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Analysis of volatile organics by supercritical fluid extraction coupled to gas chromatography I. Optimization of chromatographic parameters

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Abstract

A solid-based calibration standard (consisting of several *n*-alkanes and aromatic hydrocarbons spiked onto Tenax-TA) was successfully used to optimize the chromatographic parameters for coupled supercritical fluid extraction–gas chromatography (SFE–GC). A simple and reliable split SFE–GC system was developed utilizing a commercially septumless injector installed on a split/splitless injection port. The high gaseous flow-rate generated inside the injection port during the SFE step was accommodated for by using the correct split ratio, so that high (1 ml/min liquid CO_2) SFE flow-rates could be used. The use of thick-film columns (5 μm film thickness) and cryogenic trapping temperatures in the GC oven as low as −50°C allowed efficient trapping of species as volatile as *n*-butane, acetone and methylene chloride. The chromatograms obtained using the optimized SFE–GC technique showed good peak shapes (comparable to those obtained using a conventional split injection) and peak area reproducibilities typically $<5\%$ relative standard deviation.

1. Introduction

Directly coupling sample extraction techniques with sample analysis has recently received a significant amount of attention due to the potential of the "coupled technique" to achieve very rapid, sensitive and cost effective analysis. Coupled or on-line extraction/analysis methods generally reduce the time required for sample extraction, analyte collection and analyte concentration. Since sample preparation and handling steps are minimized, the potential for analyte loss, degradation, and/or contamination is reduced and more sensitive analyses can be achieved. Sample throughput can also be improved because the extraction and analysis occur in the same step, and on-line methods requiring less than 1 h for both extraction and gas chromatographic (GC) analysis have been reported [1–3].

Supercritical fluid extraction (SFE) is an ideal extraction technique to directly couple with capillary GC, since the gaseous effluent obtained in SFE after depressurization is compatible with GC analysis. Furthermore, using CO_2 as the supercritical fluid allows the direct use of flame ionization detection (FID) as the CO_2 does not have a FID response [4]. SFE also has the potential to extract a wide range of analytes that would normally require liquid solvent extraction

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[5,6], but avoids the problems related to introducing large volumes of liquid solvents onto a chromatographic column as the extracted analytes are introduced to the GC in the gas phase.

To achieve quantitative SFE–GC a number of experimental conditions need to be established. First, the target analyte must be efficiently extracted from the sample matrix; then the analytes must be quantitatively transferred from the SFE system to the GC; and finally, the analytes need to be chromatographically separated. The main limitation of the directly coupled SFE–GC system is with the collection and focusing of the extracted analytes and this, in turn, is related to the high gas flow-rate generated from the depressurization of the supercritical fluid. For example, using the common extraction flow of 1 ml/min of liquid CO_2 results, upon depressurization, in a CO_2 gas flow of ca. 500 ml/min. At such high gas flow-rates, poor refocusing of the analytes can occur resulting in poor peak shapes [2]. Conversely, if a low SFE flow-rate is used that results in good peak shapes, very long times might be required to completely extract the analytes from the sample [7]. An additional problem is that the analytes are extracted over a relatively long period of time (typically 10 to 30 min) compared to a typical capillary GC peak which is only about 1 s wide.

The aim of this work is to determine and optimize the experimental parameters which affect the collection and focusing of extracted analytes at the head of the chromatographic column during the extraction step in SFE–GC–FID analysis. The development and testing of a simple and reliable technique for performing split SFE–GC that utilizes a commercially available septumless injector installed on a split/splitless injection port is described. Only minor modifications to the GC instrumentation are required, and the same GC injection port can be used for liquid solvent injections or SFE–GC without conversion. A calibration mixture of *n*-alkanes and BTEX (benzene, toluene, ethylbenzene, *m*-xylene and *o*-xylene) spiked onto Tenax-TA is used to optimize the "coupling" of the SFE system to the GC system. Comparisons of peak shapes and quantitative results between split SFE–GC and conventional split injections are also presented.

2. Experimental

2.1. Instrumentation and methods

SFE–GC–FID analysis was performed using a Hewlett-Packard 5890 gas chromatograph with helium as the carrier gas. A detailed schematic of the equipment is seen in Fig. 1. Three fused-silica capillary columns were investigated for use with SFE–GC; namely, a wide-bore (30 m × 0.32 mm I.D., 5 μm film thickness) DB-1 column, a wide-bore (30 m × 0.32 mm I.D., 1 μm film thickness) DB-5 column, or a narrow-bore (20 m × 0.25 mm I.D., 0.25 μm film thickness) DB-5 column, all supplied by J & W Scientific, Folsom, CA, USA. The septum and septum cap supplied with the conventional split/splitless injection port were replaced with a septumless injector (Model SLI-M) and installed according to the manufacturer's instructions (SGE, Austin, TX, USA). The injection port and the flame ionization detector were both operated at 300°C.

Initially, the mass flow controller supplied with the GC instrument controlled the carrier gas flow-rate. However, (as discussed later) the high gas flow-rates generated during SFE–GC proved unsuitable for use with the mass flow controller (and the back-pressure regulator supplied with the 5890 GC system). The GC system was modified so that the carrier gas flow-rate was controlled by a head pressure regulator situated on the carrier gas cylinder (see Fig. 1). The column head pressure was measured with a pressure gauge installed on the carrier gas line. A toggle shut-off valve was installed on the carrier gas supply line between the head pressure regulator and the injection port to allow the carrier gas line to be closed during the SFE step. The septum purge on the injection port was also closed by installing a cap nut on the septum purge outlet. Note, no bleed from the silicone seal or O-ring situated inside the septumless injector was detected during the SFE–GC study.

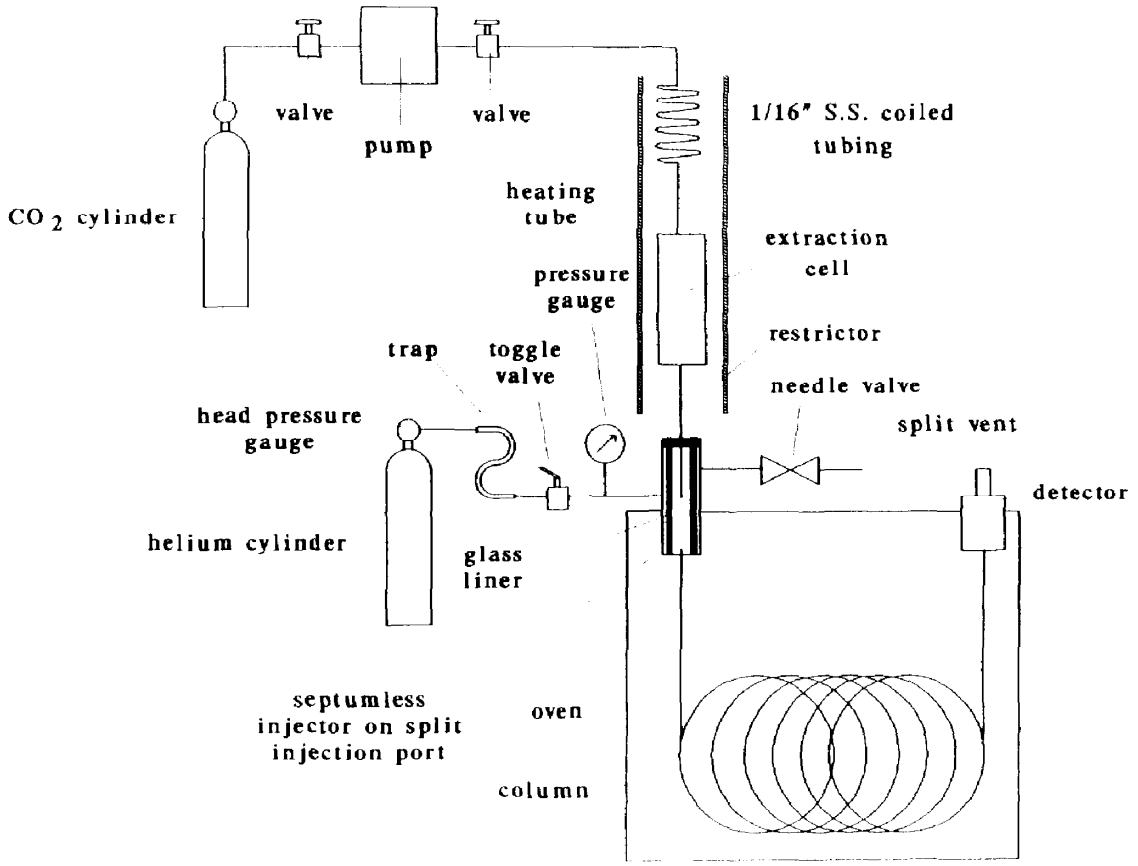

Fig. 1. Schematic diagram of the split SFE–GC–FID system. S.S. = Stainless steel; " = in. (1 in. = 2.54 cm).

The split ratio was controlled with a needle valve on the split line outlet (see Fig. 1).

Supercritical fluid extractions were performed with CO_2 (supercritical fluid grade, Scott Specialty Gases, Plumsteadville, PA, USA) and an ISCO Model 260D syringe pump (ISCO, Lincoln, NE, USA). Samples were placed in a 2.5-ml extraction cell from Keystone Scientific (Bellefonte, PA, USA). The flow-rate of the supercritical fluid through the extraction cell was controlled by 9-cm-long restrictors (15, 22, 26 or 30 μm I.D., 150 μm O.D.) cut from fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA). During the extraction, the extraction cell and a pre-equilibration coil [1 m × 0.76 mm I.D. × 1.6 mm (1/16 in.) O.D. coiled stainless-steel tubing placed before the extraction cell to pre-warm the CO_2 to the extraction temperature] were placed inside a thermostated tube heater which was situated directly above the injection port.

Split SFE–GC analysis was performed by inserting the extraction cell restrictor through the septumless injector into the injection port liner (8 cm × 6 mm O.D. × 3 mm I.D. glass tube) so that the end of the restrictor was about 3.5 cm above the chromatographic column's inlet. As the silicone seal inside the septumless injector provided a gas-tight seal around the restrictor and, as the injector septum purge was closed, the supercritical fluid effluent exiting the restrictor either went into the chromatographic column or out the split line. The split ratio used during the extraction was, therefore, controlled by simply adjusting the needle valve on the split line.

The steps used for split SFE–GC analysis were as follows: (1) the GC oven was cooled to the appropriate cryogenic trapping temperature

(from −50 to 25°C for this study); (2) the assembled extraction cell was placed inside the tube heater, the GC carrier gas supply was shut off with the toggle valve, and the extraction cell restrictor was inserted into the injection port through the septumless injector; (3) the extraction cell was pressurized with 400 atm (1 atm = 101 325 Pa) CO_2 at 60°C and the sample was extracted for 10 min (during the extraction, the CO_2 effluent was depressurized in the injection port and the analytes were trapped on the chromatographic column); (4) the extraction cell restrictor was withdrawn from the injection port and the CO_2 effluent was allowed to dissipate from the injection port and column (this usually took about 1 min). After the CO_2 had dissipated, the carrier gas was turned on and the GC analysis begun with the GC oven rapidly heated to 40°C then at 8°C/min to 300°C.

2.2. Samples and standards

A neat mixture of BTEX and C_4–C_{20} *n*-alkanes (ca. 0.5 g each) was prepared in a vial and stored at −10°C. All the hydrocarbons were supplied by Aldrich (Milwaukee, WI, USA). Note, since *n*-butane (C_4) is a gas at room temperature and pressure, it had to be cooled to a liquid to enable the analyte to be accurately added to the neat hydrocarbon mixture. To obtain liquid *n*-butane, the compressed *n*-butane gas was passed through a coiled stainless-steel tube immersed in a dewar of methanol and ice at −15°C. The liquid exiting the coiled tube was collected in a cold (ca. −15°C) 25-ml "pressure-lok" gas syringe (Supelco, Bellefonte, PA, USA) with the plunger removed for ease of fill. After ca. 10 ml of liquid *n*-butane had been collected, the plunger was reinstalled on the syringe, and ca. 0.5 g of the liquid was injected into the neat hydrocarbon mixture.

The neat hydrocarbon mixture was spiked onto several matrices, including 70–80-mesh (180–200 μm) silanized glass beads (Analab, Norwalk, CT, USA) or the sorbent resins, 60–80-mesh Tenax-TA, 60–80-mesh (180–250 μm) XAD-2, 60–80-mesh Carbosieve S-III or 20–40-mesh (420–850 μm) Carbotrap C (Supelco). The silanized glass beads were used as supplied. The sorbent resins were prepared by weighing 1 g of the sorbent resin into a 3.5-ml extraction cell, and preextracting for 30 min with 400 atm CO_2 (60°C) to remove contaminants. Each clean sorbent (400 mg) was placed inside a 2.5-ml extraction cell on a bed of 70–80-mesh silanized glass beads (100 mg), and 0.2 μl of the neat hydrocarbon mixture (ca. 9 μg of each analyte) were injected into the middle of the sorbent. The extraction cell was then sealed and either immediately connected to the SFE–GC apparatus or left for 24 h at room temperature and pressure. Once the extraction cell was connected to the apparatus, the cell was equilibrated at 60°C for 5 min, and then extracted by SFE–GC as described in the *Instrumentation and methods* section.

To quantify the recovery of the hydrocarbons from the matrices two internal standards were used depending on the matrix. For the majority of matrices *n*-decane (C_{10}) was used as the internal standard. However, occasionally the C_{10} *n*-alkane was irreversibly retained on the matrix (e.g., Carbosieve S-III) or evaporated from the matrix when aged 24 h (e.g., silanized glass beads) and, in these instances, an alternative internal standard, octahydroanthracene was used. A solution of octahydroanthracene (18 mg/ml) was prepared in methylene chloride and stored at −10°C. A 0.5- μl aliquot of the internal standard solution (9 μg of analyte) was injected onto a bed of glass beads (100 mg) situated inside a 2.5-ml extraction cell. The glass beads were then left exposed to the atmosphere for 10 min to allow the methylene chloride solvent to evaporate. Once the solvent had evaporated, the glass beads were covered with 400 mg of sorbent resin or with more glass beads, onto which was spiked the hydrocarbon mixture (9 μg of each analyte). The extraction cell was then sealed and analyzed by SFE–GC as described above. Analyte recovery was determined by comparing the split SFE–GC results to a conventional split injection, with the same amount of analytes that were spiked onto the matrices for SFE–GC analysis being injected directly into the GC–FID system.

The SFE–GC technique was further evaluated using several organic solvents including ethanol (Fisher Scientific, Fair Lawn, NJ, USA); acetone (Fisher Scientific); diethyl ether (J.T. Baker, Phillipsburg, NJ, USA); methylene chloride (Fisher Scientific); trichlorotrifluoroethane (Freon 113; Fisher Scientific); chloroform (Fisher Scientific); tetrahydrofuran (J.T. Baker); trichloroethylene (Aldrich); tetrachloroethylene (Aldrich); and chlorobenzene (Fisher Scientific). A mixture of the solvents (ca. 1 g each) and 1 g of the internal standard *n*-heptane was prepared in a brown vial and stored at −10°C. A 0.2- μl aliquot of the mixture (ca. 18 μg of each analyte) was spiked into the middle of 400 mg of Tenax-TA situated inside a 2.5-ml extraction cell. The extraction cell was then sealed, connected to the SFE–GC apparatus, and analyzed as described in the *Instrumentation and methods* section.

3. Results and discussion

3.1. Instrumental modifications required to perform SFE–GC

Split SFE–GC deposits the extracted analytes inside a conventional split injection port and, analogous to a conventional split injection, a fraction of the extracted analytes enters the chromatographic column for focusing in the stationary phase, while the remainder is flushed out the split vent [8–10]. While this approach is simple, reproducible qualitative and quantitative results require a few simple instrumental modifications. The SFE effluent can expand backwards into the carrier gas line during the extraction, because the internal volume of the vaporizing chamber (i.e., glass liner) inside the injection port is relatively small (ca. 0.5 ml) compared to the gaseous flow of the supercritical fluid (ca. 8 ml/s of gaseous CO_2 at a typical liquid CO_2 flow-rate of 1 ml/min), and the pressure generated inside the injection port during extraction can be higher than the carrier gas head pressure. To avoid the extracted analytes contaminating the carrier gas line a shut-off valve was placed near the injection port to block the GC carrier gas flow during the SFE step (Fig. 1). Even though the majority of the SFE effluent (usually ca. 95–99%) was vented out of the injection port through the split vent during the SFE–GC extraction step, the gas flow through the chromatographic column was still sometimes sufficient to extinguish the FID flame. To maintain the FID signal the flame's hydrogen flow was slightly increased, and CO_2 gas flow-rates through the GC column of 10–15 ml/min have been used without quenching the FID flame [11].

The most important modification to the normal configuration of the Hewlett-Packard 5890 GC system was the addition of a needle valve installed onto the split line to control the split flow during the SFE step. In the normal configuration of the 5890 GC system, the split flow exits through the back-pressure regulator (which opens and closes to maintain constant pressure); and, the split ratio is controlled by the amount of carrier gas supplied by the mass flow controller (which is isolated from the GC flows by the toggle shut-off valve during the SFE step). In an unmodified 5890 GC system, the high CO_2 gas flow-rate which enters the injection port during SFE causes the head pressure regulator to open (in an attempt to maintain its set-point pressure) thus, varying the split ratio during the SFE step. While this does not affect the qualitative peak shapes, the change in split ratio which can occur during the SFE step can yield poor quantitative results. Therefore, to assure a constant split ratio during the SFE step, the gas chromatograph was modified so that the split was controlled by a needle valve placed in the split outlet line rather than the normal configuration where the split flow exits through the back-pressure regulator. During the GC analysis, the column head pressure was maintained by the head-pressure regulator situated on the carrier gas inlet line (Fig. 1). This simplified system resulted in good peak area reproducibility (as discussed below) and was also easier to maintain because if the split vent became contaminated during the extraction of very dirty samples, the tubing and needle valve could be easily removed and flushed with solvent.

Because of the good sensitivity possible with SFE–GC, the purity of the entire SFE–GC system had to be rigorously controlled. Minimal valving and other devices in the lines between the carrier gas cylinder, the pump and the extraction cell were used to avoid contamination. The apparatus used in this study (Fig. 1) utilized only three valves, two of which were situated on the inlet and outlet of the pump and the third, a toggle valve, was used to cut off the carrier gas supply during the extraction. The valves were packless to avoid contamination by lubricants and extractable species from components such as O-rings. A high-purity supercritical fluid (supercritical-grade CO_2) was also used because previous studies had found that impurities in the fluid can cause artifact peaks in SFE–GC generated chromatograms [12–14]. The lack of any transfer lines between the SFE cell and the GC injection port also eliminated any carryover between samples.

Several other minor modifications were made to the SFE–GC system. The bottom of the injection port protruding into the GC oven was insulated with a lined cover (as supplied by the manufacturer) so that a sharp temperature boundary could exist between the hot injection port and cold chromatographic column. This distinct temperature gradient helped focus the analytes as a band on the top of the chromatographic column during SFE; and, without the insulated cover, chromatographic peak fronting was observed. The position of the extraction cell restrictor inside the conventional split injection port also proved to be important. The best results were obtained with the tip of the restrictor situated about half way down the glass liner (which is the same position as the tip of a conventional syringe needle). If the restrictor outlet was placed a few millimeters from the chromatographic column inlet then poor peak shapes were obtained. Conversely, if the restrictor was situated just inside the injection port, poor recoveries of high-molecular-mass analytes resulted. Since the injection port was heated to 300°C, the restrictor plugging that commonly occurs with off-line SFE [15] was eliminated entirely.

3.2. Optimizing SFE–GC peak shapes

The ability of the split SFE–GC system to yield good peak shapes was investigated by comparing conventional split injections of a test mix with SFE–GC analysis of the same quantity of test mix from the Tenax-TA resin (selected for a solid-based calibration standard as described below). The test mix contained several *n*-alkanes ranging from a gas (butane) to a solid (eicosane) and BTEX aromatics. The test mix represented the major components present in gasoline and/or diesel fuel, and was seen as an ideal mixture to use to determine the experimental parameters which affected the collection and focusing of extracted analytes on the chromatographic column during the extraction step in SFE–GC. Three experimental factors were investigated: SFE flow-rate, cryogenic trapping temperature and chromatographic column stationary phase thickness.

Previous reports have shown that successful SFE–GC is dependent on the extraction flow-rate and the extraction time [2,3,8]. High extraction flow-rates may be desirable as the potential sample size can be increased, and the extraction time decreased [2,8], though additional factors such as the kinetics and mechanisms of the extraction may also affect the extraction rate [16]. Fig. 2 shows the effect of the SFE flow-rate on the peak shapes generated by SFE–GC which was performed under identical conditions using a wide-bore thick-phase (30 m × 0.32 mm I.D., 5 μm film thickness) chromatographic column at a trapping temperature of −50°C. SFE–GC analysis using extraction flow-rates as high as 0.6 ml/min (measured as liquid CO_2 at the pump, and corresponding to a restrictor with an internal diameter of 26 μm) yielded good peak shapes. The relative peak distribution (peak ratios) obtained using extraction flow-rates up to 0.6 ml/min were essentially identical to the peak distribution from split injection, indicating that the SFE–GC did not introduce any significant splitter discrimination (Fig. 2). However, differences in the absolute peak intensities occurred based on changes in the split ratio with different SFE flow-rates as discussed below.

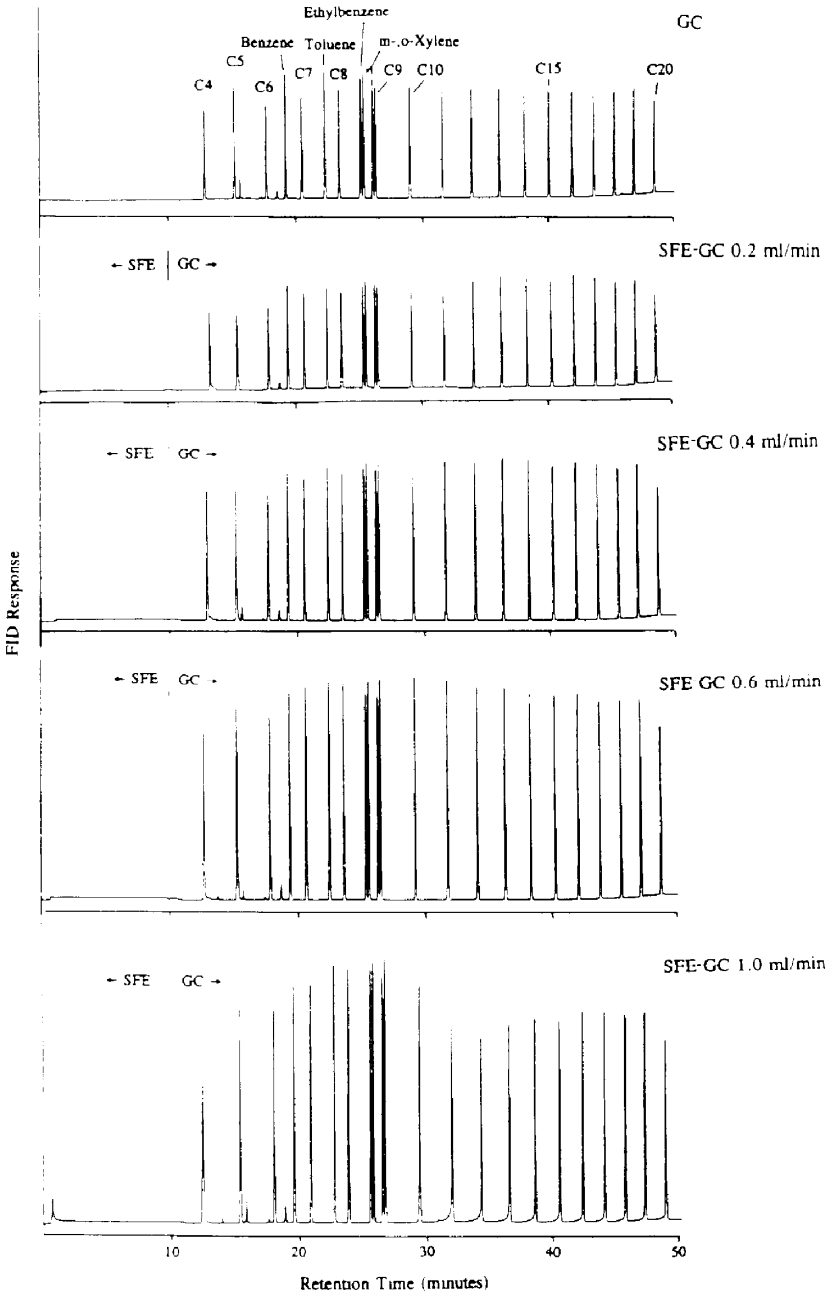

Fig. 2. Effect of SFE flow-rate on the peak shapes of BTEX and C_4–C_{20} *n*-alkanes obtained by split SFE–GC–FID. A neat BTEX–*n*-alkane mixture (0.2 μl) was either injected onto the capillary column (top chromatogram) or the mixture was spiked onto 400-mg Tenax-TA and extracted "on-line" for 10 min with 400 atm, 60°C CO_2 at an extraction flow-rate of 0.2 ml/min (9 cm × 15 μm I.D. restrictor); 0.4 ml/min (9 cm × 22 μm I.D. restrictor); 0.6 ml/min (9 cm × 26 μm I.D. restrictor); or 1.0 ml/min (9 cm × 30 μm I.D. restrictor). For the corresponding split ratios at the various extraction flow-rates see Table 1. The 30 m × 0.32 mm I.D. (5-μm film) DB-1 capillary column was kept at −50°C during the injection or extraction step. After each extraction or 10 min after the injection the GC oven was heated at ca. 50°C/min to 40°C then at 8°C/min to 300°C.

The peak widths at half-height for both the relatively volatile (*n*-butane) and non-volatile (*n*-eicosane) alkanes generated by SFE–GC were essentially identical to those obtained by split injection. The only exception was the peak widths obtained by SFE–GC at the high (1.0 ml/min) extraction flow-rate, where the volatile (*n*-butane and *n*-pentane) and the semivolatile (C_{10}–C_{20}) peak widths are about a third and a fifth (respectively) wider than those obtained by split injection. The slight peak broadening of the early eluting analytes at the high extraction flow-rate is to be expected, as these are the most volatile analytes and are, therefore, the hardest to trap. The peak broadening of the later eluting peaks is discussed below.

The symmetry or shape of the chromatographic peaks obtained by SFE–GC at moderate extraction flow-rates (0.2 to 0.6 ml/min) also compared favorably with those obtained by split injection. However, at the high extraction flow-rate (1.0 ml/min liquid CO_2) poor peak shapes were obtained for the C_{10} to C_{20} *n*-alkanes (Fig. 2). The inefficient focusing of the analytes on the chromatographic column was related to the high gas flow (ca. 590 ml/min, Table 1) resulting from the depressurization of the supercritical fluid inside the injection port. The limiting factor was not the high total volumetric flow-rate passing through the injection port, as higher injection port flow-rates could be tolerated under normal GC conditions (Table 1); instead, the poor peak shapes were associated with the high volumetric flow-rate through the chromatographic column. Peak fronting occurred when the gaseous CO_2 flow-rate through the GC column during SFE–GC exceeded ca. 9 ml/min (Table 1, Fig. 2). By increasing the split ratio so that more of the CO_2 was vented through the split vent and less entered the column, peak fronting was eliminated at the 1 ml/min extraction flow (even though the total gas flow through the injection port was still ca. 590 ml/min using the 30 μm I.D. restrictor). Therefore, the maximum possible SFE flow that can be used and still obtain good peak shapes will depend on the split ratio, that is, higher split ratios (and thus lower columns flows) allow higher SFE flow-rates.

The cryogenic trapping temperature used during the extraction step also affects the ability of SFE–GC to efficiently focus volatile analytes. Fig. 3 shows the effect of the cryogenic trapping temperature on the chromatographic peak shape generated by SFE–GC which was performed under identical conditions with a 26 μm I.D. restrictor (0.6 ml/min liquid CO_2 flow-rate) and

Table 1
Split ratio measured under GC and split SFE–GC conditions

	SFE flow-rate (ml/min)[a]	Column head pressure (p.s.i.)	Column volumetric flow (ml/min)[b]	Split vent volumetric flow (ml/min)[b]	Split ratio (column/split)
GC	–	15	5.1	789	1:155
SFE 15 μm I.D. restrictor[c]	0.18	1	0.8	86	1:107
SFE 22 μm I.D. restrictor[c]	0.38	4	2.8	267	1:95
SFE 25 μm I.D. restrictor[c]	0.58	9	5.0	400	1:80
SFE 30 μm I.D. restrictor[c]	0.96	16	9.2	577	1:63

See Fig. 2 for chromatographic results. 1 p.s.i. = 6894.76 Pa.

[a] Flow-rate measured as liquid CO_2 at pump.

[b] Flow-rate measured as volume of gas using a bubble flow meter. Column flow was measured at the detector end of the column.

[c] SFE–GC conditions: 400 atm, 60°C CO_2, wide-bore, thick-phase (30 m × 0.32 mm I.D., 5 μm film thickness), chromatographic column at −50°C.

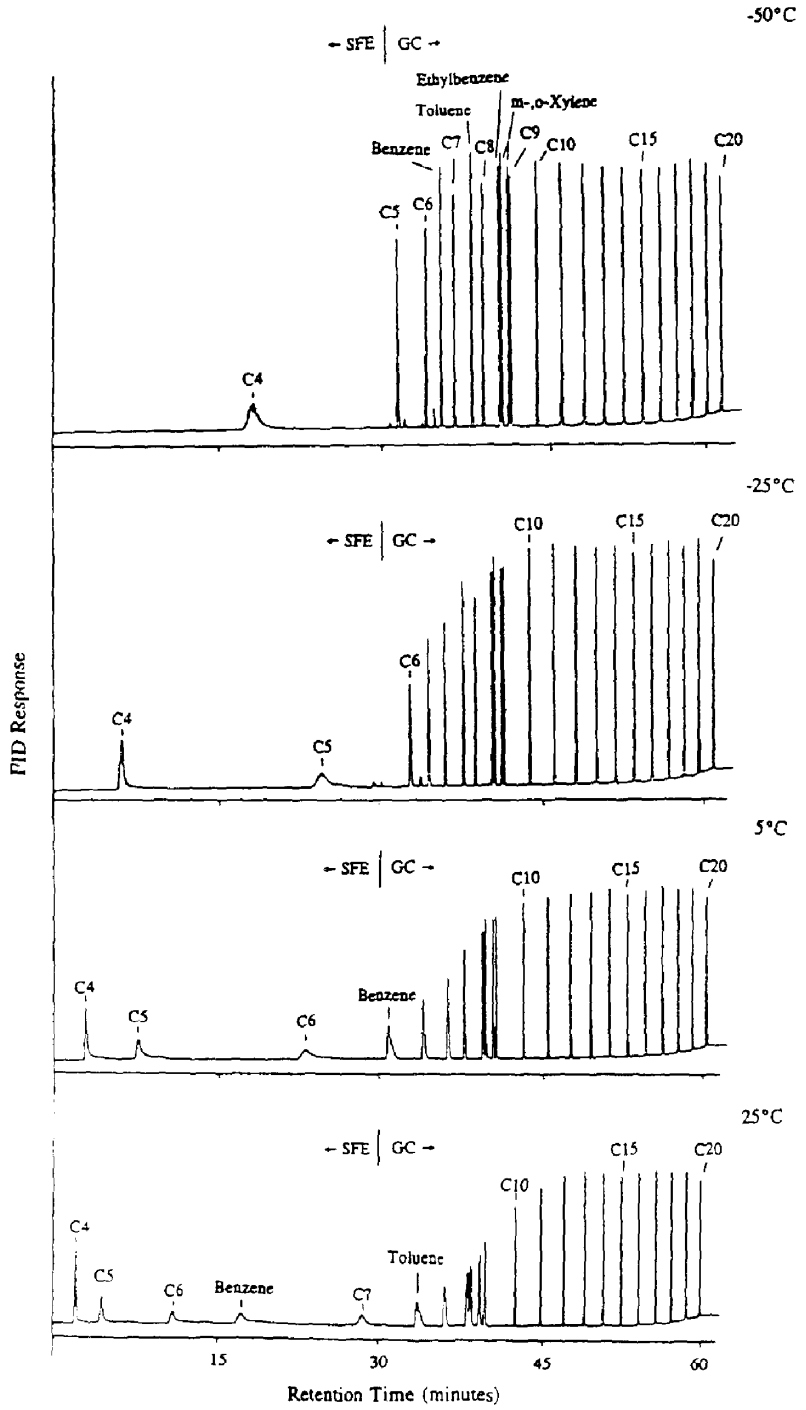

Fig. 3. Effect of the cryogenic trapping temperature on the retention of BTEX and C_4–C_{20} *n*-alkanes on a thick-film (5 μm) 30 m × 0.32 mm I.D. DB-1 capillary column during SFE–GC–FID. A neat mixture (0.2 μl) of BTEX and *n*-alkanes was extracted from Tenax-TA (400 mg) using 400 atm, 60°C CO_2 at 0.6 ml/min for 30 min. During the extraction the capillary column was maintained at a temperature of −50, −25, 5 or 25°C. After each extraction the GC oven was heated at ca. 50°C/min to 40°C then at 8°C/min to 300°C.

a wide-bore thick-phase (30 m × 0.32 mm I.D., 5 μm film thickness) chromatographic column. The coldest temperature investigated (−50°C) yielded chromatograms with the best peak shapes. However, as the SFE time was increased from 10 (Fig. 2) to 30 min (Fig. 3), the *n*-butane peak was broadened and eluted through the GC column during the SFE step. Colder trapping temperatures were not investigated as a means of increasing the trapping efficiency of *n*-butane because at lower temperatures (< −60°C) the chromatographic column stationary phase behaves as a solid [17,18]. When the cryogenic trapping temperature was raised to −25, 5 or 25°C, the trapping efficiency of the chromatographic column correspondingly decreased, and an increasing number of analytes eluted as discrete peaks through the column during the extraction step (Fig. 3). However, even with no cryogenic cooling and the column at room temperature (25°C) during the extraction step, analytes as volatile as *n*-decane could still be efficiently retained. The peak shape of the retained analytes tended to be broader for the more volatile species, but the less volatile C_{10}–C_{20} *n*-alkanes had good peak shapes at all the trapping temperatures investigated.

Finally, the effect of the capillary column stationary phase film thickness on the chromatographic peak shape was investigated (Fig. 4). Each SFE–GC analysis was performed under identical conditions with a 26 μm I.D. restrictor (0.6 ml/min liquid CO_2 flow-rate) and a cryogenic trapping temperature of −50°C. Three chromatographic columns were investigated including the wide-bore (320 μm I.D., 5 μm film thickness) DB-1 column used in the previous studies, a wide-bore (320 μm I.D., 1 μm film thickness) DB-5 column, and a narrow-bore (250 μm I.D., 0.25 μm film thickness) DB-5 column. The column with the thickest stationary phase (5 μm film thickness) was the most efficient in focusing and retaining the analytes during the extraction step (Fig. 4). The 5- μm column was able to give good peak shapes for *n*-alkanes as volatile as pentane even after 60 min of SFE. When chromatographic columns were used with thinner stationary phase thicknesses the trapping efficiency of the SFE–GC decreased, and more analytes eluted through the column during the SFE step. However, the advantage of using a thinner stationary phase is that higher-boiling components can be eluted at reasonable chromatographic temperatures. For example, the thick-film 5- μm column could resolve *n*-alkanes up to ca. C_{25} in a typical GC run, but the thin-film 0.25- μm column could resolve *n*-alkanes up to ca. C_{40} in the same analysis time. Therefore, a trade-off exists when choosing a column film thickness between the ability to efficiently trap very volatile analytes and to elute high-boiling point analytes.

3.3. Quantitative considerations for SFE–GC

The feasibility of directly coupling the SFE step with the GC analysis required the development of a calibration standard to be used to determine when quantitative SFE–GC had been achieved. A solid calibration standard was required whereby the test analytes could be easily and quantitatively spiked onto a solid matrix and then easily and quantitatively extracted from the matrix by SFE–GC, so that only the SFE–GC collection parameters were investigated, and not the SFE extraction efficiencies. To fully evaluate the potential of the on-line technique the *n*-alkane–BTEX test mixture was used. Based on the results of the peak shape studies described above, all subsequent SFE–GC analyses were performed using the 5- μm film thickness DB-1 column, a flow-rate (liquid CO_2) of 0.6 ml/min, a 10-min extraction time, and a cryogenic trapping temperature of −50°C.

Previous off-line SFE collection efficiency studies were based on the extraction of test analytes that were spiked onto relatively inert matrices [15,16]. However, unretentive matrices such as silanized glass beads proved unsuitable for use with the test mix, since a proportion of all of the more volatile analytes were lost from volatilization during the spiking process (Table 2). Thus, a more retentive matrix was required and a number of commercially available sorbent resins were investigated. Sorbent resins were seen as an ideal means of preparing a solid

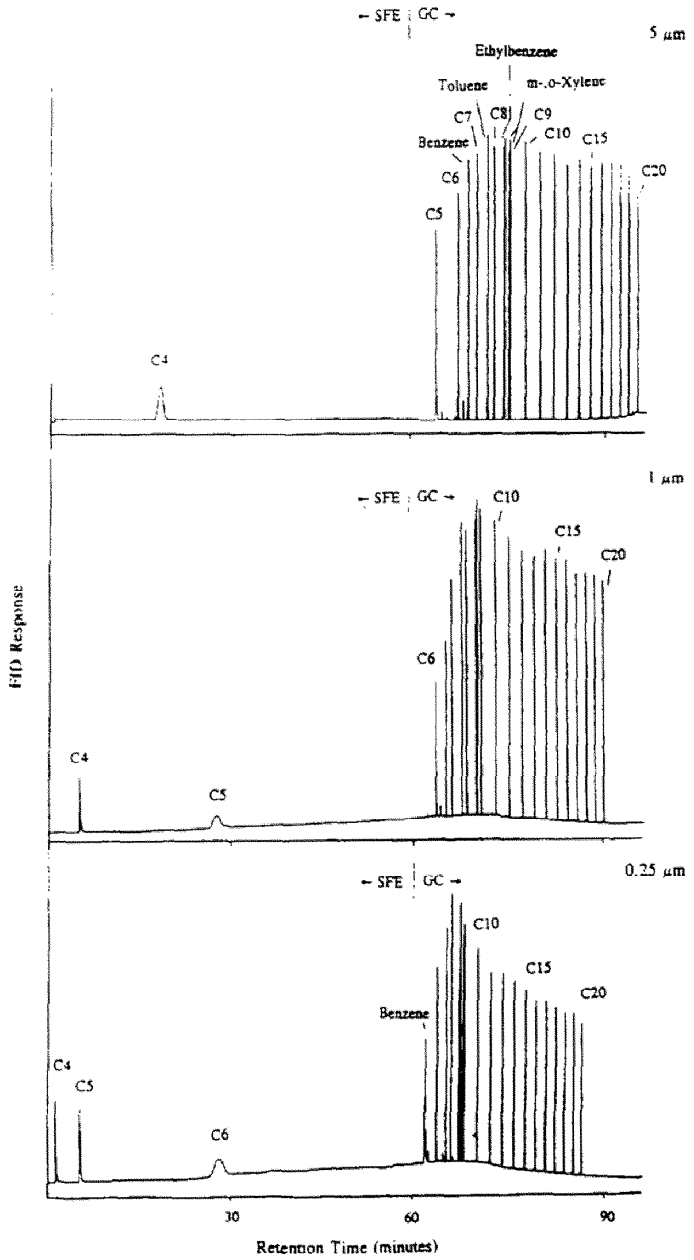

Fig. 4. Effect of capillary column stationary phase thickness on the chromatographic peak shape of BTEX and *n*-alkanes during SFE–GC–FID. A neat mixture of BTEX and C_4–C_{20} *n*-alkanes (0.2 μl) was extracted from Tenax-TA (400 mg) using 400 atm, 60°C CO_2 at 0.6 ml/min for 60 min. The extracted analytes were cryogenically focused onto a 5-μm film DB-1 capillary column (30 m × 0.32 mm I.D.); a 1-μm film DB-5 capillary column (30 m × 0.32 mm I.D.); or a 0.25-μm film DB-5 capillary column (20 m × 250 mm I.D.). After each extraction the GC oven was heated at ca. 50°C/min to 40°C then at 8°C/min to 300°C.

calibration standard since volatile and semivolatile analytes have large gas retention volumes on the resins at ambient conditions [19,20]. Furthermore, quantitative recovery of analytes from several sorbent resins has been achieved by SFE [7,21–23].

Table 2
Recovery of BTEX and C_4–C_{20} *n*-alkanes from sorbent resins using 400 atm 60°C CO_2

Analyte	Recovery (%) (R.S.D., %)[a]				
	Glass beads[b]	Carbosieve S-III[c]	Carbotrap C[b]	Tenax-TA[b]	XAD-2[b]
n-Butane (C_4)	61 (20)	49 (20)	104 (3)	109 (6)	96 (3)
n-Pentane (C_5)	69 (18)	44 (25)	96 (5)	105 (4)	91 (4)
n-Hexane (C_6)	91 (9)	30 (20)	96 (6)	103 (3)	92 (3)
Benzene	93 (8)	26 (15)	96 (6)	97 (3)	94 (2)
n-Heptane (C_7)	98 (6)	18 (12)	97 (4)	102 (3)	94 (3)
Toluene	97 (8)	6 (22)	97 (4)	97 (2)	94 (3)
n-Octane (C_8)	98 (8)	8 (23)	97 (2)	100 (3)	95 (4)
Ethylbenzene	101 (4)	4 (21)	98 (2)	96 (1)	95 (3)
m-Xylene	101 (3)	3 (12)	97 (1)	96 (1)	95 (2)
o-Xylene	101 (3)	2 (13)	98 (2)	98 (3)	94 (3)
n-Nonane (C_9)	101 (2)	3 (15)	100 (4)	101 (1)	96 (4)
n-Undecane (C_{11})	97 (5)	ND	102 (7)	102 (4)	103 (3)
n-Dodecane (C_{12})	95 (4)	ND	103 (6)	96 (4)	102 (3)
n-Tridecane (C_{13})	94 (6)	ND	88 (9)	97 (3)	97 (5)
n-Tetradecane (C_{14})	95 (4)	ND	81 (4)	97 (4)	100 (4)
n-Pentadecane (C_{15})	100 (5)	ND	83 (9)	98 (2)	93 (4)
n-Hexadecane (C_{16})	101 (5)	ND	57 (23)	95 (3)	90 (4)
n-Heptadecane (C_{17})	101 (5)	ND	31 (41)	95 (4)	95 (4)
n-Octadecane (C_{18})	100 (3)	ND	16 (39)	100 (4)	96 (10)
n-Nonadecane (C_{19})	101 (2)	ND	4 (16)	92 (7)	98 (7)
n-Eicosane (C_{20})	100 (2)	ND	2 (21)	99 (5)	95 (8)

ND = Not detected.
[a] Values in parentheses are the percent relative standard deviations of triplicate 10-min extractions.
[b] The internal standard is *n*-decane.
[c] The internal standard is octahydroanthracene.

As shown in Table 2, SFE–GC recoveries with pure CO_2 were very low from the most retentive sorbent, Carbosieve S-III. For the slightly weaker sorbent, Carbotrap C, recoveries were quantitative for all BTEX compounds and for all of the alkanes up to *n*-dodecane, but not for the more highly retained (less volatile) alkanes. The poor recoveries from the Carbotrap resins were in part related to the short, 10-min extraction times. Longer (20-min) extractions resulted in nearly quantitative recovery of all the analytes from Carbotrap C and marginally improved recoveries for Carbosieve S-III. However, the goal was to develop a solid calibration standard that could be quantitatively extracted in 10 min. Fortunately, Tenax-TA and the XAD-2 resins were ideal calibration matrices since all the test analytes (including *n*-butane) could be quantitatively spiked into the middle of the sorbent resins at room temperature and pressure without loss of the volatile compounds, and then quantitatively recovered within 10 min using SFE–GC (Table 2).

The "shelf-life" of the spiked sorbent resins was also determined, since routine use of SFE–GC for volatile hydrocarbons would be simpler if several solid calibration standards could be made at one time and stored for use throughout the working day. As shown in Table 3, if the test *n*-alkanes and BTEX components were spiked onto an inert matrix such as glass beads, over a third of the most volatile analytes were completely lost after 24 h storage inside a closed extraction cell at room temperature. The most volatile analytes (*n*-butane and *n*-pentane) also evaporated from the Carbotrap C resin, which

Table 3
Recovery of BTEX and C_4–C_{20} *n*-alkanes from sorbent resins 24 h after spiking

Analyte	Recovery (%) (R.S.D., %)[a]			
	Glass beads[b]	Carbotrap C[c]	Tenax-TA[c]	XAD-2[c]
n-Butane (C_4)	ND	ND	94 (5)	90 (6)
n-Pentane (C_5)	ND	69 (7)	109 (4)	103 (6)
n-Hexane (C_6)	ND	95 (3)	107 (3)	98 (5)
Benzene	ND	92 (4)	100 (4)	97 (4)
n-Heptane (C_7)	ND	98 (5)	98 (5)	98 (4)
Toluene	ND	100 (2)	96 (6)	98 (5)
n-Octane (C_8)	ND	98 (3)	99 (3)	99 (3)
Ethylbenzene	5 (59)	101 (3)	95 (5)	98 (4)
m-Xylene	7 (65)	101 (3)	95 (4)	98 (4)
o-Xylene	8 (69)	102 (2)	95 (4)	97 (4)
n-Nonane (C_9)	13 (71)	98 (2)	99 (1)	100 (2)
n-Decane (C_{10})	52 (53)	100 (0)	100 (0)	100 (0)
n-Undecane (C_{11})	82 (24)	102 (4)	102 (3)	102 (3)
n-Dodecane (C_{12})	95 (8)	102 (5)	100 (4)	101 (3)
n-Tridecane (C_{13})	96 (4)	101 (6)	101 (7)	98 (7)
n-Tetradecane (C_{14})	96 (6)	97 (10)	102 (9)	98 (7)
n-Pentadecane (C_{15})	94 (7)	77 (24)	102 (9)	95 (9)
n-Hexadecane (C_{16})	95 (6)	49 (25)	101 (9)	100 (7)
n-Heptadecane (C_{17})	101 (3)	28 (26)	97 (9)	103 (8)
n-Octadecane (C_{18})	95 (4)	17 (33)	98 (9)	98 (8)
n-Nonadecane (C_{19})	98 (5)	9 (38)	99 (9)	97 (9)
n-Eicosane (C_{20})	97 (7)	5 (46)	100 (11)	97 (7)

ND = Not detected.
[a] Values in parentheses are the percent relative standard deviations of triplicate 10-min extractions.
[b] The internal standard is octahydroanthracene.
[c] The internal standard is *n*-decane.

might be expected as the sorbent resin was designed to trap heavier hydrocarbons with volatilities similar to, or greater than C_8 [24]. However, the Tenax-TA and XAD-2 resins gave quantitative recoveries of the test mix after 24 h of storage, and replicate SFE–GC analysis of the aged sorbent standards produced low (1 to 11%) relative standard deviations (R.S.D.s) considering the R.S.D.s included all the possible errors associated with the spiking procedure, the storage, SFE–GC and the chromatographic peak integration. Both the spiked Tenax and XAD resins were, therefore, reliable calibration standards, but for convenience, only Tenax-TA was used to further investigate the SFE–GC collection parameters. Tenax-TA also proved to be a robust and reusable matrix, with no degradation of the sorbent observed during the study even after 30 extractions, and less than 1 ppm of detectable impurities were found by SFE–GC in the SFE-cleaned resin.

For these sorbent studies, analyte recovery was determined by comparing conventional split injections of the *n*-alkane and BTEX test mix with SFE–GC analysis of the same quantity of test mix from the sorbents. Initially, analyte recoveries were calculated by comparing the raw chromatographic peak areas from the injection and SFE–GC methods, but this proved to be unreliable as the split ratio changed between the GC and SFE–GC analysis because of the changes in total gas flow introduced into the GC injection port (Table 1) [25]. For example, a split ratio of ca. 155:1 under normal GC con-

ditions decreased to ca. 65:1 under SFE–GC conditions, even though the needle valve (which controlled the split) had not been adjusted (Table 1) and the pressure in the injection port was similar. Furthermore, the split ratio during SFE–GC increased as the SFE flow-rate decreased (Table 1). At a constant SFE flow-rate, replicate SFE–GC analyses showed good quantitative reproducibility of raw peak areas; thus, demonstrating that the split ratio remains constant under constant flow conditions and that external standardization can be used for quantitation as long as the solid calibration standards (e.g., spiked sorbents) are extracted and analyzed under identical SFE–GC conditions. However, a better approach is to use an internal standard which is added to the environmental sample (and solid calibration sorbents) since any variations in split ratio that may occur at different SFE flow conditions should not affect the ratio of analyte to internal standard peak areas. Therefore, all the analyses in this study were performed with an internal standard (e.g., decane or octahydroanthracene, depending on the sorbent) added to the sample prior to SFE.

3.4. SFE–GC analysis of common solvents

In addition to the alkane–BTEX organics used for the optimization studies, the potential application of SFE–GC for determining several volatile and semivolatile hazardous organic solvents from solid samples was also investigated. A mixture of the test solvents (ca. 18 μg of each component and 18 μg of the internal standard *n*-heptane) was spiked onto Tenax-TA, extracted at 0.6 ml/min using a 80:1 split ratio (resulting in ca. 5 ml/min flow of gaseous CO_2 through the GC column), the 5- μm film thickness DB-1 GC column, and a cryogenic trapping temperature of −50°C. Fig. 5 shows that the SFE–GC chromatograms generated using these optimized experimental parameters compared favorably with those generated by using the conventional split injection technique and the peak widths and peak symmetry of the test solvents yielded by SFE–GC were similar to those obtained by the split injection. All the solvents were quantitatively recovered from the sorbent resin, and replicate SFE–GC analyses produced very low relative standard deviations (Table 4). SFE–GC required ca. 30 min to complete including spiking the matrix, assembling the extraction cell and performing the extraction and gas chromatographic separation.

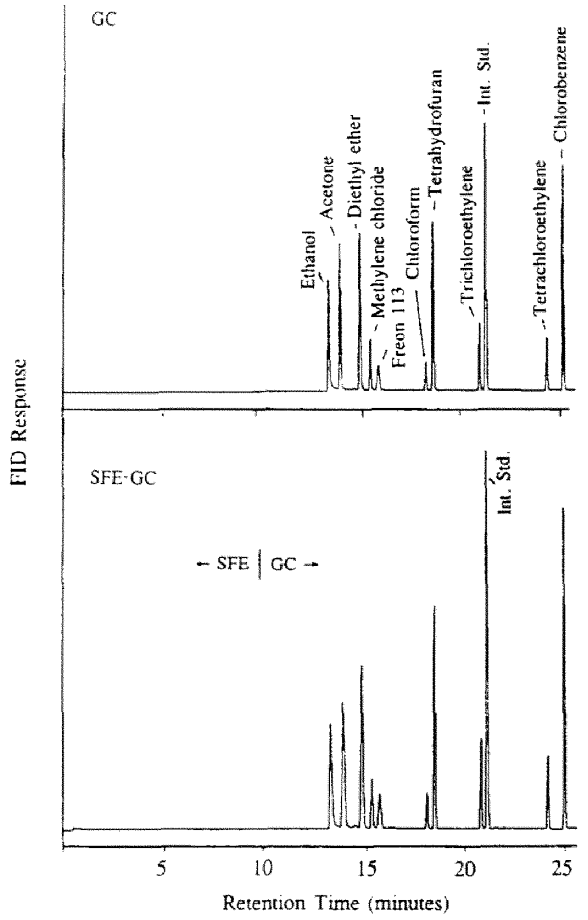

Fig. 5. Comparison of peak shapes generated using conventional GC injection of a mixture of organic solvents with those obtained using SFE–GC–FID. A mixture (0.2 μl) was either injected onto a capillary column or spiked onto Tenax-TA (400 mg) and extracted "on-line" for 10 min with 400 atm, 60°C CO_2 at 0.6 ml/min. The 30 m × 0.32 I.D. (5-μm film) DB-1 capillary column was kept at −50°C during the injection or extraction step. After the extraction or 10 min after the injection the GC oven was heated at ca. 50°C/min to 40°C then at 8°C/min to 300°C.

Table 4
Recovery of volatile organic compounds from Tenax-TA using 400 atm 60°C CO_2

Analyte	Recovery (%) (R.S.D., %)[a]
Ethanol	105 (1.3)
Acetone	101 (0.2)
Diethyl ether	102 (0.4)
Methylene chloride	98 (0.1)
Freon 113	111 (0.6)
Chloroform	96 (0.3)
Tetrahydrofuran	96 (0.3)
Trichloroethylene	97 (0.5)
Tetrachloroethylene	99 (0.7)
Chlorobenzene	101 (0.9)

See Fig. 5 for chromatographic results.
[a] Values in parentheses are the percent relative standard deviations of triplicate 10-min extractions.

4. Conclusions

A simple and reliable method has been developed for performing split SFE–GC–FID using standard GC instrumentation which has undergone minor modifications. A solid-phase calibration standard (consisting of several *n*-alkanes and BTEX spiked onto Tenax-TA) was successfully used to determine and optimize the collection efficiency and focusing of extracted analytes on the chromatographic column during the extraction step in SFE–GC analysis. The most important experimental parameters to be optimized are the cryogenic trapping temperature, the SFE flow-rate, and the column stationary phase thickness. Cooling the chromatographic column to −50°C enables analytes as volatile as *n*-butane to be focused and retained during the SFE step, and then resolved as a sharp gaussian peak in the GC analysis. The high gaseous flow-rates generated during the SFE step can be accommodated for by using the correct split ratio so a suitable column volumetric flow-rate can be obtained, and thus, 1 ml/min liquid CO_2 SFE flow-rates can routinely be used for SFE–GC analysis. Since the results of this study demonstrate that SFE–GC has the potential to determine both volatile and semi-volatile organics, a single SFE–GC analysis has good potential to replace two analyses (e.g., purge and trap for volatiles, and liquid solvent extraction for semivolatiles) when quantitative information is desired for organics having a wide range of volatility.

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