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Stability and purity of low-polarity adsorbents for coupled supercritical fluid extraction–supercritical fluid chromatography–flame ionization detection

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Abstract

Several adsorbent materials have been evaluated for their purity and stability when used and reused in on-line solid-phase adsorption (SPA)–supercritical fluid extraction (SFE)–supercritical fluid chromatography (SFC) with neat carbon dioxide as mobile phase and flame ionization as universal detection. The low-polarity adsorbents in this study were made of deactivated glass, solid and porous silica, polymeric beads and a continuous bed of polyacrylamide.

Keywords: Adsorbents; Supercritical fluid chromatography; Supercritical fluid extraction; Stearic acid methyl ester; Pentacosanoic acid methyl ester; Trilaurin; Cholesteryl palmitate; Fatty acid methyl esters

1. Introduction

The use of adsorbents in supercritical fluid extraction (SFE) was first reported for air sampling applications [1–3]. It was not until recent years that solid-phase SFE started to become more common for the analysis of liquid samples. Several types of adsorbents have, however, been used in SFE of liquid samples to date. The most common adsorbents are bare or surface-modified silica particles [4–16] or Tenax particles [3,5,6,10,15,17]. Among the polymer-based particles that have been reported, polystyrene–divinylbenzene (PS–DVB) [1,3,4,6,10,16,18], polyurethane [1,7,15,19], and polyimide particles [2] have been the most intensively studied. Materials based on carbon have also been used [1,3,5,6], as well as the silicate material Florisil

[5,6,10], glass beads [8,20,21], alumina [6,7,15], and cellulose [8]. The adsorbents discussed in these articles represent different types of bead materials, porous or solid. Lately, another group of adsorbents, i.e. extraction disks, also have gained interest [10,14,16,22,23]. More and more, adsorbent materials are used in instrumentation where SFE is coupled on-line to a chromatographic system [10,12,17]. Surface-modified adsorbents then become important in tailoring the surface chemistry for selective enrichment. It is, however, of utmost importance that the bulk adsorbent materials are compatible with the mobile phases used in both the adsorption and the desorption step.

In this study, a number of adsorbent materials with different chemical compositions and physical structures have been investigated for their suitability in on-line solid-phase adsorption (SPA)–SFE–SFC

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using samples in hydrophilic solvents and a neat-carbon dioxide mobile phase for chromatography, followed by flame ionization detection. The background signals from off-line SFE purified adsorbents and from adsorbents cleaned on-line for automated reuse, have been studied. The recovery from adsorption/extraction of 10 μl of a standard solution (2 $\mu\text{g}/\text{ml}$ each of stearic acid methyl ester, pentacosanoic acid methyl ester, trilaurin, and cholesteryl palmitate in ethanol), with a subsequent 100- μl solvent plug of ethanol, was used to comparatively evaluate the adsorbents. The set of standard solutes is considered to span a wide enough range of polarities to be useful to characterize the differences in non-polar adsorbents to be used with neat CO_2 desorption. Finally, recommendations are made with respect to suitable physical structures of the adsorbents to be used for on-line SFE–SFC analysis.

2. Experimental

2.1. Instrumentation

The analyses were performed with a supercritical fluid chromatograph, Series 600 (Dionex, Sunnyvale, CA, USA) equipped with on-line SFE and flame

ionization detector (FID). The extraction cells (0.167 ml, Keystone, Bellefonte, PA, USA) contained two stainless-steel frits, 0.5 μm pore size. The sample was injected automatically with a CMA/200 autosampler (CMA/Microdialysis, Stockholm, Sweden), and a modified LC pump (Model P-500, Pharmacia, Sollentuna, Sweden) was coupled to the autosampler as a solvent delivery system. The pump was controlled by a LC controller (LCC-500, Pharmacia). The analytes were extracted and concentrated in a cryo-trap held at -20°C using carbon dioxide (4.0 quality, AGA Gas, Sundbyberg, Sweden), prior to the chromatographic run. The analytical column was a 5 m \times 50 μm I.D. SB-Octyl-50 column, film thickness 0.25 μm (Dionex) coupled to a frit restrictor (Dionex). Carbon dioxide, SFC-grade, was purchased from Air Liquide Gas (Malmö, Sweden). Nitrogen (Plus quality, AGA Gas) was used for drying the adsorbent contained in the extraction cell. A diagram of the instrumental setup is shown in Fig. 1. The extraction cell was connected to the trap with a linear fused-silica restrictor. The restrictor was inserted into a deactivated [24] but uncoated trap and precolumn of fused-silica. This precolumn was connected to the analytical column with a glass connector (fused-silica coupler, Dionex). The entire system was controlled and data processed with a computer,

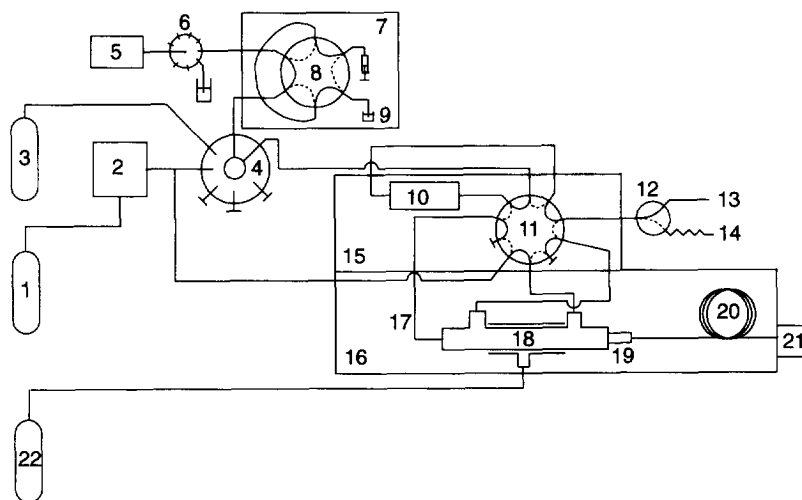


Fig. 1. Instrumentation. 1= CO_2 , SFC-grade; 2=pump; 3=nitrogen; 4=multi-position valve; 5=LCC-500 pump; 6=motor valve; 7=autosampler; 8=6-port valve; 9=sample or rinse solution; 10=extraction cell; 11=10-port valve; 12=3-port valve; 13=vent line; 14=linear restrictor; 15=SFE oven; 16=SFC oven; 17=linear restrictor; 18=cryo-trap; 19=glass connector; 20=column; 21=detector; 22= CO_2 .

Model Scandic 486 (Scandic Products, Uppsala, Sweden). The instrumentation is described elsewhere in more detail [21].

For precleaning of the adsorbents an SFE-703M extractor from Dionex was used. The adsorbents were transferred to a 2.5-ml extraction cell (Keystone) and extracted with neat or methanol-modified carbon dioxide (SFC-grade, Air Liquide Gas). A linear restrictor with a 30 μm I.D. was used (Dionex).

2.2. Adsorbents

The adsorbents used in this study were: glass beads with a particle size of 30 and 50 μm ; solid-core silica with a particle size of 25 μm (Vydac, Hesperia, CA, USA); porous silica particles with a particle size of 5 μm (Eka Nobel, Bohus, Sweden); polyacrylamide continuous bed gel (Biomedical Center, Uppsala University, Uppsala, Sweden); PS–DVB particles with different polymer proportions and particle sizes of 5 μm , 10 μm (Dyno Particles, Lillestrøm, Norway) and 300–840 μm (i.e. 20–50 mesh) (Serva, Feinbiochemica, Heidelberg, Germany); styrene-free polymer like DVB–EVB (ethylvinylbenzene) (Dionex, Sunnyvale, CA, USA); and polymethacrylate (Umeå University, Umeå, Sweden) with a particle size ranging from 105 to 149 μm . See Table 1 for further information.

2.3. Pretreatment of the adsorbents

Adsorbents made of silica (adsorbents A–F in Table 1) were dried in a nitrogen atmosphere at 250°C for 12 h. The glass material (adsorbents A and B) was deactivated with octamethylcyclotetra-siloxane, D₄, (Aldrich, Steinheim, Germany) in an ampule at 400°C for 2 h under nitrogen atmosphere. The deactivated material was thereafter cleaned with supercritical CO₂ at 450 atm (1 atm=101.3 kPa) and 50°C for 15 min, followed by an additional cleaning extraction with supercritical CO₂ at 450 atm and 100°C for 30 min. Silica particles (adsorbents C–F) were coated with D₄ in a similar reaction vessel as reported by Payne et al. [25], after which they were transferred to an ampule which was sealed under nitrogen and heated to 400°C for 2 h. The ampule was cooled to room temperature before it was opened and the material was cleaned in a three-step extraction procedure as follows: (i) methanol-modified CO₂ (5 mol%) at 450 atm and 50°C for 15 min; (ii) methanol-modified CO₂ (5 mol%) at 450 atm and 100°C for 30 min; and, finally, (iii) pure CO₂ at 450 atm and 100°C for 15 min to remove methanol from the adsorbent. If the adsorbents did not fill the extraction cell completely, the cell was filled with CO₂ cleaned glass wool, to prevent channel formation in the adsorbent. Particles made of PS–DVB (adsorbents G–L), DVB–EVB (adsorbent M), poly-

Table 1
Specifications for the evaluated adsorbents

Adsorbent	Type	Material	Particle size (μm)	Pore size (Å)	Surface area (m^2/g)	Surface modification
A	Nonporous beads	Glass: (SiO ₂) _n ; B ₂ O ₃ ; Na ₂ O	30	–	–	Methyl
B	Nonporous beads	Glass: (SiO ₂) _n ; B ₂ O ₃ ; Na ₂ O	50	–	–	Methyl
C	Beads with solid core	Silica: (SiO ₂) _n	25	300	100	Methyl
D	Porous beads	Silica: (SiO ₂) _n	5	100	310	Methyl
E	Porous beads	Silica: (SiO ₂) _n	5	150	250	Methyl
F	Porous beads	Silica: (SiO ₂) _n	5	200	210	Methyl
G	Porous beads	PS–DVB (80:20)	300–840	90	330	–
H	Porous beads	PS–DVB (80:20)	300–840	50	750	–
I	Nonporous beads	PS–DVB (98:2)	5	–	–	–
J	Nonporous beads	PS–DVB (98:2)	10	–	–	–
K	Porous beads	PS–DVB (65:35)	5	<1000	675	–
L	Porous beads	PS–DVB (65:35)	10	<5000	500	–
M	Porous beads	DVB–EVB (55:45)	4.5	230	120	–
N	Nonporous beads	Polymethacrylate	105–149	–	0.1	–
O	Continuous bed	Polyacrylamide	–	–	–	–

methacrylate (adsorbent N) and polyacrylamide (adsorbent O) were cleaned in the same way as described for the deactivated silica particles. The PS-DVB (80:20) materials, XAD-2 and XAD-4, had earlier been washed with methanol and stored in a mixture of water and methanol (50:50) prior to the extraction with supercritical CO₂. Adsorbents K and L were stored in methanol and adsorbents I and J were stored in water, when delivered from the manufacturer.

2.4. Chemicals

Pentacosanoic acid methyl ester, stearic acid methyl ester, cholesteryl palmitate, and trilaurin were obtained from Sigma (St. Louis, MO, USA). A standard solution (2 µg/ml) was prepared in ethanol. Methanol (LiChrosolv quality) was used as modifier and purchased from Merck (Darmstadt, Germany). Octamethylcyclotetrasiloxane was used for deactivation (Aldrich).

2.5. Analytical procedure

The extraction cells were loaded with cleaned (see Section 2.3) adsorbents by dry-packing except for the DVB-EVB material (adsorbent M) that was packed using a methanol slurry and the polyacrylamide gel (adsorbent O) that was packed in a water slurry. Prior to analysis, the packed cell was cleaned by SFE, in the on-line system, using carbon dioxide at 400 atm and 80°C for 10 min. The extraction was repeated until no differences were seen in two subsequent SFC chromatograms (shown in Fig. 2). A standard solution in ethanol was automatically injected onto the adsorbent; thereafter followed a drying step with nitrogen purge and extraction with supercritical CO₂ (SPA-SFE). The extraction was performed at 400 atm with neat CO₂ at 80°C for 10 min. After the SPA-SFE step, simultaneously with the on-line SFC analysis, the cell was cleaned by backflushing supercritical CO₂ at 80°C and using the same pressure program as used for the chromatography, i.e. 100–140 atm at 2 atm/min, then 140–400 atm at 10 atm/min. Another blank SFE-SFC run followed to check the memory effects from the adsorbents. The procedure with sample injection and blank run was repeated three times for each adsorbent.

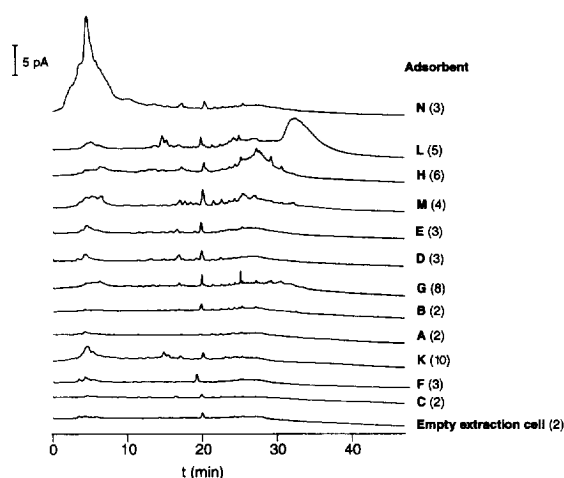


Fig. 2. Chromatograms showing the background signal for each adsorbent (A–H, K–N), and from an extraction cell containing no adsorbent. Conditions: extraction with CO₂ at 400 atm and 80°C for 10 min; chromatography with CO₂ at 80°C, 100–140 atm, 2 atm min⁻¹; 140–400 atm, 10 atm min⁻¹ (1 atm=101.3 kPa). Number of CO₂ blank extractions in the on-line system, required to obtain two runs with no differences in background level, are shown in brackets.

3. Results and discussion

An adsorbent that should be used and reused in an on-line system with universal detection has to comprise a number of specific properties of both physical and chemical character in order to ensure a satisfying operation. For example, it is important to ensure uniform accessibility to the entire surface on the adsorbent. The size of the pores could be of importance. A different degree of swelling during the adsorption and desorption steps would potentially allow sample molecules to become trapped within the particles. The kinetics also need to be fast and the process should be quantitative both for the adsorption and the desorption step. Chemical purity and stability requirements are extremely high for all conditions that will be used with the adsorbent. The physical stability of the adsorbents themselves as well as of the total packed bed is of high importance in obtaining reproducible results. In this preliminary approach, a number of adsorbents with different chemical and physical properties were tested for their feasibility to be used in solute adsorption of a standard from an alcohol solution followed by

supercritical carbon dioxide desorption and on-line post-cleaning for repetitive analysis.

The silica-based adsorbents investigated in this study were glass, solid-core silica and porous silica particles. They were deactivated with a covalently bonded and dense layer of methyl siloxane groups that effectively covers most of the otherwise highly adsorptive and unstable silanol groups. In the different solvents the silica-based particles do not swell to the same extent as the organic polymers, but there is a potential risk of entrapment of analytes in narrow deep pores. Materials having pore sizes of 100, 150, 200 and 300 Å were therefore compared. The results show that the particle size and pore size themselves do not influence the peak areas of the analytes and the reproducibility (Table 1 and Fig. 3). If the silica-based material is used for fatty acid methyl esters and trilaurin, however, larger peak areas were achieved with smaller pore size. For all four analytes in the standard it was advantageous to use a silica adsorbent with a larger surface area, resulting in larger peak areas. Fig. 4a shows an example of a chromatogram of the analytes when the silica ad-

sorbent with the largest surface area (adsorbent D) was used.

The organic polymers that have been investigated in this study were mostly different combinations of PS–DVB. DVB acts as a crosslinker, and with a higher amount of crosslinker the particles can withstand the high pressure that is expected to be preferred with the technique described. The surface of the PS–DVB particles is mainly hydrophobic and thus attracts organic analytes by Van der Waals forces [26]. Due to the aromatic rings in the bulk material, electron donor interactions (π – π) with aromates are expected [27]. A similar polymer made of DVB–EVB was also investigated. A more polar material, i.e. methacrylate particles, containing oxygen in ester bond structures was also tested. The synthesis of the non-porous co-polymer is described elsewhere [28]. A third type of organic polymer, polyacrylamide gel, was used in the formation of a so-called continuous bed [29]. These different types of polymer adsorbents represent materials with and without pores. More information about the physical properties of the adsorbents studied can be found in Table 1. A

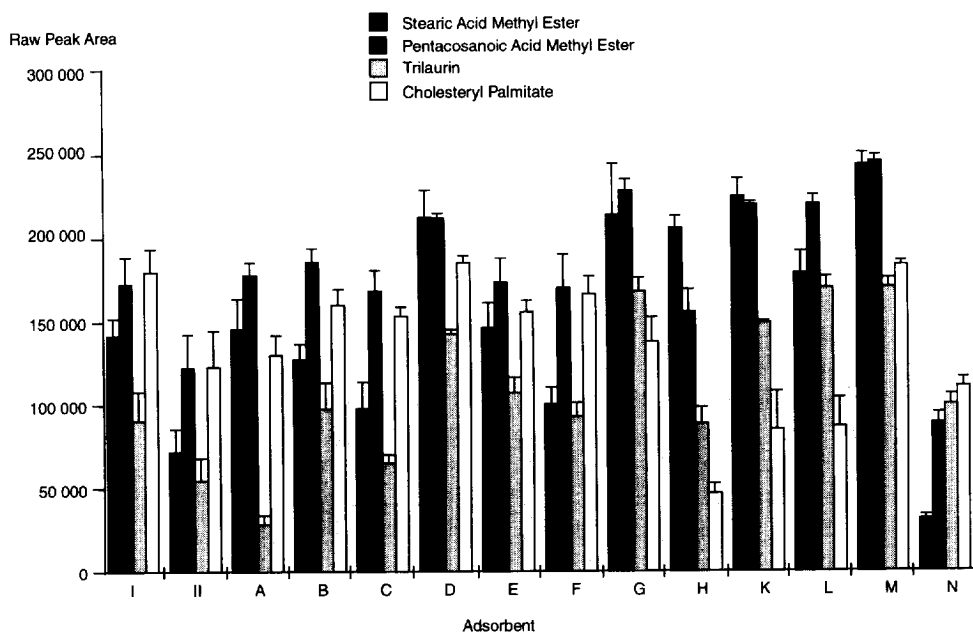


Fig. 3. Bar chart showing mean raw peak areas for the four analytes on each adsorbents (A–H, K–N), named as in Table 1. The error bars show the standard deviation from three injections. For comparison, the results from injections into an empty cell are shown (I=manual injection direct in cell, II=automatic injection).

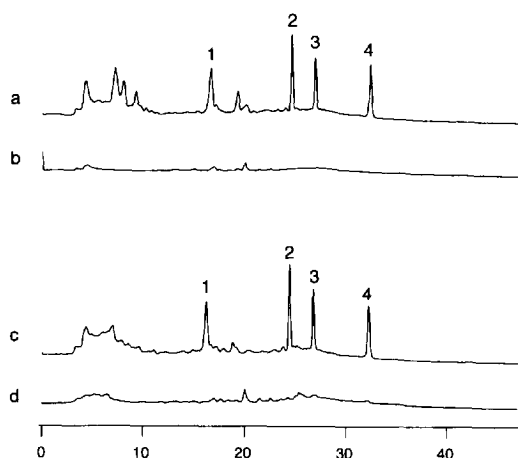


Fig. 4. Chromatograms showing the four analytes and the peak sizes in comparison to the corresponding background signal. Peaks: 1=Stearic acid methyl ester; 2=pentacosanoic acid methyl ester; 3=trilaurin; 4=cholesteryl palmitate. (a) Sample injection onto adsorbent D. (b) Blank run with adsorbent D. (c) Sample injection onto adsorbent M; (d) Blank run with adsorbent M. Conditions, see Fig. 2.

phenomenon described by Burford et al. [30], when trying to use polyacrylamide gel as drying agent in SFE, was found to be important also in the present study. They found that the gel became a hard plug after exposure to a pressure of 400 atm. They suggested that water mainly flowed along the interior extraction cell wall instead of through the dried part of the agent. Lohleit et al. encountered similar problems with Carboxypack B, which could not withstand supercritical conditions and was compressed to a block [5]. As indicated, the same phenomenon was observed in the present study where both the polyacrylamide (material O in Table 1) and the PS-DVB particles with a low degree of crosslinking (2%, materials I and J) acted in a similar way. These adsorbents did not withstand the pressure and were compressed to hard rods in the extraction cell and thus they were excluded from further tests.

The polymer adsorbents that had physical stability for supercritical fluid CO₂ cleanup, i.e. the PS-DVB adsorbents with >2% DVB, DVB-EVB and the polymethacrylate material, were used for an adsorption/desorption test. It is difficult to make any conclusions with respect to the pore size of the polymeric adsorbents, since the exact pore sizes of adsorbents K and L are unknown. The optimal

surface area was reversed compared to the silica-based material, i.e. while silica-based material gave superior performance for adsorbents with larger surface area, the DVB adsorbents gave better results for smaller surface areas. Fig. 4c shows a chromatogram of the analytes tested, when they were injected onto the DVB adsorbent with the lowest surface area (adsorbent M). If the surface area for PS-DVB was higher the release was slower and memory effects occurred. Wong et al. [3] reported that XAD-4 should be recommended as adsorbent instead of XAD-2, because of its better trapping efficiency for volatile compounds. For the application described in the present paper, XAD-2 (adsorbent G) with its larger pore size and smaller surface area would be recommended over XAD-4 (adsorbent H). This is in agreement with the results obtained by Lin et al. [15] who also found that the smaller surface area of XAD-2 gave higher recoveries than XAD-4. The extremely low surface area of the polymethacrylate material (adsorbent N) showed an interesting recovery pattern different from that of the other tested adsorbents. One explanation for the low recovery could be the actual sample breakthrough due to the low surface area of the material instead of irreversible adsorption or slow release in the desorption process. A porous particle with larger surface area might yield a higher recovery.

A bar chart of the recovery (expressed as mean raw peak area) for the four tested analytes on the different adsorbent materials is shown in Fig. 3. The standard deviation is also shown for three repetitive extraction injections performed on the same adsorbent with intermediate post-cleaning. Direct injection in an empty cell and auto-injection in an empty cell are also shown to give an indication of instrument effects on recovery. It is difficult to draw safe conclusions from these data since the flow dynamics in an empty cell is not comparable to that in a packed bed. It is significant, however, that some discrimination can be contributed to the auto-injection. In general, in the cells filled with adsorbent the fatty acid methyl esters and trilaurin gave higher recovery on the DVB polymeric adsorbents than on the other types of adsorbents. This might be due to the electron donor interactions which make it easier to adsorb the analytes from the ethanol solvent. At the same time, the recovery of cholesteryl palmitate

was lower on these materials, except for adsorbent M, compared to that found on silica-based materials. It should be noted that the PS–DVB materials showed memory effects from cholesteryl palmitate in the subsequent blank runs. It is suggested that the π – π interactions between the ring structures were too strong to be completely desorbed by the action of the fairly non-polar supercritical carbon dioxide.

It was earlier pointed out that one primary goal of the present methodology is to be able to use a universal and sensitive detector for non-polar to medium-polar analytes. With the instrumentation used in this study, it is thus important to use a clean adsorbent giving a low background signal in the FID. The impurities found in PS–DVB adsorbents are a known problem that has been discussed earlier, e.g. for XAD-2 and XAD-4 [31,32]. Also the interferences from C_{18} bonded porous silica cartridges have been discussed [33]. It has been reported how adsorbents can be precleaned with Soxhlet extraction prior to SFE [6]. In the present study we investigated if it was sufficient to preclean the adsorbents with high-density supercritical CO_2 in an off-line extractor and then directly use them in the on-line instrumentation. In the pre-cleaning off-line extraction the adsorbents were exposed to both higher pressure and higher temperature than were used in the on-line system. In addition, methanol-modified CO_2 (5 mol%) was used as extraction fluid to remove more polar impurities in the polymeric adsorbents. After the off-line cleaning, the adsorbents were transferred to the on-line system and extractions were made with pure CO_2 until no differences were seen in subsequent chromatograms. In Fig. 2 the background signals of the cleaned adsorbents are shown. In general, the silica-based materials were purer and more easily cleaned. The organic polymers needed more extractions and even after that they had a somewhat higher background signal. However, in this case the background signal did not disturb the analyte signal. The poly-methacrylate adsorbent (N) had a high background signal in the beginning of the chromatogram that could result from early-eluting monomers remaining from the polymer production. When ethanol was injected onto the adsorbents, there was often a higher background from polymer-based adsorbents than from silica-based ones. If there were any impurities

in the ethanol, from the tubings, etc., they were probably polar and because of that more easily adsorbed onto the polymer adsorbents.

After adsorption of the analytes, the purge time and pressure of nitrogen used to dry the adsorbents from residual ethanol, was set as short and low as possible to reduce the risk of loss of analyte (Table 2). If the adsorbent was not dry enough before use, a large solvent peak occurred in the chromatogram and the restrictor was periodically blocked. The silica-based adsorbents were easiest to dry, needing 2–10 min at a pressure of 10–20 p.s.i. (1 p.s.i.=6.89 kPa). The organic polymers needed a longer drying time of 10–30 min, and a nitrogen pressure of 10–40 p.s.i., probably due to their more polar character. The DVB material (M) needed only 10 min while the poly-methacrylate adsorbent (N) needed 30 min of drying with a nitrogen pressure of 40 p.s.i. to remove all solvent. That is probably another reason, in addition to the low surface area, why the recoveries were so low from that adsorbent (Fig. 3); the analytes, especially the volatile analytes, followed the nitrogen out through the vent tubing. It should be added, that if a 10- μ l water plug was injected, the drying times shown in Table 2 were long enough to prevent plugging in the cryo-trap except for adsorbent C and E.

It can be concluded that all adsorbents tested in this study are reusable for at least three times without

Table 2
Nitrogen pressure, flow and purge time used to dry each adsorbent after the SPA step

Adsorbent ^a	Pressure (p.s.i.) ^b	Flow (ml min ⁻¹) ^c	Time (min)
A	10	27	2
B	Unknown	Unknown	2
C	20	60	2
D	10	13	10
E	10	15	5
F	10	12	5
G	10	27	10
H	10	30	10
K	10	14	10
L	10	26	10
M	10	11	10
N	40	141	30

^a See Table 1 for description of each adsorbent.

^b 1 p.s.i.=6.89 kPa.

^c Measured at vent line, cf. Fig. 1, No. 13.

opening the solid-phase extraction cell. The methyl deactivated glass beads (adsorbent B) have been successfully reused for at least 60 times [21]. Adsorbents K–N showed memory effects for the late-eluting analytes; thus, if they should be reused, a different extraction procedure, or one or two blank runs are needed between sample injections. The polymeric DVB–EVB adsorbent (M) gave promising results with the highest recovery of all adsorbents, and together with the 5- μm silica particles with 100-Å pores that showed a combination of easy cleaning, high recovery and absence of memory effects, these two adsorbents were chosen as the best-performing adsorbents in this study. Fig. 4 shows chromatograms for sample injections and blank runs from the two recommended materials.

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