ELSEVIER

Journal of Chromatography A, 931 (2001) 53–65

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Quantitative coupling of supercritical fluid extraction and high-performance liquid chromatography by means of a coated open-tubular interface

Mark A. Stone, Larry T. Taylor*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0212, USA

Received 22 June 2001; received in revised form 14 August 2001; accepted 14 August 2001

Abstract

Supercritical fluid extraction was coupled on-line with reversed-phase high-performance liquid chromatography (HPLC). An open-tubular column with a 95% methyl–5% phenyl stationary phase was utilized as an interface between the two systems. This phase allowed for good analyte focusing onto the packed analytical column and exhibited low reactivity. Due to the non-polar nature of this phase there was a low tendency for analytes to be prematurely rinsed off the interface by condensed modifier. Using this approach, it was possible to transfer the extracted analytes quantitatively to the HPLC column in the presence of as much as 10% (v/v) methanol. By placing a 10 m guard column at the head of the interface, the same could be accomplished with ethanol as the modifier: allowing the extraction to be conducted entirely with non-toxic fluids. The method also allowed the use of very practical extraction parameters in terms of amount extracted, extraction flow-rate, extraction vessel volume, and extraction time.

Keywords: Supercritical fluid extraction; Interfaces, SFE–HPLC; Instrumentation

1. Introduction

On-line coupling of extraction and separation techniques is highly beneficial for trace analysis. The resulting process is far less labor intensive than off-line analysis. Opportunity for the sample to become contaminated, or for analytes to volatilize or degrade, is minimized. In addition, on-line methods offer improved sensitivity in cases wherein all extracted analytes are transferred to the separation column. The primary obstacle affecting on-line coupling is that the attached chromatographic systems cannot tolerate the large volume of liquid solvent typically used in the extraction process. Gases, by their nature, readily vaporize, hence, gas-phase methods such as purge-and-trap or thermal desorption, have always been done on-line. Unfortunately, these methods cannot recover non-volatile compounds, which prompted the need for the current research.

Burford et al. have noted that extractions with supercritical fluids are ideal for on-line work [1]. Supercritical carbon dioxide has a solvent strength approaching that of non-polar liquid solvents, therefore, it is capable of extracting a wide range of non-volatile analytes from a variety of matrices. Since supercritical carbon dioxide becomes gaseous

*Corresponding author. Tel.: +1-540-2316-680; fax: +1-540-2313-255.

E-mail address: ltaylor@vt.edu (L.T. Taylor).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01208-0

upon depressurization it is easily eliminated from the analysis system. In addition, the variable solvating power of supercritical CO_2 makes it possible to minimize the quantity of contaminant material that is extracted. This is important for methods that seek to quantitatively transfer the analytes to the analytical system, as a large quantity of co-extracted material could potentially obscure the peaks of interest and affect the performance or lifetime of the column.

Despite these advantages, there is a fundamental problem, due to the fact that the extraction portion of the system is at an elevated pressure and there is a substantial drop in density as the fluid passes from the vessel, through the outlet restrictor. This results in a flow-rate increase of the same magnitude as the density drop: approximately a factor of ~400. These excessive post-restrictor flow-rates lead to trapping problems for both off-line and on-line supercritical fluid extraction (SFE) techniques. Limitations have been placed on the extraction parameters that can be used: specifically, extraction flow-rate, extraction vessel volume, and extraction time. These limitations have been especially severe for on-line techniques that seek to accomplish quantitative transfer of the analytes to the chromatographic system. Recently, it has been demonstrated that, by maintaining an elevated pressure on the post-restrictor part of the system, solvent trapping could be accomplished much more efficiently and with virtually no loss of the collection solvent [2,4]. Similarly, on-line SFE–gas chromatography (GC) was accomplished with quantitative transfer of the extraction effluent to a megabore capillary column, by maintaining a somewhat elevated pressure in the column itself [3,4]. The advantages of pressurized trapping have been thoroughly discussed in these papers.

In a survey of the literature, only four cases were found where SFE–high-performance liquid chromatography (HPLC) (subsequently abbreviated as SFE–LC) has been accomplished with quantitative transfer of analytes to the analytical column [5–8]. Generally in these methods, a low extraction flow-rate was required or a limited number of analytes could be handled by the system. Quantitative transfer in the presence of modifier has never been demonstrated. This is unfortunate as many analytes for which LC is chosen as the separation method will require modifier during the extraction. In this paper a coated, open-tubular column was used to accomplish SFE–LC with quantitative transfer of the extracted analytes, using practical extraction parameters, and in the presence of 10% (v/v) modifier.

2. Experimental

A Prepmaster supercritical fluid extractor (Isco-Suprex, Lincoln, NE, USA) was used with a Model 1050 HPLC pump and UV detector (Hewlett-Packard, Wilmington, DE, USA). These components were interfaced by way of a six-port valve with 1/16 in. fittings (1 in.=2.54 cm) and a type E rotor (Valco, Houston, TX, USA). The temperature of the trapping column was controlled via the oven of a Model 5890 series II GC system (Hewlett-Packard). A 2.5-ml vessel was used for all extractions (Keystone Scientific, Bellefonte, PA, USA). The vessel was packed with sand and spiked with 5 μl of stock solution at the beginning of each run. Direct injection of standards onto the LC column was accomplished using a Valco injection valve with a nominal 5 μl sample loop. The actual volume of the loop was determined to be 6.71 μl, hence, a correction factor of 1.34 was applied to all results.

The valve diagrams for each step are depicted in Fig. 1. Heating tape was wrapped around the restrictor line up to 1 in. from the T/C valve. The heating tape was maintained at 120–130°C. All extractions were 25 min in length at 400 atm and 90°C using carbon dioxide as the extraction fluid (1 atm= 101325 Pa). The stainless steel line going into T/C port 6 was crimped at the end such that an extraction flow-rate of 1±0.2 ml/min was obtained. The end was then filed on the sides so that it fit into the valve port. All stainless steel lines were 1/16 in. O.D.× 0.02 in. I.D.

Analytes were trapped on 0.53 mm I.D. open-tubular columns with various wall-coated stationary phases (Restek, Bellefonte, PA, USA). The pressure at the back end of the interface was controlled by a backpressure regulator (Commonwealth Industrial Specialties, Hopewell, VA, USA). After the **dynamic extract/trap** step, the system was held in the **depressurize** stage for 60 s before beginning the separation. At the beginning of each run, the backpressure regulator was disconnected to allow expul-

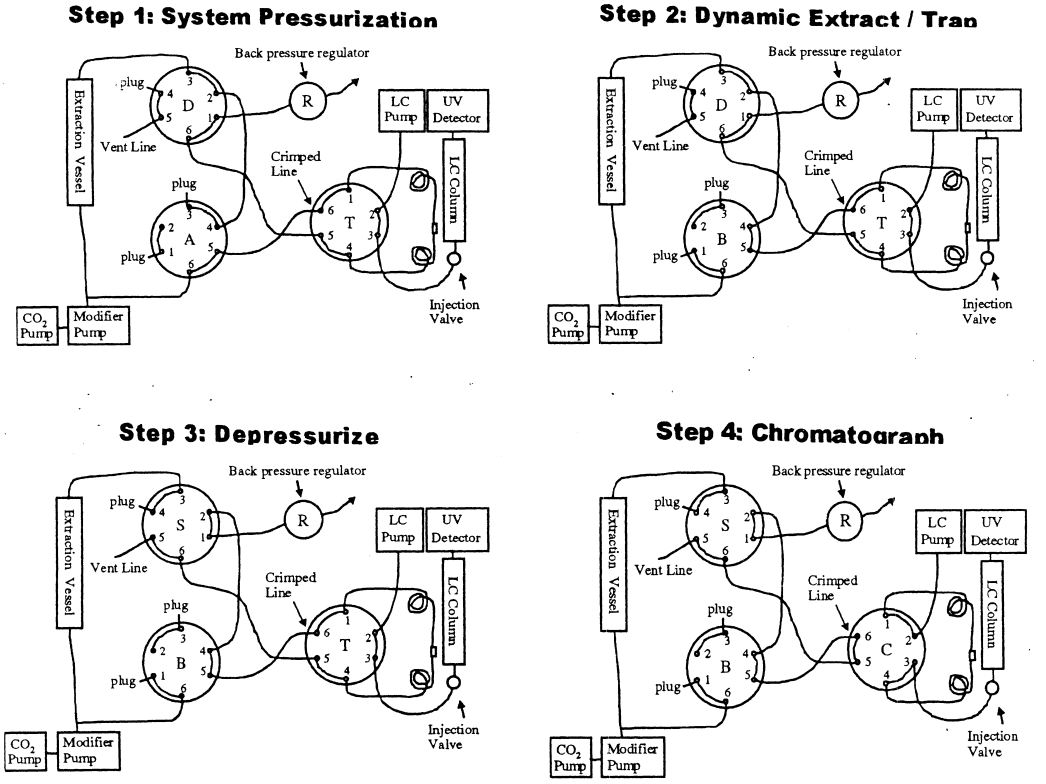

Fig. 1. Valve diagrams.

sion of the water and methanol in the interface (from the previous separation).

Two standard solutions were used during the study. One mixture contained relatively volatile analytes, and one contained semivolatile analytes. Please note the designation of some analytes as volatile and others as semivolatile is somewhat arbitrary. The analytes and concentrations in the **volatiles** mixture were 4-nitrotoluene, 400 μg/ml; 2-naphthol, 500 μg/ml; 2,6-dinitrotoluene, 400 μg/ml; benzophenone, 200 μg/ml; and fluorene, 200 μg/ml. The mixture of **semivolatiles** contained: lorazepam, temazepam, flurazepam, medazepam, all at 80 μg/ml and phenanthrene and pyrene at 17 μg/ml.

An Xterra RP_{18} column, 100×3.9 mm with 3.5 μm particles (Waters, Milford, MA, USA) was used for all analyses. The mobile phase was water–methanol (75:25, v/v) for the first 2 min, then ramped to 45:55 at 12 min, to 20:80 at 15 min, to 10:90 at 20 min and held there for the remainder of the run. No buffers or additives were added to the mobile phase. The detection wavelength was 254 nm for the volatile compounds and 240 nm for the semivolatiles.

3. Results and discussion

3.1. Background

When trying to accomplish on-line coupling of SFE and HPLC there are several issues that must be kept in mind. First, HPLC separations are generally done on packed columns. These columns generate much larger pressure gradients across them, for a given flow-rate, then is the case for the wide-bore open-tubular columns previously used for on-line SFE–GC [3,4]. Excessive pressures would be generated if the decompressed extraction effluent were

channeled directly onto these packed columns. Even in the absence of modifier, this would be a problem, as the fluid would acquire a degree of solvating power which would make trapping less efficient. However, when continuous addition of modifier is needed, such a configuration would be completely unworkable as modifier evaporation would not readily occur at the elevated pressure and a large quantity of liquid would remain, thus causing analytes to rinse prematurely off the interface. Additionally, there may be residual water in the interface from the previous separation; as water is typically present in reversed-phase eluents and has a tendency to linger in the interface due to associations with the trapping phase as well as its high boiling point. This reinforces the need for a system that facilitates rapid evaporation of liquids in the interface. As a result it will not be possible to trap the extracted analytes directly on the analytical column as was done for SFE–GC.

Given these concerns, a wide-bore open-tubular column was selected as the best method for interfacing the SFE and the HPLC systems. Only moderate pressure gradients are generated as carbon dioxide is channeled through a wide-bore open-tubular column. As a result, the pressure at the head of the interface is not drastically higher than that at the end of the interface. The fluid acquires virtually no solvating power, and the modifier (and residual water) can readily evaporate. The greater length of open-tubular columns, compared to packed columns, further aids modifier evaporation.

The use of an open-tubular interface is also advantageous with respect to another inherent difficulty of SFE–LC techniques: that of suspended gas in the HPLC mobile phase. Several groups have observed this problem [5,9–11]. Two groups have reported success in minimizing the size of the gas peak by pressurizing the detector [6,11]. During the **chromatographic** stage, liquid mobile phase pushes the carbon dioxide gas in the interface onto the analytical column. Although some gas enters the analytical column, we never have a situation where this column goes completely dry. This is a clear advantage of this configuration because when a packed column is completely dry and liquid is subsequently pushed through, a prolonged time interval is required for the gas to be completely removed from the column. Fig. 2 depicts the profile of the CO_2 peak verses different pressures maintained at the end of the trapping column. As expected when a greater pressure was maintained in the interface, a larger quantity of gas was transferred to the packed column and, hence, the CO_2 peak was larger. However, even at the higher pressures, the gas exited the column very efficiently as evidenced by the steep drop-off at the tail end of the gas peaks.

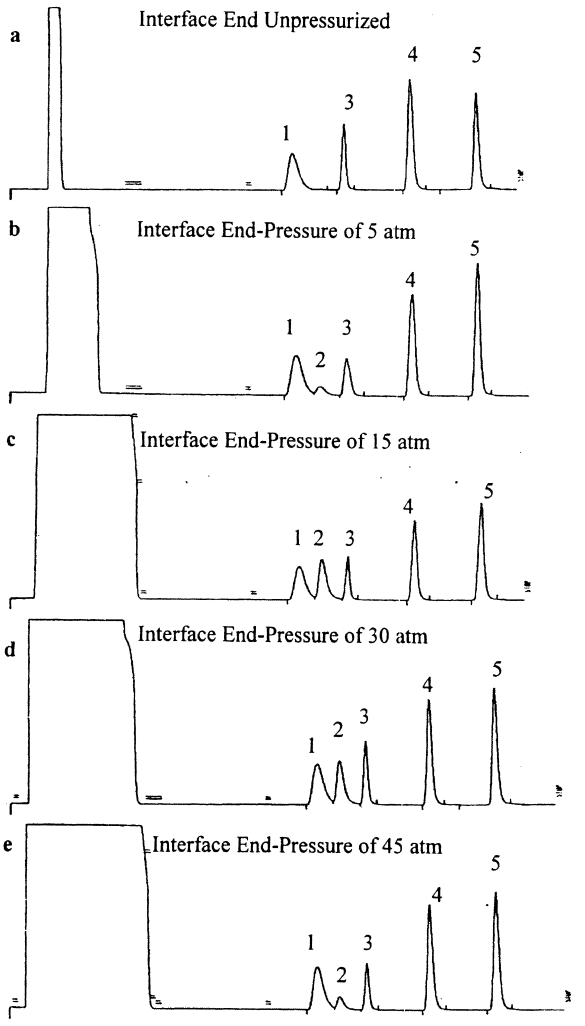

Fig. 2. SFE–LC chromatograms at different interface pressures. 2% modifier used in these runs. Peak identification: 1 = 4-nitrotoluene; 2 = 2-naphthol; 3 = 2,6-dinitrotoluene; 4 = benzophenone; 5 = fluorene.

3.2. Evaluation of different interface stationary phases

Given that an interface is needed, the issue of analyte focusing becomes critical. Adequate chromatography will only be obtained if the analytes are refocused at the head of the LC column during the beginning of the chromatographic separation. We would expect that focusing would be most efficient when the elution strength of the mobile phase is stronger in the open-tubular interface column, than on the packed analytical column. It follows that with reversed-phase LC, the best focusing would be expected with a polar stationary phase in the interface. Hence, a Wax column (crosslinked polyethylene glycol) was evaluated first. The chromatograms in Fig. 3 show that the Wax column did, in fact, allow for efficient focusing. A 10 m Wax interface was evaluated as well as a 20 m interface with a 10 m guard column. Some peak broadening was observed in both cases relative to the standard run, however, this was a fairly minor effect. An important observation here is that the size of the gas peak does not increase in proportion with the length of the interface. In Fig. 3 it can be seen that when the interface length is tripled, the width of the gas peak is less than doubled. This is because as the length of the interface increases, the pressure near the head of the interface will be higher. Hence, there is a compression effect which minimizes the differences that are observed with respect to the size of the gas peak in the chromatograms.

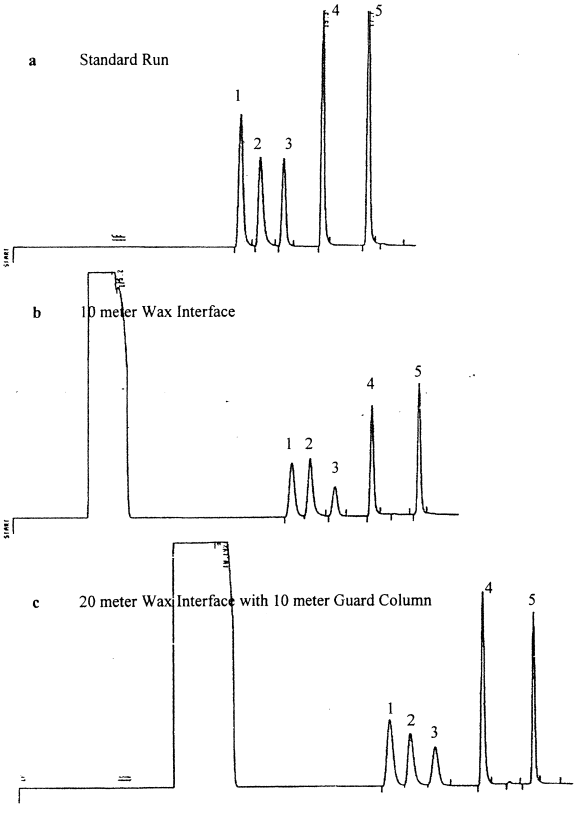

Fig. 3. Chromatograms obtained with a Wax column interface. No modifier used in these runs. Peak identification: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene.

Despite the recorded focusing efficiency, several problems were encountered with the use of a Wax column interface. First, the stationary phase was extremely unstable and recoveries dropped noticeably after only a few runs. No buffers or additives were added to the mobile phase, however, some acidity does result from the reaction of CO_2 with water. In addition, our ability to add modifier to the extraction fluid was limited. With the end of the interface unpressurized, more than 5% modifier caused analyte recoveries to drop. This was due to analytes being prematurely rinsed off the interface by unevaporated modifier: a phenomenon subsequently referred to as the **rinsing effect**. This problem became worse as the interface end-pressure was elevated. Higher pressures not only mean that evaporation of modifier (and water) will be more difficult, but also that the quantity of liquid that remains unevaporated will be present at a larger volume percentage, because the gas is compressible but the liquid is not. These data are not presented here as they have been published previously [4]. Lastly, it was also noted that an interval of time was required to remove all of the mobile phase from the Wax column. The bulk of the mobile phase was eliminated quickly, however, residual liquid continued to exit the system for a prolonged period of time (observed as a mist discharged from the end of the interface).

It was expected that susceptibility to the **rinsing effect** and a tendency to hold onto water would be problematic not just for Wax phase, but for phases of

high polarity in general. The next step then was to consider moderate to low polarity phases. MXT-50 (crosslinked 50% methyl–50% phenylpolysiloxane) and MXT-5 (crosslinked 95% methyl–5% phenylpolysiloxane) were chosen for evaluation. Preliminary work [4] demonstrated that the MXT-5 column did not trap quite as efficiently as the MXT-50 column. This is not surprising as the larger percentage of phenyl groups give the MXT-50 some additional polarity and a greater propensity to participate π–π interactions. The polarity difference also explains why the MXT-5 column was found to be less susceptible to the **rinsing effect** [4].

Surprising were the results obtained when the focusing ability of the two phases was investigated. Peak shapes were evaluated for runs where trapping was accomplished on 3, 5.5, and 8 m lengths of interface. Chromatograms are depicted in Figs. 4 and 5 for the MXT-50 and MXT-5 columns, respectively. The more polar MXT-50 phase was expected to yield better peak shapes, however, exactly the opposite

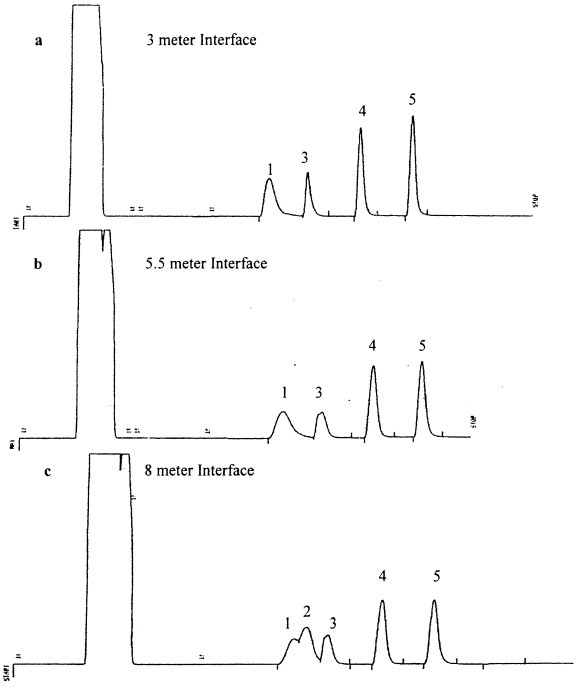

Fig. 4. Chromatograms obtained on MXT-50 interfaces of different lengths. 2% modifier used in these runs. Peak identification: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene.

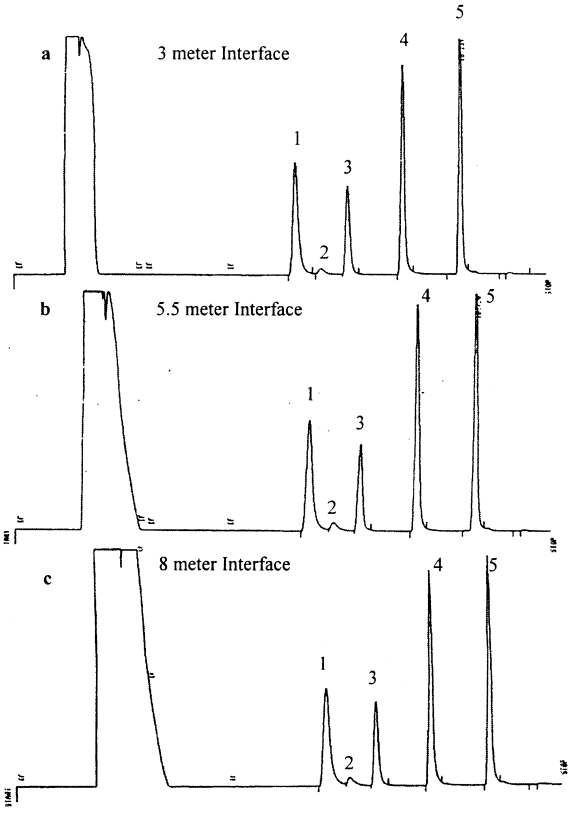

Fig. 5. Chromatograms obtained on MXT-5 interfaces of different lengths. 2% modifier used in these runs. Peak identification: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene.

was found. Peak broadening occurred with the MXT-50 phase as the length of the column increased. Conversely, with the MXT-5 phase, very little change was seen in the peak shapes as a function of the length of the interface. Apparently the MXT-5 phase has enough polarity relative to the C_{18} phase in the packed column, that efficient focusing was possible. The higher phase ratio of the interface column, as compared to that of the packed column may have helped to promote efficient focusing as well. The poor focusing observed with the MXT-50 phase is believed to be due to the fact that the larger number of π electrons on this column interacted with the analytes, through π–π interactions, and retarded their movement through the interface. It seems this phenomenon was more important than the polarity of the trapping phase.

3.3. Stability of siloxane-based phases

In the absence of any buffer, the siloxane-based phases (MXT-50 and MXT-5) were far more stable than the Wax phase. Still, there is some phase breakdown that does occur. Although changes in trapping efficiency were not observed, breakdown was evident in two ways. First, it was observed throughout the study that peak shapes periodically deteriorated. When this occurred it was found that running the analytical column backwards for 5 or 10 min restored peak shapes to their previous symmetry. This suggested that a fraction of the siloxane material that bleeds off the interface column was large enough to be retained by the frit at the head of the analytical column. When a sufficient quantity of this material builds up, it interacts with the analytes causing tailing and split peaks. This phenomenon is clearly depicted in Fig. 6 where standard runs are shown before, and immediately after, running the column backwards. The need to backwash the column was quite frequent with the MXT-50 interface and considerably less so with the MXT-5 column, suggesting that the MXT-50 column is less stable.

The suspected poor stability of the MXT-50 phase was further supported by the presence of a peak which eluted between 21 and 22 min. The peak was never present in standard runs but usually seen in SFE–LC runs. It is believed to be the fraction of siloxane bleed that does pass through the frit. The size of this peak can be used to roughly assess the extent to which the stationary phase is attacked under

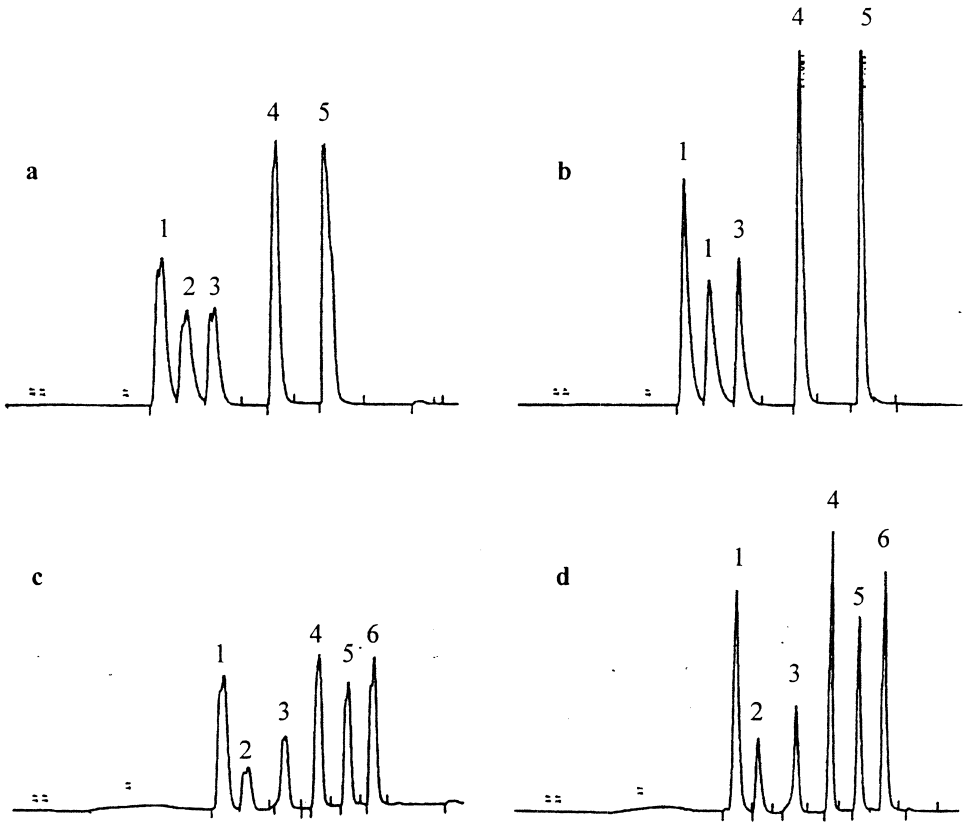

Fig. 6. Peak symmetry before and after running the column backwards. Volatile analytes prior to (a) and after (b) running the column backwards. Semivolatile analytes prior to (c) and after (d) running the column backwards. Peak identification, a and b: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene. Peak identification, c and d: 1=lorazepam; 2=temazepam; 3=fluorazepam; 4=medazepam; 5=phenanthrene; 6=pyrene.

various conditions. This bleed peak was much more pronounced with the MXT-50 then with the MXT-5 phase. Variables such as interface temperature and analyte type were also found to have a strong effect on the degree to which column bleed occurs. For example, Fig. 7a and b show that with the MXT-50 column much larger bleed peaks were observed at 30°C than at 45°C. It seems the degradation reactions require a polar medium to take place and do not occur, to any significant extent, in the gas phase. Of course the reactions we speak of here are a different type than those that occur in gas chromatography (typically at temperatures above 200°C).

Fig. 7c shows that the bleed peak was even larger when the semivolatile analytes were run. It should be noted that the attenuation setting was one unit less for semivolatile runs hence the peak height would have to be reduced by a factor of two for a fair comparison. However, it is clear that the semivolatile bleed peak would still be considerably bigger than for the volatile runs. This is not surprising as four of the compounds in this mixture are nitrogenous bases which are able to undergo acid–base type reactions

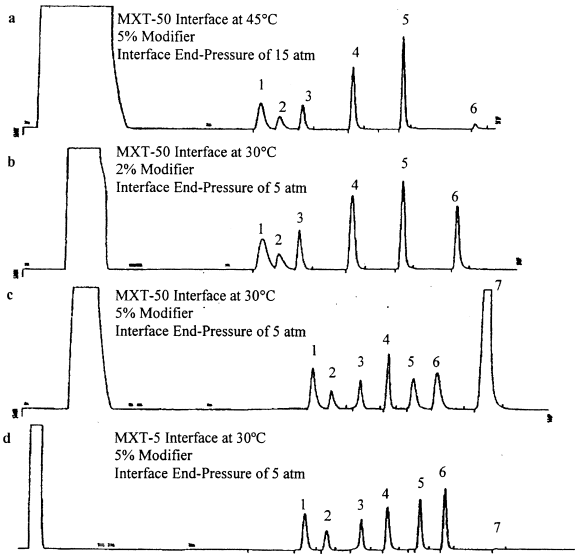

Fig. 7. Evaluation of column bleed on 3-m lengths of interface under different conditions. Peak identification, a and b: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene; 6=bleed. Peak identification, c and d: 1=lorazepam; 2=temazepam; 3=fluorazepam; 4=medazepam; 5=phenanthrene; 6=pyrene; 7=bleed.

with the MXT-50 stationary phase. In Fig. 7d it is seen that, even with the basic analytes, there was virtually no bleed peak observable on the MXT-5 column.

3.4. Use of a 15 m MXT-5 column as an interface

Summarizing the information gathered so far it seems that, in many ways, MXT-5 is the ideal trapping phase for this application. This is based on the efficient focusing that is possible as well as its low susceptibility to the **rinsing effect** in the presence of modifier, low reactivity, and low tendency to hold onto water. The MXT-5 was not the best phase from a trapping standpoint, however because focusing was so efficient, longer lengths of column can be used to make up for the less than optimal trapping.

Based on all of this information, the next step was to use a 15 m MXT-5 column with the first 4 m of the interface heated. Preliminary work [4] suggested that relatively low interface pressures should be used, hence initial evaluation was done with the end of the interface unpressurized. It is important to note that the pressure throughout most of the interface was elevated despite the fact that the end was unpressurized. Specifically, the pressure ranged from 1 atm at the end, to 4.3 atm at the head of the interface (calculated). The data are summarized in Table 1 for both volatile and semivolatile analytes, run with 10% methanol. The analyte most susceptible to rinsing was naphthol and, in fact, a very clear trend was observed for this analyte. At the higher temperatures recoveries were quantitative. Below 45°C recoveries became less quantitative. In contrast, good recoveries were obtained at room temperature for most other analytes.

The data in Table 2 summarize the results obtained when the interface end-pressure was elevated to 5 atm. Fluorene, phenanthrene, and pyrene were quantitative at all conditions. Recoveries for benzophenone, nitrotoluene, dinitrotoluene, and naphthol were below quantitative at 30°C, but quantitative at higher temperatures. This suggests that the rinsing effect was problematic at 30°C but not at the higher temperatures. What was surprising were the data obtained for the basic analytes. Previously it was observed that these analytes were less susceptible to the **rinsing effect** than naphthol. Yet here recoveries

Table 1
Effect of interface heating on trapping efficiency with the end of the interface unpressurized: percent recovery (RSD)[a]

	Interface temperature			
	Oven door open (~25°C)	35°C	45°C	55°C
2,6-Dinitrotoluene	93 (1.6)	94 (0.15)	99 (0.18)	98 (0.20)
2-Naphthol	51 (6.0)	74 (4.7)	100 (1.1)	101 (1.8)
4-Nitrotoluene	96 (0.55)	97 (0.39)	100 (0.22)	97 (0.42)
Benzophenone	97 (0.67)	97 (0.25)	101 (0.24)	97 (0.94)
Fluorene	100 (0.047)	99 (0.30)	101 (0.20)	101 (0.82)
Lorazepam	91 (5.0)	96 (4.6)	97 (6.4)	98 (7.1)
Temazepam	94 (5.0)	97 (5.3)	101 (6.0)	102 (6.8)
Fluorazepam	83 (7.3)	82 (4.9)	77 (7.4)	84 (4.1)
Medazepam	95 (7.1)	101 (6.1)	96 (6.7)	96 (6.2)
Phenanthrene	95 (0.78)	98 (1.9)	101 (0.55)	103 (1.2)
Pyrene	96 (1.4)	100 (2.2)	102 (0.39)	101 (2.0)

[a] A 15-m MXT-5 column, 10% methanol modifier, 4 m of column in oven.

for these analytes were much worse than naphthol at 30°C. Although the exact nature of the process is not well understood, it seems clear that there is some type of reactivity phenomenon occurring with the basic analytes in the interface. The fact that recoveries are worse at lower temperatures suggests that the modifier is participating in this process.

3.5. Importance of pressure and flow

When gas is forced through an open-tubular column, a pressure gradient develops that decreases, from the head, towards the end of the column. The magnitude of the pressure at a given point can be described by the Hagen–Poiseuille equation:

$$p_{\text{interface point}} = \left(F_c 16\eta L p_{EC}/\pi r^4 + p_{EC}^2\right)^{1/2} \quad (1)$$

This equation demonstrates that the pressure at a given interface point is a function of the pressure at the back end of the column (p_{EC}), the distance between the end of the column and the point of interest (L), as well as the viscosity of the gas (η), and the flow-rate, measured at the column outlet (F_c). There is a particularly strong dependence on the column's internal radius (r). Once the pressure at a given point has been calculated, the CO_2 density may then be obtained, and the flow at that point may be determined as follows:

$$\text{Flow}_{\text{interface point}} = \text{Flow}_{\text{extraction vessel}} \cdot \left(\rho_{\text{extraction vessel}}/\rho_{\text{interface point}}\right) \quad (2)$$

Values for pressure and flow have been calculated for three different points on a 25 m interface, shown in Table 3. A length of 25 m was selected as that is the longest interface that would likely be used. Values were calculated for the head of the interface, the middle of the interface (12.5 m from either end), and the end of the interface.

In Table 2 we observed a drop in extraction recovery for some analytes when the end-pressure was increased to 5 atm. At first it would seem that this was simply due to the higher pressure inhibiting

Table 2
Effect of interface heating on trapping efficiency with the end of the interface at 5 atm: percent recovery (RSD)[a]

	Interface temperature		
	30°C	45°C	55°C
2,6-Dinitrotoluene	84 (4.0)	99 (0.84)	100 (1.2)
2-Naphthol	73 (6.1)	91 (2.5)	95 (2.3)
4-Nitrotoluene	82 (2.4)	96 (0.53)	96 (0.76)
Benzophenone	87 (1.2)	97 (1.4)	96 (1.5)
Fluorene	101 (1.4)	101 (0.59)	98 (1.2)
Lorazepam	24 (19)	62 (9.4)	92 (4.3)
Temazepam	37 (16)	69 (7.2)	90 (3.9)
Fluorazepam	26 (11)	57 (4.9)	83 (4.9)
Medazepam	40 (18)	68 (7.6)	94 (4.0)
Phenanthrene	96 (0.65)	98 (1.1)	98 (0.96)
Pyrene	101 (1.7)	102 (2.1)	100 (1.6)

[a] A 15-m MXT-5 column, 10% methanol modifier, 4 m of column in oven.

Table 3
Calculated pressure and flow-rates at various points in the interface for extraction flow of 1 ml/min

Set pressure at end of column[a] (atm)	Head of interface		Middle of interface		End of interface	
	Pressure (atm)	Flow (ml/min)	Pressure (atm)	Flow (ml/min)	Pressure (atm)	Flow (ml/min)
Open	5.5	79	4.0	111	1	444
5	8.0	54	7.1	61	6	72

Calculations were based on extraction at 400 atm and 90°C with the end of the interface at 30°C. Densities were obtained from the Gas Encyclopedia [12] or by using the SF-Solver software (distributed by Isco). Values were then calculated using Eqs. (1) and (2). For all calculations a CO_2 viscosity of 0.000155 P was used.

[a] Set pressure = absolute pressure − atmospheric pressure. Equivalent to the pressure that would be set on the gauge of a backpressure regulator.

modifier evaporation. However, the calculated values in Table 4 show that increasing the column end-pressure to 5 atm only has a modest effect on the pressure at the head of the interface, where modifier evaporation is taking place. Specifically, the pressure increases from 4 to 7.1 atm. Conversely, the reduction in flow-rate is more substantial: a change of 50 ml/min would be recorded[1]. The effect of flow-rate was not studied explicitly, however, the use of a crimped stainless steel restrictor lead to some inevitable variation in flow-rate. It was consistently observed throughout the study that modifier evaporation was more efficient when the flow drifted slightly upwards and was less efficient when the flow drifted slightly downwards (with constant modifier percentage). Given this, and the values in Table 3, we believe that it was not so much the elevated pressure that caused the reduced recoveries but the resulting drop in flow-rate at the head of the interface.

Previously published calculations [4] show that when the column end-pressure is elevated, and at the same time a higher extraction flow-rate is used, it is possible to increase the flow at the head of the interface (making modifier evaporation more efficient) and simultaneously, decrease the flow towards the end of the interface (making trapping more efficient). It is important to avoid excessive pressures, hence, column end-pressures much above 5 atm are not recommended. Based on the experience gained in the course of this study, and the calculations mentioned above, it is believed that column end-pressures of 2–5 atm and flow-rates of 2–4 ml/min, with 4–5 m of guard column at the head of a 12 m interface would work well for the vast majority of analytes without a need for heating the interface. Additional research is needed to prove that these ranges are, in fact, optimal. A system with better flow control – preferably a variable restrictor – would be needed as there is too much fluctuation of the flow-rate using a crimped stainless steel restrictor.

It is strongly recommended that the use of a back-pressure regulator be avoided. Heating is required in order to keep the liquids from accumulating and the higher temperatures can damage the internal components of the regulator. Instead, the desired pressure can be established at the back end of the interface by using stainless steel tubing of specified length and inner diameters. In Table 4 the length of 0.01 in. I.D. tubing required to achieve certain pressures at the end of the interface is calculated for different flow-rates. These values were calculated using Eq. (1), and solving for *L*. Using tubing instead of a regulator also eliminates the need to unhook the regulator at the beginning of each run to eliminate the water and methanol on the interface from the previous run.

3.6. Evaluating the miscibility of the reversed-phase eluent with the MXT-5 stationary phase

Several reasons have been previously stated as to why MXT-5 is the best phase to serve as an interface for the coupling of SFE and HPLC. However, given that the phase is relatively non-polar, an obvious concern is the miscibility of the water–methanol

[1] These are the values calculated in Table 4 for the middle of a 25 m interface, i.e., 12.5 m from the end. Technically this is not a perfect match with the current situation where we are using a 15 m interface. However, the differences in pressure and flow-rate would to be minor.

Table 4
Length of tubing needed to achieve various column end-pressures[a]

Target set pressure at end of column[b] (atm)	Length of tubing required (m)				
	1 ml/min	2 ml/min	3 ml/min	4 ml/min	5 ml/min
1	2.2	1.1	0.72	0.54	0.43
2	5.8	2.9	1.9	1.4	1.2
3	11	5.4	3.6	2.7	2.2
4	17	8.6	5.8	4.3	3.5
5	25	13	8.4	6.3	5.0

[a] Values are calculated for tubing with a 0.01 in. internal diameter.
[b] Set, or gauge, pressure (not absolute pressure).

mobile phase with the stationary phase. The chromatograms in Fig. 5 show that excellent focusing was obtained with lengths up to 8 m for an MXT-5 column with a 1.5 μm film, and with 2% modifier used in the extraction. In Fig. 8 it is observed that good focusing was still obtained when the length of the column was increased to 15 m: even when the film thickness was doubled. The increase in peak widths were fairly small and may be related to the larger quantity of methanol in the longer interfaces. More methanol would strengthen the mobile phase at the beginning of the separation and, thereby, make focusing less efficient. However, when a run was made in the absence of modifier all recoveries dropped significantly except that of naphthol, which was quantitatively recovered, Fig. 8c. This occurs because, during trapping, a certain fraction of the analytes diffuse into deep regions of the stationary phase. Subsequently, when the mobile phase sweeps through the interface it can only access that fraction of the analytes that are relatively near the surface, due to its poor miscibility with the stationary phase. Naphthol is unaffected as it also is not miscible with the stationary phase and, hence, remains on the surface. This is confirmed by the fact that the first run with modifier – after two runs without – showed greater than 100% recovery for all peaks except for naphthol, Fig. 8d.

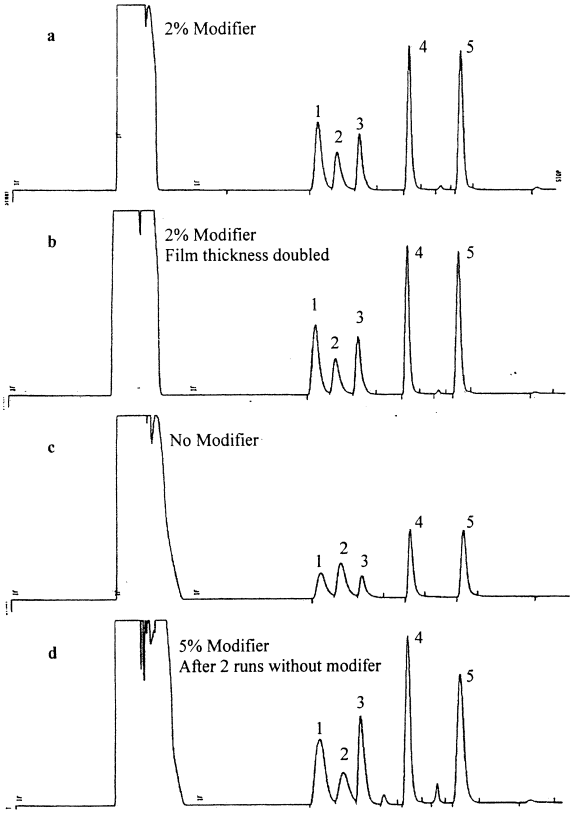

Fig. 8. Evaluating focusing on a 15-m MXT-5 interface. All runs with interface at 35°C. End of interface unpressurized. Peak identification: 1=4-nitrotoluene; 2=2-naphthol; 3=2,6-dinitrotoluene; 4=benzophenone; 5=fluorene.

When modifier is used in the extraction, the stationary phase becomes saturated with the liquid. Subsequently, when the mobile phase is introduced, this layer of modifier acts as a co-solvent between the mobile and stationary phases. One would think the fact that there is already methanol in the mobile phase would mean good results should be obtained regardless of whether modifier was used during the extraction. However, this was found not to be the case. It appears that the methanol cannot permeate the stationary phase in the presence of a large quantity of water. The conclusion is that the low polarity of the stationary phase does not present a

problem, provided that a small amount of modifier is used in the extraction.

3.7. Use of ethanol in place of methanol

Because quantitative recoveries were obtained for all analytes with 10% methanol, it was thought that the potential existed to conduct the extraction with completely non-toxic fluids if ethanol were substituted as the modifier instead of methanol. For these analyses, the first 6.5 m of the interface were placed in the oven. Despite the fact that the boiling point of ethanol is only ~13°C higher, there was a considerable difference in the **rinsing effect** that was observed between the two modifiers. As noted in Table 1, temperatures of 45°C or higher were sufficient to allow quantitative trapping of naphthol in the presence of 10% methanol. Conversely, with ethanol, 65°C was not a high enough temperature to evaporate the modifier in the interface as indicated in Table 5. However, when a 10 m guard column was added to the front end of the interface, excellent recoveries were obtained for all analytes with 10% ethanol, even at 25°C. This leads to the important conclusion that the use of a guard column has a much more substantial effect on modifier evaporation than does heating the head of the interface. Quantitative results were still obtained, for all but the basic analytes, when the back end of the column was pressurized at 5 atm.

4. Conclusions

On-line coupling of SFE and HPLC was accomplished by use of a coated open-tubular interface. This approach allowed for quantitative transfer of the analytes to the separation column even when substantial quantities of modifier were used in the extraction.

MXT-5 was found to be the preferred stationary phase for use as an interface from the standpoint of analyte focusing, the stability of the phase, its low tendency to hold onto water, and its low susceptibility to analyte losses due to rinsing. The efficient focusing allowed longer lengths of column to be used, which maximizes trapping efficiency. With a 15 m column efficient trapping of 2-naphthol was possible: the most difficult analyte evaluated from a trapping standpoint. It was possible to use ethanol in place of methanol as the extraction modifier if a 10 m guard column was placed at the head of the interface. The data obtained with ethanol as a modifier demonstrated, quite conclusively, that the use of a guard column was more effective than heating of the interface in achieving good recoveries

Table 5
Evaluation of ethanol as a modifier: percent recovery (RSD)[a]

	Interface temperature		
	65°C, 10% modifier, unpressurized	25°C, 10% modifier, unpressurized, with 10 m guard	25°C, 10% modifier, 5 atm, with 10 m guard
2,6-Dinitrotoluene	93 (1.5)	96 (0.36)	96 (0.18)
2-Naphthol	64 (13)	96 (2.5)	92 (1.6)
4-Nitrotoluene	97 (0.47)	98 (0.081)	103 (0.49)
Benzophenone	94 (1.7)	96 (0.11)	96 (0.23)
Fluorene	97 (1.2)	95 (1.3)	99 (0.24)
Lorazepam	66 (14)	102 (19)	40 (22)
Temazepam	85 (3.8)	109 (12)	47 (24)
Fluorazepam	74 (9.4)	93 (6.3)	75 (9.7)
Medazepam	47 (31)	91 (16)	25 (20)
Phenanthrene	96 (1.9)	97 (1.0)	96 (1.5)
Pyrene	95 (3.2)	97 (0.41)	95 (2.6)

[a] 6.5 m of column in oven.

in the presence of modifier. Fortunately, it has also been shown that increasing the length of the interface has only a moderate effect on the size of the gas peak in the chromatogram.

Acknowledgements

The authors would like to thank Restek Corporation for providing the metal-jacketed capillary columns that were critical to this work. We thank Keystone Scientific for donation of the extraction vessels used in this study. In addition, we would like to thank Isco-Suprex Inc. for the supercritical extractor, as well as Air Products and Chemicals Inc. for providing the carbon dioxide. Much thanks to Lynn Hunter of Renaissance Analytical for assistance in the installation of the six-port valve and for providing the necessary pin contacts. The 3M Corporation provided financial assistance during performance of the work.

References

[1] M.D. Burford, K.D. Bartle, S.B. Hawthorne, Adv. Chromatogr. 37 (1997) 163.
[2] M.A. Stone, L.T. Taylor, Anal. Chem. 72 (2000) 1268.
[3] M.A. Stone, L.T. Taylor, Anal. Chem. 72 (2000) 3085; M.A. Stone, L.T. Taylor, Anal. Chem. 72 (2000) 4529.
[4] M.A. Stone, Doctoral Dissertation, Virginia Polytechnic Institute and State University, 2001.
[5] H.J. Cortes, L.S. Green, R.M. Campbell, Anal. Chem. 63 (1991) 2719.
[6] C. Mougin, J. Dubroca, E. Barriuso, J. High Resolut. Chromatogr. 19 (1996) 700.
[7] F. Ischi, W. Haerdi, Chromatographia 41 (1995) 238.
[8] H.R. Johansen, G. Becher, T. Greibrokk, Anal. Chem. 66 (1994) 4068.
[9] M. Ashraf-Khorassani, M. Barzegar, Y. Yamini, J. High Resolut. Chromatogr. 18 (1995) 472.
[10] J.D. Brewster, R.J. Maxwell, J.W. Hampson, Anal. Chem. 65 (1993) 2137.
[11] M.H. Liu, S. Kapila, K.S. Nam, A.A. Elseewi, J. Chromatogr. 639 (1993) 151.
[12] Gas Encyclopedia, Elsevier, New York, 1976.