

# The substitution of toxicologically critical solvents in the residue analysis of pesticides

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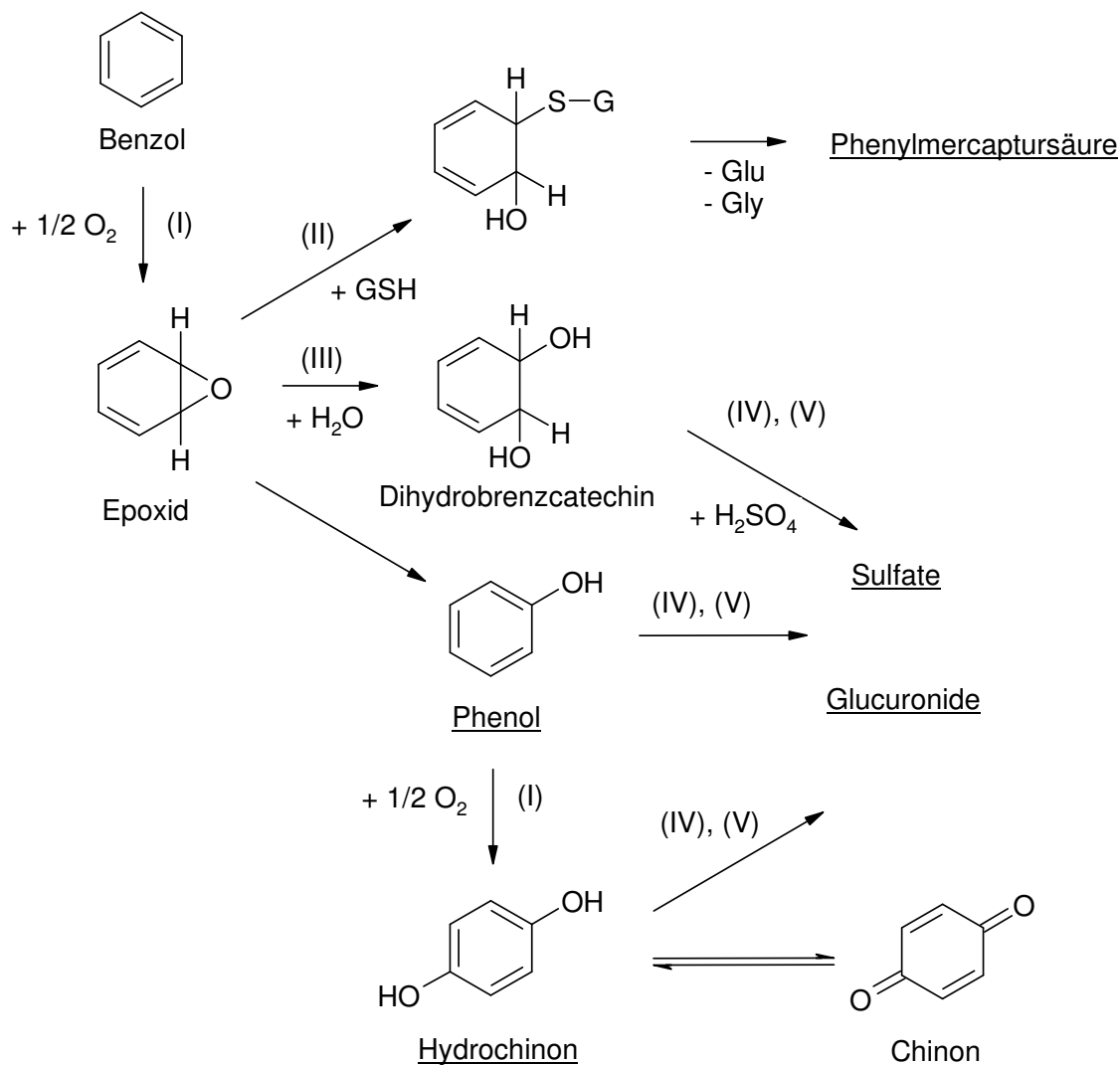
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## Overview

In residue analysis different environmental samples, such as water, soil and plant matter, are examined for pesticide residues. Analytical methods and special analytical equipment are employed to provide an accurate identification of these organic environmental pollutants and finally to determine their concentration in  $\mu\text{g dm}^{-3}$  of water or  $\mu\text{g kg}^{-1}$  of soil or plant matter. The substances examined, also known as analytes, have to be extracted from the sample using an organic solvent or solvent mixture in the first analytical step. At the same time, these analytes must be available as authentic standard solutions for comparison when determining the identity and concentration of the pesticide in the sample. Since different organic solvents are also used here, these are indispensable, versatile aids.

## Problem

The selection of a suitable solvent for the individual analysis steps is governed by the solubility of the analytes, the matrix properties of the samples and the requirements of the analytical equipment used. In residue analysis, the possible harmful or toxic effect of organic solvents is an essential criterion in the choice of solvent. For this reason, attempts are being intensified at an early stage to substitute toxicologically critical solvents with harmless ones. For example, benzene has been substituted with methylbenzene (toluene) in analytical methods. Whereas benzene is carcinogenic, as a result of metabolic conversion to its corresponding epoxide, (Figure 1), methylbenzene and its higher homologues are excreted in the urine and bile after hydroxylation of the alkyl group and conjugation with sulfur and glucuronic acid, and are regarded as non-haemolytic and thus non-carcinogenic. (Forth *et al*, 1992).



Labels: Benzol = benzene; Phenylmercaptursäure = mercaptobenzoic acid; Epoxid = epoxide; Dihydrobrenzcatechin = cyclohexa-3,5-diene-1,2-diol (dihydrobenzocatechol); Sulfate = sulfates; Phenol = phenol; Glucuronide = glucuronide; Hydrochinon = benzene-1,4-diol (hydroquinone); Chinon = cyclohexadiene-1,4-dione (quinone)

Enzymes involved in the reaction: **I:** Monooxygenases, **II:** Glutathione-S-transferase, **III:** Epoxide hydrolase, **IV:** Sulfotransferase, **V:** Glucuronyltransferase; the metabolites underlined appear in urine

**Figure1 Scheme for the metabolism of benzene in warm-blooded organisms to substances which can be excreted in the urine (Forth *et al*, 1992)**

Another example is the substitution of trichloromethane (chloroform), which has a strong narcotic effect, with dichloromethane. From a technical point of view, the lower toxicity<sup>1</sup> (the MAK value for dichloromethane is 360 mg m<sup>-3</sup> compared with 50 mg m<sup>-3</sup> for trichloromethane (chloroform); MAK = maximum workplace concentration) and non-flammability of dichloromethane spoke in favour of this solvent. Since the halogen aliphatics decree (1991), which is intended to protect the consumer from certain aliphatic chlorinated hydrocarbons, there have been many attempts to dispense with chlorinated solvents altogether. The necessity of this step was

<sup>1</sup> Nevertheless, dichloromethane is suspected of being carcinogenic (Group IIIB on the MAK list) (Falbe and Regitz, 1995).

underlined by the fact that the limit of  $0.5 \text{ mg dm}^{-3}$  for all volatile halogenated hydrocarbons in waste water (set by a decree passed in Germany to control the passing of non-household waste water into communal waste water treatment plants) is constantly exceeded by dichloromethane alone. For this reason, a number of attempts have been made to substitute this solvent.

## **Solution**

Standard analytical methods are available for many analytical tasks. The S19 method is commonly used in pesticide analysis (DFG, 1991a). The individual steps, techniques and functions are illustrated in Figure 2. This method has been used in many attempts to substitute dichloromethane as a solvent, some of these are shown in Figure 3. In the original protocol dichloromethane was used in the liquid / liquid partition. Substitution experiments concentrate on the one hand on the use of non-chlorinated solvents, and on the other on the modification of the preliminary extraction step in such a way that the liquid / liquid partition can be omitted. With the general aim of green chemistry, supercritical fluid extraction provides an analytical technique with which the use of organic solvents can be kept to a minimum.

## **Background**

Dichloromethane was used in the liquid / liquid partition because it is non-miscible with water, in order to retain pesticides in the organic phase after extraction with the water / propanone (acetone) mixture. According to its position in the middle of the eluotropic series (Table 1), in which organic solvents are compared with aluminium oxide in terms of polarity, dichloromethane with a value of  $E^0 = 0.42$ , has medium polarity, meaning that not only non-polar but also polar pesticides, and hence those more soluble in water, can be retained without loss in the organic phase. Since in the partition step propanone (acetone) can also drift partially from the water / propanone (acetone) / soil suspension to the dichloromethane phase and thus act as a solubilizer for polar residues, the use of non-polar, non-chlorinated solvents, such as cyclohexane or petroleum ether was found to be promising.

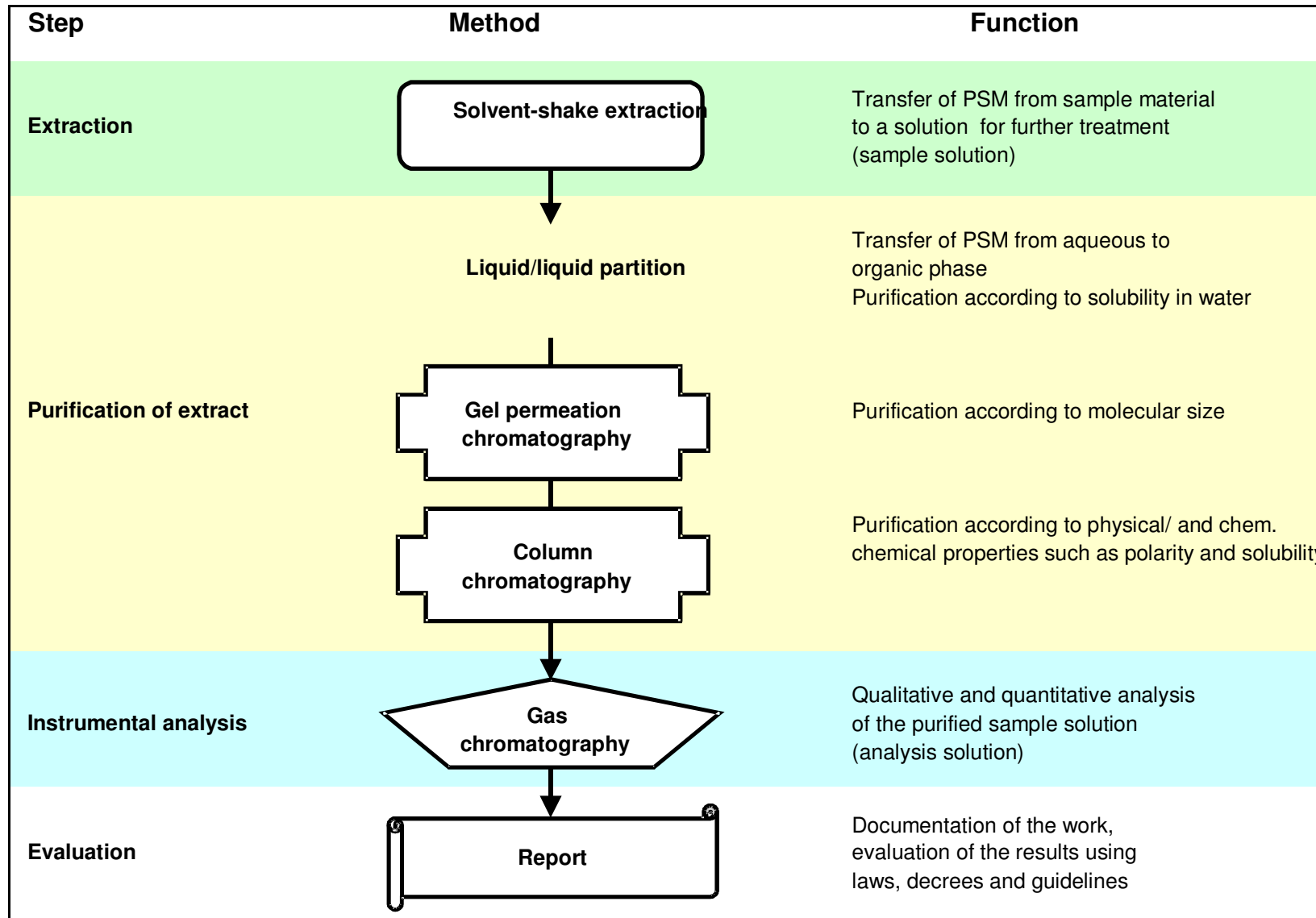


Figure 2 Principles of the S19 multi-residue method

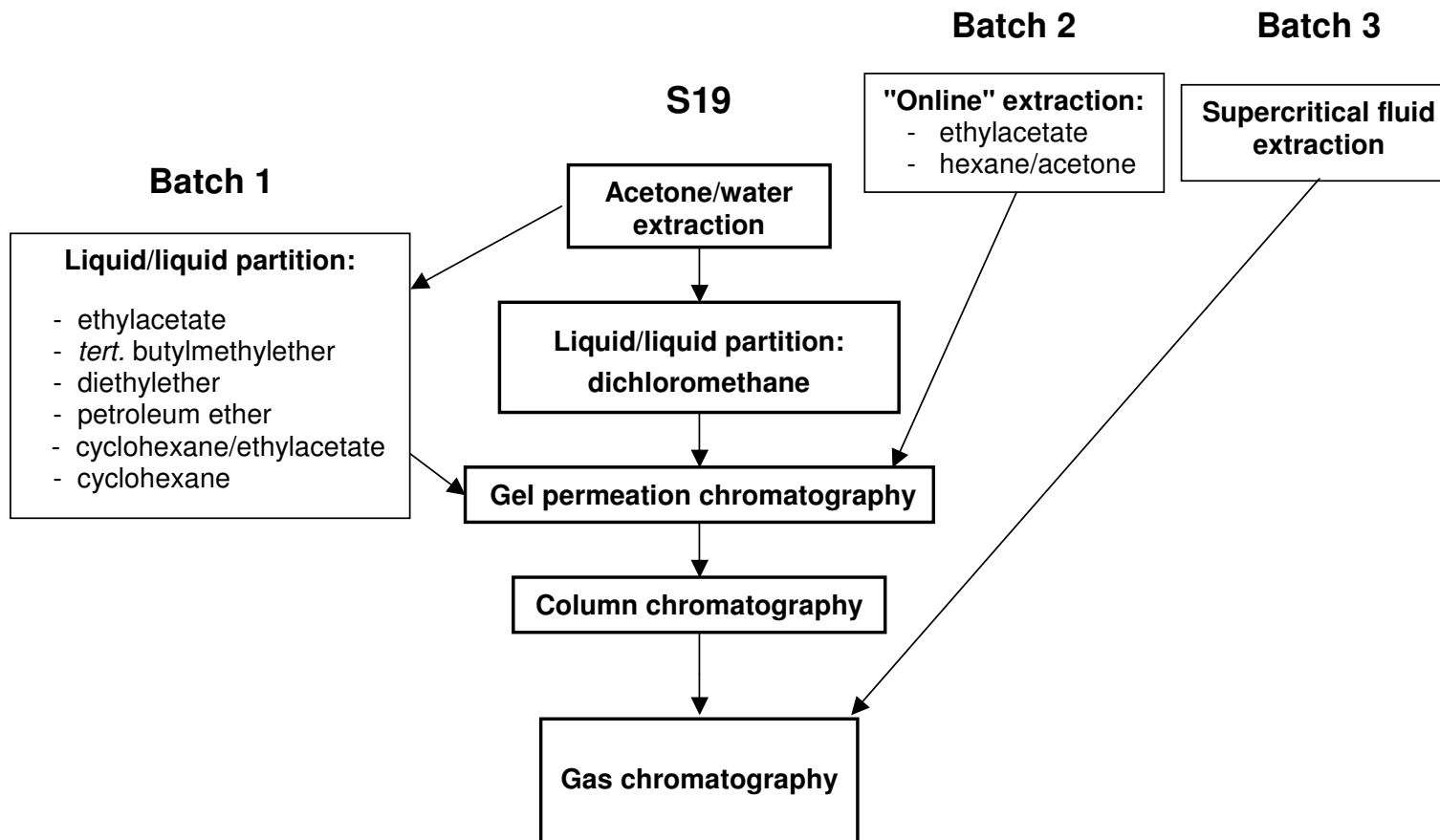


Figure 3 S19 standard method and batches used for dichloromethane substitution in pesticide analysis

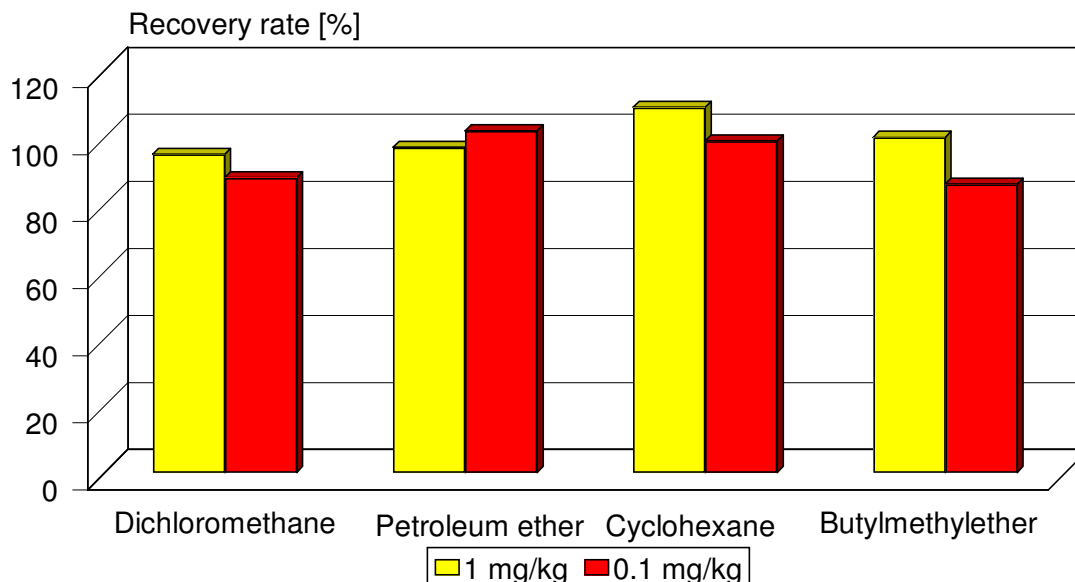
Solvent	Polarity $E^0$ ( $Al_2O_3$ )
Hexane	0.00
Petroleum ether	0.01
Cyclohexane	0.04
2-methoxy-2-methyl-propane ( <i>tert.</i> -butylmethylether)	0.20
Methylbenzene (toluene)	0.29
Benzene	0.32
Ethoxyethane (diethylether)	0.38
Trichloromethane(chloroform)	0.40
Dichloromethane	0.42
Propanone (acetone)	0.56
Ethyl ethanoate (ethyl acetate)	0.58
Ethanol	0.88
Methanol	0.95

**Table 1 The polarity of organic solvents compared with aluminium oxide: taken from the eluotropic series (Meyer, 1986)**

To avoid the liquid / liquid partition, it is not sufficient to simply dispense with water as the extracting agent, the inherent water content of samples also has to be taken into account. The latter cannot be reduced as a result of desiccation at 378 K or at room temperature. A particularly interesting variation was the solvent-free extraction using supercritical carbon dioxide. Since this has a similar polarity to hexane, an additional polar solvent was required as a modifier for the extraction of polar analytes. Finally, the analytes extracted from the supercritical medium have to be transferred to a solvent, so that organic solvents can rarely be dispensed with altogether.

### **Non-chlorinated solvents for liquid/liquid partition**

In Batch 1 solvents with different polarity are used for the substitution of dichloromethane in this liquid/liquid partition step (Figure 3). Experiments conducted by Koinecke *et al* (1994) showed that dichloromethane in soil analysis can be successfully substituted with non-polar solvents such as petroleum ether, cyclohexane or 2-methoxy-2-methyl-propane (*tert.*-butylmethylether). The important factor here is that the propanone (acetone) used as the extracting agent here at least partially enters the organic phase and thus acts as a solubilizer for pesticides which are more soluble in water, such as the insecticide carbamate Pirimicarb ( $L(H_2O) = 3060 \text{ mg dm}^{-3}$ ). Thus recovery rates in the concentration ranges  $1 \text{ mg kg}^{-1}$  and  $0.1 \text{ mg kg}^{-1}$  of dry soil of 97-109 % and 86-102 %, respectively, were found in additional experiments (Figure 4).



**Figure 4 Additional experiments for the determination of Pirimicarb in soil samples substituting dichloromethane in the liquid / liquid partition step in the S19 multi-residue method**

### Chromatography

Chromatography describes physical separation techniques in which substances with different physical and chemical properties are separated between a mobile and a stationary phase. The different interactions of the substances with the stationary phase lead to varying retention and thus to different retention times. The combination of solid, liquid and gas phases for the mobile and stationary phases has led to the development of liquid chromatography (eg paper, thin layer and column chromatography) and gas chromatography. The phase condition of the stationary phase also allows a further division into adsorption and distribution chromatography. In adsorption chromatography, separation is achieved by adsorption to a solid matrix, whereas distribution chromatography achieves separation through the dissolution of the sample between two non-miscible phases.

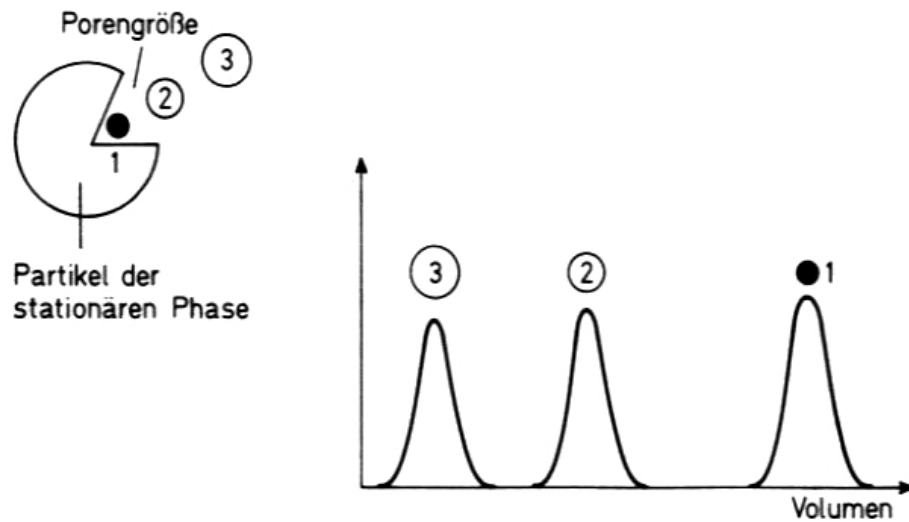
### Column chromatography

Column chromatography is an important method in residue analysis and is used as a form of adsorption chromatography for the purification of samples. The adsorbent, such as silica gel or aluminium oxide, is poured into a glass column. The sample to be purified is applied to the top of the column and the mixture to be separated is transported through the stationary phase with a solvent. This process is known as elution. The different interactions of the substances lead to substance-specific retention and thus to the separation of the substances, which are captured in different fractions and can then be analyzed.

### Gel permeation chromatography

Gel permeation chromatography is an exclusion technique, and thus a special kind of distribution chromatography. The sample applied passes through a gel bed with a defined pore size. In the process, sufficient small molecules (eg pesticides) diffuse in and out of the pores so that they are retained longer than large molecules (eg co-extracted substances from the humin fraction of soil), which cannot enter the pores.

The separation of the substance mixture then results in a sieving effect, in which the substances are separated according to molecular size. (Figure 5).



1, 2, 3: Substances with different molecular sizes

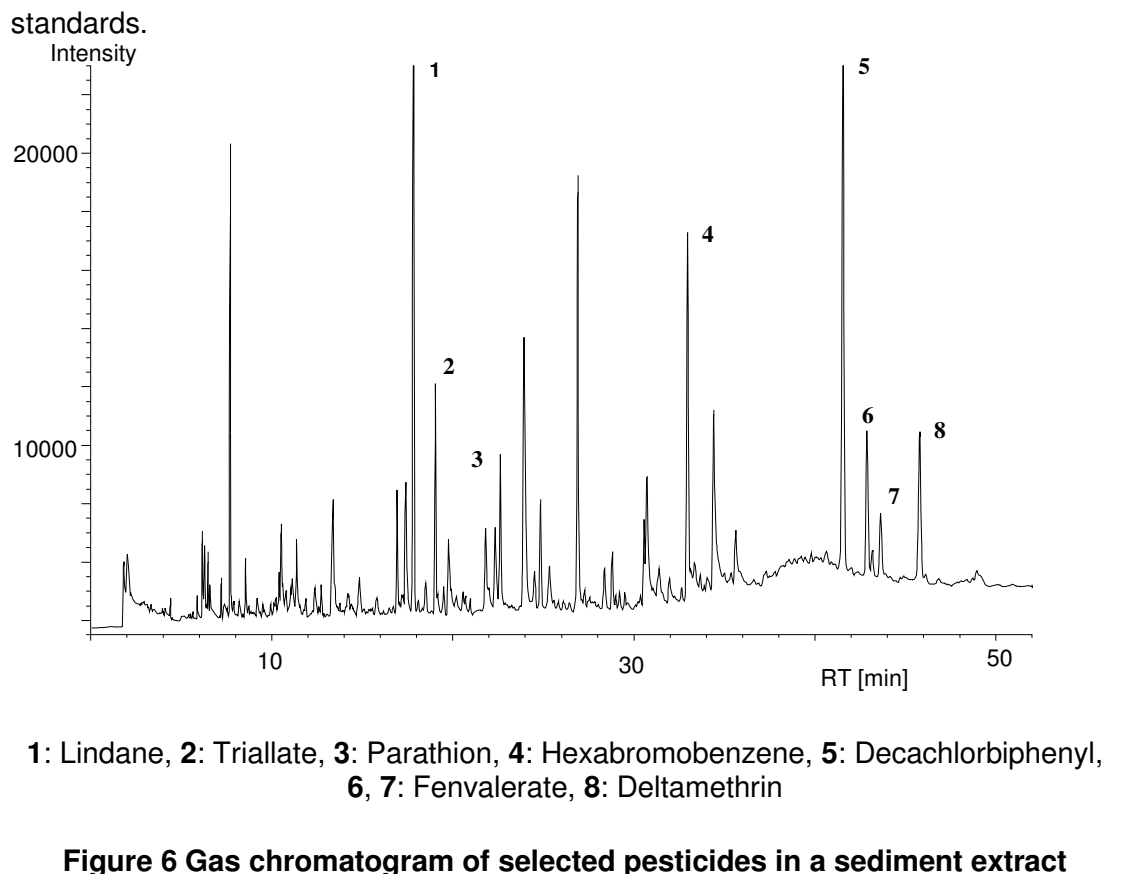
Labels: Porengröße = pore size; Partikel der stationären Phase = particles in the stationary phase; Volumen = volume

**Figure 5 The principle of gel permeation chromatography: the dependency between molecule size and elution volume**

## Gas chromatography

In gas chromatography the sample is transported in the gas phase. Substances in the sample that are not dissolved or can be detected when evaporated are transferred to the gas phase using a heated injector. The continual distribution between the mobile phase (carrier gas) and the solid and liquid stationary phase on the column separates the substances which can then be subsequently detected using a detector. The signals are recorded and displayed on a chromatogram. (Figure 6).

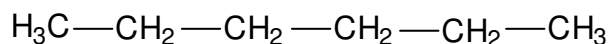
The qualitative analysis (identification) is carried out by comparing the retention times with known values from reference substances (standard solutions) which were analyzed under exactly the same conditions. Since gas chromatography requires calibration, the quantitative analysis (quantification) results from a comparison of the signal intensities of the analytes and the



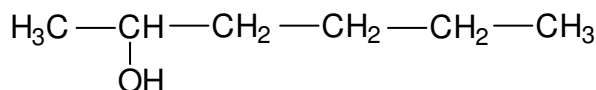
In the case of the non-polar solvents investigated which were equally suitable, cyclohexane had the added advantage that it could be used in a 1:1 mixture with ethyl ethanoate (ethyl acetate) in the subsequent purification step (gel permeation chromatography). Thus the organic phase in the liquid/liquid partition no longer has to be completely evaporated, but rather it can be made up to a final volume of 1 cm<sup>3</sup> by adding the required volume of ethyl ethanoate (ethyl acetate) and cyclohexane after it has been concentrated, and it can be used to redissolve the residue in the cyclohexane / ethyl ethanoate (ethyl acetate) mixture. As Specht *et al* (1995) showed, ethyl ethanoate (ethyl acetate) can also be used, or a mixture of ethyl ethanoate (ethyl acetate) and cyclohexane.

### "Online" extraction

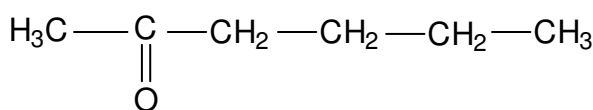
In order to avoid the time-consuming liquid/liquid partition step, in Batch 2 the extraction step was modified by selecting other solvents. In one case ethyl ethanoate (ethyl acetate) was used (Andersson und Pålsheden, 1991). In contrast to propanone (acetone) this solvent is not miscible with water. Since water is up to 8% soluble in ethyl ethanoate (ethyl acetate) however, the wettability of moist sample matrices as well as the extraction step are sufficient for many analytes. The solubility of water in ethyl ethanoate (ethyl acetate) also means however, that considerable amounts of water remain in these organic sample extracts. They have to be eliminated before subsequent analysis in several drying steps using anhydrous sodium sulfate. In order to remove traces of water, the formation of the binary azeotrope of 91.5 % ethyl ethanoate (ethyl acetate) to 8.5 % water at 70.4 °C can be used to concentrate the extract using a rotary evaporator.



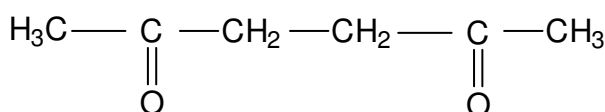
**n-Hexan**



**2-Hexanol**



**Methyl-Butyl-Keton**



**2,5-Hexandion**

Labels: n-Hexan = hexane; 2-Hexanol = hexan-2-ol; Methyl-Butyl-Keton = hexane-2-one; 2,5 – Hexandion = hexane-2,5-dione

**Figure 7 Simplified activation of hexane via hexane-2-one to the neurotoxic hexane-2,5-dione (Forth *et al*, 1992)**

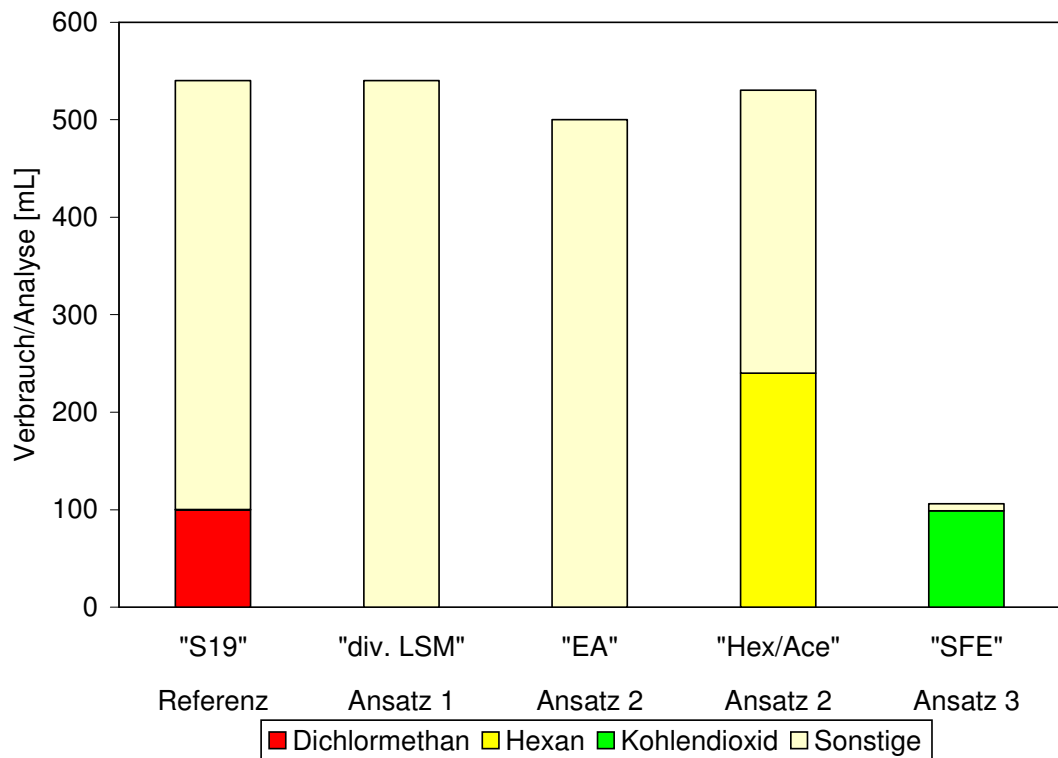
According to the modular multi-residue method for the determination of pesticide residues in food products (which, according to §35 of the Foods and Consumables Law, is an extended revision of the S19 multi-residue method), cold extraction with hexane / propanone (acetone) (2:1) can also be used as an alternative to the ethyl ethanoate (ethyl acetate) method. This column extraction technique which dates back to Ernst *et al* (1974), which is particularly suitable for foodstuffs containing fats and with a high water content, is based on pretreatment of the moist sample matrix by rubbing it with anhydrous sodium sulfate and sand. The solid mixture is poured into a glass extraction column, eluted with the hexane / propanone (acetone) mixture, purified and analyzed by GC. The chemical elimination of the water inherent in the samples reduces the labour compared with the S19 method. For this reason, the column extraction method is particularly suitable for the analysis of sediments and waste water slurries. If these samples are significantly contaminated with surfactants as surface-active solubilizers, it is often difficult or even impossible as a result of strong emulsion to achieve complete separation between aqueous and organic phases in a liquid / liquid partition.

In this extraction technique the use of hexane deserves a special mention. Even though it is an aliphatic hydrocarbon, hexane has a special status in this homologous series. Because, according to Forth *et al* (1992), hexane causes peripheral polyneuropathy in persons who were exposed to high concentrations, and had a narcotic effect (typical for benzene). The neurotoxic effect of hexane is due to a metabolic conversion specific to this hydrocarbon, which does not occur with its immediate neighbours in the homologous series, pentane and heptane. As is illustrated in Figure 7 in simplified form, without taking into account all the side-reactions of metabolism, hexane is broken down in a first oxidative step in the human body to hexan-2-ol. This is then converted to hexan-2-one (butylmethylketone) by the action of dehydrogenases. Further oxidations then lead finally to hexane-2,5-dione, which is the neurotoxic metabolite. Thus there have been many attempts to substitute hexane with heptane.

### **Supercritical fluid extraction**

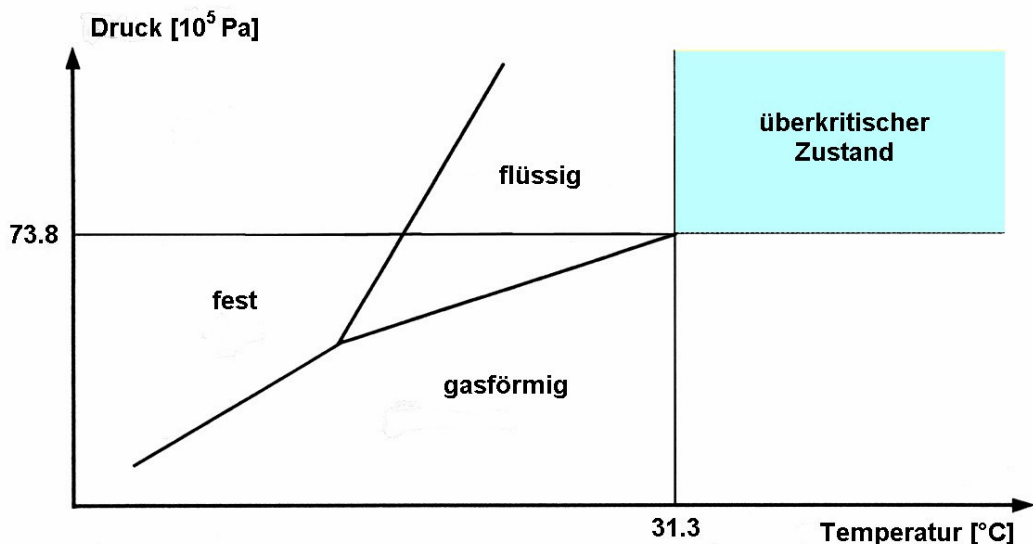
In Batch 3, the use of supercritical carbon dioxide for the extraction achieves a considerable reduction in the organic solvent (Figure 8). According to the phase diagram of carbon dioxide (Figure 9), the supercritical state is reached when a critical pressure ( $73.8 \times 10^5$  Pa) and a critical temperature (304.3 K) are exceeded. The fluid is then a mixture between liquid and gas in terms of the diffusion coefficients and viscosity.

A high extraction efficiency was also expected in residue analysis using supercritical fluid extraction with carbon dioxide. This method became well-known due to its technical significance in the decaffeination of coffee. Since carbon dioxide has a similar polarity to hexane, methanol (up to 5 vol. %) is used as a modifier to increase the extraction efficiency, in particular to release polar analytes from the different sample matrices (Koinecke *et al*, 1997, Koinecke, 1999).



Labels: Verbrauch / Analyse = Consumption / analysis; Referenz = standard; Ansatz = batch; Dichlormethan = dichloromethane; Hexan = hexane; Sonstige = others

**Figure 8 Solvent consumption in the S19 multi-residue method and the changes due to substitution of dichloromethane**

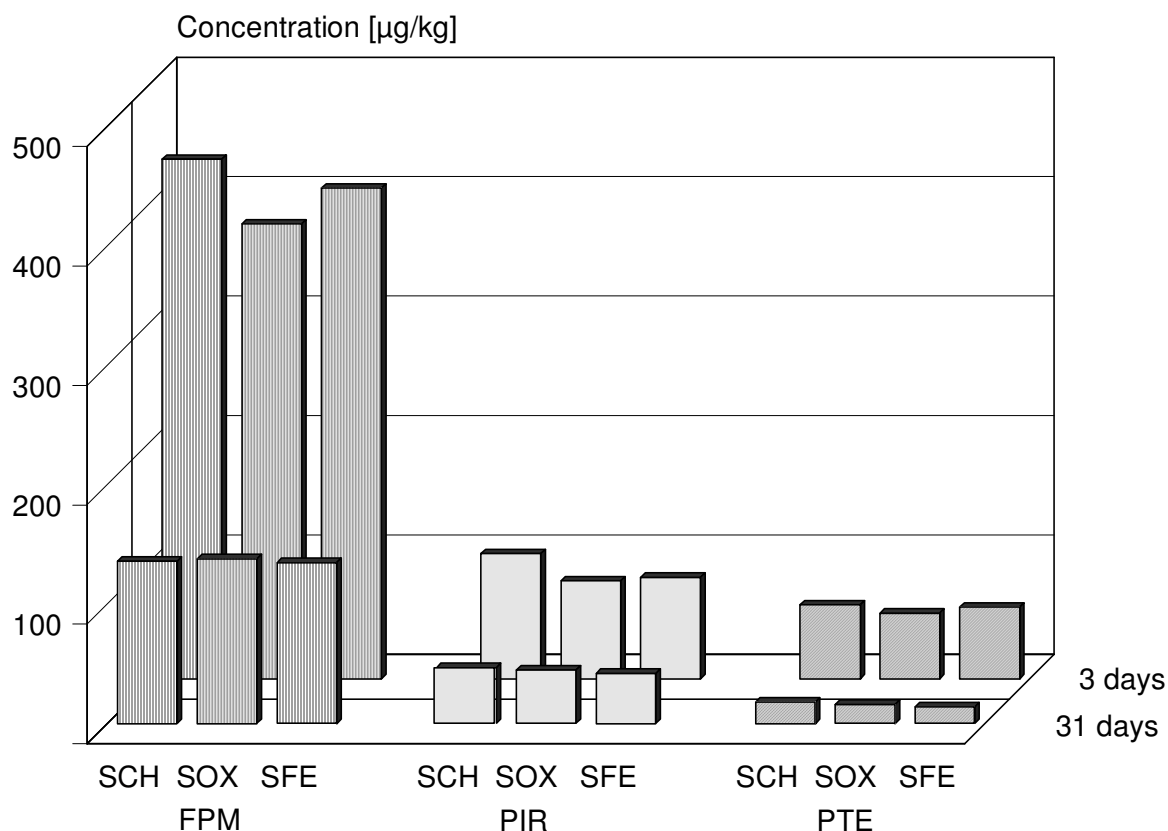


Labels: Druck = pressure; fest = solid; flüssig = liquid; gasförmig = vapour; überkritischer Zustand = supercritical state

**Figure 9 Phase diagram of carbon dioxide**

Although only a few matrix components are coextracted from complex sample matrices, such as soil, sediment sewage sludge samples, this expensive extraction technique also has significant drawbacks. As a result of the size of the extraction

cartridge, which depends on the equipment used, sample weighing is extremely limited compared with the solvent-shake or Soxhlet extraction methods, which has a negative effect on the sensitivity of the whole method. Since many tests did not show a significantly higher extraction efficiency of this method compared with the solvent-shake or Soxhlet extraction methods (Figure 10), supercritical fluid extraction has not become established in residue analysis. This was emphasized by its exclusion as a complementary method for the modular S19 multi-residue method (Kreuzig, 1998, Kreuzig *et al*, 2000).



**Figure 10 Efficiency of solvent-shake (SCH), Soxhlet (SOX) and supercritical fluid extraction (SFE) for Fenpropimorph (FPM), Pirimicarb (PIR) and Parathion (PTE) from soil samples 3 and 31 days after standard application**

## Summary and outlook

The tests carried out in different teams showed clearly that solvents used in established and routine analysis methods can be substituted successfully in line with efforts to achieve green chemistry and reduce the toxicity and negative environmental effects caused by these solvents. Positive side-effects were also observed, such as a reduction in labour and time spent carrying out these analyses. Dispensing with chlorinated solvents also simplifies the specialist, expensive disposal of waste. Attempts are also being made to miniaturize the analysis techniques in order to reduce solvent or even achieve solvent-free extraction or concentration techniques.

## Literature

Andersson, A. und Pålsheden, H. (1991): Comparison of the efficiency of different GLC multi-residue methods on crops containing pesticide residues. *Fresenius J. Anal. Chem.*, 339, 365-367.

- Anonym (1999): Untersuchung von Lebensmitteln: Modulare Multimethode zur Bestimmung von Pflanzenschutzmittel-Rückständen in Lebensmitteln. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG.
- DFG (1991a): Multimethode S19, Rückstandsanalytik von Pflanzenschutzmitteln, Organochlor- und Organophosphor-Verbindungen sowie stickstoffhaltige und andere Pflanzenschutzmittel. Mitteilung VI der Senatskommission für Pflanzen- und Vorratsschutzmittel, Methodensammlung der Arbeitsgruppe Analytik, 1-11 Lfg., VCH, Weinheim.
- DFG (1991b): XI, Statistische Beurteilung von Analysenverfahren und Analyseergebnissen. Mitteilung VI der Senatskommission für Pflanzen- und Vorratsschutzmittel, Methodensammlung der Arbeitsgruppe Analytik, 1-11 Lfg., VCH, Weinheim.
- Ernst, W., Schaefer, R.G., Goerke, H. und Eder, G. (1974): Aufarbeitung von Meerestieren für die Bestimmung von PCB, DDT, DDE, DDD,  $\gamma$ -HCH und HCB. Fresenius Z. Anal. Chem., 272, 358-363.
- Falbe, J. und Regitz, M. (1995): Römpp Chemie-Lexikon Georg Thieme Verlag, Stuttgart, New York.
- Forth, W., Henschler, D., Rummel, W. und Starke, K. (1992): Pharmakologie und Toxikologie. Wissenschaftsverlag, Mannheim, Wien, Zürich.
- Halogenaliphaten-Verordnung (1991): 1. Verordnung zum Schutz des Verbrauchers vor bestimmten aliphatischen Chlorkohlenwasserstoffen (1. Chloraliphaten-Verordnung), BGBl, 1059.
- Koinecke, A. (1999): Einsatzmöglichkeiten der Superkritischen Flüssigkeitsextraktion in der Rückstandsanalytik von Pflanzenschutzmitteln in Böden. Diss. TU Braunschweig.
- Koinecke, A., Kreuzig, R., Bahadir, M., Siebers, J. und Nolting, H.-G. (1994): Investigations on the substitution of dichloromethane in pesticide residue analysis of plant materials. Fresenius J. Anal. Chem., 349, 301-305.
- Koinecke, A., Kreuzig, R. und Bahadir, M. (1997): Effects of modifiers, adsorbents and eluents in supercritical fluid extraction (SFE) of pesticides in soils. J. Chromatogr. A, 786, 155-161.
- Kreuzig, R. (1998): Entwicklung analytischer Methoden zur Differenzierung von Abbau und Sorption als konzentrationsbestimmenden Prozessen für Pflanzenschutzmittel-Wirkstoffe in Böden. Habilitationsschrift, TU Braunschweig ISBN 3-89720-291-3.
- Kreuzig, R., Koinecke, A. und Bahadir, M. (2000): Use of supercritical fluid extraction in the analysis of pesticides in soil. J. Biochem. Biophys. Methods, 43, 403-409.
- Meyer, V. (1988): Praxis der Hochleistungsflüssigchromatographie. Verlag Moritz Diesterweg, Frankfurt.
- Schwedt, G. (1986): Chromatographische Trennmethode. Georg Thieme Verlag, Stuttgart, New York.
- Specht, W., Pelz, S. und Gilsbach, W. (1995): Gas-chromatographic determination of pesticide residues after clean-up by gel-permeation chromatography and mini-silica-gel chromatography. Fresenius J. Anal. Chem., 353, 183-190.
- Steinwandter, H. (1985): Universal 5 min on-line method for extracting and isolating pesticide residues and industrial chemicals. Fresenius Z. Anal. Chem., 332, 752-754.