CHIMIA 51 (1997) Nr. 10 (Oktober)

Chimia 51 (1997) 756–759

ISSN 0009–4293

Stereoselective Separation Combined with Mass Spectrometry: An Indispensable Tool for the Quantification and Identification of Toxins in the Ultra Trace Range

Michael Oehme*, **Ruth Baycan-Keller**, **Martin Biniosek**, **Heidi Karlsson**, **Stephan Kölliker**, **Luzius Müller**, and **David Pekar**

Abstract. A short survey is given over the main fields of interest of the recently established research group for organic analytical chemistry at the University of Basel. Organic trace analysis and structure elucidation in the pg to ng range are carried out using high resolution gas and chromatography liquid chromatography coupled to single and multiple mass spectrometry. Important ionisation techniques are electrospray, atmospheric chemical ionisation and negative ion chemical ionisation. Beside the development of isomer and enantiomer selective HRGC capillaries and techniques, the techniques mentioned above are applied to problems in the field of environmental analytical chemistry such as the determination and structure elucidation of mycotoxins and pesticides as well as atmospheric polar pollutants. Further fields are the identification of alkaloids in plant extracts and synthetic perfume compounds in detergents.

Introduction

The research group for organic analytical chemistry at the University of Basel has been recently established from scratch. The first doctoral students started in February 1996. Our research interests are partly a continuation of the research carried out when *M. Oehme* was still the head of the analytical chemistry department and a research group at the Norwegian Institute for Air Research and at the University of Tromsø, Norway during the past 15 years. At this time trace analysis methods were developed to study the environmental behaviour and dispersion of persistent polychlorinated compounds with special emphasis on vulnerable polar ecosystems. A summary of some previous work is given in [1]. A much broader field of research is planned and already on-going in Basel. However, the combination of organic analytical chemistry and environmental chemistry will still be a main field of interests.

**Correspondence*: Prof. Dr. M. Oehme
Organic Analytical Chemistry
University of Basel
Neuhausstr. 31
CH–4057 Basel
Tel.: +41 61 639 23 01
Fax: +41 61 639 23 00
E-Mail: oehme@ubaclu.unibas.ch

What Are Our Interest?

Biologically active compounds may cause effects in organisms at extremely low exposure levels. For example, pg to ng/kg body weight of anthropogenic persistent organic pollutants (POPs) such as pesticides or polychlorinated biphenyls can induce immunosuppressive or endocrine effects. Furthermore, biogenic toxines such as neurotoxins from algae (*e.g.* anatoxin, saxitoxins) or mycotoxins such as trichothecenes from fungi growing on cereals can show high acute toxicity. Toxic blue-green algae start to become a major threat of the fresh water resources within certain regions of the world such as the Northern boreal zone. Also parts of the Alps are affected (canton Tessin). The lethal dose of some neurotoxic alkaloids belonging to the anatoxin group can be in the range of 50 µg/kg body weight. Both the death of cattle as well as an intoxication of drinking water resources, *e.g.*, in the Baltic countries have been observed.

The natural toxins of interest are relatively small and polar molecules with masses below 500 u. Concentrations varying from sub-ppb to high ppb levels and the presence of homologues not been identified yet make it highly desirable to have a method which has sufficient sensitivity and allows to elucidate structures with only a few ng without isolation of the compound. High performance liquid chromatography (HPLC) combined with multiple mass spectrometry (MS^n) is, therefore, an important tool in our group which is also applied to other fields such as identification of carbonyl derivatives originating from atmospheric samples and plant alkaloids. A more detailed survey is given in one of the following subchapters.

Two other minor fields of interest deal with environmental analysis. The first one is the development of sampling and detection methods for polar volatile organic compounds (VOC) in ambient air originating from different industrial and combustion sources. Though such compounds might represent more than 30% of the totally emitted VOC, both data and suitable methods are scarce. The second one deals with the detection of persistent synthetic perfume additives in detergents which might be neurotoxic and are able to bioaccumulate in the food chain. Both subjects will be presented in more detail later.

The very low effect levels of POPs and their environmental behaviour leading to a global dispersion and a high bioaccumulation in the food chain, require detection limits in the pg range to study transport paths and to detect critical loads. Furthermore, the lower the detection limits, the earlier problems can be discovered before they are a threat to the environment.

The steric structure of a molecule has a great influence on its toxic properties. Polychlorinated bornanes, cyclodienes and aromatics belong to the POP classes being most relevant at present. The first two groups consist of hundreds of chiral structures, show both endocrine and carcinogenic properties, and are found ubiquitously in the environment and at any stage of the food chain. A study of such compounds in the environment requires techniques which allow a simultaneous isomer and enantiomer-specific determination of pg quantities. The development of such techniques based on high resolution gas chromatography (HRGC) and their application to environmental problems is one of our main tasks which can be summarised as follows:

- Development of separation systems being able to separate simultaneously a large number of isomers and enantio-

mers in air, water and biogenic samples without disturbances by matrix interferences. Detection limits should be a few pg to allow measurements both in transport media such as the atmosphere and the lowest levels of a food chain.

- Identification and isolation of compounds of toxicological interest and preparation of enantiopure reference substances for *in vitro* studies.
- Development of detection methods which have the necessary selectivity and detection limits.
- Application of such methods to real world problems and study of the parameters which influence the mobility, bioaccumulation and metabolisation of a POP.

Enantioselective Separations by HRGC in the Ultratrace Range

HRGC combined with mass spectrometry using different ionisation techniques is the method of choice to separate and detect such tiny quantities stereoselectively. Beside the development of suitable sample clean-up techniques to remove large quantities of sample matrix, the selectivity which has to be achieved by the separation system, is the biggest challenge. Modified cyclodextrins are popular chiral stationary phases for enantioselective separations by HRGC. However, when applying them for the analysis of POPs, one will experience several severe problems: The presence of a large number of chiral isomers will lead to a high risk of signal overlaps between pairs of enantiomers. Solutions of this problem are the development of tandem- or multidimensional techniques using different isomer and enantiomer-selective columns [2][3].

The enantioselective properties which are necessary to separate enantiomers are still not well understood. One of the reasons is that the synthesised alkyl-chain modified cyclodextrins often consist of a mixture of stereoisomers and homologues with different properties [4]. The characterisation of such mixtures is not easy, and we found that HPLC combined with multiple fragmentation mass spectrometry (MS^n) is the most promising technique (see also below). *Fig. 1* shows the HPLC/MS chromatogram (atmospheric pressure chemical ionisation, cation detection, (APCI(+)) of (2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin). A series of overmethylated homologues are present though the intermediate (2,6-di-*O*-methyl)-β-cyclodextrin was pure. Similar mixtures are also used for commercially available cap-

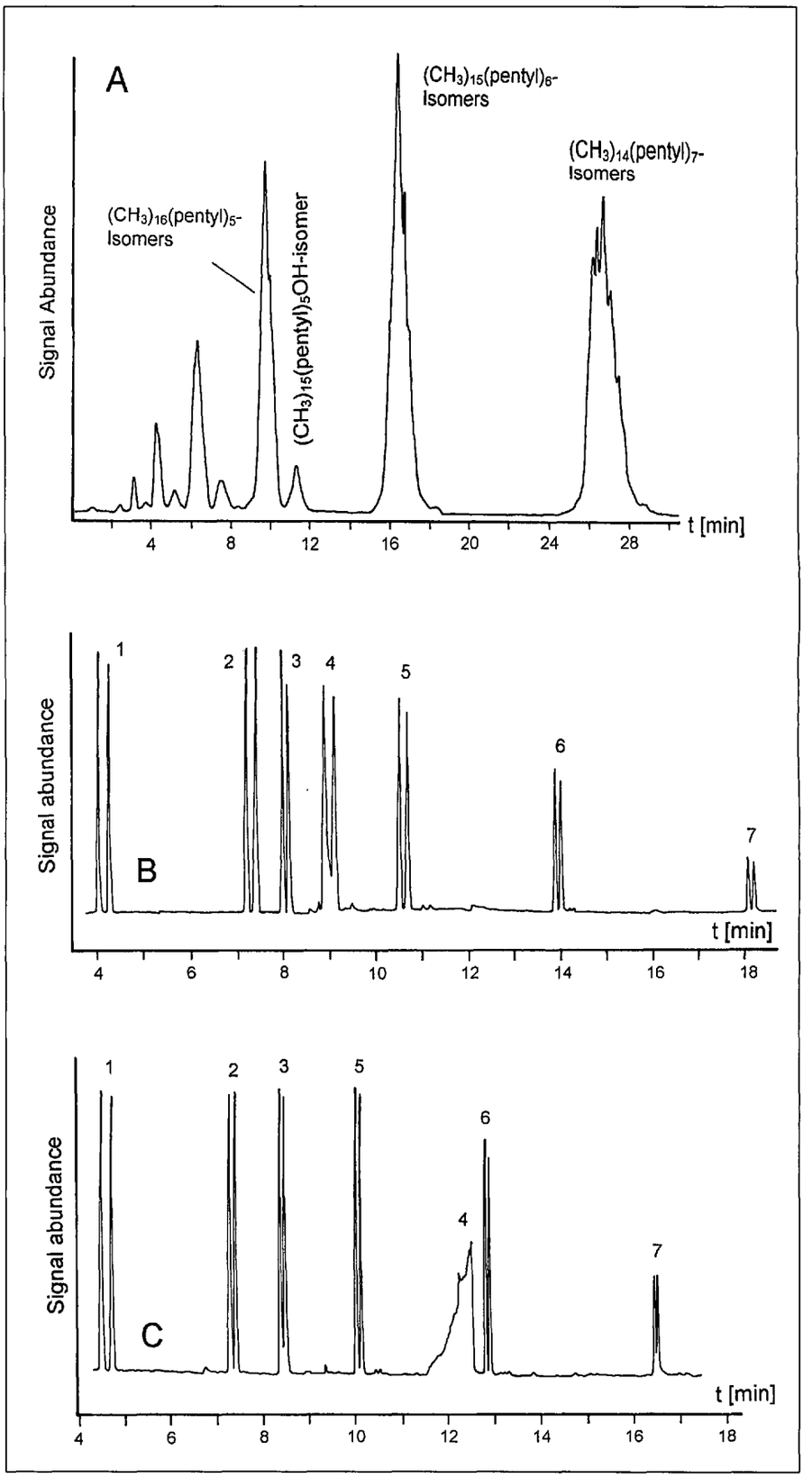

Fig. 1. A) *Full-scan chromatogram obtained by HPLC/APCI(+)-MS of (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin after normal purification by column chromatography. B) Gas chromatogram of an enantioselective capillary* (15 m × 0.25 mm i.d.) *coated with 10% of the last-eluting fraction with the correct mass spectrum in 90%* OV-1701 (see also [4]). C) *Enantioselective capillary coated with the overmethylated product eluting at 16.5 min.* The compounds in the modified *Schurig* test (see [4]) for enantioselective capillaries indicate different separation properties. 1: α-pinene, 2: methyl 2-ethylhexanoate, 3: γ-valero lactone, 4: α-phenylethylamine, 5: linanool, 6: 2-ethylhexanoic acid, 7: phenylethyleneglycol. HPLC separation: *Nucleosil* C_{18} 125 × 2 mm, 5 µm (*Macherey-Nagel*), eluent 100% methanol.

illaries. As can be seen from *Fig. 1*, these homologues show different separation properties. Batch-to-batch differences in column inertness might occur and even changes in the elution order of both isomers and enantiomers can be observed, if the cyclodextrin is not purified by preparative HPLC [4]. Therefore, a very thorough characterisation of the separation properties of each single column is required using enantiopure standards [4–6].

Furthermore, this heterogeneous composition influences also the thermostability of the stationary phase resulting in a more pronounced stationary phase bleeding and an upper temperature limit around 250°. Reactions with the stationary phase might also occur as in the case of polychlorinated bornanes. These are severe limitations for the introduction of enantioselective separations as a routine tool in environmental analysis. Compared to pharmaceutical analysis where enantioselective separations are carried out regularly, the enantiomer specific determination of POPs in environmental analysis is still in its infancy. However, it is gaining a strongly increasing interest as special sessions at environmental conferences show. Only about ten groups (including ours) are really active in this field and are faced with the interest of hundreds who would like to make use of it. Therefore, one of the major aims of our group is to make the enantioselective analysis of pg quantities a reliable and predictable tool. This includes both the improvement of the purity and stability of enantioselective phases, the development of testing procedures including the preparation of enantiopure standards, the understanding of the enantioselective separation process using polychlorinated cyclodienes and bornanes as model compounds as well as molecular modelling of the steric interactions between host and guest.

Chemical ionisation mass spectrometry has been a very important tool for us to achieve the necessary low detection limits and to improve the selectivity further. Electron capture mass spectrometry is one of the techniques which has been optimised by us throughout the years allowing also the differentiation of isomers by ion-molecule adduct formation [7]. Future plans are the use of enantioselective chemical ionisation techniques in mass spectrometry. Finally, as a major application we also study the influence of biological parameters on the enantioselective accumulation and degradation of POPs in biota. Compound group of interest are polychlorinated bornanes (toxaphene) and cyclodienes (chlordane). Both have been and are still applied as technical pesticides

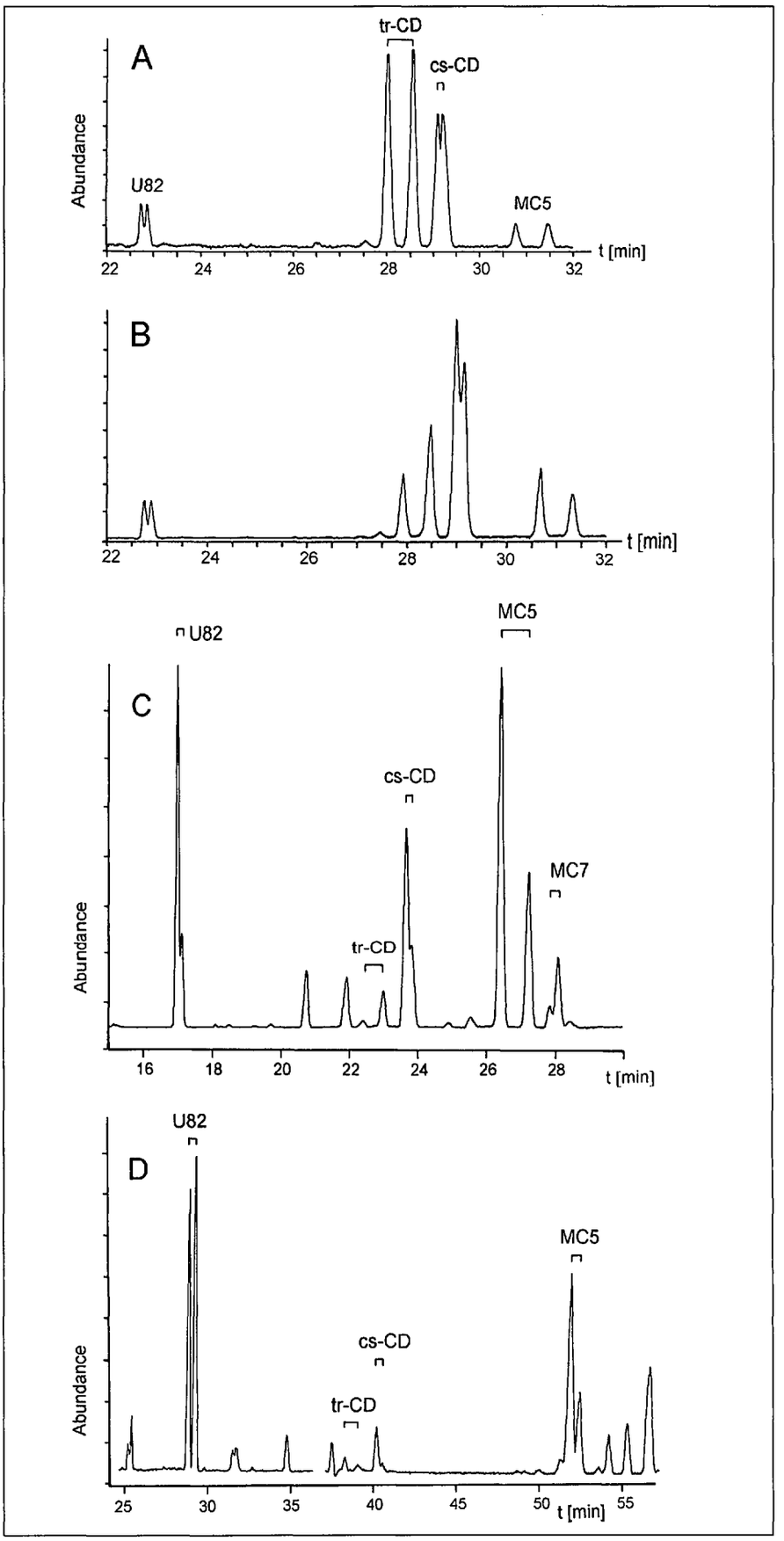

Fig. 2. *Enantioselective separation of chlordane isomers with tandem capillaries* [2]. *A*) Racemic enantiomer ratios in ambient air. *B*) Herring oil from the North Sea. *C*) Seal blubber. *D*) Human adipose tissue. Compared to *trans*- (*tr*-CD) and *cis*-chlordane (*cs*-CD) the isomers U82 and MC5 (see [2][4]) are strongly accumulated in the food chain. The isomer and enantiomer concentration ratios are very species-dependent.

in large quantities (*e.g.* $1.3 \cdot 10^6$ t for toxaphene) and consist of a hundreds of chiral congeners. Factors influencing the bioaccumulation and metabolisation of these structures are the number and position of Cl-atoms in the molecule, the trophic level of the studied organism in the food web as well as age, sex and species. Here, we found highly significant gender and species-related differences [6][8]. *Fig. 2* shows the enantioselective accumulation and metabolisation of chiral chlordane isomers in different species as an example. Furthermore, it seems that a relation might exist for polychlorinated bornanes [3] between an enantioselective degradation and the steric structure of the isomer. Based on different cooperations with groups in Canada, Sweden and the Netherlands as well as companies, we consider our activities in the field of environmental enantioselective analysis and method development to be in front worldwide.

Trace Analysis by HPLC/MS^n

Another main field of interest is structure elucidation and quantitative analysis of small polar molecules. So far, the determination and structure confirmation/elucidation of trace amounts of small polar and/or thermolabile molecules (< 500 u) is still problematic. HRGC/MS has been frequently used together with derivatization. HPLC/MS in the full scan mode has usually not low enough detection limits, and MS/MS with quadrupoles does not yield sufficient information for structure elucidation. The very recently introduced combination of HPLC with ion trap multiple fragmentation mass spectrometry (MS^n) by the company *Finnigan* has opened new perspectives. We received in Basel the very first HPLC/MS^n system in Europe about a year ago. Our intention is to develop HPLC/MS^n further and to make it an attractive method for trace analysis of, *e.g.*, biogenic toxins such as fusaria and algae/mussel toxins. In addition, these compound groups are still not fully explored, and it is expected that new homologues and related structures can be found in real samples.

HPLC/MS^n has both the necessary sensitivity to record full mass spectra with ng and sub-ng amounts and can achieve the needed selectivity by multiple MS/MS. The latter also allows to elucidate (at least partly) unknown structures employing a few ng. By consecutive fragmentations a molecule will loose side chains and functional groups until the basic skeleton or major parts are left. Related molecules with the same basic structure will then give the same mass spectrum of the corresponding fragment ion. In this way not yet known homologues and derivatives of compound group can be identified in real samples by HPLC only.

Examples of what has been studied in our group are the identification of differently substituted aconitum alkaloids and their homologues using electrospray ionisation in the positive ion mode (ESI(+)) [9]. Furthermore, APCI(–) MS/MS and MS^n were used to identify substructures being present in the mass spectra of 2,4-dinitrohydrazone (DNPH) derivatives of carbonyls formed by photochemical degradation processes in the atmosphere. The identification and quantification of such compounds are important for the understanding of atmospheric chemistry. The carbonyls are usually transformed to the more stable DNPH derivatives during sampling. Air samples contain hundreds of carbonyl compounds, and HPLC is not able to separate them sufficiently. However, MS^n allows immediately to determine important substructures and to differentiate between aldehydes and ketones, unsaturated, saturated and aromatic systems as well as straight chain and branched molecules just to mention some possibilities [10]. A fragmentation scheme for the identification of carbonyls has been established recently and is applied within an EU project studying their atmospheric formation. As a next step the suitability of HPLC/MS^n will be studied for the detection of fusaria toxins in cereals. At present our group has the lead in optimising HPLC/MS^n conditions for trace analysis. Beside instrumental aspects, critical points are the solvent and water purity, the insufficient hydrolysis resistance of stationary phases leading to increased background as well as the bleeding of additives from polymers into the separation system.

Volatile Organic Compounds in the Atmosphere and Perfume Additives

Our group is also involved in the development of monitoring methods for polar volatile organic compounds in ambient air. Sampling is based on passive diffusion samplers and quantification is carried out by capillary GC/MS. Challenges are the necessary resolution power of the chromatographic system and the contamination-free sampling and sample handling. In ambient air a large number of oxygen containing compounds such as ethers, ester and ketones have to be separated from other components. Here, the development of new separation systems based on tandem capillaries has improved the separation efficiency and, in principle, hundreds of compounds can now be determined simultaneously. Due to lack of suitable methods, the determination of polar organic compounds in ambient air has been somewhat neglected, and at present only a few groups are working actively in this field.

Finally, it has been shown that some of the artificial perfumes frequently added to washing powders and detergents are rather persistent. Some showed even neurotoxic and endocrine activities. They can already be found in the food chain and in human breast milk. The best known compound class are the artificial polycyclic musk compounds. However, several hundreds of other substances are probably also in use. Since such additives have not to be declared and no regulation exists, the analysis of detergents and the identification of both odorous compounds and their degradation products after the washing process is the only way to get an idea about the presence and use of such compounds. The development of extraction methods for perfumes in detergents is quite challenging since interfering matrix compounds with similar properties have to be eliminated. Identification is carried out by GC/MS. Recently, this work has been started in our group and will be followed up by modelling the environmental behaviour of the identified compounds.

We like to thank the *Swiss National Science Foundation* (project No. 2100-043399.95 and 2100-046665.96) and the *Fund of the Swiss Chemical Industry* in Basel for financial support.

Received: July 8, 1997

[1] M. Oehme, M. Schlabach, R. Kallenborn, J. E. Haugen, *Sci. Total Environ.* **1996**, *186*, 13.
[2] M. Oehme, R. Kallenborn, K. Wiberg, C. Rappe, *J. High Resolut. Chromatogr.* **1994**, *17*, 583.
[3] H.-J. de Geus, R. Baycan-Keller, M. Oehme, J. de Boer, U.A.Th. Brinkman, *J. High Resolut. Chromatogr.* **1997**, submitted.
[4] M. Oehme, L. Müller, H. Karlsson, *J. Chromatogr.* **1997**, *775*, 275.
[5] H. Karlsson, M. Oehme, L. Müller, *Organohalogen Compd.* **1996**, *28*, 405.
[6] M. Oehme, H. Karlsson, R. Kallenborn, *J. Toxicol. Environ. Chem.* **1997**, in press.
[7] M. Oehme, *Fresenius J. Anal. Chem.* **1994**, *350*, 544.
[8] K. Wiberg, M. Oehme, P. Haglund, H. Karlsson, M. Olsson, C. Rappe, *Mar. Pollut.* **1997**, in press.
[9] M. Oehme, S. Kölliker, *Labo* **1997**, August issue, 20.
[10] S. Kölliker, M. Oehme, *Anal. Chem.* **1997**, submitted.