APPLICATIONS OF MODIFIERS IN SUPERCRITICAL FLUID EXTRACTION AND CHROMATOGRAPHY

by

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Abstract

The use of modifiers in supercritical fluid chromatography and extraction has become quite common due to the inability of pure carbon dioxide alone to solvate many of the compounds of interest. The effects of modifiers in supercritical fluid chromatography have been more throroughly studied than the effects of modifier in supercritical fluid extraction. The effects of modifier on trapping efficiencies for off-line supercritical fluid extraction have been evaluated in this work.

Sorbent and solid phase traps were investigated with pure carbon dioxide in order to determine the effect of stationary phase identity, pretreatment, and rinse solvent on the recoveries of a test mixture of compounds of varying vapor pressure and molecular weight. The solid phase traps, which were polyethylene frits, performed as well as the sorbent traps in most cases, and significantly better than the sorbent traps in many cases. The ability to cool these traps to -20°C allowed for efficient trapping of volatile compounds without the benefit of sorptive interactions.

Sorbent and solid phase traps were then studied with the addition of 1%, 2%, 4%, and 8% methanol to the mobile phase. The sorbent trap explored consisted of 40 μm ODS packing material, while the solid phase trap consisted of 100 μm stainless steel beads. In this work trap temperatures ranged from 5-80°C. It was found that trap temperature, modifier concentration, and trap type influenced recoveries of the test mixture components.

Applications of these solid phase and sorbent traps explored were the extraction of polychlorinated biphenyls from river sediment and the extraction of the active components from a drug formulation. The separation of some compounds of

pharmaceutical interest was also explored, where the addition of modifier, and in some cases an additive, was required to elute compounds from the chromatographic column.

This thesis is dedicated to
Joseph L. Hedrick.

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Chapter 1

Introduction

The use of modifiers in supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) has become quite common. In SFC modifiers are generally added through the use of an additional pump or by using cylinders which contain premixed amounts of modifier. In SFE modifiers are added in the same way, but additionally can be placed directly on the sample matrix. There has been extensive work done to determine the role of modifiers in SFC. However, SFE is the less mature technique of the two, and much of the SFE literature concerns different samples that have been studied, rather than basic studies into the kinetics of extraction, trapping mechanisms, or the role of modifiers in extraction.

The area of trapping in off-line SFE, although it is beginning to be more intensely studied, has traditionally been ignored. In many papers the trap is not described at all, or described incompletely. If extraction recoveries are lower than expected, often the density or the polarity of the fluid are adjusted, rather than the variables concerned with the trapping process. With the advent of commercial instrumentation specifically designed for off-line SFE, which utilize different trapping modes, the study of trapping efficiency has become quite important. Although modifiers are often used in extraction, their affect on trapping efficiency is unknown. It is conceivable that the modifier, although it may increase the efficiency of the extraction, may decrease the trapping efficiency.

The goal of the work done in this laboratory over the past few years has been to characterize the behavior of solid surface and sorbent traps for use in off-line SFE. To this end the effects of the sorbent phase, sorbent and solid surface pretreatment, rinse solvents, and the extraction fluid itself have all been explored. Both types of traps

were evaluated first with pure carbon dioxide, and then with modified fluids. These sorbent and solid surface traps were then applied to a variety of real world samples. Chapter 2 contains a review of trapping mechanisms used for off-line SFE, and the evaluation of solid and sorbent phase traps with pure carbon dioxide as the extraction fluid. Chapter 3 deals with the evaluation of sorbent and solid surface traps when methanol modified carbon dioxide is used. Chapters 4 and 5 are application chapters. Chapter 4 discusses the extraction of Septra Infusion, a water/propylene glycol formulation with two active components. The extraction of PCBs from river sediment is discussed in Chapter 5. The final chapter deals with the application of modifiers in SFC to compounds of pharmaceutical interest.

Chapter 2

Collection Efficiency of Solid Phase and Sorbent Traps

Introduction

Dynamic off-line supercritical fluid extraction (SFE) is the most commonly used mode of SFE today. In this extraction mode there are two major concerns for the analyst. First, the compounds of interest must be extractable from their matrix, and second, the system being used to trap the analytes must perform efficiently. Three different types of trapping systems are commonly used for dynamic off-line SFE. The first type of trapping system that may be used is a liquid trap. This trapping mode may be the mechanically simplest way to trap an analyte. The restrictor is simply placed in a vial of liquid solvent. The analyte is trapped in the solvent, while the decompressed fluid vents to the atmosphere. The liquid solvent must be compatible with the analytes of interest, and also with the extraction fluid when modifiers are used. When CO_2 and N_2O are used as extraction fluids there can be a great deal of cooling associated with the decompression of the fluid. Because of this cooling, it is possible for the collection fluid to freeze and for small pieces of ice to clog the restrictor tip. For this reason, the restrictor is often heated. The flow of compressed fluid for liquid trapping is usually maintained at less than 1 mL/min (liquid) because upon decompression of the fluid approximately 500 mL/min of gas is produced. This large volume of gas can cause violent bubbling of the liquid collection solvent, and lead to analyte loss.

Hawthorne and coworkers[1] have shown high recoveries for PAH's from urban dust, river sediment, and fly ash when collecting into a liquid trap. The collection solvent used was methylene chloride (2 mL) spiked with 0.5 μg of 4,4'-

dichlorobiphenyl as an internal standard. The flow rate was controlled by unsing a 10 cm length of 20-30 μm fused silica as a restrictor. They reported that no significant evaporation of the methylene chloride occurred during extraction. Lopez-Avilia et al[2] reported the extraction of PAH's and organochlorine pesticides using hexane as a collection solvent. Recoveries covered a broad range (22-107%) for the PAH's. No volume of hexane was given. The collection solvent was spiked with an internal standard of terphenyl-d_{14}. A 60 cm piece of 50 μm i.d. fused silica was used to control the flow rate. When modified (10% methanol) CO_2 was used, the collection solvent was changed to either methylene chloride or methanol in order to be compatible with the extraction fluid. Alexandrou and Pawlisyzn[3] used 1.0 mL of hexane as the trap for the extraction of polychlorinated dibenzo-p-dioxins and dibenzofurans from municipal incinerator fly ash. Recoveries of these compounds ranged from 79-117% when 10% benzene was added to the CO_2. "Ice-cold" hexane was also used as the collection solvent for the extraction of polychlorinated organics from biological tissue samples.[4] At the 250 ppb level, 100% recoveries were reported for a variety of polychlorinated organics. Hexane has also been used as a collection solvent for the extraction of PCBs from whole blood and milk, triazine herbicides, thiophosphate and carbamate pesticides from dairy biomass, n-alkanes and polynuclear aromatic hydrocarbons from tissue samples, and dioxin from liver tissue.[5] Currently there are two commercially available instruments designed specifically for SFE followed by collection into a liquid organic solvent.

The second type of trapping system used is a solid surface. The surface is cryogenically cooled by the expanding extraction fluid, or by another source (CO_2 or liquid N_2). Typical solid surfaces that have been used are glass vials, stainless steel beads, and glass beads. The analytes are trapped on the cryogenically cooled surface,

and then rinsed from the surface for further analysis. Typical compressed flow rates (liquid) for this type of trapping range from 1-4 mL/min. Although faster flow rates are possible for this type of trapping as compared to trapping in a liquid, rinsing the analytes from the trap becomes a factor that does not have to be considered for liquid trapping.

Trapping of PAH's on the cooled surface of a volumetric and a round bottom flask was reported by Wright et al.[6] They found that the geometry of the vessel, and whether the vessel was sealed or open greatly affected extraction recoveries. Although both collection vessels were maintained at 0°C, collection efficiencies for the open vessel ranged from 0-8.2%. The mechanism for solute loss was attributed to solute aerosol fromation. When a sealed vessel was used collection ranged from 25-95%. McNally and Wheeler[7] also reported the use of a glass vial as a collection device for the extraction of linuron and diuron from Sassafras Soil. Extraction with pure CO_2 yielded less than 1% recovery, while addition of modifier directly to the matrix resulted in 90-100% recoveries for both compounds. Currently there are two commercially available instruments that employ this type of trapping.

The third type of trapping system used in dynamic off-line SFE is a solid phase sorbent, which most often is chromatographic packing material. The packing material provides two trapping mechanisms - cryogenic trapping and absorption. The trap is cryogenically cooled, again either by the expanding fluid or by another source. The analytes are trapped and then rinsed from the packing material with a small volume of organic solvent. Typical compressed flow rates (liquid) range from 1-4 mL/min.

Schantz et al[8] used a 5 cm section of 0.25" o.d. stainless steel tubing packed with μ-Bondapak C-18 to trap PCBs extracted from urban particulate. A second C-18 column was used in tandem to detect any breakthrough of extracted PCBs from the

first column. Analysis of the solvent rinse of the second column showed that no breakthrough had occurred. After an extraction was completed, the C-18 column was removed from the extraction apparatus and hooked up to a high pressure LC pump where it was rinsed with 40 mL of solvent. The solvent was then evaporated and the PCBs were analyzed by GC. Taylor and Hedrick [9] reported the use of solid phase extraction (SPE) tubes as traps for the extraction of phenol from water. A silica trap was used, and the results were compared to liquid trapping in a vial containing 5 mL of 50/50 methanol/water. Collection of phenol on the silica SPE tube resulted in 80% recovery with an RSD of 9%. Collection of phenol into the liquid trap resulted in a 60% recovery and a high RSD (15%) which was attributed to sample loss due to the high compressed flow rate (> 1 mL/min). Currently there is commercial instrumentation that allows for trapping on chromatographic packing material. However, at the onset of this work this instrumentation was not available.

The goal of the work reported here was to evaluate different solid phases for use as traps for dynamic off-line SFE. Commercial instrumentation was modified to allow for the use of these solid phase traps, which were in the form of solid phase extraction (SPE) tubes. The application of this system to the extraction of PCB's from river sediment will be discussed in a later chapter.

Experimental

Equipment and Chemicals

The instrument used for this work was a Suprex 200A (Pittsburgh, PA) SFC modified as previously described.[9] Specifically, the Suprex FID was not used as a detector, but as a heated block upon which the solid phase extraction tube could be mounted. Figure 1 shows a schematic of the modified FID. A plastic adapter, which

Figure 1

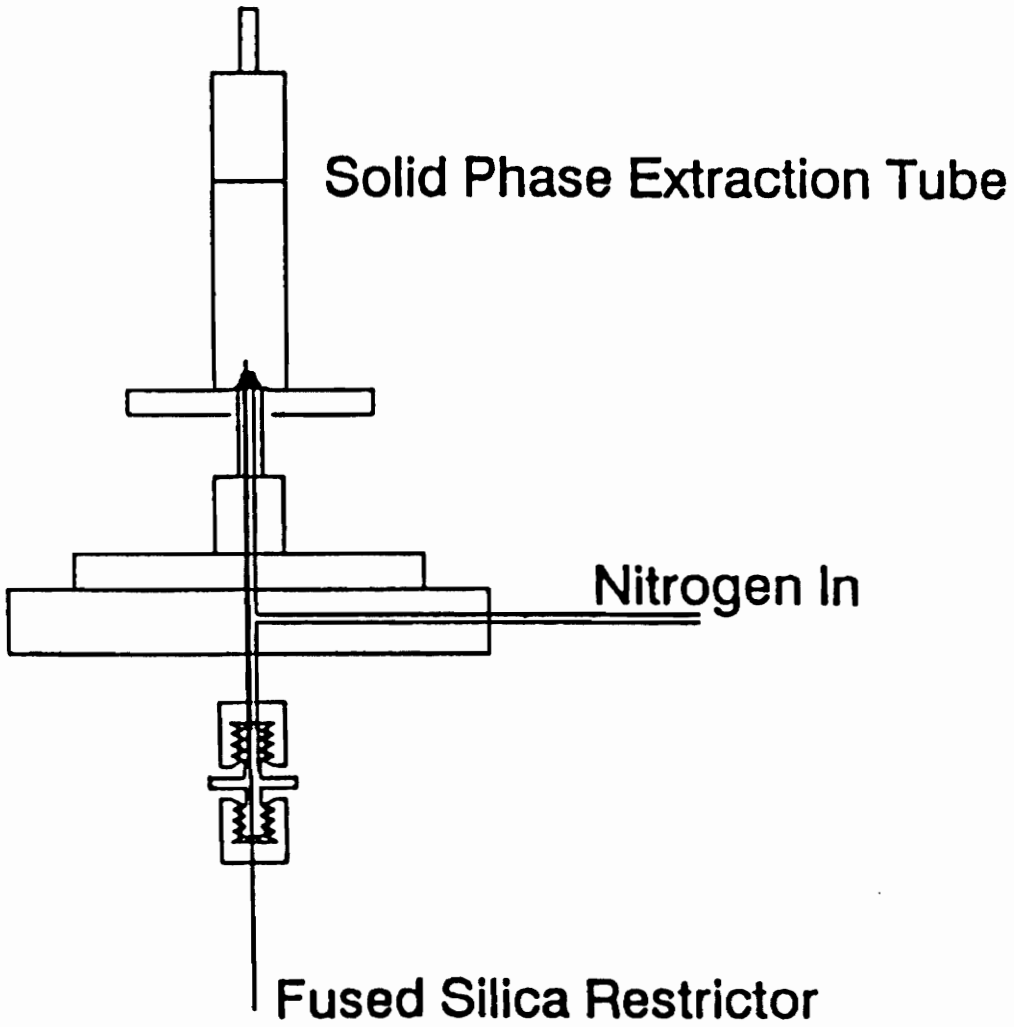


Figure 1: Schematic of modified flame ionization detector used for collection in off-line SFE experiments.

is not shown, was drilled out to fit tightly over the FID flame jet. The SPE tubes then fit onto the plastic adapter. Nitrogen was plumbed in through the hydrogen line in order to provide a flow of heated gas across the restrictor tip and therefore prevent restrictor clogging. The restrictor used in this work was a 50 μm tapered restrictor that gave a compressed flow (liquid) of approximately 2 mL/min at 300 atm. The block temperature of the FID was typically operated in the range of 50-100 °C, in order to control the temperature of the trap. The trap temperature was measured by placing a thermocouple in the space between the restrictor tip and the solid phase. These results have been reported previously.[10] It is possible to obtain trap temperatures ranging from -50 to 50 °C with this system.

Solid phase extraction tubes were purchased from Supelco (Bellefonte, PA), and contained 100 mg of 40 μm (60 Å pore size) packing material. Tubes containing only a 20 μm, 130 mg polyethylene frit (no packing material) were also purchased from Supelco. The tubes were treated before use with approximately 3 mL of the rinse solvent, followed by 3 mL of methanol. Once collection of analytes onto the trap was complete, the traps were rinsed by passing a small volume (1-2 mL) of rinse solvent through them with the aid of a vacuum. The tubes that contained only frits were rinsed without the use of a vacuum.

The phenols used in these studies were purchased from Chem Service (West Chester, PA). Acetophenone, N,N-dimethylaniline, n-decanoic acid, anthracene, naphthalene, and 2-naphthol were purchased from Aldrich Chemical Co. (Milwaukee,WI). Rinse solvents were all HPLC grade and were purchased from Fisher (Pittsburgh,PA). All carbon dioxide used was SFC grade from Scott Specialty Gases (Plumbsteadville, PA).

Trap Evaluation

Trap evaluation was performed by introducing a known concentration of sample into the extraction system, as shown in Figure 2. A 50 μL loop was used to introduce the sample. This loop was calibrated by the following process, which will be referred to afterwords as a "loop blank" experiment. The injection valve was plumbed so that the 50 μL loop was filled in the inject position with the sample of interest through a needle port. Once the loop was filled, the position of the valve was changed from load to inject. The loop was then rinsed with an organic solvent into a 2 mL autosampler vial, where internal standard was added, and then analyzed by GC. These values for the ratio of the average peak area to internal standard peak area were used as 100% values for each component. A 0.5 mL dead volume was introduced into the system in order to provide mixing of the analytes with SC-CO_2 so that the compounds were not simply mechanically pushed through the system. The traps were cryogenically cooled by the expanding CO_2 before an injection was made. A compressed flow rate (liquid) of 2 mL/min was used. The specific temperatures for each trapping study will be given with the results. Five replicate measurements were made for all traps unless otherwise stated. T-tests using pooled standard deviation were used to determine if data sets differed significantly. The results of these tests are contained in Appendix I.

The compounds used to evaluate the trapping efficiencies were phenols, and a "test mix" of varying polarity and vapor pressure. The phenol solution consisted of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 3-methyl-4-chlorophenol, 2,4-dimethylphenol, 4-nitrophenol, and pentachlorophenol dissolved in methanol at approximately 1 mg/mL per component. These analytes were transferred to the trap by putting the filled sample loop in-line with the flow of CO_2 for a period of 7 min at 300 atm and 50°C. The temperature of the collection traps was -20°C. The

Figure 2

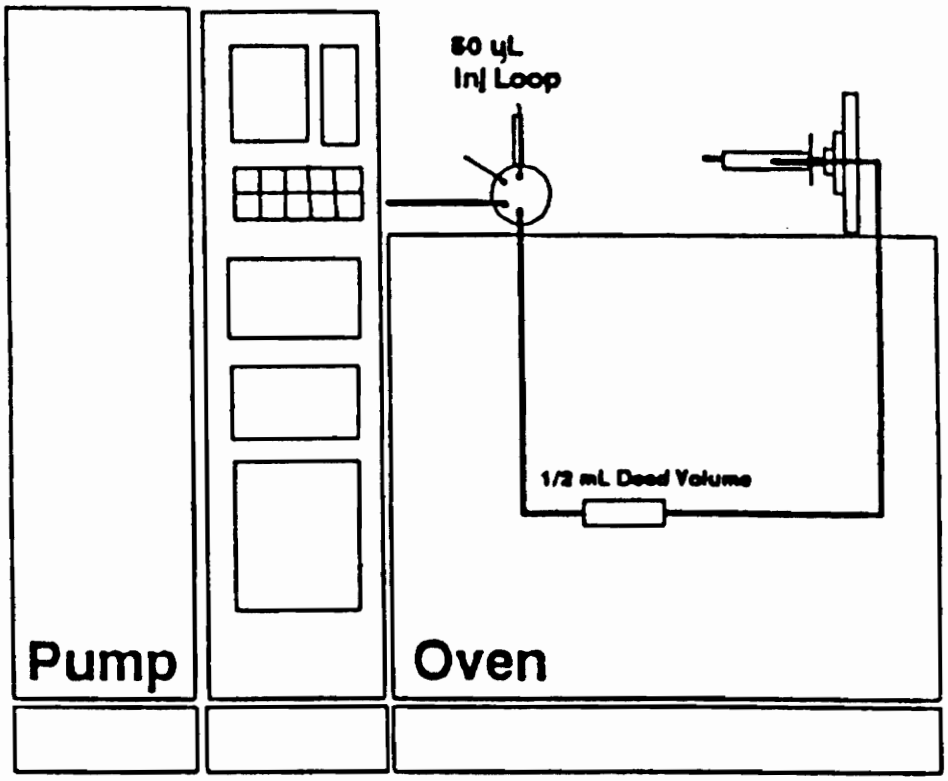


Figure 2: Schematic of the modified Suprex system used for extractions showing the use of the injection valve and the location of the 0.5 mL dead volume.

traps were rinsed with 2 mL of CH_3OH, and an internal standard (naphthalene) was added. The "test mix" consisted of acetophenone, N,N-dimethylaniline, n-decanoic acid, 2-naphthol, and n-tetracosane dissolved in CH_2Cl_2 at approximately 1 mg/mL. These "test mix" analytes were transferred to the traps by placing the filled loop in-line with the flow of CO_2 for a period of 4 min at 300 atm and 50°C. The temperature of the collection traps was -20°C. The traps were rinsed with either 2 mL of CH_2Cl_2 or CCl_4. An internal standard (anthracene) was added to the recovered analytes. The analysis of recovered analytes was then performed by gas chromatography. A 5% phenyl column (25 m x 0.20 mm i.d., df=0.33 μm) was used for all separations.

Results and Discussion

Previous Work

Previous work in this laboratory involved the evaluation of SPE tubes for trapping hydrocarbons and phenols.[10] The hydrocarbons studied were C10-C32 (excluding C30). The reverse phases studied were C18, C8 and phenyl. As expected, the C18 and C8 traps performed the best for the hydrocarbons, with recoveries in the range of 94-100%. The conclusions drawn from this work were that trapping was occurring by two mechanisms - cryotrapping and absorption. The traps were maintained at -20°C, therefore all of the less volatile hydrocarbons (C18 and above) exit the restrictor and are trapped either on the walls of the SPE tube and/or on the surface of the packing material. The more volatile hydrocarbons (C10, C12, C14, and C16), on the other hand, must be trapped by absorption. Because the polarity match between the analytes and the C8 and C18 traps is good, effective trapping of these hydrocarbons is achieved. For the absorbed compounds the phenyl trap is less efficient

due to reduced interactions with the aliphatic hydrocarbon solutes. For example, C10 was trapped at 80% on the phenyl trap while it was trapped at 100% on the C18 trap.

Phenols were also studied on the reverse phase traps, although for this work the C18 trap was not used. Recoveries ranged from 59-90% on the phenyl trap and 68-91% on the C8 trap. Of the more volatile phenols, 2-chlorophenol had the lowest recovery on both traps. Phenol, which is more volatile than 2-chlorophenol, exhibited recoveries near 80%. Pentachlorophenol had low recoveries and high RSD's on both traps. However, with pentachlorophenol, since it is very non-volatile, the low recoveries were attributed to poor rinsing. The small volume of methanol used to rinse the trap was unable to efficiently overcome the interactions betwen the solute and the stationary phase.

Normal phase traps (diol, silica, cyano, and amino) were also studied with the hydrocarbons and phenols. The recoveries for the hydrocarbons were generally lower on the normal phase traps than on the reverse phase traps, with maximum recoveries of 90%. On the silica and diol traps, the same general trend is seen as was observed on the reverse phase traps (i.e. absorption - more volatile, cryotrap - less volatile). Recoveries for the more volatile hydrocarbons (C10-C16) are lower due to the reduced interactions of these traps with the hydrocarbons. At hydrocarbons C18 and above, recoveries on the silica and diol traps are high (90%) and constant since cryotrapping becomes the dominant mechnaism. The cyano trap has low recovery for C10, but behaves more like a reverse phase trap in general, and the amino trap behaves unpredictably.

For the phenol mixture, recoveries were generally the best on the diol trap. Some of the same trends that were observed on the reverse phase traps are seen on the normal phase traps. Recoveries were generally lower for the cyano trap, again

indicating that it is performing more like the reverse phase traps. Pentachlorophenol elutes off of the cyano trap only. Since pentachlorophenol is the least volatile phenol of the phenols studied, it can be assumed that the 0% recoveries from the amino and diol traps result from the inability of the rinse solvent (methanol) to overcome the solute/stationary phase interactions.

Test Mix

The hydrocarbon and phenol trapping and rinsing studies have shown that trapping is occurring by two mechanisms and that problems can be encountered in trying to rinse the traps. Therefore, a test mix was explored (specifics of preparation are in the experimental) because these compounds cover a wide range of polarity and vapor pressure, and may be more representative of a "real world" sample. Table I lists the compounds of the test mix and some of their properties. Calibration curves were generated to ensure that the chromatographic behavior of each component used was well characterized. The concentration of the standards used for the calibration curves ranged from 6-600 ppm. All of the calibration curves were linear ($r=0.99$) with an intercept of zero, except for n-decanoic acid, which had a slightly negative intercept. The concentration of each component used in the trap evaluation study (once it is analyzed by GC) is around 25 ppm. The chromatographic behavior is therefore well characterized for these compounds over the concentration range we were working.

The loop blank experiments described earlier were used to determine 100% values for each of the components and to insure that the GC system was functioning well. The average component-to-internal standard ratio, its standard deviation and relative standard deviation for five determinations are shown in Table II. The

Table I

Physical Properties of the test mix components taken from the Merck Index

Compound	MW	mp(°C)	bp(°C)
acetophenone	120.15	20.5	202
N,N-dimethylaniline	121.18	2	193
n-decanoic acid	172.26	31.4	270
2-naphthol	144.16	122	285
n-tetracosane	398.63	125	300

Table II

Component to internal standard peak area ratio (X/IS), standard deviation (SD), and relative standard deviation (RSD) of five measurements for each test mix component for 100% determination.

Compound	X/IS	SD	RSD
acetophenone	0.303	.010	4.2
N,N-dimethylaniline	0.276	.010	4.2
n-decanoic acid	0.053	.006	11.2
2-naphthol	0.157	.006	3.9
n-tetracosane	0.175	.007	3.9

component to internal standard ratios were compared to the same ratios obtained in the trapping and rinsing studies to determine the percent recovery of each component. As can be seen from the Table, the RSDs are acceptable, showing that the GC is functioning well. The high RSD for decanoic acid is typical for tailing compounds. Each time a new solution was prepared, the loop blank experiment was repeated.

Table IIIA shows the trapping and rinsing efficiencies for the test mix on the reverse phase traps using CH_2Cl_2 as a rinse solvent. The phenyl trap performs better for this mixture than the C8 trap does. The two most volatile compounds of the mixture, acetophenone and N,N-dimethylaniline, have recoveries of 70% on the octyl trap. The recoveries for these compounds are slightly higher on the phenyl trap, where ¶-¶ interactions between these solutes and the stationary phase can occur. It is difficult to determine whether the 74% recoveries for these two compounds on the phenyl trap occur because of the inability of the trap to retain the volatiles or because of the inability of the rinse solvent to overcome these ¶-¶ interactions. N,N-dimethylaniline is a fairly hindered amine, and therefore should not have as strong interactions with the stationary phase through the basic nitrogen (i.e. hydrogen bonding) as a less hindered amine would. Decanoic acid recovery is low on both traps, although based on the behavior of decane, which is more volatile, decanoic acid should be trapped effectively. The lower recoveries in this case are believed to be due to the inability of the rinse solvent (CH_2Cl_2) to effectively overcome the solute/stationary phase interactions. Good recoveries ($\geq$90%) are achieved for 2-naphthol and n-tetracosane, the least volatile components, on the phenyl traps, with recoveries somewhat lower on the octyl traps. The carbon loading of the octyl phase is 8.0%, while the carbon loading of the phenyl trap is 5.3%. However, carbon loading comparisons are useful mainly when comparing the same type of bonded phases (i.e. octyl to octyl).

Table III

Trapping of test mix on reverse phase traps with (A) 2 mL of CH_2CL_2 rinse solvent, and (B) 2 mL of CCL_4 rinse solvent. Trap temperature was at -20°C. RSDs are based on five replicate measurements.

A

	Octyl Trap		**Phenyl Trap**	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	70.0	10.1	74.6	7.1
N,N-dimethylaniline	68.3	7.2	73.2	7.7
n-decanoic acid	43.2	18.8	55.2	16.7
2-naphthol	86.1	12.5	90.0	7.0
n-tetracosane	80.7	6.7	91.5	5.5

B

	Octyl Trap		**Phenyl Trap**	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	81.9	8.5	88.1	3.7
N,N-dimethylaniline	81.5	5.2	87.2	5.5
n-decanoic acid	1.9	3.2	17.4	2.5
2-naphthol	31.9	24.7	77.2	5.6
n-tetracosane	87.5	3.2	84.7	4.7

Therefore, no correlation between the ability to rinse the trap and carbon loading was made. In general the phenyl traps appear to be more easily rinsed that the octyl traps. The lower RSDs on the phenyl traps also support this trend.

Table IIIB shows the recoveries of the test mix on the same traps using CCl_4 as a rinse solvent. The recoveries of acetophenone and N,N-dimethylaniline increased on both traps, indicating that although they are the most volatile, they are indeed being trapped and the nature of the rinse solvent is very important in obtaining high recoveries. The most drastic difference seen with CCl_4 as a rinse solvent appears to be its inability to effectively rinse the acidic compounds (i.e. decanoic acid and naphthol) from the traps. On the octyl trap decanoic acid recovery droped from 43.2% with the CH_2Cl_2 rinse to 1.9%with the CCl_4 rinse, and naphthol recovery drops from 86.1% to 31.9%. These reductions in recovery are not as severe on the phenyl trap (55% with CH_2Cl_2 rinse to 17% with the CCl_4 rinse), again indicating that the phenyl trap can be more effectively rinsed. Naphthol recovery on the phenyl trap only decreased from 90.9% to 77.2%. These changes in recovery demonstrate clearly how vital the rinse solvent is in obtaining high extraction recoveries. For the non-acidic compounds, with the exception of n-tetracosane, the change in rinse solvent from CH_2Cl_2 to CCl_4 results in improved recoveries and RSDs. The reason for improved recoveries and reduced RSDs with CCl_4 as a rinse solvent are not immediately obvious. The density of CCl_4 at 25 °C is 1.5843 g/mL, while the density of CH_2Cl_2 at 25 °C is 1.317 g/mL.[11] The densities do not differ enough to cause significant differences in recovery. The viscosities of CCl_4 and CH_2Cl_2 are different, however, at 0.97 cP and 0.44 cP, respectively.[11] This viscosity difference may account for the observation that the traps rinse much faster with CH_2Cl_2 than with CCl_4. The slower rinsing may lead to improved recoveries for some of the components.

Table IV shows the recoveries of the test mix on the normal phase traps with CH_2Cl_2 as a rinse solvent. The normal phase traps are comparable to the reverse phase traps for this set of compounds, with the exception of decanoic acid trapping, which is 0% on the silica and amino traps. Decanoic acid is apparently absorbed by these traps so strongly that the rinse solvent is unable to overcome the analyte/trap interactions. The silica trap has higher percent recoveries for acetophenone and N,N-dimethylaniline than were achieved on the octyl traps, indicating that the absorptive interactions on the silica traps are favorable for these volatile compounds. The diol trap is the only trap that releases the decanoic acid, and it also gives the highest recovery for naphthol. The diol trap was the only normal phase trap tried with CCl_4 as a rinse solvent, and the results are also shown in Table IV. The recovery of the acidic compounds decreased on the diol trap when CCl_4 was used as a rinse solvent, just as was found on the C8 and phenyl traps. In fact, the recovery of naphthol was the lowest of all the traps for the diol trap with CCl_4 as a rinse solvent.

There are several possible reasons for less than 100% recoveries for all components off of the sorbent traps. One possible reason was that the sorbent traps were being overloaded and breakthrough was occurring. In the case of the "test mixture" 0.3 mg (1 μg/μL x 50 μL injection volume x 5 components = 250 μg) of material was loaded onto 100 mg of sorbent material. This concentration corresponds to 0.3 weight % liquid loading on the sorbent bed. Acording to Snyder and Kirkland, the loading range for silica gel (5-30 μm) is 10-30 weight % of liquid.[12] Therefore, the SPE tubes were not being overloaded. Another possible reason for less than 100% recoveries is the possibility of a synergistic effect between two compounds. If this occurred, the sorbent traps may exhibit different recoveries if a single component were being studied versus a mixture of components. However, at 0.3 weight % it is

Table IV

Trapping of test mix on normal phase traps. In all cases the rinse solvent was CH_2Cl_2, except for Diol*, where the rinse solvent was CCl_4.

	Silica Trap		**Amino Trap**	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	80.2	4.4	69.3	4.4
n,n-dimethylaniline	83.2	4.6	73.9	4.0
n-decanoic acid	0	0	0	0
2-naphthol	84.8	6.6	28.7	6.5
n-tetracosane	84.3	4.7	79.2	5.5

	Diol Trap		**Diol Trap***	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	71.1	7.0	79.5	6.8
n,n-dimethylaniline	72.1	7.6	78.8	5.3
n-decanoic acid	27.6	6.1	0.5	0.8
2-naphthol	95.6	7.0	4.5	2.8
n-tetracosane	93.4	5.4	83.1	8.3

unlikely that this is occurring.

Another possibility for these recoveries is that the compounds in the "test mix" may not be soluble in CO_2 to the extent that they were introduced into the system. Although there is limited data in the literature on the solubility of compounds in CO_2, it has been reported that acetophenone and N,N-dimethylaniline are miscible with CO_2.[13] No data were given in this paper for decanoic acid, 2-naphthol, or n-tetracosane solubility, although n-tetradecane is soluble in CO_2 at 50 weight %. Based on known structure/solubility behavior, the compounds in the test mix should all be soluble in CO_2. For each component of the test mix, 50 μg were introduced into the "extraction" system, and approximately 8 grams of liquid CO_2 (2 mL/min x 4 min x1.0 g/mL) were passed through the system. The weight percentage of component/CO_2 is approximately 0.0006%. The solubility of these compounds is therefore not exceeded.

The temperature of the traps could also have an effect on the recoveries obtained. For the work reported here, the trap temperature was not varied. However, the traps were maintained at -20°C, which is well below the melting point of the most volatile components of the test mixture (see Table I). The temperature of the trap during rinsing (room temperature) was not controlled. Percent recoveries may have been improved if the trap temperature was increased during the rinse. With the instrumentation we were using this was not possible.

Another factor that may contribute to recoveries of less than 100% is the small rinse volumes used. Recoveries may have been improved if a larger rinse volume was used. However, larger rinse volumes were not practical because the tubes that fit into the vacuum manifold to contain the rinse effluent could hold <3 mL of liquid. We also wished to avoid any further sample manipulation, such as preconcentration, so that the RSDs reflected, as much as possible, only trapping and rinsing efficiencies.

Therefore, the efficiencies obtained for the test mixture are believed to be a result of the sorbent material and the rinse solvent used. When the sorbent material and the solutes are similar, efficient trapping generally results (likes dissolve likes). The rinse solvent must be able to effectively compete with the sorbent to release the solutes.

Polyethylene Frits

The polyethylene frits (20 μm) that are used to keep the solid sorbent material in place were then used as traps for the test mix and the phenols. The objective in using the frits as traps was to determine the contribution of the packing material to the overall trapping efficiency. Table V(A) shows the recoveries for the test mix analytes using CH_2Cl_2 as a rinse solvent. The trapping efficiencies obtained on the frit compare very favorably to the trapping efficiencies obtained on the phenyl trap, which was the best trap overall for this mixture of analytes. Acetophenone recovery was 82% on the frit with the CH_2Cl_2 rinse, versus 70% on the octyl trap and 74.6% on the phenyl trap. Because the mechanism for trapping on the frit should be only cryotrapping, the lower recoveries from the octyl and phenyl traps must be due to inefficient rinsing. Acetophenone recovery on the phenyl trap with the CCl_4 rinse was the highest recovery obtained, at 88.1% with an RSD of 3.7%, and the frit recovery is comparable. N,N-dimethylaniline recovery was 79.1% on the frit, and 73.2% on the phenyl trap with CH_2Cl_2 as a rinse solvent, which, with the associated RSDs translates to no significant difference in trapping efficiency. Decanoic acid recovery on the frits is also comparable to that obtained on the phenyl and octyl traps. Recoveries of 2-naphthol and n-tetracosane are higher on the phenyl traps than on the frits. This is somewhat

Table V

Trapping of test mix on frits where (A) is CH_2Cl_2 prerinse and rinse, and (B) is CCl_4 prerinse and rinse.

A

Compounds	% Recovery	RSD
acetophenone	82.3	4.0
N,N-dimethylaniline	79.1	5.9
n-decanoic acid	51.5	17.2
2-naphthol	81.9	7.0
n-tetracosane	84.5	7.8

B

acetophenone	93.9	3.8
N,N-dimethylaniline	92.8	3.8
n-decanoic acid	97.5	10.7
2-naphthol	82.1	3.1
n-tetracosane	91.5	4.8

surprising since these compounds should cryotrap effectively and since the rinse solvent should have fewer interactions to overcome with the frits than with the sorbent trap.

The prerinse and rinse solvent for the frits was then changed to CCl_4, and the trapping efficiencies obtained are shown in Table V(B). By changing the rinse solvent to CCl_4 the recoveries of all analytes, with the exception of 2-naphthol and n-tetracosane, improved while RSDs decreased. Acetophenone and N,N-dimethylaniline recoveries were at 94% and 93% respectively, which is higher than the recoveries achieved for these compounds on any of the sorbent traps. Decanoic acid recovery improved drastically, jumping from 51.5% to 97.5% with the CCl_4 rinse.

This increase in trapping efficiency with CCl_4 as a rinse solvent was surprising. Prior to using the frits for trapping the test mix, the frits were prerinsed with CCl_4. The purpose of the prerinse was to ensure that any CCl_4-soluble species present on the traps as-received were removed. A prerinse with CH_3OH was then performed in order to be consistent with previous experiments. When CH_2Cl_2 was used as a prerinse solvent, some of it remained in the trap. When the traps were then cooled to -20°C, the CH_2Cl_2 freezes and flow through the trap stops. Although the freezing point of CH_2Cl_2 and CH_3OH differ by only a few degrees, (-95.14°C and -97.68°C respectively)[11] CH_2Cl_2 freezes when used for liquid trapping while CH_3OH does not. By rinsing the trap with methanol after the CH_2Cl_2 rinse this problem is avoided. It has been reported [14] that although CCl_4 has good properties for a slurry solvent for packing LC columns (ie: high density) once it is associated with the stationary phase it is difficult to remove. When the frits are rinsed with CCl_4 prior to use, the CCl_4 may remain in the frit, therefore providing another trapping mechanism for the solutes. In order to determine the effect of the prerinse on trapping efficiency, a systematic study was performed where the prerinse and rinse solvents were varied. The results from

this study are shown in Table VI.

The highest recoveries in general were achieved with the CCl_4 prerinse and CCl_4 rinse (D), while the lowest recoveries were obtained with the CH_2Cl_2 prerinse and rinse (A). With the exception of naphthol, the recoveries for B (the CCl_4 prerinse with the CH_2Cl_2 rinse) are the same as for C (the CH_2Cl_2 prerinse with the CCl_4 rinse). The recovery of decanoic acid is most affected by changing the prerinse/rinse solvents. With the CH_2Cl_2 prerinse and CH_2Cl_2 rinse decanoic acid recovery was 51.5%. When the prerinse and rinse solvents are not identical (ie: B and C) decanoic acid recovery is approximately 70%. In other words, the order of the solvents appears to not influence recovery in these two cases. When the prerinse and rinse solvent was CCl_4, decanoic acid recovery was 97.5%. This trend indicated that the recovery of decanoic acid from the frit seems to be a function of both the prerinse and the rinse solvent. The other acidic component, naphthol, was much less affected by the prerinse and rinse solvent combination. Naphthol recovery was constant in all cases except B. Although no mechanism is immediately obvious to account for the high naphthol recovery of B, this number nevertheless represents the average recovery for five replicate experiments. The RSD was low, therefore these data should not be discounted.

The comparison of decanoic acid and naphthol trapping on the frit versus the sorbent traps with the CCl_4 rinse and prerinse is quite interesting. Recall that decanoic acid and naphthol recoveries dropped drastically on the C8, phenyl, and diol traps when the rinse solvent was changed to CCl_4 (see Table III and Table IV). The interactions between the acidic solutes and the packing material are apparently too strong to be overcome by the CCl_4 rinse. The high recoveries achieved on the less active frits reinforces the difficulty associated with rinsing compounds off of sorbent traps.

Table VI

Comparison of trapping efficiencies on frits with varying prerinse and rinse solvent.

	A		B	
prerinse	3 ml CH_2Cl_2 3 mL CH_3OH		3 mL CCl_4 3 mL CH_3OH	
rinse	2 mL CH_2Cl_2		2 mL CH_2Cl_2	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	82.3	4.0	88.0	5.6
n,n-dimethylaniline	79.1	5.9	89.5	6.5
n-decanoic acid	51.5	17.2	74.1	13.4
2-naphthol	81.9	7.0	95.1	2.7
n-tetracosane	84.5	7.8	86.0	2.8

	C		D	
prerinse	3 mL CH_2Cl_2 3 mL CH_3OH		3 mL CCl_4 3 mL CH_3OH	
rinse	2 mL CCl_4		2 mL CCl_4	
Compound	% Recovery	RSD	% Recovery	RSD
acetophenone	89.7	6.4	93.3	3.8
n,n-dimethylaniline	89.0	6.3	92.8	3.8
n-decanoic acid	71.6	13.0	97.5	10.7
2-naphthol	81.6	8.7	82.1	3.1
n-tetracosane	92.3	10.9	91.5	4.8

The next series of compounds studied on the polyethylene frits were phenols. Table VII lists the phenols studied and some of their properties. The loop blank experiment was performed for this set of compounds and the results are shown in Table VIII. As can be seen from the Table, these compounds chromatograph well, with RSDs all less than 5%. In a previous study, the low recoveries of these phenols off of the SPE tubes containing stationary phases was attributed to poor rinsing of the traps.[10] If the phenols follow the same trend as the test mix, recoveries would be predicted to improve when frits are used as traps. In the previous work, methanol was used as a prerinse and rinse solvent, so initial experiments on the frits used methanol as the prerinse and rinse solvent. The results for this set of trapping studies are shown in Table IX. Phenol recoveries increased with decreasing vapor pressure, so that phenol recovery was the lowest (64.7%) while pentachlorophenol recovery was the highest (104.8%). Also shown in Table IX are the results reported by Taylor et al.[10] for trapping of these phenols on diol SPE tubes, which was the best sorbent trap for these compounds. The recoveries are generally higher on the diol traps and do not follow the trend of increasing recovery with decreasing volatility. The RSDs on the frits are lower than the RSDs on the diol traps, indicating that the frits are easier to rinse. The lower recoveries on the frits indicate that while they may be easily rinsed, cryotrapping may not be sufficient for trapping these phenols. The major difference between recoveries on the frits versus the diol traps is that pentachlorophenol recovery was 0% on the diol traps and 100% on the frits. Pentachlorophenol was rinsed off the frits much easier than off of the sorbent traps due to the lack of absorptive interactions, which are expected to be large on the sorbent traps because of its high acidity.

As was done with the test mix, the prerinse and rinse solvents were varied in order to determine what effect they had on phenol recovery. The results for the

Table VII

Physical properties of the phenol mixture components taken from the Merck Index.

Compound	MW	mp(°C)	bp(°C)
phenol	94.11	40.85	182
2-chlorophenol	128.56	9.3	175
2,4-dimethylphenol	122.2	26	210
2,4-dichlorophenol	163.0	45	210
3-methyl-4-chlorophenol	142.58	55.5	235
2,4,6-trichlorophenol	197.46	69	246
4-nitrophenol	139.11	114	-----*
pentachlorophenol	266.35	191	310

* sublimes

Table VIII

Component to internal standard peak area ratio (X/IS), standard deviation (SD), and relative standard deviation (RSD) of five measurements for each component of the phenol mixture for 100% determination.

Compound	X/IS	SD	RSD
phenol	0.191	.005	2.5
2-chlorophenol	0.179	.004	2.2
2,4-dimethylphenol	0.203	.004	1.8
2,4-dichlorophenol	0.098	.001	1.0
3-methyl-4-chlorophenol	0.141	.002	1.2
2,4,6-trichlorophenol	0.094	.003	3.4
4-nitrophenol	0.068	.002	3.2
pentachlorophenol	0.022	.001	4.6

Table IX

(A) Trapping efficiencies of phenol mixture on frits, and (B) trapping efficiency of phenol mixture on diol traps.

A

Compound	% Recovery	RSD
phenol	64.7	2.0
2-chlorophenol	64.7	2.5
2,4-dimethylphenol	71.9	2.5
2,4-dichlorophenol	71.7	3.3
3-methyl-4-chlorophenol	73.8	2.3
2,4,6-trichlorophenol	74.6	4.1
4-nitrophenol	77.6	3.4
pentachlorophenol	104.8	10.5

B

Compounds	% Recovery	RSD
phenol	97.2	13.0
2-chlorophenol	91.8	18.9
2,4-dimethylphenol	97.0	11.3
2,4-dichlorophenol	92.4	12.8
3-methyl-4-chlorophenol	97.0	9.6
2,4,6-trichlorophenol	86.9	14.0
4-nitrophenol	58.1	2.4
pentachlorophenol	0	0

CCl_4 prerinse and rinse, the CCl_4 prerinse/CH_3OH rinse, and the CH_3OH prerinse and rinse are shown in Table X. The prerinse and rinse solvents affect the recoveries of the more volatile phenols. For example, phenol recovery is statistically different under all three prerinse/rinse conditions. Phenol, 2-chlorophenol,and 2,4-dimethylphenol all have higher recoveries when the CCl_4 prerinse and CH_3OH rinse was used. The less volatile compounds, 2,4-dichlorophenol, 3-methyl-4-chlorophenol, and 2,4,6-trichlorophenol, show no significant difference in their recoveries when prerinse and rinse solvents were varied. The recovery of 4-nitrophenol is lowest when CCl_4 prerinse and rinse were used, and there is no difference when the prerinse solvent was varied when the rinse solvent was CH_3OH. Pentachlorophenol recovery was much higher than was observed on the diol trap. However, a low recovery (68%) was obtained for the CCl_4 prerinse/CH_3OH rinse for this compound. The reason for the low recovery under these conditions is not immediately obvious.

The trends observed for the phenols were that the more volatile phenols trapped better when CCl_4 was present on the trap, supporting the theory that CCl_4 provides an additional trapping mechanism. The prerinse and rinse solvents appear to make no difference for the less volatile phenols, with the exception of nitrophenol and pentachlorophenol, indicating that these compounds require an absorptive mechanism to be efficiently trapped.

Conclusions

The goal of finding a solid phase that will efficiently trap analytes of varying polarity and vapor pressure is quite challenging. The solid phase extraction tubes function well as traps for the phenol test mix, while the frits are more effective for the "test mix". The fact that >90% recoveries for the "test mix" from the frits was achieved belies the difficulty that can be encountered in rinsing solutes from sorbent

Table X

Trapping efficiencies on frits for phenol mixture, with (A) CCl_4 prerinse and rinse, and (B) CCl_4 prerinse and CH_3OH rinse, and (C) CH_3OH prerinse and rinse.

	A	
Compound	% Recovery	RSD
phenol	61.2	2.5
2-chlorophenol	55.0	4.1
2,4-dimethylphenol	63.9	4.7
2,4-dichlorophenol	71.3	2.1
3-methyl-4-chlorophenol	74.2	1.1
2,4,6-trichlorophenol	70.7	2.5
4-nitrophenol	71.9	2.6
pentachlorophenol	103.2	10.3

	B		C	
Compound	% Recovery	RSD	% Recovery	RSD
phenol	69.1	3.5	64.7	2.0
2-chlorophenol	68.4	4.6	64.7	2.5
2,4-dimethylphenol	73.3	4.3	71.9	2.5
2,4-dichlorophenol	71.7	5.1	71.7	3.3
3-methyl-4-chlorophenol	74.4	3.5	73.8	2.3
2,4,6-trichlorophenol	71.0	6.7	74.6	4.1
4-nitrophenol	76.4	3.3	77.6	3.4
pentachlorophenol	69.8	5.4	104.8	10.5

traps. The "test mix" is a more realistic sample since it affords a wide range of polarities. For each solute there will be an ideal trap material/rinse solvent combination, however, for real world samples that contain multiple extractable components, it is not feasible to change rinse solvents and traps for each component.

Chapter 3

The Effect of Modifiers on Collection Efficiency of Solid Surface and Sorbent Traps

Introduction

As was discussed in the previous chapter, there are two general requirements that must be met in order to successfully perform off-line analytical supercritical fluid extraction (SFE). First, the extraction parameters must be chosen correctly, given that the analyte is soluble in the extraction fluid. Second, the trapping system used must perform efficiently. For a trapping system to perform efficiently the analytes must be quantitatively trapped and released quantitatively from the trap upon rinsing.

The three types of post restrictor traps generally used for off-line SFE - solid surface, sorbent, and liquid- were described in the previous chapter, as well as some of the advantages and disadvantages of each. A systematic evaluation of several sorbent traps and a solid surface trap was also presented in the previous chapter. In all of this work only pure carbon dioxide was used. Therefore, the effect of modified fluids on the trap was not studied.

When liquid trapping is used the effect of modifier should be negligible as long as the modifier and the collection fluid are miscible, and both fluids are compatible with the method of analysis. For example, water would not be an appropriate modifier if hexane was used as the collection fluid because a two phase system would result and the analytes of interest may partition between these phases.

The effect of modifiers on solid surface and sorbent traps has not been studied. If the trap is maintained above the boiling point of the modifier, the modifier should vaporize upon contact with the trap and vent to waste. However, the analytes of

interest may not trap effectively at the temperatures required to vaporize the modifier. If the trap temperature is maintained at temperatures below the boiling point of the modifier the modifier may condense on the stationary phase and influence the trapping efficiency.

The goal of this work was to determine what effect modifier has on the trapping efficiency of solid surface and sorbent traps. Stainless steel beads were investigated as the solid surface trap and octyldecylsilane bonded silica (ODS) packing material was used for the sorbent trap. The effects of methanol concentration in carbon dioxide as well as trap temperature were explored. The effect of modifier on the trap is an extremely important parameter. Often modifier is added to a fluid in an attempt to improve extraction recoveries or decrease extraction time. However, if modifier is causing the trap to perform less efficiently than it would with a pure fluid, the benefit of adding the modifier may be reduced or lost. A search of the literature resulted in no studies regarding the influence of modifier on trapping efficiency for off-line SFE with sorbent or solid phase traps.

Experimental

A Hewlett Packard (Avondale, PA) 7680A supercritical fluid extractor was used for all work reported here. This instrument was modified so that a reproducible amount (50 μL) of sample could be introduced into the system. A six port external loop injection valve (Valco, Houston, TX) was plumbed in-line between the extraction vessel and the pressure isolation valve. A 0.5 mL dead volume was introduced after the injection valve so that mixing of the injected components and the extraction fluid would occur.

The trapping system on the 7680A consists of a temperature controlled stainless

steel housing with frits on both ends. The trap contains approximately 1 mL of either 100 μm stainless steel beads or 40 μm silica based ODS packing material. The available temperature range during both the extraction and rinse steps is from 5-80 °C. The trap was rinsed with methylene chloride, and both the trap temperature and solvent rinse volume were controlled during rinsing. The trap temperature was maintained at 30 °C during rinsing and 2.6 mL of rinse solvent were passed through the trap (two 1.3 mL fractions). Any irreproducibility in rinse solvent volume was eliminated through the use of an internal standard (anthracene) added to the 2 mL autosampler rinse vial before rinsing so that peak area ratios and not absolute peak areas could be used.

The 50 μL loop was calibrated as was described in Chapter 2 as a "loop blank" experiment. That is, the injection valve was plumbed so that the loop was filled in the load position with the sample of interest through a needle port. Once the loop was filled the position of the valve was changed from load to inject. The loop was then rinsed with methylene chloride into a 2 mL vial where anthracene (200 μL of 1 mg/mL) was added as an internal standard, and then analyzed by GC. The values for peak area ratios obtained (peak area of compound/peak area of internal standard) for five replicate "loop blank" experiments were used for 100% numbers. The relative standard deviations obtained for the peak area ratios were typically in the range of 1-2%.

The compounds used to evaluate trapping efficiency were the "test mix" compounds also used in Chaper 2. This mixture consisted of acetophenone, N,N-dimethylaniline, n-decanoic acid, 2-naphthol, and n-tetracosane dissolved in methylene chloride at approximately 1 mg/mL. The molecular weights, melting and boiling points for these compounds are listed in Chapter 2 on page 14. The test mix components were all obtained from Aldrich Chemical Co. (Milwaukee,WI).

Carbon dioxide was obtained from Air Products (Allentown, PA) while the 1%, 2%, 4%, and 8% methanol modified carbon dioxide was provided by Scott Specialty Gases (Plumsteadville,PA). Methanol modified tanks were supplied with 1500 psi of helium headspace, while the pure carbon dioxide had no headspace.

For trap evaluation the analytes were transferred to the trap by placing the filled sample loop in line with the fluid path for a period of ten minutes at a liquid flow rate of 2 mL/min at 340 bar with an oven temperature of 75 °C and a nozzle temperature of 50 °C. Recovery was monitored at trap temperatures of 5, 10, 20, 30, 40, 50, 65, and 80 °C. Three replicate "extractions" were done at each trap temperature. The extracts were analyzed by gas chromatography on a Hewlett Packard (Avondale, PA) 5890 Series II GC equipped with an autosampler. The column used was an HP-5 (5% phenyl methylsiloxane) and was 25 m X 0.2 mm with a film thickness of 0.33 μm. A purged splitless injection was used. The initial column temperature was held at 75 °C for 0.5 min and ramped to 300 °C at 25 °C/min.

Results and Discussion

Sample Introduction

Because the goal of this work was to evaluate the trapping efficiency of the ODS and stainless steel traps as a function of modifier concentration, the sample introduction system described in the Experimental section was used. With sample introduction through a sample loop the kinetics of extraction are eliminated since an actual extraction is not occurring. The test mix components could have been introduced into the extraction system by spiking them onto an inert support, such as Celite. However, this method of sample introduction dictates that extraction kinetics be considered. For example, if recovery of a test mix component was low, trapping

efficiency alone could not be blamed since that compound may simply require a larger volume of fluid to extract efficiently. The elimination of extraction kinetics therefore makes data interpretation much simpler. Note also that all of the instrumental parameters (i.e. rinse solvent, rinse volume, rinse temperature, flow rate, extraction time) have been left constant in order to study only trapping.

After extraction kinetics have been eliminated from consideration, the solubility of the test mix components must be considered. Although solubility data for specific compounds in supercritical carbon dioxide is limited, there are some basic structure/solubility correlations that are generally known. For example, the presence of multiple hydroxyl groups, acid groups, halogens, or a combination of these functional groups decreases the solubility of a compound in supercritical carbon dioxide.[15] Based on the known structure/solubility data and the absence of any clogging of the extraction system after more than 150 injections of the test mix, if is safe to assume that all of the components of the test mixture are soluble in carbon dioxide.

Another point that needs to be addressed is that these components were introduced into the trapping system as a mixture. The question of whether the trapping efficiency would differ if the components were introduced individually could be raised. These components were prepared at a concentration of 1 μg/μL (per component) in 25 mL of methylene chloride. Methylene chloride has a density of 1.317 g/mL at 25 °C. Therefore, each component is present at approximately 0.07% (w/w), leading to a total solution at approximately 0.4% (w/w). At this concentration it is more likely that a solute molecule would encounter a solvent molecule rather than another solute molecule. Note also, as reported in Chapter 2, that calibration curves over a large concentration range were prepared for these compounds, and correlation coefficients of approximately 1 were obtained. The fact that no changes in the chromatographic

behavior (i.e. the presence of additional peaks, or increases/decreases in peak area with time) were observed also indicates that it is unlikely that any reaction is occurring between test mixture components. The next issue that must be addressed is whether these compounds encounter each other on the trap material. Again, the weight percent ratio of compound to stationary phase is low (0.3%) indicating that it is unlikely that the components would encounter each other on the stationary phase.

ODS Bonded Phase Trap

The characteristics of bonded phases have been studied since these phases are the most commonly used stationary phases for HPLC. There are three general methods for the preparation of bonded phases, according to Snyder and Kirkland.[16] The first method is by esterification of silanol groups with an alcohol, or by chlorinating the silica support and then reacting with an alcohol to produce a silicate ester. These esterified phases are hydrolytically or thermally unstable. The second method used for bonded phase preparation involves Si-C bonds instead of Si-O-C bonds. In this method bonded phases are prepared by first chlorinating the silanol groups and then reacting with a Grignard reagent. A disadvantage of this method is that relatively low surface coverage is achieved. Therefore, the third and most common method is based on siloxanes (Si-O-Si-C). These bonded phases are prepared by reacting the silanol groups on the silica support with organosilane or organoalkylsilane reagents. The reagent used is dependent on the desired functionality. These bonded phases are hydrolytically stable at pH= 3-7, and satisfactory surface coverage can be obtained.

Although satisfactory surface coverage can be obtained there will still be residual acidic silanol groups that are accessible to the mobile phase and to the sample. Residual silanol sites are known to lead to tailing of chromatographic peaks. The deactivation of residual silanol groups through the addition of methanol to the mobile

phase in supercritical fluid chromatography (SFC) has been reported.[17] Addition of a small amount of modifier to the mobile phase has resulted in improvements in peak shape and reduction in retention. Because there are residual silanol groups present on the ODS trapping material used in this work two types of sorptive interactions between the solutes and the stationary phase can occur. There can be absorptive interactions between the solutes and the ODS phase (dipersive forces) and there can be adsorption between the silanol groups and the analytes (hydrogen bonding, or forces stonger than dipsersive). Although we refer to absorptive forces in this manuscript, we realize that there are adsorptive forces also occurring.

Studies of bonded stationary phases involve their use in HPLC, and recently in SFC.[18,19] The ODS material used in this work was a silica based bonded phase, which therefore has residual silanol groups. At the trap temperatures used in this work (5-80 °C) the phase should be stable since there is flow of carbon dioxide passing through it when it is heated (i.e. the phase is not being "baked") and the temperatures used were not high enough to remove any physically adsorbed water. Snyder and Kirkland report that temperatures of 200 °C under vacuum for 8-16 hours are required to remove physically adsorbed water from silica supports.[20] Therefore, it is unlikely that carbon dioxide at 80 °C would be able to dehydrate the stationary phase. The solubility of water in supercritical carbon dioxide is low[9] (0.1%), therefore it is unlikely that water would be removed by decompressed carbon dioxide passing through the trap. Therefore it will be assumed for this work that no physical changes to the bonded phase due to the temperatures used or the presence of decompressed carbon dioxide occurred.

The surface of a bonded ODS phase has been described as "brush-like" where the ODS chains would be extended into the mobile phase. One could also envision the ODS chains to overlap and cover the silica support. The physical appearance of the

ODS chains depends on the mobile phase. To our knowledge there has been no work on the appearance of these ODS chains in the presence of decompressed carbon dioxide.

Stainless Steel Beads

Because stainless steel beads are not used as stationary phases in chromatography the information regarding their behavior is somewhat limited. The stainless steel beads used in this work were 100 μm 316 stainless steel with high nickel content. These beads have low carbon content and were not subjected to acid or heat pretreatment. The mechanism of action for analyte trapping on stainless steel beads is supposed to be cryotrapping only. However, there may be an oxide layer present that could lead to some adsorption of either methanol or other analytes.

Mobile Phase

In this trapping study there is no "mobile phase" in the traditional sense, since the carbon dioxide decompresses before it reaches the trap. Therefore, the mobile phase is not a liquid or a supercritical fluid, but could probably be more accurately described as a gas or a gas doped with an organic modifier. In gas chromatography the mobile phase has no solvating ability and is therefore referred to as a carrier gas. However, in this work there may be some solvation of the test mix components with the methanol in the decompressed mobile phase, and therefore the decompressed carbon dioxide and carbon dioxide/methanol will be referred to as a mobile phase.

ODS Trap, 100% carbon dioxide

The first trap investigated was the ODS trap, which contained approximately 1.0 mL (100 mg) of packing material. Table XI lists the percent recoveries and their associated relative standard deviations for the two most volatile components of the test

Table XI

Percent recovery and RSDs for the volatile compounds with pure carbon dioxide on the ODS trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	96.3	1.7	94.7	2.7
10	102.3	2.1	100.4	2.8
20	102.2	2.8	100.4	2.8
30	58.2	3.4	61.5	3.7
40	23.6	0.6	19.2	0.8
50	27.3	0.7	21.8	0.6
65	15.4	0.6	15.1	0.7
80	9.6	0.6	10.8	0.4

mixture, acetophenone and N,N-dimethylaniline. These numbers are presented graphically in Figure 3. As can be seen from the Table and the Figure, at trap temperatures of 5,10, and 20°C high recoveries and low relative standard deviations were obtained. At a trap temperature of 30°C, however, the recoveries of the volatiles dropped to approximately 60%. At trap temperatures of 40 and 50°C recoveries of both compounds dropped to 20-25%, and finally at trap temperatures above 50°C recoveries of 10-15% were obtained. For efficient trapping of volatile compounds it therefore appears that two trapping mechanisms are required - cryotrapping and absorption. At trap temperatures from 5-20°C both mechanisms combine to result in efficient trapping. However, at higher trap temperatures absorption must become the only mechanism. For these volatile compounds efficient trapping by the ODS phase through absorptive interactions alone was not achieved. As the trap temperature increases the vapor pressure of the volatile components increases and trapping efficiency decreases.

Table XII shows the percent recoveries and relative standard deviations obtained for n-decanoic acid, 2-naphthol, and n-tetracosane on the ODS trap. These results are also shown graphically in Figure 4. As can be seen from the Table and the Figure, the behavior of the less volatile analytes is drastically different from the more volatile analytes. Decanoic acid yielded the lowest recovery (75-80%) at low trap temperatures (5-40°C). However, as the trap temperature was increased above 40°C, decanoic acid recovery improved until it reached 90% at a trap temperature of 80°C. Since the rinse conditions were constant at all trap temperatures, it can be assumed that any variation in recoveries was due to the trapping portion of the experiment. Therefore, decanoic acid traps more efficiently at higher temperatures where sorptive interactions are dominant. The dispersive forces that exist between the ODS phase and decanoic acid

Figure 3

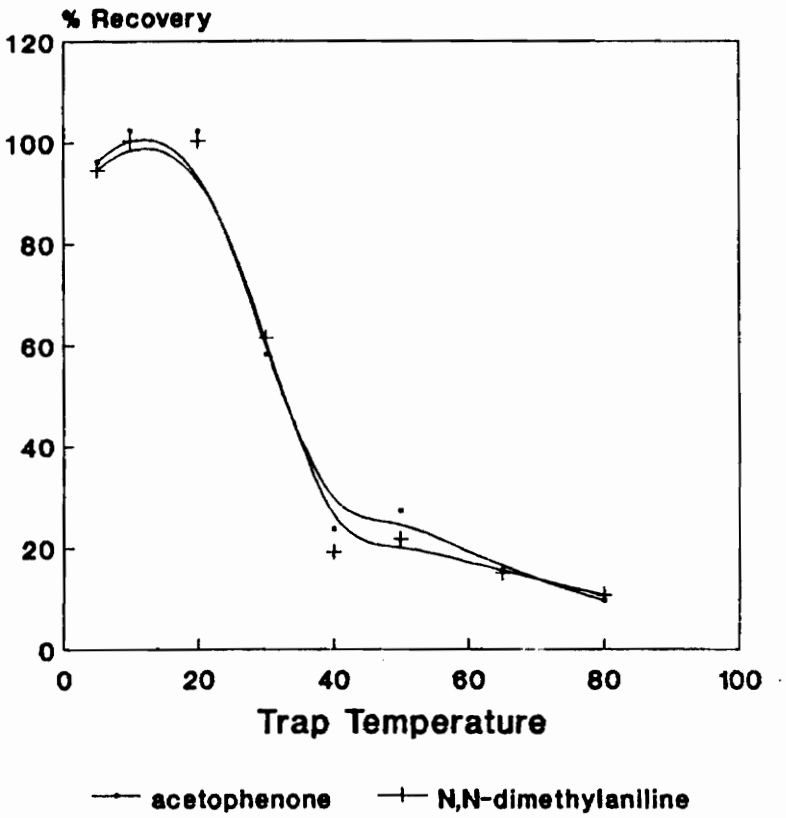


Figure 3: Percent recovery versus trap temperature for acetophenone and N,N-dimethylaniline with pure carbon dioxide as the mobile phase on the ODS trap.

Table XII

Percent recovery and RSDs for the nonvolatile compounds pure carbon dioxide on the ODS trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	75.1	3.0	94.8	2.0
10	78.2	3.4	99.8	2.5
20	76.4	3.4	99.2	2.5
30	76.7	4.8	96.6	1.6
40	80.1	3.9	94.9	1.8
50	83.4	4.9	93.4	1.5
65	85.3	4.0	92.6	5.6
80	90.1	4.6	91.3	2.4

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	97.5	1.6
10	100.7	2.7
20	99.5	1.7
30	97.7	1.1
40	98.0	2.0
50	97.7	1.1
65	97.4	4.9
80	97.1	1.7

Figure 4

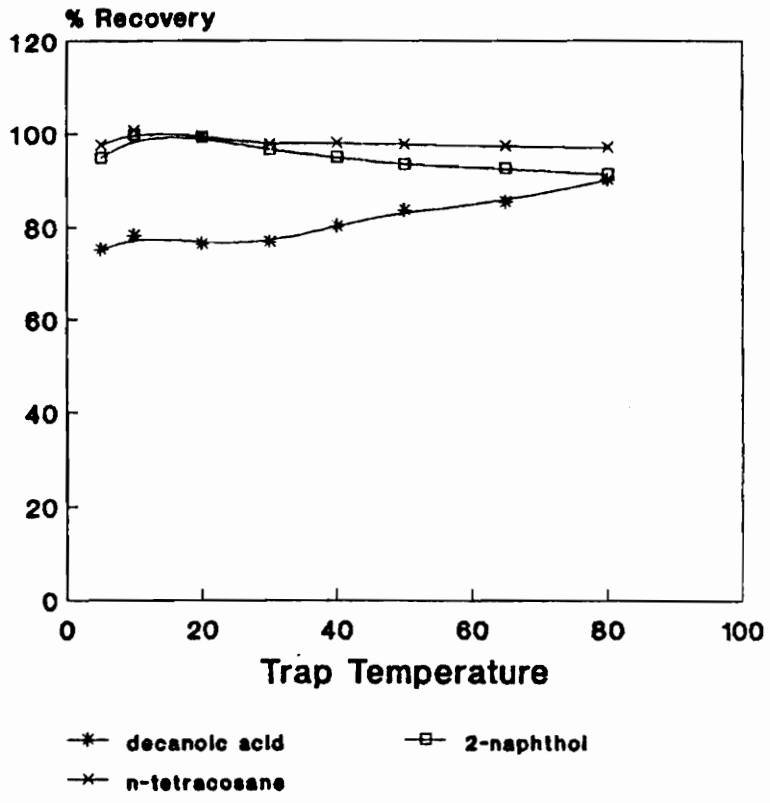


Figure 4: Percent recovery versus trap temperature for decanoic acid, 2-naphthol, and n-tetracosane with pure carbon dioxide as the mobile phase on the ODS trap.

and the hydrogen bonding that can occur between the acid group and the silanols at high temperature are apparently strong enough to result in efficient trapping.

Trapping of 2-naphthol and n-tetracosane was unaffected by trap temperature. There was no statistical difference in recovery between the 5 °C trapping study and the 80 °C trapping study for either of these compounds. These compounds are non-volatile enough to be efficiently trapped on the ODS packing material even at high temperatures. Since no cryotrapping can occur at the high trap temperatures, trapping for these nonvolatile compounds can occur in two ways - through sorptive interactions with the stationary phase and by seeing the ODS packing material as just a solid surface. The recoveries of n-tetracosane, which would be expected to have strong absorptive interactions with the ODS phase were not statistically different than those obtained over the entire temperature range on the stainless steel trap (vide infra), indicating that absorptive interactions are not necessary for efficient trapping of n-tetracosane. Recoveries of 2-naphthol differ significantly between the ODS trap and the stainless steel trap indicating that the absorptive interactions between the stationary phase and the solute lead to efficient trapping at high temperatures.

ODS Trap, 1% and 2% methanol

Figure 5 shows the percent recoveries obtained for acetophenone and N,N-dimethylaniline over the 5-80 °C temperature range with 1% and 2% methanol as the mobile phase. The percent recoveries and associated RSDs for these and all subsequent mobile phases studied are shown in Appendix II. The recoveries and RSDs of the volatiles were very good at trap temperatures from 5 to 20 °C. As was the case when pure carbon dioxide was used, recoveries of the volatiles dropped drastically when a trap temperature of 30 °C was used. However, acetophenone recovery was much more

Figure 5

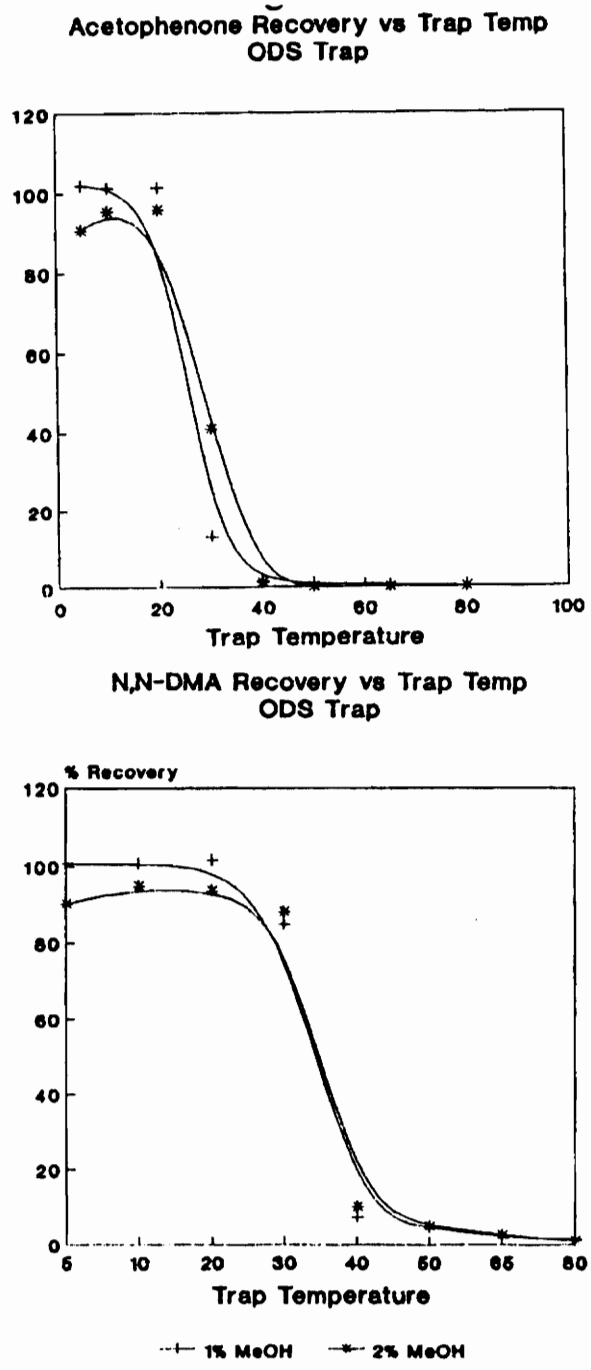


Figure 5: Percent recovery versus trap temperature for acetophenone and N,N-dimethylaniline with 1% and 2% methanol in carbon dioxide on the ODS trap.

affected by the increase in trap temperature than was N,N-dimethylaniline. Acetophenone recovery at a trap temperature of 30°C with 1% methanol was 12.9%, while N,N-dimethylaniline recovery was 84.8%. This disparity in recovery was also observed with 2% methanol. At trap temperatures of 40°C and above the recoveries of both compounds were low, with the recovery of acetophenone generally being the lowest of the two, again following the trend of decreased recovery with an increase in vapor pressure.

The recoveries versus temperature for acetophenone and N,N-dimethylaniline with pure carbon dioxide and 1% and 2% methanol in carbon dioxide were generally the same, however, there are a few differences. At trap temperatures of 40°C and above the volatiles were trapped at 10-20% when pure carbon dioxide was used. At these high trap temperatures no cryotrapping is occurring, therefore sorptive interactions are the dominant trapping mechanism. When 1% and 2% methanol were added to the mobile phase, recoveries of $<2\%$ were achieved for acetophenone and $<8\%$ for N,N-dimethlyaniline at temperature of 40°C and above. Therefore, the addition of methanol has somehow influenced the absorptive behavior for these compounds. The methanol could influence the absorption of acetophenone and N,N-dimethylaniline in three ways - it could affect the chemical characteristics of the trap, it could affect the characteristics of the mobile phase, or it could do both.

As will be discussed in Chapter 6, in supercritical fluid chromatography the addition of small amounts of organic modifier can have a drastic effect on retention. The explanation for changes in retention and improvement in peak shape with the addition of modifier are (1) deactivation of the stationary phase, and (2) increasing the solvent strength of the mobile phase. The general consensus reached in the literature is that the addition of modifiers to carbon dioxide in supercritical fluid chromatography

affects both the mobile phase strength and the characteristics of the stationary phase. Therefore, both of these effects must be examined here.

As was stated earlier, in this trapping study there is no "mobile phase" in the traditional sense, since the carbon dioxide decompresses before it reaches the trap. The mobile phase can therefore be more accurately described as a gas doped with an organic modifier. Because there may be some solvation of the test mix components with the methanol in the decompressed mobile phase, this must be considered as a partitioning mechanism.

The ODS stationary phase used in this work is silica based, and therefore has residual silanol groups, or active sites, which are slightly acidic. The disparity in recovery of acetophenone and N,N-dimethylaniline can be explained by examining the interactions of each compound with the stationary phase. Both compounds are soluble in methanol, and their boiling points are similar (Chapter 2, page 14). However, N,N-dimethylaniline would be expected to have stronger interactions with the stationary phase, especially the silanol sites, than acetophenone would, since N,N-dimethylaniline is basic. It is therefore more difficult for the methanol to remove N,N-dimethylaniline from the stationary phase than to remove acetophenone, which is not held as strongly. The differences in the strengths of interaction between the analyte and the stationary phase probably account for the large differences in recovery seen for the volatiles at 30°C.

Another aspect of trapping of volatiles that can be explained by stationary phase interactions is the reduction in recovery at high trap temperatures upon the addition of 1% or 2% methanol. The role of the methanol at these high trap temperatures may be to deactivate the silanol sites, and therefore make them unavailable for trapping the volatiles. Although the deactivation of silanol sites with methanol is thought to occur

under supercritical conditions,[3] it is not known if this deactivation could occur under the decompressed conditions that are present in this system. Therefore, the low recoveries at high temperatures are due to two things - the increase in vapor pressure of the analytes and the possible deactivation of the silanol sites.

Figure 6 shows the recoveries versus trap temperature for the nonvolatile components of the test mixture with 1% and 2% methanol in carbon dioxide. Upon first examination of the Figure it appears that there is little effect on recovery for the nonvolatiles when methanol is present over the entire temperature range. However, when pooled t-tests were performed differences at the 95% confidence level were revealed. The results of these tests are shown in Appendix III and discussed below.

Decanoic acid recovery is affected by the addition of methanol. Recoveries of decanoic acid were statistically higher at low trap temperatures (5-40°C) when 1% methanol was used as the mobile phase as compared to recoveries obtained with pure carbon dioxide. The role of the methanol at this concentration may be to form a thin film on the ODS phase, thereby providing an additional trapping mechanism in the polar methanol. As the trap temperature increases the amount of methanol on the stationary phase decreases so that recoveries do not differ significantly between pure carbon dioxide and 1% methanol at trap temperatures above 40°C. Another possible explanation for greater recoveries with 1% methanol is that the methanol present on the stationary phase at low temperatures could be interacting with the silanol sites and deactivating the stationary phase. By deactivating the silanol sites on the stationary phase the amount of decanoic acid trapped through adsorptive interactions would decrease. Since dispersive forces between the ODS phase and the hydrophobic chain of decanoic acid are weaker than the hydrogen bonding between the silanol and the acid group, methanol deactivation of the silanol sites may allow decanoic acid to be more

Figure 6

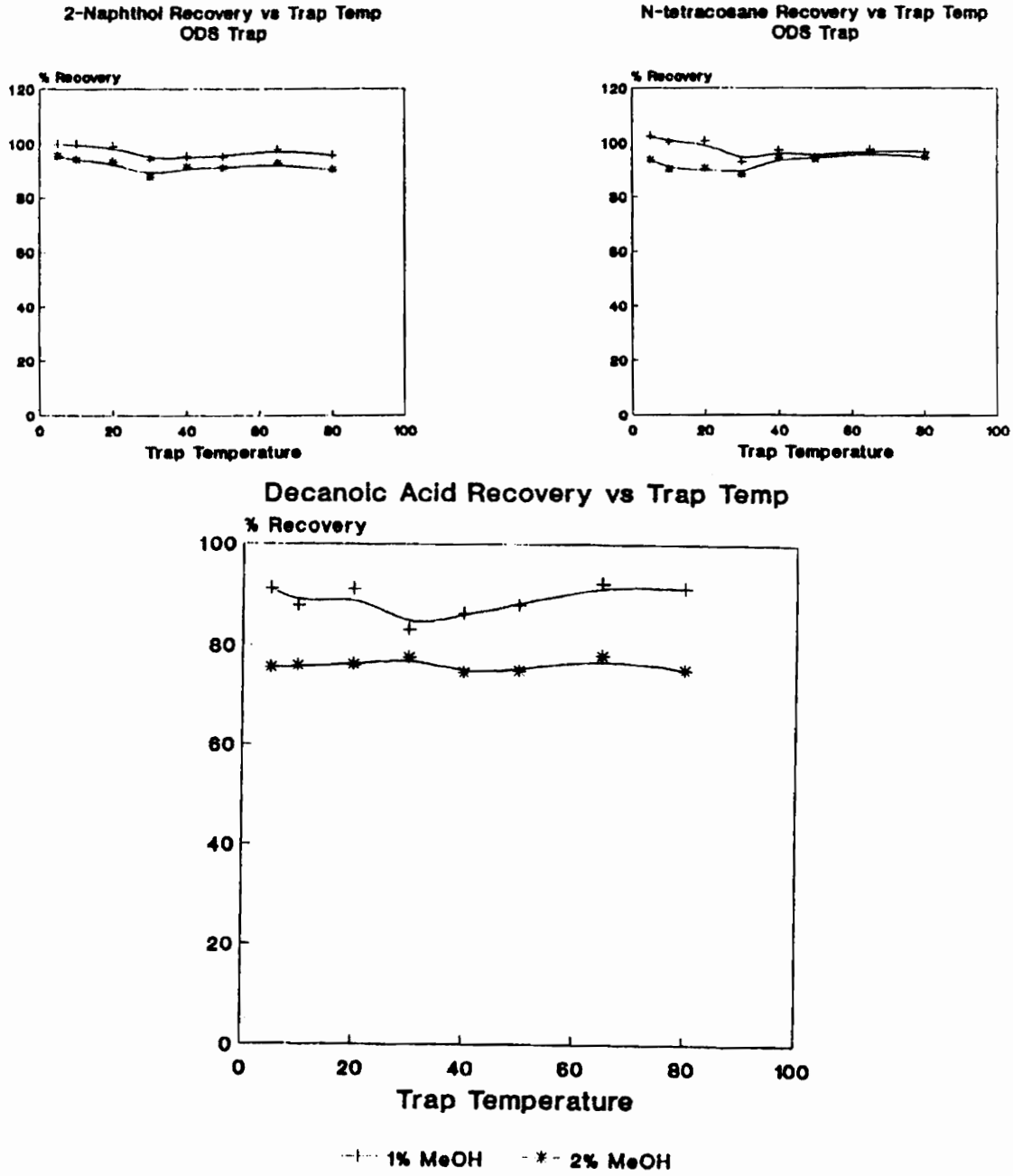


Figure 6: Percent recovery versus trap temperature for decanoic acid, 2-naphthol, and n-tetracosane on the ODS trap with 1% and 2% methanol modified carbon dioxide.

easily rinsed from the trap.

When 2% methanol was added to the mobile phase the recoveries of decanoic acid dropped in comparison to those obtained with 1% methanol over the entire temperature range. There are three possible explanations for this behavior. First, methanol could again be forming a film on the surface of the bonded phase. However, with the greater concentration of methanol the film may be thick and not mechanically stable. Therefore, as decompressed carbon dioxide passes through the trap at flow rates from 200-2000 mL/min, analyte and methanol could be mechanically pushed through the trap. The second way that 2% methanol could cause a decrease in recovery is by forming a film over the ODS stationary phase and preventing some of the dispersive interactions that lead to the efficient trapping. These sorptive interactions are necessary for efficient trapping as is evidenced by the lower recoveries obtained for decanoic acid on the stainless steel trap. The third way that methanol could be causing a decrease in decanoic acid recovery is by increasing the strength of the mobile phase and causing more decanoic acid to partition into it.

The recovery of 2-naphthol follows trends somewhat different than those observed with decanoic acid. When 2-naphthol recoveries obtained with pure carbon dioxide and 1% methanol were compared, no statistical difference was found over the entire temperature range. The vapor pressures and/or solubility in methanol of decanoic acid and 2-naphthol apparently differ enough so that decanoic acid recovery is affected by the addition of 1% methanol while 2-naphthol is not. When the recoveries of 2-naphthol with pure carbon dioxide were compared to those obtained with 2% methanol it was found that recoveries were statistically higher when pure carbon dioxide was used over the 5-40°C temperature range. At temperatures above 40°C there was no difference in recovery. Again, formation of a mechanically unstable film

of methanol that is pushed from the trap, disruption of the sorptive interactions between the analyte and the ODS phase, and partitioning to the mobile phase are all possible explanations for this behavior. Lower recoveries obtained on the stainless steel trap for 2-naphthol (vide infra) with pure carbon dioxide as compared to those obtained on the ODS trap indicate that sorptive interactions are necessary for efficient trapping.

The recoveries of n-tetracosane were unchanged when the mobile phase was changed from pure carbon dioxide to 1% methanol, as was the case with 2-naphthol. Recoveries however were greater for n-tetracosane with pure carbon dioxide than with 2% methanol in carbon dioxide. Since n-tetracosane is not soluble in methanol, methanol must be reducing the absorptive interactions between the analyte and the stationary phase. This reduction in recovery is not drastic. Recoveries ranged from 97-100% for n-tetracosane with pure carbon dioxide, and 90-95% with 2% methanol. These differences, however, are statistically significant.

ODS Trap, 4% and 8% methanol

Upon switching to higher methanol concentrations in the mobile phase the 10°C trapping study was eliminated since little difference had been observed in analyte recoveries between 20°C and 5°C. Figure 7 shows the results obtained for acetophenone and N,N-dimethylaniline over the 5-80°C temperature range. As can be seen by comparing Figure 7 and Figure 5, the trend observed with 1% and 2% methanol is no longer observed with 4% and 8% methanol in carbon dioxide. Instead of the high recoveries at low trap temperatures and low recoveries at high trap temperatures, a maximum recovery was obtained at a temperature that is dependant on the methanol concentration. With 4% methanol in the mobile phase maximum recoveries were obtained at 30°C, while a trap temperature of 50°C resulted in recovery maxima for 8% methanol.

Figure 7

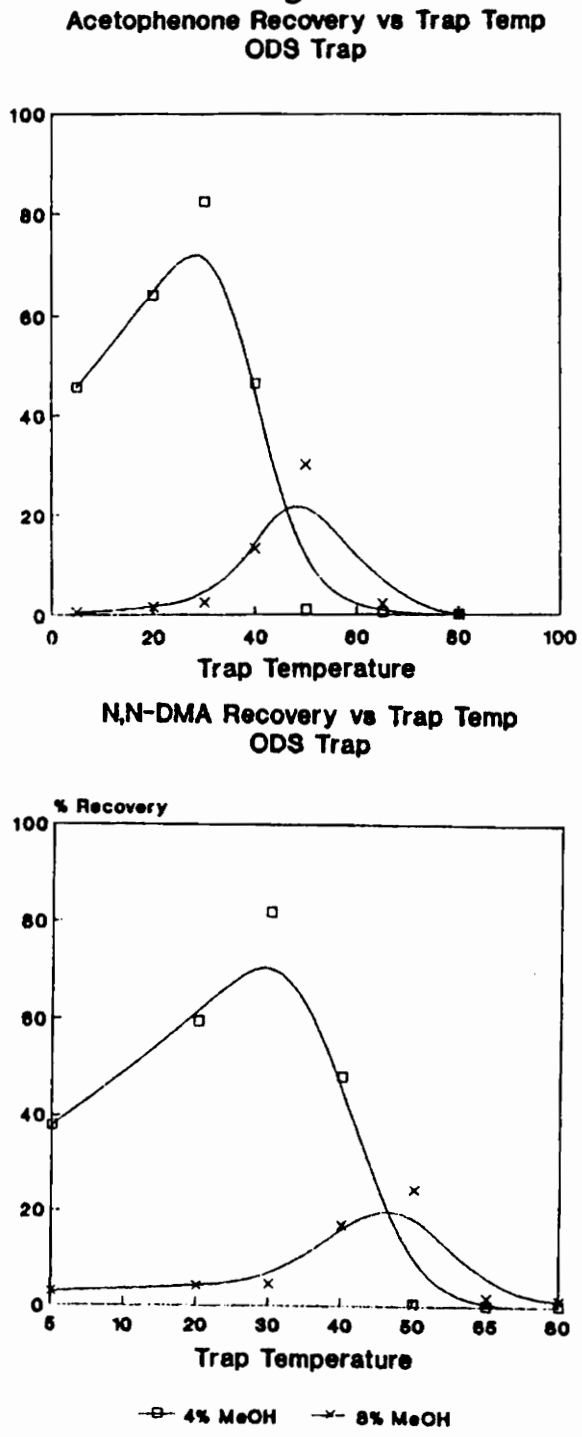


Figure 7: Percent recovery versus trap temperature for acetophenone and N,N-dimethylaniline on the ODS trap with 4% and 8% methanol modified carbon dioxide.

The shape of the percent recovery versus trap temperature curves for the volatile components are a result of two competing mechanisms. At low trap temperatures methanol condenses on the stationary phase where N,N-dimethylaniline and acetophenone dissolve in it. These components are then mechanically removed from the trap by the decompressed carbon dioxide. The compressed flow rate delivered by the pump was 2 mL/min, and the decompressed flow rate is estimated as 100-1000 times the compressed flow. As the trap temperature is increased methanol begins to vaporize, and the amount of methanol on the stationary phase decreases. The decreased amount of methanol on the stationary phase allows acetophenone and N,N-dimethylaniline to more strongly interact with the stationary phase, leading to an increase in recovery. This behavior explains the first half of the recovery versus trap temperature curve, where recovery increased with increasing temperature. At trap temperatures above the maximum recovery temperature the percent recovery curves follow the same trend seen with 100% carbon dioxide, 1% methanol, and 2% methanol for the volatiles. As trap temperature increases the vapor pressure of the analytes increases and recovery decreases. These two competing mechanisms lead to the observed maxima. The main difference between recoveries with 4% methanol and 8% methanol is that recoveries were lower with the higher methanol concentration. The maximum recoveries were obtained at 50°C with 8% methanol, as compared to 30°C with 4% methanol, indicating that higher trap temperatures were required to remove enough of the additional methanol so that sorptive interactions could occur.

Figure 8 shows the recovery versus trap temperature curves for the nonvolatile compounds with 4% and 8% methanol as the mobile phase. Again, there are differences immediately obvious when higher methanol concentrations were used as the mobile phase. First, let's examine the curves obtained for decanoic acid. When 4%

Figure 8

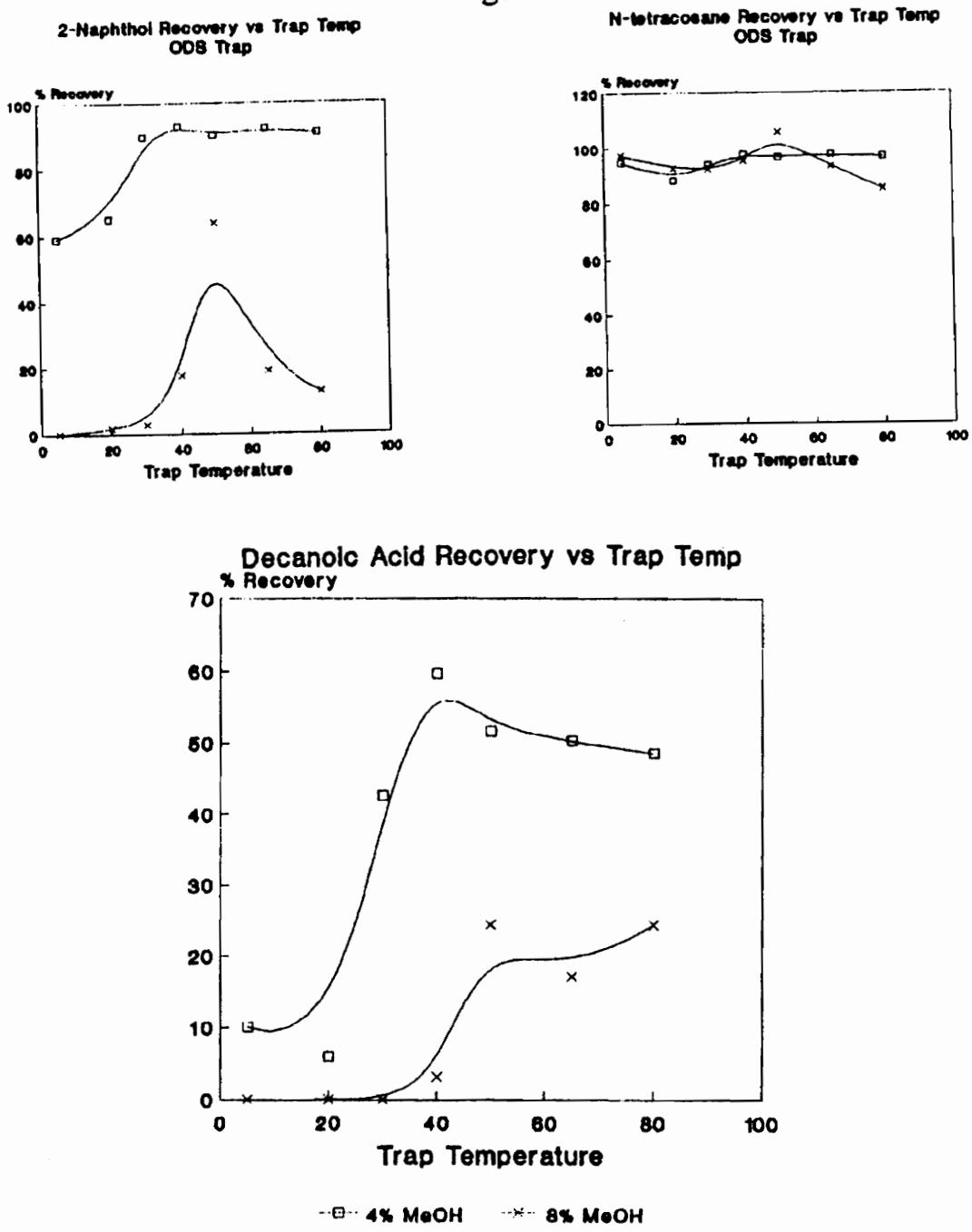


Figure 8: Percent recovery versus trap temperature for decanoic acid, 2-naphthol, and n-tetracosane on the ODS trap with 4% and 8% methanol in carbon dioxide.

methanol was used as the mobile phase the percent recovery verus trap temperature curve is similar to what was seen for the volatiles at low temperatures. That is, low initial recoveries with increased recovery as trap temperature was increased was observed. Decanoic acid is soluble in methanol and has a boiling point of 270°C, as compared to 193°C for N,N-dimethylaniline and 202°C for acetophenone. Since the boiling points of decanoic acid and the volatile components of the test mix are so different, their similar behavior may be based on the fact that decanoic acid is very soluble in methanol. The increase in recovery for decanoic acid as the trap temperature was increased from 5-40°C can be explained the same way as for the volatiles. Decanoic acid dissolves in the methanol pooled on the stationary phase and is mechanically removed from the trap by decompressed carbon dioxide. As the trap temperature increases, the amount of methanol on the stationary phase decreases and recovery increases. Unlike the volatile components, the recovery of decanoic acid did not decrease at temperatures above 40°C. Instead decanoic acid recovery remained constant, although statistically lower than what was obtained with 2% methanol. When methanol concentration was increased to 8% the same trend as was seen with 4% methanol was observed, but with lower recoveries. In fact, at trap temperatures of 5, 20, and 30°C no decanoic acid was recovered. Again, recovery became constant at trap temperatures of 50°C and greater, although these recoveries were statistically lower than those obtained with 4% methanol. The possible reasons for decreased recovery are: (1) formation of a mechanically unstable film of methanol, (2) disruption of sorptive interactions between the analyte and the stationary phase, and (3) increased partitioning to the mobile phase.

The percent recovery versus trap temperature curve for 2-naphthol shows behavior similar to that of decanoic acid. At low temperatures 2-naphthol recoveries

were low since the analyte is being mechanically pushed through the trap. When 4% methanol was used at higher trap temperatures, recoveries were not statistically different than those obtained with 2% methanol. However, when 8% methanol was used recoveries were significantly reduced. Recoveries obtained for n-tetracosane with 4% and 8% methanol were not statistically different from those obtained with 2% methanol. The drastic drops in recovery observed with the other components of the test mix with 8% methanol were not observed since n-tetracosane is not soluble in methanol.

Conclusions, ODS Trap

The addition of methanol to the mobile phase affected all components of the test mixture. The degree to which analytes were affected depended on their vapor pressure, solubility in methanol, and the specific interactions between the analyte and the stationary phase. The recovery trends observed with 1% and 2% methanol for acetophenone and N,N-dimethylaniline indicated that partitioning of these analytes was occurring, and that sorptive interactions between the analyte and stationary phase could be disrupted by the addition of methanol. Decanoic acid exhibited unique behavior in that it was the only compound that had maximum recoveries with 1% methanol. The addition of 1% methanol may have served to create a better polarity match between the analyte and the stationary phase. With higher methanol content decanoic acid exhibited behavior like both the volatile and nonvolatile components of the test mix. The unique nature of decanoic acid behavior may be due to its high solubility in methanol as compared to the solubility of 2-naphthol and n-tetracosane in methanol. 2-Naphthol behavior was not affected by 1% methanol, but was affected by higher concentrations of methanol. As was stated earlier, the three possible reasons for the decrease in recovery upon additon of methanol are: (1) formation of a mechanically unstable film

of methanol on the stationary phase, (2) disruption of the sorptive interactions between the analyte and the stationary phase, and (3) increased partitioning to the mobile phase. The behavior of 2-naphthol indicates that at low temperatures the formation of a mechanically unstable layer of methanol is the dominant mode for sample loss, while the other two mechanisms dominate at higher temperatures. The behavior of n-tetracosane is unique in that it is the only component of the test mixture that is not soluble in methanol. There was a small decrease in recovery upon addition of 2% methanol, but no further decreases were observed at higher methanol concentrations.

Stainless Steel Trap, 100% carbon dioxide

Figure 9 shows the percent recovery versus trap temperature curves obtained over the 5-80 °C range for the volatile components of the test mixture. The percent recoveries and RSDs are listed in Appendix II. Neither of the trends that were observed with the ODS trap were observed on the stainless steel trap. Acetophenone recovery at 5 °C was 11.7%, while N,N-dimethylaniline recovery was 3.6%. The difference in recoveries of these compounds at low trap temperatures reflects the slight differences in volatility of these compounds. N,N-dimethylaniline has a lower boiling point (193 °C) as compared to acetophenone, which has a boiling point of 204 °C. The large disparity in recovery that was observed with these compounds on the ODS trap was no longer seen, since the dominant mechanism for trapping on the stainless steel beads is cryotrapping, not absorption. The recoveries of the volatile analytes decreased as the trap temperature increased and the vapor pressure of the analytes increased. At a trap temperature of 80 °C there was still small amounts of acetophenone and N,N-dimethylaniline recovered, which indicates that the stainless steel beads are not totally inert and some sort of adsorption may be occurring. The stainless steel beads used for

Figure 9

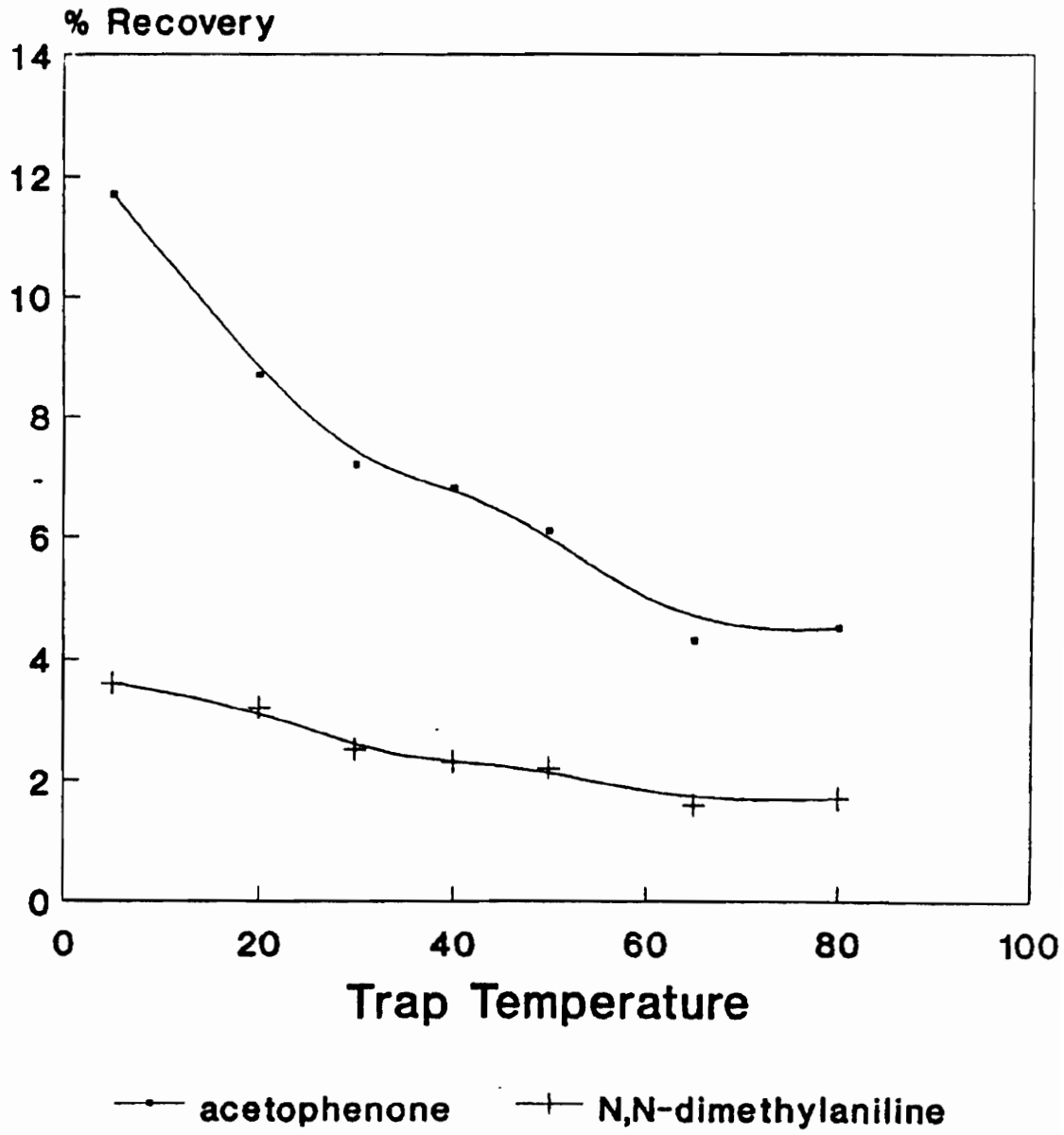


Figure 9: Percent recovery versus trap temperature for acetophenone and N,N-dimethylaniline on the stainless steel trap with pure carbon dioxide.

trap material have high nickel content, have not been heat or acid treated, and do not have high carbon content. There may be an oxide layer present on the stainless steel surface that causes surface activity.

Figure 10 shows the recoveries versus trap temperature for the nonvolatile components. Decanoic acid recovery was low at low temperatures (26.3% at 5 °C) and increased as the trap temperature increased up to 50 °C, after which recovery remained constant. This increase in recovery of decanoic acid with increasing trap temperature also was observed on the ODS trap, and indicates that it is a function of the compound rather than the trap material. Therefore, as the temperature increases the decanoic acid may be becoming more "sticky", and therefore able to trap more efficiently. The recoveries were generally higher on the ODS phase indicating that the dispersive interactions between the hydrophobic chain of decanoic acid and the ODS phase contributed to the recoveries obtained. Recoveries of 2-naphthol and n-tetracosane on stainless steel were unchanged with trap temperature, as was also the case for the ODS trap.

Stainless Steel Trap, 1% methanol

Figure 11 shows the recovery profiles for the stainless steel trap with 1% methanol as the mobile phase. The recoveries of both the volatile components and the nonvolatile components are shown on this plot, although the volatile components are shown as two points, since recovery for these compounds was obtained at 5 °C only. At trap temperatures greater than 5 °C recoveries of N,N-dimethlyaniline and acetophenone were 0%. The recoveries of the volatiles were greater at 5 °C as compared to the recoveries obtained with pure carbon dioxide. This increase in recovery is probably due to the formation of a thin film of methanol which is

Figure 10

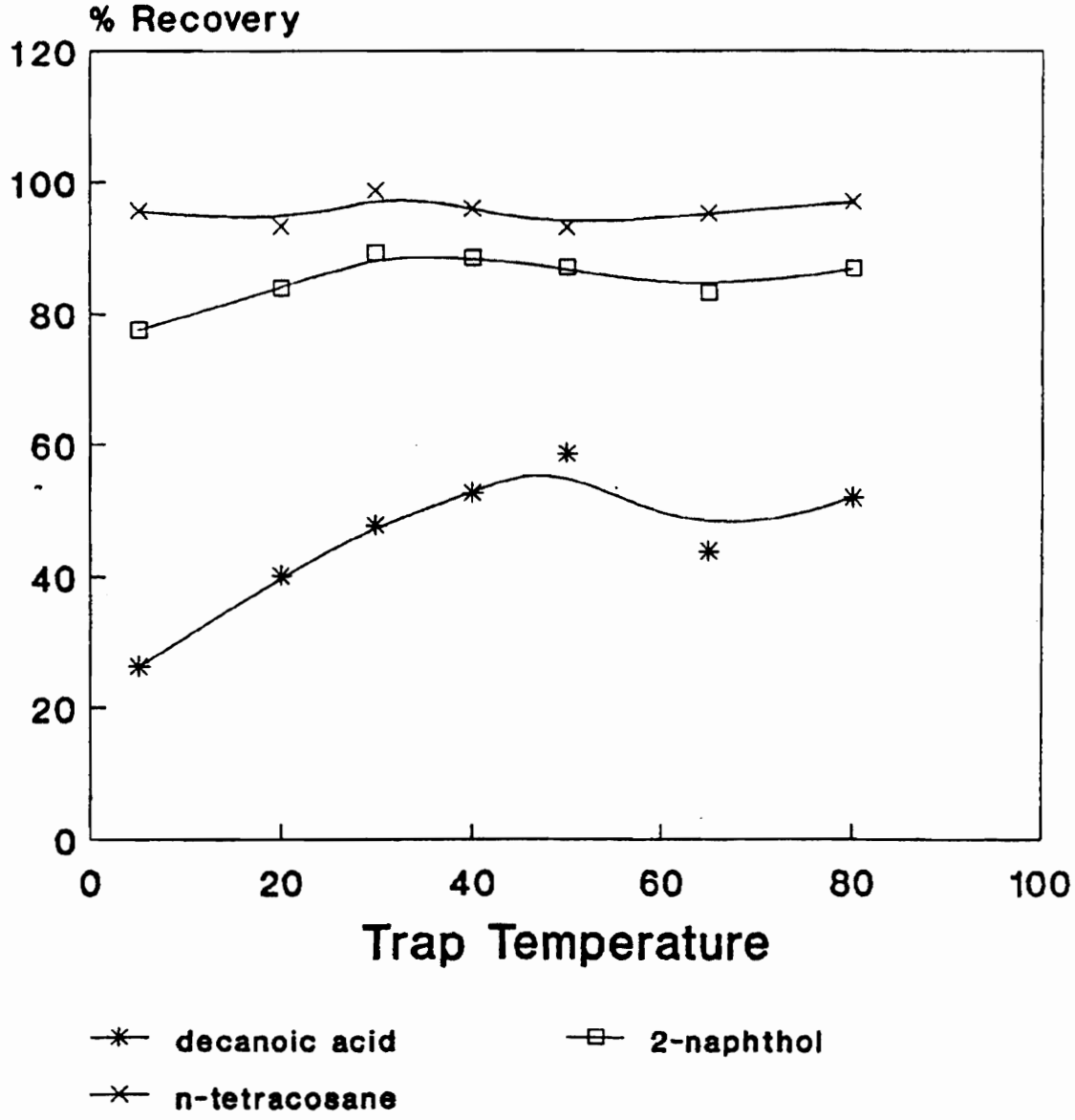


Figure 10: Percent recovery versus trap temperature for decanoic acid, 2-naphthol, and n-tetracosane on the stainless steel trap with pure carbon dioxide.

Figure 11

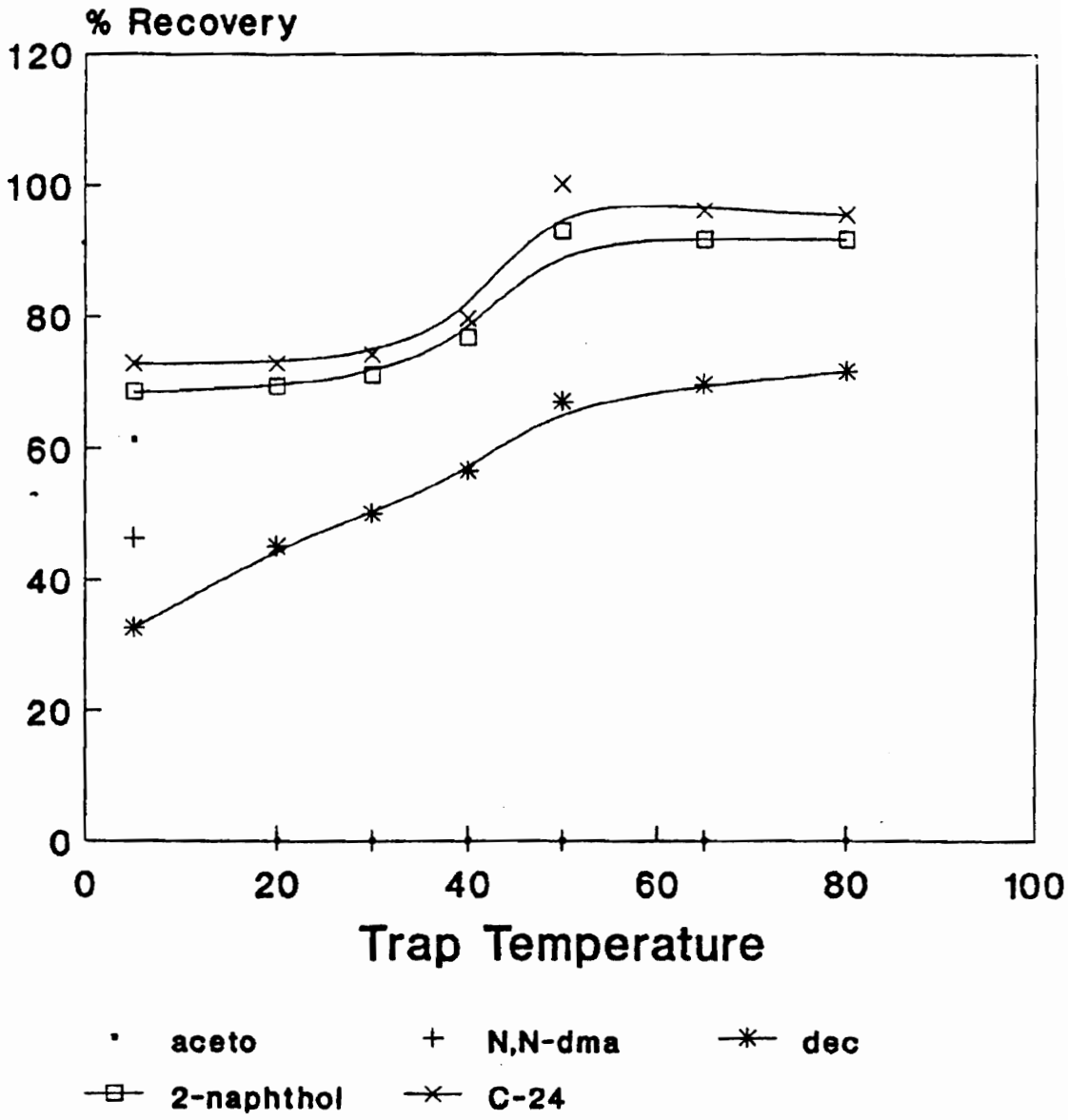


Figure 11: Percent recovery versus trap temperature for all components of the test mixture on the stainless steel trap with 1% methanol in carbon dioxide.

physisorbed to the stainless steel surface. The volatile components are soluble in methanol, and may be trapped in the thin film. Therefore, their recovery increased as compared to that obtained with pure carbon dioxide.

The recoveries of the nonvolatile components follow a trend quite different from the volatile components. The recovery of decanoic acid follows the same trend as seen before, with increased recovery as trap temperature increases. Decanoic acid recoveries were greater with 100% carbon dioxide than with 1% methanol at trap temperatures of 50°C and above. Again decanoic acid may be dissolving in methanol and mechanically pushed from the trap. Recoveries were constant from 5-40°C at 70-75% for both naphthol and n-tetracosane. Both of these compounds are fairly nonpolar, although n-tetracosane is certainly the least polar, and therefore do not trap as efficiently in the thin film of methanol as the polar analytes do. As the trap temperature is increased the amount of methanol on the solid surface decreased, and the recovery of both compounds increases to the 90-100% range.

Stainless Steel Trap, 2% and 4% methanol

Because the only compounds to be significantly affected by 1% methanol content were the volatiles, recoveries of the volatiles were determined with 2% and 4% methanol as the mobile phase at a trap temperature of 5°C. Figure 12 shows the recovery of the volatile components with pure carbon dioxide, 1%, 2%, and 4% methanol as the mobile phases. The maximum recoveries for the volatiles occurred with 1% methanol in the mobile phase, where these analytes were trapped in a thin film of methanol that coated the stainless steel beads. When 2% methanol was used as the mobile phase the recoveries of the volatiles were lower than those obtained with pure carbon dioxide. The greater concentration of methanol apparently forms a thicker film

Figure 12

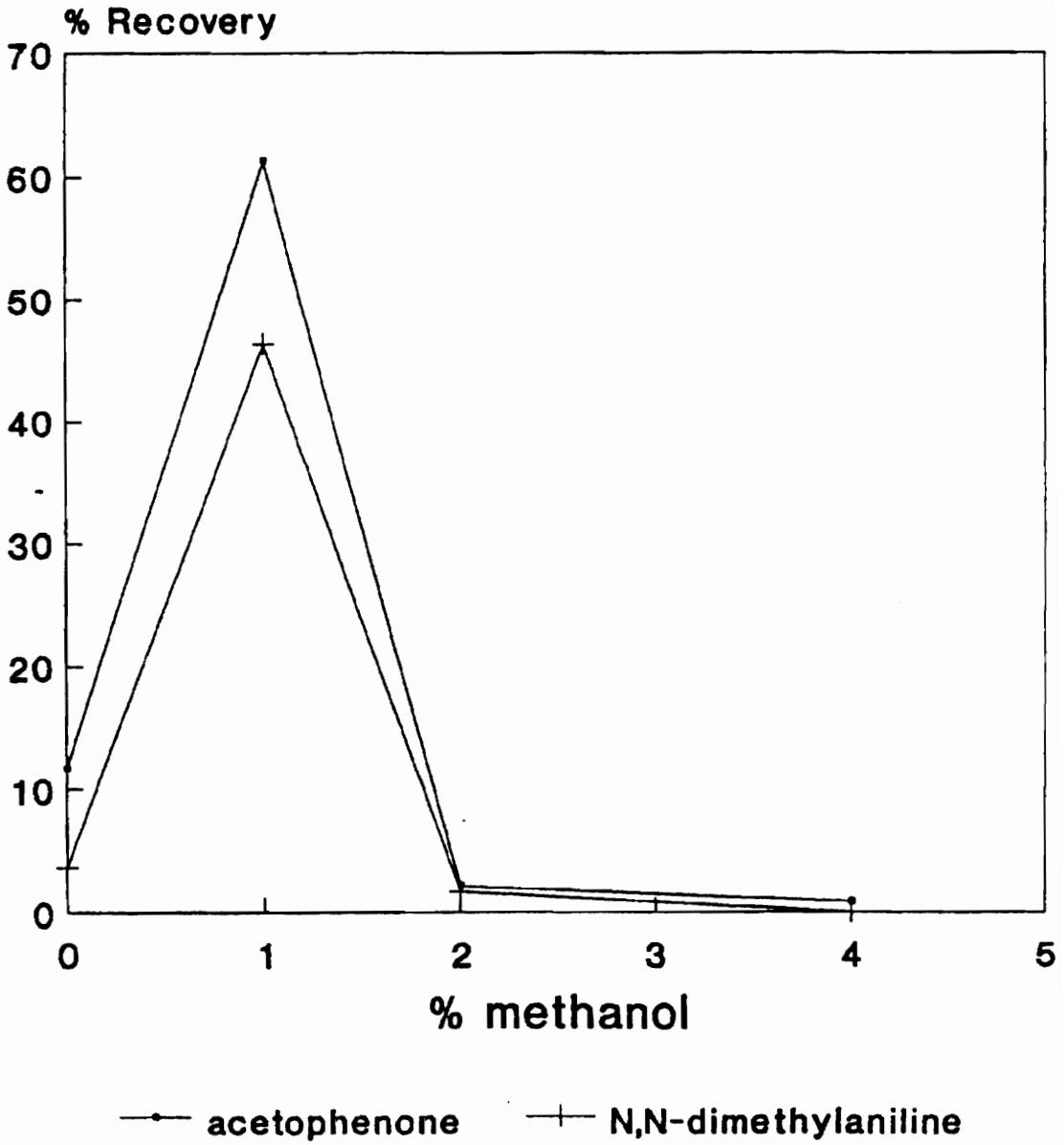


Figure 12: Percent recovery versus methanol concentration at 5 °C for acetophenone and N,N-dimethylaniline on the stainless steel trap.

that can be mechanically pushed through the trap by decompressed carbon dioxide. The volatile components that were dissolved in the methanol travel with the methanol, and exit the trap, resulting in lower recoveries. At a methanol concentration of 4%, recoveries of acetophenone and N,N-dimethylaniline are neglible, indicating that nearly all of these compounds are being mechanically removed from the trap with the methanol.

Conclusions

The effect of methanol on trapping efficiency of solid surface and sorbent traps was found to vary greatly with methanol concentration. For concentrations of methanol of 2% or less on the ODS trap, it is not necessary to maintain the trap temperature above the boiling point of the methanol. In fact, decreases in recovery for the volatile components were drastic at 30°C and above. For methanol concentrations above 2% on the ODS trap, low trap temperatures resulted in inefficient trapping due to the presence of condensed methanol on the stationary phase. However, maximum recoveries for the volatiles were not obtained at temperatures above the boiling point of methanol. Instead, recovery maxima were obtained in the 30-50°C range, depending on the methanol concentration. For the nonvolatile compounds trap temperature had no effect on recovery when 1% and 2% methanol were used. However, when greater concentrations of methanol were used trap temperatures of at least 40-50°C were required to obtain efficient trapping.

The trends observed for the stainless steel trap were very different from those observed with the ODS trap. For efficient recovery of the volatile compounds, the addition of a modifier to establish a thin film on the surface drastically improved recovery. However, the addition of too great a concentration of modifier allows for mechanical rinsing of the trap. For the nonvolatile compounds high recovery can

generally be obtained across the entire trap temperature range when 100% carbon dioxide is used. When methanol was added to the mobile phase, trap temperatures of 50°C and above were needed to eliminate methanol from the surface and achieve efficient trapping.

It is obvious from this work that the addition of modifier can have drastic effects on the ability of a trap to function. The vapor pressure of the components, their solubility in the modifier, and the type of trap material must all be considered in order to successfully trap extracted components.

Chapter 4

Application of SFE to Septra Infusion

Introduction

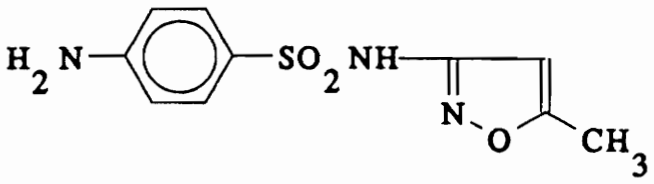
Septra Infusion is a drug formulation used to treat urinary tract infections. An infusion refers to a drug formulated to be administered intravenously. The active ingredients of Septra Infusion are trimethoprim and sulfamethoxazole, whose structures are shown in Figure 13. Sulfamethoxazole is present in the infusion at a concentration of 80 mg/mL and trimethoprim is present at 16 mg/mL. The active components are dissolved in 40% propylene glycol, 10% ethyl alcohol, and 0.3% diethanolamine, with 1% benzyl alcohol and 0.1% sodium metabisulfite added as preservatives. The goal of this work was to determine whether SFE could be used to extract these polar compounds from their polar matrix. A literature search was unsuccessful in finding reports of drugs extracted from their formulations. However, trimethoprim has been separated from other nitrogen containing compounds by SFC, although base had to be added to the mobile phase in order to elute trimethoprim cleanly.[21] Sulfamethazine, which has a structure very similar to sulfamethoxazole, has been extracted from milk.[22] Therefore, it is not unreasonable to expect that these compounds could be extracted from Septra Infusion.

Experimental

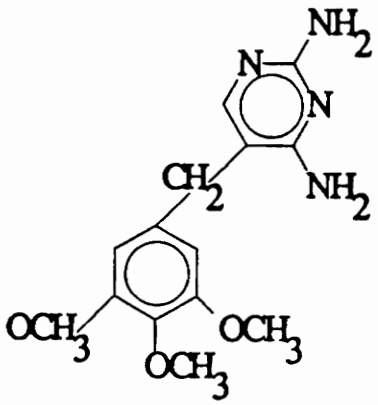
HPLC System

Sulfamethoxazole and trimethoprim were to be quantified by HPLC using UV detection at 240 nm. An LC method was provided to us by Burroughs Wellcome, however, this method was modified in order to improve peak shape and decrease the total run time. The modifications to this method will be discussed in the Results and

Figure 13



Sulfamethoxazole



Trimethoprim

Figure 13: Structures of sulfamethoxazole and trimethoprim.

Discussion section of this chapter.

A Nicolet LC-9560 (Madison,WI) delivered the mobile phase at a flow rate of 2 mL/min. A Spherisorb phenyl column (150 x 4.6 mm, 5 μm particles, Keystone Scientific, Bellefonte, PA) was used for the separation. Detection was accomplished with a Kratos Spectroflow 757 UV detector at 240 nm (cell volume=8 μL). A Rheodyne (Cotati, CA) Model 7125 injection valve equipped with a 10 μL loop was used to introduce the sample to the column.

To prepare the mobile phase 3.84 g of the sodium salt of 1-pentane sulfonic acid (Sigma, St.Louis, MO) was dissolved in 1700 mL of HPLC grade water (Fisher, Pittsburgh,PA). The pH was adjusted to 3.2 with 1 N HCl (Aldrich, St.Paul,MI). To the sulfonic acid solution, 200 mL of acetonitrile were added. The solution was then diluted to volume (2 L volumetric flask) with water. Vacuum filtration through 0.8 μm filters (Millipore, Bedford, MA) was then performed. This solution was then used in a 80:20 (v/v) mixture with acetonitrile (80% ion-pair solution/20% acetonitrile) as the mobile phase. Since there is acetonitrile present in the ion-pair solution, the 80/20 mixture actually corresponds to 72% ion-pair solution/28% acetonitrile. The mobile phase was constantly sparged with helium.

Sample Preparation

Sulfamethoxazole and trimethoprim were provided by Burroughs Wellcome, and were dissolved in a solution of 3:1 (v/v) 0.1% glacial acetic acid/acetonitrile. Septra Infusion was diluted with this same solution.

SFE System

During the course of this work two SFE systems were used - one that employed an extraction vessel that could contain liquid samples (modified Suprex 200A) and one that employed standard extraction vessels (Hewlett Packard 7680A). The Suprex

(Pittsburgh, PA) system modifications have been described in Chapter 2. With this system the extracted components were collected into a vial of liquid solvent or onto a diol solid phase extraction tube (Supelco, Bellefonte, PA). The diol SPE tube was rinsed with acetonitrile before it was used as a trap in order to remove any acetonitrile-soluble species. After extraction was complete, the tube was rinsed with 1 mL of acetonitrile, followed by 1 mL of the HPLC mobile phase. The rinse was then diluted up to the 10 mL mark in a volumetric flask with the mobile phase and analyzed. The extraction vessel outlet restrictor used with this instrument was a 60 cm length of 50 μm fused silica that was tapered to produce a condensed flow rate of 1 mL/min or less of liquid as measured at the pump. The extraction vessel used in this work has been described in the literature.[9] This vessel was designed to prevent the transport of unsolvated water, and is shown in Figure 14. The vessel was 1 cm i.d. x 10 cm in length, with a volume of 8 mL.

The second extraction system used was a Hewlett Packard 7680A (Avondale, PA). This instrument performs extraction in the off-line mode only, and uses solid surface (stainless steel beads) or solid phase (ODS or other bonded phases) trapping. For the work on Septra Infusion the stainless steel beads were used as a trap since sulfamethoxazole and trimethoprim are fairly nonvolatile. The HPLC mobile phase was used to rinse the analytes off of the trap. Since the LC method involved ion pairing, no attempt was made to use an internal standard. Ion-pairing was used because these compounds are so basic that liquid chromatography by traditional means (i.e. normal or reverse phase) does not work well. Therefore, after an extraction was complete, the trap rinses were transferred from the 2 mL autosampler vials into a 10 mL volumetric flask, and diluted to volume with the acetonitrile/acetic acid solution. Pure carbon dioxide was used for all work reported here. Carbon dioxide was

Figure 14

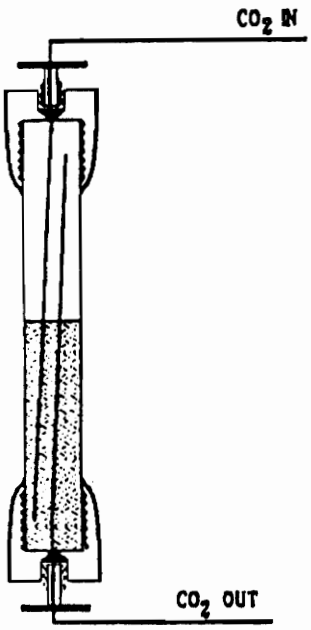


Figure 14: Aqueous extraction vessel used with modified Suprex system for off-line SFE.

provided by both Scott Specialty Gases (Plumsteadville, PA) and Air Products (Allentown, PA). There were no detectable differences between carbon dioxide supplied by the two different vendors.

Mass Spectrometer

A Hewlett Packard (Avondale, PA) 5970 quadrupole mass spectrometer interfaced to a 5890 gas chromatograph was used to identify volatile extracted components. The column used was an HP-5 (5% phenyl) and was 20 m x 320 μm in diameter.

Method Development

The method development for Septra Infusion proceeded as follows:

Step 1: Trimethoprim and sulfamethoxazole standards were run using the HPLC method provided by Burroughs Wellcome in order to determine if this method required any modification.

Step 2: A rapid extraction of Septra Infusion was performed in order to determine which of the other components, beside sulfamethoxazole and trimethoprim, were extracted by supercritical carbon dioxide.

Step 3: An actual sample of Septra Infusion was analyzed by HPLC to determine if any of the other components present coelute with the compounds of interest.

Step 4: Sulfamethoxazole and trimethoprim calibration curves were prepared to determine the working range for the LC analysis.

Step 5: Optimum extraction conditions for sulfamethoxazole and trimethoprim were determined, and extraction reproducibility was shown.

Results and Discussion

Step 1: Validation of HPLC Method with Standards

A mixture of sulfamethoxazole and trimethoprim (0.0793 g and 0.0157 g,

respectively in 250 mL) was prepared as described in the Experimental section. The mobile phase recommended to us by Burroughs Wellcome was the ion-pair solution without the additional 20% (v/v) acetonitrile. When this mobile phase was used sulfamethoxazole eluted at 4.51 minutes and trimethoprim eluted about thirty minutes later as a ghost peak with very poor peak shape. Since a gradient LC system was being used, 10% acetonitrile was mixed with the original phase on-line. The system was allowed to equilibrate, and the mixture was injected again. Both drugs eluted in under eight minutes, however, the trimethoprim peak was unacceptably broad. The 80:20 mobile phase was then tried, and with this mobile phase both compounds eluted from the column in less than five minutes, with sulfamethoxazole eluting at 1.77 minutes, and trimethoprim eluting at 3.04 minutes. Figure 15 shows the chromatogram obtained, where RSDs for peak areas of five injections were $\leq$2%. As can be seen from the Figure, the peak shapes are satisfactory and area reproducabilities are good, indicating that the LC method was acceptable. Therefore, this LC method was used during the course of this work.

Step 2: Determination of Extractable Components

To determine which components of Septra Infusion, in addition to the compounds of interest, were extractable with 100% carbon dioxide, a sample of the infusion was placed in the aqueous extraction vessel. An extraction was carried out at 350 atm and 50°C for approximately five minutes, and the extract was collected in a vial of acetonitrile. The reason that the extraction time was so short was that severe restrictor plugging occured. Nevertheless, the extract was analyzed by GC/MS and the total ion chromatogram (TIC) obtained over the mass range of 0-500 amu's is shown in Figure 16. Note that since sulfamethoxazole and trimethoprim are nonvolatile, they are not present in the TIC. The first peak corresponds to methanol and ethanol, which

Figure 15

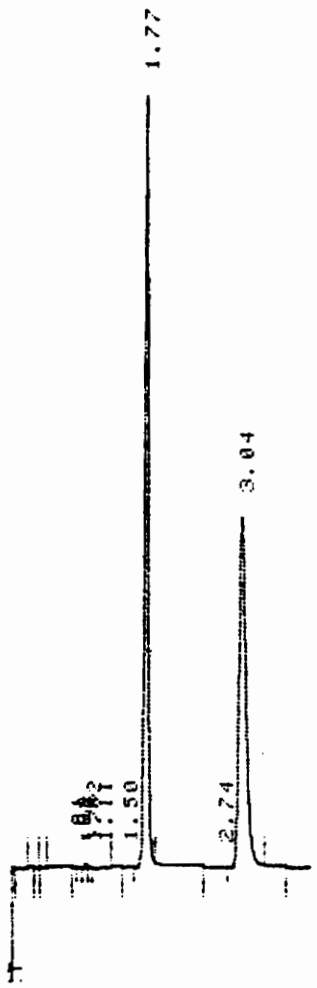


Figure 15: Chromatogram of sulfamethoxazole and trimethoprim standards using the condition described in Step 1.

Figure 16

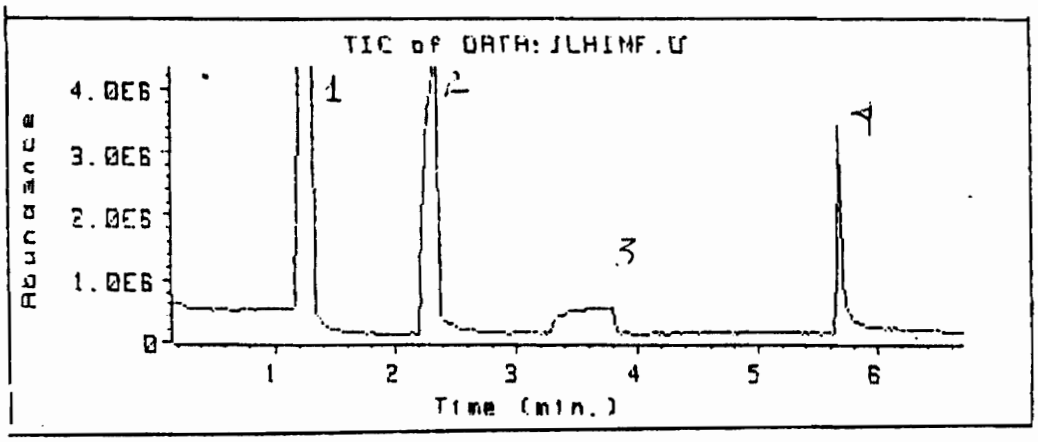


Figure 16: TIC of Septra Infusion extract with an elution order of: (1) methanol and ethanol, (2) propylene glycol, (3) air, and (4) benzyl alcohol.

coelute under the temperature program used (100°C to 300°C linearly in 20 min). The second peak corresponds to propylene glycol. The identity of Peak 2 was confirmed by comparing the spectrum obtained to a library spectrum. The third peak was due to air, and the fourth peak corresponds to benzyl alcohol. The identity of peak four was confirmed by the spectral search routine, which gave benzenemethanol as the top match. Therefore, in an extraction of only five minutes, it was determined that methanol, ethanol, benzyl alcohol and propylene glycol were all extractable by supercritical carbon dioxide. The restrictor plugging observed will be discussed in a later section.

Step 3: Validation of HPLC Method with Infusion

Because it was determined that propylene glycol and benzyl alcohol were extracted from Septra Infusion, and it was initially reported to us by Burroughs Wellcome that propylene glycol caused an interference in the chromatogram, an actual sample of Septra Infusion was injected into the LC system. A solution of 200 μL of infusion in 50 mL of the acetic acid/acetonitrile mixture was prepared. Relative standard deviations of peak area were 0.1% for sulfamethoxazole and 0.07% for trimethoprim. The relative standard deviation of the capacity factor (k') for both compounds was no more than 0.3%. However, the fact that the peak areas and retention times were reproducible does not exclude the possibility that either benzyl alcohol or propylene glycol coelute with one of the compounds of interest, but that this is not observed at 240 nm. Therefore, the detector wavelength was changed. Septra Infusion was monitored at detector wavelengths of 254, 240, 230, 220, 210, and 200 nm. At 254 nm the peak height of trimethoprim decreased (in comparison to the peak height at 240 nm), and no additional peaks were observed. At 240 and 230 nm the

chromatograms show only the expected peaks. However, at 220 nm there is a small peak present at 1.36 minutes. This peak is also present at 210 and 200 nm, and is baseline resolved from sulfamethoxazole, as is shown in Figure 17. It was suspected that this peak was due to benzyl alcohol. Standards of benzyl alcohol and propylene glycol were then prepared. When benzyl alcohol was injected into the LC system and monitored at wavelengths of 220, 210, and 200 nm a peak was observed at 1.36 minutes. The propylene glycol solution was treated in the same manner, and no detector response was observed. The lack of detector response for propylene glycol is not surprising, since propylene glycol would not be expected to have an appreciable chromophore. Therefore, the peak observed at 1.36 minutes corresponds to benzyl alcohol. Since this peak is resolved from the compounds of interest, no further adjustment to the LC method was made.

Step 4: Preparation of Calibration Curves

A stock solution containing 15.6 mg of sulfamethoxazole and 3.0 mg of trimethoprim in 50 mL of the acetic acid/acetonitrile mixture was prepared. A set of standards was then prepared by serial dilution. These standards were each injected five times, and the average peak areas were used to construct calibration curves. The calibration curves for sulfamethoxazole and trimethoprim can be seen in Figure 18. Note that the correlation coefficients are nearly 1.0 in both cases. These calibration curves were then used to determine the concentrations of the compounds of interest in any extracts.

Step 5: Determination of Extraction Conditions

In an attempt to determine the optimum extraction conditions for

Figure 17

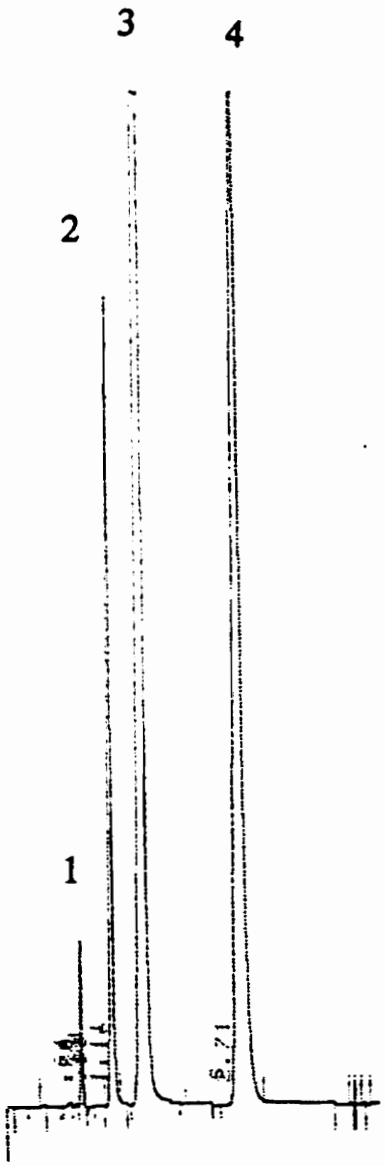


Figure 17: Chromatogram of Septra Infusion at 200 nm showing an additional peak at 1.36 minutes. Peak 1 is the refractive index change due to the injection solvent. Peak 2 is the additional peak. Peak 3 is sulfamethoxazole and Peak 4 is trimethoprim.

Figure 18

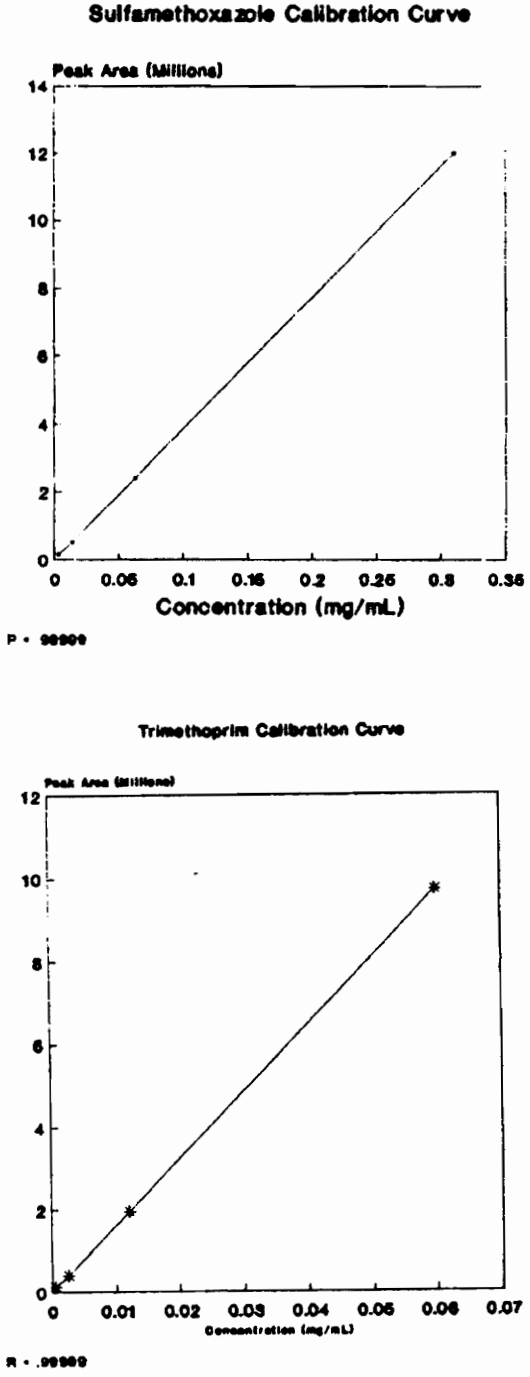


Figure 18: Calibration curves for sulfamethoxazole and trimethoprim.

sulfamethoxazole and trimethoprim directly from the infusion, a time or volume of CO_2 versus amount of extracted material plot was constructed at various densities. The first conditions chosen were P_{ext} = 350 atm, and T_{ext} = 50°C, and 5 mL of infusion were placed in the vessel. Under these conditions the restrictor plugged very rapidly. Upon depressurization a white crystalline solid was present in the extraction vessel. The contents of the vessel were filtered, and the white solid was collected and allowed to dry. The identity of the white solid was thought to be (1) polypropylene glycol, (2) the carbamate of sulfamethoxazole and/or trimethoprim, or (3) Na_2CO_3, since the pH of the infusion is adjusted to 10 with NaOH.

In order to determine which of the above, if any, were occurring, individual components were placed in the extraction vessel, and pressurized under the same conditions that had previously resulted in the white solid. A 10% (v/v) solution of propylene glycol was placed in the vessel and extracted for approximately fifteen minutes at 350 atm and 50°C. No restrictor clogging occurred, and upon depressurization no white solid was present in the extraction vessel. Next a solution of trimethoprim was prepared, and treated in the same manner. Again, no white solid formed.

In order to attempt to identify the white solid it was dissolved in the mobile phase and injected it into the LC system using the conditions under which the standards were run. The resulting chromatogram is shown in Figure 19. Immediately after running the white solid, a solution of Septra Infusion was injected, and from the retention times it could be seen that the white solid had the same retention time as sulfamethoxazole.

In order to determine why sulfamethoxazole was precipitating during extraction some rudimentary pH studies were performed. The pH of Septra Infusion is 10. We

Figure 19

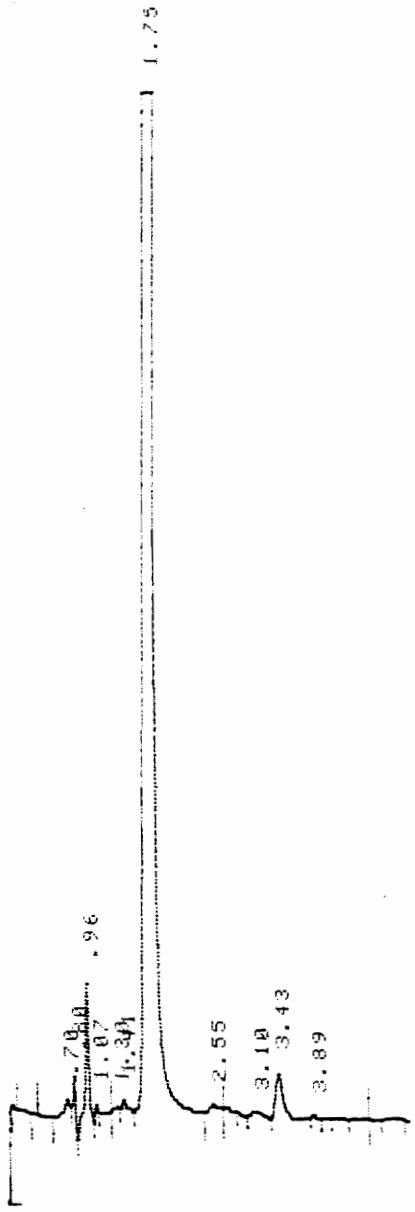


Figure 19: Chromatogram of the white solid that precipitated during extraction.

first attempted to dissolve sulfamethoxazole in distilled water at a pH of 7, but it would not go into solution. Upon adding a few drops of a solution of sodium hydroxide to the water, sulfamethoxazole went into solution. Upon adding a few drops of hydrochloric acid, sulfamethoxazole precipitated. Next the pH of distilled water was measured as carbon dioxide was bubbled through it. The pH of the water dropped from 7 to approximately 3 within 1-2 minutes. Therefore, as carbon dioxide passes through the infusion the solution becomes acidic and sulfamethoxazole falls out of solution. In order to prevent further clogging of the restrictor by sulfamethoxazole, an in-line filter was placed at the exit of the extraction vessel. After this was done, there were no further problems with restrictor clogging due to sulfamethoxazole.

Once the filter was placed in-line so that clogging was no longer a problem, the mg of sulfamethoxazole or trimethoprim versus volume of carbon dioxide was constructed. A 5 mL sample of Septra Infusion was placed in the aqueous extraction vessel. The extraction conditions were P_{ext} = 250 atm, T_{ext} = 50°C, with a trap temperature of -20°C. The diol trap was changed with every 10 mL of (liquid) carbon dioxide, as measured by the syringe pump. Changing the trap was accomplished easily through the use of a six port switching valve. Flow through the extraction vessel could be stopped in order to replace the diol trap, so that there would not be a time when there was no trap in place. In this way, 80 mL of carbon dioxide was passed through the extraction vessel. A cumulative plot of mg of sulfamethoxazole extracted versus volume of carbon dioxide is shown in Figure 20. There was a sampling error for the 40 mL point (i.e. the extract was spilled), and therefore there are two curves on the plot after this point. The lower curve results when 0 mg is used for the 40 mL point. However, for the other seven samples the amount of sulfamethoxazole extracted was fairly constant at 0.17 mg per 10 mL of carbon dioxide. If 0.17 mg rather than 0 mg

Figure 20

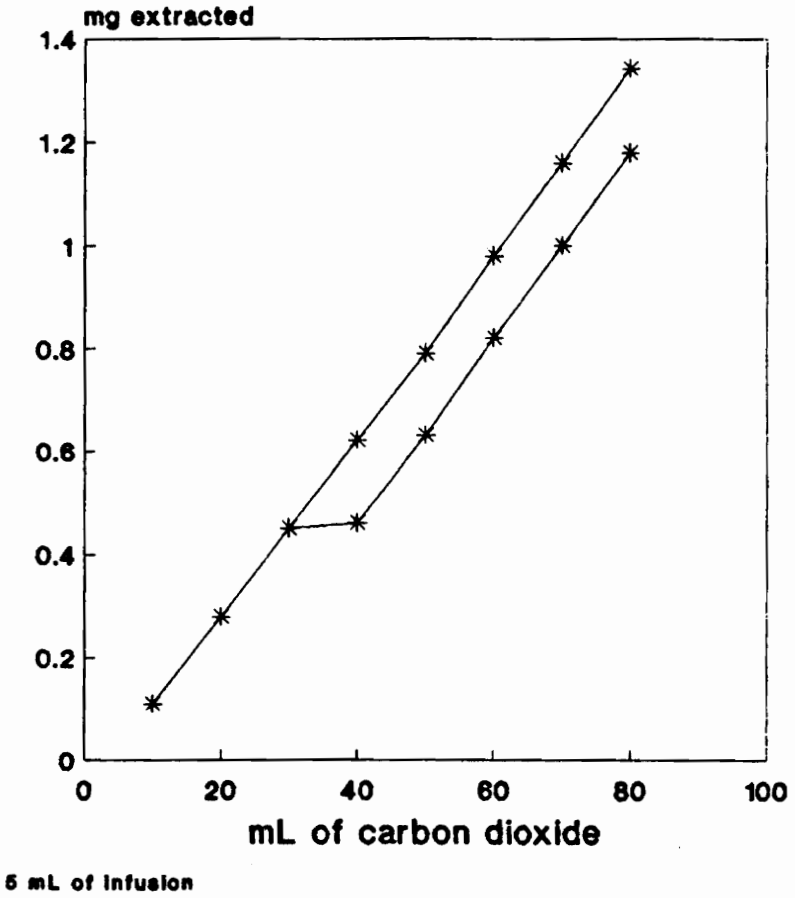


Figure 20: Extraction profile of 400 mg of sulfamethoxazole at 250 atm and 50 °C, where the diol sorbent trap was replaced every 10 mL of carbon dioxide.

is plotted for the 40 mL point the upper curve results. The total amount of sulfamethoxazole extracted under these conditions was 1.18 mg, while the amount of sulfamethoxazole present in the extraction vessel initially was 400 mg. Therefore, with 80 mL of carbon dioxide only 0.3% of the sulfamethoxazole present was extracted. At this rate of extraction, a little over 27 liters of carbon dioxide would be required to exhaustively extract sulfamethoxazole. Although these results seem somewhat discouraging, a great deal of useful information was obtained from this experiment.

First, the sample size used was impractical. A typical extraction results in micrograms (or less) of extracted material on the low end to tenths of a milligram on the high end. Therefore, 400 mg of sulfamethoxazole and 80 mg of trimethoprim is a very large amount of material to extract. However, the shape of the mass of sulfamethoxazole extracted versus volume of carbon dioxide also yields some interesting information. These curves, referred to from now on as extraction profiles, typically have three regions. A theoretical extraction profile(taken from reference 64) is shown in Figure 21. The first region of the curve is a straight line of fairly high slope. In the second region of the extraction profile the slope begins to decrease, and in the third region of the profile the curve flattens and may have a small positive slope. The extraction profile for sulfamethoxazole shows only the first region - a line of fairly high slope. In this portion of the extraction the analyte is simply being solvated by the carbon dioxide and rinsed out of the extraction vessel. When the slope of an extraction profile starts to decrease (region 2) diffusion and/or chemisorption is affecting the extraction rate. When the extraction profile flattens (region 3) the extraction is said to be diffusion limited. Therefore, after 80 mL of carbon dioxide under these conditions, the extraction of sulfamethoxazole does not become diffusion limited. However, after 80 mL of carbon dioxide no trimethoprim was detected in any of the extracts. The

Figure 21

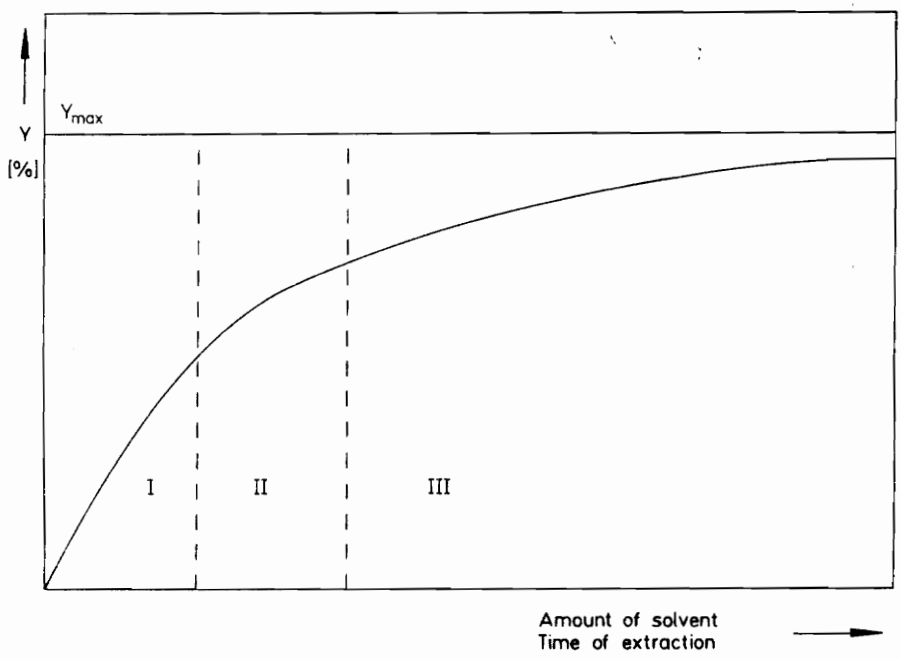


Figure 21: Theoretical extraction profile taken from Dense Gases for Extraction and Refining, by E.Stahl, K.W. Quirin, and D. Gerard, published by Springer-Verlag, New York; 1987, p.6

absense of trimethoprim could be due the large amount of sulfamethoxazole present saturating the carbon dioxide.

In order to determine whether the large amount of sulfamethoxazole present was preventing trimethoprim from being extracted the amount of infusion placed in the vessel was decreased. The extraction vessel was filled with 5 mL of water and 100 μL of infusion. At this concentration there are 8 mg of sulfamethoxazole present as compared to 400 mg in the previous experiment, and 1.6 mg of trimethoprim as compared to 80 mg previously. The extraction was carried out under the same conditions as the previous extraction, but the trap was replaced at 20 mL intervals instead of at 10 mL intervals. Figure 22 shows the extraction profile obtained. Again, the amount of sulfamethoxazole extracted was fairly constant at approximately 0.1 mg per 20 mL of liquid carbon dioxide. Note that although twice the amount of fluid was passed through the extraction vessel before the trap was changed, the amount of sulfamethoxazole collected did not double. In fact, the amount of sulfamethoxazole collected after 20 mL was less than that collected after 10 mL in the previous experiment. Under these conditions approximately 0.41 mg of sulfamethoxazole were extracted with 100 mL (liquid) of carbon dioxide, which corresponds to 5% recovery. The chromatograms of these extracts show that a very small amount of trimethoprim was extracted. The fact that less sulfamethoxazole was extracted during this extraction than in the previous extraction with greater sample size may indicate that extraction reproducibility from this matrix may problematic.

During this extraction many problems with restrictor clogging were encountered. The in-line filter prevented the transfer of particulates through the system. However, this filter can not prevent the transfer of a small amount of unsolvated water upon pressurization of the system. Water has a negative effect on

Figure 22

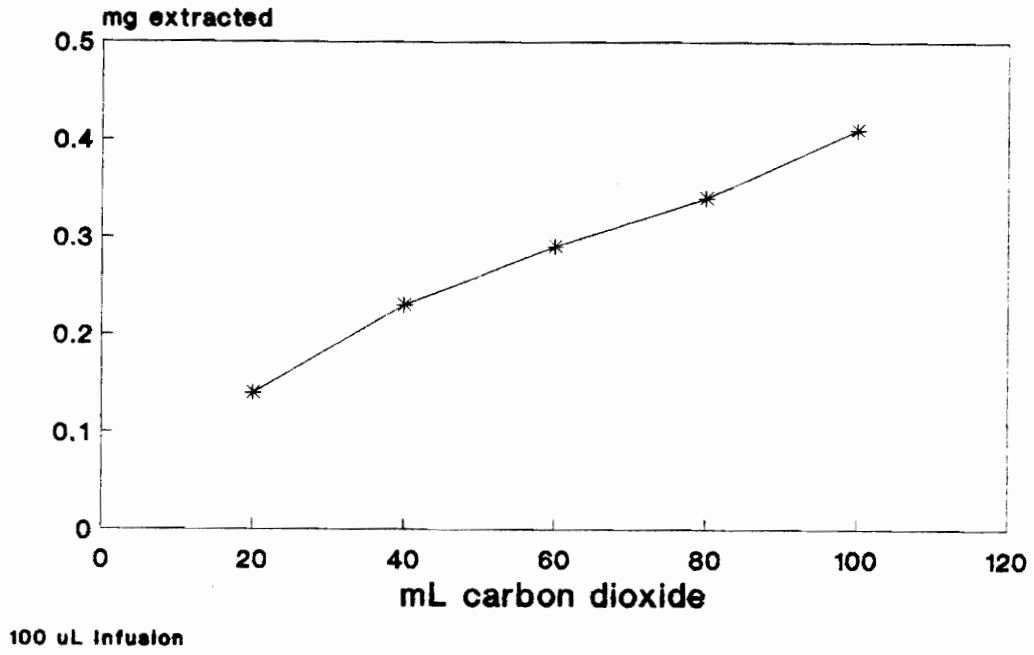


Figure 22: Extraction profile of 8 mg of sulfamethoxazole from aqueous solution under the same conditions as Figure 20, except that the trap was replaced at 20 mL intervals.

fused silica, and can cause the silica to weaken and become active. The clogging also could have been due to an improperly pulled restrictor which allowed decompression over a large range. Because of the problems encountered, in all further work Septra Infusion was spiked onto an absorbent for extraction on the HP 7680A system.

Initial experiments on this instrument involved spiking Septra Infusion onto filter paper which had been cut into strips, z-folded, and placed in a 1.5 mL extraction vessel. When the vessel was filled with filter paper the lower cap was removed and the vessel was placed on a piece of filter paper. Next, a 200 μL solution of neat Septra Infusion was spiked onto the filter paper. Once the transfer of the infusion was complete the filter paper under the vessel was examined in order to see if any breakthrough had occurred. Since no breakthrough was observed filter paper was used as a solid support for Septra Infusion.

An extraction of Septra Infusion (200 μL) was carried out at 60 °C and a density of 0.85 g/mL (P_{ext} = 329 bar). Stainless steel beads were used as the trap material since the compounds of interest are non-volatile and therefore should not require absorptive interactions for efficient trapping. The stainless steel trap was maintained at 5 °C during the extraction, while the nozzle (restrictor) was maintained at 45 °C. The flow rate of carbon dioxide (liquid) was 2 mL/min, so that 36.3 thimble volumes (1.5 mL thimble) were rinsed during the 25 minute dynamic extraction. The dynamic extraction was preceeded by a 15 minute static extraction. The trap was rinsed with the HPLC mobile phase, and the resulting extract was analyzed by LC. Under these extraction conditions 12% sulfamethoxazole and 4.8% trimethoprim recoveries were obtained. However, attempts to increase the flow rate of carbon dioxide or decrease the static extraction time resulted in clogging of the extraction system. This clogging was probably a result of the large amount of sulfamethoxazole and trimethoprim placed

on the solid support. A 200 μL spike volume corresponds to 16 mg of sulfamethoxazole and 3.2 mg of trimethoprim. Therefore, 1 mL of infusion was diluted to volume in a 10 mL volumetric flask with the acetic acid/acetonitrile solution. This diluted solution was then spiked onto Celite at the 100 μL level. Celite was used as the solid support rather than filter paper since less manipulation of the support was required. Celite can be easily placed in the vessel, while filter paper must be cut into strips and folded. At this spike level 0.8 mg of sulfamethoxazole and 0.16 mg of trimethoprim were placed in the 1.5 mL vessel. At these concentrations no plugging was observed.

A density study was conducted where recovery of sulfamethoxazole and trimethoprim were monitored at 0.4 g/mL, 0.65 g/mL, and 0.85 g/mL. Pure carbon dioxide was used as the extraction fluid at a temperature of 60 °C and a liquid flow rate of 2 mL/min. The stainless steel trap was maintained at 5 °C during extraction and elevated to 30 °C during the rinse with the LC mobile phase. The extraction time at each density was held constant at 10 minutes. Therefore, the number of times the vessel was swept at each density was different. However, the goal of this experiment was to determine the threshold densities of the compounds of interest, not to perform quantitation. The results of the density study are shown in Figure 23. As can be seen from the Figure, the threshold density of sulfamethoxazole is between 0.4 g/mL and 0.65 g/mL, while the threshold density of trimethoprim is between 0.65 g/mL and 0.85 g/mL. Based on these results, all further extractions were carried out at a density of 0.85 g/mL (329 bar at 60 °C).

Next a time study was performed in order to construct an extraction profile for these compounds, as was done earlier with the aqueous system. There is no reason to expect the extraction kinetics of these compounds from water to be identical to the

Figure 23

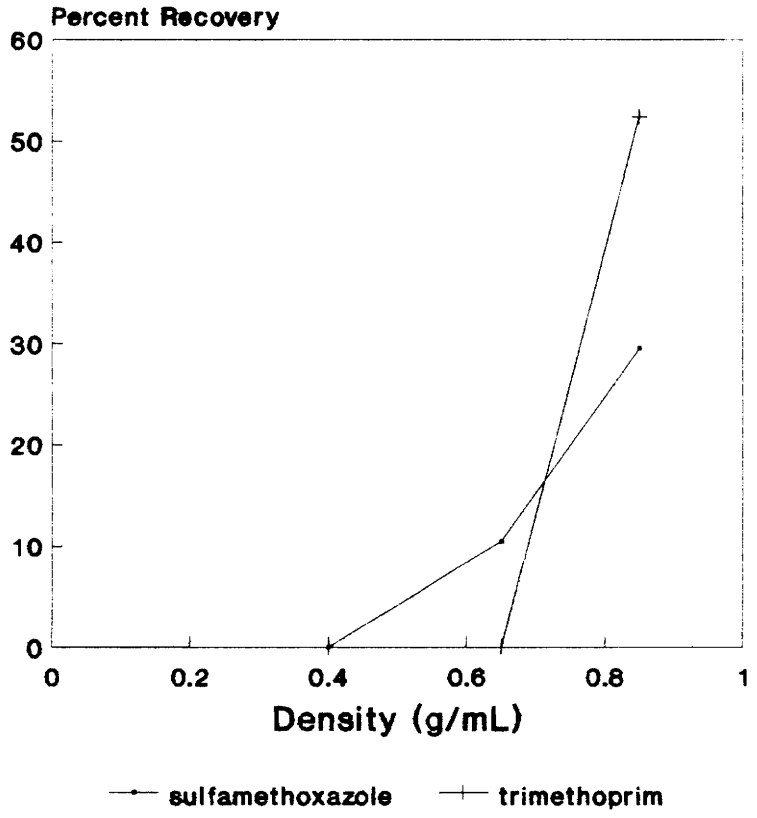


Figure 23: Density study of sulfamethoxazole and trimethoprim performed to determine the threshold densities.

extraction kinetics off of a solid support. A four step extraction was performed at 0.85 g/mL, where recovery was determined at 10 minute intervals (14.5 thimble volumes swept in each interval). Again the extraction vessel was maintained at 60°C during the extraction, while the stainless steel trap was held at 5°C. The LC mobile phase was used as the trap rinse solvent. The extraction profiles for sulfamethoxazole and trimethoprim are shown in Figure 24. As can be seen from the Figure these profiles have the expected three region shape. Recovery of sulfamethoxazole was 113% with an RSD of 14% and recovery of trimethoprim was 85% with an RSD of 10%. Note that the error associated with these recoveries is large due to the four step procedure. Since the trap was rinsed into two vials at each of the four ten minute steps, the error of 8 sample transfers, 4 dilutions to volume, the extraction itself and the subsequent LC run are all reflected in these numbers.

Finally two more samples of the 100 μL spike were extracted for 40 minutes (continuosly) under the same density conditions used for the time study. The result of all three extractions are shown in Table XIII. Note that the recoveries of sulfamethoxazole and trimethoprim are slightly lower than were achieved with the time study, however the error is decreased because there was less sample manipulation to obtain the percent recoveries.

Conclusions

The extraction of sulfamethoxazole and trimethoprim was accomplished with satisfactory percent recoveries. It is difficult to compare the extraction kinetics of these compounds from the solid Celite support to the primarily aqueous solution, since such different concentrations of analyte were used. When the aqueous extractions were carried out the extraction profiles were straight lines instead of the three portion curves

Figure 24

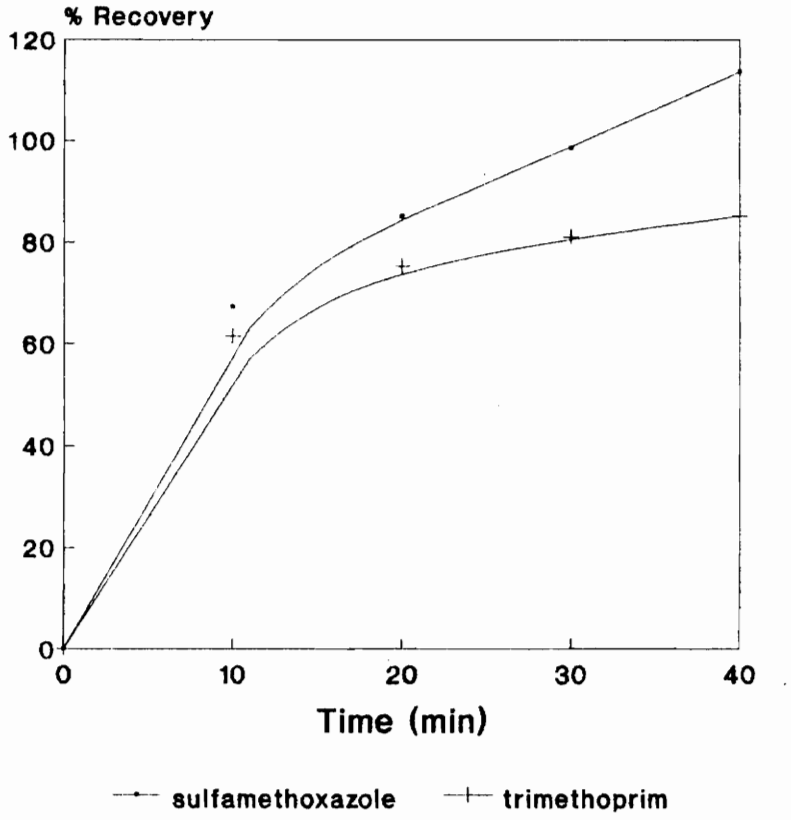


Figure 24: Extraction profile of sulfamethoxazole and trimethoprim off of Celite.

Table XIII

Recoveries and RSDs for sulfamethoxazole and trimethoprim off of Celite, where the recoveries marked by * were calculated by adding the recoveries obtained from the extraction profile. The other two recoveries represent one extraction each, but the extracts were injected into the LC system three times each.

Sulfamethoxazole		Trimethoprim	
% Recovery	RSD	% Recovery	RSD
113*	14	85*	10
95.3	1.9	85.6	1.8
90.8	1.6	87.8	4.7

that were obtained from the solid support. The ease with which this extraction was carried out once the concentrations were reduced enough to avoid saturation of the extraction fluid indicates that these compounds are extremely soluble in carbon dioxide.

Chapter 5

Application of SFE to Polychlorinated Biphenyls from River Sediment

Introduction

In recent years there had been a great deal of interest in using supercritical fluids to extract trace organics from environmental solids, such as soil, sediment, fly ash, and plant material. Supercritical fluid extraction provides advantages over traditional sample preparation techniques for these types of samples. Sample preparation for environmental solids often is done by Soxhlet extraction, which usually requires large sample sizes, large volumes of organic solvents, and long extraction times. A further disadvantage of Soxhlet extraction is that since it is carried out at the boiling point of the organic solvent, thermally labile compounds may degrade, and volatiles may be lost. Sample preparation by centrifugation or ultrasonication is also done, although for environmental samples these techniques are often less efficient than Soxhlet extraction. Thermal desorption is also used as a sample preparation technique for environmental samples, but again thermally labile compounds may degrade. Supercritical fluid extraction provides some advantages over the sample preparation techniques listed above in that extraction times are reduced as compared to those with liquid solvents due to the improved diffusivity of supercritical fluids. When supercritical carbon dioxide is used mild extraction temperatures can be used, which may prevent thermally labile compounds from degrading. Supercritical fluid extraction also eliminates that use of large volumes of organic solvents that are required for Soxhlet extraction, and supercritical solvents are also easily and cleanly removed when

pressure is decreased.

Schantz and Chesler[8] explored the extraction of polychlorinated biphenyls (PCBs) from sediment and polyaromatic hydrocarbons (PAHs) from urban particulate using supercritical carbon dioxide at a density of 0.93 g/mL. After extracting for four hours the extract was concentrated and analyzed by gas chromatography using an electron capture detector for the PCBs and a flame ionization detector for the PAHs. They compared recoveries of Arochlor 1254 from 6 g of sediment obtained by Soxhlet and by SFE and found that they achieved comparable recoveries by both methods, although the Soxhlet extraction required sixteen hours. For the extraction of PAHs from urban particulate, the values for Soxhlet and SFE were in good agreement, with the exception of benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, where values obtained by SFE were 18% and 30% higher than those obtained by Soxhlet, respectively. They concluded that for these two compounds supercritical fluid extraction was more efficient than Soxhlet extraction. They also placed a sample of urban particulate that had previously been extracted under supercritical conditions in a Soxhlet apparatus and extracted for sixteen hours with methylene chloride. When this second stage extract was analyzed by GC no PAHs were detected, indicating that the total extractable PAHs were initially removed by the supercritical fluid.

Capriel et al.[23] have studied the extraction of bound pesticide residues from soil and plant samples. They reported that studies using radioisotopes as tracers for pesticides have shown that a considerable amount of pesticide residues may remain bound after traditional liquid extraction techniques, and therefore estimates of pesticide residues in soils and plants were low. They used supercritical methanol at 150 bar and 250°C at a liquid flow rate of 1 mL/min to extract ^{14}C labeled atrazine, prometryn, deltamethrin, 2,4-D, methyl parathion, dieldrin, and carbofuran from soil, humis,

corn, and radishes. After four hours of extraction, recoveries with supercritical methanol were generally much improved over those obtained by a high temperature distillation technique. They noted that the residual ^{14}C in the extracted plant samples was significantly less than in the soil samples.

Hawthorne et al. have been very active in the study of supercritical fluid extraction of organics from environmental solids. They reported the extraction of PAHs from diesel exhaust particulates and from Tenax-GC resin.[24] Extractions were carried out at 300 atm and 45 °C for 90 minutes, and the extracted species were collected in methylene chloride. Although the recoveries of PAHs from standard reference materials obtained with pure carbon dioxide were quantitative, extraction times of 90 minutes were required. Therefore, 5% methanol in supercritical carbon dioxide was used to obtain quantitative extraction in only 30 minutes. They also investigated the use of supercritical carbon dioxide to extract PCBs, PAHs, and n-alkanes from polyurethane foam sorbents.[25] Polyurethane foam (PUF) sorbents are often used for collecting organic air pollutants. These organics are then recovered from the PUF sorbent by Soxhlet extraction. Extractions of PUF were carried out at 380 atm and 45 °C using supercritical CO_2. Recoveries for n-alkanes (C_{12},C_{16},C_{20},C_{24}) were 100%. PAH recoveries also were 100%. When PCBs were spiked on PUF, extraction times of only ten minutes were required for complete recovery. Further work by Hawthorne reported the extraction of PAHs and PCBs from sediment using supercritical nitrous oxide.[26] Analysis was done by on-line gas chromatography. Percent recoveries for the PAHs ranged from 70-113% with RSDs from 2-21%. Quantitation was not reported for the PCBs. Hawthorne et al. also reported the extraction of PAHs from river sediment and fly ash using supercritical nitrous oxide with 5% methanol modifier.[1] Recoveries for phenanthrene, pyrene, and perylene from

river sediment of 100% were achieved, while recoveries from fly ash were 102%, 74%, and 44%, respectively.

Wright et al.[27] reported the extraction of soil samples containing coal tar residues from manufactured gas plants. Manufactured gas, or town gas, was a gasoline produced by the high temperature carbonization of bituminous coal. This process was used in the 1800's, and resulted in the formation of coal tar residues, which were then burned or buried. These sites are now hazardous, and therefore it is necessary to analyze these samples. Soil samples were extracted at pressures of 300-400 bar at 125 °C for 30-90 minutes with supercritical carbon dioxide. These results were compared to results obtained by Soxhlet extraction with methylene chloride. When fourteen PAHs were spiked on soil, recoveries ranged from 44-124%, with lower molecular weight PAHs giving recoveries less than 80%. They reported that given the same sample sizes, the detection limits achievable by SFE were essentially the same as Soxhlet extraction. When ten replicate soil extractions were performed by SFE the results averaged within 10% of the Soxhlet extractions. The higher molecular weight compounds showed the greatest deviation between the two extraction methods, with SFE resulting in recoveries of half that obtained by Soxhlet.

Raymer et al.[28] reported the extraction of radiolabeled hexachlorocyclohexane, hexachlorobiphenyl, anthracene, and Parathion from Tenax-GC resin, and compared recoveries to those obtained with traditional thermal desorption. Extractions were carried out with carbon dioxide at 3000 psi and 40 °C. After fourteen void volumes were passed over the Tenax-GC trap recoveries of 81-100% were obtained, with the lowest recovery obtained for anthracene. However, when the trap was desorbed with 260 void volumes of helium, anthracene recovery was 99%. For all of the other compounds studied, recoveries were higher when supercritical carbon dioxide was

used. They concluded that desorption of polynuclear aromatics, pesticides, and PCBs from Tenax-GC using supercritical carbon dioxide was efficient and potentially more applicable to recovering compounds of lesser volatility and greater polarity. Raymer et al. also reported the desorption of the same compounds from four polyimide-based sorbent materials.[29] They reported that supercritical desorption was superior to thermal desorption, but that it was more difficult to desorb these compounds from the polyimide-based sorbents than from the Tenax-GC sorbent.

Knopf et al.[30] reported the extraction of organic hazardous waste from contaminated soils. They extracted PCBs, 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT), and toxaphene from contaminated topsoils and subsoils. Supercritical carbon dioxide at 100 atm and 40°C was passed continuously through 10 grams of soil. Recoveries of 70% were obtained for topsoil contaminated with 100 ppm DDT, and 75% for 400 ppm toxaphene in under ten minutes. The extraction of subsoil spiked with 1000 ppm PCBs resulted in recoveries of over 90% in under one minute. They also explored the effect of soil water content on the extraction of contaminated soils. The DDT/toxaphene contaminated topsoil and the PCB contaminated subsoil were prepared with 20% moisture content by weight. When the wet soils were extracted under the same conditions as the dry soils there was a slower rate of removal of contaminants, especially with the PCBs, but total recoveries were comparable. Knopf et al. also reported the use of supercritical carbon dioxide with methanol, acetone, diethylamine, and acetic acid/methanol cosolvents for the extraction of lab spiked and spill site soil samples contaminated with Arochlor 1254.[31] In both cases (lab spiked and spill site) methanol modified (5 wt%) carbon dioxide resulted in the best recoveries. Near complete removal of Arochlor 1254 from the spill site soil was achieved in 15 minutes of extraction time at 101 bar and 313 K.

Onuska and Terry reported the extraction of PCBs from Canadian sediment using SFE with on-line gas chromatography for analysis.[32] Small amounts of sediment (10-100 mg) were extracted with 100% carbon dioxide and 2% methanol in carbon dioxide at 200 atm and 60°C. The analytes were trapped on a retention gap, and then were transferred to the GC column by thermal desorption. Recoveries of approximately 100% were achieved in eight minutes. For the smallest sample size (10 mg), 20.9 ng of material was extracted, while for the larger sample size (100 mg) 201.5 ng of material was extracted. They also reported the extraction of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from sediment.[33] Using supercritical carbon dioxide at 310 atm and 40°C recovery of 2,3,7,8-TCDD was 48% in thirty minutes. The extraction fluid was then changed to nitrous oxide, and another 50 mg sediment sample was extracted. In thirty minutes under the same conditions 91% recovery of TCDD was achieved. In order to improve recoveries further 2% methanol was added to both the carbon dioxide and the nitrous oxide. A recovery of 93% in under 30 minutes was achieved with methanol/CO_2, and 100% recovery was obtained with methanol/N_2O. The effect of moisture content on the extraction of 2,3,7,8-TCDD was explored by preparing samples that contained 20% moisture and extracting under the conditions used with 2% methanol in carbon dioxide. The additional moisture resulted in a slower extraction rate, so that it was possible to achieve the same total extraction yields, but the time required was doubled.

Alexandrou and Pawliszyn reported the extraction of polychlorinated dibenzo-p-dioxins and dibenzofurans from municipal fly ash.[3] One hour of extraction with nitrous oxide at 400 atm and 40°C resulted in the recovery of over 90% of the tetrachlorodibenzo-p-dioxins compared to recoveries with a twenty hour Soxhlet extraction. Nitrous oxide was used as the extraction solvent since recoveries of 0%

were obtained for most of the compounds of interest when pure carbon dioxide was used. Further experiments with carbon dioxide indicated that the compounds of interest were soluble in carbon dioxide, but could not be removed from the fly ash matrix. Upon addition of 10% methanol to carbon dioxide recoveries were not significantly improved. Therefore, benzene was explored as a modifier. Upon addition of 10% benzene to carbon dioxide over 95% recovery of the compounds of interest were obtained. They placed 0.5 mL of benzene directly into the extraction vessel and obtained similar results. Finally, the fly ash matrix was subjected to strong acid, and extraction with pure carbon dioxide was carried out. Percent recoveries obtained were similar to those obtained with nitrous oxide and 10% benzene in carbon dioxide, once again indicating that these compounds were highly bound to the matrix.

Nielen et al.[34] reported the extraction of hexachlorobenzene, PCB-101, PCB-153, and PCB-180 off of Tenax-GC at 20 MPa and 42 °C using 11.5 mL of liquid carbon dioxide. The extractor was connected to a GC so that on-line SFE/GC/ECD could be used for analysis. Percent recoveries of 52% were reported for hexachlorobenzene, and 58%, 59%, and 63% for the PCBs, respectively. No extractions from real (non-spiked) matrices were reported.

Janda et al.[35] reported the extraction of s-triazine herbicides from sediment. The specific herbicides studied were Simazine, Atrazine, Propazine, Terbutylazine, and Cyanazine. Carbon dioxide at 230 bar and 48 °C (0.8 g/mL) was used to deliver 18 mL (liquid) carbon dioxide through 500 mg of sediment. The sediment samples were spiked with the analytes of interest. Recoveries were all greater than 80%, with the exception of Simazine, where only 42.5% recovery was achieved. Next, 20 μL of methanol were placed directly on the matrix and the extraction was repeated. Recoveries for all compounds were greater than 90%.

McNally and Wheeler reported the extraction of sulfonylurea herbicides and their metabolites from soil, plant materials, and a cell culture medium.[36] They also reported the extraction of linuron and diuron that was spiked on soil and wheat.[7] Using pure carbon dioxide no diuron or linuron was extracted from soil. When 0.2 mL of methanol were added directly to the matrix, 99% recovery was achieved for diuron in 35 minutes. Linuron recoveries of 95% were achieved in 50 minutes when 0.5 mL of ethanol were added directly onto the matrix.

Burk and Kruus[37] studied spiked soil samples at two spike levels using supercritical carbon dioxide at 40.5 °C and 31.1 MPa for 20 minutes (5 mL liquid carbon dioxide). For the soil spiked with PCB 33,77, and 153 at the high spike level (20-30 ng/g) recoveries of 103, 220, and 90% were achieved, respectively. When the same type of soil was spiked at the 0.2-0.3 ng/g level recoveries for the same PCBs under identical conditions were 76,81, and 170%, respectively. The high recovery of PCB 77 for the highest spike level was attributed to a coeluting peak. The less than 100% recoveries were thought to be obtained either because the compounds sublimed from the matrix before the extractions were performed or that the liquid trapping in dichloromethane was inefficient.

Lohleit et al.[38] used on-line SFE/GC to measure the recoveries of PCBs 28, 52, 101, 138, 153, and 180 from Spherosil XOA 200, Florisil, and C-18 adsorbents. The PCBs were spiked on these matrices at the 500 pg level, and were recovered quantitatively with a ten minute extraction time. No trends between recoveries versus adsorbent used were observed, indicating that the "matrix" had little effect on the recoveries.

The goal of this work was to identify and quantitate six PCBs from river sediment using supercritical fluid extraction. This work was undertaken as part of a

round robin study sponsored by the National Institute of Standards and Technology (NIST). As can be seen from the literature review, the extraction of PCBs from many different matrices has been reported. However, with the exception of Schantz and Chesler, and Onuska, the samples were all prepared by spiking. Samples prepared by spiking are routinely much easier to extract, and are not always an accurate representation of how the sample would exist in nature. Therefore, in this work percent recoveries were based on those obtained by Soxhlet extraction rather than on the amount of material spiked on a matrix.

Experimental

PCB standards were supplied by NIST (Gaithersburg, MD) as a 2 μg/mL (per component) solution dissolved in 2,2,4-trimethylpentane. Standards were prepared by serial dilution, and analysis was done with gas chromatography. A Hewlett Packard Series II GC (Avondale, PA) was used equipped with an ECD that was maintained at 300°C. Makeup gas of 5% methane in argon was used at a flow rate of 60 mL/min. A HP-5 (5% phenyl) column, 25 m x 0.20 mm i.d., df= 0.33 μm was used for all separations. The column was initially held at 75°C for 0.5 minutes, and then ramped at 25°C/min to 150°C, where it was held for 10 minutes. A ramp of 2°C/min was then used to reach a temperature of 250°C, and then the temperature was immediately ramped at 10°/min to the final temperature of 300°C. The column was maintained at this final temperature for 10 minutes before it was allowed to reequilibrate at 75°C. This temperature program was used for both the standards and the extracts obtained by Soxhlet and SFE.

Soxhlet Extractions

Cellulose extraction thimbles (22 x 65 mm, Whatman, Maidstone, England) were used to contain the sediment sample. These extraction thimbles, along with all the glassware, were subjected to Soxhlet for 12 hours before they were used for an actual sample. A 250 mL round bottom flask was used to contain 60 mL of benzene. For the extraction of river sediment, 2 grams of sediment as received were placed in the thimble and extracted for 20 hours. The extract was then concentrated by rotoevaporation, transferred to a 10 mL volumetric flask, and diluted to volume with benzene. This extract was used to obtain 100% numbers for the PCBs of interest.

Supercritical Fluid Extractions

The modified Suprex 200A (Pittsburgh, PA) instrument described in Chapter 2 was used for this work. The six port valve was replumbed so that flow through the extraction vessel could be stopped by changing the position of the valve. When flow was stopped, the SPE tube that was used to trap analytes could be changed without any sample loss. A 50 μm piece of fused silica was used as a restrictor, and was pulled to give a condensed flow (liquid) of 2 mL/min at 350 atm. The oven temperature was maintained at 50°C, and the block temperature was held at 75°C, resulting in a trap temperature of approximately -20°C. Approximately 2 grams of river sediment were placed in the 3.5 mL extraction vessel (Keystone Scientific, Bellefonte, PA). Diol SPE tubes (Supelco, Bellefonte, PA) were used as traps when pure carbon dioxide was used as an extraction fluid. These traps were rinsed with benzene, and then diluted to volume in a 10 mL volumetric flask. When modified fluid was used for extraction, 2 mL of toluene in a 10 mL volumetric flask were used for trapping. Pure carbon

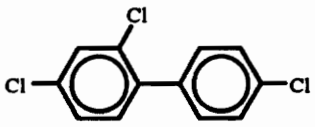
dioxide was purchased in aluminum cylinders with 1500 psi helium headspace from Scott Specialty Gases (Plumsteadville, PA), while modified fluid was prepared in-house. Toluene modified carbon dioxide was prepared by placing an injection valve in-line between the cylinder of pure carbon dioxide and the syringe pump. This valve had an external 1 mL injection loop, which was filled with toluene. During the filling process for the syringe pump this valve was turned in-line so that the toluene was passed into the syringe pump.

Results and Discussion

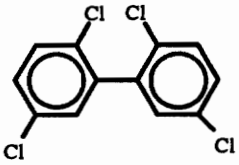
The mixture of standards supplied by NIST contained 28 PCB congeners, out of which we were interested in quantitating six. The six compounds of interest are shown in Figure 25 along with their PCB numbers. The PCBs will be referred to by number in this manuscript. Figure 26 shows the GC separation that was achieved for the PCB standards, with the PCB numbers written above each peak. Note that one of the PCBs of interest, PCB 28, is not baseline resolved from PCB 50. These two peaks could be resolved if a longer column was used, however, with a run time of 70 minutes a longer run time was considered impractical. Therefore, PCB 28 was eliminated from our study.

Standards were prepared ranging in concentration from 0.4 ppm to 3.2 ppb. Due to the non-linearity of the ECD, calibration curves were fitted to polynomial equations rather than lines. These calibration curves were used to quantitate the PCBs extracted by Soxhlet. Table XIV shows the concentration values of the PCBs of interest as obtained by Soxhlet extraction using methylene chloride (data supplied by NIST) and the concentrations we obtained for Soxhlet extraction with benzene. There is no reason to expect Soxhlet extraction with a different organic solvent to result in

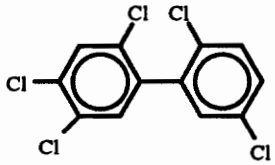
Figure 25



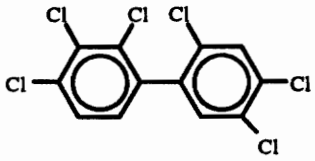
PCB 28 2,4,4'-trichlorobiphenyl



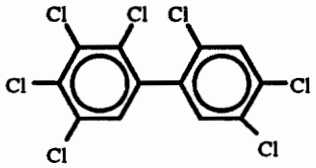
PCB 52 2,2',5,5'-tetrachlorobiphenyl



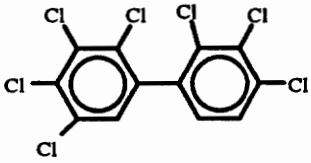
PCB 101 2,2',4,5,5'-pentachlorobiphenyl



PCB 138 2,2',3,4,4',5'-hexachlorobiphenyl



PCB 180 2,2',3,4,4',5,5'-heptachlorobiphenyl



PCB 170 2,2',3,3',4,4',5-heptachlorobiphenyl

Figure 25: Structures, congener number, and IUPAC names for the PCBs studied.

Figure 26

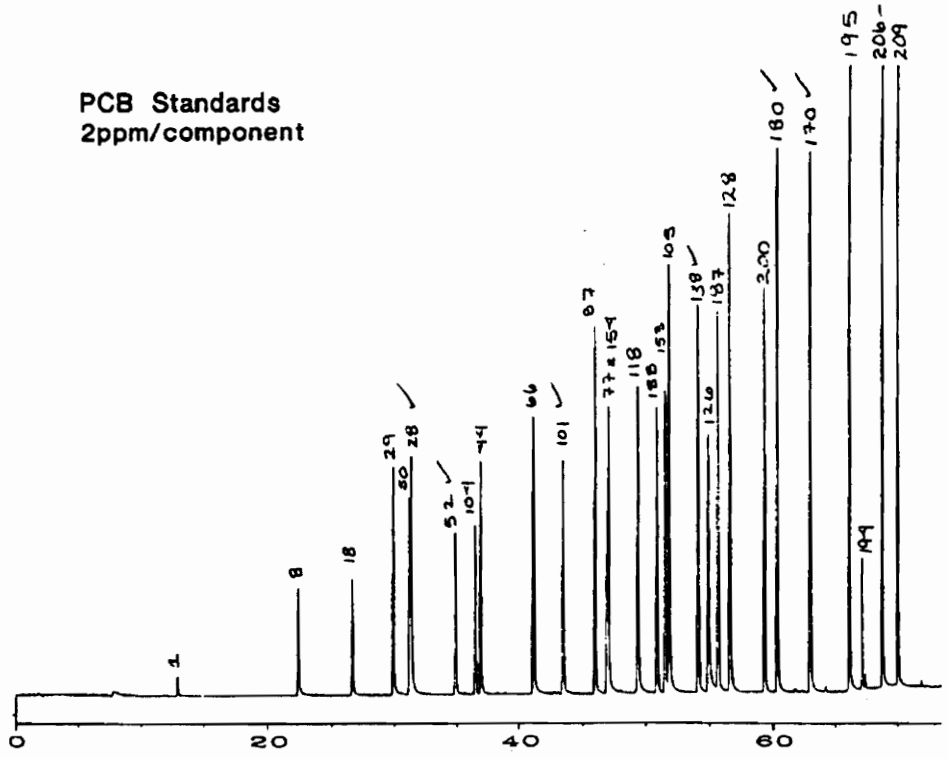


Figure 26: Separation of the PCB standards under the conditions given in the Experimental section.

Table XIV

Concentrations of PCBs in river sediment as given by NIST, and as determined by Soxhlet extraction with benzene.

Compound	NIST method	Soxhlet
	μg/g sediment	μg/g sediment
PCB 52	4.480 + .060	4.415
PCB 101	0.820 + .010	0.660
PCB 138	0.560 + .020	0.489
PCB 170	0.110 + .010	0.146
PCB 180	0.150 + .010	0.092

the same extraction recoveries. In order to identify the PCBs of interest in the Soxhlet extract of the sediment, spiking and retention time matching were tried. However, spiking and retention time matching were not effective for identifying the peaks of interest from the Soxhlet extract, since the chromatogram was so complex. The chromatogram obtained for the Soxhlet extract of sediment is shown in Figure 27. Due to the complexity of the sample, an appropriate internal standard could not be found, so all quantitation was done based on absolute peak areas. In order to identify the PCBs of interest in the extract, retention time matching and spiking was done on an extract that was much more dilute. When this extract was spiked changes in peak area and peak height were immediately obvious.

Next a sorbent phase used for trapping extracted components in the SFE mode was tested. Using the same method that was used to evaluate sorbent traps in Chapter 2, 12.5 ng of PCB 180 was introduced into the trap. Diol SPE tubes were compared to liquid trapping in benzene, and both of these trapping methods were compared to 100% values. A liquid flow rate of 2 mL/min was used when trapping on the diol phase, while a liquid flow of approximately 0.5 mL/min was used for trapping into liquid. For both trapping modes 100% recovery of PCB 180 was obtained. The RSD when trapping into benzene was 12.5% and the RSD when trapping on the diol trap was 18.3%. The larger RSD on the sorbent trap was due to the additional rinsing step that was required. However, benzene freezes when carbon dioxide expands into it, and lower flow rates are required for extraction into liquid solvents. Therefore we decided to use diol traps for all work when carbon dioxide was used as the extraction fluid. When modified carbon dioxide was used as an extraction fluid, liquid trapping was done because the effect of modifier on the sorbent trap was unknown.

Figure 27

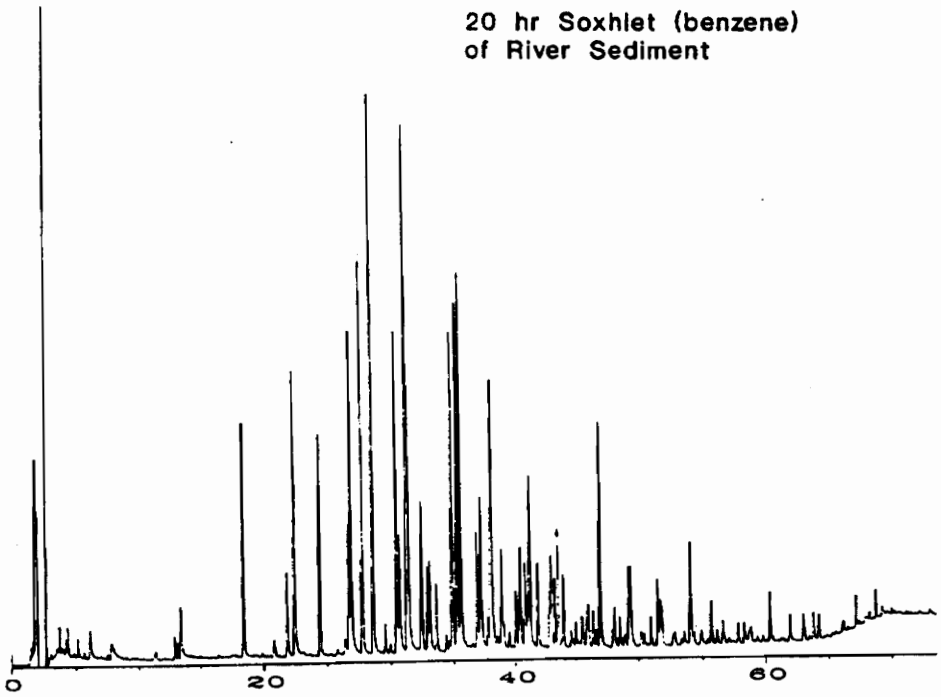


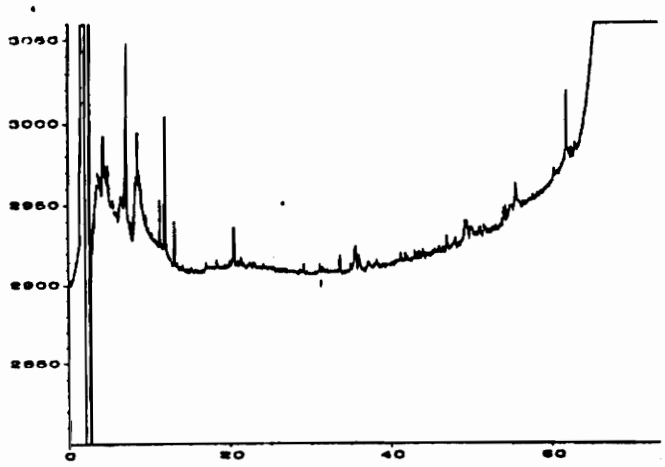
Figure 27: Separation of sediment extract obtained by benzene Soxhlet showing 144 peaks.

The ECD is sensitive to halogenated compounds, and fluorinated lubricants are sometimes used in the preparation of carbon dioxide cylinders. Therefore the background due to carbon dioxide was examined. Carbon dioxide was passed through the extraction system and collected on the diol trap and in benzene. Both "extracts" were diluted up to 10 mL and analyzed by GC. The results are shown in Figure 28 for the same volume of carbon dioxide (20 mL liquid) delivered to each trap. Examination of the magnitude of the detector response indicates that any contaminants present are at very low levels. The baseline rise for the "extract" collected in benzene is probably due to the presence of dissoved carbon dioxide and/or water in the collection fluid.

The extraction profiles for PCBs from river sediment by SFE were then explored by changing the diol trap at intervals of carbon dioxide measured by the pump as a liquid. In this way a plot of volume of carbon dioxide used versus percent recovery was generated. These curves are shown in Figure 29. There are three regions to each of these curves, which is typical for this type of data. The first is a region of steep slope where small volumes of carbon dioxide (5 mL fractions) extract the PCBs rapidly. In this region (first three points) the extraction fluid is simply rinsing the analytes off of the sediment surface and onto the trap. This region therefore represents a bulk extraction of PCBs. Between the third and sixth data points on these curves the extraction profile starts to flatten, indicating that the extraction is starting to become diffusion limited. The latter portions of these curves are fairly flat, indicating that the extraction has become diffusion or chemisorption limited. All of the PCBs, with the exception of PCB 52, become limited after approximately 50 mL of carbon dioxide have passed through the extraction vessel. PCB 52 becomes chemisorption limited after approximately 30 mL of carbon dioxide, indicating that it may be more tightly bound to the matrix than the other PCBs. PCB 52 is more volatile than the other

Figure 28

A) through trap



B) liquid collection

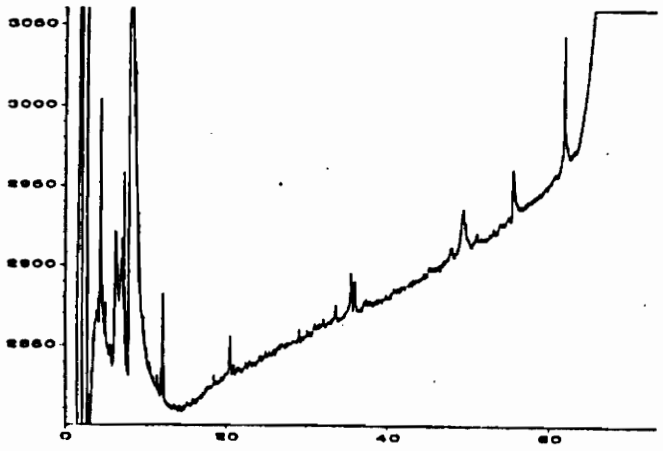


Figure 28: ECD background with (A) collected onto the diol trap, and (B) collected into benzene. GC conditions are reported in the Experimental section.

Figure 29

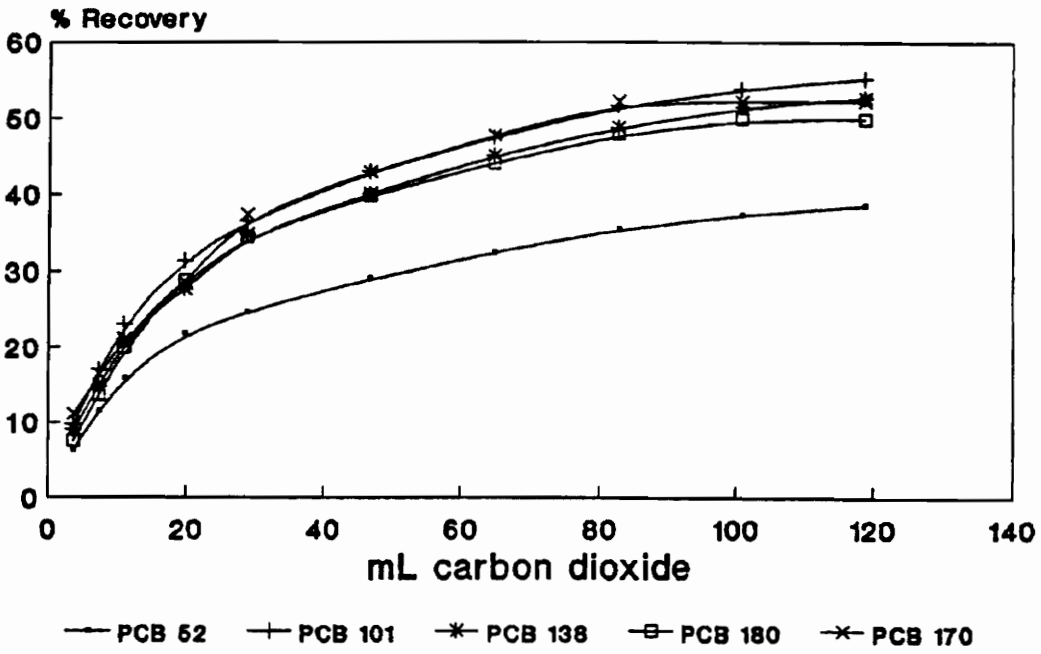


Figure 29: Extraction profile of PCBs using pure carbon dioxide at 350 atm and 50°C. Samples were taken at 2 mL intervals for the first three points, 5 mL intervals for the next two points, and 10 mL intervals for the last five points.

PCBs of interest based on its behavior in the GC, and it should be at least as soluble in carbon dioxide, if not more so, than the other PCBs. There is no obvious reason why PCB 52 would be any less accesible to the extraction fluid than the other PCBs. Therefore, its low recovery indicates that it is probably more strongly chemisorbed to the matrix than the other PCBs.

An attempt at exhaustive extraction using 160 mL of carbon dioxide was then made. The reason for using 160 mL of carbon dioxide was that a syringe pump was being used to deliver the fluid, and under the conditions of extraction, this was the maximum obtainable volume without stopping to refill the pump. The additional increase in amount of fluid delivered (as compared to the amount of fluid used for the extraction profiles) resulted in only slight changes in recovery, as are shown in Table XV. The recovery of PCB 52 was unchanged, while slight improvements in the recoveries of PCBs 138, 180, and 170 were obtained. It is obvious from this data that to obtain 100% recoveries of these PCBs, if possible at all, would require large volumes of carbon dioxide.

In much of the work reported in the Introduction of this chapter, large improvements in extraction recoveries were obtained when modified fluids were used for extraction. Because the extraction profiles obtained with pure carbon dioxide indicated that 100% recoveries would require very large volumes of carbon dioxide, toluene modified carbon dioxide was prepared as described in the Experimental so that 0.8% toluene in carbon dioxide was obtained. The extraction profiles for PCBs of interest were then determined, and are shown in Figure 30. The most immediate difference between extraction profiles with and without toluene modified fluid occurs in the first region of the curve. With the first 2 mL of toluene modified fluid, extraction recoveries are much higher than for the first 4 mL of pure carbon dioxide. Figure 31

Table XV

Percent recoveries for PCBs of interest with 100% CO_2 where the data for 114 mL comes from the extraction profile, and 160 mL were passed continuously over the sediment sample.

Compounds	114 mL CO_2	160 mL CO_2
PCB 52	47.3	44.6
PCB 101	57.8	62.0
PCB 138	54.4	62.3
PCB 180	49.9	64.7
PCB 170	52.2	65.1

Figure 30

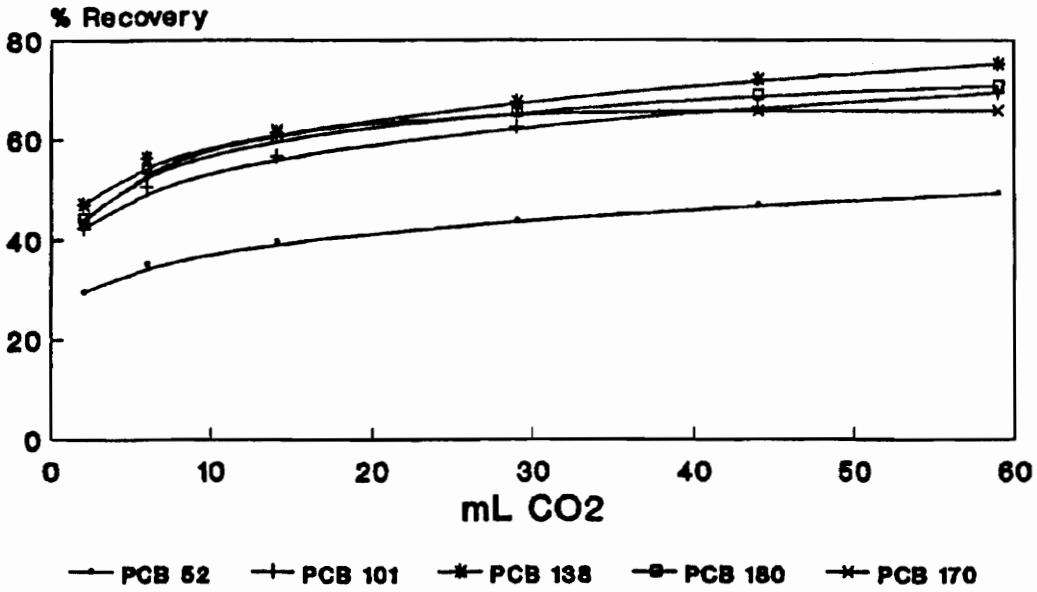


Figure 30: Extraction profile of PCBs using 0.8% toluene in carbon dioxide at 300 atm and 50°C. Samples were taken at 2,6,14,29,44, and 60 mL of liquid carbon dioxide, as measured by the syringe pump.

Figure 31

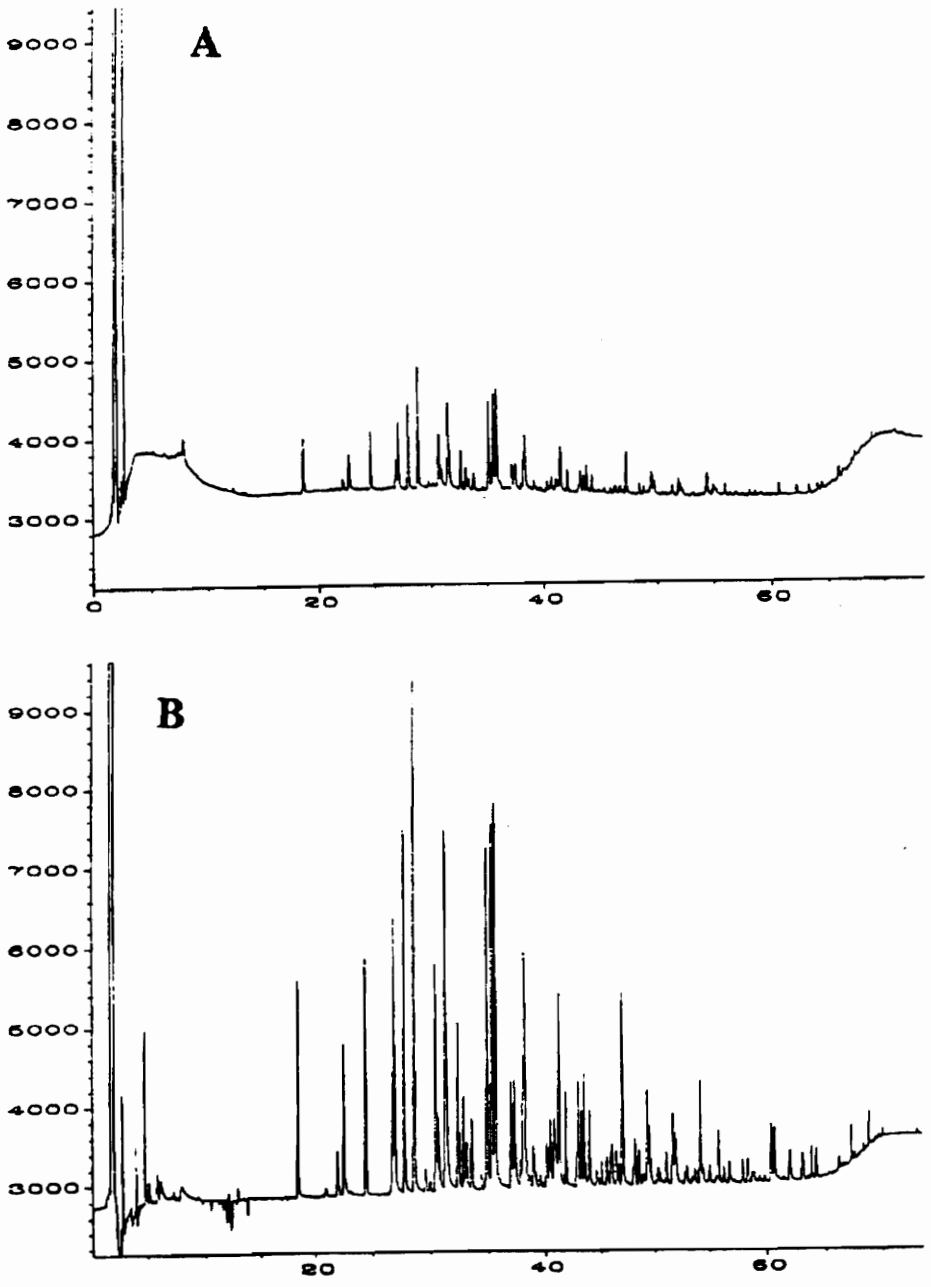


Figure 31: Chromatograms of SFE extracts of river sediment where (A) 4 mL of 100% carbon dioxide, and (B) is 2 mL of 0.8% toluene modified carbon dioxide.

shows the chromatograms obtained for the first 2 mL of toluene modified carbon dioxide and for the first 4 mL of pure carbon dioxide. Both of these chromatograms are on the same scale, so they may be readily compared. Table XVI lists the percent recoveries obtained for each of the PCBs of interest that correspond to the chromatograms in Figure 31. As can be seen from the Table, the addition of modifier affects the bulk portion of the extraction drastically. PCB 52 was recovered at the 6% level with pure carbon dioxide and jumped to 30% when modifier was added.

The role of modifiers in chromatography is explored in Chapter 6. The mechanisms of action of modifiers in SFC that have been proposed are: (1) coverage of active (silanol) sites, (2) swelling or modification of the stationary phase, (3) increasing the density of the mobile phase, and (4) increasing the solvent strength of the mobile phase. The role of modifiers in extraction can be thought of in a similar manner, except that there is no stationary phase. Instead of affecting the stationary phase, modifiers in extraction affect the matrix. Matrix effects can include swelling of the matrix, and competition with "active sites" on the matrix for compounds that are physisorbed or chemisorbed to the matrix. The effect of modifiers on the mobile phase obviously are the same in chromatography and extraction.

PCBs are soluble in supercritical carbon dioxide, as is evidenced by Knopf et al.[30] who extracted PCB spiked on subsoil and achieved 90% recovery in under 1 minute. The long extraction times and low recoveries encountered when pure carbon dioxide was used here must therefore be mainly a matrix effect. The extraction with pure carbon dioxide required 160 mL of carbon dioxide at a density of 0.902 g/mL to achieve recoveries of 50-75%, while only 60 mL of modified fluid were needed to obtain comparable recoveries. The recovery of PCB 52 was again the lowest of all the PCBs of interest, indicating that it may be absorbed more strongly by the matrix than

Table XVI

Percent recoveries of PCBs in river sediment at 350 atm and 50°C for 4 mL pure CO_2 as compared to 2 mL of 0.8% toluene modified CO_2.

Compound	4 mL CO_2	2 mL 0.8% toluene
PCB 52	6.4	29.5
PCB 101	9.7	42.3
PCB 138	9.1	47.0
PCB 180	7.6	44.2
PCB 170	11.1	43.5

the other PCBs. Although the addition of modifier seems to increase the amount of PCB 52 accessible for bulk extraction, the extraction still becomes diffusion limited rapidly. Figure 32 shows the extraction profile for PCB 52 with and without toluene modified carbon dioxide. As can be seen from the Figure the bulk extraction is more efficient with modifier, but the slopes of the two curves are quite similar. In comparison, Figure 33 shows the extraction profile of PCB 170 with and without toluene modified carbon dioxide. Again, more PCB 170 appears to be accessible to bulk extraction when toluene modified fluid is used. However, the slopes of the two lines appear different, with the toluene modified curve having a slightly steeper slope. Unfortunately there are not enough data points for the toluene curve to clearly observe the slope. However, it appears that the modifier has a more drastic effect on those PCBs that are not bound as tightly to the matrix. Table XVII lists the overall recoveries obtained from the sediment with 160 mL of pure carbon dioxide as compared to 60 mL of toluene modified carbon dioxide. Comparable recoveries were obtained, although 100 mL less fluid was used in the modified case. Table XVII also lists the masses of PCBs extracted by SFE from river sediment.

These data compare well with data reported in the literature for the extraction of PCBs. In the Introduction, only two publications were reviewed where PCBs were extracted off of a non-spiked matrix. Chesler et al. reported quantitative extraction of PCBs from sediment. They extracted 6 grams of sediment for 4 hours at 345 bar. The extraction vessel was at room temperature, so the fluid passing through the vessel was subcritical. As a restrictor they used a 66 cm piece of 60 μm fused silica, which would result in a compressed flow rate of 3-4 mL/min of carbon dioxide. Therefore, in 4 hours they passed 720-960 mL of carbon dioxide through the system. Therefore, the recoveries we achieved with pure carbon dioxide (45-65%) with only 160 mL of fluid

Figure 32

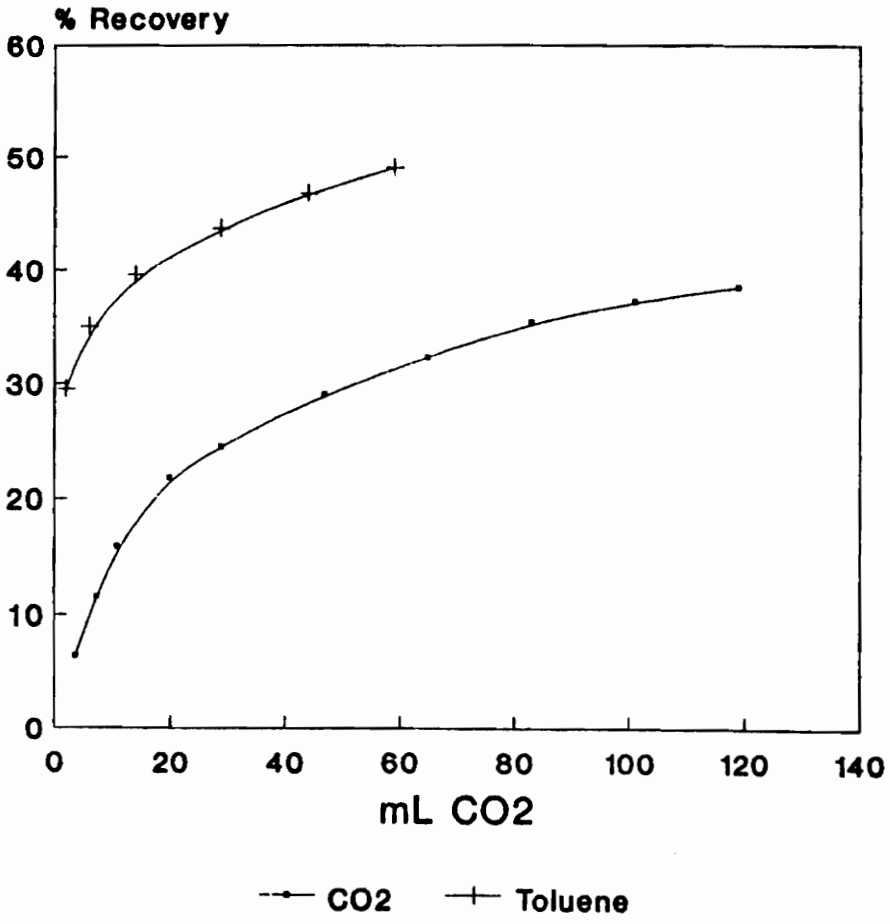


Figure 32: Extraction profile of PCB 52 with and without toluene modifier.

Figure 33

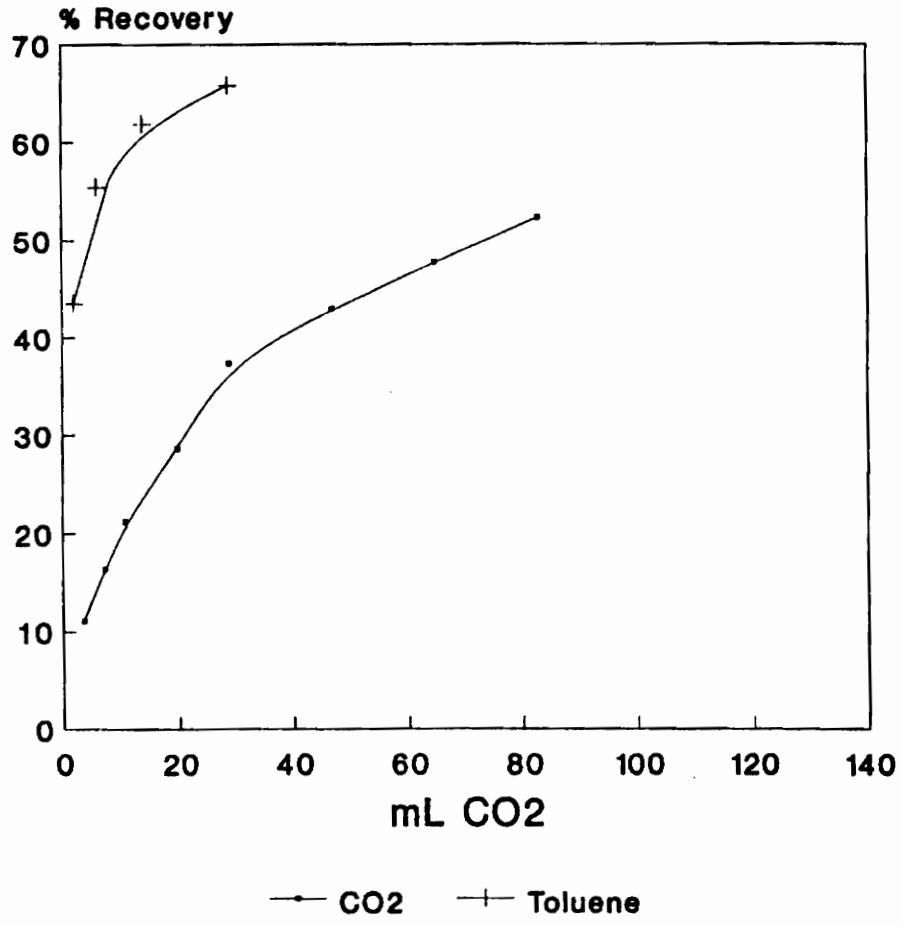


Figure 33: Extraction profile of PCB170 with and without toluene modifier.

Table XVII

Percent recoveries of the PCBs of interest with 160 mL of pure carbon dioxide as compared to 60 mL of 0.8% toluene modified fluid, and masses of extracted PCBs from river sediment for 1.9338 g of sediment for 160 mL of CO_2, and 1.9656 g of sediment for 60 mL of toluene modified fluid.

Compound	160 mL CO_2		60 mL 0.8% toluene	
	% Recovery	Mass	% Recovery	Mass
PCB 52	44.6	3.75 μg	49.1	4.20 μg
PCB 101	62.0	0.78	69.4	0.89
PCB 138	62.3	0.58	75.1	0.71
PCB 180	64.7	0.11	70.8	0.12
PCB 170	65.1	0.18	65.8	0.20

are not unreasonable.

Onuska et al. reported the extraction of a microporous sediment, where all quantitative results given were achieved with 2% methanol in carbon dioxide at 200 atm and 40°C. They reported recoveries of approximately 90% in four minutes. They report a mass flow rate of 0.354 g/min, which corresponds to a liquid flow rate of 0.5 mL/min, when divided by the density ((0.354 g/min)/(0.70 g/mL)= 0.5 mL/min). They also report the volume of their extraction vessel to be 2 mL. Therefore, in four minutes of extraction time under the conditions described, the vessel would have been cleared once. These results differ significantly from those reported by Chesler, and those reported here. In plots of percent recovery versus time, Onuska et al. report no diffusion or chemisorption limited extraction. These plots indicate that this system is performing more like a spiked system than a real sediment sample. Very rapid recoveries of PCBs off of soil, where the soil was spiked, have been reported.[10] Another possible explanation for the large differences in extraction behavior could be the character of the sediment. It has been reported that soil with higher organic matter may exhibit a wide variety of active sites that interact with polar and ionic compounds as well as weakly polar and nonionic compounds.[10] Therefore, the sediment sample studied by Onuska and the sediment sample studied by Chesler and in this work could be very different samples.

The next step in the extraction of river sediment was to add water to the sediment and determine how water affected the extraction. In the 3.5 mL vessel 2 mL of water were added to 1 gram of river sediment. After 8 mL of carbon dioxide at 350 atm and 50°C were passed through the system the diol trap was removed, rinsed, and the extract was analyzed by GC. The chromatogram obtained is shown in Figure 34. As can be seen from the Figure, only extremely small amounts of compounds were

Figure 34

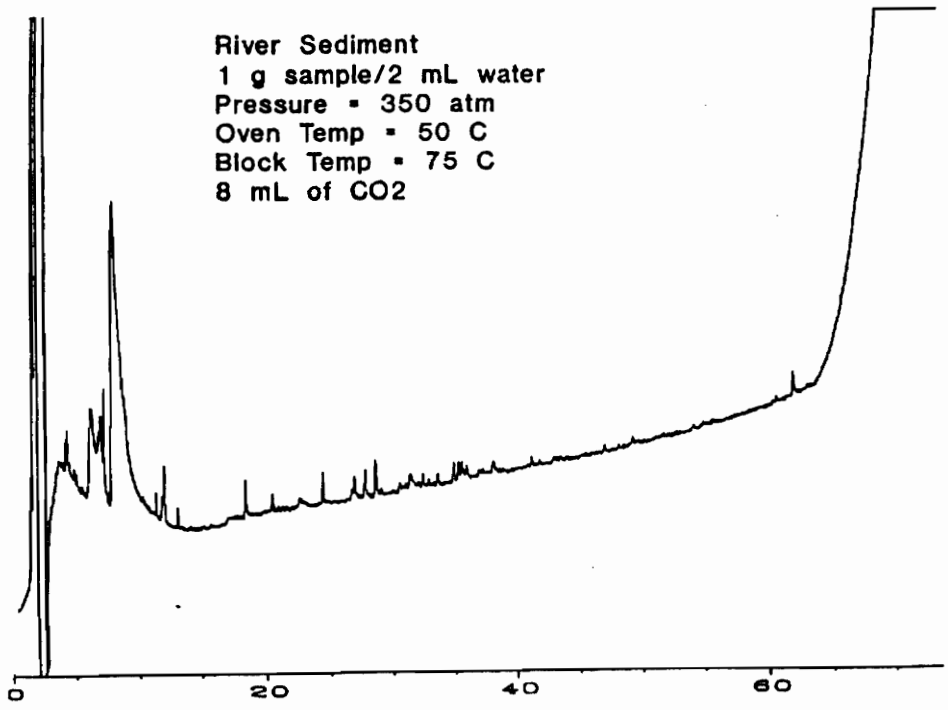


Figure 34: Chromatogram of an SFE extract (8 mL liquid CO_2) of river sediment where 2 mL of water were added to 1 gram of sediment.

extracted under these conditions. It has been reported that moisture can slow down the rate of extraction from various matrices, but that comparable recoveries could be obtained if extraction times were increased.[32] However, these samples contained 20% moisture by weight, whereas the sample we prepared had almost 200% moisture by weight. It is not surprising that extraction kinetics would be different for a moist sample as compared to a wet sample.

A 2 gram sample of estuarine sediment was also extracted by Soxhlet extraction and analyzed by GC. The chromatogram obtained is shown in Figure 35. The large, off-scale peak that elutes between 20-40 minutes is due to sulfur, which is present in estuarine sediments and gives an appreciable signal in the ECD. No attempts were made to scavenge the extracted sulfur and therefore this sample was not studied further.

Conclusions

The extraction of PCBs from river sediment resulted in recoveries of 45-60% when pure carbon dioxide was used as the extraction fluid and 50-75% when toluene modified carbon dioxide was used. These recoveries agree well with what was reported by Schantz and Chesler. This work demonstrates the differences between extracting a spiked sample and a real sample. Although recoveries less than 100% were obtained, it is probable that increasing the toluene content in the mobile phase would improve recoveries further.

Figure 35

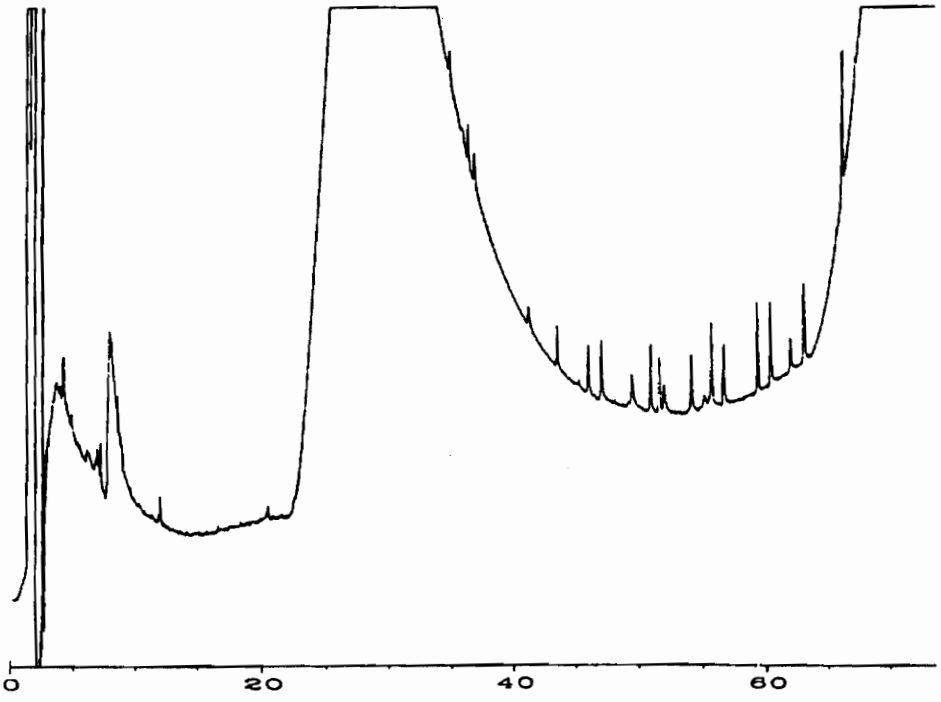


Figure 35: Chromatogram of Soxhlet extract from 2 grams of estuarine sediment.

Chapter 6

Application of Modifiers in Sub- and Supercritical Fluid Chromatography

Introduction

Although supercritical fluid chromatography (SFC) has found applications in many industries, such as petroleum[39] and food products[40], it has yet to be successfully applied to problems encountered in the pharmaceutical industry. One of the reasons SFC has been unable to solve problems encountered in this field is that the solvent strength of supercritical CO_2 is not sufficient for the elution of many polar compounds. The solvent strength of CO_2 at moderate density is similar to that of hexane and other normal hydrocarbons.[41-43] Although mobile phases other than CO_2 have been explored, none of them have all of the desirable properties of CO_2, such as low critical parameters, transparency in the FID, and a high degree of safety. Table XVIII lists fluids that have been used for SFC and some of their physical properties.[44] As can be seen from the Table, CO_2 has low critical parameters and no dipole moment. Fluids with significant dipole moments, such as NH_3, have high critical parameters and are often dangerous to work with.

The polarity of supercritical CO_2 can be increased through the addition of polar modifiers, such as methanol. Table XIX lists some of the most commonly used modifiers and their physical parameters.[45] Methanol is by far the most commonly used modifier in SFC. Acetonitrile, although its polarity index is higher than that of methanol, is less soluble in CO_2 than methanol and therefore is less frequently used.[13] Water is even less soluble in CO_2 than acetonitrile, and therefore when water is used as a modifier in CO_2, the mobile phase is usually saturated with water.

The disadvantage of adding even small amounts of modifier to carbon

Table XVIII

Physical properties of selected supercritical fluid taken from Reference 44.

Fluid	T_c(°C)	P_c(atm)	Dipole moment (debyes)
CO_2	31.3	72.9	0.00
N_2O	36.5	72.5	0.17
NH_3	132.5	112.5	1.47
SF_6	45.5	37.1	0.00
Xe	16.6	58.4	0.00

Table XIX

Frequently used modifiers and some of their physical parameters, taken from Reference 44.

Modifier	T_c(°C)	P_c(atm)	Polarity Index
methanol	239.4	79.9	5.1
acetonitrile	275	47.7	5.8
propylene carbonate	352	------	6.1
formic acid	307	------	-----
water	374.1	217.6	10.2

------- not given

dioxide is that, in most cases, the FID and the FT-IR can no longer be used as detectors. This presents a problem for pharmaceuticals, since many of the compounds of interest do not have UV chromophores. In addition, many polar materials can not be chromatographed by SFC, even with very high modifier concentrations. Another disadvantage of using a modified mobile phase for SFC is that the critical parameters of the binary phase are greater than the critical parameters of the pure fluid. Critical parameters can be calculated using equations of state, however, it has been reported that calculated parameters have been found to differ by 20% from empirical measurements.[50] If the compounds of interest are thermally labile, working at the higher temperatures required in order to produce a single phase may be a disadvantage.

Despite these disadvantges, modifiers offer many advantages that make them of interest in supercritical fluid chromatography. The addition of small amounts of modifier has been shown to drastically affect the quality of SFC separations. Compounds that can not be efficiently eluted with pure fluids can often be eluted with reduced retention times and improved peak shape when small amounts of modifiers are added. Modified carbon dioxide provides definite advantages over pure fluids of greater polarity in that the modified fluid often has lower critical parameters and is generally safer to work with. The ability to expand the polarity range of compounds that can be chromatographed using supercritical fluids through modifiers is therefore an important area of research.

Generation of Modified Phases

Although the need for modifier in separations of polar compounds is well known, the introduction of modifiers into the supercritical fluid is often not accomplished simply. Modifiers can be introduced into chromatographic systems in

three ways. First, premixed tanks of modified fluid can be purchased. These tanks can be purchased with a variety of fluids and modifier percentages, and are usually sold by weight % modifier in the pure fluid. With premixed tanks of modified fluid limited flexibility is achieved in that each time a change in the composition of the mobile phase is desired another cylinder must be purchased. The purchase of many tanks with different modifiers and modifier concentrations, which may be required for method development, can become quite expensive. One problem encountered when introducing pre-mixed modified fluid into a system is that contamination often occurs with syringe pumps. It has been observed in this laboratory that once a modifier has been introduced into a syringe pump, many rinses (>10) of pure carbon dioxide are required to remove the modifier from the pump. In some cases, it may be necessary to take the pump apart, wash the surfaces with an organic solvent, dry in an oven to remove the solvent, and replace the seals.

The second way to add modifier to a chromatographic system is to add the modifier as a liquid to the syringe pump. This is often accomplished by placing a valve in-line between the tank of fluid being used and the syringe pump. A large injection loop (1 mL) is filled with the liquid solvent of choice. The injection loop is then placed in-line while the syringe pump is filled with fluid. In this way the liquid solvent is mixed with the fluid. A volume % is obtained from the ratio of the injection loop volume to the volume of fluid in the syringe pump. Although this method is flexible, in that a variety of liquid solvents can be used and the size of the injection loop can easily be changed, the problem with contamination of the syringe pump is still present. Also, since the kinetics of mixing may be slow or unknown, the composition of the fluid may not remain constant with time. Since the solubility of many fluids in carbon dioxide is unknown, it is also possible to exceed the solubility and produce a two-phase

system in the syringe pump.

The third way to add modifiers to a chromatographic system is to use a two pump system where one pump delivers the pure fluid and the other pump delivers the liquid modifier. The two fluid streams are mixed to form the mobile phase. This method of preparing the mobile phase provides the greatest flexibility in that the mobile phase is mixed in-line. It is also an accurate way to add modifier, since the flow rate of each pump is known. Usually a reciprocating piston pump is used to deliver the pure fluid, while a syringe pump is used to deliver the modifier. One of the main concerns in mixing a liquid modifier and the supercritical fluid is that the compressibilities of the two fluids are different. The compressibilities of the fluids must be considered if a pressure gradient is to be used. Another concern is that time must be allowed for efficient mixing of the two fluids. However, much of the work using modified mobile phases has been done under isobaric conditions.

For all of the methods described above, one must be concerned with the solubility of the modifier in the supercritical fluid. If the solubility of the modifier is exceeded, a two-phase system will result, and the effect of a two-phase system on chromatography may be detrimental. The UV detector often gives an indication of whether a two-phase system is present or not, in that a very noisy signal results when two phases are present.

In the literature there are little data regarding the solubility of polar modifiers in carbon dioxide at various temperatures and pressures. Francis reported the solubility of components in liquid CO_2 at 25 °C. In this work many ternary phases were studied.[13] Macguire et al. reported that their search of the literature resulted in only two sources for solubility data for polar modifiers.[46] They reported that Kuk and Montagna investigated the solubility of ethanol in CO_2 at 40 °C and pressures from 75-200 atm,

and the solubility of isopropanol in CO_2 at high pressures.[47] The other source found reported on the solubility of acetone and ethanol in CO_2 at 30-60°C and 20-150 bar.[48] Both of these references come from the chemical engineering literature.

Macguire et al. studied the solubility of methanol, acetonitrile, and chloroform in CO_2 at temperatures ranging from -4 to 50°C and pressures ranging from 15-170 atm.[46] They determined that the concentration of methanol in CO_2 should be kept at or below 12 mole % in order to maintain a homogeneous mixture. They also determined that acetonitrile in CO_2 should be kept at concentrations below 1.6 mole%, while chloroform should be kept no higher than 1.8 mole %.

Page et al. have studied the phase behavior of various modifiers using laser light scattering.[49] They concluded that SFC should be performed above 160 atm if the column temperature exceeds 90°C when methanol concentrations of 7 mole % or above are used. They also found that propylene glycol produced a two-phase system over the entire useful pressure/temperature/composition region, and is therefore not very useful as a modifier.

Mechanism of Modifier Action

The effects of modifiers in SFC as reported in the literature are: (1) coverage of active (silanol) sites, (2) swelling or modification of the stationary phase, (3) increasing the density of the mobile phase, and (4) increasing the solvent strength of the mobile phase.[50] The mechanism of action of modifiers, however, is somewhat ambiguous as reported in the literature, and competing mechanisms (i.e. stationary phase effects vs. mobile phase effects) have been proposed.

Much of the earlier literature attributed changes in retention solely to stationary phase effects when modifier concentrations were at 1% or less. Ashraf et al. investigated nitrogen containing compounds of varying basicity on different stationary

phases, and compared the results obtained with 100% CO_2 to those obtained with 1% methanol in CO_2.[51] They noted that some of the compounds that did not elute with pure CO_2 did elute when 1% methanol was added to the mobile phase, and that peak shapes improved.

Levy and Ritchey studied PAHs with methanol, 2-methoxyethanol, 1-propanol, tetrahydrofuran, dimethylsulfoxide, and acetonitrile as modifiers.[52] The general trend observed was a decrease in capacity factor with the addition of modifier. They proposed a competing mechanism where at low concentrations of modifier (0.5-3.0 weight %) the modifier affects the stationary phase through hydrogen bonding with the silanol sites, while at higher concentrations the mobile phase polarity was affected.

Schoenmakers et al. also reported that at low modifier concentrations (0-2 %) modifier effects in packed column SFC were largely due to deactivation of residual silanol groups on the silica support.[17] They studied the adsorption isotherms of various modifiers on octadecyl-modified silica and found that the capacity factors of polar solutes at different modifier concentrations correlated well with the amount of modifier adsorbed on the surface. They concluded that the effects of low concentrations of modifier were caused by stationary phase deactivation and that the influence of mobile phase modification on the reduction of retention was negligible in comparison.

It has been reported that, in contrast to packed columns, capillary columns do not show the drastic changes in capacity factors or peak shape upon addition of small ($<2\%$) amounts of modifier.[53] These less drastic differences were attributed to the differences in the degree of deactivation of the packed column stationary phase as compared to the capillary column stationary phase. A capillary column has a smaller number of active sites present; therefore, there are less active sites for the modifier to deactivate. Capillary columns do have active sites, as it has been reported that column

coating efficiencies for small diameter capillary columns range from 20-80 % depending on the stationary phase.[50] It was also reported that higher modifier concentrations (5-20%) resulted in changes in retention on capillary columns.[53] These two papers again support the theory that modifier affects the stationary phase at low concentrations and the mobile phase at higher concentrations. However, Berger et al. contend that modifiers do not produce significantly different results on capillary and packed columns.[50] They reported that the retention of polyaromatic hydrocarbons (PAHs) has been shown to decrease 15-32% on a variety of packed columns and 26-28% on capillary columns when approximately 2% of 2-propanol or methanol was added to the mobile phase.

In order to study the effect of modifiers on the solvent strength of the mobile phase, several groups have undertaken spectroscopic studies. Solvatochromic scales, which are based on shifts in the wavelength of maximum absorption for various dyes, are commonly used to measure solvent strength. Solvatochromic parameters are influenced by the local solvent environment surrounding the probe molecule. Therefore, solvatochromic measurements should not be considered to be a bulk property. Clustering of supercritical fluids around solute molecules has been widely accepted in the chemical engineering literature. Johnston et al. reported that the partial molar volume of naphthalene reaches -10^3 mL/mol (negative) in a highly compressible supercritical fluid.[54] "The small compressible fluid molecules condense about the highly polarizable solute, such that the local density is much larger than the bulk solvent density".

Levy et al. used the azo dye 5-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC) as a solvatochromic probe.[55] They measured wavelength shifts for carbon dioxide/modifier mixtures ranging from 0.5 to 10 mole%. The modifiers studied were

propylene carbonate, 1-hexanol, dimethylacetamide, acetonitrile, 1-propanol, dimethylsulfoxide, tetrahydrofuran, methanol, and methylene chloride. The pressure was held at 348 atm (5100 psi) and the temperature was held at 40°C. They reported that all of the modifiers studied exhibited positive solvatochromism (i.e. shift to longer, lower energy wavelengths) as modifier concentrations were increased, and that the magnitude of the shift was dependent on the identity of the modifier. The largest wavelength shift reported (40-60 nm) was obtained with propylene carbonate. The major problem with this work is that no attempt was made to ensure that a single phase system was being studied. Other work has shown the limited solubility of acetonitrile and propylene carbonate in carbon dioxide would have resulted in at least a two phase system at the concentrations reported in this work.[46,49] The large wavelength shifts obtained for propylene carbonate reflect measurement of the wavelength of maximum absorption for pure propylene carbonate.

Yonker et al. measured the solvent strength of CO_2 containing various concentrations of methanol using the dye ET(30).[43] With the addition of 9.5% methanol to CO_2, the solvent strength increased to nearly that of tetrahydrofuran, and rose to more than half the solvent strength of pure methanol. Although their data indicated a large change in solvent strength, they concluded that the addition of small amounts of modifiers did not drastically alter the solvent strength, although large changes in retention were noted. These changes in retention were instead attributed to the deactivation of the stationary phase.

Johnston et al. studied acetone, methanol, ethanol, and n-octane as modifiers in CO_2 using phenol blue as a solvatochromic dye.[54] They concluded that preferential solvation of the dye by the modifier was responsible for the observed shift in wavelengths. The dye was experiencing a higher solvent strength than was present in

the bulk of the fluid due to clustering of the modifier around the dye.

Berger et al. have done work using Nile Red as a solvatochromic dye. They studied methanol, acetonitrile, methylene chloride, and tetrahydrofuran in CO_2, Freon-13, and Freon-23.[56] They found that the addition of methanol appeared to drastically affect the solvent strength of the mixture. When methanol, acetonitrile, methylene chloride, and tetrahydrofuran were studied in liquid CO_2, methanol caused the largest shift in the wavelength of maximum absorption, followed by acetonitrile > methylene chloride > tetrahydrofuran. Based on the shifts in wavelength, 5% methanol in CO_2 is equivalent to 10% acetonitrile and >20% tetrahydrofuran in CO_2. They also found that the greater the difference in solvent strength of the two fluids (modifier-fluid) the more drastic the solvent strength change. This observation again supports the theory of clustering.

Berger et al. also compared composition, pressure, and density effects for methanol in CO_2.[50] They measured the capacity factor (k') of PTH-aminobutyric acid (Aba) at constant composition while changing the density (pressure), and at constant density while the methanol composition was varied from 1.2-22.5%. They found that by changing the pressure from 76 bar to 276 bar, the capacity factor decreased from k'=30 to k'=10. By changing the methanol composition, k' changed from k'=25 to k'=2. Therefore, changing the composition of the mobile phase had a much more drastic effect on k' than was seen with a change in density or pressure.

As the proceeding literature review demonstrates, there is a great deal of conflicting interpretations of data regarding the mechanism of action of modifiers. It is clear the role of modifiers should not be divided into what occurs at low modifier concentrations versus what occurs at higher modifier concentrations. At even the lowest levels, modifiers appear to affect both the stationary and the mobile phase.

Ternary Phases

Low concentrations of very polar compounds added to a modifier have been reported in the literature. When a compound is added to the mobile phase via the modifier it is referred to as an additive.[57] Acetic, citric, chloroacetic, dichloroacetic, trichloroacetic, and trifluoroacetic acid have been studied as acidic additives,[58] and tetrabutylammonium hydroxide and isopropylamine[50] have been studied as basic additives.

Berger et al. separated mono-, di-, and trihydroxybenzoic acids on cyanopropyl, diol, and sulfonic acid columns.[57] When pure carbon dioxide was used none of the acids eluted from the columns. When methanol was added to the mobile phase, some of the acids eluted, but with very bad peak shapes. The addition of citric acid to the mobile phase allowed for the separation of ten mono-, di-, and trihydroxybenzoic acids in approximately 1.5 minutes with much improved peak shape. They concluded that the most predominant action of additives is to improve the solubility of the solute in the mobile phase and to suppress the ionization of very polar solutes. They also concluded that very polar additives interact with the active sites on a column so strongly that they can not be removed by the solutes, therefore serving to further deactivate the column. The same group also studied the elution of bases containing multiple amine groups with pKa's greater than 9 using basic additives.[50] They observed that ortho-, meta-, and para- phenylenediamines and benzyl-, dibenzyl-, and tribenzylamine were difficult to elute with good peak shape and in a reasonable amount of time using pure or methanol modified supercritical fluids. They explored seven different stationary phases, and three fluids. The fluids explored were carbon dioxide, Freon-23, and Freon-13, and these fluids were explored pure, modified with methanol, and with two basic additives

in the methanol. When pure fluids were used either the solutes did not elute, or they eluted with very poor peak shape. When modifier was added to the system the solutes eluted and peak shapes were improved, but symmetric peaks were not obtained. When TBAOH was used as an additive, peak shapes generally improved, but in some cases the peak shape was actually degraded. Isopropylamine produced much more symmetric peaks when it was used as an additive as compared to TBAOH. The same mechanisms of stationary phase deactivation, improved solubility, and suppression of ionization appear to occur with basic additives as well as acidic additives.

Mobile Phase Gradients

Gradient elution in gas chromatography refers to increasing the temperature during the chromatographic run. In liquid chromatography gradient elution refers to changing the composition of the mobile phase during the course of the separation. In supercritical fluid chromatography, gradient elution refers to changing the composition of the mobile phase with time. Therefore, a pressure, density, or temperature program would not be considered gradient elution in SFC.

The gradient separation of PTH-amino acids, accomplished using a two-pump system, has been reported.[59] The instrumentation consisted of an HP 1082B to pump the CO_2 and a syringe pump to deliver the modifier. All work was done at constant pressure, while the composition of the mobile phase was changed with time. In this work twenty-four PTH-amino acids were separated in approximately eighteen minutes. The separation of triazine and triazole herbicides on the same system was also reported.[60] A separation of eight of these compounds in under six minutes was accomplished through the use of a methanol gradient. A mobile phase composition of approximately 33% methanol was required for the last eluting compound.

A two-pump system that also allowed for pressure programming as well as mobile phase composition programming has been reported.[61] Using this system Triton X-114 and Triton X-165 were chromatographed. A comparison of Triton X-114 chromatographed isobarically with constant mobile phase composition, isobarically with a mobile phase gradient, and pressure programmed with constant mobile phase composition showed that peak shape was improved when pressure was programmed with a constant mobile phase composition.

The separation of caprolactone diol oligomers using acetonitrile as a modifier in CO_2 has been reported.[62] The acetonitrile composition was ramped from 10 to 60 % (v/v) during the course of the run. The resolution of approximately twenty oligomers was achieved. Based on the solubility data available for acetonitrile in CO_2 and on the calculated values for the critical parameters, it can be seen that during the course of the separation there is a transition from a supercritical fluid to a subcritical fluid. However, this transition did not adversely affect the chromatography.

Styrene oligomers have been separated using 1,4-dioxane as a modifier in a variety of pure fluids.[63] 1,4-Dioxane was added to propane, hexane, butane, pentane, and carbon dioxide in varying percentages in order to achieve separation of the styrene oligomers. In all cases, satisfactory separations of the oligomers were obtained, but the amount of dioxane required to achieve these separations varied depending on the pure fluid. For example, a gradient of 30 to 49 % dioxane in propane was used, while similar separation was achieved in hexane with a 5 to 20 % gradient of dioxane.

In this work a separation of seven azaarenes was developed. Varying concentrations of modifier were used, and the observed trends will be discussed. This work was undertaken because an attempt to reproduce previously published data indicated that the reported results were inaccurate. Therefore, the separation of these

compounds was explored again in order to more accurately observe the effect of modifier on their separation. The conditions necessary for analyzing seven nitrogenous bases, several of which are purine and pyrimidine-based drugs, by means of sub- and supercritical fluid chromatography were also determined. A two-pump system was used, based on the HP 1082B and the Suprex syringe pump. Elution of all of the compounds of interest was accomplished by varying the modifier concentration, and by addition of an additive to the modifier. In addition, the analysis of HCl salts, which are not soluble in carbon dioxide, was explored using on-line SFE/SFC. The goal of this work was to extend the polarity range of compounds that can be analyzed by supercritical fluid chromatography.

Experimental

Azaarenes

A Hewlett Packard 1082B liquid chromatograph modified for use with supercritical fluids was used for this work. This instrument is equipped with an HP 79875 variable wavelength detector (8 μL cell) and a Tescom back pressure regulator. This instrument has two reciprocating pumps, A and B, and allows for the use of modified fluids by utilizing premixed tanks. For this work pump A delivered pure carbon dioxide, and pump B delivered 2 % methanol in carbon dioxide. The proportion of A to B determined the composition of the mobile phase. Carbon dioxide and methanol-modified carbon dioxide were purchased in aluminum cylinders from Scott Specialty Gases (Plumsteadville, PA). The flow rate was held constant at 2 mL/min, and the backpressure regulator was set to maintain the pressure in the column at 330 bar. The oven temperature was held at 50°C. The composition of the mobile phases used were pure CO_2 (100% A), 0.6% methanol/CO_2 (70% A/30% B), 0.8%

methanol/CO_2 (60% A/40% B), and 1.0% methanol/CO_2 (50% A/50%B).

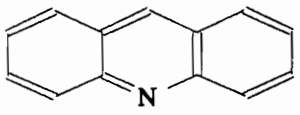
A Spherisorb amino column (250 x 4.6 mm, 5 μm particles) was used for these separations. The column was conditioned before use in order to remove the packing solvent by pumping CO_2 at high temperature (100°C) and pressure (5000 psi) through the column for 24 hours. Acridine, 5,6-benzoquinoline, 7,8-benzoquinoline, pyridine, quinoline, quinoxoline, and quinazoline, whose structures are shown in Figure 36, were purchased from Aldrich. Solutions of these compounds were prepared in methylene chloride at 0.1 μg/μL. Typically, 1-2 μL of mixture was introduced onto the column for UV detection.

Nitrogenous Bases

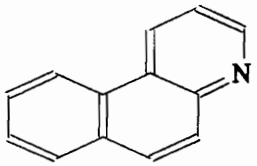
A Suprex (Pittsburgh, PA) Model 200A supercritical fluid chromatograph equipped with a flame ionization detector was employed for all studies with pure CO_2. For mobile phase gradient studies, a Hewlett Packard 1082B liquid chromatograph modified for use with supercritical fluids and equipped with an HP 79875 variable wavelength UV detector (8 μL cell) was used to deliver CO_2 to the column. A Suprex syringe pump was used as a programmable flow (5-2000μL/min) liquid modifier pump. Modifier and supercritical CO_2 were dynamically mixed in a T-mixing chamber (Lee Co., West Brook,CT) at 60°C. The modified CO_2 was then passed to the column (also maintained at 60°C), through a heat exchanger, to the UV detector, and finally to the back pressure regulator, which was fixed at 3000 psi. The schematic for this system is shown in Figure 37.

Keystone Scientific (Bellefonte, PA) Deltabond cyanopropyl columns (250 x 1 mm, 250 x 4.6 mm, 5 μm particles) were used. Carbon dioxide was obtained in aluminum cylinders with 1500 psi helium headspace from Scott Specialty Gas

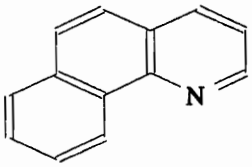
Figure 36



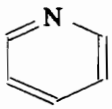
acridine



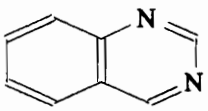
5,6-benzoquinoline



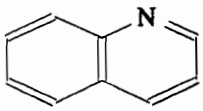
7,8-benzoquinoline



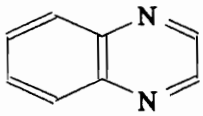
pyridine



quinazoline



quinoline



quinoxaline

Figure 36: Structures of the azaarenes separated.

Figure 37

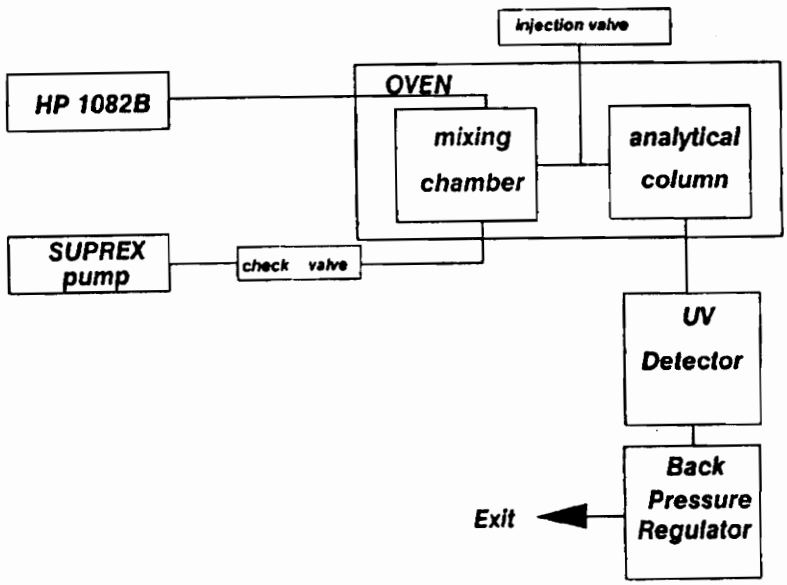


Figure 37: Schematic for the two-pump system.

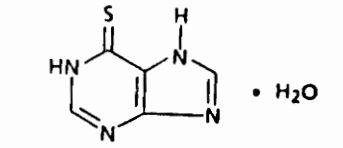
(Plumsteadville, PA). Methanol (Baker) and methanol containing 0.001 M tetrabutylammonium hydroxide served as modifiers. The nitrogenous bases were provided by Burroughs Wellcome. The compounds and their corresponding structures are shown in Figure 38. Zidovudine, also known as AZT, is the drug being used to treat AIDS. Pseudoephedrine·HCl is the active ingredient in Sudafed. Nitrogenous bases were dissolved in HPLC-grade methanol at concentrations of approximately 1 μg/μL, and typically 3-5 μL of mixture was applied to the column for UV detection. For triprolidine and pseudoephedrine, both hydrochloride salts, a static extraction with CO_2 was performed by adding a molar excess of tetrabutylammonium hydroxide to 3 mL of 1 μg/μL solutions of both compounds in water. Static extractions of the free bases were then carried out using a system previously described.[31]

Results and Discussion

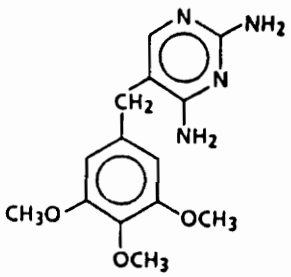
Azaarenes

Initially, 1 μL of the azaarene mixture was injected onto the amino column with 100% CO_2 as the mobile phase. After approximately seventy minutes only four peaks could be observed, with extremely poor peak shape. This column was brand new, and had been conditioned as previously described. Ashraf et al. reported the separation of the same set of compounds on an amino column using 100 % CO_2 as the mobile phase, and was able to elute all of the compounds, although peak shape and resolution were generally poor.[14] This work was probably performed on a column that had previously been deactivated through the use of modifiers and by compounds binding to the active sites. The deactivation of the column allowed the compounds to elute, although the run took forty minutes, but the poor peak shape indicated that a stronger mobile phase was required.

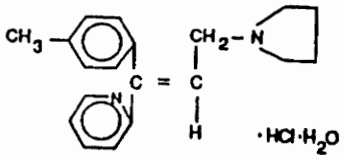
Figure 38



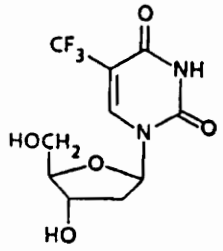
Mercaptopurine



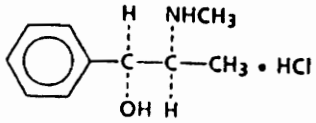
Trimethoprim



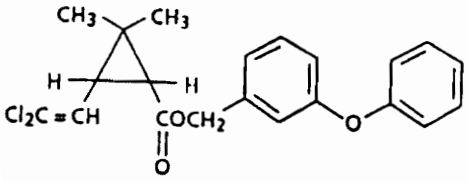
Triprolidine



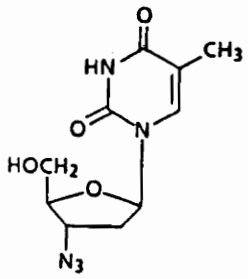
Trifluridine



Pseudoephedrine



Permethrin



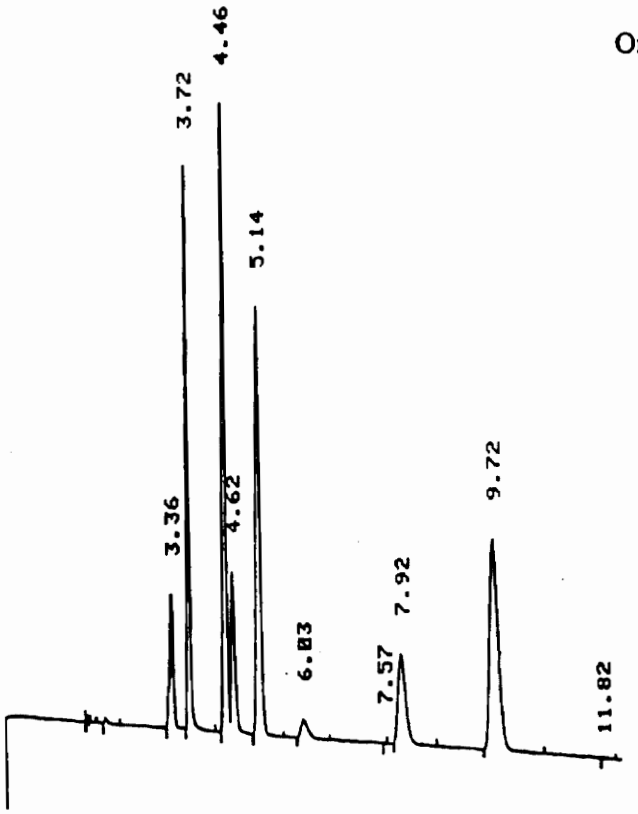
Zidovudine

Figure 38: Structures of the nitrogenous bases separated.

The methanol content in the mobile phase was increased to 0.6%, and the mixture was injected again. The chromatogram is shown in Figure 39. All seven components elute with baseline resolution in less than ten minutes. Note the order of elution of 7,8-benzoquinoline and quinazoline. These two compounds elute at 4.5 and 4.6 minutes, respectively. Next the methanol content was increased to 0.8% and the separation obtained is shown in Figure 40. The retention times of all of the components decreased. The latest eluting compound, 5,6-benzoquinoline, eluted at 9.7 minutes with 0.6% methanol, and at 8.1 minutes with 0.8% methanol. However, with 0.8% methanol, baseline resolution of all components is no longer achieved - 7,8-benzoquinoline and quinazoline now coelute. Finally the methanol content in the mobile phase was increased to 1.0%. Figure 41 shows the chromatogram obtained under these conditions. 5,6-Benzoquinoline now elutes at 6.6 minutes, as compared to 8.1 minutes with 0.8% methanol. The most interesting aspect of this separation is that once again 7,8-benzoquinoline and quinazoline are baseline resolved, however the order of elution has reversed.

This reversal in elution order may be due to two things. First, although these separations were carried out at constant pressure, they were not carried out at constant density, since changing the methanol content changes the density of the mobile phase. The methanol is also serving to deactivate the stationary phase and increase the solvent strength of the mobile phase, thereby changing the partition coefficient for these compounds. The capacity factors for all of the compounds at the three different methanol concentrations are shown in Table XX. As can be seen from the Table, the capacity factor of 7,8-benzoquinoline decreased by approximately 15% when the methanol content was increased from 0.8% to 1.0%, while the capacity factor of quinazoline decreased by 25%. Quinazoline is apparently much more affected by

Figure 39

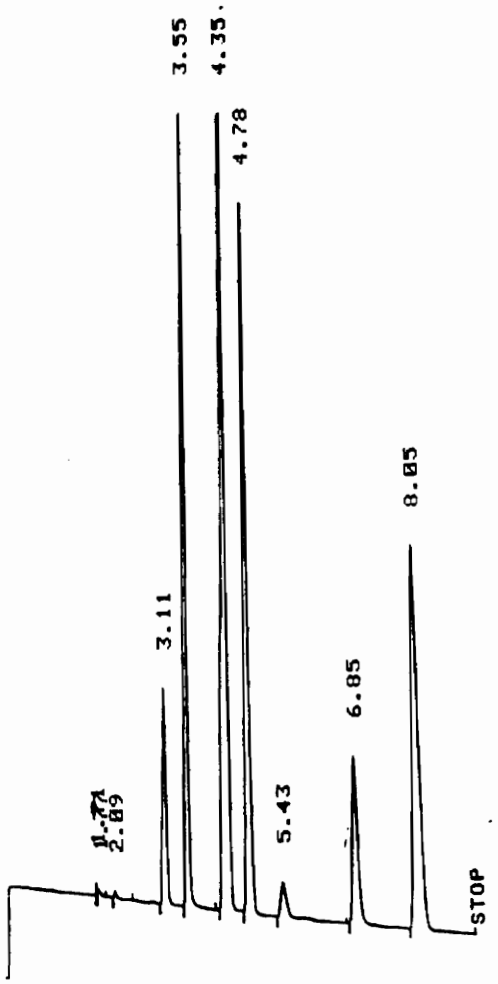


Order of Elution:

1. quinoxaline
2. pyridine
3. quinazoline
4. 7,8-benzoquinoline
5. quinoline
6. impurity
7. acridine
8. 5,6-benzoquinoline

Figure 39: Separation of azaarenes on an amino column with 0.6% methanol in carbon dioxide at 50°C and 330 bar, flow rate= 2 mL/min, with UV detection at 254 nm.

Figure 40

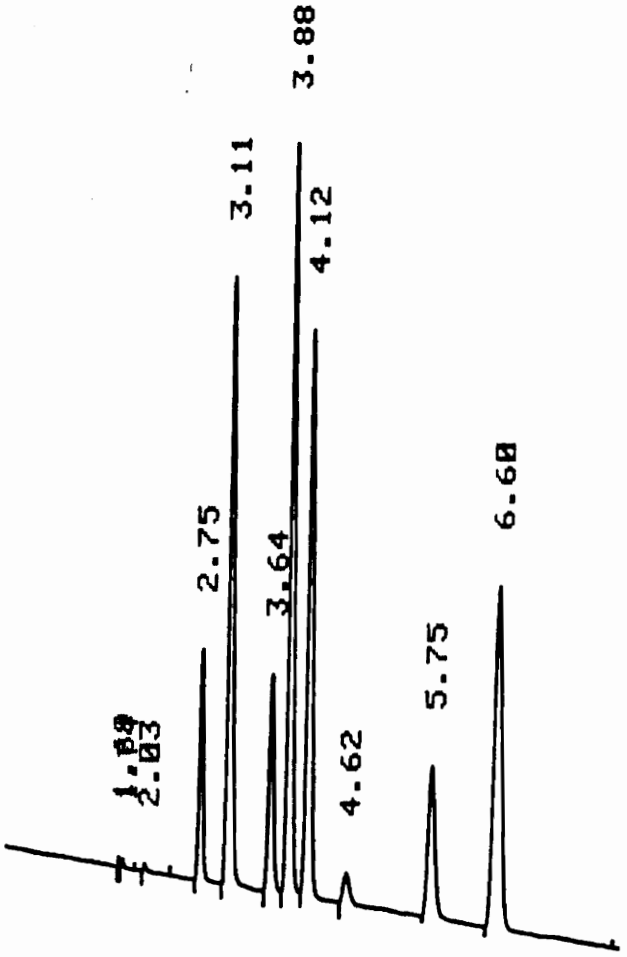


Order of Elution:

1. quinoxaline
2. pyridine
3. quinazoline/7,8-benzoquinoline
4. quinoline
5. impurity
6. acridine
7. 5,6-benzoquinoline

Figure 40: Separation of azaarenes on an amino column with 0.8% methanol in carbon dioxide at 50°C and 330 bar, flow rate= 2 mL/min, with UV detection at 254 nm.

Figure 41



Order of Elution:

1. quinoxaline
2. pyridine
3. 7,8-benzoquinoline
4. quinazoline
5. quinoline
6. impurity
7. acridine
8. 5,6-benzoquinoline

Figure 41: Separation of azaarenes on an amino column with 1.0% methanol in carbon dioxide at 50°C and 330 bar, flow rate= 2 mL/min, with UV detection at 254 nm.

Table XX

Capacity factors for azaarenes for 0.6, 0.8, and 1.0% methanol in carbon dioxide.

	0.6 %	0.8%	1.0%
quinoxaline	0.66	0.49	0.35
pyridine	0.84	0.70	0.53
7,8-benzoquinoline	1.21	1.08	0.91
quinazoline	1.29	1.08	0.79
quinoline	1.54	1.29	1.03
acridine	2.92	2.28	1.83
5,6-benzoquinoline	3.81	2.85	2.25

changes in the mobile phase strength. When the structures of quinazoline and 7,8-benzoquinoline are compared, a possible explanation for this behavior becomes apparent. Quinazoline has two nitrogens that may interact with the stationary phase. As the mobile phase strength is changed, the strength of these interactions is more drastically affected than the interactions between 7,8-benzoquinoline and the stationary phase. If the structure of 7,8-benzoquinoline is examined, it can be seen that the nitrogen is in a very sterically hindered position, and is therefore unlikely to be able to interact strongly with the stationary phase. This theory is also supported if the retention of acridine and 5,6-benzoquinoline are compared to the retention of 7,8-benzoquinoline. Acridine and 5,6-benzoquinoline are the last eluting compounds, while 7,8-benzoquinoline elutes third (or fourth), indicating that the nitrogen is less accesible to the stationary phase and retention is controlled mainly by solubility in the mobile phase for 7,8-benzoquinoline. The trends observed for the separation of the azaarenes employing modifier correspond well to those reported in the literature. The capacity factor of all of the compounds decreased with increasing methanol content, and peak shapes were generally improved. The work reported by Ashraf et al. demonstrated the need for care with respect to column deactivation caused by the use of modifiers and permanently retained species.[51] They reported that all of the azaarenes eluted with pure carbon dioxide, which does not occur. Since the conditions reported by Ashraf et al. were replicated, with the exception of the use of a new column in this work, it is probable that previous samples and modifiers/additives were permanently adsorbed onto their column, thereby leading to the inaccurate results they reported.

Nitrogenous Bases

The goal of this research was to determine the conditions, if any, under which the compounds shown in Figure 38 could be chromatographed using CO_2 as the major

component of the mobile phase. With pure CO_2 as the mobile phase at a temperature of 60 °C, all of the compounds of interest were injected singly on the microbore cyano column. The pressure was initially held at 80 atm for three minutes, then ramped to 450 atm in five minutes and held there for fifteen minutes. If no FID response was observed within twenty minutes of injection, it was assumed that the compound did not elute from the column. Under these conditions, none of the compounds that were injected eluted from the column, indicating that pure CO_2 was not a strong enough mobile phase for these compounds.

In order to determine the strength of the mobile phase that was required to elute these compounds, the mobile phase was modified with methanol using the two-pump system previously described. A constant flow rate of CO_2 at 2 mL/min was mixed with methanol pumped at 500 μL/min. The oven temperature was maintained at 60 °C, and the compounds were injected singly. The UV signal was monitored at 254 nm. Under these conditions mercaptopurine, trifluridine, zidovudine, and permethrin eluted with the solvent front, indicating that the mobile phase was so strong that there was little interaction between the solute and the stationary phase. However, trimethoprim eluted as a broad peak at approximately three minutes and tailed out to nine minutes, as is shown in Figure 42. The hydrochloride salts, pseudoephedrine and triprolidine, did not elute with methanol added to the mobile phase.

Next a solution of the four components that eluted with the solvent front was prepared. The percentage of methanol in the mobile phase was decreased from the previous single component injections so that the compounds would interact with the stationary phase. A separation of these four compounds was developed using a methanol gradient, and is shown in Figure 42. As can be seen from the Figure, the separation is rapid, occurring in less than five minutes. Although the mobile phase

Figure 42

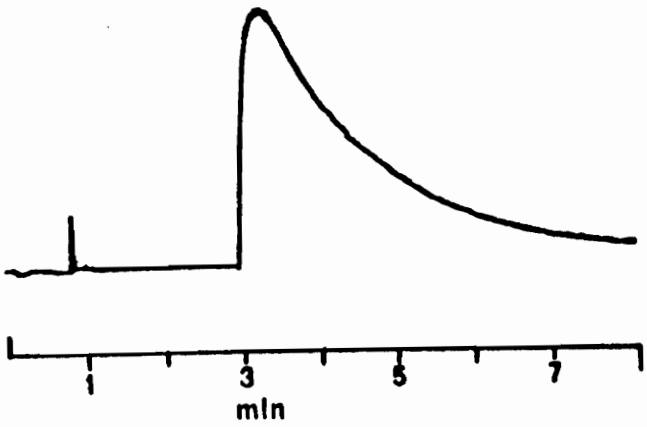


Figure 42: Chromatogram showing the tailing of trimethoprim with methanol in carbon dioxide. Flow Rate= 2 mL/min carbon dioxide, 500 μL/min methanol. Column: Delatbond CN; Temperature: 60°C; UV Detection at 254 nm.

Figure 43

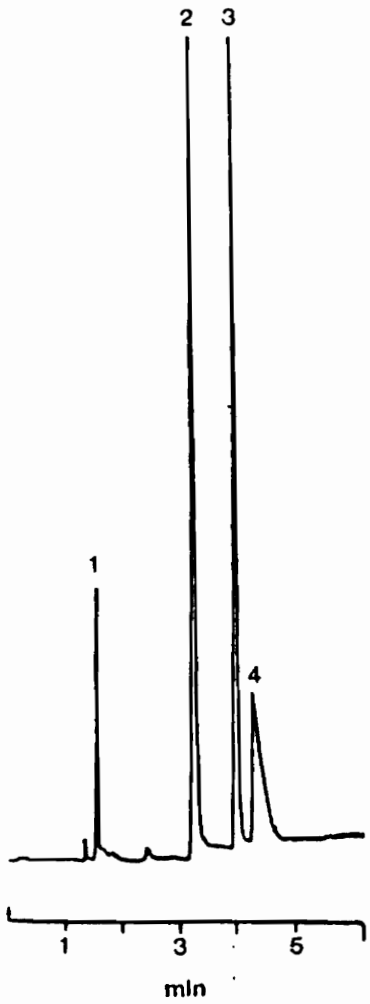


Figure 43: Separation of (1) permethrin, (2) zidovudine, (3) trifluridine, (4) mercaptopurine at a flow rate of 2 mL/min of carbon dioxide, 150 μL/min methanol for 1.5 min, up to 450 μL/min in 2.0 min, hold at 450 μL/min. Column: Deltabond CN. Oven temperature:60°C; UV detection at 254 nm.

passes into a subcritical state during the separation, no detrimental effects are seen. The baseline rise is due to the increasing modifier concentration. The last peak to elute, mercaptopurine, is a less than symmetric peak, indicating that the solubility of this compound in the mobile phase is poor. Column equilibration time with the gradient system varied depending on how much methanol was added to the mobile phase. Changing retention times for replicate injections were taken as an indication of a non-equilibrated column.

In order to elute trimethoprim from the column without severe tailing, 0.001 M tetrabutylammonium hydroxide was added to the methanol. Upon addition of this base to the mobile phase, trimethoprim eluted cleanly from the column, as is shown in Figure 44. Both the retention time and the peak shape have changed, as compared to the elution of trimethoprim with no base present in the mobile phase. The tetrabutylammonium hydroxide may be suppressing the protonation of trimethoprim and binding to active sites on the stationary phase. Figure 45 shows the separation of permethrin, zidovudine, and trimethoprim employing 0.001 M tetrabutylammonium hydroxide as an additive. Note the tailing of trimethoprim, indicating that the strength of the mobile phase may need to be increased further in order to elute this compound as a symmetric peak.

Pseudoephedrine and triprolidine did not elute from the cyano column upon addition of base to the mobile phase. In order to chromatograph these compounds, the free bases were produced, and the free bases were introduced to the chromatographic column in an on-line SFE/SFC experiment. Individual solutions of triprolidine and pseudoephedrine were prepared at 1 mg/mL in water. An 8 mL extraction vessel (1 cm i.d. x 10 cm in length) was loaded with 3 mL of either the triprolidine or pseudoephedrine solution, and a molar excess of tetrabutylammonium hydroxide. The

Figure 44

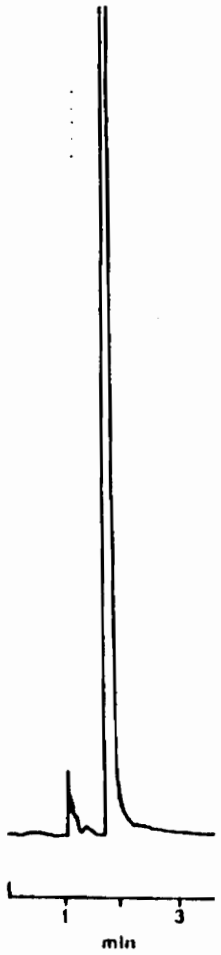


Figure 44: Chromatogram of trimethoprim with base added to the methanol modifier. Flow= 2 mL/min carbon dioxide, 500 μL/min 0.001 M tetrabutylammonium hydroxide in methanol. Column: Deltabond CN; Oven temperature: 60°C; UV detection at 254 nm.

Figure 45

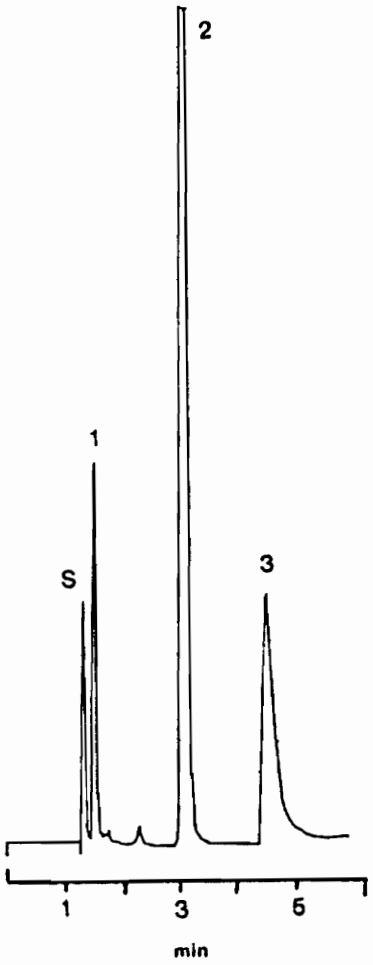


Figure 45: Separation of (1) permethrin, (2) zidovudine, and (3) trimethoprim using tetrabutylammonium hydroxide in the mobile phase. Flow rate = 2 mL/min carbon dioxide, 100 μL/min methanol/TBAOH for 2.0 min, up to 500 μL/min in 4.0 min, and hold at 500 μL/min. Column: Deltabond CN; Oven temperature: 60°C; UV detection at 254 nm.

extraction vessel was designed to minimize the transport of unsolvated water. Additional information about the extraction vessel and valving scheme used has been reported.[63] The extractions were carried out for 15-20 minutes at 5000 psi and 50°C, after which 20 μL of the extract was transferred to the head of the chromatographic column. The mobile phase consisted of CO_2/methanol/tetrabutylammonium hydroxide, and a mobile phase gradient was used. The chromatograms obtained are shown in Figures 46A and B.

Conclusions

The results show that the addition of modifier and sometimes an additive to CO_2 is often necessary if pharmaceuticals are to be eluted. The addition of additives can greatly influence chromatographic retention and peak shape through suppression of ionization and deactivation of the column. Although the purines and pyrimidines in the mixtures that were separated are not likely to be found together, the separations indicate the conditions necessary to chromatograph these types of compounds. A separation of a parent compound and its metabolites would be a more meaningful application. However, the fact that metabolites are often more polar than the parent compounds further reinforces the need for modifiers and additives.

The separations of the azaarenes clearly demonstrate the trends of decreasing capacity factor and improved peak shape that often occur with the addition of modifier. The differences between the azaarene separations reported here and those previously reported indicate that care must be taken when modifiers are used in order to obtain reproducible separations.

Figure 46

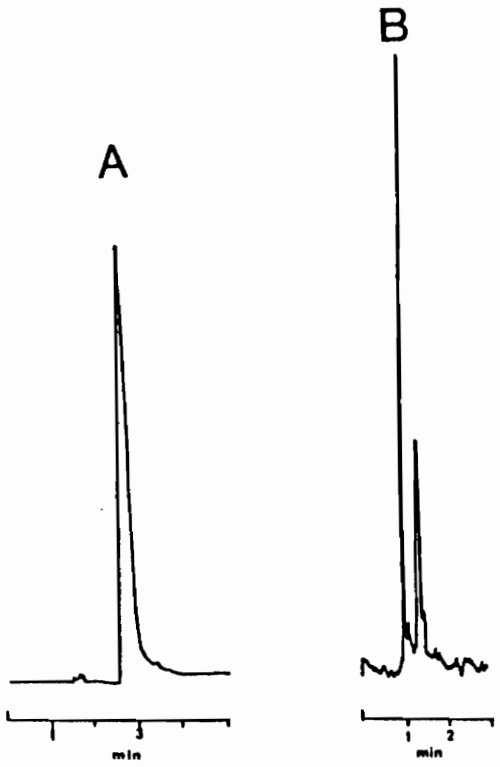


Figure 46: SFE/SFC of (A) triprolidine and (B) pseudoephedrine. Extraction Conditions: Pressure = 5000 psi (340 bar); Temperature = 50°C; Extraction Time = 15-20 minutes. Amount of extract transferred to column = 20 μL. Chromatographic Conditions: Flow Rate = 2 mL/min carbon dioxide, 150 μL/min methanol/TBAOH for 1.0 min, 150-450 μL/min in 3.0 min, hold at 450 μL/min; Column: Deltabond CN; Temperature:60°C; UV Detection at 290 nm for (A), and 254 nm for (B).

Chapter 7

Conclusions and Future Work

Conclusions

Many aspects of sorbent and solid surface traps for off-line SFE were evaluated. For the solid phase extraction tubes it was found that the choice of sorbent was critical only for the most volatile compounds where sorptive interactions were required for efficient trapping. The inability of some of the test compounds to be efficiently rinsed from the sorbent traps lead to poor recoveries. It was also found that the rinse solvent and the prerinse treatment of the traps was critical in obtaining satisfactory recoveries. The inability to heat these traps (in a controlled or automated manner) during rinsing also may have contributed to poor rinsing. For both the sorbent traps and the frits it was found that pretreatment with carbon tetrachloride significantly affected recoveries.

When the extraction system with an automated rinsing system was used (Chapter 2) with the ability to heat the trap during the rinse, the choice of rinse solvent becomes somewhat less critical. The rinse solvent must ofcourse be able to solvate the compounds of interest, but the added thermal desorption also may lead to more efficient rinsing than would be achieved at room temperature, as was the case when the solid phase extraction tubes were used.

The addition of modifier was found to have dramatic effects on recovery of the test mix components. Mobile phases consisting of 1% and 2% methanol behaved in a similar manner. At these concentrations methanol lead to partitioning of the more volatile components between the stationary phase and the mobile phase. Of the nonvolatile compounds, decanoic acid was most affected by the addition of methanol.

Methanol was shown to disrupt sorptive interactions between the test mix components and the stationary phase.

Mobile phases consisting of 4% and 8% methanol behaved similarly, and exhibited different behavior from 1% and 2% methanol, especially over the lower temperature range (5-50°C). While 1% and 2% methanol in the mobile phase appear to be behaving mainly as an organically doped gas, 4% and 8% methanol allow for condensed methanol on the stationary phase. This condensed fluid on the phase allows for mechanical movement of compounds dissolved in methanol through the trap. There is evidence in the trapping results for the volatile compounds on the stainless steel trap that a thin film of methanol can form with 1% methanol as the mobile phase. However, when the methanol content was increased above 1% the film was no longer stable and could be mechanically pushed from the trap.

The study of real world samples using solid surface and sorbent traps with pure and modified fluids shows that these traps can be efficiently and easily utilized in off-line SFE. The active components of a drug formulation were trapped on stainless steel beads. Efficient trapping, rinsing, and release of these highly basic compounds was achieved.

The extraction of PCBs from river sediment was accomplished with the use of a sorbent trap. Based on the behavior of the solid surface traps, a solid surface trap could have been efficiently used for these compounds. Although the effects of toluene on trapping efficiency were not specifically studied, at the low concentration used the effect on trapping of fairly nonvolatile compounds should be minimal.

The use of modifiers is essential if SFE is going to be successfully applied to environmental, pharmaceutical, food and other real world samples. The polarity of pure carbon dioxide is insufficient to solvate many compounds of interest. Based on

the drastic effects that are observed with modifiers in both SFC and SFE, the role of modifiers is of great interest. This work has shown that sorbent and solid phase traps can be successfully used for off-line SFE for pure and modified systems as long as trap temperature is considered.

Future Work

There is a great deal of work remaining to be done in order to completely characterize sorbent and solid surface traps for off-line SFE. The effects of modifiers other than methanol remain to be explored. Further experiments to determine the specific interactions of modifier/analyte/stationary phase are necessary. The study of more strongly acidic and basic compounds, as well as polar nonvolatiles compounds also remains to be done.

References

1 Hawthorne,S.B.; Miller,D.J. *Anal .Chem.* **1987**,59,1705-1708

2 Lopez-Avila,V.; Dodhiwala,N.S.; Beckert, W.S. *J.Chrom. Sci.* **1990**,28,468-476

3 Alexandrou,N.; Pawliszyn,J. *Anal .Chem.* **1989**,61,2770-2776

4 Nam,S.K.; Kapila,S.; Viswanath,D.S.; Clevenger,T.E.; Johansson, J.; Yanders,A.F. *Chemosphere* **1989**,19,33-38

5 Nam,S.K.; Kapila,S.; Yander,A.F.; Puri,R.K. *Chemosphere* **1990**,20,871-880

6 Wright,B.W.; Wright,C.W.; Gale,R.W.; Smith,R.D. *Anal .Chem.* **1987**,59,38-44

7 McNally,M.E.; Wheeler,J. *J .Chrom. Sci.* **1989**,27,534-539

8 Schantz,M.M; Chesler,S.N. *J .Chromatogr.* **1986**,363,397-401

9 Hedrick,J.L.; Taylor,L.T. *HRC&CC* **1990**,13,312-316

10 Mulcahey,L.J.; Hedrick,J.L.; Taylor,L.T. submitted to *Anal .Chem.*

11 Burdick and Jackson High Purity Solvent Guide, Second Edition, p.26 and 82.

12 Snyder,L.R.; Kirkland,J.J., in *Introduction of Modern Liquid Chromatography*; John Wiley & Sons, Inc.: New York 1979;p.131

13 Francis,A.W. *J .Phys .Chem.* **1954**,58,1099-1114

14 Bristow,P.A.; Brittain,P.N.; Riley,C.M.; Williamson,B.F. *J.Chromatogr.* **1977**,131,57-64

15 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L. Lee and K.E.Markides; Chromatography Conferences Inc., Provo, Utah 1990; p.320

16 Snyder,L.R.; Kirkland,J.J., in *Introduction to Modern Liquid Chromatography*; John Wiley & Sons, Inc., New York 1979; p.272-273

17 Janssen, J.G.M.; Schoenmakers, P.J.; Cramers, C.A. *HRC & CC* **1989**,12,645-651

18 Strubinger, J.R.; Song, H.; Parcher, J.F. *Anal .Chem.* **1991**,63,98-103

19 Strubinger, J.R.; Song, H.; Parcher, J.F. *Anal .Chem.* **1991**,63,104-108

20 Snyder,L.R.; Kirkland,J.J., in *Introduction to Modern Liquid Chromatography*; John Wiley & Sons, Inc., New York 1979; p.276-277

21 Mulcahey, L.J.; Taylor, L.T. *HRC & CC* **1990**,13,393-395

22 Personal Communication, Joe Hedrick

23 Capriel,P.; Haisch, A.; Khan, S.U. *J .Agric. Food. Chem.* **1986**,34,70-73

24 Hawthorne, S.B.; Miller, D.J. *J .Chrom .Sci* .**1986**,24,258-264

25 Hawthorne, S.B.; Krieger, M.S.; Miller, D.J. *Anal .Chem.* **1989**,61,736-740

26 Hawthorne, S.B.; Miller, D.J. *J .Chromatogr.* **1987**,403,63-76

27 Wright, B.W.; Wright, C.W.; Fruchter, J.S. *Energy & Fuels* **1989**,3,474-480

28 Raymer, J.H.; Pellizzari, E.D.; Cooper, S.D. *Anal .Chem.* **1987**,59,1043-1048

29 Raymer, J.H.; Pellizzari, E.D.; Cooper, S.D. *Anal .Chem.* **1987**,59,2069-2073

30 Brady, B.O.; Kao, C-P.C.; Dooley, K.M.; Knopf, F.C.; Gambrell, R.P. *Ind. Eng. Chem .Res.* **1987**,26,261-268

31 Dooley, K.M.; Ghonasgi, D.; Knopf, F.C.; Gambrell, R.P. *Environ. Prog.* **1990**,9,197-203

32 Onuska, F.I., Terry, K.A. *HRC &CC* **1989**,12,527-531

33 Onuska, F.I., Terry, K.A. *HRC &CC* **1989**,12,357-361

34 Nielen, M.W.F.; Sanderson, J.T.; Frei, R.W.; Brinkman, U.A. *J. Chromatogr.***1989**,474,388-395

35 Janda, V.; Steenbeke, G.; Sandra, P. *J .Chromatogr.* **1989**,479,200-205

36 McNally, M.E.; Wheeler, J.R. *J. Chromatogr.* **1988**,435,63-71

37 Burk, R.C.; Kruus, P. *J .Environ .Sci .Health.* **1990**,B25,553-567

38 Lohleit, M.; Hillman, R.; Bachmann, K. *Fresenius J. Anal .Chem.* **1991**,339,470-474

39 Schwartz, H.E.; Levy,J.M.; Guzowski,J.P. in *Modern Supercritical Fluid Chromatography*, ed. C.M.White; Huthig, NewYork, 1988; p.135-149

40 King,J.W. *J .Agric. Food .Chem.* **1989**,37,951

41 Hyatt,J.A. *J .Org .Chem.* **1984**,49,5097-5101

42 Sigman,M.E.; Lindley,S.M.; Leffler,J.E. *J .Am .Chem. Soc.* **1985**,107,1471-1472

43 Yonker,C.R.; Frye,S.L.; Kalnarf,D.R.; Smith,R.D. *J .Phys. Chem.* **1986**,90,3022-3026

44 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.14

45 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.100

46 Maguire,K.L.; Deniszyn,R.B., in *Modern Supercritical Fluid Chromatography*, ed. C.M.White; Huthig, New York 1988; p.45-57

47 Kuk,M.S.; Montagna,J.C., in *Chemical Engineering at Supercritical Conditions*, eds. M.E.Paulaitus, J.M.L.Penninger, and R.P.Gray; Ann Arbor Science, Ann Arbor, Michigan 1983; p.101-111

48 Panagiotopoulos,A.Z.; Reid,R.C., in *Supercritical Fluids: Chemical and Engineering Principles and Applications*, eds. T.G.Squires and M.E.Paulaitis; American Chemical Society, Washington,D.C. 1987; p.115-129

49 Page,S.H.; Goates,S.R.; Lee,M.L., in the proceeding of the International Symposium on Supercritical Fluid Chromatography and Extraction, January 1991; p.17-18

50 Berger,T.A.; Deye,J.F. *Anal .Chem.* **1990**,62,1181-1185

51 Ashraf-Khorassani,M.; Taylor,L.T., in *Modern Supercritical Fluid Chromatography*, ed. C.M.White; Huthig, NewYork, 1988; p.115-125

52 Levy,J.M.; Ritchey,W.M. *HRC & CC* **1985**,8,503-509

53 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.104-105

54 Johnston,K.P.; Kim,S.; Coombes,J., in *Supercritical Fluid Science and Technology*, ACS Symposium Series #406, ed. K.P.Johnston and J.Penninger; American Chemical Society, Washington, DC 1989; p.52-70

55 Levy,J.M.; Ritchey,W.M. *HRC&CC* **1987**,10,493-496

56 Berger,T.A.; Deye,J.F. *Anal .Chem.* **1990**,62,615-622

57 Berger,T.A.; Deye,J.F. *J. Chrom. Sci.* **1991**,29,26-30

58 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.107

59 Berger,T.A.; Deye,J.F.;Ashraf-Khorassani,M.; Taylor,L.T. *J .Chrom .Sci.* **1989**,27,105-107

60 Giorgetti,A.; Pericles,N.; Widmer, H.M.; Anton,K.; Datwyler,P. *J .Chrom. Sci.* **1989**,27,318-322

61 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.133

62 *Analytical Supercritical Fluid Chromatography and Extraction*, ed. M.L.Lee and K.E.Markides; Chromatography Conferences Inc., Provo,Utah 1990; p.135-137

63 Hedrick,J.L.; Taylor,L.T. *Anal .Chem.* **1989**,27,105-107

64 Stahl,E.; Quirin, K.W.; Gerard, D., *Dense Gases for Extraction and Refining*; Springer-Verlag, New York 1987; p.6

APPENDIX I

Statistical Analysis of Recovery Data for Chapter 2

Pooled standard deviations were calculated for any data sets that were to be compared using the formula:

$$s^2 = \{(n_1-1)s_1^2+(n_2-1)s_2^2\}/(n_1+n_2-2)$$

where

s^2 = pooled standard deviation

n_1 = number of measurements for data set 1

n_2 = number of measurements for data set 2

s_1^2 = standard deviation of data set 1, squared

s_2^2 = standard deviation of data set 2, squared

The pooled standard deviation was then used to calculate a t value using the following equation:

$$t_{calc} = (x_1 - x_2)/ (s(1/n_1 + 1/n_2))$$

where

t_{calc} = calculated t value

x_1 = average value for data set 1

x_2 = average value for data set 2

s = pooled standard deviation

The calculated t value was then compared to the t value for the 95 % confidence level taken from table A.1 in Statistics for Analytical Chemistry by Miller and Miller. If the t value calculated is greater than that in the table, the difference between the averages is significant. For all data sets compared, these tables were generated.

Table XXI

Acetophenone trapping results at the 95% confidence level for methylene chloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Diol	Amino	Octyl	Phenyl	Silica	Frit
Diol	X	No	No	No	Yes	Yes
Amino	No	X	No	No	Yes	Yes
Octyl	No	No	X	No	Yes	Yes
Phenyl	No	No	No	X	No	Yes
Silica	Yes	Yes	Yes	No	X	No

Table XXII

N,N-dimethylaniline trapping results at the 95% confidence level for methylene chloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Diol	Amino	Octyl	Phenyl	Silica	Frit
Diol	X	No	No	No	Yes	No
Amino	No	X	No	No	Yes	No
Octyl	No	No	X	No	Yes	Yes
Phenyl	No	No	No	X	Yes	No
Silica	Yes	Yes	Yes	Yes	X	No

Table XXIII

N-decanoic acid trapping results at the 95% confidence level for methylene chloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Diol	Amino	Octyl	Phenyl	Silica	Frit
Diol	X	Yes	Yes	Yes	Yes	Yes
Amino	Yes	X	Yes	Yes	No	Yes
Octyl	Yes	Yes	X	No	Yes	No
Phenyl	Yes	Yes	No	X	Yes	No
Silica	Yes	No	Yes	Yes	X	Yes

Table XXIV

2-naphthol trapping results at the 95% confidence level for methylene chloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Diol	Amino	Octyl	Phenyl	Silica	Frit
Diol	X	Yes	No	No	Yes	Yes
Amino	Yes	X	Yes	Yes	Yes	Yes
Octyl	No	Yes	X	No	No	No
Phenyl	No	Yes	No	X	No	Yes
Silica	Yes	Yes	No	No	X	No

Table XXV

N-tetracosane trapping results at the 95% confidence level for methylene chloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Diol	Amino	Octyl	Phenyl	Silica	Frit
Diol	X	Yes	Yes	No	Yes	No
Amino	Yes	X	No	Yes	No	No
Octyl	Yes	No	X	Yes	No	No
Phenyl	No	Yes	Yes	X	Yes	Yes
Silica	Yes	No	No	Yes	X	No

Table XXVI

Trapping results for the frit at the 95% confidence level for methylene chloride prerinse and rinse. Aceto= acetophenone, DMA= n,n-dimethylaniline, Dec= decanoic acid, Naph= 2-naphthol, C24= n-tetracosane. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	Aceto	DMA	Dec	Naph	C24
Aceto	X	No	Yes	No	No
DMA	No	X	Yes	No	No
Dec	Yes	Yes	X	Yes	Yes
Naph	No	No	Yes	X	No

Table XVII

Trapping results for acetophenone on the frit under different prerinse and rinse conditions. A= methylene chloride prerinse and rinse, B= carbon tetrachloride prerinse and methylene chloride rinse, C= methylene chloride prerinse and carbon tetrachloride rinse, and D= carbon tetrachloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	A	B	C	D
A	X	No	Yes	Yes
B	No	X	No	No
C	Yes	No	X	No

Table XVIII

Trapping results for N,N-dimethylaniline on the frit under different prerinse and rinse conditions. A= methylene chloride prerinse and rinse, B= carbon tetrachloride prerinse and methylene chloride rinse, C= methylene chloride prerinse and carbon tetrachloride rinse, and D= carbon tetrachloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	A	B	C	D
A	X	Yes	Yes	Yes
B	Yes	X	No	No
C	Yes	No	X	No

Table XXIX

Trapping results for n-decanoic acid on the frit under different prerinse and rinse conditions. A= methylene chloride prerinse and rinse, B= carbon tetrachloride prerinse and methylene chloride rinse, C= methylene chloride prerinse and carbon tetrachloride rinse, and D= carbon tetrachloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	A	B	C	D
A	X	Yes	Yes	Yes
B	Yes	X	No	Yes
C	Yes	No	X	Yes

Table XXX

Trapping results for 2-naphthol on the frit under different prerinse and rinse conditions. A= methylene chloride prerinse and rinse, B= carbon tetrachloride prerinse and methylene chloride rinse, C= methylene chloride prerinse and carbon tetrachloride rinse, and D= carbon tetrachloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	A	B	C	D
A	X	Yes	No	No
B	Yes	X	Yes	Yes
C	No	Yes	X	No

Table XXXI

Trapping results for n-tetracosane on the frit under different prerinse and rinse conditions. A= methylene chloride prerinse and rinse, B= carbon tetrachloride prerinse and methylene chloride rinse, C= methylene chloride prerinse and carbon tetrachloride rinse, and D= carbon tetrachloride prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no significant difference between data sets.

	A	B	C	D
A	X	No	No	No
B	No	X	No	Yes
C	No	No	X	No

Table XXXII

Trapping results for the diol trap at the 95% confidence level. The methylene chloride prerinse and rinse is compared to the carbon tetrachloride prerinse and rinse. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	No
N,N-dimethylaniline	No
n-decanoic acid	Yes
2-naphthol	Yes
n-tetracosane	No

Table XXXIII

Trapping results for the phenyl trap at the 95% confidence level. The methylene chloride prerinse and rinse is compared to the carbon tetrachloride prerinse and rinse. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	Yes
N,N-dimethylaniline	Yes
n-decanoic acid	Yes
2-naphthol	Yes
n-tetracosane	No

Table XXXIV

Trapping results for the octyl trap at the 95% confidence level. The methylene chloride prerinse and rinse is compared to the carbon tetrachloride prerinse and rinse. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	No
N,N-dimethylaniline	Yes
n-decanoic acid	Yes
2-naphthol	Yes
n-tetracosane	No

Table XXXV

Trapping results comparing the frit to the diol trap at the 95% confidence level. In both cases carbon tetrachloride was used as both the prerinse and rinse solvent. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	Yes
N,N-dimethylaniline	Yes
n-decanoic acid	Yes
2-naphthol	Yes
n-tetracosane	No

Table XXXVI

Trapping results comparing the frit to the octyl trap at the 95% confidence level. In both cases carbon tetrachloride was used as both the prerinse and rinse solvent. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	Yes
N,N-dimethylaniline	Yes
n-decanoic acid	Yes
2-naphthol	Yes
n-tetracosane	No

Table XXXVII

Trapping results comparing the frit to the phenyl trap at the 95% confidence level. In both cases carbon tetrachloride was used as both the prerinse and rinse solvent. A No in the table means that there is no significant difference between data sets, while a Yes in the table means that there is a significant difference between data sets.

	$t_{calc} > t_{table}$
acetophenone	No
N,N-dimethylaniline	No
n-decanoic acid	Yes
2-naphthol	No
n-tetracosane	Yes

Table XXXVIII

Trapping results for phenol on the frit under different prerinse and rinse conditions. A= carbon tetrachloride prerinse and rinse, B= carbon tetrachloride prerinse and methanol rinse, C= methanol prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets; a No in the table means that there is no siginificant difference between data set.

	A	B	C
A	X	Yes	Yes
B	Yes	X	Yes

Table XXXIX

Trapping results for 2-chlorophenol on the frit under different prerinse and rinse conditions. A= carbon tetrachloride prerinse and rinse, B= carbon tetrachloride prerinse and methanol rinse, C= methanol prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets, while a No in the table means that there is no siginificant difference between data set.

	A	B	C
A	X	Yes	Yes
B	Yes	X	No

Table XL

Trapping results for 2,4-dichlorophenol on the frit under different prerinse and rinse conditions. A= carbon tetrachloride prerinse and rinse, B= carbon tetrachloride prerinse and methanol rinse, C= methanol prerinse and rinse. An X in the table means that the comparison is invalid; a Yes in the table means there is a significant difference between data sets, while a No in the table means that there is no siginificant difference between data set.

	A	B	C
A	X	No	No
B	No	X	No

APPENDIX II

Percent Recoveries and Relative Standard Deviations for Test Mix Components from Chapter 3

Table XLI

Percent recovery and RSDs for the volatile compounds with 1% methanol in carbon dioxide on the ODS trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	101.9	1.8	100.7	1.8
10	101.2	1.7	100.4	1.7
20	101.3	2.0	101.6	1.9
30	12.9	1.0	84.8	3.7
40	1.4	0.3	7.4	1.2
50	0.9	0.1	4.6	0.4
65	0.6	0.1	1.9	0.1
80	0	---	1.3	0.1

Table XLII

Percent recovery and RSDs for the nonvolatile compounds for 1% methanol in carbon dioxide on the ODS trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	91.2	8.2	99.8	2.9
10	87.9	2.0	99.6	2.5
20	91.1	2.1	99.0	2.9
30	83.2	2.9	94.2	2.6
40	86.4	3.5	95.3	4.3
50	88.0	2.4	95.0	2.6
65	92.3	3.0	97.9	3.5
80	91.4	2.1	95.7	2.5

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	102.5	2.5
10	100.3	2.1
20	100.7	2.4
30	92.8	2.0
40	97.2	2.4
50	94.5	2.3
65	97.4	2.6
80	96.7	2.2

Table XLIII

Percent recovery and RSDs for the volatile compounds with 2% methanol in carbon dioxide on the ODS trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	90.6	3.8	90.2	3.6
10	95.3	1.8	94.7	1.8
20	95.7	3.5	93.6	3.6
30	40.8	1.1	88.0	1.4
40	1.1	0.2	10.1	0.4
50	0	---	4.9	0.4
65	0	---	2.4	0.4
80	0	---	1.0	0.4

Table XLIV

Percent recovery and RSDs for the nonvolatile compounds for 2% methanol in carbon dioxide on the ODS trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	75.6	13.5	95.4	4.6
10	75.9	1.4	94.0	1.3
20	76.1	4.9	93.2	4.2
30	77.6	2.0	87.7	1.8
40	74.6	2.0	91.3	1.3
50	74.9	2.0	90.8	1.8
65	78.0	4.2	92.6	3.4
80	75.2	1.4	90.3	1.3

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	93.7	5.8
10	90.1	1.8
20	90.4	4.6
30	88.3	2.3
40	94.4	1.8
50	93.9	2.4
65	96.5	3.5
80	94.7	1.8

Table XLV

Percent recovery and RSDs for the volatile compounds with 4% methanol in carbon dioxide on the ODS trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	45.7	5.5	37.9	3.7
20	64.1	4.2	59.4	2.6
30	82.4	2.3	81.7	2.1
40	46.3	1.8	48.1	1.2
50	0.9	0.2	0.4	0.1
65	0.4	0.1	0	---
80	0	---	0	---

Table XLVI

Percent recovery and RSDs for the nonvolatile compounds for 4% methanol in carbon dioxide on the ODS trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	10.1	9.2	59.2	5.1
20	5.9	2.0	65.2	3.5
30	42.6	11.9	89.8	3.4
40	59.7	4.0	92.9	2.1
50	51.6	3.8	90.4	2.1
65	50.3	2.0	92.2	2.5
80	48.5	2.3	90.9	2.1

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	94.8	3.3
20	88.0	2.9
30	93.9	3.6
40	97.4	3.0
50	96.5	3.0
65	97.4	3.5
80	96.6	3.0

Table XLVII

Percent recovery and RSDs for the volatile compounds with 8% methanol in carbon dioxide on the ODS trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	0.4	0.8	2.9	3.1
20	1.5	2.3	4.1	4.8
30	2.4	0.5	4.5	0.7
40	13.2	2.0	16.9	2.1
50	30.1	10.9	24.5	10.1
65	2.1	0.7	1.7	0.1
80	0	---	1.0	0.1

Table XLVIII

Percent recovery and RSDs for the nonvolatile compounds for 8% methanol in carbon dioxide on the ODS trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	0	---	0	---
20	0	---	1.4	2.3
30	0	---	2.7	1.4
40	3.1	0.5	17.7	2.8
50	24.4	22.0	52.7	18.6
65	17.1	4.5	19.0	1.8
80	24.4	3.7	12.7	3.1

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	97.5	2.7
20	92.5	5.1
30	92.2	2.1
40	95.0	5.1
50	105.4	3.8
65	93.0	2.6
80	84.9	1.9

Table IL

Table IL: Percent recovery and RSDs for the volatile compounds with pure carbon dioxide on the stainless steel trap.

	Acetophenone		N,N-Dimethylaniline	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	11.7	2.0	3.6	0.3
20	8.7	0.2	3.2	0.1
30	7.2	0.3	2.5	0.1
40	6.8	0.7	2.3	0.2
50	6.1	0.3	2.2	0.2
65	4.3	0.7	1.6	0.1
80	4.5	0.2	1.7	0.1

Table L

Percent recovery and RSDs for the nonvolatile compounds pure carbon dioxide on the stainless steel trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	26.3	14.9	77.5	9.0
20	40.2	2.3	83.9	2.3
30	47.7	7.5	89.2	3.6
40	52.6	3.1	88.4	3.2
50	58.6	6.7	87.0	3.6
65	43.9	6.0	83.2	3.6
80	52.0	3.1	86.9	1.0

	n-Tetracosane	
Trap Temp (°C)	% Recovery	RSD
5	95.6	2.3
20	93.3	1.9
30	98.7	3.4
40	95.9	3.4
50	93.1	3.4
65	95.3	2.0
80	97.1	2.0

Table LI

Percent recovery and RSDs for the test mix compounds with 1% methanol in carbon dioxide on the stainless steel trap.

	n-Decanoic Acid		2-Naphthol	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD
5	32.6	9.4	68.5	2.1
20	45.0	4.0	69.3	1.5
30	50.0	3.3	71.0	1.5
40	56.6	9.8	76.7	10.3
50	67.0	8.5	92.9	4.9
65	69.7	8.6	91.7	2.8
80	71.6	8.7	91.7	3.1

	n-Tetracosane		Acetophenone		N,N-DMA	
Trap Temp (°C)	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD
5	72.8	1.0	61.3	13.4	46.3	26.3
20	72.8	1.0	------		------	
30	74.1	1.0	------		------	
40	79.6	8.2	------		------	
50	100.2	2.2	------		------	
65	96.2	1.1	------		------	
80	95.5	2.2	------		------	

APPENDIX III

Statistical Analysis of Recovery Data from Chapter 3

Pooled standard deviations were calculated for any data sets that were to be compared using the formula:

$$s^2 = \{(n_1-1)s_1^2+(n_2-1)s_2^2\}/(n_1+n_2-2)$$

where

s^2 = pooled standard deviation

n_1 = number of measurements for data set 1

n_2 = number of measurements for data set 2

s_1^2 = standard deviation of data set 1, squared

s_2^2 = standard deviation of data set 2, squared

The pooled standard deviation was then used to calculate a t value using the following equation:

$$t_{calc} = (x_1 - x_2)/\cdot (s(1/n_1 + 1/n_2))$$

where

t_{calc} = calculated t value

x_1 = average value for data set 1

x_2 = average value for data set 2

s = pooled standard deviation

The calculated t value was then compared to the t value for the 95 % confidence level taken from table A.1 in Statistics for Analytical Chemistry by Miller and Miller. If the t value calculated is greater than that in the table, the difference between the averages is significant. For all data sets compared, these tables were generated.

Table LII

Decanoic acid trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means that the difference in recovery is not significant.

Trap Temp (°C)	Are recoveries with 1% MeOH > 100% CO_2?	Are recoveries with 1% MeOH > 2% MeOH
5	Yes	No
10	Yes	Yes
20	Yes	Yes
30	Yes	Yes
40	Yes	Yes
50	No	Yes
65	Yes	Yes
80	No	Yes

Table LIII

Decanoic acid trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with 2% MeOH > 4% MeOH?	Are recoveries with 4% MeOH > 8% MeOH?
5	Yes	Yes
20	Yes	Yes
30	Yes	Yes
40	Yes	Yes
50	Yes	Yes
65	Yes	Yes
80	Yes	Yes

Table LIV

Decanoic acid trapping results at the 95% confidence level with n=4 comparing recoveries on the ODS trap and stainless steel trap, and comparing 100% carbon dioxide to 1% methanol on the stainless steel trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with ODS > SS?	Are recoveries with 100% CO_2 > 1% MeOH?
5	Yes	No
20	Yes	Yes
30	Yes	No
40	Yes	No
50	Yes	Yes
65	Yes	Yes
80	Yes	Yes

Table LV

N-tetracosane trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with 100% CO_2 > 1% MeOH?	Are recoveries with 1% MeOH > 2% MeOH?
5	No	No
10	No	Yes
20	No	Yes
30	No	Yes
40	No	Yes
50	No	Yes
65	No	No
80	No	No

Trap Temp (°C)	Are recoveries with 100% CO_2 > 2% MeOH?
5	No
10	Yes
20	Yes
30	Yes
40	Yes
50	Yes
65	No
80	Yes

Table LVI

N-tetracosane trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with 2% MeOH > 4% MeOH?	Are recoveries with 4% MeOH > 8% MeOH?
5	No	No
20	No	No
30	No	No
40	No	No
50	No	No
65	No	No
80	No	Yes

Table LVII

N-tetracosane trapping results at the 95% confidence level with n=4 comparing the ODS trap to the stainless steel trap, and comparing pure and 1% methanol on the stainless steel trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with ODS > SS?	Are recoveries with 100% CO_2 > 1% MeOH?
5	No	Yes
20	Yes	Yes
30	No	Yes
40	No	Yes
50	Yes	No
65	No	No
80	No	No

Table LVIII

2-Naphthol trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Any difference in recoveries 100% CO_2 > 1% MeOH?	Are recoveries with 100% CO_2 > 2% MeOH?
5	No	No
20	No	Yes
30	No	Yes
40	No	Yes
50	No	No
65	No	No
80	No	No

Table LIX

2-Naphthol trapping results at the 95% confidence level with n=4 on the ODS trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with 2% MeOH > 4% MeOH?	Are recoveries with 4% MeOH > 8% MeOH?
5	Yes	Yes
20	Yes	Yes
30	No	Yes
40	No	Yes
50	No	Yes
65	No	Yes
80	No	Yes

Table LX

2-Naphthol trapping results at the 95% confidence level with n=4 on the stainless steel trap. A Yes in the Table means that the difference in recovery is significant, while a No in the Table means there is no significant difference between recoveries.

Trap Temp (°C)	Are recoveries with ODS > SS?	Are recoveries with 100% CO_2 > 1% MeOH?
5	Yes	No
20	Yes	Yes
30	Yes	Yes
40	Yes	Yes
50	Yes	No
65	Yes	No
80	Yes	No

Vita

Leah Jean Mulcahey was born on September 3, 1965 in Auburn, New York. She received her B.A. in chemistry in 1987 from Bucknell University in Lewisburg, Pennsylvania. In July of 1987 she began her graduate studies toward the Ph.D. in the Chemistry Department of Virginia Tech under the direction of Dr. Larry T. Taylor. She completed the requirements for the Ph.D. in October 1991.