SUPERCRITICAL FLUID EXTRACTION OF NON-TRADITIONAL MATRICES

By

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(ABSTRACT)

Supercritical extraction (SFE) has most often been linked to the use of modified or unmodified CO_2 for the recovery of relatively non-polar analytes from solid matrices. The objective of this research was to expand current supercritical fluid (SF) techniques to the recovery of analytes from non-traditional matrices. SFE with three unique matrices was completed.

Small mammals are often used in drug toxicity studies. Pharmaceutical dosage verification in the animal feed is a requirement in these studies. Atovaquone, a drug used in the treatment of aids related pneumonia, was successfully recovered from rat feed with supercritical CO_2. Drug recoveries of $\geq$ 90% were achieved with a range of drug concentrations from 0.03% to 1.1% in the feed.

The second phase of the investigation studied the recovery of acyclovir, an antiviral agent, from Zovirax® 5% ointment. This recovery required a radically different approach from all previous SFE techniques, as the analyte was completely insoluble in the supercritical fluid. This unique situation led to the development of "Inverse SFE", where the ointment matrix was extracted and the drug analyte was retained in the extraction vessel. Included in the investigation were the effects of temperature, modifier, drug recovery techniques and length of extraction. Employing a 20 minute inverse SFE, 99% of the acyclovir was recovered from the ointment.

Increasing regulation, disposal costs and environmental issues have fueled concerns over the use of chlorinated organic solvents. Currently, over 50% of the samples regulated by the EPA have liquid matrices that have been traditionally analyzed using these solvents. The implementation of solid phase extraction (SPE) has significantly reduced the amount of organic solvent utilized for the extraction of liquid matrices; however, further reduction is desired. The third phase of this work concerned the elution of a SPE disk with SF wherein the disk had been used to concentrate pollutants from fresh and brackish water. Initially, this research focused on the quantitation of polyaromatic hydrocarbons (PAHs) from distilled water. The analytes were deposited onto a solid phase extraction disk and eluted with a SF. The proper method of quantitation, relative versus absolute, was also investigated. Optimization of the SF elution in relation to time, temperature, flow rate, and pressure while maintaining quantitative recoveries was performed. A three step, 27 minute SF elution method resulted from this effort. Recoveries were ≥90% for all the 16 PAHs studied. A chamber temperature of 80°C with liquid flow rate of 2 mL/minute was employed. The study was expanded to 39 EPA Method 525.1 analytes in distilled water. Although system contamination proved to be a problem, all but four analytes were quantitatively recovered according to EPA criteria. SF elution studies of brackish water matrices from the Chesapeake Bay indicated suspended sediment was responsible for water retention on the surface of the SPE disk. The retained water interfered with analyte recovery. More thorough drying techniques resulted in the recovery all but five analytes meeting EPA criteria.

This thesis is dedicated to

Ruth Steman Messer

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It is by the efforts of many that this work has come to fruition. I would like to express my sincere gratitude to:

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Chapter I

INTRODUCTION

Historically, technological advances such as transistors and circuit boards have greatly enhanced analytical method development in the area of chromatography and spectroscopy. Liquid, gas and supercritical fluid chromatographic techniques have been described for nearly every class of compound. Infrared and mass spectroscopy have benefited from advancements such as Fourier-transform. However, improvements in sample preparation have not kept pace. Liquid-liquid and soxhlet extractions, developed over 75 years ago, are still commonly used today. It has been only in the last decade that focus has shifted to sample preparation techniques. Solid phase disk extraction, first introduced in 1989, has become the standard for extraction of liquid matrices. Supercritical extraction (SFE) has been employed for recovery of analytes from solid matrices.

A supercritical fluid (SF) is defined as any substance that is above both its critical pressure and temperature. These fluids have high diffusivity and low viscosity in comparison to their corresponding liquid state, allowing rapid mass transport and enhanced matrix penetration. SF density is related to temperature and pressure. In turn, the solvating power is directly proportional to density. Selective extractions are achieved by regulation of pressure and/or temperature. Analyte deposition onto the trap results from decompression, as most SF's are gases at ambient conditions. Carbon dioxide (CO_2) is the most common SF. Advantages of CO_2 are it is inert, non-flammable, non-toxic and has mild critical parameters. A major disadvantage of CO_2 is its non-polar nature, which makes it is less compatible with polar analytes. The final product of a SFE is a low

volume, highly concentrated solution which is in most cases, needs no further preparation prior to assay. The advantages outlined allow supercritical fluids to produce selective, efficient extractions that are suitable for trace analysis, and reduce the need for solvent disposal.

The focus of this research is to expand SFE applications by attempting analyte recoveries from atypical analyte-matrix combinations. Typical SFEs emphasize the recovery of analytes such as aliphatic hydrocarbons, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) from environmental solids, or additives from polymers. Commonly studied matrices include environmental solids, polymers and food matrices. Analytes and matrices are equally important to the extraction process.

Chapters II and III involve the SFE of pharmaceutical compounds from dramatically different matrices. Pharmaceuticals are generally polar, water soluble molecules with complex matrices. Stringent industrial standards require high quantitative levels and reproducibilities. Chapter II discusses the recovery of the aids-related pneumonia drug, atovaquone from a rat feed matrix. Drug extraction from this matrix was necessary for dosage verification. Variable percentages of drug concentrations ranged from 0.3% to > 1%. Chapter III describes the novel recovery method, "Inverse SFE". The technique recovered a highly polar analyte, acyclovir, from a SF soluble matrix. The innovation in this technique is based on retaining the analyte in the extraction vessel while the matrix is removed with a dynamic SF flow.

Increased organic solvent regulations and disposal costs provide momentum for improving sample preparation methods. Chapters IV through VI focus on the indirect recovery of trace level environmental pollutants from water. SFE serves as an elution technique for solid phase C-18 extraction disks. Advantages of this method include decreased residual organic solvents and removal of the solvent reduction step common in

SPE. Chapter IV investigates SF elution of PAHs from distilled water. Analyte recoveries are expanded to include PCBs and selected pesticides in Chapter V. A review of current literature indicates this is the first attempt to apply solid phase trapping to such a large number of analytes. Chapter VI explores application of SPE/SF elution to 39 analytes (Chapter V) in a brackish water matrix. The presence of sediment and dissolved organic compounds significantly complicated analyte recovery.

Chapter II
Quantitative Analysis of a Drug in Animal Feed Employing Sample Preparation by Supercritical Fluid Extraction

INTRODUCTION

Although supercritical fluid extraction (SFE) has been touted as a likely successor to many of the current Soxhlet and liquid-solid extractions (1), to this point SFE has largely resided in the research laboratory. Some of the properties that have brought supercritical fluids to the forefront are their high diffusivity and low viscosity (2), when compared to traditional extraction solvents. Another benefit is the ability to control the solvating power of the supercritical fluid by regulation of pressure and temperature (3). This chapter illustrates the use of SFE in quantitation of a drug substance within an animal feed matrix.

A search of the literature reveals few applications of SFE to pharmaceutical agents. Mulcahey (4) et al. showed the application of SFE for direct extraction of active ingredients from a liquid pharmaceutical matrix. Their work involved the extraction of sulfamethoxazole and trimethoprim from Septra Infusion®, which is used for treatment of urinary tract infections. They extracted the drugs in two ways. First, the Septra Infusion liquid was extracted directly using a modified extraction vessel designed to bubble supercritical fluid through the liquid sample before exiting to the trap. This method yielded very little extract due to restrictor plugging caused by precipitation of sulfamethoxazole as the solution pH was lowered by introduction of CO_2. Secondly, a method of spiking the Septra Infusion into Celite was explored followed by extraction of the drug component from the dried Celite matrix. Nearly quantitative recoveries were realized for both analytes by using the latter method.

Startin (5) et al. used supercritical CO_2 to extract four veterinary drugs from freeze dried pig kidney: trimethoprim, hexestrol, diethylstilbestrol, and denestrol. Qualitative analysis was attempted by on-line SFE/SFC/MS/MS. It was concluded from this study that the method demonstrated great potential, but detection limits were not sufficient for this particular matrix.

Richter (6) et al. discussed two applications involving modified CO_2 for extraction of pharmaceutical agents. The first application was the use of 10% chloroform modified CO_2 for the extraction of an antihistamine from a transdermal patch. The active agent was suspended in a gel under an adhesive in this matrix. Richter reported quantitative results with a relative standard deviation (RSD) of 2.7%. In a second application, sulfa drugs were extracted under various pressure and temperature conditions from spiked liver and pork samples. Incurred sulphamethazine was also extracted from pork with quantitative recoveries reported.

Locke (7) et al. and Messer (8) et al. were among the first to apply SFE to an animal feed matrix. Locke extracted menadione (Vitamin K_3) spiked at the 1 mg/g level from rat feed and reported an average recovery of 90.5% with a 2.2% RSD. To achieve these recoveries Locke employed a 20 minute static extraction using 100% CO_2 held at 8000 psi and a temperature 60°C. Trapping was achieved by allowing the supercritical CO_2 to decompress into a 6 inch X 1/4 inch outside diameter (O.D.) stainless steel tube filled with silica gel. The silica gel was then washed with 10 mL of methylene chloride.

Messer reported on the applicability of supercritical CO_2 extraction as a quantitative method for recovery of 4'-trifluoromethyl-2-biphenyl carboxylic acid spiked into a rat feed matrix at a level of 1%. Off-line extraction with solid phase trapping and solvent rinsing was utilized. An optimized method for quantitative extraction of the pure drug was initially developed with high reproducibility. Three drug/rat feed matrices were

examined. The as-received "crystalline matrix" yielded the poorest reproducibility, suggestive of a heterogeneous matrix. A laboratory prepared crystalline drug/feed matrix and a matrix prepared by spiking the animal feed with a solution of the drug gave ten-fold better precision than the "crystalline matrix".

The application of SFE for routine analysis will increase as the technology of supercritical fluids matures and the understanding of SFE deepens. One such application is the use of a commercially available instrument with quantitative supercritical fluid extraction (SFE) of trans-2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone) from a rat feed blend (Figure 1). The drug concentration in the feed ranged from 0.03% to over 1.0%. Supercritical fluid extraction was completed in approximately one hour, with recoveries of greater than 90% and RSDs generally less 5%.

EXPERIMENTAL

The extractions were performed on a Hewlett-Packard (Avondale, PA) Model 7680A Supercritical Fluid Extractor. In brief, this extractor utilizes a cryogenically cooled dual head reciprocating pump, with an upper pressure limit of 5500 psi and liquid flow rates of up to 4 mL/minute. Unique to this instrument is the use of a computer regulated variable restrictor which independently controls both flow rate and pressure. Air Products and Chemicals Inc. (Allentown, PA) supplied SFC/SFE grade CO_2 and SFC grade 2% methanol modified CO_2 was supplied by Scott Specialty Gases (Plumsteadville, PA) Analysis of the atovaquone extracts was carried out by liquid chromatography employing isocratic elution with a 4.6 X 250 mm Keystone Scientific (Bellfonte, PA) Hypersil ODS column (5 μm particle size and 120 Å pore size). The mobile phase was 780 mL/220

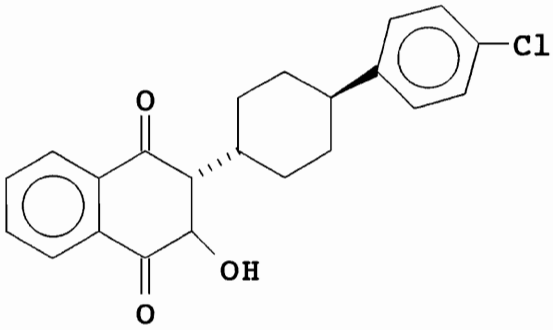


Figure 1. The structure of Atovaquone

mL/5 mL of acetonitrile/water/phosphoric acid respectively. The flow rate was 2 mL/minute. Acetonitrile and water were HPLC Grade from Fisher Scientific (Raleigh, NC). The phosphoric acid was 99.99% pure and obtained from Aldrich Chemical (Milwaukee, WI). The aqueous portion of the mobile phase was vacuum filtered using a 0.22 μm GS type filter from Millipore (Bedford, MA). A Hewlett-Packard (Avondale PA) 1050 isocratic pump was used, and connected to a Valco (Austin, TX) model EQ-60 LC injector using a 10 μL loop. A Spectro Monitor III (Houston, TX) ultraviolet (UV) detector monitoring at 254 or 220 nm and a Hewlett-Packard (Avondale, PA) model 3394A or model 3392A integrator was used. All atovaquone\rat feed samples and atovaquone standards were provided by Burroughs Wellcome Company (Research Triangle Park, NC). HPLC quantitation was determined by plotting peak area versus concentration relative to external standards of the pure drug. The calibration curve had a correlation coefficient of 0.999. Recoveries were calculated using the mass of the spiked drug.

Additional sample preparation after supercritical fluid extraction was minimal. It was found that supercritical CO_2 also extracted components of the rat feed. These compounds produced a solid precipitate when the organic rinse solvent was diluted with water to achieve the appropriate solvent strength for liquid chromatography. This precipitate was removed prior to drug analysis by syringe filtering. The filters used were Whatman® Puradisc 25 TF filters. These filters were 25 mm in diameter and constructed of PTFE membrane with polypropylene housing.

The current method of dosage verification for atovaquone is a liquid-solid extraction which uses approximately 1.5 grams of rat feed sample and 15 to 40 mL of acetonitrile as an extraction solvent. The specific amounts of extraction solvent used depend upon the drug concentration in the matrix. The rat feed was weighed into a 50 mL

polypropylene centrifuge tube, and the appropriate volume of acetonitrile was added. The sample was then placed on a horizontal shaker for 15 minutes and subsequently centrifuged for 2 minutes. An aliquot of the supernatant was then diluted to the proper HPLC solvent strength and filtered prior to analysis.

RESULTS AND DISCUSSION

Extraction Profile of Neat Drug

Extraction of atovaquone from an inert matrix was performed to determine the extraction profile of atovaquone in the absence of any matrix effects from the rat feed. Specifically, after increments of time, the dynamic extraction was interrupted, the trap was washed, and the resulting solution analyzed. The cumulative results were evaluated after the completion of a several step combination static/dynamic extraction. Through this evaluation an approximation of both drug solubility in the supercritical fluid and drug extraction kinetics could be determined.

Test samples were prepared by spiking 0.200 mL of a 1.00 mg/mL solution of atovaquone in methylene chloride onto Celite bed inside the 1.5 mL extraction vessel. These samples were dried overnight at ambient conditions to allow for evaporation of solvent. The atovaquone/Celite sample was then subjected to a five step extraction. Supercritical CO_2 was used as the extraction solvent at a liquid flow rate of 2 mL/minute for all steps. The pressure was held at 350 bar with a chamber temperature of 50°C, which translates into a supercritical fluid density of 0.90 g/mL. The trap was stainless steel beads (100 µm) held at 5°C during the extraction step, and raised to 40°C during the rinsing of the trap. Temperature of the nozzle (i.e. variable restrictor) was held at 55°C for both the extraction and rinsing phases. The stainless steel trap was washed with 1 mL

of acetonitrile after each step, with the exception of step 5, where the trap was rinsed with 3 mL of acetonitrile.

The five steps of the extraction were as follows:

Step 1: Equilibration (vessel pressurized) for 2 minutes, dynamic extraction (SF flows through the vessel) for 2 minutes with vessel volume sweeps = 2.7 (Note: a vessel volume is the amount of SF need to completely swept the vessel one time.)

Steps 2 & 3: Dynamic extraction for 3 minutes with vessel volume sweeps = 4.1

Step 4: Dynamic extraction for 4 minutes with vessel volume sweeps = 5.5

Step 5: Dynamic for 8 minutes with vessel volume sweeps = 11.0.

This produced a total dynamic extraction time of twenty minutes with 27.4 vessel volumes of supercritical CO_2.

As shown in Figure 2, the first step resulted in a recovery of 67.1%, while the second step indicated a recovery of an additional 14.5%. Therefore, over 80% of the atovaquone was extracted in the first five minutes of dynamic extraction. The third step of the extraction produced a recovery of 6.17%; while the fourth and fifth step of the extraction had a recovery of 3.17% and 3.51%, respectively. The total recovery for the extraction profile was 94.5%. The results of this extraction profile indicated several important results. First, atovaquone showed a very high solubility in supercritical CO_2. Second, the extraction kinetics were favorable. Third, the stainless steel trap was able to hold the analytes during the extraction process and efficiently release the analyte upon application of the rinse solvent.

A second extraction profile was performed using 2% methanol-modified CO_2 to determine if the recovery could be increased. This extraction was performed using the same parameters as the previous profile with the exception of trap temperature. The trap

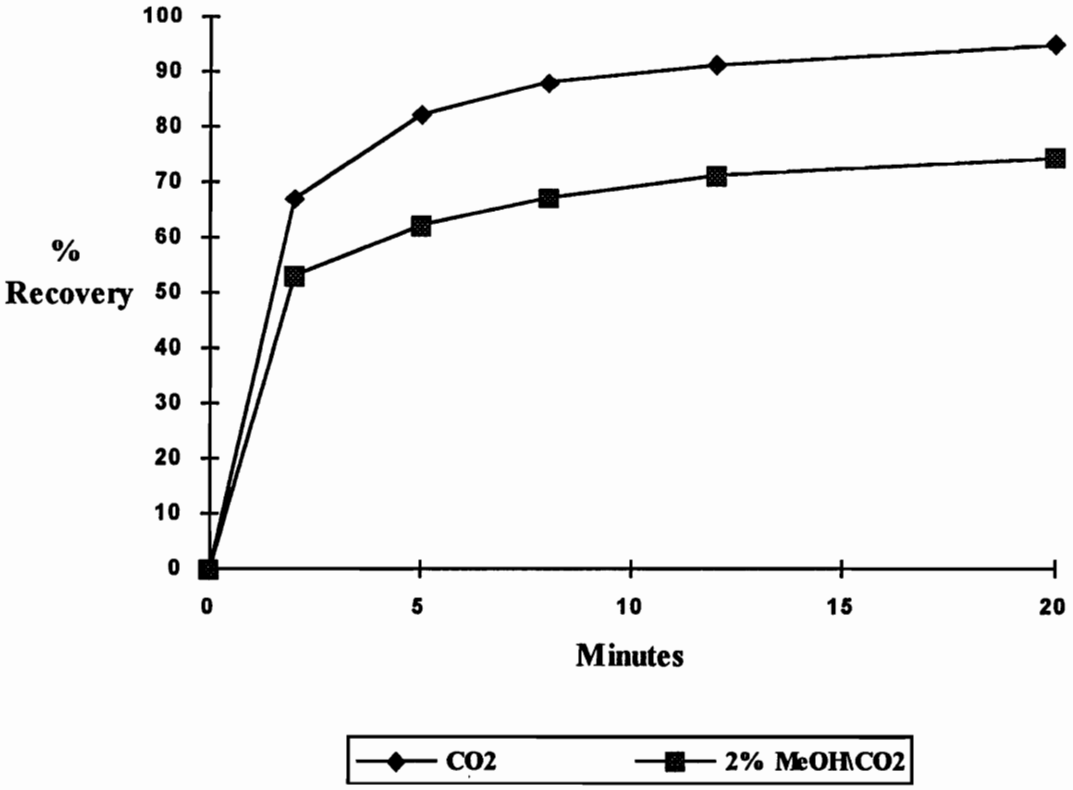


Figure 2. Extraction profile of atovaquone from Celite using 100% CO_2 and 2% methanol modified CO_2.

temperature was raised to 70°C to insure that the methanol (b.pt. 67°C) appearing in the trap was in the gaseous phase rather than in the liquid phase. Mulcahey et al. (9) have shown that methanol in the liquid phase can reduce the trapping efficiency of stainless steel beads.

Extraction recovery for the combined initial two steps was only 61.8% for the methanol-modified CO_2, compared with over 80% for the extraction using 100% CO_2. Recoveries for steps 3-5 were 5.11%, 3.68%, and 3.15% respectively, which was similar to the CO_2 results. The lower recovery with methanol-modified CO_2 was probably not due to lower solubility of the drug in the modified supercritical fluid. A more probable explanation was the loss of trapping efficiency due to the presence of liquid methanol on the stainless steel trap. Although the trap temperature was set for 70°C, this temperature was monitored at the heating jacket around the trap. The actual temperature inside the trap, after considering the effect of Joule-Thompson cooling, probably was low enough to account for the presence of liquid methanol. The stainless steel trap was inert, and therefore offered no sorption mechanism to the analytes. Given the high analyte concentration in the trap during the early steps of the extraction, even a small amount of methanol had the potential to carry a substantial amount of analyte from the trap. Although methanol was still lost from the trap in the later steps of the extraction, the trap concentration of the analyte was much lower and thus the loss was less significant.

Reproducibility of Neat Drug Extractions

The next phase of this study was to establish the reproducibility of supercritical CO_2 extraction of neat atovaquone on Celite. Reproducibility was established using triplicate extractions and the parameters as previously stated. These extractions had an

equilibration time of 2 minutes followed by a 25 minute dynamic extraction. During this period, the 1.5 mL vessel was swept 34.3 times. The trap was rinsed with two 1 mL aliquots of acetonitrile. Triplicate extraction yielded an average recovery of 100.3% with a relative standard deviation (RSD) of 5.8%. These results directed the selection of parameters to be used for the extraction of atovaquone from rat feed.

Extraction of Atovaquone from Rat Feed

The investigation of extraction of atovaquone from rat feed began with the extraction of blank rat feed. Assay of previous atovaquone extracts had used UV detection at 220 nm. The extraction of blank rat feed, however, showed an interference at this wavelength. The monitored wavelength was changed to 254 nm to minimize the effect of this interference in the extract.

This study involved extraction of atovaquone at six levels, ranging from 0.0335% to 1.1208% of drug in the rat feed. Sample sizes for extraction were either 250 mg or 500 mg depending on the spiking level. Initially, triplicate 250 mg samples of 1.1208% rat feed were extracted. Extraction parameters were as previously described for the reproducibility study. The trap rinse solvent volume was increased from 2 mL to 4 mL (i.e. 4 x1 mL aliquots) of acetonitrile as the larger amount of the drug extracted related to earlier studies. The average from this set of triplicate extractions was 98.7% recovery with a RSD of 2.3%. The next concentration of drug investigated was 0.8386%. The average recovery of atovaquone was 102.5% with a RSD = 3.2% for five replicates.

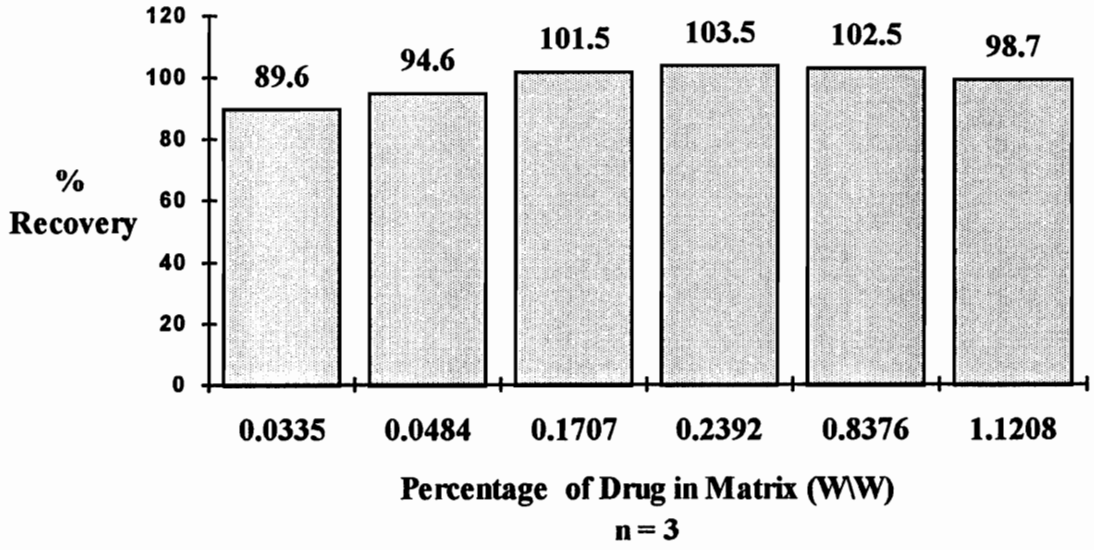


Figure 3. Supercritical CO_2 extraction of atovaquone from rat feed.

Triplicate extractions were also carried out atovaquone from rat feed spiked at levels of 0.2392%, 0.1707% and 0.0484%. Recoveries at all levels were excellent as shown in Figure 3. The final drug level investigated was 0.0335%. A large reduction in recovery was obtained, i.e. 82.4% recovery with a RSD of 4.2%, in the initial attempt . A second set of triplicate extractions was performed using identical parameters to the first set of extractions. The average recovery was 96.8 % with a RSD of 4.6%. Combining thetwo sets of extractions (n=6), the average recovery was 89.6% with the RSD increased to 9.6%.

The industry method of dosage verification for atovaquone is liquid-solid extraction employing a 1.5 gram rat feed sample and acetonitrile. Nine replicate extractions of 0.02% atovaquone from rat feed yielded typical recoveries of 83.3% with RSDs of 1.1%. Liquid-solid extractions yielded recoveries of 94.9% with relative standard deviations of 2.1% (n=11) when the concentration of drug in the matrix was increased to 2.0% .

SUMMARY

In summary, this study clearly (Figure 3) demonstrated the ability of supercritical CO_2 to quantitatively extract trans-2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone) from a rat feed matrix. Triplicate extractions produced recoveries near 95% and relative standard deviations of less than 5%, except with the lowest drug level. Comparison of results for drug SFE in Celite and rat feed indicated that atovaquone was probably not binding with the rat feed. Quantitative extraction can be achieved in approximately one hour including the rinse procedure. Supercritical fluid

extraction results were comparable to conventional liquid-solid extraction results, even though samples sizes were considerably less in the SFE studies (e.g. 1500 mg versus 250 or 500 mg).

Chapter III
Application of Supercritical Fluid Extraction (SFE) to the Quantitation of Acyclovir from Zovirax® Ointment 5%

INTRODUCTION

There are two major reasons for the slow advancement of supercritical extraction (SFE) in the pharmaceutical field. First, supercritical CO_2 the most common SF, does not possess the solvent strength necessary to efficiently extract highly polar analytes common in pharmaceutical agents; although, the use of modifiers as co-solvents can increase the solvent strength moderately. Second, progress has been impeded by the complexity of pharmaceutical matrices (10). Pharmaceutical applications of SFE include extraction of antihistamines from transdermal patches (6) and the extraction of polar drugs such as sulfamethazine from pork (11). SFE has been used for the extraction of drugs and vitamins from animal feeds, (8,12,13) drug and drug residues form animal tissue, (11,14,15) medicinal herbs (16,17) from plants and drug metabolites from plasma (18,19). Masuda (20) et al. also applied on-line SFE with supercritical fluid chromatography (SFC) for the determination of fat-soluble vitamins in a hydrophobic ointment.

This chapter assesses the feasibility of using supercritical fluids as a means of isolating a highly polar drug from its ointment carrier. Traditionally, supercritical fluids extractions are performed in an effort to remove an analyte from the matrix, and in most cases this means the extraction of several micrograms to milligrams of material. This study will discuss extraction of the matrix while leaving an unextracted insoluble analyte. The method necessitates the extraction of tens to hundreds of milligrams of matrix material. This process can be thought of as an "inverse SFE," which to this author's knowledge, has not been reported before to achieve analytical SFE.

There are five parameters that can be readily identified for a successful "inverse SFE." First, the analyte must be totally insoluble in the supercritical fluid. Second, the matrix must be readily extractable with the chosen SF. Preferably, the matrix should be totally extractable such that no residual matrix may interfere with either analyte recovery or quantitation. Third, a highly efficient washing method is necessary in order to transfer the unextractable analyte from the extraction vessel assay. Fourth, the analyte concentration in the matrix should be relatively high (>2%) which would allow a relatively small sample (<1 g) to be extracted. Finally, an assay method with low detection limits for the amount of analyte that remains behind is desired.

Discussion focuses on isolation of acyclovir (Figure 4) which is the active ingredient in Zovirax® Ointment 5%. Acyclovir is a polar, water-soluble analyte, while the ointment is hydrocarbon based. Acyclovir has been discovered to be insoluble even in 2% methanol modified CO_2, but the ointment exhibits good solubility in this SF.

EXPERIMENTAL

SFE and Quantitation

Extractions were performed on a Suprex Prepmaster (Pittsburgh, PA) at a pressure of 500 atm using a 5 mL vessel. An additional vessel was inserted into the 5 mL extraction vessel to aid in recovery of the analyte (Figure 5). The ointment sample was placed between the frits of a 1 mL empty solid phase extraction tube which was then

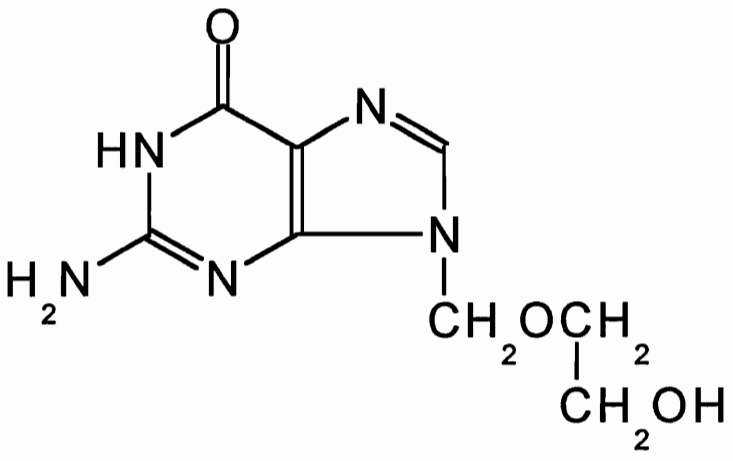


Figure 4. The structure of acyclovir

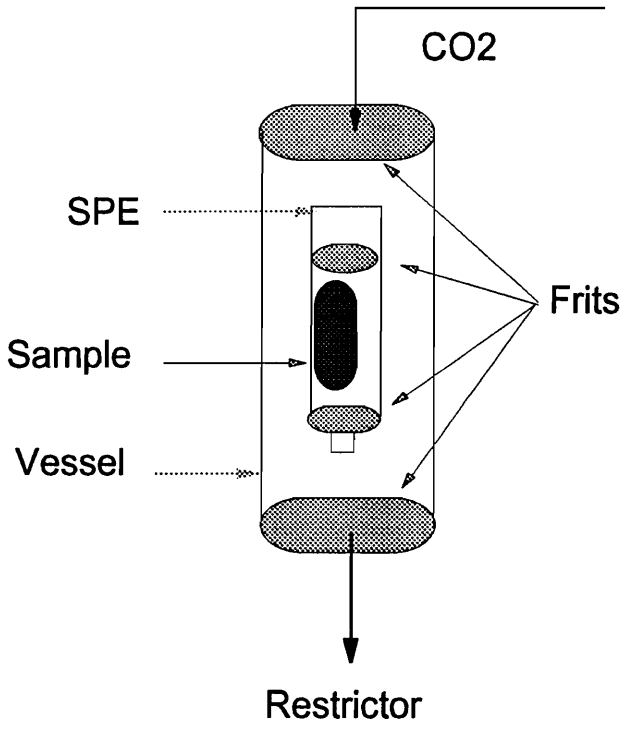


Figure 5. Inverse SFE flow path of the "vessel within a vessel".

weighed and placed inside a 5 mL extraction vessel. Analyte was recovered by washing the inner vessel utilizing a vacuum box. A second extraction flow path utilizing a 1 mL extraction vessel (Figure 6) was studied. The sample was placed in the extraction vessel beneath a layer of glass wool to reduce dead volume. The analyte was rinsed from the vessel with 8 mL of 0.01 N NaOH under vacuum. Experiments utilized 2% methanol-modified CO_2 and a labratory fabricated manual variable restrictor. A liquid CO_2 flow rate of approximately 0.9 mL/minute and a chamber temperature of 60°C were used. Assay of the drug was performed by liquid chromatography (LC). The LC method was performed on a Perkin-Elmer Series 10 pump (Norwalk, CT), a Valco model C6W injector (Houston, TX) with a 50 μL loop and a 4.6 mm i.d. X 250 mm Hypersil-ODS column from Keystone Scientific, Inc. (Bellefonte, PA) The mobile phase was 0.1% glacial acetic acid in water (v/v) with a flow rate was 3.0 mL/minute. A Hewlett Packard Series 1050 ultraviolet (UV) detector at 254 nm was used. Quantitation was determined by an external calibration curve by plotting peak area versus concentration. A correlation coefficient of 0.9998 was obtained.

Analyte Recovery

The post-extraction recovery of the analyte from the extraction vessel is critical in this technique. A 100 mg ointment sample was placed between the frits of a empty, 1 mL solid phase extraction tube which served as an extraction vessel insert (Figure 5). The inner vessel was inserted into the 5 mL extraction vessel and its matrix contents were extracted. The unextracted analyte was then washed in a dropwise fashion under vacuum

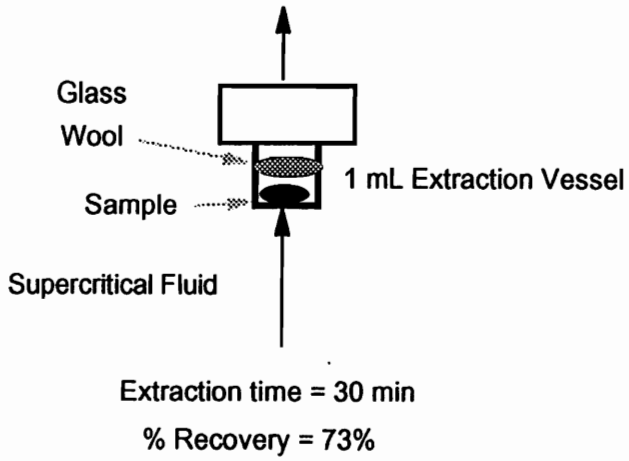


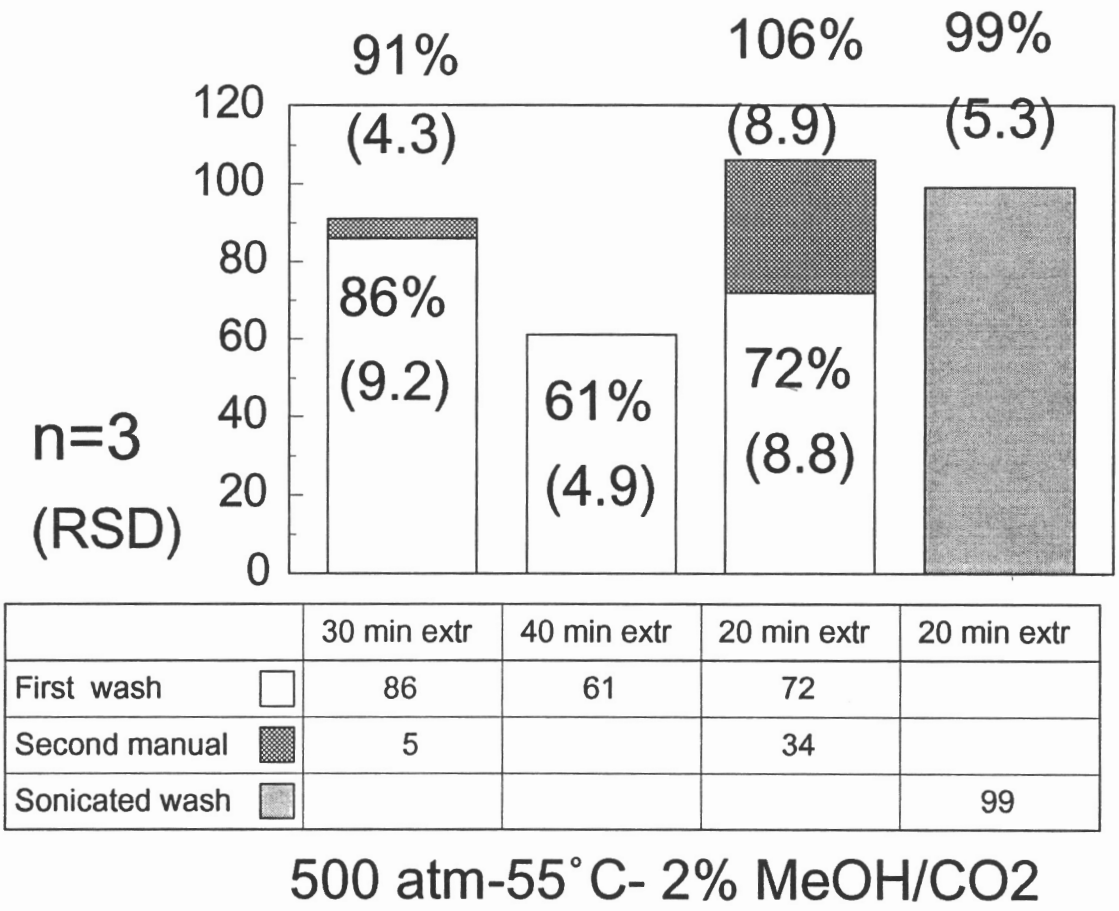
Figure 6. Alternate flow path.

with a minimum of 4 mL 0.01 N NaOH. Subsequent inspection of the cartridge showed that despite copious washing, residual acyclovir remained. The inner vessel was trimmed to approximately 1 inch prior to extraction to improve the washing method. The frits and the polypropylene inner vessel were placed in a 3.7 mL vial fully filled with 0.01 N NaOH and sonicated for 5 minutes utilizing a standard laboratory sonicator. The sonication was repeated with a fresh wash solution with both washes placed into a 10 mL volumetric flask and diluted to the mark. Recoveries were calculated based on an assumed initial concentration of 50 mg of acyclovir/g of Zovirax® Ointment 5%.

RESULTS AND DISCUSSION

SFE of Ointment Samples

The restrictor, described in the Experimental Section, had higher resistance to plugging than other available fixed restrictors as it could be manually opened if plugging occurred and the flow rate reset. Although the partial plugging and unplugging caused flow rates to vary throughout the course of the extraction, this was of little consequence. Triplicate extractions of 100 mg ointment samples were performed at 500 atm and 55°C, with extraction times of 20, 30, and 40 minutes using 2% methanol modified CO_2. The 20 minute extraction assay recovery was 72% with a relative standard deviation (RSD) of 8.8% . The 30 minute extraction resulted in an acyclovir assay of 86% with a RSD of 9.2%. The average assay for the 40 minute extraction was 61% with a RSD of 4.9% (Figure 7).



	30 min extr	40 min extr	20 min extr	20 min extr
First wash	86	61	72	
Second manual	5		34	
Sonicated wash				99

500 atm-55°C- 2% MeOH/CO2

Figure 7. Recovery of acyclovir from Zovirax® by SFE

Sonicated Wash Method

Three possible causes for the low recoveries could be postulated. First, the acyclovir may have been solubilized and extracted by the supercritical fluid. Attempted extraction of neat drug, however, confirmed the drug was insoluble at the extraction conditions. A second possibility of acyclovir loss was by mechanical transport out of the SPE tube; however no evidence of this was observed during the extraction of the neat drug. A third explanation could be that the acyclovir in the vessel insert was not being effectively transferred for subsequent analysis. A second flow path (Figure 6) was developed to investigate this hypothesis. The vessel was washed with 8 mL 0.01 N NaOH, twice the solvent previously used, following a 30 minute extraction. Although recovery was 73%, acyclovir was found on the glass wool when the vessel was opened and inspected incicating the wash method was inadequate. Reinspection, days later, of a previously washed and dried inner vessel revealed the presence of acyclovir. This residue was not evident after the initial wash. Analysis of the additional drug in the insert increased the total recovery for the 30 minute extraction from 86% to 91% with a RSD of 4.3% (Figure 6). The 20 minute recovery likewise increased from 72% to an average of 106% with a RSD of 8.9% (Figure 7). These results suggested that a revision in the washing method was needed and that a 20 minute extraction using 2% methanol modified CO_2 was sufficient to remove the matrix components.

Subsequently, a second set of triplicate, 20 minute extractions with 2% methanol modified CO_2 was performed consuming approximately 15 mL of supercritical fluid. A washing method employing sonication was performed on the inner vessel containing raffinate with two 3 mL aliquots of 0.1 N NaOH solution. Average recovery for the triplicate extractions was 99% with a RSD of 5.3%.

SUMMARY

This study confirms that acyclovir can be isolated from its matrix components by employing 2% methanol modified CO_2 in 20 minutes. Quantitative recovery of the non-extracted acyclovir from the vessel insert can be performed by implementation of a sonicated washing technique with analysis by HPLC/UV.

Chapter IV
Method Development for the Quantitation of Polyaromatic Hydrocarbons from Water Via Solid Phase Extraction with Supercritical Fluid Elution

INTRODUCTION

Environmental awareness has shifted from pollution clean-up to prevention over the last decade . An example of this change in awareness is the U.S. Environmental Protection Agency (EPA) Industrial Toxic 33-50 Program that plans to reduce the use of many traditional solvents and chemicals by 50% before 1995. Methylene chloride, which has been identified as a possible carcinogen, is among the solvents subjected to regulation. Methylene chloride has been widely used in liquid-liquid extraction of aqueous matrices. Several hundred milliliters of solvent are required for this extraction process resulting in high volume, dilute analyte solutions. Solvent reduction is required before assay of the analyte which ultimately releases the solvent into the environment.

The principles of liquid chromatography (LC) have been applied to extraction technology over the last several years. It is in this context that the concepts of solid phase extraction (SPE) and solid phase microextraction (SPME) (21) have been developed as an alternative to liquid-liquid extraction. Traditional SPE technology employs LC stationary phases to collect and concentrate analytes from solution. The analytes are then washed or eluted from the stationary phase by an organic solvent. One to five hundred milligrams of the stationary phase is placed into a polypropylene cylinder. These cylinders are a few millimeters in diameter, have volumes of 1 to 5 mL and are referred to as cartridges. These cartridges work well for small, relatively clean samples, but are subject to channeling and plugging with high particulate samples.

The solid phase extraction disk was developed to accommodate the high volume and high particulate load typical of ground and surface water samples. Disks, which are approximately 0.5 millimeter thick, resemble a membrane or filter, and are available in 47 or 90 millimeters diameters. The disks are capable of extracting large volumes of water; however, elution requires a minimum of 15 mL of solvent for clean water samples and considerably more rinse solvent for high particulate water samples to recover trapped analytes. Despite requiring significantly less organic solvent than liquid-liquid extractions, a SPE disk solvent reduction step is required before assay. Both cartridges and disks are available in several types of stationary phases enabling SPE to be applicable to many classes of analytes.

Solid phase microextraction employs a 1 cm bonded fused silica fiber for an analyte collection device. The fiber is bonded to the plunger of a GC type syringe. The fiber can be extended from the syringe needle for sampling or can be withdrawn within the needle thus allowing the fiber to be passed through the septum of a GC injector port. The fiber is extended from within the needle after it is in the injector port to allow thermal desorption of the analytes. This analysis method is reported to provide quick, solventless screening of polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) from clean water samples.

Supercritical fluid extraction (SFE) has been utilized for the extraction of many environmental matrices (1,22-25). Advantages of supercritical fluids are very low surface tension, low viscosity and high diffusivity which guarantees fast mass transfer, when compared to liquid solvents (26). Another important advantage of a SF is that solvent strength can be modified by varying the pressure and/or temperature of the fluid. Most supercritical fluids are gases at ambient conditions and have mild critical parameters thus enabling analyte recovery and concentration to occur as the SF decompresses into a pre-

arranged solvent or solid sorbent trap. Compounds possessing these parameters include nitrous oxide, butane, freon®, pentane and carbon dioxide. The explosive potential of nitrous oxide makes it too dangerous for routine extraction of organic materials as reported by Sievers (27) and Raynie (28). Carbon dioxide is the most common supercritical fluid because in addition to having mild critical parameters (P_c = 72 atm, T_c = 32°C), it is chemically inert, non-toxic and noncombustible. Supercritical fluid carbon dioxide is a non-polar solvent and therefore is best able to solvate non-polar analytes. The polarity and solvating power of SF CO_2 can be increased by adding a small amount of organic modifier which increases the polarity range of the analytes that the SF can solvate.

The application of analytical supercritical fluid extraction to environmental matrices has focused almost exclusively on solid matrices, although a few reports have appeared concerning direct liquid-fluid extraction. Hedrick et al., for example, have demonstrated a method for direct extraction of phenols, phosphonates and nitrogenous bases from water using a modified 7 mL extraction vessel (29-31). Although Hedrick's direct liquid-fluid method is useful for very small samples with highly concentrated analytes, it is not applicable to environmental water samples for the following reasons. Matrix mobility can be a problem as water is slightly soluble in SF CO_2 to approximately 0.1% (w/w) and mechanical transfer of matrix can easily occur because water is a liquid. The amount of CO_2 needed to extract a 1 liter water sample may also be prohibitive since it has been suggested that exhaustive extraction may require up to 7 sample volumes of SF CO_2 (32). Additionally, as CO_2 contacts water, the pH of the matrix is reduced which may complicate the extraction of basic analytes (4).

An alternative to direct SFE of aqueous samples is to use supercritical fluids to elute SPE disks. SFE elution reduces further the need for organic solvents and eliminates the requirement for a solvent reduction step. Supercritical fluid elution of a solid phase

disk has been successfully employed for the recovery and quantitation of two polar analytes from water, sulfometuron methyl and chlorsulfuron, (33). Recoveries >90% and relative standard deviations (RSDs) < 8% were reported using a C-8 disk as a deposition medium. This study (33) stressed the importance of matrix pH for proper deposition and release of polar analytes. The relationship of modifier and trap temperature on an inert solid phase trap was also investigated.

A study investigating the feasibility of simultaneous SF elution of a large number of analytes from SPE cartridges was initiated by Ho and Tang (34). They used a statistical 2^3 factorial experimental design to optimize the elution of 29 PAHs and organochlorine pesticides which had been fortified onto a 500 mg C-18 cartridges. The effects of pressure, temperature, and extraction time on the SF elution were studied. They concluded that these analytes could be extracted from fortified cartridges at a pressure of 350 to 400 atm with extraction times of 20 to 35 minutes. Ho and Tang stated that adding a small amount of organic modifier (methanol) directly to the analyte laden cartridge was necessary for efficient recovery of some of the PAHs. This study employed 2 mL of liquid acetone as a trap.

Tang et al. continued work in this area with an extensive study of the combined elution of polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalate esters and organochlorines contained in reagent water (32). These analytes were used to investigate the effects of temperature, extraction mode, modifier, and residual SPE trap water on the SF elution of both C-18 bonded silica disks and cartridges. Disks were more efficient than cartridges for the analytes and sample sizes studied. Extraction temperature in the 50° - 100°C range had little effect on recovery. Initially, 400 µL of methanol was added to the extraction vessel as a CO_2 modifier. Later, it was determined that the methanol did not directly enhance the extraction of analytes, but reduced fixed

capillary restrictor plugging. Many of the analytes studied by Tang et al. are from Method 525.1 analytes which concerns the extraction of semi-volatile compounds from water. The Method requires recoveries of 70-130% and RSDs <30% for fortified water samples. Applying the Method 525.1 criteria to their SFE elution, 11 of 47 analytes were outside the EPA criteria for recoveries and 7 analytes had RSDs >30%.

The overall goal of this project is to study recovery of EPA Method 525.1 analytes (40) from Chesapeake Bay water by disk SPE followed by SF elution. A preliminary study involving recovery of 16 PAHs from fortified distilled water (e.g. naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene) will be discussed in this chapter. PAHs were chosen because they possessed the entire range of analyte volatilities presented in EPA Method 525.1, and because quantitation of PAHs themselves, were of interest per EPA Method 550.1.

EXPERIMENTAL

Supercritical Fluid Extraction (SFE)

Extractions were performed on a Hewlett Packard (HP) (Avondale, PA) 7680T Supercritical Fluid Extractor (SFE) using a 7 mL vessel. The extractor was interfaced with a HP 1050 quaternary liquid chromatography (LC) modifier pump. Modifier was added to the liquid CO_2 stream via a zero dead volume tee fitting. Extracted samples were transferred to the gas chromatographic autosampler manually or by use of HP "Bridge Software" that enabled automatic sample transfer and mass spectrometric assay.

Air Products and Chemicals Inc. (Allentown, PA) provided pure (SFE/SFC grade) CO_2. Polyaromatic hydrocarbon standards were obtained from Supelco (Bellefonte, PA). All analytes were diluted to 100 ppm in acetone for matrix fortification. Solvents were at least HPLC grade or better and were obtained from Fisher Scientific (Fair Lawn, NJ)

Solid Phase Extraction (SPE)

Empore™ disks of 47 mm diameter (3M, St. Paul, MN) were used as a sorbent for deposition of analytes from water. Empore™ disk are membranes consisting of C-18 bonded silica particles meshed with a Teflon® weave. Disks were washed and conditioned to ensure proper deposition and maximum retention of analytes by removing contaminants that were introduced during the manufacturing, packaging and handling process. The wash step involved placing the disk in a filtration apparatus and adding 10 mL of methylene chloride. A slight vacuum was used to initiate the flow of solvent. The vacuum was then released and the disk is allowed to soak for three minutes. Next air was drawn through the disk for 1 minute. The disk was conditioned to solvate the C-18 chain immediately following this step. It is important to note, that the disk was not allowed to go dry during the conditioning process to insure proper solvation of the C-18 chains. Conditioning was accomplished by adding 10 mL of methanol to the disk followed by momentary application of a slight vacuum to initiate the flow of solvent. The disk was allowed to soak for 10 minutes. Ten milliliters of HPLC grade water is added to the disk while it was still covered by a thin film of methanol. The disk was ready for immediate sample deposition after approximately half of the volume was pulled through the disk. . One liter fortified distilled water (not pH adjusted) samples were passed through the Empore™ disk in approximately ten minutes using a Varian, 6 station, SPE manifold with

20-25 inches Hg vacuum. After deposition, the disks were dried by drawing air through them for 5 to 10 minutes. All extractions were performed in triplicate and all one liter fortified matrices were spiked at 2 μg/component. Naphthalene-d8, phenanthrene-d10, chrysene-d12 internal standards were added to the sample water prior to analyte deposition onto the disk, unless otherwise stated. All sample bottles were pretreated with a solution of 10% dichlorodimethylsilane in toluene to reduce analyte adhesion.

The dried Empore™ disk containing the concentrated analytes was packed into the 7 mL extraction vessel by first adding approximately ¼ inch of Ottawa sand (Fisher Scientific, Fair Lawn, NJ) to the bottom of the extraction vessel. The disk was then rolled into a cylinder shape and placed in the vessel. Finally, the remaining void volume was completely filled with sand, and the vessel inverted for extraction. See Figure 8 for SPE apparatus and SPE/SFE recovery method diagram.

Quantitation

Assay of the extracts employed a HP 5890 Series II gas chromatograph interfaced to a HP 5971A mass spectrometer (GC/MS), and a J & W (Folsom, CA) 0.25 mm i.d. X 0 m DB-5MS column with a 0.25 μm film thickness and the He carrier gas was held at a linear velocity of 36 cm/second. The GC method employed splitless injection of 2 μL with a purge after one minute and an injector temperature of 250°C. The initial GC oven temperature was as follows: initial temperature of 45°C with a 1 minute hold followed by a 45°C/minute temperature ramp to 160°C; the ramp was reduced to 6°C/minute and a final temperature of 320°C with a 1 minute hold. The MS transfer line held at 280°C. the MS was tuned in two ways prior to use; first, the standard tune followed by a decafluorotriphenyl phosphate (DFTPP) tune. The DFTPP has been specified for use

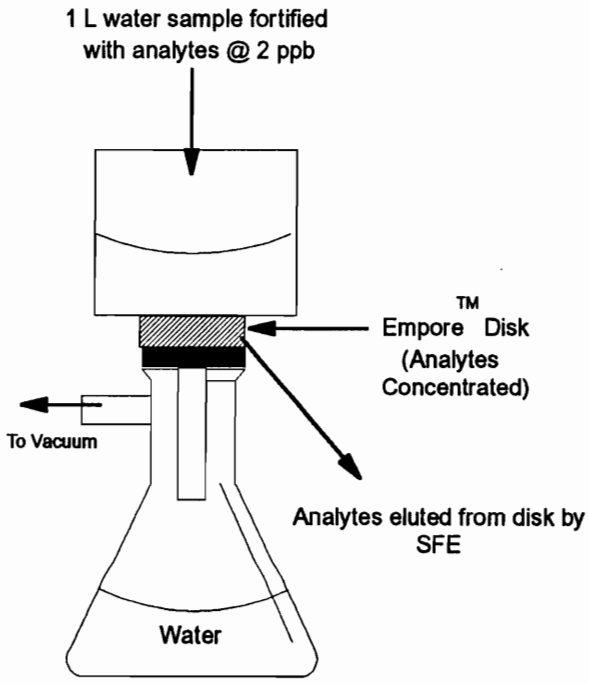


Figure 8. SPE appartus and SPE/SFE recovery method

with EPA method 525.1 and PAHs as it has increased MS sensitivity for the lower mass range. The MS scanned a range from 50 to 450 m/z for data collection; quantitation was by ion extraction. Specific ions employed for quantatition of each analyte are shown in Appendix II. Four point calibration curves with concentrations from 1 μg/mL to 10 μg/mL produced correlation coefficients of 0.995 or greater for all analytes. Analytes and internal standards were fortified into the water samples and three calibration vials. The solution in the vials was diluted to 1 mL with methylene chloride and served to determine the 100% recovery level. The daily determination of 100% levels compensated for minute changes in MS response.

Initial SFE Method

Gere et al. have previously reported on the SFE of PAHs from sediment. Their extraction strategy involved three steps and modified CO_2 (35). This extraction strategy was chosen because exclusive use of a modifier in a one step supercritical fluid extraction was believed based upon previous studies to interfere with solid phase trapping of volatile analytes (4). The Gere study has been expanded to the extraction of sorbent disks by employing the following philosophy: the first step was designed to extract and recover volatile compounds; the second step to extract less volatile compounds; and the third step to remove residual modifier from the instrument prior to the succeeding extraction.

The SFE initial conditions were as follows:

Step 1: 1 minute equilibration at 2600 psi (179 bar) with 2 mL/minute pure liquid CO_2 for 10 minutes and chamber temperature at 120°C. The ODS trap was held at 5°C during extraction and raised to 25°C for rinsing. The trap was rinsed with ≈ 0.6 mL of methylene chloride.

Step 2: 1 minute equilibration at 5100 psi (352 bar) using 4 mL/minute 5% (v/v) acetone modified CO_2 for 30 minutes and chamber temperature at 120°C. Trap temperature increased to 80°C during the extraction. The trap was not rinsed at the end of this step.

Step 3: 1 minute equilibration using 4 mL/min. unmodified CO_2 for 10 minutes. Trap temperature at 5°C during extraction and 25°C during the rinse cycle employing 0.6 mL methylene chloride as trap rinse. The total extraction time was **53 minutes.**

RESULTS AND DISCUSSION

Quantitation--Absolute versus Relative

The initial investigation studied the difference in absolute versus relative quantitation methods. For absolute quantitation, the internal standards were added post extraction to the SFE trap rinse solution. Absolute quantitation refers to the amount of analyte recovered from a combination of the extraction step, trap step and rinse step. On the other hand, with relative quantitation the internal standards are added to the water sample bottle prior to SPE/SFE. Analyte loss during the extraction process can be compensated for by selecting internal standards that act chemically similar to the analytes. For example, if analytes loss is due to adherence to the sample container, an equivalent amount of internal standard is also lost. Analytes, therefore, are quantified relative to the actual amount in the water rather than to the amount spiked into the rinse. Generally, SFE of solids and semi-solids is quantitated on an absolute scale, while water samples are quantitated on a relative scale. Sample bottles which contain the water are rinsed with extracting solvent as part of the disk elution process in order to remove any PAHs which might have adhered to the walls of the flask, while SPE cartridges or disks are being eluted with traditional solvents. This type of step is not possible with SFE elution

The method described in "Experimental" was used for elution of the disk in the initial study. Recoveries of the low molecular weight PAHs were quantitative within the EPA guidelines (70-130%) using absolute quantitation (i.e. adding internal standards to the SFE trap rinse); however, recoveries of the high molecular weight PAHs were only about 50% and RSDs were high (Table I). Subsequent rinsing of the sample bottles with a chlorinated solvent showed that significant amounts of PAHs had adhered to the glass, with recoveries of the bottle alone at nearly 40% for the high molecular weight PAHs (Table II).

Initial SPE/SFE

Employing the relative quantitation method and the exact same SPE/SFE procedure, recoveries for all PAHs (except anthracene) were above 80% (Table III). The low recovery for anthracene even for relative quantification is believed to be related to the the disk drying procedure. The disk was dried for up to 10 minutes at 20-25 inches Hg in initial experiments. It has been reported, that anthracene is much more susceptible to photo-oxidation when placed on silica gel (36). Later, the drying time was held rigidly to 5 minutes which led to quantitative recoveries for anthracene (Table V). On the basis of these results, the PAHs were quantified by the relative method since our interest was the quantity of PAHs actually in the water.

Attempts were made to more fully optimize the SF elution method relative to the initial 53 minute extraction while maintaining quantitative (>90) recoveries for all analytes. Parameters of interest were flow rate, temperature, pressure and time. The initial goal was to reduce the SF elution time by ten minutes. This was accomplished by reducing the second step from 30 to 27 minutes and the third step from 10 to 3 minutes. This elution

Table I. SF elution of PAHs from water via C-18 disk @ 2 ug/component applying absolute quantitation.

Compound	%Recovery	RSD
Naphthalene	91	5.0
Acenaphthylene	82	4.5
Acenaphthene	87	5.7
Fluorene	83	10
Phenanthrene	85	15
Anthracene	68	18
Fluoranthene	77	19
Pyrene	77	18
Benzo[a]anthracene	53	14
Chrysene	52	15
Benzo[b]fluoranthene	55	17
Benzo[k]fluoranthene	51	15
Benzo[a]pyrene	49	15
Indeno[1,2,3-cd]pyrene	47	23
Dibenzo[a,h]anthracene	50	19
Benzo[g,h,i]perylene	49	13
n=3 Average	66	14

SFE conditions:

Step 1: 1 minute equilibration @ 2600 psi, 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 1 minute equilibration @ 5100 psi, 30 minute dynamic extraction with 5% acetone modified CO_2 @ 4 mL/minute. No rinse. Trap at 80°C for extraction.
Step 3: 1 minute equilibration @ 5100 psi, 10 minute dynamic extraction with CO_2 @ 4 mL/minute. The ODS trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 120°C.

Table II. Recoveries of PAHs from empty sample bottles via methylene chloride rinse after SPE of fortified water sample.

Compound	%Recovery	RSD
Naphthalene	0	-
Acenaphthylene	0	-
Acenaphthene	0	-
Fluorene	5	7.8
Phenanthrene	8	14
Anthracene	20	15
Fluoranthene	17	24
Pyrene	17	26
Benzo[a]anthracene	35	12
Chrysene	38	15
Benzo[b]fluoranthene	36	10
Benzo[k]fluoranthene	37	12
Benzo[a]pyrene	35	14
Indeno[1,2,3-cd]pyrene	23	24
Dibenzo[a,h]anthracene	32	16
Benzo[g,h,i]perylene	15	37

Table III. SF elution of PAHs from water via C-18 disk @ 2 ug/component applying relative quantitation.

Compound	%Recovery	RSD
Naphthalene	106	2.1
Acenaphthylene	96	11
Acenaphthene	105	6.2
Fluorene	114	4.5
Phenanthrene	98	4.5
Anthracene	58	1.6
Fluoranthene	103	2.1
Pyrene	96	0.7
Benzo[a]anthracene	105	5.4
Chrysene	100	8.4
Benzo[b]fluoranthene	97	9.1
Benzo[k]fluoranthene	97	9.7
Benzo[a]pyrene	91	10
Indeno[1,2,3-cd]pyrene	85	8.0
Dibenzo[a,h]anthracene	80	9.9
Benzo[g,h,i]perylene	91	9.6
n=3 Average	95	6.6

SFE conditions:

Step 1: 1 minute equilibration @ 2600 psi, 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 1 minute equilibration @ 5100 psi, 30 minute dynamic extraction with 5% acetone modified CO_2 @ 4 mL/minute. No rinse. Trap at 80°C for extraction.
Step 3: 1 minute equilibration @ 5100 psi, 10 minute dynamic extraction with CO_2 @ 4 mL/minute. The ODS trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held 120°C.

method provided an average recovery and RSD of 90% and 4.9% respectively. With the exception of anthracene, recoveries and RSDs for all analytes were well within the acceptable range (Table IV). With the exception of anthracene, there was no statistical difference between the numbers in Tables III and IV (61). Next, an attempt to further reduce the SF elution time to 33 minutes was made by decreasing the second step from 27 to 17 minutes. All other parameters remained constant. This experiment failed to produce quantitative recoveries for all analytes.

Effects of Temperature and Flow

The effects of changes in chamber temperature and flow rate were next studied employing the **43 minute** SF elution method. An extraction temperature of 120°C which was used in the initial 43 minute extraction has been reported by Tang et al. (34) to be unnecessary. Lowering the temperature to 80°C and keeping all other parameters constant gave PAHs recoveries ≥94% and RSDs <6%. Total amount of CO_2 used was reduced by lowering the flow rate in the second step from 4 mL/minute to 2 mL/minute while maintaining chamber temperature at 80°C. Recoveries for all the compounds were acceptable, but RSDs were significantly higher than in the previous experiment (Table V).
It was concluded that any further reduction in extraction time or flow rate under these conditions would probably adversely affect the recoveries and RSDs.

Table IV. 43 minute SF elution of PAHs from water via C-18 disk @ 2 ug/component with chamber temperature @ 120°C

Compound	%Recovery	RSD
Naphthalene	101	0.9
Acenaphthylene	94	4.7
Acenaphthene	107	3.8
Fluorene	116	2.2
Phenanthrene	95	0.9
Anthracene	52	3.5
Fluoranthene	96	2.9
Pyrene	93	3.4
Benzo[a]anthracene	96	0.8
Chrysene	97	2.9
Benzo[b]fluoranthene	87	1.5
Benzo[k]fluoranthene	85	3.7
Benzo[a]pyrene	77	7.0
Indeno[1,2,3-cd]pyrene	83	13
Dibenzo[a,h]anthracene	70	16
Benzo[g,h,i]perylene	99	10
n=3 Average	90	4.9

SFE conditions:
Step 1: Pressure @ 2600 psi, 1 minute equilibration, 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: Pressure @ 5100 psi, 1 minute equilibration, 27 minute dynamic extraction with 5% acetone modified CO_2 @ 4 mL/minute. Trap at 80°C for extraction. No rinse.
Step 3: Pressure @ 5100 psi, 1 minute equilibration, 3 minute dynamic extraction with CO_2 @ 4 mL/minute. Trap at 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 120°C throughout.
Total SF elution: 43 minutes.

TableV. 43 minute SF elution of PAHs from water via C-18 disk @ 2 ug/component with chamber temperature @ 80°C

Compound	%Recovery	RSD
Naphthalene	108	3.8
Acenaphthylene	103	2.8
Acenaphthene	105	2.2
Fluorene	116	2.6
Phenanthrene	103	1.7
Anthracene	94	2.1
Fluoranthene	103	1.1
Pyrene	97	0.4
Benzo[a]anthracene	100	1.3
Chrysene	109	14
Benzo[b]fluoranthene	120	8.4
Benzo[k]fluoranthene	98	11
Benzo[a]pyrene	98	3.6
Indeno[1,2,3-cd]pyrene	110	17
Dibenzo[a,h]anthracene	94	13
Benzo[g,h,i]perylene	98	8.3
n=3 Average	103	5.8

SFE conditions:

Step 1: Pressure @ 2600 psi, 1 minute equilibration, 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).

Step 2: Pressure @ 5100 psi, 1 minute equilibration, 27 minute dynamic extraction with 5% acetone modified CO_2 @ 4 mL/minute. No rinse. Trap at 80°C for extraction.

Step 3: Pressure @ 5100 psi, 1 minute equilibration, 3 minute dynamic extraction with CO_2 @ 4 mL/minute. Trap at 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). **Chamber temperature was reduced from 120° to 80°C** throughout.

Total SF elution: 43 minutes.

Effects of Density

An amended approach was considered to further optimize the SFE method. Initially, the first step was designed to remove only the most volatile, low molecular weight analytes, (i.e. those analytes that may be lost by a solid phase trap if the extraction employed a modified CO_2 fluid). This was initially accomplished by using a moderate extraction pressure (2600 psi). However, use of a higher pressure (e.g. greater density) during this step should enable extraction of not only the lower molecular weight analytes but also the middle and some higher molecular weight analytes as well. The second step of the SF elution should recover the remaining higher molecular weight or matrix bound analytes. The third step of the SF elution would again serve to remove residual modifier from the instrument. The chamber temperature was held at 80°C throughout the elution. Compared to the method described earlier (Table III), the combination of higher pressure and lower temperature will cause the CO_2 density to increase in the first step from ≈ 0.35g/mL to ≈0.81g/mL and in the second and third steps from ≈0.65 g/mL to ≈0.81 g/mL. This should significantly increase the solvating power of the fluid and thus decrease the extraction time. The new method was as follows:

Step 1: 1 minute equilibration time, pressure at 5500 psi, with a CO_2 flow rate 2 mL/minute for 10 minutes. The ODS trap was held at 5°C for extraction and raised to 25°C for rinsing with 0.6 mL of methylene chloride.

Step 2: 1 minute equilibration time, pressure at 5500 psi with a 5% (v\v) acetone modified CO_2 flow rate 2 mL/minute for 10 minutes. The ODS trap was held at 80°C for extraction. The trap was not rinsed after this step.

Step 3: CO_2 (5500 psi) flow rate 4 mL/minute for 3 minutes. The ODS trap was held at 80°C for extraction and lowered to 25°C for rinsing with 0.6 mL of methylene chloride. Chamber temperature was held at 80°C throughout the extraction. Total extraction time was **25 minutes**.

The results of this elution method were quite good except for the compounds with molecular weight > 275 amu (Table VI). The average recovery was 95% with an average RSD of 4.4%.

To obtain quick, quantitative recovery for all PAHs the preceding **25 minute** SF elution method was further optimized by removal of all equilibration times, increasing the second step from 10 to 15 minutes and decreasing the third step from 3 to 2 minutes. Total extraction time was **27 minutes**. The average recovery was 99% with an average RSD of 4.6% (Table VII). This method consumed 2.7 mL of acetone for fluid modification and 1.2 mL of methylene chloride for trap rinsing.

Hildebrand Solubility Parameters

In many environmental samples, such as soot, sludge or sediment, it is probable that several different types of active sites exist on the matrix, consequently prediction of analyte/matrix interaction is near impossible. In our case a clean moist C-18 disk afforded a relatively simple matrix. It is speculated that Van der Waal interactions between the matrix and the analytes were significant. Recovery of analytes from the C-18 disk comparable to the elution of analytes from a liquid chromatographic (LC) column was considered as they possess similar matrix properties. LC theory implies that SF fluid solvent strength is fundamental in the recovery of the analytes. The Hildebrand solubility parameter is a measure of solvent strength that can be used to predict the compatibility of the solvent and a solute. A solubility parameter difference of $\pm$ 2 between the solute and solvent is considered to be reasonable for SFE (37). At ambient conditions, PAHs have a Hildebrand solubility parameter of approximately 10, but the parameter decreases as

Table VI. 25 minute SF elution of PAHs from water via C-18 disk @ 2 ug/component with chamber temperature @ 80°C

Compound	%Recovery	RSD
Naphthalene	109	3.0
Acenaphthylene	111	3.5
Acenaphthene	112	3.5
Fluorene	108	2.7
Phenanthrene	93	8.3
Anthracene	90	1.6
Fluoranthene	105	4.9
Pyrene	102	1.8
Benzo[a]anthracene	94	3.5
Chrysene	95	2.2
Benzo[b]fluoranthene	105	4.4
Benzo[k]fluoranthene	93	1.3
Benzo[a]pyrene	90	6.4
Indeno[1,2,3-cd]pyrene	66	7.9
Dibenzo[a,h]anthracene	75	5.5
Benzo[g,h,i]perylene	77	11
n=3 Average	95	4.4

SFE conditions:
Step 1: 1 minute equilibration, 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 1 minute equilibration, 10 minute dynamic extraction with 5% acetone modified CO_2 @ 2 mL/minute. No rinse. Trap at 80°C for extraction.
Step 3: 3 minute dynamic extraction with CO_2 @ 4 mL/minute. Trap at 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 80°C throughout with pressure held at 5500 psi.
Total SF elution time: 25 minutes.

TableVII. 27 minute SF elution of PAHs from water via C-18 disk @ 2 ug/component with chamber temperature @ 80°C

Compound	%Recovery	RSD
Naphthalene	102	2.2
Acenaphthylene	110	3.3
Acenaphthene	105	1.3
Fluorene	106	2.9
Phenanthrene	92	8.4
Anthracene	91	8.2
Fluoranthene	105	1.6
Pyrene	100	1.0
Benzo[a]anthracene	95	6.0
Chrysene	96	5.6
Benzo[b]fluoranthene	98	4.0
Benzo[k]fluoranthene	100	1.1
Benzo[a]pyrene	101	3.7
Indeno[1,2,3-cd]pyrene	91	7.9
Dibenzo[a,h]anthracene	95	5.8
Benzo[g,h,i]perylene	99	12
n=3 Average	99	4.6

SFE conditions:
Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute. Trap at 5°C for extraction, raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 10 minute dynamic extraction with 5% acetone modified CO_2 @ 2 mL/minute. No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute. The ODS trap was held @ 80°C for extraction, reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 80°C, pressure at 5500 psi throughout.
Total SF elution time: 27 minutes.

temperature increases. (1,38,39). In step 1 of the initial SF elution method, a pressure of 2600 psi and temperature of 120°C was used. At these conditions CO_2 has a solubility parameter of 3.0 and probably only extracted the very low molecular weight PAHs (38). By increasing the pressure to 5500 psi and retaining the temperature at 120°C, the SF solubility parameter was increased to 5.8 (39). Hence, the solubility parameter of the solvent nearly doubled while the solubility parameter of the analytes was held constant. At 5500 psi and 80°C, the solubility parameter was 6.9. At this temperature and solubility parameter, the ability of the SF to extract the larger molecular weight range of PAHs significantly increased. In the second step of the optimized elution method, the use of 5% (v/v) acetone modified CO_2 at 80°C increased the solubility parameter to 8.6. This enabled extraction the of highest molecular weight PAHs. Additionally, it could be speculated that the acetone modified CO_2 was more able to penetrate through residual water that may have been trapping analytes within or on the disk. Finally without temperature control of the solid phase trap, accumulation of PAHs which vary considerably in volatility would not have been possible under variable extraction conditions.

SUMMARY

The recovery and elution of PAHs from water has been achieved by supercritical fluid elution of a SPE disk. This method proved to be significant as it was not only effective but reduced the amount of solvent traditionally required for analyte elution by 75% and eliminated the solvent reduction step. Although this work was conducted using distilled water, there should be no difficulty applying this method to drinking water

samples. Future goals include expansion of this method to all EPA Method 525.1 analytes from surface water samples as well as to marine and estuarine waters.

Chapter V
Recovery of EPA Method 525.1 Analytes from Reagent Water Via Solid Phase Deposition Followed by Supercritical Fluid Elution

INTRODUCTION

The initial phase of the project involved development of a three step SF elution method for the recovery of 16 PAHs from distilled water. All 16 PAHs were recovered at a level > 90% with RSDs < 12%. The first step employed CO_2 as a solvent and was designed to recover the more volatile PAHs. The second step used 5% acetone modified CO_2, and was intended to recover the remaining, less volatile analytes. The third step utilized CO_2, and was designed to remove the residual organic modifier from the extractor. The solid phase C-18 trap was rinsed with 0.6 mL of methylene chloride after the first and third steps. This entire method used 1.2 mL of methylene chloride for trap rinsing and 1.5 mL of acetone for the organic modifier.

The next phase of the research focused on the recovery of 21 semi-volatile analytes such as organochlorines, phthalates and adipate esters. These analytes have not been previously recovered via SPE/SF elution. Subsequently, recovery of 39 EPA Method 525.1 analytes will be discussed including PAHs, PCBs and Test Mix 2.

EPA Method 525.1

The general purpose of EPA Method 525.1 is to specify a procedure for the determination of 43 semi-volatile organic compounds in drinking water at any stage of treatment. The analytes included in this method are selected PAHs, PCBs,

organochlorines, and phthalate and adipate esters. A brief description of the procedure follows.

Both organic analytes and internal standards (IS) are concentrated from a 1 L water sample by passing the sample through a pre-conditioned cartridge or disk containing C-18 bonded silica. Three five mL portions of methylene chloride were used for analyte recovery. Each portion was first used to rinse the sample bottle, and then passed through the C-18 membrane. Residual water was removed from the extraction solvent by passing through anhydrous sodium sulfate. Prior to assay by GC/MS, the elution solvent was reduced under a nitrogen stream to approximately 1 mL. The criteria for EPA Method 525.1 (spiked water samples) require recoveries of 70% to 130% and RSDs < 30%.

EPA Method 525.1 -- Potential Problems

EPA Method 525.1 has been used for the recovery of analytes from several types of water matrices. It has been reported that for high particulate water matrices, up to 60 mL of solvent may be required to elute the cartridge or disk for recovery of the analytes (8). EPA Method 525.1 also references several problem compounds such as phthalate and adipate esters. These esters are widely used commercially as plasticizers and are present in variable quantities in blanks; therefore, quantitation at or below 2 ppb is difficult. Because the level of contamination varies significantly from sample to sample, background subtraction is not recommended.

Other problem compounds that have been identified are pentachlorophenol (PCP) and hexachlorocyclopentadiene (HCCP). PCP is a strong acid and may elute as a broad GC peak (Figure 9, peak 8); however, this was determined not to be a problem. The

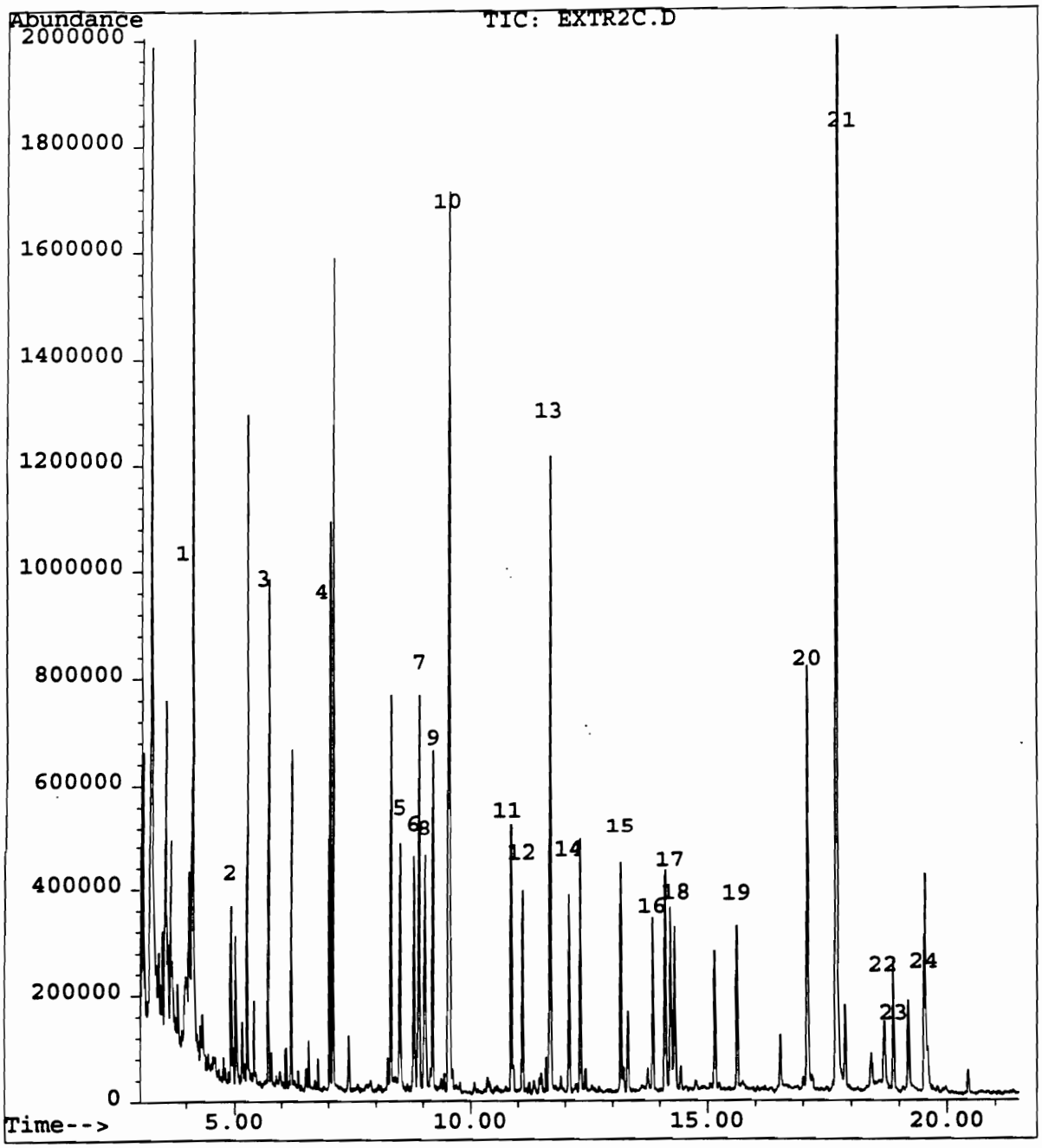


Figure 9. Total ion chromatogram assay of Test Mix 2 via solid phase extraction followed by SF elution. Non-identified peaks are co-extactants and/or interferences. See Table VIII for peak identification.

Table VIII. Test Mix 2 GC/MS retention times

Peak No.	Compound	Retention Time (Min)
1.	Naphthalene-d8--IS	4.12
2.	Hexachlorocyclopentadiene	4.94
3.	Dimethylphthalate	5.73
4.	Diethylphthalate	7.02
5.	Hexachlorobenzene	8.51
6.	Simazine	8.81
7.	Atrazine	8.92
8.	Pentachlorophenol	9.04
9.	Lindane	9.21
10.	Phenanthrene-d10--IS	9.56
11.	Alachlor	10.86
12.	Heptachlor	11.10
13.	Di-N-butylphthalate	11.69
14.	Aldrin	12.09
15.	Heptachlor epoxide	13.18
16.	Gamma-chlordane	13.86
17.	Trans-chlordane	14.23
18.	Trans-nonachlor	14.32
19.	Edrin	15.63
20.	Butylbenzylphthalate	17.10
21.	Di(2-ethylhexyl)adipate	17.71
22.	Chrysene-d12--IS	18.69
23.	Methoxychlor	18.89
24.	Di(2-ethylhexyl)phathalate	19.54

IS = Internal Standard

presence of water in the extraction solvent may also result in high recoveries. Trace amounts of water are believed to change the injection characterics of PCP in the GC (40). HCCP, on the other hand, is susceptible to photochemical and thermal decomposition which would cause recovery to be low.

EXPERIMENTAL

Extractions were performed on a Hewlett Packard (HP) (Avondale, PA) 7680T Supercritical Fluid Extractor (SFE) using a 7 mL vessel. The extractor was interfaced with a HP 1050 quaternary liquid chromatography (LC) modifier pump. Modifier was added to the liquid CO_2 stream via a zero dead volume tee fitting. Air Products and Chemicals Inc. (Allentown, PA) provided pure (SFE/SFC grade) CO_2. Analytes and internal standards were obtained from ChemService (Bellfonte, PA) or from the EPA repository (Cincinnati, OH). All analytes were diluted to 10 ppm in acetone for matrix fortification. Empore™ disks of 47 mm diameter (3M, St. Paul, MN) were used as a sorbent for deposition of analytes from water. The disks were prepared for use according to manufacture's specification

Solvents were at least HPLC grade or better and were obtained from Fisher Scientific (Fair Lawn, NJ). Assay of the extracts employed a HP 5890 Series II gas chromatograph interfaced to a HP 5971A mass spectrometer (GC/MS), and a J & W (Folsom, CA) 0.25 mm i.d. X 30 m DB-5MS column with a 0.25 µm film thickness and He carrier gas.

One liter of fortified distilled water or high performance liquid chromatograph (HPLC) grade water (pH = 2) was passed through the Empore™ disk in approximately

ten minutes. Analytes were deposited via a 6 station SPE manifold (Varian, Spring Park, CA) that was evacuated to 20-25 inches Hg by an electric vacuum pump. (Supelco, Bellefonte, PA). After deposition, the disks were dried by drawing air through them for 5 minutes. Extractions were performed in triplicate. All one liter fortified matrices were spiked at 2 µg/component. Naphthalene-d8, phenanthrene-d10, chrysene-d12 internal standards (EPA Depository, Cincinnati, OH) were added to the sample water before analyte deposition onto the disk. All sample bottles were pretreated with a solution of 10% dichlorodimethylsilane in toluene to reduced analyte adhesion (45).

The dried Empore™ disk containing the concentrated analytes was packed into the 7 mL extraction vessel by initially adding approximately ¼ inch of Ottawa sand (Fisher Scientific, Fair Lawn, NJ) to the bottom of the extraction vessel. The disk was then rolled into a cylinder shape and placed in the vessel. Finally, the remaining void volume was completely filled with sand, and the vessel inverted for extraction.

Procedures for Recovery

The emphasis of this research was on the development of a SF elution method for EPA Method 525.1 analytes. It is important to note that this analyte recovery method requires a multi-step procedure with each step being as critical as the next. The entire procedure is outlined below.

Water Preparation

Steps 1-6 (Table IX) address the water sample preparation procedures required to provide appropriate levels of analytes and internal standards for deposition onto the disk.

Although steps 1,2,5,6 are self explanatory, steps 3 and 4 necessitate clarification. The reduction of pH in step 3 is required to inhibit biological growth in water samples, thus preventing the destruction of analytes by biological means. The addition of methanol in step 4 is designed to ensure proper flow paths through the disk by "wetting" the bonded C-18. This procedure assists in preventing channeling of water through the disk.

Analyte Concentration on the Disk

Analytes are concentrated onto the disk from the water in steps 7, 8 and 9 (Table IX). Step 7 removes contaminates that may have been introduced during manufacturing, packaging, etc of the disk. Solvation refers to the "wetting" of the bonded C-18. Rinsing with water is performed to remove excess methanol. Step 8 refers to analyte deposition: the water sample is passed through the disk under a vacuum of 20-25 inches Hg. A 1 L sample of distilled water can be passed through the disk in approximately 10 minutes, while a high particulate sample may exceed 3 hours. After the water sample is deposited, the disk is dried by passing air through the disk for 5 minutes. The drying process is performed to remove excess water from the disk that may interfere with the elution process.

SF Elution

After the analytes have been concentrated onto the disk, the SF elution process removes the analytes from the disk and ultimately affords a finished solution ready for assay. The SF elution can be broken down into 3 sub-steps as illustrated above in Table IX, Step 11. The first substep removes the analytes from the disk. This process is

Table IX. Procedure for recovery of EPA Method 525.1 analytes employing supercritical fluid elution

1. Measure the 1L sample.
2. Fill the sample bottle.
3. Acidify the water to pH 2.
4. Add 5 mL of methanol.
5. Spike the analytes.
6. Spike the internal standards.
7. Prepare the disk:
    a) Wash with 10 mL of methylene chloride.
    b) Solvate with 10 mL of methanol.
    c) Rinse with 10 mL of HPLC grade water.
8. Deposit the analyte onto the disk.
9. Dry the disk.
10. Insert the disk into the SF vessel.
11. SF elution:
    a) Remove analytes from the disk by SF.
    b) Trap the analytes.
    c) Rinse the trap.
12. Assay by GC/MS.

controlled by the interaction of the analytes with the disk, as well as the amount, density, temperature and type of SF solvent utilized. The second substep involves solid phase trapping of the eluted analytes. Parameters affecting solid phase trapping include the type of solid phase, the temperature of the trap, and the chemical and physical characteristics of the analytes. Trapping will be discussed in greater detail later in the chapter. Finally, after the analytes have been trapped they must be eluted from the trap by a solvent. To avoid a subsequent solvent reduction step, only a small volume of solvent should be employed. No further treatment of the trap eluent is required before assay.

A three step SF elution similar to that used for PAHs was found to be most successful and an outline of the method is described below. Parameters that are highlighted varied throughout the course of the investigation. Specific conditions for these variables will be further defined for individual experiments as needed. All other parameters were held constant.

General SF Elution Method

Step 1: Designed for the recovery of the most volatile analytes. **0-1 minute equilibration time** at 5500 psi followed by a 10 minute extraction with a flow of 2 mL/minute (liquid) of CO_2. ODS trap temperature was held at **≤5°C** during the extraction and increased to 25°C for rinsing with 0.6 mL of methylene chloride.

Step 2: Designed for the recovery of less volatile analytes. **0-1 minute equilibration time** at 5500 psi followed by a **≥10 minutes** extraction with **≥5%** acetone modified CO_2 at a flow rate of 2 mL/minute (liquid). The trap was held at 80°C during the extraction and was not rinsed.

Step 3: Designed for the removal of residual organic modifier from the extractor. **0-1 minute equilibration** at 5500 psi followed by a **≤3** minutes extraction time with a flow rate of 4 mL/minute of pure CO_2 (liquid). The ODS trap was held at 80°C during the extraction and reduced to 25°C. The chamber temperature was

held at ≥80°C with nozzle temperature at 60°C throughout the extraction. The nozzle temperature was then reduced to 25°C for rinsing.

GC/MS

Assay of the extracts was achieved by GC/MS. The chromatograph separates extract solution into individual components. The MS quantifies and identifies each of the components by defining both retention time and ion extraction of the spectra.

RESULTS AND DISCUSSION

Preliminary Study -- Recovery of Organochlorines and Phthalates

The twenty-one compounds initially investigated in this phase of the work are listed in Table VIII and will be designated as "Test Mix 2." The retention times and peak number specifications correspond to the chromatogram shown in Figure 9. Organochlorine compounds used as pesticides and wood preservatives, and common phthalates and adipates used as plasticizers, were included in this study. Each analyte was spiked into distilled water at a concentration of 4 ppb/component with internal standards at 8 ppb/component. Recoveries and RSDs were evaluated on the basis of EPA 525.1 Method criteria. The SF elution method employed here was previously used during the development of a final method for the recovery of PAHs (Table VI, Chapter IV). The variables noted in the General SF Elution Method are defined below:

Supercritical Fluid Elution Method

Step 1: 1 minute equilibration time at 5500 psi with a 10 minute extraction time.

Step 2: 1 minute equilibration time at 5500 psi with a 10 minute time extraction using 5% acetone modified CO_2.

Step 3: 1 minute equilibration at 5500 psi with a 3 minute extraction time. Chamber temperature was held at 80°C throughout. All other parameters are as outlined in the Experimental section .

Results from this study are shown in Table X. Satisfactory recoveries were obtained for 14 of the analytes. Compounds that produced unsatisfactory results included hexachlorocyclopentadiene (HCCP) and hexachlorobenzene (HCB), with recoveries of 51% and 58% and RSDs of 11% and 21% respectively. High recoveries were obtained for alachlor (162%) and for several of the common plasticizer compounds. Di-n-butylphthalate, butylbenzylphthalate, and di(2-ethylhexyl)adipate produced recoveries of 200%, 151% and 328% respectively. The high recoveries for these analytes resulted from contamination of our water supply. Steps taken to reduce these contaminants will be discussed in a later section.

Both HCCP and HCB compounds had short GC retention times which indicate they are relatively volatile compounds. HCCP and HCB compounds are more polar than the PAHs studied here but with similar retention times, therefore these two compounds may have less interaction with the C-18 trap. Their low recoveries can be attibuted to two possible factors: inadequate solid phase trapping after the elution from the disk, or insufficient solubility in the SF resulting in poor extraction.

Table X. Recovery of phthalate and organochlorines from water via C-18 disk with subseqent SF elution @ 4 ug/component.

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	51*	11
Dimethylphthalate	97	7.4
Diethylphthalate	118	9.6
Hexachlorobenzene	58*	21
Simazine	73	8.9
Atrazine	109	7.3
Pentachlorophenol	117	10
Lindane	111	6.6
Alachlor	162*	8.9
Heptachlor	103	8.4
Di-N-butylphthalate	200*	7.6
Aldrin	93	8.8
Heptachlor epoxide	120	10
Gamma-chlordane	116	16
Trans-chlordane	100	9.8
Trans-nonachlor	100	5.4
Edrin	118	39*
Butylbenzylphthalate	151*	7.8
Di(2-ethylhexyl)adipate	328*	43*
Methoxychlor	113	12
Di(2-ethylhexyl)phathalate	91	8.9

SFE conditions:

Step 1: 1 minute equilibration, 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at 5°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).

Step 2: 1 minute equilibration, 10 minute dynamic extraction with 5% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.

Step 3: 3 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap at 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 80°C with pressure held at 5500 psi throughout .

Total SF elution time: 25 minutes.

n=3

* = Outside EPA Criteria

Solid Phase Trapping

As stated previously, the first step of the SF elution method is designed to recover the more volatile compounds. Therefore, a strategy for improving the recovery of HCCP and HCB could focus on improving their trapping. Solid phase trapping can employ inert or active surfaces. Materials considered inert for the purposes of solid phase trapping are glass and stainless steel beads. Liquid chromatographic column packing materials are among the most popular active solid phase traps. Although the C-18 phase is the most popular, other materials include C-8, cyano and amino phases. The C-18 packing which is frequently used as a general purpose trap, interacts with analytes through dispersion forces. The interactions are greatest with non-polar, polarizable analytes such as aliphatic and polyaromatic hydrocarbons.

Another important parameter for solid phase trapping is temperature. (4) High temperatures may be necessary when utilizing modified CO_2. A trap temperature in excess of the boiling point of the organic modifier, will force the modifier into a gaseous phase thus preventing the condensed liquid organic modifier from stripping analytes from the trap. The use of below ambient trap temperatures is called cryo-trapping, and can be useful in increasing recovery of volatile analytes. Cryo-trapping is simply the use of reduced temperature to retain volatile analytes on a solid phase surface by reducing the vapor pressure of the analyte. Cryo-trapping can be used in conjunction with an inert or a non-inert surface. Cryo-trapping can aid in the recovery of volatile non-polar and polar analytes on C-18 traps.

The supercritical fluid extractor employed for this study was capable of trap temperatures from -30°C to 120°C. A C-18 trap at a temperature of 5°C was utilized in the first step of the SF elution method described here. Reducing the temperature of the

trap from 5°C to -20°C favored the cryo-trapping mechanism and should aid in the trapping of HCH and HCCP. Therefore, subsequent extractions utilized a first step trap temperature of -20°C.

Interferences and Contamination

As discussed previously, several plasticizer "analytes" were recovered in excess of the EPA stated criteria. Blank recoveries, those without fortified internal standards and analytes, showed a substantial amount of background contamination of plasticizers. In order to understand the possible source(s) of contamination, a detailed examination of the extraction and quantitation processes was necessary. Although contamination is a common reason for recoveries above 100%, it is not the only explanation. A discussion of high recoveries follows.

Recoveries in Excess of 100%

Improper Internal Standards

Assay of extracts detected several analytes with recoveries that exceeded the acceptable level of 130%. There can be at least two reasons for recoveries of greater than 100%. One possibility is contamination with the analyte of interest from the water, glassware etc. used. Another explanation could be the use of an inappropriate internal standard (IS). When internal standards are used for calibration, the calibration curve is a plot of :

(response of analyte) / (response of IS)
versus
(mass of analyte) / (mass of IS).

An example of a sample calibration curve is shown in Figure 10. Calibration curves are produced by analysis of a series of solutions containing a range of analyte masses and a set mass of internal standard. For example 10, 20, 30, and 40 μg of analytes would be added to 4 respective identical volumetric flasks. Then 10 μg internal standard would be added to each flask and diluted to the mark. Therefore the ratio of (mass of analyte) / (mass of IS) is 1, 2, 3, and 4, respectively. Assay of solutions result in responses for analyte and IS. The ratio of (response of analyte) / (response of IS) is calculated and plotted versus the ratio of the mass. See Figure 10.

Quantitation is based on the ratio of analyte response to internal standard response. When analytes are quantified in a relative manner (i.e. IS is added to the sample bottle prior to deposition) as they are in EPA method 525.1, the internal standards are assumed to act in a physically similar manner to their assigned analytes. Relative quantitation is used in extractions to compensate for analyte loss during the extraction process. If the internal standards are lost in the same manner and quantity, then the ratio of internal standard to analytes remains unchanged. On the other hand, if the internal standards are lost to a greater extent, for example by adhering to the sample bottle, the analyte recovery will increase relative to the internal standard, and a recovery of greater than 100% will be achieved. It has been demonstrated that the internal standards selected in EPA Method 525.1 are appropriate for the analytes of interest (40).

Contamination

The other possibility for an analyte recovery over 100% is the foreign introduction to the instrumental or deposition systems of one of the analytes that is to be analyzed. The three analytes in question are common plasticizers and are ubiquitous in the environ-

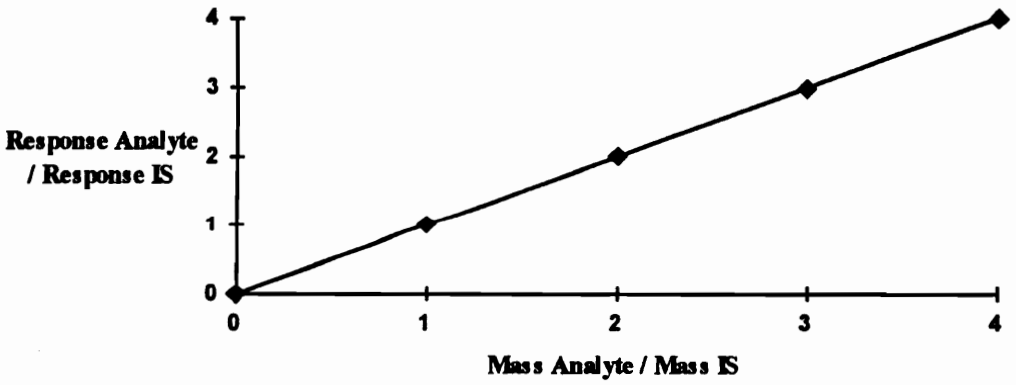


Figure 10. Idealized calibration curve

ment. Di-n-butyl phthalate, di(2-ethylhexyl) adipate, and butylbenzyl phthalate produced recoveries of 200%, 328% and 151% respectively when utilizing SF elution and GC/MS quantitation (Table X). As previously stated, subsequent blank extractions confirmed the presence of these analytes as contaminants in the system. Ironically, one source of phthalate and adipate contamination was in the solid phase extraction disks. The initial wash step of the disk preparation was thought to be designed to remove these contaminants (Table IX, step 7a). Because of the ubiquitous nature of these compounds, EPA Method 525.1 sets the limit of detection of these compounds on the basis of background levels due to environmental contamination i.e. quantitation becomes unreliable due to the present of contamination. (40). Some of these compounds, particularly the di(2-ethylhexyl) adipate, were detected in our previous work dealing with development of the SF elution method for PAHs from water. These compounds, however, did not interfere with assay of PAH analytes because they possessed different GC retention times.

A systematic investigation of the assay equipment, sample concentration method, and extraction system was conducted to ascertain possible point sources for apparent phthalate and adipate contamination. Although contamination can enter the process at any point, several important components of the recovery process have been identified. These components are: 1) gas chromatograph/mass spectrometer, 2) solvents, 3) supercritical fluid extractor, and 4) SPE deposition apparatus.

Removal of Contamination

GC/MS

The GC/MS instrumentation was checked first for contamination. A blank run (air injection) on the GC/MS confirmed the instrument to be free of contamination. A smooth baseline without peaks was observed.

Solvents

Next, the solvents used in the SPE/SF elution procedure were evaluated for contamination. A 10 mL aliquot of each of the solvents (methanol, acetone and methylene chloride) used during disk preparation and SF elution was taken and concentrated to a volume of approximately 1 mL. GC/MS analysis scanning from 50-450 m/z found the solvent concentrates to be free of extraneous peaks. The purity of the solvent was monitored repeatedly as reservoirs of solvents were depleted and replaced. The three solvents were next utilized for analysis of other components of the extraction apparatus following the confirmation of their purity.

Supercritical Fluid Extractor

A blank extraction (e.g., empty 7 mL vessel) of the supercritical fluid extractor revealed the presence of several contaminants. Many of these contaminants were identified by GC/MS as a series aliphatic alkanes. The source of the alkane contamination was traced to the SFE/SFC grade CO_2. Replacement of the contaminated CO_2 tank

eliminated these impuritites. SF extraction performed on the Ottawa sand used to pack the extraction vessel was also found to be free of interferences by GC/MS analysis.

Besides contamination from the CO_2 tank, an additional unidentified contaminant was also discovered in the SFE blank. This evidenced as an early eluting GC peak. Although the mass spectrometer could not positively identify the contaminant, the retention time and mass spectrum confirmed that the compound was not an EPA Method 525.1 analyte. Further investigation found that the contaminant was present in the SFE rinse system. It was eliminated by replacing the rinse systems' solvent reservoir bottle.

To this point, the clean-up investigation checked and/or removed from consideration the CG/MS, the solvents and the supercritical fluid extractor as possible point sources of contamination. Thus steps 10 through 12 of the "Procedure for Recovery" in Table IX were eliminated. Contamination therefore must be introduced during the deposition of the analytes onto the C-18 disk (i.e. steps 7 and 9).

The Disk

The disk resembles a membrane with a diameter of 47 mm and a thickness of 0.5 mm. The membrane disk is constructed by bonding aliphatic C-18 to 8 μm silica particles. The silica particles are bonded together by Teflon strands into a tight membrane. The function of the C-18 disk is to capture analytes from the water matrix by employing the dispersion forces characteristic of the C-18 chains. Prior to deposition of the analytes onto C-18 the disk was placed in the filtration apparatus, washed with 10 mL of with methylene chloride, solvated with 10 mL of methanol, and finally, the excess methanol was rinsed from the disk with 10 mL of HPLC water. The disk was then dried by passing air through by vacuum for 1 minute. Assay of the disk subsequently revealed significant

levels of all three of the plasticizer contaminants discussed earlier. This established either the deposition apparatus or the disk as potential sources of contamination.

Deposition Apparatus

The deposition apparatus consisted of a six station stainless steel manifold with glass funnel/disk holders. The holder consisted of a top funnel section, a screen like disk support, and a lower section. The lower section connected to the manifold on one end and held the disk support on the other end. The lower section was placed onto the manifold by means of a large ground glass joint, designed to be inserted over the throat of the stainless steel manifold. The disk support was a Teflon® coated, stainless steel screen that was seated into the lower section. The disk is placed on top of the disk support and was held in place by clamping the top funnel onto the lower section. Vacuum for each station (funnel) was controlled by a Teflon® stopcock at the base of the manifold. In a larger scheme, the manifold was part of the vacuum system. The vacuum system consisted of the manifold, the waste water reservoir, the water trap and the vacuum pump. Each of these components, 1) manifold, 2) reservoir, 3) trap, and 4) pump were connected in series by rubber tubing.

Although the use of rubber tubing is standard, it was a potential source of plasticizer contamination. Therefore, most of it was removed and replaced with copper tubing. A small section of rubber tubing between the trap and vacuum pump was not replaced as it was relatively distant from the manifold. Improperly venting the system could transport contaminants, via "back flushing," from other components into the manifold and contaminate the disk. After replacing the rubber tubing, a disk was again prepared as previously described, and SF elution was performed. The results showed no

improvement in the level of contamination. This indicated that the contaminants found on the disk were not the result of "back flushing" into the manifold and contaminating the glassware.

Glassware and Manifold

As the tubing did not appear to be a source of contamination, the glassware and manifold were investigated. After every use, the glassware was washed thoroughly with soap and hot water, followed by copious rinsing with distilled water. The water rinse was followed by an acetone rinse. In an effort to reduce the possibility of contamination, the manifold was disassembled and cleaned in this same manner. Each Teflon® stopcock was removed and cleaned individually, as was each of the stainless steel funnel holders. The manifold base was also rinsed with acetone. After drying, the apparatus was reassembled. The level of contamination was reduced as was ascertained by SF elution of a prepared disk.

As stated previously, the disk itself was a source of phthalate and adipate contamination, and therefore it was important to obtain information on the level of contamination that could be attributed directly to the disk. Employing 10 mL of methylene chloride, the disk itself was extracted by placing it in a vial with the solvent and shaking for 10 minutes. The disk was then removed, and the solvent volume was reduced under a nitrogen stream to approximately 1 mL. Analysis by GC/MS revealed phthalate contamination at a level insufficient to account for all the contamination noted.

Thus far, the SFE and GC/MS have been cleaned and removed as possible sources of contamination and interferences. Despite vigorous cleaning of the manifold and associated glassware, plasticizer contamination still remained. As noted earlier, the EPA

Method 525.1 states that glassware must be thoroughly cleaned with soap and hot water and rinsed with copious amounts of distilled water. The glassware must then be rinsed with organic solvent and stored in solvent rinsed aluminum foil. Industrial guidelines also suggest that it may be necessary to periodically heat glassware in a muffle furnace at 400°C from 30 minutes to 4 hours to control contamination. Unfortunately, an oven capable of 400°C was not initially available in our laboratory. As it became more apparent that the glassware was a source of contamination, several alternative non-heating cleaning techniques were attempted.

One non-heating technique used to reduce contamination is to perform of spot cleaning in addition, to the standard cleaning technique. This involved cleaning targeted areas of glassware that were difficult to clean by traditional methods and therefore were likely to harbor contaminants. The suspected trouble spot was the seat in which the disk support was held. This area was identified for two reasons. First, the physical conformation made it difficult to clean, and second, the close approximation of the seat to the disk made the transfer of contaminants to the disk possible. Spot cleaning was conducted by scrubbing the seat with a cotton swab and methylene chloride. Assay of the eluted disk indicated this "non-heating technique" did not eliminate the contaminant from the glassware. Another non-heating cleaning technique was to soak the glassware in a highly oxidative solution of 0.6% chromic acid in concentrated sulfuric acid for 12 hours. After subsequent washing and rinsing of the glassware, assay of a prepared disk showed contamination was still present.

Concurrent with the investigation of non-heating cleaning techniques, an oven capable of producing temperatures of 400° C was obtained. Since the non-heating methods of decontamination were unsuccessful, glassware was heated to 400°C for 4 hours in an attempt to remove any contaminates. The glassware was again washed,

rinsed, and checked for contamination. Although this cleaning technique did not completely remove the contamination, a marked improvement was noted.

The decontamination procedures discussed thus far eliminated unwanted aliphatic compounds from the SF fluid tank and undefined contamination from the SF rinse system. The plasticizer contamination was reduced but not removed. It became apparent that despite dealing with a limited system, (i.e., glassware and manifold) the plasticizer contaminants were not from a small number of point sources, but rather from a systematic source. Therefore, the search for possible sources of plasticizer contamination was expanded to a larger scale. It was discovered that the pipes that carried all the water to the lab, including the distilled water, were constructed of plastic. Thus, laboratory water was discovered to be the likely source of contamination. This implied that washing the glassware in the usual manner could contaminate them, yet washing was unavoidable. It became readily apparent that it was probably not possible to eliminate all sources of plasticizer contamination from the system. What was needed was to control contamination at its lowest possible level and continue with the project at hand.

Contamination Control

The discovery that all water sources in the laboratory were a potential source of plasticizer contamination required the following steps to be taken to minimize their effect. First, all sample deposition glassware was periodically heated to a minimum of 400°C for 4 hours. Glassware was washed with hot water and soap, and rinsed with copious amounts of tap water and distilled water as described in Method 525.1. An additional rinse in HPLC grade water was introduced followed by a final soak in acetone, before glassware was stored in solvent rinsed aluminum foil.

Sample bottles were periodically treated with a 10% solution of dichlorodimethylsilane in toluene to reduce the adherence of hydrophobic compounds to the glass. The bottles were washed by filling them with hot tap water and soap, followed by 15 minutes of sonication. The bottles were then rinsed with copious amounts of distilled water, HPLC grade water and two, 20 mL portions of acetone. All glassware was re-rinsed with acetone prior to use. HPLC grade water was used to replace laboratory sample water to eliminate the possibility of contamination.

Summary: Recovery of Test Mix 2

Six of the 21 analytes studied in this SF elution were recovered at unacceptable levels. Three of these recoveries were high due to contaminants in the systems. Steps to minimize these contaminants were outlined above. Two other analytes, HCB and HCCP, were recovered below acceptable levels. In an attempt to increase the recoveries of these two analytes the trap temperature will be reduced from 5°C to -20°C in the first step of the SF elution in the next phase of research. For unknown reasons, alachlor also produced a recovery of 162%, and edrin produced a RSD of 43%. Nevertheless, based of the positive results of this experiment, SF elution was then attempted using 39 of the Method 525.1 analytes incorporating the revisions noted.

Recovery of EPA Method 525.1 Analytes at 4 ppb

Since supercritical fluid elution has successfully recovered 16 polyaromatic hydrocarbons as well as the compounds in Test Mix 2 the next goal was to recovered a combination of Test Mix 2, PAHs and a group of polychlorinated biphenyls (PCB) in a

single extraction from a spiked reagent water sample via SPE/SF elution. This group of 39 analytes is included in EPA method 525.1. Table XI lists the internal standards used and the analytes investigated. Peak number desingations and retention times correspond to the chromatogram shown in Figure 11. A brief outline of the optimized SF elution method is as follows:

Step 1: No equilibration time with a 10 minute dynamic extraction at 5500 psi and a trap temperature at -20°C for extraction.

Step 2: No equilibration time with a 10 minute dynamic extraction at 5500 psi using 5% acetone modified CO_2.

Step 3: No equilibration time with a 2 minute dynamic extraction at 5500 psi .

All other parameters were as outlined in the Experimental section, General SF Elution Method.

Reagent water samples (1L) were spiked at 4 ppb with analytes and 8 ppb with internal standards. The results are shown in Table XII. Thirteen of the analytes were outside of the 70% to 130% range for recovery, 14 compounds had RSDs higher than the acceptable limit of 30%. Additionally, several other analytes produced RSDs in excess of 20%, which was much higher than any experiments thus far.

As the recovery of these analytes from drinking water employing the C-18 disk is a routine EPA Method, it was assumed that the analytes were successfully trapped on the disks from water. The total analyte mass was well below the manufacturers' stated 5 mg disk loading capacity. Several of the analytes produced recoveries of 140% or greater. Erratic recoveries were therefore apparently a result of the SF elution process. Most of the high molecular weight analytes were quantitated by using chrysene-d12 as an internal standard which was spiked at a level of 8 ppb in reagent water. The high recoveries for

Table XI. GC/MS retention times of EPA Method 525.1 analytes

	Compound	Retention Time (min)
1.	Naphthalene-d*--IS	4.12
2.	Hexachlorocyclopentadiene	4.88
3.	Dimethylphthalate	5.88
4.	Acenaphthylene	5.89
5.	Diethylphthalate	6.95
6.	Fluorene	7.15
7.	Hexachlorobenzene	8.42
8.	Simazine	8.72
9.	Atrazine	8.83
10.	Pentachlorophenol	8.95
11.	Lindane	9.11
12	Phenanthrene-d12--IS	9.46
13.	Phenanthrene	9.52
14.	Anthracene	9.67
15.	Trichlorobiphenyl	10.61
16.	Alachlor	10.76
17.	Heptachlor	11.00
18.	Di-N-butylphthalate	11.59
19.	Aldrin	11.98
20.	Heptachlor epoxide	13.06
21.	Gamma-chlordane	13.74
22.	Pentachlorobiphenyl	13.94
23.	Pyrene	14.06
24.	Alpha-chlordane	14.10
25.	Trans-nonachlor	14.18
26.	Edrin	15.50
27.	Hexachlorobiphenyl	16.35
28.	Butylbenzylphthalate	16.98
29.	Di(2-ethylhexyl)adipate	17.58
30.	Benzo[a]anthracene	18.53
31.	Chrysene-d12--IS	18.57
32	Chrysene	18.68
33.	Methoxychlor	18.76
34.	Di(2-ethylhexyl)phathalate	19.47
35.	Heptachlorobiphenyl	19.84
36.	Octachlorobiphenyl	21.04
37.	Benzo[b]fluoranthrene	22.38
38.	Benzo[k]fluoranthrene	22.47
39.	Benzo[a]pyrene	23.41
40.	Indeno[1,2,3,cd,]pyrene	26.80
41.	Dibenzo[a,h]anthracene	26.93
42.	Benzo[g,h,i]perylene	27.49

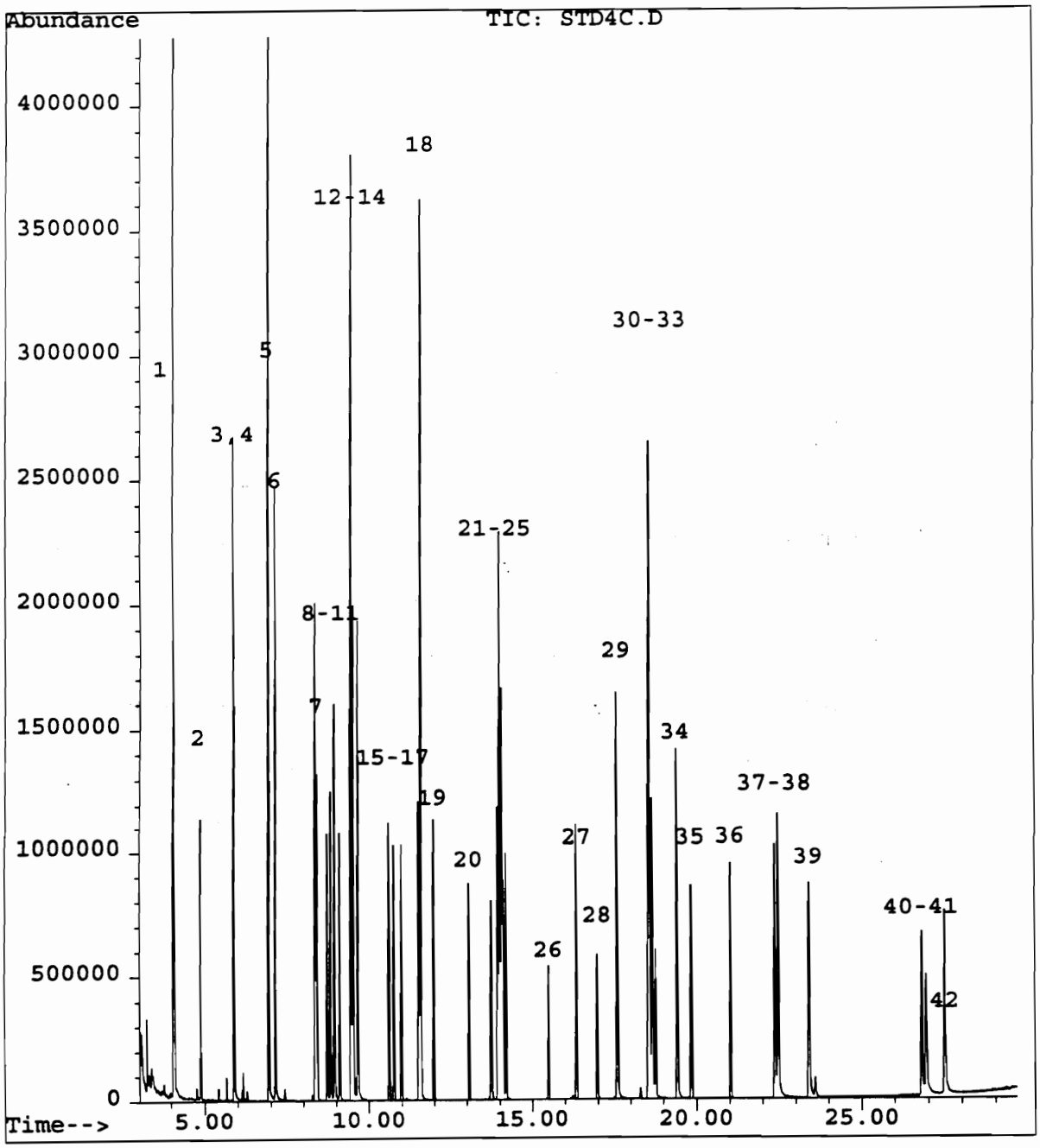


Figure 11. Total ion chromatogram of internal standards and EPA 525.1 analytes See Table XI for peak identification.

Table XII. Recovery of Method 525.1 analytes from water via C-18 disk with subsequent 27 minute SF elution @ 2 ug/component

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	56*	12
Dimethylphthalate	108	5.7
Acenaphthylene	106	2.2
Diethylphthalate	120	1.9
Fluorene	114	1.7
Hexachlorobenzene	73	1.8
Simazine	48*	28
Atrazine	76	20
Pentachlorophenol	130	4.1
Lindane	118	4.6
Phenanthrene	110	7.5
Anthracene	95	8.7
Trichlorobiphenyl	73	5.1
Alachlor	115	16
Heptachlor	67*	12
Di-N-butylphthalate	122	8.4
Aldrin	61*	5.5
Heptachlor epoxide	93	10
Gamma-chlordane	67*	8.6
Pentachlorobiphenyl	61*	7.6
Pyrene	85	2.5
Alpha-chlordane	66*	12
Trans-nonachlor	89	19
Edrin	68*	48*
Hexachlorobiphenyl	57*	8.2
Butylbenzylphthalate	102	14
Di(2-ethylhexyl)adipate	45*	27
Benzo[a]anthracene	143*	17
Chrysene	72	134*
Methoxychlor	149*	28
Di(2-ethylhexyl)phathalate	160*	16
Heptachlorobiphenyl	125	20
Octachlorobiphenyl	128	25
Benzo[b]fluoranthrene	121	20
Benzo[k]fluoranthrene	124	15
Benzo[a]pyrene	92	26
Indeno[1,2,3,cd,]pyrene	88	67*
Dibenzo[a,h]anthracene	73	42*
Benzo[g,h,i]perylene	71	35*

SFE conditions:
Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 15 minute dynamic extraction with 5% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 80°C with pressure at 5500 psi throughout. n=3.
Total SF elution time 27 minutes.
* = Outside EPA Criteria

this group of analytes indicated that the internal standard, chrysene-d12, was not being extracted in as quantitative a manner as the analytes. It is readily accepted that "true" recoveries of 140% from a spiked sample are not possible. These excessively high recoveries may also imply that other analytes quantitated by chrysene-d12 are skewed high. For example, the 85% reported for the benzo[a]pyrene may be skewed high such that the "true recovery" could be in the range of 50%.

Spiking Level Effects

Several factors can affect the recovery of analytes such as the SF elution method, the choice of internal standards, and perhaps the spiking level. In retrospect, increasing the spiking level from 2 ppb/component, as used in the PAH study, to 4 ppb/component in this experiment, may have been unwise. The higher spiking level was employed in an attempt to decrease relative error in the experiment, but the trap capacity may have been exceeded.

Several other factors that may have adversely affected the recovery of analytes must be considered. The implications of limited aqueous analyte solubility will be discussed in a later section. A second possible concern was the ability for a limited amount of SF solvent to remove the higher mass of analytes present from the disk. The SF elution method was optimized for 16 PAHs at 2 ppb/component and resulted in a total mass of 32 μg of analytes to be recovered. In contrast, 39 analytes at 4 ppb resulted in a total analyte mass of 156 μg. A third factor was the ability of 1.2 mL of rinse solvent to remove the analytes from the trap. Reducing the spiking level to 2 ppb/component would obviously reduce the level of concern for all these factors.

Aqueous Solubility

Solubility limitations were a particular concern for analytes with log octanol-water partition coefficient (K_{ow}) greater than 5 when spiking at the 4 ppb level. These compounds are only sparsely soluble in water. Water solubilities of these compounds are determined for the individual compound in water, therefore synergistic effects are not considered. Aqueous solubility for a mixture of isomers of the same compound, or a mixture of compounds of the same class, may reduce the solubility of individual compounds. (41)

Adherence Problems

The higher 4 ppb concentration in water may increase the tendency of the analytes to adhere to the sample bottles and apparatus. The analytes that possess a log $K_{ow} > 5$ include: benzo[g,h,i]perylene, dibenzo[a,h] anthracene, indeno[1,2,3,cd,] pyrene, benzo[a]fluoranthracene, benzo[k]fluoranthracene, chrysene, benzo[a]anthracene and the hepta and octa chlororinated biphenyl. The results from the previous SF elution were unacceptable in terms of recoveries and reproducibility. The higher spiking levels may have adversely affected the SF elution due to inadequate analyte solubility in water, inadequate SF elution solvent, or inadequate trap rinse solvent. The extraction therefore was repeated at a 2 ppb/component spike level. Recoveries from SF elution at the 2 ppb level however were poor. Thirteen analytes fell outside the acceptable 70% to 130% range and five analytes had RSDs > 30%. Additionally, four of the high molecular weight PAHs produced extremely high RSDs. The erratic recoveries and high RSDs suggested that the disk was probably not efficiently eluted. Three approaches can be used to enchance

elution of analytes from the disk: first is to use of more solvent; second is to use a stronger solvent; and third is, a combination of these two approaches.

Elution Effects- An Increase in Solvent and Solvating Power

Due to the extreme number of analytes out of range and the high RSDs, the third option was employed, i.e. using greater quantities of a stronger SF solvent. Most of the analytes with unacceptable recoveries or erratic RSDs were the less volatile analytes. Therefore, the length of the second extraction step was also increased from 15 to 23 minutes, resulting in an increase in modified CO_2(l) from 30 mL to 46 mL.

An increase in solvating power of the fluid was also desired. This was easily achieved by increasing the percentage of organic modifier in the fluid. Hence, the percentage of acetone in the supercritical fluid was raised to 8% (v/v) which increased the Hildebrand solubility parameter from 8.6 to 9.0 (39). The combination of increased extraction time and percentage of modifier increased the amount of acetone modifier available for disk elution from 1.5 mL to 3.7 mL The SF elution method employed is shown below

SF Elution Method:

Step 1: No equilibration time with 10 minute dynamic extraction time at 5500 psi of CO_2 at a flow rate of 2 mL/minute. The ODS trap was held at -20°C during the extraction and raised to 25°C for rinsing.

Step 2: No equilibration time with a 23 minute dynamic extraction at 5500 psi of 8%(v/v) modified CO_2 and a flow rate of 2 mL/minutes.

Step 3: No equilibration time with a 2 minute dynamic extraction at 5500 psi CO_2 and a flow rate of 4 mL/minute.

This SF elution method showed improved recoveries when compared with previous experiments. As expected, a low recovery remained for HCCP, since the increase in modifier used in step 2 would not have affected this analyte. Pentachlorophenol (PCP) produced a recovery of 138%. High recoveries for pentachlorophenol have previously been caused by small amounts of water in the GC/MS injection solvent (42,43). The heavy PAHs continued to produce low recoveries although all the RSDs were less than the EPA's criteria of 30% (Table XIII). Three analytes were depleted from our stock and consequently, were not investigated. Next it was reasoned that the solubility of PAHs in a SF CO_2 can be enhanced by an increase in chamber temperature. Subsequently, this could increase the recoveries of the PAHs without modifying other parameters.(44)

Elution Effects-- An Increase in Temperature

SF elution using identical parameters with the exception of increasing the chamber temperature to 110°C was attempted. All analytes produced RSDs ≤11% with only 3 analytes out of the acceptable range (Table XIV). The three analytes were HCCP, PCP, and endrin. The reason for the high recovery for PCP has been discussed previously. HCCP consistently produced low recoveries despite attempts at cryo-trapping. Endrin produced a recovery of 69%, but because endrin had been quantitatively recovered previously, this recovery was considered to be anomalous.

Table XIII. Recovery of Method 525.1 analytes from water via C-18 disk with subsequent 35 minute SF elution @ 2 ug/component and chamber temperature at 80°C

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	35*	9.0
Dimethylphthalate	121	6.5
Acenaphthylene	100	5.0
Diethylphthalate	122	1.2
Fluorene	126	20
Hexachlorobenzene	90	7.9
Simazine	116	1.4
Atrazine	110	5.8
Pentachlorophenol	138*	12
Lindane	122	7.1
Phenanthrene	105	6.1
Anthracene	102	5.9
Trichlorobiphenyl	86	7.5
Alachlor	107	6.5
Heptachlor	87	5.7
Di-N-butylphthalate	131*	5.7
Aldrin	73	8.0
Heptachlor epoxide	101	12
Gamma-chlordane	88	7.0
Pentachlorobiphenyl	82	9.9
Pyrene	92	8.1
Alpha-chlordane	87	8.4
Trans-nonachlor	ni	
Edrin	114	3.7
Hexachlorobiphenyl	74	10
Butylbenzylphthalate	ni	
Di(2-ethylhexyl)adipate	66*	14
Benzo[a]anthracene	88	11
Chrysene	91	13
Methoxychlor	116	8.7
Di(2-ethylhexyl)phathalate	125	26
Heptachlorobiphenyl	89	14
Octachlorobiphenyl	94	9.6
Benzo[b]fluoranthrene	77	22
Benzo[k]fluoranthrene	75	15
Benzo[a]pyrene	70	17
Indeno[1,2,3,cd,]pyrene	ni	
Dibenzo[a,h]anthracene	61*	26
Benzo[g,h,i]perylene	59*	11

SFE conditions:
Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 23 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 80°C with pressure at 5500 psi throughout. n = 3.
Total SF elution time: 35 minutes.
* = Outside EPA Criteria ni = not investigated

Table XIV. 35 minute SF elution of Method 525.1 analytes from water via C-18 disk @ 2 ug/component and chamber temerature of 110°C

Compound	Percent Average	RSD
Hexachlorocyclopentadiene	35*	4.0
Dimethylphthalate	136*	9.6
Acenaphthylene	97	9.6
Diethylphthalate	117	9.8
Fluorene	104	8.8
Hexachlorobenzene	86	7.8
Simazine	118	11
Atrazine	116	11
Pentachlorophenol	158*	14
Lindane	118	13
Phenanthrene	101	9.2
Anthracene	99	9.3
Trichlorobiphenyl	82	7.8
Alachlor	111	9.7
Heptachlor	82	10
Di-N-butylphthalate	127	11
Aldrin	69*	5.7
Heptachlor epoxide	98	5.1
Gamma-chlordane	82	6.4
Pentachlorobiphenyl	76	5.9
Pyrene	89	8.4
Alpha-chlordane	81	8.0
Trans-nonachlor	ni	
Edrin	115	11
Hexachlorobiphenyl	72	8.8
Butylbenzylphthalate	ni	
Di(2-ethylhexyl)adipate	74	10
Benzo[a]anthracene	92	3.3
Chrysene	94	5.3
Methoxychlor	123	4.8
Di(2-ethylhexyl)phathalate	113	11
Heptachlorobiphenyl	91	6.6
Octachlorobiphenyl	90	6.9
Benzo[b]fluoranthrene	89	13
Benzo[k]fluoranthrene	82	4.7
Benzo[a]pyrene	91	4.3
Indeno[1,2,3,cd,]pyrene	ni	
Dibenzo[a,h]anthracene	86	5.1
Benzo[g,h,i]perylene	78	15

SFE conditions:
Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).
Step 2: 23 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held at 110°C with pressure at 5500 psi throughout. n = 3.
Total SF elution time: 35 minutes.
* = Outside EPA Criteria ni = not investigated

SUMMARY

The recovery of EPA Method 525.1 analytes from water was complicated by the ubiquitous presence of plasticizer contamination. Steps were taken to reduce the contamination to a minimal level. The steps included heating of glassware to 400°C, washing with soap and water, followed by rinsing with water and an organic solvent. The direct transfer of the SF elution method developed for recovery of PAHs to EPA Method 525.1 analytes from water was not possible. Many of the analytes in the Method are more polar than the PAHs used in the initial study. Therefore, it was necessary to lengthen the elution time and increase the amount of acetone modifier used. These modifications afforded recoveries for most of the analytes at acceptable levels with the exception of the high molecular weight PAHs. Quantitative recovery of PAH analytes required that the chamber temperature be increased to 110°C. The SF elution method detailed in Table XIV, successfully recovered all but three analytes investigated. The next phase of this research involved the application of this technique to authenic salt water matrices.

Chapter VI
Recovery of EPA Method 525.1 Analytes from Brackish Water Via Solid Phase Deposition Followed by Supercritical Fluid Elution

INTRODUCTION

The second phase of this investigation (Chapter V) involved the recovery of 39 EPA method 525.1 analytes from reagent water by SPE/SF elution. The recovery utilized a three step SF elution process: step one was designed to recover the most volatile compounds using CO_2 as a SF; step two employed acetone modified CO_2 to recover the less volatile compounds; the final step was designed to remove residual modifier solvent from the SF extractor prior to subsequent extractions. The method utilized a solid phase C-18 trap, consumed 1.2 mL of methylene chloride for rinsing the trap and 3.7 mL of acetone as a modifier. The SF elution method recovered all but four of the analytes within the criteria stated for Method 525.1.

The successful recovery of EPA Method 525.1 analytes from reagent water provided the ground work for the attempt to recover these analytes from brackish water. The water matrices of interest were estuarine and marine sources from the Chesapeake Bay. A literature search revealed the application of solid phase extraction to brackish water matrices has been limited. No literature cited recovery of 525.1 analytes by SPE from brackish water. The focus of most brackish water research thus far has been on the recovery of pesticides such as triazines, organophosphorus, carbamates, phenylureas and organochlorines (46-49). A discussion of these studies follows.

Hinckley, et al. were among the first to investigate the recovery of pesticides from river and brackish water matrices (46). A collection of 11 organochlorines, organophosphates and pyrethoroids were assayed by gas chromatography/electron capture detection in their study. The sea water matrices were collected from the South Carolina coast and had a salinity of 33-35 parts per thousand. Sample volumes were from 1-4 liters and were fortified from 14 to 125 ng/L per component. All samples were prefiltered with a glass fiber trap to remove particulate matter. The analytes were deposited onto C-8 bonded silica cartridges after filtration, and eluted with 3 mL of a solution diethyl ether-hexane (50%/50%). Recoveries ranged from 85% to 115% with RSDs $\leq$ 21% for all analytes.

Hinckley, et al.also reported that several of the analytes were substantially retained on the experimental apparatus. Cis-chlordane, trans-chlordane and fenvalerate were sorbed on the glass prefilter at levels of 14%, 18%, and 28% respectively. Additionally, the same analytes were retained on the sample bottles at levels of 13%, 18%, and 54% respectively. Therefore, the total recoveries were based on the sum of analytes recovered from the prefilter, the bottle and the cartridge.

Barcelo et al. (47) reported on the recovery of seven pesticides and five metabolite products from river and artificial seawater. C-18 Empore™ disks were utilized for the recovery of the analytes. Methanol was used as the elution solvent. Assay was performed by liquid chromatography with diode array detection.

Barcelo, et al. (47) prefiltered their samples with a 0.45 μm filter. The analytes of interest were polar pesticides and possessed a log K_{OW} of approximately 2. Prefiltering was justified because the distribution of the analytes in the water versus sorbed to particulate matter was a ratio of 99.5 to 0.5. Barcelo also stated that if the log K_{OW} was approximately 6, large amounts of analytes would adhere to the particulate matter.

Prefiltering permitted a 5 liter water sample to be processed in 2.5 hours. Pesticides investigated included atrazine and simazine, parathion-ethyl, propanil, linuron, aldicarb and carbofuran. The atrazine decomposes by the loss of ethyl or isopropyl groups resulting in the formation of deethyl or deisopropyl atrazine. The decomposition products, deethyl- and deisopropylatrazine, aldicarb sulfoxide, aldicarb sulfone and 3-hydroxy-7-phenol carbofuran were also studied. This study involved fresh river water samples which were fortified at levels of 0.2 μg/L and 5 μg/L. Seawater was fortified at 20 μg/L. Recoveries for river water were ≥ 80% for most of the pesticides. Exceptions identified were carbofuran and aldicarb. Carbofuran was recovered at 74% and 60% for the fortification levels of 5 μg/L and 0.2 μg/L respectively; aldicarb was recovered at 70% fortified at 0.2 μg/L. The more polar pesticide metabolites were recovered ≤ 35%. The study of simulated sea water was reduced to 7 analytes including the five pesticides successfully recovered in the previous study (simazine, atrazine, propanil, parathion-ethyl and linuron) and two additional atrazine metabolites. Recoveries for the pesticides ranged from 85% to 135%. Atrazine was quantitated at 135% due to interference in the chromatogram. The atrazine metabolites were recovered ≤ 10%.

In a later study, Barcelo, et al. verified the above results employing liquid chromatography coupled with UV-visible spectroscopy and thermospray-mass spectrometry (48). This study also evaluated both methanol and acetonitrile as disk elution solvents and showed that acetonitrile produced higher recoveries and similar RSDs for the analytes. The recovery of pesticides fortified at 25 μg/L from seawater was again in excess of 83%. The recovery of metabolites were ≤ 10%, probably due to analyte breakthrough during solid phase deposition.

In 1993, Barcelo, et al. (49) addressed two issues concerning the implementation of C-18 disks for the analysis of pesticides from brackish water: (a) the possible

interferences present in C-18 Empore™ disks; and (b) the analysis of chloroatrazines from estuary water as salinity levels change . Barcelo reported possible interferences contained in the C-18 Empore™ disks included C-19 alkane and the antioxidant, Nonox A. Additionally, several plasticizer contaminants were identified including diethyl phthalate, dibutyl phthalate, butyl-2-ethylhexyl phthalate and di(2-ethylhexyl) phthalate. It was reported that the plasticizer contamination in the disk probably caused systematic errors when using EPA Method 525.1 and/or 506 resulting in recoveries of 150% for the plasticizer analytes.

The change in concentration of atrazine, simazine, and de-ethylatrazine as a function of salinity was also investigated. The study employed 5 liter estuary and sea water samples with salinity levels from 0 to 34 parts per thousand. Each water sample was pre-filtered with a 0.7 μm glass filter and again with a 0.45 μm glass filter to remove particulate matter. Simazine and atrazine are herbicides and are transported by runoff into water systems. It was hypothesized that as the fresh water was diluted by the seawater, the concentration of the analytes would steady decrease as the level of salinity increased. The data verified the expected trend, with the exception of two inflexion points around 3% and 10% salinity. The inflexion points were believed to be caused by incomplete mixing of water in the estuary zone. Barcelo reported an estimated limit of detection of 0.02 ng/L (signal to noise ratio = 3) for GC/MS with single ion monitoring.

The application of SPE/SF elution to brackish water matrices has been limited to the recovery of organotin compounds (50). The strong toxicity of the organotins to the coastal environments coupled with their more frequent usage over the past decade has intensified the need for monitoring these compounds.

Alzaga et al. were the first to apply SPE/SF elution to a seawater matrix. The study employed SPE followed by *in situ* derivatization and elution of the disk by SFE.

Both simulated and real sea water samples of 0.5 to 1 liter in volume were utilized in this study. Analytes of interest were monobutyltin (MTM), dibutyltin (DBT), and tributyltin (TBT). The basic format of the experiment was as follows:

1. Extract analytes by SPE using C-18 Empore™ disks.
2. Air dry disk for 60 minutes.
3. Derivatize with ethyl magnesium chloride for 15 minutes.
4. Evaporate derivatization solvent.
5. SFE at 40°C with 10 MPa CO_2 for 30 minutes.
6. Assay by gas chromatography with flame photometric detection (FPD) or by mass spectrometry.

The analytes of interest were spiked into simulated sea water at the low μg/L level to assess recovery and reproducibility . Recoveries of the derivatized MTB, DBT, and TBT were 69.9%, 91.8%, and 102.0% with RSDs of 17.4%, 6.6% and 8.2% respectively. The analytes through the entire recovery process were calculated by employing GC/MS assay. The limits of detection (LOD) of MBT, DBT, TBT were 16 ng/L, 7 ng/L and 6 ng/L respectively, assuming a 1000 mL water sample was concentrated to a 0.5 mL extract.

The emphasis of research was the recovery of EPA Method 525.1 analytes from brackish water matrices. As compared with the reagent water study in Chapter V, the brackish water matrix included naturally occurring suspended sediment and dissolved organic carbons (DOC) in the sample. Most of the previous studies involved pesticides and none of the studies attempted to incorporate a high number of analytes in a single assay as has been attempt in the work to be presented. It should be noted that in most brackish water studies, the samples were pre-filtered to removed the sediment. As stated

previously, pre-filtering of samples is justifiable if the analytes exhibit log $K_{ow} \leq 2$ (1). EPA Method 525.1 analytes include compounds with log K_{ow} as high as 6, therefore analytes loss due to adherence to sediment was deemed to be a real possibility therefore prefiltering was not be advisable for these analytes (46).

EXPERIMENTAL

Brackish water was collected from both marine and estuarine sources in the area of the Chesapeake Bay. Two different marine water samples were utilized for the experiments. Both samples were collected from Rudee Inlet (Virginia Beach, VA) and had a salt content of approximately 30 parts per thousand. The first marine water sample was collected and stored for nearly a year. Storage temperatures varied due to a refrigeration failure. This sample will be referred to as Marine Water I. This marine water was subsequently replaced with a new sample, referred to as Marine Water II, which was stored at 4°C until fortification. Estuarine water was collected at the same time as Marine Water II, from Gloucester Point, VA and had a salt content of approximately 22 parts per thousand. All samples contained a non-specified amount of suspended sediment. Samples were collected in 4 liter solvent rinsed, amber glass collection bottles and were immediately acidified to a pH of 2 after collection.

The procedures outlined in Table IX, Chapter V, Step 1 required modification given the amount of sediment contained in the water samples. In the work described in Chapters 4 and 5, the volume of all water samples were pre-measured with a 1 L. volumetric flask prior to use filling the sample bottle (Table IX, Chapter V). It was observed that sediment from Marine Water II was adhering to the volumetric flask, therefore the measured step was eliminated. One liter sample bottles were directly filled

after shaking the 4 liter collection bottle contents. The 1 liter sample bottle was rinsed with 20 mL of reagent water to remove the sediment. This rinse water was then used to rinse sediment from the sides of the collection funnels. Rational for this procedural change will be outlined in the "Results and Discussion". All other experimental parameters for the recovery of 525.1 analytes remained as outlined in the previous chapter.

RESULTS AND DISCUSSION

Marine Water I

The first brackish water matrix studied was Marine Water I. It was assumed that the salt content in the matrix was relatively stable, but the organic sediment may have degraded, given the extended length of time the Marine Water I sample was stored. Therefore, this matrix was investigated only on an interim basis until a fresh sample could be obtained from the Chesapeake Bay.

The SF elution method optimized for the recovery of EPA Method 525.1 analytes from reagent water was applied to the fortified Marine Water I samples. Results (Table XV) indicated that eight of the analytes were out of the range for quantitative recovery in Marine Water I sample as compared to four analytes in the reagent water. Marine Water I sample had only 2 analytes with RSDs > 30%. Recovery and quantitation concerns regarding pentachlorophenol (PCP), hexachlorocyclopentadiene (HCCP), and dimethylphthalate have been previously discussed. The remaining five analytes that failed to meet EPA criteria were recovered at <70% which indicated that the analytes were less available to SF elution with this matrix. A SF elution method that incorporated a longer second extraction step of 30 minutes produced very similar results. No further

Table XV. Recovery of Method 525.1 analytes from Marine Sample I via C-18 disk with subsequent SF elution @ 2 ug/component.

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	33*	49*
Dimethylphthalate	139*	35*
Acenaphthylene	89	12
Diethylphthalate	103	7.8
Fluorene	112	17
Hexachlorobenzene	73	10
Simazine	127	26
Atrazine	128	17
Pentachlorophenol	164*	19
Lindane	100	12
Phenanthrene	105	6.8
Anthracene	96	9.2
Trichlorobiphenyl	74	5.9
Alachlor	113	16
Heptachlor	70	14
Di-N-butylphthalate	115	19
Aldrin	64*	9.0
Heptachlor epoxide	98	15
Gamma-chlordane	66*	9.0
Pentachlorobiphenyl	61*	3.9
Pyrene	92	8.7
Alpha-chlordane	70	7.4
Trans-nonachlor	76	4.9
Edrin	116	21
Hexachlorobiphenyl	57*	9.2
Butylbenzylphthalate	108	21
Di(2-ethylhexyl)adipate	55*	17
Benzo[a]anthracene	87	10
Chrysene	87	8.1
Methoxychlor	107	19
Di(2-ethylhexyl)phthalate	100	16
Heptachlorobiphenyl	76	13
Octachlorobiphenyl	70	11
Benzo[b]fluoranthrene	89	20
Benzo[k]fluoranthrene	78	18
Benzo[a]pyrene	80	22
Indeno[1,2,3,cd,]pyrene	102	26
Dibenzo[a,h]anthracene	85	26
Benzo[g,h,i]perylene	79	29

SFE conditions:

Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL).

Step 2: 23 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse.

Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 110°C, and pressure was held at 5500 psi throughout. n=3. **Total SF elution time 35 minutes.** *= Outside EPA Criteria

investigation of this sample was performed since fresh Chesapeake Bay water (Marine Water II) was obtained.

Marine Water II

Recovery of Method 525.1 analytes from the Marine Water II sample was then attempted. The SF elution method was modified slightly since the previous method was not completely successful when applied to the Marine Water I sample. The chamber temperature was reduced from 120°C to 80°C in the first step and increased from 110°C to 120° in the final two steps. The temperature reduction increased the density of the SF fluid in the first step from 0.64 g/mL to 0.81g/mL. The increase in density corresponded to increased solvating power, and theoretically, allowed for more of the analytes to be recovered in the first step. A brief synopsis of the SF elution method follows:

SF Elution Method for Marine Water II (Modification I)

Step 1: No equilibration time. 10 minute extraction at 5500 psi with a trap temperature at -20°C for the extraction. Chamber temperature at 80°C.

Step 2: No equilibration time. 23 minute extraction at 5500 psi using 8% acetone modified CO_2. Chamber temperature at 120°C.

Step 3: No equilibration time. 2 minute extraction at 5500 psi. Chamber temperature at 120°C.

All other parameters remained as outlined in the General SF Elution Method, Chapter IV.

The SF elution method (Modification I) is identical to the method employed for analyte recovery from reagent water differing only in the chamber temperatures used. The recoveries from the marine water II matrix, however, were substantially less. Results shown in Table XVI, placed 25 of the compounds out of the criteria for recovery with 2

Table XVI. Recovery of Method 525.1 analytes from Marine Sample II via C-18 disk with subsequent 35 minute SF elution @ 2 ug/component.

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	84	13
Dimethylphthalate	351*	12
Acenaphthlene	136*	4.1
Diethylphthalate	120	1.9
Fluorene	191*	9.4
Hexachlorobenzene	93	5.3
Simazine	53*	57*
Atrazine	55*	8.2
Pentachlorophenol	306*	8.1
Lindane	244*	8.0
Phenanthrene	82	17
Anthracene	71	11
Trichlorobiphenyl	47*	6.5
Alachlor	110	6.8
Heptachlor	57*	1.8
Di-N-butylphthalate	100	6.9
Aldrin	39*	7.8
Heptachlor epoxide	80	1.9
Gamma-chlordane	40*	8.8
Pentachlorobiphenyl	33*	3.9
Pyrene	66*	5.5
Alpha-chlordane	41*	7.4
Trans-nonachlor	39*	7.1
Edrin	97	1.6
Hexachlorobiphenyl	30*	5.1
Butylbenzylphthalate	82	7.2
Di(2-ethylhexyl)adipate	31*	9.6
Benzo[a]anthracene	73	13
Chrysene	53*	70*
Methoxychlor	116	11
Di(2-ethylhexyl)phathalate	194*	12
Heptachlorobiphenyl	62*	9.5
Octachlorobiphenyl	63*	6.7
Benzo[b]fluoranthrene	74	18
Benzo[k]fluoranthrene	78	18
Benzo[a]pyrene	56*	24
Indeno[1,2,3,cd,]pyrene	62*	19
Dibenzo[a,h]anthracene	56*	20
Benzo[g,h,i]perylene	54*	15

SFE conditions:
Step 1: 10 minute dynamic extraction with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 80°C.
Step 2: 23 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap held @ 80°C for extraction, reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature held @ 80°C in steps 2 and 3 with pressure at 5500 psi throughout. n=3. **Total SF elution time 35 minutes.**
* = Outside EPA Criteria

compounds producing RSDs of ≥30%. The decreased recoveries may be attributed to a matrix effect. Marine water matrix contains suspended sediment, salts and dissolved organic carbons (DOC's) not contained in the reagent water. Many DOC's fall into a humic substance category. It has been widely noted that many of the analytes of interest adhere to sediment (52-55) or humic material (56-60). Consequently, the adherence of the analytes to the sediment or to the humic material may lower the recovery.

Suspended Sediment

The effects of suspended sediment were directly observable. The sediment coated the surface of the disk as the marine water passed through it. This had two effects: first, the sample flow rate through the disk was significantly reduced resulting in a 1.5 to 2 hour analyte deposition time, while deposition time for reagent water was approximately 10 minutes; second, the sediment on the disk appeared to interfere with disk drying . After deposition, the disk was dried by passing room air through the disk for five minutes under a vacuum of 25 inches of Hg. The sediment appeared to retain more water in the disk. The combination of water and sediment produced a slimy coating on the surface of the disk. Water on the disk could block access of the supercritical fluid to the analytes and prevent elution of the analytes.

Suspended sediment adhered to the sides of 1 liter volumetric flask used for measuring samples and to the neck of the deposition funnel as well. Therefore, samples were poured directly, rather than premeasured, into the 1 liter sample bottles to minimize the loss of sediment. The bottles were rinsed with reagent water after sample deposition, This same water was used to rinse sediment from the sides of the deposition funnel. This water was filtered through the disk.

Humic Materials

Humic materials contain a complex mixture of compounds that represent products of microbial degradation, chemical polymerization and oxidation (51). Many of the humic materials were also dissolved in the sample water so their effects upon the extraction were not directly observable. Interaction of the analytes with humic materials are unknown.

Attempts to Enhance Recovery Part II: Effect of Modifiers

Continuing to employ the same three step extraction method described thus far, a survey of the effects of three different modifiers on the recovery of analytes was performed. The three modifiers employed were 5% acetone, 8% acetone, and a mixed modifier composed of 4% methylene chloride/1% methanol. The mixed modifier was chosen because it had been proven to be effective in the recovery of PAHs from sediment (35). The SFE method utilized for the recovery of PAHs from sediment employed a lower pressure in the initial step. The lower pressure increases the diffusivity of the fluid, which may be helpful in penetration of the "slime" covering on the disk.

The results are shown in Table XVII. Data interpretation must be guarded as each SF elution method was only performed once; however, some general observations can be made. Depending on the modifier used 12 to 16 analytes failed to meet the requirements for recovery, compared to 25 in the previous recovery. The SF elution parameters are noted below:

Table XVII. Effect of Modifier I on recovery of Method 525.1 analytes from Marine Sample II via C-18 disk with subsequent 42 minute SF elution @ 2 ug/component.

	Percent Recovery				
Compound	A	B	C	Average	RSD
Hexachlorocyclopentadiene	45*	35*	59*	46*	26
Dimethylphthalate	142*	151*	141*	145*	3.6
Acenaphthlene	108	97	97	101	6.2
Diethylphthalate	123	144*	141*	136*	8.7
Fluorene	122	110	139*	124*	12
Hexachlorobenzene	81	106	105	97	15
Simazine	81	76	70	76	7.0
Atrazine	129	131*	123	128	3.1
Pentachlorophenol	248*	239*	227*	238*	4.3
Lindane	175*	156*	142*	156*	10
Phenanthrene	97	100	94	97	3.1
Anthracene	87	90	91	89	2.5
Trichlorobiphenyl	78	77	77	77	0.8
Alachlor	108	116	112	112	3.6
Heptachlor	67*	76	77	73	7.4
Di-N-butylphthalate	131*	132*	119	127	5.7
Aldrin	65*	63*	68*	65*	4.2
Heptachlor epoxide	120	100	100	107	11
Gamma-chlordane	68*	67*	71	69*	3.0
Pentachlorobiphenyl	62*	64*	64*	64*	2.1
Pyrene	83	91	87	87	4.7
Alpha-chlordane	64*	73	74	70	7.9
Trans-nonachlor	66*	57*	69*	64*	9.3
Edrin	188*	166*	152*	169*	11
Hexachlorobiphenyl	58*	56*	62*	59*	6.3
Butylbenzylphthalate	109	102	110	107	4.3
Di(2-ethylhexyl)adipate	60*	59*	71	63*	10
Benzo[a]anthracene	96	75	89	87	12
Chrysene	98	77	90	88	12
Methoxychlor	124	105	91	106	16
Di(2-ethylhexyl)phathalate	168*	139*	163*	157*	9.8
Heptachlorobiphenyl	93	74	77	81	13
Octachlorobiphenyl	89	71	73	78	13
Benzo[b]fluoranthrene	111	80	91	94	17
Benzo[k]fluoranthrene	101	85	92	93	8.5
Benzo[a]pyrene	86	76	86	83	7.6
Indeno[1,2,3,cd,]pyrene	105	82	98	95	12
Dibenzo[a,h]anthracene	80	69*	91	80	14
Benzo[g,h,i]perylene	97	80	88	88	10

SFE conditions:

Step 1: 10 minute dynamic extraction, pressure at 2250 psi with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 80°C. Modifier used: column A- 4% Methylene chloride/1% methanol, B: 5% Acetone, C: 8% Acetone

Step 2: 30 minute dynamic extraction with varied modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction. **Step 3:** 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 120°C throughout, pressure was held at 5500 psi steps 2 and 3. n=3. **Total SF elution time: 42 minutes.**

* = Outside EPA Criteria

SF Elution Method (Modification II)

Step 1: No equilibration time. 10 minute extraction at 2250 psi with a trap temperature at -20°C for extraction.

Step 2: No equilibration time. 30 minute extraction at 5500 psi using varied modifier CO_2.

Step 3: No equilibration time. 2 minute extraction at 5500 psi.

All other parameters remained as outlined in the General SF Elution Method, Chapter V.

None of the three different modifiers could be judged as superior, however the method employing 8% acetone modifier produced the fewest unacceptable recoveries. The majority of the RSDs ≤ 12% when these data were considered as a set. The low RSDs indicated that recoveries may not be dictated by the SF elution method, but by some other parameter. The brackish water matrix introduced several new parameters to the recoveries as discussed previously. One of the parameters thought to be an interference was the additional water trapped in the sediment on the disk (43). The water was thought to prevent the SF from properly penetrating the disk thus inhibiting recovery of the analytes. More convincing evidence was that overpressure errors caused the instrument to abort several elutions. These errors were caused by plugging in the extraction vessel, clearly demonstrating restricted flow through the region. No such plugging had been experienced in the reagent water matrix. Improving the disk drying method then became the immediate focus. An overview of disk drying methods attempted follows:

1. Increasing vacuum disk drying time.
2. Increasing modifier.
3. Introducing dessicant.

Vacuum Drying

A different post-analyte deposition drying technique was employed to attempt to eliminate the excess water on the disk. Ho suggested that improved drying could be achieved by passing a stream of nitrogen through the disk under a vacuum of 15 inches of Hg for 10 minutes (43). The nitrogen stream also removed the remote possibility of oxidation of analytes from contact with the air (43). The SF elution method employed was identical to the previous elution method (Modification II) utilizing 8% acetone. See Table XVIII for results. Nine of the compounds were outside of the EPA criteria. Five of the nine compounds could be considered problem compounds; three were phthalates, the other two were HCB and HCCP. Consequently, only 4 of 39 compounds were out of range without an explanation. The three highest molecular weight PAHs produced RSDs of >30%. These results indicated that a longer disk drying time produced positive effects on the recovery of analytes. Moderately volatile analytes with unacceptable recoveries may be positively influenced by returning the chamber pressure from 2250 psi to 5500 psi resulting in an increased density in the first step of the extraction.

Effect of Modifier

Method development was continued employing an estuary water matrix since the supply of Marine Water II matrix was limited. Differences between the two matrices were minimal. Salinity of the estuary water was ≈2% with marine water at ≈3%. The estuary water may have contained slightly more sediment than the marine water. It was also desirable that the analyte recovery method be appropriate for both matrices. It took 2 to 2.5 hours to pass the estuary water matrix through the Empore™ disk, which was slightly longer than that required for the Marine II matrix. This was indicative of larger amounts

Table XVIII. Recovery of Method 525.1 analytes from Marine Sample II via C-18 disk with subsequent SF elution @ 2 ug/component and nitrogen drying

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	68*	18
Dimethylphthalate	165*	5.9
Acenaphthlene	103	5.0
Diethylphthalate	155*	6.7
Fluorene	136*	11
Hexachlorobenzene	165*	19
Simazine	41*	6.0
Atrazine	72	3.9
Pentachlorophenol	258*	8.7
Lindane	186*	10
Phenanthrene	97	3.1
Anthracene	83	7.2
Trichlorobiphenyl	92	14
Alachlor	102	6.1
Heptachlor	84	15
Di-N-butylphthalate	126	3.4
Aldrin	75	24
Heptachlor epoxide	98	6.2
Gamma-chlordane	95	19
Pentachlorobiphenyl	89	23
Pyrene	93	8.6
Alpha-chlordane	92	16
Trans-nonachlor	89	18
Edrin	108	25
Hexachlorobiphenyl	86	26
Butylbenzylphthalate	97	33*
Di(2-ethylhexyl)adipate	93	25
Benzo[a]anthracene	95	20
Chrysene	92	20
Methoxychlor	120	12
Di(2-ethylhexyl)phathalate	153*	17
Heptachlorobiphenyl	106	25
Octachlorobiphenyl	104	27
Benzo[b]fluoranthrene	91	21
Benzo[k]fluoranthrene	91	21
Benzo[a]pyrene	79	34*
Indeno[1,2,3,cd,]pyrene	87	30*
Dibenzo[a,h]anthracene	85	34*
Benzo[g,h,i]perylene	81	24

SFE conditions:

Step 1: 10 minute dynamic extraction, pressure at 2250 psi with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 80°C.

Step 2: 30 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.

Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 120°C throughout, pressure was held at 5500 psi in steps 2 and 3 . n=3.

Total SF elution time: 42 minutes. * = Outside EPA Criteria

of sediment in the samples that could potentially interfere with sample recovery. The disk was dried for 10 minutes under a nitrogen stream as described earlier. This matrix was subjected to two SF elutions methods. The methods differed by the amount of acetone modifier used in the SF elution; the first employed 8% acetone, the second used 16% acetone. It was hypothesized that 16% acetone may act as a drying agent thus allowing increased analyte recovery. The higher percent modifier may have a greater capacity to break up the slime covering the disk after analyte deposition and thus work as a drying agent. The method is outlined below:

SF Elution Method (Modification III)

Step 1: No equilibration time. 10 minute extraction time at 5500 psi of CO_2 with a flow rate of 2 mL/minute. The ODS trap was held at -20°C during the extraction and raised to 25°C for rinsing. Chamber temperature held constant at 120°C.

Step 2: No equilibration time. 23 minute extraction at 5500 psi of 8%(v/v) or 16% (v/v) modified CO_2 with a flow rate of 2 mL/minutes. Chamber temperature held constant at 120°C.

Step 3: No equilibration time. 2 minute extraction at 5500 psi CO_2 with a flow rate of 4 mL/minute. Chamber temperature held constant at 120°C.

The results are illustrated in Table XIX. Dimethylphthalate apparently degraded in the standard and therefore was not investigated. Both of the SF elution methods produced inconsistent and erratic results. The method employing 8% acetone produced recoveries with 10 analytes not meeting EPA criteria; the 16% acetone method resulted in 12 analytes failing criteria disproving the assumption that the higher percent acetone modifier would increase recoveries. Continual inconsistencies, despite numerous changes in the SF elution method implied that the controlling factor was not the SF elution method. The addition of sediment to a matrix can have several implications including: (a) an extended period of time for sample deposition, (b) adherence of the sediment to glassware, and (c) sorption of the analytes to the sediment.

Table XIX. Effect of modifier II on recovery of Method 525.1 analytes from Estuary water via C-18 disk with subsequent SF elution @ 2 ug/component.

	8% Acetone		16% Acetone	
Compound	Recovery	RSD	Recovery	RSD
Hexachlorocyclopentadiene	74	8.3	61*	8.6
Dimethylphthalate	ni		ni	
Acenaphthlene	99	3.3	113	16
Diethylphthalate	107	6.7	140*	25
Fluorene	97	4.4	117	16
Hexachlorobenzene	83	9.5	95	36*
Simazine	65	4.0	93	29
Atrazine	97	9.5	91	86*
Pentachlorophenol	140*	13	214*	36*
Lindane	98	9.8	127	30
Phenanthrene	96	2.1	107	11
Anthracene	76	1.7	81	10
Trichlorobiphenyl	80	2.4	81	15
Alachlor	106	4.8	134*	11
Heptachlor	76	12	84	12
Di-N-butylphthalate	113	3.0	135*	14
Aldrin	65*	8.8	65*	19
Heptachlor epoxide	106	3.1	109	13
Gamma-chlordane	72	12	71	19
Pentachlorobiphenyl	64*	17	64*	19
Pyrene	80	8.4	87	21
Alpha-chlordane	70	9.7	71	19
Trans-nonachlor	64*	12	67*	25*
Edrin	88	52	107*	13
Hexachlorobiphenyl	65*	25	62*	24
Butylbenzylphthalate	102	9.9	125	19
Di(2-ethylhexyl)adipate	559*	52*	582*	104*
Benzo[a]anthracene	90	17	92	9.5
Chrysene	90	17	92	9.2
Methoxychlor	76	52*	67*	66*
Di(2-ethylhexyl)phathalate	116	16	138*	10
Heptachlorobiphenyl	82	22	78	9.7
Octachlorobiphenyl	85	22	86	11
Benzo[b]fluoranthrene	85	18	91	13
Benzo[k]fluoranthrene	85	18	88	10
Benzo[a]pyrene	68*	19	71	18
Indeno[1,2,3,cd,]pyrene	65*	33*	74	24
Dibenzo[a,h]anthracene	69*	31*	67*	67*
Benzo[g,h,i]perylene	61*	25	68*	24

SFE conditions:

Step 1: 10 minute dynamic extraction, pressure at 5500 psi with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 120°C. Modifier used: 8% Acetone or 16% Acetone **Step 2:** 30 minute dynamic extraction with varied modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction. **Step 3:** 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 120°C throughout. n=3. **Total SF elution time: 42 minutes.** * = Outside EPA Criteria ni = not investigated

Possible interference mechanisms of the suspended sediment on the SF elution are illustrated in Figure 12. The first mechanism describes surface interaction where a layer of sediment is trapped between the water layer and the disk. The water/sediment layer inhibits SF flow through the disk which reduces the fluid's access to the analytes. The SF would only have restricted access to the disk from the bottom side. A second mechanism could involve the partial blocking of the disk by a sediment/water plug. The plug would again block access of the SF solvent to the disk but could also contain analytes that are sorbed or bound to the sediment. The plug could also maintain water on the surface or interstitially which would interfere with SF elution.

Desiccant Disk Drying

Inhibiting the free flow of SF through the matrix could result in random analyte loss and give rise to an explanation for the random recoveries. If either of the proposed mechanisms are correct, then a more effective drying method should allow for greater SF access to the analytes. An additional drying step was incorporated in order to test this theory. The Empore™ disks were held overnight in fresh calcium sulfate desiccant. The disks remained damp but no longer appeared saturated after desiccation. No changes were made in the SF elution method using 8% acetone modifier.

The results from the recovery are reported in Table XX. Five analytes did not meet the criteria for recoveries however 4 of these analytes are phthalates. High recoveries for the phthalates are most probably due to contamination. No definitive explanation for the atrazine recovery could be determined. All RSDs were ≤12% indicating consistent recoveries for all analytes. These results confirmed that the moisture

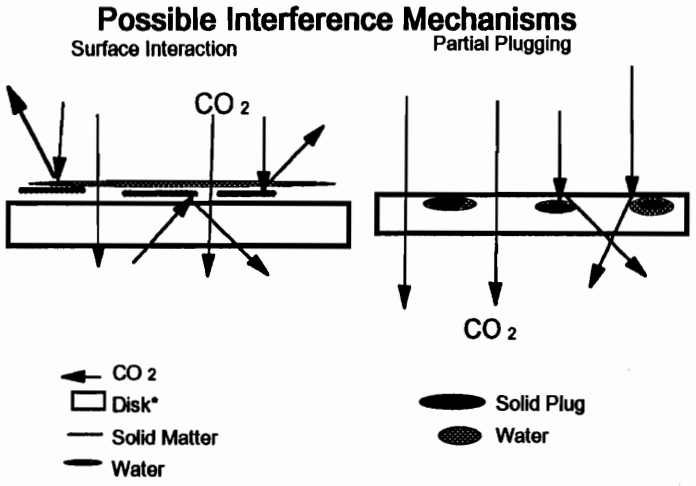


Figure 12. Possible interference mechanisms that may reduce analyte recoveries.

Table XX. Recovery of Method 525.1 analytes from Estuary water via C-18 disk with subsequent SF elution @ 2 ug/component and desiccant drying

Compound	Percent Recovery	RSD
Hexachlorocyclopentadiene	80	4.9
Dimethylphthalate	116	3.2
Acenapthlene	107	3.4
Diethylphthalate	145*	8.6
Fluorene	110	12
Hexachlorobenzene	109	9.0
Simazine	111	1.4
Atrazine	134*	6.0
Pentachlorophenol	ni	
Lindane	126	4.5
Phenanthrene	105	4.6
Anthracene	86	1.9
Trichlorobiphenyl	98	7.6
Alachlor	126	7.2
Heptachlor	106	6.6
Di-N-butylphthalate	192*	4.5
Aldrin	92	6.4
Heptachlor epoxide	119	3.6
Gamma-chlordane	108	3.9
Pentachlorobiphenyl	115	5.0
Pyrene	114	0.4
Alpha-chlordane	108	3.9
Trans-nonachlor	112	9.0
Edrin	ni	
Hexachlorobiphenyl	105	4.1
Butylbenzylphthalate	140*	3.8
Di(2-ethylhexyl)adipate	128	6.7
Benzo[a]anthracene	103	4.9
Chrysene	103	4.9
Methoxychlor	99	3.9
Di(2-ethylhexyl)phathalate	151*	6.3
Heptachlorobiphenyl	101	5.5
Octachlorobiphenyl	110	5.5
Benzo[b]fluoranthrene	109	9.1
Benzo[k]fluoranthrene	105	5.9
Benzo[a]pyrene	91	2.8
Indeno[1,2,3,cd,]pyrene	91	7.0
Dibenzo[a,h]anthracene	98	1.6
Benzo[g,h,i]perylene	96	1.9

SFE conditions:
Step 1: 10 minute dynamic extraction, pressure at 5500 psi with CO_2 @ 2 mL/minute (liquid). Trap at -20°C for extraction and raised to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature at 120°C.
Step 2: 30 minute dynamic extraction with 8% acetone modified CO_2 @ 2 mL/minute (liquid). No rinse. Trap at 80°C for extraction.
Step 3: 2 minute dynamic extraction with CO_2 @ 4 mL/minute (liquid). Trap was held @ 80°C for extraction and reduced to 25°C for rinsing with $MeCl_2$ (0.6 mL). Chamber temperature was held 120°C throughout, pressure was held at 5500 psi in steps 2 and 3 . n=3.
Total SF elution time: 42 minutes.
* = Outside EPA Criteria, ni = not investigated

content of the disk is important in the recovery of the analytes, (i.e. when the moisture content is reduced, the SF elution method is sufficient to recover the analytes). Endrin and pentachlorophenol were not investigated due to degradation.

An identical recovery was attempted using the Marine Water II matrix. The recoveries for this SF elution were not as impressive. A total of 6 analytes did not meet recovery criteria; 4 of these were "problem compounds" including PCP, HCCP and two phthalates. Explanation for deviation from accepted recoveries has been discussed previously in Chapter V. Atrazine and lindane were the only compounds that produced unacceptable recoveries without explanation. Five analytes had RSDs > 30%. The results may indicate that the dessicant used should have been re-dried before using as moisture retained in the disk could effect analyte recovery.

SUMMARY

The SF elution of EPA Method 525.1 analytes can be accomplished providing proper drying of the disk precedes the elution. The moisture content appears to combine with the sediment to inhibit the recovery of the analytes. If the disk is properly dried, a three step, 42 minute SF elution is adequate. The SF elution method employed a 10 minute first step with CO_2, a 30 minute second step with 8% (v/v) acetone modified CO_2 and a 2 minute third step utilizing CO_2. A 1.2 mL portion of methylene chloride was used as a trap rinse solvent along with 4.8 mL of acetone as a modifier. The final extract required no further preparation prior to assay.

Chapter VII

CONCLUSION

This study demonstrated expanded applications of SFE for the recovery of analytes from non-traditional matrices. Three distinct analyte/matrix combinations were examined: (1) atovaquone from rat feed, (2) acyclovir from ointment, and (3) multiple semi-volatile analytes from distilled and brackish water sources.

SFE feasibility for atovaquone was initially evaluated by employing an extraction profile. The extraction profile enabled an estimate of the solubility and the extraction kinetics of the analyte in the SF. A second extraction profile drug produced a lower analyte recovery when 2% methanol modified CO_2 was employed versus unmodified CO_2. The lower recovery was attributed to the organic modifier interfering with analyte trapping (4). The study further illustrated the ability of SFE to recover an analyte over a large range of concentrations. High quantitative results were obtained for all concentration levels, and were comparable to the traditional extraction method.

The recovery of acyclovir from Zovirax® Ointment 5% presented a unique situation. Acyclovir is a polar molecule, insoluble in modified CO_2; while the ointment base was soluble in the SF. Therefore, the focus of this study was to recover the analyte by removing the matrix. Two critical steps were developed in order to quantitatively recover the analyte. First, the analyte had to be retained in the extraction vessel by introduction of an inner vessel and second, a highly efficient analyte transfer was required. The transfer of analyte was achieved by sonication of the inner vessel in an aqueous solution. A 20 minute SF extraction employing 2% methanol modified CO_2 produced an average recovery of 99% with RSDs of < 6%. Inverse SFE should be applicable to other cream and ointment matrices for the recovery of SF insoluble analytes.

Supercritical fluid extraction was also applied to the recovery of semi-volatile analytes from water via solid phase disk extraction. A three step SFE method was developed which allowed for the used of organic modifier in conjunction with quantitative solid phase trapping of analytes. The first step employed SF CO_2 to recover the most volatile analytes; while the second step employed acetone modified CO_2 to recover analytes not extracted initially. The final step removed residual modifier from the SFE. A group of 16 PAHs fortified in distilled water @ 2 ppb was investigated. A preliminary study was conducted to determine the proper technique for quantitation; absolute versus relative. It was concluded that relative quantitation was necessary due to analyte adherence to glassware. A 27 minute SF elution method was subsequently developed and produced recoveries >90%. SF elution reduced organic solvent use by 75% and eliminated the solvent reduction procedure.

The study of SPE/SF elution was expanded to 38 of EPA Method 525.1 analytes, including PAHs, PCBs, phthalates, and organochlorines. Solid Phase trapping had not previously been successfully applied to such a large group of analytes. Quantitation of the phthalates was found to be problematic due to ubiquitous contamination. The SF elutions were evaluated in accordance to EPA criteria, i.e. recoveries of 70-130% and RSDs <30%. The additional analytes necessitated an increase in the SF elution time to 35 minutes. All but four analytes were within the stated criteria.

Application of SPE/SF elution to real, brackish water samples, obtained from the Chesapeake Bay, was also investigated. The brackish water samples contained suspended sediment, dissolved organic matter and salt. The sediment made post-deposition drying of the SPE disk difficult and the residual water interfered with analyte recovery. Attempts to dry the disk included increasing the vacuum drying time, use of a higher percent of modifier and desiccation. The best results were obtained by first drying the disk for 10

minutes under a stream of nitrogen, followed by overnight dessication, and finally, SF elution. The SF method required a 3 step, 42 minute extraction which would reduce organic solvent use by 60% compared to traditional elution. All but five analytes met EPA criteria when fresh dessicant was used to dry the disk; four of the unacceptable recoveries were presumably due to plasticizer contamination. The results indicated that SF elution of analytes from a brackish water matrix was feasible, but an optimized disk drying method must be developed before SPE/SF elution can become a routine process.

Chapter VIII

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Chapter IX
APPENDIX I

Statistics for Chapter IV-Pooled t-Test

The pooled t-test is employed to test if there is a statistical difference between the means of two data sets (61). In order to perform this test the Null Hypothesis (H_O) must be set and tested. The Null is that the two means are equal. The test is based on a pooled standard deviation (s). The pooled standard deviation is calculated by:

$$s^2 = \{(n1 - 1)\, s_1^2 + (n_2 - 1)\}/\, (n_1 + n_2 - 2)$$

Where

n_1 = number of measurements in data set 1

n_2 = number of measurements in data set 2

s_1 = standard deviation in data set 1

s_1 = standard deviation in data set 1

n = $n_1 + n_2 - 2$ = number of degrees of freedom

The calculated value of t is derived from the following equation:

$$t_{cal} = \{(mean_1) - (mean_2) / s\, (1/n_1 + 1/n_2)^{1/2}$$

The absolute value of t calculated (t_{cal}) is compared to the table value of t (t_{tab}). If (t_{cal}) < (t_{tab}) the null hypothesis is accepted. According to *Statistics for Analytical Chemist*, the (t_{tab}) with four degrees of freedom at the 99% confidence level is 4.60 (p.191).

Table XXI - Pool t Test for the Comparison for Tables III and IV

$$H_O : (mean_1) = (mean_2)$$

Compound	t_{cal}
Naphthalene	3.60
Acenaphthylene	0.30
Acenaphthylene	0.50
Fluorene	0.51
Phenanthrene	1.16
Anthracene	5.09
Fluoranthene	3.44
Pyrene	1.61
Benzo[a]anthracene	2.73
Chrysene	0.52
Benzo[b]fluoranthene	1.94
Benzo[k]fluoranthene	2.10
Benzo[a]pyrene	2.29
Indeno[1,2,3-cd]pyrene	0.27
Dibenzo[a,h]anthracene	1.26
Benzo[g,h,i]perylene	1.05

$t_{tab} = 4.60$, therefore null hypothesis is accepted for all analytes with the exception of anthracene.

Chapter X
APPENDIX II

Table XXII. Quantitation Ions Employed for GC/MS

Compound	Quantitation Ion (m/z)
Hexachlorocyclopentadiene	237
Dimethylphthalate	163
Acenaphthylene	152
Diethylphthalate	149
Fluorene	166
Hexachlorobenzene	284
Simazine	201
Atrazine	200
Pentachlorophenol	326
Lindane	181
Phenanthrene	178
Anthracene	178
Trichlorobiphenyl	256
Alachlor	160
Heptachlor	100
Di-N-butylphthalate	149
Aldrin	66
Heptachlor epoxide	81
Gamma-chlordane	375
Pentachlorobiphenyl	326
Pyrene	202
Alpha-chlordane	375
Trans-nonachlor	409
Edrin	81
Hexachlorobiphenyl	360
Butylbenzylphthalate	149
Di(2-ethylhexyl)adipate	129
Benzo[a]anthracene	228
Chrysene	228
Methoxychlor	227
Di(2-ethylhexyl)phthalate	149
Heptachlorobiphenyl	394
Octachlorobiphenyl	430
Benzo[b]fluoranthrene	252
Benzo[k]fluoranthrene	252
Benzo[a]pyrene	252
Indeno[1,2,3,cd,]pyrene	276
Dibenzo[a,h]anthracene	278
Benzo[g,h,i]perylene	276

Chapter XI
APPENDIX III

Future Work

The data and discussion presented here proved the feasibility of the use of SPE/SF elution for a large number of analytes from several difficult, real water matrices. A more efficient method of disk drying is necessary before SF elution can be accepted by the EPA as a standard procedure. The drying method should be rapid (i.e. < 30 minutes) and consistent. Several possible techniques for future research are: add a sorbent, such as Hydromatrix™, directly into the vessel; replace calcium sulfate used in the dessicator with a more active compound such as phosphorus chloride; or use a combination of the above. The SF elution could be further optimized employing the method described in this chapter, if an acceptable drying technique is developed.

VITA

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