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Behavior and elimination of pesticide residues during supercritical carbon dioxide extraction of essential oils of spice plants and analysis of pesticides in high-lipid-content plant extracts.

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List of abbreviations

CCC	Counter current chromatography
CCD	Counter current distribution
GC	Gas chromatography
GC-ECD / NPD	Gas chromatography coupled with electron capture detector / nitrogen phosphorous detector
GC-MS	Gas chromatography coupled with mass spectrometry
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
HSCCC	High speed coil counter current chromatography
HSMLCCC	High speed multi-layer coil counter current chromatography
MS	Mass spectrometer
PPC	Paprika permeation chromatography
SF	Supercritical fluid
SFE	Supercritical fluid extraction
SFC	Supercritical fluid chromatography
SECS	Simultaneous extraction and chromatographhic separation
SECD	Simultaneous extraction and chromatographhic discrimination

I. INTRODUCTION

The conventional method of extracting vegetable oils is done either mechanically by pressing out or squeezing out oils from the parts of plant they are contained or by means of solvent extraction. A combination of both methods is often employed, especially for the extraction of oils from raw materials with high oil contents.

Total extraction of oil is achievable only by means of a solvent extraction. Less than 1 % oil remains in the extraction cake. Hexane is mostly employed as extraction solvent for the industrial-scale production of oil. After the extraction, the solvent is separated from both the raw oil and the extraction cake by various means such as distillation and refining of the oil. Alternatively, oil can be extracted with supercritical fluids (SFs) [1-16, 87].

With SFE technology several extraction steps of the classical refining process are combined. One of the advantages of extraction with supercritical CO_2 is that it provides a high quality extract that can be used without refining (i.e. removal of lecithins, free fatty acids, mucins, bad odours, bitter contaminants, etc from the extract) for human food. Therefore oils, for example from coconut, peanut, soybean and sunflower seeds are extracted with CO_2 at a pressure of 280-350 bar and temperatures close to the T_c (45-50 °C). In food and pharmaceutical industries, the SFE technology is reported in the selective extraction of fish and animal oils with certain physiological functions (removal of unwanted cholesterol from egg yolk at high temperature and pressure). SFE technology is also used in decaffeination of coffee beans, extraction of beer hops, spices and flavors.

Table 1, for example, shows the total amount of paprika raw material imported by the spice company Raps & Co from around the world in 1995. About 25 % of the raw material were found to be not suitable, even for the fluid carbon dioxide extraction, because of high contamination with PCBs, organophosphorous and chlorinated pesticides.

Table 1. Paprika raw material imported in 1995 by the spice company Raps & Co, Kulmbach-Germany [17].

Provenience (Country)	Import (% of weight)	No. of shipments	No. of reclamations (% of weight)	Main objections
Hungary	32	560	25 (ca. 5%)	ethylene oxide, chloro ethanol
Spain	24	475	35 (ca. 7 %)	Chloro pesticides, PCBs, organophosphorous pesticides
South Africa	5	120	0	
Israel	10	255	15 (ca. 2%)	Chloro pesticides, PCBs
Greece	8	210	45 (ca. 4%)	Chloro pesticides, pyrethroids
Turkey	6	181	30 (ca 4%)	ethylene oxide
Others	15	385	154 (ca. 8 %)	Chloro pesticides
∑	100	2185	324 (ca.25 %)	

The industrial supercritical extraction technology employs parameters that include temperatures in the range of 40-70 °C and pressures in the range of 250-1000 bar. [2-15]. Due to the higher diffusivity and lower viscosity of CO_2 under the aforementioned conditions, fluid carbon dioxide has excellent transport qualities, which means that the extracting power is generally higher, and, as a result, more components are extracted, which, of course, could include pesticide residues of variable substance classes.

In order to ascertain the extractibility of pesticide residues from samples and a possible carry-over into the extract, the knowledge of pesticide behavior under subcritical, critical and supercritical conditions becomes very vital. The more contaminated a raw material is, the higher is the probability of pesticide enrichment in the extract.

Prior to now, however, the quality of plant extracts from fluid carbon dioxide has been determined only on the basis of the sensory qualities. Quality evaluation on the basis of pesticide contamination level has received virtually no consideration up till now. However, due to dietary demand (allergens-free foods) or due to new social trends (such as organic foods), there is a consumer's demand for residue-free food. This, coupled with governmental regulations and legislations, especially in the EC to eliminate export-import restrictions, has resulted to a pressure on companies to accommodate both the new societal trends and governmental regulations in the production of food and pharmaceuticals. The experiences of Raps & Co, a manufacturer of spice extracts, have shown that the usual pesticide residues control analysis of raw materials meant for supercritical fluid extraction is not effective enough to eliminate every contaminated raw material. In order to continue to lay claim on the production of quality extracts, Raps & Co decided to develop and optimize methods that can enable the production of pesticide-free CO_2 extracts.

With the production of pesticide-free extracts as an objective in focus, this research work commenced with the extraction of spice imports from different countries firstly by conventional method and followed by fluid CO_2 extraction. The qualities of extracts from both extraction methods were investigated.

The work proceeded with the investigation of various parameters influencing pesticides behavior during fluid carbon dioxide extraction. The knowledge gained therefrom, was utilized for an on-line reduction or eventual elimination of pesticide residues during the extraction procedure.

II. THEORETICAL PART

1. The supercritical fluid extraction (SFE) technology

1. 1. Physico-chemical fundamentals of supercritical fluids

The temperature-pressure phase diagram of a pure substance is characterised by regions in which the substances occur as a single phase [solid (s), liquid (l) or gaseous (g)]. The boundaries of these regions are indicated with curves that stand for regions of coexistence of two phases (s-g, s-l and l-g) that are involved in sublimation, melting and vaporization equilibria, respectively. These regions intersect at a point known as the triple point (TP) where the solid, liquid and gaseous phase coexist in equilibrium.

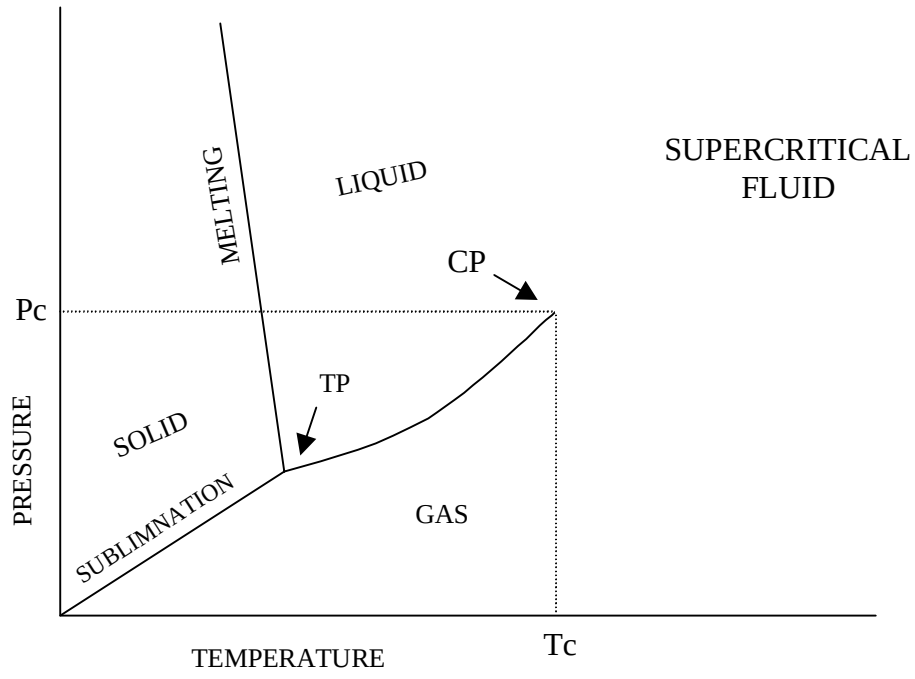


Figure 1. Solid-liquid-gas supercritical fluid phase diagram. (TP = triple point, P_c = critcal pressure; T_c = critical temperature; CP = critical point) [18].

The temperature (t) and pressure (p) at which the liquid and gaseous phase coexist in equilibrium gives the vapor pressure curve. It divides the P, T plane into two regions; a region where the liquid is the stable phase and a region where the gas is the stable phase. Enthalpy change is necessary for a phase transformation and at a constant pressure the temperature, at which a phase transition occurs, is known as transition temperature. At a transition temperature for each pressure and in the absence of external influences, two phases can indefinitely coexist.

On the phase diagram, the coexistence curve, i.e the equilibrium between two phases is either infinity or intercepts another coexistence curve. Unlike the liquid-gas equilibrium, the vapor pressure curve here has an abrupt break at a point called the critical point (CP). This point is described as a point in the phase diagram designated by a critical temperature (T_c) and a critical pressure (P_c) beyond which no liquification will take place on raising the pressure and no gas will be formed on increasing the temperature. Taking these properties into consideration, a supercritical fluid, therefore, is defined as a fluid that is compressed beyond its critical pressure and heated beyond its critical temperature.

Increase in temperature will result to an increased pressure at which the liquid and vapor phases coexist on the vapor pressure curve. Temperature increase at a constant pressure results to a decrease in density of liquid and gaseous phases. At a constant temperature and pressure the density of the liquid and gas are identical and the phases indistinguishable. Beyond this temperature and pressure the liquid and gaseous phases exist as a single phase. The region of pressures and temperatures above P_c and T_c is known as the supercritical region [18].

A given final supercritical state can be reached either by starting from a point within the liquid region, increasing the pressure above P_c and then raising the temperature above T_c until the state denoted by CP is reached or by starting from a point in the vapor region, heating the substance above its T_c and then raising the pressure above its critical va lues.

Carbon dioxide, which is mostly used in high pressure extraction, has the following critical data (Table 2):

Table 2. Critical data of carbon dioxide

P_c = 73.79 bar
T_c = 31.04 °C
δ_c = 0.468 g / cm^3

In the supercritical region the interactions between the fluid and solute molecules are accumulated resulting to enhanced dissolving properties.

Table 3. Properties of supercritical CO_2 as compared with those of ordinary gases and liquids.

	Density ($g\ cm^{-3}$)	Viscosity ($g\ cm^{-1}\ s^{-1}$)	Diffusion coefficient ($cm^2\ s^{-1}$)
Gases	$(0.1\text{-}2)10^{-3}$	$(1\text{-}3)\ 10^{-4}$	0.1-0.4
Supercritical CO_2 Tc, Pc	0.47	3×10^{-4}	7×10^{-4}
Tc, 6 Pc	1.0	1×10^{-3}	2×10^{-4}
Liquids	0.6-1.6	$(0.2\text{-}3)\ 10^{-2}$	$(0.2\text{-}2)\ 10^{-5}$

6 Pc = 442.74 bar

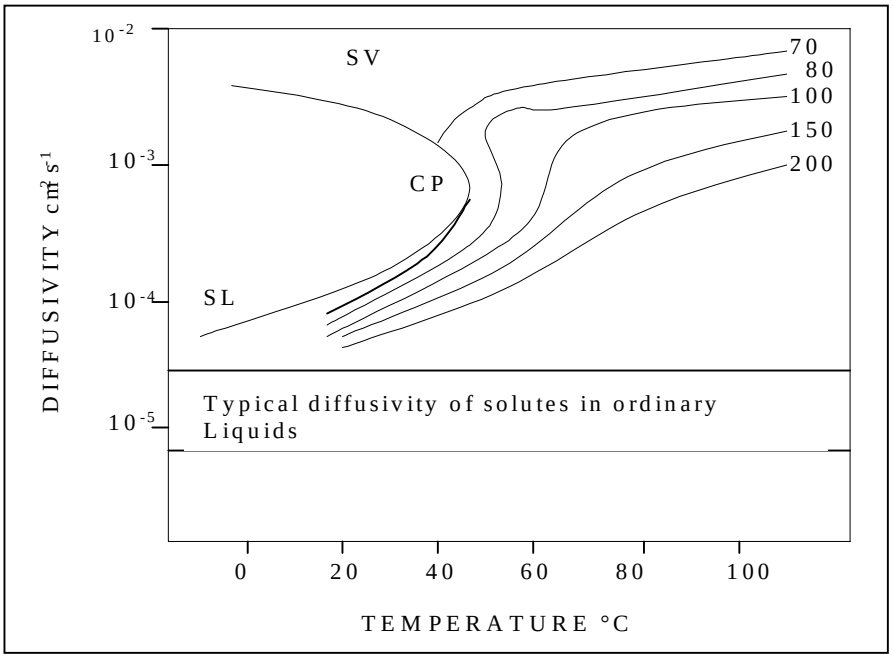


Figure 2. Variation of the diffusivity in CO_2 as a function of temperature at several pressures; SV = saturated vapor; CP = critical point; SL = saturated liquid [19].

Diffusivity (diffusion constant) is an important parameter for the fluid penetration of the extraction. Mass transfer from matrix into the extraction fluid is more efficient and faster than with conventional liquid solvent (Fig. 2). The dissolving power of a supercritical fluid is actually not better than that of liquid solvents. It approaches that of a liquid solvent only when the density is high enough. The maximum solubility in most liquids is higher than in supercritical fluids (SFs).

Therefore in conclusion, in terms of dissolving power the supercritical fluids offer no advantage over liquid solvents, but other properties such as the excellent mass transfer make the SF extraction a superior method. The rate at which extraction can be accomplished is determined by the rate of mass transfer. Solute diffusivities in supercritical fluids are by one order of magnitude (10^{-4} against10^{-5} $cm^2 \times s^{-1}$) higher than those of liquid solvents and their viscosity is lower by one order of magnitude (10^{-4} against 10^{-3} $Ns \times m^{-2}$) hence their mass transfer properties are more favorable than those of liquids (compare also Table 3). SFE is, therefore, much faster than liquid extraction. Supercritical extraction can be accomplished within some fractions of an hour while liquid extraction usually lasts several hours.
From the thermodynamic point of view, SFs have densities that are 100-1000 times higher than those of gases, which gives them a solvating strength closer to that of liquids. The density depends on pressure and temperature. The change in density of SFs with pressure at constant temperature is non-linear.

The isothermal variation of the density of SFs with pressure, that is typically non-linear, is also depicted on the phase diagram with reduced variables. A reduced variable is defined as the quotient of any variable in consideration and the critical equivalent of the same variable.

$$Tr = \frac{T}{Tc}, \quad \mathrm{Pr} = \frac{P}{Pc} \quad \delta r = \frac{\delta}{\delta c};$$

δ = Density; T = Temperature; P = Pressure
where $T = T_c$ and $P = P_c$, this means T_r or $P_r = 1$.

In other words, the critical point results from a triple intersection where the three reduced variables coincide and are in unity. In a supercritical state, the values of two variables are above unity and a subcritical state exists, if one variable is less than unity.

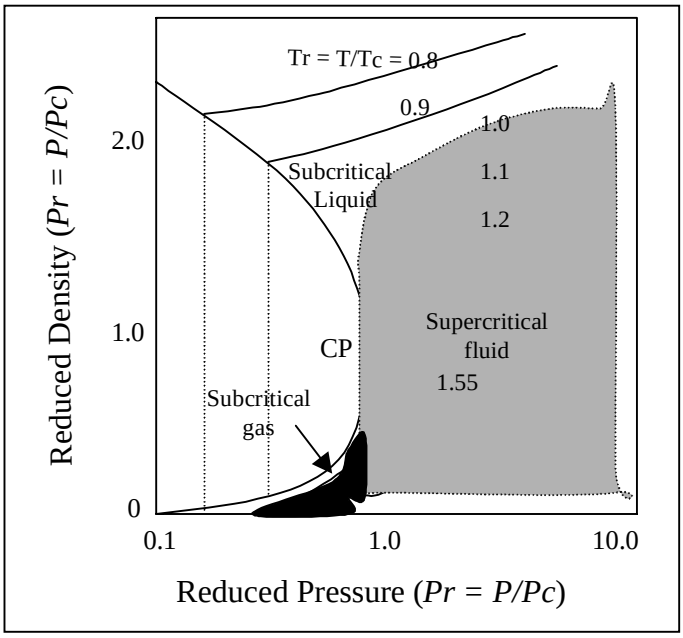


Figure 3. Two-dimensional reduced density/reduced pressure graph including several reduced temperature isotherms. (CP = critical point, the fairly shaded area above shows the supercritical and the dark shaded area shows the subcritical gas region and the unshaded subcritical liquid region [18]).

The pressure-density diagram (Fig.3) can be interpreted as follows:

a. The critical point emanates from a triple intersection where the three variables are unity

b. In the supercritical region, the density increases sharply at a constant temperature with an increasing pressure, also at a constant pressure the density decreases with an increasing temperature.

c. Near the critical point, a small pressure rise results in a sharp increase in the solvent density. This is depicted on the diagram by the steep slope of the curves. The slope of the curves also decreases sharply with increasing distance from the critical point.

d. The immediate region above the critical point is the region of highest density change. Therefore, any minimal variation in temperature or pressure in this region will have more effect on the density.

e. Useful for the extraction is the dark-shaded area at the bottom of the diagram and on the left side of the supercritical region. Although the slopes of the curves in this region are not so steep as in the supercritical region, the density varies significantly enough with pressure for extraction purposes. The dissolving power of conventional liquid solvents in comparison with SFs is independent of the pressure, since the liquid density is not affected by this variable. But the density of SFs can be altered over a wide range by changing the pressure or temperature or both.

f. Selective extraction of substance groups can be accomplished by an isothermal or isobar manipulation of the density. At constant temperature the dissolution of non-polar and scarcely polar analytes will be enhanced by low pressure, while polar and high-molecular weight solutes will be easier at high pressures.

Reduced isotherms are then in a supercritical state, if the values are above unity and lie within the region of reduced presure, also with values above unity. Therefore, on the shaded areas in the diagram, the most significant changes in pressure are within the reduced temperatures between 1.0 and 1.1 in Fig. 3 (40-62 °C in Table 4) and reduced pressure between 1 - 4.5 in Fig. 3 (73-370 bar in Table 4). This is the region where supercritical fluid extraction is executed. Technically, parameters within this region are easily achievable. Supercritical fluids have greater dissolving (solvation) strength within densities in the boundaries of 0.47g cm^{-3} and 0.95 g cm^{-3}.

Table 4. The dependency of the density of CO_2 on pressure and temperature.

Temperature	40 °C (313 K)	62 °C (335 K)	100 °C (373 K)	250 °C (523 K)
73 bar	0.22	0.17	0.13	0.11
405 bar	0.96	0.89	0.76	0.62

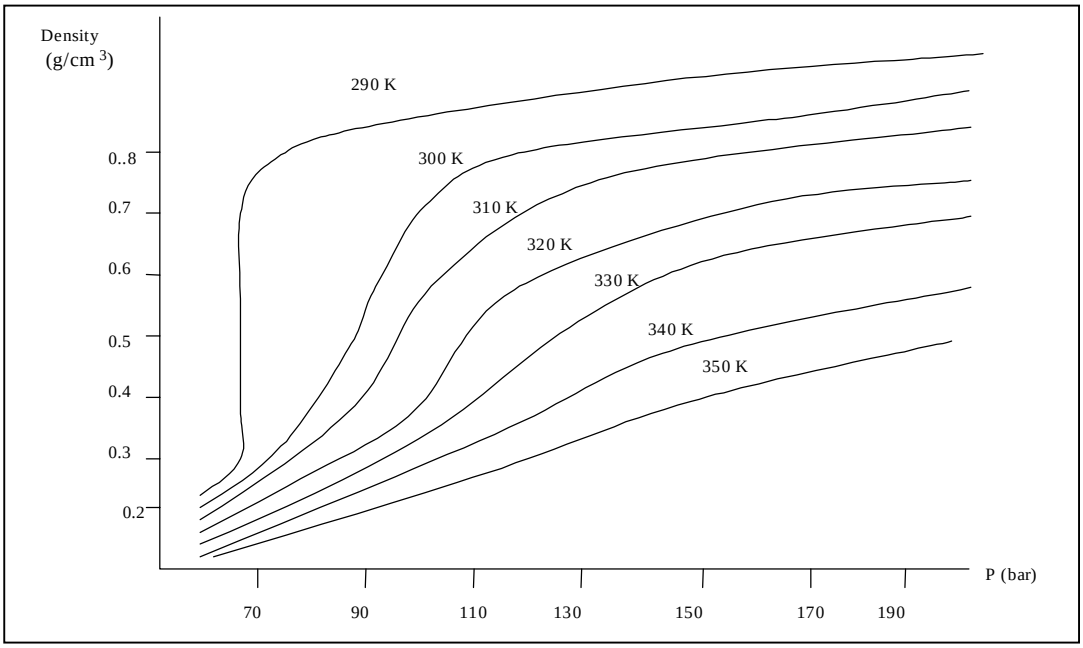


Figure 4. Density expressed as a function of pressure.

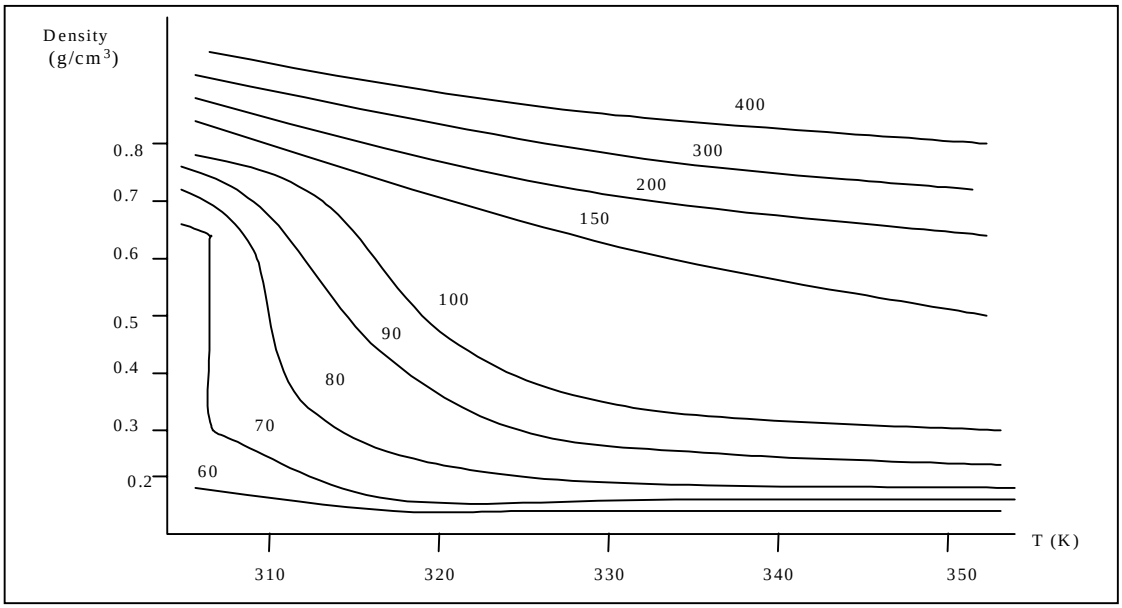


Figure 5. Density expressed as a function of temperature.

Figs. 4 and 5 and Table 4 show that in the vicinity of the critical point there is an isocore decrease in density with an increase in temperature and an isothermal increase in density with an increase in pressure.

Viscosity is like the diffusion coefficient a function of pressure and temperature. However, the dependency on these variables is not very much expressed. Increased pressure results in increased SF viscosity and hence in diminished solute diffusivity and transport phenomena, but most often in increased solubility by increased density.

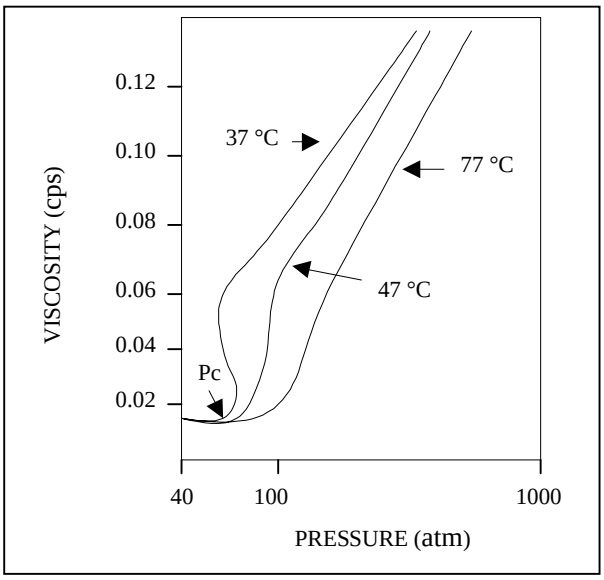


Figure 6. Variation of the viscocity of CO_2 with pressure at different temperatures (Pc = critical pressure [19, 19*].

The viscosity of the supercritical fluid lies between those of liquids and gases (Fig. 6, Table 5). It is 5-20 times lower than that of ordinary liquids. From the kinetic point of view, therefore, SFs have better hydrodynamic properties (penetratibility) than liquids. Low viscosity invariably means low surface tension, hence penetration into sample matrices is more rapid.

The slope of the diffusivity-temperature isobars, on the one hand, increases with decreasing temperature and pressure, on the other hand, the diffusivity range of ordinary liquids runs parallel to the temperature axis. Therefore, the following conclusions can be made:

a. The diffusivity of a solute in SF is higher than in an ordinary fluid (Table 5).
b. Diffusivity of SF decreases with increase in pressure.
c. Diffusivity increases with increase in temperature, especially in the region near the critical point.
d. The rate of mass transfer is determined by the diffusion coefficient. The higher the diffusion coefficient of a solute in a fluid is, the faster is the mass transfer.

Table 5. Some parameters of supercritical CO_2 compared with n-hexane, methylene chloride and methanol [20].

	Density [g cm^{-3}]	Viscosity [m s^{-1} * 10^7]	Diffusion of benzoic acid in [m^2 s^{-1} * 10^9]
CO^2	0.749	1.00	6.0
n-Hexane	0.660	4.45	4.0
CH_2Cl_2	1.326	3.09	2.9
MeOH	0.791	6.91	1.8

The solubility of substances in a supercritical fluid depends also on the dielectric constant $\in$. The $\in$-values of some solvents under normal conditions are shown in Table 6.

Table 6. The $\in$-value of some solvents under normal conditions [19, 19*].

Substance	$\in$ [T]
Water	78.4 [298 K]
Ethanol	24.3 [298 K]
n-Hexane	1.89 [293 K]

∈ is proportional to temperature and pressure. Thus, the value decreases with increase in temperature and decreasing pressure (Fig. 7). At 1000 °C and a density of around 1 g mL^{-1}, water, for example, has a dielectric constant of ca. 12, at 0 °C and the same density the value is 90 and at the critical point the value is 6. Due to its low dielectric constant at high temperatures, water exerts a weak electrostatic potential between ions, so that dissolved ions can freely form ion-pairs. Although water is a conventional polar solvent, it behaves as a non-polar solvent at high temperature. This property is partly responsible for the ability of water to dissolve non-polar organic compounds [18].

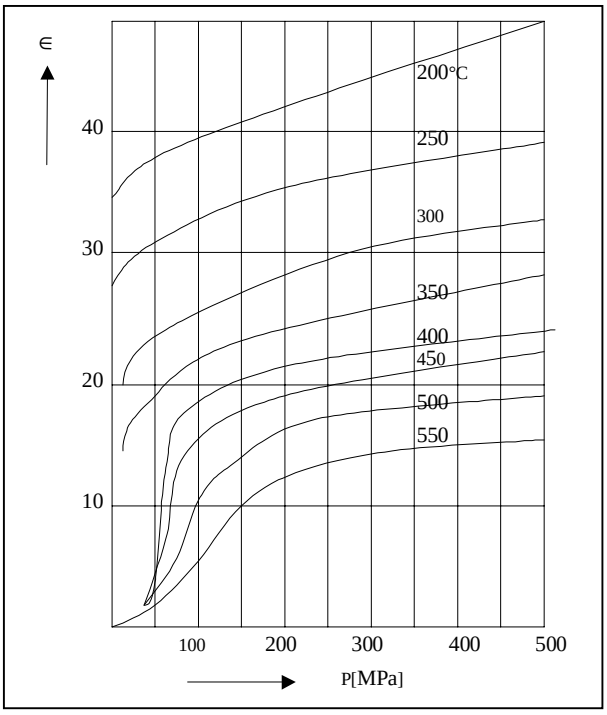


Figure 7. The dependency of ∈-value (dielectric constant) of water on temperature and pressure [21].

In comparison, the dielectric constant ∈ of carbon dioxide increases with pressure (Fig. 8). In a dense state, at 200 bar and 40 °C, ∈ carbon dioxide is ca. 1.5, which is comparable with a highly non-polar solvent, hence it can be used in dissolving non-polar substances. At 400 bar the value of ∈ increases to 1.6. Further pressure increase results only in a minor increase to 1.8 (T = 323 K), This value is almost the same as that of hexane (1.89 at 20 °C).

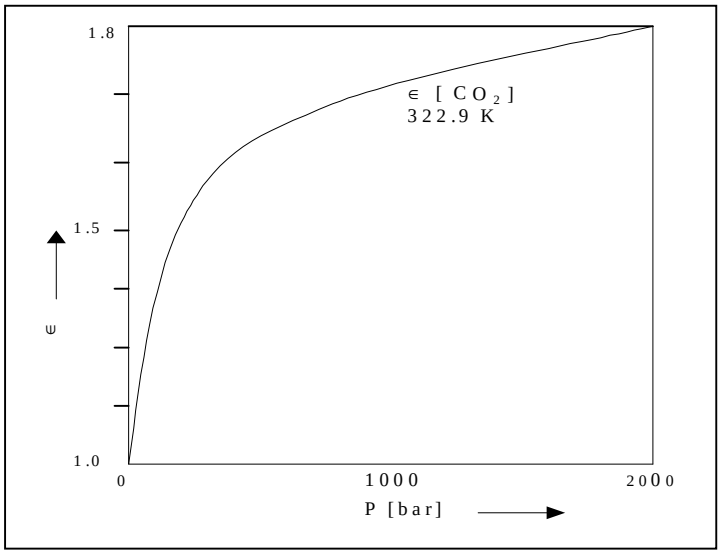


Figure 8. The dependency of dielectric constant on pressure [22]

Critical data do vary with substances. The variation is more pronounced with the following examples: supercritical water exists only at very high critical data (T_C: 647.3 K, P_C: 221.1 bar) while supercritical helium is obtainable at 5.3 K and 2.29 bar.

1. 1. 1. Evaluation of thermodynamic behavior of supercritical fluid

A good understanding of thermodynamic behavior of supercritical fluid and components contained therein is necessary for the development of effective supercritical fluid extraction processes. The phase equilibria of a system that is made up of more than one component, that are being considered in this section, will help to understand the complexity of supercritical extraction of real sample.

1. 1. 2 Phase diagram of simple binary systems

The thermodynamics of processes involving mixtures are generally very complex and a prediction of phase equilibria of a complex mixture in a high-presure equilibrium is even more difficult. However, a closer look at phase diagrams of binary mixtures will be helpful in

grasping SF extraction processes of such mixtures. For this purpose, phase rules are applied. Gibbs expressed phase rule as follows:

$$F = K + 2 - P$$

where F = the degree of freedom, i.e the number of independent variables that must be specified to describe properly the thermodynamic state of a mixture; K = the number of components and P = the number of phases.

It follows that the phases in a binary system are presentable on a three-dimensional graph and that any additional phase results to a unit reduction of the degree of freedom in the mixture.
A three-dimensional phase (Ptx) diagram such as the one in Fig. 9 is required for good description of a binary system.

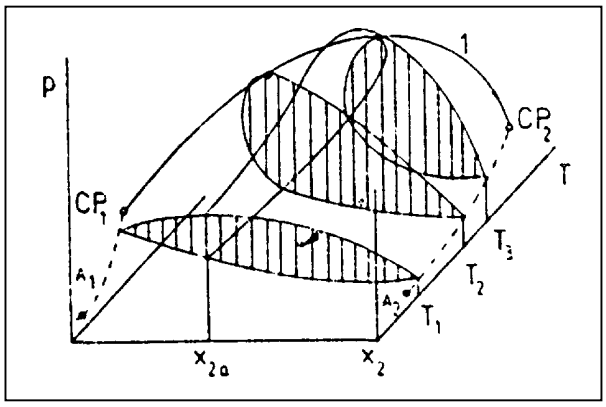


Figure 9. Three-dimensional pressure (P), temperature (T), mole fraction (x) graph for a two-component system (PTx diagram) [25].

Figure 9 shows two totally miscible and chemically very similar components (e.g CO_2 / n-hexane). CP_1 and CP_2 represent their respective critical points. The line from A_1 represents the vapor pressure of the more volatile solvent (1) while that from A_2 represents the vapor pressure of the less volatile solute. X_{2a} represents the mole fraction of the mixture. The two components influence each other's critical point when coexisting in the mixture. The line

joining the critical points CP_1 and CP_2 is known as the critical curve. This curve has moveable boarders, which means that any variation in the composition of the mixture X_{2a} at any condition, will result in a change of the critical point of the mixture. The cross-section outline on the graph provides information about the composition of the binary system gas / liquid under isothermal conditions. The border to liquid phase is shown on the graph as the area in front, that lies above the imaginary line A_1-CP_1, CP_1-CP_2, CP_2-A_2 and the one below represents the border to the gaseous phase. The coexistence of two phases is represented by the surface bound by the two vapor pressure lines of the two phases.

Figure 10 depicts a simplified form of the phase diagram in Fig. 9. Fig 10a shows that there are generally three possible ways the critical line or curve can join the critical points of the pure components CP1 and CP2, namely a direct connection (curve 2), via a minimum (curve 3) and maximum (curve 1) connection. The cross-section shows the inside of a heterogenous (liquid and vapor) phase system.

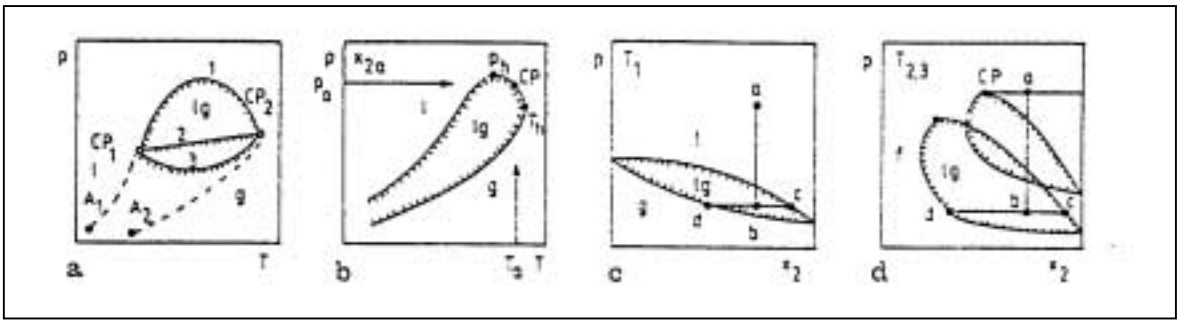


Figure 10. Two-dimensional projections of Fig. 9 (l = liguid, g = gaseous, s = solid, f = fluid)

At any temperature and pressure above each critical curve, the two components are totally miscible, hence a binary supercritical fluid exists. However, in the case of curve 1, the mixture can be heterogeneous instead of a homogeneous supercritical fluid at a pressure above both critical pressures and a homogeneous binary supercritical state is attainable at a pressure below the two critical pressures in the case of curve 3. These three cases show that the individual critical conditions of individual components in a mixture cannot be extrapolated to the mixture in question. This is more so in the region near the critical point.

In Fig. 10b the surface obtained from a mixture with constant composition x_{2a} is presented on a PT plane and P_h and T_h are the highest positions that can be attained in a binary phase system. The region of supercritical fluid begins from the point where the critical curve intercepts the mole fraction at the right upper corner of the graph.

Figure 10c shows the isothermal segment of a projection at a given temperature T_1. Due to the fact that the temperature for the segment lies below the critical curve, there is non-existence of supercritical phase. Hence, one can speak of a normal PT-phase-diagram of a binary mixture with the melting line above and the boiling line below. Since the vapor pressure of the two components still exists, the outward ends of the curves are regarded to lie on the ordinate of this projection. Therefore, if from position ***a*** on the liquid homogeneous phase, the pressure is isothermally reduced, the phase will break into a two phase-system. The vapor phase will have the composition x_c, the liquid phase is composed of components with x_d as the mole fraction.

Fig 10d shows that a supercritical fluid can be achieved only when the isothermal segment of the projection in a P,x-plane intersects the critical curve. In the P,x-plane (Fig. 9) in which $T_{c1} < T_2 < T_3 < T_{c2,}$ one critical point is obtained for the binary system, respectively. Above this point, the two components are completely miscible throughout the composition range. This means that either of the components can function as a solvent and dissolve the other. Isothermal decompression of the homogeneous phase at ***a*** will lead to a good separation of the two components. If depressurisation is applied befor the pressure ordinate can reach the two-phase region, it results in the enrichment of the liquid phase with substance 2 (the dissolved component) ($x_2 \leq 1$).

1. 1. 3 The phase diagram of a complex binary system

In view of fluid extraction of natural substances such as spices, the behavior of a binary mixture that is composed of a non-volatile solid (component 2) and a light supercritical fluid (component 1) is looked at here more closely. The PTx-diagram for this type of a binary mixture is shown in Fig. 11.

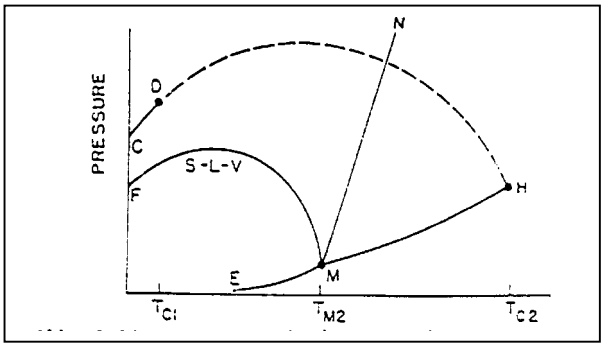


Figure 11. P,T-phase diagram of a complex binary mixture [24, see25*[2]]

The three-dimensional projection is shown in Fig. 12. Characteristically for this system is the occurrence of a three-phase curve (FM) in addition to the critical curve (DH), due to a fall in the melting point M from (2), (which is analogue to an ebullioscopy that results from dissolving supercritical (1) in (2).

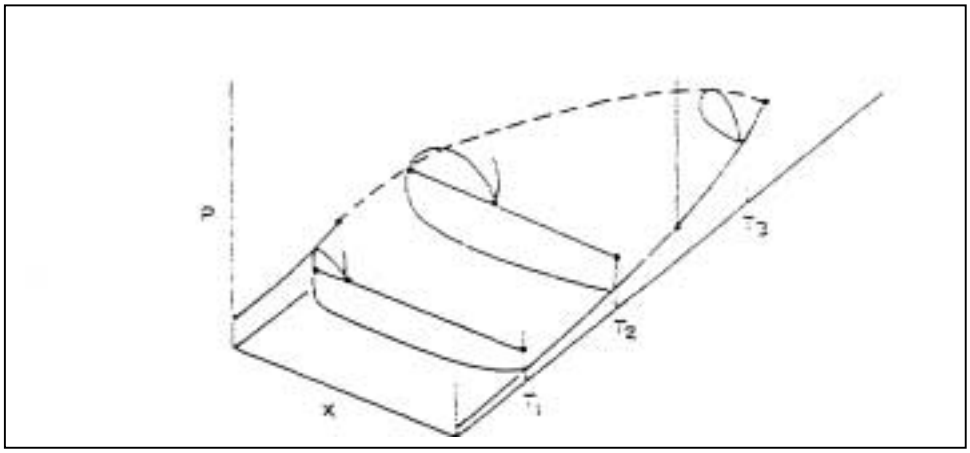


Figure 12. P,T,x-phase-diagram of a complex binary mixture [25, see*[2]]

Different isothermal segments that ly before T_{c1}, but between T_{c1} and T_{c2} are also presented in this three-dimensional diagram. However, the projection is very complex, due to the additional three-phase curve (s-l-g-curve).

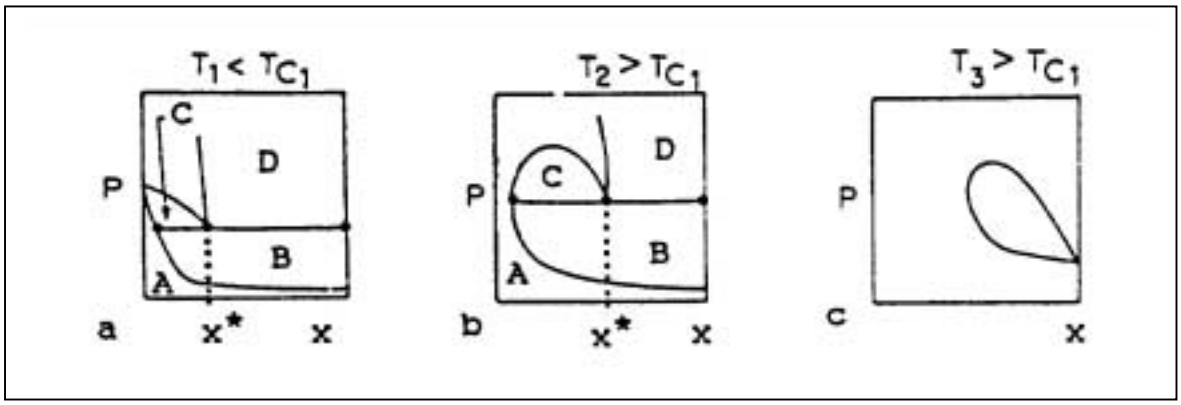


Figure 13. P, x-isothermal segments out of Fig.12.

In Fig. 13a, the isothermal segment below the critical temperature of the more volatile component 1 is shown to be an almost horizontal three-phase-line (curve) of solid-liquid-gas. Leftwards from x* and at low pressure, a homogeneous gas phase (A) exists in all ratios of the mole fraction. A minimal increase in pressure here results to an intersection of the sublimation curve of the solid (2) (this is the region of solid (2)-gas mixture). The more volatile component solidifies by a futher pressure increase, resulting in the formation of a liquid-gas-system (C) that stretches from the pressure coordinate of component (1) to the composition x*. An increase in the concentration of (2) i.e the mole fraction > x*, leads to the formation of a two-phase region (D) {liquid-solid (2)}. However, if the mole fraction is < x*, one phase system will be formed, in which (2) is dissolved in (1). In this case, there is no critical phase.

The isothermal segment projection at a temperature $T_2 > T_{c1}$ (Fig. 13b) shows more clearly the following major difference to Fig 13a:

- The two-phase region is no longer joined to the ordinate,
- The mole fraction that separates the two systems, fall into (1) by too high concentration of (2) which in turn is caused by higher temperature. The effect is that the two-phase system (D) is much smaller and the two-phase region (C) bigger than at temperature T_1.
- The highest pressure in the two-phase system (C) ends at the critical point. This means that a supercritical composition is the only existing composition in the system.

Figure 13d shows that at a temperature T_3 (bigger than the melting temperature of the less volatile component (2), the behavior of the phases is anologue to the cases presented in 1. 1. 2.

The behaviour of a binary system consisting of substances that are chemically and physically different is considered in this section.

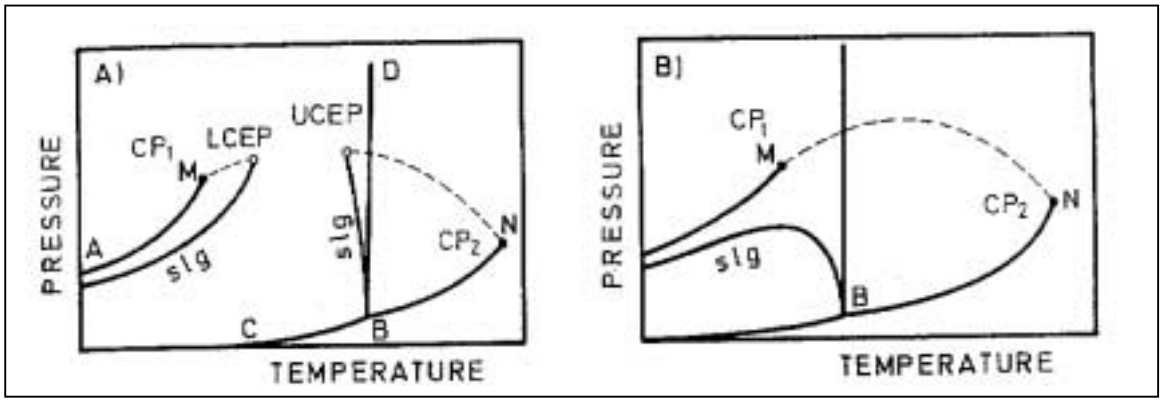


Figure 14. P,T,x-phase diagram for a mixture consisting of a light component (1) and a heavy non-volatile solid (2). (A) Immisicible and (B) miscible molten solid and supercritical fluid [18].

Figure 14 is the P,T-diagram of a binary mixture that consists of a heavy non-volatile solid (component 2) and a light supercritical fluid (component 1) with AM and BN representing the vapor pressure curves for the pure components, BD representing the melting point and CB the sublimation curve for component 2; CP1 and CP2 denote the critical points for the pure components.

The solid component has a melting point of T_{m2} that is higher than the critical temperature of the light component T_{C1}. In addition, the molecular size, form, structure and critical conditions of the two components differ to a great extent.

The major difference between the binary systems of mixtures of different compositions and the one that has been discussed previously is the double three-phase curve. While two vapor pressures for the two components remain the same, their critical conditions show two

branches of the critical mixing curve: one starts at the critical point of the heavy component (CP_2) and intercepts the freezing point depression curve (s-l-g three-phase curve) at an upper critical end point (UCEP). The other starts at the critical point of the light component (CP_1) and intercepts the s-l-g curve at a lower critical end point (LCEP). Freezing point depression of the solid arises from the solubility of the light component in the heavy liquid. If such solubility is high enough, the s-l-g curve starts at the melting point of the heavy component and extends undisruptedly to the lower temperatures. The critical mixing curve also joins the critical points of both components without break (Fig. 14). In a case where component 1 is only slightly soluble in the heavy liquid phase, the depression of the melting point of component 2 is quite small. Th s-l-g curve in Fig. 14 rises steeply and intercepts the critical mixing curve at both the LCEP and the UCEP. At these critcal end points, the liquid and the gaseous phase in the s-l-g line converge on a single fluid phase in the presence of excess solid. The extraction of solids with solvents in a supercritical state may occur in the gas-solid region within the two branches of the s-l-g curve. Three-dimensional P,T,x-diagrams for elaborate explanation of the P,T-projections are discussed at length in [25, 25*[2], 25*[3]].

1. 1. 4. The phase diagram of a ternary system

A ternary system is described at best by applying the Gibbs´ phase-rule to a four-dimensional graph of the system. For clearity purposes, two parameters are always held constant. A typical ternary system projection with constant pressure and temperature is shown in Fig. 15.

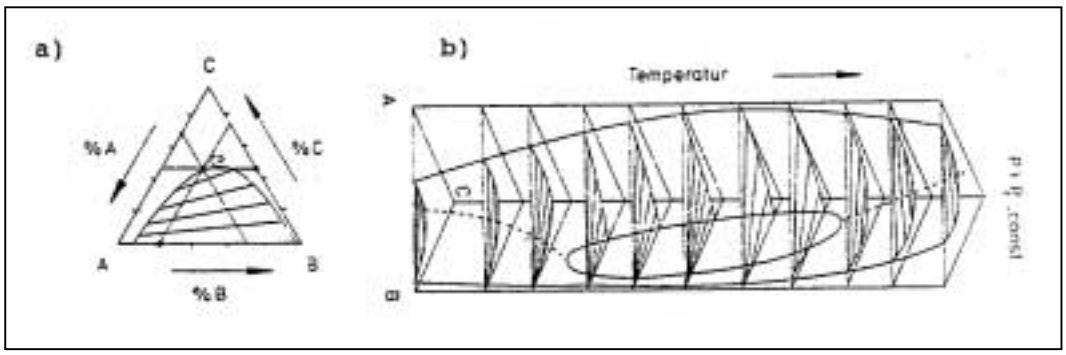


Figure 15. The projection of a ternary system [25].

The composition of each component of a ternary phase system can be determined by drawing a parallel that goes through the joining point of the other two components and meets the third side of the triangle on which the component in question lies (Fig. 15a). The concentration of component A, therefore, will be equivalent to 100 % of angle A of the triangle. The concentrations at CP will be approximately the following: 30 % A, 20 % B, 50 % C and the outline of the cross section has a mixed composition. The addition of another variable to the phase projection will result to a column-like projection with a triangular base (Fig. 15b).

The ternary phase system is applicable especially for such fluid extractions in which modifiers are employed. Modifiers (or entrainers) are usually organic additives to binary systems that enhance the solvent power of supercritical fluids by raising their average polarity. The role of modifiers in fluid extraction with CO_2 is to modify this extraction fluid that semi-polar compounds can be extracted. Zhuse et al [26] reported in 1958 the use of entrainers for extraction.

The modifying effect of entrainers is based on their ability to either initiate a hydrogen bond, dipole-dipole or an acid-base interaction with the extractants. Generally, a small amount (usually less than 5 %) of mostly polar solvents such as water, methanol, THF or isopropanol are employed [25, 26]. Berger [26] reported a density reduction at pressures above 80 bar if a modifier concentration (up to 28 %) is employed. The modifying effect of water, a non-toxic solvent, was investigated in this work.

1. 1. 5. Behavior of substances under phase equilibrium conditions

Elaborate experiments are necessary for the determination of substance solubility in a supercritical fluid in a state of equilibrium. Two methods are generally employed:

Dynamic approach

A certain amount of sample is weighed into an extractor vessel. The vesssel is covered with metal diaphragms (≤ 10 µm bore). Thereafter, a definite amount of extraction fluid is

connected in a circuit with the vessel. The circuit connection ensures equilibrium of the phases. Upon the establishment of an equilibrium, aa aliquot of fluid is taken away and the content is quantitatively determined.

Static approach

A definite sample amount is weighed into an extractor vessel with a transparent viewing cell. The extraction fluid is brought to a supercritical state, the parameters are varied until the two-phase system changes into a single-phase system. The whole system is, at this point, in a state of equilibruim and a quantum of fluid is taken away for quantitative determination of the content.

A mathematical theoretical approach is also applied. This approach is based on two principles in which supercritical fluid is regarded either as a compressed gas or an expanded liquid. Even this theoretical approach is not possible without experimental determination of some data. The first principle is based on the fact that the solubility of a solid depends on its vapor pressure. However, theoretical values differ greatly (up to 10^{18} [158]) from experimental results. This approach has been described in detail by many authors [119-161].

1. 1. 6. Conditions for solubility of substances in supercritical CO_2

The solubility of a substance, as has been stated already, depends on the density of the supercritical fluid which in turn is a function of pressure and temperature. This relationship can be elaborated upon by examining the solubility isotherm of benzoic acid.

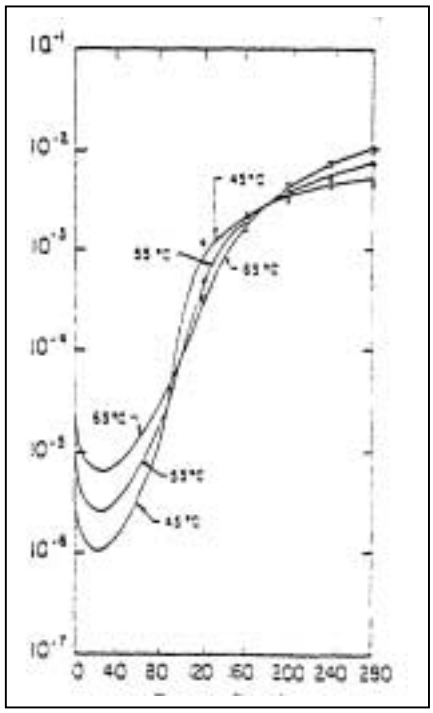


Figure 16. Solubility isotherms of benzoic acid in supercritical CO_2 [27].

Fig. 16 demonstrates that the origin of the isotherms on the graph lies on the abscis, which is within the region of a subcritical fluid. The fluid is still gaseous and the solubility is determined by the vapor pressure of benzoic acid and the pressure of carbon dioxide. An increase in pressure to about 30 bar results to a decrease in the gas-gas-interaction and the solubility sinks as a result. The density increases again with increase in pressure. A jump in the increase is observed on reaching a pressure of about 85 bar, which is the region of supercritical CO_2. Any little increase in pressure along this region results to a rapid increase in density, which in turn means an increase in solubility. This phenomenon continues till ca 140 bar. Around 150 bar the compressibility of the fluid begins to diminish, which manifests itself on the solubility curve that flattens up. The maximum is reached at 1800 bar.

The effect of temperature is more apparent, if isotherms at 45 °C and 65 °C are compared. A higher vapor pressure exists for the less soluble component at the higher temperature. This means, at lower pressure, gaseous benzoic acid is dissolved in gaseous CO_2. Upon reaching the supercritical region, the density increases, and invariably the solvent strength increases. In

this region, density is the major determining factor on solubility. At a particular pressure (180 bar) the vapor pressure increases for the less volatile component, becomes predominant again and there is a solubility shift in favor of the high temperature isotherm.

The observed behavior is characteristically especially at high extraction temperatures for non-polar compounds such as fats, oils or waxes. The increase in solubility can be explained thus: a temperature increase by an increasing pressure has hardly any effect on density change, however, it causes an exponential increase in vapor pressure of solubilized components.

1. 1. 7. General criteria for solubility of a substance in a supercritical fluid

The following general rules can be drawn from the aforementioned factors that enhance solubility in supercritical CO_2 :

A. Density is the principal variable determinant of the solvent strength of supercritical fluids. Solubility increases with increasing solvent density.
   The solubility also increases with increasing pressure. The increase is more pronounced near the critical point as a result of remarkable changes in solvent density.

B. The solubility increases, remains constant or decreases with increase in the temperature at a constant pressure, depending on the predominant factor, viz. the solute vapor pressure or the solvent density.
   i) At low pressures, the solubility decreases with temperature (this is due to the decrease in density with temperature)
   ii) At high pressure, it increases markedly with temperature (this is at the dense, less readily compressible region and results from the vapor pressure of solid).
   The maximum solubility increases with increasing temperature at high pressures.

C. Substances with larger vapor pressure, lower molecular weight and low polarity show a better solubility in supercritical CO_2.

D. The effect of density on solubility is utilizable for a selective extraction by varying the temperature and pressure.

E. Modifiers enhance the solubility and selectivity of substances in a supercritical fluid. However, the optimum modifier and concentration usually have to be determined experimentally.

F. Polar modifiers such as water, acetone or methanol enhance the extraction of semi-polar substances with non-polar carbon dioxide.

1. 1. 8. The use of supercritical fluid CO_2 for extraction

The advantages of supercritical fluid CO_2 as extraction medium are as follows:

- Critical data of CO_2 are achieveable with low energy input. Moreso, low critical temperature ensures mild extraction conditions that can be used especially for the extraction of labile natural compounds from plants.
- It is sterile and poses no toxicological concerns
- It is ideal especially for food and pharmaceuticals production since no residues are left in the extract after extraction process.
- Fluid CO_2 is cleaner and less hazardous since it is non-toxic and uninflammable, and unlike n-hexane or ether, it poses no fire risk and leaves no environmental harzardous wastes.
- CO_2 is chemically stable and inert, as a result, and does not react with extraction samples, except strong bases.
- It protects samples against oxidation.
- It is an abundant extraction medium hence very economical. It results to no additional waste disposal expenses etc.

1. 1. 9. Disadvantages of supercritical CO_2 for extraction

The SFE technology is not widely used due to the high costs involved in acquifing the extraction equipment. Highly trained personnel is required for the use of SFE technology. Only few disadvantages can be mentioned with SF CO_2 as extraction medium.

1. 2. Areas of application of supercritical fluid CO_2 extraction

Supercritical carbon dioxide extraction has been gaining ground today as an effective extraction method in the food and pharmaceutical industries, mostly due to the less health and environmental hazards involved.

The following are the major areas in which supercritical fluid CO_2 can be employed: Extraction and refining of edible oils and fats, extraction of animal fats, removal of Cholesterol from oils and food, decaffeination, extraction of hops and spices [27-32].

Extraction of spices

Supercritical carbon dioxide extraction method is used to extract essential oils, pungent and color components from spices. These extracts are used as fragrances and for coloration in cosmetic industries or for the production of pharmaceutical products. The spice extracts can be processed further, also with SFE technology, by selectively extracting any compound of interest, e.g. antioxidant active compounds. Other fields of application are the production of micro-encapsulated flavors and other food ingredients.

1. 3 Supercritical carbon dioxide extraction

For supercritical extraction contact between the supercritical fluid and sample has to be established. This can be done either by employing a static, a dynamic extraction mode or a combination of both.

1. 3. 1 Dynamic extraction

In a dynamic extraction, fresh fluid CO_2 is continuously pumped through the extractor vessel containing the sample. In analogue to a chromatographic process, a permanent state of equilibrium is maintained during the flow of supercritical medium through the extraction vessel. The longer fluid CO_2 is allowed to flow through, the more an exhaustive extraction into the collecting vessel is assured. However, according to Nernst partition rule, a near 100 % yield is possible only by means of exponential extension of the extraction duration [34].
The main advantage of this mode of extraction is the possibility to continually manipulate pressure and /or temperature, which invariably means a manipulation of density and solubility to achieve a certain extraction purpose. By means of density variation, substances with variable solubilities in fluid CO_2 are thus separated in a fractional extraction mode.

1. 3. 2 Static extraction

For the static extraction, the sample is soaked in a large and sufficient amount of fluid medium. The flow of the fluid is halted over a certain period of time to achieve an equilibrium under definite conditions. Then the valve is opened and the extraction medium is allowed to flow to the separator vessel. The penetration time is the single most important aspect of static mode of extraction, especially in a case where analytes cannot be readily removed from the matrix. Therefore, for a compact or dense sample, a slow extraction will be ideal. The volume of the extractor vessel is a limiting factor here hence exhaustive extraction of the analyte is seldomly realized in a static extraction. Lancas et al [33] compared both extraction modes in the experimental extraction of PAHs from carbon. He discovered that the only difference is that the static mode gave a low yield and lasted longer, otherwise there was no difference in the quality of the extracts obtained. The best results can be obtained by a combination of both methods.

1. 3. 3 The efficiency of SFE technology

Lipophilic components of food, such as fats and oils of plant and of animal origin usually are separated by means of a thermally aided conventional solvent extraction. In most cases, hazardous organic solvents are often employed in large quantities. After the extraction process, the solvents are separated from the extract by distillation.

In both extraction and solvent separation process, the extract is subjected to a constant effect of high temperature. This usually results to thermal degradation of thermolabile components of the extracts, which in turn leads to the formation of artifacts or off-flavors. This affects the quality of the extract. The presence of solvent residues in the extract is another problem associated with the production of extracts with organic solvents. Due to the increasing awareness of environmental problems, the solvent extraction of certain flavors, essential oils and fats, or separation of complex mixtures is being replaced more and more by high pressure extraction.

1. 4. The spices (Historical aspects)

The use of vegetable additives, now known as spices and herbs, dates back to a few millenia before written records. No sooner than their arrival at the coast of West Africa did the European colonialists develop appetite for aroma, bitterness and purgency of the local spices that include “Ose Ndi Igbo” or the ashanti pepper (*Piper guineense*) and brought these spices to Europe. The use of spices and high quality oils was mentioned in the bible and many spices such as sringaveara (ginger), haridara (tumeric), marica (black pepper) were mentioned in the ancient sanskrit texts of India, dated by recent research to at least 4000 to 5000 years BC [34-35]. Although these texts presented philosophical precepts, they, however, give descriptions of land and life of the people including the use of food additives to enhance the appeal and acceptability of food and their nutritional values. With the advent of civilization, these food cultures came to be refined in formulations, cooking and presentation.

1. 4. 1. Modern agricultural practice as source of pesticide contamination

In Africa, Asia, and South America from where these spices mainly originate, the traditional method of cultivation of the spice plants was basically peasantry in nature. The local farmers produced enough for the local needs. However, as demand increased especially from abroad, there was the need to produce more. Plantations were established where only these crops were cultivated in large quantities. The monocultural agricultural practice that was intensive in nature heralded the practice of crop protection. To ensure such benefits as regular and stable harvest and improve the quality of the harvest, chemical plant protection was inevitable. Pesticides were applied for different purposes and at different times of the year. Efforts were made with little success to obtain enough information about pesticides use in countries from where the spices are imported [36]. However, according to the information from the Food and Drug Administration, "Bayerische Landesanstalt für Bodenkultur und Pflanzenbau" and "Zentrallaboatorium Deutscher Apotheker", the pesticides that are often identified in pesticide residue analysis can be grouped into insecticides and herbicides.

There are four major groups of insecticides, namely, organohalogens, n-methylcarbamates, organophosphates and Pyrethroids, that are used for agricultural purposes. Hence their residues are found in foods, pharmaceutical products and all types of environmental samples.
Herbicides can be grouped into different categories based on chemical classes, properties and usage. The pre-emergent, non-selective herbicides are the most heavily used herbicides. Normally they are used to clear fields before planting. Triazines and acetamides belong to this category as well as contact herbicides such as glyphosate and paraquat. There are many publications on supercritical fluid extraction of triazines, especially from soil matrices.

Selective herbicides such as sulfonyl ureas, imidazolinones, and phenoxy acids are used during the growing season for weed control. Due to their early-season usage or low usage near harvest, high solubility in water, and rapid degradation rates, herbicides are seldomly found in foods with the exception of the *non-polar dinitroanilines*. Also with the exception of dinitroaniline herbicides, class-specific methods are commonly employed for their analysis.

Fungicides are employed in a lot of ways against spoilage of produce in storage and to extend their shelf life. Since they are often applied lately in the growing season or even after harvest, they are the most commonly detected pesticides in many fruits and vegetables**.** For the same reason, (benz) imidazoles, triazoles, phthalimides, and similar classes of fungicides are not very much of concern in environmental samples. Multiresidue analysis of these types of fungicides in food using GC may be very difficult. HPLC gives better peak shapes for these fungicides and is used for their analysis [36-39].

1. 4. 1. 2. The sources of PCBs as contaminants in spices

Polychlorinated biphenyls (PCBs) are a class of 209 non-polar, chlorinated hydrocarbon congeners with a biphenyl nucleus on which one to ten of the hydrogens have been replaced by chlorine. PCBs are no pesticides but were marketed for use in various applications, including dielectric fluids in capacitors and transformers, heat transfer fluids, hydraulic fluids, lubricating and cutting oils, and also as additives in printing inks, paints, dedusting agents, **pesticides**, copying paper and carbon-less copy ("NCR") paper, adhesives, sealants and plastics. PCBs are used as synergistic additives in pesticides to enhance their effects.

Since PCBs are not readily degradable in the environment after disposal or dissemination and are lipophilic, they are persistent and tend to bioaccumulate. The usage in inks and other open-ended applications resulted in widespread, low-level release to the environment, while the closed and controlled uses such as dielectrics within electrical equipment can and has resulted in environmental release because of spills, improper handling, or improper disposal. Most of such releases tend to be localized near the use site, in landfills or elsewhere and can often result in relatively high, localized concentration. Due to gradual environmental aided distribution and other means of distribution PCBs have spread from the initial contamination sites to non-contaminated areas. It is shown to be nearly a ubiquitously environmental pollutant, occurring in most human and animal adipose samples, milk, sediment, and numerous other matrices that include spices [40].

A list of pesticides that are often identified in vegetables during regular pesticide residue analysis by Landesuntersuchungsamt (LUA) [41] show possible pesticide residues to be expected in spices. These pesticides were used as a guide for the residue analysis and SFE method development.

Organophosphates	**Organohalogens**
Azinophos methyl	PCB's (28, 52, 77, 101, 138, 153, 180)
Azinophos ethyl	2,4.Dichlorophenol
Bromophos ethyl	Pentachlorphenol
Bromophos methyl	HCB
Chloropyriphos methyl	∝-HCH
Diazinon	β-HCH
Malathion	γ-HCH (Lindan)
Parathion methyl	DDT
Parathion ethyl	DDE
	DDD
Triazine derivatives	Heptachlor
Atrazine	cis-Heptachlorepoxide
Simazine	trans-Heptachlorepoxide
Sencor	∝-Endosulfane
Terbutylazine	β-Endosulfane
	Aldrin
Dithiocarbamates	Dieldrin
As total sum	Cis-Nonachlor
	Trans-Nonachlor

Table 7. List of pesticides that are identified on a regular basis in residue analyses of LUA [41].

1. 4. 2. The most important spices

Spice plants such as pepper (black and white), paprika, rosemary, corriander,and macis are known for their essential oils. Essential oils are mixtures of volatile hydrocarbons (aldehydes, alcohols, esters, terpenes), are less water-soluble and can be found in all plant organs.

1. 4. 2. 1. Pepper (*Piper nigrum* L.) {*Piperaceae*}

Pepper is grown today in all tropical regions of the world that include India, Indonesia, Philippines, Westindies, Brazil, Nigeria, East Africa and Madagascar.

The pepper plant is a perennial climbing shrub. It has a woody stem that is swollen at the joints. As a grown plant, the pepper shrub can reach a height of 15 m and is supported by numerous rootlets that cling to any support around the plant. The leaves are alternated, broad-ovate, accuminated, coriaceous, smooth petiolated and dark green. It has very small whitish flowers that are borne in pendulous, dense catkin-like spikes. The fruit is small and berry-like and nearly globular, with a single seed and sessile. The fruit is green at an early stage, yellow as it develops and turns red when ripe. The plant produces fruits from the seventh year up to the fifteenth. The harvest is done manually by gathering the spikes from the pepper shrubs as soon as the berries begin to turn red. Thereafter the berries are dried. Drying is carried out by spreading the berries on mats in the full sun or on fire. At the end *black pepper* is obtained. Black pepper has dark brown to black berries with a thin pericarp that encloses a single seed with a hollow center. The perisperm of the seed is horny in the outer part and floury around the central cavity. At the apex of the seed lies the embryo embedded in an endosperm. Black pepper possesses a characteristic penetrating aromatic flavor and a hot, biting very pungent taste. In contrast *white pepper* is produced from the fully riped red berries deprived of the greater part of the pericarp or the outer coating. This is achieved by keeping the ripe berries in moist heaps or in sacks submerged in running water for about 3 days. This fermentation process softens the pericarp, which then can entirely be removed either by washing the berries or trampling. Thereafter the grey-white berries are spread out on mats and allowed to dry in the sun for a couple of days.

The spicy constituents of pepper that are contained in the perisperm are composed of 1-2,5 % essential oil and terpenes. It contains also the following alkaloids; 5-9 % peperin and 0.8 % chavicin (a stereoisomer of peperin). The pungency of pepper is based on the stereoisomerism of chavicin and peperin. Pepper corns or ground pepper are used for spicing up meat sausages, soups, sauces, salads etc [42-43].

1. 4. 2. 2. Paprika (*Capsicum annuum*) {Solanaceae}

From the European point of view, the origin of paprika is according to Franke [ ] the tropical Americas from where it was brought to Europe by the Spaniards. As a result, paprika is also called Spanish pepper to differentiate it from the original pepper from Asia. Besides Spain in Europe, paprika has been grown in Hungary since the 16th century. However, before the European colonization era in Africa, paprika was among the spices known to have been grown in Africa. Today African countries export paprika under trade names such as Mombasa, Nigerian, Congo etc.

Capsicum annuum is an annual or a perennial plant. There are many varieties and diverse forms. Today however, paprika is grown in temperate and tropical regions of the world. The fruit, a podlike berry, with 2 to 4 locules that are united in the upper part and divided below, varies in shape and size and contains small seeds and a discoid. It is a herbaceous or semiwoody plant with more or less angular branched stems. The leaves are alternated, entire, daccuminated and petiolated, dark green on the upper surface and paler on the lower

The spice paprika is the finely ground product of mature, dried fruits of *Capsicum annuum* L. The following categories of paprika are commercially available: sweet, semisweet and pungent. There are also various grades of each type.

Paprika contains the alkaloid capsaicin which develops in the epidermis of the placenta as oily excreet and is deposited in the cuticular where it cristallizes. Capsaicin can also be found in secretion walls (cuticular walls) and in the pericarp. Capsaicin content of paprika varies from 0.3-0.5 % depending on the sort and the climatic conditions. Besides capsaicin, paprika contains essential oils. The fruits contain in addition red carotinoides (capsanthin, α-carotin, violaxanthin, cryptoxanthin and capsorubin) as well as the antibioticum capsicidin (0.02-0.03 %). The pungency of ground paprika increases with increase in cuticular walls, seeds and placenta contents. The Hungarian type is categorized into the edible that is fairly red in color and “Dulce” or the noble sweet (none pungent), “Agridulc” or semi sweet (half- pungent) and “Picante”, the rose-paprika (pungent), and the very pungent that is brownish-red in color.

Sweet and semi-sweet paprika varieties are manufactured with the seeds and adhering placenta tissues that are macerated and washed in water to deprive them of part of the pungent alkaloid capsaicin. The color of the grade one paprika is rich-red and that of the grade 2 less intense red. Lower quality paprika varies in color ranging from brick-red to brownish-red and brownish-yellow. Paprika is used in goulash meat, soups, potato chips etc [42-43].

1. 4. 2. 3. Rosemary (*Rosmarinus officinalis* L.) {*Labiatae*}

Rosemary is grown in South-European countries. It is a woody evergreen shrub that can grow to a height of 1 m. The leaves are brownish-green, sessile, linear and slightly curved, with revolute margins, about 6–25 mm long, with a prominent midrib that is concealed by marginal curling of the leaf.

Rosemary leaves have a tea-like fragrance. When squeezed, they produce an agreeable, aromatic flavor with camphoraceous note. The taste can be characterized as aromatic, pungent, somewhat bitter and slightly camphoraceous. The characteristic flavor of rosemary is due to the 1-2 % essential oil content that is composed of pinen, camphene, borneol, campher and cineol. It is used for medicinal purposes and to spice up fish sauces, soups, salads and sausages. The fragrance is also used in liquor, soap and other cosmetics [42-43].

1. 4. 2. 4. Coriander [*Coriandrum sativum*] {*Umbelliferae}*

Coriander is reported to be native to the Orient. It is, however, grown today in Morocco, the Balkans, Russia and in Western Europe. It is an annual herb that can grow to a height of about 50 cm. The lower leaves are broad and deeply segmented or lobed and the upper leaves are composed of very narrow, linear segments. The flowers are small, pinkish and are produced in compound umbels. The fruit schizocarps are spherical and ridged. Dried coriander fruit is a schizocarp that is almost globular in form, yellowish-brown, 3 to 5 mm in diameter, with 10 straight and 10 wavy longitudinal ridges, a depression at the base, 5 small teeth and a conical stylopodium at the apex. The pericarp encloses a single seed with a small embryo embedded in the endosperm toward the apex of the seed. Dried coriander fruits have a fragrant odor and a pleasantly aromatic taste due to the 0.2 - 1 % essential oil constituent. The essential oil is composed of 60 – 70 % linalool and ca. 20 % terpenes.

Coriander is used in the production of sausage spice such as curry powder and in confectionarys, sauces, liquors etc [42-43].

1. 4. 2. 5. Macis (*Myristica fragrans* Hout) {*Myristicaceae*}

The mace of commerce is produced from the nutmeg tree. It is from the crimson aril that covers the shell-enclosed nutmeg. The aril is removed by opening it from the top and turning it back, after which it is flattened out either by hand or between boards. It is then sun-dried. Mace as well as nutmeg contains 30 % fatty oil, 30 % starch and 7-16 % essential oils. The essential oil is composed of 80 % α-pinene, camphene and other terpenes such as borneol, linalool, 8 % myristicin and other phenols. Mace oil is used as fragrance in liquor and in perfume industry. Both nutmeg and mace are used to spice up soups, meat and vegetable products, confectionarys and sausages [42-43].

1. 4. 2. 6. Supercritcal fluid extraction of spices

A two-step extraction method is usually used for the extraction of spices. In the first step, dry SF-CO_2 is used to extract essential oils. In the second step, water-saturated CO_2 is employed for extraction of flavor components. This approach is not necessary with those spices where flavor plays little or no role, for example paprika. The pungent components can be extracted with a single step approach. SF extracts have the advantages of being sterile and as a result can be used for longer periods of time.

1. 4. 3. Paprika, a representative of spices with high-lipid-content

1. 4. 3. 1 Oil content of Paprika *(hexane extract)*

Paprika essential oil from solvent extraction is dark-red in color, viscous and soluble in fat, oils and organic solvents. The characteristics are given in Table 8.

Table 8. Physical and chemical characteristics of paprika oil [44].

Characteristics	Value
Refractive index (25 °C)	1.4780
Specific mass (25 °C)	0.9212
Acidity Nr.	16.28
Iodine Nr.	142.2
Saponification Nr.	180

Based on the effectiveness of the extraction process, paprika extracts either do or do not have the typical paprika aroma. Paprika extracts from organic solvent extraction have high contents of unsaturated fatty acids and tocopherols (0.9 – 1.0 %) and a total carotinoid content of 4.3 % [44,44*[v], 44*[g]]. Table 9 shows extract (oleoresins) compositions from different paprika species

1. 4. 3. 2. Oil compositions of spice extracts

Table 9. Extract (oleoresins) compositions from different paprika species [44]

	Capsaicinoids [%]	Coloring substances [%]	Fat and other substances [%]
Oleoresin capsicum (high pungency)	3-6	1	90
Oleoresin red pepper (low pungency)	1-3	3	85-90
Oleoresin paprika (little or no pungency)	0.5-1.0	10-15	80-85

1. 4. 3. 3. The importance of paprika components for the food and pharmaceutical industries

The colorants of paprika are more important than the pungent for the food and pharmaceutical industries. The various carotinoid compounds are either used as natural coloring substances for food, cosmetics and pharmaceutical products.

2. Physico-chemical properties of relevant pesticides.

Table 10. Solubility of organophosphate pesticides and triazine herbicides in water

Pesticides	Log K_{OW}	Solubility range in water (mg L^{-1})
Organophosphates		10^{-1}-10^{6}
Diazinon		
Parathion-methyl	3.81	
Parathion-ethyl		
Malathion	2.89	
Bromophos-methyl	5.07	
Bromophos-ethyl		
Azinophos-methyl	2.96	
Azinophos-ethyl		
Chlorpyriphos-methyl	4.24	
Chlorpyriphos-ethyl	4.7	
Fenitrithion	3.43	
Pirimiphos-methyl	4.1	
Triazines		10^{0}-10^{4}

Table 11. Physico-chemical properties of some typical chlorohydrocarbon pesticides [18].

Compounds	Solubility in lipids (g L^{-1})	Aqueous solubility (mg L^{-1})	Log K_{OW}
4,4'-DDT	330	3.36	6.36
Heptachlor	1000	50	4.11
Dieldrin	3700	200	3.88
Lindane	800	130	2.67

2. 1. Legislation / regulations on pesticides

The tolerance levels are set by the regulatory authority to control the pesticide use and to make sure that the residues are kept as low as possible. There are local, regional and international regulations on pesticides.

2. 1. 1. The German pesticide residue regulations

Pesticide residues in food in general are regulated in Germany by the food and essential products control law ["Lebensmittel und Bedarfsgegenstände (LMBG)"] and the pesticide residue control act ["Pflanzenschutzmittel Höchstmengen-Verordnung (PHmV)"].
LMBG and PHmV offer, in a nutshell, the following provisions:
Food should not be made commercially available,

- if it contains pesticide residues above the tolerance level of the pesticide in question.
- if it contains more than 0.01 mg Kg^{-1} of banned pesticide residues or a pesticide residue that was not specifically registered to be used for food production, unless another tolerance level is stated in paragraph 3 of the pesticide residue control law Nr.1 § 3 of RHmV.

The concentration of pesticide residues that are listed in the table of substance tolerance level, but are not specifically named for the particular food involved, should not be above one tenth of the lowest stated tolerance level of the pesticide in question. The concentration level of 0.01 mg Kg^{-1} applied, should the "one tenth" rule to give a lower concentration level. *The rule exempts spices, raw coffee, tea, tea-like products and oil-bean-seed, which can contain higher levels*

Any pesticide residue listed for crop protection, but not named in this act, can be regulated with § 8 LMBG. This law states that it is forbidden to produce, process or make food commercially available for other people in such a way that its consumption or when consumed will be detrimental to human health [46].

In the past, pesticide tolerance level act (RHmV) for food is reviewed in several year intervals. Today the RHmV novellation is carried out yearly. This is the reason why every residue analyst that works in the area of food research should regularly acquaint himself or herself with the current regulations.

Decision on the tolerance level of any residue is based on the most current information available and, any limit that is agreed upon is expected not to have any ill effect on the health of consumers. From this perspective, a tolerance level is set in such a way that when the pesticide is rightly used and in accordance with good agricultural practices and observing the required interval recommended between uses, the level will not be exceeded.

Additional informations are available in some cases in the tolerance level regulations, stating whether the level is only for the active substance or it includes the metabolites (for example, malathion with its metabolite malathion-oxone). In other cases, active substances are grouped together with their metabolites or degradation products of active substances, (degradation in plants or during the analysis; e.g. carbendazim that can be produced by benomyl and thiophanate methyl). Tolerance limits for some active substances or a group of substances take into consideration that only the derivates or degradation products are analyzable, and the difficulty involved. For example, phenyl urea herbicides together with certain degradation products can be determined as chloroaniline; another example are the dithiocarbamate fungicides, whose residues are analyzed by producing CS_2 with the help of concentrated acid. These and other details have to be taken care of if the food being analyzed for a possible tolerance level violation. This is important especially in the multiresidue methods of analysis, where metabolites are either not or insuffici ently accounted for.

2. 1. 2. International Regulations

The national residue tolerance level can vary from one country to the other. This affects the international trade in the sense that it could be misused as trade barriers in trade between countries. As a result of this, efforts are being made on the regional level, (e.g. by the

European Union) and on the international level, (by WHO and F.A.O.) to eliminate these barriers through regional and international harmonization. However, due to conflicting interests, it has proved itself as not going to be easy [38].

The first step to establish a regional residues tolerance level in Europe was undertaken in 1976, when the first European list of residues tolerance level was published. The list was binding for all countries of the European Union without nullifying the nationally established tolerance level for respective countries; the national level can be lower but not above the European regional levels. Since the European Union has no single trade and commerce policies with other countries outside the region, lower residues tolerance limits by individual countries can be misused as trade barriers to protect local producers.

In a bid to harmonize the residues tolerance level worldwide, the F.A.O and the WHO have decided in 1962 to work together in the context of a Joint Food Standards Program to establish international norms. This is a sort of a worldwide recognized law; for food it is known as **Codex Alimentarius**. The aim was to enable a free exchange of food and health commodities. Commission on Codex Alimentarius with over 122 member countries was established to carry out this program. The codex committee on pesticide residues (CCPR) that was established in 1966, which since then has been meeting yearly in the Netherlands, was established as a working program to overlook the international harmonization of pesticide residue tolerance levels.

The major functions are as follows:

- to establish the maximum residue limits (MRL) in foods
- to set pesticide assessment priorities for the JMPR
- to give suggestions on method of sample collection and analysis of residues

The scientific aspect of suggestions on the maximum residue limits for the CCPR are worked out by independent F.A.O and WHO-commissioned experts. They have been meeting yearly either in Rome (F.A.O) or in Geneva (WHO) since 1965 as FAO/WHO Joint Meeting on Pesticide Residues and the Environmental (JMPR). In its yearly program, the committee

considers 20 to 30 substances that could be used worldwide as pesticides in agricultural practices and can cause residue problems in food. The JMPR receives information materials on the amount and behavior of the residues in food commodity, analytical method for the control of such residues in food and about the toxicology of the residue in question from the pesticide producers and from other sources.

The JMPR experts evaluate the information materials based on scientific criteria and make suggestions on ADI level and the MRLs for a specific food and other food commodities. After deliberation on the JMPR suggestions, the CCPR either accepts or returns it to the JMPR for further considerations and making use of new suggestions as the case may be. The results of the JMPR toxicological evaluations are not deliberated on, rather is regarded as an separate suggestion from a scientific gremium.

Suggestions from the JMPR and the CCPR are important for many developing countries with no individual research centers or that can not afford accurate toxicological and residues analysis. The results of the JMPR which are published in monographies can be used by the developing countries to facilitate their decision on licensing of new pesticides in their respective countries [38].

2. 2 Problem of contaminants in spice extracts

Till date there is not known any report about pesticides in commercially available spice extracts. However, there is a higher probability that extracts from contaminated raw material will contain residues of the contaminants. Table 12 shows the maximum residue limits of pesticides, herbicides and PCBs in spices [45].

A very low limit is designated especially for the organohalogens that can be accumulated in mammalian organs. In the experimental part, a quality assessment of the paprika extracts from SFE and conventional extraction methods was made based on these contamination levels.

Table 12. Maximum residue limits of organochlorine pesticides, triazine herbicides and PCBs in spices.

Pesticides	**Maximum residue limits in spices [mg Kg^{-1}]**	**Pesticides / PCBs**	**Maximum residue limits in spices [mg Kg^{-1}]**
Organophosphates		**Organohalogenes**	
Azinophos methyl	0.05	PCB's (28, 52, 77, 101, 138, 153, 180)	0.1
Azinophos ethyl	0.05	2,4.Dichlorophenol	0.1
Bromophos ethyl	0.05	Pentachlorphenol	
Bromophos methyl	0.01	HCB	0.1
Chloropyriphos methyl	0.05	HCH-Isomers, including β-HCH but excluding Lindane	0.2
Diazinon	0.02		
Malathion	0.05	γ-HCH (Lindane)	0.2
Parathion methyl	0.1	DDT, DDE, DDD Sum of p,p-DDT, o,p-DDT, p, p-DDE and p, p'-TDE (DDD) calculated as DDT	1
Parathion ethyl	0.1		
Triazine derivatives		Heptachlor	0.1
Atrazine	0.1	cis-Heptachloroepoxide; trans-Heptachloroepoxide	Calculated as Heptachloro-epoxide 0.01
Simazine	0.1		
Sencor		∝-and β-Endosulfane calculated as Endosulfan	0.05
Terbutylazine	0.05		
		Aldrin	0.1
Dithiocarbamates		Dieldrin	0.01
As total sum	0.05	cis-Nonachlor	
		Trans-Nonachlor	

2. 2. 1. Pesticide residues in supercritical carbon dioxide extracts

There is virtually no report on quality of extracts from supercritical carbon dioxide extraction that is based on pesticide contamination level. The few reported works are on hop. They show that there is basically no contamination problem with hop extracts. However, there are reports

from the analytical SFE that compared the extraction efficiencies of solvent extraction with supercritical fluid CO_2, in which the later was shown to be generally more efficient by producing higher yield [46]. The very qualities of supercritical fluid CO_2 such as low surface tension that assures higher penetrability, higher diffusivity for better carrying properties, and high solvating powers that ensure its higher extracting power result in high quality extracts. Nonetheless, the very qualities of fluid CO_2 that ensure effective carry-over of lipophilic flavors or oils could also lead to an extraction of lipophilic residues. It is, therefore, necessary to investigate whether there is really a tendency for accumulation of such substances in the extracts.

3. Standard instrumental analysis of pesticide residues

3. 1 Pesticides analysis

The analytical work-up should be based on the selectivity of the technique used for the determination. For example, a good clean-up is necessary if a relatively non-specific UV absorption is employed. A good clean-up is also necessary, for example with thin layer chromatography.

If gas chromatography is employed for substance determination, then the detector used determines extent of the clean-up. The highly selective flame photometric detector (FPD), on the one hand, requires practically no clean-up, however, due to possible damage to the column, sample clean up is usually carried out. On the other hand, ECD that registers several non-pesticide sample components, requires a thoroughly cleaned extract. If in place of a packed column, a capillary column is used, there are less chances that interference peaks could be interpreted as pesticides, due to their separation efficiency. However, interferents can also depend on the required sensitivity of the detector used. For example, if a control analysis is carried out to determine whether a regulation on tolerance level is kept, then it will be enough to work within the boundaries of 0.5 mg Kg^{-1} requiring less sensitive detectors and at the same time is not be prone to interference. It is possible only in very few instances to utilize one analytical method to specifically analyze certain pesticides or a small group of pesticides e.g. the determination of dithiocarbamate fungicides as CS_2. A specific method may be used if only one substance is involved.

3. 2 The traditional multiresidue methods (standard work-up for spices) [47-49]

Homogenization

Homogenization is normally carried out in liquid medium in order to limit degradation and volatilization processes and to allow the use of salts, acids and bases and different partitioning techniques.

Extraction

High selectivity can be achieved by using liquid based extraction and cleanup by means of liquid-liquid partitioning and chromatographic means. But these types of extraction and the associated solvent concentration steps are time-consuming, require space and specialization equipment and add to the expense. However, many aspects of liquid-based extraction can be automated with sample size reduction, thereby reducing the associated limitations with traditional multiresidue methods.

Partitioning and Clean-up

The next step after the extraction of pesticide residues from the sample is to clean the residues from plant co-extractives. Generally, about 100 g of plant sample is extracted. The extract contains about 100-500 mg of natural plant components, which can be reduced to 1-10 mg with the help of an effective clean-up method. GPC is often employed [84-88].

Determination

The determination of the residues in the pre-cleaned extract by a selective method of analysis due to plant co-extractives is required. If the residue concentration in the sample is 0.1 mg Kg^{-1}, the concentration of the residue in the cleaned extract will be only 10 µg with 100 to 1000 times more co-extractives.

The choice of analytical method is based on the problem involved. For example, if the degradation of a certain pesticide active substance in plant or in the soil is to be determined quantitatively, the knowledge of the chemical and physical properties can be utilized to develop a clean-up method that can remove sample components which may cause interferences during the analysis. Almost all the chemical properties of a molecule can be utilized for the determination, e.g. a functional group that can exhibit an absorption maximum or support color reaction, a hetero atom that can be detected by a selective detector such as an ECD, or exhibition of unique biological activity. Whatever method may be chosen, the aim always remains a successful quantification of the substances identified.

In effect, not only the pesticide active substance to be always determined, but also the properties of the determinable metabolites (functional group, biological activity etc) are targeted. If it is only the active substance or the metabolite that should be determined, then the final identification should apply a chromatographic method to differentiate the substances (chlorohydrocarbons [50-55], PCBs [56-60], organophosphates [61-65], pyrethroids [66-67], triazines [68-83]).

3. 2. 1 Residues identity confirmation possibilities

Analytical result obtained with certain analytical methods conveys only the information about the several properties of the analyzed substance which tends to behave like a certain pesticide, and that the analyst can make the assumption with a greater probability that both substances are identical. However, identity clearification is rather difficult.
Unexpected disturbances from sample co-extractives, metabolites, reaction products of pesticides and sample components or other sources can result to wrongful interpretations in the residue analysis (PCB peaks, for example, have been interpreted as DDT or DDE for many years). Moreover, a quantitative analysis makes sense only by a correct identification, because some detectors like ECDs give different signals to compounds with various structures.

GC should only be used for substance identification if two columns of different polarities are employed. The suitability of the second column can be ascertained by a comparison of a graphical presentation of the relative retention times of the substance groups in question. A near linear graph shows that the two columns are unsuitable and the more the non-linearity is, manifested the more the possibility of employing the second column for some sort of GC confirmation. More reliable are retention times on capillary columns due to their high separation efficiency.
The less the quantity of the sample or residues involved, the more is the need for result confirmation steps. At the same time the identification and the confirmation should be carried out by separate analytical methods (the usage of different separation columns, identification

systems or making a derivate of the substance). The following aspects depend on the same properties of a substance: partition coefficient (p-value) and R_f-value in the partition chromatography, R_f-value in thin layer chromatography and rate of elution in the adsorption chromatographic column, and retention time on identical columns in GC. These values corelate to each other, and as a result, they give no new information on the substance identity.
In a good analytical practice, a substance is then declared as identified only after it has been confirmed by other independent methods such as GC-MS etc.
In practical terms, certain pesticide-substrate combinations cannot be anticipated, could be a useful hint, since, on the one hand, certain pesticide active substances cannot occur in a sample or undergo further reactions. For instance, parathion or heptachlor are not expected to occur in meat samples, instead, the metabolite, paraoxon or heptachlor epoxide is. On the other hand, one should not conclude that certain pesticides occur only in the areas for which they are approved. Important among different methods of pesticide confirmation are those that are suitable for large groups of substances [79-81].

3. 2. 2 Problems involved in residue analysis

Pesticides and their metabolites occur in food commodities generally in trace concentrations in the magnitude of 0.01 mg Kg^{-1}. The analysis involves the identification and quantification of the residues that usually occur in trace concentration together with enormous amounts of natural components of the sample.

4. Alternative methods of pesticide analysis

4. 1 Pesticide analysis by means of SFE and SFE-SFC methods

Analytical supercritical fluid extraction employs CO_2 and other gases at high pressure and moderate temperature instead of an organic solvent. Beyond the critical point, the properties of supercritical fluids resemble those of liquid and gas. Supercritical fluids have higher diffusivity and lower viscosity than liquids that approach those of gases, which contribute to their superior extraction and separation capabilities. The solvent strength of the fluids depends on the chemical composition of the fluid and the fluid density, which can be controlled by temperature and pressure. The analyst can control extraction and chromatographic separation process by manipulating the temperature, pressure and the composition of the fluid.

A typical analytical SFE apparatus consists of a pump for delivery of the supercritical fluid to a tubular cell packed with 1 g or less of sample, a sample collection vessel and a restrictor to maintain pressure through the system. In addition to the extraction advantages, most of the SFE solvents are non-toxic and evaporate at ambient conditions, thus reducing post-extraction concentration problems, laboratory safety and waste disposal concerns.

The major disadvantage of SFE is the need for a special equipment. However, the accelerated extraction (min) and the possibility to conduct the SFE on-line with a supercritical fluid chromatography (SFC) or gas chromatography system make up for this.

4. 1. 1. Relevant examples of analytical supercritical extraction of pesticides

Several SFE methods for organochlorine insecticides that ranged from selective extraction, in-line cleanup (in-line separation of insecticides from fat) and post-SFE clean up have been reported by King et al [46]. The on-line/in-line pesticide separation from sample lipids is particularly of interest in the sense that some inherent principles can be used for this research. If a low density was used for a selective extraction of the insecticides with a reduced lipid co-extractive an incomplete extraction of non-polar pesticides is obtained

Another SFE approach of interest was the in-line clean-up method applied in the separation of pesticides from fat tissue matrices. It was shown to be a very selective technique and was used for the first time by Franke et al [89]. This involves placing a sorbent between the sample and the restrictor. Based on the sorbent, analytes and the SFE conditions, either the pesticides or the lipids are retained by the sorbent. In the analytical scale SFE, where pesticides are the compounds of interest, it will be convenient to retain the lipids on the sorbent and to let the pesticides pass through for analysis. Murugaverl et al [90] did just that using diol-C_{18} for the determination of carbamate insecticides in beef and chicken. Silica and alumina also proved suitable for non-polar pesticides. However, any approach that will retain pesticides and let the extract pass through will serve best for the purpose of this research.

Supercritical fluid extraction of herbicides may be difficult. The extraction of relatively polar compounds containing amines, amides, imides and similar structures that can easily be charged, can pose difficulties in SFE. It is worthy of note that all the crop matrices employed for the fluid extraction have negligible water content. Water seems to have played a role in the extraction of these polar fungicides [91-93]. Their natural tendency to remain in the extraction cake can be harnessed for the production of pesticide-free extracts.

SFE of Herbicides

Most of the reported studies on the supercritical extraction of herbicides employed certain extraction aids that will not be acceptable in this study. Such extraction aids include ion-pairing or derivatisation agents that are applied prior or during the extraction to aid the extraction. Other measures that are taken to enhance the extraction of this class of substances are sample pretreatment with acid prior to extraction or the use of methanol as a modifier. (Chlorophenoxy acid herbicides in various matrices in the SFE of 2,4-D and similar herbicides ion-pairing or derivatization agents used prior or during the extraction were found to aid the extraction).

Therefore, since most of the extraction aids are toxic, focus was only on those supercritical extraction approaches where no hazardous chemicals and only CO_2 as the extraction fluid was employed. Lancas et al [94] employed only CO_2 for the extraction of pyridazinone herbicides

(norflurazon from cotton seed). The extraction parameters are 100 atm, 70 °C (0.26 g ml^{-1} CO_2 density) and 5 min of dynamic extraction time. In his research work, Berger [95] developed SFC conditions for separation of phenylureas. He reported the degradation of these herbicides in supercritical carbon dioxide at moderate temperature [95]. This finding may be of relevance to the study of pesticides carry-over in extracts in the sense that the degradation of the pesticides will result to a reduced or a non carry-over.

Howard and Taylor [96] reported on the extraction conditions of the sulfonyl urea herbicides. They observed that celite and filter paper retain these herbicides to a larger extent than cotton, probably due to the greater surface area of those materials. Matrix particle size can lead to the reduction of pesticides in extracts. The small particle size of the sample provides a large surface area on which the analytes can be reabsorbed. The continuous desorption and reabsorption of the residues on the sample surface area will delay their partition into the extraction fluid and may, consequently, prevent their elution into the extract.

In their study of the extractability of ^{14}C-labelled sulfonylurea in soil, Krieger et al [97] concluded that supercritical fluid carbon dioxide was not suitable for the extraction. Instead, water as an extractant gave 7-fold recoveries (75 %) than supercritical fluid carbon dioxide at nearly maximum density (0.97 g mL^{-1}) for the commercial instrument used. Higher recovery was achieved at a maximum temperature and pressure, by using modifiers, but recoveries of less than 60 % were achieved. Other authors concluded that extraction with supercritical carbon dioxide is not suitable for these and other types of polar herbicides in real samples. The extraction of such types of herbicides should be done with water-based systems controlled by pH value [97-135].

4. 2. Effective clean-up method for multiresidue analysis of pesticides in plant extracts with high lipid content.

The determination of pesticides in fatty samples is executed by residue analysis methods with varying efficiencies. The efficiency of the clean-up step plays an important role in the quality

of the results obtained. In many cases therefore, it is necessary to have additional steps, especially if the polarities of plant components in a complex matrix do not allow certain clean-up steps such as gel permeation chromatography (GPC) for an interference-free GC analysis. Wax components of plant extracts sometimes modify their consistence in such a way that GPC is insufficient for a quantitative GC analysis (for example paprika extract). Here, fatty acids in high concentrations were co-eluted with substances such as PCBs and organohalogens in trace concentrations. An optimal clean-up method for paprika extracts and similar samples has to be realized.

4. 3. Countercurrent Chromatography (CCC)

Countercurrent chromatography (CCC) is a relatively new form of separation method that is based on differential solubilities or partition coefficients of molecules between two immiscible phases.

It integrates two forms of classical partition methods: the counter current distribution (CCD) and the liquid-liquid partition chromatography (LC). CCC yields high partition efficiency by a continuous elution, as does LC. At the same time it possesses the good sample recovery, excellent reproducibility and high purity of fractions that are inherent to CCD.

As a highly selective method it can be used for the separation of molecules that are chemically similar by harnessing the different partition coefficients of the molecules in immiscible phases. The selectivity can be raised either by varying the phase composition or variation of temperature.

CCC is similar to such separation techniques as paper chromatography, high-resolution column chromatography, high performance liquid chromatography (HPLC) in the sense that the separation principle is based on liquid-liquid partitioning but unlike them it uses no solid support. The liquid stationary phase is retained in the column by an acceleration field rather than by solid support matrix. The advantages of this separation technique include the elimination of irreversible adsorption of the analyte on the solid support matrix, usage in the preparative (mg-g) range especially for polar and labile organic compounds and biparticulate materials such as cell and cell fragments, and suitability for pre-concentration of trace substances (for example in pesticide residues analysis). Almost any two-phase system,

aqueous or non-aqueous can be employed. Such problems as the loss of samples by an irreversible adsorption on the solid phase and chemical degradation of compounds are either minimal or non-existent [137-143].

4. 3. 1. CCC apparatus and principles of operation

The CCC device employed here for the investigations belongs to the family of coil planet centrifuges. It consists of multiple layers of coiled PTFE tubing coaxially coiled around a spool-shaped holder of 15 cm diameter. The coil column rotated around its axis and at the same time revolved around the central axis of the device. This resulted in a synchronous planetary motion where the two solvent phases employed are subjected to a rapid countercurrent flow along the length of the coil with the upper phase travelling towards the innermost head-end and the lower phase toward the outermost tail-end of the column. During the flow towards both ends, the phases are subjected to a multiple mixing and re-mixing that culminates in a hydrodynamic equilibrium. Under equilibrium, over 80 % of the total column space is covered by the stationary phase and the mobile phase could be pumped at a high flow rate without having any effect on the retention level of the stationary phase [144].

4. 3. 2 General chromatographic principles [126-151]

Novak and Janak [130] have described chromatography as a separation method based on the differential migration of solutes through a system of two phases, one of which is mobile. Therefore, a chromatographic system can be classified according to the state of the aggregation of the phases, the physical arrangement of the phases, and the mechanism underlying the distribution equilibrium. The following chromatographic systems can be derived from solid, liquid and gaseous phases: liquid-liquid, liquid-solid, gas-liquid, and gas-solid, and from these only liquid-liquid and liquid-solid can be regarded as liquid chromatography and, again it is only the principle of liquid-liquid chromatography directly applicable to countercurrent chromatography.

Chromatographic phases can be arranged physically in columnar or planar systems and the columnar systems can be further divided depending on the geometry of the chromatographic column support (packed columns, capillary columns etc).

Novak and Janak [141] regarded the various ways of achieving stationary phase in the liquid-liquid chromatography as an important feature. Phases are formed in the countercurrent chromatography either by surface tension as in droplet countercurrent chromatography (DCCC) or by centrifugal force as in rotation locular countercurrent chromatography (RLCCC).

They divided chromatographic systems into two categories, adsorption chromatography as two-dimensional in which the solute-solvent interactions take place on the surface of the phase and partition chromatography as three-dimensional in which solute-solvent interactions take place in the interior of the phase [141, 142-150].

Three types of operations can be carried out in a chromatographic system, namely elution, frontal and displacement chromatography (with a solid adsorbent). Components are recovered in the elute and displacement chromatography either by elution from the column or by stopping the run before zones reach the exit and extracting portions of the column packing. Displacement and frontal methods are of historical importance while the modern liquid chromatography with open tubular columns, HPLC, and CCC based on the elution method.

4. 4. Analysis of radio-labelled substances by means of a liquid scintillation counting (LSC)

Liquid scintillation counting (LSC) is an anlytical technique, which is defined by the incorporation of the radiolabelled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into emitted photons.

The technique of LSC is based on the following well-known physical phenomena: the ß-particle and photon production (products of nuclear decay). Nuclear decay that results to radiation is a highly complex process involving the laws of relativity that will not be dealt with here. Generally, the elements at the lower end of the atomic table contain an equal number of protons and neutrons in the nucleus and as the atomic weight increases in the

atomic table, the number of neutrons exceeds the number of protons. The elemens become unstable with such an imbalance in the number of protons and neutrons in their nucleus and thus resulting to a rearrangment. During this rearrangement, a particle is given off, representing a decay or a radioactive event and energy is released in the form of radiation.

The decay of the radioactive isotope, for example of ^{14}C, results in the emission of two particles, an electron (β-particle) and a neutrino (η-particle). These two particles are released simultaneously and carry the decay energy from the nucleus. This energy is characteristic for the radionuclide. In the case of ^{14}C, the beta maximum energy is 156 kiloelectron volts (KeV). The only relevance the neutrino has, is that it carries away some of the decay energy, otherwise it is of no interest in liquid scintillation counting. The 156 KeV is shared randomly between the β-particle and the η-particle. This means that the β-particle can possess any energy between 0 and 156 KeV. As a kinetic energy, the β-particle dissipates its energy by collision in the medium in which it is released. In a relatively dense medium, the ^{14}C β-particle will travel only short distances before all its kinetic enrgy is dissipated. The energy is absorbed by the medium in three forms, heat, ionozation, and excitation of the molecules of the solution. Excitation of the solution molecule is of interest in the liquid scintillation technique. The solution should also serve as a solvent for the sample in order to ensure efficient transfer of energy between the β-particle and the solution. Since the excited solvent molecules are not readily recognized, the scintillation solution (the cocktail) consists of a mixture of a solvent and a solute. The excited solvent molecules can transfer energy to one another and also to the fluor solute. An excited solvent molecule, which transfers its energy to a solute molecule, disturbs the orbital electron cloud of the solute raising it to a state of excitation. As the exited orbital electrons of the solute molecule return to the ground state, a radiation occurs, in this case the radiation is a photon of light. Thus, a single ß-particle will manifest its presence by colliding with solvent molecules, resulting to the excitation of many fluor molecules. The total number of photons from the excited fluor molecules constitute the scintillation. To a first approximation this is a linear conversion of energy into photons and, therefore, the intensity of light in the scintillation is proportional to the initial energy of the β-particle. Referring again to the case of ^{14}C, the scintillation intensity will range from 0 to the maximum possible for a particle of 156 KeV. The scintillation solution converts the kinetic energy of the β-particle into light energy [152].

Photon detection: Placing a glass or plastic vial containing the radiolabelled analyte with the scintillation solution into a dark enclosure allows the photon intensity to be observed. The relative photon intensity is dependent on many factors, including the type of liquid scintillation cocktail, radiolabelled analyte, other solutes dissolved along with the analyte, sample vial material, quantity of radiolabelled analyte, maximum energy of emission, and other chemically related phenomena affecting radiochemical distribution. The nature of the nuclear and chemical interactions produce emitted photons in the ultraviolet region of the electromagnetic energy spectrum, typically with low intensity. The detection of the emitted photons is by means of a photosensitive device (photomultiplier tubes (PMT)) that amplifies the light, and transforms the detected photons into electrical pulses.

Quenching: The sample and the scintillator are dissolved in a solvent, that allows energy to be transferred. Any factor which reduces the efficiency of the energy transfer or causes the absorbtion of photons results in quenchingeffects in the sample. There are two main types of quench, namely chemical and color quench.

Chemical quench causes non-radioactive dissipation of energy before being converted to photons. Chemical quench occurs during the transfer of energy from the solvent to the scintillator. Color quench is an attenuation of the photons of light. The photons produced are absorbed or scattered by the color in the solution, resulting in reduced light output available for measurement by the PMTs. The collective effect of quench is a reduction in the number of photons produced and therfore detected. Counting efficiency is affected by the degree of quenching in the sample. Thus, to determine absolute sample activity (decay per minute (DPM)), it is necessary to measure the level of quench of the samples firstly, and then make the necessary corrections for the measured reduction in counting efficiencies.

Measurement of Quenching: It is possible to measure quench accurately via high-resolution spectral analysis. The first method is the spectral index of the sample (SIS) which uses the sample isotope spectrum to monitor quenching of the solution. The second method is the transformed spectral index of the external standard (tSIE) which is calculated from the control spectrum induced in the scintillation cocktail by an external ^{133}Ba gamma source. Both SIS and tSIE are used as quench indicating parameters (QIPs).

Quench curve: A quench standard curve is a series of standards in which the absolute radioactivity (DPM) per vial is constant and the amount of quench increases from vial to vial. A quench curve uses the relationship between counting efficiency and QIP to correct the measured CPM (counts per minute) to DPM (disintegrations per minute or absolute activity).

III. EXPERIMENTAL PART

1. Chemicals, Standards, Materials and Methods

1.1 Chemicals

1. 1.1 Solvents for extraction (Purity: Pestanal grade and/or distilled)

- Acetone
- Cyclohexane
- Methylene chloride
- Ethyl acetate
- n-Hexane
- Isooctane
- Toluene
- Water for residue analysis

1. 2. Pesticide Reference Standards

The following reference pesticides and PCB standards were supplied by Dr. Ehrenstorfer GmbH (Augsburg, FRG):

- **Organochloropesticides (**reference standard mix 14)

Aldrin	Endrin
p, p-DDT	Heptachlor
p, p-DDE	trans-Heptachlorepoxide
o, p-DDT	Hexachlorobenzene
p, p-DDT	α-HCH
Dieldrin	β-HCH
α-Endosulfane	γ-HCH (Lindane)
β-Endosulfane	Methoxychlorine

- **Polychlorinated Biphenyl (PCB) Standards**

2, 4, 4´-Trichlorobiphenyl (PCB 28)

2, 2´, 5, 5´-Tetrachlorobiphenyl (PCB 52)

3,3´, 4, 4´- Tetrachlorobiphenyl (PCB 77)

2, 2´, 4, 5, 5´-Pentachlorobiphenyl (PCB101)

2,2´, 3, 4,4´, 5-Hexachlorobiphenyl (PCB 138)

2,2,4,4´, 5´5-Hexachlorobiphenyl (PCB 153)

2,2´, 3, 4´, 5, 5´-Heptachlorobiphenyl (PCB 180)

- **Organophosphorous Standards**

Diazinone	Azinophos methyl
Parathion methyl	Azinophos ethyl
Parathion ethyl	Chloropyriphos methyl
Malathione	Chloropyriphos ethyl
Bromophos ethyl	Fenithrothion
Bromophos methyl	Pirimiphos methyl

- **Triazine Standards and Pyrethroids**

Triazine Standards	**Pyrethroids**
Simazine	cis-Permethrine
Atrazine	trans-Cypermethrine
Terbutylazine	Fenvalerate
Metribuzine	Deltamethrine

1. 2.1. Pesticide Standards for the internal standard quantification method (Internal Standard Solutions (ISS))

PCB Nr 88	ISS for polychlorinated biphenyls
Bromophos methyl	ISS for the organophosphates
Isodrine	ISS for chlorohydrocarbons
Fenvalerate	ISS for pyrethroids
Atrazine:	ISS for triazines

1. 3. Materials

Filter aids:	Celite 545 (Fischer Scientific)
Blender:	2L Blender (Warring Products Div., New Hartford, NJ)
Mill:	Muelle vermahler with 2 mm Sieve, IKA Germany
Filter paper:	Black band filter paper that was thoroughly purified with cyclohexane-methylene chloride mixture (2:1)

Vacuum rotary evaporator with jacketed coil condenser

The apparatus was cleaned by rotary evaporation of system with 300 mL acetone prior to use (standard rotary evaporator VV 2000; Heidolph-Elektro GmbH + Co KG, Kelheim, Germany)

Sodium Chloride:	p. a.
Glass wool:	purified with methylene chloride

Generally, equipment and glassware were purified with acetone before use.

Spice Samples:	Dried Spanish paprika with native pesticide contamination (charge of 08.11.94), grounded with a Retsch grinding mill to 2 mm particle size
SFE extract:	SFE extract of Spanish paprika from 0.8 mm particle size pulver, (extracted at 360 bar, 70 °C) all supplied by RAPS & Co, Kulmbach, Germany.
Paprika SFE-cake:	0.8 mm particle size; supplied by RAPS & Co, Kulmbach, Germany

1. 3. 1. Mini silica gel column chromatography

Purification of silica gel:	200 g silica gel 60 (Merck, 70-230 mesh) was Soxhlet-purified by extraction with acetonitrile/methylene chloride (40:60)

Activation of silica gel:	Purified silica gel 60 (Merck 70 - 230 mesh) was then oven-dried at 130 °C for 6 h, cooled and stored in an air-tight bottle or a desiccator until use.
Deactivation of silica gel:	100 g of activated silica gel was deactivated one time by dropping 1.5 ml high grade water from a burette to a shaken beaker glass
Acid treated Celite 545	Celite 545 was treated with sulfuric acid until there is no longer black coloration. Thereafter it was washed and dried.

H_2SO_4-treated silica gel column:

60 g purified silica gel were thoroughly mixed with 40 g concentrated acid. The mixture was allowed to dry in the air and subsequently stored to be used later.

20 g were packed into a glass column (i. d. = 20 mm), followed by a 1-2 cm layer of a mixture of anhydrous Na_2SO_4 and acid washed Celite 545

1. 3. 2. Elution solvent for the mini silica gel column

Elution solvent 1	hexane
Elution solvent 2	n-hexane / toluene (65 + 35)
Elution solvent 3	toluene
Elution solvent 4	toluene / ethyl acetate (95 + 5)
Elution solvent 5	toluene / acetone (8 + 2)

1. 3. 3 Preparation of the mini silica gel columns

A glass column (7 mm i.d., 230 mm long) with a protruding elongated end was prepared firstly by tightly covering the column outlet with glass wool and packing 1 g deactivated silica gel into it and placing 5-10 mm anhydrous sodium sulfate on the silica gel and covering it with glass wool.

Solvent system: acetonitrile/petroleum ether/water (100:100:5).

All solvents were from Sigma Aldrich (residue analysis grade Pestanal). All pesticide standards were supplied by Euronorm standard institute, Technical University Munich, Freising-Weihenstephan or by Dr. Ehrenstorfer, Augsburg. Paprika powder with 8 % water content was supplied by Raps & Co, Kulmbach.

1. 4 Instrumental analysis conditions

1. 4. 1. HRGC-ECD / NPD analysis

Chrompack CP-9002 GC; temperature programme: 100 °C (1 min), 15°C min^{-1} to 150 °C (1 min), ramped at 2 °C min^{-1} to 250 °C (10 min); Inj.: 250 °C; ECD: 280 °C; Column: CP-Sil-8 CB;, 25 m x 0.32 mm x 0,42 µm; Carrier gas: H_2, 45 kPa; Split ratio: 1:10. Chromatograms of reference pesticide standards are shown below.

1. 4. 2. HRGC-MS analysis

GC-MS-Interface

Gas chromatograph: HP-GC 5890 series II

Mass spectrometer: Finnigan 8200-Mass spectrometer

Datasystem: MASPEC Data system for windows (MSS, Manchester), Vers. 2.11, Nist-Library;

Methods/Programme:

Finnigan 8200 coupled with HP-GC 5890 series 2; 70 ev, cathode 2 A, emission 1 mA, 3 kV accelerating voltage, 1.8 kV multiplier voltage; mass range 33-400 amu (1sec/decade, 0.2sec inter scan delay), calibration with FC43; MASPEC Data system for windows (MSS, Manchester), Vers. 2.11, Nist-Library; Injector: 230°C, Transferline: 230°C, Ion source: 250°C; Capillary column: fused silica, cross-linked SE54; 30m x 0.25mm x 0.25 µm; Temperature programme: initial 50 °C, 1 °C min^{-1} to 70 °C, 5 °C min^{-1} to 150 °C, 10 °C min^{-1} to 260 °C, 60 min hold.

1. 4. 3. HPLC analysis

HPLC: Beckman 166 double pump HPLC system

Detector: UV

Data system: IBM-System-Gold.

1. 4. 4 GPC System

A glass column (25 mm i. d., 400 mm length; Pharmacia) slurry-packed with Bio-beads SX-3 resin (ca. 45 g, 200-400 mesh), that was swollen in ethyl acetate / cylohexane (1:1) and compressed to a bed length of 350 mm equipped with a 5 mL Rheodyne sample loop

Solvent elution mixture for GPC:	Ethyl acetate / cylohexane (1:1)
GPC elution rate:	4 mL min^{-1}

1. 4. 5 High speed multilayer coil countercurrent chromatography (CCC)

CCC-System:	Ito multi-layer coil separator-extractor (A product of P. C. Inc., Potomac)
Column1:	PTFE coil (i.d. 1.6 mm) for acetonitrile-petroleum ether partitioning.
Column 2:	self-made metal coil (i.d. 1.6 mm)
Solvent capacity:	130 mL and 400 mL
Speed:	800 r. p. m.
Mode:	normal / reverse

1. 4. 6 Liquid scintillation measuring equipment

A liquid scintillation measuring equipment (L. Packard Canberra, Heidelberg, FRG) was used.

1. 4. 7. SFE-Equipment

SFE-Equipment:	A NOVA SWISS SFE equipment shown in Fig. 19. See chapter 1.6.1 for technical data of the SFE equipment.
Flow-metering-system:	Type: V100-140.18; with a floating metal-ball of 9.525 mm density in a 140 mm graduated glass .(An open flow metring system with a calibrated flow measuring instrument made by Vögtlin)
Calibration:	Flow hight (in mm); thus 25 mm = 9.215 Norminal Liter min^{-1} at 20 °C; Deensity: 0.00198 g cm^{-3}; Viscosity: 0.0138 mPa s^{-1}

1.5. Methods

1. 5. 1 Determination of pesticide contamination levels of spices from different countries

Piper nigrum, (black and white), *Capsicum annuum*, *Rosmarinus officinalis*, *Coriandrum sativum*, *Myristica fragrans* from Malaysian, Brazil, South Africa, Morocco, Hungary, India, Russia and China were subjected to a multiresidue pesticide analysis according to procedures in chapters **1. 5. 4. – 1. 5. 7**. Preliminary experiments to determine the effect of particle size on the extractability of pesticides from sample was also conducted. In addition, the possibility of pesticide reduction by sun-induced degradation of pesticides (chapters **1. 5. 2** and **1. 5. 3**.).

1. 5. 2. Influence of sample homogenization / non-homogenization on the extractability of pesticides from chili (solvent extraction)

Two experiments were carried out to investigate the effect of homogenization on the extractability of pesticides from paprika samples. In the first experiment paprika flakes without pre-homogenization, were subjected to a homogenization in acetone. For the second experiment, paprika flakes were pre-homogenized with spice mill with a 2 mm sieve (i.e. 2 mm particle size), before the powder was subjected to homogenization and extraction in acetone by means of a Waring laboratory blender.

1. 5. 3. Sun radiation experiment with *Capsicum annuum*

Paprika berries contained in a large cristalline tray were placed in the summer sun for 28 hrs. Thereafter the berries were subjected to a multiresidue pesticide analysis according to the procedures in chapters **1. 5. 4. –1. 5.7**.

1. 5. 4. Work-up methodology for the multiresidue pesticide analysis

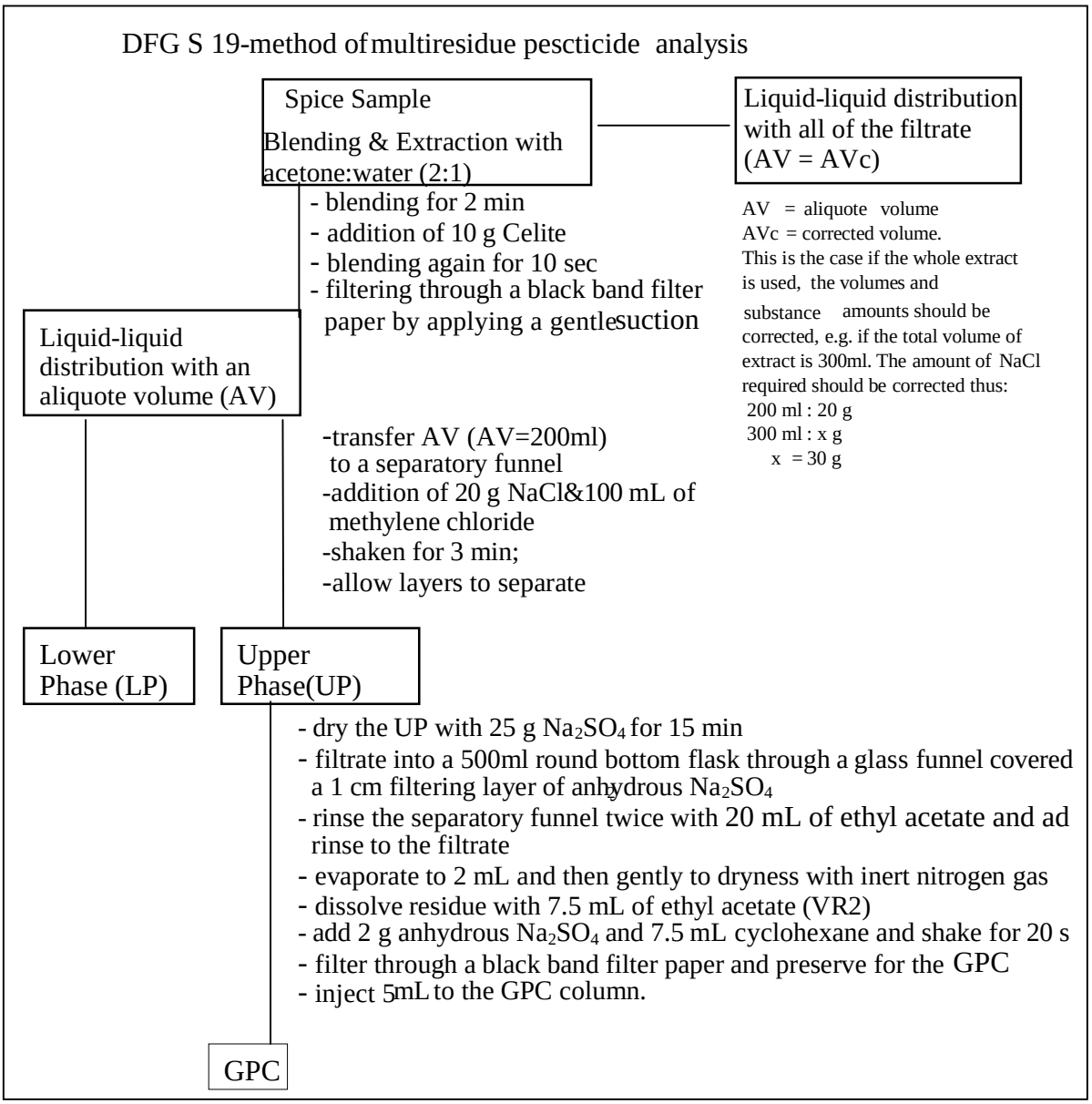


Figure 17. DFG S19-method of multiresidue pesticide analysis showing liquid-liquid distribution with an aliquot (1) of filtrate and all of the filtrate (2).

The work-up according to the sketch above was carried out as described in chapters **1. 5.–1. 7**.

1. 5. 5. Liquid solvent extraction work-up

100 g of air dried plant was weighed into a 2L speed-blender jar (high form). Water was added to the sample to bring the total amount of water to 100 g. Volume (v) of water required depends on the water content of the dried sample matrix and is calculated thus:

v =100 – % water in sample × sample amount / 100

Water content of dried paprika = 8 %; 92 mL of water were added to the sample

After the required amount of water was added, the jar content was stirred and allowed to percolate at room temperature for 10 min, thereafter 200 mL of acetone was added and homogenized with a Warring Blender for 2 min. After the addition of 10 g celite it was blended again for 10 sec, and filtered with a gentle suction through a Buchner funnel fitted with a black band filter paper.

Liquid -Liquid Partitioning

200 mL of the filtrate (or the whole filtrate AV, in the case of a very low analyte concentration) were transferred to a separatory funnel and 20 g sodium chloride was added to prevent the formation of an emulsion. The funnel was shaken vigorously for 3 min. After the addition of 100 mL of methylene chloride, the separatory funnel was shaken again for 2 min and the layer was allowed to separate. The lower water phase was dumped while the organic phase was dried with ca 25 g anhydrous sodium sulfate for 15 min. Thereafter, it was drained through a funnel covered with glass wool and a 1 cm layer of anhydrous sodium sulfate into a 500 mL round bottom flask. The separatory funnel and the filter were rinsed twice with 20mL ethyl acetate. The combined extracts were evaporated to 2 mL using a vacuum rotary evaporator at 30-40 °C and 260 mbar. The remaining 2 mL were gently evaporated with nitrogen gas.

This residue was dissolved with 7.5 mL of ethyl acetate by gently swirling by hand. After the addition of 2g anhydrous sodium sulfate and swirling, 7.5 mL of cyclohexane was added and shaken for 20 sec. The solution was filtered through a black band filter paper. The filtrate was to be used later for GPC.

1. 5. 6. GPC clean-up procedure

The filtrate was loaded via a 5mL Rheodyne sample loop onto a GPC column. The eluting solvent, ethyl acetate-cyclohexane (1:1), was pumped at a rate of 4 ml min^{-1} by means of a Beckman HPLC pump. The first 100 mL of eluent were discarded and the following 80 mL were collected. The collected eluate was reduced to 1 ml by a slow rotary evaporation at 35 – 40 °C under a reduced pressure of 260 mbar. The reduced eluate volume was transferred into a measuring cylinder by means of a pipette and filled up to 5 mL with ethyl acetate (GPC eluent). The method was validated by evaluating the dump / collect volume for each plant extract and butter fat in relation to the elution behavior of the PCBs, triazines, organochlorines, organophosphates and pyrethroids.

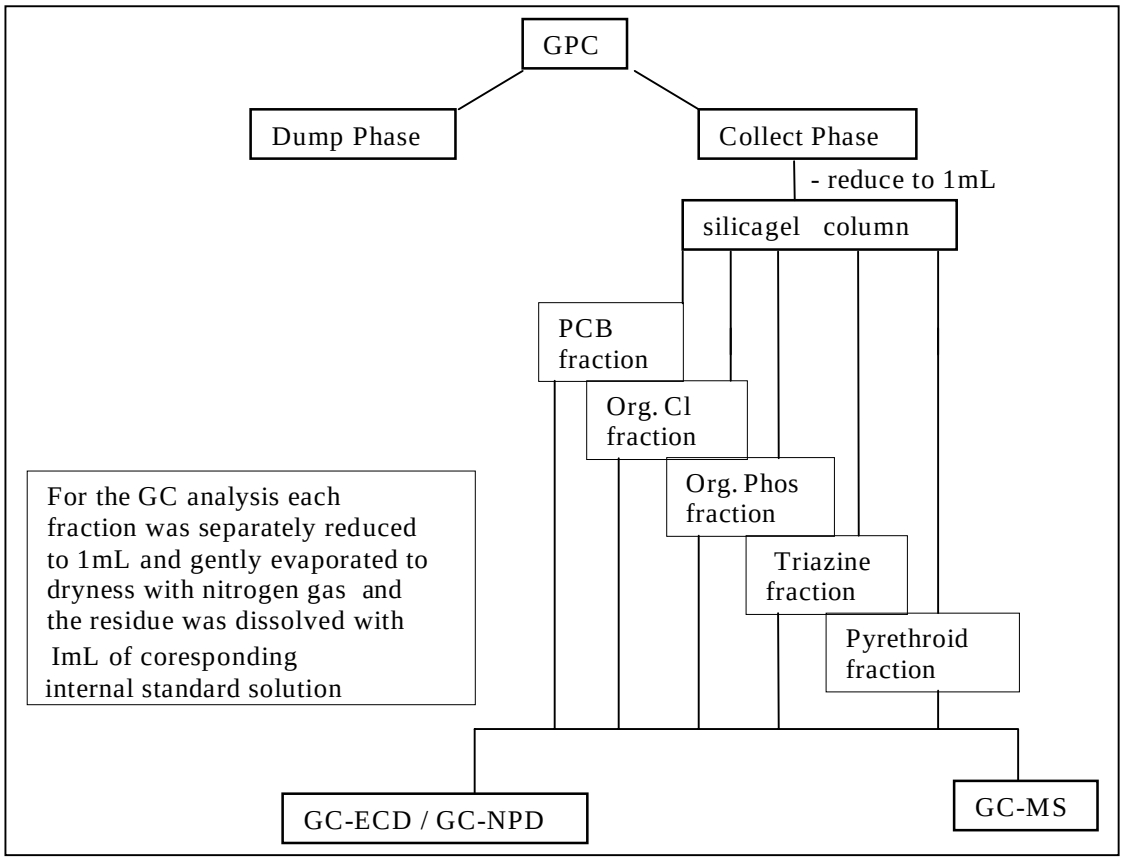


Figure 18. Gel permeation chromatography (GPC), mini silica gel fractionation, GC-ECD/NPD and GC-MS analysis

1. 5. 7. Mini silica gel chromatography / fractionation of paprika extracts

2.5 mL of GPC eluent were pipetted to a 100 mL long-neck round-bottom-flask and 5 mL isooctane were added. The mixture was reduced to 1 mL by rotary evaporation (slow rotation, shallow immersion). To make sure that ethyl acetate was completely evaporated, 5 mL of isooctane were added for a second time and reduced once more to 1mL by rotary evaporation and nitrogen gas (Isooctane solution).

Prior to use, the mini silica gel column was washed with 5 mL of hexane.With the outlet still open, the isooctane solution was transferred to the column by means of a pipette and the flask rinsed with 1 mL hexane and the rinse was equally transferred to the column. As soon as the hexane meniscus touched the top of the column fillings, the tap was closed and a 10 mL measuring cylinder was placed beneath the column to collect the eluent. The fractionation of pesticides into different classes progressed in the following way:

A 2 mL portion of hexane was added to the flask, swirled and the rinse transferred to the column. After opening the tap, the column was then eluted with 6 mL of elution 1 (hexane). The tap was closed thereafter, and the recovered fraction marked as Fraction 0 (PCBs, HCB). A new 10 mL measuring cylinder for the eluent-collection was placed under the column. 2 mL portion of elution solvent 1 was added to the flask, swirled and the rinse transferred to the column. The tap was closed thereafter and the rest fractions were eluted in the same manner with the following eluates:

eluate 1: n-hexane / toluene (65 / 35) = Fraction 1.
2: toluene = Fraction 2.
3: toluene / ethyl acetate (95 / 5) = Fraction 3.
4: toluene / acetone (8 / 2) = Fraction 4.

The collected fractions were separately evaporated to ca. 1 mL on a rotary evaporator and with nitrogen gas to dryness. The residues of each fraction were collected with 1 mL of corresponding Internal Standard Solution (ISS) which was later used for the GC analysis.

1. 5. 7. 1. Sulfuric acid clean up

1 g of the acid-treated-silica gel that was prepared according to chapter 1.3.1 (experimental part) was loaded onto a silica gel column. Then the collected fraction 1 and 2 from chapter 1.5.7 was eluted through the column. The eluent was washed with 10 ml water-hexane-mixture. Thereafter it was dried with anhydrous sodium sulfate. After concentrating to about 1 mL, the remaining solvent was removed with nitrogen gas. The residues of each fraction were collected with 1 mL of corresponding Internal Standard Solution (ISS) and subjected to a GC analysis.

1. 5. 7. 2 Determination of pesticide residues in extracts from solvent extraction

See chapter **1. 5. 1 - 1. 5. 7**

1. 5. 7. 3. Determination of pesticide residues in extracts from fluid CO_2 extraction

(see chapter **1. 5. 4 - 1. 5. 7**.)

4 g of SFE paprika extract were weighed into a 2 L speed-blender jar (high form). 92 mL of water and 200 mL of acetone were added to the sample and stirred. For the work-up procedure and analysis see chapters **1. 5. 1**. – **1. 5. 4**.

1. 5. 8. Quality comparison between extracts from liquid solvent extraction and supercritical CO_2 extraction based on contamination level

Quality evaluation of both extracts was made by instrumental analyses described earlier in this section comparing their levels of contamination.

1.6. Supercritical fluid extraction

The supercritical fluid extraction was conducted with a NOVA SWISS SFE equipment shown in Fig. 19. The subsequent flow directions are shown in Fig. 20.

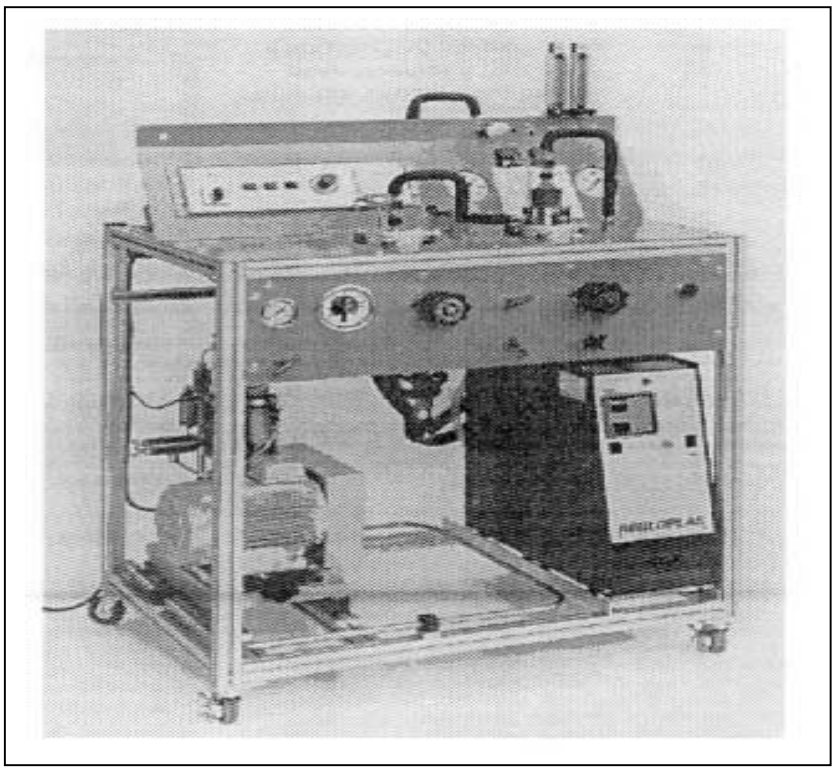


Figure 19. SFE extraction equipment used for spices extraction experiments.

1. 6. 1. Technical data of the SFE equipment

Name:	laboratory scale equipment		
Article Nr.:	567.1001		
Manufacturer:	NOVA WERKE AG, Vogelsangstrasse 24, CH-8207 Effretikon		
Technical data:			
Extractor:	Pressure	max.	500 bar
	Temperature	max.	120 °C
	Volume		200 mL
Separator:	Pressure	max.	100 bar
	Temperature	max.	120 °C
	Volume		135 mL
Extractor and separator vessels are heat-isolated.			
Compressor:	Exit pressure	max.	500 bar
	Entry pressure	min.	20 bar
		max.	200 bar
	Normal working pressure		50 bar
Extraction medium:	Gaseous CO_2 (supplied from a 50 L technical grade gas cylinder)		
Protective units:	Burst membrane	Compressor	500 bar
		Extractor	500 bar
		Separator	150 bar
	Protective valve	Flow meter	3-5 bar
Temperature indication unit:	Eurotherm temperature sensors with Indicators TI-1 and TI-2		
Connection data:			
Electrical connections:	3×400 VAC / 50 Hz with zero conductor and PE		
Water connection:	max. 8 bar		
Heating:	Oil and electricity as heat transmitters		

1. 6. 3 Method of operation

As it can be seen in both figures (Figs. 19 and 20), the heating of the extractor and the separator vessels was initially done by means of a single thermobath TB. The flow directions diagram (Fig. 20) shows that the extractor and separator vessels are serially heated. The serial connection of the heating line is as follows: (TB $\rightarrow$ HE-1 $\rightarrow$ E $\rightarrow$ FMV $\rightarrow$ HE-2 $\rightarrow$ S $\rightarrow$ PCV-2 $\rightarrow$ HE-3 $\rightarrow$ V-5 $\rightarrow$TB).

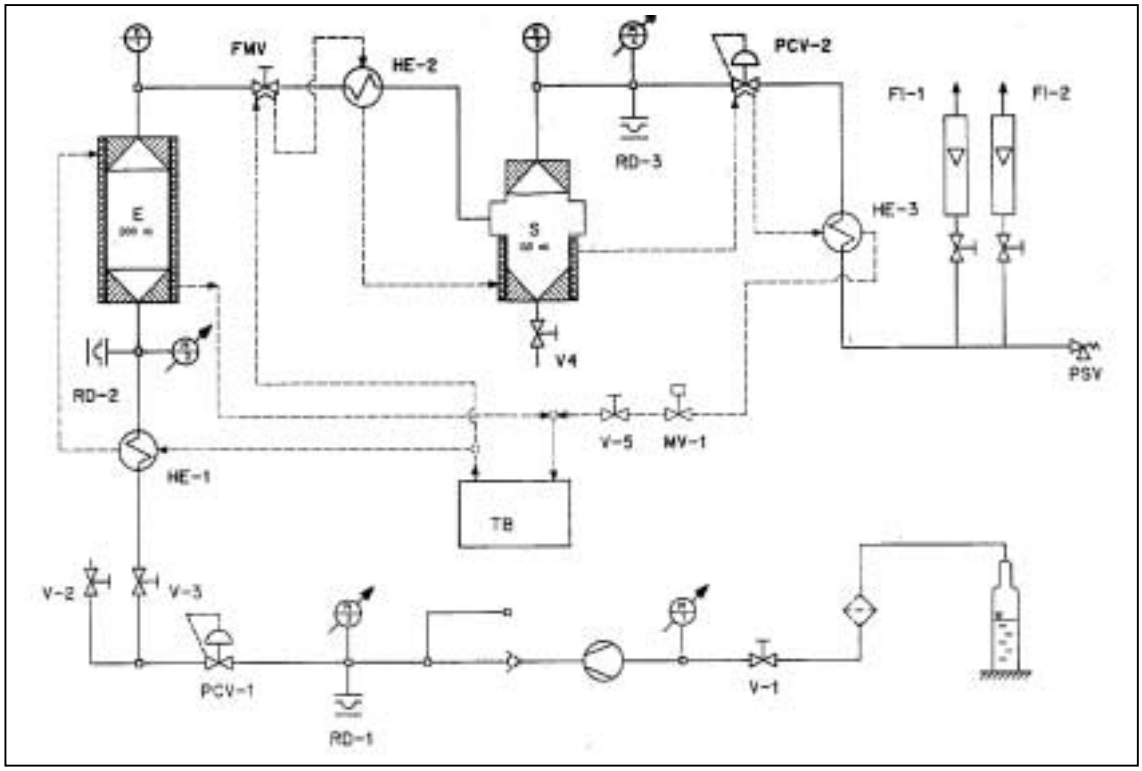


Figure 20. Bench-top CO_2 extraction machine showing the original flow directions

1. 6. 4. Extraction preparations

- Water flow was turned on.
- The gas cylinder via the high pressure tube was connected.
- Valve V-1 and fine metering valve (FMV) were closed.
- The valves V-2, V-3 and V-4 were opened upon turning the knobs anti-clockwise. Valve V-5 for the heat circulation was left open all through the extraction duration.
- Then the main switch was turned on and the compressor degassed.
- Thereafter, the extractor that was filled with sample and the clean separator were connected.

1. 6. 5. Extraction begin

- The heating system was switched on.
- The Regloplas thermo baths TB-1 and TB-2 for heat regulation were switched on, and the required temperatures for the extractor and separator were set.
- After the extractor and separator temperatures have been attained, the gas cylinder was slowly opened and the valves V-3 and V-4 were closed. In addition, valve V-2 was opened for a dynamic extraction. Fluid flow was controlled by the regulators FL-1 (for small flow), and FL-2 (for lager flow).
- Gaseous CO_2 was let out of the gas cylinder with 50 bar exit pressure (PI-1) into the compressor. The CO_2 comes out of the compressor as a fluid with an outlet pressure of nearly 1000 bar (CO_2 compression occurs in a single step process: the piston that was powered by conventional crank force, either in a downward movement enlarges or compresses in an upward movement the compartment that was filled with oil under a membrane). This resulted in a continuous compression of 50 bar pressured gas contained therein and ejection as soon as 1000 bar back pressure is attained).
- Required working pressures for extraction and separation were set and regulated by means of Tescom valves and contact manometers PI-1 or PI-4. The *pre-pressure* for the extractor was set roughly on the Tescom contact manometer (ca. 50 bar above the required constant and electrically switched extraction pressure).

1. 6. 6. Extraction stop

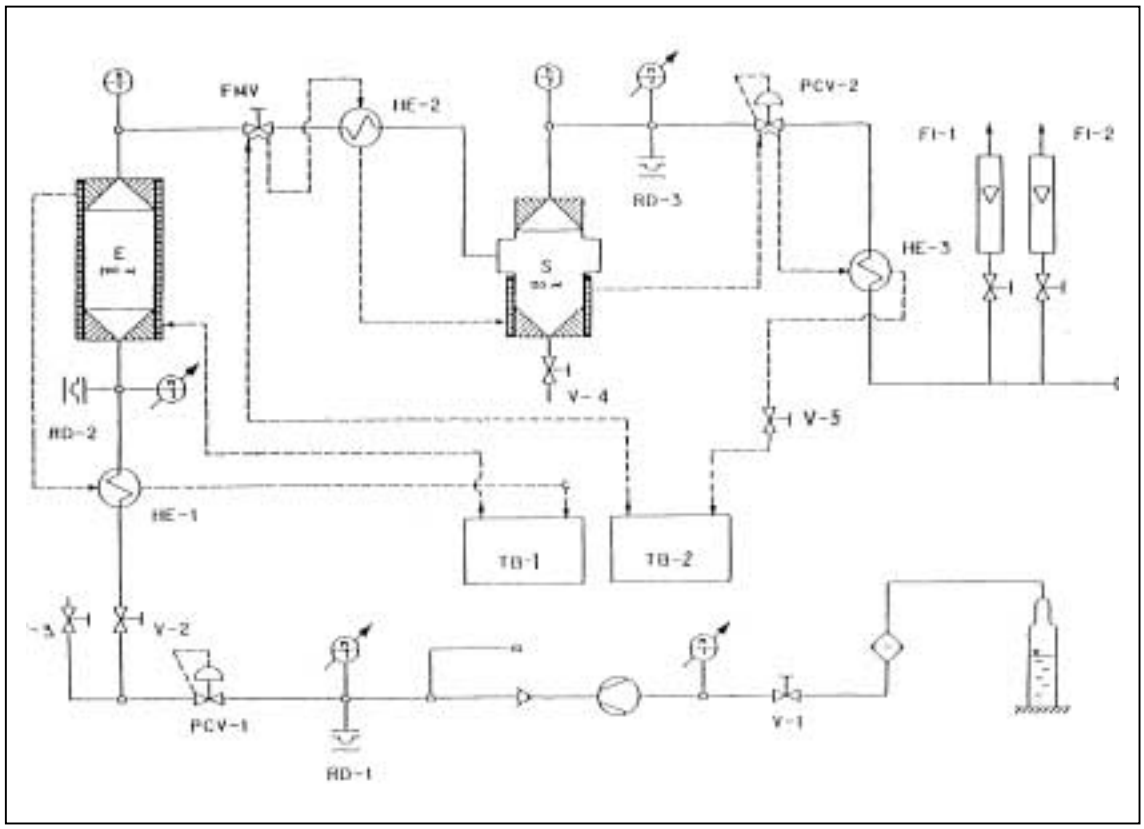
Each extraction process was usually brought to an end by turning off the compressor, and additionally by the subsequent steps:

- The valve V-1 was closed.
- The temperature was set to 20°C on the Regloplas temperature regulator.
- Collection of CO_2 extract from the separator by opening valve V-4.
- The extractor was depressurized by means of valve V-3.
- The gas cylinder was closed.
- Valves V-1, V-2, V-3, V-4 and FMV were gradually opened.

- The heating system was turned off as soon as the Regloplas indicated a temperature of ca. 30 °C, after which the main switch was turned off. At the end the separator vessel and the extract lines were dismantled and cleaned.

1. 6. 7 Parallel heating circuits for extractor and separator (see Fig. 21)

A second thermobath was installed to heat the separator and the extractor separately. The extractor E is separately heated in a close heating circuit TB-1 → HE-1 →E →TB-1 by means of themobath TB-1 and the separator S is likewise heated separately by means of thermobath TB-2 in a close circuit of TB-2 → HE-2 →S → PCV-2 → V-5 → TB. The two heating circuits were parallel to one another.



PI: Pressure indicator,
RD: Rupture disc
PCV: Pressure control valve
HE: Heat exchanger
TI: Temperature Indicator
E: Extractor
FMV: Fine metering valve
S: Separator
TB: Thermobath

Figure 21.a Bench-top CO_2 extraction machine showing the new flow directions from extractor and separator thermobaths.

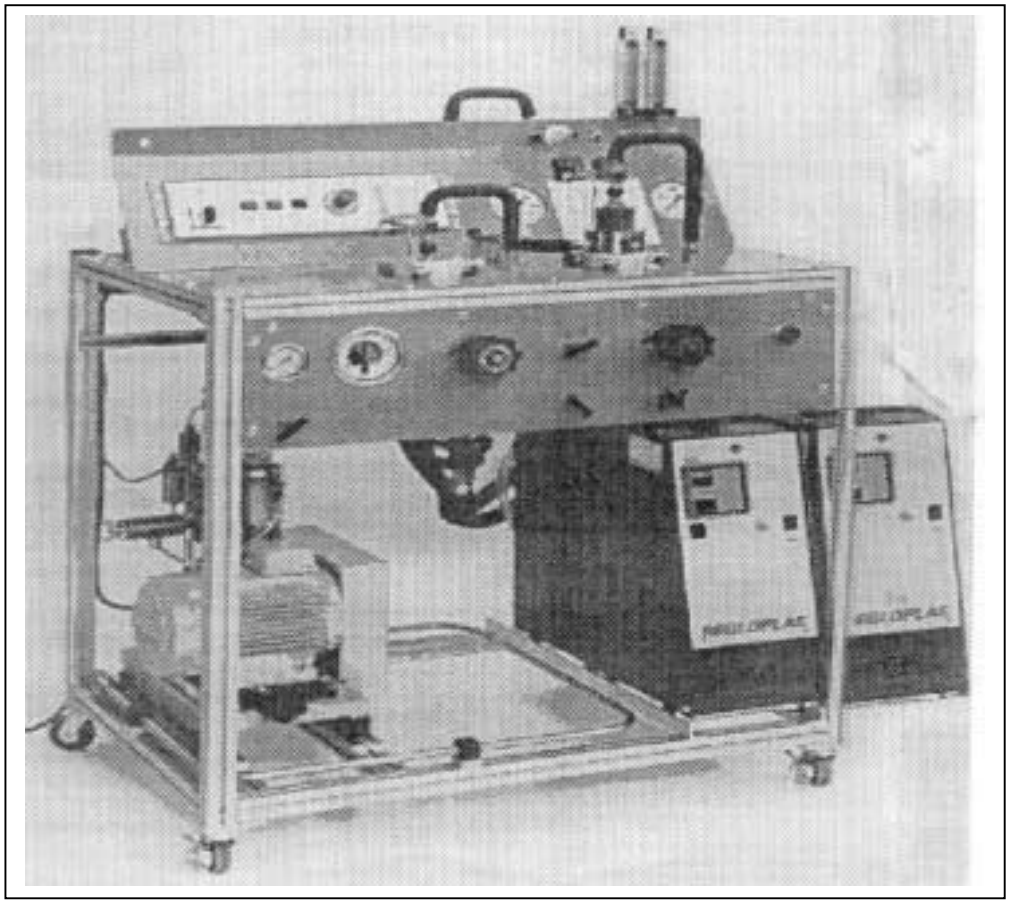


Figure 21.b Bench-top CO_2 extraction machine showing two thermobaths that enables separate regulation of the extractor and separator temperatures

1. 6. 8 Problems involved in regulating the pressure

Pressure regulation for the extractor was done by means of a Tescom pressure regulator. The pressure in the regulator is sustained by making sure it is fluid-tight by means of a soft polyethylene or polypropylene seal (cmpare Nr 007 in Fig. 22).

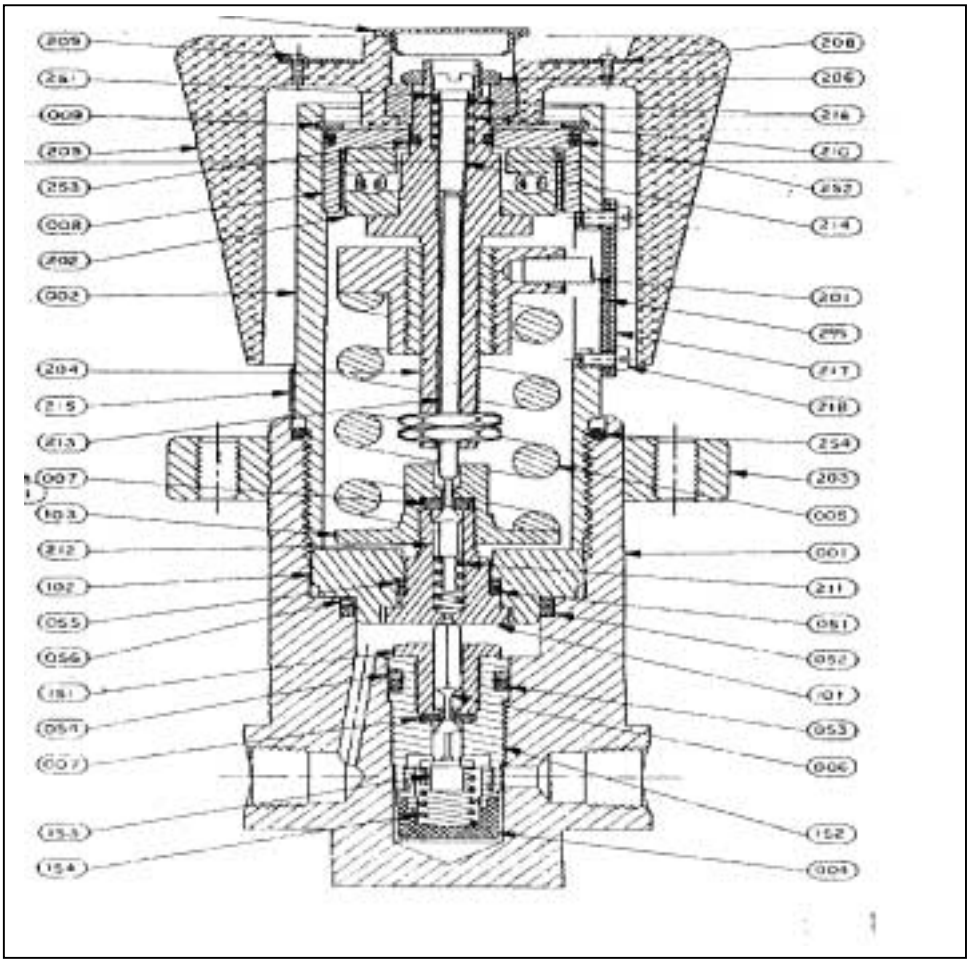


Figure. 22. Tescom pressure regulators of the SFE equipment.

The pressure regulatos very often lost their fluid-tightness and fluid CO_2 exuded from 214 (Fig. 22) upon pressure increase. This caused drop in pressure, hence extraction process was disrupted. The exuding fluid caused formation of ice crystals upon CO_2 compression. The prevailing cryogenic conditions obviously caused to the seals to become hard and brittle and they were damaged easily upon contact with the piston “X” during pressure build-up by means of the handle wheel.

1. 6. 9 Regulating the temperature in the extractor and separator

Extractor and separator temperatures are regulated by electrically powered thermostated oil baths (TB-1 and TB-2, Fig. 21). The hot oil with excellent heat capacity was pumped to different places that need to be thermally regulated. To prevent loss of heat energy, the extractor and separator chambers and the connecting lines to thermobath and to and from both chambers are heat-isolated. Extractor temperature is measured by means of a thermoelement placed on the exit of the extractor vessel, while separator temperature is measured by means of another thermoelement placed on the entrance of the separator vessel. Both temperatures are displayed electronically by means of digital Eurotherm temperature units. All things being equal, the thermobath working temperature should be the same as extractor and separator temperature. However, due to the positions of the thermoelementsat both vesses, the working temperatures inside the vessels cannot be exactly measured.

1. 6. 10 Fluid CO_2 extraction procedures

The extractor vessel was filled void-volume-free with 100 g dried paprika powder. The vessel was then placed in the thermo-controlled extraction chamber and allowed to attain the extraction temperature. The extraction process for each experiment started after the extraction vessel attained the working conditions.

1. 6. 11. Experiments to determine the effect of temperature and pressure on reduction / elimination of pesticides

Extraction parameters

Particle Size:	0.8 mm and 2 mm
Sample size:	100 g
Packing density:	densely pressed
Extraction mode:	dynamic
Extraction fluid:	CO_2
Extractionpressure, temperature and flow rate:	see Table 13
Modifier:	none
Dynamic extraction time:	30.45 min

Table 13. Extraction parameters

Experiment (Nr.)	1	2	3	4	5	6	7	8	9	10
Pressure(bar)	350	350	350	200	200	200	200	100	100	100
Temperature(°C)	70	60	30	70	60	70	30	70	30	80
Flowheight (mm) [L min^{-1}]	20-55 [8-20]	20-40 [8-14]	35-40 [12-14]	35-40* [12-14]	35-100* [12-37]	35-100 [12-37]	35-50 [12-18]	60-75 [22-27]	20-35 [8-12]	100 [36]

Experiment(Nr.).	11	12	13	14	15	16	17	18	19	20
Pressure [bar]	100	100	100	70	70	70	70	70		
Temp.[°C]	70	60	30	70	30	70*	60*	30		
Flow height(mm) [L min^{-1}]	45-50 [16-18]	60-75 [22-27]	20-38 [8-12]	50-100 [18-36]	50-75 [18-27]	60-80 [22-29]	55-75 [20-27]	25-35 [9-12]		

* = parameter at which only water was extracted;

Flow height = flow rate; the flow height was measured with a standardized flowmeter (Vögtlin GmbH, FRG). See **chapter 1.4.7** for conversion and details)

The dynamic extraction time initially was 45 min and was reduced to 30 min in the later experiments. For all extractions, the separator vessel was kept at 40 °C and 60 bar. The search for optimum parameters and extraction mode to achieve the set goal proceeded with the following investigations:

1. 6. 12. Selective extraction of water as a possible means for pesticide residue elimination

Extraction parameters

Particle Size: 0.8 mm and 2 mm).

Sample size: 100 g

Packing density: densely pressed

Extraction mode: dynamic

Extraction fluid: CO_2

Flow rate: see Table 13.

Modifier: none

Phase I:

Pressure and temperature: 100 bar, 70 °C; *100 bar, 60 °C*; 70 bar, 70 °C; 70 bar, 60 °C

Dynamic extraction time: 15 min

Phase II:

Pressure: 350 bar

Temperature: 70 °C

Dynamic extraction time: 30 min

The parameters at which water alone was extractable (see **1. 6. 11**) were employed for this investigation. In a two-phase extraction process, dried paprika (with 8 % residual water content) was extracted at 200 bar 70 °C; 200 60 °C; 100 bar, 70 °C; *100 bar, 60 °C*; 70 bar, 70 °C, and 70 bar, 60 °C for 15 min, respectively.

Thereafter the compressor was turned off, and the valves from and to the extraction vessel were closed. With the extractor vessel kept under pressure, the separator vessel was depressurized, emptied, cleaned and reconnected. The connection lines between the separator and the extractor were also cleaned and reconnected. After opening the valves to and from the extractor vessel, the compressor was restarted and the second/main phase of extraction at 350 bar and 70 °C followed.

1. 6. 13. Water as modifier

Water was added to the paprika samples to bring the total water content to 12 %. Extraction was subsequently conducted by making use of the following parameters:

Particle Size: 0.8 mm and 2 mm).

Sample size: 100 g

Packing density: densely pressed

Extraction mode: dynamic

Extraction fluid: CO_2

Flow rate: 60-75 mm (22.12-27.65 L min^{-1})

Modifier : water (total amount ad. 12 %)

Pressure:	100 bar
Temperature:	60 °C
Dynamic extraction time:	45 min.

1. 6. 14. Experiments to determine the extractability of polar pesticides (SFE of paprika that was spiked with ^{14}C-terbutylazine)

Particle Size:	2 mm
Sample size:	100 g
Packing density:	densely pressed
Extraction mode:	dynamic
Extraction fluid:	CO_2
Flow rate:	35-55 mm (12.90-20.27 L min^{-1})
Modifier :	none
Pressure:	350 bar and 200 bar
Temperature:	70 °C
Dynamic extraction time:	45 min

Procedure:

^{14}C-labelled terbutylazine (300.000 dpm*) was added to about 15 mL acetone. This mixture was later used for the fortification of the paprika sample. The sample was fortified by wetting it with the standard mixture. Thereafter it was allowed to dry in the air. The dried sample was quantitatively transferred into the extractor vessel and subjected to a supercritical extraction with the parameters given above. After the extraction, the extract was analyzed by means of a liquid scintillation counting (see **chapter 3.3**).

* = disintegrations per minute; this means the number of radioactive disintegrations per unit time; there are 2.2 E6 disintegrations per minute in a microCurie.

1. 6. 15. Analysis of ^{14}C-labelled terbutylazine in SFE extract of paprika by means of a liquid scintillation counting (LSC) equipment

The SFE extract (**chapter 1.8)** was diluted in 5 mL acetone in oder to prevent a color quench from carotinoids in paprika. 0.5 mL and 1 mL were injected into 20 mL super polyethylene sample bottles (Packard). After the addition of 15 mL cocktail (Ultimate Gold XR), the mixture was properly shaken. The measurement was carried out within one hour.

The results obtained were corrected by means of a quench value for a ^{14}C-standard in various concentrations of paprika.

1. 6. 16. Experiments to determine the duration of exhaustive extraction

Particle Size:	0.8 mm and 2 mm
Sample size:	100 g
Packing density:	densely pressed
Extraction mode:	dynamic
Extraction fluid:	CO_2
Flow rate:	35-55 mm (12.90-20.27 L min^{-1})
Modifier :	none
Pressure:	350 bar
Temperature:	70 °C
Dynamic extraction time:	45 min

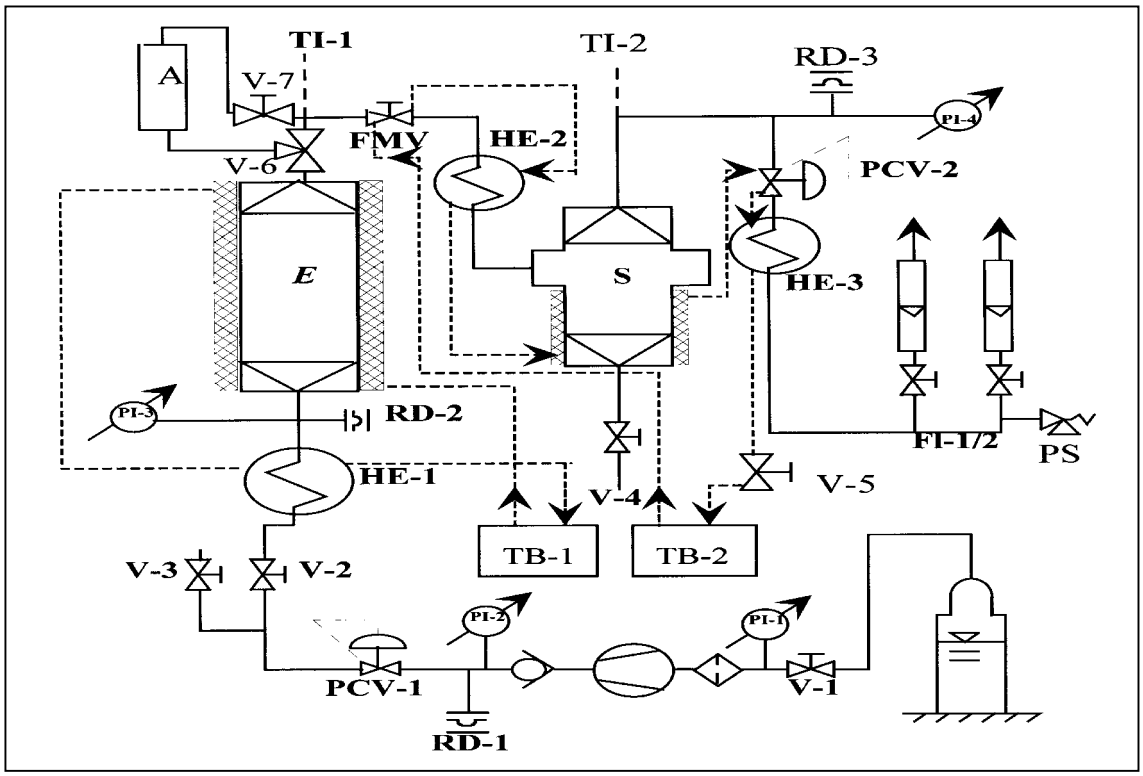
For the chosen parameters extraction experiments were carried out for tentative extraction times that ranged from 45 to 15 min. Beginning with extraction duration of 45 min and proceeding in decreasing mode, series of extractions were conducted for various extraction times at a constant pressure of 350 bar and a constant temperature of 70 °C. 100 g of dried paprika were employed as in the other investigations (see the extraction yield-timegraph, Fig. 30 in Results section.)

1. 6. 17. Determination of the extraction time for a high-quality yield

Particle Size:	2 mm (*spiked with 64 ppb organochlorine pesticides)*
Sample size:	100 g
Packing density:	densely pressed
Extraction mode:	dynamic
Extraction fluid:	CO_2
Flow rate:	35-55 mm (12-20 L min^{-1})
Modifier :	none
Pressure:	350 bar
Temperature:	70 °C
Dynamic extraction time:	45 min

1. 6. 18. Simultaneous-extraction and chromatographic separation (SFECS)

Particle Size:	0.8 mm and 2 mm
Sample size:	100 g
Packing density:	densely pressed
Extraction mode:	dynamic
Extraction fluid:	CO_2
Flow rate:	35-60 mm flow height (13-22 NL min^{-1})
Modifier:	none
Pressure:	350 bar
Temperature:	70 °C
Dynamic extraction time:	45 min
Experiment I:	Chromatographic vessel A: length 69 cm, i.d. 1 cm, densely packed with 10.91 g dry silica gel
Experiment II:	Chromatographic vessel A: length 69 cm, i.d. 1 cm, densely packed with dry and solvent-cleaned paprika flakes



PI: RD: Rupture disc, FMV: Fine metering valve
PCV: Pressure control valve S: Separator
HE: Heat exchanger TB: Thermobath
TI: Temperature Indicator A: Chromatographic column

Figure 23. Bench-top CO_2 extraction machine showing the chromatographic column (A).

Experiment I

At first, the extraction via a chromatographic vessel coupled to the main extractor vessel was conducted. The temperature-controlled chromatographic vessel was densely filled with 9 g silica gel, and at the end of the test extraction, chromatographic separation of color compounds of paprika (carotenoids) into yellow, orange and red colors were observed.

Experiment II

For the second experiment, the column was densely packed with paprika extraction cake particles (totally extracted paprika flakes) instead.

1. 6. 19. Investigating the chromatographic seperation of pesticides on a column with paprika cakes as column material

SFECS phenomenon observed in chapter **1.6.18** was further investigated by a series of experiments. An empty GPC column was filled with paprika extraction cake pre-cleaned by 6 hrs Soxhlet extraction. After conditioning the column with the elution solvent, 1 g paprika extract spiked with 22 pesticides was loaded onto the PPC column and chromatographed by elution with ethyl acetate:cyclo-hexane (1:1). The thrilling results of a chromatographic principle termed here as "Paprika permeation chromatography" (PPC) are shown in the results section

1. 6. 20. The applicability of high speed multilayer coil countercurrent chromatography (HSML CCC) as a clean up step in multiresidue analysis of lipophilic pesticides in lipophilic samples

The applicability of high speed multilayer coil countercurrent chromatography (HSML CCC) as a clean-up step in multiresidue analysis of lipophilic pesticides in lipophilic samples was investigated with the experiments described in chapters **1.6.21 – 1.6.26** .

1. 6. 21. The solubility of petroleum ether 40-60 °C (PE) and paprika extract in acetonitrile (ACN) with variable water content at room temperature

Solubility of PE in anhydrous ACN and aqueous ACN

ACN : PE : H_2O

(1.) 100 : 100 : 0 ⇒ ACN = Solvent ***1a***, PE = solvent ***1b***

(2.) 100 : 100 : 5 ⇒ aqueous ACN-phase = Solvent **2a**, PE-phase = 3. solvent **2b**

(3.) 100 : 100 : 10 ⇒ aqueous ACN-phase = Solvent ***3a***, PE = solvent ***3b***

(4.) 100 : 100 : 15 ⇒ aqueous ACN-phase = Solvent ***4a***, PE = solvent ***4b***

(5.) 100 : 100 : 20 ⇒ aqueous ACN-phase = Solvent ***5a***, PE = solvent ***5b***

(6.) 100 : 100 : 25 ⇒ aqueous ACN-phase = Solvent ***6a***, PE = solvent ***6b***

(7.) 100 : 100 : 30 ⇒ aqueous ACN-phase = Solvent ***7a***, PE = solvent ***7b***

Solvent 1a and 1b were prepared as follows: 100 ml of ACN and PE were poured in a separatory funnel. After closing the funnel with a stopper, the mixture was shaken vigorously. It was allowed to settle for 1 min. Thereafter, the two phases (lower acetonitrile phase and the upper PE phase) were separated into two 150 mL graduated cylinders. The percentage solubility of PE in ACN was ascertained by determining the volume of each solvent.
Solvents 2a – 7b were prepared thus: The acetonitrile solvent mixtures with various amount of water were mutually saturated with petroleum ether in a separatory funnel. The resulting solvent (ACN = solvent ***a*** and PE = solvent ***b***) were employed for the subsequent experiments.
See table **25** for results
Solubility of paprika in anhydrous ACN and aqueous ACN

100ml of solvent ***1b*** were transferred into a separatory funnel. After dissolving 1g of paprika extract, solvent ***1a*** was added. The mixture was thoroughly shaken and allowed to settle for 1 min. Thereafter, the two phases (lower acetonitrile phase and upper PE phase) were separated into two 150 mL measuring cylinders. The percentage solubility of paprika in anhydrous ACN was determined gravimetrically. See table **25** for results

The solubility of paprika in solvent systems 2a-7b was determined as above-mentioned.

1. 6. 22. Partitioning of pesticide standards in acetonitrile-petroleum ether solvent system on a PTFE-column-operated-HSMLCCCC

41 pesticide standards of various substance classes were used as model substances, namely (PCBs, triazine herbicides, pyrethroids, organochlorines and organophosphates).

Experiment 1.

The distribution pattern of these pesticides and PCBs in ACN-PE solvent systems were ascertained by HSML CCC according to **1.6.21**

1. 6. 23. Procedures of HSMLCCC

Chromatographic parameters

Upper phase: petroleum ether (40-60 °C)

Lower phase: aqueous acetonitrile (5% water content)

Column: PTFE coil (i.d. 1.6 mm)

Volume: 120 ml

Speed: 800 r. p. m

Flow rate: 4 ml min^{-1}

Sample load: 0.5 g

Mode: normal

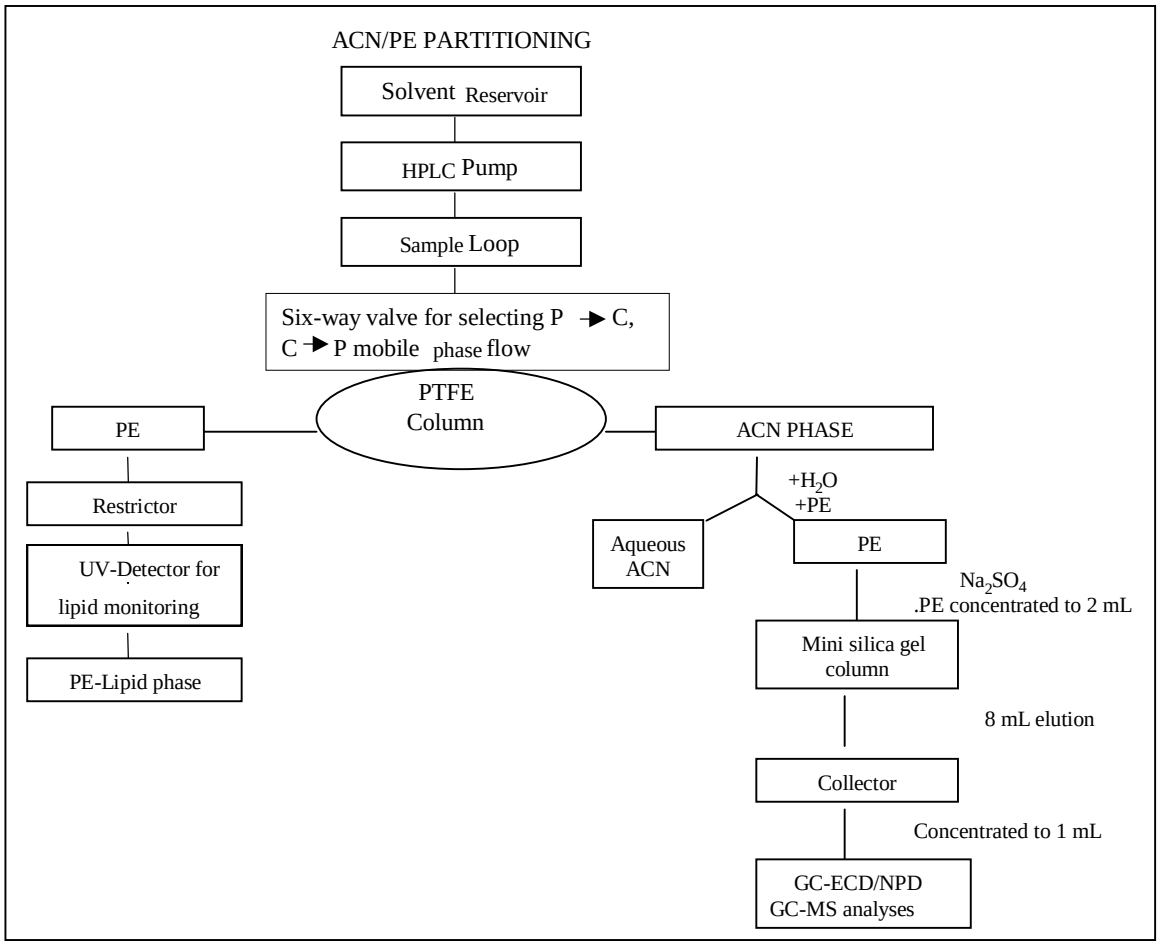


Figure 24. Acetonitrile-petroleum ether partitioning of a PTFE-column operated CCC

The solvent system for the CCC was prepared according to **1.6.21**. The column was filled with the aqueous acetonitrile (stationary) phase by pumping through to the tail end. 500 mg of the paprika extract dissolved in 10 ml of petroleum ether (40-60 °C) were loaded onto the equipment by means of a four-way Rheodyne injection valve. A second six-way valve was installed for a possible reversed mode of operation. The column was rotated at 800 r.p.m for 5 min, thereafter the mobile petroleum ether phase was pumped into the column at a rate of 5 ml min^{-1} and rotated for 10 min before the sample was injected by switching the Rheodyne valve to the injection position. The lipid eluent was UV-monitored at 230 nm. The partitioning was completed after the mobile phase was totally pumped through the column.

The stationary phase was then pressured out of the column with nitrogen gas (0.5 bar). The collected aqueous acetonitrile was used either for the on-line PE←ACN-back-partitioning described in chapter **1.6.25** or for a partioning into methylen chloride organic phase. For a CH_2Cl_2 partitioning an equal volume of methylene chloride was poured into the collected aqueous acetonitrile. Water originating from aqueous acetonitrile and insoluble in the new mixture was separated by drying with anhydrous sodium sulfate. The remaining organic phase was evaporated to 2 ml, gently dried with nitrogen and finally collected in 5 ml of hexane (isolate a). Isolate **a** was either separated into different substance classes on a silica gel column or when necessary, subjected to further cleaning.

(I) Isolate ***a*** from ***experiment 1*** was separated into five fractions that were analyzed directly by GC-ECD.

(II) Isolate ***a*** from ***experiment 2*** was separated into five fractions. The PCBs and the organochlorine fractions were subjected to a further sulfuric acid cleanup by means of a H_2SO_4-treated silica gel column.

(III) Isolate ***a*** from ***experiment 2*** was further cleaned by means of a H_2SO_4-treated silica gel column coupled to HSML CCC (chapter **1.5.7.1**). The cleaned isolate (isolate ***b***) was subsequently analyzed with GC-ECD.

1. 6. 24. Multiresidue pesticide analysis of paprika extract with HSML CCC as a clean-up step (*Chromatographic parameters* see chapter **1.6.23)**

The column was filled with the aqueous acetonitrile stationary phase (solvent system 2**a** prepared according to **1.6.21**) by pumping through to the tail end.
500 mg of paprika extract dissolved in 10 ml of petroleum ether (solvent system 2**b** prepared according to **1.6.21**) were loaded onto the equipment by means of a four-way Rheodyne injection valve. The CCC system was made to rotate at 800 r.p.m. for 5 min. Thereafter the mobile petroleum ether phase was pumped into the column at a rate of 5 ml min^{-1} and allowed to rotate for 10 min before the sample was injected by switching the Rheodyne valve to the injection position. The lipid eluent was UV-monitored at 230 nm. The partitioning was completed after the mobile phase was totally pumped through the column. The stationary phase was then washed out of the column with pressurized nitrogen gas (0.5 bar). An equal volume of methylene chloride was poured into the collected aqueous acetonitrile. Water originating from aqueous acetonitrile and insoluble in the new mixture was separated, by drying with anhydrous sodium sulfate. The remaining organic phase was evaporated to 2 ml, gently dried with nitrogen and finally collected in 1 ml of hexane.

1. 6. 25. Investigating the possibility of on-line HSMLCCC back-partitioning with an acid-treated silica gel column interface.

Upper phase:	petroleum ether (PE) (40-60 %)		
Lower phase:	aqueous acetonitrile (ACN) (5 % H_2O)		
Column:	PTFE coil (i.d. 1.6 mm)	Volume:	120 ml
Speed:	800 r. p. m	Flow rate:	4 mL min^{-1}
Sample load:	0.5 g	Mode:	normal

PE→ACN partitioning: For the steps involved in extracting pesticide residues from samples dissolved in PE into ACN see **1.6.23 and 1.6.24**.

On-line PE←ACN-back-partitioning: The ACN stationary phase obtained chapter **1.6.23** and figure **24**) was washed out of the column with nitrogen gas (0.5 bar) into 200 mL of water.

The same column (or a second column) was filled with petroleum ether, (now employed as stationary phase). Rotation was still a forward mode, however, the mobile heavy phase was pumped in head to tail direction. (backward mode can be used as well). The equipment was made to rotate at 800 r. p. m before the back extraction began. It was allowed to rotate for 5 min, thereafter the acetonitrile-water mixture (as a mobile phase) was pumped into the column at a rate of 5ml min^{-1}. The back partitioning was completed after the heavy mobile phase was completely pumped through the column. The PE stationary phase was then washed out of the column with nitrogen (0.5 bar) and reduced as usual to 2 mL. The reduced PE-back extract was subjected to a sulfuric acid degradation.

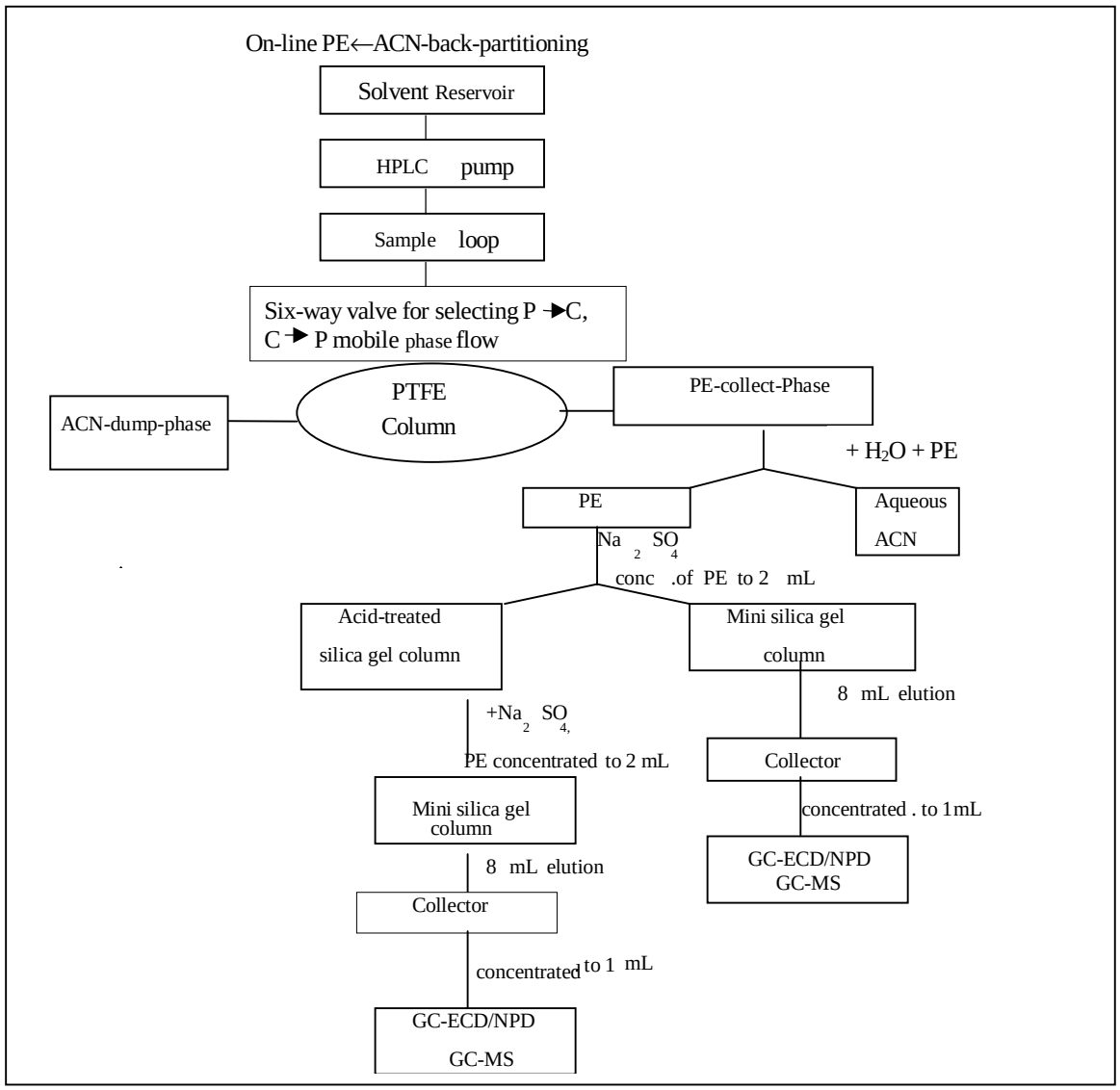


Figure 25. On-line PE←ACN-back-partitioning with HSML CCC interfaced with an acid-treated silica gel column.

HSCCC-acid treated silica gel column interface:

The PE stationary phase that was pressured out of the column with nitrogen (0.5. bar) was transferred online into a sulfuric acid-treated silica gel column. At the end of the transfer, the column was washed with ultra pure water and eluted with PE-methylene chloride (1:1) (for PCB).

The eluted organic phase was evaporated to 2 ml, gently dried with nitrogen and finally collected in 1 ml of hexane. The analysis was done by means of GC-ECD and GC-MS.

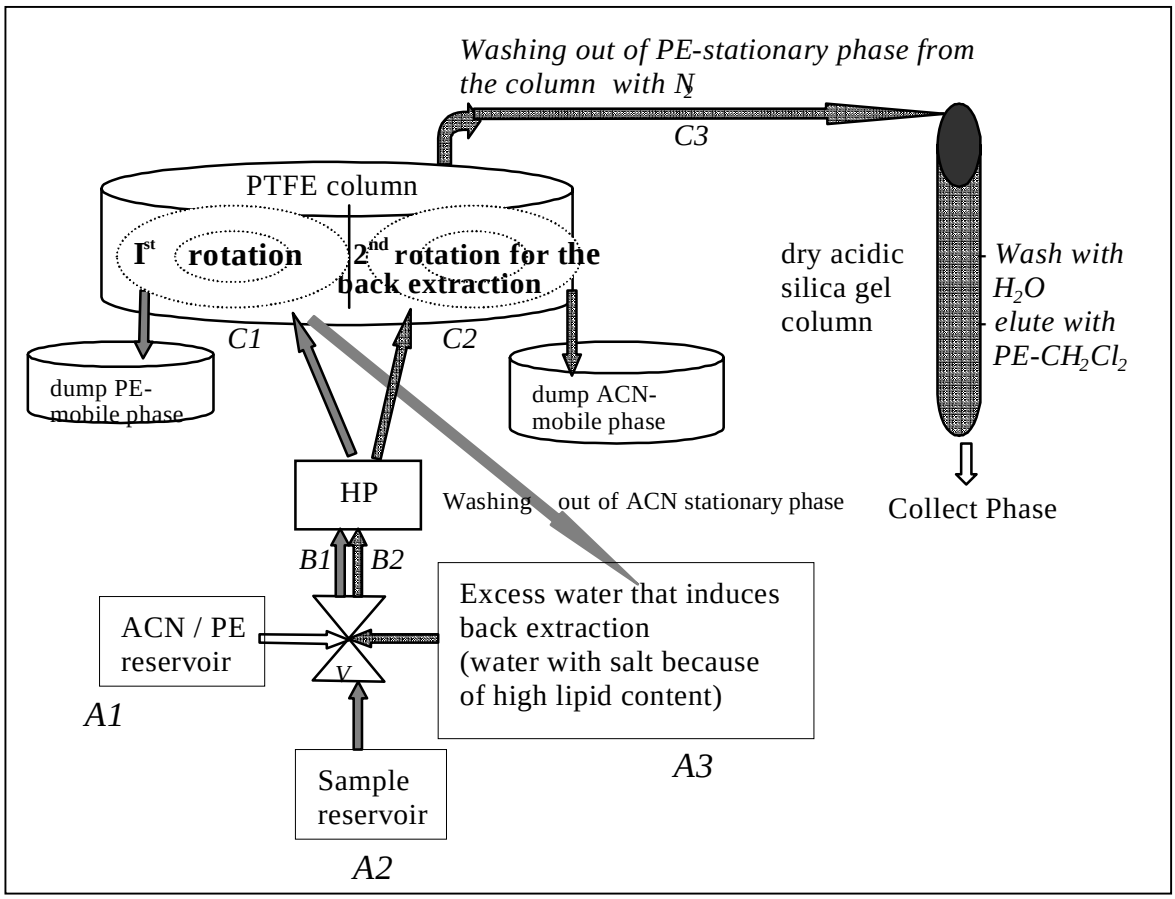


Figure 26. Pathways for partitioning of pesticides from PE into ACN, on-line back extraction of pesticides from ACN- into PE-phase and on-line acid degradation process (*V* = connecting Rheodyne valve; HP = HPLC pump; extraction path way = A1/A2 V.B1 HP C1; back extraction pathway = C1-A3 V B2 HP C2)

1. 6. 26. The solubility of petroleum ether 40-60 °C (PE) and paprika extract in dimethylformamide (DMF) with variable water content at room temperature

100 ml of solvent ***1b*** (see chapter **1.6.21**) were pipetted into a separatory funnel. After dissolving 1g of paprika extract, solvent ***1a*** (see chapter **3. 1**) was added. The mixture was thoroughly shaken and allowed to settle for 1 min. Thereafter, the two phases (lower DMF phase and upper PE phase) were separated into two 150 mL measuring cylinders. Then the percentage solubility of paprika in DMF was determined gravimetrically.

1. 6. 27. Determination of water content

The water content of the paprika samples was determined by means of an azeotropic distillation. The results are in line with the values determined by Vesper [45] according to Karl Fischer method (for details see [44 and 44*^{v}]).

1. 6. 28. Determination of total fat content

The total fat content was determined using the German Food and Drug Administration official standardized method Nr. 35 according to Weibull-Stoldt (see [44, 44*v and 44*g).

Digestion: 1.0 g Paprika was weighed into a 400 mL cylinder glass. 100 mL HCl and some boiling stone was added and the content stirred with a glass rod and covered with a glass plate. The mixture was brought to boiling while it was stired intermitently and allowed to boil without bubbling for 1hr. Thereafter, 100 mL of hot destilled water was added to the digested paprika and filtered through a prewetted Fold-filter-paper. The glass rod, glass plate, and the filter paper were washed with water untill no more chlorid ion was detectable in the wash water. (Chlorid ion detection was carried out by means of $AgNO_3$ solution). A pre-wetted fold-filter-paper was transfered to a glass plate and dried at 103 °C for 2 hours in a laboratory dryer.

Extraction: the extraction of the lipid from the filter paper followed by putting the now completely dried fold-filter-paper into an extraction hull. The glass plate and the 400 mL cylinder glass that was used for HCl-digestion were wiped with a cotton piece that was wetted with diethylen ether. The piece of cotton was likewise transfered to the extraction hull. The shull with its content was subjected to Soxhlett extraction and the fat content in percentage was determined gravimetrically.

IV RESULTS

1. 1. Influence of particle size on the extractability of pesticides from chili flakes by solvent extraction

Preliminary experiments to ascertain the contamination levels of spice imports analyzed were carried out to determine the influence of grinding (reduction of particle size) on the extractability of pesticides from the samples. The investigation of the influence of particle size on the extractability of pesticides from dried chili was taken as a representative sample. The results are presented in Table 14

Table 14. Influence of particle size on the extractability of pesticides from dried chili.

	Not-grounded chili (India)	Grounded chili (India), 0.8 mm	Chili powder (grounded in India); 1 mm
α-HCH	75 ppb	80 ppb	80 ppb
β-HCH	29 ppb	26 ppb	22 ppb
Lindane	26 ppb	29 ppb	25 ppb
o, p-DDE	16 ppb	18 ppb	12 ppb
p, p-DDE	26 ppb	27 ppb	19 ppb
o, p-DDT	18 ppb	20 ppb	15 ppb
o, p-DDT	8 ppb	8 ppb	6 ppb
α-Endosulfane	17 ppb	17 ppb	12 ppb
β-Endosulfane	25 ppb	25 ppb	17 ppb
Cypermethrine	3.5 ppm	3.5 ppm	3.0 ppm
Fenvalerate	185 ppb	180 ppb	90 ppb
Ethyl-Chloropyriphos	100 ppb	100 ppb	70 ppb

The results in Table 14 clearly show that the actual difference in pesticide concentration in both grounded and not-grounded chili samples is within the margin of analytical error of 20 % and hence negligible.

1. 2. Pesticide concentration in *Capsicum annuum* before and after sun radiation

Table 15. Pesticide concentration in *Capsicum annuum* before and after sun radiation.

	Sample before sun radiation	Sample after sun radiation
α-Endosulfane	0.2 ppm	0.2 ppm
β-Endosulfane	0.4 ppm	0.5 ppm
Ethyl-Chloropyriphos	0.9 ppm	0.9 ppm
cis-, trans-Permethrine	10 ppb	10 ppb
Cypermethrine	20 ppb	45 ppb
Deltamethrine	130 ppb	110 ppb

These experiments were conducted to determine if a sun drying process may lead to a degradation of contaminants and eventually could result to a reduction of pesticides in contaminated spice samples (see experimentall section , chapter **1.5.3**). Although experiments have shown that the climatic conditions (for example sun exposure) can lead to a degradation of pesticides in the nature [38-40], the result in Table 15 reflect that 28 hours of sun radiation was not enough to effect any reasonable degradation and reduction of these pesticides.

Spice samples were prepared and analyzed by GC-ECD as described in chapters **1.5.4 – 1.5.7** of the experimental section. The results are presented in Table 16.

1. 3. Pesticide contamination levels of spices from different countries

Table 16. Pesticide contamination levels of spices from different countries.

Samples (Provenience)	PCBs (ppb)#	Organochloro Pesticides (ppb)#	Organophos-phates (ppb)	Pyrethroids (ppb)#	Triazines (ppb)#
1. White Pepper (Muntok, 04. 07. 95)	n. d.	15 ppb p,p-DDT 25 ppb β/γ-HCH 12 ppb cis-Hepta-chloroepoxide	n. d.	n. d.	n. d.
2. Paprika (Negev, Israel; 30.03.95)	ca. 1.5 ppb (153)	90 ppb α-Endosulfane 150 ppb β-Endosulfane	40 ppb Ethyl-chlor-pyriphos	n. d.	30 ppb Metri-buzine
4. Rosmary (Morocco, 30.03.95)	ca. 0.5 ppb (153)	0.4 ppb p,p-DDE 0.9 ppb Dieldrin	n. d.	n. d.	n. d.
5. Coriander (Hungary, 1994)	n. d.	0.5 ppb Hepta-chlor 0.7 ppb β / γ-HCH	n. d.	n. d.	n. d.
6.Nosampleassigned	-	-	-	-	-
7. Paprika (S. Africa, 22.2.95)	1 ppb (28) 1 ppb (52)	0.5 ppb p,p-DDE	n. d.	n. d.	n. d.
8. Black Pepper (Malaysia, 24.08.95)	n. d.	10 ppb p,p-DDE 10 ppb o,p-DDE	n. d.	n. d.	n. d.
9. Rosmary (Spain, 1994)	n. d.	10 ppb α-Endo-sulfane 15 ppb-DDE 20 ppb Dieldrin	n. d.	n. d.	n. d.
11.White pepper (Malaysia, 21.08.95)	3 ppb (101) 2.5 ppb (153) 3 ppb (138)	10 ppb-DDE 1.5 ppb HCB 4 ppb o,p-DDE 7 ppb p,p-DDE	n. d.	n. d.	n. d.
12. Black pepper Brazil, 11.07.95	n. d.	0.4 ppb p,p-DDE 10 ppb p,p-DDT	n. d.	n. d.	n. d.
13. Paprika (Spain, 08.11.94)	n. d.	230 ppb α-Endo sulfane 360 ppb β-Endo sulfane	900 ppb Ethyl-chlor-pyriphos 16 ppb Azinipho s ethyl	130 ppb Delta-methrine 20 ppb Cyper-metrine 18 ppb Per-methrine	6 ppb Simazin.

= Average concentration of two determinations

Table 16, continued

Sample	PCBs (ppb)	Organochlor-pesticides (ppb)#	Organophos-phate (ppb)#.	Pyrethroids (ppb)#	Triazines (ppb)#
14. Black Pepper (Malabar)	n. d	20 ppb α-HCH 13 ppb β / γ-HCH	n. d.	n. d.	n. d.
15. Paprika (Morocco, 16.03.95)	n. d	20 ppb p,p-DDE 2 ppb cis-Heptachloro-epoxide	20 ppb Ethyl-chlorpyripho		
16. Coriander (Egypt,1994 harvest)	n. d	n. d.	n. d	n. d.	n. d.
17. White Pepper (Muntok; 27.12.94)	n. d	6 ppb β / γ-HCH	n. d	n. d.	n. d.
18. Black Pepper (Vietnam, 01.08.95)	n. d	1 ppb p,p-DDT 1 ppb HCB 6 ppb p,p-DDT 10 ppb β-Endosulfane	n. d	n. d.	n. d.
19. Paprika (Hungary,1994)	n. d	15 ppb p,p-DDE 7 ppb β / γ-HCH	n. d	n. d.	n. d.
20. Rosmary (Spain, oil-free)	n. d	70 ppb α-Endosulfane 60 ppb β-Endosulfane 10 ppb β / γ-HCH	5 ppb Ethyl-chlorpyripho s	n. d.	n. d.
21. Mace	n. d	1 ppb p,p-DDE 3 ppb p,p-DDT	Malathione	n. d.	n. d.
22. Paprika(Queens, South Africa; 180 ASTA)	n. d	55 ppb α-Endo-sulfane 110 ppb β-Endo-sulfane 100 ppb Fenvalerate 2 ppb Lindane	n.d.	70 ppb Fen-valerate	
23. Paprika (Kalosai, South Africa)	n. d	20 ppb α- 30 ppb β-Endosulfane 3 ppb DDE	5 ppb Ethylchlor-pyriphos	20 ppb Fenvalerate	
24. Paprika (Israel, 100 ASTA)	n. d	15 ppb α-Endosulfane 65 ppb β-Endosulfane 220 ppb Cypermethrine	n. d.	240 ppb Cypermethri n	
30. Paprika (Israel, 160 ASTA)	n. d.	20 ppb α-Endosulfane 80 ppb β / γ-HCH 55 ppb Cypermethrine		55 ppb Cyper-methrine	

n. d. = not detected

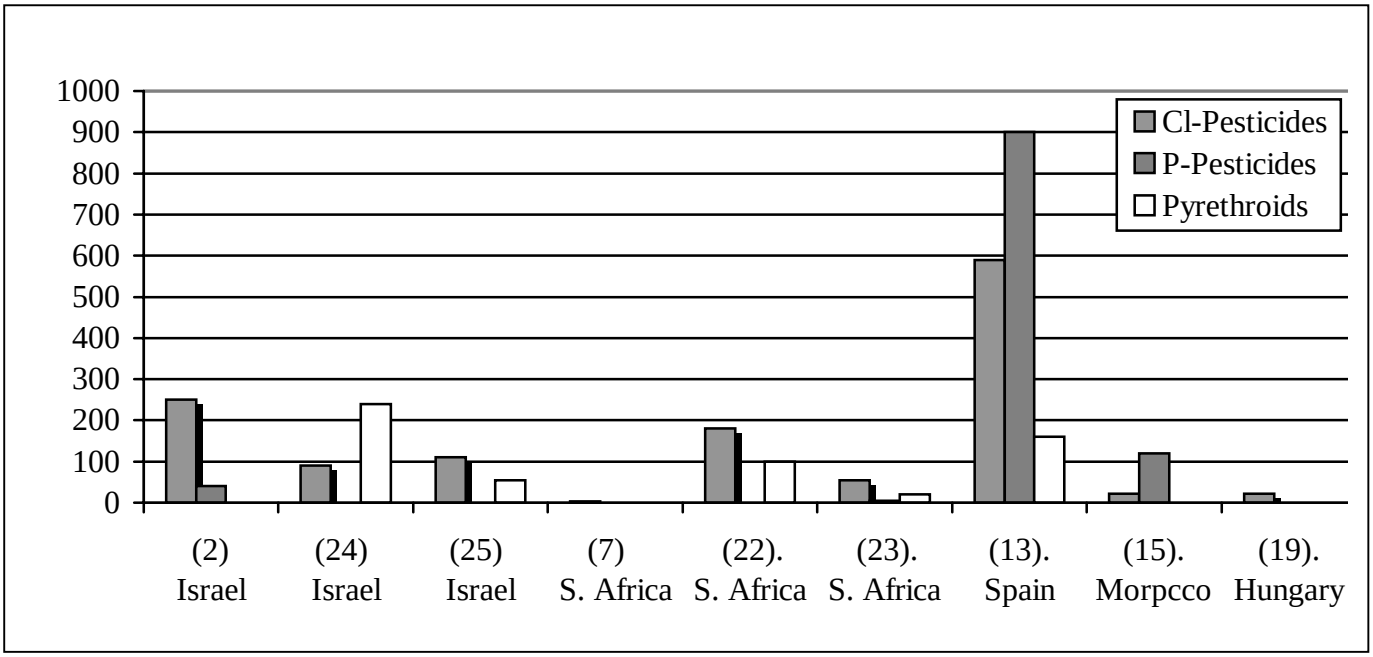


Figure 27. Graphical presentation of the contamination levels of paprika proveniences from different countries (taken from Table16)

The results of the conventional solvent extraction are shown in Table **16** and Fig. 27. Spanish paprika (13) is the most contaminated sample. Spanish and Israelian paprika samples have a considerable high level of α-endosulfane, β-endosulfane and cypermethrine. Traces of the toxicological persistent isomers of lindane (β-HCH and γ-HCH) were detected in Muntok pepper and Hungarian coriander. Most of the spices contain DDT and its metabolites (DDE and DDD) in trace concentrations. Although DDT and its metabolites are known to be ubiquitously found contaminants [38], they were not detected in Chinese pepper and Russian coriander. Traces of cis- and trans-heptachlorepoxide were found in Muntok pepper, and traces of dieldrin were identified in Hungarian coriander as well (Table 16). Spices from China and Russia were free of pesticide contamination while Israelian and European spices are the most contaminated. Spices from African countries have low pesticide contamination levels. However, the contamination levels for all spices are within the maximum concentration limit.

After careful observation of the data in Table 16, paprika samples were identified as the spices with the highest contamination in comparison to other spice varieties. Therefore, paprika from different countries were further compared and the results presented in Fig. 28. It can be seen clearly that Spanish followed by Israelian paprika samples were highly contaminated. With a total concentration of ca. 600 μg kg^{-1} the concentration of organochlorine pesticides was just above the maximum limit of 0.5 mg kg^{-1}. This implies that a strong possibility for pesticide enrichment exists if the sample is extracted by means of a solvent / fluid with a stronger solvatation strength (for example SF CO_2).

1. 4 Comparison of conventional solvent extraction and supercritical CO_2 extraction (350 bar, 70 °C) of paprika (*Capsicum annuum*)

The comparison as presented on table **17** shows the existence of different extractabilities with convention solvent or CO_2 are employed as extraction medium.

Table 17. Comparison of conventional solvent extraction and supercritical CO_2 extraction (350 bar, 70 °C) of *Capsicum annuum.*

Sample / Source	Liquid solvent extraction	SFE Extract	Enrichment Factor
Paprika Queens, South Africa (180 ASTA)	α-Endosulfane: 55 ppb β -Endosulfane: 110 ppb Fenvalerate: 100 ppb Lindane: 2 ppb	1.4 ppm 2.3 ppm 2.1 ppm 50 ppb	25 21 21 25
Paprika Kalosai, South Africa (180 ASTA)	α-Endosulfane: 20 ppb β -Endosulfane: 30 ppb DDE: 3 ppb	900 ppb 1.5 ppm 160 ppb	45 50 53
Paprika Israel (100 ASTA)	α-Endosulfane: 15 ppb β -Endosulfane: 65 ppb Cypermethrine: 220 ppb	130 ppb 620 ppb 2.2 ppm	9 10 10
Paprika Israel (160 ASTA)	α-Endosulfane: 20 ppb β / γ-HCH: 80 ppb Cypermethrine: 55 ppb	145 ppb 760 ppb 0.5 ppm	7 10 9
Paprika Spain	α-Endosulfane: 55 ppb β-Endosulfane: 0.11 ppm Cypermethrine: 18 ppb	1.4 ppm 2.3 ppm 510 ppb	25 20 28

The most contaminated spice samples of conventional solvent extraction (compare Table 16, Figs. 27 and 28) were extracted with supercritical carbon dioxide by making use of parameters commonly employed in the industrial extraction procedures. A comparison of the results from both extraction methods is presented in Table 17.

As it can be seen, all pesticides identified were 7 to 53-fold enriched in the SFE samples compared to the concentrations in conventional solvent extracts. Similar enrichment factors of α-endosulfane and β-endosulfane in the SFE extracts of the other paprika proveniences from South African, Israel and Spain can be observed (Table 17). Also a remarkable enrichment of pyrethroids could be noticed in these SFE extracts of South African, Israelian, and Spanish paprika.

2. Results of supercritical carbon dioxide extraction

2. 1. Parallel heating circuits for extractor and separator vessels

The SFE extraction plant (Fig. 19) was purchased with a single thermostat in which heat-conducting oil was transported by means of serial connection lines from the thermostat to the extractor and separator.

A second thermobath (Fig. 21) was installed to heat the extractor and separator separately with closed heating circuits (separator, thermobath 1: TB-1 → HE-1 →E →TB-1 and extractor, new thermobath 2: TB-2 → HE-2 →S → PCV-2 → V-5 → TB-2).

With the parallel connection of the two heating circuits the extraction process could be timely shortened and the quality of the experiment was improved.

2. 2. Solution to the problem involved in regulating the pressure by means of a contact manometer

The problem with pressure-drop during CO_2 extraction process was alleviated by means of the following treatments that helped to sustain the pressure constant in the separator during compressor pulsation:

- Periodic exchange of the parts of the Tescom valves, especially the polypropylene seals, that were responsible for high-pressure fluid-tightness.

- The pre-pressure in the electrically switched contact/control manometer of the compressor was set about 60 bars above the necessary constant working pressure in the separator.

2. 3. Precision of temperature control in the double-walled extractor, separator, fine metering valve and stainless steel tubes of the SFE plant used (Fig. 21)

The temperatures in the extractor and separator vessels have been additionally controlled by a separate and highly sensitive thermoelement. These measurements are carried out inorder that the heat capacity of the two thermostating circuits (TB-1 and TB-2) was enough to maintain the wanted constant temperatures during extraction. A precise digital and non-varying measurement with the originally installed thermoelements was not possible because of their positions at the extractor exit and separator entrance with the highest supercritical flow. They have to be replaced by thermoelements placed inside of both vessels.

2. 4 Supercritical fluid CO_2 extraction of natively contaminated Spanish Capsicum annnum samples (effects of various extraction parameters on the extractability of pesticides and PCBs; see Tables 18-23).

Table 18. Various extraction parameters and their effects on the extractability of PCB 180, lindane, α- and β-endosulfane.

				PCB 180 (ppb)			Lindane (ppb)			α-Endosulfane (ppb)			β-Endosulfane (ppb)		
Exp.	*	P [bar]	T [°C]	Solv. Extr	SFE	F	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F
cont.				128.3			53.7			23.4			34.2		
1**		350	70		+	-		1500	27		314	13		686	20
2		350	60		+	-		976	18		240	10		604	18
3		350	30		-	-		628	12		204	99		480	14
4	*	200	70		61	0.4		454	8.5		84	3.6		128	3.7
5	*	200	60		56	0.4		401	7.5		31	1.3		99	2.9
6		200	70		+	-		500	9.3		112	4.8		142	4.2
7		200	30		+	-		44	0.8		14	0.5		19	0.6
8		100	70		-	-		-			-	-		-	-
9		100	30		-	-		-			+	-		-	-
10	*	100	80		-	-		+			+	-		-	-
11	*	100	70		-	-		-			-	-		-	-
12	*	100	60		-	-		-			-	-		-	-
13	*	100	30		-	-		-			+	-		-	-
14		70	70		-	-		-			+	-		-	-
15		70	30		-	-		-			-	-		-	-
16	*	70	70		+	-		-			+	-		-	-
17	*	70	60		+	-		-			+	-		-	-
18	*	70	30		-	-		-			-	-		-	-

Cont. = control sample used for extraction; * = spiked samples PCB and Aldrine 128.3 ppb; Atrazine and Bromophosethyl 1283 ppb; ** = 0.8 mm and 2 mm sample particle size were used for the experiments and the results were nearly the same; - = not detected; solv. extr. = solvent extraction; t = trace; F = enrichment factor; SFE = supercritical fluid extraction; P = pressure; T = temperature. All results were average values from double analytical determinations. SFE Extraction: 4× 350bar/70°C; 3×350brr/30; Rest double;

Table 19. Various extraction parameters (pressure, temperature and water fortification) and their effects on the extracttability of permethrine, cypermethrine and deltamethrine.

				Aldrine (ppb)			Permethrine (ppb)			Cypermethrine (ppb)			Deltamethrine (ppb)		
Exp.	*	P [bar]	T [°C]	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F
cont.				128.3			10.7			18			103		
1		350	70	-		-		308	29		510	28		3090	30
2		350	60	-		-	10.7	279	26		460	26		2740	27
3		350	30	-		-	10.7	213	20		367	20		2109	20
4	*	200	70		560	4.4	10.7	53	5.5		109	6.1		580	5.6
5	*	200	60		546	4.3	10.7	41	3.8		72	4.0		497	4.8
6		200	70	-	-	-	10.7	50	4.7		103	50.7		539	5.2
7		200	30	-	-	-	10.7	8.3	0.8		9.6	0.5		118	1.1
8		100	70	-	-	-	10.7	-	-		-	-		+	-
9		100	30	-	-	-	10.7	-	-		-	-		-	-
10	*	100	80		100	0.8	10.7	-	-		-	-		-	-
11	*	100	70		60	0.5	10.7	-	-		-	-		-	-
12	*	100	60		52	0.4	10.7	-	-		-	-		+	-
13	*	100	30		20	0.2	10.7	-	-		-	-		+	-
14		70	70		-	-	10.7	-	-		-	-		-	-
15		70	30		-	-	10.7	-	-		-	-		-	-
16	*	70	70		12	0.09	10.7	-	-		-	-		+	-
17	*	70	60		9	0.07	10.7	-	-		-	-		+	-
18	*	70	30		3	0.02	10.7	-			-	-		+	-

* = for further explanations of spike concentrations and abbreveations see legend of Table 18

Table 20. Various extraction parameters (pressure, temperature and water fortification and their effects on the extractibility of bromophos ethyl, atrazine and metribuzine.

				Bromophos ethyl (ppb)			Atrazine (ppb)			Metribuzine (ppb)		
Exp.	*	P [bar]	T [°C]	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F	Solv. Extr.	SFE	F
cont.				104								
1		350	70		1684	16	+	-	-	+	+	-
2		350	60		1320	13	+	-	-	+	+	-
3		350	30		1680	16	+	-	-	+	+	-
4	*	200	70		2400	1.7	1283	3849	3.0	+	+	-
5	*	200	60		1920	1.4	1283	3412	2.7	+	+	-
6		200	70		320	3.1	+	+	-	+	+	-
7		200	30		59	0.6	+	+	-	+	+	-
8		100	70		-	-	+	-	-	+	-	-
9		100	30		-	-	+	-	-	+	-	-
10	*	100	80		25	0.02	1283	8983	7.0	+	+	-
11	*	100	70		20	0.01	1283	3979	7.0	+	+	-
12	*	100	60		18	0.01	1283	7702	6.0	+	-	-
13	*	100	30		21	0.01	1283	4490	3.5	+	-	-
14		70	70		-	-	+	+	-	+	-	-
15		70	30		-	-	+	+	-	+	+	-
16	*	70	70		12	0.01	1283	10481	8.2	+	+	-
17	*	70	60		14	0.01	1283	7407	5.7	+	-	-
18	*	70	30		5	0.004	1283	8230	6.4	+	+	-

* = spiked with pesticide standard; for further details see Table 18.

Table 21. The influence of temperature and pressure on the discrimination of pesticides Lindane, α-Endosulfane, β-Endosulfane) during supercritical fluid CO_2 extraction of natively contaminated Spanish paprika and **the effect on the sensory quality of the extract*.**

Nr	P (bar)	T (°C)	Sensory observations	Extr. Yield (g)	Lindane (γ-HCH) (ppb)	α-Endo-sulfane (ppb)	β-Endo-sulfane (ppb)
1	350	70	Color: dark red extract, Odor: intensive-paprika Taste: intensive paprika	5.53	1500	313.5	686
2	350	60	Color: dark red extract Odor: intensive-paprika Taste: intensive paprika	4.80	967	239.7	604
3	350	30	Color: red extract Odor: intensive-paprika Taste: intensive paprika	5.00	628	204.0	480
4	200	70	yellow, viscous oil phase & clear water phase	4.08	500	112.1	142
spiked	200	60	water and yellow oil phase			13.6	
5	200	30	reddish thick extract	4.25	44		19
6	100	70	Clear water	4.00	-	-	-
spiked	100	60	Clear water phase	4.00	-		
7	100	30	yellowish red thick oil	2.37	-	+	-
8	70	70	opaque & watery	0.48	-		-
9 spiked	70	60	water phase only, pungent paprika odor			+	
	70	30	lightly viscous yellow oil	1.06	-	-	-

* = The definition of quality as ascribed here to paprika extract was based on the end use of the extract largely for coloration and less for taste of food products. Therefore, the characteristic color of Paprika is dark red extract. (additionally the odor is characterized as intensive-paprika odor , and taste as intensive paprika taste).

The effects of temperature and pressure on the extraction behavior of different pesticides (organochloropesticides, organophosphates, pyrethroids and triazines) are shown in Tables 18-22. As it could be seen, temperature was used as a parameter to control fluid density at any given pressure.

This approach was employed because of the overriding effect of temperature on the solubility of both the wanted and unwanted extract components and on the sensory quality of the extract.
At high temperature and pressure (70 °C / 350 bar), the extract yield was high but more pesticide residues were extracted as well.

The reduction of the extraction temperature to 60 °C resulted to no considerable loss in yield, but to a reduction of lindane below tolerance level and to a further reduction of already low concentrations of α- and β-endosulfane, permethrine and cypermethrine (Table 21). Although the concentration of deltamethrine was reduced, it was still above the tolerance level.
With the extraction temperature of 30 °C there was a further reduction of pesticide residues in the extract, an unchanged sensory value of extract and only 0.53% loss in yield. Deltamethrine carry-over in the extract depreciated further, but the level was still above the tolerance level.

Table 22. The influence of temperature and pressure on yield and quality* of extracts from supercritical fluid CO_2 extraction of Spanish paprika.

Nr	P (bar)	T (°C)	Sensory observation For experiments ***I*** & (***II***)	% yield for experiment ***I*** & (***II***)	% Av. Yield
1	350	70	Color: dark red extract, Odor: intensive-paprika Taste: intensive paprika Consistence: paprika extract	5.53 (5.45)	5.49
2	350	60	Color: dark red extract, Odor: intensive-paprika Taste: intensive paprika Consistence: paprika extract	5.00 (5.29)	5.15
3	350	30	Color: red extract, Odor: intensive-paprika Taste: intensive paprika Consistence: paprika extract	4.80 (5.05)	4.93
4	200	70	yellow, viscous oil phase & clear water phase (water phase volume is twice theat of oil phase)	4.08 (6.00)	5.04
spiked	200	60	water and yellow oil phase	4.98 (4.55)	4.77
5	200	30	reddish thick extract	4.25 (3.27; 3.56; 4.13)	3.80
6	100	70	clear water	4.00; (4.00)	4.00
spiked	100	60	clear water phase	4.00; (4.00)	4.00
7	100	30	yellowish red thick oil	2.37	
8	70	70	opaque & watery	0.48; (1.53)	1.01
9	70	60	water phase only, pungent paprika odor	0.23	0.23
	70	30	lightly viscous yellow oil	1.06	1.06

* = see legend of table 21 for the description of the quality parameter

The effects of temperature and pressure on total quantity and sensory variations of SFE extracts are elucidated and presented inTables 21 and 22. Only at an elevated pressure of 350 bar and at temperatures between 30 and 70 °C extracts with real sensory qualities of fresh paprika were obtained. Interestingly enough, at 350 bar and at a reduced temperature of 30 °C, the extract obtained has the same sensorial characteristics than that obtained at 70 °C. At 200 bar and 30 °C a thick reddish extract was obtained. However, it was not quite clear why at 200 bar and 60 °C an extract was obtained composed of water and a yellowish oily phase. Tables 18-21 show that under the extraction conditions used pesticide residues were considerably reduced.

2. 5 Results of the selective extractions of water- soluble and partially water-soluble pesticides

The results presented in Tables 21 and 22, show that water was extracted or desorbed at the following parameters: (200 bar and 70 °C; 200 bar and 60 °C; 100 bar and 70 °C; 100 bar and 60 °C; 70 bar and 70 °C). The residue analysis of the extracted water showed the presence of metribuzine. Traces of cypermethrine and deltamethrine were also identified in the watery extract.

These parameters were applied in the subsequent experiments for a selective elimination of metribuzine and other contaminants. Since the SFE extraction plant used was equipped with only one separator and has no separate waste pathway, a selective extraction approach was employed. The selective extraction process was carried out in an intermittent extraction mode, i. e, at the end of the first phase of the extraction, the separator vessel was disconnected, emptied, cleaned and reconnected for the second phase of the extraction. The second phase of the extraction was done under practical/industrial conditions (350 bar and 70 °C). In both extraction phases metribuzine, cypermethrine and deltamethrine were extracted. The reduction of these substances in the first phase of the extraction resulted in this wide range of extraction parameters has to be clarified by more focused investigations of pesticide extractability within this range of extraction parameters.

2. 6. Result of the use of water as a modifier in SFE experiments.

Table 23. Supercritical fluid extraction of paprika (sample I = water content 8%; sample II = spiked with water to 12% water content; extractions were carried out at 350 bar and 70 °C).

Pesticides	Solvent extract		SFE extract		Enrichment	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
α-Endosulfane	90 ppb	230 ppb	1900 2200*	5200 ppb	21 24*	23
β-Endosulfane	150 ppb	360 ppb	3300 ppb 3600*	9600ppb	22 24*	26
Chlorpyriphos	>100 ppb	900 ppb	2400 ppb 2600 ppb*	23000 ppb	24 26	26
Deltamethrine	n. d.	130 ppb	n. d.	3100 ppb	-	23
Cypermethrine	n. d.	20 ppb	n.d.	800 ppb	-	40
Permethrine	n. d.	10 ppb	n.d.	400 ppb	-	40
Control analysis of the extraction cake						
α-Endosulfane		1.5 µg		9,6 µg		111 µg
β-Endosulfane		2.5 µg		16.7 µg		192 µg
Chlorpyriphos		2.2 µg		12.1 µg		143 µg

* = sample spiked with water (Total water content of the sample = 12%); n. d. = not determined; - = not detected

The result in **Table 23** demonstrate that water as modifier has only little effect on the concentration of residues that can be extracted at the parameters used (70 °C and 350 bar). However, at 100 bar and between 60 – 70 °C, 4 g clear water were extracted from the dried supercritical CO_2. Therefore, the process by which water was obtained during the supercritical extraction can be characterized as a desorptive one.

2. 7. The duration of an exhaustive SF extraction.

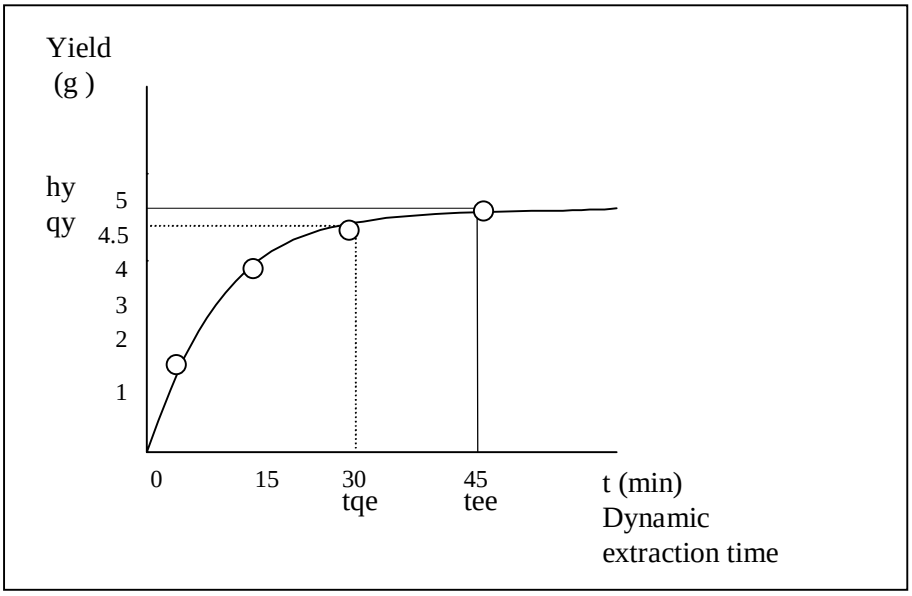


Figure 29. Relationship between the extraction time and the yield of SFE (**tee** = exhaustive yield; **tqe** = high quality yield; hy = high yield; qy = quality yield). The extractable amount of extract in a given time was gravimetrically determined.

The time-yield graph in **Fig. 29** shows the different yields obtained during extraction processes with various extraction times ranging from 30 to 45 min. For the time **tee** (Fig. 29), a total yield of 5 g was obtained. The extension of extraction time gave no further increase in yield. 45 min was the optimal extraction time for which a maximum of yield could be achieved.

2. 8. The extraction time for a high qualitative yield and a decrease of pesticide carry-over

After an extraction time of 30 min (***tqe***, compare **Fig. 29**) 4.5 g of extract were obtained. The residue analysis of the extracts and the control analysis of the extraction cakes showed that a reduction of the dynamic extraction time from 45 to 30 min resulted to a minimal loss in yield

(average 5%). But the reduced extraction time caused a reduction of the amount of pesticides in the extract and a higher recovery of the pesticides in the remaining SFE cake (Table 23). A reduced extraction time possibly avoids desorption of naturally occurring pesticides because their lipid-accompanying elution has been delayed either as a result of adsorption phenomena onto the fine particle surfaces of the sample or due to their molecular size.

2. 9. The influence of parameters such as particle size and extraction time on the extractability of pesticides.

No considerable effect of particle size alone on the extractability of pesticides could be noticed at 70 °C and 350 bar. This, however, could be easily explained by the drastic extraction parameters employed and, additionally, due to too long extraction times.
Nonetheless, a cumulative reduction of pesticide residues was observed when fine particle size in association with high packing density, high extraction pressure, moderate dynamic extraction temperature and extraction time were interrelatedly employed.

2. 10. Determination of the extractibility of polar pesticides by liquid scintillation counting (LSC) of a SFE paprika sample spiked with ^{14}C-terbutylazine.

Table 24. Extractability of polar pesticides (SFE of paprika spiked with ^{14}C-terbutylazine).

Spike amount (Control analysis)		3.000.000 dpm* (absolute)	
Amount counted prior to SF CO_2 extraction		300.000 dpm	
Amount extracted (350 bar, 70 °C)	Experiment 1	117.270 dpm	Average: 137.515 (45.8 %)
	Experiment 2	114.173 dpm	
	Experiment 3	158.716 dpm	
	Experiment 4	161.938 dpm	
Amount extracted (200 bar, 70 °C)	Experiment 1	221.914 dpm	Average: 237.823 79.3 %
	Experiment 2	253.731 dpm	

*dpm = disintegrations per minute

300.000 dpm of ^{14}C-terbutylazine used as a representative of polar pesticides have been counted in a spiked paprika sample prior to supercritical fluid extraction. 137.515 dpm (44.8 % of total dosage in spiked sample) were measured after an SF extraction at 350 bar and 70 °C. With extraction parameters of 200 bar and 70 °C 237.823 dpm (79.3% of spiked ^{14}C-terbutylazine have been extracted) were counted. These impressive results confirm the extractability of polar substances at both *hydrophilic extraction* as well as industrial extraction conditions.

2. 11. Duration of an extraction via a by-passed chromatographic vessel.

Exhaustive SFE (5 g extract out of 100 g paprika sample) took about 5 min more time to pass also through a metallic chromatographic vessel (1cm × 9 cm) that was additionally coupled to the extraction vessel (35 min were determined for a high-quality extraction). The extraction time was adjusted to this result. For more details see also the chapters dealing with paprika permeation column (PPC) used for retention of pesticides during SFE of paprika samples.

2. 12. Simultaneous supercritical fluid extraction and chromatographic separation (SFECS) of paprika color components

Different coloration zones were observed in the extraction cake remeining in extraction vessel at the end of each CO_2 extraction: from pale to yellow and orange till red from vessel basis to upper parts and exit of extractor vessel (upstream extraction). The degree of redness depended on the extraction pressure employed and the duration of dynamic extraction.

the extractor vessel and more striking coloration zones were obtained. The separation of paprika colors on this column (yellow, orange and red colors) was comparable to the separation colors obtained on a normal GPC column filled with polystyrol beads.

In another experiment the chromatographic vessel was densely packed with paprika extraction cake (0.8 mm particle size), again placed after the extractor vessel and similar observations could be made.

All these observations pointed out that a chromatographic separation that is predominantly based on the molecular weight of the sample colorants. This means that possibly a simultaneous discrimination of pesticide residues from lipids and other macro compounds

during supercritical fluid extraction is conceivable (see the following chapter: comparison of pesticides elution pattern on a normal GPC and a paprika permeation chromatographic (PPC) column.

2. 13. Pesticide elution pattern of paprika permeation chromatography (PPC)

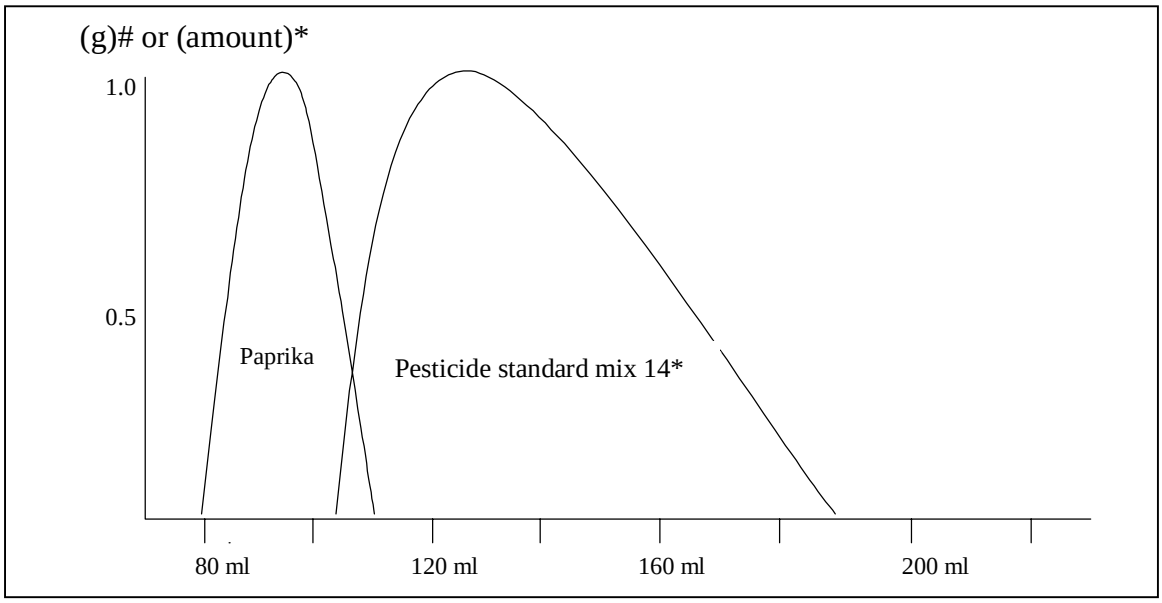


Figure 30. Elution pattern of paprika extract and organochlor pesticides on a **paprika permeation chromatographic** (**PPC**) column. # = The amount of paprika extract eluted in each fraction was detrermined gravimetrically; * = GC-ECD determination of the pesticide eluted in each fraction. For individual substances see chapter 1.2 of the experimental part

The elution pattern of paprika extract and organochlor pesticides on a PPC is presented in Fig. 30. It was thrilling to observe that the elution pattern of pesticides and lipids on PPC columns (see chromatogramms on **Figures. 31-33**) resembled that on a GPC column (compare chromatogramms on **figures 34-43**).

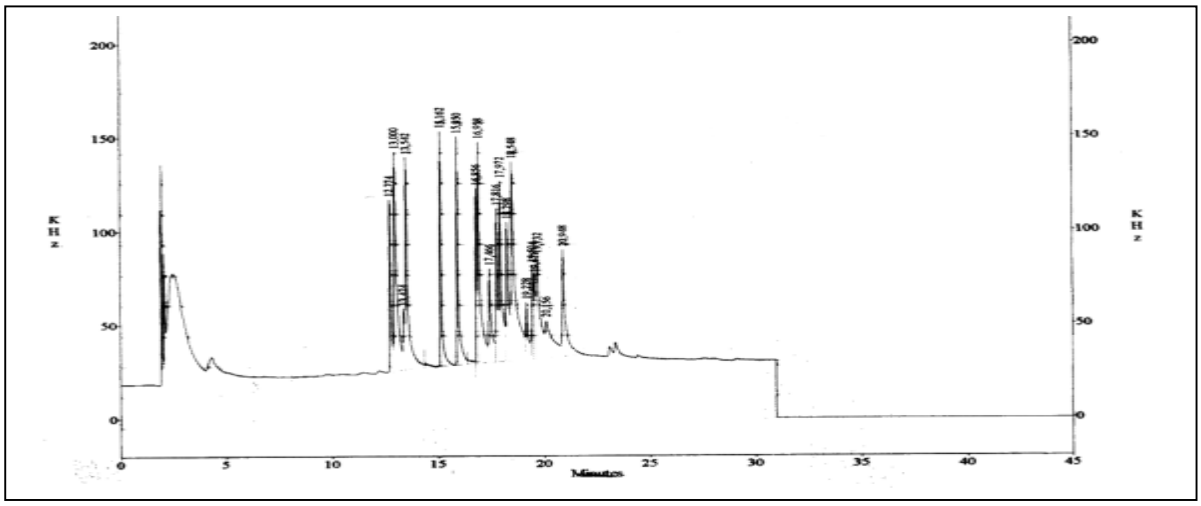


Figure 31. GC-ECD determination of organochlor pesticide standard 14*.

(concentration: 64.15 ppb) See material and method for the temperature program.

* = Organochloropesticides **(**reference standard mix 14) and retention time (figure 31)

Substance	*Rt* (min)	Substance	*Rt* (min)
1. α-HCH	12.77	11. Dieldrin,	17.97
2. HCB	13.00	12. o,p-DDD	18.29
3 β-HCH	13.42	13. Endrin	18.54
4. γ-HCH (Lindane)	13.52	14. β-Endosulfane	19.22
5. Heptachlor	15.16	15. p,p-DDD	19.50
6. Aldrin	15.95	16. o, p-DDT	19.61
7. tr-Hepatachlorepoxid	16.85	17. p, p-DDT	19.73
8. α-Endosulfane	16.95	18. p, p-DDT	20.94
9. Dieldrin	17.46	17. Methoxychlorine	24.5
10. p, p-DDE	17.81		

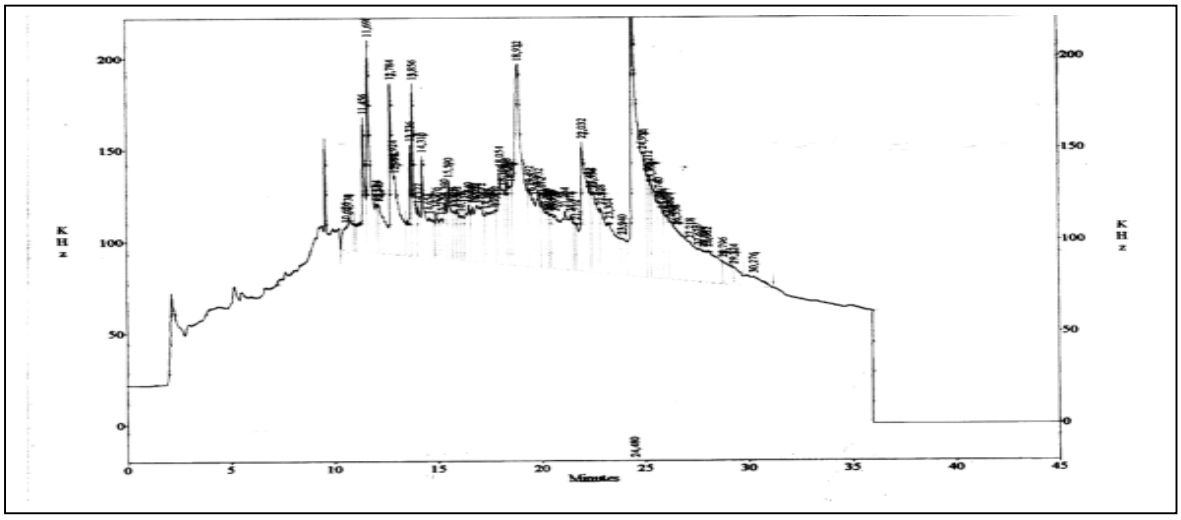


Figure 32. PPC fraction 50-100 ml = dump fraction; Investigation of elution pattern of organochlor pesticides on a PPC column.

Shown on figure 32 here are the elution patterns of Mix 14-pesticides on a paprika permeation column. GC-ECD determination of the pesticide eluted in each fraction. (Pesticide concentration: 64 ppb). Compare also the elution patterns of Mix 14 pesticides on GPC (compare **figure 34-43**).

In comparison, on a GPC with bio-beads as column material, lipids were also eluted in the corresponding dump fraction. The GC-MS analysis showed that the detected peaks on figure 32 were are mostly long chain alkanes, fatty acid esters etc., were from the paprika column material.

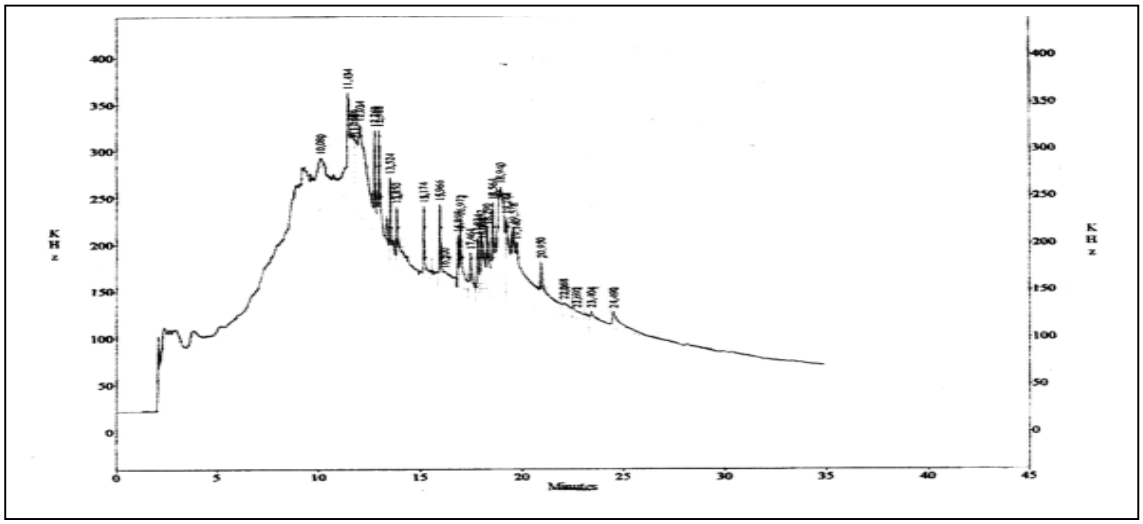


Figure 33. PPC fractions 100-190; Investigation of elution pattern of organochlor pesticides Shown on figure 33 here are the elution patterns of Mix 14 pesticides on a paprika permeation column. GC-ECD determination of the pesticide eluted in each fraction (Pesticide concentration: 64 ppb). For substance retention time and peaks description see **figure 31**

Pesticide residues were eluted in the fractions from 100-190ml (Fig. 33). The elution pattern obtained was similar to the collect fraction of GPC (See **Fig. 34 - 43**).

This was regarded as an indication of separation of components in the pattern of size exclusion chromatography prevalent in gel permeation chromatography and a conclusion was made that a discrimination of pesticide residues from lipids and other macro compounds is based predominantly on molecular weight.

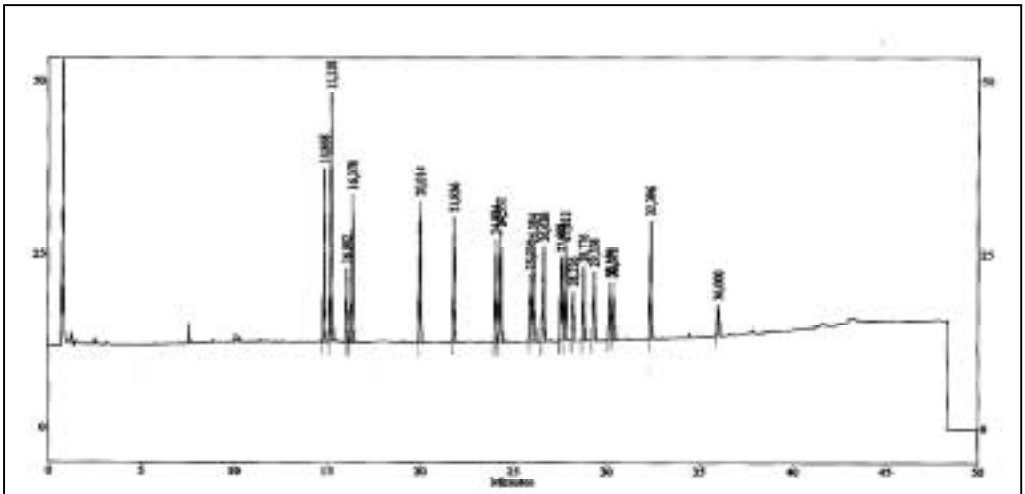
3. Work-up for multiresidue pesticide analysis

3. 1 Liquid solvent extraction work-up

The success of residue analysis depends very much on the effectiveness of the clean-up steps employed. This is more so if the analytes in question are found only in trace concentration in the samples.

3. 2. GPC clean-up procedure

The results of the experiment to determine the pesticide-collect-fraction and lipid-dump-fraction are presented on of figure 34-37



Pk Nm	Rt	Area
a-HCH	14,87	78902
HCB	15,23	122039
b-HCH	16,08	36680
LINDAN	16,38	75881
HEPTACL	20,01	83643
ALDRIN	21,84	75332
c-HEPTCLEPOX	24,08	66886
t-HEPTACLEPOX	24,33	70851
o,p-DDE	25,94	43812
a-ENDOSULFAN	26,08	65203
t-NONACL	26,64	66182
DIELDRIN	27,59	55892
o,p-DDE	27,81	62160
o,p-DDD	28,23	31865
ENDRIN	28,78	50397
b-ENDOSULFAN	29,34	48794
c-NONACL	30,20	35630
o,p-DDT	30,37	40703
p,p-DDT	32,40	82817
METHOXYCL	36,00	20175

Figure 34. GC-ECD Chromatogram of organochlorine pesticide standard substances. Concentration: 16 ppb

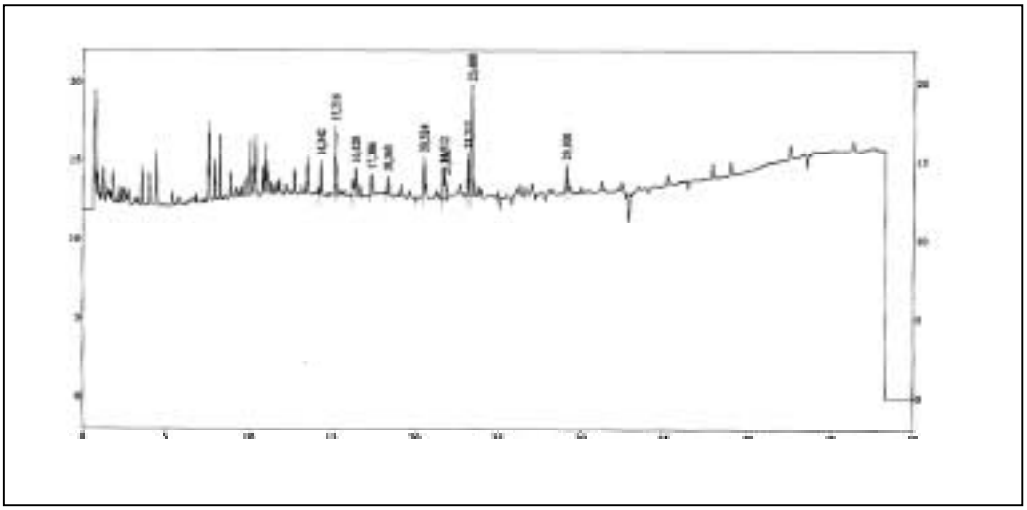


Figure 35. GPC fraction 1-50 mL = dump fraction; the elution pattern of organochlor pesticides. The identified peaks here are impurities GC-ECD determination of the pesticide eluted in each fraction.

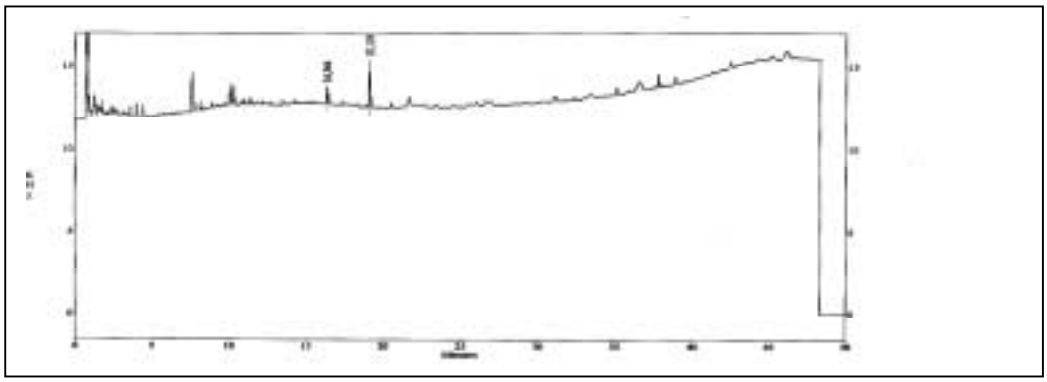


Figure 36. GPC fraction 78-86 = dump fraction; the elution pattern of organochlor pesticides. The identified peaks here are impurities. GC-ECD determination of the pesticide eluted in each fraction.

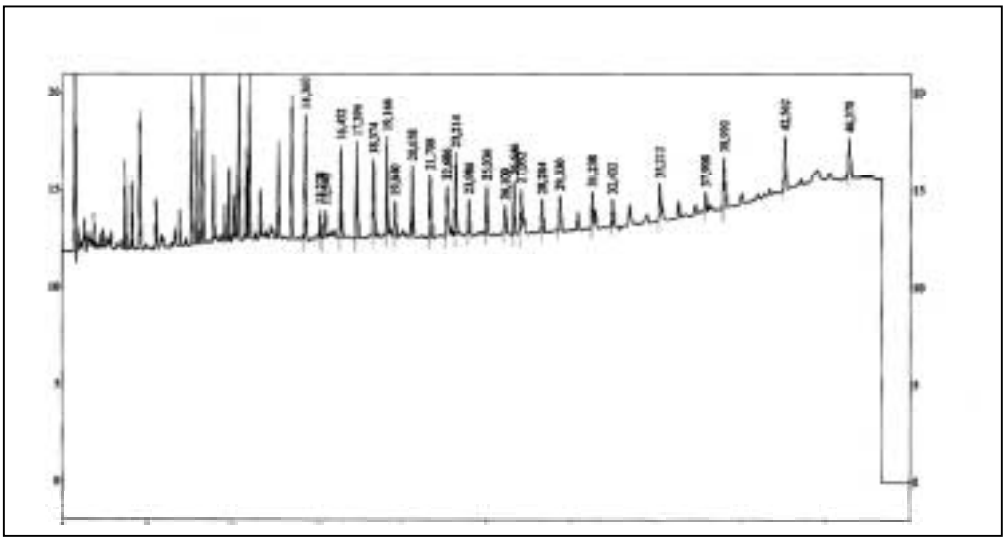


Figure 37. GPC fraction 86-94; the elution pattern of organochlor pesticides.
The chromatogramm shows the elution of impurities and the onset of the elution of Mix 14 pesticides standards. GC-ECD determination of the pesticide eluted in each fraction.

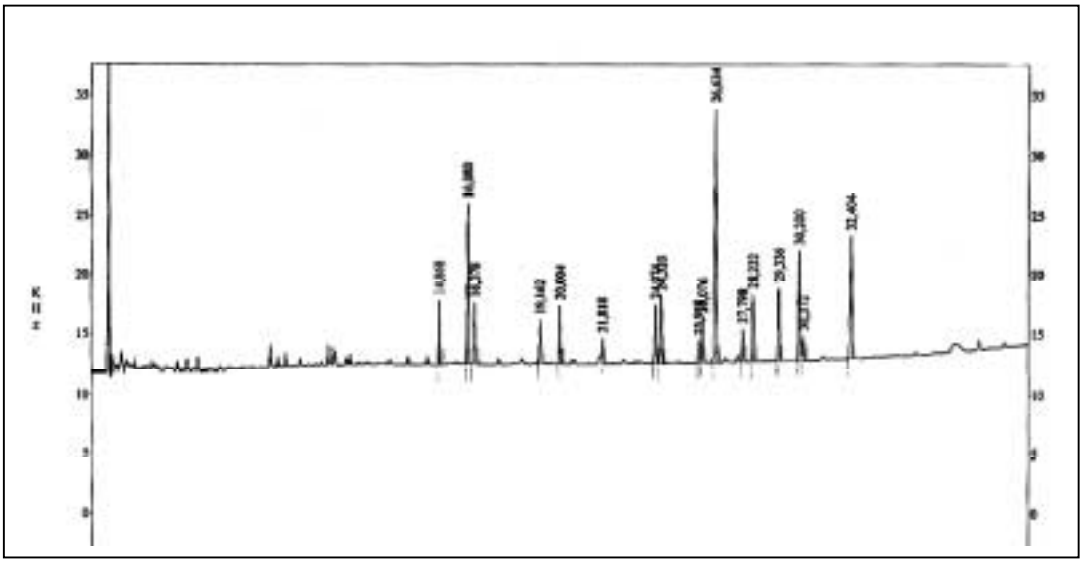


Figure 38. GPC fraction 94-102; the elution pattern of organochlor pesticides. As shown on the chromatogramm a sharp elution of Mix 14 pesticides standards can be observed. GC-ECD determination of the pesticide eluted in each fraction.

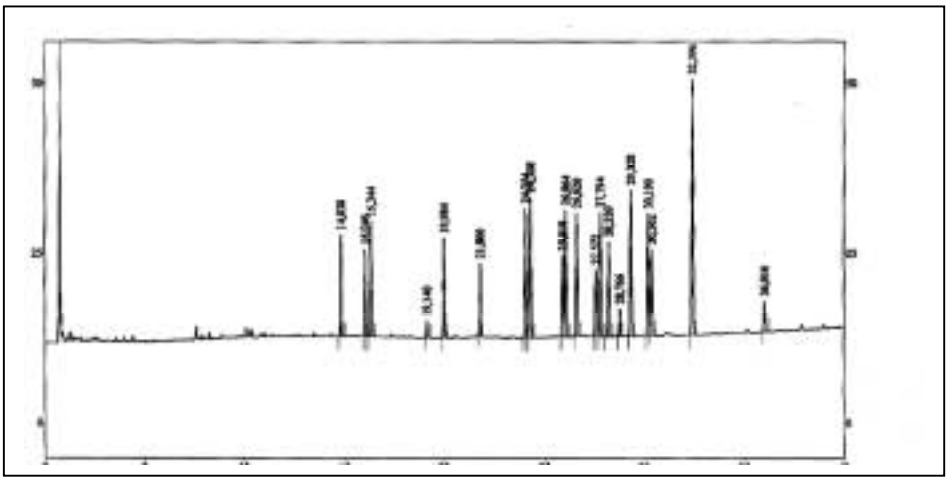


Figure 39. GPC fraction 102-110; the elution pattern of organochlor pesticides. The chromatogramm shows a sharp elution of Mix 14 pesticides standards continued in these fractions. GC-ECD determination of the pesticide eluted in each fraction .

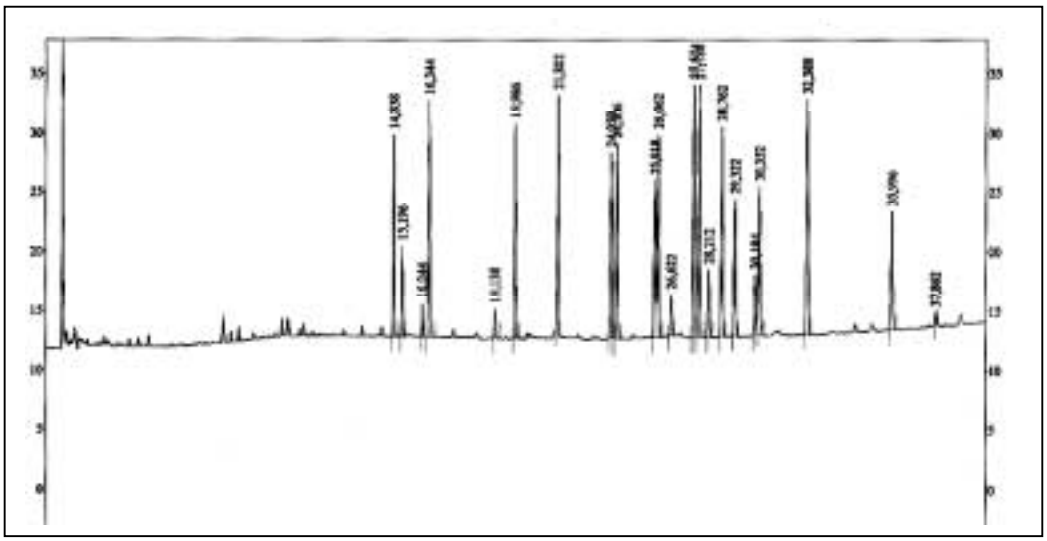


Figure 40. GPC fraction 110-118; the elution pattern of organochlor pesticides. The chromatogramm shows a sharp elution of Mix 14 pesticides standards continued in these fractions. GC-ECD determination of the pesticide eluted in each fraction .

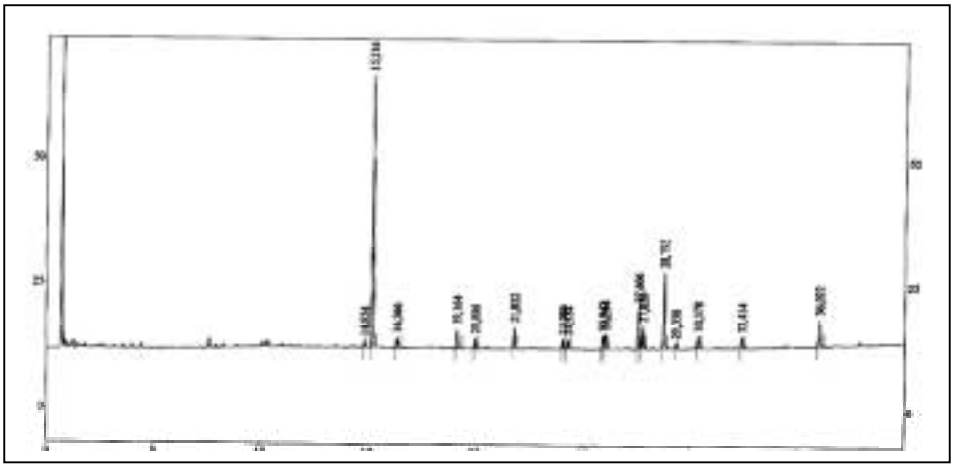


Figure 41. GPC fraction 118-126; the elution pattern of organochlor pesticides. The diminished peak height of the pesticide is an indication of the end of the elution of Mix 14 pesticides standards. GC-ECD determination of the pesticide eluted in each fraction.

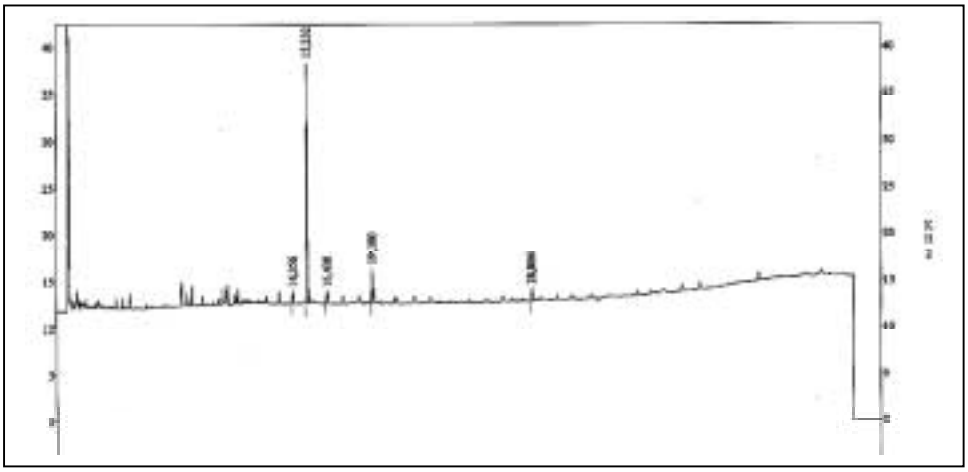


Figure 42. GPC fraction 126-134; the elution pattern of organochlor pesticides. Only HCB (*Rt*: 15:23), Aldrin (*Rt*: 16:42), Endrin (Rt: 28:18) out of Mix 14 pesticides standards were eluted in these fractions. GC-ECD determination of the pesticide eluted in each fraction.

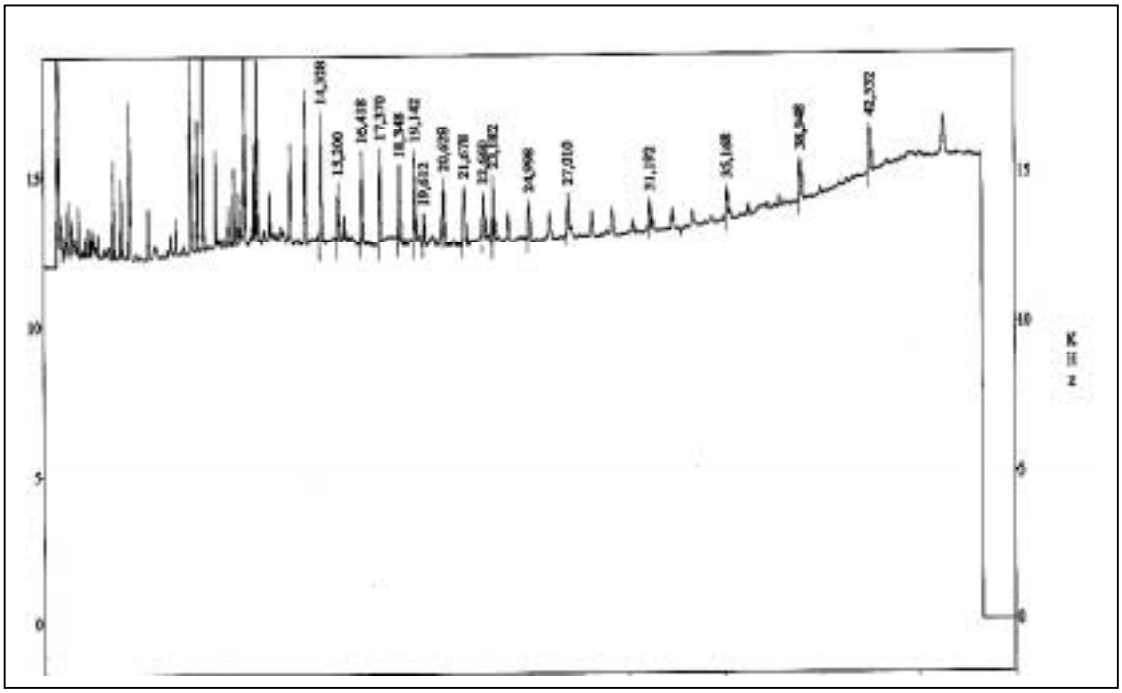


Figure 43. GPC fraction 134-142; the elution pattern of organochlor pesticides.

This chromatogramm is identical with that in **Figure 37.** GPC fraction 86-94. The chromatogramm here as the one on figure 37 shows the elution of impurities. This is hence the indication of the end of the elution of Mix 14 pesticides standards. GC-ECD determination of the pesticide eluted in each fraction.

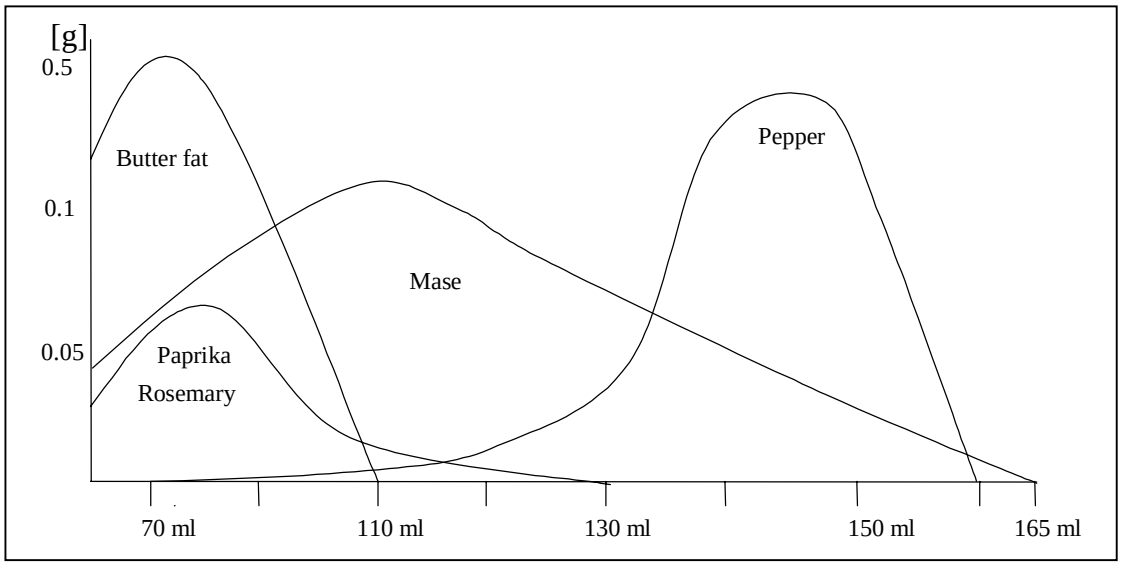


Figure 44. The elution pattern of butter fat, paprika, rosemary, mace and pepper on a GPC bio beads-column. The amount of paprika extract eluted in each fraction was detrermined gravimetrically.

The different elution patterns of the extracts (Fig. 34) demonstrate that the separation of lipids from the pesticides is not possible in the cases of pepper. The clean-up of rosemary and paprika was more effective although 30 % of the oily extract eluted into the pesticide fraction. The suitability of GPC for lipid clean-up was reexamined by determining the elution pattern of 1g butter fat on a GPC column under the same conditions. There was a 100 % separation of the butter fat fraction from the pesticide fraction. This means that the unsatisfactory separation of the plant extracts from the pesticide fraction can only be attributed to their compositions rather than to the efficacy of the GPC system. In order to increase pesticide retention on GPC column, a slow elution (2 ml min^{-1}) was tried with no success. This showed that the separation efficacy in this case does not depend on the flow rate of the GPC column.

3. 3. Mini silica gel chromatography / fractionation

There was no additional clean-up effect achieved by means of this chromatographic method. The activity of the mini silica gel used, enabled only the fractionation of the pesticides more or less into their respective classes.

3. 4. The Inadequacy of GPC clean-up and mini silica gel fractionation

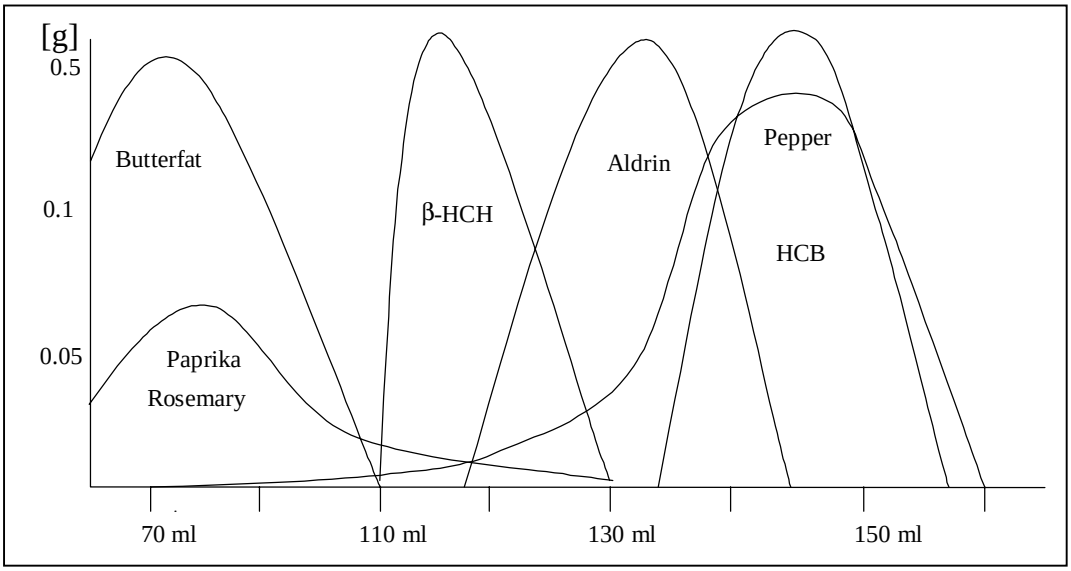


Figure 45. The elution pattern of butter fat, spice extracts (SFE) and some earlier eluting pesticides on a GPC column.
= The amount of paprika extract eluted in each fraction was detrermined gravimetrically.
* = GC-ECD determination of the pesticide eluted in each fraction.

The different elution patterns obtained on the GPC column highlighted the different physical properties and chemical composition of these spices. Taking these differences into consideration means that there is no universal clean-up method for all spice and medicinal plants, hence suitable GPC parameters (dump, collect volume, possibility of an additional clean-up step) for each plant extract should always be examined prior to the the main analyses.

The fractionation of the pesticides into five classes on a column of silica gel with 1.5 % less adsorbent activity also resulted to a non-considerable clean-up for the remaining interfering lipids from the GPC fractionation.

Due to the insufficient GPC clean-up, only the first three silica gel column fractions were analyzed with less interferences. In some cases (e.g. pepper) and after establishing that no sulfuric acid degradable pesticides were present, an additional sulfuric acid clean-up was carried out. The following fractions that were eluted with polar solvents gave at first no useful chromatograms, as a result of co-eluted substances that include lipids, colorings, waxes and wax alcohols and above all, flavor substances with low molecular weight. These co-eluents caused not only interferencing peaks but also influenced the separation efficiency of the GC capillary column. Overnight heating of the GC colunm at elevated temperature was necessary before it could be used for subsequent analyses. The introduction of a 5 m long methyl-deactivated fused silica retention gap alleviated this problem. In view of these interferents despite all the clean-up steps and the use of specific detectors, the confirmation of the identified substances by means of a mass spectrometer was absolutely necessary.

3.5. Counter current chromatographic cleanup for the lipophylic pesticides in lipids.

The search for an effective cleanup led to the experiments with CCC. The applicability of a high speed multilayer coil countercurrent chromatography (HSML CCC) as a clean-up step in multiresidue analysis of lipophilic pesticides in lipophilic samples was tested firstly by determining to what extent petroleum ether is miscible with acetonitrile in a acetonitrile-petrol ether (ACN-PE) solvent system.

3.6. The solubility of petroleum ether 40-60 °C (PE) and paprika extract in acetonitrile (ACN) with variable water content at room temperature

Table **25** shows that petroleum ether is soluble up to 17 % and paprika extract up to 5 % in acetonitrile at room temperature. A distribution of 1g paprika with this solvent system means that up to 50 mg lipid will be partitioned together with pesticide residues into the acetonitrile phase. Without further cleanup step, this sample solution with this amount of lipid proved unsuitable for a GC-ECD analysis.

Table 25: The solubility of petroleum ether 40-60 °C (PE) and paprika extract in acetonitrile with variable water content at room temperature.

Solvent system Nr.	Solvent composition	% Solubility of PE	% Solubility of paprika extract
1	ACN : PE : H_2O		
2	100 : 100 : 0	17	5
3	100 : 100 : 5	13	4
4	100 : 100 : 10	10	3.8
5	100 : 100 : 15	9	3.5
6	100 : 100 : 20	5	3
7	100 : 100 : 25	4	2
8	100 : 100 : 30	4	2

To raise the ***in***solubility of PE and paprika extract in ACN, the surface tension between the two solvents was increased by increasing their polarity differences. This was achieved by introducing water, a polar solvent as the third component to the solvent system. An addition of 5 % water to the solvent system decreased the solubility of PE in ACN from 17 % to 13 %. However, there was only 1 % reduction in solubility of paprika extract in ACN. With 30 % water content there was only 4 % solubility of PE and 2 % paprika extract in aqueous CAN (Table 25).

3. 7 Partitioning of pesticide standards in acetonitrile-petroleum ether solvent system in a PTFE-column operated HSML CCC.

41 pesticide standards from various substance classes (PCBs, triazine herbicides, pyrethroids, organochlorines and organophosphates) were used as model substances. Triazines, pyrethroids and most of the organophosphorous substances used were distributed in the aqueous acetonitrile phase. With more water in the solvent system, more polar pesticides (traizines, organophosphorous pesticides) and less non-polar PCBs and pesticides are partitioned into the aqueous ACN phase. This means that the more water is added the less suitable is ACN for a multiclass and multiresidue analysis. A more favorable distribution of pesticides was obtained with solvent systems 1 and 2.

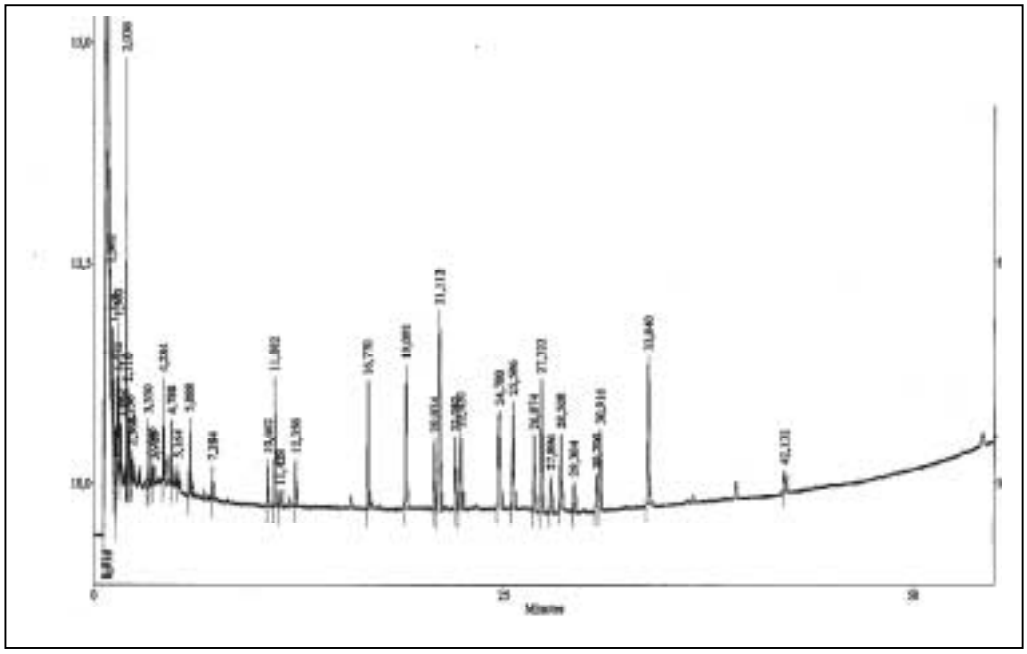


Figure 46. GC-ECD Chromatogram of organochlorine pesticide standard substances. (Spike concentration: 32 ppb)

Substance	*Rt* (min)	Substance	*Rt* (min)
1. α-HCH	10.71	11. t-Nonachlor:	25:67
2. HCB	11.13	12. Dieldrin	26:96
3 β-HCH	12.06	10. p, p-DDE	27.40
4. γ-HCH (Lindane)	12:42	12. o,p-DDD	27:97
5. Heptachlor	16.84	13. β-Endosulfane	28:59
6. Aldrin	19.16	14. Endrin	29:40
7. Isodrin:	21:17	15.c-Nonachlor	30:77
8. c-Hepatachlorepoxid	22:16	16. o, p-DDT	30.98
9. tr-Hepatachlorepoxid	22:49	18. p, p-DDT	33:91
10. o,p-DDE/α-Endosulfane:	24:84	17. Methoxychlorine	39:25

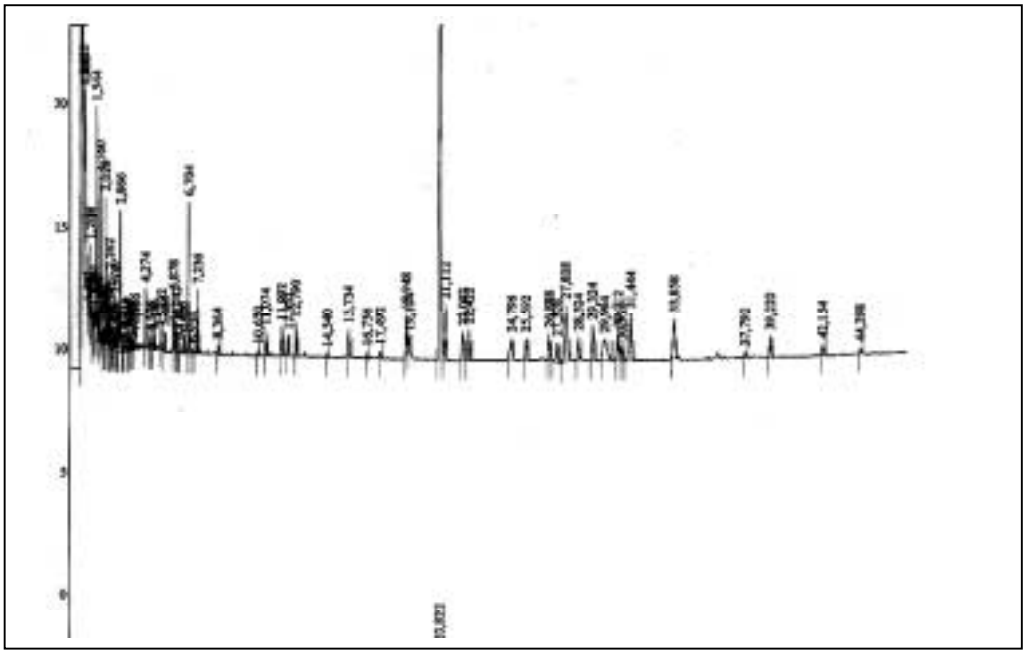


Figure 47. Aqueous ACN-phase of the counter current chromatographic distribution of organochlor pesticides in 5% ACN/PE-phase. (Spike concentration: 32 ppb)

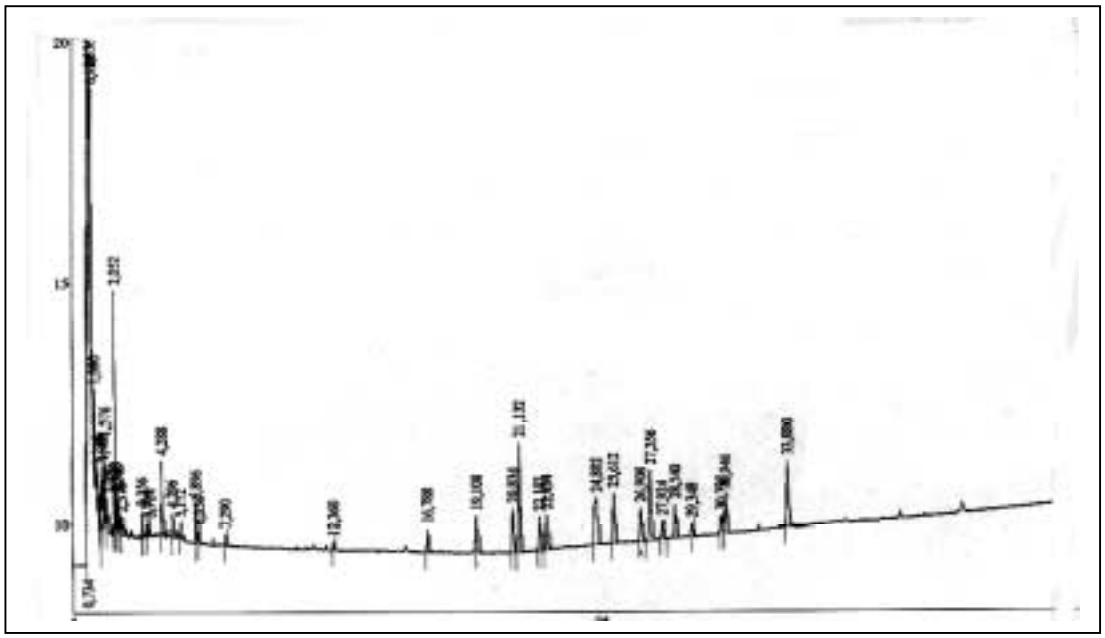


Figure 48. PE-phase of the counter current chromatographic distribution of organochlor pesticides in 5% ACN/PE-phase. (Spike concentration: 32 ppb)

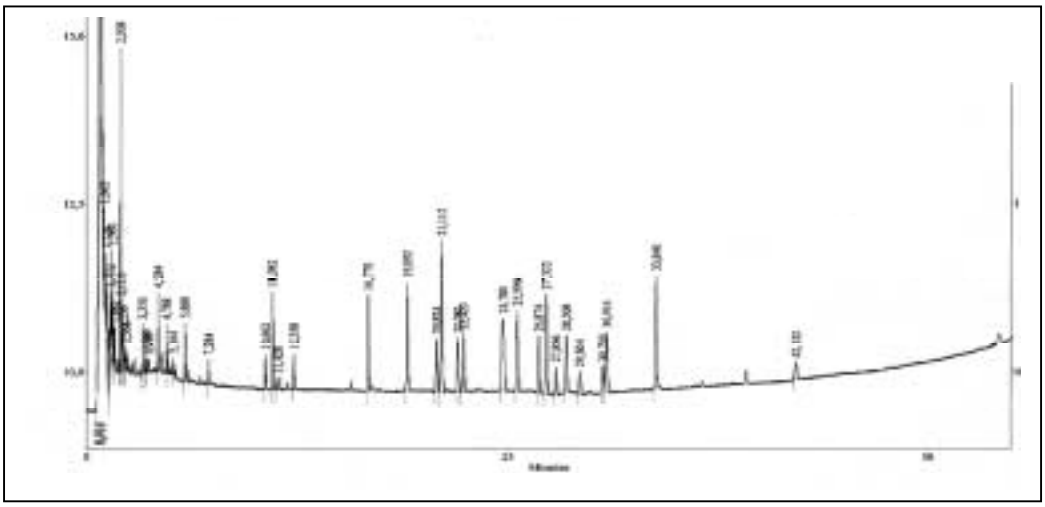


Figure 49. ACN-phase of the counter current chromatographic distribution of organochlor pesticides in ACN/PE/10 % H_20 solvent system. (Spike concentration: 32 ppb). A GC-ECD-determination. See the legends of figure 46 for the corresponding pesticide name to the retention time

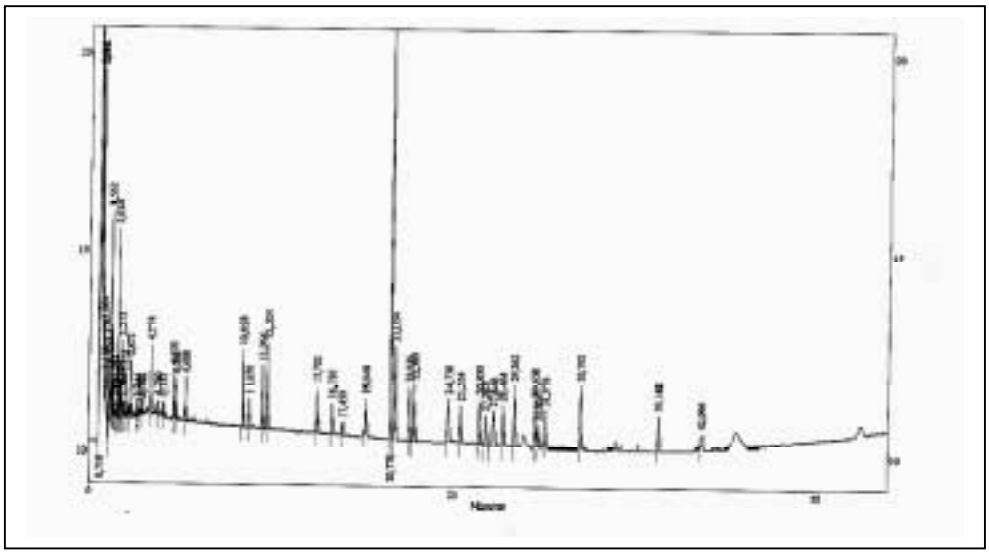


Figure 50. PE-phase of the counter current chromatographic distribution of organochlor pesticides in ACN/PE/10 % H_20 solvent system. A GC-ECD-determination. See the legends of figure 46 for the corresponding pesticide name to the retention time

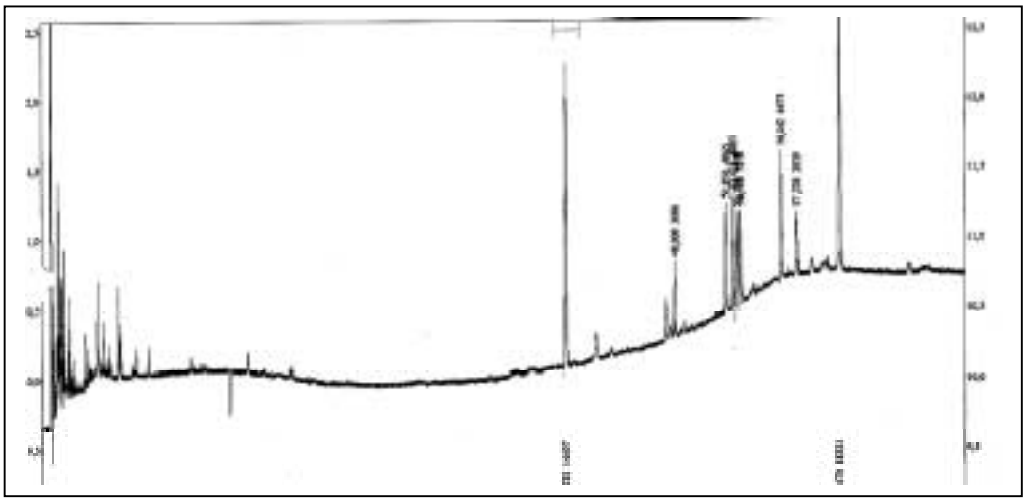


Figure 51. GC-ECD Chromatogram of pyrethroid pesticide standard substances.

(Spike concentration: 128.3 ppb)

Substance	*Rt*	Substance	*Rt*
Fenpropathrin	39:73	Cypermethrin	51:94/52:48
cis-Permethrin	47.41		52:77 / 53:11
trans-Permethrin	48:00	Fenvalerat	56:11 / 57:22/
		Deltamethrin	60:54

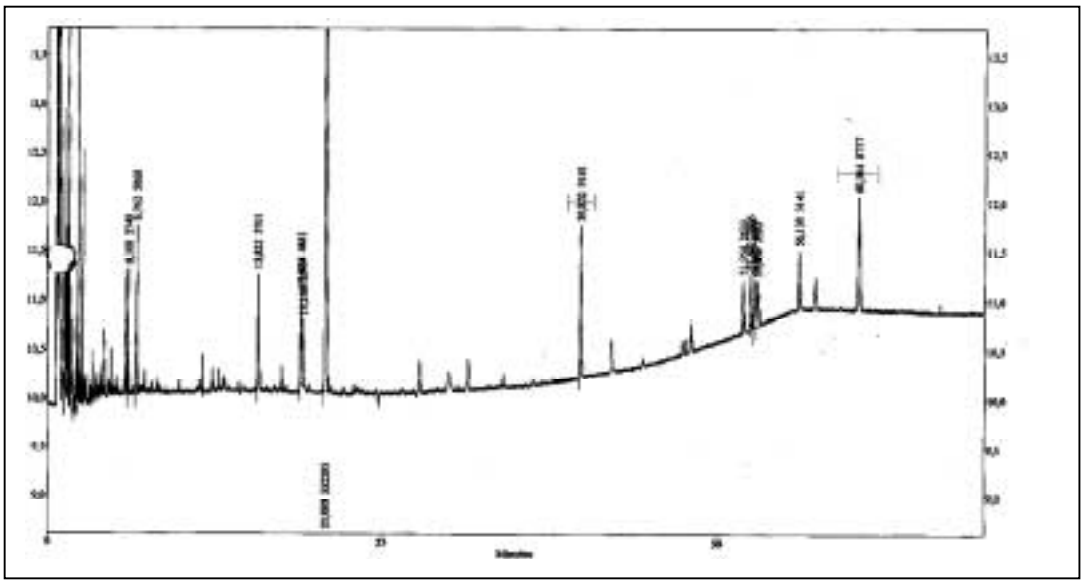


Figure 52. ACN-phase of the counter current chromatographic distribution of organochlor pesticides in ACN / PE / 5 % H_20 solvent system. (Spike concentration: 128.3 ppb). A GC-ECD-determination. See the legends of figure 51 for the corresponding pesticide name to the retention time

Figure 52 shows a very good distribution of pyrethroid pesticide in the aqueous ACN-phase

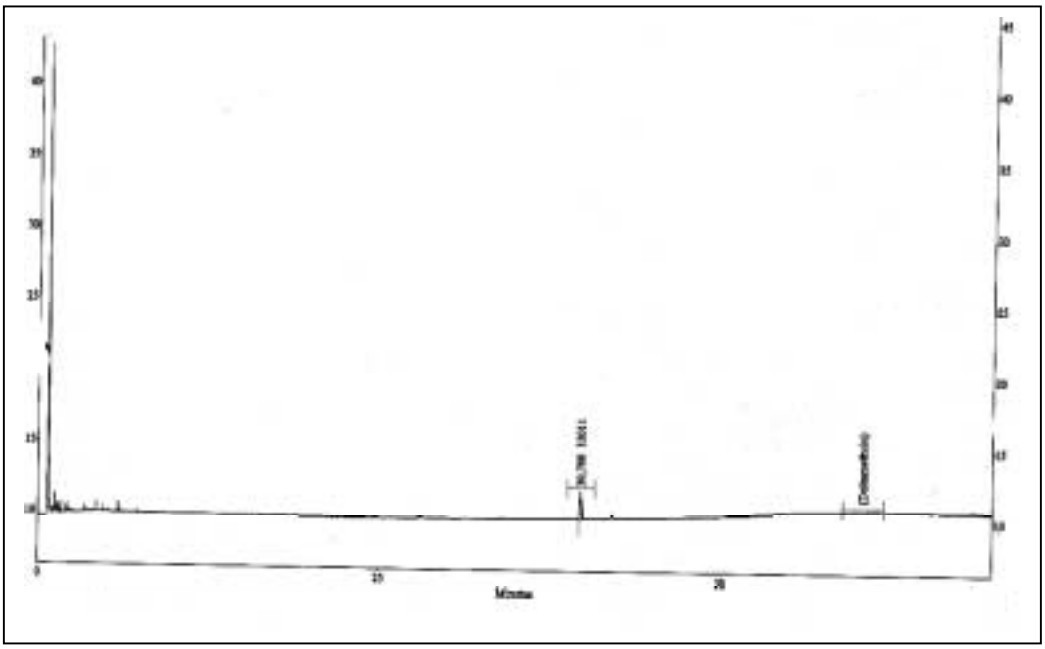


Figure 53. PE-phase of the counter current chromatographic distribution of organochlor pesticides in ACN / PE / 5 % H_20 solvent system. (Spike concentration: 128.3 ppb). A GC-ECD-determination. See the legends of figure 51 for the corresponding pesticide name to the retention time

Figure **53** shows that almost no pyrethroid substance was distributed PE-phase. This means the investigated pyrethroid substances have a favorably distribution behavior in the aqueous ACN phase employed. The percentage distribution of organochlor insecticides, triazine herbicides, organophosphate pesticides in aqueous ACN phase are presented on **table 26**.

Table 26: Partitioning of pesticide standards into acetonitrile phase with 5 ml water content.

Substances	**%**	**Substances**	**%**
Organohalogens		**Triazine Herbicides**	
Aldrine	+ + + + -	Atrazine	+ + + + +
Dieldrine	+ + + + -	Desethylatrazine	+ + + + +
o,p-DDD	+ + + + -	Isopropylatrazine	+ + + + +
p,p-DDE	+ + - - -	Metribuzine	+ + + + +
o,p-DDT	+ + - -	Terbutylatrazine	+ + + + +
p,p-DDT	+ + + - -		
o,p-DDE	+ + + - -	**Organophosphates**	
Endrin	+ + + + -	Azinophos ethyl	
α-Endosulfane	+ + - - -	Azinophos methyl	+ + + + +
β-Endosulfane	+ + - - -	Bromophos ethyl	+ + + + +
Hexachlorbenzine	+ + - -	Bromophos methyl	+ + - -
α-Hexachlorohexane	+ + --	Chloropyriphos ethyl	+ + + - -
Trans-Nonachlor	+ + - -	Chloropyriphos methyl	+ + - - -
β-Hexachlorohexane	+ + + +	Diazinone	+ + - - -
Isodrine	+ + - -	Parathion ethyl	+ + - - -
Heptachlor	+ + + + -	Parathion methyl	+ + + - -
cis-Heptachlorepoxide	+ + - -		
trans-Heptachlorepoxide	+ + + - -	**Pyrethroid insecticides**	
Lindane (γ-HCH)	+ + - -	Cypermethrine	+ + + + +
Methoxychlor	+ + + + +	Deltamethrine	+ + + + +
cis-Nonachlor	+ + + + -	Fenvalerate	+ + + + +
		cis-Permethrine	+ + + + +
		Trans-Permethrine	+ + + + +

+ + + + + 100%; + + + + - 80 - 95%; + + + - - 65-75%; + + - - 50%; + + - - - 30-45; + - - - - 10-20%.

Since the aim was to partition as many classes of pesticides and as less lipids as possible (see **Table 25**) into acetonitrile, the solvent composition with 5 ml water content was the best compromise to partition polar triazines, pyrethroids, and most of the organophosphorous and organohalogen compounds (**Table 26**). See also **figures 47-53** The distribution of real paprika sample is presented in **chapter 3.8** and on **figure 54**.

Triazines, pyrethroids and most of the organophosphorous substances used were distributed in the aqueous acetonitrile phase. Due to the fact that this solvent system was too polar for non-polar pesticides (hexachlorbenzene, p,p-DDE and other organochlorine compounds with unfavorable distribution in acetonitrile phase, Table 26), they were partly or completely partitioned into the petroleum ether phase. PCBs were completely partitioned into the petroleum ether dump phase and organohalogens with less favorable distribution in acetonitrile phase were cleaned up by additional sulfuric acid degradation.

3.8 Multiresidue pesticide analysis of *real* paprika extracts with HSML CCC.

To ascertain the effectiveness of a CCC clean up, the isolate ***a*** (ACN collect phase) from CCC cleanup was without further clean up subjected to a GC-ECD analysis.

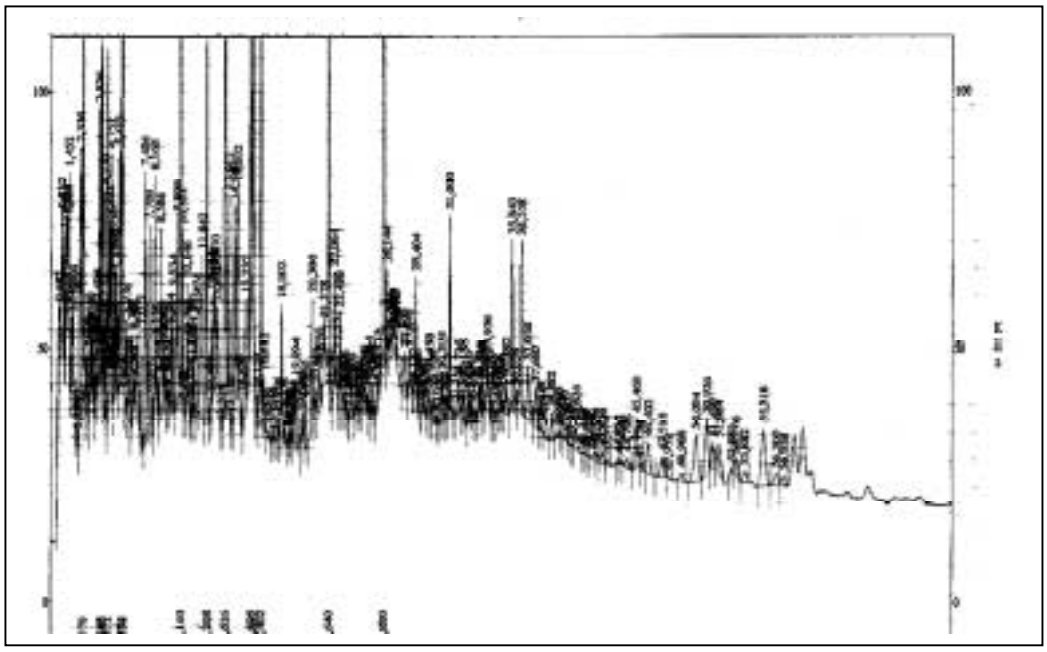


Figure 54. aqueous ACN-phase of the counter current chromatographic distribution of paprika extract in ACN / PE / 5 % H_20 solvent system. A GC-ECD-qualitative determination.

Substance	Rt	Substance	Rt
The PCBs are masked by Long chain		Permethrin:	47:51
Alkanes, long chain Alkenes, long chain			47:95
fatty acid:		Cypermethrin:	50:09
α-Endosulfane:	26:00		50:93
β-Endosulfane:	31:00		51:43
			51:88
		Deltamethrin:	59:09

Shown on figure 54 is a *qualitative* GC-ECD analysis of a CCC isolate ***a*** (ACN collect phase). A GPC eluate as we know is not suitable for a direct GC-ECD analysis of paprika extract. In contrast, the chromatogram shown on figure 54 shows that the CCC clean up resulted to a relatively cleaner eluate for a direct GC-ECD analysis, especially for the group of pyrethroid pesticides. However, for other groups of pesticides (especially the PCBs and organochlor pesticides that are masked by peaks of long chain alkanes and alkenes and long chain fatty acids), additional clean up were necessary. The GC analysis of PCBs and organohalogens with less favorable distribution in acetonitrile phase that were partitioned into PE phase (isolate ***b***) was only possible after a further clean-up step by means of sulfuric acid degradation.

3.9. ACN-PE cleanup with HSCCC - interfaced with an acid-treated silica gel column.

In situations where partition coefficients of some pesticides were not favorable enough to ensure a partition into the aqueous acetonitrile phase of an ACN-PE solvent system, the petroleum ether phase containing some organochlor pesticide and PCBs were further cleaned by means of sulfuric acid-treated silica gel column and gas chromatographicaly analyzed. A qualitative GC-FID analysis of fraction 1 of the acid-treated silica gel fraction of of aqueous ACN-phase of counter current chromatographic distribution of paprika extract is shown on figure 55.

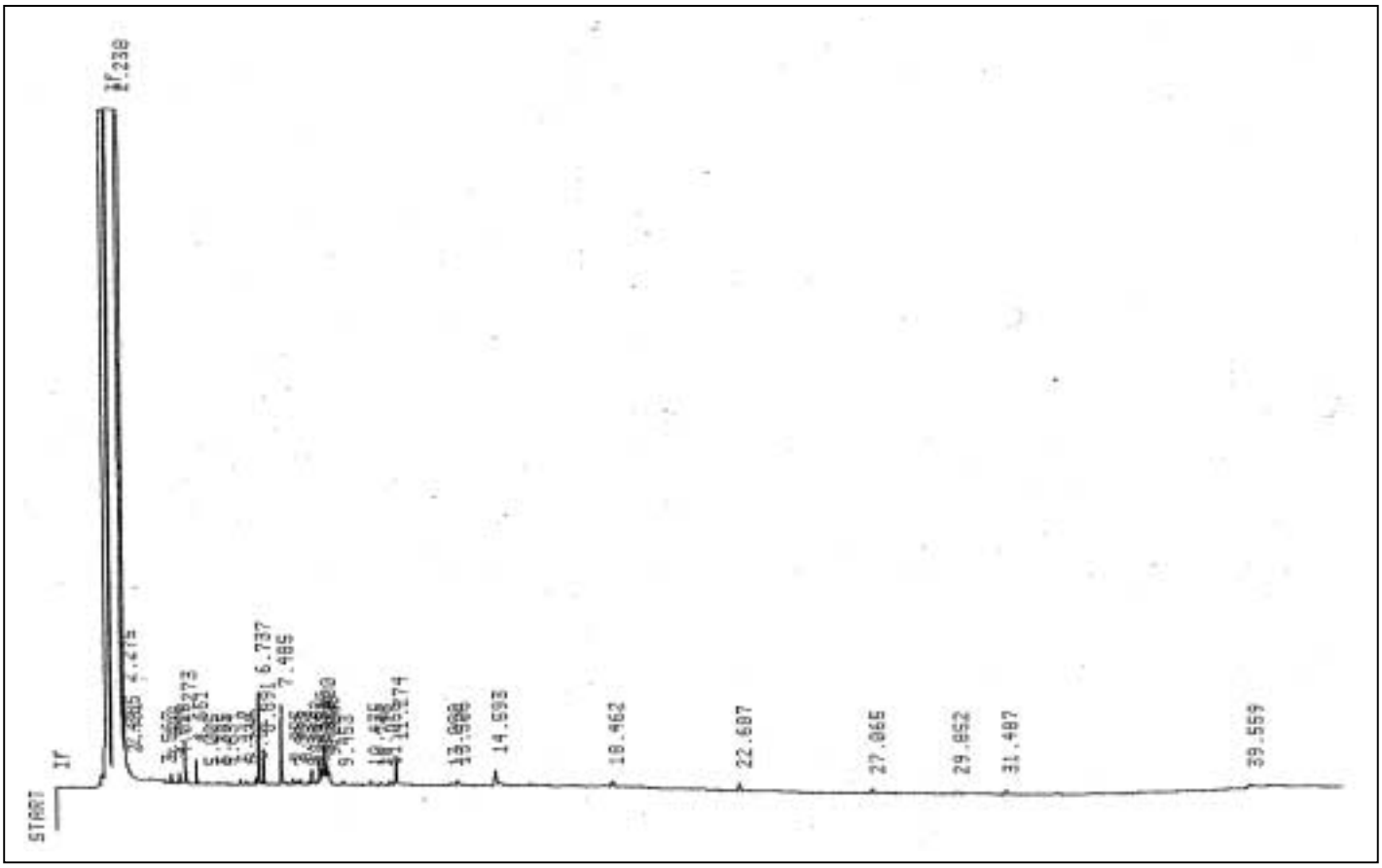


Figure 55. Paprika SFE-Extract. The PE-phase of CCC clean up was eluted on an acid-treated-silica-gel column to produce. GC analyzable PCB-fraction. A GC-FID-determination. 1 µl injected. Programm: 50/1-70-150/5-2-260

Substance	Rt	Substance	Rt
Tetrachlorbiphenyl:	22:58	Pentachlorbiphenyl:	29:85

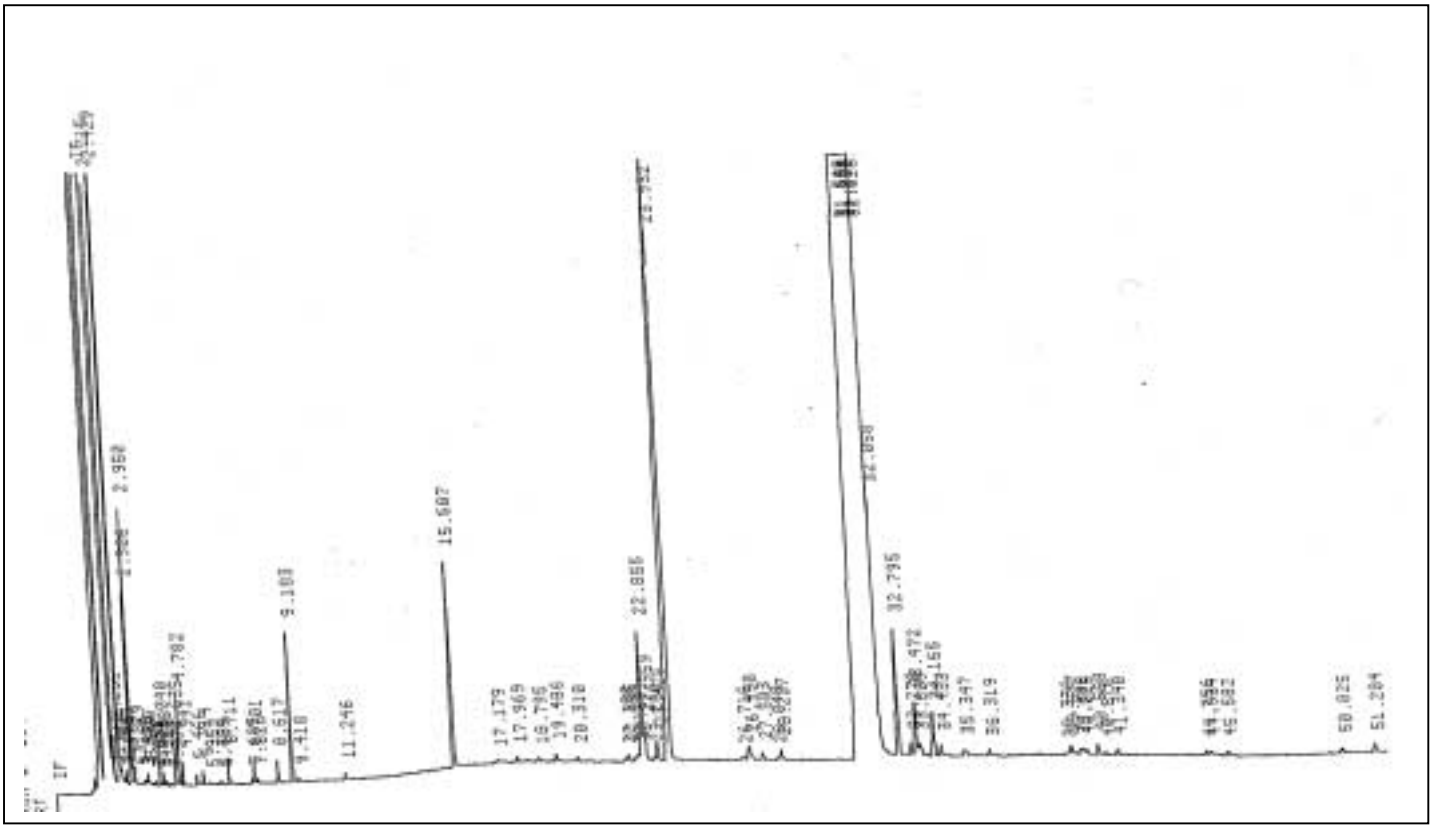


Figure 56. Silica gel eluate of aqueous ACN-phase of CCC-distribution of paprika extract. A GC-FID-determination. 1 µl injected. Programm: 50/1-70-150/5-2-260

Substance	Rt	Substance	Rt
Lindane:	15:07	α-Endosulfane:	31:50
Chlorpyriphos ethyl:	23:93		

The chromatogramms on **figures 55** and **56** show that a combination of counter current chromatography and acid treated silica gel column can be used for the analysis of high lipid plant extract such as paprika. GC-MS analysis of remaining fractions are shown on figures.

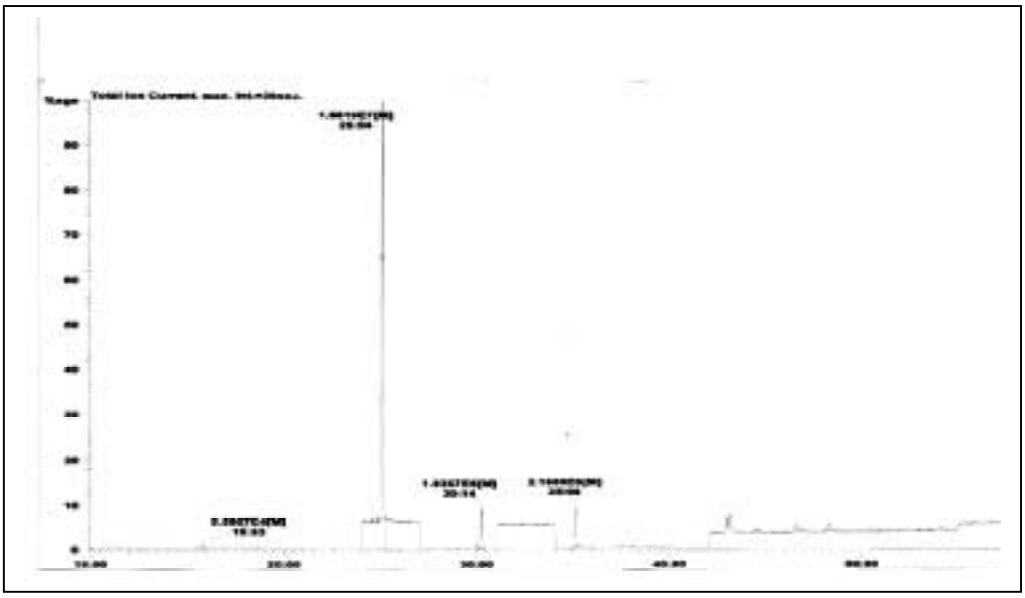


Figure 57a. Silica gel fraction 1: Total Ion current showing Lindan (15.53); Chlorpyriphos ethyl (Rt:25:04); α-Endosulfan (30.14); β-Endosulfan (35.08). Flagging = Peak Area & Rt. For the concentration of the respective analyte see Tabl. 16.

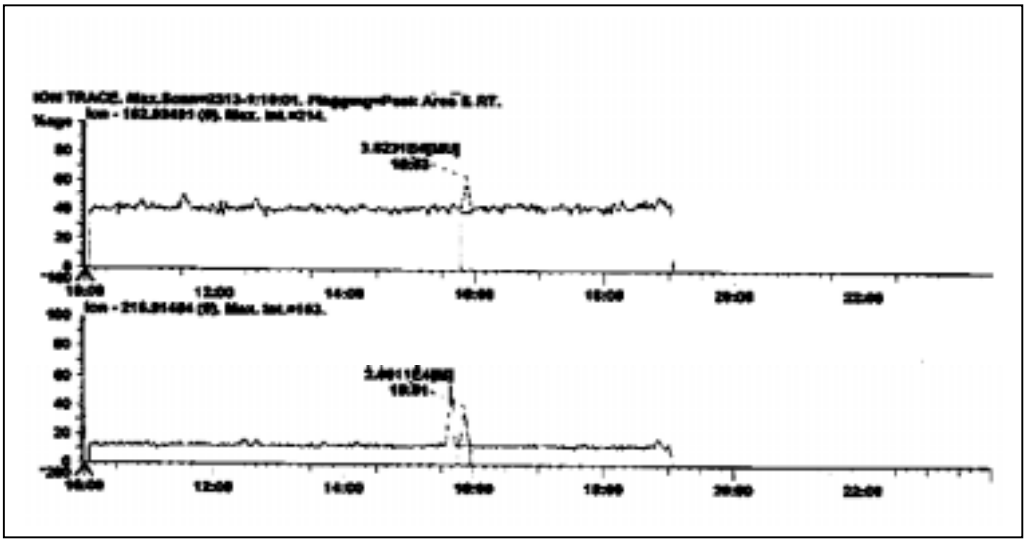


Figure 57b. Lindan Ion trace (15.53) m/e =182.93

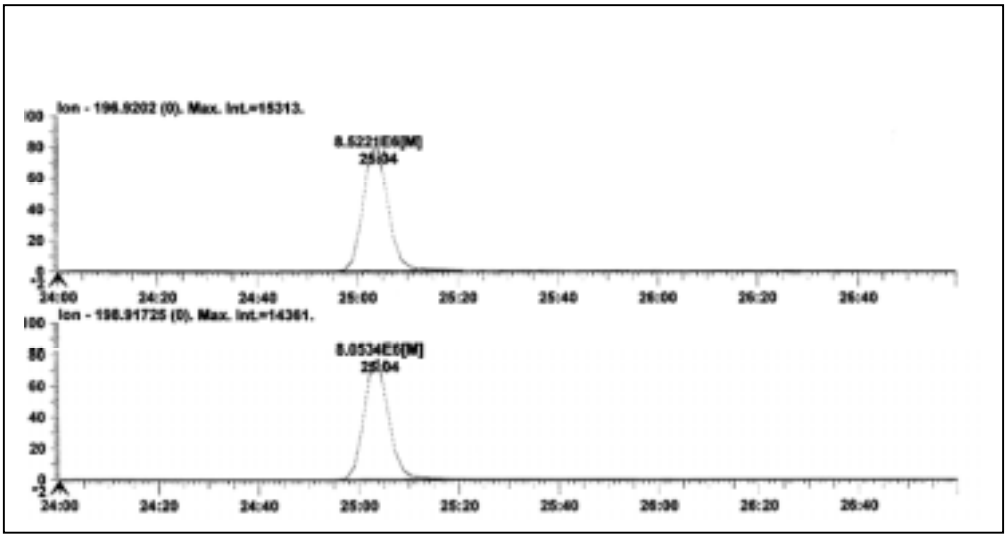


Figure 57c. Chloropyrphos-ethyl-ion trace RT= (25.04), m/e = 124.98

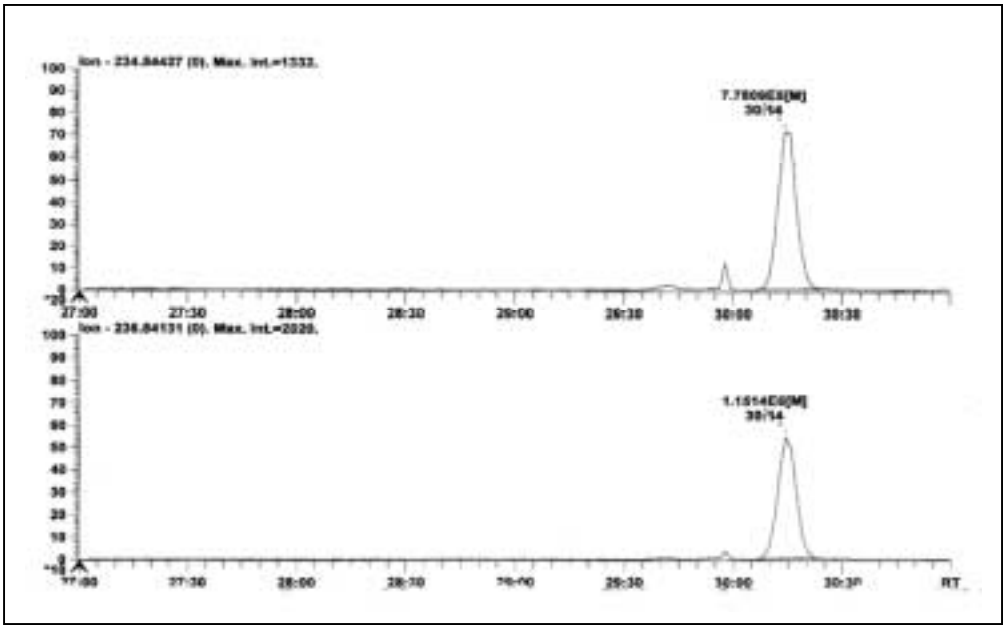


Figure 57d α-Endosulfan-ion trace (30.14), m/e = 234.84

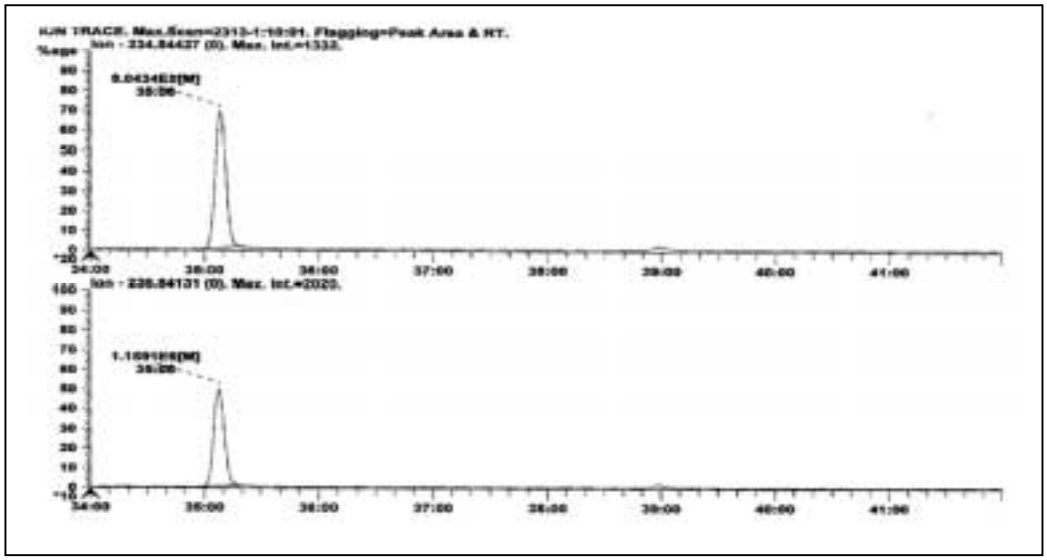


Figure 57e β-Endosulfan-ion trace (35.08), m/e = 236.84

3.10. The solubility of petroleum ether 40-60 °C (PE) and paprika extract in dimethylformamide (DMF) with variable water content at room temperature.

Although the solubility of petroleum ether in DMF decreased by 4% with 5 ml water content, there was only 1% decrease in the solubility of paprika extract in DMF (Table 27). A considerable increase of the water content up to 30 ml finally decreased the solubility of petroleum ether by 13 % and of that paprika extract by 3 % in DMF **(Table 27)**.

Table 27. The solubility of petroleum ether 40-60 °C (PE) and paprika extract in dimethylformamide with variable water content at room temperature.

Solvent composition	% Solubility of PE in DMF	% Solubility of paprika extract in DMF
DMF : PE : H_2O		
100 : 100 : 0	16	4
100 : 100 : 5	12	3
100 : 100 : 10	11	2.8
100 : 100 : 15	8	2.4
100 : 100 : 20	4	2.8
100 : 100 : 25	3	1.5
100 : 100 : 30	3	1.5

V. DISCUSSION

In the first part of this work, chili, paprika, rosmary, coriander and mace imports from India, South Africa, Morocco, Graneda and Spain were extracted conventionally with an acetone-water mixture. The multiresidue pesticide analysis showed that spices from China and Russia were free of pesticide contamination while Israelian and European spices were the most contaminated. Spices from African countries were of low pesticide contamination. Due to a high contamination level, the Spanish paprika narrowly meets the pesticide requirements. The contamination levels for the rest of the spices analyzed were within the maximum concentration limit allowed.

The Spanish paprika with the highest level of contaminants was later subjected to a supercritical fluid carbon dioxide extraction. A comparison between the levels of pesticides in CO_2 extracts and those of the conventional liquid solvent extraction was made and the qualities of both extracts were evaluated based upon their level of pesticide residues contamination. In CO_2 extracts a 7 to 53-fold enrichment of all the identified pesticides compared to that from the convention solvent extraction was observed. This result is in agreement with that of Aharonson et al [46]. In their report on SFE of sample inherent residues, they indicated that the SFE results were higher than that of traditional solvent extraction. Solvent extracts of paprika proveniences from South African, Israel and Spain contained α-endosulfane and β-endosulfane in concentration levels that were within the maximum limit. However, extracting the same samples with fluid CO_2, produced higher concentrations that were above the maximum tolerance limit of 0.1mg Kg^{-1} for spices [45].

The same effects were found for the concentration of pyrethroids in SFE extracts of South African, Israel and Spanish paprika. Conventional solvent extracts were also within the tolerance limit.

The results of this investigation underscore a fact already known in analytical SFE. Pesticide residues are extracted more effectively with fluid CO_2 than with liquid solvents [46]. Therefore, it can be said that the enrichment of valuable components is responsible for the high quality of SFE extracts, but the enrichment of contaminants undermines this high quality.

In the second part of this work the possibilities to achieve a below-tolerance-level reduction or an elimination of pesticide residues in extracts during supercritical fluid extraction were investigated. For this purpose, Spanish paprika that was found to have a high level of native pesticide contamination, was subjected to supercritical carbon dioxide extraction using variable extraction modes and parameters.

Due to overriding effects on the solubility of both the wanted and unwanted substances and on the sensory quality of the extract, temperature was used as a parameter to control fluid density at any given pressure.

At elevated temperature and pressure (70 °C / 350 bar), extract yield was generally high. It is no surprise that the industrial SFE employs this drastic parameters in order to maximize yield. However, this investigation has shown that although the yield was high at 70 °C, more pesticides residues were extracted as well.

Lowering the extraction temperature to 60 °C, resulted to non-considerable loss in yield, but to a below tolerance-level of lindane and to a further reduction of the already low concentrations of α- and β-endosulfane, permethrine and cypermethrine (Table 21). Although the concentration of deltamethrine was reduced, it was still above the tolerance level.

By furhter lowering the temperature to 30 °C, there was also a further reduction of the pesticides residue carry-over, an unchanged sensory value of the extract and only 0.53% loss

in yield. Although deltamethrine carry-over in the extract depreciated further, the concentration was still above the tolerance level because of its initially very high concentration. This can be explained by considering the fact that the concentration at the onset was excessively high. By decreasing the extraction temperature, generally a systematic reduction pattern was clearly noticed.

This investigation has shown that at 350 bar and at a mild temperature of 30 °C it is possible to obtain CO_2 extracts that have nearly identical sensory qualities (especially color) to those obtained at elevated temperatures (up to 70 °C) that can be used as colarant. At relatively low pressures (200 bar) and low temperatures (30 °C) a thick reddish extract with less quantities of pesticide residues was obtained.

In addition to temperature and pressure manipulation, a selective discrimination of the pesticides was also employed for the same purpose, by investigating the effect of water on the extractability of contaminants and the possibilities for a selective extraction of more or less water-soluble or water-modifiable pesticides and metabolites.

Water, as it is known, can modify the polarity of fluid CO_2 in such a way that polar substances are dissolved therein. The usage of water as modifier in conjunction with the extraction parameters of 70 °C and 350 bar had no considerable effect on the level of pesticide-carry over. However, water/watery extract was extracted or desorbed at the following parameters: 200 bar and 70 °C; 200 bar and 60 °C; 100 bar and 70 °C; 100 bar and 60 °C; 70 bar and 70 °C. The residue analysis of the extracted water/watery extract showed the presence of metribuzine. Traces of cypermethrine and deltamethrine were also identified in the watery extract.

The spike experiments with ^{14}C-terbutylazine confirmed the extractability of polar pesticides at (200 bar / 70°C) as well as with industrially practised ones (350 bar / 70 °C).

At 100 bar and between 60 – 70 °C, 4 g clear water were extracted or desorbed from the dried paprika samples. This means that half of the 8 % water content of the dried paprika sample were extracted under these conditions. However, Lehotay reported that according to Kuk et al [46] water is only 0.3 % soluble in supercritical CO_2. Therefore, this process by which more than 0.3 % water was obtained during the fluid CO_2 extraction can be characterized as a a mere physical process by which water is pressured out of the matrix. The extractability of polar water can also be attributed to a more apparent effect: higher temperature causes a higher vapor pressure for the less soluble components. The increased water vapor was then desorbed or pressured out of the sample by means of the extraction fluid. Extraction time in min is another most important factor that contributes to the desorption of pesticides into supercritical CO_2 extracs. It can be argued that the longer the extraction time at any given flow rate, the more of the later eluting pesticide residues are eventually extracted. To prevent this effect, an optimal dynamic extraction time of 30 min for a high yield with high quality was investigated.

Afterwards the extraction time for an exhaustive CO_2 extraction (5 g extract from 100 g paprika sample) was determined to be 45 min. Further extension gave no longer increase in yield. The reduction of the extraction time coupled with the reduction of the extraction temperature enhanced the the reduction of pesticide carry over into the extract

In the case of non-polar pesticides, where solubility is not a limiting factor, it has been argued that back partitioning due to adsorption onto the fine particles of the matrix could lead to less pesticides being partitioned into the extract [46, 92]. Therefore, 2 mm and also 0.8 mm particle size samples were employed to investigate the effect of a larger surface area as a possible means of avoiding the residues from being extracted.

But no considerable effect of particle size alone on the extractability of the pesticides at 70 °C and 350 bar could be noticed. A possible explanation might be that those pesticides, actually absorbed onto the available active surfaces, were desorbed by the drastic extraction parameters employed (70 °C, 350 bar, 45 min).

During all the supercritical fluid extraction experiments of paprika various coloration zones that ranged from pale to red were repeatedly observed in the remaining extraction cakes. This led to the conclusion that chromatographic separation of the extractants occurs even within

the extractor vessel (pale coloration in the inlet region of the vessel which means total extraction; reddish zones with growing color intensity towards the upper part and exit or upper part of the extraction vessel which means incomplete elution and separation). The degree of redness also depended on the extraction parameters employed and the duration of dynamic extraction.

These observations necessitated the suspicion of the occurance of a phenomenon termed here as “Simultaneous fluid extraction and chromatographic separation (SFECS)” within the extraction vessel. In a control experiments a narrow chromatographic vessel containing silica gel was placed just after the extractor vessel. The separation of paprika colorants on this column into zones of yellow, orange, and red color zones was comparable to the separation obtained on a normal GPC column with polystyrol beads. Similar observation were made when the aforementioned by-passed metallic chromatographic vessel was densely packed with paprika extraction cake (0.8 mm particle size). Further experiments were conducted to compare the pesticide elution patterns of a normal GPC column and on a column with paprika extraction cake particles as column material. This column is termed here as “paprika permeation chromatographic column” (PPC). On a PPC column the pesticides were eluted into the fractions 100-190 ml. The elution pattern correlated with the “collect fraction” of GPC. Therefore, the occurance of a zonal chromatograhpy in which paprika colors are separated in different fronts is thus established and the discrimination of pesticide residue from lipids and other macromolecules in manner that is comparable to the size exclusion chromatography was thus established. Hence it has been established that the by-passed metallic chromatographic vessel with paprika cake as column material discriminate pesticide from lipids and other macro compounds during fluid CO_2 extraction by a way of size exclusion chromatographyic principle.

Furthermore, the sharpness of the separation zones is attributable to the vessel geometry (1cm i.d. $\times$ 9.5 cm height of bed; chromatographic vessel and 5 cm i.d. $\times$ 10 cm height of bed; main extractor vesse). According to Furton et al [46] higher extraction efficiencies (exhaustive extraction) of PCBs were obtained with short, broad extraction cells because the fluid linear velocity decreases as the cell diameter increases. A decrease in fluid linear velocity (decrease

in flow rate) results in increased extraction recoveries (as a result of an extended contact between the supercritical fluid and the sample). Since the aim of this investigation was to achieve a very low to zero recovery of contaminants, an ideal cell geometry will be the one that supports the extractions of wanted extractants while limiting the extractability of contaminants. Andersen et al [146] reported an improved extraction recovery for long-chain hydrocarbons (*n*-octadecane and *n*-eicosane) by decreasing the extraction cell diameter (which means increasing the linear velocity). They explained the increase to be due to a better mass transfer within the cell due to an increase in turbulence in the fluid flow pattern inside the extraction cell. Therefore, in this investigation, a combination of a narrow-bore chromatographic column and a wide-bore extraction vessel was employed based on this theory. This leads to a good extraction of sample components and a simultaneous on-line separation of lipids and pesticides with a favored early elution of macro molecular lipids.
Stalling et al[12]designed an SFE-GPC equipment and Taylor et al [45] actually performed a chromatographic separation based upon this concept by employing supercritical CO_2 as a mobile phase. The PPC experiments performed in this research work were also a mimick of GPC separation with a metallic column that could withstand high pressure of SFE for the decontamination of a consumable extract. Paprika extraction cake particles such as the GPC bio-beads are macromolecular, hence similar chromatographic separation was obtained.

Therefore, subsequent SF CO_2 extraction was carried out via a metalic chromatographic column coupled to the extractor vessel. The PPC column was used as a confirmation step for the observations made in the same manner by Furton et al [46]. They confirmed the results of their experiments on the extractability of PCBs that were spiked onto octadecylsilane (C_{18}) sorbents by correlating them with chromatographic data obtained in experiments with similar sorbents.
The simultaneous fluid extraction chromatographic separation (SFECS) explained above, was utilized for the reduction or elimination of pesticide-carryover. During SFE experiments, a high sample bed in the extraction vessel, fine particle sizes, high packing densities and, most importantly, extraction temperature, extraction pressure and extraction time, were chosen and

manipulated to guarantee the size exclusion chromatographic principle inherent in GPC with the aim of achieving pesticides residue discrimination.

By all considerations, focus was kept on ensuring that the sample treatment before and during the extraction results to a product that is suitable for human consumption. Therefore, the use of modifying, ion-pairing, derivatizing agents and salts for the extraction of polar substances as used in the analytical-scale environmental SFE were not employed, since their usage would have entailed either the introduction of hazardous chemicals or resulted to negative effects on flavor, color, taste or consistence of the end product (theoretically, polar compounds can be extracted with a non-polar fluid CO_2 only by means of a modifier). In this investigation however, metribuzine, a polar substance, was extracted without the addition of a modifier. This result is in contradiction to the theory of non-extractability of polar substances with fluid CO_2. The non extractability of polar substances by non-polar medium such as fluid CO_2 is more pronounced in a simple binary or ternary system with fluid CO_2 as a component. However, the more complex the system is, the more the likelyhood that some compound could have modifying effect on the fluid CO_2 phase that polar compounds are then extractable.

This is the case with the SFE of paprika. It is a complex system that is composed of fluid CO_2, water vapor, lipids, fatty acids, long chain alcohols (waxes), flavor compounds, etc.

In as system such as this some components have the ability to go into some sort of bondage with CO_2 molecules. Lipophilic substances acting as surfactants link polar with non-polar compounds) thus modifying the polarity of CO_2 that also polar substances are extracted (e. g. metribuzine).

A possible explanation for the extratability of metribuzine, terbutylatrazin and some other polar substances, against the rule though, could be that non-polar sides of waxes and fatty acids are attached to CO_2 molecules, while the polar side with alcohol group or acid group to are attached to metribuzine and water molecules via a hydrogen-oxygen-hydrogen-bond. Therefore, within this state of fluid CO_2 the extraction of metribuzine or any other polar and semi-polar substance may be possible.

In the remaining part of this work a countercurrent chromatography-based cleanup method for the multiresidue analysis of lipophylic pesticides in lipophylic plant extracts was developed as a solution of the major analytical problems encountered in these investigations: the GC-ECD analysis of non-polar pesticide residues in paprika and mace extracts with more than 85 % lipid content. The quantitative determination of pesticides in such fatty samples is executed by residue analysts with varying efficiencies. The efficiency of the clean-up steps plays an important role in the quality of the results obtained. In many cases therefore, it is necessary to have additional steps, especially if the polarities of plant components in a complex matrix do not allow certain clean-up steps such as gel permeation chromatography (GPC) for an interference-free GC analysis. For example, the consistence of paprika extract is modified by waxes and other components in addition to those with low molecular weight such as flavor compounds in such a way that the official German analytical method of the "Deutsche Forschungsgemeinschaft" (DFG S19-Method) [47] with GPC as a clean-up step proved to be insufficient for an interference-free GC analysis.

The search for an optimum clean-up method for paprika extracts led to the experiments with counter current chromatography (CCC) [125-149]. Here a chromatographic clean-up step with a partial ***ternary*** solvent system (acetonitrile/petroleum ether 40-60 °C/water) allowed the partitioning of pesticides of different classes. Since petroleum ether is partially soluble in acetonitrile, the lipid fraction, which is completely soluble in petroleum ether is invariably partially soluble in acetonitrile. Therefore, a reduction of the solubility of petroleum ether in acetonitrile should equally mean a reduction of the solubility of lipids in acetonitrile. This reduction was achieved by the addition of water to the solvent system thereby raising the surface tension of the two phases by increasing their polarity differences. Unlike the

partitioning by means of a separatory funnel, that requires series of successive partitioning processes, the high speed multi-layer coil countercurrent chromatography (HSMLCCCC) proved to be more efficient in a number of ways: a long column means a high number of theoretical plates guaranteeing an efficient partitioning; the high speed involved here ensures that the partitioning is accomplished in a relatively short period, the large sample capacity ensures the clean-up of large sample volumes, especially for the detection of trace substances

in the lower ppb or ppt range. The absence of a solid phase such as aluminum oxide reduces the danger of low recovery due to irreversible adsorption on the stationary phase.

The preliminary results of the experiments with pesticide standards have been published in the 245th proceedings of the American Chemical Society [158]. However the analysis of real paprika extract showed that due to the relatively high distribution of paprika extract in 5 % aqueous ACN, the direct GC analysis of CCC was done with interefences. Although the interference was less if compared to GPC eluate. However the elution CCC-ACN eluate on sulfuric acid-treated silica gel column resulted to a clean sample suitable for DC analysis. What can be deduced from this experiment thus far is that a multiclass pesticide analysis can be best accomplished by employing a substance-group-analysis (organohalogens on the one hand, organophosphor, triazines and pyrethroids on the other hand). Accordingly, an aliquot of a sample that representing each of the substance group was partitioned into aqueous ACN (5 % water for the organohalogene and 15-30 % water for triazine, organophosphor and pyrethroids). The organohalogene subsatnce group was futher cleaned by an acid digestion by means of sulfuric acid-treated-silica gel column. The search for a better solvent system (with a favorable partition coefficient especially for organohalogenes) continued and lead to the experiment with dimethylformamide (DMF).

The solubility of petroleum ether 40-60 °C (PE) and paprika extract in aqueous DMF at room temperature suggests that a far more better clean-up can be achieved with a DMF/PE solvent system. However, the corrosiveness of DMF on teflon column restricted its applicability at this stage of countercurrent chromatography. The elimination of this problem that is associated with the aggressiveness of DMF will involve a partial or total reconstruction of the countercurrent chromatographic equipment. In pre-experiments a selfmade stainless steel column was used instead of the PTFE-column). This was a novel introduction of metal column in countercurrent chromatography. The continuation of this experiment was accomplished in the proceeded postdoctoral research project and therefore was beyound the scope of a doctoral research work.

VI. CONCLUSIONS

The results of the pesticide residue analysis showed that spice extracts from conventional liquid solvent extraction are less contaminated than those of fluid CO_2. While the contamination level in the liquid solvent extracts are in the lower ppb level and hence far much below the tolerance level, there was up to 53-fold enrichment of pesticide residues in extracts from supercritical carbon dioxide extraction. This invariably means that the extracting power of fluid CO_2 is up to 53 times higher than that of liquid solvent used. The enrichment of pesticides above tolerance level in SFE extracts emphasizes the need to include ”pesticide contamination level” to the criteria for quality assessment of SF extracts and the need for an integrated quality assurance in SFE technology of food and pharmaceutical production with emphasis on production of pesticide-free fluid CO_2 extracts.

Due to the problem of contaminants enrichment in SFE extracts, care must be taken using these extracts to spice up meat and dishes. However, dilution effect of the pesticides is more than sufficient to reach concentrations below tolerance level if the extracts are further used as raw material in the production of food and pharmaceuticals.

This investigation has shown that the production of high quality SF CO_2 extracts free of certain pesticide residues or with concentrations that are below tolerance level is also feasible.

To achieve this, the following steps were applied:

- If the raw material originates from a region of high pesticide usage or if a control analysis of the raw material prior to supercritical fluid extraction shows indications of pesticide contamination, then instead of the normally applied exhaustive extraction in the industrial SFE, a modified SF extraction procedure should be carried out with emphasis on elimination or employment of a selective extraction as a first extraction phase to eliminate rest water that could modify the polarity of fluid CO_2 and pesticides or their metabolites thereby

- making them soluble in fluid CO_2. In this case, separate waste pathways (by-passed chromatographic vessel) and a second separator for the water extract are recommended to avoid a high contamination of extracts and to save the time spent for cleaning the separator vessel before reuse.

- Actual elimination of pesticide residues and a reduction below the tolerance level was attained for all but one pesticides with practically no loss in yield and no negative sensory effect at temperatures between 30-50 °C and a pressure of 350 bar. Therefore, moderate temperature and high pressure should be the parameters of choice to achieve both high yield with less or no contaminants

- In addition to high extraction pressure and moderate extraction temperature the following parameters, when employed interrelatedly, enhance a selective extraction of lipids and wanted extractants of paprika matrix:

By ensuring that the sample are of fine particle size (0.8 mm or less instead of 2 mm) a larger surface area for the interaction of the residues with the active sites are created. There are more than enough reports on the relationship between surface area and the residue retention on samples. Due to the increased interaction between the residues and the sample materials, the desorption rate is expected to be lowered which in turn should delay or prevent the extraction of pesticidesthereby favoring the discrimination of residues from the lipids.
The effect of vessel geometry on the extraction efficiency are widely reported. The extraction vessel used here can be described as moderately slender and long. This geometry facilitated a dense package and a sufficient bed height of the sample material and was actually the first step in introducing a zonal chromatographic influence during the extraction process, in which lipid eluted first and eventually delayed the carry-over of pesticide residue into the extract.
The introduction of an interface of the extraction vessel and a paprika permeation chromatographic column should be a method of choice for an on-line discrimination of pesticide by means of size exclusion chromatographic principles.

The dynamic extraction time of 30 min should be enough for a good yield. The extraction time should not be overstretched unnecessarily to avoid an eventual desorption or carry over of pesticides.

Analytically, the different elution patterns obtained on the GPC highlighted the different physical and chemical composition of these spices and medicinal useful plants. Taking these differences into consideration means that there is no universal clean up method for all spice and medicinal plants, hence suitable GPC parameters (dump, collect volume, possibility of an additional clean up step) for each plant extract should always be determined prior to executing the main analyses.

Conclusively, although the "DFG S19-Methode"(the German official method of pesticide residue analysis) is a very good method for the analysis of pesticide residues in *animal lipids* such as butter, it has however proved ineffective for the analysis of lipophylic pesticide residue in *plant extracts* such as those from paprika, mace and other plants with high lipid content. The DFG S19 method employs the GPC as a clean-up step, which was rather ineffective in discriminating low molecular weight pesticide residues from low molecular weight flavor substances and fatty acids that are present in paprika extracts. Due to the co-elution of the substances, the determination and quantitation of the mini silica gel fractions of all PCBs was practically impossible and the determination of some of the early eluting organohalogens, triazines and organophosphates was very problematic and painstaking. A far much effective clean-up method with subsequent less problematic GC analysis was achieved with a clean-up by means of high speed multilayer coil countercurrent chromatography with aqueous acetonitrile and petroleum ether as solvent system. However this solvent system gave a less favorable partioning for the organohalogens.

Further experiments indicated that yet much more better separation efficiency of the pesticides from lipid interferents was achieved with a DMF / PE solvent system. However, the advantages of this solvent system can only be fully utilized by finding a solution to the problem of PTFE (Teflon) coil permeation associated with DMF. The with teflon column permeation was solved by a novel introduction of metal column in countercurrent

chromatography. The experiment with DMF solvent system and metal column is to be concluded as a postdoctoral research work and will be published later.

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VII. Appendages:

(I) SFE 350 bar at 70 °C (Spain-paprika-extract)

(FRC:0)

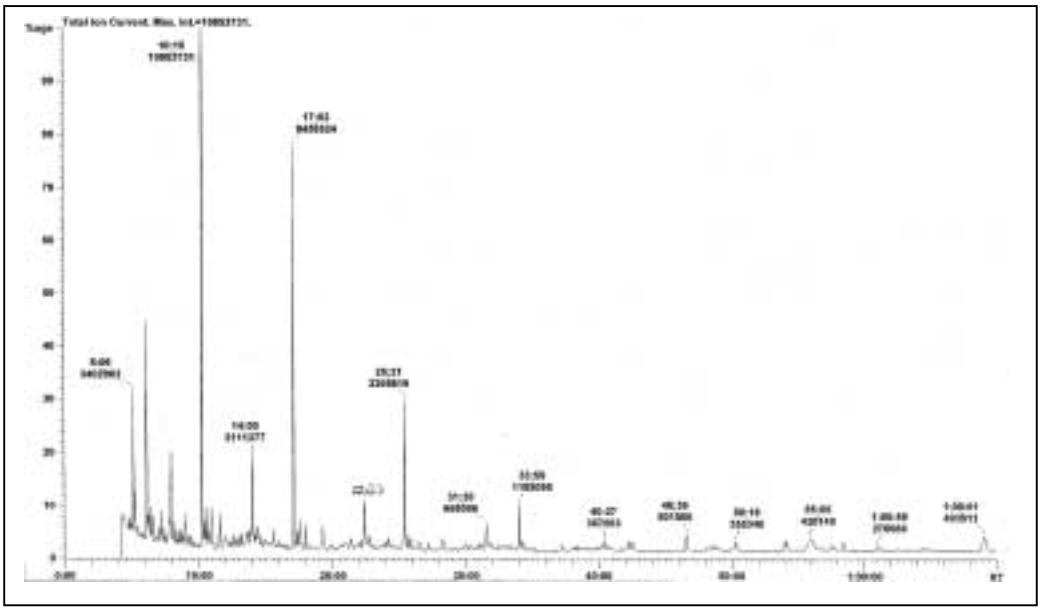


Figure 1a. Silica gel fraction 0a: Total Ion current showing long chain Alkanes, Alkenes interferences. Flagging = Rt & Int.

Substance	Rt	Substance	Rt
135-91;150	5:06	Heptadecane:	11:31
Tetradecene:	6:06	Heptadecane:	14:00
Tetracane:	6:10	Octadecene:	17:00
Pentadecane:	7:58	Dococene:	33:59
Hexadecene:	10:15		
Hexadecane:	10:25		

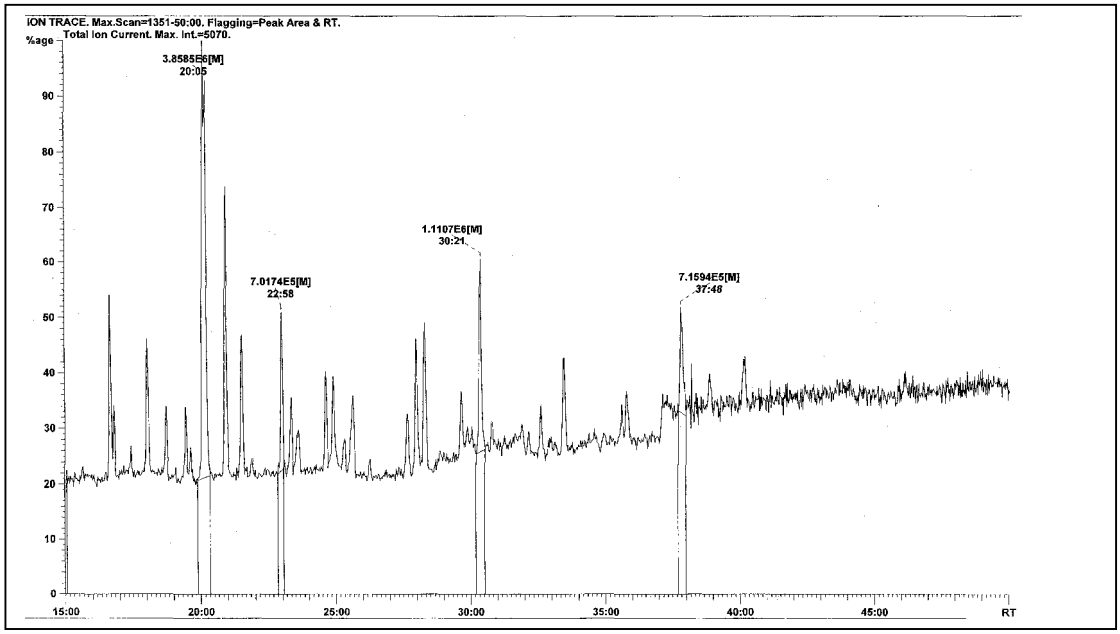


Figure2: Silica gel fraction 0a: Total Ion Current showing Polychlorbiphenyls (PCBs Flagging = Peak Area & Rt.

Substance	Rt	Substance	Rt
Trichlorbiphenyl (PCB 28):	20:05	Pentachlorbiphenyl(PCB 101):	30:21
Tetrabiphenyl (PCB 52):	22:58	Hexachlorbiphenyl (PCB 52):	22:58

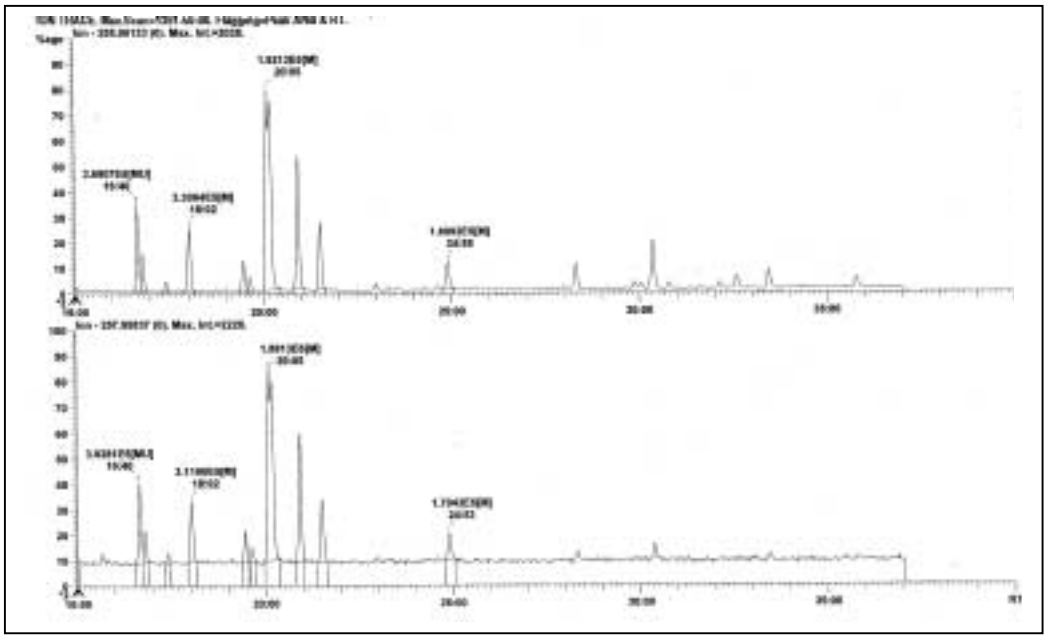


Figure 3. SIM: Ion trace for Trichlorbiphenyl (PCB Nr 28) showing ion 255.96 in sample and 257.95 as a library possible match. Flagging = Peak Area & Rt.

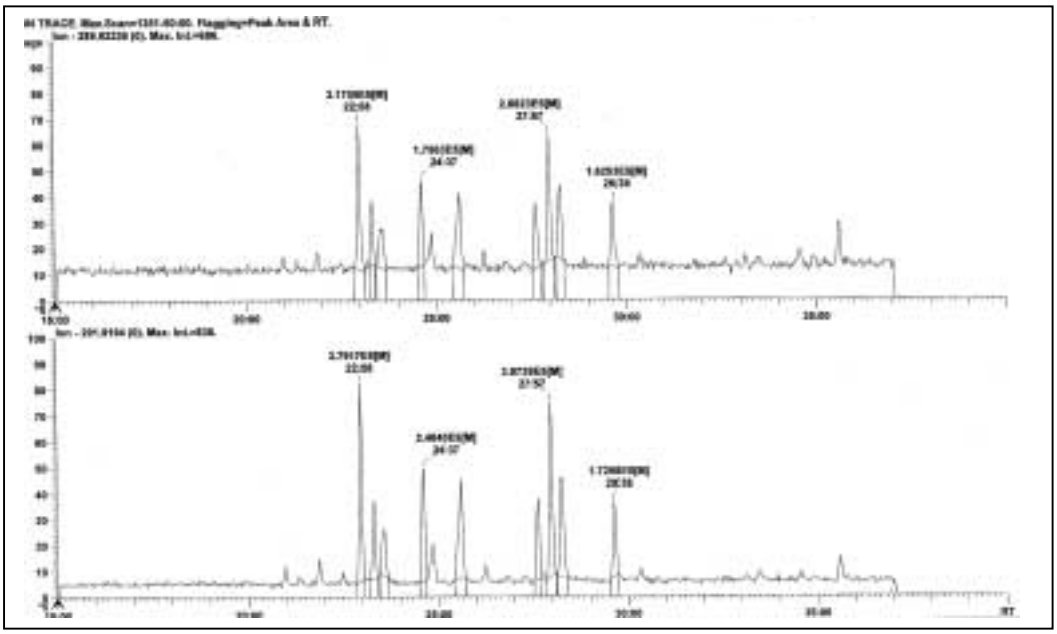


Figure 4. SIM: Ion trace for Tetrachlorbiphenyl (PCB Nr 52) showing ion 289.92 in sample and 291.91 as a library possible match. Flagging = Peak Area & Rt.

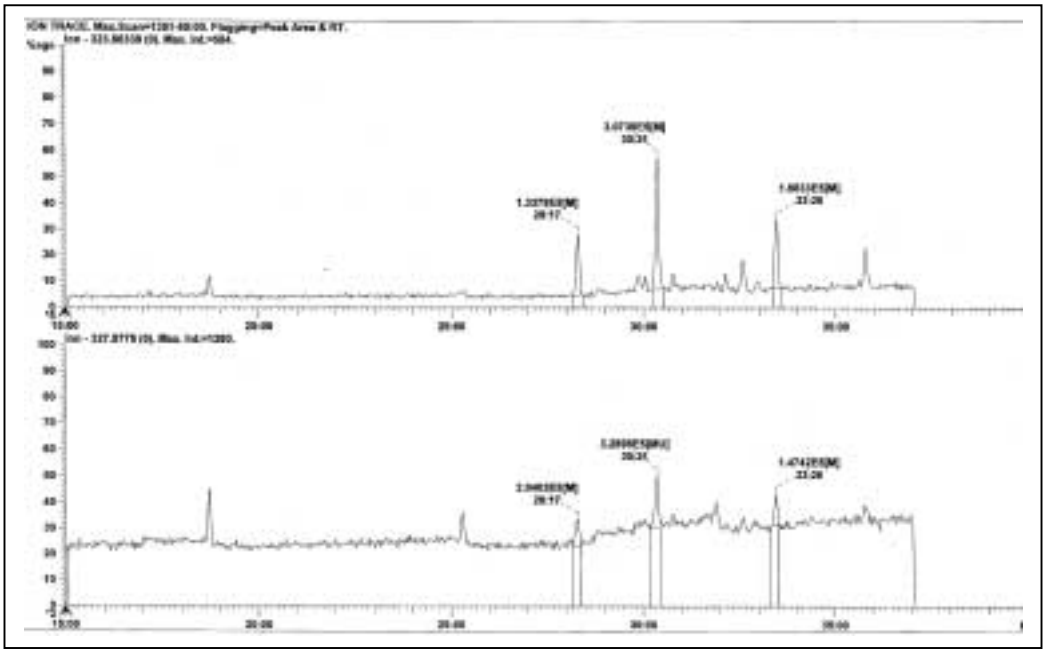


Figure 5. SIM: Ion trace for Pentachlorbiphenyl (PCB Nr 101) showing ion 232.88 in sample and 327.88 as a library possible match. Flagging = Peak Area & Rt.

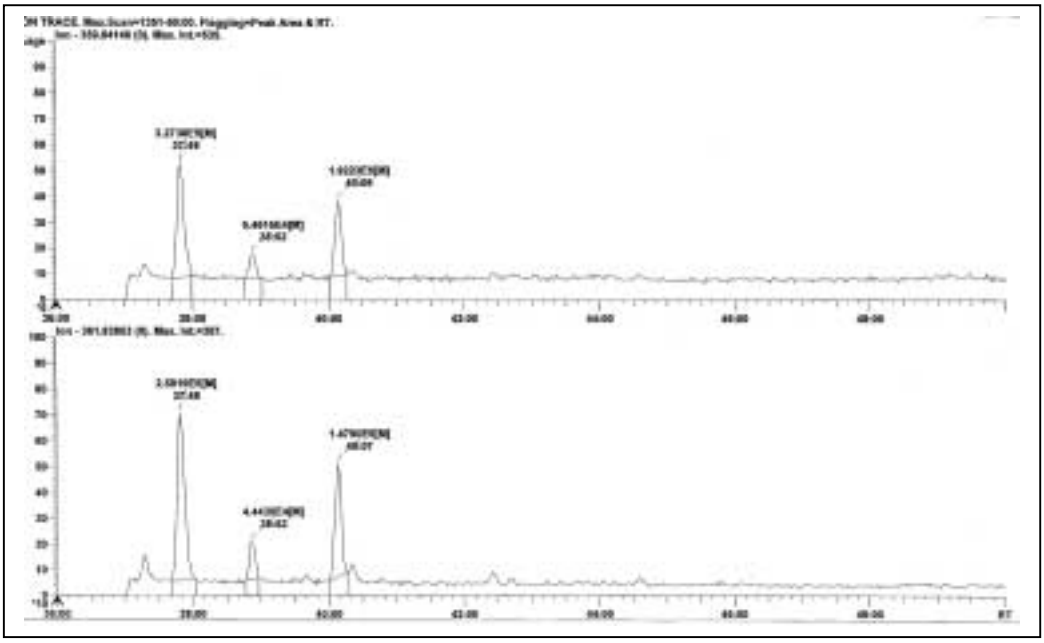


Figure 6. SIM: Ion trace Hexachlorbiphenyl (PCB Nr 153) showing Ion 359.84 in sample and 361.83 a library possible match. Flagging = Peak Area & Rt.

Fraction I

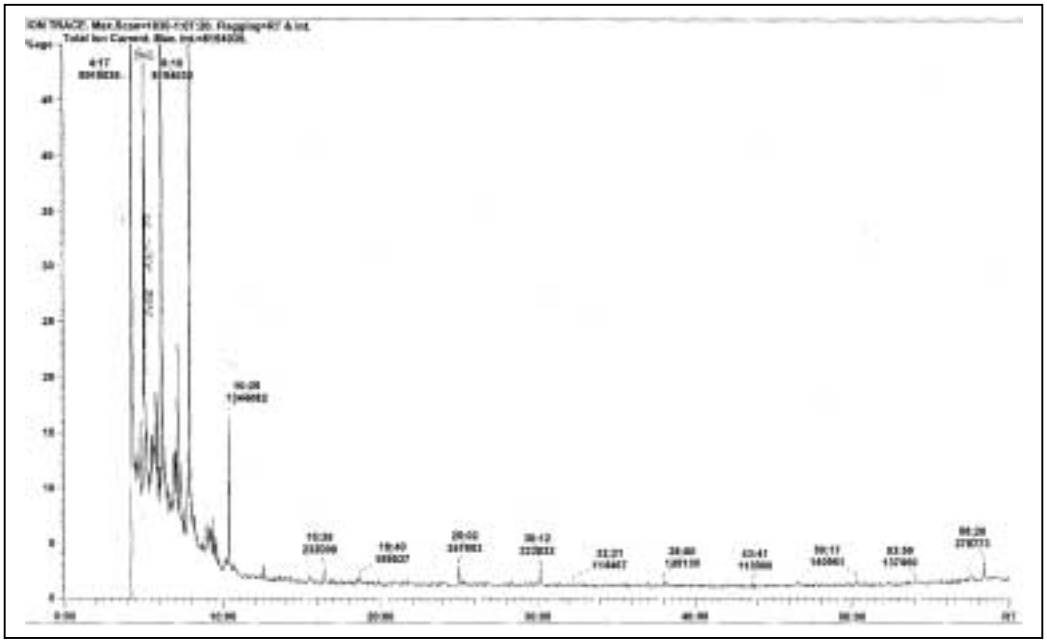


Figure 7 Silica gel fraction 1: Total Ion current showing primarily long chain alkanes Interferences.

Substance	Rt	Substance	Rt
Cyclohexane:	4:145:06	Tetradecane:	6:10
Tridecane:	5:02	Pentadecane:	7:56
135-77-162:	5:06	Hexadecane:	10:25

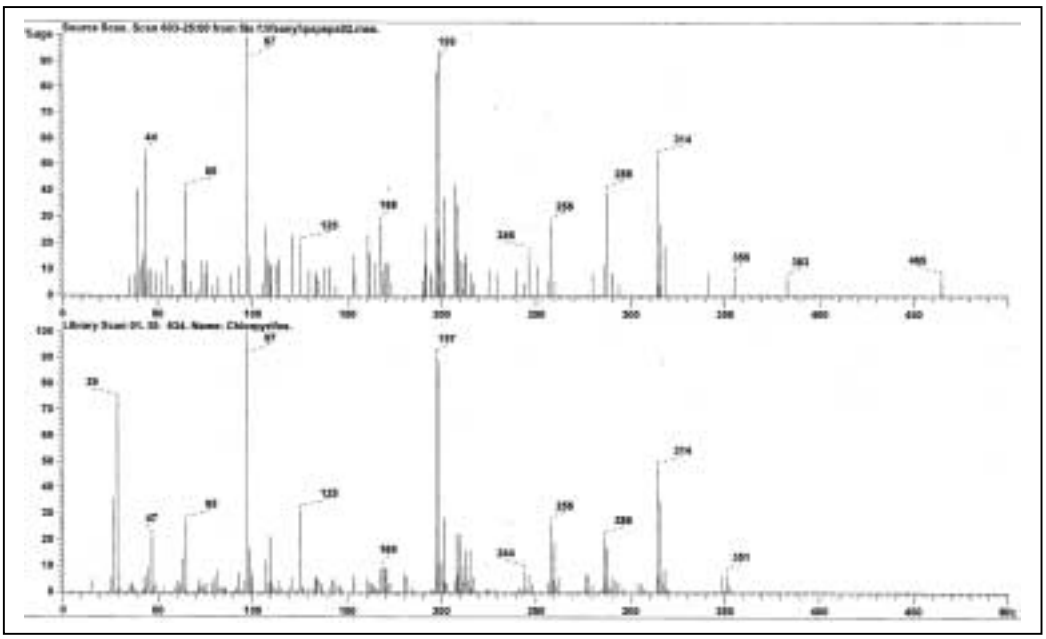


Figure 8. Silica gel fraction 1. Upper Scan graph: Chlorpyriphos ethyl; lower scan graph: Library possible match. Flagging = m/z

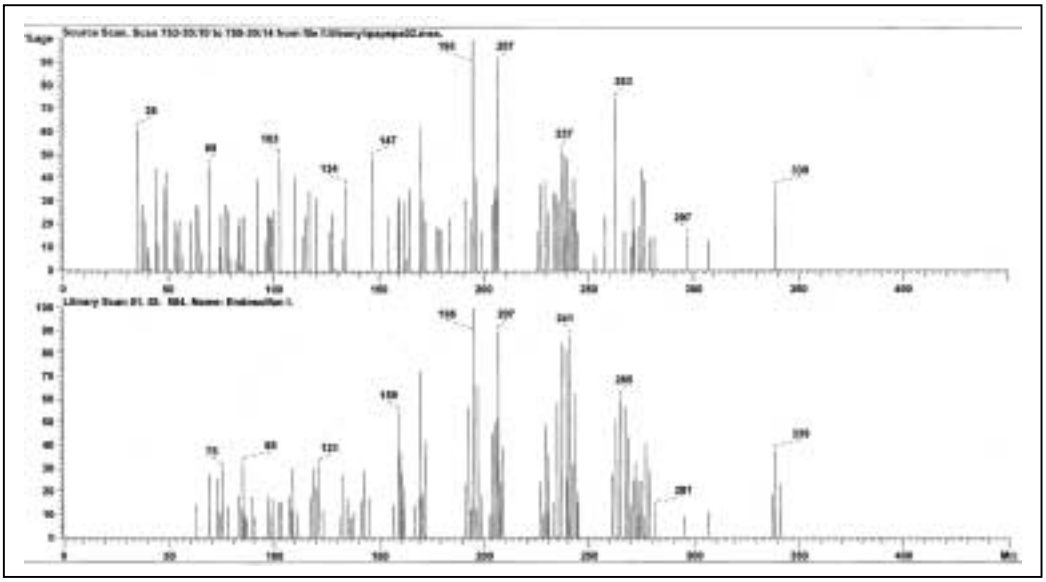


Figure 9. Silica gel fraction 1. MS: Upper Scan graph: α-Endosulfane; lower scan graph: Library possible match. Flagging = m/z

Fraction 2.

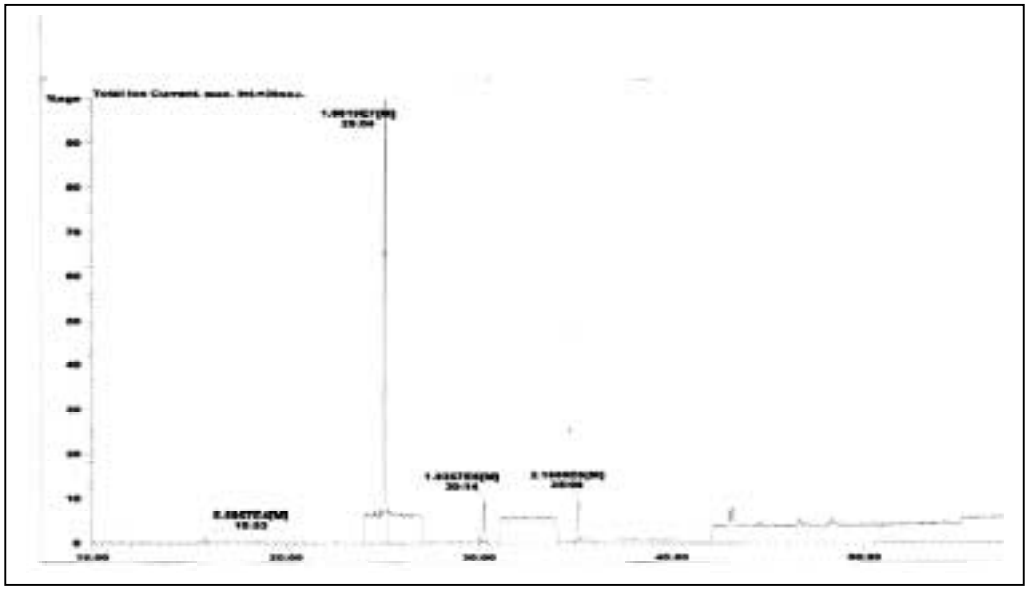


Figure11. Silica gel fraction 2 Ion trace: Total Ion current;
MS- Methods: Iμl spll. 50/1-70-150/5-2-260; *Rt* 15:53 = Lindane; 25:04 = Chloropyriphos
30:14 = α-Endosulfane; 35:08 = β-Endosulfane

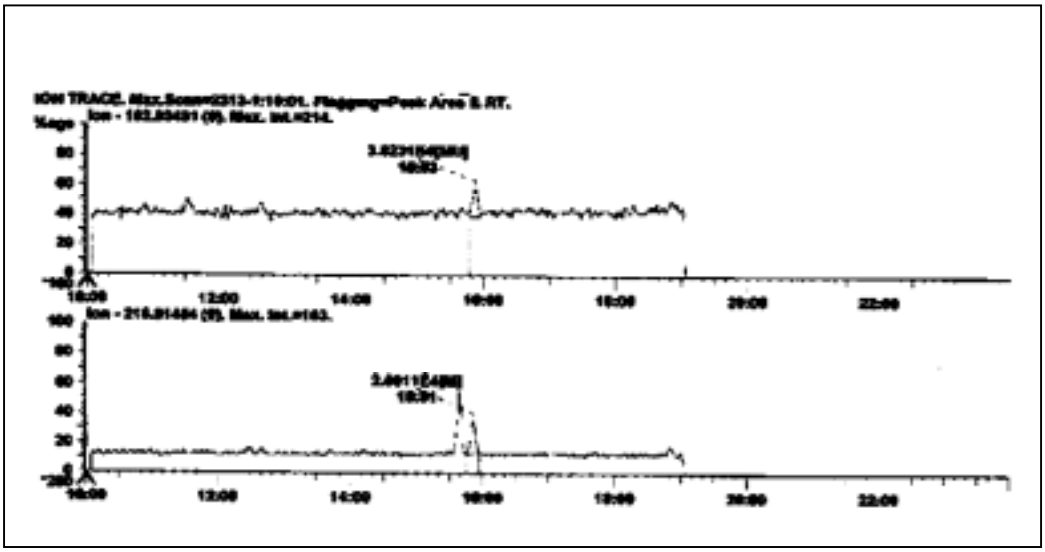


Figure12. Silica gel fraction 2: Ion trace: Total Ion current showing Ions 182.93 and 216.91 γ-Lindane; Cyclohexane 1,2,3,4,5,6-hexachloro (MW: 288)

$$288 \xrightarrow[-Cl]{} 253 \qquad C_6H_6Cl_5 \qquad = Rt{:}15{:}53$$

$$253 \xrightarrow[-HCH]{} 217 \qquad C_6H_5Cl_4 \qquad = Rt{:}15{:}51$$

$$217 \xrightarrow[-HCH]{} 181 \qquad C_6H_4Cl_3$$

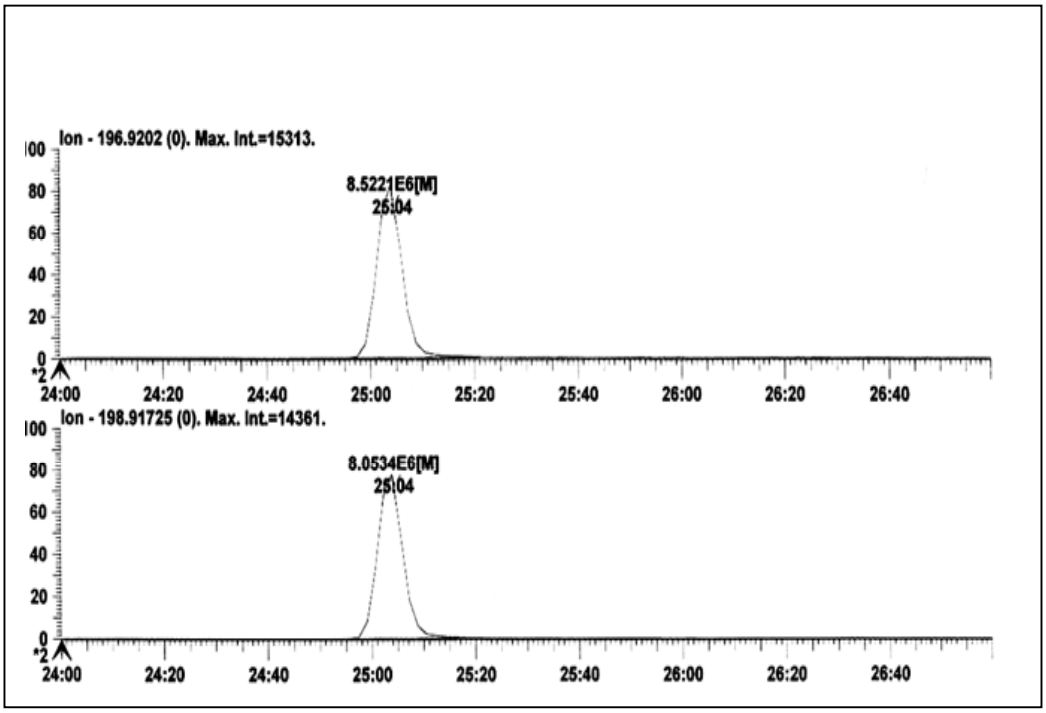


Figure 13. Silica gel fraction 2: Ion trace: Total Ion current showing Ions196.92 and 198.91 Chloropyrfos; MW: 349; Formula: $C_9H_{11}Cl_3NO_3PS$

m/z 349 $\xrightarrow[-Cl_3]{}$ 244; 244 $\xrightarrow[-C_2H_5]{}$ 215; 215 $\xrightarrow[-CH_4]{}$ 199 = *Rt*:25:04

199 $\xrightarrow[-H_2]{}$ 197 = *Rt*:25:04

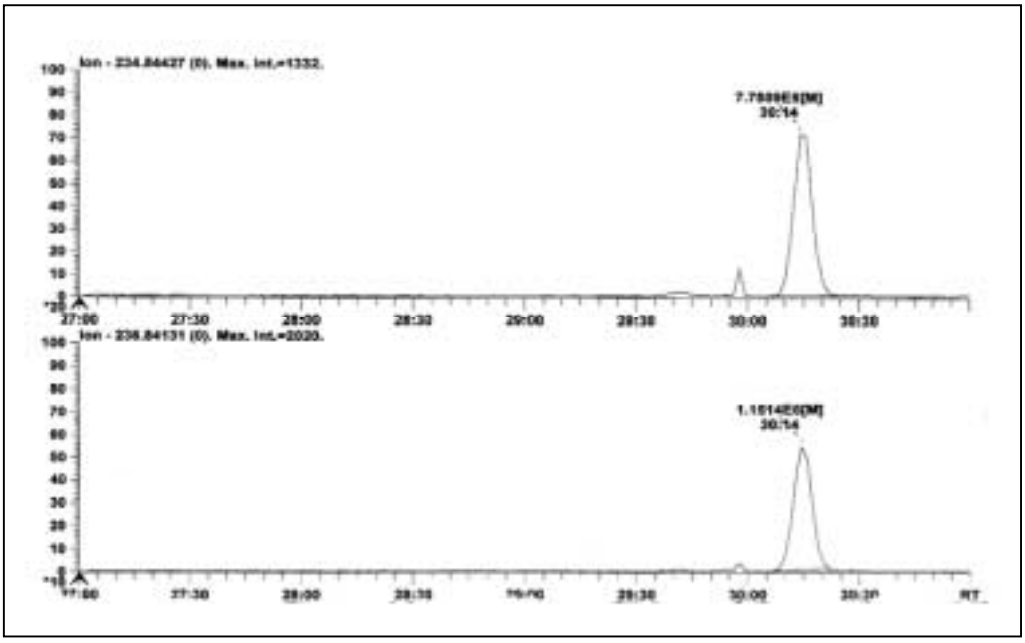


Figure 14. Silica gel fraction 2: Ion trace: Total Ion current showing Ions 234.84 and 235.00
α-Endosulfane; MW: 404; m/e 404 $\xrightarrow[-C_4H_6SO_3]{}$ 236 C_5Cl_5 = *Rt*:30:14

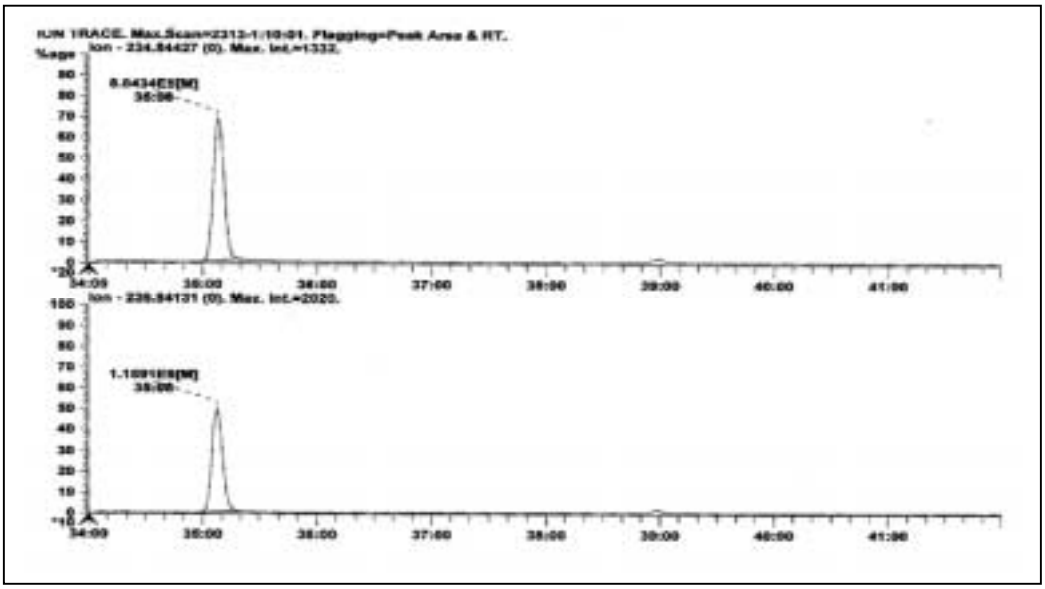


Figure 15. Silica gel fraction 2: Ion trace: Total Ion current showing Ions 234.84 and 236.84
β-Endosulfane.
MW: 404; m/e 404 $\xrightarrow[-C_4H_6SO_3]{}$ 236 C_5Cl_5 = *Rt*:35:08

(Frc 3)

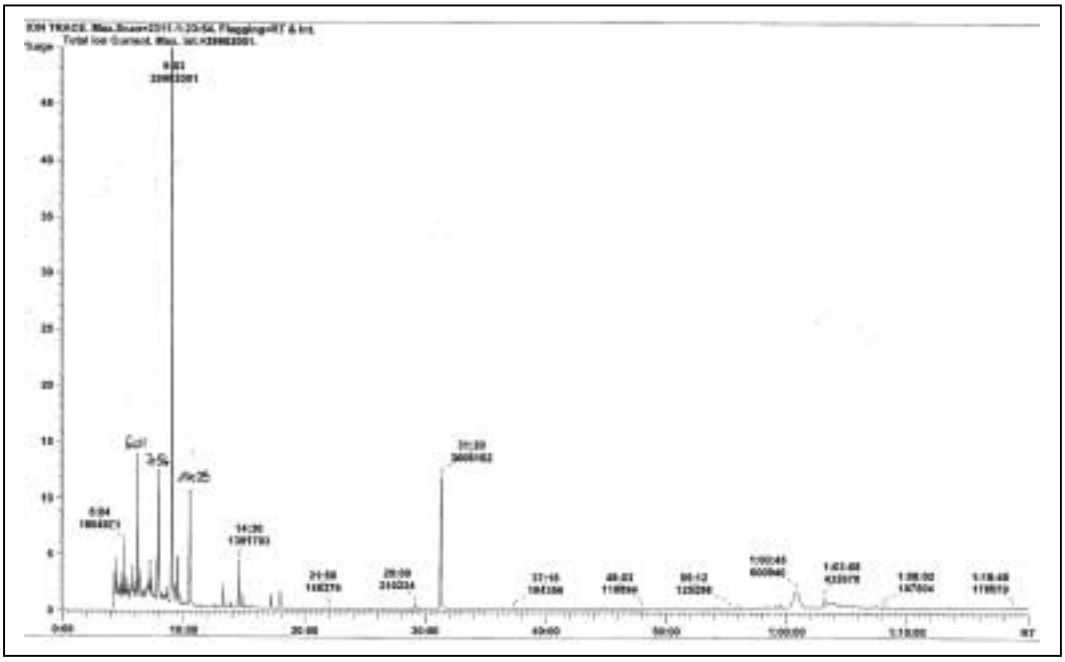


Figure 16. Silica gel fraction 3: Total Ion current showing primarily long chain alkanes Flagging = Rt & Int.

Substance	Rt	Substance	Rt
135-150,91:	5:06	Hexadecane methylate:	22:26
Tetradecane:	6:11	Linol methylate:	29:40
Pentadecane:	7:54	Octadecene methylate:	29:57
Myristicine:	8:36	Octadecane methylate:	31:07
Hexadecane:	10:36	57;112,71;70;43-256;239:	46:35
Tetradecane methylate:	14:26	57;112,71;70;43-284-267:	54:09

Fraction 4

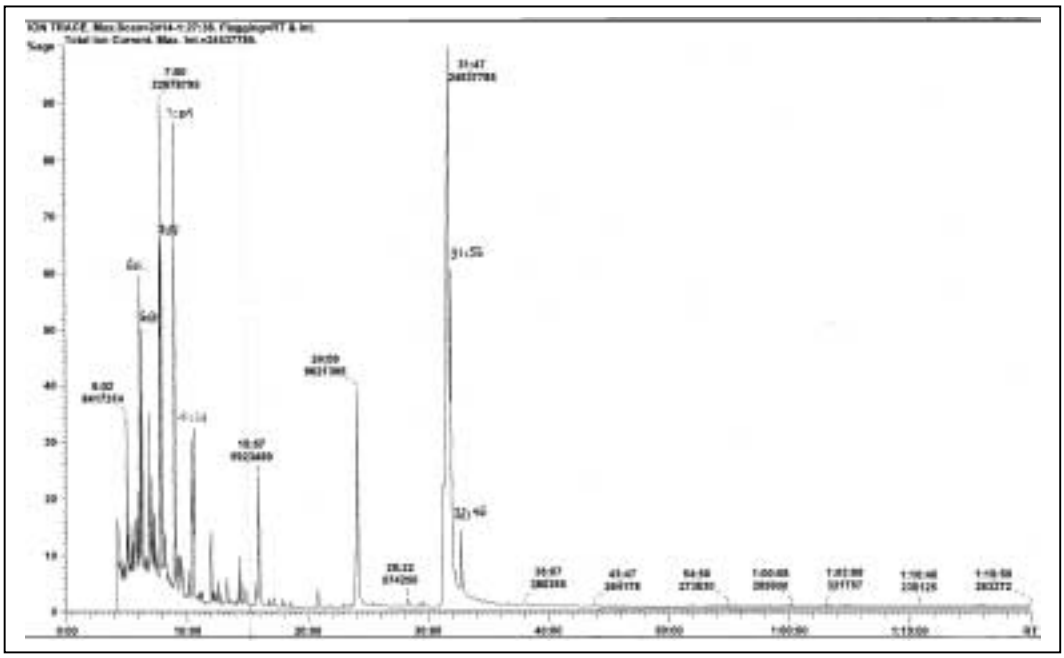


Figure 17. Silica gel fraction 4: Ion trace / Total Ion Current: Total Ion current showing primarily long chain alkanes and long chain fatty acids

Substance	Rt	Substance	Rt
Tetradecane:	6:09	150;179:	12:00
Vanillin:	6:20	43;111;178-196:	15:57
Acetovanillon:	7:50	Hexadecanic acid:	24:09
Pentadecane:	7:54	Linolic acid:	31:56
Hexadecane	10:24	Petroselin acid:	31:56
151;43;110;194:	10:35	Octadecanic acid:	32:46

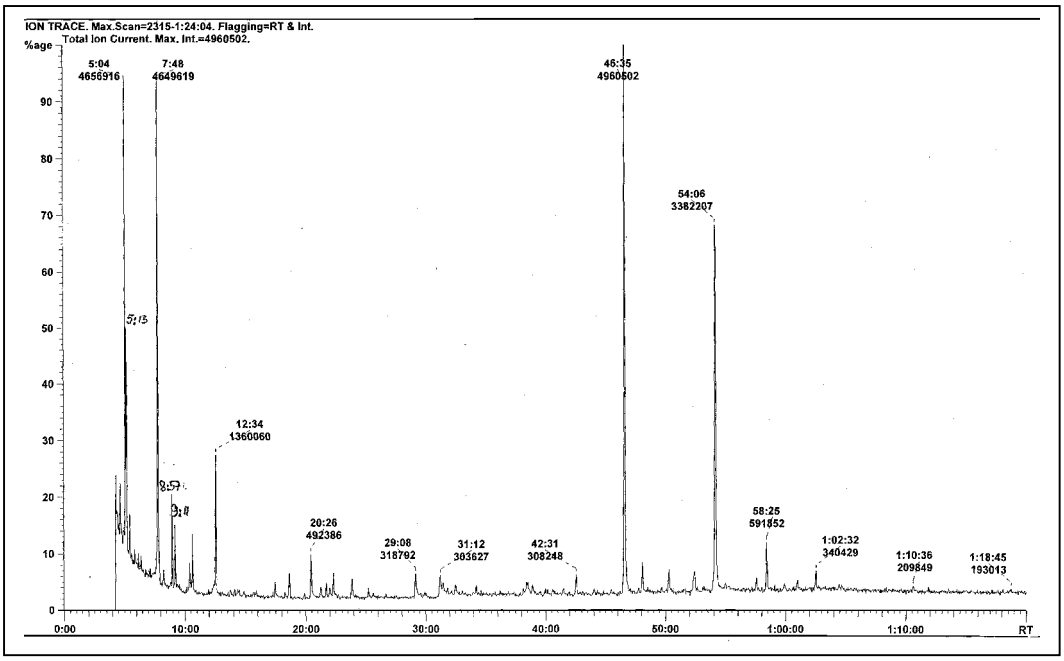


Figure 18. Silica gel fraction 5: Ion trace / Total Ion Current: Total Ion current showing primarily long chain alkanes and long chain fatty acids

Substance	Rt	Substance	Rt
135-150;91:	5:04	Hexadecanol:	20:26
Piracetane:	5:13	Octadecanol:	29:08
73:	7:48	Tetracosan:	42:31
Dihydroactinidiolid:	8:57	Hexadecanic acid octylester:	46:35
Elemicin:	9:11	Octadecanic acid octyletser:	54:06
Tetradecanol:	12:34		

(I) Convention solvent extraction (Spain-paprika-extract)

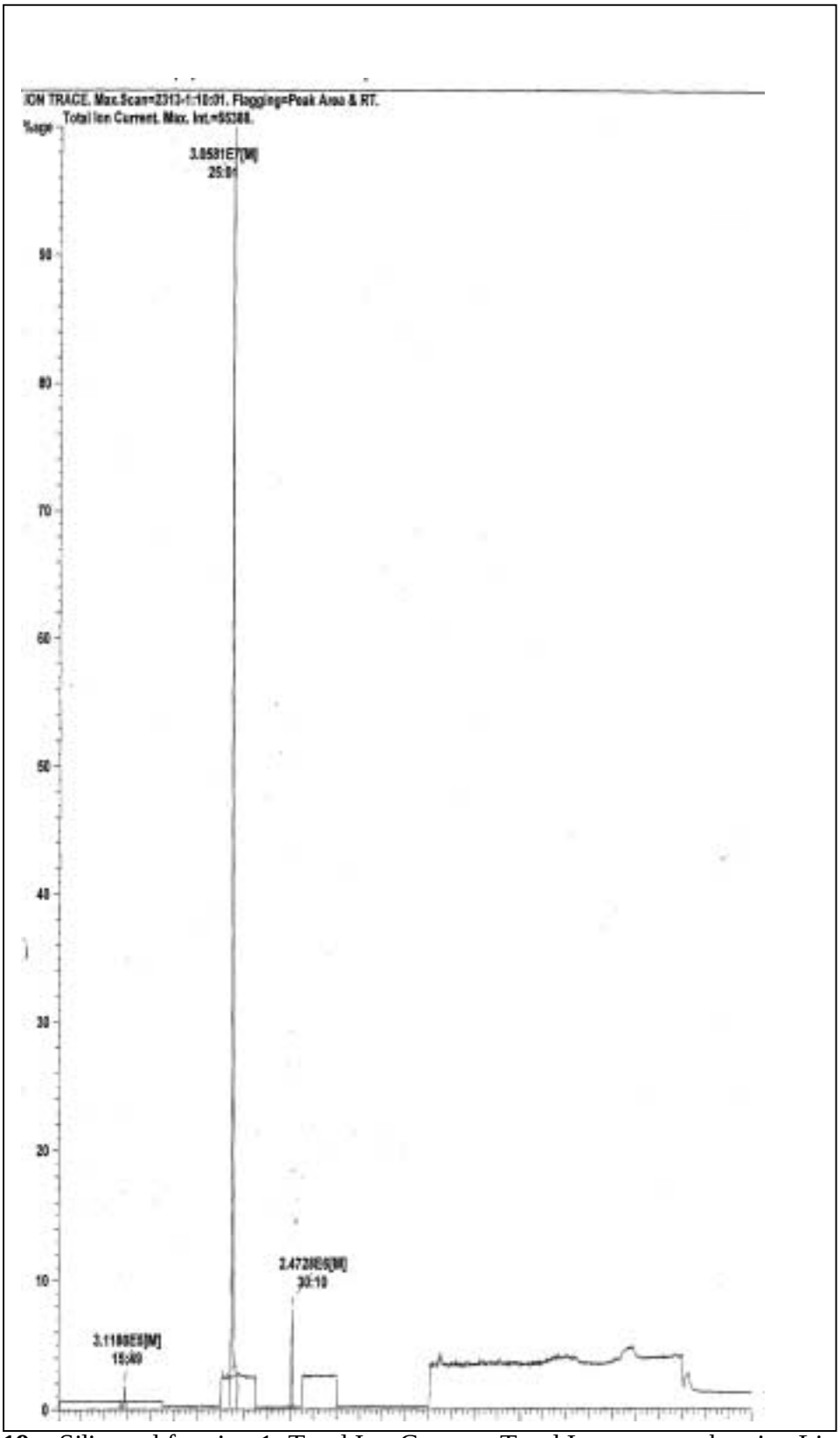


Figure 19a. Silica gel fraction 1: Total Ion Current: Total Ion current showing Lindane (Rt: 15:49), Chlorpyriphos ethyl (Rt: 25:01) and α-Endosulfane (Rt: 30:10)
Flagging = Peak Area & Rt

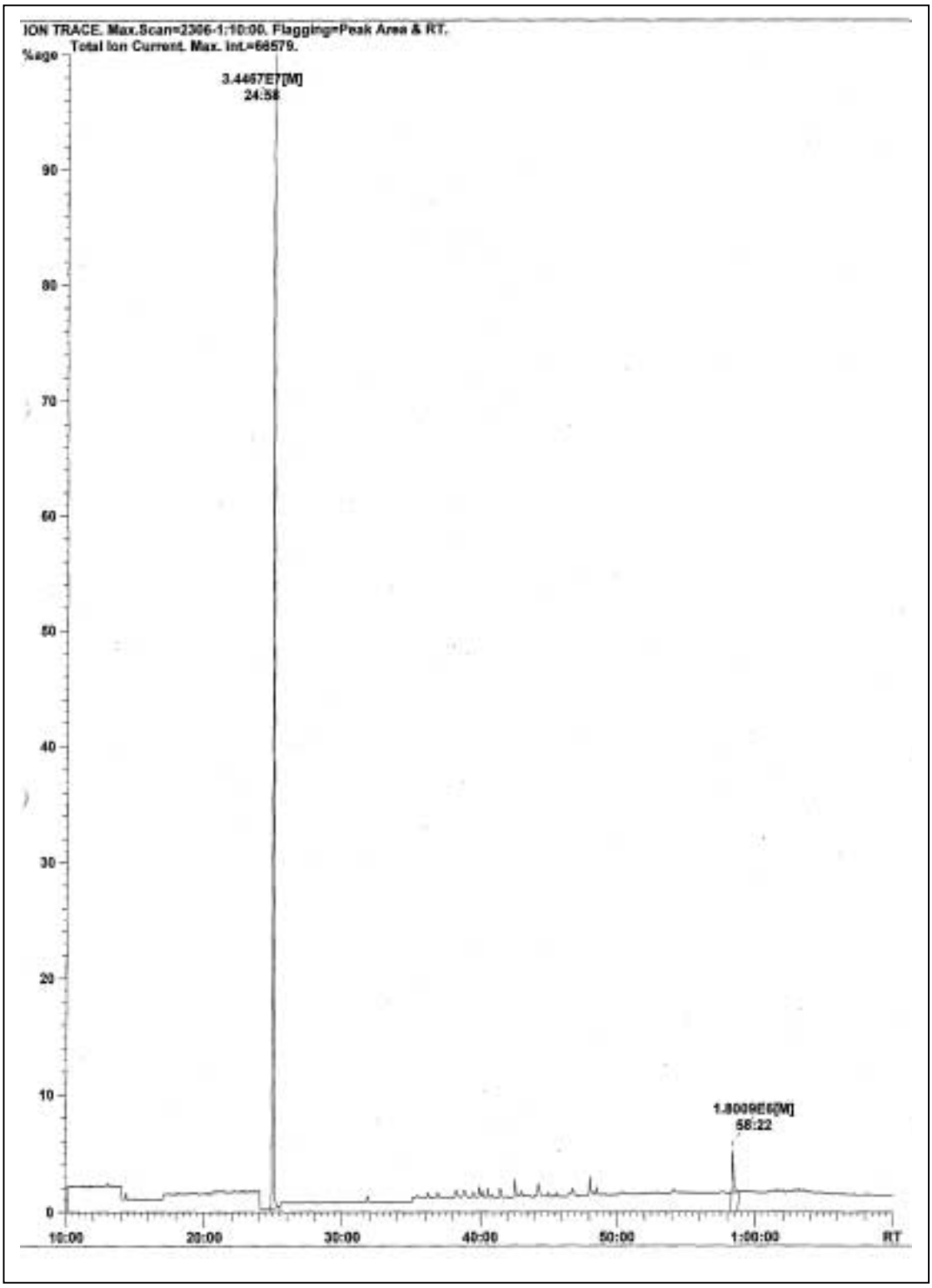
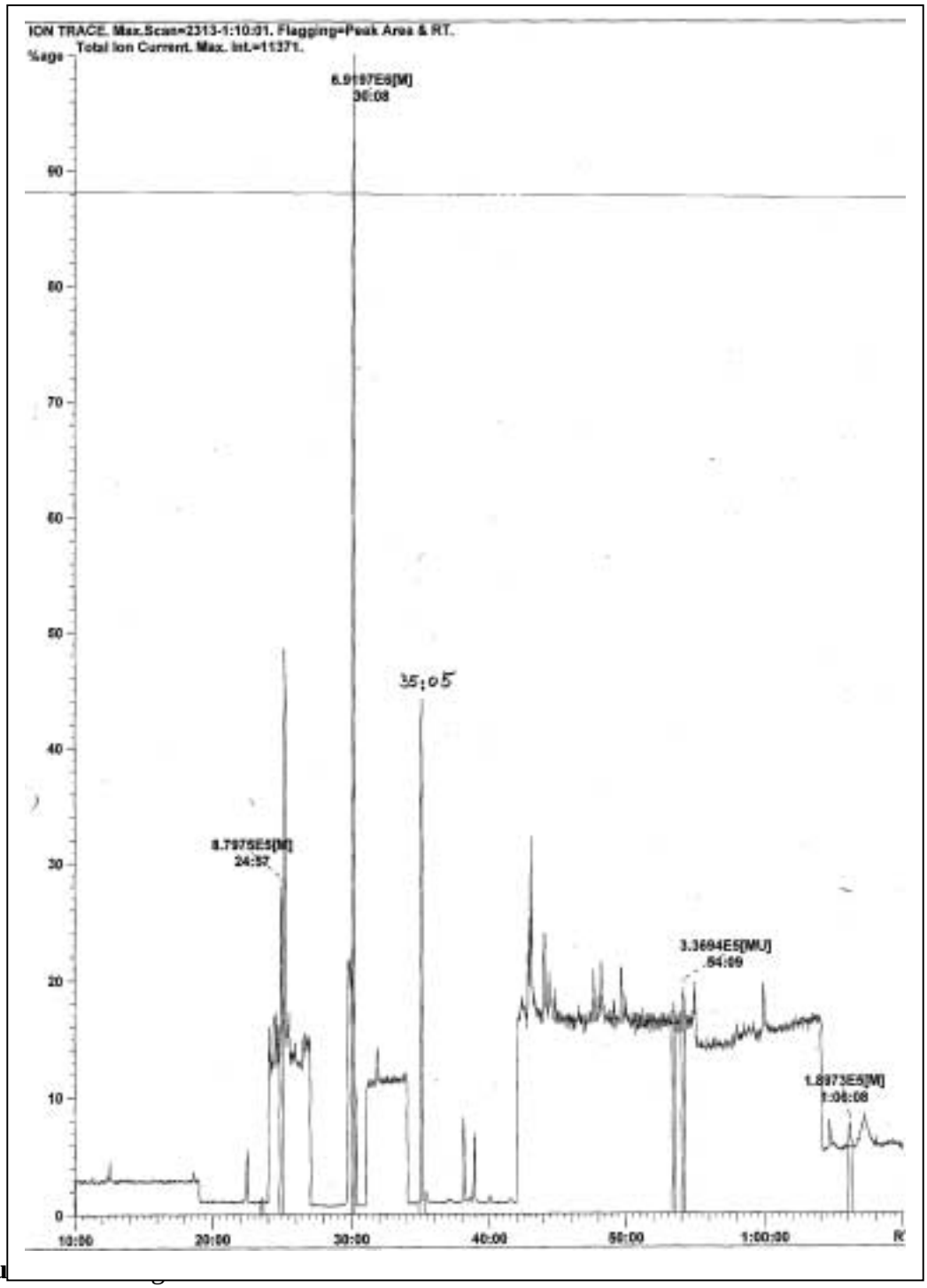


Figure 19b. Silica gel fraction 1: Total Ion Current: Total Ion current showing Chlorpyriphos ethyl (Rt:15:49) and Azinophos ethyl (58:22). Flagging = Peak Area & Rt. For the concentration of the respective analyte see Tabl. 16.

Fraction. 2



Figu

Substance	Rt	Substance	Rt
Pirimiphos methyl:	22:10	β-Endosulfane:	35:05
Chlorpyriphos ethyl:	24:57	Permethrin:	54:09
α-Endosulfane:	30:01	Deltamethrin:	66:09

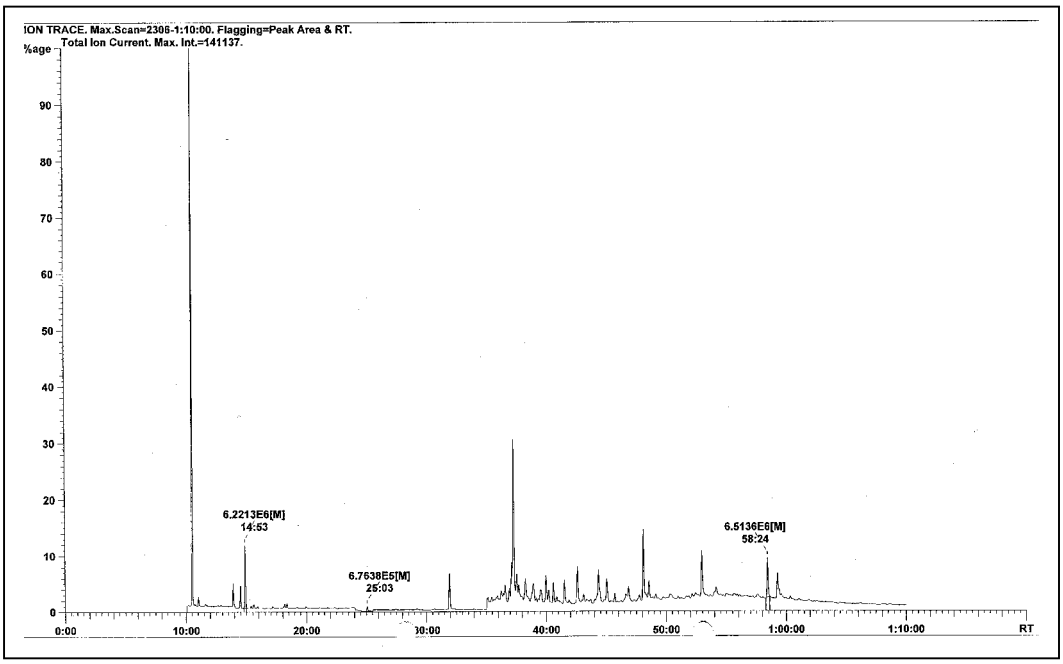
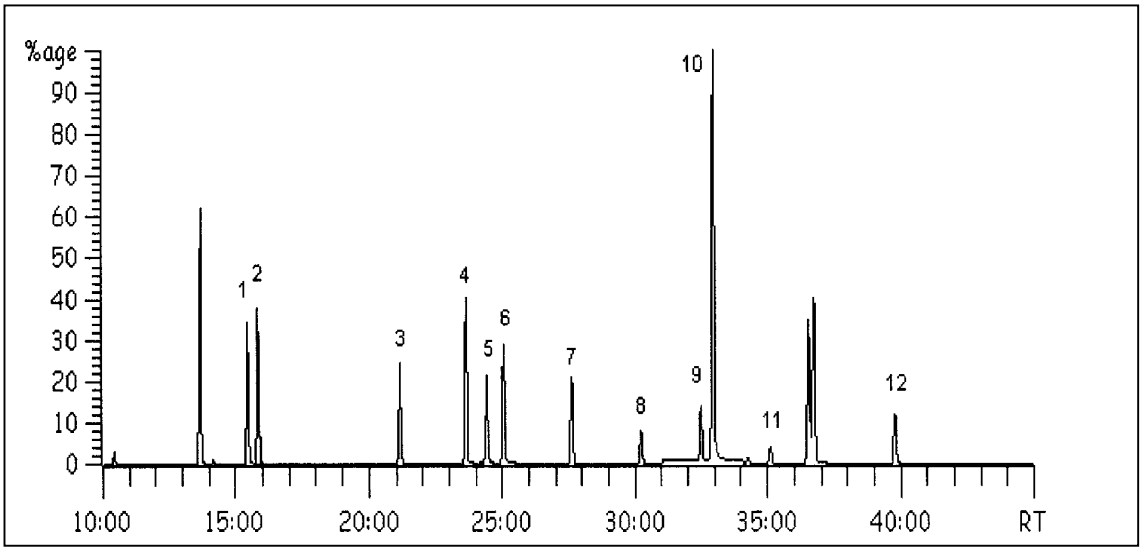


Figure 21. Silica gel fraction 1: Total Ion current showing Simazin (Rt: 14:53), Chlorpyriphos ethyl (Rt:25:03), Azinphos ethyl (58:24). Flagging = Peak Area & Rt. See Tabl. 16 for the concentration for the respective analyte.

Organochlor and organophor pesticide standard substances

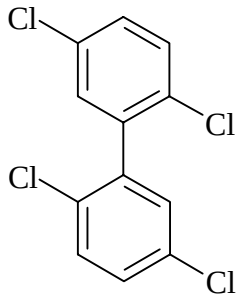


Method: 50 °C /1min-70-150°C/5-2-260

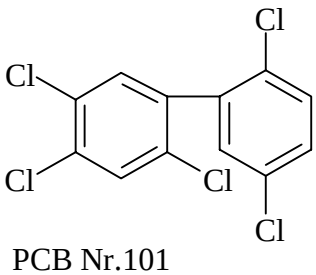
Substance	*Rt* (min)	Substance	*Rt* (min)
α-Endosulfan:	15:26	Heptachlorepoxid	27:36
β-Endosulfan:	15:50	α-Endosulfan,:	30:13
Heptachlor:	21:10	Dieödrin:	32:29
Pirimiphos-methyl:	23:38	P,p-DDE:	32:56
Malathion	24:25	β-Endosulfan:	35:07
Chlorpyriphos ethyl	25:02	p,p-DDT:	39:47

Chemical Structures of the identified Pesticides and PCB's

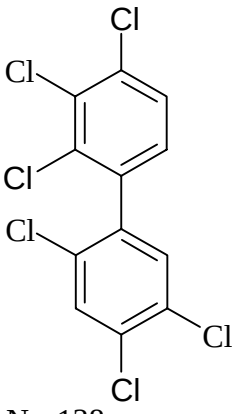
1. Polychlorbiphenyls (PCB's)



PCB Nr. 52
2,2,5,5'-Tetrachlorobiphenyl

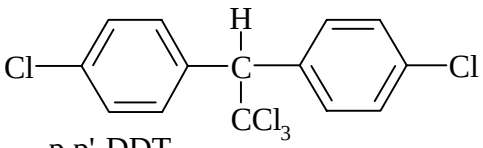


PCB Nr.101
2,2',4,5,5'-Pentachlorbiphenyl

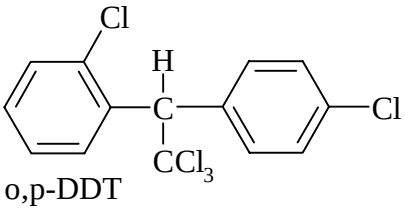


PCB Nr. 138
2,2',3,4,4'5-Hexachlorobiphenyl

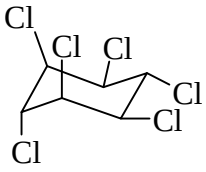
2. Organochlor pesticides



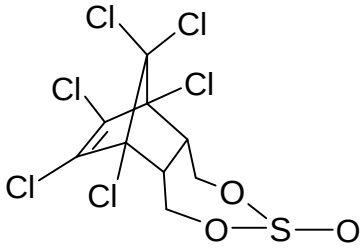
p,p'-DDT



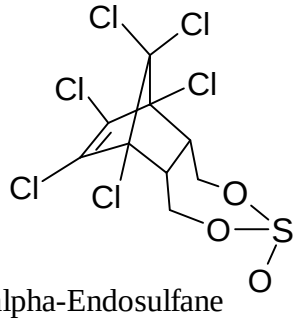
o,p-DDT



Lindane
gamma-1,2,3,4,5,6-Hexachlorocyclohexane
Positions of Cl-Atom: a,a,a,e,e,e

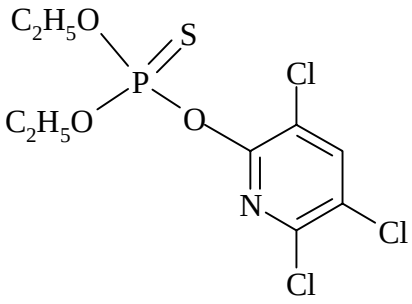


beta-Endosulfane

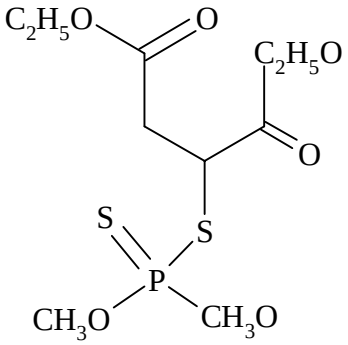


alpha-Endosulfane

3. Organophosphates

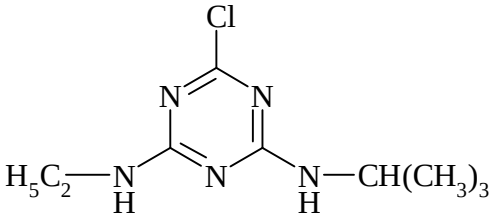


Chlorpyrifos ethyl



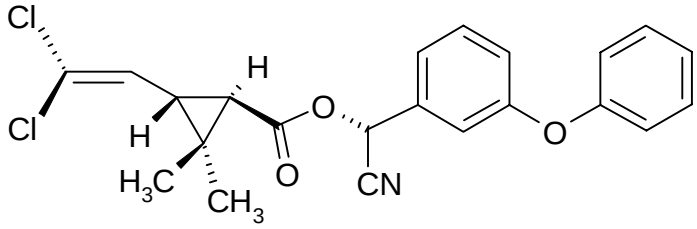
Malathion

4. Triazines

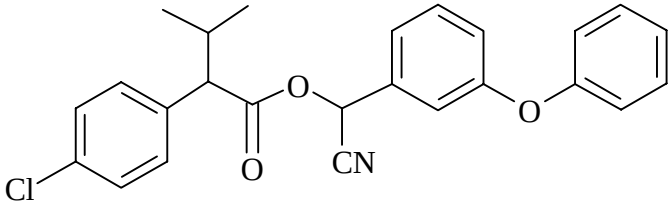


Terbutylazine

5. Pyrethroids



Cypermethrine



Fenvalerate