

Isomeric pairs: (a) and (b) are isomers, as are (c) and (d). Each has the same mass but different structures and hence are different compounds.

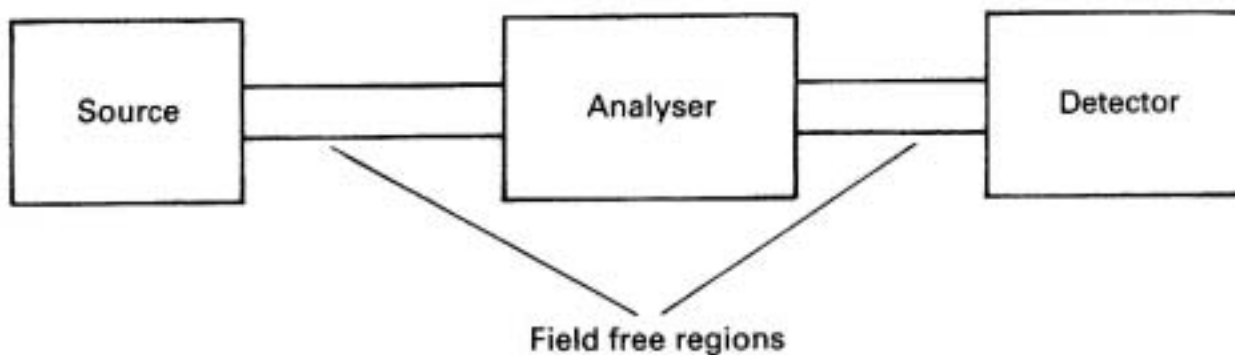
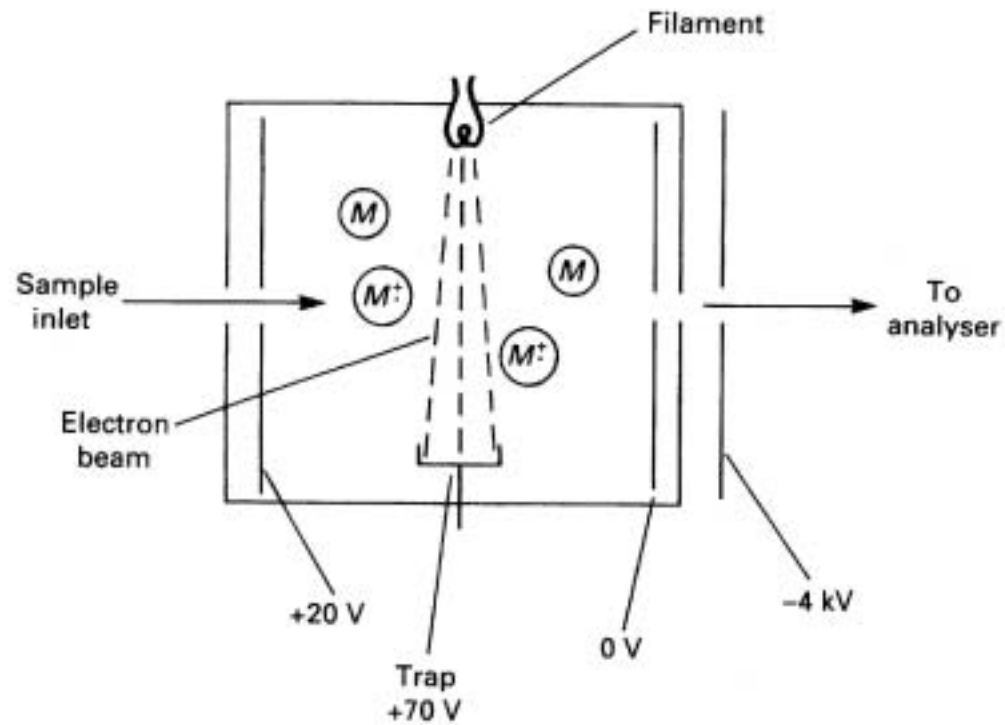
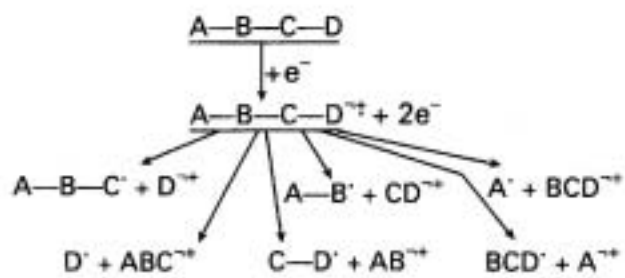


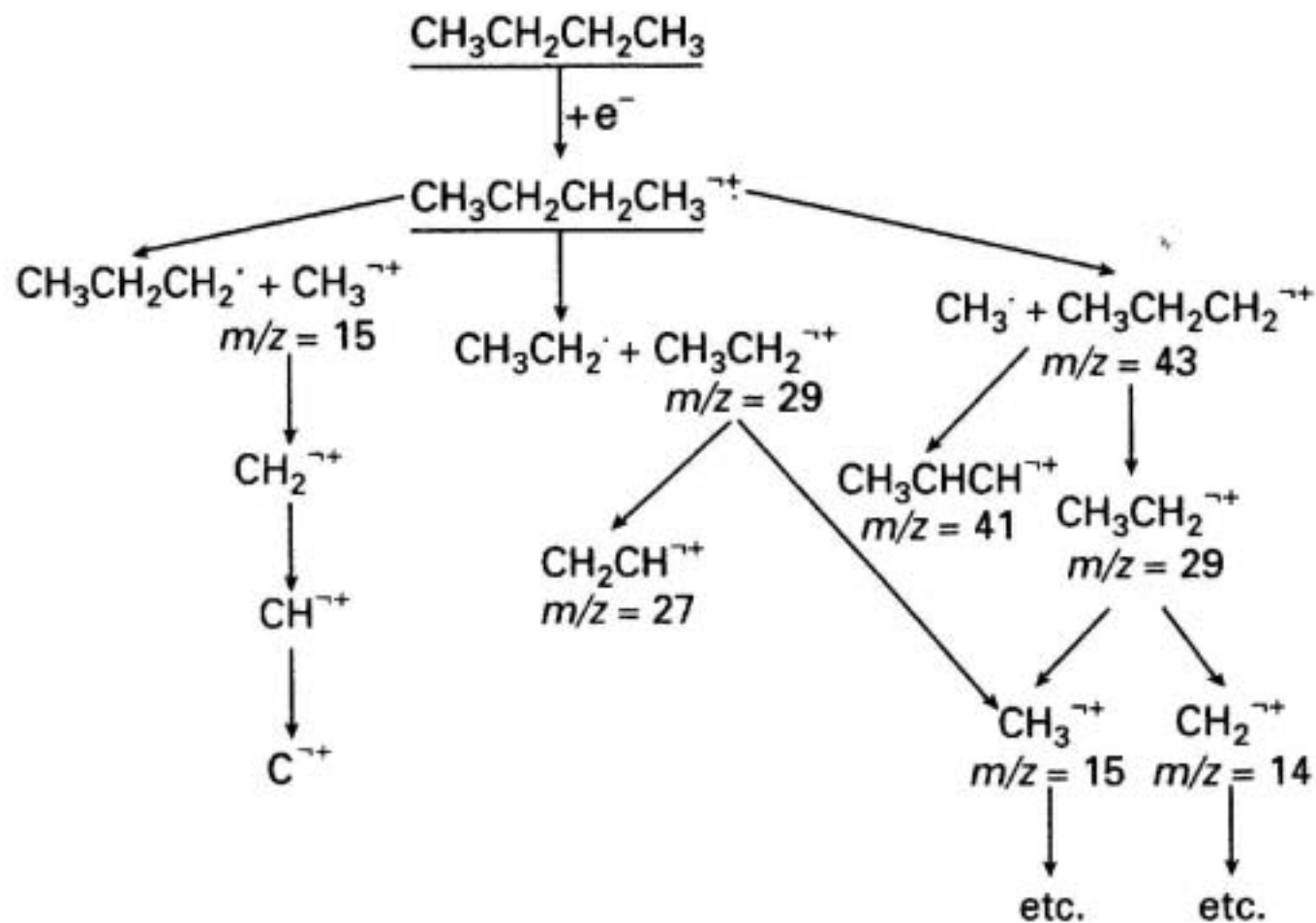
Diagram of a mass spectrometer. The whole system is kept under high vacuum of the order of 10^{-5} torr.



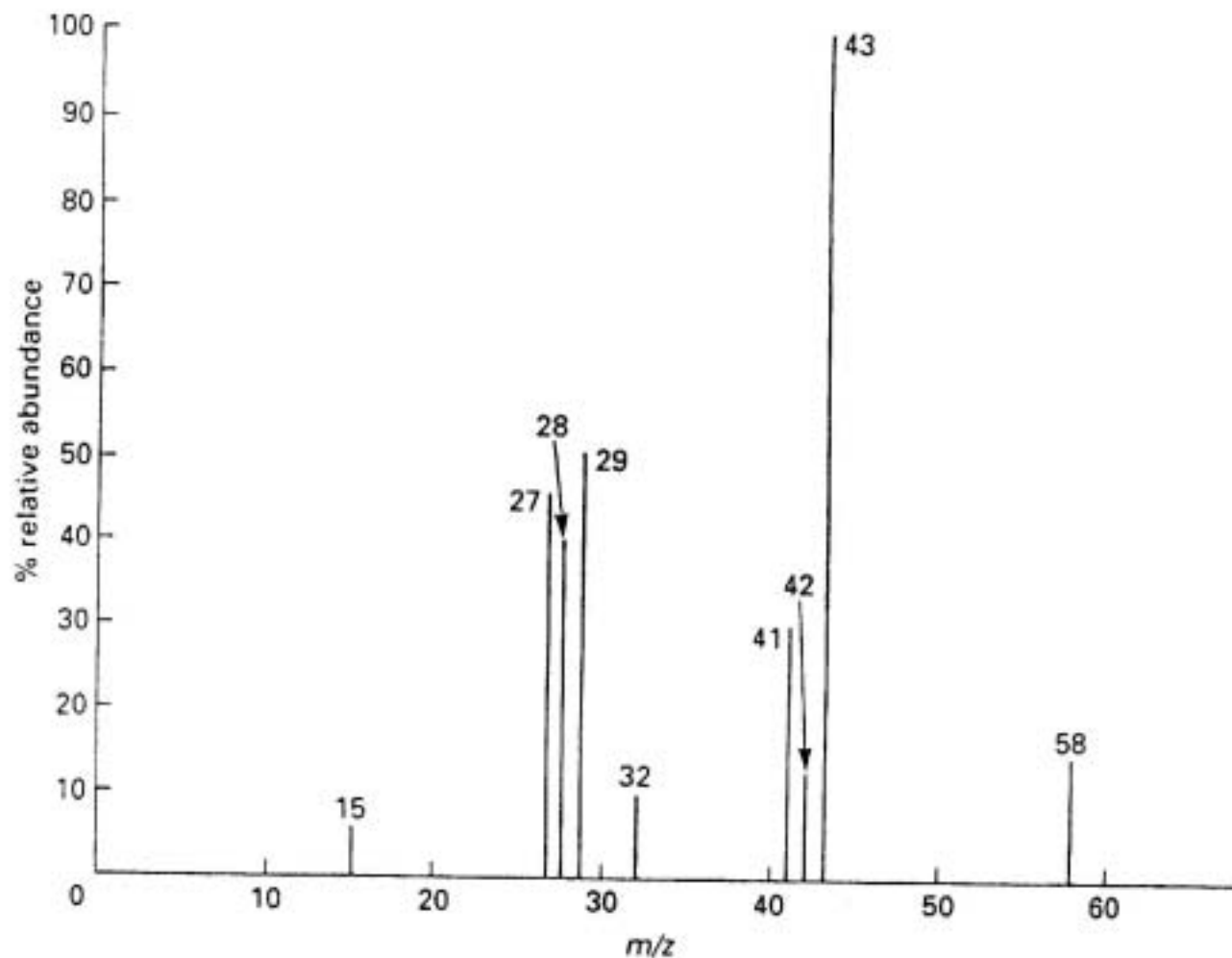
Electron impact ionisation source.



Fragmentation processes in a hypothetical species.



Fragmentation pathways in *n*-butane.

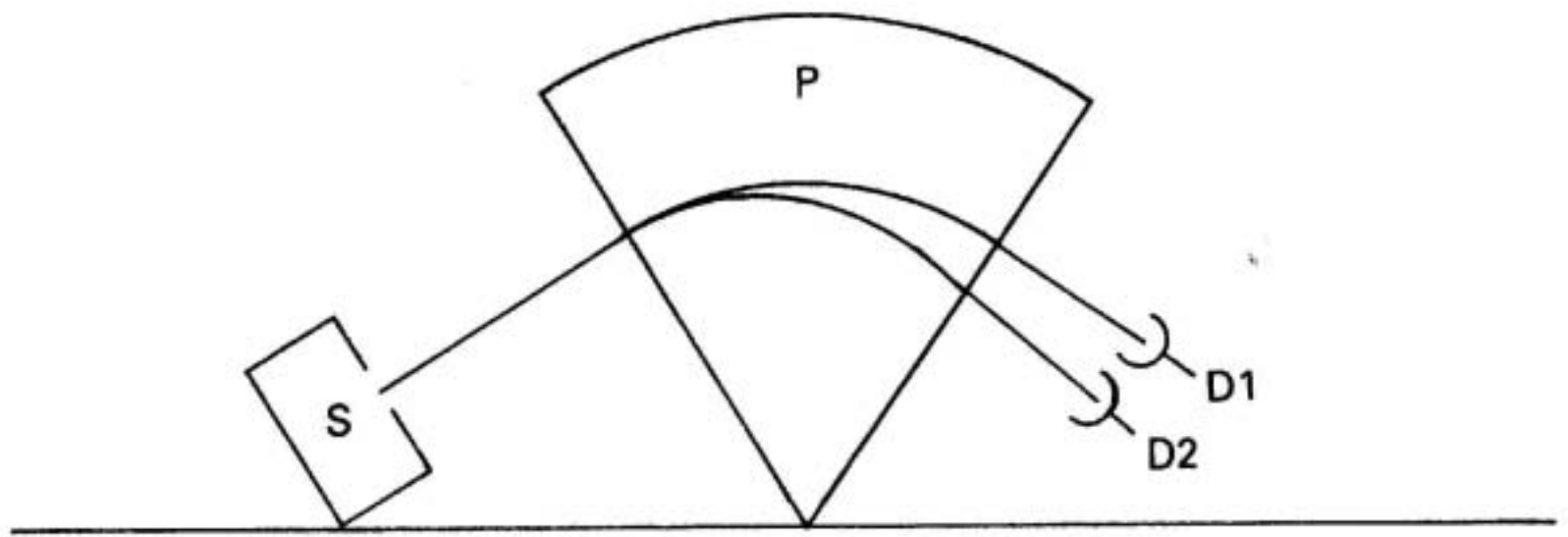


. Electron impact ionisation spectrum of *n*-butane. Relative abundance is a scale calibrated from 0 to 100%. The largest peak in the spectrum is set at 100% (base peak) and all the others calculated in proportion as a percentage. Spectra produced in this way are said to be normalised, are machine independent and hence are directly comparable. Conversely, spectra whose ordinates are labelled as intensity are absolute spectra.

Probability of occurrence of different isotopic compositions

No of ^{13}C atoms present	% probability of occurrence
0 (all ^{12}C)	95.641
1	4.2863
2	0.0720
3	0.0005
4	0.0000
	<hr/>
	99.9998

There have to be four C atoms of one kind or another so that the total percentage adds up to 100.



A single-sector mass spectrometer for isotope ratio studies. S, source; P, magnetic pole; D, detectors.

Applications of ^{13}C labelling for IRMS measurements

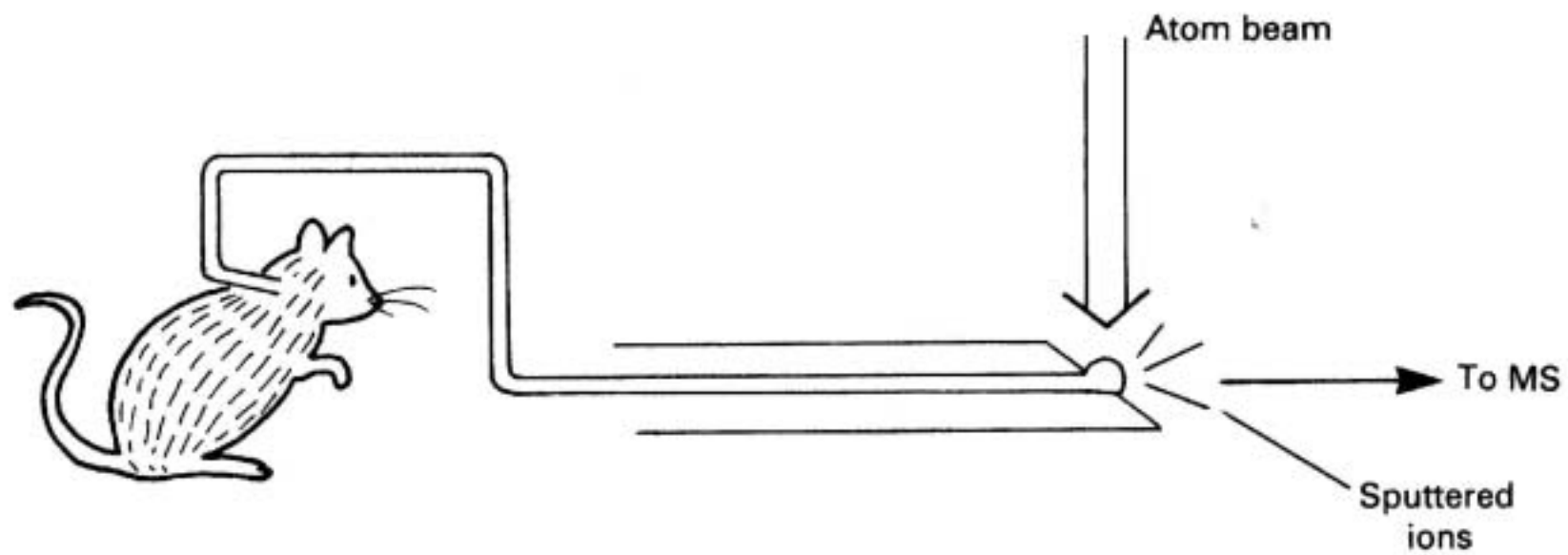
^{13}C compound	Application
Triolein	Fat malabsorption
Bicarbonate	Energy expenditure
Lactose	Lactase deficiency
Glucose	Carbohydrate metabolism
Palmitate	Fatty acid oxidation
Galactose	Liver enzyme function

Метод **ФАВ** (*Fast atom bombardment*) был создан в 1981 году и открыл новую эру в биологической масс-спектрометрии.

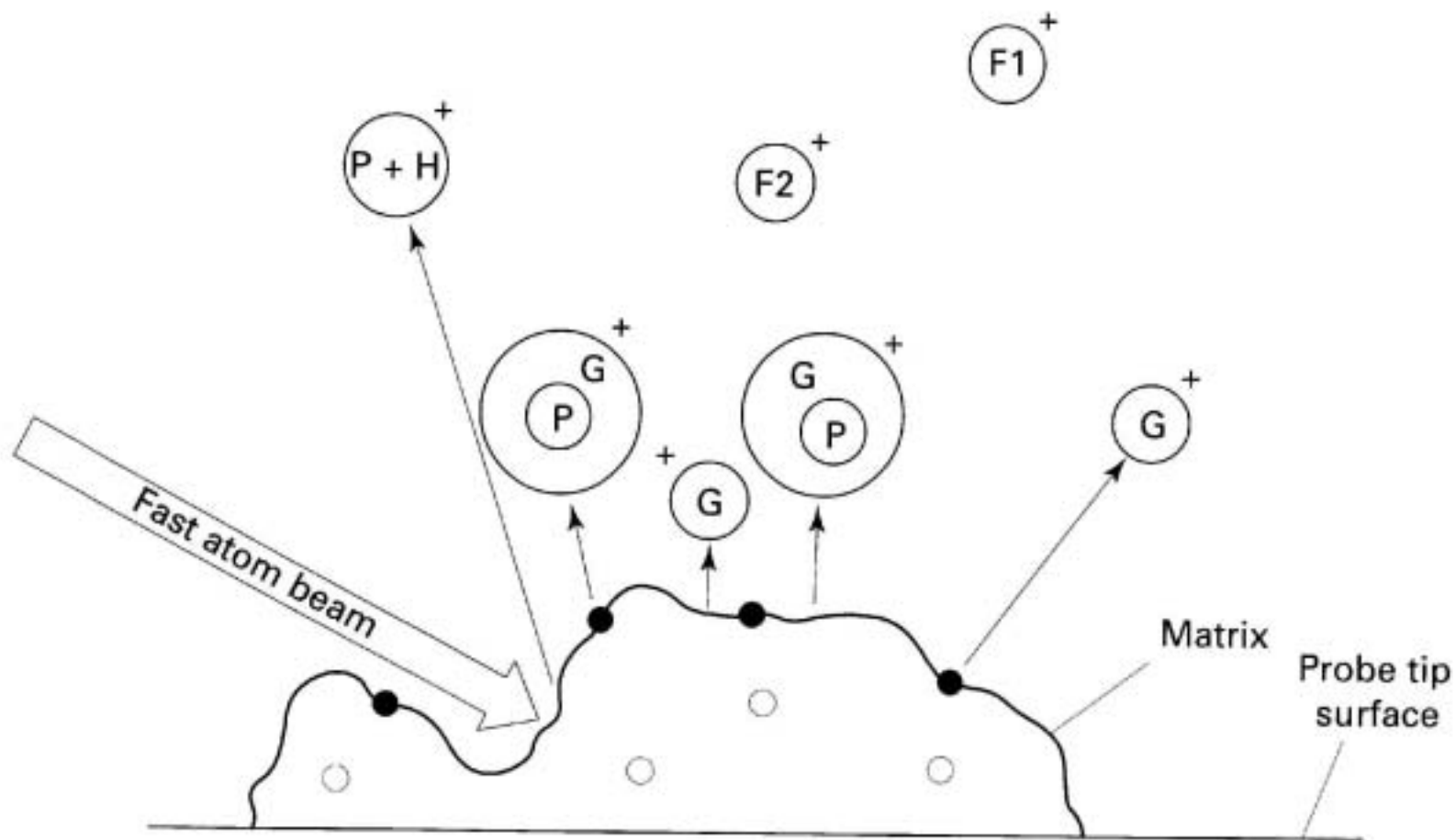
- Ионизацию можно проводить из раствора
- Поверхность раствора постоянно обновляется, что предотвращает дополнительное химическое разложение на поверхности, свойственное твердым образцам.

Проблемы: распределение между поверхностью и объемом. То что в объеме может хуже ионизироваться, а находящиеся на поверхности тоже могут изменить потенциал ионизации. Особенно это проявляется в анализе пептидов и белков.

Различают статический метод и комбинацию с HPLC и капиллярным электрофорезом.

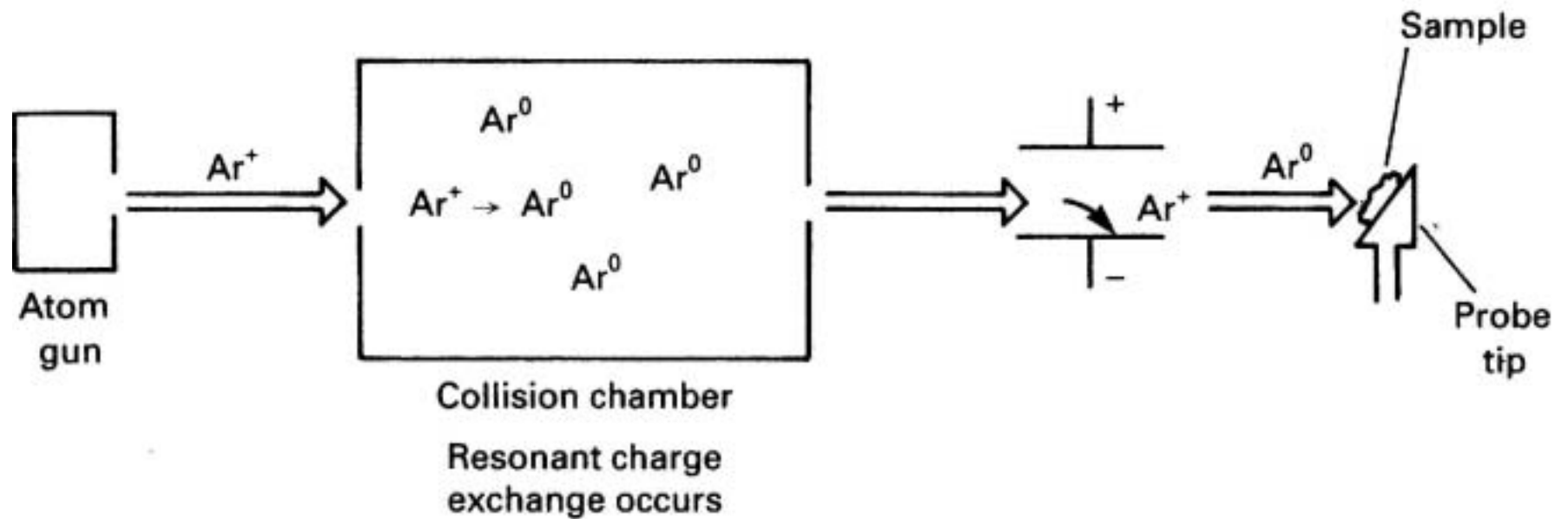


In vivo CF-FAB-MS experiment.

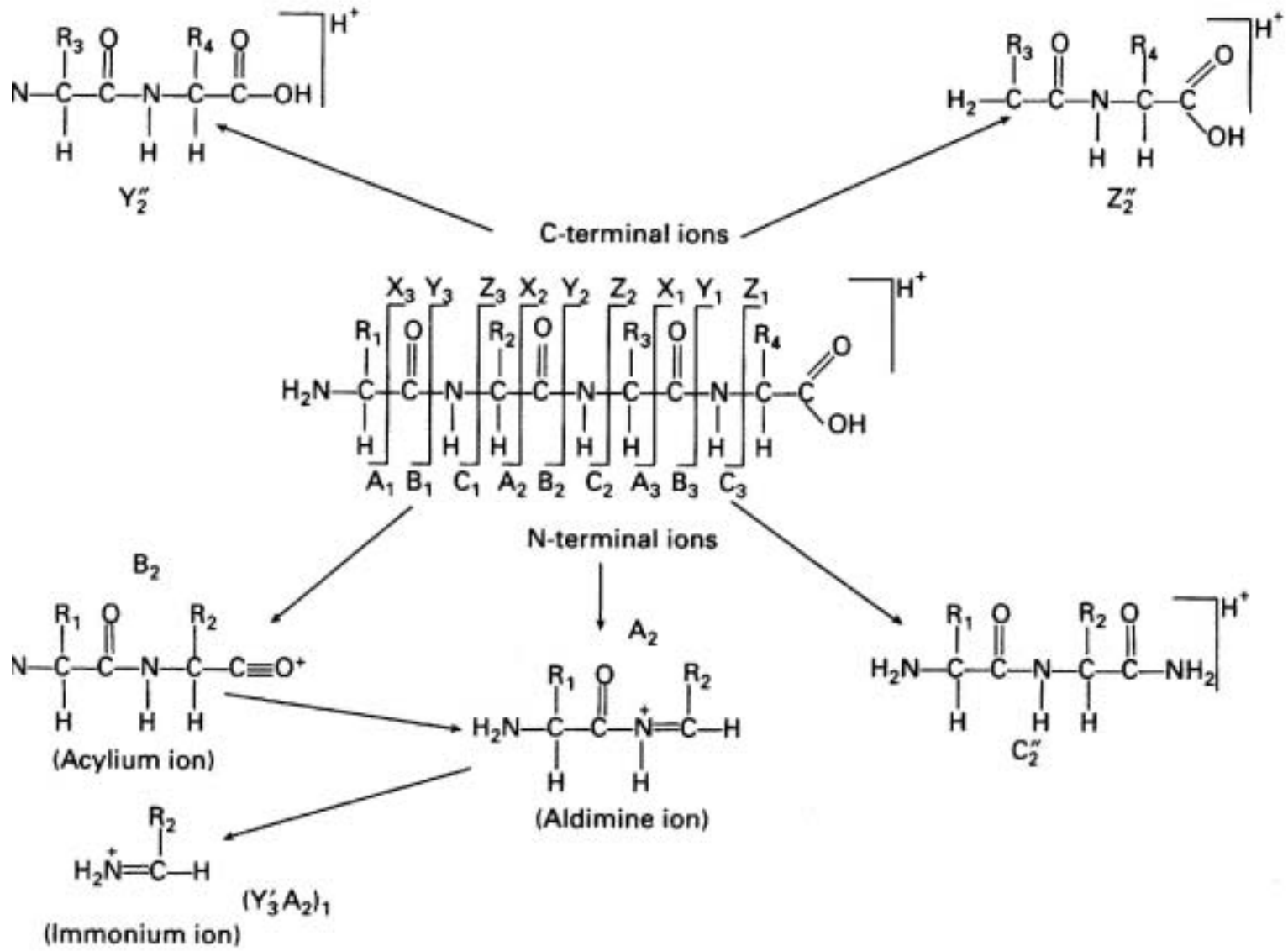


Sputtering phenomenon from liquid matrix during bombardment with fast atoms. G, matrix; $P + G^+$, parent solvated with matrix; $P + H^+$, pseudomolecular ion; F_1 , F_2 , fragment ions.

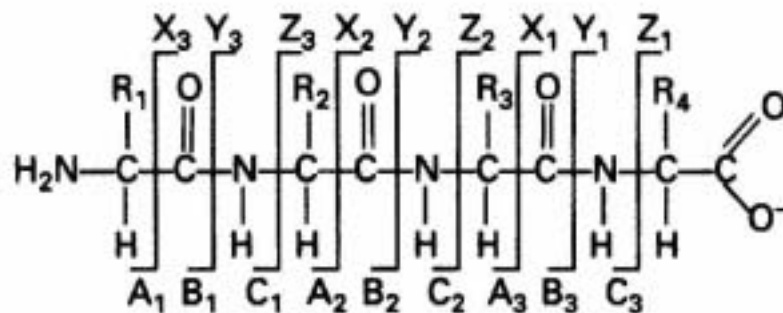
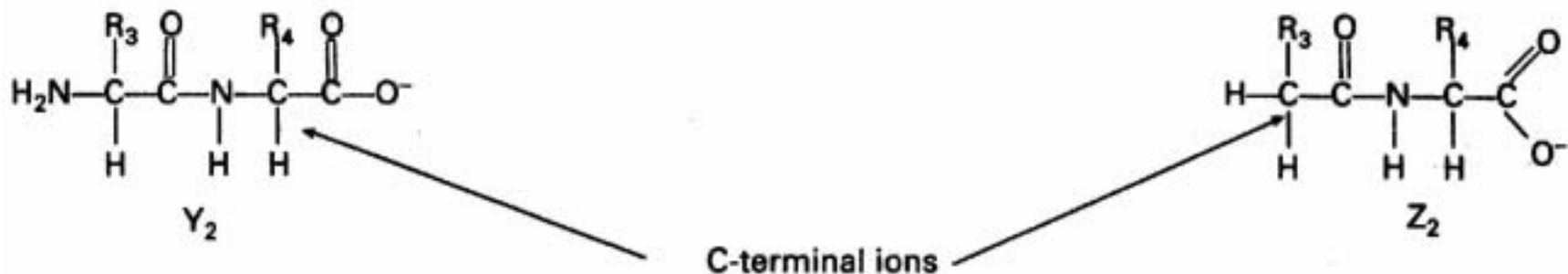
Mass spectrometric techniques



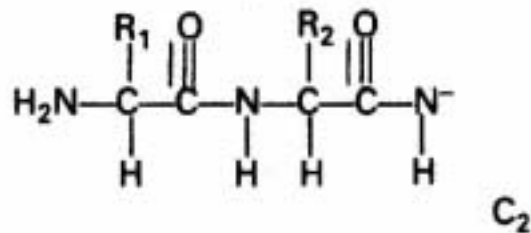
Generation of beams of fast atoms. Ar^0 , argon atoms; Ar^+ , argon ions.



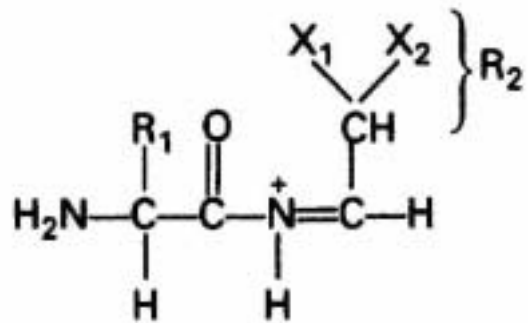
Fragmentation of an idealised peptide (protonated).



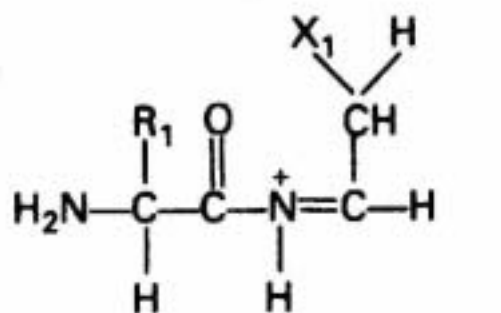
N-terminal ions



Fragmentation of an idealised peptide (deprotonated).

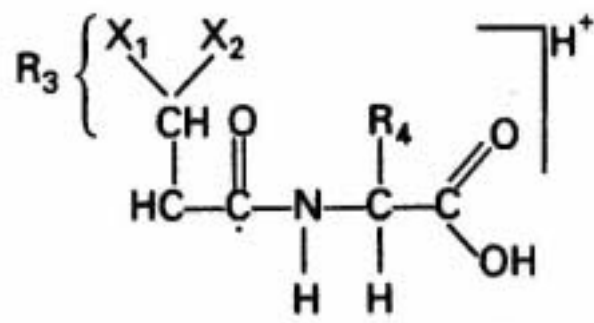


A_2

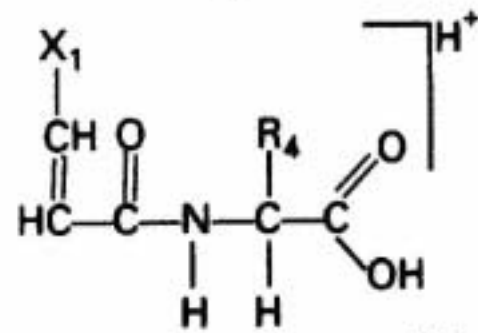


D'_2

N-terminal (A_n) ion
Loss of 42 indicates Leu
Loss of 28 indicates Ile



Z'_2

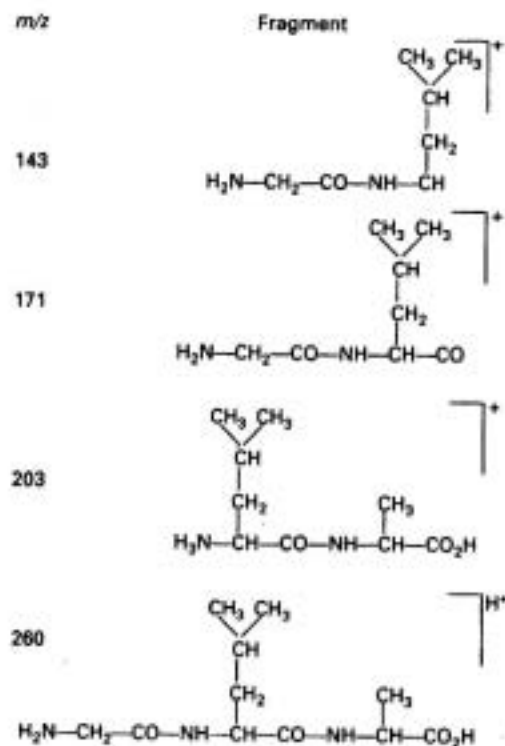
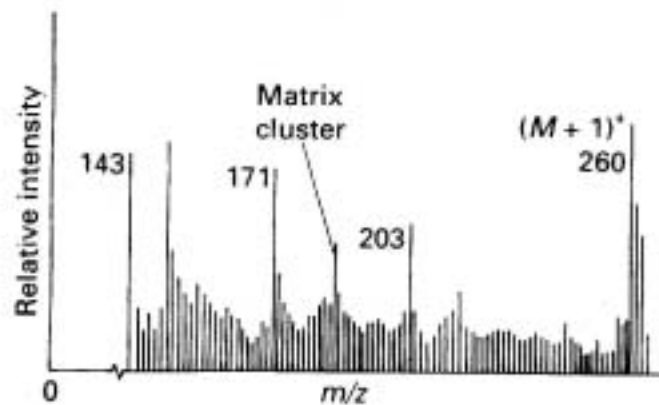


W'_2

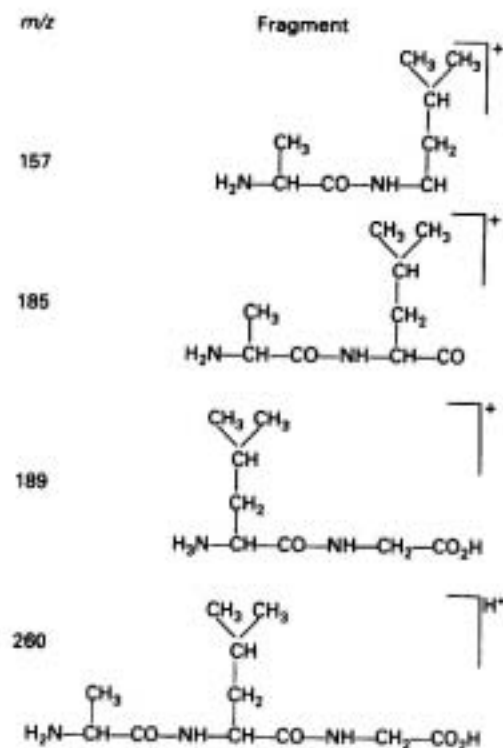
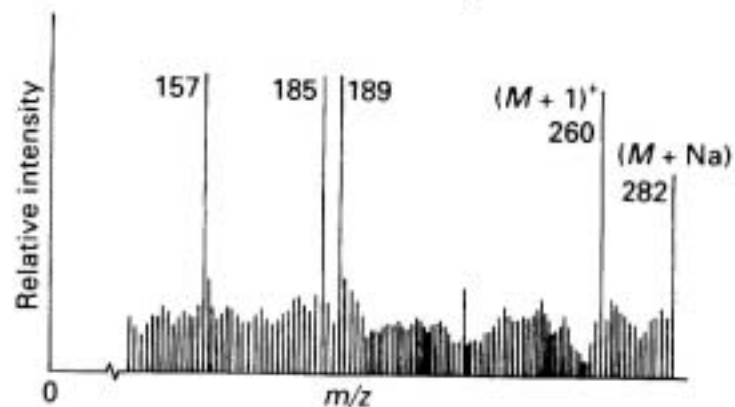
C-terminal (Z'_n) ion
Loss of 43 indicates Leu
Loss of 29 indicates Ile

D- and W-type ions allowing the distinction between isomeric amino acids.

(a) (Gly-Leu-Ala)



(b) (Ala-Leu-Gly)



. FAB mass spectra of two isomeric tripeptides of alanine, glycine and leucine.

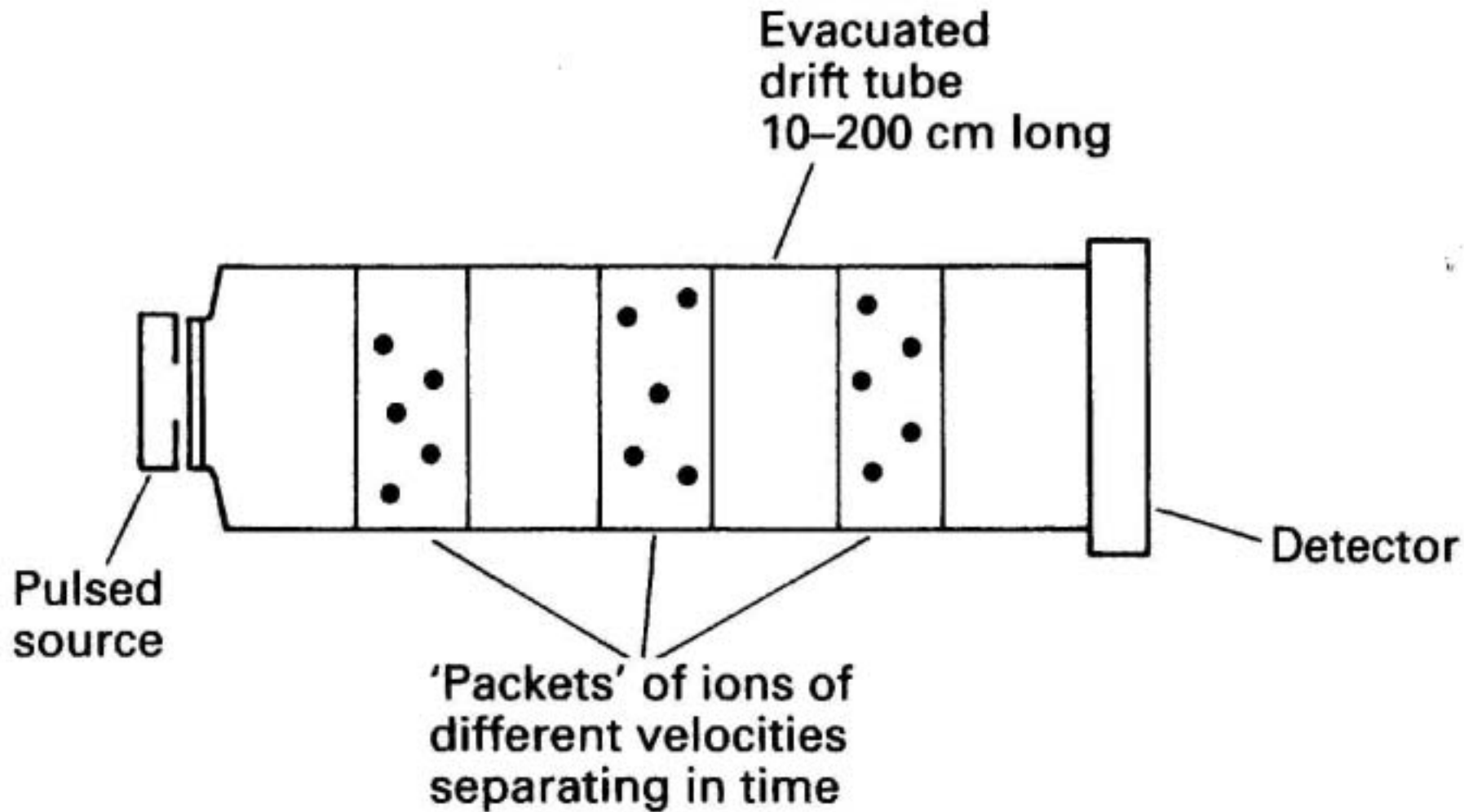
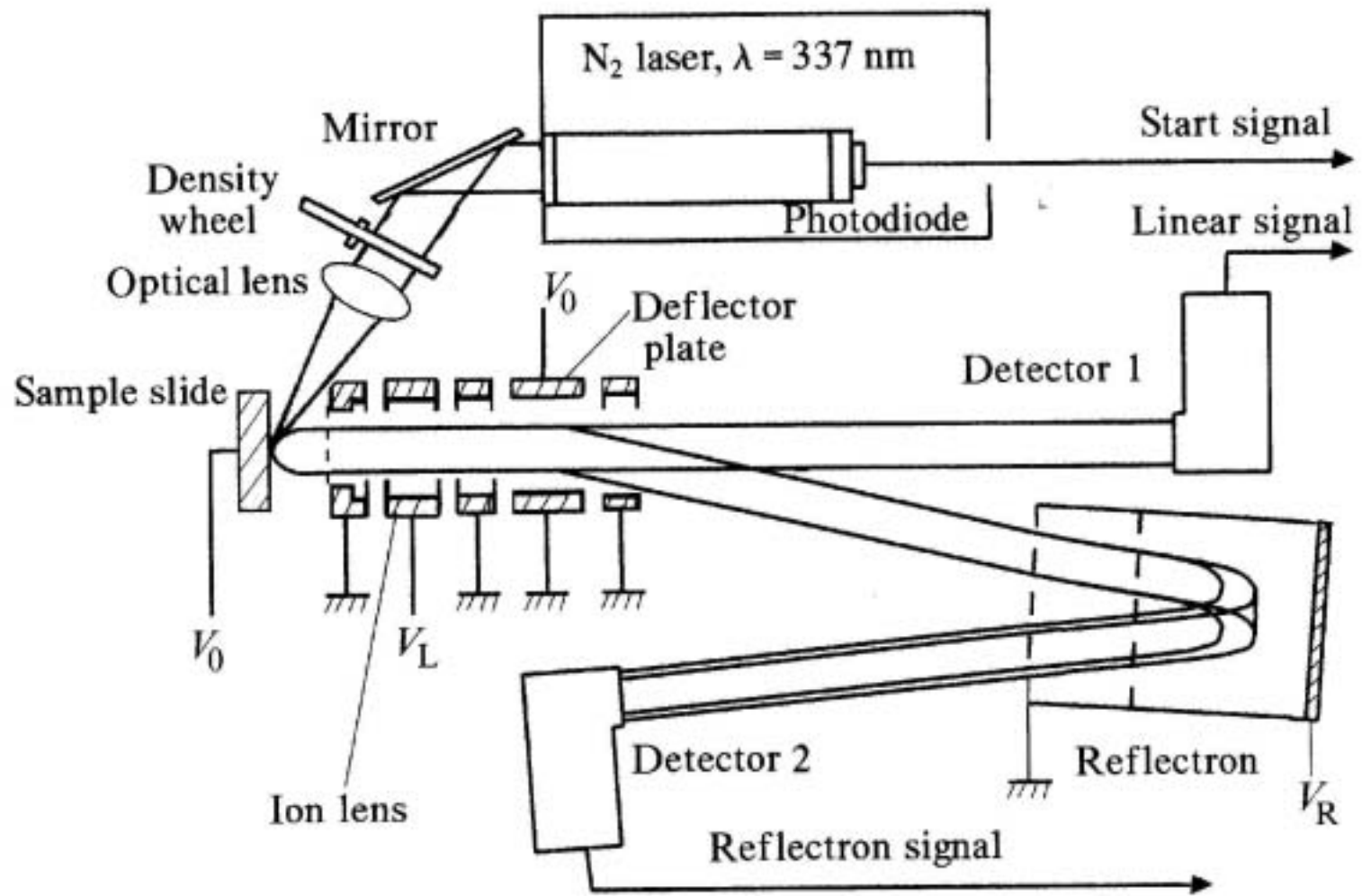
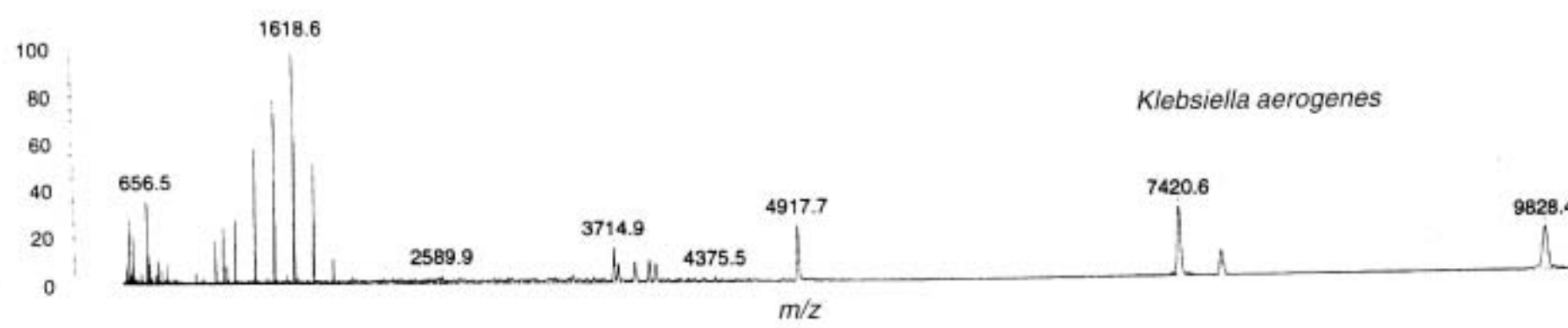
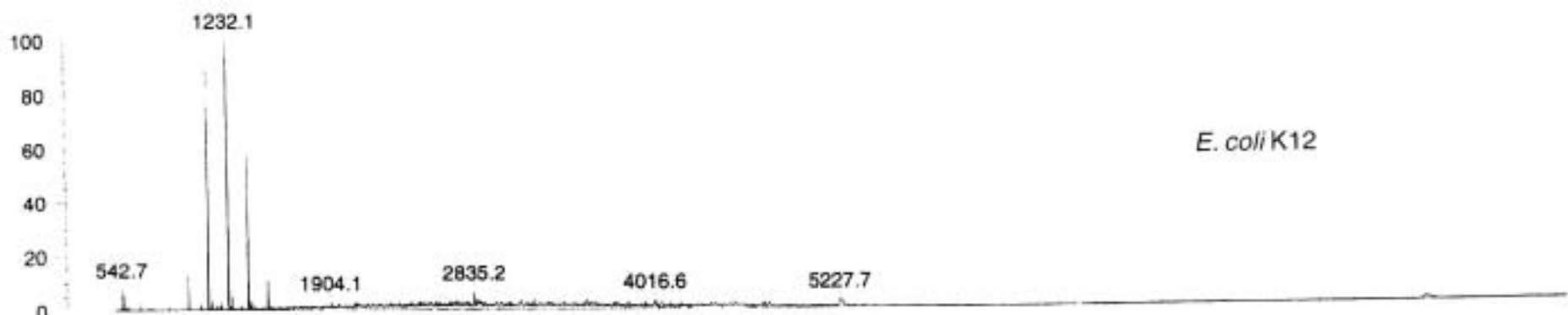
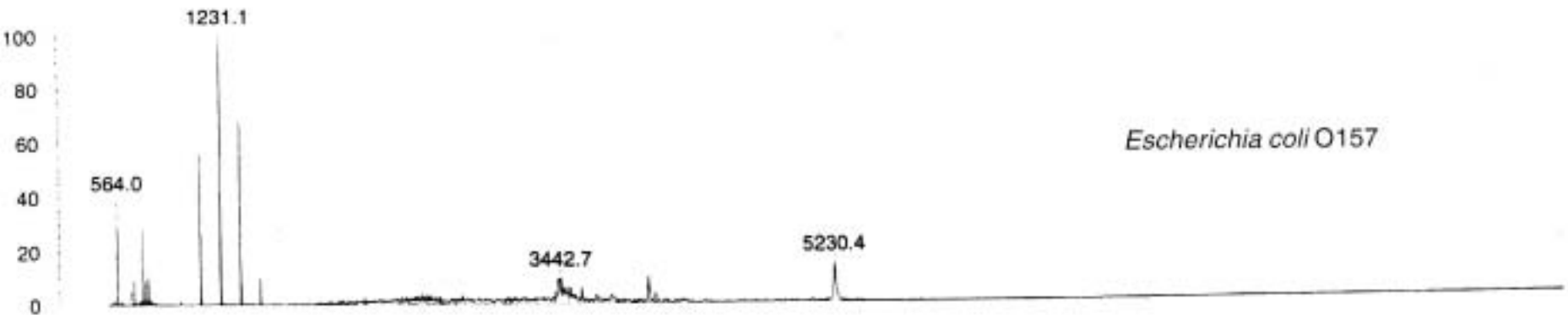


Diagram of the time-of-flight analyser.



Kratos Kompact MALDI-TOF Instrument. V_0 , ground voltage; V_L , lens voltage; V_R , reference voltage; N_2 , nitrogen. (Reproduced by permission of Kratos Analytical Ltd, Manchester.)



Comparison of MALDI-TOF-mass spectra.

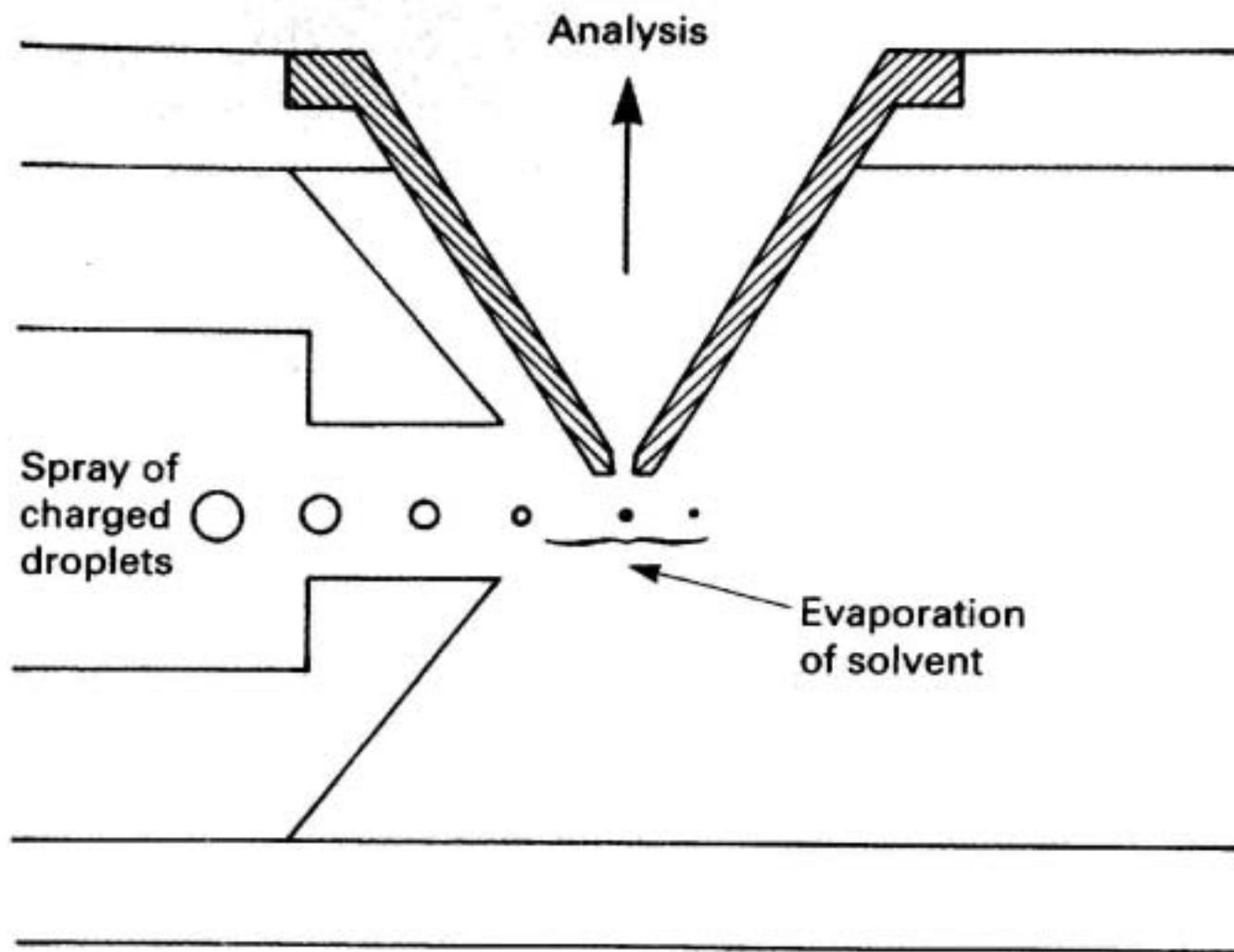


Diagram of the thermospray source. The shading denotes the cross-section through the funnel-shaped orifice.

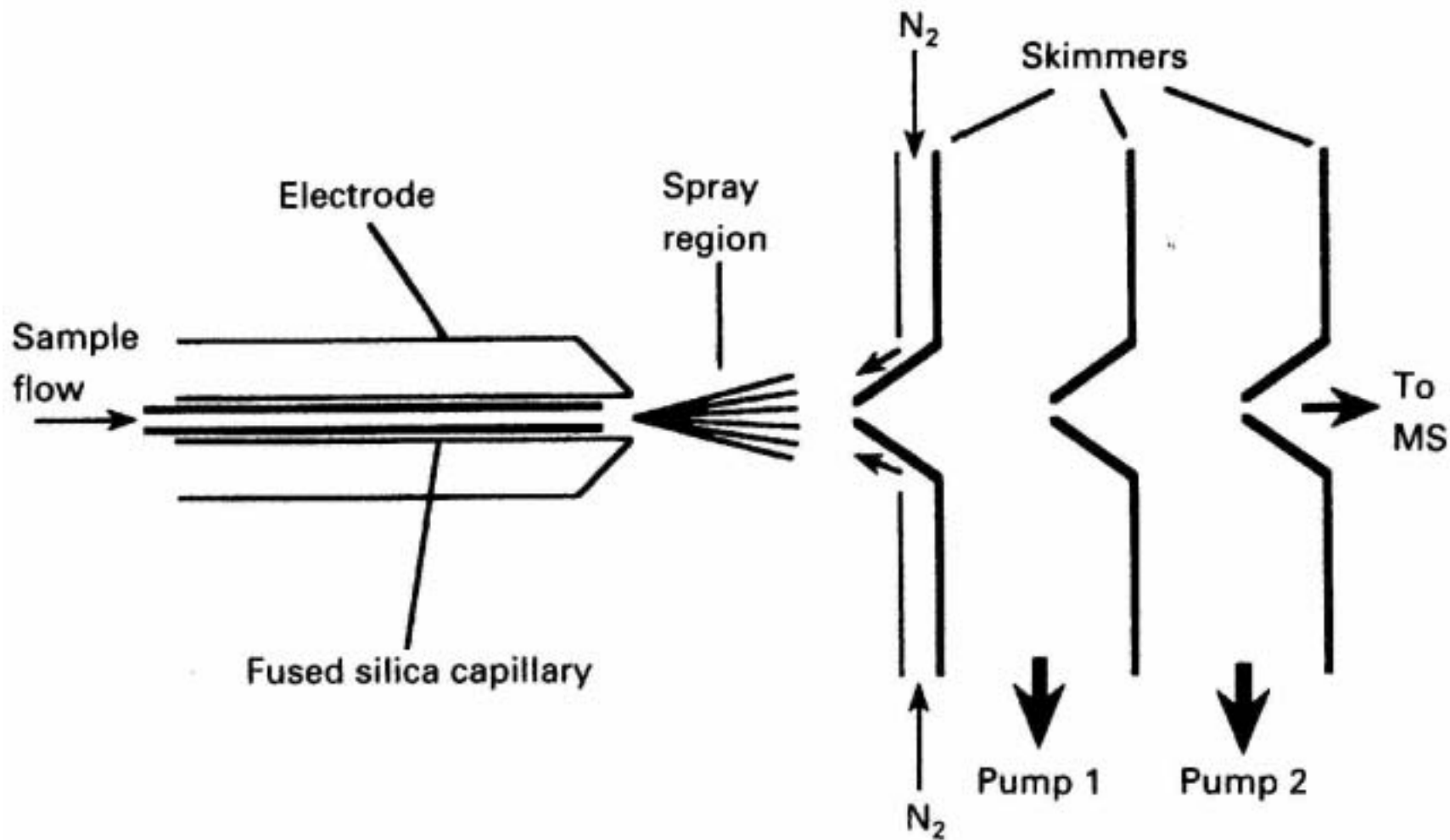


Diagram of the electrospray ionisation source. MS mass spectrometer.

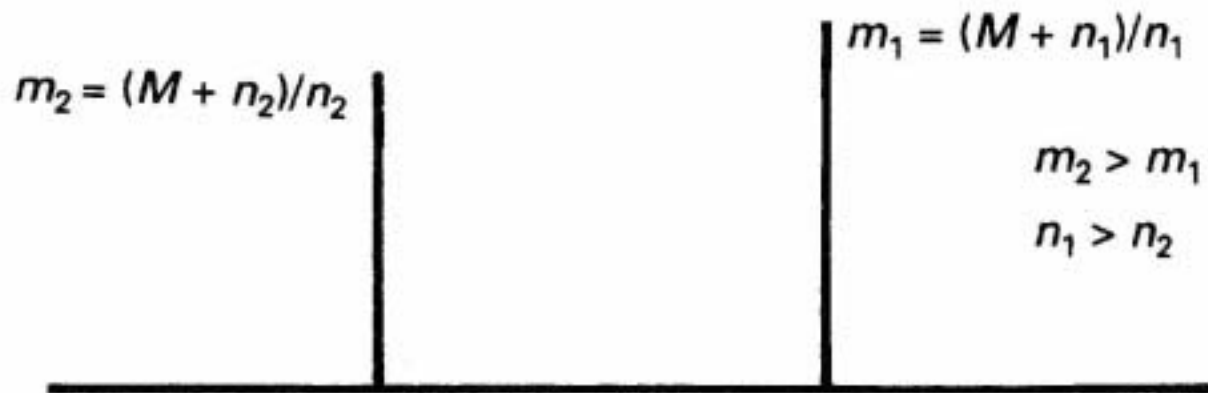
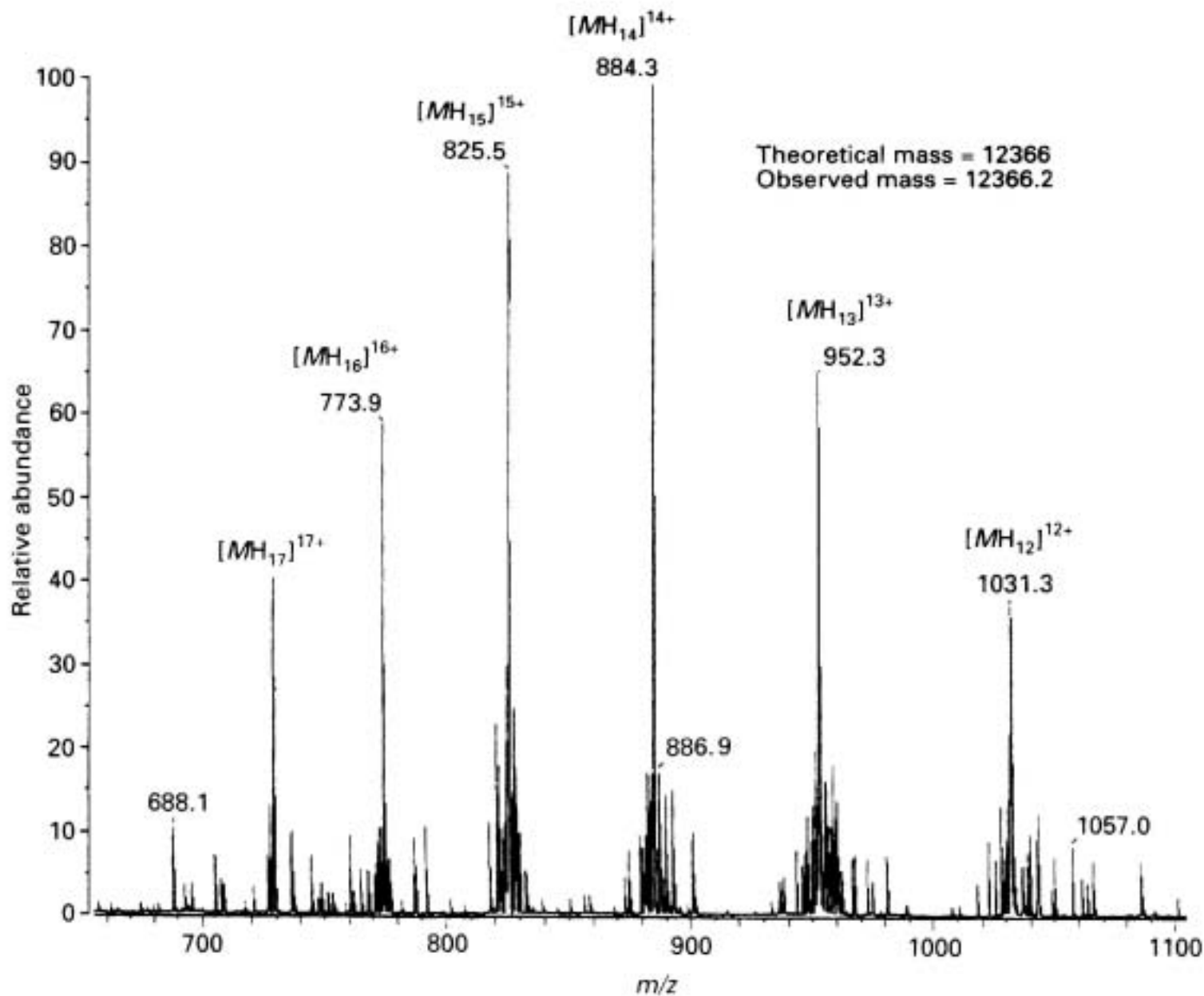


Diagram of two hypothetical multiply charged peaks in an ESI spectrum. It is assumed that the ions are adducts of neutral molecule and protons. If $n_1 = n_2 + 1$; then $n_2 = (m_1 - 1)/(m_2 - m_1)$ and $M = n_2(m_2 - 1)$, which is equal to the mass of the neutral molecule. m_1 and m_2 are the recorded masses (equivalent to the m/z values). n_1 and n_2 are the number of charges (z values) or protons added, respectively. By taking peaks in pairs, from the recorded masses, n_2 can be calculated and hence M . A range of values may be obtained for M and an average value calculated.



m/z spectrum of multiply charged cytochrome *c*. Applying the algebra stated in the caption to Fig. 11.21 and remembering that *z* values must be integers:

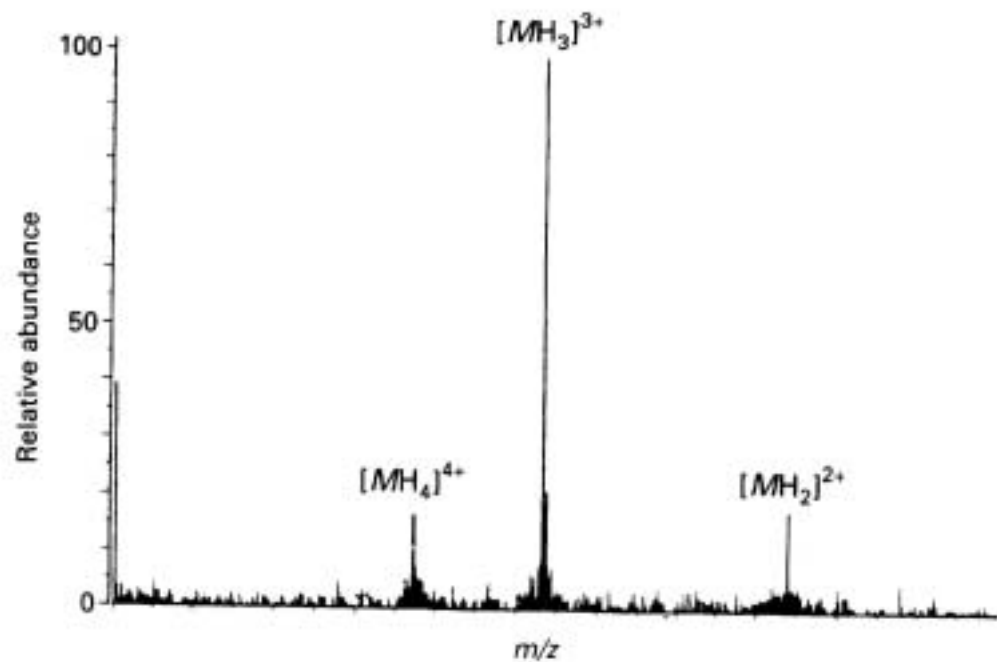


Fig. 11.23. m/z spectrum of multiply charged insulin B chain (oxidised). (Reproduced by kind permission of JEOL (UK) Ltd, JEOL House, Welwyn Garden City.)

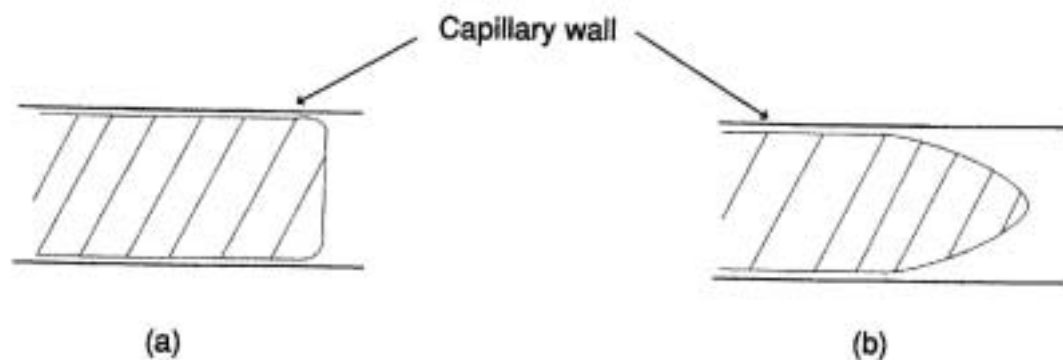
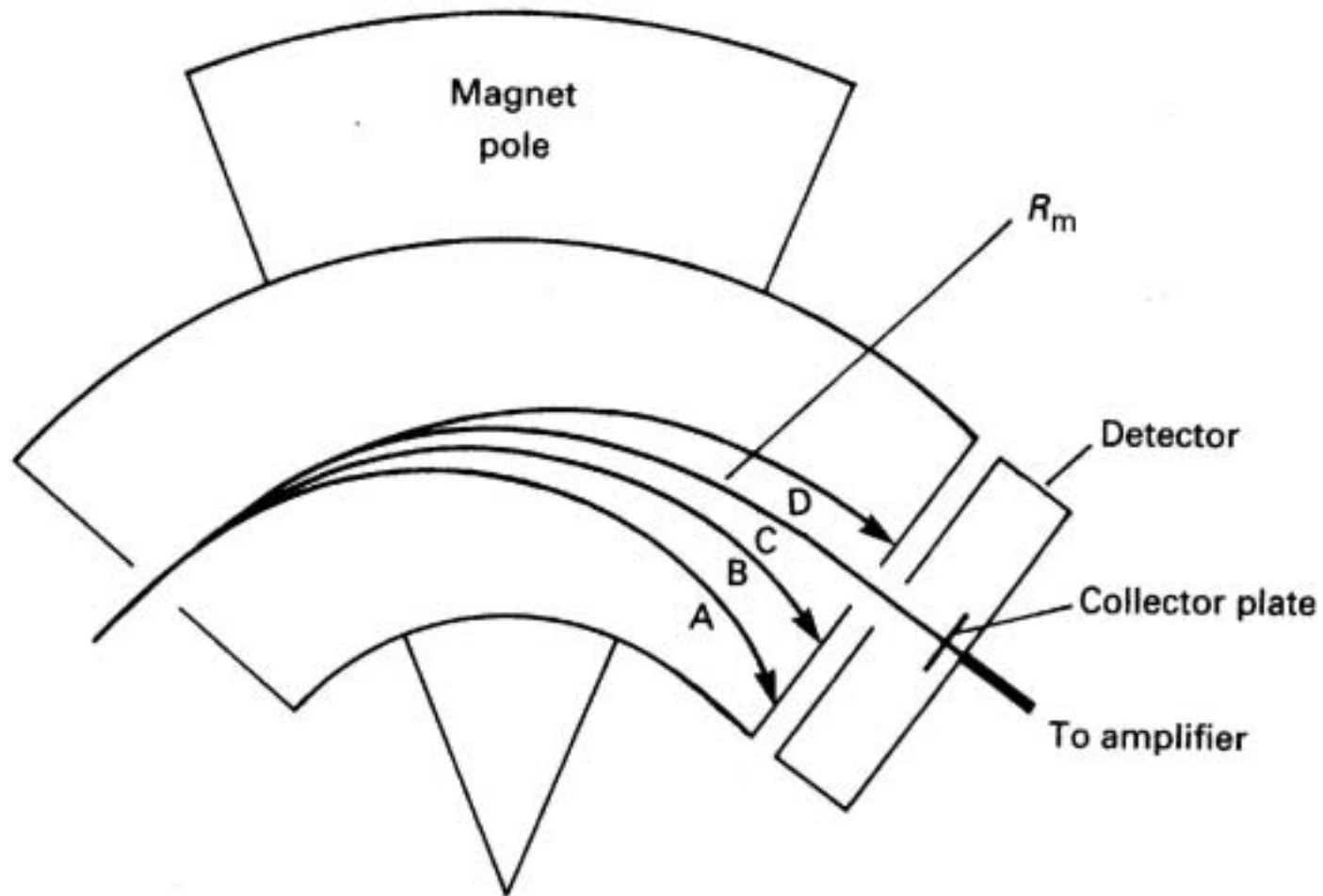


Fig. 11.24. Flow profiles: (a) electroosmotic, flat; (b) Poiseuille, bullet.



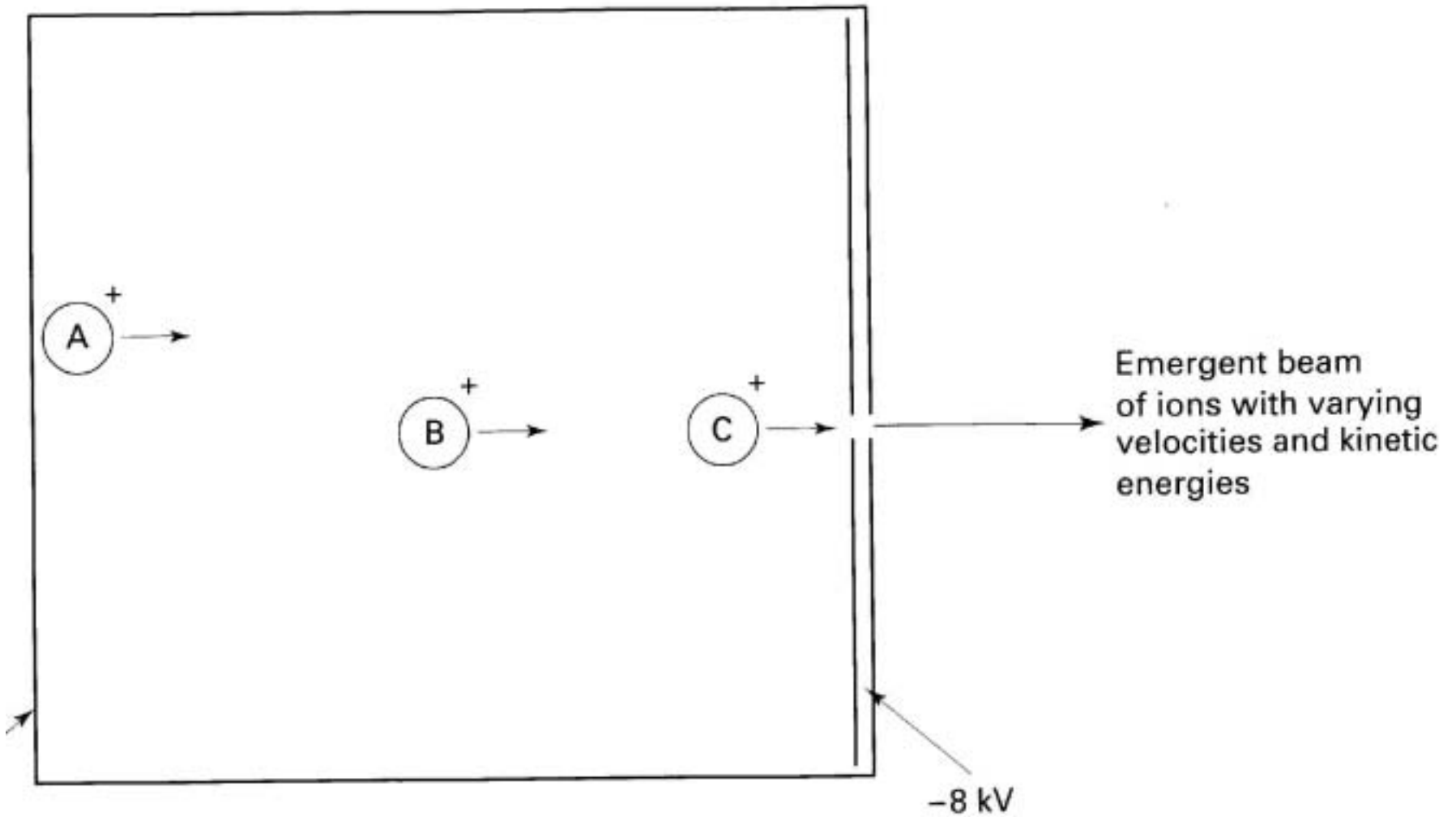
Representative of ion generation between acceleration plates. Only ions following a trajectory of radius R_m will be focused. A, B and D are currently defocused. By altering the field, other ions can be forced to travel along R_m and be focused.

$$m/z = B^2 R^2 / 2V$$

B – плотность потока магнитного поля

R – радиус траектории

V – ускоряющее напряжение, используемое вне
источника ионов

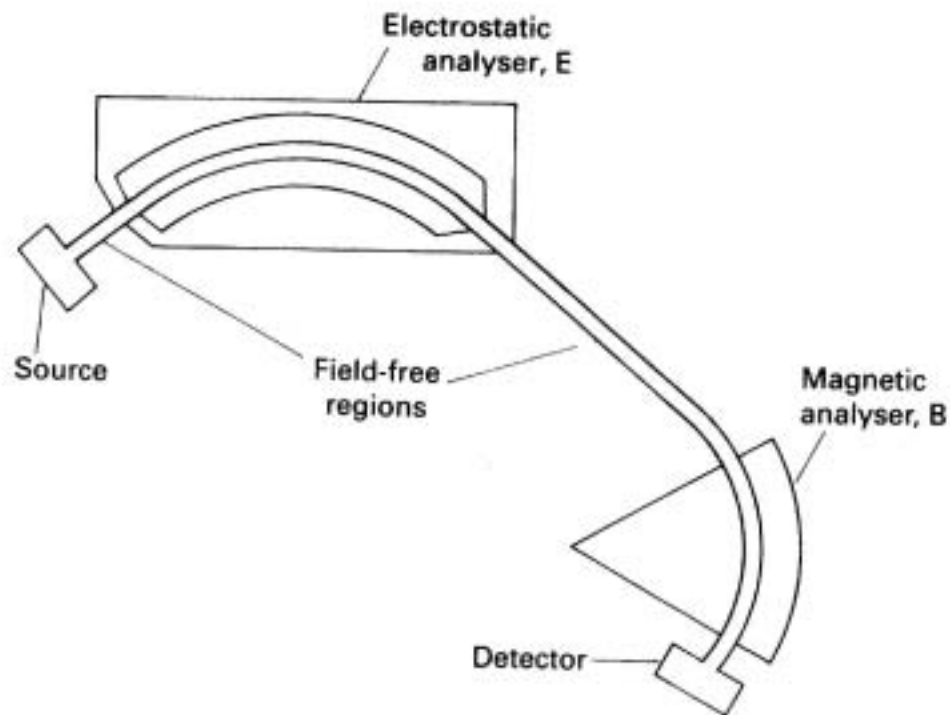


Ions arising at different points in space between the acceleration plates.

$$R_e = 2V/E$$

V – ускоряющее напряжение в источнике ионов

E – напряженность электростатического поля в анализаторе

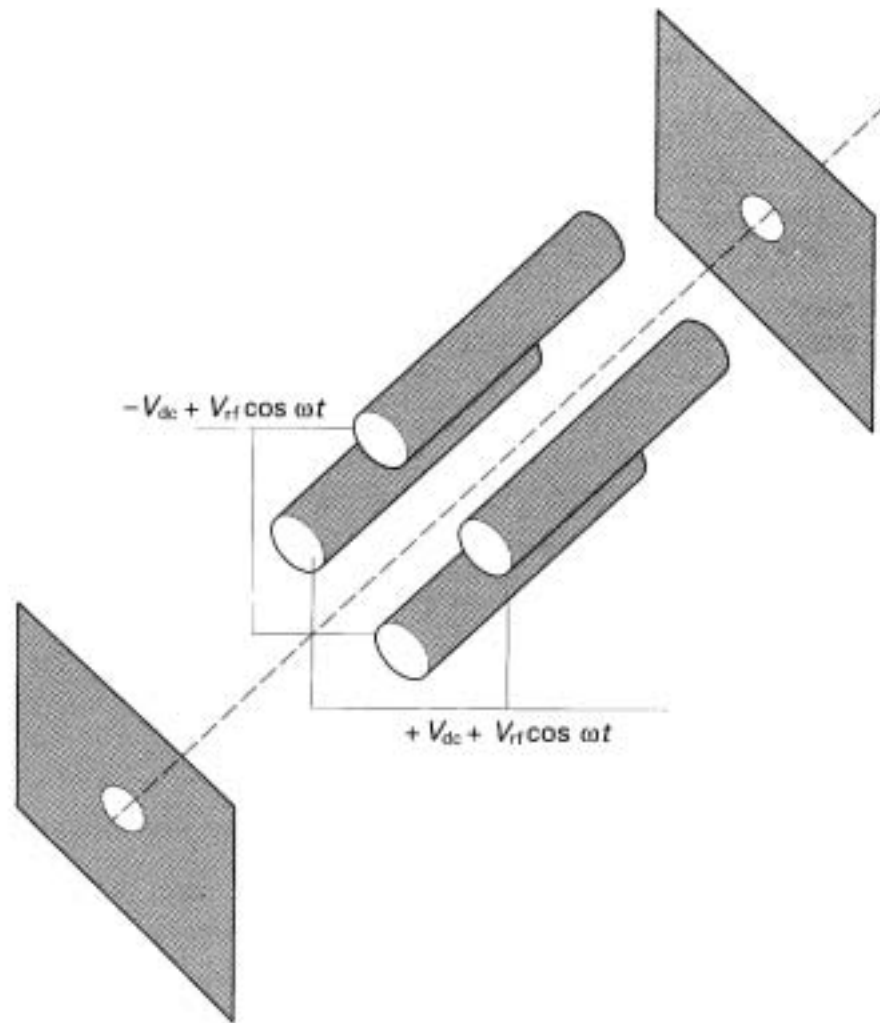


Double focusing mass spectrometer.

Nominal and accurate masses of neutral loss groups in fragmentation

Neutral group lost	Nominal mass	Accurate mass
CO (carbonyl)	28	27.994914
C ₂ H ₄ (double bond fragment)	28	28.031299
N ₂ (diazo, in some drugs)	28	28.006158
(CH ₂ N) (nitrile or isonitrile)	28	28.018732 ^a
(CNH ₂) (amino-carbon)		

^aThe last two examples cannot be distinguished by accurate mass measurement.



Quadrupole mass filter. In the quadrupole mass filter one opposite pair of rods has a negative DC voltage, $-V_{dc}$, applied and the other pair a positive DC voltage, $+V_{dc}$. There is also a superimposed radiofrequency (RF) voltage, $V_{rf} \cos \omega t$, which is 180° out of phase between rod pairs. Mass filtering occurs as these voltages are scanned but the ratio DC to RF is kept constant. In spatial tandem mass spectrometry, Section 11.10.1 and Fig. 11.34, quadrupole collision cells are used which are RF-only devices. No DC voltages are applied and no mass filtering occurs in these cases.

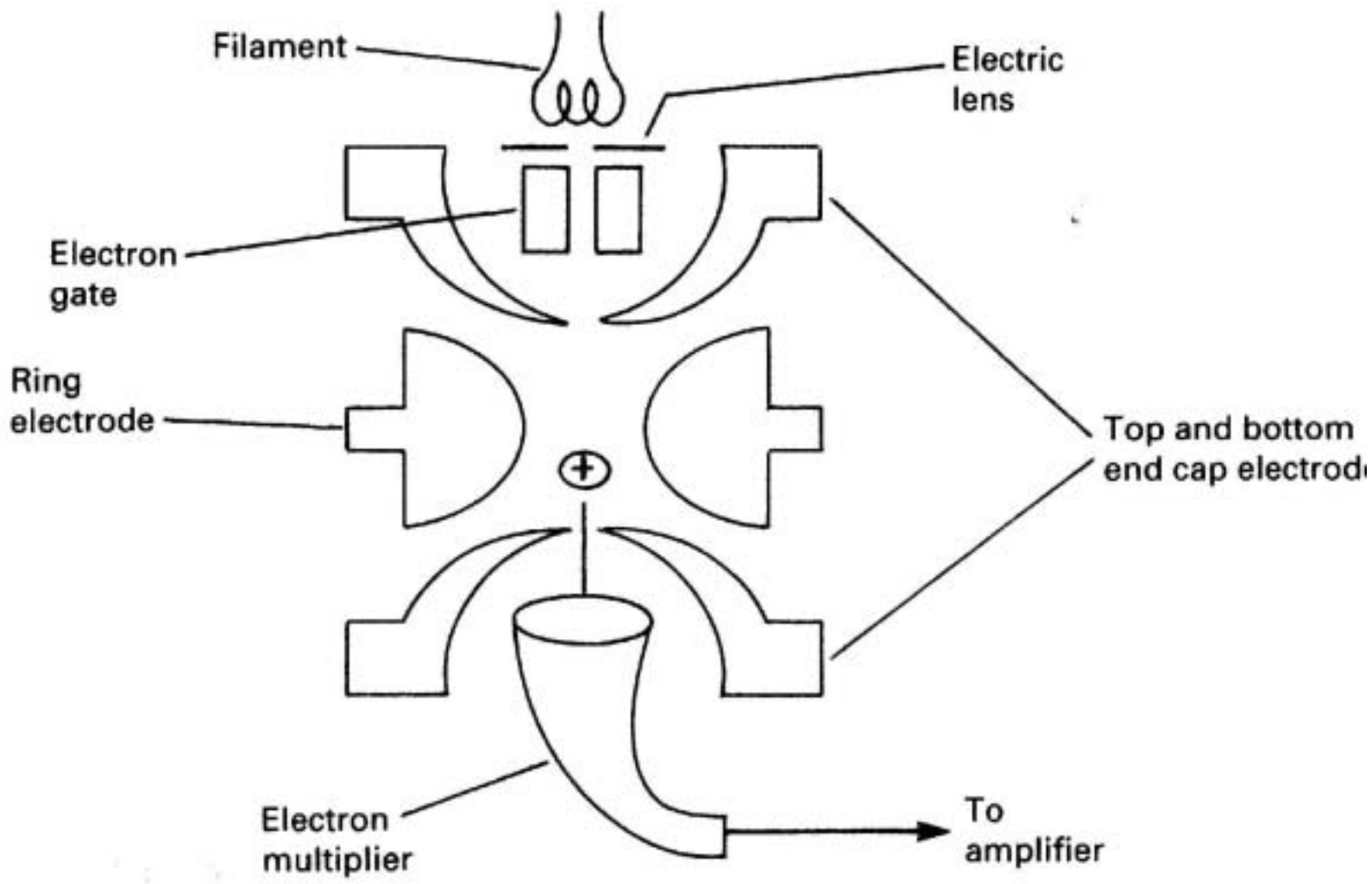
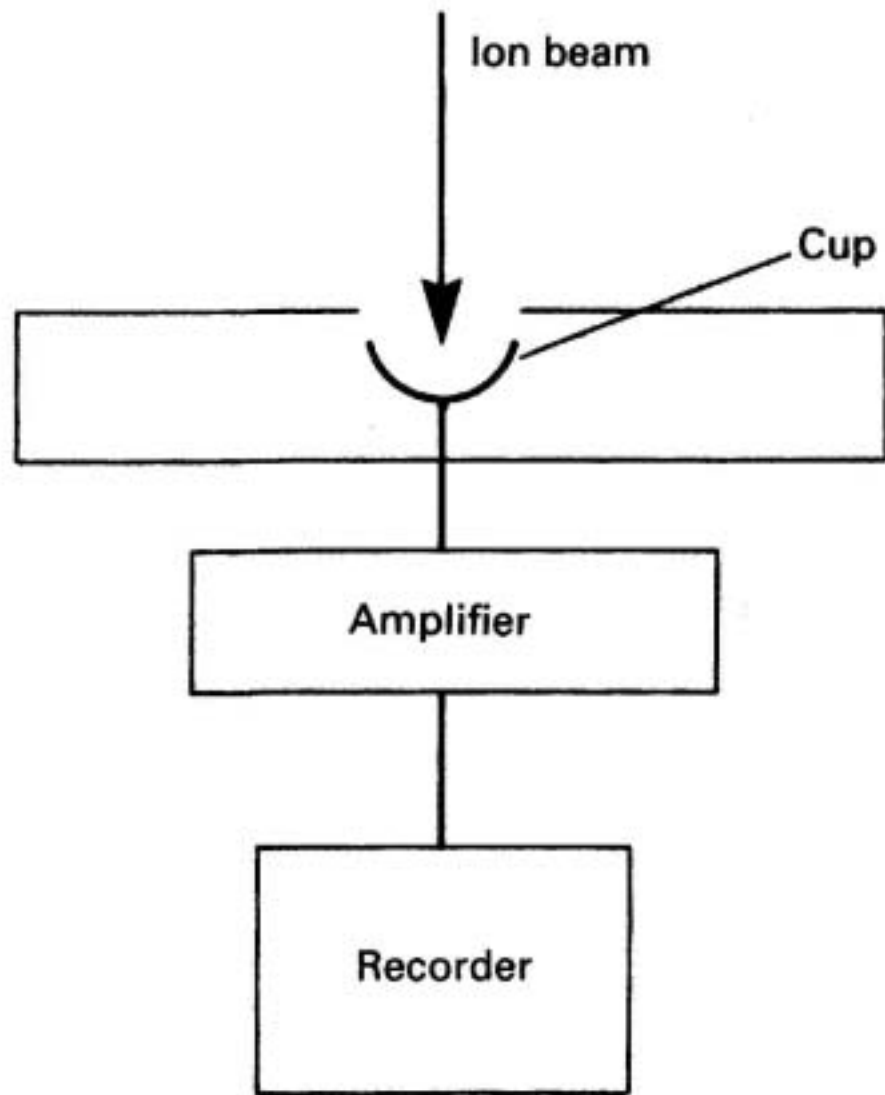
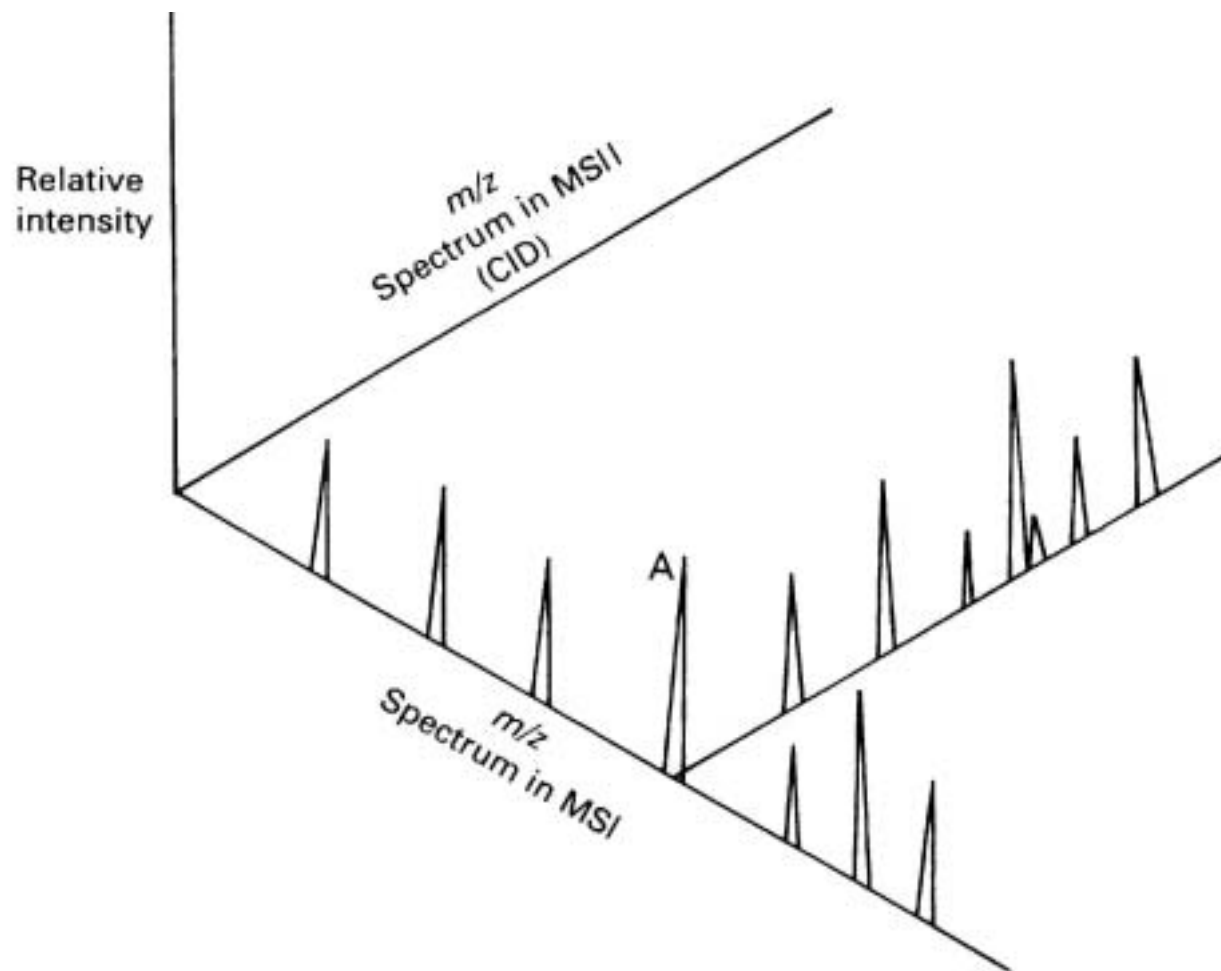


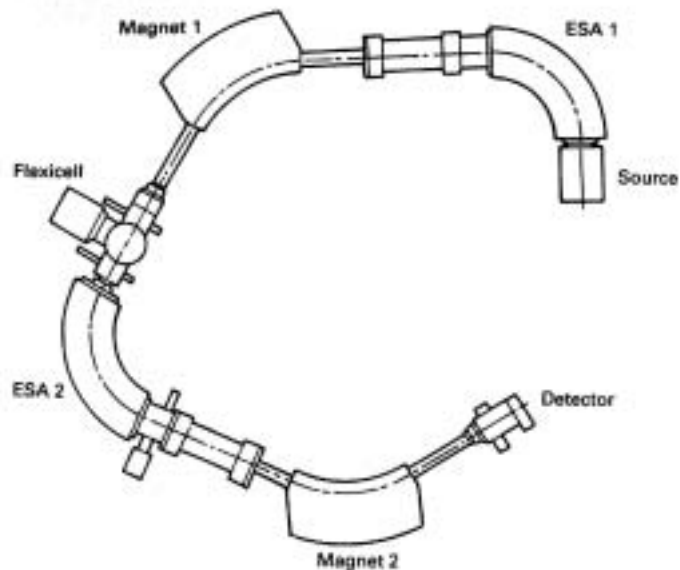
Diagram of the ion trap.



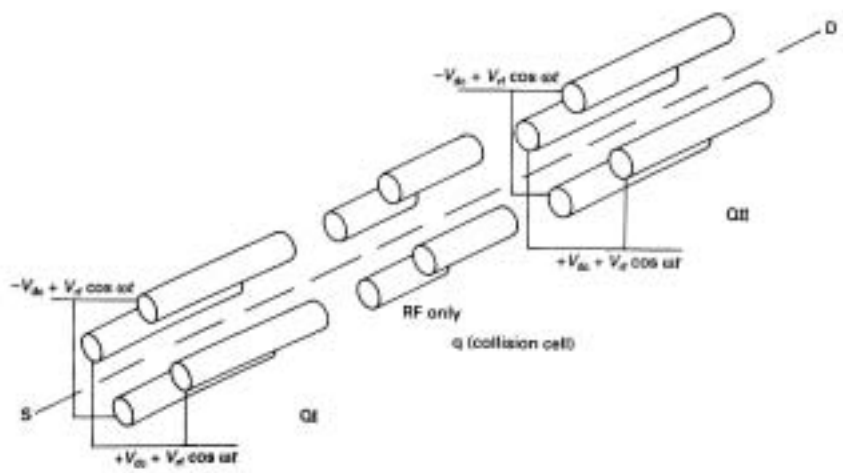
Simplified diagram of the Faraday cup.



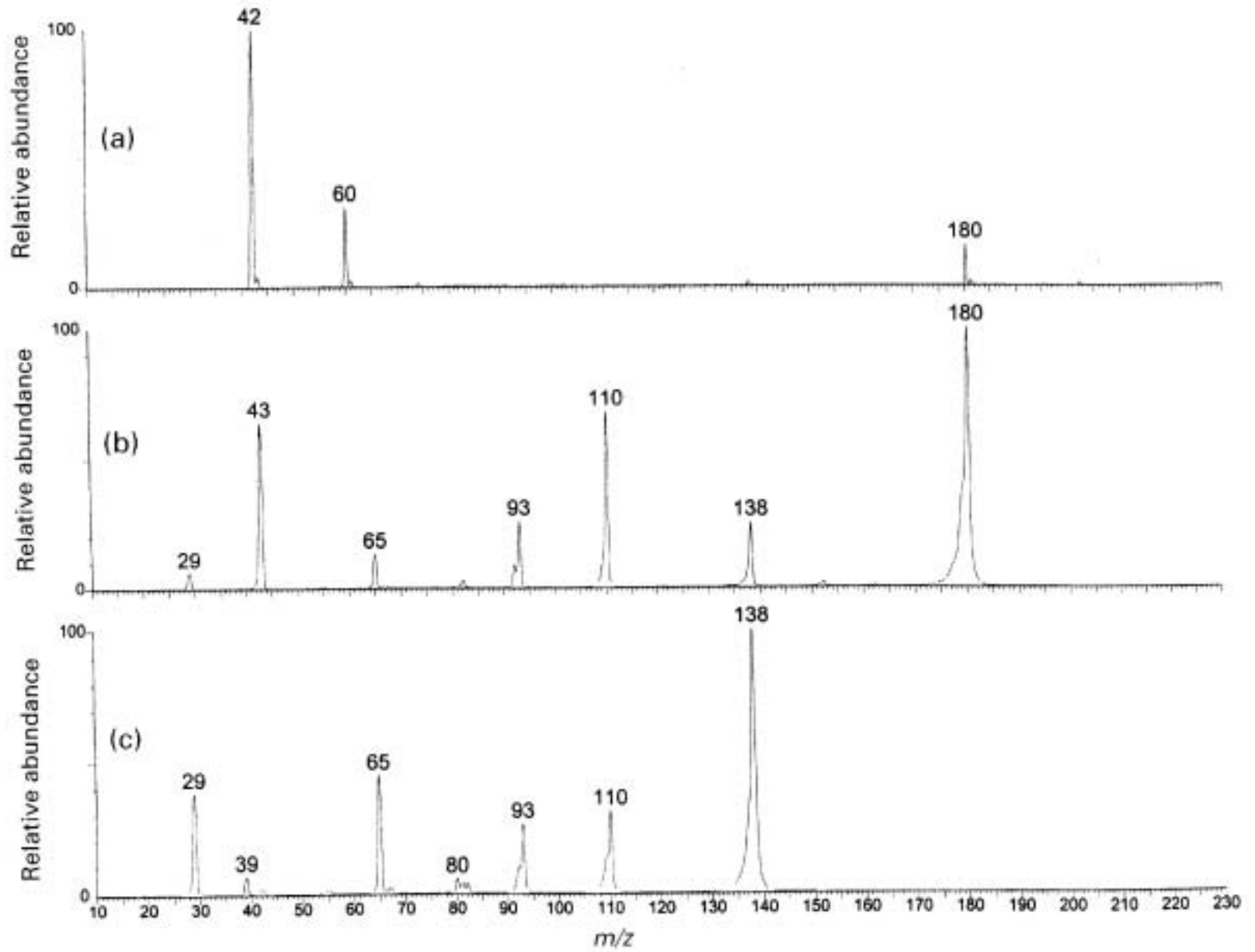
Two-dimensional mass spectrometry spectra obtained in a MSII experiment. The first mass spectrum is generated in mass spectrometer I (MSI) and is shown along the appropriately labelled axis. A particular peak, for example that marked A, is selected and the packet of ions comprising this peak is focused into the collision cell where CID occurs. The m/z spectrum corresponding to these decomposition fragments is analysed in MSII. This CID mass spectrum is shown along the isometric axis parallel to the axis labelled spectrum in MSII.



Kratos Concept II H H four-sector instrument. This design includes two forward geometry sector mass spectrometers linked through a collision cell, termed a flexicell by this manufacturer. MSI is represented by ESA 1 and magnet 1 and is where the appropriate first mass spectrum is obtained. Collisionally induced decomposition occurs in the flexicell and the products analysed in MSII, which incorporates ESA 2 and magnet 2. (Reproduced by kind permission of Kratos Analytical, Manchester.)



Arrangement in triple quadrupole systems. S, source; D, detector; RF, radiofrequency.



Single and multiple mass spectra of phenacetin.

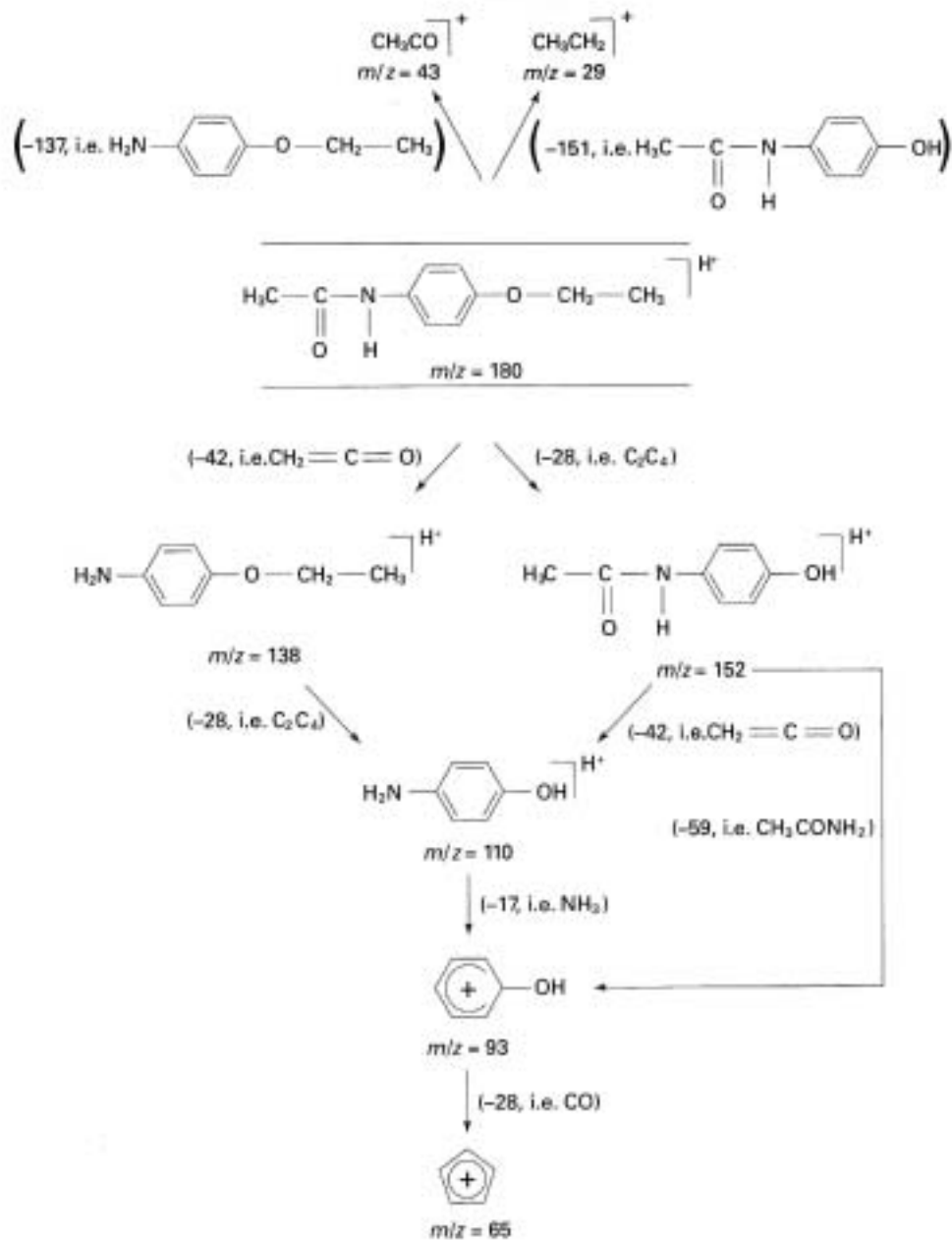


Fig. 11.36. Fragmentation pathways for phenacetin.

Gordon D.B. Mass spectrometric techniques.
In: Principles and Techniques of practical Biochemistry.
K.Wilson & J.Walker Eds. Cambridge University Press,
Cambridge, 2000

Proteome Research: Mass Spectrometry. P. James Ed.
Springer-Verlag, Berlin-Heidelberg-New York, 2001

The API Book. Perkin-Elmer SCIEX.