Variables affecting the supercritical fluid extraction of analytes from octadecylsilane solid-phase sorbents

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ABSTRACT

The relative effects of the numerous variables which can influence the supercritical fluid extraction (SFE) efficiencies of analytes from solid-phase extraction (SPE) sorbents is discussed. SFE efficiencies are not only influenced by experimental variables such as temperature and pressure (density), but also by sample/matrix variables, extraction vessel variables and collection variables. The significance of many of these variables are underrated, and studies aimed at quantitative comparisons of these variables have generally focused on extraction of analytes from endogenous matrices, rather than SPE sorbents such as those evaluated here. The relative effects of temperature and density have been quantitatively compared for the extraction of polycyclic aromatic hydrocarbons and methoxychlor from octadecylsilane sorbents. Under the conditions studied, the effect of temperature was found to be of equal importance to that of density, and either could be used to vary the recovery of analytes over an extremely wide range. A more thorough knowledge of the relative effects of all of the controllable variables should facilitate optimization of SFE of analytes from SPE sorbents for maximum selectivity as well as maximum overall recoveries.

INTRODUCTION

Solid-phase extraction (SPE) has rapidly established itself as an important sample preparation technique for both matrix simplification and trace enrichment in a variety of clinical and environmental applications. One of the most commonly used SPE sorbents is the reversed-phase octadecylsilane sorbent used in numerous clinical and environmental applications, including the analysis of drugs, essential oils, food preservatives, vitamins, plasticizers, pesticides, steroids, hydrocarbons, toxins, etc.

[1–7]. SPE techniques have numerous advantages over liquid–liquid extraction techniques, including ease of automation, reduced cost, reduced solvent use and higher sample throughput. However, SPE techniques still suffer from numerous problems, particularly when applied to the analysis of biological specimens [3]. The chemical background from impurities, contaminants, antioxidants, etc., often observed when using SPE, can interfere in the subsequent analysis of the sample, as well as reduce the lifetime of chromatographic columns [8–10]. Running blanks and cleaning of the cartridges to minimize interferences diminishes sample throughput and adds to solvent consumption and processing costs. Many of these problems can potentially be minimized by the use of highly selective elution solvents which can elute the analyte of interest while

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leaving the matrix interferences. Supercritical fluids have unique solvent properties which may make them ideal candidates for selective elution of target analytes without increased solvent consumption or reduced sample throughput.

Supercritical fluid extraction (SFE) has proven to be a powerful alternative to conventional liquid extraction methods, such as Soxhlet extraction, particularly in environmental applications [11,12]. Most of the applications which have emerged recently have involved the extraction of analytes directly from endogenous solid and liquid matrices, although the gaseous trapping of analytes onto various adsorbents with subsequent recovery by SFE has also been investigated [11–13]. Solid-phase sorbents have been used in combined SFE–supercritical fluid chromatography (SFC) techniques where the sorbent acts as a chromatographic column with selective elution of target analytes [14–16]. Alternatively, solid-phase sorbents have been used as efficient traps for off-line SFE [17]. In this paper, we discuss the potential for the use of supercritical fluids as elution solvents to enhance the selectivity, as well as overall recovery, of analytes trapped onto SPE sorbents. Subsequent SFE of analytes can be used on-line, combined with gas, liquid or supercritical fluid chromatography, to provide a complete sample extraction and analysis system, or, more commonly, can be employed off-line with sorbent or solvent trapping of the analytes. On-line methods provide the greatest sensitivity and, in principle, should be more accurate due to the reduced sample handling. Off-line methods often allow the greatest experimental flexibility and the possibility of multiple analysis of the extract regardless of the sample conditions. For these reasons, off-line methods are the most common.

Supercritical fluids possess unique physicochemical properties which make them attractive as alternative extraction solvents to liquids currently used. Supercritical fluids have low viscosities and zero surface tension which allows for very efficient penetration into macroporous materials such as those used in SPE. Additionally, the significantly higher diffusivities of solutes in supercritical fluids provides for more rapid transport out of the sorbent bed. The greatest advantage of supercritical fluids, however, is the fact that they have densities (and often solvating powers) comparable to that of liquids, which can be continuously varied by as much as an order of magnitude by varying the temperature and pressure of the extraction vessel. This paper continues a systematic, experimental investigation of the effects that controllable variables have on the supercritical fluid elution of analytes from SPE sorbents. This work focuses on the off-line solvent collection of polycyclic aromatic hydrocarbons (PAHs) and methoxychlor eluted from octadecylsilane sorbents, and a discussion of the major variables affecting achievable recoveries by this method.

EXPERIMENTAL

All standards were obtained from Aldrich (Milwaukee, WI, USA) and used without further purification. PrepSep (Fisher Scientific, Orlando, FL, USA) octadecylsilane (C_{18}) solid phase extraction cartridges were used in this study. A stock packing containing 200 ppm of the standards on C_{18} was prepared by slow evaporation of a standard chloroform solution. The SFE apparatus used has been described previously [18]. SFE-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, USA) was used for all of the extractions. In order to accurately quantify the effects of temperature and density (pressure at constant temperature), care was taken to ensure that all of the other major controllable experimental variables were kept constant and precisely measured. Numerous extractions were performed and only those extractions with the same flow-rates (0.60 ml/min), extraction times (5.3 min) and total volumes (3.2 ml of liquid carbon dioxide) were compared for temperature and density effects. Flow-rates were controlled by varying the length of linear restrictors fabricated from 40 μm I.D. x 375 μm O.D. fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA). Extractions were performed on packings contained in a 1.0 x 1.0 cm I.D. vessel. Extraction conditions were purposely chosen to yield less than quantitative recovery of the analytes to allow for comparisons of the different extraction conditions. Analytes were collected into methylene chloride with fluorene added as an external standard. Identification and quantitation of the analytes were performed using a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with a flame ionization detection.

RESULTS AND DISCUSSION

The elution of analytes from SPE sorbents is controlled by a variety of interrelated factors, including the affinity of the analytes for the sorbent, the vapor pressure of the analytes and the solubility and diffusion coefficient of the analytes in the supercritical fluid. In addition, SFE efficiencies are controlled by a complex relationship between many experimental variables, some of which are listed in Table I. Although it is well established that, to a first approximation, the solvent power of a supercritical fluid is related to its density, the relative effects of many of the other controllable SFE variables are poorly understood. The list in Table I was tabulated from observations in the authors' laboratory for the SFE of analytes from octadecylsilane sorbents. Some of the relative effects observed for octadecylsilane sorbents are different from those seen for other SPE sorbents and may be different from those seen for the extraction of analytes from endogenous solid matrices as discussed below. Much additional work is needed to distinguish which of these variables are significant and their relative effects for the multitude of possible analyte–sorbent(matrix) combinations. The variables affecting SFE of analytes from sorbents can be broken down into four main areas, namely, experimental variables, sample/matrix variables, extraction vessel variables and, finally, collection variables. Each group from Table I is discussed in more detail below.

Experimental variables

The experimental variables are those which can be continuously varied during an extraction to maximize selectivity, as well as overall recoveries. These variables often have the largest effect on observed recoveries and will be discussed first. Carbon dioxide is the primary fluid used in most SFE applications because it has low critical points ($T_c = 31.3°C$, $P_c = 1070$ p.s.i.), is non-toxic, non-flammable, odorless, readily available in high purity, inexpensive and eliminates solvent waste disposal problems. Unfortunately, carbon dioxide has one severe problem; it is non-polar and, therefore, of limited value for very polar or ionic analytes. There are three possible approaches to overcoming the polarity problem of carbon dioxide. First, one can choose a more polar supercritical fluid (*e.g.* $CHClF_2$, N_2O), although at the expense of many of the advantages stated above [19]. A second approach, which is more commonly used, involves the addition of a small volume percent of an organic solvent modifier to increase the solvent strength/selectivity of the carbon dioxide. A third approach, which can be combined with pure or modified carbon dioxide SFE, is to chemically derivatize the analyte *in situ* to a more extractable form. In this approach, the analyte is simultaneously derivatized and extracted by static SFE, followed by dynamic extraction and analyte collection. This approach has been applied with success to the extraction of a variety of analytes directly from environmental ma-

TABLE I

VARIABLES WHICH MAY AFFECT THE SUPERCRITICAL FLUID ELUTION OF ANALYTES FROM SPE SORBENTS EMPLOYING OFF-LINE SOLVENT COLLECTION

| Experimental variables	Sample/matrix variables	Extraction vessel variables	Collection variables
Primary fluid	Sorbent type	Extraction vessel dimensions	Solvent type
Fluid modifiers	Analyte type	Extraction vessel size	Solvent temperature
Static SFE/derivatization	Sorbent condition	Extraction vessel dead volume	Solvent volume
Density (pressure)	Weight of sorbent	Extraction vessel orientation	Fluid flow-rate
Temperature	Analyte concentration		
Total volume of extraction fluid	Co-extractants present		
Fluid flow-rate	Sorbent particle size		
Extraction time			
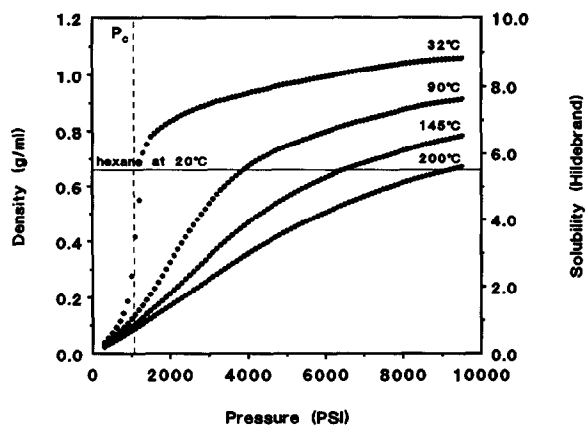
Fig. 1. Plot of carbon dioxide density (and calculated Hildebrand solubility parameter) versus temperature and pressure. The density of hexane at 20°C is shown for comparison.

trices as well as from analytes collected on Empore C_{18} sorbent disks [20].

The next controllable experimental variables which can have tremendous effects on achievable recoveries are density (at a constant temperature) and temperature (at a constant density). The temperature is an extremely powerful variable, often underrated in optimization schemes. Typical densities and Hildebrand solubility parameters for supercritical carbon dioxide are shown in Fig. 1 at various temperatures and pressures (data calculated with SF Solver software, Isco, Lincoln, NE, USA), illustrating the extremely wide range of solubilities available at a variety of temperatures. The relative effect of these two variables are illustrated later in this paper for the elution of PAHs and methoxychlor from octadecylsilane SPE sorbents. In general, maximum recoveries are achieved by maximizing these two variables, although careful optimization of temperature and density allows for pre-elution of potential SPE contaminants and highly selective elution of analytes [14,18]. The extraction of analytes by deposition onto SPE sorbents, followed by elution with supercritical fluids, can be called SFC since chromatographic processes are involved in the selectivity of the extraction, although, experimentally, this technique more closely resembles SFE. The final two controllable experimental variables are the extraction time and fluid flow-rate, which

yield the total elution volume of supercritical fluid. For the analytes studied here, we have generally found that the total volume of the extraction fluid used is more important than the extraction time. It is important to note that this is the opposite of the effect reported for the SFE of environmental solids where kinetic effects may dominate and the total extraction time is often one of the most important variables [21].

Sample/matrix variables

Sample/matrix variables can be extremely important in achievable elution of analytes from solid-phase sorbents. The type of analyte and the type of sorbent/matrix will often dictate the feasibility of quantitative recovery by SFE. In addition, the particle size and the condition of the sorbent (moisture content, pH, etc.), as well as the concentration of the analyte and any co-extractants present can significantly affect observed recoveries. Finally, the weight of sorbent extracted can affect SFE recoveries and relates to other experimental and extraction vessel variables, such as the total volume of extraction fluid used and/or the total extraction time, as well as extraction cell dead volume at a constant extraction cell size. These sample/matrix variables are often dictated by the SPE conditions chosen and are often more difficult to adjust for maximizing selectivity/overall recoveries using supercritical fluids.

Extraction vessel variables

The third set of variables in Table I relate to the design of the extraction vessel. We have previously published the significant effect that the dimensions of the extraction vessel (I.D. to length) can have on the elution of PAHs and methoxychlor from octadecylsilane SPE sorbents [18,22]. These results are different than those observed for the SFE of analytes directly from environmental solids where no effect has been seen [23]. A more thorough discussion of the effect of cell dimensions and comparisons for different SPE matrix/analyte types has recently been published [24]. The size of the extraction vessel combined with the sample size determine the amount of dead volume within the vessel. In some cases, for the SFE of PAHs from octadecylsilane supports, we have observed increases in recoveries when dead volume was present in the extraction

vessel compared to when it was completely filled. Again, this is in conflict with observations for the SFE of analytes directly from environmental solids where lower recoveries have been observed when significant dead volume is present [25]. Clearly, the relative effects of SFE variables for the recovery of analytes from endogenous matrices cannot be directly applied to predict relative effects of SFE of solid-phase sorbents. The orientation of the extraction vessel (vertical or horizontal) may also affect observed recoveries depending on whether the supercritical fluid enters the top or the bottom of the vessel (in the vertical orientation) and if there is dead volume in the vessel. Since these variables generally have only small effects on achievable recoveries and may actually decrease reproducibility, prudent practice is to always completely fill the extraction vessel.

Collection variables

The final variables which must be considered are those related to the final trapping of the extracted analyte in the supercritical fluid. One of the most common forms of analyte collection is to decompress the supercritical fluid directly into a liquid solvent. Improper control of collection variables can result in losses improperly attributed to poor SFE efficiencies [23]. The analytes must have a high solubility in the chosen collection solvent and a sufficient solvent volume (*e.g.* > 1 ml) must be present to efficiently trap the target analytes. The solvent temperature should be maintained at a low enough temperature to prevent volatilization losses of analytes while at a high enough temperature to prevent freezing of the solvent (due to the cooling effect from the rapid expansion of the supercritical carbon dioxide) or blocking of the restrictor. Finally, the flow-rate of the supercritical fluid is often limited to *ca.* <2 ml/min (and ideally <1 ml/min) due to the purging and solvent disruption encountered with solvent trapping at very high flow-rates. Purging losses can become significant particularly for more volatile analytes using long extraction times. The ideal collection conditions are dependent on the analyte and co-extractants. For non-volatile analytes, such as the 4–6 ring PAHs and methoxychlor studied here, 1 ml of methylene chloride was adequate to quantitatively trap these analytes.

Relative effects of temperature and density

Data for the supercritical carbon dioxide elution of methoxychlor, pyrene, perylene and benzo[*ghi*]perylene from octadecylsilane sorbents are given in Table II. It is important to note that conditions were chosen to deliberately yield recoveries of at least 1% and less than 100% for these analytes to allow for a quantitative relative comparison between these two variables. Quantitative recoveries

TABLE II

RECOVERY OF ANALYTES FROM OCTADECYL-BONDED PACKINGS USING VARIOUS DENSITIES AT VARIOUS TEMPERATURES AND DENSITIES

Variables			Recovery of analytes (%) (average S.D. = 1.2)			
CO_2 volume (ml)	Density (g/ml)	Temperature (°C)	Methoxychlor	Pyrene	Perylene	Benzo[*ghi*]perylene
7.5	0.70	40.0	37.6	22.1	1.9	0.2
7.5	0.70	50.0	69.1	39.2	7.4	1.9
7.5	0.70	60.0	75.2	60.1	13.7	4.1
7.5	0.70	80.0	94.3	78.7	24.1	7.8
7.5	0.70	100.0	102.1	80.9	47.8	27.4
3.0	0.40	100.0	25.4	26.6	5.6	0.6
3.0	0.50	100.0	38.8	41.4	9.2	2.6
3.0	0.60	100.0	59.0	50.8	14.6	6.6
3.0	0.70	100.0	80.7	70.2	22.2	4.0

of these analytes were readily achieved by maximizing the temperature and/or density of the supercritical fluid. Selectivity tuning of supercritical fluid elution of analytes, however, requires a quantitative measure of the relative effects of each controllable variable. Recoveries increased in direct proportion to the density of supercritical carbon dioxide (at a constant temperature of 100°C) whereas there was an approximately linear logarithmic increase in recovery with the inverse of the temperature (at a constant density of 0.70). Linear least squares regression analysis of these data yields the following equations:

$$\text{Recovery}_{\text{methoxychlor}} = 186.1d - 51.38$$
$$(r^2 = 0.99) \tag{1}$$

$$\text{Recovery}_{\text{methoxychlor}} = \exp[5.336 - 63.25(t^{-1})]$$
$$(r^2 = 0.92) \tag{2}$$

$$\text{Recovery}_{\text{pyrene}} = 140.5d - 29.99$$
$$(r^2 = 0.98) \tag{3}$$

$$\text{Recovery}_{\text{pyrene}} = \exp[5.429 - 89.43(t^{-1})]$$
$$(r^2 = 0.95) \tag{4}$$

$$\text{Recovery}_{\text{perylene}} = 55.20d - 17.46$$
$$(r^2 = 0.97) \tag{5}$$

$$\text{Recovery}_{\text{perylene}} = \exp[5.902 - 204.4(t^{-1})]$$
$$(r^2 = 0.98) \tag{6}$$

$$\text{Recovery}_{\text{benzo}[ghi]\text{perylene}} = 33.90d - 13.57$$
$$(r^2 = 0.98) \tag{7}$$

$$\text{Recovery}_{\text{benzo}[ghi]\text{perylene}} = \exp[6.236 - 301.4(t^{-1})]$$
$$(r^2 = 0.96) \tag{8}$$

where d = density and t = temperature.

Using the above equations, it is possible to compare the maximum effect of temperature and density on recoveries of these analytes. The data, as well as the curves generated from the above equations, are shown in Fig. 2 for perylene and pyrene over a density range of 0.20 g/ml (1500 psi at 100°C) to 0.90 g/ml (10 000 p.s.i. at 100°C) and just above the critical temperature, 32°C, to 142°C. It is obvious that the effect of temperature is as great as, or in some cases greater than, that of density over the ranges compared. The great selectivity of this technique is apparent when one considers that recoveries can be increased from 0 to 100% simply by varying either the temperature or the density of the
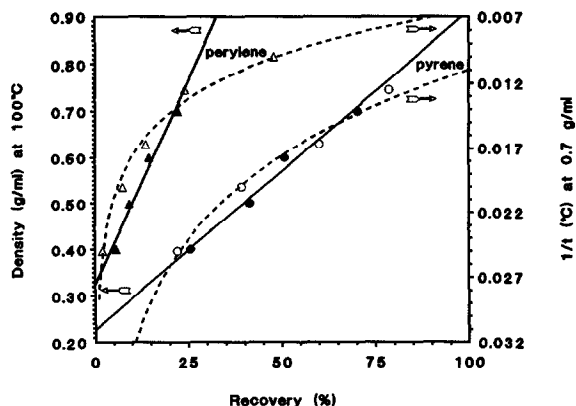

Fig. 2. Plot of the actual recoveries and calculated recoveries of perylene and pyrene from octadecysilane sorbents as a function of supercritical carbon dioxide density (solid lines) and inverse of the temperature (dashed lines).

supercritical carbon dioxide. The effect of temperature is sometimes underrated in SFE optimization schemes and appeared to be particularly important when employing supercritical fluids as SPE elution solvents. Increasing the supercritical fluid temperature (at a constant density) generally enhances the solubility, vapor pressure and the diffusion coefficient of the analyte in the supercritical fluid, as well as reducing the affinity of the analyte for sorptive sites on the SPE matrix.

CONCLUSIONS

The use of supercritical fluids for the selective extraction of compounds from solid-phase sorbents has great potential. Current limitations arise from our limited understanding of relative effects and interrelationships between the large number of experimental variables controlling the supercritical fluid elution of analytes from SPE sorbents, as well as the relatively expensive instrumentation required for SFE. As our understanding of these variables increases, our ability to fine tune SFE for highly selective extractions, as well as maximum overall recoveries should increase dramatically. In addition, the ease of automation of SFE techniques and reduced solvent consumption could potentially make supercritical fluids cost-effective alternatives to conventional liquid solvents. The use of supercritical fluids in combination with SPE will likely have a bright

future due to the following potential advantages of supercritical fluids over conventional solvents: (1) highly selective pre-elution of interferents by simple changes in temperature/density of the supercritical fluid; (2) more selective extraction of target analytes from matrix; (3) straightforward on-line/chromatographic analysis can increase recoveries, with decreased analysis times; (4) rapid simultaneous derivatization/extraction possible; (5) methodology may be directly applied to some samples with minimal sample preparation (*e.g.* biological tissues) with the potential for developing completely automated SFE–SPE(SFC)–SFE methods.

ACKNOWLEDGEMENT

REFERENCES

1 C. F. Poole and S. K. Poole, *Chromatography Today*, Elsevier, Amsterdam, 1991.
2 I. D. Wilson, E. D. Morgan and S. J. Murphy, *Anal. Chim. Acta*, 236 (1990) 145.
3 K. G. Furton and J. Rein, *Anal. Chim. Acta*, 236 (1990) 99.
4 J. M. Vinuesa, J. C. M. Cortes, C. I. Canas and G. F. Perez, *J. Chromatogr.*, 472 (1989) 365–370.
5 G. A. Junk and J. J. Richard, *Anal. Chem.*, 60 (1988) 451.
6 O. Suzuki, H. Seno and T. Kumazawa, *J. Forensic Sci.*, 33 (1988) 1249.
7 M. J. Shepherd, M. Holmes and J. Gilbert, *J. Chromatogr.*, 354 (1986) 305.
8 G. A. Junk, M. J. Avery and J. J. Richard, *Anal. Chem.*, 60 (1988) 1347.
9 H. D. Rood, *Anal. Chim. Acta*, 236 (1990) 115.
10 W. E. Johnson, N. J. Fendinger and J. R. Plimmer, *Anal. Chem.*, 63 (1991) 1510.
11 S. B. Hawthorne, *Anal. Chem.*, 62 (1990) 633A.
12 V. Lopez-Avila, N. S. Dodhiwala and W. F. Beckert, *J. Chromatogr. Sci.*, 28 (1990) 468.
13 J. M. Wong, N. Y. Kado and P. A. Kuzmicky, *Anal. Chem.*, 63 (1991) 1644.
14 J. M. Levy, R. A. Cavalier, T. N. Bosch, A. F. Rynaski and W. E. Huhak, *J. Chromatogr. Sci.*, 27 (1989) 341.
15 E. D. Ramsey, J. R. Perkins and D. E. Games, *J. Chromatogr.*, 464 (1989) 353.
16 J. E. France, J. W. King and J. M. Snyder, *J. Agric. Food Chem.*, 39 (1991) 1871.
17 L. J. Mulcahey, J. L. Hendrick and L. T. Taylor, *Anal. Chem.*, 63 (1991) 2225.
18 J. Rein, C. M. Cork and K. G. Furton, *J. Chromatogr.*, 545 (1991) 149.
19 S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and M. D. Burford, *Anal. Chem.*, 64 (1992) 1614.
20 S. B. Hawthorne, D. J. Miller, D. E. Nivens and D. C. White, *Anal. Chem.*, 64 (1992) 405.
21 K. D. Bartle, A. A. Clifford, S. B. Hawthorne, J. J. Langenfeld, D. J. Miller and R. Robinson, *J. Supercrit. Fluids*, 3 (1990) 143.
22 K. G. Furton and J. Rein, *Chromatographia*, 31 (1991) 297.
23 J. J. Langenfeld, M. D. Burford, S. B. Hawthorne and D. J. Miller, *J. Chromatogr.*, 594 (1992) 297.
24 K. G. Furton and Q. Lin, *Chromatographia*, 34 (1992) in press.
25 S. B. Hawthorne, personal communication.