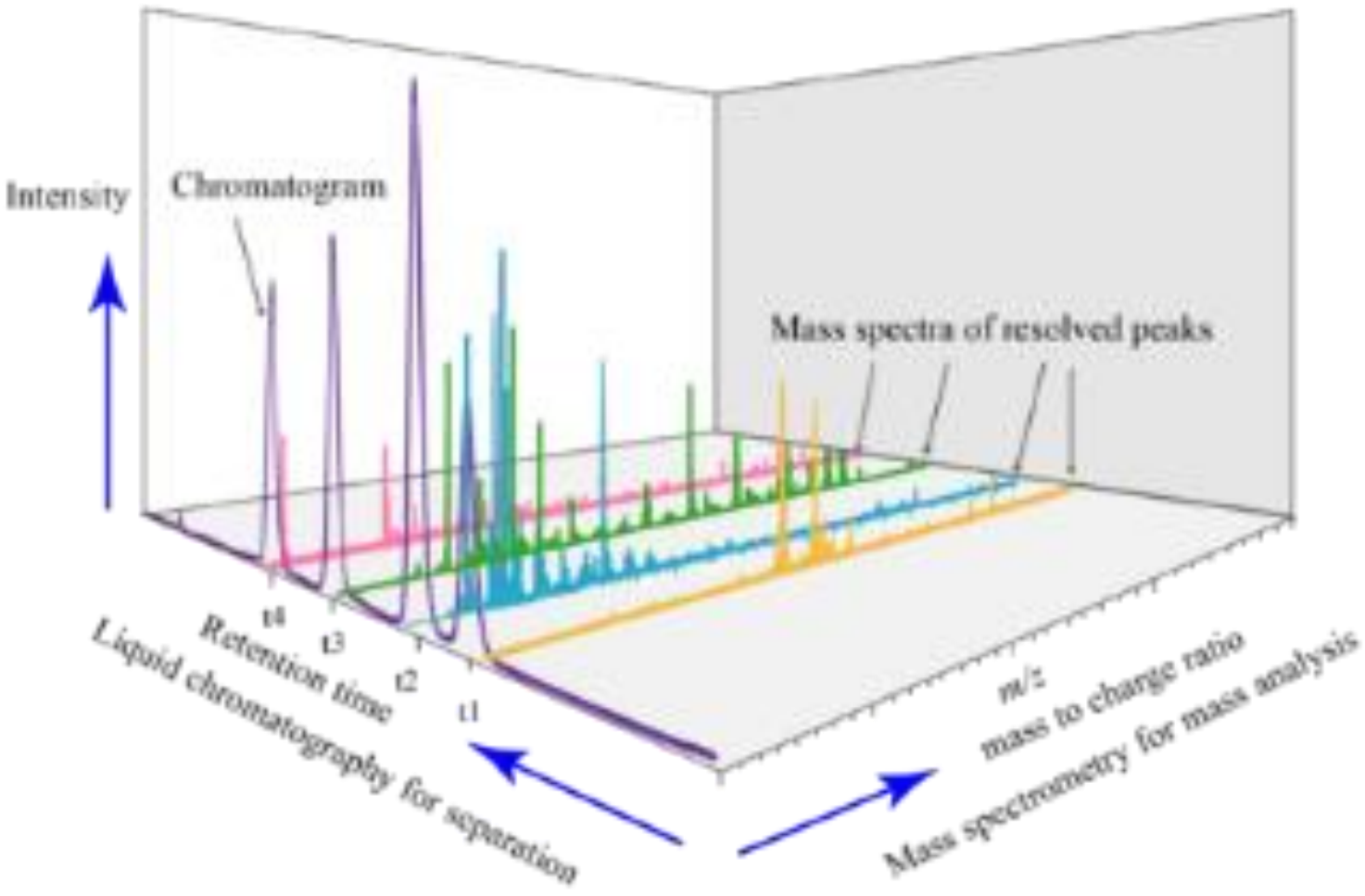
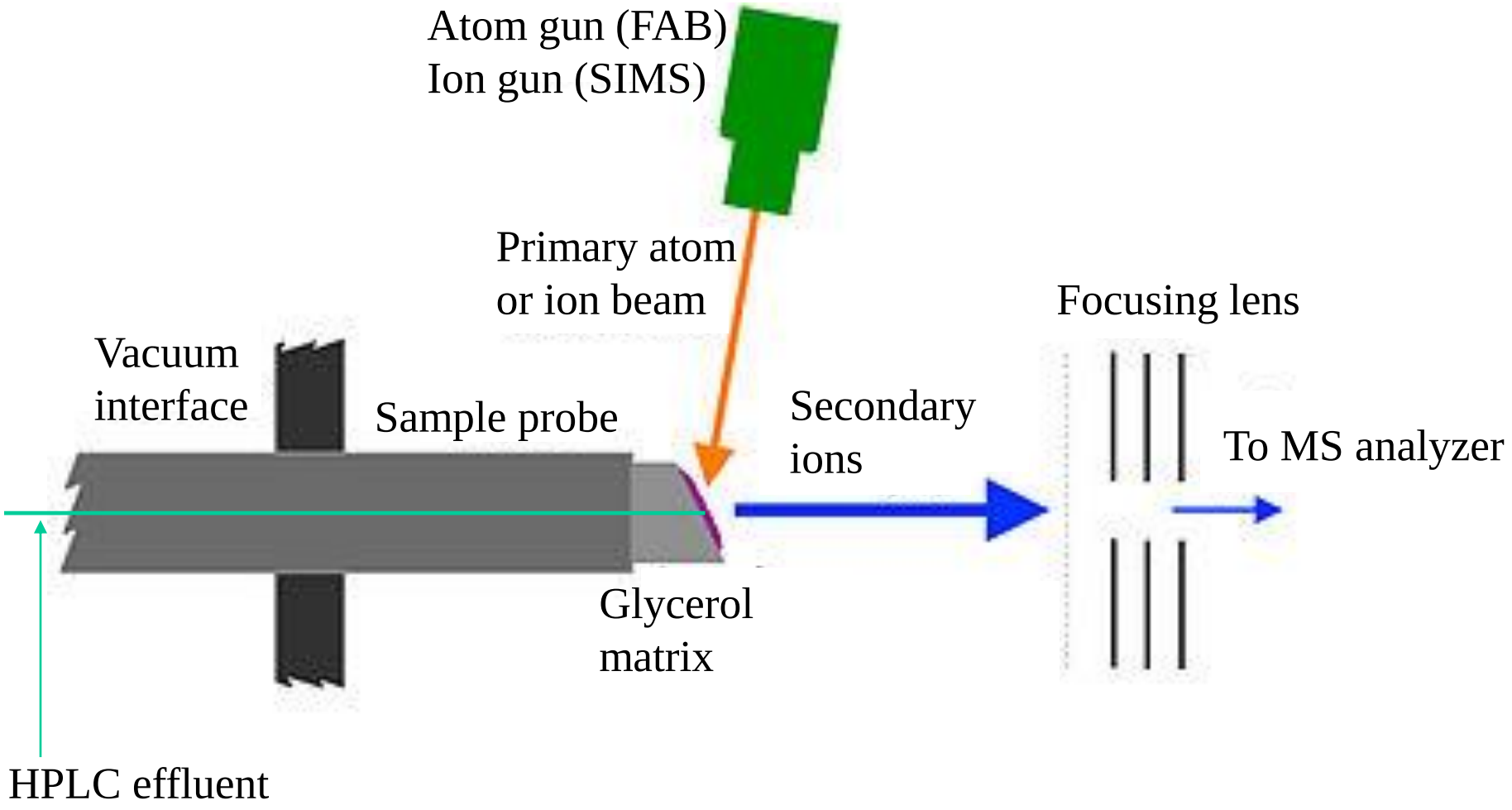
HPLC-MS

LC-MS interfaces

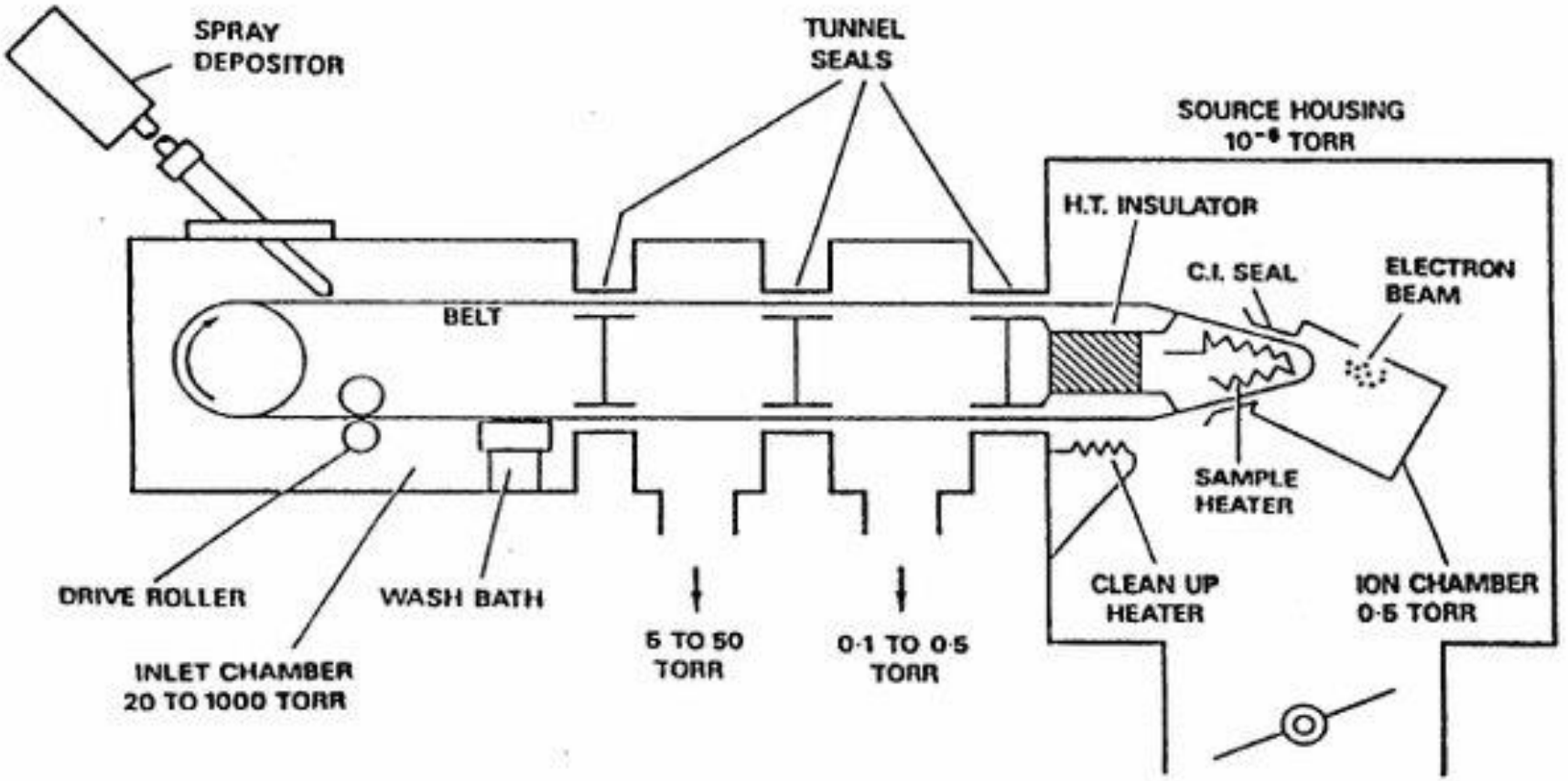
- Continuous-flow secondary ion mass spectrometry (SIMS)
  Continuous-flow fast atom bombardment (FAB)
- Moving belt interface
- Atmospheric pressure chemical ionization (APCI)
- Electrospray ionization (ESI)


Intensity
Chromatogram
Mass spectra of resolved peaks
t4
t3
t2
t1
Retention time
Liquid chromatography for separation
m/z
mass to charge ratio
Mass spectrometry for mass analysis

Continuous-flow SIMS or FAB

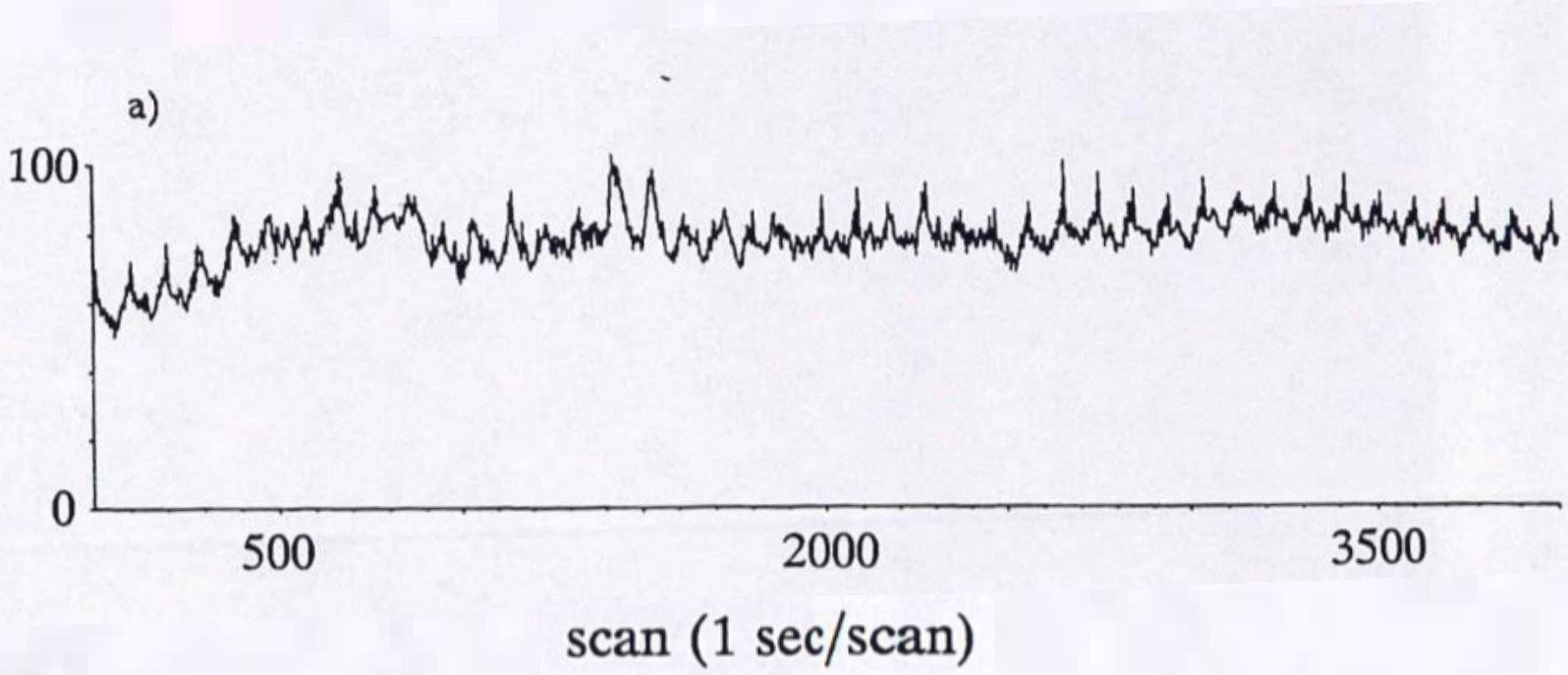


Moving belt interface for EI/CI-MS

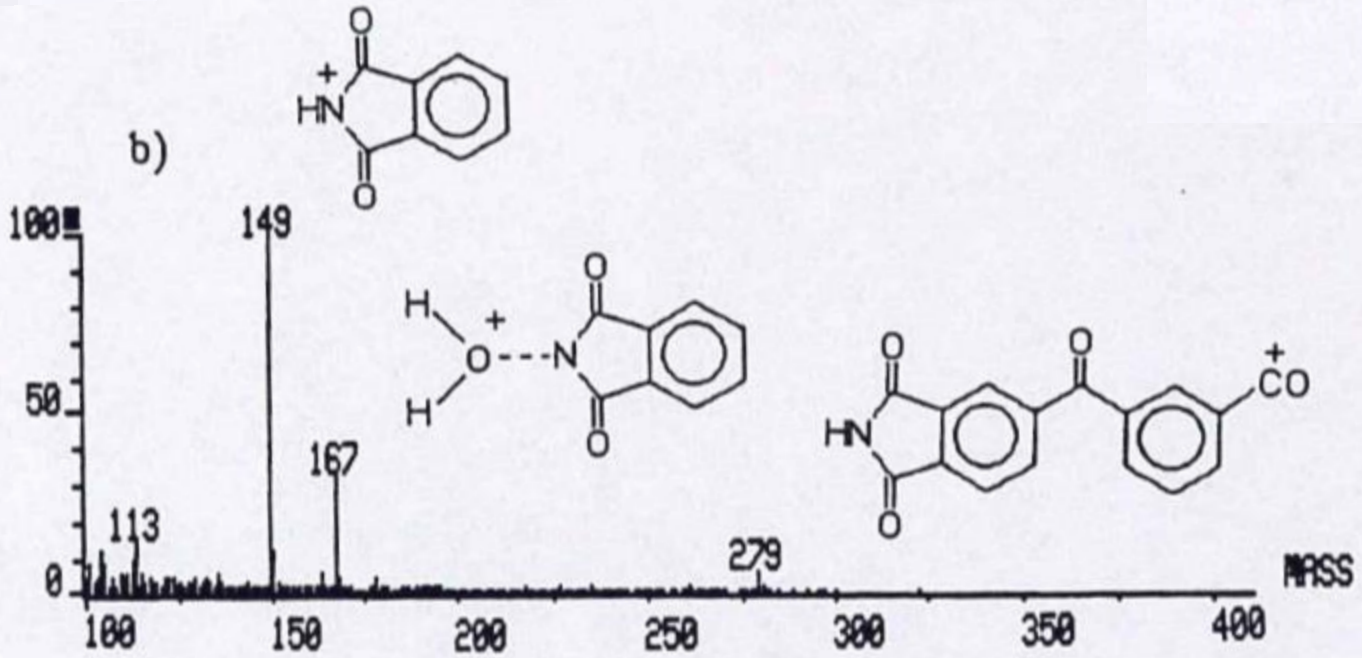


https://www.researchgate.net/publication/257329197_Coupling_Mass_Spectrometry_with_Liquid_Chromatography_for_Petroleum_Researc/figures?lo=1

Total ion current, belt only

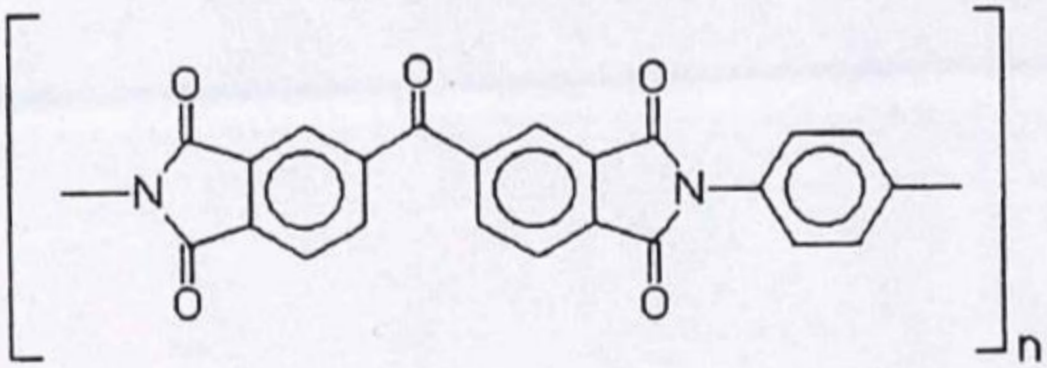


b)

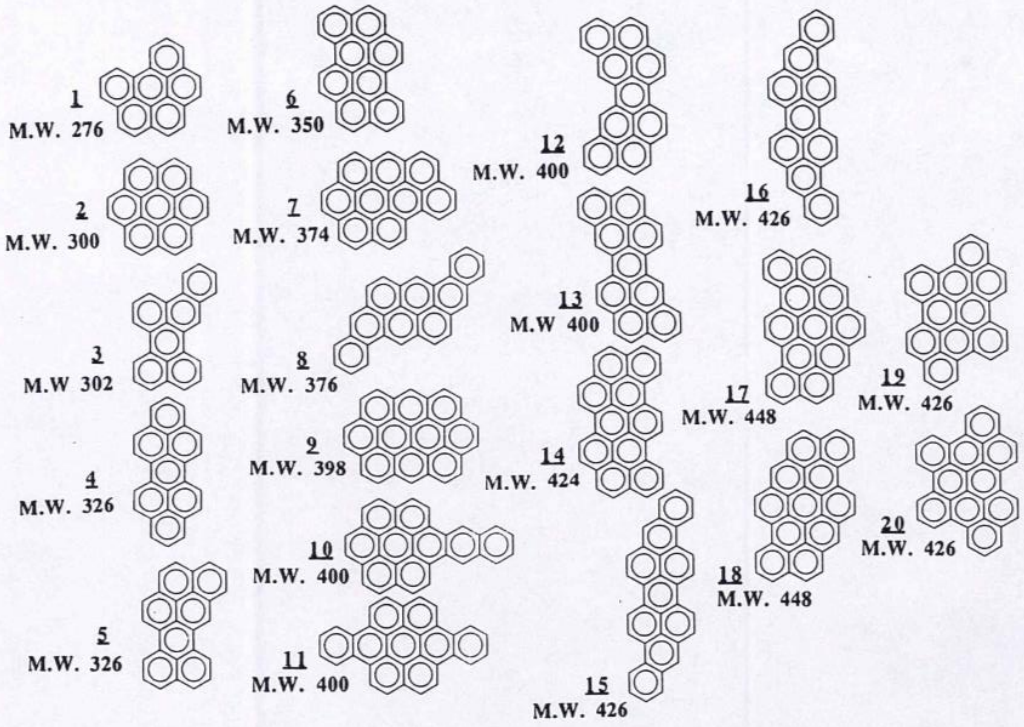


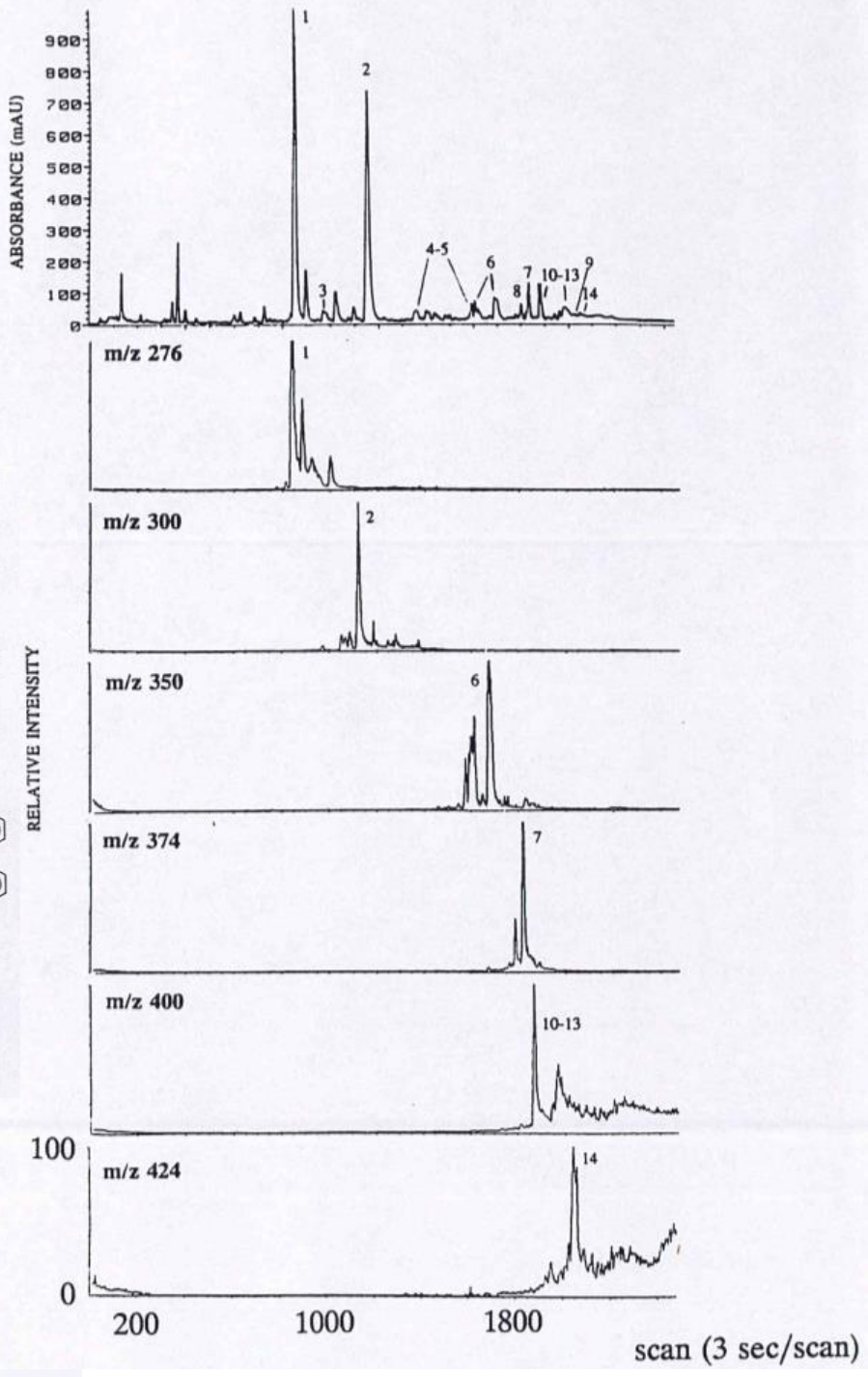
Structure of polyimide

c)

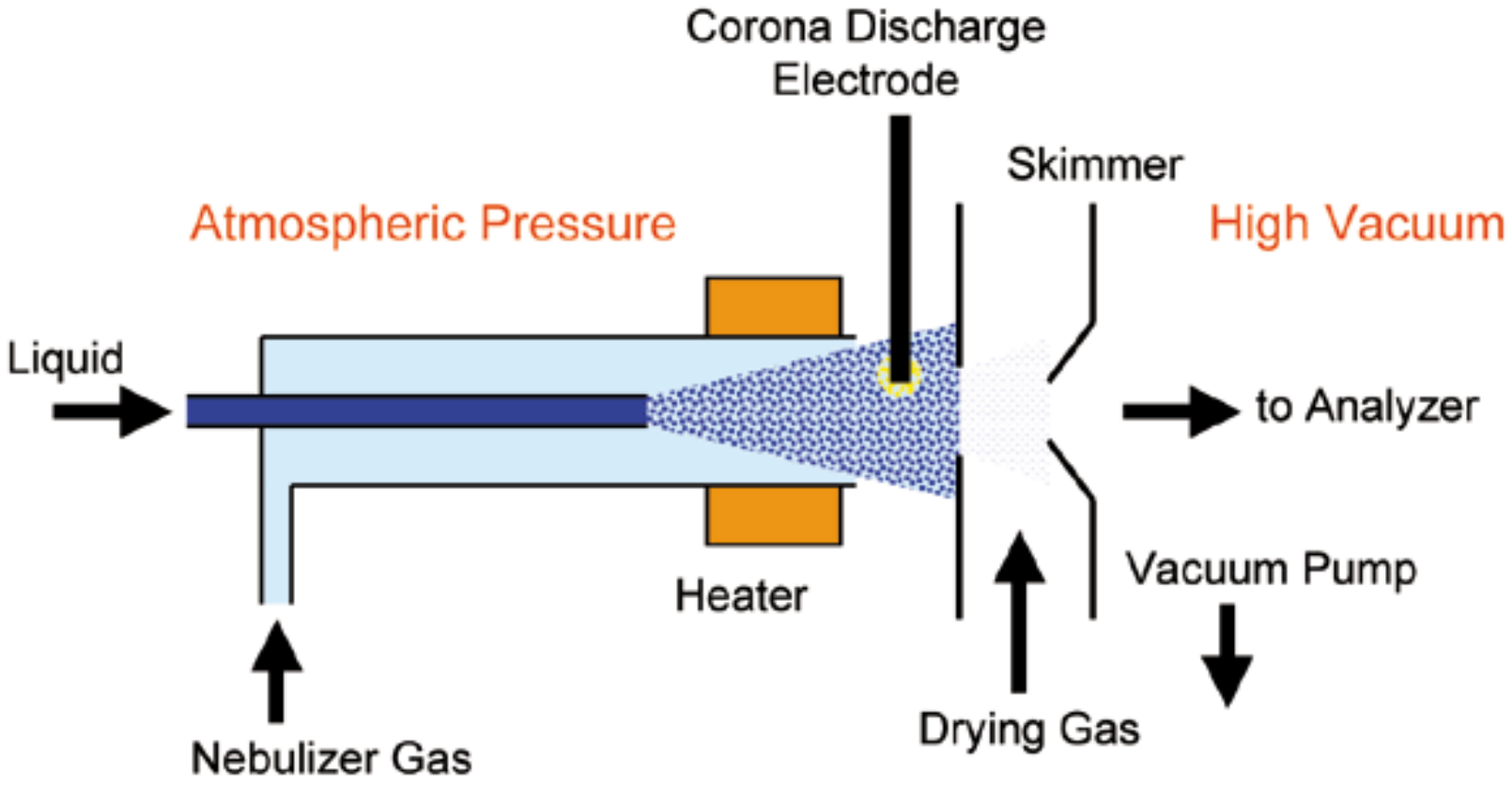


UV (254 nm) and SIC for PACs

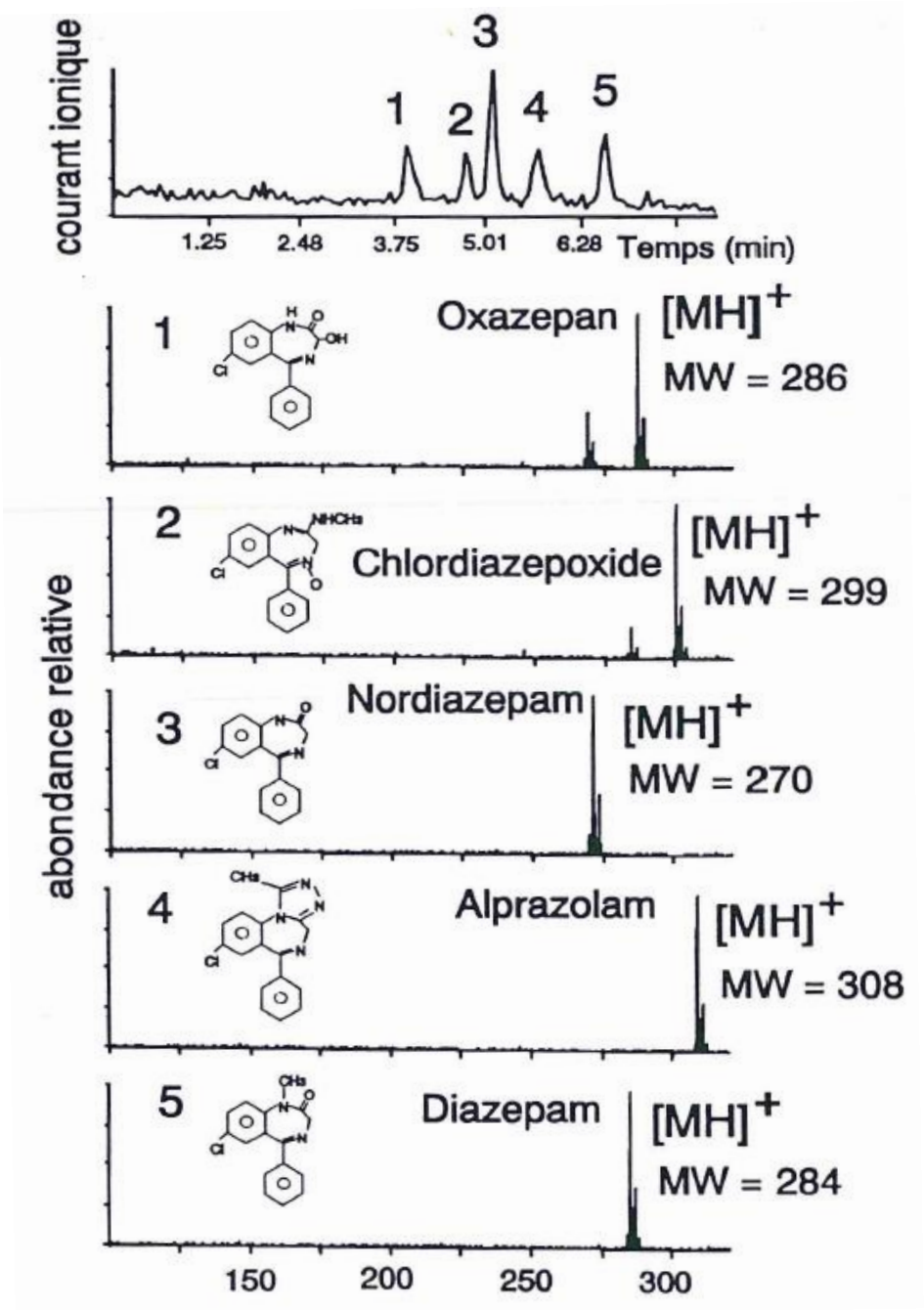




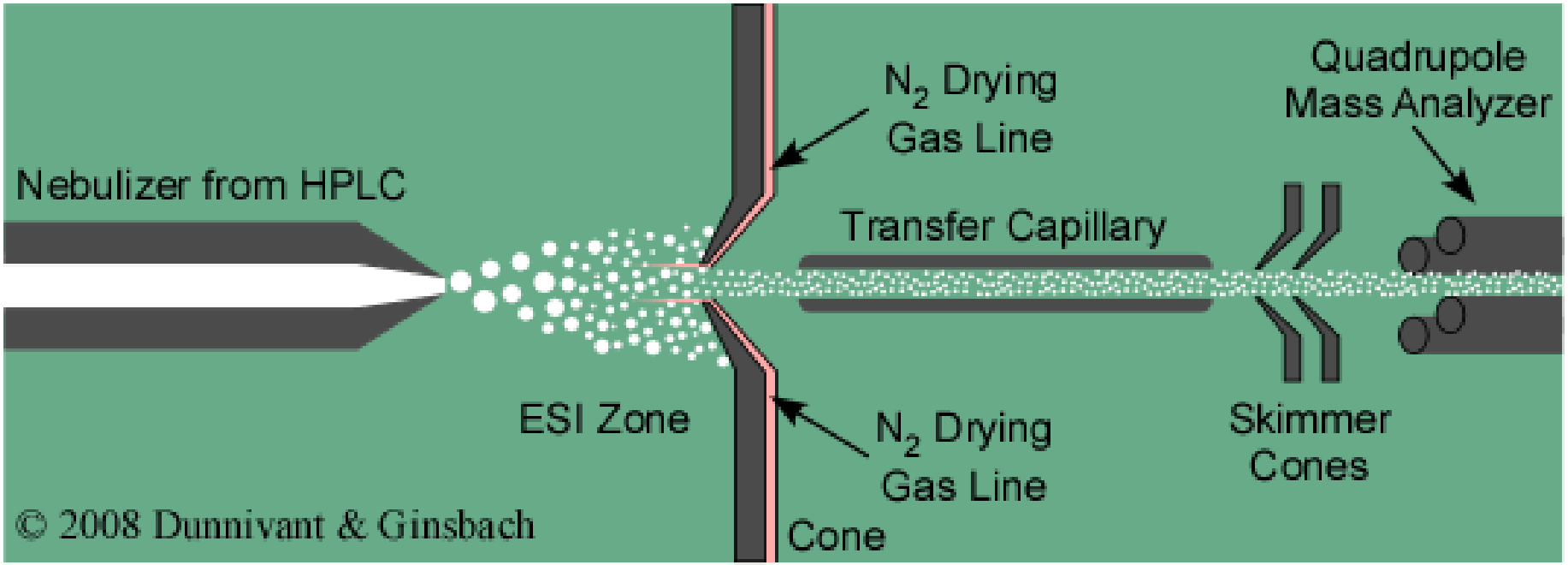
Atmospheric pressure CI interface



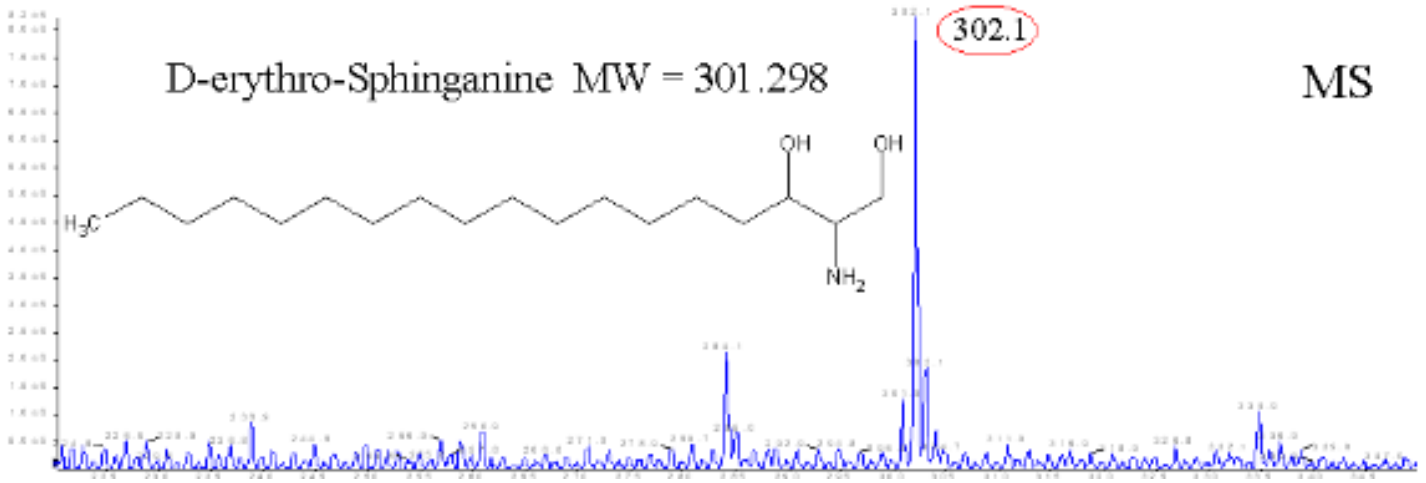
RP-HPLC/MS of benzodiazepines (APCI)



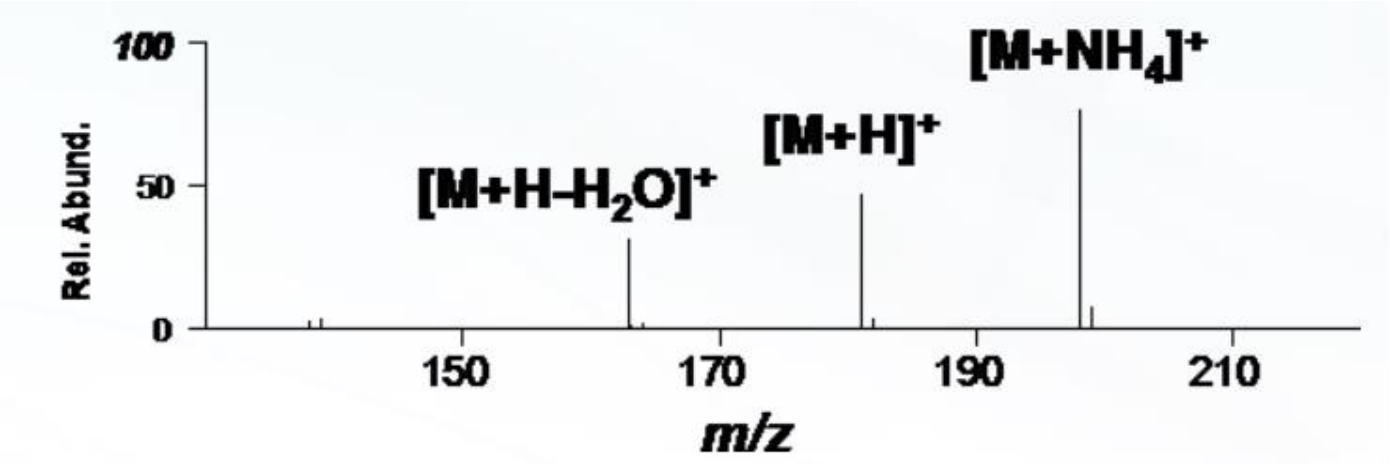
Electrospray ionization interface



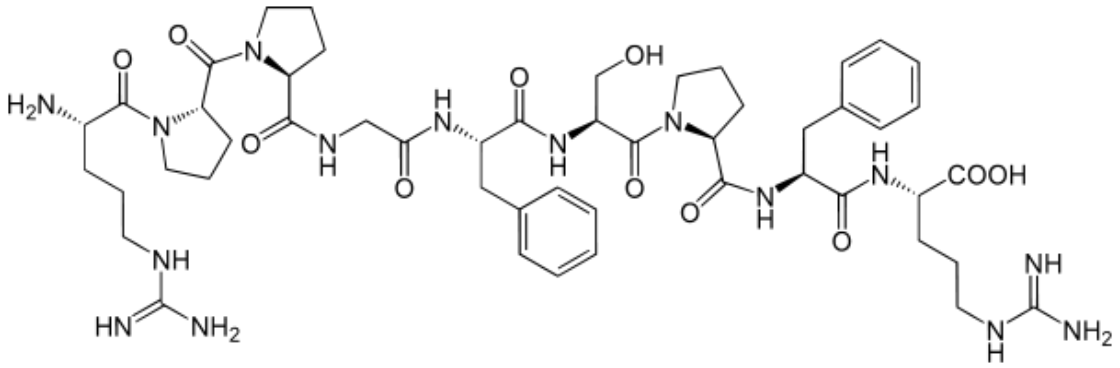
ESI-MS of small molecules

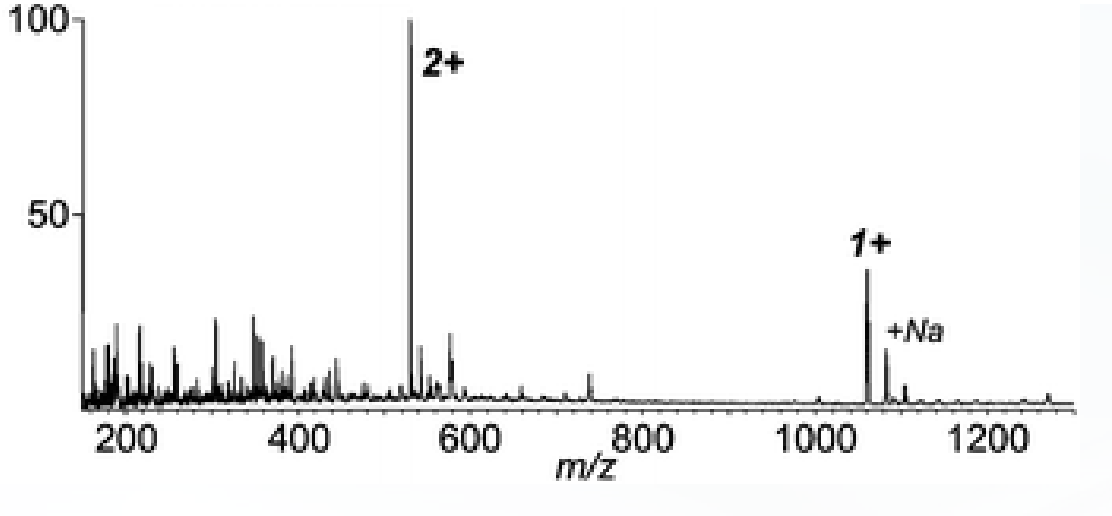


Aspirin

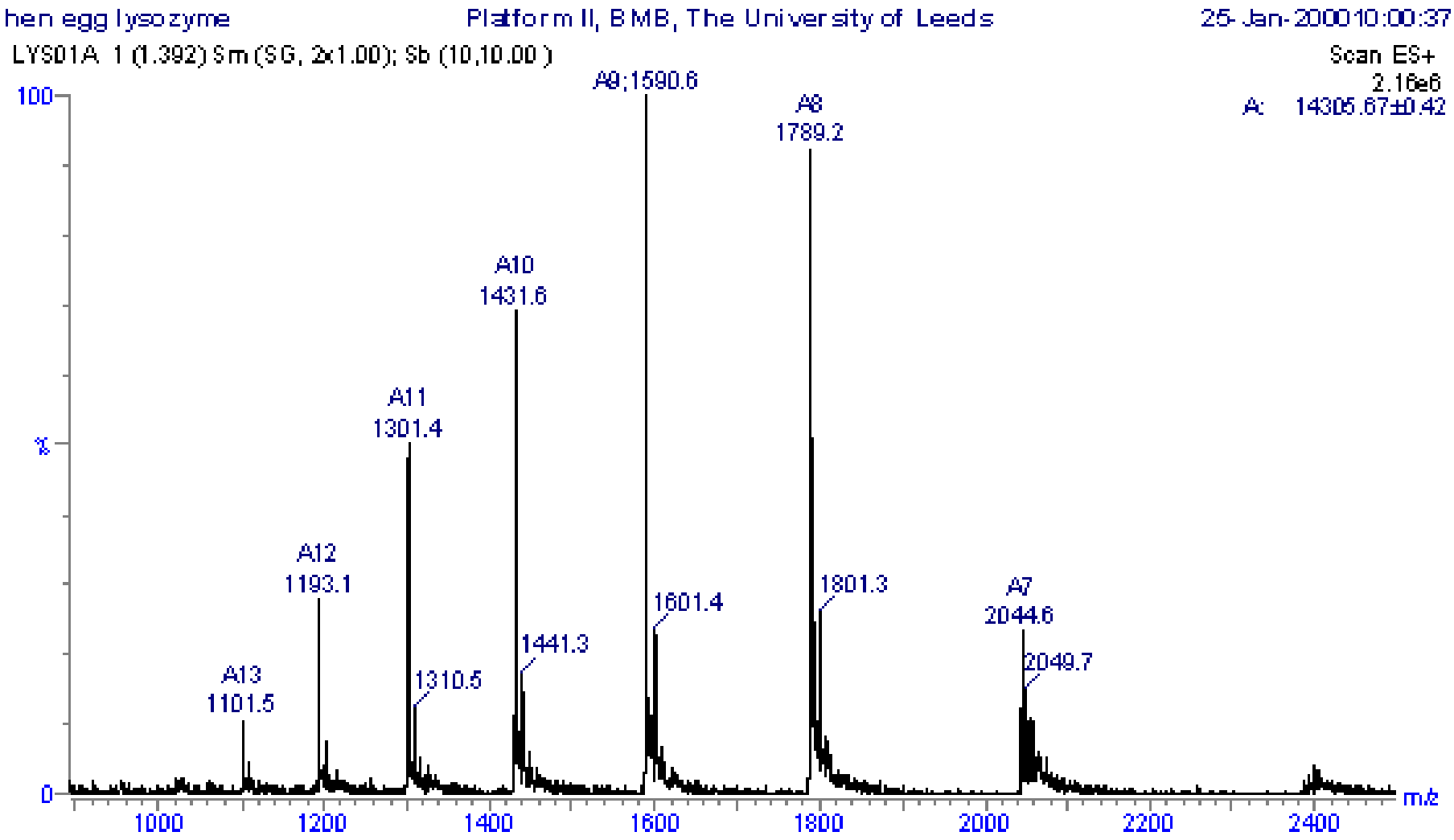


ESI-MS of larger molecules



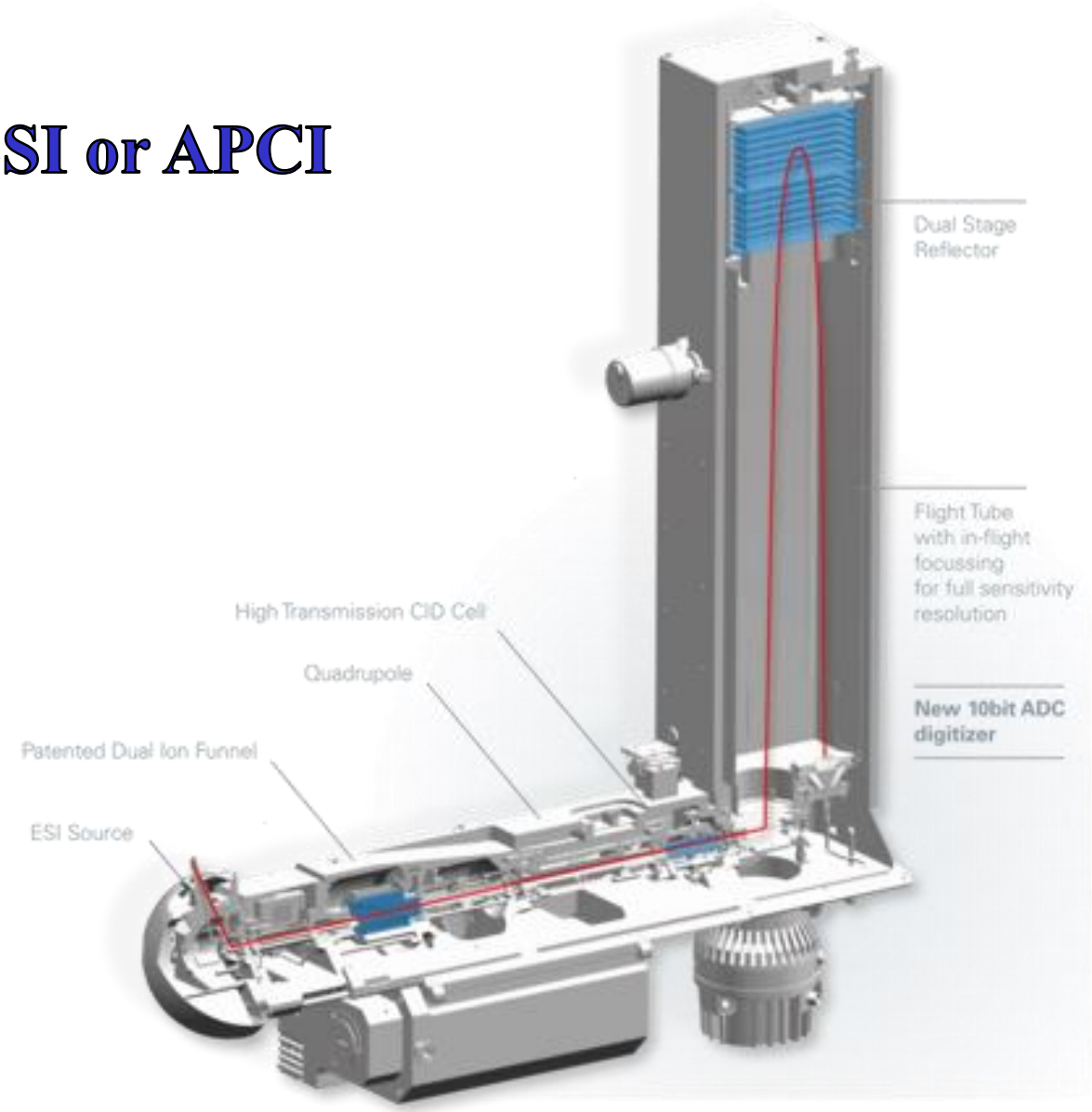


ESI-MS of a large protein



In MCAL: quadrupole-time-of-flight tandem analyzer

ESI or APCI



https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/o-tof/compact/technical-details.html

Anal Bioanal Chem (2010) 398:769–777
DOI 10.1007/s00216-010-3931-1

ORIGINAL PAPER

Application of LC–TOF MS to analysis of hemoglobin acetaldehyde adducts in alcohol detoxification patients

Stefan W. Toennes · Moritz G. Wagner · Gerold F. Kauert

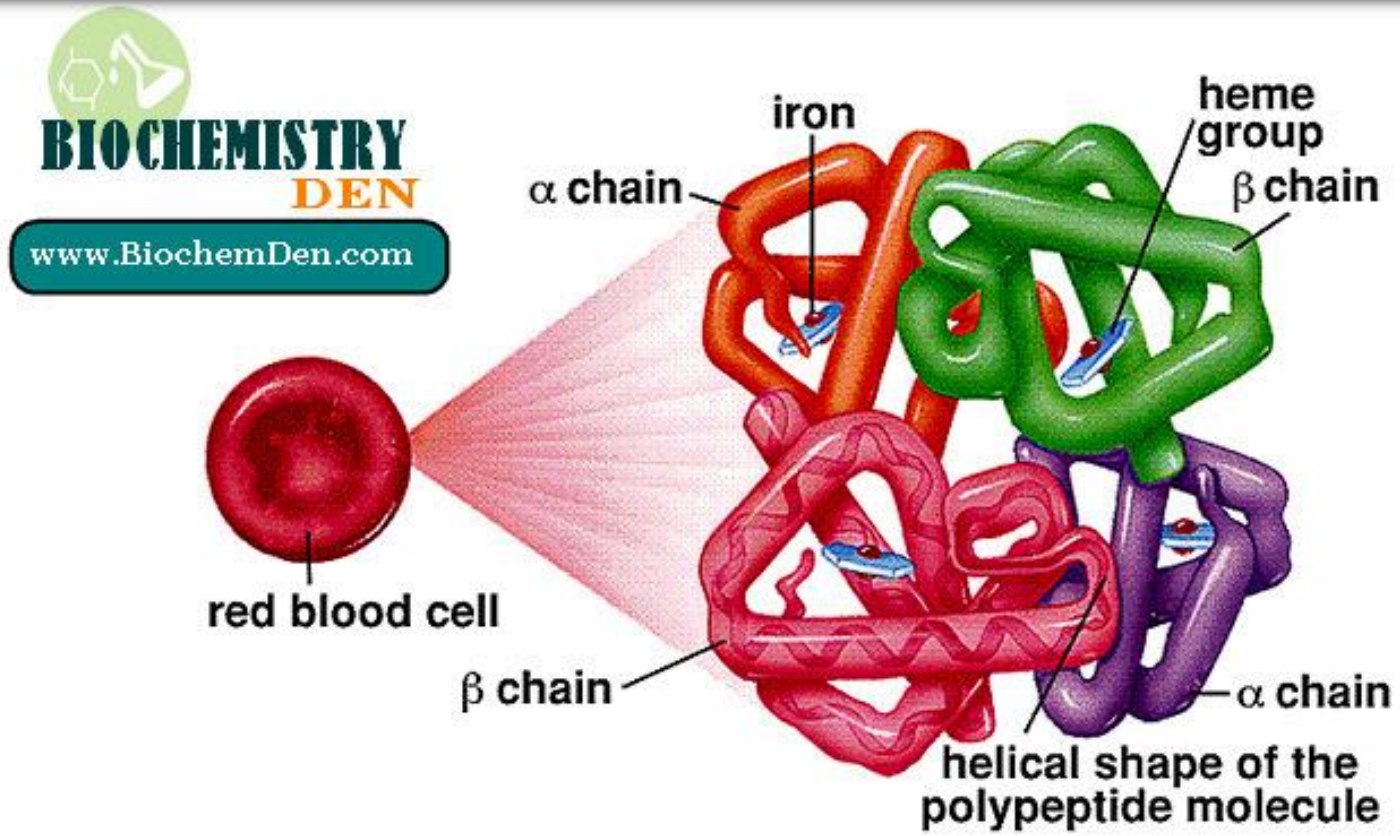
RATIONALE:

- Acetaldehyde, as first oxidative metabolite of ethanol, binds *in vivo* to a wide variety of molecules, e.g. to albumin, low density lipoproteins, ribonuclease, nucleosides, and hemoglobin (Hb).

- Acetaldehyde adducts of hemoglobin are regarded as potential markers of ethanol consumption.

- **Hemoglobin** from blood samples of alcohol addicts before and during detoxification was obtained, analyzed and compared with hemoglobin taken from children.

HEMOGLOBIN MOLECULE



https://www.biochemden.com/hemoglobin/

EXPERIMENTAL:

Red blood cells were lysed and filtered.

Hemolysate (200 μL, containing approximately 20 mg of Hb) was mixed with an equal volume of 80:20 acetonitrile–water containing 0.2% TFA to denature Hb.

This was centrifuged at 16000 g for 10 min.

Of the supernatant, 0.5 μL was analyzed by LC–TOF MS using a linear gradient with 0.1% TFA in water as solvent A and acetonitrile as solvent B.

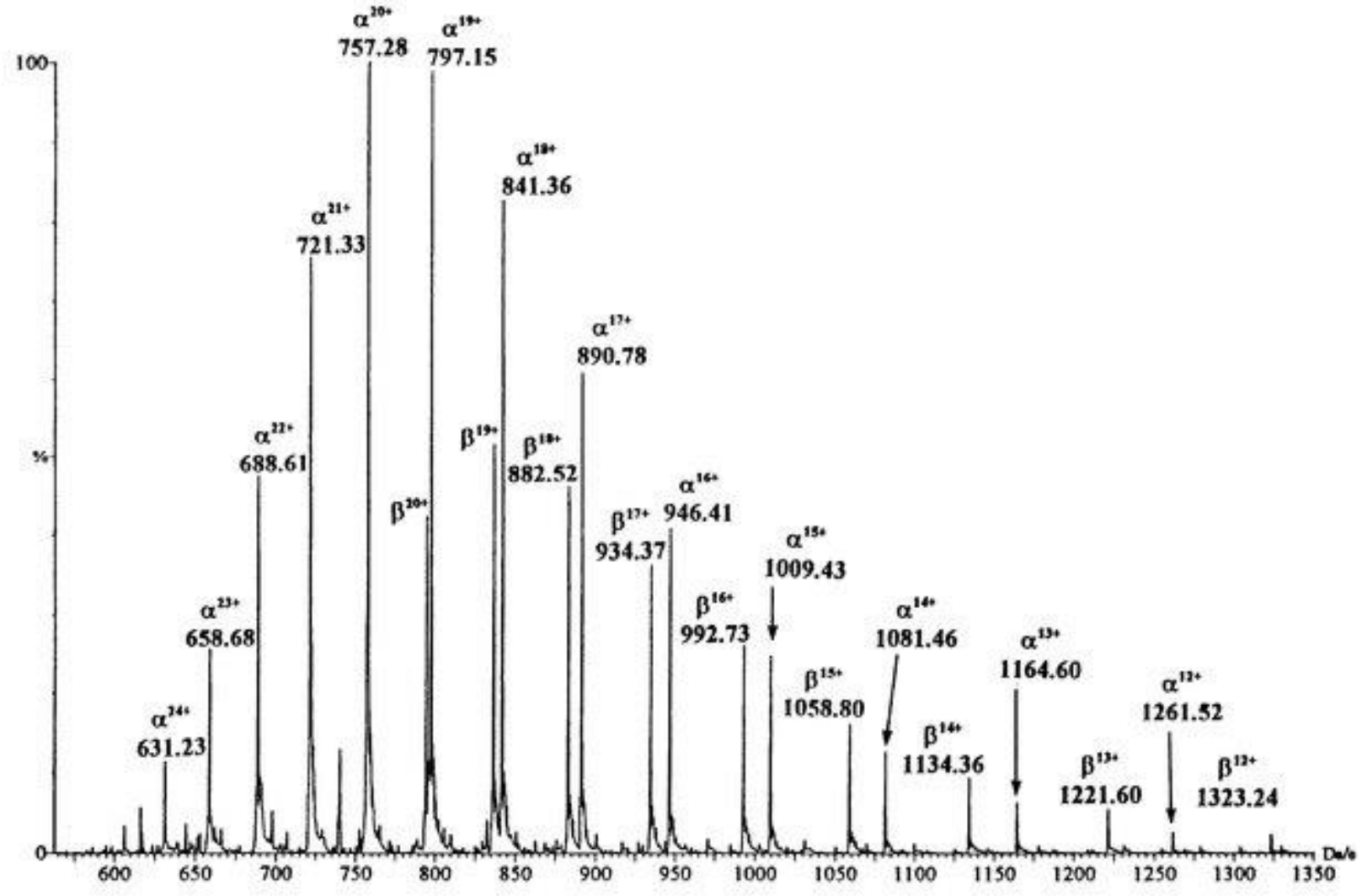
The % B was increased from 40% to 50% in 10 min, followed by a washing step with 100% B for 2 min.

The flow rate was 0.4 mL/min and the column was operated at 50 °C.

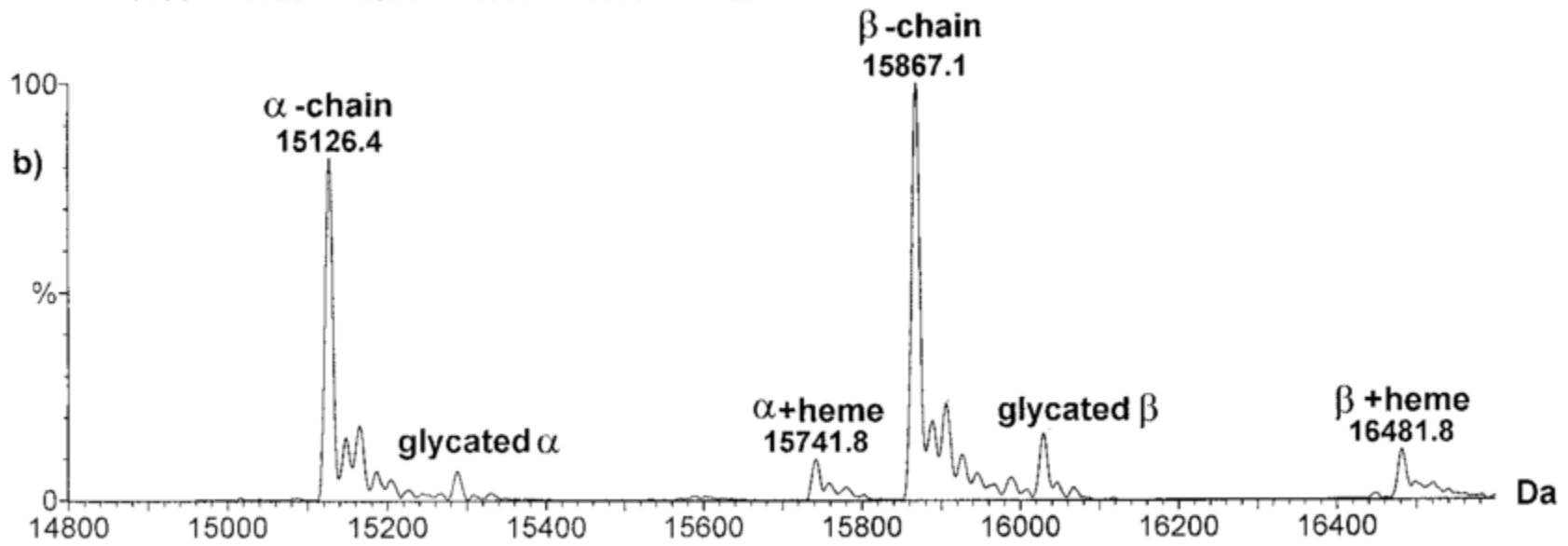
Column: C18-Ether column (100×2.0 mm, 3 μm particle size)

MS: ESI-TOF (Agilent)

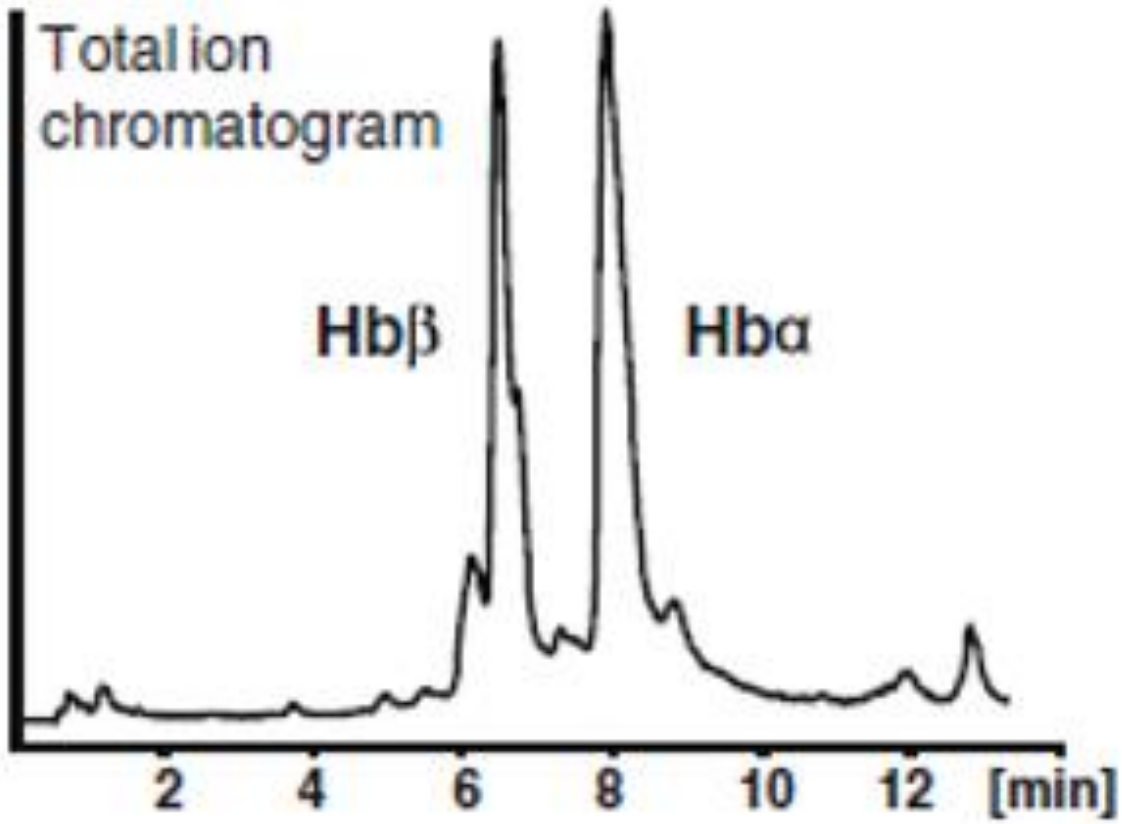
ESI-MS spectrum of normal Hb alpha + beta chains



Deconvoluted ESI-MS spectrum of normal Hb alpha + beta chains



LC-MS TIC



Deconvoluted ESI mass spectrum averaged from 6-9 min range

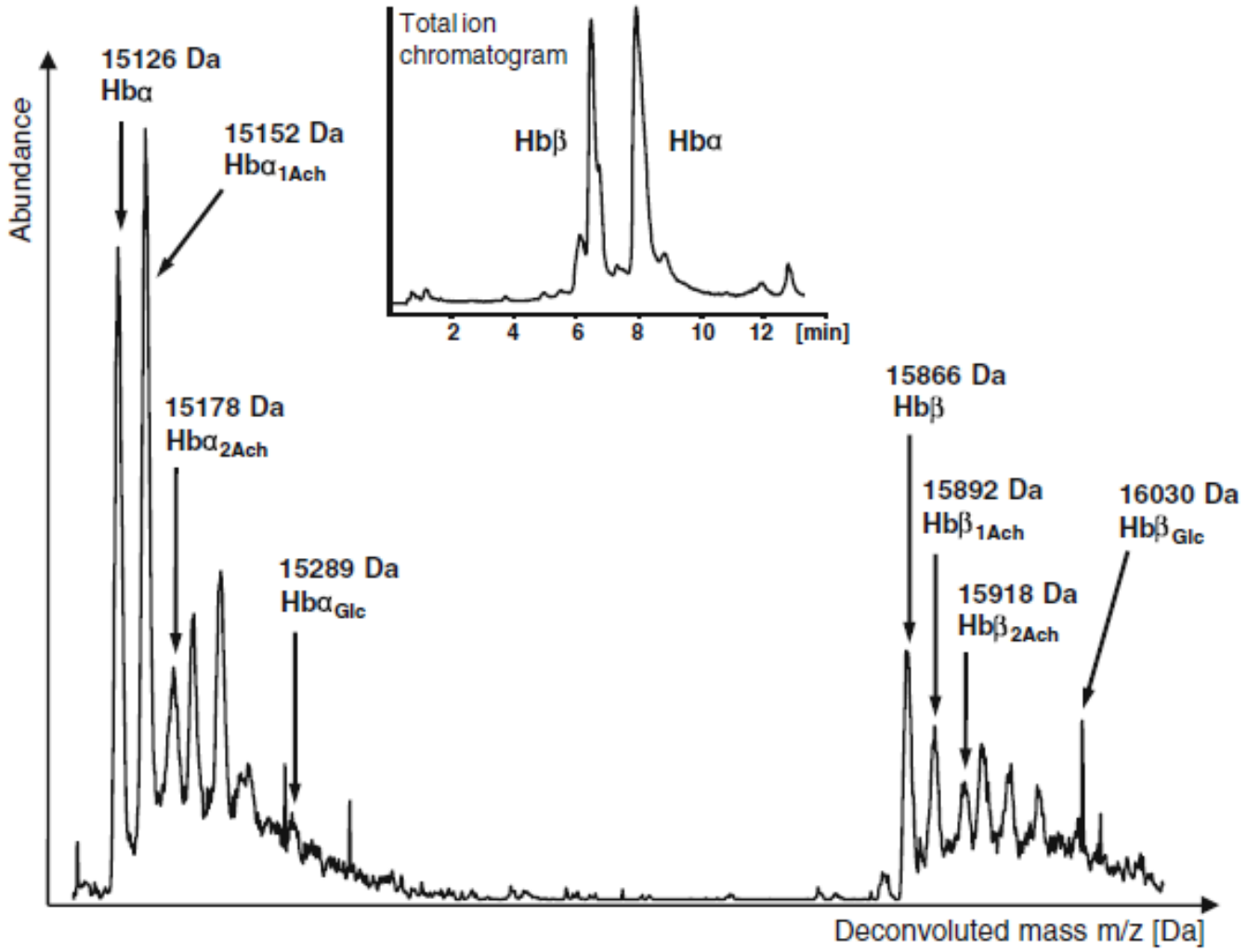


Fig. 1 Deconvoluted mass peaks resulting from the mass spectra in the retention time range 6–9 min; the total-ion chromatogram of the hemoglobin chain separation is shown in the *inset*. The hemoglobin alpha (Hbα) and beta (Hbβ) chains and their modified homologues (glycosylation, *Glc*; and acetaldehyde modification, *Ach*) are annotated

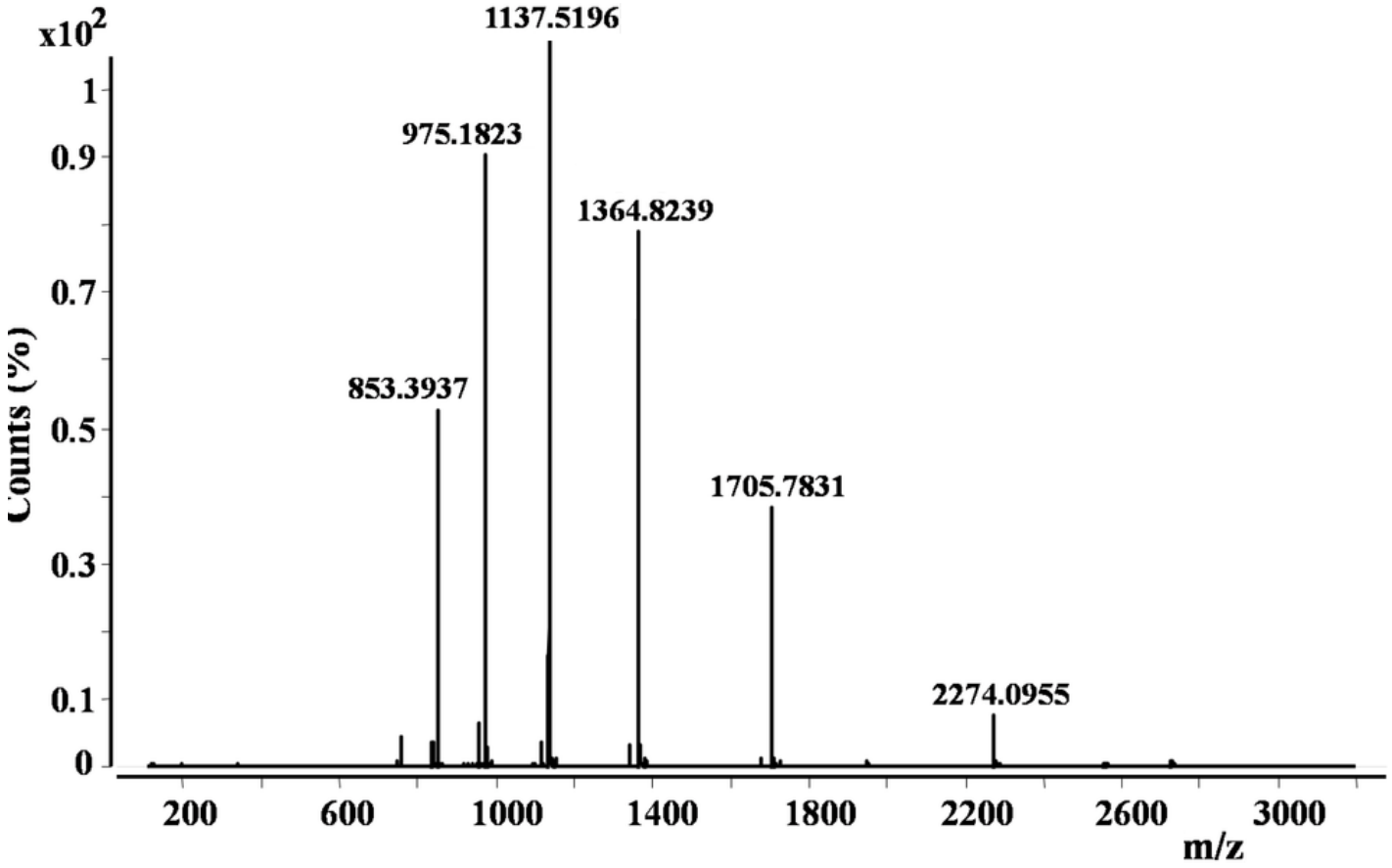
Agilent 6200 Series Accurate-Mass Time-of-Flight (TOF) LC/MS

Summary LC-MS

- Good for liquid and solid samples
- All size of molecules
- Normal, reversed phase LC possible
- UV/fluorescence detector in series or parallel
- Very specific owing to mass measurement

Questions

1. Calculate the molecular mass of the compound that produced this positive mode electrospray spectrum:



2. a) In LC-MS, reversed-phase HPLC is much more used than normal phase HPLC. Discuss one possible reason for this fact.

b) In LC-MS why is the flow split after the column?