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Supercritical Fluid Extraction and Chromatography Using a Lee Scientific Series 600 SFE/SFC System

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SUPERCRITICAL FLUID EXTRACTION AND CHROMATOGRAPHY USING A LEE SCIENTIFIC SERIES 600 SFE/SFC SYSTEM

A Thesis

by

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ABSTRACT

Supercritical Fluid Extraction and Chromatography Using a Lee Scientific Series 600 SFE/SFC System (May 1992)

Timothy Scott Green, B.S., Texas A&M University

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The Lee Scientific Series 600 supercritical fluid extractor and chromatography system has been evaluated for quantitative analytical chromatography and quantitative on- and off-line extraction using benzo(a)pyrene, pentachlorophenol, and naphthalene spiked silica samples. The silica was spiked by adding chemical/solvent solutions with known chemical mass to the silica, tumbling, drying under a hood at room temperature, and then remixing. The samples were split so other researchers could perform the traditional Soxhlet and tecator extraction procedures on the same material. The supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) was conducted with high purity carbon dioxide as the carrier fluid and mobile phase. On-line extraction utilized a cryofocusing tee on the instrument to immobilize and concentrate the analytes during dynamic extraction of the sample. After the extraction was complete, the tee was thawed and the analytes impulse loaded on the chromatographic column. Off-line extraction carried the extractant through a pressure restrictor and into a collection vial, or vials, of methylene chloride solvent,

where the analytes dissolved into aqueous phase. The solvent solution was then concentrated and analyzed by SFC.

The instrument was able to complete quantitative chromatography satisfactorily, but unable to perform reproducible quantitative on- or off-line extractions as configured. The on-line extraction problems center in the cryofocusing tee, which is unable to maintain a constant temperature as set up, altering the trapping efficiency during, and between, extractions. The off-line extraction difficulties revolve around the expansion of the supercritical extractant to atmospheric pressure through the pressure restrictor and the subsequent trapping of the analytes into the aqueous phase. Crystallization of B(a)P and PCP in the pressure restrictor was encountered. The solvent trapping schemes were unable to transform all the analytes to aqueous phase, most notably with naphthalene.

ACKNOWLEDGEMENT

This study could not have been completed without the assistance, guidance and support of many people. I am grateful to Dr. James S. Bonner, chairman of my advisory committee, for providing the vision and special funding to keep this research moving forward. I was fortunate to have committee members truly interested in my work and who provided advice and suggestions in their specialty areas during the past seventeen months. As committee members, Dr. Robin Autenrieth, Dr. K.C. Donnelly, and Dr. Aydin Akgerman each made special contributions to both my research and broader learning experiences. I also appreciate the assistance of Dr. Can Erkey and Henry Heubner during the project. Lee Scientific chemist Shawn Ludlow provided invaluable support and information throughout the project.

I also wish to thank the United States Air Force for providing the time to continue my education. I am excited to share what I have learned and address environmental issues in the Air Force community.

I am grateful to my parents, Margie and Don Lee, for planting within me a continual desire to learn. Most of all, I want to thank Susan, my wife, who has been my ardent supporter and encourager for many years. She is always ready to listen. Her love and support make life exciting and joyous.

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CHAPTER I

INTRODUCTION

Liquid extraction techniques for removing contaminants, such as polycyclic aromatic hydrocarbons (PAHs), from solid matrices, such as soil or biomass, are commonly used in laboratory work. The U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) program is currently evaluating application of such extractions as site remediation alternatives to incineration and land disposal. In laboratories, various liquid extractions are currently the only EPA approved methods of quantitative extraction. Supercritical Fluid Extraction (SFE) is extraction performed above the critical temperature and pressure of the solvent, enhancing its solvent powers. Because of this improved solvent power and available non-toxic SFE solvents, SFE can normally be completed in much less time than liquid extraction, without solvent waste disposal problems. These broad advantages make SFE an attractive alternative for study when compared with traditional techniques, both in the laboratory and at contaminated waste sites.

The format used in this proposal follows the guidelines set by Journal of Environmental Engineering, American Society of Civil Engineers.

FACTORS OF DEVELOPMENT

Supercritical Fluid technology is based on behavior of a compound when temperature and pressure are elevated beyond it's critical point as defined by a phase diagram. Figure 1 illustrates a generic phase diagram for a pure component. In this state the compound is denser than gases, but behaves like a liquid. These two properties improve mass transfer properties and make supercritical fluids (SCFs) ideal solvents.

SCF technology is not new, but has never fully developed because of cost and lack of practical applications. Rising energy costs and heightened concerns about the impact that chemicals and wastes have on the environment spurred SCF development. McHugh and Krukonis (1986) state that SCF development as a separation technique is the result of:

1. Significantly increased energy costs over the previous twenty years, producing a comparable increase in cost of traditional, energy intensive separation techniques, such as distillation;

2. Increased government regulation of industrial solvents, such as chlorinated hydrocarbons. Non-toxic SCF solvents, such as carbon dioxide, are viable alternatives because they are not strictly regulated and are used up in-process, eliminating disposal costs and responsibilities;

3. Continually changing and increasingly stringent pollution control legislation. These changes, and impending future changes, have caused industry to look at alternative means of treatment;

4. Rising performance requirements of solvents, which more traditional techniques and materials can no longer meet.

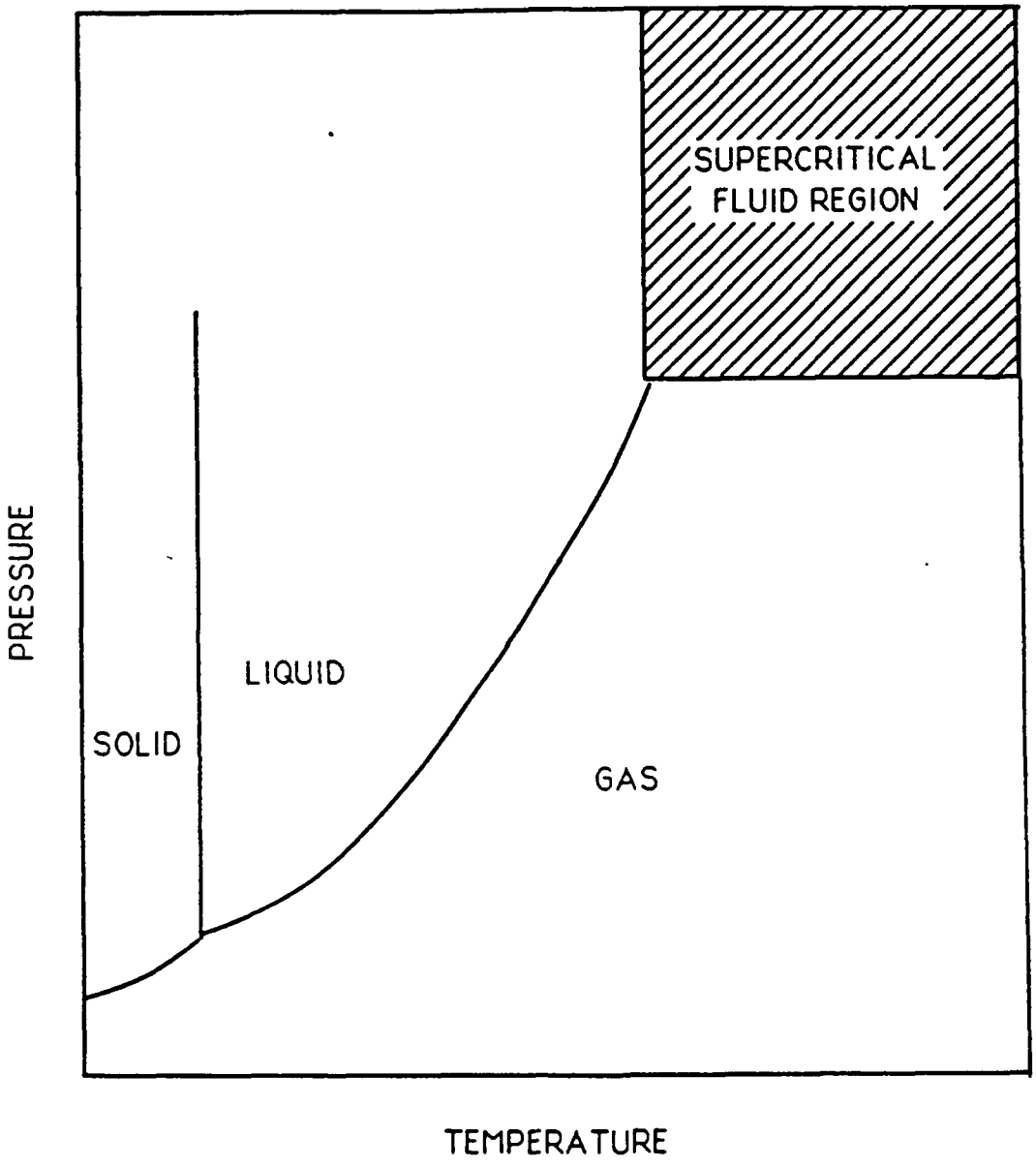

Figure 1. Pressure-Temperature Diagram for a Pure Component (McHugh and Krukonis, 1986, reprinted with permission).

Since McHugh's 1986 book was published, government regulation of solvents and wastes has become the primary reason for a large increase in research into environmental application of SCF technology.

BACKGROUND

Origins

The first work with SCFs as a solvent was presented by Hannay and Hogarth to the Royal Society of London in 1879 (Hannay and Hogarth, 1879). Hannay and Hogarth studied the solubility of cobalt and iron chlorides in supercritical ethanol. They elevated their ethanol-chlorides solution temperature above 234°C, the critical temperature of ethanol, and found that chlorides dissolved as pressure increased and precipitated when pressure decreased back to initial pressure.

Naphthalene-ethylene systems (naphthalene as the solute, ethylene as the SCF) have been studied extensively. In 1948 Diepen and Scheffer (see McHugh and Krukonis, 1986) published a classic paper on solubility and phase behavior of NAP in supercritical ethylene. They continued to study and publish papers on high-pressure phase-behavior through the 1960s. In the 1960s many other researchers began to study NAP solubility in a number of SCF solvents and produced a large amount of data on the subject (McHugh and Krukonis, 1986).

The solvent properties of liquid CO_2 were studied extensively to determine the solubilities of 261 compounds in near critical, liquid CO_2 (Francis, 1954). Classes of organic compounds studied include aliphatics, aromatics, heterocyclics, and compounds with differing functional groups. His work with liquid CO_2 can be used to determine the potential for SFE with CO_2, since anything soluble in liquid CO_2 will be soluble in supercritical carbon dioxide ($SCCO_2$).

Current Applications

Currently, SCF's are used primarily as chromatography mobile phases in supercritical fluid chromatography (SFC) and as industrial solvents. Industrial commercial applications include coffee decaffeination by Hag Aktiengeselschaft in Bremen, West Germany (Coenen and Kriegel, 1984), nicotine removal from tobacco, and extraction of hops and spices. Carbon dioxide is the primary compound used as a SFC mobile phase (Sanagi and Smith, 1988). In addition to solvent powers of the CO_2, it returns to its gas phase, which is non-toxic, upon exiting the system, leaving only the extracted and trapped material to be disposed. In chromatographic applications, the material of interest is often destroyed during destructive detection techniques. Decreased disposal requirements become particularly attractive when working with hazardous wastes, which must be handled carefully and

disposed in accordance with Federal, state, and local regulations.

SCFs have been used in separating many compounds from various systems. Materials analyzed range from PAHs to carboxylic acids to pesticides and foods (Sanagi and Smith, 1988). The analysis is primarily through SFC. The high pressures required by SCFs also speeds transport of the eluite. However, this does not mean chromatography is better and faster at higher and higher pressures. There are practical and physical property limits which create an optimal pressure for separation. Because of increased speed, longer columns can be used for additional separation while still completing analysis in less time than high pressure liquid chromatography (HPLC) and gas chromatography (GC) (Sanagi and Smith, 1988).

Laboratory scale SFE has begun receiving substantial interest in the analytical and environmental communities. Commercial analytical scale SCF extractors are available on the marketplace and competing for customers based on increased extraction speed decreased disposal requirements. There are currently no EPA approved SFE methods because a sufficient database comparing extraction methods does not exist. The EPA is working to increase the data base and publish analytical SFE methods in the near future (Lesnik, 1991). Chemical engineers are also studying benchtop and commercial SFE work for its potential application to site

remediation. Akgerman and Erkey have already proposed one such application for further research (Akgerman and Erkey, 1990).

OBJECTIVES

The principle objective of this research was to determine the capabilities and limitations of the Lee Scientific Series 600 Supercritical Fluid Extractor and Chromatography system to perform quantitative SFE and SFC. To assess the ability of the instrument, benzo(a)pyrene (B(a)P), naphthalene (NAP) and pentachloraphenol (PCP) were extracted from spiked silica in the series 600 extraction cell. These compounds were selected because they represent two classes of compounds, polyaromatic hydrocarbons and chlorinated compounds, and other researchers have published work indicating these compounds can be successfully extracted.

One of the research goals was to determine if the instrument could perform quantitative on-line extraction, the biggest challenge of the instrument. The ability to perform on-line quantitative extraction would offer great advantages to environmental chemists because of reduced sample handling and associated errors, as well as reductions in extraction and analysis times, and solvent waste generation.

A second goal of the research was to learn if the instrument is able to conduct quantitative off-line

extraction and trapping as designed. Although this method is less advantageous overall than on-line extraction, it would still reduce the sample handling and extraction time. Off-line extraction does offer an almost unlimited ability to manipulate the resultant sample for use with each sample matrix's most desirable analytical instrument.

CHAPTER II

LITERATURE REVIEW

SFE and SFC have recently received a great deal of attention by researchers because of the tremendous application potential in many fields. Publications of the past decade are both fundamental and application oriented. Fundamental work reviews physical properties, basic applications and theory, such as viscosity behavior (McHugh and Krukonis, 1986), solubilities (McHugh and Paulaitis, 1980; Kurnik and Reid, 1982; Kuk and Montagna, 1983), extraction cell geometry (Furton and Rein, 1991), general chromatographic applications (Lee and Markides, 1987) and application of chromatography theory to extraction (Erkey and Akgerman, 1990).

Researchers have also examined specific applications and problems, such as polychlorinated biphenyl extraction (PCB) (Onuska and Terry, 1989; Krukonis, 1989), ways to couple extraction and analysis (Christensen, 1985; Hawthorne and Miller, 1987; Levy and Rosselli, 1989; Levy et al., 1990; Raymer and Velez, 1991) or the effects of different column types on SFC (Wheeler and McNally, 1989; Onuska et.al., 1990). There have been so many advances made in the fundamental understanding of SCF processes, overview articles have begun to appear more frequently and in a wide variety of

literature (Hawthorne, 1990; Katauskas and Goldner, 1991; Green and Bonner, 1991). The literature reviewed here focuses on analytical applications for environmental research.

PRINCIPLES OF SUPERCRITICAL FLUID EXTRACTION

As described previously, a solvent's SCF phase exists beyond its critical temperature and pressure. Figure 1 illustrates the phase and how it falls between liquid and gas phases. As one would expect, it has transitional and mass transfer properties between the two phases. Its density is close to that of a liquid. This allows a solvent to "push through" and "wash" a compound. Mass transfer properties, viscosity and diffusivity, improve by an order of magnitude over those of the liquid phase. A comparison of these properties is shown in Table 1 (Pauliatis et al., 1983). The mass transfer properties are also improved by the almost zero surface tension of SCFs, which allows greater micropore penetration of solid matrices (McHugh and Krukonis, 1986).

Table 1. Average Mass Transfer Properties of Gas, SCF, and Liquid Phases.

Property	Gas	SCF*	Liquid
Density (kg/m	1	700	1000
Viscosity (Ns/m^2)	10^{-5}	10^{-4}	10^{-3}
Diffusion Coef (cm^2/s)	10^{-1}	10^{-4}	10^{-5}

* At $T_r = 1$ and $P_r = 2$

Carbon Dioxide

The most commonly used supercritical fluid is carbon dioxide, involved in almost all SCF studies. The variety of solutes and SCF solvents studied in the 1980s increased as our understanding of high pressure behavior and desire for alternative solvents or products rose. Other solvents, such as nitrous oxide, ammonia, freon and xenon can be used as SCFs as well (Sakaki et al., 1990; Ashraf-Khorassani and Taylor, 1990; Li et al., 1990), but are not as safe, cheap, available, and odorfree as CO_2 (Katauskas and Goldner, 1991). Many applications and research efforts have included modifiers mixed in the CO_2 to improve performance for specific application. In general the solvent modifier changes the mobile phase density producing an effect similar to that of increasing the density of pure CO_2 (Janssen et al., 1989). These modifications were developed as our knowledge base expanded. Several specialty gas companies routinely sell CO_2 with modifiers in the gas, such as $SCCO_2$ with 5% methanol or xylene.

Solvent Capacity

Advantages of SFE over liquid extraction (Schantz and Chesler, 1986) include controlling the solvent power of the SCF by adjusting the pressure. Liquid solvent power can only be altered by varying the temperature or solvent composition. Physical parameters affecting solving power of an SCF include

density, diffusivity, and viscosity (Chesler and Schantz, 1986). Fluids with high diffusivity and low viscosity provide the most efficient means of mass transfer in extractions.

The extracting potential of carbon dioxide is primarily a function of density. Solvent properties increase as density increases (Chesler and Schantz, 1986). Density of carbon dioxide increases with increasing pressure and decreases with increasing temperatures as shown in Figure 2 (Angus et al., 1976). Once in the supercritical region, increasing temperature decreases solving power because density will decrease. High compressibility of carbon dioxide (large density changes with small pressure change) provides broad selectivity of solvent power.

Figures 3 and 4 show diffusivity and viscosity of carbon dioxide versus pressure, contributing factors for determining total solving power. Diffusivity decreases and viscosity increases with increasing pressure, which inhibit mass transfer. However, the diffusivity for solutes in organic liquids included in Figure 3 (Paulaitis et al., 1983, referenced by McHugh and Krukonis, 1986) show that diffusivity of carbon dioxide can be from 1 to 2 orders of magnitude greater than that of organic liquid solutes. Figure 4 shows the variability of carbon dioxide viscosity, which at high pressure is an order of magnitude below typical

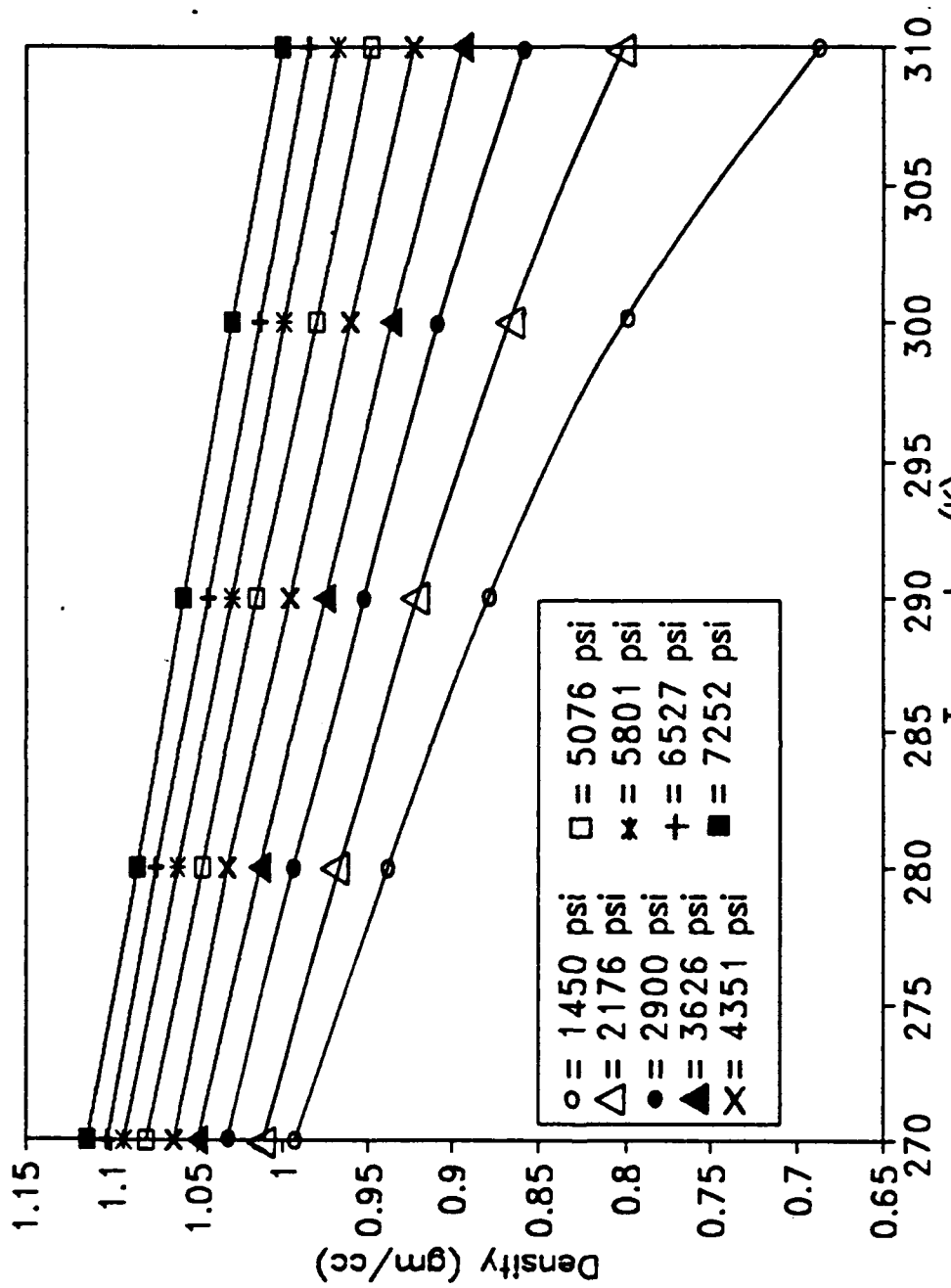

Figure 2. Carbon Dioxide Density as a Function of Pressure (adapted from Angus et al., 1976)

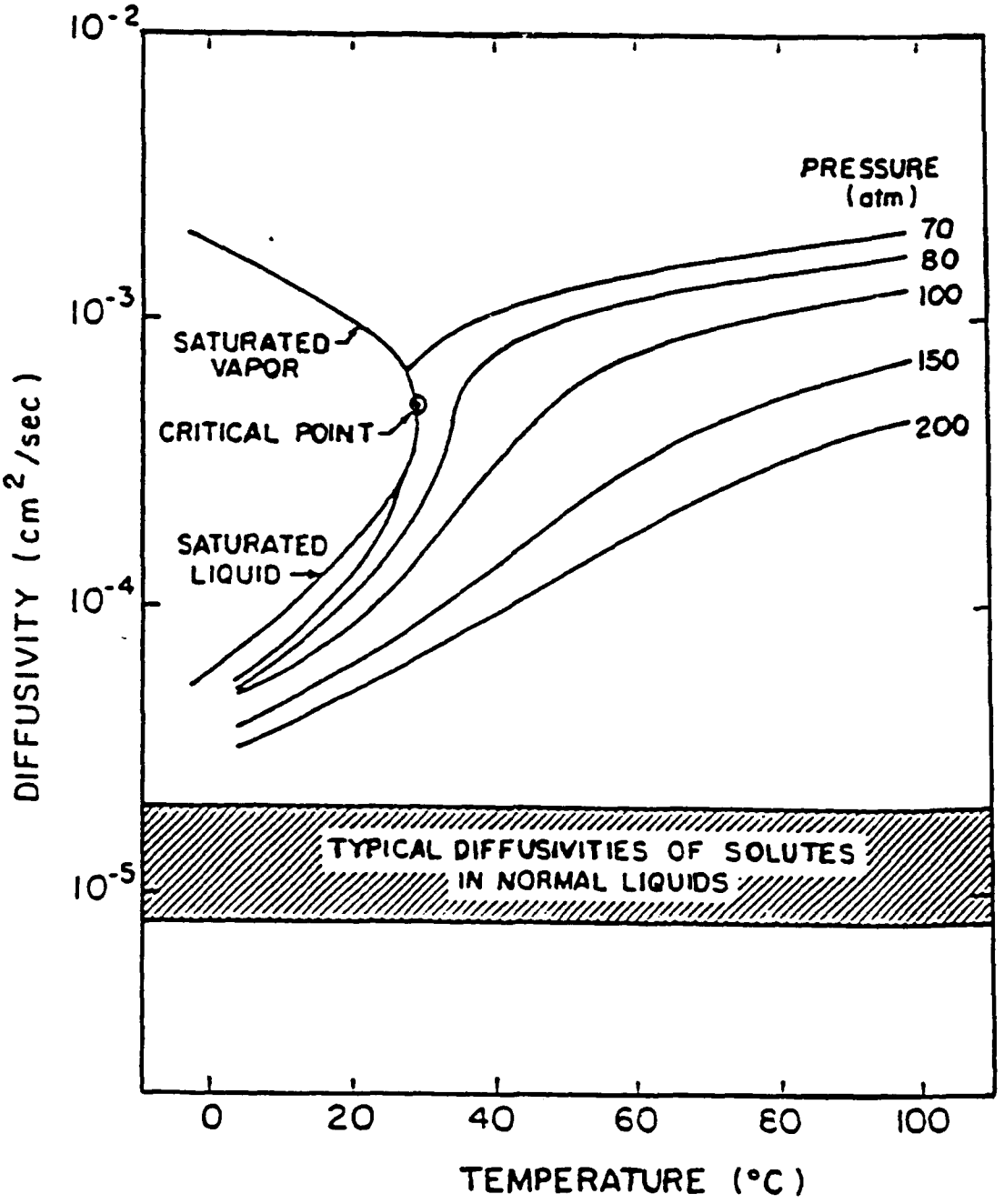

Figure 3. Carbon Dioxide Diffusivity Behavior (McHugh and Krukonis, 1986, reprinted with permission).

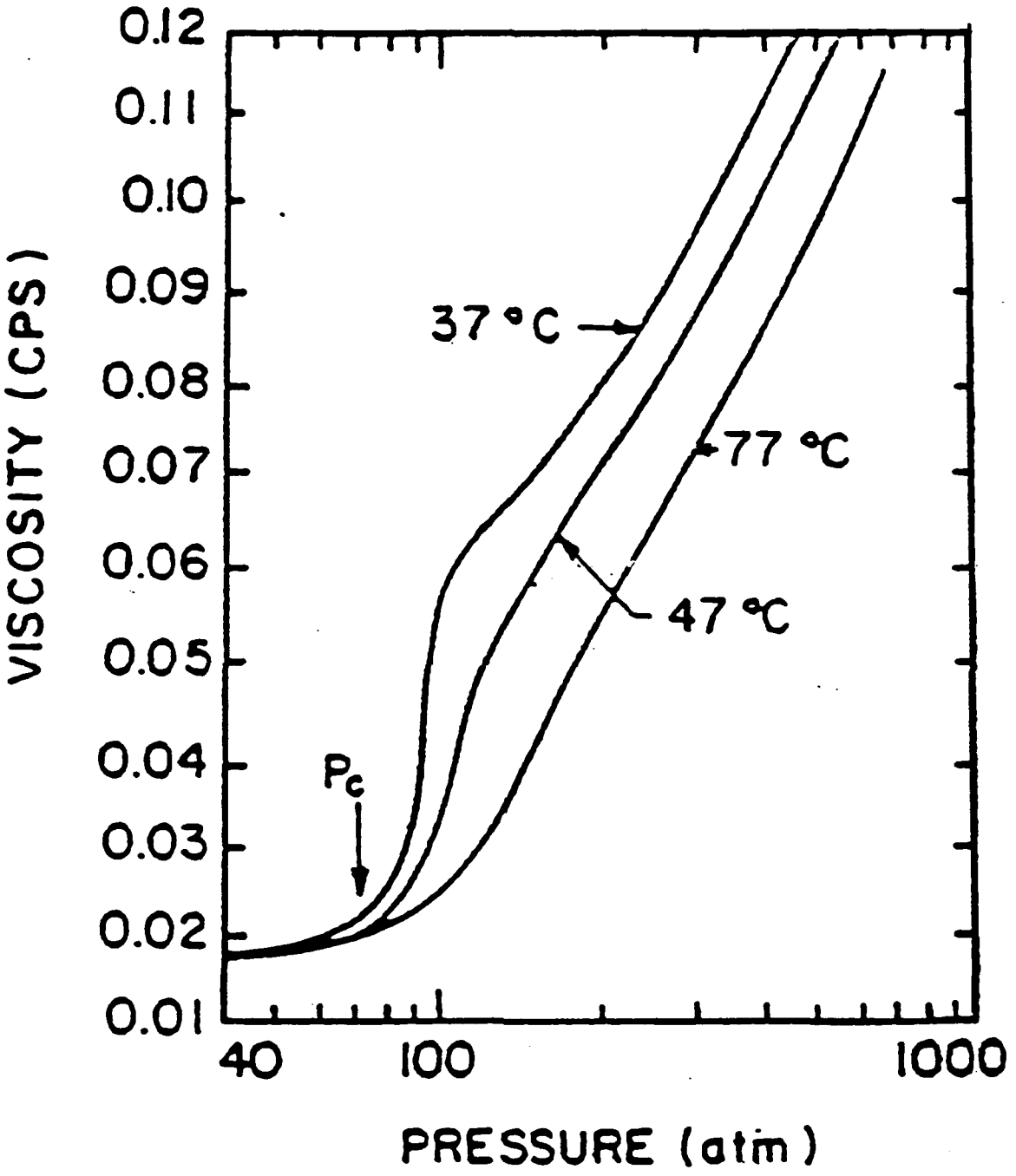

Figure 4. Carbon Dioxide Viscosity Behavior (McHugh and Krukonis, 1986, reprinted with permission).

viscosities of liquid organic solvents (de Filippi et al., 1980 in McHugh and Krukonis, 1986).

EXTRACTION OF POLLUTANTS FROM SOLID MATRICES

The goal of treatment oriented extraction is to remove pollutants from waste streams. Of particular interest is the ability of SFE to remove pollutants, such as pesticides or PCBs, from contaminated soils. Characteristics of SCFs which are valuable for rapid and quantitative extraction and recovery of organic pollutants from environmental samples include selectivity of extraction by adjusting pressure and ability to concentrate the extracted species at ambient or sub-ambient temperatures (Hawthorne and Miller, 1986). The extracted species can be concentrated to prevent loss of volatile analyte species due to relatively high SCF volatility.

Hawthorne and Miller (1986) studied SFE with diesel exhaust collected onto filters, National Bureau of Standards standard reference material (SRM) 1650 (diesel exhaust particulate) and biosludge from an activated sludge pilot wastewater treatment plant for waste produced during lignite coal gasification. Duplicate 5 mg samples were extracted using a SFT Model 250-TMP supercritical fluid pumping system (Lee Scientific) and triplicates of the resultant extract solution analyzed using GC/MS. The 5 mg samples are typical of the sample sizes used in most analytical SFE work. These

small sample sizes can create difficulty in obtaining repeatable homogeneous samples from true environmental samples rather than lab spikes.

The researchers found PAHs can be extracted rapidly (relative to liquid extractions, which may take several hours or days) and quantitatively from solid samples and Tenax-sorbent traps. When a five percent methanol modifier was added to the carbon dioxide for further work with the SRM, recovery time decreased from 90 to 30 minutes. The SRM components (fluoranthene, pyrene, benz[a]anthracene, and B(a)P) were completely recovered to within certified ranges. PAHs were also successfully extracted from test biosludge.

Class selective extraction by pressure variance was also investigated. While, in general, SFE simplifies extraction, class selectivity would further simplify analysis by reducing the number of different analytes passing through the chromatographic instrument. A SRM 1659 sample was extracted at low pressure (75 atm at 45°C) for five minutes and then at high pressure (300 atm at 45°C) for ninety minutes. The low pressure extraction removed approximately 85% of the alkanes while retaining over 90% of the PAHs until the high pressure extraction. This suggests that samples with high concentrations of extractable organics, such as the alkanes, which make chromatograph interpretation difficult but are of little toxicological significance, may be removed without fractionation methods. Fractionation following extraction

takes time and can be a source of sample contaminant loss prior to analysis.

Richards and Campbell (1991) conducted a comparison study of SFE with Soxhlet and sonication extraction, methods commonly used today, for removal of sixteen environmentally significant semivolatile organic compounds. Analytical reference standards and spiked soil samples were used in the extractions. The extraction and trapping set-up shown in Figure 5 was developed during their research. The contents of the two collection flasks were combined, evaporated down to 1 ml and analyzed. Across the range of compounds extracted, they found SFE using $SCCO_2$ with 2% methanol, faster and more efficient than both Soxhlet and sonication techniques. SFE also required less solvent, roughly 15 mls

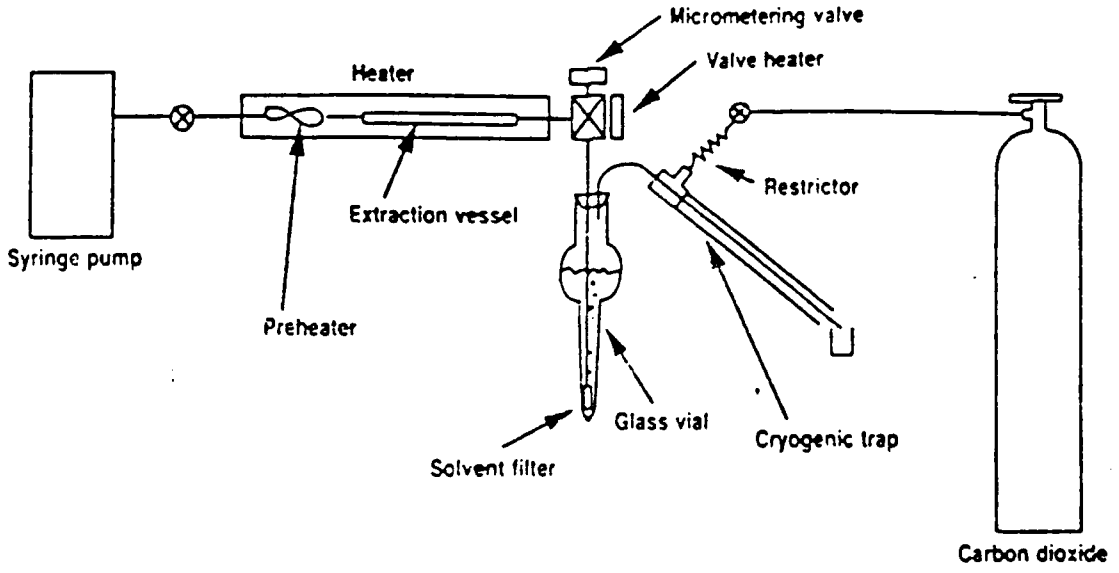

Figure 5. Schematic of Richards and Campbell's SFE Apparatus (adapted from Richards and Campbell, 1991).

for each extraction as opposed to several hundred milliliters per extraction for Soxhlet and sonication.

The study provides impetus for additional research because of the positive results and the limitation of the methods used, specifically spiked samples and the specially fabricated (non-manufactured) trapping apparatus. Extraction of pollutants from spiked samples may not realistically portray what occurs for extraction of pollutants from matrices which have been exposed for many years. The majority of EPA Superfund cleanup sites have been exposed to pollutants for decades. The ability to extract material from these sites is of primary interest. Another concern is the ability to develop methods using existing "off-the-shelf" capabilities reproducible between contract analytical laboratories.

RESTRICTORS AND OFF-LINE ANALYTE TRAPPING

Chromatographic restrictors serve two basic functions, to control the flow rate and facilitate the reduction of the carrier fluid pressure to atmospheric pressure at the discharge point. This pressure drop causes the carrier fluid to change phases, which, in turn, may cause the analytes of interest to crystallize out of the new liquid phase and onto the walls of the restrictor rather than pass into the atmosphere, collection vessel, or detector field. The

ability to eliminate such crystallization is crucial to obtaining quantitative extraction efficiency data.

When the restrictors are placed in solvent containing collectors, the solvent cools the restrictor, which can promote crystallization and produce slowed or even zero flow. Even across the phase change brought about by the pressure drop to atmosphere, maintaining higher temperatures can help reduce crystallization and sustain flow. There are two fundamentally different approaches to solving this problem found in the literature. The first is to maintain elevated restrictor temperatures and chill the solvent collecting the restrictor discharges. This decreases the bubble size and flow rate in the solvent, improving mass transfer capabilities and reducing solvent loss from volatilization (McNair and Frazier, 1991). The second approach is to add a post-extraction solvent flush of the pressure restrictor by combining the extractant and solvent prior to the mixtures entry into the pressure restrictor (Thomson and Chesney, 1991).

McNair and Frazier (1991) concentrated on delivering the analytes to the bottom of a single chilled vial of solvent. The specialty vial developed is cooled and requires the use of a resistively heated restrictor as shown in Figure 6. B(a)P was extracted from spiked glass beads into an empty vial, a vial with methylene chloride (MeCl) and from spiked soil into MeCl. The recoveries were 15, 88, and 88%

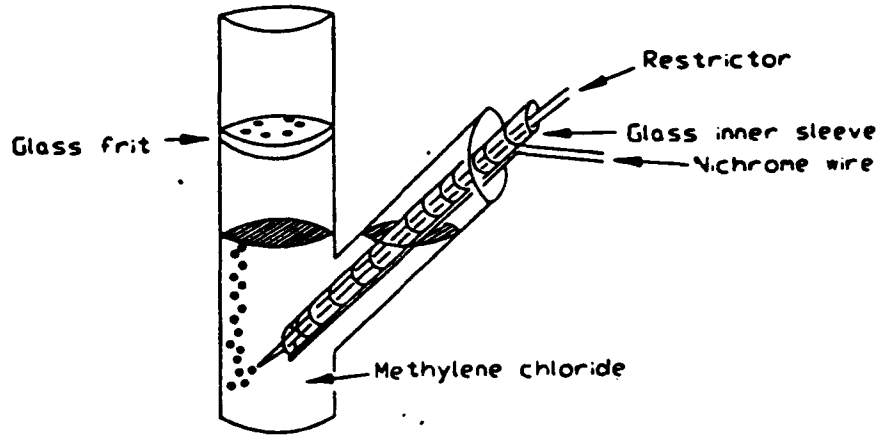

Figure 6. Resistively heated SFE collector (adapted from McNair and Frazier, 1991).

respectively. The high recoveries in MeCl show a great deal of potential for this relatively simple recovery system.

Lee Scientific began marketing a Model 703 eight cell off-line supercritical fluid extractor in 1991. They solved the heating/cooling problem by heating a stainless steel restrictor, placing it inside a stainless steel block, and submerging the block in a chilled collector vial. The restrictor remains heated and the solvent stays chilled. Lee Scientific personnel believe the system is a significant step forward because it can extract eight cells at one time and the results should be uniform and reproducible between laboratories (Lee Scientific, 1991).

An apparatus to add post-extraction solvent flush to the supercritical extractant prior to entering the pressure

restrictor was developed by Thomson and Chesney (1991). This was developed because of unpredictable precipitation of coextractants within their pressure restrictor during off-line extraction of crops, such as barley seed and barley straw, for pesticide analysis. The precipitation was reducing, and often stopping, the extraction flow. By pumping isooctane into the supercritical extractant immediately prior to the pressure restrictor, precipitation in the restrictor was minimized and no longer obstructed or significantly decreased flow during the extraction. The goal of this research was to be able to maintain flow through the extraction cell. Quantification of extraction efficiencies was not included.

Figure 5 illustrates the restrictor and trapping apparatus used by Richards and Campbell (1991) to successfully move the analytes through a system and trap them. Rather than chilling the solvent, they chose to leave it at room temperature and cryogenically trap the analytes vented in a gaseous phase from the solvent. The narrow "column" of solvent increases the pathway length of the bubbles to increase the recovery in the solvent. As discussed previously, they found this SFE apparatus to be faster and more efficient than both Soxhlet and sonication.

CHAPTER III

METHODS AND MATERIALS

To provide a direct comparison of selected "routine" laboratory extraction method efficiencies, two research groups jointly prepared a series of "spiked" samples of B(a)P, Pentachlorophenol (PCP), and NAP on both silica and Weswood loam soil. The selected extraction procedures, SFE, Soxhlet, and Tecator, were carried out on subsamples of the prepared sample mixtures. The Soxhlet and Tecator research group strictly followed EPA protocols during Soxhlet and Tecator extractions. During SFE method development, different methods were tried and the extractions compared on a chromatography response basis. The greater the response, the higher the analyte recovery. Quantification was conducted after method development. The Weswood loam samples were not extracted using SFE because of relatively poor SFE recoveries from the silica samples.

EXPERIMENTAL APPARATUS

A Lee Scientific Series 600 supercritical fluid chromatography unit with an on-line supercritical fluid extractor was purchased in 1991 to enter the growing SFE/SFC research field and as a research tool for existing work. Figure 7 is the general schematic of the overall system. A

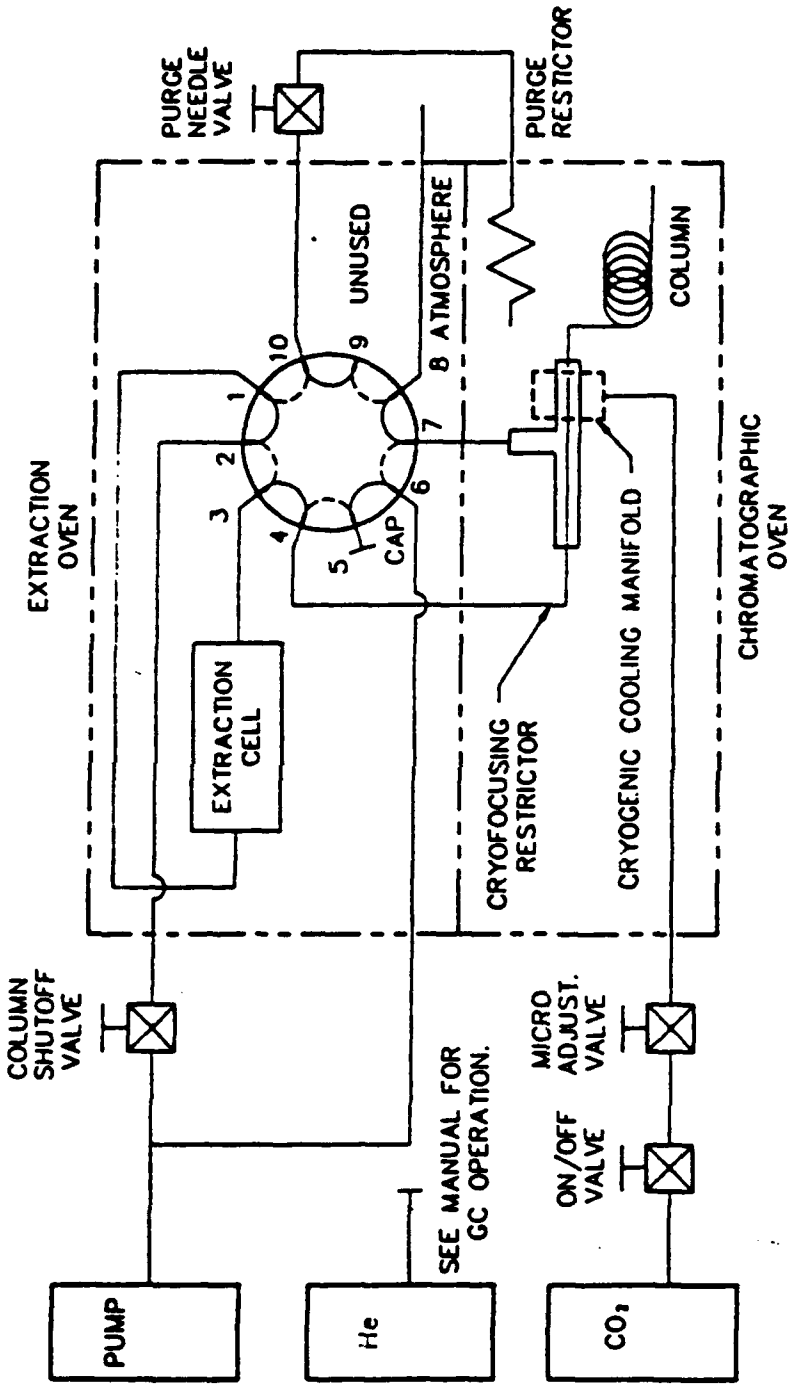

Figure 7. Schematic of Lee Scientific Series 600 SFE/SFC Instrumentation (adapted from Lee Scientific, 1988).

flame ionization detector (FID) was used for analysis. The unit offers a choice of either on-line SFE/SFC-FID through a cryofocusing apparatus or off-line extraction into a vial or tube appropriate for the extraction. The collected solution can then be analyzed using any analytical instrument the operator chooses. This system was selected because of its advertised ability to quantitatively extract, cryofocus, and analyze compounds. The most notable advantages offered by such a system are reduced sample preparation, extraction, analysis time and manpower requirements. The biggest disadvantage is the inability to conduct multiple extractions simultaneously as with some off-line extraction instruments.

Scott Specialty Gas SFC grade CO_2 was plumbed to the system syringe pump as the carrier fluid for both extraction and chromatography. Carbon dioxide was selected because it is the most widely used and amended supercritical fluid. The pump pressure range used in programming was 85 to 400 atmospheres (atm) (1250 to 5900 psi). In extraction mode the carrier fluid passes from the pump lines into port 2 of the ten port valve and out port 1 to the extraction cell. The cell can be placed in a temperature controlled block for heating. The carrier fluid exits the cell and reenters the ten port valve at port 3. It exits port 4 into a stainless steel line and is the passed into a restrictor. The restrictor differed, depending on the extraction technique, on- or off-line.

On-line Extraction

If on-line extraction was being performed, the restrictor, 25 μm internal diameter (ID) silica tubing (Lee Scientific, PN 015244), passed into a stainless steel cryofocusing tee containing a cryogenic cooling manifold (Figure 8). The extracted molecules were immobilized in the restrictor by freezing action of the cyrocoolant. The carrier CO_2 passed through the tee and exited into a stainless steel line carrying it back to the ten port valve. This "waste" CO_2 entered port 7 of the valve and exited port 8, where it was vented through a stainless steel line into the atmosphere. The "vent" also served to monitor the flow through the cell. If there was no flow from the vent line, there was no flow through the cell. The primary causes of low or no flow through the cell were large molecules slowing or stopping flow in the restrictors and excessive cooling in the cryofocusing restrictor due to poor adjustment of the coolant, bone dry CO_2.

When the extraction was completed, carrier fluid flow was shutoff using the column shutoff valve on the control panel and the pressure released from the cell by cracking the port 3 holding nut. Column shutoff valve is an inappropriate and misleading name for this valve because it controls flow to the extraction cell, not the column! The cryofocusing coolant was shutoff (flow through the restrictor to move the now concentrated molecules was stopped and pressure vented)

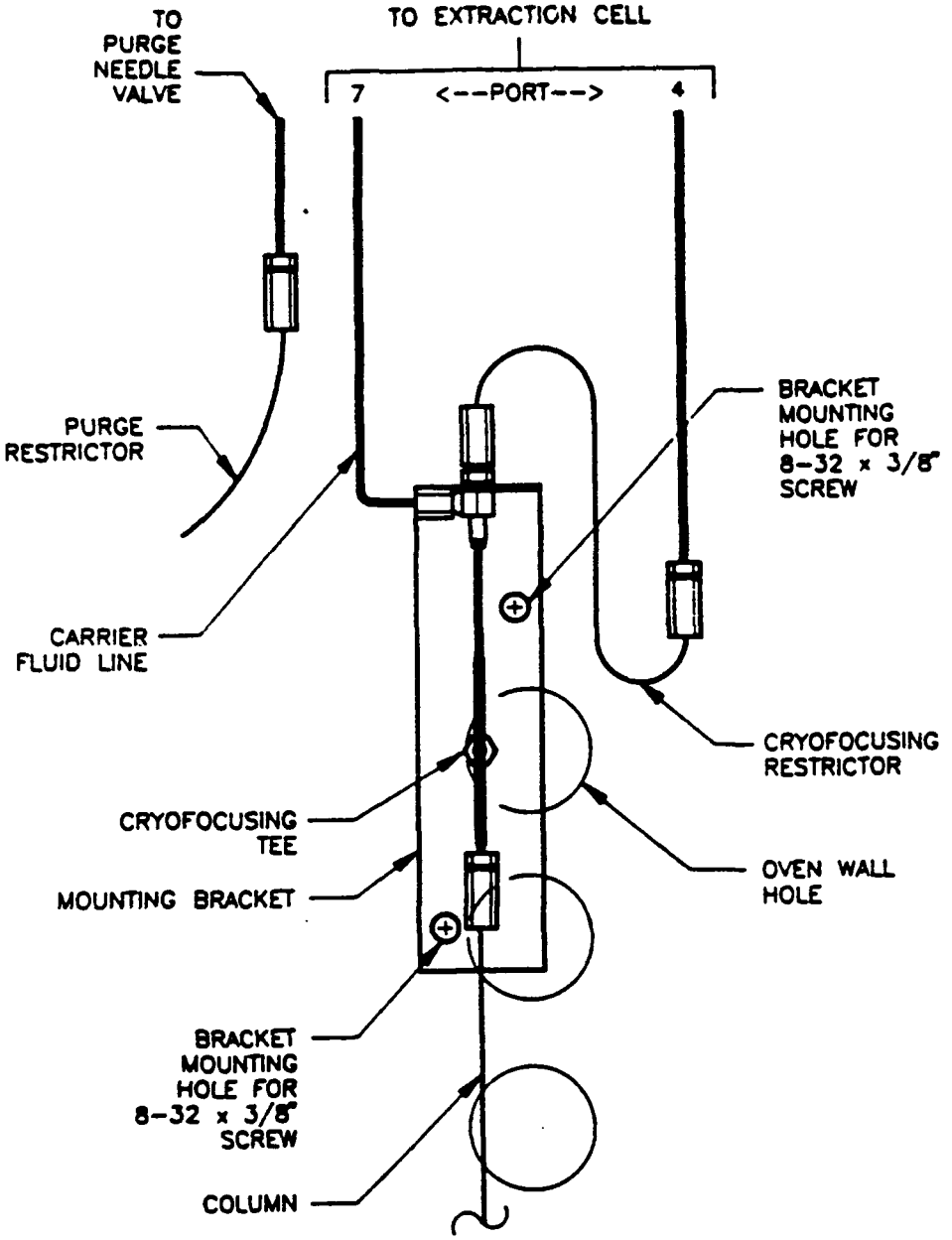

Figure 8. Cryofocusing Tee Detail (adapted from Lee Scientific, 1988).

and the chromatography program initialized. This set the oven and FID temperatures and the carrier fluid pressure to the first values in the chromatography program to be used. The extraction cell may be emptied, cleaned or rinsed with a solvent and reinstalled at this time or later during the chromatography run. The cell was always emptied and cleaned at this time.

When the program settings were reached and the cryofocusing tee thawed, the ten port valve was switched to column position and the chromatography program started simultaneously. In the column position with the column shutoff valve closed there was no flow to the extraction cell and the carrier fluid goes from the pump into port 6 and out port 7 into the cryofocusing tee where it sweeps the previously immobilized molecules onto the column as an impulse load.

Immediately following the impulse loading of the column and start of the chromatography run, the column shutoff and purge needle valves were opened to allow $SCCO_2$ through the cell for additional cleaning. This could be done at any time during the run, however, a temporary pressure drop results from the sudden split in the $SCCO_2$ flow. Beginning the cell "cleanup" at the start of the chromatography run each time, the methods are repeatable. During the "cleanup", the ten port valve was in column position, so the flow from the pump entered the ten port valve at port 2 as before, but exited

from port 3 to the extraction cell. This is opposite of the extraction flow through the cell. The fluid exits the cell and goes into port 1. The waste fluid then flows out port 10 into the purge line and, when the purge needle valve is open, through the purge restrictor to the atmosphere. When the purge valve is closed, there is no flow through the cell.

Off-line Extraction into Solvent

When off-line extraction into solvent was conducted, 15 μm internal diameter silica (Lee Scientific, PN 015299) tubing served as the restrictor and carried the extractant into a vial containing $MeCl_2$, where the extracted compounds were dissolved into aqueous solvent phase and the CO_2 vented to atmosphere. Small internal diameter tubing was selected to minimize the bubble size produced in the solvent and maximize the gas/solvent interface, thereby increasing trapping efficiency.

Several solvent trapping setups were used to achieve maximum recovery for the instrumentation used. The primary vial series, shown in Figure 9, was a set of five 5 ml micro reaction vessels (Supelco, PN 3-3299) with Teflon lined septa (Supelco, PN 2-3269). The vials were connected with 200 μm ID silica tubing (Lee Scientific, PN 015302). The tubing was also used to make a small vent port in vial 5 as well. 30 ml vials (I-Chem Research, PN S226-0040) were also used as collectors in some parts of the work.

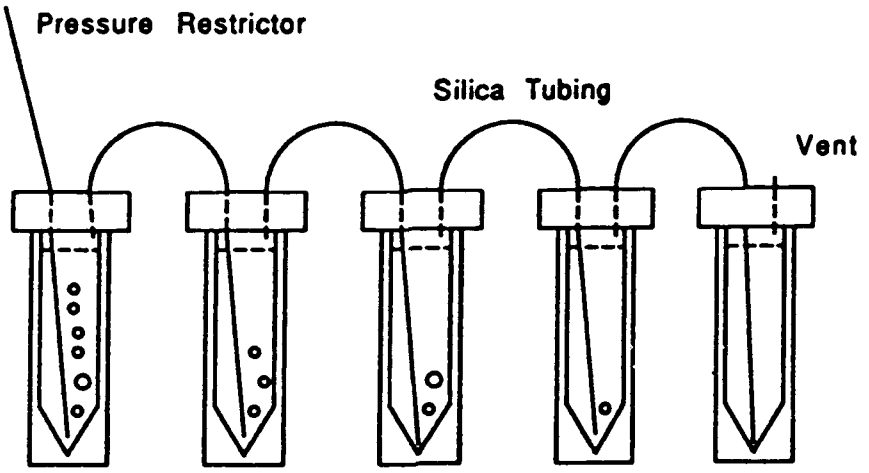

Figure 9. Five 5 ml Vial Collection Series.

Each spiked preparation sample was extracted for 60 minutes at 350 atmospheres and either 50° C (B(a)P) or 37° C (all others). To obtain the quantity of mass required for analysis, three extraction cell loads of approximately 0.8 grams each, were extracted into the same set of vials. The lines were cleaned and any precipitated contaminant in the lines recovered using a standard cleanup procedure. The cell was emptied at the conclusion of the third extraction. 25 μls of $MeCl_2$ was injected in the cell and extracted at room temperature and 350 atm for five minutes. The cleanup was conducted at room temperature so both the CO_2 and $MeCl_2$ would remain in the liquid phase and repeated three times. The cell was then sonicated in $MeCl_2$ for 3 min and allowed to dry. The recovery for each set of vials is reported as an average recovery efficiency because three cells are extracted into

one set of vials. The solvent solutions were prepared for analysis as described in the analysis preparation section.

Off-line Extraction into Chilled Vials

The second off-line extraction setup utilized a 100 μm stainless steel restrictor heated to 220° C. to reduce or prevent crystallization in the restrictor line. From the extraction cell, the $SCCO_2$ entered the ten port valve at port 3 and exits port 4 as before. The installed component stainless steel line which carried the solution to the silica restrictor was replaced with a stainless steel line connecting to a 96 cm (38 in) 100 μm ID stainless steel restrictor (Alltech, PN 31212). The end of the restrictor was crimped to increase the pressure drop and slow the flow as the carrier solution exited the restrictor. Prior to being crimped, the flow at 100 atm (1470 psi) was approximately 6 mls/min. Such a fast flow could not be maintained by the syringe pump nor allow effective trapping. Flow maintained for extractions was approximately 5 to 15 mls/hour, depending on the programmed pressure and the degree of restrictor crimping.

The stainless steel line from port 4 was insulated using insulation wrap and the restrictor heated to 220 and 240° C with heat tape (Fisher, PN). The heat tape was controlled using a variable voltage regulator (Staco Energy Products Co., Type 3PN1010) and set at 30 amps to maintain 220° C on

the line during extractions. Line cleanups were conducted at 32 amps and 240° C. The heat tape was wrapped with insulation wrap to maintain the temperature in the restrictor above the melting point of B(a)P and PCP, theoretically preventing their crystallization along the walls of the restrictor. The extraction cell was maintained at 50° C for both the B(a)P and PCP extraction sets. Initial restrictor cleanup was a 1.5 hour extraction at 125 atm with cell and restrictor temperatures at 50 and 240° C. A second, "long," cleanup followed poor recoveries and recovery of an unidentified compound from empty cell and silica blank extractions. The "long" cleanup was five hours of empty cell extraction at 250 atm with cell and restrictor temperatures at 50 and 240° C.

Figure 10 shows the 30 ml collection vial in an ice bath in an insulated cup with a styrofoam insulation "cap" over the ice. The heated restrictor was placed into the mouth of the vial and held in place, off the glass, by the insulation wrap, which was in contact with the mouth of the vial. This contact did cause a heat gradient through the vial from the mouth, which was hot, to the bottom, which was coldest. The CO_2 exiting the restrictor flowed into the glass walls of the vial. Portions of the B(a)P and PCP in the CO_2 either precipitated immediately when cooled and stuck to the glass walls or remained vaporized and were carried out of the vial.

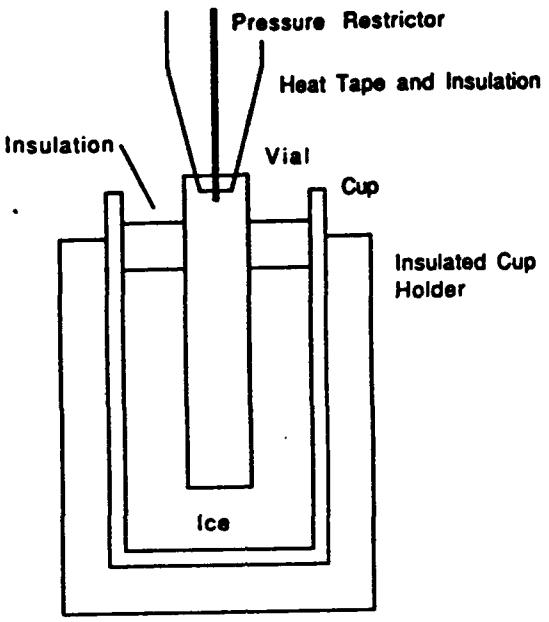

Figure 10. Chilled 30 ml Vial Set-up.

The extractions were from the same spiked silica preparations used throughout. As with the extractions into solvent, a series of three cells were extracted. The B(a)P series was broken into two sets, extraction from 0-30 minutes and from 30-60 minutes. The PCP series was three 30 minute extractions. The cleanup from both sets were collected at the conclusion of both sets of extractions. The cleanup was conducted after all lines and cells returned to room temperature. The liquid extraction cleanup procedure as the same as the extraction into solvent except for the extraction pressure, which was changed to 100 atm for the "chilled vial" line cleanup procedure.

Analytical Equipment

SFC was the analytical tool used because of the desire to perform on-line extraction and chromatography. The Series 600 SFC system is composed of a syringe pump, a Series 600 computer controller (Lee Scientific, Series 600 Controller), a Valco GC Split/Splitless Injector (Lee Scientific, PN 013190), an oven to house the column and cryofocusing tee, an FID detector (Lee Scientific FID3, PN 013124). A Lee Scientific ten meter SB-Methyl-100 50 μm ID column (Lee Scientific, PN 015002) was used in this research. The SFC/SFE controller was interfaced with an 386 IBM compatible personal computer with a Dionex Advanced Computer Interface 450 (Dionex, Model AI-450) to enhance data collection and reduction. Dionex AI-450 computer software running in a Windows 3.0 environment was used for data collection.

The Valco split/splitless injector was used in a time-split mode. The sample loop in the injector valve was loaded using a 100 μl blunt tip syringe (Unimetrics, Lee Scientific, PN 011116). The injector valve is actuated by zero grade helium for column loading. The helium rotates the valve to the column position where the carrier fluid sweeps the sample onto the column. Helium then rotates the loop back into it loading position. The longer the valve is in column position, the greater the sample volume loaded on the column. The standard injection time in this research was 0.05 seconds.

EXPERIMENTAL METHODS

Sample Spiking Procedure

The contaminants of interest were "spiked" onto silica by mixing solutions containing the contaminants into the previously washed silica samples. The silica (Fisher, PN S-151-10) was triple washed with methylene chloride followed by distilled water. The liquid was decanted off after each wash and the silica allowed to air dry after all six washes. 500 gram lots of the silica were then poured into twelve jars (I-Chem, PN 320-0500) to be spiked.

Concentrated stock solutions of B(a)P, PCP, and NAP were prepared using dry chemicals. 370 mg of B(A)P (Sigma, PN B1760) was diluted into 250 mls of acetone to create a 1480 ppm stock solution. Similarly, 460 mg PCP (Sigma, PN P1045) and 368 mg of NAP (Sigma, PN N2380) were diluted into 250 ml solutions of methanol for 1840 and 1470 ppm stock solutions. To prepare the spiking solutions, the mass of contaminant for each spike and the corresponding volume of stock solution was calculated. The volume of stock solution required was placed in a 100 ml volumetric flask and the flask brought up to volume with the appropriate solvent. The solution was then poured into the 500 gram silica sample. The resulting moisture content of the resulting mixture was approximately at field capacity and allowed good tumbling and mixing.

The spiked sample was then sealed, tumbled, dried, and shaken. Teflon thread tape was placed around the jar threads and the jar capped. Sealant tape was wrapped around lid/jar interface to create an additional seal. The jar was then placed in a plastic container, sealed and rolled for a minimum of 36 hours on an automatic roller. They were rolled to create a uniform application of the solution. The jars were removed, opened and placed under a fume hood overnight to evaporate the solvent. Heating was not used to minimize volatilization, a significant problem with NAP. Even without heating, the capillary action and contaminant affinity for the solvents cause the chemicals to be "pulled" toward the sample surface. This created a visibly nonhomogeneous sample. The jars were resealed and placed on an automatic shaker for a minimum of two hours to thoroughly remix the sample. The samples were stored in a refrigerator at 4° C until extractions were conducted. A detailed step-by-step sample spiking procedure is provided in Appendix C.

Off-line Extract Solution Preparation

The vials of solvent mixture generated during off-line extraction, as shown in figure 5, were concentrated to a 0.2 ml solution by evaporation, combination and reconstitution. The vials were placed under a fume hood and the $MeCl_2$ volatilized as nitrogen gas was blown in the headspace above the solution. When vials 2-4 were reduced to approximately

1 ml, :h was mixed using a Vortex mixer and then added to vial 1. The reduced vial 5 contents were mixed and used as a rinse for vials 4-2 respectively and finally added to vial 1. One ml of $MeCl_2$ was then added to vial 5 and the rinse procedure repeated.

The combined solvent in vial 1 was completely volatilized to leave the compounds of interest along the glass walls. The solution was reconstituted with 0.2 mls of $MeCl_2$. Vial 1 was placed on the mixer again to ensure the solvent swept across the vial walls to pull the trapped compound back into solution. This procedure was selected to mirror the traditional solvent solution reduction and reconstitution technique used in the Soxhlet, sonication, and Tecator procedures and offers an additional benefit to off-line SFE application. It ensures the analyzed solution is not supersaturated with CO_2 from the discharge into the solvent, which has been shown to hinder reproducibility in GC (Swanson and Richter, 1990). SFC would not be impacted by such supersaturation, but this provides the flexibility to split samples with GC users.

The extractions into the 30 ml vials of solvent were prepared in the same manner. The initial preparation step following off-line extraction into the chilled glass vials was to add 2 mls of $MeCl_2$ and thoroughly rinse down the inner glass walls. The solution was then prepared as the others.

Analytical Methods

Unique chromatography programs were developed for each set of solutions to take advantage of the potential speed offered by SFC. Pressure programming was the primary variable used in peak separation and temperature programming was used in the last portion of each run to improve column cleanup between sample extractions. The FID temperature was maintained at 300° C. Programming was developed with the intent to retain solvent in the 10 meter column between eight and ten minutes to achieve adequate resolution. Oven temperature during all separations was 75 and 125° during cleanup. The initial pump pressure was 85 atm for injection runs and either 85 or 100 atm for on-line SFE/SFC runs. The actual chromatography programs are provided in appendix B.

For each set of analyses, EPA certified standards from NSI Environmental Solutions, listed in Table 2, were used to make serial dilutions for five or six point standard curves. The sample analysis run sets began the standards calibration. Spiking solution samples taken from the solutions applied to the silica were analyzed to determine the actual compound loading on the silica. Extracted samples were run to determine the compound recovery. If the number of samples run between the two standard curves reached nine, a standard was again injected to ensure the calibration curve remained applicable. When four or more samples were analyzed during an analytical set, a five point calibration curve was

repeated at the end of the set. Points from the two calibration sets were averaged to determine the final calibration curve.

Table 2. Certified Standards from NSI Environmental Solutions.

Compound	Concentration (μg/ml)	Catalog Number
Naphthalene	1000±100	0053
Pentachlorophenol	5000±500	0062
Benzo(a)pyrene	1000±100	0071

CALCULATIONS

Sample Preparation

Equation 1 was used to determine the volume of prepared stock solution to apply to each silica sample.

(1)

$$mls = T\left(\frac{\mu gChem}{gSilica}\right)(500 gSilica)\left(\frac{mg}{10^3 \mu g}\right)\frac{1}{C}\left(\frac{mlSol}{mgChem}\right)$$

Where: T=target concentration (μg/g)
C=stock solution concentration (mg/ml)
Chem=chemical of interest
Sol=stock solution

Extraction CO_2 Required

Equation 2 was used to make a rough calculation of the theoretical volume of CO_2 required for the extraction of B(a)P from an empty cell. The calculation can also be used to

estimate the volume of CO_2 required to remove B(a)P from silica, which should not exhibit any binding or matrix effects requiring a significant increase in the volume of CO_2. Close approximations for molecular weights, solubility and extracted mass simplify the calculation. Estimate of B(a)P solubility in CO_2, 10^{-3}, provided by Erkey (1991).

(2)

$$mlCO_2 = \left(\frac{1 mlCO_2}{1 gCO_2}\right)\left(\frac{44 gCO_2}{moleCO_2}\right)\left(\frac{moleCO_2}{10^{-3} moleB(a)P}\right)\left(\frac{moleB(a)P}{250 gB(a)P}\right)\left(\frac{g}{10^6 \mu g}\right)$$

$$= 1.8 * 10^{-4} \text{ ml } CO_2$$

All extractions performed exceeded this very small theoretical volume by five orders of magnitude.

Analytical Calculations

Analytical calculations were based on linear equations for standard curves. Linear regression, performed using Quatro Pro 1.0 spreadsheet software, of the 5 and 6 point standard curves provided the slope and y-intercepts for the equations. Equations 3, 4, 5 and 6 were used to determine the concentration of the injected solution, concentration of the silica mix, theoretical mass in the subsamples extracted and mass extracted and recovered.

(3)

$$conc(solution) = \frac{area - b}{m} = \left(\frac{mgChem}{1Sol}\right) = ppm(solution)$$

Where: area = integrated area under the chromatogram peak
m = slope
b = y-intercept

(4)

$$Conc(mix) = \left(\frac{C.mgChem}{1Sol}\right)\left(\frac{10^3\mu g}{mg}\right)\left(\frac{1}{10^3 ml}\right)\left(\frac{X.mls(added)}{Y.gSilica}\right) = ppm(mi$$

Where: X = mls stock solution added to silica
Y = grams silica in sample mix

(5)

$$\mu gChem(theoretical) = \left(\frac{Z.\mu gChem}{gSilica}\right)(V.gSilica)$$

Where: Z = concentration (ppm) of silica mix
V = grams of sample extracted

(6)

$$\mu gChem(recovered) = \left(\frac{0.2mlSolv}{Sample}\right)\left(\frac{area-b}{m}\right)$$

CHAPTER IV

RESULTS AND DISCUSSION

The objectives of the research focused the ability of the instrument, as configured, to perform qualitative and qualitative work. Qualitative work is important in many fields, such as drug or food testing. In an environmental setting such qualitative work is generally of less value, although still usefull. One such qualititive application might be to detect the presence or absence of a compound which by itself would alter the hazardous waste classification of a mixture. However, most environmental applications address the concentration of a given substance. For example, many potential carcinogenic compounds occur naturally in non-hazardous concentrations throughout our environment. Knowing the concentration of a compound is essential to determining the potential dose and effects on plant and animal life. As a result, there are fewer environmental extraction applications associated with a qualitative instrument. The following discussion focuses on the quantitative aspects of the research.

SAMPLE MIX PREPARATION AND CELL LOADING

Creating a homogeneous silica/contaminant mix and taking uniform sub-samples from the prepared mix was essential to obtaining quantitative extraction results. The spiking

method selected was the best available with existing resources. The biggest problem associated with the silica spiking is the very reason silica was selected, there are no sites for contaminant binding.

The lack of binding sites should result in 100% contaminant recoveries, but it also means the contaminant in solution will be pulled through the sample to the surface by capillary action as the solvent (methanol or acetone) is pulled through the sample and evaporated into the hood. Some of the chemical may not have crystallized in the sample, but instead been pulled into the hood in a gaseous phase with the solvent.

The sample mixes were shaken to remix the silica and create a uniform distribution of the contaminates. However, the sub-sample size used in SFE cell was an average of only 0.8 grams, meaning even small scale distribution variances could have a large impact on extraction efficiencies. This problem is not encountered when conducting the more traditional extraction methods because larger samples, such as 30 grams or more, can be used. This means the degree of uniformity of the silica samples may play a role in the variability of the results between SFE runs. When determining SFE efficiencies, the averaging of three cell extractions should reduce the impact of such variability, but probably does not eliminate it.

To determine the quality of extractions, the SFE results are compared with the "rough estimates" of recoveries from the Soxhlet and Tecator extractions (Huebner, 1992). The initial recovery estimates for NAP have been below detection limits. BAP and PCP recoveries range from 70 to 90%.

CHROMATOGRAPHY DEVELOPMENT AND RESULTS

The development of chromatographic methods was straightforward. The SB-Methyl-100 column was selected because of its ability to separate the three compounds of interest and potential as a column for future applications. The speed of the carrier flow through the column reduced method development time and produced methods faster than those used for the same compounds with the GC used by the "joint" team. For example, B(a)P eluted from the GC column after 30 min, as opposed to less than 13 minutes for the SFC injections. Separation of the three components was easy to achieve and control by adjusting the pressure ramp of the carrier fluid. Standard injection calibration curves produced R^2s ranging from 0.985 to 0.999 and averaged 0.992. This was a very nice chromatography system to work with, easy to program, monitor, and manipulate.

ON-LINE METHOD DEVELOPMENT

Initial research centered around on-line extraction and analysis of silica spiked with 100 ppm B(a)P and standard

B(A)P/methanol solutions to maximize the extraction efficiency of the system. The objective, to maximize the mass extracted, was monitored by chromatography. The area under the curve increased as the mass of B(a)P extracted and analyzed increased. B(a)P was selected at this stage because it is a high molecular weight PAH and its role as a carcinogen. Additionally, it was expected to be the most difficult of the three compounds to extract.

Extraction programming was also straightforward and improvements to extraction efficiency seemed to be made by adjustments to the temperature and pressure. To quantify the extraction efficiencies, a B(a)P/methanol standard solution was injected into to cell in increasing volumes (1 μl to 4 μl) or increasing concentrations, the methanol evaporated and the cell extracted.

Results

It soon became apparent on-line extraction was not repeatable, even though the response generally increased as the mass loading increased. It was difficult to reproduce results from one set of extractions to a second, duplicate set. One very notable exception was a set of near duplicate back-to-back extractions summarized in Table 3 with the averages plotted in Figure 11. This was one of the extraction sets conducted with primarily visual cryocooling control, with the coolant flow adjusted to try and maintain

Table 3. Best Set of Back-to-Back Extraction Series of B(a)P Standards in the On-Line Development Process.

μg B(a)P	Set 1 Area	Set 2 Area	Avg Area	% Dev
0.050	NA	360,000	360,000	NA
0.10	690,000	670,000	680,000	1.5
0.15	NA	830,000	830,000	NA
0.20	630,000	770,000	700,000	10
0.30	1,200,000	NA	1,200,000	
0.40	1,900,000	NA	1,900,000	

a uniform "ice" layer on the cryocooling tee. These results showed a great deal of promise because of the uniform increase in analyte extracted and analyzed as the concentraction of the sample increased. The calculated R^2 of the data was 0.911 and created hopes of a breakthrough in reproducibility. Unfortunately, the results could not be duplicated. Typical on-line extraction results are represented by a set of triplicate standard extractions of 2 μl of 250 ppm B(a)P/methanol solution (0.5 μg absolute mass of B(a)P) with resultant areas of 710,000, 1,100,000, and 940,000. The standard deviation of this set of data is 200,000 (22%).

Possible Causes of Non-repeatability

The non-repeatability of on-line extraction efficiencies is not associated with the silica samples because liquid

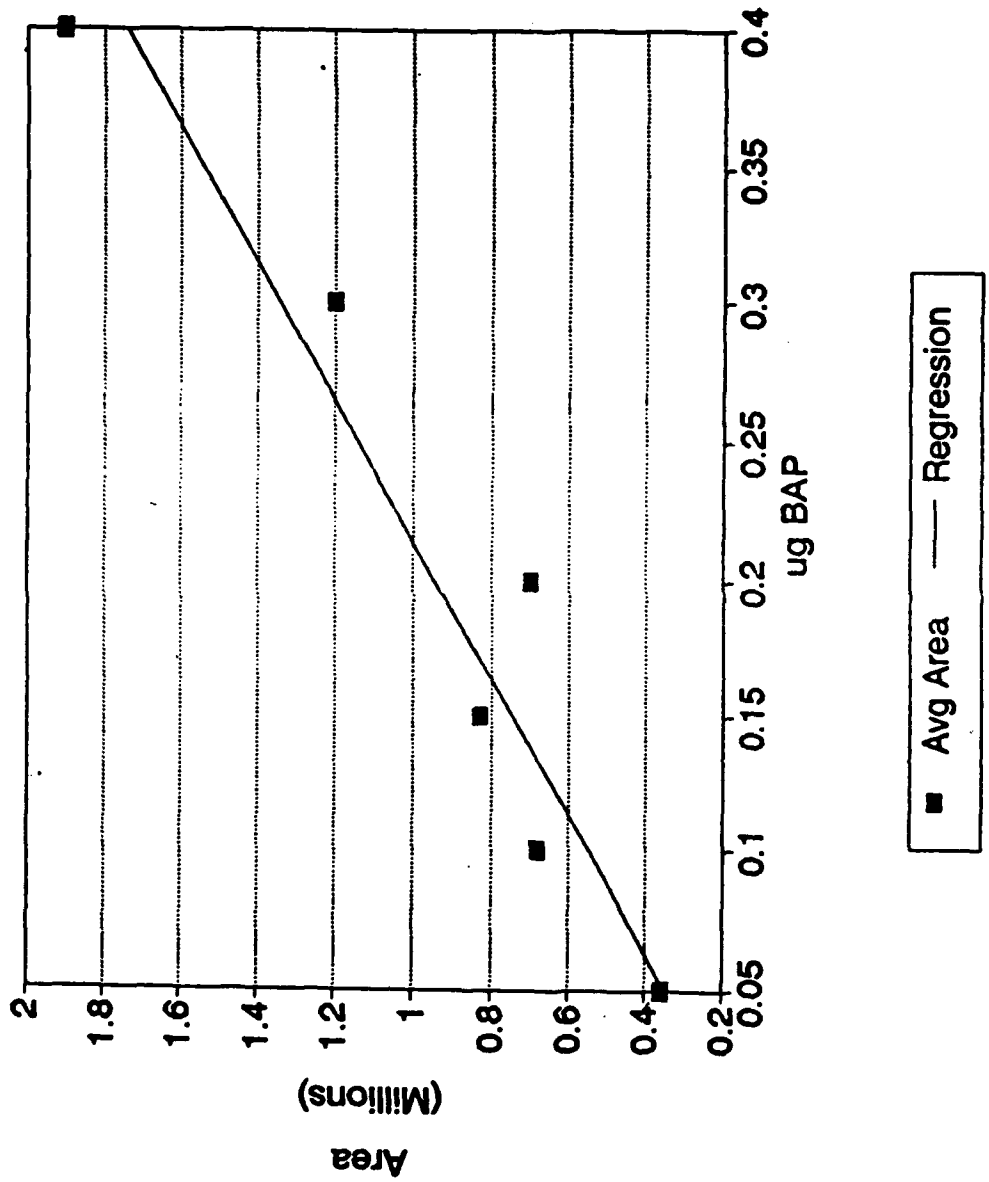

Figure 11. Average Values From the Best Set of Back-to-Back B(a)P Extraction Series in the On-Line Development Process.

standards were being extracted at this stage. Because standards were used, the primary sources of error considered were non-repeatable loading and non-repeatable analyte immobilization in the cryofocusing tee. Analytes not immobilized in the tee would have passed into the discharge line and either crystallized or been passed into the atmosphere.

Based upon previous lab work and current lab techniques, the the cryofocusing unit was considered the primary source of error and attention was centered on this step of the process. The biggest problem with the tee, as designed, is the inability to control the cooling temperature of the tee. The coolant (Bone dry CO_2) flow is regulated visually to maintain a white "ice" layer on the carrier fluid flow line (See Figure 8). The temperature of this layer will vary from run-to-run, even if all flow valves are readjusted to the same setting each time.

The extraction system users manual (Lee Scientific, 1988) notes that "It has been shown that the temperature in the cryofocusing tee has a profound effect on the trapping efficiency." The manual also states selective analyte trapping can be achieved by adjusting the cooling level of the tee. The results seem to substantiate this because the neither the tee temperature nor the B(a)P mass captured were constant. The "best" data set was obtained when the efforts to maintain a near constant temperature by visual observation

were probably successful. The same methods were used before and after this data set unsuccessfully.

The best way to improve the tee cooling would be develop a computer controlled flow meter based on the temperature of the tee. Another, possibly easier improvement would be to replace the cryocooling tee with a temperature based, computer controlled cryofocusing devise already marketed by a manufacturer. If this is not possible, one way to improve visual temperature control would be to develop a three dimensional marking system on the tee itself. This would allow the user to try and reproduce the dimensions of the ice layer on the tee. While this would be an improvement, it will not allow the user to control the actual temperature in the tee, nor will it allow the user to control the density of the ice layer or the shape it forms on the tee. Though an even distribution of the coolant is designed, the oven temperature, humidity, and flow all seem to impact the shape and development of the ice layer, which is not always uniform.

The ice layer develops most uniformly at high flow rates, which then cause the line to freeze and stops all flow through the tee. Increasing the flow to develop a uniform layer and then reducing it to prevent freezing alters the layer. The outermost portions tend to liquify or go into a gas phase. The liquified portion flows toward the center of the tee and refreezes. This alters the uniform texture and

shape of the layer and possibly creates temperature gradients within the tee. Monitoring and adjusting the coolant flow during extraction is crucial to maintaining continuous carrier fluid flow and analyte capture.

OFF-LINE METHOD DEVELOPMENT AND RESULTS

Because of the potential shown by the on-line extraction set-up, quantitative off-line extraction with no instrument modification was attempted. Quantitative off-line extraction and contaminant trapping with no instrument modification of B(a)P, PCP, and NAP was unsuccessful. The instrument is designed for off-line extraction and trapping as described in the Methods Off-Line Extraction section. The results of the off-line extraction/trapping, with the oven at 32° C, and subsequent analysis are summarized in Table 4. The percent

Table 4. Off-line Extraction Recoveries.

Compound	Silica Conc (ppm)	Mass Ext (μg)	Mass Rec (μg)	% Recover
BAP	14 20 85	34 48 210	31 33 18	90 68 8.7
PCP	7.9 26 89	20 62 210	5.9 7.5 85	30 12 40
NAP	3.1 16 55 55*	7.6 40 140 140*	0 0 0 2.4*	0 0 0 1.8*

* Extractions performed into 30 ml vial

recovered is an average because three subsamples were extracted into a single trapping solution to obtain a theoretical absolute mass within detection limits.

B(a)P extraction efficiency decreases as the B(a)P loading increases. The recovery from the low concentration sample (14ppm) was 90%. The sample recoveries from the 20 and 85 ppm spikes were 68% and 8.7% respectively. This individual data set can suggest two things, a time factor is involved and/or crystallization within the restrictor increases as the concentration of B(a)P increases. The extraction of the 14 and 20 ppm samples result in approximately the same absolute mass recovered, 31 and 33 μg. At first glance, this could indicate extraction time for the 20 ppm sample was inadequate and the B(a)P was being extracted at a constant rate. The 85 ppm sample extraction does not support this because the absolute mass recovered actually decreased to 18 μg with the higher loading. If it were soley a time factor, the absolute mass would have been close to 32 μg. It is possible a time factor could be at work with low concentration samples, but there is not enough data to strongly support this supposition. The PCP sample extractions differed from the B(a)P extractions because the absolute mass recovered increased from 5.9 to 7.5 to 85 μg from the 7.9, 26 and 89 ppm samples respectively. The efficiencies of the extractions were 30, 12 and 40%. The middle concentration sample does not seem to fit well and may

illustrate a non-reproducable aspect of the trapping and recovery set up. The 30 and 40% recoveries from the low and high concentraction spikes are reasonably close in value, but fall way below desired efficiencies of 80 to 90% as obtainable from Soxhlet and sonication extractions.

NAP was not recovered by the 5 ml vial series. The volatile nature of the compound made this the most difficult compound to trap into aqueous phase, but also meant no apparant crystalization problems in the restrictors. Because of this, the NAP extractant solution was bubbled into a vial containing 30 mls of $MeCl_2$. No clogging or flow restriction problems were encountered and a small amount of NAP was recovered. Another source of possibly significant NAP loss was the solvent volatilization step, where the solution was concentrated onto the silica. This could be significant because neither the Soxhlet or sonication NAP extractantions recovered NAP.

During cleanup of the primary restrictor (five minute extraction of 30 mls $MeCl_2$ at room temperature through the cell,repeated three times) the bubble size and flow rate would both visibly increase during the third extraction. The probable cause of this increased flow is the removal of contaminant crystals and increased flow. The cleanup extractant was trapped in the vial series used during the extraction set.

Problems associated with off-line trapping center on keeping the restrictor heated to maintain the supercritical phase as long as possible and reduce the area over which crystallization is possible as well as keeping the trapping solution cool enough to minimize bubble size and velocity through the solvent. These two objectives require two different environments within the same trapping vessel or apparatus as discussed in Chapter II.

To try and improve the trapping efficiency without modifying the instrument set-up, several alternate methods were studied. Heating the oven to 36 and 40° C improved and maintained extraction flow, but resulted in excessive solvent losses and poor trapping as large bubbles quickly flowed through the solvent. Chilling the vial to any extent to slow the flow and reduce the size of bubbles through the solvent to improve mass transfer efficiency resulted in total loss of flow as the contaminants crystallized in the restrictor and block the flow. Running the restrictor out an oven port and into the vial also failed. The oven was heated to 250° C to maintain the restrictor temperature in the oven and the vial was kept at room temperature, 28° C. Flow could not be maintained with this set-up.

The optimum set-up continued to be with the oven at 32° C and a restrictor of minimum length running into the vials. The bubbling action did create some level of cooling for the solvent and flow could be maintained without excessive

solvent loss. After 30 minutes of extraction the first and second 5 ml vials generally lost close to 1 ml each, which was replaced and the extraction continued.

A larger sample vial, 30 mls, was used to possibly improve trapping because the bubble pathway was more than double that of the 5 ml vials. The B(a)P and PCP samples plugged the restrictor after a few seconds of flow. The greater solvent depth cooled the restrictor too much, probably causing crystallization which resulted in total loss of flow.

Off-Line Restrictor Modification

The problems associated with quantitative extraction are in trapping the extracted contaminants. One possible method briefly studied was to replace the restrictor set-up designed and bring a stainless steel restrictor from the ten-port valve to the trapping vial. The heated restrictor line discharged into the empty "iced" 30 ml vial. Theoretically, the discharged contaminant crystallizes and sticks to the vial walls. This was also unsuccessful, initially recoveries of B(a)P were BDL. Subsequent recoveries, following an extensive five hour cleanup (250 atm, 50° C cell, 250° C restrictor), totalled approximately 52 μg of the initial 800 μg pure B(a)P load (6.5%). The problems associated with this method centered on three aspects of the restrictor; its unclean inner surface, the temperature drop at the point of

discharge and the possibility the flow is simply too fast for the vial to retain the analyte from the aerosol type discharge.

The restrictor was new and had been cleaned with solvents at the factory. When conducting initial cleaning and flow tests, some type of contaminant was crystallized on the glass vial. This material was never fully removed and could have bound some of the contaminant to the restrictor walls. The cleaning methods used relied on liquid extraction of $MeCl_2$ through the system at room temperature and long empty cell extractions at high temperature (restrictor at 220 to 250° C) and pressure (125 and 250 atm). A possible cleaning method not tried is to connect the restrictor to a High Pressure Liquid Chromatography (HPLC) unit and force a larger volume of $MeCl_2$ through the restrictor using the HPLC pump.

One of the goals of this brief feasibility study was to maintain the temperature of the restrictor above the melting point of B(a)P (177° C) all the way to the end of the restrictor using heat and insulation tape. Exposing the tip of the restrictor to the vial atmosphere resulted in a temperature drop to 150° C at the tip. This temperature drop allows the B(a)P to crystallize in the "pinched" tip of the restrictor. The solution to this problem is to secure a heat source close enough to the tip that the temperature will remain above 177° C at the tip.

The solution to the discharge flow into an empty container for trapping is more difficult to solve, as illustrated by the work of McNair and Frazier. One possible scheme using an empty initial collector is to seal it and trap non crystallized analytes in a second, solvent containing collector. Obstacles to this would be to develop a seal and transfer line capable of withstanding the high temperature of the discharge restrictor.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The Lee Scientific SFE/SFC Series 600 instrument was unable to produce repeatable quantitative on-line or off-line extractions as configured. Preliminary Soxhlet and sonication extraction recoveries ranged from 70 to 90 percent (Heubner, 1992), significantly better than the 9 to 90 percent recoveries from the same samples using off-line SFE. However, the instrument is valuable for qualitative extraction and a user friendly quantitative SFC analytical tool. SFC will prove to be superior to GC and HPLC in both method development and analysis speed for selected applications. The priciple problems with the SFC in the future will be analyte and detector compatibility and sensitivity. There are only a few detectors which can currently be used by the series 600 system, none of which are the ideal detector, electron capture, for PCBs.

The potential for quantitative extraction demonstrated by selected extraction results justify additional research into modifying the extraction trapping mechanisms, especially for off-line work. Previous research by McNair and Frazier (1991) provide a good starting point to improving the efficiency of the instrument. The disappointing results for quantitative extraction with the instrument are not a reflection on the fundamental technology, but on the

quantitative extraction application of the instrumentation. This limitation was recognized by Lee Scientific, so they developed the 703 off-line extractor. The technology continues to have broad appeal as we learn more about the properties and application of SCFs. Subsequent research with the instrument should focus on improving the extraction efficiency of on- and off-line extraction and center of the cryofocusing tee and pressure restrictor/trapping setup. To provide continuity with this research, the following recommendations are made:

1. Extract pure chemicals in the extraction cell prior to extraction from silica matrices. This will ensure all of the measured compound is loaded for extraction and eliminate nonhomogeneous sample concerns. Such loading will also enable the researcher to quantify each individual extraction, rather than conducting multiple extractions into a single collector.

2. Carry out method development with each compound separately. Each behave differently and unique problems associated with them, as illustrated by the vastly different problems posed by B(a)P and NAP.

3. Develop or purchase a computer controlled cryofocusing tee apparatus capable of maintaining constant temperatures

during extraction. A constant temperature will create reproducable conditions and should lead to reproducible results.

4. Review literature immediately prior to beginning off-line experiments and select the most promising pressure restrictor/analyte trapping setup suitable to the Series 600 system for adaption and development. A good review is essential because the extraction setups discussed in the literature review were all published in 1991 and more will follow. The McNair and Frazier resistively heated setup in Figure 6 seems to be ideal for adaption and development at this time.

NOTATION

area = integrated area under the chromatogram peak

b = y-intercept

BAP = benzo(a)pyrene

C = stock solution concentration (mg/ml)

Chem = chemical of interest

CO_2 = carbon dioxide

m = slope

Sol = stock solution

T = target concentration (μg/g)

V = grams of sample extracted

X = mls stock solution added to silica

Y = grams silica in sample mix

Z = concentration (ppm) of silica mix

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APPENDIX A

CHROMATOGRAPHY PROGRAMS

Table A-1. B(a)P SFC Program.

	Mode	Ramp Rate (atm/min)	Final Pressure (atm)	Hold Time (min)	Run Time (min)
Pump	Initial	100	85	0.00	0.00
	Injection			0.05	0.00
	Hold			4.00	4.00
	Ramp	25	300	0.00	12.60
	Ramp	50	400	4.00	18.60
	Quit	-100	85	0.00	21.75
		(°C/min)	(°C)	(min)	(min)
Temp	Initial		75	0.00	0.00
	Hold			15.00	15.00
	Ramp	50	125	4.00	20.00
	Ramp	-50	75	0.75	21.75
	Quit		75	0.00	21.75

Table A-2. Naphthalene SFC Program.

	Mode	Final Rate (atm/min)	Hold Temp (atm)	Total Time (min)	Run Time (min)
Pump	Initial	-100	85	0.00	0.00
	Injection			0.50	0.00
	Hold			4.00	4.00
	Ramp	10	130	2.50	11.00
	Ramp	25	225	0.00	14.80
	Ramp	100	400	1.00	17.55
	Quit	-100	85	0.00	20.70
		(°C/min)	(°C)	(min)	(min)
Temp	Initial		75	0.00	0.00
	Hold			15.00	15.00
	Ramp	50	125	3.00	19.00
	Ramp	-50	75	0.70	20.70
	Quit		75	0.00	20.70

Table A-3. PCP SFC Program.

	Mode	Ramp Rate (atm/min)	Final Pressure (atm)	Hold Time (min)	Total Time (min)
Pump	Initial	-100	85	0.00	0.00
	Injection			0.50	0.00
	Hold			9.00	9.00
	Ramp	10	130	2.50	16.00
	Ramp	25	300	0.00	22.80
	Ramp	50	400	1.00	25.80
	Quit	-100	85	0.00	28.95
		(°C/min)	(°C)	(min)	(min)
Temp	Initial		75	0.00	0.00
	Hold			16.80	16.80
	Ramp	50	125	7.00	24.80
	Ramp	-50	75	3.15	28.75
	Quit			0.00	28.95

Table A-4. Mix SFC Program.

	Mode	Ramp Rate (atm/min)	Final Pressure (atm)	Hold Time (min)	Total Time (min)
Pump	Initial	-100	85	0.00	0.00
	Injection			0.50	0.00
	Hold			7.00	7.00
	Ramp	10	130	2.50	14.00
	Ramp	25	300	2.00	22.00
	Ramp	50	400	4.00	28.40
	Quit	-100	85	0.00	31.55
		(°C/min)	(°C)	(min)	(min)
Temp	Initial		75	0.00	0.00
	Hold			22.00	22.00
	Ramp	50	125	7.00	30.00
	Ramp	-50	75	0.55	31.55
	Quit			0.00	31.55

APPENDIX B

DATA ANALYSIS:

STANDARDS, CHEMICAL ADDITIONS AND EXTRACTIONS

Appendix B contains a series of tables representing the chromatography series associated with each set of off-line extractions used. The tables are divided into three sections. The top section contains the standard curves (pre and post analysis), their average and the resultant regression cf the average used to develop equations. The middle of the table contains the equation used to calculate the concentration (ppm) of the injected solution and the equation used to convert the solution concentration to mass recovered (μg).

The bottom of each table is also divided, with the upper portion containing the "A" samples and bottom the "E". The "A" samples are actual samples of the spiking solutions placed on the silica and the chemical recovery column represents the concentration in ppm. The "E" samples are from the extracted silica samples referenced in the body of the thesis. The far right column, silica sample ppm, contains the actual concentration of the silica sample based upon the "A" sample, spiking solution, results, as are the chemical "Mass" figures.

Following each table is the graphical representation of the standards and the regression equation.

Table B-1. Off-Line B(a)P Extraction into Vials.

Standard Curves (Pre and Post)

B(a)P (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
62.5	5063	3922	4492.5
125	26155	30687	28421
250	75545	93732	84638.5
500	245353	287017	266185
1000	501600	444310	472955

Regression Output:

Constant		-19229.6
Std Err of Y Est		24519.75
R Squared		0.986655
No. of Observations		6
Degrees of Freedom		4
	108950	
X Coefficient(s)	501.7134	
Std Err of Coef.	29.17405	

Equation: ppm B(a)P = (area + 19300)/502
Equation: ug B(a)P recovered = 0.2*(ppm B(a)P recovered)

Sample ID	Sample Mass (mg)	B(a)P Mass (ug)	Area (count)	B(a)P Recov	% Recov	Silica Sample PPM
				(ppm)		
BAP10A	NA	NA	15172	68.8		
BAP25A	NA	NA	30056	98.5		
BAP100	NA	NA	193706	425.2		
				(ug)		
BAP10S	2.49	34.3	57000	30.9	90.3	13.8
BAP25S	2.43	47.9	61229	32.5	67.9	19.7
BAP100S	2.47	210.0	22393	18.2	8.7	85.0
Known	NA	NA	34560	22.7		114
K+BAP1	NA	NA	46424	27.1		135

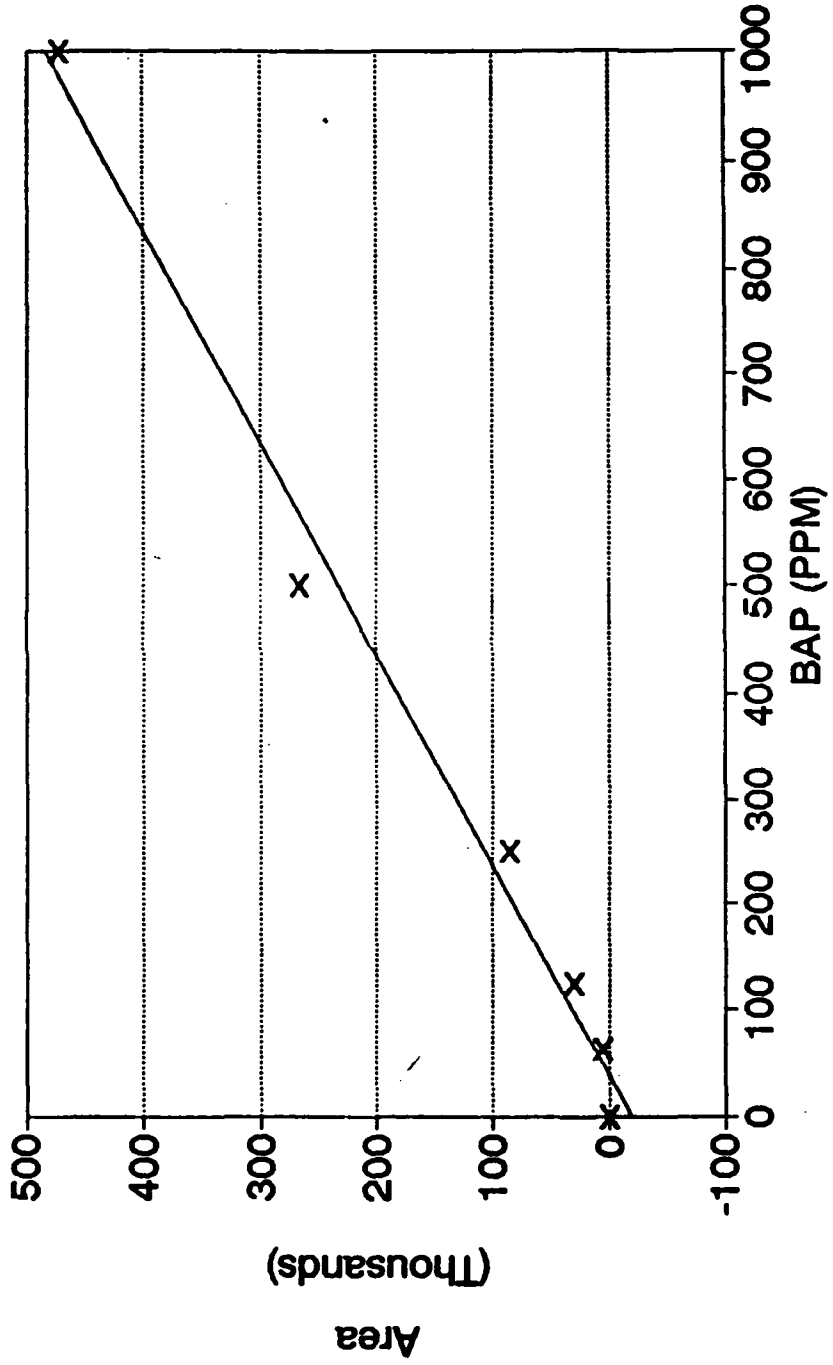

Figure B-1. Off-Line B(a)P Extraction Standards and Regression.

Table B-2. Off-Line B(a)P Extraction from Mix into Vials.

Standard Curves (Pre and Post)

B(a)P (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
62.5	10966	17670	14318
125	41436	44784	43110
250	119548	155029	137289
500	302181	271264	286723

Regression Output:

Constant		-16148.8
Std Err of Y Est		13881.12
R Squared		0.989817
No. of Observations		5
Degrees of Freedom		3
108950		
X Coefficient(s)	599.662	
Std Err of Coef.	35.11676	

Equation: ppm B(a)P = (area + 16100)/600
Equation: ug B(a)P recovered = 0.2*(ppm B(a)P recovered)

Sample ID	Sample Mass (g)	Nap_ Mass (ug)	Area (count)	B(a)P Recov (ppm)	% Recov	Silica Sample PPM
MIX10A	NA	NA	0	5.4		
MIX25A	NA	NA	13043	9.7		
MIX100	NA	NA	122877	46.3		
				(ug)		
MIX10E	2.38	2.6	18882	11.7	456.5	1.1
MIX25E	2.49	4.8	56816	24.3	502.4	1.9
MIX100E	2.42	22.4	162480	59.5	265.5	9.3

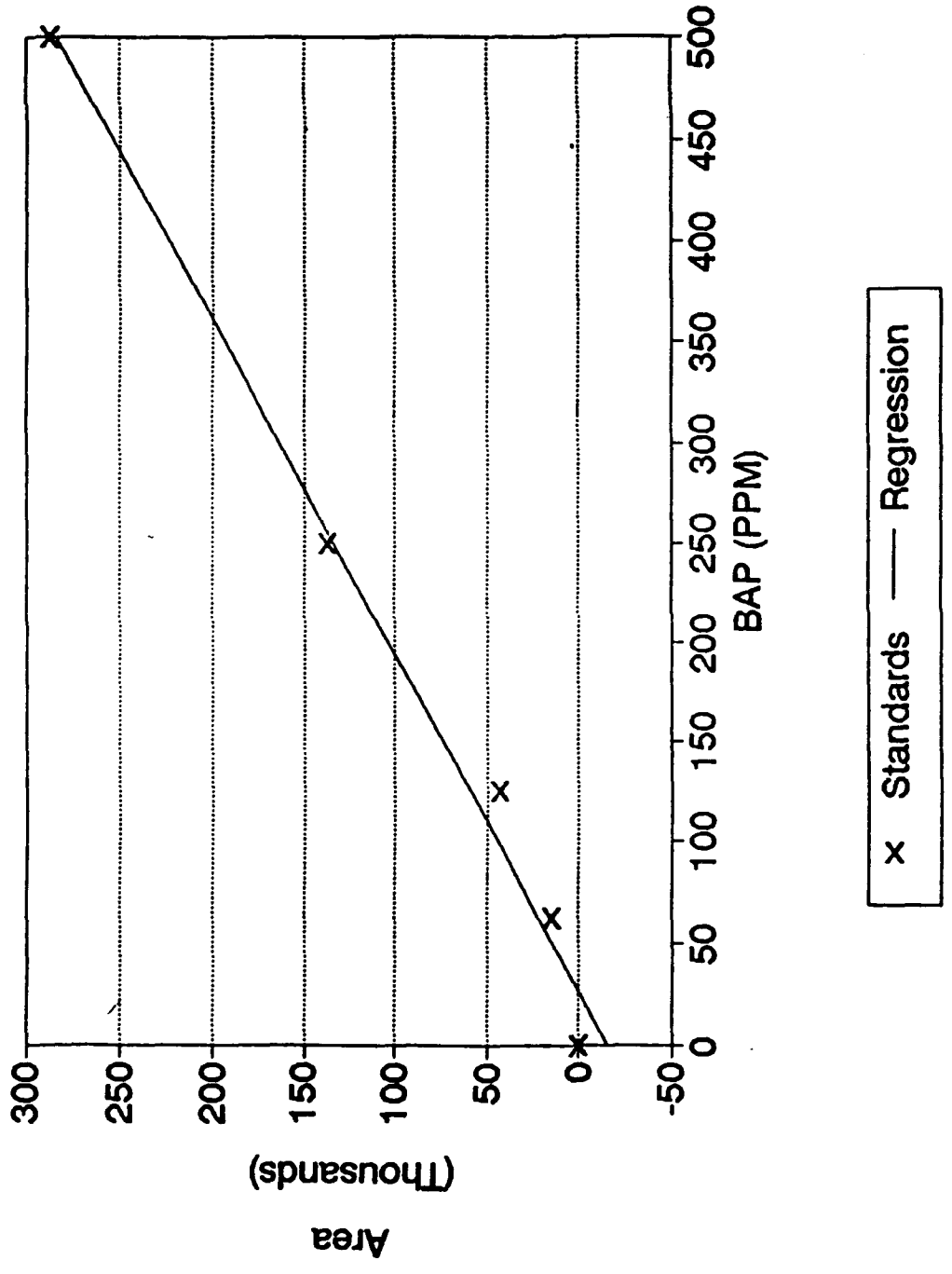

Figure B-2. Off-Line B(a)P Extraction from Mix Standards and Regression.

Table B-3. Off-Line Naphthalene Extraction into Vials.

Standard Curves (Pre and Post)

Nap (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
21		11267	11267
43	17952	22805	20379
133	67237	84060	75649
275	128259	125749	127004
550	232248	308904	270576
1000	448515	462970	455743

Regression Output:

Constant		4809.937
Std Err of Y Est		8996.747
R Squared		0.99764
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	458.4307	
Std Err of Coef.	9.97158	

Equation: ppm Nap = (area - 10637)/461

Sample ID	Sample Mass (g)	Nap_ Mass (ug)	Area (count)	Nap Recov	% Recov	Silica Sample PPM
				(ppm)		
NAPA10	NA		17675	15.3		
NAPA25	NA		48354	81.8		
NAPA10	NA		138028	276.3		
				(ug)		
NAPE10	2.48	7.6	0	0.0	0.0	3.1
NAPE25	2.45	40.1	0	0.0	0.0	16.4
NAPE10	2.51	138.7	0	0.0	0.0	55.3
NAPKA	NA	NA	210068	478.8 PPM		NA
*500 PPM STD + 100 PPM Extract (100 ul each)						NA

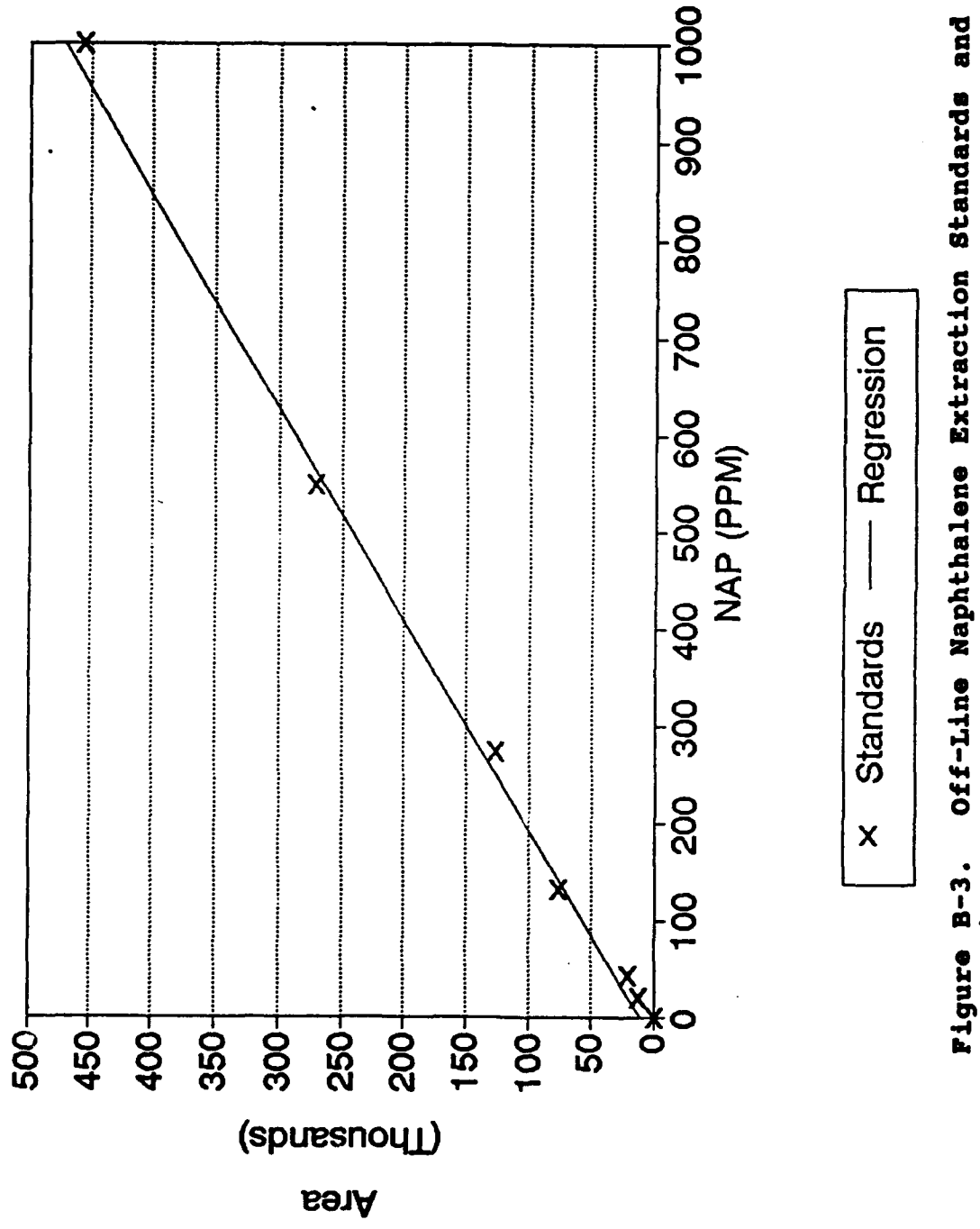

Figure B-3. Off-Line Naphthalene Extraction Standards and Regression.

Table B-4. Off-Line Naphthalene Extraction from Mix into Vials.

Standard Curves (Pre and Post)

Nap (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
32	17485	17552	17519
63	32975	34858	33917
125	73112	87725	80419
250	148968	148968	148968
333	206836	208543	207690

Regression Output:

Constant		-1742.38
Std Err of Y Est		3985.224
R Squared		0.998102
No. of Observations		6
Degrees of Freedom		4
X Coefficient(s)	621.3764	
Std Err of Coef.	13.54713	

Equation: ppm Nap = (area + 1740)/621

Sample ID	Sample Mass (g)	Nap_ Mass (ug)	Area (count)	Nap Recov (ppm)	% Recov	Silica Sample PPM
MIX10A	NA	NA	21372	7.4		
MIX25A	NA	NA	50039	16.7		
MIX100	NA	NA	183215	59.6		
				(ug)		
MIX10E	2.38	3.5	NA	NA	NA	1.5
MIX25E	2.49	8.3	NA	NA	NA	3.3
MIX100E	2.42	28.8	NA	NA	NA	11.9
NAP100	2.47	136	5570	2.4	1.7	55.3

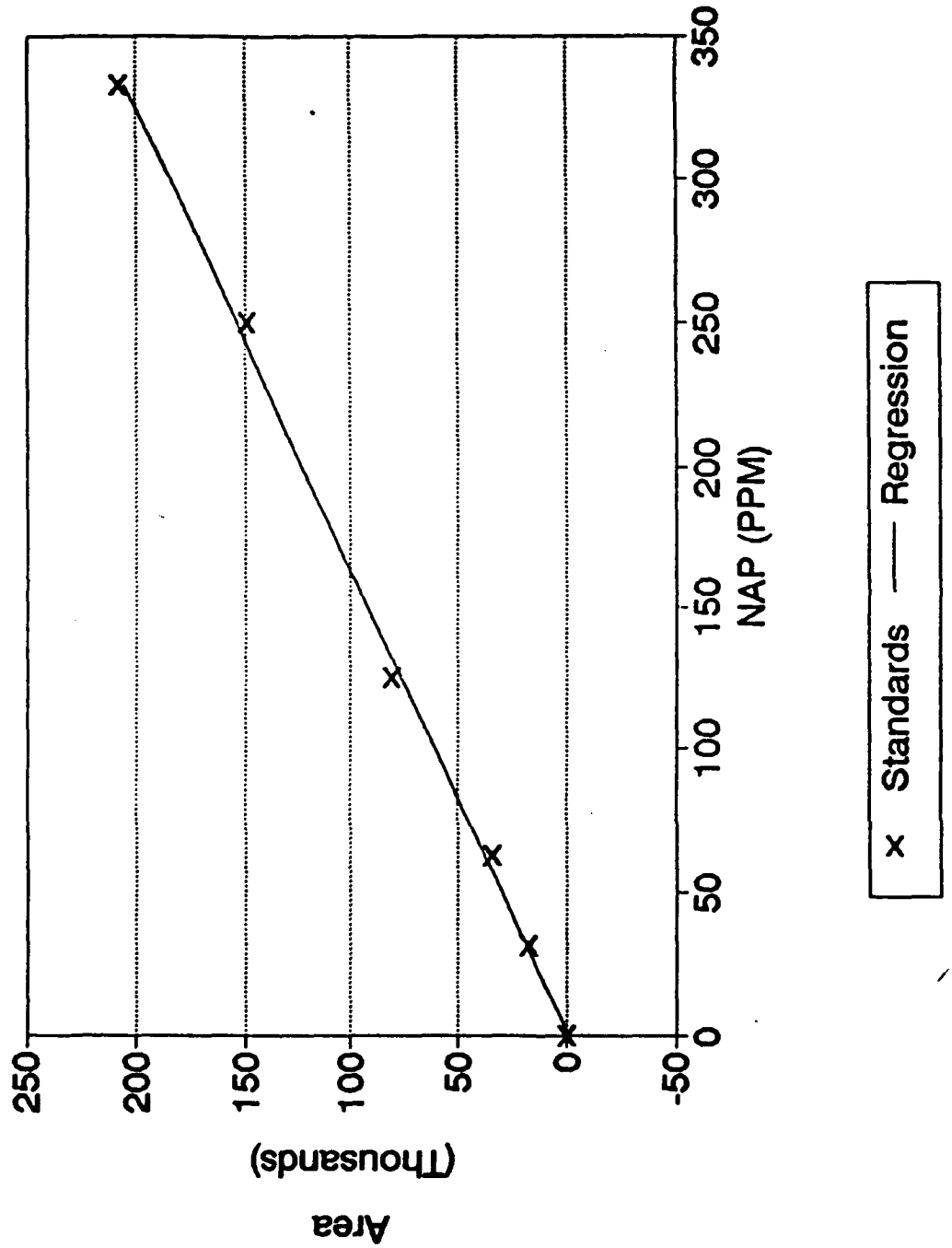

Figure B-4. Off-Line Naphthalene Extraction from Mix Standards and Regression.

Table B-5. Off-Line PCP Extraction into Vials.

Standard Curves (Pre and Post)

PCP (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
50	10918	10284	10601
100	19908	22002	20955
250	NA	44114	44114
500	75468	70290	72879
1000	117237	NA	117237

Regression Output:

Constant		3919.215
Std Err of Y Est		4051.642
R Squared		0.985496
No. of Observations		5
Degrees of Freedom		3
	108950	
X Coefficient(s)	143.2808	
Std Err of Coef.	10.03546	

Equation: ppm PCP = (area - 3920)/143

Equation: ug PCP recovered = (0.2)*(ppm PCP recovered)

Sample ID	Sample Mass (g)	Nap_ Mass (ug)	Area (count)	PCP Recov (ppm)	% Recov	Silica Sample PPM
PCP10A	NA	NA	9566	39.5		
PCP25A	NA	NA	22420	129		
PCP100A	NA	NA	67636	446		
				(ug)		
PCP10E	2.49	19.7	8171	5.9	30.2	0.0
PCP25E	2.38	61.6	9415	7.7	12.5	25.9
PCP100E	2.40	213.9	126014	85.4	39.9	89.1

* Used 0.1 mls to reconstitute

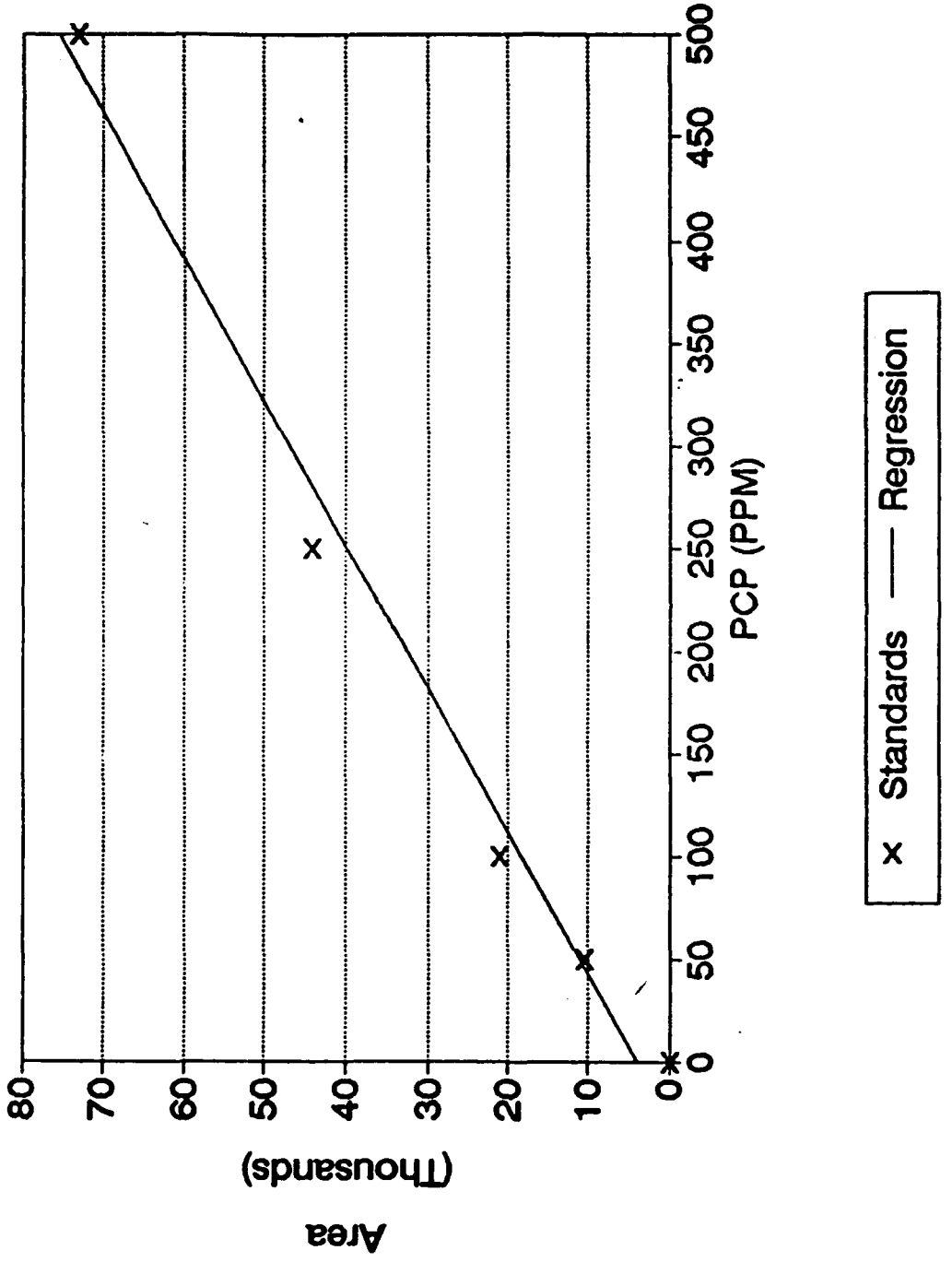

Figure B-5. Off-Line PCP Extraction Standards and Regression.

Table B-6. Off-Line PCP Extraction from Mix into Vials.

Standard Curves (Pre and Post)

PCP (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0	0	0
32	4058	4538	4298
63	10341	13375	11858
125	28406	35675	32041
250	67602	72812	70207
666	182229	198148	190189

Regression Output:

Constant		-3633.73
Std Err of Y Est		2452.387
R Squared		0.999089
No. of Observations		6
Degrees of Freedom		4
40825		
X Coefficient(s)	290.9682	
Std Err of Coef.	4.392464	

Equation: ppm PCP = (area + 3630)/291
Equation: ug PCP recovered = ppm PCP

Sample ID	Sample Mass (g)	Nap_ Mass (ug)	Area (count)	Nap Recov (ppm)	% Recov	Silica Sample PPM
MIX10A	NA	NA	19262	16		
MIX25A	NA	NA	6331	6.8		
MIX100	NA	NA	41329	30.9		
				(ug)		
MIX10E	2.38	7.5	3677	5.0	67.1	3.1
MIX25E	2.49	3.4	2991	4.6	133.5	1.4
MIX100E	2.42	15.0	41927	31.3	209.4	6.2

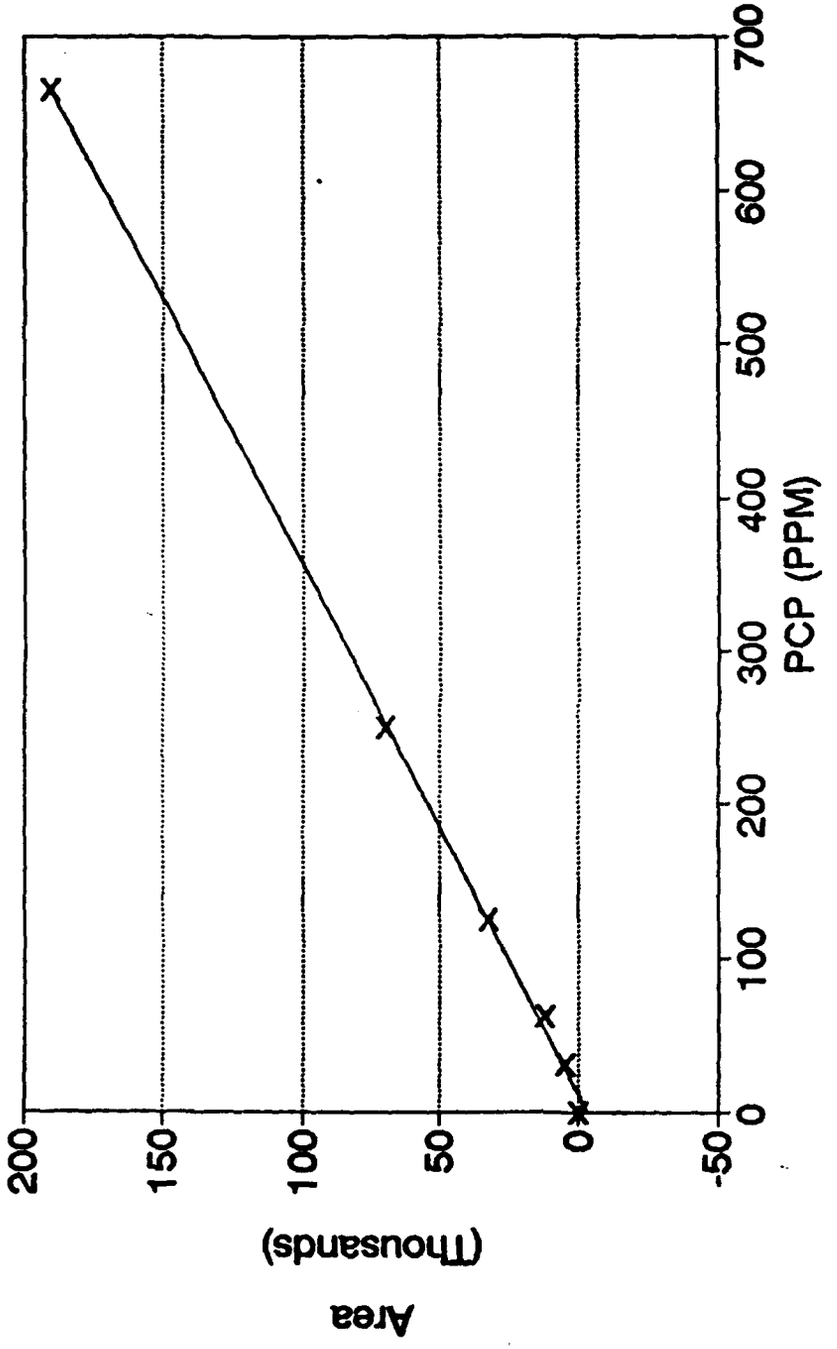

Figure B-6. Off-Line PCP Extraction from Mix Standards and Regression.

Table B-7. Off-Line PCP Extraction into Chilled Vials.

Standard Curves (Pre and Post)

PCP (ppm)	Area 1 (count)	Area 2 (count)	Avg Area
0	0		0
50	1988		1988
100	10024		10024
250	36129		36129
500	87662		87662

Regression Output:

Constant		-5438.74
Std Err of Y Est		4453.057
R Squared		0.988995
No. of Observations		5
Degrees of Freedom		3
108950		
X Coefficient(s)	181.1074	
Std Err of Coef.	11.02972	

Equation: ppm PCP = (area + 5440)/181
Equation: ug PCP recovered = 0.2*(ppm B(a)P recovered)

Sample ID	Sample Mass (mg)	PCP Mass (ug)	Area (count)	PCP Recov (ug)	% %Recov	Silica Sample PPM
PCPCV1	2.44	217	42964	53.5	24.6	89.1
PCPCV1	2.44	217	49356	60.5	27.9	89.1
PCPCVC	NA	NA	2204	8.4		

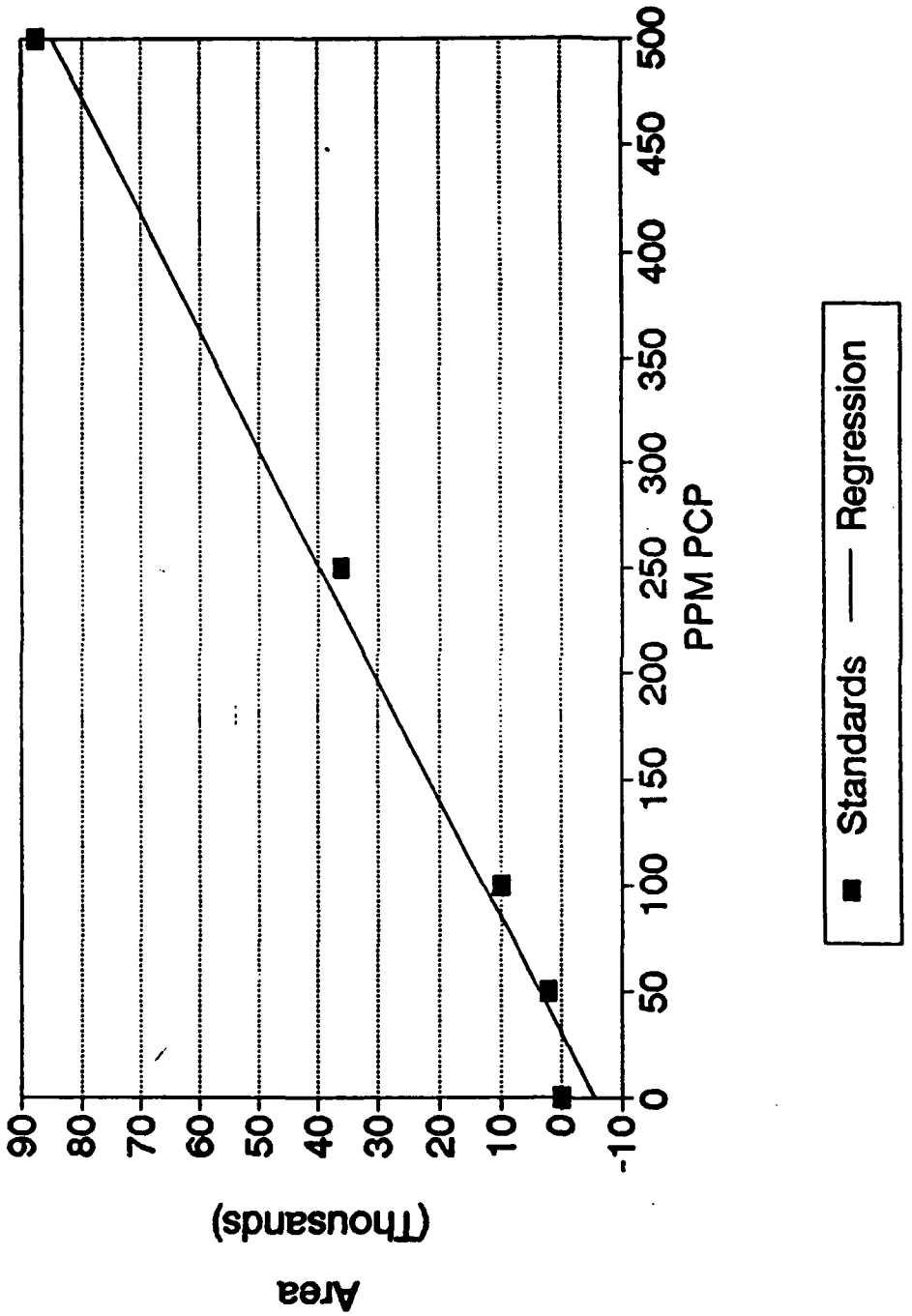

Figure B-7. Off-Line PCP Extraction into Chilled Vials Standards and Regression.

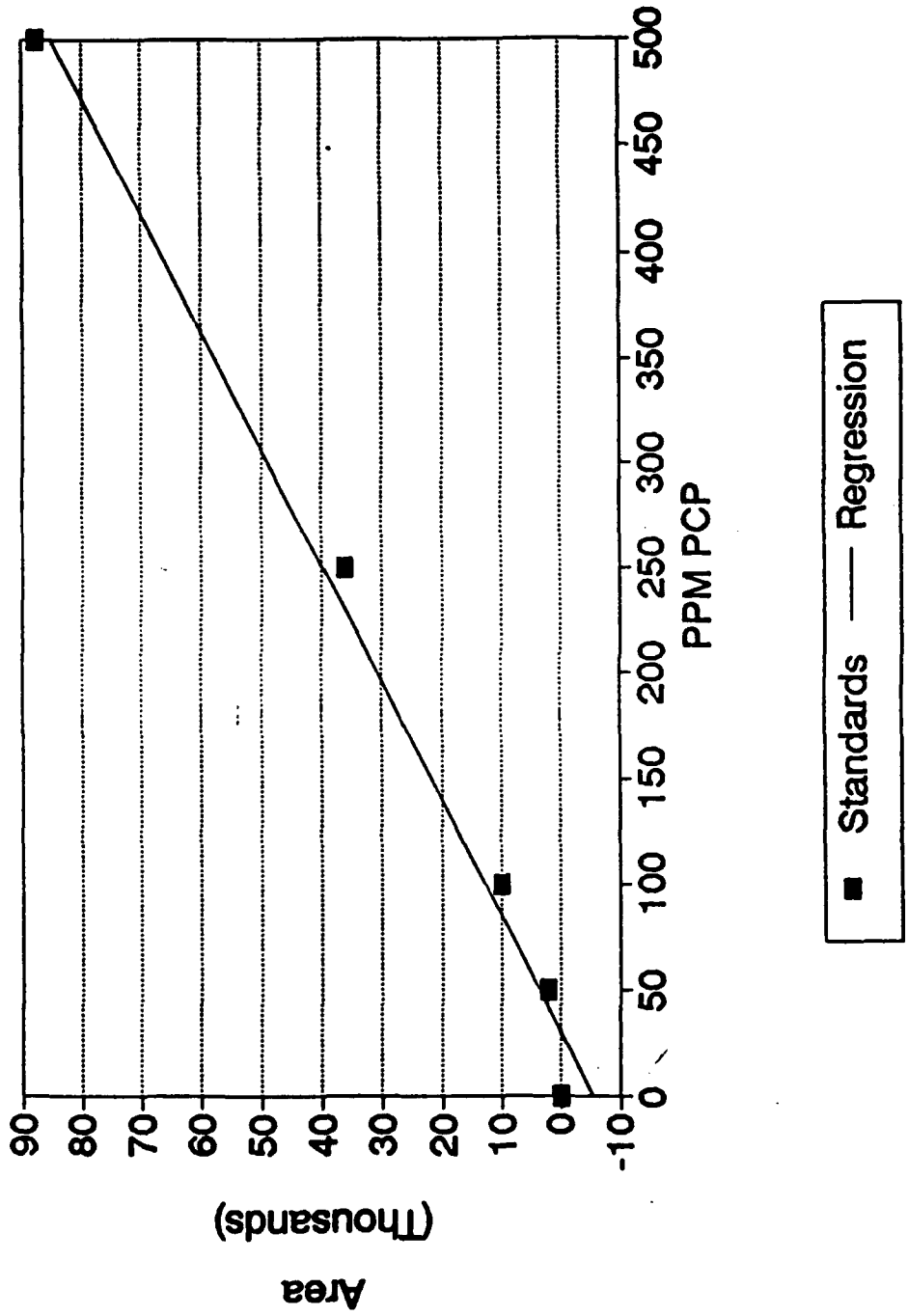

Figure B-7. Off-Line PCP Extraction into Chilled Vials Standards and Regression.

APPENDIX C

SAND/SEDIMENT SPIKING PROCEDURES

1. When working with the pure chemicals a lab coat, one pair of latex gloves and one pair of vinyl gloves over top of the latex will be the minimum protection used.

2. Prepare a work area in the restricted access room vent-hood. (Room entry procedures are clearly defined and access limited to authorized personnel.)

   a. Unlock and remove the appropriate chemical storage desiccator from the toxic chemical refrigerator. Place the desiccator to the side in the hood. Allow the chemicals to come to room temperature in the desiccator as the work area is prepared.

   b. Line the bottom of the hood work area with absorbent paper.

   c. Line a lipped tray with absorbent paper.

   d. Place the tray and auxiliary equipment, such as the heater/stir plate, on the absorbent paper in the vent-hood.

   e. Gather all required glassware, utensils, pipettes, overpacks, and solvent.

   f. Label all items appropriately. (ALL B(a)P items must have "Cancer Suspect" stickers.)

   g. Prepare a disposal bag and tape it to the vent-hood for use during work.

3. Ensure the "In use" and "cancer agent" signs are in place on the outer and inner doorways.

4. Weigh Chemical - (For either a concentrated stock solution or a one time application solution)

   a. Tare a foil-capped beaker containing a stir bar using a mass balance in the main laboratory area.

   b. Under the restricted access vent-hood, place slightly more than the required mass of chemical required in the beaker.)Getting slightly more than required prevents the need to repeat the

procedure.) Immediately close the chemical bottle and recover the beaker with the original foil.

c. Change the outer vinyl gloves. (Change them after each exposure to the pure chemical or when coming in contact with solvent).

d. Place the beaker in an over pack (a plastic jar with a screw top lid) for "transport" to the mass balance. The overpack will be used in each subsequent step requiring the movement of the beaker or the final sand/sediment mixtures.

e. Weigh the beaker, stir-bar, chemical, foil set-up on the same mass balance used previously.

f. Calculate the mass of the chemical.

5. Determine the volume of solution required to prepare the desired concentration in the final application. (NOTE: This is not the final concentration of the soil mixture. It provides the mass of contiminant needed for the soil mixture. The predetermined volume of solvent used creates a moisture content in the sand/sediment mixture just below field capacity. Because more chemical was used than required by this volume, more solvent will be required for the preparation than will be applied to the sand/sediment mixture.)

6. Under the restricted access vent-hood, pipette the calculated solvent volume into the beaker. Stir the mixture as required to create a homogeneous mixture with all the chemical in solution. (Perhaps overnight)

7. While the mixture is stirring, prepare a second work area in the fume-hood next to the gas chromatography unit in the main laboratory. (Absorbent paper, materials, labels, etc)

8. Place the homogeneous solvent/chemical mixture in the overpack and move it to the second vent-hood.

9. Pipette the required volume of solvent mixture into the premeasured sand/sediment. Mix thoroughly as the solvent is added. Cap the jar and mix vigorously. Place the remaining solvent mixture in a vial for chromatographic analysis.

10. Place the mixture jar on rollers and roll the jar for a minimum of 36 hours.

11. Evaporate the solvent under the restricted access fume hood overnight.

APPENDIX D

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March 27, 1991

Permissions Editor
Butterworth Publishers
80 Montvale Avenue
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This letter is to request permission to reprint four figures from Supercritical Fluid Extraction, Principles and Practice by McHugh and Krukonis published by Butterworths in 1986. I wish to use Figures:

1.1	Pressure-temperature diagram
1.5	Diffusivity behavior of carbon dioxide
1.6	Viscosity behavior of carbon dioxide
2.1(a)	Schematic diagram of Hannay and Hogarth's apparatus

with "(from McHugh and Krukonis, 1986, reprinted with permission)" as the courtesy line.

The figures will be used in "Environmental Application of Supercritical Fluid Extraction", to be published next fall or witner in Dangerous Properties of Industrial Materials Report published by Van Nostrand Reinhold. I also intend to use them in my thesis (untitled) to be published at Texas A&M. If you have any questions about the paper or the intended use of the figures, please contact myself or the coauthor, Dr. James Bonner, at:

Lt Timothy Green
Environmental Division
Civil Engineering Department
Texas A&M University
College Station, TX 77843
(409) 845-1419

We look forward to your response.

Respectfully,

Timothy S. Green, 1Lt, USAF

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4/2/91 Date

For BUTTERWORTH-HEINEMANN, STONEHAM, MA

VITA

Timothy Scott Green was born 23 September 1963 in Midland, Texas. In the fall of 1982 Capt. Green entered Texas A&M University, where he spent four years in the Corps of Cadets and Fighting Texas Aggie Band. His awards and honors while at A&M include Distinguished Student, Aggie Band Outstanding Freshman, Corps of Cadets Outstanding Sophomore as well as membership in the Ross Volunteers and Chi Epsilon. He completed his B.S. degree in Civil Engineering at Texas A&M and earned a regular commission in 1986. While awaiting his first assignment, he began graduate studies in pavements.

In October 1987 Captain Green entered active duty at Laughlin AFB in Del Rio, Texas, where his duties included Environmental Coordinator, Pavements Engineer and exercise evaluator. He was very active in base organizations and received many honors while at Laughlin. His awards include the 1990 Air Training Command (ATC) Federal Environmental Engineer of the Year, 1989 ATC Outstanding Engineering Military Manager of the Year and 1989 Laughlin AFB Company Grade Officer of the Year. Capt. Green returned to Texas A&M in August 1990 to begin graduate work in environmental engineering. He is married to the former Susan Moseley and was stationed at Center for Environmental Excellence at Brooks AFB in San Antonio in January 1992. His permanent address is 19707 Encino Way, San Antonio, TX 78259.

ABSTRACT

Supercritical Fluid Extraction and Chromatography Using a Lee Scientific Series 600 SFE/SFC System (May 1992)

Timothy Scott Green, B.S., Texas A&M University

Chair of Advisory Committee: Dr. James S. Bonner

The Lee Scientific Series 600 supercritical fluid extractor and chromatography system has been evaluated for quantitative analytical chromatography and quantitative on- and off-line extraction using benzo(a)pyrene, pentachlorophenol, and naphthalene spiked silica samples. The silica was spiked by adding chemical/solvent solutions with known chemical mass to the silica, tumbling, drying under a hood at room temperature, and then remixing. The samples were split so other researchers could perform the traditional Soxhlet and tecator extraction procedures on the same material. The supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) was conducted with high purity carbon dioxide as the carrier fluid and mobile phase. On-line extraction utilized a cryofocusing tee on the instrument to immobilize and concentrate the analytes during dynamic extraction of the sample. After the extraction was complete, the tee was thawed and the analytes impulse loaded on the chromatographic column. Off-line extraction carried the extractant through a pressure restrictor and into a collection vial, or vials, of methylene chloride solvent,

where the analytes dissolved into aqueous phase. The solvent solution was then concentrated and analyzed by SFC.

The instrument was able to complete quantitative chromatography satisfactorily, but unable to perform reproducible quantitative on- or off-line extractions as configured. The on-line extraction problems center in the cryofocusing tee, which is unable to maintain a constant temperature as set up, altering the trapping efficiency during, and between, extractions. The off-line extraction difficulties revolve around the expansion of the supercritical extractant to atmospheric pressure through the pressure restrictor and the subsequent trapping of the analytes into the aqueous phase. Crystallization of B(a)P and PCP in the pressure restrictor was encountered. The solvent trapping schemes were unable to transform all the analytes to aqueous phase, most notably with naphthalene.