General Remarks

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The use of gas chromatography-mass spectrometry in biological monitoring

Contents

Abbreviations

ADBI	4-Acetyl-1,1-dimethyl-6-*tert*.-butyldihydroindene
AHDI	6-Acetyl-1,1,2,3,3,5-hexamethyldihydroindene
AHTN	7-Acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene
CI	Chemical ionisation
CAD	Collision-activated decomposition
CID	Collision-induced dissociation
DCP	Dichlorophenol

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DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
ECD	Electron capture detector
EI	Electron impact ionisation
eV	Electron volt
FID	Flame ionisation detector
FPD	Flame photometric detector
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HHCB	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyrane
HMEPMU	1-(4-(1-Hydroxy-1-methylethyl)phenyl)-3-methylurea
HPLC	High performance liquid chromatography
IP	Ionisation potential
m/z	Mass/charge ratio
MALDI	Matrix-assisted laser desorption ionisation
MEV	N-Methylvaline
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MSD	Mass selective detector
MS/MS	Tandem mass spectrometer
NCI	Negative chemical ionisation
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyls
PCI	Positive chemical ionisation
PFPTH	Pentafluorophenylthiohydantoin
PND	Phosphorus nitrogen detector
rpm	Revolutions per minute
SIM	Selected ion monitoring
SRM	Selected reaction monitoring
TIC	Total ion chromatogram
TCD	Thermal conductivity detector
TCP	Trichlorophenol
TeCP	Tetrachlorophenol

1 Introduction

In recent years gas chromatography coupled with mass spectrometric detection (GC/MS) has become a standard technique for the assay of organic compounds and their metabolites in biological material. Whereas capillary gas chromatography has already been in use for some time, e.g. in combination with flame ionisation detectors (FID), electron capture detectors (ECD) or substance-specific detectors (such as the phos-

phorus nitrogen detector, PND), mass spectrometry has become established as a routine technique only as a consequence of price reductions in the last ten years. This is especially true for the widely used "quadrupole" instruments with a performance that is often sufficient to meet the requirements of investigations in the fields of occupational and environmental medicine. However, in special cases more powerful systems, which are technically more complex and more expensive are required, and such systems have been employed only in relatively few laboratories to date.

With the establishment of GC/MS occupational and environmental medicine now have a technique that permits specific, sensitive and reproducible measurement in the trace range, i.e. in the ppm, ppt and sub-ppt range. Thus mass spectrometry is the only detection method combined with gas chromatography to meet all the key requirements for the qualitative and quantitative trace analysis of organic compounds. However, the most important limitation of GC/MS analysis arises from the coupling of mass spectrometry with gas chromatography, i.e. the range of substances that can be analysed is restricted to those substances that can be vaporised without being decomposed.

Gas chromatography has already been comprehensively described as a separation method in combination with various detectors in the "Analyses of Hazardous Substances in Biological Materials" series [1]. Therefore this review of GC/MS analysis focuses on mass spectrometry as a detection method. In addition to the presentation of the underlying physico-chemical principles and current instrumental technology, special aspects of the coupling and method development are dealt with here.

2 Mass spectrometry in occupational and environmental chemical analysis

The origins of mass spectrometric analysis can be traced back to the studies of Thomson on the separation and detection of the neon isotopes 20 and 22 in 1910 [2]. Four years earlier Tswett had first successfully performed the chromatographic separation of leaf pigments on calcium carbonate [3]. This principle in the form of liquid chromatography (HPLC) and gas chromatography (GC) is one of the most efficient and versatile separation methods, and advances continue to be made in its development. Mass spectrometry was predominantly used for investigations in nuclear physics, e.g. for the separation of isotopes, until after the Second World War. Electron impact ionisation, ion focusing by means of electromagnetic lenses, ion separation in an electromagnetic field and detection by means of photoplates were developed in this context [4].

The publications of McLafferty et al. in the early 1960s triggered systematic research on fragmentation reactions and their assignment to intramolecular and intermolecular reaction mechanisms. Instrumental techniques were also developed during that period and they have been continually improved since then, e.g. electrical and magnetic sector field separation for high resolution, quadrupole analysers and time-of-flight mass spectrometers. Today access to these developments is no longer restricted to highly specialised research laboratories. Towards the end of the 1980s simple GC/MS sys-

tems with packed separation columns were devised and made affordable for a wide range of users [4].

With the introduction of fused silica capillary columns and the technical advances in mass spectrometry for routine investigations, GC/MS analysis has made great progress in every aspect (e.g. reduced space requirements, powerful vacuum systems, ionisation techniques), and it is now in widespread use. However, mass spectrometry has prevailed as a standard procedure in occupational and environmental medicine only in recent years. This tendency is clearly demonstrated in the series "Analysis of Hazardous Substances in Biological Materials" by the Working Group of the Deutsche Forschungsgemeinschaft's Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (Fig. 1).

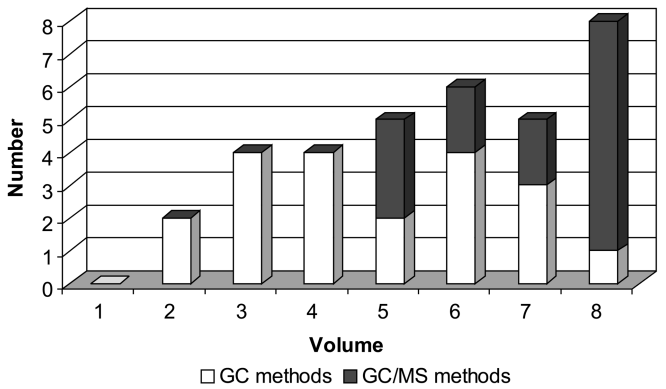

Fig. 1. GC and GC/MS methods in this series

Gas chromatography, generally in combination with flame ionisation or electron capture detectors, was described as a separation method for biological monitoring from the very beginning of the series. From 1975 to 1981 an average of five GC methods were published in each volume. The first GC/MS method to be included in the German loose-leaf collection of methods was the assay of thiodiacetic acid, a metabolite of vinyl chloride, in 1982 [5]. In this case gas chromatography was still performed using a packed column.

In general, collective methods for GC analysis, e.g. to determine aromatic, aliphatic and chlorinated hydrocarbons in blood by means of headspace gas chromatography, were published until the 2nd volume in this series in 1988. Since then the number of GC methods has risen with every volume, but further GC/MS methods were only included in the 5th volume of this collection in 1996. Seven of the eight GC methods published in the 8th volume in 2003 were based on the use of a mass spectrometer as a detector.

Mass spectrometry is of special significance for biological monitoring on account of its high substance specificity and its great power of detection. As limit values have been reduced at the workplace and on account of the generally low levels of expo-

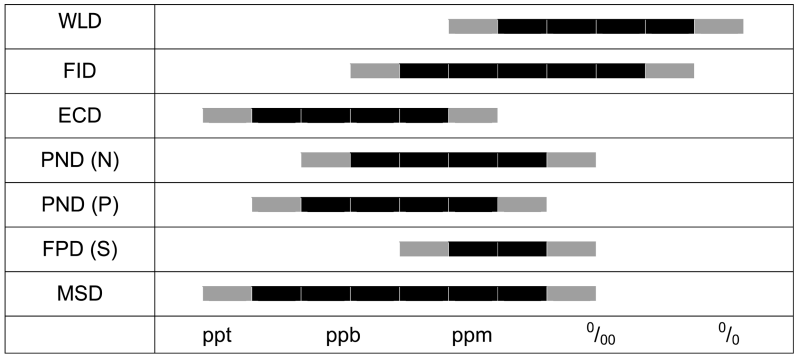

Fig. 2. Working ranges of various detectors used with gas chromatography (TCD thermal conductivity detector, FID flame ionisation detector, ECD electron capture detector, PND phosphorus nitrogen detector, FPD flame photometric detector, MSD mass selective detector) (according to [6])

sure to xenobiotics from environmental sources, demand has continued to rise for methods to enable sure and reliable determination of the correspondingly lower substance concentrations in the body. Although other detectors achieve detection limits of magnitudes similar to those of a mass spectrometer (Fig. 2), GC/MS exhibits a relatively wide dynamic working range in which the signals show a linear relationship with respect to the substance concentration [2].

However, the instrumental parameters of mass spectrometry can be optimised to ensure compatibility with the target molecule to a large extent, and therein lies the decisive practical advantage of this technique. Molecules or molecular fragments can even be selectively enriched in the mass filter by means of the "ion trap" technique before detection. This technical option enables the measured signal to be intensified, while the analytical background noise is minimised. The combination of gas chromatographic separation on capillary columns with mass spectrometry permits trace analysis in complex matrices, e.g. in blood and urine, which are the samples of choice for investigations in the fields of occupational and environmental medicine.

The methods that have been published in this series to date were therefore developed for the assay of such compounds of relevance at the workplace and in the environment, which are present in very low concentrations and for which the risk of interference from concomitant substances in the matrix is very high (Table 1). This applies particularly to the analysis of dioxins and furans, organochlorine compounds and "protein adducts" (N-terminal alkylated globins, aromatic amines).

A literature search on the use of GC/MS for biological monitoring since 2000 (keywords: GC-MS, biological monitoring, urine, blood) showed a similar focus on this application (Table 2). In addition to the aromatic amines, which are detected in their acetylated form in urine or as haemoglobin adducts from a complex matrix (e.g. Kaaria et al. 2001 [21]; Sabbioni et al. 2001 [22]; Weiss and Angerer 2002 [23]; Sennbro et al. 2003 [24]), various procedures for the assay of polychlorinated biphenyls, dioxins, furans and other organochlorine compounds have been described (e.g. Dmitrovic et al. 2002 [25]; Turci et al. 2003 [27]; Focant et al. 2004 [28]).

Table 1. GC/MS methods in this series (including the 8th volume) (DCP: dichlorophenol, TCP: trichlorophenol, TeCP: tetrachlorophenol, DDT: dichlorodiphenyltrichloroethane, DDE: dichlorodiphenyldichloroethene, HCB: hexachlorobenzene, HCH: hexachlorocyclohexane, HMEPMU: 1-(4-(1-hydroxy-1-methylethyl)-phenyl)-3-methylurea, PCB: polychlorinated biphenyls)

Parameter	Working material	Author
Thiodiacetic acid	Vinyl chloride	Müller (1982) [5]
S-Phenylmercapturic acid	Benzene	Müller and Jeske (1996) [7]
Haemoglobin adducts of alkylating compounds	Ethylene oxide, acrylnitrile, methylating substances	van Sittert et al. (1996) [8]
Chlorophenoxycarboxylic acids	Chlorophenoxycarboxylic acids	Krämer et al. (1996) [9]
Pyrethroid metabolites	Pyrethroids	Angerer et al. (1996) [10]
Pentachlorophenol	Pentachlorophenol	Hoppe (1999) [11]
2,4-, 2,5-, 2,6-DCP 2,3,4-, 2,4,5-, 2,4,6-TCP 2,3,4,6-TeCP	Chlorophenols	Angerer (2001) [12]
Haemoglobin adducts of aromatic amines	Aromatic amines	Lewalter and Gries (2001) [13]
N-Benzylvaline	Benzylchloride	Lewalter et al. (2003) [14]
Cotinine	Nicotine	Müller (2003) [15]
Hexamethylene diisocyanate and hexamethylenediamine	Hexamethylene diisocyanate, hexamethylenediamine	Lewalter et al. (2003) [16]
HMEPMU	Isoproturon	Bader et al. (2003) [17]
DDT, DDE, HCB, α-, β-, γ-HCH, PCB 28, 52, 101, 138, 153, 180	Organochlorine compounds	Hoppe and Weiss (2003) [18]
Dioxins, furans, PCB	Dioxins, furans, PCB	Ball (2003) [19]
Cyclophosphamide, Ifosfamide	Oxazaphosphorines	Hauff and Schierl (2003) [20]

The GC/MS technique has proved particularly successful for determining environmental exposure, for example to pesticides and their metabolites, on account of its great power of detection combined with its high substance specificity (e.g. Hardt and Angerer 2000 [29]; Perry et al. 2001 [30]; Wittke et al. 2001 [31]; Heudorf and Angerer 2001 [32]; Leng et al. 2003 [33]).

In certain cases GC/MS has also been used to improve analytical specificity, for instance in the case of the metabolites of the BTX aromatic compounds (e.g. Laurens et al. 2002 [34]; Barbieri et al. 2002 [35]; Jacobson and McLean 2003 [36]) or inhalation anaesthetics (e.g. Accorsi et al. 2001 [37]; Gentili et al. 2004 [38]).

Table 2 clearly shows that the "quadrupole technique" is by far the most frequently applied analytical variation (see Section 4.4) of mass spectrometry used to assay hazardous substances and their metabolites. At present quadrupole analytical instruments offer an optimum compromise between the high performance of a GC/MS system

Table 2. Publications on the use of gas chromatography-mass spectrometry in the field of biological monitoring since 2000 (Searches based on: PubMed service of the U.S. National Library of Medicine, Bethesda, MD, USA. URL: *http://www.ncbi.nlm.nih.gov/entrez/query.fcgi*, keywords: biological monitoring, GC-MS, urine, blood)

Analyte(s)	Matrix	Detection	Author(s)
Aniline	Haemoglobin	Quadrupole	Zwirner-Baier et al. 2003 [40]
Aromatic amines from diisocyanates	Urine	Quadrupole	Kaaria et al. 2001 [21] Rosenberg et al. 2002 [41] Sennbro et al. 2003 [24]
Aromatic amines from toluene diisocyanate	Haemoglobin	Quadrupole	Sabbioni et al. 2001 [22]
Aromatic amines and metabolites of the nitroaromatic compounds	Urine	Quadrupole	Weiss & Angerer 2002 [23]
Organic arsenic compounds	Haemoglobin	Quadrupole	Fidder et al. 2000 [42]
Atrazine	Urine	Quadrupole	Perry et al. 2001 [30]
Benzene	Urine	Quadrupole	Prado et al. 2004 [43]
Bromoxynil	Urine	Quadrupole	Semchuk et al. 2004 [44]
Metabolites of the BTX aromatic compounds	Urine	Quadrupole	Laurens et al. 2002 [34] Szucs et al. 2002 [45] Jacobson & McLean 2003 [36]
1,3-Butadiene	Haemoglobin	Quadrupole	van Sittert et al. 2000 [46] Begemann et al. 2001 [47]
Butoxyethoxyacetic acid	Urine	Quadrupole	Göen et al. 2002 [48]
Dialkylphosphates	Urine	Quadrupole	Hardt & Angerer 2000 [29]
Dichloroanilines from pesticides	Urine	Quadrupole MS/MS	Wittke et al. 2001 [31]
2,4-Dichlorophenoxy-acetic acid	Urine	Quadrupole	Hughes et al. 2001 [49]
Dimethylphenyl-mercapturic acids	Urine	Quadrupole	Gonzalez-Reche et al. 2003 [50]
Dioxins, furans and poly-chlorinated biphenyls	Hair	High-resolution	Nakao et al. 2002 [39]
Hydroxyterpenes	Urine	Quadrupole	Sandner et al. 2002 [51]
Inhalation anaesthetics	Urine	Quadrupole	Accorsi et al. 2001 [37] Accorsi et al. 2003 [52] Gentili et al. 2004 [38]
Ethylenethiourea	Urine	Quadrupole	Fustinoni et al. 2005 [53]
5-Hydroxy-N-methyl-2-pyrrolidone 2-Hydroxy-N-methylsuccinimide	Urine	Quadrupole	Åkesson & Jönsson 2000 [54] Akrill et al. 2002 a [55] Jönsson & Åkesson 2003 [56]
Nitroaromatic compounds	Urine	Quadrupole	Letzel et al. 2003 [57]
Nitroglycerin	Urine	Quadrupole	Akrill et al. 2002 b [58]

Table 2 (continued)

Analyte(s)	Matrix	Detection	Author(s)
Opioids	Urine	Quadrupole	van Nimmen et al. 2004 [59]
Organochlorine compounds	Serum	Ion trap	Moreno Frias et al. 2004 [60]
Metabolites of the organophosphates	Urine	Quadrupole	Heudorf and Angerer 2001 [32]
Phenylenediamine	Blood, urine	Ion trap	Stambouli et al. 2004 [61]
S-Phenylmercapturic acid	Urine	Quadrupole	Aston et al. 2002 [62]
Polybrominated diphenyl ethers	Plasma, serum, milk	Quadrupole, high-resolution	Thomsen et al. 2002 [26]
Polychlorinated biphenyls	Serum	Quadrupole	Turci et al. 2003 [27]
Polychlorinated biphenyls	Serum	Time-of-flight	Focant et al. 2004 [28]
Polychlorinated biphenyls and various pesticides	Serum	Quadrupole	Dmitrovic et al. 2002 [25]
Polychlorinated biphenyls and organophosphates	Blood	Quadrupole	Liu & Pleil 2002 [63]
Polychlorinated biphenyls and endocrine-affecting pesticides	Serum	Ion trap	Martinez Vidal et al. 2002 [64]
Polycyclic aromatic hydrocarbons (PAH)	Urine	Quadrupole	Waidyanatha et al. 2003 [65]
PAH metabolites	Urine	High-resolution	Smith et al. 2002 [66]
Pyrethroid metabolites	Urine	Quadrupole	Elflein et al. 2003 [67] Hardt & Angerer 2003 [68] Leng et al. 2003 [33]
m-Toluidine	Urine	Quadrupole	Schettgen et al. 2001 [69]
Toxaphenes	Serum	High-resolution	Barr et al. 2004 [70]
trans,trans-Muconic acid	Urine	Quadrupole	Barbieri et al. 2002 [35]
Trichloroethylene	Urine	Quadrupole	Imbriani et al. 2001 [71]

and the necessary investment and maintenance costs. Other mass filters, such as ion traps and MS/MS, are rarely used for biological monitoring, while there is no practical alternative to high-resolution sector field mass spectrometry for differentiation of complex mixtures of structural isomers (e.g. dioxins, furans, PCB and polybrominated diphenyl ethers) (e.g. Nakao et al. 2002 [39]; Turci et al. 2003 [27]; Ball 2003 [19]; Focant et al. 2004 [28]; Thomsen et al. [26]).

One advantage of mass spectrometry over conventional detection methods is the possibility of including an isotope-labelled reference substance to clearly establish the identity of the analyte and at the same time to serve as an optimum internal standard to compensate for losses due to processing.

However, it must be emphasised in this context that despite the option of "suppressing" the analytical background interference effectively using the GC/MS technique, sample preparation (extraction, work-up, derivatisation, etc.) still plays a key role in an analytical method. The reduced effort required for sample treatment was long regarded as an advantage of GC/MS. In fact, some manufacturers promote their instruments with explicit claims of the efficient elimination of undesirable matrix constituents. But concomitant substances from the work-up, by-products of derivatisation reactions or contaminants in solvents can cause rapid contamination of the GC/MS system (e.g. injector, capillary column, interface, ion source, analyser). In the case of matrix constituents being co-eluted with the analyte a "quenching" effect may also occur, e.g. due to competitive ionisation reactions [4].
In the most unfavourable case the affected component groups have to be laboriously cleaned after only a few injections. Moreover, increasing contamination lowers both the reproducibility of the system and its useful operational life. Therefore GC/MS analysis does not differ significantly from other detection methods with regard to general method development. The specificity and sensitivity of GC/MS can be utilised to achieve low detection limits and substance-characteristic measurement signals only after optimum sample preparation.

3 Principle of gas chromatography/mass spectrometry

Gas chromatography/mass spectrometry (GC/MS) is a so-called "coupled technique", in which two fundamentally independent and separately functioning systems are linked together (Fig. 3). In a simplified view, the gas chromatograph represents only a "sample inlet system" for a mass spectrometer compared with other detectors (e.g. FID, ECD, PND, TCD) that can be operated only in combination with a gas chromatograph. The greatest technical problem of coupling gas chromatography and mass spectrometry is the pressure difference between the two systems: the components to be separated are transported by a stream of carrier gas at 0.5 to 2 mL/min in the GC and this leads to a column pressure of approx. 1 to 2 bar at the end of the column.
In contrast, typical mass spectrometers require a stable high vacuum of 10^{-5} to 10^{-6} mbar. Therefore pressure adjustment is necessary at the interface between the gas

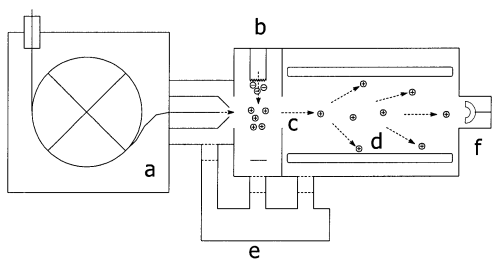

Fig. 3. Schematic structure of a GC/MS (a = gas chromatograph, b = ion source, c = ion focusing, d = mass filter, e = vacuum system, f = detector)

chromatograph and the mass spectrometer. However, this adjustment should have little effect on the gas chromatographic separation of components and, at the same time, should admit as many sample molecules as possible into the mass spectrometer. This is primarily achieved by devices for distributing the volume flow rate or by efficient carrier gas extraction using vacuum pumps.
After gas chromatographic separation (Fig. 3 a), ionisation and excitation of the molecules to be analysed are caused e.g. by electron bombardment or intermolecular charge exchange (Fig. 3 b). Then an acceleration voltage focuses the positively or negatively charged ions (depending on the ionisation method) emitted from the ion source in the direction of an electromagnetic field in the analyser of the mass spectrometer (Fig. 3 c). There the ions are deflected from their flight path to a greater or lesser degree, depending on their mass/charge ratio (m/z) (Fig. 3 d).
This deflection is utilised for ion separation, as only molecules with certain mass/charge ratios are allowed to pass to the detector. The high vacuum of at least 10^{-5} mbar (Fig. 3 e) minimises undesirable subsidiary reactions, e.g. charge exchanges as a result of intermolecular impact and reactions as a result of collision with the walls. The ions finally reach a detector that converts the intensity of the ionic beam into an electrically processable signal (Fig. 3 f). In addition to the charged molecule ions, substance-characteristic fragments are formed as a result of rapid chemical decomposition reactions. The total information obtained (molecule ion + fragment ions) provide indications of the chemical structure of the ionised compound. Thus the GC/MS system yields important substance-specific data for the identification of target components in addition to the gas chromatographic retention time.
GC/MS systems can be described on the basis of six characteristic component groups:

1. Gas chromatograph (for the separation of substance mixtures)
2. Interface (between the gas chromatograph and the mass spectrometer)
3. Ion source (to generate electrically charged and excited molecules)
4. Ion focusing and mass filters (for ion separation)
5. Detector (to record the ions)
6. Vacuum pumps (to generate a high vacuum in the mass spectrometer).

The technology of these components (with the exception of the gas chromatograph) is presented, and the analytically relevant aspects are discussed in the following sections. For a more detailed consideration of individual topics, especially of fragmentation reactions and spectral interpretation, the reader is referred to relevant review articles and textbooks [2, 4, 72–74].

4 Component groups of the mass spectrometer

4.1 Coupling (interface)

Coupling a gas chromatograph to the high vacuum of the mass spectrometer poses special technical problems on account of the pressure difference between the two systems. Depending on the dimensions of the installed separation column and the pressure of the carrier gas at the injector, the volume flow rate is between approximately 0.5 mL/min and 4 mL/min with a pressure difference of several orders of magnitude between the capillary outlet of the gas chromatographic separation column and the high vacuum of the mass spectrometer. Most of the vacuum pumps normally used in GC/MS systems are set to operate at extraction rates of about 2 mL/min. High-performance vacuum pumps must be used for instruments with the option of chemical ionisation or for high flow rates, in some cases the ion source and the analyser are equipped with separate vacuum pumps.
In older GC/MS systems with packed columns and less powerful vacuum technology the carrier gas stream had to be diminished before entering the ion source by "open" coupling of two columns or by the use of gas flow separators (jet and membrane separators). However, "direct" coupling prevailed after the introduction of capillary columns with their lower gas flow rates. In this technique the end of the capillary column is positioned immediately before the ion source or is inserted directly into the ion source. Direct coupling is advantageous for trace analysis, as practically all the analyte in a sample reaches the ion source with the carrier gas, and thus high sensitivity is achieved. The sample volume is primarily determined by the injection technique of the gas chromatograph (e.g. unpulsed and pulsed split/splitless injection, on-column injection, thermal desorption).
Direct coupling also has disadvantages, the most serious of which, from a practical point of view, is the inevitable ventilation of the entire GC/MS system when replacing the column. Each time it is opened, the mass spectrometer can be contaminated with dust, fibres or gases from the ambient air in the laboratory, which may lead to mechanical damage to the vacuum pumps. After the instrument is put into operation, undiscovered leaks may cause corrosion of the heated parts and may impair ionisation efficiency.
Two aspects must be considered, regardless of the type of the interface:

1. No "cold" or "active" sites may be present in the interface. Therefore the temperature must be permanently at least 10 °C higher than the final temperature of the analytical separation column. This can cause problems, if columns with a low operational temperature and notable "column bleeding" are used. Therefore most manufacturers offer "low-bleed" or MS columns, which are specially designed for GC/MS analysis.
2. The vacuum system must be adjusted to suit the carrier gas stream. This is practically always the case in modern instruments, and when capillary columns are used. When an instrument is purchased, it is advisable to select the most powerful vacuum pump available (most manufacturers offer two or three alternatives), as

the quality and constancy of the high vacuum has a direct influence on the sensitivity of the mass spectrometric analysis.

4.2 Vacuum system

Most GC/MS techniques require a high vacuum in the ion source, in the analyser and at the detector. The following factors make this necessary:

(a) *Intermolecular reactions and collisions with the walls must be minimised by ensuring that the mean free path of the molecules is as long as possible.* Each impact of an ion with another molecule or with the surfaces in the mass spectrometer leads to a deflection in the flight path and to a reduction in the ion beam reaching the detector. The mean free path should be approximately equivalent to the distance between the ion source and the detector. Therefore the greater the distance between the ion source and the detector the lower the pressure that is required. This distance is about 50 cm in modern quadrupole instruments, the operational vacuum is approximately 10^{-6} mbar.

(b) *The background flow of molecules from the air (e.g. due to leaks) must be kept to a minimum.* Atmospheric gases, such as nitrogen, oxygen, argon and water vapour may be ionised in the mass spectrometer and be accelerated towards the detector. The resulting background causes a higher baseline and lowers the sensitivity of the detector. In addition, the signals cause interference to analysis in the lower mass range (18 to 40 m/z).

(c) *It is essential to avoid electrical discharges.* In some cases potentials of several kilovolts are present in the ion source, at the focusing apertures and in the analyser of the mass spectrometer. High gas pressure (e.g. due to leaks) or surface soiling can lead to discharges, short circuits and considerable damage to the instrument.

(d) *The incandescent filament in the ion source reacts sensitively to overheating.* On account of the enhanced thermal conductivity at a higher pressure in the ion source the current at the electron-generating incandescent filament is automatically regulated in order to maintain a constant emission of thermal electrons. The elevated current leads to more rapid wear and tear of the filament, and in extreme cases the filament burns out. In addition to leaks, the solvent peak after a sample injection leads to higher pressure in the ion source. For this reason all GC/MS instruments allow a "solvent delay", i.e. the ion source is switched on only when the solvent peak has passed through the instrument and the gas pressure has fallen to about 10^{-6} mbar again. The "solvent delay" to protect the filament is an important parameter of a GC/MS method.

As a rule, the vacuum system of a GC/MS is composed of two components: a mechanical "pre-vacuum pump" initially generates an underpressure of the magnitude of 10^{-2} to 10^{-3} mbar. Then a high-performance pump switches on automatically to attain the final vacuum of 10^{-5} to 10^{-6} mbar. The pre-vacuum pump is normally a rotary-vane pump, which pumps the gas from the space to be evacuated by means of

Table 3. Advantages and disadvantages of typical high-vacuum pumps

	Oil diffusion pump	Turbomolecular pump
Advantages	• Inexpensive • Low maintenance (only oil change) • Pump capacity increases as the molecule size decreases (favourable for carrier gases and solvents)	• Higher pump performance • Vacuum is achieved more rapidly • Short residual running time after switching off e.g. to replace the column
Disadvantages	• Poorly volatile solvents can condense in the oil and reduce the performance • Risk of contamination of the MS due to rising oil vapours • Long cooling phase	• Complex mechanical parts, expensive to replace the bearings • Sensitive to mechanical stress and dust • Pump capacity increases as the molecule size increases (unfavourable for carrier gas and solvents)

two eccentric rotors in series. Either oil diffusion pumps or turbomolecular pumps are used to create the high vacuum of 10^{-5} to 10^{-6} mbar (Table 3). Both pumps require a pre-vacuum of about 10^{-2} mbar in order to function.

The oil diffusion pump is directly coupled via a high-vacuum flange to the mass spectrometer and it has a connection to the pre-pump on its lower end. The pre-pump creates the initial vacuum, while a poorly volatile synthetic oil is being heated in a bottom sump of the diffusion pump. The hot oil vapours ascend in a central tube and subsequently diffuse at high speed from downwards-facing nozzles with a small diameter into the pre-vacuum. This supersonic stream of gas carries over residual gas molecules in the direction of the connected pre-vacuum pump. While the oil vapours condense on the cooled walls of the pump and flow back into the bottom sump, the residual gas molecules are sucked through the pre-pump. Residual pressures of up to 10^{-8} mbar can be achieved using oil diffusion pumps.

Turbomolecular pumps function on the principle of the suction turbine: gas molecules at the inlet of the pump are accelerated by impact with very rapidly rotating inclined lamellae (approx. 60,000 rpm) in the direction of the pre-pump and there they are sucked away. Fixed blades inclined in the opposite direction are installed between two adjacent lamellar rotors in each case, thus forming the "stator", and these blades prevent the molecules diffusing back. Turbomolecular pumps can attain lower final pressures than oil diffusion pumps (10^{-10} mbar).

A basic rule of thumb is that the vacuum pumps for a GC/MS should rather be "over-dimensional", i.e. as powerful as possible. This is primarily due to the fact that there is a close correlation between the residual gas pressure in the mass spectrometer and the sensitivity of the instrument. In addition, it is difficult to seal a GC/MS system effectively on account of the many connections and valves, so a higher output than expected is generally necessary.

Typical carrier gas flow rates in capillary gas chromatography of 1 to 2 mL/min require a pump output of approx. 100 to 150 L/s in the high-vacuum range. Either an oil diffusion pump or a simple turbomolecular pump can achieve this performance.

However, if higher carrier gas flow rates are required or chemical ionisation with additional reactant gas is used, a suitable turbomolecular pump with a performance well over 200 L/min is the only appropriate option. In addition, a pre-pump with as high a capacity as possible should also be selected (more than 10 m^3/h). In the case of chemical ionisation it is particularly advantageous for the analyser and the ion source to be in separate components, as up to 1 mbar pressure is created in the ion source, while a high vacuum must be maintained in the mass filter. For this reason high-performance instruments are equipped with two separate high-vacuum pumps, one for the ion source and one for the mass filter area.
At this point the importance of manometers in the mass spectrometer should be emphasised. The most important function of a manometer is to indicate leaks after changing the set-up, replacing the column or maintenance work. In the case of chemical ionisation and the ion trap technique the maintenance of an optimum pressure in the ion source is of key significance for the reproducibility of the analysis, as it directly influences the ionisation yield. Therefore, from the practical point of view, a manometer on the mass spectrometer is an instrument for technical quality assurance.
The "Penning gauging head" (cold cathode) and the "ionisation tube" according to Bayard-Alpert (hot cathode) are typical manometers used for GC/MS. In the Penning gauging head, gas molecules are ionised in a high-voltage field by electrons emitted from a cylindrical cathode and deflected spirally in the magnetic field (increasing the effective path length and thus the probability of ionisation). In the ionisation tube the electrons are emitted from a hot tungsten wire. The electrons migrate several times through a spiral, positively charged grid before landing on its surface. Cations that are formed by impact with residual gas molecules cause an ion flow. The ion flow is proportional to the residual gas concentration in both the Penning gauging heads and in the ionisation tube. The hot cathode measurement is more accurate than the Penning set-up, but the filament is more sensitive to overheating and the tube becomes easily soiled when it is not switched on.

4.3 Ion sources

The ion source is the first typical component group of a GC/MS system. Simply expressed, the components eluted from the analytical separation column or from the interface are ionised in the ion source by electron bombardment. The resulting radical cations and cations are accelerated out of the ion source and focused by electrostatic "lenses" towards the mass filter. In addition to this "classical" electron impact ionisation (EI), chemical ionisation (CI) has also become established as a standard procedure in GC/MS analysis in the fields of occupational and environmental medicine. In this case the ions are formed by charge exchange with a reactant gas that has itself been previously ionised by electron impact. Chemical ionisation can generate cations as well as anions, and therefore a differentiation is made between "positive chemical ionisation" (PCI) and "negative chemical ionisation" (NCI).

4.3.1 Electron impact ionisation (EI)

In principle, the ion source for electron impact ionisation consists of a small hollow space with a volume of less than 1 cm^3 that houses the inlet for the analytical separation column or for the interface, an incandescent filament made of tungsten or rhenium oxide, the target (an anode situated opposite), and an outlet to the mass filter or to the ion focus (generally at an angle of 90° to the sample inlet). The outlet apertures consist of an extractor, a discharger and one or more ion focuses. A potential is applied between the extractor and a repeller situated at the opposite side of the ion source, which causes positively charged molecules to be accelerated towards the system of apertures and out of the ion source (Fig. 4).
The ion source temperature of approx. 200 to 250 °C generally represents a compromise: on the one hand the thermal energy of the molecules should be kept as low as possible to enable efficient ionisation and to minimise the number of collisions between molecules and with the walls within the relatively small volume of the ion

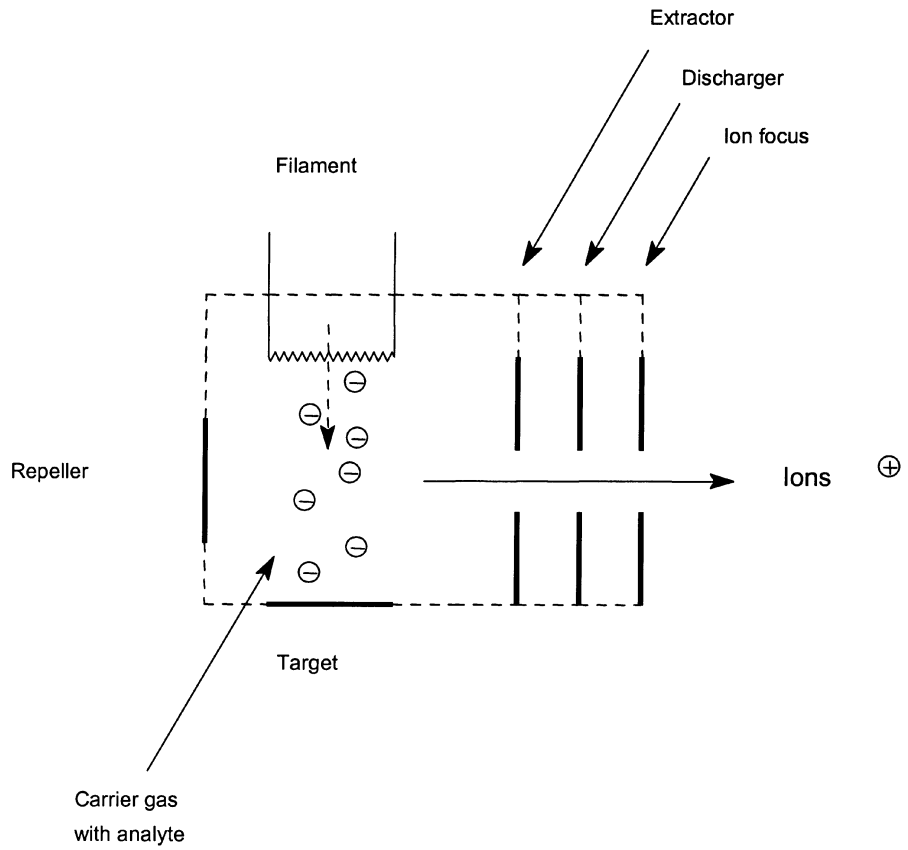

Fig. 4. Schematic set-up of an electron impact ion source

source, on the other hand no "cold" sites should be present to avoid changes to the surface due to substance deposits.
The filament is heated by a current of 200 to 300 μA for ionisation, and high-energy electrons are emitted and accelerated by a voltage towards the opposite electrode. The energy of these electrons is usually given in electron volts (eV), whereby 1 eV is equivalent to the energy taken up by an elemental charge while traversing a potential difference of 1 volt. The emitted electrons collide with the sample molecules on the way to the opposite electrode and can displace an electron from the highest occupied molecular orbital there, thus generating two free electrons at a lower energy and a radical cation from the collision:

$$\mathrm{M} + \text{electron}\ (70\,\mathrm{eV}) \rightarrow \mathrm{M}^{*+} + 2\,\text{electrons}$$

The ionised molecules are accelerated out of the ion source by electrical repulsion from the repeller anode towards the aperture and lens system, comprising an extractor (cathode opposite the repeller), a discharger (to discharge defocused ions) and one or more ion focuses. The ion beam is focused in the following mass filter by the ion focus.
The first ionisation potential IP of a molecule must be exceeded in order to generate cations. Organic compounds exhibit an IP of 8 to 13 electron volts. Above this threshold the ionisation yield rises continuously and reaches a saturation level at 40 to 60 eV. However, "saturation" in this context means a yield of only about 0.1% of the molecules.
Uncharged molecules are removed from the mass spectrometer by the vacuum pumps. Electron energies of 70 eV are typically used in order to improve the ionisation yield for a wide range of substances. However, such values, which considerably exceed the typical ionisation potentials of organic compounds, cause the excessive impact energy to convert the molecule into excited oscillation states, consequently covalent bonds are broken and fragmentation of the molecule ensues. On principle, the extent of fragmentation can be reduced by lowering the kinetic energy of the impact electrons. However, the ionisation yield falls by several orders of magnitude in this case so that "low-electron volt spectra" are only useful for qualitative purposes, e.g. for identification of the molecule ion.
Interpretation of fragmentation reactions cannot be reviewed in detail here on account of the large number of different mechanisms and special cases. Section 6 gives an overview of more detailed sources of literature.
Mass spectrometry is eminently suitable for quantitative trace analysis thanks to fragmentation:

– Fragmentation reactions are specific for substances and, as a rule, they permit definitive identification of a substance. This also applies with reservations to structural isomers that yield almost identical fragments and must initially be separated by gas chromatography in most cases. While other GC detectors primarily enable identification by means of the retention time, mass spectrometric data provide direct indications of the chemical structure of a compound.

– Fragmentation reactions are readily reproducible. The molecular geometry does not change during the ionisation phase (10^{-15} to 10^{-16} s) (Franck-Condon principle) [75]. Thus the fragmentation pattern of a substance is always identical under constant ionisation conditions. A certain variation is caused only by the thermal energy dispersion of the individual molecules.

4.3.2 Chemical Ionisation (CI)

Chemical ionisation is a procedure for generating ions using less excess kinetic energy than is required for electron impact ionisation. Ions are formed by charge exchange during collision of the sample molecules with a reactant gas previously ionised by electron impact. In contrast to the EI ion source, intermolecular collisions are desirable and are promoted by a closed design and a higher gas pressure (0.1 to 1 mbar). As both positively and negatively charged ions can be generated by the appropriate choice of ionisation conditions, these procedures are known as positive or negative chemical ionisation (PCI or NCI) [76, 77].

4.3.3 Positive Chemical Ionisation (PCI)

Methane, which is frequently utilised as a reactant gas, is used here as an example for the reactions during chemical ionisation. Initially "primary" radical cations are generated (Fig. 5) from methane molecules by electron bombardment in the ion source. Higher potentials of approx. 100 to 150 eV can be applied to the incandescent cathode to enhance the ionisation yield. Reactant gas molecules are largely ionised due to the high methane vapour pressure and the associated "excess of methane".

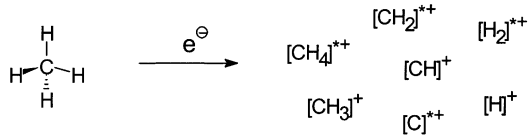

Fig. 5. Generation of radical cations and cations during ionisation of methane

In addition to the primary ions, collisions of the reactant gas molecules with each other result in the generation of "secondary" ions (Fig. 6). Together with the primary ions they form a relatively stable plasma.

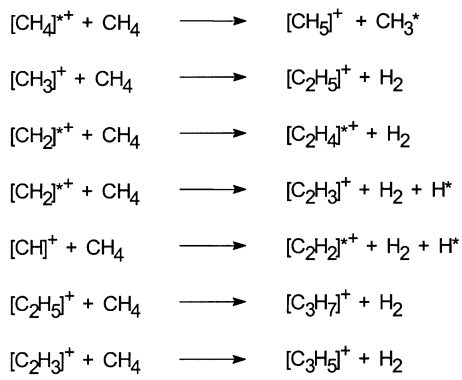

Fig. 6. Formation of secondary ions during chemical ionisation with methane

The following mechanisms occur due to collisions with sample molecules:

– *Proton transfer*
Sample molecules M can act as Brönstedt bases and accept protons from secondary reactant gas ions RH^+ (e.g. CH_5^+, $C_2H_5^+$, $C_3H_7^+$, NH_4^+, H_3^+). The proton is transferred to the molecule with the highest proton affinity (PA). Therefore the selection of the reactant gas already determines the sample molecules that can be ionised. Table 4 shows the reactant gases most frequently used for chemical ionisation:
Methane is a very efficient reactant gas on account of its relatively low proton affinity (cf. Table 4). In contrast, e.g. isobutane and ammonia lead to considerably "gentler" ionisation. In these cases proton transfer is accompanied by a low excess of energy. Thus fragmentation of the sample molecule is only slight and it occurs with high selectivity.

Table 4. Reactant gases for chemical ionisation (according to [2])

Gas	Proton affinity [kcal/mol]	Reactant ion
Hydrogen	100	H_3^+
Methane	127	CH_5^+
Ethene	160	$C_2H_5^+$
Water vapour	165	H_3O^+
Hydrogen sulphide	171	H_3S^+
Methanol	182	$CH_3OH_2^+$
Isobutane	195	*tert*.-$C_4H_9^+$
Ammonia	207	NH_4^+

Table 5. Proton affinities of selected organic compounds (according to [2, 4])

Compound	Proton affinity [kcal/mol]	Compound	Proton affinity [kcal/mol]
Ethane	121	Methyl sulphide	185
Methyl chloride	165	Nitroethane	185
Trifluoroacetic acid	167	Methyl cyanide	186
Formic acid	175	Toluene	187
Benzene	178	Xylene	187
Cyclopropane	179	Acetic acid	188
Propylene	179	Dimethyl ether	190
Methylcyclopropane	180	Isopropanol	190
Nitromethane	180	2-Butanol	197
Methanol	182	Ethyl acetate	198
Acetaldehyde	185	Acetone	202

Table 5 shows that (with the exception of ethane) all the selected compounds can be chemically ionised using methane, while e.g. methanol is especially suitable for heteroatomic and aromatic compounds.
Ammonia is an extremely selective reactant gas, e.g. for aromatic amines and phthalic acid esters, on account of its high proton affinity. As in the case of hydrogen sulphide and water vapour, it must be considered that ammonia has a corrosive effect on the sensitive surfaces in the ion source and in the mass filter. Therefore these gases cannot be recommended for constant use, and the gas supply installations and pressure reducer must be made of stainless steel.

– *Hydride loss*
When reactant gas ions are formed, species with high hydride (H^-) affinity are created, e.g. CH_3^+, CH_5^+, $C_2H_5^+$. The reactant gas can wrest a hydride ion away from molecules containing hydrogen, leaving a corresponding cation. The exothermal nature of the reaction results in an intensive fragmentation of the $[M\text{-}H]^+$-ions. Hydride loss and proton transfer (see above) often occur simultaneously.

– *Cation addition*
As it is thermodynamically favourable, the addition of cations to the sample molecule is a frequently occurring gas phase reaction. This mechanism is particularly prominent in the case of methane. $[C_2H_5^+]$ and $[C_3H_5^+]$ ions are added to form $[M+29]^+$ and $[M+41]^+$ molecule adducts. Therefore together with proton transfer a characteristic addition pattern is observed with an intensity distribution of about 100 : 10 : 2 for (M + 1), (M + 29) and (M + 41), which is observed only in the molecule ion in this form (cf. Fig. 6 b). PCI is therefore an important method for identification of the molecule ions in a compound.

– *Charge transfer*
  While the charge donors in the mechanisms described above are protons or hydride ions and a "classical" acid-base reaction takes place, "charge transfer" is based on an electron transfer, i.e. an "oxidation effect". A noble gas or another chemically inert compound (e.g. nitrogen, carbon dioxide, carbon monoxide) is initially ionised with the help of electron impact. The sample molecule receives its charge by electron transfer from the sample molecule to the reactant gas cation.

This ionisation method is especially "gentle" and results in low fragmentation, as there is little difference between the recombination energy (e.g. He: 24.6 eV, Ar: 15.8 eV, CO: 14.0 eV, CO_2: 13.8 eV, Xe: 12.1 eV, NO: 9.3 eV, benzene: 9.3 eV) and the ionisation potential of the (generally organic) compounds. Therefore only a slight excess energy is transferred to the sample molecule cation. The fragmentation pattern is similar to low-electron volt spectra, but the ionisation yield is higher.

4.3.4 Negative Chemical Ionisation (NCI)

Negatively charged ions can be detected by reversal of the electrical polarity in the ion source and at the detector [78, 79]. However, anions are generated only to a limited extent, even during electron impact ionisation, and the ions thus created are hardly useful for detection because of the large excess of radical cations and cations. However, chemical ionisation can promote the formation of negatively charged ions that can be utilised for analysis.
With the exception of the "ion-molecule reactions", the reactant gas primarily serves to generate slow "thermal" electrons. As in the case of electron impact ionisation the reactant gas is bombarded with high-energy electrons (>200 eV) that are emitted from an incandescent filament. Low-energy electrons result from the impact between the electrons from the incandescent filament and the reactant gas. In the case of methane the following reaction takes place:

$$CH_4 + \text{electron}^-_{(230\,\text{eV})} \rightarrow CH_4^{*+} + 2\,\text{electrons}^-_{(\text{thermal})}$$

The energy of the released electrons is only a few electron volts, therefore they can be "captured" by the sample molecules. Subsequent reactions and fragmentation occur to a considerably lesser extent than in the case of electron impact ionisation. Negative chemical ionisation can have a considerably higher detection capacity than electron impact ionisation and PCI:

– NCI exhibits a high selectivity for "electron-trapping" compounds (e.g. halogen-containing and other heteroatomic compounds) and electron-deficient aromatic compounds. The sensitivity can be improved by two orders of magnitude compared with EI and PCI, and it lies in the range of that achieved by an electron capture detector (ECD).

– No reactant gas anions are formed in the case of NCI, and as a consequence background interference is very low, e.g. compared to positive chemical ionisation.

On principle, the gases already mentioned in the section on positive chemical ionisation can also be utilised for NCI, as the high energy of the emitted electrons guarantees a good ionisation yield. However, in contrast to PCI, the identity of the gases is not important for the actual ionisation of the sample molecules, so the reactant gas can be selected on the grounds of safety and the ease of technical installation.
The released electrons can lead to negative ionisation of the sample molecules via various mechanisms:

– *Electron capture*
  Ionisation due to electron capture is the quantitatively most important reaction in the NCI mode: thermal electrons are incorporated into unoccupied molecular orbitals and lead to the formation of radical anions:

$$M + \text{electron}^- \rightarrow M^{*-}$$

This reaction is reversible, i.e. in the worst-case scenario the radical anion simply releases the charge it has previously acquired ("spontaneous discharge"). This process proceeds very rapidly, leading to an equilibrium being established between the ionised and the uncharged sample molecules. The higher the electron affinity of the sample molecule, the more the equilibrium lies on the side of the radical anions. For this reason NCI mass spectrometry, like electron capture detection (ECD), is particularly suitable for halogen-containing and heteroatomic compounds. But stabilisation of the anions can also be achieved by fragmentation that is observed as a secondary reaction of NCI:

– *Dissociative electron capture*
  Radical anions are stabilised by homolytic bond cleavage in the case of dissociative electron capture:

$$MX + \text{electron}^-_{(\text{thermal})} \rightarrow M^* + X^-$$

This reaction is also reversible in principle and proceeds rapidly so that a substance-specific equilibrium is established in the ion source.

– *Ion pairing*
  Ion pairing results in stabilisation of the radical anion due to heterogenic fragmentation and release of the thermal electron. Therefore it is a combination of "spontaneous discharge" and dissociation:

$$MX + \text{electron}^-_{(\text{thermal})} \rightarrow M^+ + X^- + \text{electron}^-_{(\text{thermal})}$$

– *Ion-molecule reactions*
Ion-molecule reactions occur particularly frequently in the presence of oxygen, water or other electron-affinative compounds in the ion source:

$$M + X^- \rightarrow MX^-$$

Initially ionisation of the interfering component (X) occurs and its radical anion or anion associates with the sample molecule. This reaction is also known as "ion adduct formation" and it is an undesirable reaction that competes with electron capture. The different types of ionisation and their effect on the mass spectrum of a compound can be illustrated using N-methylvaline as an example (Figs. 7 a to 7 c). N-Methylvaline (MEV) is an amino acid that is formed by methylation of the free N-terminus of a haemoglobin chain in the erythrocytes. The parameter can be used for biological monitoring [8]. MEV is cleaved from the globin chain by means of a "modified Edman degradation" and converted to a pentafluorophenylthiohydantoin (PFPTH) derivative [80]. The electron impact mass spectrum of N-methylvaline-PFPTH is shown in Figure 7 a, in which the most important fragments are marked. The EI spectrum exhibits

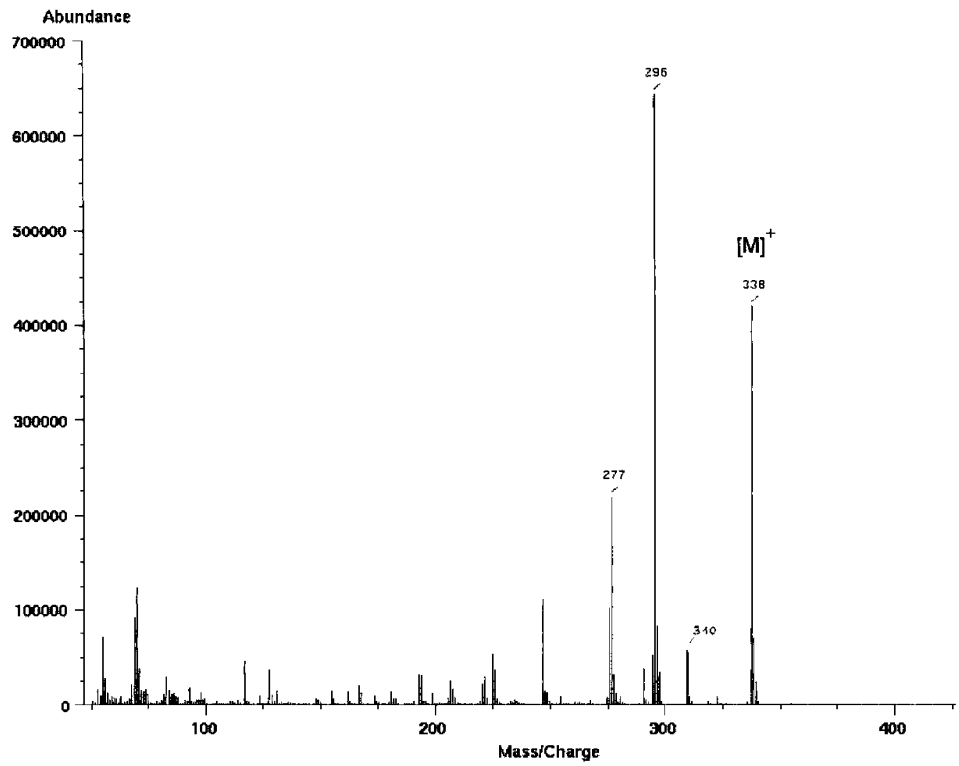

Fig. 7 a. Electron impact mass spectrum of N-methylvaline-PFPTH

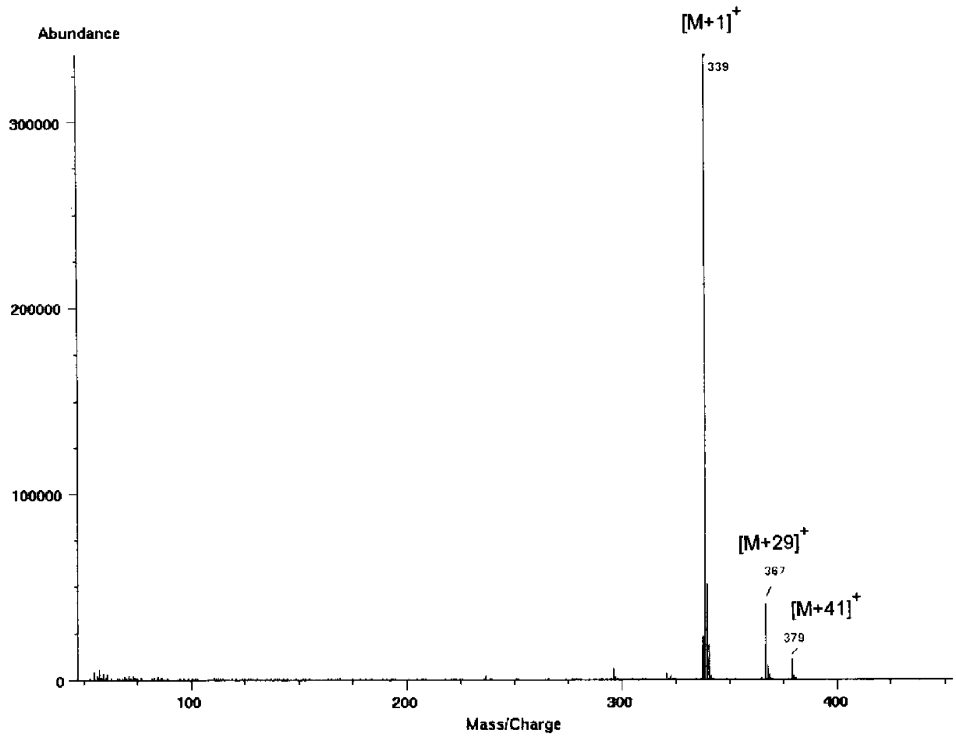

Fig. 7 b. PCI mass spectrum of N-methylvaline-PFPTH

marked fragmentation in addition to the molecule ion at 338 m/z. The main fragments can be explained by typical reaction mechanisms (McLafferty rearrangement ($338 \rightarrow 296$), CO cleavage in the α-position to yield a heteroatomic fragment ($338 \rightarrow 310$), loss of a fluoride ion ($296 \rightarrow 277$)). The molecule ion 338 m/z exhibits a peak intensity of about 500,000 relative units.

When the same sample is analysed with positive chemical ionisation using methane (Fig. 7 b), it is evident that the molecule ion hardly fragments. In addition to the protonated molecule ion [M+1] with a m/z of 339, the only other products of importance to be formed are the adducts [M+29] (molecule ion+C_2H_5) and [M+41] (molecule ion+C_3H_5]. Both adducts confirm that the molecule ion has the atomic mass or the m/z ratio of 338. However, the intensity of the main signal is only approx. 350,000 relative units, which shows a reduction in the sensitivity compared with analysis using electron impact ionisation.

The degree of fragmentation in the case of negative chemical ionisation (Fig. 7 c) is similar to that caused by electron impact ionisation. The main signal at 337 m/z is generated by a ion-molecule reaction (proton transfer). The intensity of this signal is approx. 1,200,000 relative units.

Compared to electron impact ionisation, the calculated sensitivity is increased by a factor of 2. In practice, however, the detection limit is improved by a factor of 5 on

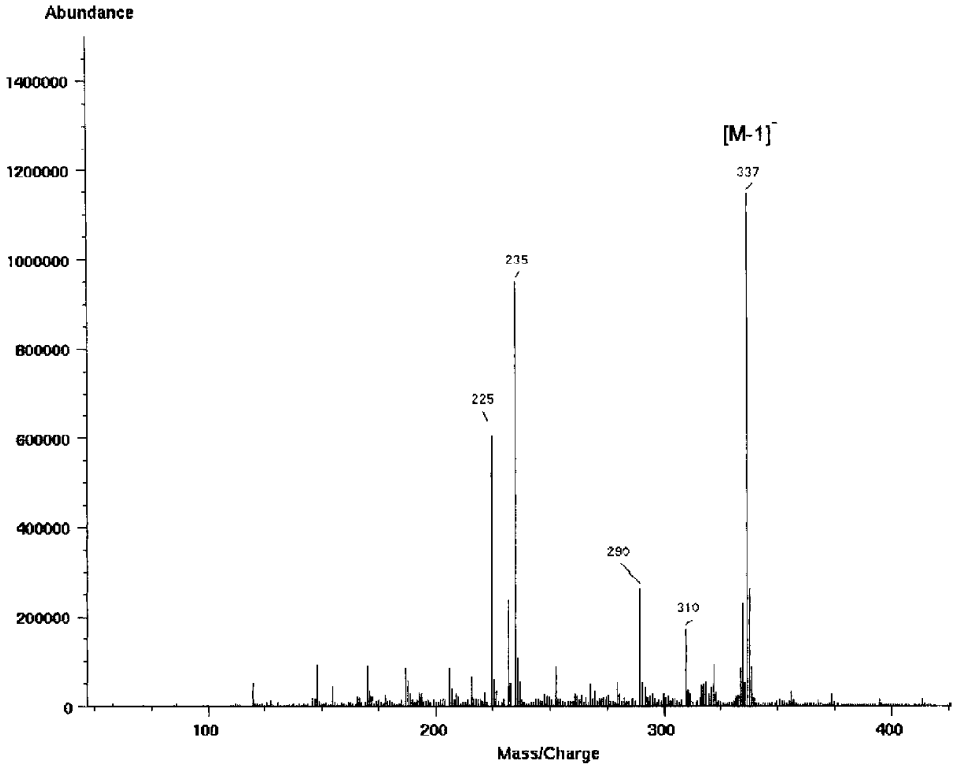

Fig. 7c. NCI mass spectrum of N-methylvaline-PFPTH

account of the more favourable signal/background ratio for NCI MS in the case of the PFPTH derivatives of alkylated amino acids.

4.4 Mass filter

After the ion source the second most important component group of a mass spectrometer is the mass filter. The charged molecules are deflected from their flight path by means of electromagnetic fields. This deflection depends on the mass or, more exactly expressed, on the mass/charge ratio of the molecule, and leads the ions either to electrically charged surfaces within the mass filter where they discharge and are removed by the high-vacuum pumps, or deflects them towards the detector where they are recorded. The deflection of the flight path can be achieved by electric and magnetic fields with different geometrical designs. In practice several designs have become established: quadrupole, ion trap and sector field instruments. All these designs of mass filters are based on the principle of separation of accelerated ions according to their mass/charge ratio.

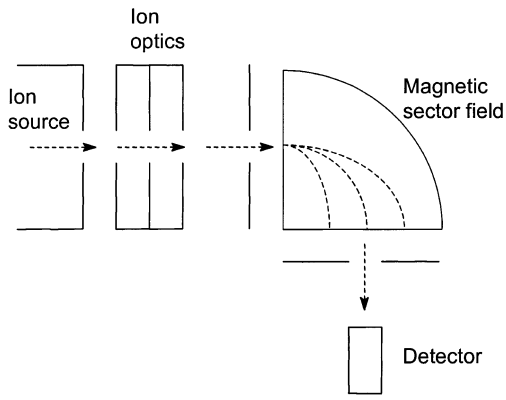

Fig. 8. Deflection of an ion beam by a magnetic sector field

When linearly accelerated, electrically charged molecules reach a magnetic field that is directed at right angles to their flight path and the molecules are deflected in a circular path [72]. This deflection is schematically illustrated in Figure 8. The kinetic energy of these ions is determined by the voltage with which they are accelerated out of the ion source:

$$e\,U = \frac{1}{2}\,mv^2$$

(e = ion charge, U = acceleration voltage, m = molecular mass, v = velocity).
The focused ion beam is bent in the magnetic field H and follows a trajectory in which the strength of the magnetic field is compensated by the centripetal force of the accelerated ions:

$$H\,e\,v = m\,v^2\,r^{-1}$$

The deflection radius is therefore expressed as:

$$r = (m\,v)/(e\,H)$$

It is directly proportional to the mass of the charged ion, its velocity and the strength of the magnetic field. Regardless of the type of ionisation, small molecules usually generate singly charged molecule ions. Thus the ionic charge e is equivalent to the single elemental charge of 1. When combined, these equations give the "basic mass spectrometric equation":

$$m/z = (r^2\,H^2)/(2\,U)\,.$$

There is a constant relationship between the mass/charge ratio and the square of the deflection radius when the acceleration potential and the strength of the magnetic field are kept constant:

$$m/z = r^2 \text{ const.}$$

When $z = 1$ it follows that the deflection radius is proportional to the mass.
This relationship can be utilised for spatial separation of the various ions or charged molecular fragments in the magnetic field of the analyser: only ions with specific m/z ratios are deflected in a circular path in the magnetic sector field, at the end of which the mass spectrometric detector is installed. Any sections of the mass spectrum of a compound or individual substance-characteristic fragments can be investigated by varying the strength of the magnetic field or the acceleration voltage.
The performance of a mass filter is characterised by three factors:

- mass range
- scan speed
- and resolution.

The maximum accessible mass range of the filter primarily depends on the available strength of the magnetic field: while practically all mass spectrometers permit ion detection in the range below 250 to 300 m/z, instruments with weak magnetic fields show an increasing discrimination in the case of higher masses.
For a given sector field geometrical design each magnetic field has a maximum m/z ratio, above which deflection to the detector is no longer possible. Furthermore, the kinetic energy of the molecules in the ion beam exhibits dispersion to a greater or lesser degree: the higher the mass of the ion to be deflected, the more noticeable the differences in kinetic energy. An increasing proportion of the ion beam is not focused with sufficient precision on the detector and the intensity of the signal decreases. This effect causes an increasing distortion in the spectrum in the range of the higher masses, and the relative intensity of the heavier fragments or of the molecule ion seems to diminish.
The stronger the magnetic field, the more precisely the heavier ions with higher dispersion can be deflected. Instruments with an accessible mass range of up to 1000 m/z are especially suitable for use in the fields of occupational and environmental medicine. Organic compounds or their metabolites, even in the form of their volatile derivatives, are seldom heavier than 500 to 600 m/z, so most GC/MS systems meet the stated requirements. Instruments with wider mass ranges up to 2000 m/z are more suitable for applications in the area of protein and DNA analysis. However, in these areas HPLC (high performance liquid chromatography) instruments and special techniques for sample injection are generally required (e.g. matrix-assisted laser desorption ionisation, MALDI), as the compounds to be investigated are no longer volatile enough to be separated by gas chromatography and transported into the mass spectrometer.
The complete mass spectrum of a compound is usually recorded by continually varying the strength of the magnetic field. The result is known as a “scan”. This investigation can be carried out only if the intensity of the ion beam remains constant throughout the duration of one or several scans and the concentration of the compound to be investigated is high enough to generate a signal at the detector. While the latter condition is relatively simple to fulfil (a substance quantity of approx. 1 to

10 ng is required in the ion source, which is equivalent to an injection volume of 1 μL at a concentration of 1 to 10 mg/L), the scan speed and scan rate (number of spectral recordings per second) depend on the instrument. They are largely determined by the speed of the magnetic field variation, the response time of the ion detector and the speed of data transfer and data processing.

As a rule, a component separated by gas chromatography is eluted from the separation column after about 5 to 20 seconds. Approx. 15 to 20 individual measurement points or complete scans are required in order to portray a reproducible and quantitatively evaluable peak. As a consequence of these general requirements, the mass filter must perform at a scan rate of at least 1 s^{-1}. Modern GC/MS systems attain scan rates of about 2 to 3 s^{-1} for a mass range of 300 m/z. A scan analysis is graphically depicted by the GC/MS in such a manner that the intensities of the individually recorded masses are added together to give a total signal. Therefore each complete scan yields only one individual value. A seemingly uninterrupted line, similar to a "typical" gas chromatogram and known as a "total ion chromatogram" (TIC), results by continuous recording of several scans per second and by plotting the signals as a function of time.

When closely scrutinised, each "point" of the signal line represents a complete mass spectrum and thus contributes to the identification of the relevant analyte. In addition, individual substance-specific signals can be isolated from the spectral set and can be reconstructed to give a chromatogram. In this way interfering components can be largely suppressed, at least visually.

The narrower the mass range selected, the more frequently a complete scan can be carried out. This is particularly desirable for the definitive identification of a component. However, the sensitivity of the scan technique is low compared with other gas chromatographic detection methods (e.g. flame ionisation). As already mentioned above, the lowest concentration for scan mass spectrometry is about 1 mg/L. The scan technique is inadequate to deal with most investigations in occupational medicine and almost all the questions posed in environmental medicine.

However, in many cases it is not necessary to record complete mass spectra for substance identification. In addition to the gas chromatographic retention time, which represents an important identification characteristic of a compound, the m/z ratio of the molecule ion provides further significant substance-specific information. Moreover, most compounds fragment in a very characteristic and reproducible manner, so that individual ions may be sufficient to enable clear identification of components. This principle is used in the "selected ion monitoring" (SIM) technique.

Only selected ions reach the detector in the SIM mode, i.e. the strength of the magnetic field is varied discontinuously and optimised for the m/z ratio of the ions in question. Two or three individual ions (molecule ion and characteristic or high-intensity fragments) are generally sufficient, together with the retention time, to ensure definitive identification of the component. The advantage of this technique is that the time available to record an ion is distinctly greater than in the scan mode.

Thus at most four seconds per fragment are available for measurement at an elution duration of 12 seconds and with three fragments to be recorded. If this time is divided by the time required for the desired number of measured points, i.e. 20, only 200 milliseconds remain per measurement point and ion. The duration of the switch-

over from one m/z ratio to the next must be considered (approx. 25 ms), resulting in a total of 175 milliseconds per measurement point and ion.

The measurement time per ion is considerably shorter in the scan mode: recording 20 scans over a range of 300 mass units during an elution duration of 12 seconds leads to a scan rate of 0.6 s^{-1}, i.e. 2 milliseconds of measurement time are available for each ion per scan. This is equivalent to about 1% of the measurement time in the SIM mode. The intensity of an individual ion signal is two orders of magnitude higher in the SIM mode than when a scan is recorded.

Figure 9 (top) shows the gas chromatogram of a urine extract that was investigated to determine a urea herbicide (retention time 17.87 min). The pertinent electron impact mass spectrum (Fig. 9 (middle)) exhibits a main fragment with an m/z ratio of

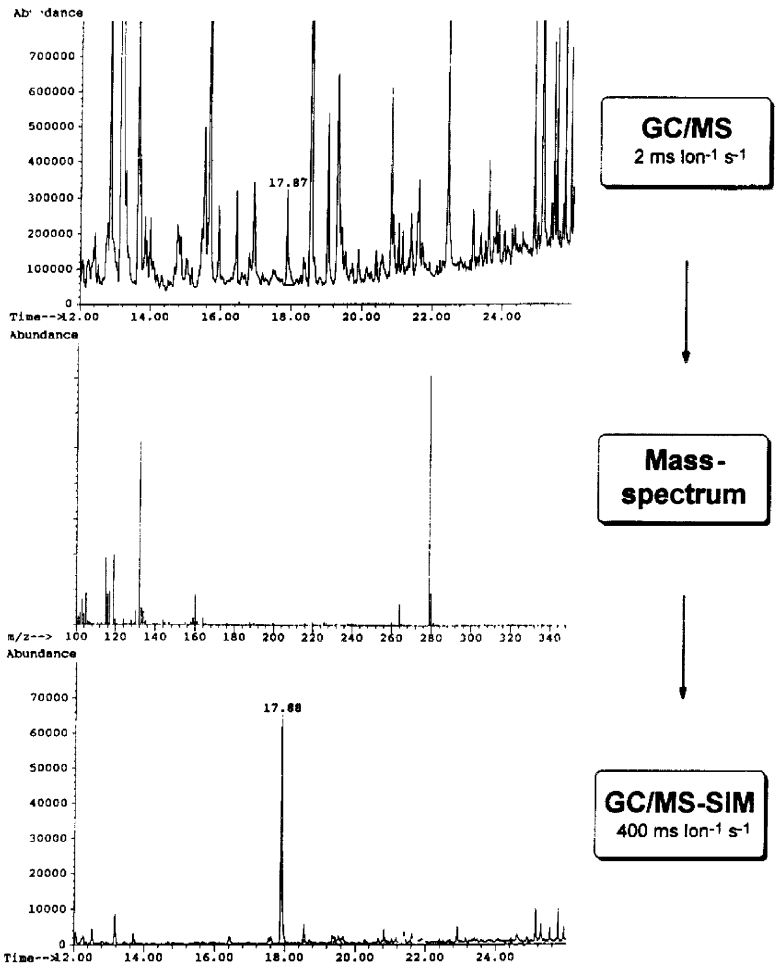

Fig. 9. Switchover from the scan mode to selected ion monitoring

132 in addition to the molecule ion at 279 m/z. A considerably higher selectivity (less interfering components of weaker intensity) and an improved signal/background ratio are achieved by switching the mass spectrometer to a SIM analysis of these two ions (Fig. 9 (bottom)).
The distinct improvement in sensitivity using the SIM technique is accompanied by a relatively slight loss of structural information. The mass range between 160 m/z and 260 m/z yields no relevant information, but requires half of the measurement time. The SIM mode permits the substance to be identified with a sufficiently high degree of certainty on the basis of fewer selected ions.
The following criteria apply when selecting suitable SIM ions:

1. The selectivity of the SIM procedure gradually decreases as the masses become lower (m/z < 100), as many compounds are fragmented in this range. On principle, either the molecule ion or fragments with higher and therefore more substance-specific masses should be selected. Furthermore, many compounds undergo only one or two main fragmentation reactions that are easy to interpret and permit direct conclusions on the nature of the molecule ion (e.g. α-cleavage, McLafferty rearrangement, CO loss, *tert*.-butyl cleavage, etc.).
2. It is advisable to record at least two, preferably three, ions per analyte. On the one hand, analysis of several characteristic fragments enhances the chances of identifying the analyte, on the other the relative ratio of the signal intensities can provide evidence to enable definition of a background interference. As fragmentation reactions are readily reproducible, any deviation of the signal intensities from a specified quotient (e.g. ion A : ion B : ion C) can indicate an interfering component that has not been separated by gas chromatography and may even prevent erroneous identification. Each user must define the limit at which a deviation represents an unacceptable interference to the analysis according to his/her own requirements. The ion trace ratio becomes increasingly inexact, in particular at low concentrations. The signal/background noise ratio (3 : 1), which is frequently defined as the detection limit, is not suitable in this case, particularly as the detection limit must be based on the fragment with the weakest signal.
3. Wherever possible, fragments of strong intensity should be measured for SIM analysis. The detection limit of the method depends directly on the strength of the signal at the detector. Therefore it is advisable to select a "quantifier" (an ion that is used to calculate the analytical result) and "qualifiers" (two or three ions that are measured to confirm the identity of the analyte; the relative intensity ratios serve as a check and they should be less than 10%) from the main fragments.
4. If possible, ions should be chosen that are not subject to any interference from concomitant components which yield the same fragments.

A further advantage of the SIM technique is that the unspecific background is very efficiently suppressed by the high selectivity of the procedure, thus enhancing sensitivity compared with scan recording due to improvement of the signal/background ratio.
In certain investigations it is also important to have a high mass resolution in order to achieve more specific and sensitive detection. The resolution power of a mass

spectrometer is defined as the ability to separately detect ions with slight differences in their molecular mass. The resolution R is expressed mathematically as follows

$$R = m/\Delta m$$

Analogous to the definition of gas chromatographic separation, complete mass spectrometric separation is given, when the overlap (the "valley" between two peaks) of two signals of equal height is less than 10% of the peak height. For example, in order to separate masses of 1000 and 1001, a resolution of 1000/1 = 1000 is required. The typical resolution power of low-resolution mass spectrometers is 1000 to 2000, while high-resolution systems achieve R-values of 10,000 or more.
The resolution R of a magnetic sector field instrument is constant over the entire mass range: the distance between two neighbouring signals in the low mass range is therefore relatively large. In contrast, the distance Δm between two signals is constant in quadrupole instruments and ion traps, so the resolution is poorer at lower masses. However, in practice these instruments are adjusted by the manufacturers for constant resolution that is defined by the 10% criterion.
As already mentioned, three technically different types of mass filters capable of different performances are used at present:

4.4.1 Magnetic sector field instruments

The deflection of electrically charged molecules in a magnetic field described above is a "classical" technique of mass spectrometry (Fig. 8). Although the basic principle has been retained, magnetic sector field instruments have become the most powerful of the mass spectrometers as result of many improvements.
However, the molecules accelerated out of the ion source do not move homogeneously in a single defined direction, but are subject to a certain scattering (directional dispersion). Moreover, the kinetic energy of the molecules or molecular fragments has a normal distribution under given external conditions (energy dispersion). While the directional dispersion can be compensated by the "lens effect" of the magnetic sector field, the kinetic energy scatter causes broadening and relative weakening of the ion beam. The mass resolution of a magnetic sector field is also determined by the scatter of the kinetic energy of ions with an identical m/z ratio. Simple instruments use electrostatic apertures for ion focusing. This technique achieves resolutions between 5000 and 10,000 and has the advantage of a relatively simple instrumental set-up. However, it results in considerable loss of sensitivity due to screening of a part of the ion beam.
In "double-focusing" sector field instruments (Fig. 10) a curved electrostatic field is introduced either in front of or after the magnetic field in order to reduce energy dispersion. This field separates the ions with the same m/z ratio but different kinetic energies and also focuses them in the same manner as a lens: the electrostatic field deflects slower ions more strongly than faster ones, thus resulting in spatial separation.

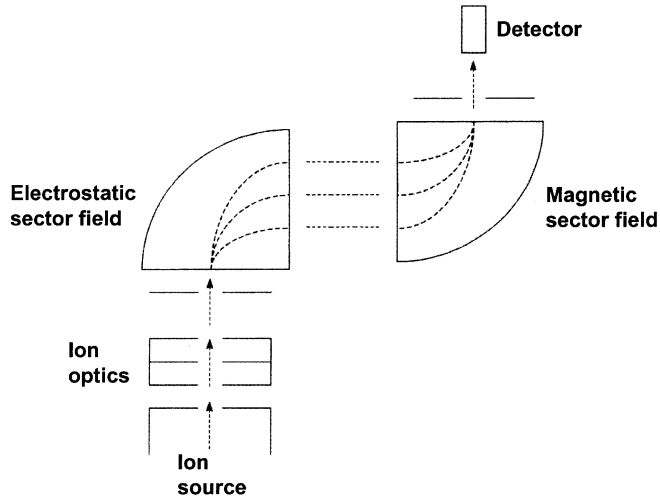

Fig. 10. Schematic illustration of a double-focusing sector field instrument

The directional focusing and the velocity dispersing properties of the sector fields can be harmonised with each other by the sequential arrangement in such a manner that ion beam dispersion is compensated to the greatest possible degree. All ions with the same m/z ratio are focused at the detector regardless of their energy dispersion. Depending on the arrangement of the electrostatic analyser and of the magnetic sector, we differentiate between the Nier-Johnson geometry (first the magnetic field, then the electrostatic analyser; semicircular ionic beam path) and the Mattauch-Herzog geometry (first the electrostatic analyser, then the magnetic field; sigmoid ion beam path). In addition to a higher sensitivity, double-focusing sector field instruments also achieve a considerably higher resolution compared with the simple sector field technique (R = 100,000).

4.4.2 Quadrupole instrument

Quadrupole mass filters utilise the interaction between the ion beam and an oscillating electric field. A quadrupole analyser consists of a total of four parallel rod electrodes that are arranged in a square and point in the longitudinal direction. In each case opposite poles are coupled by a direct voltage U and an alternating voltage with an amplitude of V_0 so that the resulting potential difference V_{total} for each pair of poles is

$$V_{total} = U \pm V_0 \times \cos(\omega \times t)$$

(U = direct voltage, V_0 = alternating voltage, ω = angle velocity, t = time) (Fig. 11).

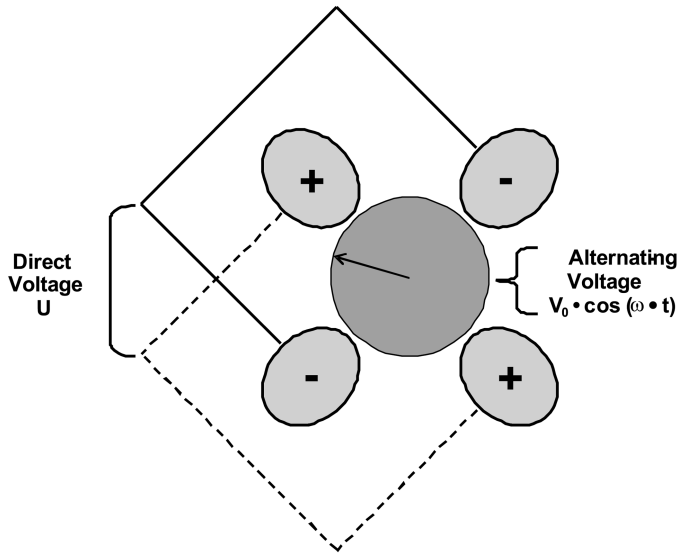

Fig. 11. Principle of the quadrupole analyser

When ions reach the alternating electric field, their flight path is influenced in a complex manner. The resulting flight paths are described by the "Mathieu equations". These are differential equations that describe the oscillation of an ion in a three-dimensional space or, more exactly, in a three-dimensional alternating electric field. Only those ions in resonance with the alternating electric field exhibit a stable flight path and pass through the mass filter. This depends on the mass of the ion and on the selected direct and alternating voltages. To generalise, it can be stated that ions with a small m/z ratio are especially influenced by the alternating voltage, while the heavier ions (high m/z) are subject to deflection by the direct voltage. The ions are not accelerated towards the detector in the quadrupole. Their path is determined by the strength of the direct voltage or alternating voltage, the radius of the alternating electromagnetic field and the frequency of the alternating voltage. Like the cycle frequency of the alternating voltage, the quadrupole dimensions are pre-determined by the design. Therefore in practice the flight path of the ions is determined only by the potentials U and V.
When specific voltages are set, only ions with certain m/z ratios pass through the quadrupole, whereas all other ions either collide with the quadrupole rods and discharge or exhibit an unstable flight path and leave the mass filter between the poles.
Quadrupole instruments have several interesting properties:

– They are more compact and cheaper than sector field instruments, and they are very robust.
– As the ions are intended to take the longest possible path through the mass filter, the electrical acceleration voltages in the quadrupole are considerably lower that

in the sector field instrument (5 to 30 volts compared with 5 to 10,000 volts). There is therefore less danger of high voltage surges in the ion source.
– As no magnetic fields are necessary, the scan rate of a quadrupole instrument is higher than that of a sector field instrument. Between 2 and 3 mass spectra of about 300 m/z units can be recorded per second.

However, the operational range of a quadrupole instrument is narrower than that of a sector field instrument. The mass resolution is also lower. The resolution of a quadrupole depends ultimately on the number of oscillations of the ions during their passage through the quadrupole: the higher the number, the better the mass resolution. The lower the acceleration voltage that is set in the ion source, the longer the ions remain in the quadrupole and the more oscillations they exhibit. On the other hand, reduction of the acceleration voltage leads to discrimination of the transmission rate in favour of higher masses, leading to a decrease in sensitivity. Quadrupole instruments are therefore adjusted to a mass resolution of 1000 to 2000 by the manufacturer, which represents a compromise between resolution and sensitivity.

4.4.3 Ion traps

The "ion trap" represents a special form of the quadrupole. The electric field is also generated by applying a direct and an alternating voltage, but the ion trap consists of a central "ring electrode" and two "end cap" electrodes that enclose the ring volume from above and below (Fig. 12).

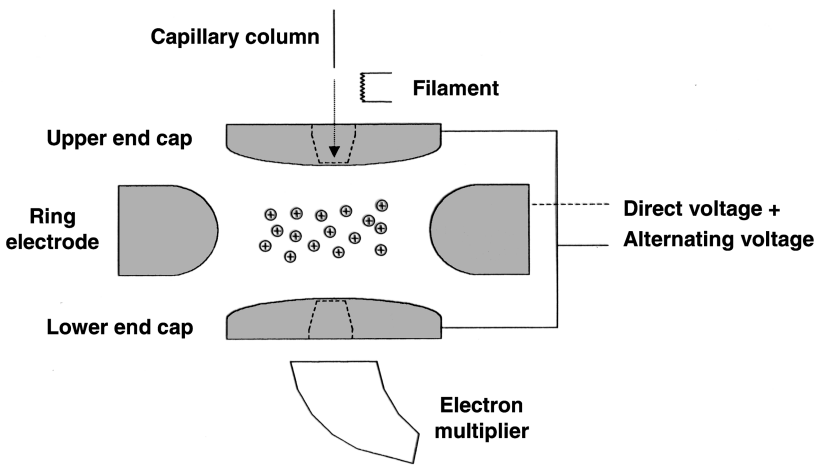

Fig. 12. Principle of the ion trap

Sample molecules are ionised in the resulting hollow space by electrons from an incandescent wire and then accelerated by alternating electric fields in complex trajectories. Thus the ion trap is successively "filled" initially, e.g. with ionised sample molecules that have been previously separated from a mixture by gas chromatography. It is possible to select which ions adopt stable or unstable flight paths within the trap by varying the voltages. In contrast to the quadrupole, in which the ions with stable trajectories impact on the detector and those with unstable trajectories lead to discharge, ions with stable flight paths remain in the trap while the ions of interest are accelerated towards a detector, generally through an aperture in the lower end cap electrode [81].

The most important differences between ion traps and quadrupole or sector field instruments arise from the different procedures for sample ionisation and subsequent detection:

- ions can be "enriched" in the trap, i.e. the time in which the sample molecules flow into the trap with the carrier gas can be varied within certain parameters. As in quadrupole and sector field MS, the areas of components separated by gas chromatography must be defined by several measurement points. No enrichment takes place in instruments that are in continuous operation. The intensity of the subsequently generated ion beam can only be as high as the intensity of the non-ionised sample molecule stream.
- ionisation occurs only when a previously defined optimum "sample concentration" has been reached in the trap. For this purpose the mass spectrometer carries out a brief "pre-scan" before each measurement. In quadrupole and sector field instruments ionisation occurs continuously and independently from the mass concentration in the ion source.
- in the trap all the ions to be detected are sorted very rapidly according to their mass, accelerated towards the detector and recorded. In quadrupole and sector field instruments only one type of ion with a certain m/z ratio is recorded. When a mass spectrum of 1 to 500 m/z is recorded at a scan rate of 2 s^{-1}, 1000 masses per second can be analysed, which is equivalent to a measurement duration of 1 millisecond per ion. The measurement duration per ion in an ion trap mass spectrometer comprises practically the entire ionisation period of the enriched sample (up to 25 milliseconds), as all the enriched ions of a certain m/z ratio are recorded in the subsequent scan.

As a result of the system differences, an ion trap mass spectrometer exhibits considerably higher sensitivity than a quadrupole or a sector field instrument when recording mass spectra. The difference becomes even greater the broader the range scanned because the measurement time per ion diminishes as the scan range increases in continuously operating instruments, while it remains practically unchanged in the case of ion traps. Conversely, an ion trap mass spectrometer permits the recording of complete mass spectra in concentration ranges in which quadrupole and also sector field instruments already have to operate in the selected ion monitoring mode (SIM) (0.01 to 0.1 mg/L). This aspect is particularly advantageous when the component under investigation is present in the sample only in very small concentrations and an unam-

biguous identification is required. Moreover, ion trap mass spectrometry also permits investigation of unknown samples, i.e. screening of samples on the basis of complete mass spectra. Thus substances that were not originally sought can be detected in the trace range. This is not possible in the case of the SIM technique, which operates on the principle of the selection of previously known substance-characteristic ions.
However, ion trap mass spectrometry also has disadvantages compared with the quadrupole and sector field techniques:

– the typical mass resolution of ion traps is between 500 and 1000. Thus more exact analysis, e.g. of isotope distribution, usually proves difficult.
– like the correct selection of the sample concentration, optimisation of the duration of sample enrichment in the ion trap and ionisation depend to a large extent on the experience of the analyst. The theory and practice of ion trap mass spectrometry are more complicated than in the case of the quadrupole technique, especially in combination with chemical ionisation or tandem mass spectrometry (see below).
– ion traps show poorer repeatability than quadrupoles. Whereas the repeatability in quadrupole or sector field analysis is less than 2% (multiple injection of one sample), it can be up to 10% in ion trap MS, depending on the consistency of the sample. This imprecision is caused by the complex processes in the ion trap and the complicated pressure and flow control technique. One possible way of compensating for these effects is to use isotope-labelled compounds as internal standards: provided they have the same chemical structure, these analytes behave exactly like the unlabelled target compounds in the mass spectrometer. Fluctuations in the signal intensity can be compensated mathematically. However, a prerequisite of this method is the availability of isotope-labelled compounds.

4.4.4 Tandem mass spectrometry (MS/MS)

As described above, ion trap mass spectrometry permits very efficient suppression of matrix constituents or interfering components. However, the sensitivity of this procedure is not considerably better than that of quadrupole MS in the SIM-mode, while the reproducibility is rather poorer. A considerable improvement in the signal/background ratio and thus in sensitivity can be achieved by means of tandem mass spectrometry (tandem MS, MS/MS). Moreover, this technique is being increasingly used as a detector in conjunction with high performance liquid chromatography (HPLC).
On principle, quadrupole mass spectrometers, sector field instruments and ion traps are all suitable for tandem MS. In the first case described above the individual steps of the mass spectrometric analysis take place sequentially at different sites in the mass spectrometer (separation of the parent ions in the first quadrupole, absorption of impact energy in the collision cell, separation of the daughter ions in the second quadrupole or in the magnetic sector field). This technique is therefore known as “tandem in space”. In ion trap instruments the processes take place in chronological order at the same site, i.e. in the trap. Accordingly, this technique is called “tandem in time” (Fig. 13).

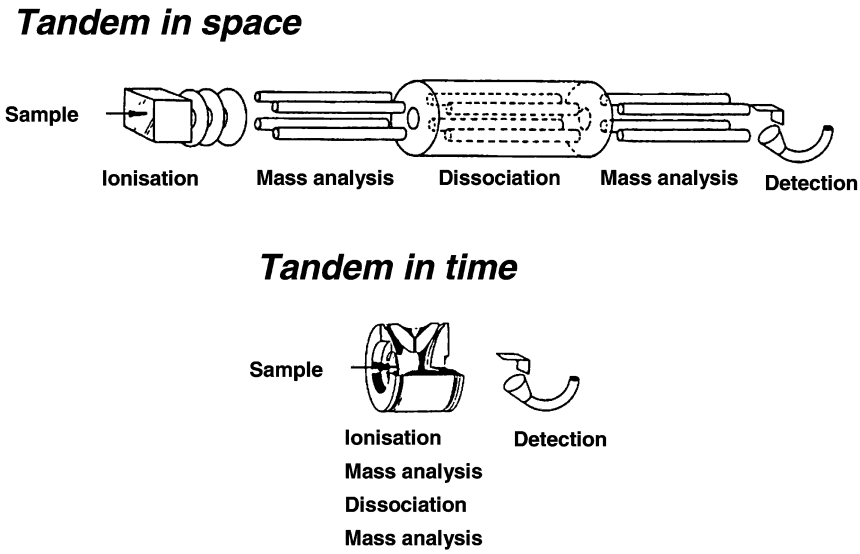

Fig. 13. Principle of tandem mass spectrometry with sequentially arranged quadrupoles ("tandem in space") and ion traps ("tandem in time")

In tandem mass spectrometry with quadrupoles the sample molecules transported by the carrier gas into the ion source are initially ionised and accelerated into the first mass filter. There one ion with a specific m/z ratio or a certain m/z range is separated as already described for the SIM or scan technique. However, the selected ions are not detected after leaving the quadrupole, but enter a second quadrupole, in which they are induced to further fragment due to high energy impact with an inert gas (reduction of the mean free path due to increase in pressure).
This process is normally known as "collision-induced dissociation" (CID) or "collision-activated decomposition" (CAD) and generates "daughter ions". Daughter ions are formed from molecule ion fragments that are called parent ions or precursor ions in this context. The spectrum of the daughter ions is recorded in the second mass filter (scan mode). Alternatively, the analysis can also be restricted to individual, characteristic daughter ions (selected ion monitoring mode). Tandem mass spectrometry therefore enables the separation of undesirable components as part of a mass selective clean-up in the first step and allows selection of substance-specific fragments or mass ranges in the second step.
The sequential arrangement of two quadrupoles for separation according to the m/z ratio permits free variation of the scan and SIM techniques:

– A combination of the first quadrupole running in the SIM mode and the second quadrupole in the scan mode enables clarification or confirmation of the structure of a compound present in the trace concentration range in a complex matrix. A parent ion (e.g. a molecule ion or a characteristic fragment) is initially isolated, then further fragmentation is induced by collision, leading to the formation of

daughter ions. The scan of these reaction products (daughter ion scan) generally yields substance-specific structural information.
- The SIM-SIM combination permits detection of substance-specific daughter ions with the highest possible intensity, i.e. it is especially suitable for highly specific and highly sensitive detection. In trace analysis it is normally used to detect an individual daughter ion (selected reaction monitoring, SRM) or a few fragments (multiple reaction monitoring, MRM).
- Only certain daughter ions that have been cleaved from a parent ion with a similar structure (parent ion scan) are recorded using the scan-SIM combination. Thus substance groups with certain characteristic structures or fragmentation reactions can be identified.
- The scan-scan combination is used for a "neutral loss scan", i.e. it can be used to identify all the parent ions from which neutral fragments of a certain mass are lost. For this purpose both quadrupoles are operated in the scan mode, but the mass range is shifted to correspond with the neutral fragment, e.g. a McLafferty product. The detector then displays only daughter ions whose parent ion shows a loss of the relevant neutral fragment.

In general, a "gentle" initial ionisation, e.g. by means of PCI or NCI, is preferable in tandem mass spectrometry. The intensive fragmentation that ions undergo as a result of electron impact ionisation leads to a low intensity of the individual ions. As interfering components are mainly separated by the first quadrupole, a lower fragmentation rate with higher ion intensities is more favourable. The collision cell in continuously operating tandem mass spectrometers is frequently composed of a quadrupole or octopole rod system in which only one alternating electrical voltage is applied to focus the ion beam. For this reason these instruments are also known as "triple quadrupole" systems. As a rule, a noble gas (helium, argon, xenon) or nitrogen with a pressure of about 10^{-4} mbar is used as the reactant gas for the collision. The energies transferred during collision are in the range of 50 to 100 eV.

In ion traps the ion selection processes and the collisions within the trap are staggered in time. After the primary ionisation, the ions with masses higher and lower than those desired are then removed from the source by appropriate adjustment of the voltage. An alternating voltage that is in resonance with the circulation frequency of the ions is subsequently applied between the end caps. The ions are kinetically excited in this manner and fragment when they collide with the carrier gas atoms (usually helium). The resulting daughter ions are retained in the trap and can be subsequently detected in the scan or SIM mode.

For quadrupole and sector field instruments as well as ion traps the yield of the CID process is of key importance. While the impact energy and the pressure have to be exactly optimised in the continuously operating instruments to avoid e.g. dispersion effects at excessive pressures, the duration and the strength of the resonance voltage must be optimised in the ion trap.

Compared with the triple quadrupole instrument, the ion trap technique exhibits a higher fragmentation yield and a better daughter ion transmission to the detector. Furthermore, complete mass spectra can be recorded, as in the conventional ion trap

technique, even in the trace range, while triple quadrupole instruments must already be operated in the SIM mode for comparable sensitivity. The accessibility of a broader mass range and the possibility of performing the parent ion and neutral loss scans are the most important advantages of the triple quadrupole instruments. In addition, as already mentioned when the conventional technique was described, the reproducibility of the tandem technique is considerably better in the case of quadrupole and sector field instruments. However, these shortcomings of the ion trap technique can be partly compensated by isotope-labelled standards, and their use is particularly recommended in this case.

Tandem MS instruments have rarely been used for detection in routine analysis of xenobiotics in biological material. Therefore no example is yet to be found in the Deutsche Forschungsgemeinschaft's collection of methods in this series. The first application of the MS/MS technique was a procedure to assay polycyclic musk compounds (PMC) in blood completed in 2003 [82].

This example demonstrates the superior signal/background ratio of tandem MS compared with single quadrupole mass spectrometry when used for the analysis of samples from complex matrices (Fig. 14). The signal/background ratio increases from 217 to 1048, thus the sensitivity is enhanced by a factor of five.

This method also shows another important advantage of tandem MS over the conventional quadrupole technique, the possibility of recording complete fragment mass spectra, even close to the detection limit (Fig. 15).

To date ion trap tandem MS has not been used for certain applications, e.g. chlorinated compounds, because only slight further fragmentation of the parent ions was possible. However, the ion trap technique can be distinctly superior to the quadrupole technique in the case of readily fragmentable analytes such as the polycyclic musk compounds. If the polycyclic musk compounds are enriched by a factor of 50 during processing, detection limits of 0.1 µg/L can be achieved using the ion trap technique in the MS/MS mode. This is only a fifth of the detection limit achieved by MS/MS detection with a triple quadrupole mass spectrometer and with the same sample preparation (Figs. 16, 17).

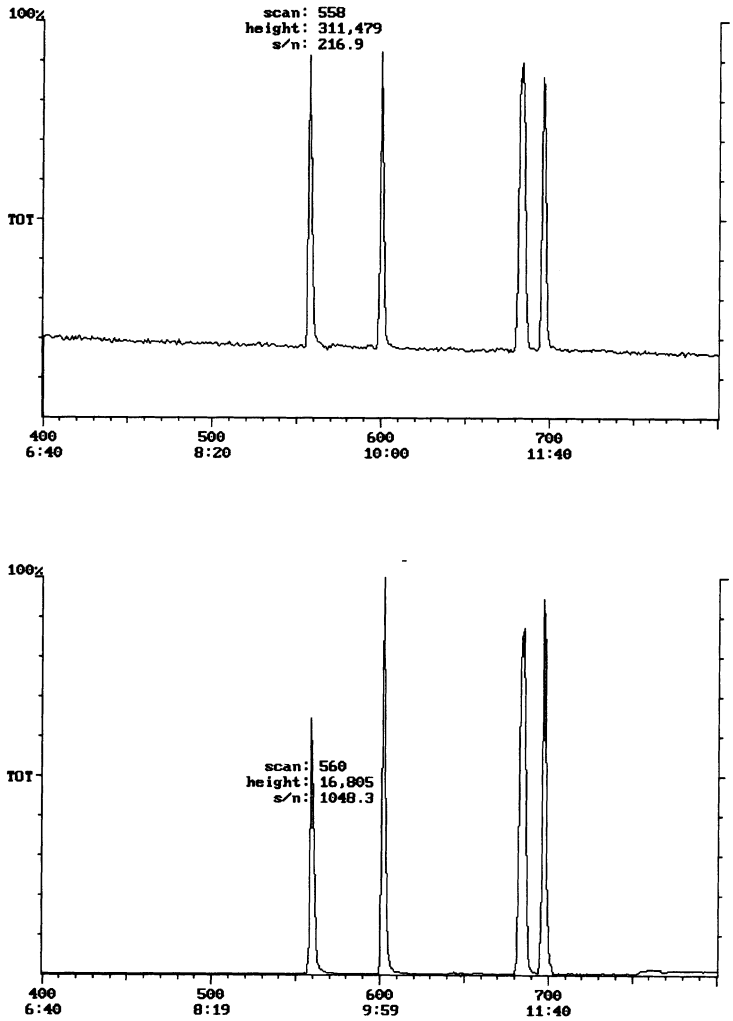

Fig. 14. Total ion chromatogram in the MS mode (above) and total ion chromatogram of the daughter ions of selected parent ions in the MS/MS mode (below) of a mixture of the polycyclic musk compounds ADBI (4-acetyl-1,1-dimethyl-6-*tert*.-butyldihydroindene), AHDI (6-acetyl-1,1,2,3,3,5-hexamethyldihydroindene), HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyrane) and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene) (in the order of elution, concentration: 1 mg/L (equivalent to 20 µg/L in blood)), detected by an ion trap mass spectrometer (Varian Saturn 3)

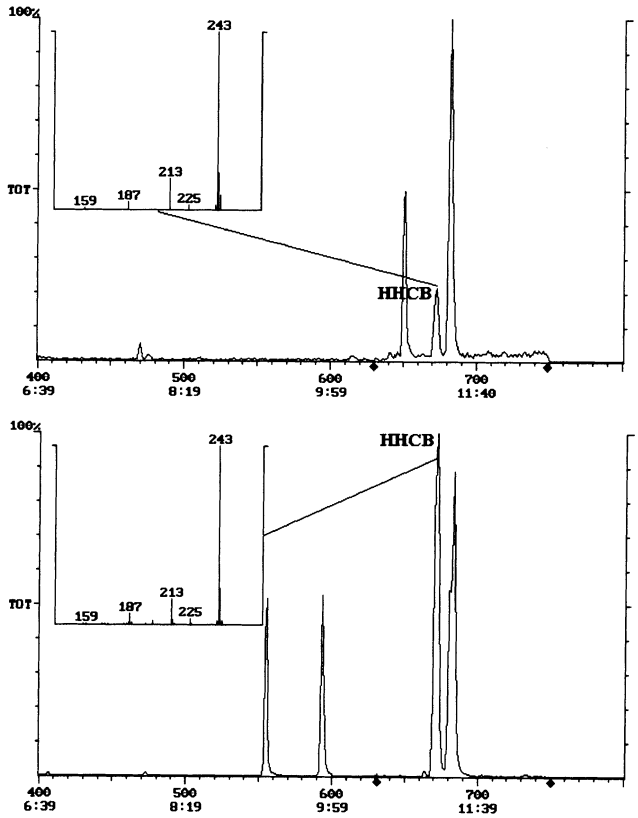

Fig. 15. Daughter ion chromatograms and mass spectra after MS/MS (above: HHCB spectrum of a processed blood sample (0.12 µg/L), below: HHCB spectrum of a calibration standard (4 µg/L)

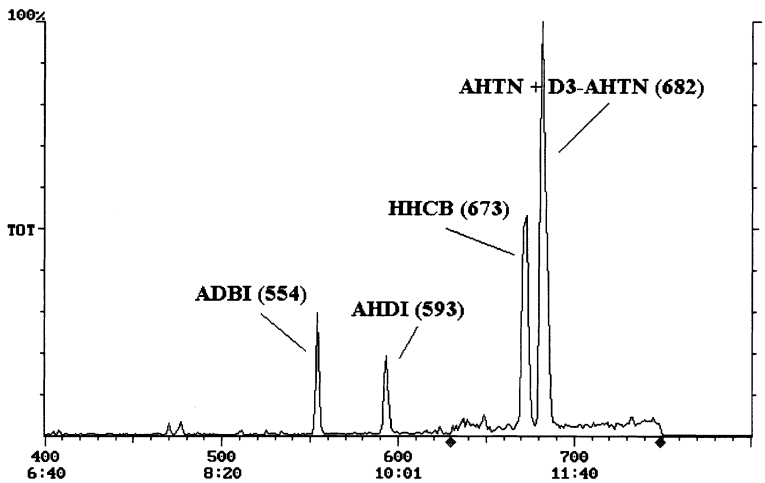

Fig. 16. Daughter ion chromatogram (full scan) following ion trap MS/MS of a blood sample spiked with 1 µg/L each of AHDI, HHCB and AHTN (D_3-AHTN: internal standard: 4 µg/L) (fragmentation on the y-axis)

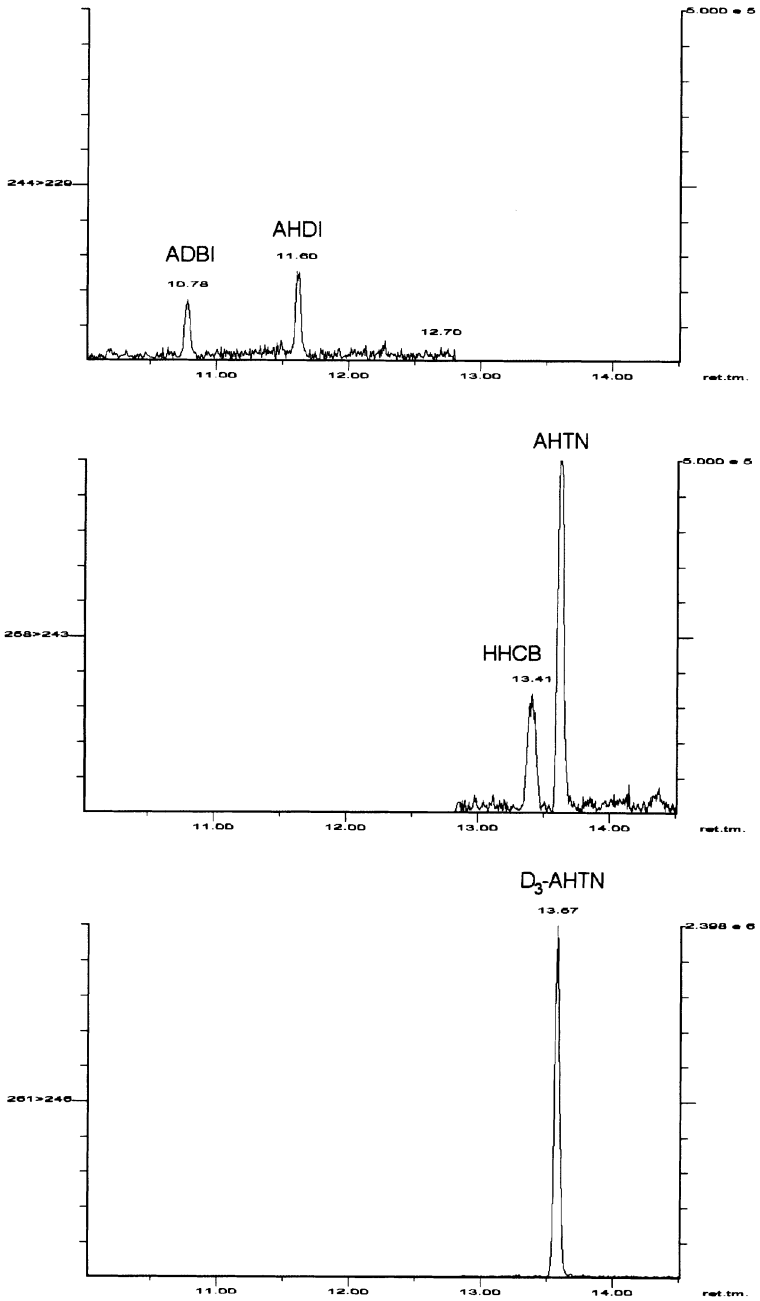

Fig. 17. Daughter ion chromatograms (full scan) following triple quadrupole MS/MS of blood sample spiked with 5 µg/L each of ADBI, AHDI, HHCB and AHTN (D_3-AHTN: internal standard: 5 µg/L) (fragmentation on the y-axis)

4.5 Detectors

Ions generated in the ion source and selected in the mass filter are normally detected by a secondary electron multiplier (SEM) or a photon multiplier.

All electron multipliers function according to the same principle (Fig. 18). A beam of positive ions that hits an impact plate coated with copper/beryllium oxide (conversion dynode) causes the release of "primary electrons". These electrons are accelerated by an electrical potential (1 to 3 kV) towards a further dynode and release further electrons ("secondary electrons"). Dynodes (about 10 to 15 dynodes) arranged in sequence generate an electron cascade that is converted as an electrical potential by an amplifier to a measurable signal (Fig. 18 a). The amplification achieved by an electron multiplier is in the magnitude of 10^4 to 10^7 secondary electrons per primary electron. In the case of a photon multiplier the electrons pass a plate containing phosphorus that emits photons in the direction of a photocathode. The release of electrons from the alkaline metal coating there also results in a measurable current as a signal.

So-called "channeltrons" are shaped like a horn with the larger aperture pointing towards the mass filter (Fig. 18 b). An insulating coating of lead oxide enables a large potential difference to be applied between both ends of the multiplier. In this case the electron cascade travels continuously from the entrance of the channeltron to the amplifier. This design is more compact and cheaper than the electron multiplier with individual dynodes and is therefore very frequently used.

Changes in the multiplier voltage influence the measurement signal to a large extent: a reduction of 200 volts can cause the intensity to diminish by 90%. This is particu-

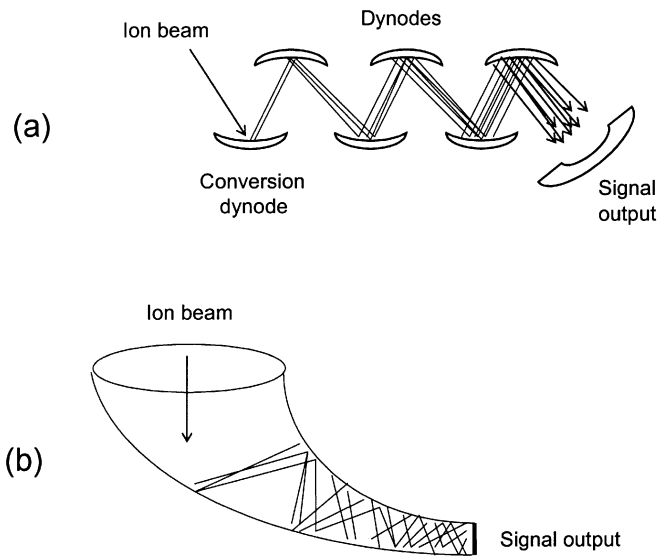

Fig. 18. Principle of the electron multiplier (a = dynodes, b = channeltron)

larly interesting if the samples to be investigated have relatively high unavoidable concentrations that would lead to overloading of the multiplier or to a signal outside the linear range (about 5 to 6 orders of magnitude). On the other hand an increase in the multiplier voltage in order to enhance the measurement signal or to improve the detection limit is advisable only if the background noise is low or can be further reduced by the selection of the general mass spectrometric conditions (suppression of the background signal, ion selection, measurement time per ion, etc.); otherwise amplifying the signals also increases the background noise.
Depending on the frequency of use, the operational life of an SEM is about 1 to 3 years. As electron multipliers have a finite number of secondary electrons that can be released, the number decreases with each cascade. The state of the multiplier can be relatively easily assessed by the voltage that has to be set by the GC/MS system during autotuning to reach the standard specifications. Normally the electron multiplier voltage in a new instrument is about 1000 to 1200 volts. This voltage rises to a maximum of 3000 volts with increasing use of the electron multiplier. In this case the multiplier must be replaced. However, a rise in the voltage can also be caused by a contaminated ion source that gives a poor ionisation yield. In this case the electron multiplier adjusts the voltage to a high value that amplifies even small ion beams to a signal of a previously set intensity. If the voltage is readjusted to the range below 1500 volts after the ion source has been cleaned, the multiplier is still functional. If the voltage remains high despite optimal maintenance of the ion source, the electron multiplier must be replaced. Wherever possible, only "clean" samples with low concentrations should be injected in order to prolong the useful life of a multiplier. In addition, data recording can be limited to the retention time range of interest in the gas chromatogram. Most GC/MS systems permit the multiplier to be switched off before and after this time interval.

5 Summary

Gas chromatography in combination with mass spectrometry is one of the key detection techniques used in occupational and environmental medicine. Lower exposure to xenobiotic substances at the workplace and increasing interest in exposure from the environment have fuelled the need for both sensitive and also substance-specific methods for biological monitoring in concentrations as low as the ppt range.
On the one hand, GC/MS meets these requirements with its broad dynamic working range of several orders of magnitude and its high reproducibility (detector stability, ionisation yield, fragmentation pattern). On the other, the mass spectrometer provides considerably more information on the chemical structure of the relevant analytes than other detectors normally used with gas chromatography. Complete mass spectra, even at low concentrations in the ppm range, can be recorded by suitable selection of the instrumental parameters, thus enabling clear identification of substances. As a rule, the detection limit of a method can be improved by up to two orders of magnitude by limiting the analysis to a few specific molecule or fragment ions in the selected ion monitoring mode, which usually poses no problems. At present four mass

spectrometric techniques are mainly used in occupational and environmental medicine: single quadrupole MS, sector field MS, triple quadrupole MS ("tandem in space") and ion trap MS ("tandem in time)". From a technical point of view the difference between these mass spectrometers lies above all in their resolution power and their sensitivity. Both properties are especially important in the analysis of isomer mixtures (e.g. polychlorinated biphenyls, dioxins and furans) and in very low concentration ranges, in which analytical detection is limited by interfering components of the matrix or other analytes present in excess.

Sector field mass spectrometry is remarkable for its very high resolution, while the main advantage of ion traps lies in their ability to record complete mass spectra, even in the trace range. Triple quadrupole instruments permit a very efficient suppression of undesirable signals or matrix components, and their robustness is comparable with that of quadrupole instruments.

Quadrupole GC/MS, which is regarded as a standard technique on account of its current widespread application, has reached its limits in the above-mentioned cases. However, quadrupole instruments are suitable for most investigations in occupational and environmental medicine on account of their considerably simpler instrumental technology, their high ionisation and mass filter stability, and the possibility of "classical" spectra interpretation. This is especially true as the detection power and selectivity can be further enhanced by the use of chemical ionisation. The CI mode permits a "gentle" ionisation of sample molecules, so fragmentation reactions can be suppressed to a large extent. In the case of analysis of compounds containing halogens or aromatic substances, sensitivity can be improved by detecting negatively charged ions to include the range accessible to an electron capture detector.

The selection of the optimum mass spectrometer for an analysis in occupational or environmental medicine principally depends on the particular application: the methods already published in this series can and should provide important pointers to the development of further biological monitoring parameters on the basis of GC/MS.

6 References

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