INVESTIGATION INTO THE QUANTITATIVE ASPECTS OF SUPERCRITICAL FLUIDS AS MOBILE PHASES FOR CHROMATOGRAPHY AND EXTRACTION

BY

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ABSTRACT

Supercritical fluids were introduced as mobile phases for chromatography and extractions in the early sixties. Over the past decade the technique has received increased attention, largely owing to the introduction of several commercial instruments and to the ease of adapting available commercial equipment for use in sfe and sfc. This dissertation examines the use of supercritical CO_2 as a mobile phase for chromatography and extraction. The first chapter explores the problems and provides background for the research. The physical properties of supercritical fluids are described and pertinent recent research is identified and summarized. The following chapter presents the design of three interrelated studies that investigate *in-situ* concentration, quantitative aspects of sfc, and quantitative aspects of sfe. The first of the three studies examined the exploitation of the innate properties of a supercritical fluid. Because the mechanism of elution with supercritical fluid mobile phases is based on solvation, it was possible in this study to concentrate analytes at the head of the supercritical zone. Concentration was followed by supercritical fluid chromatography. This allowed the analysis of sample

components at part per billion levels. The use of supercritical fluids as mobile phases for chromatography and the quantitative nature of sfc with flame ionization detection was examined in the second study. Under numerous detector and injector configurations, a distinct decrease in response factors with an increase in carbon number was evident. Results showed that the decrease in response factors was related to an increase in CO_2 flow through the detector. The magnitude of the decrease was multivariably dependent. The third study dealt with the quantitative aspects of using a supercritical 0obile phase for the extraction of PNAs and pesticides from several matrices including contaminated soil. Results showed that sfe is a reliable, easy, and efficient (> 85% recovery) method of removing trace materials from contaminated soil. Implementation of a simple resistively heated collector was used to circumvent the problems of extracting damp matrices such as soil. The results and conclusions are presented in the final two chapters.

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INTRODUCTION

PHYSICAL PROPERTIES

SuperCritical fluids (SCF) are substances that have been elevated above their critical pressure and temperature. They are neither a gas nor a liquid, but have physical properties that lie between those exhibited by gases and liquids. Figure 1 is a phase diagram for a pure compound with the supercritical zone crosshatched in the upper right hand corner. The critical temperature is shown as Tc and the critical pressure as Pc. Table 1 (1-3) compares typical values for three important properties--viscosity, diffusivity, and density--of gases, liquids, and supercritical fluids.

VISCOSITY. Supercritical fluids exhibit viscosity values closest to gases. This permits supercritical fluids to be used as mobile phases in open tubular columns and packed columns because of the low to moderate pressure drop across either column. In contrast, liquids as mobile phases have viscosities on the average of 100 times larger. A gaseous mobile phase would show the smallest pressure drop across any type of column.

DIFFUSIVITY. The diffusivity, as measured by the diffusion coefficients (Dm), of each of the three mobile phases shows that SCFs exhibit diffusivities ca. 1000 times smaller than gases, yet 10 times greater than in liquids, as seen in Figure 2 (2). Thus, the permeability of the supercritical fluids is lower than gases but higher than liquids. Solute mass transfers are higher in the supercritical state than in liquids, and consequently, peak widths tend to be narrower with SCFs as the mobile phase. As a consequence of the

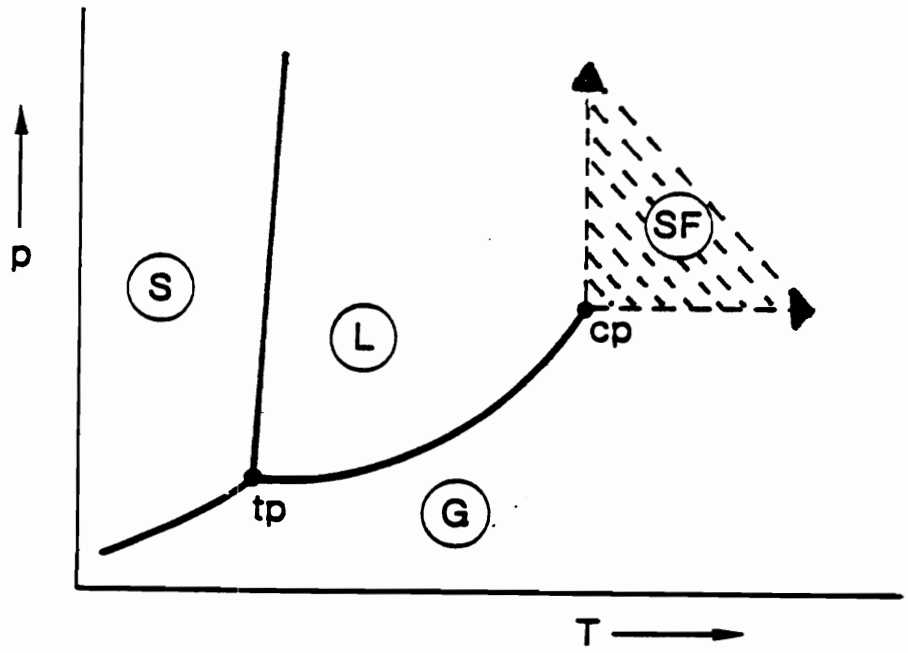

Figure 1. **PHASE DIAGRAM FOR A PURE COMPONENT.**

P.Shoenmakers,'Mobile and Stationary Phases for SuperCritical Fluid Chromatography', Philips R&D, Private Communication

TABLE 1

CHROMATOGRAPHIC MOBILE PHASE PROPERTIES

MOBILE PHASE	DENSITY (g/ml)	VISCOCITY (POISE)	DIFFUSIVITY (CM2/S)
GAS	0.001	5E-5 - 3.5E-4	0.01-1.0
SCF	0.2-0.9	2E-4 - 1E-3	3.3E-4 - 1E-5
LIQUID	0.8 - 1.0	0.003 - 0.024	5E-6 - 2E-5

Lauer, H., McMangill. D., Board, R., Anal. Chem., 55, 1370, 1983

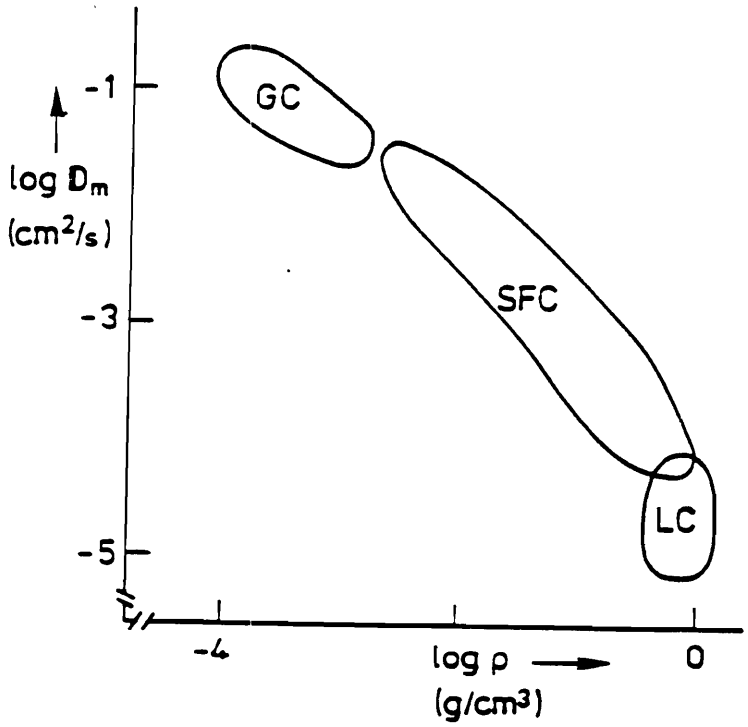

Figure 2. **ILLUSTRATION OF DIFFUSION COEFFICIENTS FOR COMMON MOBILE PHASES**

P.Shoenmakers,'Mobile and Stationary Phases for SuperCritical Fluid Chromatography', Philips R&D Private Communication

diffusivities, the optimal linear velocities and the speed of analysis for the three mobile phases are: gases > SCFs > liquids.

DENSITY. Liquids, at a density of 1.0 g/mL, are ca. 1000 times as dense as gases, whereas supercritical fluids' densities range from 0.1 to 0.9 g/mL. The densities of supercritical fluids are a function of their pressure; as the pressure is increased, the density increases. As the density increases the ability of the fluid to solvate analytes also increases. Temperature is another parameter that affects the density of supercritical fluids. Figure 3 shows the effect that temperature has on density as a function of pressure. These two physical parameters lead to a very unique and highly exploited property of SCF: the solvent strength of the fluid can be altered simply by changing the pressure or the temperature of the mobile phase. In contrast, to affect the solvating power of liquids, in HPLC the chemical composition of the solvent system must be changed; to affect similar chromatographic changes in gas chromatographic systems, a temperature ramp is used. Consequently, the gap between chromatographic systems utilizing liquids and gases is bridged because a supercritical fluid mobile phase can be applied to analytes that are difficult or unable to be processed by either liquid or gas chromatography. These compounds include thermally labile analytes that do not possess an extended chromophore.

The discussion of the physical properties of supercritical fluids points to the obvious conclusion that they make excellent mobile phases for chromatography. Their properties constitute the "best of both worlds" and give rise to a mobile phase that is solvating in nature, yet gas-like in chromatographic properties.

The substances that can be used for a supercritical fluid are determined by the pressure and temperature capability of the system that is being utilized. All that is

Figure 3. **THE EFFECT OF TEMPERATURE ON DENSITY AND PRESSURE FOR CO2**

Phelps, D., Amoco R&D, Private Communication

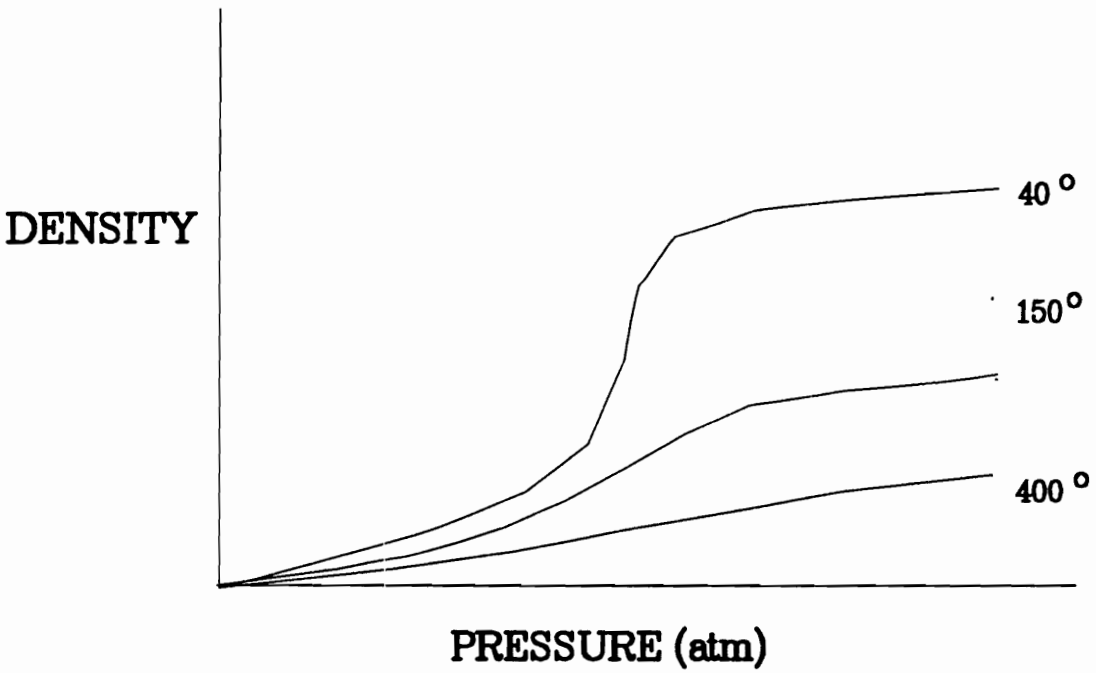

Figure 3. **THE EFFECT OF TEMPERATURE ON DENSITY AND PRESSURE FOR CO2**

Pheips, D., Amoco R&D, Private Communication

required is that the system operate above the critical temperature and critical pressure. Table 2 lists some mobile phases that are commonly used in SFC. The first listed is CO_2; it comprises >90% of the mobile phases currently employed. CO_2 is used for several reasons. Its toxicity level is one of the lowest on the list, and its critical points are easily accessed by common GC and HPLC equipment. Figure 4 is the triple point diagram for pure CO_2 which shows the Pc to be 73 atm. and the Tc to be 31°C. These critical parameters can be reached easily by slight modifications of gas and liquid chromatographic equipment (8). Another advantage is that CO_2 is a gas at ambient temperature and pressure. This is especially relevant in the area of supercritical extractions, where the mobile phase used for the separation dissipates upon exiting the restrictor and only the analyte(s) remain.

There is, of course, a critical drawback to using CO_2 as the mobile phase--its extreme low polarizabililty decreases the range of samples that are soluble. However, the solubilizing power of CO_2 can be increased by adding modifiers (methanol, water) and additives (formic acid), which greatly enhance the polarity of CO_2 (9-13).

APPLICATION TO ANALYTICAL CHEMISTRY

SCFs can be applied to many of the problems that face analytical chemists; three in particular are explored in this dissertation: trace analysis, the quantitation of the composition of crude oils and petroleum distillates, and the extraction of trace contaminants in soil with a supercritical fluid mobile phase.

TRACE ANALYSIS. Although trace analysis is an integral process of analytical chemistry, environmental concerns, spurred by the increased production of highly toxic

TABLE 2

PHYSICAL PARAMETERS OF SUPERCRITICAL FLUID

FLUID	CRITICAL TEMPERATURE T_c (^{0}C)	CRITICAL PRESSURE T_p (atm)	DIPOLE MOMENT μ
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
FREON	111.8	40.7	0.17

Hoyer, G., Chemtech, July 1985

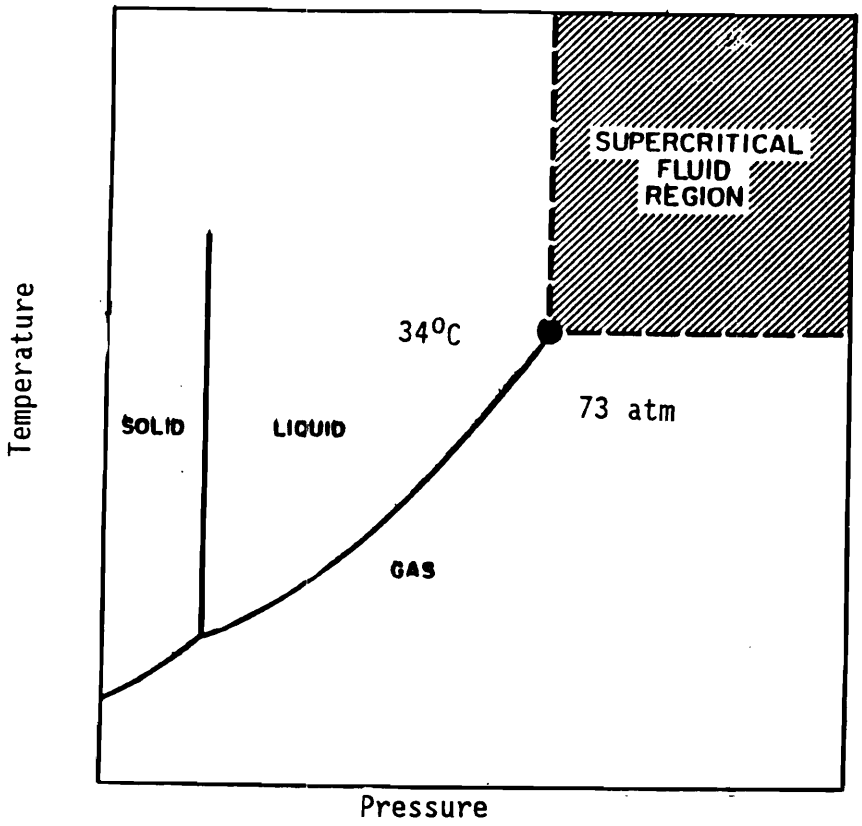

Figure 4. **PHASE DIAGRAM FOR PURE CARBON DIOXIDE**

Jentoft, R. Gouw, T., Anal. Chem., 44, 681, 1972

and carcinogenic compounds, have increased its importance. Trace analysis is usually performed in a "wet" fashion; that is, several extractions are performed, and each is followed by a concentration step.

One method that has little or no sample handling is on-column or *in-situ* concentration. This refers to condensing the analyte of interest at the head of the column from a dilute solution injected onto the column. The idea is similar to cyrogenic focusing in gas chromatography (14,15) and solvent focusing or analyte enrichment techniques in liquid chromatography (16,17). Solvation is the principle mechanism of retention in supercritical fluid mobile phase (18,19). Because solvation is a function of density, if a dilute solution of analyte is injected into a SFC system at a density below the elution density, then the analyte will be condensed at the head of the column. The elution density refers to that density at which a particular analyte elutes from the column. Elution pressure can be used in place of elution density as long as the temperature of the system remains constant. This permits for multiple injections (assuming that the solvent elutes at this low pressure) of the solution into the system. After the multiple injections, the system pressure can be elevated above the elution pressure of the analyte and chromatography can be continued in a normal fashion. This concept will be explored later in this dissertation in an investigation of several environmentally important compounds.

QUANTITATION. The composition of crude oils and petroleum distillates is another important issue of concern, particularly to chemists in the petrochemical field. One technique that is used extensively in the petrochemical industry is simulated distillation (SIMDIS). SIMDIS (20) is a technique that can show not only boiling point distribution, but other physical properties of petroleum products. It is based on a

relationship between retention time and boiling point. With conventional capillary gas chromatography using short columns and oven temperatures up to 450°C, the boiling range of chromatographed compounds reaches ca. 1400°F (21,22). It has been reported that petrochemical samples will degrade or "crack", above 360°C, although some reports have stated that this temperature is as low as 250°C. In 1986 and 1987, Schwartz and Brownlee (23,24,25) showed that SFC gives SIMDIS of components up to a boiling point of 1426°F, with an analysis temperature of only 100°C. The TGA analysis in the same paper reports sample degradation starting at 370°C. Using capillary SFC and a flame ionization detector, the authors reported relative response factors that are constant over the range of compounds used in their study (Table 3). They also showed good agreement between the SFC analysis and the fluorescent indicator adsorption method (ASTM D1319). Smith and Wright (26) demonstrated good resolution in the separation of middle distillate fuels. In another paper on oil residue group type analysis, Griebrokk and Lundanes (27) reported good separation using packed or capillary columns. Their quantitative study showed that SFC results for the separation of the sample into saturated, aromatic, and polar compounds compared well with the results from the tandem technique of HPLC gravimetric analysis (Table 4). Lee and Campbell (28) showed the ability of supercritical fluids to perform fractionation on petroleum and coal derived mixtures with the actual analysis carried out on a capillary gas chromatograph. Lee et al. (29) used supercritical fluid fractionation with FID detection for separating the sulfur heterocycles out of a catalytically cracked petroleum sample. In both of these papers there was no quantitative data. Using on-line multidimensional SFC/GC and employing the technique of heart cutting, Levy and Guaowski (30) showed group type separations with the SFC followed by excellent high resolution gas chromatographic

TABLE 3

RESPONSE FACTORS FOR CAPILLARY SFC WITH FLAME IONIZATION DETECTION

COMPOUND	VOLUME IN TEST MIXTURE (μL)	RELATIVE RESPONSE FACTOR
PENTANE	200	0.95
TRIMETHYLPENTANE	400	1.00
NONANE	200	0.96
HEPTENE	200	0.97
NONENE	200	0.95
BENZENE	200	1.24
TOLUENE	200	1.24
t-BUTYLBENZENE	200	1.06

Schwartz, H., Brownlee, R., J. Chrom., 653, 77, 1986

TABLE 4

COMPARISON OF WEIGHT (LC) AND FID AREA (SFC) OF THE SATURATED, AROMATIC, AND POLAR FUNCTIONS OF TWO HIGH BOILING RESIDUES OF NORTH SEA OIL

FRACTION	OIL A		OIL B	
	weight %	FID area %	weight %	FID area %
Saturated	44.5	45.6 ± 0.6	57.6	59.6 ± 0.6
Aromatic	35.4	35.3 ± 1.1	28.7	27.1 ± 0.6
Polar	20.0	19.1 ± 1.2	13.6	13.2 ± 1.1

Griebrokk, T., Lundanes, E., J. Chrom., 349, 439, 1985

analysis with FID detection of the heart cut. Another contribution from Lee (31), which gave results similar to the Schwartz paper (23), showed fairly linear response factors for several model compounds. Although these studies presented some elegant work with quantitation, the molecular weight range was not large.

EXTRACTIONS WITH A SUPERCRITICAL FLUID. SuperCritical fluids are excellent mobile phases for extractions. Supercritical fluid extraction is not a new technique (33), but it is now entering a revitalized period of use in the food industry (34-38) and in analytical chemistry, as evidenced by the devotion of an entire issue of the Journal of Chromatographic Science to extractions with supercritical fluids (39) and the appearance of several recent review articles and chapters dealing with supercritical extraction (40). The use of supercritical extraction for the recovery of organic pollutants has been the subject of several elegant analyses. Hawthorne and Miller (41) showed excellent results of supercritical fluid extraction of several polynuclear aromatics from several matrices, including Tenax-GC traps and air-dried soils. In another publication, Hawthorne and Miller (42) demonstrated the ability of a supercritical fluid to extract polynuclear aromatics from several matrices, with efficiencies above 95%. They also showed that some of the higher molecular weight PNAs could not be extracted from the matrices using pure CO_2; rather, a mobile phase modifier was required. In these two studies the analysis was accornplished with off-line capillary gas chromatography. In a 1989 article, Hawthorne, Miller, and Krieger (43) used directly coupled SFE/GC to evaluate the technique for extracting and analyzing organic analytes from several matrices. Another article on multidimensional supercritical extraction by Levy (44) showed excellent recoveries, > 90%, for polynuclear aromatics extracted from alumina beads with SFE/GC on-line and off-line. Several other articles (45-48) also addressed

this technique. Schantz and Chesler (49) showed recoveries of > 88% for several polynuclear aromatics using CO_2 for extractions from dried sediment. They trapped the extractate inside an octadecyl column (after the extraction cell and restrictor) and analyzed it by LC. In the case of GC analysis, the effluent from the second column was first concentrated and than analyzed. This work, done with pesticides and involving supercritical fluids as the mobile phase, dealt mainly with chromatographic separations (50). The on-line and off-line techniques showed the power of extractions of differant analytes with supercritical fluids.

The research undertaken for this dissertation explored each of the three problems described above. The first objective was to investigate the validity and ease of using *in-situ* concentration. The second was to investigate the feasibility of using a home-built system to study the reproducibility and quantitation of normal paraffins over a range of C_{10} through C_{60} carbon units, which represents the light-mid range of a crude oil. The reproducibilities were measured by relative standard deviation and the response factors were defined as the area counts divided by the concentration. The response factors for a non-split LC type of injection should be comparable to the results of on-column injection in gas chromatography (32). The F test using pooled standard deviations was employed to look for significant differences in groups of data. The last objective was to determine the practicality of using SFE for the recovery of polynuclear aromatics and pesticides using a unique resistively heated collection device designed to circumvent the problem of plugging typically experienced with damp soils or troublesome matrices. The final step in this last objective was to use supercritical fluid extraction on a damp waste land fill soil sample and to use capillary gas chromatography flame ionization and mass spectral detection for identification. The three studies undertaken to address these objectives are

described in the next chapter; the instrumentation and experimental design are presented for each study in turn.

EXPERIMENTAL

A. *In-situ* CONCENTRATION

Instrumentation. Most of the instrumentation used in this dissertation was assembled in house. Figure 5 shows a schematic of the system that was used in all three studies. The mobile phase was CO_2 (SFC grade), obtained from a stainless steel tank fitted with a dip tube from Scott Specialty Gas (Plumsteadville, Pa.). The mobile phase was passed through a 300 mL stainless steel high pressure bomb filled with 50% zeolites (Fisher Scientific, Fairlawn, N.J.) and 50% activated charcoal (Supelco, Bellefonte, Pa.). The pump was an Isco model 500 μLC syringe pump (Lincoln, Ne.) that was controlled by an analogue pressure controller (built by the Chemistry Department electronics shop, Virginia Tech) that allowed pressures up to 10,000 psi with three available pressure ramps and three isoconfertic points. The mobile phase, once it was pressurized, passed into a Valco four port high pressure 0.1 μL internal loop LC injection valve (VICI Houston, Tx.). The CO_2 moved into the heated zone, which in all experiments was a GC oven, where it became supercritical. All connecting tubing to this point was 0.001 inch stainless steel tubing (Supelco, Bellefonte, Pa.). The column used for the *in-situ* concentration and the quantitative study was a reversed phase octadecyl microbore column (1mm X 15cm) from Keystone Scientific (Bellefonte, Pa.). The *in-situ* concentration study used a 15 μm I.D. by 15 cm section of untreated fused silica from Polymicro Technologies (Phoenix, Az.) as the column restrictor. This was positioned ca. 2 cm below the flame. The detector used was the flame ionization unit on a Perkin-

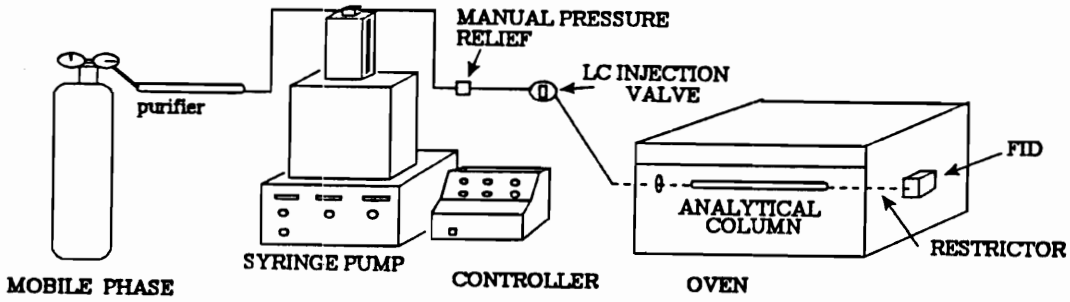

Figure 5. **INSTRUMENTAL SCHEMATIC FOR SUPERCRITICAL FLUID CHROMATOGRAPHY**

Elmer model 2000 gas chromatograph (Norwalk, Ct.). The conditions for the detector fuel gas flow rates were 350 and 35 mL/min air and hydrogen respectively; the detector temperature was 350°C.

Sample Preparation. Eicosane, 157 mg, (Aldrich, Milwaukee, Wi) was added to 10 mL (13.25 g) methylene chloride to make a 1.19% solution which was injected at 1070 psi; the pressure increased to 5000 psi at a rate of 100 psi/min. The 1.19% was serially diluted 1:10 six consecutive times with methylene chloride to give a 11.9 ppb solution.

For the *in-situ* concentration study the 11.9 ppb solution was injected six times while holding the system pressure at 1070 psi. This was accomplished by injecting, waiting for 10-15 seconds while the sample valve was flushed with CO_2, returning the valve to the load position, and repeating the procedure five times. After the injections were completed the pressure was increased at a rate of 100 psi/min to 5000 psi.

Pentachlorophenol (PCP)(92.7 mg) was added to 10 mL (13.25 g) of methylene chloride to make a 0.7% solution that was chromatographed from 1070 psi to 5000 psi at 100 psi/min to insure that no analyte signal was seen. The 0.7% solution was serially diluted 1:10 six times consecutively to give a 7 ppb solution.

The 7 ppb solution of pentachlorophenol was injected seven times using the procedure for the eicosane *in-situ* concentration technique. Once the seven injections were complete the pressure was increased from 1070 psi to 5000 psi at a rate of 100 psi/min.

Dursban (53 mg) was added to 10 mL of methylene chloride to give a solution that was 0.4%. This was chromatographed as was the 0.7% solution of PCP and then serially diluted 1:10 six times consequently to give a 4 ppb solution of Dursban in CH_2Cl_2.

The 4 ppb solution of Dursban was injected six consecutive times using the on-column concentration technique used in the eicosane and pentachlorophenol experiments. Once the six injections were complete the pressure was raised at 100 psi/min to a value of 5000 psi. The pressure was taken to 5000 psi for each analyte in order to chromatograph each compound, regardless of its elution pressure, without having to change chromatographic conditions.

B. QUANTITATION

Instrumentation. The instrumentation for the quantitation study varied depending on the experiment. The mobile phase, pump, controller, and internal loop injection valve were the same as in the *in-situ* concentration study. Along with the PE model 2000 two other gas chromatographs were used, the Hewlett-Packard (Avondale, Pa.) model 5880 and the Hewlett-Packard model 5790 with FID. The fourth system used was the commercially available Suprex model 200A SFC system, (Pittsburgh, Pa.). All restrictors were made from untreated polyimide coated fused silica from Polymicro (Phoenix, Az.), and used in various lengths and diameters, depending upon the individual experiment.

Normal paraffins were chosen for the standards components in the quantitation study because of their presence in most petrochemical samples and their easy availability in high purity. Solutions of hydrocarbons were made by dissolving the paraffin (Aldrich Chemical Co., Milwaukee, Wi.) in carbon disulfide (Aldrich Chemical Co., Milwaukee,

Wi.); 25.3 mg of eicosane (C_{20}) was dissolved in 100 mL of carbon disulfide to give a 200 ppm solution.

The first quantitative experiments performed focused on the reproducibility of the home-built supercritical fluid chromatographic system. This was accomplished by single component injection, single pressure chromatographic runs. The injection pressures were determined by injecting 0.1 μL of a 200 ppm solution of the analyte in CS_2 at 1070 psi followed by a pressure ramp of 100 psi/min to a final pressure of 5000 psi. As the analyte eluted, the pressure displayed on the Isco pump was recorded as the elution pressure.

The reproducibility was measured as the relative standard deviation (RSD) of analyte peak areas over a specified number of injections. The quantitative evaluation of the system was accomplished by calculating response factors (RF) of the analytes (the response factor is the peak area divided by the concentration in ppm).

During the initial reproducibility investigation, the instrument was configured (Figure 5) with a Hewlett-Packard model 5880 GC; the column restrictor was a 15 μm I.D. by 10 cm section of untreated fused silica, placed ca. 2 cm below the flame.

Sample Preparation. Eicosane, C_{20}, as a 200 ppm solution in carbon disulfide, was chromatographed six times at a pressure of 2100 psi with column oven and detector temperatures of 75 and 375°C, respectively.

To extend the molecular weight range of the study, seven injections of tetracontane, C_{40}, as a 200 ppm solution in CS_2 at 2650 psi were made followed by pentacontane, C_{50}, as a 200 ppm solution in CS_2 at 3150 psi, and finally five chromatographic runs of a 200 ppm solution of hexacontane, C_{60}, at 3500 psi. The oven and detector temperatures were 75°C and 375°C respectively.

Because the reproducibility of peak areas was poor, the single component, single injection pressure study was continued to include the effect of using two detector temperatures. Pentacontane (200 ppm) was injected three times at a detector temperature of 375°C and five times at a detector temperature of 400°C. All injections were made at 3100 psi with an oven temperature of 75°C.

The effect of injection pressure on the peak area reproducibility was studied using single component injections at two different pressures. With oven and detector temperatures of 75 and 400°C, respectively, pentacontane was chromatographed five times at 2400 psi and six times at 3400 psi, and tetracontane, 200 ppm, was chromatographed five times at 2100 psi and five times at 3000 psi.

To increase the breadth of the peak area reproduciblity study and to mimic more closely a petrochemical sample, a paraffin standard, C_{10}, C_{20}, C_{30}, C_{40}, C_{50}, C_{60}, was made up in carbon disulfide with each component at a concentration of ca. 200 ppm. This solution was chromatographed three times by injecting at 1070 psi and raising the pressure to 5000 psi at 80 psi/min, with the oven and detector temperatures at 75 and 400°C, respectively. To study the effect of pressure rate increase, the standard sample was chromatographed six times under indentical instrumental conditions, while the rate of pressure increase was changed to 120 psi/min. Continuing this study, four injections at a rate of 170 psi/min and three injections at a rate of 200 psi/min were carried out.

Although the previous experiment gave a "best" pressure rate increase, the reproducibilities of peak areas were still unacceptable when compared to those obtained with capillary GC. Thinking that the concentration of the standard might affect the reproducibility, two ten-fold serial dilutions of C_{10}-C_{60} standard were made, resulting in solutions containing 20 ppm and 2 ppm of the hydrocarbon standard. These were

individually chromatographed three times by injecting at 1070 psi and increasing the pressure at 170 psi/min to 5000 psi. The 2 ppm solution, although showing more linear response factors than did the more concentrated solution, gave poor peak area reproducibility.

In response to observations of the physical nature of the flame during the concentration study, the 15μm by 10 cm fused silica restrictor was replaced with a 29 μm I.D. by 100 cm section of untreated fused silica to decrease the velocity of the CO_2 exiting the restrictor, thus reducing the flame disturbance. To further stabilize the flame, the detector jet was bored out to a diameter of 0.022 inches and the hydrogen fuel gas flow rate was changed from 40 mL/min to 63 mL/min while the air remained at 310 mL/min. The restrictor was placed ca. 2 cm from the flame and the C_{10}-C_{60} standard (20 ppm) was chromatographed three times by injecting at 1070 psi and increasing the pressure at 170 psi/min to 5000 psi with an oven temperature of 150°C. The oven temperature was increased from 75°C to reduce the effects of density change during the pressure increase. The results showed the reproducibility of the peak area increased in comparison to the chromatographic analysis using the 15 μm by 10 cm restrictor.

Three additional restrictor positions were studied to investigate possible effects on reproducibility. The instrument and chromatographic settings from the previous experiment were used and the restrictor was placed 1.7 cm below the flame; the C_{10}-C_{60} standard, 20 ppm, was chromatographed three times from 1070 to 5000 psi at a rate of 170 psi/min. The standard mixture was then injected three times with the restrictor 1.9 cm below the flame and three times with the restrictor 2.1 cm below the flame. This experiment showed no significant difference between a restrictor position of 1.7 and 1.9 cm below the flame.

Because the flame gas flows for normal GC operation might not be optimized for SFC conditions, the effect of an increase in the fuel gas air flow rate was investigated by increasing the air flow from 310 mL/min to 385 mL/min while the other instrumental parameters remained unchanged from the previous experiment (restrictor position 1.7 cm below the flame). The 20 ppm C_{10}-C_{60} standard was injected at 1070 psi and the pressure increased at 170 psi/min to 5000 psi. The result showed a decrease in peak area reproducibility when compared to the chromatographic runs made with the lower air flow.

During the previous experiments it was observed that the physical characteristics of the flame changed during a chromatographic run, even though several parameters were altered. One parameter that could have been responsible was the CO_2 flow rate. To investigate changes in the CO_2 flow rate throughout the chromatographic run, the flow rate from the restrictor was monitored by turning the fuel gas off and monitoring the outflow of expanded gas from the detector. The oven and detector temperatures were held at 150° and 400°C respectively. This showed that the CO_2 flow rate did increase during a chromatographic run. This is logical since a higher pressure would compress a gas more, producing a larger volumetric flow rate at the eluting pressure (atmospheric).

To investigate the effect of the increasing CO_2 flow rate on the response factors, supercritical CO_2 make-up gas was added to detector. The C_{10}-C_{60} standard, 20 ppm, was chromatographed without CO_2 make-up gas using instrumental conditions from the previous experiment. With the same instrumental conditions, 29 mL/min of supercritical CO_2 make-up gas was added to the detector, and the C_{10}-C_{60} standard was chromatographed three times. This was followed by three runs under indentical conditions of the standard while 35 mL/min of make-up was added.

This experiment showed a response factor dependence on the flow rate of CO_2 through the detector. Investigating the possibility that detector geometry plays a significant role, an H-P model 5790 was used as the column oven and detector with the restrictor placed 1.7 cm below the flame and the collector ring of the detector 2.5 mm above the flame jet (manufacturers prescribed position). The 20 ppm C_{10}-C_{60} standard was injected three times at 1070 psi and the pressure increased to 5000 psi at 170 psi/min. The collector ring was lowered to 1.8 mm above the flame jet to test for a significant change in the peak area reproducibility and response factors. Using the same experimental procedure, the standard mixture was chromatographed three times. No significant change in the RFs or peak area reproducibility was noted.

In an earlier experiment, several parameters were changed to create a more robust flame; one of these changes was the increase of flame jet diameter. The result of that experiment showed increased reproducibility and a better linearization of the RFs. To further investigate the significance of the flame jet diameter, the Hewlett-Packard model 5880 was set-up with the best conditions to date: 29 μm I.D. by 100 cm restrictor positioned 1.7 cm below the flame jet and fuel gas flow rates of 63 and 310 mL/min for H_2 and air. The detector jet was further bored out to a diameter of 0.1 inches and the oven and detector temperatures set at 150 and 400°C. The C_{10}-C_{60} standard, 20 ppm, was chromatographed three times from 1070 and 5000 psi at a rate of 170 psi/min. No improvement was found over the previous conditions.

Using the 0.022 inch detector jet and the previous instrumental and chromatographic conditions, the quantitation of a "real world" sample was attempted using an internal standard and a response factor calibration curve. "Light fraction three" is a distillation fraction of a whole oil that encompasses compounds with carbon numbers of ca. C_{25}-C_{40}.

This fraction as a 0.05% solution in CS_2 with an eicosane spike of 0.019% was chromatographed from 1070 psi to 5000 psi at a rate of 170 psi/min.

The response factor of the C_{20} spike did not match those found in earlier experiments. To investigate the possibility that the concentration of the spike and sample were playimg a role, light fraction three and the eicosane spike were diluted with carbon disulfide to give the following solution concentrations, 1.05, 0.105, 0.019, 0.0078, 0.0019 and 0.0002%. These solutions were chromatographed from 1070 to 5000 psi at 170 psi/min.

The previous experiment showed the H-P 5880 detector to have a dependence on the analyte concentration. To study a new type of detector, the Suprex 200A SFC was configured with the best conditions to date. A 29 μm I.D. by 100 cm section of untreated fused silica was used as the restrictor and positioned 1.7 cm below the flame while the hydrogen and air fuel gas flows were set at 63 and 310 mL/min, respectively, and the oven and detector temperatures at 150 and 400°C. The C_{10}-C_{60} standard, 20 ppm, was chromatographed six times from 1070 psi to 5000 psi at a pressure increase of 170 psi/min.

No significant difference in the response factors was noted while implementing the new detector, and rather than testing another detector design, an alternative type of restrictor was investigated. Using the Suprex SFC system with the optimum conditions found to date (i.e., the same temperature and gas flows as in the previous experiment), a pulled (tapered) 50 μm I.D. section of untreated fused silica (Figure 6) replaced the 29 μm I.D. by 100 cm linear restrictor and was placed 1.7 cm below the flame. The pulled restrictor was made by heating an untreated 10 cm by 50 μm piece of fused silica with an oxybutane torch and slowly pulling the fused silica to a taper. The flow rate exiting the restrictor was controlled by cutting off different lengths of the tapered end. The 20 ppm C_{10}-C_{60}

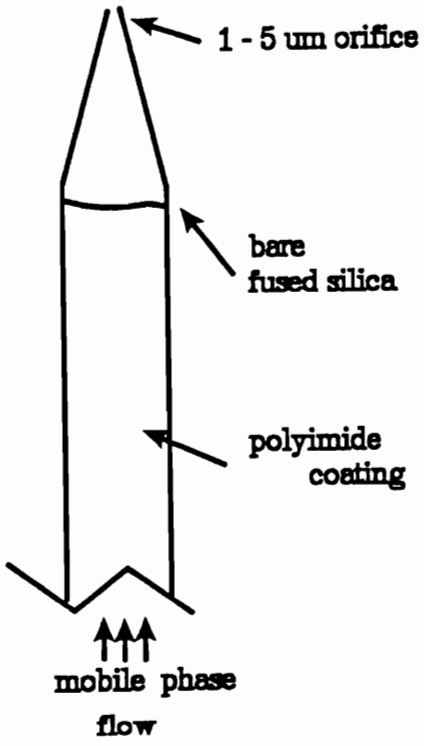

Figure 6. **SCHEMATIC REPRESENTATION OF A PULLED OR TAPORED RESTRICTOR**

standard was chromatographed three times from 1070 psi to 5000 psi at 170 psi/min with the results showing no improvement in response factors and a decrease in peak area reproducibility.

Because the experiments focusing on the detector end of the system had not solved the response factor problem or improved the reproducibility to that of a capillary GC, the injection system was investigated next. An HPLC type of on-column injector (Figure 7) was fitted to the Perkin-Elmer model 2000. This was accomplished by replacing one of the detector modules on the PE 2000 with the stop flow injector (SFI). With the top frit of the column removed, the column was attached as shown in Figure 7. The operation of the stop flow injector (Figure 8) showed that the flow of CO_2 was first stopped at valve 1; the stop slide was moved to the open position and the syringe was inserted 4 cm into the apparatus. This allowed the direct deposition of the analyte solution on the head of the column packing. When this was accomplished, the slide (Figure 7) was moved to the off position and CO_2 flow resumed. Using the chromatographic and instrumental conditions set in the previous experiment, 0.1 μl of the 20 ppm C_{10}-C_{60} standard was deposited on the head of the packing material in the column with a 1μL syringe and chromatographed from 1070 to 5000 psi at a rate of 170 psi/min. This was repeated three times. Reproducibilities were higher than in the previous experiment; however, a problem with the linearity of the response factors was evident as well.

C. EXTRACTION

Instrumentation. The instrumentation for the extractions was basically the same as that described in the previous two sections. Figure 9 shows the flow path of the CO_2 through

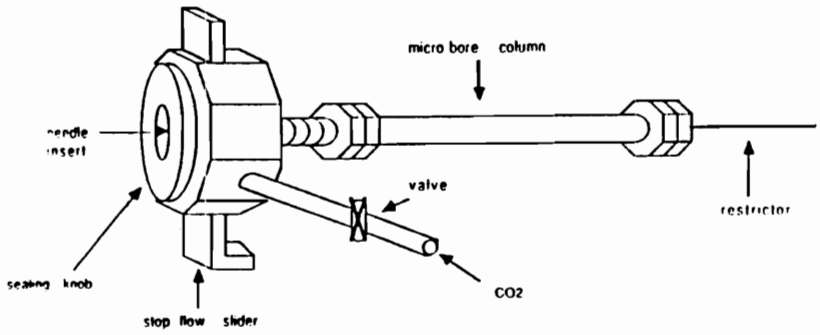

Figure 7. **SCHEMATIC REPRESENTATION OF AN ON-COLUMN INJECTOR FOR SFC**

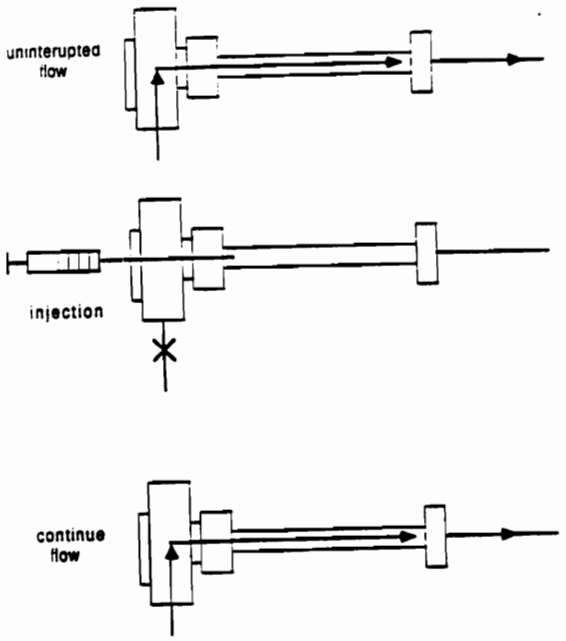

Figure 8. **SCHEMATIC REPRESENTATION OF THE MOBILE PHASE FLOW DIAGRAM FOR THE ON-COLUMN INJECTOR**

the instrument as set up for extractions. After being cleaned by molecular sieves and activated charcoal, and pressurized in the syringe pump, the CO_2 moves from the T valve into a Dickie (Auburn, IL.) high pressure on/off valve. From this point the extraction fluid moves into the Perkin-Elmer (Norwalk, Ct.) model 2000 gas chromatograph where it became supercritical. The CO_2 traveled into the extraction vessel, which was an ISCO (Lincoln, Ne.) stainless steel 4.6 mm I.D. by 10 cm column blank in the first experiments and a Dupont (Wilmington, De.) stainless steel 10 mm I.D. by 25 cm column blank in the soil extractions. The extraction matrix used depended upon the experiment. Ferro (Cleveland, Oh.) Microglass glass beads, 254 microns, and uncontaminated soil (Virginia Tech.) were utilized in the standard experiments, while contaminated soil (Virginia Tech.) was used in the soil extraction portion. After passing through the extraction vessel, the CO_2 traveled into the 50 μm I.D. by 15 cm tapered fused silica restrictor (PolyMicro Technologies, Phoenix, Az.) via a Valco (Houston, Tx.) zero dead volume butt connector and into the collector. The collector underwent several metamorphoses during the extractions, starting with the blank 0.5 dram boroslilicate vial (Supelco, Bellefonte, PA.), then moving to a pyrex collection vessel (see Figure 10) containing Baker (Phillipsburg, N.J.) HPLC grade methylene chloride, and ending with the resistively heated collector (figure 11) containing methylene chloride. This pyrex glass heated collector (Virginia Tech glass shop) was used in the extraction studies of soils. In all extractions where the CO_2 was bubbled through the accumulator, even though there was cooling due to the Joule-Thompson effect, there was some loss of liquid due to evaporation. To alleviate this problem and maintain a constant volume of solvent, an EM Science (Gibbstown, N.Y.), MACS 100 single piston reciprocating pump delivered 20 μL/min methylene chloride into the collector. The oven temperature was held constant

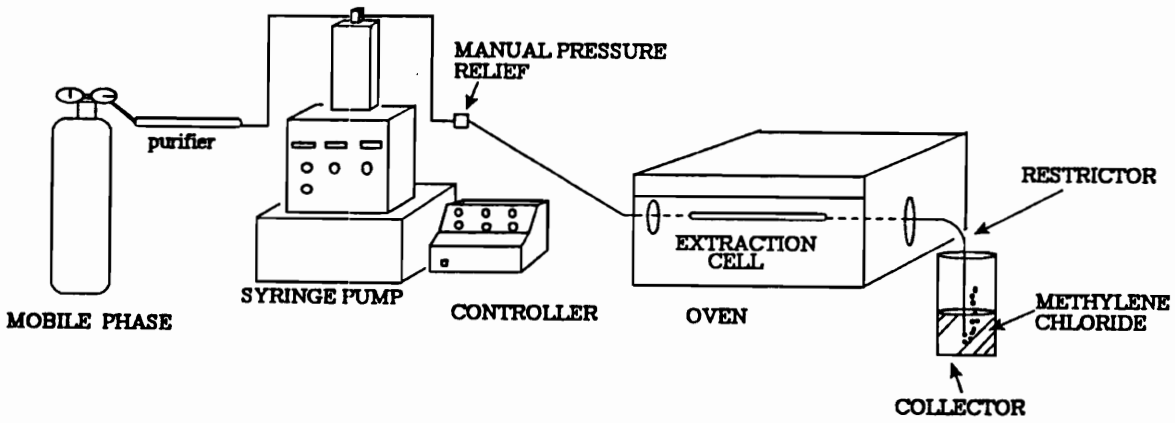

Figure 9. **SCHEMATIC REPRESENTATION OF THE INSTRUMENT FOR SUPERCRITICAL FLUID EXTRACTION**

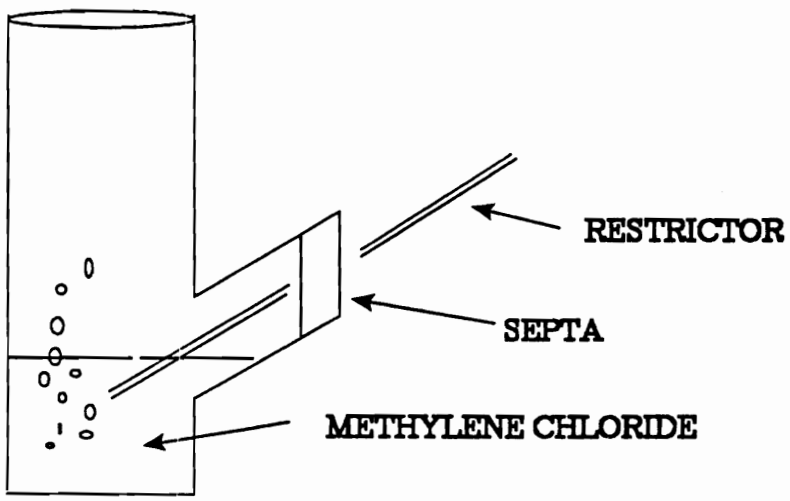

Figure 10. **SUPERCRITICAL FLUID EXTRACTION COLLECTOR**

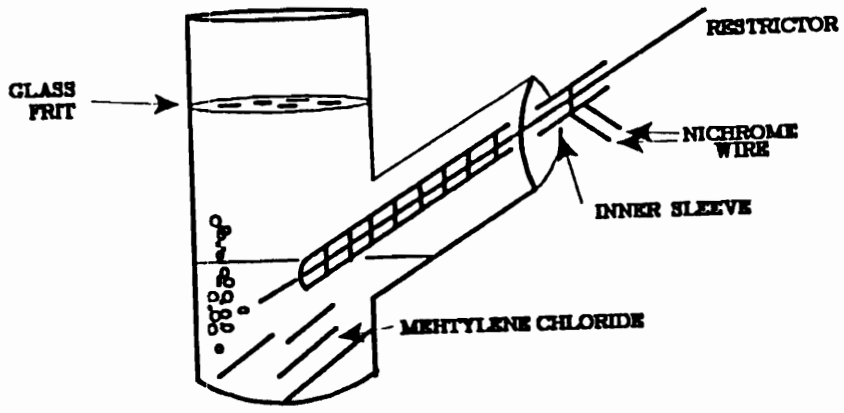

Figure 11. **RESISTIVELY HEATED SUPERCRITICAL FLUID EXTRACTION COLLECTOR**

at 60°C in all of the extractions for several reasons. At this temperature the higher densities are reached at a lower pressure than if the oven were run at 100 or 150°C. In addition the study involved the use of a supercritical fluid mobile phase as the extractor. A solvation mechanism is responsible for the removal of analytes. However, if the temperature of the oven is raised too high, volitization could also play a role in the separation.. The analyses for the fractions collected were done off-line on a Hewlett-Packard (Avondale, Pa.) model 5890 gas chromatograph with a Perkin Elmer (Norwalk, CT.) 530 μm I.D. by 25 μm fused silica capillary column with a stationary phase of 95% methyl, 5% phenyl polysiloxane, 1 μm film thickness. The gas chromatograph had an oven temperature profile of 50°C to 275°C at 7°C/min. The detector and injector temperatures were 275 and 300°C, respectively; the hydrogen and air fuel gas flows were 32 and 360 mL/min, respectively; the hydrogen carrier gas linear velocity was 40 cm/sec and the sample was run splitless (valve closed for 1 min).

Sample Preparation. The polynuclear aromatics (Figure 12) that were chosen as standards in the supercritical extraction study were selected because of their carcinogenicity as well as their widespread distribution in soils. The pesticides, Dursban and pentachlorophenol (Figure 12), were chosen because of their environmental importance in nature--Dursban especially has wide use as a pesticide. In the first experiment both types of standards were extracted from a glass bead matrix. The glass beads, which were unsilanized, were used because of their high activity and convenience.

Twenty-five microliters of a 0.38 mg/mL solution of anthracene [9.5 mg of anthracene (Aldrich Chemical Company Inc., Milwaukee, Wi.) in 25 mL HPLC grade methylene chloride] were deposited onto 1.9 grams of the glass bead matrix in the 4.6 mm I.D. by 10

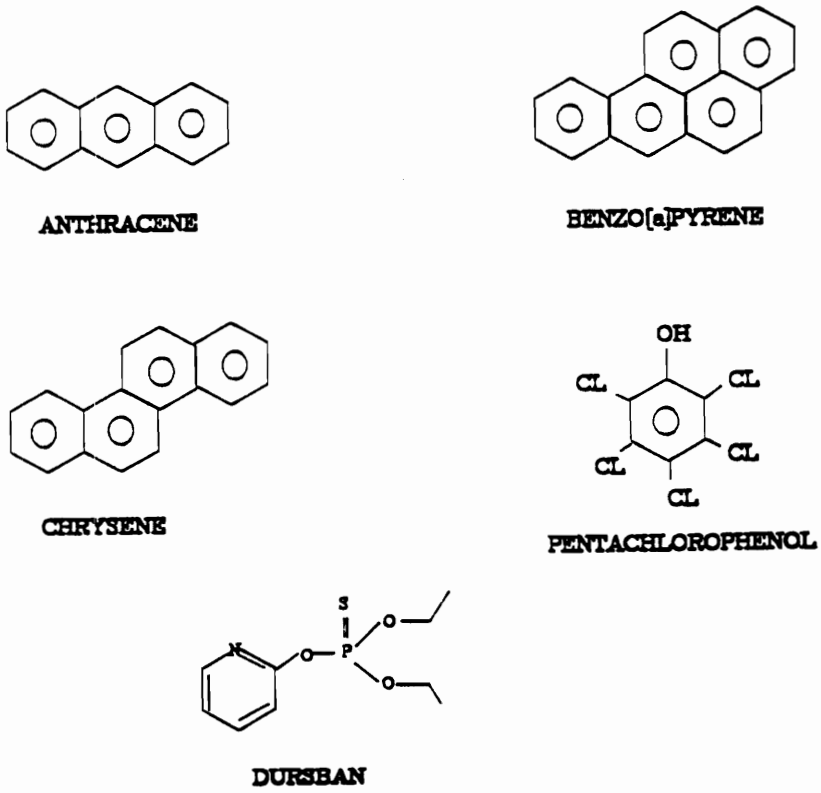

Figure 12. **POLYNUCLEAR AROMATICS AND PESTICIDES USED FOR THE MODEL COMPOUNDS IN THE SUPERCRITICAL FLUID EXTRACTION INVESTIGATION**

cm stainless steel column to give 5 ppm of anthracene. The CO_2 pressure was raised from 1070 psi to 5000 psi at 100 psi/min where it remained for 10 minutes while the oven was held at 60°C. The extractate was collected in a blank vial. After the extraction was complete, it was diluted with 1.2 mL of methylene chloride and chromatographed off-line on the Hewlett Packard 5890 gas chromatograph.

Twenty-five microliters of a 0.8 mg/mL solution of chrysene [20 mg of chyrsene (Aldrich Chemical Company, Milwaukee, Wi) in 25 mL of Baker HPLC grade methylene chloride] were deposited on the microclass glass bead matrix in the 4.6 mm I.D. by 10 cm stainless steel extraction vessel to give 10 ppm of chrysene. The CO_2 pressure was raised from 1070 psi to 5000 psi, at a rate of 100 psi/min, and was held for 10 minutes. The extracted material was collected in a blank 0.5 dram glass vial. When the extraction was complete it was diluted with 1.7 mL of methylene chloride. The quantitative analysis was completed using off-line capillary gas chromatography.

The last of the polynuclear aromatics that was investigated was Benzo[a]pyrene. Twenty five microliters of 0.7 mg/mL solution of Benzo[a]pyrene (17.4 mg of Benzo[a]pyrene in 25 mL of HPLC methylene chloride) were deposited on the glass beads (9 ppm), again using the same extraction vessel as in earlier experiments, and extracted, using the blank collection vial in the same fashion as in the previous experiment. After the extraction was complete, the analyte was diluted with 1.4 mL of methylene chloride and analyzed off-line using capillary gas chromatography under the same conditions as in the previous experiment.

The extraction efficiencies of the PNAs from the glass beads were poor, with all three recoveries below 30%. To see if solubility or something connected with the extraction

was the problem, or if the sample itself was causing the difficulty, a different class of compound was investigated--pesticides.

The first pesticide that was examined was the pyridine based phoshpothioate, Dursban. Twenty microliters of a 0.7 mg/mL methylene chloride solution of Dursban (17.4 mg of Dursban in 25 mL of Baker methylene chloride) were deposited on the glass bead matrix, 7 ppm, with the 4.6 mm I.D. by 10 cm column blank acting as the extraction vessel. Using the same extraction conditions as in the previous experiments, the extractate was collected in the 0.5 dram blank vial and quantitatively analyzed off-line by capillary gas chromatography with identical conditions as before.

The pesticide extractions, with recovery efficiencies worse than the PNAs, showed that the poor efficiencies did not result from a direct structural correlation but rather were caused by an extraction or collection problem. In each case the matrix was extracted a second time to insure that no material was left in the extraction vessel. No detectable analyte was found in the second extractate. To change the conditions of collection, the collector was modified (Figure 10) and filled with methylene chloride. Trapping the analyte was accomplished by placing the tapered end of the restrictor below the level of the methylene chloride and bubbling the analyte laden CO_2 through the liquid.

The first trial of the liquid accumulator (a term used to specify the material in the collection apparatus) consisted of deposition of 25μL of a 0.8 mg/mL methylene chloride solution of chyrsene onto the glass beads. Using methylene chloride as the accumulator in the modified pyrex collector, the glass beads were extracted from an initial pressure of 1070 psi to a final pressure of 5000 psi at 100 psi/min with a 10 minute hold time at 5000 psi. The analyte, in 0.32 mL of methylene chloride, was analyzed off-line with capillary gas chromatography using conditions identical to the previous experiments.

The second extraction of chyrsene into the liquid accumulator was identical to the previous experiment except for the amount of accumulator utilized: 0.7 mL. The last two experiments showed that the problem had been in the collection method rather than in the extraction. To investigate further, the other two PNAs were extracted with the pesticide Dursban.

Twenty-five microliters of 0.38 mg/mL methylene chloride solution of anthracene were deposited on the glass bead matrix (5 ppm), utilizing the 4.6 mm I.D. blank column extraction vessel. The supercritical extraction was carried out at an oven temperature of 60°C; the pressure program was 1070 psi to 5000 psi at a rate of 100 psi/min with a 10 min isoconfertic point at 5000 psi. The accumulator was methylene chloride, and the final volume being 1.3 mL. The quantitative analysis was carried out with the same conditions as used in the previous experiments.

The second trial of the extraction efficiency of anthracene was carried out with a deposition of 25 μL of a 0.38 ng/mL methylene chloride solution on the glass beads. The extraction was carried out under indentical chromatographic and instrumental conditions as the previous extraction of anthracene. The analysis again was carried out by capillary gas chromatography; the final volume of accumulator was 1.3 mL.

The third polynuclear aromatic, benzo[a]pyrene, was deposited on the glass beads to give 9 ppm (25 μL of a 0.7 mg/mL methylene chloride) solution. The extraction was carried out under identical chromatographic conditions as those for anthracene extractions. The analysis was carried out in the same manner as the anthracene extractions, except that the final dilution of the analyte was 1.5 mL methylene chloride.

In the second trial for the extraction efficiency of benzo[a]pyrene, the chromatographic and instrumental conditions for the extraction and off-line analysis were identical to the

first trial for benzo[a]pyrene. The final volume of methylene chloride was, however, different than the first--1.2 mL instead of 1.5 mL.

The three PNA extractions, all with efficiencies above 85%, proved that the problem that occured in the initial investigation was a collection problem, at least for the aromatics. To complete the investigation, Dursban as a solution in methylene chloride was extracted twice using methylene chloride as the accumulator.

Fifty microliters of the methylene chloride solution (0.66 mg/mL) of Dursban were deposited on the glass beads matrix (17 ppm) and extracted at 60°C by pressure programming from 1070 psi to 5000 psi at 100 psi/min with a ten minute isochoric period at 5000 psi. The final volume of methylene chloride in the collection apparatus was 1.2 mL and was analyzed off-line by capillary gas chromatography using an oven temperature profile of 50°C to 275°C at 7°C/min with an injection of 1 ul. The detector and injector were at 275 and 300°C, and the hydrogen carrier flow was at 35 cm/sec.

The second trial for the extraction efficiency of Dursban consisted of the deposition of 50 μL of the 0.66 mg/mL methylene chloride solution onto the glass bead matrix. Extraction was accomplished with a pressure program of 1070 psi to 5000 psi with a rate increase of 100 psi/min and a ten minute isochoric point at 5000 psi. The gas chromatographic off-line analysis was executed under conditions identical to the first Dursban extraction.

The two pesticide trials showed that in those extractions using the blank vial as the collector, the collection step was the most critical problem associated with the poor extraction efficiencies.

The next step in the extraction study was to use a matrix that was more environmentally relevant. The soil was taken from the ground outside the Virginia Tech chemistry

department and was pre-extracted with the equivalent of 25 mL of liquid CO_2 at 5000 psi with the oven temperature at 60°C before use. The standards used in the extraction efficiencies from soils were identical to those used in the glass bead extraction efficiency study, with the addition of a second pesticide, pentachlorophenol. During the initial runs of the soil sample it was observed that the restrictor was being plugged by the formation of ice. The restrictor was warmed above ambient temperature and the flow resumed. To prevent further ice plugs from forming, the collector was modified by adding resistive heating. This was accomplished by using a 30 cm piece of nichrome wire that was wrapped around a 100 mm I.D. by 65 mm long inner glass sleeve (Figure 11) which, when 15 volts were applied with a variable voltage source, gave an inner glass sleeve temperature of 65°C. The inner glass sleeve was protected from the rest of the collector by a 4 mm I.D. by 58 mm long section of pyrex. The outer glass sleeve was held to the pyrex collector by a teflon tape wrap.

Twenty-five microliters of a 0.55 mg/mL methylene chloride solution (13.75 mg anthracene in 25 mL Baker HPLC grade methylene chloride) of anthracene were deposited on 2 grams of soil to give a 7 ppm sample. The CO_2 pressure was raised from 1070 to 5000 psi at a rate of 100 psi/min, with a ten minute isochoric point at 5000 psi. The resistively heated collector with 1.7 mL of Baker HPLC grade methylene chloride was used as the accumulator. The off-line quantitative capillary gas chromatograph used an oven profile of 50°C to 275°C at rate of 7°C/min with a 1 μL injection. The detector and injector were at 275 and 300°C, respectively, with the average linear carrier gas velocity for helium of 35cm/sec.

The second trial of the anthracene extraction was accomplished by depositing 25 μL of the 0.55 mg/mL methylene chloride solution on the soil (7 ppm) and extracting in the

same manner. The only difference was the total volume of methylene chloride used (2.7 mL) as the accumulator. The off-line capillary gas chromatography was executed as described earlier. The results of the first extractions from soil (both > 98% recovery) showed excellent results. To complete this study, the remaining two standards were extracted from the same soil.

Twenty-five microliters of 0.8 mg/mL methylene chloride solution of chrysene were deposited on 2 grams of soil. The extraction mobile phase, CO_2 was pressure programmed from 1070 psi to 5000 psi at a rate increase of 100 psi/min with a ten minute isochoric plateau at 5000 psi. The volume of accumulator in the resistively heated collector was 2.3 mL. The off-line quantitative gas chromatographic analysis was carried out as described earlier.

The second trial for the chyrsene extraction was identical to the first, except that the volume of accumulator in the collector was 2 mL. The off-line GC analysis also was identical to the earlier trials. The results for the two chrysene trials showed excellent efficiency with an average value of 100%.

The third PNA tested was benzo[a]pyrene. Twenty-five microliters of the 0.7 mg/mL methylene chloride were deposited on the soil (8 ppm) and extracted from 1070 psi to 5000 psi at a rate increase of 100 psi/min with a ten minute isochoric hold at 5000 psi. The final volume of the accumulator in the heated collection tube was 2.5 mL. The off-line gas chromatographic analysis was carried out in an identical fashion as that for chrysene.

The second test of the extraction efficiency of benzo[a]pyrene from soil was executed by depositing 25 μL of the 0.7 mg/mL methylene chloride solution on the soil matrix and extracting by ramping the pressure from 1070 psi to 5000 psi by 100 psi/min with a 10 min

hold at 5000 psi. The off-line analysis was carried out as described earlier. The average recovery for the benzo[a]pyrene was 89%. This result and those for chrysene and anthracene evidenced an increase from the recovery efficiencies from the glass bead extractions. To complete this study, Dursban and pentachlorophenol were extracted from soil with an average recovery of 86 and 91%, respectively.

The PNAs and pesticides showed recoveries exceeding 85%. An important relationship would be to know the percent removal of analyte as a function of CO_2 volume.

Fifty microliters of a 0.4 mg/mL solution of anthracene were deposited on 2 grams of soil (10 ppm). The anthracene was extracted at 5000 psi with 10 mL of liquid CO_2. The extraction was continued, replacing the accumulator in the resistively heated collector, for an additional 10 mL of $CO_{2,(l)}$. Both fractions were analyzed off-line by capillary gas chromatography.

Since the supercritical extraction showed excellent recovery for PNAs and pesticides from soil, the next step was to use a "real" world sample. A sample of contaminated Virginia soil was obtained and was investigated not only for total contaminants but especially for the PNAs that have been studied in the Virginia Tech laboratory.

Thirteen grams of soil were placed in the 10 mm I.D. by 25 cm extraction vessel and extracted at 5000 psi with a total of 15 mL of CO_2. The final volume of the accumulator was 0.29 mL of methylene chloride. The off-line GC analysis was performed as described earlier.

The results of the contaminated soil extraction showed ca. 60 peaks (Figure 33). The retention times from capillary gas chromatography of three PNA standards (anthracene 19.7 min, chrysene 38.8 min, and benzo[a]pyrene 33.7 min) were compared to the soil extract for preliminary confirmation of these analytes in the soil. Three peaks of the

extraction matched the three standard times; the next step was to run standard spikes. Spiking was carried out by placing new soil in the extraction vessel and depositing the standard solutions of the PNAs onto the soil in three separate trials.

To the 13 grams of soil, 100 μL of the anthracene solution was added. The extraction was carried out by pressuring the system to 5000 psi and holding the system isochorically for 12 mL of $CO_{2(1)}$, and collecting the extractate in the accumulator in the heated collection tube.

This procedure was carried out two more times, once with 100 μL of the chrysene standard and once with the benzo[a]pyrene standard. In all three cases the off-line gas chromatographic analysis was done by programming the oven from 50 to 275°C at 7°C/min, with the injector and detector temperatures at 275 and 300°C, respectively.

The results confirmed the presence of the three PNAs in the soil. Final confirmation was made by matching the mass spectra of the soil extract with the mass spectra of the three PNA standards. This was done using the Hewlett-Packard 5890 gas chromatograph and 5970 mass selective detector with a temperature program from 35 to 275°C to 7°C/min.

RESULTS AND DISCUSSION

A. *In-situ* Concentration

The idea for *in-situ* concentration of trace quantities seemed logical from the basic mechanism of SFC separation. An analyte only moves through the column when the density of the system is above the elution density of the analyte. If the mobile phase is kept below the elution density for the analyte in question, then that analyte should remain at the head of the column. Accordingly, several injections could be made with the *in-situ* concentration of a very dilute solution and ultra trace analysis could be performed.

The first compound that was tried was a normal paraffin, eicosane (C_{20}). The pressure was maintained at 1070 psi for ten minutes and the open temperature was held constant at 60°C. After injection the pressure was increased at a rate of 100 psi/min to 5000 psi. Figure 13 shows the result of a single injection of a 7 ppb solution. The resultant chromatogram showed no peak at the appropriate time. Figure 13 also shows the peak resulting from six injections of the same solution using the *in-situ* concentration technique. After each injection, the valve was swept with CO_2 for 10-15 seconds to insure that the entire contents of the valve were removed. Not shown in Figure 13 are the six solvent peaks. Because the technique has proven itself as viable with a simple analyte, the next compounds chosen were those that are more relevant to environmental studies.

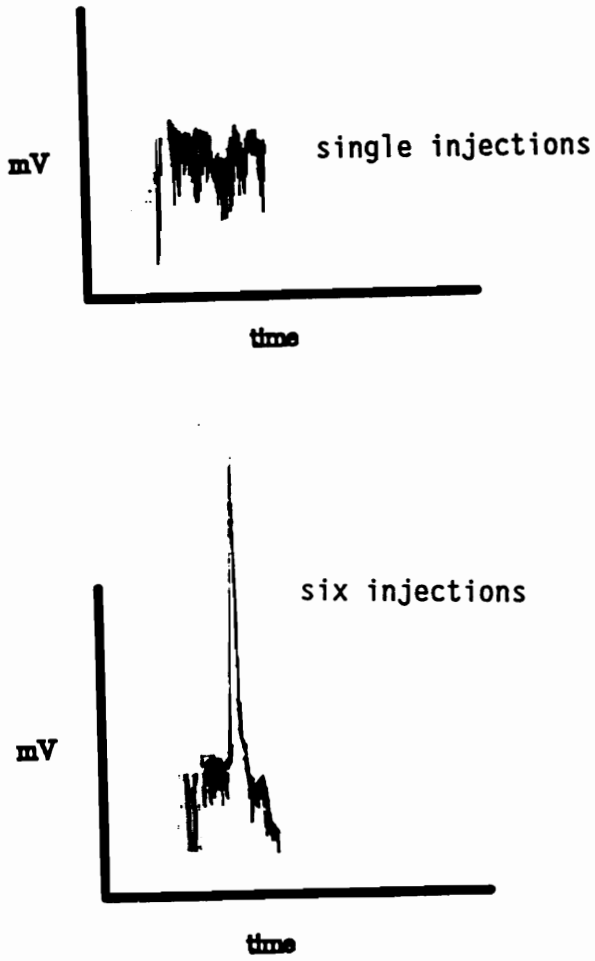

Figure 13. **SINGLE AND MULTIPLE INJECITONS OF A SEVEN ppb EICOSANE SOLUTION**

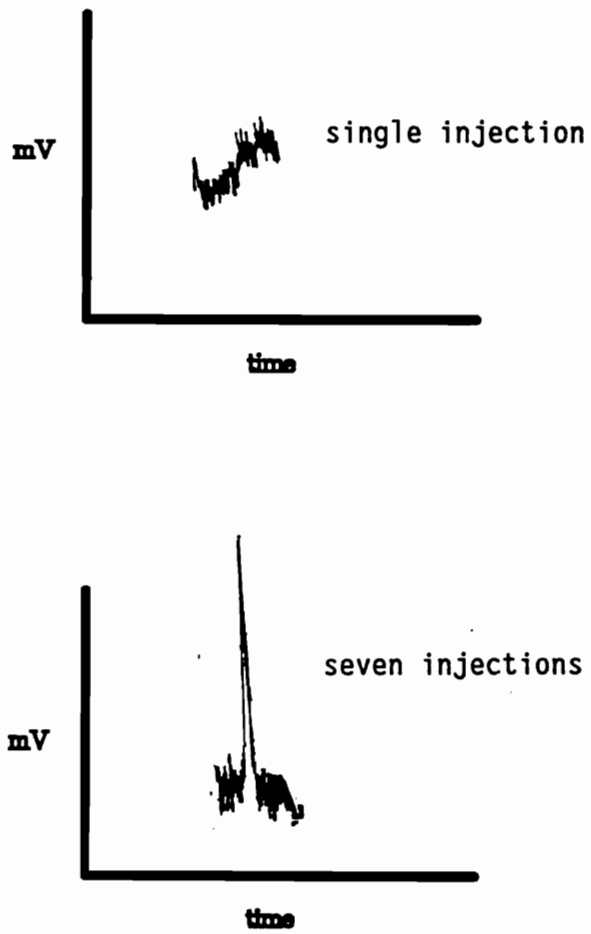

Figure 14. **SINGLE AND MULTIPLE INJECITONS OF A OF A SEVEN ppb SOLUTION OF PENTACHLOROPHENOL**

Figure 12 gives the structure for the two pesticides that were used, pentachlorophenol and Dursban. Again, with one injection of 7 ppb solution and with the same system parameters as with eicosane, no peak was seen. Seven injections of the 7 ppb solution of pentachlorophenol gave a peak that was greater than two times the noise level with the *in-situ* concentration technique (Figure 14).

The last pesticide that was investigated, Dursban, gave the same type of results (Figure 15) with the same system parameters used previously. A single injection gave no peak; injecting six times gave a peak that was 2 times the noise level.

The implications of this *in-situ* concentration technique are far reaching. It is a technique that extends the range of SFC to the ppb level for trace analysis.

B. Quantitation

During the initial tests for reproducibility utilizing single component/single pressure chromatography, it became apparent that the column restrictor had a critical position with respect to the flame. If the restrictor was placed too near the flame, the flame was extinguished. Conversely, if the restrictor was placed too far from the flame, > 3 cm, severe spiking occurred during the run. It was believed that spiking is the result of CO_2 particulate formation in the detector, which is a consequence of the Joule-Thompson cooling caused by the rapid expansion of the CO_2.

The first studies on reproducibility were made isochorically with the restrictor 2 cm below the flame. The pressure, 2100 psi, used in the single component injections of eicosane was obtained from the "elution pressure" experiments. The results of these chromatographic runs showed that when the pressure used was 1000 psi lower than the

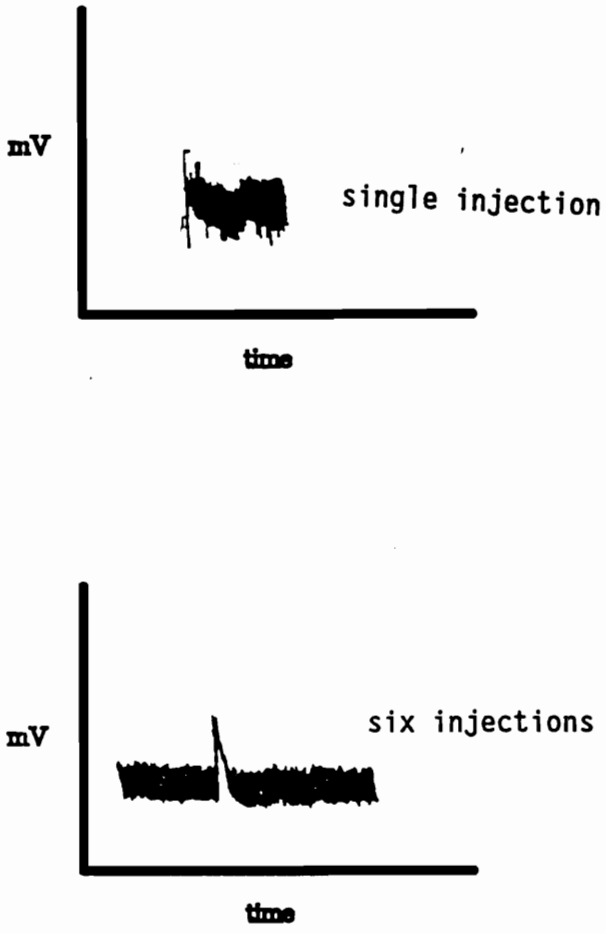

Figure 15. **SINGLE AND MULTIPLE INJECITONS OF A FOUR ppb SOLUTION OF DURSBAN**

elution pressure, the analyte eluted; however, the peak was very broad and elution time was as long as forty-five minutes. When the column pressure at the time of injection was greater than the elution pressure, the peak eluted on the tail of the solvent peak, which made quantitation impossible.

The result (Table 5) of the six replicate injections of eicosane at a single pressure showed a relative standard deviation (RSD) of 5%, which is a reasonable value when compared to a value of 0.5 to 3% RSD for GC or HPLC (depending on sample type and concentration)(52). The logical extension of this experiment was to extend the analyte molecular weight range, since a major advantage of SFC is its ability to elute higher molecular weight analytes. This served a twofold purpose: to test the reproducibility of higher molecular weight components at higher injection pressures, and to mimic more closely the type of components that are found in a petrochemical sample. Table 6 gives the results of C_{40}, C_{50}, and C_{60} hydrocarbons, injected at 2650, 3150, and 3500 psi, respectively. The RSDs for the three components were 4,7, and 11%, respectively. Statistically, using the F test, at a 90% confidence interval there was no difference between the C_{40} and C_{50}, and the C_{50} and C_{60} standard deviations. There was, however, an appreciable difference in precision of the C_{40} and C_{60} standards.

During the elution of the C_{50} and C_{60} an interesting phenomena ocurred. As the components eluted from the column into the detector the pump flow rate (as read from the digital display of the Isco μ500 LC) decreased. After the peak passed completely into the detector the flow rate rebounded to its original position. It was proposed that the presence of the analytes, possibly condensing from their previously solvated states, changed the density of the mobile phase and thereby decreased the CO_2 flow rate. To see if the mobile phase and the analyte in the restrictor were affected by the temperature,

TABLE 5

REPRODUCIBILITY OF EICOSANE PEAK AREA

2100 PSI, 60°C

RUN	AREA COUNT
1	1,563
2	1,600
3	1,511
4	1,491
5	1,025
6	1,545
mean	1456
standard deviation	21
RSD	5%

TABLE 6

REPRODUCIBLITIES OF HYDROCARBON PEAK AREAS AT CONSTANT PRESSURE

RUN	C_{40}(2650psi)	C_{50}(3150psi)	C_{60}(3500psi)
1	**168**	**209**	**171**
2	**177**	**209**	**159**
3	**179**	**220**	**202**
4	**178**	**182**	**168**
5	**178**	**208**	**202**
6	**189**	**221**	*
7	**168**	**196**	*
mean	177	206	181
standard deviation	7	13	20
RSD	4%	7%	11%

* no measurements taken

the detector temperature was increased to 400°C. The results (Table 7) show that a 350°C FID gave an RSD of 11%, while raising the temperature lowered the value to 5%. Statistically, again using the F test at a 90% CI, the two measurements were not significantly different. It was noted, however, that the increase in detector temperature reduced the flow rate change that was observed during the peak elutions. Because the 400°C isotherm on the pressure vs. density curve (Figure 3) was at a lower density range than the 350°C isotherm, it was apparent that the analyte was condensing out of the supercritical state and was relying on volatilization to move into the flame.

To investigate the effect of detector temperature on the flow rate and to see if a further increase in detector temperature gave a significant difference in RSDs, an increase of the detector temperature was needed. The geometry of the Hewlett-Packard model 5880 FID has the fused silica restrictor traveling upward through the center of the flame jet (Figure 16), which is also the path of the hydrogen fuel gas. Because the restrictor is not in contact with the wall of the flame jet, it is heated by thermal convection and may be at a lower temperature than the detector block. To increase the restrictor temperature the hydrogen fuel line (pre-detector) was heated to 400°C. This resulted in an extremely unstable flame that would not remain lit because the lower mass flow of the hydrogen at the higher temperature. Consequently, the air flow to the detector had to be lowered to 100 mL/min in order for the flame to remain lit. This was consistent with the idea that too little hydrogen was flowing into the detector. At this low level of air, the flame was positioned ca. 5-6 cm above the flame tip, which resulted in extremely poor sensitivity. These findings reiterate the fact that a detector's geometry requires specific detector flow rates to achieve a flame that will remain stable.

TABLE 7

REPRODUCIBILITY OF PENTACONTANE AT TWO DETECTOR TEMPERATURES AT A CONSTANT PRESSURE

RUN	375°C	400°C
1	1262	1537
2	1441	1579
3	1556	1691
4	*	1691
5	*	1710
mean	1420	1634
standard deviation	148	75
RSD	**11%**	**5%**

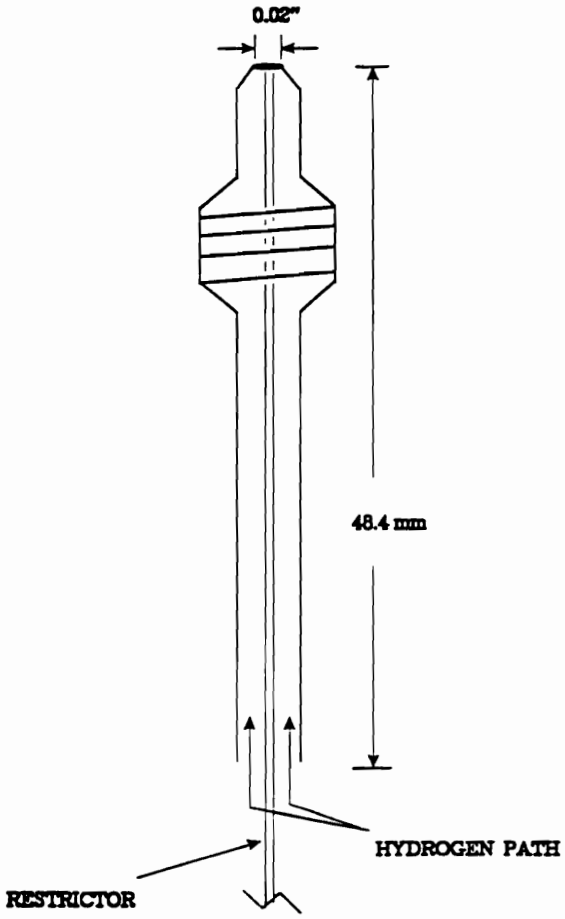

Figure 16. **HEWLETT–PACKARD MODEL 5880 GAS CHROMATOGRAPH FLAME JET SCHEMATIC**

After heating the hydrogen line, the instrument was returned to its original state and the eicosane standard solution was injected to test the integrity of the system. Spiking was observed; the physical shape of the flame was elliptical. The restrictor was removed and examined under an optical microscope, and it was discovered that it had been shattered and no longer terminated in a flat surface. Squaring the end returned the system to its original state.

The reproducibility (5-7%) to this point seemed reasonable for a standard at a single pressure. The next logical step was to examine the effect of injection pressure on reproducibility. Pentacontane, C_{50}, was chromatographed at two pressures, 2400.and 3400 psi; the results (Table 8) showed that at the higher pressure the RSD increased from 5% to 16%. Using the F test at 0.90 CI, the results were significantly different. The differentiation leads to the hypothesis that either the flow rate or the injection pressure had an effect on the reproducibility. To test this, tetracontane (C_{40}) was chromatographed at two pressures, 2100 and 3000 psi, with the results (Table 9) showing no statistical difference (RSDs of 5 and 4%, respectively). The last two experiments gave contradictory data. In the case of pentacontane, the injection pressure did seem to have an effect on peak area, whereas in the case of tetracontane, the injection pressure apparently did not play a role. Further investigation revealed that with injections at pressures above 3300 psi, a pressure "wave" occurred in the system resulting in a small peak on the chromatogram. The capacity factor of the pressure peak changed and sometimes co-eluted with the higher molecular weight analyte, resulting in differences in peak area and consequently high RSD. These results clearly showed that with the current instrument set-up, higher pressure injections are not possible.

TABLE 8

REPRODUCIBILITY OF PENTACOSANE AT 2400 psi AND 3400 psi

RUN	**2400psi**	**3400psi**
1	248	359
2	240	301
3	227	228
4	261	336
5	248	264
6	*	295
mean	245	297
standard deviation	12	47
RSD	**5%**	**16%**

* no measurement taken

TABLE 9

REPRODUCIBILITY OF TETRACONTANE AT 2100 psi AND 3000 psi

RUN	2100psi	3000psi
1	184	295
2	206	269
3	212	287
4	209	296
5	202	277
mean	202	285
standard deviation	10	11
RSD	**5%**	**4%**

The second portion of the quantitative study investigated the response factors and reproducibilities of a homologous series, which should, for a non-split injection technique, have the same value. The standard used was the result of a collaborative project between VPI Chemistry Department and Amoco Corporation Research and Development, Tulsa, Oklahoma. Amoco was interested in the feasibility of using SFC for distilled and crude oils. In order to mimic the paraffinic components of a distilled, or crude, petrochemical sample, the standard was made up of C_{10}, C_{20}, C_{30}, C_{40}, C_{50}, and C_{60} hydrocarbons in carbon disulfide. By employing this type of standard sample, the feasibility and reproducibility of a homebuilt SFC system could be investigated over a wide range of molecular weights, and a direct comparison of the chromatographic results from SFC and from simulated distillation by high temperature GC could be made.

To elute the standard in a reasonable time a pressure gradient (or ramp) had to be employed. The initial pressure ramp was 80 psi/min from 1070 psi to 5000 psi. The reproducibilities for the standard mixture (Table 10) showed very poor results when compared to the single component runs. The RSDs for the six components were 8, 20, 20, 18, 12, and 32%, respectively, with no apparent trend in the reproducibilities. One difference between the constant pressure runs and those with a pressure ramp was that the flow rate through the column changed during the pressure ramp.

If the pressure ramp were to be increased while the initial and final pressures remained constant, the flow rate through the system would have to increase. To investigate the effect that changes in flow rate had on the reproducibilities, ramps were run at 120, 170, and 200 psi/min. During this investigation, the restrictor position remained constant at 1.7 cm below the flame. The best reproducibilities, although still not good, were seen at a programming rate of 170 psi/min (Tables 11, 12, 13). This conclusion was verified

TABLE 10

REPRODUCIBILITY OF ALKANE STANDARD MIXTURE AT A PRESSURE RAMP OF 80 psi/min AND AN OVEN TEMPERATURE OF 75°C

	C_{10}	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	62	256	255	175	68	20
	57	201	184	135	54	36
	67	300	270	193	65	24
mean	63	253	237	168	63	27
standard deviation	4	50	46	29	7	9
RSD	**8%**	**20%**	**20%**	**18%**	**12%**	**32%**

TABLE 11

REPRODUCIBILITY OF ALKANE STANDARD MIXTURE AT A PRESSURE RAMP OF 120psi/min AND AN OVEN TEMPERATURE OF 75°C

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	308	300	195	98	50
	294	297	202	102	36
	268	289	235	212	133
	307	301	272	159	101
	299	295	239	180	119
	314	316	267	214	111
	307	282	198	162	85
mean	300	298	230	161	91
standard deviation	15	11	32	47	36
RSD	**5%**	**4%**	**14%**	**29%**	**40%**

TABLE 12

REPRODUCIBILITY OF ALKANE STANDARD MIXTURE AT A PRESSURE OF 170 psi/min AND AN OVEN TEMPERATURE OF 75°C

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	320	288	217	221	120
	280	289	218	224	114
	245	263	187	164	110
	287	269	219	224	148
mean	284	278	211	209	123
standard deviation	31	14	15	30	16
RSD	**11%**	**5%**	**7%**	**14%**	**13%**

TABLE 13

REPRODUCIBILITY OF ALKANE STANDARD MIXTURE AT A PRESSURE RAMP OF 200 psi/min AND AN OVEN TEMPERATURE OF 75°C

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	254	257	166	152	66
	126	164	138	176	150
	252	259	190	135	87
mean	211	227	165	155	102
standard deviation	73	54	26	21	43
RSD	**35%**	**24%**	**16%**	**13%**	**43%**

statistically by a 0.90 CI F test utilizing standard pools; no significant difference was evident between the 80 psi/min and the 120 psi/min with standard deviations of 75 and 71%. There was, however, a significant difference between 120 psi/min and 170 psi/min with standard deviations of 71 and 50%, respectively. The difference between 170 psi/min and 200 psi/min was significant with standard deviations of 50 and 108%, respectively. The RSDs for the 170 psi/min increase were 18, 11, 5, 7, 14, and 13%, respectively, and were acceptable for routine applications of SFC. The calculated response factors for the ramp runs are summarized in Table 14 and shown in Figures 17-20. The scatter of response factors showed that there was a large discrepancy across the molecular weight range; as the molecular weight increased from C_{20}-C_{60}, the response factors decreased. The difference from C_{20} to C_{60} in response factors was approximately 45%.

At this point, a peak area reproducibility problem coupled with the problem of decreasing response factors with increasing molecular weight existed.

It was noted earlier that a change in the flow rate occurred during peak elution of higher molecular weight paraffins. The increase in detector temperature from 350 to 400°C solved this problem. In the earlier investigation the paramount concern was the reproducibility of the system and not the response factors. If in that study there had been a constant loss of high molecular weight material in the restrictor or in the flame itself, the reproducibilities would have been good but the RFs would have been lower. To investigate, two dilutions of the standard C_{10}-C_{60} mixture, 1:10 and 1:100, were chromatographed from 1070 to 5000 psi at a rate of 170 psi/min. The use of 170 psi/min was based on the results of the last set of experiments. The reproducibilities were still very poor. The 1:10 dilution reproducibilities (Table 15) were 10, 9, 19, 25, and 33%,

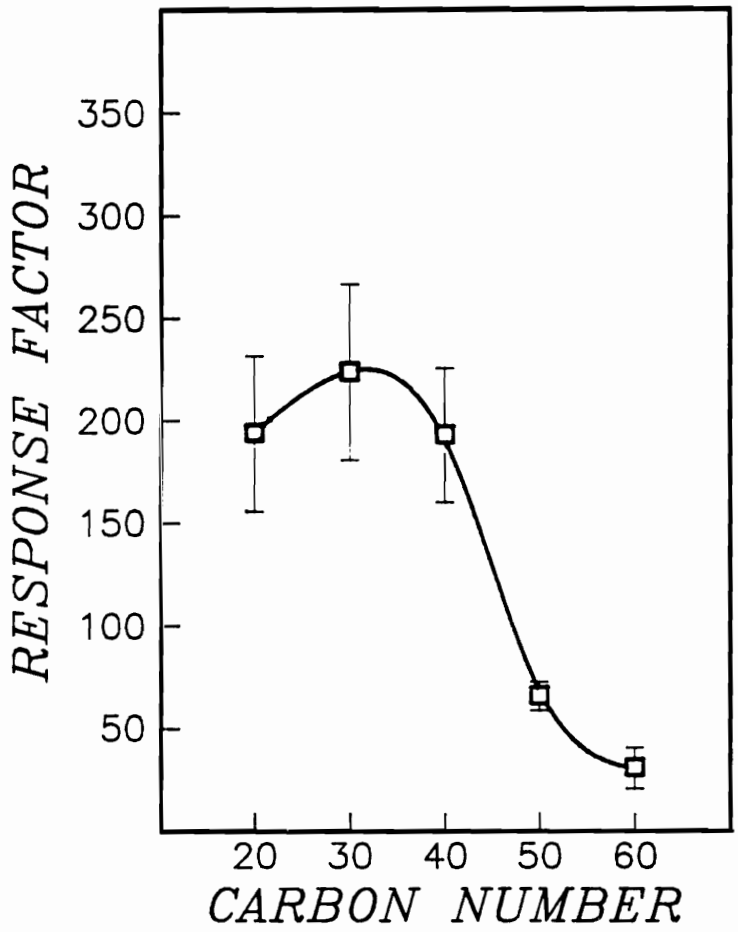

Figure 17. **RESPONSE FACTORS FOR A RAMP RATE OF 80 PSI/MIN**

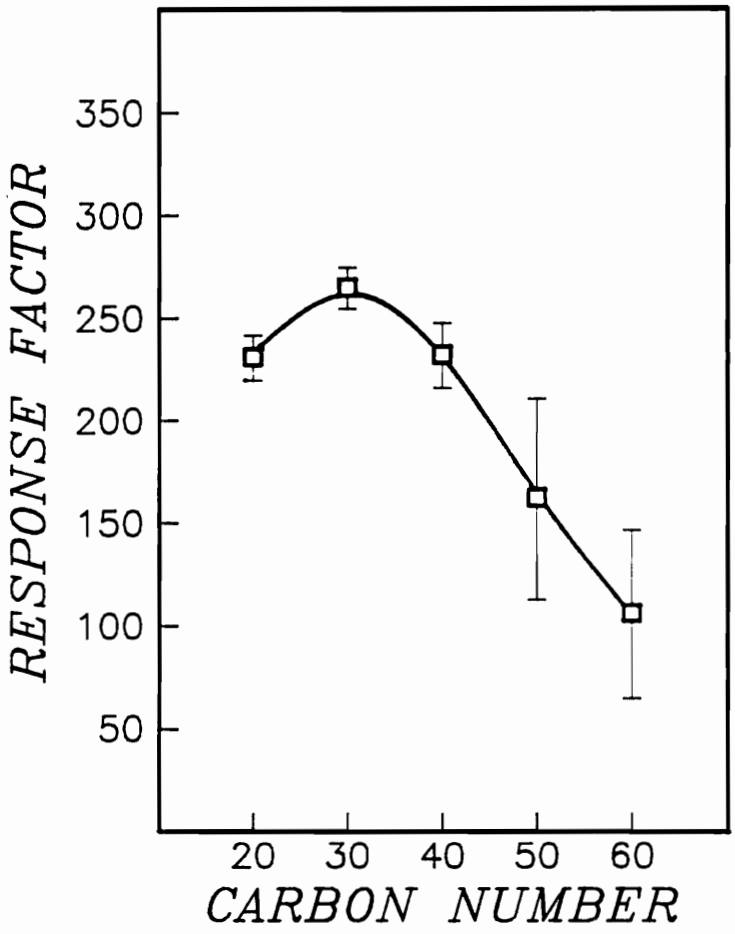

Figure 18. **RESPONSE FACTOR FOR A RAMP RATE OF 120 PSI/MIN**

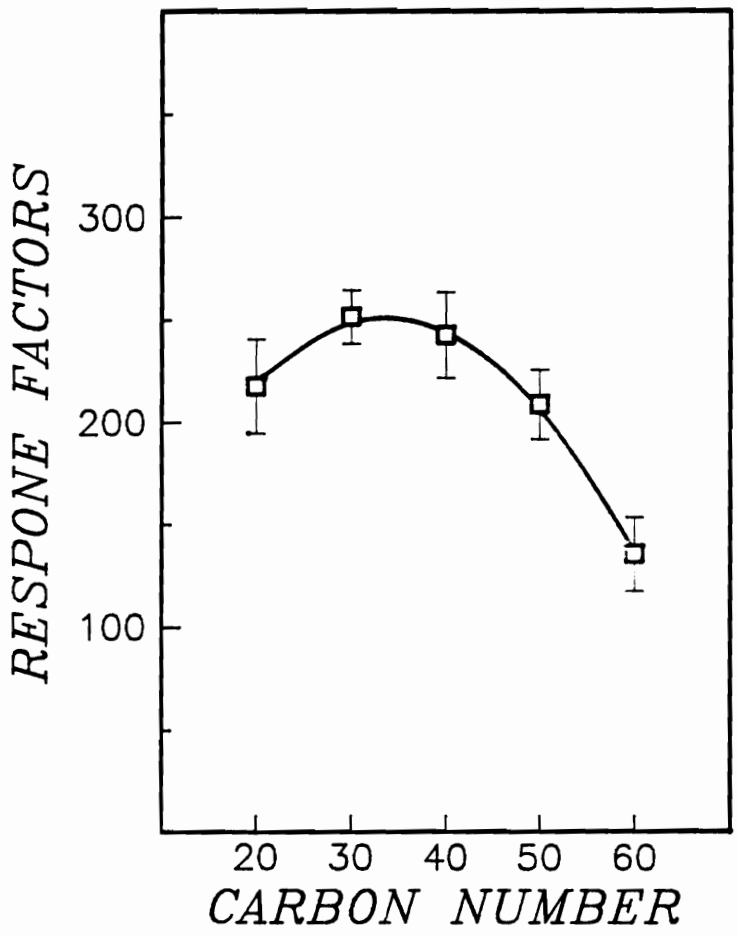

Figure 19. **RESPONSE FACTORS FOR A RAMP RATE OF 170 PSI/MIN**

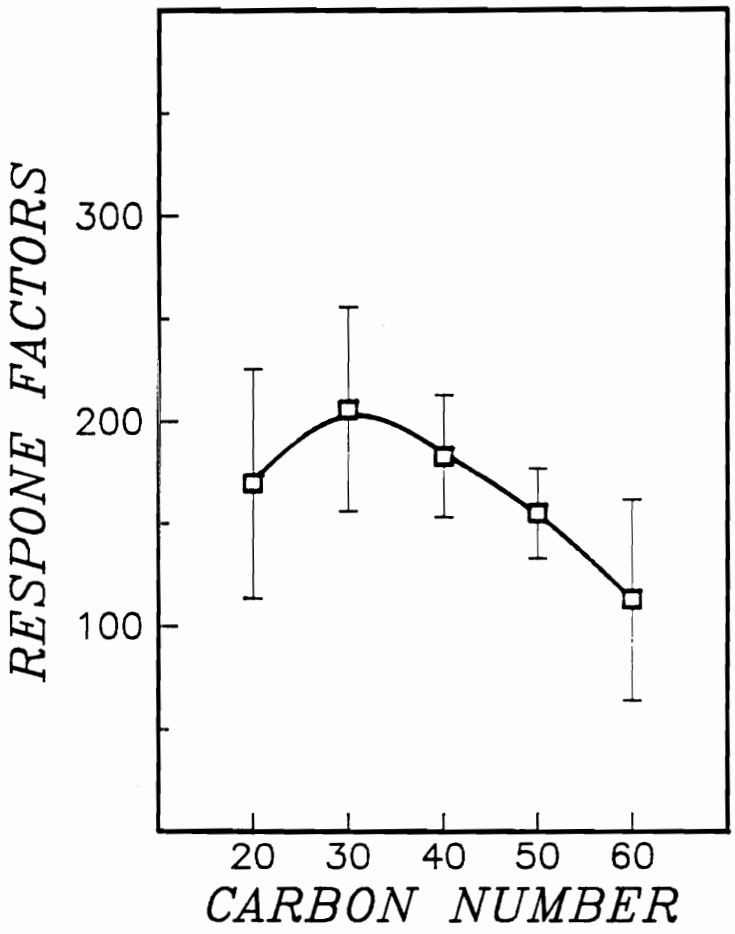

Figure 20. **RESPONSE FACTORS FOR A RAMP RATE OF 200 PSI/MIN**

TABLE 14

CALCULATED RESPONSE FACTORS FOR THE FOUR PRESSURE RAMPS

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
80 psi/min	194	224	193	66	31
120 psi/min	230	264	252	163	106
170 psi/min	218	252	234	209	136
200 psi/min	170	206	183	155	113

TABLE 15

REPRODUCIBILITY FOR ALKANE STANDARD MIXTURE, 1:10 DILUTION (20ppm)

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	46	43	73	59	59
	49	40	68	41	26
	48	45	56	54	63
	39	36	47	55	49
mean	46	41	61	55	49
standard deviation	5	4	12	8	16
RSD	**10%**	**9%**	**19%**	**15%**	**33%**

respectively. In the 1:100 dilution of the standard, 2 ppm, the RSDs (Table 16) ranged from 9% for C_{40} to 66% for C_{50}. Table 17 shows the response factors for the three concentrations of alkane mixtures. In both instances the reproducibilities were extremely poor.

Results of the previous experiments showed that changing the pressure programming rate and the analyte concentration did not reduce the RSDs to a reasonable level. These results, in conjunction with results of earlier investigations centering on the physical aspects of the flame, influenced the decision to investigate the detector. Specifically, in earlier experiments the flame was seen to change in size and in intensity throughout a pressure program. To minimize the disturbance of the flame, several instrumental criteria could be adjusted: the restrictor, column temperature, fuel gas flow rates, or the detector jet itself. Investigations were carried out into the effect that each of these criteria had on the peak area reproducibilities and response factors.

The short 15 μm linear restrictor was replaced by a 29 μm I.D. by 100 cm piece of untreated fused silica. The larger bore fused silica was used to decrease the linear velocity of the exiting gas, thus reducing the effect on the flame. When the 29 μm I.D. by 100 cm restrictor was placed 2 cm below the flame, the flame fluttered and blew out at ca. 2500 psi. When the restrictor was moved to greater than 3 cm below the flame, spiking occurred. Because the flame still showed instability, the second criteria was changed. The hydrogen fuel gas flow was increased from 45 mL/min to 63 mL/min, with the air flow remaining at 310 mL/min. When this was done the diameter of the flame increased and consequently was affected to a lesser degree by the flow rate of CO_2. The pressure was then ramped at 170 psi/min from 1070 to 5000 psi. However, the flame still fluttered above 3500 psi, and another adjustment was clearly needed. The effect of temperature

TABLE 16

REPRODUCIBILITY FOR ALKANE STANDARD MIXTURE, 1:100 DILUTION (2 ppm)

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	3.3	3.0	18.8	9.4	1.3
	3.2	2.8	18.6	5.1	4.7
	4.5	5.0	21.7	5.6	5.7
mean	3.7	3.6	19.7	3.9	3.9
standard deviation	0.7	1.2	1.7	2.6	2.3
RSD	**19%**	**33%**	**9%**	**66%**	**59%**

TABLE 17

RESPONSE FACTORS FOR THREE DIFFERENT CONCENTRATIONS OF ALKANE MIXTURE

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
stock (0.2%)	187	175	163	76	47
1:10 dilution	142	141	225	174	170
1:100 dilution	116	125		130	135

on the pressure vs. density curve (Figure 3) showed that as the system temperature increased the linearity of the isotherms increased. Because it was necessary to use a pressure ramp during the chromatographic runs, the oven temperature was raised to 150°C. As the pressure was increased linearily in the column, the density also increased in a linear fashion. This should have given a more uniform CO_2 flow change in the detector during the ramp, thus negating any effects that had been caused by sudden increases in density. With these alterations completed, the physical appearance of the flame still changed slowly throughout a programmed pressure run from 1070 to 5000 psi. The change was in the flame diameter and intensity: as the pressure increased the flame diameter decreased in size and the edges of the flame became more defined; a consequence of the H-P flame jet design. To produce a more robust flame that did not change during a run, the detector jet was drilled out to 0.052 inches from 0.022 inches (Figure 21), resulting in a flame that had a larger diameter than seen in the normal H-P flame jets. These changes, when used additively, created a flame that did not flutter during a run and did not change size and intensity as drastically as before the modifications.

Using the 29 μm I.D. by 100 cm restrictor, the wide bore jet, and the hydrogen flow rate at 63 mL/min, a chromatographic run was carried out from 1070 psi to 5000 psi at a rate of 170 psi/min (giving good flame stability throughout) with the 20 ppm standard mixture (Table 18). The RSDs were all below 10%, with the average 7%. In this run the restrictor was positioned 2.6 cm below the flame (lower than in earlier experiments). To investigate the effect of restrictor position on the peak area reproducibility and the response factors, several restrictor positions were investigated.

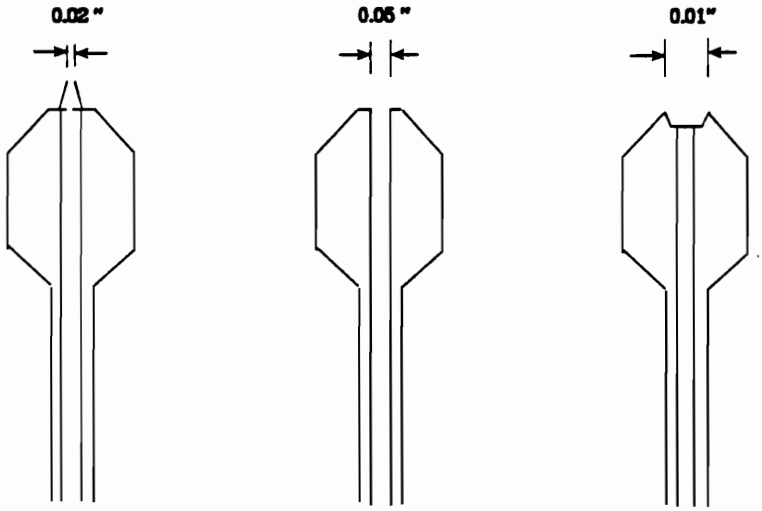

Figure 21. **HEWLETT–PACKARD MODEL 5880 GAS CHROMATOGRAPH FLAME JETS USED DURING SFC**

TABLE 18

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 2.6 cm BELOW THE FLAME

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	49	25	19	20	15
	57	25	20	22	14
	65	29	22	22	19
mean	53	25	20	21	16
standard deviation	4.3	2.5	1.5	1.2	2.6
RSD	**8%**	**10%**	**7%**	**6%**	**16%**

The instrumental conditions of the last experiment were used--hydrogen and air flow at 63 and 310 mL/min, and the detector temperature at 400°C. The restrictor positions investigated were 1.7, 1.9, and 2.1 cm below the flame. The results of chromatographic runs from 1070 psi to 5000 psi at 170 psi/min of the C_{20}-C_{60} standard (Tables 19, 20, 21, 22) showed that statistically, using pooled standard deviations with a 0.9 CI, the difference between the restrictor at 1.9 and 2.1 cm below the flame was significant, but the difference between the restrictor at 1.7 and 1.9 cm below the flame was not statistically significant. The summarized results of the reproducibilities from this test are shown in Figure 22. Because there was no statistical difference between 1.7 and 1.9 cm, 1.7 cm below the flame was chosen as the restrictor position used in the remaining experiments.

Another change that was made was the enlargement of the flame jet. To investigate further the effect of the increased size of the flame jet on the peak area reproducibilities and response factors, the flame jet was bored out from 0.022 inches to ca. 0.1 inches. Using the instrumental conditions from the previous experiment, the C_{20}-C_{60} hydrocarbon standard was chromatographed from 1070 psi to 5000 psi at a rate of 170 psi/min. The lower molecular weights showed RSDs below 5%, whereas the C_{50} and C_{60} showed RSDs of 26 and 29%, respectively. The response factors (Figure 23) were lower than those from earlier experiments. Although showing a decrease in RFs with an increase in molecular weight, they did show more linear response than the earlier 0.05 inche flame jet (Figure 24). Conceivably, the flame jet could be enlarged even more; however, with the decrease in sensitivity seen with increasing jet inside diameter, the resultant detector would show poor sensitivity. *It appeared from these studies that wider flame jets are necessary to accommodate the higher volumetric flow*

TABLE 19

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 1.7 cm BELOW THE FLAME

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	139	95	59	18	18
	137	90	58	18	14
	148	94	57	17	14
mean	141	93	59	18	17
standard deviation	7	6	1.3	1	1
RSD	**4%**	**3%**	**2%**	**6%**	**6%**

TABLE 20

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 1.9 cm BELOW THE FLAME

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	100	70.2	47.7	31.5	25.5
	106.5	59.2	37.8	26.6	22.5
	111.3	68.8	44.1	28.0	19.1
mean	105.9	66.1	43.2	28.7	22.4
standard deviation	5.7	5.9	5.0	2.4	3.1
RSD	**5%**	**9%**	**12%**	**9%**	**14%**

TABLE 21

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 2.1 cm BELOW THE FLAME

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	166.2	88.6	64.0	25.5	42.1
	150.7	101.9	61.4	25.2	21.7
	134.7	87.4	57.5	21.7	21.2
mean	150.5	92.6	61.0	23.8	28.3
standard deviation	15.7	8.0	3.2	1.8	11.9
RSD	**11%**	**9%**	**5%**	**8%**	**42%**

TABLE 22

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 1.7 cm BELOW THE FLAME AND A 0.1" BORE FLAME JET

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	71.0	54.1	34.5	12.9	11.2
	69.2	49.7	33.2	8.7	7.0
	70.9	50.9	36.6	8.1	6.8
mean	70.4	51.6	34.7	9.9	8.3
standard deviation	1.0	2.2	1.7	2.6	2.5
RSD	**2%**	**4%**	**5%**	**26%**	**30%**

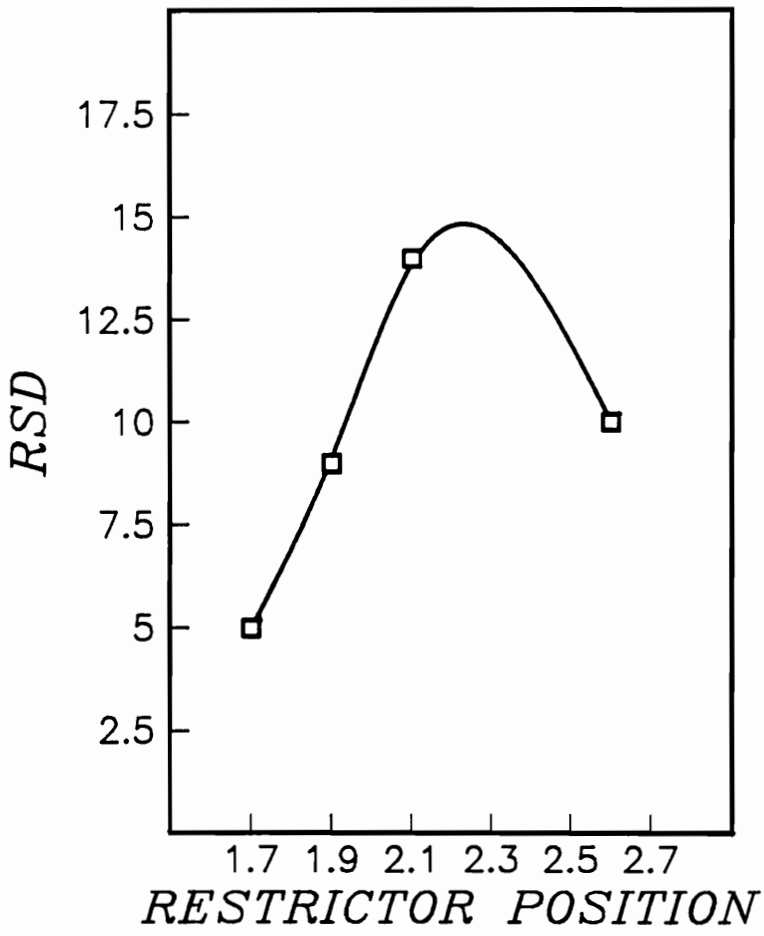

Figure 22. **EFFECT OF RESTRICTOR POSITION ON REPRODUCIBILITY OF A HYDROCARBON MIXTURE**

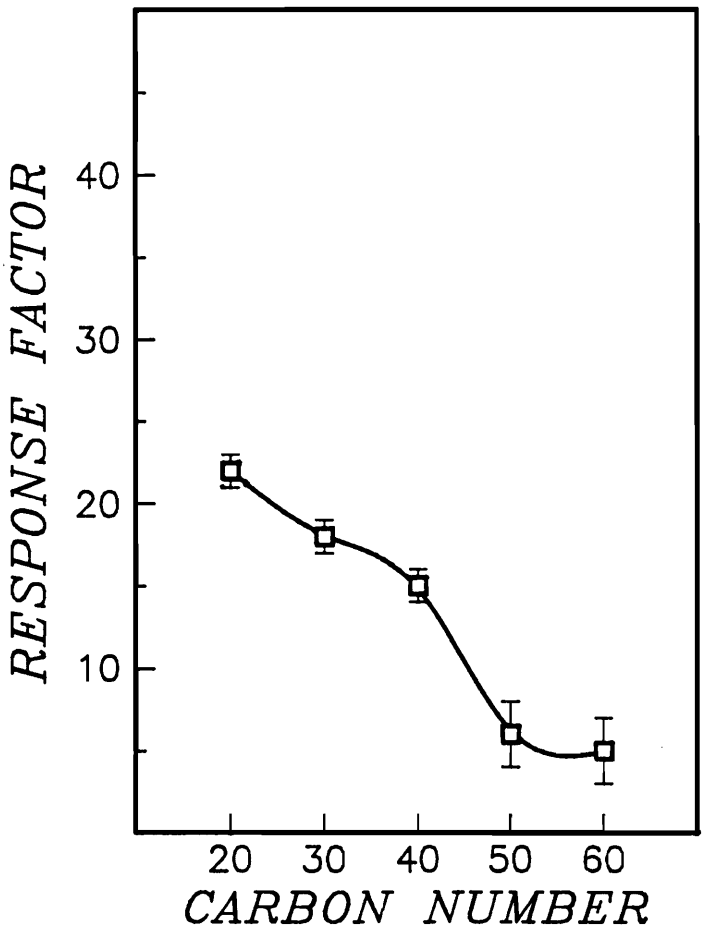

Figure 23. **RESPONSE FACTORS vs. CARBON NUMBER USING A 0.1" BORE CAPILLARY FLAME JET**

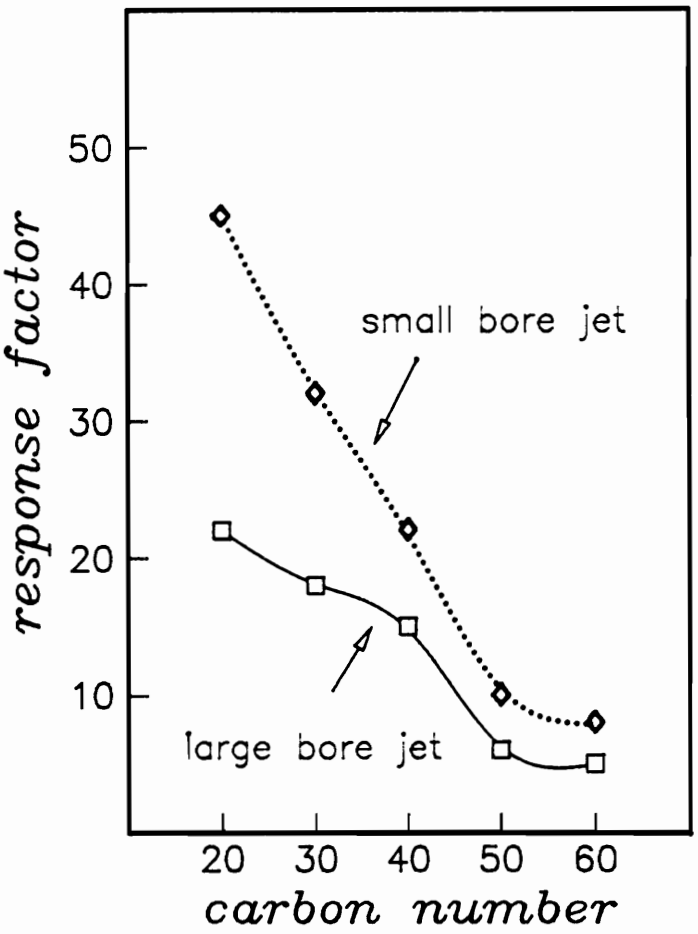

Figure 24. **A COMPARISON OF RESPONSE FACTOR vs. CARBON NUMBER FOR LARGE AND SMALL BORE CAPILLARY FLAME JETS**

rates obtained from packed SFC columns; however other parameters such as gas flow rates, electrode surface area, electrode position, and polarizing voltage may have to be changed to maintain FID sensitivity and stability. Unfortunately these studies are beyond the capabilities available at VPI.

In the initial investigations hydrogen flow rate was increased to 63 mL/min to produce a more stable flame. Higher flow rates of hydrogen resulted in a flame that was difficult to light and unstable. Lower hydrogen flow rates resulted in a flame that was extinguished at ca. 1500 psi. To study the effect of air flow rate on reproducibility and linearity, air flow was increased from 310 to 384 mL/min. The C_{20}-C_{60} standard was chromatographed from 1070 to 5000 psi at a rate of 170 psi/min; the results (Table 23) showed reproducibilities (11%) that were significantly higher than those found with the lower detector air flow. The response factors (Figure 25) showed no significant increase in linearity.

At this point the wide bore (29 μm I.D.) long (100 cm) restrictor was positioned 1.7 cm below the flame and the detector was at 400°C. The hydrogen and air flow were 63 and 310 mL/min, respectively, and the pressure rate increase was 170 psi/min. This configuration showed area reproducibilities below 10% that, although not as low as GC or HPLC, were reasonable. Although the RSDs were reasonable, the question of why there was a decrease in response factors with increasing molecular weight remained. The results of earlier observations showed that the flame changes its size and shape, and, as a result, its chemistry, with an increase in column pressure. These effects were minimized by changing the fuel gas flows and the jet size to give a more robust system, yet the effect of the flow rate of CO_2 itself had not been studied. To investigate the effect that CO_2 flow rate had on the response factors, the volume of CO_2 exiting the detector was

TABLE 23

REPRODUCIBILITY OF STANDARD ALKANE MIXTURE WITH THE RESTRICTOR 1.7 cm BELOW THE FLAME AND THE AIR FLOW AT 384 ml/min

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
	135.9	86.8	55.1	13.6	14.4
	147.8	99.1	62.0	16.4	15.0
	129.9	79.4	52.2	11.2	11.3
	143.7	95.8	60.0	14.7	*
mean	139.3	90.3	57.3	14.0	13.6
standard deviation	7.9	9.0	4.4	2.1	2.0
RSD	**6%**	**10%**	**8%**	**16%**	**15%**

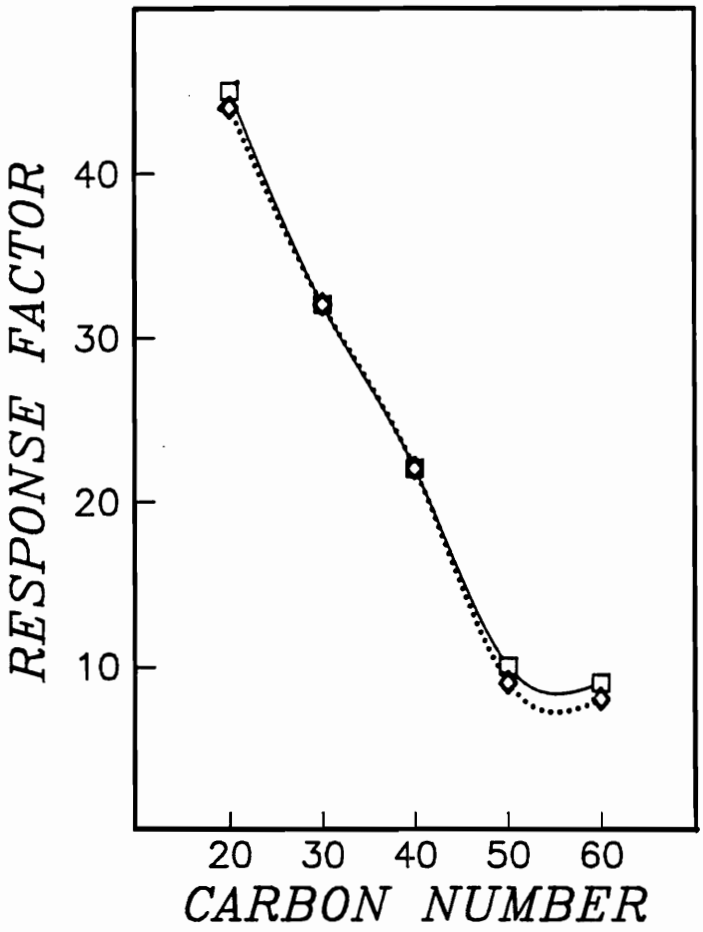

Figure 25. RESPONSE FACTORS vs. CARBON NUMBER FOR DETECTOR AIR FLOWS OF 310 AND 384 ml/min

monitored during a normal chromatographic run from 1070 psi to 5000 psi at a ramp of 170 psi/min. The result of this experiment (Figure 26) showed that the flow rate of CO_2 ranged from 35 mL/min at 1070 psi to 145 mL/min at 5000 psi. Make-up gas (supercritical CO_2) was added post column and the detector signal monitored to identify the effect that this phenomena had on the response factors. Response factors for the C_{10}-C_{60} hydrocarbon standard, 20 ppm, at 1070 psi followed by a 170 psi/min ramp to 5000 psi without make-up flow, are shown in Figure 27, and response factors with make-up flows of 29 and 35 mL/min are given in Figure 30. Higher make-up flows could not be studied because at the higher flows, 45 mL/min, the flame was blown out. *The response factors decreased as the amount of CO_2 increased (Figure 28).* This indicated a problem for quantitative SFC analysis while using FID detection and pressure gradients.

The response factor data from the CO_2 make-up gas study could be rationalized if the response factors of the standard hydrocarbons were correlated to the specific volume of CO_2 in the system. Eicosane eluted at 2000 psi, a flow rate of 50 mL/min and a response factor of 50. Tricontane eluted at 3000 psi, which corresponded to 80 mL/min in the SFC system, and had a response factor of 30. When 30 mL/min of make-up gas was added to the detector, the eicosane eluted with an exit flow rate of 80 mL/min. Not surprisingly, the response factor for the eicosane now corresponded to the original value of the tricontane, 30. This indicated, that although the system showed reasonable reproducibility, the response factors showed a dependence on the total CO_2 flow rate in our detector. The dependence of the response factors on the flame detector geometry suggested the need for a detector that has a geometry and flow system specifically designed for SFC. Another alternative would be to design a restrictor that would maintain a constant CO_2 flow rate during pressure programming.

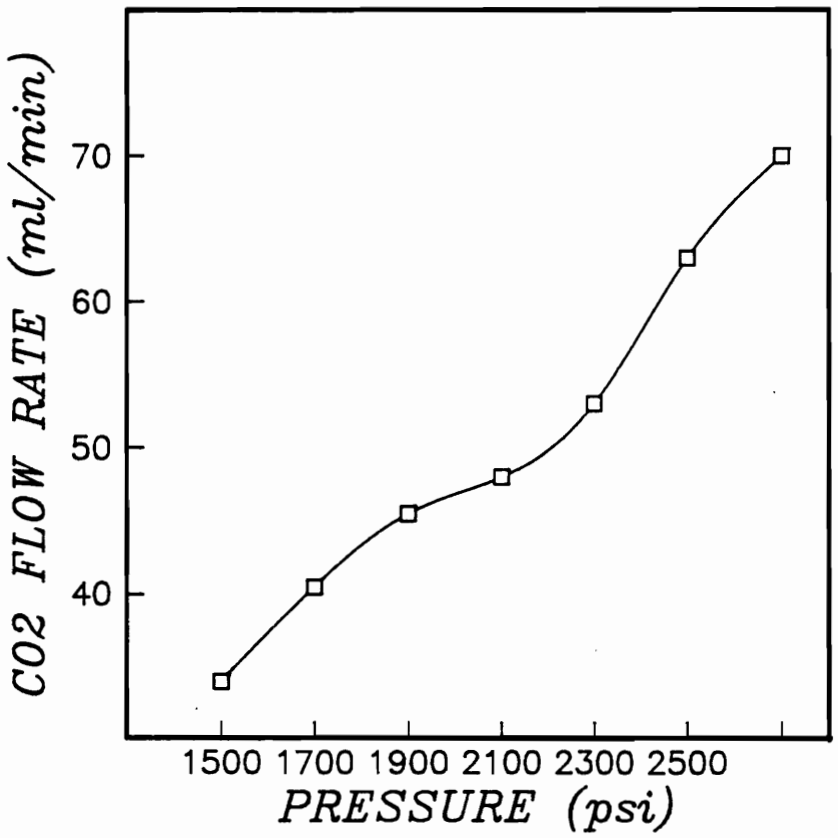

Figure 26. **CO2 GAS VOLUME FLOW RATE AS A FUNCTION OF SYSTEM PRESSURE**

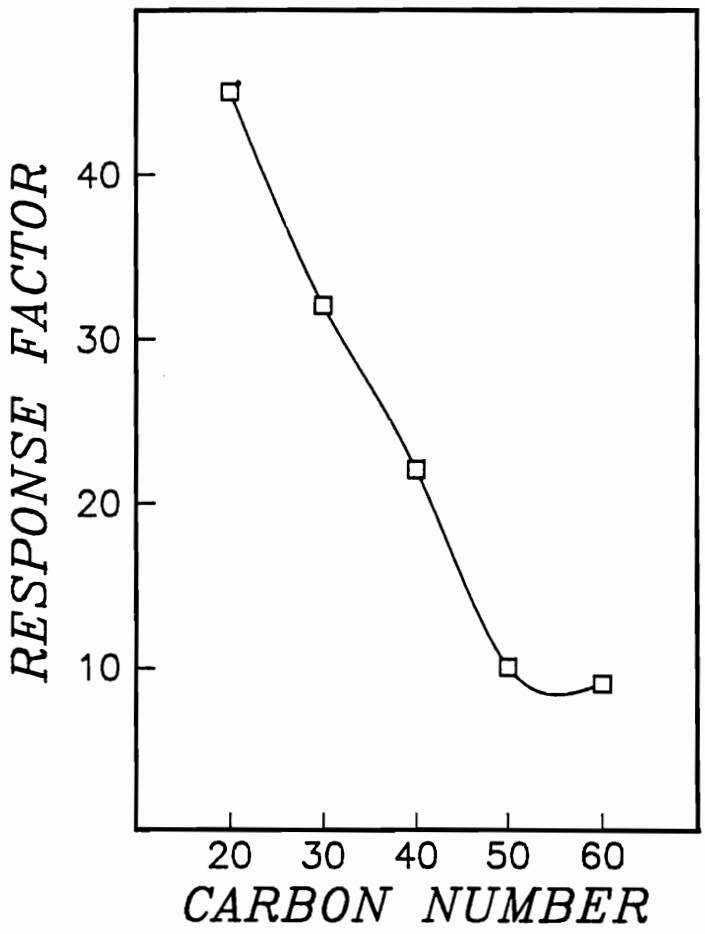

Figure 27. **RESPONSE FACTOR vs. CARBON NUMBER WIHTOUT SUPERCRITICAL CO2 MAKE UP GAS FLOW**

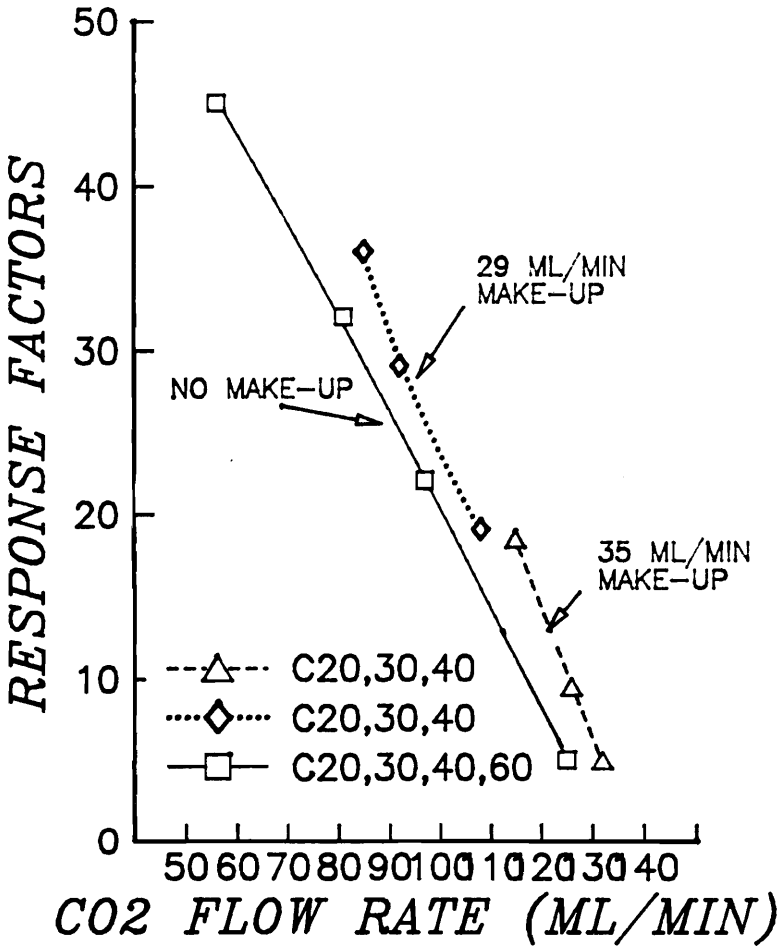

Figure 28. **RESPONSE FACTORS vs. CARBON NUMBER UTILIZING SUPERCRITICAL CO_2 AS A MAKE UP GAS COMPARED TO RESPONSE FACTORS OBTAINED WITHOUT MAKE UP FLOW**

To investigate the possibility that a different type of GC detector would be amenable to the increasing CO_2 flow rates, a Hewlett-Packard model 5790 gas chromatograph was evaluated. This detector has a collector electrode that can be moved vertically (relative to the flame jet). The investigation of this detector focused on the collector electrode position--two positions were chosen to look for a major change in peak area reproducibility. The collector electrode was set at 2.5 mm above the flame jet; the prescribed setting, and the fuel gas flows were set identical to those used with the H-P model 5880 GC. The 20 ppm standard was chromatographed from 1070 psi to 5000 psi at 170 psi/min. The resulting RSDs were higher than those found with the Hewlett-Packard 5880 detector. The collector electrode was then moved to 1.8 mm above the jet tip. The results of a chromatographic run from 1070 psi to 5000 psi at 170 psi/min produced no C_{50} and C_{60} peaks and reproducibility was no better than before. The response factors gave the characteristic drop as molecular weight increased. The geometry of the H-P model 5790 gas chromatograph was not the answer to the problem of decreasing response factors.

The instrument was configured to operate in the most favorable way to date. The wide bore (29 μM) long restrictor (100 cm) was positioned 1.7 cm below the flame, the hydrogen flow was set at 63 mL/min, the air flow was set at 340 mL/min, and the pressure rate increased to 170 psi/min. Light distillation fraction 3 was prepared at a concentration of 0.5% and a C_{20} spike at 0.19%. The distillation fraction and standard were chromatographed from 1070 psi to 5000 psi at a rate of 170 psi/min. The peak area reproducibility for the oil fraction was 4% while the RSD for the eicosane was lower than it was in the earlier experiments under identical chromatographic and instrumental conditions. Several concentrations of samples with spikes were made in carbon disulfide

and chromatographed and the effect that concentration had on response factors was examined. *The results (Table 24 and Figure 29) showed a concentration dependency that was not seen in the earlier C_{10}-C_{60} standard chromatographic runs where three 1:10 serial dilutions (200 ppm, 20 ppm, 2 ppm) were used.* In the earlier C_{10}-C_{60} standard chromatographic runs the total area was 1 X 10^6 microvolts and this was distributed over 6 discreet peaks over 25 minutes; in the real samples the area was 10 X 10^6 microvolts in a period of eight minutes. With the increased amount of analyte moving into the detector, the flame was saturated by the high concentrations, which would result in a concentration to concentration non-linear response. Lower response factors were seen for the higher concentration solutions.

To study a different type of detector, a commercially available instrument, a Suprex (Pittsburgh, Pa) model 200 was used with the 29 μm by 100 cm restrictor positioned 1.7 cm below the flame. The hydrogen and air flow rates were set at 63 and 310 mL/min. Higher air and hydrogen flows were attempted; they did not support the flame but extinguished it between 1500 and 2000 psi. The peak area RSDs (Table 25) for the 20 ppm standard all fell below 10%; however, the response factors (Figure 30) decreased by 21% with increasing carbon number.

The three commercially available FID detectors that were tested did not produce peak area reproducibilities below 10% and the response factors still showed a decrease with increasing molecular weights. Another possibility was to use a different type of restrictor: a pulled restrictor (Figure 6). It was made by heating a piece of 100 μm fused silica and pulling it to a taper. The taper was than cut off to give the desired orifice and, subsequently, the desired CO_2 flow rate through the column and exiting the restrictor. One problem with this type of restrictor is the reproducibility of flow rate from one

TABLE 24

RESPONSE FACTORS FOR C_{20} SPIKE IN A LIGHT OIL DISTILLATION FRACTION

CONCENTRATION (%)	RESPONSE FACTOR
10.5	2.0
1.05	17.6
0.019	22.9
0.0078	25.8
0.0019	27.0
0.0002	28.0

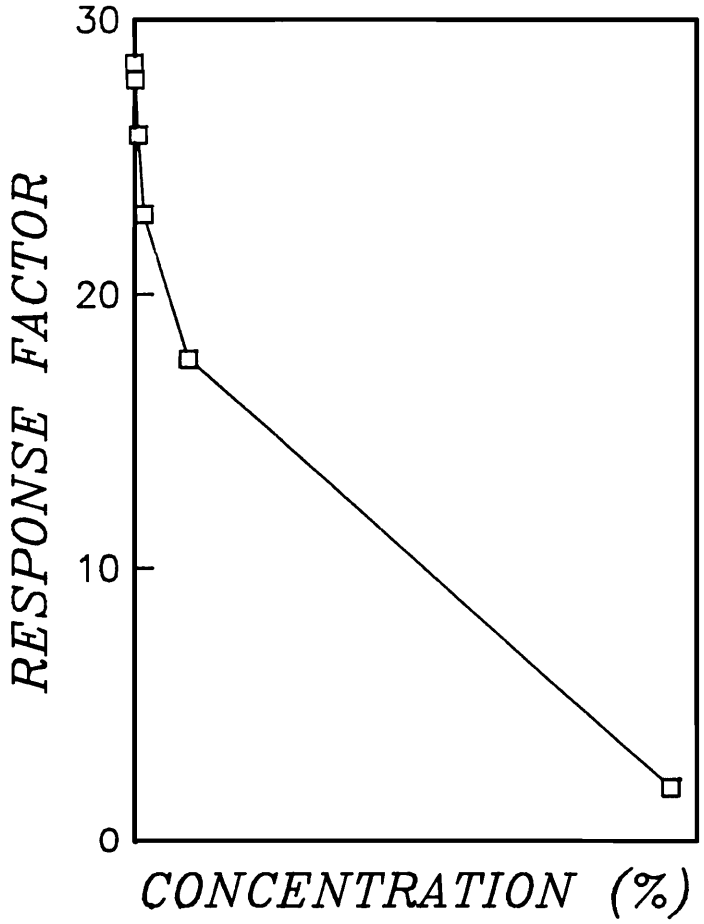

Figure 29. **RESPONSE FACTOR vs. CONCENTRATION FOR FIVE DIFFERENT CONCENTRATIONS OF EICOSANE**

TABLE 25

RESPONSE FACTORS AND REPRODUCIBILITY USING THE STANDARD ALKANE MIXTURE FOR SUPREX DETECTOR USING THE 30 um by 100 cm RESTRICTOR

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
RESPONSE FACTOR	31.3	27.6	16.8	9.9	6.7
RSD	**8%**	**6%**	**8%**	**3%**	**7%**

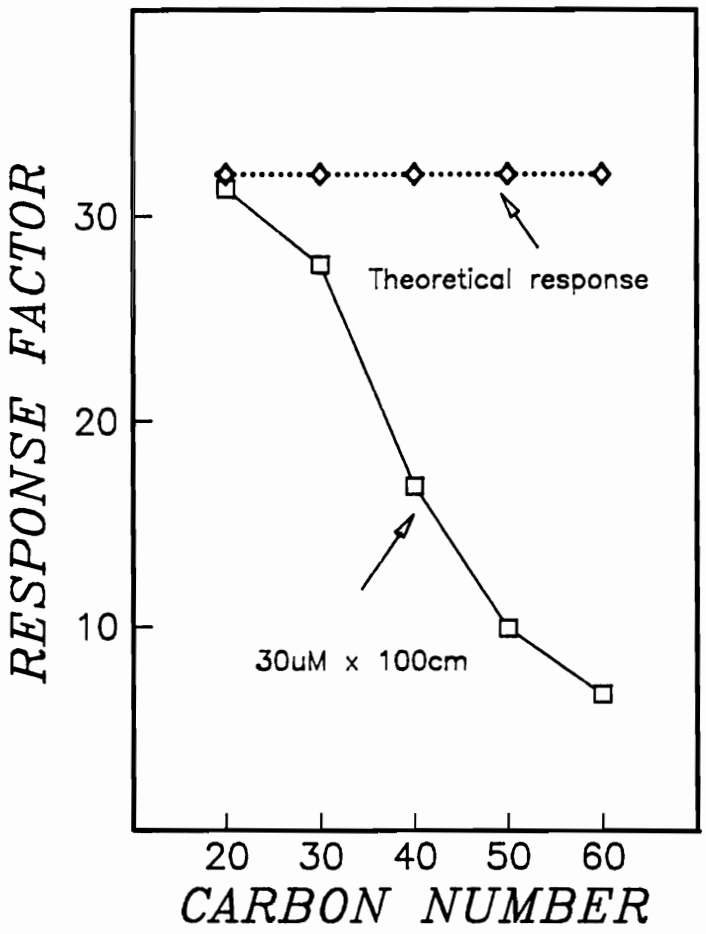

Figure 30. **RESPONSE FACTOR vs. CARBON NUMBER FOR A SUPREX COMMERCIAL SYSTEM AND FOR A THEORETICAL CURVE**

restrictor to another. With the H_2 and air flow at the optimum, the alkane standard C_{20}-C_{60} was run with a pressure ramp from 1100 to 5000 psi with an increase of 170 psi/min. The reproducibilities (Table 21) show RSDs of 7, 6, 7, 18, and 17 for the standard mixture, which is comparable only at the lower molecular weights when compared to the 29 μm I.D. by 100 cm restrictor. Higher molecular weights showed poorer reproducibility with the pulled restrictor. *The response factors (Table 26, Figure 31) showed that the pulled restrictor has the better linearity; however, the problem of reproducibility at the higher molecular weights still existed.*

The FIDs used so far had shown a range of peak area reproducibilities. In the case of the H-P model 5880 with the 29 μm I.D. by 100 cm restrictor, the RSDs were good, with an average of 5%. However, the response factors decreased with increasing molecular weight. When the system parameters were changed to give more linear response factors, the RSDs increased, particularly in the higher molecular weight range.

The investigation of detector parameters and restrictors was the main thrust of this study. Another possible cause for non-linear response factors could be the injector. Possibly the higher molecular weight analytes were not being moved into the supercritical zone by the liquid CO_2. Blank runs with pure CO_2 after standard component runs in the previous systems showed no peaks; indicating possible adsorption in the system. It has been shown that the solubility of compounds increases with increasing temperature, so the next logical step was to heat the injector. Unfortunately, the solvent, carbon disulfide, is extremely volatile and flammable. In order to heat the injection port, a non-volatile solvent that solvates the entire molecular weight range had to be used. Those common solvents that have a higher boiling point than CS_2 - tetrahydrofuran, xylene,

TABLE 26

RESPONSE FACTORS AND REPRODUCIBILITY USING THE STANDARD ALKANE MIXTURE FOR SUPREX DETECTOR USING THE PULLED RESTRICTOR

	C_{20}	C_{30}	C_{40}	C_{50}	C_{60}
RESPONSE FACTOR	17	15.7	12.7	10.0	7.6
RSD	**7%**	**6%**	**7%**	**17%**	**17%**

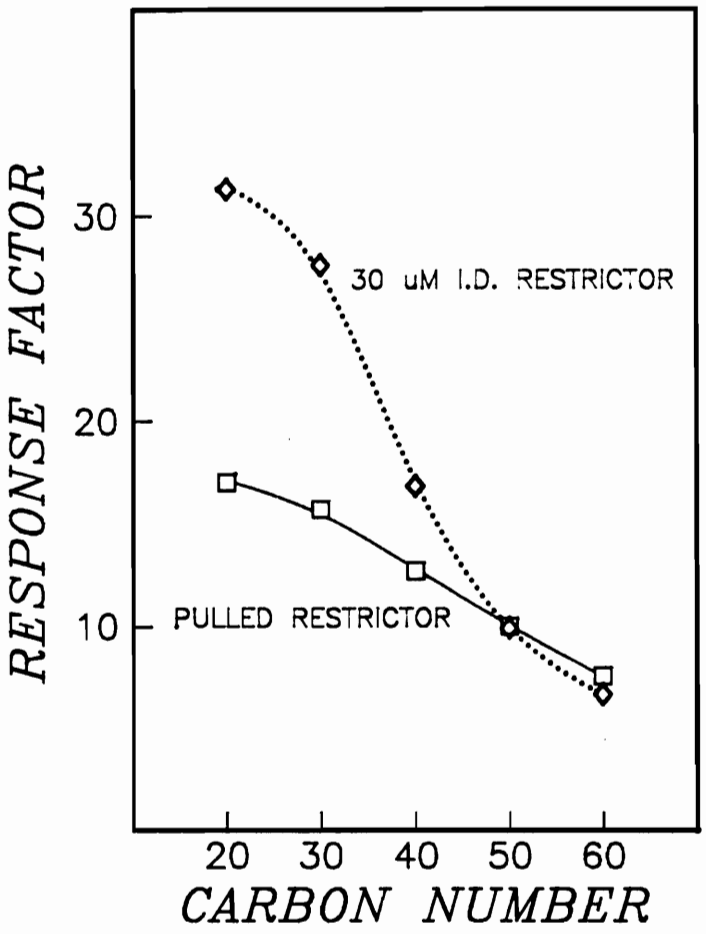

Figure 31. **RESPONSE FACTOR vs. CARBON NUMBER FOR A PULLED RESTRICTOR USING A SUPREX INSTRUMENT**

dimethylsulfoxide - did not solvate the higher molecular compounds even at elevated temperatures.

The next step was to use a system which does not require an injector. An obvious solution was to deposit the analyte directly on the head of the column. Figure 7 shows a stop flow apparatus developed to make on-column injections possibile, and figure 8 shows the flow patterns for the apparatus. The top of the column frit was removed to make the direct deposition possible, therefore depressurization and column bed deformation were possible problems. The response factors for the standard mixture chromatographed under normal conditions still showed a decrease with an increase in carbon number. The C_{30} and C_{40} had the same response factors, but C_{50} and C_{60} factors decreased. *The result of this experiment showed that the problem may still exist in the restrictor of FID and, consequently, a detector that could handle high flow rates needed to be tested.* Unfortunately, facilities to design and build an FID specifically for large flow rates common to SFC were not available at VPI, and the quantitation project was discontinued.

C. Extraction

Continuing the investigation into the quantitative aspects of supercritical fluids using a home-built system led from the chromatographic arena into the area of supercritical extractions (SFE). SFE, as discussed in the introduction, is an area that has been receiving increased attention (52) as a method to remove various analytes from any number of matricies while keeping the temperature of the operation at a level where

thermal degradation does not usually occur. The analytes that were chosen as standards in this study represent two classes of compounds, relatively non-polar polynuclear aromatics and the more polar pesticides (Figure 11). To study the efficiency of analyte removal using SFE, two matrices were used during the study. The first matrix, glass beads, was easily cleaned and obtained, whereas the second matrix, soil, was much more practical and environmentally relevant. Waste landfills are one of the most widely used means for the deposit of hazardous chemicals in the USA, thus analysis of soil samples is particularly relevant. This study was aimed at investigating the feasibility of using SFE to extract polynuclear aromatics and pesiticides from soil blanks and waste landfill soil samples.

The results of SFE of model compounds from a glass bead matrix into the blank vial collector (Table 27) showed extremely poor recovery, *nothing above 32%*. The low efficiency could have resulted from two factors: the extraction process itself or the collection efficiency. A loss of 70% in the extraction process from the glass beads seemed unlikely, since the polynuclear aromatics had all been chromatographed easily by SFC without problems with solubility. In contrast, the low results for the pesticide standard, Dursban, could have been the result of strongly bonding interactions such as hydrogen bonding with the matrix surface.

To examine the possibility of low collection efficiencies, the collector was partially filled with methylene chloride (as the accumulator). The collection apparatus was changed from the blank 0.5 dram vial to that shown in Figure 32. Initial extractions with the new collector were tried without holding the restrictor in place, however, the restrictor frequently came in contact with the collector wall, froze to the wall and broke. Another slight modification resulted in a collector that could hold the fused silica restrictor in

TABLE 27

RECOVERY EFFICIENCIES OF STANDARD PNA'S AND A PESTICIDE FROM GLASS BEADS USING A BLANK VIAL AS THE COLLECTOR

COMPOUND	% RECOVERY
ANTHRACENE	32
CHRYSENE	10
BENZO[a]PYRENE	15
DURSBAN	6

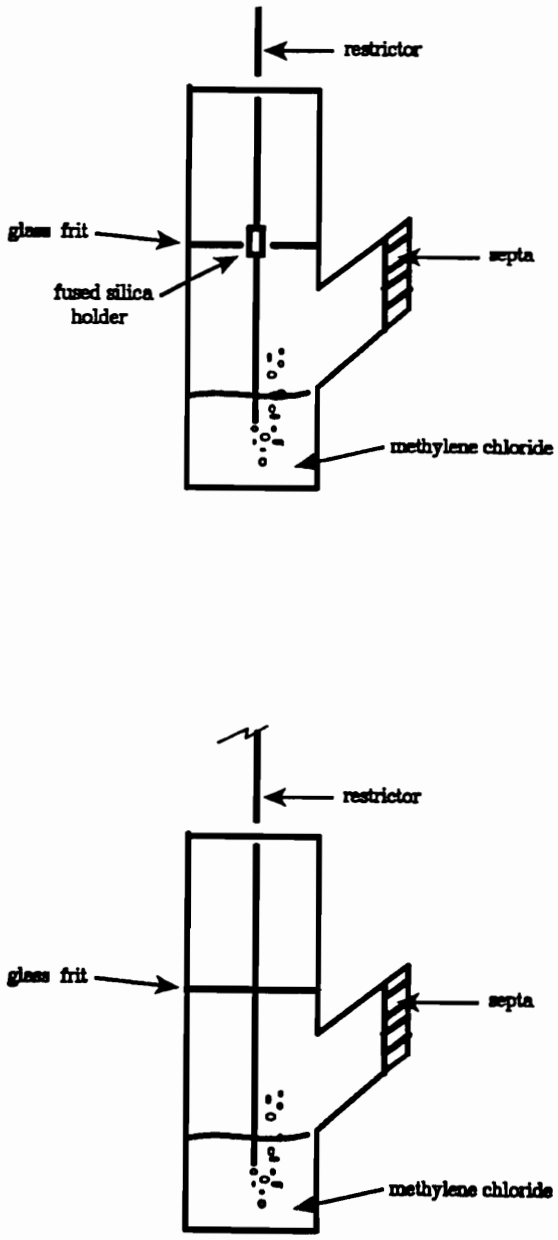

Figure 32. **SUPERCRITICAL FLUID EXTRACTION COLLECTOR WITH AND WITHOUT RESTRICTOR RETAINER**

place during the extraction (Figure 32). During the initial extractions with the revised collector, the evaporation of the methylene chloride, a result of the strong turbulence caused by the CO_2 bubbles, became a problem. The evaporation occurred in spite of the cooling due to the Joule-Thompson effect (thermodynamic cooling owing to the rapid volumetric expansion of a material). To alleviate any problem with excess evaporation, 20 μL/min of methylene chloride was added via the MACCS single piston reciprocating pump.

The results of the polynuclear aromatic extractions from a glass bead matrix using the liquid accumulator in the pyrex collector were excellent (Table 28); the average of two trials for anthracene was 97%, chrysene was 83% and benzo[a]pyrene was 88%. The average efficieny for the extractions of Dursban was 75%, which, although not as high as the PNAs, was a substantial increase over the efficiency when the empty collector was used. To ensure that all of the standard was removed in the SFE, a second identical extraction was performed. The careful analysis of these extractates showed no analyte present.

The recoveries proved that the problem had been in the collection efficiency, not in the extraction efficiency. The I.D. of the restrictor used during these extractions was a 20 μm I.D. fused silica with the exit orifice ca. 2 um. Partly because of this narrowing, the mobile phase exited the restrictor at such a high velocity that it blew a majority of the desolvated analyte out of the blank vial instead of depositing it on the walls. *The results of investigation showed that the extraction process worked very well with the glass bead matrix providing that a liquid accumulator was used.*

The next step in the extraction study was to change to a soil matrix. The soil used in the standard extraction study was taken from the ground outside of the chemistry department

TABLE 28

EXTRACTION AND RECOVERY EFFICIENCY OF STANDARD PNA'S AND A PESTICIDE FROM GLASS BEADS USING METHYLENE CHLORIDE IN THE COLLECTION APPARATUS

COMPOUND	TRIAL #1	#2
	% RECOVERY	
ANTHRACENE	97	97
CHRYSENE	85	82
BENZO[a]PYRENE	88	89
DURSBAN	76	74

at Virginia Tech and was cleaned by supercritical extraction before use. When the soil was first used as a matrix, the restrictor plugged. The restrictor was gently warmed to 50°C; however, the flow did not resume. Examination by optical microscope revealed a white crystalline substance constricted flow through the restrictor. The restrictor's inside diameter was enlarged from 20 μm to 50 um. When the 50 μm I.D. restrictor was used there was still a problem with ice formation in the tip of the restrictor. This was verified by removing the restrictor from the accumulator and gently warming it, which immediately restored the flow. In order to prevent plugging by ice formation, the collection device was further modified to allow warming of the restrictor. This was accomplished by resistively heating, indirectly, the restrictor (Figure 11). Plugging was not a problem after implementation of this device.

The results of the extraction of the three PNAs from the soil matrix (Table 29) showed even better results than those obtained with the glass bead matrix. *The average recovery efficiencies for two runs were 99, 95, and 88%, for anthracene, chrysene, and benzo[a]pyrene, respectively. The pesticide, Dursban, also showed an increase in recovery efficiency from 75% (glass beads) to 88% (soil). A second pesticide, pentachlorophenol, gave an average recovery of 93% from the soil.* The increase in recoveries from glass beads to soils could be attributed to the fact that the glass beads, which were used unsilanized, were very active, whereas the soil, because it had been pre-extracted, had less active sites for adsorption.

In these studies the standards had all been extracted using a pressure gradient rather than a single pressure. This was done for convenience. The results of extracting a single standard, anthracene, at a single pressure revealed that at a pressure of 5000 psi, a 20 ppm anthracene standard was 85% extracted and recovered with the first 10 mL of CO_2

TABLE 29

EXTRACTION AND RECOVERY EFFICIENCY OF STANDARD PNA'S AND TWO PESTICIDES FROM SOIL USING METHYLENE CHLORIDE IN THE COLLECTION APPARATUS

COMPOUND	TRIAL #1	#2
	% RECOVERY	
ANTHRACENE	100	99
CHRYSENE	97	93
BENZO[a]PYRENE	89	88
DURSBAN	86	86
PENTACHLOROPHENOL	91	94

and the remaining 15% was extracted with the next 10 mL. All of the extractions up to this point were in the 20-50 ppm range. Extending this range, using pentachlorophenol, to 200 ppb showed an 83% recovery (Figure 47) from a soil matrix using 10 mL CO_2. However, 20 ppb did not give meaningful recovery from the soil matrix.

The technique of supercritical extractions from soils showed excellent results with the in-house built system, demonstrating that the system, with subtle changes in hardware, could be used for SFC and SFE. The next step was to extract and identify the materials from an unknown soil matrix, using the techniques developed with glass beads and soils. Soil samples were taken from a contaminated landfill near Virginia Tech.

The results of SFE of a 10 mm I.D. by 25 cm column blank with 13 grams of soil followed by capillary GC (Figure 33) were excellent and showed that *ca. 60 different compounds were isolated from the contaminated soil.* The identification of anthracene, chrysene, and benzo[a]pyrene in the contaminated soil was a *three-step process.* The first step was preliminary identification by retention times. Figure 34 shows where the three PNAs from the study would elute using the retention times of standards run under identical conditions. *This figure showed that according to the preliminary retention time data all three of the polynuclear aromatics were present in the sample.* The second step was to spike the pre-extracted soil with the analytes. This was followed by extraction and gas chromatographic analysis to see if the spikes corroborated the retention time conclusions. *The result of the spike study (Figures 35, 36) showed that the three peaks which matched retention times also matched the spikes.* The third piece of evidence needed for the conformation was mass spectral data.

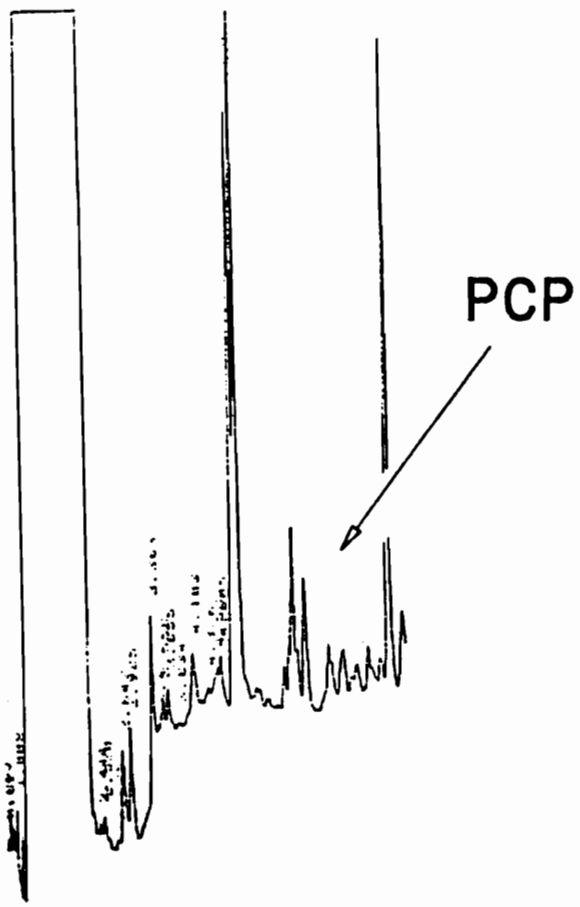

Figure 47. **RESULT OF THE SUPERCRITICAL FLUID EXTRACTION OF 200 ppb OF PENTACHLOROPHENOL**

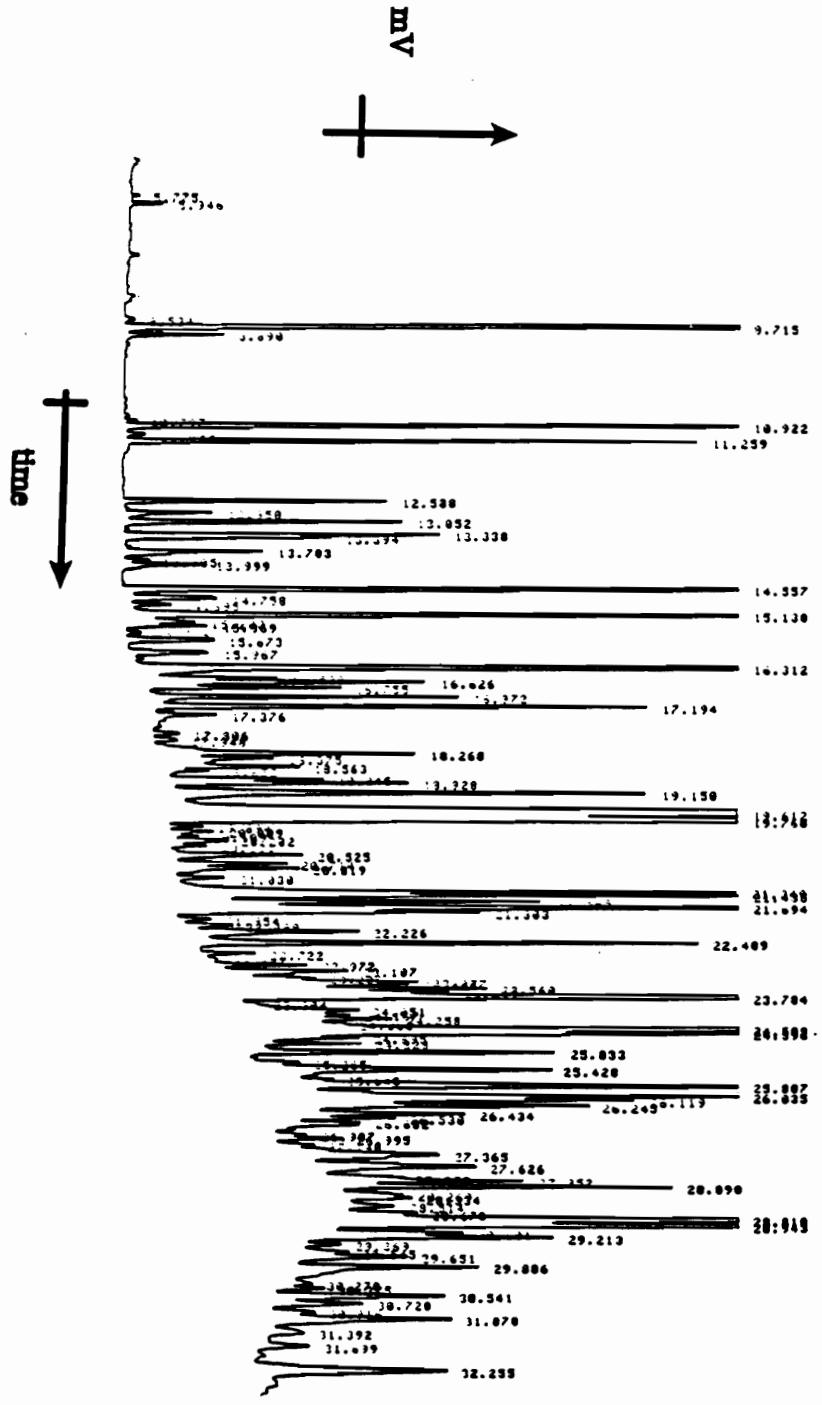

Figure 33. **CAPILLARY GC TRACE OF A SUPERCRITICAL FLUID EXTRACTION OF 13 GRAMS OF LAND FILL SOIL**

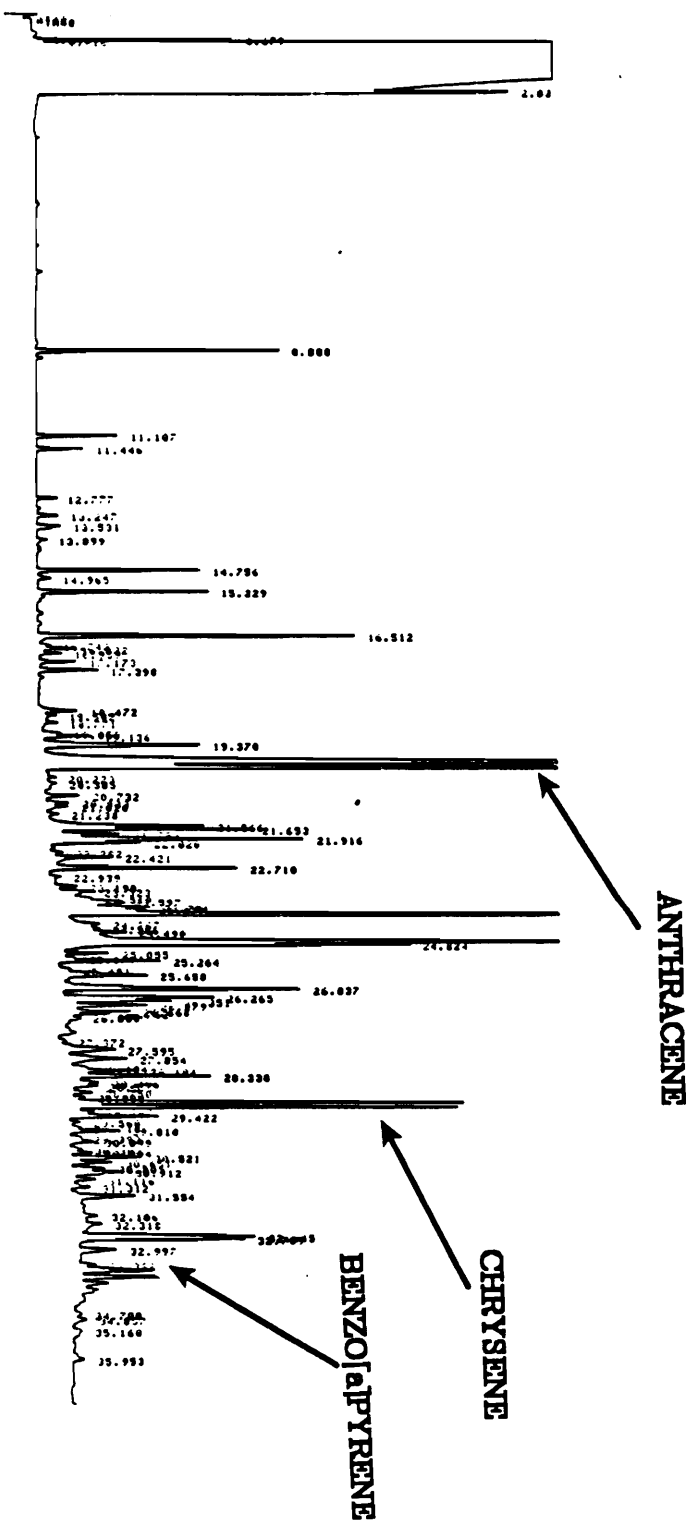

Figure 34. **RETENTION POSITIONS OF THREE POLYNUCLEAR AROMATICS IN THE CAPILLARY GC TRACE OF OF 13 GRAMS OF SFE EXTRACTED LAND FILL SOIL**

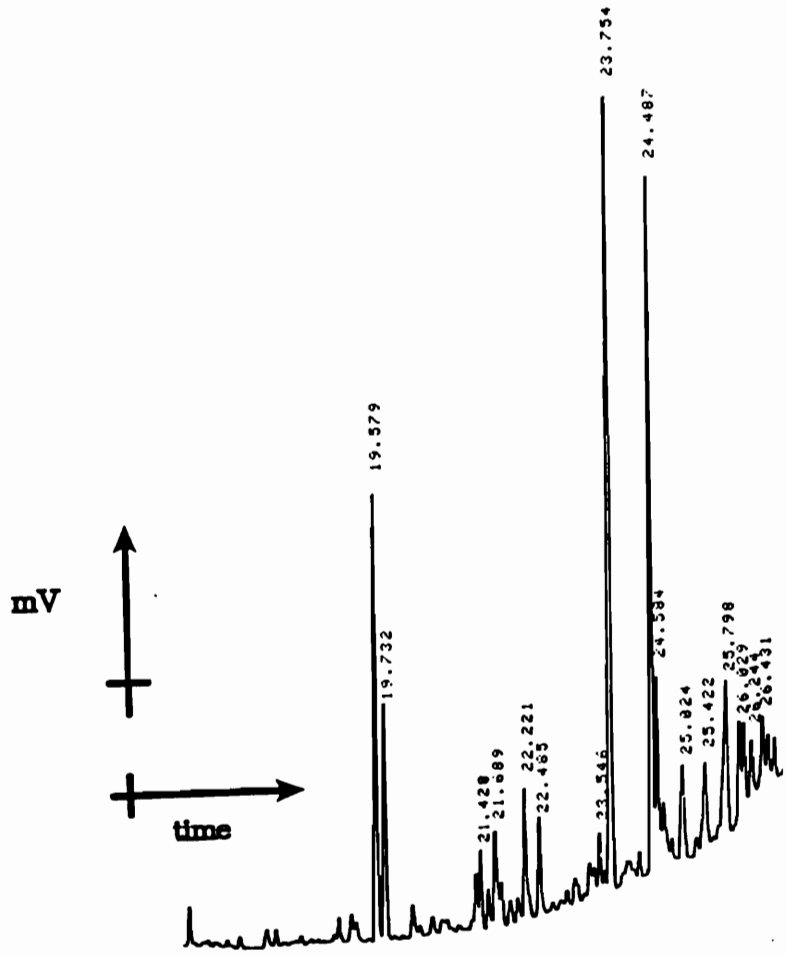

Figure 35. **CAPILLARY GC TRACE OF UNSPIKED EXTRACTED SOIL**

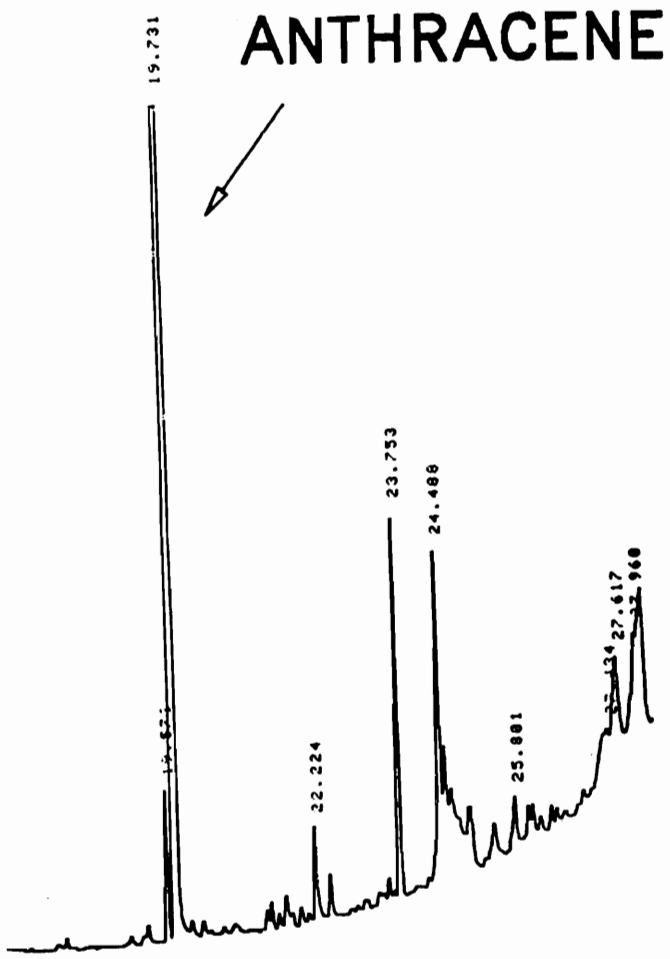

Figure 36. **CAPILLARY GC TRACE OF ANTHRACENE SPIKED SFE EXTRACTED LAND FILL SOIL**

The mass spectral data was taken on a Hewlett-Packard 5890/5970 GCMS unit. The mass spectral configuration and GC temperatures and oven profiles are listed in Tables 30 and 31. The total ion chromatogram (Figure 37) showed the same basic pattern as the capillary gas chromatograph although the resolution was not as high. Because the column used in the MS system was different than that used in the GC sysyem, the retention times were dissimilar; however, the pattern of the total ion chromatograph and the GC trace could be used to identify the peaks of interest. *Using mass spectra of the three standards and comparing those of the mass spectra of the relevent peaks in the total ion chromatogram (Figures 38-46), those peaks identified by retention time and by spiking could be confirmed as anthracene, chrysene, and benzo[a]pyrene.*

Using a stepwise analytical approach to the identification of unknown peaks of a supercritical extraction of contaminated soil led to their confirmation using both chromatographic retention times and mass spectral analysis.

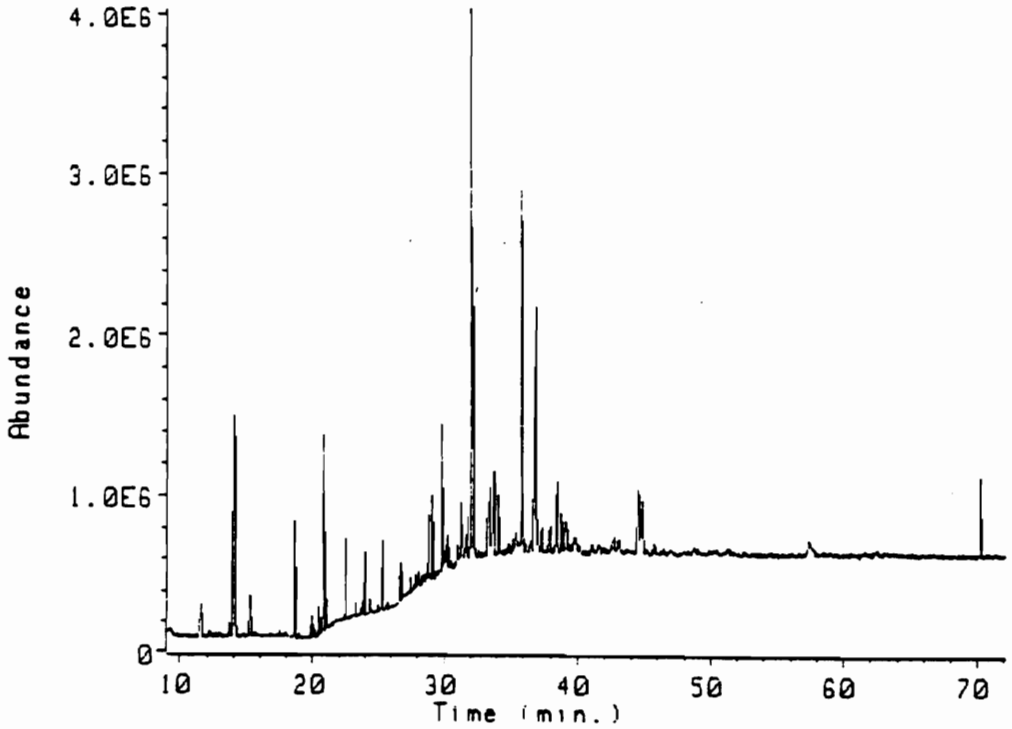

Figure 37. **TOTAL ION CHROMATOGRAM (TIC) FROM A GC/MS OF SUPERCRITICAL FLUID EXTRACTED LAND FILL SOIL**

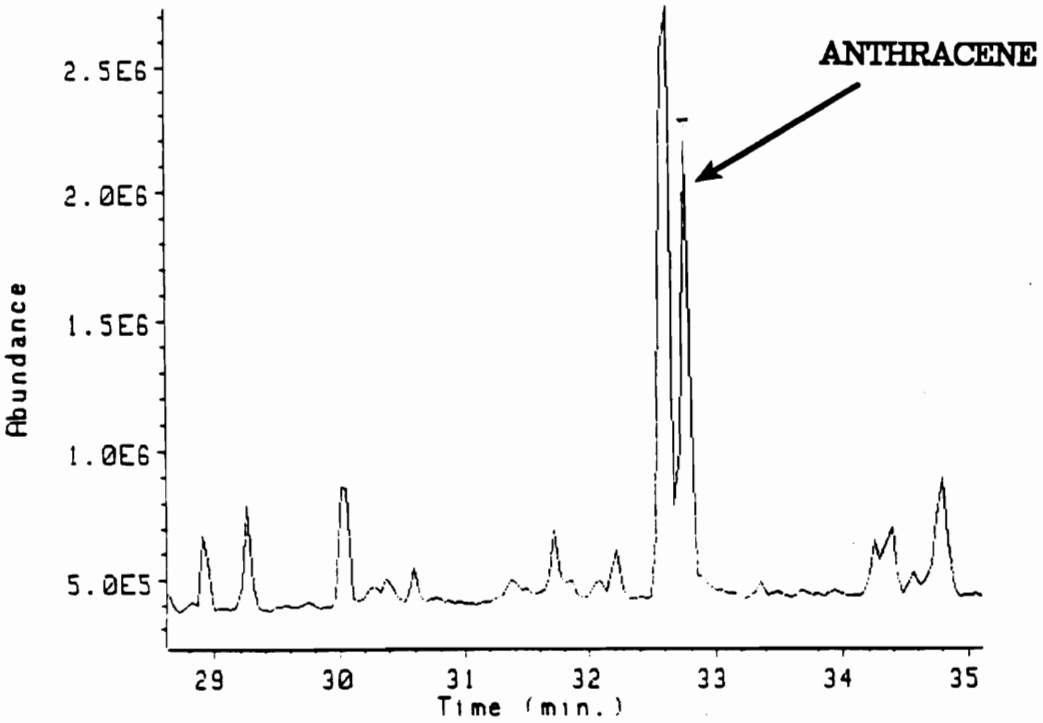

Figure 38. **SELECTED REGION OF THE TIC OF EXTRACTED LAND FILL SOIL**

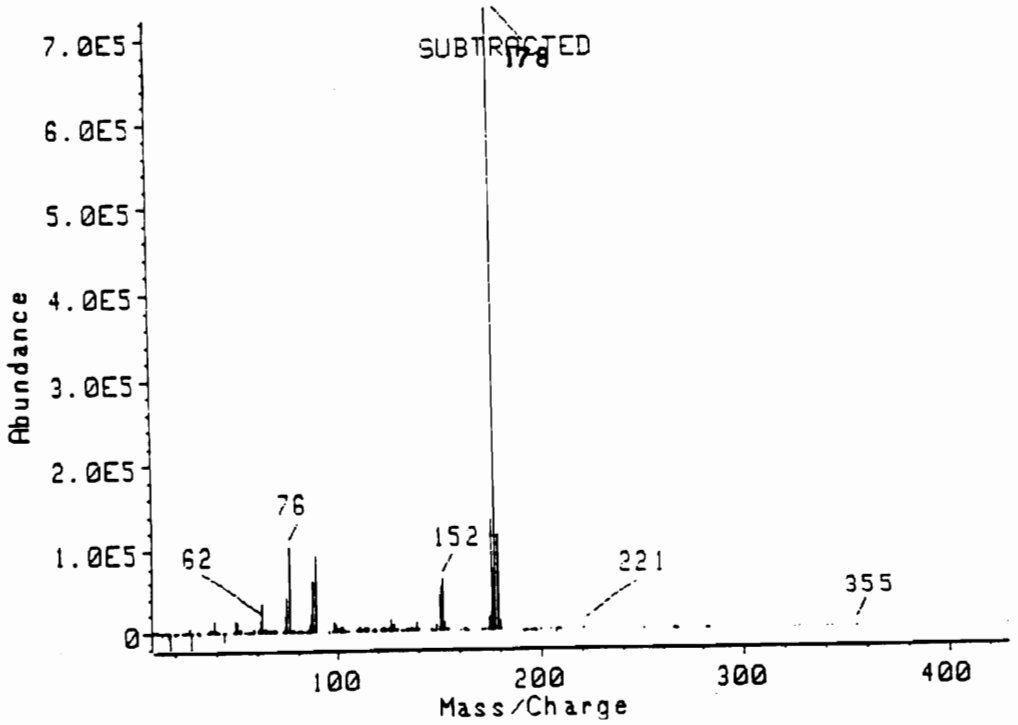

Figure 39. **MASS SPECTRA OF THE PEAK CORRESPONDING TO THE RETENTION TIME OF ANTHRACENE**

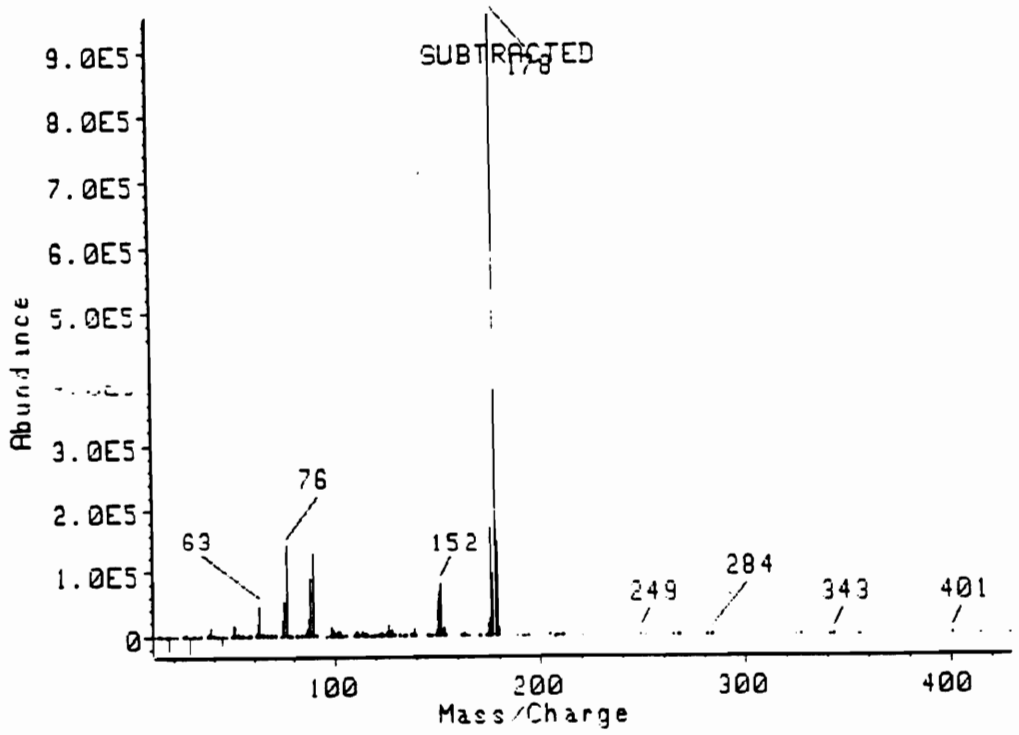

Figure 40. **MASS SPECTRUM OF THE ANTHRACENE STANDARD**

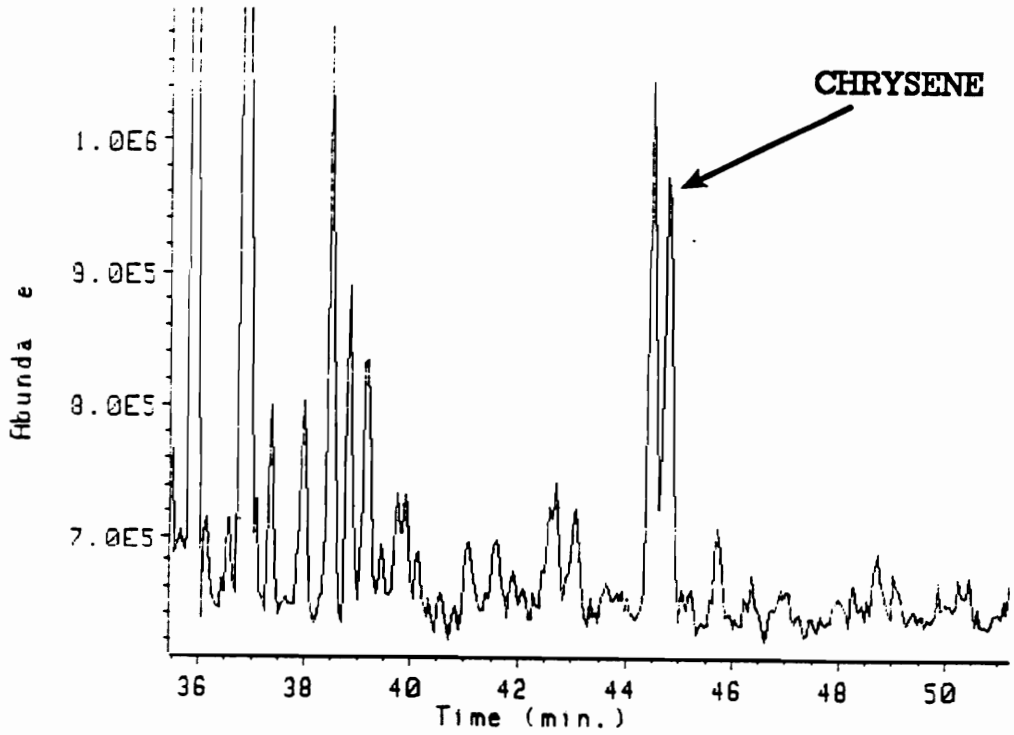

Figure 41. **SELECTED REGION OF THE TIC OF EXTRACTED SOIL CORRESPONDING TO THE K' OF CHRYSENE**

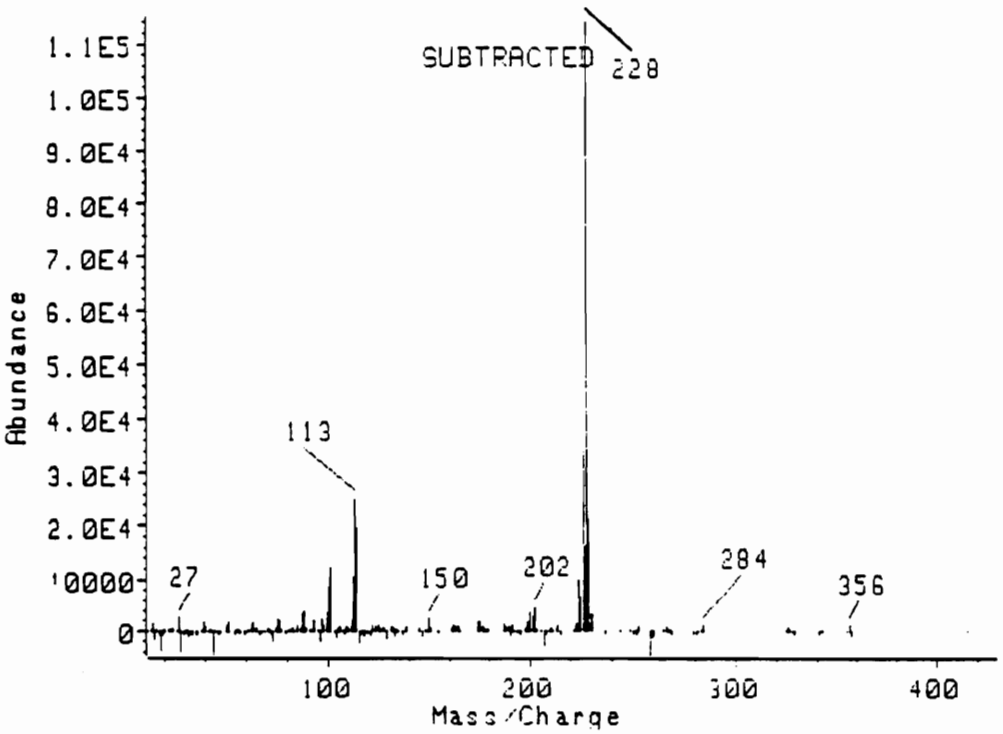

Figure 42. **MASS SPECTRUM OF THE PEAK CORRESPONDING TO THE k' OF CHRYSENE**

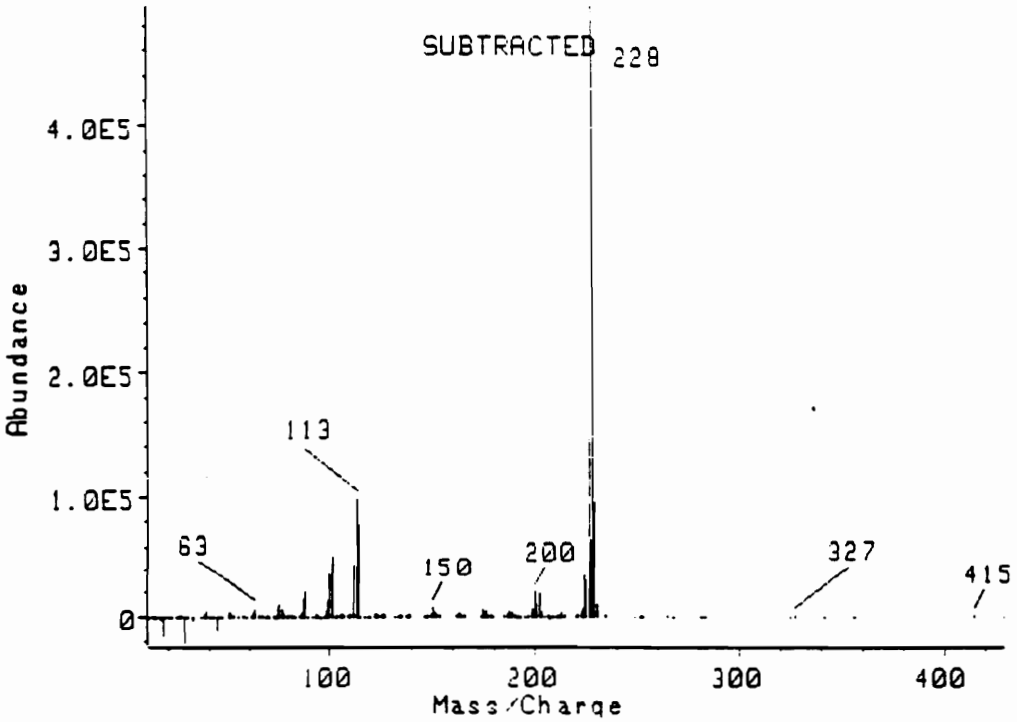

Figure 43. **MASS SPECTRUM OF THE CHRYSENE STANDARD**

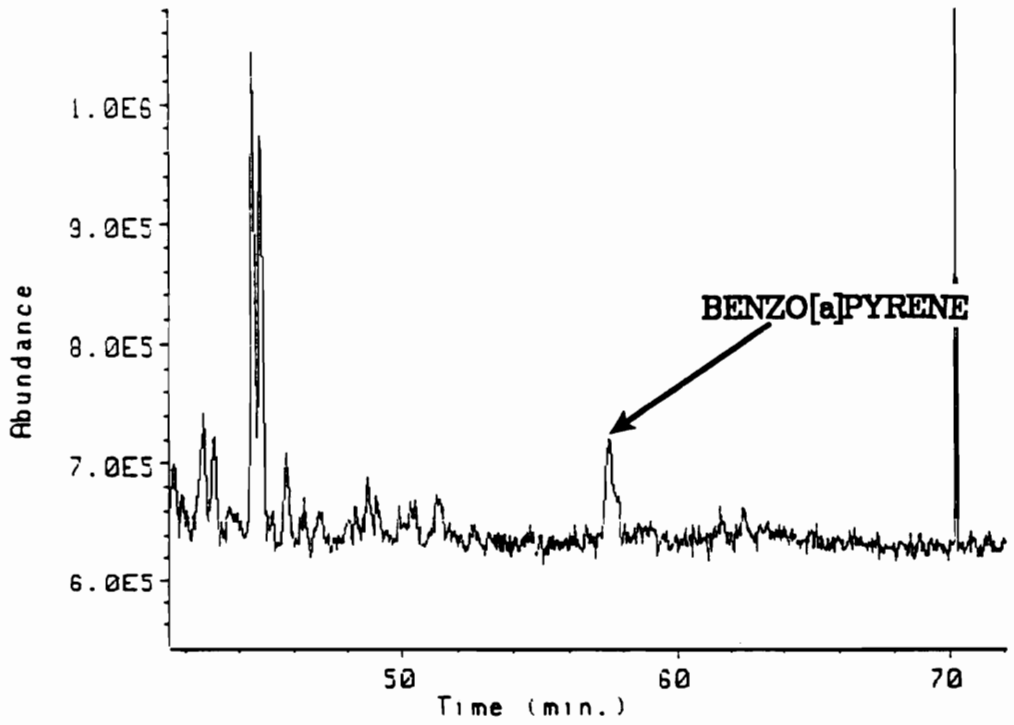

Figure 44. **SELECTED REGION OF THE TIC CORRESPONDING TO THE RETENTION TIME OF BENZO[a]PYRENE**

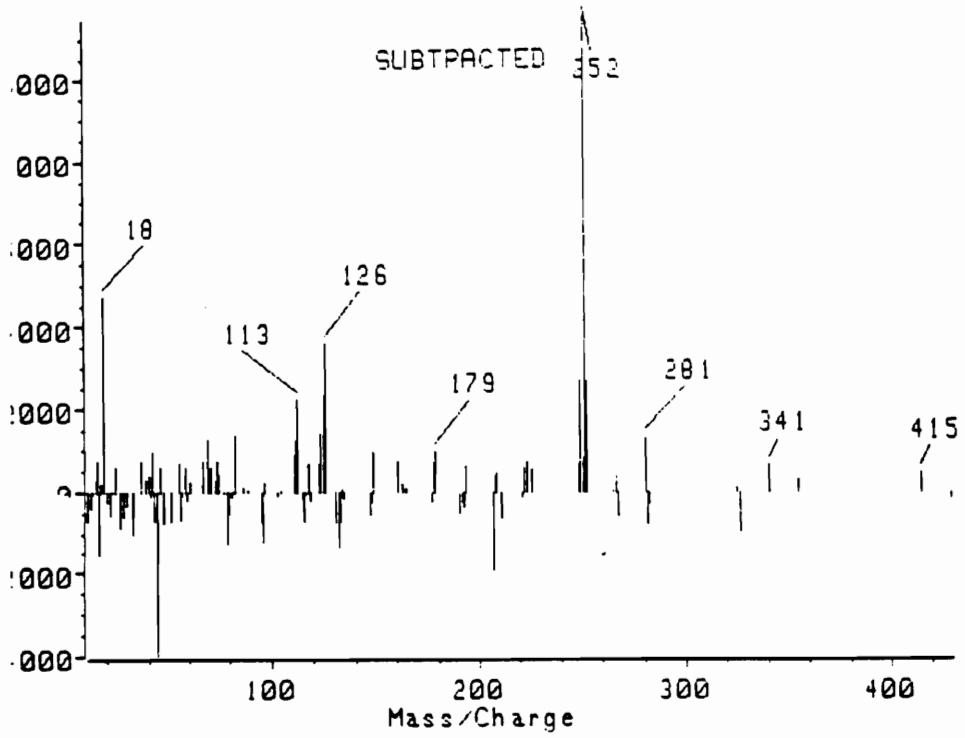

Figure 45. **MASS SPECTRM OF THE PEAK IDENTIFIED AS BENZO[e]PYRENE**

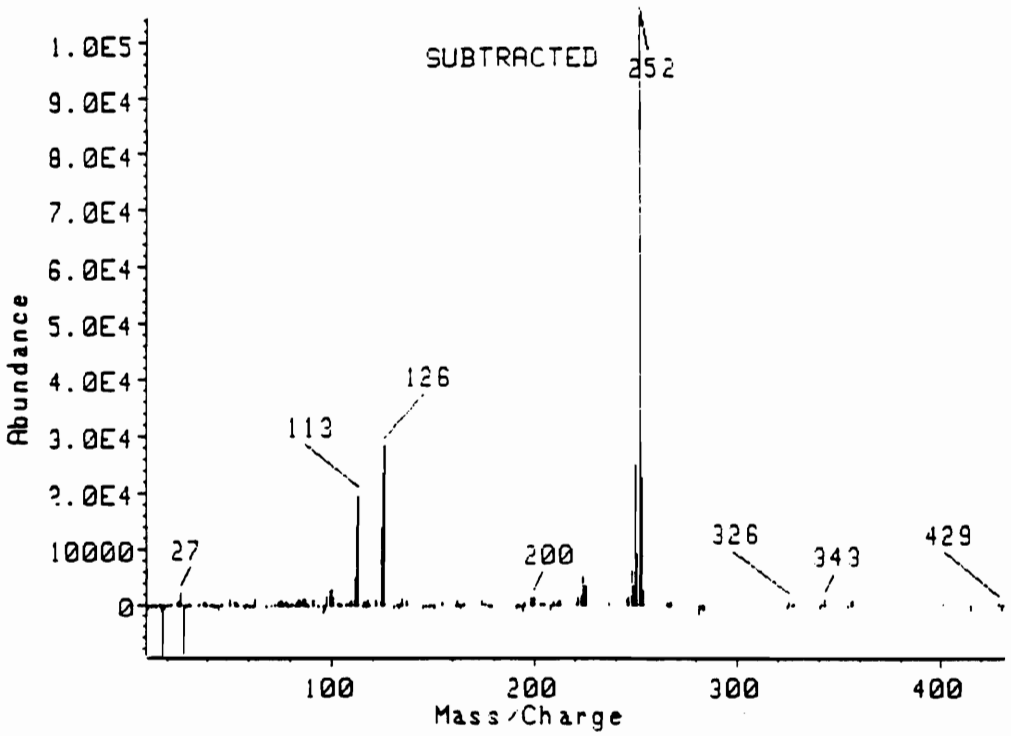

Figure 46. **MASS SPECTRM OF BENZO[a]PYRENE STANDARD**

CONCLUSIONS

The research for this dissertation explored the feasibility of using a home-built supercritical chromatographic system for investigating; on-column concentration (*in-situ* concentration), aspects of SFC quantitation by FID, chromatography of a wide molecular weight range of paraffins, and supercritical extractions of polynuclear aromatics and pesticides from waste landfill soils.

In the first study, on-column or *in-situ* concentration, the innate properties of a supercritical fluid were exploited as a means of concentrating an analyte from a dilute solution at the head of the chromatographic column. Because a solvation mechanism is essential for migration, analytes remain condensed on the column if the supercritical fluid is not dense enough to affect solvation. This physiochemical property (remaining below the solvation density), in combination with remaining below the volatilization point, allows the chromatographer to concentrate an analyte from a dilute solution. The results of this study proved the ability to concentrate an analyte at ppb levels on the column, by repeated injections of dilute solutions at mobile phase densities below the elution density. This was confirmed with analysis of 4 ppb Dursban, 7 ppb pentachlorophenol, and 7 ppb eicosane.

The second portion of the dissertation research concerned quantitative results (precision and linearity of response factors) using the home-built system and commercial FIDs. The first set of experiments in this study explored supercritical fluid

chromatography as a viable alternative to high temperature gas chromatography for the analysis of petrochemical samples, particularly paraffin standards that are components of a middle weight crude oil. The standard mix used was C_{20}-C_{60} normal paraffins. This standard not only allowed the experimental range of molecular weights to be relatively large, it also mimicked the paraffinic portion of a petrochemical sample quite well.

The reproducibilities generated in this set of experiments were not as good as capillary gas chromatography data. Flame instability hindered the attempt to make the system reproducible. Stability was improved using a wide bore (29μm I.D), long (100 cm) restrictor coupled with a modified detector jet and using a slight modification to the hydrogen flow to the detector. These changes resulted in a flame that was more robust. Another problem was the poor linear response and precision of FIDs owing to changes in CO_2 flow during the pressure ramp. The reproducibilities, as reported, were not as good with capillary gas chromatography, but were kept below 10%, with an average of 7%.

The second set of experiments in the quantitative study concerned the response factors for the individual components in the standard test mixture. After the reproducibility had been brought close to that of gas chromatography, the thrust of the study turned to the problem of the response factors. Unfortunately, with the system set up for maximum reproducibilty, the response factors decreased with increasing carbon number (i.e., less response for higher molecular weights). Subsequent studies showed this to be a consequence of the increased CO_2 flow through the column as the pressure increased. In fact, it was shown that there was an inverse correlation between the volumetric CO_2 flow into the detector and the response factors: the higher the flow, the lower the response factor. Obviously, the increased flow was affecting the flame response characteristics, making the flame less efficient at higher flows. Alternate detector geometries were

studied, but did not solve the problem. Pulled or tapered restrictors were also investigated, with no improvement shown. One experiment was designed to see if the problem lied in the injector. Replacing the LC type injector with an on-column injector showed no apparent linearization of the response factors. What was needed (unfortunately, the facilities do not exist at VPI to manufacture one) was a detector that has a geometry that allows for the high flow rates that accompany microbore supercritical fluid chromatography.

The final study involved the feasibility of using supercritical extractions with a resistively heated collector for trace PNAs and pesiticides from soil. The results from the supercritical fluid extraction of polynuclear aromatics and pesticides showed excellent recoveries from glass beads and soil matrices, above 88% for the model compounds studied. When damp soil was used it was imperative to use the resistively heated collector to prevent condensation of moisture in the restrictor tip, along with the 50μm I.D. (instead of the 20μm I.D.) fused silica tubing.

The concentrations used throughout the standard study were in the 2-10 ppm range and showed excellent results. The results with the 20 ppm solution showed an average recovery of 90%; average recovery dropped to 82% with the 20 ppb solutions and no significant recovery was seen with the 2 ppb standard.

Because the extraction of the standards resulted in excellent recoveries the focus of the study was shifted to look at real world samples. SFE of a waste landfill soil showed more than 60 peaks by off-line capillary GC, most of which were in the sub ppm range. This soil was shown to contain anthracene, chrysene, and benzo[a]pyrene; these findings were confirmed by capillary GC retention time, spiking, and mass spectral analysis.

Supercritical fluid extraction shows considerable promise not only in the extraction of analytes from soils and other solid matrices, as substantiated in this dissertation, but also for extractions from liquids (53). The use of supercritical fluids as both mobile phases for chromatography and extraction media has an exciting future. The innate physical properties of the fluid make it ideal for applications in both areas.

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VITAE

James Owen Frazier was born on January 17, 1960 in Hutchinson, Kansas to John and Clara Frazier. He was raised in Garden City, Kansas and attended school through the age of 18 in the public school system. In 1978, he attended the University of Kansas where he obtained degrees in chemistry and biochemistry. After undergraduate school, he moved to Virginia where he attended graduate school in chemistry. From 1983 to 1986, he worked for Tomas Hudlicky and obtained a Masters in synthetic organic chemistry, specifically, the synthesis of pyrrolizidine alkaloids. After his stint in organic, he moved into analytical chemistry where he studied under the auspices of Professor H.M.McNair in the area of supercritical fluids. He graduated in 1989 and is now working for American Cyanamid R&D in Princeton, New Jersey.