Edited by Lothar Matter

WILEY-VCH

Food and Environmental Analysis by Capillary Gas Chromatography

Hints for Practical Use

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Foreword

Advances in important areas of environmental and food analysis would be unthinkable without gas chromatography in capillary columns. Many years elapsed between the introduction of capillary columns (open-tubular columns, Golay 1958) into gas chromatographic analysis and the widespread adoption of this technique in practice. The reasons for this delay lay in the difficulties of column technology, instrumentation, and methodology encountered in the use of very narrow capillaries with their very small sample capacity. The process of acceptance received a considerable boost through the introduction of fused silica capillaries (Dandeneau, 1978). Thanks to the column geometry, capillary GC is a miniaturized separating analytical process. In general, GC can only be used for volatile compounds or those which can be vaporized without decomposition at high temperatures. Because of the low sample capacity of systems with capillary columns, environmental analysis with high resolution chromatographic methods places stringent demands on the separation, detection, and identification of analytes in matrices of complex composition. This is particularly true of samples containing very small concentrations of the toxic substances to be detected and determined. Such analysis is possible only with special sample introduction techniques. Detection with the necessary very low detection limits for toxic compounds can be accomplished with comparative ease on use of the ionization detectors available in GC.

Even after sophisticated sample preparation, precise and accurate analyses of trace components in difficult matrices are possible only on optimal use of the best instruments and modern analytical methods of capillary GC.

Particular difficulties are encountered, as in all analytical chromatographic techniques, in forensically reliable identification of target compounds. These can only be overcome by use of hyphenated techniques, preferentially GS/MS.

This book, containing a collection of important applications, will be of great value to those engaged in the practice of environmental analysis. It demonstrates, for a number of typical examples of food and environmental analyses, how modern GC analysis with capillary columns can be successfully applied to this area.

Mülheim an der Ruhr *Prof. Dr. Dr. Gerhard Schomburg*

Preface

The objective of this book differs from that of most other publications on capillary gas chromatography. Instead of presenting all known methods of determination, it focuses on those which are important to the user. The book does not aim to transform the reader into a capillary GC expert, but instead addresses those readers who use capillary gas chromatography because of its various applications, i.e. practitioners. This is therefore *a book by practitioners for practitioners.*

The content is based on a number of meetings held by the German Chemical Society on the same topic and comprises selected presentations delivered on those occasions.

Well known and experienced authors have contributed their knowledge and experience to the individual chapters. All the applications described have been tried and tested and are reproducible on adherence to the basis rules of chromatography.

I wish to express my gratitude to the authors for their readiness to contribute to this work.

Special thanks goes to my family, without whose understanding this book would never have been published.

Dinslaken, May 1997 *Lothar Matter*

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Lothar Matter

1.1 Introduction

High resolution capillary gas chromatography is the analytical method of choice for organic trace analysis in foods and in the environment. Thanks to the wide range of detection methods (flame ionization, electron capture, nitrogen/phosphorus, flame photometric, mass selective, and ion trap detection, to name but a few) it is possible too determine a variety of "contaminants/impurities", both qualitatively and quantitatively.

Capillary gas chromatography is fast, highly sensitive, and accurate. It is one of the most powerful of all separation methods. However, its use calls for knowledge and skill; in other words, the dream of a "black box" or a single push-button instrument into which a sample is placed and which promptly yields the correct results with nothing more ado, will remain a dream for ever.

Finding the appropriate solution for the analytical task at hand requires some degree of experience and a critical approach to the material. The analyst should/must be aware of the properties, composition, and pitfalls of the sample to be analyzed in order to choose and optimize the right analytical method. The generally valid rules of chromatography must be strictly applied in the field of capillary gas chromatography if useful results are to be obtained. If newly published work of interest is to be repeated then all the gas chromatographic parameters given in the publication must also be repeated. Details such as the temperature program, the carrier gas used, column length, column diameter, film thickness, phase, etc., play an important role in the description

of the method. This information is essential not only for evaluation of the method but also for the analyst interested in using the method [1-1]. However, most publications, including many recent ones, fall far short of this mark [1-2, 1-3, 1-4].

The common remark or statement "just inject" should be consigned to the past.

1.2 Rules of Chromatography

In my opinion the most important rules of chromatography are the following (no claim is made to completeness of this list):

- Sample introduction
- Selection of the "right" stationary phase
- Column length
- Column internal diameter
- Film thickness
- Carrier gas
- Clean separation of the components to be analyzed from the matrix

1.2.1 Sample Introduction

Correct sample introduction is a prerequisite for successful chromatographic analysis. Even today the manner is which this is performed is a matter of sometimes heated discussion. Back in 1983 Pretorius and Bertsch wrote: "If the column is described as the heart of chromatography, then sample introduction must be its Achilles tendon" [1-5]. One can only concur with this statement.

The requirements to be fulfilled by a sample introduction system can be described as follows [see 1-1]:

The injection method should not have a discriminatory effect on individual substances. Direct or indirect sample transfer (via an intermediate step) must occur "linearly" into the column, i.e. the original mutual ratios of the components of the sample must remain constant, at least for the substances to be determined.

- *Accuracy:* This important demand is often misunderstood and replaced by good reproducibility. A small standard deviation does not rule out systematic errors/incorrect results.
- *Thermal and/or catalytic decomposition:* The risk of decomposition and /or rearrangement on active surfaces should be reduced to a minimum, or, whenever possible, completely eliminated. If a septum is used, it is subject to constant thermal and mechanical stress and can also influence the chromatographic result by releasing volatile components (plasticizers ...).
- *No band broadening:* The separation performance of the capillary column should not be impaired to any significant extent by the injection method employed.
- *Reproducible retention times:* Unequivocal identification of retention times and exact reproducibility of sample components in a complex matrix go hand in hand.
- *Contamination:* Entry of non-volatile or only slightly volatile sample components into the actual separation system leads to a drop in column performance through peak broadening, shortens the lifetime of the capillary column, or exerts other detrimental effects.

In the area of trace analysis it is necessary to transfer the substances with *minimal possible losses* into the system and to generate peaks that are as high as possible and have steep flanks. We shall not consider the various sample introduction techniques, such as split/splitless, PTV, and direct (or on-column) injection because they are described at length in the literature. However, mention should be made of cold on-column injection according to G. Schomburg and later modified by K. Grob which, in my opinion, represents the most reliable and most accurate sample introduction technique for high resolution capillary gas chromatography [1-6, 1-7].

All other sample introduction systems in which the sample is first heated up in the injector and then transferred in vaporized form to the capillary column are subject to the danger of pyrolysis of sample components [1-8]. They constitute sources of constant misinterpretation in organic trace analysis. The popular determination of hydrocarbons can be cited as an example. Figure 1-1 (see p. 4) shows the result of a quantitative determination of hydrocarbons in a solution with a known content of eicosane, which was part of a round robin test for the recognition of irradiated chicken, pork, and beef by gas chromatographic identification of volatile hydrocarbons [1-9].

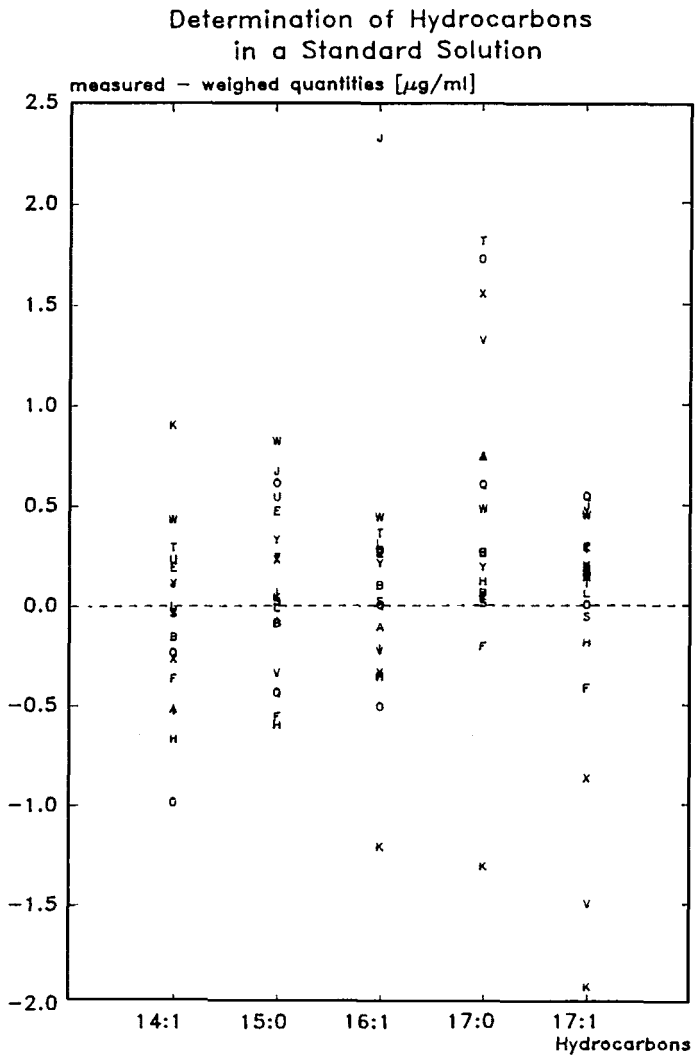

Fig. 1-1: Determination of hydrocarbons in a standard solution.

The figure shows the differences between the values found (mean of several determinations) and the actual amounts present. Of the 18 participating laboratories, only 4 reported values for all hydrocarbons which were very close to the actual values. It can also be said that the rules of chromatography were observed; the GC analysts knew what they were doing.

1.2.2 Choice of the "Right" Stationary Phase

No generally valid rules exist for the selection of the right stationary phase for a given analytical problem. However, experience shows the following guidelines to apply: like dissolves in like. This means that the stationary phase should have a polarity similar to that of the substances to be separated (apolar phases for apolar substances, high boiling samples require high temperature stable phases). Apolar phases are more stable towards oxidative processes than polar ones. Inappropriate storage or leaks in operation of a capillary column (and hence uncontrolled entry of air) are "forgiven" more readily by apolar phases than by polar ones.

Wherever possible, stationary phases which contain a functional group giving a detector response should be avoided. Thus cyanopropyl phases should not be used with a nitrogen/phosphorus detector, or trifluoropropyl phases with the electron capture detector. The signals of both these detectors would be swamped by the normal level of bleeding of the column [1-10].

1.2.3 Column Length

The column length is dependent upon the degree of difficulty of the separation problem. The only general recommendation that can be made is that the column should not be longer than necessary for the problem at hand. The shortest column permitting a good separation should be selected. It is inefficient to use excessively long columns for samples containing only few components requiring determination. The use of capillaries of lengths exceeding 60 or more meters lies in the separation of complex mixtures such as polychlorinated biphenyls.

1.2.4 Column Diameter

The internal diameter of columns used in capillary gas chromatography ranges between 0.53 mm (megabore columns) via standard columns with 0.32 mm to columns with 0.10 mm i.d. (microbore columns). Microbore columns have a high separation performance per unit time but require state-of-the-art instrumentation and are not easy to handle.

The normal standard columns (0.32 mm i.d.) or so-called narrow-bore capillaries (0.25 mm i.d.) are used in most laboratories.

So-called megabore columns (0.53 mm i.d.) have recently come into use as alternatives to packed columns. After examining all the facts supposedly favoring megabore capillaries, K. Grob and P. Frech came to the conclusion

that the widely propagated positive arguments do not stand up to critical examination. [1-11]. All the separation problems for which megabore capillaries are recommended are better solved on standard columns. Perhaps the great popularity of megabore columns lies in their ability to transform a packed column chromatograph into a capillary column instrument. The user thus has a capillary gas chromatograph, i.e. a state-of-the-art instrument.

Mere reduction of the internal column diameter from 0.32 to 0.25 mm can reduce the analysis time in the case of complex samples.

1.2.5 Film Thickness

Playing with the film thickness of the stationary phase shows users possible approaches to mastery of their analyses or separation problems. Owing to interaction between phase and sample, a "thick" film is suitable for separation of highly volatile substances. Sample capacity is greatly increased, i.e. the amount of substance which can be chromatographed without peak deformation increases. Such thick film columns (film thickness greater than 1 μm) permit working at higher temperatures but are subject to greater phase bleeding at the upper temperature limit.

A thin film column exhibits minimal bleeding at the upper temperature limit, a low sample capacity, and is highly suitable for low volatility components with high boiling points. The duration of analysis and peak broadening are minimized in the case of thin film capillaries (film thickness 0.1 μm).

1.2.6 Carrier Gas

The nature and flow rate of the carrier gas through the capillary are of immense importance in a gas chromatographic separation. The flow rate must be reproducibly and accurately adjustable and measurable in order to exploit the separation power and maximum performance of the column. Slight deviations in carrier gas flow have a pronounced influence on the separation. The carrier gases commonly used today are nitrogen, helium, and hydrogen, with hydrogen demonstrably giving the best results (also in the author's own laboratory) [1-12]. Hydrogen carrier gas extends the range of application, e.g. for high temperature gas chromatography, and is preferred over helium. In view of the fact that hydrogen is unreservedly accepted as combustion gas for the FID, objections still raised by "analysts" to hydrogen carrier gas are irrational and totally unfounded if a few basic safety rules are observed [1-13].

Clean separation of the components to be analyzed from the matrix

This attitude must become part of the very make-up of every analyst concerned with high resolution capillary gas chromatography.

1.3 Applications

1.3.1 Determination of Pentachlorophenol in Wood

The hazard to health posed by pentachlorophenol (PCP)as a wood impregnation agent led to the withdrawal of this substance from wood preservatives. German legislation laid down a limit of 5 mg/kg for products contaminated with PCP as a result of treatment. Only the part affected by treatment is relevant for determination of the concentration [1-14]. Impregnated or coated wooden ceilings are considered here as an example.

Figure 1-2 shows a section of a chromatogram of treated wood recorded by HRCGC-ITD (high resolution capillary gas chromatography with ion trap detection) after prior extraction and methylation [1-15]. The methylated, and hence more readily chromatographed, pentachloromethoxybenzene is seen to be separated from a contaminant in the mass range 278–282. Exact identification requires not only agreement between the mass fragments of the sample and those of authentic PCP standard but also the same retention time (mass fragments of PCP ester: 237–239; 265–267; 280–282).

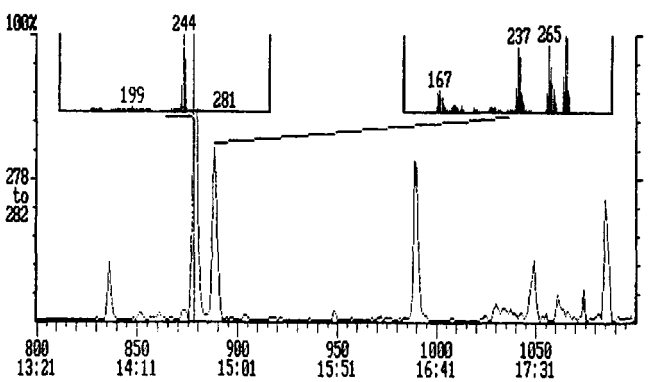

Fig. 1-2: PCP determination (HRCGC-ITD) in a sample of wood. Column: 60 m × 0.25 mm i.d., DB-5, 0.1 µm film thickness/helium. 0.5 µl injection volume, splitless. TP: 90 °C (1 min), then up to 260 °C at 10 °/min, 260 °C 20 min isothermal.

For ECD detection additional column chromatographic purification on Florisil or silica gel is recommended (removal of interfering "colorants" and polar components).

1.3.2 Subjectivity of the Senses – Objectivity of Capillary GC

Sensory testing is still the principal method used for the testing of foods. Fortunately, such tasting is of a subjective nature, i.e. everybody experiences the characteristics of taste and odor in a difference way. Among other techniques; objective headspace capillary gas chromatography can serve as a backup for such subjective findings. For example, pentane and hexanal arise during the processing and storage of fat-containing food samples as a result of oxidative degradation of unsaturated fatty acids. They therefore represent key components for assessing rancidity of foods [1-16]. A significant correlation was found between the organoleptic parameters (odor, taste, color) and the pentane content of edible fats under various storage conditions [1-17]. It should be noted that pentane represents an indicator for the oxidative rancidity of an edible oil [1-18]. The same effect of the interplay between subjective senses and objective headspace GC is illustrated in Figure 1-3. Here the shelf life of cooked ham (sealed in aluminum-plastic) is being examined. One package is tasted on receipt of the sample (part being weighed out for headspace GC analysis and frozen); the other package after appropriate storage until expiry of the minimum shelf life. Comparison of the sensory with the gas chromatographic findings unequivocally shows whether the shelf life was correct or too long. As another example of the objective confirmation of a subjective sensory finding is provided by the so-called "medicine" taste.

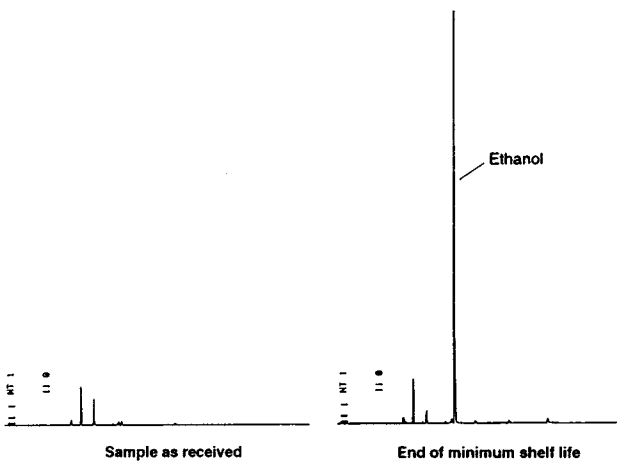

Fig. 1-3: Headspace analysis of cooked ham. Column: 60 m × 0.32 mm i.d., DB-Wax, 0.25 µm film thickness/helium. 500 µl injection volume, split, 60 °C isothermal.

A hibiscus tea infusion was found by a consumer and the author to have a significant " medicine-like" taste. After extraction and enrichment on a solid phase column, dichlorophenols could be identified as the reason for the sensory finding [1-19].

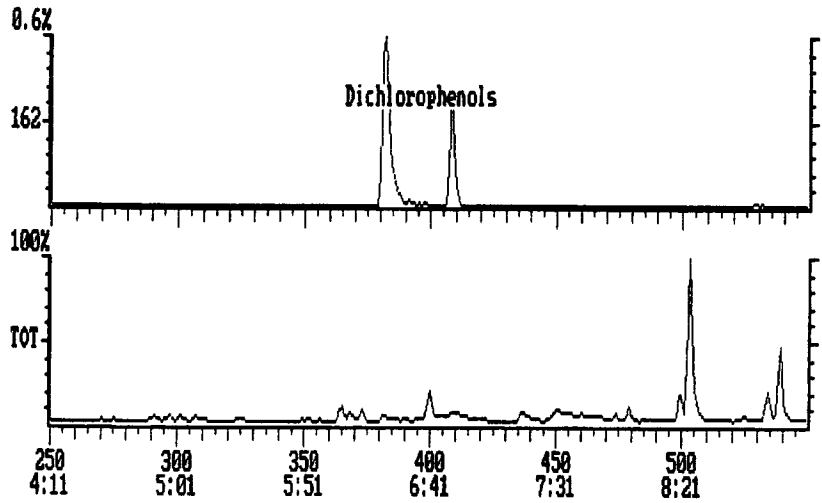

Fig. 1-4: TIC chromatogram of a tee infusion (HRGC-ITD). Column: 30 m × 0.32 mm i.d., DB-5, 0.1 µm film thickness/helium. 0.5 µl splitless. TP: 40 °C (1 min), then up to 260 °C at 10 °/min, 260 °C (20 min) isothermal.

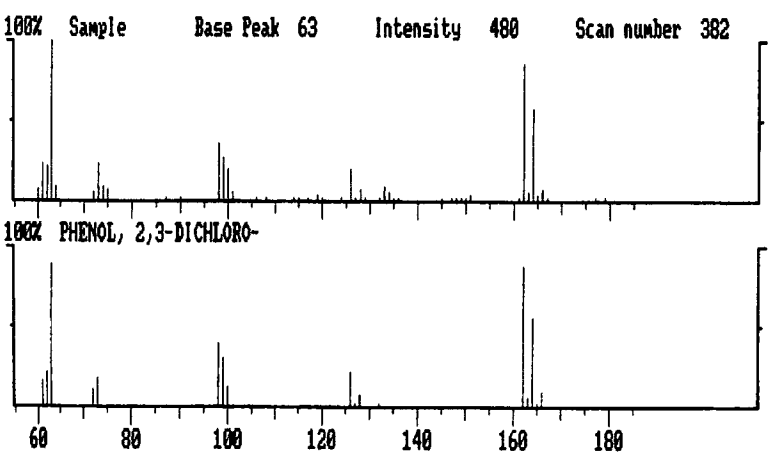

Fig. 1-5: Mass spectrum of dichlorophenol – comparison sample/library search.

Figure 1-4 shows the total ion chromatogram of the analyzed tea extract in the bottom part, and just the mass 162 at the top. The library search shows good agreement with dichlorophenols (Fig. 1-5).

1.3.3 Determination of Bromocyclene and Musk Xylene in Fish

Bromocyclene is used as active substance in veterinary preparations as an ectoparasitic (trade name "Alugan"). It is not (yet) permitted for use with fish. In 1992 this substance was discovered in trout in the course of country-wide monitoring of foods for organic pollutants (such as PCB) conducted by the German Federal Office of Health [1-20]. A second compound was also found to be present, viz. musk xylene. Separation of the two substances was straightforward on observation of the rules of chromatography. Other aromatic nitro musk compounds are also readily separated and determined.

Musk xylene, ketone, tibetene, muscene, and ambrette musk are synthetic compounds not found in nature. They are used in cosmetics and detergents and cleaners and thus enter natural bodies of water and thence the food chain.

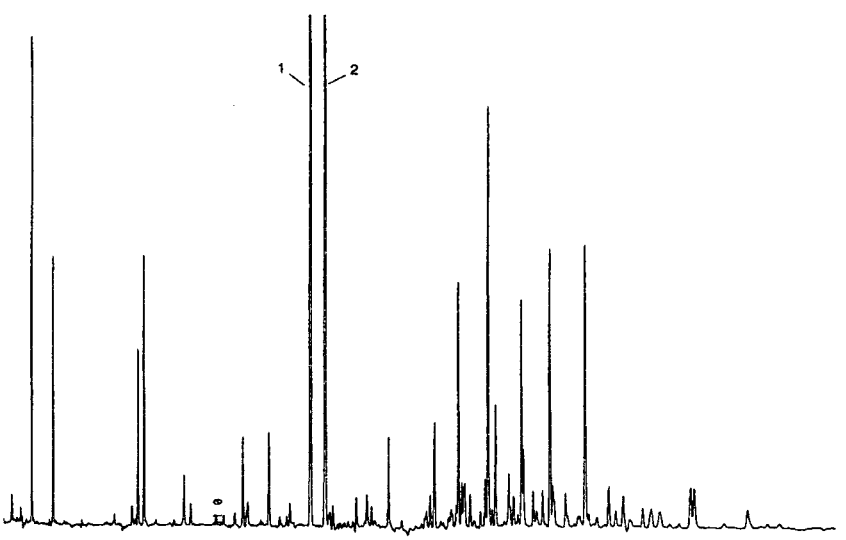

Fig. 1-6: Organochlorine residues (HRGC-ECD) in a rainbow trout: (1) bromocyclene; (2) musk xylene. Column: 60 m × 0.25 mm i.d., DB-5, 0.1 μm film thickness/hydrogen. 0.5 μl splitless. TP: 55 °C (1 min) then up to 145 °C at 30 °/min, 145 °C 10 min isothermal, then up to 225 °C at 2 °/min, 225 °C 20 min isothermal.

Owing to their lipophilic nature, bioaccumulation, and persistence they are to be regarded as ubiquitous environmental contaminants. Figure 1-6 shows an ECD chromatogram of a concentrated fat extract of a rainbow trout. The two largest peaks, viz. bromocyclene and musk xylene, appear in the middle of the chromatogram. The other compounds are organopesticides and PCB. If traces of musk xylene suddenly appear in a blank run, it can be assumed that a musk-containing cosmetic is responsible [1-12].

1.3.4 Separation of 33 Organochloropesticides

The following example is intended to demonstrate observation of the chromatographic rules. Figure 1.7 shows the separation of 33 organochloropesticides in trace amounts ranging between 50 and 200 picogram, depending upon the substance.

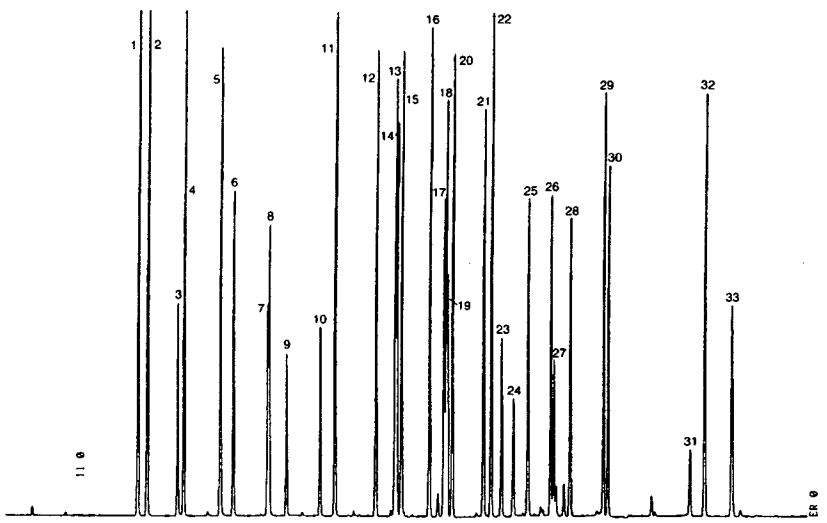

Fig. 1-7: Separation (HRGC-ECD) of organochloropesticides: 1: α-hexachlorocyclohexane; 2: hexachlorobenzene; 3: β-hexachlorocyclohexane; 4: lindane (γ-hexachlorocyclohexane); 5: δ-hexachlorocyclohexane; 6: ε-hexachlorocyclohexane; 7: PCB 31; 8: PCB 28; 9: heptachlor; 10: PCB 52; 11: aldrin; 12: isodrin; 13: *cis*-heptachlor epoxide; 14: oxichlordan; 15: *trans*-heptachlor epoxide; 16: *cis*-chlordan; 17: *o,p*-DDE; 18: α-endosulfan; 19: PCB 101; 20: *trans*-chlordan; 21: dieldrin; 22: *p,p*-DDE; 23: o,p-DDD; 24: β-endosulfan; 25: *o,p*-DDT; 26: *p,p*-DDD; 27: ethion; 28: PCB 153; 29: *p,p*-DDT; 30: PCB 138; 31: methoxichlor; 32: PCB 180; 33: mirex.
Column: 60 m × 0.25 mm i.d., DB-5, 0.1 μm film thickness/hydrogen (temperature program , see Fig. 1-6).

The chromatogram recorded with temperature programming shows only negligible, if any, baseline drift. The peaks show no tailing. The sequence of the individual compounds is valid only for the capillary column used and the specific temperature program.

1.3.5 Sense and Nonsense of Analyzing PAH-Contaminated Soils [1-22]

Polycyclic aromatic hydrocarbons are compounds with a number of fused aromatic rings. They are of both natural and anthropogenic origin and are formed by free-radical mechanisms, especially on incomplete combustion or pyrolysis of organic material.

The compounds present in emission condensates or tar products can be divided into fractions of certain substance classes:

1. PAH with 2 and 3 rings
2. PAH with 4 or more rings
3. Thiaaranes
4. Nitro-PAH
5. Phenols
6. Carbazoles
7. Azaarenes
8. Aromatic amines

In animal experiments the first group shows a low carcinogenicity but a high acute toxicity. The situation is reversed with the higher PAH of the second group: low toxicity, high carcinogenicity. Of importance in the toxicological assessment are the synergic effects arising on combination of various PAH. Thus higher doses of carcinogenic PAH often lead to inhibitory action; in contrast, combinations of hardly effective individual doses can lead to potentiation.

Studies performed on the individual compounds of the 8 classes of substances in animal experiments reveal extremely high carcinogenicity in some cases (e.g. for benzo[*a*]pyrene or naphthylamine). Epidemiological studies confirm the results of animal experiments. Recently, increased attention has been

focused not only on "classical" PAH but also on the phenols, quinones, and diols of PAH with regard to their carcinogenic action. However, their complex nature still precludes a clear picture of their toxicity. It should therefore be noted that not only the content of individual substances but the relative amounts of PAH determine their biological effects.

In order to take full account of these toxicological aspects both the concentrations of the individual substances and sum parameters of primary PAH are considered. The primary substances correspond to the 16 EPA and 6 TVO parameters. The sum parameters permit very good assessment of the hazard caused directly by soils.

In order to assess the hazard to ground water posed by PAH-contaminated soils, the pollutants in the filtrate of a 24 h eluate are studied. Once again, the two sum parameters (6 TVO and 16 EPA parameters) are used. Assignment of the soils to various classes of disposal sites is also undertaken on the basis of these parameters. However, since we are dealing mainly with the higher fused and thus poorly water-soluble PAH, only very low concentrations of the compounds are to be expected in the eluate.

What is the situation with the other compounds?

To determine this, the original substance and eluates from highly contaminated soil (sum of TVO-PAH > 1 g) were investigated by GC-ITD with and without clean-up. The instrumentation parameters were: 25 mm × 0.32 mm i.d., FS SE 54, 0.25 μm film thickness, splitless injection (1 μl), 300 °C, temperature program from 90 °C (1 min) at 15 °/min to 280 °C (15 min), ITD 800, mass range from 30 to 400 amu.

The results are shown in Figures 1-8 to 1-11.

Accordingly, after clean-up the original substance contains in addition to the TVO and EPA-PAH further alkylated PAH and lower concentrations of numerous N-, O-, and S-containing aromatic substances. In the eluate without clean-up the PAH are present to a much smaller extent (low solubility in water). However, if we consider the heteroaromatics, they represent the main burden in the eluate. After column clean-up of the eluate the same amounts of PAH are still present but the heteroaromatics are now only present in low traces.

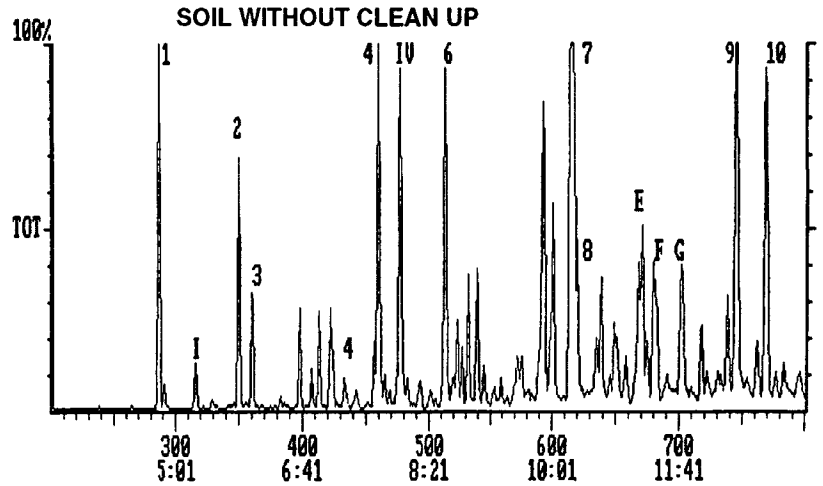

Fig. 1-8: TIC chromatogram of a PAH-contaminated soil without clean-up.
For Figures 1-8 to 1-11:
1: Naphthalene; 2: 2-methylnapthalene; 3: 1-methylnapthalene; 4: acenaphthylene; 5: acenaphthene; 6: fluorene; 7: phenanthrene; 8: anthracene; 9: fluoranthrene; 10: pyrene.
I: quinoline; II: quinoline; III: methylquinoline; IV: dibenzofuran; V: N-containing aromatic; VI: O-containing aromatic; VII: O-containing aromatic; VIII: O-containing aromatic; IX: benzoquinoline; X: pyridine derivative.
A: S-containing aromatic; B: methylquinoline; C: N-containing aromatic; D: S-containing aromatic; E–G: alkylated PAH.

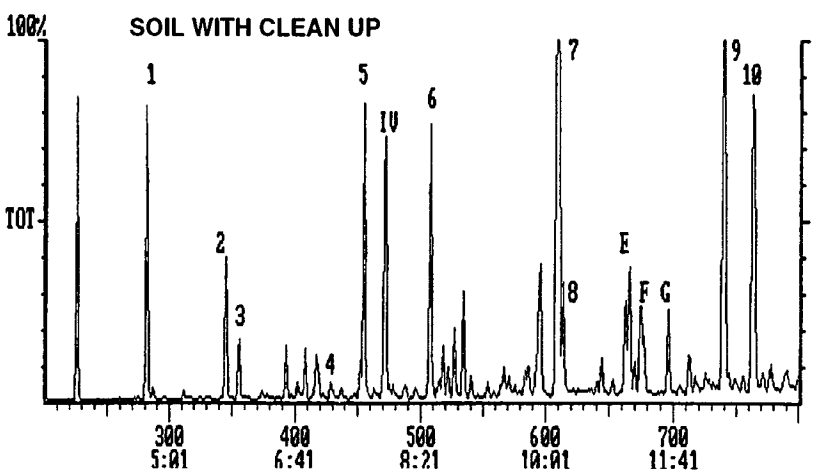

Fig. 1-9: TIC chromatogram of a PAH-contaminated soil with clean-up.

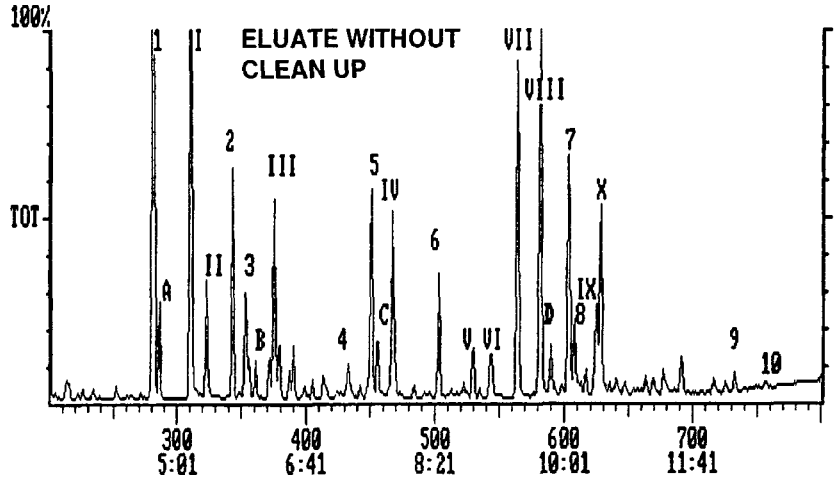

Fig. 1-10: TIC chromatogram of a PAH-contaminated soil eluate without clean-up.

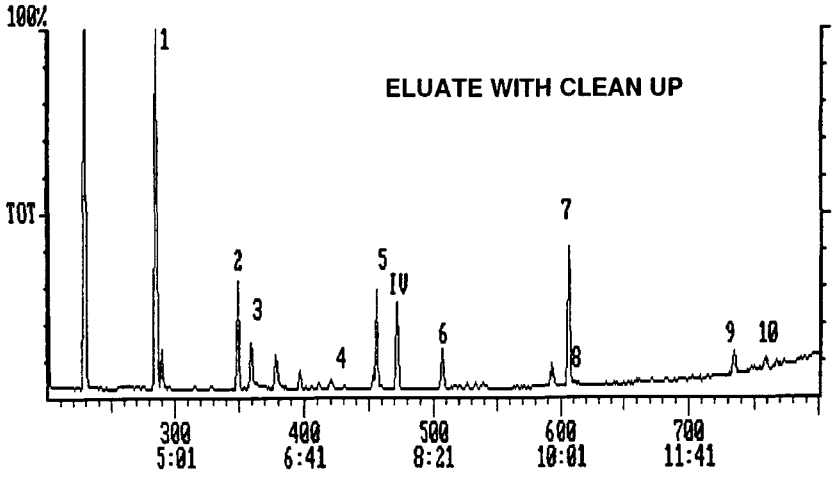

Fig. 1-11: TIC chromatogram of a PAH-contaminated soil eluate with clean-up.

To summarize, it must be stated that estimation of the hazard posed to ground water by contaminated soil through a 24-h eluate and the PAH sum parameters is a rather unfortunate choice. The PAH studies mask the actual potential hazard posed by contaminated materials. The main burden in the eluate is presented by the more water-soluble heteroaromatics and *not* by the PAH. Ecotoxicological studies of the eluate should therefore also take account of the PAH analogs which have so far attracted little if any attention. There is little detailed information about the toxicity of the heteroaromatics. Their better degradability suggests a lower toxicity than that of PAH; however, a toxic action cannot be excluded [1-23].

1.3.6 Detection of Irradiated Fat-Containing Foods

Irradiation of some fat-containing foods such as meat can be detected quickly and unequivocally by GC-FID of GC-MS. On treatment of triglycerides with ionizing radiation, cleavage of chemical bonds occurs in primary and secondary reactions. Radiolysis products are formed which have one carbon atom (C_{n-1}) or two carbon atoms less (C_{n-2}) than the original fatty acids of the triglyceride. Given a knowledge of the fatty acid composition of the sample being investigated the principal radiolysis products can be predicted. After

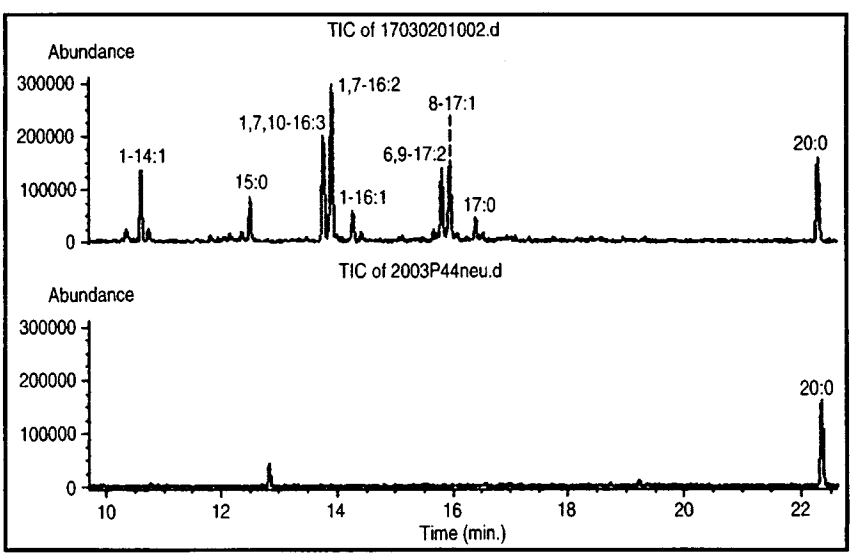

Fig. 1-12: TIC chromatogram of irradiated (3 kGy) and non-irradiated chicken meat (fat fraction).

collecting the fat the apolar fraction is isolated and the hydrocarbons determined by gas chromatography [1-9]. Unequivocal identification requires strict application of the rules of gas chromatography. Figure 1-12 shows the radiation-induced hydrocarbons from the fat fraction of irradiated chicken meat, and Figure 1-13 those in irradiated pork and beef [1-24].

The clear appearance of "new" hydrocarbons underscores the applicability of this method.

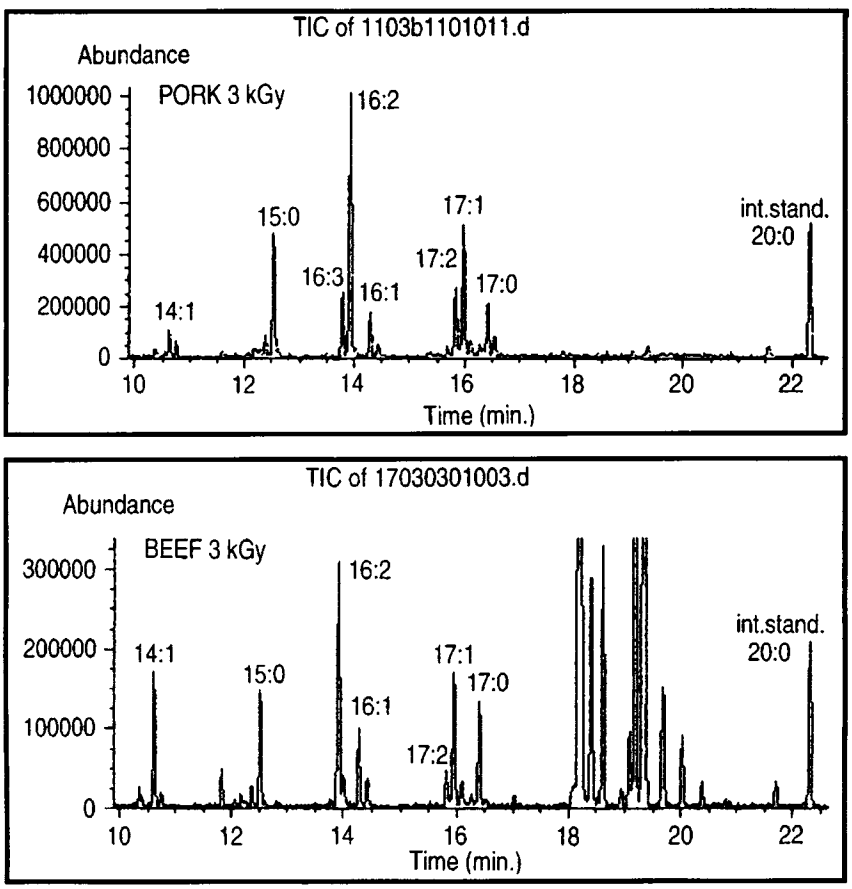

Fig. 1-13: TIC chromatogram of irradiated pork and beef (fat fraction).

1.3.7 Distinction between Animal Species in Heated Products

Species differentiation in raw and heated foods is nowadays performed almost exclusively by electrophoretic methods. However, in heated foods and food mixtures this method does not provide unequivocal evidence for the animal species used. If only fat is available then these methods fail. In this case, capillary gas chromatographic fatty acid analysis (observing the rules of chromatography) is the method of choice [1-25]. The following examples support this claim.

1.3.7.1 Detection of the Addition of Pork Dripping to Goose Dripping

Figure 1-14 shows the fatty acid methyl ester chromatogram of pure goose dripping.

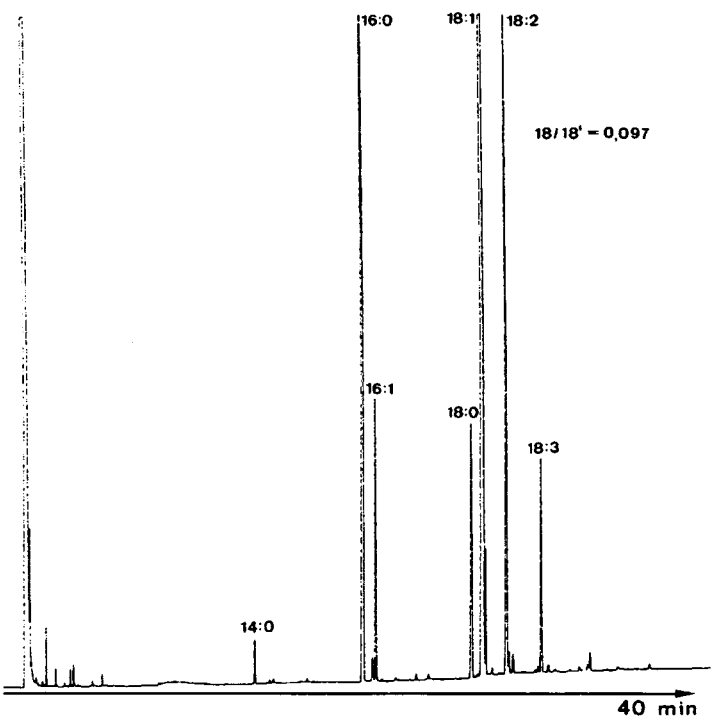

Fig. 1-14: Range of fatty acids (HRGC-FID) present in pure goose dripping. See Figure 1-22 for GC conditions.

Calculation of the ratio of stearic acid to oleic acid reveals the admixture of pork dripping (to improve the spreading properties). While pure goose dripping shows a ratio of up to 0.13, obligatory labeling indicating addition of 10% pork dripping is required if this ratio is exceeded.

1.3.7.2 Detection of the Addition of Cow's Milk to Sheep and Goat's Milk Products

Adulteration of sheep or goat's milk cheese by addition of cow's milk is proven by means of the ratio of myristoleic to pentadecanoic acid (see [1-25]). In a 100% pure sheep or goat's milk cheese this ratio is 0.20 at most, while a cheese prepared from cow's milk shows a value of about 1.

The fatty acid methyl ester spectrum of a pure sheep's milk cheese is shown in Figure 1-15 while Figure 1-16 (see p. 20) indicates the addition of cow's

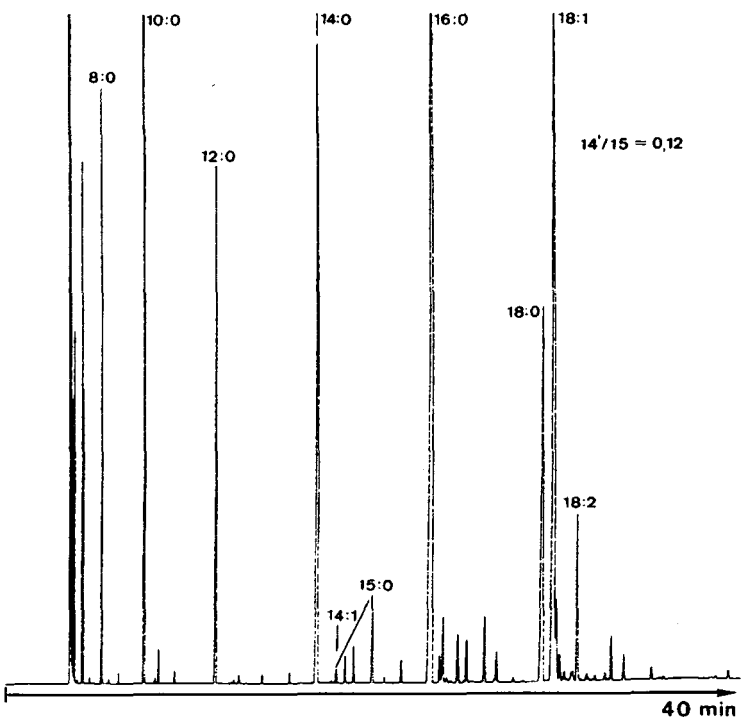

Fig. 1-15: Range of fatty acids (HRGC-FID) present in pure sheep's milk cheese. See Figure 1-22 for GC conditions.

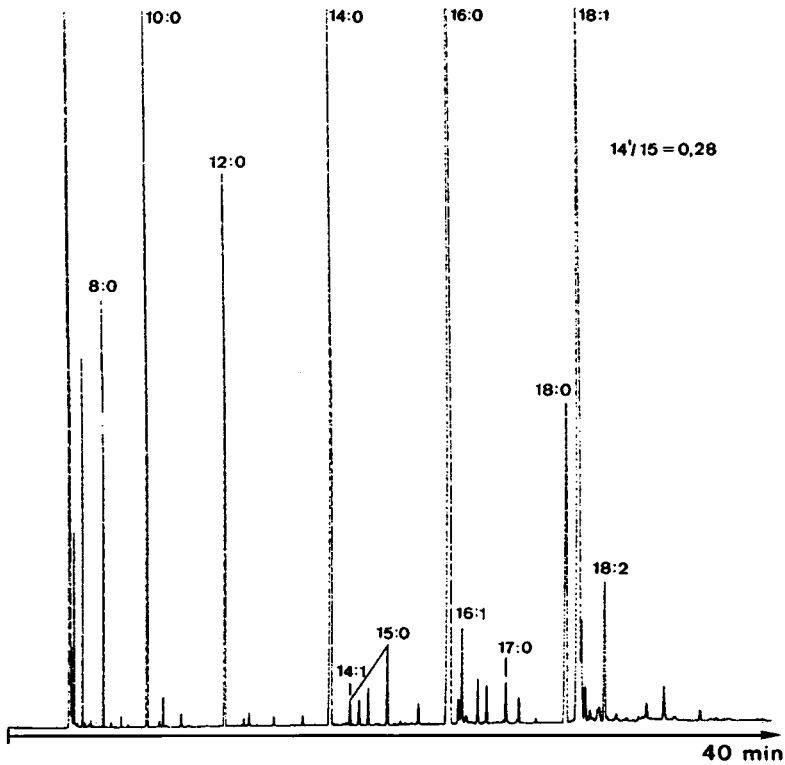

Fig. 1-16: Range of fatty acids (HRGC-FID) present in sheep's milk cheese adulterated with cow's milk. See Figure 1-22 for GC conditions.

milk although the product was declared as 100% sheep's milk cheese. The ratio lies above 0.20.

Effects of different fodder, breed, mode of cheese maturation, etc. play only a minor role [1-26, 1-27]. However, such investigations require unequivocal separation of the fatty acids, i.e. rigorous adherence to the rules of chromatography.

Confirmation of the capillary GC findings is possible, for example, via the β-carotene content of the cheese. Sheep and goat's milk cheese exhibit only low β-carotene contents (<1 μg/kg) whereas cow's milk contains more than 1000 μg/kg of β-carotene. Admixture of cow's milk is indicated by β-carotene contents in excess of 100 μg/kg [1-28].

1.3.7.3 Distinction between Beef and Pork Products

In distinguishing between pork and beef (raw, heated, individually or together) eicosadienoic acid (C20:2) provides sure proof of pig as animal species [1-29]. It is present in amounts up to 0.7% (of total fatty acids) in pork fat but only ≤0.05% in beef fat. Figure 1-17 shows the range of fatty acids present in pork products. While pork fat contains only traces of myristoleic and pentadecanoic acids (≤0.05%) beef contains much higher amounts of these fatty acids.

Linoleic acid can serve as a further distinguishing factor (beef < 2%, pork 8–12%); however, different feeds may exert an influence here.

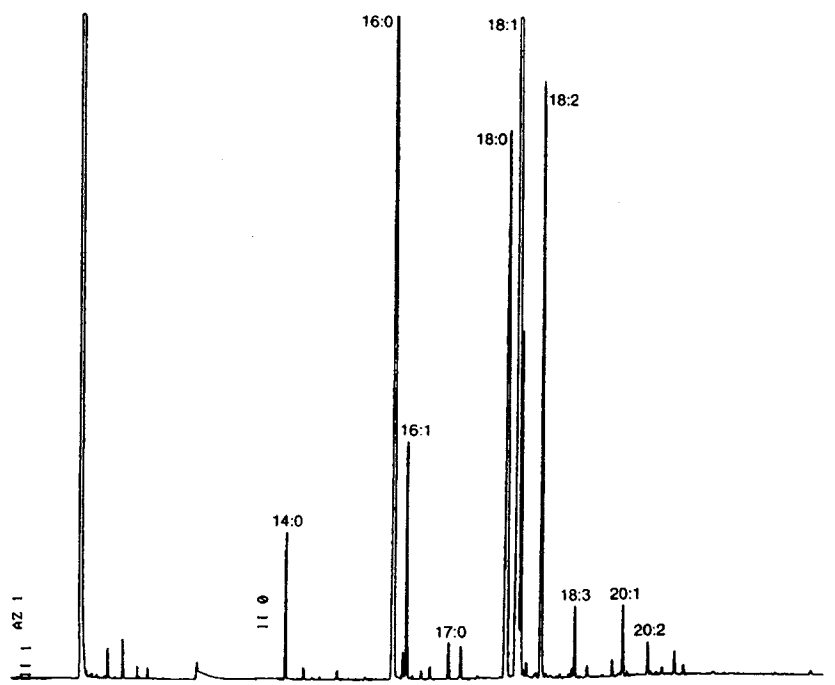

Fig. 1-17: Range of fatty acids (HRGC-FID) present in pork. See Figure 1-22 for GC conditions.

1.3.7.4 Distinction between Hutch Rabbit and Wild Hare

This example demonstrates a distinction between animal species caused, among other things, by feeding differences, and can also serve as evidence in the case of incorrect labeling. Figure 1-18 sows this difference.

While the fatty acid spectrum of hutch rabbits contains higher amounts of linoleic acid, the wild hare exhibits a fatty acid distribution in which linolenic acid predominates [see 1-25].

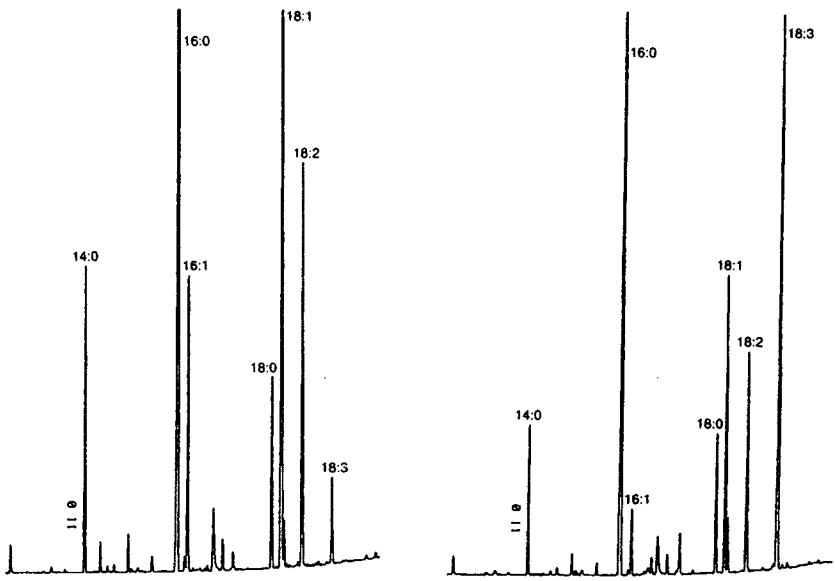

Fig. 1-18: Part of the range of fatty acids (HRGC-FID) present in hutch rabbit (left) and wild hare (right). See Figure 1-22 for GC conditions.

1.3.7.5 Distinction between Domesticated Pig and Wild Boar, and between Veal and Beef

Figure 1-19 indicates the difference between domesticated pig and wild boar and between veal and beef as bar charts.

The ratio of heptadecanoic to palmitic acid (C17:0/C16:0) × 100 indicates which animal species is present (genuine wild boar).

A distinction between chicken and turkey meat is also possible with the aid of this ratio. Figure 1-20 shows the fatty acid methyl esters present in a chicken meat product.

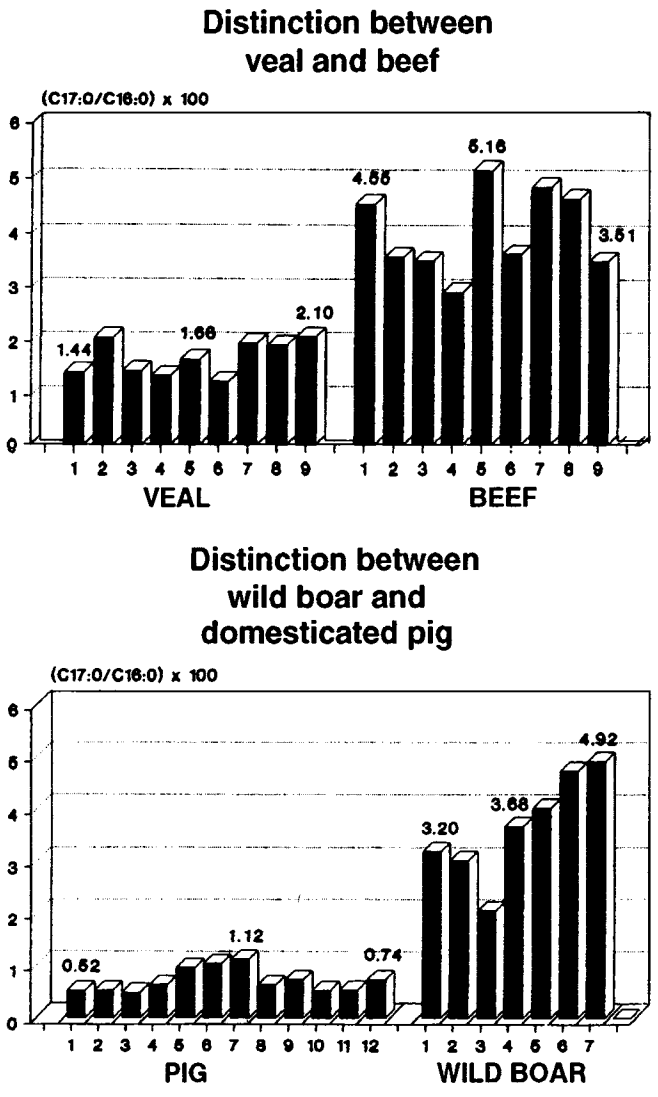

Fig. 1-19: Distinction between domesticated pig and wild boar, and veal and beef. See Figure 1-22 for GC conditions.

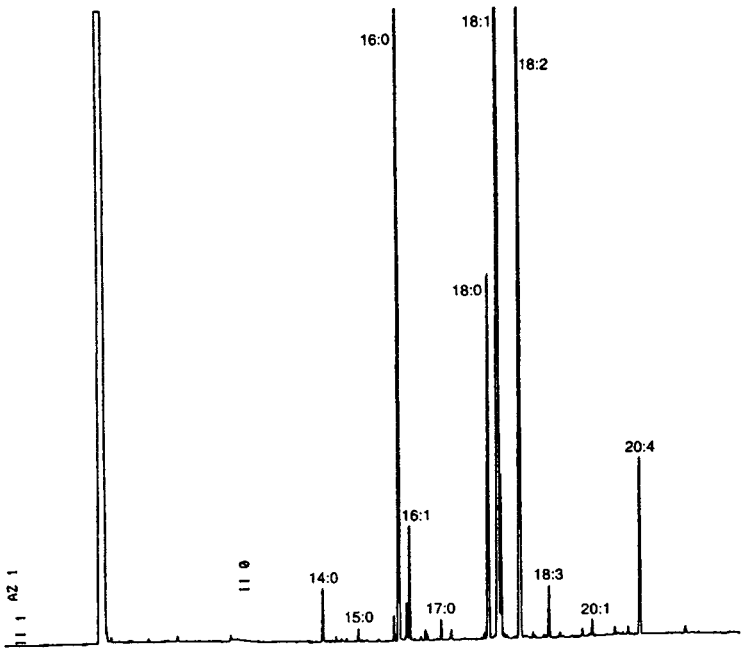

Fig. 1-20: Range of fatty acids (HRGC-FID) present in chicken meat. See Figure 1-22 for GC conditions.

1.3.7.6 Distinction between Game Species

Differences between game species suitable for their identification are shown in Figures 1-21 and 1-22.

These are fatty acid patterns from free living game animals from various federal states of Germany (North Rhine-Westphalia, Rhineland-Palatinate, Brandenburg, etc.) and from Poland. The range from myristic acid to palmitic acid underlines the differences between the individual game animals.

1.3.8 Determination of Germination Inhibitors (IPC/CIPC) in Potatoes

Propham (IPC) and chlorpropham (CIPC) are selective herbicides and are also used to inhibit germination in potatoes (as inhibitors of photosynthesis and mitosis). Fast and reliable determination can be performed after hexane extraction of homogenized potatoes, filtration, and capillary gas chromatography with ion trap detection [1-31]. Adherence to the rules of chromatography is of cardinal importance and a prerequisite for the success of the method.

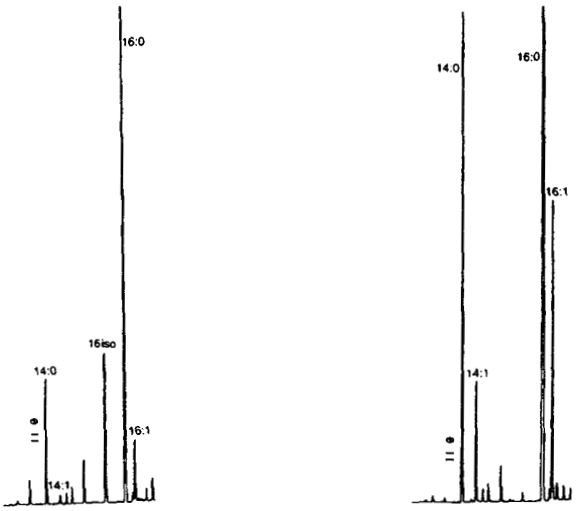

Fig. 1-21: Part of the range of fatty acids (HRGC-FID) present in fallow deer (*Dama dama* L., left) and red deer (*Cervus elaphus* L., right). See Figure 1-22 for GC conditions.

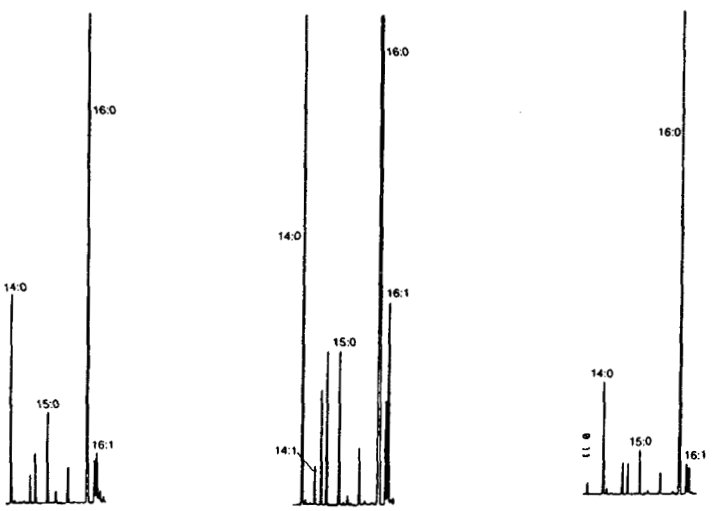

Fig. 1-22: Part of the range of fatty acids (HRGC-FID) present in roe deer (*Capreolus capreolus* L., left), red deer (*Cervus elaphus* L., center), and wild sheep (right). GC conditions for Figures 1-14–1-22: column 60 m × 0.25 mm i.d., DB-Wax, film thickness 0.15 µm/hydrogen. 0.5 µl split injection. TP: 130 °C up to 230 °C at 3 °/min, 230 °C 10 min isothermal.

Figure 1-23 shows the total ion current chromatogram of a potato sample containing the two inhibitors.

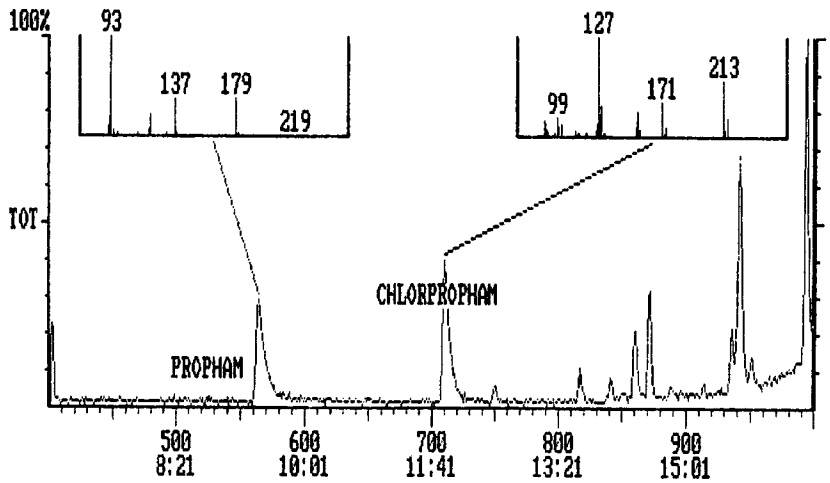

Fig. 1-23: TIC chromatogram of a potato sample containing propham and chlorpropham. Column 30 m × 0.31 mm i.d., DB-5, film thickness 0.1 μm/helium. 0.5 μl splitless, 55 °C (1 min), then up to 260 °C at 10 °/min, 260 °C 10 min isothermal.

1.3.9 Interpretation of Chromatograms – An Example

Examination of a real-life chromatogram reveals further information beyond the analytical task at hand. Recognition of such interrelationships, however, requires familiarity with analytical methods and background knowledge (acquired by reading the "right" literature). Figure 1-24 shows a TIC chromatogram of the analysis of PAH in soil samples. Alongside traces of PAH compounds, a compound/class of compounds is seen to be present which exhibits heading (gradual rise of peak). The same phenomenon is seen in the analysis of PCBs in sewage sludges (by ECD detection). In both cases we are dealing with molecular sulfur S_8, which can serve the analyst as an indicator of the use of sewage sludge [1-32].

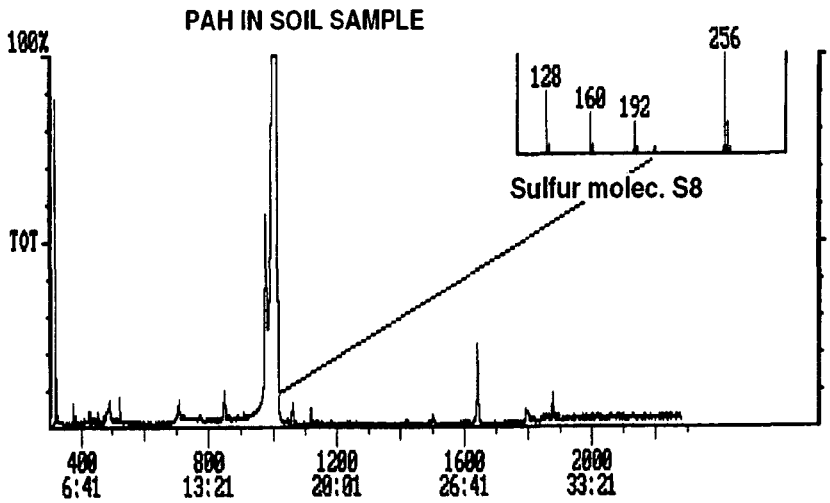

Fig. 1-24: TIC chromatogram of a soil sample for polycyclic aromatic hydrocarbons. Column: 30 m x 0.32 mm i.d., SE 54, film thickness 0.1 µm/hydrogen. 0.5 µl splitless, 90 °C (1 min), then up to 260 °C at 5 °/min, 260 °C 20 min isothermal.

1.4 Summary

The applications listed impressively demonstrate that the analytical error associated with gas chromatography can be minimized on observation and implementation of the rules of chromatography. Particularly in the area of trace analysis, the analyst must exercise an enhanced degree of critical awareness.

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Food and Environmental Analysis by Capillary Gas Chromatography
Lothar Matter

2 GC/MS Determination of Residues and Contaminants

Peter Fürst

2.1 Introduction

During recent years problems associated with residues and environmental contaminants have increasingly been the focus of public attention since modern instrumental analysis has meanwhile provided the means of performing measurements in concentration ranges of ng/kg (ppt) and even pg/kg (ppq). This extreme sensitivity now permits detection of many substances which were formerly also present in biological samples but could not be determined because of the lack of appropriate methods.

An important contribution to this rapid development has undoubtedly come from the advent of temperature stable quartz capillary columns offering very high separation performance and from the drop in price of mass spectrometers thanks to the availability of new technologies (quadrupole, ion trap). Mass spectrometry has meanwhile become an indispensable tool in the hands of the analyst since, in combination with high resolution capillary gas chromatography (GC/MS), it offers an extremely powerful method of assuring analytical data and for identifying unknown substances in complex mixtures. Particularly in the area of residue analysis it has often been observed in the past that use of multiple methods in chromatograms obtained with electron capture or thermionic detectors gave rise to signals whose retention times failed to correspond to any of the standard substances present in the laboratory, even when large numbers thereof were available. In such cases, mass spectrometric analysis can provide valuable information about whether a given peak is actually an active component or perhaps only a characteristic constituent which also generates a signal in specific detectors owing to its very high concentration.

Moreover, effective analysis of aromatic substances, essential oils, and fragrance components in cosmetics would nowadays be almost unthinkable without use of GC/MS coupling.

2.2 Recording Mass Spectra

The principle of mass spectrometry consists in generation of ions in an appropriate manner, separating them according to their mass and charge in a separation system, and ultimately recording them according to mass and abundance in the form of a mass spectrum [2-1 to 2-10].

Two different approaches may be distinguished in the operation of combined gas chromatography/mass spectrometry (GC/MS):

- Recording of mass spectra, often referred to as full scan operation, and
- Recording of mass fragmentograms, also termed selected ion monitoring (SIM), selected ion recording (SIR), or multiple ion detection (MID).

2.2.1 Full Scan

If mass spectrometry is to be used to identify unknown compounds, it is generally necessary to recorded complete mass spectra. In identification of unknowns the use of different ionization techniques can prove extremely helpful because the mass spectra obtained vary considerably according to the ionization technique employed. Each spectrum contains a wealth if information which, taken together, can provide valuable pointers to the identity of the unknown substance.

The method of ionization has a substantial influence on the attainable sensitivity and on the specificity of the mass spectrometric determination. Although numerous ionization techniques have been described in the literature, the principal methods used in food analysis are electron impact ionization (EI) as well as positive chemical ionization (PCI) and negative chemical ionization (NCI). If, for example, ionization is performed with the aid of electron impact, the conventional and most widespread kind of ion generation, then 1–10 ng of substance is generally required for recording the mass spectrum. This means that the concentration, often in the picogram range, is frequently insufficient in EI operation for identification of unknown substances or for confirmation of analytical data obtained with highly sensitive detectors such as the electron capture detector (ECD) or the nitrogen/phos-

phorus detector (NPD, TSD). In such cases the identification of the unknown substance can utilize other ionization techniques, such as chemical ionization.

Chemical ionization belongs to the so-called soft ionization techniques [2-10 to 2-12]. During analysis a reactant gas, usually methane or isobutane, is continuously fed into the source of the mass spectrometer. Due to the large excess of reactant gas, ionization of the gas first takes place; in a secondary reaction this then ionizes the eluate flowing from the gas chromatograph into the mass spectrometer. In this way, spectra are obtained which are generally characterized by a very stable molecular ion peak and show only few fragment ions. With the aid of this technique it is possible, for example, to perform molecular weight determinations of compounds whose instability precludes formation of a molecular ion peak under the conditions of electron impact ionization.

If the compounds to be analyzed contain electron-withdrawing functional groups, generation of negative ions may occur through charge transfer on chemical ionization. This phenomenon can be readily observed with halogen-containing pesticides and environmental contaminants which, under appropriate conditions, give high ion yields and can thus be detected with great sensitivity. In many cases picogram amounts already suffice for recording a complete spectrum. However, special mass spectrometric equipment is required for recording negative ions. The main advantage of this analytical technique is that compounds with suitable structures can be determined very selectively with a sensitivity comparable with the electron capture detector. This accounts for the increasing importance of this analytical technique for solving problems relating to analyses of residues.

In the structure elucidation of unknown compounds, extensive MS libraries available on electronic media prove to be an enormous help. Such modern spectral collections (NIST, NBS/Wiley) often contain not only more than 80000 mass spectra but also other important characteristics such as CAS numbers and in some cases even structural formulae of the pertinent substances (Fig. 2-1). Comparisons of an unknown spectrum performed with the aid of a computer, e.g. with the 80000 compounds of the combined NBS/Wiley library, generally take between a few seconds and about one minute. If the mass spectrum of the unknown substance is not in the library, modern instruments also permit automatic computer interpretation of the unknown structure. In this context, mention should be made of the "self-training interpretive and retrieval system (STIRS)" software package developed by McLafferty et al., which effectively supports the analyst in interpreting unknown mass spectra [2-13, 2-14].

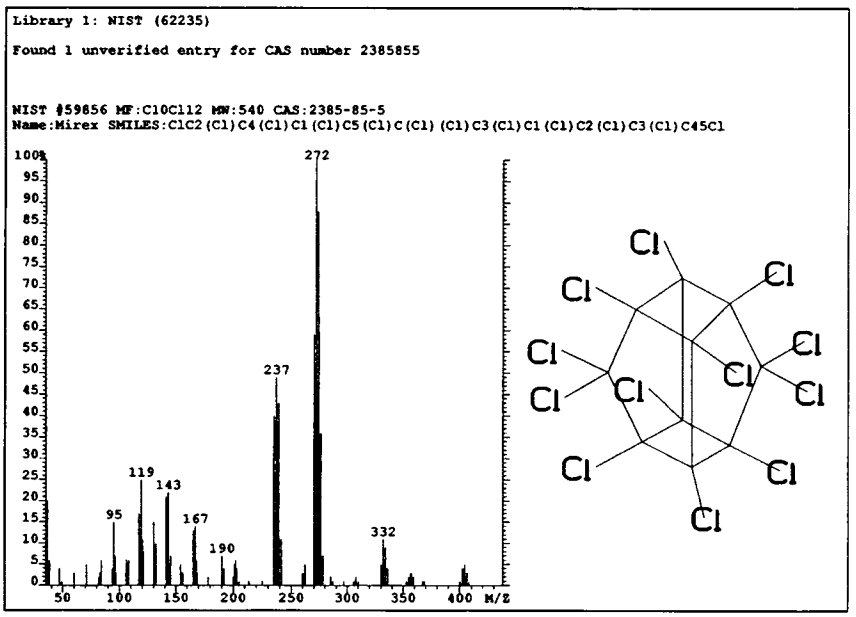


Fig. 2-1: Data for the pesticide Mirex taken from the NIST-MS library.

2.2.2 Mass Fragmentography

Mass fragmentography (SIM = selected ion monitoring, SIR = selected ion recording, MID = multiple ion detection) represents the second most important technique of combined capillary GC/MS. This procedure is always used when a specific search is made for certain substances and/or if quantitation is to be performed. Under such circumstances the mass spectrometer serves as a kind of mass-selective detector. In this technique highly characteristic fragments are selected from the mass spectrum of the substance to be investigated, with preference being given above all to fragments of high mass and/or high intensity. In the actual analysis, attention is no longer focused on entire mass ranges but on a few predetermined mass fragments. This greatly enhances the sensitivity because the measuring time per fragment is substantially prolonged. In addition, very clear chromatograms generally result because only those compounds are shown which contain at least one of the selected fragments.

Compared with conventional detectors, this approach yields not only the retention time but also important information about the presence of characteristic mass fragments, which greatly enhances the selectivity of the procedure and hence the reliability of the results.

If mass fragmentography is used with chemical ionization a better signal-to-noise ratio is obtained than with electron impact ionization because the mass spectrometric signal is no longer distributed over numerous fragments but consists mainly of the molecular ion. This also increases the selectivity because measurements at high masses are less subject to the influence of matrix effects.

A further advantage of GC/MS techniques is that ^{13}C-labeled or deuterated compounds can be added to the sample as ideal internal standards at the start of analysis. Since these substances behave just like the native analytes very accurate information is obtained about losses incurred during sample preparation. Although not always completely separable by gas chromatography, they are readily distinguished by their different mass fragments.

2.3 Applications of GC/MS to the Analysis of Residues and Contaminants in Foods

A number of examples given below will serve to illustrate the potential of combined capillary gas chromatography/mass spectrometry, particularly in combination with various recording and ionization techniques in the trace analysis of residues and contaminants.

2.3.1 Identification of Unknown Substances and Confirmation of Analytical Data by Recording Complete Mass Spectra

2.3.1.1 Determination of Triazine Herbicides in Drinking Water

Legal limits of 0.1 µg/l have been set for individual organic chemical substances used as pesticides and their principal toxic degradation products in the regulations governing drinking water and water for use in the food processing industry; the corresponding limit for the sum of all such above substances is 0.5 µg/l. Checks on whether these extremely low values are fulfilled requires extremely powerful analytical methods. Since triazine herbicides contain nitrogen, gas chromatographic methods using a NP detector are highly suitable for their detection alongside HPLC methods. Thanks to the pronounced selectivity of this detector extracts of well water generally give very clear chromatograms with only few peaks.

Owing to the widespread use of triazine herbicides, particularly in maize cultivation, the limiting values of atrizine and simazine, both meanwhile prohibited in Germany, were repeatedly exceeded in well water samples during past years. On acquisition of confirmatory evidence for one such purported finding it was established that the substance present was neither atrizine nor simazine but tris(2-chloroethyl)phosphate, a flame retardant frequently used in paints and varnishes and which almost co-elutes with atrazine and simazine on non-polar stationary phases and is also detected with great sensitivity by a NP detector owing to it phosphate group [2-15]. The gas-chromatographic separation of these substances on a 30 m DB-5 capillary column (0.10 μm film thickness, 0.25 mm internal diameter) is shown in Figure 2.2. This chromatogram demonstrates that that the spurious finding was actually due to tris(2-chloroethyl)phosphate. It also underscores the need for confirmation of positive results on a second column of different polarity or by use of a different detector system. Thus, even in the simultaneous presence of triazine herbicides and the flame retardant, GC/MS not only permits the pertinent substances to be distinguished but also to be quantified. The differing mass spectra of atrazine and tris(2-chloroethyl)phosphate are shown in Figure 2-3.

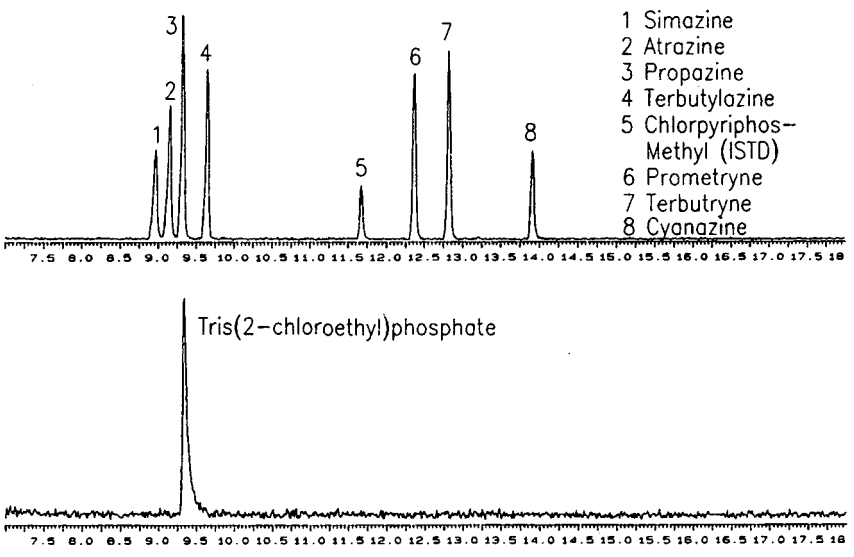


Fig. 2.2: Gas chromatographic separation of triazine herbicides and tris(2-chloroethyl)phosphate ion a 30 m DB-5 capillary column.

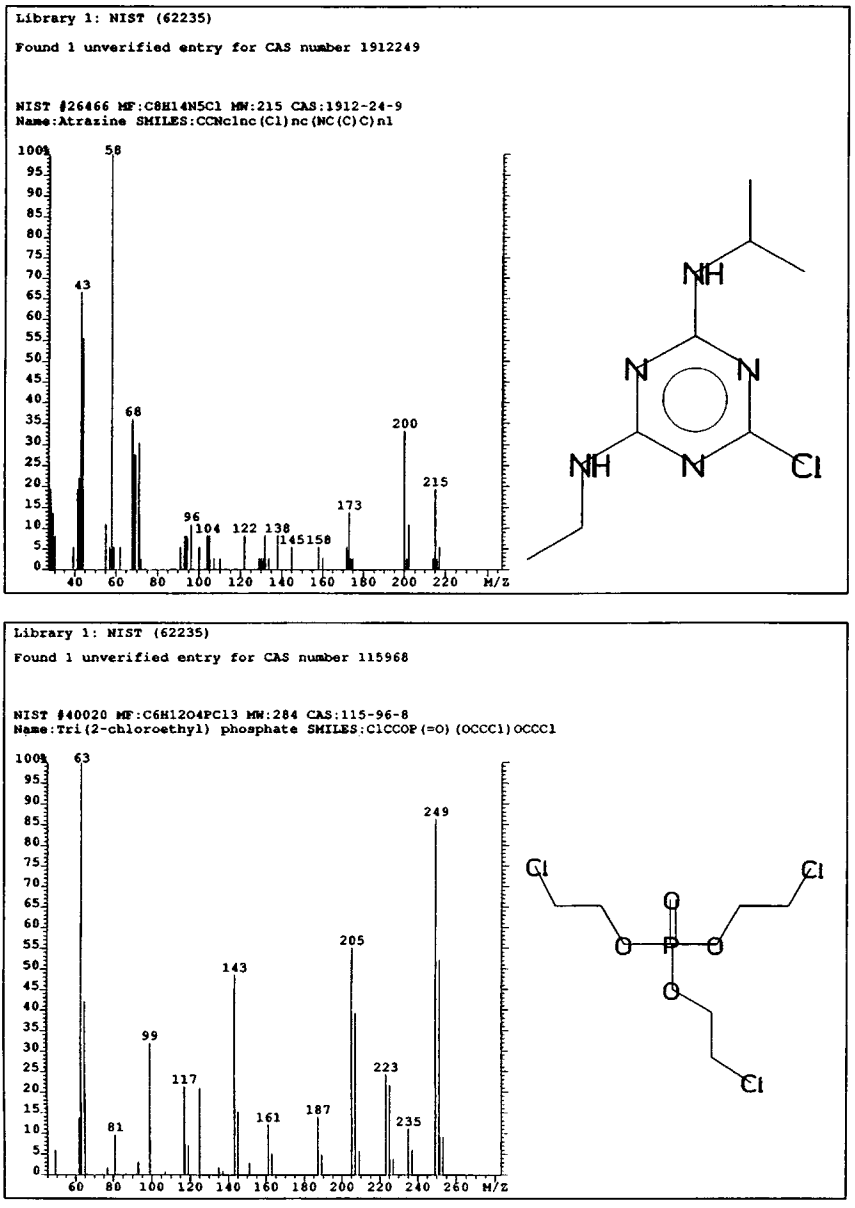


Fig. 2.3: Distinction between atrazine and tris(2-chloroethyl)phosphate by means of differing mass spectra.

2.3.1.2 Confirmation of Chlorpyriphos-Ethyl in Oranges

Figure 2-4 shows the total ion current of an orange extract as a function of the ionization technique. Of interest is the size ratio of the asterisked peak relative to the other peaks of the respective chromatograms. It is clearly seen that that the peak in question is greater in the NCI spectrum than in the other two. This observation by itself strongly suggests that this peak is due to a halogenated compound. A subsequent library search of the mass spectrum confirmed the suspicion expressed after ECD analysis that it is the phosphoric ester insecticide chlorpyriphos-ethyl, also known as dursban.

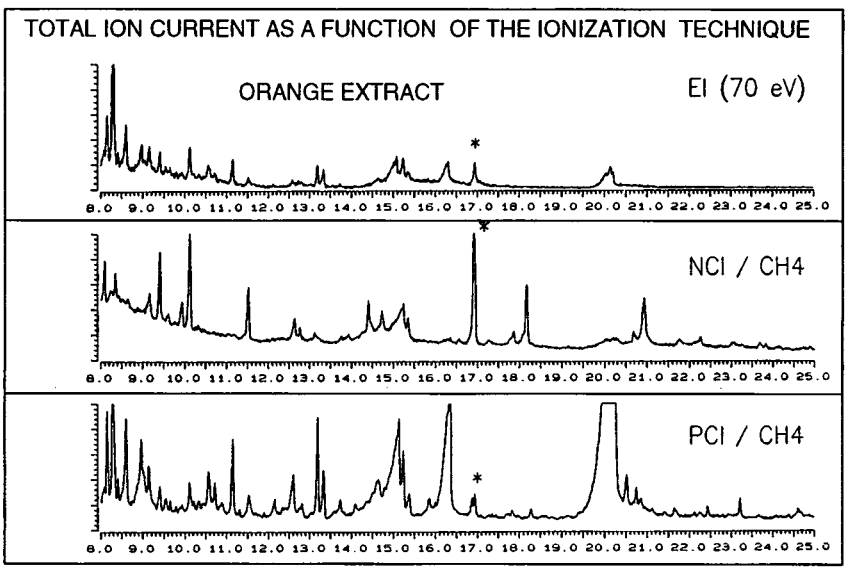


Fig. 2-4: GC/MS chromatograms of an orange extract obtained with different ionization techniques.

The resulting mass spectra differ substantially depending upon the ionization technique employed. This is demonstrated by Figure 2-5 for the example of chlorpyriphos-ethyl. Each spectrum contains a wealth of information which taken together provide great help in interpretation. It is clear from the example that the molecular ion is very weak in the electron impact ionization spectrum owing to the instability of the compound. However, the characteristic fragments clearly reveal important molecular structures.

In contrast, positive chemical ionization clearly shows the protonated molecular peak at m/e 350 with its characteristic isotopic pattern due to the three

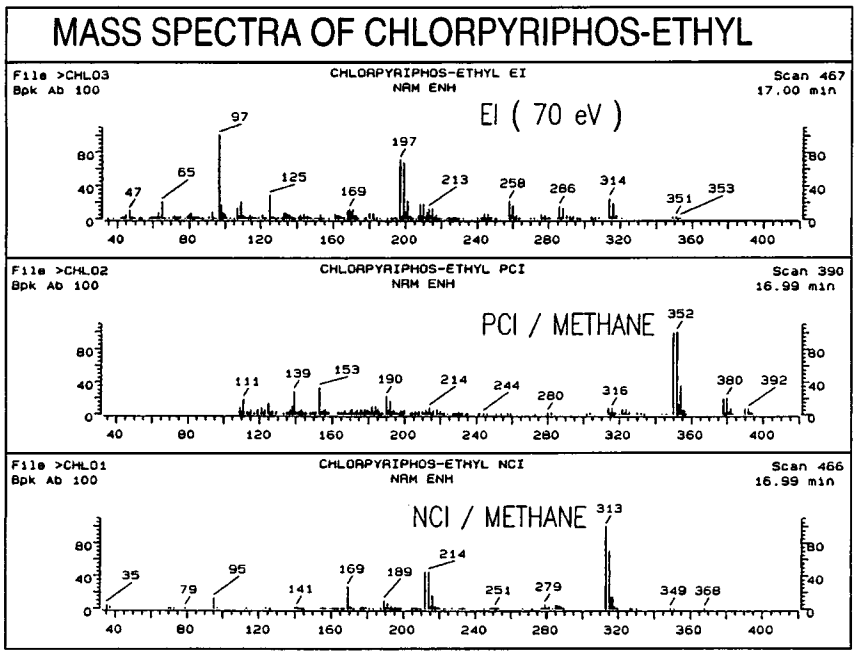


Fig. 2-5: Mass spectra of chlorpyriphos-ethyl obtained with different ionization techniques.

halogen atoms. The higher fragments at m/e 380 and 392 are due to addition reactions with the methane used as reactant gas.

Finally, the bottom spectrum, recorded with the aid of negative chemical ionization, affords important information about the presence of halogen atoms, i.e. of three chlorine atoms in the present case.

2.3.1.3 Detection of Dichloran in Nutrition

Figure 2-6 shows mass spectrometric confirmation of detection of dichloran in nutrition. In this case the absolute concentration was so low that confirmation was performed not via electron impact ionization but with the aid of negative chemical ionization. The total ion current is shown in the top chromatogram. In its selectivity the chromatogram is seen to resemble an ECD run. Below the NCI spectra of the unknown peak and of dichloran are shown. Owing to the good agreement of the spectra and the other chromatographic data the presence of the fungicide dichloran can be regarded as assured.

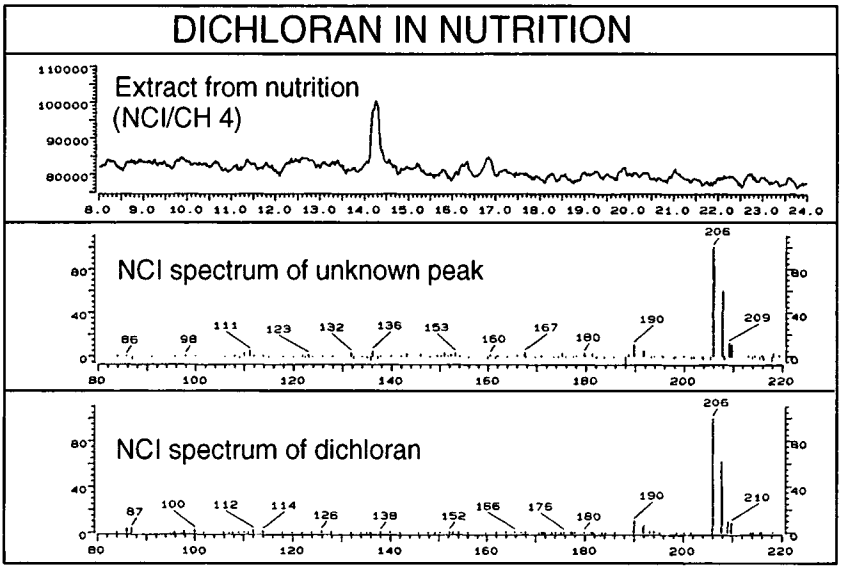


Fig. 2-6: Confirmation of the presence of dichloran in nutrition.
Top: NCI total ion current chromatogram.
Middle: NCI spectrum of the unknown peak.
Bottom: NCI spectrum of dichloran.

2.3.1.4 Identification of Propenophos in Tomatoes

Figure 2-7 shows the mass spectrometric analysis of a tomato extract which, on residue analysis, gave an unknown ECD peak which failed to match any of the standards available in the laboratory. The upper part of the Figure shows the total ion current of the sample as obtained with the aid of electron impact ionization. Compared to the previous example, the EI technique showed a far lower selectivity. While NCI runs display similarities to ECD chromatograms, the EI runs are roughly comparable with chromatograms obtained with a flame ionization detector. For this reason, the chromatogram of Figure 2-7 also shows numerous constituent substances which are not recorded by an electron capture detector. The mass spectrum of the asterisked unknown substance is shown in the lower part of the figure.

Although the MS library used contained 80000 mass spectra the search for this compound was unsuccessful. A chemical ionization mass spectrum was therefore also recorded (Fig. 2-8). The NCI spectrum clearly shows that the substance contains at least one chorine and one bromine atom, recognizable

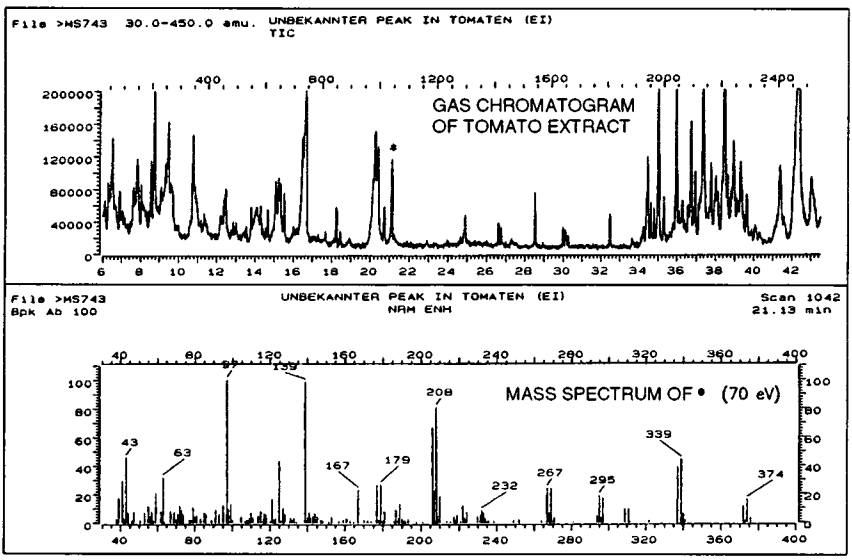


Fig. 2-7: GC/MS analysis of a tomato extract. Above: EI total ion current chromatogram. Below: Mass spectrum of the unknown peak.

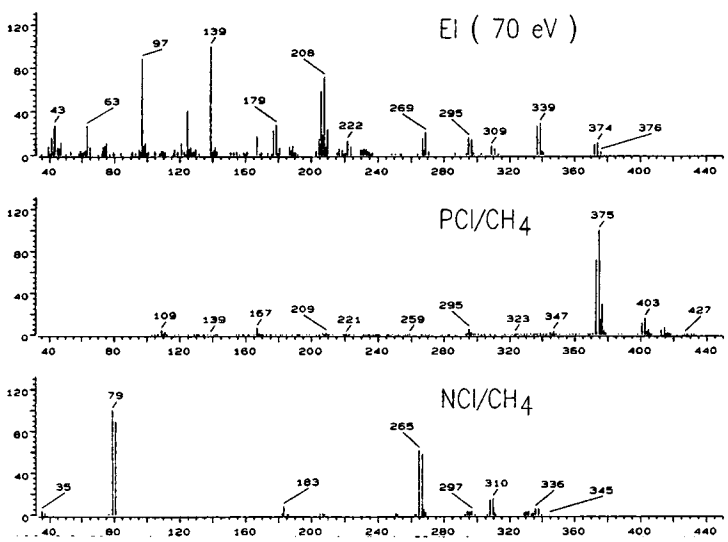


Fig. 2-8: Mass spectra of the unknown peak as a function of the ionization technique. Top: Electron impact ionization (70 eV). Middle: Positive chemical ionization (CH_4). Bottom: Negative chemical ionization (CH_4).

from the fragments m/e 35, 37, 79, and 81. The mass spectrum obtained with positive chemical ionization shows that the molecular weight is probably 372 owing to the appearance of the protonated molecular peak at m/e 373. Moreover, the characteristic isotopic pattern indicates the presence of only one bromine and only one chlorine atom. The higher masses are due to addition reactions with methane.

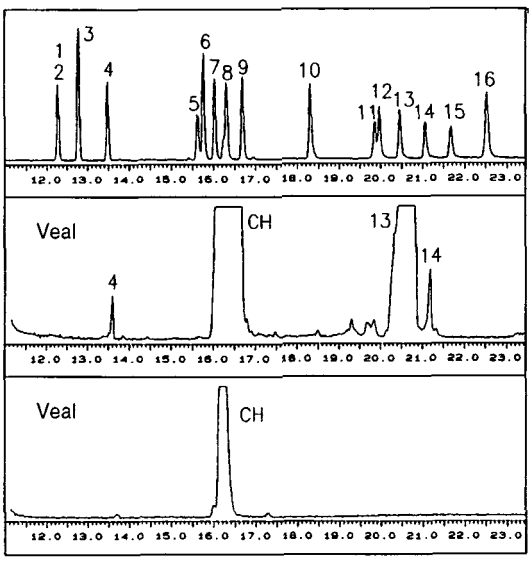
The EI spectrum also permits deduction of the presence of a phosphorothionate in the substance.

Combination of the information forthcoming from the three mass spectra ultimately suggested that the unknown substance could be the phosphoric ester propenophos, an insecticide which is not permitted in Germany. This finding was subsequently confirmed on acquisition of the substance and comparison of the spectra.

2.3.1.5 Determination of Hormone Derivatives in Veal

In recent years there have been repeated findings of fattening aids in veal. Mention should be made of synthetic anabolics (DES, dienestrol, hexestrol), natural hormones (testosterone, estradiol, progesterone), hormone esters (testosterone propionate and cypionate, estradiol valerate and benzoate), and also β-sympathomimetics (clenbuterol, salbutamol). GC/MS has proved effective in clarification of such cases because it not only provides fast information about the composition of implants frequently found in muscular tissue during veterinary inspection of veal carcasses but is indispensable for identification of the active substances in the region of entry. In most cases sufficient substance was present for identification from the total mass spectrum. The total ion current chromatogram of 16 important hormone derivatives and one positive and one negative veal sample are shown in Figure 2-9. Gas chromatographic separation was performed with a 30 m DB-1 capillary column (0.10 μm film thickness, 0.32 mm internal diameter). Mass spectrometric recording of the total ion current chromatograms was performed in the EI mode. The upper chromatogram shows the separation of 16 common hormone derivatives. Although testosterone acetate and nortestosterone propionate coelute under the chosen conditions, in the case of a positive finding a distinction between these two compounds would readily be possible on the basis of their differing mass spectra.

The middle chromatogram shows a positive hormone detection in veal. In this case, testosterone propionate, testosterone cypionate, and nortestosterone decanoate were found at an injection site which had aroused suspicion during



Hormones in Veal

1	Testosterone acetate
2	Nortestosterone propionate
3	Norethisterone acetate
4	Testosterone propionate
5	Estradiol valerate
6	Estradiol dipropionate
7	Medroxyprogesterone acetate
8	Megestrol acetate
9	Testosterone isocapronate
10	Testosterone oenanthate
11	Estradiol benzoate
12	Testosterone benzoate
13	Testosterone cypionate
14	Nortestosterone decanoate
15	Testosterone decanoate
16	Estradiol undecylate
CH	Cholesterol

Fig. 2-9: GC/MS analysis of veal for hormone derivatives.
Top: Standard solution.
Middle: Positive hormone detection in veal.
Bottom: Negative hormone detection in veal.

veterinary inspection; this mixture corresponds to an illegal hormone cocktail widely used around the end of the 1980's. It is seen from the chromatogram that cholesterol, which was not removed during sample treatment, coincides with megestrol acetate and testosterone isocapronate. However, this has no influence on the determination of the two hormone derivatives since the mass spectra of the three substances show such enormous differences that the substance-specific fragmentograms required for substance identification can be subsequently reconstructed from the total ion current chromatogram. The bottom chromatogram shown a veal extract in which no hormone derivatives could be detected.

2.3.1.6 Detection of Neuroleptics in Pork

Neuroleptics number among the psychopharmaceuticals whose calming, aggression-reducing, and sedating action make them useful especially for treatment of pigs shortly before slaughter in order to reduce stress and the risk of heart failure during transportation to the abattoir. If these are permitted drugs, possible restrictions on their use and prescribed waiting times must be observed. Testing for observation of waiting times is performed mainly by radioimmunological, HPLC, and GC/MS techniques, with mass fragmen-

tography generally being performed in the last case owing to the low concentrations expected.

In some cases, on non-observation of the required waiting periods, the concentration of neuroleptics in the meat samples to be analyzed is so high that analysis is also possible by recording of a total mass spectrum. Figure 2-10 shows the gas chromatographic separation (DB-1 capillary, 0.10 μm film thickness, 0.32 mm internal diameter) of the principal phenothiazine and butyrophenone derivatives used as neuroleptics. Figure 2-11 (see p. 43) shows the confirmation of a positive acepromazine finding in pork. The upper chromatogram is the total ion current recorded with the aid of electron impact ionization. The peak marked with a "1" is unequivocally due to acepromazine, as follows from comparison of the mass spectrum with that of the reference substance. There is also another peak in the total ion current chromatogram which was not shown by the preceding mass fragmentography because it does not contain any of the fragments selected for measurement. The close resemblance of the mass spectrum to that of acepromazine suggest that this substance may be a metabolite of acepromazine.

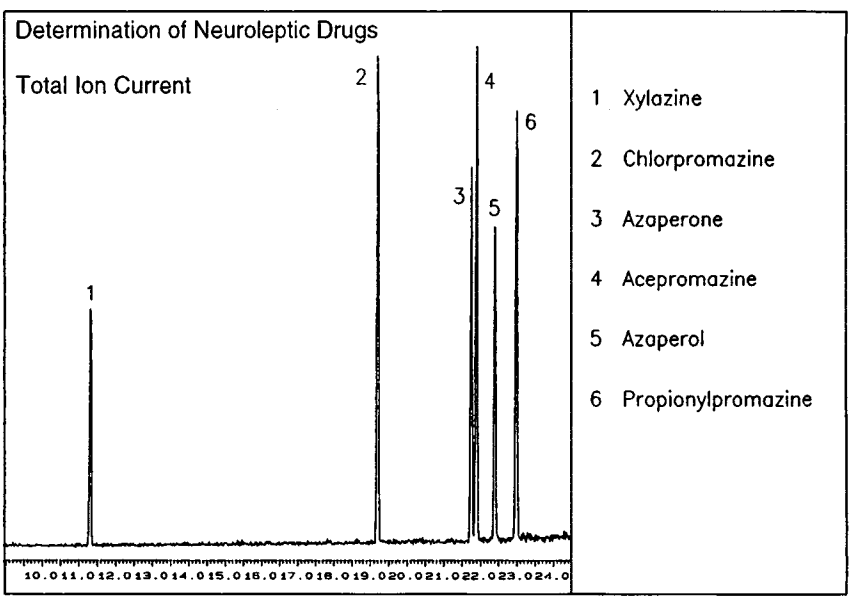


Fig. 2-10: Gas chromatographic separation of the principal neuroleptics on a DB-1 capillary.

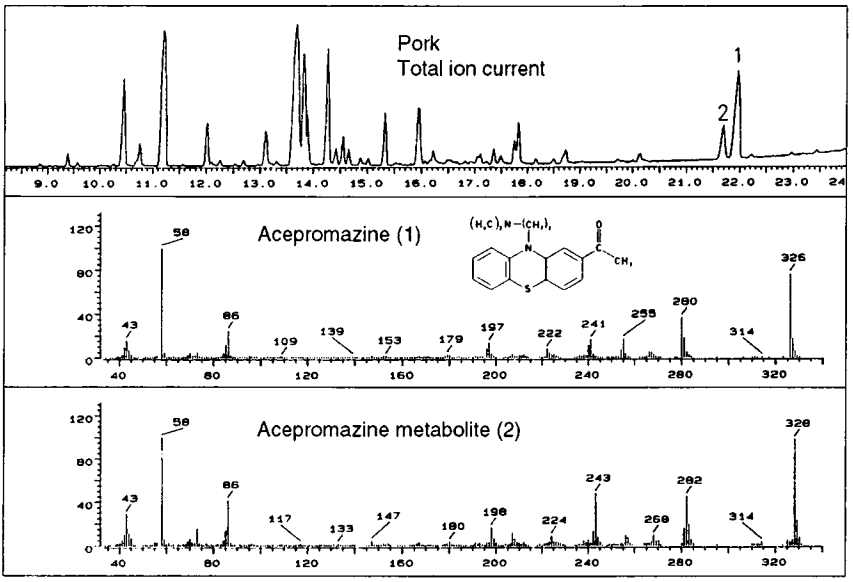


Fig. 2-11: Confirmation of the detection of acepromazine in pork.
Top: EI total ion current chromatogram.
Middle: Mass spectrum of acepromazine (peak 1).
Bottom: Mass spectrum of an acepromazine metabolite (peak 2).

This example demonstrates the scope and also the limitations of GC/MS analysis. Thus although it is possible to determine substances selectively and with high precision in very low trace concentrations by selection of mass fragments with the aid of mass fragmentography, yet if the extracts analyzed contain substances, such as the acepromazine metabolite, which yield none of the preselected fragments, then they go undetected and valuable information may be lost. Depending upon the problem at hand, recording of a total ion current chromatogram with the possibility of obtaining complete mass spectra may prove superior to mass fragmentography, although the sensitivity is significantly lower.

2.3.2 Detection and Determination of Residues and Contaminants with the Aid of Mass Fragmentography

As already mentioned above, mass fragmentography provides a means of determining substances selectively and with high precision in very low trace concentrations. An essential prerequisite is an accurate knowledge of the mass spectrum of the analyte to be determined. This technique is therefore always

used when samples are to be analyzed for specific active substances or groups of active substances. The following examples are presented to show the possibilities and limitations offered by this analytical technique in the determination of residues and contaminants in foods.

2.3.2.1 Determination of Polychlorinated Dibenzodioxins and Dibenzofurans

Polychlorinated dibenzodioxins (PCDD) and dibenzofurans (PCDF) belong to the class of aromatic ethers with one to eight chlorine atoms. Depending upon their degree of chlorination and the substitution pattern in the aromatic rings, 75 PCCD and 135 PCDF, also known as congeners, can be distinguished. Both classes of compounds are environmental contaminants which occur not only in numerous industrial products as undesired components but may also be formed in incomplete combustion processes. Owing to the wide range of possible sources PCDD and PCDF have meanwhile become ubiquitous in the environment. As in the case of DDT and other persistent organohalogen compounds this leads to accumulation in adipose tissue and to enrichment in the food chain. Interestingly, of the 210 congeners almost only the 2,3,7,8-chlorine substituted compounds are found in human samples and foods of animal origin, insofar as they derive from mammalian species.

The toxicity of the individual congeners differs considerably. Thus 2,3,7,8-tetrachlorodibenzodioxin (TCDD) is found to have an acute toxicity 100–1000 times greater than 1,2,3,8-TCDD. In general, it can be said that congeners having chlorine atoms in positions 2,3,7, and 8, and which also possess a vicinal proton (dirty dozen), will show the highest toxicity. It therefore follows that isomer-specific PCDD and PCDF analysis is absolutely essential in order to obtain reliable data as a basis for risk assessment.

Owing to the low contents in foods and biological samples, which generally lie in the ng/kg (ppt) or pg/kg (ppq) range, and high demands on accuracy and precision, the only method coming into question for PCDD and PCDF analysis is combined capillary gas chromatography/mass spectrometry. Although capillary columns permit high separation performance it is not yet possible to separate all PCDD and PCDF congeners on a single column. The choice of column is determined largely by the matrix. Thus non-polar temperature stable phases are used for analysis of human samples and foods of mammalian origin because such samples generally contain only 2,3,7,8-chlorine-substituted congeners. If, in contrast, foods of plant origin or animal samples from polluted areas, such as cow's milk from the vicinity of point sources, are to be examined, then more selective phases must be used for gas

chromatographic separation which permit unequivocal separation of toxic 2,3,7,8-chlorine-substituted congeners from less toxic compounds. The cyanosilicone phases SP 2331, CP-SIL 88, CPS-2, DB-Dioxin, as well as the liquid-crystalline polysiloxane phases (SB smectic capillaries) can be mentioned as examples. Figure 2-12 shows the elution order of all 22 TCDD isomers from a CP-SIL 88 and an OV-17 capillary. The most toxic isomer, 2,3,7,8-TCDD, clearly overlaps with 1,2,7,9-TCDD on the OV-17 capillary (above), but can be determined without interference on the CP-SIL 88 column (below).

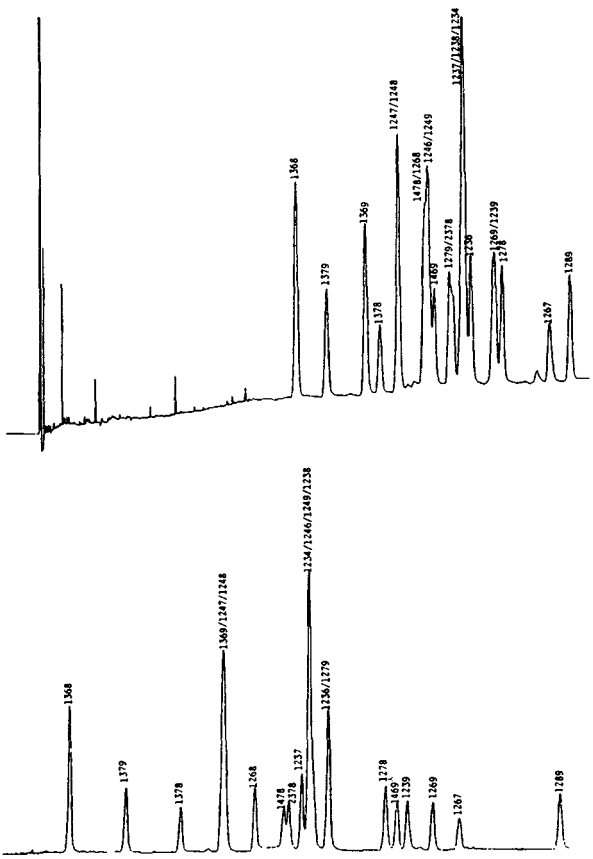


Fig. 2-12: Order of elution of all 22 TCDD isomers from an OV-17 (above) and a CP-SIL 88 capillary (below) [2-16].

Depending upon the problem, mass spectrometric analysis can either be performed with low resolution (LRMS, quadrupole, ion trap instruments) or with high resolution (HRMS) instruments. Owing to the low PCDD/PCDF contents in foods and biological samples analysis is performed almost exclusively by mass fragmentography. In fact, PCDD/PCDF analysis serves to demonstrate progress in instrumental analysis. Table 2-1 shows that the sensitivity of detection of 2,3,7,8-TCDD has increased by a factor of some 20,000 since 1967. This progress means that modern high resolution mass spectrometers are able to detect 30 femtograms of 2,3,7,8-TCDD at a resolution of R = 10,000 and a signal-to-noise ratio of >10:1.

Table 2-1. Increase in analytical detection sensitivity for 2,3,7,8-TCDD between 1967 and 1988.

Year	Method	Detection limit (pg)
1967	GC/FID (packed column)	500
1973	GC/MS (packed column)	300
1976	GC/MS-SIM (capillary column)	200
1984	GC/MS-SIM (capillary column)	2
1988	GC/HRMS (capillary column)	0.03

A substantial increase in selectivity and sensitivity (detection limit around 1 picogram) on use of quadrupole or low resolution instruments operating in an EI mode can be achieved by use of mass fragmentography in combination with negative chemical ionization (NCI). Thus the absolute detection limit based on a signal-to-noise ratio of 10:1 for congeners occurring in human samples lies between 50 and 500 femtogram. However, 2,3,7,8-TCDD represents an exception because it shows an insufficient response under the conditions of negative chemical ionization and therefore has to be determined at trace levels with the aid of electron impact ionization.

Alongside the nature of the reactant gas, considerable importance attaches to the reactant gas pressure and the temperature of the ion source in NCI measurements. Optimization of these parameters permits considerable variation of the selectivity and sensitivity for individual substances. Thus on use of methane as reactant gas 2,3,7,8-TCDD gives the lowest response of all 22 tetrachlorodibenzodioxins but the strongest response on use of methane/N_2O [2-17]. However, the absolute detection limit is still about 25 picogram, making this procedure too insensitive for trace determinations in biological samples. A drawback of NCI measurements is that, as in the case of ECD, the response depends not only on the number of halogen atoms but also on their

position in the molecule. Therefore, accurate quantitative determinations are only possible if the compounds to be analyzed are available as defined reference substances, permitting determination of substance-specific response factors.

Figure 2-13 shows typical mass fragmentograms of a human milk extract [2-18]. While the determination of TCDD (m/e 319.9) is performed with the aid of a mass selective detector in the EI mode, determination of the other congeners was undertaken in the NCI mode with methane as reactant gas. Since only 2,3,7,8-chloro-substituted congeners occur in human milk, both analyses were performed on a 30 m DB-5 capillary (0.10 µm film thickness, 0.25 mm internal diameter). The peaks marked "A–C" in the dioxin fragmentograms originate from the corresponding ^{13}C-labeled furans with the same degree of chlorination. Since some of them show the same nominal masses in the isotope pattern of their molecular peak as native dioxins having the same chlorine content, they are also included in the dioxin fragmentograms if analysis is performed with low-resolution or quadrupole mass spectrometers.

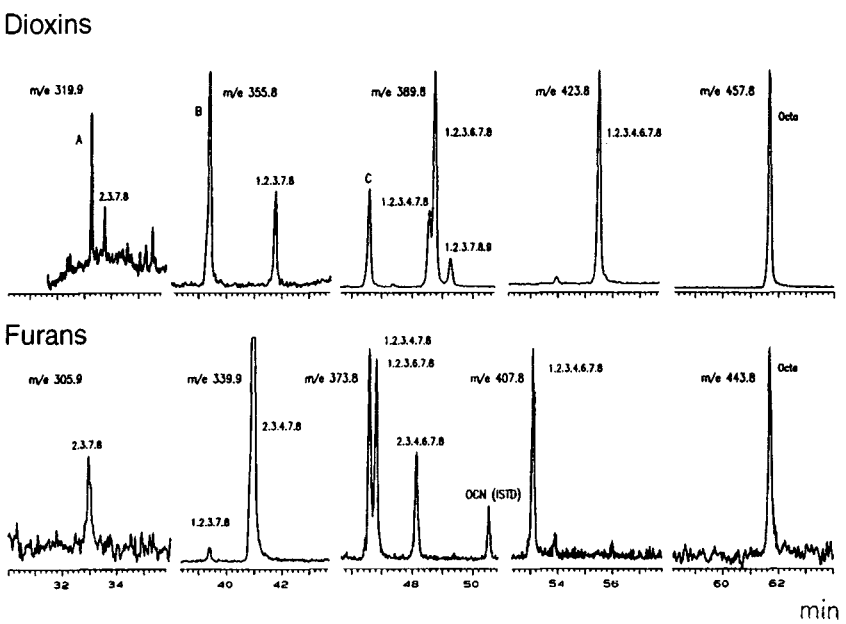


Fig. 2-13: PCDD/PCDF mass fragmentograms of a human milk extract, recorded in the EI and NCI mode, respectively, with a quadrupole instrument.

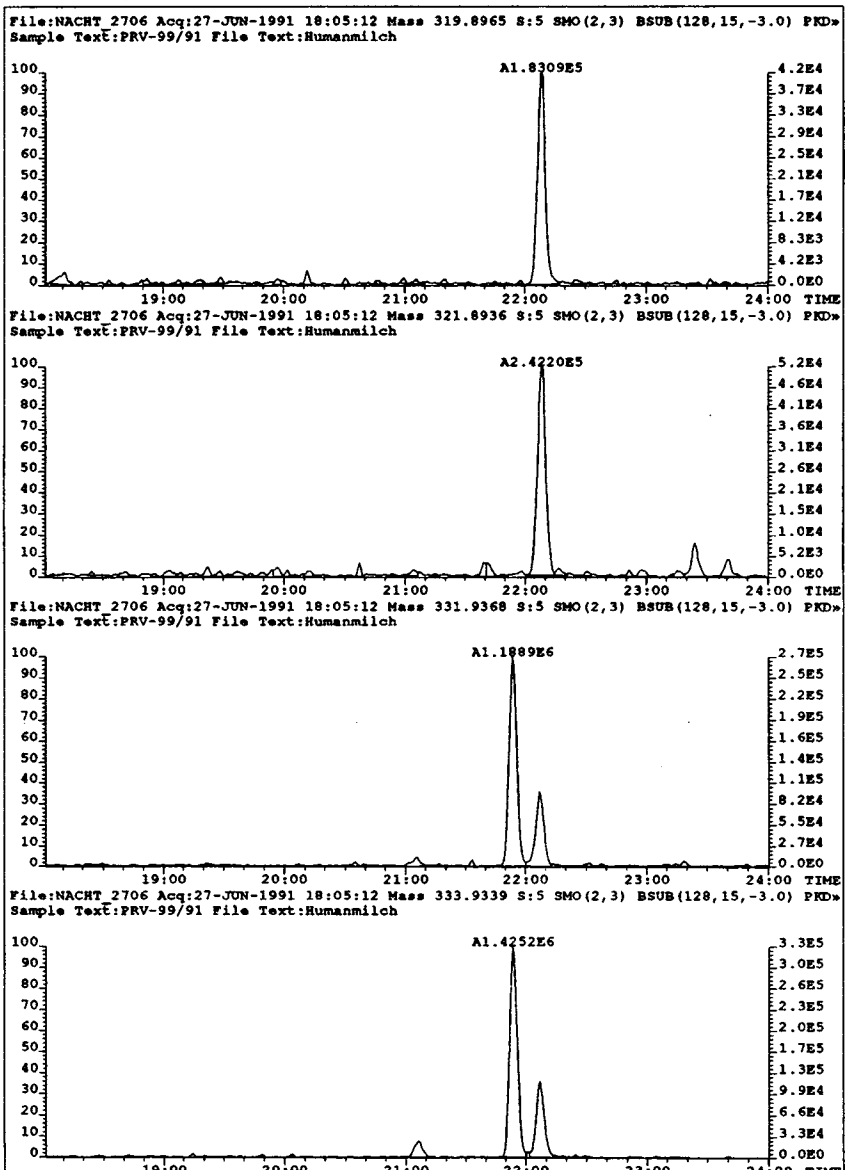


Fig. 2-14: Determination of 2,3,7,8-TCDD in human milk by high resolution mass fragmentography on a DB-5 capillary.

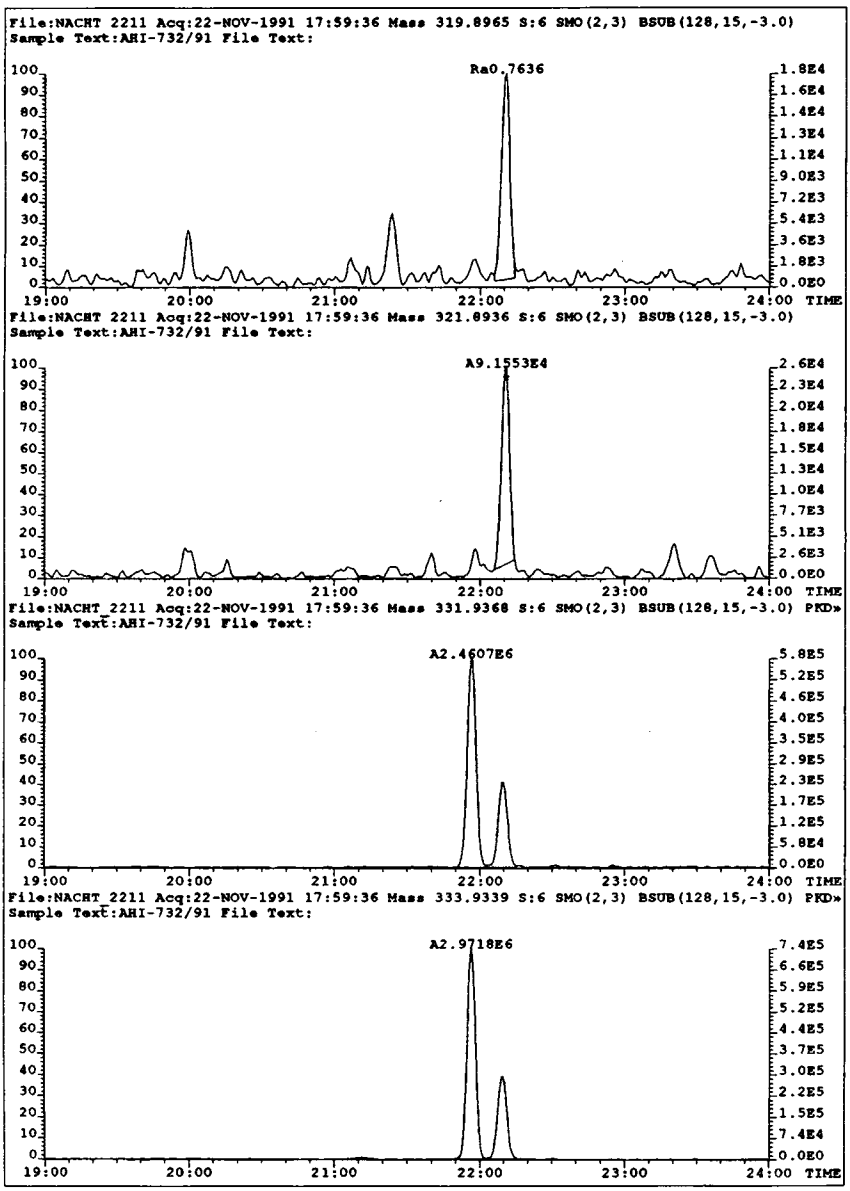


Fig. 2-15: Determination of 2,3,7,8-TCDD in cow's milk by high resolution mass fragmentography on a DB-5 capillary.

The content of 2,3,7,8-TCDD in this sample is ca. 2 pg/g (ppt) of fat. The fragmentogram for this congener (m/e 319.9) indicates that this content represents the lower limit of the practical working range on use of low-resolution and quadrupole systems.

In contrast, much greater sensitivity can be achieved on performing mass fragmentography with the aid of high-resolution mass spectrometry. Figure 2-14 (see p. 48) shows the determination of 2,3,7,8-TCDD in human milk. In this case analysis was accomplished by capillary gas chromatography/high-resolution mass spectrometry with the aid of mass fragmentography in the EI mode. A DB-5 capillary column (0.10 μm film thickness, 0.25 mm internal diameter) was again used for gas chromatography. While the top two chromatograms display the fragmentograms of the exact masses m/e 319.8965 and 321.8936 selected for native 2,3,7,8-TCDD, the bottom two chromatograms depict the corresponding fragmentograms for the ^{13}C-labeled tetrachlorodibenzodioxins, with the second peak arising from ^{13}C-2,3,7,8-TCDD. At the start of sample preparation 25 picograms of this substance is added to the sample. The peak eluted shortly before corresponds to ^{13}C-1,2,3,4-TCDD, a substance which is added to the extracts in a quantity of 50 picograms prior to the final concentration step. The use of these two internal standards provides extremely accurate information about the losses incurred during the analysis. The completeness of separation of these two substances also quickly reveals whether the separation performance of the GC column used is sufficient for the analytical sample.

The advantage of high-resolution mass fragmentography is apparent from the fact that, in contrast to the previous example, the ^{13}C-2,3,7,8-TCDD is no longer visible in the mass trace for native 2,3,7,8-TCDD and that the sensitivity of detection is considerably improved by stronger elimination of the background. This sensitivity enhancement even permits detection of 2,3,7,8-TCDD in cow's milk not originating from contaminated regions. Figure 2-15 (see p. 49) shows the analysis of a cow's milk sample from a commercial outlet, in which a 2,3,7,8-TCDD content of 0.20 pg/g (ppt) of fat was detected. In spite of this very low concentration the measured ratio of 0.7636 for the peak areas for the selected fragments shows very good agreement with the theoretical value of 0.77. The measuring conditions corresponded to those used for analysis of human milk.

Whereas temperature-stable apolar silicone phases can readily be used for the analysis of human samples because only 2,3,7,8-chlorine substituted compounds are present, the analysis of foods of plant origin requires more selective separation phases permitting unequivocal separation of the toxic

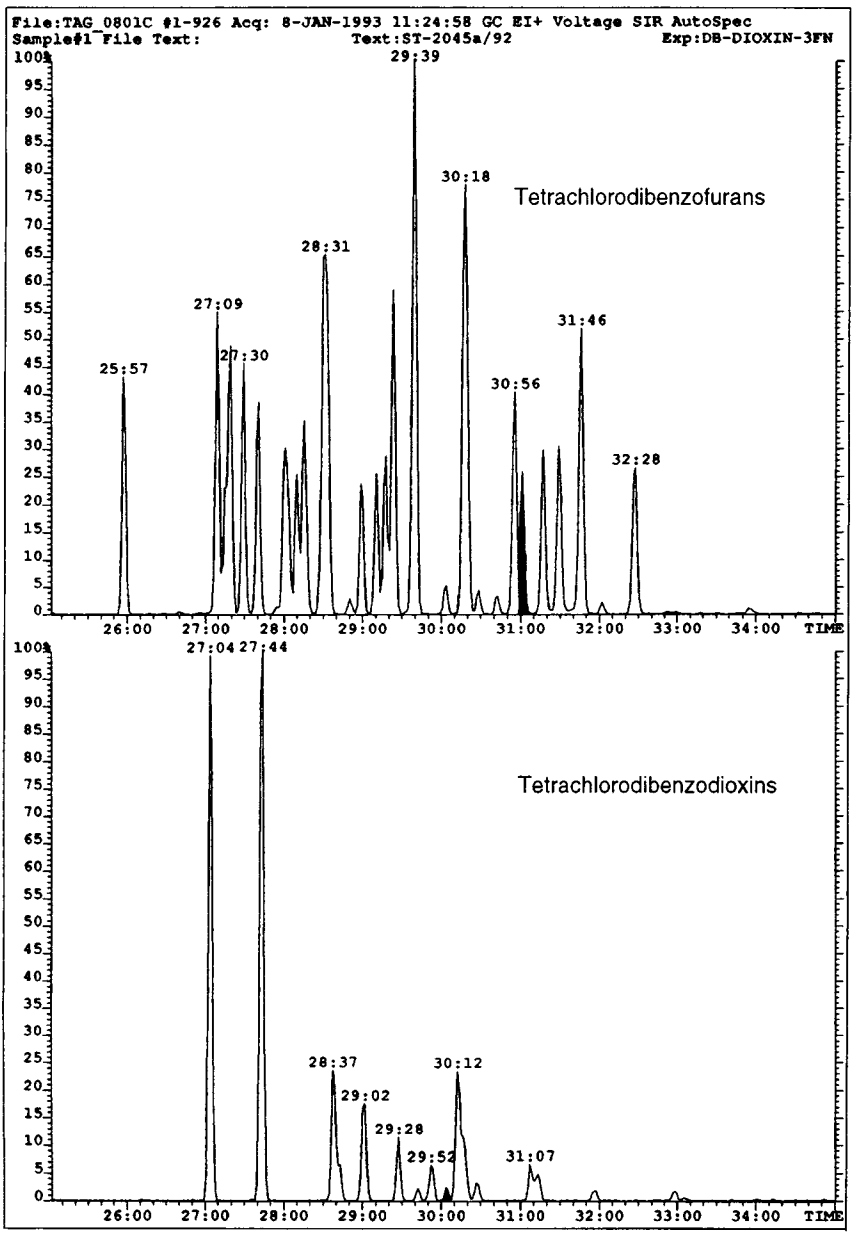


Fig. 2-16: Determination of tetrachlorodibenzofurans (top) and tetrachlorobenzodioxins (bottom) in a sample of kale on a DB-Dioxin capillary.

components from the less toxic ones. Figure 2-16 (see p. 51) demonstrates this for the example of the determination tetrachlorodibenzofurans (above) and tetrachlorobenzodioxins (below) in a sample of kale. The gas chromatographic separation was accomplished with a DB-Dioxin capillary. For the sake of clarity only one fragmentogram is included for each group of homologs. The peaks for the two particularly relevant 2,3,7,8-chlorine substituted congeners are shown in black. In contrast to the human and cow's milk samples this extract shows many more peaks. This is because the longer growing time and the large surface area enable the plant to take up airborne pollutants like a sponge over a long period, with practically no metabolization or degradation occurring. This property makes kale an excellent bioindicator, whose analysis very quickly affords information about air pollution, particularly with organic pollutants.

The chromatograms clearly show that the combination of gas chromatography and mass spectrometry permits highly selective determination of polychlorinated dibenzodioxins and dibenzofurans even in complex matrices. This was also the result found by an international round robin experiment in which cow's milk samples of various PCDD/PCDF contents were analyzed with high accuracy and reproducibility [2-19].

2.3.2.2 Determination of PCB and PCB Substitutes

A knowledge of the potential hazard posed by polychlorinated biphenyls (PCB) has in the past prompted increased efforts to replace these compounds by less toxic products. A class of substances whose good technical properties have permitted their extensive use as PCB substitutes, particularly in mining, are the tetrachlorobenzyltoluenes (TCBT). TCBT are the principal components of products commercially available as Ugilec 141 and Ugilec T. Although use in mining is designated as a closed application, not inconsiderable release nevertheless does occur, with hydraulic oils entering the environment via mine waste water or ventilation plant.

The determination of PCB in foods is generally performed isomer-specifically with the aid of an electron capture detector (ECD). Errors can occur if tetrachlorobenzyltoluenes are also present. Since PCB and TCBT have comparable polarities it is very difficult to selectively separate the two classes of substance from each other. Moreover, the two classes exhibit similar behavior on analysis by gas chromatography, leading to peak overlapping. Figure 2-17 depicts chromatograms of a mixture of Clophen A30/A60, selected individual isomers of PCB, and Ugilec 141, each separated on a DB-5 capillary. The figure shows that the TCBT lies in the retention time window of the PCB.

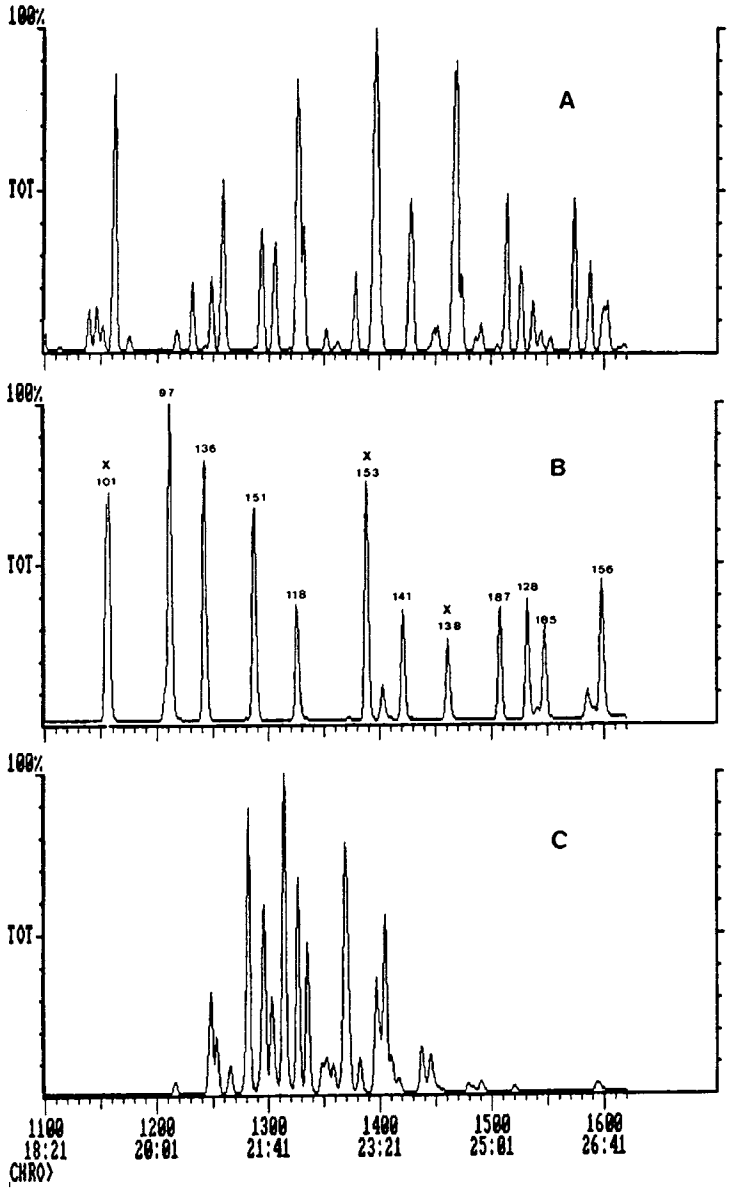


Figure 2-17: Gas chromatographic separation of a mixture of Clophene A30/A60 (top), selected individual isomers of PCB (middle), and Ugilec 141 (bottom) on a DB-5 capillary.

Above all, mention should be made of the PCB congener with Ballschmiter number 153, for which a limiting value is stated in German maximum pollutant level legislation. The joint presence of TCBT and PCB in a food sample would thus inevitably lead to erroneous results if PCB 153 is determined by GC/ECD.

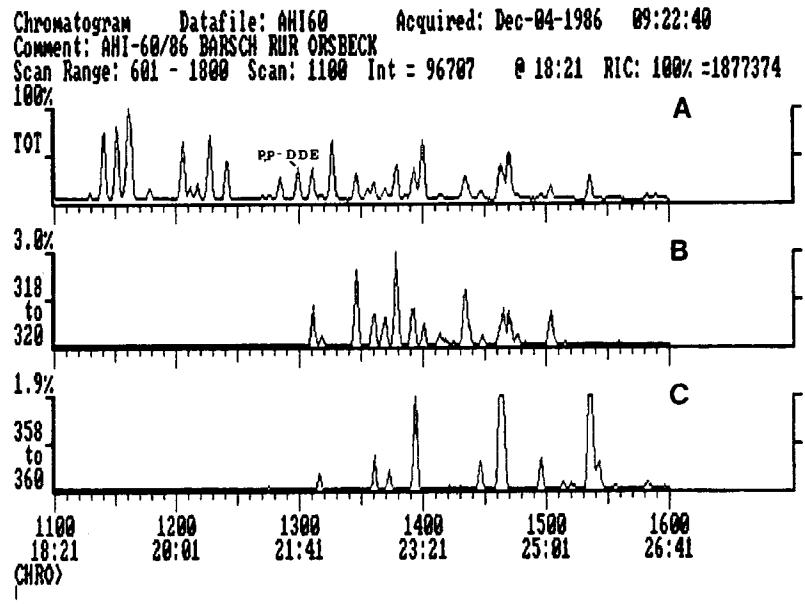


Figure 2-18: Determination of PCB and TCBT in a perch extract.
Top: Total ion current chromatogram.
Middle: Fragmentogram for tetrachlorobenzyltoluenes.
Bottom: Fragmentogram for hexachlorobiphenyls.

Since PCB and TCBT have different masses, a mass spectrometer can be used as mass-selective detector in their analysis, with the determination of one class of substances succeeding well even in the presence of a large excess of the other compounds. Figure 2-18 demonstrates this for the example of a perch extract from the River Rur, Germany. While the upper chromatogram shows the total ion current recorded by the mass spectrometer, the two lower fragmentograms show the typical patterns of tetrachlorobenzyltoluenes and hexachlorobiphenyls. In this case analysis was performed with an ion trap detector.

The fact that tetrachlorobenzyltoluene contents up to 25 mg/kg of edible material can be determined in fish from mining areas [2-20, 2-21] gives good reason for rethinking the use of these PCB substitutes.

2.3.2.3 Determination of Polybrominated Flame Retardants

Polybrominated biphenyls (PBB) and biphenyl ethers (PBBE) are of considerable importance as flame retardants for plastics and textiles. Figure 2-19 shows the structural formulae of the principal active components. Their uses range from additives in plastics, making aircraft components and TV cabinets fire resistant, to impregnation of textiles such as theater curtains or floor coverings. Particular interest has recently been focused on polybrominated flame retardants because the combustion of plastics with PBB or PBBE additives can lead to the formation of polybrominated dibenzodioxins and dibenzofurans.

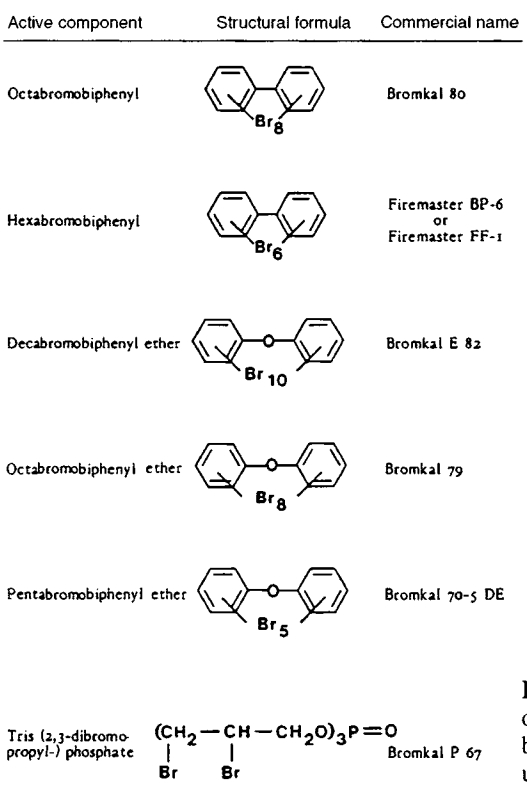


Figure 2-19. Structural formulae of the principal polybrominated biphenyls and biphenyl ethers used as flame retardants.

In contrast to polychlorinated biphenyls, polybrominated biphenyls have only been studied to a very limited extent, presumably because of the low amounts produced and the resulting low concentrations to be expected, although they show a persistence comparable with that of PCB and a high degree of bioaccumulation. They are also comparable to PCB with regard to their toxicity, with those congeners showing an enhanced toxic potential which are substituted in both *para* positions and in at least one *meta* position of the phenyl ring [2-22].

It therefore follows that the analytical procedure selected for determination of PBB and PBBE must not only be sensitive and highly selective, to assure reliable results also in the presence of an expected excess of other halogenated environmental contaminants, but must also be in a position to determine the relevant compounds in congener-specific manner. Since the analytes concerned are comparatively apolar substances, sometimes with very high molecular weights, the only stationary phases suitable for coating the separation capillaries are the thermostable apolar silicone phases.

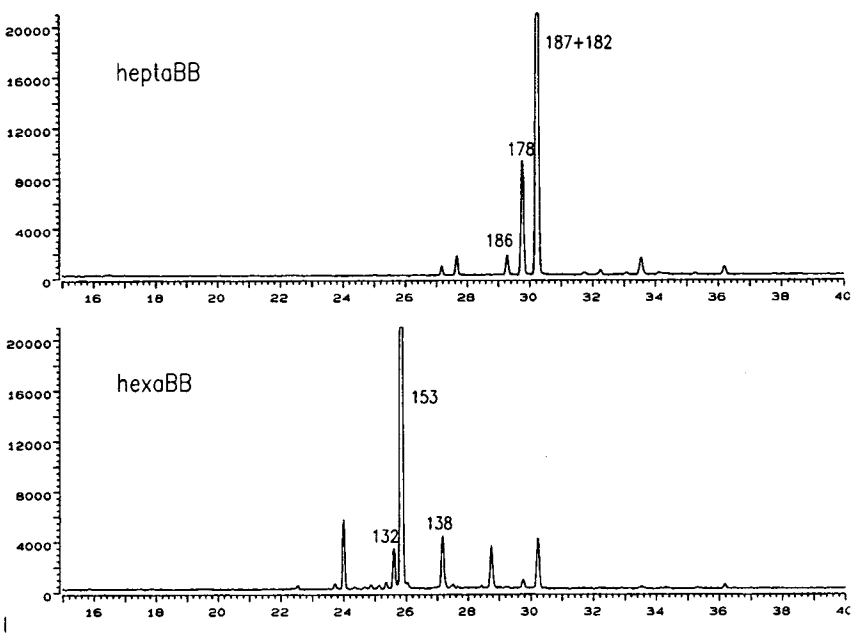


Figure 2-20: Detection of heptabromobiphenyls (above) and hexabromobiphenyls (below) in human milk by mass fragmentography in the NCI mode.

Since a large excess of PCB must always be expected in the analysis of foods of animal origin, the danger of error in the determination of PBB and PBBE is always particularly great on use of an electron capture detector. In contrast, combined GC/MS with the aid of mass fragmentography using negative chemical ionization represents a far more selective approach. The NCI technique proves especially useful because brominated aromatic compounds are detected with great sensitivity. Use of this analytical technique permits reliable determination of ppt levels of PBB and PBBE in foods and biological samples even in the presence of a large excess of PCB [2-23, 2-24]. Figure 2-20 demonstrates this for the example of determination of hexa- and heptabromobiphenyls in human milk [2-23]. The contents of these compounds lie between 0.03 and 1 ng/g (ppb) of fat. Here 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB 153) is the predominant congener. This is not surprising since this compound is also the principal component in the flame retarding agents Firemaster BP-6 and FF-1. It is also known from PCB studies that chlorine substitution in positions 2,2′,4,4′,5,5′ leads to particularly pronounced bioaccumulation in mammalian tissue.

2.3.2.4 Determination of Polychlorinated Terpenes

Insecticides designated as toxaphene, camphechlor, strobane, and melipax all contain polychlorinated terpenes as active components, with the industrial product toxaphene having the greatest importance. After its commercial introduction in 1945 it became one of the most widely used insecticides of the 19960s and 1970s, particularly in the USA. Owing to its toxicity and environmental persistence, production and use have been drastically reduced in most western countries since the end of the 1970s. Thus the use of toxaphene was prohibited in West Germany as long ago as 1971. In contrast, toxaphene is still widely used in Eastern Europe and in some third-world countries, especially in cotton production.

Owing to the widespread use of toxaphene combined with its pronounced persistence, polychlorinated terpenes meanwhile appear to be ubiquitous like many other lipophilic insecticides. This is also apparent from reports of toxaphene at mg/kg levels in cod liver oil [2-25].

The analytical determination of polychlorinated terpenes is made difficult by the large number of possible congeners with different numbers of chlorine atoms. Thus toxaphene consists of at least 202 individual components, among which polychlorinated bornane accounts for 76% [2-26]. Moreover, the likewise widespread PCB and other organochlorine pesticides can lead to serious interference in the analysis of foods, making reliable analytical

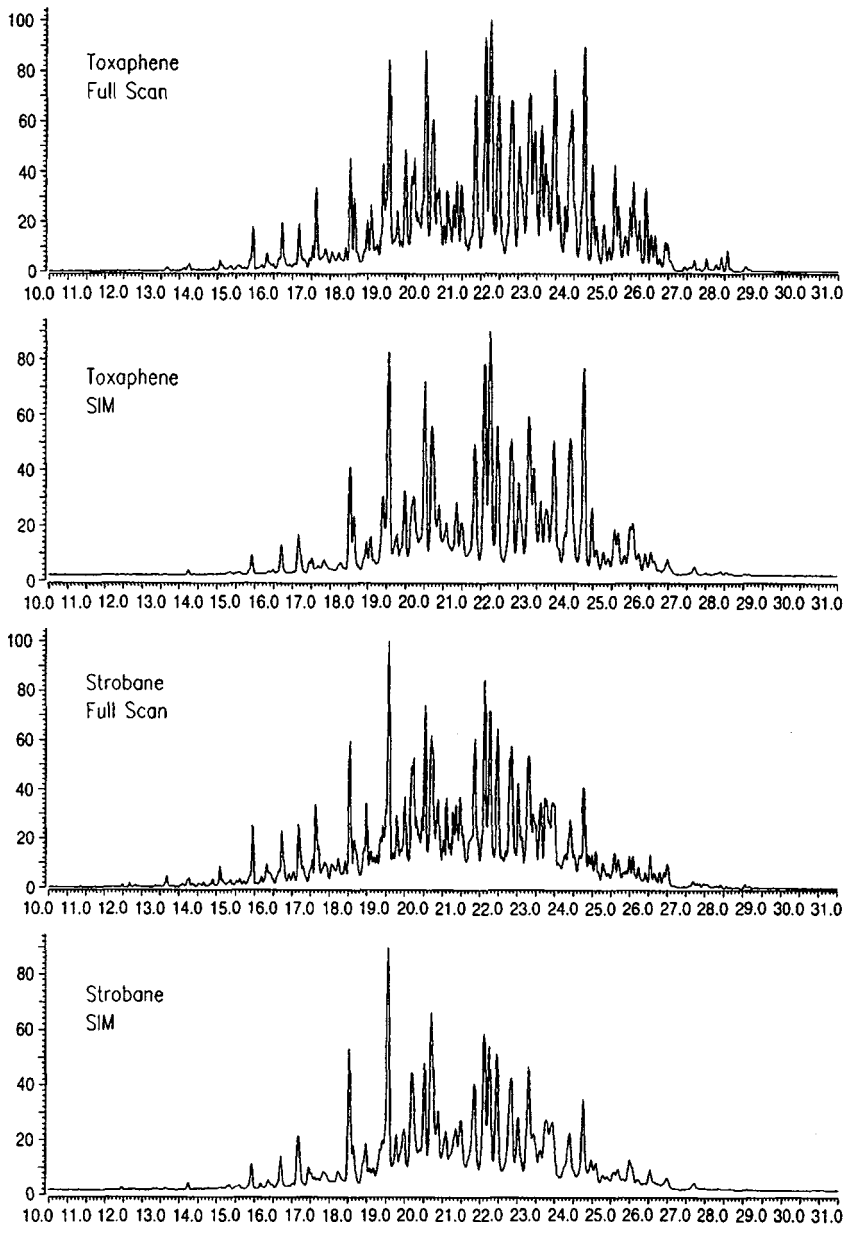


Figure 2-21: GC/MS/NCI analyses of toxaphene and strobane by recording the ion current (full scan) and by mass fragmentography (SIM).

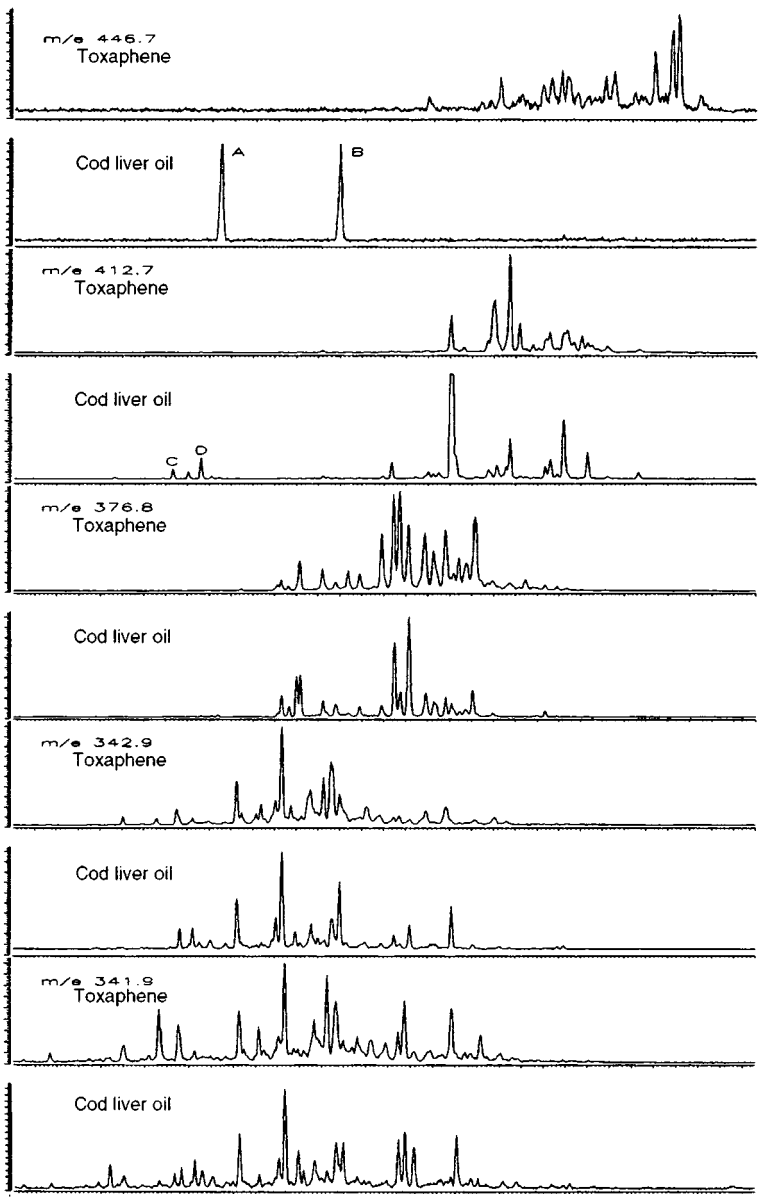


Figure 2-22: GC/MS/NCI mass fragmentograms for hexa- and deca-chloroterpenes of atechnical toxaphene mixture and a cod liver oil sample.

determination by GC/ECD problematical. However, the GC/MS procedure of Swackhamer et al. [2-27] in slightly modified form [2-28] has proved highly suitable for analysis of this class of compounds. Use of mass fragmentography in combination with negative chemical ionization permits highly sensitive determination of polychlorinated terpenes, even in the presence of other persistent insecticides and PCB. Since the polychlorinated terpenes of toxaphene are mainly bornanes and bornenes containing six to ten chlorine atoms, two characteristic fragments are selected for each group of isomers. A look at the total ion current chromatograms of technical mixtures of toxaphene and strobane shown in Figure 2-21 and at the mass fragmentograms obtained after recording of the selected masses clearly reveals that the major portion of the toxaphene and strobane components are found by this method.

On uptake of toxaphene by living organisms it is metabolized to form a mixture of substances deviating substantially in percentage composition from the original product prior to uptake. Figure 2-22 shows this for the example of the fragmentograms of the deca- to hexachloroterpenes of a toxaphene standard and a cod liver oil sample. The intensity differences for the various isomers of a given degree of chlorination between the toxaphene standard and the cod liver oil are considerable in some cases, which is undoubtedly due to different extents of metabolization of the individual polychlorinated terpenes in the fish organism.

The peaks marked "A–D" are due to *cis*- and *trans*-nonachlor and *cis*- and *trans*-chlordan, which are also detected by the analytical method without interfering with the analysis of the polychlorinated terpenes. Since authentic individual compounds are available for these analyses, accurate quantification can be performed.

In contrast, exact quantitative determination of the individual polychlorinated terpenes is not yet possible since authentic samples of the individual compounds are not commercially available. Thus, at present it is only possible to estimate the amounts present in foods on the basis of a commercially available polychloroterpene-based insecticide [2-28]. Some individual polychlorinated terpenes have meanwhile (1997) become available from Promochem Ltd.

2.3.2.5 Determination of Benzo[a]pyrene in Smoked Meat and Meat Products

Legislation pertaining to meat and meat products limits the average content of benzo[*a*]pyrene in smoked meat products and meat products containing a proportion of smoked foods to 1 μg/kg (ppb). Owing to the very low maxi-

mum amount permitted, very sensitive methods of determination are necessary for the analysis of benzo[*a*]pyrene residues in foods. The official monitoring of foods uses mainly high performance liquid chromatography, exploiting the good fluorescing properties of polycyclic aromatic compounds. Some gas chromatographic methods have also been described. It is a drawback that benzo[*a*]pyrene contains no functional groups permitting determination with selective detectors such as the electron capture detector or the thermionic nitrogen detector. Instead, a flame ionization detector (FID) has to be used; the very modest selectivity of this detector necessitates enormous effort in purification of the extract.

A very elegant method of determining small traces of benzo[*a*]pyrene in foods with high selectivity is the combination of capillary gas chromatography with mass spectrometry using the SIM technique. Furthermore, mass spectrometric determination has the added advantage that deuterated substances can be added directly as internal standards to the samples prior to sample preparation. The bottom part of Figure 2-23 shows the mass spectrum of benzo[*a*]pyrene. One clearly sees the stable peak of the molecular ion at m/e 252. Fragments of low mass only appear at low intensity. Owing to this fact, polycyclic

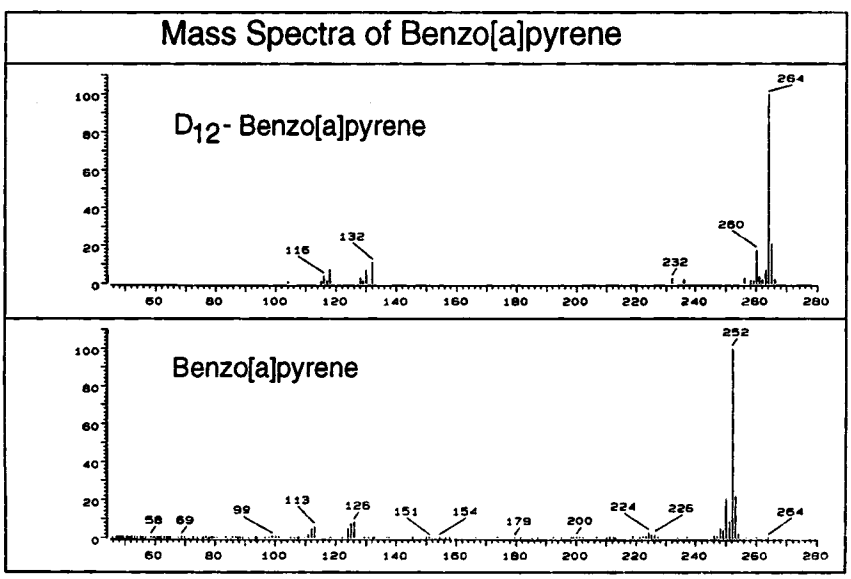


Figure 2-23: Mass spectra of deuterated (above) and native benzo[*a*]pyrene (below).

aromatic compounds are generally very suitable for analyses by GC/MS employing the SIM technique. This is due to the principle of this technique: characteristic masses are selected from the mass spectra of the substances to be analyzed. In view of the high selectivity together with the high sensitivity such fragments are above all those of high intensity and/or mass. These requirements are ideally fulfilled by substances such as benzo[*a*]pyrene because its molecular ion is also the fragment with the greatest intensity (base peak).

The upper part of Figure 2-23 shows the mass spectrum of deuterated benzo[*a*]pyrene. Since the twelve hydrogen atoms of the native compound have been replaced by deuterium, the molecular peak is shifted by twelve mass units to m/e 264. Thus addition of a known amount of deuterated benzo[*a*]pyrene to the sample provides valuable information about losses occurring during analysis.

The procedure used for clean-up of the extracts was adapted from one that is widely used in the analysis of environmentally relevant pollutants. It employs a small column packed with specially treated activated charcoal, in this case Carbopack C (from Supelco). This activated charcoal selectively retains planar and co-planar compounds whereas non-planar ones can pass through the column unhindered. Clean-up steps using such activated charcoals play a major role in the analysis of polychlorinated dibenzodioxins and dibenzofurans which can be selectively separated from the non-planar PCBs in this way.

As a rule the extract is applied to the activated charcoal in an organic solvent which may not be aromatic or planar. The non-planar compounds are then removed using all kinds of solvents except the planar solvents mentioned above. The planar fraction is subsequently eluted from the column with toluene. Since the charcoal is a very finely divided material which can readily block the column, it has proved beneficial to disperse the adsorbent is a glass fiber filter or to mix it with a support material such as Celite 545.

This procedure developed for dioxin analysis can also be used for the determination of polycyclic aromatic compounds.

Figure 2-24 shows typical fragmentograms obtained on analysis of smoke-cured Black Forest ham. The upper trace is the fragmentogram of mass m/e 264 shown practically only by the deuterated benzo[*a*]pyrene used as internal standard. In contrast, the lower fragmentogram of mass m/e 252 corresponds not only to benzo[*a*]pyrene but clearly also to benzo[*e*]pyrene

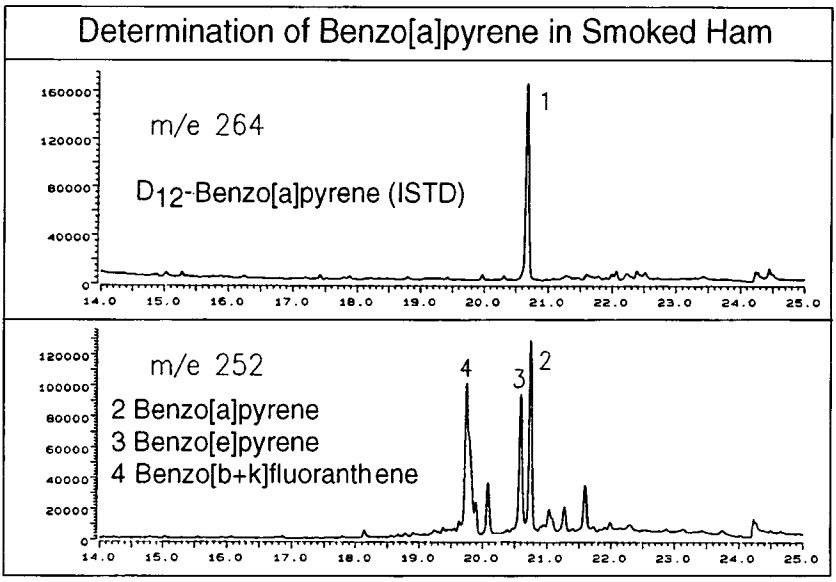


Figure 2-24: Determination of benzo[*a*]pyrene in smoke-cured Black Forest ham.
Above: Fragmentogram for m/e 264.
Below: Fragmentogram for m/e 252.

and to benzo[*b*+*k*]fluoranthene, because they also have a molecular weight of 252 and are thus also detected using SIM. These fragmentograms containing few peaks clearly demonstrate that the combination of sample clean-up with activated charcoal and GC/MS coupling offers an excellent approach to the selective and sensitive determination of polycyclic aromatic compounds for an acceptable expenditure of time and effort.

2.3.2.6 Determination of Chloramphenicol in Foods of Animal Origin

Thanks to its good bacteriostatic action towards numerous Gram-positive and Gram-negative bacteria chloramphenicol (CAP) has in recent years come into widespread use in factory farming. Since even small amounts of chloramphenicol cannot be regarded as entirely safe for humans, legislation relating to the presence of pharmacologically active substances has set maximum chloramphenicol levels of 1 or 10 μg/kg in a number of foods.

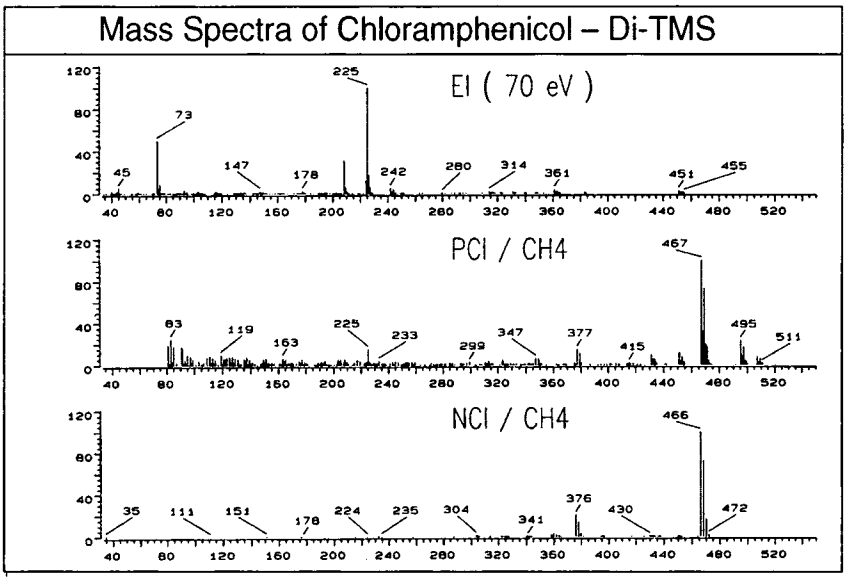


Figure 2-25: Mass spectra of the di-TMS chloramphenicol.
Top: Electron impact ionization (70 eV).
Middle: Positive chemical ionization (CH_4).
Bottom: Negative chemical ionization (CH_4).

Since the molecule of CAP contains two chlorine atoms, ECD and especially GC/MS with negative chemical ionization are suitable for its detection after silylation [2-29].

On observation of certain derivatization conditions it is possible to convert chloramphenicol into the doubly silylated product [2-30]. The mass spectra obtained for this substance on use of various ionization techniques are shown in Figure 2-25. The mass spectra are seen to differ considerably. Owing to the instability of the molecular ion there is clearly no fragment at m/e 466 in the EI spectrum. In contrast, positive chemical ionization generates a stable protonated molecular ion at m/e 467 with a characteristic isotope pattern indicative of two chlorine atoms. The higher fragments arise from addition products of the methane used as reactant gas. Similarly to the PCI spectrum, the bottom spectrum recorded with the aid of negative chemical ionization shows practically only the molecular peak at m/e 466 with its characteristic isotope pattern. In contrast to positive chemical ionization, negative chemical ionization combined with mass fragmentography results in an extremely high

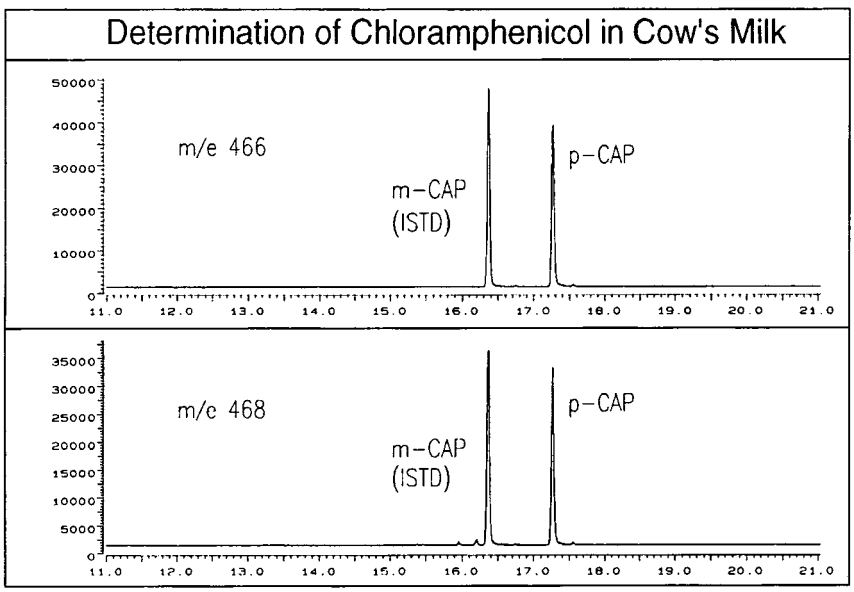


Figure 2-26: GC/MS/NCI determination of chloramphenicol in cow's milk.

sensitivity with an absolute value of ca. 200 femtograms on recording of five masses.

Owing to this extreme sensitivity combined with high selectivity, negative chemical ionization appears to be the method of choice for GC/MS determination of very low trace levels of CAP.

The internal standard used is *meta*-chloramphenicol which differs from the therapeutically utilized substance by having its nitro group in the *meta* rather than the *para* position.

Figure 2-26 shows the analysis of a sample of cow's milk in which a CAP content of 0.87 µg/l (ppb) could be determined. The figure indicates that combined capillary GC/MS together with negative chemical ionization permits reliable determination of chloramphenicol residues in foods of animal origin also in the concentration range below 1 µg/kg (ppb) without interference from any accompanying substances present in the matrix. The lower limit of the practical working range lies between 0.001 and 0.01 µg/kg, depending upon the substrate [2-29].

2.3.2.7 Determination of β-Sympathomimetics in Meat and Urine

β-Sympathomimetics are used both in human and in veterinary medicine as highly effective drugs for the treatment of acute and chronic ailments of the respiratory tract. Treatment of animals with large amounts of these substances can also lead to stimulation of the β-2 receptors of adipose tissue and thus ultimately to an improvement in the muscle/fat ratio as desired in the fattening process.

Use of β-sympathomimetics for fattening purposes is prohibited in Germany.

After the illegal use of clenbuterol for fattening of calves and pigs had been discovered in the German state of North Rhine-Westphalia in 1988 [2-31] it was also possible to prove the widespread illegal use of salbutamol as fattening aid in animal production in August 1989 [2-32]. Analysis was performed by GC/MS using positive chemical ionization.

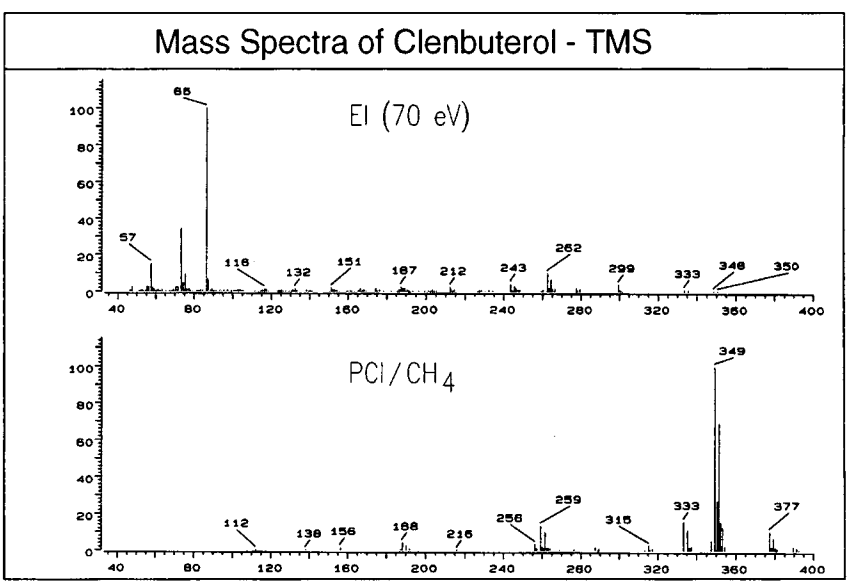


Figure 2-27: Mass spectra of the TMS derivative of clenbuterol.
Above: Electron impact ionization (70 eV).
Below: Positive chemical ionization (CH_4).

Since clenbuterol, like chloramphenicol, contains two chlorine atoms in its molecule, an attempt was also made to utilize negative chemical ionization with methane as reactant gas. Contrary to expectation, no informative mass spectra were obtained with this technique.

Figure 2-27 shows the mass spectra obtained for silylated clenbuterol after electron impact ionization (EI) and positive chemical ionization (PCI). While the EI spectrum only gives intense fragments in the lower mass range, the $(M+H)^+$ fragment in the PCI spectrum is seen to be the base peak. In addition to the characteristic isotope pattern indicative of two chlorine atoms, higher fragments are also seen which arise from addition products formed with the reactant gas. Thus, compared to electron impact ionization, positive chemical ionization offers the benefit of higher selectivity since measurement can be performed at higher masses with the aid of the SIM technique.

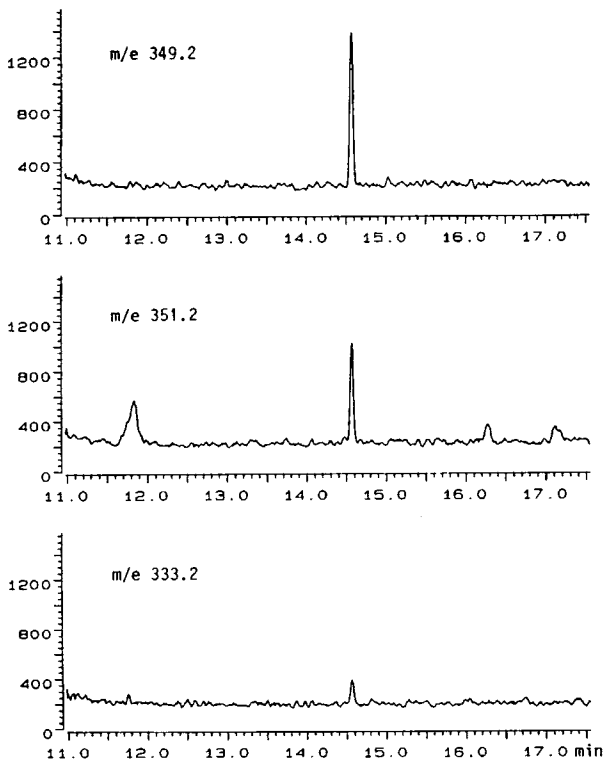


Figure 2-28: GC/MS/PCI determination of clenbuterol in pig's liver by mass fragmentography with three fragments.

Figure 2-28 shows the fragmentograms of three masses characteristic of clenbuterol-TMS from the analysis of a pig's liver sample, which was found to have a clenbuterol content of 5.2 µg/kg (ppb). Since all the signals appear at the appropriate retention times and the ratio of their relative intensities also agrees with those of the reference substance, the determination of clenbuterol can be regarded as assured.

The determination of salbutamol in calf urine is shown in Figure 2-29. The salbutamol content in this case was 1.2 µg/l. While the detection of salbutamol was based on fragments at m/e 440.4 and 456.4, among others, the internal standard clenbuterol-D9 was recorded via the fragment m/e 358.2. Since veterinary use of salbutamol is not permitted in Germany, this finding must indicate illegal administration of the drug.

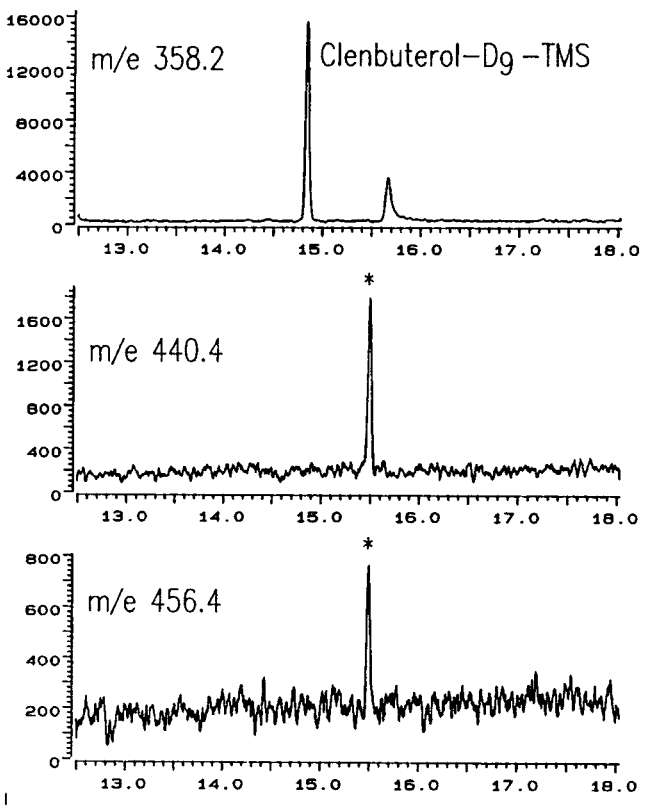


Figure 2-29: GC/MS/PCI determination of salbutamol in calf urine.

2.3.2.8 Determination of Diethylsilbestrol in Meat by GC/LRMS and GC/HRMS

In recent years GC/MS using mass fragmentography has played a special role in the analysis of foods for residues of anabolic steroids, such as diethylstilbestrol (DES), dienestrol, hexestrol, and also ethynylestradiol. Prior to gas chromatographic analysis the substances first have to be transformed into volatile derivatives; silylation is especially suitable for this purpose since the resulting products are not only thermally stable but – with the exception of the symmetrical molecule of hexestrol – also give intense fragments in the region of the molecular ion peak.

The criteria for performing these analyses are set out in the Appendix to the final decision of the commission of July 14th, 1987, regarding the determination of residues of compounds with hormonal and thyreostatic properties (87/410/EEC)". In addition to a number of definitions, specifications of accuracy and precision, quality criteria are also given in Section II for evaluation of the results. The criteria for determination of an analyte by gas chromatography/low resolution mass spectrometry (GC/LRMS) prescribe, among other things, that the deviation of the retention time of the analyte from that of the standard may not exceed ± 5 seconds. In recording the mass spectrum all the ions must originate from one analyte eluting at a single retention time. For each substance at least two (preferably more) fragments must be recorded, with the molecular ion also being included where possible. Finally, the deviation of the relative intensities of the fragments of the analyte from those of the standard should not exceed ± 20% for chemical ionization and ± 10% for electron impact ionization.

Figure 2-30 shows the fragmentograms of a meat extract which was analyzed in 1992 within the framework of a certification study for the Community Bureau of Reference of the EC (BCR). Analysis was performed in this case by GC/MS with low-resolution mass fragmentography (GC/LRMS), with a DB-5 capillary being used for the gas chromatographic separation.

While the top chromatogram depicts the trace for the mass m/e 418.3 selected for the internal standard DES-D_6, the two lower traces show the fragmentograms for masses 412.3 and 383.2, which are characteristic for the silylated native DES. The figure demonstrates that *cis*-DES can be unequivocally determined since the retention time and also the ratio of the two selected fragments are in agreement with the values for the reference substance. In contrast, *trans*-DES coincides with co-extracted interfering substances which were not completely removed during sample treatment, thus precluding its

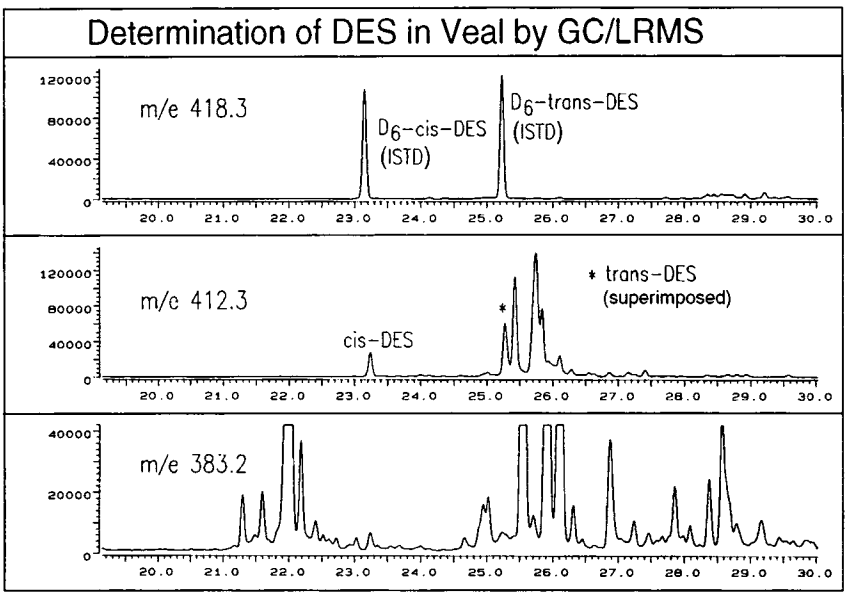


Figure 2-30: Determination of DES in meat by GC/MS with low-resolution mass fragmentography.

determination under the conditions of the above-mentioned EC quality criteria. Unequivocal determination of this compound is, however, possible by high-resolution mass fragmentography (GC/HRMS) since although the interfering substances, like the silylated DES, have a nominal mass of m/e 418.3 they differ sufficiently from the analyte in the millimass range. Hence, increasing the resolution to R = 10000 suffices for distinction to be made between these substances. Figure 2-31 demonstrates this for the same meat extract which was already analyzed with the aid of low-resolution mass fragmentography. At a resolution of R = 10000 the exact masses of 418.2630, 412.2254, and 383.1863 were selected for measurement. The figure shows that there is no longer any significant interference. Moreover, thanks to the extensive elimination of the background the sensitivity could be greatly improved. Since all the criteria for determination of an analyte by gas chromatography/high-resolution mass spectrometry stipulated in the decision of the EC Commission are fulfilled, the determination of *cis*- and *trans*-DES in this sample can be regarded as assured.

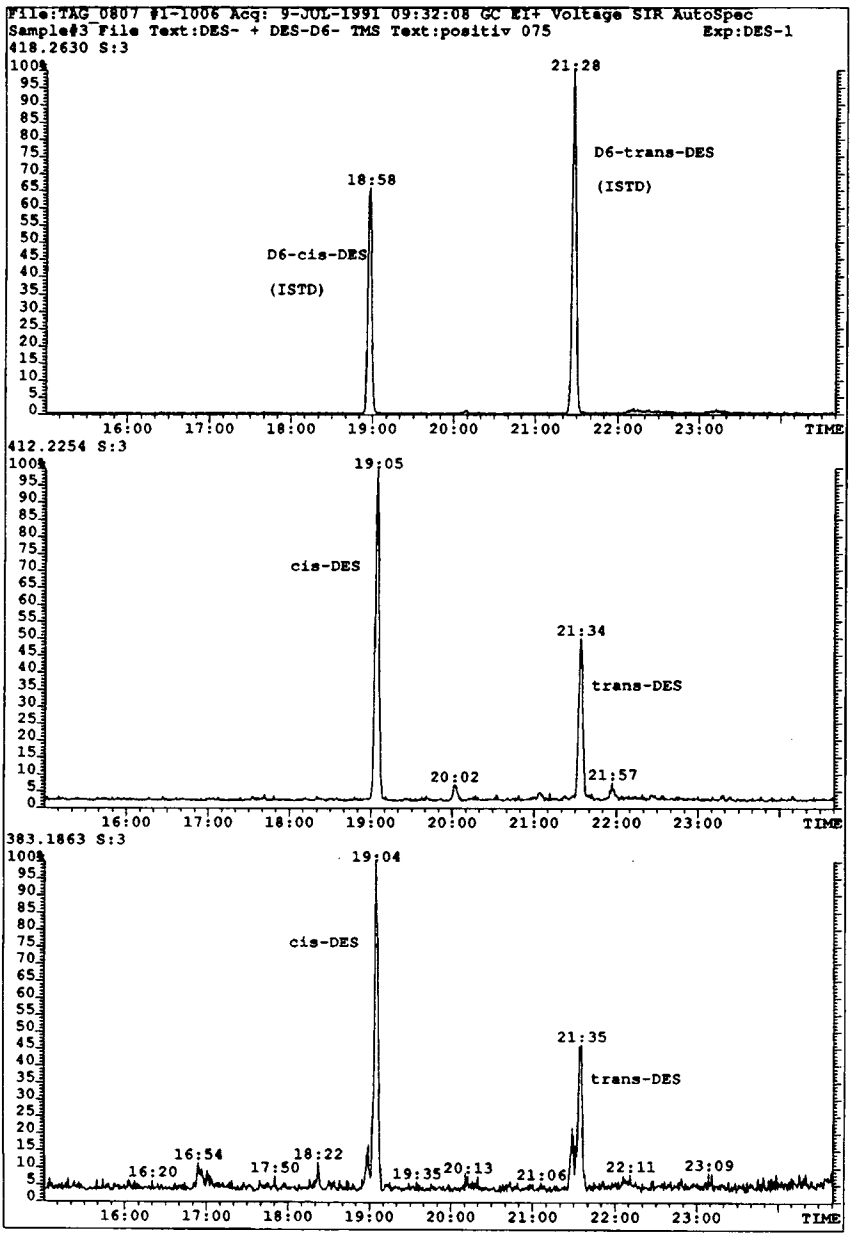


Figure 2-31: Determination of DES in meat by GC/MS with high-resolution mass fragmentography.

2.4 Summary

The examples shown above demonstrate the possibilities offered by GC/MS in combination with various ionization and recording techniques, above all in the analysis of residues and contaminants in foods. This analytical technique can not only be used for identification of unknown substances but is also particularly suitable for specific determination of organic compounds in very low trace amounts. This also applies to analytes without functional groups, and which therefore cannot be analyzed with the aid of specific detectors such as the ECD and the NPD. However, working in these low concentration ranges imposes rigorous demands not only on the instrumentation but also on the analyst's critical attitude towards his own results, which frequently provide the basis for far-reaching administrative decisions with costly consequences. In this context, the implementation of quality assurance measures in analytical laboratories on the basis of the OECD Fundamentals of Good Laboratory Practice (GLP) and the European Standards 45000 will acquire increasing importance in the next few years.

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Food and Environmental Analysis by Capillary Gas Chromatography
Lothar Matter

3 Determination of PAH in Foods

Karl Speer

3.1 Introduction

In the article entitled "The Cancer of the Scrotum" published in 1775, Percivall Pott reported on the increased incidence of cancer of the scrotum among chimney sweeps. He suspected the presence of certain compounds in soot which he considered responsible for causing this disease. The resulting research activities led to the discovery of a new class of substances: polycyclic aromatic hydrocarbons (PAH). Even today, over 200 years later, research on the origin, occurrence, and toxicology of PAH continues.

Polycyclic aromatic hydrocarbons are produced on incomplete combustion of organic materials. In addition to natural sources such as forest and prairie fires, anthropogenic activities are chiefly responsible for the formation of PAH. Above all, mention should be made of power generation and space heating as well as industrial emissions and also automotive emissions.

The straight-chain, branched, or cyclic hydrocarbons are formed mainly by free radical reactions in which acetylene plays the key role; higher condensed hydrocarbons are then formed by polymerization and ring closure.

Over 100 polycyclic aromatic hydrocarbons have been found so far; the compounds always occur as complex mixtures, never as individual compounds. Most of them prove to be carcinogenic and mutagenic in animal experiments. This is particularly true of the best-known compound among the polycyclic aromatic hydrocarbon, viz. benzo[*a*]pyrene, whose pronounced toxicity and thorough investigation make it a key substance in this group.

Owing to their mode of formation, PAH are almost ubiquitous and enter our foods via the air and soil.

Some of the PAH found in our food are a consequence of the method of preparation. Thus, in particular, grilling or barbecuing and also smoking – one of the oldest methods of preserving foods – can lead to considerable contamination of the products if not performed correctly.

The major portion of polycyclic aromatic hydrocarbons is not consumed via grilled or smoked foods but via cereal products (Dennis et al. [3-2]). Oils and fats and vegetables are also strongly contaminated with PAH (Figure 3-1).

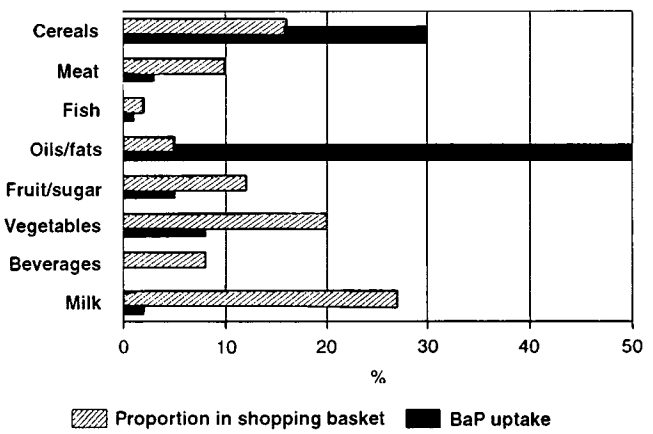

Fig. 3-1: Proportions of foods in shopping basket and uptake of benzo[*a*]pyrene (see data published by Dennis et al., 1983).

German legislators have limited the content of PAH after the manner of a preventative consumer health protection program in only a few regulations of food law. In drinking water maximum amounts have been stipulated for six PAH, viz. fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene; in regulations relating to meat and cheese only the maximum content of benzo[*a*]pyrene is stipulated.

Investigations of the PAH burden of foods should not be limited to the determination of benzo[*a*]pyrene as key substance but should be extended to cover a series of other selected PAH.

The recommendations of the American Environmental Protection Agency provide a basis for selecting the PAH components to be determined. As a rule, both the "light" PAH phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, and triphenylene (Figure 3-2) and the "heavy" PAH benzo[*a*]fluoranthene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene, dibenzo[*ah*]anthracene, and dibenzo[*ac*]anthracene are analyzed (Figure 3-3).

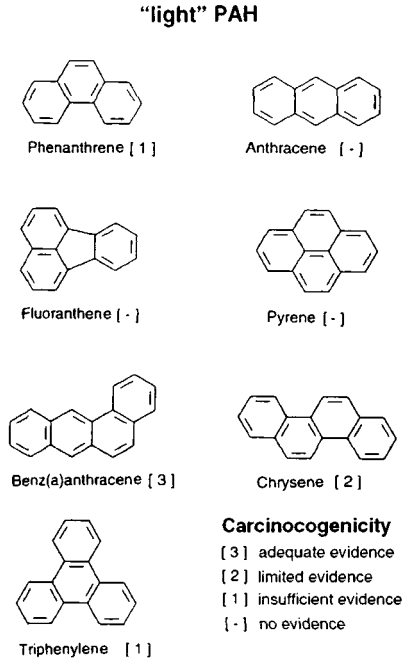

Fig. 3-2: Structural formulae of "light" PAH.

The division into "light" and "heavy" PAH was undertaken by Wendt [3-3]; he collected compounds with three or four rings in the former group and compounds with five and more rings in the latter. Toxicological assessment of the individual compounds was performed by the International Agency for Research on Cancer (IARC) [3-4], a Lyon-based organization belonging to the WHO. Apart from benz[*a*]anthracene, there is insufficient if any evidence of carcinogenicity for most of the "light" compounds. In contrast, among the "heavy" polycyclic compounds there is generally at least limited or even sufficient evidence of carcinogenicity if benzo[*e*]pyrene, perylene, and

benzo[*ghi*]perylene are disregarded. Thus the classification of PAH according to their molecular size also makes toxicological sense.

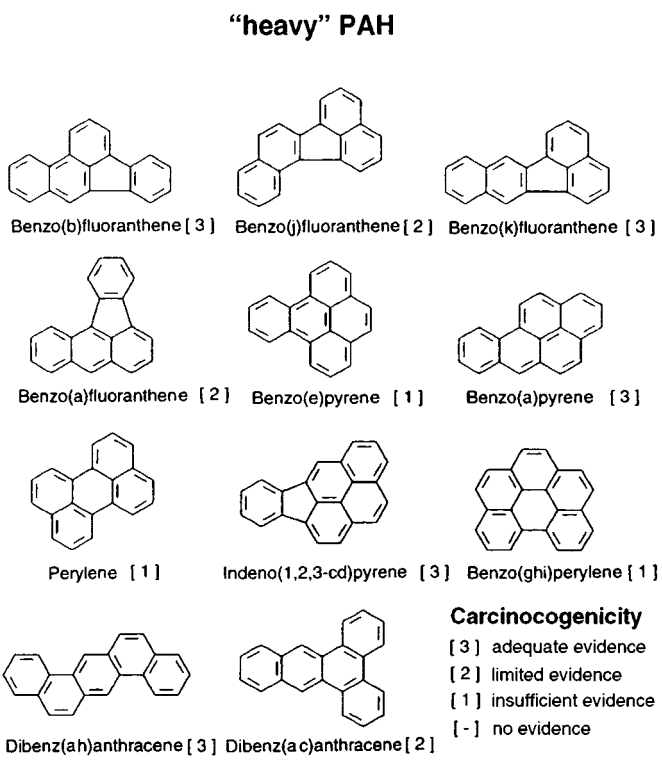

Fig. 3-3: Structural formulae of "heavy" PAH.

The following sections describe analytical procedures for the determination of selected PAH in the foodstuffs oils and fats, vegetables, fish products, mollusks, and in coffee and tea, and presents a number of analytical results.

3.2 PAH in Oils and Fats

The presence of PAH in vegetable oils and fats was first pointed out by Jung and Morand [3-5, 3-6] in 1962. A number of other publications followed [3-7 to 3-22] which were concerned, among other things, with the source of contamination of vegetable oils. While fish oils are often contaminated by irresponsible handling of mineral oils, the polycyclic compounds get into vegetable oils by drying of the oil-bearing seeds over an open fire.

Thanks to the work of Biernoth and Rost [3-8] it is known that most of the polycyclic compounds can be removed on refining the oils. Deodorization or steam treatment of the oil permits extensive removal mainly of "light" PAH; elimination of "heavy" PAH requires treatment with activated charcoal.

The effect of refining is demonstrated for a coconut oil. While the total PAH content of the raw coconut oil was approximately 2000 μg/kg, after treatment with steam and activated charcoal only 10 μg/kg was detected in the oil (Sagredos et al. [3-19]. Hence the polycyclic compounds can be effectively removed, leaving only slight residues, by fat refining processes.

As a result of the "back-to-nature" and "bio" movement, native, i.e. untreated and hence unrefined, vegetable fats and oils have recently become increasingly popular. According to the German regulations applicable to vegetable oils and fats these native oils may only be washed, dried, and filtered, but not deacidified, bleached, or deodorized. Only steaming – i.e. steam treatment to deactivate fat-degrading enzymes – has been permitted for most native oils, according to a circular issued by the Federal German Minister of the Interior [3-24].

It is therefore appropriate to test these unrefined oils for PAH, especially heavy PAH.

3.2.1 Analysis of Fats and Oils

Various methods are adopted for the *separation of PAH* from fats and accompanying materials.

Potthast and Eigner [3-25] developed a procedure in which the sample material is first treated with an equal volume of chloroform. After addition of sodium sulfate and celite, the substances are thoroughly mixed and applied to a chromatographic column after removal of the chloroform. The polycyclic compounds are then eluted with propylene carbonate; the lipids remain on the column since they are insoluble in this solvent.

Other authors [3-11, 3-12, 3-13, 3-26, 3-27] utilize the phenomenon published as long ago as 1938 by Brock and later studied in depth by Weil-Malherbe that the water solubility of PAH is improved by addition of caffeine-formic acid. Stijve and Diserens [3-18] describe a method in which the major portion of the oil is bound to Calfo E.

Isolation of the PAH from vegetable oils does not require hydrolysis of the sample material if performed according to Grimmer and Böhnke [3-10]. Lipid removal is accomplished by liquid-liquid extraction of the oil dissolved in cyclohexane with a mixture of dimethylformamide and water (9:1). Due to this step the the amount of fat in the original extract is reduced to about 0.2%.

Purification of the extracts obtained by liquid-liquid partitioning is generally accomplished by column chromatography. Dennis and co-workers [3-2] and Gertz [3-26, 3-27] perform this clean-up with SEP-PAH cartridges, whereas Welling and Kaandorp [3-16] use chromatography on XAD-2.

Activated alumina is also used [3-52] but the method that has proved to be particularly valuable is the use of silica of defined water content as described by Grimmer and Böhnke.

The clean-up steps described generally prove adequate for quantitative analysis of polycyclic compounds by HPLC with fluorescence detection.

If determination is to be performed by capillary gas chromatography with a flame ionization detector (FID), or on use of mass spectrometry in the multiple ion selection (MIS) mode, it is frequently necessary to undertake further clean-up of the sample solution. Thus Kolarovic and Traitler [3-13] clean-up their samples on HPTLC plates prior to determination by capillary GC (FID).

Grimmer and Böhnke suggest column chromatographic clean-up on Sephadex LH-20; the particular advantage of this approach is that the retention volumes to be isolated are independent of the water content of the elution agent.

Determination of PAH can nowadays often be accomplished by high pressure liquid chromatography with fluorescence detection thanks to the development of suitable stationary phases. HPLC is readily applicable to the analysis of drinking water and air and for studies on automotive exhaust gases; however, the use of capillary gas chromatography-mass spectrometry is recommended for the determination of PAH in foods, i.e. in complex matrices. This analytical technique permits selective determination of PAH, with utilization of the MIS method also making it selective.

The *analytical procedure* for determination of PAH in vegetable oils and fats presented below is based on the method developed by Grimmer and Böhnke [3-10] (Figure 3-4).

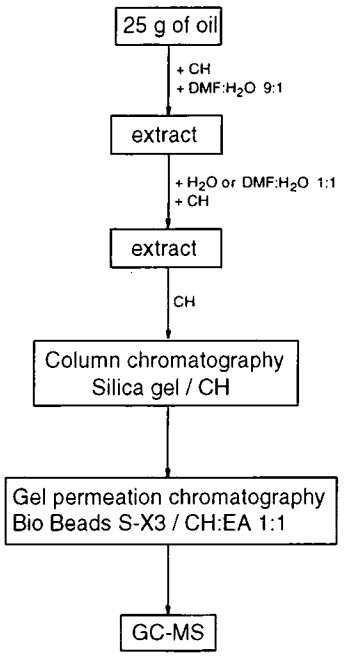

Fig. 3-4: Analysis scheme "oils and fats". CH = cyclohexane; EA = ethyl acetate; DMF = dimethylformamide.

After addition of the internal standards phenanthrene-d_{10}, benzo[*e*]pyrene-d_{12}, and perylene-d_{12}, the homogeneous sample material is dissolved directly in cyclohexane and subjected to repeated liquid-liquid extraction in the system dimethylformamide-cyclohexane-water. In this way about 99.8% of the oil can be removed.

The sample extract is then cleaned-up on an activated silica gel column to remove chlorophyll and other polar substances. Deviating from the procedure of Grimmer and Böhnke, further clean-up is performed automatically with Biobeads S-X3 in an "Autoprep". Most institutes concerned with pesticide residue analysis have such a unit at their disposal; the eluent – cyclohexane/ethyl acetate (1:1); flow rate 5 ml/min – can be maintained so that only the waste and collect phases have to be adapted to the new analytical goal. The concentrated eluate is then used for quantitative determination of the individual PAH. The capillary gas chromatogram of an olive oil extract after clean-up is shown in Figure 3-5.

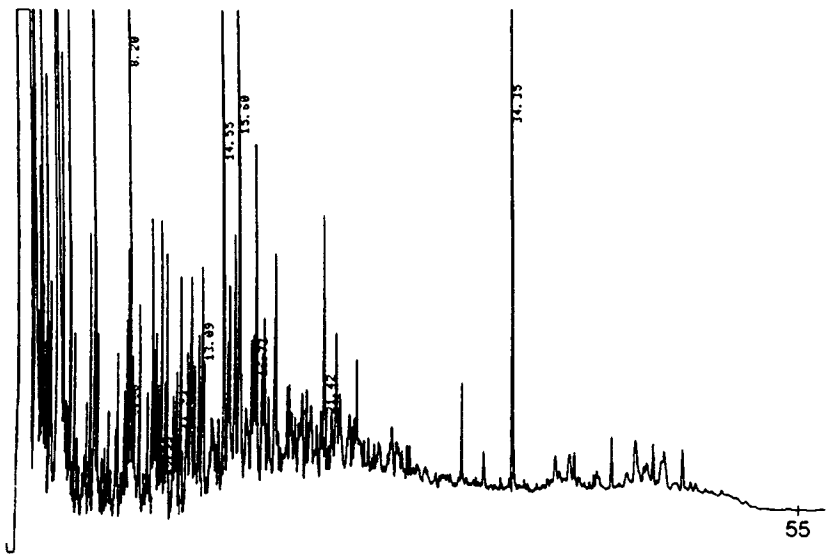

Fig. 3-5: Capillary gas chromatogram of an olive oil extract (detector: FID).

This complex chromatogram suffices to demonstrate that the determination of PAH with flame ionization detection is very difficult and not without attendant error. Of much greater selectivity and sensitivity is mass spectrometry utilizing the multiple ion detection technique. In this approach, the full scale spectrum, which generally requires 1 to 10 ng of a substance, is not recorded, but only the mass fragment ions typical for the compound in question. Determination of a component is regarded as confirmed if the retention times and the intensity ratios of the characteristic mass fragment ions of the sample and the standard are in agreement. Thus for a starting amount of oil of 25 g and a final volume of 500–100 µl contents of below 0.2 µg/kg of substrate can still be determined.

The mass fragment ions to be selected for the individual polycyclic compounds are taken from the work of Tuominen and co-workers [3-28] and are listed in the section entitled "Procedures".

In addition, GC/MS determinations also permit the use of deuterated standard substances; these compounds are ideal standards because they do not occur in nature, on the one hand, and because they behave in exactly the same way as the analytes during sample preparation, on the other. This permits work-up related losses to be taken into consideration.

Studies have shown that it is not necessary to add the corresponding deuterated compound for each component to be determined prior to sample preparation. Instead, the addition of three substances is sufficient for quantitative determination of the compounds shown (see Figures 3-2 and 3-3). The reason is that the response is almost the same for all substances on electron impact ionization (EI). Thus the concentration of phenanthrene and anthracene in the sample can be determined with the aid of phenanthrene-d_{10} and all other components either via the content of benzo[*e*]pyrene-d_{12} or via the content of perylene-d_{12}. Although the native compounds elute almost together with the deuterated compounds on gas chromatography (Figure 3-6) reliable quantification is possible via the different mass fragment ions. For example, the benzofluoranthenes, the benzopyrenes, and perylene are determined in the mass fragment ion range of *m/z* 251 to *m/z* 253; in contrast, the deuterated species benzo[*e*]pyrene-d_{12} and perylene-d_{12} are evaluated in the mass fragment ion range of *m/z* 263 to *m/z* 265.

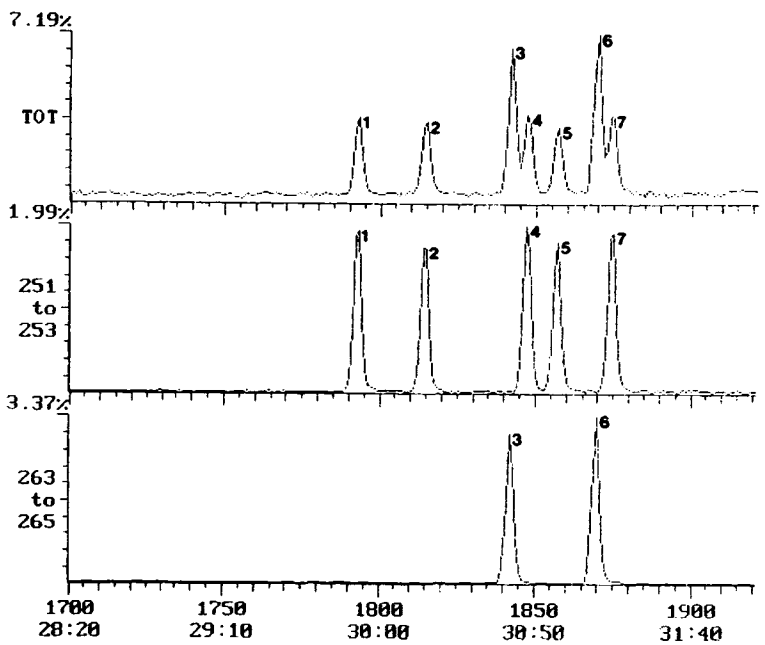

Fig. 3-6: Mass fragmentograms: TOT total ion current, range of ions 251–253, and range of ions 263–265. (1) benzo[*b*]fluoranthene, (2) benzo[*a*]fluoranthene, (3) benzo[*e*]pyrene-d_{12}, (4) benzo[*e*]pyrene, (5) benzo[*a*]pyrene, (6) perylene-d_{12}, (7) perylene.

The absolute values of the limit of detection for the individual polycyclic compounds listed in Figures 3-2 and 3-3 are around 50 pg on use of a MAT 112 sector field mass spectrometer in routine operation. The same order of magnitude is also obtained on determination of polycyclic compounds with an ITS 40 mass spectrometer. In order to obtain correct and reproducible results the intensities of individual mass fragment ions are evaluated in the case of the sector field system whereas it is advisable to consider the entire mass range of quantification in the case of the ITS 40 instrument (see Procedures).

A number of polycyclic compounds also cause problems on determination by GC/MS. Chrysene and triphenylene as well as the three benzofluoranthenes (*b,j,k*) cannot be separated on the DB-5 column used, so that only a sum total can be given; the same also applies to the substances dibenz[*ah*]anthracene and dibenz[*ac*]anthracene.

Although Fernandez and Bayona [3-29] have achieved baseline separation of the three benzofluoranthenes on a specially coated fused silica capillary, the stationary phase used is not thermally stable and is thus of little value for routine analysis.

3.2.2 Injection Systems

On-column injection has proved favorable for the analysis of PAH. Systems can also be used which in which the point of injection initially lies above the oven and is later moved into the oven by pushing down a kind of telescopic device. In spite of higher temperatures – these may lie between 90 and 110 °C – good separations of the individual components are achieved within short analysis times.

In order to avoid constant shortening of the analytical column, installation of a precolumn deactivated with 1,3-diphenyl-1,1,3,3-tetramethyldisilazane is recommended. Its length should be at least one meter, but can lie between 2.5 and 3 meters. Pre-columns and analytical columns can be joined almost dead-volume free by commercially available press-fit unions.

A PTV injector is also suitable for analysis of PAH. The glass wool frequently present in the glass liner should be removed.

3.2.3 Results

Numerous native sunflower seed oils, maize oils, safflower oils, and olive oils were investigated [3-20]. The number of samples is shown in Figure 3-7 above the corresponding bar. Also given are the highest and the lowest value and the median value.

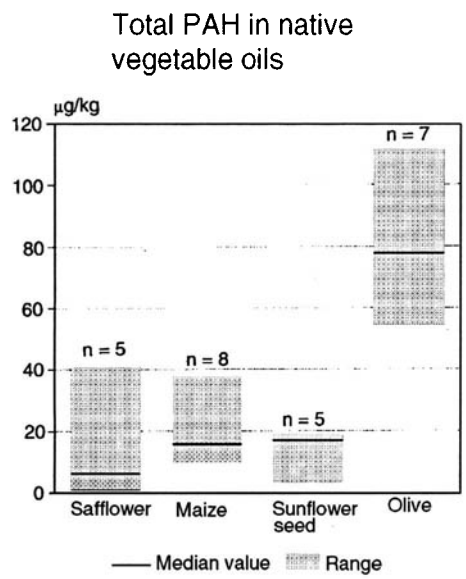

Fig. 3-7: Results for "total PAH".

Evaluation of the results obtained from vegetable oils and fats is difficult in the absence of any legal requirements. There is still neither a maximum permissible value for the sum of certain PAH nor a maximum permissible content of benzo[*a*]pyrene. Pragmatic limits have therefore been proposed for refined oils by Wendt. Thus the sum of selected PAH should not exceed 25 µg/kg and the content of "heavy" PAH should be below 5 µg/kg. These limits were also used here for assessment of the native oils.

Native sunflower seed oil and maize oil fulfilled the requirements of Wendt. However, higher PAH contents were found for safflower oil, wheat germ oil, and also olive oils. Remarkably, olive oils contain small amounts of "heavy" PAH relative to the total PAH content; for example, the contents of benzo[*a*]pyrene were only between 0.2–1.2 µg/kg. In contrast to the other oils

with a high PAH burden the "light" PAH make a disproportionately high contribution to the total PAH content of olive oils (Figure 3-8).

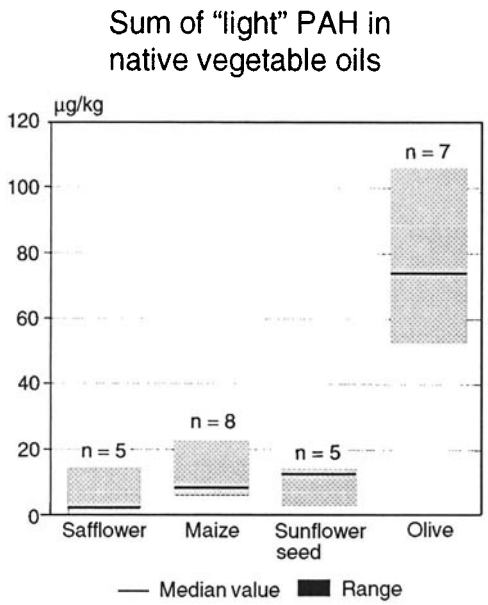

Fig. 3-8: Results "light" PAH.

Figure 3-9 shows some mass fragmentograms of an olive oil and a safflower oil. The ratios of "light" PAH to "heavy" PAH in these two oils differ considerably. The following explanation is forthcoming: In accord with the guidelines for native oils, the sample of safflower oil has been subjected to steaming which removes most of the "light" polycyclic compounds from the oil [3-8, 3-22].

In contrast, native olive oil may not be steamed owing to the International Olive Oil Agreement and thus has the typical high content of "light" PAH.

This example clearly shows how important it is not only to determine benzo[*a*]pyrene as key substance but also to undertake a PAH profile analysis, particularly since this involves no extra analytical work when using the analytical procedure presented here.

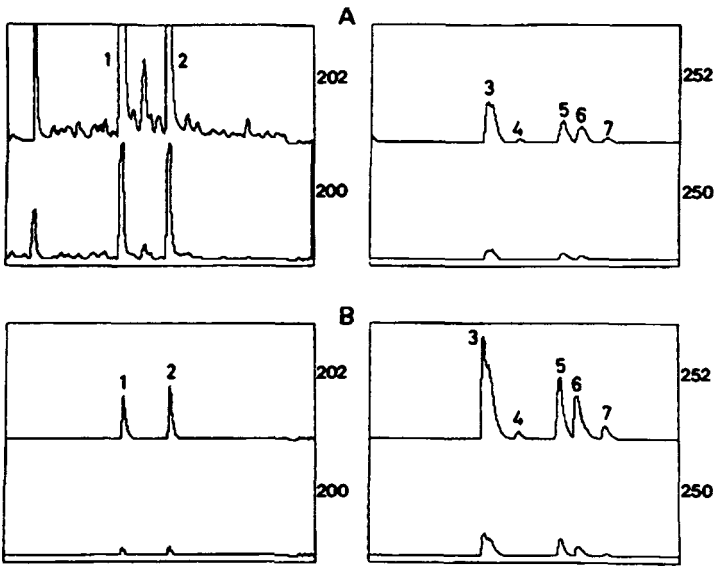

Fig. 3-9: Mass fragmentograms of (A) olive oil and (B) safflower oil. (1) Fluoranthene, (2) pyrene, (3) benzo[*b,j,k*]fluoranthene, (4) benzo[*a*]fluoranthene, (5) benzo[*b*]pyrene, (6) benzo[*a*]pyrene, (7) perylene.

Owing to the analytical results obtained in 1988 various native vegetable oils and some cod liver oil samples were again examined in 1992. The results are presented in Table 3-1. The sesame and sunflower seed oils and the walnut oil satisfied requirements, i.e. the sum of all PAH lies below 25 µg/kg and the sum of "heavy" PAH below 5 µg/kg.

The PAH contents of olive oils has fallen significantly since 1988; at levels of 26 and 37 µg/kg the pragmatic limits are only slightly exceeded. However, since in this case the "light" PAH – and hence the compounds that are less decisive for a toxicological assessment of the oil – make a large contribution to the total, the potential hazard arising from the consumption of olive oil can be regarded as low.

In two of the three wheat germ oils investigated the total amount of PAH is 28 and 32 µg/kg, respectively, and thus lies slightly above the limit. While this is due mainly to the content of "light" PAH in the case of wheat germ oil 2, a significantly higher content of "heavy" PAH is found in wheat germ oil 1. At 10 µg/kg for the sum of "heavy" PAH and 1.4 µg/kg for benzo[*a*]pyrene the highest values of the series are found for this oil.

Table 3-1: PAH in native vegetable oils and cod liver oil.

Sample	Cod liver oil 1	Cod liver oil 2	Cod liver oil 3	Wheat germ oil 1	Wheat germ oil 2	Wheat germ oil 3	Walnut oil 1
Phenanthrene	2.9	4.4	1.6	9.6	17.5	5.3	12.9
Anthracene	0.2	0.6	0.1	0.6	1.2	0.3	0.9
Fluoranthene	2.1	2.0	1.1	1.8	5.0	3.1	3.4
Pyrene	1.7	1.7	0.9	1.7	3.1	3.0	3.3
Benz[*a*]anthracene	0.5	0.5	0.3	0.4	0.7	0.6	0.5
Chrysene/Triphenylene	1.0	1.2	0.7	3.9	1.3	1.4	1.2
Sum of light PAH	8.4	10.4	4.7	18.0	28.8	13.7	22.2
Benzo[*b,j,k*]fluoranthenee	1.2	1.0	1.0	3.9	1.3	1.1	0.8
Benzo[*a*]fluoranthene	<0.1	0.2	0.1	0.3	0.1	0.1	<0.1
Benzo[*e*]pyrene	0.4	0.4	0.3	2.7	0.5	0.5	0.4
Benzo[*a*]pyrene	0.3	0.3	0.3	1.4	0.4	0.4	0.3
Perylene	0.1	0.1	0.1	0.4	0.1	0.1	<0.1
Indeno[1,2,3-*cd*]pyrene	0.4	0.3	0.3	0.6	0.4	0.3	0.2
Dibenz[*ah,ac*]anthracene	0.2	<0.1	0.1	0.3	0.1	0.1	<0.1
Benzo[*ghi*]perylene	0.6	0.5	0.4	0.7	0.5	0.5	0.3
Sum of heavy PAH	3.2	2.8	2.6	10.3	3.4	3.1	2.0
Sum of PAH	11.6	13.2	7.3	28.3	32.2	16.8	24.2

Sample	Sunfl. seed oil 1	Sunfl. seed oil 2	Sesame oil 1	Sesame oil 2	Olive oil 1	Olive oil 2
Phenanthrene	5.3	5.3	14.7	9.8	11.5	14.4
Anthracene	0.4	0.5	0.5	0.4	0.5	0.6
Fluoranthene	3.4	2.0	2.9	1.9	4.6	6.6
Pyrene	3.1	2.1	2.2	1.4	4.8	8.6
Benz[*a*]anthracene	0.6	0.2	0.4	0.1	0.4	0.5
Chrysene/Triphenylene	1.1	0.5	1.4	0.5	2.0	2.8
Sum of light PAH	13.9	10.6	22.1	14.1	23.8	33.5
Benzo[*b,j,k*]fluoranthene	0.9	0.4	0.8	0.3	0.8	1.2
Benzo[*a*]fluoranthene	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Benzo[*e*]pyrene	0.4	0.2	0.4	0.1	0.4	0.7
Benzo[*a*]pyrene	0.3	0.2	0.2	<0.1	0.3	0.3
Perylene	0.1	0.1	<0.1	<0.1	<0.1	<0.1
Indeno[1,2,3-*cd*]pyrene	0.3	0.1	0.3	0.1	0.3	0.4
Dibenz[*ah,ac*]anthracene	0.2	<0.1	0.1	<0.1	<0.1	0.2
Benzo[*ghi*]perylene	0.4	0.2	0.4	0.1	0.6	0.7
Sum of heavy PAH	2.6	1.2	2.2	0.6	2.4	3.6
Sum of PAH	16.5	11.8	24.3	14.7	26.2	37.1

Low PAH contents are found for the three cod liver oil samples. The sum of the "heavy" PAH lies in the range found for vegetable oils, while the contents of "light" PAH are significantly lower. Thus it may be concluded that the cod liver oil samples have been treated.

3.3 PAH in Vegetables

Vegetables are frequently contaminated with polycyclic compounds through exposure to the air and the soil [3-30 to 3-43]. According to studies by Fritz [3-30 to 3-32] and Dennis et al. [3-2], humans ingest a disproportionately large amount of benzo[*a*]pyrene through consumption of vegetables, particularly when they are cultivated in the immediate vicinity of a source of emission.

Linne and Martens cultivated mushrooms and carrots on soils loaded to different extents with polycyclic compounds and analyzed them after harvesting for PAH. It was found that the mushrooms did not contain detectable amounts of aromatic compounds in their fruiting bodies, even when grown on soils containing very high amounts of polycyclics (e.g. benzo[*a*]pyrene > 1000 ppb). In the experiments on carrots, on the other hand, the PAH contents of both the roots and the tops was found to depend upon the content of polycyclic compounds in the soil; thus cultivation of carrots in soil containing composted municipal waste, which has a PAH content 20- to 40-fold higher than uncontaminated soil, led to an increase in the polycyclic compound content by the same factor.

Larsen [3-37] determined the content of selected polycyclic compounds in lettuces grown close to a road carrying heavy traffic. He was able to show that the level of traffic affected the PAH content of the plants up to a distance of 25 m from the road.

3.3.1 Analysis

In contrast to oils and fats, vegetables do not represent a homogeneous matrix (Figure 3-10). Moreover, the polycyclic compounds are mainly bound to particles, thus necessitating hydrolysis of the sample material.

The solution obtained by hydrolysis is extracted with cyclohexane and the extract is concentrated. The extensive clean-up with dimethylformamide/water necessary for analysis of vegetable oils and fats is not required for the determination of PAH in vegetables. Maier and Aubort report similar findings [3-42].

However, some clean-up steps are necessary prior to GC/MS analysis. Depending upon the vegetable, this is accomplished by gel permeation chromatography (GPC) on Bio Beads S-X3 or high pressure liquid chromatography on silica gel.

The faster and more straightforward clean-up by GPC is adequate for extracts of iceberg lettuce, lettuce, kohlrabi, white cabbage, spinach, and cauliflower.

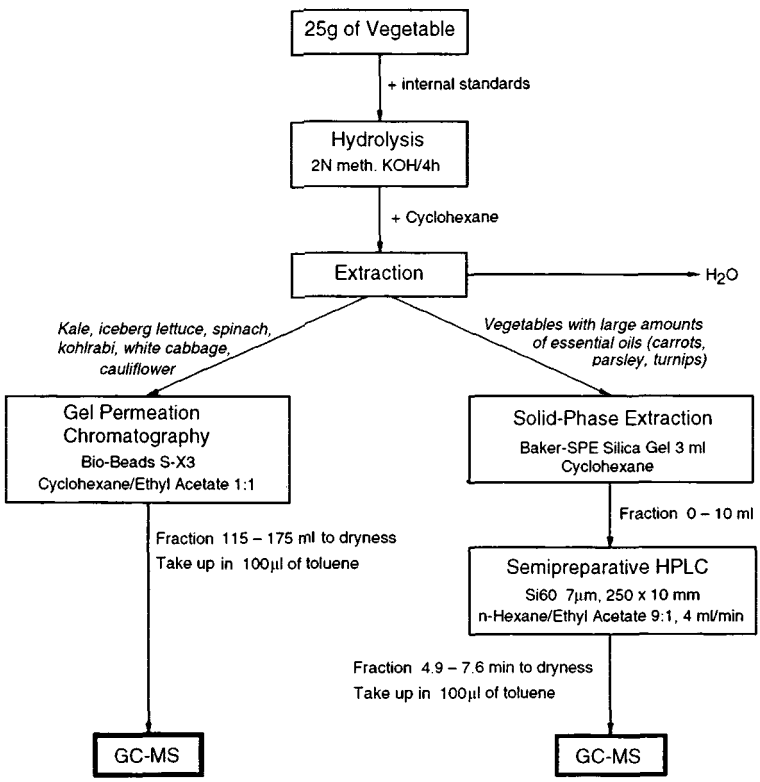

Fig. 3-10: Sample treatment scheme for vegetables.

If the sample material contains large amounts of carotenoids, chlorophylls, or essential oils, as do carrots and parsley, then clean-up of the sample extracts is preferably performed by HPLC. Before doing so, however, the cyclohexane extracts should be subjected to column liquid chromatography on silica gel. This can also be done with disposable 3-ml silica gel columns, thus greatly reducing the analytical effort required.

Figure 3-11 shows the chromatograms of a carrot sample after various stages of clean-up. It is clearly seen that HPLC on silica gel (chromatogram A) offers significant advantages for removal of essential oils compared to gel permeation chromatography on Bio Beads (chromatogram B).

In the case of kale and also apple samples with their wax-like cuticular layers, clean-up of the sample should definitely be performed by GPC on Bio Beads S-X3.

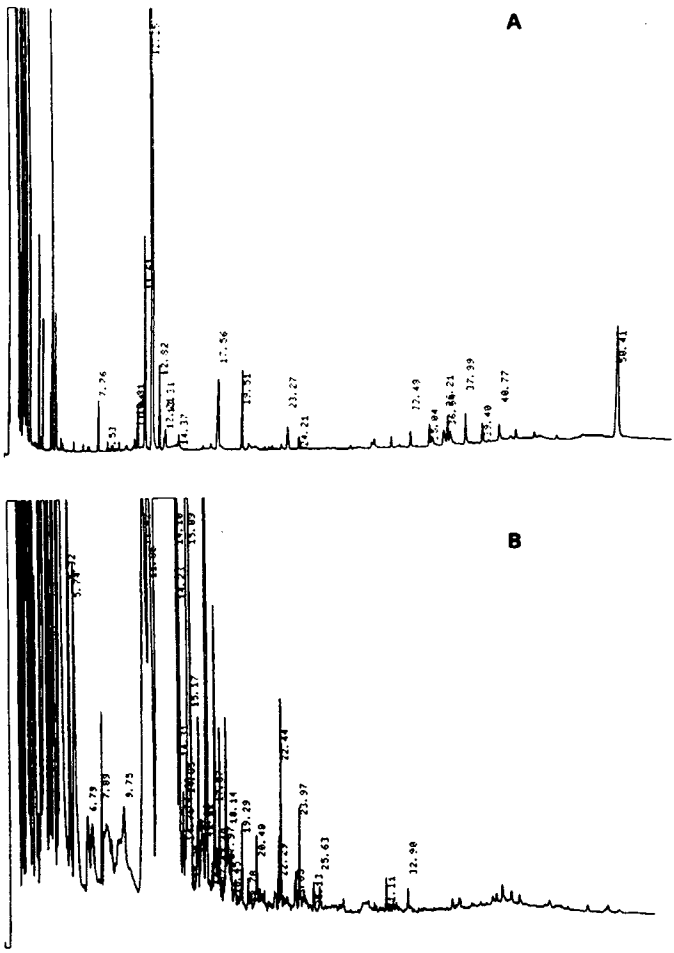

Fig. 3-11: Carrot sample after (A) HPLC clean-up, (B) GPC clean-up.

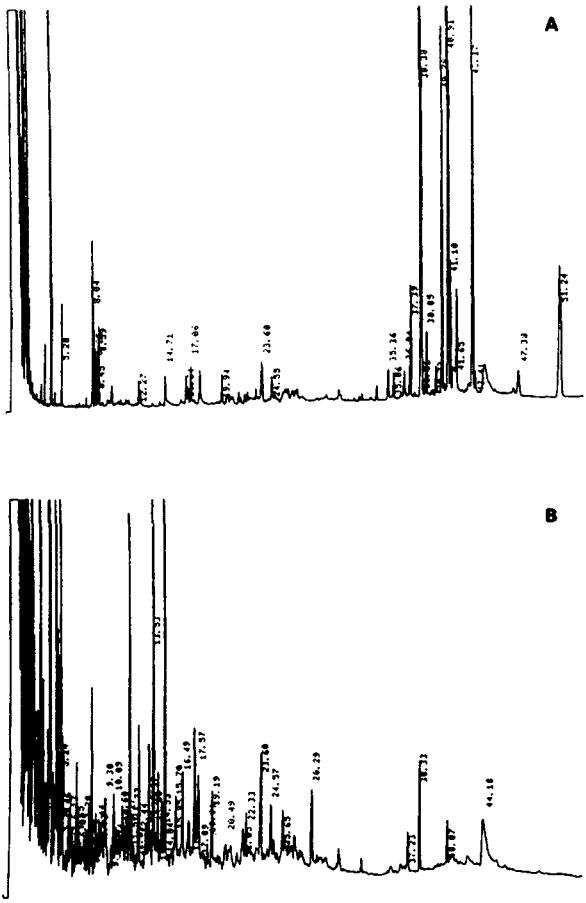

Fig. 3-12: Kale sample after (A) HPLC clean-up, (B) GPC clean-up.

In Figure 3-12 the front region of the FID chromatogram B of a kale sample contains a number of signals; however, no effects on the PAH measured with the aid of the MIS technique could be observed. Chromatogram A recorded for the same kale sample shows fewer signals in the front region after clean-up by HPLC, yet several interfering peaks do appear in the elution region of benzofluoranthenes, benzopyrenes, and perylene. Above all, this greatly impairs the determination of perylene, thus often precluding correct determination of the quantitative result. Therefore, extracts of kale samples should always be subjected to clean-up by gel permeation chromatography on Bio Beads S-X3.

3.3.2 Results

The methods presents were used to analyze various species of vegetables for PAH [3-43]. A selection of the results obtained in these studies is presented in Table 3-2.

Only insignificant amounts of polycyclic compounds were found in the leaves and nerve of the leaves of kohlrabi; no PAH could be detected in the bulbous stem. It therefore follows that there is no direct transfer of polycyclic via the roots to the stem. Likewise in the case of cauliflower, iceberg lettuce (inside leaves), turnips, and white cabbage no evidence was found for uptake of polycyclic compounds by the parts eaten as food via the roots.

As in the case of kohlrabi, only the outer leaves of white cabbage were slightly contaminated. In contrast, parsley and kale were contaminated to a much greater extent. Carrots and parsnips were found to contain PAH which had clearly entered the outer regions of the plant via the soil. Peeling of the surface of the roots removed the polycyclic compounds. The content of PAH in kale, lettuce, and spinach is hardly reduced by washing, meaning that air and soil contamination has a direct effect on these vegetables.

Figure 3-13 compares the PAH profiles of a "Carrot, leaves" sample (exposed to the air) and a "Carrot, roots" sample.

While the profiles of the "heavy" PAH components were comparable for leaves and roots, significantly higher levels of the "light" PAH components fluoranthene and pyrene were found in the tops than in the roots.

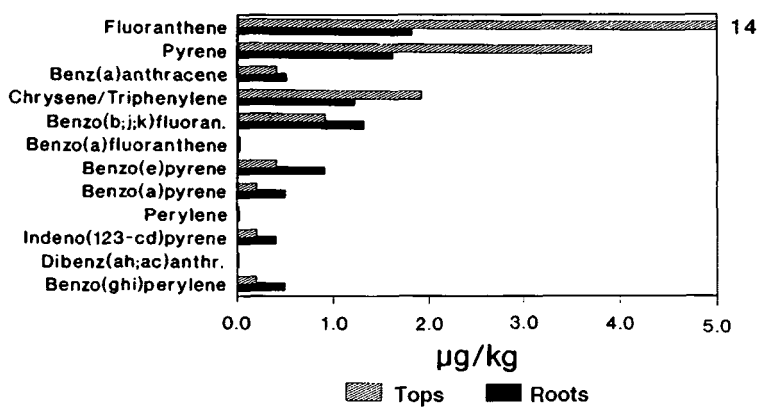

Fig. 3-13. PAH in carrots: tops and unpeeled roots.

Table 3-2: PAH in vegetables.

	Parsely			
	Leaves		Roots	
	unwashed	washed	unwashed	peeled
Phenanthrene	29.9	21.3	4.0	0.8
Anthracene	1.2	0.4	0.3	<0.1
Fluoranthene	7.7	3.3	4.0	0.3
Pyrene	5.1	1.7	3.6	0.1
Benz[*a*]anthracene	2.1	0.3	1.6	<0.1
Chrysene/Triphenylene	3.6	0.9	2.9	<0.1
Sum of light PAH	49.6	27.9	16.4	1.2
Benzo[*b,j,k*]fluoranthene	5.6	1.1	4.0	<0.1
Benzo[*a*]fluoranthene	0.5	0.1	0.4	<0.1
Benzo[*e*]pyrene	3.0	0.5	2.6	<0.1
Benzo[*a*]pyrene	2.0	0.2	1.4	<0.1
Perylene	0.6	0.1	0.4	<0.1
Indeno[1,2,3-*cd*]pyrene	1.7	0.2	1.4	<0.1
Dibenz[*ah,ac*]anthracene	0.3	<0.1	0.2	<0.1
Benzo[*ghi*]perylene	2.1	0.3	1.8	<0.1
Sum of heavy PAH	15.8	2.5	12.2	
Sum of PAH	65.4	30.4	28.6	1.2

	Carrots			
	Tops	Upper half	Lower half	Root hairs
Phenanthrene	41.3	<0.1	<0.1	3.3
Anthracene	0.7	<0.1	<0.1	0.1
Fluoranthene	13.7	<0.1	<0.1	1.8
Pyrene	3.7	<0.1	<0.1	1.6
Benz[*a*]anthracene	0.4	<0.1	<0.1	0.5
Chrysene/Triphenylene	1.9	<0.1	<0.1	1.2
Sum of light PAH	61.7			8.5
Benzo[*b,j,k*]fluoranthene	0.9	<0.1	<0.1	1.3
Benzo[*a*]fluoranthene	<0.1	<0.1	<0.1	<0.1
Benzo[*e*]pyrene	0.4	<0.1	<0.1	0.9
Benzo[*a*]pyrene	0.2	<0.1	<0.1	0.5
Perylene	<0.1	<0.1	<0.1	<0.1
Indeno[1,2,3-*cd*]pyrene	0.2	<0.1	<0.1	0.4
Dibenz[*ah,ac*]anthracene	<0.1	<0.1	<0.1	<0.1
Benzo[*ghi*]perylene	0.2	<0.1	<0.1	0.5
Sum of heavy PAH	1.9			3.6
Sum of PAH	63.6			12.1

3.4 PAH in Fish Products and Mollusks

Foods products smoked in older plant are often contaminated with PAH [3-44 to 3-51]. Problems occur above all if the curing process is uncontrolled, as it is in many traditional smoking chambers where the smoke is produced directly below the food products. In more recent smoking units various parameters, such as the smoke concentration, temperature, etc., can be controlled in such a way as to ensure that the PAH burden of the products can be reduced to an acceptable level. However, since such units are not yet in universal use, analysis of smoked products for PAH is justified.

3.4.1 Analysis

As in the case of vegetables, the sample material has to be hydrolyzed (Figure 3-14). The cyclohexane extract is subjected to clean-up by chromatography on silica gel and Bio Beads S-X3 and the polycyclic compounds analyzed by GC/MS.

In addition to non-oily fish, mussels, and oysters the analytical procedure can also be applied to meat products. However, samples with a high fat content require highly time consuming extraction with dimethylformamide/water.

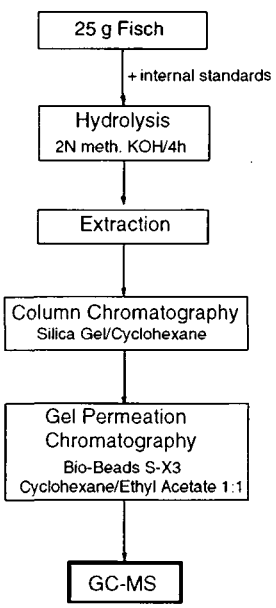

Fig. 3-14. Clean-up scheme for meat products and mollusks.

3.4.2 Results

Studies on oysters and mussels revealed high contents of in some cases (Table 3-3). In one sample of "oysters in oil" benzo[*a*]pyrene levels of 12 µg/kg were found for the mussel flesh and even 76 µg/kg for the oil.

Figure 3-15 compares some mass fragmentograms for a sample of "smoked eel" with those of a sample of "fresh oysters". Significant differences are seen both for the components phenanthrene and anthracene and for benzopyrenes and perylene. High concentrations of the "light" PAH components phenanthrene and anthracene were detected in smoked eel; in addition, benzo[*a*]fluoranthene and perylene as well as benzo[*e*]pyrene and benzo[*a*]pyrene were found in similar concentrations.

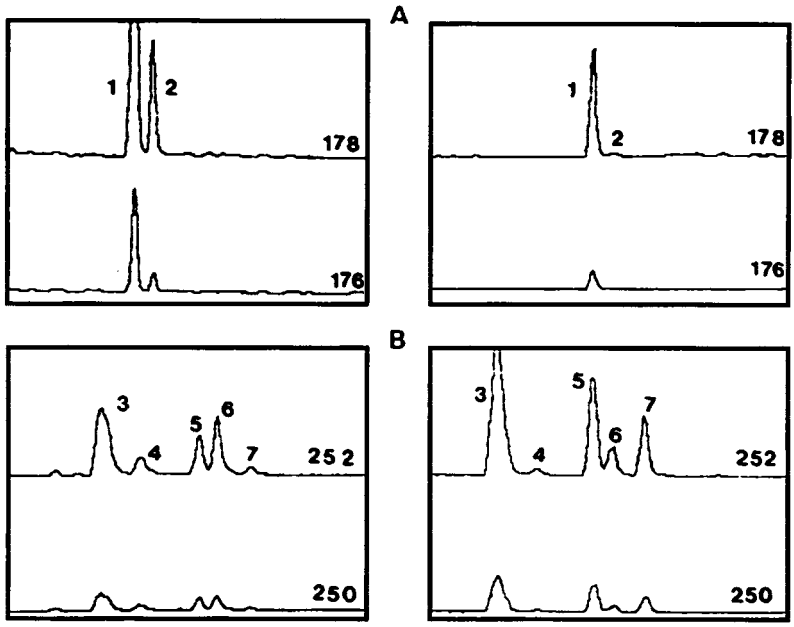

Fig. 3-15: Mass fragmentograms of (A) "smoked eel" and (B) "fresh oysters". (1) phenanthrene, (2) anthracene, (3) benzo[*b,j,k*]fluoranthene, (4) benzo[*a*]fluoranthene, (5) benzo[*e*]pyrene, (6) benzo[*a*]pyrene, (7) perylene.

Tab. 3-3: PAH in oysters and mussels.

	Fresh oysters		
Origin	Sylt	France	unknown
Phenanthrene	2.1	4.2	3.4
Anthracene	<0.1	0.6	0.3
Fluoranthene	5.1	17.5	5.6
Pyrene	3.1	12.4	3.2
Benz[*a*]anthracene	1.1	3.0	0.8
Chrysene/Triphenylene	7.2	8.8	3.2
Sum of light PAH	18.6	46.5	16.5
Benzo[*b,j,k*]fluoranthene	12.2	10.2	4.5
Benzo[*a*]fluoranthene	<0.1	0.2	<0.1
Benzo[*e*]pyrene	6.3	5.5	2.4
Benzo[*a*]pyrene	0.6	1.0	0.4
Perylene	2.7	0.5	0.2
Indeno[1,2,3-*cd*]pyrene	0.6	0.5	0.3
Dibenz[*ah,ac*]anthracene	0.2	0.2	0.1
Benzo[*ghi*]perylene	0.8	0.8	0.4
Sum of heavy PAH	23.4	18.9	8.3
Sum of PAH	42.0	65.4	24.8

	Mussels			
Origin	Denmark	Korea	Germany 1	Germany 2
Phenanthrene	3.5	19.6	1.9	12.9
Anthracene	<0.1	1.9	0.1	1.2
Fluoranthene	10.5	13.5	4.5	18.7
Pyrene	7.9	5.5	3.2	11.2
Benz[*a*]anthracene	5.7	0.8	1.9	3.6
Chrysene/Triphenylene	13.8	3.8	5.3	7.1
Sum of light PAH	41.4	45.1	16.9	54.7
Benzo[*b,j,k*]fluoranthene	9.8	1.2	4.8	9.0
Benzo[*a*]fluoranthene	0.4	<0.1	0.2	0.4
Benzo[*e*]pyrene	5.3	0.7	2.6	6.6
Benzo[*a*]pyrene	1.7	0.3	0.8	1.5
Perylene	3.1	0.1	0.5	n.a.*
Indeno[1,2,3-cd]pyrene	1.2	0.2	1.0	1.2
Dibenz[*ah,ac*]anthracene	0.5	<0.1	0.3	0.3
Benzo[*ghi*]perylene	1.5	0.3	1.2	2.1
Sum of heavy PAH	23.5	2.8	11.4	21.1
Sum of PAH	64.9	47.9	28.3	75.8

* not available

Only small amounts of phenanthrene and anthracene could be found in the unsmoked oyster sample; moreover, significantly more perylene than benzo[*a*]fluoranthene and benzo[*e*]pyrene than benzo[*a*]pyrene were determined. Thus the determination of PAH profiles permits a distinction to be made between PAH contamination arising from maritime sources and from smoke.

3.5 PAH in Coffee

With an average annual per capita consumption of approx. 180 liters, coffee is the most important beverage in Germany. In the interests of public health this beverage warrants great interest with regard to its potential content of pollutants [3-53 to 3-58].

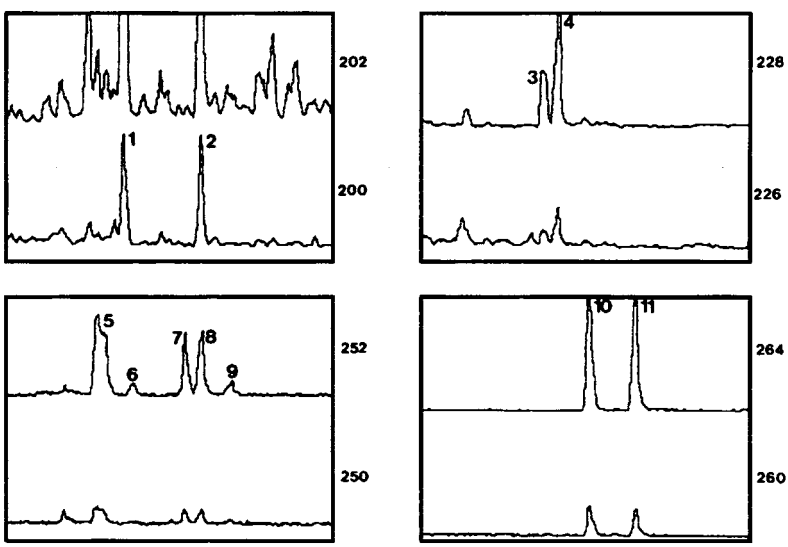

Fig. 3-16: Mass fragmentograms of a roasted coffee extract. (1) fluoranthene, (2) pyrene, (3) benzo[*a*]anthracene, (4) chrysene/triphenylene, (5) benzo[*b,j,k*]fluoranthene, (6) benzo[*a*]fluoranthene, (7) benzo[*e*]pyrene, (8) benzo[*a*]pyrene, (9) perylene, (10) benzo[*e*]pyrene-d_{12}, (11) perylene-d_{12}.

Table 3-4: PAH in coffee.

	Raw coffee		Roasted coffee			
Sample	1	2	1	2	3	4
Fluoranthene	3.4	8.0	5.3	21.1	10.7	4.3
Pyrene	2.8	8.1	5.9	26.8	13.2	5.2
Benz[a]anthracene	0.1	1.4	0.3	1.4	0.6	0.2
Chrysene/Triphenylene	0.7	3.0	1.6	2.8	1.8	0.9
Sum of light PAH	7.0	20.5	13.1	52.1	26.3	10.6
Benzo[b,j,k]fluoranthene	0.3	1.9	0.3	1.6	0.9	0.3
Benzo[a]fluoranthene	<0.1	0.2	<0.1	0.2	0.1	<0.1
Benzo[e]pyrene	<0.1	0.8	0.2	0.8	0.4	0.2
Benzo[a]pyrene	0.1	0.9	0.1	1.0	0.4	0.2
Perylene	<0.1	0.3	<0.1	0.2	0.1	<0.1
Indeno[1,2,3-cd]pyrene	<0.1	0.5	<0.1	0.5	0.2	0.1
Dibenz[ah,ac]anthracene	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
Benzo[ghi]perylene	<0.1	0.6	0.1	0.8	0.3	0.1
Sum of heavy PAH	0.4	5.3	0.7	5.1	2.4	0.9
Sum of PAH	7.4	25.8	13.8	57.2	28.7	11.5

	Espresso coffees				Soluble coffees	
Sample	1	2	3	4	1	2
Fluoranthene	14.9	7.5	5.8	6.3	0.3	0.6
Pyrene	16.7	7.9	6.7	6.4	0.2	0.8
Benz[*a*]anthracene	1.7	0.5	0.5	0.7	<0.1	0.1
Chrysene/Triphenylene	4.3	2.0	2.5	5.5	<0.1	0.2
Sum of light PAH	37.6	17.9	15.5	18.9	0.5	1.7
Benzo[*b,j,k*]fluoranthene	1.8	0.6	0.5	0.9	<0.1	<0.1
Benzo[*a*]fluoranthene	0.1	<0.1	<0.1	0.1	<0.1	<0.1
Benzo[*e*]pyrene	0.7	0.2	0.2	0.7	<0.1	<0.1
Benzo[*a*]pyrene	0.8	0.3	0.2	0.2	<0.1	<0.1
Perylene	0.2	<0.1	<0.1	0.2	<0.1	<0.1
Indeno[1,2,3-*cd*]pyrene	0.4	0.2	0.1	0.1	<0.1	<0.1
Dibenz[*ah,ac*]anthracene	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Benzo[*ghi*]perylene	0.8	0.3	0.2	0.3	<0.1	<0.1
Sum of heavy PAH	4.8	1.6	1.2	2.5		
Sum of PAH	42.4	19.5	16.7	21.4	0.5	1.7

Coffee is often roasted by direct heating. The roasted coffee beans therefore come into direct contact with combustion residues, leading to a probable contamination with PAH.

Hence numerous samples of raw and roasted coffee from various sources were analyzed. Samples were taken at time intervals in order to account for any time-based influences. In addition, a number of espresso coffees, which are roasted particularly strongly, and several soluble coffees were also analyzed.

3.5.1 Analysis

The polycyclic compounds were determined, after prior hydrolysis of the sample material and clean-up of the extracts on silica gel and Bio Beads S-X3, by GC/MS using methods analogous to those employed for analysis of fish samples (Procedure "Coffee").

Some mass fragmentograms of a roasted coffee sample are shown in Figure 3-16. It is seen that individual compounds can be selectively determined after appropriate clean-up.

3.5.2 Results

The results [3-58] compared in part in Table 3-4 show that the PAH contents of the various roasted coffees almost all lie within the same order of magnitude. On comparing the PAH contents of the unroasted coffee with those of the roasted product it is seen that it is not so much the roasting process but the PAH content of the raw material which determines the content in the roasted product.

The PAH contents of espresso coffees–although very strongly roasted–lie in the same range as the other roasted coffees considered here. Expectedly, the contents of the individual PAH in soluble coffee are low and in many cases lie below the limit of detection of 0.1 μg/kg.

3.6 PAH in Tea

Remarkably high contents of benzo[*a*]pyrene (BAP) in tea and especially in maté tea were reported by Ruschenberg and Jahr [3-54].

Black tea (17)	0.5 – 25.7	µg/kg BAP
Smoked tea (10)	5.6 – 77.2	µg/kg BAP
Maté leaves, green (19)	56 – 532	µg/kg BAP
Maté leaves, roasted (12)	146 – 714	µg/kg BAP

(number of samples given in parentheses)

3.6.1 Analysis

In order to obtain useful chromatograms from tea samples in spite of the numerous extractable substances present, semipreparative HPLC should be used as clean-up method after hydrolysis of the sample A silica gel Si 60 (7 µm) column serves as stationary phase; *n*-hexane/ethyl acetate (9:1) proved to be a suitable mobile phase. If sufficiently small fractions are cut under these conditions, then the benzo[*a*]pyrene can be determine without interference by gas chromatography with an FID after concentration of the sample. Figure 3-17 shows how the clean-up effect can be enhanced by the isolation of smaller and smaller fractions. However, fractionation in small time windows requires good reproducibility of chromatographic separation.

3.6.2 Results

The results compiled in Tables 3-4 and 3-5 demonstrate the significantly higher contamination of tea with benzo[*a*]pyrene as compared to coffee. However, estimates of the possible health hazard depend not upon the content of polycyclic compounds in the product but rather on the amount of polycyclic compounds transferred the beverage. These transfers have been adequately studied by several groups (Ruschenberg and Jahr [3-54], Hischenhuber and Stijve [3-56], Kruijf, Schouten, and van der Stegen [3-57]). Thus only 1–2% of the PAH are transferred to the beverage.

Compared to other foods, humans ingest only insignificant amounts of polycyclic aromatic hydrocarbons by drinking coffee and tea.

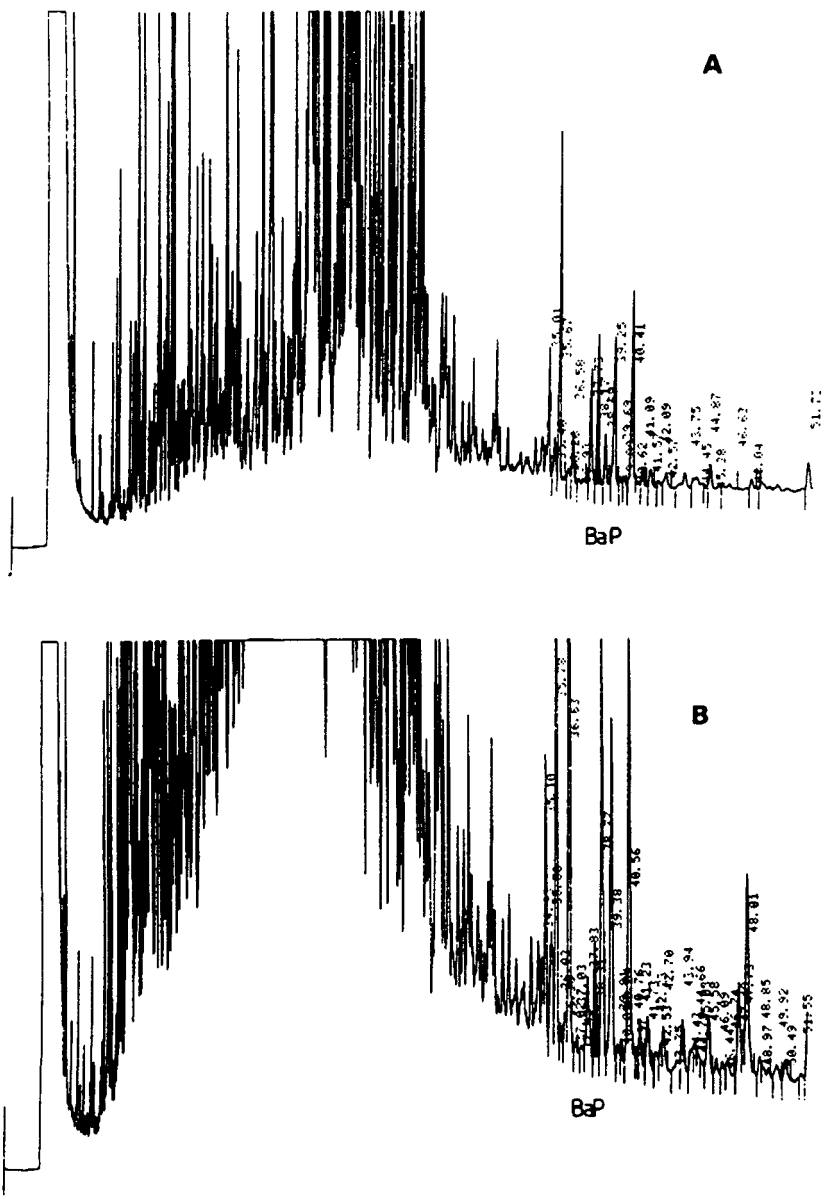

Fig. 3-17: Capillary gas chromatograms of a tea sample after differing fractionation. Fractionation time (A) 4 min, (B) 5 min.

Tab. 3-5: Benzo[*a*]pyrene in tea.

Tea	Benzo[*a*]pyrene [µg/kg]
Russian blend 1	12. 4
Russian blend 2	5.0
Russian blend 3	3.2
Chinese tea 1	20.1
Chinese tea 2	17.6
Chinese tea 3	15.2
Chinese tea 4	10.8
Chinese tea 5	3.8
Russian smoked tea	14.5
Chinese smoked tea	4.8

3.7 Analytical Procedures for Determination of PAH in Foods

3.7.1 Chemicals

- Standard substances (0.5 ng/µl in toluene): phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, triphenylene, benzofluoranthenes *a,b,j,* and *k*, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[1,2,3-*cd*]pyrene, dibenz[*ah,ac*]anthracene, benzo[*ghi*]perylene
- internal standard solution (0.5 ng/µl in toluene) of phenanthrene-d_{10}, benzo[*e*]pyrene-d_{12}, perylene-d_{12}
- cyclohexane, nanograde
- toluene, nanograde
- dimethylformamide p.a.
- *n*-hexane, nanograde
- Bio Beads S-X3
- water, bidistilled
- Na_2SO_4 p.a.: calcined (12 h, 550 °C), keep in a desiccator, seal ground glass joints with parafilm

- 2N methanolic potassium hydroxide: 112 g KOH p.a. in 100 ml bidistilled water, make up to 1000 ml with methanol
- methanol/bidistilled water (9:1; *v/v*)
- cyclohexane/ethyl acetate (1/1; *v/v*) as GPC eluent
- methanol/bidistilled water (1/1; *v/v*), 2% in sodium sulfate: dissolve 20 g of sodium sulfate in 500 ml of bidistilled water, mix with 500 ml of methanol
- dimethylformamide/bidistilled water (9/1; *v/v*): place 225 ml of DMF in a 250-ml mixing cylinder, add 25 ml of bidistilled water; shake vigorously until thorough mixing has taken place (any schlieren formed must disperse)
- 1% sodium sulfate solution: place 100 ml of bidistilled water in a 100 ml shaking cylinder and add 1 g of Na_2SO_4 that has been calcined at 550 °C; shake the cylinder until the sodium sulfate has dissolved (the above sequence should be observed since the calcined sodium sulfate otherwise forms lumps resisting dissolution)
- silica gel (SiOH) cartridges (3 ml)
- silica gel 60 for column chromatography, particle size 0.063–0.2 μm, 70–230 mesh ASTM. Conditioning: calcine for 12 h at 550 °C; pre-cool for several hours at 125 °C in a drying cabinet; cool in a desiccator; place weekly requirements in an iodine-number flask, adjust to 15 % wt.-% with bidistilled water, shake vigorously for 30 min in a shaker; then allow to stand for 24 h in a desiccator, seal ground glass joints with parafilm.

3.7.2 Glass Apparatus and Aids

Brown glass should be used whenever possible; wash all glass apparatus with cyclohexane prior to use.

For Hydrolysis

- electric hot plate with sand bath
- reflux condenser
- 250-ml round bottom flask
- glass beads
- pumice stone
- 1-ml enzyme test pipette

For PAH Extraction

- Büchner funnel, plate diameter 73 mm, content 220 ml
- circular filter diameter 70 mm
- vacuum pump with controller
- Witt pot
- 500-ml separatory funnel
- 100-ml separatory funnel
- 250-ml glass beakers
- 100-ml measuring cylinder
- 500-ml round bottom flask

For Clean-up on Silica Gel

- 250-ml iodine number flasks, parafilm
- 200 × 10 mm chromatography tube with a 250-ml reservoir and PTFE stopcock
- glass wool, silanized
- 10-ml or 20-ml glass beakers
- Pasteur pipettes, long and short
- 10-ml measuring cylinder
- 250-ml round bottom flask

For Clean-up on GPC Columns

- 10-ml graduated (0.5 ml) test tubes with ground stoppers
- 500-ml round bottom flask
- long Pasteur pipettes
- Glass vials, 4 ml, brown glass with screw caps and PTFE septa
- Reacti-Therm block with N_2 flow (washing bottles filled with H_2SO_4 and with toluene)

3.7.3 Procedure P1: Fish Products and Mollusks

P1-1 Hydrolysis

- 25 g homogenized sample in a 250-ml Soxhlet flask is treated with 100 ml of 2N methanolic potassium hydroxide
- add 4 to 6 glass beads and 4 to 6 small pumice stones

- add 1 ml of internal standard solution (0.1 ng/µl)
- hydrolyze for four hours under reflux

P1-2 Extraction

- transfer the warm solution with 100 ml of methanol/bidistilled water (9/1;*v/v*) to a 500-ml brown glass separatory funnel; the reflux condenser is washed out with approx. 30 ml and the Soxhlet flask with the remaining 70 ml
- extract the united aqueous phases by shaking (2 min each time) with 2 × 100 ml of cyclohexane
- add a few ml of ethanol if necessary to improve phase separation
- extract the united cyclohexane extracts by shaking with 100 ml of methanol/bidistilled water (1/1;*v/v*)
- extract the cyclohexane phase with 100 of bidistilled water
- transfer the cyclohexane phase to a 500-ml round bottom flask with 5 g of calcined sodium sulfate
- allow to stand overnight in a refrigerator, or at for 2 h, until the solution is clear

P1-3 Clean-up on Silica Gel

Preparation of the Sample Extract

- carefully transfer the cyclohexane extract dried over sodium sulfate to a second 500-ml round bottom flask
- wash out the first round bottom flask with approx. 3 × 20 ml of cyclohexane
- evaporate united volumes of cyclohexane down to approx. 1 ml (*not to dryness*) in a rotary evaporator at 40 to 50 °C

Preparation of the Silica Gel Column

- close the bottom end of the column with a small glass wool plug
- weigh 5 g of conditioned silica gel into a glass beaker
- slurry with cyclohexane (*brief sonication*)
- transfer to column
- tap column to ensure uniform packing density (*essential to watch for air bubbles*)

- allow cyclohexane to drain to just above the surface of the silica gel
- again fill column with cyclohexane up to neck of reservoir, *making sure that the silica gel is not disturbed*
- allow cyclohexane to drain to just above the surface of the silica gel
- again fill column with cyclohexane up to neck of reservoir
- cover silica gel with 0.5 g of calcined sodium sulfate prior to draining
- before using the column drain off cyclohexane until the level reaches the sodium sulfate layer

Execution

- transfer concentrated extract to the prepared column with a Pasteur pipette
- place 10-ml measuring cylinder below the column exit
- drain off liquid until level reaches the sodium sulfate layer
- repeat rinsing twice
- immediately the 10-ml mark of the measuring cylinder has been reached close the column, *discard 10-ml volume*
- place a 250-ml round bottom flask under the exit of the column
- elute the PAH from the column with 75 ml of cyclohexane

There is no need to check the drop rate during conditioning or elution because only a low drop rate is attained with a 5 g silica gel packing. *Overnight elution is possible.*

P1-4 Clean-up on a GPC Column

- evaporate eluate from silica gel column down to approx. 1–2 ml (*not to dryness*) in a rotary evaporator at 40–45 °C
- transfer the residue with a Pasteur pipette to a graduated 10 ml test tube with ground glass stopper
- rinse out the round bottom flask three times with cyclohexane until a total volume of 5 ml of cyclohexane sample extract is present in the test tube
- rinse round bottom flask with approx. 5 ml of ethyl acetate and likewise transfer to the test tube
- make up to the 10 ml mark with ethyl acetate

GPC Conditions

Column material:	Bio Beads S-X3
Column parameters:	filling height: 39 cm; column ID: 2.5 cm
Sample loop:	5 ml
Eluent:	cyclohexane/ethyl acetate (1/1; *v/v*)
Flow rate:	5 ml/min
Dump:	30 min
Collect:	40 min

Solutions for the GC/MS Measurements

- evaporate GPC eluate to approx. 0.5–1 ml (*not to dryness*) in a rotary evaporator at 40–45 °C
- transfer the extract with a long Pasteur pipette into a 4-ml vial which has previously been rinsed with *n*-hexane or cyclohexane
- rinse out round bottom flask with 3 × 1 ml of cyclohexane and evaporate the united sample solutions *just to dryness* in as weak stream of N_2 at 50 °C
- take up residue in 0.1 ml of toluene (enzyme test pipette), seal vial, shake, and sonicate briefly

P1-5 GC/MS Measurement

Instrument:	**MAT 112**
Column:	30 m DB-5, 0.25 mm i.d., 0.25 µm film
Temp. program:	110 °C at 5 °/min to 280 °C hold for 20 min
Carrier gas:	helium, 0.7 bar
Injection:	1 µl, cold on-column
Transfer line:	260 °C
Source temp.	250 °C
Ionization:	EI, 0.1 A
Scanning condition:	MID mode
Phenanthrene, anthracene:	*m/z* 178; *m/z* 176
Phenanthrene-d_{10}:	*m/z* 188; *m/z* 184
Fluoranthene, pyrene:	*m/z* 202; *m/z* 200
Benz[*a*]anthracene, chrysene/triphenylene:	*m/z* 228; *m/z* 226
Benzofluoranthenes *b,j,k,a*:	*m/z* 252; *m/z* 250
Benzo[*e*]pyrene, benzo[*a*]pyrene, perylene:	*m/z* 252; *m/z* 250
Benzo[*e*]pyrene-d_{12}, perylene-d_{12}:	*m/z* 264; *m/z* 260
Indeno[1,2,3-*cd*]pyrene:	*m/z* 276; *m/z* 277

Dibenz[*ah,ac*]anthracene:	*m/z* 278; *m/z* 279
Benzo[*ghi*]perylene:	*m/z* 276; *m/z* 277
Instrument:	**ITS 40**
Column:	30 m DB-5, 0.25 mm i.d., 0.25 μm film
Temp. program:	70 °C, hold for 1 min, 7 °/min to 280 °C, hold for 20 min
Carrier gas:	helium, 0.7 bar
Injection:	1 μl, split 40 s closed
Transfer line:	260 °C
Source temp.	250 °C
Ionization:	EI
Phenanthrene, anthracene:	177–179; 175–177
Phenanthrene-d_{10}:	187–189; 183–185
Fluoranthene, pyrene:	201–203; 199–201
Benz[a]anthracene, chrysene/triphenylene:	227–229; 225–227
Benzofluoranthenes *b,j,k,a*:	251–253; 259–261
Benzo[*e*]pyrene, benzo[*a*]pyrene, perylene:	251–253; 249–251
Benzo[*e*]pyrene-d_{12}, perylene-d_{12}:	263–265; 259–261
Indeno[1,2,3-*cd*]pyrene:	275–277
Dibenz[*ah,ac*]anthracene:	277–279
Benzo[*ghi*]perylene:	275–277

3.7.4 Procedure P2: Vegetables

P2-1 Hydrolysis

See procedure P1-1

P2-2 Extraction

See Procedure P1-2

P2-3A Clean-up on GPCV Column

This clean-up step is essential for plant material with wax-containing cuticular plants such as kale; use of GPC is also recommended as a means of saving time for cabbage, cauliflower, spinach, lettuce.

- concentrate the cyclohexane extract to approx. 2 ml in a rotary evaporator (bath temperature 40 °C)
- transfer the solution to a 10-ml cylinder and concentrate to a few ml in a stream of N_2
- make up to exactly 5 ml with cyclohexane
- added exactly 5 ml of ethyl acetate and shake
- apply 5 ml of this solution to the GPC column

GPC Conditions

See Procedure P1-4

P2-3B Clean-up by Semipreparative HPLC after Prior Clean-up on a Disposable Silica Gel Column (Bakerbond-SPE 3 ml)

This clean-up step is recommended above all when essential oils have to be removed together with chlorophyll and carotenoids from the sample matrix, e.g. with parsley and carrots.

Preparation of the Disposable Separation Columns

- activate by washing with 5 ml of cyclohexane and placing in a slight vacuum

Execution

- apply approx. 2 ml of sample solution concentrated according to Procedure P2-2 to the prepared column
- wash sample vessel with a total volume of 1 ml of cyclohexane
- elute the PAH with a further 7 ml of cyclohexane under slight vacuum
- cautiously evaporate the entire eluate (approx. 10 ml) just to dryness in a stream of N_2
- take up the residue in 2 ml of HPLC eluent

Semi-preparative HPLC

Autosampler:	LDC Milton Roy ASI 150
Pump:	LDC Milton Roy constaMetric III
UV detection:	LDC Milton Roy SpectroMonitor D
Fraction collector:	ISCO Foxy

Integrator:	Spectra Physics 4290
Stationary phase:	LiChrosorb Si 60 (7 μm), Merck
Column dimensions:	10 × 250 mm
Mobile phase:	*n*-Hexane/ethyl acetate (9/1; *v/v*)
Flow rate:	4 ml/min
Injection volume:	1.0 ml
PAH fraction:	4.9–7.6 min

- cautiously evaporate the fractionated solution just to dryness in a stream of N_2
- take up residue in 50 or 100 μl of toluene

P2-4 GC/MS Measurement

See Procedure P1-5.

3.7.5 Procedure P3: Vegetable Oils and Fats

P3-1 Extraction

- weigh approx. 25 g of oil into a 100-ml brown glass flask
- add 25 ml of cyclohexane and rotate (schlieren should no longer be observable)
- place 25 ml of cyclohexane in a 250-ml brown glass separatory funnel
- transfer oil solution to the separatory funnel
- wash out flask with 2 × 25 ml of cyclohexane
- transfer washings to the separatory funnel
- add 1 ml of internal standard solution
- shake vigorously
- add 50 ml of DMF/bidistilled water (9/1; *v/v*)
- shake well for 2 min
- after phase separation (time required approx. 35 min; turbidity of one or both phases can be neglected) run off the DMF/bidistilled water phase into a 500-ml separatory funnel
- repeat extraction with 2 × 25 ml DMF/bidistilled water
- combine the DMF/bidistilled water phases
- mix the DMF/bidistilled water phase with 100 ml of 1% aqueous Na_2SO_4 solution

- shake vigorously, carefully allow air to escape, wait for about 5 min
- add 50 ml of cyclohexane
- shake for 2 min
- after phase separation (time required approx. 35 min; turbidity can be neglected) run off the DMF/bidistilled water phase into a 500-ml separatory funnel and again extract for 2 min with 35 ml of cyclohexane
- after phase separation transfer the cyclohexane phase from the first separatory funnel to the second one in such a way that no mixing occurs
- run off the DMF/bidistilled water phase into the first separatory funnel and again extract for 2 min with 35 ml of cyclohexane
- after phase separation discard the DMF/bidistilled water phase
- run off the cyclohexane phase into the second separatory funnel, wash out the first separatory funnel with 40 ml of bidistilled water, rinse, and add to the cyclohexane phase in the second separatory funnel
- shake vigorously for 2 min
- allow to stand overnight for phase separation
- discard the washing water and repeat the washing process with 40 ml of bidistilled water. (The phase separation for the first washing step may be very time consuming; emulsions are formed which are difficult to separate. While addition of ethanol speeds up phase separation, the separation achieved is usually incomplete. Leaving to stand overnight is therefore recommended. The following approach can be adopted in particularly obstinate cases: 10 ml of a 10% solution of sodium sulfate are first added and the mixture is shaken vigorously. If phase separation is not seen to improve within a few minutes, the aqueous phase and the generally creamy emulsion are drained into the empty 500-ml separatory funnel. 50 ml of bidistilled water is added and the mixture is shaken vigorously. The creamy consistency of the aqueous phase disappears and a clearly recognizable phase boundary appears.)
- run the cyclohexane phase into a 250-ml brown glass flask
- wash out the separatory funnel with 2 × 15 ml of cyclohexane, place cyclohexane in the first separatory funnel, shake, run off into the second separatory funnel, after shaking with cyclohexane, combine in brown glass round bottom flask
- add 2.5g of calcined sodium sulfate, stopper flask, and shake vigorously
- keep at least 2 h in refrigerator (or overnight) until the supernatant is clear

- carefully decant the solution into a 250-ml brown glass flask
- wash the flask containing sodium sulfate with 3 × 15 ml of cyclohexane, decanting each time
- evaporate the combined extracts almost to dryness in a rotary evaporator
- make up to 1 ml with cyclohexane

P3-2 Clean-up on Silica Gel

See Procedure P1-3

P3-3 Clean-up on GPC Column

See Procedure P1-4

P3-4 GC/MS Measurements

See Procedure P1-5

3.7.6 Procedure P4: Coffee

P4-1 Hydrolysis

Roasted Coffee (Ground)

- weigh 25 g of coffee into a 250 ml Soxhlet flask
- add 100 ml of 2 N methanolic potassium hydroxide
- introduce 6 to 8 glass beads and 6 to 8 small pumice stones
- add 1 ml of internal standard solution (0.1 ng/µl)
- hydrolyze for four hours under reflux

Soluble Coffee (Ground with Pestle and Mortar)

- weigh 25 g of coffee into a 500 ml brown glass round bottom flask
- add 200 ml of 2 N methanolic potassium hydroxide
- introduce 6 to 8 glass beads and 6 to 8 small pumice stones

- add 1 ml of internal standard solution (0.1 ng/µl)
- hydrolyze for four hours under reflux

P4-2 Extraction

Roasted Coffee

- wash a Büchner funnel with round filter and separatory funnel with approx. 30 ml of cyclohexane
- transfer the warm solution obtained by P4-1 with 100 ml of methanol/bidistilled water (9/1; *v/v*) via the Büchner funnel with precisely fitting round filter into a 500-ml brown glass separatory funnel, rinsing the reflux condenser with about 30 ml and the Soxhlet flask with the other 70 ml (it is essential to apply *full vacuum* before passing the sample through the filter; otherwise coffee particles could enter the separatory funnel)
- after the liquid has been completely drawn through the Soxhlet flask is again washed with 30 ml of methanol/bidistilled water (9/1; *v/v*) (ultrasonic bath) and the washings poured onto the filter cake.
- again draw off all liquid (*full vacuum*)
- extract the aqueous phase with 100 ml of cyclohexane for 2 min (add a few ml of ethanol if necessary to improve phase separation)
- after draining off the aqueous phase into a 250-ml glass beaker, the cyclohexane phase is poured out of the top of the separatory funnel into a second separatory funnel (otherwise the extract is too contaminated)
- repeat the extraction of the aqueous phase with cyclohexane, having previously washed out the glass beaker portionwise the 100 ml of cyclohexane
- extract the combined cyclohexane extracts with 100 ml of methanol/bidistilled water (1/1; *v/v*). *The procedure may be interrupted here*
- extract the cyclohexane phase with 100 ml of bidistilled water
- transfer the cyclohexane phase into a 500-ml round bottom flask with 5 g of calcined sodium sulfate
- place in a refrigerator overnight

Soluble Coffee

The following changes should be made to the above procedure for roasted coffee:

- transfer the hydrolyzate with 150 ml of methanol/bidistilled water (9/1; *v/v*) via the Büchner funnel into a 1000-ml separatory funnel
- extract the combined aqueous phases with 2 × 150 ml of cyclohexane
- then treat the cyclohexane phases as for "roasted coffee"

P4-3 Clean-up on Silica Gel Column

See Procedure P1-3

P4-4 Clean-up on GPC Column

See Procedure P1-4

P4-5 GC/MS Measurements

See Procedure P1-5

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Food and Environmental Analysis by Capillary Gas Chromatography
Lothar Matter

4 The Mass Spectrometer as Detector in Capillary GC

Possibilities of Electron Impact (EI) and Chemical Ionization (CI) in Coupling with Capillary Gas Chromatography

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4.1 Classical Detectors versus Mass Spectrometry

Classical GC detectors such as the thermal conductivity (TCD) and the flame ionization detector (FID) afford results which are typically derived as retention time and peak area from the chromatogram. Additional information can be obtained via the selective element specific detectors such as the electron capture detector (ECD), the nitrogen phosphorus detector (NPD), the oxygen-specific FID (OFID), or the atomic emission detector (AED). Qualitative analysis is thus based only *indirectly* on the determination of retention indices and their comparison with those obtained from known standards.

A change of polarity of the analytical column used (change of the capacity factor k') generally serves as additional confirmatory evidence on agreement of retention times. In practice, this is accomplished by installing the two capillaries of differing polarities in an injector and performing parallel detection. On use of a suitable chromatography data system, the differing retention indices recorded for the different columns can be evaluated for substance identification, according to a prior calibration. In spite of the high information content for mixtures with few components, multicomponent analyses (e.g. for pesticide analysis and for industrial solvents) show an interfering number of

overlapping peaks which can lead to a false positive identification. This situation can be remedied by applying detection processes which provide a *direct* link to the eluting components.

Direct structure related data and thus substance-specific results in gas chromatographic detection are nowadays obtained only by use of a mass spectrometer. In capillary gas chromatography with a mass spectrometer as detector mass spectra are continuously recorded with the 'retention data'. The scanning rate is so fast that even the fastest peaks, such as those produced by split injection of highly volatile chlorinated hydrocarbons, are reliably detected and recorded. Thus a mass spectrum is available for further evaluation at every point of the chromatogram.

Mass selective detection by recording single masses is a special case in this context. Selected ion monitoring (SIM) and multiple ion detection (MID) reduce the mass spectrometer to a mass selective detector for just a few expected compounds selected prior to analysis. The underlying technical reason for the measuring technique is the lack of sensitivity of quadrupole systems in the recording of complete mass spectra, which is overcome by the predetermination of the detection mass(es). Reports of false positive findings in real-life samples are on the increase. Even the recording of up to three typical masses is not always sufficient to reliably confirm the presence of certain active substances in various matrices.

On GC/MS coupling with ion-trap mass spectrometers chromatograms are recorded which permit substance identification in proven manner via retention data. In addition, GC/MS analyses with an ion-trap detector typically show a complete, substance-specific mass spectrum at any desired point of the chromatogram. This is of particular value precisely for residue analyses at trace levels since no limitations regarding the expected substances have to be made prior to analysis; instead, all components present in the sample will be recorded together with their spectra. Comparison with commercial spectral libraries (such the Wiley/NBS Library, 5th Edition with over 146,000 spectra and structures) is even possible in trace analysis and rapidly provides reliable information about the identity of a component in question.

Exploitation of substance-specific spectral information is increasing the reliability of quantitative determinations. On recording data in the full scan mode, spectral comparison is first undertaken at the calibrated retention time in order to determine the identity of the substances. Only on identification of the expected component is conventional peak integration performed; however, MS detection assures interference-free integration even in cases of

co-elution of components by utilizing selective mass chromatograms. In this way, a whole set of chromatograms can be automatically searched for analytes and quantified (target compound analysis). The calculation of contents is performed with the aid of external and internal standards, the standard addition procedure, or the 100% method, available as a commonly used calculation procedures in most data systems.

Today, mass spectrometric detection in the form of coupled GC/MS has become a powerful and highly reliable routine method for environmental analysis, food analysis, and toxicological applications. Because it records the complete mass spectra, coupled GC/MS meanwhile vies with classical GC detection and also MS detection with selected ion monitoring as confirmatory and reference procedure.

4.2 Mass Spectrometry

Mass spectrometers of widely differing performance levels are currently used for coupling with capillary gas chromatography. The detectable mass range is already largely determined by the use of gas chromatography as method of separation – the substances must be vaporizable. In contrast, the sensitivity and resolving power of the mass spectrometer serving as detector can be varied within wide limits. This is of considerable importance for certain tasks. The resolving power in mass spectrometry refers to the ability of an instrument to separate adjacent mass signals (ions) according to their m/z (mass-to-charge) ratios. Determination of the resolution also requires consideration of the nature of the separation system. High resolution is generally considered to mean that C, H, N, O multiplets can be distinguished (resolution > 10,000); in contrast, low resolution refers to the ability to separate according to nominal masses (integral mass numbers; uniform mass resolution) [4-1].

4.2.1 High Resolution Mass Spectrometers

The trajectories of ions of various m/z values in double focusing sector-field instruments differ because of the influence exerted by a magnetic and electric field. Slit systems eliminate certain ion trajectories and continuous change of the instrumental parameters such as the accelerating voltage permit recording of mass spectra. The width of the ion beams is determined by an exit slit at the ion source. To allow recording of ions of different masses in close proximity the beams may overlap only very slightly or not at all. The resolution A of adjacent signals in a sector instrument is calculated according to:

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

where m is the mass and Δm is the distance to the adjacent mass. In accordance with this formula the resolution is dimensionless.

What mass spectrometric resolution will be required, for example, to obtain distinguishable signals of carbon monoxide (CO), nitrogen (N_2), and ethene (C_2H_4)? These can enter the ion source of a mass spectrometer via a transfer line. Table 4-1 shows the exact masses and the nominal masses of these compounds.

Table 4-1: Exact masses and nominal masses of CO, C_2H_4, and N_2

Substance	Nominal mass	Exact mass
CO	*m/z* 28	*m/z* 27.994910
C_2H_4	*m/z* 28	*m/z* 29.006148
N_2	*m/z* 28	*m/z* 28.031296

MS separation of jointly occurring CO and N_2 requires a resolution of at least 2500 according to the above formula. All MS instruments of low resolution rely on a gas chromatographic pre-separation of the components (CO, C_2H_4, and N_2 successively enter the ion source of the MS). This is the case for all quadrupole and ion-trap instruments.

A mass spectrum recorded with a sector-field instrument characteristically has a constant resolution A over the entire mass range. In accordance with the resolution formula the distance Δm between the mass signals for water (*m/z* = 17/18) is significantly larger than for signals in the higher mass range. The maximum attainable resolution is characterized by the slit system and the quality of the ion optics of the sector-field instrument. Given sufficient precision of measurement, determination of the exact masses can reveal the empirical formula of a molecular ion (and of fragment ions).

GC/MS coupling with high resolution double focusing sector-field mass spectrometers is used in special analytical situations. The high selectivity of detection of precise masses (SIM/MID mode) is required in order to rule out false positive results to be expected on use of low resolution mass spectrometers. Recording of precise masses, such as *m/z* 321.8937 instead of the nominal

mass of 322 for 2,3,7,8-TCDD, only becomes possible with the high resolution offered by sector-field instruments which eliminate known interferences. Extremely low detection limits of less than 10 fg are then obtained which afford the necessary certainty for important decisions. High resolution is therefore still needed for confirmation of positive screening results.

4.2.2 Low Resolution Mass Spectrometers

A mass spectrum recorded with a quadrupole or ion-trap mass spectrometer shows a characteristic difference. The distance Δm between two mass signals remains constant over the entire mass range. It is immaterial how large the recordable mass range is. The water peak (m/z = 17/18) is therefore just as broad and well separated as the masses in the upper mass range.

According to the formula $A = m/\Delta m$ where Δm = constant the consequence for these low resolution instruments would be that the resolution A is directly proportional to m, the mass range. At a peak distance of normally 1 mass unit the resolving power would be small in the lower mass range (e.g. $A \approx 18$ for water) and greater in the higher mass range. Thus the formula $A = m/\Delta m$ fails to give useful data for quadrupole and ion-trap instruments and is therefore inapplicable. The optical resolution seen on the screen is the same in the upper and in the lower mass ranges. This constant resolving power over the entire mass range is set for the instrument by the manufacturer and is the same for all types of instrument and for all manufacturers. The peak width is adjusted such that the distance between two nominal mass signals is one mass unit (1 u = 1000 mu) with a valley between the signals of 10% at most. High resolution of the kind obtained with sector-field instruments is physically impossible for quadrupoles and in its infancy for ion traps. The analyzers for quadrupole and ion-trap instruments function on the same mathematical basis (Paul/Steinwedel, Bonn 1954) and therefore exhibit the same resolution properties. The practically attainable resolving power which is constant over the entire mass range is therefore referred to as "uniform mass resolution".

The labeling of line spectra on the display should be regarded completely independently of the resolution. By definition a mass speak with uniform mass resolution has a base width of one mass unit or 1000 mu. This position of the apex of the peak can be precisely calculated. Mention of 1/10 mass unit such as sometime occurs in the literature merely creates the impression of a resolution that is higher than the uniform mass resolution. Simultaneously appearing components of different exact mass but with the same nominal mass, such as may occur in GC/MS through co-eluates, matrices, column bleeding, etc., cannot be separated from one another by the resolving power

of the quadrupole or ion-trap analyzer (see Table 4-1: N_2, CO, C_2H_4). The position of the center of gravity (or centroid) has a value that completely defies interpretation due to superposition of matrix and other components. This provides no basis for calculation of empirical formulae! Depending upon the manufacturer, the labeling of the spectra can range between purely nominal masses and numbers with several places after the decimal point and can usually be changed by the user.

Every MS data system uses mass spectra with nominal masses for internal processing during background subtraction and library searches. The spectra of all MS libraries likewise contain whole number spectra.

Low resolution mass spectrometers have a very wide range of applications as detectors for gas chromatography. Such detection is suitable for the entire field of organic analysis. The pronounced sensitivity and high information content of mass spectra are good reasons for the universal application of GC/MS analytical instruments.

4.3 Ionization Processes

Nowadays the fused silica capillaries of all GC/MS instruments terminate directly or via an open interface at the ion source. The ionization necessary for detection affords typical molecular and fragment ions for each substance. The qualitative and quantitative composition of the ions can be seen in the recorded mass spectrum. The generation of the spectrum from the structure and clearly also the identity of the substance are reflected in characteristic manner.

4.3.1 Electron Impact Ionization

Ionization by electron impact (EI) is found as standard process in GC/MS instruments. The ionization energy of 70 eV is nowadays also standard for all commercial instruments. Only few benchtop instruments still permit the user to select the ionization energy for special applications. In sector-field instruments in particular use was frequently made of the possibility of recording low energy spectra with a high molecular information content by lowering the ionization energy to about 15 eV. Use of this technique has become progressively rarer since the introduction of chemical ionization.

The ionization process of electron impact ionization can be understood in terms of a wave model or a corpuscular model. The present day theory is based on interaction of the high energy electron radiation with the outer electrons

of a molecule. Uptake of energy leads via loss of an electron to formation of a molecular ion M^+. This molecular ion possesses an unpaired electron, and is therefore a radical ion. The excess energy leads to rotation and to vibrations of ligands of the molecule. The ensuing fragmentation processes depend upon the amount of excess energy and the ability of the molecule to stabilize [4-2].

The energy required for ionization of organic molecules is small compared to the energy of 70 eV effectively used, generally lying below 13 eV. The standard value of 70 eV valid for many years was adopted not only to optimize the signal intensity but above all to ensure comparability of spectra. Assuming maximum energy transfer, a multiple of the energy required for primary ionization to M^+ remains as excess energy in the molecule. As a consequence, intense fragmentation reactions occur and reduce the abundance of M^+ ions in the ionization region (ion source). There is a concomitant increase in formation of stable fragment ions.

The fragmentation and rearrangement processes are now largely understood. The fragmentation rules serve for manual interpretation of mass spectra or for identification of unknown substances. The mass spectrum is thus a quantitative record of the ionization processes by the analyzer system.

The mass spectrum is represented graphically as a line diagram. The mass number, or more accurately the mass/charge ratio m/z, is shown on the horizontal axis. Since we only expect singly positively charged ions (with a few known exceptions, such as PAH), this axis is known colloquially as the mass scale and indicates the mass of an ion. The vertical intensity scale shows the abundance of an ion under the selected ionization conditions. The scale usually indicates a percentage of the base peak (100% intensity) or measured intensity value (counts).

On fragmentation or rearrangement of a molecular ion M^+ (carrying just a single charge) neutral entities may also be set free. The analyzer cannot record these uncharged particles. However, the difference between the molecule ion and the fragment ion (or precursor fragment) reveals the mass of this neutral particle.

On operation of ion-trap instruments in the electron impact (EI) mode a switchable electron beam continues to produce ions until the capacity of the analyzer has been reached and the mass spectrum is recorded [4-3]. The time required for recording a complete mass spectrum lies only in the millisecond range. This mode of operation permits detection of complete mass spectra even at the lowest trace levels.

The examination of lemons for phosphoric esters is shown here as an example [4-4]. A typical GC trace of the work-up extract is shown in Figure 4-1. The NPD detector used affords a "clean" chromatogram containing only few peaks with the internal standard as the principal component. A substance is detected at slightly lower intensity which is identified as Quinalphos on the basis of its retention time of 22.85 min. The peak is free of all superposition and the analysis seems to have reached a reliable conclusion.

Routine examination with an ion-trap GC/MS system shows a different picture (Figure 4.2). A wealth of matrix peaks is present the retention range in question. The Quinalphos peak has a shoulder on its left side and is closely

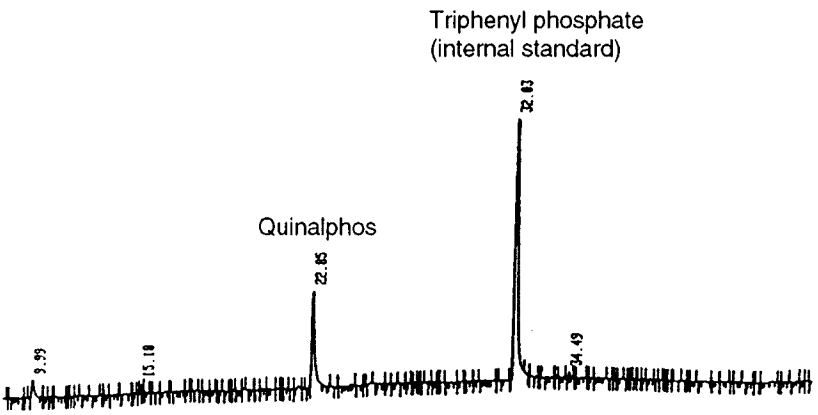


Fig: 4-1: Analysis for phosphoric esters, NPD trace with Quinalphos peak and triphenyl phosphate as internal standard.

followed by a lower intensity component. The peak spectrum obtained after removal of the flanks can be confirmed as Quinalphos by comparison with a library spectrum. Exact investigation of the mass spectrum reveals coelution of a second active substance which remained hidden in the NPD analysis (see Section 4.5.1.2). This demonstrates the power of recording complete mass spectra with the ion-trap analyzer. In the total ion current chromatogram all the plant protection agents eluting from the column are recorded and can be unequivocally identified via the mass spectrum.

4.3.2 Chemical Ionization

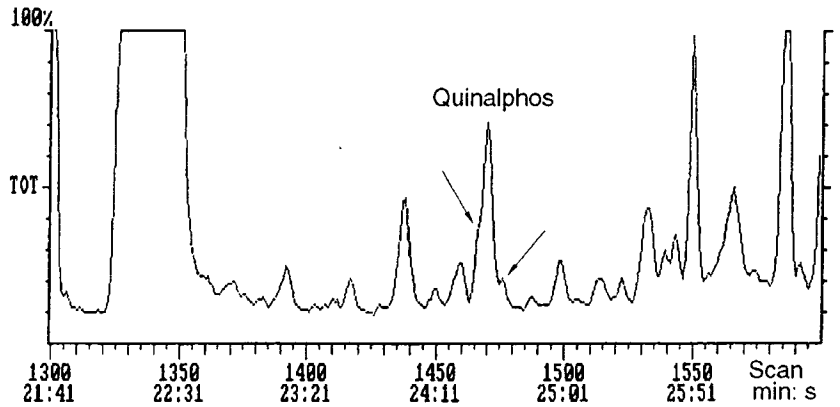


Fig: 4-2: Analysis for phosphoric esters, ion-trap GC/MS trace with Quinalphos peak at scan 1469.

Use of the EI mode does, however, come up against limitations in practice. More complex molecules often fragment to give very small entities. Molecular ions are only formed with stable structures (such as PCBs, PAHs); however, they are essential for confirmation of presumed structures, because "related" compounds may well generate the same fragments. The molecular ion is often present only at very low intensity and is often hard to isolated from the background noise (matrix) in the case of small samples, or it undergoes complete fragmentation and is not recorded in the mass spectrum. In quantitative analysis it may happen that the preselected quantification mass suddenly lies in an area of chemical noise. Integration of the peak is inadequate owing to the low signal-to-noise ratio or is completely impossible.

Chemical ionization can remedy this situation. Use of a reactant gas (such as methane, methanol, isobutane, ammonia) reduces the ionization energy. Stable molecular ions are formed which are detected with high intensities.

Environmentally relevant, toxicologically or pharmacologically active substances are frequently seen to contain polar groups or multiple halogen substitution. These structural features can be selectively exploited by protonation or electron capture in CI [4-7].

As an example, Figure 4-3 shows the EI and CI spectra of the phosphoric ester Tolclofos-Methyl. The base peak in the EI spectrum shows the presence of

one Cl atom from the isotopic pattern. Loss of methyl leads to *m/z* 250. Is *m/z* 265 the nominal molecular mass?

The CI spectrum shows a protonated ion at *m/z* 301, hence the nominal molecular weight could be 300 u. The isotopic pattern clearly indicates the presence of *two* chlorine atoms. Thus the EI and CI spectra provide complementary information. Tolclofos-Methyl apparently undergoes complete fragmentation on EI ionization with loss of a chlorine atom to give *m/z* 265 as $(M - 35)^+$; however, the molecular ion is not to be seen. In contrast, this fragmentation does not occur in the CI mode. Addition of a proton retains the complete structure and forms a quasimolecular ion $(M + H)^+$.

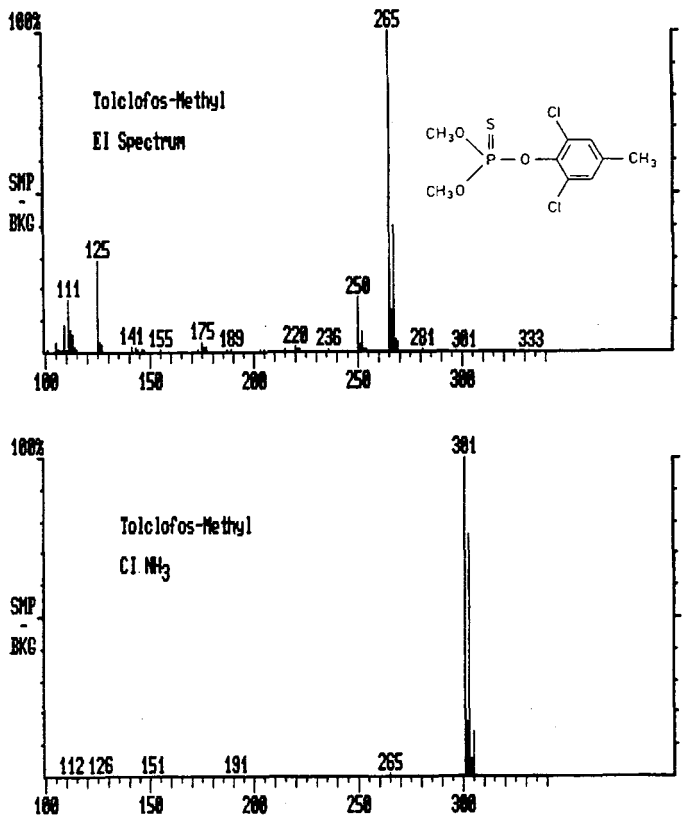


Fig: 4-3: EI and CI(NH_3) spectra of Tolclofos-Methyl.

In contrast to electron impact ionization, the term *chemical ionization* covers all soft ionization techniques which take place through the intermediacy of a reactant gas and its reactant ions in the form of an exothermic chemical reaction in the gas phase [4-7]. The products formed are stable positive or negative ions. The principle of chemical ionization was first described by Munson and Field in 1966 [4-8]. Chemical ionization uses much less energy for ionization of the molecule M. The CI spectra therefore show fewer fragments or none at all, and therefore usually give clear information about molecular weight.

The use of chemical ionization is valuable in structure elucidation and in the confirmation or determination of molecular weights, and also for elucidation of significant structural groups. Utilization of the CI reactions of various reactant gases can introduce additional selectivity into mass spectrometric detection, e.g. indication of active substances in a transparent hydrocarbon matrix. Quantification can be performed selectively, with great sensitivity, and through choice of an appropriate quantification mass in a molecular weight range devoid of interference by the low molecular weight matrix. The spectrum of analytical possibilities is not limited to the basic reactions presented. Instead, a wide range of gas phase organic chemical reactions is there to be exploited.

4.3.2.1 Principle of Chemical Ionization

Two reaction steps are necessary in chemical ionization (Figure 4-4). In a *primary reaction* electron bombardment of the reactant gas introduced pro-

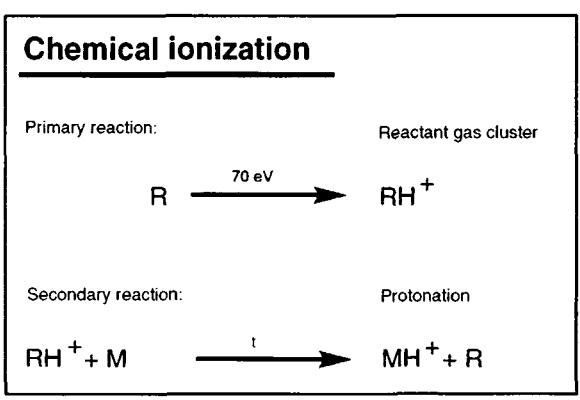


Fig. 4-4: Primary and secondary reaction in chemical ionization (protonation).

duces a stable cluster of reactant ions. The composition of the reactant gas cluster is typical for the reactant gas used. Since only an extremely small amount of gas corresponding to about 10^{-5} torr partial pressure of the CI gas is required in the ion-trap analyzer, the cluster is displayed on the instrument control monitor for adjustment. In quadrupole and sector-field instruments a reactant gas pressure of about 1 torr is set thanks to use of a specially designed ion source. In the ensuing *secondary reaction* the molecules M of the GC eluate react with the ions of the reactant gas cluster. The ionic reaction products are detected and displayed as the CI spectrum.

The appearance of the CI spectrum depends largely on the secondary reaction. Only exothermic spontaneous reactions afford CI spectra. In the case of protonation (Figure 4-4) this means that the proton affinity PA of M has to be greater than the PA of R, the reactant gas. Thus the choice of reactant gas R determines the extent of the energy released and transferred to the molecule M. This in turn determines the extent of subsequent fragmentation. Moreover, the question of selectivity is also determined by the choice of reactant gas. If PA(R) > PA(M) no protonation reaction will occur. Such selectivity can be achieved on use of ammonia. The frequently occurring undesired sample matrix of hydrocarbons remains transparent on use of ammonia as reactant gas, whereas active substances such as pesticides show up with a high signal-to-noise ratio.

Numerous reactions of different types can be exploited for chemical ionization. Four types of reaction have special significance in the formation of positive ions. After collision of the reactants the course of the CI reaction initially proceeds via formation of a transition complex $M{\cdot}R^+$. Subsequent reaction or continued existence of the transition complex decides the type of reaction considered below.

The formation of negative ions generally occurs in one of two very different ways. A negatively charged quasimolecular ion $(M - H)^-$ can be formed in a chemical reaction while a negative molecular ion M^- can arise by addition of an electron. The latter route resembles the mode of operation of an ECD. Other reaction pathways have little importance in analytical practice.

4.3.2.1.1 Proton Transfer (Protonation)

Protonation is the most widely utilized reaction in positive chemical ionization. It leads to formation of the quasimolecular ion $(M + H)^+$, which can undergo fragmentation:

$$M + RH^+ \rightarrow M + H^+ + R$$

Methane, water, methanol, isobutane, or ammonia are generally used as protonating reactant gas (Table 4-2). Methanol assumes a middle position with regard to fragmentation and selectivity, methane which is designated as a "hard" reactant gas is less selective. Typical "soft" CI gases are isobutane and ammonia, with the latter gas having proved extremely valuable in ion-trap spectrometers. Interfering $(NH_3)_x{\cdot}NH_4^+$ clusters, such as occur under the higher operating pressure conditions in quadrupole and sector-field instruments, are not observed in ion-trap instruments.

Table 4-2: Reactant gases for protonation reactions (according to H. Budzikiewicz, Massenspectrometrie, VCH Publishers 1992, [4-1]).

Gas	Reactant 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-$C_4H_{10}^+$	807
NH_3	NH_4^+	840

The CI spectra obtained by protonation show the quasimolecular ion $(M + H)^+$. Possible fragmentations start from the $(M + H)^+$ ion. Thus, for example, elimination of water will appear as M – 17 in the spectrum, arising from $(M + H)^+ - H_2O$!

Confirmation of the quasimolecular ion is often provided by the appearance of small amounts of addition products of the reactant gas; for example, in the case of methane $(M + H)^+$ may be accompanied by $(M + 29)^+$ and $(M + 41)^+$ (see methane) and with ammonia varying intensities of $(M + 18)^+$ accompany $(M + H)^+$ (see ammonia).

4.3.2.1.2 Hydride Abstraction

In this reaction a hydride ion is transferred from the hydrogen-containing molecule M to the reactant ion R^+:

$$M + R^+ \rightarrow RH + (M - H)^+$$

For example, this process is observed on use of methane, with the $C_2H_5^+$ ion (*m/z* 29) present in the methane cluster abstracting hydride from alkyl chains.

4.3.2.1.3 Charge Transfer

Charge transfer reaction forms a molecular ion with a reduced number of electrons. Accordingly, the fragmentation is qualitatively comparable with an EI spectrum. The extent of fragmentation is determined by the ionization potential IP of the reactant gas.

$$M + R^+ \rightarrow R + M^+$$

The ionization potentials of most organic compounds lie below 13 eV. By choice of the reactant gas (Table 4-3), the occurrence of a fragmentation can be steered between an individual molecular ion in the spectrum and appearance of an EI-like spectrum. Commonly used reactant gases are benzene, nitrogen, carbon monoxide, nitrogen monoxide, and argon.

Table 4-3: Reactant gases for charge transfer (according to H. Budzikiewicz, Massenspectrometrie, VCH Publishers 1992, [4-1]).

Gas	Reactant 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

On use of methane, superimposed charge transfer reactions can be observed especially with molecules having low proton affinities. Typical classes of compounds are chlorinated pesticides such as lindane, heptachlor, endosulfan, etc., and also chlorinated aromatic compounds such as PCBs.

4.3.2.1.4 Adduct Formation

If the transition complex mentioned above does not dissociate it becomes visible in the spectrum:

$$M + R^+ \rightarrow (M + R)^+$$

This effect is not of great systematic analytical usefulness. Pronounced adduct formation is always observed in protonation reactions in which the proton affinities of the participating components differ only slightly. Higher reactant gas pressures favor the effect through collision stabilization.

A $(M + R)^+$ ion is often not recognized immediately. However, when it is recognized it affords information just as valuable as a quasimolecular ion formed by protonation. Cluster ions of this kind sometimes interfere with spectral interpretation, particularly when neutral particles split off unrecognized from the transition complex.

4.3.2.1.5 Electron Capture

Addition of electrons to electronegative compounds is known in classical GC detection with the electron capture detector (ECD). Comparable processes and potential applications apply to the capture of electrons in the ion source of a mass spectrometer.

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

On use of a reactant gas electrons with a kinetic energy approaching 0 eV are generated at high pressure. Methane is often used at a source pressure of about 1 torr. These "thermal" electrons can add onto compounds with high electronegativities and the negatively charged molecular ion undergo stabilization by collision with neutral reactant gas molecules [4-9].

This kind of ionization is limited to the special ion source designs of quadrupole and sector-field instruments because of the need to provide a high population of thermal electrons. Today's ion-trap instruments do not permit such operation.

In practice, it has become apparent that only certain substances can be detected to advantage on ionization by electron capture. All compounds containing either halogens, nitro, keto, methoxy, or other electron-withdrawing functional groups can be detected in principle. However, in comparison with EI ionization a response enhancement is only observed from 6 chlorine atoms per molecular upwards and the attainable detection limits for less strongly halogenated compounds become poorer. EI ionization is therefore the preferred method of the analysis of TCDDs/TCDFs (tetrachlorodibenzodioxins/-furans).

However, of great utility is the high selectivity of the procedure, which like ECD makes even strong contamination of a sample extract, e.g. by hydrocarbons, appear transparent.

4.3.2.1.6 Proton Abstraction

Reaction with reactant ions which abstract a proton from an acidic sample molecule produces negatively charged quasimolecular ions.

$$M + R^- \rightarrow RH + (M - H)^-$$

Possible reactant ions are, for example, OH^- ions, which are formed from a reactant gas mixture of CH_4 and N_2O. This extremely sensitive reaction can be utilized wherever readily accessible acidic hydrogen atoms are present in the structure of an active substance, such as phenolic OH groups, keto-enol tautomers, etc.

4.3.2.2 Reactant Gas Systems

4.3.2.2.1 Methane

One of the longest known and best studied reactant gases is methane. As a "hard" reactant gas it has been replaced in most analytical applications by "softer" ones.

The reactant gas cluster of methane is produced by a multistep reaction which forms two dominant reactant gases ions at *m/z* 17 ands 29; a third species of *m/z* 41 occurs at low intensity:

CH_4 at 70 eV			$\rightarrow$	CH_4^+, CH_3^+, CH_2^+, etc.					
CH_4^+	+	CH_4	$\rightarrow$	CH_5^+	+	CH_3			50%
CH_3^+	+	CH_4	$\rightarrow$	$C_2H_5^+$	+	H_2			48%
CH_2^+	+	CH_4	$\rightarrow$	$C_2H_3^+$	+	H_2	+	$H^\cdot$	
$C_2H_3^+$	+	CH_4	$\rightarrow$	$C_3H_5^+$	+	H_2			2%

Good CI conditions are described on reaching a ratio of *m/z* 17 to 16 of 10:1. The pressure setting of the methane is shown by experience to be correct when the ions *m/z* 17 and 29 become predominant and attain roughly the same height at good resolution. The ion *m/z* should also be recognizable in the cluster at low intensity.

Applications of methane lie mainly in protonation reactions and to some degree also in charge transfer. With substances of low proton affinity methane often offers the "last chance" of recording CI spectra. The adduct ions $(M + C_2H_5)^+ = (M + 29)^+$ and $(M + C_3H_5)^+ = (M + 41)^+$ provide reliable confirmation of molecular mass interpretation.

4.3.2.2.2 Methanol

The low vapor pressure of methanol make it ideally suited for CI in ion-trap instruments. No pressure regulators, no gas cylinders, and no long gas lines are necessary. Connection of a glass flask containing methanol directly to the corresponding CI connection is sufficient. Analytical grade methanol is available in any laboratory. The low vapor pressure of methanol precludes its use with quadrupole and sector-field spectrometers because these instruments require a significantly higher pressure (about 1 torr) for supplying the specially designed CI ion source.

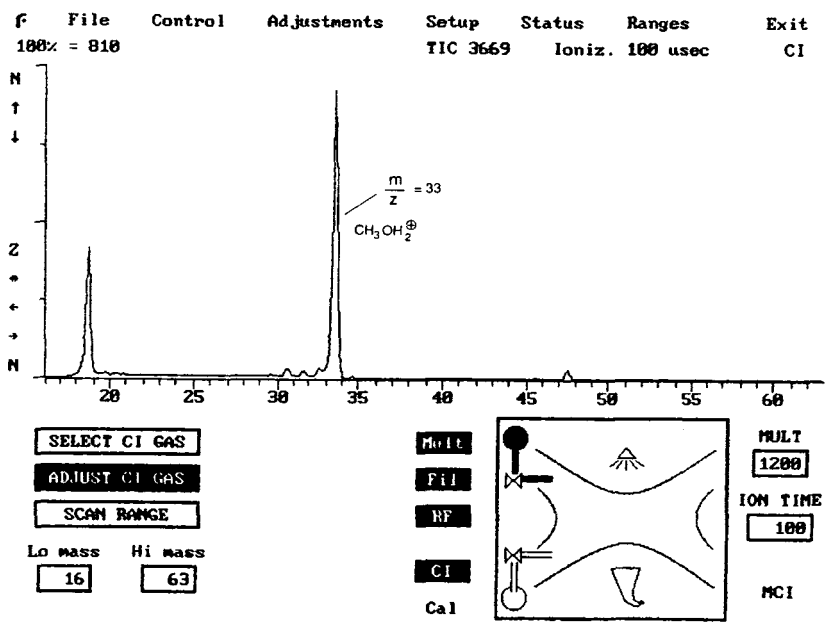


Fig. 4-5: Reactant gas adjustment for methanol on a MAGNUM ion-trap system.

The reactant ion formed is $(CH_3OH{\cdot}H)^+$ (Figure 4-5), which is set to a high intensity at good resolution. The appearance of a peak at *m/z* 47 indicates formation of the dimer after loss of water (dimethyl ether), which is formed at sufficiently high methanol concentrations. It is not necessary for functioning as a protonating CI gas.

The main application of methanol is typically protonation. A broad spectrum of compound classes of is accessible to methanol thanks to its medium level of proton affinity. It is therefore well suited for initial CI measurements on hitherto unstudied compounds. While the medium proton affinity of methanol does not yield any pronounced selectivity, substances of predominantly alkyl character nevertheless remain transparent. Accompanying fragmentations are of low intensity.

4.3.2.2.3 Isobutane

Chemical ionization with isobutane, like that with methane, has been known for many years.

In the reactant gas cluster tertiary butyl cations of *m/z* 57 are formed, which are responsible for the "soft" character of the reactant gas. The range of applications embraces protonation reactions of multifunctional and polar compounds. Isobutane has also proved valuable for the analysis of silylated derivatives. The selectivity of isobutane is high; fragmentations play a minor role.

4.3.2.2.4 Ammonia

Ammonia is known as a very "soft" reactant gas. The selectivity is correspondingly high, and is often utilized in the residue analysis of many active substances. Fragmentation reactions occur to only a small extent in ammonia CI (see Figure 4-3).

In ion-trap instruments only the ammonium ion NH_4^+ of mass *m/z* 18 is formed in the reactant gas cluster. Adduct formation with NH_4^+ can occur with substances which differ only slightly from NH_3 in their proton affinity. Corresponding signals can serve for confirmation of the molecular mass interpretation.

Formation and addition of higher $(NH_3)_n \cdot NH_4^+$ clusters as known for quadrupole and sector-field instruments with source pressures of around 1 torr

and which can impair interpretation and quantification, is not observed with ion-trap instruments.

4.3.3 Aspects of Switching between EI and CI

The value of chemical ionization can readily be demonstrated in the analysis of plant protection agents. The pronounced variety of fragmentation of many of the substance relevant for residue analysis, e.g. pesticides with their many functional groups, generally leads to complete loss of molecular information in the EI spectrum. In contrast, recording of CI spectra affords unequivocal information about the molecular weight. Combination of the information from EI and CI permits identification of substances and assignment of a hitherto unknown GC peak.

In addition, the use of chemical ionization has further advantages in securing the required detection limits and quantitative determinations. The broad range of fragmentation of pesticides already mentioned spreads the available total ion current over many different molecular fragments, each one of which should stand out from the matrix to be detected. Chemical ionization concentrates the substance reaching the detector to just a few species, generally to just the molecular or quasimolecular ion, which brings about a significant improvement of the detection limit in practice.

The effect is supported on use of an ion-trap analyzer by utilizing the entire storage capacity solely for these $M^+/(M + H)^+$ species. On operation in the EI mode, all the fragments formed share the same capacity. Quadrupole and sector-field instruments therefore generally employ SIM/MID in the CI mode.

Purposeful exploitation of additional selectivities attains better signal-to-noise ratios for all active substance peaks by eliminating the generally hydrocarbon-rich matrix. This also means that lower detection limits are achieved in CI operation than with EI ionization.

The benefit for qualitative analysis lies in the sure identification of substances and in confirmation of structural proposals from library searches. The molecular ion contains all the isotopic information of the compound and should plausibly explain all EI fragments (see Figure 4.3, Tolclofos-Methyl).

Quantitative determinations are favored by chemical ionization because the quantification mass can be placed in the upper mass range free from noise and other interference to integration. The improved detection limit in the CI

mode is also achieved by concentration of the ion current of a substance to just one ion, which is detected with high intensity.

4.3.3.1 Quadrupole and Sector Field Spectrometers

In instruments such as quadrupole and sector-field spectrometers, an ion source pressure of about 1 torr in an environment of $10^{-5}-10^{-7}$ torr is required to start the CI reaction and assure a sufficient conversion rate. To this end, the EI ion source is replaced by a specially designed CI source, which must be completely closed except for the few openings for the GC column, the electron beam, and the ion exit. Combination sources with mechanical devices for sealing off the EI from the CI source have so far only been accepted in sector-field instruments. In the miniaturized quadrupole sources the danger of slight leaks is high. As a consequence, mixed EI/CI spectra are produced.

For this reason, particular effort must be devoted to the conversion, pumping, and calibration of the CI source in electron beam instruments. The high current of reactant gas also leads to rapid contamination of the entire analyzer and thus to additional cleaning operations in order to regain the original sensitivity of the system.

4.3.3.2 Ion Trap Spectrometers

All ion-trap mass spectrometers are immediately suitable for CI operation without any need for conversion. Owing to its mode of operation as a storage mass spectrometer an extremely low reactant gas pressure is required. Adjustment is performed by a special needle valve which is operated at minimal leakage rate and only has to maintain a partial pressure of about 10^{-5} torr. The total pressure of the ion-trap analyzer of about $10^{-4}-10^{-3}$ torr remains unaffected. There is no need at all for all the mechanical devices still required for sealing ion sources in beam instruments.

Switching between the EI and CI modes is performed by keying in a command or by the selected data recording sequence in automatic operation. The ion-trap analyzer is switched internally to the CI scan function without any changes being made to the analyzer.

The triggering moment for the start of the CI reaction is the availability of reactant ions due to alteration of the operating parameters and the allowance of a brief reaction phase in the ion-trap analyzer. The scan function used in ion-trap instruments when operated in the CI mode therefore shows two clearly recognizable plateaus, which correspond directly to the primary and

secondary reactions. On completion of the secondary reaction the generated and stored product ions are detected by the mass scan and the CI spectrum recorded. The course of the scan function is represented in Figure 4-6 as the voltage profile of the ring electrode of the ion-trap analyzer. In spite of the presence of reactant gas the classical EI spectra can be recorded in the EI mode. Only on switching to the CI operating parameters does the desired chemical ionization become possible.

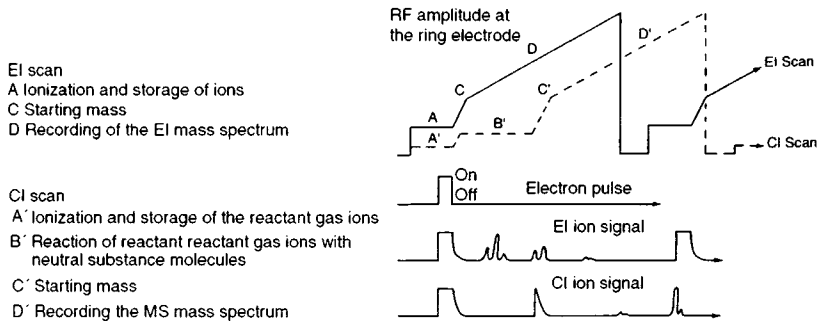


Fig. 4-6: Scanning profiles in the EI and CI modes.

On working with autosamplers it is therefore readily possible to switch back and forth between EI and CI operation such that each sample is measured in both the EI and the CI mode. In this way, both of the ionization processes can be routinely utilized. The danger of additional contamination by CI gas can be neglected because of the extremely low supply of reactant gas in ion-trap instruments; this mode of operation can therefore be pursued without sacrificing quality.

4.4 Special Aspects of GC/MS Coupling

4.4.1 Choice of Carrier Gas

It is no secret that use of hydrogen as carrier gas significantly improves the separation performance of a GC instrument, lowers the elution temperatures of compounds, and permits shorter analysis times through higher flow rates. Of decisive importance for this improvement of analytical performance is the favorable course of the Van-Deemter curve for hydrogen.

With regard to mass spectrometry the use of hydrogen means that a correspondingly higher volume flow has to be handled to maintain the vacuum. In this case the pump system and the type of analyzer decide the advantages and disadvantages.

The performance ratings of the turbomolecular pumps generally used nowadays are specified for pumping of a nitrogen atmosphere. Use of helium already lowers the pump performance. And the use of hydrogen causes a further reduction in efficiency. The reason for the pronounced drop in performance in the case of hydrogen is its low molecular weight. This reduced pump performance leads to a measurable increase in pressure in the analyzer, which in ion beam instruments has a negative effect on transmission and hence on instrument sensitivity. The effect can be compensated by using higher performance pumps or by connection of additional pumps. Owing to their storage effect, this behavior is not observed with ion-trap instruments. With hydrogen as carrier gas, it is advantageous to use oil diffusion pumps whose pumping performance is generally independent of molecular weight and therefore just as high for helium and hydrogen.

In addition, it should also be borne in mind that hydrogen is a reactive gas prone to effect hydrogenation of the substances being analyzed. This effect is already known from gas chromatography. Reactions in heated injectors can lead to the appearance of interfering peaks. In GC/MS additional ion source reactions are also known to produce hydrogenation products. Changing to helium as carrier gas will quickly reveal such occurrences.

4.4.2 Choice of Capillary Column

The choice of capillary columns available for general and specific applications is nowadays greater than ever. Certain limitations apply with regard to internal diameter and length on use in GC/MS systems with direct coupling. Here the limiting factors are generally the performance of the MS vacuum system and the design of the ion source. While most quadrupole GC/MS systems are designed for a carrier gas flow of up to about 1 ml/min, ion-trap systems can be operated up to about 3 ml/min (manufacturer's information should be noted). Hence standard columns of 15 m or more in length and with an internal diameter of 0.25 mm (narrow bore) can be used without problems in all GC/MS instruments.

Use of columns with 0.32 mm internal diameter (wide bore) whose greater film thickness make them specially desirable for analysis of highly volatile compounds (LH hydrocarbons, BTEX, etc.) is not feasible in all GC/MS

instruments. As a compromise, only flow rates below the optimum can generally be used. Wide bore columns of 25 m and greater length can be used without restrictions with ion-trap systems.

Use of megabore columns of 0.53 mm internal diameter is impossible with directly coupled GC/MS. This kind of column is recommended as a replacement for packed columns in various protocols issued by the United States Environmental Protection Agency.

With regard to column bleeding there are no fundamental restrictions in the choice of stationary phase and film thickness at elevated temperatures. However, pronounced column bleeding does impair the attainable detection limits (signal-to-noise ratios) in both the full scan and the SIM/MID modes and should therefore be avoided.

4.4.3 GC/MS Coupling Techniques

4.4.3.1 Open Split

Many analysts will be familiar with open split coupling from the infancy of the GC/MS technique. The task of the open split was to balance incompatible flow rates, and particularly to eliminate the solvent peak and other main components in order to protect the ion source against contamination and the resulting drop in sensitivity. These former constraints have now receded in to the background with the advent of today's fused silica capillaries and maintenance-friendly ion source designs. However, many advantages of open split coupling (Figure 4-7) are still used today.

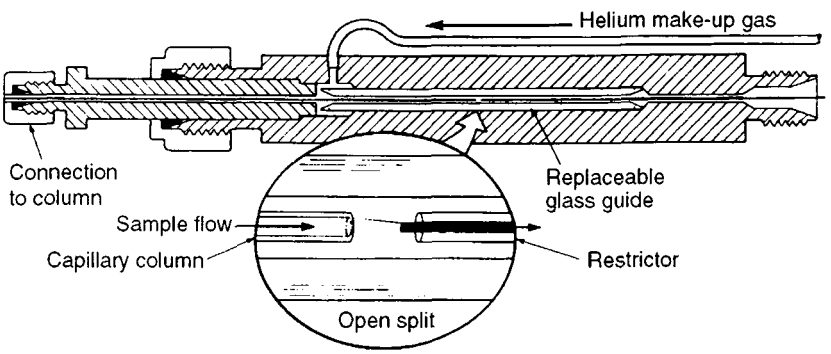


Fig. 4-7: Section through an open-split coupling, Finnigan MAT 800 (ion-trap spectrometer).

Advantages of open-split coupling:

- The retention times of classical detectors such as FID, ECD, etc. also remain unchanged in GC/MS and permit comparison of chromatograms.
- At the end of the column, splitting to an additional element specific detector such as ECD, NPD can take place, which provides information complementary to the MS.
- The capillary column can easily be replaced, generally without ventilation of the mass spectrometer and thus permitting rapid resumption of work.
- The choice of GC conditions (column length, diameter, and flow rate) can be optimized for the task at hand, independently of the MS system.
- Connection of wide bore, megabore, and packed columns is possible. Excess eluate not drawn into the spectrometer can be split off.
- A constant flow rate independent of the oven temperature enters the mass spectrometer, permitting precise optimization of the ion source.

Disadvantages of open-split coupling:

- The coupling site is at atmospheric pressure and has to be surrounded by carrier gas to prevent entry of air. Further screwed unions are necessary which lead to interfering leaks.
- If a positive split ratio occurs as a result of the balance between column flow and vacuum performance of the mass spectrometer then the sensitivity of the system is reduced.
- On inappropriate handling, e.g. entry of particles for sealing ferrules or imperfect cut of the end of the column, the gas chromatographic separation is impaired.

4.4.3.2 Direct Coupling

The direct route of GC eluate into the mass spectrometer without hindrance is assured by inserting the end of the separation column in the ion source. For this reason, direct coupling is nowadays regarded as the ideal approach. The pumping performance of modern mass spectrometers is adapted to the commonly used flow rates of gas chromatographs.

Advantages of direct coupling:

- Uncomplicated construction and handling.
- Uniform, unperturbed passage of substances from the GC injector to the ion source.

Disadvantages of direct coupling:

- The MS instrument must by cooled down and ventilated when the column is changed.
- The carrier gas flow is not constant in the ion source, but depends upon the selected GC temperature program.
- The vacuum in the mass spectrometer influences the separation on the GC column and shortens the retention times compared to FID, ECD, etc.
- High interface temperatures limit the lifetimes and inertness of sensitive types of stationary phase films, such as Carbowax, OV 1710, etc.
- The choice of column and adjustment of the optimum flow rate are limited by the maximum carrier gas flow for the mass spectrometer.

4.4.3.3 Separator Techniques

Special separators are no longer required for coupling capillary gas chromatography with mass spectrometry. Only those GC methods still using packed columns with flow rates of around 10 ml/min (or use megabore columns instead) cannot be coupled to mass spectrometers without separators.

The jet separator (Biemann-Watson separator) formerly used in many cases works according to the principle of diffusion of small gas molecules out of the transmission axis, which are then drawn off by a rotary vane pump. Owing to their greater inertia, heavier molecules reach the ion source of the mass spectrometer directly through the transfer capillary. As a rule, the use of separators is associated with a loss of sensitivity and therefore regarded as past history since the widespread use of the capillary column technique for GC/MS residue analysis.

4.5 Evaluation of GC/MS Analyses

4.5.1 Presentation of the Chromatograms

The chromatograms recorded by GC/MS are conventionally presented as plots of intensity against retention times. Nevertheless, there are considerable differences between these and chromatograms produced by classical detectors.

4.5.1.1 Total Ion Current

In GC/MS the intensity axis is designated as TOT or total ion current, or as the RIC or reconstructed ion chromatogram. Both terms describe the mode of representation determined by the method of detection. The mass spectrometer records spectra over the selected mass range at a constant scanning rate and affords a three dimensional data field comprising retention time, mass scale, and intensity. The signal size equivalent to that of FID detection is not directly available. A comparable total intensity at one scan point results from summation of all intensities of the mass spectrum recorded at this point. The peak intensity therefore corresponds to the number of measured ions.

The representation of a GC/MS chromatogram (TOT/RIC) with the peak intensities shown therein is therefore strongly dependent upon the mass range covered. Repeated GC/MS analysis of one and the same sample leads on choice of mass scans of different widths over the baseline of the total ion current leads to peaks of differing heights. In this context, the starting mass of the scan exerts a considerable influence. The consequence is a more or less pronounced recording of the non-specific background, which is reflected in a higher or lower baseline of the total ion current chromatogram. Peaks of the same concentration are therefore displayed with differing signal-to-noise ratios in the total ion current. In spite of differing representations of the substance peaks the sensitivity of the GC/MS system clearly does not change.

In the case of data recording by SIM/MID the intensity profile of the selected masses is indicated. All substances providing signals at the selected masses due to fragment or molecular ions are shown as a peak. No mass spectrum is available for checking identity. The relative intensity of two or three specific lines serves as qualification characteristic.

4.5.1.2 Mass Chromatograms

An informative assessment of signal-to-noise ratios of certain peaks is performed with the aid of mass chromatograms of substance specific ions (fragment/molecular ions). The three-dimensional data field of GC/MS analyses in the full scan mode of operation permits not only the determination of a total ion intensity at a scanning point. Selective representation of individual components is achieved by individual representation of the intensities of certain selected ions from the recorded data field and an intensity/time trace is plotted.

Evaluation of these mass chromatograms permits exact determination of the detection limit via the signal-to-noise ratio of a substance-specific ion of a compound. In the SIM/MID mode of operation this ion would be detected, but there would be no access to a complete mass spectrum. In complex chromatograms of real-life samples, mass chromatograms provide a key to isolation of co-eluting components and their unambiguous quantification.

The analysis of lemons for residues of plant protection agent serves as an example illustrating the discovery of a co-elution situation by full-scan recording with an ion-trap detector with subsequent evaluation of the mass chromatograms.

Routine monitoring with an ion-trap GC/MS system presents a different picture from analysis with the element-specific NP detector. A number of different peaks adorn the retention range in which the NPD showed quinalphos as active substance. The quinalphos peak shows a shoulder on its left flank and is closely followed by a component of less intensity. In the mass chromatogram of the characteristic individual masses (fragment ions) a form of presentation can be derived from the total ion current which shows an additional eluting active substance (Figure 4-8). In contrast to NP detection, GC/MS analysis clearly reveals the presence of a co-eluting second component, viz. chlorfenvinphos, after evaluation of the mass chromatograms and the recorded mass spectra.

4.5.2 Identification by Library Searches

One of the major strengths of mass spectrometry is the immediate availability of information about the eluting components. Careful extraction of the substance-specific signals from the chromatogram plays a decisive role in reliable elucidation of identity. Thus recording the identity of mass spectra which are as complete as possible is essential for the identification or confirmation of individual GC peaks.

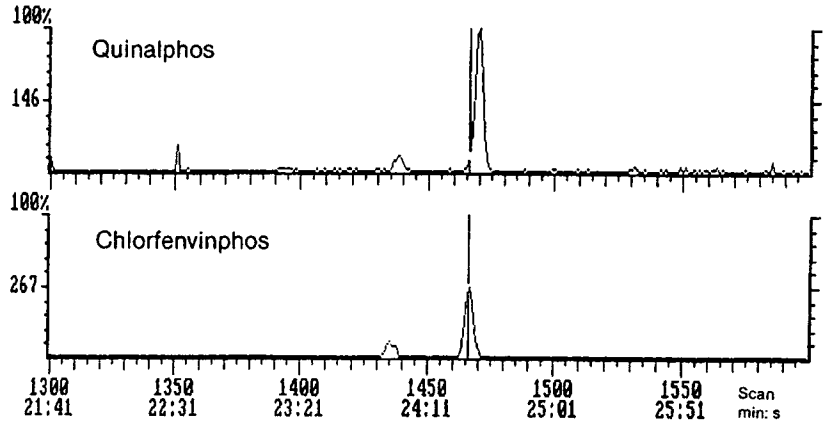


Fig. 4-8: Mass chromatograms of the co-elution plant protection agent quinalphos on mass trace *m/z* 146 and chlorfenvinphos *m/z* 267.

As shown above, working with mass chromatograms can reveal cases of co-elution. The mass chromatograms of dominant ions also provide important information by way of their maximization behavior. Their formation from the same structure can only be assumed if their intensity profiles show exactly the same time course. The only exception is that of ideal simultaneous elution. If differing peak maxima result for different ions then co-elution components must be assumed.

4.5.2.1 Extraction of Spectra

Subtraction of the background or co-elution spectra from in front of or behind a GC peak in question cleanly extracts the mass spectrum of the substance sought from the chromatogram. All the substances co-eluting with an unknown, including the matrix components and column bleed appear as chemical background. Distinguishing between substance signals and background and elimination of the latter from the substance spectrum is of decisive importance for successful spectral comparison in library searches. This approach is adopted to identify the active substance in the above example of GC/MS analysis of lemons for pesticide residues.

4.5.2.2 Reference Mass Spectra

In EI ionization at 70 eV the extent of fragmentation reactions observed for most organic compounds is independent of the manufacturer-specific design of the ion source. This assures comparability of the spectra generated when

compiled in spectral libraries. All commercially available libraries of mass spectra were recorded under standard conditions and permit comparison of the fragmentation pattern of a unknown substance with spectra included in the library. The great value of EI spectra lies in their fragmentation patterns. All search processes through spectral libraries are (still) based on EI spectra. Introduction of the highly reproducible technique of *advanced chemical ionization* led to the first appearance of a commercial CI spectral library covering 300 pesticides (Finnigan MAT 1992).

The available reference mass spectral libraries can be divided into general, very extensive collections, and smaller, highly specialized task-oriented compilations.

The most comprehensive commercial library of spectra is undoubtedly *The Wiley/NBS Registry of Mass Spectral Data*, whose origins data back to the year 1963. The 6th Edition contains over 220000 spectra. Owing to their primary use for the PBM search procedure (see Section 4.5.2.3.2) several spectra are sometimes included for a given substance. This accounts for different recording conditions. In addition to the mass spectra, the database also contains structural formulae, names of commercial products, and trivial names, as well as reference sources. Development of the database is in the hands of Professor McLafferty, Cornell University, New York.

Also of general importance is the very widely used NIST library originally based on a collection undertaken by the US Environmental Protection Agency and the National Institutes of Health in the early 1970s. It was first published as the NBS library containing about 25000 spectra by S.R. Heller and G.W.A. Milne in 1978. The current version contains more than 64000 mass spectra.

Specialized libraries aim to cover clearly defined areas of application. The advantage of these purposely limited libraries lies in their concentration on substances leading to reasonable interpretations only in a specific area of work. Maintenance of these collections is generally easier, and updates appear at shorter intervals. Well known commercial libraries include the *Pfleger/Mauer/Weber-Spektrensammlung* with 4370 entries from the fields of pharmacology and toxicology, whose importance derives from inclusion of valuable additional information such as details of metabolites, indications, and sample processing. A remarkable *Collection of Terpene Spectra* has been published by R.P: Adams. Some 570 compounds are included, with their retention times. Further specialized libraries include compilations of pesticide spectra. The most extensive collection is currently W. Ockels' *Pestizid-Bibliothek* containing some 600 entries.

4.5.2.3 Library Search Procedures

The widespread use of mass spectrometers as detectors in capillary gas chromatography has rekindled discussions of the effectiveness of the procedures used in library searches. As a result of the differences in software used in today's benchtop GC/MS systems two search procedures effectively share the market: INCOS and PBM. The SISCOM procedure (Search for Identical and Similar Compounds) developed by Henneberg/Weimann, which is distinguished by its excellent performance in computer-aided interpretation of mass spectra [4-10], is limited to use in data systems of a medium level of technology.

In general, library search procedures are expected to identify an unknown substance. However, it is better to regard the result of a search procedure as indicating similarity between the reference and the unknown spectrum. Further information confirming identity, such as retention time, sample processing, and additional spectral data should always be consulted as well. In particular, the limited coverage of the libraries should always be borne in mind [4-11].

Procedures for establishing spectral similarity are based of very different considerations. The INCOS and PBM procedures nevertheless have the common goal of suggesting substances permitting elucidation of an unknown spectrum [4-12]. These two algorithms predominate in the qualitative evaluation of spectra from sector-field, quadrupole , and ion-trap GC/MS systems.

4.5.2.3.1 The INCOS Search Procedure

In the early 1970s the former company Incos presented a search procedure based on principles of pattern recognition which also utilizes components of classical interpretation techniques and can reliably handle data from different types of mass spectrometers. The early years of GFC/MS coupling were dominated by the rapid development of quadrupole instruments which, owing to their high scanning speeds compared to those of the sector-field instruments of the day, were ideally suited for coupling with gas chromatography. However, the spectral libraries available at the time had been produced of the basis of the then established sector-field instruments – as are in fact today's spectral databases.

Since its introduction, the INCOS procedure [4-13] has been able to handle the characteristics of both kinds of analyzer techniques. The INCOS approach has been used almost unchanged since the 1970s and is known for its accuracy and complete independence of the type of instrument used.

After significance weighting (square root of the product of mass and intensity) and data reduction through a "noise filter" and a "redundancy filter" the extensive database is subjected to a very coarse pre-search for candidates suitable for the actual pattern comparison. The qualitative presence of up to 8 of the most significant mass signals serves as an important prior criterion. It is assumed that only those reference spectra containing at least 8 of the most significant masses come into question at all for identification. Depending upon optional information entered by the user, spectra with less than 8 coinciding mass signals are also included. Reference spectra with only a small number of coinciding mass lines or whose molecular weights do not agree with an optional data entry are excluded from the list of possible suggestions.

In the INCOS algorithm the main search is the decisive step in which the candidates found in the pre-search are compared with the unknown spectrum and a series of suggestions in the order of their likelihood is generated. Of critical importance for the instrument-independence of the INCOS procedure, and hence for its high accuracy, is a process known as "local normalization".

This local normalization introduced an important component into the search procedure which is comparable with visual comparison of two patterns. Individual ion clusters and isotope patterns are compared with each other in a local mass window. The central mass of such as window in the reference spectrum is compared with the intensity of this mass in the unknown spectrum in order to assess the agreement of the line pattern on the right and left thereof. In this way, the near region of a mass signal is investigated and the coincidence, for example, of isotope patterns (Cl, Br, S, Si, etc.) and fragmentation reactions is evaluated.

The advantage of this procedure is that deviations in relative intensities in the overall spectrum do not influence the result of the search. Variation in relative intensities of signals in a mass spectrum can arise, for example, from different choice of spectra from the rising or falling peak flank in the case of quadrupole or sector-field instruments , and by alteration of the tuning parameters of the ion source or its increasing contamination.

Local normalization is the reason why all spectral libraries working with the INCOS procedure contain only one mass spectrum per substance.

As a consequence of local normalization, two values are determined for spectral comparison. The FIT value is a measure of the quality with which the reference spectrum is present in the unknown spectrum. The opposite view, i.e. the extent to which the unknown spectrum is present in the pertinent

Table 4-4: Results of an INCOS library search.

FIT	RFIT	PUR	Evaluation
High	high	high	identity or isomer highly likely
High	low	low	identity possible, as is a homologous substance, co-elution, noise
Low	high	low	spectrum possibly incomplete through too narrow a mass range

reference spectrum, is expressed as the RFIT or reverse fit value. The combination of the two values indicates the purity of the unknown spectrum (Table 4-4). If the FIT value is high in a given case and the RFIT value significantly lower, then it can be assumed that the measured spectrum contained considerably more lines than the reference spectrum concerned. Mass fragmentography or background subtraction should be used to establish whether a co-eluate, chemical noise, or the presence of a homologous substance, or other reasons are responsible for the occurrence of the additional lines.

The results of library searches for co-eluting pesticides quinalphos and chlorfenvinphos are shown in Figures 4-9 and 4-10.

All the candidates found in the pre-search are processed as described in the main search. The result comprises lists sorted according to PURity, FIT, and RFIT. Initial sorting according to PURity is recommended because this value provides the best information about possible identity. Further sorting by FIT values offers additional suggestions generally aid further identification steps with valuable information about partial structure or whether the unknown belongs to a particular class of chemicals.

The difference between a reference spectrum and the measured unknown spectrum can serve as basis for further manual processing. A renewed library search can be undertaken with the remaining portion of the spectrum. In some cases this can reveal simultaneous co-elution of components, which is observed even on the most careful application of capillary gas chromatography in the case of complex samples, for example in environmental analysis.

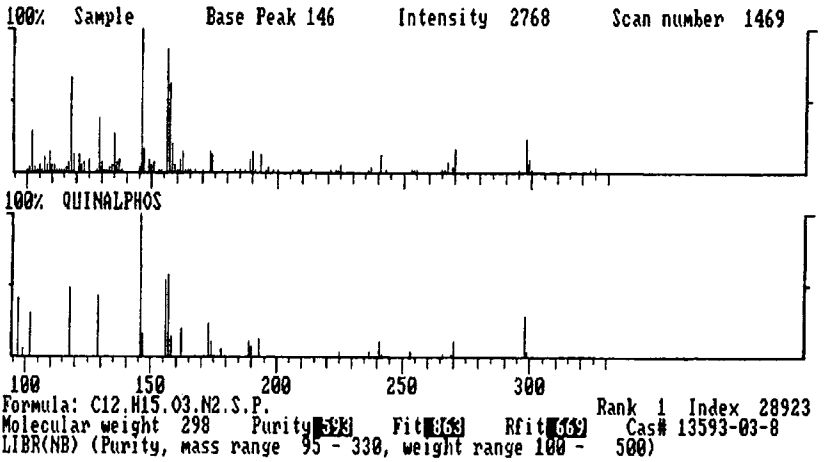


Fig. 4-9: Identification of quinalphos by the NBS library gives a FIT value 863 (INCOS).

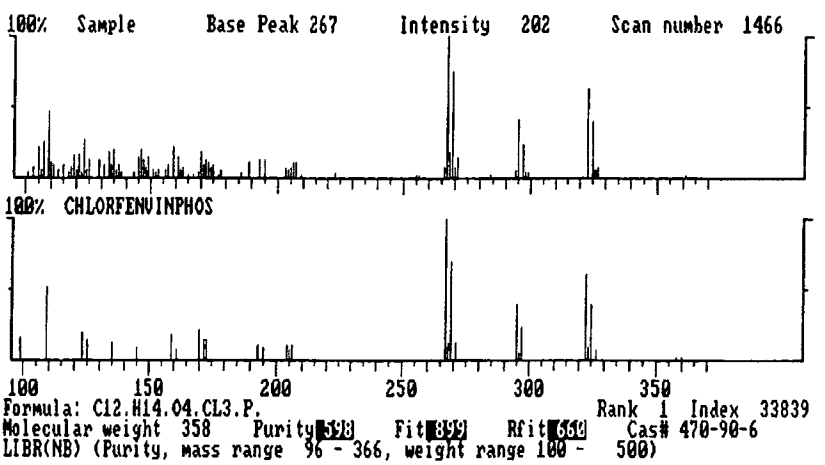


Fig. 4-10: Identification of chlorfenvinphos by the NBS library gives a FIT value 899 (INCOS).

4.5.2.3.2 The PBM Search Procedure

The PBM (probability based match) algorithm relies on an entirely different approach [4-14]. The statistical method developed by Professor McLafferty permits statement of the probable identity of a substance suggestion. This statement is determined on the basis of the spectral database used, which should be as extensive as possible for this procedure. The search procedure was likewise developed in the 1970s at Cornell University. In the ensuing years parts of the PBM procedure were adapted for use on personal computers, also under the name PBM but generally only comprising the less effective "pure search" mode. At the beginning of the 1990s, however, a PC version known as Benchtop/PBM became available which now includes the extremely powerful "mixture search" mode for the data systems of commercially available GC/MS systems.

In the PBM search procedure a significance weighting is also performed (sum of mass m and intensity I). The pre-search of the PBM procedure has a similar orientation to the nature of the significance weighting. The reference spectra are sorted in the order of their maximum significance in the database used for the PBM. For a given significance of an unknown spectrum a set of equally significant reference spectra can be selected. In this connection, the term depth of search refers to the selection of a number of sets of mass spectra, which, starting with $(m + I)_{max}$ reach, say, depth 3 with $(m + I)_{max\text{-}3}$. This increases the number of possible candidates for the main search.

The PBM main search can proceed in one of two ways. In the "pure search" mode only the fragments of the unknown spectrum are sought in the reference spectra and compared (forward search). This approach assumes that the mass spectra are free of matrix components and superposition, which will only occur in simple separations from medium concentration ranges upwards.

The "mixture search" mode first examines whether the mass signals of the reference spectrum are present in the unknown spectrum (reverse search). Local normalization analogous to that of the INCOS procedure is only included from Version 3.0 upwards (1993). During the course of processing, spectrum subtraction is performed between each one of the spectra selected in the pre-search and the unknown spectrum. The result of the subtraction is in turn compared with the remaining candidates of the pre-search and agreement criteria recorded. This accounts for the fact that even in high resolution gas chromatography mixed spectra will arise through matrix effects or co-elution. On use of the ion-trap technique in particular full scan information of numerous components is available in parallel for evaluation.

In the PBM search procedure the results are sorted on the basis of probabilities determined during the course of spectral comparison (Table 4-5). The prime objective is to provide a statement (Class I) about the identity of a proposal. The probability (Class IV) that a compound is present with structural characteristics which have little or no influence in the appearance of the mass spectrum means that the user is given a value for consideration which clearly indicates that the mass spectrum in question can hardly be unequivocally identified by mass spectrometry alone owing to its rather non-specific fragments.

In practice it is seen that the score of the PBM search depends upon the quality of the spectra. This is determined by the recording parameters of the instrument, the choice of spectrum in the peak (rising/falling flank, maximum), scan times, and differences between instruments. This is the reason why several spectra of the same substance from different sources are often present in libraries for PBM.

Table 4-5: Results of benchtop/PBM library search.

Class I	Probability that a suggestion is identical or a stereoisomer
Class IV	Probability that a compound is present, which shows structural differences from the reference having little influence on the pattern of the mass spectrum
% contamination	Gives the number of ions which are not present in the reference

4.6 Applications

The applications shown here illustrate the extremely wide range of applications of the mass spectrometer as detector in capillary gas chromatography. The flexible utilization of full-scan data recording in the EI and CI modes and the various aspects of data evaluation as employed in routine use are demonstrated. This section does not include examples of SIM analysis which are evaluated in the same way as chromatograms recorded with classical detectors.

4.6.1 Determination of Substituted Phenols in Drinking Water

Even in very low concentrations substituted phenols in drinking water lead to unpleasant odor and taste. Various phenolic components can arise from humic acids on chlorination of drinking water.

In order to determine substituted phenols (alkyl-, chloro-, and bromophenols, chloroalkylphenols, higher phenols) they are acetylated in the water sample and concentrated on C_{18} solid phases [4-15]. The detection limits lie between 1 and 10 ng/L, depending on the degree of substitution.

GC conditions: PTV cold sampling system 60 °C (0.05 min), 20 °/s to 250 °C (6 min); RT_X1701 capillary column, 30 m × 0.25 mm × 0.1 µm/helium, temperature program, 60 °C (1 min), 5 °/min to 280 °C (4.5 min).

MS parameters: ITS40-GC/MS system, EI/CI ionization, reactant gas methane, methanol, scan range 50–300 u, scan rate 1 s^{-1}, direct coupling 280 °C.

Figure 4-11 shows the chromatogram (total ion current) of a substituted phenol acetate standard (Table 4-6). Evaluation of the individual components proceeds via the selective mass traces (Figure 4-12) shown with *m/z* 122 for the alkylphenols, *m/z* 162 for the dichlorophenols, and *m/z* 196 for the trichlorophenols.

On use of an ion-trap mass spectrometer the phenolic compounds can still be detected from the mass spectrum even at the very low detection limits given. Analysis of a water sample (Figure 4-13) via the total ion current chromatogram and a mass trace is illustrated for the alkylphenols at *m/z* 122.

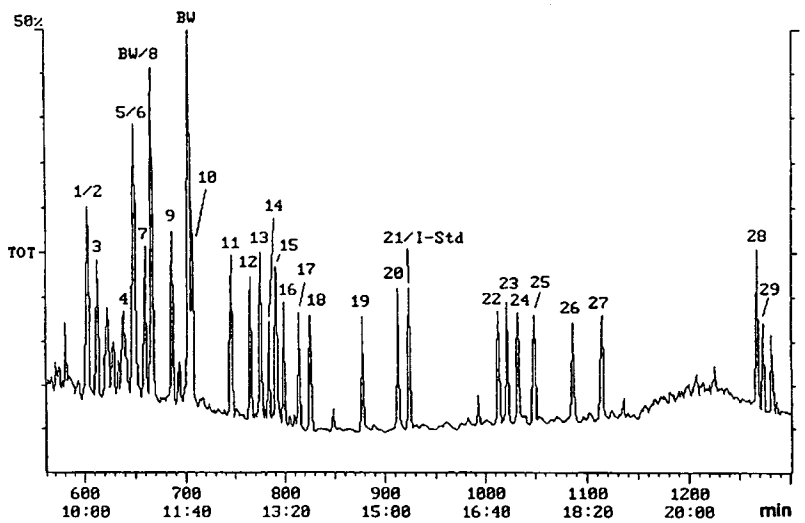


Fig. 4-11: GC/MS chromatogram of a standard mixture of phenol acetates.

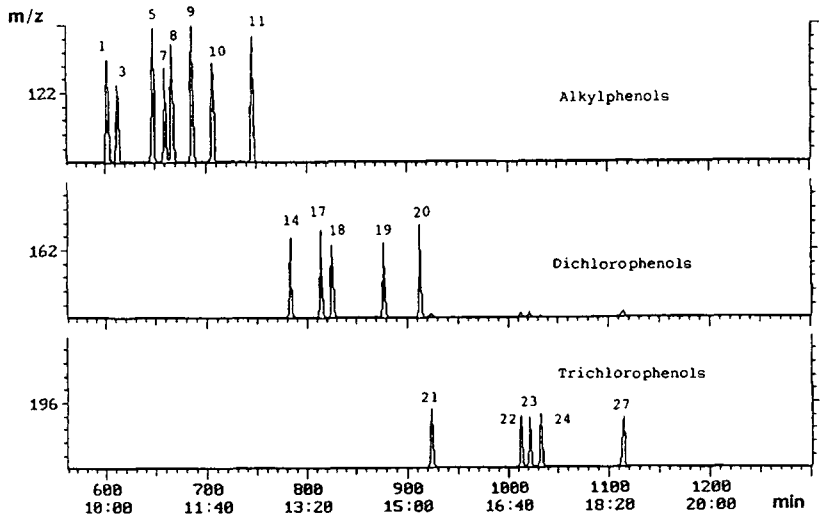


Fig. 4-12: Mass chromatograms of alkylphenols, dichlorophenols, and trichlorophenols.

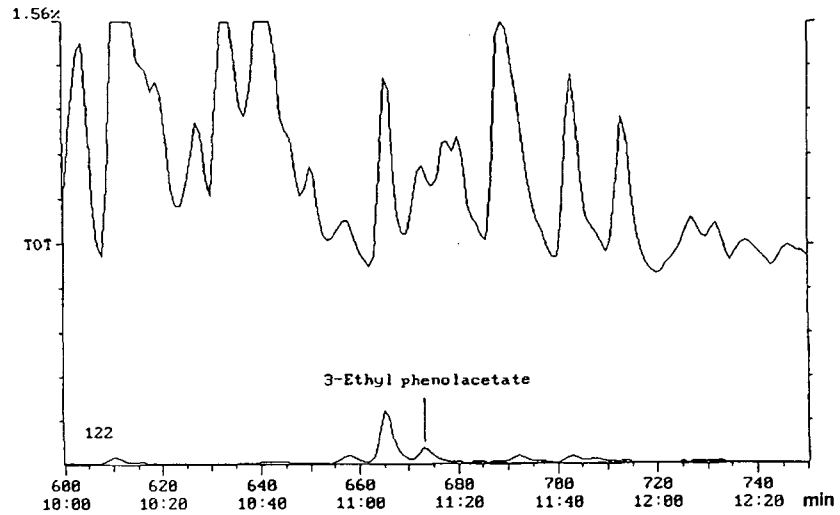


Fig. 4-13: Total ion current of a water sample with evaluation via the mass trace *m/z* 122 for alkylphenol acetates.

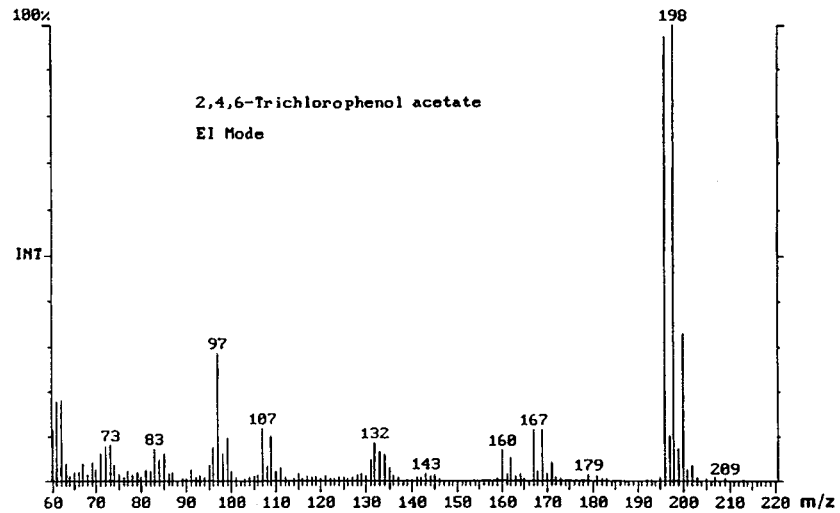


Fig. 4-14: EI mass spectrum of 2,4,6-trichlorophenol acetate.

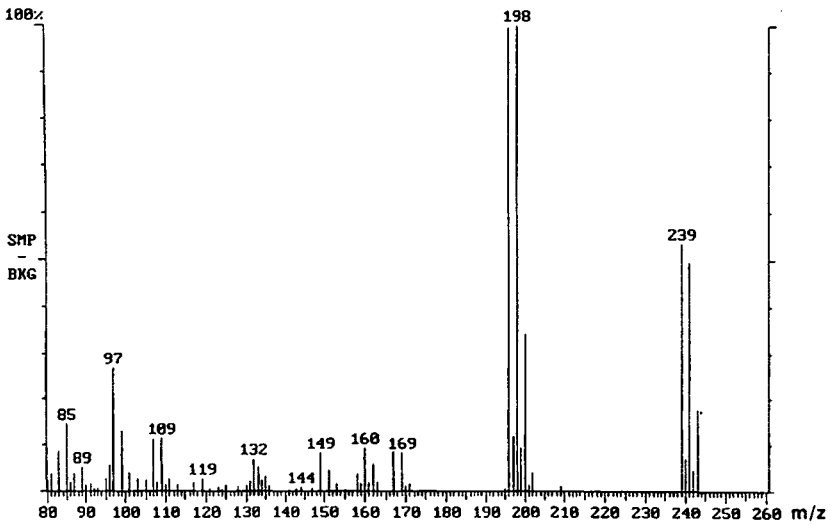


Fig. 4-15: CI mass spectrum of 2,4,6-trichlorophenol acetate.

Table 4-6: Composition of the standard in Fig. 4-11.

1	2,6-Dimethylphenol	16	4-Chloro-3-methylphenol
2	2-Chlorophenol	17	2,4-Dichlorophenol
3	2-Ethylphenol	18	3,5-Dichlorophenol
4	3-Chlorophenol	19	2,3-Dichlorophenol
5	2,5-Dimethylphenol	20	3,4-Dichlorophenol
6	4-Chlorophenol	21	2,4,6-Trichlorophenol
7	2,4-Dimethylphenol	22	2,3,6-Trichlorophenol
8	3-Ethylphenol	23	2,3,5-Trichlorophenol
9	3,5-Dimethylphenol	24	2,4,5-Trichlorophenol
10	2,3-Dimethylphenol	25	2,6-Dibromophenol
11	3,4-Dimethylphenol	26	2,4-Dibromophenol
12	2-Chloro-5-methylphenol	27	2,3,4-Trichlorophenol
13	4-Chloro-2-methylphenol	28	2,4,6-Tribromophenol
14	2,6-Dichlorophenol	29	4,6-Dichlororesorcinol
15	4-Bromophenol		

The specific mass chromatogram permits initial localization of the individual compounds. In spite of numerous matrix components elution of the alkylphenols is clearly shown.

Identification of the compounds is accomplished via the EI spectrum with confirmation from the pertinent CI spectrum. The EI and the CI spectrum are shown (Figures 4-14 and 4-15) for the trichlorophenol acetate. The molecular ion at *m/z* 238 is not detected in the EI mode. However, the intense chlorine isotope pattern *m/z* 196/198 is available for specific quantification. The CI spectrum of the compound shows the quasimolecular ion $(M + H)^+$ at *m/z* 239, which is formed by protonation with methane or methanol and confirms the suspected identity.

4.6.2 Determination of PCB, PAH, and Ugilec in Soil

The widespread distribution of polychlorinated biphenyls (PCB) and polycyclic aromatic hydrocarbons (PAH) in the environment makes the routine determination of these multicomponent mixtures very important. PCB substitutes such as Ugilec [4-16] represent a further challenge to detection methods. On using GC/MS, it is possible to determine PCB, PAH, and Ugilec simultaneously in a single analytical run [4-17]. This relies on the quality of the gas chromatographic separation and parallel mass spectrometric detection

of all components with their complete mass spectrum. The large number of components precludes SIM analysis.

The sample preparation methods used for far for PAH are suitable for such mixtures. PCB and Ugilec are also readily detectable by GC/MS methods in the extracts. As in EPA method 8270 deuterated analogs of PAH are used as internal standards. The deuterated standards behave like the PAH during sample preparation and chromatography. Since they elute almost simultaneously their used is restricted to GC/MS analysis.

GC conditions: PTV cold sampling system 50 °C, 12 °/s to 320 °C (15 min); HT 5 capillary column, 25 m × 0.22 mm × 0.1 µm/hydrogen, temperature program 80 °C (1 min), 20 °/min to 380 °C (1 min), 20 °/min to 280 °C (4.5 min).
MS parameters: ITD 800 MS system, EI ionization, scan range 120–400 u, scan rate 1 s^{-1}, direct coupling to GC, transfer line 320 °C.

A typical PAH chromatogram is shown in Figure 4-16. The mass trace *m/z* 252 shows the elution sequence of the isomers benzofluoranthenes, benzopyrenes, and of perylene (Figure 4-17) as a detail. Separation of the PCB mixture under the same conditions is shown in Figure 4-18; the individual components are labeled according to the Ballschmiter nomenclature. Mention should be made in this context of separation of the PCB isomers 31 and 28, which are resolved right the way down to the baseline.

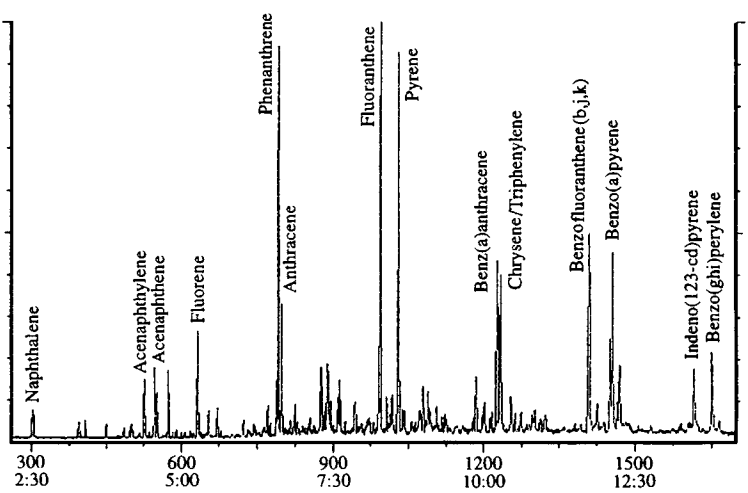


Fig. 4-16: Typical PAH analysis (total ion current) on the HT 5 capillary column.

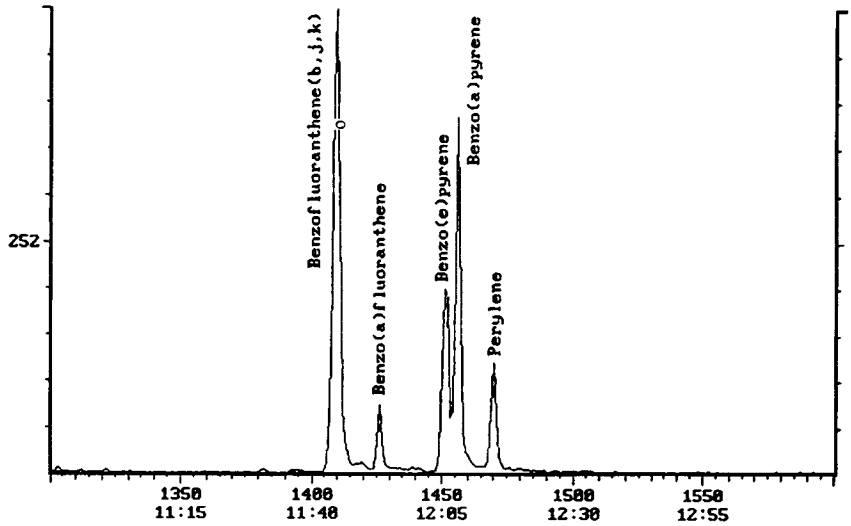


Fig. 4-17: Mass chromatogram for *m/z* 252 in the PAH analysis.

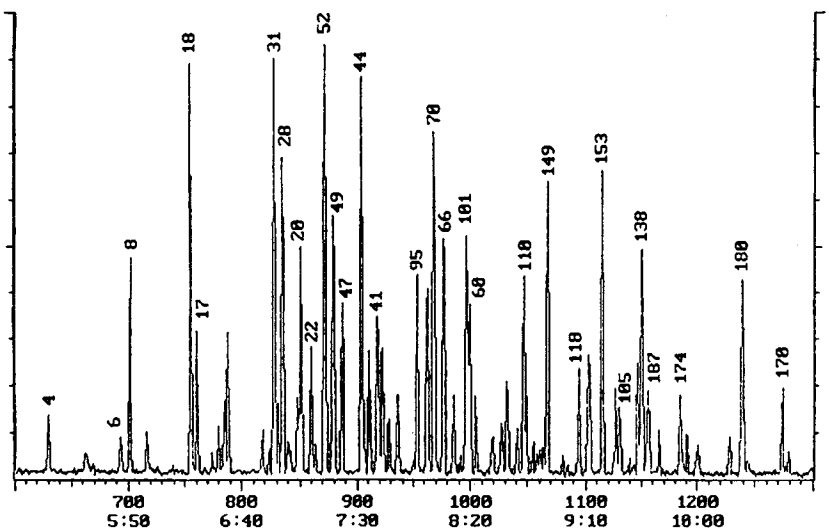
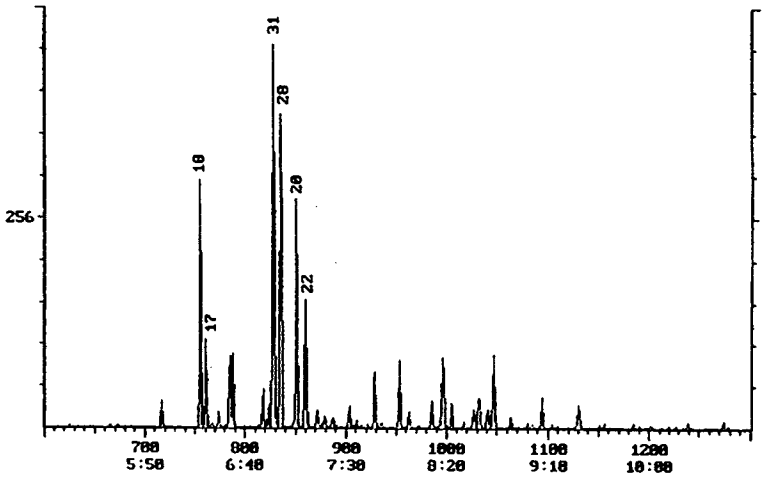


Fig. 4-18: Typical PCB analysis (total ion current) on the HT 5 capillary column.



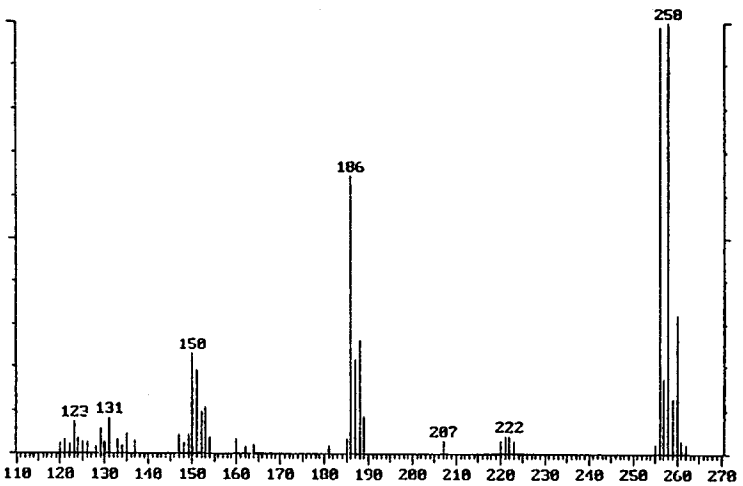
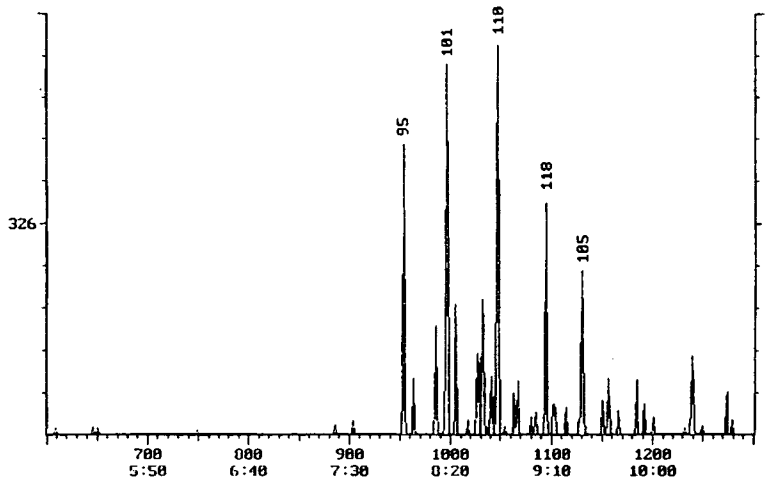


Fig. 4-19: Elution region of trichlorophenyls (*m/z* 256) and mass spectrum of a trichlorophenyl (PCB 28).



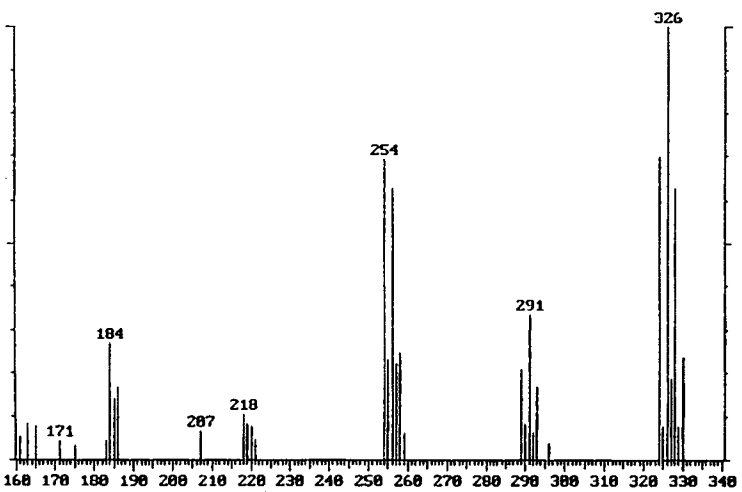
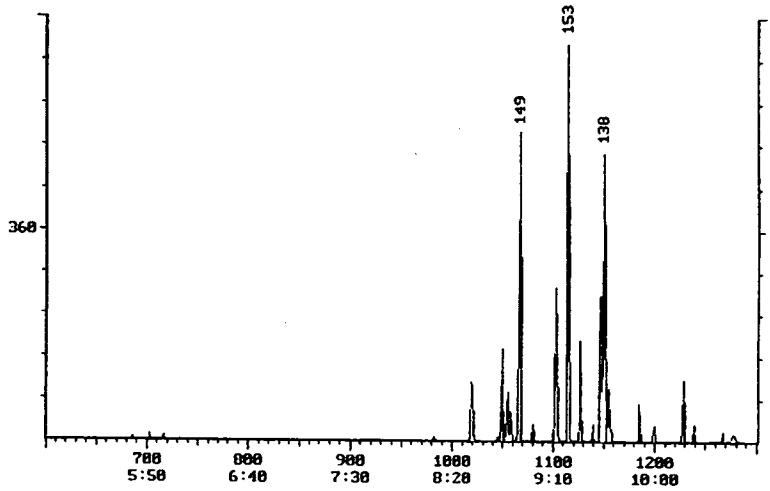


Fig. 4-20: Elution region of pentachlorophenyls (*m/z* 326) and mass spectrum of a pentachlorophenyl (PCB 101).



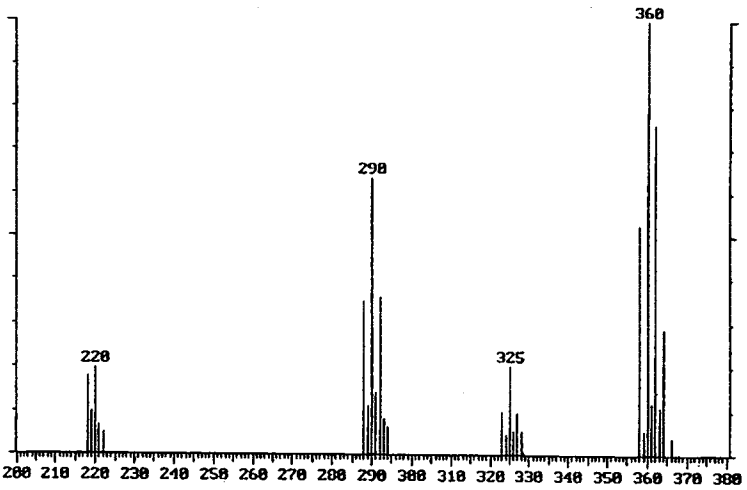


Fig. 4-21: Elution region of hexachlorophenyls (*m/z* 360) and mass spectrum of a hexachlorophenyl (PCB 153).

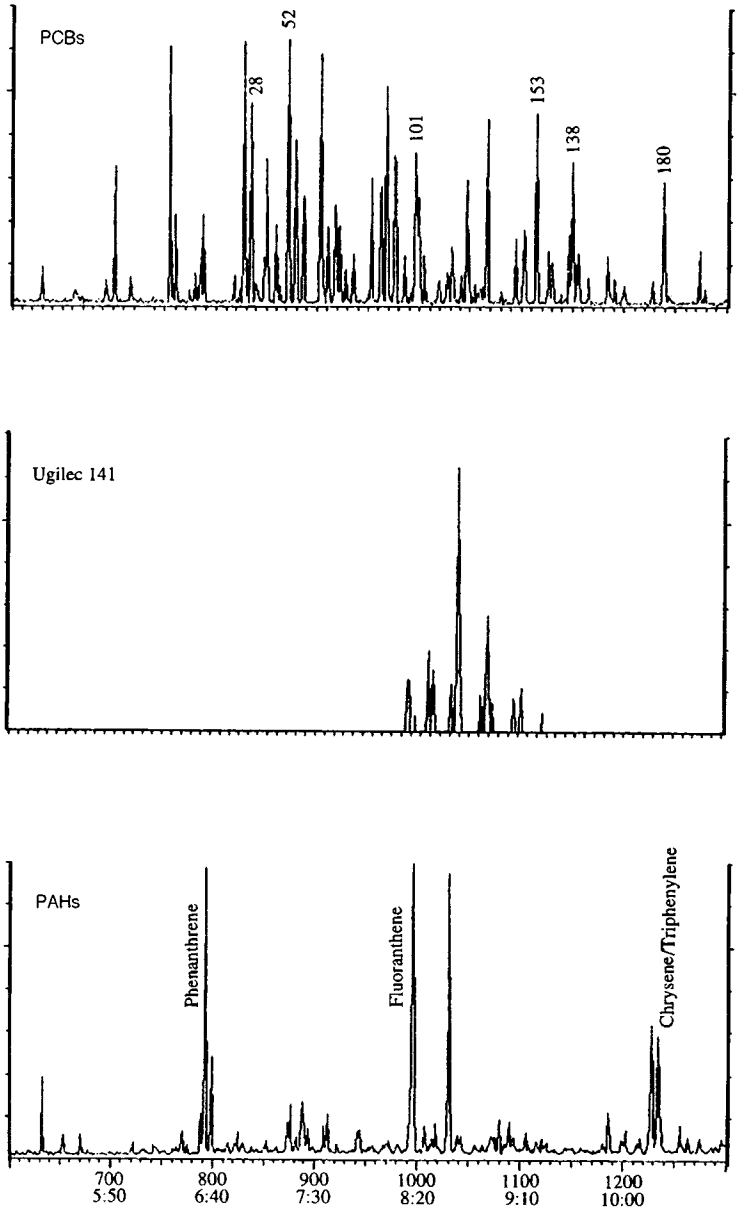


Fig. 4-22: Retention behavior of PCB, Ugilec, and PAH on a HT 5 capillary column, parallel ion trap GC/MS, parallel ion trap GC/MS detection.

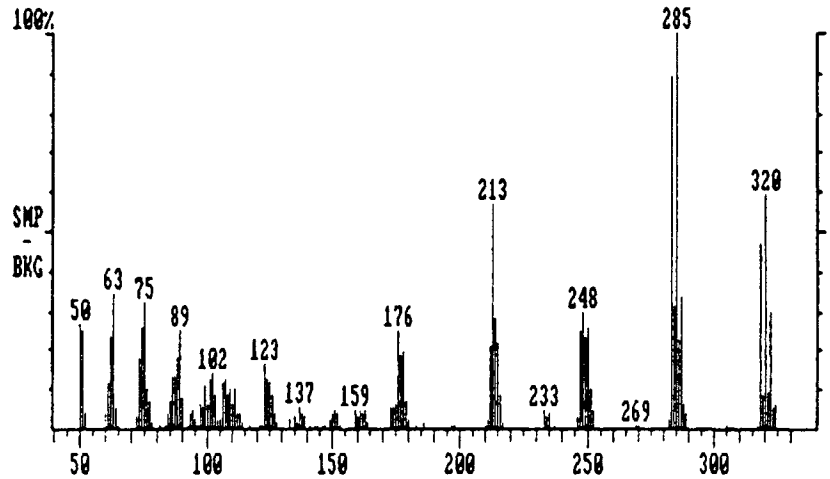


Fig. 4-23: EI mass spectrum of tetrachlorobenzyltoluene isomers (Ugilec).

Through appropriate choice of specific mass chromatograms the elution of the individual degrees of chlorination can be followed. Figures 4-19 to 4-21 show one characteristic spectrum each for the groups of trichlorobiphenyls, pentachlorobiphenyls, and hexachlorobiphenyls.

The retention behavior of the PCB in comparison to PAH and the PCB substitute Ugilec is shown for the selected analytical conditions in Figure 4-22. The way in which Ugilec covers the retention range of penta- (above about PCB 101) and hexachlorobiphenyls (up to about PCB 153) is clearly seen. Thanks to the readily distinguishable mass spectrum (Figure 2-23) Ugilec can be unequivocally determined by the ion trap GC/MS system in the presence of PCB and PAH.

4.6.3 Determination of PAH in Urban Air

Polycyclic aromatic compounds arise in all incidences of incomplete combustion of organic matter. In areas of conurbation the concentration of PAH exceed natural levels several-fold. Among the large class of PAH, 11 substances are known to be powerful carcinogens and 10 less powerful ones.

For determination of PAH in urban air [4-18] dust filters were extracted with toluene, the extract was cleaned-up by solid phase extraction (benzenesulfonic acid/silica gel) and analyzed by GC/MS.

GC conditions: SPI injector, 110 °C, 300 °/min to 30 °C (45 min); DB 5 capillary column, 30 × 0.25 mm × 0.25 µm/helium, temperature program 100 °C (3 min), 10 °/min to 200 °C, 5 °/min to 310 °C (10 min).

MS parameters: ITS40 GC/MS system, EI ionization, scan range 100–520 u, scan rate 1 s^{-1}, direct coupling 280 °C.

The entire chromatogram (total ion current) of a dust filter sample is shown in Figure 4-24. A section is shown in Figure 4-25 for the retention time range of 10 min to 13 min, with the mass trace *m/z* 182 being depicted under the total ion current. In this range dimethylbiphenyl isomers elute which were identified by comparison of their mass spectrum (Figure 4-26) with the NIST library (Figure 4-27). Since the isomers have almost identical spectra, mass spectrometry cannot be used for assignment of the isomers.

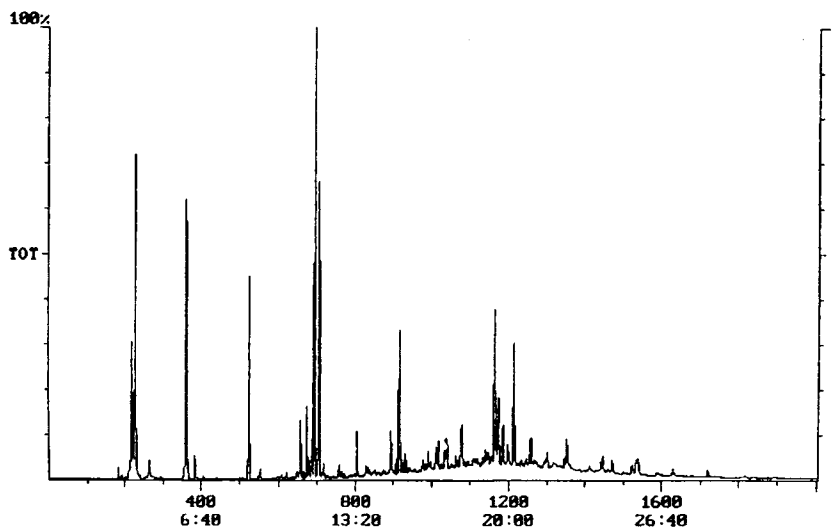


Fig. 4-24: Total ion current chromatogram of a dust filter extract.

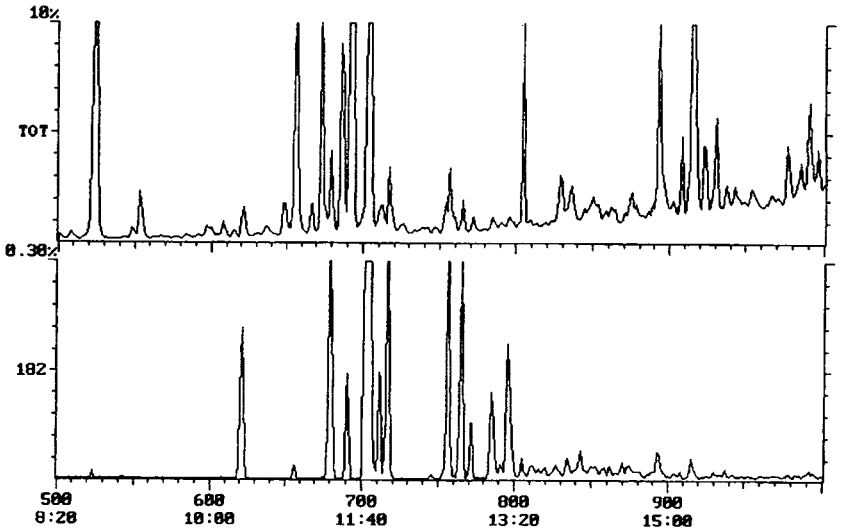


Fig. 4-25: Analysis for PAH via the mass chromatogram *m/z* 182.

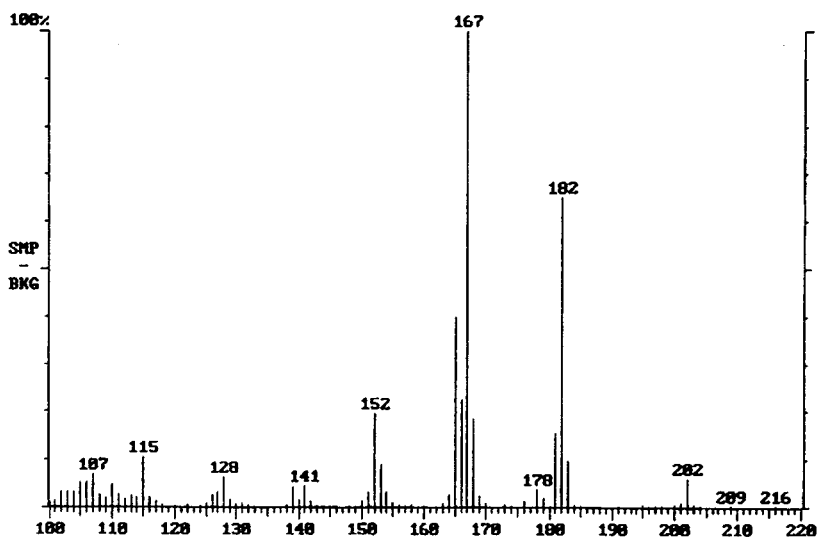


Fig. 4-26: EI mass spectrum of a dimethylbiphenyl isomer.

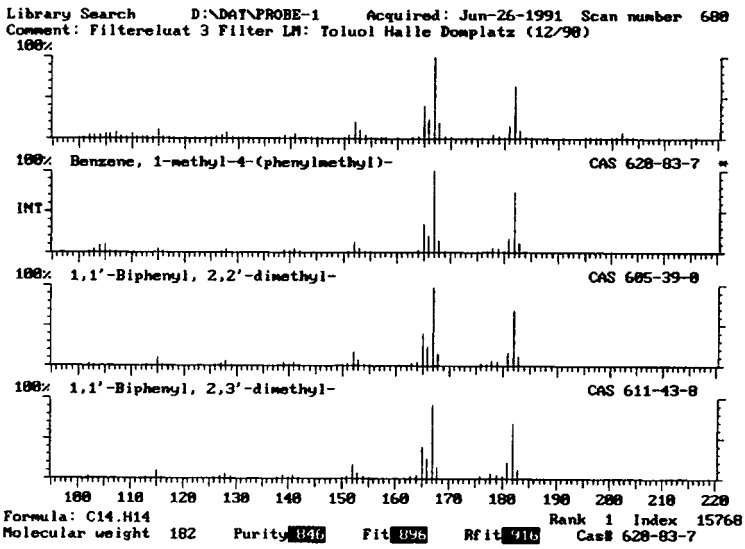


Fig. 4-27: Identification through an INCOS library search (NIST library).

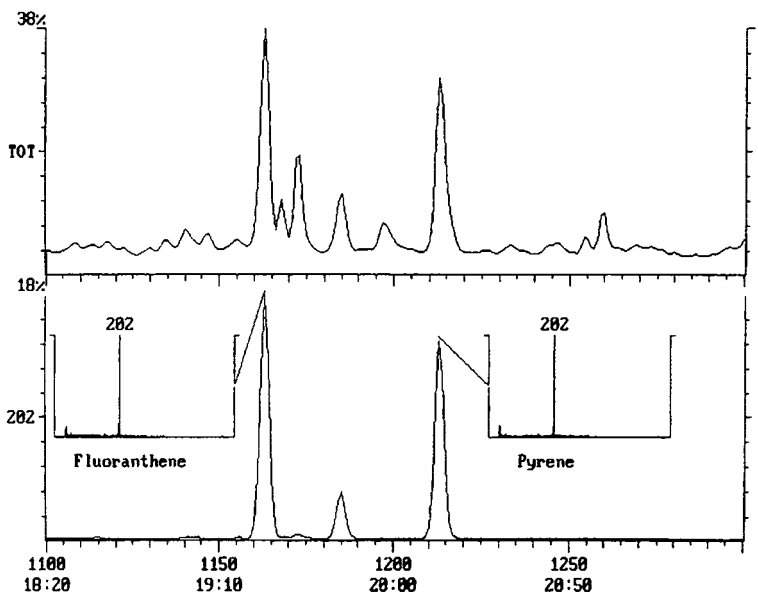


Fig. 4-28: Analysis for PAH via the mass chromatogram *m/z* 202 with the mass spectra of fluoranthene and pyrene.

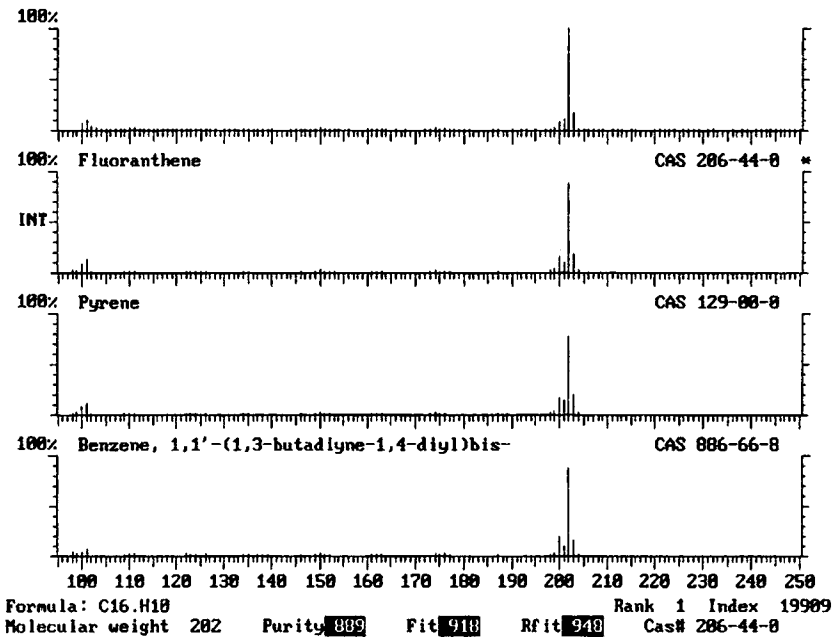


Fig. 4-29: Identification through an INCOS library search (NIST library).

A similar situation is shown for fluoranthene and pyrene in Figure 4-28. The elution of the components is shown by the specific mass chromatogram *m/z* 202. Owing to the pronounced similarity of their spectra, the result of the library search (Figure 4-29) suggests both isomers with a high degree of correspondence (FIT > 900). Such cases require a combination of retention time and mass spectrum for identification.

4.6.4 Determination of Clenbuterol

Clenbuterol is not only used for doping in competitive sport. It is a recognized human and veterinary drug, but is also misused as an aid in fattening animals for slaughter. Identification of clenbuterol in meat requires analytical method offering a high degree of certainty in the trace range. For this reason, GC/MS with chemical ionization has become the established method for clenbuterol.

Prior to GC/MS analysis [4-19] clenbuterol is derivatives with hexamethyldisilazane to form the trimethylsilyl compound (clenbuterol-TMS). Methane is used as reactant gas.

GC conditions: Injector 270 °C isothermal, splitless, DB 5 capillary column, 30 m × 0.25 mm × 0.1 μm/helium, temperature program, 80 °C (1 min), 20 °/min to 160 °C, 10 °/min to 250 °C (1 min).

MS parameters: ITS40-GC/MS system, CI ionization, manifold temperature 180 °C, reactant gas methane, scan range 120–400 u, scan rate 1 s^{-1}, direct coupling 280 °C.

The CI mass spectrum of clenbuterol-TMS (Figure 4-30) shows the quasi-molecular ion $(M + H)^+$ *m/z* 349 with the isotope pattern of two Cl atoms as base peak. The fragmentation occurring is unavoidable on use of methane as reactant gas.

In a typical CI chromatogram (Figure 4-31) the elution of clenbuterol-TMS is clearly evident from the specific mass trace *m/z* 349/351. These ions are also used in quantification. Figure 4-32 shows the calibration of clenbuterol-TMS in the range of 25 pg to 2 ng as the amount injected.

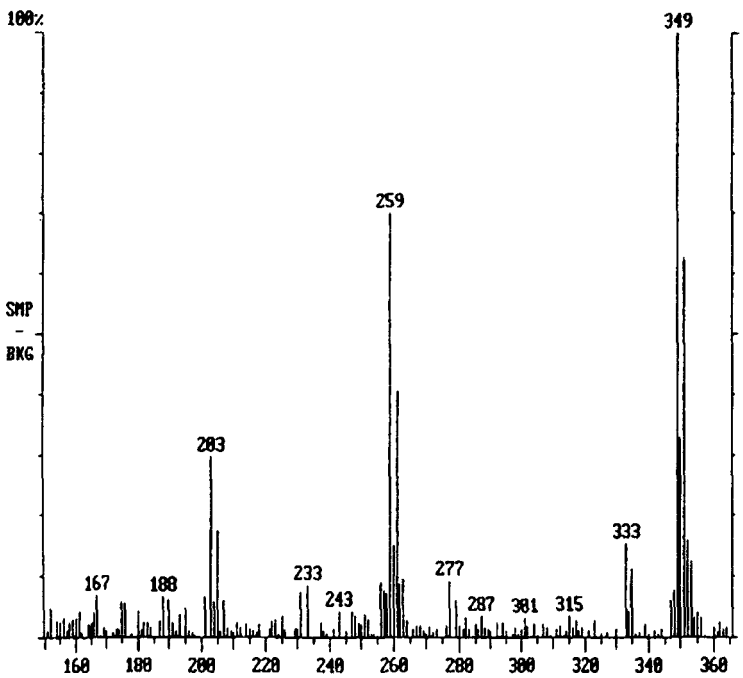


Fig. 4-30: CI mass spectrum of clenbuterol-TMS, reactant gas methane.

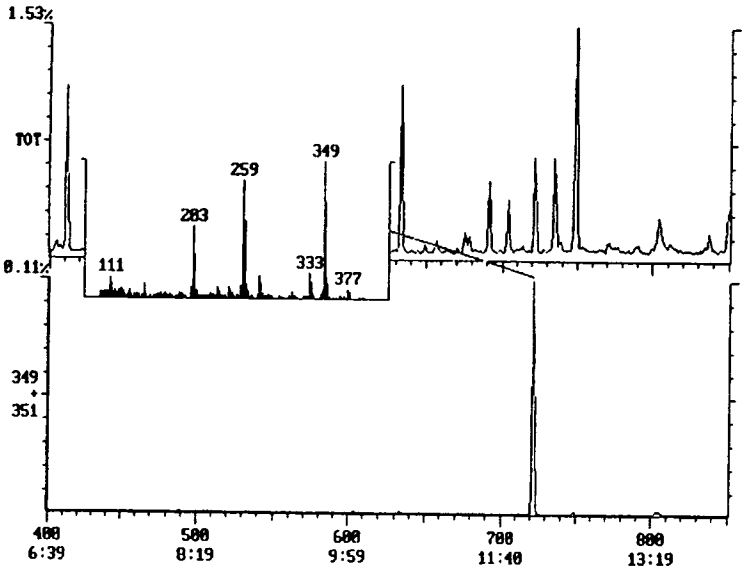


Fig. 4-31: Total ion current and mass chromatogram of a clenbuterol analysis, CI methane.

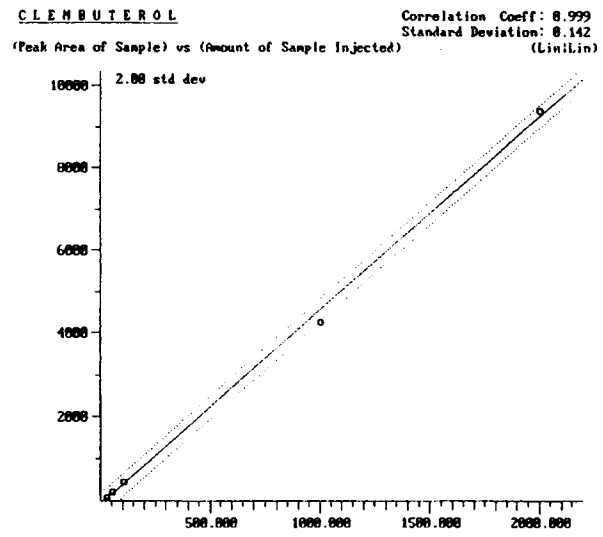


Fig. 4-32: Calibration function for clenbuterol determination by CI-GC/MS.

4.7 Summary

The mass spectrometer represents an extremely powerful and flexible detection system for high resolution gas chromatography. Depending upon the choice of analyzer, exact mass determinations, screening, and multicomponent analysis are all possible with the appropriate degree of precision and reliability for the task at hand. The broad range is partly due to the universal nature of EI ionization. In addition, extremely specific detection tasks become possible through the choice of high resolution or chemical ionization techniques.

Today's ideal GC/MS coupling techniques provide latitude for use of the optimum conditions for the formerly separate procedures – open and direct coupling – without analytical restrictions arising from the coupling. Rapid advances in computing have rendered fast automatic extraction and processing of analytical data from the huge volumes of information of GC/MS feasible. Combination of the evaluation of direct substance information with specific quantification provides gas chromatography with a reference procedure without equal in other chromatographic disciplines.

Acknowledgment

I am grateful to B. Rönnefahrt of the Public Analyst's Office of the City of Hamm for providing the results of an analysis of lemon for plant protection agents in the EI and CI modes, and to Dr. Schlett of Gelsenwasser AG, Gelsenkirchen, for analytical data relating to phenol analysis in drinking water.

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