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Utilization of fat retainers in supercritical fluid extraction for the selective extraction of polychlorinated biphenyls from a model fat sample

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Abstract

Two common fat retainers used in supercritical fluid extraction – basic alumina and the silica based adsorbent Florisil – were investigated using lard fat as model material. With a fat retainer in the extraction cell it was possible to obtain fat-free time windows. Activation by heating did not influence the length of the time windows, while deactivation of the retainers with 10% water (w/w) drastically decreased the fat retaining capabilities. The influence of modifier addition was also investigated. Finally, a method was developed, where basic alumina was utilized to selectively extract polychlorinated biphenyls (PCBs) from a model fat sample, containing PCBs, triglycerides and phospholipids. The PCBs could be quantitatively extracted in a totally fat-free time window.

Keywords: Fat retainers; Polychlorinated biphenyls

1. Introduction

Selective extraction in supercritical fluid extraction (SFE) is an important research area. The selective extraction of trace compounds in presence of extractable major sample components offer special problems. One example of this is fat components like mono-, di- and triglycerides and sterols which are easily dissolved in supercritical carbon dioxide [1]. The solubility increases with increasing temperature and density (pressure) [2]. While extracting analytes from matrices with a high fat content, some co-extraction of matrix components are inevitable, though undesirable since it might lead to problems in the final analysis. Especially in gas chromatography (GC), large amounts of injected fat may cause problems in the injector and at the top of the column [3,4]. Using mass spectrometry (MS), contamination of the ion source may impair the analytical performance [5]. The presence of substances with low vapor pressure can affect the evaporation and hereby the transfer of analytes onto the analytical column, leading to discrimination of some compounds, when using the split injection technique [6]. Low precision was found to be related to injection of large amounts

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Table 1
Short review of publications utilizing fat retainers (1 atm=101 325 Pa; 1 p.s.i.=6894.76 Pa)

	Sample type	Analytes	Sample weight	Adsorbent	Adsorbent weight (g)	Activation, Temperature, Time, Deactivation	Density, Pressure, Temperature, Flow	Extraction time/ volume	Method of analysis	Result	Misc.	Ref.
1	Chicken fat	Organochlorine pesticides	50 μl?	Neutral aluminia I (Brockman)	1.4	– – 5% Water	– 190–270 bar 40°C 1 ml/min	20 min	GC–ECD	No further sample clean-up needed	Injection with sample loop	[1]
2	Lard fat	Organochlorine pesticides	50 μl?	Silica, PN 51900, Millipore (Waters)	0.5	– – –	See above	See above	GC–ECD	No further sample clean-up needed	Injection with sample loop	[1]
3	Soy bean oil, pork lard, rendered bacon and beef fat	Diuron, alachlor, bendiocarb, carbaryl	3–8 mg	Octadecyl, 40 μm, 40 Å pore size, Sep-Pak extraction tubes	–	– – –	0.2–0.6 g/ml – 100°C 40–70 ml/min[a]	–	SFC–FID	No further sample clean-up needed	In some cases co-elution of FFA and sterols	[13]
4	Cod liver/fillet crab claw/hepatopancreas	PCB	0.15–1.7 g	Basic aluminia, 70–230 mesh (Merck)	1–10	– – –	0.69 g/ml 14.5 MPa 60°C 133 ml/min[a]	50 min	GC–ECD (on line)	No further sample clean-up needed. No negative effect on recovery or chromatography	Cod liver (19% fat) Cod fillet (0.2–1%) Crab claw (0.3%) Crab hepatopancreas (9%)	[14]
5	Human milk, blood serum and crab hepatopancreas	Planar PCBs	5 ml serum 5 ml milk 0.25 g crab	Basic aluminia, 70–230 mesh, (Merck)	0.5	190°C Overnight –	– 14.5 MPa – –	–	GC–ECD GC–MS	No further sample clean-up needed		[15]
6	Human adipose tissue	Chlorinated pesticides, PCB	0.064–0.89 g	Neutral aluminia, 20–200 μm, activity II (Brockman)	1	– – –	– 330 atm 50°C –	10 min stat. 20 min dyn. 20 min dyn. (modifier)	GC–ECD	Fat present in extracts. Further clean-up needed	Extraction fluid: CO_2+5% dichloromethane	[16]

7	Fish, chicken egg	PCB	2 g	Florisil, 60–100 mesh (US Silica)	5	120°C >170 h –	– 6000 p.s.i. 65°C 320–820 ml/min[a]	–	GC–ECD	No further sample clean-up needed	Several other adsorbents tested	[17]
8	Fish	PCB	1 g	Basic aluminia activity I, 80–200 mesh (Brockman)	4	200°C Overnight –	0.71 g/ml 5000 p.s.i. 100°C 2.5 ml/min	0.5 min stat. 20 min dyn.	GC–ECD GC–MS	Fat present in extracts. Further clean-up needed	Carp (38% fat) Trout (23%)	[18]
9	Fish	PCB	1 g	Neutral aluminia, 150 mesh, neutral, (Fluka)	6	150°C – –	– 350 atm 150°C 3 ml/min	10 min stat. 30 min dyn.	GC–ECD	No further sample clean-up needed		[19]
10	Sow fat	Androstenone	0.3 g	Basic aluminia, 0.063–200 mm (Merck)	–	– – –	0.7–0.9 g/ml 115–334 bar 40–100°C 2.5–4 ml/min	10 min stat. 30 min dyn.	GC–MS	Cleaner extracts, but decreased recovery of analyte	>95% No adsorbent used in final method	[5]
11	Human adipose tissue	PCB	0.2 g	Basic aluminia, 5016 A (Fluka)	2.5–3.4	– – –	0.5 or 0.9 g/ml 91 or 281 bar 40°C –	9.4 min (0.5 g/ml) 17.2 min (0.9 g/ml)	GC–MS	No further sample clean-up needed		[20]
12	Human adipose tissue	PCB, PCDD, PCDF	1 g	Basic aluminia, 5016 A (Fluka)	5	– – –	0.9 g/ml 281 bar 40°C 2 ml/min	34 min dyn.	GC–MS	No further sample clean-up needed		[21]

[a] Decompressed. In all applications, neat carbon dioxide was used if not stated otherwise.

of fat on a GC system equipped with an electron-capture detection (ECD) system [7].

One way to improve this situation is to use a post-extraction clean-up step to separate co-extractants from the analytes. This step is often performed by solid-phase extraction (SPE) using disposable cartridges or column liquid chromatography [8–10].

Different approaches to achieve sufficient selectivity in the SFE step alone by adjusting the conditions in the extraction cell have been presented. Sometimes even careful adjustment of the extraction parameters with respect to minimal co-extraction of fat components is sufficient [5]. However, when the extractability of analytes and matrix are similar, this approach is inadequate.

Another possible route is to derivatize the analyte [11] or the matrix components [12] to change the solubility of the analyte in relation to the matrix components. However, derivatization has not yet been tested for fat containing matrices.

Until now, most work aiming to achieve sufficient selectivity in a single SFE step for the analytes towards fat components have been focused on the addition of an adsorbent that retains the matrix components. Table 1 gives a review of important work in this direction.

One striking feature in this table is the great variety and complexity in the parameters chosen to approach a quantitative retaining of the fat components. Two basically different concepts are utilized; one where the adsorbent is used downstream the extraction cell, and one where the adsorbent is added directly into the cell. The first approach has been used by Johansen and co-workers in three papers [14,15,22] dealing with on-line SFE–GC and SFE–high-performance liquid chromatography (HPLC) to determine polychlorinated biphenyls (PCBs) in various fat containing matrices like fish, crab, human milk and human blood serum. In all these investigations, basic alumina was placed at the outlet of the extraction cell or in a separate column directly after the extraction cell. Between 70 and 96% of the PCBs were extracted from fish and crab tissue with basic alumina as the lipid adsorbent. On-line extraction–clean-up of human milk and blood serum also showed high recoveries, comparable to those obtained with liquid extraction and off-line clean-up using sulfuric acid and gel permeation chromatography (GPC). In one of these publications, the work was extended to deal with planar PCBs present at very low levels. This on-line method gave results comparable to solvent extraction and was claimed to be advantageous since the addition of sulfuric acid and a GPC separation were made redundant. In another early paper a combined on-line SFE–SPE–SFC method was presented [13], where extracted material was fractionated on a column packed with different sorbents. Test compounds like acetone and hexane were spiked and extracted from soybean oil within 5–20 min, while glycerides were retained for 60 min using C_{18} as sorbent. However, when using animal fat some interfering compounds (probably sterols) co-eluted with the analytes.

The latter concept was used by France et al. [1], who investigated both neutral alumina and silica as adsorbents in SFE for the determination of organochlorine pesticides in fat. A comparison between the conventional method and the on-line column clean-up showed no statistical difference at the 95% confidence limit using either neutral alumina or silica. The same concept of adding basic alumina as a fat retainer directly in the extraction cell, has also been utilized with success by van Bavel and co-workers for the extraction of PCBs, PCDDs, PCDFs and pesticides from human adipose tissue [20,21]. The extracts were ready for analysis without further clean-up. Similar results were achieved by Hale and Gaylor [19], who extracted PCBs from fish tissue. In their final procedure, 6 g of neutral alumina was used for the extraction of 1 g lyophilized fish tissue (24 or 40% lipid content) giving an amount co-extracted lipids less than 0.1%. The PCB recoveries did not significantly differ from values obtained on the same samples in two other investigations where other methodologies were used for the extraction. Lee et al. [18] also made a small investigation of how the amount of basic alumina affected the amount of lipids and PCBs extracted from two fish species containing 23 or 38% lipids. For a 1-g fish sample with 1 g basic alumina added, the amount of lipids extracted were 7 and 15%, respectively. This was further decreased by adding 4 g of basic alumina, to less than 0.1% and 2.2%, respectively. The recoveries for PCBs were as high as 95 and 97% compared to Soxhlet extraction, respectively. Due to the dimensions of the thimble, no more adsorbents

could be added meaning that in some cases they had to perform an off-line Florisil column clean-up. Alley and Lu [17] tested six different adsorbents in the extraction of PCBs from chicken egg or fish and found Florisil to be the best for their purposes. In spite of the fairly frequent use of fat retainers, illustrated in Table 1, the deeper understanding of the fat retaining process has only been given limited attention. In this paper we have studied the possibility to improve selectivity in SFE for fat containing samples by using fat retaining adsorbents in the extraction cell. The aim of this study is a thorough investigation of such separation systems with respect to their ability to create sufficiently wide time windows where target analytes can be extracted without further need for sample processing. Finally, a method was developed, utilizing basic alumina, for the selective extraction of PCBs from a model fat sample, consisting of PCBs, lard fat and a phospholipid mixture obtained from egg yolk. The applicability of the developed method on real-world samples is left open for further studies.

2. Materials and methods

2.1. Supercritical fluid extraction

All extractions were performed on a Hewlett-Packard 7680T SFE unit (Wilmington, DE, USA). Hewlett-Packard standard 7-ml extraction thimbles were used in all experiments. The analytes were collected on a Hewlett-Packard standard trap packed with octadecyl silica (ODS, 0.6 g). The flow-rate of the extraction fluid was set to 2 ml/min in all experiments. The density was set to 0.90 g/ml, and the extraction temperature was held at 40°C throughout. When extracting lard fat with pure carbon dioxide, the trap was kept at 40°C, but in some cases, when modifier was added to the extraction, it was increased to 80°C. In the final method, the PCBs were trapped at 10°C. Lard fat and PCBs were eluted from the trap with cyclohexane and collected in pre-weighed standard (1.8 ml) sample glass vials (Chromacol, Welwyn Garden City, UK). The rinse volume was determined gravimetrically. The nozzle temperature of the SFE unit was always set 5°C above the trap temperature.

2.2. Chemicals

All gases used – carbon dioxide 4.8 (99.998%) and carbon dioxide (food quality) – were purchased from Aga (Stockholm, Sweden). Cyclohexane and methanol of HPLC quality were delivered by Lab Scan (Dublin, Ireland). Basic aluminia was supplied by Fluka (Buchs, Switzerland). Florisil was delivered by Supelco (Bellafonte, PA, USA). Stainless steel beads were kindly donated by Anval (Torshälla, Sweden), lard fat by the Swedish Meat Research Institute (Kävlinge, Sweden) and purified soybean oil from Pharmacia & Upjohn (Stockholm, Sweden).

A commercially available PCB Isomer Calibration Mix (Accustandard, New Haven, CT, USA), containing eight different congeners (PCB IUPAC Nos. 5, 29, 50, 87, 154, 188, 200, 209) was diluted to between 200 and 1000 ppm with cyclohexane. A 50-μl volume of the diluted standard mixture was added to the extraction thimble. PCB 30 (Accustandard), dissolved in cyclohexane was used as internal standard. The internal standard was added directly to the capped vials into which the extracted PCBs were eluted.

2.3. Sample handling

Initially, two types of sample supports were tested. In the preliminary extraction experiments, dental tampons were spiked with fat, but since it turned out that the tampons contained extractable fat, stainless steel beads were used for further work. The solid fat was melted on a water bath and pipetted directly onto 6 g (2 ml) of stainless steel beads in the pre-weighed extraction thimble. The thimble was weighed again and the amount of applied fat was calculated.

2.3.1. Packing of the extraction thimble in the initial fat retainer characterization

During the initial characterization of the fat retainers, a glass tube (when necessary) was first inserted in the extraction thimble to minimize the void volume and to support a sintered glass filter. The stainless steel beads were then poured into the thimble followed by addition of the fat sample. The retainer, when used, was loaded on top of the fat and the steel beads. To ensure a non-leaking thimble, a

small gap of 2 mm was left between the adsorbent and the top thimble cap. This sample loading procedure was used throughout this work if not stated otherwise.

2.3.2. Packing of the extraction thimble in the final PCB method

In the final method, the thimble was packed with 6 g of stainless steel beads, without any glass tube or sintered glass filter present in the thimble. PCBs, triglycerides and phospholipids were added on top of the beads, in the mentioned order. Between 2 and 4 g of basic alumina (Fluka) was then applied on top of the beads. During the intitial characterization of the two fat retainers (above), the thimble was always completely filled, but when no glass tube was used in the set-up of the extraction thimble, about 3 ml of dead volume was obtained above the alumina. In order to keep the alumina in place, a stainless steel spring with a metal frit was therefore designed (Chemical Centre Workshop, Lund, Sweden). The spring pressed down the frit towards the alumina in the desired way. To visually check the process inside the extraction thimble, an extraction chamber of sapphire was utilized. With a fast pressurization at 52 bar, without the spring-loaded frit in place, it was found that the adsorbent was forced towards the outlet of the cell leaving a dead volume above the stainless steel beads. This might create problems with the retention of the fat during the initial PCB extraction step due to channel formation when the retainer is allowed to ''float'' freely in a large dead volume. However with the spring in place, any fat retention problems can be interpreted without the need to consider these type of effects.

2.4. Fat quantitation

In the initial fat retainer characterization experiments, extracted fat was determined both by weighing the extraction cell and by analyzing the fraction collected on the trap. Determination of the collected fraction was done either by weighing the whole eluate or a part of it as described below. Hence it was possible to distinguish between problems caused by incompleteness of the extraction procedure or by insufficient trapping efficiency. All quantitative fat analysis was performed by using a Sartorius MC1 RC 210D balance (Sartorius, Göttingen, Germany).

2.4.1. Quantitation of extracted fat

By weighing the extraction thimble before and after the extraction, the remaining fat could be determined. Prior to weighing, the extraction thimble was ventilated for a couple of minutes to get rid of the remaining carbon dioxide.

2.4.2. Quantitation of trapped fat

Trapped fat was quantified by two methods. Normally, when no modifier was used, 100 μl of the eluate from the trap was pipetted onto preweighed micro slides using a SMI pipett (SMI Liquid Handling Products, Miami, FL, USA). Evaporation of solvent was performed by placing the micro slides in a fume hood over night. The micro slides were then weighed and the fat content was calculated as the difference. When using modifier, the whole fraction collected in a small 2-ml vial (weight ca. 2.8 g) was evaporated and the fat content was determined gravimetrically.

2.5. PCB quantitation

After the PCBs were trapped, they were eluted with 1.8 ml cyclohexane into top-capped vials. The PCBs were analyzed on a Hewlett-Packard GC 5890 system equipped with a HP 5972 MS system. The samples were injected with a HP 7673 GC/STC autoinjector at 250°C, 73 kPa, splitless mode. The injection volume was 2 μl. Helium 5.6 (Aga) was used as carrier gas with the flow set to 1 ml/min. Temperature programming was 100°C isothermal for 2 min, increasing to 200°C at a rate of 40°C/min and held for 5 min, followed by an increase to 230°C at a rate of 10°C/min and a subsequent increase to 300°C at a rate of 50°C/min, held for 4 min. The PCBs were analyzed in the selected ion monitoring (SIM) mode, based on the two most intensive fragments from each congener. The interface temperature was set to 280°C, and the electron multiplicator voltage was constant at 2000 V.

3. Results and discussion

3.1. Fat quantitation

To check if the weight of the micro slides and of the vials with applied fat varied over time, standard

solutions of lard fat dissolved in cyclohexane were analyzed as described above. For the micro slides 1, 2 and 5 mg were pipetted. The weight of five replicates of each amount was measured two times a day during a 4-day period. For fat samples of 1 and 2 mg, the relative standard deviation (RSD) was below 10%, while the 5 mg sample had an RSD of less than 3%. In the sample vials, 5 and 50 mg of fat was applied resulting in an RSD of less than 3%.

3.2. Choice of sample support

In some preliminary experiments, two types of fat samples (soybean oil and lard fat) and two types of sample support (dental tampons and stainless steel beads) were tested. Dental tampons had previously been proven to be a suitable sample support, when handling large amounts of fat [5]. A comparison of 500 mg samples applied on both types of sample supports is shown in Fig. 1. Also included in this figure, is a comparison between amount extracted (measured from thimble weight), and amount trapped fat (determined by weighing the trap eluate).

From Fig. 1 it follows that the extraction times are much shorter using stainless steel beads, due to loss of the chromatographic effects caused by the cellulose in the dental tampons. The chromatography effect is more pronounced for soybean oil than for lard fat. This is probably due to stronger interactions between the unsaturated fats in the oil and the cellulose matrix. It should also be noted that profiles for extracted and trapped fat agree well for dental tampons. Using stainless steel beads no difference between the extracted amounts of fat and oil can be detected, indicating that lard fat and soybean oil are unaffected by the matrix. An important observation is the decrease in recovery for both trapped lard fat (84%) and soybean oil (85%), which can be traced to trapping problems. It has been demonstrated that the Hewlett-Packard standard trap packed with ODS can hold approximately 100 mg of triglycerides using a single extraction step [23]. For extraction of larger fat samples on stainless steel beads, a fractionated extraction–elution procedure must be used [23] to reduce the amount extracted fat in each step.

The final choice of sample support was stainless

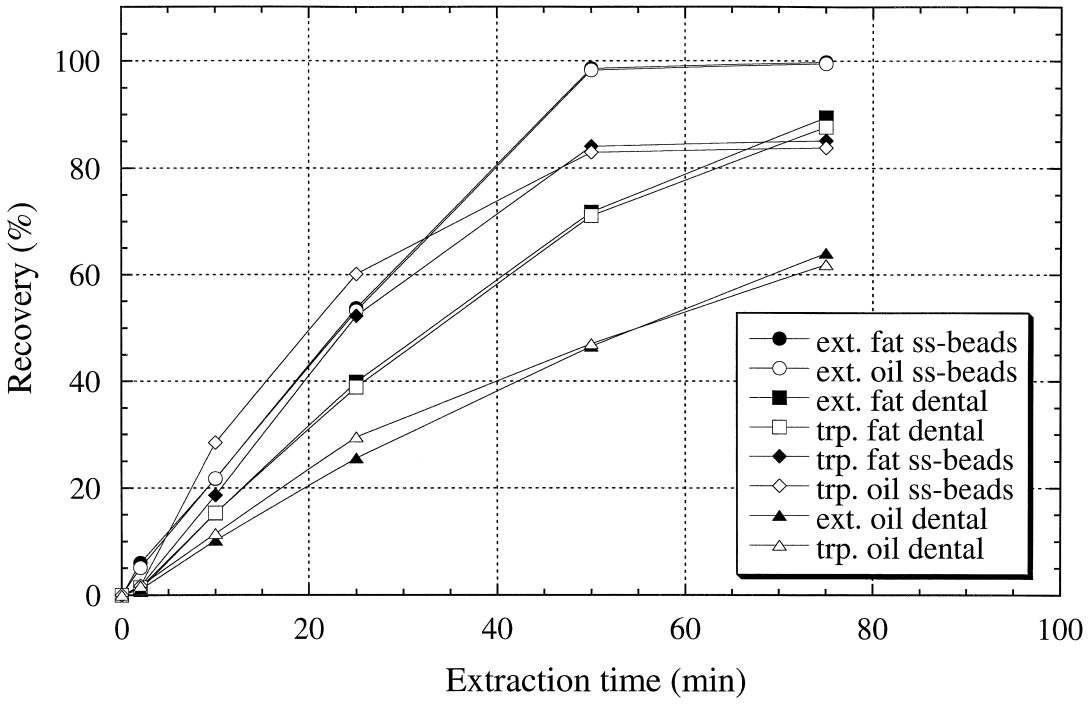


Fig. 1. Lard fat or soybean oil extracted from dental tampons and stainless steel beads. Extraction conditions: 276 bar (0.90 g/ml), 40°C, 2 ml/min. Nozzle and trap temperatures, 45°C and 40°C, respectively.

steel beads due to their inertness giving shorter extraction times. Another reason for not choosing dental tampons was that blank extractions of these indicated that the material contained relatively large amounts of fat, which was confirmed by GC–MS analysis. This would cause problems when extracting 100 mg samples or less.

Lard fat was the final choice of model sample. Lard fat is not in a liquid state at room temperature, and hence will not pour out from the extraction cell as soybean oil has a tendency to do.

3.3. Performance of fat retainers

3.3.1. Untreated fat retainers

In the first experiments no pre-treatments of the fat retainers (basic alumina and Florisil) were done. The adsorbents were taken directly from the containers in which they were delivered. Conditions published in the literature concerning extraction, gives values in the interval of 4–6 g of basic alumina [5,13,20,21] or 2.5 g of Florisil [17] per gram of fat sample. The mechanisms of fat retention were investigated by varying the amount of retainer and fat, respectively. In this study, the amount of Florisil was 0.8–3 g, when the fat amount was 500 mg. Accordingly, the amount of basic alumina was 4.5 g, when the amount of fat was varied from 500 to 1000 mg. It should be noted that 3 g of Florisil has approximately the same volume as 4.5 g of basic alumina.

The effects on the extraction process of varying amounts of Florisil at constant amount of fat are shown in Fig. 2.

The time for the start of fat extraction, defined here as the time when >1% fat is found in the collection vessel, gives a time window of only a few minutes for 800 mg Florisil. This value is increased to 60 min with a retainer amount of 3 g. With low amounts of retainer material the determination of the time windows before the fat starts to elute from the extraction cell is uncertain. For a 500 mg sample, using 1.5 g of Florisil a time window of 23 min is determined with an RSD of 32% (n=8). The precision is improved by using 3 g of Florisil for a 1000 mg sample, where the time window of 58 min is determined with an RSD of 8% (n=3).

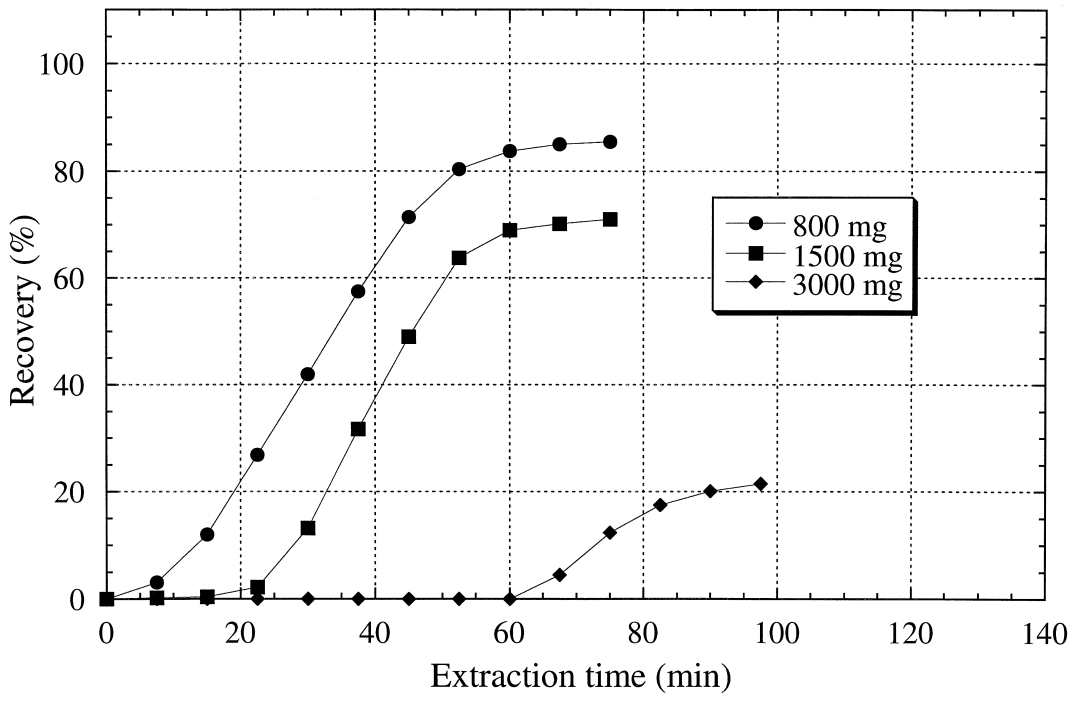


Fig. 2. Extraction profiles for 500 mg sample extracted through 800, 1500 and 3000 mg Florisil®. Extraction parameters as in Fig. 1.

Experiments performed using basic alumina as the adsorbent show similar results. With 2 g of basic alumina (500 mg fat sample) and the same extraction parameter as for Florisil, the time window was 19 min with an RSD of 57% ($n=8$). With 4.5 g basic alumina (1000 mg fat sample) the corresponding value was 47 min with an RSD of 7% ($n=5$). From these data, it is evident that for the same volume of retainer, Florisil is somewhat more efficient.

In the different investigations presented in Table 1, the extraction times are normally between 20 and 40 min for 1 g fat samples in combination with approximately 5 g of the adsorbent. From our results above follows that the ratio between the sample and the retainer material should be at least in the order of 1:3 (w/w), in order to get a fat-free time window with a length of approximately 20 min.

Fig. 3 shows the influence on the extraction profiles of different amounts of fat with constant amount of adsorbent. The reason for using a large amount of adsorbent (4.5 g) is that this gives more stable values of the fat keeping capability, and thus more well defined profiles.

From Fig. 3, the fat keeping capabilities were determined to 95, 95 and 97 mg/g for 500, 750 or 1000 mg fat, respectively. Obviously, with enough amount of adsorbent the relative fat retaining properties is practically constant. Thus, when extracting 500 mg of fat using 4.5 g of basic alumina only a fraction corresponding to 4% (w/w) is eluted from the adsorbent within 90 min. Reducing the amount of basic alumina to 2 g reduces the fat keeping capability to 69 mg/g. The reason for this lower value and the impaired precision while using less amount of retainer, is probably the higher probability for dissolved fat material to elute through channels in a shorter adsorbent bed. Occasionally, probably when the packing process in the extraction cell has progressed in a favorable fashion, the fat keeping capability showed no difference between 1.5 g and 3 g Florisil for 500 mg samples.

All the experiments above have concerned basic

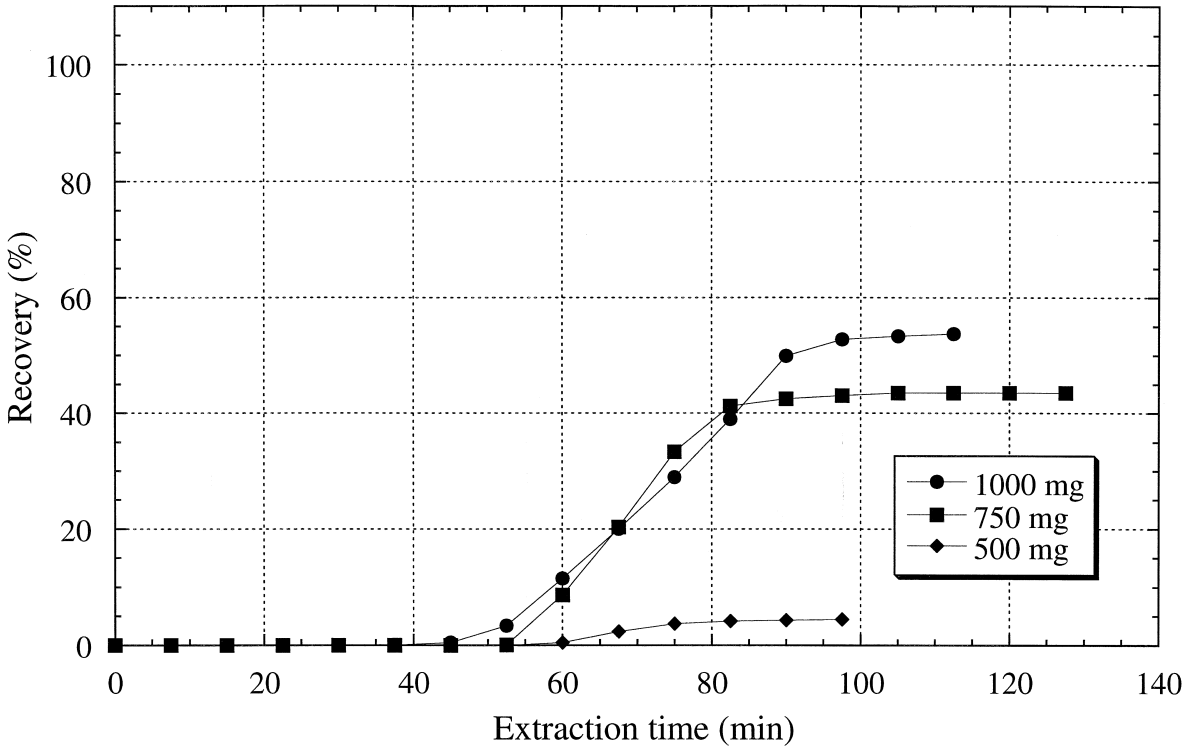


Fig. 3. Extraction profiles for 500, 750 and 1000 mg samples extracted through 4.5 g basic alumina. Extraction parameters as in Fig. 1.

alumina. We have also made similar extractions using both neutral or acidic alumina. The extraction profiles were very similar to the one obtained for basic alumina. However, the fat-free time windows are slightly different with 40, 47 and 52 min for acidic, basic and neutral alumina, respectively.

3.3.2. Activated fat retainers

As can be seen in Table 1, some of the researchers either activate or deactivate their adsorbents prior to use. However, no obvious trend regarding activation temperature can be seen in the listed examples in Table 1. Hence, the effects of heating basic alumina and Florisil for different time intervals were investigated. A temperature of 300°C was chosen. This temperature is significantly higher than temperatures reported in Table 1, which was expected to increase the differences between activated and non activated adsorbent. The heating time was 0, 3, 24, 36 and 58 h. In all cases, the adsorbents were taken directly from the oven to minimize any uptake of water.

The most interesting observation was that the time window differences between activated and nonactivated were very limited. For activated basic alumina the time window was 44 min (RSD=7%) which is a 3 min decrease in comparison with non activated. The time window for Florisil increases from 50 min (RSD=17%) for non activated to 60 min for activated. Secondly, the amount of fat permanently hold by the adsorbents was relatively constant and independent of the heating time. For 3, 24, 36 and 58 h the fat keeping ability was 117, 101, 108 and 112 mg/g for basic alumina, and 96, 134, 131 and 120 mg/g for Florisil, respectively. From these data, it can be concluded that neither the fat-free time window nor the fat keeping ability is markedly changed by activation of the adsorbents.

3.3.3. Deactivated fat retainers

In the publication by France et al. [1], basic alumina was deactivated by adding 5% (w/w) water. To investigate the behavior of fat on deactivated basic alumina and Florisil the adsorbents were treated with 10% (w/w) water. The results for a 1000 mg fat sample (4.5 g basic alumina or 3 g Florisil) are presented in Fig. 4.

For both adsorbents the time window and fat keeping ability was decreased more than 50%, demonstrating that water has a pronounced counteracting effect on the retardation of fat. Thus, to use a fat retainer in an efficient way, the amount of water should be kept at a low level. If water is released from the sample during the extraction process, a water adsorbing material needs to be added in the extraction cell between the sample and the fat retainer to keep its retaining at a sufficient level. This problem has previously been solved by adding basic aluminia directly after the sample and placing the fat retaining adsorbent in an separate cell down stream the extraction cell [14].

3.3.4. Modifier influence

As shown above, a fat retaining material can be used in the extraction cell to make a selective extraction possible of target analytes dissolved in the fat matrix. However, there is a risk that the analyte will interact strongly with the matrix. To break such interactions a polar modifier can be used. Addition of a modifier will generally decrease the interaction between target analytes as well as of fat components and the retaining material. Additionally, selectivity changes between different target compounds and fat components can be expected when using different modifiers. This is illustrated in Fig. 5a and b.

The extraction profiles in Fig. 5 shows that also with a modifier in the extraction fluid fat-free time windows can be obtained. The extraction profiles for the different modifiers are very similar (almost superimposing), for the two different adsorbents. It is merely the starting points for the fat extraction which slightly differ. The only modifier, where the starting points differ considerably for the two investigated adsorbents, is for 1% methanol. The higher capacity for hydrogen bonding in Florisil compared to basic alumina, makes the release of fat components from the adsorption sites less efficient resulting in a larger fat-free time window for the former adsorbent.

The extraction rate is depending both on the solubility of the target compound in the extraction fluid and the ability of the modifier to release the target compounds from the matrix by breaking interactions between these compounds and the matrix. As expected with adsorbents as basic alumina and Florisil, where hydrogen bonding is an important characteristics, the fat-free time windows decreases

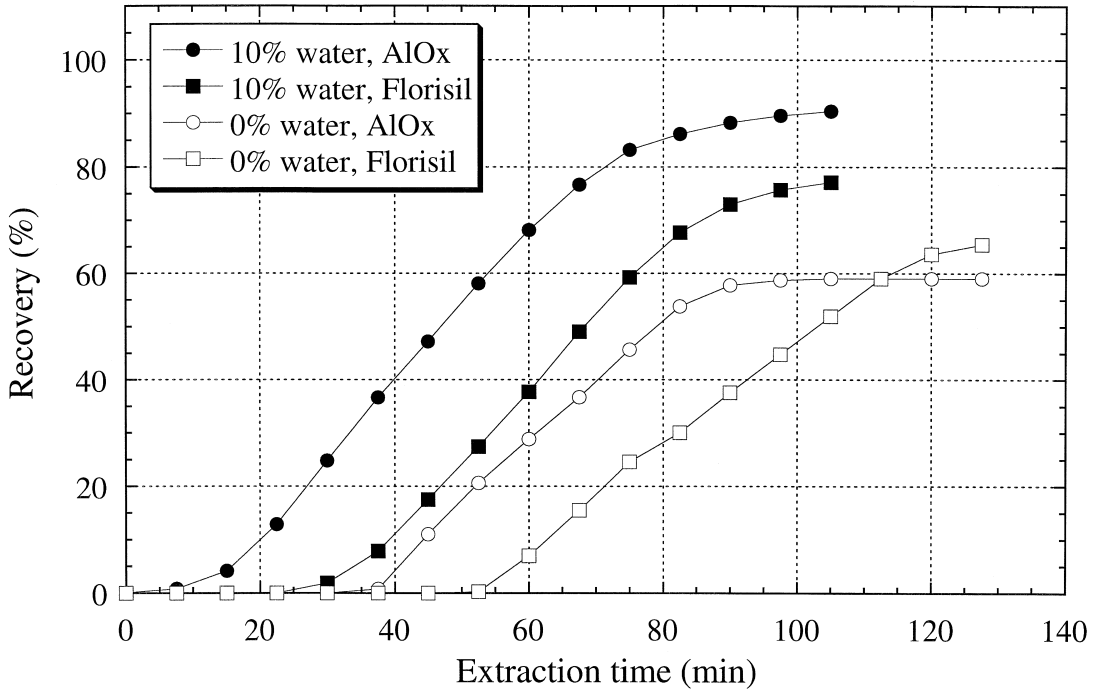


Fig. 4. Effects of deactivating adsorbents with water. Extraction profiles for 1000 mg samples extracted through (a) 4.5 g basic alumina or (b) 3.0 g Florisil. Extraction parameters as in Fig. 1, (n=2).

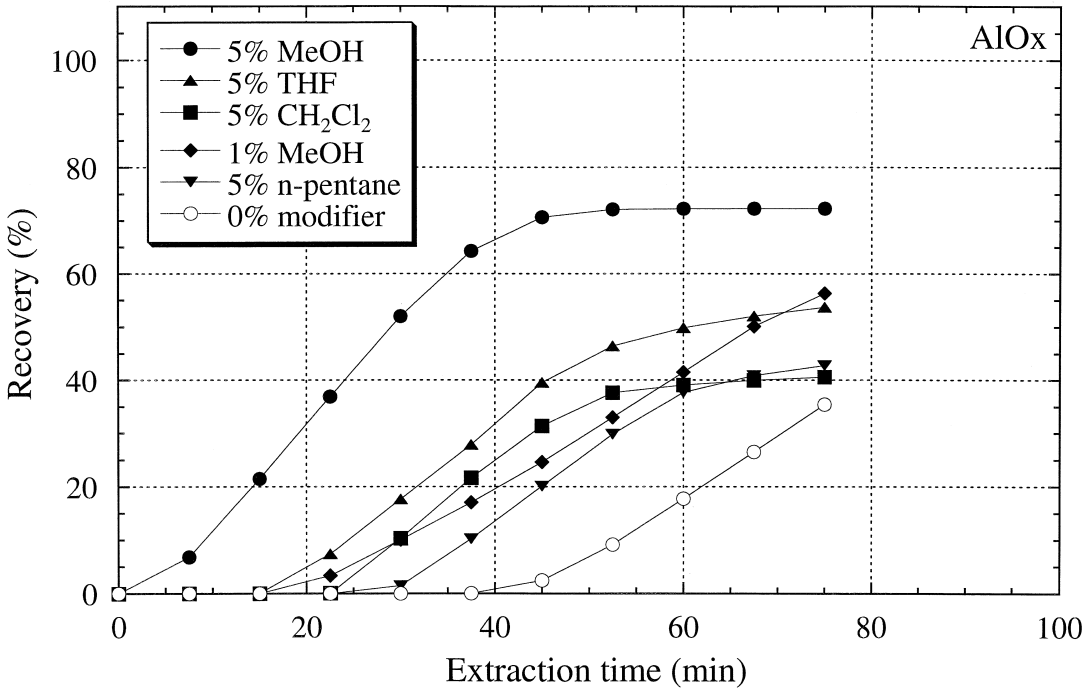
with increasing hydrogen bonding ability of the modifier.

Generally, in the modifier systems investigated above breaking the interactions between analytes and matrix seems to bee more important than the solubility of the analyte in the supercritical fluid. However, one exception is *n*-pentane with Florisil as adsorbent, where the fat-free time window actually is shorter for 5% *n*-pentane than with 1% methanol as modifier.

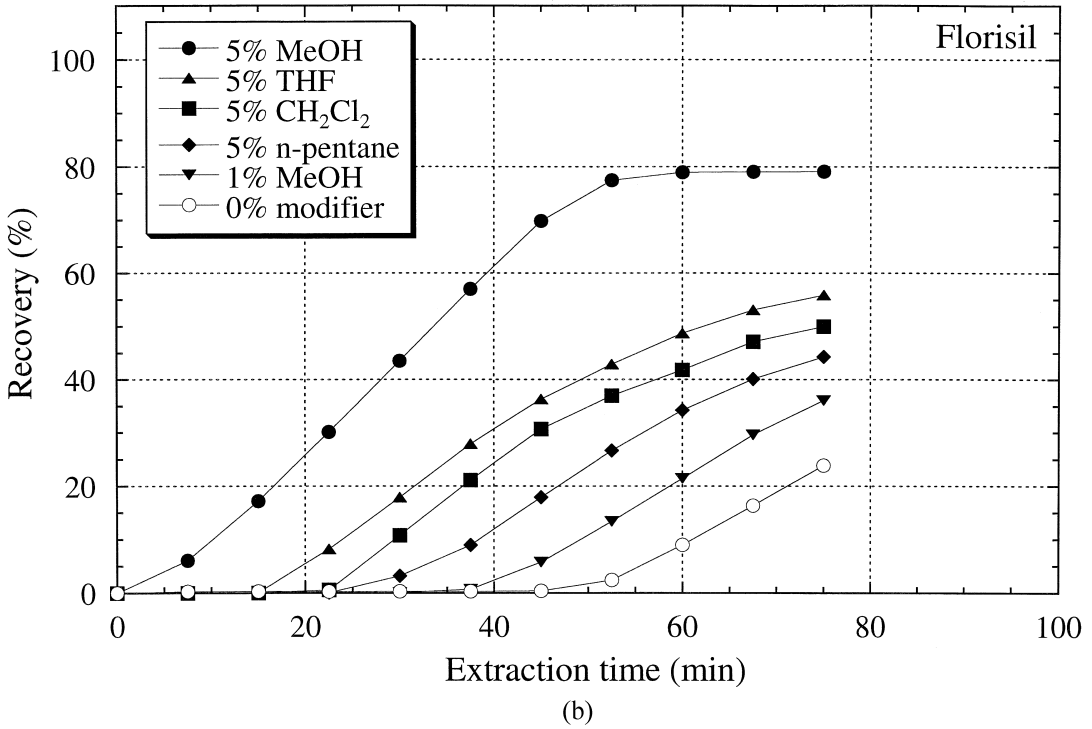
The reason for the extraction profiles leveling out at a recovery of 70–80% when using 5% methanol for both basic alumina and Florisil is trapping problems. As shown elsewhere [23] a normal C_{18} trap for the Hewlett-Packard equipment can accommodate 100 mg. To prevent the breakthrough problem, the trap should be eluted when not more than 80% of the capacity is achieved. In the experiments above 1000 mg fat was used, which means that at least in steps 2–6 in the extraction profiles for 5% methanol the extracted amount is higher than 100 mg. Also for the other profiles, a careful examination reveals that an extracted amount of 100 mg may occasionally have been exceeded. This does not change anything essential in the discussion above. Profiles based on extracted amounts rather than on recovery would of course give a somewhat steeper slope.

3.4. Selective extraction of PCBs from a model fat sample

In order to verify that PCBs could be selectively extracted through a fat retainer in the presence of lipids, a model fat sample was utilized. Basic alumina was used as fat retainer, and the model fat sample consisted of PCBs, lard fat and a phospholipid mixture obtained from egg yolk. In order to have a high selectivity towards interfering compounds, including traces of lipids, GC–MS was chosen for the final determination step. With the rapid development in benchtop MS, where detection limits are constantly decreasing, it can be anticipated



(a)



(b)

Fig. 5. Influence of modifiers on extraction time for basic (a) 4.5 g alumina and (b) 3 g Florisil with a sample size of 1000 mg lard fat. Nozzle and trap temperatures, 85°C and 80°C, respectively, otherwise extraction conditions as in Fig. 1, (n=2).

that in a near future GC–MS will substitute GC–ECD in many environmental applications.

3.4.1. Extraction of PCBs through alumina

The first thing to study is the behavior of PCBs when extracted through a fat retainer. The PCB mixture was dropped on stainless steel beads and alumina was loaded on top. Initial experiments demonstrated that an extraction time in the order of 15 to 20 min would be suitable for extracting PCBs with varying degree of chlorination. To further study the influence of alumina on the different congeners, new experiments were performed at the same extraction conditions, choosing 15 min as the extraction time. The results are presented in Table 2.

The results clearly demonstrate that PCBs ranging from di- to decachlorinated can be quantitatively recovered within 15 min, passing through 2 or 3 g of fat retainer (basic alumina).

3.4.2. Influence of lipids on PCB recovery

To study the effects on PCB recovery, when lipids are present in the extraction cell, 100 mg of lard fat was dropped on top of the applied PCBs. This was followed by addition of 20 mg phospholipids, before applying alumina. The results are presented in Table 2.

From the data it can be seen that with a 20 min extraction step, the recoveries are between 90 and 110% for the various PCBs present in the model fat sample. No large differences in recoveries are observed when a total of 120 mg fat (100 mg triglycerides and 20 mg phospholipids) is added to the extraction cell as compared to the data obtained without fat present in the extraction cell. The extracts obtained for 2 and 3 g of alumina were nearly free of interfering fat, even though 11 mg (RSD=10%, $n=3$) and 7 mg (RSD=18%, $n=3$) were co-eluted when 2 and 3 g of alumina were used, respectively. Despite the fact that 2 g of alumina should be able to hold up to 200 mg fat (see discussion above), this might only be true if the fat retainer column is long enough. In fact it was demonstrated above that the fat keeping ability was lowered from ca. 95 mg/g when using 4.5 g alumina, to 69 mg/g when using 2 g of alumina. Additionally in the experiments presented here (as compared to the results above), phospholipids are present in the extraction thimble. These interact more strongly with the adsorbent than triglycerides, and efficiently competes with triglycerides for active sites. Consequently, being on the limit of how much fat 2 g of alumina can retain in this system (ca. 140 mg), and the presence of 20 mg phospholipids, it is not unlikely that even when 3 g of alumina is used, small amounts of triglycerides are able to pass the alumina during the first 20 min. However by increasing the amount alumina to 4 g a completely fat-free extraction could be obtained. This is further confirmed by the good long-term stability of the MS system where several hundred samples can be injected without any need of cleaning the ion source. This clearly demonstrates that PCBs with varying degree of chlorination can be quantitatively extracted without interfering lipids present in the extracts (Table 2).

Table 2
Recovery of eight PCBs extracted through 2, 3 and 4 g of alumina for 15 and 20 min, with and without lipids present in the extraction cell

PCB no.	Recovery (%) 2 g alumina 15 min, no fat	RSD (%) ($n=6$)	Recovery (%) 3 g alumina 15 min, no fat	RSD (%) ($n=3$)	Recovery (%) 2 g alumina 20 min, fat[a]	RSD (%) ($n=8$)	Recovery (%) 3 g alumina 20 min, fat[a]	RSD (%) ($n=6$)	Recovery (%) 4 g alumina 20 min, fat[a]	RSD (%) ($n=3$)
5	103	4	96	7	95	9	96	7	102	3
29	107	5	93	6	100	10	93	6	102	4
50	97	4	96	4	94	10	96	4	95	1
87	104	4	99	4	96	11	99	4	110	9
154	98	3	102	6	92	11	102	6	106	1
188	98	5	103	9	91	11	103	9	109	3
200	96	5	105	10	90	11	105	10	108	2
209	93	5	104	10	95	10	104	10	109[b]	–

[a] PCBs extracted through 100 mg triglycerides and 20 mg phospholipids.
[b] $n=1$.

4. Conclusion

With adsorbents in the extraction cell fat-free time windows can be obtained. The size is depending on the type and amount of adsorbent as well as on the solvent strength of any modifier added. With increasing ability to compete with the analyte for the active sites on the adsorbent the length of these time windows decrease. However, with an optimization of the extraction procedure it is possible to selectively extract PCBs from the fat samples with a minimum of sample handling. It must be stressed though that more research is of need, where the developed method should be applied on real-world samples.

Acknowledgements

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References

[1] J.E. France, J.W. King, J.M. Snyder, J. Agric. Food Chem. 39 (1991) 1871.
[2] D.R. Gere, C.R. Knipe, P. Castelli, J. Hedrick, L.G.R. Frank, H. Schulenberg-Schell, R. Schuster, L. Doherty, J. Orolin, H.B. Lee, J. Chromatogr. Sci. 31 (1993) 246.
[3] K. Grob Jr., J. Chromatogr. 287 (1984) 1.
[4] K. Grob Jr., M. Bossard, J. Chromatogr. 294 (1984) 64.
[5] M. Mågård, H.E.B. Berg, V. Tagesson, M.L.G. Järemo, L.L.H. Karlsson, L.J.E. Mathiasson, M. Bonneau, J. Hansen-Møller, J. Agric. Food Chem. 43 (1995) 114.
[6] V. Ferreira, A. Escudero, J. Salafranca, P. Fernández, J. Cacho, J. Chromatogr. 655 (1993) 257–266.
[7] M.L. Hopper, J. High Resolut. Chromatogr. 10 (1987) 620–622.
[8] M. Holcomb, H.C. Thompson Jr., W.M. Cooper, M.L. Hopper, J. Supercrit. Fluids 9 (1996) 118.
[9] S.L. Taylor, J.W. King, J.I. Greer, J.L. Richard, J. Food Prot. 60 (1997) 698.
[10] Y.Y. Wigfield, J. Selwyn, S. Khan, R. McDowell, Chemosphere 32 (1996) 841.
[11] S.B. Hawthorne, D.J. Miller, D.E. Nivens, D.C. White, Anal. Chem. 64 (1992) 405.
[12] J.W. Hills, H.H. Hill, J. Chromatogr. Sci. 31 (1993) 6.
[13] B. Murugaverl, K.J. Voorhees, J. Microcol. Sep. 3 (1991) 11.
[14] H.R. Johansen, G. Becher, T. Greibrokk, Fresenius' J. Anal. Chem. 344 (1992) 486.
[15] H.R. Johansen, G. Becher, T. Greibrokk, Anal. Chem. 66 (1994) 4068.
[16] M.V. Djordjevic, D. Hoffmann, J. Fan, B. Prokopczyk, M.L. Citron, S.D. Stellman, Carcinogenesis 15 (1994) 2581.
[17] E.G. Alley, G. Lu, J. AOAC Int. 78 (1995) 1051.
[18] H.-B. Lee, T.E. Peart, A.J. Niimi, C.R. Knipe, J. AOAC Int. 78 (1995) 437.
[19] R.C. Hale, M.O. Gaylor, Environ. Sci. Technol. 29 (1995) 1043.
[20] B. van Bavel, P. Dahl, L. Karlsson, L. Hardell, C. Rappe, G. Lindstroem, Chemosphere 30 (1995) 1229.
[21] B. van Bavel, M. Järemo, L. Karlsson, G. Lindstroem, Anal. Chem. 68 (1996) 1279.
[22] H.R. Johansen, C. Thorstensen, T. Greibrokk, G. Becher, J. High Resolut. Chromatogr. 16 (1993) 148–152.
[23] L. Mathiasson, E. Björklund, P. Persson, M. Järemo, J. Chromatogr. A, submitted for publication.