2
Fundamentals

2.1
Sample Preparation

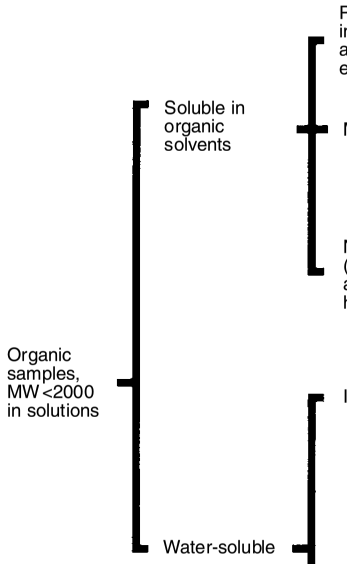
The preparation of analysis samples is today already an integral part of practical GC/MS analysis. The current trend is clearly directed to automated instrumental techniques and limits manual work to the essential. The concentration processes in this development are of particular importance for coupling with capillary GC/MS, as in trace analysis the limited sample capacity of capillary columns must be compensated for. It is therefore necessary both that overloading of the stationary phase by the matrix is avoided and that the limits of mass spectrometric detection are taken into consideration. To optimise separation on a capillary column, strongly interfering components of the matrix must be removed before applying an extract. The primarily universal character of the mass spectrometer poses conditions on the preparation of a sample which are to some extent more demanding than those of an element-specific detector, such as ECD or NPD unless highly selective techniques as MS/MS or high resolution accurate measurements are applied. The clean-up and analyte concentration, which forms part of sample preparation, must therefore in principle always be regarded as a necessary preparative step for GC/MS analysis. The differences in the concentration ranges between various samples, differences between the volatility of the analytes and that of the matrix and the varying chemical nature of the substances are important for the choice of a suitable sample preparation procedure.

Off-line techniques (as opposed to on-line coupling or hyphenated techniques) have the particular advantage that samples can be processed in parallel and the extracts can be subjected to other analytical processes besides GC/MS. On-line techniques have the special advantage of sequential processing of the samples without intermediate manual steps. The on-line clean-up allows an optimal time overlap which gives the sample preparation the same amount of time as the analysis of the preceding sample. This permits maximum use of the instrument and automatic operation.

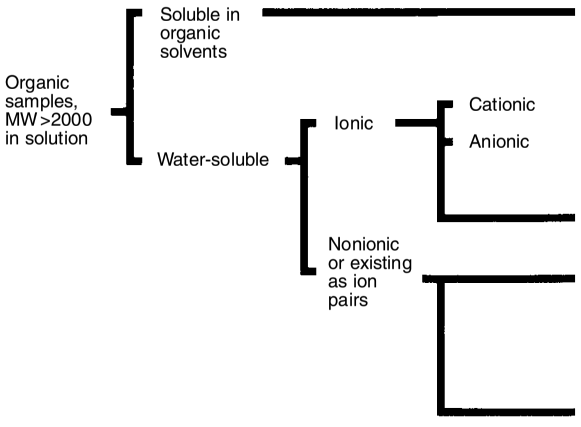
On-line processes generally offer potential for higher analytical quality through lower contamination from the laboratory environment and, for smaller sample sizes, lower detection limits with lower material losses. Frequently total sample transfer is possible without taking aliquots or diluting. Volatility differences between the sample and the matrix allow, for example, the use of extraction techniques such as the static or dynamic (purge and trap) headspace techniques as typical GC/MS coupling techniques. These are already used as on-line techniques in many laboratories. Where the volatility of the analytes is insufficient, other

Handbook of GC/MS: Fundamentals and Applications, Second Edition. Hans-Joachim Hübschmann

ISBN: 978-3-527-31427-0



Separating mechanism[1]	Column[2]	Eluent[3) 4)]
NPC	Cyano (CN) Diol (COHCOH) Amino (NH_2) 1°,2°-amino (NH_2NH)	Hexane Chloroform Dichloromethane Acetone Methanol
LSC	Kieselgur (SiOH) Silica gel (SiOH) Florisil® (SiO_n) Aluminium oxide (Al_2O_3)	Hexane Chloroform Dichloromethane Ethyl acetate
RPC	Octadecyl (C_{18}) Octyl (C_8) Cyclohexyl (CH) Phenyl (C_6H_5) Cyano (CN)	Hexane Dichloromethane Acetone Acetonitrile Methanol Water
IEC	Cyano (CN) Carboxylic acid (COOH) Benzenesulfonic acid ($C_6H_5SO_3H$) Amino (NH_2) 1°,2°-amino (NH_2NH) Quaternary ammonium (N^+)	Acids Bases Buffers
NPC	Cyano (CN) Diol (COHCOH) Amino (NH_2) 1°,2°-amino (NH_2NH)	Hexane Chloroform Dichloromethane Acetone Methanol
LSC	Kieselgur (SiOH) Silica gel (SiOH) Florisil® (SiO_n) Aluminium oxide (Al_2O_3)	Hexane Chloroform Dichloromethane Ethyl acetate Methanol
RPC	Octadecyl (C_{18}) Octyl (C_8) Cyclohexyl (CH) Phenyl (C_6H_5) Cyano (CN)	Hexane Dichloromethane Acetone Acetonitrile Methanol Water



Soluble in organic solvents	RPC	Butyl (C_4) Wide pore 250 Å	Hexane Dichloromethane Acetone Acetonitrile Methanol Water
Water-soluble / Ionic / Cationic	IEC	Carboxylic acid (COOH) Wide pore 250 Å PEI (NH) Wide pore 250 Å	Aqueous buffer
Water-soluble / Ionic / Anionic	SEC	Sephadex® G-25	Aqueous buffer
Nonionic or existing as ion pairs	RPC	Butyl (C_4) Wide pore 250 Å	Hexane Dichloromethane Acetone Acetonitrile Methanol Water
Nonionic or existing as ion pairs	SEC	Sephadex® G-25	Aqueous buffer
Trace metals in solution	IEC	Carboxylic acid (COOH)	Low pH Aqueous 1–8 N HCl
		Benzenesulfonic acid ($C_6H_5SO_3H$) Amino (NH_2) Quaternary ammonium (N^+)	Strongly chelating (thiourea)
Metal chelates	RPC	Octadecyl (C_{18}) Octyl (C_8) Cyclohexyl (CH) Phenyl (C_6H_5) Cyano (CN)	Hexane Dichloromethane Acetone Acetonitrile Methanol Water

[1] Separating mechanisms
LSC = liquid solid chromatography (adsorption)
NPC = normal phase chromatography (bonded phase separation)
RPC = reverse phase chromatography (bonded phase separation)
IEC = ion exchange chromatography (bonded phase ion exchange)
SEC = size exclusion chromatography

[2] The columns are listed in order of increasing polarity
[3] The eluents are listed in order of increasing polarity
[4] Selective elution can be carried out by mixing two or more solvents to achieve different degrees of polarity

Fig. 2.1 Key to choosing SPE columns and eluents. The choice of the SPE phase depends on the molecular solubility of the sample in a particular medium and on its polarity. The sample matrix is not considered (J. T. Baker).

extraction procedures e. g. thermal extraction, pyrolysis or online SPE techniques are being increasingly used on-line. Solid phase extraction in the form of microextraction, LC/GC coupling, or extraction with supercritical fluids show high analytical potential here.

2.1.1 Solid Phase Extraction

From the middle of the 1980s solid phase extraction (SPE) began to revolutionise the enrichment, extraction and clean-up of analytical samples. Following the motto 'The separating funnel is a museum piece', the time-consuming and arduous liquid/liquid extraction has increasingly been displaced from the analytical laboratory. Today the euphoria of the rapid and simple preparation with disposable columns has lessened as a result of a realistic consideration of their performance levels and limitations. A particular advantage over the classical liquid/liquid partition is the low consumption of expensive and sometimes harmful solvents. The amount of apparatus and space required is low for SPE. Parallel processing of several samples is therefore quite possible. Besides an efficient clean-up, the necessary concentration of the analyte frequently required for GC/MS is achieved by solid phase extraction.

In solid phase extraction strong retention of the analyte is required, which prevents migration through the carrier bed during sample application and washing. Specific interactions between the substances being analysed and the chosen adsorption material are exploited to achieve retention of the analytes and removal of the matrix. An extract which is ready for analysis is obtained by changing the eluents. The extract can then be used directly for GC and GC/MS in most cases. The choice of column materials permits the exploitation of the separating mechanisms of adsorption chromatography, normal-phase and reversed-phase chromatography, and also ion exchange and size exclusion chromatography (Fig. 2.1).

The physical extraction process, which takes place between the liquid phase (the liquid sample containing the dissolved analytes) and the solid phase (the adsorption material) is common to all solid phase extractions. The analytes are usually extracted successfully because the interactions between them and the solid phase are stronger than those with the solvent or the matrix components. After the sample solution has been applied to the solid phase bed, the analytes become enriched on the surface of the SPE material. All other sample components pass unhindered through the bed and can be washed out. The maximum sample volume that can be applied is limited by the breakthrough volume of the analyte. Elution is achieved by changing the solvent. For this there must be a stronger interaction between the elution solvent and the analyte than between the latter and the solid phase. The elution volume should be as small as possible to prevent subsequent solvent evaporation.

In analytical practice two solid phase extraction processes have become established. Cartridges are mostly preferred for liquid samples (Figs. 2.2 and 2.3). If the GC/MS analysis reveals high contents of plasticisers, the plastic material of the packed columns must first be considered and in special cases a change to glass columns must be made. For sample preparation using slurries or turbid water, which rapidly lead to deposits on the packed columns, SPE disks should be used. Their use is similar to that of cartridges. Additional contamination, e. g. by plasticisers, can be ruled out for residue analysis in this case (Fig. 2.4).

A large number of different interactions are exploited for solid phase extraction (Fig. 2.2). Selective extractions can be achieved by a suitable choice of adsorption materials. If the eluate is used for GC/MS the detection characteristics of the mass spectrometer in particular

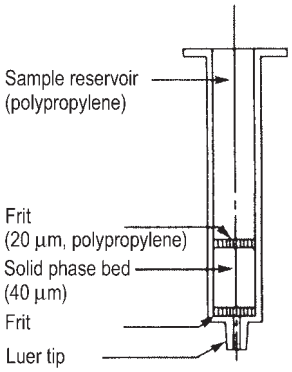


Fig. 2.2 Construction of a packed column for solid phase extraction (J. T. Baker).

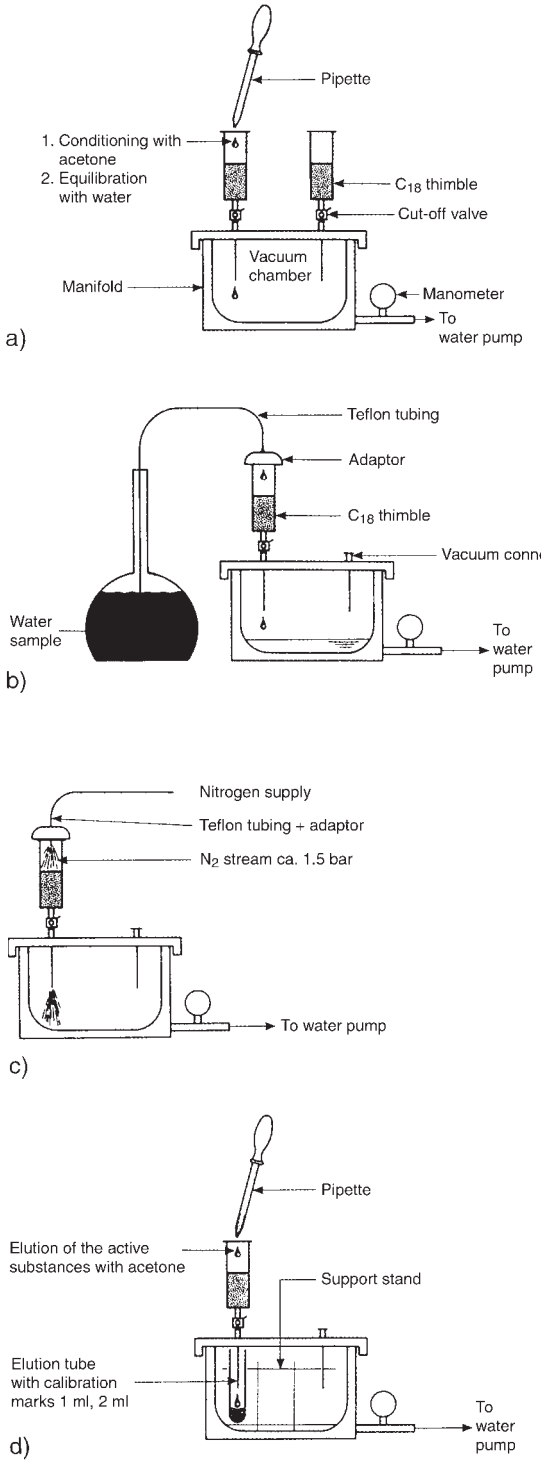


Fig. 2.3 Enrichment of a water sample on solid phase cartridges with C_{18}-material (Hein/Kunze 1994):
(a) conditioning,
(b) loading (extraction)/washing,
(c) drying,
(d) elution of active substances.

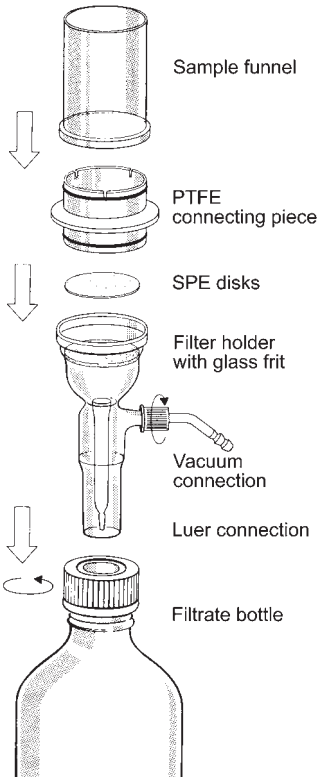


Fig. 2.4 Apparatus for solid phase extraction with SPE disks (J. T. Baker).

must be taken into account. Unlike an electron capture detector (ECD), which is still widely used as a selective detector for substances with a high halogen content in environmental analysis, a GC/MS system can be used to detect a wide range of nonhalogenated substances. The use of a selected ion monitoring technique (SIM, MID) therefore requires better purification of the extracts obtained by SPE. In the case of the processing of dioxins and PCBs from waste oil, for example, a silica gel column charged with sulfuric acid is also necessary. Extensive oxidation of the nonspecific hydrocarbon matrix is thus achieved. The quality and reproducibility of SPE depends on criteria comparable to those which apply to column materials used in HPLC.

2.1.1.1 **Solid Phase Microextraction**

The solvent-free extraction technique *solid phase microextraction* (SPME) recently developed by Prof. J. Pawliszyn (University of Waterloo, Ontario, Canada) is an important step towards the instrumentation and automation of the solid phase extraction technique for on-line sample preparation and introduction to GC/MS. It involves exposing a fused silica fibre coated with a liquid polymeric material to a sample containing the analyte. The typical dimensions of a fibre are 1 cm × 100 μm. The analyte diffuses from gaseous or liquid samples into the fibre surface and partitions into the coating according to the first partition coefficient. The

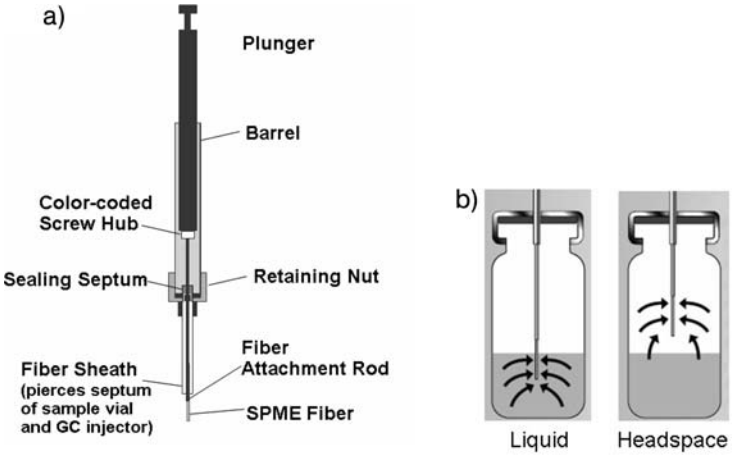


Fig. 2.5 (a) SPME plunger for autosampler use; (b) SPME principle for liquid and headspace application.

agitated sample can be heated during the sampling process if desired to achieve maximum recovery and precision for quantitative assays. After equilibrium is established, the fibre with the analyte is withdrawn from the sample and transferred into a GC injector system manually or via an autosampler. The analyte is desorbed thermally from the coating.

SPME offers several advantages for sample preparation including reduced time per sample, less manual sample manipulation resulting in an increased sample throughput and, in addition, the elimination of organic solvents and reduced analyte loss.

An SPME unit consists of a length of fused silica fibre coated with a phase similar to those used in chromatography columns. The phase can be mixed with solid adsorbents, e.g. divinylbenzene polymers, templated resins or porous carbons. The fibre is attached to a stainless steel plunger in a protective holder used manually or in a specially prepared autosampler. The plunger on the syringe retracts the fibre for storage and piercing septa, and exposes the fibre for extraction and desorption of the sample. A spring in the assembly keeps the fibre retracted, reducing the chance of it being damaged (Fig. 2.5 a and b).

The design of a portable, disposable SPME holder with a sealing mechanism gives flexibility and ease of use for on-site sampling. The sample can be extracted and stored by placing the tip of the fibre needle in a septum. The lightweight disposable holders can be used for the lifetime of the fibre. SPME could also be used as an indoor air sampling device for GC and GC/MS analysis. For this technique to be successful, the sample must be stable to storage.

A major shortcoming of SPME is the lack of fibres that are polar enough to extract very polar or ionic species from aqueous solutions without first changing the nature of the species through prior derivatisation. Ionic, polar and involatile species have to be derivatised to GC-amenable species before SPME extraction.

Since the introduction of SPME in 1993, a variety of applications have been established, including the analysis of volatile analytes and gases consisting of small molecules. However, SPME was limited in its ability to retain and concentrate these small molecules in the fibre coating. Equilibria were obtained rapidly and distribution constants were low, which resulted in high minimum detection limits. The thickness of the phase is important in capturing

small molecules, but a stronger adsorbing mechanism is also needed. The ability to coat porous carbons on a fibre has enabled SPME to be used for the analysis of small molecules at trace levels.

The amount of analyte extracted is dependent upon the distribution constant. The higher the distribution constant, the higher is the quantity of analyte extracted. Generally a thicker film is required to retain small molecules and a thinner film is used for larger molecules with high distribution constants. The polarity of the fibre and the type of coating can also increase the distribution constant (Table 2.1).

Table 2.1 Types of SPME fibres currently available.

Nonpolar fibres

- Cellulose acetate/polyvinylchloride (PVC), alkane selective (Farajzadeh 2003)
- Powdered activated carbon (PAC), for BTEX and halocarbons (Shutao 2006)
- Polydimethylsiloxane (PDMS), 7, 30, 100 μm coating

Bipolar fibres

- Carboxen/Polydimethylsiloxane(CAR/PDMS), 75, 85 μm coating
- Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), 30, 50 μm coating
- Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), 30, 50 μm coating
- Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), 65 μm coating

Polar fibres

- Carbowax/Divinylbenzene (CW/DVB), 70 μm coating
- Carbowax/Templated Resin (CW/TPR), 50 μm coating
- Polyacrylate (PA), 85 μm coating

Blended fibre coatings contain porous material, such as divinylbenzene (DVB) and Carboxen, blended in either PDMS or Carbowax.

Table 2.2 Summary of method performance for several analysis examples

Parameter	Formaldehyde	Triton-X-100	Phenylurea pesticides	Amphetamines
LOD	2–40 ppb	1.57 μg/L	< 5 μg/L	1.5 ng/mL
Precision	2–7%	2–15%	1.6–5.6%	9–15%
Linear range	50–3250 ppb	0.1–100 mg/L	10–10 000 μg/L	5–2000 ng/mL
Linearity (r_2)	0.9995	0.990	0.990	0.998
Sampling time	10–300 s	50 min	8 min	15 min

The principle of solid phase micro extraction recently found its extension in the stir bar sorptive extraction (SBSE). The extraction is performed with a glass coated magnetic stir bar which is coated with polydiemthylsiloxane (PDMS). The difference to SPME is the capacity of the sorptive PDMS phase. While in the SPME fibre a volume of about 15 μL is used, with SBSE a significantly enlarged volume of up to 125 μL is available. The larger volume of sorption phase provides a better phase ratio with increased recovery resulting in

up to 250 fold lowered detection limits. Many samples can be extracted at the same time, typically extraction times run up to 60 min. Transfer to the GC is achieved by thermal desorption of the stir bar in a suitable injection system. Thermolabile compounds can be alternatively dissolved by liquids (SBSE/LD). The excellent sensitivity and reproducibility has been demonstrated in the multi-residue detection of endocrine disrupting chemicals in drinking water (Serodio 2004).

2.1.2 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) has the potential of replacing conventional methods of sample preparation involving liquid/liquid and liquid/solid extractions (the current soxhlet extraction steps) in many areas of application. The soxhlet extraction (Fig. 2.6) has, up to now, been the process of choice for extracting involatile organic compounds from solid matrices, such as soil, sewage sludge or other materials.

The prerequisite for the soxhlet extraction with organic solvents is a sample which is as anhydrous as possible. Time-consuming drying of the sample (freeze drying) is therefore necessary. The time required for a soxhlet extraction is, however, ca. 15–30 h for many samples in environmental analysis. This leads to the running of many extraction columns in parallel. The requirement for pure solvents (several hundred ml) is therefore high.

While the development of chromatographic separation methods has made enormous advances in recent years, equivalent developments in sample preparation, which keep up with

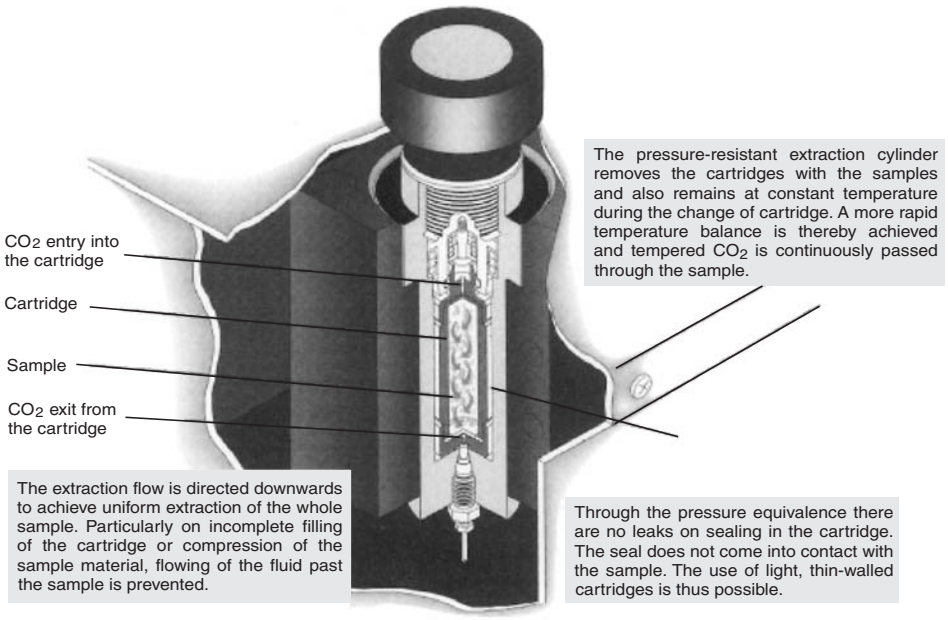


Fig. 2.6 SFE extraction system employing the principle of pressure equivalence (the cartridge with the sample is in a pressure-resistant cylinder, the internal and external pressures on the cartridge are kept equal, ISCO).

the possibilities of modern GC and GC/MS systems, have not been made. The technical process for extraction of natural products (e. g. plants/perfume oils, coffee/caffeine) with supercritical carbon dioxide has already been used for a long time and is particularly well known in the area of perfumery for its pure and high-value extracts.

Only in recent the years has the process achieved increased importance for instrumental analysis. SFE is a rapid and economical extraction process with high percentage recovery which gives sample extracts which can be used for residue analysis usually without further concentration or clean-up. SFE works within the cycle time of typical GC and GC/MS analyses, can be automated, and avoids the production of waste solvent in the laboratory. The EPA (US Environmental Protection Agency) is working on the replacement of extraction processes with dichloromethane and freons used up till now, with particular emphasis on environmental analysis. The first EPA method published was the determination of the total hydrocarbon content of soil using SFE sample preparation (EPA # 3560). The EPA processes for the determination of polyaromatic hydrocarbons (EPA # 3561) and of organochlorine pesticides and PCBs (EPA # 3562) using SFE extraction and detection with GC/MS systems soon followed. In the fundamental work of Lehotay and Eller on SFE pesticide extraction, carbamates, triazines, phosphoric acid esters and pyrethroids were all analysed using a GC/MS multimethod.

Supercritical fluids are extraction agents which are above their critical pressures and temperatures during the extraction phase. The fluids used for SFE (usually carbon dioxide) provide particularly favourable conditions for extraction (Fig. 2.7). The particular properties of a supercritical fluid arise from a combination of gaseous sample penetration, liquid-like solubilising capacity and substance transport (Table 2.3). The solubilising capacity of supercritical fluids reaches that of the liquid solvents when their density is raised. The maximum solubility of an organic compound can, nevertheless, be higher in a liquid solvent than in a supercritical phase. The solubility plays an important role in the efficiency of a technical process. However, in residue analysis this parameter is of no practical importance because of the extremely low concentration of the analytes.

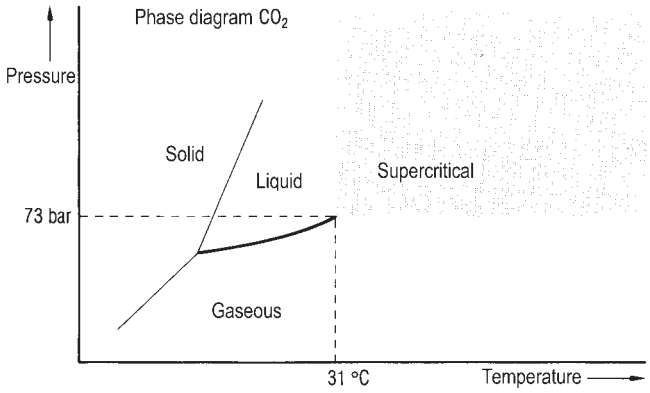


Fig. 2.7 Phase diagram.

Physical data for CO_2:		Critical data:	
Mp	–78.5 °C	Pressure	73.8 bar
Bp	–56.6 °C	Temperature	31.1 °C
Vapour pressure	57.3 bar at 20 °C	Density	0.464 g/L

Table 2.3 Comparison of the physical properties of different aggregate states (in orders of magnitude).

Phase	Density [$g \cdot cm^3$]	Diffusion [$cm^2 \cdot s^{-1}$]	Viscosity [$g \cdot cm^{-1} \cdot s^{-1}$]
Gas	10^{-3}	10^{-1}	10^{-4}
Supercritical fluid	0.1–1.0	10^{-3}–10^{-4}	10^{-3}–10^{-4}
Liquid	1	$<10^{-5}$	10^{-2}

The rate of extraction with a supercritical fluid is determined by the substance transport limits. Since supercritical fluids have diffusion coefficients an order of magnitude higher and viscosities an order of magnitude lower than those of liquid extraction agents, SFE extractions can be carried out in a much shorter time than, for example, the classical soxhlet extraction. Quantitative SFE extractions are typically finished in ca. 10–60 min, while normal solvent extractions for the same quantity of sample take several hours, often even overnight.

The solubilising capacity in an SFE step can easily be controlled via the density of the supercritical fluid, whereas the solubilising capacity of a liquid extraction agent is essentially constant. The density of the supercritical fluid is determined by the choice of pressure and temperature during the extraction. Raising the extraction temperature to ca. 60–80 °C also has a favourable effect on the swelling properties of the sample matrix. High-boiling polyaromatic hydrocarbons are even extracted from real life samples at temperatures above 150 °C. This makes it necessary to use pressures of above 500 bar to achieve maximum extraction.

At constant temperature low pressures favour the extraction of less polar analytes. The continuation of the extraction at higher pressures then elutes more polar and higher molecular weight substances. This allows the optimisation of the extraction for a certain class of compound by programming the change in pressure. In this way extracts are produced by SFE which are ready for analysis and generally require no further clean-up or concentration. The SFE extracts are often cleaner than those obtained by classical solvent extraction. Selective extractions and programmed optimisation are possible through the collection of extract fractions.

Carbon dioxide, which is currently used as the standard extraction agent in SFE, is gaseous under normal conditions and evaporates from the extracts after depressurising the supercritical fluid. Various techniques are used to obtain the extract. An alternative to simply passing the extract into open, unheated vessels involves freezing out the extracts in a cold trap. Here the restrictor is heated to ca. 150 °C and the extract is frozen out in an empty tube or one filled with adsorbent. The necessary cooling is achieved using liquid CO_2 on the outside of the trap. For many volatile substances this type of receiver leads to abnormally low values for quantitative analyses because of aerosol formation. The direct collection of the extract by adsorption on solid material (e.g. C_{18}) or passing the extract by means of a heated restrictor into a cooled, pressurised solvent has proved successful in many areas of application. The extraction of samples containing fats can also be carried out reliably using this method. Where adsorption is used, elution with a suitable solvent is carried out after the extraction as with solid phase extraction (SPE). If the extract is passed directly into a cooled solvent, the contents of the receiver can be further processed immediately. The choice of the appropriate collection technique is critical when modifiers are used. Even the addition of 10% of the

modifier results in the capacity of the cold trap being exceeded. Where the extract is collected on C_{18} cartridges increasing quantities of modifier can lead to premature desorption, because, in general, good solvents for the analyte are used as modifiers.

The extracts thus obtained can be analysed directly using GC, GC/MS or HPLC. Only in the processing of samples containing fats is the removal of the fats necessary before the subsequent analysis. This can be readily achieved using SPE. On the other hand, extracts from liquid extractions must be subjected to a clean-up before analysis and also concentrated because of their high dilution. The steps require additional time and are often the cause of low percentage recoveries.

Carbon dioxide has further advantages as a supercritical fluid. It is nontoxic, inert, can be obtained in high purity, and is clearly more economical with regard to the costs of obtaining it and disposing of it than liquid halogenated extraction agents. Because of the critical temperature of CO_2 (31 °C) SFE can be carried out at low temperatures so that thermolabile compounds can be extracted.

The mode of function of the analytical SFE unit (Fig. 2.8) has clear parallels with that of an HPLC unit. An analytical SFE unit consists of a pump, the thermostat (oven) in which the sample is situated in an extraction thimble, and a collector for the extract.

Carbon dioxide of the highest purity ('for SFE/SFC', 'SFE grade', total purity < 99.9998 vol. %) is used as the extraction agent and is available from stock from all major gas suppliers. The pumping unit consists of a syringe or piston pump. The liquid CO_2 is pumped out of the storage bottle, via a riser and compressed. Piston pumps require CO_2 bottles with an excess helium pressure (helium headspace ca. 120 bar when full). On the high pressure side the liquid modifier is passed into the compressed CO_2 with a suitable pump.

The extraction vessels for SFE are either situated in pressure-resistant cells (pressure equivalent extraction) or are the pressurised cells themselves. Nonextractable plastics or aluminium with a low thermal mass are used as the construction materials. Pressure-resistant vessels are made exclusively from stainless steel and are specified to resist up to the range of 500 bar or higher. The extraction vessels should be filled as completely as possible with the sample to avoid empty spaces. They should be extracted in a vertical position to prevent the CO_2 from flowing past the sample. Carbon dioxide flow from above to below has been shown to be most favourable, as even with compression homogeneous penetration of the sample on the base of the extraction chamber is guaranteed. For analytical purposes extraction thimbles with volumes of 0.5 mL to 50 mL are usual. The cap of the extraction vessels consists of a stainless steel frit with a pore size of 2 μm. In the case of pressure-resistant stainless steel vessels frits made of PEEK material are used as seals for the screw-on caps.

The CO_2 converted into a critical state through compression and variation of the temperature of the oven is passed continuously through the sample. In a static extraction step the extraction thimble is first filled with the supercritical fluid. At the selected oven temperature and through the effects of a selected modifier, in this step of ca. 15–30 min the sample matrix is digested and partition of the analytes between the matrix and the fluid is achieved. The swelling of the matrix has been shown to be of critical importance for real life samples as it leads to higher and more stable the yields of extract.

The dynamic extraction step is introduced by using a 6-way valve (Fig. 2.8). In this step supercritical CO_2 is passed continuously through the equilibrated sample and under the chosen conditions extractable analytes are transported out of the extraction chamber.

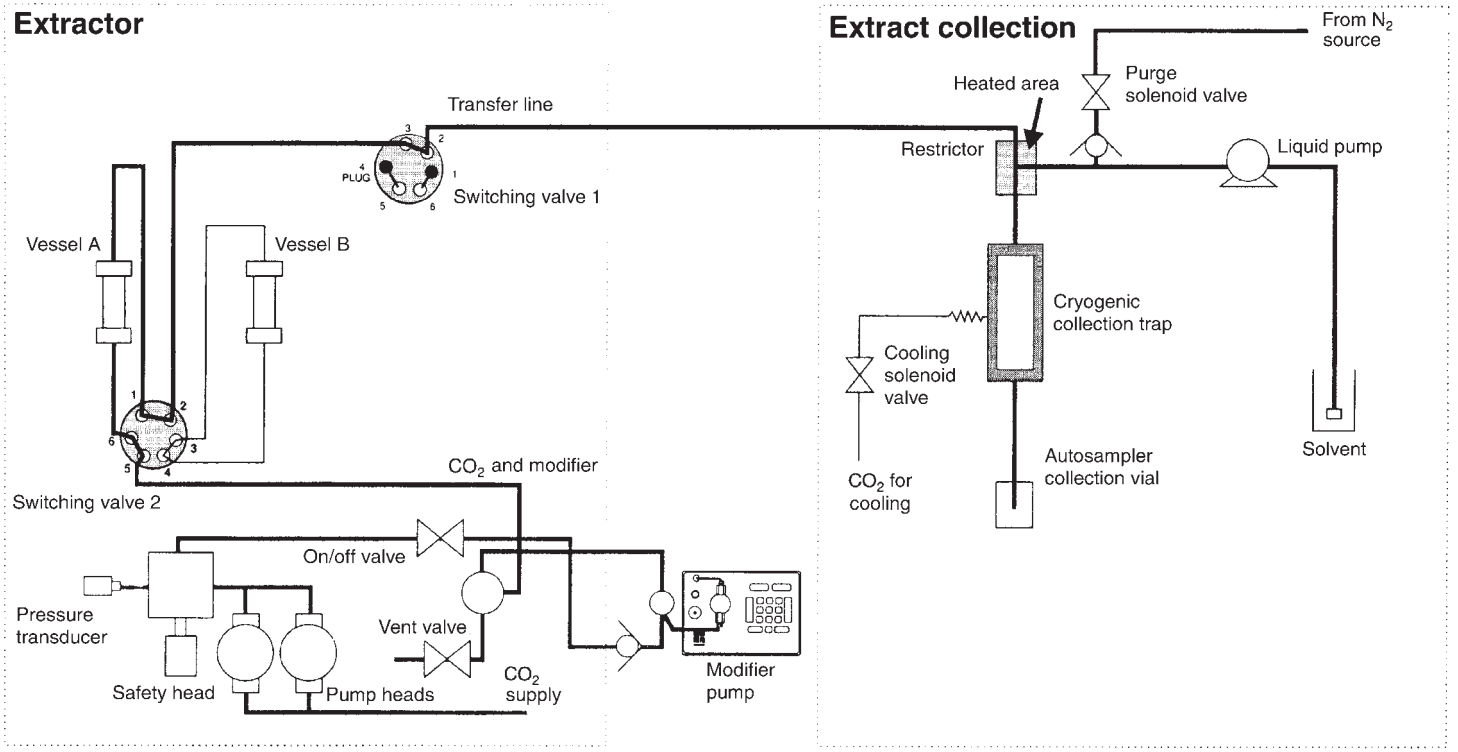


Fig. 2.8 Flow diagram of an SFE unit with cold trap and fraction collection, off-line coupling to GC (after Suprex).

The pressure ratios in the extraction chamber and in the tube carrying the extract are maintained by a restrictor at the end of the carrier tube. The dynamic extraction is quantitative and thus purifies the system for the next sample. The time required depends on the sample volume chosen.

At the restrictor the supercritical CO_2 is depressurised and the sample extract collected. A wide variety of restrictor constructions have been used with varying degrees of success. The choice of restrictor significantly affects the sample throughput of the SFE unit, the reproducibility of the process, and the breadth of application of SFE with regard to volatility of the analytes and the use of predried or moist samples. The earlier constructions with steel or fused silica restrictors with a defined opening size (e.g. integral restrictors) have not proved successful. The depressurising of the CO_2 usually took place in small glass vials. These were filled with solvent into which the restrictor was dipped. A portion of the volatile substances was lost and the necessary heat of evaporation for depressurising was not supplied adequately. All samples which were not carefully freeze-dried led here to a sudden freezing out of the extracted water and thus to frequent blocking of the restrictor. Consequently at this stage of the development the results of the process had low reproducibility.

A significant advance in restrictor technology came with the introduction of variable restrictors (Fig. 2.9). The width of the opening of the variable restrictor is controlled by the flow rate and has a fine adjustment mechanism in order to achieve a constant flow of the supercritical fluid. Diminutions in flow with simultaneous deposition of the matrix or particles at the restriction are thus counteracted. In this construction the restriction is situated

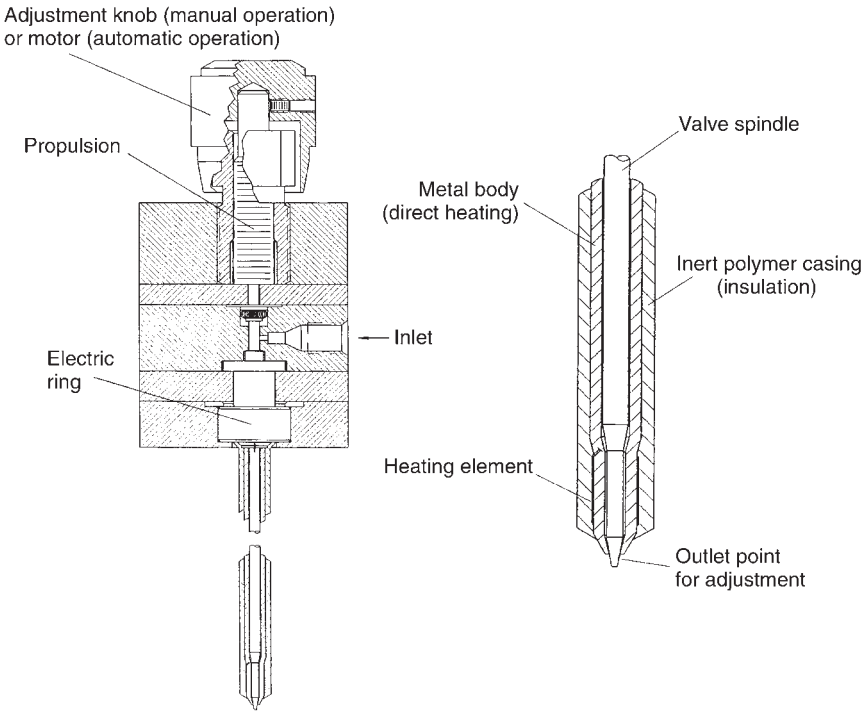


Fig. 2.9 Coaxial heater, variable restrictor for SFE (ISCO).

in a zone which can be heated to 200 °C, which completely prevents water from freezing out and provides the necessary energy for the depressurising of the CO_2.

Polar solvents, such as water, methanol, acetone, ethyl acetate or even toluene are predominantly used as modifiers in SFE (Table 2.4). The modifiers are added to swell the matrix and improve its surface activity and to increase the polarity of the CO_2. In many cases the use of modifiers leads to an increase in the yield of the extraction and even ionic compounds can be quantitatively extracted. The choice of modifier and the adjustment of its concentration are usually carried out empirically. Experience shows that modifiers which are themselves good solvents for the analytes can also be used successfully in SFE (Tables 2.5 and 2.6). Changes in the critical parameters of the fluid on addition of the modifier should be taken into account. Suitable software programs for calculating the quantities have been available (e.g. SFE-Solver, ISCO Corp., Lincoln, NE, USA). While methanol is the most widely used modifier, ethyl acetate, for example, has been shown to be particularly effective in the extraction of illegal drugs from hair (see Section 4.30).

The modifier can be added in two different ways. The simplest involves the direct addition of the modifier to the sample in the extraction vessel. As many samples, in particular most foodstuffs, already have a high water content, in such cases lyophilising the sample is not necessary. Here a suitable restrictor is necessary. The water contained in the sample can be used as the modifier for the extraction and the addition of other modifiers is unnecessary. In other cases a few mL of the modifier are added to the sample. The direct addition procedure is suitable for all extractions which employ the static extraction step.

By using an additional modifier pump (HPLC pump, syringe pump) a preselected quantity of modifier can be mixed continuously with the supercritical carbon dioxide on the high pressure side. This elegant procedure which is generally controlled by the system's software in modern instruments allows both the static step and also the continuous dynamic extraction in the presence of the modifier.

In SFE the sample quantity used has a pronounced effect on the concentrations of the analytes in the extract and on the length of the extraction. For residue analysis using SFE, sample volumes of up to 10 mL are generally used. Larger quantities of sample increase the time required for the quantitative extraction and also increase the CO_2 consumption, which is ca. three to ten times the empty volume of the extraction thimble. Further shortening of the extraction time and lowering of the sample volume is possible using on-line extraction.

On coupling with GC and GC/MS the possible water content of the extracts must be taken into consideration. The GC column must be chosen so that water can be chromatographed as a peak and does not affect the determination of the analytes. All stationary phases of medium and high polarity are suitable for this purpose. Generally it must be assumed that if there is a constant water background the response of the analytes in the mass spectrometer will be strongly impaired. An accumulation with decreasing response factors can be observed in the course of a day with ion sources with unheated lens systems.

On-line coupling with GC/MS can be easily realised with a cold injection system (e.g. PTV, programmable temperature vaporiser. Here a fused silica column as a restriction and the transfer line from the 6-way valve of the SFE unit are connected to the injector (see Fig. 2.8). The injector can be filled with a small quantity of an adsorption material, e.g. Tenax, depending on the task required. During the extraction the injector is kept at a low temperature with the split open, to ensure the expansion of the supercritical CO_2 and the trapping of the analyte. At the end of the extraction the 6-way valve of the SFE unit switches the carrier gas into

Table 2.4 Development of an SFE method for the extraction of PCDDs from fly ash (after Onuschka).

Experiment no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pressure	350	400	400	350	400	400	400	400	350	350	400	350	350	400
Replicate number	2	1	1	2	1	2	1	3	2	2	3	2	2	2
Supercritical fluid	N_2O	CO_2	N_2O	N_2O	CO_2	N_2O	N_2O	N_2O	CO_2	N_2O	N_2O	N_2O	N_2O	N_2O
Modifier identity	M	–	T	M	T	T	T	M	M	M	M	M	M	M
Pretreatment	–	F	F	–	F	F	F	HCl	T	T	T	E	–	–
Time-static [min]	–	64	–	120	64	64	32	64	40	40	60	50	40	64
Time-dynamic [min]	60	16	60	30	16	16	8	16	10	10	12	16	10	16
Program no.	B	A	B	C	A	A	A	A	C	C	C	D	A	A

Remarks:

Codes:
- M methanol 2%
- T toluene 5%
- F formic acid
- E pre-extraction with ethylene
- HCl hydrochloric acid treatment

Program A 2 min static equilibration repeated 8 times followed by 30 seconds of leaching and recharging with a fresh supercritical fluid. This program, which takes 20 min to complete the 15 step run, was usually repeated 2 to 4 times.

Program B Dynamic leaching only.

Program C 20 steps static extraction; 20 min static equilibration time + 3 min purging, usually repeated 2 to 6 times. 20 step program consisting of 14 min static extraction followed by 30 seconds of leaching supplied 13 times. The program requires a total of 20 min and was repeated 2 to 6 times.

Program D 12 steps static extraction; 15 min equilibration time + 3 min purging. 12 steps static extraction; 6 static steps, the first one lasting 5 min, the rest are set for 2 min duration. Leaching steps are set for 30 seconds. This program was repeated up to 7 times.

Table 2.5 Extraction yields from the SFE of PCBs for selected modifiers (after Langenfeld, Hawthorne et al.).

PCB isomer no.	1	2	3	4
18–2,2′,5	3.46 (2)	71 (12)	61 (1)	56 (4)
28–2,4,4′	2.21 (5)	63 (3)	72 (1)	65 (5)
52–2,2′,5,5′	4.48 (1)	71 (4)	74 (1)	70 (8)
44–2,2′,3,5′	1.07 (11)	94 (14)	101 (1)	88 (10)
66–2,3′,4,4′	0.93 (1)	68 (4)	73 (1)	66 (1)
101–2,2′,4,5,5′	0.82 (1)	84 (3)	87 (1)	81 (4)
118–2,3′,4,4′,5	0.51 (2)	87 (4)	92 (2)	84 (5)
138–2,2′,3,4,4′,5′	0.57 (2)	93 (2)	95 (4)	79 (7)
180–2,2′,3,4,4′,5,5′	0.16 (6)	106 (1)	109 (2)	99 (8)

(1) Certified contents in µg/g of a sediment sample according to NIST, determined by two sequential Soxhlet extractions of 16 h.

% recovery (% RSD) for the addition of the modifiers
(2) 10% methanol
(3) 10% methanol/toluene 1:1
(4) 1% acetic acid

SFE conditions: ISCO model 260D and SFX 2–10, 400 bar, 80 °C, 0.5 g sediment, 2.5 mL extraction cell, extraction agent CO_2, addition of modifier to the extraction cell (10% addition corresponds to 2.5 µL modifier), 5 min static and 10 min dynamic extraction.

Table 2.6 Extraction yields from the SFE of polyaromatic hydrocarbons for selected modifiers (after Langenfeld, Hawthorne et al.).

Polyaromatic hydrocarbon	1	2	3	4	5
Phenanthrene	4.5 (7)	99 (5)	100 (2)	238 (7)	265 (3)
Fluoranthene	7.1 (7)	84 (7)	96 (2)	175 (5)	195 (3)
Pyrene	7.2 (7)	69 (7)	81 (2)	138 (3)	151 (2)
Benzo[a]anthracene	2.6 (12)	63 (3)	94 (4)	97 (6)	89 (1)
Chrysene + triphenylene	5.3 (5)	67 (2)	90 (4)	56 (5)	67 (2)
Benzo[b,k]fluoranthene	8.2 (5)	55 (3)	108 (8)	66 (1)	67 (1)
Benzo[a]pyrene	2.9 (17)	20 (8)	60 (8)	32 (1)	34 (1)
Benzo[ghi]perylene	4.5 (24)	7 (5)	26 (11)	16 (4)	13 (11)
Indeno[1,2,3-cd]pyrene	3.3 (15)	14 (7)	57 (13)	35 (10)	28 (9)

(1) Certified contents in µg/g of a dust sample (urban air particulate matter) according to NIST, determined by soxhlet extraction over 48 h.

% recovery (% RSD) for the addition of modifiers
(2) 10% methanol
(3) 10% toluene
(4) 10% methanol/toluene 1:1
(5) 10% acetic acid

SFE conditions: ISCO model 260D and SFX 2-10, 400 bar, 80 °C, 0.3 g dust, 2.5 mL extraction cell, extraction agent CO_2, addition of modifier to the extraction cell (10% addition corresponds to 2.5 µL modifier), 5 min static and 10 min dynamic extraction.

Note: The high recoveries for some polyaromatic hydrocarbons are real and were verified using blank values and carry-over experiments.

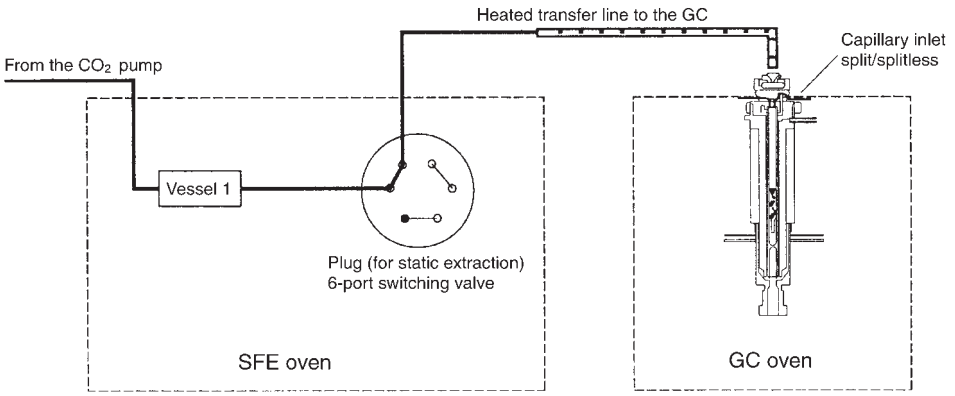


Fig. 2.10 On-line coupling of SFE with GC and GC/MS via a heated transferline (connection directly at the 6-port switching valve) and PTV cold injection system.

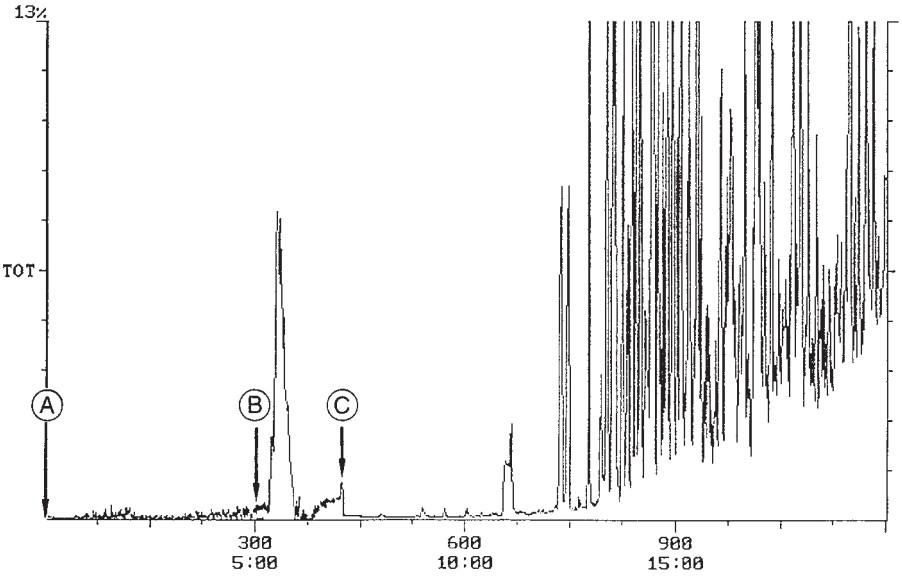


Fig. 2.11 Course of on-line SFE/GC/MS coupling for a polyaromatic hydrocarbon analysis
(A) Start of the dynamic extraction and data recording. Injector cold, split open
(B) End of the dynamic extraction and start of the injection Injector heats up, split shut
(C) Start of the GC temperature program Injector hot, split open

the transfer line to prevent further passage of CO_2 on to the GC column. Injection requires controlled heating of the injector with the split closed and the start-up of the analysis program (Fig. 2.11).

The principal advantages of on-line SFE are that the manual steps between extraction and analysis are omitted and high sensitivity is achieved. All the extract is transferred quantitatively to the chromatography column. The quantity of sample with a particular expected ana-

lyte content applied must be modified according to the capacity of the GC column. As far as the mass spectrometer is concerned, no special measures need to be taken on account of the on-line coupling. The quality of the analysis which can be achieved corresponds without limitations to the requirements of residue analysis.

The example of the extraction of polychlorinated dibenzodioxins (PCDDs) from fly ash clearly shows the comparison between the conventional soxhlet extraction and the way in which the current method is progressing. For the extraction 25 mg portions of homogenised fly ash were weighed out. The determination of the PCDDs was carried out with labelled internal standards using high resolution GC/MS. In the experiments presented here the variables sample pretreatment, fluid, modifier, pressure, and extraction time and program were systematically varied (Figs. 2.12 and 2.13).

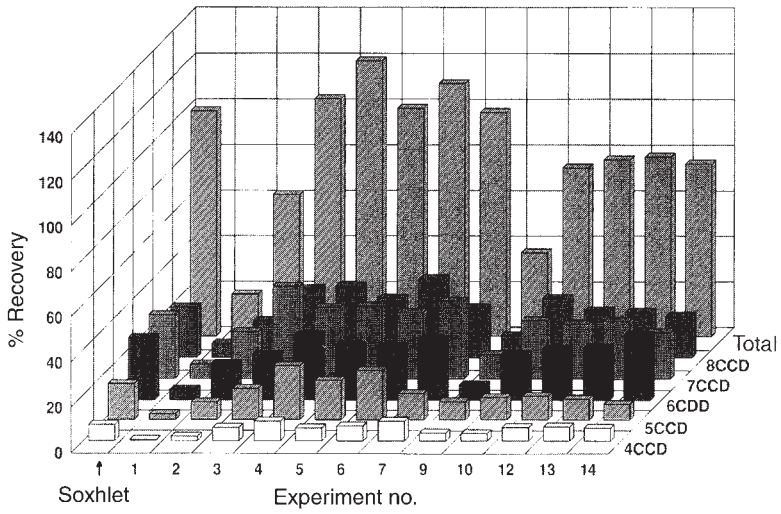


Fig. 2.12 Recoveries in SFE for PCDD extraction from fly ash compared with Soxhlet extraction (after Onuschka).

Besides the analytical aspects, the ecological and economic aspects of SFE are also important. An environmental analysis technique which produces large quantities of potentially environmentally hazardous waste is paradoxical and not acceptable in the long term. At the same time the laboratory personnel have to handle smaller quantities of harmful solvents. The critical analysis of the costs during one year shows that the SFE procedure for sample preparation in the routine laboratory reduces the cost to two-thirds that of the conventional soxhlet extraction.

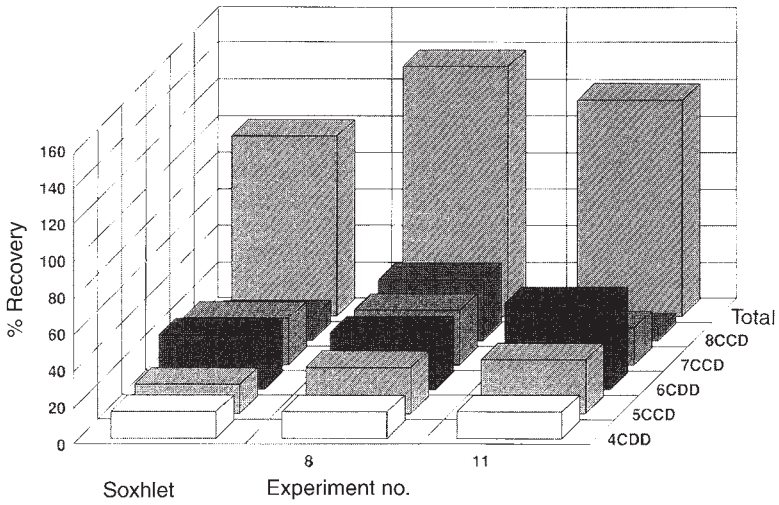


Fig. 2.13 Experiments 8 and 11 of the PCBD extraction from Table 2.6 compared with the Soxhlet extraction (after Onuschka).

2.1.3 Pressurized Fluid Extraction

The trend to automatized instrumental extraction methods has also reached the time consuming Soxhlet extraction process. A comparison of modern extraction methods like microwave assisted extraction (MAE), supercritical fluid extraction (SFE), solid phase micro extraction (SPME) and pressurized fluid extraction (PFE) with the traditional Soxhlet method, finds that PFE has a widespread application due to a number of positive features, including the most practical ones: ease-of-use, versatility and reproducibility. There are both strong analytical and economical advantages with PFE; the high extraction efficiency, savings in solvent consumption (and waste generation) and the short extraction times led to a steadily growing area of applications also for biological samples (Wenzel 1998, Li 2004). PFE also limits glassware handling, and facilitates a safe laboratory working environment.

Pressurized Fluid Extraction (PFE) is the general term for an automated technique for extracting solid and semisolid samples with liquid, organic or aqueous solvents. The PFE method is also marketed under the term "Accelerated Solvent Extraction" (ASE) by Dionex Corp., USA, covered by a number of international patents (Dionex 2006). PFE takes advantage of a high energy regime for the extraction process provided by high pressures at high temperatures. Special care has to be taken concerning thermolabile components for which a cold extraction method as the microwave assisted extraction might be the appropriate alternative. Automated PFE instrumentation allows the independent control of temperature and pressure conditions for each sample cell. This control is critical for a high analyte recovery and reproducibility.

At the typical PFE conditions the solvent characteristics change compared to the traditional Soxhlet technique and allow an improved separation of target analytes from the matrix (Hubert 2001). For instance the extraction of relatively polar phenols from soil sam-

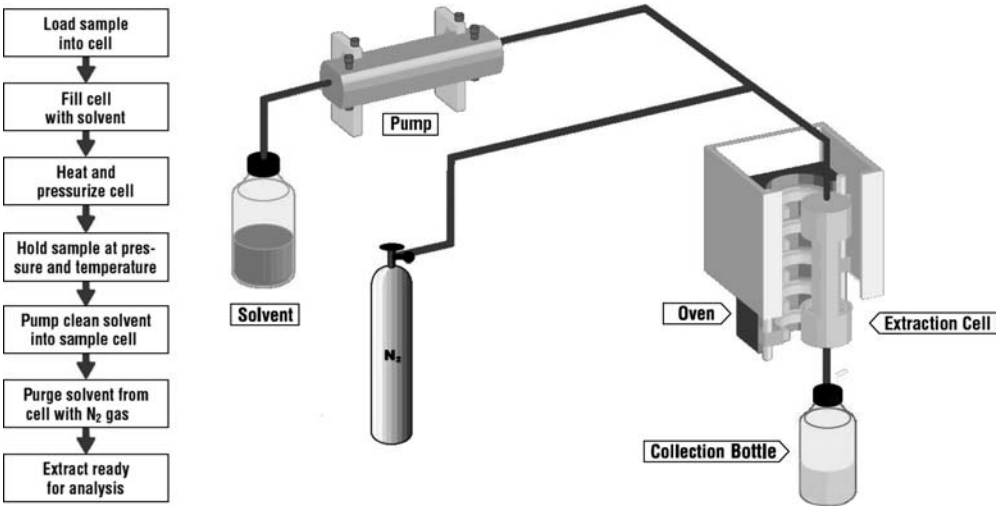


Fig. 2.14 Schematics of a pressurized extraction unit (courtesy Dionex Corp.).

ples is possible using the non polar solvent n-hexane. PFE extractions typically use the known organic solvents n-hexane, cyclo-hexane, toluene, dichloromethane, methanol, acetone as applied in Soxhlet methods. For biological samples and the extraction of water soluble components also water based media in mixtures with organic solvents are applied (Curren 2002). This variation gives access to polar compounds for subsequent LC/MS analysis. Due to a mostly sharp matrix/analyte separation by PFE, the collected extracts require less time consuming clean-up typically employing SPE or GPC steps.

Traditional techniques, like Soxhlet, can take 4–48 hours. With PFE, analyte recoveries equivalent to those obtained using traditional extraction methods can be achieved in only 15 to 30 minutes. Although PFE uses the same aqueous and organic solvents as traditional extraction methods, it uses them more efficiently. A typical PFE extraction is done using 50–150 mL of solvent, see Table 2.7 for comparison.

Table 2.7 Comparison of standard laboratory extraction techniques.

Techniques	Solvent volume per sample [mL]	Extraction time per sample [h]	Cost per sample [a] (in US $)
Manual Soxhlet	200–500	4–48	23.50
Automated Soxhlet	50–100	1–4	16.25
Sonication	100–300	0.5–1	20.75
SFE	8–50	0.5–2	17.60
Microwave	50–100	0.5–1	15.25
ASE	15–40	0.2–0.3	11.20

a) Cost per sample in US $ based on 2000 samples per year, average values, based on US comparison (Dionex 2006)

Approximately 75% of all PFE extractions are completed in less than 20 minutes using the standard PSE extraction conditions (100 °C, 1500 psi). But, PFE extractions are matrix dependent and require further optimization when applied to different matrices. Method development starts with standard conditions and evaluates both the recovery in the first step and the result of a second extraction of the given new sample. Also the initial application of a standard matrix e. g. sand is a helpful step. If these initial parameters don't provide the recoveries desired, the temperature is increased to improve the efficiency of the extraction. Adding static cycles, increasing static time, and selecting a different solvent are additional variables that can be used to optimize a method.

Recommended Extraction Conditions (US EPA 1998)

For semivolatiles, organophosphorus pesticides, organochlorine pesticides, herbicides, and PCBs:

Oven temperature:	100 °C
Pressure:	1500–2000 psi
Static time:	5 min (after 5 min pre-heat equilibration)
Flush volume:	60% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static cycles:	1

For PCDDs/PCDFs:

Oven temperature:	150–175 °C
Pressure:	1500–2000 psi
Static time:	5–10 min (after 5 min pre-heat equilibration)
Flush volume:	60–75% of the cell volume
Nitrogen purge:	60 sec at 150 psi (purge time may be extended for larger cells)
Static cycles:	2 or 3

PFE extraction is accepted for use in U.S. EPA SW-846 Method 3545A, which can be used in place of Methods 3540, 3541, 3550, and 8151. Method 3545A can be applied to the extraction of base/neutrals and acids (BNAs), chlorinated pesticides and herbicides, polychlorinated biphenyls (PCBs), organophosphorus pesticides, dioxins and furans and total petroleum hydrocarbons (TPH).

Scope and Application of EPA Method 3545A

1.1 Method 3545A is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and waste solids. The method uses elevated temperature (100–180 °C) and pressure (1500–2000 psi) to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time than the Soxhlet procedure. This procedure was developed and validated on a commercially available, automated extraction system.

1.2 This method is applicable to the extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, PCBs, and PCDDs/PCDFs, which may then be analyzed by a variety of chromatographic procedures.

(US EPA Method 3545A, Jan 1998)

2.1.4 Online Liquid Chromatography Clean-up

The necessary effort of sample preparation in trace analysis is driving cost and limiting productivity not only in the GC/MS laboratory today. Especially a series of manual steps for extraction, evaporation and transfer involved are responsible for long analysis times, high cost and also the reason for contaminations and analyte losses. Coupling LC clean-up on-line with the GC separation opens a great potential for a significant reduction in cost per sample, full automation and high productivity for standardized analyses. An on-line coupling of GPC or HPLC as clean-up procedure is a mature technique first described by Majors 1980. Commercial solutions are available since more than 20 years and established especially for routine analysis in many laboratories covering a wide range of applications e.g. for pesticides, PCBs, dioxins or plasticizers (Grob 1990, De Paolo 1992, Jongenotter 1999). A specific advantage for trace analyses is the concentrated eluate allowing lower detection limits and reproducibility. High extract dilutions introducing additional contaminations with a subsequent need for evaporation as known from Soxhlett or SPE extractions are avoided.

The instrumental set-up is built by an additional LC system with autosampler, HPLC separation column and switching valve in front of the GC (see Fig. 2.15). An on-line LC detector is necessary during method development with spiked samples. The GC is equipped with an injector suitable for large volume injections as a regular PTV equipped with a packed insert liner and LVI vent valve or an on-column injector with retention gap and solvent vapor exit. Conventional liquid extraction techniques can be replaced with this setup completely and run fully automated for large sample series.

The significantly higher separation efficiency in GPC or regular HPLC columns (number of available theoretical plates) can be exploited for group type separations. Eluents applied for the on-line clean-up need to be compatible with gas chromatography as typical for normal phase LC (reversed phase separations need fraction collection and solvent exchange). The analyte containing fractions of the LC eluate are cut via a sample loop, or more flexible for

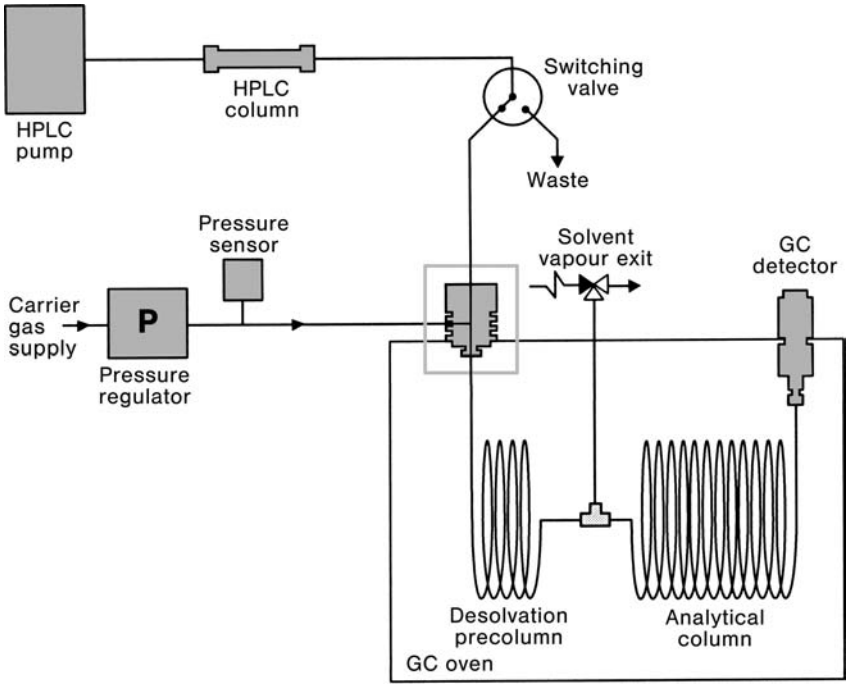


Fig. 2.15 Schematic of an on-line LC clean-up GC system (Trestianu 1996)

optimization, using a time controlled transfer to the GC injector. Typically, injection volumes of 100 µL to 1000 µL are employed using concurrent solvent evaporation or retention gap large volume injection (LVI) techniques as described in Section 2.2.3 GC/MS Inlet Systems.

2.1.5 Headspace Techniques

One of the most elegant possibilities for instrumental sample preparation and sample transfer for GC/MS systems is the use of the headspace technique (Fig. 2.16). Here all the frequently expensive steps, such as extraction of the sample, clean-up and concentration are dispensed with. Using the headspace technique the volatile substances in the sample are separated from the matrix. The latter is not volatile under the conditions of the analysis. The tightly closed sample vessels, which, for example, are used for the static headspace procedure, can frequently even be filled at the sampling location. The danger of false results (loss of analyte) as a result of transportation and further processing is thus reduced.

The extraction of the analytes is based on the partition of the very and moderately volatile substances between the matrix and the gas phase above the sample. After the partition equilibrium has been set up the gas phase contains a qualitatively and quantitatively representative cross-section of the sample and is therefore used for analysing the components to be determined. All involatile components remain in the headspace vial and are not analysed. For this reason the coupling of headspace instruments and GC/MS systems is particularly fa-

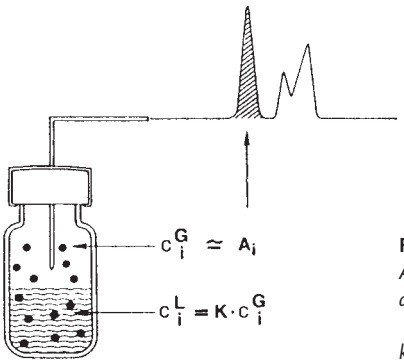


Fig. 2.16 Principle of headspace analysis (after Kolb)
A_i area of the GC signal of the i^{th} component.
c_{Gi}, c_{Li} concentrations in the gas and liquid phases respectively
k' partition coefficient

vourable. Since the interfering organic matrix is not involved, a longer duty cycle of the instrument and outstanding sensitivity are achieved. Furthermore headspace analyses are easily and reliably automated for this reason and achieve a higher sample throughput in a 24-hour operating period.

There are limitations to the coupling of headspace analysis with GC/MS systems if moisture has to be driven out of the sample. In certain cases water can impair the focusing of volatile components at the beginning of the GC column. Impairment of the GC resolution can be counteracted by choosing suitable polar and thick film GC columns, by removing the water before injection of the sample, or by using a simple column connection.

It is also known that water affects the stability of the ion source of the mass spectrometer detector, which nowadays is becoming ever smaller. In the case of repeated analyses the effects are manifested by a marked response loss and poor reproducibility. In such cases special precautions must be taken, in particular in the choice of ion source parameters.

The headspace technique is very flexible and can be applied to the most widely differing sample qualities. Liquid or solid sample matrices are generally used, but gaseous samples can also be analysed readily and precisely using this method. Both qualitative and, in particular, quantitative determinations are carried out coupled to GC/MS systems.

There are two methods of analysing the volatiles of a sample which have very different requirements concerning the instrumentation: the static and dynamic (purge and trap) headspace techniques. The areas of use overlap partially but the strengths of the two methods are demonstrated in the different types of applications.

2.1.5.1 **Static Headspace Technique**

The term headspace analysis was coined in the early 1960s when the analysis of substances with odours and aromas in the headspace of tins of food was developed. The equilibrium of volatile substances between a sample matrix and the gas phase above it in a closed static system is the basis for static headspace chromatography (HSGC). The term headspace is often used without the word static, e. g. headspace analyses, headspace sampler etc.

An equilibrium is set up in the distribution of the substances being analysed between the sample and the gas phase. The concentration of the substances in the gas phase then remains constant. An aliquot is taken from the gas phase and is fed into the GC/MS system via a transfer line (Fig. 2.17).

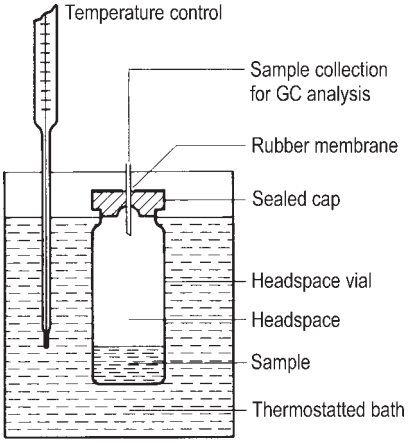


Fig. 2.17 Sample handling in the static headspace method (after Hachenberg).

The static headspace method is therefore an indirect analysis procedure, requiring special care in performing quantitative determinations. The position of the equilibrium depends on the analysis parameters (e.g. temperature) and also on the sample matrix itself. The matrix-dependence of the procedure can be counteracted in various ways. The matrix can be standardised, e.g. by addition of Na_2SO_4 or Na_2CO_3. Other possibilities include the addition method, internal standardisation, or the multiple headspace extraction procedure (MHE) published by Kolb in 1981 (Fig. 2.18).

Static Headspace Analysis

In static headspace analysis the samples are taken from a closed static system (closed headspace bottle) after the thermodynamic equilibrium (partition) between the liquid/solid matrix and the headspace above it has been established.

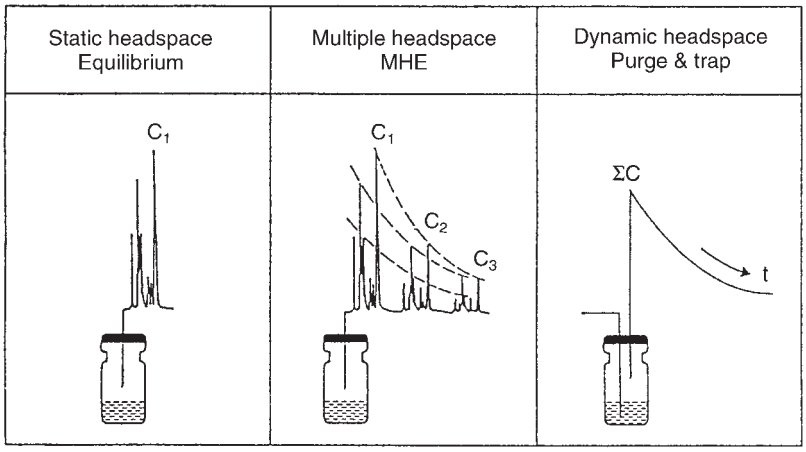


Fig. 2.18 Gas extraction techniques (after Kolb).

For coupling of HSGC with mass spectrometry the internal standard procedure has proved particularly successful for quantitative analyses. Besides the headspace-specific effects, possible variations in the MS detection are also compensated for. The best possible precision is thus achieved for the whole procedure. The MHE procedure can be used in the same way.

In static headspace analysis the partition coefficient of the analytes is used to assess and plan the method. For the partition coefficient k of a volatile compound the following equation is valid:

$$k = \frac{c_S}{c_G} = \frac{\text{concentration in the sample}}{\text{concentration in the gas phase}} \tag{1}$$

The partition coefficient depends on the temperature at equilibrium. This must therefore be kept constant for all measurements.

Rearranging the equation gives:

$$c_S = k \cdot c_G \tag{2}$$

As the peak area A determined in the GC/MS analysis is proportional to the concentration of the substance in the gas phase c_G, the following is valid:

$$A \approx c_G = \frac{1}{k} \cdot c_S \tag{3}$$

To be able to calculate back to the concentration of a substance c_0 in the original sample, the mass equilibrium must be referred to:

$$M_0 = M_S + M_G \tag{4}$$

The quantity M_0 of the volatile substance in the original sample has been divided at equilibrium into a portion in the gas phase M_G and another in the sample matrix M_S. Replacing M by the product of the concentration c and volume V gives:

$$c_0 \cdot V_0 = c_S \cdot V_S + c_G \cdot V_G \tag{5}$$

By using the definition of the partition coefficient given in equation (2), the unknown parameter c_S can be replaced by $k \cdot c_G$:

$$c_0 \cdot V_0 = k \cdot c_G \cdot V_S + c_G \cdot V_G \tag{6a}$$

$$c_0 = c_G \cdot \frac{V_S}{V_0} \cdot \left(k + \frac{V_G}{V_S}\right) \tag{6b}$$

The starting concentration c_0 of the sample, assuming $V_0 = V_S$, is given by:

$$c_0 = c_G \cdot \left(k + \frac{V_G}{V_S}\right) \tag{7}$$

As the peak area determined is proportional to the concentration of the volatile substance in the gas phase c_G, the following equation is valid, corresponding to the proportionality between the gas phase and the peak area:

$$c_0 \approx A \cdot (k + \beta) \tag{8}$$

whereby β is the phase ratio V_G/V_S (see equation (7)) and therefore describes the degree of filling of the headspace vessel (Ettre, Kolb 1991).

The effects on the sensitivity of a static headspace analysis can easily be derived from the ratio given in equation (8):

$$A \approx c_0 \cdot \frac{1}{k + \beta} \quad \text{with } \beta = V_G/V_S \tag{9}$$

The peak area determined from a given concentration c_0 of a component depends on the partition coefficient k and the sample volume V_S (through the phase ratio β).

For substances with high partition coefficients (e.g. ethanol, isopropanol, dioxan in water) the sample volume has no effect on the peak area determined. However, for substances with small partition coefficients (e.g. cyclohexane, trichloroethylene, xylene in water), the phase ratio β determines the headspace sensitivity. Doubling the quantity of sample leads in this case to a doubling of the peak area (Fig. 2.19). For all quantitative determinations of compounds with small partition coefficients, an exact filling volume must be maintained.

The sensitivity of detection in static headspace analysis can also be increased through the lowering of the capacity factor k by setting up the equilibrium at a higher temperature. Substances with high partition coefficients profit particularly from higher equilibration tempera-

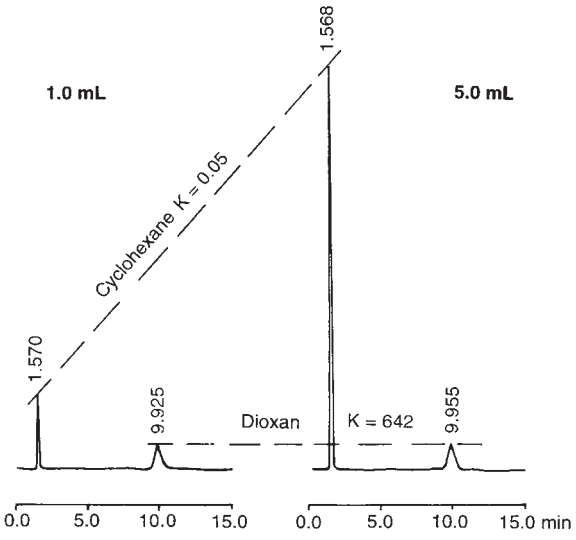


Fig. 2.19 Sample volume and sensitivity in the static headspace for cyclohexane ($K = 0.05$) and dioxan ($K = 642$) with sample volumes of 1 mL and 5 mL respectively (after Kolb).

tures (Table 2.8, e.g. alcohols in water). Raising the temperature is, however, limited by an increased matrix evaporation (water) and the danger of bursting the sample vessel or the seal.

Changes in the sample matrix can also affect the partition coefficient. For samples with high water contents an electrolyte can be added to effect a salting out process (Fig. 2.20). For example, for the determination of ethanol in water the addition of NH_4Cl gives a twofold and addition of K_2CO_3 an eightfold increase in the sensitivity of detection. The headspace sensitivity can also be raised by the addition of nonelectrolytes. In the determination of residual monomers in polystyrene dissolved in DMF, adding increasing quantities of water, e.g.

Table 2.8 Partition coefficients of selected compounds in water (after Kolb).

Substance	40 °C	60 °C	80 °C
Tetrachloroethane	1.5	1.3	0.9
1,1,1-Trichloroethane	1.6	1.5	1.2
Toluene	2.8	1.6	1.3
o-Xylene	2.4	1.3	1.0
Cyclohexane	0.07	0.05	0.02
n-Hexane	0.14	0.04	< 0.01
Ethyl acetate	62.4	29.3	17.5
n-Butyl acetate	31.4	13.6	7.6
Isopropanol	825	286	117
Methyl isobutyl ketone	54.3	22.8	11.8
Dioxan	1618	642	288
n-Butanol	647	238	99

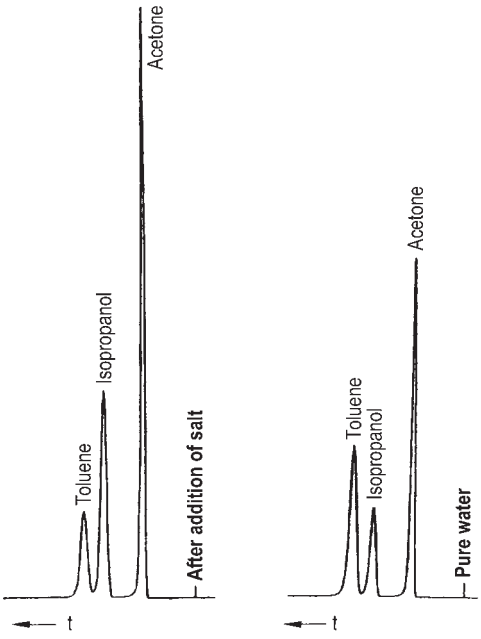


Fig. 2.20 Matrix effects in static headspace. The effect of salting out on polar substances (after Kolb).

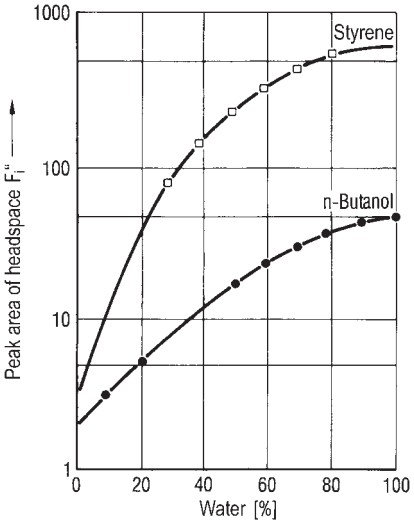


Fig. 2.21 Increasing water concentrations in the determination of styrene as a residual monomer lead to a sharp increase in response (after Hachenberg).

in the determination of styrene and butanol, leads to an increase in peak area for styrene by a factor of 160 and for n-butanol by a factor of only 25 (Fig. 2.21).

To shorten the equilibration time the most up-to-date headspace samplers have devices available for mixing the samples. For liquid samples vigorous mixing strongly increases the phase boundary area and guarantees rapid delivery of analyte-rich sample material to the phase boundary.

Teflon-coated stirring rods have not proved successful because of losses through adsorption and cross-contamination. However, shaking devices which mix the sample in the headspace bottle through vertical or rotational movement have proved effective (Table 2.9). A particularly refined shaker uses changing excitation frequencies of 2–10 Hz to achieve optimal mixing of the contents at each degree of filling (Fig. 2.22).

The resulting equilibration times are in the region of 10 min on using shaking devices compared with 45–60 min without mixing.

In addition quantitative measurements are more reliable. The use of shaking devices in static headspace techniques lowers the relative standard deviations to less than 2%.

Table 2.9 Comparison of the analyses with/without shaking of the headspace sample (volatile halogenated hydrocarbons), average values in ppb, standard deviations (after Tekmar).

Substance	Without shaking			With shaking		
Ethylbenzene	353	18	(5.2%)	472	8	(1.7%)
Toluene	336	20	(5.9%)	411	4	(1.0%)
o-Xylene	324	13	(4.1%)	400	7	(1.8%)
Benzene	326	18	(5.4%)	372	5	(1.3%)
1,3-Dichlorobenzene	225	13	(5.6%)	255	5	(2.1%)
1,2,4-Trichlorobenzene	207	9	(4.2%)	225	6	(2.5%)
Bromobenzene	213	11	(5.2%)	220	5	(2.1%)

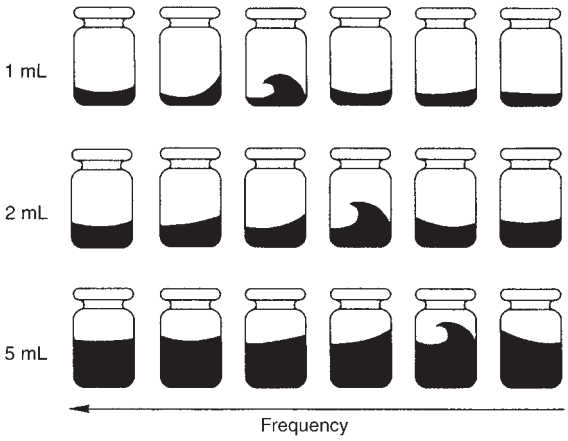


Fig. 2.22 Effect of variable shaking frequencies (2–10 Hz) on different depths of filling in headspace vessels (Perkin Elmer).

Static Headspace Injection Techniques

- *Pressure Balanced Injection* (Fig. 2.23):
  Variable quantity injected: can be controlled (programmed) by the length of the injection process, injection volume = injection time x flow rate of the GC column, no drop in pressure to atmospheric pressure, depressurising to initial pressure of column.
  Change in pressure in the headspace bottle: reproducible pressure build-up with carrier gas, mixing, initial pressure in column maintained during sample injection.
  Sample losses: none known.
- *Syringe Injection* (Fig. 2.24):
  Variable injection volume through pump action: easily controlled, drop in pressure to atmospheric on transferring the syringe to the injector.
  Pressure change in the headspace bottle: varies with the action of the syringe plunger (volume removed) on sample removal from the sealed bottle, compensation by injection of carrier gas necessary.
  Sample losses: possible through condensation on depressurising to atmospheric pressure, losses through evaporation from the syringe after it has been removed from the septum cap of the bottle, in comparison the largest surface contact with the sample.
- *Sample Loop* (Fig. 2.25):
  Quantity injected on the instrument side: changes in the volume injected by disconnection of the sample loop, drop in pressure to atmospheric on filling the sample loop.
  Pressure change in the headspace bottle: complete depressurising of the pressure built up in the bottle through tempering, the expansion volume must be a multiple of the sample loop to be able to fill it reproducibly.
  Sample losses: for larger sample loops attention must be paid to dilution with carrier gas during the pressure build-up phase, possible condensation on depressurising to atmospheric pressure.

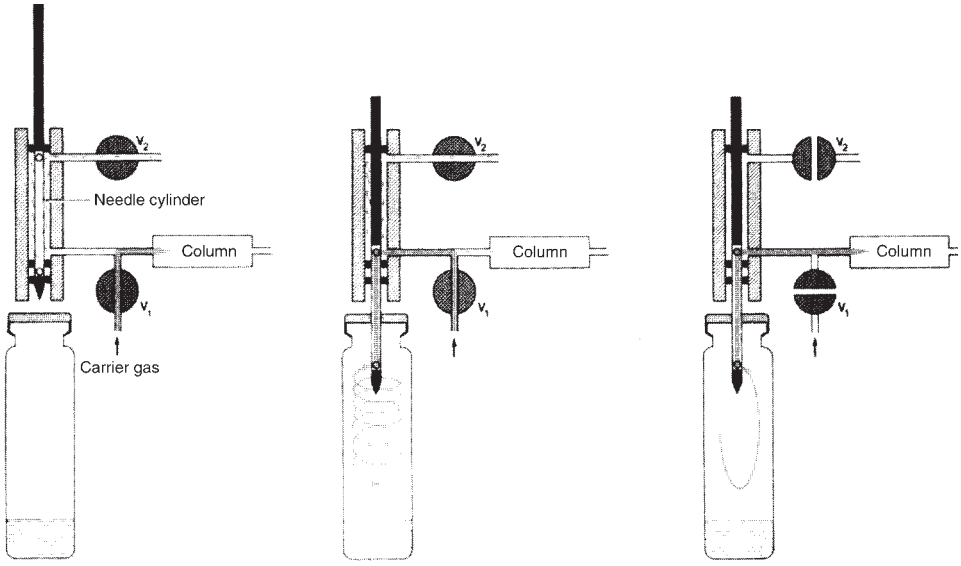


Fig. 2.23 Injection techniques for static headspace: the principle of pressure balanced injection (Perkin Elmer).

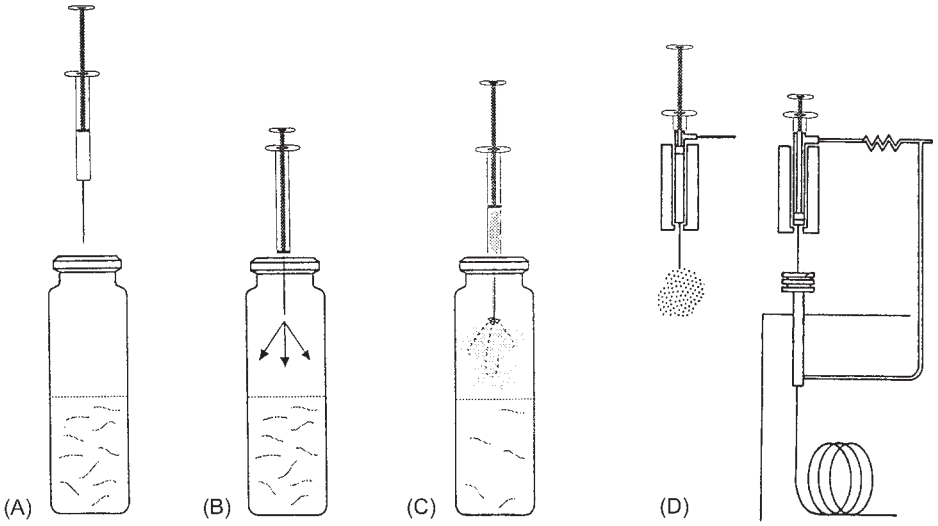


Fig. 2.24 Injection techniques for static headspace: the principle of transfer with a gastight syringe. (A) Sample heating, (B) Pressure, (C) Sampling, (D) Inject.

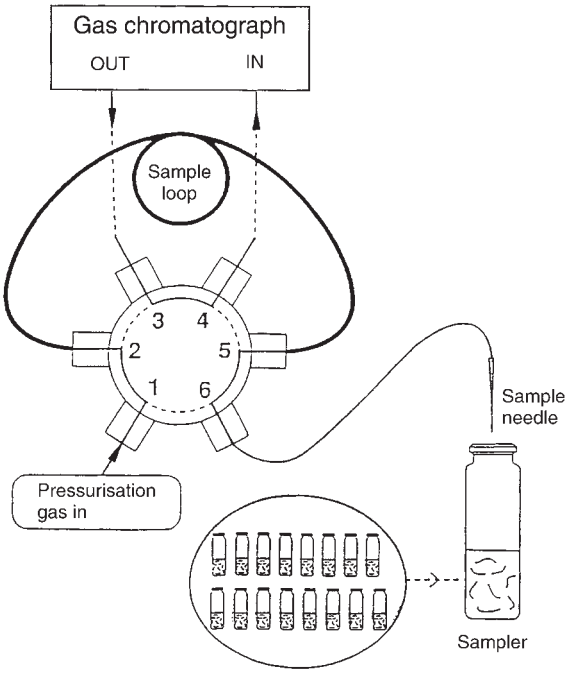


Fig. 2.25 Injection techniques for static headspace: the principle of application with a sample loop (Tekmar).
Sequence:
1 Heat sample at a precise temperature over a set period of time
2 Pressurise with carrier gas
3 Fill the sample loop
4 Inject by switching the 6-port switching valve

2.1.5.2 **Dynamic Headspace Technique (Purge and Trap)**

The trace analysis of volatile organic compounds (VOCs, volatile organic carbons) is of continual interest, e.g. in environmental monitoring, in outgasing studies on packaging material or in the analysis of flavours and fragrances. The dynamic headspace technique, known as purge and trap (PAT), is a process in which highly and moderately volatile organic compounds are continuously extracted from a matrix and concentrated in an adsorption trap. The substances driven out of the trap by thermal desorption reach the gas chromatograph as a concentrated sample plug where they are finally separated and detected.

Early purge and trap applications in the 1960s already involved the analysis of body fluids. In the 1970s purge and trap became known because of the increasing requirement for the testing of drinking water for volatile halogenated hydrocarbons. The analysis of drinking water for the determination of a large number of these compounds in ppq quantities (concentrations of less than 1 µg/L) only became possible through the concentration process which was part of purge and trap gas chromatography (PAT-GC). This technique is now frequently used to detect residues of volatile organic compounds in the environment and in the analysis of liquid and solid foodstuffs. In particular, the coupling with GC/MS systems allows its use as a multicomponent process for the automatic analysis of large series of samples.

An analytically interesting variant of the procedure is the fine dispersion of liquid samples in a carrier gas stream. This so-called spray and trap process uses the high surface area of the sample droplets for an effective gas extraction. The procedure also works very well with foaming samples. A detergent content of up to 0.1% does not affect the extraction result. The spray and trap process is therefore particularly suitable for use with mobile GC/MS analyses.

Modes of Operation of Purge and Trap Systems

A purge and trap analysis procedure consists of three main steps:

1. Purge phase with simultaneous concentration
2. Desorption phase
3. Baking out phase

1. The Purge Phase

During the purge phase the volatile organic components are driven out of the matrix. The purge gas (He or N_2) is finely divided in the case of liquid samples (drinking water, waste water) by passing through a special frit in the base of a U-tube (frit sparger, Fig. 2.26A). The surface of the liquid can be greatly increased by the presence of very small gas bubbles and the contact between the liquid sample and the purge gas maximised.

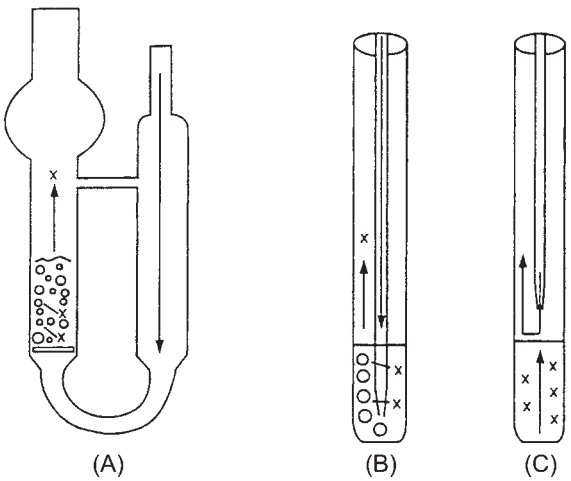


Fig. 2.26 Possibilities for sample introduction in purge and trap.
(A) U tube with/without frits (*fritless/frit sparger*) for water samples.
(B) Sample vessel (*needle sparger*) for water and soil samples (solids).
(C) Sample vessel (*needle sparger*) for foaming samples for determination of the headspace sweep.

The purge gas extracts the analytes from the sample and transports them to the trap. The analytes are retained in this trap and concentrated while the purge gas passes out through the vent. The desorption gas, which comes from the carrier gas provision of the GC, enters this phase via the 6-port switching valve in the gas chromatograph and maintains the constant gas flow for the column (Fig. 2.27).

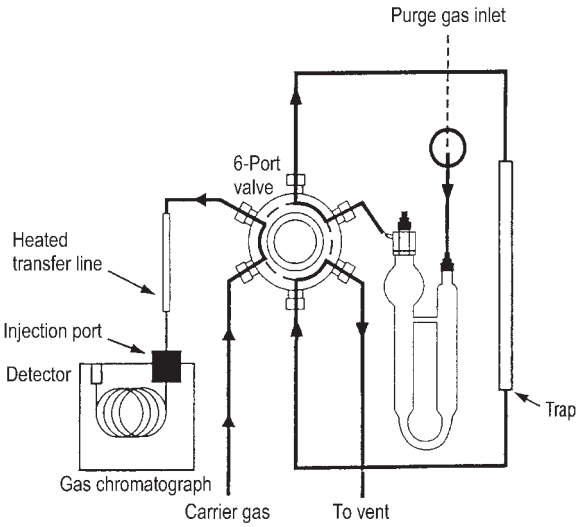


Fig. 2.27 Gas flow schematics of purge & trap-GC coupling, switching of phases at the 6-port switching valve. Bold line: Purge and baking out phase; dotted line: Desorption phase.

Solid samples are analysed in special vessels (needle sparger, see Fig. 2.26 B and C) into which a needle with side openings is dipped. For foaming samples the headspace sweep technique can be used.

The total quantity of volatile organic compounds removed from the sample depends on the purge volume. The purge volume is the product of the purge flow rate and the purge time. Many environmental samples are analysed at a purge volume of 440 mL. This value is achieved using a flow rate of 40 mL/min and a purge time of 11 min. A purge flow rate of 40 mL/min gives optimal purge efficiency. Changes in the purge volume should consequently only be made after adjusting the purge time. Although a purge volume of 440 mL is optimal in most cases, some samples may require larger purge volumes for adequate sensitivity to be reached (Fig. 2.28).

The purge efficiency is defined as that quantity of the analytes which is purged from a sample with a defined quantity of gas. It depends upon various factors. Among them are: purge volume, sample temperature, and nature of the sparger (needle or frit), the nature of the substances to be analysed and that of the matrix. The purge efficiency has a direct effect on the percentage recovery (the quantity of analyte reaching the detector).

Control of Purge Gas Pressure During the Purge Phase

The adsorption and chromatographic separation of volatile halogenated hydrocarbons is improved significantly by regulating the pressure of the purge gas during the purge phase. By additional back pressure control during this phase, a very sharp adsorption band is formed in the trap, from which, in particular, the highly volatile components profit, since a broader distribution does not occur. The danger of the analytes passing through the trap is almost completely excluded under the given conditions.

During the desorption phase the narrow adsorption band determines the quality of the sample transfer to the capillary column. The result is clearly improved peak symmetry,

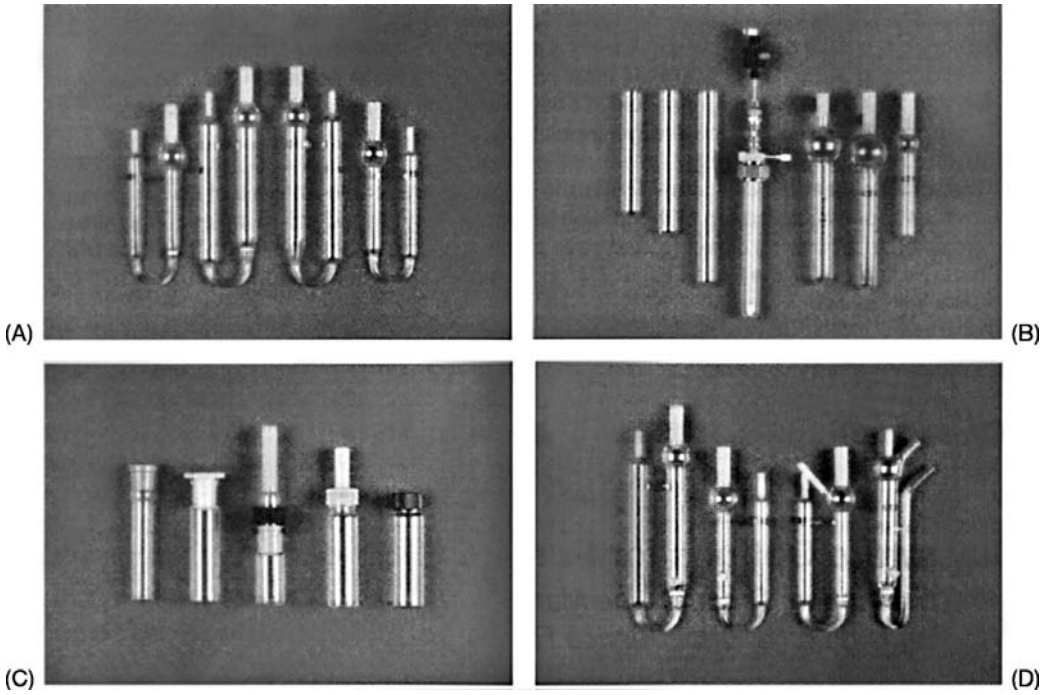


Fig. 2.28 Glass apparatus for the purge and trap technique (Tekmar).
(A) U-tube with/without frit (5 mL and 25 mL sizes).
(B) Needle sparger: left: single use vessels, middle: glass needle with frits, right: vessels with foam retention, 5 mL, 20 mL and 25 mL volumes.
(C) Special glass vessels: 25 mL flat-bottomed flask, 40 mL flask with seal, 20 mL glass (two parts) with connector, 40 mL glass with flange, 40 mL screw top glass.
(D) U-tube for connection to automatic sample dispensers: left: 25 mL and 5 mL vessel with side inlet, right: 5 mL vessel with upper inlet for sample heating, 25 mL special model with side inlet.

and thus better GC resolution and an improvement in the sensitivity of the whole procedure.

The Dry Purge Phase
To remove water from a hydrophobic adsorption trap (e.g. with a Tenax filling) a dry purge phase is introduced. During this step most of the water condensed in the trap is blown out by dry carrier gas. Purge times of ca. 6 min are typical.

2. The Desorption Phase

During the desorption phase the trap is heated and subjected to a backflush with carrier gas. The reversal of the direction of the gas flow is important in order to desorb the analytes in the opposite direction to the concentration by the trap. In this way narrow peak bands are obtained.

The time and temperature of the desorption phase affect the chromatography of the substances to be analysed. The desorption time should be as short as possible but sufficient to

transfer the components quantitatively on to the GC column. Most of the analytes are transferred to the GC column during the first minute of the desorption step. The desorption time is generally 4 min.

The temperature of the desorption step depends on the type of adsorbent in the trap (Table 2.10). The most widely used adsorbent, Tenax, desorbs very efficiently at 180 °C without forming decomposition products. The peak shape of compounds eluting early can be improved by inserting a desorb-preheat step. Here the trap is preheated to a temperature near the desorption temperature before the valve is switched for desorption and before the gas flows freely through the trap. Gas is not passed through the trap during the preheating step, but the analytes are nevertheless desorbed from the carrier material. When the gas stream is passed through the trap after switching the valve, it purges the substances from the trap in a concentrated carrier gas cloud. Highly volatile compounds which are not focused at the beginning of the column thus give rise to a narrower peak shape. A preheating temperature of 5 °C below the desorption temperature has been found to be favourable.

VOCARB material, which is used in all current applications involving volatile halogenated hydrocarbons, can be desorbed at higher temperatures than Tenax (up to 290 °C). At higher desorption temperatures, however, the possibility of catalytic decomposition of some substances must be taken into account (see also Section 2.1.5).

Moisture Removal

Water is driven out of aqueous or moist samples, most of which is disposed of by the dry purge step, particularly in Tenax adsorption traps. Residual moisture would be transferred to the GC column during the desorption step. As the resolution of highly volatile substances on capillary columns would be impaired and the detection by the mass spectrometer would be affected, additional devices are used to remove the water. A moisture control system (MCS) found as a dedicated device in earlier purge or trap systems guaranteed maximum efficiency and retention of the residual moisture during the desorption process (Fig. 2.29, see p. 47).

If the dew point for water is reached in the MCS, a stationary water phase is formed which, for example, for BTEX and volatile halogenated hydrocarbons, the analytes can pass through unaffected. Polar components can also pass through the MCS system at moderate temperatures with a short retardation. When the desorption phase is finished, the tubing of the MCS is dried by baking out in the countercurrent (Fig. 2.30, see p. 48).

In particular, where the purge and trap technique is used in capillary gas chromatography and when using ECD or mass spectrometers as detectors, reliable removal of the moisture driven out is necessary.

3. Baking Out Phase

When required, the trap can be subjected to a baking out phase (trap-bake mode) after the desorption of the analytes. During desorption involatile organic compounds are released from the trap at elevated temperatures and driven off. Baking out conditions the trap material for the next analysis.

Table 2.10 List of trap materials used in the purge and trap procedure with details of applications and recommended analysis parameters.

Trap No.	Adsorbent	Application	Drying possible?	Drying time [min]	Desorb pre-heat [°C]	Desorb temp. [°C]	Bake-out temp. [°C]	Bake-out time [min]	Conditioning temp. [°C] time [min]	Remarks
1	Tenax	All substances down to CH_2Cl_2	Yes	2–6	220	225	230	7–10	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
2	Tenax Silica gel	All substances except freons	No	–	220	225	230	10–12	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
3	Tenax Silica gel Activated charcoal	All substances including freons	No	–	220	225	230	10–12	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
4	Tenax Activated charcoal	All substances down to CH_2Cl_2 and gases	No	–	220	225	230	7–10	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
5	OV-1 Tenax Silica gel Activated charcoal	All substances including freons	No	–	220	225	230	10–12	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
6	OV-1 Tenax Silica gel	All substances except freons	No	–	220	225	230	10–12	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene

Table 2.10 (continued)

Trap No.	Adsorbent	Application	Drying possible?	Drying time [min]	Desorb pre-heat [°C]	Desorb temp. [°C]	Bake-out temp. [°C]	Bake-out time [min]	Conditioning temp. [°C] time [min]	Remarks
7	OV-1 Tenax	All substances down to CH_2Cl_2	Yes	2–6	220	225	230	7–10	230 10	Low response with brominated substances, high back pressure, background with benzene, toluene, ethylbenzene
8	Carbopak B Carbosieve SIII	All substances including freons	Yes	11	245	250	260	4–10	260 20–30	Losses of CCl_4
9	VOCARB 3000	All substances including freons	Yes	1–3	245	250	260	4	290 4 h	Response factors see Table 2.11
10	VOCARB 4000	All substances including freons	Yes	1–3	245	250	260	4	270 4 h	Low response with chlorinated compounds, high back pressure, quantitative losses of chloroethyl vinyl ethers
11	BTEXTRAP Carbopak B Carbopak C	All substances including freons	Yes	1–3	245	250	260	4	270	–
12	Tenax GR Graphpac-D	All substances including freons	Yes	1–4	245	250	260	12	–	–

Trap nos. 1–8: Tekmar; trap nos. 9–11: SUPELCO; trap no. 12: Alltech

Table 2.11 Response factors and standard deviations for components with EPA method 624 using a VOCARB 3000 trap and 5 ml of sample (250 °C desorption temperature, reference bromochloromethane/difluorobenzene, concentrations in ppb, Supelco).

Compound	20	50	100	150	200	Average	Standard deviation	% Relative standard deviation
Methyl chloride	0.933	0.962	0.984	0.906	1.287	1.014	0.139	13.7
Vinyl chloride	1.037	1.167	1.466	1.536	1.333	1.308	0.185	14.1
Methyl bromide	1.018	1.257	1.079	1.077	1.290	1.144	0.108	9.5
Ethyl chloride	0.419	0.582	0.503	0.557	0.536	0.519	0.056	10.9
Trichlorofluoromethane	0.972	1.533	1.266	1.491	1.507	1.354	0.213	15.8
1,1-Dichloroethylene	0.873	1.207	1.203	1.264	1.180	1.145	0.139	12.1
Dichloromethane	1.550	1.134	1.231	1.325	1.11	1.270	0.159	12.5
1,2-Dichloroethylene	0.973	0.991	0.944	1.124	1.007	1.008	0.062	6.1
1,1-Dichloroethane	2.064	2.093	2.013	2.186	2.175	2.106	0.066	3.1
Chloroform	2.513	2.368	2.193	2.419	2.373	2.373	0.104	4.4
Tetrachloroethane	1.205	1.472	1.302	1.325	1.476	1.356	0.105	7.7
Carbon tetrachloride	1.067	1.426	1.350	1.240	1.335	1.284	0.124	9.6
Benzene	0.947	0.949	0.906	0.973	1.024	0.960	0.039	4.0
1,2-Dichloroethane	0.046	0.045	0.047	0.050	0.049	0.047	0.002	4.2
Trichloroethylene	0.412	0.495	0.485	0.504	0.540	0.487	0.042	8.6
1,2-Dichloropropane	0.535	0.530	0.513	0.537	0.549	0.533	0.012	2.2
Bromodichloromethane	0.082	0.080	0.084	0.088	0.087	0.084	0.003	3.4
2-Chloroethyl vinyl ether	0.043	0.039	0.043	0.047	0.041	0.043	0.003	6.2
cis-1,3-Dichloropropene	0.898	0.977	1.001	1.086	1.082	1.009	0.070	6.9
Toluene	0.861	1.208	1.141	1.250	1.283	1.149	0.151	13.2
trans-1,3-Dichloropropene	0.312	0.300	0.280	0.299	0.289	0.296	0.011	3.7
1,1,2-Trichloroethane	0.593	0.486	0.488	0.526	0.507	0.520	0.039	7.5
Tetrachloroethylene	0.364	0.488	0.492	0.497	0.558	0.480	0.063	13.2
Dibromochloromethane	0.818	0.712	0.731	0.787	0.785	0.767	0.039	5.1
Chlorobenzene	1.135	1.116	1.117	1.183	1.242	1.159	0.048	4.2
Ethylbenzene	0.561	0.508	0.476	0.504	0.543	0.518	0.030	5.8
Bromoform	1.070	0.882	0.920	1.013	0.918	0.961	0.070	7.3
1,1,2,2-Tetrachloroethane	0.082	0.069	0.070	0.078	0.065	0.073	0.006	8.4
1,3-Dichlorobenzene	1.277	1.215	1.237	1.338	1.451	1.304	0.085	6.5
1,4-Dichlorobenzene	1.397	1.279	1.291	1.391	1.509	1.374	0.084	6.1
1,2-Dichlorobenzene	1.351	1.233	1.188	1.284	1.350	1.281	0.064	5.0

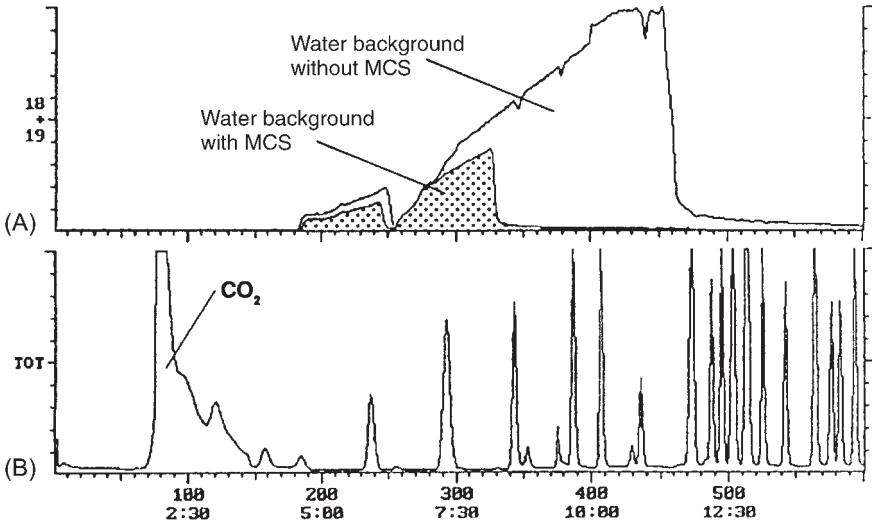


Fig. 2.29 GC/MS analysis using purge and trap.
(A) Mass chromatogram for water (m/z 18+19) with and without removal (without MCS) of the moisture driven out.
(B) Total ion chromatogram with volatile substances (volatile halogenated hydrocarbons) after removal of the water (with MCS).

Coupling of Purge and Trap with GC/MS Systems

There are two fundamentally different possibilities for the installation of a purge and trap instrument coupled to a GC/MS system, which depend on the areas of use and the number of purge and trap samples to be processed per day.

In many laboratories the most flexible arrangement involves connecting a purge and trap system to the gas supply of the GC injector. The purge and trap concentrator is connected in such a way that either manual or automatic syringe injection can still be carried out. The carrier gas is passed from the GC carrier gas regulation to the central 6-port valve of the purge and trap system (see Fig. 2.27). From here the carrier gas flows back through the continuously heated transfer line to the injector of the gas chromatograph. A particular advantage of this type of installation lies in the fact that the injector can still be used for liquid samples. This allows the manual injection of control samples or the operation of liquid autosamplers. In addition it guarantees that the whole additional tubing system remains free from contamination even when the purge and trap instrument is on standby.

When the sample is transferred to the GC column it should be ensured that adequate focusing of the analytes is achieved. For this purpose GC columns are available with film thicknesses of ca. 1.8 µm or more, which have already been used for the analysis of volatile halogenated hydrocarbons using GC/MS systems (types 502, 624 or volatiles). The injector system is operated using a split ratio which can be selected depending on the detection limit required.

A complete splitless injection of the analytes on to the GC column can only be achieved with cryofocusing (see also Section 2.2.3.7). Here the column is connected to the transfer

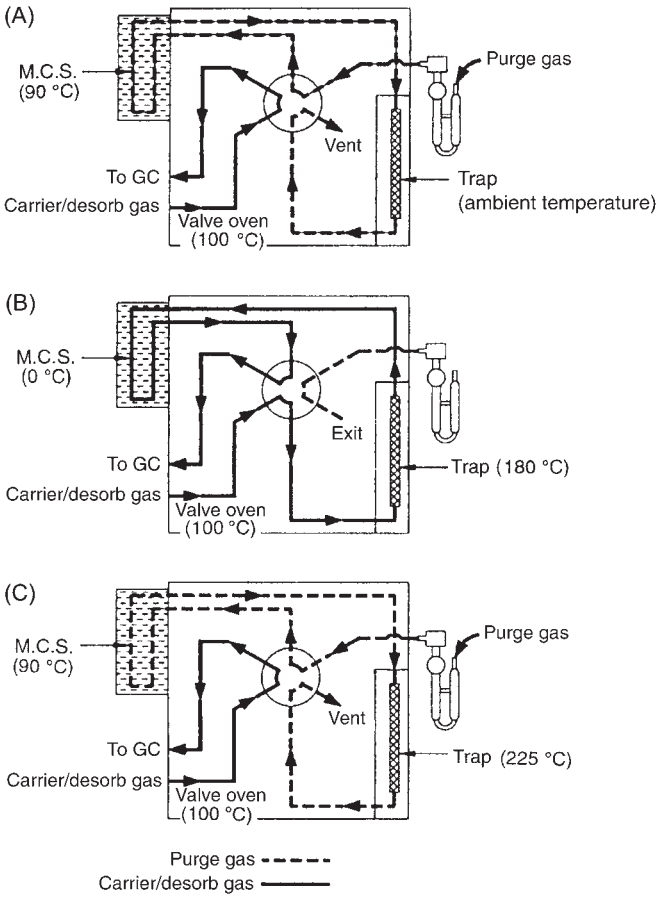


Fig. 2.30 Scheme showing the three phases of the purge and trap cycle with water removal (MCS, Tekmar). (A) Purge phase, (B) Desorption phase, (C) Baking out phase

line or the central 6-port valve of the purge and trap apparatus in such a way that the beginning of the column passes through a zone ca. 10 cm long which can be cooled by an external cooling agent, such as liquid CO_2 or N_2, to –30 °C to –150 °C. All components which reach the GC column after desorption from the trap can be frozen out in a narrow band at the beginning of the analytical column. This enables the highest sensitivities to be achieved, in particular with mass spectrometers. As large quantities of water are also concentrated together with the analytes in the case of moist or aqueous samples, removal of moisture in the desorption phase is particularly important with this type of coupling. This can, for example, be effected by means of a moisture control system (MCS) (Fig. 2.30). Insufficient removal of water leads to the deposition of ice and blockage of the capillaries. The consequences are poor focusing or complete failure of the instrument.

2.1.5.3 Headspace versus Purge and Trap

Both instrumental extraction techniques have specific advantages and disadvantages when coupled to GC and GC/MS. This should be taken into consideration when choosing an analysis procedure. In particular, the nature of the sample material, the concentration range for the measurement and the work required to automate the analyses for large numbers of samples play a significant role. The recovery and the partition coefficient, and thus the sensitivity which can be achieved, are relevant to the analytical assessment of the procedure. For both procedures it must be possible to vaporise the substances being analysed below 150 °C and then to partition them in the gas phase. The vapour pressure and solubility of the analytes, as well as the extraction temperature, affect both procedures (Fig. 2.31).

How then do the techniques differ? For this the terms recovery and sensitivity must be defined. For both methods, the recovery depends on the vapour pressure, the solubility and the temperature. The effects of temperature can be dealt with because it is easy to increase the vapour pressure of a compound by raising the temperature during the vaporisation step. With the purge and trap technique the term *percentage recovery* is used. This is the amount of a compound which reaches the gas chromatograph for analysis relative to the amount which was originally present in the sample. If a sample contains 100 pg benzene and 90 pg reach the GC column, the *percentage recovery* is 90%. In the static headspace technique, a simple expression like this cannot be used because it is possible to use a large number of types of vial, injection techniques and injection volumes, which always apply aliquots to the analysis.

The commonly used term connected with the static headspace method is the *partition coefficient*, as mentioned above. The partition coefficient is defined as the quantity of a compound in the sample divided by the quantity in the vapour phase. Therefore, the smaller the partition coefficient is, the higher the sensitivity. It should be noted that a partition coefficient is only valid for the analysis parameters at which it has been determined. These include the temperature, the size of the sample vial, the quantity of sample (weight and

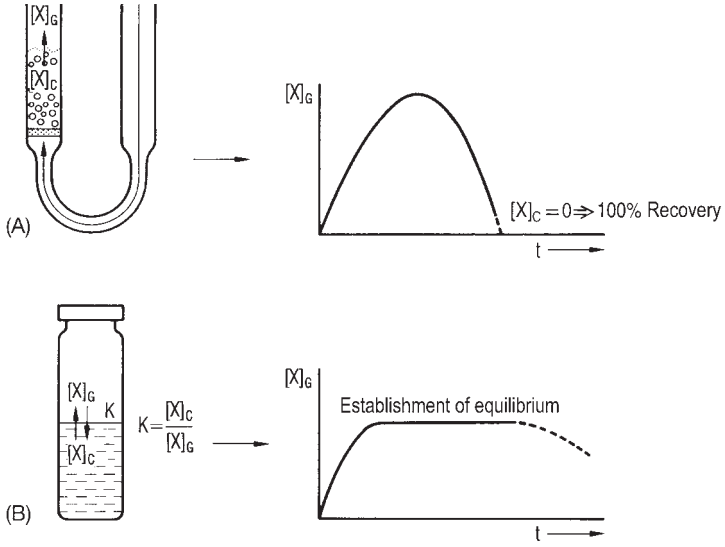


Fig. 2.31 Comparison of the purge and trap and static headspace techniques.
(A) Purge and trap, (B) Static headspace

volume), the nature of the matrix and the size of the headspace. After the partition coefficient, the quantity injected is the next parameter affecting headspace sensitivity. The quantity injected is limited by a range of factors. For example, only a limited quantity can be removed from the headspace of a closed vessel. Attempts to remove a larger quantity of sample vapour would lead to a partial vacuum in the sample vessel. This is extraordinarily difficult to reproduce. Furthermore, only a limited quantity can be injected on to a GC column without causing peak broadening. For larger quantities of sample cryofocusing is necessary. Capillary columns require cold trapping at injection quantities of more than ca. 200 µL.

An alternative injection system involves pressure balanced injection. Here a needle is passed through the septum into the headspace in order to create pressure in the headspace vial which is at least as high as the column pre-pressure. After equilibrium has been established this pressure is released during injection on to the GC column over a short programmable time interval. This allows larger quantities to be injected. It is impossible to measure the exact quantity injected; however, the reproducibility of this method is extremely high.

First Example: Volatile Halogenated Hydrocarbons

A comparison with actual concentration values makes the differences between the static headspace and purge and trap techniques very clear. The percentage recovery for the purge and trap technique is, for example, for an environmental sample of volatile halogenated hydrocarbons: 95% for chloroform, 92% for bromodichloromethane, 87% for chlorodibromomethane and 71% for bromoform. For a sample of 5 mL, which contains 1 ppb of each substance (i. e. a total quantity of 5 ng of each compound), 4.75 ng, 4.6 ng, 4.35 ng and 3.55 ng are recovered. In a typical static headspace system with a sample vessel of 21 mL containing 15 mL of sample at 70 °C the partition coefficients for the corresponding volatile halogenated hydrocarbons are: 0.3, 0.9, 1.5 and 3.0. This means that the quantities in the 5 ml of headspace are: 11.5 ng, 7.9 ng, 6.0 ng and 3.8 ng. On injection of 20 µL of the headspace gas mixture on to a standard capillary column, the quantities injected are: 0.05 ng, 0.03 ng, 0.02 ng and 0.015 ng. For a larger injection (0.5 mL) using cryofocusing, the quantities injected are: 1.2 ng, 0.8 ng, 0.6 ng, and 0.4 ng. The purge and trap technique is therefore more sensitive than the static headspace procedure for these volatile halogenated hydrocarbons by factors of 4.1, 5.8, 7.2 and 9.3 (Table 2.12).

Static Headspace	**Dynamic Headspace**
Headspace	Purge and trap
Extraction: waiting for establishment of equilibrium	Continuous disturbance of the equilibrium by the purge gas
Intermediate steps: closed vessel at constant temperature	Enrichment of the substances driven off in a trap
Sample injection: Removal of a preselected volume from the headspace	Thermal desorption from the trap

Table 2.12 Lower application limits [µg/L] for headspace and purge and trap techniques in the analysis of water from the river Rhine (after Willemsen, Gerke, Krabbe).

Substance	Lower application limits [µg/L] for headspace	Lower application limits [µg/L] for purge and trap
Dichloromethane	1	0.5
Chloroform	0.1	0.05
1,1,1-Trichloroethane	0.1	0.02
1,2-Dichloroethane	5	0.5
Carbon tetrachloride	0.1	0.02
Trichloroethylene	0.1	0.05
Bromodichloromethane	0.1	0.02
1,1,2-Trichloroethane	0.5	0.5
Dibromochloromethane	0.1	0.05
Tetrachloroethylene	0.1	0.02
1,1,2,2-Tetrachloroethane	0.1	0.02
Bromoform	0.1	0.2
1,1,2,2-Tetrachloroethane	0.1	0.02
Benzene	5	0.1
Toluene	5	0.05
Chlorobenzene	5	0.02
Ethylbenzene	5	0.1
m-Xylene	5	0.05
p-Xylene	5	0.05
o-Xylene	5	0.1
Triethylamine	–	0.5
Tetrahydrofuran	–	0.5
1,3,5-Trioxan	–	0.5

Second Example: Cooking Oils

For compounds with poorer recoveries, e.g. in the analysis of free aldehydes in cooking oils, the difference is even clearer. At 150 °C the purge and trap analysis gives recoveries of 47%, 59% and 55% for butanal, 2-hexenal and nonan. For a sample of 0.5 mL, containing 100 ppb of the compounds, 24 ng, 30 ng and 28 ng are recovered. In the static headspace analysis, the partition coefficients for these compounds at 200 °C are all higher than 200. Assuming the value of 200, the quantity of each of these compounds in 5 mL of headspace is 0.7 ng. For an injection of 0.5 mL the quantity injected is therefore only 0.07 ng. The differences in sensitivity favouring purge and trap analysis are therefore 343, 428 and 400!

Third Example: Residual Solvents in Plastic Sheeting

Comparable ratios are obtained in the analysis of a solid sample, for example, the analysis of residual solvents in a technical product. A run using the purge and trap technique and 10 mL of sample at 150 °C gave a recovery of 63% for toluene. The sample contained 1.6 ppm, which corresponds to a quantity of 101 ng. The partition coefficient in the static headspace technique at 150 °C (for a sample of 1 g) is 95. The quantity of residual solvent in 19 mL of headspace is therefore 17 ng. For an injection of 0.5 mL, 0.4 ng are injected. The quantity injected is therefore smaller by a factor of 250 than that in the purge and trap analy-

sis. Furthermore, the reproducibility of this analysis was 7% for the purge and trap technique and 32% for the static headspace analysis (relative standard deviation).

The theoretically achievable or effectively necessary sensitivity is not the only factor deciding the choice of procedure. The specific interactions between the analytes and the matrix, the performance of the detectors available, and the legally required detection limits play a more important role.

Besides the sensitivity, there are other aspects which must be taken into account when comparing the purge and trap and static headspace techniques.

The static headspace technique is very simple and relatively quick. The procedure is well documented in the literature, and for many applications the sensitivity is more than adequate, so that its use is usually favoured over that of the purge and trap technique. There are areas of application where good results are obtained with the static headspace technique which cannot be improved upon by the purge and trap method. These include: the determination of alcohol in blood, of free fatty acids in cell cultures, of ethanol in fermentation units or drinks, and residual water in polymers. This also applies to studies on the determination of ionisation constants of acids and bases and the investigation of gas phase equilibria.

However, for many other samples specific problems besides sensitivity arise on use of the static headspace technique, which can be overcome using the purge and trap procedure. In every static headspace system all the compounds present in the headspace are injected, not only the organic analytes. This means that an air peak is also obtained. All air contaminants (a widely occurring problem in many laboratories) are visible and oxygen can impair the service life of the capillary column at high temperatures. In addition a larger quantity of water is injected than in the purge and trap method. For drinks containing carbonic acid CO_2 can lead to the build-up of excess pressure. Even at room temperature an undesirably high pressure can build up in the sample vials, which leads to flooding of the GC column during the analysis. In addition, a very large quantity of CO_2 is injected. Heating the sample enhances these effects. For safety reasons sample vials with safety caps to guard against excess pressure have to be used in these cases. Dust particles lead to further problems in the case of powder samples. To achieve the necessary sensitivity for an analysis, the sample often has to be heated to a temperature which is higher than that necessary in the purge and trap technique. This can lead to thermal decomposition, which is frequently observed with foodstuffs. In addition, oxygen cannot be eliminated from the sample before heating in a static headspace system. It is therefore impossible to prevent oxidation of the sample contents. During the thermostatting phase additional problems arise as a result of the septum used. Substances emitted from the septum (e.g. CS_2 from butyl rubber septums) falsify the chromatogram. The permeability of the septum to oxygen presents a further hazard.

Advantages of the Static Headspace Technique

- The static headspace can be easily automated. All commercial headspace samplers operate automatically for 20, 40 or 100 samples. For manual qualitative preliminary samples a gas-tight syringe is satisfactory.
- Samples, which tend to foam or contain unexpectedly high concentrations of analyte, do not generally lead to faults or cross-contamination.
- All sample matrices (solid, liquid or gaseous) can be used directly usually without expensive sample preparation.

- Headspace samplers are often readily portable and, when required, can be rapidly connected to different types of GC instruments.
- The sample vessels (special headspace vials with caps) are only intended for single use. There is no additional workload of cleaning glass equipment and hence cross-contamination does not occur.
- Headspace vials can be filled and sealed at the sampling point outside the laboratory in certain cases. This dispenses with transfer of the sample material and eliminates the possibility of loss of sample. Moreover, the danger of inclusion of contamination from the laboratory environment is reduced.
- Because of the high degree of automation, the cost of analysing an individual sample is kept low.

Disadvantages of the Static Headspace Technique

- On filling headspace vials a corresponding quantity of air is enclosed in the vial unless filling is carried out under an inert gas atmosphere. However, this is very expensive. During thermostatting undesirable side reactions can occur as a result of atmospheric oxygen in the sample.
- During injection from headspace vials air regularly gets into the GC system and can affect sensitive column materials.
- In the case of moist or aqueous samples, a considerable quantity of water vapour gets on to the column. This requires special measures to ensure the integrity of the early eluting peaks.
- On use of mass spectrometers special attention must be paid to the stability of ion sources. On insufficient heating the surface of the source and the lens system become increasingly coated with moisture in the course of the work (caused by the injection of water vapour). As a result the focusing of the mass spectrometer can change and this can impair quantitative work.
- Quantitation is matrix-dependent. Standardisation measures are necessary, such as addition of carbonate, internal standards and MHE procedures.
- The ability of substances to be analysed is limited by the maximum possible filling of the headspace vials and by the partition coefficients. For small partition coefficients larger quantities of sample do not lead to an increase in sensitivity. If the maximum possible equilibration temperature is being used, it is almost impossible to increase the sensitivity further.
- The quantity injected is limited and where the sensitivity is insufficient, multiple extraction with cryofocusing is necessary.
- Undesired blank values can be obtained through contaminated air (laboratory air) which gets into the headspace vial or through bleeding of the septum caps.
- Excess pressure can cause headspace vials to burst (e.g. with drinks containing carbonic acid or high equilibration temperatures). This always puts instruments out of operation for long periods and results in considerable clean-up costs. Only the use of special vial caps (with spring rings) together with special sealed vials which can release excess pressure into the atmosphere prevents bursting.

Advantages of the Purge and Trap Technique

- The sample quantity (maximum 25–50 ml) can easily be adapted to give the required sensitivity.
- A pre-purge step can remove atmospheric oxygen from the sample, even at room temperature if required.
- No septum or other permeable material is placed in the way of the sample.
- Substances with high partition coefficients in the static headspace can be determined with good yields.
- There are no excess pressure problems. The entire gas stream is passed pressure-free through the trap to the vent.
- Water vapour can be kept completely out of the GC/MS system by means of a dry purge step and a moisture control system (MCS).
- In automatic operations mixing in an internal standard without manual measures is quite straightforward.

Disadvantages of the Purge and Trap Technique

- Foaming samples require special treatment, the headspace sweep technique.
- The purge vessels (made of glass) must be cleaned carefully. Economical single-use vessels are currently only available in polymer materials.
- Larger quantities of sample require longer purge times.
- For highly contaminated samples the breakthrough volume of the trap must be taken into consideration. If the baking out step is inadequate, the danger of a carry-over exists.
- Coupling with capillary GC necessitates the use of small split ratios or cryofocusing.

2.1.6 Adsorptive Enrichment and Thermodesorption

To determine volatile organic compounds, GC/MS coupling is the method of choice. In the analysis of air or gas samples an extraordinarily large number of components of the most widely differing classes of compounds and over a very wide concentration range have to be considered. Usually, the concentration of the substances of interest is too low for the direct measurement of an air sample, and therefore enrichment on suitable adsorbents is necessary. The concentration on solid adsorption material allows the accumulation of organic components from large volumes of gas. Typical areas of use include soil air, workplace monitoring, gases from landfill sites, air pollution, and air inside buildings.

Besides a high storage capacity to achieve high breakthrough volumes (BTV), the adsorption material is expected to have a low affinity for water (air moisture). This is not only important for GC/MS coupling. Neither water nor CO_2 should have a negative effect on the breakthrough volume of the organic components. The surrounding air generally has a high moisture content. However, particular precautions must be taken in the case of combustion gases. The desorption of the enriched components from the carrier materials used should be complete and without thermal changes.

The adsorption material used must have a high thermal stability and must not contribute to the background of the analysis. The expected adsorption and stability properties should not change for a large number of analyses, even on repeated use.

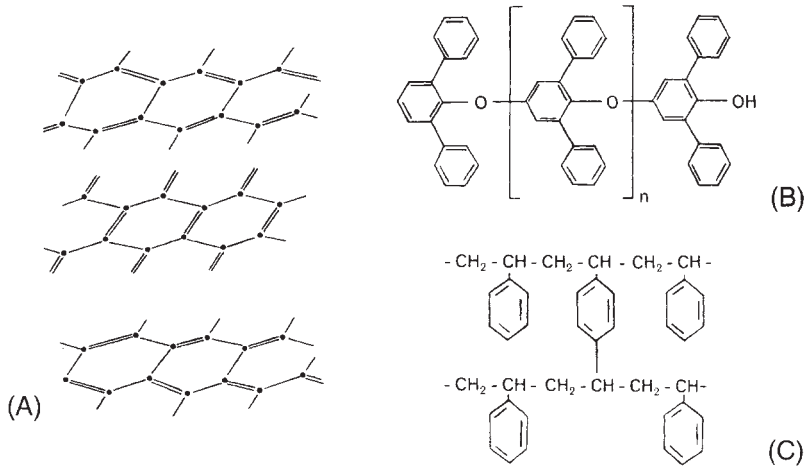


Fig. 2.32 Surface model for common adsorbents (Supelco).
(A) Carbotrap, surface area ca. 100 m^2/g, uniform charge distribution over all carbon atom centres.
(B) Tenax (2,6-diphenyl-p-phenylene oxide), surface area ca. 24 m^2/g, nonuniform charge distribution, the charge is essentially localised on the oxygen atoms. (Tenax-TA has replaced Tenax-GC as a new material of higher purity; Tenax-GR is a graphitised modification).
(C) Amberlite XAD-2, surface area ca. 300 m^2/g, nonuniform charge distribution, less polar than Tenax (XAD-4, ca. 800 m^2/g).

In air analysis adsorption materials, such as Tenax, Carbotrap and XAD resins, are generally used (see Fig. 2.32 and Table 2.13). Pure activated charcoal is indeed an outstanding adsorbent for all organic compounds; however, these can only be sufficiently desorbed when displaced using liquid solvents. Complete thermal desorption requires extremely high temperatures (>600 °C). This can lead to pyrolytic decomposition of the organic compounds which are then no longer detected in the residue analysis.

A recent development for retaining the high adsorptivity of activated charcoal with simultaneously favourable desorption properties involves the use of graphitised carbon black (Carbotrap, Carboxen). Its surface consists of graphite crystals. The associated hydrophobic properties and the exploitation of nonspecific interactions have led to wide use of these carrier materials.

Like Tenax, graphitised carbon blacks also have a low affinity for water. These adsorption materials can be dried with a dry gas stream, e. g. the GC carrier gas (in the direction of adsorption!), without significant loss of material (dry purge).

VOCARB traps are special combinations of the adsorption materials Carbopack (graphitised carbon black, GCB) and Carboxen (carbon molecular sieve). These combinations have been optimised in the form of VOCARB 3000 and VOCARB 4000 for volatile and involatile compounds respectively, corresponding to the EPA methods 624/1624 and 542.2. VOCARB 4000 exhibits higher adsorptivity for less volatile components, such as naphthalenes and trichlorobenzenes. However, it shows catalytic activity towards 2-chloroethyl vinyl ether (complete degradation!), 2,2-dichloropropane, bromoform and methyl bromide.

Volatile polar components are enriched on highly polar carrier materials. The combination of Tenax TA and silica gel has proved particularly successful for the enrichment of polar compounds.

Table 2.13 Adsorption materials and frequently described areas of use.

XAD-4	C_1/C_2-chlorinated hydrocarbons R11 halogenated narcotics vinyl chloride ethylene oxide styrene
Tenax TA 35-60 mesh	for boiling points 80–200 °C C_1/C_2-chlorinated hydrocarbons general solvents, volatile halogenated hydrocarbons BTEX phenols
Porapak	high-boiling, nonpolar substances halogenated narcotics ethylene oxide
Carbotrap/VOCARB	for boiling points –15 to –120 °C C_1/C_2-chlorinated hydrocarbons volatile halogenated hydrocarbons BTEX styrene
Molecular sieve 5 Å	N_2O

To cover a wider range of molecular sizes various adsorption materials are combined with one another. For example, the Carbotrap multibed adsorption tube (Fig. 2.33) consists of three materials: Carbotrap C with its low surface area of 12 m^2/g is used to enrich high molecular weight components, such as alkylbenzenes, polyaromatic hydrocarbons or PCBs, directly at the inlet. All the more volatile substances pass through to the subsequent layers. A layer of Carbotrap particles (Carbotrap B) separated by glass wool is characterised by the higher particle size of 20/40 mesh. Volatile organic substances, in particular, are excellently adsorbed on Carbotrap and thermally desorbed with high recoveries. To adsorb C_2-hydrocarbons Carbosieve S-III material with a particularly high surface area of 800 m^2/g and a pore

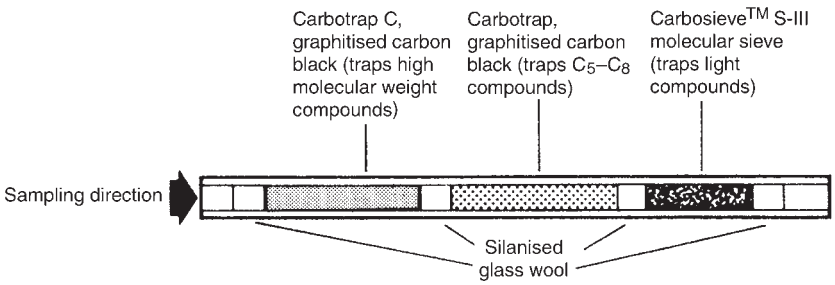


Fig. 2.33 Multibed adsorption/desorption tube Carbotrap 300 (Supelco).
Carbotrap, Carbotrap C: graphitised carbon black, GCB, surface area ca. 12 m^2/g.
Carbosieve S-III: carbon molecular sieve, surface area ca. 800 m^2/g, pore size 15–40 Å

Table 2.14 Breakthrough volumes [L] for Carbotrap 300 adsorption/desorption tubes (Supelco).

Substance	Carbosieve S-III	Carbotrap	Carbotrap C
Vinyl chloride	158		
Chloroform		1.1 [a)]	
1,2.Dichloroethane		0.4	
1,1,1-Trichloroethane		2.7 [a)]	
Carbon tetrachloride		4.7 [a)]	
1,2.Dichloropropane		6.8	
Trichloroethylene		2.5	
Bromoform		1.7	
Tetrachloroethylene		2.2	
Chlorobenzene		316	
n-Heptane		262	
1-Heptene		284 [a)]	
Benzene		2.3	
Toluene		130	
Ethylbenzene			12.9
p-Xylene			11.2
m-Xylene			11.0 [a)]
o-Xylene			11.0 [a)]
Cumene			27.8 [a)]

a) Theoretical value.

size of 15–40 Å is placed at the end of the adsorption tube (patent, BASF AG, Ludwigshafen) (Table 2.14). The hydrophobic properties of the Carbosieve material allow its use in atmospheres with high moisture contents.

2.1.6.1 **Sample Collection**

Sample collection can be either passive or active (Fig. 2.34). For passive collection diffusion tubes with special dimensions are used. The content of substances in the surrounding air is integrated, taking the collection time into account.

Active collection devices require a calibrated pump with which a predetermined volume is drawn through the adsorption tube. Having estimated the expected concentrations, for example for indoor air 100 mL/min and for outdoor air 1000 mL/min, the air is drawn through the prepared adsorption tube over a period of 4 h. After sample collection the adsorption tube must be closed tightly to exclude additional uncontrolled contamination.

Brown and Purnell carried out thorough investigations on the determination of breakthrough volumes. The latter generally vary widely with the collection rate. On use of Tenax as the adsorbent the ideal collection rate is 50 mL/min, in any case, however, between 5 and 600 mL/min. Moisture does not affect the breakthrough volumes with Tenax (unlike other porous materials). Furthermore, sample collection is greatly affected by temperature. An increase in temperature increases the breakthrough volume (ca. every 10 K doubles the volume).

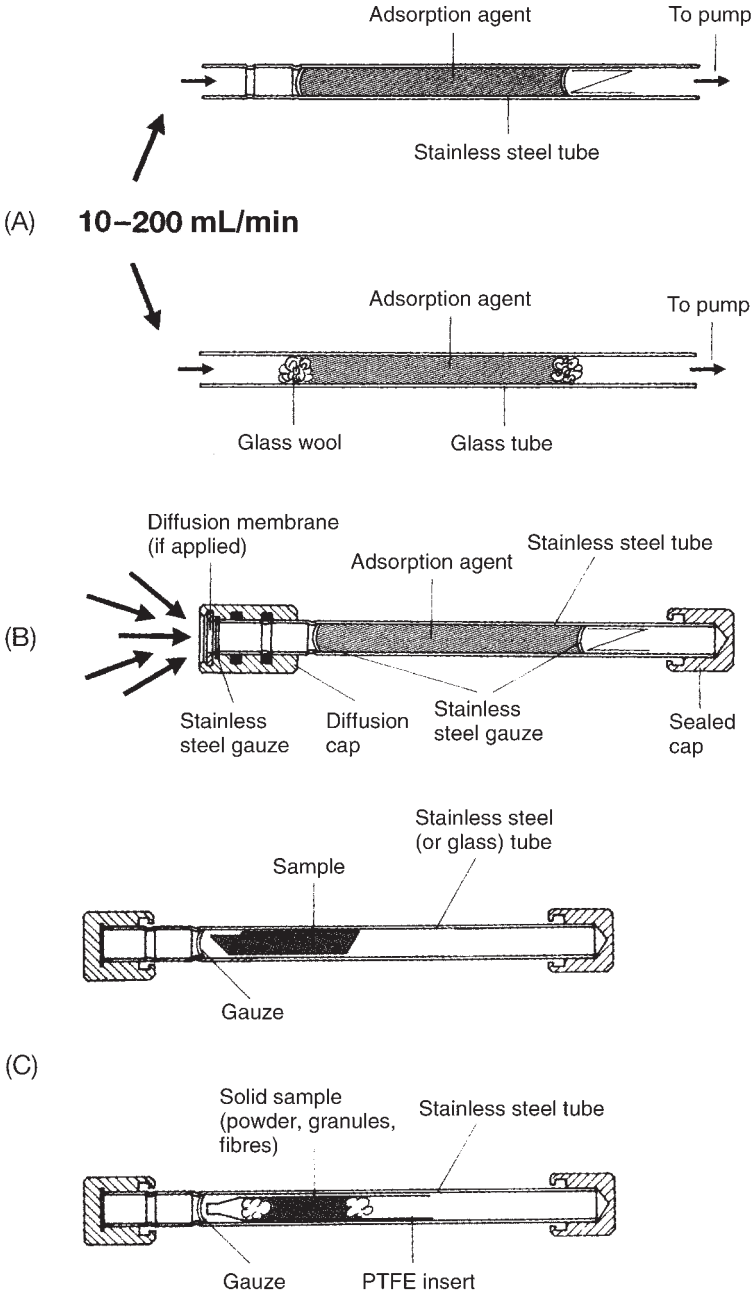


Fig. 2.34 Sample collection with thermodesorption tubes (Perkin-Elmer).
(A) Active sample collection with pump (e.g. personal air sampler),
(B) Passive sample collection by diffusion,
(C) Direct introduction of solid samples

2.1.6.2 **Calibration**

In the calibration of thermodesorption tubes the same conditions should predominate as in sample collection. Methods such as the liquid application of a calibration solution to the adsorption materials or the comparison with direct injections have been shown to be unsatisfactory. Alternatively Certified Reference Standards (CRS) are available (e.g. Markes Int., UK). CRS tubes are recommended in many key standard methods (e.g. US EPA Method TO-17) for auditing purposes and as a means of establishing analytical quality control. CRS tubes are often certified traceable to primary standards, have a minimum shelf life of typically 6 month. They are available ready for use with concentrations varying from 10 ng to 100 μg per component. Chromatograms from a shipping blank, and an example analysis of a CRS tube should be supplied along with the CRS Certificate.

CRS tubes are available loaded with benzene, toluene and xylene at levels of 25 ng or 1 çg per component; TO-17 standards at 25 ng per component of benzene, toluene, xylene, dichloromethane, 1,1,1-trichloroethane, 1,2,4-trimethylbenzene, methyl-t-butyl ether, butanol, ethyl acetate, methylethyl ketone. Custom CRS tubes are also available on a variety of sorbents for a wide range of compounds from different vendors.

The process for the preparation of standard atmospheres by continuous injection into a regulated air stream has been described in the VDI guidelines (Fig. 2.35). In this process an individual component or a mixture of the substances to be determined is continuously charged to an injector through which air is passed (complementary gas), using a thermostatted syringe burette. The air quantity (up to 500 ml/min) is adjusted using a mass flow

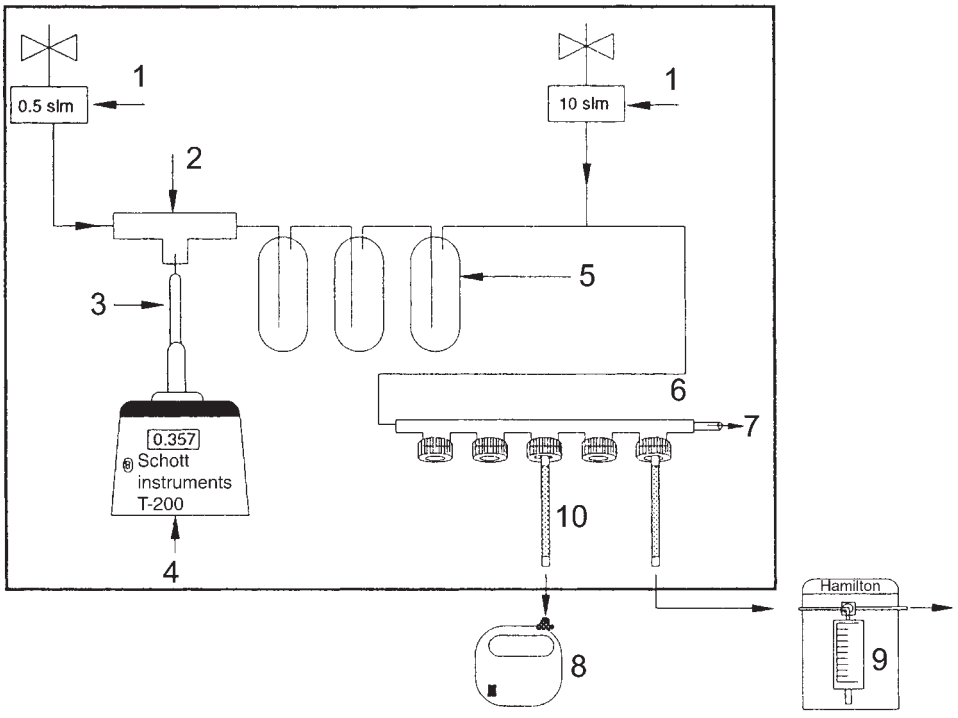


Fig. 2.35 Principle of a calibration unit for thermodesorption.

meter. The complementary gas can be diluted by mixing with a second air stream (dilution gas up to 10 l/min). Moistening the gas can be carried out inside or outside the apparatus. In this way concentrations in the ppm range can be generated. For further dilution, e. g. for calibration of pollution measurements, a separate dilution stage is necessary. The gas samples to be tested are drawn out of the calibration station into a glass tube with several outlets. In this case active or passive sample collection is possible. Continuous injection has the advantage that the preparation of mixtures is very flexible (Tables 2.15 and 2.16).

Table 2.15 Evaluation of test tubes which were prepared using the calibration unit by continuous injection ($n = 10$).

Component	Mean value	Standard deviation	Relative standard deviation [%]
1,1,1-Trichlorethane	562.6	4.95	0.88
Dichloromethane	538.6	7.93	1.47
Benzene	753.3	9.48	1.25
Trichloroethylene	627.3	16.7	2.67
Chloroform	626.6	12.1	1.94
Tetrachloroethylene	698.2	6.11	0.88
Toluene	1074	6.88	0.64
Ethylbenzene	358.1	2.91	0.81
p-Xylene	736.3	4.85	0.66
m-Xylene	731.6	4.77	0.65
Styrene	755.8	4.60	0.61
o-Xylene	389.5	3.49	0.89

Table 2.16 Analysis results of BTEX determination of certified samples after calibration with the calibration unit.

Mass [µg]	Benzene	Toluene	m-Xylene
Measured value	1.071	1.136	1.042
Required value (certified)	1.053	1.125	1.043
Standard deviation (certified)	0.014	0.015	0.015

2.1.6.3 **Desorption**

The elution of the organic compounds collected involves extraction by a solvent (displacement) or thermal desorption. Pentane, CS_2 or benzyl alcohol are generally used as extraction solvents. CS_2 is very suitable for activated charcoal, but cannot be used with polymeric materials, such as Tenax or Amberlite XAD, because decomposition occurs. As a result of displacement with solvents the sample is extensively diluted, which can lead to problems with the detection limits on mass spectrometric detection. With solvents additional contamination can occur. The extracts are usually applied as solutions. The readily automated static head-

space technique can also be used for sample injection. This procedure has also proved to be effective for desorption using polar solvents, such as benzyl alcohol or ethylene glycol monophenyl ether (1% solution in water after Krebs).

In thermal desorption, the concentrated volatile components are released by rapid heating of the adsorption tube and after preliminary focusing, usually within the instrument, are injected into the GC/MS system for analysis (Table 2.17). Automated thermodesorption gives better sensitivity, precision and accuracy in the analysis. The number of manual steps in sample processing is reduced. Through frequent re-use of the adsorption tube and complete elimination of solvents from the analysis procedure, a significant lowering of cost per sample is achieved.

Table 2.17 Desorption temperatures for common adsorption materials and possible interfering components which can be detected by GC/MS.

Adsorbent	Desorption	Maximum temperature	Interfering components
Carbotrap	Up to 330 °C	> 400 °C	Not determined
Tenax	150–250 °C	375 °C	Benzene, toluene, trichloroethylene
Molecular sieve	250 °C	350 °C	Not determined
Porapak	200 °C	250 °C	Benzene, xylene, styrene
VOCARB 3000	250 °C	> 400 °C	Not determined
VOCARB 4000	250 °C	> 400 °C	Not determined
XAD-2/4	150 °C	230 °C	Benzene, xylene, styrene

Thermodesorption has now become a routine procedure because of program-controlled samplers. The individual steps are prescribed by the user in the control program and monitored internally by the instrument. For the sequential processing of a large number of samples, autosamplers with capacities of up to 100 adsorption tubes are commercially available.

The adsorption tubes are fitted with temporary Teflon caps, septums or sealed by use of SafeLok technique, a patented check valve design diffusion locking technology at either end of the tube (Markes Inc., UK). The sealing of the tubes protects the sample from ingress or loss of volatiles at all stages of the monitoring process. Tubes using check valves are not suitable for diffusive monitoring. The Teflon caps are removed inside the instrument before measurement and the adsorption tube is inserted into the desorption oven. Before the measurement is carried out the tightness of the seal is tested by monitoring an appropriate carrier gas pressure for a short time. Carrier gas is passed through the adsorption tubes in the desorption oven at temperatures of up to 400 °C (in the reverse direction to the adsorption!). The components released are stored in a cold trap inside the apparatus. The sample is transferred to the GC column by rapidly heating the cold trap. This two-stage desorption and the use of the multiple split technique enable the measuring range to be adapted to a wide range of substance concentrations. The sample quantity can be adapted to the capacity of the capillary column used through suitable split ratios both before and after the internal cold trap (Fig. 2.36).

Thermodesorption is now usually carried out automatically. The high performance achieved does not mean that it has no disadvantages. A gas sample in an adsorption tube for-

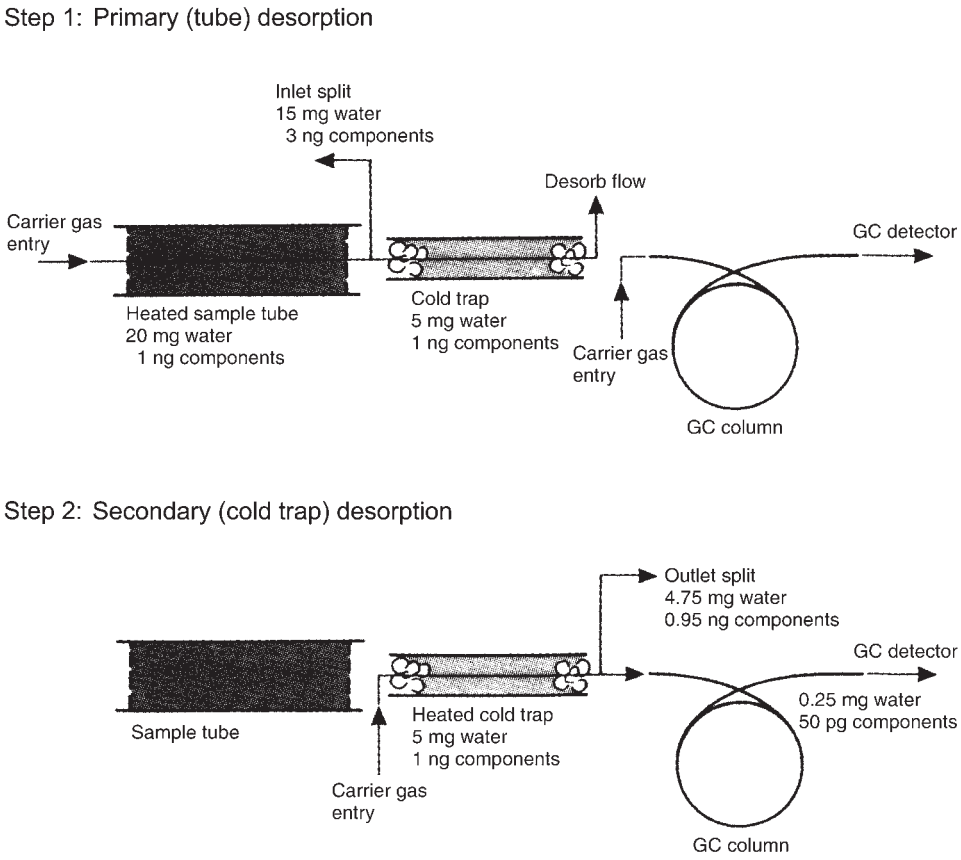


Fig. 2.36 Multiple split technique for thermodesorption (Perkin-Elmer).

merly always was unique. With a recent development by Markes International SecureTD-Q with a quantitative re-collection of a split flow for repeat analysis it overcomes the historical one-shot limitation of thermal desorption methods and simplifies method/data validation.

For high throughput applications an electronic tube tagging using RFID tube tags is available for industry standard sorbent tubes and 4.5-inch (DAAMS) tubes.

Possible and, for certain carrier materials, already known decomposition reactions have already been mentioned. For this reason another method is favoured by the EPA for air analysis. Passivated, nickel-coated canisters (ca. 2 L, maximum up to 15 L) are evacuated for sample collection. The samples collected on opening the canister can be measured several times in the laboratory. Suitable samplers are used, which are connected on-line with GC/MS. Cryofocusing is used to concentrate the analytes from the volumes collected. If required, the sample can be dried with a semipermeable membrane (Nafion drier) or by condensation of the water (moisture control system, see also Section 2.1.4.2). Adsorption materials are not used in these processes.

2.1.7
Pyrolysis and Thermal Extraction

The use of pyrolysis extends the area of use of GC/MS coupling to samples which cannot be separated by GC because they cannot be desorbed from a matrix or evaporated without decomposition. In analytical pyrolysis a large quantity of energy is passed into a sample so that fragments which can be gas chromatographed are formed reproducibly. The pyrolysis reaction initially involves thermal cleavage of C-C bonds, e. g. in the case of polymers. Thermally induced chemical reactions within the pyrolysis product are undesired side reactions and can be prevented by reaching the pyrolysis temperature as rapidly as possible. The reactions initiated by the pyrolysis are temperature-dependent. To produce a reproducible and quantifiable mixture of pyrolysis products, the heating rates and pyrolysis temperatures, in particular, should be kept constant. The sample and its contents can be characterised using the chromatographic sample trace (pyrogram) or by the mass spectroscopic identification of individual pyrolysis products.

The use of pyrolysis apparatus with GC/MS systems imposes particular requirements on them. The sample quantity applied must correspond to the capacity of commercial fused silica capillary columns. It is usually in the µg range or less. By selecting a suitable split ratio peak equivalents for the pyrolysis products can be achieved in the middle ng range. These lie completely within the range of modern GC/MS systems. The small sample quantities are also favourable for analytical pyrolysis in another aspect. The reproducibility of the procedure increases as the sample quantity is lowered as more rapid heat transport through the sample is possible (Fig. 2.37). Side reactions in the sample itself and as a result of reactive pyrolysis products are increasingly eliminated.

Because of the small sample quantities and the high reproducibility of the results, analytical pyrolysis has experienced a renaissance in recent years. Both in the analysis of polymers

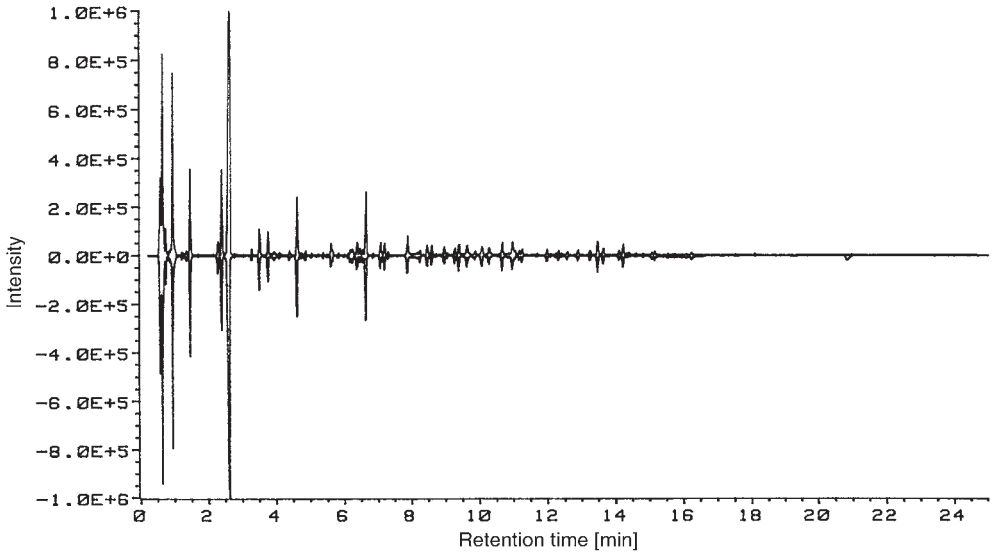


Fig. 2.37 Reproducibility of the pyrolysis of an automotive paint on two consecutive days using a foil pyrolyser coupled to GC/MS, mirrored representation (Steger, Audi AG).

with regard to quality, composition and stability, and in the areas of environmental analysis, foodstuffs analysis and forensic science, pyrolysis has become an important analytical tool, the significance of which has been increased immensely by coupling with GC/MS.

Analytical pyrolysis is currently dominated by two different processes: high frequency pyrolysis (Curie point pyrolysis) and foil pyrolysis. The processes differ principally through the different means of energy input and the different temperature rise times (TRT). Both pyrolysis processes can easily be connected to GC and GC/MS systems. The reactors currently used are constructed so they can be placed on top of GC injectors (split operation) and can be installed for short term use only if required.

Analytical Pyrolysis Procedures

Procedure	Foil	High frequency
Carrier	Pt foil	Fe/Ni alloys
Pyrolysis temperature	Can be freely selected up to 1400 °C	Fixed Curie temperatures
TRT	<8 ms	ca. 30 to 100 ms

2.1.7.1 Foil Pyrolysis

In foil pyrolysis the sample is applied to a thin platinum foil (Fig. 2.38). The thermal mass of this device is extremely low. After application of a heating current any desired temperature up to ca. 1400 °C can be achieved within milliseconds. The extremely high heating rate results in high reproducibility. The temperature of the Pt foil can be controlled by its resistance. However, the temperature can be measured and controlled more precisely and rapidly from the radiation emitted by the Pt foil. An exact calibration of the pyrolysis temperature can be carried out and the course of the pyrolysis is recorded by this feedback alone. Besides endothermic pyrolyses, exothermic processes can also be detected and recorded.

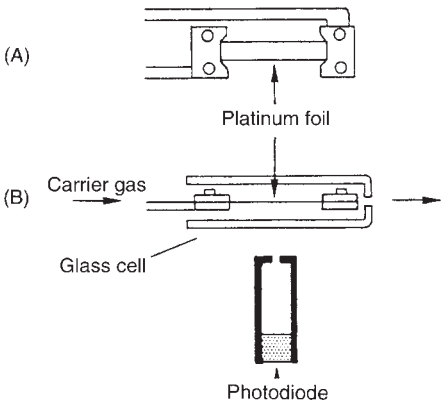


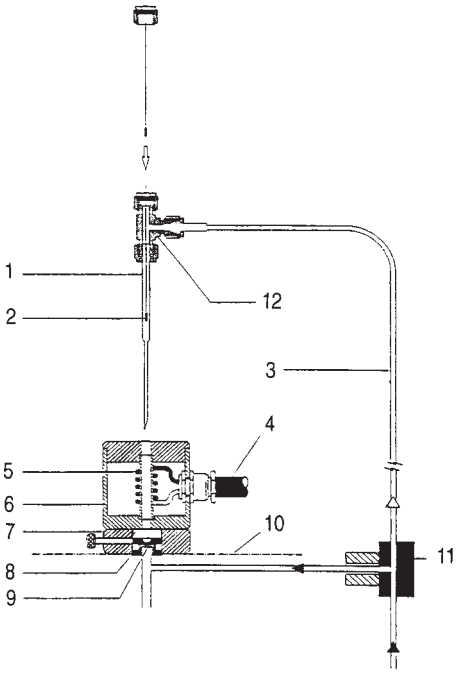
Fig. 2.38 Scheme of a Pt foil pyrolyser with temperature control by means of fibre optic cable (Pyrola).
(A) View of the Pt foil with carrier gas and current inlets.
(B) Side view of the pyrolysis cell with the glass cell (ca. 2 mL volume, pyrex) and photodiode under the Pt foil for calibration and monitoring of the pyrolysis temperature.

Pyrolysis Nomenclature

- Pyrolysis:
  A chemical degradation reaction initiated by thermal energy alone.
- Oxidative pyrolysis:
  A pyrolysis which is carried out in an oxidative atmosphere (e.g. O_2).
- Pyrolysate:
  The total products of a pyrolysis.
- Analytical pyrolysis:
  The characterisation of materials or of a chemical process by instrumental analysis of the pyrolysate.
- Applied pyrolysis:
  The production of commercially usable materials by pyrolysis.
- Temperature/time profile (TTP):
  The graph of temperature against time for an individual pyrolysis experiment.
- Temperature rise time (TRT):
  The time required by a pyrolyser to reach the pyrolysis temperature from the start time.
- Flash pyrolysis:
  A pyrolysis which is carried out with a short temperature rise time to achieve a constant final temperature.
- Continuous pyrolyser:
  A pyrolyser where the sample is placed in a preheated reactor.
- Pulse pyrolyser:
  A pyrolyser where the sample is placed in a cold reactor and then rapidly heated.
- Foil pyrolyser:
  A pyrolyser where the sample is applied to metal foil or band which is directly heated as a result of its resistance.
- Curie point pyrolyser:
  A pyrolyser with a ferromagnetic sample carrier which is heated inductively to its Curie point.
- Temperature-programmed pyrolysis:
  A pyrolysis where the sample is heated at a controlled rate over a range of temperatures at which pyrolysis occurs.
- Sequential pyrolysis:
  Pyrolysis where the sample is repeatedly pyrolysed for a short time under identical conditions (kinetic studies).
- Fractionated pyrolysis:
  A pyrolysis where the sample is pyrolysed under different conditions in order to investigate different sample fractions.
- Pyrogram:
  The chromatogram (GC, GC/MS) or spectrum (MS) of a pyrolysate.

2.1.7.2 **Curie Point Pyrolysis**

High frequency pyrolysis uses the known property of ferromagnetic alloys of losing their magnetism spontaneously above the Curie temperature (Curie point). At this temperature a large number of properties change, such as the electrical resistance or the specific heat. Above the Curie temperature ferromagnetic substances exhibit paramagnetic properties. The possibility of reaching a defined and constant temperature using the Curie point was first realised by W. Simon in 1965. In a high frequency field a ferromagnetic alloy does not absorb any more energy above its Curie point and remains at this temperature. As the Curie temperature is substance-dependent, another Curie temperature can be used by changing the material. If a sample is applied to a ferromagnetic material and is heated in an energy-rich high frequency field (Fig. 2.39), the pyrolysis takes place at the temperature determined by the choice of alloy and thus its Curie point. The temperature rise curves for various metals and alloys are shown in Fig. 2.40.



1 Glass pyrolysis injector
2 Ferromagnetic sample carrier
3 Teflon tubing for carrier gas inlet
4 Connection to high frequency generator
5 Induction coil
6 Aluminium casing
7 Adaptor piece for GC injector
8 GC injector
9 GC septum
10 Cover for GC oven
11 Carrier gas valve for switching to pyrolysis
12 3-Way screwed connection

Fig. 2.39 Pyrolysis injector (Curie point hydrolysis) (after Fischer).

In practice sample carriers in the form of loops, coils or simple wires made of different alloys at fixed temperature intervals are used. A disadvantage of Curie point pyrolysis is the longer temperature rise time of ca. 30–100 ms required to reach the Curie point compared with foil pyrolysis. There are also effects due to the not completely inert surface of the sample carrier. They manifest themselves in the inadequate reproducibility of the pyrolysis process for analytical purposes. Copolymers with thermally reactive functional groups (e. g. free OH, NH_2 or COOH groups) cannot be analysed by Curie point pyrolysis.

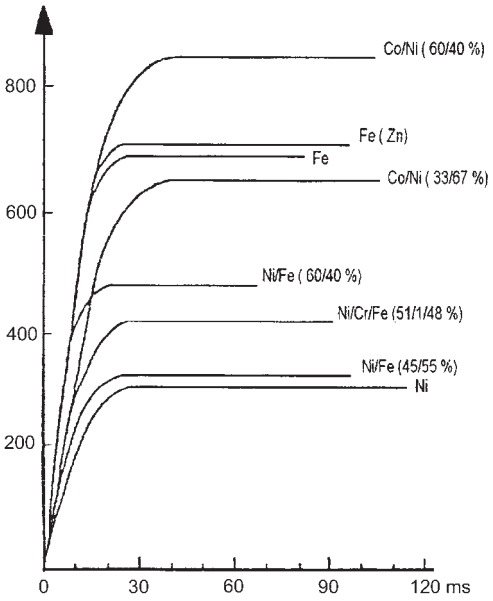


Fig. 2.40 Curie temperatures and temperature/time profiles of various ferromagnetic materials (after Simon).

For the analytical assessment of the coupling of pyrolysers with GC and GC/MS systems, a high-boiling mixture of cholesterol with n-alkanes has been proposed (Gassiot-Matas). Figure 2.41 shows the evaporation of cholesterol (500 ng) with the C_{34}- and C_{36}-n-alkanes (50 ng of each). Before the intact cholesterol is detected, its dehydration product appears. The intensity of this peak increases with small sample quantities and increasing temperatures in the region of substance transfer in the GC injector. The high peak symmetries of the signals of the alkanes, the cholesterol and its dehydration product indicate that the coupling is functioning well.

There are various possibilities for the evaluation of pyrograms obtained with a GC/MS system. With classical FID detection the pyrogram pattern is only compared with known standards. However, with GC/MS systems the mass spectra of the individual pyrolysis products can be evaluated. By using libraries of spectra, substances and substance groups can be identified. GC/MS pyrograms can be selectively investigated for trace components even in complex separating situations by using the characteristic mass fragments of minor components. The comparison of sample patterns becomes meaningful through the choice of mass chromatograms of substance-specific fragment ions. GC/MS pyrograms with its full pattern information can be stored and compared for identity or similarity. Suitable software systems are commercially available providing a numerical measure on similarity instead a visual inspection only (Chromsearch, Axel Semrau, Germany; Xaminer, Thermo Fisher, USA).

In the library search for the spectra of pyrolysis products special care must be taken. Commercial spectral libraries consist of spectra of particular substances which have been fully characterized, which is not the case for the majority of pyrolysis products. Classical mass spectrometry fragmentation rules apply for interpretation of search results. Depending on the sample material, however, an extremely large number of reaction products are formed on pyrolysis, which cannot be completely separated even under the best GC conditions. If the detection is sensitive enough this situation is shown clearly by the mass chromatogram. Also, for polymers there is a typical appearance of homologous fragments, which must also be taken

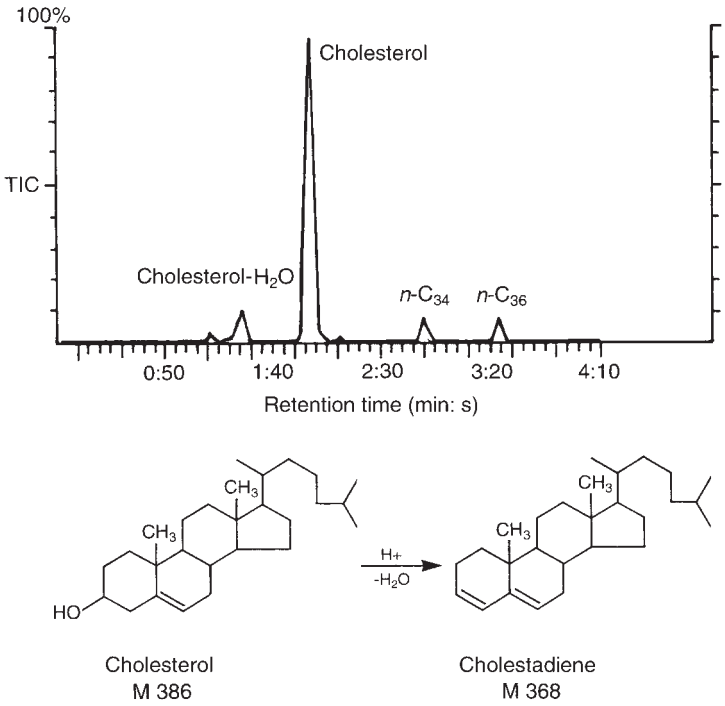


Fig. 2.41 Analysis of cholesterol and n-alkanes (C_{34}, C_{36}) for testing the pyrolysis coupling to the GC injector (after Richards 1988).
Conditions: GC column 4 m × 0.22 mm ID × 0.25 µm CP-Sil5, 200–320 °C, 30 °C/min, GC-ITD

into account. These series can also be shown easily using mass chromatograms through the choice of suitable fragment ions.

Quantitative determinations using pyrolysis benefit particularly from the selectivity of GC/MS detection. The precision is comparable to that of liquid injections.

2.1.7.3 **Thermal Extraction**

Besides the processes of foil and Curie point pyrolysis described, thermal extraction (Fig. 2.42) covers the area between pyrolysis and thermodesorption. In a thermal extraction device the sample (manual or automatic) is placed in a quartz oven, which can be operated at a constant temperature of up to 750 °C. This allows both the thermal extraction of volatile components from solid samples as well as their pyrolysis. Other technical solutions allow the temperature control of the pyrolysis chamber for a thermal desorption step with collection of the evaporated material either on a dedicated trap (e.g. Markes Inc., UK; Frontier Laboratories, Japan) or in the GC injector (PTV with sorbent, PYROL AB, Sweden).

The difference between thermal extraction and the analytical pyrolysis systems described above lies in the consideration of the sample quantity. The typical capacity of a thermal extractor is between 0.1 mg and ca. 500 mg sample material. This allows weighing out and reweighing. Even inhomogeneous materials can be investigated in this way.

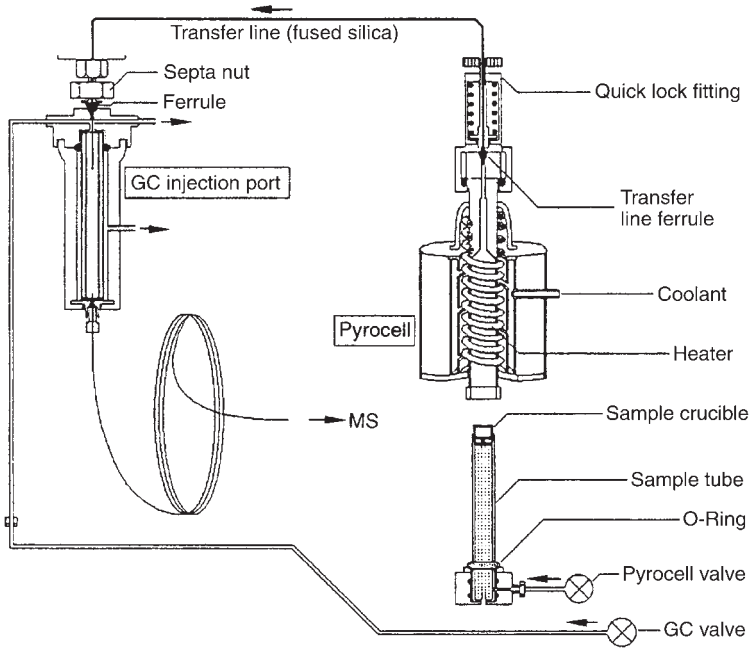


Fig. 2.42 Thermal extractor, cross section and coupling to a GC injector (after Ruska).

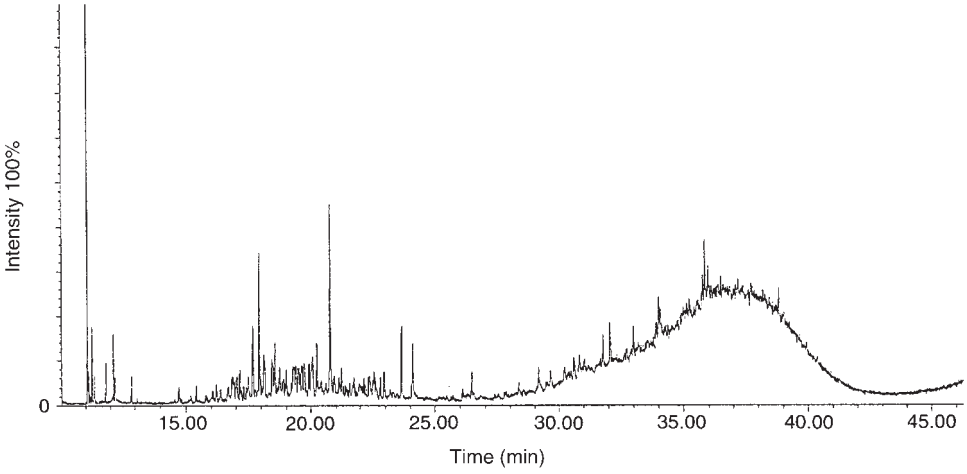


Fig. 2.43 GC/MS analysis of volatile components of a floor covering by thermal extraction at 80 °C (after Ruska).

The wide temperature range allows a broad spectrum of applications. The stripping of volatile compounds, e.g. solvents, volatile halogenated hydrocarbons and BTEX, is carried out typically below 80 °C (Fig. 2.43). At temperatures of up to 350 °C involatile compounds are released from the sample, for example from plastics or composite materials. Pyrolytic cleavage reactions begin at temperatures above 350 °C. Because of the large sample quanti-

ties and the indirect heating of the sample in an oven, the temperature in the sample increases relatively slowly. The pyrograms obtained can be compared with pyrolysis patterns which take precedence.

In the field of environmental analysis thermal extraction is proposed by an EPA method for the quantitative analysis of semivolatile compounds from solid sample materials. The method EPA 8275 is a thermal extraction capillary GC/MS procedure for the rapid and quantitative determination of targeted PCBs and PAHs in soils, sludges and solid wastes (EPA 1996).

2.2 Gas Chromatography

2.2.1 Fast Gas Chromatography

The pressure of workload in modern analytical laboratories necessitates an increased throughput of samples and hence method development is largely focused on productivity. However, speed of chromatographic analysis and enhancement of peak separation generally require analytical method optimization in opposite directions. Basically, two adjacent approaches are increasingly used to combine advancements in speed and peak separation. In the existing conventional GC ovens, the use of narrow bore columns below 0.1 mm i.d. offers a viable practical "Fast GC" solution for almost every GC and GC/MS with electronic pressure regulation. Alternatively the installation of cartridges for direct column heating are today commonly used even for „Ultra Fast" GC separations.

A recent publication has studied the performance of different columns featuring different lengths and internal diameters at different heating rates and with different carrier gas types and flow rates (Facchetti 2002).

2.2.1.1 Fast Chromatography

The term "Fast GC" compares to the conventionally used fused silica columns of typical dimensions with lengths of 30 m or longer and inner diameters of 0.25 mm or higher. These column types became standard since the introduction of the fused silica capillaries used for most applications in GC/MS. With improved and partially automated sample preparation methods (see e. g. Section 2.1.3), GC analysis time is becoming the rate determining step for productivity in many laboratories. A reduction of GC/MS analysis time is possible with the utilization of alternatively available techniques using a reduced column length and inner diameter with appropriate film thicknesses and oven temperature programming.

A very good example describing the progress in GC separation technology and advancement in GC productivity is given with an optimisation strategy starting from a packed column separation (Facchetti 2005). The sample used for this comparison is the 16 component standard mixture used in EPA 610 analysis of polynuclear aromatic hydrocarbons (PAH). The EPA method is antiquated and describes a 45 minute analysis using a packed column containing OV17. All columns used in this example were 5% phenyl polysiphenylene-siloxane coated Trace TR-5MS columns (Thermo Fisher Scientific, Bellefonte, PA, USA) with di-

Table 2.18 Compounds in the EPA 610 standard on packed column.
Note: Four pairs of peaks remain unresolved using the EPA 610 packed column technique, marked with *.

Component	Retention time [min]
1. Naphthalene	4.50
2. Acenaphthylene	10.40
3. Acenaphthene	10.80
4. Fluorene	12.60
5. Phenanthrene	15.90*
6. Anthracene	15.90*
7. Fluoranthene	19.80
8. Pyrene	20.60
9. Benzo(a)anthracene	24.70*
10. Chrysene	24.70*
11. Benzo(b)fluoranthene	28.00*
12. Benzo(k)fluoranthene	28.00*
13. Benzo (a) pyrene	29.40
14. Dibenzo(a,h)anthracene	36.20*
15. Indeno(1,2,3-cd)pyrene	36.20*
16. Benzo(ghi)perylene	38.60

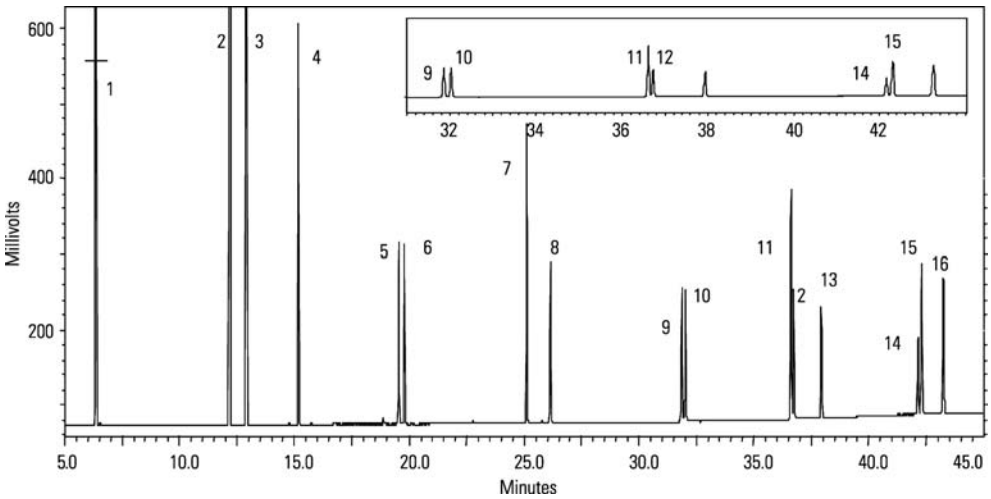


Fig. 2.44 Simulation of a packed column separation performed on a 30 m capillary column. Carrier gas has been changed from nitrogen to helium and compared with a 3% OV17 packed column, the 5% phenylmethylpolysiloxane capillary column separates 4 previously unresolved peak pairs in a similar run time. Column: 30 m Trace TR-5MS; ID 0.25 mm, Film 0.25 µm; Initial Temp: 100 °C; Rate 1: 5 °C/min to 300 °C; Carrier Gas: Helium; Gas Flow: 1.5 mL/min. (constant flow); Detector: FID; Split Ratio: 50:1; Injection vol.: 1.0 µL by TriPlus auto sampler; **Sample run rate is 1 per hour** (includes oven cooling)

Table 2.19 Variables affecting GC run time.

Parameter	Run time effects
Temperature ramp rate	Higher starting temperature and steeper heating rate → peaks elute faster
Column length	Shorter column → shorter run time
Carrier gas flow rate	Each gas type has an optimum linear flow range within which speed can be adjusted
Film thickness	Thicker film → more interaction of solutes → longer run time Thinner film → Fast Chromatography
Column internal diameter	Reducing internal diameter increases column efficiency → shorter run-times using "Fast Chromatography"
Ultra fast technology	Combination of short column, small internal diameter, thin film, special ultra fast heating coil for ultra fast separations

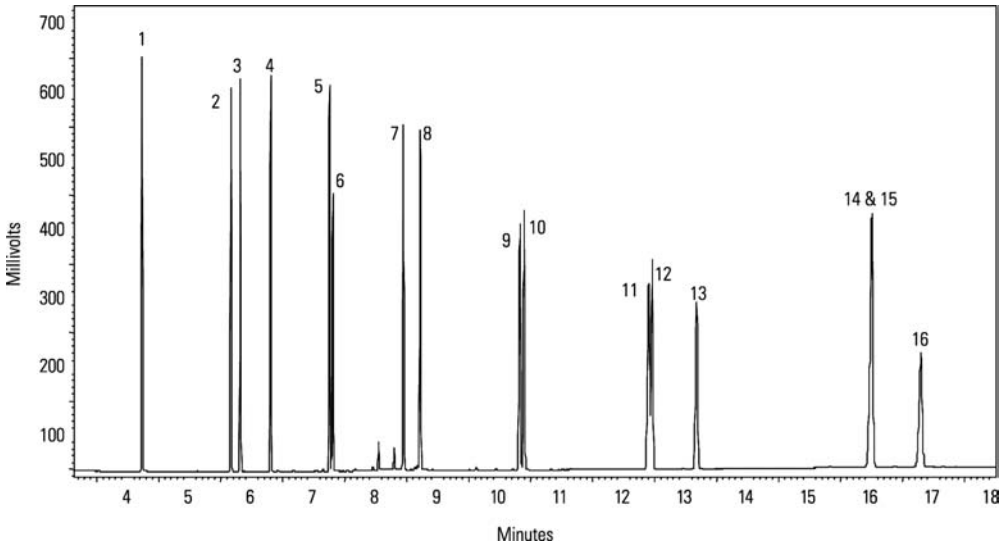


Fig. 2.45 Optimisation of the oven program has cut the run time by more than half without changing any other parameters. There is some loss of resolution between peaks 14 and 15 compared with the 45 min run, however the extra specificity of a MS detector would resolve these peaks.
Column: 30 m Trace TR-5MS; ID 0.25 mm, Film 0.25 µm; Initial Temp: 90 °C hold 1 min; Rate 1: 25 °C/min to 290 °C; Rate 2: 4 °C/min to 320 °C hold 5 min; Carrier Gas: Helium; Gas Flow: 1.5 mL/min. (constant flow); Detector: FID; Split Ratio: 50:1; Injection vol.: 0.5 µL manual injection; **Sample run rate is 2 per hour** (includes oven cooling).

mensions as specified with each chromatogram. Helium is the carrier gas in each case. Flow rates and oven programs were optimized to the column dimensions. The chromatograms were run in either constant flow or constant pressure mode as indicated using a Trace Ultra GC with a TriPlus autosampler (Thermo Fisher Scientific, Milan, Italy) fitted with a FID detector.

Initially, capillary GC methods attempted to simulate the packed column method with a run time of 45 minutes. The example below in Fig. 2.44 shows a capillary separation where the oven program is slowed down to keep the run time similar to the packed column method, and helium has been substituted for the original nitrogen carrier gas. However, with the increasing pressures on analysis time, and the flexibility within the EPA 610 method, it has been possible to greatly reduce run time by altering simple GC variables.

A number of variables within the GC setup can be manipulated to alter the run time, bearing in mind the need to maintain resolution, elution order and sensitivity as outlined in Table 2.19.

The first strategy for reducing run time should be to modify the oven ramp by increasing the ramp where the peaks are well separated with large retention time spaces and applying a slow ramp where extra separation is required (see Fig. 2.45).

Reducing column length is the second strategy for reducing run time. Note the need to increase the oven ramp rate in order to elute the high boiling materials, whilst retaining peak shape (see Fig. 2.46 with the switch to a 15 m column).

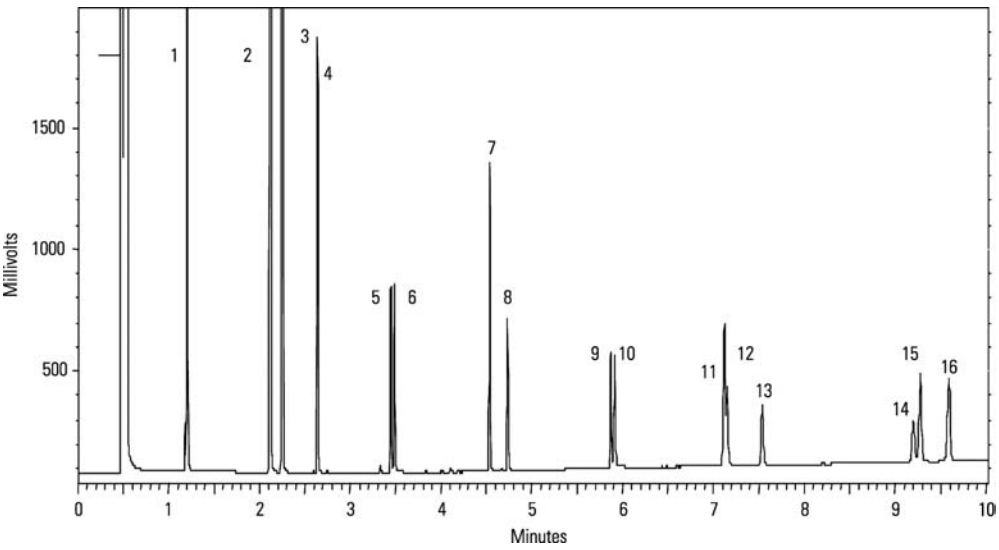


Fig. 2.46 Illustration of the effect of simply reducing column length by half (15 m) using constant flow reducing the run time to 10 min.
Column: 15m Trace TR-5MS; ID 0.25 mm, Film 0.25 µm; Initial Temp: 120 °C hold 0.2 min; Rate 1: 25 °C/min to 260 °C; Rate 2: 7 °C/min to 300 °C hold 3 min; Carrier Gas: Helium; Gas Flow: 1.5 mL/min constant flow; Detector: FID; Split Ratio: 50:1; Injection vol.: 1.0 µL by TriPlus auto sampler; **Sample run rate is 4 per hour** (includes oven cooling).

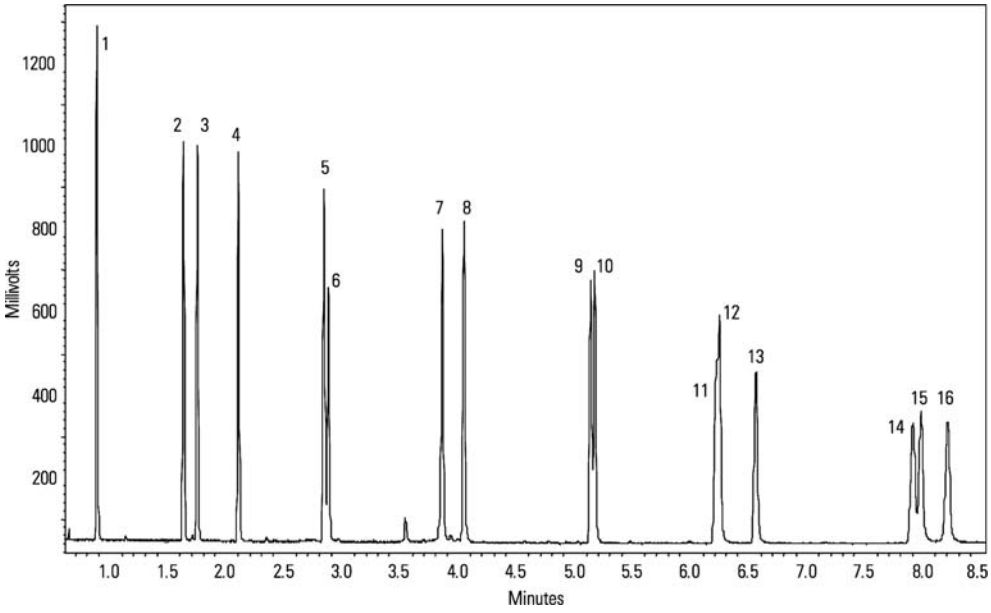


Fig. 2.47 Further reduction of the column length by half to 7 m but with larger ID, illustrates the effect of film thickness on run time. The larger ID makes the column less efficient. Therefore to maintain the resolution a similar oven program is needed, making the analysis time only slightly shorter than on the 15 m column. Column: 7 m Trace TR-5MS; ID 0.32 mm, Film 0.25 µm; Initial Temp: 120 °C hold 1 min; Rate 1: 25 °C/min to 250 °C; Rate 2: 10 °C/min to 300 °C hold 5 min; Gas Flow: 1.5 mL/min constant flow mode; Carrier Gas: Helium; Detector: FID; Split Ratio: 50:1; Injection vol.: 0.5 µL manual injection; **Sample run rate is 5 per hour** (includes oven cooling).

2.2.1.2 **Ultra Fast Chromatography**

The term Ultra Fast Gas Chromatography describes an advanced column heating technique that is taking advantage of short, narrow bore capillary columns, and very high temperature programming rates. This technique offers to shorten the analysis time by a factor up to 30 compared to conventional capillary gas chromatography. Ultra fast GC conditions typically apply the use of short 0.1 mm i.d. narrow-bore capillary columns of 2.5 to 5 meters in length with elevated heating rates of 100 to 1200 °C/min providing peak widths of 100 ms or less, see Fig. 2.49. At this point the limiting factor for sample throughput becomes the ability of the column to cool quickly enough between runs. In addition to a fast heating/cooling regime, a fast conventional or MS detection with an acquisition rate better than 25 Hz for reliable peak identification is required in, e.g. a restricted scan, SIM/MID or TOF mode, to achieve the necessary sampling rate across individual peaks. Currently, TOF and the fast scanning quadrupole technologies are the only MS technologies that are able to provide the required speed of detection for a ultra fast GC/MS at data rates of 50 Hz or better with a partial or full mass spectral information.

The special instrumentation required for ultra fast chromatography comprises a dedicated ultra fast column module (UFM), see Fig. 2.51, comprising a specially assembled fused silica column for direct resistive heating, wrapped with a heating element and a temperature

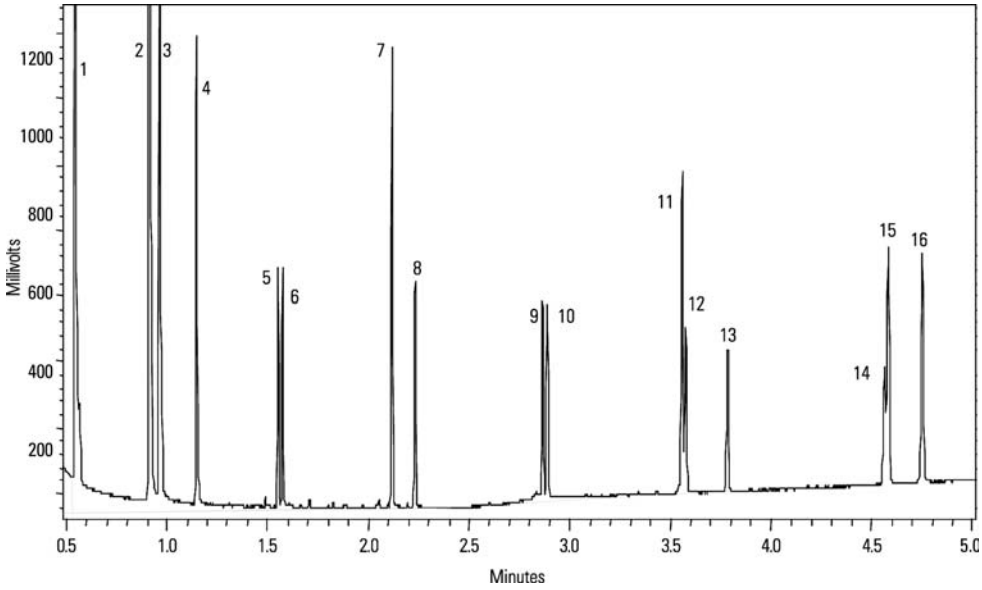


Fig. 2.48 The combination of using a narrow 0.1 mm column coated with a thinner film is the strategy for fast analysis. In comparison with the 7 m 0.25 µm film column, the longer 10 m x 0.1 µm film is faster. In addition, using constant pressure has enhanced the analysis speed down to under 5 minutes. The effect of running at constant pressure is to greatly speed up the linear velocity at the beginning of the run where the peaks are well separated. Later in the run, when the temperature is high, the velocity is lower allowing more dwell time on the column for the later peaks which elute close together.

sensor, see Fig. 2.52 (Facchetti 2002). The assembly is held in a compact metal cage to be installed inside the regular GC oven (which is not active during ultra fast operation). Temperature programming rates can be achieved as high as 1200 °C/min. UFM modules are commercially available providing different column lengths and diameters.

Another compelling example in Fig. 2.53 shows the ultra fast analysis of a mineral oil performed in only 2 minutes still maintaining enough resolution to separate pristane and phytane from n-C17 and n-C18.

2.2.2 Two Dimensional Gas Chromatography

The search for innovative solutions to increase the limiting peak capacities in gas chromatography came up many years ago with two dimensional separations. The development of instrumental techniques focused in the beginning on the transfer of specific peak regions of interest, the unresolved "humps", to a second separation column. This technique, widely known as "heart cutting", found many successful applications in solving critical research oriented projects but its use was not widespread in routine laboratories

The objective to increase the peak capacity for the resolution of entire complex chromatograms and not only of few discrete congested areas could be satisfied first with the introduction of a comprehensive two-dimensional gas chromatography (GCxGC), the most powerful

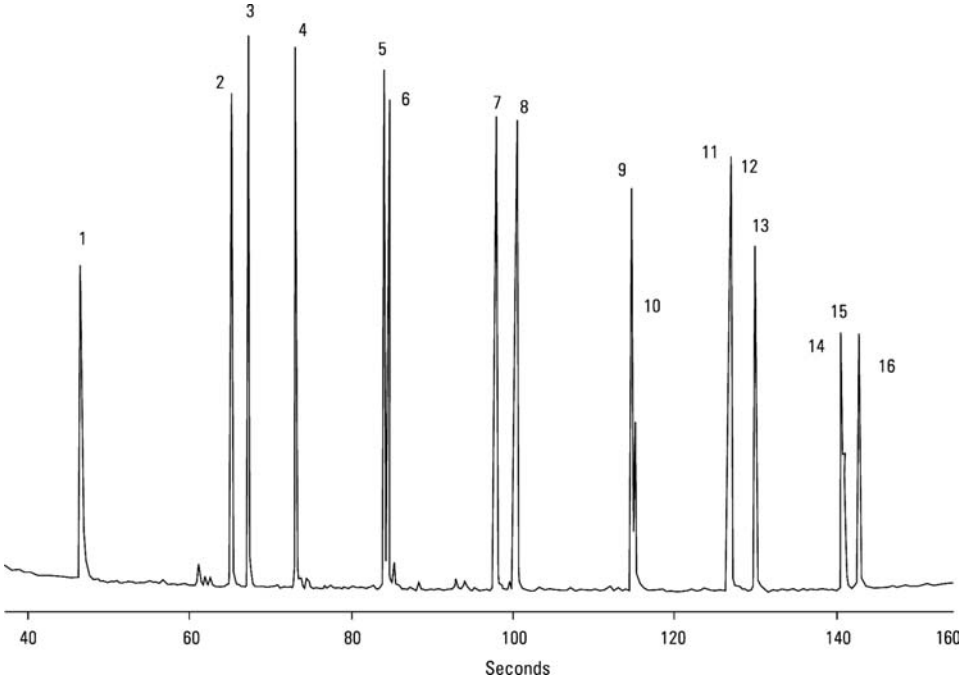


Fig. 2.49 Ultra Fast column and detector can run sample in just 160 seconds improving sample throughput to approx 12 per hour.; Column: 5 m Ultra Fast TR5-MS; ID 0.1 mm, Film 0.1 µm; Flow Rate: 1.0 mL/min; Carrier Gas: Hydrogen; Oven: 40 °C hold 0.3 min; Rate 1: 2 °C/s to 330 °C; **Sample run rate is 12 per hour** (includes column cooling).

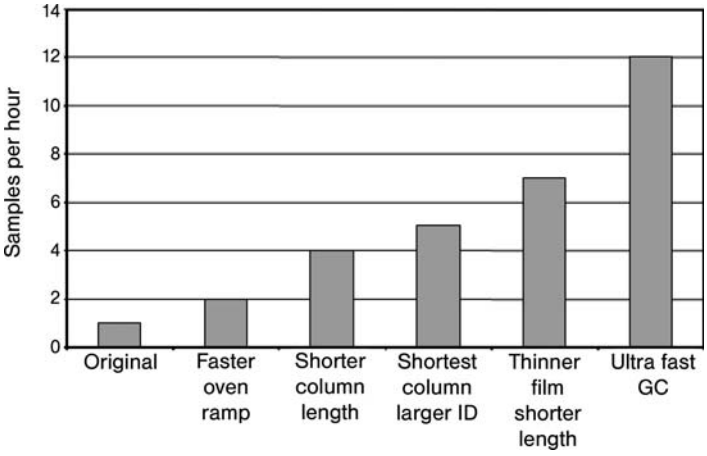


Fig. 2.50 Increased sample throughput by progress in column technology.

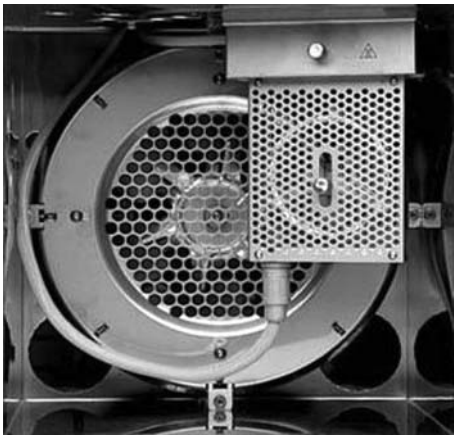


Fig. 2.51 UFM column module installed in a regular GC oven (Mega, Legnano, Italy; TRACE GC Ultra, Thermo Fisher Scientific, Milan, Italy).

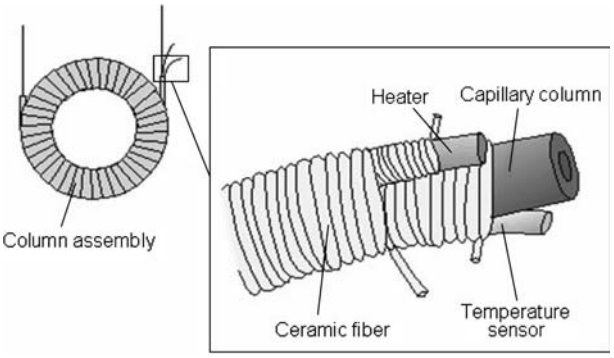


Fig. 2.52 Column wrapping detail with heating element and temperature sensor for ultra fast capillary chromatography.

two-dimensional solution today (Liu 1991). While the initial ideas of multidimensional GC relied on the use of two regular capillary columns (MDGC, selectivity tuning), current comprehensive two-dimensional chromatography benefits from the alignment with fast GC on the second separation dimension (Beens 2000, Bertsch 2000). The result is a significantly improved separation power. With the commercial availability of GC×GC instrumentation this technique found avid utilization for a widespread range of applications within the first few years (Marriott 2003).

Particularly for the separation of complex mixtures, GC×GC showed far greater resolution and a significant boost in signal to noise, with no increase in analysis times. Further, the hyphenation of GC×GC with MS detection providing three independent analytical dimensions made this technique ideal for the measurement of organic components within complex samples such as those from environmental, petrochemical and biological analysis. The significantly increased chromatographic resolution in GC×GC allows separation of

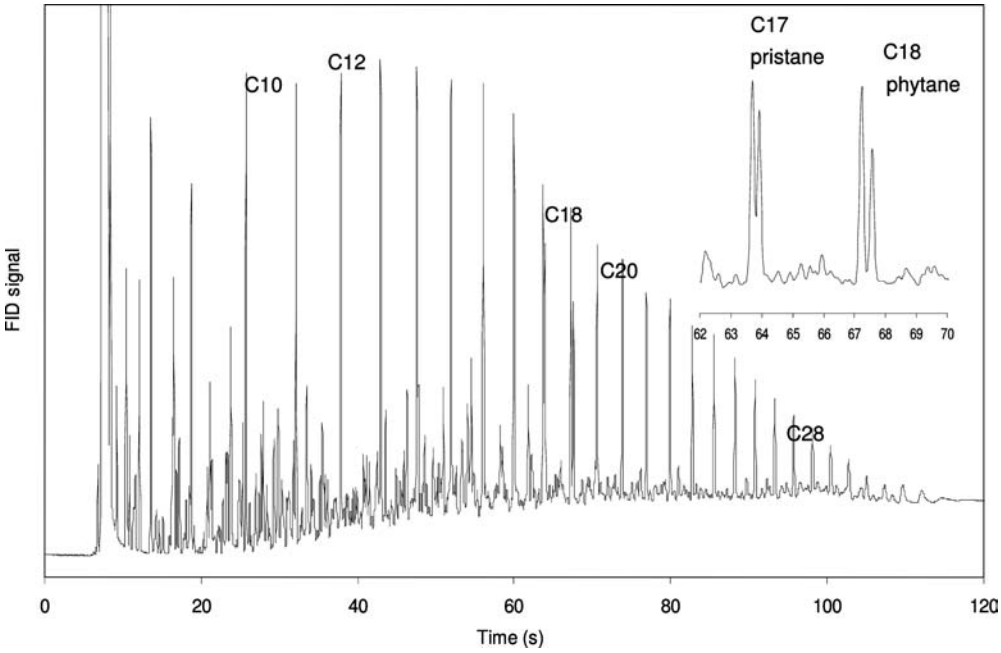


Fig. 2.53 Ultra Fast GC chromatogram of a mineral oil sample. The chromatogram was obtained using a 5 m, 0.1 mm ID, 0.1 µm film thickness SE54 column with a temperature program from 40 (12 s) to 350 °C (6 s) at 180 °C/min.

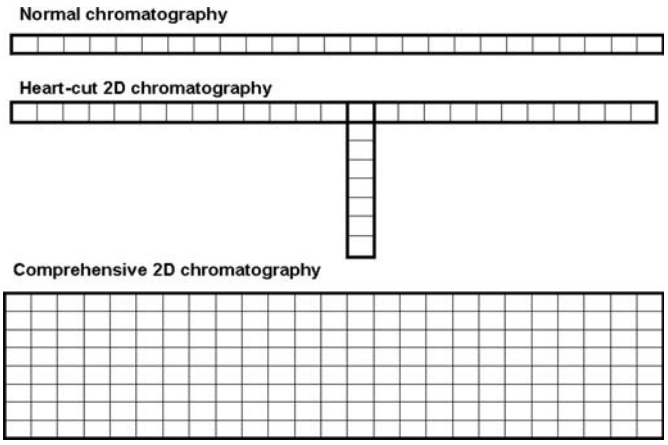
many previously undetectable components. Fast scanning mass spectrometers are required to maintain the excellent chromatographic resolution, provided today with full scan data by time-of-flight MS, or in selected ion monitoring mode also by quadrupole or high resolution MS systems. More than 15,000 peaks could be detected in an ambient air sample from the city of Augsburg by thermal desorption comprehensive two-dimensional GC with time-of-flight MS detection (Welthagen 2003).

The great advantage of the combination of multiple chromatographic steps is the increase in peak capacity. Peak capacity is the maximum number of peaks that can be resolved in a given time frame. The more peaks a combination of techniques is able to resolve, the more complex samples can be analysed. When a sample is separated using two dissimilar columns, the maximum peak capacity Φ_{max} will be the product of the individual column's peak capacity Φ_n.

$$\Phi_{max} = \Phi_1 \times \Phi_2$$

For example, if each separation mode generates peak capacities of 1100 in the first dimension and 30 in the second, the theoretical peak capacity of the 2D experiment will be 33,000, a huge gain in separation space, which would theoretically compare to

the separation power of a 12,000 m column in the normal single dimension analysis. To achieve this gain, however, the two techniques should be totally orthogonal, that is, based upon completely different separation mechanisms.



Comparison of peak capacities in normal, heart-cut and comprehensive two-dimensional chromatography

2.2.2.1 **Heart Cutting**

The goal of heart cutting is the increase of peak capacity for target substances in congested regions of unresolved compounds. Two capillary columns are connected in series, typically by means a valveless flow switching system. One or more short retention time slices are sampled onto a second separation column. A valveless switching device is ideally suited for high speed flow switching. It is based on the principle of pressure balancing and was introduced by Deans already in 1968. This technique found a wider recognition first with state-of-the-art EPC units, which allowed the integration of column switching as part of regular GC methods for routine applications (Deans 1968, MacNamara 2003, Majors 2005).

Heart cutting requires a sample specific individual setup of the analysis strategy. The sections of interest have to be previously identified and selected by retention time from the first chromatogram to be "cut" into the second column. The choice of column length is application specific and does not interfere with the "cutting" process. The second dimension column can be of the same but recommended of a different film polarity. A monitor detector is required, typically a universal FID, which continuously observes the separation on the first dimension. A second detector, often a MS detector, acquires data from the 2nd dimension in now "higher" chromatographic resolution.

2.2.2.2 **Comprehensive GC×GC**

True multidimensional chromatography requires two independent (orthogonal) separations mechanisms and the conservation of the first separation into the 2nd dimension. Compre-

hensive GC×GC today is the most developed and most powerful multi-dimensional chromatographic technique. The technique has been widely accepted and applied to the analysis of complex mixtures. Commercial instrumentation is available at a mature technological standard for routine application (see Fig. 2.54).

In contrast to heart cutting, the complete first dimension effluent is separated in slices (which can be interpreted as a continuous sequence of cuts) and forms a three dimensional data space on a second dimension with the retention times in both dimensions and the peak intensities by classical or MS detection. No monitor detector for the first dimension, or prior identification of an individual retention time window is required, and no increase in total analysis time is involved.

The timing regime of GC×GC should be relatively slow with a regular capillary column for the first dimension and a short fast GC column for the second dimension separation. The second dimension column should be of a different polarity than the first one to provide different kind of interactions in substance class separations. Typically, non polar columns

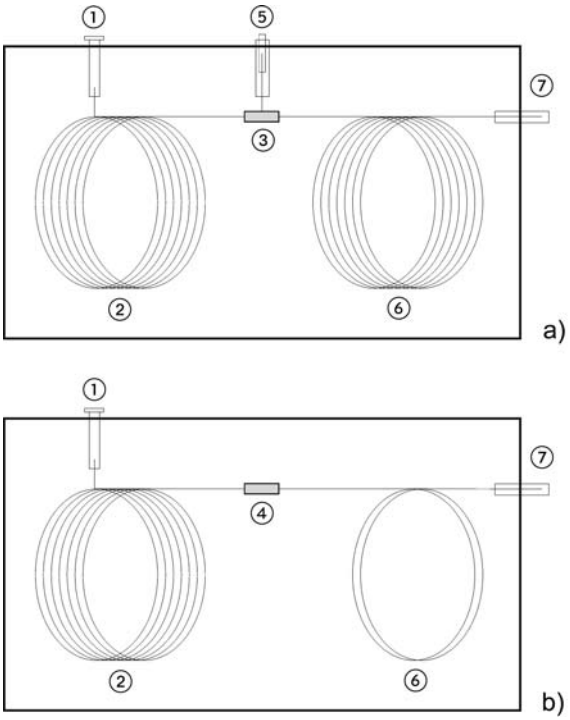
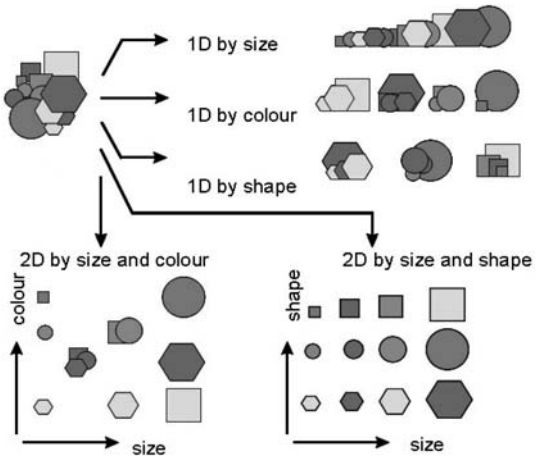


Fig. 2.54 Two-dimensional GC Principles.
a) Heartcutting with monitor detector. b) Comprehensive GC×GC.
1 Injector
2 Primary separation column, 1st dimension
3 Flow switching device
4 Modulator device
5 Monitor detector
6 Secondary separation column, 2nd dimension
7 MS transfer line or conventional detector, 2nd dimension

are used in the first dimension for a separation along the substance boiling points. Polar film interactions characterize the further enhanced separation on the 2nd dimension, which takes place at an only low temperature gradient, due to the speed of elution. Column lengths in the 2nd dimension are as short as 1–2 m for fast GC conditions (see also Section 2.2.1). In total, the duration of analysis in GC×GC is not increased compared to conventional techniques and the same sample throughput can be achieved.

The operation of the interface between the both columns, called the modulator, is key to GC×GC. The modulator operates in an alternating trap and inject mode with a modulation frequency related to the fast second dimension separation. Short elution sequences are integrated from the first column and injected for the second dimension separation. Each injection pulse generates a high speed chromatogram.



Use of Multi-Dimensionality in Chromatographic Separation (Blomberg)

The Key Features of GC×GC

Significantly increased resolution power
Significantly increased peak capacity
Significantly increased signal/noise ratios

The Key Benefits of GC×GC

Lower detection limits
Separation of substance classes
Productive, no increase of separation time
Same overall analysis time as conventional separations
Same sample throughput as conventional techniques

The collection of a large series of second dimension chromatograms results in a large amplification of the column peak capacities as the first dimension peaks are distributed on multiple fast second dimension chromatograms. The effect of increased separation can be visua-

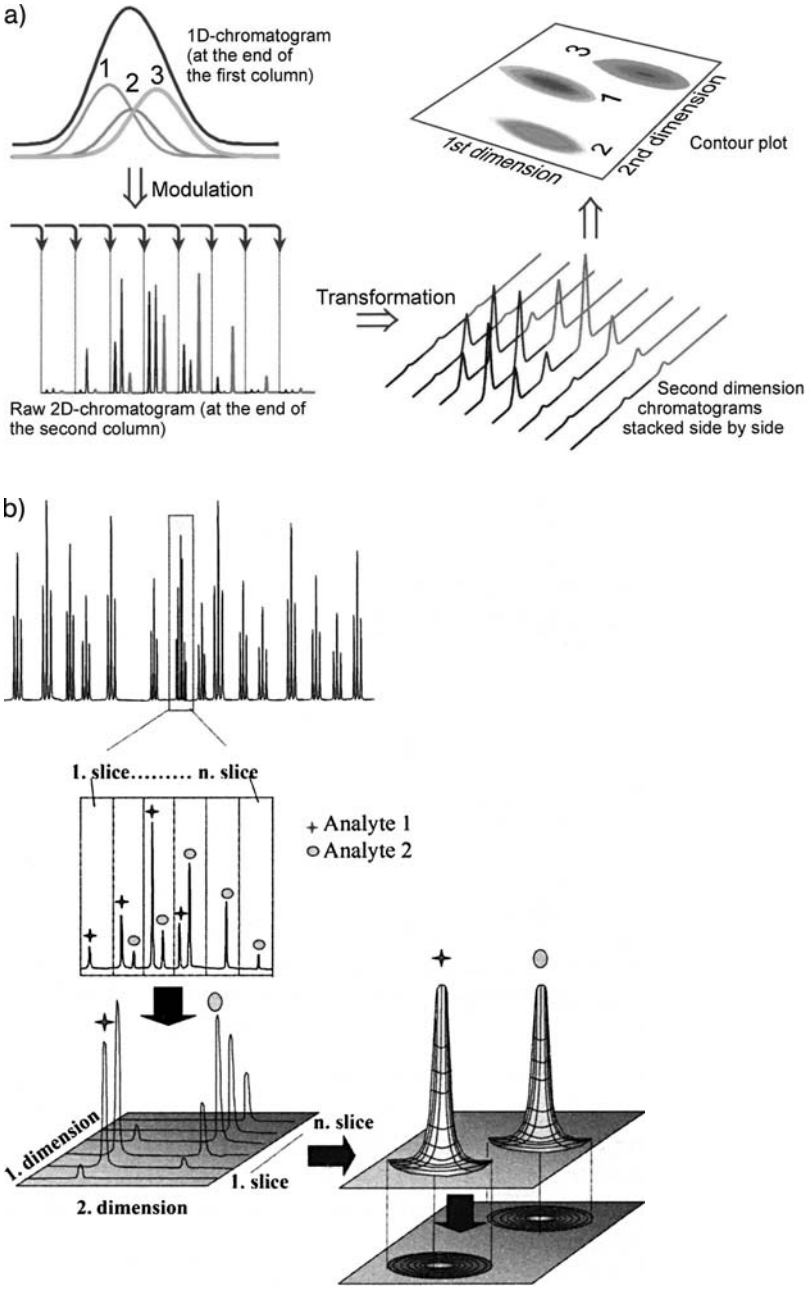


Fig. 2.55 (a) Visualisation of the principle of multidimensional GC separation with the final generation of the contour plot of an unresolved coelution of 3 substances in the 1st dimension (Cavagnino 2003); (b) Construction of a 3-dimensional contour plot for analytes 1 and 2 from a modulated chromatogram (Kellner 2004).

lized best with 3D contour or peak apex plots, illustrating best the commonly used term "comprehensive" chromatography, see Fig. 2.55 a. (Cavagnino 2003). The very high separation power offered by the comprehensive two-dimensional gas chromatography allows, even in the case of complex matrices, a good separation of target compounds from the interferences with a significant increase in signal to noise. This emerging technology has substantially enhanced the chromatographic resolution of complex samples and proven its great potential to further expand the capabilities of modern GC/MS systems.

2.2.2.3 **Modulation**

The key to successful application of GC×GC is the ability to trap and modulate efficiently the first column effluent into the second dimension. It is important to modulate faster than the peak width of first dimension peaks, so that multiple second dimension peaks (slices) are obtained (see Fig. 2.55 a). The moderator unit has to provide minimized bandwidths in sample transfer to retain the first dimension resolution and also allow the rapid remobilization. Practically a high peak compression is achieved by trapping the effluent from the first column on a short band inside of the modulator, being responsible for the significant increase of S/N values (Patterson 2006). This requires a high frequency handling of the column effluent with a reproducible trapping and controlled substance release. Practically applied modulation frequencies range from 4 to 6 s duration depending on the 2nd dimension column parameters.

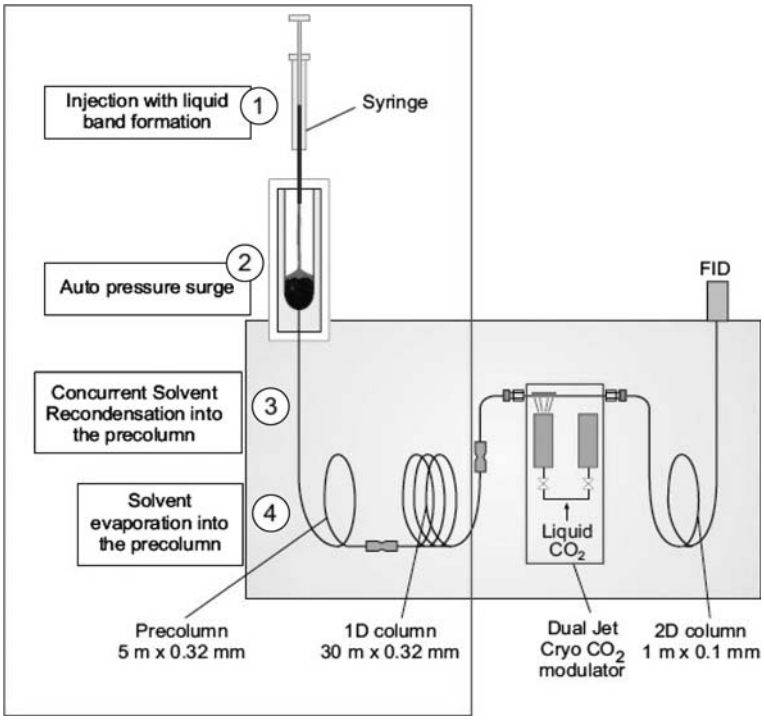


Fig. 2.56 Comprehensive GC schematics using LVSI and dual jet cryo modulator (Cavagnino 2003).

Thermal modulator solutions for substance trapping are:

- Trapping by thick stationary film and heat pulse to release substances.
- Cryogenic zone placed over a modulator capillary. Substances are released when removed and resume movement by oven temperature. Also dual jet cryo modulators have been introduced and commercially available to decouple the processes of collection from the 1st column and sampling into the 2nd dimension, see Figs. 2.57 and 2.58 a (Beens 1998). The operation principle of a dual cryo jet modulator is illustrated in Fig. 2.58 b (Kellner 2004).
- Jet-pulsed modulators, synchronizing hot and cold jet pulses.
- Flow switching valve solutions, used mainly for heart cutting purposes.

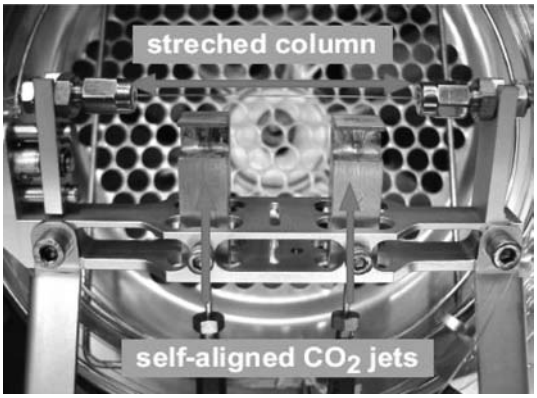


Fig. 2.57 Dual jet cryo modulator, using a stretched column region (Cavagnino 2003).

The availability of commercial instrumentation providing method controlled modulators, able to cool via cryojets, chilled air, liqu. CO_2 or liqu. N_2 and heat the trapping areas for injection rapidly, has made GC × GC straightforwardly applicable today to perform routinely.

2.2.2.4 **Detection**

The high speed conditions of the secondary column deliver very sharp peaks of typically 200 ms base width or below and hence require fast detectors with an appropriate high detection rate. Due to the strong peak band compression obtained during the modulation step, low detection limits are reached. Fast scanning quadrupole MS (scan speed >10,000 Da/s) have been applied by scanning a restricted mass range with an acquisition rate of 25 Hz as a cost effective alternative to TOF-MS for compound identification and quantitation (Mondello 2007). Even sector mass spectrometers have been used successfully in the fast SIM/MID technique, with excellent sensitivity in environmental trace analysis (Patterson 2006). TOF detection and fast scanning quadrupole instruments in a restricted mass range offer full scan analysis capabilities allowing a detailed peak deconvolution for the generation of pure mass spectra also for unresolved trace components, see Fig. 2.59 (also see Section on "Deconvolution").

With regard to sensitivity and specificity GC×GC/TOF-MS has proven to compare well with the classical HRGC/HRMS methods. Isotope ratio measurements of the most intense ions for both natives and isotopically labelled internal standards ensured the required spe-

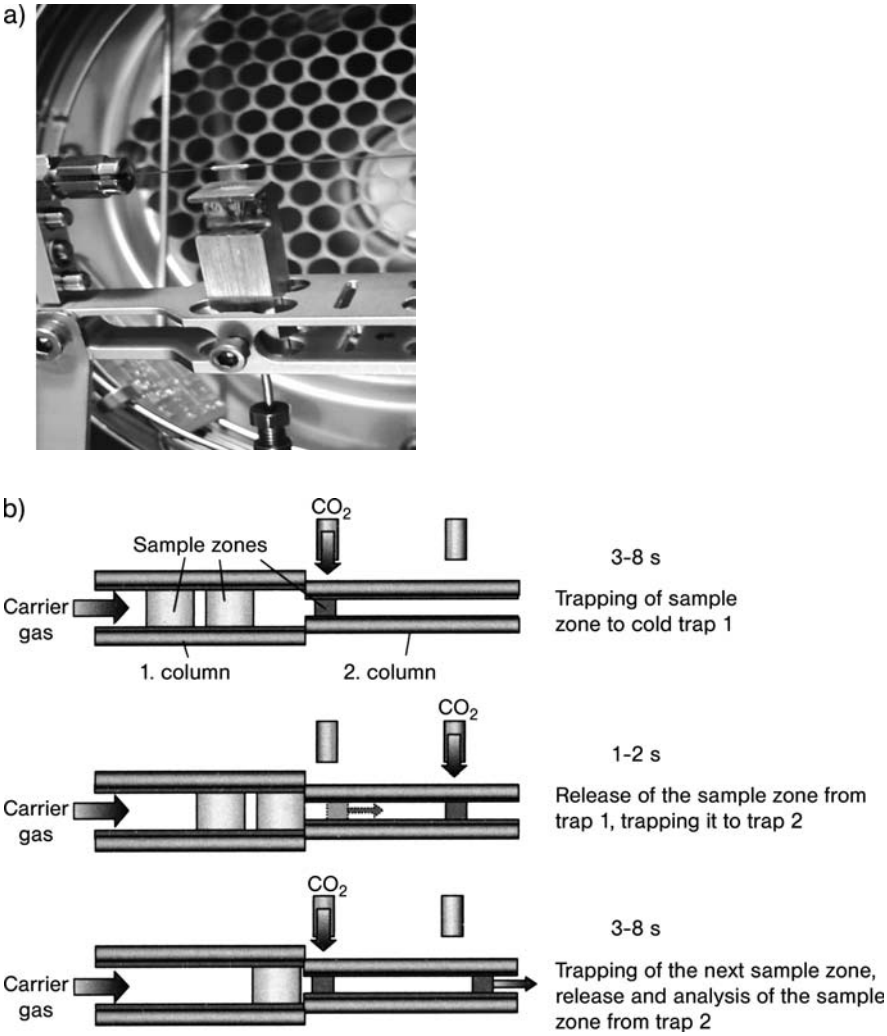


Fig. 2.58 (a) Dual jet cryo modulator in trapping operation on the first column (Cavagnino 2003). (b) Principle of dual jet cryo modulation (Kellner 2004).

cificity. Potentially interfering matrix compounds are kept away from the compounds to be measured in the two-dimensional chromatographic space (Focant 2004).

2.2.2.5 **Data Handling**

Data systems for comprehensive GC require special tools extending the available features of most current GC/MS software suites. Because of the 3D matrix of multiple chromatograms and conventional or MS detection, special tools are required to display and evaluate comprehensive GC separations. The time/response data streams are converted to a matrix format for the two-dimensional contour plot providing a colour code of peak in-

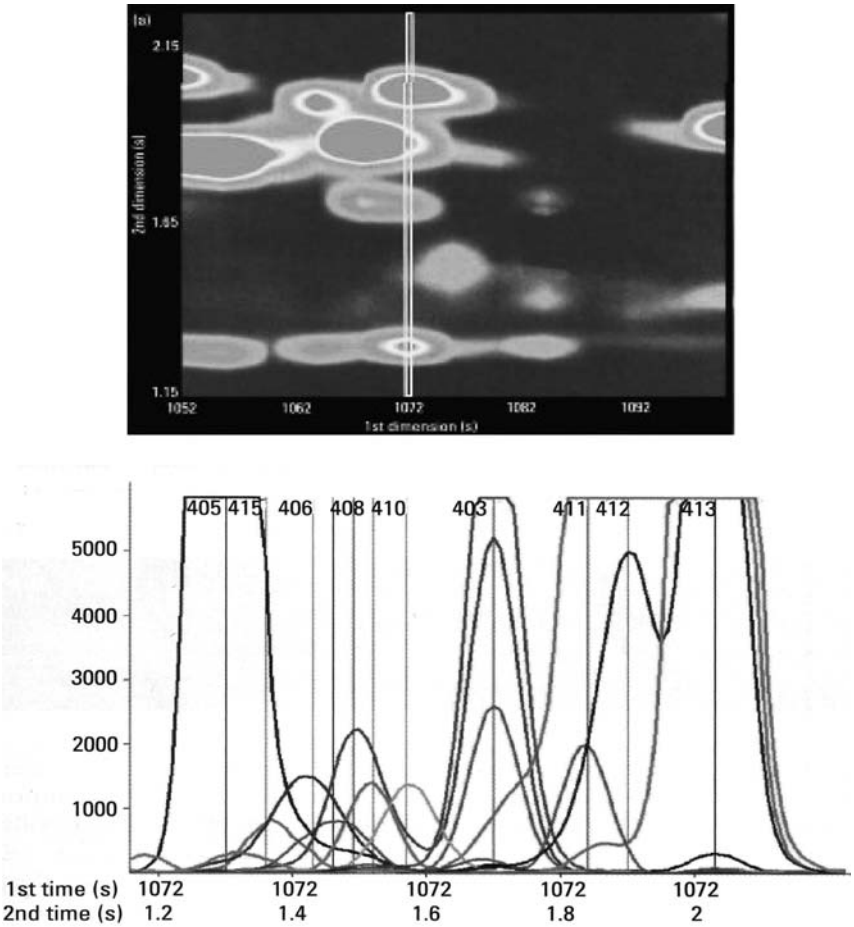


Fig. 2.59 Peak deconvolution in full scan GCxGC/TOF-MS (Dimandja 2004, reprinted with permission from Analytical Chemistry, Copyright American Chemical Society).
Top: 3D contour plot, scan 1072 highlighted.
Bottom: scan 1072, each vertical indicates a deconvoluted compound.

tensities (Fig. 2.59), or the three-dimensional surface plot generation (Fig. 2.60), for visualization.

Integrated software programs are commercially available providing total peak areas also for quantification purposes (Cavagnino 2003, Reichenbach 2004). Dedicated to the comprehensive qualitative sample characterization with GC × GC/TOF-MS analyses, as well as the quantitative analysis of specific mixture analytes, a GC/MS software package is commercially available (Leco 2005).

Advanced chemometric processing options are an active area of current research and will advance the GC × GC technology in the near future. In comprehensive GC using TOF detection (GC × GC/MS), data also can be visualized and processed as an image. Each resolved compound produces a two-dimensional peak and can be visually distinguished through pseudo-

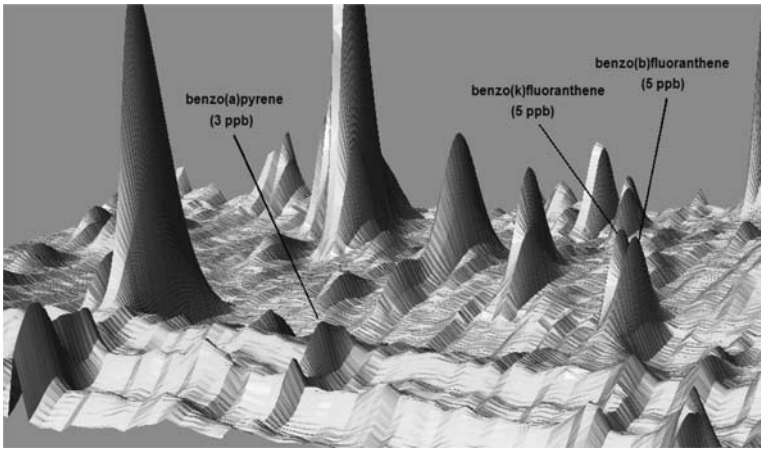


Fig. 2.60 3D Peak view of a GCxGC analysis of a cigarette smoke extract (Cavagnino 2003).

Columns set:	
Precolumn:	5 m deactivated retention gap, 0.32 mm i.d.
1st dimension column:	RTX-5 30 m, 0.32 mm i.d., 0.25 µm df
2nd dimension column:	BPX-50 1 m, 0.1 mm i.d., 0.1 µm df
Carrier:	Helium
Operative conditions:	
Oven:	90 °C (8 min) to 310 °C @ 3 °C/min
Prog. Flow:	2.5 mL/min (4min) to 0.8 mL/min @ 5 mL/min/min
Inj. Vol.:	30 µL splitless
Splitless time:	0.8 min
Moderator:	CO_2 Dual-Jet modulator
Modulation time:	6 s
Data system:	HyperChrom for acquisition and data processing

colour mapping, generating a three-dimensional surface. Digital image processing methods, such as creating a difference image (by subtraction of chromatograms) or addition image (by addition of chromatograms in different colours) will provide insights previously not available to analytical samples. For GC×GC/MS, mass spectral searching can be used in conjunction with pattern matching (Hollingsworth 2006). Those features demonstrate the great potential of GC×GC/TOFMS with its large peak capacity at a largely improved speed of analysis.

2.2.2.6 **Moving Capillary Stream Switching**

Another often used valveless flow switching device for heart cutting purposes or switching the flow direction to different detectors is the Moving Capillary Stream Switching system (MCSS). The MCSS is a miniaturized and very effective flow switching system based on the position of capillary column ends in a small transfer tube. The column effluent is not directed by pressure variations but "delivered" to the required column by moving the end of the delivering column close to the column outlet of choice into an auxiliary carrier gas stream, see Fig. 2.61 (Sulzbach 1990).

Up to five capillary columns can be installed to a small (a few cm long) hollow tip of glass, the "glass dome" (see Fig. 2.61), which is located inside the GC oven. The column ends are

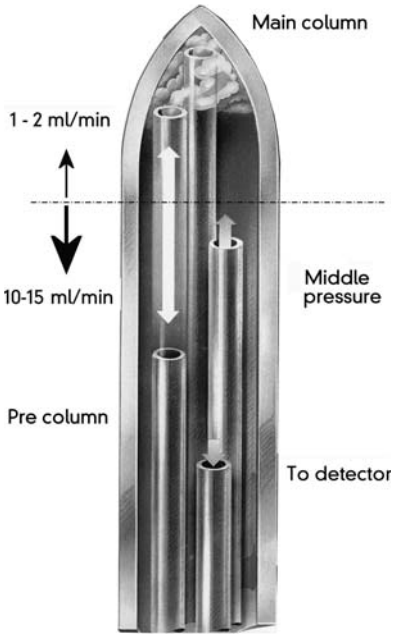


Fig. 2.61 Visualisation of MCSS glass dome operation – the flow of the movable pre-column is directed to the main column (reprinted with permission of Brechbuehler AG, Switzerland).

positioned at different locations inside the glass dome. The delivering pre-column inlet is movable in position over a length of about 1 cm. One of the installed columns is used for a variable make up flow of carrier gas from a second independent regulation. This make-up gas facilitates the parallel coupling of MS detectors in parallel to classical detectors or IRMS to prevent the access of the ions source vacuum to the split region. Instead of an additional detector the additional line can also be used for monitoring the middle pressure between the two columns. Commercial products are available for multidimensional GC covering a variable split range.

The analyte carrier stream fed into the dome by the pre-column can be split into a variable stream upwards, serving a second analytical column, and another stream downwards depending on the position of the pre-column end, e.g., to another column outlet, which can lead to a monitor detector. If the delivering column is pushed fully up, the eluate enters the second column and quantitatively transfers the analytes eluted in that position. There is no change of the pressure conditions in the whole switching system during the transfer period. Therefore, the carrier gas flow rates through both of the columns are always kept stable. Hence there is no influence of the number or duration of switching processes on the retention times on either column. Analytes can only contact inert glass surfaces, fused silica and the column coating; likewise when the sample is injected in any heated injector. Furthermore, the system is free of diffusion and maintains the chromatographic resolution of the pre-column. No influences from ambient atmosphere could be observed using ECD, MS or IRMS detection.

The movement of the pre-column is electrically actuated from outside of the GC oven, completely automated and sequence controlled through the time functions of the GC (see

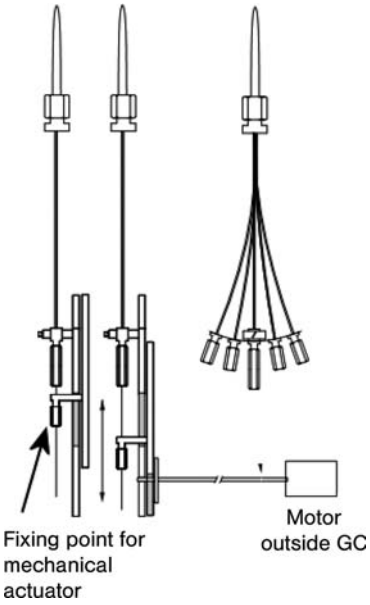


Fig. 2.62 MCSS actuation by external stepper motor.

Fig. 2.62). The MCSS can be used for a number of tasks affording the increased power of multidimensional heart-cutting GC separation (Fig. 2.63). The range of applications covers the classical MDGC in a single or double oven concept, as well as detector switching, back-flush, preparative GC and more.

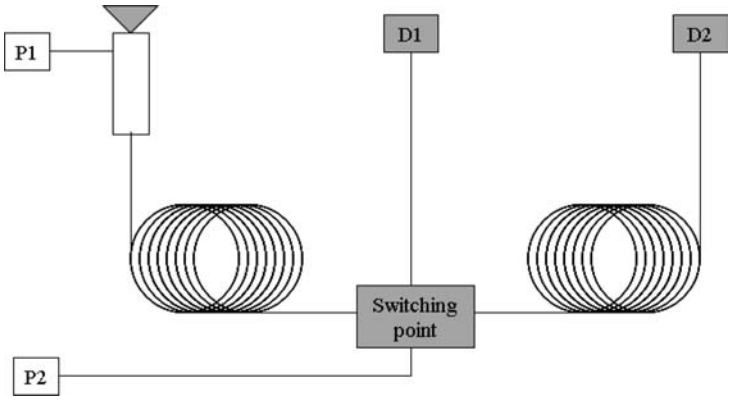


Fig. 2.63 Column Switching Schematics with pre-column, MCSS flow switch at the switch point and main separation column (P1, P2 carrier gas regulations, D1 monitor detector, D2 main detector).

MCSS Applications

Chiral separations of essential oils (FID/MS)
Classification of fuels (FID)
Matrix exclusion (FID/PND/ECD/MS)
Sniffing devices in parallel to detectors (MS)
Coplanar PCBs (double ECD/MS/IRMS)
Pesticides (ECD)
Preparative sampling (Microprep Trap)
All kinds of GC-IRMS applications
Parallel GC-MS-IRMS detection
and many others

2.2.3 GC/MS Sample Inlet Systems

Much less attention was paid to this area at the time when packed columns were used. On-column injection was the state of the art and was in no way a limiting factor for the quality of the chromatographic separation. With the introduction of capillary techniques in the form of glass or fused silica capillaries, high resolution gas chromatography (HRGC) maintained its presence in laboratories and GC and GC/MS made a great technological advance. Many well known names in chromatography are associated with important contributions to sample injection: Desty, Ettre, Grob, Halasz, Poy, Schomburg and Vogt among others. The exploitation of the high separating capacity of capillary columns now requires perfect control of a problem-orientated sample injection technique.

According to Schomburg, sample injection should satisfy the following requirements:

- Achieving the optimal efficiency of the column.
- Achieving a high signal/noise ratio through peaks which are as steep as possible in order to be certain of the detection and quantitative determination of trace components at sufficient resolution (no band broadening).
- Avoidance of any change in the quantitative composition of the original sample (systematic errors, accuracy).
- Avoidance of statistical errors which are too high for the absolute and relative peak areas (precision).
- Avoidance of thermal and/or catalytic decomposition or chemical reaction of sample components.
- Sample components which cannot be evaporated must not reach the column or must be removed easily (guard column). Involatile sample components lead to decreases in separating capacity through peak broadening, and shortening of the service life of the capillary column.
- In the area of trace analysis it is necessary to transfer the substances to be analysed to the separating system with as little loss as possible. Here the injection of larger sample volumes (up to >100 μL) is desirable.

- Simple handling, service and maintenance of the sample injection system play an important role in routine applications.
- The possibility of automation of the injection is important, not only for large numbers of samples, but also as automatic injection is superior to manual injection for achieving a low standard deviation.

Careless injection of a sample extract frequently overlooks the outstanding possibilities of the capillary technique. In all modern GC and GC/MS systems, sample injection is of fundamental importance for the quality of the chromatographic analysis. Poor injection cannot be compensated for even by the choice of the best column material. This also applies to the choice of detectors. The use of a mass spectrometer as a detector can be much more powerful if the chromatography is of the best quality. In fact the use of GC/MS systems shows that as soon as the GC is coupled to an MS system, the chromatography is rapidly degraded to an inlet route. Effort is put too quickly into the optimisation of the parameters of the MS detector without exploiting the much wider possibilities of the GC. Each GC/MS system is only as good as the chromatography allows!

The starting point for the discussion of sample inlet systems is the target of creating a sample zone at the top of the capillary column for the start of the chromatography which is as narrow as possible. This narrow sample band principally determines the quality of the chromatography as the peak shape at the end of the separation cannot be better (narrower, more symmetrical etc.) than at the beginning. As an explanatory model, chromatography can be described as a chain of distillation plates. The number of separation steps (number of plates) of a column is used by many column producers as a measure of the separating capacity of a column. In this sense sample injection means application to the first plate of the column. In capillary GC the volume of such a plate is less than 0.01 µl. The sample extracts used in trace analysis are generally very dilute, making larger quantities of solvent (>1 µl) necessary. This shows the importance of sample injection techniques. In this connection Pretorius and Bertsch should be cited: "When the column is described as the heart of chromatography, sample injection can be identified as the Achilles heel. It is the least understood and the most confusing aspect of modern gas chromatography."

In practice, different types of injectors are used (see Table 2.17). The sample injection systems are classified as hot or cold according to their function. A separate section is dedicated to direct on-column injection.

2.2.3.1 **Carrier Gas Regulation**

The carrier gas pressure regulation of GC injectors found in commercial instrumentation follows two different principles which influence injection modes and the adaptation of automated sample preparation devices. As most of the modern GC instruments are equipped with electronic flow control units (EPC) the sensor control loop must be taken special care of when modifying the carrier gas routes through external devices such as headspace, purge & trap or thermal desorption units. In these installations the carrier gas flow is often directed from the GC regulation to the external device and again returned to the GC injector.

Also, when applying large volume injection techniques, knowledge of the individual pressure regulation scheme is required for successful operation.

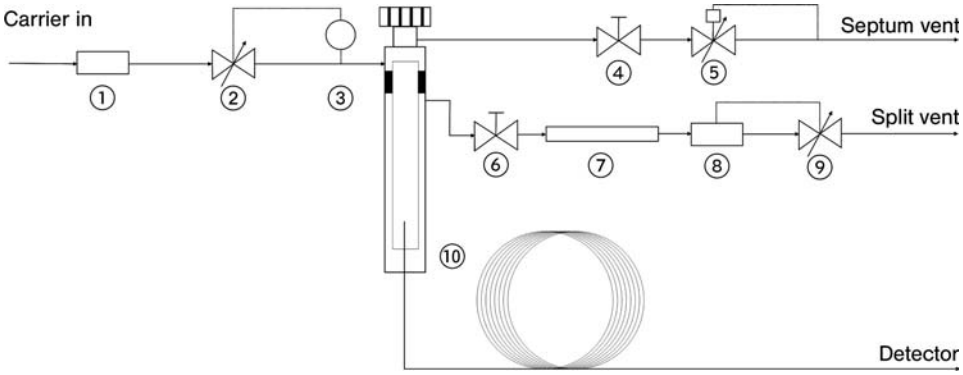


Fig. 2.64 Injector forward pressure regulation.

1 Carrier gas inlet filter
2 Proportional control valve
3 Electronic pressure sensor
4 Septum purge on/off valve
5 Septum purge regulator
6 Split on/off valve
7 Split outlet filter cartridge
8 Electronic flow sensor
9 Proportional control valve
10 Injector with column installed

Forward Pressure Regulation

The classical control of column flow rates is achieved by a "forward pressure" regulation. The inlet carrier gas flow is regulated by the electronic pressure control valve in front of the injector. The pressure sensor is installed close to the injector body and hence to the column head for fast feedback on the regulation either in the carrier gas supply line to the injector, the septum purge or split exit line. The split exit usually is regulated further down the line by a separate flow regulating unit (see Fig. 2.64). An in-line filter cartridge is typically used to prevent the lines from accumulating trace contaminations in the carrier gas, which needs to be exchanged frequently.

The forward injector pressure regulation is the most simple and versatile regulation design used in all splitless injection modes as well as the cold on-column injector. It uniquely allows the large volume injection in the split/splitless mode using the concurrent solvent recondensation (CSR) and the closure of the septum vent during the splitless phase. Also split and splitless injections for narrow bore columns, as used in Fast GC , benefit from the forward pressure regulation with a stable and very reproducible regulation at low carrier gas flow that is not usually possible with mechanical or electronic mass flow controllers. Also, the switch of injection techniques in one injector e. g. the PTV body between regular split/splitless and on-column injection with a retention gap is possible without any modification of the injector gas flow regulation.

The adaptation of an external device is straightforward, with the pressure sensor installed close to the injector head which is generally the case with commercial forward regulated GC instruments. If the pressure sensor is installed in the split line of the injector, it has to be moved close to the inlet to provide a short carrier gas regulation loop. Carrier gas flow and pressure in the external device is controlled by the EPC module of the GC.

Large volume injections are also straightforward using forward pressure regulated injectors. Typically, the regular split/splitless device can be used for large volume injections of up to 10 or 50 µL without any hardware modification, exploiting the concurrent solvent recondensation effect. For injections up to 10 µL, only the injection volume programming of the autosampler and the oven program has to be adjusted accordingly.

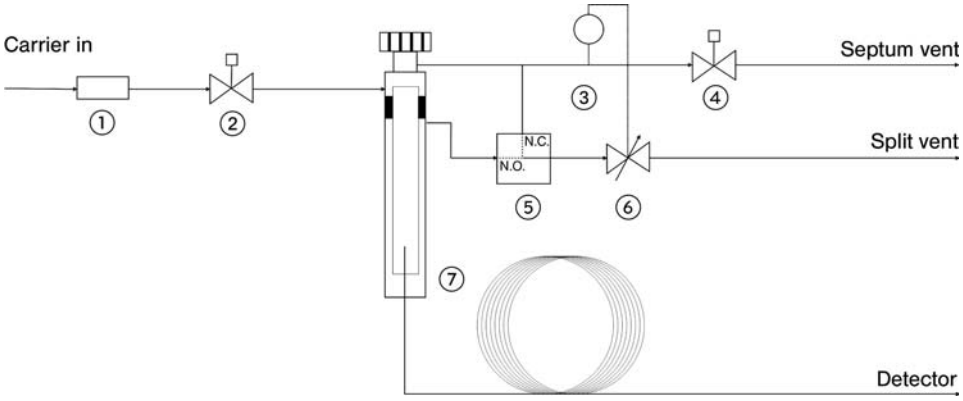


Fig. 2.65 Injector back pressure regulation.
1 Carrier gas inlet filter
2 Mass flow regulator
3 Electronic pressure sensor
4 Septum purge regulator
5 Solenoid valve
6 Electronic pressure control valve
7 Injector with column installed

Back Pressure Regulation

The term "back pressure" describes the position of the electronic pressure control valve behind the injector in the split exit line, in combination with a mass flow controller in front of the injector (see Fig. 2.65). In this widely used carrier gas regulation scheme for split/splitless injectors, the pressure sensor is typically found in the septum purge line close to the injector body to insure a pressure measurement close to the column head. A filter cartridge may be used to protect the regulation unit from any carrier gas contamination.

The total carrier gas flow of the back pressure regulated injector is set by the mass flow controller and is typically held constant independently of the column head pressure. The column flow is then adjusted and kept constant by varying the split exit flow according to the required column flow conditions. This allows the exact and independent setting of split flows from column flows. However, when the backpressure regulated inlet is used in split mode, a large amount of sample passes through the regulation in the split exit line. This mode of operation requires special care using a filter cartridge with frequent exchange to prevent the system from flow restrictions, chemical attack and, finally, clogging.

When adapting an external inlet device, the outlet of the mass flow controller is directed on the shortest route to the external device. A sample gas line from the external device is returned to the injector either using the regular carrier inlet, or by means of a needle through the septum. The pressure sensor must be relocated from the split line close to the electronic pressure control valve to achieve correct flows and sufficient pressure control stability with a short feedback loop due to the additional flow restrictions in the external device. The carrier gas flow through the external device is now regulated by the mass flow controller. The GC inlet pressure at the injector and the effective column flow is regulated independently by the EPC module through the electronic pressure control valve.

Alternatively, an external auxiliary EPC module for feeding the external device may be recommended.

2.2.3.2 **The Microseal Septum**

The Merlin Microseal Septa is a unique long-life replacement for the conventional silicon septa on split/splitless and PTV injectors. Functionally, the Microseal provides a two step sealing mechanism (see Fig. 2.66): A double O–ring type top wiper rib around the syringe needle improves resistance to particulate contamination, and a spring assisted duckbill seals the injection port. The Microseal can be easily installed on many injector types by removing the existing septa nut without any modifications. It can be used at injection port temperatures up to 400 °C. It is commercially available for operation up to 30 psi or up to 100 psi with the high pressure series.

The seal is made from Viton material, a high temperature resistant fluorocarbon elastomer also providing high resistance to wear. It greatly reduces shedding of septum particles into the injection port liner. Because the syringe needle does not pierce a septum layer, it is eliminating a major source of silicon septum bleed and ghost peaks. Due to this sealing mechanism it is especially suited for trace analysis applications requiring particular low background conditions.

The Microseal is usable in either manual or autosampler applications with 0.63 mm diameter (23 ga) blunt tip syringes. The low syringe insertion force makes it ideal for manual injections. The longer lifetime for many thousands of injections reduces the chances for septum leaks especially during extended autosampler runs.

Microseal is a trademark of Merlin Instrument Company.

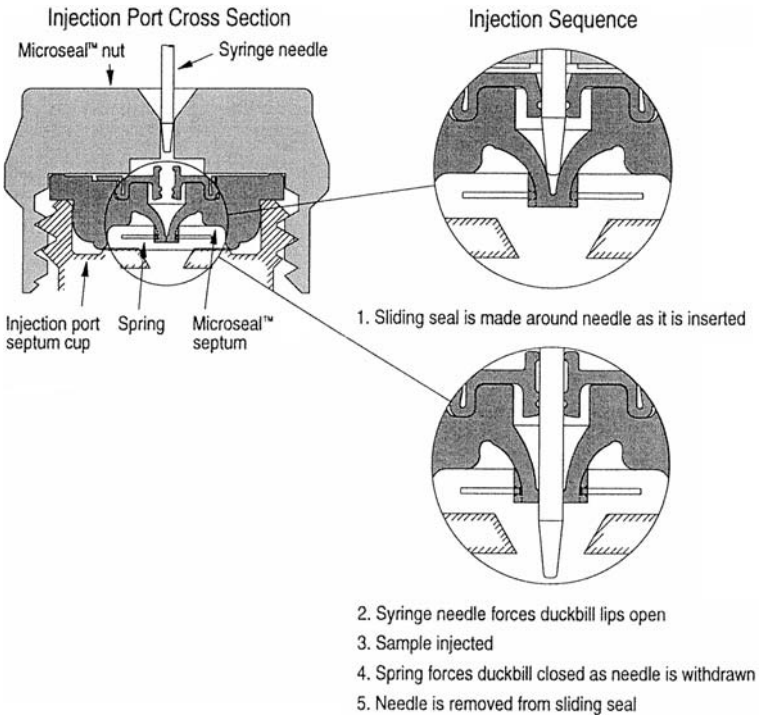


Fig. 2.66 2-Step injection process with the Merlin Microseal. Top: the needle is first penetrating the dust wiper rib; bottom: the needle is penetrating the spring loaded duck bill seal into the injector liner (Merlin Instr. Comp.).

2.2.3.3 Hot Sample Injection

Classical injection techniques involve applying the sample solution in constantly heated injectors. Both the solvent and the dissolved sample evaporate in an evaporation tube specially fitted for the purpose (insert) and mix with the carrier gas. Temperatures of ca. 200 °C to above 300 °C are used for evaporation. The operating procedures of split injection and total sample transfer (splitless) differ according to whether there is partial or complete transfer of the solvent/sample on to the column.

The problem of discrimination on injection into hot injectors arises with the question of what is the best injection technique. Figure 2.67 shows the effects of various injection techniques on the discrimination between various alkanes. While in the chain length range of up to ca. C_{16} hardly any differences are observed, discrimination can be avoided for higher-boiling, long-chain compounds by a suitable choice of injection technique. For example, filling the injection syringe with a solvent/derivatising agent plug before drawing up the sample and observing the needle temperature during a short period after insertion into the injector.

- *Solvent Flush/Hot Needle:* This is the injection procedure of choice for hot injectors and gives favourable discrimination properties. Circa 0.5–1 µl solvent or derivatising agent are first taken up in the syringe, then 0.5 µl air and finally the sample extract (sandwich technique). Before the injection the liquid plug is drawn up into the body of the syringe. The volumes can thus be read better on the scale. The injection involves first inserting the needle into the injector, waiting for the needle to warm up (ca. 2 s) and then rapidly injecting the sample. The solvent flush/hot needle procedure can be carried out manually or using a programmable autosampler.
- *Hot Needle:* This technique is definitely the most frequently used variant. Only the sample extract is drawn up into the syringe; the plug is thus drawn up into the body of the syringe

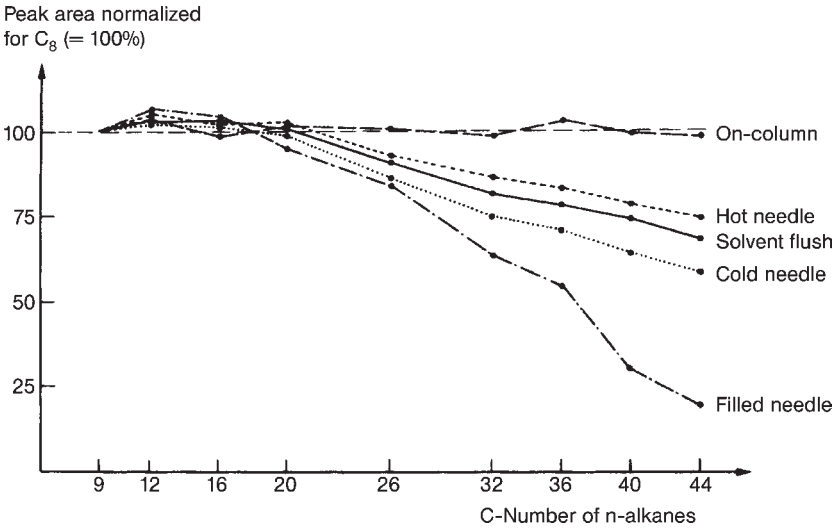


Fig. 2.67 Discrimination among alkanes using different injection techniques (Grob 2001). The peak areas are normalised to C_8 = 100 %.

so that the volume can be read off on the scale. After inserting the needle, there is a pause for warming up and then the sample is rapidly injected.

- *Solvent Flush/Cold Needle:* Here the sandwich technique described above is used to fill the syringe. The injection is, however, carried out very quickly (usually using an autosampler) without waiting for the injection needle to warm up. This technique is most used with filled liners (e.g. glass wool, absorbent) allowing the formation of a liquid band inside the insert liner for the deposit of the liquid sample in the adsorbent at the bottom part of the liner. Evaporating liquid from the adsorbent leads to a local temperature decrease keeping the analytes focused in a small region for the transfer to the column.
- *Cold Needle:* Only the sample extract is drawn up into the syringe; the plug is held in the body of the syringe in such a way that the volume on the scale can be read. The injection is carried out very rapidly (usually with an autosampler) without waiting for the needle to warm up allowing liquid band formation with packed liners as well.
- *Filled Needle:* This injection procedure is no longer up-to-date and should be avoided with hot vaporizing injectors. It is associated with certain types of syringe which, on measuring out the sample extracts, can only allow the liquid plug into the injection needle. Warning: certain automatic liquid sample injectors use this procedure.

Split Injection

After evaporation of the liquid sample in the insert, with the split technique the sample/carrier gas stream is divided. The larger, variable portion leaves the injector via the split exit and the smaller portion passes on to the column. The split ratios can be adapted within wide limits to the sample concentration, the sensitivity of the detector and the capacity of the capillary column used. Typical split ratios mostly lie in the range 1 : 10 to 1 : 100 or more. The start values for the temperature program of a GC oven are independent of the injection procedure using the split technique. If the oven temperature is kept below the boiling point of the solvent at a given pressure, the re-concentration of the solvent into the column needs to be considered when calculating the split ratio. In this case more sample enters into the column and consequently the split ratio is not as calculated by the measured flow ratios. For that reason it is a good practice to keep the column temperature sufficiently high to avoid any recondensation during the split injection, if the split ratio is important.

For concentrated samples the variation of the split ratio and the volume applied represents the simplest method of matching the quantity of substance to the column load and to the linearity of the detector. Even in residue analysis the split technique is not unimportant. By increasing the split stream the carrier gas velocity in the injector is increased and this allows the highly accelerated transport of the sample cloud past the orifice of the column. This permits a very narrow sample zone to be applied to the column. To optimise the process the possibility of a split injection at a split ratio of less than 1 : 10 should also be considered. In particular, on coupling with static headspace or purge and trap techniques better peak profiles and shorter analysis times are achieved. The smallest split ratio which can be used depends on the internal volume of the insert. For liquid injection in hot split mode in general a large diameter liner for a reduced carrier gas speed is required to allow the sample to vaporize and mix with carrier gas before reaching the split point. Small diameter liners can induce a partial splitting as liquid that produce non repeatable data.

A disadvantage of the split injection technique is the uncontrollable discrimination with regard to the sample composition. This applies particularly to samples with a wide boiling point range. Quantitation with an external standard is particularly badly affected by this. Because of the deviation of the effective split ratio from that set up, this value should not be used in the calculation. Quantitation with an internal standard or alternatively the standard addition procedure must be used.

Total Sample Transfer (Splitless Injection)

With the total sample transfer technique the sample is injected into the hot injector with the split valve closed (Fig. 2.68). The volume of the injector insert must be able to hold the solvent/sample vapour cloud completely. Because of this special insert liners (vaporiser) are recommended for splitless operations. Depending on the insert used and the solvent, there is a maximum injection volume which allows the vapour to be held in the insert. Inserts which are too small lead, on explosive evaporation of the solvent, to expansion of the sample vapour beyond the inserts into the cold regions of the injector, and are causing a probable loss by the septum purge. Pressure waves of subsequent injections are bringing back deposited material as carry over from the split line into the next analysis. Inserts which are too

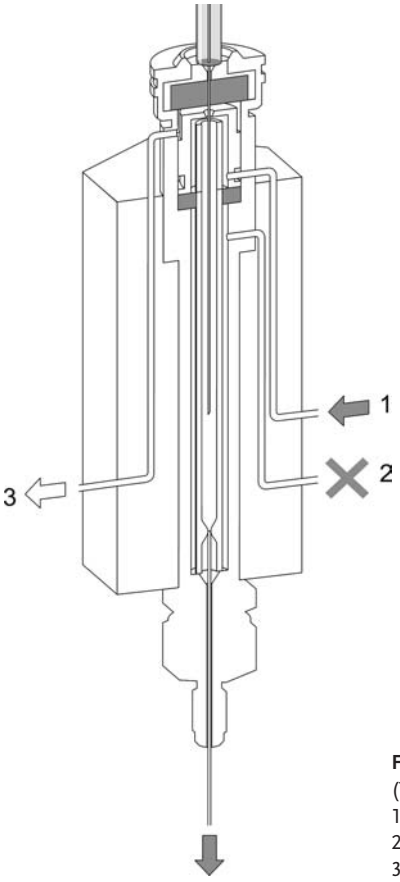


Fig. 2.68 Hot split/splitless injector (Thermo Fisher Scientific).
1 Carrier gas inlet
2 Split exit line
3 Septum purge line

wide lead to significant dilution of the sample cloud and thus to prolonged transfer times and losses through diffusion. A recent compromise made by many manufacturers involves insert volumes of 1 mL for injection volumes of about 1–2 µL. Splitless injections require an optimized needle length as stated by the manufacturer that defines the point of evaporation inside the liner. The septum flush should not be completely closed even with splitless injection. With many commercial SSL injectors the closure of the septum purge is optional and can be used to favor the auto-pressure surge. For application of surge pressures during injection the septum purge will be closed. On correct choice of insert the sample cloud does not reach the septum so that the low purge flow does not have any effect on the injection itself.

The carrier gas flushes the sample cloud continuously from the injector on to the column. This process generally takes ca. 30–90 s for complete transfer depending on the insert volume, sample volume and carrier gas flow. There is an exponential decrease in concentration caused by mixing and dilution with the carrier gas. Longer transfer times are generally not advisable because the sample band becomes broadened on the column. Ideal transfer times allow three to five times the volume of the insert to be transferred to the column. This process is favoured by high carrier gas flow rates and an increased head pressure during the transfer process (surge pressure). Hydrogen is preferred to helium as the carrier gas with respect to sample injection. For the same reason a column diameter of 0.32 mm is preferred for the splitless technique compared to narrower diameters, in order to be able to use optimal flow rates in the injector. In the same way a pressure surge step is available in modern GC instrumentation using electronic pressure regulation to improve the sample transfer.

As the transfer times are long compared with the split injection and lead to a considerable distribution of the sample cloud on the column, the resulting band broadening must be counteracted by a suitable temperature of the column oven. Only at start temperatures below the boiling point of the solvent sufficient refocusing of the sample is achieved because of the solvent effect. A rule of thumb is that the solvent effect operates best at an oven temperature of 10–15 °C below the boiling point of the solvent. The solvent condensing on the column walls acts temporarily as an auxiliary phase, accelerates the transfer of the sample cloud on to the column, holds the sample components and focuses them at the beginning of the column with increasing evaporation of solvent into the carrier gas stream (solvent peak). Here the use of "GC compatible solvents" that are miscible with the stationary phase becomes important. "Incompatible" solvents do not generate a suitable solvent effect and need to be avoided or applied in split mode. Only after the sample transfer is complete the split valve is opened until the end of the analysis to prevent the further entry of sample material or contaminants on to the column. The splitless technique therefore requires working with temperature programs. Because of the almost complete transfer of the sample on to the column, total sample transfer is the method of choice for residue analysis.

Because of the longer residence times in the injector, with the splitless technique there is an increased risk of thermal or catalytic decomposition of labile components. There are losses through adsorption on the surface of the insert, which can usually be counteracted by suitable deactivation. Much more frequently there is an (often intended) deposit of involatile sample residues in the insert or septum particles collect. This makes it necessary to regularly check and clean the insert.

Concurrent Solvent Recondensation

The large volume injection possible on a regular splitless injector did not garner much attention until the transfer mechanism of the sample vapour into the column involved was further investigated and fully understood. The concurrent solvent recondensation technique (CSR) permits the injection of increased sample amounts up to 50 µL by using a conventional split/splitless injector with forward pressure regulation. This can be achieved in a very simple and straightforward way, since all processes are self-regulating. Moreover, the technique is robust towards contaminants and therefore it is suitable when complex samples have to be injected in larger amounts (Magni 2003).

Due to the recondensation of the solvent inside of the capillary column causing a pressure drop at the beginning of the column, a strong pressure difference occurs between column and injector liner that significantly speeds up the sample transfer. The sample and solvent vapour is "sucked down" from the insert by the lower pressure inside of the column and continuously condensed to form the liquid band at the beginning of the column (see Fig. 2.69). For proper operation of the CSR technique the SSL injector has to be operated in the forward pressure regulation. A back pressure regulation would compensate for the occurring inlet pressures causing severe sample loss.

Sample volumes of up to 10 µL can be injected by using the regular 0.25 mm capillary columns while sample volumes of up to 50 µL require the wider diameter of an empty retention gap with 0.53 mm i.d. to accept the complete amount of liquid injected and some silanized glass wool in the insert liner (focus liner).

The practical benefit of CSR is the flexibility for the injection of a wider range of diluted sample volumes reducing significantly the time for extract pre-concentration. As the CSR injection is performed with the regular SSL injector hardware without any modifications, only the autosampler injection volume, when using regular 10 µL syringes, has to be programmed in the methods. For increasing volumes the initial isothermal time needs to be extended accordingly up to the end of the solvent evaporation from the pre-column. The increased solvent vapor cloud needs to leave the condensation region (band formation) before starting the oven program. The remaining oven temperature program remains unchanged.

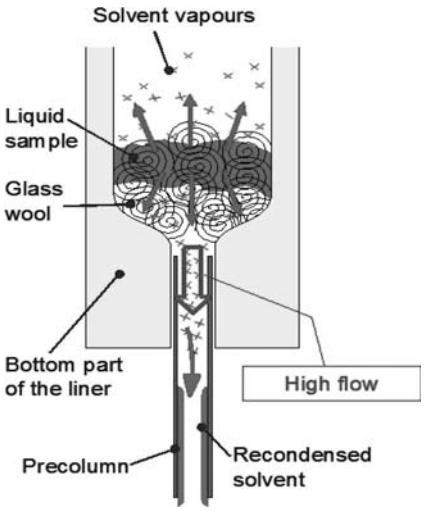


Fig. 2.69 Principle of concurrent solvent recondensation in splitless injection (Magni 2003).

Concurrent Solvent Recondensation
Key steps (see Fig. 2.69)

Restricted evaporation rate:
Injection with liquid band formation: The collection of the liquid on the glass wool allows a slow evaporation from a "single" droplet.

Increased transfer rate:
Auto-pressure surge due to the large amount of solvent: A temporary increase of the inlet pressure rapidly drives the vapours into the column where the recondensation process starts. The concurrent solvent recondensation in the pre-column is generating a strong suction effect.

Solvent evaporation in the pre-column:
The oven temperature is kept below the boiling point up to the end of solvent evaporation from the pre-column.

2.2.3.4 Cold Injection Systems

The cold sample injection technique involves injecting the liquid 'cold' sample directly on to the column (on-column, see Section 2.2.1.4) or into a specially constructed vaporiser. The sample extract is ejected from the syringe needle in liquid form into the insert at temperatures which are well below the boiling point of the solvent. Heating only begins after the syringe needle has been removed from the injection zone. In the area of residue analysis programmed temperature vaporiser (PTV) cold injection systems for split and splitless injection are becoming more widely used, as are on-column systems for exclusively splitless injection.

The cold injection of a liquid sample eliminates the selective evaporation from the syringe needle in all systems, which, in the case of hot injection procedures, leads to discrimination against high-boiling components. Discrimination is also avoided as a result of explosive evaporation of the solvent in hot injectors. Colder regions of the injection system, such as the septum area or the tubing leading to the split valve, can serve as expansion areas if the permitted injection volume is exceeded and can retain individual sample components through adsorption. The individual concepts of cold injection differ in the transfer of the sample to the column and in the possibility for using the split exit. There are definite advantages and limitations for the operation of injectors in practice and for the areas of use envisaged. These exist both between different cold injection techniques and in comparison with hot injection techniques.

The PTV Cold Injection System

The temperature-programmed evaporation with split or splitless operations using the currently available injectors is based on the systematic work of Poy (1981) and Schomburg (1981) (Figs. 2.70 and 2.71). In particular, emphasis was placed on the precise and accurate execution of quantitative analyses of complex mixtures with a wide boiling point range. Particularly at the beginning of the experiments, absence of discrimination for substances up to above C_{60} was documented and its suitability for involatile substances, such as polyaromatic

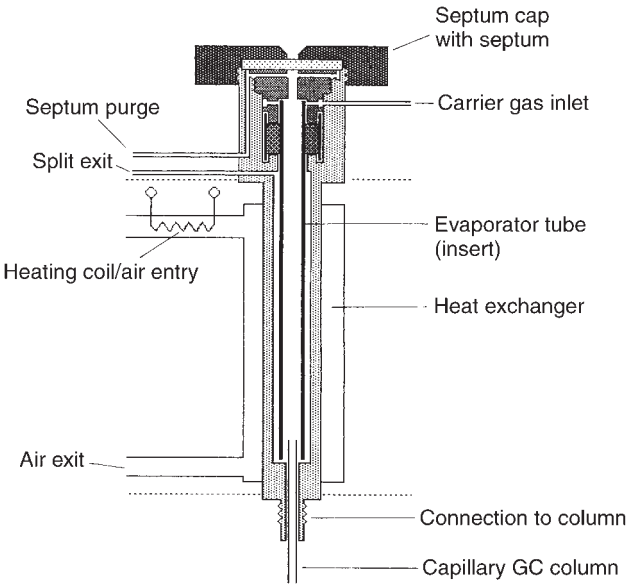


Fig. 2.70 PTV with air as the heating medium from the design by Poy.

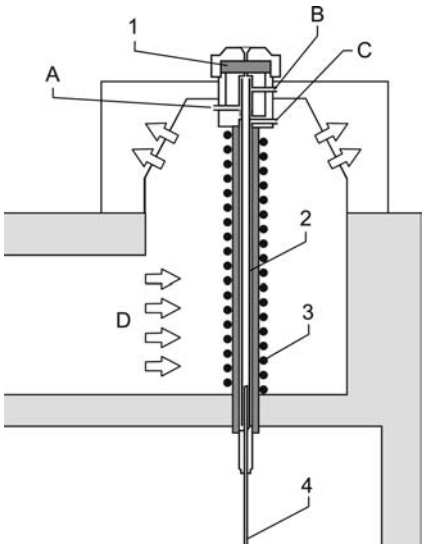


Fig. 2.71 PTV split/splitless injector with direct heating (Thermo Fisher Scientific).

A Carrier gas inlet	1 Septum
B Septum purge line	2 Injector body
C Split exit line	3 Direct heating
D Active cooling	4 Analytical column

hydrocarbons, was stressed. Examples of the analysis of triglycerides demonstrate the injection of samples up to above C_{50} and the analysis of crude oil fractions up to C_{90}! The PTV process combines the advantages of the hot and on-column injection techniques.

There are many advantages of cold split and splitless sample injection:

- Discrimination as a result of fractionated evaporation effects from the syringe needle does not occur.
- A defined volume of liquid can be injected reproducibly.
- The sample components evaporate as a result of controlled heating of the injection area in the order of their boiling points. The solvent evaporates first and leaves the sample components in the injection area without causing a distribution of the analytes in the injector as a result of explosive evaporation.
- Aerosol and droplet formation is avoided through fractional evaporation.
- As the evaporator does not have to take up the complete expansion volume of the injected sample solution, smaller inserts with smaller internal volumes can be used. The consequently more rapid transfer to the column lowers band broadening of the peaks and thus improves the signal/noise ratio.
- If the boiling points of the sample components and the solvent differ by more than 100 °C larger sample volumes (up to more than 100 µl) can be injected (solvent split).
- Impurities and residues which cannot be evaporated do not get on to the column.
- With concentrated samples the possibility can be used of adapting the injection to the capacity of the column and the dynamic range of the detector by selecting the split ratio.

Currently the PTV cold injection technique is mainly used for residue analysis in the areas of plant protection agents, pharmaceuticals, polyaromatic hydrocarbons, brominated flame retardants, dioxins and PCBs. However, the enrichment of volatile halogenated hydrocarbons, the direct analysis of water and the formation of derivatives in the injector also demonstrate the broad versatility of the PTV type injector.

The PTV Injection Procedure

PTV Total Sample Transfer

The splitless injection for the injection of a maximum sample equivalent on to the column is the standard requirement for residue analysis. The sample is taken up in a suitable solvent and injected at low temperature. The split valve is closed. The PTV temperature at injection should correspond to the boiling point of the solvent at 1013 mbar. During the injection phase the oven temperature is kept below the PTV temperature. It should be below the boiling point of the solvent in order to exploit the necessary solvent effect according to Grob for focusing the substances at the beginning of the column (Fig. 2.72). If the focusing of the substances is unsuccessful or insufficient, the peaks of the components eluting early are broad and are detected with a low signal/noise ratio.

The injector is heated a few seconds after the injection when the solvent has already evaporated and has reached the column (Fig. 2.73). Typically this time interval is between 5 and 30 s. For high-boiling substances in particular longer residence times have been found to be favourable. The heating rate should be moderate in order to achieve a smooth evaporation of the sample components required for transfer to the column. Heating rates of ca. 200–

300 °C/min have proved to be suitable. The optimal heating rate depends on the dimensions of the insert liner and the flow rate of the carrier gas in the insert.

PTV Total Sample Transfer

- Split valve closed
- PTV at the boiling point of the solvent
- Oven temperature below the boiling point of the solvent
- Start of PTV heating ca. 10 s after injection
- Start of the GC temperature program ca. 30–120 s after injection
- PTV remains hot until the end of the analysis

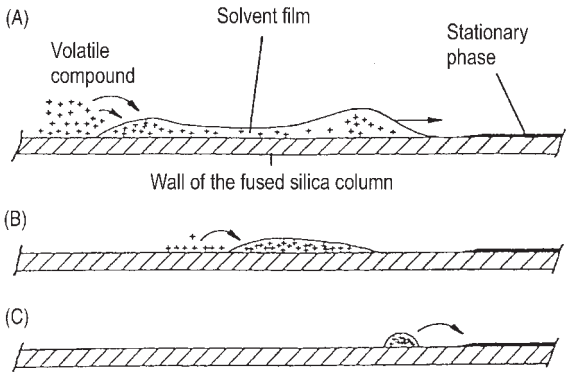


Fig. 2.72 Refocusing by means of the solvent effect in splitless injection (after Grob).
(A) Condensation of solvent at the beginning of the capillary column by lowering the oven temperature below the boiling point. The condensed solvent acts as a stationary phase and dissolves the analytes. At the same time this front migrates and evaporates in the carrier gas flow.
(B) The continuous evaporation of the solvent film concentrates the analytes on to a narrow ring (band) in the column. For this process no stationary phase at the beginning of the column is necessary.
(C) The substances concentrated in a narrow band meet the stationary phase. The separation begins with a sharp band.

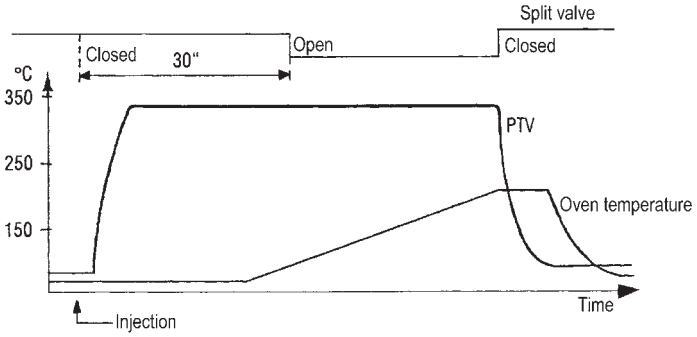


Fig. 2.73 PTV total sample transfer (splitless injection).

Filling the insert with silanised glass wool has proved effective for the absorption of the sample liquid and for rapid heat exchange. However, the glass wool clearly contributes to enlarging the active surface area of the injector and should therefore only be used in the analysis of noncritical compounds (alkanes, chlorinated hydrocarbons etc.). For the injection of polar or basic components an empty deactivated insert is recommended.

After the transfer of the substances to be analysed to the column, which is complete after ca. 30–120 s, the temperature program of the GC oven is started and the injector purged of any remaining residues by opening the split valve. If required, a second PTV heating ramp can follow to bake out the insert at elevated temperatures. The high injection temperature of the PTV is retained until the end of the analysis to keep the injector free from possible adsorptions and the accumulation of impurities from the carrier gas inlet tubing. The cooling of the PTV is adjusted so that both the PTV and the oven are ready for the next analysis at the same time.

A special form of the PTV cold injection system consists of the column and insert connected via a press-fit attachment. Total sample transfer is possible in the same way as the classical PTV injection, but no split is present to allow flushing of the injection area after evaporation. Injectors of this type can only be used for total sample transfer (e. g. SPI injector, Fig. 2.74).

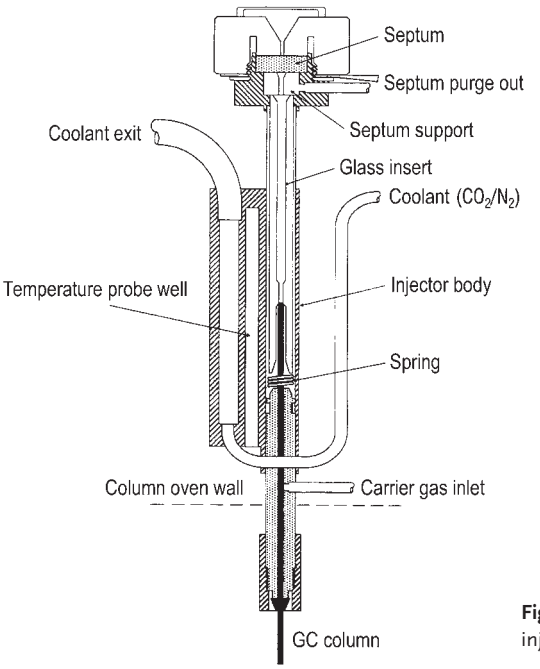


Fig. 2.74 Septum-equipped programmable injector, SPI (Finnigan/Varian).

PTV Split Injection

In this mode of operation the split valve is open throughout (Fig. 2.75). In classical sample application this mode of injection is suitable for concentrated solutions, whereby the column loading can be adapted to its capacity and the nature of the detector by regulating the split flow. Cold injection systems are characterised by the particular dimensions of the inserts. This allows a high carrier gas flow rate at the split point. Compared to hot split injectors,

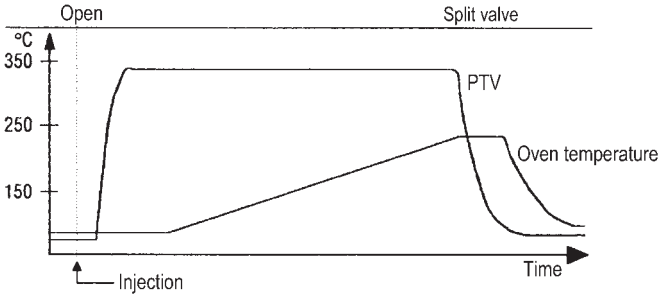


Fig. 2.75 PTV split injection.

smaller split ratios of ca. 1:5 are possible. In an individual case it is possible to work in the split mode and thus achieve better signal/noise ratios and shorter analysis times, compared with total sample transfer

PTV Split Injection

- Split valve open
- PTV below the boiling point of the solvent
- Start of PTV heating ca. 10 s after injection
- Oven temperature to be chosen freely
- PTV remains hot until the end of the analysis

All other PTV adjustments of time and temperature are completely unchanged compared with total sample transfer! Of particular importance for the split injection using the PTV cold injection system is the fact that the sample is injected into the cold injector (see also Section 2.2.3.5). The choice of start temperature of the GC oven is now no longer coupled to the boiling point of the solvent because of the solvent effect and is chosen according to the retention conditions.

PTV Solvent Split Injection

This technique is particularly suitable for residue analysis for the injection of solutions of diluted extracts and with low concentrations, which would undergo loss of analyte on further concentration (Fig. 2.76). The solvent split mode allows most efficient sample throughput shortening time-consuming pre-concentration steps. With suitable parameter settings very large solvent quantities, also for a fraction collection from an online sample preparation and being limited only by practical considerations, can be applied.

To inject larger sample volumes (from ca. 2 µl to well over 100 µl) it is advantageous for the insert to be filled at least with silanised glass wool or packing of Tenax, Chromosorb Supelcoport or another inert carrier material ca. 0.5–1 cm wide. The split valve is open during the injection phase. The PTV is kept at a temperature which corresponds to the boiling point of the solvent. The oven temperature is below the PTV temperature and thus below the boiling point of the solvent. The maximum injection rate depends upon how much solvent per unit time can be evaporated in the insert and carried out through the split tubing by the carrier gas. The at-once injection of large volumes can best be tested easily by apply-

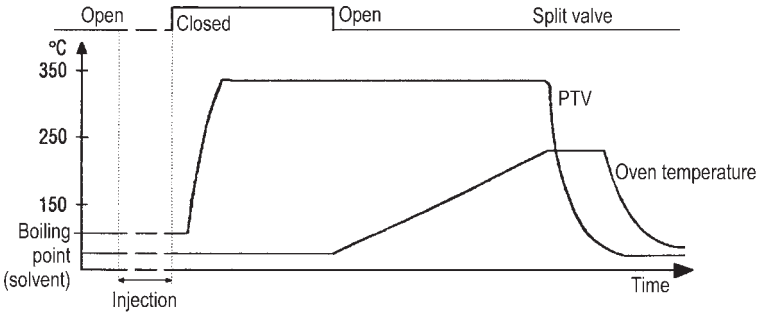


Fig. 2.76 PTV solvent split injection.

ing the desired amount of solvent with the liner outside of the injector. During injection a high split flow of >100 mL/min is recommended to focus the analytes on the packing material by local consumption of evaporation heat. For ease of method development of the injection the data recording of the GC or GC/MS system is started so that the course of the solvent peak can be followed (Fig. 2.77).

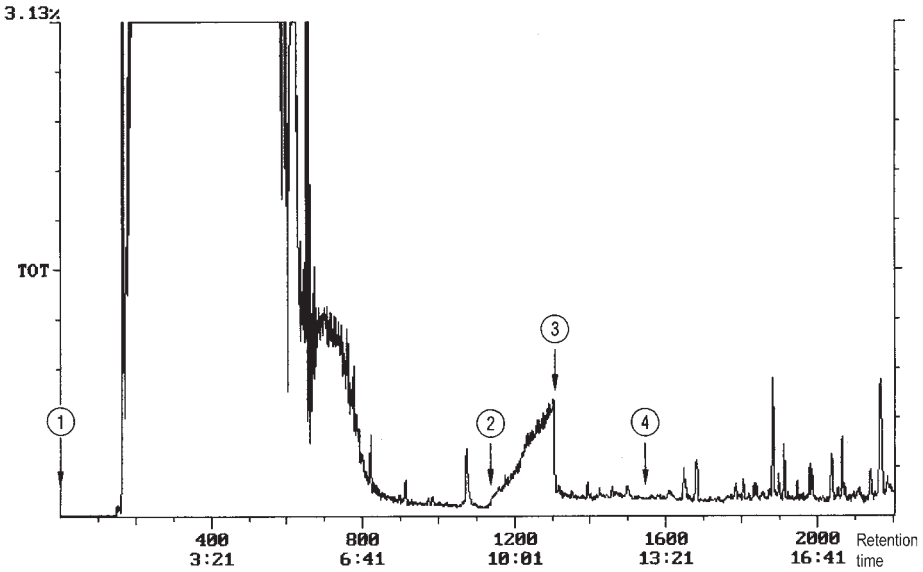


Fig. 2.77 The course of an analysis with large quantities of solvent for the PTV cold injection system during method development: 100 µL PCB solution (200 pg/µL). After the dead time a broad solvent peak is registered. After the solvent peak is eluted, the split valve is closed and the PTV heated up. A smaller roughly triangular solvent peak is produced from adsorbed material. After injection the split valve is opened again and the temperature program started. The peaks eluting show good resolution and are free from tailing.

1 Start of the injection: split open
2 Start of PTV heating: split closed
3 Baking out the PTV: split open
4 Start of the GC temperature program: split open
and typically the start of the data acquisition

When quantities of more than 10 µL are applied, the split valve is only closed when the end of the solvent peak begins to show on the display, meaning that the injector is free of most of the solvent. After the split valve has been closed, the PTV injector can be heated up as usual. After ca. 30–120 s the transfer of the enriched sample from the insert to the column is complete and the GC temperature program can be started. The split valve then opens and the PTV is held at the injection temperature until the end of the analysis.

The PTV solvent split mode also allows the derivatisation of substances in the injector. This procedure simplifies sample preparation considerably. The sample extract is treated with the derivatising agent (e.g. TMSH for methylation) and injected into the PTV. The excess solvent is blown out during the solvent split phase. The derivatisation reaction takes place during the heating phase and the derivatised substances pass on to the column.

PTV Large Volume Injection

The PTV large volume injection mode (LVI-PTV) allows the repeated automated injection of sample volumes in the range up to 100 µL and even more. The large volume mode requires that the sample components are less volatile than the used solvent. The LVI-PTV operation needs the additional installation of a heated solvent split valve to prevent solvent vapour to condensate and plug the split exhaust line. The operation is further facilitated by a backflush valve below of the injector system which is recommended to prevent large amounts of solvent vapour entering the analytical column and for cleaning the injector during the heat-off step after injection.

As described in the previous section for PTV solvent split injections, a wide liner needs to be filled either by silanised quartz wool or other suitable packing material to retain the sample and prevent solvent from rinsing through the liner. The solvent capacity of the liner can easily be tested outside of the injector by adding the intended amount of solvent. No rinsing of the solvent may be observed when holding the insert liner upright on a sheet of paper.

Table 2.20 Effective solvent boiling points for different inlet pressures in °C.

Solvent	BP standard	100 kPa	200 kPa	300 kPa	400 kPa	500 kPa
iso-Pentane	28	49	65	77	87	98
Diethylether	35	54	72	84	93	101
n-Pentane	35	57	72	84	93	102
Dichloromethane	40	60	75	87	96	105
MTBE	55	72	91	104	118	124
Methylacetate	57	72	91	104	115	124
Chloroform	61	81	97	109	121	130
Methanol	65	82	97	107	116	124
n-Hexane	69	95	111	124	136	145
Ethylacetate	77	97	114	126	136	145
Cyclohexane	81	106	122	137	149	159

Comparison of large volume injection methods

	LV-On-column with solvent vapor exit	LV-PTV with solvent split	LV-Split/Splitless with concurrent solvent recondensation
Typical injection volume	150 µL	100 µL	30 µL
Robust versus complex matrix	No	Yes	Yes
Volatiles analysis	Yes	Need optimization	Yes
Suitability for thermolabile and actives compounds	High	Medium	Medium
Solvent vented	Yes	Yes	No
Requires uncoated pre-column	Yes	No*	Yes
Number of parameters	Medium	Large	Small**
Software assisted set-up	Yes	No	Yes

* It can be necessary if large amount of solvent is retained for improving volatiles recovery.
** Up to 10 µL with regular 0.25 ID columns.

PTV Cryo-enrichment

This injection technique is suitable for trace analysis and for concentrating volatile compounds, which are present in large volumes of gas, for example in gas sampling, thermodesorption or the headspace technique. Sample transfer into the injector is carried out while the PTV is in trapping mode. For this the insert is filled with a small quantity (ca. 1–2 mg, ca. 1–3 cm wide) of Tenax, Carbosieve or another thermally stable adsorbent. The PTV injector is cooled with liquid CO_2 or nitrogen until injection. During the injection the split valve is open as in the solvent split mode. Like the total sample transfer method, the oven temperature is kept correspondingly low in order to focus the components at the beginning of the column.

The gaseous sample is passed slowly through the injector and the organic substances retained on the adsorbent. Before injection the PTV can be heated to a low temperature with the split still open to dry the Tenax material if required. For transfer of the sample to the GC column, the split valve is closed and the PTV is heated to effect the total sample transfer of the concentrated components. After ca. 30–120 s the temperature program of the oven is started and the split valve is opened (Fig. 2.79). The PTV remains at the same temperature until the end of the analysis. This last step is particularly important in cryo-enrichment because the cooled adsorbent could become enriched with residual impurities from the carrier gas, which would lead to ghost peaks in the subsequent analysis.

2.2.3.5 **Injection Volumes**

For the discussion of the optimal injection volumes for hot and cold injection systems, the use of a 25 m long capillary column with an internal diameter of 0.32 mm is assumed, which operates with a linear carrier gas velocity of 30 cm/s. Under these conditions a carrier gas flow of ca. 2 mL/min through the column is expected. For the total sample transfer

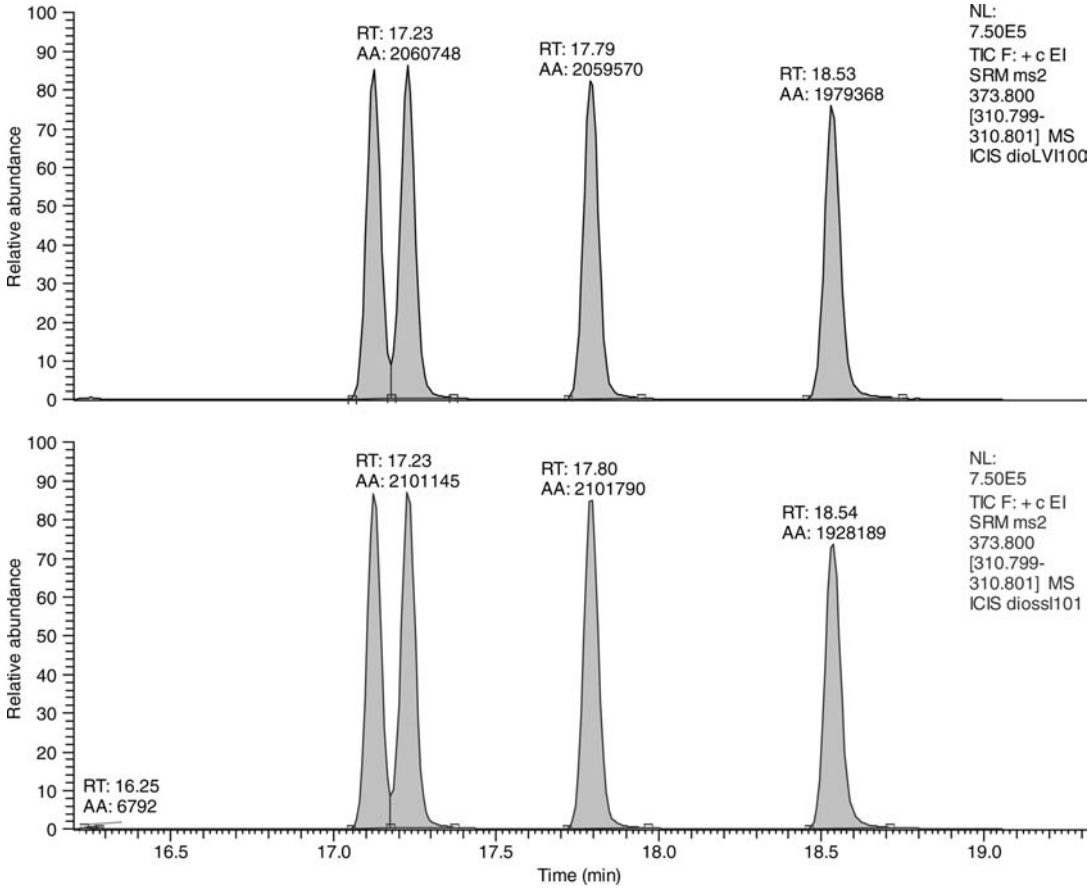


Fig. 2.78 Comparison of regular and large volume injection.
EPA 1613 CS3 standard, mass chromatograms of the hexachlorofuran congener peaks with retention time and peak area.
Thermo Scientific TSQ Quantum GC, column: TR-5MS, 30 m × 0.25 mm ID × 0.1 µm film
Trace GC with PTV: packed liner with deactivated glass wool
Top: 80 µL PTV-LVI injection of the 1 : 80 diluted standard solution
Bottom: 1 µL PTV splitless injection of the undiluted standard solution

mode the carrier gas velocity for both types of injector can be determined by considering the insert volumes in each case. The expansion volumes of the solvents used must also be taken into account. These are shown in Table 2.21.

To determine the time required to flush the contents of the injector completely on to the column at least twice the insert volume is applied, corresponding to a yield of 90–95 %. Table 2.22 gives details for hot and cold injectors.

These considerations make it clear that the choice of capillary column and the appropriate injection parameters and volumes only allow the maximum performance of both hot and cold injection systems to be exploited. In particular, cold injection systems function less well if they are improperly used as hot injectors with solvent volumes which are normally used

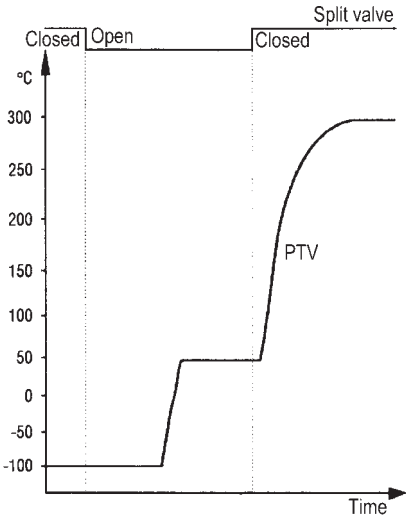


Fig. 2.79 PTV cryo-enrichment at –100 °C (with an optional fractionation step here at 50 °C) and injection with total sample transfer.

Table 2.21 Typical solvent expansion volumes.

Injection volume	H_2O	CS_2	CH_2Cl_2	Hexane	iso-Octane
0.1 µL	142 µL	42 µL	40 µL	20 µL	16 µL
0.5 µL	710 µL	212 µL	200 µL	98 µL	78 µL
1.0 µL	1420 µL	423 µL	401 µL	195 µL	155 µL
2.0 µL	2840 µL	846 µL	802 µL	390 µL	310 µL
3.0 µL	4260 µL	1270 µL	1200 µL	585 µL	465 µL
4.0 µL	5680 µL	1690 µL	1600 µL	780 µL	620 µL
5.0 µL	7100 µL	2120 µL	2000 µL	975 µL	775 µL

The expansion volumes given here refer to an injection temperature of 250 °C and a column pressure of 0.7 bar (10 psi). The values for other temperatures and pressures can be calculated according to $V_{Exp.} = 1/P \cdot n \cdot R \cdot T$.

with all commercial hot split or splitless injectors. The probability that the sample cloud will expand beyond the insert increases with the volatility of the solvent. This then leads to losses and tailing as a result of interactions with active surfaces. This would not occur if the PTV were used according to the instructions.

For hot injectors capillary columns with an internal diameter of 0.32 mm are more suitable, as here the transfer of the sample from the injector to the column takes place without diffusion and a more rapid injection is permitted. However, on coupling with MS detectors, the flow rate of ca. 2 ml/min can exceed the maximum compatible flow rate in some quadrupole systems. Columns with an internal diameter of 0.25 mm can be used as an alternative and then should be used in hot injectors with a narrow 2.0 mm internal diameter insert and an injection volume of 0.5 µl, to obtain optimal results. If inserts with a wide internal diameter (4 mm) are used, the solvent effect must be particularly exploited for focusing. It leads to accelerated emptying of the insert through solvent recondensation and a slip-stream

Table 2.22 Injection times for hot and cold injection (GC column 25 m × 0.32 mm).

Injector type	Hot injector	Cold injection system
Split valve	Closed	Closed
Internal diameter of insert	4 mm	2 mm
Interior volume	1 mL	300 µL
Flow rate	0.3 cm/s [a)]	1 cm/s
Minimum time with split closed	60 s [b)]	18 s
Maximum injection volume (250 °C)		
CH_2Cl_2	2 µL	(0.5 µL) [c)]
Hexane	5 µL	(1 µL)

a) This low flow rate already causes diffusion of the analytes injected against the carrier gas stream. The peaks are broader and the signal/noise ratio is poorer.
b) On use of a 25 m × 0.25 mm internal diameter column the carrier gas rate in the insert of the hot injector is only ca. 0.1 cm/s (4 mm insert) and would require a period with the split closed of at least 3 min. On the other hand the cold injection system only requires the split to be closed for at least 54 s.
c) The given injection volumes are valid for the case where a sample is injected into a heated cold injection system which contradicts the use envisaged for the construction. The injection volumes for cold injection are much higher.

in the direction of the injector. Furthermore, the start temperature of the program must be well below the boiling point of the solvent and there should be an isotherm of 1–3 min at the beginning of the oven program.

The use of capillary columns of 25 m in length and 0.25 mm internal diameter can be recommended without limitations for cold injection systems. The coupling to a mass spectrometer is particularly favourable because of the generally low flow rates. Also, the injection of larger sample volumes is straightforward and does not impair the operation of the mass spectrometer. For these reasons the use of cold injection systems for GC/MS is particularly recommended. This applies all the more to the throughput of large numbers of samples, as here automation of the system is indispensable. Unlike on-column injectors, cold injection systems can be used with autosamplers without special modifications.

In spite of the many advantages of the cold injection system, in practice there are also limits to its use (Table 2.23). These include the analysis of particularly thermally labile substances. Because of the low injection temperature cold injection systems should be particularly suitable for labile substances. However, during the heating phase, the residence times of the substances in the insert are long enough to initiate thermal decomposition. In this case, only on-column injection can be used because it completely avoids external evaporation of the sample for transfer to the column (see Section 2.2.1.4). To test for thermal decomposition, Donike suggested the injection of a mixture of the same quantities of fatty acid TMS esters (C_{10} to C_{22} thermolabile) and n-alkanes (C_{12} to C_{32} thermally stable). If no thermal decomposition takes place, all the substances should appear with the same intensity.

Table 2.23 Choice of a suitable injector system.

Characteristics of the sample	(1) Hot split	(2) Hot splitless	(3) PTV split	(4) PTV splitless	(5) PTV solvent split	(6) On-column
Concentrated samples	+	–	+	–	–	–
Trace analysis	–	+	–	+	+	+
Extreme dilution	–	–	–	–	+	+
Narrow boiling range	+	+	+	+	+	+
Wide boiling range	–	≈	+	+	–	+
Volatile substances	+	+	+	+	–	–
Involatile substances	–	≈	+	+	+	+
With involatile matrix	+	+	+	+	+	–
Thermally labile substances	≈	–	≈	≈	≈	+
Can be automated	+	+	+	+	+	+

+ recommended, ≈ can be used, – not recommended

2.2.3.6 **On-column Injection**

In the era of packed columns on-column injection (although not known as such at the time) was the state of the art. The difference between that and the present procedure lies in coping with the small diameters of capillary columns which have caused the term "filigree" to be applied to the on-column technique. Schomburg introduced the first on-column injector for capillary columns in 1977 under the designation of direct injection and described the process of sample injection very precisely. A year later a variant using syringe injection was developed by Grob. Today's commercial on-column injection involves injecting the sample directly on to the column (internal diameter 0.32 mm) in liquid form using a standard syringe with a 75 mm long steel canula of 0.23 mm external diameter. More favourable dimensioning is possible on use of a retention gap with an internal diameter of 0.53 mm, which can even be used with standard canulas. The use of retention gaps allows autosamplers to be employed for on-column sample injection.

The injector itself does not have a complicated construction and can be serviced easily and safely. The carrier gas feed is situated and the capillary fixed centrally in the lower section. The middle section carries a rotating valve as a seal and in the upper section there is the needle entry point which allows central introduction of the syringe needle. In all types of operation the whole injector block remains cold and warming by the oven is also prevented by a surrounding air stream.

Small sample volumes of less than 10 µL can be injected directly on to a capillary column. Larger volumes require a deactivated retention gap of appropriate dimensions (Fig. 2.80). The retention gap is connected to the column with connectors with no dead volume (e.g. press fit connectors). For injection, the column should be operated at a high flow rate (ca. 2–3 mL/min for He). During the injection the oven temperature must be below the effective boiling point of the solvent. This corresponds to a temperature of ca. 10–15 °C above the boiling point of the solvent under normal conditions because of the pressure ratios in the

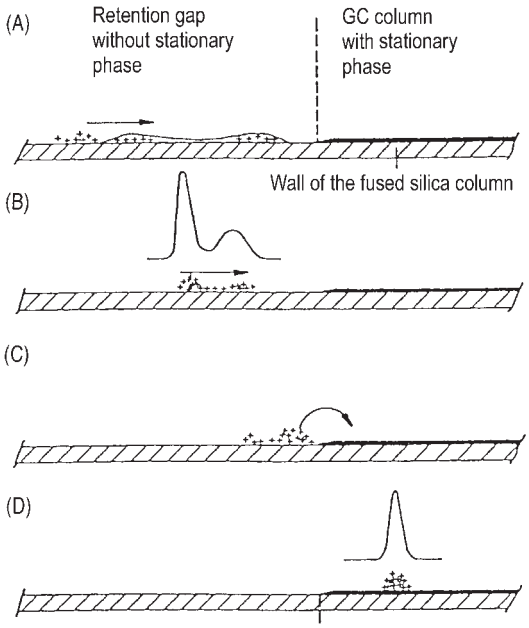


Fig. 2.80 Formation of the start band on use of a retention gap (after Grob).
(A) Starting situation after injection: the retention gap is wetted by the sample extract.
(B) The solvent evaporates and leaves an undefined substance distribution in the retention gap.
(C) The substance reaches the stationary phase of the GC column. Considerable retention corresponding to the capacity factor of the column begins.
(D) The analytes injected with the sample have been concentrated in a narrow band at the beginning of the column; this is the starting point for chromatographic separation.

on-column injector. As a rule of thumb, the boiling point increases by 1 °C for every 0.1 bar of pre-pressure. The injection is carried out by pressing the syringe plunger down rapidly (ca. 15–20 µL/s) to avoid the liquid being sucked up between the needle and the wall of the column through capillary effects, otherwise the subsequent removal of the syringe would result in loss of sample. For larger sample volumes, from ca. 20–30 µL, the injection rate must, however, be lowered (to ca. 5 µL/s) as the liquid plug causes increasing resistance and reversed movement of the liquid must be avoided (Fig. 2.81). The temperature program with the heating ramp can then begin when the solvent peak is clearly decreasing.

On-column Injection

- Flow rate ca. 2–3 mL/min
- Oven temperature at the effective boiling point of the solvent (normal boiling point plus 1 °C per 0.1 bar pre-pressure)
- Rapid injection of small volumes
- Oven temperature to be kept at the evaporation temperature
- Only start the heating program after elution of the solvent peak

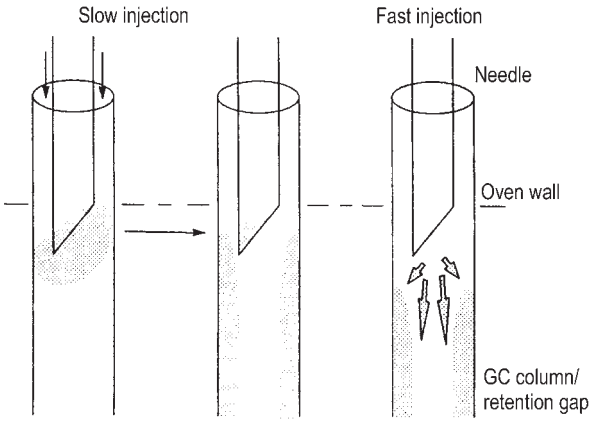


Fig. 2.81 Slow and fast on-column injections. For the slow injection the exterior of the needle is contaminated and part of the sample is lost.

The on-column technique has the following advantages over the cold injection system:

- The danger of thermal decomposition during the injection is practically eliminated. A substance evaporates at the lowest possible temperature and is heated to its elution temperature at the maximum.
- Only on-column injection allows the quantitative and reproducible sample injection on to the column without losses. Involatile substances, in particular, are transferred totally without discrimination.
- Defined volumes can be injected with high reproducibility. Standard deviations of ca. 1% can be achieved.
- The injection volumes can be varied within wide ranges without additional optimisation. At the beginning of the heating program, only the duration of elution of the solvent peak needs to be taken into account.

For use in GC/MS systems attention should be paid to the injection at the necessary high flow rates in connection with the maximum permitted carrier gas loading of the mass spectrometer. As the injection system is always cold, moisture can accumulate from samples or insufficiently purified carrier gas (Fig. 2.82). Because of this, the sensitivity of the mass spectrometer could be reduced. The effect is easy to detect because the mass spectrometer continually registers the water background and because the on-column injector can easily be opened at a particular time so that the carrier gas can pass out via the splitter.

If the water background decreases after the dead time, this effect must be taken into consideration during planning of the analysis, as the response behaviour of substances can change during the working day.

Other system-related limitations to the on-column technique for certain types of sample are:

- Concentrated samples may not be injected on-column. In these cases preliminary dilution is necessary or the use of the split injector is better (minimum on-column injection volume 0.2 μL).
- Samples containing a matrix rapidly lead to quality depletion. Here a retention gap is absolutely necessary. However, impurities in the retention gap quickly give rise to

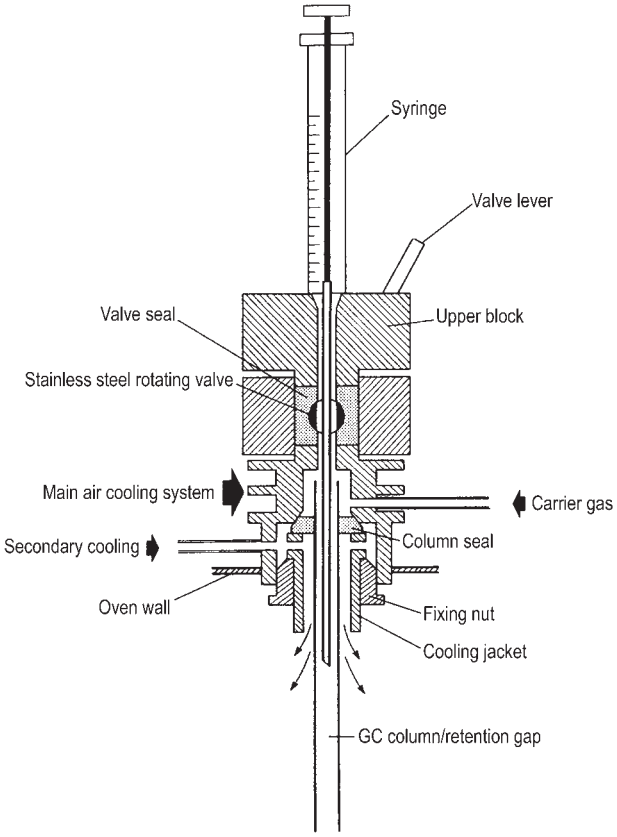


Fig. 2.82 On-column injector (Carlo Erba).

adsorption and peak broadening because of the small capacity, so that changing it regularly is necessary. For dirty samples hot or PTV sample injection is preferred.

- Samples containing volatile components are not easy to control, as focusing by the solvent effect is inadequate under certain circumstances. If changing the solvent does not help, changing to a hot or cold split or splitless system is recommended.

PTV On-column Injection

A regular PTV injector can also successfully be used for on-column injections. This method allows the straightforward automation of on-column injections using regular autosamplers with standard syringes. The insert liner used needs to be equipped with a restrictor at the top. This restrictor functions as a needle guide into the column. Syringes with the regular 0.47 o.d. needles can be used allowing the direct injection into wide-bore columns or retention gaps (pre-columns) of equivalent dimensions e. g. 0.53 mm i.d. For this purpose the column inlet is pushed up until it gets positioned at the bottom side of the restrictor site of the insert liner.

In PTV on-column mode, the injector body and the column oven is set for injection to a temperature below the solvent boiling point (see Table 2.20). After a short injection time of up to 20 s the injector is heated up for sample transfer. The oven program is started right

after the completed sample transfer into the analytical column. The split valve remains opened with a low flow rate of only a few mL/min. The PTV temperature is maintained high throughout the chromatographic run as usual.

2.2.3.7 **Cryofocusing**

Cryofocusing should not be regarded as an independent injection system, but nevertheless should be treated individually in the list of injectors because the static headspace and purge and trap systems can be coupled directly to a cryofocusing unit as a GC injector. Furthermore, some thermodesorption systems already contain a cryofocusing unit. Many simpler instruments, however, do not, and require external focusing to ensure their proper function. Generally, for the direct analysis of air or gases from indoor rooms or at the workplace, or of emissions, a cryofocusing unit is required for concentration and injection on to the GC column.

Packed columns can effectively concentrate the substances contained in gaseous samples at the beginning of the column because of their high sample capacity. Capillary columns under normal working conditions cannot form sufficiently narrow initial bands for the substances to be detected from the gas volumes being handled in direct air analysis or on heating up traps (purge and trap, thermodesorption) because of their comparatively low sample capacity. Additional effective cooling is necessary. The entire oven space can be cooled, but this has a major disadvantage: the requirement in terms of time and cooling agents is immense.

A special cryofocusing unit cools down the beginning of the GC column and allows on-column focusing of the analytes without requiring a retention gap. The column film present improves the efficiency at the same time by acting as an adsorbent. For this purpose the capillary column is inserted into a 1/16 inch stainless steel tube via an opening in the

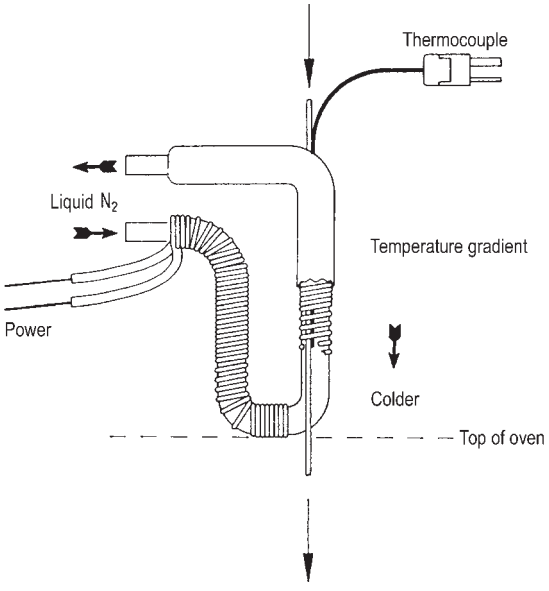


Fig. 2.83 Cut away diagram of a cryofocusing unit for use with fused silica capillary columns (Tekmar).

oven lid of the chromatograph and is connected via a connecting piece to the transfer line of the sampler (e. g. headspace, purge and trap, canister). This stainless steel tube is firmly welded to a cold finger and a thermoelement (Fig. 2.83). The tube is also surrounded by a differential heating coil which heats the inlet side more rapidly than the exit to the GC oven. In this way a uniform temperature gradient is guaranteed in all the phases of the operation. Because of the gradient, on cooling, e. g. with liquid nitrogen, all the analytes are focused into a narrow band at the beginning of the capillary column, as the substances migrate more slowly at the start of the band than at the end. After concentration and focusing, the chromatographic separation starts with the heating up of this region. On careful control of the gradient the heating rate does not affect the efficiency of the column (heating rates between 100 and 2000 °C/min).

Because of on-column focusing the cryotrap has the same sample capacity as the column has for these analytes. A breakthrough of the cryotrap as a result of too high an analyte concentration can be prevented by changing the column. Overloading the column would, in any case, result in poor separation. The diagram in Fig. 2.84 gives the temperatures at which a breakthrough of the analytes must be reckoned with in cryofocusing. Larger film thicknesses and internal diameters permit higher focusing temperatures which favour the mobilisation of the analytes from the column film.

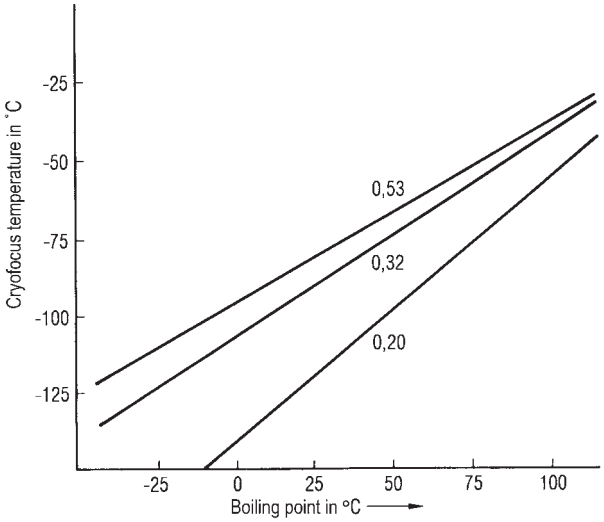


Fig. 2.84 Breakthrough temperatures in cryofocusing for various column internal diameters as a function of the boiling point of the analyte (Tekmar).
Film thicknesses:
0.25 µm at 0.20 mm internal diameter
1.0 µm at 0.32 mm internal diameter
3.0 µm at 0.53 mm internal diameter

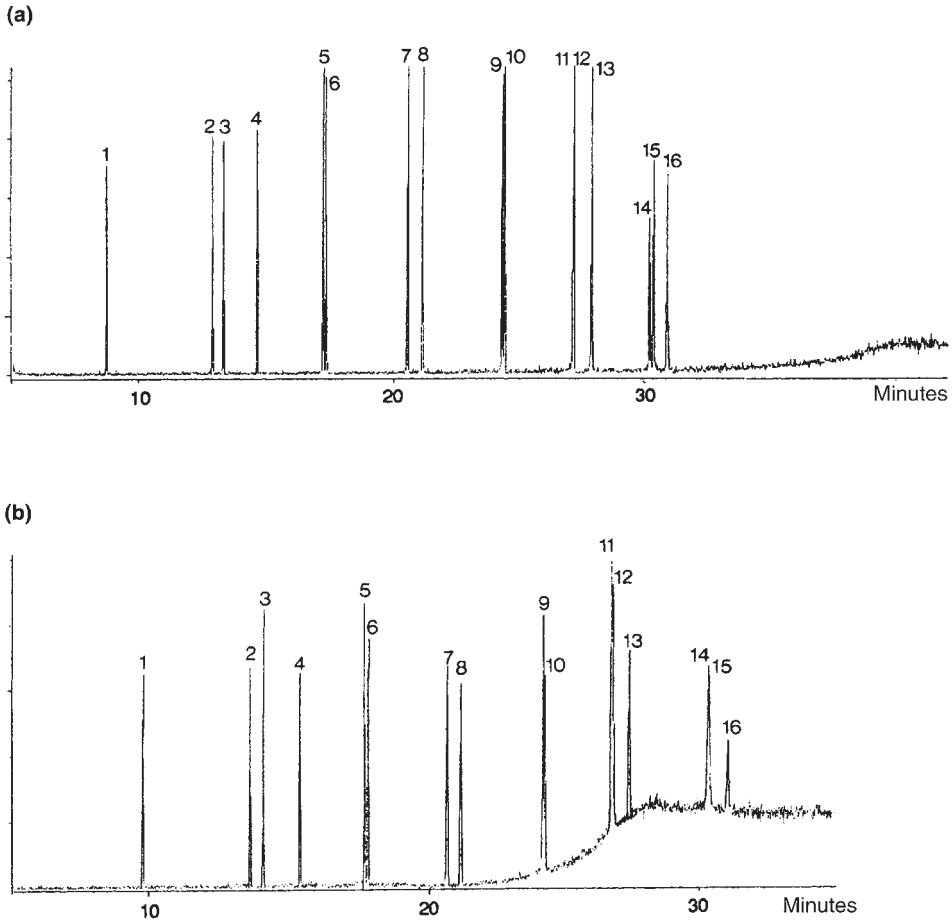
2.2.4
Capillary Columns

There are no hard and fast rules for the choice of column for GC/MS coupling. The choice of the correct phase is made on the usual criteria: "like dissolves like". If substances exhibit no interaction with the stationary phase, there will be no retention and the substances leave the column in the dead time. The polarity of the stationary phase should correspond to the polarity of the substances being separated (see Table 2.24, pp. 120–127). Less polar substances are better separated on nonpolar phases and vice versa

Change to More Strongly Polar Stationary Phases

- Weaker retention of nonpolar compounds
- Stronger retention of polar compounds
- Shift of compounds with specific interactions

For coupling with mass spectrometry (GC/MS), the carrier gas flow and the specific noise of the column (column bleed) are included in the criteria governing the choice of column. When considering the optimal carrier gas flow, the maximum loading of the mass spectrometer must be taken into account. The limit for small benchtop mass spectrometers with quadrupole analysers is frequently ca. 1–2 mL/min. Larger instruments can generally tolerate higher loads because of their more powerful pumping systems. Ion trap mass spectrometers can be operated with a carrier gas flow of up to 3 mL/min and even higher with an external ion source. These conditions limit the column diameter which can be used. There can be no compromises concerning column bleed in GC/MS. Column bleed generally contributes to chemical noise where MS is used as the mass-dependent detector, and curtails the detection limits. The optimisation of a particular signal/noise ratio can also be effected in GC/MS by selecting particularly thermally stable stationary phases with a low tendency to bleed. For use in residue analysis, stationary phases for high-temperature applications have proved particularly useful (Figs. 2.85 and 2.86). Besides the phase itself, the film thickness also plays an important role. Thinner films and shorter columns have a lower tendency to cause column bleed.



Polynuclear aromatic hydrocarbons

(a)	**Phase:**	**HT5, 0.10 µm film thickness**	**(b)**	**Phase:**	**BP1, 0.25 µm film thickness**
	Column:	**25 m × 0.22 mm ID**		**Column:**	**25 m × 0.22 mm ID**
	Start temperature:	50 °C, 2 min		Start temperature:	50 °C, 2 min
	Program rate:	10 °C/min		Program rate:	10 °C/min
	Final temperature:	**420 °C**, 5 min		Final temperature:	**300 °C**, 10 min
	Detector:	HP 5971 MS		Detector:	HP 5971 MS
	Scan range:	35–550 amu		Scan range:	35–550 amu
	Injection:	Split 50 : 1		Injection:	Split 50 : 1

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Pyrene
8. Fluoranthene
9. Chrysene
10. Benzo(a)anthracene
11. Benzo(b)fluoranthene
12. Benzo(k)fluoranthene
13. Benzo(a)pyrene
14. Indeno(1,2,3,-cd)pyrene
15. Dibenzo(ah)anthracene
16. Benzo(ghi)perylene
    (0.5 ng per component)

Fig. 2.85 Comparison of a conventional silicone phase (lower trace: SGE BP1, dimethylsiloxane) with a high temperature phase (upper trace: SGE HT5, siloxane-carborane) using a polyaromatic hydrocarbon standard (SGE). The long temperature program for the high temperature phase up to 420 °C allows the elution of components 14, 15 and 16 during the heating-up phase at an elution temperature of ca. 350 °C. Sharp narrow peaks with low column bleed give better detection conditions for high-boiling substances (GC/MS system HP-MSD 5971).

Table 2.24 Composition of stationary phases for fused silica capillary columns with column designations for comparable phases from different manufacturers, arranged in order of increasing polarity, and columns with special phases (selection).

Phase composition	Agilent/J&W
	HP-1, HP-101, HP-1MS, Ultra-1,
100% dimethyl polysiloxane	DB-1, DB-1MS, DB-1ht, SE-30
	HP-5, HP-5MS, PAS-5, DB-5,
95% dimethyl/5% diphenyl polysiloxane	DB-5.625, DB-5ht, SE-54
95% dimethyl/5% diphenyl polysilarylene	HP-5TA, DB-5ms
6% cyanopropylphenyl/94% dimethyl polysiloxane	HP-1301, HP-624, DB-1301, DB-624
80% dimethyl/20% diphenyl polysiloxane	–
65% dimethyl/35% diphenyl polysiloxane	HP-35, HP-35MS, DB-35
14% cyanopropylphenyl/86% dimethyl polysiloxane	HP-1701, PAS-1701, DB-1701
trifluoropropylmethyl polysiloxane	DB-210, DB-200
50% dimethyl/50% diphenyl polysiloxane	DB-17, DB-17HT, DB-608
100% methylphenyl polysiloxane	HP-17, HP-50+
35% dimethyl/65% diphenyl polysiloxane	–
50% cyanopropylmethyl/50% phenylmethyl polysiloxane	HP-225, DB-225
polyethylene glycol (PEG)	HP-20M, Inno Wax, DB-Wax, Carbowax 20M, HP-Wax, DB-Wasetr
PEG for amines and basic compounds	CAM
PEG for acidic compounds	HP-FFAP, DB-FFAP, OV-351
90% biscyanopropyl/10% cyanopropylphenyl polysiloxane	–
70% biscyanopropyl/30% cyanopropylphenyl polysiloxane	–
biscyanopropyl polysiloxane	–
permethylated β-cyclodextrin	Cyclodex-β
1,2,3-tris(cyanoethoxy)propane	–
proprietary phase	–
phenyl polycarborane-siloxane	–
proprietary phase	DB-XLB
Column features	
built in guard column	DuraGuard
Silcosteel-treated stainless steel	ProSteel

Alltech	Macherey-Nagel	PerkinElmer	Phenomenex	Quadrex
AT-1, SE-30				
AT-1MS, EC-1	Optima 1, Optima-1ms	Elite-1	ZB-1	007-1
AT-5, SE-54				
AT-5	Optima 5, Optima-5ms	PE-2	ZB-5	007-2
–	–	–	ZB-5MS	–
AT-624	Optima 1301, Optima 624	–	ZB-624	007-1301
AT-20	–	PE-7	–	007-7
AT-35, AT-35MS, EC-35	–	Elite-35, Elite-35ms	ZB-35	007-11
AT-1701	Optima 1701	PE-1701	ZB-1701	007-1707
AT-210	Optima 210	–	–	007-210
–			–	
AT-50, AT-50MS	Optima 17	PE-17	ZB-50	007-17
–	–	–	–	400-65HT, 007-65HT
AT-225	Optima 225	PE-225	–	007-225
AT-Wax, Carbowax,				
AT-WAXMS	Permabond CW 20M, Optima WAX	PE-CW	ZB-WAX	007-CW
–	–	–	–	–
AT-1000, FFAP	Permabond FFAP, Optima FFAP	PE-FFAP	–	007-FFAP
AT-Silar	–	–	–	007-23
–	–	–	–	–
–	–	–	–	–
Chiraldex-β	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	Guardian	–
–	–	–	–	Ultra-Alloy

Table 2.24 (continued)

Phase composition	Restek	SGE
100% dimethyl polysiloxane	Rxi-1ms, Rtx-1, Rtx-1MS, Stx-1HT	–
95% dimethyl/5% diphenyl polysiloxane	Rxi-5ms, Rtx-5, Rtx-5MA, XTI-5, Stx-5HT	BP-5
95% dimethyl/5% diphenyl polysilarylene	Rtx-5Sil MS	BPX-5
6% cyanopropylphenyl/94% dimethyl polysiloxane	Rtx-1301, Rtx-624	BP-624
80% dimethyl/20% diphenyl polysiloxane	Rtx-20	–
65% dimethyl/35% diphenyl polysiloxane	Rtx-35, Rtx-35MS	BPX-35, BPX-608
14% cyanopropylphenyl/86% dimethyl polysiloxane	Rtx-1701	BP-1701
trifluoropropylmethyl polysiloxane	Rtx-200, Rtx-200MS	–
50% dimethyl/50% diphenyl polysiloxane	Rtx-17	–
100% methylphenyl polysiloxane	Rtx-50	BPX-50
35% dimethyl/65% diphenyl polysiloxane	Rtx-65, Rtx-65TG	–
50% cyanopropylmethyl/50% phenylmethyl polysiloxane	Rtx-225	BP-225
polyethylene glycol (PEG)	Stabilwax, Rtx-WAX	BP-20, SolGelWAX
PEG for amines and basic compounds	Stabilwax-DB	–
PEG for acidic compounds	Stabilwax-DA	BP-21
90% biscyanopropyl/10% cyanopropylphenyl polysiloxane	Rtx-2330	–
70% biscyanopropyl/30% cyanopropylphenyl polysiloxane		BPX-70
biscyanopropyl polysiloxane	Rt-2560	–
permethylated β-cyclodextrin	Rt-βDEXm	Cydex-β
1,2,3-tris(cyanoethoxy)propane	Rt-TCEP	–
proprietary phase	Rtx-440	HT-5
phenyl polycarborane-siloxane	Stx-500	–
proprietary phase	Rtx-XLB	–
Column features		
built in guard column	Integra-Guard	–
Silcosteel-treated stainless steel	MXT	AlumaClad

Supelco	Thermo Scientific	USP Nomenclature	Varian/Chrompack
Equity-1, SPB-1, SP-2100			VF-1MS, CP Sil 5 CB
SPB-1 Sulfur, SE-30, MDN-1	TR-1, TR-1MS	G1, G2, G38	CP Sil 5 CB MS
Equity-5, SPB-5, PTE-5, SE-54, SAC-5			VF-5MS, CP Sil 8 CB,
PTE-5 QTM, MDN-5,	TR-5	G27, G36, G41	CP Sil 8 CB MS
MDN-5S	TR-5MS	–	VF-5MS
SPB-1301	TR-V1	G43	–
SPB-20,VOCOL	–	G28, G32	–
SPB-35, SPB-608, MDN-35	TR-35MS	G42	VF-35MS
SPB-1701	TR-1701	G46	CP Sil 19 CB
–	–	G6	VF-200MS
–	–		
SP-2250, SPB-50	TR-50MS	G3	CP Sil 24 CB
–	–	G17	TAP-CB
–	–	G7, G19	CP Sil 43 CB
Supelcowax-10, Carbowax PEG 20M	TR-WAX, TR-WaxMS	G14, G15, G16, G20, G39	CP Wax 52 CB
Carbowax-Amine	–	–	CP Wax 51
Nukol, SP-1000	TR-FFAP	G25, G35	CP Wax 58 CB
SP-2330, SP-2331, SP-2380	–	G48	CP Sil 84
–	TR-FAME		–
SP-2560	–		–
β-DEX	–	–	CP-Cyclodextrin β
TCEP	–	–	CP-TCEP
–	–	–	–
–	–	–	–
–	–	–	–
–	–	–	EZ-Guard
Metallon	–	–	Ultimetal

Table 2.24 (continued)

Application	Agilent/J&W
Organic analysis-EPA methods 502.2, 524.2, 601, 602, 624, 8010, 8020,	–
Organic analysis-EPA methods 502.2, 524.2, 601, 602, 624, 8010, 8020,	HP-624, HP-VOC, DB-624, DB-502.2, DB-VRX
Organochlorine pesticides-EPA methods 8081, 608, and CLP pesticide	–
Organophosphorus pesticides-EPA method 8141A	–
Organochlorine pesticides-EPA methods 8081, 608, and CLP pesticide	HP-5, PAS-5, DB-5, DB-35, DB-608, HP-608,
	PAS-1701, DB-1701, DB-17, HP 50, HP-35
ASTM test method D2887	DB-2887
PONA analysis	HP-PONA, DB-Petro
Simulated distillation	–
Amines and basic compounds	–
Fatty Acid Methyl Esters (FAMES) (70% cyanopropyl polysilphenyl-siloxane)	–
Blood alcohol analysis	DB-ALC1, DB-ALC2
Residual solvents in pharmaceuticals	–
Residual solvents in pharmaceuticals	–
Fragrances and flavors	HP-20M, Carbowax 20M
Explosives (8% phenyl polycarbonane-siloxane)	–
Dioxins and furans	–
PCB congeners	–

Alltech	Macherey-Nagel	PerkinElmer	Phenomenex	Quadrex
–	–	–	–	–
AT-624	–	PE-502	OV-624	007-624, 007-502
–	–	–	–	–
–	–	–	–	–
AT-5, AT-35, AT 50,			ZB-5, ZB-35	007-2, 007-608,
AT-Pesticides	–	PE-2, PE-608, PE-1701	ZB-1701, ZB-50	007-17, 007-1701
–	–	–	–	007-1-10V-1.0F
AT-Petro	–	–	–	007-1-10V-0.5F
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–
–	–	–	–	007-CW
–	–	–	–	–
–	–	–	–	–
–	–	–	–	–

Table 2.24 (continued)

Application	Restek
Organic analysis-EPA methods 502.2, 524.2, 601, 602, 624, 8010, 8020,	Rtx-VMS, Trx-VGC
Organic analysis-EPA methods 502.2, 524.2, 601, 602, 624, 8010, 8020,	Rtx-VRX, Rtx-502.2, Rtx-624, Rtx-Volatiles
	Rtx-CLPesticides, Rtx-CLPesticides2,
Organochlorine pesticides-EPA methods 8081, 608, and CLP pesticide	Stx-CLPesticides, Stx-CLPesticides2
Organophosphorus pesticides-EPA method 8141A	Rtx-OPPesticides, Rtx-OPPesticides2
Organochlorine pesticides-EPA methods 8081, 608, and CLP pesticide	Rtx-5, Rtx-35, Rtx-50, Rtx-1701
ASTM test method D2887	Rtx-2887
PONA analysis	Rtx-1PONA
Simulated distillation	MXT-500 Sim Dist
Amines and basic compounds	Rtx-5 Amine
Fatty Acid Methyl Esters (FAMES) (70% cyanopropyl polysilphenyl-siloxane)	FAMEWAX
Blood alcohol analysis	Rtx-BAC1, Rtx-BAC2
Residual solvents in pharmaceuticals	Rtx-G27
Residual solvents in pharmaceuticals	Rtx-G43
Fragrances and flavors	Rt-CW20M F&F
Explosives (8% phenyl polycarbonane-siloxane)	Rtx-TNT, Rtx-TNT2
Dioxins and furans	Rtx-Dioxin, Rtx-Dioxin2
PCB congeners	Rtx-PCB

SGE	Supelco	Thermo Scientific	USP	Varian/Chrompack
–	–	TR-524	–	–
–	VOCOL, SPB-624	TR-524	–	CP Sil 13 CB
–	–	TR-5MS	–	–
–	–	TR-5MS	–	–
	SPB-5, SPB-608,	TR-5, TR-35MS,		
BP-5, BP-10, BP-608	SPB-1701	TR-50MS, TR-1701	G3	CP Sil 8 CB, CP Sil 19 CB
	Petrocol 2887,			
–	Petrocol EX2887	TR-SimDist	–	CP-SimDist-CB
BP1-PONA	Petrocol DH	–	–	CP Sil PONA CB
HT5	–	–	–	–
–	PTA-5	–	G50	–
–	Omegawax	TR-FAME	–	–
–	–	–	–	–
–	–	–	G27	–
–	OVI-G43	TR-V1	G43	–
BP-20M	–	TR-WaxMS	–	–
–	–	TR-8095	–	–
–	–	–	–	–
–	–	–	–	–

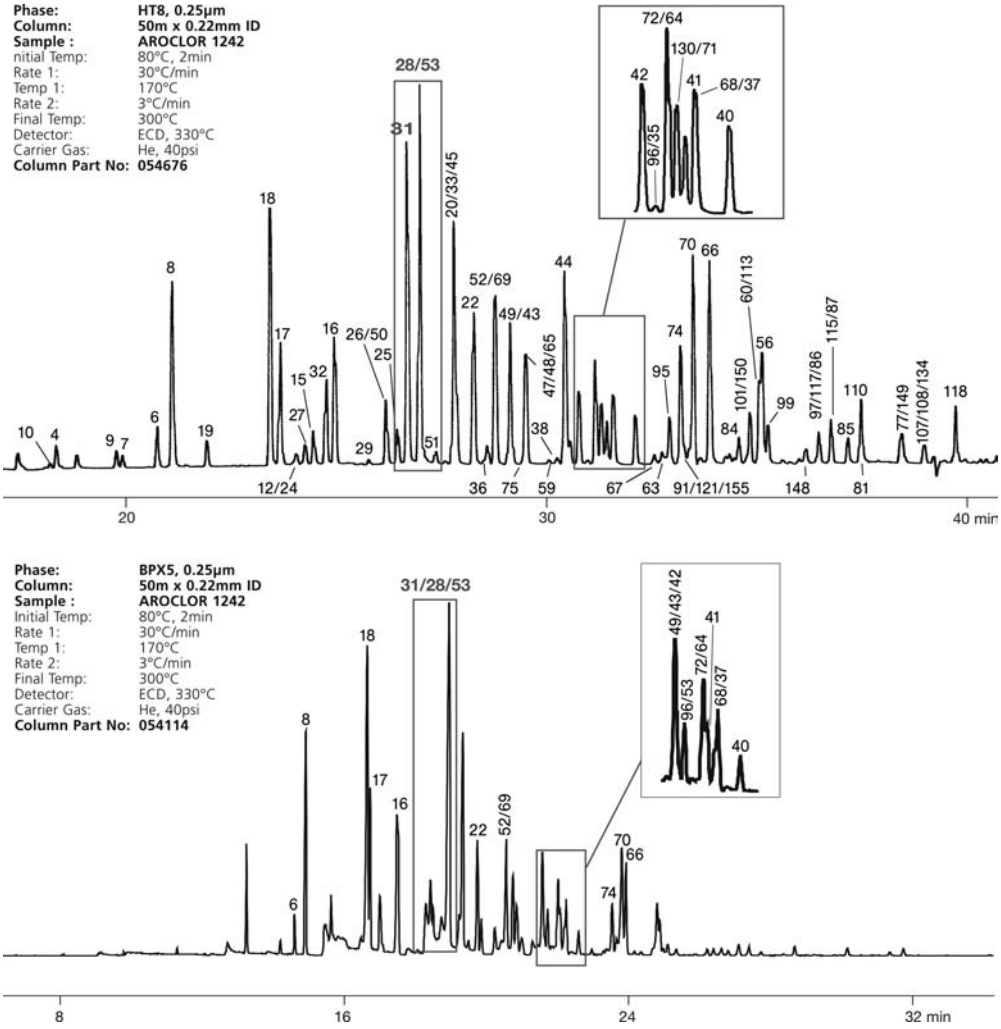


Fig. 2.86 An example of the different selectivities of stationary phases for the Aroclor mixture 1242 (see in particular the separation of the critical congeners 31, 28 and 53 (Courtesy SGE))
Top: Carborane phase – HT8 50 m × 0.22 mm × 0.25 µm
Bottom: 5%-Phenyl phase – BPX5 50 m × 0.22 mm × 0.25 µm

2.2.4.1 **Sample Capacity**

The sample capacity is the maximum quantity of an analyte with which the phase can be loaded (Table 2.25). An overloaded column exhibits peak fronting, i.e. an asymmetrical peak which has a gentle gradient on the front and a sharp slope on the back side. This effect can increase until a triangular peak shape is obtained, a so-called "shark fin". Overloading occurs rapidly if a column of the wrong polarity is chosen. The capacity of a column depends on the internal diameter, the film thickness and the solubility of a substance in the phase.

Table 2.25 Sample capacities for common column diameters.

Internal diameter	0.18 mm	0.25 mm	0.32 mm	0.53 mm
Film thickness	0.20 µm	0.25 µm	0.25 µm	1.00 µm
⇒ **Sample capacity**	<50 ng	50–100 ng	400–500 ng	1000–2000 ng
Theoretical plates per metre of column	5300	3300	2700	1600
Optimal flow rate at				
20 cm/s helium	0.3 mL/min	0.7 mL/min	1.2 mL/min	2.6 mL/min
40 cm/s hydrogen	0.6 mL/min	1.4 mL/min	2.4 mL/min	5.2 mL/min

Sample Capacity

- Increases with internal diameter
- Increases with film thickness
- Increases with solubility

2.2.4.2 **Internal Diameter**

The internal diameter of a column used in capillary gas chromatography varies from 0.1 mm (microbore capillary) via 0.18 mm and 0.25 mm (narrow bore) and the standard columns with 0.32 mm to 0.53 mm (megabore, halfmil). For direct coupling with mass spectrometers, in practice only the columns up to 0.32 mm internal diameter are used. Megabore columns are mainly used for retention gaps (deactivated, no stationary phase) or to replace packed columns in specially designed GC instruments.

The internal diameter affects the resolving power and the analysis time (Fig. 2.87). Basically, at constant film thicknesses lower internal diameters are preferred in order to achieve higher chromatographic resolution. As the flow per unit time decreases at a particular carrier gas velocity, the analysis time increases. In the case of complex mixtures, changing to a column with a smaller internal diameter gives better separation of critical pairs of compounds (Table 2.26). In practice it has been shown that even changing from 0.25 mm internal diameter to 0.20 mm allows an improvement in the separation of, for example, PCBs. For sample application to narrow bore columns certain conditions must be adhered to, depending on the type of injector (see Section 2.2.3).

Table 2.26 Effect of column diameter and linear carrier gas velocity on the flow rate.

Internal diameter	**Linear carrier gas velocity**		**Flow rate**	
	He	H_2	He	H_2
0.18 mm	30–45 cm/s	45–60 cm/s	0.5–0.7 mL/min	0.7–0.9 mL/min
0.25 mm	**30–45 cm/s**	**45–60 cm/s**	**0.9–1.3 mL/min**	**1.3–1.8 mL/min**
0.32 mm	30–45 cm/s	45–60 cm/s	1.4–2.2 mL/min	2.2–2.8 mL/min
0.54 mm	30–45 cm/s	45–60 cm/s	4.0–6.0 mL/min	6.0–7.9 mL/min

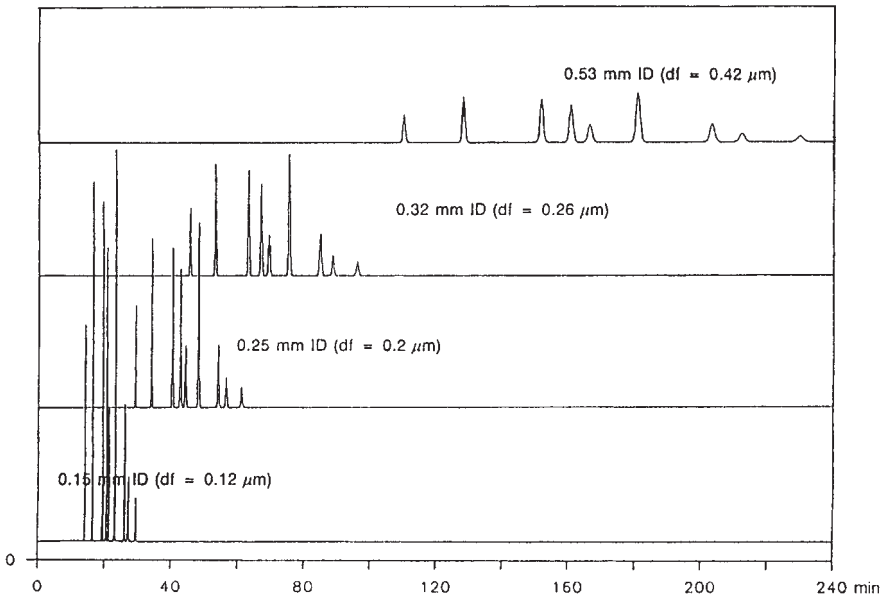


Fig. 2.87 Effect of increasing column internal diameter together with increasing film thickness on peak height and retention time. (The columns have the same phase ratio!) (Chrompack).

Smaller Internal Diameter (at Identical Film Thickness)

- Increases the resolution
- Increases the analysis times

2.2.4.3 Film Thickness

The variation in the film thickness at a given internal diameter and column length gives the user the possibility of carrying out special separation tasks. As a rule, thick films are used for volatile compounds and thin films for high-boilers and trace analysis. Thick film columns with coatings of more than 1.0 µm can separate extremely low boiling compounds well, e.g. volatile halogenated hydrocarbons. Through the large increase in capacity with thicker films, it is even possible to dispense with additional oven cooling during injection in the analysis of volatile halogenated hydrocarbons using headspace or purge and trap. Start temperatures above room temperature are usual. However, thick film columns exhibit severe column bleed at elevated temperatures.

For the residue analysis of all other substances, thin film columns with coating thicknesses of ca. 0.1 µm have proved to be very effective in GC/MS. Thin film columns give narrow rapid peaks and can be used in higher temperature ranges without significant column bleed. The elution temperatures of the compounds decrease with thin films and, at the same program duration, the analysis can be extended to compounds with higher molecular weights and thermolabile compounds e.g. the decabromodiphenylether (PBDE 209). The

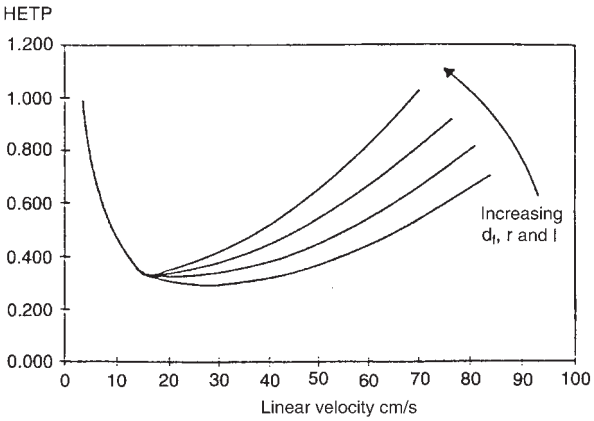


Fig. 2.88 Effect of film thickness (d_f), internal diameter (r) and length (l) on the van Deemter curves for helium as the carrier gas.

duration of the analysis for a given compound becomes shorter, but the capacity of the column decreases also limiting the load for matrix samples (Fig. 2.88).

Increasing Film Thicknesses

- Improve the resolution of volatile compounds
- Increase the analysis time
- Increase the elution temperatures

The Relationship Between Film Thickness and Internal Diameter

The phase ratio of a capillary column is determined by the ratio of the volume of the gaseous mobile phase (internal volume) to the volume of the stationary phase (coating). From Table 2.27 the phase ratio can be read off for each combination of film thickness and internal diameter (assuming the same film and the same column length). High values mean good separation; the same values show combinations with the same separating capacity. For GC/MS optimal separations can be planned and also other conditions, such as carrier gas flow and column bleed, can be taken into consideration. To achieve better separation it is possible to change to a smaller film thickness at the same internal diameter or to keep the film thickness and choose a higher internal diameter. For example, a Fast GC column of 0.18 mm ID and 0.10 µm film has almost the double phase ratio than the commonly used 0.25 mm ID column with 0.25 µm film. The phase ratio is tripled when switching to a 0.25 mm ID column with 0.1 µm film which is typical for trace analysis applications. Using Table 2.27 the separation efficiency can easily be optimized to the required conditions.

2.2.4.4 Column Length

The analytical column should be as short as possible. The most common lengths for standard columns are 30 or 60 m. Greater lengths are not necessary in residue analysis with GC/MS systems even for separating complex PCB or volatile halogenated hydrocarbon mixtures.

Table 2.27 Effect of column diameter and film thickness on the phase ratio.

Internal diameter	Film thickness							
	0.10 µm	0.25 µm	0.50 µm	1.0 µm	1.50 µm	2.0 µm	3.0 µm	5.0 µm
0.18 mm	450	180	90	45	30	23	15	9
0.25 mm	**625**	**250**	**125**	**63**	**42**	**31**	**21**	**13**
0.32 mm	800	320	160	80	53	40	27	16
0.53 mm	1325	530	265	128	88	66	43	27

Shorter columns would be desirable for simpler separations, but they are with the same diameter at the limit of the maximum flow for the mass spectrometer used. Here the switch to fast GC applications using smaller diameters should be considered. Doubling the column length only results in an improvement in the separation by a factor of 1.4 ($\sqrt{2}$) while the analysis time is doubled (and the cost of the column also!). For isothermal chromatography, the retention time is directly proportional to the column length while with programmed operations the retention time is essentially determined by the elution temperature of a compound. On changing to a longer column, the temperature program should always be optimised again to achieve optimal retention times.

Doubling the Column Length

- Resolution only increases by a factor of 1.4
- Costs are doubled
- Retention times are doubled
- Sample throughput (productivity) is cut by half
- The temperature program must be optimised again

2.2.4.5 Adjusting the Carrier Gas Flow

The maximum separating capacity of a capillary column can only be exploited with an optimised carrier gas flow. With direct GC/MS coupling the carrier gas flow is affected slightly by the vacuum on the detector side. In practice the adjustment is no different from that in classical GC systems. Only with instruments with electronic pressure control (EPC) is adjustment necessary for direct coupling with MS. The separating efficiency of a capillary column is given as theoretical plates in chromatographic terminology (see Section 2.2.5). A high analytical separating capacity is always accompanied by a large number of plates (number of separation steps) so the height equivalent to a theoretical plate (HETP) decreases. In van Deemter curves, the HETP is plotted against the carrier gas velocity (not flow!). The minimum of one of these curves gives the optimal adjustment for a particular carrier gas for isothermal operations.

For helium the optimal carrier gas velocity for standard columns is ca. 24 cm/s. As the viscosity of the carrier gas increases in the course of a temperature program, in practice at

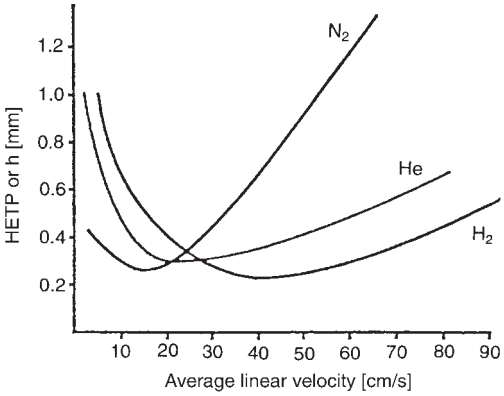


Fig. 2.89 van Deemter curves for nitrogen, helium and hydrogen as the carrier gas. The values refer to a standard column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (Restek).

the lower start temperature a higher velocity is used (ca. 30 cm/s). Velocities which are too high lower the efficiency.

With hydrogen as the carrier gas a much higher velocity (>40 cm/s) can be used, which leads to significant shortening of the analysis time. Furthermore, with hydrogen the right hand branch of the van Deemter curve is very flat, so that a further increase in the gas velocity is possible without impairing the efficiency (Figs. 2.89 and 2.90). The separating efficiency is retained and time is gained. Because of the often very limited pumping capacity in commercial GC/MS systems (see Section 2.4.1), little use can be made of these advantages with hydrogen. Caused by the expected shortage of the natural helium supply and the already increasing prices hydrogen becomes an economical alternative. Hydrogen generators are available for on-site premium quality supply.

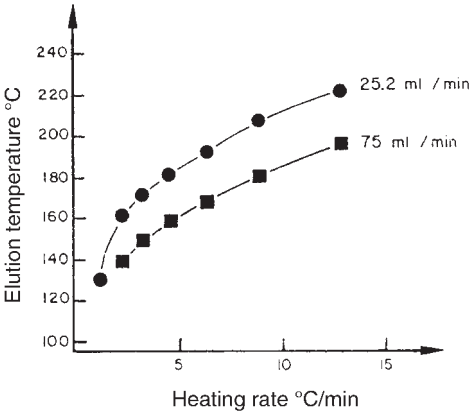
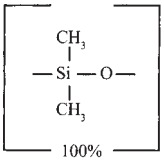


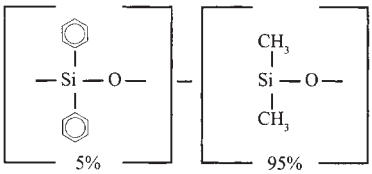
Fig. 2.90 The effect of heating rate and carrier gas flow on the elution temperature (retention temperature) (after Karasek).

2.2.4.6 Properties of Stationary Phases



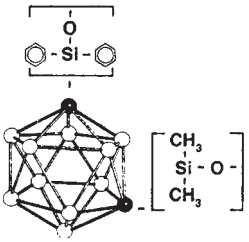
Polarity: least polar bonded phase
Use: boiling point separations for solvents, petroleum products, pharmaceuticals
Properties: minimum temperature –60 °C
maximum temperature 340–430 °C
helix structure

Fig. 2.91 100% Dimethyl-polysiloxane



Polarity: nonpolar, bonded phase
Use: boiling point, point separations for aromatic compounds, environmental samples, flavours, aromatic hydrocarbons
Properties: minimum temperature –60 °C
maximum temperature 340 °C

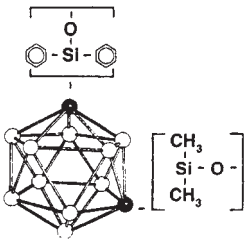
Fig. 2.92 5% Diphenyl-95% dimethyl-polysiloxane



Use:
- ideal for GC/MS coupling because of very low bleeding
- all environmental samples
- all medium and high molecular weight substances
- polyaromatic hydrocarbons, PCBs, waxes, triglycerides

Properties: minimum temperature –10 °C
maximum temperature 480 °C (highest operating temperature of all stationary phases, aluminium coated, 370 °C polyimide coated), high temperature phase

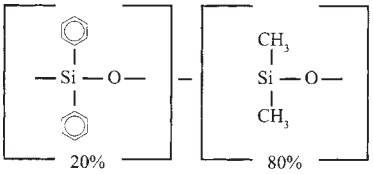
Fig. 2.93 Siloxane-carborane, comparable to 5% phenyl



Polarity: weakly polar, similar to 8% phenylsiloxane
Use:
- ideal for GC/MS coupling because of very low bleeding
- all environmental samples, can be used universally
- volatile halogenated hydrocarbons, solvents – polyaromatic hydrocarbons, pesticides, only column that separates all PCB congeners

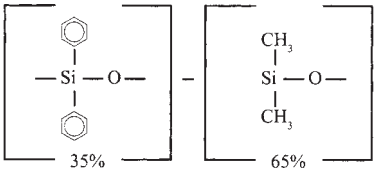
Properties: minimum temperature –20 °C
maximum temperature 370 °C

Fig. 2.94 Siloxane-carborane, comparable to 8% phenyl



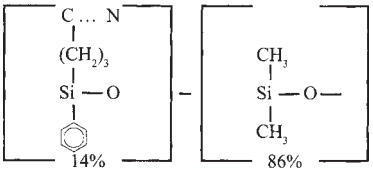
Polarity: slightly polar
Use: for volatile compounds
Properties: minimum temperature –20 °C
maximum temperature 310 °C

Fig. 2.95 20% Diphenyl-80% dimethyl-polysiloxane



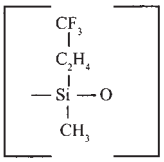
Polarity: intermediately polar
Use: pesticides, PCBs, amines
Properties: minimum temperature 20 °C
maximum temperature 300 °C

Fig. 2.96 35% Diphenyl-65% dimethyl-polysiloxane



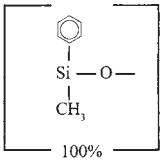
Polarity: intermediately polar
Use: pesticides, PCBs, alcohols, oxygenates
Properties: minimum temperature –20 °C
maximum temperature 280 °C

Fig. 2.97 14% Cyanopropylphenyl-86% dimethyl-polysiloxane



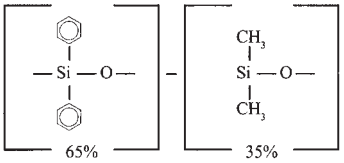
Polarity: selective for lone pair electrons
Use: environmental samples, solvents, freons
Properties: minimum temperature –20 °C
maximum temperature 360 °C

Fig. 2.98 100% Trifluoropropylmethyl-polysiloxane



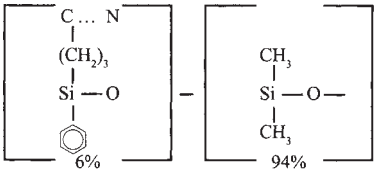
Polarity: intermediately polar
Use: triglycerides, phthalic acid esters, PAH etc.
Properties: minimum temperature 25 °C
maximum temperature 340 °C

Fig. 2.99 50% Diphenyl-50% dimethyl-polysiloxane



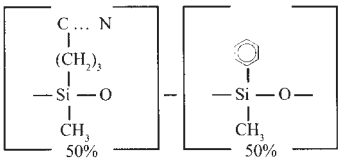
Polarity: medium polarity
Use: triglycerides, free fatty acids, terpenes
Properties: minimum temperature 50 °C
maximum temperature 340 °C

Fig. 2.100 65% Diphenyl-35% dimethyl-polysiloxane



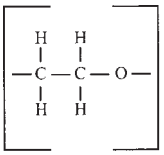
Polarity: medium polarity
Use: pesticides, PCBs, alcohols, oxygen-containing compounds, volatile organic compounds, drugs etc.
Properties: minimum temperature –20 °C
maximum temperature 280 °C

Fig. 2.101 6% Cyanoprophylphenyl-94% dimethyl-polysiloxane



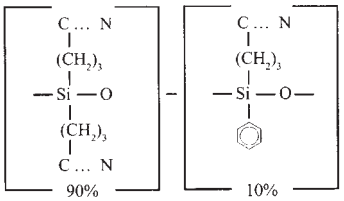
Polarity: polar phase
Use: fatty acid methyl esters (FAMES), carbohydrates
Properties: minimum temperature 40 °C
maximum temperature 220 °C

Fig. 2.102 50% Cyanopropylmethyl-50% phenylmethyl-polysiloxane



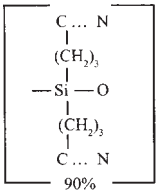
Polarity: polar phase
Use: fatty acid methyl esters (FAMES), terpenes, acids, amines, solvents
Properties: minimum temperature 40 °C
maximum temperature 280 °C

Fig. 2.103 100% Carbowax polyethyleneglycol 20M



Polarity: very polar
Use: cis/trans isomers
Properties: minimum temperature 0 °C
maximum temperature 275 °C

Fig. 2.104 90% Biscyanopropyl-10% phenylcyanopropyl-polysiloxane



Polarity: very polar
Use: fatty acid methyl esters (FAMES)
Properties: minimum temperature 20 °C
maximum temperature 250 °C

Fig. 2.105 100% Biscyanopropyl-polysiloxane

2.2.5 Chromatography Parameters

All chromatography processes are based on the multiple repetition of a separation process, such as the continuous dynamic partition of the components between two phases.

In a model chromatography can be regarded as a continuous repetition of partition steps. The starting point is the partition of a substance between two phases in a separating funnel. Suppose a series of separating funnels is set up which all contain the same quantity of phase 1. As this phase remains in the separating funnels, it is known as the stationary phase.

The sample is placed in the first separating funnel dissolved in a second phase (the auxiliary phase). After establishing equilibrium through shaking, phase 2 is transferred to separating funnel 2. The auxiliary phase thereby becomes the mobile phase. Fresh mobile phase is placed in the first separating funnel etc. (Fig. 2.106).

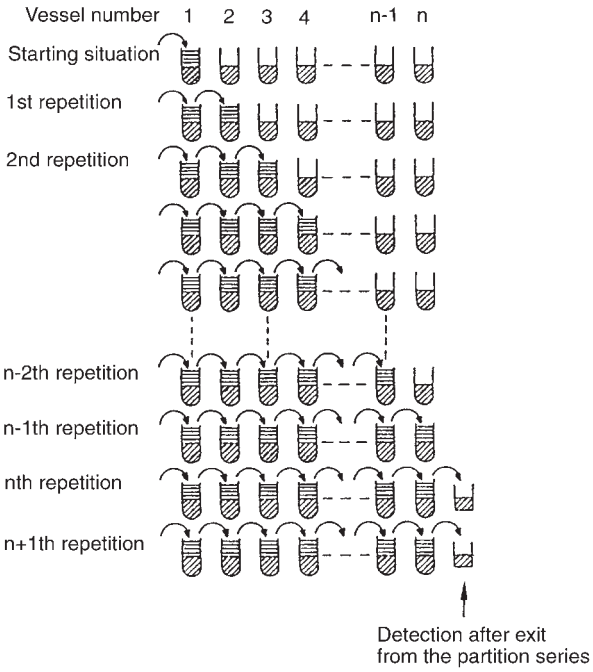


Fig. 2.106 Partition series: mode of operation with two auxiliary phases.

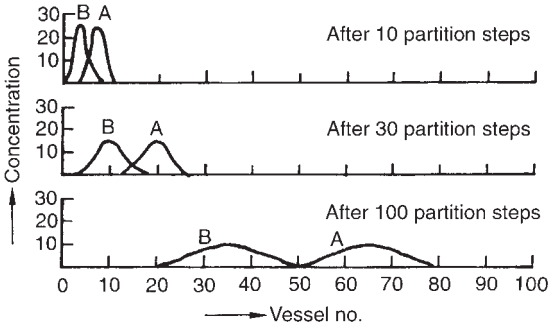


Fig. 2.107 Partition of substances A and B after 10, 30 and 100 separating steps.
After 10 steps A and B are hardly separated, after 30 steps quite well and after 100 steps practically completely. The two substances are partitioned among an ever increasing number of vessels and the concentrations decrease more and more ($\alpha_A = 2 : \alpha_B = 0.5$).

The results of this type of partition with 100 vessels and two substances A and B are shown in Fig. 2.107. The prerequisite for this is the validity of the Nernst equation. For detection the concentrations of A and B in the vessels are determined.

With the model described, so many separating steps are carried out that the mobile phase leaves the system of 100 vessels and the individual components A and B are removed, one after the other, from the series of vessels. This process is known as elution.

2.2.5.1 The Chromatogram and its Meaning

The substances eluted are transported by the mobile phase to the detector and are registered as Gaussian curves (peaks). The peaks give qualitative and quantitative information on the mixture investigated.

Qualitative: The retention time is the time elapsing between injection of the sample and the appearance of the maximum of the signal. The retention time of a component is always constant under the same chromatographic conditions. A peak can therefore be identified by a comparison of the retention time with a standard (pure substance).

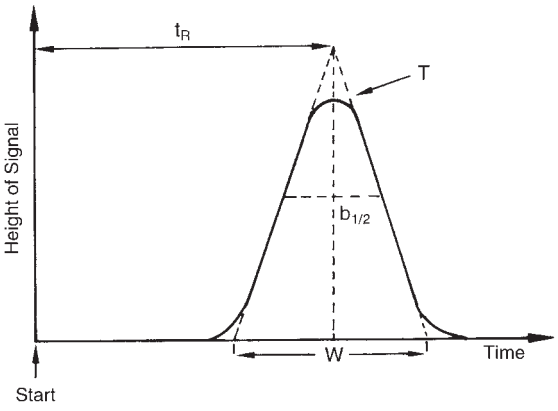


Fig. 2.108 Parameters determined for an elution peak.
t_R retention time
$b_{1/2}$ half width
W base width

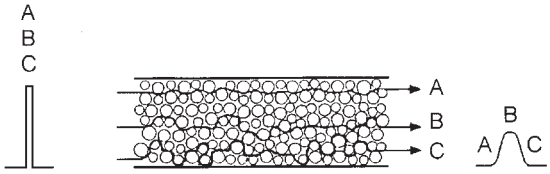


Fig. 2.109 Eddy diffusion in packed columns (multipath effect).

Quantitative: The height and area of a peak is proportional to the quantity of substance injected. Unknown quantities of substance can be determined by a comparison of the peak areas (or heights) with known concentrations.

In the ideal case the peaks eluting are in the shape of a Gaussian distribution (bell-shaped curve, Fig. 2.108). A very simple explanation of this shape is the different paths taken by the molecules through the separating system (multipath effect), which is caused by diffusion processes (Eddy diffusion) (Fig. 2.109).

Under defined conditions the time required for elution of a substance A or B at the end of the separating system, the **retention time** t_R, is characteristic of the substance. It is measured from the start (sample injection) to the peak maximum (Fig. 2.110).

At a constant flow rate t_R is directly proportional to the retention volume V_R.

$$V_R = t_R \cdot F \tag{10}$$

where F = flow rate in mL/min

The retention volume shows how much mobile phase has passed through the separating system until half of the substance has eluted (peak maximum!).

2.2.5.2 Capacity Factor k'

The retention time t_R depends on the flow rate of the mobile phase and the length of the column. If the mobile phase moves slowly or the column is long, t_0 is large and so is t_R. Thus t_R is not suitable for the comparative characterisation of a substance, e. g. between two laboratories.

It is better to use the capacity factor, also known as the k' value, which relates the net retention time t'_R to the dead time:

$$k' = \frac{t'_R}{t_0} = \frac{t_R - t_0}{t_0} \tag{11}$$

Thus, the k' value is independent of the column length and the flow rate of the mobile phase and represents the molar ratio of a particular component in the stationary and mobile phases. Large k' values mean long analysis times.

The k' value is related to the partition coefficient K as follows:

$$k' = K \cdot \frac{V_l}{V_g} \tag{12}$$

where V_l = volume of the stationary phase
V_g = volume of the mobile phase

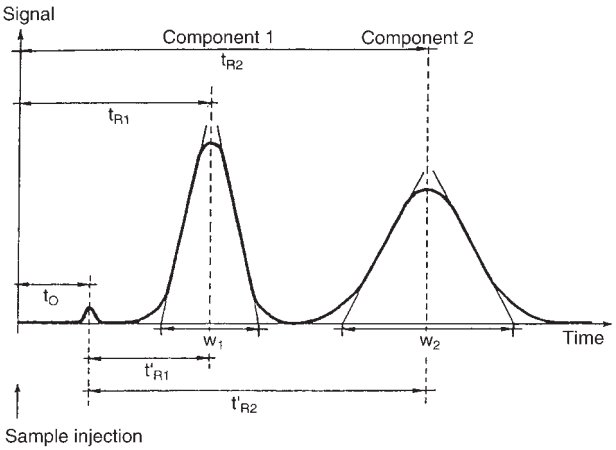


Fig. 2.110 The chromatogram and its parameters.

W *Peak width* of a peak. $W = 4\ \sigma$ with σ = the standard deviatiion of the Gaussian peak.

t_0 *Dead time* of the column; the time which the mobile phase requires to pass through the column. The linear velocity u of the solvent is calculated from

$$u = \frac{L}{t_0} \quad \text{with } L = \text{length of the column}$$

A substance which is not retarded, i. e. a substance which is not held by the stationary phase, appears at t_0 at the detector.

t_R *Retention time*: the time between the injection of a substance and the recording of a peak maximum.

t'_R *Net retention time.* From the diagram it can be seen that $t_R = t_0 + t'_R$.
t_0 is the same for all eluted substances and is therefore the residence time in the mobile phase. The substances separated differ in their residence times in the stationary phase t'_R. The longer a substance stays in the stationary phase, the later it is eluted.

The capacity factor is therefore directly proportional to the volume of the stationary phase (or for adsorbents, their specific surface area in m^2/g).

α is a measure of the relative retention and is given by:

$$\alpha = \frac{k'_2}{k'_1} = \frac{K_2}{K_1} \qquad (k'_2 > k'_1) \tag{13}$$

In the case where $\alpha = 1$, the two components 1 and 2 are not separated because they have the same k' values.

The relative retention α is thus a measure of the selectivity of a column and can be manipulated by choice of a suitable stationary phase. (In principle this is also true for the choice of the mobile phase, but in GC/MS helium or hydrogen are, in fact, always used.)

2.2.5.3 **Chromatographic Resolution**

A second model, the theory of plates, was developed by Martin and Synge in 1941. This is based on the functioning of a fractionating column, then as now a widely used separation technique. It is assumed that the equilibrium between two phases on each plate of the column has been fully established. Using the plate theory, mathematical relationships can be

derived from the chromatogram, which are a practical measure of the sharpness of the separation and the resolving power.

The chromatography column is divided up into theoretical plates, i. e. into column sections in the flow direction, the separating capacity of each one corresponding to a theoretical plate. The length of each section of column is called the height equivalent to a theoretical plate (HETP). The HETP value is calculated from the length of the column L divided by the number of theoretical plates N:

$$\text{HETP} = \frac{L}{N} \qquad \text{in mm} \tag{14}$$

The number of theoretical plates is calculated from the shape of the eluted peak. In the separating funnel model it is shown that with an increasing number of partition steps the substance partitions itself between a larger number of vessels. A separation system giving sharp separation concentrates the substance band into a few vessels or plates. The more plates there are in a separation system, the sharper the eluted peaks.

The number of theoretical plates N is calculated from the peak profile. The retention time t_R at the peak maximum and the width at the base of the peak measured as the distance between the cutting points of the tangents to the inflection points with the base line are determined from the chromatogram (see Fig. 2.108).

$$N = 16 \cdot \left(\frac{t_g}{W}\right)^2 \tag{15}$$

where t_R = retention time
W = peak width

For asymmetric peaks the half width (the peak width at half height) is used:

$$N = 8\ \ln 2 \cdot \left(\frac{t_g}{W_n}\right)^2 \tag{16}$$

where t_R = retention time
W_n = peak width at half height

Consequence: A column is more effective, the more theoretical plates it has (Fig. 2.111).

The width of a peak in the chromatogram determines the resolution of two components at a given distance between the peak maxima (Fig. 2.112). The resolution R is used to assess the quality of the separation:

$$R \approx \frac{\text{retention difference}}{\text{peak width}} \tag{17}$$

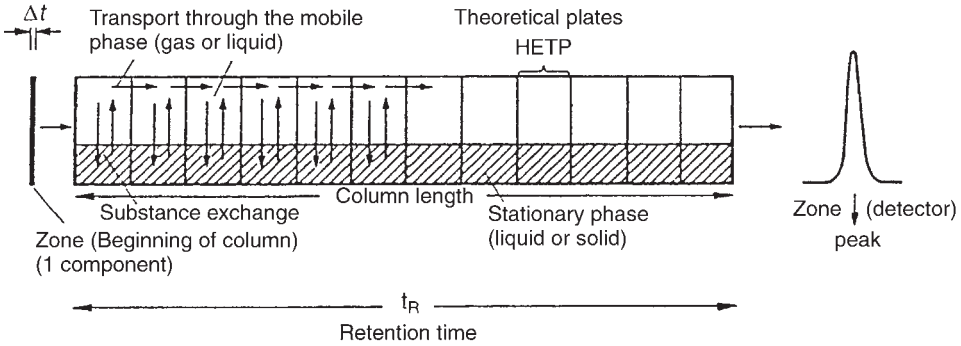


Fig. 2.111 Substance exchange and transport in a chromatography column are optimal when there are as many phase transfers as possible with the smallest possible expansion of the given zones (after Schomburg).

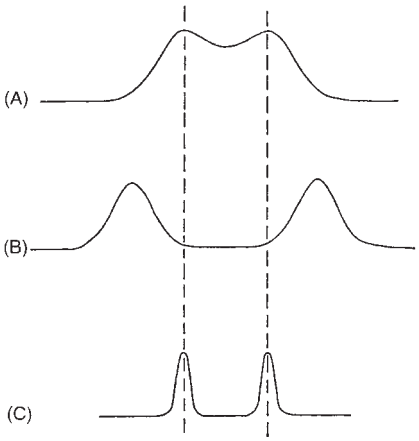


Fig. 2.112 Resolution.
(A/C) Peaks with the same retention time
(A/B) Peaks with the same peak width
(B/C) Separation with the same resolution

The resolution R of two neighbouring peaks is defined as the quotient of the distance between the two peak maxima, i.e. the difference between the two retention times t_R and the arithmetic mean of the two peak widths:

$$R = 2 \cdot \frac{t_{R2} - t_{R1}}{W_1 + W_2} = 1.198 \cdot \frac{t_{R2} - t_{R1}}{W_{h1} + W_{h2}}$$

where W_h = peak width at half height

Figure 2.113 shows what one can expect optically from a value for R calculated in this way. At a resolution of 1,0 the peaks are not completely separated, but it can definitely be seen that there are two components. The tangents to the inflection points just touch each other and the peak areas only overlap by 2%.

For the precise determination of the peak width the tangents to the inflection points can be drawn in manually (Fig. 2.114). For a critical pair, e.g. stearic acid (C_{18-0}) and oleic acid (C_{18-1}) the construction of the tangents is shown in Fig. 2.115.

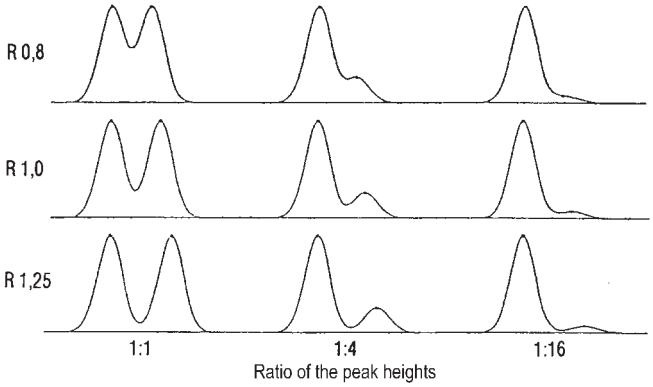


Fig. 2.113 Resolution of two neighbouring peaks (after Snyder and Kirkland).

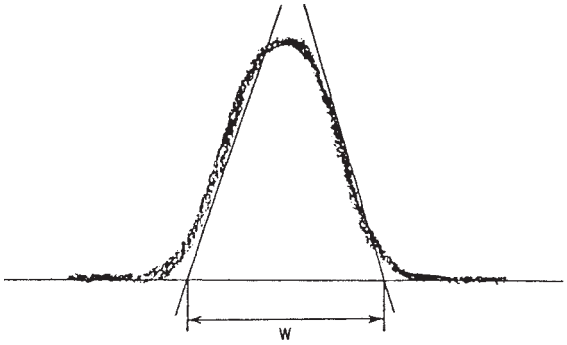


Fig. 2.114 Manual determination of the peak width using tangents to the inflection points.

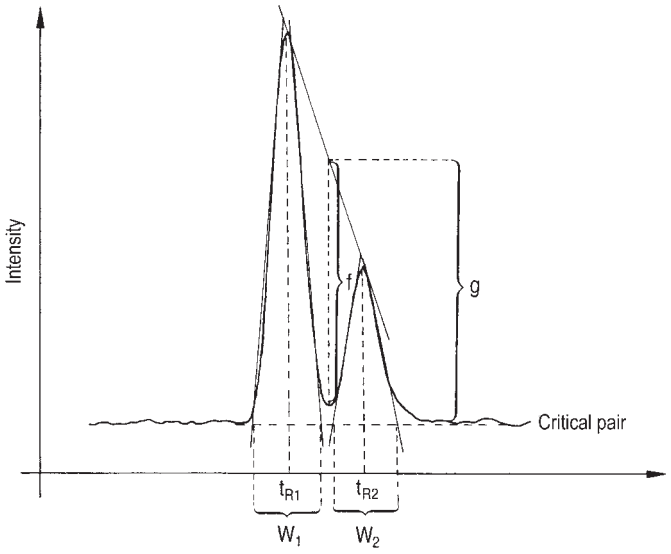


Fig. 2.115 Determination of the resolution and peak widths for a critical pair.

2.2.5.4 Factors Affecting the Resolution

Rearranging the resolution equation and putting in the capacity factor $k' = (t_R - t_0)/t_0$, the selectivity factor $\alpha = k'_2/k'_1$ and the number of theoretical plates N gives an important basic equation for all chromatographic elution processes.

The resolution R is related to the selectivity α (relative retention), the number of theoretical plates N and the capacity factor k' by:

$$R = \underbrace{\frac{1}{4}(\alpha - 1)}_{\text{I.}} \cdot \underbrace{\frac{k'}{1 + k'}}_{\text{II.}} \cdot \underbrace{\sqrt{N}}_{\text{III.}} \tag{18}$$

The Selectivity Term

R is directly proportional to $(\alpha - 1)$. An increase in the ratio of the partition coefficients leads to a sharp improvement in the resolution, which can be achieved, for example, by changing the polarity of the stationary phase for substances of different polarities.

As the selectivity generally decreases with increasing temperature, difficult separations must be carried out at as low a temperature as possible.

The change in the selectivity is the most effective of the possible measures for improving the resolution. As shown in Table 2.28, more plates are required to achieve the desired resolution when α is small.

Table 2.28 Relationship between relative retention α and the chromatographic resolution R.

Relative retention α	$R = 1.0$	$R = 1.5$
1.005	650 000 plates	1 450 000 plates
1.01	163 000	367 000
1.05	7 100	16 000
1.10	3 700	8 400
1.25	400	900
1.50	140	320
2.0	65	145

Figure 2.116 shows the effect of relative retention and number of plates on the separation of two neighbouring peaks:

- At high relative retention the number of theoretical plates in the column does not need to be large to achieve satisfactory resolution (a). The column is poor but the system is selective.
- A high relative retention and large number of theoretical plates give a resolution which is higher than the optimum. The analysis is unnecessarily long (b).
- At the same (small) number of theoretical plates as in (a), but at a smaller relative retention, the resolution is strongly reduced (c).
- If the relative retention is small, a large number of theoretical plates are required to give a satisfactory resolution (d).

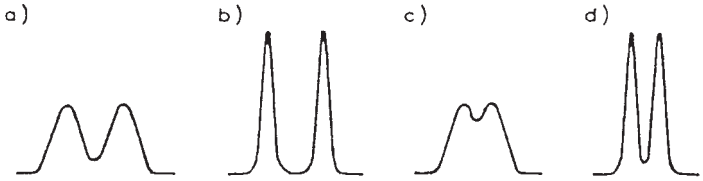


Fig. 2.116 Relative retention, number of plates and resolution.

The Retardation Term

Here the resolution is directly proportional to the residence time of a component in the stationary phase based on the total retention time. If the components only stayed in the mobile phase ($k' = 0$!) there would be no separation.

For very volatile or low molecular weight nonpolar substances there are only weak interactions with the stationary phase. Thus at a low k' value the denominator $(1 + k')$ of the term is large compared with k' and R is therefore small.

This also applies to columns with a small quantity of stationary phase and column temperatures which are too high. To improve the resolution a larger content of stationary phase can be chosen (greater film thickness).

The Dispersion Term

The number of plates N characterises the performance of a column (Fig. 2.117). However the resolution R only increases with the square root of N. As N is directly proportional to the column length L, the performance is only proportional to the square root of the column length.

Doubling the column length therefore only increases the resolution by a factor of 1.4. Since the retention time t_R is proportional to the column length, for an improvement in the resolution by a factor of 1.4, the analysis time is doubled.

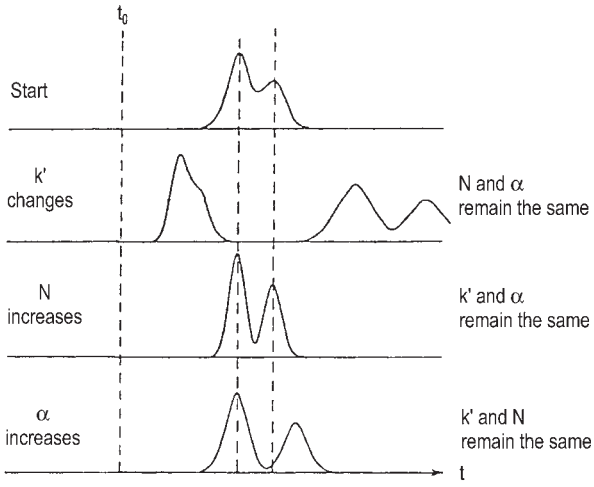


Fig. 2.117 Effect of capacity factor, number of plates and relative retention on the chromatogram (after L. R. Snyder and J. J. Kirkland).

2.2.5.5 Maximum Sample Capacity

The maximum sample capacity can be derived from the equations concerning the resolution. Under ideal conditions (see Fig. 2.115):

$$\frac{f}{g} = 100\% \tag{19}$$

where f = the area under the line connecting the peak maxima
g = the height of the connecting line above the base line, measured in the valley between the peaks

The maximum sample capacity of a column is reached if f/g falls below 90% for a critical pair. If too much sample material is applied to a column, the k' value and the peak width are no longer independent of the size of the sample, which ultimately affects the identification and the quantitation of the results (Fig. 2.118).

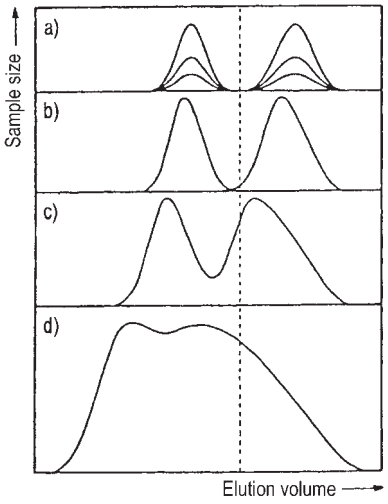


Fig. 2.118 Change in the chromatogram with increasing sample size (after L. R. Snyder and J. J. Kirkland).
(a) Constant k' values
(b–d) Increasing changes to the retention behaviour through overloading

2.2.5.6 Peak Symmetry

In exact quantitative work (integration of the peak areas) a maximum asymmetry must not be exceeded, otherwise there will be errors in determining the cut-off point of the peak with the base line.

For practical reasons the peak symmetry T is determined at a height of 10% of the total peak height (Fig. 2.119):

$$T = \frac{b_{0.1}}{a_{0.1}} \tag{20}$$

where $a_{0.1}$ = distance from the peak front to the maximum measured at 0.1 h
$b_{0.1}$ = distance from the maximum to the end of the peak measured at 0.1 h

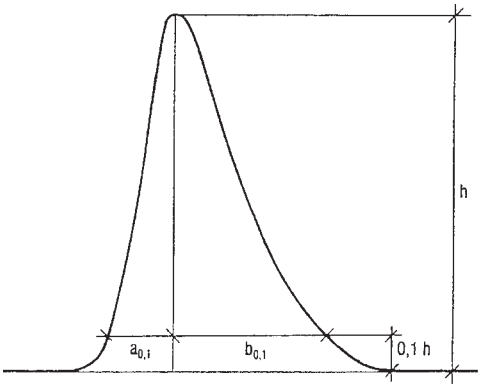


Fig. 2.119 Asymmetric peak.

T should ideally be 1.0 for a symmetrical peak, but for a practical quality measure it should not be greater than 2.5. If the tailing exceeds higher values, there will be errors in the quantitative area measurement because the point where the peak reaches the base line is very difficult to determine.

2.2.5.7 **Optimisation of Flow**

The flow of the mobile phase affects the rate of substance transport through the stationary phase. High flow rates allow rapid separation. However, the efficiency is reduced because of the slower exchange of substances between the stationary and mobile phases and leads to peak broadening. On the other hand, peak broadening caused by diffusion of the components within the mobile phase is only hindered by increasing the flow rate.

The aim of flow optimisation for given column properties at a given temperature and with a given carrier gas is to find the flow rate which gives either the maximum number of separation steps or at adequate efficiency the shortest possible analysis time.

The height equivalent to a theoretical plate (HETP) and the number of theoretical plates *N* depends on the flow rate of the mobile phase *u* according to van Deemter. The linear flow rate *u* of the mobile phase is calculated from the chromatogram:

$$u = \frac{L}{t_0} \tag{21}$$

with L = length of the column in cm
t_0 = dead time in s

The following affect the optimum flow rate:

1. The *Eddy diffusion* (Fig. 2.120) on the peak broadening. This effect is independent of flow and, naturally, for packed columns only, dependent on the nature of the packing material and the density of packing. For open capillary (tabular) columns, Eddy diffusion does not occur.
2. The *axial diffusion* on peak broadening. This diffusion occurs in and against the direction of flow and decreases with increasing flow rate.

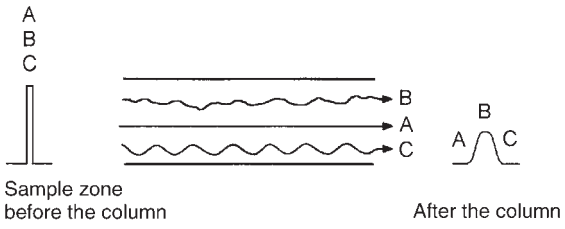


Fig. 2.120 Path differences for lamina flow in capillary columns (multipath effect, caused by turbulence at high flow rates).

3. Incomplete partition equilibrium. The transfer of analyte between the stationary and mobile phases only has a finite rate relative to that of the mobile phase, corresponding to the diffusion rates. The contribution to peak broadening increases with increasing flow rate of the mobile phase.

For the maximum efficiency of the separation (Fig. 2.121) a flow rate $u_{\min}$ must be chosen as a compromise between these opposing effects. The position of the minimum is affected by:

- the quantity of the stationary phase (e. g. film thickness),
- the particle size of the packing material (for packed columns),
- the diameter of the column,
- the nature of the mobile phase (diffusion coefficient, viscosity).

For practical reasons the effective flow rates in GC/MS analyses are set above the optimum flow rates for increased speed and sample throughput. In this case the right branch of the van Deempter graph needs to be considered where the slope of the curve is low (Fig. 2.121). The small loss in column resolution by increased flow rates is more than compensated by the advantage of a short analysis time which holds true for helium and especially for hydrogen. Constant flow rate conditions maintain chromatographic peak resolution in ramped temperature programs.

In particular in GC×GC the flow rate of the second short column is accepted to be significantly above the ideal flow rate using the direct connection to the modulator. A split device

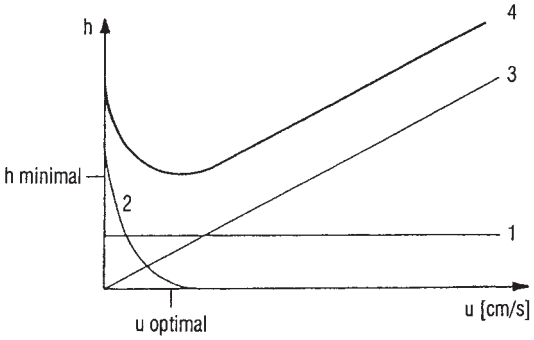


Fig. 2.121 The van Deemter curve.
1 Proportion of Eddy diffusion and flow distribution at band broadening
2 Proportion of longitudinal diffusion
3 Proportion of substance exchange phenomena
4 Resulting curve H(u), called the van Deemter curve

would be needed between modulator and second column for the independent adjustment of the flow rate. Most GC×GC application do not use a flow adjustment for the 2nd column which is due to the cut in substance concentration and hence sensitivity of the method, and, this is most probably the most important factor, the overwhelming increase in peak separation and S/N by using a high flow compromise.

Definition of Chromatographic Parameters

Carrier gas velocity	$v = L/t_0$	The average linear carrier gas velocity has an optimal value for each column with the lowest possible height equivalent to a theoretical plate (see van Deemter); v is independent of temperature. L = length of column t_0 = dead time
Partition coefficient	$K = c_l / c_g$	Concentration of the substance in the stationary phase (liquid) divided by the concentration in the mobile phase (gas). K is constant for a particular substance in a given chromatographic system.
	$K = k' \cdot \beta$	K is also expressed as the product of the capacity ratio (k') and the phase ratio (β).
Capacity ratio (partition ratio)	$k' = K \cdot V_l / V_g$	Determines the retention time of a compound. V_l = volume of the stationary phase V_g = volume of the gaseous mobile phase
	$k' = t_R - t_0 / t_0$	t_R = retention time of the substance t_0 = dead time
Phase ratio	$\beta = r/2d_f$	r = internal column radius d_f = film thickness
Number of theoretical plates	$N = 5.54\,(t_R / W_h)^2$	The number of theoretical plates is a measure of the efficiency of a column. The value depends on the nature of the substance and is valid for isothermal work. N = number of theoretical plates t_R = retention time of the substance W_h = peak width at half height

Height equivalent to a theoretical plate (HETP)	$h = L/N$	Is a measure of the efficiency of a column independent of its length. L = length of the column N = number of theoretical plates
Resolution	$R = 2\,(t_j - t_i)/(W_j + W_i)$	Gives the resolving power of a column with regard to the separation of components i and j (isothermally). t_i = retention time of substance i t_j = retention time of substance j $W_{i,j}$ = peak width at half height of substances i, j
Separation factor	$\alpha = k'_j/k'_i$	Measure of the separation of the substances i, j
Trennzahl number	$TZ = \dfrac{t_{R(x+1)} - t_{R(x)}}{W_{h(x+1)} + W_{h(x)}} - 1$	The trennzahl number is, like the resolution, a means of assessing the efficiency of a column and is also used for temperature-programmed work. TZ gives the number of components which can be resolved between two homologous n-alkanes.
Effective plates	$N_{eff.} = 5.54\,((t_{R(i)} - t_0)\,/\,W_{h(i)})$	The effective number of theoretical plates takes the dead volume of the column into account.
Retention volume	$V_R = t_R \cdot F$	Gives the carrier gas volume required for elution of a given component. F = carrier gas flow
Kovats index	$KI = 100 \cdot c + 100\,\dfrac{\log(t'_R)_x - \log(t'_R)_c}{\log(t'_R)_{c+1} - \log(t'_R)_c}$	The Kovats index is used for isothermal work. t'_R = corrected retention times for standards and substances $t'_R = t_R - t_0$
Modified Kovats index	$RI = 100 \cdot c + 100\,\dfrac{(t'_R)_x - (t'_R)_c}{(t'_R)_{c+1} - (t'_R)_c}$	The modified Kovats index according to van den Dool and Kratz is used with temperature programming.

2.2.6 Classical Detectors for GC/MS Systems

Classical detectors are important for the consideration of GC/MS coupling if an additional specific means of detection is to be introduced parallel to mass spectrometry. The parallel coupling of a flame ionisation detector (FID) does not lead to results which are complementary to those of mass spectrometry, as both detection processes give practically identical chromatograms as the response factors for most of the organic substances are comparable. Parallel detection with a thermal conductivity detector is not used in practice as the mass spectrometric analysis of gases is generally carried out with special instruments (RGA, residual gas analyser, mass range < 100 μ).

Additional information can, however, be obtained with element-specific detectors. The detection limits which can be achieved with an electron capture detector (ECD) or a nitrogen/phosphorus detector (NPD) are usually comparable to those attainable using a mass spectrometer. On dividing up the carrier gas flow, the ratio of the two parts must be considered when planning such a setup. Normally a larger proportion is passed into the mass spectrometer so that in residue analysis low concentrations of substances do not fall below the detection limit. The use of such flow dividers for quantitative determinations must be checked in an individual case, as a constant division cannot be expected for all boiling point ranges.

Applications can cover the rapid screening e.g. on the intensity of halogenated compounds using an ECD with an intelligent decision for a subsequent MS analysis of the same positively screened sample for a mass selective quantitation.

2.2.6.1 FID

With the flame ionisation detector (FID) the substances to be detected are burned in a hydrogen flame and are thus partially ionised (Table 2.29). As the jet is at a negative potential, positive ions are neutralised. The corresponding electrons are captured at the ring-shaped collector electrode to give a signal current (Fig. 2.122). The electrode is at a potential which is ca. 200 V more positive than the jet.

Table 2.29 Reactions in the FID.

Pyrolysis:	CH_3^o, CH_2^o, CH^o, C^o
Excited radicals:	O_2^*, OH^*
Ionisation:	$CH_2^o + OH^* \rightarrow CH_3O^+ + e^-$ $CH^o + OH^* \rightarrow CH_2O^+ + e^-$ $CH^o + O_2^* \rightarrow CHO_2^+ + e^-$ $C^o + OH^* \rightarrow CHO^+ + e^-$

Provided that only hydrogen burns in the flame, only radical reactions occur. No ions are formed. If organic substances with C-H and C-C bonds get into the flame, they are first pyrolysed. The carbon-containing radicals are oxidised by oxygen and the OH radicals formed in

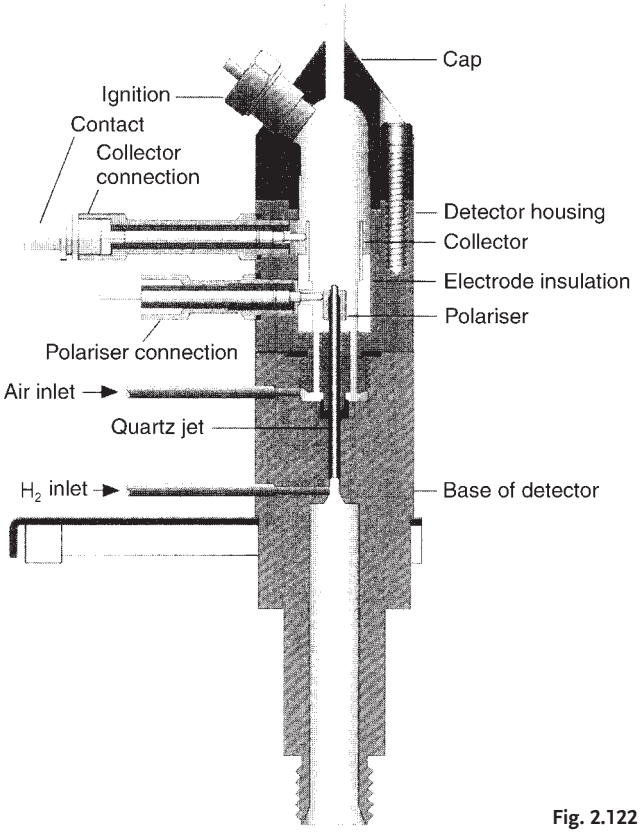


Fig. 2.122 Construction of an FID (Finnigan).

the flame. The excitation energy leads to ionisation of the oxidation products. Only substances with at least one C-H or C-C bond are detected, but not permanent gases, carbon tetrachloride or water.

If a reactor (hydrogenator, methaniser) is connected before the FID, the latter can be converted into an extremely sensitive detector for permanent gases such as CO and CO_2. The oxygen specific detector (O-FID) uses two reactors. In the first hydrocarbons are decomposed into carbon, hydrogen and carbon monoxide at above 1300 °C. CO is then converted into methane in the hydrogenation reactor and detected with the FID. With the O-FID, for example, oxygen-containing components in fuels can be detected.

FID **F**lame **I**onisation **D**etector

Universal detector

Advantages: High dynamics
High sensitivity
Robust

Use:	Hydrocarbons, e. g. fuels, odorous substances, BTX, polyaromatic hydrocarbons etc Comparison of diesel with petrol Important all round detector
Limits:	As it is universal, its performance is poor for trace analysis in complex matrices Low response for highly chlorinated or brominated substances.

2.2.6.2 **NPD**

A nitrogen-phosphorous detector (NPD) is a modified FID which contains a source of alkali situated between the jet and the collector electrode on a Pt wire, for the specific detection of nitrogen or phosphorus (Fig. 2.123).

The alkali beads are heated to red heat both electrically and in the flame and are excited to alkali emission. They are always at a negative potential compared with the collector electrode. For the detection of phosphorus the jet is earthed. The electrons emitted by the hydrocarbon parts of the molecule cannot exceed the negative potential of the beads and do not

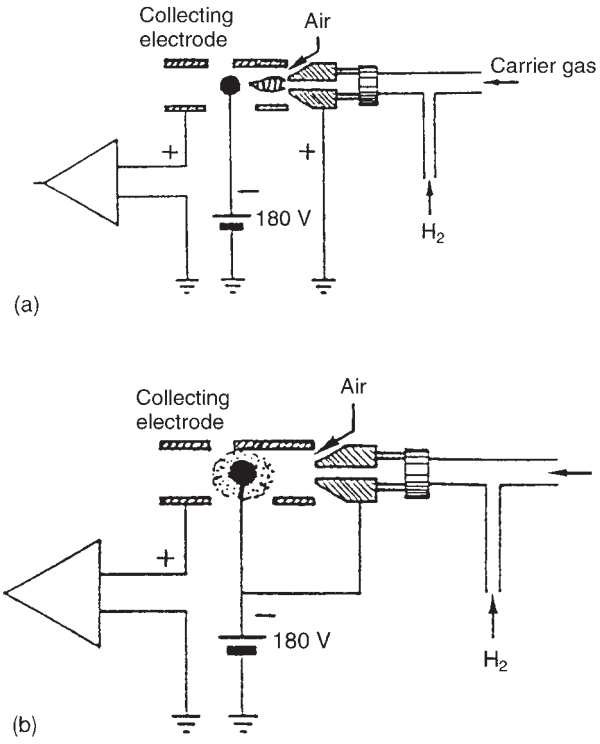


Fig. 2.123 (a) Diagram of an NPD (P operation); (b) diagram of an NPD (N operation).

Table 2.30 Reactions with P compounds in the NPD.

$\bar{\underline{O}} = \overset{\bullet}{\underline{P}}$	+	A*	→	$[\bar{\underline{O}} = \bar{\underline{P}}]^-$	+	A^+
$\bar{\underline{O}} = \overset{\bullet}{P} = \bar{\underline{O}}$	+	A*	→	$[\bar{\underline{O}} = P = \bar{\underline{O}}]^-$	+	A^+
$[\bar{\underline{O}} = \bar{\underline{P}}]^-$	+	OH°	→	HPO_2	+	e^-
$[\bar{\underline{O}} = P = \bar{\underline{O}}]^-$	+	OH°	→	HPO_3	+	e^-
HPO_3	+	H_2O	→	H_3PO_4		

A = alkali

reach the collector electrode, but are earthed. The electrons from the specific alkali reaction reach the collector electrode unhindered (Table 2.30).

Phosphorus-containing substances are first converted in the flame into phosphorus oxides with an uneven number of electrons. Anions formed in the alkali reaction by the addition of an electron are now oxidised by OH radicals. The electrons added are now released and produce a signal current.

Like phosphorus, nitrogen has an uneven number of electrons. Under the reducing conditions of the flame cyanide and cyanate radicals are formed, which can undergo the alkali reaction (Table 2.31). For this the input of hydrogen and air are reduced. Instead of the flame the hydrogen burns in the form of a cold plasma around the electrically heated alkali beads.

Table 2.31 Reactions with N compounds in the NPD.

Pyrolysis of CNC compounds			→	$C \equiv N|^\circ$		
CN°	+	A*	→	CN^-	+	A*
CN^-	+	H°	→	HCN	+	e^-
CN^-	+	OH°	→	HCNO	+	e^-

A = alkali

In order to form the required cyanide and cyanate radicals the C-N structure must already be present in the molecule. Nitro compounds are detected, but not nitrate esters, ammonia or nitrogen oxides. By taking part in the alkali reaction the cyanide radical receives an electron. Cyanide ions are formed, which react with other radicals to give neutral species. The electron released provides the detector signal.

NPD **N**itrogen/**P**hosphorus **D**etector

Specific detector

Advantages:	High selectivity and sensitivity Ideal for trace analyses
Use:	Only for N- and P-containing compounds Plant protection agents Chemical warfare gases, explosives Pharmaceuticals
Limits:	Additional detector for ECD or MS Quantitative measurements with an internal standard are recommended To some extent time-consuming optimisation of the Rb beads

2.2.6.3 **ECD**

The electron capture detector (ECD) consists of an ionisation chamber, which contains a nickel plate, on the surface of which a thin layer of the radioactive isotope ^{63}Ni has been applied (ca. 10–15 mC, Fig. 2.124). The carrier gas (N_2 or Ar/10% methane) is first ionised by the β radiation. The free electrons migrate towards the collector electrode and provide the background current of the detector. Substances with electronegative groups reduce the background current by capturing electrons and forming negative molecular ions. The main reactions in the ECD are dissociative electron capture (Table 2.32) and electron capture (Table 2.33, see also Section 2.3.4.2). Negative molecular ions can recombine with positive carrier gas ions.

Electron capture is more effective, the slower the electrons move. For this reason a more sensitive ECD is now operated using pulsed DC voltage. By changing the pulse frequency the background current generated by the electrons is kept constant. The pulse frequency thus becomes the actual detector signal.

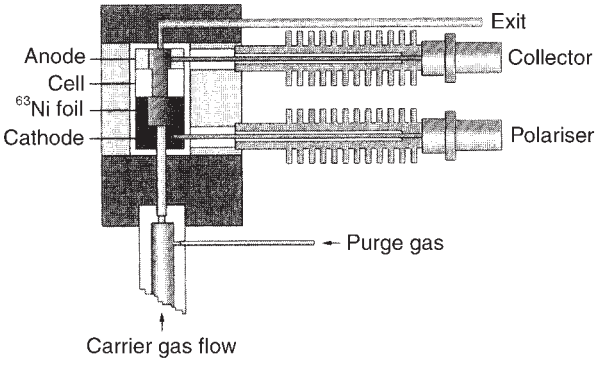


Fig. 2.124 Construction of an ECD (Finnigan).

Table 2.32 Substance reactions in the ECD.

1. Dissociative electron capture
   $AB + e^- \rightarrow A^0 + B^-$
2. Addition of an electron
   $AB + e^- \rightarrow (AB^-)^*$

Table 2.33 Basic reactions in the ECD.

$CG \xrightarrow{\beta} CG^+ + e^-$

Electron capture

$M + e^- \rightarrow M^-$

Recombination

$M^- + CG^+ \rightarrow M^o + CG$

CG = Carrier gas

ECD **E**lectron **C**apture **D**etector

Specific detector

The ECD reacts with all electronegative elements and functional groups, such as -F, -Cl, -Br, $-OCH_3$, $-NO_2$ with a high response. All hydrocarbons (generally the matrix) remain transparent, although present.

Advantages:	Selectivity for Cl, Br, methoxy, nitro groups Transparency of all hydrocarbons (= matrix) High sensitivity
Disadvantages:	Radioactive radiator, therefore handling authorisation necessary Sensitive to misuse Mobile only under limited conditions
Use:	Typical detector for environmental analysis Ideal for trace analysis Plant protection agents PCBs, dioxins Volatile halogenated hydrocarbons, freons
Limits:	Substances with low halogen contents (Cl_1, Cl_2) only have a low response (Table 2.34) Volatile halogenated hydrocarbons with low chlorine levels are better detected with FID or MS Limited dynamics Multipoint calibration necessary

Table 2.34 Conversion rates in the ECD for molecules with different degrees of chlorination.

Molecule	Conversion rate [$cm^3 \cdot mol^{-1} \cdot s^{-1}$]	Main product
CH_2Cl_2	1×10^{-11}	Cl^-
$CHCl_3$	4×10^{-9}	Cl^-
CCl_4	4×10^{-7}	Cl^-
CH_3CCl_3	1×10^{-8}	Cl^-
$CH_2ClCHCl_2$	1×10^{-10}	Cl^-
CF_4	7×10^{-13}	M^-
CF_3Cl	4×10^{-10}	Cl^-
CF_3Br	1×10^{-8}	Br^-
CF_2Br_2	2×10^{-7}	Br^-
C_6F_6	9×10^{-8}	M^-
$C_6F_5CF_3$	2×10^{-7}	M^-
$C_6F_{11}CF_3$	2×10^{-7}	M^-
SF_6	4×10^{-7}	M^-
Azulene	3×10^{-8}	M^-
Nitrobenzene	1×10^{-9}	M^-
1,4-Naphthoquinone	7×10^{-9}	M^-

2.2.6.4 PID

The photo ionization detector (PID) operates on the principle of energy absorption of photons emitted by a UV lamp in the detector housing. The absorbed energy leads to ionization of the molecule by release of an electron from the excited molecule M* according to Eq. (1).

$$M + h \cdot \nu \rightarrow M^* \rightarrow M^+ + e^- \quad (1)$$

The high selectivity of the PID is based on the different energy levels of the emitted emission lines by the used UV lamps. The different types of lamps are filled either with argon, hydrogen, krypton or other gases to cover an ionisation energy range from 8.4 to 11.8 eV. Ionisation occurs only if the ionization potential of the molecules M is lower than the energy level of the emitted UV bands.

The number of ions produced is proportional to the absorption coefficient of the molecule and the intensity of the lamp.

Lamp type	Typical applications
8.4 eV	Amines, PAH
9.6 eV	Volatile aromatics, BTEX
10.2 eV	General application
11.8 eV	Aldehydes, ketones

The UV lamp is easily removable for exchange to applications requiring different selectivity. While the lamp housing is kept relatively cool (<100 °C) the quartz ionisation chamber

containing the two measurement electrodes is the heated part of the PID (>300 °C). It is closed by a quartz or alkalifluoride window for entry of the UV light. Due to the non-destructive nature of the PID a second detector can be put in series or is mounted on top, see Fig. 2.125 with a non-selective FID.

The PID is mainly used for the analysis of aromatic pollutants, e.g. BTEX or PAH, or halogenated compounds in environmental applications. Many EPA methods e.g. 602, 502 or 503.1 cover priority pollutants in surface or drinking water. Typical for the PID is the detection of impurities air, also with mobile detectors. This is due to the fact that the energy of the UV-lamp is sufficient to ionize the majority of organic air contaminants, but insufficient to ionize the air components oxygen, nitrogen, water, argon and carbon dioxide.

PID **P**hotoi**o**nisation **D**etector

Universal and selective detector

The energy-rich radiation of a UV lamp (Fig. 2.125) ionises the substances to be analysed more or less selectively, depending on the energy content (Table 2.35); measurement of the overall ion flow.

Advantages:	The selectivity can be chosen, see Fig. 2.126 High sensitivity No gas supply is required Robust, no maintenance required Ideal for mobile and field analyses
Use:	Field analysis of volatile halogenated hydrocarbons, BTEX, polyaromatic hydrocarbons etc.
Limits:	Only for substances with low ionisation potentials (≤ 10 eV) Consult manufacturers' data

Table 2.35 Ionisation potentials of selected analytes.

Substance	Ionisation potential [eV]	Substance	Ionisation potential [eV]
Helium	24.59	Isobutyraldehyde	9.74
Argon	15.76	Propene	9.73
Nitrogen	15.58	Acetone	9.69
Hydrogen	15.43	Benzene	9.25
Methane	12.98	Methyl isothiocyanate	9.25
Ethane	11.65	N, N-Dimethylformamide	9.12
Ethylene	11.41	2-Iodobutane	9.09
2-Chlorobutane	10.65	Toluene	8.82
Acetylene	10.52	n-Butylamine	8.72
n-Hexane	10.18	o-Xylene	8.56
2-Bromobutane	9.98	Phenol	8.50
n-Butyl acetate	9.97		

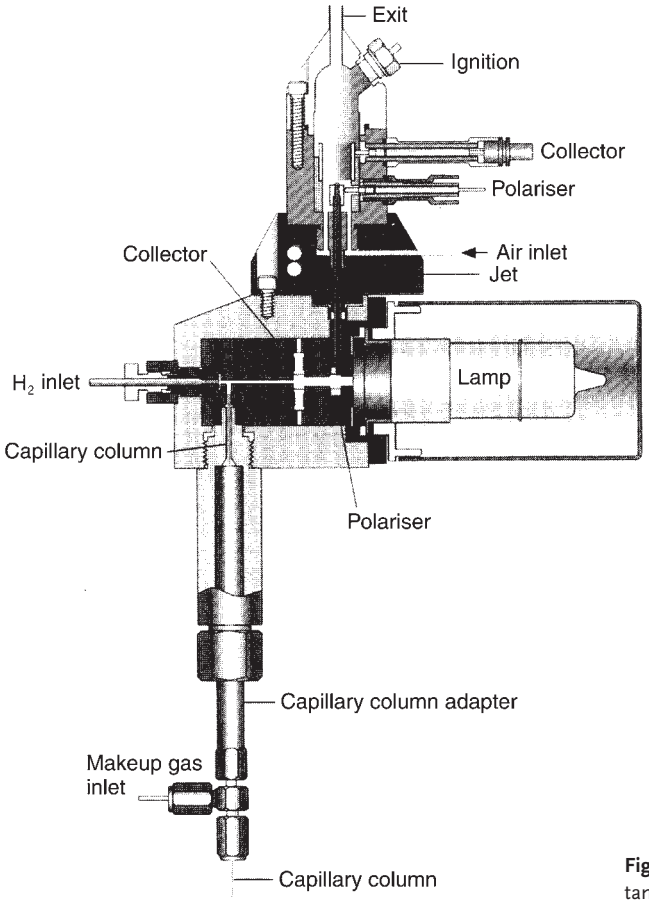


Fig. 2.125 Construction of a PID/FID tandem detector (Finnigan).

2.2.6.5 **ELCD**

In the electrolytical conductivity detector (ELCD) the eluate from the GC column passes into a Ni reactor in which all substances are completely oxidised or reduced at an elevated temperature (ca. 1000 °C).

The products dissociate in circulating water which flows through a cell where the conductivity is measured between two Pt spirals (Fig 2.127). The change in conductivity is the measurement signal. Ionic compounds can be measured without using the reactor.

The Hall detector has a comparable function to the ELCD and is a typical detector for packed or halfmil columns (0.53 mm internal diameter) because of the large volume of its measuring cell. Because of the latter significant peak tailing occurs with capillary columns. Special constructions for use with normal bore columns (< 0.32 mm internal diameter) are available with special instructions.

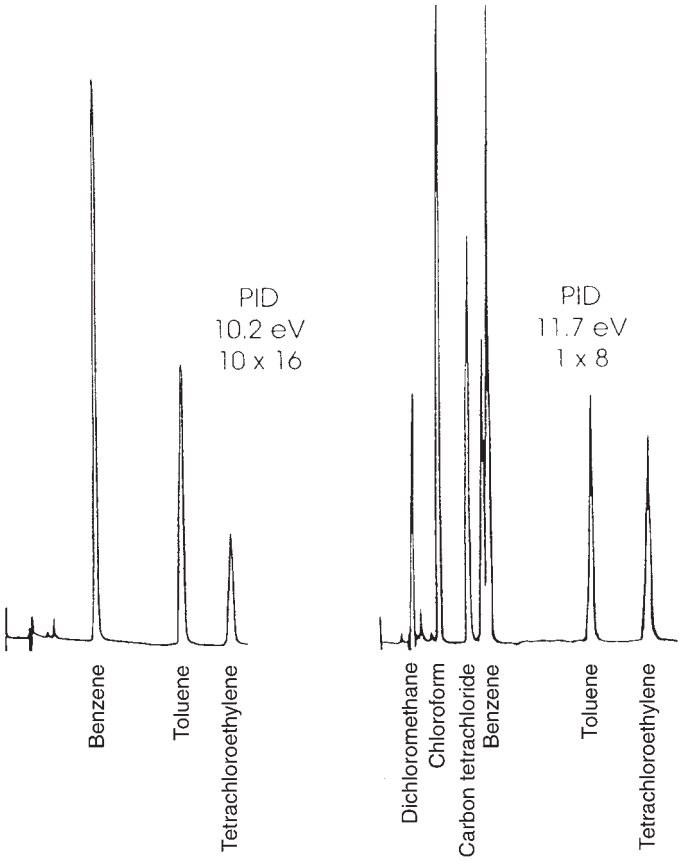


Fig. 2.126 Selectivity of the PID at 10.2 eV and 11.7 eV for a mixture of aromatic and chlorinated hydrocarbons (HNU).

ELCD **E**lectrolytical **C**onductivity **D**etector

Selective detector

Advantages:
- Can be used for capillary chromatography.
- Selectivity can be chosen for halogens, amines, nitrogen, sulfur.
- High sensitivity at high selectivity.
- Can be used for halogen detection without a source of radioactivity, therefore no authorisation necessary.
- Simple calibration, as the response is directly proportional to the number of heteroatoms in the analyte, e. g. the proportion of Cl in the molecule.

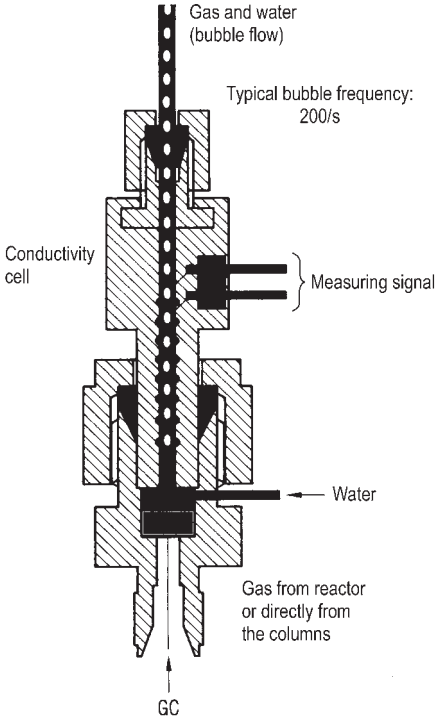


Fig. 2.127 Construction of an ELCD (patent Fraunhofer Gesellschaft, Munich).

Use:	• Environmental analysis, e. g. volatile halogenated hydrocarbons, PCBs. • Selective detection of amines, e. g. in packagings or foodstuffs. • Determination of sulfur-containing components.
Limits:	• The sensitivity in the halogen mode just reaches that of ECD, so that its use is particularly favourable in association with concentration procedures, such as purge and trap or thermodesorption.

2.2.6.6 **FPD**

Flamephotometric detectors (FPD) are used as one- or two-flame detectors. In a hydrogen-rich flame P- and S-containing radicals are in an excited transition state. On passing to the ground state a characteristic band spectrum is emitted (S: 394 nm, P: 526 nm). The flame emissions initiated by the eluting analytes (chemiluminescence) are determined using an optical filter and amplified by a photomultiplier (Fig. 2.128).

The analytical capability of the FPD can be expanded by connecting a second photomultiplier tube with a different optical filter on the same detector base, e. g. to monitor P and S containing substances in parallel.

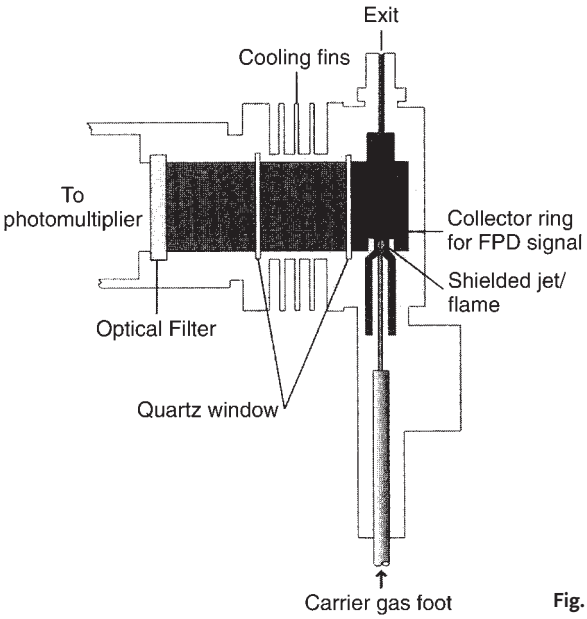


Fig. 2.128 Construction of an FPD (Finnigan).

FPD **F**lame**p**hotometric **D**etector

Selective detector

Advantages:
- In phosphorus mode, comparable selectivity to FID with high dynamics.
- High selectivity in sulfur mode.

Use:
- Mostly selective for sulfur and phosphorus compounds (e. g. plant protection agents).
- Detection of sulfur compounds in a complex matrix.

Limits:
- Adjustment of the combustion gases important for reproducibility and selectivity.
- In sulfur mode quenching effect possible because of too high a hydrocarbon matrix (double flame necessary).
- Sensitivity in sulfur mode not always sufficient for trace analysis.

2.2.6.7 **PDD**

The pulsed discharge detector (PDD) is a universal and highly sensitive non-radioactive and non-destructive detector, also known as a helium photoionisation detector. It is based on the principle of the photoionisation by radiation arising from the transition of diatomic helium to the dissociative ground state (Fig. 2.129).

The response to organic compounds is linear over five orders of magnitude with minimum detectable quantities in the low picogram range. The response to fixed gases is positive with minimum detectable quantities in the low ppb range. The performance of the detector is negatively affected by the presence of any impurities in the gas flows (carrier, discharge), therefore, the use of a high quality grade of helium (99.999% pure or better) as carrier and discharge gases is strongly recommended. Because even the highest quality carrier gas may contain some water vapour and fixed gas impurities, a helium purifier is typically included as part of the detector system.

The PDD detector consists of a quartz cell supplied from the top with ultra pure helium as discharge gas that reaches the discharge zone consisting of two of electrodes connected to a high voltage pulses generator (Pulsed Discharge Module). The eluates from the column, flowing counter to the flow of helium from the discharge zone, are ionized by photons at high energy arising from metastable Helium generated into the discharge zone. The resulting electrons are accelerated and measured as electrical signal by the collector electrode. The discharge and carrier gas flows are opposite. For this reason, it is necessary that the discharge gas flow is greater than the carrier gas flow to avoid that the eluates from the column reach the discharge zone with consequent discharge electrodes contamination.

The discharge and carrier gas flow out together from the bottom of the cell where it is possible to measure the sum of both at the outlet on the back of the instrument.

The PDD chromatograms show a great similarity to the classical FID detector and offers comparable performance without the use of a flame or combustible gases. The PDD in helium photoionisation mode is an excellent replacement for flame ionization detectors in petrochemical or refinery environments, where the flame and use of hydrogen can be problematic. In addition, when the helium discharge gas is doped with a suitable noble gas, such as argon, krypton, or xenon (depending on the desired cutoff point), the PDD can function as a specific photoionisation detector for selective determination of aliphatics, aromatics, amines, as well as other species.

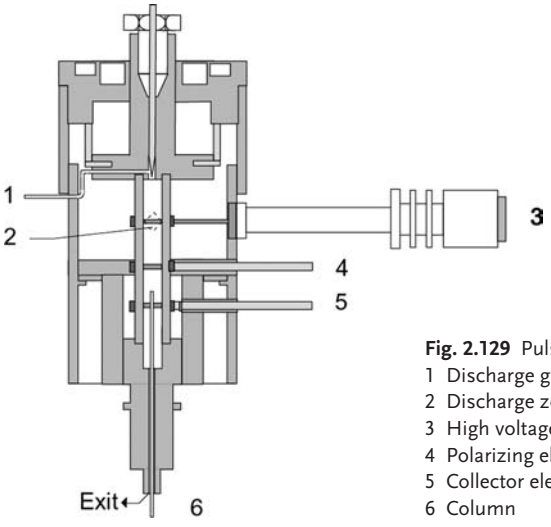


Fig. 2.129 Pulsed discharge detector (PDD) in cutaway view.
1 Discharge gas (helium) inlet
2 Discharge zone
3 High voltage discharge electrode
4 Polarizing electrode (Bias)
5 Collector electrode (signal)
6 Column

Some PDD detectors also offer an electron capture mode being selective for monitoring high electron affinity compounds such as freons, chlorinated pesticides, and other halogen compounds. For this type of compound, the minimum detectable quantity (MDQ) is at the femtogram (10–15) or picogram (10–12) level. The PDD is similar in sensitivity and response characteristics to a conventional radioactive ECD, and can be operated at temperatures up to 400 °C. For operation in this mode, He and CH_4 are introduced just upstream from the column exit.

2.2.6.8 Connection of Classical Detectors Parallel to the Mass Spectrometer

In principle there are two possibilities for operating another detector parallel to the mass spectrometer. The sample can be already divided in the injector and passed through two identical columns. The second more difficult but controllable solution is the split of the eluate at the end of the column.

The division of the sample on to two identical columns can be realised easily and carried out very reliably, but different retention times need to be considered due to the vacuum impact on the MS side. Since the capacities of the two columns are additive, the quantity of sample injected can be adjusted in order to make use of the operating range of the mass spectrometer. Two standard columns can be installed for most injectors without further adaptation being necessary. In the simplest case the connection can be made using ferrules with two holes in them. It must be ensured that there is a good seal. A better connection involves an adaptor piece with a separate screw-in joint for each column. With this construction reliable positioning of the columns in the injector is also possible. Suitable adaptors can be obtained for all common injectors.

In GC/MS the division of the flow at the end of the column requires a considerable higher effort because the direct coupling of the branch to the mass spectrometer causes reduced pressure in the split. For this reason the use of a simple Y piece is seldom possible with standard columns. The consequence would be a reversed flow through the detector connected in parallel caused by the wide vacuum impact of the MS into the column. The effect is equivalent to a leakage of air into the mass spectrometer. A split at the end of the column must therefore be carried out with high flow rates or using a makeup gas. Precise split of the eluent is possible, for example, using the glass cap cross divider (Fig. 2.130). Here the column, the transfer capillaries to the mass spectrometer and the parallel detector and a makeup gas inlet all meet. By choosing the internal diameter and the position of the end of the column in the glass cap (also known as the glass dome) the ratio of the split can easily be adjusted. The advantage of this solution lies in the free choice of column so that small internal diameters with comparatively low flow rates can also be used.

The calculation of gas flow rates through outlet splitters with fixed restrictors is desirable to have a means of estimating the rate of gas flow through a length of fixed restrictor tubing of specified dimensions. Conversely, for other applications it is necessary to estimate the length and ID of restriction tubing required to yield a desired flow rate at some specified head pressure. Within limits, the following calculation can be used for this purpose.

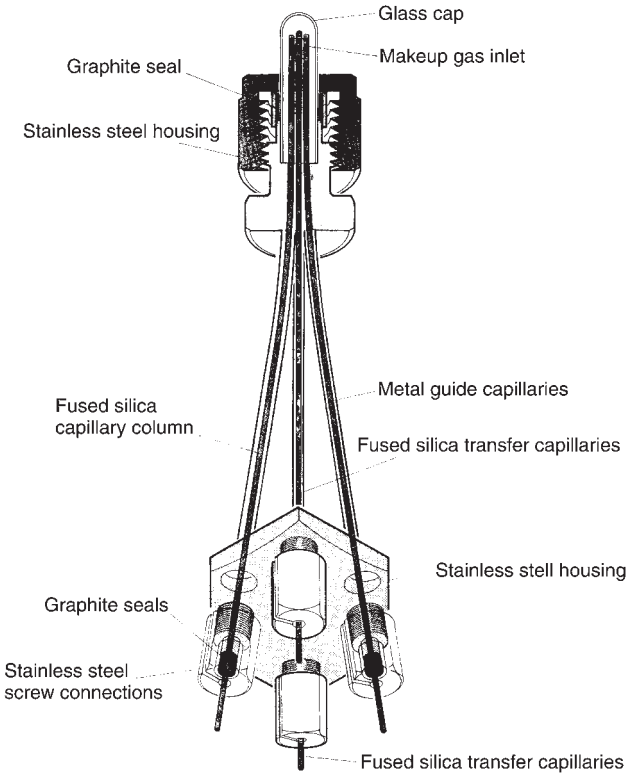


Fig. 2.130 Flow divider after Kaiser, Rieder (Seekamp Company, Werkhoff Splitter, Glass Cap Cross).

$$V = \frac{\pi \cdot P \cdot r^4 \cdot (7.5)}{l \cdot \eta}$$

with:

V = volumetric gas flow rate [cm^3/min]
P = pressure differential across the tube [dynes/cm]
= (PSI) · (68 947.6)
r = tube radius [cm]
l = tube length [cm]
η = gas viscosity [poise (dyne-seconds/cm^2)]

One of the obvious limitations to this calculation is the critical nature of the radius measurement. Since this is a fourth power term, small errors in the ID measurement can result in relatively large errors in the flow rate. This becomes more critical with smaller diameter tubes (i.e. less than 100 micron). A 100× microscope (if available) is a convenient tool for determining the exact ID of a particular length of restriction tubing.

The gas viscosity values can be determined from the graph of gas viscosity against temperature for hydrogen, helium and nitrogen (Fig. 2.131).

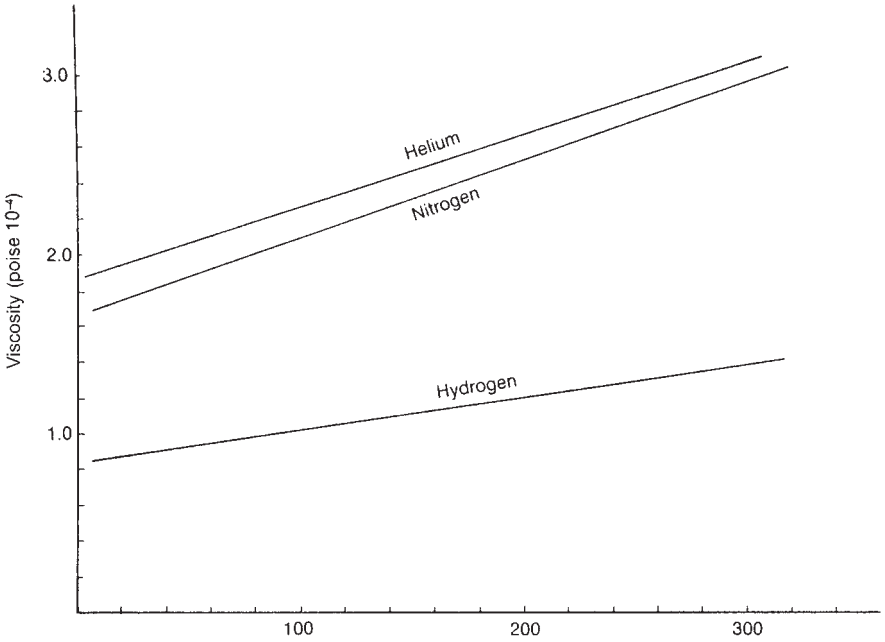


Fig. 2.131 Graphs to determine viscosity for hydrogen, helium and nitrogen.

2.3 Mass Spectrometry

"Looking back on my work in MS over the last 40 years, I believe that my major contribution has been to help convince myself, as well as other mass spectrometrists and chemists in general, that the things that happen to a molecule in the mass spectrometer are in fact chemistry, not voodoo; and that mass spectrometrists are, in fact, chemists and not shamans".

Seymour Meyerson
Research Dept., Amoco Corp.

Mass spectrometers are instruments for producing and analysing mixtures of ions for components of differing mass and for exact mass determination. The substances to be analysed are fed to an ion source and ionised. In GC/MS systems there is continuous transport of substances by the carrier gas into the ion source. Mass spectrometers basically differ in the construction of the analyser as a beam or ion storage instrument. The performance of a mass spectrometric analyser is determined by the resolving power for differentiation between masses with small differences, the mass range and the transmission required to achieve high detection sensitivity.

2.3.1 Resolving Power and Resolution in Mass Spectrometry

The resolving power of a mass spectrometer describes the smallest mass differences which can be separated by the mass analyser. Resolution and resolution power in mass spectrometry today are defined differently depending on the analyser or instrument type, without the indication of the definition employed. The new IUPAC definitions of terms used in mass spectrometry provide a precise definition.

Mass Resolution

Smallest mass difference Δm between two equal magnitude peaks so that the valley between them is a specified fraction of the peak height.

Mass Resolving Power

In a mass spectrum, the observed mass divided by the difference between two masses that can be separated: m/Δm. The procedure by which Δm was obtained and the mass at which the measurement was made shall be reported as full width at half maximum (FWHM) or 10% valley.

The mass resolving power R can be calculated by the comparison of two adjacent mass peaks m_1 and m_2 of about equal height. R is defined as

$$m_1/\Delta m$$

with m_1 the lower value of two adjacent mass peaks. Δm of the two peaks is taken at an overlay (valley) of 10% or 50%.

In the 10% valley definition, the height from the baseline to the junction point of the two peaks is 10% of the full height of the two peaks. Each peak at this point is contributing 5% to the height of the valley.

Because it is difficult to get two mass spectral peaks of equal height adjacent to one another the practical method of calculating Äm as typically done by the instruments software is using a single mass peak of a reference compound. The peak width is measured in 5% height for the 10% valley definition and at 50% peak height for the FWHM definition. The resolving power calculated using the FWHM method gives values for R that are about twice that determined by the 10% valley method. This is due to the intercept theorems calculation in a triangle in which the ratios of height to width are equal:

$$h_1/w_1 = h_2/w_2$$

$$w_2 = (h_2/h_1) \times w_1$$

and as the peak height above 5% intensity compares to the half peak height very close by a factor of 2:

$$h_2 \sim 2 \times h_1$$

following

$$w_2 \approx (2 \times h_1/h_1)\ x\ w_1$$
$$\approx 2 \times w_1$$

for
h1 = half peak height
w1 = peak width at 50 % height
h2 = peak height above 5 % to top
w2 = peak width at 5 %

following for the mass peak width that

$$\Delta m\ (5\ \%\ \text{peak height}) \approx 2 \times \Delta m\ (50\ \%\ \text{peak height})$$

consequently

$$R\ (10\ \%\ \text{valley}) \times 2 \approx R\ (\text{FWHM})$$

In practice a resolution power R 60,000 at 10 % valley compares directly to a specification of R 120,000 at FWHM.

The mass resolving power for magnetic sector instruments is historically given with a 10 % valley definition. A mass peak in high resolution magnetic sector MS is typically triangular with this analyser type. The peak width in 5 % height is of valuable diagnostic use for non optimal analyser conditions. Therefore this method provides an excellent measure for the quality of the peak shape together with the resolution power which would not be available at the half maximum condition. The typical broad peak base was initially observed in time of flight mass spectrometers and caused the 10 % valley definition would only calculate poor resolution values for this type of analyser. The more practical approach to describe the resolution power of TOF analysers is a measurement at half peak height, or as it is usually stated, at the FWHM. Both resolution power conditions compare by a factor of two as outlined above.

Constant Resolution Power Over the Mass Range

Double focusing mass spectrometers, using both electric and magnetic fields to separate ions, and time-of-flight analyzers (TOF), operate at constant mass resolving power. At a resolving power of 1000, these instruments separate ions of m/z 1000 and m/z 1001. In this example Δm is 1 and M is 1000, therefore R = 1000/1 or 1000. For practical use, this property of constant resolving power over the entire mass range means, that with a resolving power of 1000, values of 0.1 Da can be separated at m/z 100, i. e. R = 1000 = m/Δm = 100/0.1. This implies that the visible distance of mass peaks on the m/z scale decreases with increasing m/z, see Fig. 2.132.

High resolution mass spectrometers are using data system controlled split systems at the entrance and exit of the ion beam to and from the analyser for resolution adjustment. Practically, this can be done either manually or under software control. The adjustment is performed first by closing the entrance slit to get a flat top peak. Next the exit slit is closed accordingly until the intensity starts to decrease and a triangular peak is formed (see Fig. 2.133). No other adjustments in the focussing conditions are necessary to achieve the resolution setting.

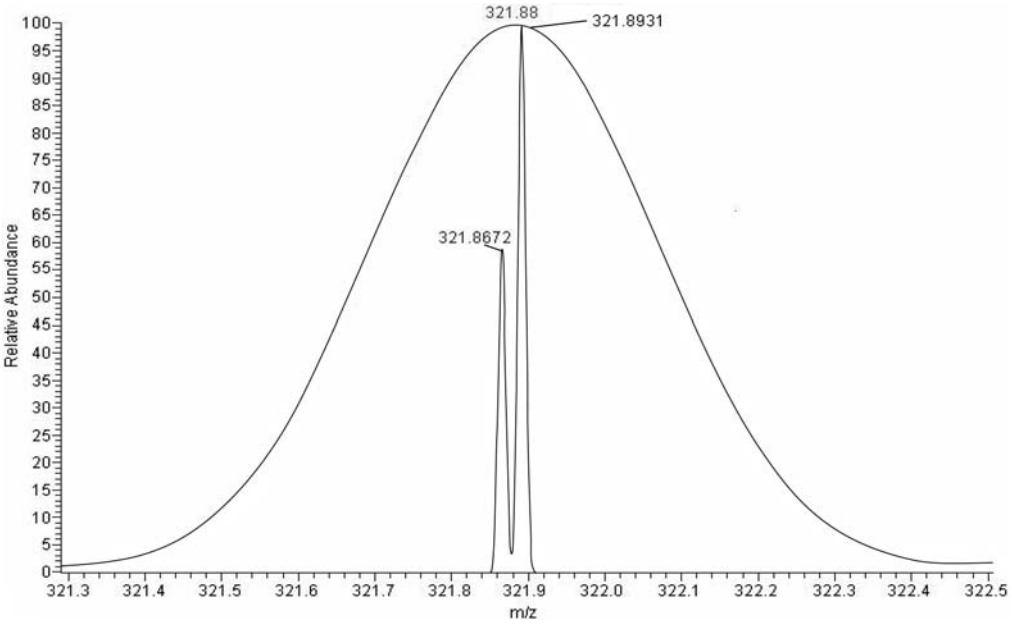


Fig. 2.132 Low and high resolved 2,3,7,8-Tetrachlorodibenzodioxin mass peak, m/z 321.8931 at R = 10,000 (10% valley). The background interference of m/z 321.8672 cannot be resolved at low resolution.

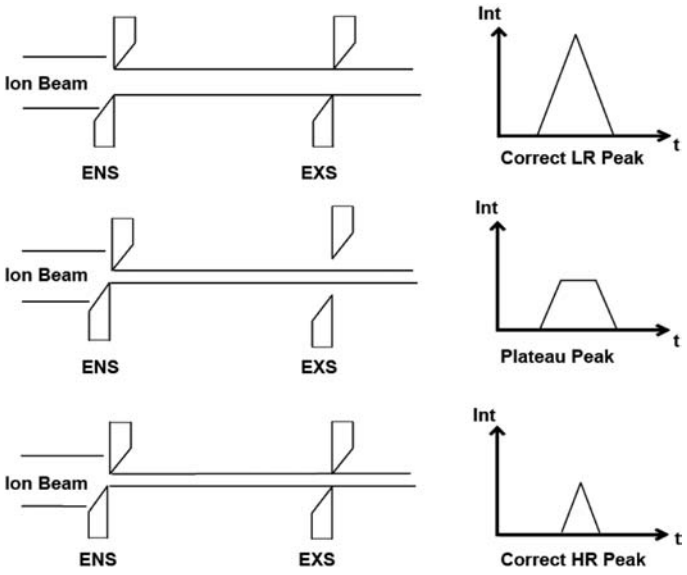


Fig. 2.133 Principle of the high resolution adjustment (ENS entrance slit, EXS exit slit).

Constant Resolution Over the Mass Range

Quadrupole and ion trap mass analysers show constant peak widths and mass differences over the entire mass range, hence both analyser types operate at constant mass resolution with increasing resolving power (see Fig. 2.145). This means, that the ability to separate ions at m/z 100 and m/z 1000 is the same. If Δm is 1 mass unit at m/z 100, the resolution at m/z 100 is 1 and the resolving power R is 100/1 or 100. If Δm is 1 at m/z 1000, the resolution at m/z 1000 is also 1, but the resolving power R at m/z 1000 is 1000/1 or 1000. Consequently, these types of analysers operate at increasing resolving power with increasing m/z value. Accordingly, the maximum resolving power, which is usually specified with a commercial quadrupole system, is dependent on the maximum specified mass range of the employed quadrupole hardware. The maximum resolution obtained with commercial quadrupole systems is typically at about 0.7 to 1.0 (FWHM) constant Da throughout the entire mass range, only hyperbolic shaped quadrupole rods of a certain length allow for narrower mass peak widths down to 0.1 Da, see Fig. 2.135, without losing ion transmission.

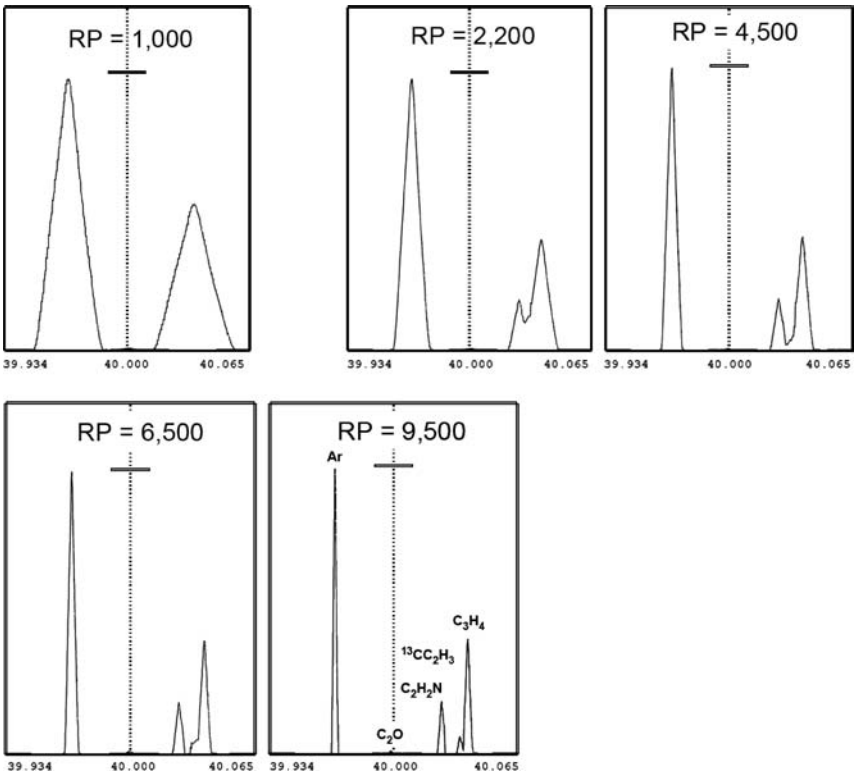


Fig. 2.134 Example of the separation of the two compounds $^{13}C_1C_2H_3$ (M = 40.0268) and C_3H_4 (M = 40.0313): R = 9500 for m = 40.0 Da; Δm = 0.0045 Da.

Ar^+	39.9624 Da
C_2O^+	39.9949 Da
$C_2H_2N^+$	40.0187 Da
$^{13}C_1C_2H_3^+$	40.0268 Da
$C_3H_4^+$	40.0313 Da

"High Resolution" with Quadrupole Analysers

Already in 1968 a publication by Dawson and Whetten dealt with the resolution capabilities of quadrupoles (Dawson 1968). The theoretical investigation covered round rods as well as hyperbolic rods and indicated the higher resolution potential of quadrupole analysers using hyperbolic rods (see Fig. 2.134). Especially with precisely machined hyperbolic rods sufficient ion transmission is achieved even at higher resolution power, making this technology especially useful for target compound analysis.

The maximum resolution power of quadrupole analysers depends on the number of cycles an ion is exposed to the electromagnetic fields inside of the quadrupole assembly, hence of the length of the rods.

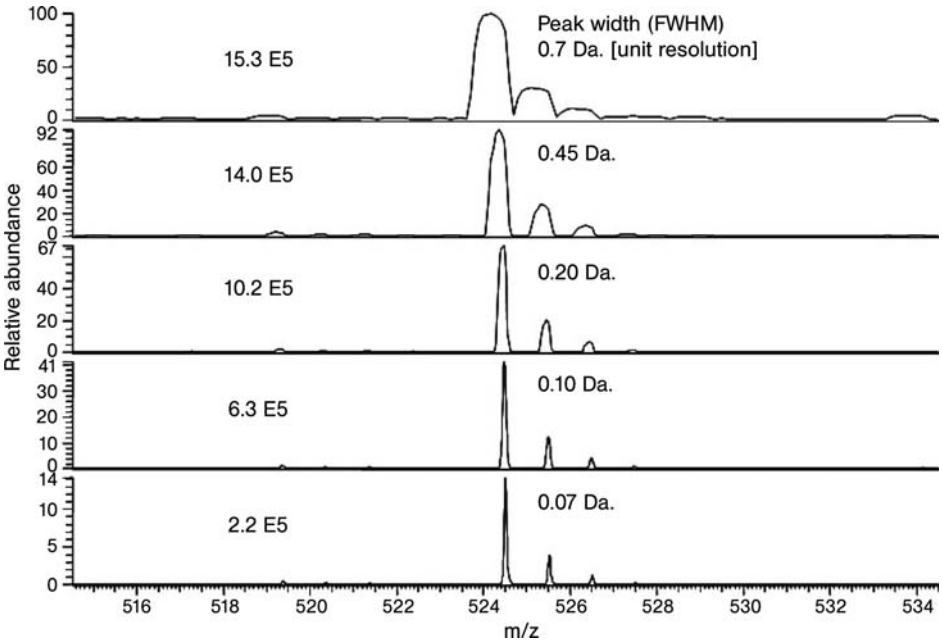


Fig. 2.135 Increased peak resolution of hyperbolic quadrupoles see Fig 2.136. Note the decrease in peak hight of factor 2 at 0.1 Da peak width compared to unit resolution (courtesy Thermo Fisher Scientific).

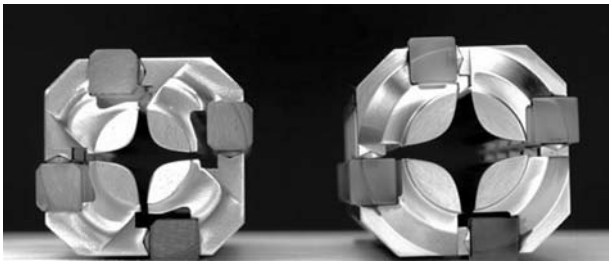


Fig. 2.136 Low resolution (left, TSQ 7000) and higher resolution (right, TSQ Quantum) quadrupole analysers (courtesy Thermo Fisher Scientific).

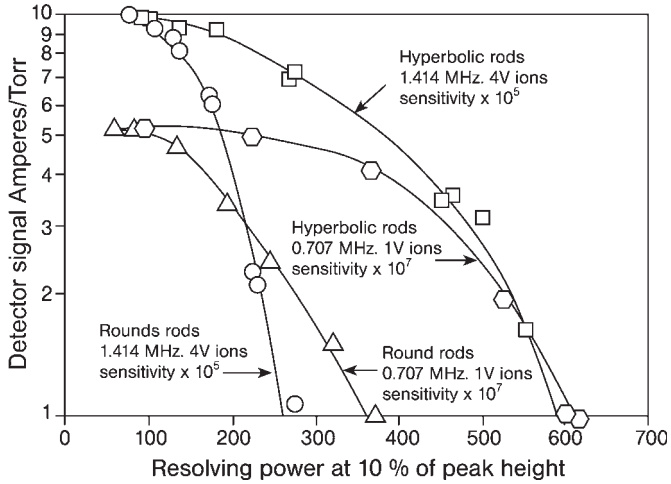


Fig. 2.137 Ion transmission of round and hyperbolic quadrupole systems (resolving power ag. ion transmission), first documented investigation by Brubaker 1968 (two curves left: round rods; two curves right: hyperbolic rods).

A recent development for "higher resolution" quadrupole analysers has been published and commercialized for a selected ion monitoring technique in MS/MS named „H-SRM", high resolution selected reaction monitoring. Hyperbolic quadrupole rods of a true hyperbolic pole face, high-precision 4-section design with a rod length of approx. 25 cm length are employed in triple quadrupole GC and LC/MS/MS systems for the selective quantification of target compounds. The increased field radius of 6 mm provides a significantly increased ion transmission for trace analysis (HyperQuad technology, patented Thermo Fisher Scientific).

By operating the first quadrupole in higher resolution mode the selectivity for MS/MS analysis of a parent target ion within a complex matrix is significantly increased. Higher S/N values for the product ion peak lead to significantly lowered method detection limits (MDL). Using the hyperbolic quadrupole rods the reduction from 0.7 Da peak width to 0.1 Da reduces overall signal intensity only by 30 % but S/N increases by a factor better than 10. In many cases at low levels it even first allows to detect the target peak well above the background. By using round rods the drop in signal intensity would be close to 100 %, and would lose all sample signal. Hence round rods cannot be applied for a higher resolved and higher selective detection mode.

To eliminate the uncertainty associated with possible mass drifts in H-SRM acquisitions, algorithms that correct the calibration table for mass position and peak width have been developed (Jemal 2003, Liu 2006). Data are obtained from H-SRM quantitation at a peak width setting of 0.1 Da at FWHM for the precursor ion. Deviations detected for mass position or peak width in internal standards are used to adjust the calibration. The data is then used to determine the deviations if any for all target calibrant ions. The calibration correction method is submitted and executed within the sequence of data acquisition. A scan-by-scan calibration correction method (CCM) has been applied to accommodate a variety of factors that influence precursor ion mass drift and ensure H-SRM performance eliminating signal

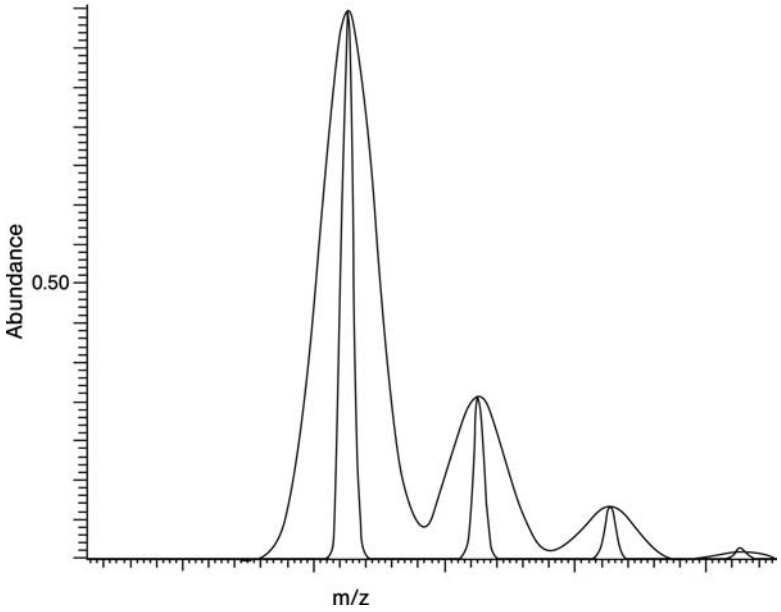


Fig. 2.138 Comparison of peak widths at 0.7 u and 0.1 u at FWHP with resolution power of R 10000 at m/z 1000.

roll-off. With real life samples exhibiting strong matrix backgrounds a significant increase in S/N is achieved by using the H-SRM technique.

Analytical benefits of highly resolving quadrupole systems in GC/MS/MS analysis are the separation of ions with the same nominal m/z value for inceased selectivity, in particular the high resolution precursor ion selection for MS/MS mode.

Table 2.36 Increase in ion transmission for low and higher resolution quadrupoles at 0.2 da mass peak width FWHM, 100% equals 0.7 FWHM (Jemal 2003).

Quadrupole type	SIM	SRM
Higher resolution	70%	53%
Low resolution	11%	0.15%

Mass Analysers for Accurate Mass Measurement

Mass analyser	Maximum resolution power (FWHM)	Mass accuracy	Dynamic range	Inlet methods	MS/MS capability
Time-of-flight	5000–15 000	5 ppm	10^2–10^3	GC/MS, LC/MS	in hybrid systems
Hyperbolic triple quadrupole	10 000	5 ppm	10^4–10^6	GC/MS, LC/MS	typical application
Magnetic sector	120 000–150 000	2 ppm	10^5–10^6	GC/MS, LC/MS, Direct Inlets	limited
FT orbitrap	500 000	<1 ppm	10^3–10^4	LC/MS	extensive use
FT ion cyclotron resonance	2 000 000	<0,2 ppm	10^3–10^4	LC/MS	extensive use

2.3.1.1 High Resolution

The flight paths of ions with different m/z values follow a different course as a result of the magnetic and electric field in a magnetic sector instrument (Fig. 2.139). Splitting systems can mask ion beams. Spectra can be recorded by continually changing the parameters of the instrument, e. g. the acceleration voltage. The width of the ion beam is determined by the source slit at the ion source. The beams must not overlap, or only to a very small extent so that ions of different masses can be registered consecutively (Fig. 2.140).

The resolution A of neighbouring signals (Fig. 2.141) for magnetic sector instruments is calculated according to

$$A = \frac{m}{\Delta m} \tag{22}$$

with
m = mass and
Δm = distance between neighbouring masses

According to this formula the resolution is dimensionless.

By determination of the exact mass the empirical formula of a molecular ion (and of fragment ions) can be determined if the precision of the measurement is high enough (Fig. 2.142). The more the mass increases, the more interference can arise.

A selection of the large number of realistic chemical formulae for the mass 310 ($C_{22}H_{46}$, M 310.3599) is shown in Fig. 2.143. To differentiate between the individual signals a precision in the mass determination of 2 ppm would be necessary.

It is characteristic for a mass spectrum produced by a magnetic sector instrument that the resolution A is constant over the whole mass range. According to the formula for the resolution, the distance between the mass signals, Δm, for water (m = 17/18) is much greater than for signals in the upper mass range. The maximum resolution that can be achieved characterises the slit system and the quality of the ion optics of the magnetic sector instrument.

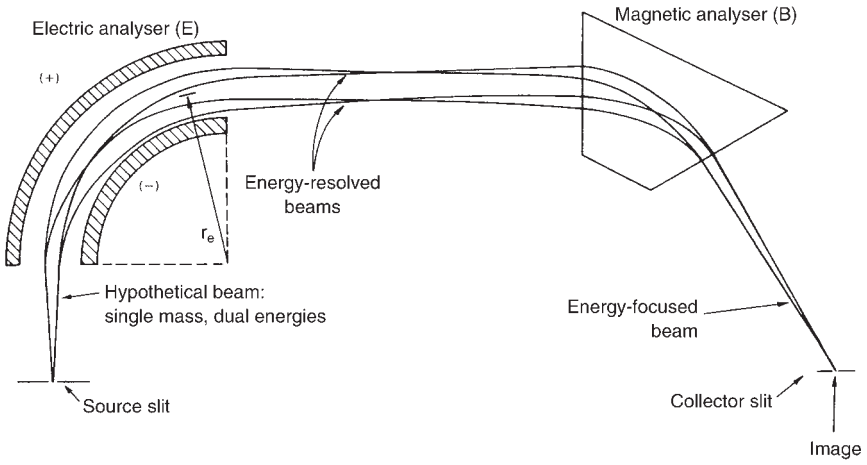


Fig. 2.139 Principle of the double focusing magnetic sector mass spectrometer (Nier Johnson geometry: EB).

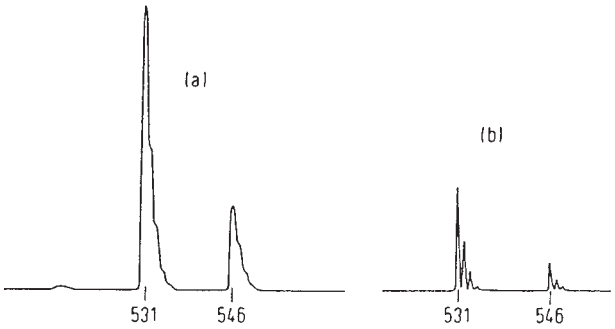


Fig. 2.140 Section of a poorly resolved spectrum (a) and of a better, more highly resolved one (b) which, however, results in a lowering of intensity of the signals (after Budzikiewicz 1980).

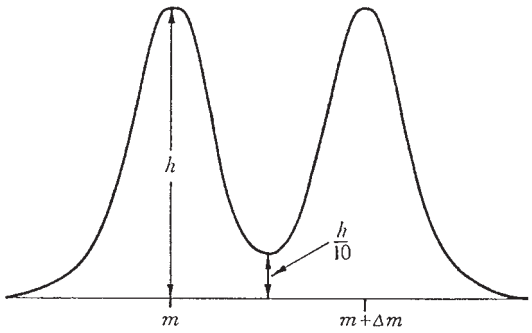


Fig. 2.141 General resolution conditions for two mass signals – 10% trough definition (after Budzikiewicz 1980).

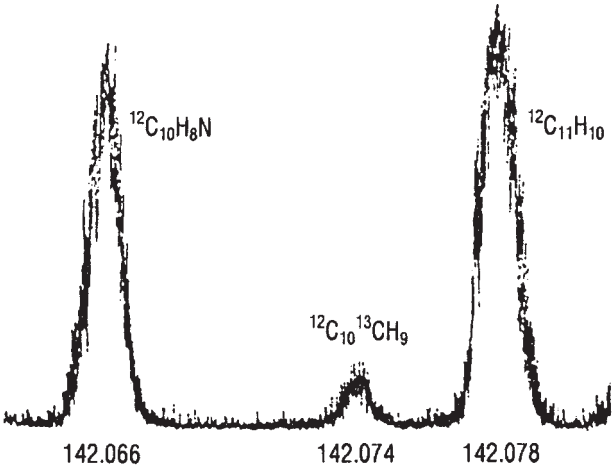


Fig. 2.142 Diagram showing mass signals in a high resolution spectrum at $A = 61\,000$ (Finnigan MAT 90).

Example of the Calculation of the Necessary Minimum Resolution

What mass spectroscopic resolution is required to obtain the signals of carbon monoxide (CO), nitrogen (N_2) and ethylene (C_2H_4) which are passed through suitable tubing into the ion source of a mass spectrometer?

Substance	Nominal mass	Exact mass
CO	m/z 28	m/z 27.994910
C_2H_4	m/z 28	m/z 28.006148
N_2	m/z 28	m/z 28.031296

For MS separation of CO and N_2, which appear at the same time in accordance with the formula given above, a resolving power of at least 2500 is necessary (see also Fig. 2.134).
All MS systems with low resolution need preliminary GC separation of the components (CO, C_2H_4 and N_2 would then arrive one after the other in the ion source of an MS). This is the case for all ion trap and quadrupole instruments.

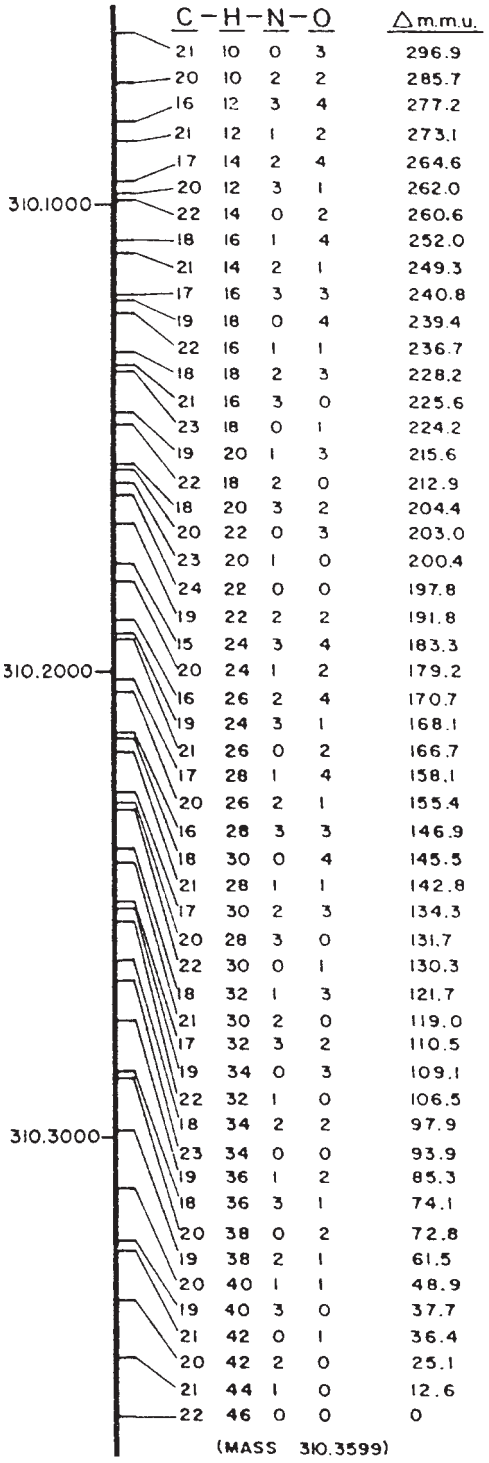


Fig. 2.143 Exact masses of chemically realistic empirical formulae consisting of C, H, N (≤ 3), O (≤ 5) given in deviations (Δ mu) from the molecular mass of $C_{22}H_{46}$ m/z 310 (after McLafferty 1993).

2.3.1.2 Unit Mass Resolution

A mass spectrum obtained with an ion trap or quadrupole mass spectrometer (Figs. 2.144 and 2.147) shows another characteristic: the distance between two mass signals and their signal widths are constant over the whole mass range! How far the instrument can scan to higher masses is therefore unimportant. The quadrupole/ion trap peaks of water (m/z = 17/18) are the same width and have the same separation as the masses in the upper mass range (Fig. 2.145).

Since the distance between two signals, Δm, is constant over the whole mass range for these instruments, the formula $A = m/\Delta m$ would have the result that the resolution A would

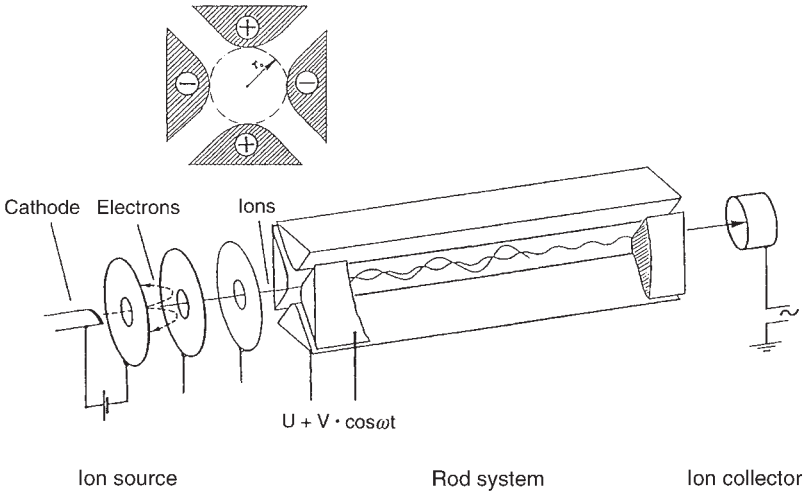


Fig. 2.144 Diagram of a quadrupole mass spectrometer.

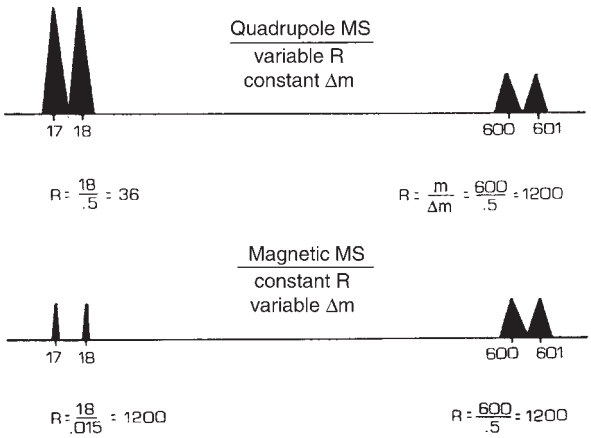


Fig. 2.145 Comparison of the principles of the mass spectra obtained from quadrupole/ion trap and magnetic sector analysers.

be directly proportional to the highest possible mass m (Fig. 2.146). At a peak separation of one mass unit the resolving power in the lower mass range would be small using the formula for the resolution (e.g. for water $A \approx 18$) and in the upper mass range higher (e.g. $A \approx 614$ for FC43).

The following conclusions may be drawn from these facts concerning the assessment of quadrupole and ion trap instruments:

1. The formula $A = m/\Delta m$ does not give any meaningful figures for quadrupole and ion trap instruments and therefore cannot be used in every case.
2. The visible optical resolution (on the screen) is the same in the upper and lower mass ranges. It can easily be seen that the signals corresponding to whole numbers are well separated. This corresponds in practice to the maximum possible resolution for quadrupole and ion trap instruments.
3. This resolving power, which is constant over the whole mass range, is set up by the manufacturer in the electronics of the instrument and is the same for all types and manufacturers. The peak width is chosen in such a way that the distance between two neighbouring nominal mass signals corresponds to one mass unit (1 u = 1000 mu). High resolution, as in the magnetic sector instrument, is not possible for quadrupole and ion trap instruments within the framework of the scan technique used. Because all quadrupole and ion trap instruments provide the same quality of nominal mass peak resolution they are said to work at unit mass resolution (see Fig. 2.146).

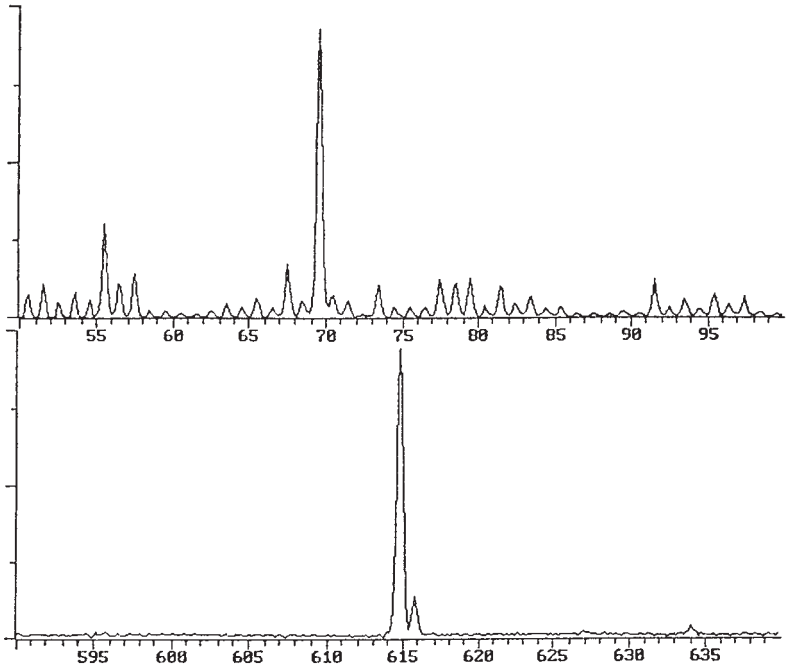


Fig. 2.146 Diagram of the mass signals obtained using an ion trap analyser in the lower and upper mass ranges (calibration standard FC43).

4. The mass range of quadrupole and ion trap instruments varies but still has no effect on the resolving power.

Frequently the terms mass range and unit mass resolution are mixed up when giving a quality criterion for a mass range above 1000 u for a quadrupole instrument (it is not obvious that a mass range up to 4000 u, for example, is always accompanied by unit mass resolution). The effective attainable resolution for a real measurable signal of a reference compound is accurate and meaningful.

The different types of analyser for quadrupole rod and quadrupole ion trap instruments function on the same mathematical basis (Paul/Steinwedel 1953 and patent specification 1956) and therefore show the same resolution properties (Fig. 2.147).

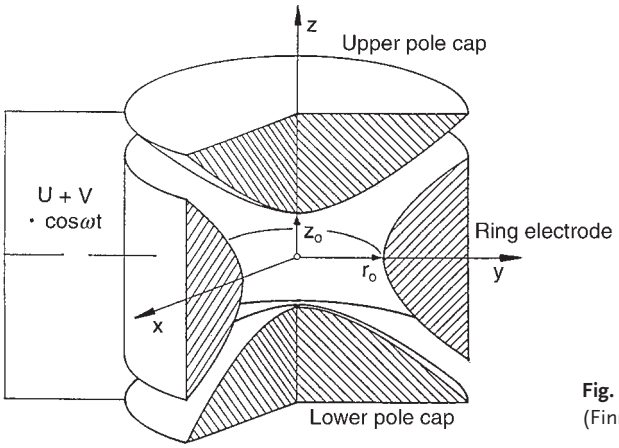


Fig. 2.147 Diagram of an ion trap analyser (Finnigan).

The display of line spectra on the screen must be considered completely separately from the resolution of the analyser. By definition a mass peak with unit mass resolution has a base width of one mass unit or 1000 mu. On the other hand the position of the top of the mass peak (centroid) can be calculated exactly. Data sometimes given with one or several digits of a mass unit gives the false impression of a resolution higher than unit mass resolution. Components appearing at the same time at the ion source with signals of the same nominal mass, which can naturally occur in GC/MS (as a result of co-eluates, the matrix, column bleed etc) cannot be separated at unit mass resolving power of the quadrupole and ion trap analysers (see Section 2.3.1.2). The position of the centroid can therefore not be used for any sensible evaluation. *In no case is this the basis for the calculation of a possible empirical formula*! Depending on the manufacturer, the labelling of the spectra can be found with pure nominal masses to several decimal places and can usually be altered by the user.

It has been observed with many benchtop instruments that the unavoidable contamination of ion volumes and lenses, the changes in the carrier gas flow as a result of temperature programs, and temperature drift of the ion source during the operation cause the position of the peak maximum to shift by several tenths of a mass during a short time. Adequate mass stability is therefore only given for the requirement of unit mass resolution. Each MS data system uses whole number mass spectra (nominal masses) for internal processing by back-

ground subtraction and library searching. The spectra of all MS libraries also only contain whole mass numbers!

2.3.1.3 High and Low Resolution in the Case of Dioxin Analysis

Can dioxin analysis be carried out with quadrupole and ion trap instruments?

Yes! But with what sort of quality? *The main problem is the resolving power.* The sensitivity of quadrupole and ion trap instruments is adequate for many applications. However, the selectivity is not.

It is possible to make mistakes on using selected ion monitoring techniques (SIM). These types of mistakes are smaller for full scan data collection with ion traps and on using MS/MS techniques, as usually a complete spectrum and a product ion spectrum are available for further confirmation. Errors caused by false positive peak detection and poor detection limits as a result of matrix overlap, which cannot be totally excluded, generally limit the use of low resolution mass spectrometers. For screening tests positive results should be followed by high resolution GC/MS for confirmation.

The high resolution and mass precision of the magnetic sector instrument allows the accurate mass to be recorded (e.g. 2,3,7,8-TCDD at m/z 321.8937 instead of a nominal mass of 322) and thus masks the known interference effects (Table 2.37). As a result very high selectivity with very low detection limits of < 10 fg are achieved, which gives the necessary assurance for making decisions with serious implications (Fig. 2.148).

Table 2.37 Possible interference with the masses m/z 319.8965 and 321.8936 relevant for 2,3,7,8-TCDD and the minimum analyser resolution required for separation.

Compound	Formula	m/z[a)]	Resolution needed for separation
Tetrachlorobenzyltoluene	$C_{12}H_8Cl_4$	319.9508	5900
Nonachlorobiphenyl	$C_{12}HCl_9$	321.8491	7300
Pentachlorobiphenylene	$C_{12}H_3Cl_5$	321.8677	12500
Heptachlorobiphenyl	$C_{12}H_3Cl_7$	321.8678	13000
Hydroxytetrachlorodibenzofuran	$C_{12}H_4O_2Cl_4$	321.8936	cannot be resolved
DDE	$C_{14}H_9Cl_5$	321.9292	9100
DDT	$C_{14}H_9Cl_5$	321.9292	9100
Tetrachloromethoxybiphenyl	$C_{13}H_8OCl_4$	321.9299	8900

a) of the interfering ion

High resolution is therefore also required to verify positive screening results in dioxin analysis (see also Section 4.25). A comparison of the two spectrometric methods of the lower and higher resolution SIM techniques is shown in Fig. 2.149 for the mass traces in the detection of TCDF traces.

The resolution comparison with respect to different analyser technologies used in dioxin analysis is shown in Figs. 2.150 to 2.152 with simulated ideal mass spectra of the TCDD iso-

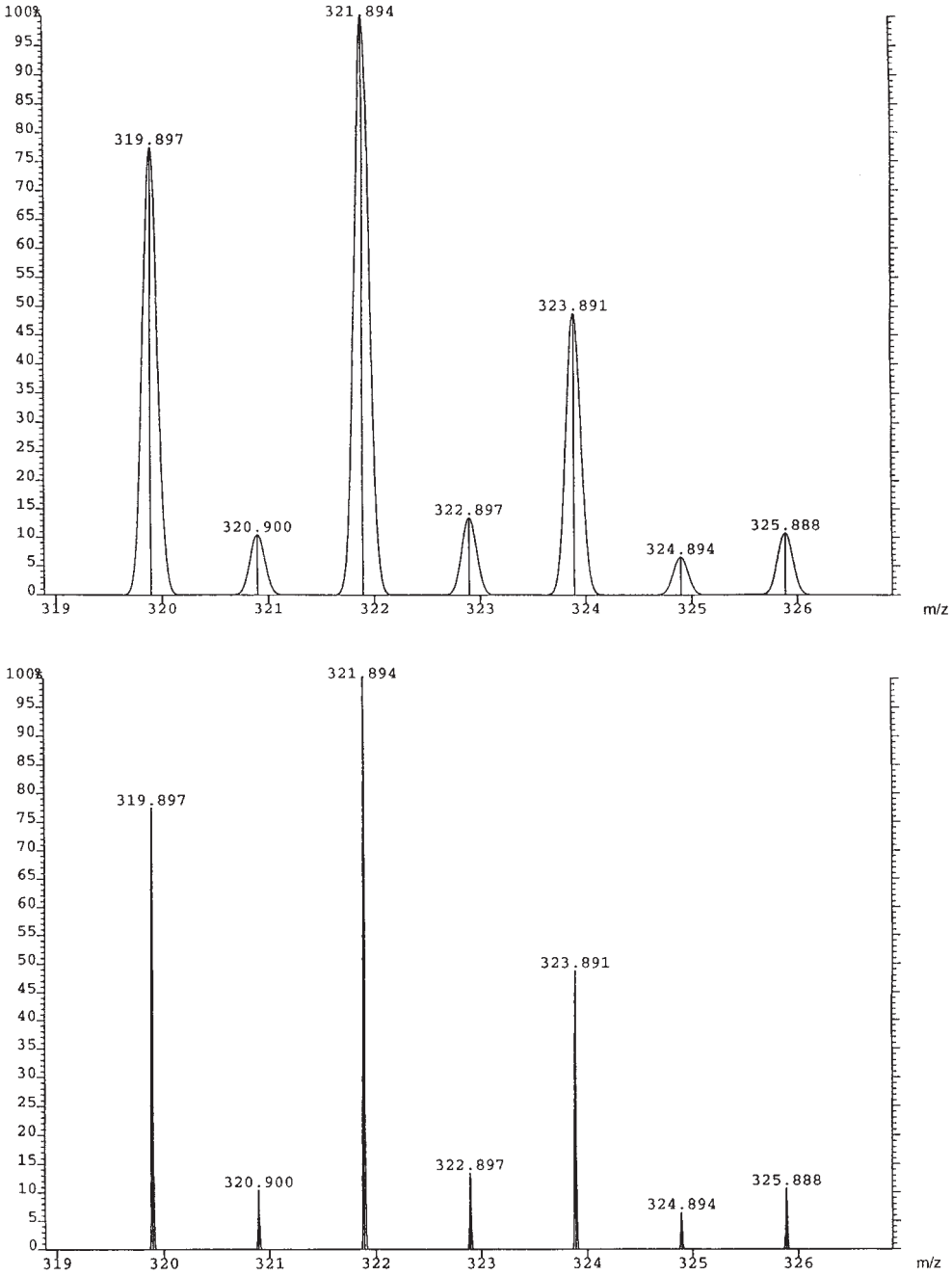


Fig. 2.148 The isotope pattern for 2,3,7,8-TCDD using a double focusing magnetic sector instrument with peak widths for resolutions of 1000 (above) and 10000 (below) (after Fürst).

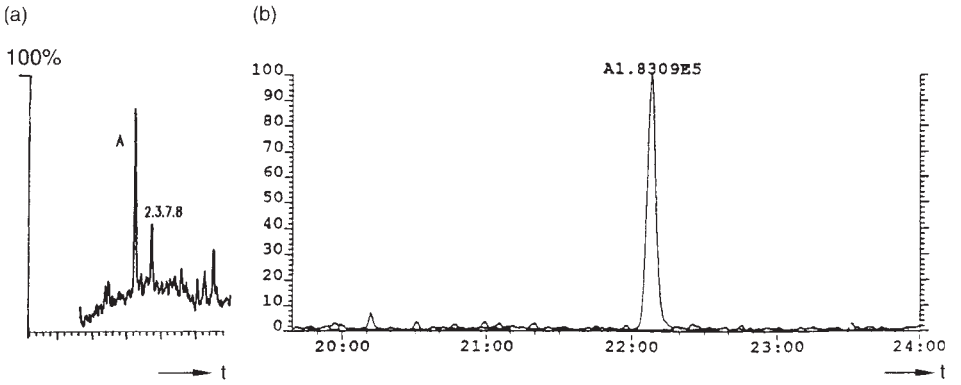


Fig. 2.149 Comparison of 2,3,7,8-TCDF traces in analyses using a low resolution quadrupole GC/MS system (a = m/z 319.9) and a high resolution GC/MS system (b = m/z 319.8965). Both chromatograms were run on an identical human milk sample. The component marked with A in the quadrupole chromatogram is an interfering component (after Fürst).

tope pattern in the molecular peak region. In practice, for real sample measurements a significant background contribution has to be kept in mind. The characteristic of the quadrupole analyser for unit mass resolution (Fig. 2.150) shows the mass peaks of the isotope pattern with the base peak width of 1 Da. The highly resolving quadrupole technology is able to reduce the peak width down to 0.4 Da with a remarkable good mass separation (Fig. 2.150). Time-of-flight analysers show very tall peaks but typically with a broad peak base. For that reason the TOF resolution power is measured at the half peak height. At the very good TOF resolution of 7,000 FPHW (frequently named hrTOF) the peak base still is wide over almost one full mass window (Fig. 2.151). Only the high resolution magnetic sector instruments provide with distinct difference the required resolution power of 10,000 for ultimate selectivity (Fig. 2.152).

2.3.2 Time-of-Flight Analyser

The concept of time-of-flight (TOF) mass spectrometry was proposed already in 1946 by William E. Stephens of the University of Pennsylvania (Borman 1998). In a TOF analyser, ions are separated by differences in their velocities as they fly down a field free drift region towards a collector in the order of their increasing mass-to-charge ratio (see Fig. 2.153). With that principle, TOF MS is probably the simplest method of mass measurement. TOF MS is fast, offers a high duty cycle with the parallel detection of ions and is theoretically unlimited in mass range. Due to the speed of detection and its inherent sensitivity by the parallel detection of a complete ion packet, it is specially suited for a full scan chromatographic detection, and, it is capable of running fast GC because of its high sampling rates of up to more than hundred spectra per second. TOF MS is also widely used for the determination of large biomolecules (electrospray ionisation, ESI; matrix assisted laser desorption, MALDI), among many other low and high molecular weight applications. TOF and Quadrupole-TOF analysers are inherently low resolution instruments. With current technologies the resolution power achieved is up to about 15,000 FWHM (Webb 2004).

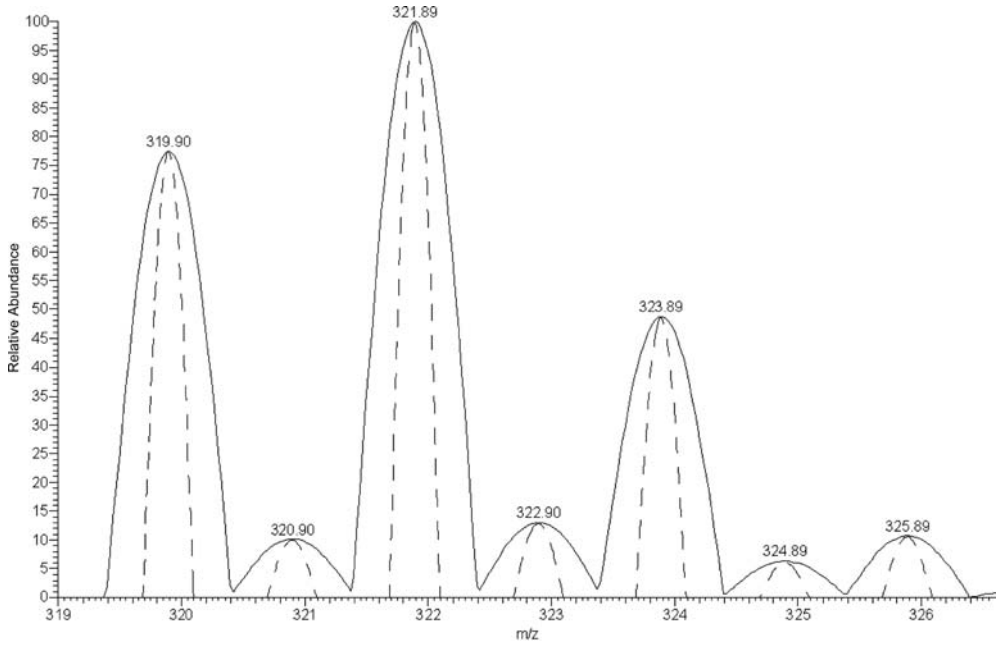


Fig. 2.150 Quadrupole mass resolution at 1 Da and 0.4 Da in H-SRM mode (TCDD isotope pattern).

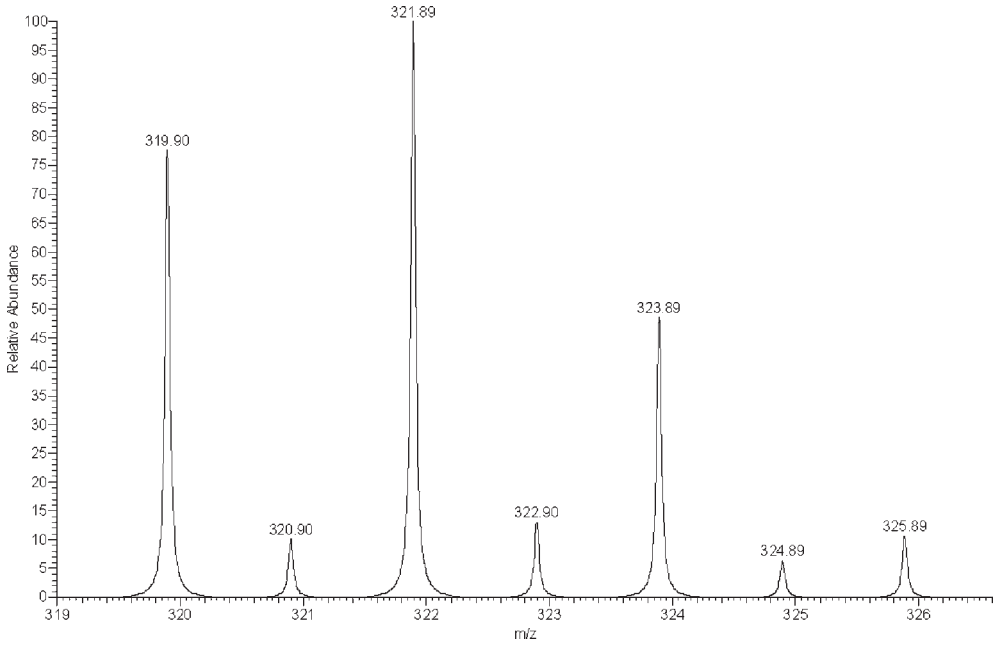


Fig. 2.151 TOF mass resolution at 7000 FPHW (TCDD isotope pattern)

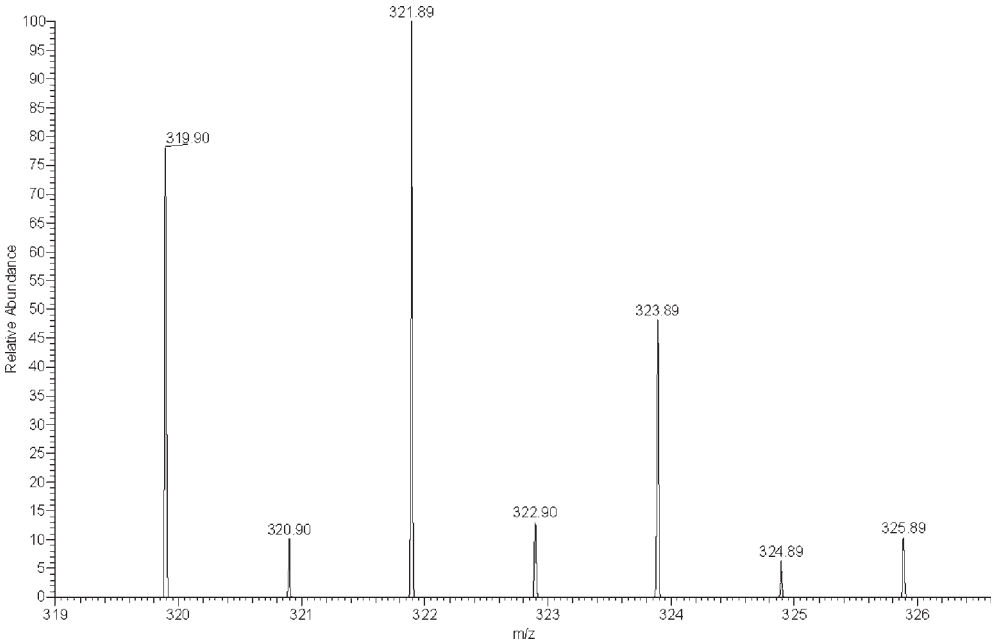


Fig. 2.152 High resolution magnetic sector analyser, mass resolution at 10,000 at 10% valley (TCDD isotope pattern).

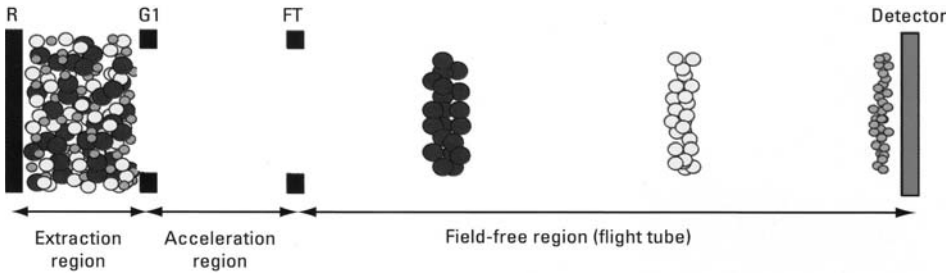


Fig. 2.153 Time-of-flight operating principle (McClenathan 2004) (reprinted with permission from the American Chemical Society)

TOF instruments were first designed and constructed starting in the late 1940s. Key advances were made by William C. Wiley and I.H. McLaren of the Bendix Corp., Detroit, USA, the first company to commercialize TOF mass spectrometers. According to pharmacology professor Robert J. Cotter of the Johns Hopkins University School of Medicine, Wiley and McLaren "devised a time-lag focusing scheme that improved mass resolution by simultaneously correcting for the initial spatial and kinetic energy distributions of the ions. Mass resolution was also greatly improved by the 1974 invention by Boris A. Mamyrin of the Physical-Technical Institute, Leningrad, Soviet Union of the reflectron, which corrects for the effects of the kinetic energy distribution of the ions." (Birmingham 2005).

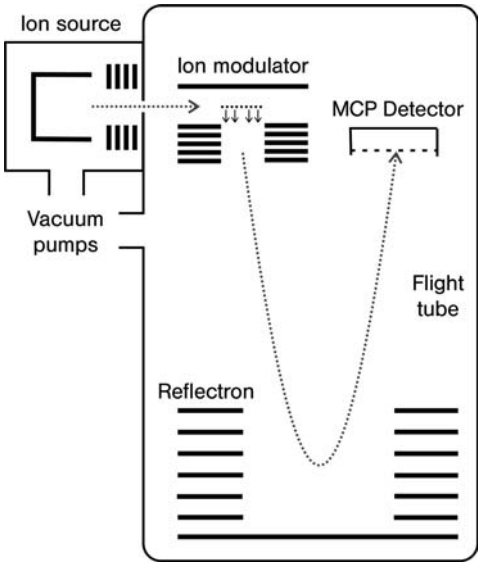


Fig. 2.154 TOF mass spectrometer with GC ion source, orthogonal ion modulation into the TOF analyser and MCP detection, dotted lines indicate the ion flight path.

The schematics of a TOF analyser is shown in Fig. 2.154. Ions are introduced as pulsed packets either directly from the GC or LC ion source of the instrument, or with hyphenated instruments, from a previous analyser (e.g. quadrupole-time-of-flight hybrid, Q-TOF). Ions from the ion source are accelerated and focused into a parallel beam that continuously enters the ion modulator region of the TOF analyser. Initially the modulator region is fieldfree and ions continue to move in their original direction. A pulsed electric field is applied at a frequency of several kilohertz (kHz) across a modulator gap of several mm width, pushing ions in an orthogonal direction (to their initial movement) into an accelerating section of several keV. The modulator pulses serve as trigger for recording spectra at the detector.

All ions in the pulsed ion packet receive the same initial kinetic energy, $E_{kin} = {}^1/_2\, mv^2$. Lighter ions travel faster and reach the detector earlier. As the ions enter and move down the fieldfree drift zone, they are separated by their mass to charge ratio in time. Commercial GC and LC/MS TOF instruments are usually equipped with a reflectron. This "ion mirror" is focusing the kinetic energy distribution, originating from the small initial velocity differences due to different spatial start positions of the ions when getting pulsed from the modulator into the drift tube (The mass resolution in general in TOF-MS is limited by the initial spatial and velocity spreads). The ratio of velocities components in the two orthogonal directions of movement from source and modulator is selected such that ions are directed to the centre of the ion mirror and get focused on a horizontal multichannel plate detector plane (MCP). The reflectron typically consists of a series of lenses with increasing potential pushing the ions back in a slight angle into the direction of the detector. Ions of higher velocity (energy) penetrate deeper into the mirror. Hence, the ion packet is getting focused in space and in time for increased mass resolution. All ions reaching the detector are recorded explaining the inherent high sensitivity in full scan mode of TOF analysers. Duty cycles reached with orthogonal ion acceleration instruments vary in a range of 5% to 30% depending on the mass range and limited by the slowest (heaviest) ion moving across the TOF mass analyser, covering the range between quadrupole and ion trap analysers.

The TOF mass separation is characterized by the following basic equation:

$$m/z = 2e \cdot E \cdot s\,(t/d)^2$$

with

m/z	mass-to-charge ratio
e	elementary charge
E	extraction pulse potential
s	length of ion acceleration, over which E is effective
t	measured flight time of an ion, triggered by pulse E
d	length of field free drift zone

The fast data acquisition rates make the TOF analyser the ideal mass detector for fast GC and comprehensive GC×GC. Despite of the described inherent sensitivity of TOF MS in full scan analysis some fundamental trade-offs in terms of response and spectral quality have to be considered when setting up TOF experiments. In contrast to the expectation that higher acquisition rates strongly support the deconvolution of coeluting compounds, the average ion abundance is dropping with increased scan rates and limiting the useful dynamic range. With the increase of the scan rate to 50 scans/s the ion abundance significantly drops to about 10% of a 5 scans/s intensity (see Fig. 2.153). Minor components may be left unrecognised. Hence, typical acquisition rates with current GC/TOFMS instrumentation are 20–50 Hz, with up to 100 Hz in fast GC or GC×GC applications.

Also the spectral quality has to be taken in account when setting up GC/TOF-MS methods for deconvolution experiments. Spectral skewing, as known from slow scanning quadrupole or sector mass spectrometers due to the increasing or decreasing substance intensity in the transient GC peak, does not occur in the ion package detection of TOF instruments. But, TOF spectral quality is limited by high data acquisition rates. There is still the general rule valid that increased measurement times support the spectrum dynamic range and hence its

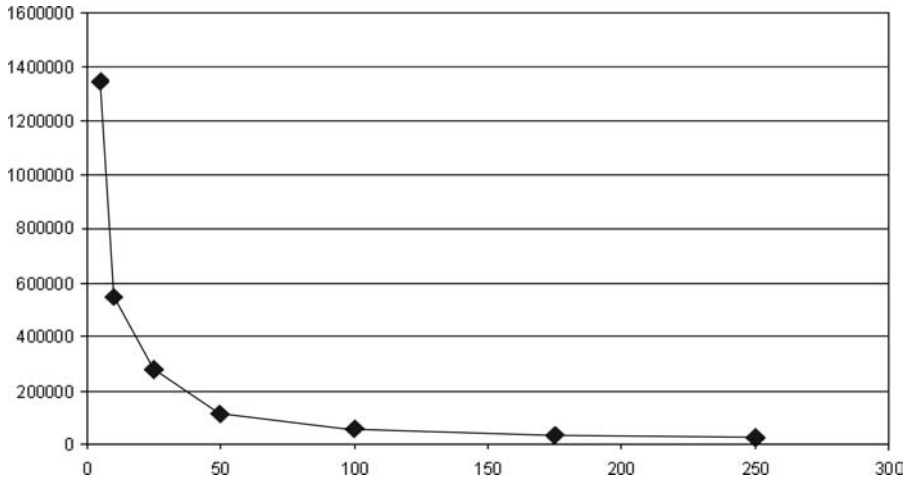


Fig. 2.155 Ion abundance dependence from the scan rate in TOF MS (counts vs. scan rate in spectra/s) (Meruva 2000).

quality. The effect can be demonstrated when comparing the acquired spectra at increasing scan rate against the reference spectra of the NIST library. The fit values of similarity match drop significantly with scan rates above 25 resp. 50 scan/s, see Table 2.38 for an experiment on two common pesticides.

Table 2.38 Average similarity match values depending on the data acquisition rate of TOF mass spectra vs. NIST library for disulfoton and diazinon. The average values of five replicate spectra from independent chromatographic runs are listed, except where low match quality did not provide identification.

Acquisition rate (spectra/s)	Disulfoton (fit value)	Diazinon (fit value)
5	884	852
10	876	851
25	837	827 [a]
50	820	623 [a]
100	735	583
175	697 [b]	626 [b]
250	660 [b]	586 [b]

a) Based on four replicate spectra.
b) Based on three replicate spectra.

2.3.3 Isotope Ratio Monitoring GC/MS

The measurement of isotope ratios was the first application of mass spectrometry. In 1907 Thomson for the first time showed the parabola mass spectrum of a Neon sample with his newly developed mass spectrometer. Later, in 1919, Aston concluded that the observed lines reveal the isotopes 20 and 22 of Neon, he later also discovered the isotope 21 of Ne with only 0.3 at% abundance. The term "isotope" was coined independently by Frederick Soddy. He observed substances with identical chemical behaviour but different atomic mass in the decay of natural radioactive elements and received 1921 the Nobel Prize in Chemistry for his investigations into the origin and nature of isotopes. The term "Isotope" is derived from the Greek "isos" for equal and "topos" for the place in the table of elements. Consequently the development in mass spectrometry in the first half of last century was dominated by elemental analysis with the determination of the elemental isotope ratios facilitated by further improved mass spectrometer systems with higher resolving power as introduced by Alfred Nier. He carried out the first measurements on $^{13}C/^{12}C$ abundance ratios in natural samples. Already in 1938 Nier studied bacterial metabolism by using ^{13}C as the tracer.

The following investigations by Alfred Nier formed the foundation for today's high precision isotope ratio mass spectrometry (IRMS). In contrast to mass spectrometric organic structure elucidation and target compound quantitation, IRMS is providing a different analytical dimension of precision data. Isotope ratio MS provides information on the physiochemical history, the origin or authenticity of a sample and is essential today in multiple

areas of research and control such as food, life sciences, forensics, material quality control, geology or climate research, just to name the most important applications today.

Isotope ratio monitoring techniques using continuous flow sample introduction via gas chromatography had been introduced in the 1970s and developed into a mature analytical technology very quickly. Already in 1976, the first approach hyphenating a capillary GC with a magnetic sector MS for the systematic measurement of isotope ratios was published (Sano 1976). The continuous flow determination of individual compound $^{13}C/^{12}C$ ratios was introduced by Matthews and Hayes in 1978 and the term "isotope ratio monitoring GC/MS" was coined, today commonly abbreviated "irm-GC/MS" (Matthews 1978). The full range of $^{15}N/^{14}N$, $^{18}O/^{16}O$ and most importantly H/D determinations lasted until its publication in 1998 (Brand 1994, Heuer 1998, Hilkert 1998). Today irm-GC/MS is the established analytical methodology for delivering the precise ratios of the stable isotopes of the elements H, N, C, and O being the major constituents of organic matter. Sulfur is due to its only low abundance in organic molecules currently not amenable to irm-GC/MS analysis. A compelling example was presented by Ehleringer on the geographical origin of cocaine from South America applied to determine the distribution of illicit drugs (Ehleringer 2000, Bradley 2002). Organic compounds containing these elements can be quantitatively converted into simple gases for mass spectrometric analysis e. g. H_2, N_2, CO, CO_2, O_2, SO_2. For that conversion to simple gases, integrating and providing the full isotope information of a substance (in contrast to a fragmented organic mass spectrum) also the term "gas isotope ratio mass spectrometry" GIRMS is used frequently also including irm-GC/MS applications.

The Principles of Isotope Ratio Monitoring

Isotope ratios, although tabulated with average values for all elements, are not constant. All phase transition processes, transport mechanisms and enzymatic or chemical reactions are dependent on the physical properties of the reaction partners, i. e. most importantly the mass of the molecule involved in the process or chemical reaction, for their kinetic properties.

The high precision quantitative data on isotope ratios obtained are not absolute quantitation data. Natural isotope ratios exhibit only small but meaningful variations that are measured with highest precision relative to a known standard. In most of the applications in stable isotope analysis the differences in isotopic ratios between samples is of much more interest and significance than the absolute amount in a given sample. The isotope ratio is independent of the amount of material measured (and at the low end only determined by the available ion statistics). The relative ratio measurement can be accomplished in the required and even higher precision, which is at least one order of magnitude higher than the determination of absolute values.

Notations in irm-GC/MS

The small abundance differences of stable isotopes are best represented by the delta notation (4) in which the stable isotope abundance is expressed relative to an isotope standard, measured in the same run for reference:

$$\delta = (R_{sample}/R_{std} - 1) \times 1000\ [‰] \quad (4)$$

with R the molar ratio of the heavy to the common (light) isotope of an element e. g. R = $^{13}C/^{12}C$ or D/H or $^{18}O/^{16}O$. A graphical representation of meaning the δ-value is given in

Table 2.39 Natural abundances of light stable isotopes relevant to stable isotope ratio mass spectrometry.

Element	Isotope	Atomic weight	Relative abundance %	Elemental relative mass difference	Molecular relative mass difference %	Terrestrial range ‰	Terrestrial range ppm	Technical precision ‰	Technical precision ppm
Hydrogen	1H	1,0078	99,9840	D/H	1HD/1H1H				
Deuterium	2H (D)	2,0141	0,0156	(2/1)	(3/2)	700	109	1	0,16
				100%	**50%**				
Boron	10B	10,0129	19,7	11B/10B		60			
	11B	11,0093	8,3	**10%**					
Carbon	12C	12,0000	98,892	13C/12C	13C16O16O/12C16O16O				
	13C	13,0034	1,108	(13/12)	(45/44)	100	1123	0,05	0,56
				8,3%	**2,3%**				
Nitrogen	14N	14,0031	99.635	15N/14N	15N14N/14N14N				
	15N	15,0001	0,365	(15/14)	(29/28)	50	181	0,10	0,72
				7.1%	**3,6%**				
Oxygen	16O	15,9949	100	18O/16O	12C16O18O/12C16O16O				
	17O	16,9991	0,037	(18/16)	(46/44)				
	18O	17,9992	0,204	**12.5%**	**4.6%**	100	200	0,10	0,20
Silicon	28Si	28	92,21	29Si/28Si	29Si19F3/28Si19F3	6			
	29Si	29	4,7	(29 / 28)	(86 / 85)				
	30Si	30	3,09	**3,6%**	**1,2%**				
Sulphur	32S	32	95,02	34S/32S	34S16O16O/32S16O16O				
	33S	33	0,76						
	34S	34	4,22	(34/32)	(66/64)	100	4580	0,20	9,16
	35S	36	0,014	**6,3%**	**3,1%**				
Chlorine	35Cl	35	75,77	37Cl/35Cl	12CH337Cl/12CH335Cl	10		0,10	
	37Cl	37	24,23	(37/35)	(52/50)				
				5,7%	**4.0%**				

From Gilles St.Jean, Basic Principles in Stable Geochemistry, IRMS Short Course, 9th Canadian CF-IRMS Workshop 2002.

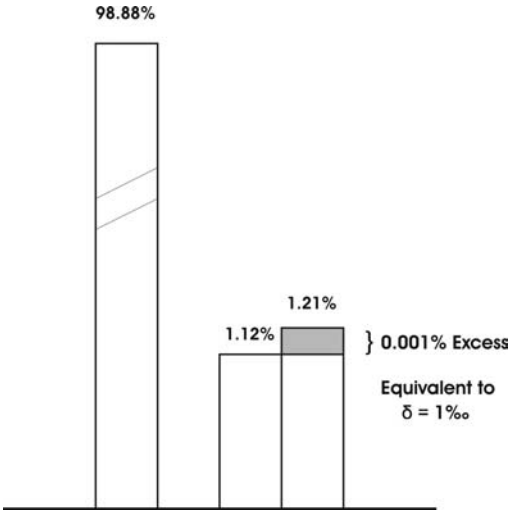


Fig. 2.156 Graphical representation of the δ-notation for a ^{13}C variation of 1‰.

Fig. 2.156 for the variation of the ^{13}C isotope proportion of carbon. δ-Values can be positive or negative indicating a higher or lower abundance of the major isotope in the sample than in the reference.

Other systems in use for expressing isotope ratios are ppm (part per million, relative value), at% (atom percent, absolute value), and APE (atom percent excess, relative value) as the most common terminology in biomedical tracer studies. The choice is dependent primarily on the specific field of application e.g. with high degrees of enrichments being very much different from natural abundances. Also typical in this field are historical traditions in the use of different notation systems. Values of at% and δ-notation can be converted as follows:

$$\text{at\%} = \frac{R_{st} \cdot (\delta/1000 + 1)}{1 + R_{st} \cdot (\delta/1000 + 1)} \cdot 100$$

with R_{st} absolute standard ratio

Isotopic Fractionation

Isotope effects, as they can be observed in phase transition or dissociation reactions are usually the result of incomplete processes in diffusive or equilibrium fractionation. This effect is caused by different translational velocities of the lighter and heavier molecule through a medium or across a phase boundary. As the kinetic energy at a given temperature is the same for all gas molecules, the kinetic energy equation

$$E_{kin} = {}^1/_2\ m \cdot v^2$$

applies for both molecules $^{12}CO_2$ and $^{13}CO_2$ with their respective masses 44 and 45 u:

$${}^1/_2\ (44 \cdot v_a^2) = {}^1/_2\ (45 \cdot v_b^2)$$

resulting in

$$v_a/v_b = \sqrt{(45/44)} = 1.0113$$

The velocity ratio of both CO_2 species explains that the average velocity of $^{12}CO_2$ is 1.13% higher than that of the heavier molecule.

Example: Boiling water will lose primarily the light $^{1}H_2{}^{16}O$ molecules as can be seen in Fig. 2.157. Heavier water molecules will consequently be concentrated in the liquid; the vapour will become depleted. The reversed processes are observed during condensation, e.g., during the formation of raindrops from humid air. As the extent of this process is temperature dependent isotopic "thermometers" are formed and ultimately isotopic „signatures" of materials and processes are created.

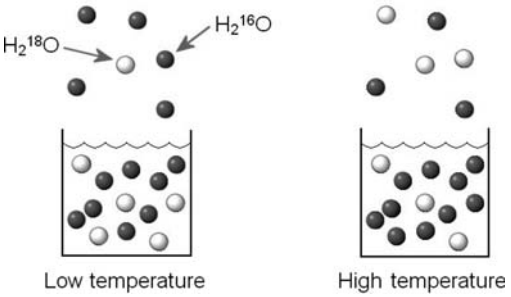


Fig. 2.157 Alteration of the isotope ratio during the evaporation of water resulting in a different $^{16}O/^{18}O$ ratio at low and high temperatures in vapour as well as in the liquid.

Equilibrium isotope effects usually are associated with phase transition processes like evaporation, diffusion or dissociation reactions. When occurring during sample preparation, incomplete phase transition processes lead to severe alteration of the initial isotope ratio. In fact, and that is of highest importance for all sample preparation steps in IRMS, only complete conversion reactions are acceptable to maintain the integrity of the original isotope ratio of the sample.

Isotope effects are also observed in chemical and biochemical reactions (kinetic fractionation). Of particular significance is the isotope effect occurring during enzymatic reactions with a general depletion in the heavier isotope being key to uncover metabolic processes. Chemical bonds to the heavier isotope are stronger, more stable and need higher dissociation energies in chemical reactions due to the different vibrational energy levels involved. Hence, the rate of enzymatic reaction is faster with the light isotope leading to differences in the abundance between substrate and product, unless the substrate is fully consumed, which is not the case in cellular steady state equilibria. Kinetic fractionation effects also have to be considered when employing compound derivatisation steps (e.g. silylation, methylation) during sample preparation for GC application (Meyer-Augenstein 1997).

The natural variations in isotopic abundances can be large, depending on the relative elemental mass differences: hydrogen (100%) > oxygen (12.5%) > carbon (8.3%) > nitrogen (7.1%), see also Table 2.39. An overview of the isotopic variations found in natural compounds is given in Figs. 2.158 to 2.161.

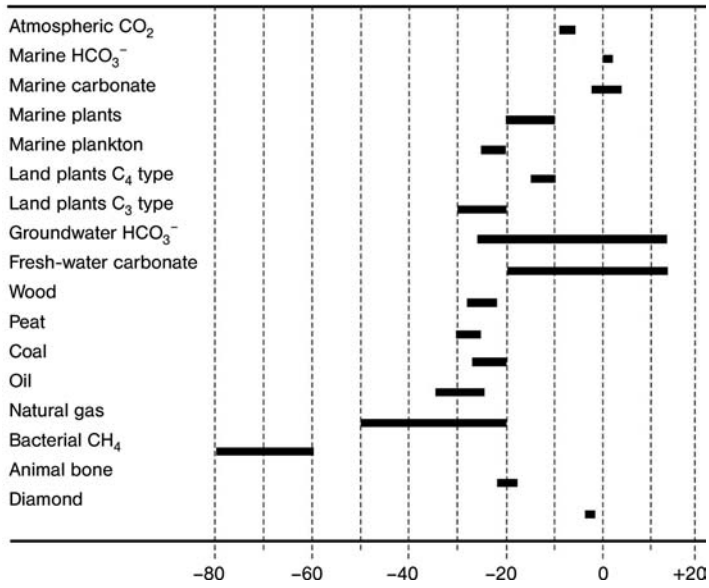


Fig. 2.158 $\delta^{13}C$ variations in natural compounds ($\delta^{13}C$ VPDB ‰ scale) (de Vries 2000, courtesy IAEA).

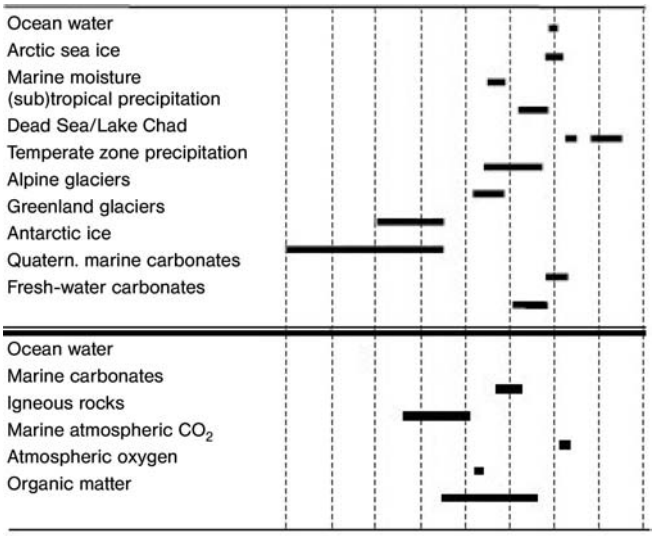


Fig. 2.159 $\delta^{18}O$ variations in natural compounds (top: $\delta^{18}O$ VSMOW ‰ scale for waters, $\delta^{18}O$ VPDB ‰ scale for carbonates, bottom: $\delta^{18}O$ VSMOW ‰ scale) (de Vries 2000, courtesy IAEA).

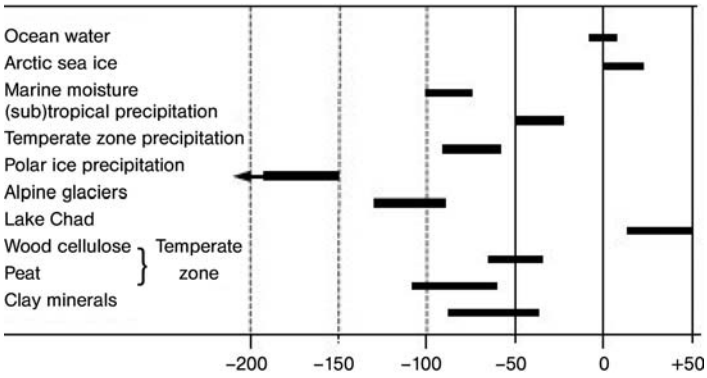


Fig. 2.160 δ^2H variations in natural compounds (δ^2H VSMOW ‰ scale) (de Vries 2000, courtesy IAEA).

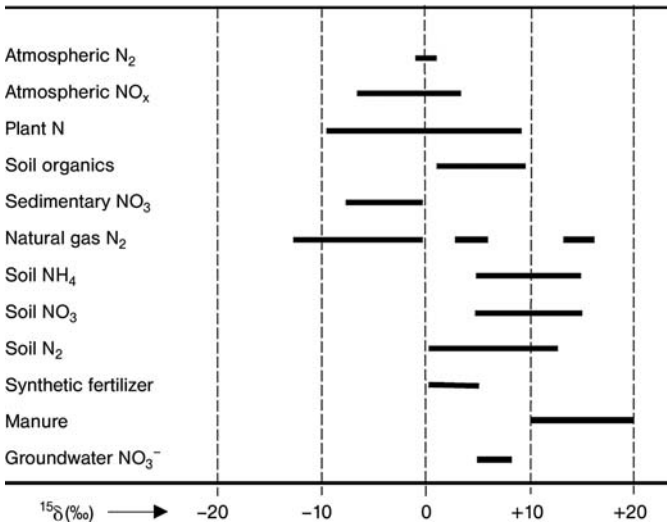


Fig. 2.161 δ^{15}N variations in natural compounds (δ^{15}N Air ‰ scale) (de Vries 2000, courtesy IAEA).

irm-GC/MS Technology

irm-GC/MS is applied to obtain compound specific data after a GC separation of mixtures in contrast to bulk analytical data from an elemental analyser system. The bulk analysis delivers the average isotope ratio within a certain volume of sample material. The entire sample is converted into simple gases using conventional elemental analysers (EA) or high temperature conversion elemental analysers (TC/EA). In contrast to a bulk analysis, irm-GC/MS delivers specific isotope ratio data in the low picomolar range of individual compounds after a conventional capillary GC separation (see Fig. 2.162).

The most important step in irm-GC/MS is the conversion of the eluting compounds into simple measurement gases like CO_2, N_2, CO, and H_2. In irm-GC/MS as a continuous flow application, this conversion is achieved on-line within the helium carrier gas stream while

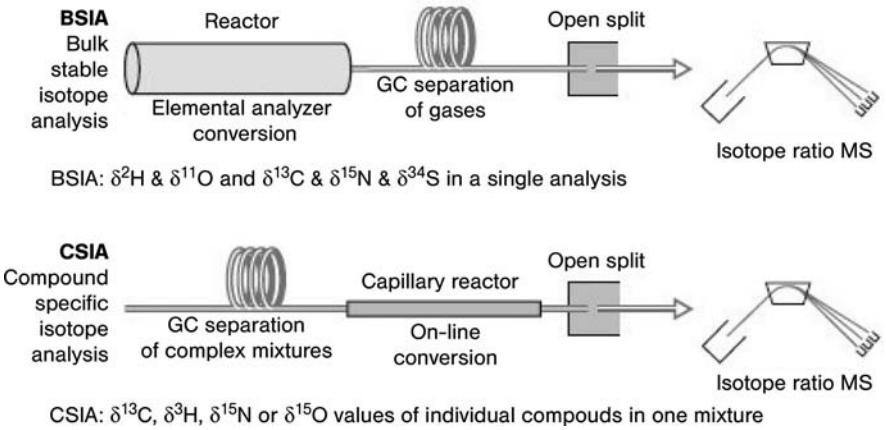


Fig. 2.162 Basic scheme of bulk sample (BSIA) vs. compound specific (CSIA) isotope analysis (courtesy Thermo Fisher Scientific).
BSIA: IRMS is coupled with an EA: The complete sample is first converted to simple gases followed by their chromatographic separation.
CSIA: IRMS is coupled with a GC: The sample components are first separated by capillary chromatography and then individually converted to the measurement gases.

preserving the chromatographic pattern of the sample. The products of the conversion are then fed by the carrier gas stream into the isotope ratio MS.

It is important to note that during chromatographic separation on regular fused silica capillary columns, a separation of the different isotopically substituted species of a compound already takes place. Due to the lower molar volume of the heavier components and the resulting differences in mobile/stationary phase interactions, the heavier components elute slightly earlier (Matucha 1991). This separation effect can be observed also in "organic" GC/MS analyses when having a closer look at the peak top retention times of the ^{13}C carbon isotope mass trace. irm-GC/MS makes special use of the chromatographic isotope separation effect by displaying the typical S-shaped ratio traces during analysis indicating the substances of interest (see Figs. 2.163 and 2.164).

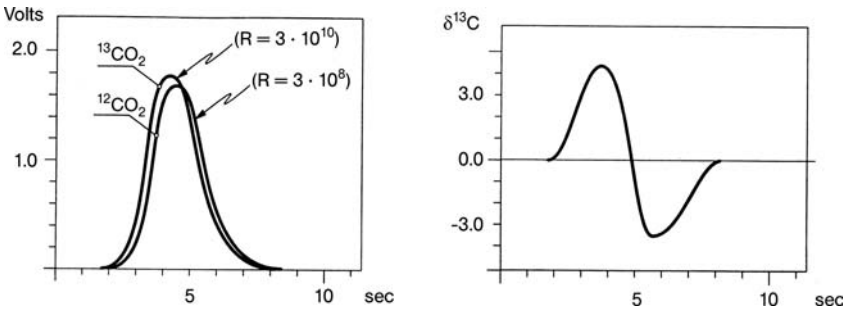


Fig. 2.163 Chromatographic elution profiles of CO_2 with natural ^{13}C abundance resulting in the typical S-shaped curvature when displaying the calculated δ-values (left: note the intensity difference of the isotopes with the choice of amplification resistors R in a difference of two orders of magnitude).

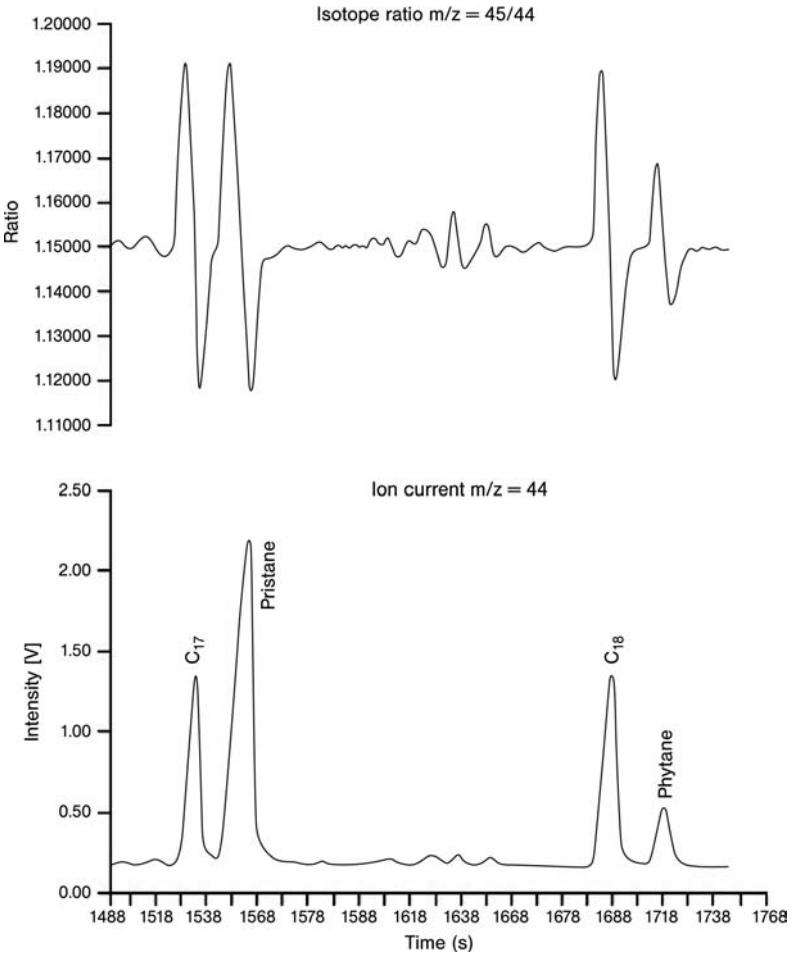


Fig. 2.164 irm-GC/MS analysis of the crude oil steroid biomarkers pristane and phytane with the $\delta^{13}C$ elution profile on top.

The Open Split Interface

For high precision isotope ratio determination, the IRMS ion source pressure must be kept absolutely constant. For this reason, it is mandatory in all continuous flow applications coupled to an isotope ratio MS, to keep the open split interface at atmospheric pressure. The open split coupling eliminates the isotopic fractionation due to an extended impact of the high vacuum of the mass spectrometer into the chromatography and conversion reactors. The basic principle of an open split is to pick up a part of the helium/sample stream by a transfer capillary from a pressureless environment, i.e. an open tube or a wide capillary, and transfer it into the IRMS (see Fig. 2.165). The He stream added by a separate capillary ensures protection from ambient air. Retracting the transfer capillary into a zone of pure helium allows cutting off parts of the chromatogram. In all modes, a constant flow of helium into the isotope ratio MS, and consequently constant ion source conditions are maintained.

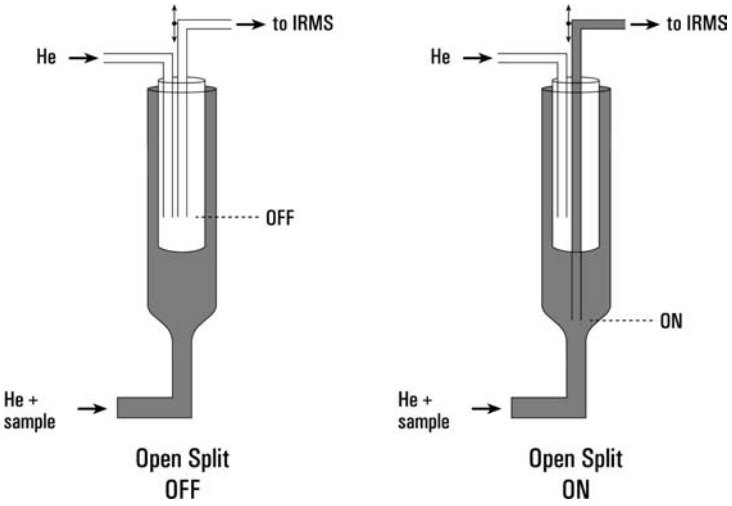


Fig. 2.165 Open split interface to IRMS, effected by moving the transfer capillary (to IRMS) from the column inlet to the He sample flow region (courtesy Thermo Fisher Scientific).

The valve-free open split is absolutely inert and does not create any pressure waves during switching.

In addition, the open split interface also offers an automatic peak dilution capability. Due to the transfer of the analyte gases in a helium stream more than one interface can be coupled in parallel for alternate use to an isotope ratio MS via separate needle valves.

Compound Specific Isotope Analysis by On-line Sample Combustion and Conversion

irm-GC/MS is amenable to all GC-volatile organic compounds down to the low picomole range. A capillary design of oxidation and high temperature conversion reactors is required to guarantee the integrity of GC resolution. Table 2.40 gives an overview of the techniques available today with the achievable precision values by state of the art IRMS technology.

Compound specific isotope analysis (CSIA) is one of the newest (but today a mature and steadily expanding) fields of isotope analysis. The first GC combustion system for $\delta^{13}C$ determination was commercially introduced in 1988 by Finnigan MAT Bremen, Germany. This

Table 2.40 Analytical methods used in compound specific isotope analysis.

Element ratio	Measured species	Method	Temperature	Analytical precision [a]
$\delta^{13}C$	CO_2	Combustion	up to 1000 °C	^{13}C 0.06 ‰, 0.02 ‰/nA
$\delta^{15}N$	N_2	Combustion	up to 1000 °C	^{15}N 0.06 ‰, 0.02 ‰/nA
$\delta^{18}O$	CO	Pyrolysis	≥ 1450 °C	^{18}O 0.15 ‰, 0.04 ‰/nA
$\delta^{2}H$	H_2	Pyrolysis	≥ 1280 °C	^{2}H 0.50 ‰, 0.20 ‰/nA

a) 10 pulses of reference gas (amplitude 3V, for H_2 5V) δ notation.

technique combines the resolution of capillary GC with the high precision of isotope ratio mass spectrometry (IRMS). In 1992, the capabilities for analysis of $\delta^{15}N$ and in 1996 for $\delta^{18}O$ were added. Quantitative pyrolysis by high temperature conversion (GC-TC IRMS) for δD analyses was introduced in 1998 introducing an energy discrimination filter in front of the HD collector at m/z 3 for the suppression of $^4He^+$ ions from the carrier gas interfering with the HD^+ signal (Hilkert 1999).

On-line Combustion for $\delta^{13}C$ and $\delta^{15}N$ Determination

All compounds eluting from a GC column are oxidized in a capillary reactor to form CO_2, N_2, and H_2O as a by-product at 940 to 1000°C. NO_x produced in the oxidation reactor is reduced to N_2 in a subsequent capillary reduction reactor. The H_2O formed in the oxidation process is removed by an on-line Nafion dryer, a maintenance-free water removal system. For the analysis of $\delta^{15}N$, all CO_2 is retained in a liquid nitrogen trap in order to avoid interferences from CO on the identical masses before transfer into the IRMS through the movable capillary open split. CO is generated in small amounts as a side reaction during the ionisation process from CO_2 in the ion source of the MS. A detailed schematic of the on-line combustion setup is given in Fig. 2.166.

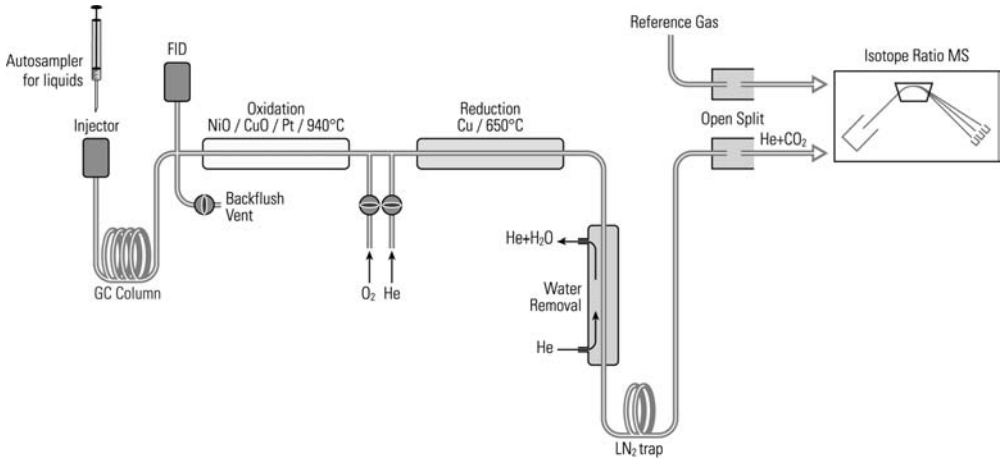


Fig. 2.166 On-line oxidation of compounds eluting from the GC for the production of CO_2 and N_2 for IRMS measurement (courtesy Thermo Fisher Scientific).

The Oxidation Reactor

The quantitative oxidation of all organic compounds eluting from the GC column, including the refractory methane, is performed at temperatures up to 1000 °C. The reactor consists of a capillary ceramic tube loaded with twisted Ni, Cu, and Pt wires. The resulting internal volume compares to a capillary column and secures the integrity of the chromatographic separation. The reactor can be charged and recharged automatically with O_2 added to a backflush flow every 2–3 days, depending on the operating conditions. The built-in backflush system reverses the flow through the oxidation reactor towards an exit directly after the GC column. The backflush is activated during the analysis to cut off an eluting solvent peak in

front of the oxidation furnace by flow switching. All valves have to kept outside of the analytical flow path to maintain optimum GC performance.

The Reduction Reactor

The reduction reactor is typically comprising of copper material and operated at 650 °C to remove any O_2 bleed from the oxidation reactor and to convert any produced NO_x into N_2. It is of the same capillary design as the oxidation reactor.

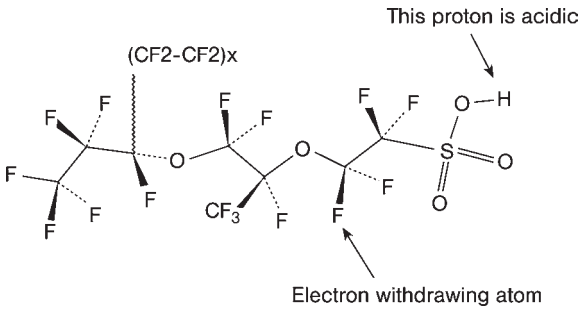
Water Removal

Water produced during the oxidation reaction is removed through a 300 µm inner diameter Nafion capillary, which is dried by a counter current of He on the outside (Fig. 2.167). The water removal adds no dead volume and is maintenance-free.

On-line Water Removal From a He Stream Using Nafion

Water is removed from a He sample stream by a gas tight but hygroscopic Nafion tubing. The sample flow containing He, CO_2 and H_2O passes through the Nafion tubing which is mounted co-axially inside a glass tube. This glass tube, and therefore the outer surface of the Nafion tube, is constantly kept dry by a He flow of approximately 8 to 10 mL/min. Due to the water gradient through the Nafion membrane wall any water in the sample flow will move through the membrane. A dry gas comprising of only He and CO_2 results which is fed to the ion source of the mass spectrometer.

Structure of Nafion:



Properties:

Nafion is the combination of a stable Teflon backbone with acidic sulfonic groups. It is highly conductive to cations, making it ideal for many membrane applications. The Teflon backbone interlaced with ionic sulfonate groups gives Nafion a high operating temperature, e.g. up to 190 °C. It is selectively and highly permeable to water. The degree of hydration of the Nafion membrane directly affects its ion conductivity and overall morphology. See also: http://de.wikipedia.org/wiki/Nafion

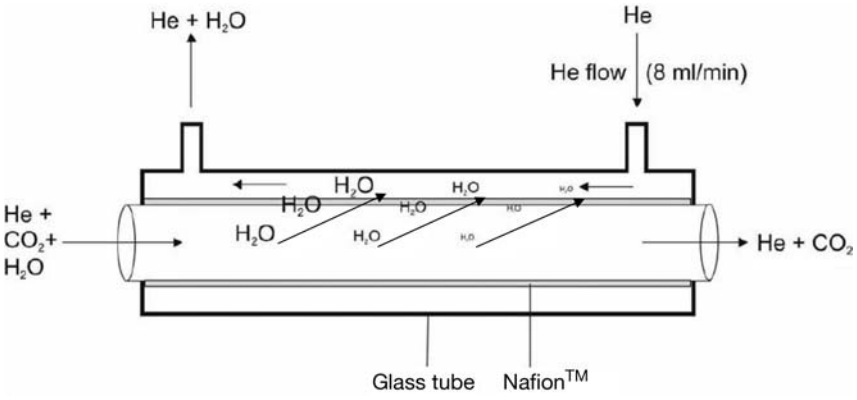


Fig. 2.167 Principle of on-line removal of water from a He stream using a Nafion membrane (courtesy Thermo Fisher Scientific).

The Liquid Nitrogen Trap

For the analysis of $\delta^{15}N$, all the CO_2 must be removed quantitatively to avoid an interference of CO^+ produced in from CO_2 in the ion source with the N^{2+} analyte. This is achieved by immersing the deactivated fused silica capillary between the water removal and the open split into a liquid nitrogen bath. The trapped CO_2 is easily released after the measurement series with no risk of CO_2 contamination of the ion source by using the movable open split.

On-line High Temperature Conversion for δ^2H and $\delta^{18}O$ Determination

A quantitative pyrolysis by high temperature conversion of organic matter is applied for the conversion of organic oxygen and hydrogen to form the measurement gases CO and H_2 for the determination of $\delta^{18}O$ or δD (see Fig. 2.169). This process requires an inert and reductive environment to prevent any O or H containing material from reacting or exchanging with the analyte.

For the determination of $\delta^{18}O$, the analyte must not contact the ceramic tube that is used to protect against air. For the conversion to CO the pyrolysis takes place in an inert platinum inlay of the reactor. Due to the catalytic properties of the platinum, the reaction can be performed at 1280 °C. For the determination of δD from organic compounds, the reaction is performed in an empty ceramic tube at 1450 °C. Such high temperatures are required to ensure a quantitative conversion.

Typically an on-line high temperature reactor is mounted in parallel to a combustion reactor at the GC oven. The complete setup for on-line high temperature conversion is given in Fig. 2.170. A water removal step has no effect here on the dry analyte gas.

Mass Spectrometer for Isotope Ratio Analysis

Mass spectrometers employed for isotope ratio measurements are dedicated non-scanning, static magnetic sector mass spectrometer systems. The ion source is particularly optimized by a "closed source" design for the ionisation of gaseous compounds at very high ion production efficiency of 500–1000 molecules/ion at a high response linearity (Brand 2004). After extraction from the source region, the ions are typically accelerated by 2.5 to 10 kV to form an ion beam which enters the magnetic sector analyser through the entrance slit (see Fig. 2.172).

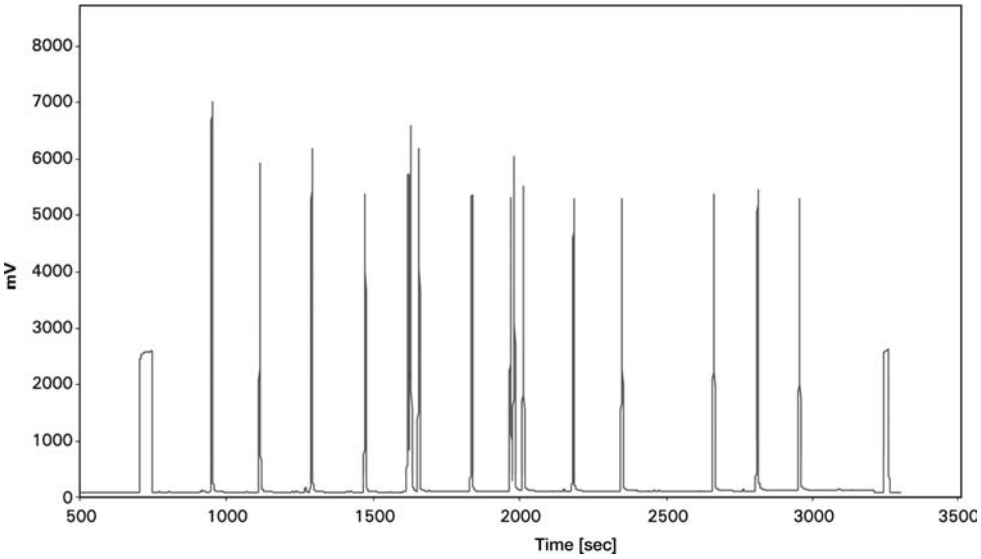


Fig. 2.168 irm-GC-C/MS chromatogram of a fatty acid methyl ester (FAME) sample after on-line combustion at 940 °C. The rectangular peaks in the beginning and at the end of the chromatogram are the CO_2 reference gas injection peaks (courtesy Thermo Fisher Scientific).

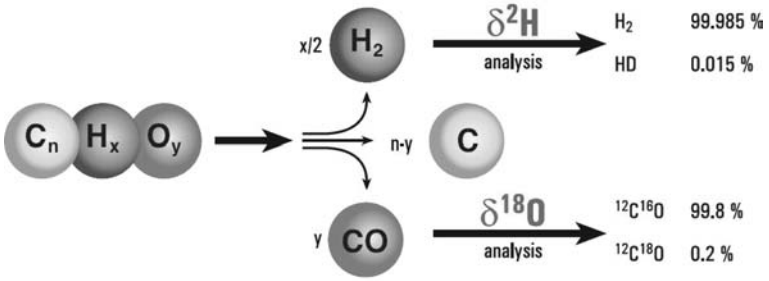


Fig. 2.169 Principle of high temperature conversion (courtesy Thermo Fisher Scientific).

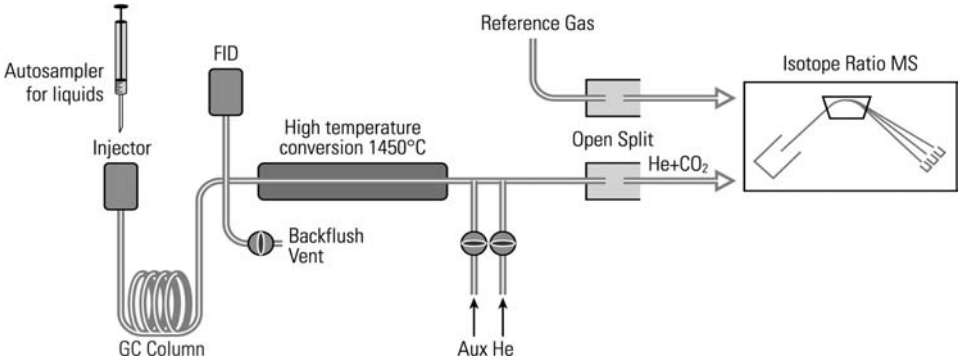


Fig. 2.170 On-line high temperature conversion of compounds eluting from the GC for the production of CO and H_2 for IRMS measurement (courtesy Thermo Fisher Scientific).

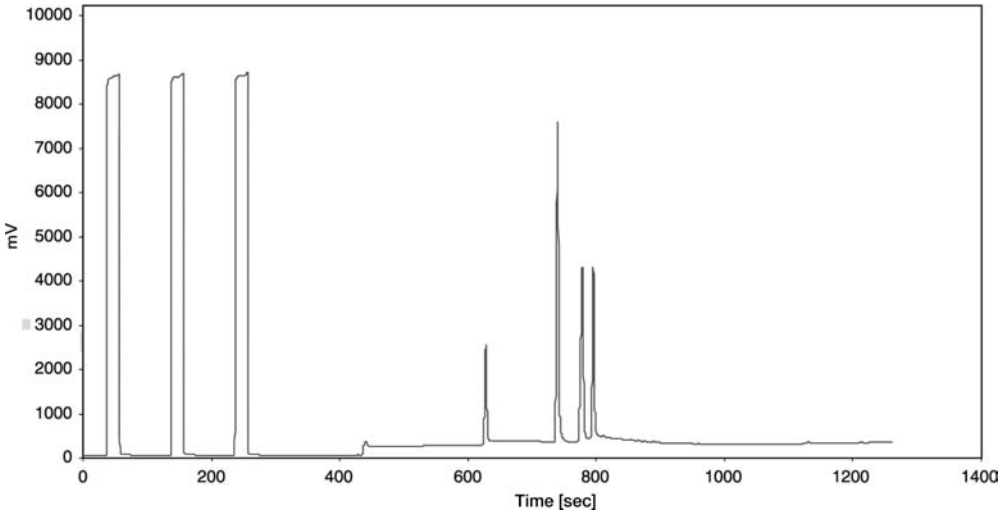


Fig. 2.171 irm-GC-TC/MS chromatogram of flavour components using high temperature conversion with three CO reference gas pulses at start (courtesy Thermo Fisher Scientific).

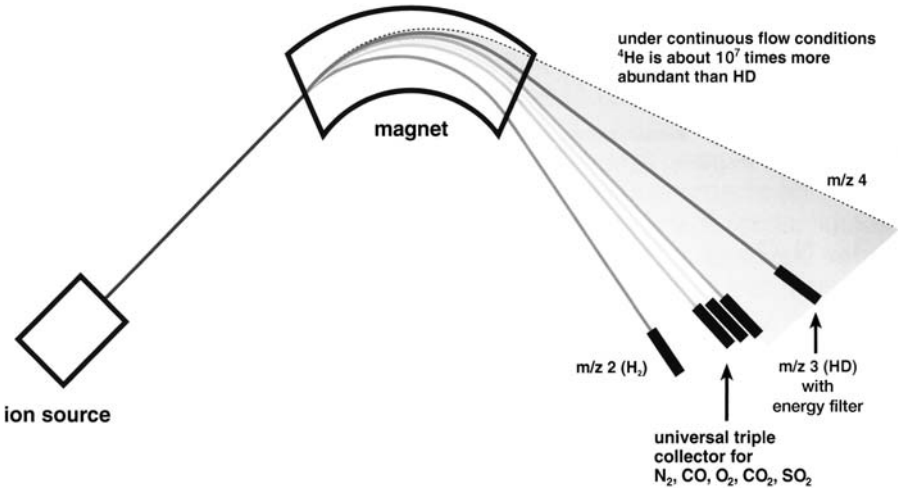


Fig. 2.172 Isotope ratio mass spectrometer with ion source, magnet and the array of Faraday cups for simultaneous isotope detection (courtesy Thermo Fisher Scientific).

The ion currents of the isotopes are measured simultaneously at the individual m/z values by discrete Faraday cups mounted behind a grounded slit (see Fig. 2.173). An array of specially designed deep Faraday cups for quantitative measurement is precisely positioned along the optical focus plane representing the typical isotope mass cluster to be determined.

Only the simultaneous measurement of the isotope ion currents with dedicated Faraday cups for each isotope using individual amplifier electronics, cancels out ion beam fluctuations due to temperature drifts or electron beam variations and provides the required preci-

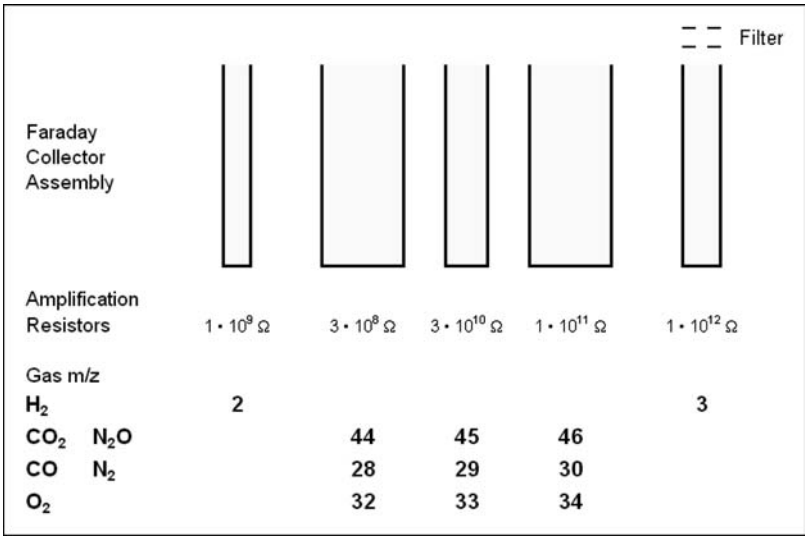


Fig. 2.173 Typical Faraday cup arrangement for measurement of the isotope ratios of the most common gas species (below of the cup symbols the values of the amplification resistors are given; the HD cup is shown to be equipped with a kinetic energy filter to prevent from excess $^4He^+$).

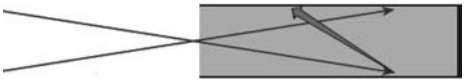


Fig. 2.174 Cross section of a Faraday cup for isotope ratio measurement, the large depth of the graphite cup prevents from losses caused by scattering. Arrows represent the ion beam with a focus point at the exit slit in front of the Faraday cup (courtesy Thermo Fisher Scientific).

sion (see Fig. 2.174, also refer to Table 2.39). Current instrumentation allows for irm-GC/MS analyses of organic compounds down to the low picomole range.

Isobaric interferences from other isotope species at the target masses require the measurement of more masses than just the targeted isotope ratio for a necessary correction. For the measurement of $\delta^{13}C$, three collectors at m/z 44, 45, and 46 are necessary (see Table 2.40). Algorithms for a fully automated correction of isobaric ion contributions are implemented in modern isotope ratio MS data systems e.g. for the isobaric interferences of ^{17}O and ^{13}C on m/z 46 of CO_2, see Table 2.41 (Craig 1957). Other possible interferences like CO on N_2, N_2O on CO_2 can be taken care of by the interface technology.

Table 2.41 Isobaric interferences when measuring $\delta^{13}C$ from CO_2.

m/z	Ion composition
44	$^{12}C^{16}O^{16}O$
45	$^{13}C^{16}O^{16}O$, $^{12}C^{16}O^{17}O$
46	$^{12}C^{16}O^{18}O$, $^{12}C^{17}O^{17}O$, $^{13}C^{16}O^{17}O$

The IT Principle

Standardisation in isotope ratio monitoring measurements should be done exclusively using the principle of "Identical Treatment of reference and sample material", the "IT Principle". Mostly, isotopic referencing is made with a co-injected peak of standard gas.

Injection of Reference Gases

The measurement of isotope ratios requires that sample gases be measured relative to a reference gas of a known isotope ratio. For the purpose of sample-standard referencing in irm-GC/MS, cylinders of calibrated reference gases, the laboratory standard of H_2, CO_2, N_2, or CO are used for an extended period of time. This referencing procedure turned out to be most economic and precise compared to the addition of an internal standard to the sample, also providing necessary quality assurance purposes. An inert, fused silica capillary supplies

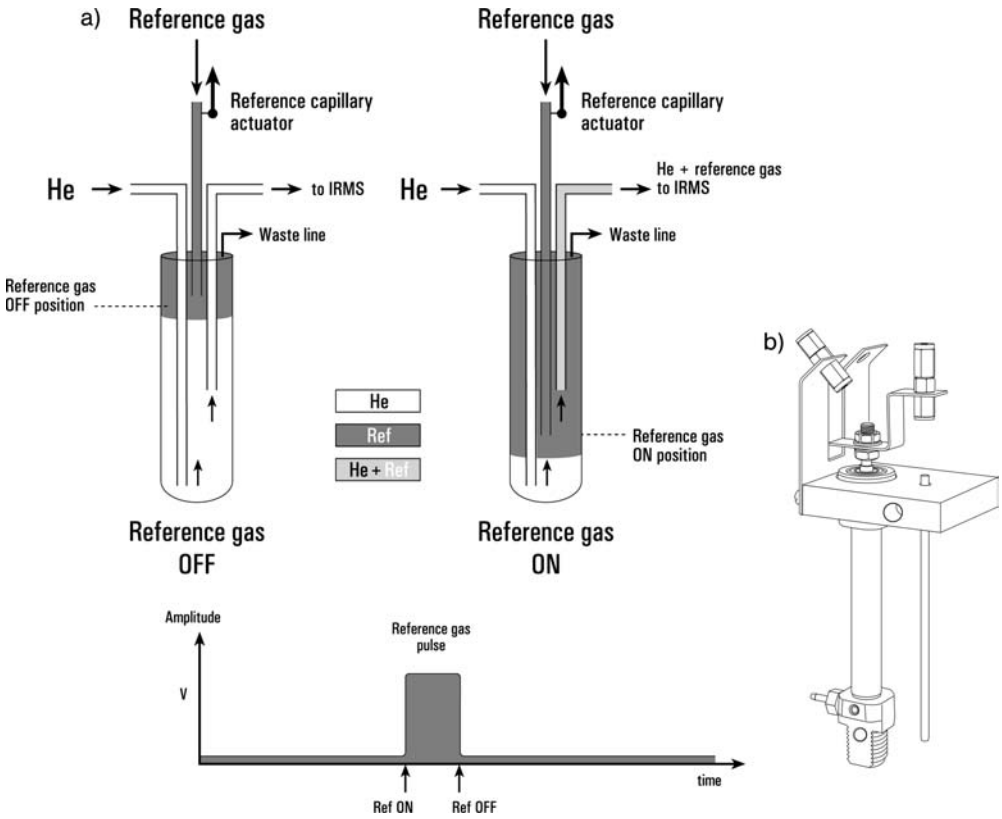


Fig. 2.175 (a) Reference gas injection port in irm-GC/MS implemented as an open coupling between GC column and IRMS ion source. (b) Reference gas injection port design: left side pneumatic drive, right side valve tube for fused silica columns, top column connectors for fixed (left) and movable (right) columns (courtesy Thermo Fisher Scientific).

the reference gas in the µL/min range into a miniaturized mixing chamber, the reference gas injection port (see Fig. 2.175 a and b). This capillary is lowered under software control into the mixing chamber for e. g. 20 s, creating a He reference gas mixture which is fed into the IRMS source via a second independent gas line. This generates a rectangular, flat top gas peak without changing any pressures or gas flows in the ion source. Reference gases used are pure nitrogen (N_2) carbon dioxide (CO_2), hydrogen (H_2), and carbon monoxide (CO).

Isotope Reference Materials

In IRMS the measurement of isotope ratios requires the samples to be measured relative to a reference of a known isotope ratio. This is the only means to achieve the required precision level of <1.5 ppm for e. g. the $^{13}C/^{12}C$ isotope ratio, which is 0.15 ‰ in the commonly used δ-notation. The employed isotopic reference scales (see Figs. 2.158 to 2.161) are arbitrarily defined by the community relative to the isotope ratio of a selected primary reference material for a given element.

Reference materials are available through the International Atomic Energy Agency (IAEA) in Vienna, Austria. The most commonly used and recognized international reference materials for stable isotope ratio analysis of natural abundances are given in Table 2.42. The abbreviations used with a preceding „V" as in „"VSMOW" refer to the reference materials prepared by the IAEA in Vienna compliant with the regular consultants meetings (for a detailed discussion see Groening 2004).

Table 2.42 IRMS primary reference materials for irm-GC/MS. Absolute isotope ratios R with 1σ standard uncertainties.

H	R = 0.00015576 ±0.000005	VSMOW	Standard Mean Ocean Water
C	R = 0.011224 ±0.000028	VPDB	Pee Dee Belemnita
N	R = 0.0036765	Air	Atmospheric Air
	R = 0.003663 ±0.000005	NSVEC	N standard by Jung and Svec
O	R = 0.0020052 ±0.000045	VSMOW	Standard Mean Ocean Water [a)]

a) As a general rule $\delta^{18}O$ data of carbonates and CO_2 gas are reported against VPDB whereas $\delta^{18}O$ data of all other materials should be reported vs. VSMOW.

VSMOW

Oceans contain almost 97% of the water on Earth and have a uniform isotope distribution. Oceans are the major sink in the hydrological cycle. The SMOW standard was initially proposed by Craig 1961 as a concept for the origin of the scale and was calculated from an average of samples taken from different oceans. The reference water VSMOW was prepared by the IAEA in 1968 with the same $\delta^{18}O$ and a –0.2‰ lighter δD as defined by SMOW.

VPDB

Pee Dee Belemnita is $CaCO_3$ of the rostrum of a bellemnite (*Belemnita americana*) from the Pee Dee Formation of South Carolina, USA. The PDB reference material has been exhausted for a long time. The new VPDB reference material was anchored by the IAEA with a fixed δ value so that it corresponds nominally to the previous PDB scale.

Atmospheric Air
Nitrogen in atmospheric air has a very homogeneous isotope composition. The atmosphere is the main nitrogen sink and the largest terrestrial nitrogen reservoir. NSVEC is the reference material initially prepared by Jung and Svec, Iowa State University, USA.

The available IAEA reference materials are intended to calibrate local laboratory standards and not for continuous quality control purposes (Groening 2004). Every laboratory should prepare for routine work long lasting laboratory specific standards with similar characteristics to the used references, the working standards, which are calibrated against the primary reference materials.

For comparison to results of other laboratories, the raw data of the mass spectrometer are converted into VSMOW or VPDB (Boato 1960). Due to the relative measurement to a standard ratio, the formula contains a product term besides the sum of the δ-values:

$\delta_1 = \delta$ (sample/reference)	measured in the lab
$\delta_2 = \delta$ (reference/standard)	known lab calibration
$\delta_3 = \delta$ (sample/standard)	unknown

$$\delta_3 = \delta_1 + \delta_2 + 10^{-3} \cdot \delta_1 \cdot \delta_2$$

2.3.4 Ionisation Procedures

2.3.4.1 Electron Impact Ionisation

Electron impact ionisation (EI) is the standard process in all GC/MS instruments. An ionisation energy of 70 eV is currently used in all commercial instruments. Only a few benchtop instruments still allow the user to adjust the ionisation energy for specific purposes. In particular, for magnetic sector instruments the ionisation energy can be lowered to ca. 15 eV. This allows EI spectra with a high proportion of molecular information to be obtained (*low voltage ionisation*) (Fig. 2.176). The technique has decreased in importance due to its inherently low sensitivity since the introduction of chemical ionisation.

The process of electron impact ionisation can be explained by a wave or a particle model. The current theory is based on the interaction between the energy-rich electron beam with the outer electrons of a molecule. Energy absorption initially leads to the formation of a molecular ion M^+ by loss of an electron. The excess energy causes excitation in the rotational and vibrational energy levels of this radical cation. The subsequent processes of fragmentation depend on the amount of excess energy and the capacity of the molecule for internal stabilisation. The concept of localised charge according to Budzikiewicz empirically describes the fragmentations. The concept was developed from the observation that bonds near heteroatoms (N, O, S; Fig. 2.177) or π electron systems are cleaved preferentially in molecular ions. This is attributed to the fact that a positive or negative charge is stabilised by an electronegative structure element in the molecule or one favouring mesomerism. Bond breaking can be predicted by subsequent electron migrations or rearrangement. These types of process include α-cleavage, allyl cleavage, benzyl cleavage and the McLafferty rearrangement (see also Section 3.2.5).

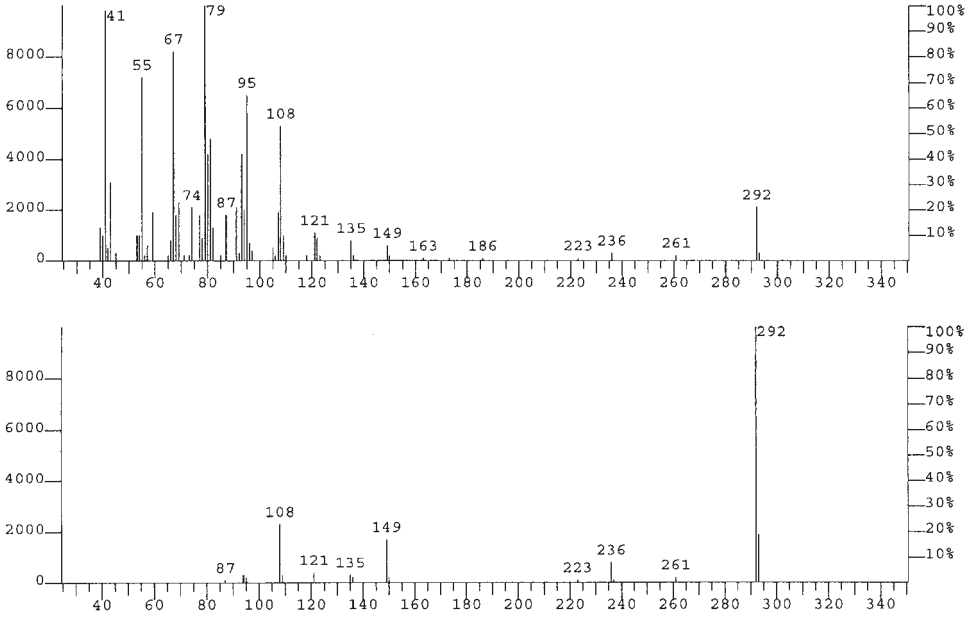


Fig. 2.176 EI spectra of methyl linolenate, $C_{17}H_{29}COOCH_3$ (after Spiteller).
Recording conditions: direct inlet, above 70 eV, below 17 eV.

The energy necessary for ionisation of organic molecules is lower than the effective applied energy of 70 eV and is usually less than 15 eV (Table 2.43). The EI operation of all MS instruments at the high ionisation of 70 eV was established with regard to sensitivity (ion yield) and the comparability of the mass spectra obtained.

Table 2.43 First ionisation potentials [eV] of selected substances.

Helium	24.6	Pentane	10.34
Nitrogen	15.3	Nitrobenzene	10.18
Carbon dioxide	13.8	Benzene	9.56
Oxygen	12.5	Toluene	9.18
Propane	11.07	Chlorobenzene	9.07
1-Chloropropane	10.82	Propylamine	8.78
Butane	10.63	Aniline	8.32

Assuming a constant substance stream into an ion source, Fig. 2.178 shows the change in intensity of the signal with increasing ionisation energy. The steep rise of the signal intensity only begins when the ionisation potential (IP) is reached. Low measurable intensities just below it are produced as a result of the inhomogeneous composition of the electron beam. Generally the increase in signal intensity continues with increasing ionisation energy until a plateau is reached. A further increase in the ionisation energy is now indicated by a

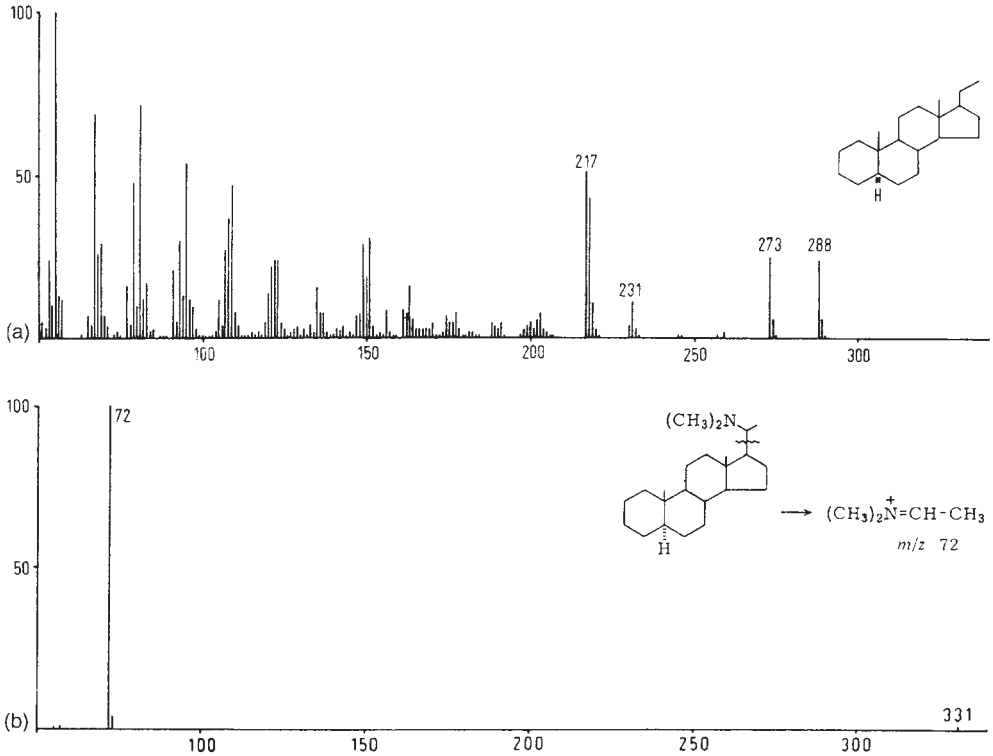


Fig. 2.177 Effect of structural features on the appearance of mass spectra – concept of localised charge (after Budzikiewicz).
(a) Mass spectrum of 5α-pregnane.
(b) Mass spectrum of 20-dimethylamino-5α-pregnane. The α-cleavage of the amino group dominates in the spectrum; information on the structure of the sterane unit is completely absent!

slight decrease in the signal intensity. An electron with an energy of 50 eV has a velocity of 4.2×10^6 m/s and crosses a molecular diameter of a few Ångstroms in ca. 10^{-16} s!

Further increases in the signal intensity are therefore not achieved via the ionisation energy with beam instruments, but by using measures to increase the density and the dispersion of the electron beam. The application of pairs of magnets to the ion source can be used, for example.

The standard ionisation energy of 70 eV which has been established for many years is also aimed at making mass spectra comparable. At an ionisation energy of 70 eV energy, which is in excess of that required for ionisation to M^+, remains as excess energy in the molecule, assuming maximum energy transfer. As a result fragmentation reactions occur and lead to an immediate decrease in the concentration of M^+ ions in the ion source. At the same time stable fragment ions are increasingly formed.

The fragmentation and rearrangement processes are now extensively known. They serve as fragmentation rules for the manual interpretation of mass spectra and thus for the identification of unknown substances. Each mass spectrum is the quantitative analysis by the analyser system of the processes occurring during ionisation. It is recorded as a line diagram.

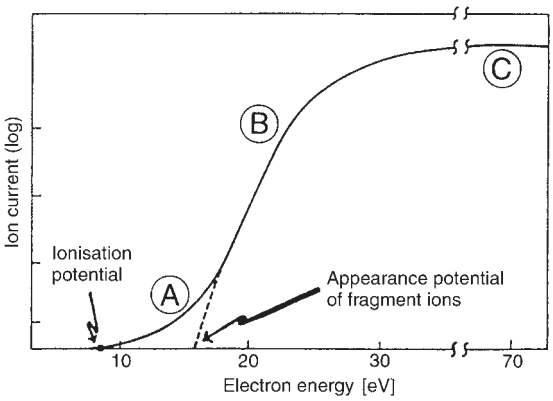
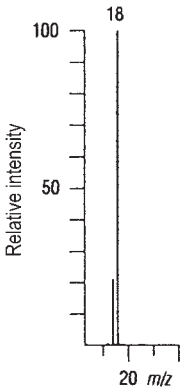


Fig. 2.178 Increase in the ion current with increasing electron energy (after Frigerio).
(A) Threshold region after reaching the appearance potential; molecular ions are mainly produced here.
(B) Build-up region with increasing production of fragment ions.
(C) Routine operation, stable formation of fragment ions.

What does a mass spectrum mean? In the graphical representation the mass to charge ratio m/z is plotted along the horizontal line. As ions with unit charge are generally involved in GC/MS with a few exceptions (e.g. polyaromatic hydrocarbons), this axis is generally taken as the mass scale and gives the molar mass of an ion (see Fig. 2.179). The intensity scale shows the frequency of occurrence of an ion under the chosen ionisation conditions. The scale is usually given both in percentages relative to the base peak (100% intensity) and in measured intensity values (counts). As neutral particles are lost in the fragmentation or rearrangement of a molecular ion M^+ and cannot be detected by the analyser, the mass of these neutral particles is deduced from the difference between the fragment ions and the molecular ion (or precursor fragments) (Fig. 2.180).



Introduction

Learning how to identify a simple molecule from its electron-ionization (EI) mass spectrum is much easier than from other types of spectra. The mass spectrum shows the mass of the molecule and the masses of pieces from it. Thus the chemist does not have to learn anything new – the approach is similar to an arithmetic brain-teaser. Try one and see.

In the bar-graph form of a spectrum the abscissa indicates the mass (actually m/z, the ratio of mass to the number of charges on the ions employed) and the ordinate indicates the relative intensity. If you need a hint, remember the atomic weigths of hydrogen and oxygen are 1 and 16 respectively."

Prof. McLafferty, Interpretation of Mass Spectra (1993)

Fig. 2.179 McLafferty's unknown spectrum.

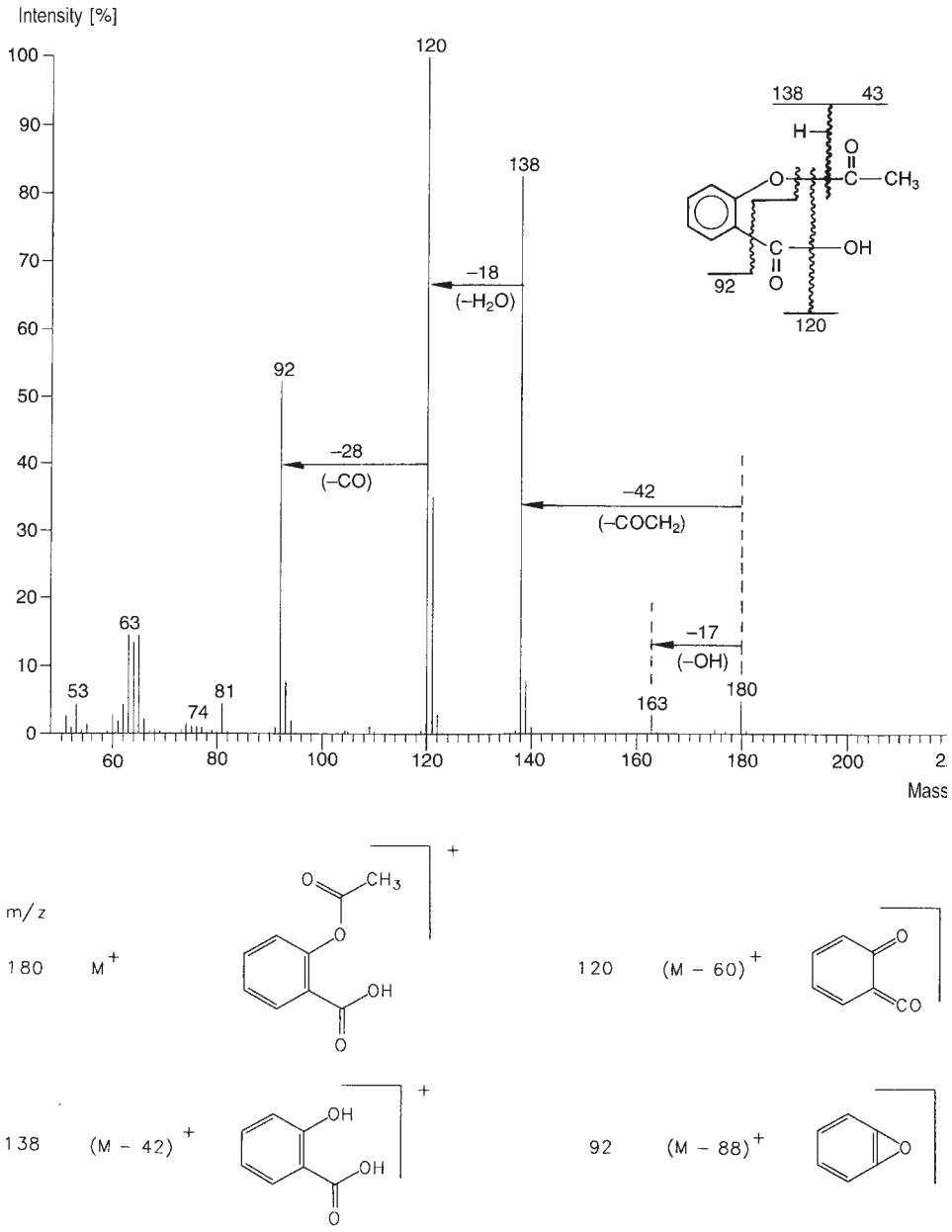


Fig. 2.180 A typical line spectrum in organic mass spectrometry with a molecular ion, fragment ions and the loss of neutral particles (acetylsalicylic acid, $C_9H_8O_4$, M 180).

In EI at 70 eV the extent of the fragmentation reactions observed for most organic compounds is independent of the construction of the ion source. For building up libraries of spectra the comparability of the mass spectra produced is thus ensured. All commercially available libraries of mass spectra are run under these standard conditions and allow the fragmentation pattern of an unknown substance to be compared with the spectra available in the library (see Section 3.2.4).

The Time Aspect in the Formation and Determination of Ions in Mass Spectrometry

• Flight time of an electron through an organic molecule (70 eV)	10^{-16} s
• Formation of the molecular ion M^+ (EI)	10^{-12} s
• Fragmentation reactions finished	10^{-9} s
• Rearrangement reactions finished	10^{-6}–10^{-7} s
• Lifetime of metastable ions	10^{-3}–10^{-6} s
Flight times of ions:	
• magnetic sector analyser	10^{-5} s
• quadrupole analyser	10^{-4} s
• ion trap analyser (storage times)	10^{-2}–10^{-6} s

Types of Ions in Mass Spectrometry

- Molecular ion:
  The unfragmented positive or negatively charged ion with a mass equal to the molecular mass and of a radical nature because of the unpaired electron.
- Quasimolecular ion:
  Ions associated with the molecular mass which are formed through chemical ionisation e.g. as $(M + H)^+$, $(M - H)^+$ or $(M - H)^-$ and are not radicals.
- Adduct ions:
  Ions which are formed through addition of charged species e.g. $(M + NH_4)^+$ through chemical ionisation with ammonia as the CI gas.
- Fragment ions:
  Ions formed by cleavage of one or more bonds.
- Rearrangement products:
  Ions which are formed following bond cleavage and migration of an atom (see McLafferty rearrangement).
- Metastable ions:
  Ions (m_1) which lose neutral species (m_2) during the time of flight through the magnetic sector analyser and are detected with mass $m^* = (m_2)^2/m_1$.
- Base ion:
  This ion gives the highest signal (100 %) (base peak) in a mass spectrum.

2.3.4.2 Chemical Ionisation

In electron impact ionisation (EI) molecular ions M^+ are first produced through bombardment of the molecule M by high energy electrons (70 eV). The high excess energy in M^+ (the ionisation potential of organic molecules is below 15 eV) leads to unimolecular fragmentation into fragment ions ($F1^+$, $F2^+$, ...) and uncharged species.

The EI mass spectrum shows the fragmentation pattern. The nature (m/z value) and frequency (intensity %) of the fragments can be read directly from the line spectrum. The loss of neutral particles is shown by the difference between the molecular ion and the fragments formed from it.

Which line in the EI spectrum is the molecular ion? Only a few molecules give dominant M^+ ions, e.g. aromatics and their derivatives, such as PCBs and dioxins. The molecular ion is frequently only present with a low intensity and with the small quantities of sample applied, as is the case with GC/MS, can only be identified with difficulty among the noise (matrix), or it fragments completely and cannot be seen in the spectrum.

Figure 2.181, which shows the EI/CI spectra of the phosphoric acid ester Tolclofos-methyl, is an example of this. The base peak in the EI spectrum shows a Cl atom. Loss of a methyl group $(M - 15)^+$ gives m/z 250. Is m/z 265 the nominal molecular mass? The CI spectrum shows m/z 301 for a protonated ion so the nominal molecular mass could be 300 u. The isotope pattern of two Cl atoms is also visible.

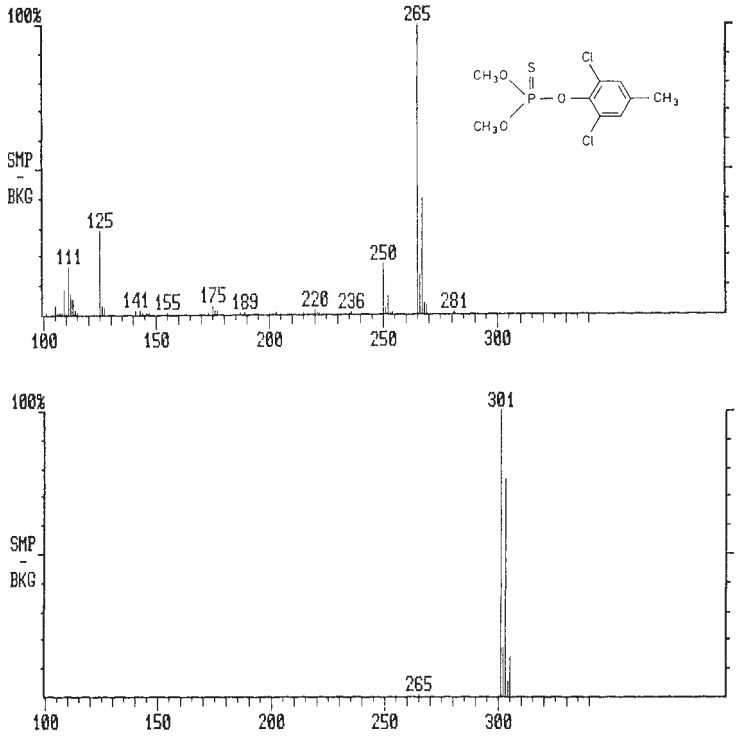


Fig. 2.181 EI and CI (NH_3) spectra of Tolclofos-methyl.

How can both EI and CI spectra be completed? Obviously Tolclofos fragments completely in EI by loss of a Cl atom to m/z 265 as $(M-35)^+$. With CI this fragmentation does not occur. The attachment of a proton retains the complete molecule with formation of the quasimolecular ion $(M+H)^+$.

The importance of EI spectra for identification and structure confirmation is due to the fragmentation pattern. All searches through libraries of spectra are based on EI spectra. With the introduction of the chemical ionisation capabilities for internal ionisation ion trap systems, a commercial CI library of spectra with more than 300 pesticides was first introduced by Finnigan.

The term chemical ionisation, unlike EI, covers all soft ionisation techniques which involve an exothermic chemical reaction in the gas phase mediated by a reagent gas and its reagent ions. Stable positive or negative ions are formed as products. Unlike the molecular ions of EI ionisation, the quasimolecular ions of CI are not radicals.

The principle of chemical ionisation was first described by Munson and Field in 1966. CI has now developed into a widely used technique for structural determination and quantitation in GC/MS. Instead of an open, easily evacuated ion source, a closed ion volume is necessary for carrying out chemical ionisation. In the high vacuum environment of the ion source, a reagent gas pressure of ca. 1 Torr must be maintained to achieve the desired CI reactions. Depending on the construction of the instrument, either changing the ion volume, an expensive change of the whole ion source or only a software switch is necessary (Fig. 2.182). Through the straightforward technical realisation in the case of combination ion sources and through the broadening of the use of ion trap mass spectrometers, CI has now become established in residue and environmental analysis, even for routine methods.

Chemical ionisation uses considerably less energy for ionising the molecule M. CI spectra therefore have fewer or no fragments and thus generally give important information on the molecule itself.

The use of chemical ionisation is helpful in structure determination, confirmation or determination of molecular weights, and also in the determination of significant substructures. Additional selectivity can be introduced into mass spectrometric detection by using the CI reaction of certain reagent gases, e.g. the detection of active substances with a transparent hydrocarbon matrix. Analyses can be quantified selectively, with high sensitivity and unaffected by the low molecular weight matrix, by the choice of a quantitation mass in the upper molecular weight range. The spectrum of analytical possibilities with CI is not limited to the basic reactions described briefly here. Furthermore, it opens up the whole field of chemical reactions in the gas phase.

The Principle of Chemical Ionisation

In CI two reaction steps are always necessary. In the *primary reaction* a stable cluster of reagent ions is produced from the reagent gas through electron bombardment. The composition of the reagent gas cluster is typical for the gas used. The cluster formed usually shows up on the screen for adjustment.

In the *secondary reaction* the molecule M in the GC eluate reacts with the ions in the reagent gas cluster. The ionic reaction products are detected and displayed as the CI spectrum. It is the secondary reaction which determines the appearance of the spectrum. Only exothermic reactions give CI spectra. In the case of protonation this means that the proton affinity PA of M must be higher than that of the reagent gas PA(R) (Fig. 2.183 and Table 2.44).

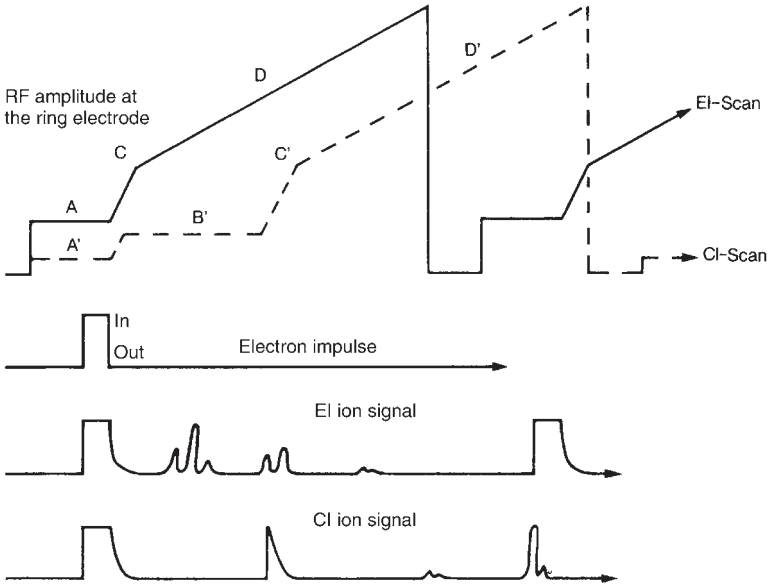


Fig. 2.182 Switching between the EI and CI scan functions in the case of an ion trap analyser with internal ionisation (Finnigan).
EI scan:
A Ionisation and storage of ions
C Starting mass
D Recording of an EI mass spectrum
CI scan:
A′ Ionisation and storage of reagent gas ions
B′ Reaction of reagent gas ions with neutral substance molecules
C′ Starting mass
D′ Recording of a CI mass spectrum

Through the choice of the reagent gas R, the quantity of energy transferred to the molecule M and thus the degree of possible fragmentation and the question of selectivity can be controlled. If PA(R) is higher than PA(M), no protonation occurs. When a nonspecific hydrocarbon matrix is present, this leads to transparency of the background, while active substances, such as plant protection agents, appear with high signal/noise ratios.

Primary reaction: Reagent gas cluster

$$R \xrightarrow{70\ eV} RH^+$$

Secondary reaction: Protonation

$$RH^+ + M \xrightarrow{t} MH^+ + R$$

Fig. 2.183 Primary and secondary reactions in protonation.

Table 2.44 Proton affinities of some simple compounds.

Aliphatic amines			
NH_3	857	n-Pr_2NH	951
$MeNH_2$	895	i-Pr_2NH	957
$EtNH_2$	907	n-Bu_2NH	955
n-$PrNH_2$	913	i-Bu_2NH	956
i-$PrNH_2$	917	s-Bu_2NH	965
n-$BuNH_2$	915	Me_3N	938
i-$BuNH_2$	918	Et_3N	966
s-$BuNH_2$	922	n-Pr_3N	976
t-$BuNH_2$	925	n-Bu_3N	981
n-Amyl-NH_2	918	Me_2EtN	947
Neopentyl-NH_2	920	$MeEt_2N$	957
t-Amyl-NH_2	929	Et_2-n-PrN	970
n-Hexyl-NH_2	920	Pyrrolidine	938
Cyclohexyl-NH_2	925	Piperidine	942
Me_2NH	922	N-methylpyrrolidine	952
MeEtNH	930	N-methylpiperidine	956
Et_2NH	941	$Me_3Si(CH_2)_3NMe_2$	966
Oxides and sulfides			
H_2O	723	n-Bu_2O	852
MeOH	773	i-PrO-t-Bu	873
EtOH	795	n-$Pentyl_2O$	858
n-PrOH	800	Tetrahydrofuran	834
t-BuOH	815	Tetrahydropyran	839
Me_2O	807	H_2S	738
MeOEt	844	MeSH	788
Et_2O	838	Me_2S	839
i-PrOEt	850	MeSEt	851
n-Pr_2O	848	Et_2S	859
i-Pr_2O	861	i-Pr_2S	875
t-BuOMe	852	H_2Se	742
Disubstituted alkanes			
$NH_2CH_2CH_2NH_2$	947	$Me_2N(CH_2)_6NMe_2$	1041
$NH_2CH_2CH_2CH_2NH_2$	973	$NH_2CH_2CH_2OMe$	933
$NH_2CH_2CH_2CH_2CH_2NH_2$	995	$NH_2CH_2CH_2CH_2OH$	952
$NH_2(CH_2)_5NH_2$	986	$NH_2(CH_2)_6OH$	966
$NH_2(CH_2)_6NH_2$	989	$NH_2CH_2CH_2CH_2F$	928
$Me_2NCH_2CH_2NMe_2$	996	$NH_2CH_2CH_2CH_2Cl$	928
$Me_2NCH_2CH_2CH_2NH_2$	1002	$OHCH_2CH_2CH_2CH_2OH$	886
$Me_2NCH_2CH_2CH_2NMe_2$	1012	2,4-Pentanedione	886
$Me_2N(CH_2)_4NMe_2$	1028		

Table 2.44 (continued)

Substituted alkylamines and alcohols			
$CH_2FCH_2NH_2$	890	CF_3NMe_2	815
$CHF_2CH_2NH_2$	871	CHF_2CH_2OH	755
$CF_3CH_2NH_2$	850	CF_3CH_2OH	731
$(CF_3)_3CNH_2$	800	CCl_3CH_2OH	760
$CF_3CH_2NHCH_3$	880	Piperazine	936
$CF_3CH_2CH_2NH_2$	885	1,4-Dioxan	811
$CF_3CH_2CH_2CH_2NH_2$	900	Morpholine	915
$CH_2FCH_2CH_2NH_2$	914		
Unsaturated amines and anilines			
$CH_2{=}CHCH_2NH_2$	905	m-$CH_3OC_6H_4NH_2$	906
Cyclo-$C_3H_5NH_2$	899	p-$CH_3OC_6H_4NH_2$	899
$CH_2{=}C(CH_3)CH_2NH_2$	912	p-$ClC_6H_4NH_2$	876
$HC{\equiv}CCH_2NH_2$	884	m-$ClC_6H_4NH_2$	872
$(CH_2{=}CCH_3CH_2)_3N$	964	m-$FC_6H_4NH_2$	870
$(CH_2{=}CHCH_2)_3N$	958	C_6H_5NHMe	912
$HC{\equiv}CCH_2)_3N$	916	$C_6H_5NMe_2$	935
$C_6H_5NH_2$	884	$C_6H_5CH_2NH_2$	918
m-$CH_3C_6H_4NH_2$	896	$C_6H_5CH_2NMe_2$	953
p-$CH_3C_6H_4NH_2$	896		
Other N, O, P, S compounds			
Aziridine	902	$CH_2{=}CHCN$	802
Me_2NH	922	ClCN	759
N-Methylaziridine	926	BrCN	770
Me_3N	938	CCl_3CN	760
MeNHEt	930	CH_2ClCN	773
2-Methylaziridine	916	CH_2ClCH_2CN	795
Pyridine	921	$NCCH_2CN$	757
Piperidine	942	i-PrCN	819
MeCH=NEt	931	n-BuCN	818
n-PrCH=NEt	942	cyclo-C_3H_6CN	824
$Me_2C{=}NEt$	959	Ethylene oxide	793
HCN	748	Oxetane	823
CH_3NH_2	895	CH_2O	741
MeCN	798	MeCHO	790
EtCN	806	$Me_2C{=}O$	824
n-PrCN	810	Thiirane	818

Table 2.44 (continued)

Carbonyl compounds, iminoethers and hydrazines			
EtCHO	800	CF_3CO_2-n-Bu	782
n-PrCHO	809	$HCO_2CH_2CF_3$	767
n-BuCHO	808	$NCCO_2Et$	767
i-PrCHO	808	$CF_3CO_2CH_2CH_2F$	764
Cyclopentanone	835	$(MeO)_2C{=}O$	837
HCO_2Me	796	HCO_2H	764
HCO_2Et	812	$MeCO_2H$	797
HCO_2-n-Pr	816	$EtCO_2H$	808
HCO_2-n-Bu	818	FCH_2CO_2H	781
$MeCO_2Me$	828	$ClCH_2CO_2H$	779
$MeCO_2Et$	841	CF_3CO_2H	736
$MeCO_2$-n-Pr	844	n-PrNHCHO	878
CF_3CO_2Me	765	Me_2NCHO	888
CF_3CO_2Et	777	$MeNHNH_2$	895
CF_3C0_2-n-Pr	781		
Substituted pyridines			
Pyridine	921	4-CF_3-pyridine	890
4-Me-pyridine	935	4-CN-pyridine	880
4-Et-pyridine	939	4-CHO-pyridine	900
4-t-Bu-pyridine	945	4-$COCH_3$-pyridine	909
2,4-diMe-pyridine	948	4-Cl-pyridine	910
2,4-di-t-Bu-pyridine	967	4-MeO-pyridine	947
4-Vinylpyridine	933	4-NH_2 pyridine	961
Bases weaker than water			
HF	468	CO_2	530
H_2	422	CH_4	536
O_2	423	N_2O	567
Kr	424	CO	581
N_2	475	C_2H_6	551
Xe	477		

All data in [kJ/mol] calculated for proton affinities PA(M) at 25 °C corresponding to the reaction $M + H^+ \rightarrow MH^+$ (after Aue and Bowers 1979)

For chemical ionisation many types of reaction can be used analytically. In gas phase reactions, not only positive, but also negative ions can be formed. GC/MS systems currently commercially available usually only detect positive ions (positive chemical ionisation, PCI). To detect negative ions (negative chemical ionisation, NCI), special equipment to reverse the polarity of the analyser potential and a multiplier with a conversion dynode are required. Specially equipped instruments also allow the simultaneous detection of positive and nega-

tive ions produced by CI. The alternating reversal of polarity during scanning (pulsed positive ion negative ion chemical ionisation, PPINICI) produces two complementary data files from one analysis.

1. Positive Chemical Ionisation

Essentially four types of reaction contribute to the formation of positive ions. As in all CI reactions, reaction partners meet in the gas phase and form a transfer complex $M \cdot R^+$. In the following types of reaction the transfer complex is either retained or reacts further.

Protonation

Protonation is the most frequently used reaction in positive chemical ionisation. Protonation leads to the formation of the quasimolecular ion $(M + H)^+$, which can then undergo fragmentation:

$$M + RH^+ \rightarrow MH^+ + R$$

Normally methane, water, methanol, isobutane or ammonia are used as protonating reagent gases (Table 2.45). Methanol occupies a middle position with regard to fragmentation and selectivity. Methane is less selective and is designated a hard CI gas. Isobutane and ammonia are typical soft CI gases.

Table 2.45 Reagent gases for proton transfer.

Gas	Reagent ion	PA [kJ/mol]
H_2	H_3^+	422
CH_4	CH_5^+	527
H_2O	H_3O^+	706
CH_3OH	$CH_3OH_2^+$	761
i-C_4H_{10}	t-$C_4H_9^+$	807
NH_3	NH_4^+	840

The CI spectra formed through protonation show the quasimolecular ions $(M + H)^+$. Fragmentations start with this ion. For example, loss of water shows up as M-17 in the spectrum, formed through $(M + H)^+ - H_2O$!

The existence of the quasimolecular ion is often indicated by low signals from addition products of the reagent gas. In the case of methane, besides $(M + H)^+$, $(M + 29)^+$ and $(M + 41)^+$ appear (see methane), and for ammonia, besides $(M + H)^+$, $(M + 18)^+$ with varying intensity (see ammonia).

Hydride Abstraction

In this reaction a hydride ion (H^-) is transferred from the substance molecule to the reagent ion:

$$M + R^+ \rightarrow RH + (M-H)^+$$

This process is observed, for example, in the use of methane when the C_2H_5 ion (m/z 29) contained in the methane cluster abstracts hydride ions from alkyl chains.

With methane as the reagent gas, both protonation and hydride abstraction can occur, depending on the reaction partner M. The quasimolecular ion obtained is either $(M-H)^+$ or $(M + H)^+$. In charge exchange reactions M^+ is also formed.

Charge Exchange

The charge exchange reaction gives a radical molecular ion with an odd number of electrons as in electron impact ionisation. Accordingly, the quality of the fragmentation is comparable to that of an EI spectrum. The extent of fragmentation is determined by the ionisation potential IP of the reagent gas.

$$M + R^+ \rightarrow R + M^+$$

The ionisation potentials of most organic compounds are below 15 eV. Through the choice of reagent gas it can be controlled whether only the molecular ion appears in the spectrum or whether, and how extensively fragmentation occurs. In the extreme case spectra similar to those with EI are obtained. Common reagent gases for ionisation by charge exchange are benzene, nitrogen, carbon monoxide, nitric oxide or argon (Table 2.46).

Table 2.46 Reagent gases for charge exchange reactions.

Gas	Reagent ion	IP [eV]
C_6H_6	$C_6H_6^+$	9.3
Xe	Xe^+	12.1
CO_2	CO_2^+	13.8
CO	CO^+	14.0
N_2	N_2^+	15.3
Ar	Ar^+	15.8
He	He^+	24.6

In the use of methane, charge exchange reactions as well as protonations can be observed, in particular for molecules with low proton affinities.

Adduct Formation

If the transition complex described above does not dissociate, the adduct is visible in the spectrum:

$$M + R^+ \rightarrow (M + R)^+$$

This effect is seldom made use of in GC/MS analysis in contrast to being the prevailing reaction in ESI-LC/MS, but must be taken into account on evaluating CI spectra. The enhanced formation of adducts is always observed with intentional protonation reactions

where differences in the proton affinity of the participating species are small. High reagent gas pressure in the ion source favours the effect by stabilising collisions.

Frequently an $(M + R)^+$ ion is not immediately recognised, but can give information which is as valuable as that from the quasimolecular ion formed by protonation. Cluster ions of this type nevertheless sometimes make interpretation of spectra more difficult, particularly when the transition complex does not lose immediately recognisable neutral species.

2. Negative Chemical Ionisation

Negative ions are also formed during ionisation in mass spectrometry even under EI conditions, but their yield is so extremely low that it is of no use analytically. The intentional production of negative ions can take place by addition of thermal electrons (analogous to an ECD), by charge exchange or by extraction of acidic hydrogen atoms.

Charge Transfer

The ionisation of the sample molecule is achieved by the transfer of an electron between the reagent ion and the molecule M

$$M + R^- \rightarrow M^- + R$$

The reaction can only take place if the electron affinity EA of the analyte M is greater than that of the electron donor R.

$$EA\ (M) > EA\ (R)$$

In practical analysis charge transfer to form negative ions is less important (unlike the formation of positive ions by charge exchange, see above).

Proton Abstraction

Proton transfer in negative chemical ionisation can be understood as proton abstraction from the sample molecule. In this way all substances with acidic hydrogens, e.g. alcohols, phenols or ketones, can undergo soft ionisation.

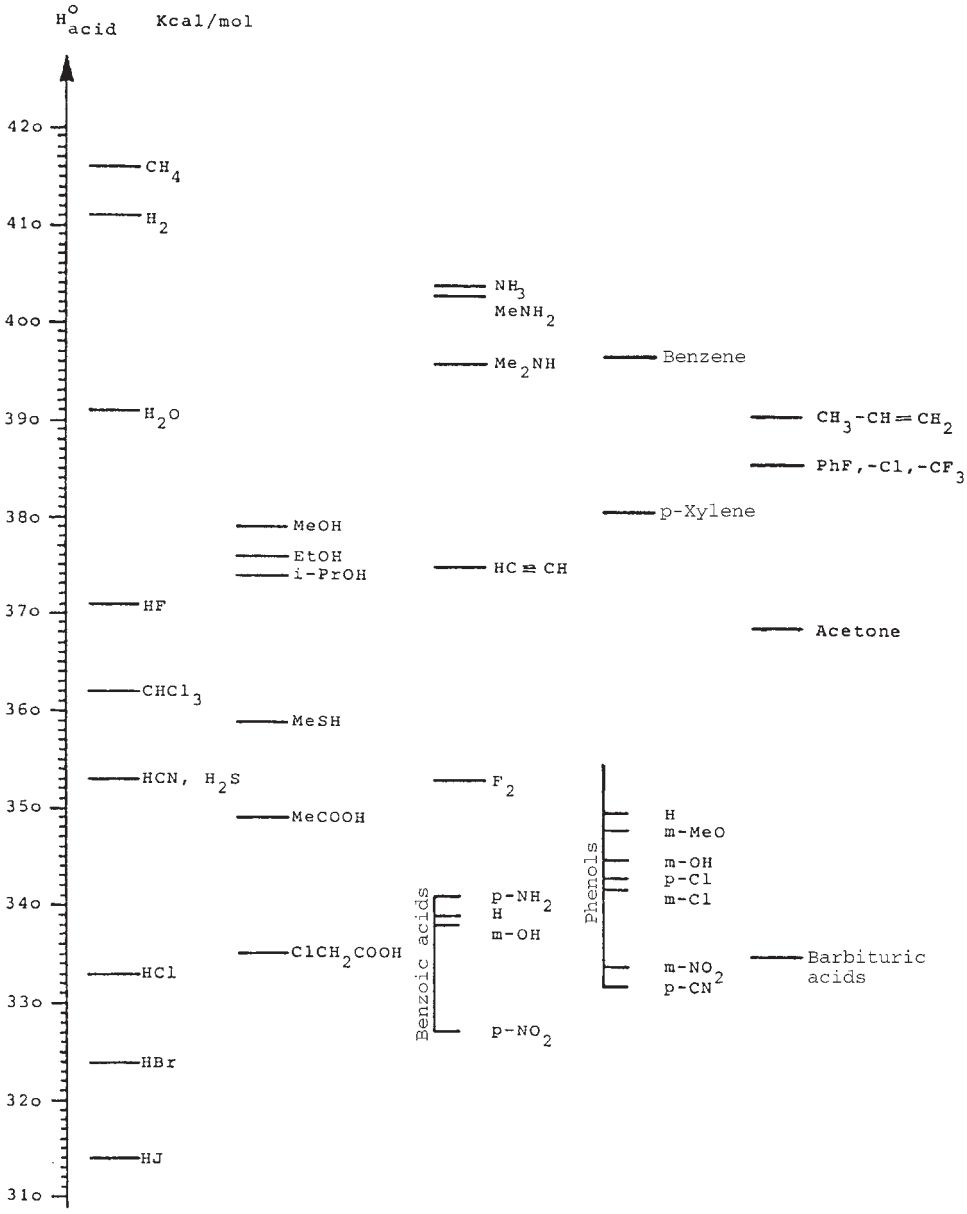
$$M + R^- \rightarrow (M{-}H)^- + RH$$

Proton abstraction only occurs if the proton affinity of the reagent gas ion is higher than that of the conjugate base of the analyte molecule. A strong base, e.g. OH^-, is used as the reagent for ionisation. Substances which are more basic than the reagent are not ionised.

Reagent gases and organic compounds were arranged in order of gas phase acidity by Bartmess and McIver in 1979. The order corresponds to the reaction enthalpy of the dissociation of their functional groups into a proton and the corresponding base. Table 2.47 can be used for controlling the selectivity via proton abstraction by choosing suitable reagent gases.

Extensive fragmentation reactions can be excluded by proton abstraction. The energy released in the exothermic reaction is essentially localised in the new compound RH. The anion formed does not contain any excess energy for extensive fragmentation.

Table 2.47 Scale of gas phase acidities (after Bartmess and McIver 1979).



Reagent Ion Capture

The capture of negative reagent gas ions was described in the early 1970s by Manfred von Ardenne and coworkers for the analysis of long-chain aliphatic hydrocarbons with hydroxyl ions.

$$M + R^- \rightarrow M \cdot R^-$$

Besides associative addition, with weak bases adduct formation can lead to a new covalent bond. The ions formed are more stable than the comparable association products.

Substitution reactions, analogous to an S_N2 substitution in solution, occur more frequently in the gas phase because of poor solvation and low activation energy. Many aromatics give a peak at $(M + 15)^-$, which can be attributed to substitution of H by an O^- radical. Substitution reactions are also known for fluorides and chlorides. Fluoride is the stronger nucleophile and displaces chloride from alkyl halides.

Electron Capture

Electron capture with formation of negative ions is the NCI ionisation process most frequently used in GC/MS analysis. There is a direct analogy with the behaviour of substances in ECD and the areas of use may also be compared. The commonly used term ECD-MS indicates the parallel mechanisms and applications. With negative chemical ionisation the lowest detection limits in organic mass spectrometry have been reached (Fig. 2.184). The detection of 100 ag octafluoronaphthalene corresponds to the detection of ca. 200 000 molecules!

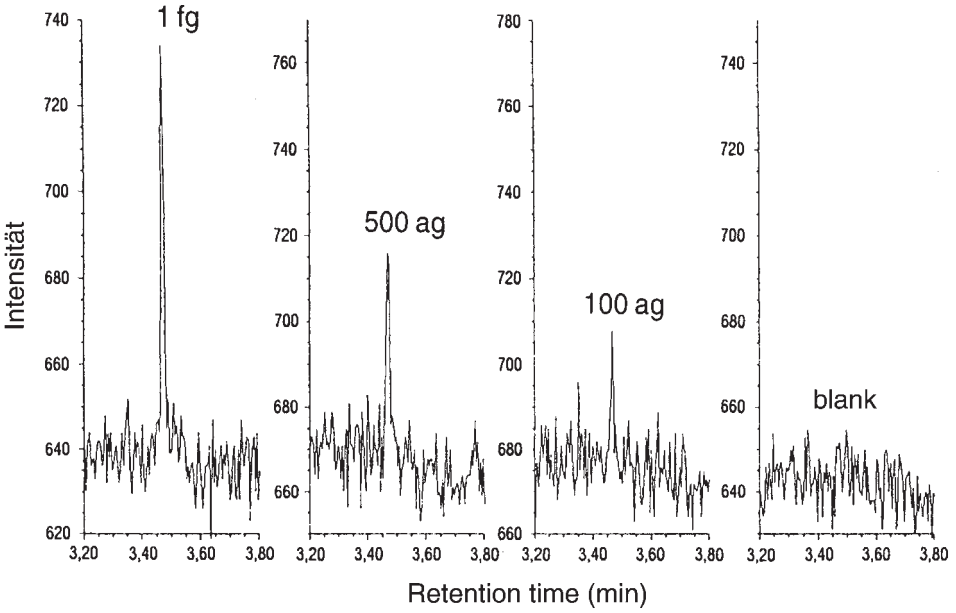


Fig. 2.184 GC/MS detection of traces of 10^{-15} to 10^{-16} g octafluoronaphthalene by NCI detection of the molecular ion m/z 272 (after McLafferty and Michnowicz 1992).

At the same energy electrons in the CI plasma have a much higher velocity (mobility) than those of the heavier positive reagent ions.

$$E = m/2 \cdot v^2 \qquad m(e^-) = 9.12 \cdot 10^{-28}\ g$$
$$m(CH_5^+) = 2.83 \cdot 10^{-22}\ g$$

Electron capture as an ionisation method is 100–1000 times more sensitive than ion/molecule reactions limited by diffusion. For substances with high electron affinities, higher sensitivities can be achieved than with positive chemical ionisation. NCI permits the detection of trace components in complex biological matrices (Fig. 2.185). Substances which have a high NCI response typically have a high proportion of halogen or nitro groups. In practice it has been shown that from ca. 5–6 halogen atoms in the molecule detection with NCI gives a higher specific response than that using EI (Fig. 2.186). For this reason, in analysis of polychlorinated dioxins and furans, although chlorinated, EI is the predominant method, in particular in the detection of 2,3,7,8-TCDD in trace analysis.

Another feature of NCI measurements is the fact that, like ECD, the response depends not only on the number of halogen atoms, but also on their position in the molecule. Precise quantitative determinations are therefore only possible with defined reference systems via the determination of specific response factors.

The key to sensitive detection of negative ions lies in the production of a sufficiently high population of thermal electrons. The extent of formation of M^- at sufficient electron density depends on the electron affinity of the sample molecule, the energy spectrum of the electron

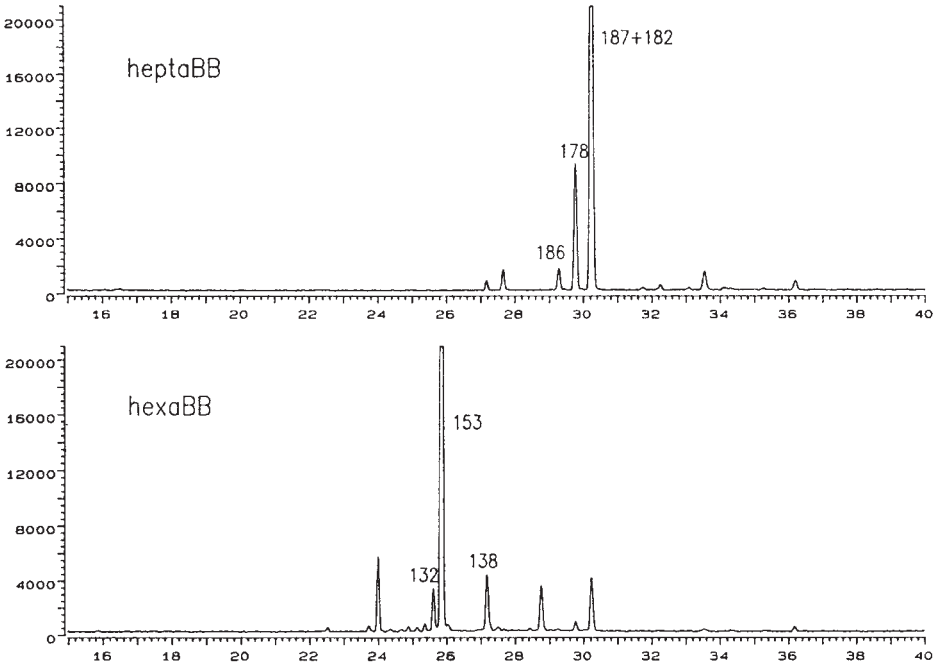


Fig. 2.185 Detection of heptabromobiphenylene (above) and hexabromobiphenylene (below) in human milk by NCI (after Fürst 1994).

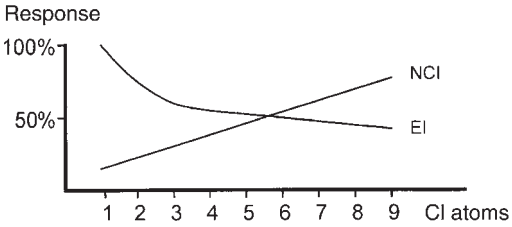


Fig. 2.186 Response dependence in the EI and NCI modes of the increasing number of Cl atoms in the molecule. The decrease in the response in the EI mode with increasing Cl content is caused by splitting of the overall signal by individual isotopic masses. In the NCI mode the response increases with increasing Cl content through the increase in the electronegativity for electrons capture (analogous to an ECD).

population and the frequency with which molecular anions collide with neutral particles and become stabilised (collision stabilisation). Even with an ion trap analyser, using external ionisation additions to give M^- can be utilised analytically. The storage of electrons in the ion trap itself is not possible because of their low mass (internal ionisation).

For residue analysis, derivatisation with perfluorinated reagents (e.g. heptafluorobutyric anhydride, perfluorobenzoyl chloride) in association with NCI is becoming important. Besides being easier to chromatograph, the compounds concerned have higher electron affinities allowing sensitive detection.

3. Reagent Gas Systems

Methane

Methane is one of the longest known and best studied reagent gases. As a hard reagent gas it has now been replaced by softer ones in many areas of analysis.

The reagent gas cluster of methane (Fig. 2.187) is formed by a multistep reaction, which gives two dominant reagent gas ions with m/z 17 and 29, and in lower intensity an ion with m/z 41.

CH_4 at 70 eV	$\rightarrow$ CH_4^+, CH_3^+, CH_2, CH^+	and others	
$CH_4^+ + CH_4$	$\rightarrow$ $CH_5^+ + CH_3$	m/z 17	50%
$CH_3^+ + CH_4$	$\rightarrow$ $C_2H_5^+ + H_2$	m/z 29	48%
$CH_2^+ + CH_4$	$\rightarrow$ $C_2H_3^+ + H_2 + H$		
$C_2H_3^+ + CH_4$	$\rightarrow$ $C_3H_5^+ + H_2$	m/z 41	2%

Good CI conditions are achieved if a ratio of m/z 17 to m/z 16 of 10:1 is set up. Experience shows that the correct methane pressure is that at which the ions m/z 17 and 29 dominate in the reagent gas cluster and have approximately the same height with good resolution. The ion m/z 41 should also be recognisable with lower intensity.

Methane is mainly used as the reagent gas in protonation reactions, charge exchange processes (PCI), and in pure form or as a mixture with N_2O in the formation of negative ions (NCI). In protonation methane is a hard reagent gas. For substances with lower proton affinity methane frequently provides the final possibility of obtaining CI spectra. The adduct ions $(M + C_2H_5)^+ = (M + 29)^+$ and $(M + C_3H_5)^+ = (M + 41)^+$ formed by the methane cluster help to confirm the molecular mass interpretation.

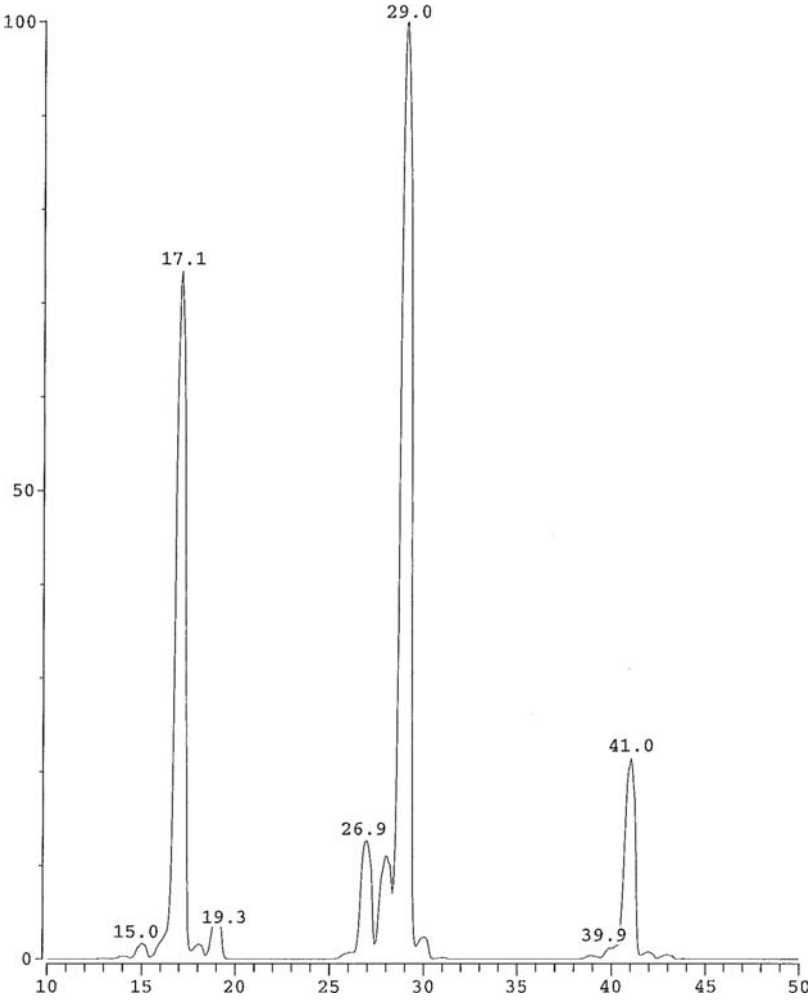


Fig. 2.187 Reagent gas cluster with methane.

Methanol

Because of its low vapour pressure, methanol is ideal for CI in ion trap instruments with internal ionisation. Neither pressure regulators nor cylinders or a long tubing system are required. The connection of a glass flask or a closed tube containing methanol directly on to the CI inlet is sufficient. In addition every laboratory has methanol available. Also for ion traps using external ionisation and quadrupole instruments liquid Cl devices are commercially available.

$(CH_3OH \cdot H)^+$ is formed as the reagent ion, which is adjusted to high intensity with good resolution (Fig. 2.188). The appearance of a peak at m/z 47 shows the dimer formed by loss of water (dimethyl ether), which is only produced at sufficiently high methanol concentrations. It does not function as a protonating reagent ion, but its appearance shows that the pressure adjustment is correct.

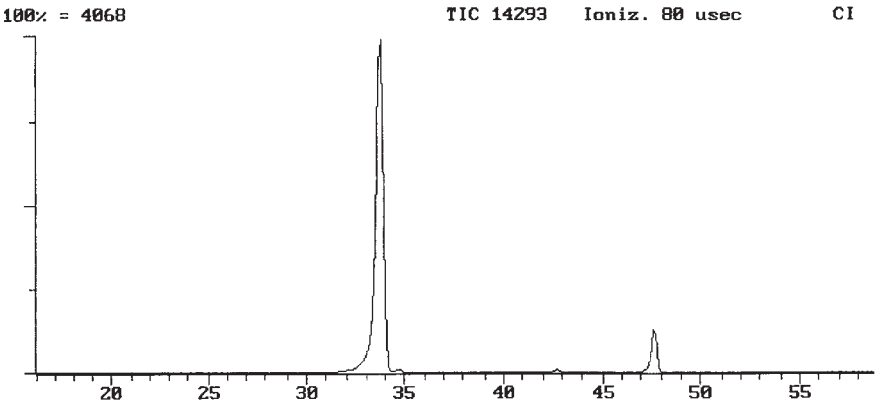


Fig. 2.188 Methanol as reagent gas (ion trap with internal ionisation).

Methanol is used exclusively for protonation. Because of its medium proton affinity, methanol allows a broad spectrum of classes of compounds to be determined. It is therefore suitable for a preliminary CI measurement of compounds not previously investigated. The medium proton affinity does not give any pronounced selectivity. However, substances with predominantly alkyl character remain transparent. Fragments have low intensities.

Water

For most mass spectroscopists water is a problematic substance. However, as a reagent gas, water has extraordinary properties. Because of the high conversion rate into H_3O^+ ions, and its low proton affinity, water achieves a high response for many compounds when used as the reagent gas. The spectra obtained usually have few fragments and concentrate the ion beam on a dominant ion.

When water is used as the reagent gas (Fig. 2.189), the intensity of the H_3O^+ ion should be as high as possible. With ion trap instruments with internal ionisation, no additional equipment is required. However a short tube length should be used for good adjustment.

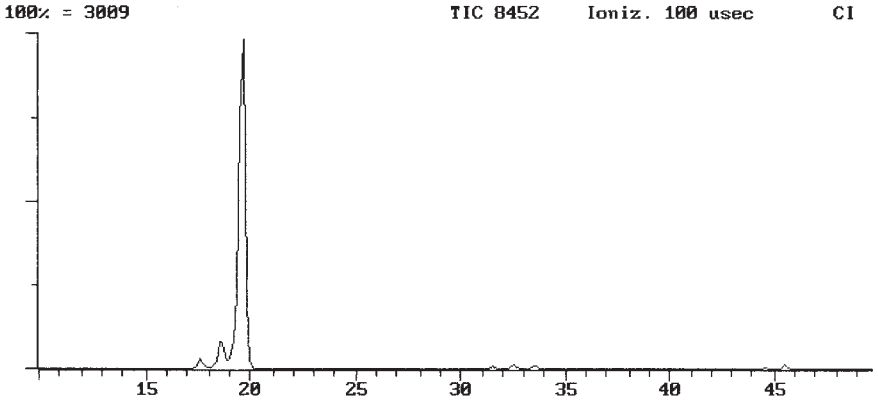


Fig. 2.189 Water as reagent gas (ion trap with internal ionisation).

For instruments with high pressure CI ion sources, the use of a heated reservoir and completely heated inlet tubing are imperative. Quadrupole instruments and ion traps using external ionisation require a special liquid CI device with heated CI gas reservoir and inlet lines.

The use of water is universal. In the determination of polyaromatic hydrocarbons considerable increases in response compared with EI detectors are found. Analytical procedures have even been published for nitroaromatics. Water can also be used successfully as a reagent gas for screening small molecules, e.g. volatile halogenated hydrocarbons (industrial solvents), as it does not interfere with the low scan range for these substances.

Isobutane

Like that of methane, chemical ionisation with isobutane has been known and well documented for years. The t-butyl cation (m/z 57) is formed in the reagent gas cluster and is responsible for the soft character of the reagent gas (Fig 2.190).

Isobutane is used for protonation reactions of multifunctional and polar compounds. Its selectivity is high and there is very little fragmentation. In practice, significant coating of the ion source through soot formation has been reported, which can even lead to dousing of the filament.

This effect depends on the adjustment and on the instrument. In such cases ammonia can be used instead.

Ammonia

To supply the CI system with ammonia a steel cylinder with a special reducing valve is necessary. Because of the aggressive properties of the gas, the entire tubing system must be made of stainless steel. In ion trap instruments only the ammonium ion NH_4^+ with mass m/z 18 is formed in the reagent gas cluster. At higher pressures adducts of the ammonium ion with ammonia $(NH_3)_n \cdot NH_4^+$ can be formed in the ion source.

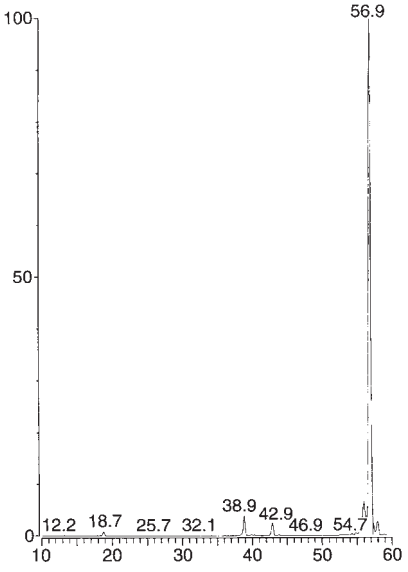


Fig. 2.190 iso-Butane as reagent gas.

Warning: Very often the ammonium ion is confused with water. Freshly installed reagent gas tubing generally has an intense water background with high intensities at m/z 18 and 19 as H_2O^+/H_3O^+. Clean tubing and correctly adjusted NH_3 CI gas shows no intensity at mass m/z 19 (Fig 2.191)!

Ammonia is a very soft reagent gas for protonation. The selectivity is correspondingly high, which is made use of in the residue analysis of many active substances. Fragmentation reactions only occur to a small extent with ammonia CI.

Adduct formation with NH_4^+ occurs with substances where the proton affinity differs little from that of NH_3 and can be used to confirm the molecular mass interpretation. In these cases the formation and addition of higher $(NH_3)_n \cdot NH_4^+$ clusters is observed with instruments with threshold pressures of ca. 1 Torr. Interpretation and quantitation can thus be impaired with such compounds.

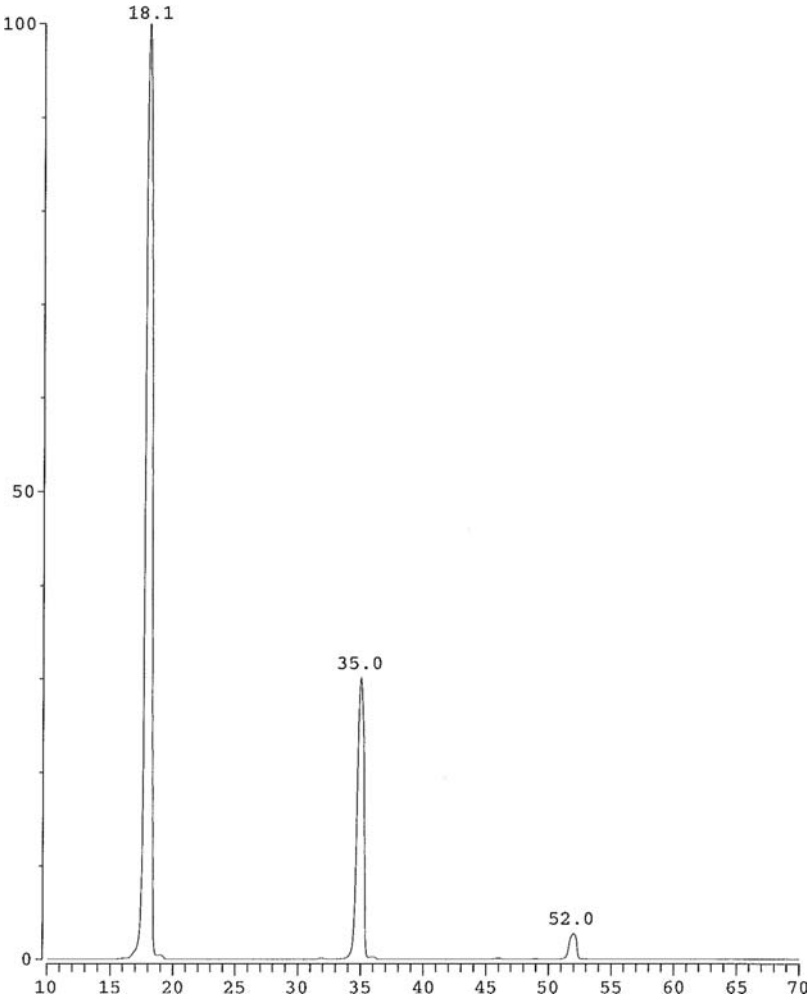


Fig. 2.191 Ammonia as reagent gas.

4. Aspects of Switching Between EI and CI

Quadrupole and Magnetic Sector Instruments

To initiate CI reactions and to guarantee a sufficient conversion rate, an ion source pressure of ca. 1 Torr in an analyser environment of 10^{-5}–10^{-7} Torr is necessary for beam instruments. For this, the EI ion source is replaced by a special CI source, which must have a gas-tight connection to the GC column, the electron beam and the ion exit in order to maintain the pressure in these areas.

Combination sources with mechanical devices for sealing the EI to the CI source have so far only proved successful with magnetic sector instruments. With the very small quadrupole sources, there is a significant danger of small leaks. As a consequence the response is below optimised sources and EI/CI mixed spectra may be produced.

Increased effort is required for conversion, pumping out and calibrating the CI source in beam instruments. Because of the high pressure, the reagent gas also leads to rapid contamination of the ion source and thus to additional cleaning measures in order to restore the original sensitivity of the EI system. Readily exchangeable ion volumes have been shown to be ideal for CI applications. This permits a high CI quality to be attained and, after a rapid exchange, unaffected EI conditions to be restored.

Ion Trap Instruments

Ion trap mass spectrometers with internal ionisation can be used immediately for CI without conversion. Because of their mode of operation as storage mass spectrometers, only an extremely low reagent gas pressure is necessary for instruments with internal ionisation. The pressure is adjusted by means of a special needle valve which is operated at low leakage rates and maintains a partial pressure of only about 10^{-5} Torr in the analyser. The overall pressure of the ion trap analyser of about 10^{-4}–10^{-3} Torr remains unaffected by it. CI conditions thus set up give rise to the term low pressure CI. Compared to the conventional ion source used in high pressure CI, in protonation reactions, for example, a clear dependence of the CI reaction on the proton affinities of the reaction partners is observed. Collision stabilisation of the products formed does not occur with low pressure CI. This explains why "high pressure" CI-typical adduct ions are not formed here, which would confirm the identification of the (quasi)molecular ion (e.g. with methane besides $(M + H)^+$, also M + 29 and M + 41 are expected). The determination of ECD-active substances by electron capture is not possible with low pressure CI.

Switching between EI and CI modes in an ion trap analyser with internal ionisation takes place with a keyboard command or through the scheduled data acquisition sequence in automatic operations. All mechanical devices necessary in beam instruments are dispensed with completely. The ion trap analyser is switched to a CI scan function internally without effecting mechanical changes to the analyser itself.

The CI reaction is initiated when the reagent ions are made ready by changing the operating parameters and a short reaction phase has taken place in the ion trap analyser. The scan function used in the CI mode with ion trap instruments (see Fig. 2.182) clearly shows two plateaus which directly correspond to the primary and secondary reactions. After the end of the secondary reaction the product ions, which have been produced and stored, are determined by the mass scan and the CI spectrum registered. In spite of the presence of the reagent gas, typical EI spectra can therefore be registered in the EI mode.

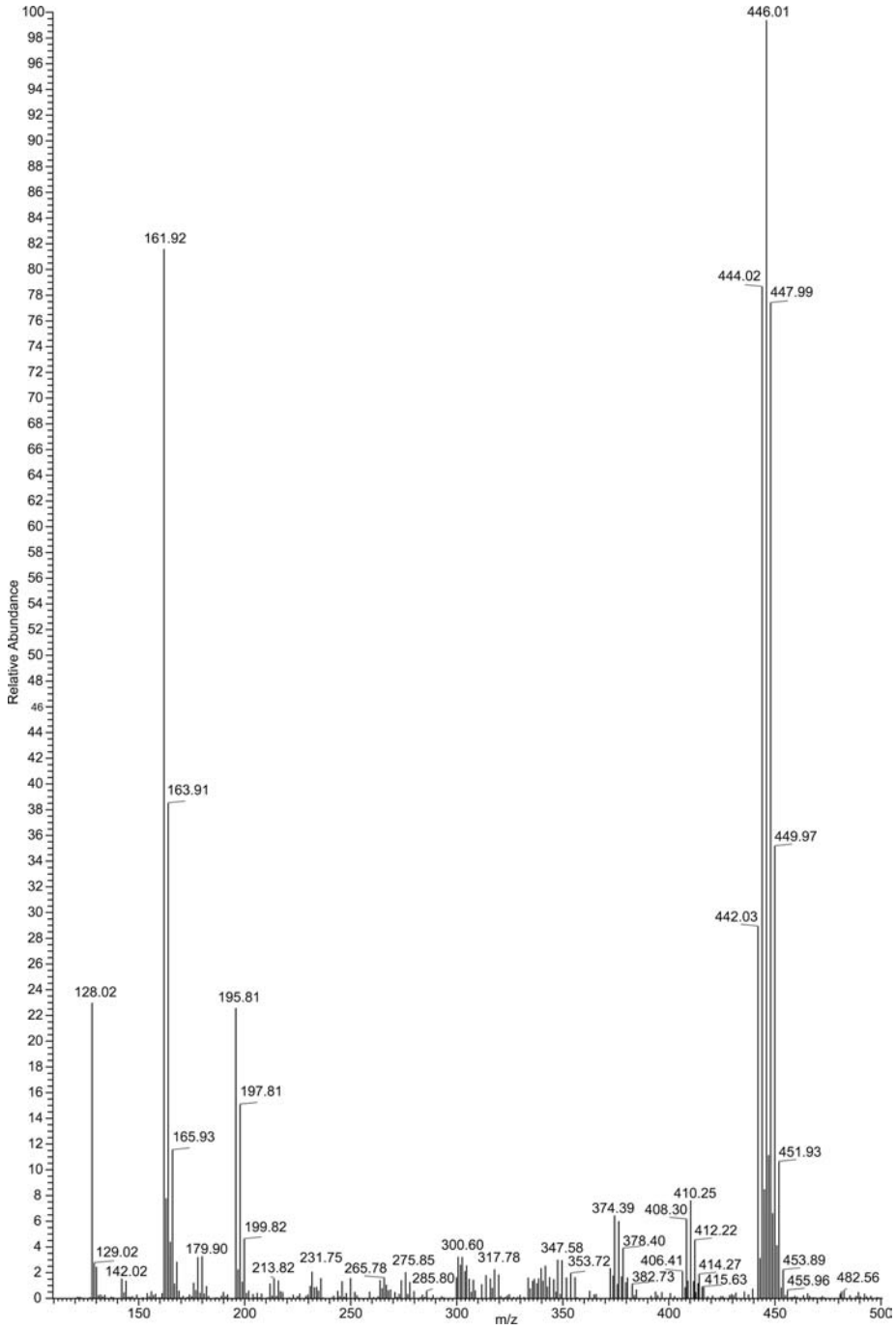


Fig. 2.192 NCI spectrum of a Toxaphen component (Parlar 69, polychlorinated bornane). The typical fragmentation known from EI ionisation is absent, the total ion current is concentrated on the molecular ion range (Theobald 2007).

The desired chemical ionisation is made possible by simply switching to the CI operating parameters.

On using autosamplers it is therefore possible to switch alternately between EI and CI data acquisition and thus use both ionisation processes routinely in automatic operations. The danger of additional contamination by CI gas does not occur with ion trap instruments because of the extremely small reagent gas input and allows this mode of operation to be run without impairing the quality.

Ion trap instruments with external ionisation have an ion source with a conventional construction. Changing between EI and CI ionisation takes place by changing the ion volume. Chemical ionisation in the classical manner of high pressure CI is thus carried out and thus the formation of negative ions by electron capture (NCI) in association with an ion trap analyser is made possible (Fig. 2.192).

2.3.5 Measuring Techniques in GC/MS

In data acquisition by the mass spectrometer there is a significant difference between detection of the complete spectrum (full scan) and the recording of individual masses (SIM, selected ion monitoring; MID multiple ion detection; SRM/MRM selected/multiple reaction monitoring). Particularly with continually operating spectrometers (ion beam instruments: magnetic sector MS, quadrupole MS) there are large differences between these two recording techniques with respect to selectivity, sensitivity and information content. For spectrometers with storage facilities (ion storage: ion trap MS, Orbitrap/ICR-MS) these differences are less strongly pronounced. Besides one-stage types of analyser (GC/MS), multistage mass spectrometers (GC/MS/MS) are playing an increasingly important role in residue target compound analysis and structure determination. With the MS/MS technique (multidimensional mass spectrometry), which is available in both beam instruments and ion storage mass spectrometers, much more analytical information and a high structure related selectivity for target compound quantitations can be obtained.

2.3.5.1 Detection of the Complete Spectrum (Full Scan)

The continuous recording of mass spectra (full scan) and the simultaneous determination of the retention time allow the identification of analytes by comparison with libraries of mass spectra. With beam instruments it should be noted that the sensitivity required for recording the spectrum depends on the efficiency of the ion source, the transmission through the analyser and, most particularly, on the dwell time of the ions. The dwell time per mass is given by the width of the mass scan (e. g. 50–550 u) and the scan rate of the chromatogram (e. g. 500 ms). From this a scan rate of 1000 u/s is calculated. Effective scan rates of modern quadrupole instruments exceed 11 000 u/s. Each mass from the selected mass range is measured only once during a scan over a short period (here 1 ms/u, Fig. 2.193). All other ions formed from the substance in parallel in the ion source are not detected during the mass scan (quadrupole as mass filter). Typical sensitivities for most compounds with benchtop quadrupole systems lie in the region of 1 ng of substance or less. Prolonging the scan time can increase the sensitivity of these systems for full scan operation (Fig. 2.193). However, there is an upper limit because of the rate of the chromatography. In practice for coupling

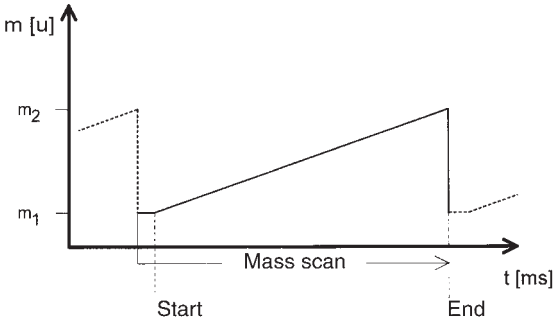


Fig. 2.193 Scan function of the quadrupole analyser: each mass between the start of the scan (m_1) and the end (m_2) is only registered once during the scan.

with capillary gas chromatography, scan rates of 0.5–1 s are used. For quantitative determinations it should be ensured that the scan rate of the chromatographic peak is adequate in order to determine the area and height correctly (see also Section 3.3). The SIM/MID mode is usually chosen to increase the sensitivity and scan rates of quadrupole systems for this reason (see also Section 2.3.5.2).

In ion storage mass spectrometers all the ions produced on ionisation of a substance are detected in parallel. The mode of function is opposite to the filter character of beam instruments and particularly strong when integrating weak ion beams (Table 2.48). All the ions formed are collected in a first step in the ion trap. At the end of the storage phase the ions, sorted according to mass during the scan, are directed to the multiplier. This process can take place very rapidly (Fig. 2.194). The scan rates of ion trap mass spectrometers are higher than 11 000 u/s. Typical sensitivities for full spectra in ion trap mass spectrometers are in the low pg range.

Table 2.48 Duty cycle for ion trap and beam instruments.

Scan range	Dwell time per mass		Sensitivity ratio
	Ion trap	Quadrupole	Ion trap/Quadrupole
[u]	[s/u]	[s/u]	
1	0.83	1	0.8
3	0.82	0.33	2.5
10	0.79	0.1	8
30	0.71	0.033	21
100	0.52	0.01	52
300	0.30	0.0033	91

The longer dwell time per mass leads to the highest sensitivity in the recording of complete mass spectra with ion trap instruments. Compared with beam instruments an increase in the duty cycle is achieved, depending on the mass range, of up to a factor of 100 and higher.

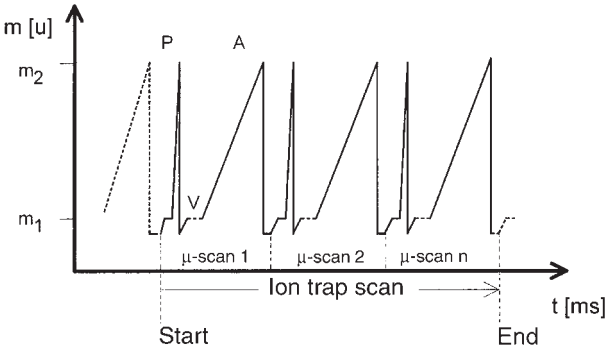


Fig. 2.194 Scan function of the ion trap analyser: within an ion trap scan, several μ-scans (three μ-scans shown here) are carried out and their spectra added before storage to disk.
P = pre-scan
A = analytical scan
V = variable ionisation time (AGC, automatic gain control)
(With ion trap instruments with external ionisation V stands for the length of the storage phase, ion injection time; for internal ionisation ion trap V stands for the ionisation time).

Dwell Times *t* Per Ion in Full Scan Acquisition With Ion Trap and Quadrupole MS

Mass range 50–550 u (500 masses wide), scan rate 500 ms

Ion trap MS	**Quadrupole MS**
Ion storage during ionisation. Ionisation time can vary, typically up to $t = 25$ ms at simultaneous storage of *all* ions formed.	$t = 500$ u/500 ms $= 1$ ms/u
Detection of *all* stored ions.	In a scan each type of ion is measured for only 1 ms; only a minute quantity of the ions formed are detected.

2.3.5.2 Recording Individual Masses (SIM/MID)

In the use of conventional mass spectrometers (beam instruments), the detection limit in the full scan mode is frequently insufficient for residue analysis because the analyser only has a very short dwell time per ion available during the scan. Additional sensitivity is achieved by dividing the same dwell time between a few selected ions by means of individual mass recording (SIM, MID) (Table 2.49 and Fig. 2.195).

At the same time a higher scan rate can be chosen so that chromatographic peaks can be plotted more precisely. The SIM technique is used exclusively for quantifying data on known target compounds, especially in trace analysis.

The mode of operation of a GC/MS system as a mass-selective detector requires the selection of certain ions (fragments, molecular ions), so that the desired analytes can be detected selectively. Other compounds contained in the sample besides those chosen for analysis re-

Table 2.49 Dwell times per ion and relative sensitivity in SIM analysis (for beam instruments) at constant scan rates.

Number of SIM ions	Total scan time[1] [ms]	Total voltage setting time[2] [ms]	Effective dwell time per ion[3] [ms]	Relative sensitivity[4] [%]
1	500	2	498	100
2	500	4	248	50
3	500	6	165	33
4	500	8	123	25
10	500	20	48	10
20	500	40	23	5
30	500	60	15	3
40	500	80	11	3
50	500	100	8	2
For comparison full scan:				
500	500	2	1	1

1) The total scan time is determined by the necessary scan rate of the chromatogram and is held constant.
2) Total voltage setting times are necessary in order to adjust the mass filter for the subsequent SIM masses. The actual times necessary can vary slightly depending on the type of instrument.
3) Duty cycle/ion.
4) The relative sensitivity is directly proportional to the effective dwell time per ion.

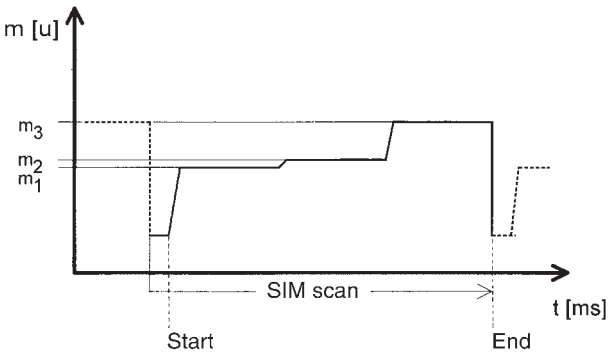


Fig. 2.195 SIM scan with a quadrupole analyser: the total scan time is divided here into the three individual masses m_1, m_2, and m_3 with correspondingly long dwell times.

main undetected. Thus the matrix present in large quantities in trace analysis is masked out, as are analytes whose appearance is not expected or planned. In the choice of masses required for detection it is assumed that for three selective signals in the fragmentation pattern per substance a secure basis for a yes/no decision can be found in spite of variations in the retention times (SIM, selected ion monitoring, MID, multiple ion detection). Identification of substances by comparison with spectral libraries is no longer possible. The relative intensities of

the selected ions serve as quality criteria (qualifiers) (1 ion – no criterion, 2 ions – 1 criterion, 3 ions – 3 criteria!). This process for detecting compounds can be affected by errors through shifts in retention times caused by the matrix. In residue analysis it is known that with SIM analysis false positive findings occur in ca. 10% of the samples. Recently positive SIM data have been confirmed in the same way as positive results from classical GC detectors by running a complete mass spectrum of the analytes suspected. Confirmation of positive results, and statistically of negative results as well, is required by international directives either by full scan, ion ratios or HRMS.

Gain in Sensitivity Using SIM/MID

A typical SIM data acquisition of 5 selected masses at a scan rate of 0.5 s is given as a typical example:

	Ion trap MS	**Quadrupole MS**
Dwell time per ion:	Identical ionisation procedure to that with full scan, however selective and parallel or sequential storage of the selected SIM ions.	At a scan time of 500 ms the effective dwell time per SIM mass is divided up as t = 500 ms/ 5 masses = 100 ms/mass.
Function:	The ion trap is filled exclusively with the ions with the selected masses. If the capacity of the ion trap is not used up completely, the storage phase ends after a given time (ms).	To measure an SIM mass the quadrupole spends 100 times longer on one mass compared with full scan, and thus permits a dwell time which is 100 times longer for the selected ions to be achieved.
Sensitivity:	The gain in sensitivity is most marked with matrix-containing samples, as the length of the storage phase still mainly depends on the appearance of the selected SIM ions in the sample and is not shortened by a high concentration of matrix ions.	Theoretically the sensitivity increases by a factor of 100. In practice for real samples a factor of 30–50 compared with full scan is achieved.
Consequences for trace analysis:	Ion trap systems already give very high sensitivity in the full scan mode. Samples with high concentrations of matrix and detection limits below the pg level require the SIM technique (MS/MS is recommended).	Quadrupole systems require the SIM mode to achieve adequate sensitivity.

Confirmation:	For 3 SIM masses by 3 intensity criteria (qualifiers), with MS/MS by means of the product ion spectra.	For 3 SIM masses by 3 intensity criteria (qualifiers), check of positive results after further concentration by the full scan technique or external confirmation with an ion trap instrument (see Table 2.49).

SIM Set-up

1. Choice of column and program optimisation for optimal GC separation, paying particular attention to analytes with similar fragmentation patterns.
2. Full scan analysis of an average substance concentration to determine the selective ions (SIM masses, 2–3 ions/component); special matrix conditions are to be taken into account.
3. Determination of the retention times of the individual components.
4. Establishment of the data acquisition interval (time window) for the individual SIM descriptors.
5. Test analysis of a low standard (or better, a matrix spike) and possible optimisation (SIM masses, separation conditions).

Planning an analysis in the SIM/MID mode first requires a standard run in the full scan mode to determine both the retention times and the mass signals necessary for the SIM selection (Tables 2.50 and 2.51). As the gain in sensitivity achieved in individual mass recording with beam instruments is only possible on detection of a few ions, for the analysis of several compounds the group of masses detected must be adjusted. The more components there are to be detected, the more frequently and precisely must the descriptors be adjusted. Multicomponent analyses, such as the MAGIC-60 analysis with purge and trap (volatile halogenated hydrocarbons see Section 4.5), cannot be dealt easily with in the SIM mode.

The use of SIM analysis with ion trap mass spectrometers has also been developed. Through special control of the analyser (waveform ion isolation) during the ionisation phase only the preselected ions of analytical interest are stored (SIS, selective ion storage). This technique allows the detection of selected ions in ion storage mass spectrometers in spite of the presence of complex matrices or the co-elution of another component in high concentration. As the storage capacity of the ion trap analyser is only used for a few ions instead of for a full spectrum, extremely low detection limits are possible (< 1 pg/component) and the usable dynamic range of the analyser is extended considerably. Unlike conventional SIM operations with beam instruments, the detection sensitivity only alters slightly with the number of selected ions using the ion trap SIM technique (Fig. 2.196). For the SIM technique the sensitivity depends almost exclusively on the ionisation time. The SIM technique with ion trap instruments is regarded as a necessary prerequisite for carrying out MS/MS detection.

Table 2.50 Characteristic ions (m/z values) for selected polycondensed aromatics and their alkyl derivatives (in the elution sequence for methylsilicone phases).

Benzene-d_6	92, 94	Pyrene	202
Benzene	77, 78	Methylfluoranthene	215, 216
Toluene-d_8	98, 100	Benzofluorene	215, 216
Toluene	91, 92	Phenylanthracene	252, 253, 254
Ethylbenzene	91, 106	Benzanthracene	228
Dimethylbenzene	91, 106	Chrysene-d_{12}	240
Methylethylbenzene	105, 120	Chrysene	228
Trimethylbenzene	105, 120	Methylchrysene	242
Diethylbenzene	105, 119, 134	Dimethylbenz[a]anthracene	239, 241, 256
Naphthalene-d_8	136	Benzo[b]fluoranthene	252
Naphthalene	128	Benzo[j]fluoranthene	252
Methylnaphthalene	141, 142	Benzo[k]fluoranthene	252
Azulene	128	Benzo[e]pyrene	252
Acenaphthene	154	Benzo[a]pyrene	252
Biphenyl	154	Perylene-d_{12}	264
Dimethylnaphthalene	141, 155, 156	Perylene	252
Acenaphthene-d_{10}	162, 164	Methylcholanthrene	268
Acenaphthene	152	Diphenylanthracene	330
Dibenzofuran	139, 168	Indeno[1,2,3-cd]pyrene	276
Dibenzodioxin	184	Dibenzanthracene	278
Fluorene	165, 166	Benzo[b]chrysene	278
Dihydroanthracene	178, 179, 180	Benzo[g,h,i]perylene	276
Phenanthrene-d_{10}	188	Anthanthrene	276
Phenanthrene	178	Dibenzo[a,l]pyrene	302
Anthracene	178	Coronene	300
Methylphenanthrene	191, 192	Dibenzo[a,i]pyrene	302
Methylanthracene	191, 192	Dibenzo[a,h]pyrene	302
Phenylnaphthalene	204	Rubicene	326
Dimethylphenanthrene	191, 206	Hexaphene	328
Fluoranthene	202	Benzo[a]coronene	350

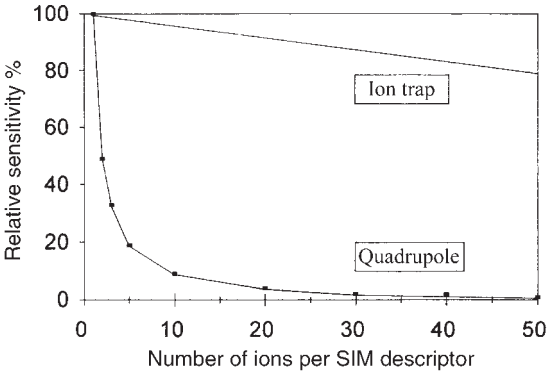


Fig. 2.196 Comparison of the SIS (ion trap analyser) and the SIM (quadrupole analyser) techniques based on the effective dwell time per ion (relative sensitivity).

Table 2.51 Main fragments and relative intensities for pesticides and some of their derivatives (DFG 1992).

Compound	Molar mass	Main fragment m/z (intensities)											
		1		2		3		4		5		6	
Acephate	183	43	(100)	44	(88)	136	(80)	94	(58)	47	(56)	95	(32)
Alaclor	269	45	(100)	188	(23)	160	(18)	77	(7)	146	(6)	224	(6)
Aldicarb	190	41	(100)	86	(89)	58	(85)	85	(61)	87	(50)	44	(50)
Aldrin	362	66	(100)	91	(50)	79	(47)	263	(42)	65	(35)	101	(34)
Allethrin	302	123	(100)	79	(40)	43	(32)	81	(31)	91	(29)	136	(27)
Atrazine	215	43	(100)	58	(84)	44	(75)	200	(69)	68	(43)	215	(40)
Azinphos-methyl	317	77	(100)	160	(77)	132	(67)	44	(30)	105	(29)	104	(27)
Barban	257	51	(100)	153	(76)	87	(66)	222	(44)	52	(43)	63	(43)
Benzazolin methyl ester	257	170	(100)	134	(75)	198	(74)	257	(73)	172	(40)	200	(31)
Bendiocarb	223	151	(100)	126	(58)	166	(48)	51	(19)	58	(18)	43	(17)
Bromacil	260	205	(100)	207	(75)	42	(25)	70	(16)	206	(16)	162	(12)
Bromacil N-methyl derivative	274	219	(100)	221	(68)	41	(45)	188	(41)	190	(40)	56	(37)
Bromophos	364	331	(100)	125	(91)	329	(80)	79	(57)	109	(53)	93	(45)
Bromophos-ethyl	392	97	(100)	65	(35)	303	(32)	125	(28)	359	(27)	109	(27)
Bromoxynil methyl ether	289	289	(100)	88	(77)	276	(67)	289	(55)	293	(53)	248	(50)
Captafol	347	79	(100)	80	(42)	77	(28)	78	(19)	151	(17)	51	(13)
Captan	299	79	(100)	80	(61)	77	(56)	44	(44)	78	(37)	149	(34)
Carbaryl	201	144	(100)	115	(82)	116	(48)	57	(31)	58	(20)	63	(20)
Carbendazim	191	159	(100)	191	(57)	103	(38)	104	(37)	52	(32)	51	(29)
Carbetamid	236	119	(100)	72	(54)	91	(44)	45	(38)	64	(37)	74	(29)
Carbofuran	221	164	(100)	149	(70)	41	(27)	58	(25)	131	(25)	122	(25)
Chlorbromuron	292	61	(100)	46	(24)	62	(11)	63	(10)	60	(9)	124	(8)
Chlorbufam	223	53	(100)	127	(20)	51	(13)	164	(13)	223	(13)	70	(10)
cis-Chlordane	406	373	(100)	375	(84)	377	(46)	371	(39)	44	(36)	109	(36)
trans-Chlordane	406	373	(100)	375	(93)	377	(53)	371	(47)	272	(36)	237	(30)
Chlorfenprop-methyl	232	125	(100)	165	(64)	75	(46)	196	(43)	51	(43)	101	(37)
Chlorfenvinphos	358	81	(100)	267	(73)	109	(55)	269	(47)	323	(26)	91	(23)
Chloridazon	221	77	(100)	221	(60)	88	(37)	220	(35)	51	(26)	105	(24)
Chloroneb	206	191	(100)	193	(61)	206	(60)	53	(57)	208	(39)	141	(35)
Chlorotoluron	212	72	(100)	44	(29)	167	(28)	132	(25)	45	(20)	77	(11)
3-Chloro-4-methylaniline (GC degradation product of Chlorotoluron)	141	141	(100)	140	(37)	106	(68)	142	(36)	143	(28)	77	(25)
Chloroxuron	290	72	(100)	245	(37)	44	(31)	75	(21)	45	(19)	63	(16)
Chloropropham	213	43	(100)	127	(49)	41	(35)	45	(20)	44	(18)	129	(16)
Chlorpyrifos	349	97	(100)	195	(59)	199	(53)	65	(27)	47	(23)	314	(21)
Chlorthal-dimethyl	330	301	(100)	299	(81)	303	(47)	332	(29)	142	(26)	221	(24)
Chlorthiamid	205	170	(100)	60	(61)	171	(50)	172	(49)	205	(35)	173	(29)
Cinerin I	316	123	(100)	43	(35)	93	(33)	121	(27)	81	(27)	150	(27)
Cinerin II	360	107	(100)	93	(57)	121	(53)	91	(50)	149	(35)	105	(33)
Cyanazine	240	44	(100)	43	(60)	68	(60)	212	(48)	41	(47)	42	(34)
Cypermethrin	415	163	(100)	181	(79)	165	(68)	91	(41)	77	(33)	51	(29)
2,4-DB methyl ester	262	101	(100)	59	(95)	41	(39)	162	(36)	69	(28)	63	(25)

Table 2.51 (continued)

Compound	Molar mass	Main fragment m/z (intensities)											
		1		2		3		4		5		6	
Dalapon	142	43	(100)	61	(81)	62	(67)	97	(59)	45	(59)	44	(47)
Dazomet	162	162	(100)	42	(87)	89	(79)	44	(73)	76	(59)	43	(53)
Demetron-S-methyl	230	88	(100)	60	(50)	109	(24)	142	(17)	79	(14)	47	(11)
Desmetryn	213	213	(100)	57	(67)	58	(66)	198	(58)	82	(44)	171	(39)
Dialifos	393	208	(100)	210	(31)	76	(20)	173	(17)	209	(12)	357	(10)
Di-allate	269	43	(100)	86	(62)	41	(38)	44	(25)	42	(24)	70	(19)
Diazinon	304	137	(100)	179	(74)	152	(65)	93	(47)	153	(42)	199	(39)
Dicamba methyl ester	234	203	(100)	205	(60)	234	(27)	188	(26)	97	(21)	201	(20)
Dichlobenil	171	171	(100)	173	(62)	100	(31)	136	(24)	75	(24)	50	(19)
Dichlofenthion	314	97	(100)	279	(92)	223	(90)	109	(67)	162	(53)	251	(46)
Dichlofluanid	332	123	(100)	92	(33)	224	(29)	167	(27)	63	(23)	77	(22)
2,4-D isooctyl ester	332	43	(100)	57	(98)	41	(76)	55	(54)	71	(41)	69	(27)
2,4-D methyl ester	234	199	(100)	45	(97)	175	(94)	145	(70)	111	(69)	109	(68)
Dichlorprop isooctyl ester	346	43	(100)	57	(83)	41	(61)	71	(48)	55	(47)	162	(41)
Dichlorprop methyl ester	248	162	(100)	164	(80)	59	(62)	189	(56)	63	(39)	191	(35)
Dichlorvos	220	109	(100)	185	(18)	79	(17)	187	(6)	145	(6)	47	(5)
Dicofol	368	139	(100)	111	(39)	141	(33)	75	(18)	83	(17)	251	(16)
o,p′-DDT	352	235	(100)	237	(59)	165	(33)	236	(16)	199	(12)	75	(12)
p,p′-DDT	352	235	(100)	237	(58)	165	(37)	236	(16)	75	(12)	239	(11)
Dieldrin	378	79	(100)	82	(32)	81	(30)	263	(17)	77	(17)	108	(14)
Dimethirimol methyl ether	223	180	(100)	223	(23)	181	(10)	224	(3)	42	(2)	109	(2)
Dimethoate	229	87	(100)	93	(76)	125	(56)	58	(40)	47	(39)	63	(33)
DNOC methyl ether	212	182	(100)	165	(74)	89	(69)	90	(57)	212	(48)	51	(47)
Dinoterb methyl ether	254	239	(100)	209	(41)	43	(36)	91	(35)	77	(33)	254	(33)
Dioxacarb	223	121	(100)	122	(62)	166	(46)	165	(42)	73	(35)	45	(31)
Diphenamid	239	72	(100)	167	(86)	165	(42)	239	(21)	152	(17)	168	(14)
Disulfoton	274	88	(100)	89	(43)	61	(40)	60	(39)	97	(36)	65	(23)
Diuron	232	72	(100)	44	(34)	73	(25)	42	(20)	232	(19)	187	(13)
Dodine	227	43	(100)	73	(80)	59	(52)	55	(47)	72	(46)	100	(46)
Endosulfan	404	195	(100)	36	(95)	237	(91)	41	(89)	24	(79)	75	(78)
Endrin	378	67	(100)	81	(67)	263	(59)	36	(58)	79	(47)	82	(41)
Ethiofencarb	225	107	(100)	69	(48)	77	(29)	41	(26)	81	(21)	45	(17)
Ethirimol	209	166	(100)	209	(17)	167	(14)	96	(12)	194	(4)	55	(2)
Ethirimol methyl ether	223	180	(100)	223	(23)	85	(14)	181	(12)	55	(10)	96	(9)
Etrimfos	292	125	(100)	292	(91)	181	(90)	47	(84)	153	(84)	56	(73)
Fenarimol	330	139	(100)	107	(95)	111	(40)	219	(39)	141	(33)	251	(31)
Fenitrothion	277	125	(100)	109	(92)	79	(62)	47	(57)	63	(44)	93	(40)
Fenoprop isooctyl ester	380	57	(100)	43	(94)	41	(85)	196	(63)	71	(60)	198	(59)
Fenoprop methyl ester	282	196	(100)	198	(89)	59	(82)	55	(36)	87	(34)	223	(31)
Fenuron	164	72	(100)	164	(27)	119	(24)	91	(22)	42	(14)	44	(11)
Flamprop-isopropyl	363	105	(100)	77	(44)	276	(21)	106	(18)	278	(7)	51	(5)
Flamprop-methyl	335	105	(100)	77	(46)	276	(20)	106	(14)	230	(12)	44	(11)
Formothion	257	93	(100)	125	(89)	126	(68)	42	(49)	47	(48)	87	(40)

Table 2.51 (continued)

Compound	Molar mass	Main fragment m/z (intensities)											
		1		2		3		4		5		6	
Heptachlor	370	100	(100)	272	(81)	274	(42)	237	(33)	102	(33)		
Iodofenphos	412	125	(100)	377	(78)	47	(64)	79	(59)	93	(54)	109	(49)
loxynil isooctyl ether	483	127	(100)	57	(96)	41	(34)	43	(33)	55	(26)	37	(16)
loxynil methyl ether	385	385	(100)	243	(56)	370	(41)	127	(13)	386	(10)	88	(9)
Isoproturon	206	146	(100)	72	(54)	44	(35)	128	(29)	45	(28)	161	(25)
Jasmolin I	330	123	(100)	43	(52)	55	(34)	93	(25)	91	(24)	81	(23)
Jasmolin II	374	107	(100)	91	(69)	135	(69)	93	(67)	55	(66)	121	(58)
Lenacil	234	153	(100)	154	(20)	110	(15)	109	(15)	152	(13)	136	(10)
Lenacil N-methyl derivative	248	167	(100)	166	(45)	168	(12)	165	(12)	124	(9)	123	(6)
Lindane	288	181	(100)	183	(97)	109	(89)	219	(86)	111	(75)	217	(68)
Linuron	248	61	(100)	187	(43)	189	(29)	124	(28)	46	(28)	44	(23)
MCPB isooctyl ester	340	87	(100)	57	(81)	43	(62)	71	(45)	41	(42)	69	(29)
MCPB methyl ester	242	101	(100)	59	(70)	77	(40)	107	(25)	41	(22)	142	(20)
Malathion	330	125	(100)	93	(96)	127	(75)	173	(55)	158	(37)	99	(35)
Mecoprop isooctyl ester	326	43	(100)	57	(94)	169	(77)	41	(70)	142	(69)	55	(52)
Mecoprop methyl ester	228	169	(100)	143	(79)	59	(58)	141	(57)	228	(54)	107	(50)
Metamitron	202	104	(100)	202	(66)	42	(42)	174	(35)	77	(24)	103	(19)
Methabenzthiazuron	221	164	(100)	136	(73)	135	(69)	163	(42)	69	(30)	58	(25)
Methazole	260	44	(100)	161	(44)	124	(36)	187	(31)	159	(24)	163	(23)
Methidathion	302	85	(100)	145	(90)	93	(32)	125	(22)	47	(21)	58	(20)
Methiocarb	225	168	(100)	153	(84)	45	(40)	109	(37)	91	(31)	58	(21)
Methomyl	162	44	(100)	58	(81)	105	(69)	45	(59)	42	(55)	47	(52)
Metobromuron	258	61	(100)	46	(43)	60	(15)	91	(13)	258	(13)	170	(12)
Metoxuron	228	72	(100)	44	(27)	183	(23)	228	(22)	45	(21)	73	(15)
Metribuzin	214	198	(100)	41	(78)	57	(54)	43	(39)	47	(38)	74	(36)
Mevinphos	224	127	(100)	192	(30)	109	(27)	67	(20)	43	(8)	193	(7)
Monocrotophos	223	127	(100)	67	(25)	97	(23)	109	(14)	58	(14)	192	(13)
Monolinuron	214	61	(1003	126	(63)	153	(42)	214	(34)	46	(29)	125	(25)
Napropamide	271	72	(100)	100	(81)	128	(62)	44	(55)	115	(41)	127	(36)
Nicotine	162	84	(100)	133	(21)	42	(18)	162	(17)	161	(15)	105	(9)
Nitrofen	283	283	(100)	285	(67)	202	(55)	50	(55)	139	(37)	63	(37)
Nuarimol	314	107	(100)	235	(91)	203	(85)	139	(60)	123	(46)	95	(35)
Omethoat	213	110	(100)	156	(83)	79	(39)	109	(32)	58	(30)	47	(21)
Oxadiazon	344	43	(100)	175	(92)	57	(84)	177	(60)	42	(35)	258	(22)
Parathion	291	97	(100)	109	(90)	291	(57)	139	(47)	125	(41)	137	(39)
Parathion-methyl	263	109	(100)	125	(80)	263	(56)	79	(26)	63	(18)	93	(18)
Pendimethalin	281	252	(100)	43	(53)	57	(43)	41	(41)	281	(37)	253	(34)
Permethrin	390	183	(100)	163	(100)	165	(25)	44	(15)	184	(15)	91	(13)
Phenmedipham	300	133	(100)	104	(52)	132	(34)	91	(34)	165	(31)	44	(27)
Phosalone	367	182	(100)	121	(48)	97	(36)	184	(32)	154	(24)	111	(24)
Pirimicarb	238	72	(100)	166	(85)	42	(63)	44	(44)	43	(24)	238	(23)
Pirimiphos-ethyl	333	168	(100)	318	(94)	152	(88)	304	(79)	180	(73)	42	(71)
Pirimiphos-methyl	305	290	(100)	276	(93)	125	(69)	305	(53)	233	(44)	42	(41)
Propachlor	211	120	(100)	77	(66)	93	(36)	43	(35)	51	(30)	41	(27)
Propanil	217	161	(100)	163	(70)	57	(64)	217	(16)	165	(11)	219	(9)

Table 2.51 (continued)

Compound	Molar mass	Main fragment m/z (intensities)											
		1		2		3		4		5		6	
Propham	179	43	(100)	93	(88)	41	(42)	120	(24)	65	(24)	137	(23)
Propoxur	209	110	(100)	152	(47)	43	(28)	58	(27)	41	(21)	111	(20)
Pyrethrin I	328	123	(100)	43	(62)	91	(58)	81	(47)	105	(45)	55	(43)
Pyrethrin II	372	91	(100)	133	(70)	161	(55)	117	(48)	107	(47)	160	(43)
Quintozene	293	142	(100)	237	(96)	44	(75)	214	(67)	107	(62)	212	(61)
Resmethrin	338	123	(100)	171	(67)	128	(52)	143	(49)	81	(38)	91	(28)
Simazine	201	201	(100)	44	(96)	186	(72)	68	(63)	173	(57)	96	(40)
Tecnazene	259	203	(100)	201	(69)	108	(69)	215	(60)	44	(57)	213	(51)
Terbacil	216	160	(100)	161	(99)	117	(69)	42	(45)	41	(41)	162	(37)
Terbacil N-methyl derivative	230	56	(100)	174	(79)	175	(31)	57	(24)	176	(23)	41	(20)
Tetrachlorvinphos	364	109	(100)	329	(48)	331	(42)	79	(20)	333	(14)	93	(9)
Tetrasul	322	252	(100)	254	(67)	324	(51)	108	(49)	75	(40)	322	(40)
Thiabendazole	201	201	(100)	174	(72)	63	(12)	202	(11)	64	(11)	65	(9)
Thiofanox	218	57	(100)	42	(75)	68	(39)	61	(38)	55	(34)	47	(33)
Thiometon	246	88	(100)	60	(63)	125	(56)	61	(52)	47	(49)	93	(47)
Thiophanat-methyl	342	44	(100)	73	(97)	159	(89)	191	(80)	86	(72)	150	(71)
Thiram	240	88	(100)	42	(25)	44	(20)	208	(18)	73	(15)	45	(10)
Tri-allate	303	43	(100)	86	(73)	41	(43)	42	(31)	70	(23)	44	(21)
Trichlorfon	256	109	(100)	79	(34)	47	(26)	44	(20)	185	(17)	80	(8)
Tridemorph	297	128	(100)	43	(26)	42	(18)	44	(13)	129	(11)	55	(5)
Trietazine	229	200	(100)	43	(81)	186	(52)	229	(52)	214	(50)	42	(48)
Trifluralin	335	43	(100)	264	(33)	306	(32)	57	(7)	42	(6)	290	(5)
Vamidothion	287	87	(100)	58	(47)	44	(40)	61	(29)	59	(26)	60	(25)
Vinclozolin	285	54	(100)	53	(93)	43	(82)	124	(65)	212	(63)	187	(61)

The data refer to EI ionisation at 70 eV. The relative intensities can depend in individual cases an the type of mass spectrometer or mass-selective detector used. For confirmation mass spectra should be consulted which were run under identical instrumental conditions.

Example of Selected Ion Monitoring
for PCB analysis taking into account the PCB replacement product Ugilec T

The analysis strategy shown here has the aim of determining a PCB pattern as completely as possible in the relevant degrees of chlorination and to test in parallel for the possible presence of Ugilec T (tetrachlorobenzyltoluenes/trichlorobenzene). Three individual masses per time window for every two degrees of chlorination are planned for the selected SIM descriptors (scan width ± 0.25 u based on the centroid determined in the full scan mode) to determine the overlapping retention ranges of the individual degrees of chlorination (Fig. 2.197). A staggered mass determination is thus obtained (Fig. 2.198).

No.	Substance	SIM masses [m/z]	Staggered time window [min:s]
1	Trichlorobenzenes	180/182/184	Start – 8:44
2	Cl_3-PCBs	256/258/260	8:44–14:30
3	Cl_4-PCBs	290/292/294	8:44–15:52
4	Cl_5-PCBs	324/326/328	14:30–18:34
5	Ugilec	318/320/322	15:52–20:15
6	Cl_6-PCBs	358/360/362	15:52–21:36
7	Cl_7-PCBs	392/394/396	18:34–23:38
8	Cl_8-PCBs	428/430/432	20:15–30:00
9	Cl_{10}-PCB	496/498/500	23:58–30:00

(The times refer to a capillary column J&W DB5 30 m × 0.25 mm × 0.25 µm.
Program: 60 °C – 2 min, 20 °/min to 180 °C, 5 °/min to 290 °C – 5 min. Splitless injection at 280 °C, column pressure 1 bar He. GC/MS system Finnigan INCOS 50. Sample: Aroclor 1240/1260 + Ugilec T, total concentration of the mixture ca. 10 ng/µl.)

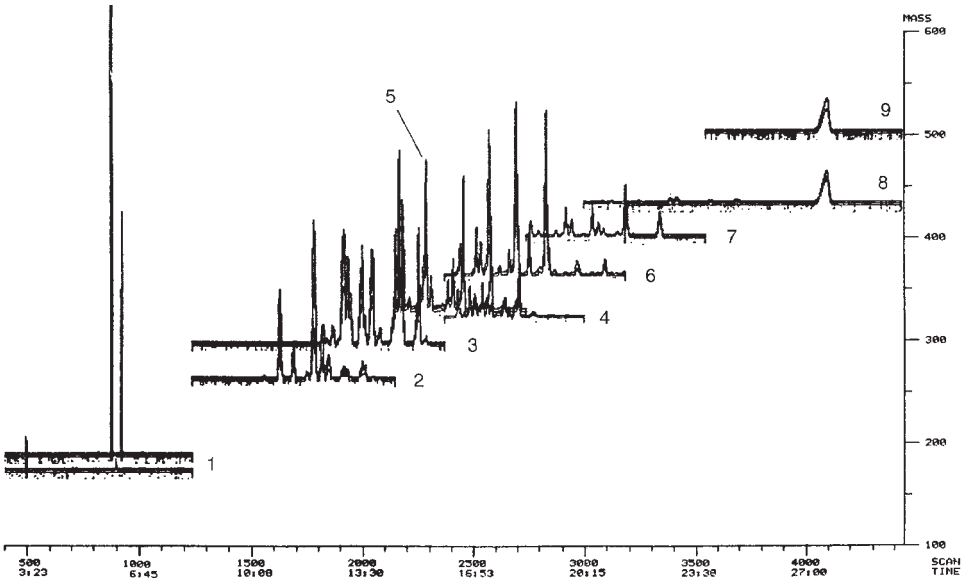


Fig. 2.197 MID chart of a PCB/Ugilec analysis.
In each case two degrees of chlorination of the PCBs and Ugilec T were detected in parallel, each with three masses. The overlapping MID descriptors were switched in such a way that each degree of chlorination was detected in two consecutive time windows (see text).

As an SIM/MID analysis is limited to the detection of certain ions in small mass ranges and the comparability of a complete spectrum for a library search is not required, on tuning the analyser a mass-based optimisation to a transmission which is as high as possible is un-

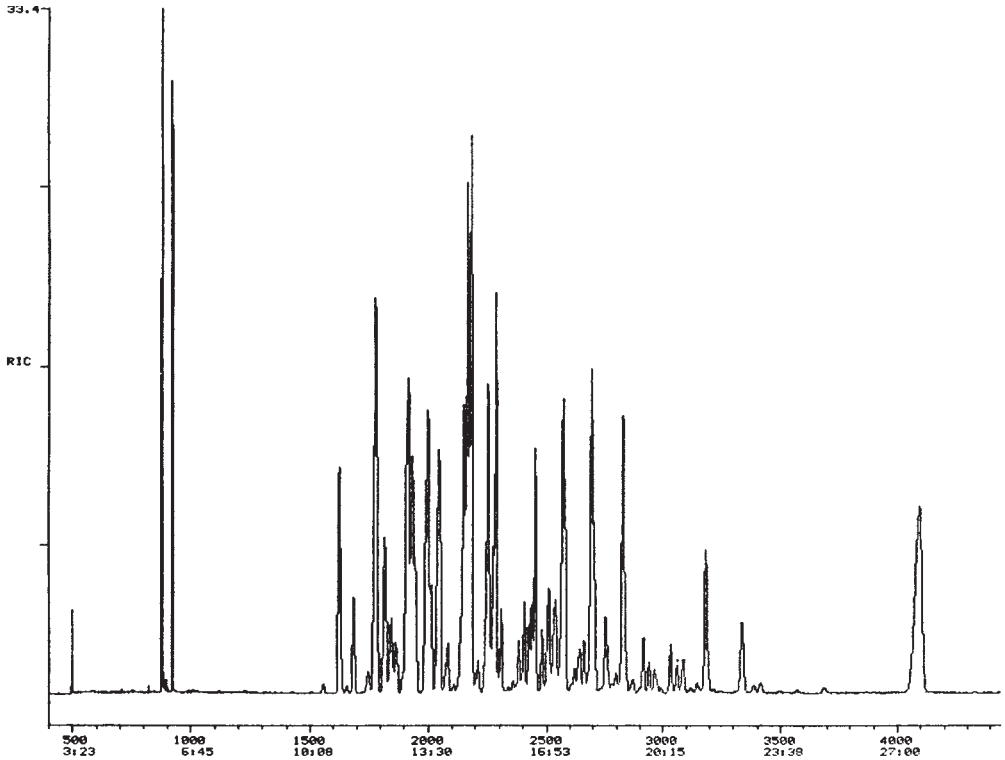


Fig. 2.198 Chromatogram (RIC) compiled after termination of the PCB/Ugilec T analysis in the MID mode from Fig. 2.197.

dertaken. Standard tuning aims to produce a balanced spectrum which corresponds to the data in a reference list for the reference substance FC43 (perfluorotributylamine), in order to guarantee good comparability of the spectra run with those in the library. Certain EPA methods require the source tuning using BFB (4-bromofluorobenzene) or DFTPP (decafluorotriphenylphosphine) for compliance with predefined mass intensities as set in the operating procedure. The position of a mass spectrum standard does not need to be adhered to in SIM analysis as no comparisons are made between spectra, but the relative intensities, e.g. of isotope patterns, must be evaluated. In the optimisation of the ion source special attention should therefore be paid to the masses (or the mass range) involved in SIM data acquisition. The source and lens potentials should then be selected manually so that a nearby fragment of the reference compound or an ion produced by column bleed (GC temperature ca. 200 °C) can be detected with the highest intensity but good resolution. In this way a significant additional increase in sensitivity can be achieved with quadrupole analysers for the SIM mode.

Figure 2.199 shows the chromatogram of a PCB standard as the result of a typical SIM routine analysis. In this case two masses are chosen as SIM masses for each PCB isomer. The switching points of the individual descriptors are recognised as steps in the base line. The different base line heights arise as a result of the different contributions of the chemical

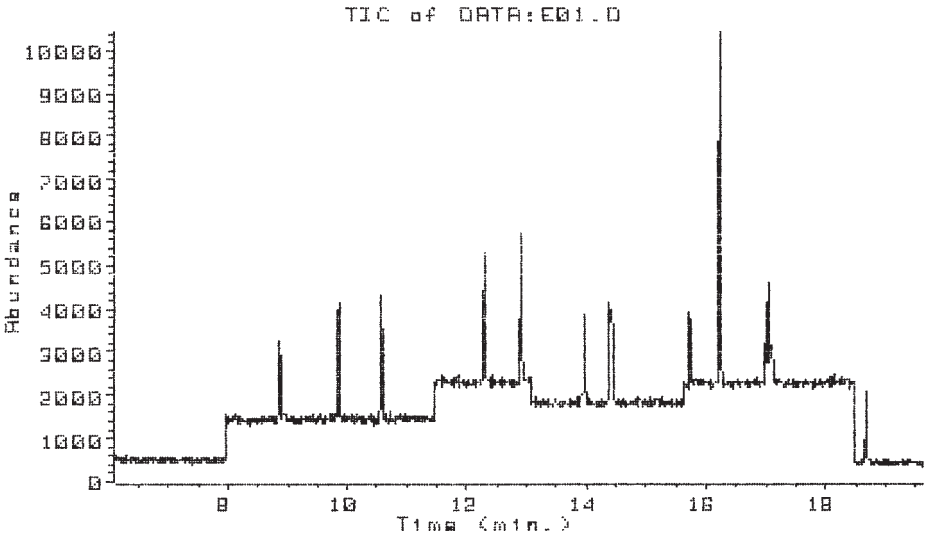


Fig. 2.199 Example of a typical PCB analysis in the SIM mode. The steps in the base line show the switching of the SIM descriptors.

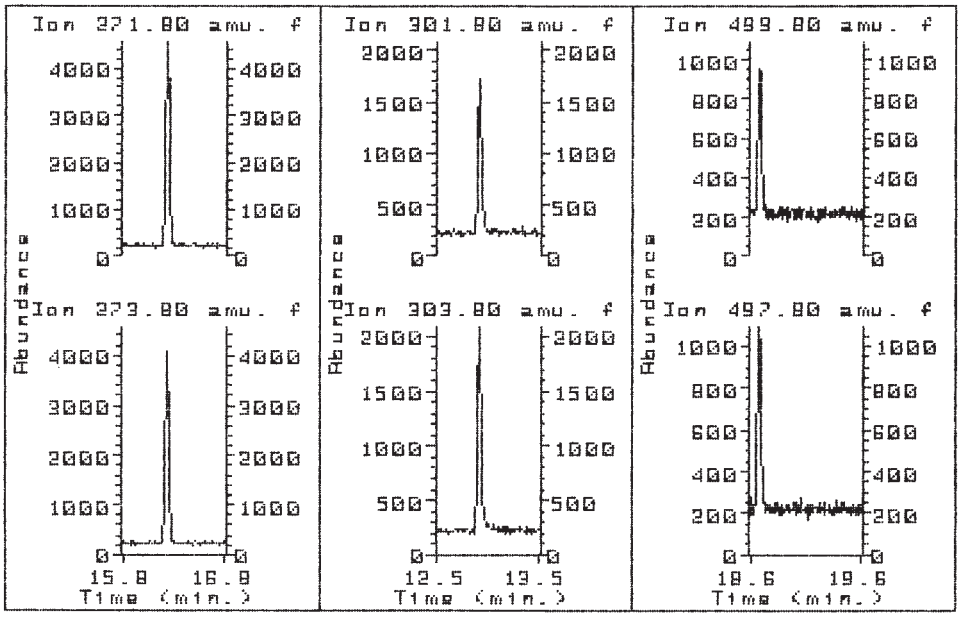


Fig. 2.200 Evaluation of the PCB analysis from Fig. 2-138 by showing the peaks at specific mass traces (see text).

noise to these signals. To control the evaluation, the substance signals can be represented as peaks in the expected retention time windows (Fig. 2.200). A deviation from the calibrated retention time (Fig. 2.200, right segment with the masses m/z 499.8 and 497.8) leads to a shift of the peak from the middle to the edge of the window and should be a reason for further checking. If qualifiers are present (e.g. isotope patterns), these should be checked using the relative intensities as a line spectrum, if possible (Fig. 2.201), or as superimposed mass traces (Fig. 2.202). In the region of the detection limit the noise width should be taken into account in the test of agreement.

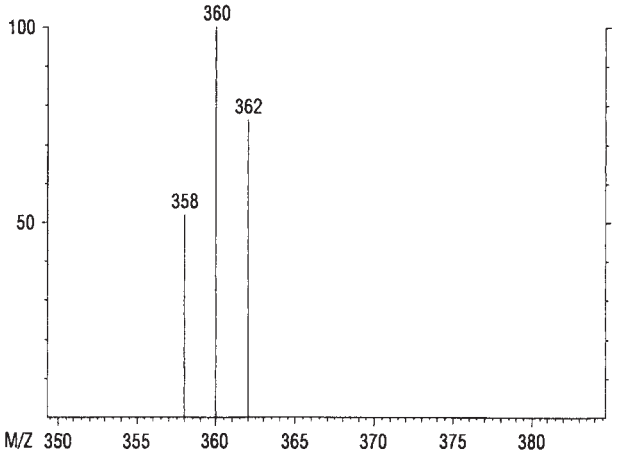


Fig. 2.201 Evaluation of isotope patterns from an MID analysis of hexachlorobiphenyl by comparison of relative intensities (shown as a bar graph spectrum).

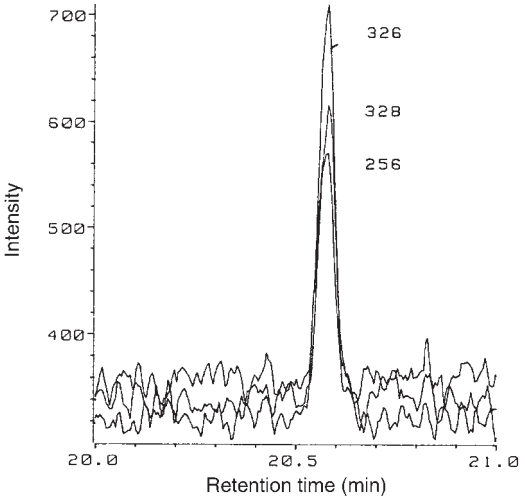


Fig. 2.202 Test of PCB isotope patterns (PCB 101, pentachlorobiphenyl) in the range of the detection limit (10 pg, S/N ca. 4:1) after SIM analysis (shown as mass traces).

2.3.5.3 High Resolution Accurate Mass MID Data Acquisition

Target compound analysis using high mass resolution e. g. for polychlorinated dioxins, furans (PCDD/F), pesticides, persistent organic pollutants (POP) or pharmaceutical residues are typically performed by monitoring the compound specific accurate mass ions at the expected retention time for each analyte. High Resolution GC/MS target compound applications benefit from a unique technical feature referred to as the lock-mass technique for performing multiple ion detection (MID) analyses. The lock mass technique provides ease of use, combined with a maximum quantitative precision and certainty in analyte confirmation.

The basic equation for sector mass spectrometers

$$m/z = c \cdot B^2/V$$

with
c = the instrument constant
B = magnetic field strength
V = acceleration voltage

shows that mass calibrations are feasible either at constant acceleration voltage by calibrating the magnetic field or vice versa.

For MID data acquisition, the fixed magnet setting with a variable acceleration voltage is typically used. The mass calibration for MID data acquisition follows a special procedure during the data acquisition in the form of a scan inherent mass calibration. This mass calibration is performed during MID analysis in every scan before acquisition of the target compound intensities. Most recent developments use two reference masses below and above the target masses referenced as the "lock-plus-cali mass technique" or in short "lock mode". It provides optimum mass accuracy for peak detection even in difficult chromatographic situations. The scan-to-scan mass calibration provides the best confidence for the acquired analytical data and basically is the accepted feature for the requirement of HRGC/HRMS as a confirmation method (EPA Method 1613 b).

The scan inherent mass calibration process is performed by the instrument control in the background without being noticed by the operator. In particular, it provides superior stability especially for high sample throughput with extended runtimes. A reference compound is leaked continuously from the reference inlet system during the GC run into the ion source. Typically perfluoro-tributylamine (FC43) is used as reference compound in HRGC/HRMS for dioxin analysis. Other reference compounds may be used to suit individual experimental conditions.

The exact ion masses of the reference compound are used in the MID acquisition windows for internal calibration. For the lock-and-cali-mass technique, two ions of the reference substance are individually selected for each MID window; one mass which is close, but below the analyte target mass, and the second, which is slightly above the analyte target masses. Although both reference masses are used for the internal calibration, it became common practice to name the lower reference mass the "lock mass" and the upper reference mass the "calibration mass".

During the MID scan the mass spectrometer is parking (locking) the magnetic field strength at the start of each MID window and then performing the mass calibration using the lock and calibration masses followed by the acquisition of the target and internal standard mass intensities.

Lock-Mass Technique

At the start of each MID retention time window, the magnet is automatically set to one mass (Da) below the lowest mass found in the MID descriptor. The magnet is parked, or "locked" and remains with this setting throughout the entire MID window. All analyzer jumps to the calibration and target compound masses are done by fast electrical jumps of the acceleration voltage.

Scan Inherent Calibration

The lock mass (L) is scanned in a small mass window starting below the mass peak by slowly decreasing the ion source acceleration voltage (see Fig. 2.203, ①). The mass resolution of the lock mass peak is calculated and written to the data file. Using the lock mass setting, a second reference mass is used for building the MID mass calibration. The calibration mass is checked by an electrical jump. A fine adjustment of the electrical calibration is made based on this measurement (see Fig. 2.203, ②). The electrical "jump" (see Fig. 2.203, ③) is very fast and takes only a few milliseconds. The dwell times for the sufficiently intense reference ions are very short.

The resulting electrical calibration is used for subsequent MID data acquisition.

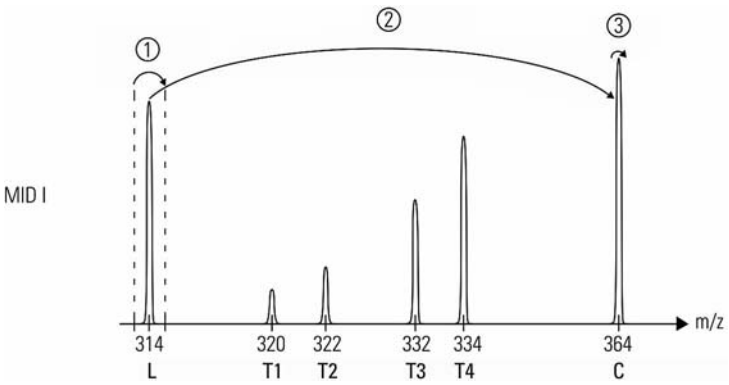


Fig. 2.203 Mass detection scheme in HRMS MID calibration. The arrows show the sequence of measurement in the mass calibration steps (the magnet is "locked" in this example at m/z 313).

①	Magnet locking and "lock mass" sweep, mass calibration and resolution determination	
②	Electrical jump to calibration mass	
③	Calibration mass sweep and mass calibration	
L	from FC43	lock mass (L), m/z 313.983364
C	from FC43	calibration mass (C), m/z 363.980170
T1, T2	from native TCDD	analyte target masses m/z 319.895992, 321.893042
T3, T4	from ^{13}C-TCDD	internal standard masses m/z 331.936250, 333.933300

Data Acquisition

With the updated and exact mass calibration settings, the analyzer now sets the acceleration voltage to the masses of the target ions. The intensity of each ion is measured based on a preset dwell time (see Fig. 2.204, ④). The dwell times to measure the analyte target ion intensities are significantly longer than the lock or calibration mass ions dwell times. This is done to achieve the optimum detection sensitivity for each analyte ion. The exact positioning on the top of the target ion mass peak allows for higher dwell times, significantly increased

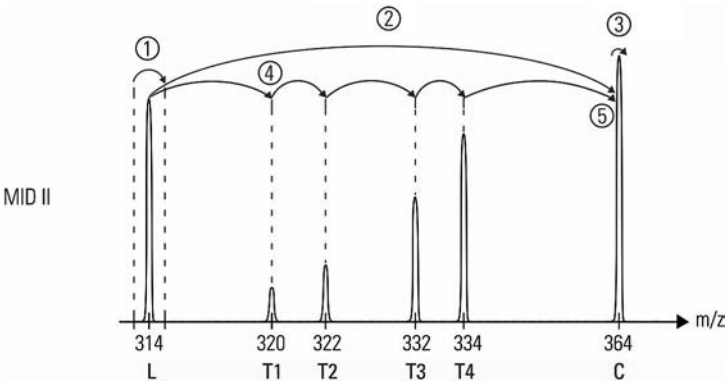


Fig. 2.204 Mass detection scheme in HRMS MID data acquisition. The arrows show the sequence of measurement in the target compound and internal standard data acquisition (legend for the mass scale see Fig. 2.203).
④ Consecutive electrical jumps to target and internal standard masses
⑤ Electrical jump to calibration mass, mass calibration

sensitivity, and higher S/N values compared to sweep scan techniques still used in older technology HRMS systems. It is important to note that the lock-plus-cali mass technique extends the dynamic range significantly into the lower concentration range.

Advantages of the Lock-plus-Cali Mass Technique

The lock-plus-cali mass calibration technique provides extremely stable conditions for data acquisitions of long sequences even over days, e. g., over the weekend and usually includes the performance documentation for quality control documented in the data file.

The electrical jumps of the acceleration voltage are very fast, and provide an excellent instrument duty cycle. Any outside influences from incidental background ions, long term drift or minute electronics fluctuations are taken care of and do not influence the result.

Both, the lock and cali masses are monitored in parallel during the run providing an excellent confirmation of system stability for data certainty. Together with the constant resolution monitoring, this technique provides the required traceability in MID data analysis.

Other acquisition techniques have been formerly used employing just one lock mass position. This technique requires a separate pre-run electrical mass calibration and does not allow a scan inherent correction of the mass position which may arise due to long term drifts of the analyzer during data acquisition.

As a consequence, with the one mass lock techniques, the mass jumps are less precise with increasing run times. Deviations from the peak top position when acquiring data at the peak slope result in less sensitivity, less reproducibility and poor isotope ratio confirmation.

The lock-plus-cali mass technique has proven to be superior in achieving lower LOQs and higher S/N values in high resolution MID. Figure 2.205 shows the typical chromatogram display of dioxin analysis with the TCDD target masses as well as the ^{13}C internal standard masses. In addition, the continuously monitored FC43 lock and cali masses are displayed as constant mass traces. Both traces are of valuable diagnostic use and confirm the correct measurement of the target compounds.

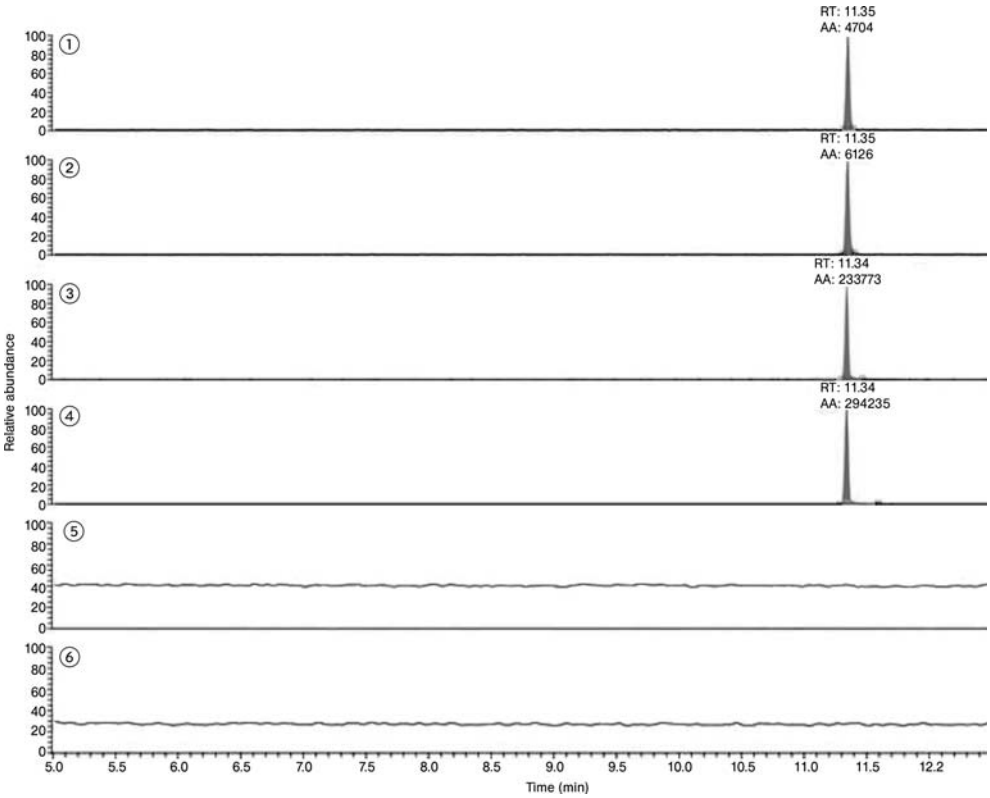


Fig. 2.205 Resulting mass chromatograms of a TCDD standard solution at 100 fg/µL (DB-5MS 60 m x 0.25 µm x 0.1 µm); ① Ratio mass of 2,3,7,8-tcdd (native) m/z 319.8960; ② Quan mass of 2,3,7,8-tcdd (native) m/z 321.8930; ③ Ratio mass of 2,3,7,8–13C12-TCDD (ISTD) m/z 331.9362; ④ Quan mass of 2,3,7,8–13C12-TCDD (ISTD) m/z 333.9333; ⑤ Lock mass of FC43 m/z 313.983364; ⑥ Cali mass of FC43 m/z 363.980170.

Setup of the MID Descriptor

The MID descriptor for the data acquisition contains all the information required by the HRMS mass analyzer. Included in each descriptor is the retention time information for switching between different target ions, the exact mass calibration, the target masses to be acquired, and the corresponding dwell times.

A sample chromatogram usually facilitates the setting of the retention time windows, as shown Fig. 2.206. The sample chromatogram is used to optimize the GC component separation and set the MID windows before data analysis, and usually consists of a higher concentration standard mix.

MID Cycle Time

In order to provide a representative and reproducible GC peak integration, the total MID cycle time on the chromatographic time scale should allow for the acquisition of 8 to 10 data points over a chromatographic peak. The cycle time has a direct influence on the available measurement time for each ion (dwell time). If the MID cycle time is too short,

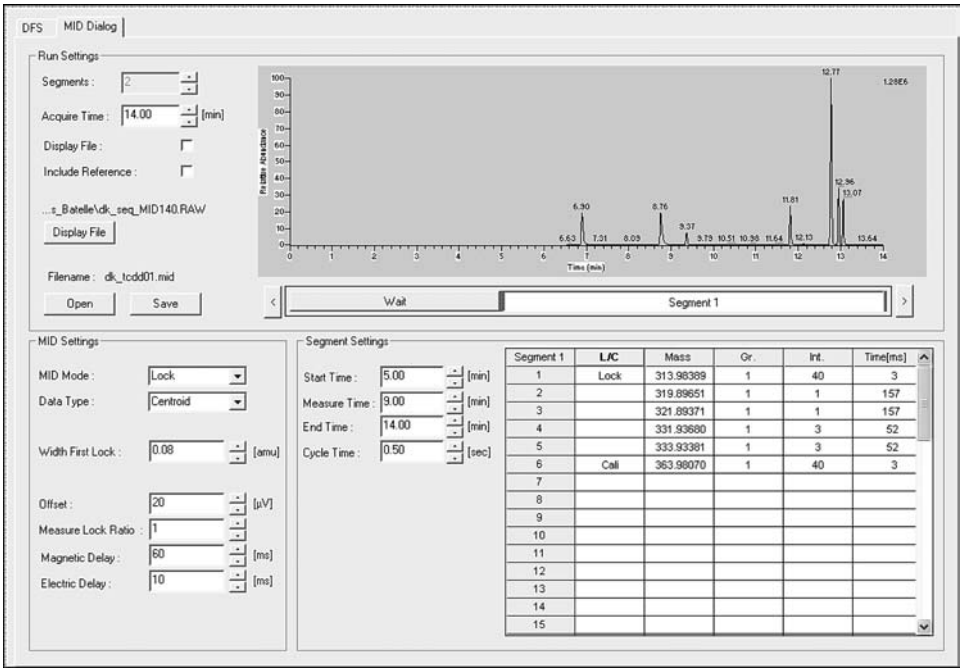


Fig. 2.206 MID editor with sample chromatogram (top) and target mass list and duty cycles for the highlighted retention time window (bottom right).

the sensitivity of the instrument is compromised, too high values lead to a poor GC peak definition.

2.3.6 MS/MS – Tandem Mass Spectrometry

"Can atomic particles be stored in a cage without material walls?

This question is already quite old. The physicist Lichtenberg from Göttingen wrote in his notebook at the end of the 18th century: "I think it is a sad situation that in the whole area of chemistry we cannot freely suspend the individual components of matter." This situation lasted until 1953. At that time we succeeded, in Bonn, in freely suspending electrically charged atoms, i.e. ions, and electrons using high frequency electric fields, so-called multipole fields. We called such an arrangement an ion cage."

Prof. Wolfgang Paul
in a lecture at the Cologne Lindenthal Institute in 1991

(from: Wolfgang Paul, A Cage for Atomic Particles – a basis for precision measurements in navigation, geophysics and chemistry, Frankfurter Allgemeine Zeitung, Wednesday 15th December 1993 (291) N4)

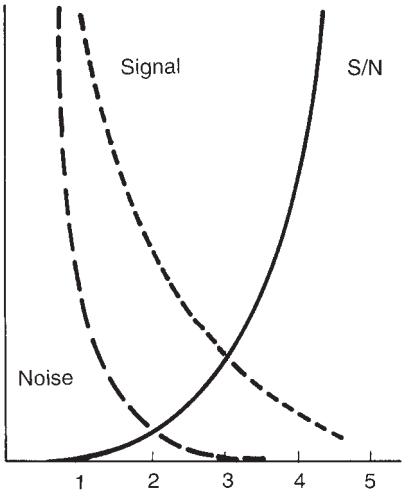


Fig. 2.207 Relationship between signal, noise and the number of analytical steps.

As part of the further development of instrumental techniques in mass spectrometry, MS/MS analysis has become the method of choice for trace analysis in complex matrices. Most of the current applications involve the determination of substances in the ppb and ppt ranges in samples of urine, blood and animal or plant tissues and in many environmental analyses. In addition the determination of molecular structures is an important area of application for multidimensional mass spectrometry.

As an analytical background to the use of the GC/MS/MS technique in residue analysis it should be noted that the signal/noise ratio increases with the number of analytical steps (Fig. 2.207). Clean-up steps lower the potential signal intensity. The sequence of wet chemical or instrumental sample preparation steps can easily lead to the situation whereby, as a consequence of processing losses, a substance can no longer be detected. From this consideration, the first separation step (MS^1) in a GC/MS/MS systen can be regarded as a mass-specific clean-up in the analysis of extracts with large quantities of matrix. After the subsequent induced fragmentation of selected ions, an analyte is identified using the characteristic mass spectrum of the product ions or it is quantified using the structure selective fragment ions for target compound quantitation in difficult matrices.

When is MS/MS Used?

- The sample matrix contributes significantly to the chemical background noise in SIM.
- Co-elution with isobaric impurities occurs.
- The structures of the compounds are unknown.
- Quantitation with the highest possible sensitivity in difficult matrices is necessary.
- The SIM analysis requires additional confirmation.

MS/MS Scan Techniques

Scan mode MS_1	Scan mode MS_2	Result	Application
Single ion	Scan	Product ion spectrum (MS/MS spectrum)	Identification and confirmation of compounds, structure determination
Single ion	Single ion	Individual intensities of product ions (selected reaction monitoring, SRM)	Highly selective and highly sensitive target compound quantitation with complex matrices, e.g. pesticides
Scan	Single ion	Precursor masses of certain fragments (precursor ion scan)	Specific analysis of compounds (classes of substance) with common structural features, screening, e.g. crude oil biomarker
Scan	Scan-NL	Precursor ions, which undergo loss of neutral particles with NL u (neutral loss scan)	Specific analysis of compounds (classes of substance) with common functional groups/structural features, e.g. loss of COCl from PCDD/PCDF

Soft ionisation techniques instead of electron impact ionisation are the preferred ionisation processes for MS/MS analysis. Although fragmentation in the EI mode of GC/MS is desirable for substance identification, frequently only low selectivity and sensitivity are achieved in complex matrices. Soft ionisation techniques, such as chemical ionisation, concentrate the ion flow to a few intense ions which can form a good starting point for MS/MS analysis. For this reason the HPLC coupling techniques atmospheric pressure CI (APCI) and electrospray (ESI) are also in the forefront of the development and extension of MS/MS analysis. The use of the GC/MS/MS technique, which can be carried out with positive and negative chemical ionisation (PCI and NCI), will in the future dominate the multicomponent target compound methods for trace analyses.

The information content of the GC/MS/MS technique was already evaluated in 1983 by Richard A. Yost (University of Florida, codeveloper of the MS/MS technique). Based on the theoretical task of detecting one of the five million substances catalogued at that time by the Chemical Abstracts Service, a minimum information content of 23 bits ($\log_2(5 \cdot 10^6)$) was required for the result of the chosen analysis procedure and the MS procedures available were evaluated accordingly. The calculation showed that capillary GC/MS/MS can give 1000 times more information than the traditional GC/MS procedure (Table 2.52)!

For the instrumental technique required for tandem mass spectrometry, as in the consideration of the resolving power, the main differences lie between the performances of the magnetic sector and quadrupole ion trap instruments (see Section 2.3.1). For coupling with GC and HPLC, triple-quadrupole instruments have been used since the 1980s. Ion trap MS/MS instruments have been used in research since the middle of the 1980s and are now being used in routine residue analysis. Tandem magnetic sector instruments are mainly used in research and development for mass spectrometry. Much higher energies (keV range) can be used to induce fragmentation. For the selection of precursor ions and/or the detection of the product ion spectrum high resolution capabilities can be used.

Table 2.52 Information content of mass spectroscopic techniques (after Yost 1983, Kaiser 1978).

Technique	P	Factor
MS [a)]	$1.2 \cdot 10^4$	0.002
Packed GC/MS [b)]	$7.8 \cdot 10^5$	0.12
Capillary GC/MS [c)]	$\mathbf{6.6 \cdot 10^6}$	**1**
MS/MS	$1.2 \cdot 10^7$	2
Packed GC/MS/MS	$7.8 \cdot 10^8$	118
Capillary GC/MS/MS	$6.6 \cdot 10^9$	1000

a) MS: 1000 u, unit mass resolution, maximum intensity 2^{12}
b) Packed GC: $2 \cdot 10^3$ theoretical plates, 30 min separating time
c) Capillary GC: $1 \cdot 10^5$ theoretical plates, 60 min separating time

MS/MS Tandem Mass Spectrometry

- Ionisation of the sample (EI, CI and other methods).
- Selection of a precursor ion.
- Collision-induced dissociation (CID) to product ions.
- Mass analysis of product ions (product ion scan).
- Detection as a complete product ion spectrum or preselected individual masses (selected reaction monitoring, SRM).

Ion trap mass spectrometry offers a new extension to the instrumentation used in tandem mass spectrometry. The methods for carrying out MS/MS analyses differ significantly from those involving triple-quadrupole instruments and reflect the mode of operation as storage mass spectrometers rather than beam instruments.

Tandem mass spectrometry consists of several consecutive processes. The GC peak with all the components (analytes, co-eluents, matrix, column bleed etc) reaching the ion source is first ionised. From the resulting mixture of ions the precursor ion with a particular m/z value is selected in the first step (MS^1). This ion can in principle be formed from different molecules (structures) and, even with different empirical formulae, they can be of the same nominal m/z value. The MS^1 step is identical to SIM analysis in traditional GC/MS, which, for the reasons mentioned above, cannot rule out false positive signals. Fragmentation of the precursor ions to product ions occurs in a collisional behind MS^1 through collisions of the selected ions with neutral gas molecules (CID, collision induced dissociation, or CAD, collision activated decomposition). In this collision process the kinetic energy of the precursor ions (quadrupole: ca. 10–100 eV, magnetic sector: >1 keV) is converted into internal energy (in an ion trap typically <6 eV), which leads to a substance-specific fragmentation by cleavage or rearrangement of bonds and the loss of neutral particles. A mixture of product ions with lower m/z values is formed. Following this fragmentation step, a second mass spectrometric separation (MS^2) is necessary for the mass analysis of the product ions. The spectrum of the product ions is finally detected as the product ion or MS/MS spectrum of this compound.

With regard to existing analysis procedures, for target compound quantitation high speed and flexibility in the choice of precursor ions is necessary. For the analysis with internal standards (e. g. deuterated standards) and multi-component methods, the fast switch between multiple precursor ions is necessary to achieve a sufficiently high data rate for a reliable peak definition of all coeluting compounds, in particular for SRM quantitations.

Besides recording a spectrum for the product ions (product ion scan), or of an individual mass (SRM), two additional MS/MS scan techniques give valuable analysis data. By linking the scans in MS_2 and MS_1, very specific and targeted analysis routes are possible. In the precursor ion scan, the first mass analyser (MS_1) is scanned over a preselected mass range. All the ions in this mass range reach the collision chamber and form product ions (CID). The second mass analyser (MS_2) is held constant for a specific fragment. Only emerging ions of MS^1 which form the selected fragment are recorded. This recording technique makes the identification of substances of related structure, which lead to common fragments in the mass spectrometer, easier (e. g. biomarkers in crude oil characterisation, drug metabolites).

In neutral loss scan all precursor ions, which lose a particular neutral particle (that otherwise cannot be detected in MS), are detected. Both mass analysers scan, but with a constant preadjusted mass difference, which corresponds to the mass of the neutral particle lost. This analysis technique is particularly meaningful if molecules contain the same functional groups (e. g. metabolites as acids, glucuronides or sulfates). In this way it is possible to identify the starting ions which are characterised by the loss of a common structural element. Both MS/MS scan techniques can be used for substance-class-specific detection in triple-quadrupole systems. Ion trap systems allow the mapping of these processes by linking the scans between separate stages of MS in time.

Mode of Operation of Tandem Mass Spectrometers

In tandem mass spectrometry using quadrupole or magnetic sector instruments, the various steps take place in different locations in the beam path of the instrument. The term "tandem-in-space" (R. Yost) has been coined to show how they differ from ion storage mass spectrometers (Fig. 2.208). In the ion trap analyser, a typical storage mass spectrometer, these

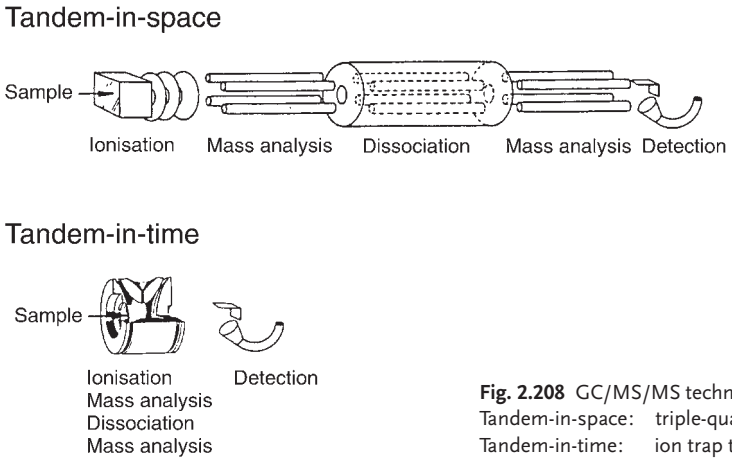


Fig. 2.208 GC/MS/MS techniques.
Tandem-in-space: triple-quadrupole technique
Tandem-in-time: ion trap technique

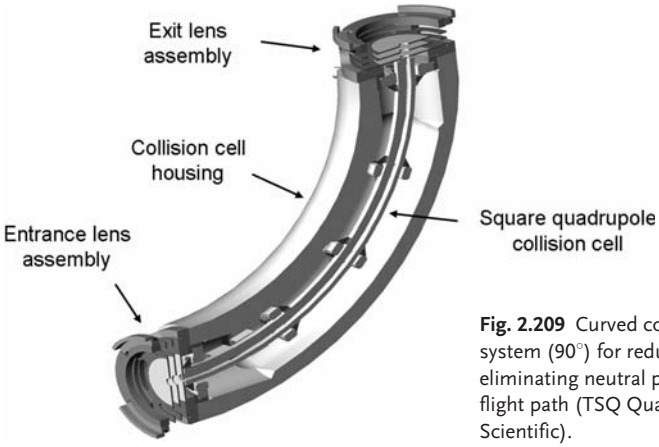


Fig. 2.209 Curved collision cell of a triple quadrupole system (90°) for reduction of non-specific noise by eliminating neutral particles and photons from the ion flight path (TSQ Quantum; courtesy Thermo Fisher Scientific).

processes take place in the same location, but consecutively. Richard Yost has described these as "tandem-in-time".

In a triple-quadrupole mass spectrometer (or other beam instruments, Fig. 2.209), an ion beam is passed continuously through the analyser from the ion source. The selection of precursor ions and collision-induced dissociation take place in dedicated devices of the analyser (Q_1, Q_2) independent of time. The only time-dependent process is the mass scan in the second mass analyser (Q_3, MS_2) for the recording of the product ion spectrum (Fig 2.210). An enclosed quadrupole device (Q_2) typically consisting of square rods is used. Formerly also hexapole or octapole rod systems have been employed. The collision cell is operated in an RF only made without mass seperating but ion focusing capabilities. (The mass filters MS_1 (Q_1) and MS_2 (Q_3) are operated at a constant RF/DC ratio!). Helium, nitrogen, argon or xenon at pressures of ca. 10^{-3} to 10^{-4} Torr are used as the collision gas. Heavier collision gases increase the yield of product ions. The collision energies are of the order of up to 100 eV and are typically controlled by a preceding lens stack, see Fig. 2.209.

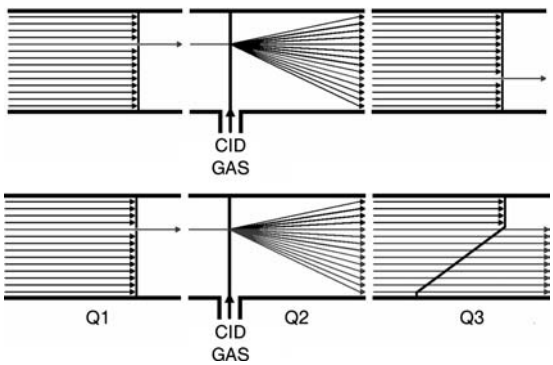


Fig. 2.210 Modes of operation of a triple quadrupole mass spectrometer.
Q_1 = first mass separating quad, mass selection of the precursor ion
Q_2 = collision cell, CID process
Q_3 = second mass separating quad, SRM detection (top) or product ion scan (bottom)

In instruments with ion trap analysers, the selection of the precursor ions, the collision-induced dissociation and the analysis of the product ions occur in the same place, but with time control via a sequence of frequency and voltage values at the end caps and ring electrode of the analyser (scan function see Fig. 2.211). The systems with internal or external ionisation differ in complexity and ease of calibration. In the case of internal ionisation the sample spectrum is first produced in the ion trap analyser and stored. The precursor ion m_p is then selected by ejecting ions above and below m_p from the trap by applying a multifrequency signal at the end caps (waveform). Ion trap systems with external ionisation can employ waveforms during injection of the ions into the analyser from the external source for isolation of the precursor ion in MS/MS mode or for isolation of the desired mass scan range in other scan modes. In this way a longer storage phase and a more rapid scan rate can be used for GC/MS in the MS/MS and SIM modes.

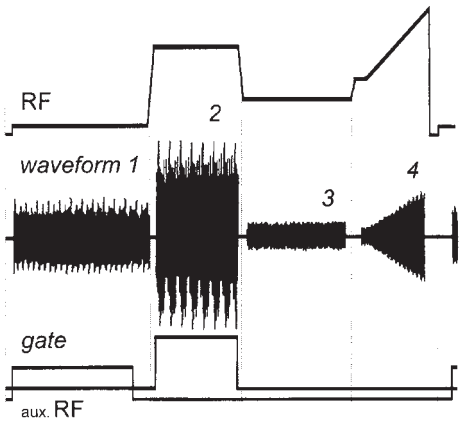


Fig. 2.211 MS/MS scan function of the ion trap analyser with external ionisation.
1 A gate switches the ion beam for transfer to the analyser, selection of the precursor ion m_p by a special frequency spectrum (ionisation waveform), variable ion injection time up to maximum use of the storage capacity.
2 One or more m/z values are then isolated using a synthesised frequency spectrum (isolation waveform). This phase corresponds to the SIM mode.
3 Collision induced dissociation (CID) by selective excitation to the secular frequency (activation waveform) of the precursor ion, storage of the product ions formed at a low RF value without exciting them further.
4 Product ion scan at a scan rate of ca. 5500 u/s (resonance ejection waveform), detection of the ions by the multiplier.

Collision-induced dissociation is initiated by an additional AC voltage at the end cap electrodes of the ion trap analyser. If the frequency of the AC voltage corresponds to the secular frequency of the selected ions, there is an uptake of kinetic energy by resonance. The collisions of the precursor ions m_p excited in this way with the helium molecules present are sufficiently energy-rich to effect fragmentation. The collision energy is determined by the level of the applied AC voltage. In the collisions the kinetic energy is converted into internal energy and used up in bond cleavage. The product ions formed are stored in this phase at low RF values of the ring electrode and are detected at the end of the CID phase by a normal mass scan. The time required for these processes is in the lower ms range and, at a high scan rate for the GC,

allows the separate monitoring of the deuterated internal standard at the same time. With the realisation of tandem-in-time MS/MS an aspect of particular importance for the efficiency of the process lies in the reliable choice of the frequencies for precursor ion selection and excitation. Instruments with external ion sources permit a calibration of the frequency scale which is generally carried out during the automatic tuning. In this way the MS/MS operation is analogous to that of the SIM mode in that only the masses of the desired precursor ions m_p need to be known. In the case of instruments with internal ionisation, where the GC carrier can cause pressure fluctuations within the analyser, the empirical determination of excitation frequencies and the broad band excitation of a mass window is necessary.

The efficiency of the CID process is of critical importance for the use of GC/MS/MS in residue analysis. In beam instruments optimisation of the collision energy (via the potential of Q_2) and the collision gas pressure is necessary. The optimisation is limited by scattering effects at high chamber pressures and subsequent fragmentation of product ions. Modern collision cells are manufactured from square quadrupole rods which provide superior ion transmission characteristics even at extended length compared to former quadrupole or hexapole cells eliminating the formerly observed loss of ions due to the collision processes (see Fig. 2.209). The now possible extended length of collision cells provides an effective fragmentation process for the generation of the product ion spectrum. Typical collision energies for SRM quantitations are in the range of 10 to 25 eV. Cross-talk from different precursors to the same product ion is effectively eliminated by an active cleaning step between the scans (adds to the interscan time). Encapsulated collision cells using square quadrupoles are typically operated at up to 4 mbar Ar or N_2 pressure for highly sensitive target compound residue analysis.

With the ion trap analyser the energy absorbed by the ions depends on the duration of the resonance conditions for the absorption of kinetic energy and on the voltage level. The pressure of the helium buffer gas can play a role in the collision energy, but is not typically adjusted. Typical values for the induction phase are 3–15 ms and 500–1000 mV. With the ion trap technique there is higher efficiency in the fragmentation and transmission to product ions (Johnson and Yost 1990). In addition the ion trap technique has clear advantages because of the sensitivity resulting from the storage technique, which allows recording of complete product ion spectra even below the pg range.

Modern developments in ion trap technology include the automated determination of collision energies (ACE). This technique makes it easier to set up MS/MS methods by not requiring manual optimization of the collision energy for every compound of a large set of targets. The instrument uses an empirical calibration scheme to calculate an optimum collision energy comprising all of the known instrument parameters as precursor mass and damping gas flow. Three collision energy values can be provided to check results. The middle one typically is the optimum calculated from the instrument parameters. The found levels cover the range of optimum collision energies for compounds of varying ion chemistry. In practice a very good correlation with manual optimization e.g. for pesticides is achieved.

Low mass MS/MS fragments, that are cut from the spectrum by the former regular ion trap operation are stored and scanned for MS/MS spectra using the pulsed q-value dissociation technique (PQD). Normal CID in ion traps is done at a particular excitation q value defining the effective collision energy. Higher q values provide higher collision energy values by getting the precursor ions moving faster but cut the low mass end from the spectrum. PQD works by first exciting the precursor ion at high q value for a short period of time

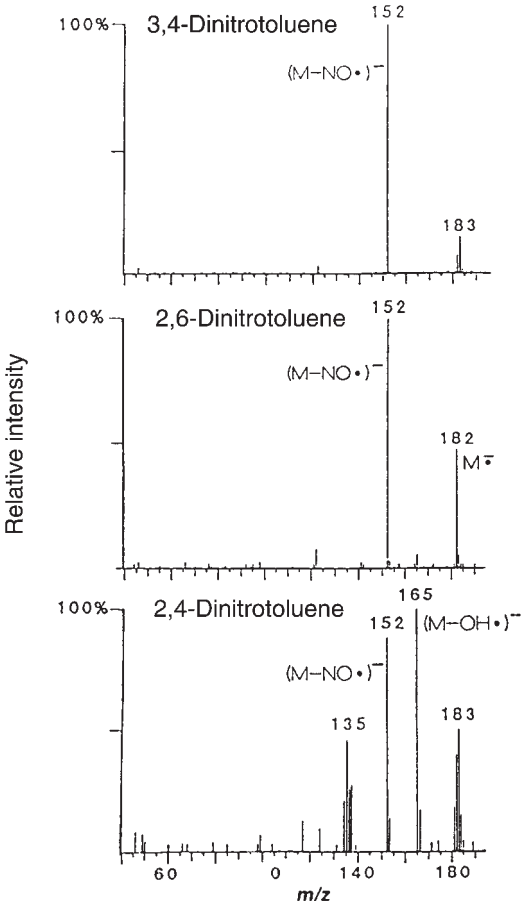


Fig. 2.212 NCI-MS/MS: Differentiation between dinitrotoluene isomers by comparison of the product ion spectra (ion trap analyser, reagent gas water, detection of negative ions) (after Brodbelt and Cooks 1988).

(50–100 μs) using a high collision energy as determined by ACE. During the induced fragmentation the q value is lowered significantly to store the full range of product ions. The result is that also low m/z fragments ions are being trapped. Therefore PQD is primarily useful as a qualitative tool for structure elucidation, but also finds utility in quantitative applications where mainly and intense low m/z fragment ions are observed as known for many N containing compounds e.g. amines. PQD is a patented technique (US 6,949,743 & 7,102,129) that was first used on the ion traps from Thermo Fisher Scientific, San Jose, CA, USA.

Today comparable results are available for both the triple-quadrupole and ion trap analysers. It is typical for the product ion spectra obtained with ion trap mass spectrometers that the intensities of the precursor ions are significantly reduced due to efficient CID. The product ion spectra show only a few well defined but intense product ions derived directly from the precursor. Further fragmentation of product ions usually does not occur as the m/z of the precursor ion is excited exclusively. Triple quadrupole instruments show less efficiency in the CID process resulting in a higher precursor ion signal. Primary product ions fragment consecutively in the collision cell and produce additional signals in the product ion spectrum. The efficiency of ion trap instruments is also reported to be higher than that of

magnetic sector instruments. The mass range and the scan techniques of precursor scan and neutral loss scan make triple-quadrupole instruments suitable for applications outside the range of pure GC/MS use.

When evaluating MS/MS spectra it should be noted that no isotope intensities appear in the product ion spectrum (independent of the type of analyser used). During selection of the precursor ion for the CID process, naturally occurring isotope mixtures are separated and isolated. The formation of product ions is usually achieved by the loss of common neutral species. The interpretation of these spectra is generally straightforward and less complex compared with EI spectra. When comparing product ion spectra of different instruments the acquisition parameter used must be taken into account. In particular with beam instruments the recording parameters can be reflected in the relative intensities of the spectra. On the other hand, rearrangements and isomerisations are possible, which can lead to the same product ion spectra.

Structure Selective Detection Using MS/MS Transitions

Triple quadrupole as well as ion trap mass spectrometers besides structure elucidation find their major application in quantitation of target compounds in difficult matrices. The increased selectivity when observing specific transitions from a precursor ion to a structural related product ion provides highly confident analyses with excellent LOQs even in matrix samples. In the selected reaction monitoring mode (SRM) an intense precursor ion from the spectrum of the target compound is selected in the first quadrupole Q1, fragmented in the collision cell, and monitored on a selective product ion for quantitation, see the principle given graphically in Fig. 2.210. The high selectivity of the SRM method is controlled by the mass resolution of the first quadrupole Q1. While round rod quadrupoles are working at unit mass resolution, hyperbolic quadrupoles typically are set to 0.7 Da peak width at FPHW as standard and are operated in the highly resolved H-SRM mode for maximum selectivity even at narrow peak widths of 0.4 Da FPHW (patented by Thermo Fisher), also refer to Section 2.3.1.

Analogue to the SIM analysis mode used with single quadrupole instrumentation the SRM mode omits full spectral information for substance confirmation. A structure selective characteristic of the assay is given by the mass difference of the transition monitored. Typically, two independent transitions together with the chromatographic retention time provide the positive confirmation of the occurrence of a particular compound (Council Directive 96/23/EC). State-of-the-art triple quadrupole instrumentation provide transition times as low as 1–2 ms offering the potential for screening of a large number of compounds in a given chromatographic window e. g. for multicomponent pesticide analysis (see Section 4.14).

Data Dependant Data Acquisition

The advanced electronic capabilities of modern triple quadrupole instruments offer additional features for delivering compound specific information even during SRM quantitation analyses. Depending on the quality of a scan currently being acquired, the mode of the data acquisition for a following scan (data point) can be switched to acquire a full product ion spectrum. The level of product ion intensity that triggers the switch of acquisition mode is set method specific. As result one data point in the substance peak is used to generate and acquire the compound product ion spectrum (see Fig. 2.113). This spectrum provides the full structural information about the detected substance and is available for library search

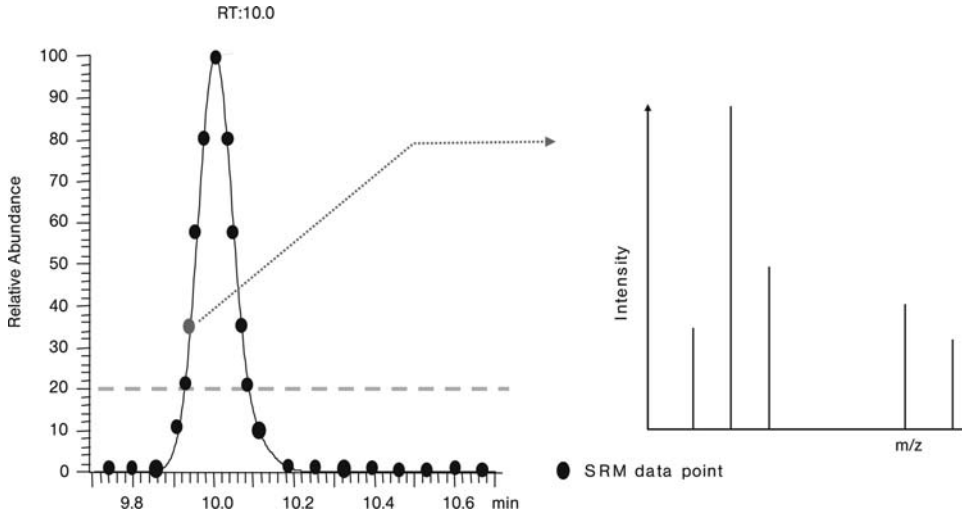


Fig. 2.213 Data dependent data acquisition scheme. The first SRM scan intensity above a user defined threshold (left, dotted line) is acquired as MS/MS product ion spectrum (right).

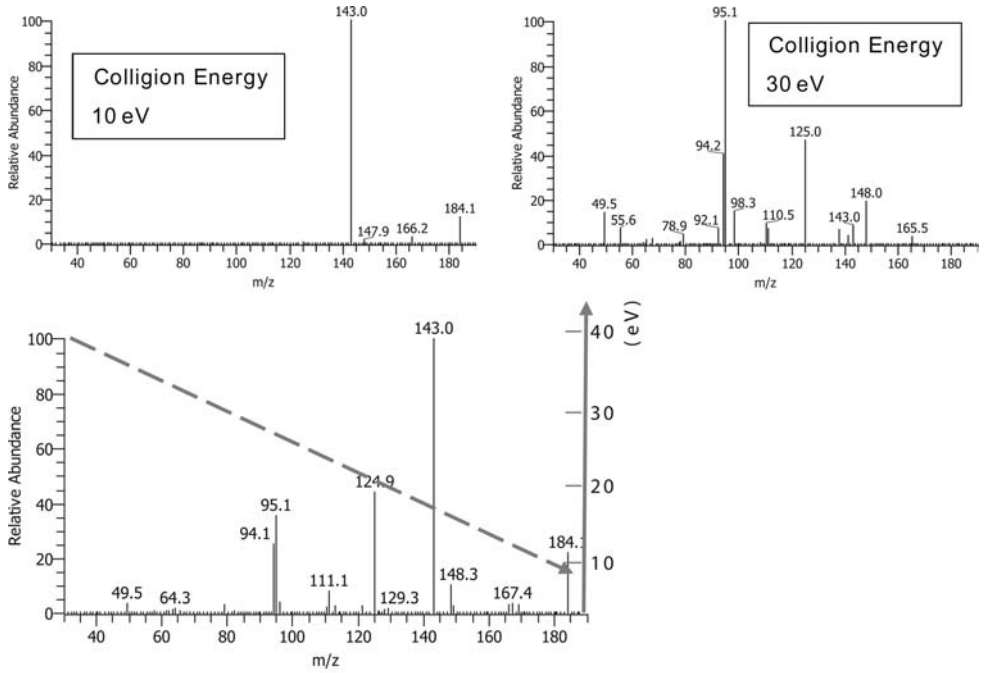


Fig. 2.214 Decreasing collision energy ramp in MS/MS mode provides information rich product ion spectrum.

and compound confirmation. Using the data dependent acquisition mode the final data file contains both quantitative as well as qualitative information.

For the generation of the MS/MS product ion spectrum the applied collision energy has substantial impact on the information content of the spectrum, represented by the occurrence and intensities of fragment and precursor ions. The maximum information on the compound structure is achieved by variation of the collision energy during the product ion scan. High collision energies lead to the generation of low mass fragments while lower collision energies provide the favoured main fragments and still some visible intensity of the precursor ion. The instrumental approach to this solution is the variation of the collision energy during the scan from a high energy to a low energy level by ramping down the ion acceleration voltage using the lenses located at the entrance of the ion beam into the collision cell (see Fig. 2.209). The resulting product ion spectrum consequently delivers the full available information about the structural characteristics of an unknown or the compound to be confirmed (see Fig. 2.114).

2.3.7 Mass Calibration

To operate a GC/MS system calibration of the mass scale is necessary. The calibration converts the voltage or time values into m/z values by controlling the analyser during a mass scan. For the calibration of the mass scale a mass spectrum of a known chemical compound is where both the fragments (m/z values) and their intensities are known and stored in the data system in the form of a reference table.

With modern GC/MS systems performing an up-to-date mass calibration is generally the final process in a tuning or autotuning program. This is preceded by a series of necessary adjustments and optimisations of the ion source, focusing, resolution and detection, which affect the position of the mass calibration. Tuning the lens potential particularly affects the transmission in individual mass areas. In particular, with beam instruments focusing must be adapted to the intensities of the reference substances in order to obtain the intensity pattern of the reference spectrum (see Section 2.3.5.2). The m/z values contained in a stored reference table are localised by the calibration program in the spectrum of the reference compound measured. The relevant centroid of the reference peak is calculated and correlated with the operation of the analyser. Using the stored reference table a precise calibration function for the whole mass range of the instrument can be calculated. The actual state of the mass spectrometer at the end of the tuning procedure is thus taken into account. The data are plotted graphically and are available to the user for assessment and documentation. For quadrupole and ion trap instruments the calibration graph is linear (Fig. 2.215), whereas with magnetic sector instruments the graph is exponential (Fig. 2.216).

Perfluorinated compounds are usually used as calibration standards (Table 2.53). Because of their high molecular weights the volatility of these compounds is sufficient to allow controllable leakage into the ion source. In addition, fluorine has a negative mass defect (^{19}F = 18.9984022), so that the fragments of these standards are below of the corresponding nominal mass and can easily be separated from a possible background of hydrocarbons with positive mass defects. The requirements of the reference substance are determined by the type of analyser. Quadrupole and ion trap instruments are calibrated with FC43 (PFTBA, perfluorotributylamine; Table 2.55 and Fig. 2.217) independent of their available mass

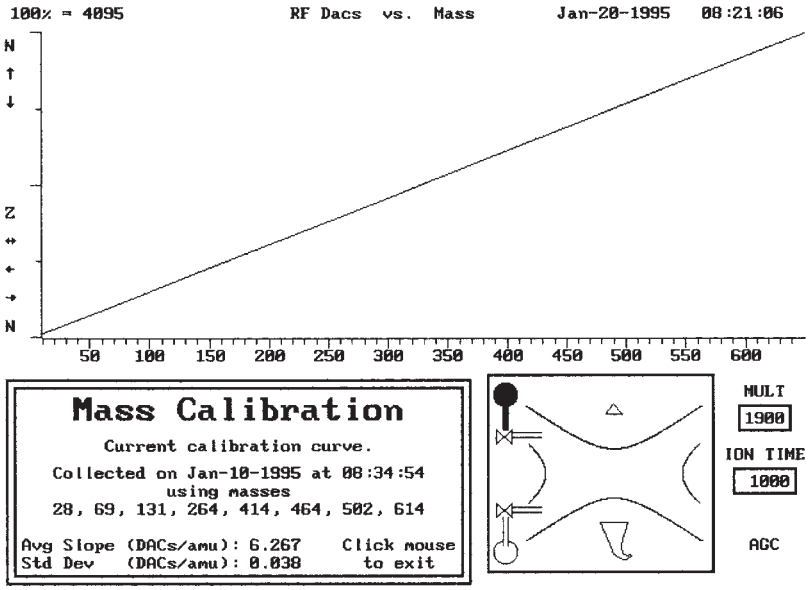


Fig. 2.215 Linear mass calibration of the quadrupol ion trap analyser (voltage of ring electrode against m/z value).

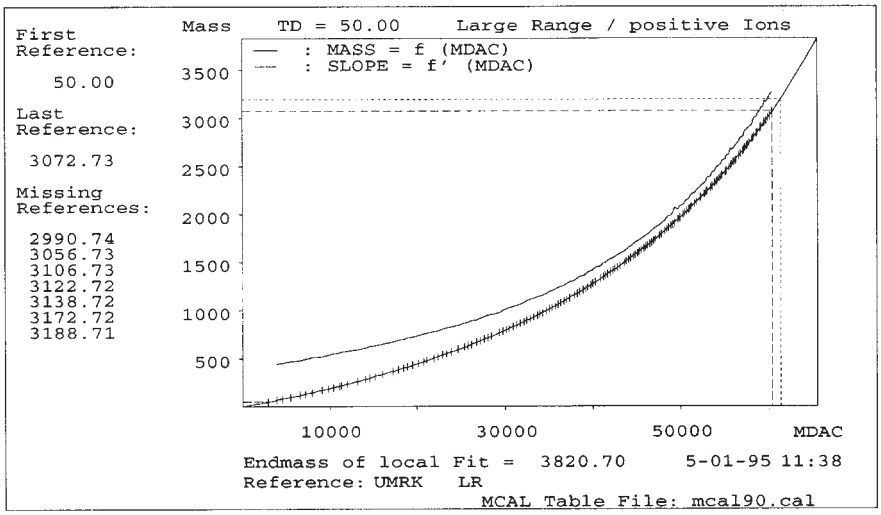


Fig. 2.216 Exponential mass calibration of the magnetic sector analyser.

range. For the compliance to a series of EPA methods and other regulations the targeted source tuning according to the specific manufacturers guidelines is required, see Table 2.54 (Eichelberger 1975). This for instance refers to EPA methods 501.3, 524.2, 8260B, CLP-SOW for the determination of volatiles using 4-bromofluorbenzene (BFB) and the EPA methods 625, 1625, 8250, 8270 on base/neutrals/acids or semivolatiles referring to difluorotriphenyl-

Table 2.53 Calibration substances and their areas of use.

Name	Formula	M	m/z max.	Instrument used	
				Magnetic sector	Quadrupole/ion trap
FC43 [1)]	$C_{12}F_{27}N$	671	614	Up to 620	Up to more than 1000
FC5311	$C_{14}F_{24}$	624	624	Up to 620	Up to more than 1000
PFK [2)]	–	–	1017	Up to 1000	Up to more than 1000
Perfluorinated triazines					
(C_7) [3)]	$C_{24}F_{45}N_3$	1185	1185	800–1200	–
(C_9) [4)]	$C_{30}F_{57}N_3$	1485	1485	800–1500	–
Fomblin [5)]	$(OCF(CF_3)CF_2)_x-(OCF_2)_y$			Up to 2500	–
CsI [6)]	–	–	15 981	High mass range	–

1) Perfluorotributylamine, 2) Perfluorokerosene, 3) Perfluorotriheptylazine, 4) Perfluorotrinonyltriazine, 5) Poly(perfluoropropylene oxide) (also used as diffusion pump oil), 6) Caesium iodide

Table 2.54 Ion abundance criteria for BFB and DFTPP target tuning.

Compound	m/z	Ion abundance criteria (relative abundance)	
BFB	50	15 to 40%	of mass 95
	75	30 to 60% (1)	of mass 95
	95	100%	base peak
	96	5 to 9%	of mass 95
	173	<2%	of mass 174
	174	>50	of mass 95
	175	5 to 9%	of mass 174
	176	>95% but <101%	of mass 174
	177	5 to 9%	of mass 176
(1) 30–80% of mass 95 for EPA 524.2			
DFTPP	51	30–60%	of mass 198
	68	<2%	of mass 69
	70	<2%	of mass 69
	127	40–60%	of mass 198
	197	<1%	of mass 198
	198	100%	base peak
	199	5–9%	of mass 198
	275	10–30%	of mass 198
	365	> 1%	of mass 198
	441	present	of mass 443
	442	> 40%	of mass 198
	443	17–23%	of mass 442

Table 2.55 (a) Reference table FC43/PFTBA (EI, intensities >1%, quadrupole instrument).

Exact mass [u]	Intensity [%]	Formula	Exact mass [u]	Intensity [%]	Formula
68.9947	100.0	CF_3^+	225.9898	2.0	$C_5NF_8^+$
92.9947	2.0	$C_3F_3^+$	263.9866	27.0	$C_5NF_{10}^+$
99.9931	19.0	$C_2F_4^+$	313.9834	3.0	$C_6NF_{12}^+$
113.9961	11.0	$C_2NF_4^+$	351.9802	4.0	$C_6NF_{14}^+$
118.9915	16.0	$C_2F_5^+$	363.9802	1.0	$C_7NF_{14}^+$
130.9915	72.0	$C_3F_5^+$	375.9802	1.0	$C_8NF_{14}^+$
149.9899	4.0	$C_3F_6^+$	401.9770	2.0	$C_7NF_{16}^+$
168.9883	7.0	$C_3F_7^+$	413.9770	9.0	$C_8NF_{16}^+$
175.9929	3.0	$C_4NF_6^+$	425.9770	1.0	$C_9NF_{16}^+$
180.9883	3.0	$C_4F_7^+$	463.9738	3.0	$C_9NF_{18}^+$
213.9898	2.0	$C_4NF_8^+$	501.9706	8.0	$C_9NF_{20}^+$
218.9851	78.0	$C_4F_9^+$	613.9642	2.0	$C_{12}NF_{24}^+$

Table 2.55 (b) Reference table FC43/PFTBA (EI, intensities >0.1%, high resolution magnetic sector instrument).

Exact mass [u]	Intensity [%]	Formula	Exact mass [u]	Intensity [%]	Formula
4.00206		He^+	230.98508	1.1	$C_5F_9^+$
14.01510		CH_2^+	242.98508	0.0	$C_6F_9^+$
18.01002		H_2O^+	263.98656	37.6	$C_5NF_{10}^+$
28.00560		N_2^+	275.98656	0.2	$C_6NF_{10}^+$
30.99786		CF^+	280.98189	0.1	$C_6F_{11}^+$
31.98928		O_2^+	294.98496	0.2	$C_6NF_{11}^+$
39.96184		Ar^+	313.98336	2.7	$C_6NF_{12}^+$
43.98928		CO_2^+	325.98336	0.3	$C_7NF_{12}^+$
49.99626	3.0	CF_2^+	344.98177	0.0	$C_7NF_{13}^+$
68.99466	50.7	CF_3^+	351.98017	1.7	$C_6NF_{14}^+$
75.99933	1.0	$C_2NF_2^+$	363.98017	1.0	$C_7NF_{14}^+$
80.99466	1.2	$C_2F_3^+$	375.98017	1.2	$C_8NF_{14}^+$
92.99466	1.0	$C_3F_3^+$	401.97698	2.0	$C_7NF_{16}^+$
99.99306	3.9	$C_2F_4^+$	413.97698	10.8	$C_8NF_{16}^+$
113.99614	3.5	$C_2NF_4^+$	425.97698	4.0	$C_9NF_{16}^+$
118.99147	5.9	$C_2F_5^+$	451.97378	0.6	$C_8NF_{18}^+$
130.99147	49.8	$C_3F_5^+$	463.97378	7.3	$C_9NF_{18}^+$
149.98987	2.1	$C_3F_6^+$	475.97378	0.0	$C_{10}NF_{18}^+$
168.98827	5.1	$C_3F_7^+$	501.97059	22.7	$C_9NF_{20}^+$
175.99295	1.3	$C_4NF_6^+$	513.97059	0.1	$C_{10}NF_{20}^+$
180.98827	1.3	$C_4F_7^+$	525.97059	0.1	$C_{11}NF_{20}^+$
199.98668	0.2	$C_4F_8^+$	551.96740	0.0	$C_{10}NF_{22}^+$
213.98975	2.5	$C_4NF_8^+$	563.96740	0.1	$C_{11}NF_{22}^+$
218.98508	100.0	$C_4F_9^+$	575.96740	1.3	$C_{12}NF_{22}^+$
225.98975	1.4	$C_5NF_8^+$	613.96420	3.6	$C_{12}NF_{24}^+$

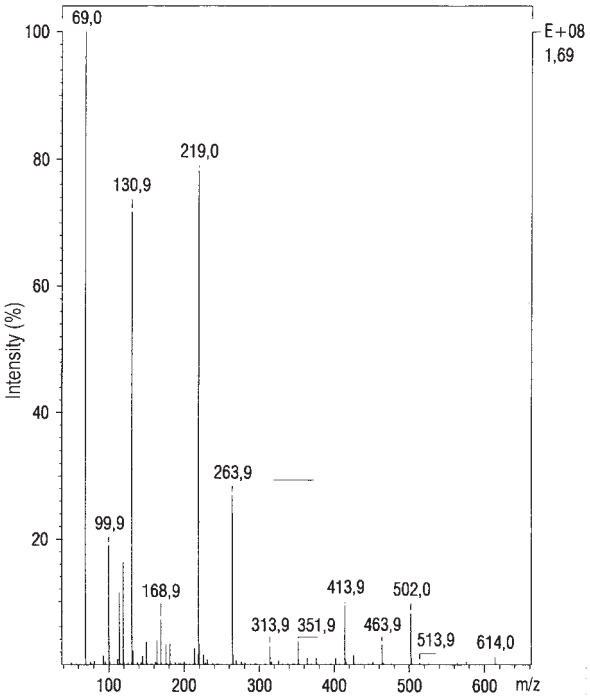


Fig. 2.217 FC43/PFTBA spectrum (Finnigan TSQ 700, EI ionisation, Q3).

phosphine (DFTPP) as tuning compounds providing consistent and instrument independent ion ratio profiles for quantitation. The precision of the mass scan and the linearity of the calibration allow the line to be extrapolated beyond the highest fragment which can be determined, which is m/z 614 for FC43. For magnetic sector instruments the use of PFK (perfluorokerosene; Table 2.56, Fig. 2.218) has proved successful besides FC43. It is particularly suitable for magnetic field calibration as it gives signals at regular intervals up to over m/z 1000. Also perfluorophenanthrene (FC5311) is frequently used as an alternative calibration compound to FC-43 which covers about the same mass range (see Table 2.57). Higher mass ranges can be calibrated using perfluorinated alkyltriazines or caesium iodide. Reference tables frequently take also account of the masses from the lower mass range, such as He, N_2, O_2, Ar or CO_2, which always form part of the background spectrum.

The calculated reference masses in the given tables are based on the following values for isotopic masses: ^{1}H 1.0078250321 u, ^{4}He 4.0026032497 u, ^{12}C 12.0000000000 u, ^{14}N 14.0030740052 u, ^{16}O 15.9949146221 u, ^{19}F 18.9984032000 u, and ^{40}Ar 39.9623831230 u. The mass of the electron 0.00054857991 u was taken into account for the calculation of the ionic masses (Audi 1995, Mohr 1999).

To record high resolution data for manual work peak matching is employed. At a given magnetic field strength one or two reference peaks and an ion of the substance being analysed are alternately shown on a screen. By changing the acceleration voltage (and the electric field coupled to it) the peaks are superimposed. From the known mass and voltage difference the exact mass of the substance peak is determined. This process is an area of the solid probe technique, as here the substance signal can be held constant over a long period.

Table 2.56 Reference table for PFK (perfluorokerosene), EI ionisation high resolution magnetic sector instrument.

Exact mass [u]	Intensity [%]	Formula	Exact mass [u]	Intensity [%]	Formula
4.002055		He^+	492.969112	1.10	$C_{11}F_{19}^+$
14.015101		CH_2^+	504.969112	0.65	$C_{12}F_{19}^+$
18.010016		H_2O^+	516.969112	0.50	$C_{13}F_{19}^+$
28.005599		N_2^+	530.965919	0.70	$C_{11}F_{21}^+$
30.007855	3.80	CF^+	542.965919	0.60	$C_{12}F_{21}^+$
31.989281		O_2^+	554.965919	0.50	$C_{13}F_{21}^+$
39.961835		Ar^+	566.965919	0.60	$C_{14}F_{21}^+$
51.004083	6.70	CHF_2^+	580.962725	0.70	$C_{12}F_{23}^+$
68.994661	100.00	CF_3^+	592.962725	0.65	$C_{13}F_{23}^+$
80.994661	0.50	$C_2F_3^+$	604.962725	0.60	$C_{14}F_{23}^+$
92.994661	3.30	$C_3F_3^+$	616.962725	0.50	$C_{15}F_{23}^+$
99.993064	5.60	$C_2F_4^+$	630.959531	0.50	$C_{13}F_{25}^+$
113.000889	0.02	$C_3HF_4^+$	642.959531	0.50	$C_{14}F_{25}^+$
118.991467	26.40	$C_2F_5^+$	654.959531	0.55	$C_{15}F_{25}^+$
130.991467	24.00	$C_3F_5^+$	666.959531	0.50	$C_{16}F_{25}^+$
142.991467	1.90	$C_4F_5^+$	680.956338	0.20	$C_{14}F_{27}^+$
154.991467	1.40	$C_5F_5^+$	692.956338	0.25	$C_{15}F_{27}^+$
168.988274	17.00	$C_3F_7^+$	704.956338	0.40	$C_{16}F_{27}^+$
180.988274	8.75	$C_4F_7^+$	716.956338	0.25	$C_{17}F_{27}^+$
192.988274	8.30	$C_5F_7^+$	730.953144	0.20	$C_{15}F_{29}^+$
204.988274	1.50	$C_6F_7^+$	742.953144	0.25	$C_{16}F_{29}^+$
218.985080	8.60	$C_4F_9^+$	754.953144	0.50	$C_{17}F_{29}^+$
230.985080	8.80	$C_5F_9^+$	766.953144	0.20	$C_{18}F_{29}^+$
242.985080	3.80	$C_6F_9^+$	780.949951	0.25	$C_{16}F_{31}^+$
254.985080	1.20	$C_7F_9^+$	792.949951	0.30	$C_{17}F_{31}^+$
268.981887	4.00	$C_5F_{11}^+$	804.949951	0.15	$C_{18}F_{31}^+$
280.981887	6.00	$C_6F_{11}^+$	816.949951	0.05	$C_{19}F_{31}^+$
292.981887	2.70	$C_7F_{11}^+$	830.946757	0.10	$C_{17}F_{33}^+$
304.981887	1.00	$C_8F_{11}^+$	842.946757	0.10	$C_{18}F_{33}^+$
318.978693	2.00	$C_6F_{13}^+$	854.946757	0.10	$C_{19}F_{33}^+$
330.978693	3.70	$C_7F_{13}^+$	866.946757	0.05	$C_{20}F_{33}^+$
342.978693	1.80	$C_8F_{13}^+$	880.943563	0.10	$C_{18}F_{35}^+$
354.978693	0.90	$C_9F_{13}^+$	892.943563	0.10	$C_{19}F_{35}^+$
368.975499	0.80	$C_7F_{15}^+$	904.943563	0.05	$C_{20}F_{35}^+$
380.975499	2.30	$C_8F_{15}^+$	916.943563	0.05	$C_{21}F_{35}^+$
392.975499	1.10	$C_9F_{15}^+$	930.940370	0.05	$C_{19}F_{37}^+$
404.975499	1.00	$C_{10}F_{15}^+$	942.940370	0.05	$C_{20}F_{37}^+$
416.975499	0.55	$C_{11}F_{15}^+$	954.940370	0.05	$C_{21}F_{37}^+$
430.972306	1.85	$C_9F_{17}^+$	966.940370	0.05	$C_{22}F_{37}^+$
442.972306	1.20	$C_{10}F_{17}^+$	980.937176	0.05	$C_{20}F_{39}^+$
454.972306	0.80	$C_{11}F_{17}^+$	992.937176	0.05	$C_{21}F_{39}^+$
466.972306	0.50	$C_{12}F_{17}^+$	1004.937176	0.05	$C_{22}F_{39}^+$
480.969112	1.40	$C_{10}F_{19}^+$	1016.937176	0.05	$C_{23}F_{39}^+$

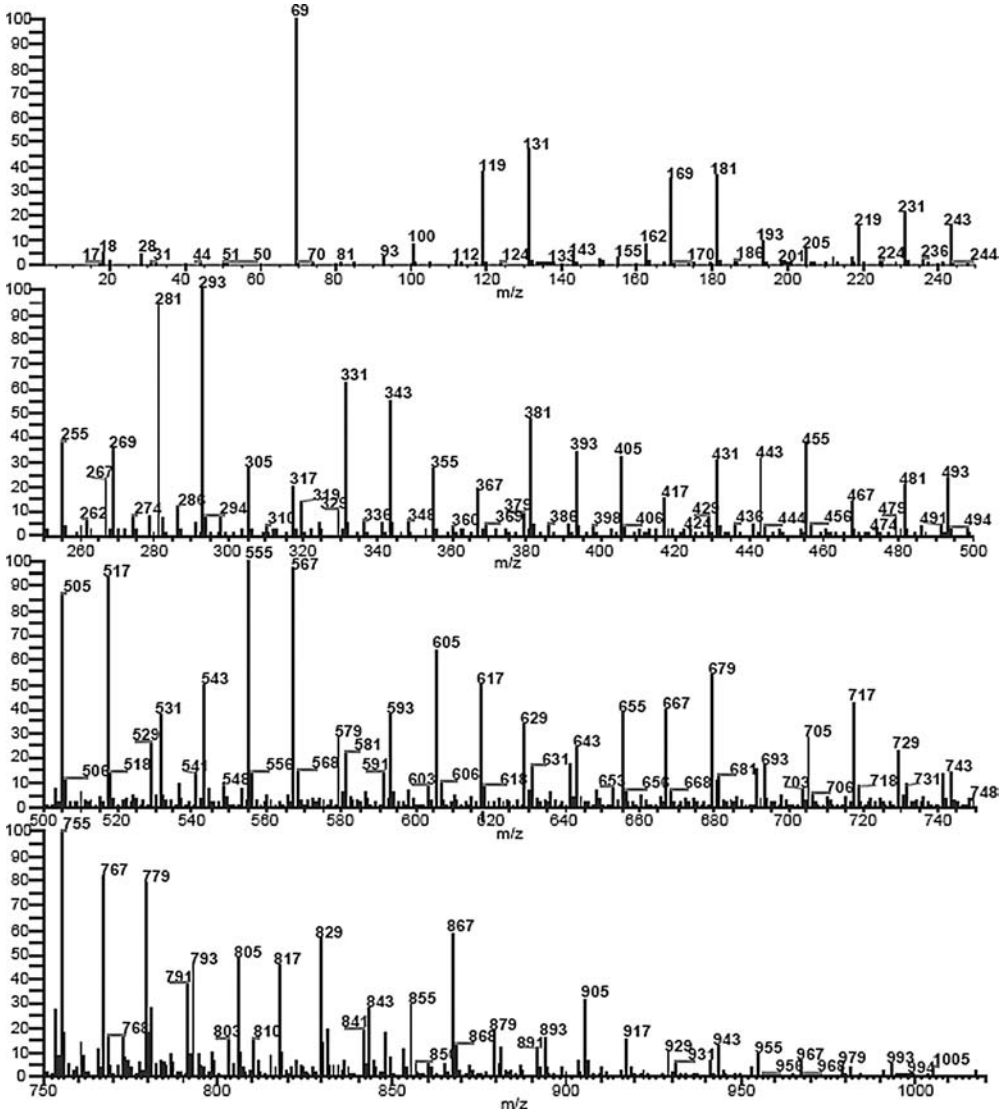


Fig. 2.218 PFK spectrum (Finnigan MAT 95, EI ionisation, B scan).

For GC/MS systems an internal scanwise calibration (scan-to-scan) by control of the data system is employed (internal mass calibration). At a given resolution (e.g. 10 000), a known reference is used which has been fed in at the same time. The analyser is positioned on the exact mass of the substance ion to be analysed relative to the measured centroid of the known reference. At the beginning of the next scan the exact position of the centroid of the reference mass is determined again and is used as a new basis for the next scan.

The usability of the calibration depends on the type of instrument and can last for a period of a few hours to several days or weeks. All tuning parameters, in particular the adjustment

of the ion source, affect the calibration as described above. Special attention should be paid to the temperature of the source. Ion sources which do not contain any internal heating are heated by the cathode in the course of the operation. This results in a significant drift in the calibration over one day. Regular mass calibration is therefore recommended also to comply with the lab internal QA/QC procedures. Heated ion sources exhibit high stability which lasts for weeks and months.

The carrier gas flow of the GC has a pronounced effect on the position of the calibration. Ion sources with small volumes and also ion trap instruments with internal ionisation show a significant drift of several tenths of a mass unit if the carrier gas flow rate is significantly changed by a temperature program. Calibration at an average elution temperature, the use of an open split, or equipping the gas chromatograph with electronic pressure programming (EPC, electronic pressure control) for analysis at constant flow are imperative in this case. Severe contamination of the ion source or the ion optics also affects the calibration. However, reduced transmission of such an instrument should force cleaning to be carried out in good time.

For analyses with differing scan rates using magnetic instruments calibrations are carried out at the different rates which are required for the subsequent measurements. For scan rates which differ significantly, a mass drift can otherwise occur between calibration and measurement. Calibrations of quadrupole and ion trap instruments are practically independent of the scan rate.

Depending on the type of instrument, a new mass calibration is required if the ionisation process is changed. While for ion trap instruments switching from EI to CI ionisation is pos-

Table 2.57 EI positive ion spectra for perfluorophenanthrene (FC5311).

m/z	Rel. abundance %	m/z	Rel. abundance %
55	1.8	219	2.7
56	1.1	231	4.1
57	3.0	243	7.7
69	100.0	255	2.1
70	1.2	267	2.6
93	6.0	286	2.1
94	1.3	293	7.7
100	11.1	305	1.1
112	1.5	317	2.3
119	16.8	331	1.1
124	1.5	343	1.2
131	47.1	367	1.9
143	5.3	405	4.2
155	3.1	455	18.3
162	5.6	505	4.4
169	7.7	517	1.2
181	11.2	555	4.6
193	6.7	605	1.6
205	2.8	624	1.0
217	2.1	–	–

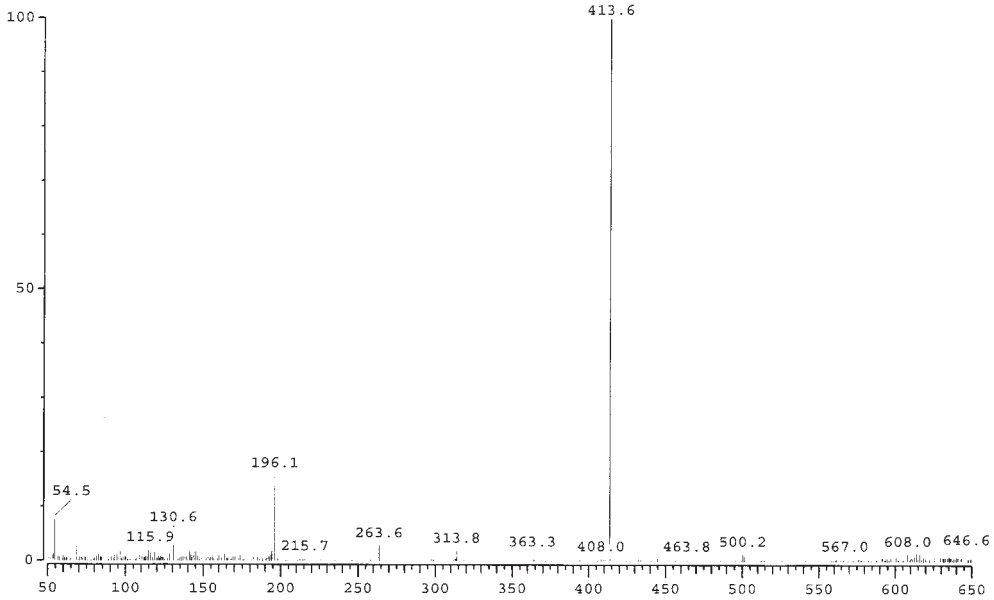


Fig. 2.219 FC43/PFTBA spectrum in PCI mode (CI gas methane, Finnigan GCQ).

sible without alterations to the analyser, with other types switching of the ion source or changing the ion volume are required. For an optimised CI reaction a lower source temperature is frequently used compared with the EI mode. After these changes have been made, a new mass calibration is necessary. This involves running a CI spectrum of the reference substance and consulting the CI reference table (Table 2.58). The perfluorinated reference substances can be used for both positive and negative chemical ionisation (PCI and NCI) (see Figs. 2.219 and 2.220). The intensities given in the CI reference tables can also be used here to optimise the adjustment of the reagent gas.

For reasons of quality control mass calibration should be carried out regularly and should be documented with a print-out (see Figs. 2.215 and 2.216).

2.4 Special Aspects of GC/MS Coupling

2.4.1 Vacuum Systems

Many benchtop quadrupole GC/MS systems are designed for flow rates of about 1 mL/min and are therefore suited for use with normal bore capillary columns (internal diameter 0.25 mm). Larger column diameters, however, can usually only be used with limitations. Even higher performance vacuum systems cannot improve on this value, as the design of the ion sources may be optimised for a particular carrier gas flow. Ion trap GC/MS systems with internal ionisation can tolerate a carrier gas flow of up to 3 mL/min. Normal and wide bore capillaries can be used with these instruments (see also Section 2.2.4).

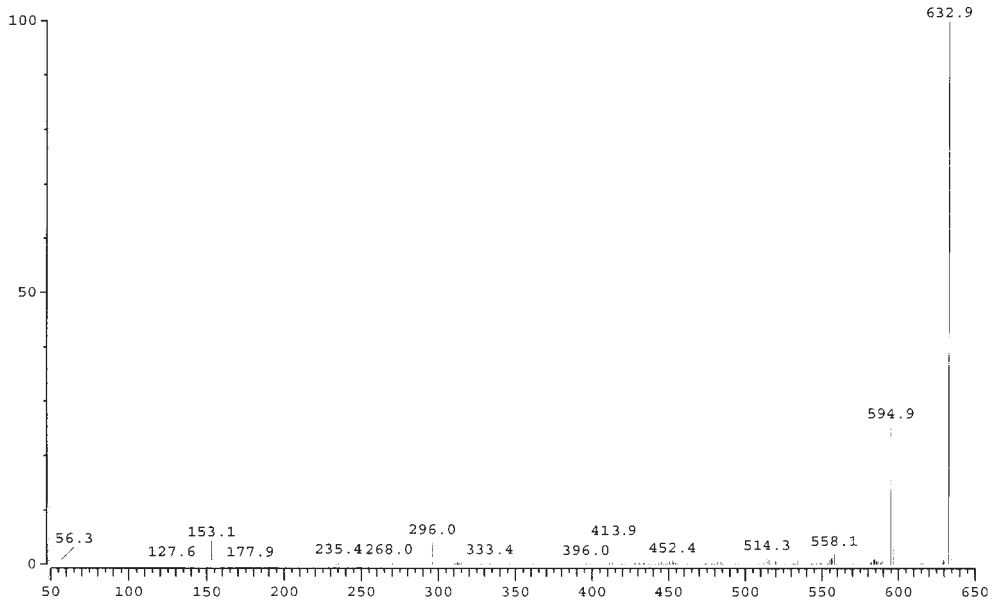


Fig. 2.220 FC43/PFTBA spectrum in NCI mode (CI gas methane, Finnigan GCQ).

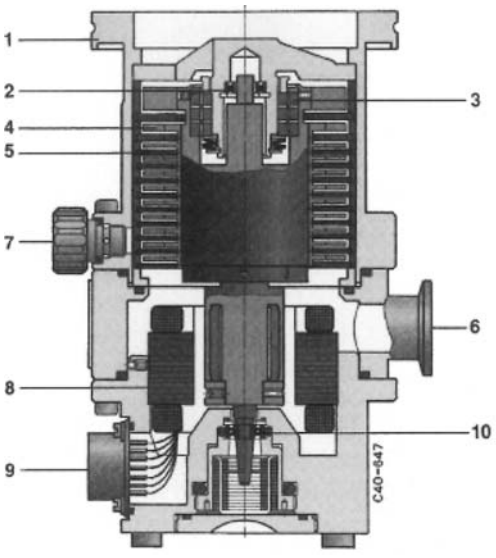
Table 2.58 Reference table for PFK (perfluorokerosene) in NCI mode (CI gas ammonia)

Exact mass	Intensity [%]	Formula	Exact mass	Intensity [%]	Formula
168.9888	2	C_3F_7	530.9664	11	$C_{11}F_{21}$
211.9872	5	C_5F_8	535.9680	8	$C_{13}F_{20}$
218.9856	8	C_4F_9	542.9664	13	$C_{12}F_{21}$
230.9856	26	C_5F_9	554.9664	21	$C_{13}F_{21}$
249.9840	3	C_5F_{10}	573.9648	17	$C_{13}F_{22}$
261.9840	40	C_6F_{10}	585.9648	20	$C_{14}F_{22}$
280.9824	81	C_6F_{11}	604.9633	31	C_4F_{23}
292.9824	12	C_7F_{11}	611.9617	13	$C_{13}F_{24}$
311.9808	51	C_7F_{12}	623.9617	42	$C_{14}F_{24}$
330.9792	100	CF_{13}	635.9617	32	$C_{15}F_{24}$
342.9792	32	C_8F_{13}	654.9601	21	$C_{15}F_{25}$
361.9776	35	C_8F_{14}	661.9585	13	$C_{14}F_{26}$
380.9760	63	C_8F_{15}	673.9585	37	$C_{15}F_{26}$
392.9760	48	C_9F_{15}	685.9585	21	$C_{16}F_{26}$
411.9744	16	C_9F_{16}	699.9553	7	$C_{14}F_{28}$
423.9744	15	$C_{10}F_{16}$	704.9569	6	$C_{16}F_{27}$
430.9728	39	C_9F_{17}	711.9553	8	$C_{15}F_{28}$
442.9728	36	$C_{10}F_{17}$	723.9553	12	$C_{16}F_{28}$
454.9728	16	$C_{11}F_{17}$	735.9553	6	$C_{17}F_{28}$
473.9712	11	$C_{11}F_{18}$	750.9601	4	$C_{23}F_{25}$
480.9696	19	$C_{10}F_{19}$	761.9521	2	$C_{16}F_{30}$
492.9696	23	$C_{11}F_{19}$	773.9521	3	$C_{17}F_{30}$
504.9696	18	$C_{12}F_{19}$	787.9489	3	$C_{15}F_{32}$
516.9696	7	$C_{13}F_{19}$	799.9489	1	C_6F_{32}
523.9680	7	$C_{12}F_{20}$			

The carrier gas for GC/MS is either helium or hydrogen. It is well known that the use of hydrogen significantly improves the performance of the GC, lowers the elution temperatures of compounds, and permits shorter analysis times because of higher flow rates. The more favourable van Deemter curve for hydrogen (see Fig. 2.88) accounts for these improvements in analytical performance. As far as mass spectrometry is concerned, when hydrogen is used the mass spectrometer requires a higher vacuum capacity and thus a more powerful pumping system. The type of analyser and the pumping system determine the advantages and disadvantages.

For the turbo molecular pumps mostly used at present the given rating is defined for pumping out a nitrogen atmosphere. The important performance data of turbo molecular pumps is the compression ratio. The compression ratio describes the ratio of the outlet pressure (forepump) of a particular gas to the inlet pressure (MS). The turbo molecular pump gives a completely background-free high vacuum and exhibits excellent start-up properties, which is important for benchtop instruments or those for mobile use. The use of helium lowers the performance of the pump. The compression ratio (e.g. for the Balzer TPH 062) decreases from 10^8 for nitrogen to $7 \cdot 10^3$ for helium, then to $6 \cdot 10^2$ for hydrogen. The reason for the much lower performance with hydrogen is its low molecular weight and high diffusion rate.

A turbo molecular pump essentially consists of the rotor and a stator (Fig. 2.221). Rotating and stationary discs are arranged alternately. All the discs have diagonal channels, whereby the channels on the rotor disc are arranged so that they mirror the positions of the channels of the stator discs. Each channel of the disc forms an elementary molecular pump. All the channels on the disc are arranged in parallel. A rotor disc together with a stator disc forms a single pump stage which produces a certain compression. The pumping process is such that a gas molecule which meets the rotor acquires a velocity component in the direction of rotation of the rotor in addition to its existing velocity. The final velocity and the direction in which the molecule continues to move are determined from the vector sum of the two velo-



1 High vacuum connection (MS)
2 Emergency bearing
3 Permanent magnet bearing
4 Rotor
5 Stator
6 Forevacuum connection
7 Flooding connection
8 Motor
9 Electrical connection (control instrument)
10 High precision ball bearings with ceramic spheres

Fig. 2.221 Construction of a turbo molecular pump with ceramic ball bearings and permanent magnet bearings for use in mass spectrometry (after Balzers).

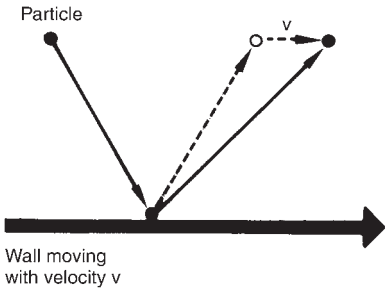


Fig. 2.222 Principle of the molecular pump (after Balzers).

cities (Fig. 2.222). The thermal motion of a molecule, which is initially undirected, is converted into directed motion when the molecule enters the pump. An individual pumping step only produces a compression of about 30. Several consecutive pumping steps, which reinforce each other's action, lead to very high compression rates.

The reduction in the performance of the pump on using hydrogen leads to a measurable increase in pressure in the analyser. Through collisions of substance ions with gas particles on their path through the analyser of the mass spectrometer, the transmission and thus the sensitivity of the instrument is reduced in the case of beam instruments (Fig. 2.223). The mean free path L of an ion is calculated according to:

$$L = p^{-1} \cdot 5 \cdot 10^{-3}\ [\text{cm}] \tag{23}$$

The effect can be compensated for by using higher performance pumps or additional pumps (differentially pumped systems for source and analyser). Ion trap instruments do not exhibit this behaviour because of their ion storage principle.

When hydrogen is used as the carrier gas in gas chromatography the use of oil diffusion pumps is an advantage. The pump capacity is largely independent of the molecular weight

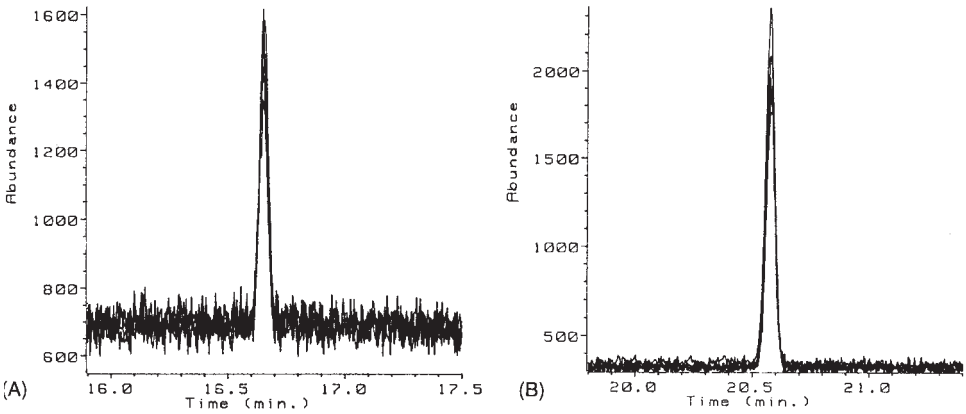
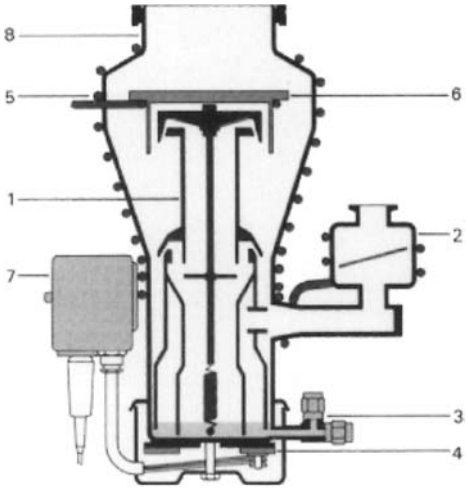


Fig. 2.223 Effect of the carrier gas on the signal/noise ratio in the quadrupole GC/MS (after Schulz).
PCB 101, 50 pg, SIM m/z 256, 326, 328.
(A) Carrier gas hydrogen, S/N 4:1. (B) Carrier gas helium, S/N 19:1



1 Jet system made of pressed aluminium components
2 Forevacuum baffle
3 Fuel top-up/measurement
4 Heating
5 Water cooling
6 Optically sealed baffle
7 Thermal protection switch/connection
8 Stainless steel pump casing

Fig. 2.224 Construction of an oil diffusion pump (after Balzers).

and is therefore very suitable for hydrogen and helium. Oil diffusion pumps also have long service lives and are economical as no movable parts are required. The pump operates using a propellant which is evaporated on a heating plate (Fig. 2.224). The propellant vapour is forced downwards via a baffle and back into a fluid reservoir. Gas molecules diffuse into the propellant stream and are conveyed deeper into the pump. They are finally evacuated by a forepump. Perfluoropolyethers (e. g. Fomblin) or polyphenyl ethers (e. g. Santovac S) are now used exclusively as diffusion pump fluids in mass spectrometry. However, the favourable operation of the pump results in disadvantages for the operation of the mass spectrometer. Because a heating plate is used, the diffusion pump starts sluggishly and can only be vented again after cooling. While older models require water cooling, modern diffusion pumps for GC/MS instruments are air-cooled by a ventilator so that heating and cool-down times of 30 min and longer are unavoidable. Propellant vapour can easily lead to a permanent background in the mass spectrometer, which makes the use of a cooled baffle necessary, depending on the construction. The detection of negative ions in particular can be affected by the use of fluorinated polymers.

Furthermore, it should be noted that, as a reactive gas, hydrogen can hydrogenate the substances being analysed. This effect has already been known from gas chromatography for many years. Reactions in hot injectors can lead to the appearance of unexpected by-products. In GC/MS ion sources reactions leading to hydrogenation products are also known. These cases are easy to identify by changing to helium as the carrier gas.

Both turbo molecular pumps and oil diffusion pumps require a mechanical forepump, as the compression is not sufficient to work against atmospheric pressure. Rotating vane pumps are generally used as forepumps (Fig. 2.225). Mineral oils are used as the operating fluid. Oil vapours from the rotating vane pump passing into the vacuum tubing to the turbo pump are visible in the mass spectrometer as a hydrocarbon background, in particular, on frequent venting of the system. Special devices for separation or removal are necessary. As an alternative for the production of a hydrocarbon-free forevacuum, spiro molecular pumps can be used. These pumps can be run dry without the use of oil. Their function involves centrifugal acceleration of the gas molecules through several pumping steps like the turbo mo-

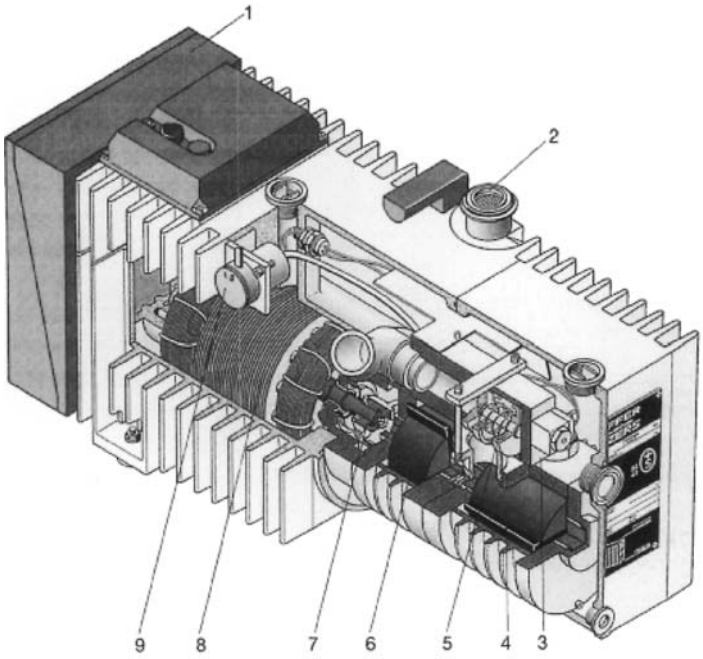


Fig. 2.225 Cross-section of a two-stage rotary vane vacuum pump (after Balzers).

1 Start-up control	4 Pump stage 1	7 Motor coupling
2 Exhaust	5 High vacuum safety valve	8 Motor
3 Aeration valve	6 Pump stage 2	9 Gas ballast valve

lecular pump. For the start-up an integrated membrane pump is used. In particular for mobile use of GC/MS systems and in other cases where systems are frequently disconnected from the mains electricity, spiro molecular pumps have proved useful.

2.4.2 GC/MS Interface Solutions

2.4.2.1 Open Split Coupling

Open split coupling has been known to many spectroscopists from the earliest days of the GC/MS technique. The challenge of open split coupling lay in the balancing of incompatible flow rates, particularly in the cutting out of the solvent peak or other unwanted main components (Fig. 2.226). The cathode was thus protected from damage and the ion source from contamination and the associated reduction in sensitivity thus prevented. These initial problems have now receded completely into the background thanks to fused silica capillaries and easily maintained ion source constructions.

Many advantages of open split coupling are nevertheless frequently exploited in various applications.

The rapid change of GC columns and the desire of the analyst to have a further detector besides the mass spectrometer after the split are now priorities. Because of the different pressure ratios a makeup gas now usually has to be added (Fig. 2.227). If the carrier gas

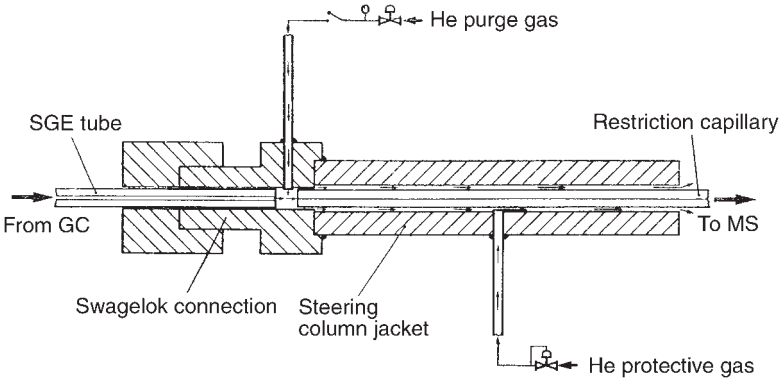


Fig. 2.226 Lengthways section of an open coupling for the expulsion of solvents (after Abraham).

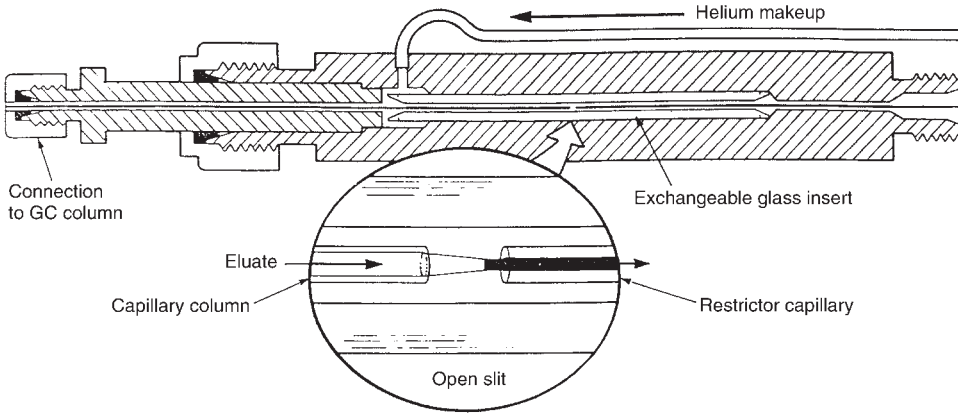


Fig. 2.227 Lengthways section of an open coupling for complete transfer of substance with controllable He makeup (ITD 800, Finnigan).

stream through the column is not sufficient, a considerable air leak can appear if the detector is only coupled by means of a T-piece. In this case the mass spectrometer sucks air through the detector into the ion source via the T-piece. By adding a makeup gas at the split point, these situations can be reliably prevented. The split situated after the column can thus be adapted rapidly to different column types and split ratios.

Advantages of Open Split Coupling

- The retention times of classical detectors, such as FID or ECD, are retained in the GC/MS and allow the direct comparison of chromatograms.
- At the end of the column a split can allow the use of an additional element-specific detector, such as ECD or NPD, and thus give information additional to that given by the mass spectrometer.
- The capillary column can be readily exchanged, usually without venting the mass spectrometer, allowing rapid resumption of work.

- The choice of GC conditions (column length, diameter, flow rate) can be optimised to give the best possible separation independent of the mass spectrometer.
- The use of wide bore, megabore and even packed columns (gas analysis) is possible. The excess eluate not sucked into the mass spectrometer is passed out via the split.
- A constant flow of material, which is independent of the oven temperature of the gas chromatograph, reaches the mass spectrometer. This allows precise optimisation of the ion source.

Disadvantages of Open Split Coupling

- The split point is at atmospheric pressure. To prevent the penetration of air the split point must be flushed with carrier gas. The additional screw joints can give rise to damaging leaks.
- If, because of the balance of column flow and suction capacity of the mass spectrometer, there is a positive split ratio, the sensitivity of the system decreases.
- On improper handling, e.g. on penetration of particles from the sealing ferrule, or a poor cut area at the end of the column, the quality of the GC is impaired (tailing).

2.4.2.2 Direct Coupling

If the end of the column is inserted right into the ion source of the mass spectrometer, the eluate reaches the mass spectrometer directly and unhindered. The entire eluate thus reaches the ion source undivided (Fig. 2.228). For this reason direct coupling is regarded as ideal for residue analysis. The pumping capacity of modern mass spectrometers is adjusted to be compatible with the usual flow rates of regular 0.25 mm and 0.32 mm ID capillary columns.

The end part of the capillary column which is inserted into the ion source requires particular attention to be paid to the construction of the GC/MS interface. The effect of the vacuum on the open end of the column causes molecular flow (as opposed to viscous flow in the column itself) with an increased number of wall collisions. According to the systematic investigations of Henneberg and Schomburg, there is possible adsorption on the column walls at the entry of the capillary into the ion source. It should therefore be ensured that there is uniform heating at this location in particular. Heating the interface too strongly can cause thermal decomposition caused by an activation of the column wall and a reduction in chromatographic performance.

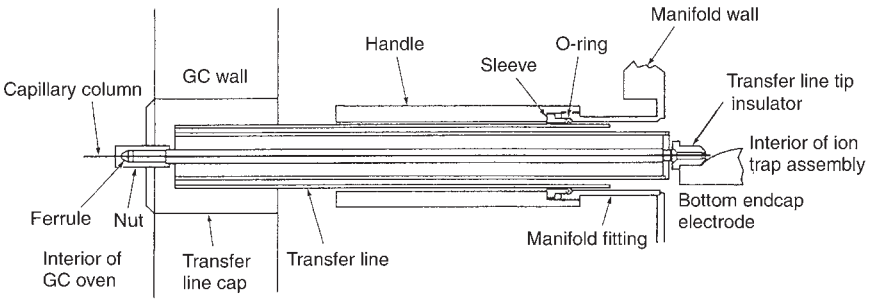


Fig. 2.228 Cross-section of a direct coupling (Finnigan).

Advantages of Direct Coupling

- Uncomplicated construction and handling.
- Single route for the substance from the GC injector to the ion source via a capillary column.
- Reliable vacuum seal at the point where the column is screwed in.

Disadvantages of Direct Coupling

- To change the column the mass spectrometer must be cooled and vented.
- The carrier gas flow into the ion source is not constant as it depends on the GC temperature program chosen and the column dimensions. Working under constant flow conditions (EPC) is recommended.
- The vacuum in the MS system affects the GC column and shortens the retention times in comparison with FID or ECD, for example. A direct comparison with these chromatograms is not straightforward.
- Constantly high interface temperatures limit the lifetime and inertness of sensitive types of column film, such as Carbowax 1701. It is essential to be aware of and adhere to the maximum continuous temperature of a particular GC column.
- The choice of column and adjustment of optimal flow rate is limited by the maximum carrier gas flow for the mass spectrometer.

2.4.2.3 Separator Techniques

For the coupling of capillary gas chromatography with mass spectrometry, special separators are no longer necessary. Only GC systems, which still involve packed columns with flow rates of ca. 10 mL/min or the use of halfmil capillary columns (internal diameter 0.53 mm), cannot be coupled to mass spectrometers without a separator. Separators are inserted between the column and the mass spectrometer to selectively lower the carrier gas load. This procedure, however, involves considerable loss of analyte.

The one-step jet separator (Biemann-Watson separator) frequently used with packed columns, operates according to the principle of diffusion of the smaller carrier gas molecules away from the transmission axis. These are evacuated by an additional rotating vane pump. A reduction in the carrier gas flow of up to ca. 1:100 can be used. Heavier molecules reach the ion source of the mass spectrometer via a transfer capillary. The use of separators involves the loss of ca. 40–80% of the sample material and since capillary column techniques have become widely used for GC/MS residue analysis, they should only be regarded as having historical interest.

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