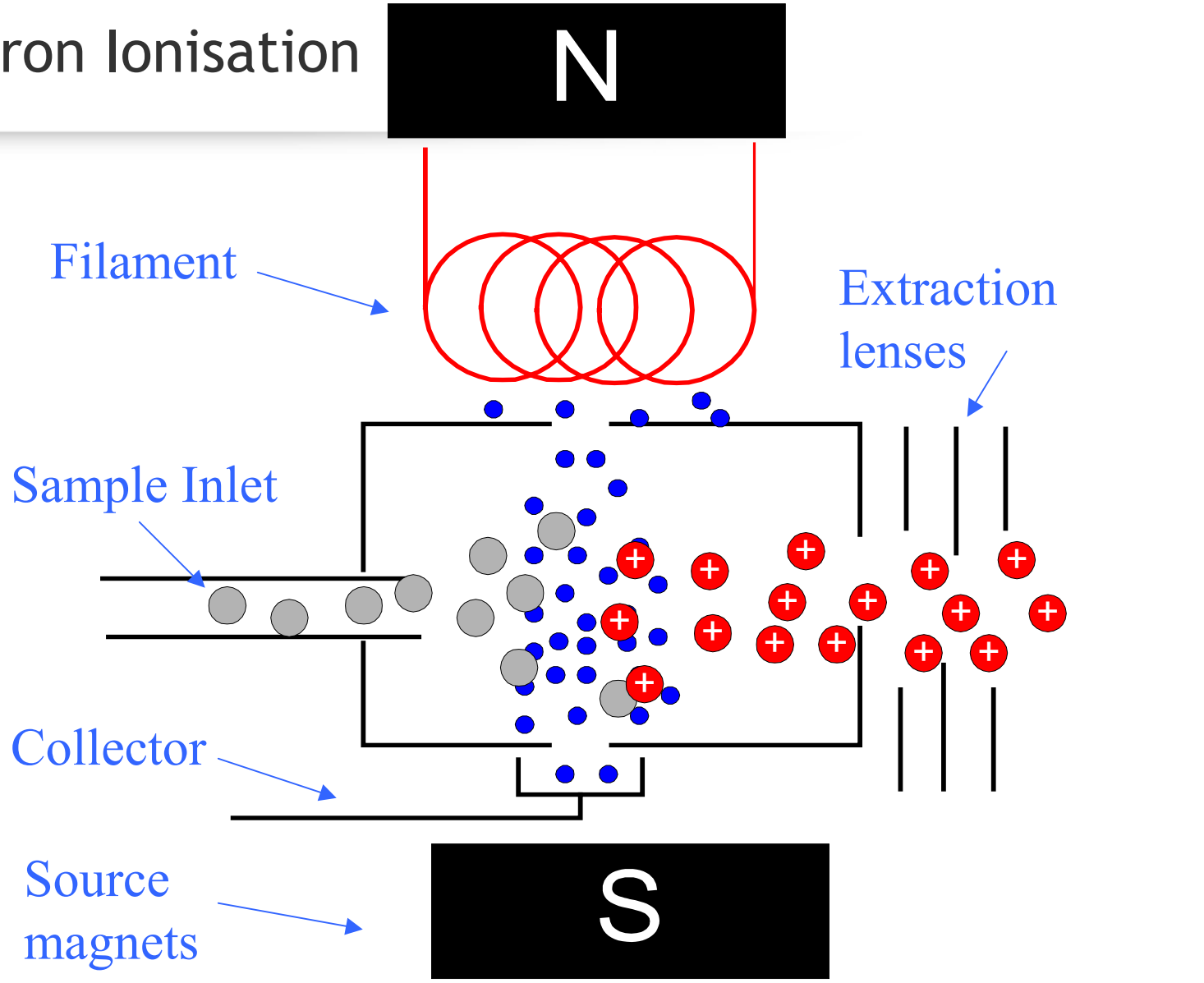
Electron Ionisation



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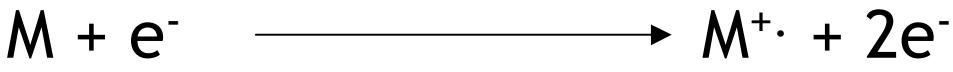
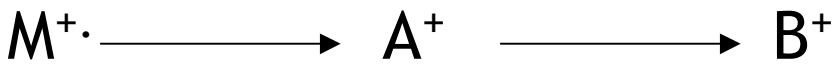
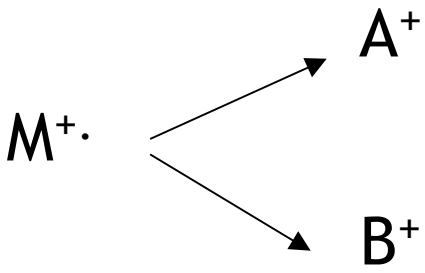
Electron Ionisation



Fragmentation





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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

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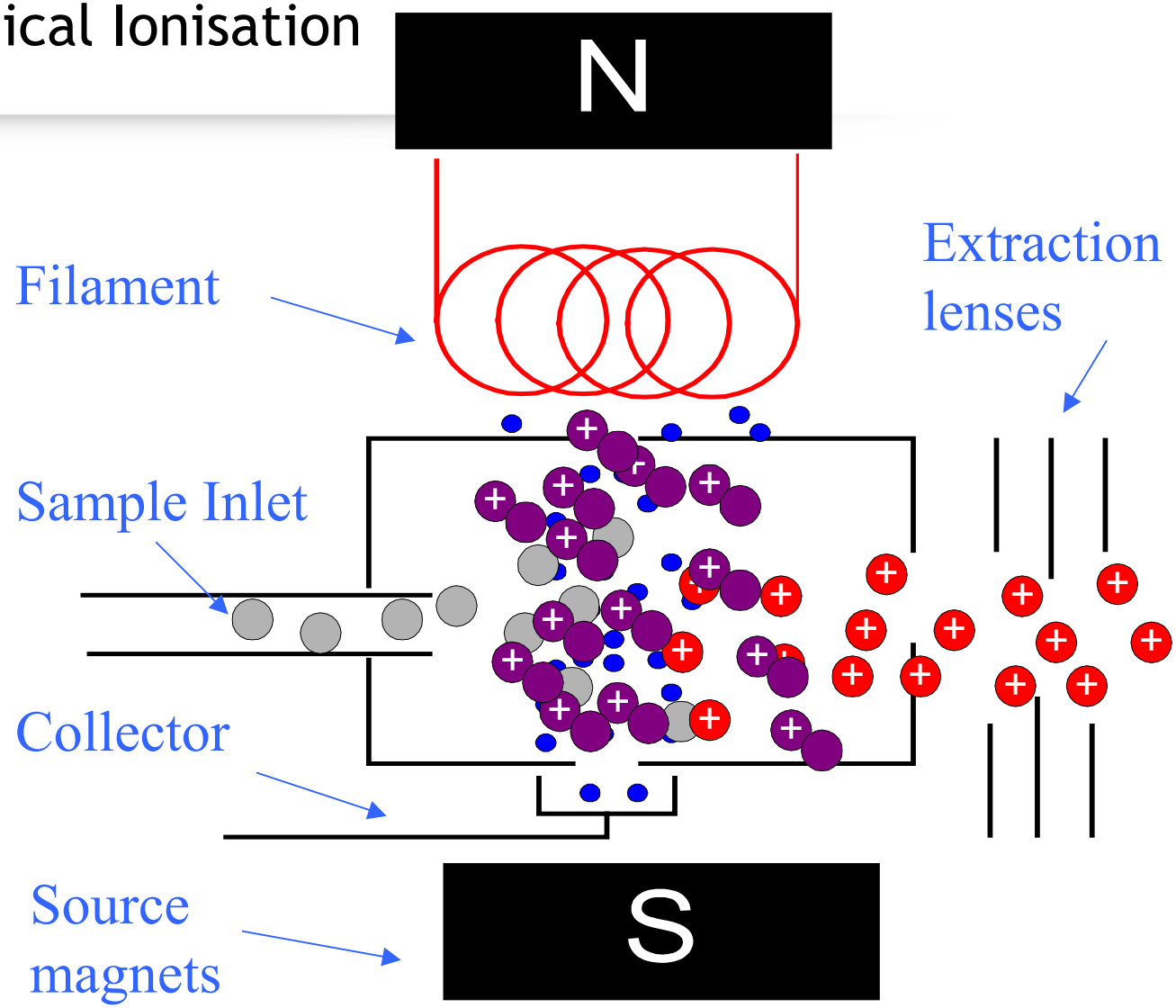
Chemical Ionisation

- 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.

Thermo Finnigan

Chemical Ionisation



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Chemical Ionisation

Primary ions

$$CH_4 + e^- \longrightarrow CH_4^{+\cdot} + 2e^-$$

$$CH_4^{+\cdot} \longrightarrow CH_3^+ + H\cdot$$

Secondary ions

$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH_3\cdot$$

$$CH_3^+ + CH_4 \longrightarrow C_2H_5^+ + H_2$$

Proton donation

$$CH_5^+ + M \longrightarrow CH_4 + MH^+$$

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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

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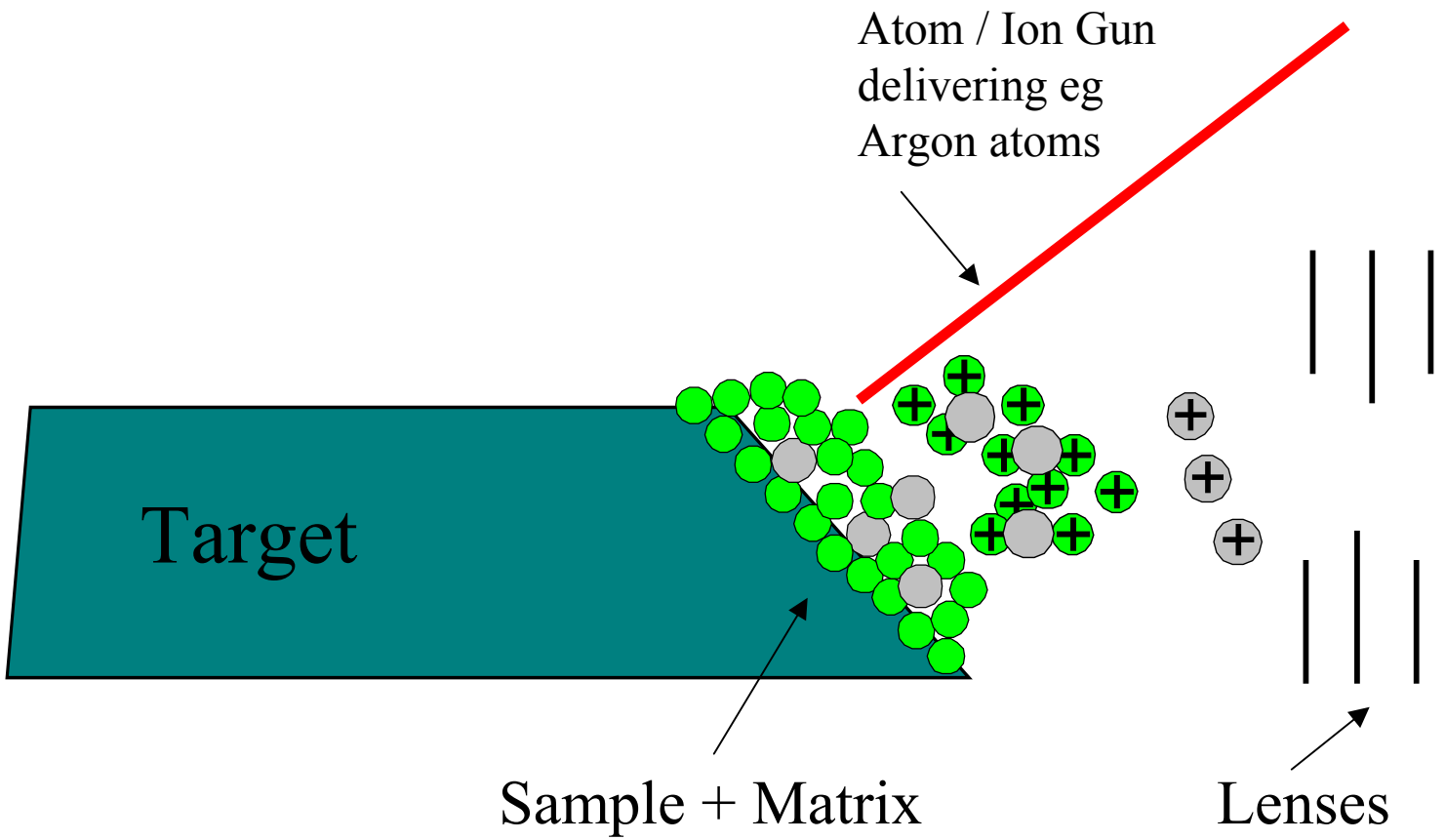
FAB Source



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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

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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

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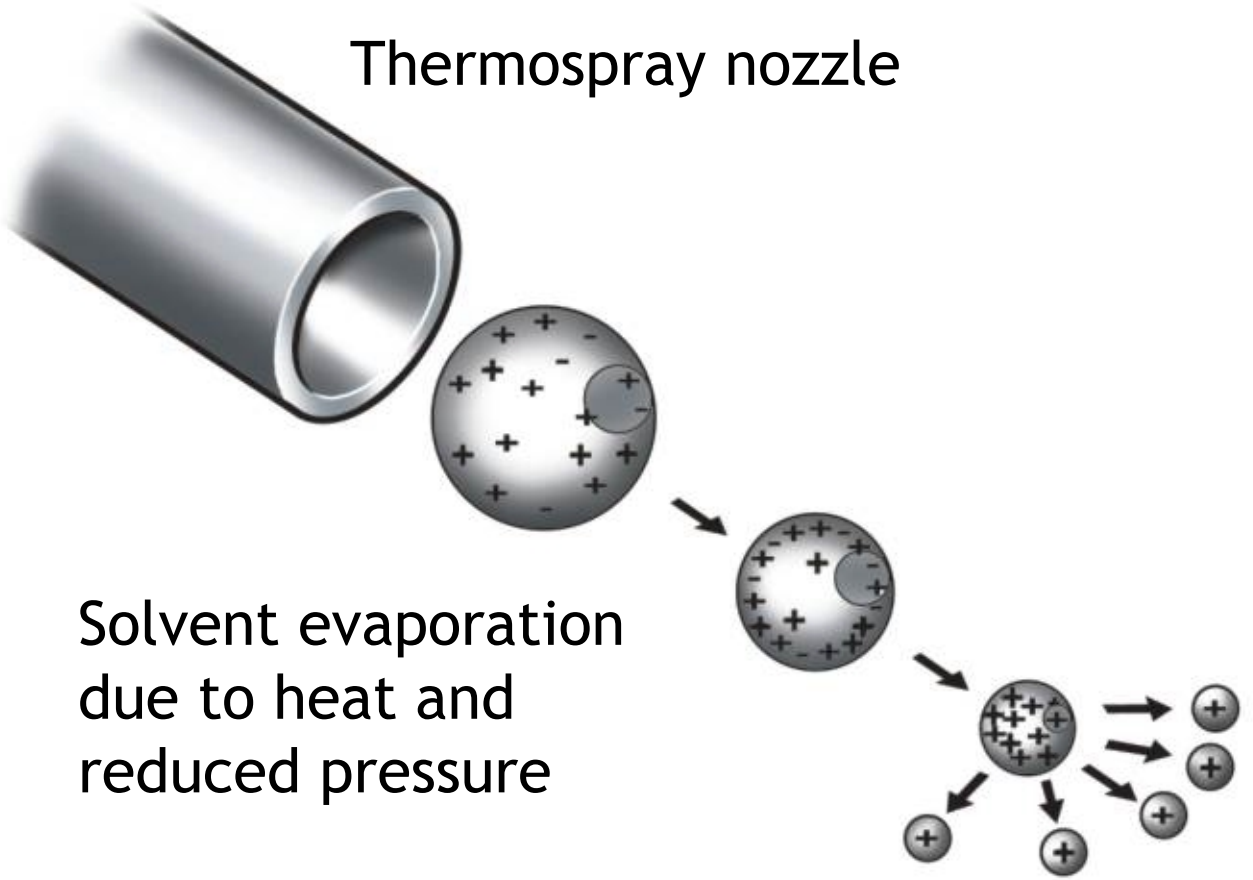
Thermospray Process



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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

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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

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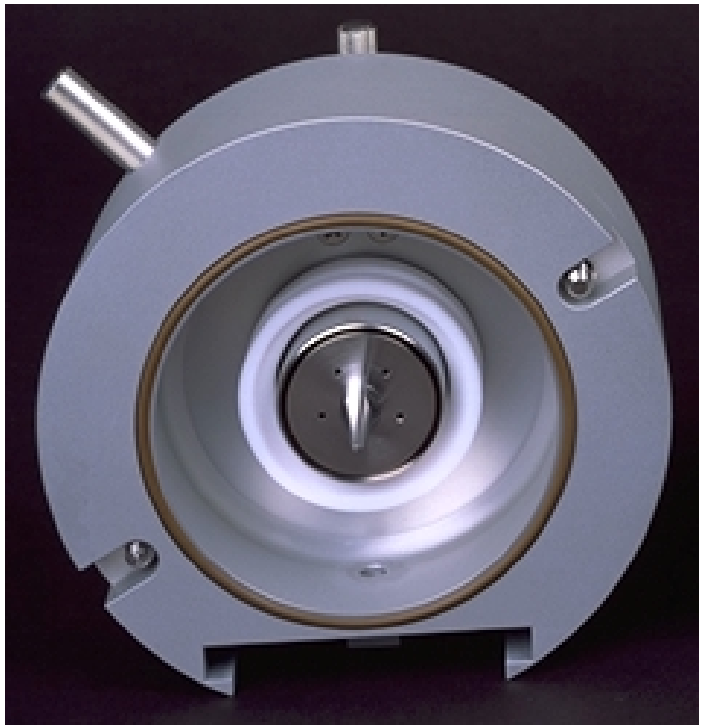
Electrospray

- 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 ul/min, nanospray (10 nL/min – 2 uL/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

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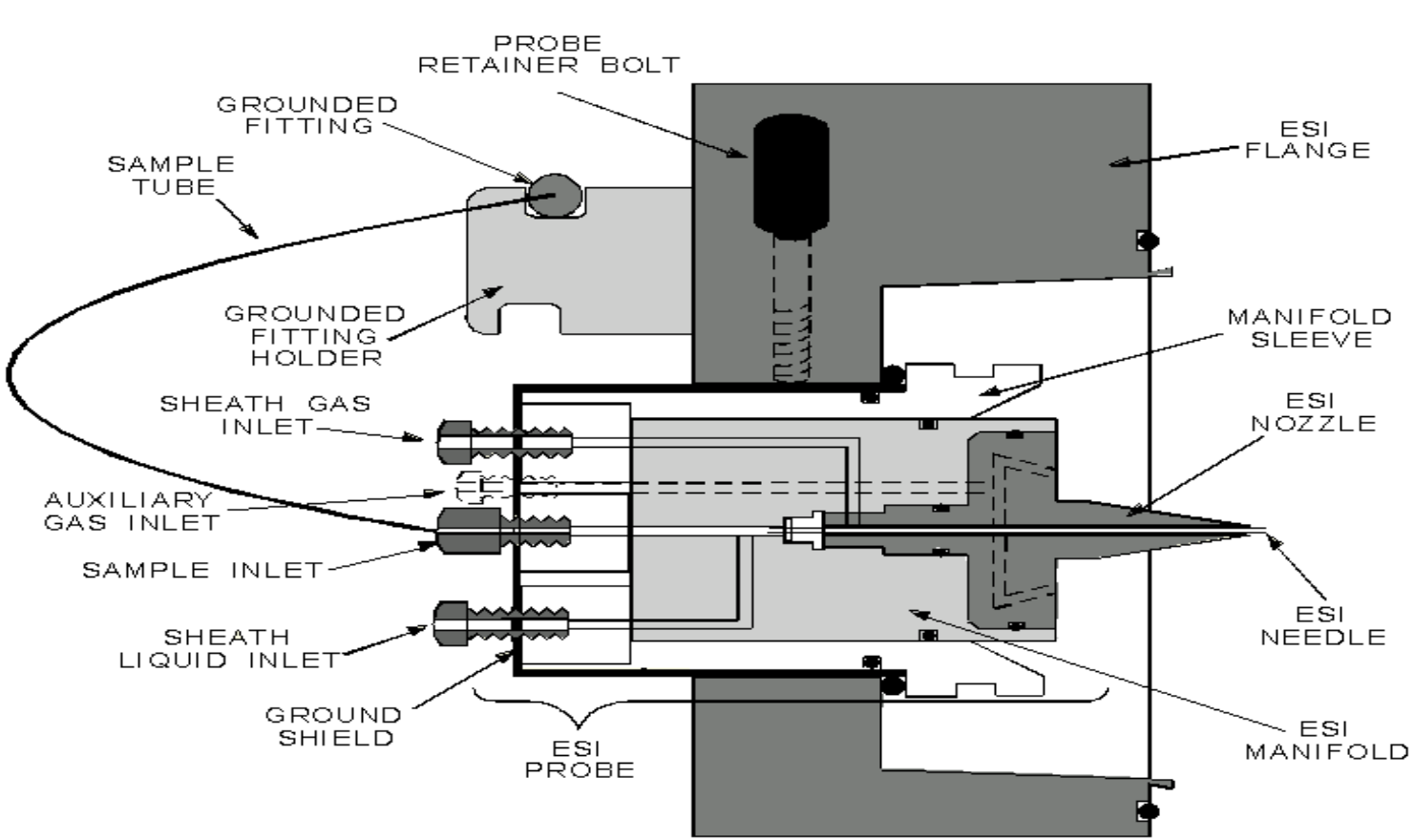
ESI Probe



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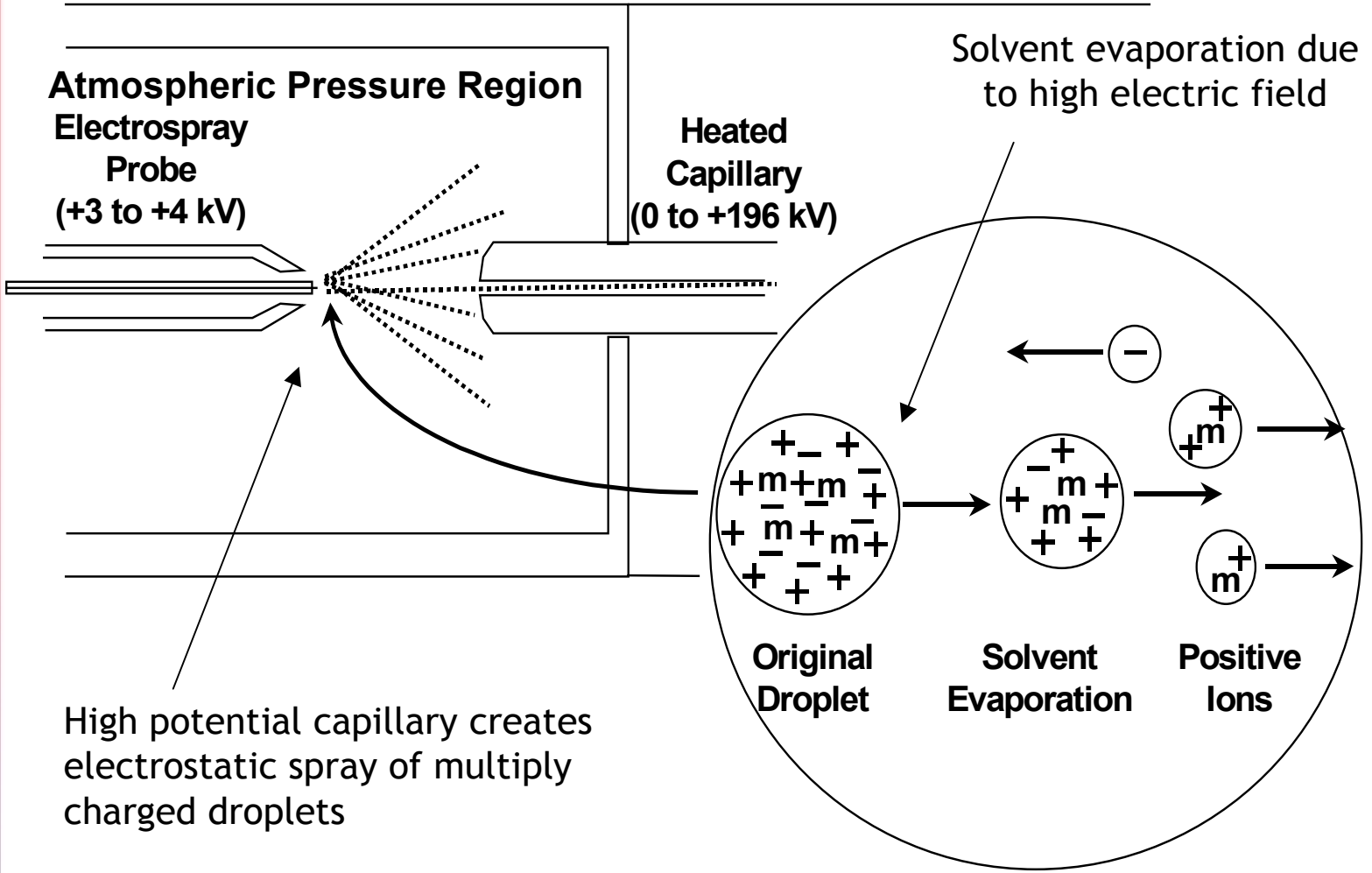
Electrospray Process



High potential capillary creates electrostatic spray of multiply charged droplets

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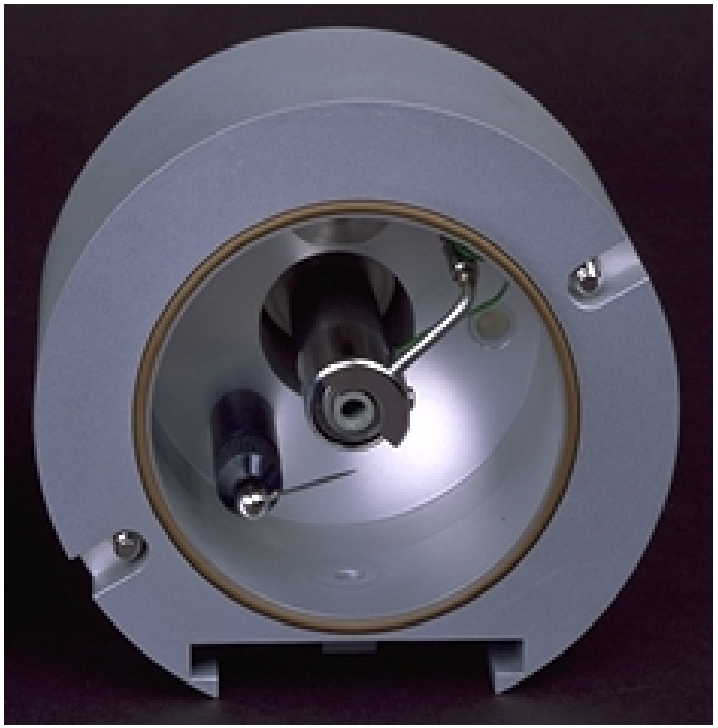
APCI

- 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 ^{o}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

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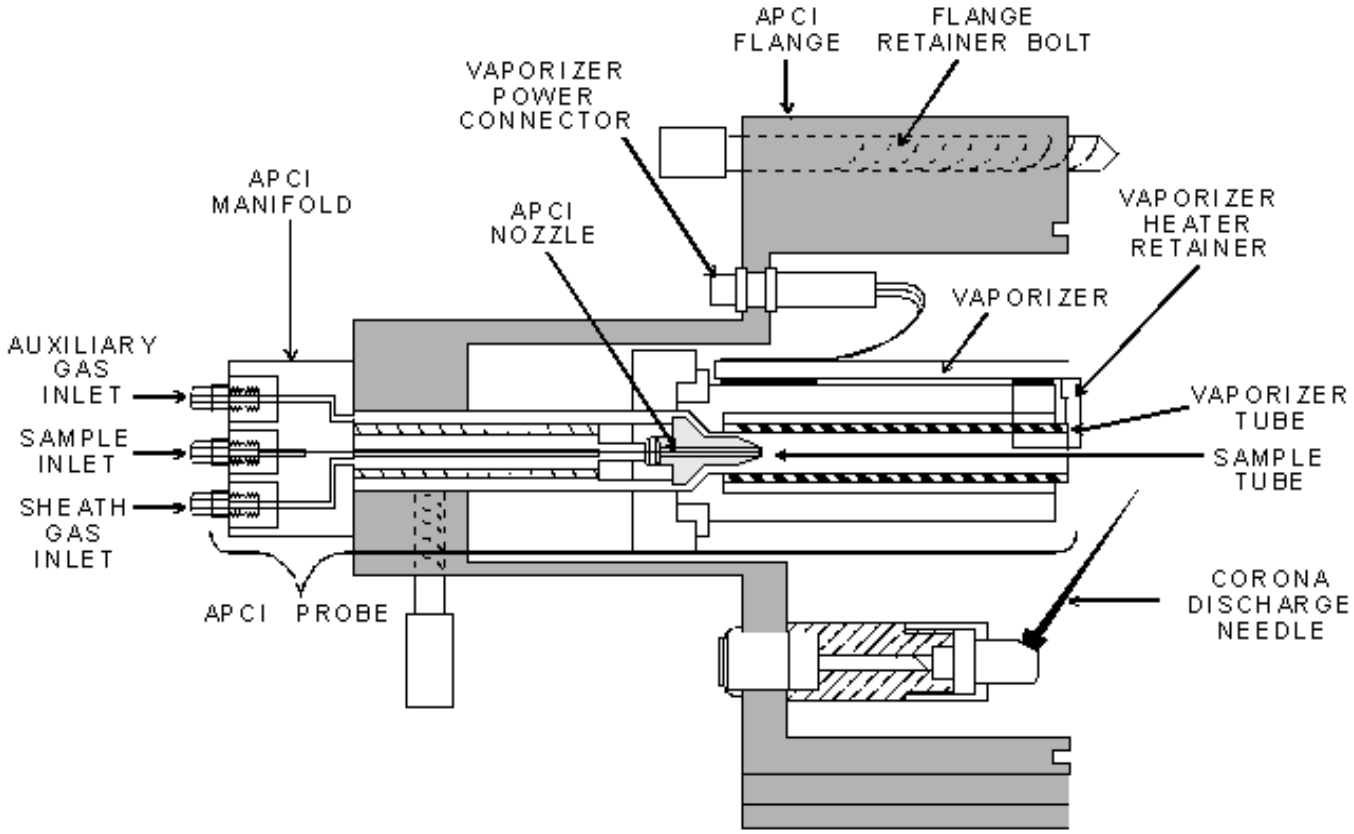
APCI Probe



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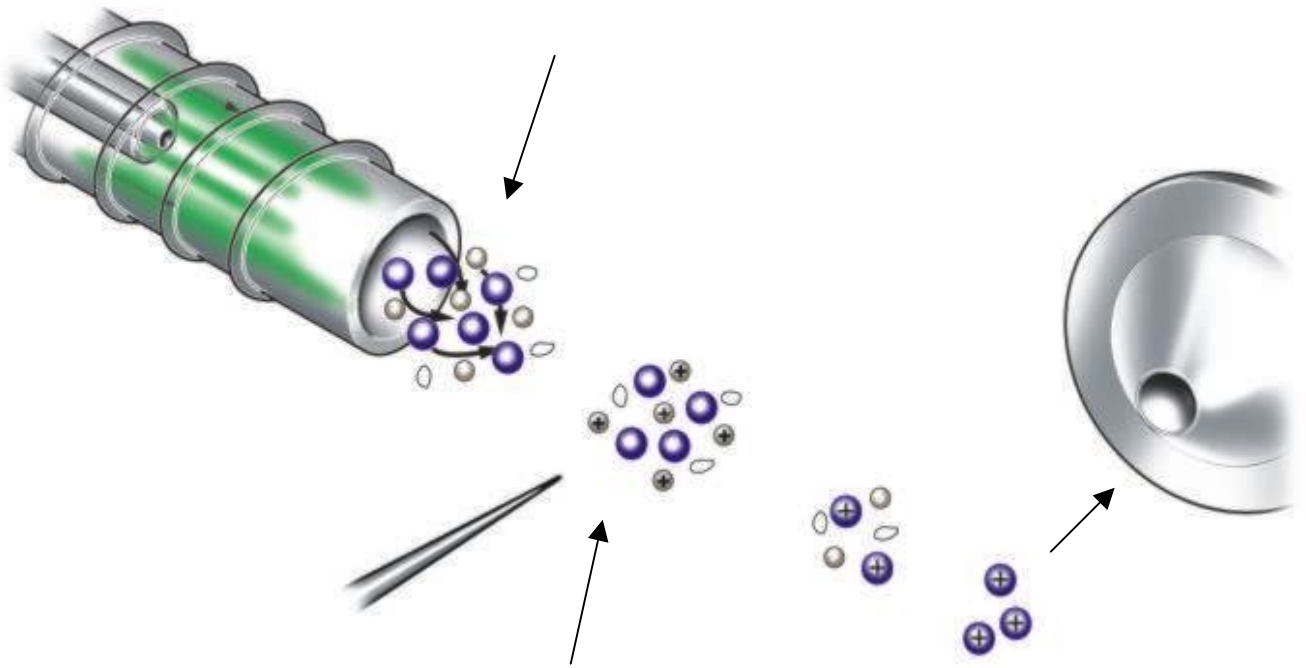
APCI Process

LC eluent evaporated
from heated vaporiser



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

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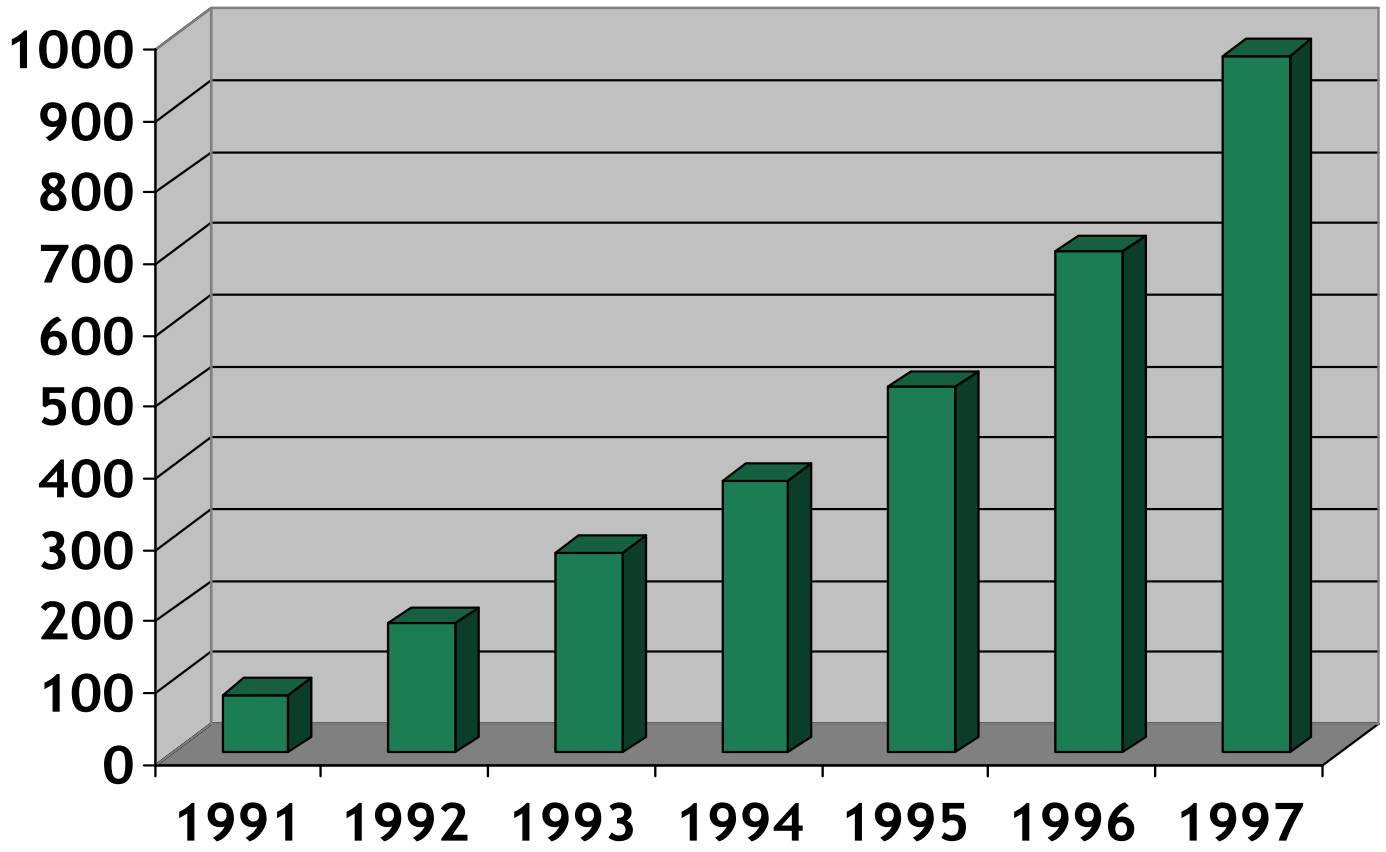
API Publications



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, H_2O,
  - 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

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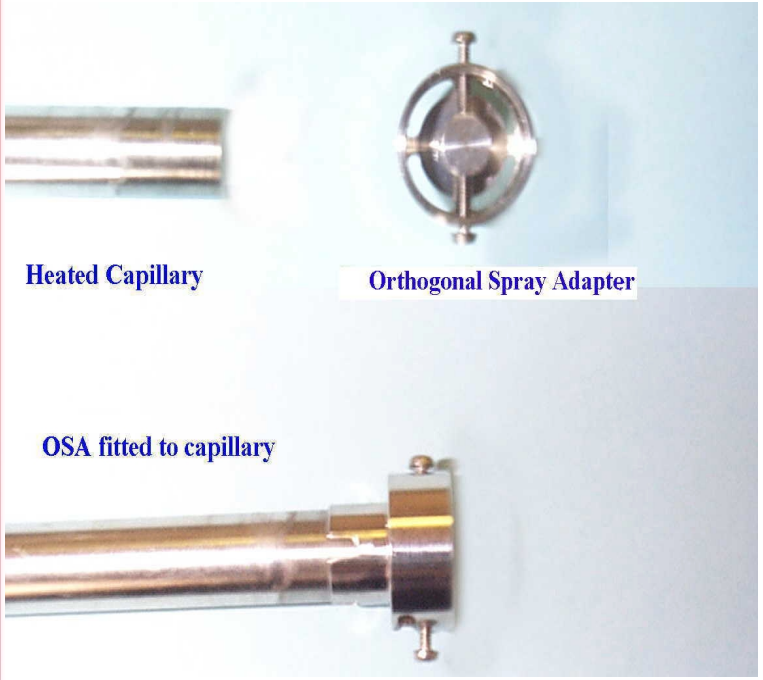
Orthogonal Sampling Adaptor



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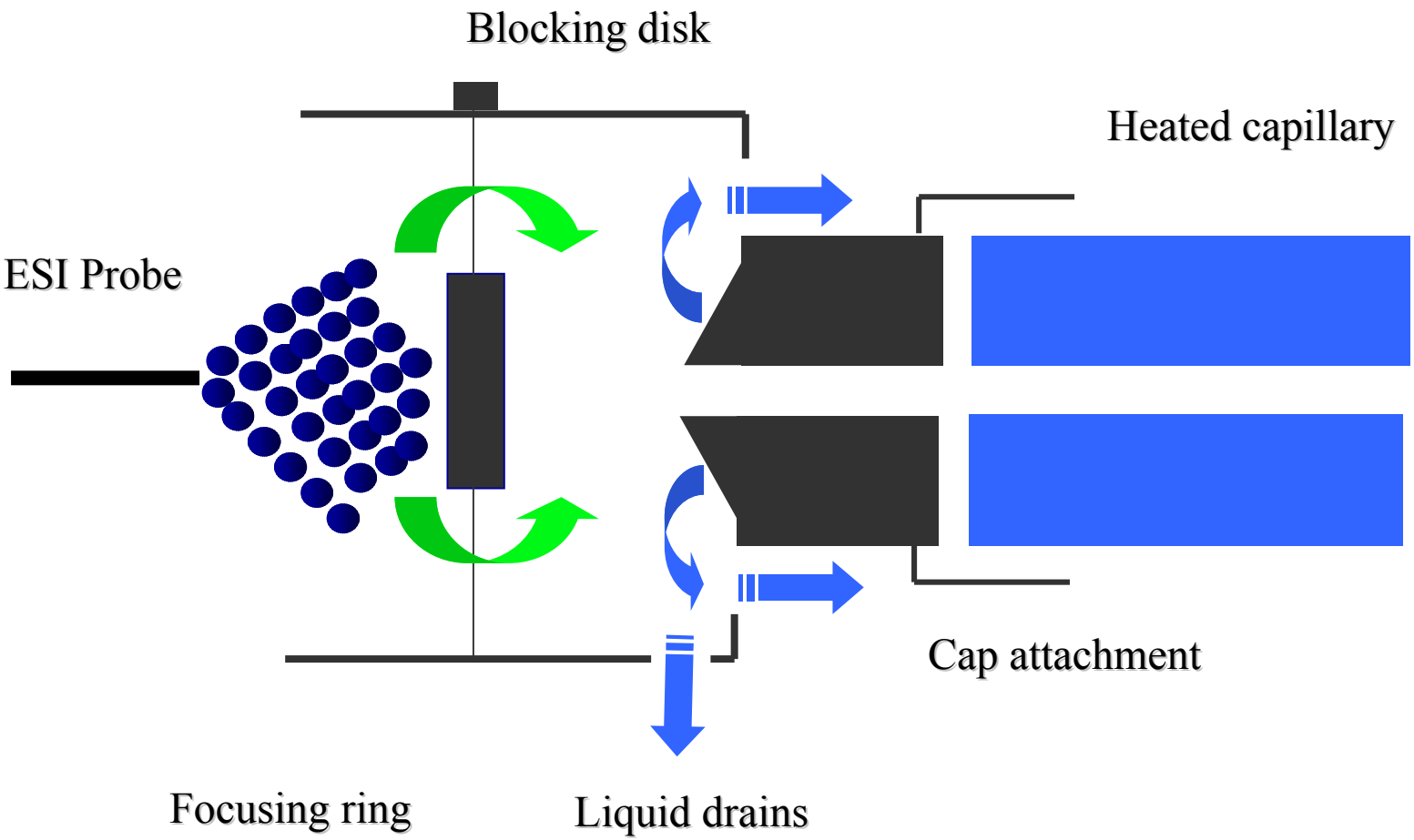
Orthogonal Sampling Adaptor



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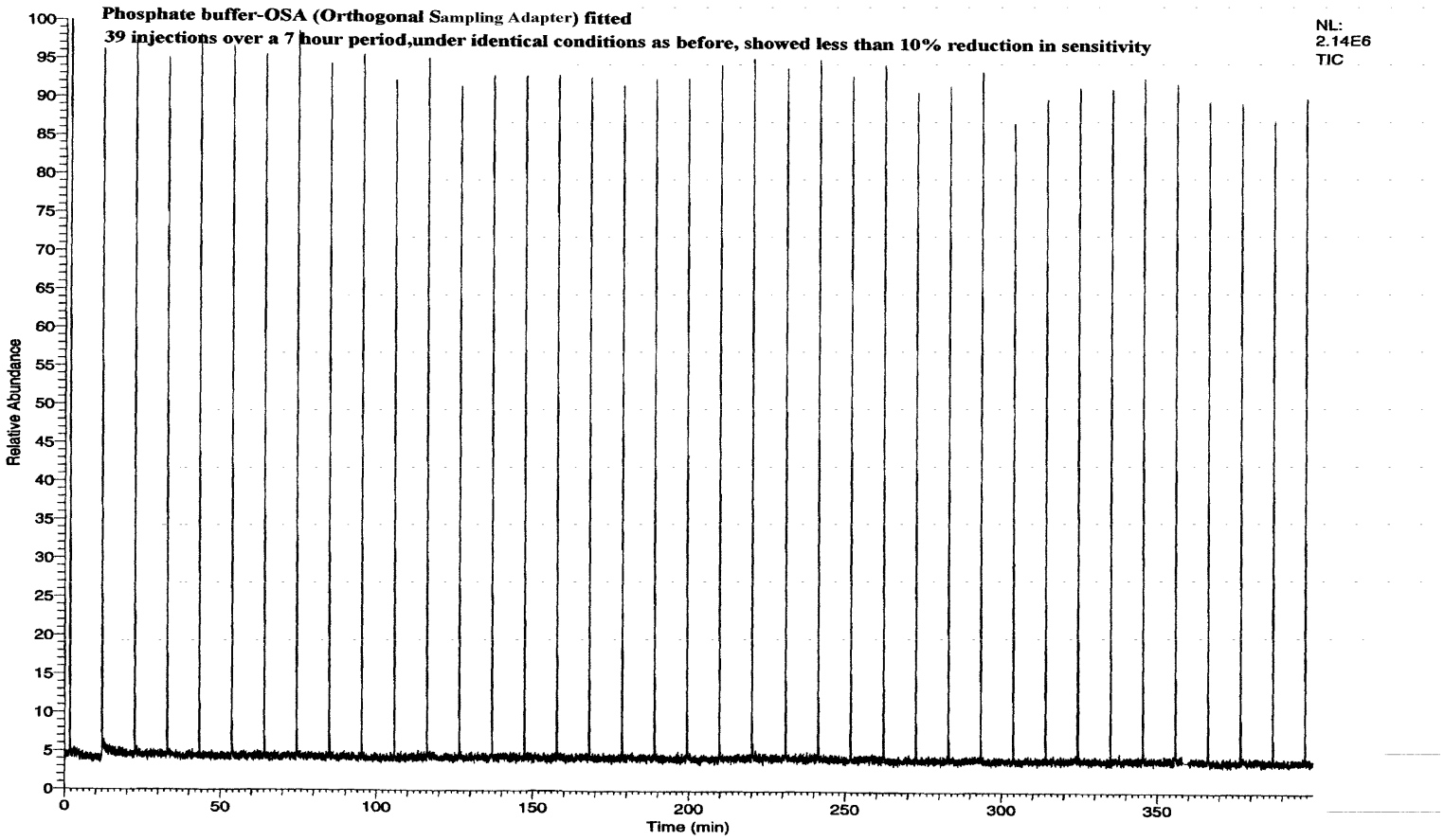
OSA fitted – 10mM Phosphate solution



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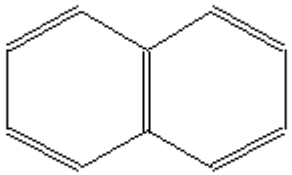
Problems

How would you analyse this compound ?

Naphthalene



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

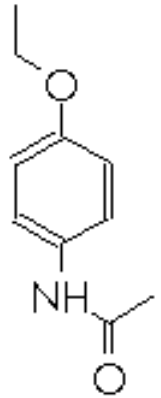
A: EI, GC/MS

Thermo Finnigan

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

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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 ?

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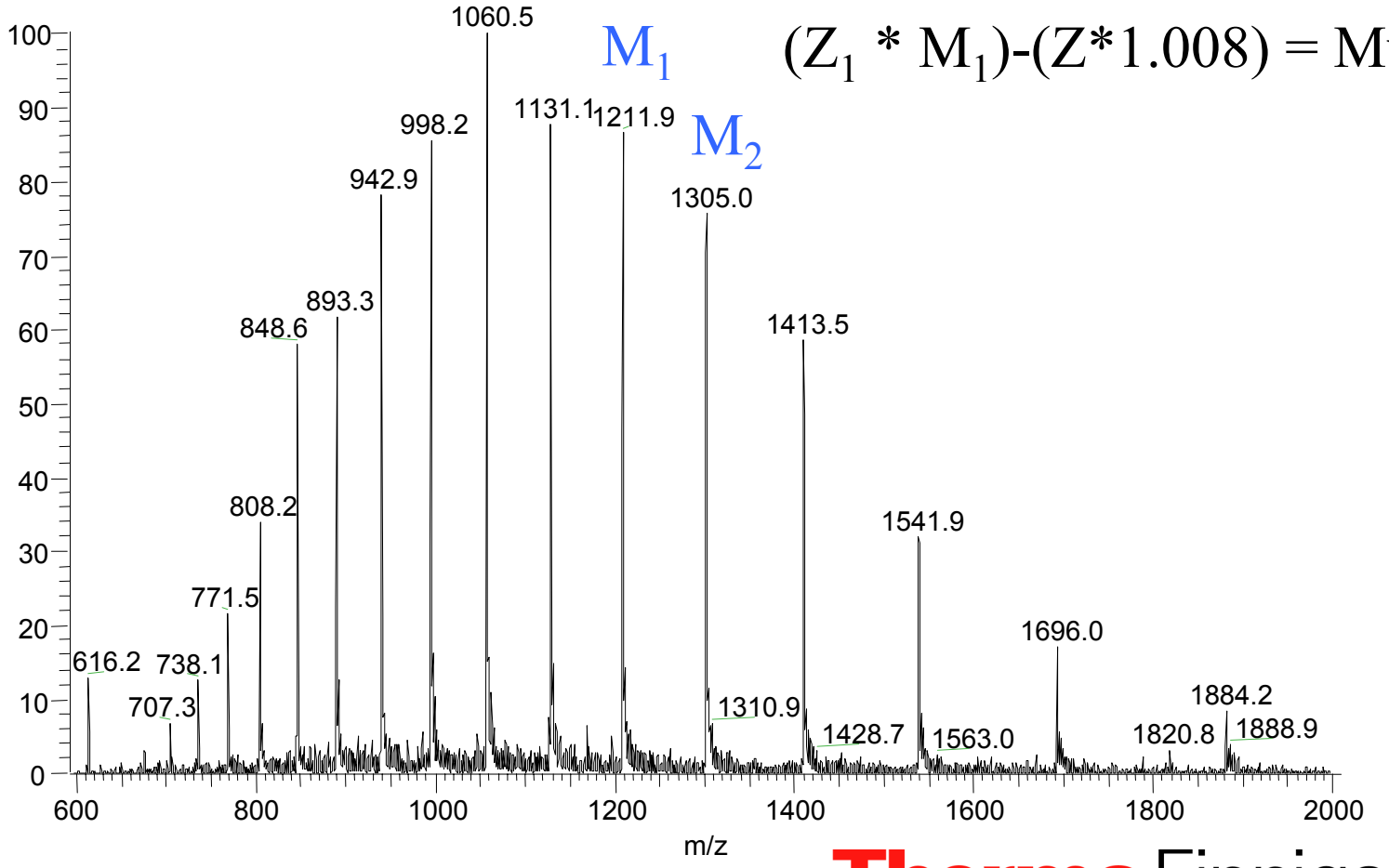
Multiply charged myoglobin ions from ESI

$$(M_2\text{-}1.008)\ /M_1\text{-}M_2 = Z_1$$

$$(Z_1 * M_1)\text{-}(Z*1.008) = \text{Mwt}$$



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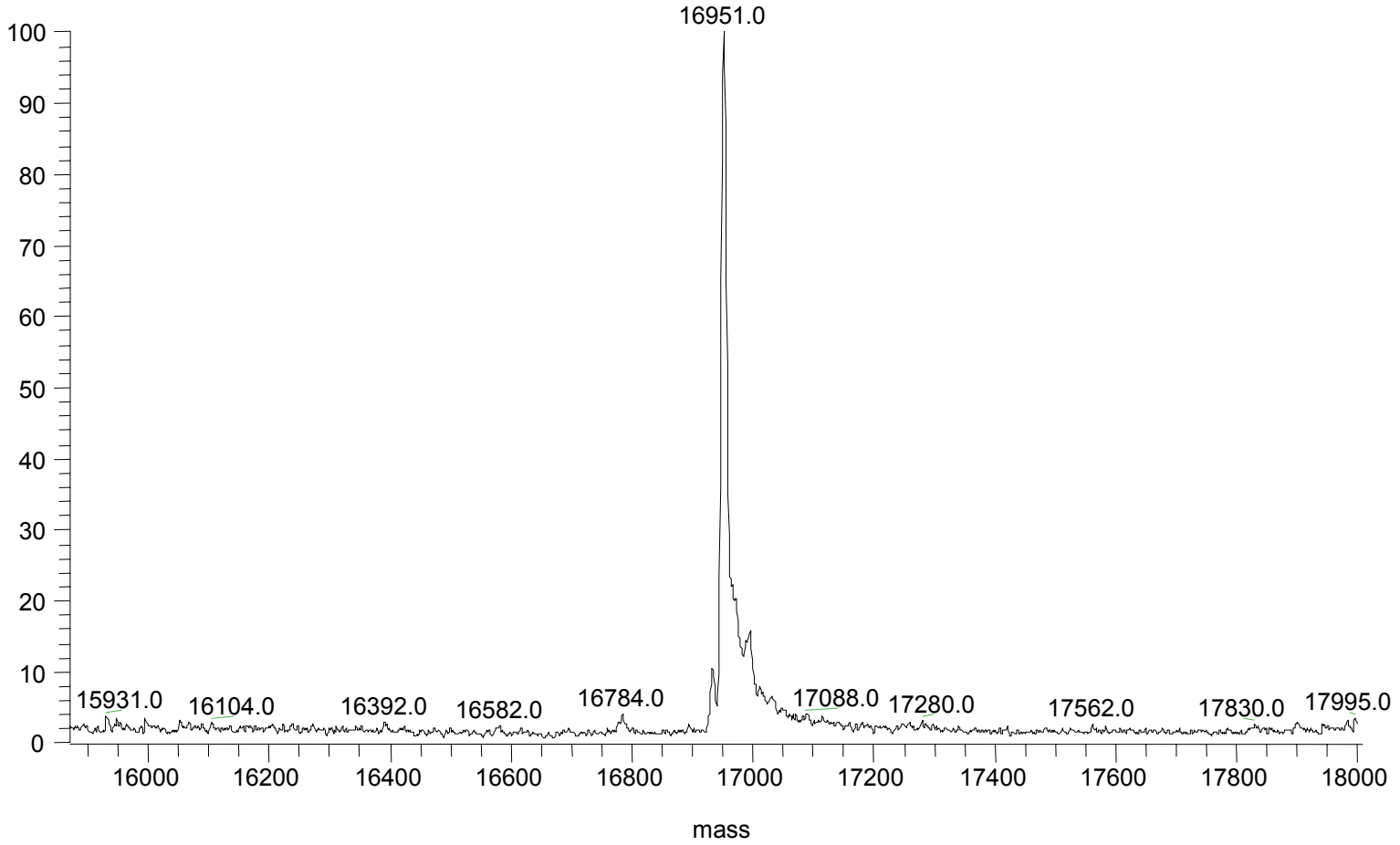
Deconvoluted myoglobin spectrum



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