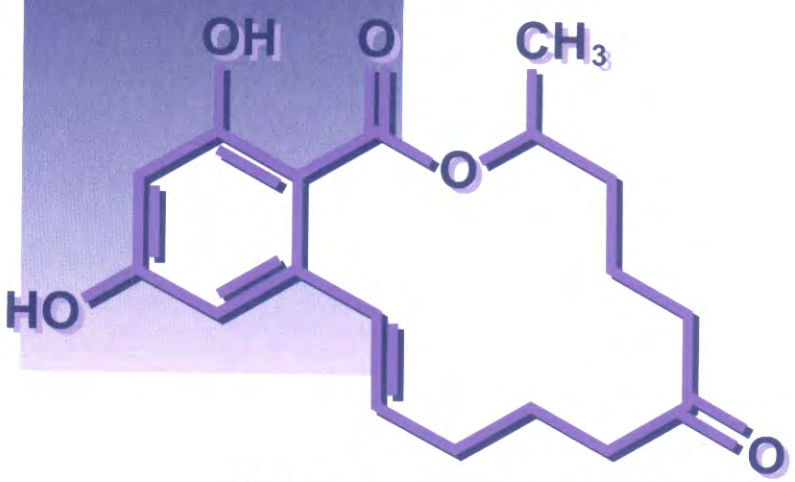
RSC FOOD ANALYSIS MONOGRAPHS

RS•C
ROYAL SOCIETY OF CHEMISTRY



Mass Spectrometry of Natural Substances in Food

F. MELLON, R. SELF and J.R. STARTIN

series editor PETER S. BELTON

MASS SPECTROMETRY OF NATURAL SUBSTANCES IN FOODS

RSC Food Analysis Monographs

Series Editor: P.S. Belton, *The Institute of Food Research, Norwich, UK.*

The aim of this series is to provide guidance and advice to the practising food analyst. It is intended to be a series of day-to-day guides for the laboratory worker, rather than library books for occasional reference. The series will form a comprehensive set of monographs providing the current state of the art on food analysis.

Dietary Fibre Analysis
by David A.T. Southgate, *Formerly of the AFRC Institute of Food Research, Norwich, UK.*

Quality in the Food Analysis Laboratory
by Roger Wood, *Joint Food Safety and Standards Group, MAFF, Norwich, UK*, Anders Nilsson, *National Food Administration, Uppsala, Sweden,* and Harriet Wallin, *VTT Biotechnology and Food Research, Espoo, Finland*

Chromatography and Capillary Electrophoresis in Food Analysis
by Hilmer Sørensen, Susanne Sørensen and Charlotte Bjergegaard, *Royal Veterinary and Agricultural University, Frederiksberg, Denmark,* and Søren Michaelsen, *Novo Nordisk A/S, Denmark*

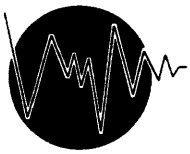
Mass Spectrometry of Natural Substances in Foods
by Fred A. Mellon, *The Institute of Food Research, Norwich, UK,* Ron Self, *University of East Anglia, Norwich, UK,* and James R. Startin, *Central Science Laboratory, York, UK*

How to obtain future titles on publication

A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately upon publication. For further information, please write to:

Turpin Distribution Services Ltd.
Blackhorse Road
Letchworth
Herts. SG6 1HN

Telephone: Letchworth (01462) 672555



RSC
FOOD
ANALYSIS
MONOGRAPHS

Mass Spectrometry of Natural Substances in Foods

Fred A. Mellon
Institute of Food Research, Norwich, UK

Ron Self
University of East Anglia, Norwich, UK

James R. Startin
Central Science Laboratory, Sand Hutton, York, UK

RS•C
ROYAL SOCIETY OF CHEMISTRY

ISBN 0-85404-571-6

A catalogue record for this book is available from the British Library.

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF, UK

For further information see our web site at www.rsc.org

Typeset by Paston Prepress Ltd, Beccles, Suffolk, NR34 9QG
Printed & bound in Great Britain by TJ International Ltd, Padstow, Cornwall

Preface

Mass spectrometry has been a pre-eminent analytical technique in food science from an early stage in its development. Flavour chemists, for example, were quick to realise the potential of combined gas chromatography/mass spectrometry (GC/MS) for determining the very complex mixtures of volatile compounds in food aromas. The subsequent introduction of powerful data acquisition and processing systems, including automated library search techniques, ensured that the information content of the large quantities of data generated by GC/MS instruments was exploited fully. These early successes were the foundation of an increasingly diverse range of applications, utilising many different mass spectrometric techniques, in other areas of food science.

Mass spectrometry's unparalleled combination of sensitivity and selectivity (especially when allied to separation techniques such as GC and HPLC) ensured its early adoption as a key analytical technique for monitoring chemical aspects of food safety. Initially, this was exemplified by the determination of anthropogenic compounds in foods (pesticide and veterinary drug residues, for example). These types of application are not discussed in this book, which is devoted to natural substances in foods. However, applications to the analysis of naturally occurring defensive compounds in food plants and naturally occurring substances that are either detrimental or beneficial to health are introduced and described.

Mass spectrometry has evolved rapidly in the last two decades. The introduction of cheap open-access instrumentation has ensured that mass spectrometry is no longer confined to research laboratories but is now used routinely in many analytical laboratories for compositional analysis. The last few years have also witnessed the revolution in analysis of molecules of biological interest brought about by the introduction of soft ionisation techniques such as matrix-assisted laser desorption (MALDI) and electrospray ionisation (ESI). This has particular implications for food quality and safety. The ability to analyse biopolymers, more or less routinely, is beginning to have a major impact on the study of food structure components (oligosaccharides, for example) and food proteins (including allergens). Furthermore, the rapidly developing field of proteomics, where advanced mass spectrometric techniques are pivotal, has tremendous potential in the study of food pathogens and food components produced from genetically modified organisms. The ability to measure high molecular weights accurately is also benefiting research into naturally occurring food antibiotics and food allergens. The development of ESI and the related atmospheric

pressure chemical ionisation (APCI) source has also brought routine LC/MS techniques within the reach of many food and nutrition laboratories. This is revolutionising the study of micronutrient metabolism, of non-volatile taste and flavour components and of biologically active, naturally occurring non-nutrients, to identify just a few examples.

Inorganic mass spectrometry, particularly inductively coupled plasma mass spectrometry (ICP-MS), is another discipline that is making a major contribution to food science. In addition to its role in food toxicology (not discussed in this book), ICP-MS is used in a growing number of laboratories to study the metabolism of nutrient minerals, including iron, zinc, calcium, copper and selenium. This is helping to fill major gaps in our knowledge of the amounts of these minerals needed to maintain optimal health (not simply to prevent deficiencies). Furthermore, inorganic mass spectrometry is also helping to elucidate the influence of the food matrix on the absorption and metabolism of mineral nutrients.

Metabolic studies of organic food components have benefited from improvements in the performance and flexibility of high precision isotope ratio mass spectrometers (used for measuring ^{13}C, ^{18}O and ^{15}N isotopes). These developments are reflected in increased activity in the field of metabolic studies in humans, particularly those involving isotopically labelled macronutrients.

One of our main aims has been to convey the depth and breadth of mass spectrometric applications to natural substances in foods. After opening chapters that introduce the principles and practice of mass spectrometry, we cover applications in flavour analysis, and the determination of non-nutrient biologically active natural substances in foods. We go on to discuss the analysis and metabolism of amino acids, peptides, proteins, lipids, sugars, carbohydrates and vitamins, with separate chapters on mineral and macronutrient metabolism and techniques of pyrolysis mass spectrometry. Numerous references are given to specific analyses, to encourage the reader who wishes to pursue these applications in greater depth. We hope that this volume will be a useful resource to food scientists, food analysts and researchers into the composition of foods and into nutrition and food safety.

Fred A Mellon
Ron Self
James R Startin
Norwich and York, 1999

Contents

Glossary of Abbreviations

AE	Appearance Energy
AMS	Accelerator Mass Spectrometry
APCI	Atmospheric Pressure Chemical Ionisation
CAD	Collisionally Activated Decomposition (≡ CID)
CE/MS	Combined Capillary Electrophoresis/Mass Spectrometry
CI	Chemical Ionisation
CID	Collision Induced Dissociation
DCI	Desorption Chemical Ionisation
DEI	Desorption Electron Ionisation
DLI	Direct Liquid Introduction
EI	Electron Ionisation
ESI	Electrospray
FAB	Fast Atom Bombardment (also FABMS)
FD	Field Desorption (also FDMS)
FFR(1, 2. . .)	First (second. . ., *etc.*) Field Free Region
FI	Field Ionisation (also FIMS)
FTICR	Fourier Transform Ion Cyclotron Resonance
FTMS	Fourier Transform Mass Spectrometry
GC/C/IRMS	Gas Chromatography/Combustion/Isotope Ratio Mass Spectrometry
GC/MS	Combined Gas Chromatography/Mass Spectrometry
GIRMS	Gas Isotope Ratio Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IE	Ionisation Energy
ITMS	Ion Trap Mass Spectrometer
LC/MS	Combined High Performance Liquid Chromatography/Mass Spectrometry
LD	Laser Desorption
LSIMS	Liquid Secondary Ion Mass Spectrometry
MALDI	Matrix Assisted Laser Desorption Ionisation
MECC	Micellar Electrokinetic Capillary Chromatography
MPI	Multiphoton Ionisation
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry (or Mass Spectrometer)
MS/MS	Tandem Mass Spectrometry

NICI	Negative Ion Chemical Ionisation
PDMS	Plasma Desorption Mass Spectrometry
Py/MS	Pyrolysis Mass Spectrometry
R	Resolution
REMPI	Resonance Enhanced Multiphoton Ionisation
SFC/MS	Combined Supercritical Fluid Chromatography/Mass Spectrometry
SFI/MS	Combined Supercritical Fluid Injection/Mass Spectrometry
SID	Surface Induced Dissociation
SIM	Selected Ion Monitoring
SIMS	Secondary Ion Mass Spectrometry
SRM	Selected Reaction Monitoring
TIC	Total Ion Current
TIMS	Thermal Ionisation Mass Spectrometry
TLC/MS	Combined Thin Layer Chromatography/Mass Spectrometry
ToF	Time of Flight
TSP	Thermospray
VOC	Volatile Organic Compounds

CHAPTER 1

Introduction to Principles and Practice of Mass Spectrometry

1 History of Mass Spectrometry

According to the International Union of Pure and Applied Chemistry definition, 'Mass spectrometry is the branch of science dealing with all aspects of mass spectroscopes and the results obtained with these instruments.'[1] It evolved from research in particle physics at the turn of the century, with Goldstein discovering positively charged 'rays' in 1886 and Wien (1898) studying their electric and magnetic properties. In the early 1900s J.J. Thomson built his 'parabola mass spectrograph' to measure the charge to mass ratio (z/m) for several ionic species. In the expression z/m, z is the charge number, *i.e.* the total charge on an ion divided by the elementary charge (e), and m is the nucleon number, *i.e.* the sum of the total number of protons and neutrons in an atom, molecule or ion. In modern mass spectrometry, the parameter measured is m/z, rather than z/m: the unit of m/z was recently designated the thomson (Th).

Aston continued the work at Cambridge and built instruments that helped him to establish the presence of isotopes. He was subsequently able to measure the atomic mass of most elements with sufficient accuracy to be able to calculate the 'packing fraction' of their atomic nuclei. The packing fraction is the difference between the accurate atomic mass of the isotope and the nearest whole number divided by the mass number, also known as the mass defect. Aston also obtained accurate measurements of the ratios of the stable isotopes of many of the known elements.

At the end of this exciting period of development, Aston was convinced that much of the potential of mass spectrometry had been exploited. It was not until the 1940s that the technique was put to work in elucidating organic structures in the petroleum industry. Ionisation was effected by electron 'impact' [now called electron ionisation (EI)] for those molecules that could withstand vaporisation into the heated and evacuated ion source without decomposition. This limited the practical mass range to less than 1000 daltons (Da)* but yielded useful

* The dalton (Da) is the unit of mass (also known as the mass unit, u) and is 1/12 of the mass of ^{12}C (defined as 12.000000).

fragmentations for structure elucidation (see Chapter 2). By choosing to work with 70 electron volt (eV) electrons many ions were formed with internal energies far in excess of the ionisation energy (IE)†. These ions decompose rapidly to produce lower mass (fragment) ions and neutral radicals or molecules.

During the 1950s, commercial instruments were being built and new applications discovered. One of the earliest of these was the identification of low molecular weight volatile food flavour compounds. Ten years later, the powerful combination of electron ionisation mass spectrometry (EIMS) with gas chromatography (GC/MS) led to an explosion of applications where mass spectrometry was used in qualitative and quantitative, chemical and biochemical studies. GC/MS instruments produced enormous amounts of data, which were best handled by computers, and data acquisition and processing methods were devised. In 1966, Munson and Field described chemical ionisation (CI). This technique increased the yield of ions representative of the molecular weight of volatile molecules through interactions with reagent gas ions (*e.g.* CH_5^+ ions from methane) with little excess energy.[2] Other 'soft' ionisation techniques such as field desorption (FD) and particle desorption methods based upon ion generation by Cf-252 fast fission products [plasma desorption, (PDMS)] were introduced during the 1970s for involatile compounds. At the same time (and in response to these developments) the instrumental mass range was increased to cope with the larger sample molecule ions now entering the gas phase. This process accelerated in the 1980s with the introduction of Fast Atom Bombardment (FAB) ionisation. FAB was the first ionisation technique to enable biologists and biochemists *routinely* to obtain molecular weight information on complex, labile biomolecules, including polypeptides and small proteins.

Ionisation from the liquid state, followed by evaporation/desolvation of charged droplets, includes techniques such as ion spray, thermospray (TSP) and electrospray ionisation (ESI). These methods differed mainly in the manner in which ionisation was initiated. Multiply charged molecular ions could be formed under ESI, facilitating the measurement of high molecular masses, even on conventional instruments (*i.e.* those with a mass range up to 2000 or 4000 Th). More efficient pumping systems were required to cope with the increased gas volumes generated by vaporising liquids.

Separation techniques such as liquid chromatography and capillary electrophoresis coupled to mass spectrometry (LC/MS and CE/MS respectively) have extended the advantages first associated with the analysis of volatile compounds by GC/MS to compounds of low volatility and high molecular weight. Tandem mass spectrometry (MS/MS) collision-induced dissociation (CID), focal-plane array detectors, ion traps and hybrid instruments are providing a high sensitivity structure elucidation facility for involatile compounds similar to that provided by EIMS of volatiles. Recently, Laser Desorption (LD), and especially Matrix Assisted Laser Desorption Ionisation (MALDI), combined

† Ionisation energy – minimum energy of excitation of an atom or molecule required to remove an electron in order to produce a positively charged ion.

with Time-of-Flight (ToF) mass analysis has extended the practical mass range to over 300 000 Da, producing mainly singly-charged molecular ions. Fourier transform mass spectrometry (FTMS), also known as Fourier transform ion cyclotron resonance (FTICR) mass spectrometry is only slowly entering the commercial area.

Only a brief introduction to the principles and practice of mass spectrometry is given herein. The reader is directed towards more general textbooks, *e.g.* Throck Watson[3] or Rose and Johnstone,[4] for a more detailed discussion of the principles and practice of mass spectrometry. Beynon and Brenton[5] lucidly introduce the physical aspects of mass spectrometry and ionisation in the gas phase. Finally, the latest volume by McLafferty and Turecek[6] is recommended for a very thorough introduction to the interpretation of organic mass spectra.

Mass Spectrometry – a definition

> Mass spectrometry is the study of systems causing the formation of gaseous ions, with or without fragmentation, which are then characterised by their mass to charge ratios (m/z) and relative abundances.

Mass spectrometry is unlike most other forms of spectroscopy or spectrometry that are concerned with non-destructive interactions between molecules and electromagnetic radiation. This is because mass spectrometry is the study of the effect of ionising energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species. Although sample is consumed destructively by the mass spectrometer the technique is very sensitive and only trace amounts of material are used in the analysis. A mass spectrometer converts sample molecules into ions in the gas phase, separates them according to their mass to charge ratio (m/z) and sequentially records the individual ion current intensities at each mass – the mass spectrum. If these ion current intensities are drawn in histogram form taking the most intense ion current as 100%, the values of m/z versus percentage relative intensity (%RI) is called a line diagram, *e.g.* Figure 1.1.

2 Ionisation of Molecules

Several types of mass spectrometry can generate ions representative of the mass of the sample molecule. These are described below and, because ionisation is often intimately linked to sample introduction techniques, this topic is also discussed.

Production of Molecular Ions

If a quantity of energy equivalent to the IE of the molecule is supplied under EI conditions, a molecular ion is formed that is a radical ion denoted as $M^{+\bullet}$. There are several ways of forming molecular ions.

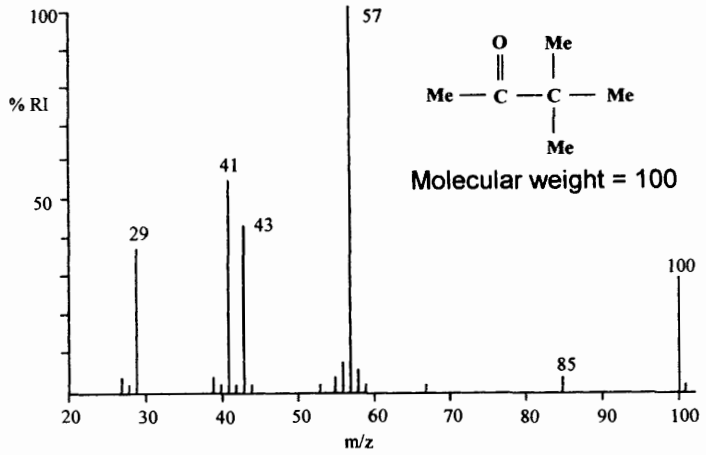


Figure 1.1 *Electron ionisation mass spectrum of methyl t-butyl ketone*

Electron Ionisation (EI)

70 eV electrons passing through the ionisation chamber from the filament to the trap, under the control of the magnetic field, interact with volatilised sample molecules, which enter their path (Figure 1.2).

The temperature of volatilisation can be varied from ambient to $>400\,^{\circ}C$ in a vacuum of about 10^{-3} Pa. Interaction of the sample molecule with an energetic electron removes one (and sometimes two or more) electrons from the valence

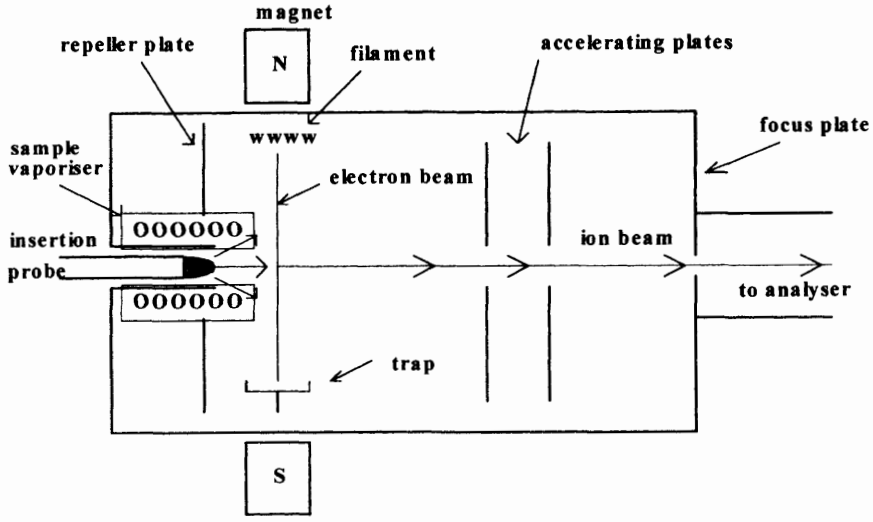


Figure 1.2 *Schematic diagram of an Electron Ionisation source*

orbitals. During this process, an excess of energy can be transferred to the newly formed positively charged 'radical ion'.

$$M \xrightarrow{e^-} M^{+\cdot} + 2e^- \tag{1.1}$$

Since the IEs of most organic molecules lie in the range 8–12 eV, the excess energy can cause bond dissociation (fragmentation) within the ion. The distribution of energy among the ions formed, and the ensuing pattern of fragmentation produced from different chemical structures, depends on several factors. Two of these, chemical bond lability and fragment ion stability, form a basis for the qualitative interpretation of mass spectra and are discussed in Chapter 2. The efficiency of the ionisation process in relation to the electron beam energy used is shown in Figure 1.3.

Most organic molecules are ionised by 8–12 eV and an additional 1–6 eV will dissociate any cleavable bonds. The choice of 70 eV electrons for conventional EI mass spectrometry (a) ensures efficient transfer of enough excess energy to induce structurally informative fragmentation and (b) is in the plateau region of the figure, where it is easier to generate reproducible mass spectra. Although 70 eV is the ionisation energy of choice in EI, it is important to note that not all this energy is transferred to molecules during ionisation.

In principle, a spectrum free from fragmentation can be obtained by lowering the energy of the bombarding electrons to values close to the IE. However, the ionisation efficiency is also lowered by a factor of 200–300, so that large quantities of sample would be required for successful analysis. Some molecules with little excess energy can dissipate this by stabilisation among their degrees of vibrational freedom. These ions will be detected as molecular ions in the mass spectrum. Other ions will apportion the energy to cleave bonds to produce well-stabilised fragment ions. Many organic compounds decompose at the elevated temperatures required for vaporisation into the conventional EI source and others do not produce stable molecular ions under normal EI conditions. In these cases, alternative ionisation methods are available.

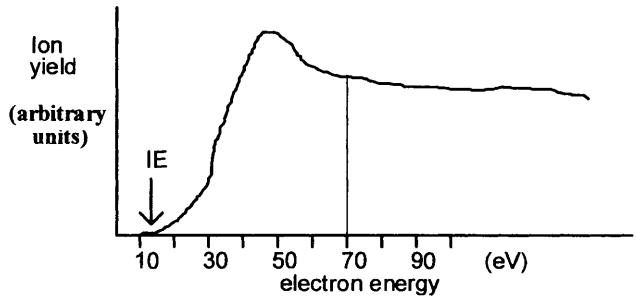


Figure 1.3 *Typical variation in ion yield from an organic compound with increasing electron beam energy*

'In-beam' (Desorption) Electron Ionisation

For the improved ionisation of relatively involatile but stable molecules, a special probe is used to place the sample very close to the electron beam. This technique, commonly known as desorption EI (DEI), has been successful with a limited range of previously intractable biomolecules, for example cyclic peptides and some glycosides.

Chemical Derivatisation

The preparation of chemical derivatives will increase the volatility and (sometimes) reduce the IE of polar compounds, allowing EI to generate stable molecular ions. Organic acids, fatty acids, *etc.* can be analysed by EIMS of their methyl esters. Many chemical classes can be rendered volatile through the preparation of a variety of silylated products that enable mass and structural information to be obtained. Special chemical derivatives can be made that yield characteristic fragmentation properties, thereby aiding analysis. An example is the preparation of *t*-butyldimethylsilyl derivatives of steroids. These yield an abundant $[M - 57]^+$ ion (generated by loss of C_4H_9) that is especially useful in quantitative measurements.

The Particle Beam Interface

The particle beam interface (Figure 1.4) is a sample introduction rather than an ionisation technique in which the incoming liquid sample, *e.g.* HPLC eluent, is nebulised with helium gas to form an aerosol of solvent droplets.

The stream of liquid droplets is allowed to desolvate at ambient temperature in a chamber at the reduced pressure provided by vacuum pump 1. The

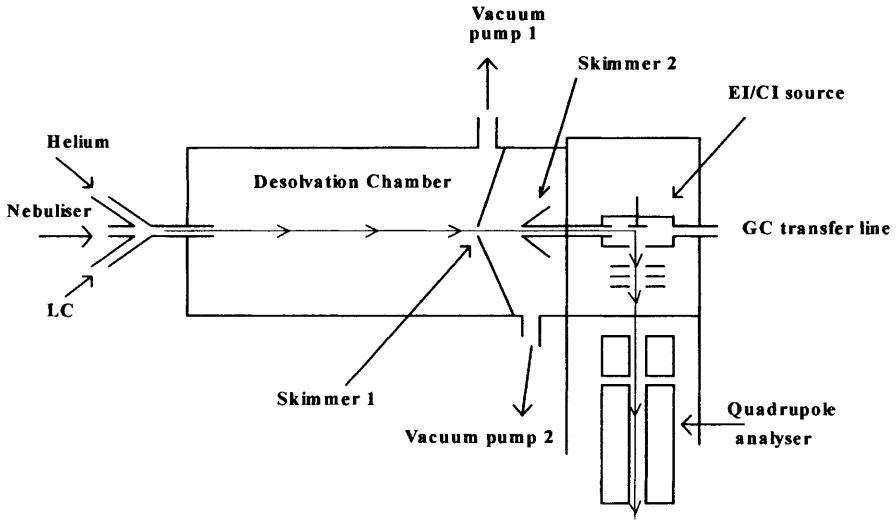


Figure 1.4 *Schematic diagram of a particle beam LC/MS interface*

remaining molecules then expand into the second evacuated region (provided by vacuum pump 2) through skimmer 1, where a supersonic jet (molecular beam) is formed. Skimmer 2 allows the molecular beam, containing heavy sample particles, to pass through into the ionisation chamber for subsequent electron, or chemical ionisation. The lighter helium and solvent particles are skimmed off and removed by vacuum pump 2. Although particle beam systems appear to be an ideal method for ionising involatile compounds, the technique lacks sensitivity and is unsuitable for polar, thermally labile molecules.

Production of Protonated Molecules and Adduct Ions

Methods that generate ions representative of the molecular weight of less volatile and thermally labile molecules are generally based on two main principles. Firstly, reaction and thermal equilibration with a reagent gas. Secondly, direct desorption from a liquid or solid matrix during or prior to ionisation. Under these conditions, ion/molecule reactions are generally responsible for the ionisation process.

Chemical Ionisation (CI)

In CI, chemical reactions occur between thermally equilibrated reagent ions and sample molecules. The conditions required are: a large excess (10^4:1) of reagent gas (R) to sample molecules (M), necessitating the use of higher energy electrons (500 eV) at the resultant source pressures in the range of 10–150 Pa. Primary ions are formed in the dense gas:

$$R + e^- \longrightarrow R^{+\bullet} \tag{1.2}$$

These primary ions react with more reagent gas to form stable, reactive secondary cations (even-electron species) by collisional hydrogen transfer:

$$R^{+\bullet} + R \longrightarrow RH^+ + (R - H)^\bullet \tag{1.3}$$

If methane is the reagent gas, CH_5^+ (a Lewis acid) is produced. This ion reacts strongly with organic molecules (at around 20 Pa pressure) by proton or hydride transfer reactions, generating stable, protonated molecules. Other reagent ions formed during methane CI include $C_2H_5^+$, $C_2H_3^+$ and $C_3H_5^+$, although CH_5^+ and $C_2H_5^+$ are the predominant species.

Protonation

Protonation occurs when the proton affinity of the sample molecules is higher than that of the reagent gas:

$$RH^+ + M \longrightarrow R + MH^+ \tag{1.4}$$

Hydride Ion Abstraction (Dissociative Proton Transfer)

This is common for samples with lower proton affinities than the reagent gas, *e.g.* alkanes:

$$RH^+ + C_{10}H_{22} \longrightarrow C_{10}H_{21}^+ + R + H_2 \tag{1.5}$$

Charge Exchange

Monoatomic reactant gas ions have no vibrational degrees of freedom and therefore the ionisation energy, *e.g.* 15.755 eV for argon, is all transferred to the colliding molecule; a useful property for energetic and kinetic studies. Charge exchange can also be used selectively to ionise particular compound classes.

$$R^{+\bullet} + M \longrightarrow R + M^{+\bullet} \tag{1.6}$$

Other popular CI reagent gases include isobutane, which yields mainly $C_4H_9^+$, and ammonia, which yields NH_4^+ ions. Depending on the acidity of the reactant secondary ion and the basicity of the sample molecule, adduct ions (electrophilic addition) can be formed

$$M + C_2H_5^+ \longrightarrow [M + C_2H_5]^+ \tag{1.7}$$

$$M + NH_4^+ \longrightarrow [M + NH_4]^+ \tag{1.8}$$

Negative Ion Chemical Ionisation

Various types of reaction can take place according to the nature of the reagent gas used, *e.g.* a mixture of hydrocarbon and water (95:5) can produce negatively-charged sample ions by the following reactions:

$$H_2O + e^- \longrightarrow OH^- + H \tag{1.9}$$

$$AH + OH^- \longrightarrow A^- + H_2O \tag{1.10}$$

$$M + HO^- \longrightarrow MOH^- \tag{1.11}$$

Other possible reagent gases include hydrocarbon/organic halide/oxygen (which produces Cl^- and O^- attachment ions) and fluorocarbons yielding negative ions by hydride abstraction and fluorine attachment. Negative ions can also be formed from suitable sample molecules by electron capture processes. In this case, the reagent gas acts as a moderator, generating thermal electrons that can then attach to molecules with high electron affinities forming negative radical ions.

$$M + e^- \textit{(thermal)} \longrightarrow M^{-\bullet} \tag{1.12}$$

Negative ion CI, especially electron capture ionisation, can be two to three orders of magnitude more sensitive than positive ion CI for electronegative molecules. It is therefore especially useful in the quantitative determination of trace substances that have, or that through the production of suitable derivatives are induced to have, electron-capturing properties.

Alternate CI-EI (ACE)

Electron and chemical ionisation produce complementary information on volatilisable samples. This is particularly useful when analysing compounds in complex mixtures by high-resolution capillary column gas chromatography. Although it is possible to run the same mixture twice, once by CI and once by EI, it can be difficult to replicate the chromatograms exactly. Furthermore, the time for each analysis is at least doubled. However, if the conditions in the source can be changed rapidly from EI to CI and back again during the elution time of the GC peak then the two spectra are known to be related and consecutive. ACE sources have a cycle time of around 1 second to record two spectra. If the GC peak is at least 4 seconds wide at the base it is possible to obtain two sets of data as the component of interest elutes (Figure 1.5).

Chemical ionisation can be performed with carefully selected reagent gases to reveal, through specific chemical reactions, something of the nature of the unknown sample. However, the sample must withstand transfer to the gaseous phase and is therefore only suitable for thermally stable, volatile molecules.

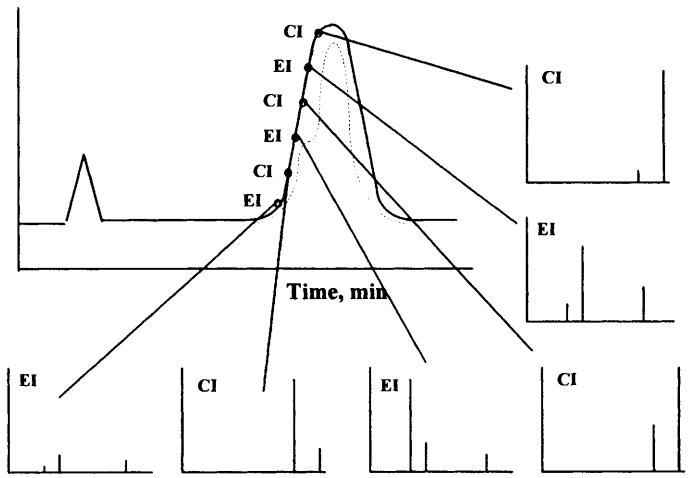


Figure 1.5 *A schematic representation of alternate Chemical Ionisation/Electron Ionisation*

Rapid Heating (Desorption CI)

Probes fitted with special rapid-heating elements produce gas-phase ions by rapid desorption or thermal evaporation while the sample is in intimate contact with the CI reagent gas. Using this technique it is possible to desorb labile samples into the gas phase whilst minimising thermal decomposition.

Atmospheric Pressure Ionisation

In a classical experiment, a ^{63}Ni β-source was used to generate primary electrons at atmospheric pressure.[7] These electrons were allowed to collide with, for example, benzene vapour. The ionised benzene reacts with very low pressures of sample molecules and the ensuing ions are leaked into the mass spectrometer's vacuum system for analysis. Very high sensitivities have been recorded for pure samples.

Atmospheric Pressure Chemical Ionisation (APCI)

In APCI a flow of liquid, typically an HPLC eluent, is induced to form a spray in a pneumatic (usually nitrogen powered) nebuliser. The emerging plume of liquid droplets is generated in atmosphere and is directed towards a corona discharge electrode that is maintained typically at 1–3.5 kV. This is positioned close to a small-diameter orifice leading to the high vacuum region containing the mass analyser. A nozzle and skimmer arrangement allows solvent ions to be pumped away, so that only desolvated ions are admitted into the analyser. A flow of warm nitrogen gas is passed across the front of the sampling orifice to ensure declustering of solvent/analyte ion complexes and to ensure that all the liquid has evaporated (see Figures 1.6 and 1.7). Ions formed from the solvent in the corona discharge plasma react with analyte molecules to generate protonated or deprotonated molecules ($[M + H]^+$ or $[M - H]^-$), in a similar manner to conventional CI.

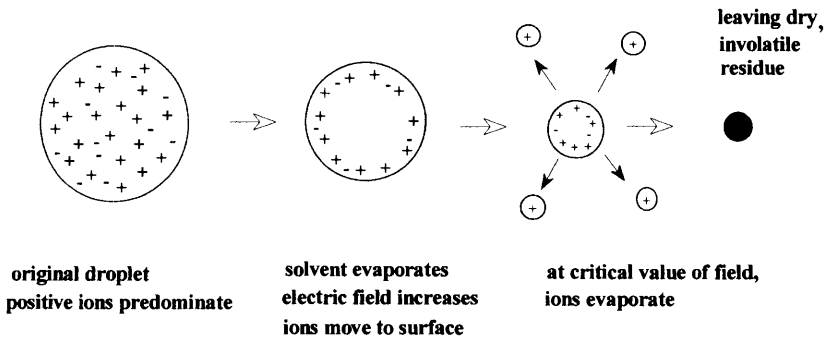


Figure 1.6 *Schematic representation of the electrospray ionisation process*

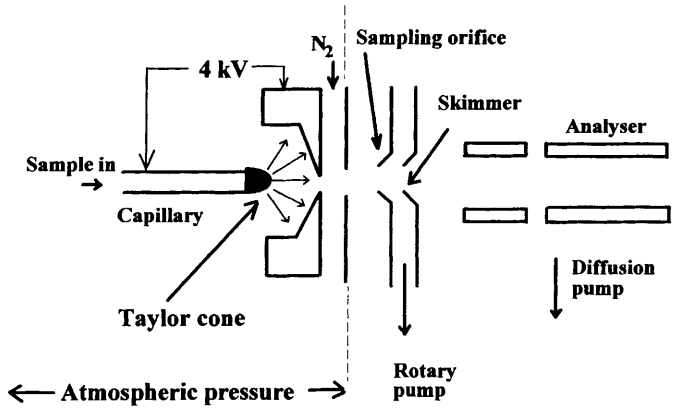


Figure 1.7 *Schematic diagram of an electrospray ionisation source*

Electrospray Ionisation (ESI)

The liquid flow from the HPLC pump enters a small diameter stainless steel capillary, which is maintained at a voltage of 3000–4000 V, A plume of charged liquid droplets is formed (the Taylor cone). Ions are produced by an evaporation mechanism and ionised particles are transported into the mass spectrometer vacuum via a pumped nozzle-skimmer arrangement (Figure 1.7). A curtain of nitrogen gas, which also aids evaporation of the charged droplets, prevents cluster ion formation. The ions are sampled through an orifice into a mass analyser. ESI and (to a lesser extent) its companion technique APCI have revolutionised mass spectrometry by allowing mass spectra to be obtained routinely on polar, involatile molecules. Even high molecular weight biomolecules can be analysed by ESI. Proteins, for example, yield a series of multiply charged ions well within the mass range of conventional mass spectrometers. These signals can be transformed mathematically to yield the molecular weight of the sample (Figure 1.8).

Thermospray Ionisation (TSP)

Thermospray was essentially a forerunner of APCI in which the eluent from the HPLC column was heated in a capillary tube until it formed a spray of liquid droplets that could be directed towards a discharge electrode for ionisation. Alternatively, addition of volatile buffers to the solvent enabled polar samples to be ionised directly from the solution phase, without application of an external ionising technique. The technique was never particularly routine (the phrase 'spray and pray' was commonplace in the mass spectrometry community) and has effectively been superseded by the more robust and sensitive ESI and APCI methods.

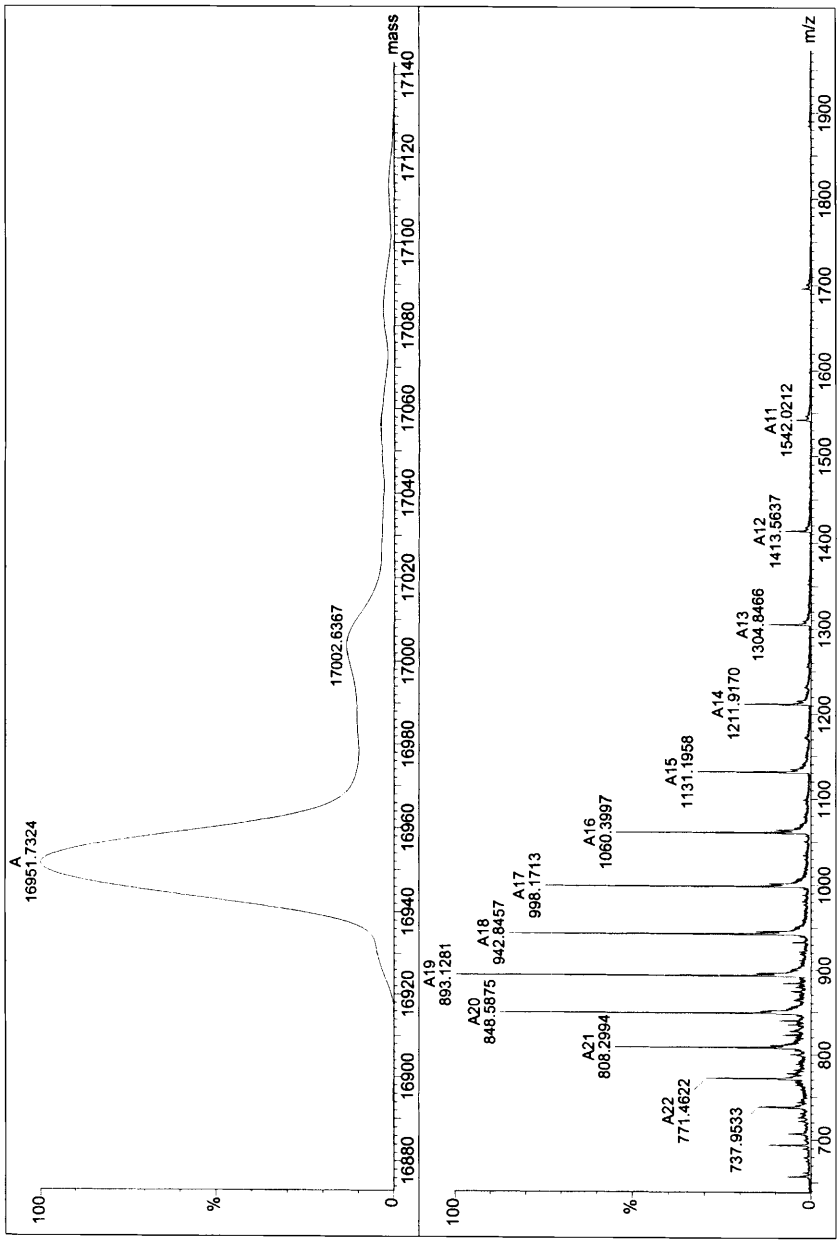


Figure 1.8 *Transformation of the mass spectrum of a multiply charged protein (horse heart myoglobin) to the molecular mass of the sample*

Laser Ionisation (Laser Desorption)

Short but intense pulses of photons cause very rapid surface heating (1000 °C in nanoseconds) with associated thermal ion formation. This effect is useful in surface studies since the beam tends not to penetrate below the surface of the sample.

Matrix Assisted Laser Desorption Ionisation (MALDI)

When used in conjunction with time-of-flight mass analysis (Section 3), MALDI is one of the favoured methods for the production of protonated molecules and adduct ions from large biomolecules (Figure 1.9).

The sample is mixed intimately with a liquid matrix solution of say, sinapinic acid, chosen for its ability to absorb and dissipate energy at the laser wavelength. It is thought that the matrix then passes transverse vibrational energy to the sample molecules in quanta sufficient to desorb them with virtually no excess energy for fragmentation. The technique is particularly useful for mass measuring large biomolecules, especially proteins, accurately and generates (mainly) $[M + H]^+$, $[M - H]^-$ or adduct ions.

Field Ionisation (FI) and Field Desorption (FD)

Molecules close to, or absorbed on surfaces of high curvature such as tips, blades, *etc.*, subjected to intense electric fields (10^7–10^8 V cm^{-1}) are ionised by

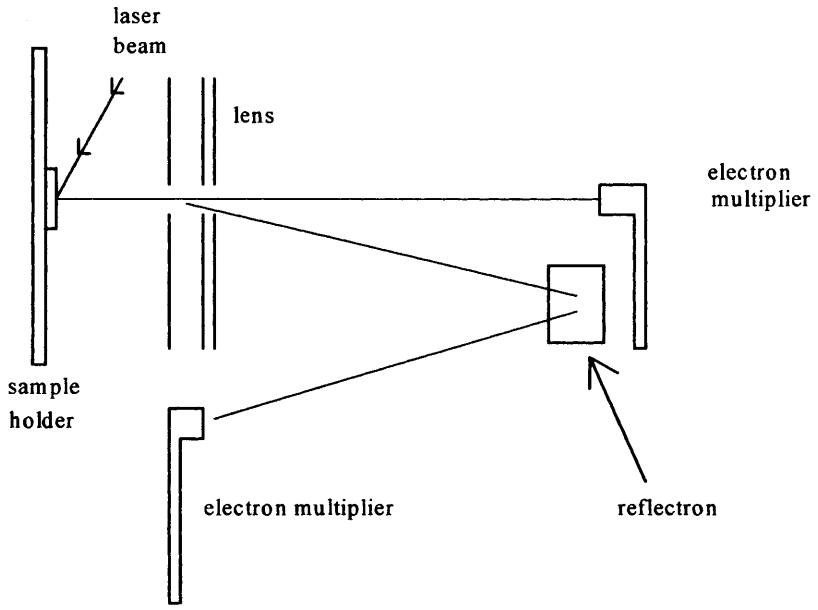


Figure 1.9 *Schematic representation of the MALDI-TOF mass spectrometer (reflectron type)*

quantum tunnelling of valence electrons from the molecule to the metal surface. The FI technique can be used for gas phase experiments, but lacks sensitivity, whereas the FD method for condensed phase samples has been highly successful in skilled hands. FD is not commonly used in modern mass spectrometry laboratories as MALDI and ESI are generally preferred for analysing polar and/or large molecules. However, the method is undergoing a new lease of life for selected applications because the introduction of wide-angle array detectors has allowed recording of the transient ion currents generated by many sample types under FD conditions.

Fission Fragment Ionisation or Plasma Desorption Mass Spectrometry (PDMS)

Pulses of californium-252 fission fragments pass through a nickel foil on which sample molecules have been deposited. The thermal shock vaporises mobile impurity ions (H^+, Na^+ and H^-). These ions interact with involatile sample molecules near the thermal shock, converting them into ions. ToF mass spectrometry, with its wide mass range and compatibility with pulse ionisation techniques, was an ideal partner for the PDMS ion source. PDMS was the most successful technique for large biomolecules (up to ~ 50 kDa) until the advent of MALDI and ESI. Samples adsorbed on nitrocellulose were found to yield singly- and multiply-charged ions with very little fragmentation. An additional advantage of the nitrocellulose surface was the ease of applying a water washing procedure, which removed inorganic impurities. PDMS has now been superseded by MALDI techniques.

Secondary Ion Mass Spectrometry (SIMS)

A beam of ions is made to collide with the sample deposited on a metal surface. Secondary ions ejected from the surface (sample) are accelerated and analysed. Protonated molecules and sodium adduct ions have been seen from a variety of organic compounds.

Liquid SIMS (LSIMS) or Fast Atom Bombardment (FAB)

Analyte samples are dissolved in a viscous, relatively involatile, liquid matrix and are bombarded with a beam of atoms or ions with kilovolt translational energies. The nature of the projectile is not particularly important and therefore the use of neutral atoms or the equivalent ion beams (*e.g.* Xe atoms or Cs ions) (Figure 1.10) produce similar spectra. The most common matrix used in FAB is glycerol, a useful solvent for many biomolecules.

In the high-pressure region above the surface of the matrix, ion–molecule reactions similar to those that occur during chemical ionisation create a variety of protonated molecules and adduct ions from the molecules emerging from the condensed phase. The method is suitable for polar molecules up to ~ 20 kDa. The higher-energy Cs ion beam used in LSIMS yields more abundant molecular

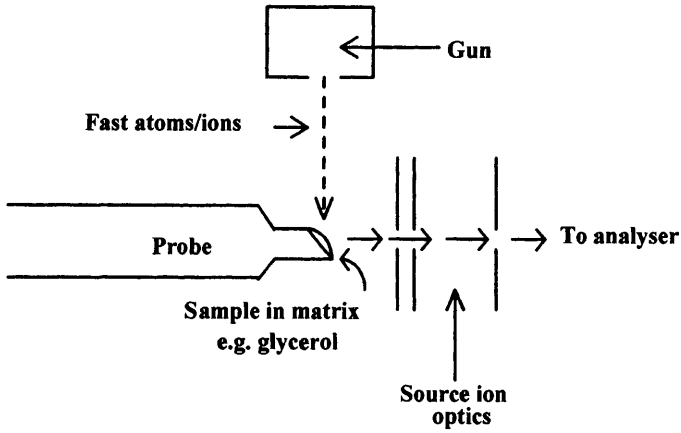


Figure 1.10 *Schematic representation of a FAB/LSIMS ion source*

and fragment ions than conventional FAB ion sources. However, in other respects the spectra are very similar.

Photon and Multiphoton Ionisation (MPI)

Photoionisation using classical light sources has been used for many years but suffers from the disadvantage of producing very low intensity ionisation, much lower than EI for example. MPI with pulsed tunable dye lasers demonstrates combined sensitivity and selectivity superior to other methods. MPI can be considered in two stages:-

(1) n photons combine (coherently) to excite the sample molecule to a real, intermediate electronic state (a resonance-enhanced process) and
(2) the irradiation of this intermediate with m photons (sequentially) leading to its ionisation.

When the laser is not tuned to a real intermediate state (non-resonant state), nearly negligible MPI occurs, demonstrating the high selectivity. When the sample molecules are concentrated into the laser beam, very high ionisation efficiencies (up to 100%) can be experienced. Finally, by increasing the power density of the laser it is possible to change from the 'soft' molecular ion spectrum to one resembling the classical EI spectrum, dominated by fragmentation.

Resonance Ionisation

A primary source of atomisation is subjected to irradiation from a tunable dye laser, thereby yielding selective multiphoton absorption and resonance-enhanced ionisation of only certain atoms in the matrix. For example, tuning a laser to 271.9 nm will produce ions of Fe out of a mixed organic and inorganic

matrix. Such specificity is invaluable in elemental analyses of environmental samples, or in the determination of specific isotopes, for example the long-lived radioactive metabolic tracer isotope ^{41}Ca.

Inorganic Mass Spectrometry Ionisation Techniques

Although several different inorganic ionisation methods are available, only two have been widely used in food sciences, mainly in studies of mineral metabolism or in multi-element analysis. The relevant techniques are described below.

Thermal Ionisation

Thermal Ionisation Mass Spectrometry (TIMS) is based upon the generation of atomic or molecular ions at the surface of an electrically heated filament.[8] It is generally accepted to be the practical mass spectrometric method that yields the most precise and accurate measurements of stable isotope ratios of inorganic elements. Its use in the food and nutritional sciences is comparatively recent, compared with well-established applications in such fields as geology, geochronology and nuclear science. It is most commonly applied to studies of the absorption and metabolism of inorganic nutrients. However, it is also used as a reference technique for quantifying nutritional and toxic trace elements in foods by isotope dilution mass spectrometry (IDMS).

A double-filament thermal ionisation source is shown in Figure 1.11. The double-filament ion source enables separation of the evaporation and ionisation processes and is useful for determining elements that evaporate at low

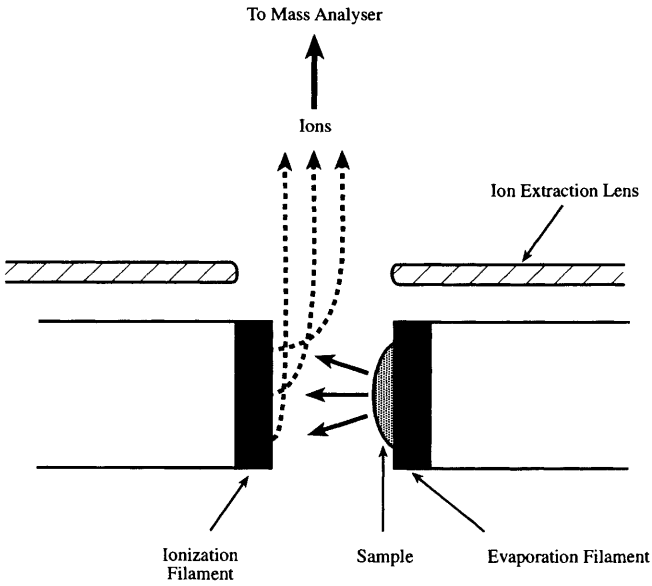


Figure 1.11 *Schematic diagram of a double-filament thermal ionisation source*

temperatures, but require high ionisation temperatures (Ca, for example). Single filament assemblies, where evaporation and ionisation take place from the same filament, are used for elements that have similar evaporation and ionisation temperatures. Positive or negative ions can be formed and mass analysed, depending on the nature of the analyte. Ions desorbed from the filament are extracted and focused by electrostatic lenses into either a magnetic sector or a quadrupole mass analyser.

High positive ion yields are obtained for elements with low ionisation energy and high negative ion yields for elements that possess a high electron affinity. Most inorganic elements evaporate and ionise inside a temperature range of 800–2000 °C. Consequently, filaments must be fabricated from high melting point filament materials such as rhenium, tantalum or tungsten. Metals with first ionisation energies greater than 7 eV frequently require the addition of chemicals to the filament to enhance ion yields. For example, silica gel and phosphoric or boric acid, or (for Fe) aluminium salts. Negative ion yields from elements such as selenium can be increased by the addition of $La(NO_3)_3$ or $Ba(OH)_2$.

Inductively Coupled Plasma

The ionisation region of an inductively coupled plasma mass spectrometer (ICP-MS) comprises a high temperature (typically *ca.* 8000 K) self-sustaining electrical discharge, applied *via* a high frequency induction coil, in a flow of argon gas at atmospheric pressure.[9–13] A schematic diagram of an ICP-MS ion source is shown in Figure 1.12.

Samples are most commonly introduced into the plasma in nebulised solutions flowing at rates of 0.5–1 ml min^{-1} and are ionised efficiently by the high-temperature plasma. Alternative sample introduction techniques, including electrothermal vaporisation, laser desorption, hydride generation,

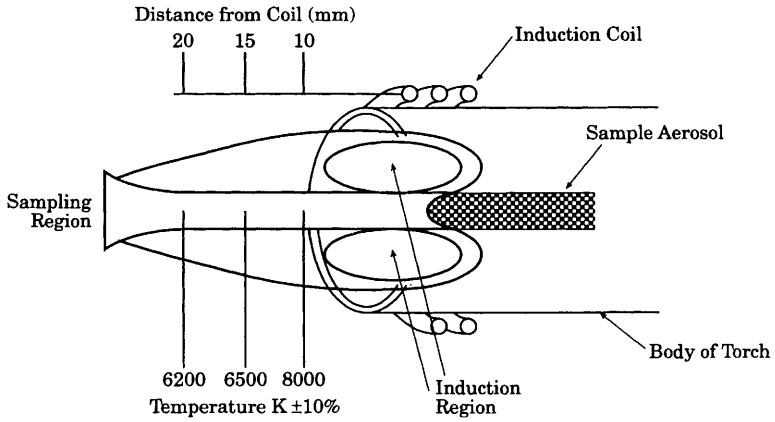


Figure 1.12 *Schematic diagram of an ICP/MS ion source*
(Redrawn from a diagram supplied by VG Elemental, with permission)

electrospray and several types of combined chromatography/mass spectrometry, are also available. A useful summary of alternative methods of sample introduction for plasma mass spectrometry has been published.[14]

Ionisation efficiency approaches 100% for most elements of interest to food and nutrition scientists. The plasma forms a supersonic jet in the differentially pumped region behind the sampling cone. The jet is sampled by a skimmer cone, yielding additional pressure reduction, and ions are then allowed to enter the mass analyser. This is usually a quadrupole device, however high resolution magnetic sector mass spectrometers may be used instead.[15–17] These are useful to resolve isobaric interferences, for example by separating $^{40}ArO^+$ and $^{56}Fe^+$ that nominally appear in the same mass channel (m/z 56).

A recent innovation has been the use of collision cells to remove argon and argide monatomic and polyatomic interference ions from the mass spectrum.[18,19] This technology has been used with both quadrupole and magnetic sector instruments and is particularly useful for increasing the accuracy and precision of measurement of certain elements and isotopes. For example, the important nutritional element selenium suffers from an abundant interference peak from $^{40}Ar_2^+$ in the ^{80}Se mass channel. A properly tuned collision cell can eliminate this and related peaks by charge exchange, allowing all isotopes of Se to be measured accurately. It is also possible to reduce the effects of other, non-argide polyatomics, although collision cell conditions for removing these interferents are different from those for removing argides, *i.e.* it is generally not possible to remove argide and non-argide species simultaneously.

ICP-MS permits multi-element detection and measurement of isotope ratios. The technique can be used to determine nutritional and toxic inorganic elements in foods, or to conduct metabolic studies using enriched stable isotopes. A schematic diagram of a complete ICP-MS instrument is shown in Figure 1.13.

3 Ion Separation

The most popular methods of separating the sample ion beam into beams of different masses are described briefly in this section. For a more comprehensive description of ion separation techniques, consult the recommended texts.[3,4,20]

Time-of-Flight (ToF)

The drift velocity of an ion is dependent on its mass and therefore, as the name suggests, the 'time-of-flight' in a field-free chamber is a measure of ion mass. ToF is ideally suited to very rapid sampling rates in, for example, fast GC/MS, and the analysis of large molecular weight peptides and proteins by pulsed ionisation methods such as PDMS or MALDI. Laser ionisation is well suited to ToF analysis since the start time of a laser pulse can be determined accurately and synchronised with arrival time of the pulse at the detector. Early ToF instruments were limited by their intrinsically low resolution. However, the development of reflectron and delayed extraction techniques has increased the performance (particularly the resolution) of ToF instruments.

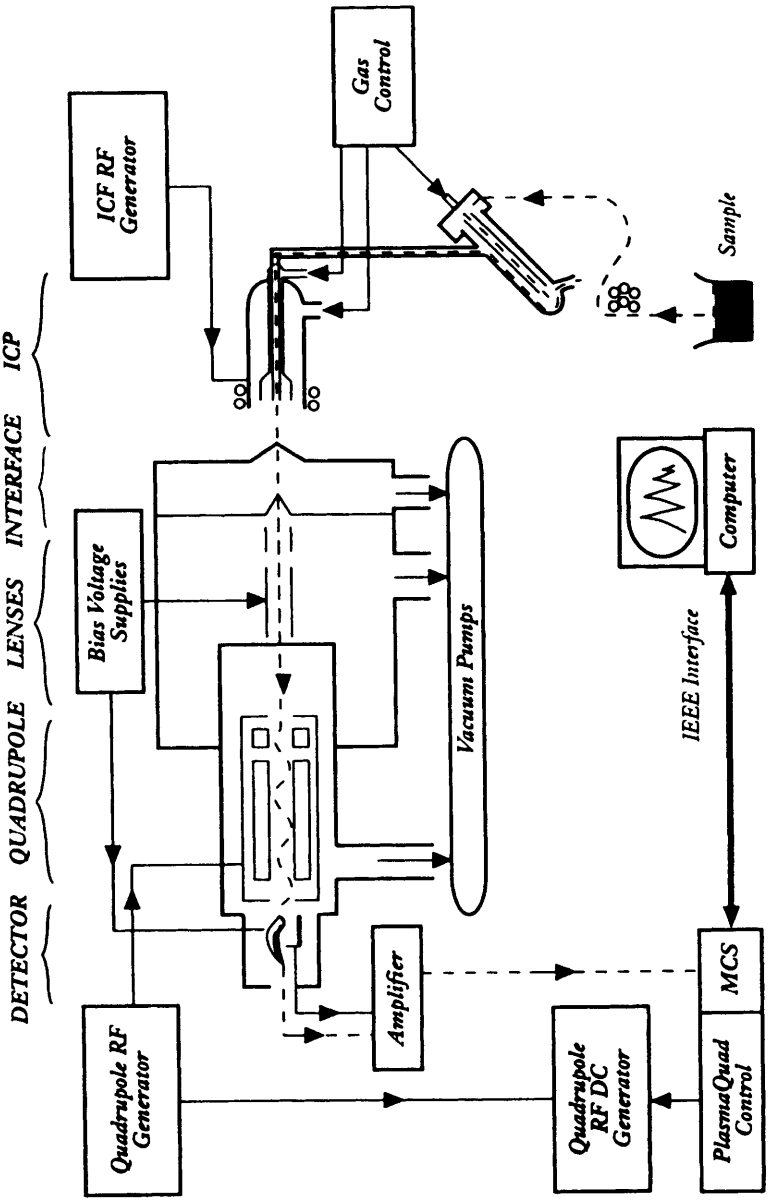


Figure 1.13 *Schematic diagram of an VG PlasmaQuad ICP/MS instrument* (Reproduced from material supplied by VG Elemental)