

COUPLED SOLID PHASE EXTRACTION-SUPERCRITICAL FLUID  
EXTRACTION ON-LINE GAS CHROMATOGRAPHY OF EXPLOSIVES  
FROM WATER

by

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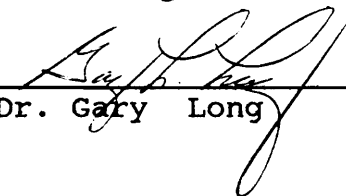
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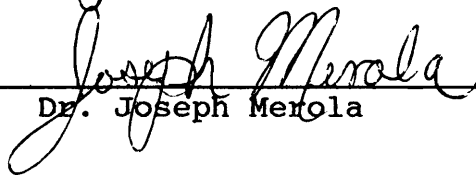
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(ABSTRACT)

A method has been developed for the quantitative extraction of nitrotoluenes (2,6-dinitrotoluene, 2,4-dinitrotoluene, and trinitrotoluene) from water. Three types of solid sorbents were investigated: two 47 mm Empore disks™ - octadecylsilane (C18) and styrene-divinylbenzene (SDVB); and one Bakerbond spe\*™ Phenyl stationary phase. The phenyl sorbent yielded the highest recoveries. The average SPE recoveries for spike standards ranged from 80 to 95 percent for Millipore water and 55 to 95 percent from well and surface water in the low ppb and ppt levels. After the nitrotoluenes were trapped on the solid sorbents they were quantitatively eluted by first doping the bed with toluene and then extracting with supercritical carbon dioxide. Doping with toluene was found to increase the rate of extraction. The extracts were analyzed off-line via GC-ECD using an internal standard. Extraction losses are due to analyte break through, and not from poor SFE recoveries. This demonstrates that supercritical fluid extraction is a

suitable elution technique for analytes trapped on solid phase extraction (SPE) cartridges.

A method has also been developed and evaluated for the direct on-line coupling of SPE to GC. SPE-SFE-GC-ECD analysis eliminates off-line collection and subsequent handling of hazardous materials. SFE is an ideal means of directly coupling SPE to GC, since carbon dioxide is a gas at ambient temperatures and pressures and thus easily removed. One potential problem for SPE-SFE on-line GC is the presence of residual water trapped on the active sites of the bonded silica sorbent. The presence of water can interfere with the cryogenic trapping of the analytes on the capillary GC column. The water becomes ice at cryogenic temperatures and in large quantities blocks the GC column. This problem has been avoided by using a split injection interface previously described by Hawthorne. The quantitative reproducibility of this interface will be investigated for nanogram quantities of nitroaromatics.

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This Dissertation is dedicated to  
Scarlett O'Brien  
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**CHAPTER I**

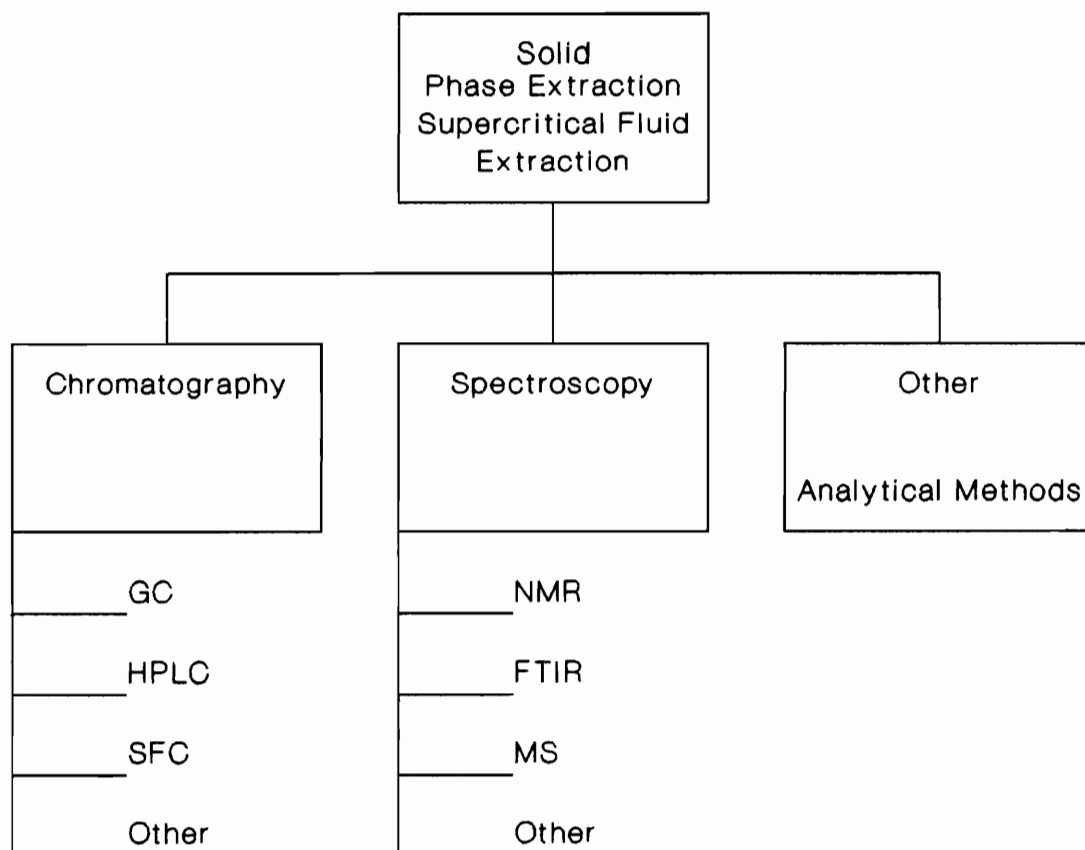
**INTRODUCTION  
and  
LITERATURE REVIEW**

## INTRODUCTION

## PURPOSE

In recent years, there has been growing concern over the routine use of hazardous solvents for sample extraction and preconcentration. Both the cost of solvent disposal and the potential danger to the analyst and the environment during sample preconcentration and solvent disposal have encouraged alternative methods of extraction. Two techniques, supercritical fluid extraction (SFE) and solid phase extraction (SPE), address these solvent concerns and are being investigated. Both techniques are used independently for the cleanup or concentration of analytes, prior to further analysis (Fig. 1). The main use of SFE is the extraction of samples from a solid or semi-solid matrix. SPE focuses more on the extraction of an analyte from a liquid by adsorption of the analyte to a solid sorbent.

The coupling of these two techniques is an ideal method for the quantitative transfer of analytes from difficult solid matrices to a capillary gas chromatography (GC) column. The purpose of this work is to investigate the use of supercritical carbon dioxide as an elution solvent for analytes, isolated from water, that have been adsorbed onto a solid sorbent. Both off-line and on-line SPE-SFE-GC will be studied.



**Figure 1.** Analyte flow chart. Once sample is prepared a multitude of analytical techniques are available for analysis.

## SUPERCRITICAL FLUID EXTRACTION

## HISTORICAL

The development of supercritical fluid extraction (SFE) is associated with that of supercritical fluid chromatography [1]. This close relationship became apparent when early researchers related the gas chromatographic carrier gas and column operating temperatures to the partition coefficient and capacity factors [2,3,4]. The possibility of improving the migration rate of high molecular weight analytes by using supercritical fluids was reported and demonstrated by Giddings et. al. in the mid to late 1960s [5,6], In spite of these early observations, the use of SFE for analytical sample preparation did not flourish until the mid-1980s [1].

The first report dealing with the solubility of solutes in supercritical fluids was by Hannay and Horgarth in 1879 [7]. Their experiments were performed in small diameter glass tubes, where they could observe the changes in the solubility of inorganic salts - cobalt chloride, potassium iodide, and potassium bromide - in ethanol at a temperature above the critical temperature (234°C) as the pressure was changed. An increase in pressure caused the analytes to dissolve and a decrease caused the compounds to precipitate [8]. This early work was reviewed by Booth and Bidwell in 1949 [9]. Although the analytical potential of SFE lay



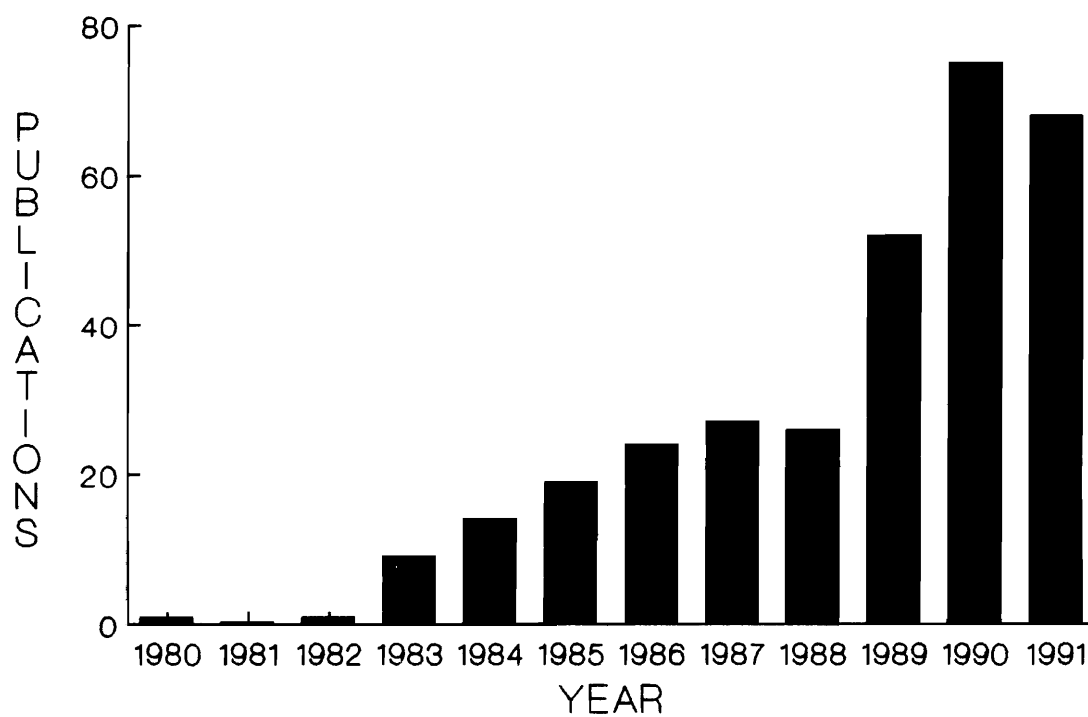
dormant for another 30 years, petroleum engineers recognized the phenomena of retrograde condensation relatively early in the 1940's [10].

Throughout the 1940's and 50's the solvent properties of liquefied gases were studied. One of these studies was prepared and published by Francis in 1954 [11]. He accumulated ternary phase diagrams for liquid carbon dioxide with organic and inorganic compounds along with the predicted solubilities of 261 compounds in near critical carbon dioxide. Even though these studies were performed in liquid carbon dioxide ( $\sim 25^{\circ}\text{C}$  and 950 psig) the results are general and provide information on supercritical carbon dioxide extraction, because analytes soluble in liquid carbon dioxide will also be soluble in supercritical carbon dioxide [8].

The use of SFE as a processing technique was not realized until Zosel's United States patent in 1976 [12]. From this point forward there has been a steady growth in the number of SFE applications in the engineering field. The publication of a book by McHugh and Krukoni in 1986 acknowledged the acceptance of SFE in the engineering community [8].

Stahl and Schilz are recognized as major contributors in demonstrating the potential of analytical SFE. Their work, published in 1976, involved the coupling of SFE with

thin layer chromatography. However, the actual birth of analytical scale SFE is difficult to pinpoint for two reasons. First, analytical scale SFE lacks a universal definition. Second, SFE evolved simultaneously with several different technical disciplines [1]. The acceptance of SFE is evidenced by the publication of several books on the subject [1,8,13,14], a drastic increase in the number of publications from 1980 to 1992 (Fig. 2)[15], and in 1989 the first international meeting emerged entitled, "The International Symposium on Supercritical Fluid Chromatography and Extraction," which focussed on the study of supercritical fluid sciences.



**Figure 2.** Number of publications, with SFE or Supercritical Fluid Extraction in the title, per year [15].

## SUPERCRITICAL FLUIDS

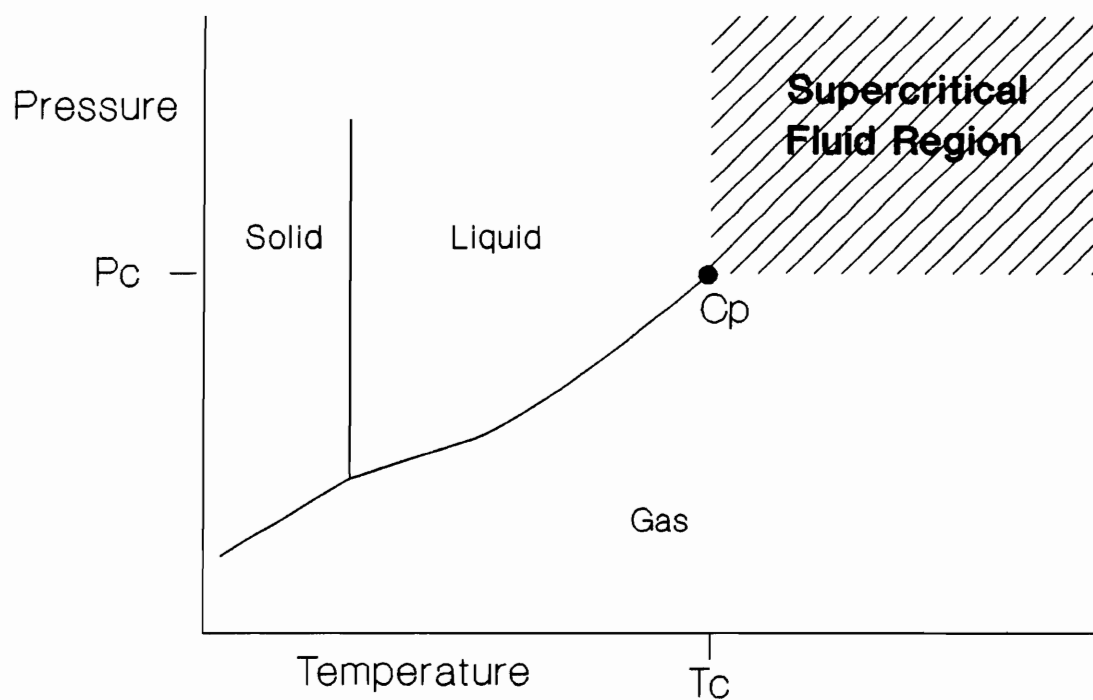
Supercritical fluids (SFs) are dense gases above their critical temperature and pressure, possessing gas like viscosities and diffusivities, and having densities and solvating properties that approach those of a liquid (Table I) [16]. Figure 3 is a typical phase diagram representing the three different phases of a pure compound, with the shaded area representing the supercritical fluid region. Above the critical temperature an increase in pressure will not drive the fluid into the liquid phase [17].

The properties of SFs make them ideal for extracting analytes from solid matrices such as soils, agricultural products, foods, and solid sorbents. Supercritical fluids have the ability to maximize the extraction selectivity by controlling the temperature and pressure of the supercritical fluid (Fig. 4) [1]. Initially, the solubility of an analyte in a subcritical gas is dependent on solute vapor pressure, thus the solubility of the analyte in the gas first decreases with a rise in pressure reaching a point of minimum solubility. As the gas is compressed into the critical phase there is a rapid increase in analyte solubility as the fluid density increases. The increase in solubility ends at a maximum pressure that is determined by the extraction temperature. Any additional increase in pressure will only slightly increase analyte solubility.

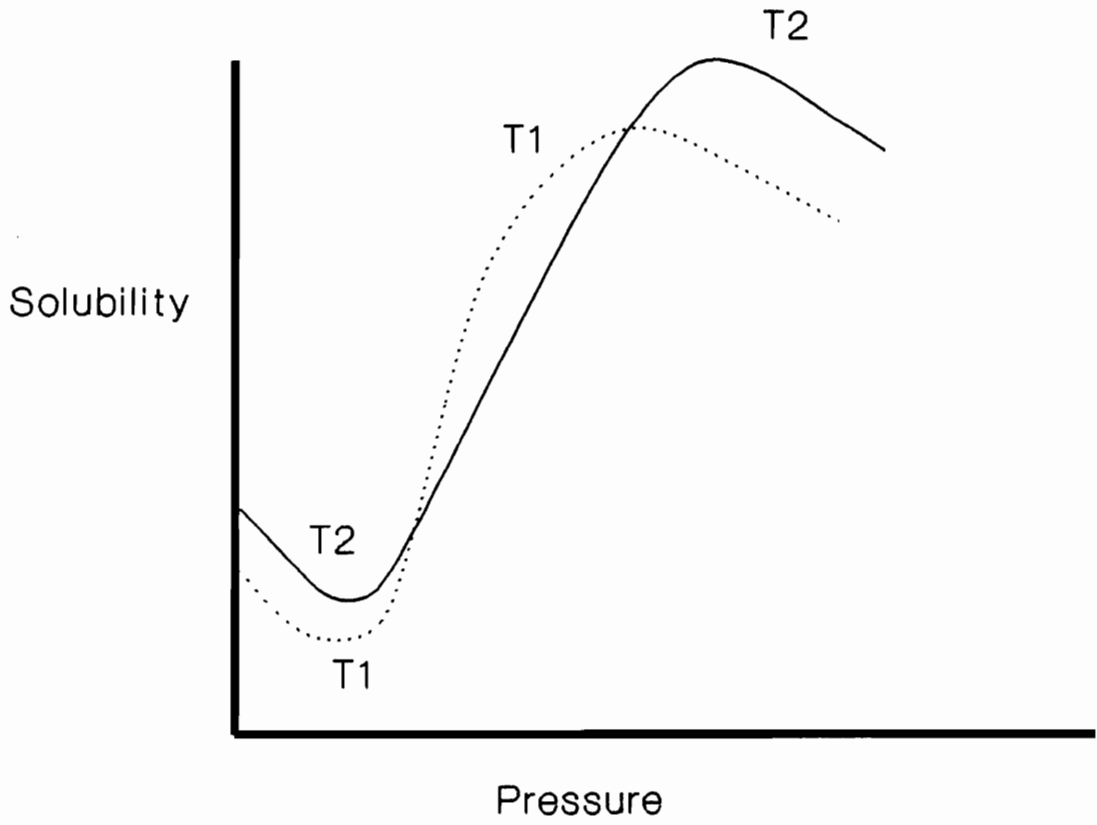
TABLE I

PHYSICAL PROPERTIES OF THE PHASES [16]

Phase	Density (g/cm <sup>3</sup> )	Viscosity (poise)	Diffusivity (cm <sup>2</sup> /S)
Gas	0.001	$5 \times 10^{-5}$ - $3.5 \times 10^{-4}$	0.01 - 1.0
Supercritical Fluid	0.2-0.9	$2 \times 10^{-4}$ - $1 \times 10^{-3}$	$3.3 \times 10^{-4}$ - $1 \times 10^{-5}$
Liquid	0.8-1.0	0.003 - 0.024	$5 \times 10^{-6}$ - $2 \times 10^{-5}$



**Figure 3.** Generalized phase diagram for a pure compound.  $P_c$  = Critical Pressure,  $T_c$  = Critical Temperature, and  $C_p$  = Critical point [17].



**Figure 4.** Generalized solubility isotherm as a function of pressure.  $T_1 > T_2$ . Adapted from [1].

Also, in some cases a higher extraction temperature will result in an increase in analyte solubility [18].

At least two factors play a role in the extractability of an analyte from a solid matrix by SFE. First, the analyte must be soluble in the supercritical fluid. Second, the analyte solvent interactions must be more energetically favorable than those of the analyte and the matrix.

To determine if the analyte is soluble in the SF, a knowledge of the physical properties of the analyte is helpful. The melting point of the solid can be vital, since analytes tend to be more soluble in SFs in their liquid states. Above the melting point, the mass transfer of the analyte into the SF is improved along with analyte solubility because the cohesive forces of the liquid are less than those of the solid. In addition, the vapor pressure can play a role in the solubility of an analyte, especially for multi-component systems [19]. Information on analysis of the analytes by supercritical fluid chromatography may be helpful in determining the analyte solubility in a supercritical fluid [20].

If the analyte is soluble in a SF, yet cannot be extracted from the matrix, the analyte matrix interactions may be too strong. The problem may be overcome by the addition of modifiers to the supercritical fluid, or by the direct addition of a modifier to the extraction vessel.



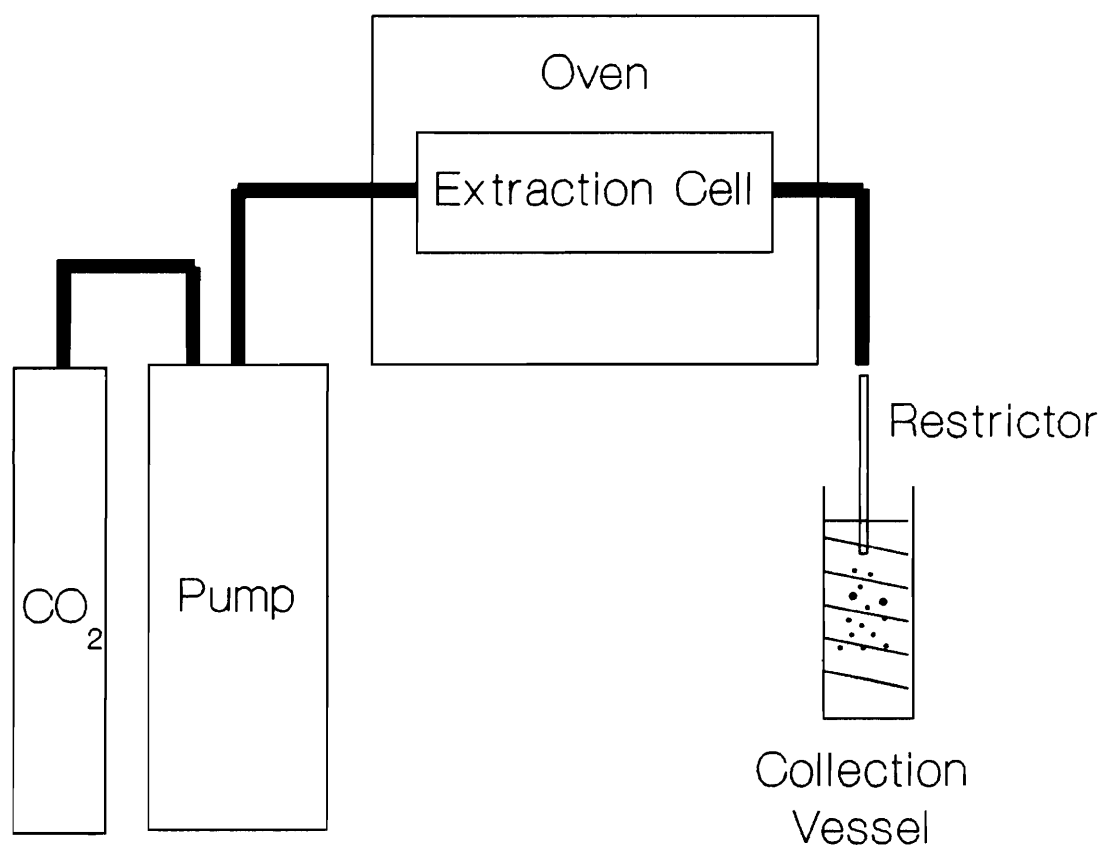
Several papers dealing with the use of modifiers for supercritical fluid chromatography (SFC) [21,22,23] and SFE have been published [24,25,26].

Modifiers have two basic effects on the SFE of analytes from a matrix. They can increase the solvating power of the SF or modifiers can interact with the surface of the matrix displacing the analyte into the SF. To distinguish between the two types of modifiers, they are commonly termed solvent modifiers and matrix modifiers, respectively.

#### SUPERCRITICAL FLUID EXTRACTION

There are two basic modes of supercritical fluid extraction: static and dynamic. Both will be discussed in the following sections. The basic instrumentation required for both modes of SFE is similar. Figure 5 illustrates the minimum hardware required to perform a supercritical fluid extraction. The components of a system are as follows: 1) a source of high purity fluid with an attainable critical temperature and pressure; 2) a high pressure delivery system; 3) an oven; 4) an extraction vessel; 5) a restrictor; and 6) a sample collector.

Several fluids have been used as supercritical solvents (Table II) [27] The most common solvent is carbon dioxide because it has critical values that are easy



**Figure 5.** Basic components of a supercritical fluid extraction system.

TABLE II

PHYSICAL PARAMETERS OF SUPERCRITICAL FLUIDS [27]

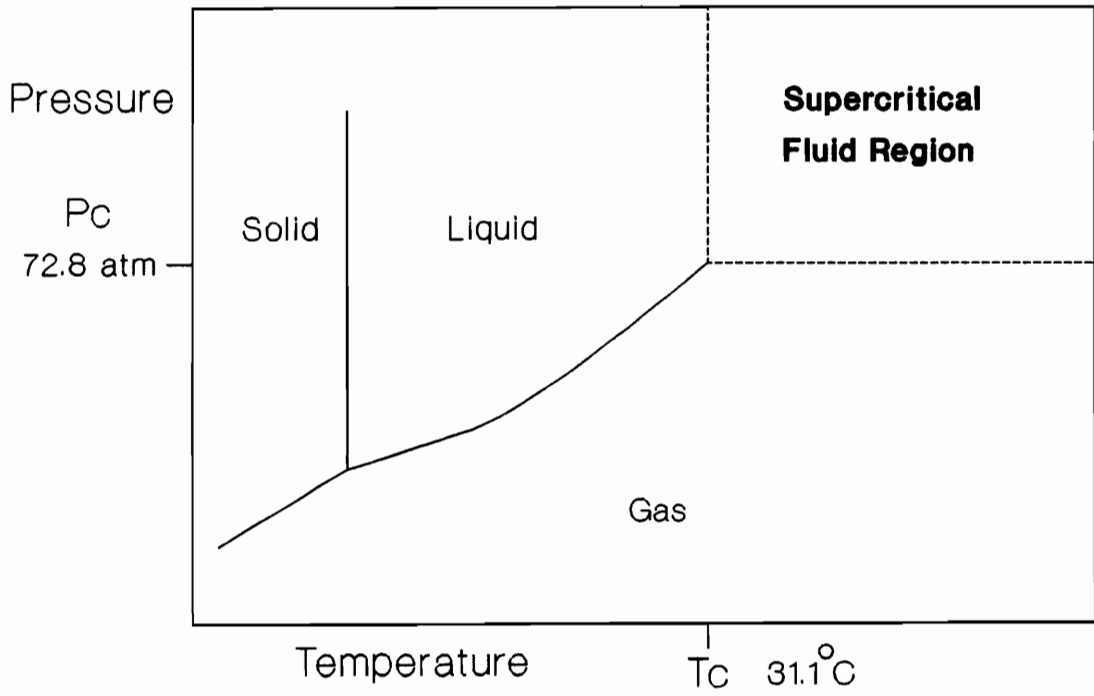
Fluid	Critical Temperature (°C)	Critical Pressure (atm)	Dipole Moment (D)
Carbon dioxide	31.3	72.9	0
Nitrous oxide	36.5	72.5	0.51
Ammonia	132.5	112.5	1.65
Pentane	196.6	33.3	0
Sulfur hexafluoride	45.5	37.1	0
Freon	111.8	40.7	0.17
Xenon	16.6	58.4	0

to obtain (Fig. 6) [28], is non-toxic, becomes a gas at ambient temperatures and pressures, is inexpensive and is mutually soluble with many organic compounds. Carbon dioxide can be obtained as a liquid from cylinders equipped with a dip tube. The cylinder head space is often pressurized with 1500 psi of helium, which conveniently allows the liquid to be transferred to a delivery system.

The delivery system can be either a syringe pump, reciprocating piston pump or gas compressor. Both syringe and reciprocating piston pumps are available on analytical scale commercial instrumentation. Isco Model 260D Syringe Pump (Isco, Inc., Lincoln NE) and Hewlett Packard 7680A Supercritical Fluid Extractor (HP, Avondale, PA), are examples of each pump, respectively. These are used for analytical scale extractions, while gas compressors are typically used for large scale extractions [29]. The pumps should be able to deliver pressures up to 10,000 psi.

To attain critical temperatures the extraction cell is placed inside an oven. The oven can be a commercial SFE oven, GC oven, or a home built heating device. One example of a home built heating device is a pipe oven that will be described in detail in the Experimental section.

The sample matrix is usually placed inside a stainless steel extraction vessel. Extraction vessels are available in a variety of shapes and sizes and are typically

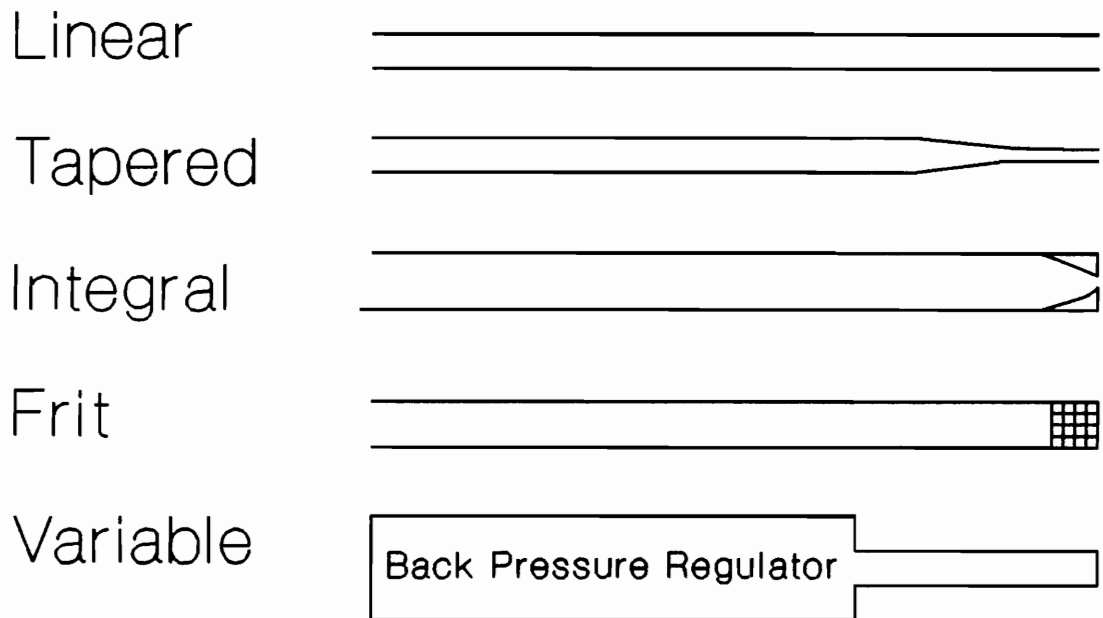


**Figure 6.** Phase diagram for pure carbon dioxide. Adapted from [28].

made of stainless steel thereby able to withstand pressures of 350 - 680 atm. Sample sizes range from 1 mg to several hundred grams [30,31,32]. Typical quantitative SFE samples are less than 10 g [33]. Ideally, the extraction cell should be large enough to hold the sample, yet leave little dead volume in the extraction vessel. Any void volume in the extraction cell will increase the time required to flush the analytes from the vessel.

The pressure in the system is maintained by a restrictor after the extraction vessel. Typical restrictors include: 1) Tapered restrictor which is a 10-cm, 50- $\mu\text{m}$  i.d. piece of fused silica capillary that has been drawn at the end to an internal diameter of 5 - 7- $\mu\text{m}$ ; 2) Linear restrictor, typically 5 - 15 cm in length, 10 - 30  $\mu\text{m}$  i. d. piece of fused silica depending on the flow rate desired; 3) Integral restrictor, a piece of fused silica for which the end is melted into a ball and then filed down until the proper diameter is reached; 4) Frit restrictor, a piece of fused silica that has the end plugged with porous silica wool; and 5) Back pressure regulator, which is commercially available. Figure 7 illustrates these various restrictors [27]. For SFE, linear restrictors, tapered restrictors and back pressure regulators are most commonly used.

After the analytes are extracted they must be trapped in order to perform the appropriate analyses.



**Figure 7.** Restrictors available for supercritical fluid extraction [27].

Several trapping techniques are currently employed. These techniques include solvent trapping [34,35] (Fig. 8), solid sorbent traps [24] (Fig. 9), and direct on-line trapping.

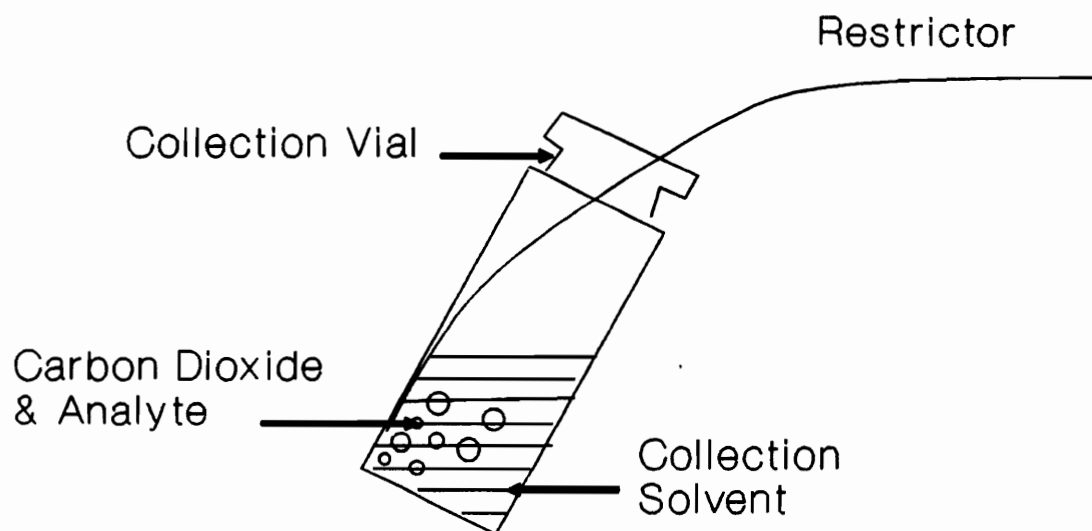
## MODES OF SUPERCRITICAL FLUID EXTRACTION

### DYNAMIC AND STATIC EXTRACTION

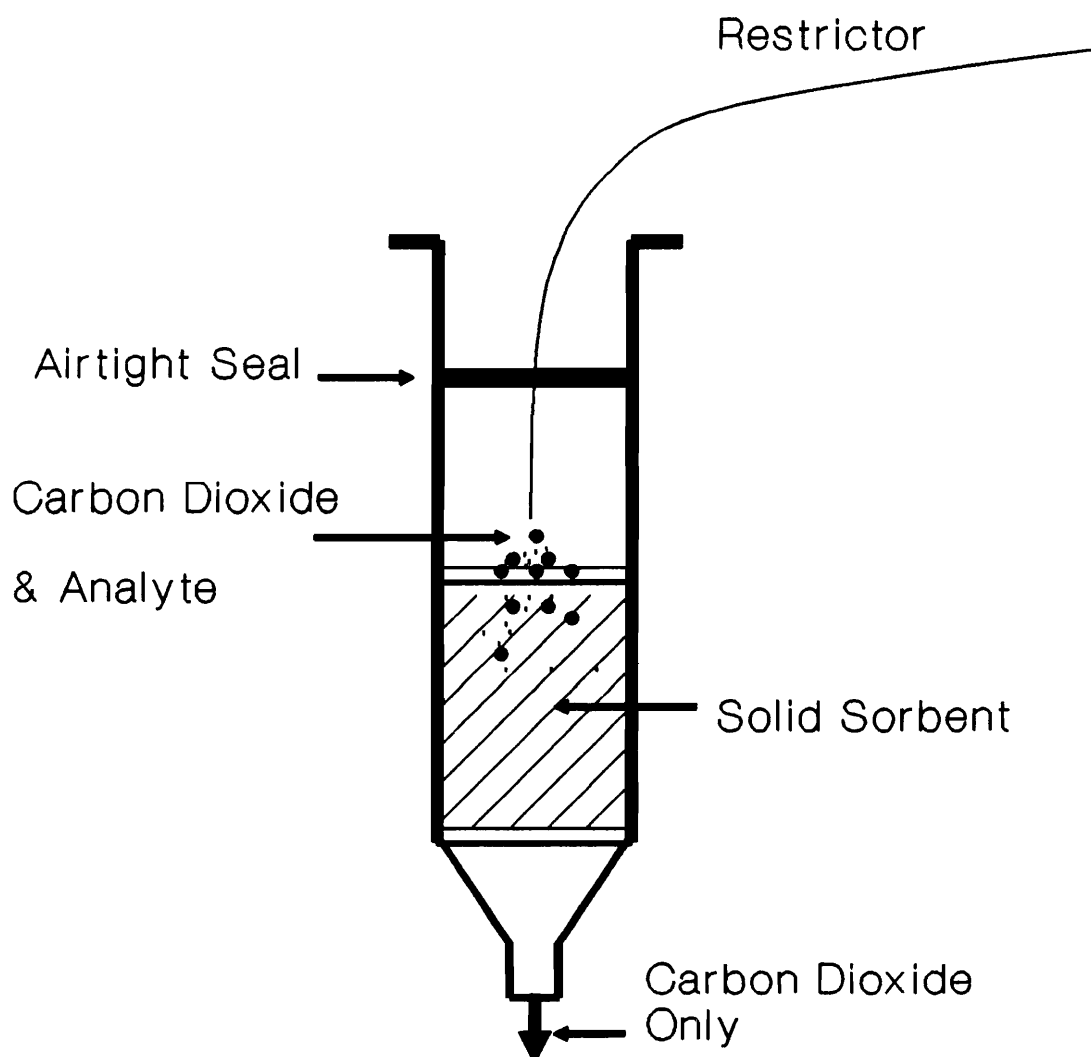
Two modes of SFE, dynamic and static, are currently employed by the analytical chemist (Fig. 10) [36]. In the dynamic mode, the sample matrix is continuously flushed with fresh supercritical fluids, which pass through the sample matrix, solvate the analytes, and carry them to a trap where the analytes are collected.

The extraction profile for a typical dynamic extraction is depicted in Figure 11 [1]. An extraction is divided into three different regions. In Region I, the equilibrium controlled phase, the analytes are rapidly extracted from the matrix with the rate of extraction depending on the solubility of the analytes in the SF, the rate at which the SF passes through the extraction vessel, and the void volume of the extraction cell. Region I is the linear portion of the curve. Region II is the transition phase, where the extraction starts to become diffusion limited because the analyte on the surface of the matrix has been swept out of the vessel. The result is a decrease in

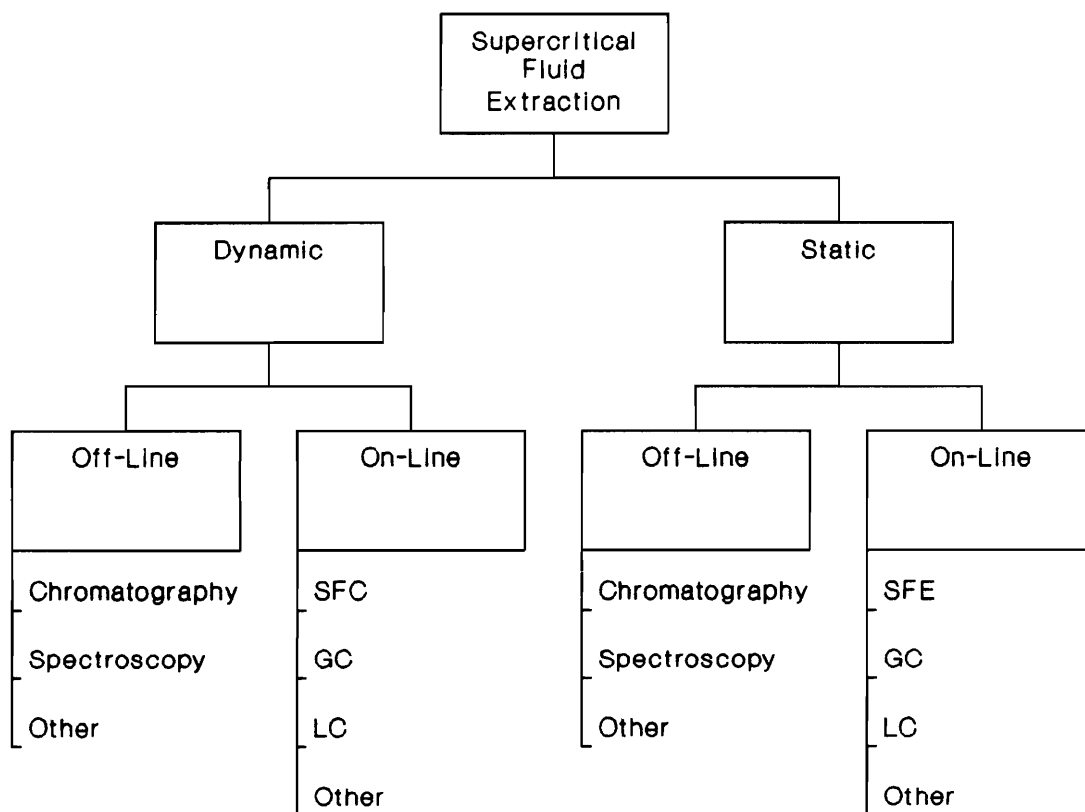




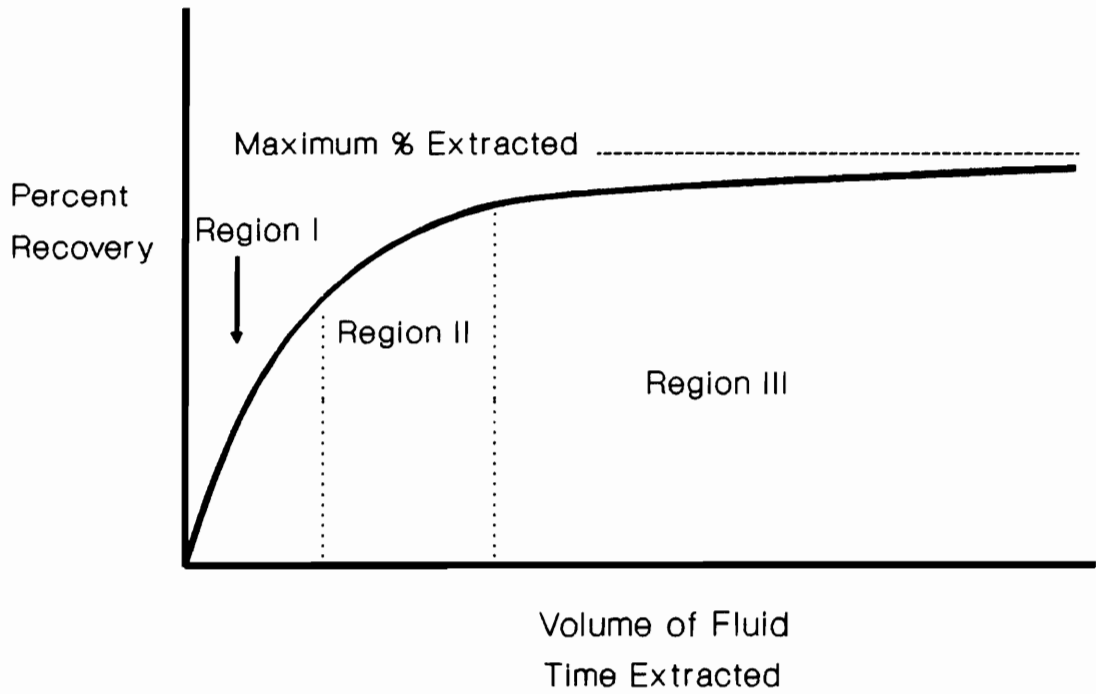
**Figure 8.** Collection vial commonly used for solvent trapping in supercritical fluid extraction to trap analytes.



**Figure 9.** Solid sorbent trap used to collect analytes in supercritical fluid extraction.



**Figure 10.** Modes, dynamic and static, of supercritical fluid extraction and their analysis techniques [36].



**Figure 11.** Generalized extraction curve of percent solute extracted as a function of volume of extraction fluid or time. Adapted from [1].

the rate of extraction as the rate becomes diffusion limited. Finally, in Region III, the diffusion controlled phase of the extraction, the extraction rate is controlled by the diffusion rate and mobility of the analyte within the sample matrix along with its desorption rate from the surface of the matrix.

In the static mode, the sample to be extracted is placed into an extraction thimble, filled with a supercritical fluid at the appropriate temperature and pressure, and allowed to stand for a period of time. When the extraction is complete the supercritical fluid is released through a trap to collect the analytes.

Static extraction allows analytes with slow mass transfer time to be solvated by the SF. Also, the use of a known concentration of modifier is possible by direct addition of the modifier to the extraction cell. The main limitation of static extraction is its inability to perform an exhaustive extraction. This situation is due to the equilibrium of the analyte between the matrix and SF which results in a loss of analyte during depressurization. Consequently, it is often necessary to perform multiple static extraction, much like the traditional liquid /liquid extraction.

## OFF-LINE VERSUS ON-LINE SFE

When developing a supercritical fluid extraction method, off-line SFE is the method of choice, because it is simpler than on-line SFE and does not require knowledge of chromatographic methods. However, an attractive property of SFE is its ability to easily interface with other analytical techniques such as supercritical fluid chromatography [37,38,39,40], high performance liquid chromatography [41] and gas chromatography [42,43,44,45,46,47,48]. On-line SFE has several advantages over off-line SFE: 1) The analyte is transferred directly to the analytical method allowing the greatest sample concentration to be introduced; 2) Additional handling of toxic chemicals in their most concentrated form is eliminated; 3) The analyte is not subject to contaminants which may be present in the collection vessels and collection solvent; 4) Chemisorption of the analyte to the surface of the collection vessel and subsequent loss of analyte is not possible; and 5) On-line analysis often provides faster results than off-line analysis [49].

Along with these advantages there are several concerns that should be mentioned. The major limitation of on-line SFE is that once the analysis is completed the analyte is gone and further analysis is not possible. Off-line SFE

allows the analyst to perform a variety of analyses on the extractants. Also, when using on-line SFE, the concentration of contaminants in the SF is increased along with the analyte of interest, therefore the SF used needs to be very pure. Finally, on-line analysis requires the dedication of an instrument, a situation which may not be economically feasible.

#### ON-LINE SFE/GC

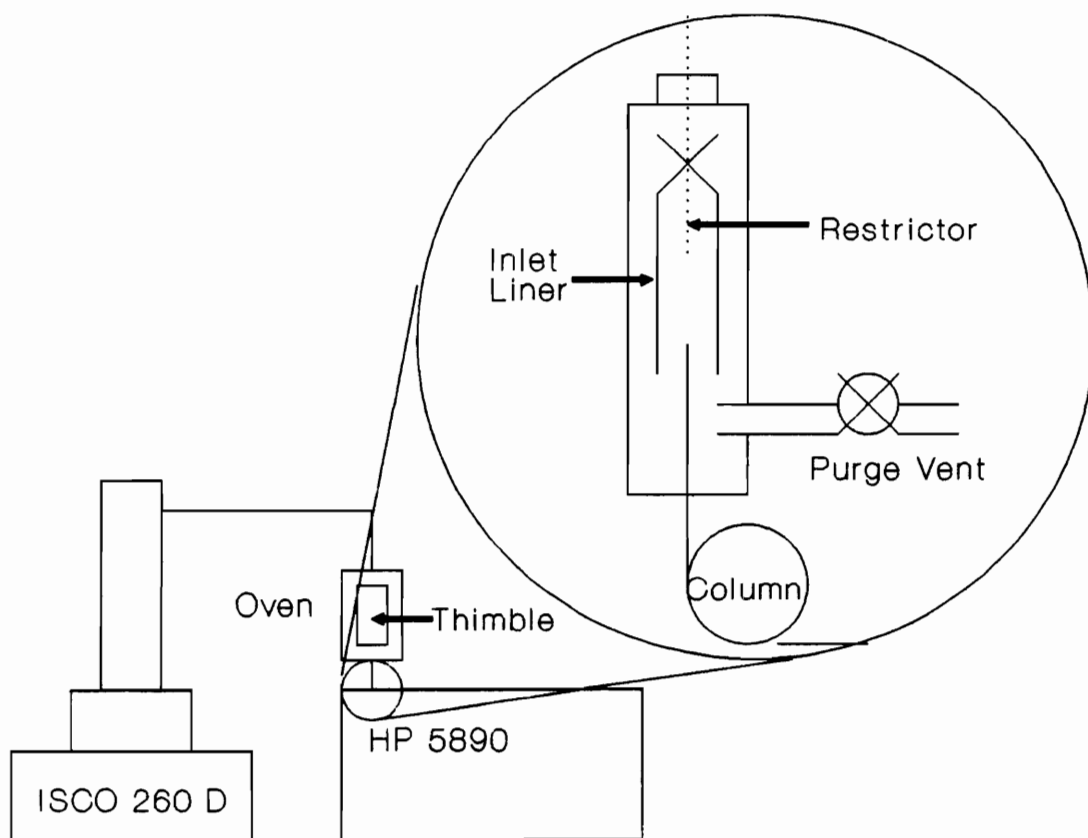
The focus of this discussion is the use of SFs for directly interfacing SPE to capillary gas chromatography. Several different types of on-line interfaces have been developed. All of these follow the same basic procedures, supercritical fluid extraction, depressurization and venting of the extraction solvent, collection and focusing of the analyte in an accumulator or on the GC column, and finally transfer of the analyte to the GC [1]. One method of interfacing SFE to GC involves the use of an accumulator device external to the GC [42,50,51,52]. The extracted analytes are trapped onto a solid sorbent then transferred to the GC column by an additional SFE step, thermal desorption, or solvent displacement via a retention gap.

More commonly the analyte is trapped directly on the capillary column. This method can be accomplished by

depressurization of the supercritical fluid directly into the capillary column [28,44,45,46,53]; in a retention gap [48]; or through a standard split injection port (Fig. 12) [54]. These techniques use the capillary stationary phase and cryofocusing to trap the analytes.

For the extraction of analytes from matrices containing large amounts of water, the use of a split method has several advantages. First, water that can interfere with the trapping of the analytes in a direct capillary on-column interface is split in the heated injection chamber reducing the amount transferred to the capillary column to an allowable level [54]. Second, any contaminants in the sample will be split thereby reducing the potential for interfering peaks during analysis. The analytes will also be split, however, the concentration of the analyte transferred to the capillary column will still be greater than for off-line collection. Third, by placing the restrictor in the heated injection port, restrictor plugging is drastically reduced. For this reason a heated split interface was chosen for the direct interface of SPE to GC via SFE.





**Figure 12.** Schematic of an On-Line SFE-GC system, using a split interface.

## SOLID PHASE EXTRACTION

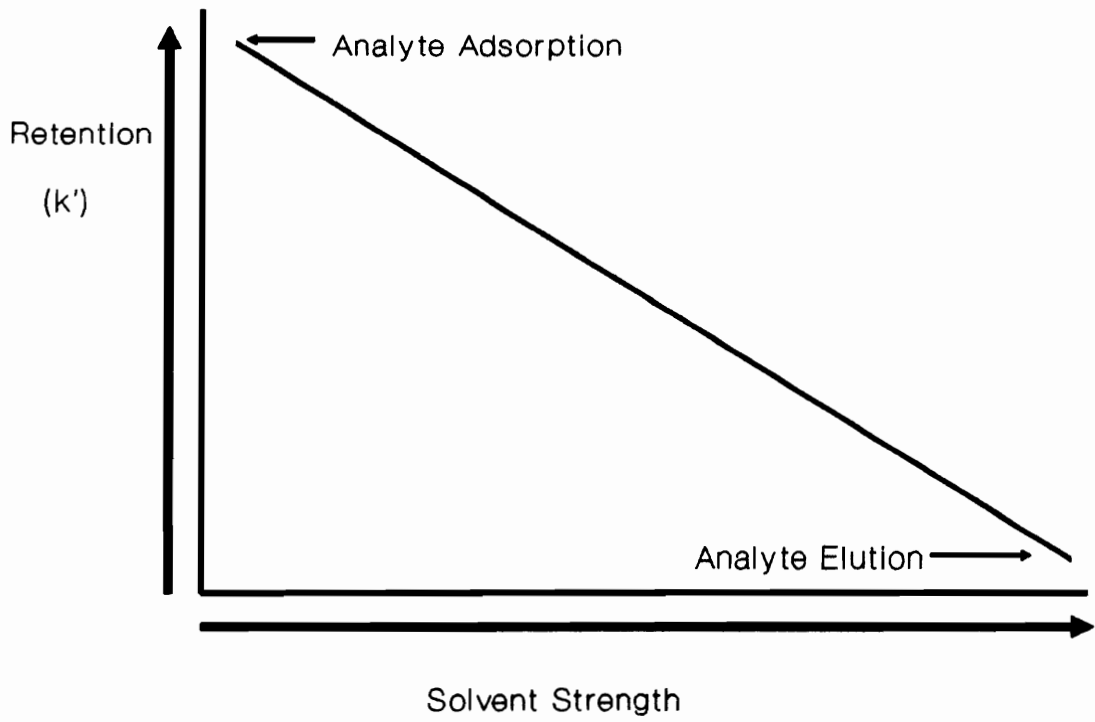
### HISTORICAL

The first successful attempts to characterize organics present in water, by trapping the analyte on a carbon column and eluting them with an organic solvent, were reported in the 1950s [55]. The use of commercial solid phase extraction columns (SPE) to trap analytes from liquids was introduced in the late 1970s and their use has grown rapidly, in particular the use of silica gel bonded phases [56].

### INTRODUCTION

Solid phase extraction applies the basic principles of liquid chromatography to trap an analyte on a solid sorbent from a liquid matrix for concentration or clean-up prior to analysis [57]. The analytes solvated in a weak solvent are trapped on a solid sorbent under conditions of high capacity factor and then eluted with a small volume of strong solvent (low capacity factor) (Fig. 13) [58].

The mechanism for SPE is similar to that of liquid liquid extraction (LLE). For both LLE and SPE the partition ratio ( $K$ ) of the analytes between the organic phase (solid sorbent) and aqueous matrix determines the amount of analyte extracted [59]. For strongly hydrophobic compounds where the partition ratio is greater than  $10^3$ , nearly one hundred



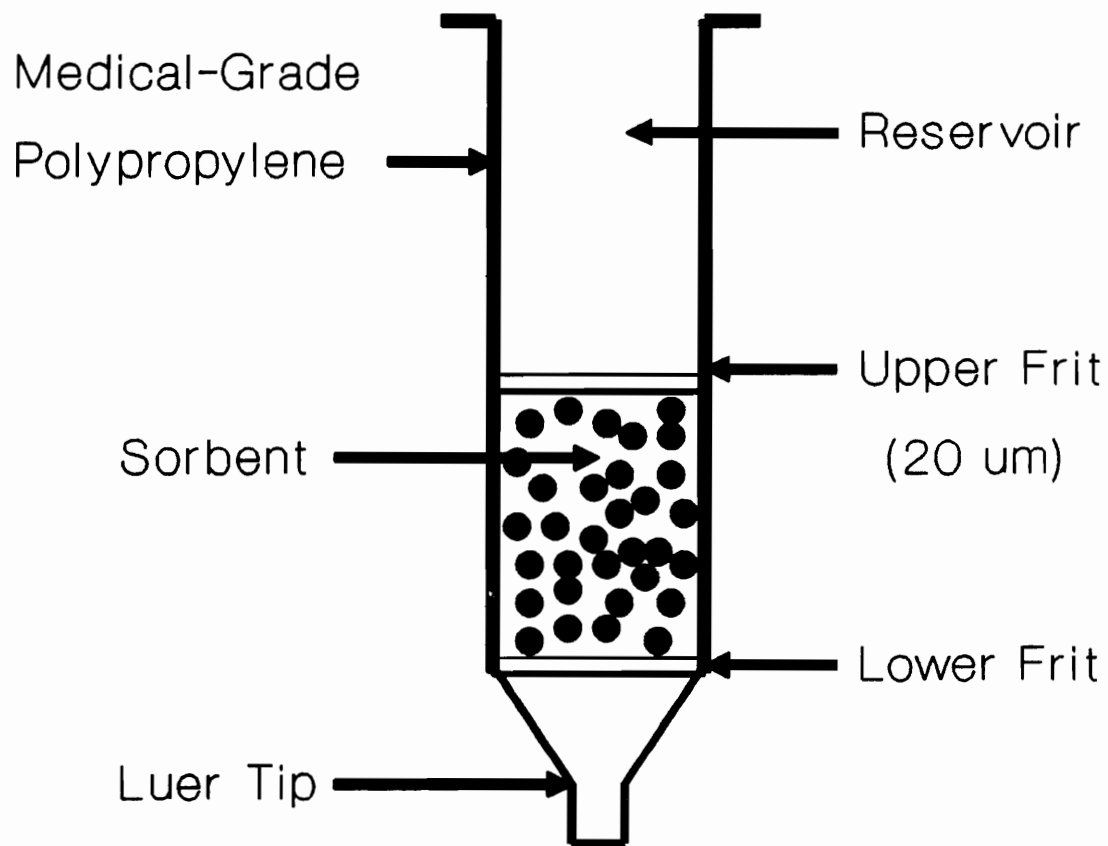
**Figure 13.** Graph of retention versus elution solvent strength for a solid phase extraction sorbent [56].

percent of the analytes will be adsorbed onto the sorbent.

For semi-polar compounds the analytes will be slightly soluble in the aqueous matrix and thus have less favorable partition ratios resulting in lower recoveries. Overcoming this problem may be possible by taking the precautions described below in the section entitled SOLID PHASE EXTRACTION.

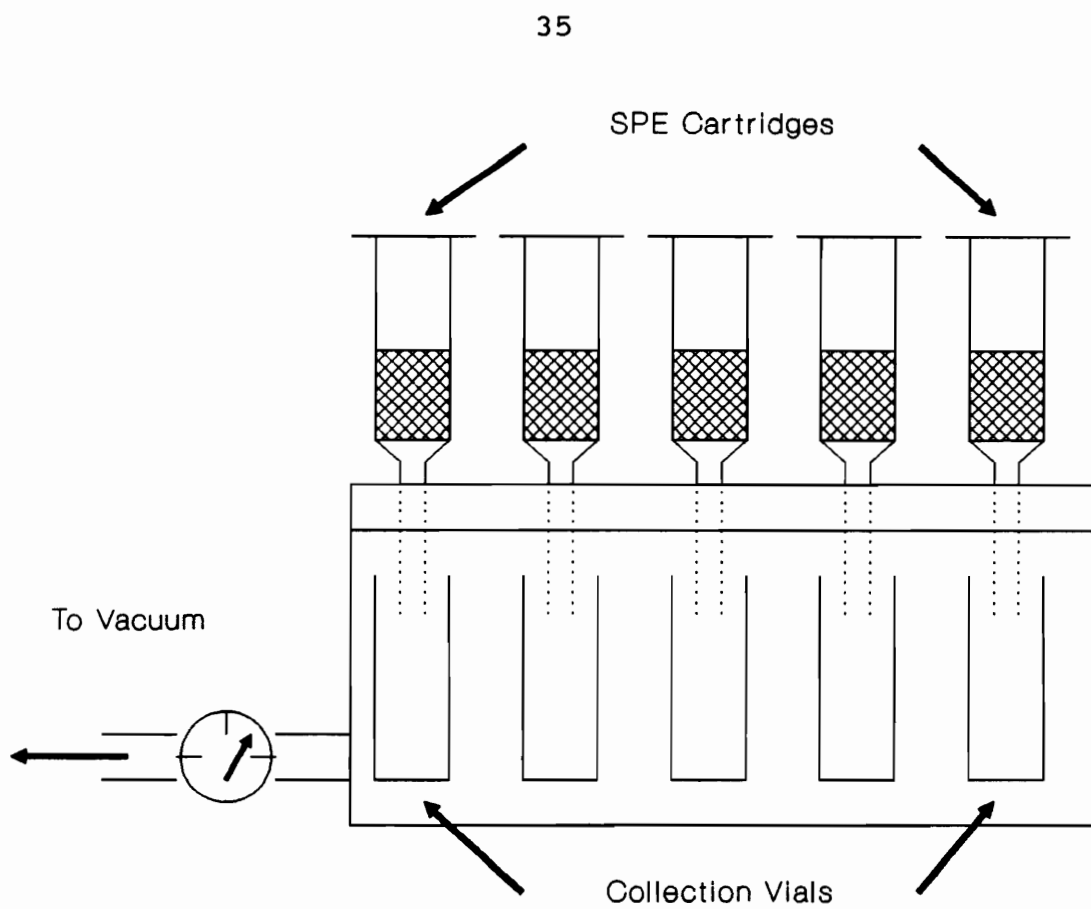
Because the solid sorbent in SPE can replace the organic solvents used in liquid/liquid extraction, SPE is being used in place of LLE. Also, for many practical reasons solid phase extraction is replacing LLE as the method of choice for isolating analyte from a liquid matrix [60]. Some of the reasons for the switch to SPE include:

- 1) The ability to sample in the field. Typically large volumes of aqueous samples are required to achieve the preconcentration necessary to reach detectable concentrations of analyte. Often this will require the transportation of several liters of liquid in glass or plastic containers back to the laboratory. When using SPE columns the preconcentration steps can be performed in the field leaving only the small cartridges to transport (Fig. 14) [61];
- 2) Simplicity. Solid phase extraction can be performed using a syringe and a single SPE cartridge. If the initial SPE attempts are successful, then more sophisticated multiple cartridge systems can be used



**Figure 14.** Solid Phase Extraction Cartridge. Typical dimensions for a 3 ml cartridge are 5.5 cm X 1 cm. Adapted from [59].


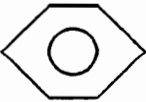
(Fig. 15); 3) Emulsion formation, which is one of the greatest drawbacks of LLE for waste water and biological liquids, is rarely a problem for SPE; 4) Safety, this technique reduces the volume of hazardous organic extraction solvents the operator is exposed to; 5) Lower costs, the reduced solvent volumes decrease the cost of solvent purchase and disposal. Also, SPE cartridges are relatively inexpensive, around a few dollars per cartridge, and can be disposed of with the costs still being 5 to 10 times less than that for LLE [62]. When necessary the cartridges can be reused provided the samples are not too contaminated; 6) Flexibility exceeds that of LLE, for LLE only hydrophobic extraction solvents may be used while the solvents available for SPE are almost limitless. Also, the wide selection of available sorbents provides the ability to maximize selectivity ( $\alpha$ ) (Table III) [63]. Disposable SPE columns allow a quick and simple means for the isolation of a variety of compounds from water matrices. Often the analyst can find a method of analysis in the application notes provided by the SPE vendors [64,65] or a method can be easily developed by having a knowledge of previous HPLC analysis of the compounds; and 7) SPE cartridges are made of medical grade polypropylene, therefore, they are clean and reduce the potential for contamination which may occur from poorly cleaned glassware.



**Figure 15. Schematic of Baker-10 spe™ Extraction System. Allows ten simultaneous extractions.**

TABLE III

SELECTED BONDED SILICA SORBENTS FOR SOLID PHASE EXTRACTION  
[64].

<b>Non-Polar Sorbent</b>	<b>Structure</b>	<b>Abbreviation</b>
Octadecyl	-Si-C <sub>18</sub> H <sub>37</sub>	C18
Octyl	-Si-C <sub>8</sub> H <sub>17</sub>	C8
Cyclohexyl	-Si- 	CH
Phenyl	-Si- 	PH



## SOLID PHASE EXTRACTION

The adsorption of an analyte from a water matrix requires several steps, these include - sorbent activation or conditioning, sample addition, washing, drying and elution (Fig. 16) [67]. Conditioning of the sorbent ensures maximum interaction of the bonded silica sorbent with the analytes present in the liquid matrix. Typically 5 - 10 bed volumes of strong solvent or the elution solvent are passed through the sorbent bed by means of an aspirator, i.e. for octadecylsilane (C18), hexane would be an appropriate solvent. In addition to sorbent activation this will remove any residual contaminants that might be present on the sorbent. The activation solvent is then removed and replaced with an intermediate solvent, usually methanol. Finally, the bed is rinsed with water prior to the addition of sample. It is important that the bed is not over rinsed with water or the bonded silica sorbent will no longer be wetted resulting in low recoveries.

Sample addition can be accomplished by either pushing or pulling the liquid through the sorbent bed. One hundred percent recovery of the analyte may be possible without additional sample preparation. However, if necessary, several steps can be taken to improve the trapping efficiency of the analyte during sample addition. Changing

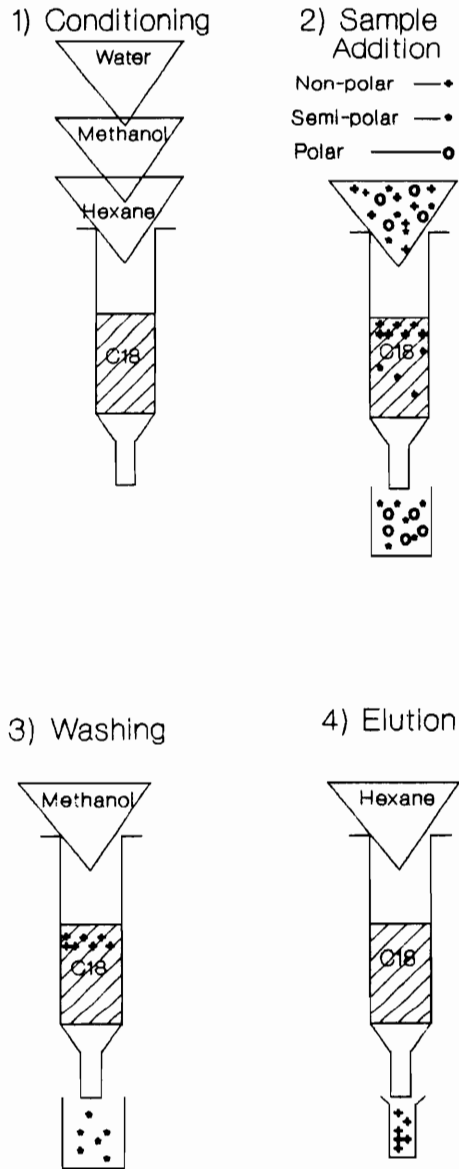


Figure 16. Steps involved in Solid Phase Extraction

the stationary phase will change the selectivity and may result in better extraction. In order to increase the adsorption efficiency of the solid sorbent, the analyte water interactions must be weakened. This can be accomplished for non-dissociating compounds by increasing the ionic strength of the aqueous matrix, thus increasing the partition ratio. This phenomena is commonly called "salting out" and is accomplished by adding electrolytes such as sodium chloride and potassium sulfate to the aqueous matrix [59,60]. For ionic analytes a pH adjustment may be necessary to convert the analytes to neutral species. For complex samples it may be necessary to increase the surface area of the sorbent available to the analytes. This increase can be accomplished by, either increasing the amount of sorbent [59], or decreasing the sample volume which in turn will increase the sorbent to sample ratio. The addition of 1-5 percent methanol to the aqueous sample will keep the bed solvated and may improve analyte recoveries.

If necessary the bed can be washed with a weak solvent to remove interfering contaminants. At least 20 bed volumes of wash solvent must be able to pass through the sorbent bed without eluting the analyte of interest. This step is often eliminated because it can result in a loss of analyte.

Drying the sorbent can be accomplished in several

ways. Water can be removed from the sorbent, by passing air through the sorbent using positive or negative pressure [66], by a stream of nitrogen [67], by using a centrifuge [68] or by placing the sorbent in a desiccator for a period of time [69]. It is very important to determine if this step will result in loss of analyte when the analyte is volatile [70].

The analytes are eluted by a small volume of strong solvent. A general rule of thumb for solvent volume is elution of the analytes by two aliquots of strong solvent using one microliter of solvent for every one mg of sorbent. However, several milliliters may be required to completely elute the analytes.

#### ON-LINE SPE

The use of SPE cartridges or precolumns for automated sample preparation interfaced with HPLC has been well documented [71,72,73] . Some vendors have introduced on-line equipment such as the AASP™ from Varian (Analytichem International, Harbor City, CA). Interfacing SPE with HPLC does not present a problem since the elution solvent used in SPE is often similar to that used for HPLC analysis.

For on-line SPE-GC a retention gap is used to remove the relatively large volumes of solvent used to elute the

analytes from the SPE sorbent [67]. This method can be difficult and cumbersome because typical injection volumes for capillary gas chromatography range from 1  $\mu\text{L}$  to 5  $\mu\text{L}$ , where the volume of SPE elution solvents range from a few hundred microliters to several milliliters. The situation can be alleviated by using supercritical fluid carbon dioxide as an elution solvent for SPE.

The use of supercritical carbon dioxide to elute the analyte allows the analyte to be trapped in a small amount of solvent, without placing restrictions or limits on the amount of carbon dioxide required. Furthermore, this approach allows the analyte to be trapped in a solvent appropriate for gas chromatographic analysis. Most importantly supercritical fluid extraction will allow direct on-line GC analysis using a split injection technique developed by Hawthorne [43].

#### SUPERCRITICAL FLUID EXTRACTION OF SOLID SORBENTS

Several studies have shown that supercritical carbon dioxide can rapidly elute organics, which have been trapped from air, from solid sorbents [74,75,76]. The major difference between this work and previous studies is the presence of residual water trapped on the active sites of the two bonded silica sorbents - C18 Empore™ Disks and Bakerbond™ phenyl sorbent. The water may either act as a

polar mobile phase modifier or hinder the ability of the carbon dioxide to extract semi-polar analytes of interest. The high recoveries found in this work support the first theory.

#### NITROAROMATICS

Nitroaromatics such as 2,6-dinitrotoluene (2,6-DNT), 2,4-dinitrotoluene (2,4-DNT), and trinitrotoluene (TNT) are contaminants that may be found in ground water around explosive manufacturing plants and disposal sites [77]. A single manufacturer can generate as much as 500,000 gallons of wastewater per day [78].

Nitrotoluenes are readily absorbed through the skin by contact. These compounds can cause anaemia, methemoglobinemia, cyanosis and liver damage [79]. The Environmental Protection Agency (EPA) [80] and Oak Ridge National Laboratories (ORNL) have set detection limits of 7 ppt, 100 ppt, and 1 ppb for 2,6-DNT, 2,4-DNT, and TNT, respectively in drinking water [81]. It has been shown that these limits can be met for ground and drinking water by liquid/liquid extraction [82]. A number of studies have been performed investigating the isolation and/or analysis of nitroaromatics from a variety of water sources [83,84,85,86].

The purpose of this work is to investigate the use of

supercritical carbon dioxide as the elution solvent for nitroaromatics trapped on a SPE solid sorbent. Two different recoveries will be discussed. First, the elution of the nitroaromatics from the SPE cartridges by supercritical fluid extraction was determined and second, the trapping efficiency of the analytes from water onto the solid sorbents was investigated. The eluted nitroaromatics were trapped from the carbon dioxide in toluene and off-line analysis was performed via GC-ECD. Finally on-line SPE-SFE-GC-ECD analysis was thoroughly investigated to determine if the technique is quantitative and reproducible.

## **CHAPTER II**

### **EXPERIMENTAL**



## EXPERIMENTAL

### MATERIALS

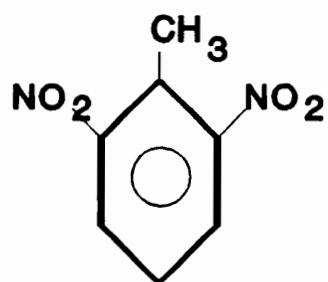
Solvents such as methanol, acetone and toluene were obtained from Fisher Scientific (Pittsburgh, PA) or Aldrich Chemical Co. (Milwaukee, WI). Standards for 2,4-DNT and 2,6-DNT were obtained from Aldrich Chemical Co. (Milwaukee, WI); m-dinitrobenzene was purchased from Fisher Scientific Co. (Fair Lawn, NJ) and the TNT and NG were provided by Naval EOD Technology Center, (Indian Head, MD) (Fig. 17). Supercritical solvents (carbon dioxide SFC and SFE grade) were obtained from Scott Specialty Gases (Plumsteadville, PA) or Air Products (Allentown, PA). Solid sorbents, Bakerbond™ phenyl and Empore™ Extraction Disks - octadecylsilane (C18) and styrene-divinylbenzene (SDVB) were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and 3M Analytical Research Laboratory (ST. Paul, MN), respectively.

## INSTRUMENTATION AND METHODS

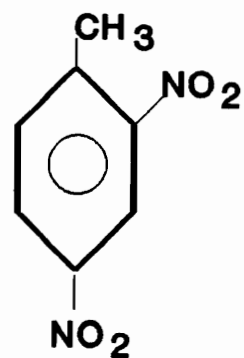
### SOLID PHASE EXTRACTION

#### BAKERBOND™ PHENYL

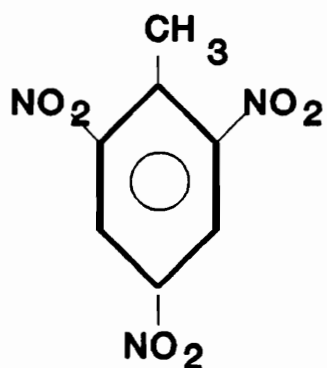
For the Bakerbond™ phenyl sorbent, solid phase extraction was performed using a Baker-10 Extraction System,



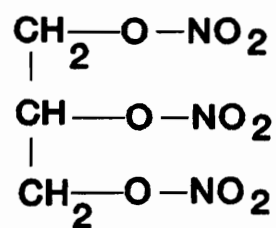
2,6-Dinitrotoluene  
(2,6-DNT)



2,4-Dinitrotoluene  
(2,4DNT)



Trinitrotoluene  
(TNT)

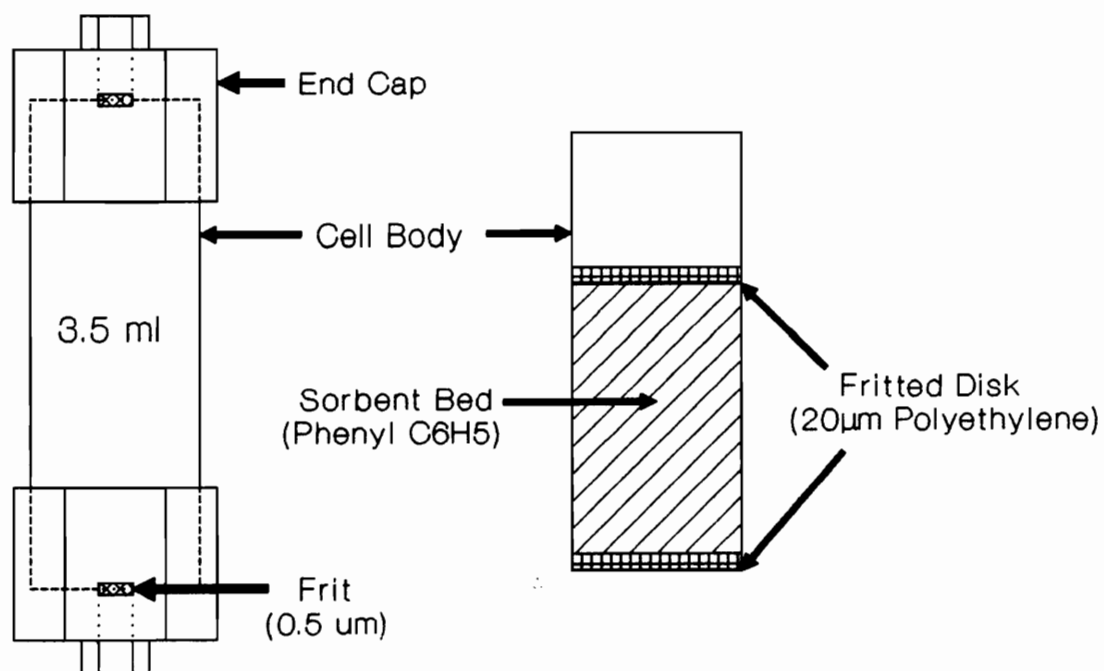


Nitroglycerine  
(NG)

**Figure 17. Chemical Structures of Explosives studied**

J. T. Baker Chemical Co. (Phillipsburg, NJ). The Bakerbond™ phenyl sorbent was removed from two 500 mg SPE cartridges and packed directly into a 3.5 mL supercritical fluid extraction vessel, Keystone Scientific, Inc. (Bellefonte, PA), that was modified to hold the solid support during the SPE sample addition step (Fig. 18). To allow the water sample to be pulled rapidly through the sorbent bed by means of an aspirator, the 0.5  $\mu\text{m}$  end frits were removed and two 20  $\mu\text{m}$  polyethylene fritted disks were placed inside the cell body to support the Bakerbond™ phenyl sorbent. These polyethylene frits were those removed from the SPE cartridges. So that the frits would fit securely inside the SFE vessel, they were compressed in a vice to expand their diameter. Prior to SFE the 0.5  $\mu\text{m}$  frits were replaced (Fig. 18).

The conditioning and sample addition steps for the Bakerbond™ phenyl sorbent were then performed. The first step was conditioning. In each case, the phenyl sorbent was preextracted using the standard SFE conditions given in the section titled Supercritical Fluid Extraction. Prior to preextraction the sorbent was wetted with Millipore™ water and doped with 0.5 mL of toluene. After SFE the 0.5  $\mu\text{m}$  frits were removed from the extraction vessel. The vessel was then mounted on the Baker-10 extraction system and fitted with a 75 mL reservoir. The luer tip of the



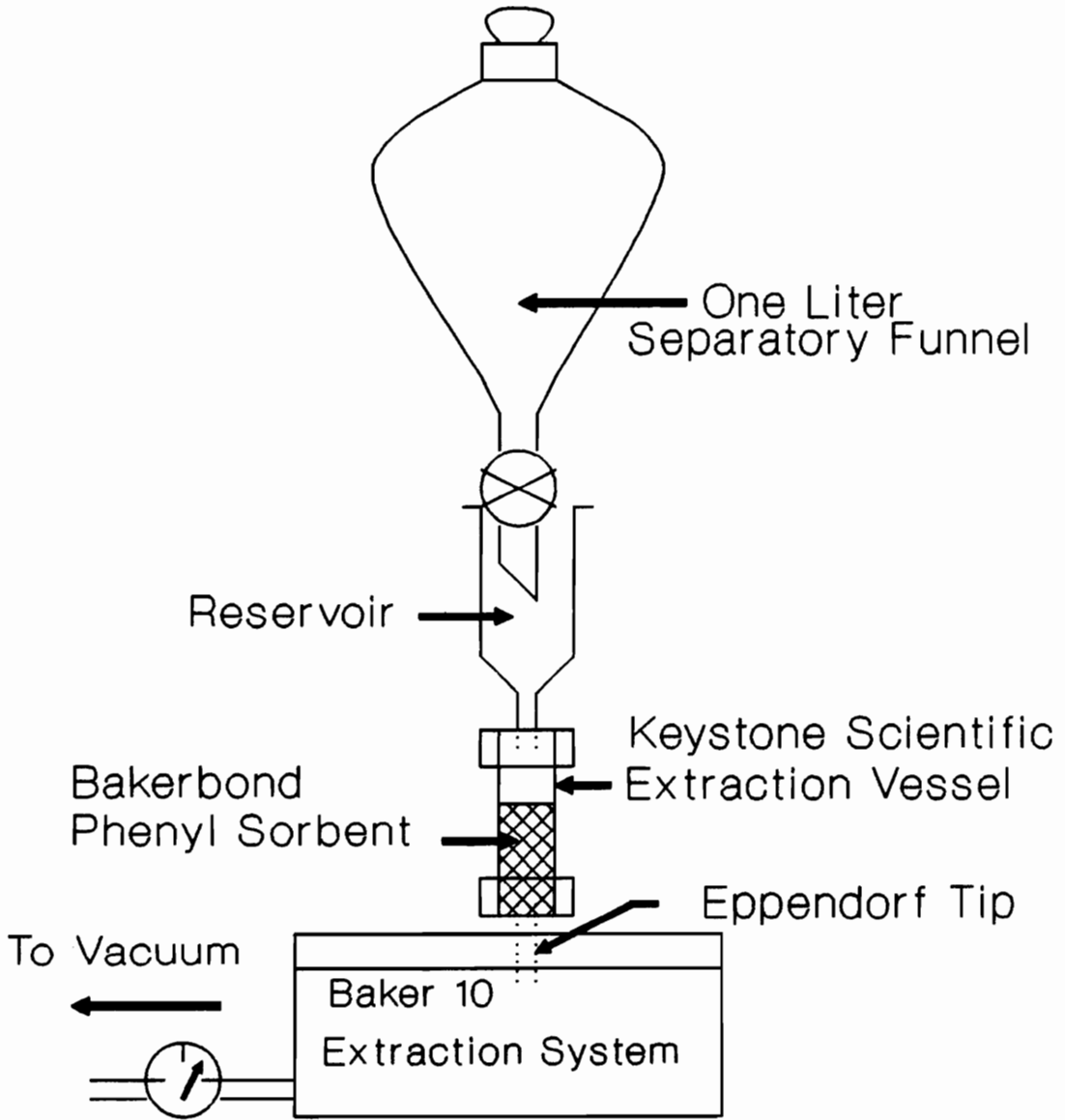
**Figure 18.** Schematic of modified 3.5 mL supercritical fluid extraction vessel. (Keystone Scientific, Bellefonte, PA)

reservoir fit tightly inside the SFE extraction vessel where the 0.5  $\mu\text{m}$  frit was located. To connect the SFE vessel to the Baker-10 Extraction System, a 1000  $\mu\text{L}$  Ependorff (Brinkmann Instruments, Inc., Websterbury, NY) pipet tip was cut to fit inside the extraction cell and Baker-10 SPE extraction system lure tip receptacle forming a leak free seal. The sorbent was then wetted with 5 to 10 mL of acetone and/or 5 to 10 mL of methanol followed by 5 mL Millipore™ water.

In the next step, sample addition, the appropriate volume of water sample was placed in a one liter separatory funnel. By capping the funnel with an air tight seal and placing the tip of the funnel into the reservoir an automatic gravity feed was created (Fig. 19). Each water sample was spiked with the appropriate amount of analyte, which was dissolved in methanol, shaken, and then aspirated through the sorbent bed with a vacuum pressure of 15 psi. For each SPE extraction, the water was salted with 20 mL saturated sodium sulfate solution per liter of water.

The third step was washing. The wash step was eliminated except when selectivity studies were performed, then the wash solvent was supercritical carbon dioxide at densities lower than that required to elute the analytes of interest.

The final step was elution. After the water sample was

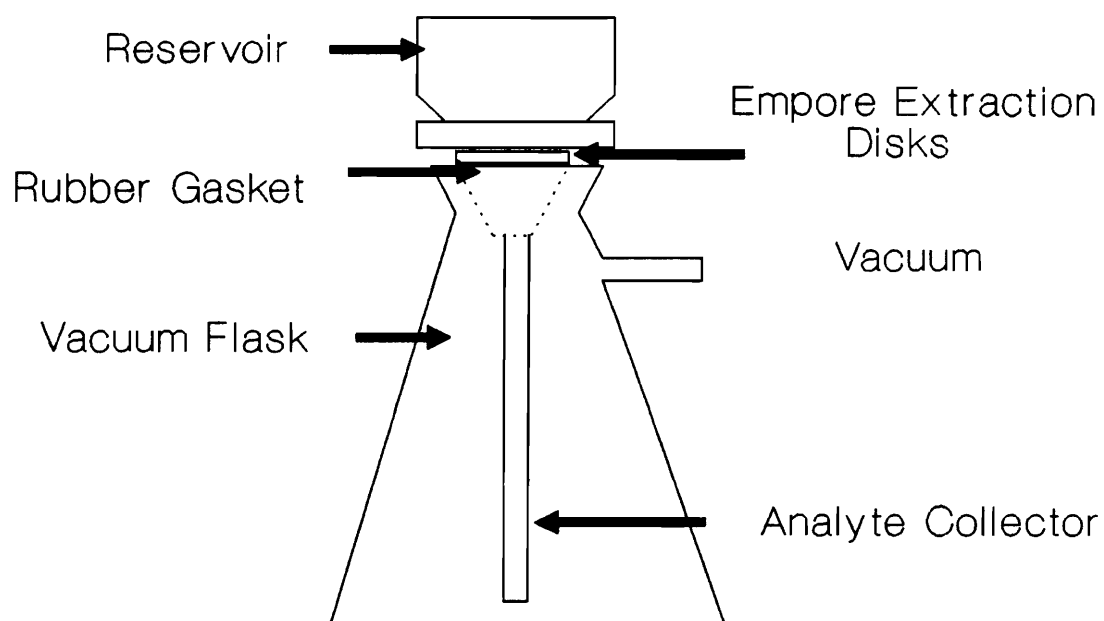


**Figure 19.** Schematic of SPE-SFE vacuum/gravity feed for aqueous samples.

aspirated through the sorbent bed. The bed was allowed to dry, under vacuum, for no less than 15 minutes. The sample was then eluted by supercritical fluid extraction.

#### EMPORE™ EXTRACTION DISKS

A Millipore™ filter holder, Millipore™ Co. (Bedford, Massachusetts) was used for the octadecylsilane (C18) and styrene-divinylbenzene (SDVB) Empore™ extraction disks, 3M Analytical Research Laboratory, (St. Paul MN) (Fig. 20). The conditioning and sample addition steps for the Empore™ extraction disks consisted of the following steps: 1) Conditioning, to minimize handling of the Empore™ extraction disks prior to SPE, the disks were not preextracted with supercritical carbon dioxide. The sorbent was then washed with 10 mL acetone and 10 mL methanol followed by 10 mL Millipore™ water; 2) Sample addition, the appropriate volume of water sample was placed in a one liter separatory funnel. Each water sample was spiked with the appropriate amount of analyte, dissolved in methanol, spiked with an additional 5 mL methanol per liter of water, shaken and then aspirated through the Empore™ extraction disks with a vacuum pressure of 15 psi.; 3) Washing, this step was eliminated to prevent unwanted loss of analyte; 4) Elution, after the water sample was aspirated through the Empore™ extraction disks, the bed was allowed to dry, under vacuum



**Figure 20.** Schematic Millipore™ extraction system used for the Empore™ Extraction Disk.

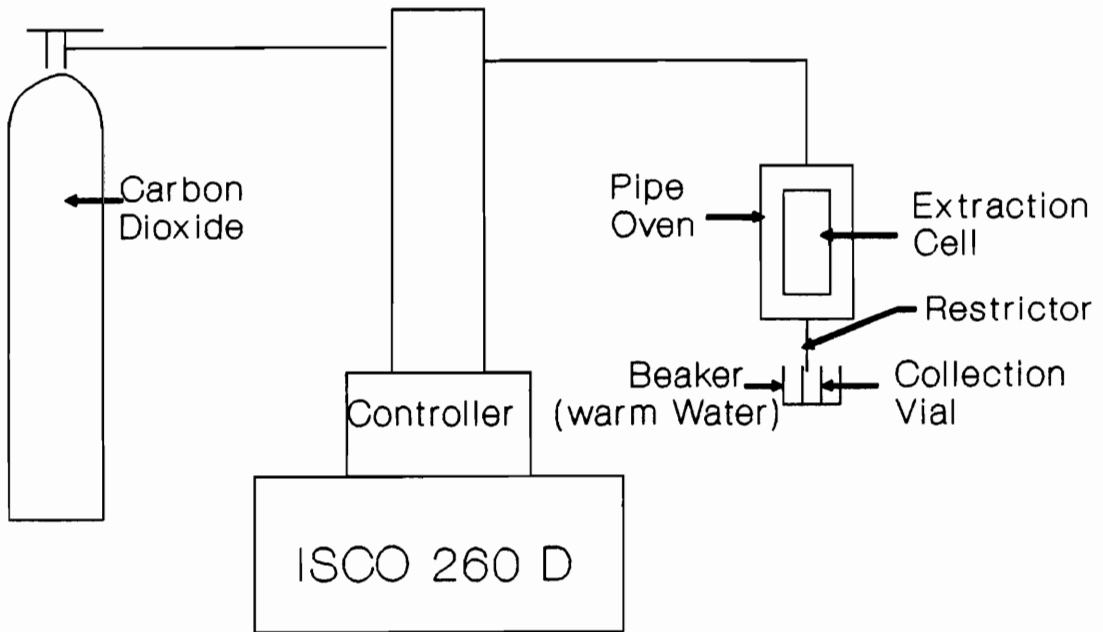


for no less than 15 minutes. A 3.5 mL Keystone Scientific extraction vessel was also used for the SFE of the Empore™ extraction disks. In this case the disk was rolled up after the SPE procedure and placed inside the extraction vessel and extracted by supercritical fluid extraction.

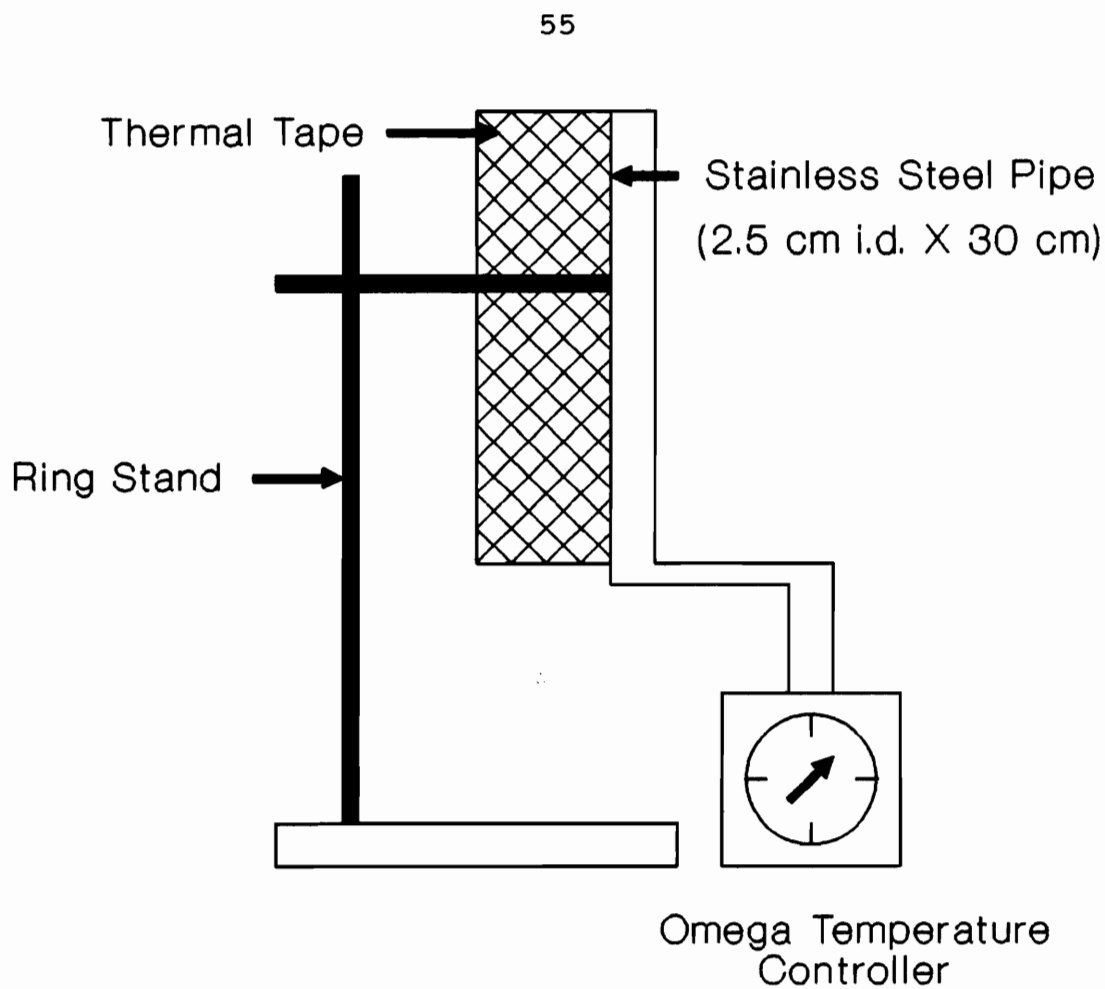
#### SUPERCRITICAL FLUID EXTRACTION

All the supercritical fluid extractions were performed using an Isco Model 260D syringe pump, Isco, Inc. (Lincoln, NE) using either SFC or SFE grade carbon dioxide, from Scott Specialty Gases (Plumsteadville, PA) or Air Products and Chemicals Incorporated (Allentown, PA) (Fig. 21). The extraction vessel, Keystone Scientific, Inc. (Bellefonte, PA), which was described above, was maintained at constant temperature by placing the cell inside a metal pipe wrapped with thermal tape, Omega Engineering Inc. (Stanford, CT). The temperature of the thermal tape was controlled by an Omega temperature controller, Omega Engineering Inc. (Stanford, CT). (Fig. 22) [43].

SFE conditions were identical for off-line and on-line extractions, except for the extraction pressure which was 400 atm and 350 atm, respectively. The temperature was held constant at 75°C, with the SFE lasting for a maximum of 15 minutes (approximately 15 mL of liquid carbon dioxide). There was a five minute thermal equilibration period prior



**Figure 21.** Schematic of Isco Model 260D syringe pump and home built supercritical fluid extraction instrumentations.



**Figure 22.** Schematic of supercritical fluid extraction pipe oven.

to the introduction of carbon dioxide for each extraction to insure supercritical conditions at the start of the extraction. Pressure was maintained with a fused silica linear restrictor, Polymicro Technologies Incorporated (Phoenix, AZ). Restrictors were 10 to 15 cm in length with an internal diameter of 30  $\mu\text{m}$ . The average carbon dioxide liquid flow rate was 1 mL/min. A toluene (3 mL - 4 mL) solvent trap was used for all off-line sample collection. The solvent was evaporated down to one or two milliliters then the internal standard (1,3-dinitrotoluene or 1,3,5-trichlorobenzene) was added and the samples were analyzed by GC-ECD.

#### ANALYSIS

A Hewlett Packard (Avondale, PA) 5890 gas chromatograph was used to analyze the SPE/SFE extracts for both off-line and on-line analysis. The GC was equipped with an electron capture detector (ECD), flame ionization detector (FID) and split/splitless injector. Instrument accessories included a 3396 integrator, manual septumless injector (SLIM), and a 7673A automatic injector.

The GC was not equipped with cryofocusing, therefore, the oven was cooled to below ambient temperature by one of the following methods, depending on the column temperature needed: 1) For a column temperature of 25 °C, the oven was

filled with two 600 mL beakers of ice. This procedure allowed the oven temperature to be set to 25 °C and to cool itself; 2) For a column temperature of 0 °C, the first 20 centimeters of the capillary column were coiled through 300 mL of a salt water ice bath in a 600 mL beaker; or 3) For sub-zero temperatures, the column was coiled through 300 mL of an acetone dry-ice bath that was also prepared in a 600 mL beaker. The beaker was insulated with plastic foam to reduce the amount of heat loss. By monitoring the temperature with an electronic thermocouple and periodically adding the appropriate amount of dry-ice the temperature was maintained to within one degree of the target temperature.

Grade 5.0 Helium and Nitrogen, Airco (Murray Hill, NJ) were used as the carrier and ECD make-up gases respectively. In addition, a Supelco (Bellefonte, PA) Heated Gas Purifier was placed in line with both the carrier and make-up gases, to insure their purity.

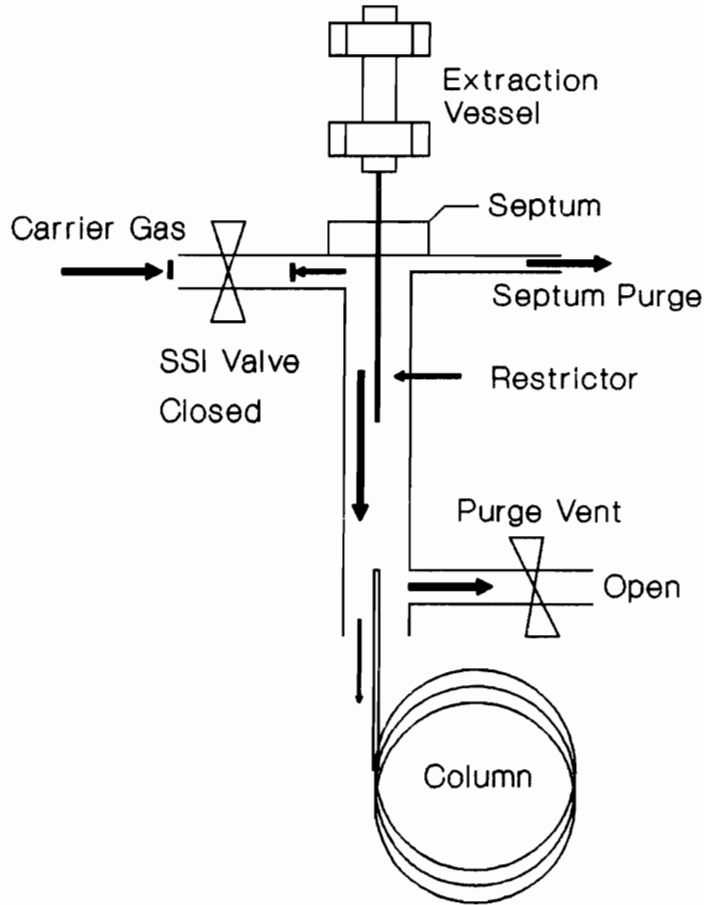
The capillary GC columns used for both off and on-line analysis were either Hewlett Packard - Ultra 1 (25 m, 320  $\mu\text{m}$  internal diameter., 0.17  $\mu\text{m}$  film thickness) or J & W Scientific - 122-5011 (15 m, 250  $\mu\text{m}$  internal diameter, 0.1  $\mu\text{m}$  film thickness). The instrument conditions are given with each chromatogram presented in the results and discussion section.

## ON-LINE ANALYSIS

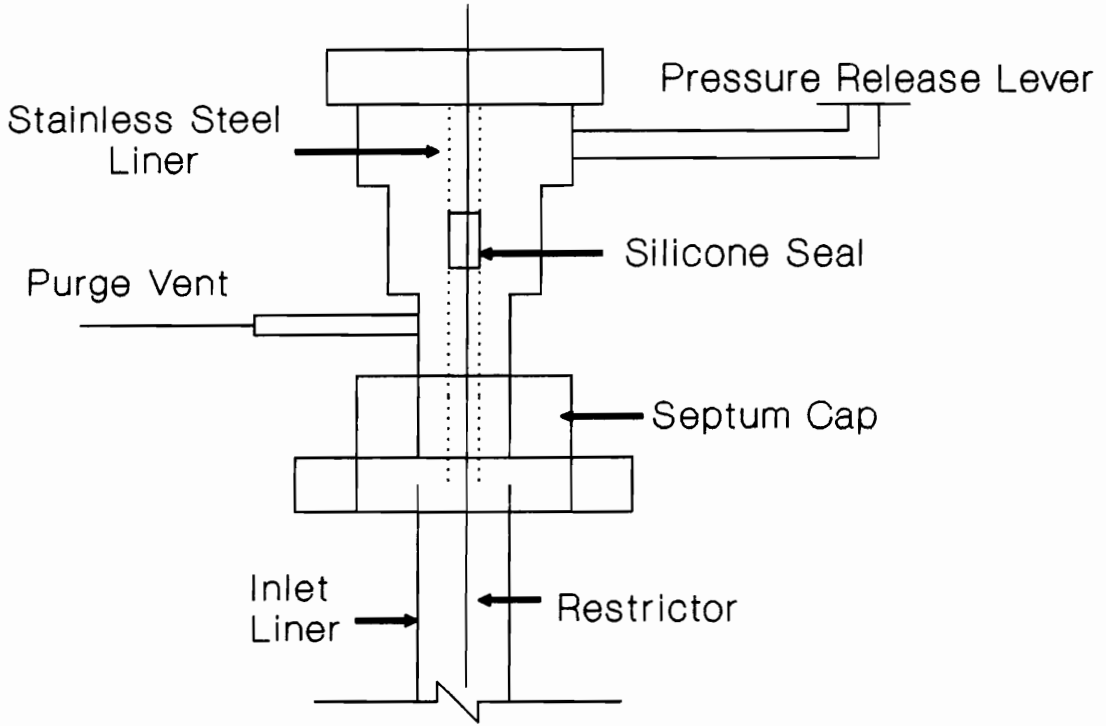
## INTERFACE

To directly couple SFE to Capillary GC the extraction vessel was mounted directly over the Hewlett Packard split/splitless injection port (Fig. 23). This can be accomplished by using the pipe oven described in the section entitled Experimental; Supercritical Fluid Extraction. Consequently, the fused silica linear restrictor (15 cm, 30  $\mu\text{m}$  i.d.) can be inserted directly into the injection port. The restrictor was inserted to exactly the same position for all on-line supercritical fluid extractions by measuring the length to be inserted and marking the restrictor with "Liquid Paper" (whiteout). To allow the the restrictor to be easily inserted and removed from the GC injector, the injection port was fitted with a manual septumless injector (SLIM), Scientific Glass Engineering (Austin, Texas) (Fig. 24). The expanding fluid will exit out of any available orifice. In order to prevent back flush into the carrier gas supply line a two way SSI valve was placed in the carrier gas inlet line (Fig. 23).

Supercritical fluid extraction was then performed exactly as it was for off-line SFE without modifier added to the extraction vessel. However, the eluent exits the linear restrictor directly into the inlet liner of the injection port. The carbon dioxide and analytes are then split



**Figure 23.** Schematic of split injection port during SFE and analyte trapping.



**Figure 24.** Schematic of manual septumless injector (SLIM), Scientific Glass Engineering (Austin, Texas).



onto the capillary GC column or swept out of the purge vent. Due to the large amount of carbon dioxide gas generated during the supercritical fluid extraction, the purge vent is opened as far as possible to prevent pressure build up in the injection port. The split ratio or amount of sample that is introduced to the capillary column can be regulated by controlling the column head pressure.

After the SFE extraction was completed, the carrier gas valve was opened and the carbon dioxide was swept out of the capillary column in about 30 seconds. The ECD background signal on the chromatograph dropped from about 45 mV down to approximately 25 mV when the column is purged of carbon dioxide. The temperature bath, or beakers of ice, were then removed from the oven. Each GC run had a 3 min hold time at 33°C, to allow any water, volatile contaminants, or remaining carbon dioxide to be removed from the column. A ternary temperature gradient was then employed to resolve the components and remove the late eluting contaminants. The exact temperature program is provided with each chromatogram illustrated.

#### PREPARATION OF STANDARDS

The individual stock and diluted standards were prepared in methanol. The 2,6-DNT, 2,4-DNT and TNT stock solutions were prepared in 100 or 250 volumetric flasks

having a concentration of 40 ng/ $\mu$ L. The mixed standards concentration used to prepare controls in toluene and to spike water samples were typically 13.3 ng/ $\mu$ L of 2,6-DNT, 2,4-DNT, and TNT and were prepared in 4 of 7.7 mL vials using a 1000  $\mu$ L Eppendorf™ pipet. Controls and standards were prepared using a 50  $\mu$ L or 100  $\mu$ L Hamilton Syringe, Microliter™ (Reno, Nev). In every analysis the same syringe was used to prepare the control and water sample to reduce the potential for error. Internal standards (m-dinitrotoluene and 1,3,5-trichlorobenzene) were also prepared in methanol with stock concentrations of 75 ng/ $\mu$ L and 40 ng/ $\mu$ L, respectively. The internal standards were diluted to attain the appropriate response for analysis and in every case the internal standard was introduced with the same syringe from the same stock dilution. Controls were prepared fresh daily and all solutions were refrigerated when not in use.

## **CHAPTER III**

### **RESULTS AND DISCUSSION**

## RESULTS AND DISCUSSION

The purpose of this work was to investigate the use of supercritical carbon dioxide as the elution solvent for nitroaromatics trapped on SPE cartridges. Most importantly supercritical fluid extraction would allow direct on-line SPE-SFE-GC analysis using a split injection interface [43]. The results will be discussed first for the off-line analysis and then later for the on-line SPE-SFE-GC.

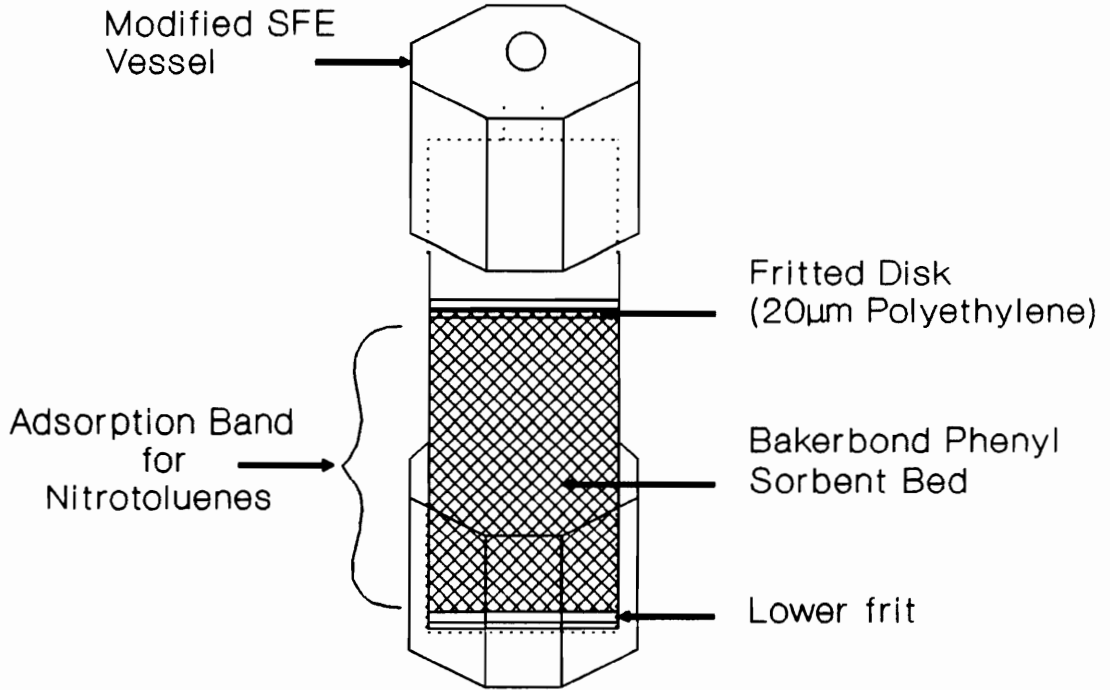
Two different off-line recoveries will be discussed, the elution of the nitroaromatics from the SPE cartridges by supercritical fluid extraction and then the trapping efficiency of the Bakerbond™ phenyl solid sorbent for nitroaromatics in organic free, surface and well water. The eluted nitroaromatics were trapped from the carbon dioxide in toluene and off-line analysis was performed via GC-ECD.

Supercritical carbon dioxide was shown to be a suitable elution solvent for semi-polar analytes from an SPE bonded silica sorbent. On-line SPE-SFE-GC-ECD analysis was thoroughly investigated to determine if the technique is quantitative and reproducible.

OFF-LINE SOLID PHASE EXTRACTION - SUPERCRITICAL FLUID  
EXTRACTION

Three types of solid sorbents were thoroughly investigated: Bakerbond™ Phenyl stationary phase and two 47 mm Empore™ extraction disks - octadecylsilane (C18) and styrene-divinylbenzene (SDVB).

The volume of carbon dioxide and time required to completely elute the analytes from the Bakerbond™ Phenyl sorbent by SFE were determined. A 500 mL aliquot of Millipore™ water was spiked with 1 $\mu$ g each of 2,6-DNT, 2,4-DNT, and TNT. Initial SFE conditions were determined using 500 mL of water. The analyte will breakthrough the sorbent bed with this volume of water, and the adsorption band should spread over the entire length of the sorbent bed and the analytes will have maximum time to diffuse into the pores of the phenyl solid sorbent (Fig. 25). Diffusion of the analyte from within the pores of the stationary phase will be the limiting factor in the time required to complete the supercritical fluid extraction [1]. Initial SFE conditions were set at 400 atm and 40 °C, with a 25  $\mu$ m x 15 cm linear restrictor. The first attempts to extract the analyte resulted in an unacceptably high number of plugged restrictors, over fifty percent. This was a result of the residual water left on the Bakerbond™ phenyl support. Even though the solid sorbent was dried under

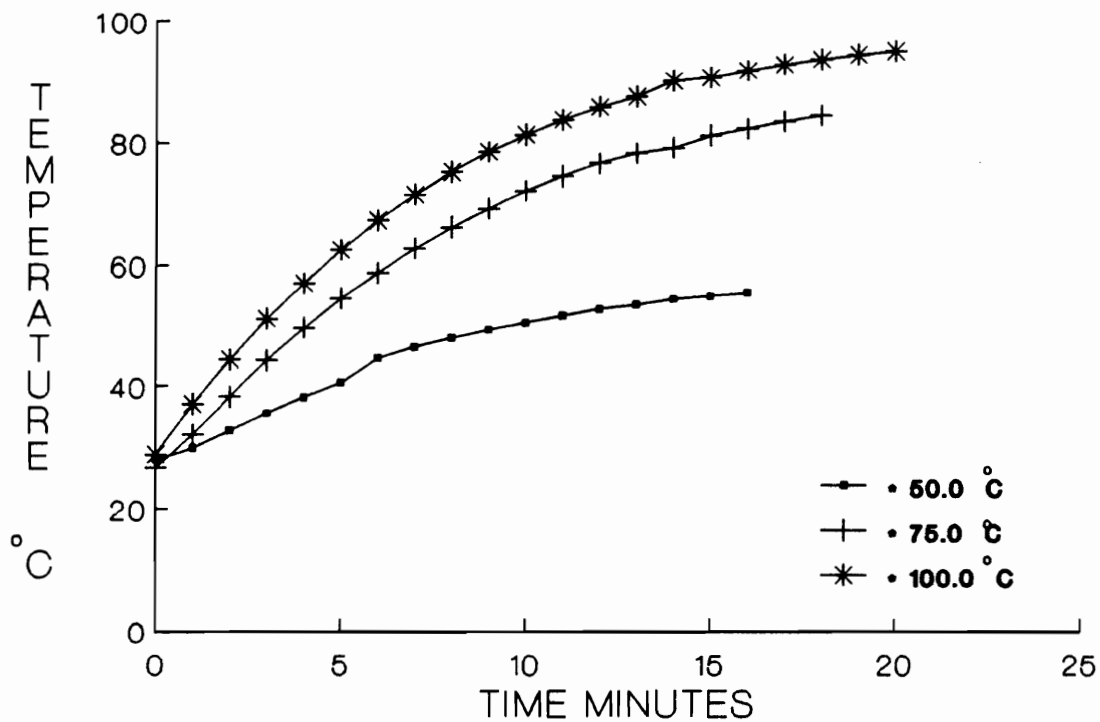


**Figure 25.** Schematic of modified SFE vessel showing the adsorption band for nitrotoluenes on Bakerbond™ phenyl sorbent, from 500 mL organic free water.

vacuum for a minimum of 15 minutes, residual water was still present on the unbound silanol sites of the Bakerbond™ phenyl solid support. The supercritical carbon dioxide physically pushed the water into the restrictor forming a plug.

To prevent a water plug from forming, several precautions were taken. First, the internal diameter of the restrictor was increased from 25  $\mu\text{m}$  to 30  $\mu\text{m}$ , thus increasing the amount of water necessary to plug the restrictor. This change reduced the number of plugs but there were still too many to make the technique feasible. Second, the extraction temperature was raised from 40°C to 75°C to increase the solubility of water in supercritical carbon dioxide [87].

To insure that the temperature throughout the extraction vessel was above the critical temperature, the heating rate of the extraction vessel was determined for three different thermal controller temperature settings - 50°C, 75°C, and 100°C. A thermocouple was placed in the center of a 3.5 mL Keystone Scientific supercritical fluid extraction vessel that was filled with moist Bakerbond™ phenyl stationary phase. From the plot of temperature versus time shown in Figure 26 an initial equilibration time of five minutes was chosen with the temperature controller set at 75°C. In addition to the preheating time period,



**Figure 26.** Rate of heating for a 3.6 mL Keystone Scientific Extraction vessel filled with damp Bakerbond™ phenyl sorbent. \* Temperature setting of thermal controller.



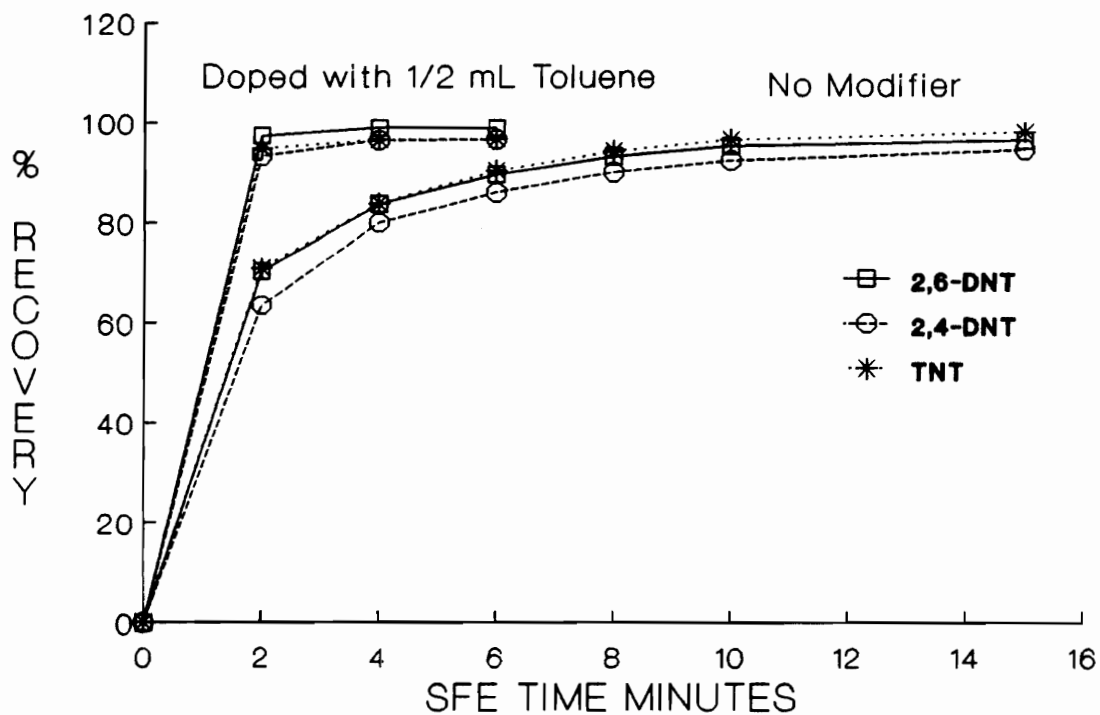
a 1 meter preheat coil was placed before the extraction vessel and inside the pipe oven. This was done to insure that the carbon dioxide was at the target temperature when it entered the extraction vessel. The thermal equilibration period drastically reduced the amount of plugging to less than one in ten. Third, the collection vessel and restrictor were preheated just prior to and during SFE. The restrictor can be heated by a Heat Gun, Master Appliance Corp. (Racine, Wis). Heating the restrictor, which is typically at room temperature, allows supercritical conditions to be maintained and premature precipitation of the analyte and desolvation of the water is prevented.

Due to the Joule - Thomson effect, the carbon dioxide gas cools as it exits the restrictor and rapidly expands. Consequently, solute crystals or ice may form at the end of the restrictor causing the orifice to plug. The collection vessel can be heated by a thermal block controlled by a thermistor or by placing it in a beaker of warm water. Doing this heats the solvent which in turn heats the end of the restrictor and prevents it from plugging.

After the restrictor plugging problems were eliminated, practical extraction conditions were determined. This was accomplished by performing kinetic studies to determine the time period required for a quantitative SFE of the nitrotoluenes, which were adsorbed from water, from the

Bakerbond™ phenyl sorbent. The rate of analyte elution was investigated, with and without a chemical modifier added directly to the extraction cell before SFE. The plot of extraction rate in Figure 27 for the unmodified carbon dioxide follows a standard exponential decrease as predicted by the general extraction curve [1]. The analyte exhibits classical Phase I quasi-equilibrium conditions for the first 2 min of the extraction. Then, the curve starts to become concave with respect to time as the factors controlling the extraction rate change from equilibrium to the diffusion rate, mobility of the analyte within the sample matrix and its desorption rate from the surface of the matrix. The extraction is complete in less than 15 minutes.

The plot in Figure 27 for the toluene modified extraction indicates much faster extraction kinetics. The toluene first solvates the nitrotoluenes then both the toluene and analyte are extracted from the cell by the supercritical carbon dioxide. This is indicated by the rapid extraction of the analytes from the Bakerbond™ phenyl solid sorbent and relatively small transition and diffusion-controlled phases. Hence, the recovery data plotted in Figure 27 indicate that the addition of 500  $\mu$ L of toluene directly to the extraction vessel before extraction increases the rate of extraction. However, there is no



**Figure 27.** Supercritical fluid extraction kinetics for 1  $\mu\text{g}$  of each nitrotoluenes from 1 g of Bakerbond<sup>TM</sup> phenyl sorbent. After adsorption from 500 mL organic free water.

statistical difference between the final recoveries observed with and without addition of toluene to the SFE extraction cell.

The total SFE recovery in Figure 27 is based on experiments where 50 mL of water was spiked with 1  $\mu$ g each of 2,6-DNT, 2,4-DNT and TNT then isolated on the Bakerbond™ phenyl SPE sorbent and extracted using the same SFE conditions as for the kinetic studies. However, the analytes were collected in one vial for the entire extraction and the total recoveries were essentially 100 % for both the non-modified and modified extraction (Table IV and V). Under these conditions (discussed below) there is no analyte breakthrough on the Bakerbond™ phenyl solid sorbent during the isolation of the nitroaromatics from the water sample. Therefore, the true quantitative SFE recovery of the analyte from the solid sorbent is known. A modifier was added to the extraction cell in all subsequent off-line SFE extractions because it was simple, it added no additional time to the procedure, and it improved the rate of extraction.

TABLE IV

NON-MODIFIED SPE-SFE PERCENT RECOVERY FOR NITROTOLUENES (1  $\mu\text{g}$ /each) FROM 50 mL OF ORGANIC FREE WATER. NO MODIFIER WAS ADDED TO THE EXTRACTION VESSEL.

Analyte	Percent Recovery	% RSD
2,6-Dinitrotoluene	96	2.5
2,4-Dinitrotoluene	95	3
Trinitrotoluene	98	4

n = 7, %RSD = Percent Relative Standard Deviation

TABLE V

MODIFIED SPE-SFE PERCENT RECOVERY FOR NITROTOLUENES (1  $\mu\text{g}$ ) FROM 50 ML OF ORGANIC FREE WATER. TOLUENE (0.5 mL) MODIFIER ADDED TO THE EXTRACTION VESSEL.

Analyte	Percent Recovery	% RSD
2,6-Dinitrotoluene	99	2
2,4-Dinitrotoluene	97	1.5
Trinitrotoluene	97	3

n = 6, %RSD = Percent Relative Standard Deviation

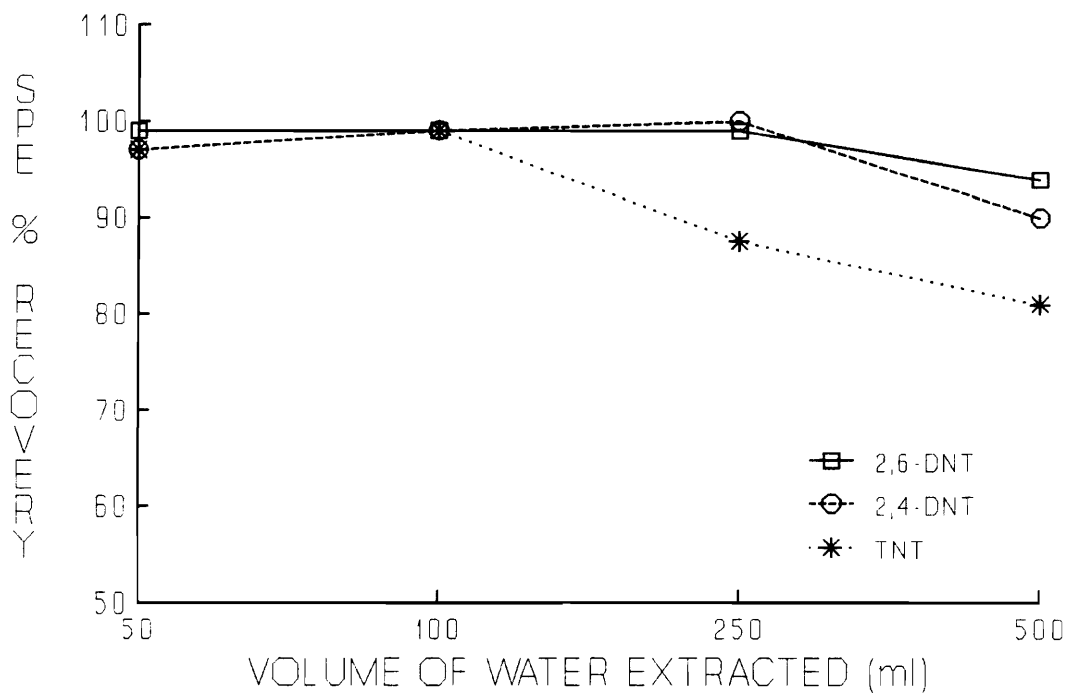
**BAKERBOND™ PHENYL BREAKTHROUGH VOLUMES**

To use SPE for the preconcentration of semi-polar analytes from water, the volume of water required to carry the analyte through the solid sorbent needs to be determined. This volume of water is termed the breakthrough volume.

The SPE breakthrough volume for each analyte on the Bakerbond™ phenyl sorbent was found by calculating the percent recovery of the analytes from four different volumes of organic free water. Each volume of water was spiked with 1 µg of 2,6- DNT, 2,4-DNT and TNT; then passed through the phenyl sorbent; then extracted by SFE; and finally analyzed off-line by GC-ECD. The SPE recovery data plotted in Fig. 28 indicates that the compounds begin to show significant breakthrough between 250 and 500 mL of water. However, all three analytes continue to show acceptable recoveries from 500 mL of water.

**EMPORE EXTRACTION DISKS**

In addition to the Bakerbond™ phenyl sorbent, octadecylsilane (C18) and styrene-divinylbenzene (SDVB) Empore™ disks were also investigated. Empore™ extraction disks are composed of an adsorbent particle entrapped in a PTFE matrix, in a ratio of 9 to 1, respectively. Empore™ disks were investigated for their speed of analysis and not



**Figure 28.** SPE-SFE recovery as a function of volume of water for 1  $\mu\text{g}$  of 2,6-dinitrotoluene, (2,6-DNT), 2,4-dinitrotoluene, (2,4-DNT) and Trinitrotoluene (TNT) on 1 g Bakerbond™ phenyl solid sorbent. Values below 90 % indicate breakthrough volumes.



their selectivity, which will be the same for Empore™ disks and cartridges packed with the same solid sorbents. The geometry of the extraction disks (47 mm X 0.5 mm) allows rapid flow of the water sample through the disks, due to the extremely low back pressure (1-2 mL/min/cm<sup>2</sup> - deionized water at 25 °C). Since the Empore™ disk is 90 percent spherical 8 μm diameter particles, there is no channeling of the water flow through the disks (Fig. 29). The disks are also cleaner than traditional irregularly shaped solid sorbents, compatible with all organic solvents and stable from a pH of 2 to 7. The pH range may be extended for short exposure time. The burst strength (supported) is 100 psi.

So that a comparison between the Bakerbond™ phenyl sorbent and the Empore™ extraction disks could be made, the nitrotoluenes (1 μg) were adsorbed from 500 mL of organic free water for both disks. When low recoveries were obtained, the nitrotoluenes (1 μg each of 2,6-DNT, 2,4-DNT, and TNT) were extracted from 10 mL of organic free water to determine whether the low recoveries were from analyte breakthrough on the extraction disks or from low SFE. A small volume of water, 10 mL, was spiked with 1 ug of each nitroaromatic and passed through the disk, since spiking an analyte evenly across a 47 mm by 0.5 mm disk is very difficult. The SPE-SFE recovery for the isolation of 1 μg of the analytes from the C18 and Empore™ extraction disks

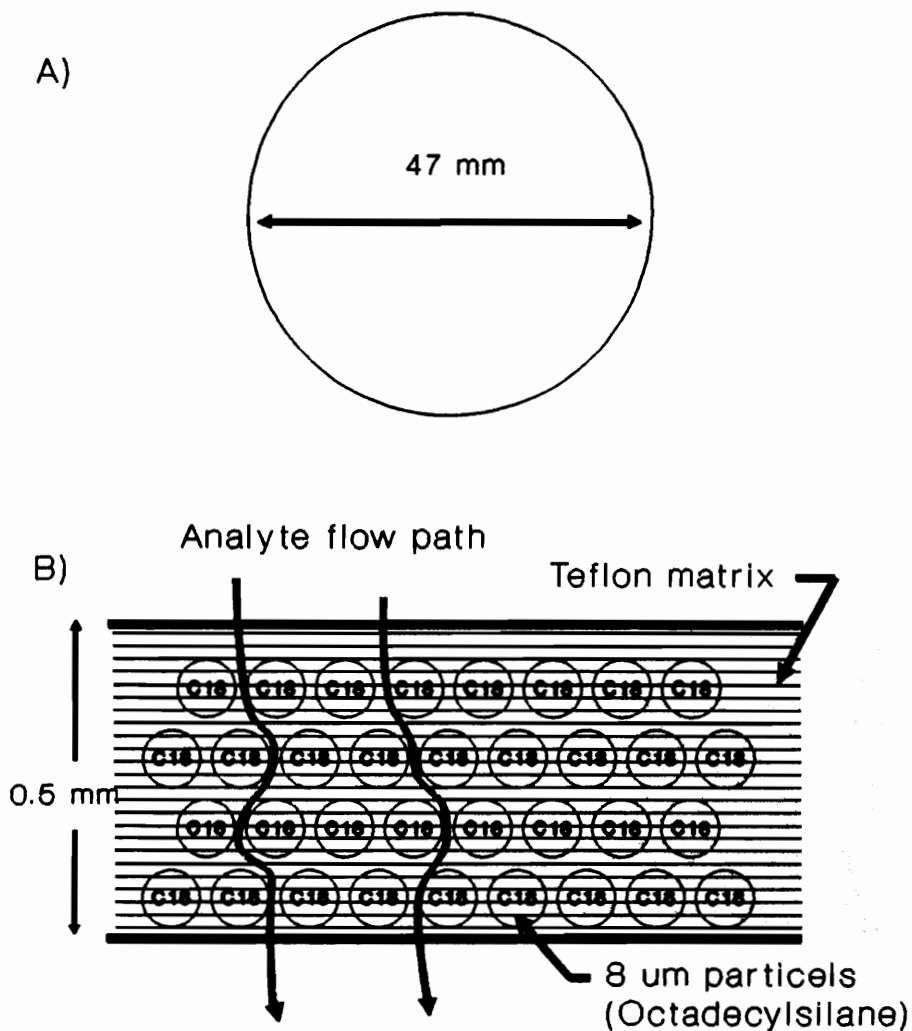


Figure 29. A) Diameter of the Empore™ extraction disks, B) Cross-sectional view of the extraction disks illustrating the analyte flow path; analytes must come in contact with sorbent material before exiting the disk, i.e. no channeling.

for two volumes (10 and 500 mL) of water are listed in Table VI and VII.

The SFE conditions for the Empore™ disks were identical to those used for traditional SPE solid sorbents. However, there was a difference in the extraction procedure, because the geometry of the Empore™ extraction disks does not allow the disk to be placed in the extraction vessel during the SPE step. Thus, after the compounds were adsorbed from the water onto the Empore™ extraction disks, the disk was rolled up with a pair of tweezers and placed inside a 3.5 mL Keystone Scientific extraction vessel and extracted for 15 minutes using SFE. In addition, to minimize handling of the extraction disk, there was no preextraction with supercritical fluid carbon dioxide.

Each Empore™ extraction disk was extracted a second time by SFE or placed in a 7.7 mL vial with three mL of toluene and shaken overnight. Both of the second extractions produced from 1 to 5 percent additional analyte. This additional recovery is included in the total SPE-SFE recoveries reported in Table VI and VII. This additional extraction was necessary because the rolled up disk, had a channel lengthwise down the center of the extraction vessel. This geometry is not ideal because it requires the analyte to diffuse from the disk into the flow of the carbon dioxide stream.

TABLE VI

## RECOVERY FOR EMPORE™ C18 EXTRACTION DISKS

RECOVERY INCLUDES SPE-SFE AND AN ADDITIONAL SFE OR LIQUID EXTRACTION OF THE EMPORE™ DISKS

Percent recovery for 1 ug spikes of 2,6-dinitrobenzene, 2,4-dinitrotoluene, and trinitrotoluene from Millipore™ water.  
± = Standard Deviation, n = 3.

Analyte	Volume of Water	
	10 mL	500 mL
2,6-Dinitrotoluene	91.5 ± 5	73 ± 3
2,4-Dinitrotoluene	91 ± 5	74 ± 1
Trinitrotoluene	85 ± 7	49 ± 6

TABLE VII

## RECOVERY FOR EMPORE™ SDVB EXTRACTION DISKS

RECOVERY INCLUDES SPE-SFE AND AN ADDITIONAL SFE OR LIQUID EXTRACTION OF THE EMPORE™ DISKS

Percent recovery for 1 ug spikes of 2,6-dinitrobenzene, 2,4-dinitrotoluene, and trinitrotoluene from Millipore™ water.  
± = Standard Deviation, n = 3.

Analyte	Volume of Water	
	10 mL	500 mL
2,6-Dinitrotoluene	92 ± 3	91 ± 4
2,4-Dinitrotoluene	95 ± 6	86 ± 3
Trinitrotoluene	62.5 ± 8	48 ± 4

The SDVB disk showed acceptable SPE-SFE recovery for the dinitro compounds  $91 \pm 4 \%$  for 2,6-dinitrotoluene and  $86 \pm 3 \%$  for 2,4-dinitrotoluene. However, recovery of TNT was poor  $48 \pm 4 \%$  and showed rapid breakthrough. Also, all three of the analytes showed poor recovery from 500 mL of water for the C18 Empore™ disks. For this reason no additional studies were performed on these adsorbents. The results for the SDVB disks are in agreement with those previously reported for the recovery of nitrophenols from water using the SDVB extraction disks [59].

#### SURFACE AND WELL WATER

To test this method in a more realistic environment where additional organic compounds are present, 500 mL of two surface waters, the Red River (I and II) and the English Coulee, and one well water (Grand Forks County, ND) sample were spiked with  $1 \mu\text{g}$  each of 2,6-DNT, 2,4-DNT, and TNT. The spiked water samples were then extracted by SPE-SFE using the Bakerbond™ phenyl sorbent. The additional organic compounds can affect the total analyte recovery for SPE in several ways. First, the additional organic components will compete with the analytes for adsorption sites on the phenyl stationary phase. This might overload the sorbent and cause the analytes to breakthrough early from the sorbent bed. Second, the organic compounds can associate with target

compounds forming a complex. The complex can then irreversibly adsorb to the sorbent, or pass through the sorbent bed due to a low affinity of the complex for the sorbent [42]. The total recoveries for the SPE-SFE of 2,6-DNT, 2,4-DNT, and TNT from two surface and one well water samples ranged from 54 % to 92 % (details in Table VIII). The two Red River water samples I and II are from the same river and collected in the same location. Due to the high recoveries from the first set of samples (Red River I) which was unexpected due to the presence of additional dissolved organics or particulate substances, a second set of samples was run (Red River II). Figure 30 illustrates a typical GC-ECD chromatogram for this analysis. The low total recoveries for the SPE-SFE of the nitroaromatics from Red River II, English Coulee and well water indicate that the additional organic compounds overloaded the solid sorbent and caused the analytes to breakthrough the Bakerbond™ phenyl sorbent.

To confirm that the low recoveries were due to overload of the sorbent and not due to poor SFE, two of the phenyl extraction cells were placed in series during the SPE preconcentration step. When the second cell was extracted by SFE it was found to contain 30 percent of the analytes, confirming breakthrough. To confirm the presence of additional organic compounds, the extract from the first

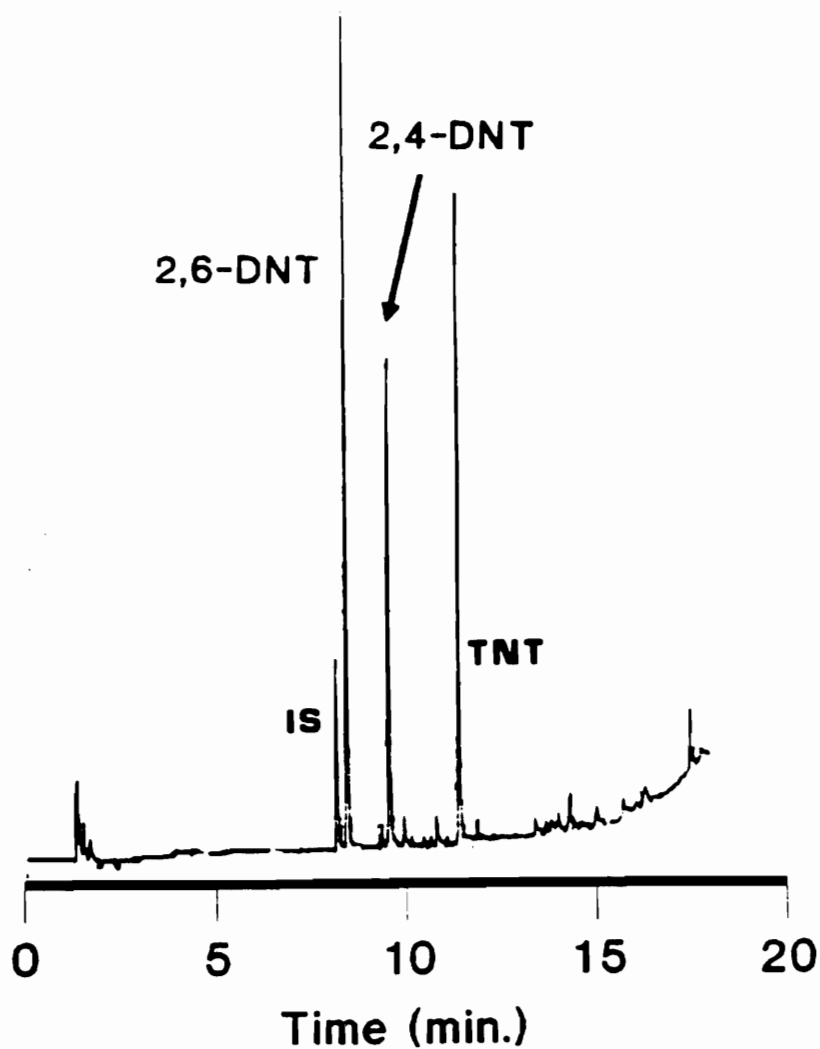
TABLE VIII

## SPE-SFE TOTAL RECOVERY FROM SURFACE AND WELL WATER

Percent recovery for 1  $\mu\text{g}$  spike of 2,6-dinitrotoluene, (2,6-DNT), 2,4-dinitrotoluene, (2,4-DNT) and trinitrotoluene (TNT) from 500 mL of water; sorbent Bakerbond™ phenyl. n = 3,  $\pm$  = Standard Deviation.

Water Source	Analyte		
	2,6-DNT	2,4-DNT	TNT
Millipore™	94 $\pm$ 0.5	90 $\pm$ 2	81 $\pm$ 1
Red River I	92 $\pm$ 6	87 $\pm$ 11	87 $\pm$ 6
Red River II	58 $\pm$ 10	52 $\pm$ 9	57 $\pm$ 12
English Coulee	75 $\pm$ 14	69 $\pm$ 12	69 $\pm$ 11
Well Water (Grand Forks, ND)	62 $\pm$ 10	54 $\pm$ 8	63 $\pm$ 10





**Figure 30.** Spiked, Red River water ECD chromatogram of SPE-SFE off line GC-ECD Split Ratio 50:1; Column: HP Ultra-1 (25 m x .32 mm x .17  $\mu$ m Film) Carrier Gas: Helium 34 cm/sec; Oven 85°C for 1 min. 7.5°C/min. to 140, then 20°C/min. to 250°C.

vessel was analyzed via GC-FID (Fig. 31) and several unknown organic compounds were found. Low recoveries of analytes due to dissolved organic compounds have been previously reported [88].

#### TRACE RECOVERIES

To meet the EPA guidelines (Table IX) [12], analysis of the three nitroaromatic compounds was run at ppt levels in water. Table X lists the concentrations and total SPE-SFE percent recovery of the analytes from 500 mL of spiked Millipore™ and Red River water samples. The percent recoveries (95 % 2,6-DNT, and 86 % TNT for Millipore™ and 83 % 2,6-DNT and 66 % TNT for Red River) are in agreement with those at the ppb level (Table VI). The 2,4-DNT recoveries are low or nonexistent due to a coeluting peak (Fig. 32 and 33).

Due to the sensitivity of the ECD detector and low levels of nitrotoluenes, minimization of all sources of contamination was essential. The contamination due to the SFE collection solvent (toluene) and a Red River Blank are illustrated in Figure 34 and 35, respectively. Several of the contaminants detected by the ECD were from the carbon dioxide solvent or the system, or the water samples. A comparison between SFE and SFC grade carbon dioxide Scott Specialty Gasses (Plumsteadville, PA) was made and no

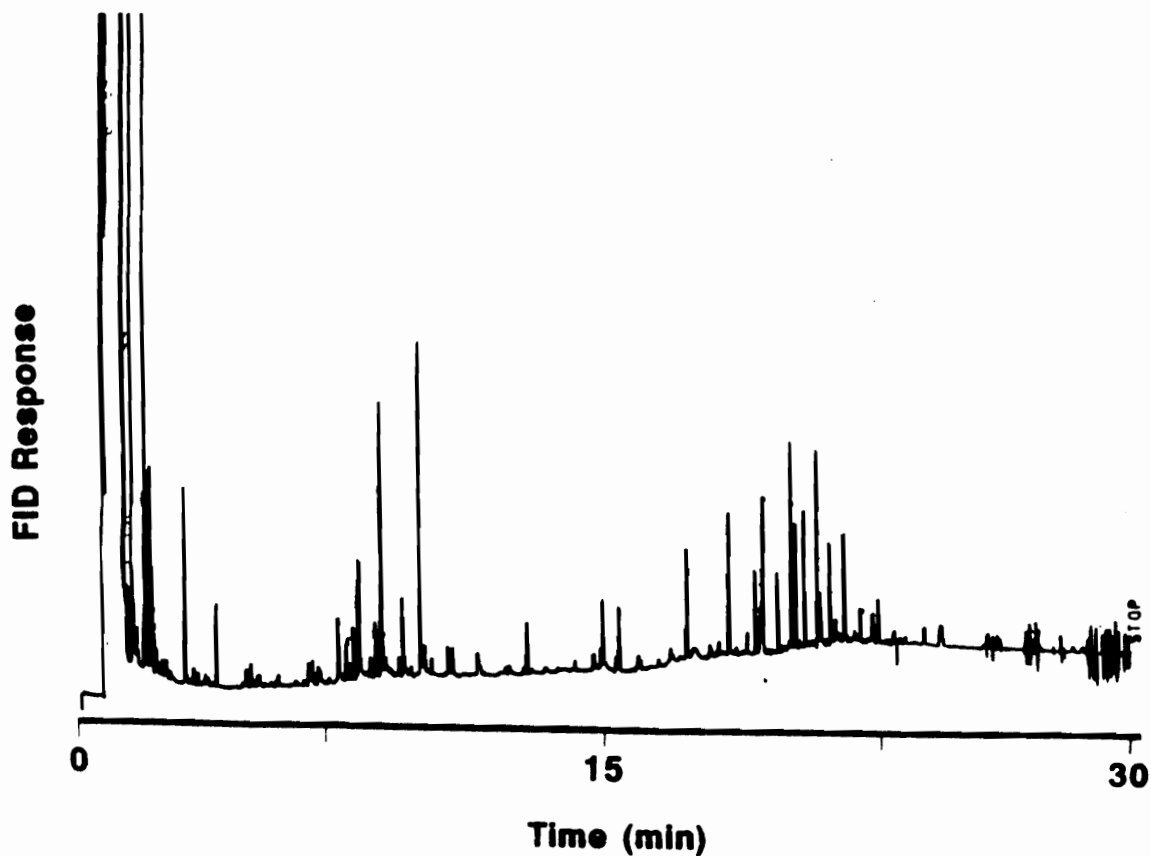


Figure 31. Spiked, Red River water FID chromatogram.  
Column: HP Ultra 5 (25 m x 0.32 mm x 0.17  $\mu$ m film). Carrier: Helium 34 cm/sec, Injection: Splitless 30 Sec, Oven 85°C for 1 min. 7.5°C/min. to 170, then 20°C/min. to 300 for 10 min.

TABLE IX

SUGGESTED DRINKING WATER LIMITS FOR NITROAROMATICS.

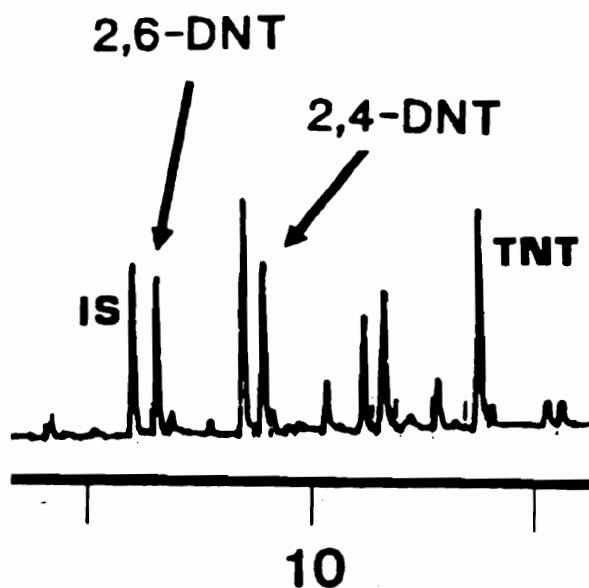
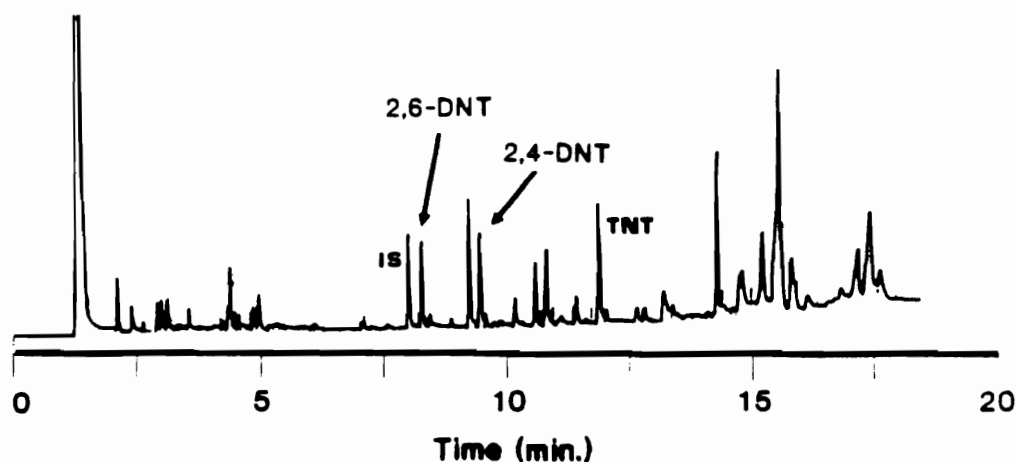
Analyte	LIMITS $\mu\text{g/L}$
2,6- Dinitrotoluene	0.007
2,4-Dinitrotoluene	0.1
Trinitrotoluene	1.0

TABLE X

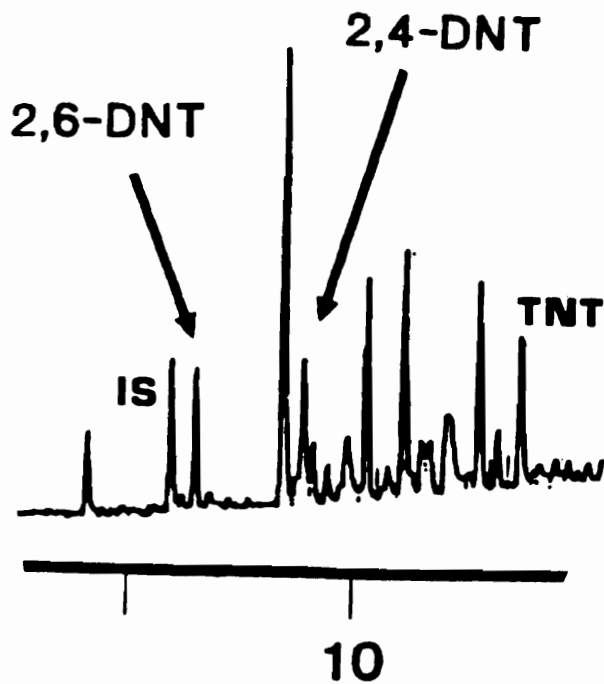
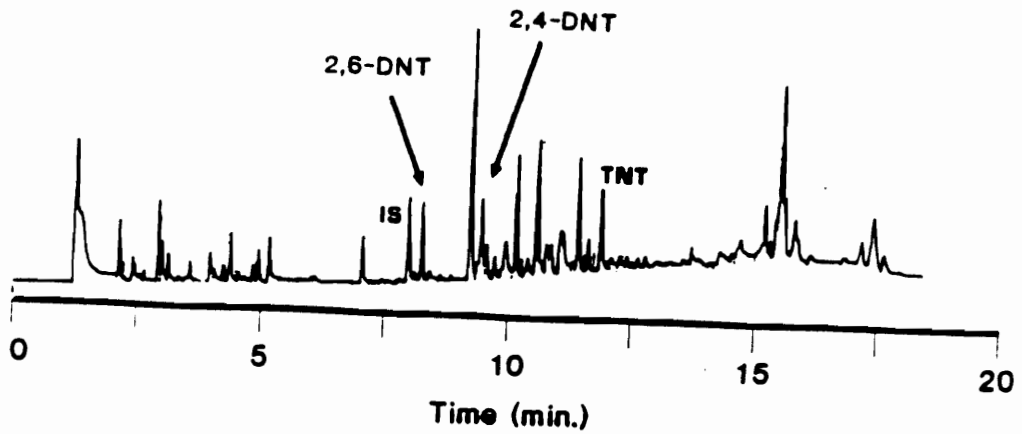
## TRACE SPE-SFE RECOVERIES

Percent recovery for spikes of 7 ppt 2,6-dinitrotoluene, 25 ppt 2,4-dinitrotoluene, and 25 ppt trinitrotoluene from 500 mL water; sorbent Bakerbond™ phenyl . RSD based on three extractions. \*\* Coeluting peak.

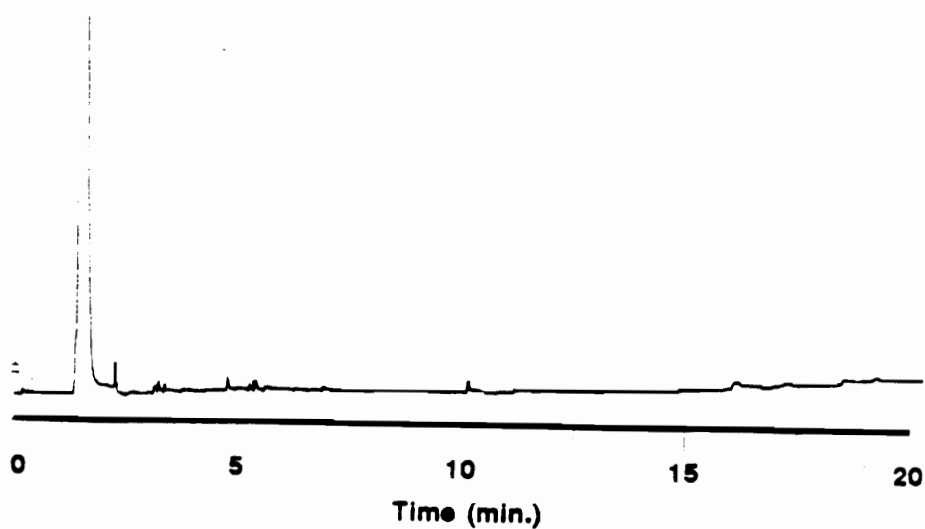
Analytes	Water Source	
	Millipore™	Red River II
2,6-Dinitrotoluene (3.5 ng)	95 ± 10	86 ± 8
2,4-Dinitrotoluene (12.5 ng)	**78 ± 9	**
Trinitrotoluene (12.5 ng)	83 ± 9	66 ± 6



**Figure 32.** Spiked, Millipore™ Water - 7ppt 2,6-Dinitrotoluene, 25 ppt 2,4-Dinitrotoluene, and 25 ppt Trinitrotoluene. The internal standard, IS, is m-Dinitrotoluene. Column: HP Ultra 1, 25m x 0.32 mm x 0.17  $\mu$ m; Carrier: Helium at 34 cm/sec.; Splitless 15 sec. purge off. Oven: 85°C for 1 min, 7.5°C/min to 170°C, the 20°C/min to 250. Detector: ECD 250°C.

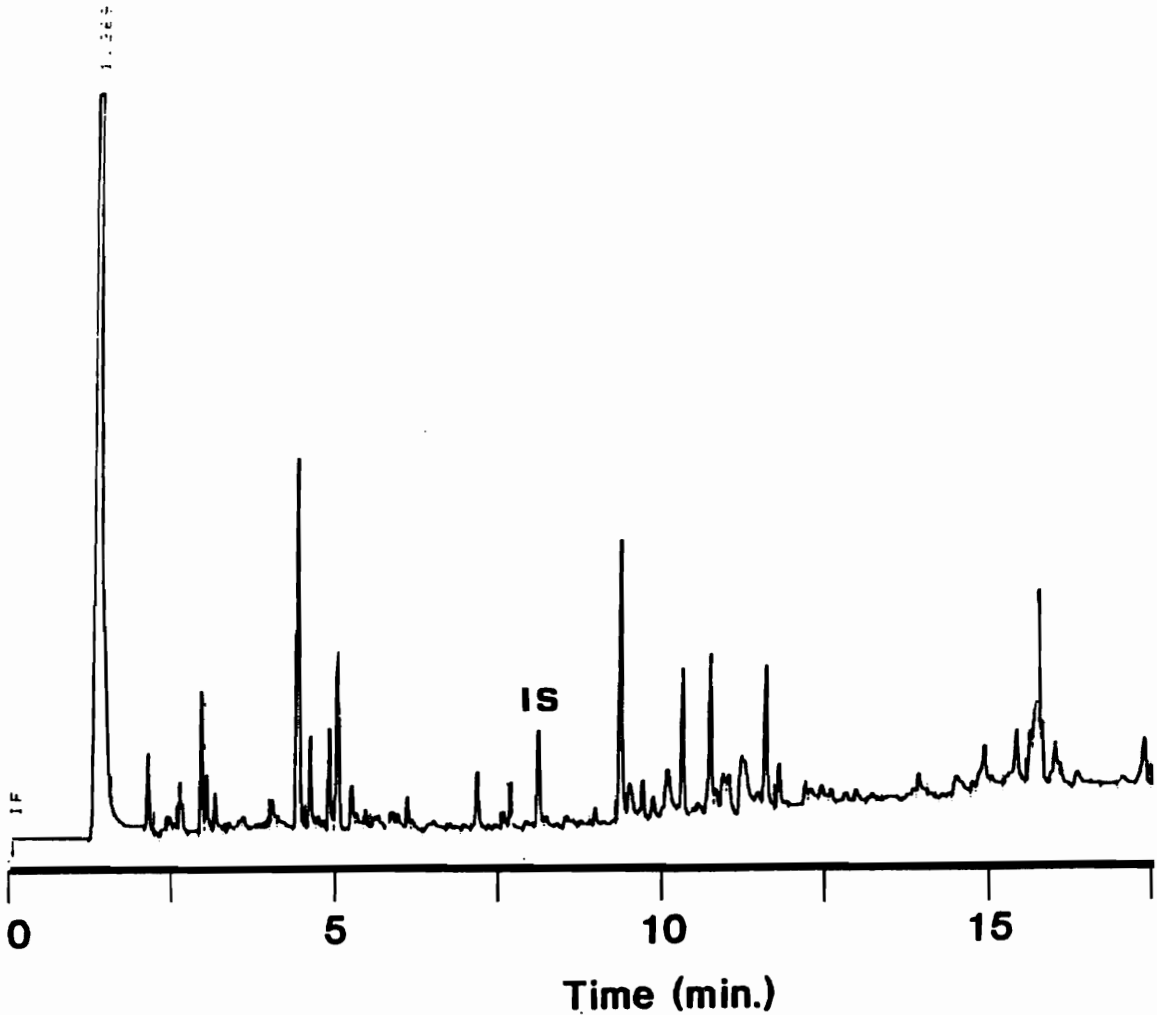


**Figure 33.** Spiked, Red River water - 7ppt 2,6-Dinitrotoluene, 25 ppt 2,4-Dinitrotoluene, and 25 ppt Trinitrotoluene. The internal standard, IS, is m-Dinitrotoluene. Column: HP Ultra 1, 25m x 0.32 mm x 0.17  $\mu$ m,; Carrier: Helium at 34 cm/sec.; Splitless 15 sec. purge off. Oven: 85°C for 1 min, 7.5°C/min to 170°C, the 20°C/min to 250. Detector: ECD 250°C.



**Figure 34.** Toluene Blank. Column: HP Ultra 1, 25m x 0.32 mm x 0.17  $\mu\text{m}$ ; Carrier: Helium at 34 cm/sec.; Splitless 15 sec. purge off. Oven: 85°C for 1 min, 7.5°C/min to 170°C, the 20°C/min to 250. Detector: ECD 250°C.





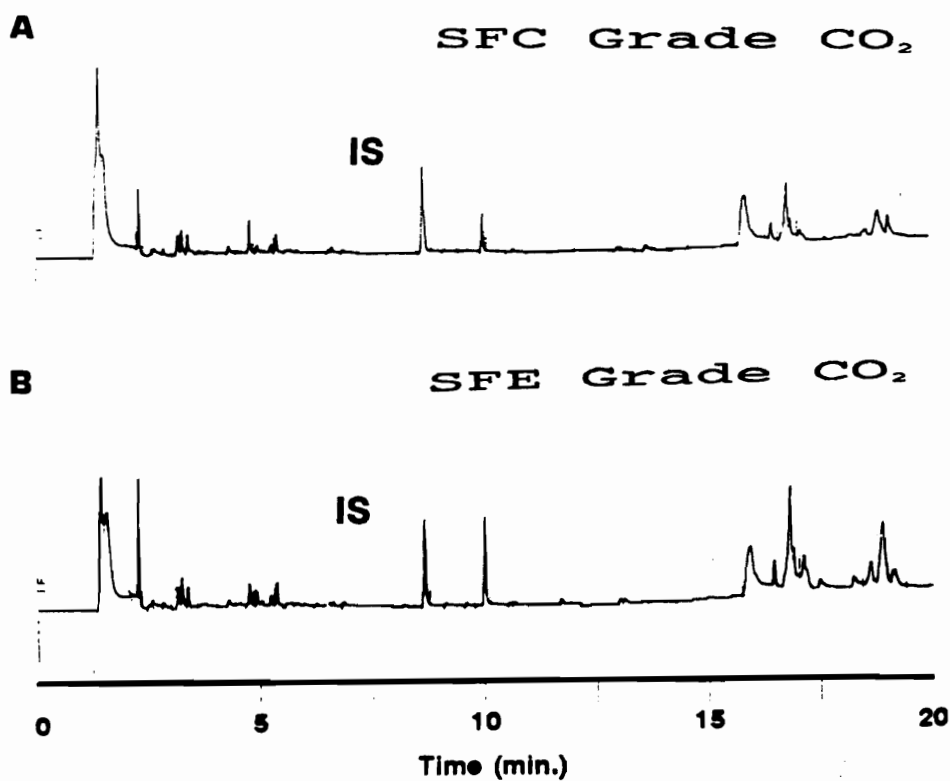
**Figure 35.** Red River Water Blank (500 ml). Collection in toluene, The internal standard (IS) is m-Dinitrotoluene 23 pg/ $\mu$ l. Column: HP Ultra 1, 25m x 0.32 mm x 0.17  $\mu$ m; Carrier: Helium at 34 cm/sec.; Splitless 15 sec. purge off. Oven: 85°C for 1 min, 7.5°C/min to 170°C, the 20°C/min to 250. Detector: ECD 250°C.

difference was observed between the two which indicates that the contaminants are probably from the contaminated extraction system and not the SFE Grade carbon dioxide (Fig. 36).

#### SELECTIVITY

One goal of sample preparation is to be able to selectively extract analytes into classes or groups based on their chemical and physical characteristics. By combination of SPE and SFE this should be possible. Solid phase extraction can be controlled by changing both the stationary phase and the extraction solvent. However, due to the solvent strengths of liquids used, it is often difficult to separate compounds soluble in a wide range of solvents. The selectivity or solvating power of supercritical fluids is controlled by changing the fluid density [33].

To demonstrate that the SPE solid sorbent can be preextracted with SF carbon dioxide at low densities to remove alkanes, and then quantitatively extracted at high densities to recover the nitroaromatics, fifty milliliters of Nanopure™ water was quantitatively spiked with 1 µg of 2,6-DNT, 2,4-DNT, and TNT and qualitatively spiked with a series of alkanes - (hexane through octadecane). The water sample was then passed through 1 g of Bakerbond™ phenyl sorbent which should trap both the nitrotouluenes and some



**Figure 36.** A) SFC Grade carbon dioxide, B) SFE Grade carbon dioxide. Collection in three mL of toluene, Pressure 400 atm, 15 mL liquid Carbon Dioxide. GC Analysis: Injection volume 1  $\mu$ L splitless 15 sec., Column: HP Ultra 1, 25m x 0.32 mm x 0.17  $\mu$ m; Carrier: Helium at 34 cm/sec.; Splitless 15 sec. purge off. Oven: 85°C for 1 min, 7.5°C/min to 170°C, the 20°C/min to 250. Detector: ECD 250°C.

of the alkanes. Phenyl sorbent will not trap alkanes as efficiently as octadecylsilane (C18), however it will trap an appreciable amount of the alkanes. The sorbent was then extracted twice with supercritical fluid carbon dioxide at densities of  $\sim .25 \text{ g/cm}^3$  and  $\sim .8 \text{ g/cm}^3$ , respectively.

In the low density SF extraction the alkanes were eluted and collected in methylene chloride. The extraction was performed at a pressure of 100 atm, temperature of  $75^\circ\text{C}$ , for 30 min. After the alkanes were eluted the collection solvent was changed to toluene and the pressure was raised to 350 atms. The Bakerbond™ phenyl sorbent was then extracted for 15 minutes with no modifier added to the extraction vessel.

The two extracts, alkanes and nitroaromatics, were then analyzed by GC-FID and ECD, respectively (Fig. 37 and 38). The SPE-SFE selective extractions procedure was repeated four times to determine if the SF extraction of the nitroaromatics was reproducible. The recovery results (essentially 100 %) from the selective extraction are similar to those for the non-selective extractions where no prewash step was performed (Table XI).

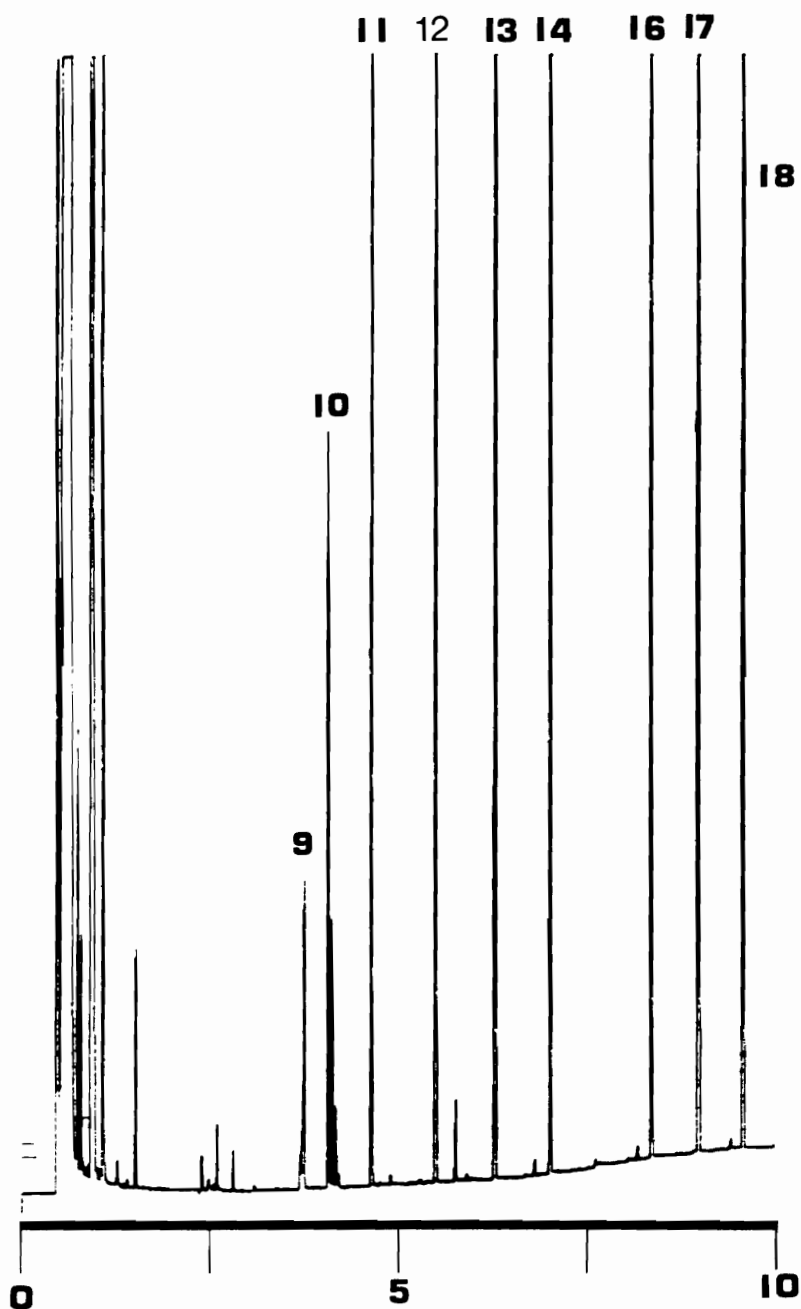


Figure 37. Chromatogram of Selective Recovery of Alkanes at Low Density (.25g/ml) Column: HP-5 (12 m x .2 mm id x .33  $\mu$ m film), Initial Temperature 50°C for 1 min, 20°C/min. to 250°C. Detector FID 350°C.

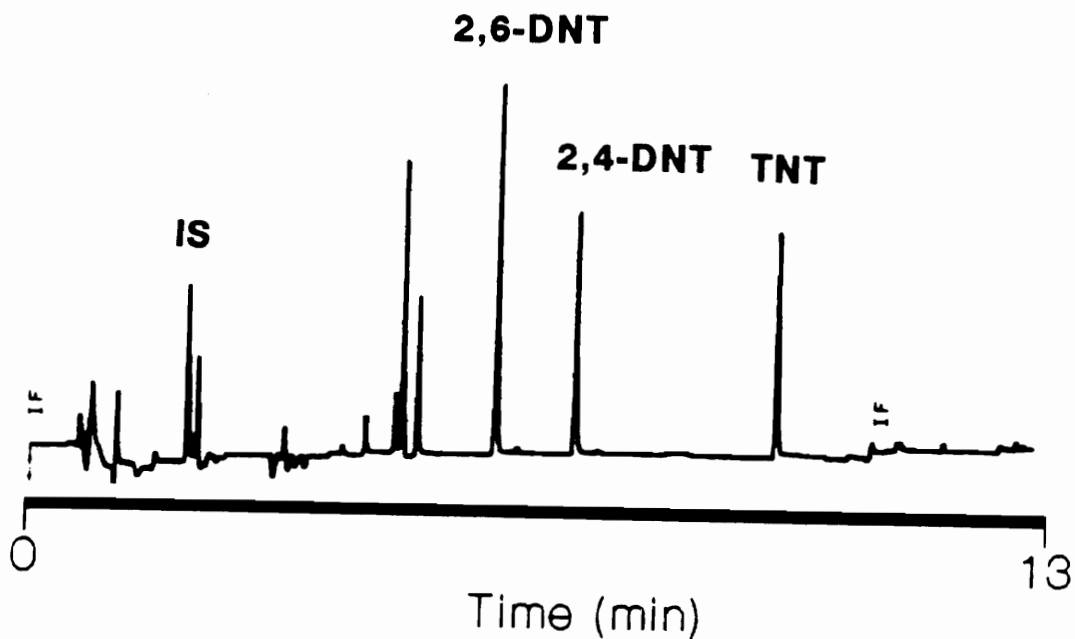


Figure 38. High Density SFE, Chromatogram of Nitrotoluene. (0.8 g/cm<sup>3</sup>) Sorbent, per-washed with low density (0.25 g/cm<sup>3</sup>) carbon dioxide. Column J & W Scientific (122-5011), Initial temperature 85°C, hold 1 min., 7.5°C/min to 155°C, then 70°C/min to 300°C. ECD 350°C.

TABLE XI

RECOVERY OF NITROTOLUENES EXTRACTED FROM BAKERBOND™ PHENYL SORBENT AT HIGH DENSITY.

Analytes	Percent Recovery	
	High Density	No wash (From Table IV)
2,6-Dinitrotoluene	99.8 ± 4	96.5 ± 2.5
2,4-Dinitrotoluene	97.4 ± 4.5	95 ± 3
Trinitrotoluene	96.3 ± 4	98 ± 4
Number of Extractions (n)	4	7

## ON-LINE ANALYSIS

On-line SFE analyses offer several advantages over traditional methods of sample preparation/concentration. First, additional handling of toxic compounds is eliminated. Second, the losses inherent in sample handling, such as adsorption to the walls of the sample vial or container, loss of volatile components during sample concentration steps, and the degradation of analytes with time or interaction with the solvent are eliminated. Third, the collection solvent is absent, hence the amount of analyte transferred to the GC column is increased, even when using a split interface. For example when an analyte is in 1 mL of solvent, and 1  $\mu$ L of sample is injected, only 0.1 % of the sample is introduced into the capillary column. A split injection interface with a 50:1 split ratio will provide a 20 fold increase in sample concentration. Finally, the use of hazardous elution solvents is eliminated.

Traditionally SPE has been coupled to GC by a retention gap. This procedure handles well the large amounts of solvents used to elute the analytes from the solid sorbents [65]. To facilitate a good SPE-GC interface using a retention gap the solvent should: 1) solvate the analytes and deposit them in a narrow band on the capillary column stationary phase; 2) be compatible with the GC detector; 3) have a lower boiling point than the analytes of interest;



4) have a low polarity to efficiently wet the retention gap surface under solvent flooding conditions; 5) have a polarity similar to that of the GC stationary phase to enhance phase-soaking characteristics; and 6) be free from contaminants [89,90]. Supercritical fluid carbon dioxide meets all these requirements making SPE-SFE uniquely suited for on-line analysis of volatile components via capillary gas chromatography. Since carbon dioxide is a gas at ambient temperatures and pressures, SFE is an ideal means of directly coupling SPE to GC, because the transfer solvent, supercritical fluid carbon dioxide, is easily removed.

The main problem encountered when directly coupling SPE (using bonded silica stationary phases) to GC is the presence of residual water in the SPE sorbent. A large portion of this water will be removed during SFE and consequently transferred to the capillary column where it can interfere with the trapping of the analytes during cryofocusing. Large amounts of water can also interfere with the electron capture detector (ECD). The use of a standard Hewlett Packard split/splitless injector equipped with a manual septumless injector (SLIM), for a split interface, will reduce the amount of water transferred to the column, while allowing a sufficient amount of analyte to be trapped on the column [53]. For this reason a split

interface was chosen for the SPE-SFE-GC study.

#### FEASIBILITY STUDIES

The use of supercritical fluid extraction as an interface between solid phase extraction and capillary gas chromatography (GC) has been investigated. Nitroglycerin and three nitroaromatic compounds - 2,6-dinitrotoluene, 2,4-dinitrotoluene and trinitrotoluene have been extracted and analyzed from spiked water samples by on-line SPE-SFE-GC-ECD. These studies were designed to determine if a quantitatively reproducible transfer of analyte from a bonded silica solid sorbent to a capillary GC can be accomplished using a supercritical fluid, in the presence of large portions of water.

Four different parameters of on-line split SPE-SFE-GC-ECD were investigated: 1) The appropriate split ratio was determined by performing a series of on-line SPE-SFE-GC-ECD extractions at several different head pressures; 2) The effect of trapping temperature on peak shape for the internal standard (1,3,5-trichlorobenzene), NG, 2,6-DNT, 2,4-DNT and TNT; 3) A comparison of two standard inlet liners, inverted cup and open tube, to determine which provides the greatest precision; and 4) The linearity of on-line split SPE-SFE-GC-ECD analysis for trace quantities of nitroaromatics.

When optimizing a system the analyst should study the effect of changing only one parameter at a time. However, when optimizing the first parameter the other initial parameters must be set at some initial value. The initial conditions for the parameters studied for the split on-line interface are listed in Table XII. These conditions were chosen based on a knowledge of sample inlet methods for capillary gas chromatography. The injection port temperature was set at 220°C to counteract the Joule-Thomson cooling of the carbon dioxide as it expands into the inlet liner. Also, at this temperature, TNT will not thermally degrade in the injection port [91]. An inverted cup injection liner was chosen since it provides the best precision for split injection of most analytes into a capillary column [92]. Finally, a trapping temperature of -30°C should provide the best focusing of the analytes, provided the column is not plugged with ice from the residual water. The studies mentioned above are designed to investigate each of these parameters individually to determine if they are the optimum conditions for on-line SPE-SFE-GC.

TABLE XII

## INITIAL CONDITIONS FOR ON-LINE SPE-SFE-GC

Parameter Studied	Initial Setting
Split Ratio	100:1
Injection Port Temperature	220 °C
Inlet Liner	Inverted Cup
Trapping Temperature	- 30 °C

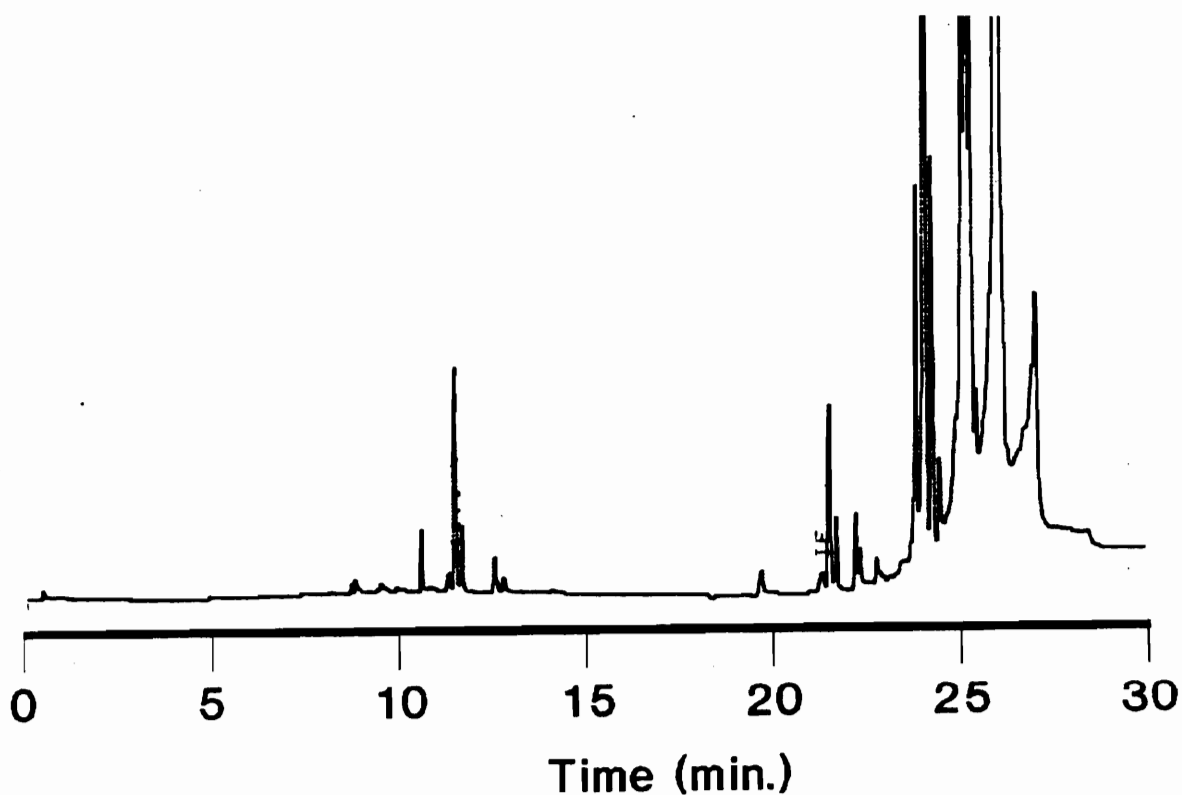
## FLUID PURITY

Before these studies were made the SFE system was checked for contaminants because SPE-SFE-GC will efficiently trap contaminants from the sample, the system and the carbon dioxide. SFE grade carbon dioxide was used for all on-line SFE-GC-ECD analyses. When performing on-line analysis, the contaminants that can be found in the SFE system will affect the ability to detect trace levels of analytes.

An on-line split SFE blank was run to determine if the contaminants in the system or Nanopure™ water would interfere with the on-line analysis of the nitroaromatics. Fifty milliliters of Nanopure™ water was passed through 1 g of Bakerbond™ phenyl sorbent following the procedure described in Chapter II. Then an on-line SPE-SFE-GC extraction was performed (Chapter II) with the initial settings listed in Table XII. Fortunately, the contaminants noted in Figure 39 did not coelute with the nitroaromatics and thus, a series of studies could be performed to determine the effectiveness of SPE-SFE-GC.

## SPLIT RATIO

In any trace capillary chromatographic analysis it is the goal of the analyst to transfer as much of the analyte to the capillary column as possible. In traditional GC



**Figure 39.** On-Line SPE-SFE-CGC System and Water Blank (50ml). Column: HP Ultra 1 (25 m x 0.32  $\mu$ l x 0.17 Film) Carrier: Helium, 34 cm/sec; Injector: 220°C, Split 120/1; Trapping Temperature -30°C; Analysis: Oven 30°C, 3 min. Hold, Rate A: 20°C/min. to 120°C, Rate B: 1°C/min. to 133°C, Rate C: 40°C/min. to 300; Detector: ECD 300°C

split injection, the operator can control the amount of sample introduced to the capillary column by adjusting the purge flow. The split ratio cannot be controlled this way with on-line SPE-SFE-GC because the purge vent is left open to prevent pressure from building up in the injector. Therefore, the split ratio is set by controlling the capillary column head pressure.

The proper head pressure is important in on-line split SFE for two reasons: it determines the split ratio and the carrier gas linear velocity. Table XIII lists the split ratios and area counts (for TNT) at five different head pressure settings. One on-line extraction was performed at each pressure, 5 psi, 8 psi, 10 psi, 15 psi, and 20 psi. As expected the highest pressure provided the greatest sensitivity for TNT. However, due to the system constraints the carrier gas head pressure must be the same for SFE and GC analysis. Pressure controller and pressure was maintained using a manually controlled analog pressure gauge. To attain the proper carrier gas linear velocity and reproduce it from run to run, the head pressure gauge must be set and not changed. This fact means the head pressure for analyte collection must be the same as that for GC analysis. At a head pressure of 20 psi, the contaminants are not resolved from NG, 2,6-DNT and 2,4-DNT because the carrier gas linear velocity (80 cm/sec) is too fast [93].

TABLE XIII

EFFECT OF COLUMN HEAD PRESSURE ON SPLIT RATIO AND LINEAR VELOCITY.

Column Head Pressure (psi)	Split Ratio	TNT Area Counts	Linear Velocity (cm/sec)	Retention Time (TNT)
5	390:1	56591	21	25.5
8	220:1	91946	33	21.2
10	170:1	112205	41.5	17.36
15	100:1	183061	61	13.28
20	70:1	226456	80.5	12.745

Column: HP Ultra 1 (25 m X 0.32 mm X 0.17  $\mu$ m Film), Split Flow ( $F_s = 428$  mL/min)



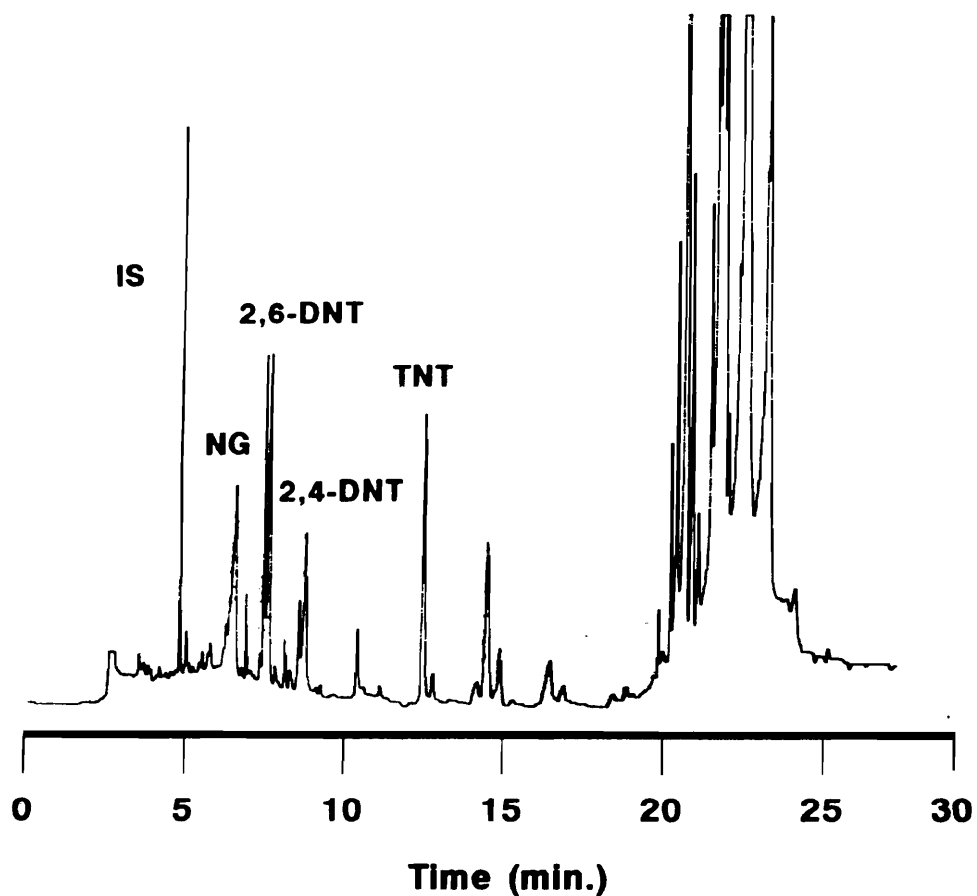
To resolve these analytes from the contaminants, the linear velocity must be closer to 35 cm/sec (Fig. 40 and 41).

Based on these test runs a head pressure of 8 or 10 psi was found to provide a suitable compromise between the amount of analyte introduced to the capillary column (split ratio between 100:1 and 200:1) and the carrier gas linear velocity ( $\mu = 33$  cm/s or 40 cm/sec).

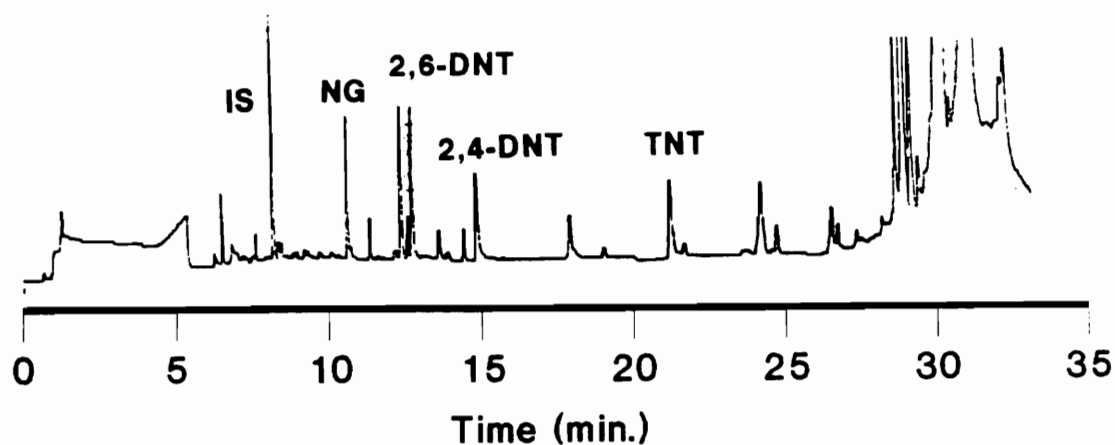
Once a pressure was chosen for a particular study it was held constant for the entire study. With the projected increased use of electronic pressure programmers in the future the two parameters may be independently controlled.

#### TRAPPING TEMPERATURE

Cryogenic sample focusing will condense vapors introduced to the capillary column as narrow bands at the head of the column. To efficiently focus analytes into a narrow band at the head of the capillary column the trapping temperature should be at least 150°C below the boiling point of the solutes. Cryofocusing is not dependent on the chromatographic processes, only a surface on which the vapors can condense is needed. However, when thermal focusing takes place in a capillary column it is aided by



**Figure 40.** Too High Head Pressure (20 psi.) Column: HP Ultra 1 (25 m x 0.32  $\mu$ l x 0.17 Film) Carrier: Helium, 33 cm/sec; Injector: 220°C, Split 220:1; Trapping Temperature -30°C; Analysis: Oven 30°C, 3 min. Hold, Rate A: 20°C/min. to 120°C, Rate B: 1°C/min. to 138°C, Rate 40°C/min to 300°C; Detector: ECD 300°C.

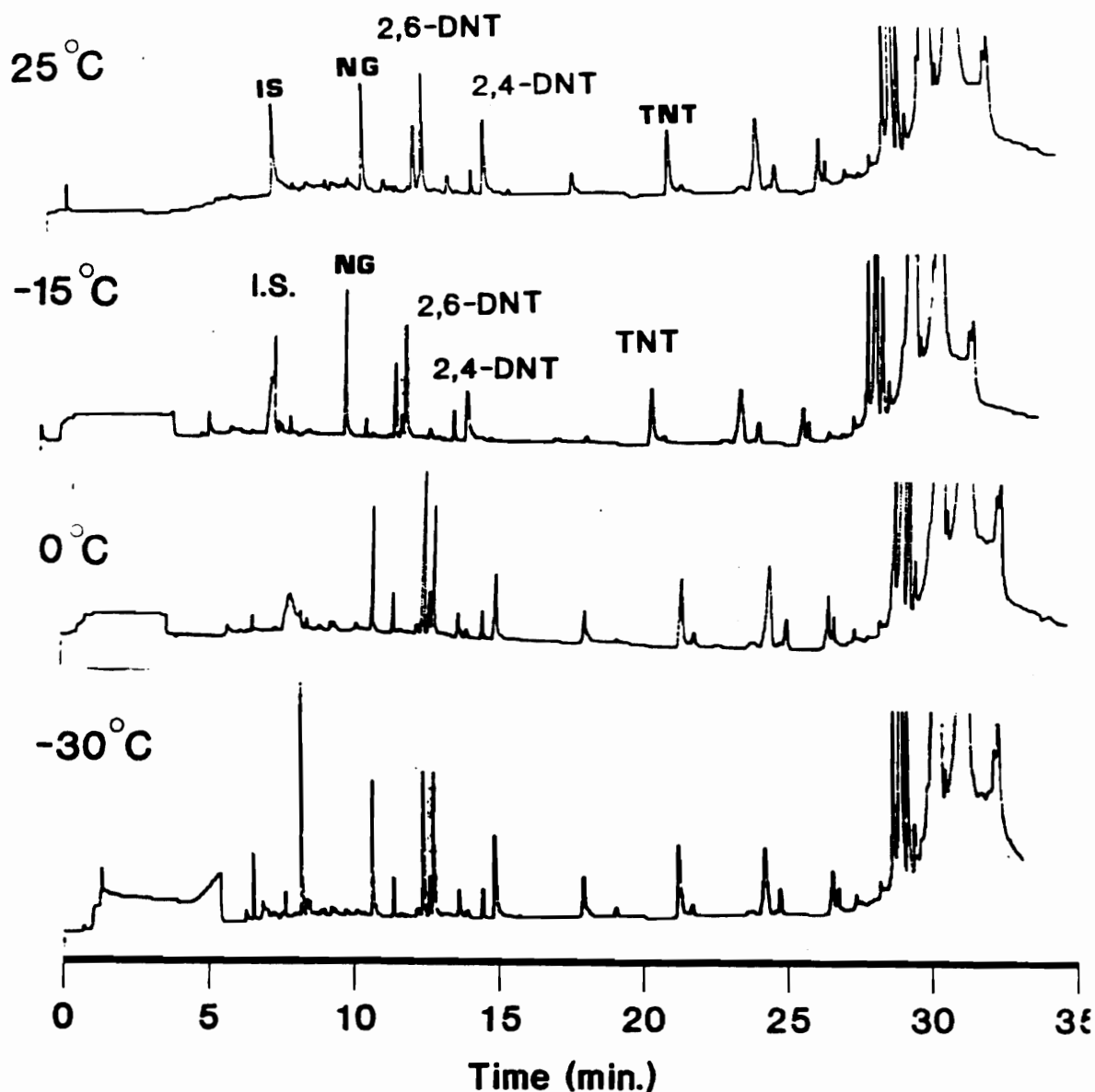


**Figure 41.** Optimum Head Pressure. (10 psi.), Column: HP Ultra 1 (25 m x 0.32  $\mu$ l x 0.17 Film) Carrier: Helium, 34 cm/sec; Injector: 220°C, Split 120/1; Trapping Temperature 25°C; Analysis: Oven 30°C, 3 min. Hold, Rate A: 20°C/min. to 120°C, Rate B: 1°C/min. to 138 °C, Rate B 40°C/min to 300 °C; Detector: ECD 300°C.

stationary phase focusing. Once the analytes are condensed at the head of the column they will not migrate further until they are vaporized [94].

The nitrotoluenes were trapped and focused at the head of the column at four different temperatures, - 25°C, 0°C, -15°C, and -30°C to determine the optimum temperature for trapping. For each study 50 mL of Nanopure™ water was spiked with 20 ng of nitroglycerine and 5 ng of 2,6-dinitrotoluene, 2,4-dinitrotoluene, and trinitrotoluene. Then the spiked water was passed through 1 g of Bakerbond™ phenyl solid sorbent by means of a vacuum aspirator. The sorbent was allowed to dry for 15 min under vacuum. The sorbent was then spiked with the internal standard and eluted by SFE using the conditions previously described. The gas chromatograph was not furnished with a cryogenic cooling system, so the temperature was controlled using the methods described in Chapter II.

As shown in the series of chromatograms in Figure 42 a trapping temperature of - 30 °C provides the sharpest peak for the internal standard (1,3,5-trichlorobenzene) while the trapping temperature of 0 °C produced the broadest peak shape. One would expect the peak shape to improve as the trapping temperature decreases,



**Figure 42.** Effect of Trapping Temperature on Peak Shape. Coupled SPE-SFE On-Line GC-ECD of 20 ng Nitroglycerin (NG), 5ng 2,6-Dinitrotoluene (2,6-DNT), 5ng 2,4-Dinitrotoluene (2,4-DNT), and 5ng Trinitrotoluene (TNT). 20 ng IS 1,3,5-trichlorobenzene. Split Injection 200/1 using an inverted cup liner. Trapping temperatures 25°C, 0°C, -15°C, and -30°C. Carrier: He 30 cm/sec. Oven: 30°C for 3 min. then 20°C/min to 120°C then 1°C/min. to 138°C then 40°C/min to 300°C.

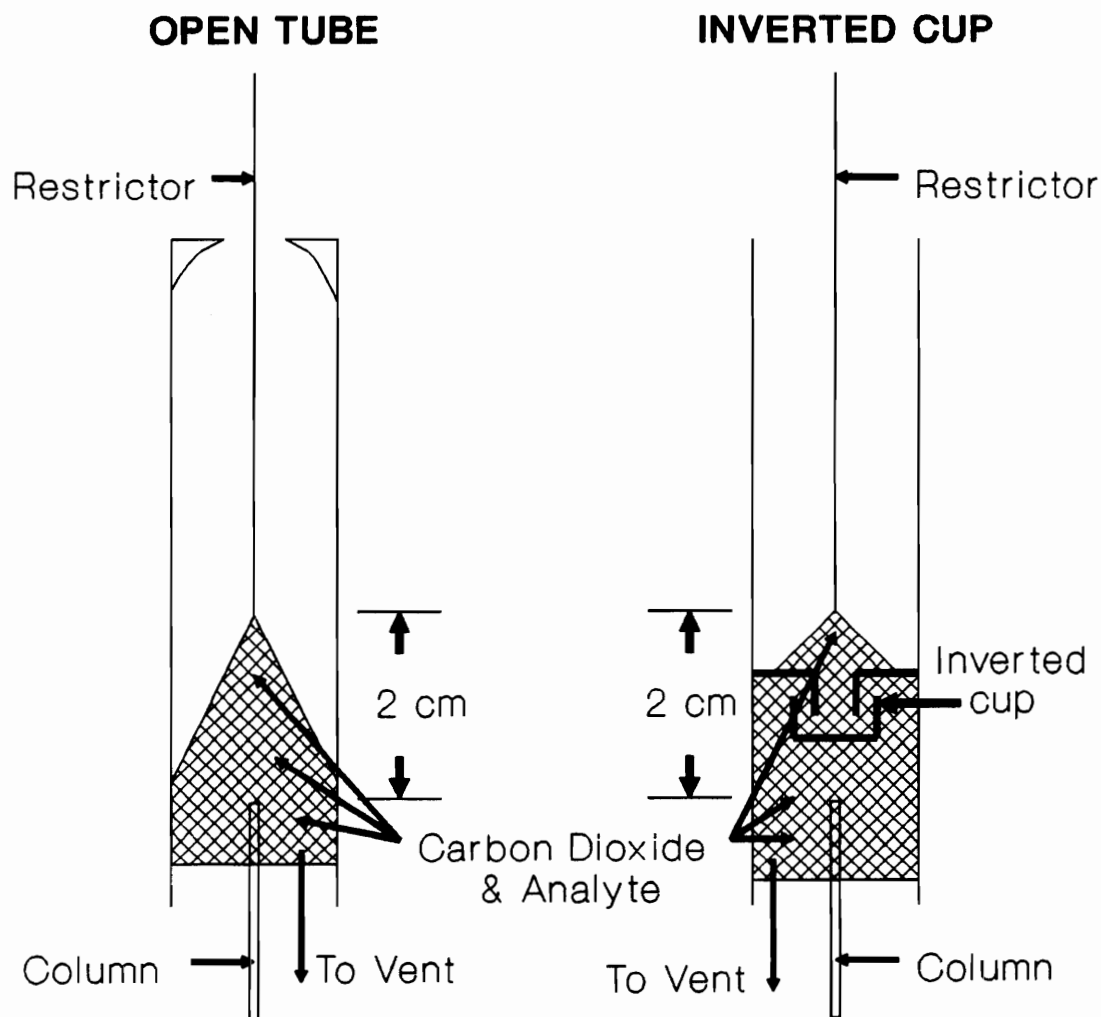
but only the internal standard was drastically effected by change in trapping temperature. This may be due to the effect of water. Because of residual water, the internal standard showed some tailing at 25 °C and as the trapping temperature decreased, the internal standard peak shape initially broadened, but then resharpended at -30°C (Fig. 42).

At a trapping temperature of 25°C most of the water can be swept through the non-polar column by the carbon dioxide during SFE because the water has no affinity for the non-polar stationary phase (Methyl Silicone Gum). At 0°C a greater amount of water is condensed on the stationary phase than at 25°C and thus interferes with the focusing of the internal standard which is more volatile than the other analytes. Because the nitroaromatic compounds have a lower vapor pressure, the water is removed from the column before they start to migrate. When the trapping temperature is reduced to -30°C the analyte is sharply focused at the head of the column and separated from the water which has a lower affinity for the nonpolar stationary phase. Based on the peak shape of the analytes, a trapping temperature of -30°C was chosen for the rest of the studies because the peaks are sharper than at the higher temperatures.

## EFFECT OF INLET LINERS ON PRECISION

Previous on-line SFE-GC analysis using split injection has been performed using a straight glass liner [43]. To determine if the type of inlet liner affects the precision, two standard Hewlett Packard sample inlet liners, a Goose Neck straight open glass tube (Resteck) and a standard inverted cup glass liner (Hewlett Packard, Avandale, PA) (Fig. 43) were investigated. Fifty mL of Nanopure™ water was spiked with 20 ng of nitroglycerine and 5 ng of 2,6-dinitrotoluene, 2,4-dinitrotoluene, and trinitrotoluene, then passed through 1 g of Bakerbond™ extraction sorbent by means of a vacuum aspirator. The sorbent was allowed to dry for 15 minutes under vacuum. The sorbent was then spiked with the internal standard and eluted by SFE using the conditions described in Chapter II. The trapping temperature was  $-30^{\circ}\text{C}$  as determined in the previous study and the head pressure gauge was set at 10 psi.

The percent relative standard deviations (%RSDs) presented in Table XIV indicate that the inverted cup liner (6.7 and 4.4 %RSD for NG and 2,6-DNT, respectively) appears to be more precise than the straight tube (15.3 and 9.0 %RSD for NG and 2,6-DNT, respectively). However, after performing F tests on the standard deviation data, a statistical difference only exists for the nitroglycerine and 2,6-DNT. The apparent difference can be attributed to



**Figure 43.** Schematic of inlet liners. A) Open Tube,  
 B) Inverted Cup, Once the carbon dioxide  
 passes  
 the column it is swept out the purge vent.



TABLE XIV.

EFFECT OF INLET LINER ON PRECISION USING AN INTERNAL STANDARD.

Percent relative standard deviation (%RAD) for spike of Nitroglycerine, 2,6-dinitrotoluene, (2,6-DNT), 2,4-dinitrotoluene, (2,4-DNT) and trinitrotoluene (TNT) from 50 mL of water; sorbent Bakerbond™ phenyl; Internal standard, IS, 1,3,5-trichlorobenzene. %RSD based on (n) extractions.

Inlet Liner	n	NG (20 ng)	2,6-DNT (5 ng)	2,4-DNT (5 ng)	TNT (5 ng)
Inverted Cup	5	6.7	4.4	4.3	4.8
Open Tube	7	15.3	9.0	4.2	6.5

the flow of the carbon dioxide and analyte in the inlet liner. With the inverted cup liner the carbon dioxide and analyte are forced to make contact with a hot glass surface before being split into the capillary column or swept out of the purge vent. The physical barrier between the restrictor and the capillary column prevents any analyte from being blown past the end of the capillary column and causes turbulent mixing of the analyte. The analyte then enters the capillary column under conditions similar to those for a standard GC syringe injection. When the open tube liner is used the analytes and carbon dioxide are sprayed directly into the capillary column decreasing the split ratio, thus increasing the amount of analyte trapped on the capillary column. Table XV lists the average area counts for the analytes along with the %RSD with out using an internal standard. The amount of contaminants was also increased due to the direct spray of the SFE eluent into the capillary column. These interferences coeluted with the NG and 2,6-DNT, causing integration problems. Thus, the increased %RSD for the open tube liner over the inverted cup liner is not from discrimination in the liner. This is evidenced by the similarity in the % RSD for the internal standards and TNT which have no coeluting peaks using either liner.

For further studies an inverted cup liner was chosen. This was based on the appearance of an improvement in

TABLE XV

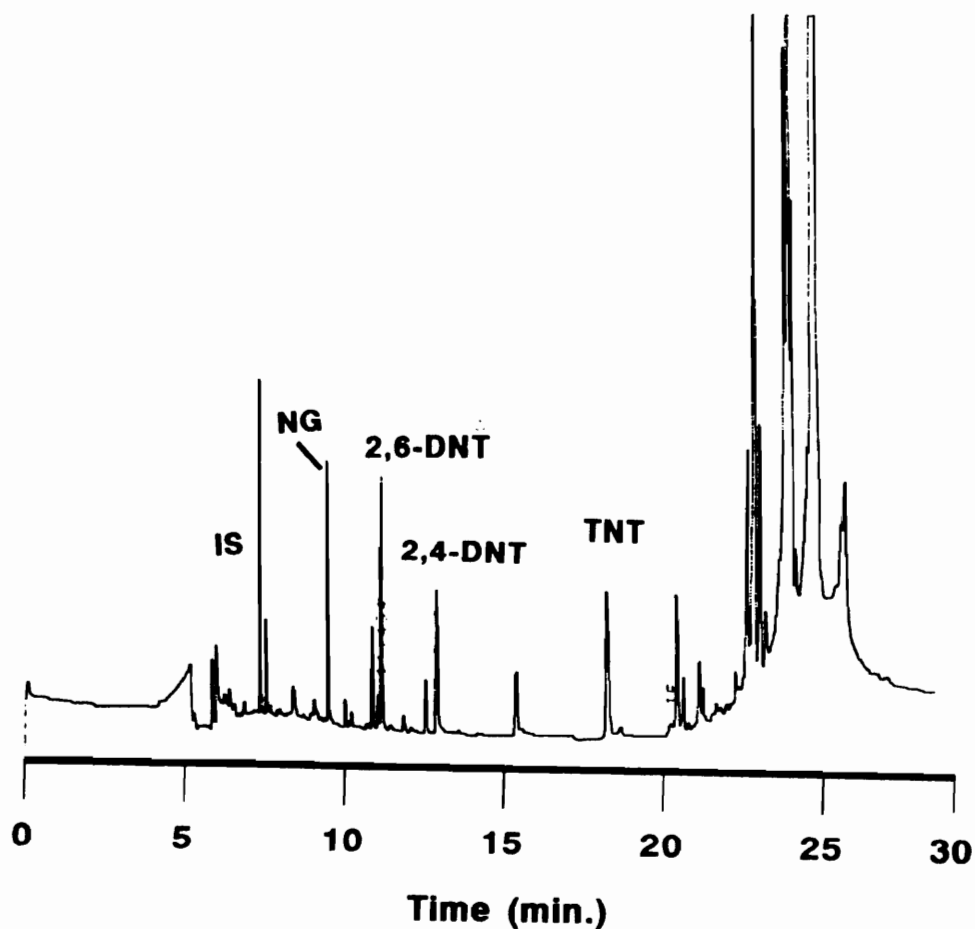
EFFECT OF INLET LINER ON AREA COUNTS AND PRECISION WITHOUT USING AN INTERNAL STANDARD.

Average area counts and percent relative standard deviations (%RSD) for spike of Nitroglycerine, 2,6-dinitrotoluene, (2,6-DNT), 2,4-dinitrotoluene, (2,4-DNT) and trinitrotoluene (TNT) from 50 mL of water; Sorbent, Bakerbond™ phenyl; Internal standard, IS, 1,3,5-trichlorobenzene. %RSD based on (n) extractions.

Analyte	Inverted Cup		Open Tube	
	Average Area Counts	%RSD	Average Area Counts	%RSD
IS (20 ng)	96000	4.8	125000	4.8
NG (20 ng)	88000	8.9	129000	17.4
2,6-DNT (5 ng)	113000	6.7	167000	11.7
2,4-DNT (5 ng)	87000	5.1	122000	7.5
TNT (5 ng)	125000	4.1	163000	6.5
n	5		7	

precision for the NG and 2,6-DNT and not the average area counts for each liner. The use of an inverted cup liner also resulted in a cleaner chromatogram (Fig. 44 and 45).

Precision with an internal standard was determined, as well as, precision without the internal standard. The results were unexpected but encouraging, there was no significant difference between the precision with and without an internal standard (Table XVI). The standard deviations for the on-line SPE-SFE-GC-ECD analysis of the nitroaromatics are similar to those found in SPE-SFE off-line GC-ECD analysis. This demonstrates reproducibility of on-line SPE-SFE-GC-ECD. As there was no difference, we chose to use an internal standard.



**Figure 44.** Inverted Cup Chromatogram. Coupled SPE-SFE On-Line GC-ECD of 20 ng IS, 20 ng, Nitroglycerin (NG), 5ng 2,6-Dinitrotoluene (2,6-DNT), 5ng 2,4-Dinitrotoluene (2,4-DNT), and 5ng Trinitrotoluene (TNT). 20 ng IS 1,3,5,-trichlorobenzene. Split Injection 200/1 using an inverted cup liner. Trapping temperature:  $-30^{\circ}\text{C}$ . Carrier: He 30 cm/sec. Oven:  $30^{\circ}\text{C}$  for 3 min. then  $20^{\circ}\text{C}/\text{min}$  to  $120^{\circ}\text{C}$  then  $1^{\circ}\text{C}/\text{min}$ . to  $138^{\circ}\text{C}$  then  $40^{\circ}\text{C}/\text{min}$  to  $300^{\circ}\text{C}$ .

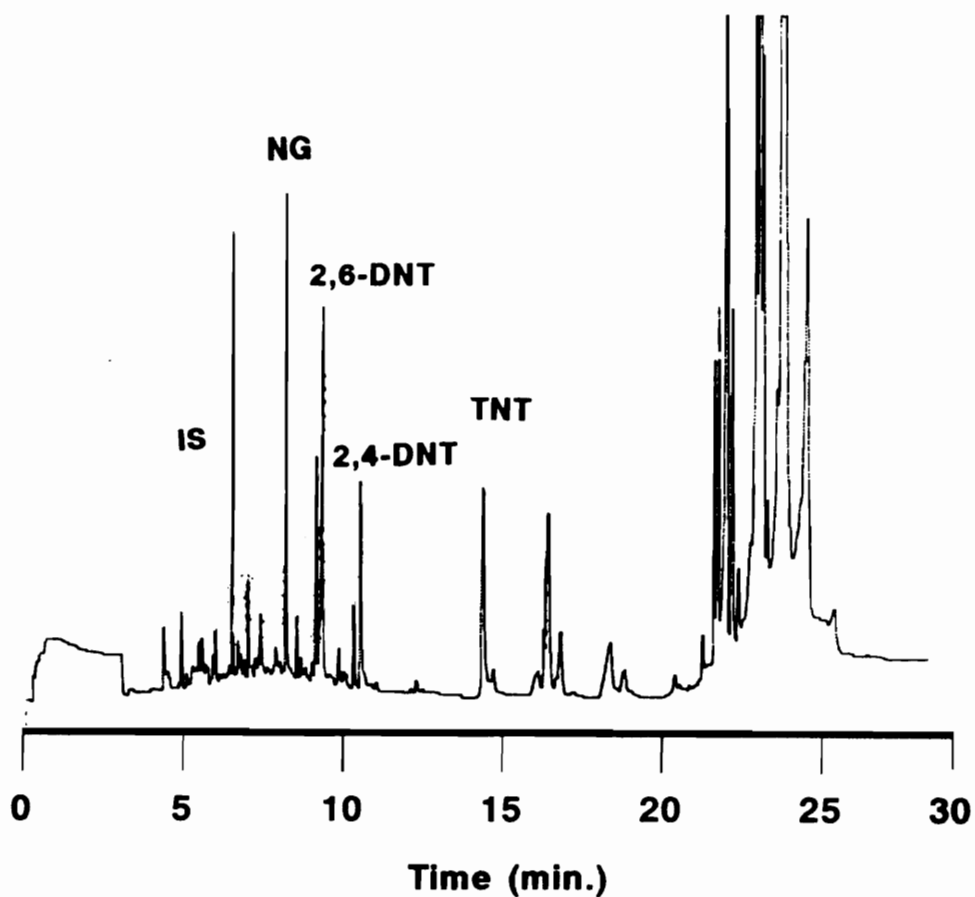


Figure 45. Open Tube Chromatogram. Coupled SPE-SFE On-Line GC-ECD of 20 ng IS, 20 ng, Nitroglycerin (NG), 5ng 2,6-Dinitrotoluene (2,6-DNT), 5ng 2,4-Dinitrotoluene (2,4-DNT), and 5ng Trinitrotoluene (TNT). 20 ng IS 1,3,5,-trichlorobenzene. Split Injection 200/1 using an inverted cup liner. Trapping temperature:  $-30^{\circ}\text{C}$ . Carrier: He 30 cm/sec. Oven:  $30^{\circ}\text{C}$  for 3 min. then  $20^{\circ}\text{C}/\text{min}$  to  $120^{\circ}\text{C}$  then  $1^{\circ}\text{C}/\text{min}$ . to  $138^{\circ}\text{C}$  then  $40^{\circ}\text{C}/\text{min}$  to  $300^{\circ}\text{C}$ .

TABLE XVI.

EXTERNAL STANDARD VERSUS INTERNAL STANDARD FOR AN INVERTED CUP LINER.

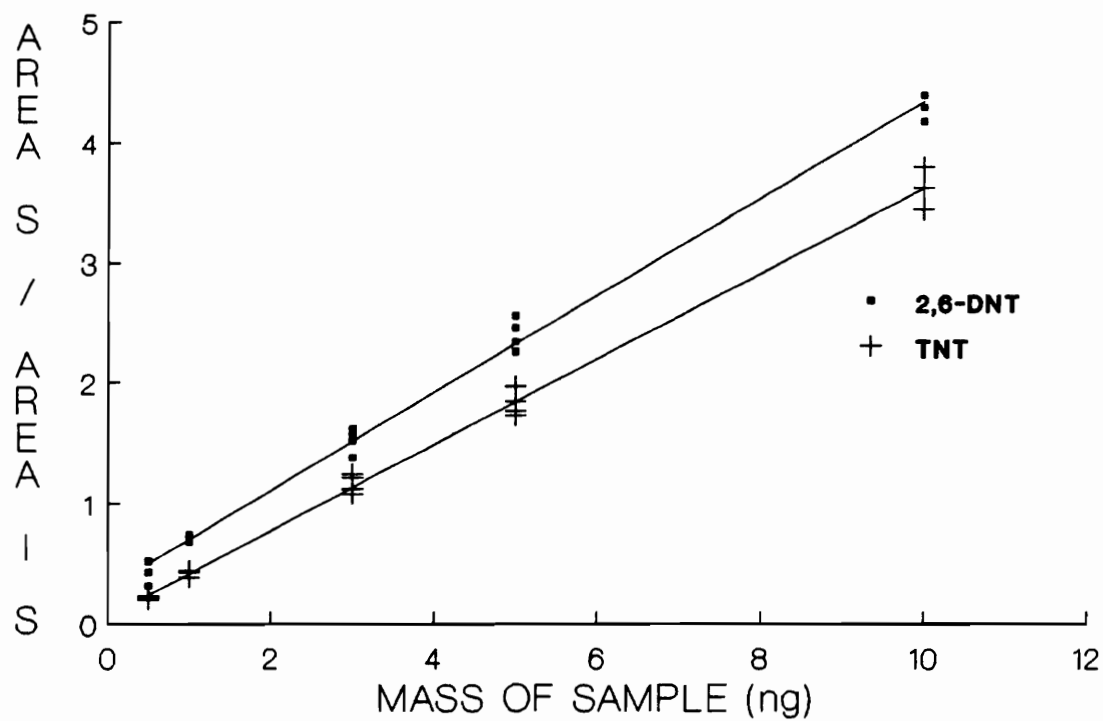
Percent Relative Standard Deviation for spike of 1,3,5-trichlorobenzene (IS), Nitroglycerine, 2,6-dinitrotoluene, (2,6-DNT), 2,4-dinitrotoluene, (2,4-DNT) and trinitrotoluene (TNT) from 50 mL of water; Sorbent, Bakerbond™ phenyl. %RSD based on (n) extractions.

Inverted cup Liner	n	IS (20 ng)	NG (20 ng)	2,6-DNT (5 ng)	2,4-DNT (5 ng)	TNT (5 ng)
External Standard	5	4.8	8.9	6.6	5.1	4.1
Internal Standard	5		6.7	4.4	4.3	4.8

## QUANTITATION

Finally, for this technique to be practical, the on-line transfer must be quantitative. The mass of sample transferred to the capillary column must be proportional to the amount of analyte trapped on the bonded silica solid sorbent. To test this, a calibration curve was constructed from spiked controls (Fig. 46). Several controls were prepared by spiking 50 mL of Nanopure™ water with 0.5 ng, 1 ng, 3 ng, 5 ng, and 10 ng of 2,4-DNT, 2,6-DNT and TNT (in methanol). A volume of 50 mL of Nanopure™ water was chosen because there is no analyte breakthrough. The analytes were trapped on phenyl sorbent and then transferred to the GC by SFE. The SFE conditions were identical to those previously reported. Based on the previous studies, the GC conditions consisted of a column head pressure of 8 psi, a trapping temperature of - 30°C and a standard split inverted cup was used for the inlet liner. Three or four SPE-SFE-GC analyses were performed at each concentration of the nitroaromatic compounds and a calibration curve was constructed. The results plotted in Figure 46 indicate good linearity. The  $r^2$  value for 2,6-DNT and TNT were 0.991 and 0.995, respectively (15 degrees of freedom for each), thus demonstrating that a quantitative calibration curve can be obtained for on-line SPE-SFE-GC-ECD using controlled standards.





**Figure 46.** Calibration curve for 2,6-Dinitrotoluene and Trinitrotoluene. Area sample/ area internal standard versus mass of sample. The  $r^2$  are 0.991 and 0.995, respectively. (15 degrees of freedom for each)

## STATISTICS

f-Test: Determines if there is a difference in precision.

$$F = \frac{S_1^2}{S_2^2}$$

First construct hypothesis:

$$H_0 = S_1 = S_2 \quad \text{or} \quad H_1 = S_1 > S_2$$

Calculate F ( $F_{ca1}$ ) and compare to F value from table ( $F_{table}$ )

[95]:

If  $F_{ca1} > F_{table}$  then  $H_1 = S_1 > S_2$

If  $F_{ca1} < F_{table}$  then  $H_0 = S_1 = S_2$

## COMPARISON OF LINERS

For Nitroglycerine

Open Tube Liner

$$F = \frac{.15^2}{.062^2}$$

$n_1 = 7, \quad S = .15$

Inverted Cup Liner

$n_2 = 5 \quad S = .062$

$$F_{ca1} = 5.8$$

$$F_{table} = 4.88 \text{ (95\% confidence)}$$

$$F_{ca1} > F_{table}$$

$$\text{Therefore: } H_1 = S_1 > S_2$$

There is a Statistical difference between the inverted cup liner and the open tube liner for nitroglycerine.

For 2,6-Dinitrotoluene

Open Tube Liner

$$F = \frac{.12^2}{.052^2}$$

$n_1 = 7, S = .12$

Inverted Cup Liner

$n_2 = 5, S = .052$

$$F_{Ca1} = 5.3$$

$$F_{table} = 4.88 \text{ (95\% confidence)}$$

$$F_{Ca1} > F_{table}$$

$$\text{Therefore } H_0 = S_1 > S_2$$

There is a statistical difference between the inverted cup liner and the open tube liner for 2,6-dinitrotoluene.

## CHAPTER IV

## CONCLUSIONS

## CONCLUSIONS

This study reports results of the coupling of solid phase extraction to supercritical fluid extraction for the purpose of coupling SPE directly to capillary gas chromatography. I investigated the use of supercritical carbon dioxide as an elution solvent for both bonded silica and polymeric sorbents for analytes trapped from water. Three solid sorbents, Bakerbond™ phenyl solid sorbent and two Empore™ extraction disks; Octadecylsilane (C18) and Styrene-divinylbenzene (SDVB) were tested.

The Bakerbond™ phenyl sorbent provided the best recoveries of the nitroaromatics from 500 mL of organic free water, greater than 80 % for TNT and greater than 90 % for the dinitrotoluenes. Of the two Empore™ extraction disks, the SDVB disk yielded the higher recoveries, approximately 90 % for the dinitrotoluenes and 50 % for TNT. The C18 Empore™ disk recoveries were approximately 74 % for both dinitrotoluenes and 50 % for TNT. Not only did the Bakerbond™ phenyl provide the highest recovery for TNT, but it was easier to handle since the extraction cell can be prepacked with the sorbent prior to the SPE step. This eliminates handling of the sorbent once the analytes are trapped.

The supercritical fluid extraction efficiency of

nitroaromatics from the solid sorbent was investigated with and without chemical modifier added directly to the extraction vessel. The kinetic studies showed that a toluene modifier increased the rate of extraction, although, it did not improve the amount of analyte extracted from the solid sorbent. Both the modified and unmodified supercritical fluid extractions showed essentially 100 percent recovery for a one microgram quantity of all the analytes trapped on one gram of the Bakerbond™ phenyl sorbent in under 15 minutes.

A method was developed for the SPE extraction of nitroaromatics at two parts per billion (ppb) from organic free water with the Bakerbond™ phenyl sorbent having the highest recoveries of  $94 \pm 0.5\%$ ,  $90 \pm 2\%$ , and  $81 \pm 1\%$ , for 2,6-DNT, 2,4-DNT, and TNT, respectively. When the extraction efficiencies of SPE-SFE for nitroaromatics were investigated for surface and well water samples, recoveries were considerably lower (54 to 92 percent). The low recoveries were due to additional organic compounds in the surface water that competed for adsorption sites on the solid sorbent, overloaded the solid sorbent and caused the nitroaromatics to breakthrough early.

Trace quantities of nitroaromatics (low parts per trillion [ppt]) were also extracted from organic free water ( $95 \pm 10\%$  for 2,6-DNT,  $78 \pm 2\%$  for 2,4-DNT and  $83 \pm 9\%$  for

TNT) and surface water ( $86 \pm 8\%$  for 2,6-DNT and  $66 \pm 6\%$  for TNT). The recoveries were similar to those at ppb levels with slightly higher standard deviations which are to be expected. The low or nonrecoveries for 2,4-DNT are a result of interfering contaminant peaks that are not a problem when analyzing at higher concentrations.

The ability to use supercritical fluids for clean up or fractionation of the analytes trapped on the solid sorbent was also demonstrated. A low density supercritical fluid extraction,  $\sim 0.25 \text{ g/cm}^3$ , was performed to qualitatively remove a series of alkanes. Then the nitroaromatics were extracted at a higher density,  $\sim 0.8 \text{ g/cm}^3$ , to quantitatively recover the nitroaromatics. The recoveries were similar to those for simple high density off-line extractions.

The off-line SPE-SFE studies showed that the supercritical fluid, carbon dioxide, is a suitable elution solvent for nitroaromatics from bonded silica solid phase extraction sorbents. Also, the standard deviation for off-line SPE-SFE extraction of analytes from organic free water was less than or equal to 5 percent, which is typical for SPE, SFE, and manual split injection GC analysis of semi-polar compounds.

The most valuable aspect of coupling SPE to SFE is the ability to interface SPE directly to capillary gas chromatography. Since carbon dioxide is a gas at ambient

temperatures, it is an ideal solvent for this interface. It eliminates the need for a retention gap which is traditionally used to handle the normally large amounts of organic solvents used to elute analytes from solid sorbents [66].

The main problem associated with SPE-SFE-GC is the presence of residual water trapped on the active sites of the bonded silica support. This problem was successfully eliminated by employing the split interface developed by S. B. Hawthorne for the extraction of damp soils [74].

Three different aspects of on-line split SPE-SFE-GC-ECD were investigated: 1) The effect of trapping temperature on peak shape for 1,3,5-trichlorobenzene (the internal standard), nitroglycerine, 2,6-DNT, 2,4-DNT and TNT; 2) A comparison of two standard inlet liners to determine which liner is more precise; and, 3) The linearity of on-line split SPE-SFE-GC-ECD analysis for trace quantities of nitroaromatics.

After comparing the peak shape for the internal standard at four different temperatures  $-25^{\circ}\text{C}$ ,  $0^{\circ}\text{C}$ ,  $-15^{\circ}\text{C}$ , and  $-30^{\circ}\text{C}$ , a trapping temperature of  $-30^{\circ}\text{C}$  was chosen for additional studies. The most volatile compound, 1,3,5-trichlorobenzene, was affected the most by the trapping temperature. Therefore, the trapping temperature chosen was based on its peak shape. The interferences caused by the



small amounts of water that did enter the capillary column during SFE and analyte trapping were resolved from the internal standard at this temperature.

A comparison was made between two standard inlet liners, an open tube liner and an inverted cup liner. Comparisons were made with and without an internal standard. The results implied an increase in precision for the inverted cup liner. However, there was a statistical difference for only two of the four analytes.

In addition to a precision comparison of the inlet liners, the reproducibility for the SPE-SFE-GC of nanogram quantities of nitroaromatics from organic free water was determined. The relative percent standard deviation for nanogram quantities of nitroglycerine and nitrotoluenes ranged from 4 to 10 percent. There was no significant difference with or without an internal standard. These results are similar to those obtained in off-line SFE. The low relative standard deviations for the external standard demonstrates the robustness of the technique. However, use of an internal standard is still recommended, because in principle it offers advantages over the external standard.

A calibration curve was constructed for the nitroaromatics using 50 mL spiked controls ranging from 500 pg to 10 ng of each nitroaromatic. The results demonstrate the ability to generate a linear calibration curve ( $r^2 =$

0.995). This shows the quantitative transfer of nitroaromatics trapped from aqueous samples to a solid sorbent to the capillary column by SFE. It vividly demonstrates the utility of the approach for quantitative analysis at trace levels.

The use of supercritical fluids, that are gases at ambient conditions to interface solid phase extraction with capillary gas chromatography is reproducible, quantitative and straight forward for semi-polar compounds. The increased use of supercritical fluids to couple analytical techniques along with the inherent need to automate analytical sample preparation will make on-line SPE-SFE techniques more common in the future.

**CHAPTER V**

**LITERATURE CITED**

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**APPENDIX A**

**SAMPLING**

## SAMPLING THE ENVIRONMENT

Because this dissertation focuses on the feasibility of coupling SPE to CGC via SFE for environmental samples, the importance, of collecting and handling environmental samples will be discussed. Sample handling has been considered the weak point in chromatography and analytical chemistry in general [1,2]. One common misconception is that samples can be collected simply and then brought to the laboratory for analysis. Often this attitude will result in the contamination of the sample, and the wasting of both time and resources. Several aspects of sample collection that the analyst must be familiar with will be discussed - regulations, statistics, sampling containers, sampling devices and sample preservation.

## REGULATIONS

The analyst should be aware of and follow the regulations that govern the selected jurisdiction and the proposed investigation. Some of these regulations may be included in city ordinances, sewage district reports, and treatment and land fill facility guidelines. Water quality boards, health departments, and the United States Environmental Protection Agency (USEPA) can also supply necessary information regarding sampling regulations [3,4].

## SAMPLING STATISTICS

When collecting samples it is often impossible to analyze the entire population or bulk sample. Therefore, the analyst must collect a limited number of samples that will represent the whole. To be able to generalize to the whole, the samples taken must be random. For the samples to be statistically random all members of the population must have an equal chance of being collected. Only then will the calculations that measure the characteristics of the population be valid.

A population is often described by its mean. In order for the population estimate of the mean to be accurate, the sample statistics used in the formulas must be from randomly collected samples. The formula for the confidence limits of the mean is:

$$\mu = \bar{x} \pm t \left( \frac{s}{\sqrt{n}} \right)$$

where  $\bar{x}$  is the sample mean,  $t$  is the confidence interval's value from student's  $t$  table,  $s$  is the standard deviation of the sample, and  $n$  equals the sample size. The appropriate  $t$  value is dependent on the degrees of freedom ( $n-1$ ) and the degree of confidence required.

Two main factors contribute to total variance of the sample ( $s^2$ ):

$$s^2 = (\sigma_0^2 + \sigma_1^2)$$

The first is sampling variance ( $\sigma_0^2$ ). No two samples will be exactly the same. The second is measurement error ( $\sigma_1^2$ ). The sample variance can be reduced by taking a larger number of samples and by using precise instrumentation.

When sampling from bulk samples such as liquids and soils, there is a standard nomenclature that is used to define the type or part of sample described. This eliminates confusion as to whether the analyst is referring to a small sample taken from bulk which is a sample increment or a group of sample increments which is called a gross sample.

Bulk samples such as soils are inherently non-homogenous and liquids can be non-homogenous on a molecular scale due to concentration gradients. This inhomogeneity can only be detected by sampling the bulk as if it were a series of cells and each one is randomly chosen using a random numbering method. One strategy for sampling bulk materials is to take  $n$  sample increments and blend them prior to analysis ( $m$  replicate measurements). The variance

of the mixed samples  $s^2 = \left( \frac{\sigma_0^2}{m} + \frac{\sigma_1^2}{n} \right)$  can then be checked

against the variance for the sample increments, where the means of the increments have been averaged. Of the two methods, the second one is more precise because it requires a greater number of measurements. However, the first method is usually more economical and thus, provided it gives the precision needed, it will usually be chosen [5,6].

#### EQUIPMENT AND CONTAINERS

The choice of collection devices and sample containers can be a crucial element in the collection of environmental samples. The container may have an effect on the representativeness of the sample. Table I lists several different collection devices available to the analyst along with the typical matrix they apply to. Table I contains some of the more common sample containers, typically the best sampling devices are constructed from polytetrafluoroethylene (PTFE, or Teflon™ [DuPont]), glass and stainless steel. These materials have been shown to be the most inert with respect to the adsorption and desorption of inorganic compounds [7]. One draw back of these materials is their intolerance to stress. Glass will

TABLE I

SAMPLING DEVICES: MATERIALS, APPLICATIONS, AND ANALYTE  
COMPATIBILITIES [8]

Matrix type	Sampling device	Material(s) of construction	Appropriate analytes
Ground water (From wells; low volumes)	Bottom emptying bailer	PTFE	All organics, metals, inorganics
Ground water (From wells; low volumes)	Kemmerer sampler	Stainless steel, Silicone, or other rubber seals	Volatile organics, most metals and inorganics
Ground water (From wells)	Bladder pump	Stainless steel and PTFE	All organics, most metals and inorganics
Surface/waste water	Direct fill bottle dipper	Glass or plastic bottles attached	All organics (glass), metals and inorganics (plastic)
Surface/waste water	automatic composite sampler	PTFE-lined tubing, glass containers	All organics except volatiles, all metals, and inorganics
Surface and shallow soils	Hand corer or trowel	Stainless steel	All organics, most metals, all inorganics
Surface and shallow soils	Plastic Scoop	PVC	All metals, all inorganics
Deeper soils	Power-assisted coring or driver samplers	Stainless steel or brass liners	All organics, most metals, all inorganics

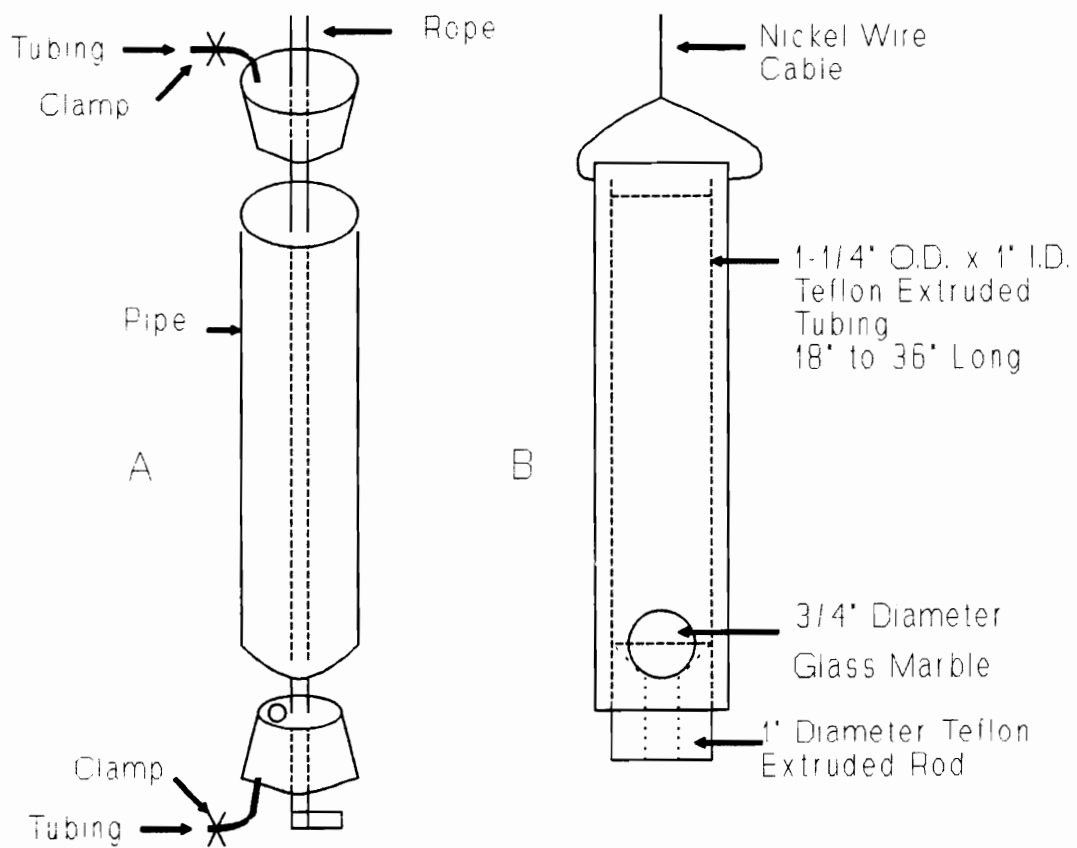


shatter easily and PTFE deforms. When force is required to collect samples, high grade stainless steel is generally preferred. Stainless steel sampling devices usually do not present problems with organic compounds, but they may introduce trace metals such as chromium, iron, nickel and molybdenum. Several other materials such as polyvinyl chloride, polypropylene, and polyethylene are frequently used for sample collection when the contaminants are known and do not interfere with the analyses [8].

#### COLLECTING LIQUID SAMPLES

The most typical liquid samples investigated are aqueous ranging from tap waters to sewage. Of the aqueous sampling methods tap water sampling is the easiest because the analyst can simply fill the container at the tap. To sample from a well the water can be collected directly from a purge pump provided the analytes and the pump materials are compatible [9].

The use of a bailer is also common, bailers are easy to use and one of the oldest water sampling devices. Bailers can be constructed from a weighted bottler or capped length of pipe on a line which allows the bailer to be lowered and raised by hand. Figure 1 shows two types of bailers, a teflon bailer and a modified Kemmerer Sampler that is commonly used for surface and ground water. Bailers have



**Figure 1. A) Modified Kemmerer Sampler; B) Teflon Bailer [10].**

several advantages over other samplers: 1) They can be constructed from a variety of materials; 2) They are convenient and affordable allowing a separate bailer for each well reducing cross contamination; 3) They require no external power source; and 4) They have a low surface to volume ratio which makes them ideal for volatile organics. The bailer does have a few drawbacks. It is sometimes difficult to transfer the sample from the bailer to a sample bottle without aeration of the sample. The equipment must also be properly cleaned before reuse or cross contamination will result [10,11].

Collection of surface water from waste streams can also be accomplished by dipping the sample bottle directly into the source by hand. This method of sampling is called Grab Sampling. To collect a grab sample, the analyst must take some precautions to prevent the introduction of contamination into the sample bottle. First a sterile bottle must be obtained and the inside of the cap, bottle and rim of mouth must not be touched. Hold the container securely at the base with one hand and rapidly submerge the bottle mouth down into the water avoiding the surface scum. Orient the mouth of the bottle upstream toward the current flow, positioning it away from the collector's hand, the shore, the boat or collecting platform. To extend the reach of the analyst and prevent interruption of the stream, an

extension pole may be helpful.

In many cases preservatives are necessary, a glass container may be used to collect the sample which is then transferred to containers with preservatives. The same devices that are used for well sampling can also be used for open surface waters. Table I also lists some sampling devices along with the material applications and analyte compatibility.

#### SAMPLE PRESERVATION

Once collected, the sample may need to be preserved against biological action, hydrolysis of chemical compounds and complexes, and loss of volatile compounds in the sample. The addition of chemicals and cooling the sample through refrigeration and freezing are the typical methods of sample preservation [10].

One type of chemical preservation is pH control. So that the metal ions will stay dissolved in the water when performing metal analysis of water samples, the pH is kept below two by the addition of concentrated nitric acid. For convenience, chemical preservatives that can be added directly to the sampling bottle before the sample is added are preferred. This allows the sample to mix with the preservatives without a delay. If the preservatives for one analyte interfere with another compound of interest,

separate samples for the two analytes must be collected [12].

Cooling the sample is a common practice when samples are collected in the field. Cooling the sample does not interfere with any analytical methods since no chemicals are added to the sample. However, cooling does not preserve the integrity for all of the possible variables in a sample.

Several preservation studies have investigated the use of freezing as a long term method of sample holding [13,14]. Due to changes that are imposed upon the solid components present in the water, both the filterable and non filterable, when they are frozen and then thawed, it is necessary to perform a high speed homogenization of the sample prior to analysis. Freezing is only acceptable for certain analyses and is not a general preservation method. Table II lists the containers, preservatives and holding times for a few select classes of organic compounds [10].

#### SUMMARY

To achieve accurate results when analyzing environmental samples it is important to be familiar with the regulatory agencies that govern the jurisdiction of the investigation. Care must be taken to collect a random sample that represents the whole. To prevent loss of sample components and degradation of the sample, the proper

collection devices and sample containers must be used. When necessary the analyst can use preservation methods such as the addition of chemicals or cooling of the sample. As illustrated by the preceding details, sample collection is a process that involves careful thought and planning and if these factors are overlooked, time and energy will be wasted.

TABLE II

CONTAINERS PRESERVATIVES AND HOLDING TIMES FOR A FEW SELECT CLASSES OF ORGANIC COMPOUNDS [8].

Organic Test	Container	Preservative	Maximum Holding Time
Purgeable halocarbons	Glass Teflon™ Lined Septum	Cool, 4 °C 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	14 Days
Purgeable aromatics	Glass Teflon™ Lined Septum	Cool, 4 °C 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> HCl to pH<2	14 days
Acrolein and acrylonitrile	Glass Teflon™ Lined Septum	Cool, 4 °C 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Adjust pH to 4-5	14 Days
Phenols	Glass Teflon™ Lined Septum	Cool, 4 °C 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	7 days until extraction, 40 days after extraction.

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## VITA

Gregory Carlton Slack was born on June 22, 1963 in Sussex, New Jersey and grew up in Eden, New York. He began his academic studies at the State University of New York at Morrisville. After graduating in 1983 with an Associate's degree in chemistry, he then went to the State University of New York at Potsdam and received a B.A. degree in chemistry. He then started graduate studies at the University of Vermont, but when his advisor resigned from the university he transferred to Virginia Tech in the fall of 1987 to begin doctoral work.

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