SUPERCRITICAL FLUID EXTRACTION OF ORGANICS IN ROCKS AND URBAN DUST AND IDENTIFICATION OF ORGANIC COMPOUNDS IN SEWAGE SLUDGE PRODUCTS AND SOILS

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1995

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

25 April 95 — Date

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April 25, 1995 — Date

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ABSTRACT

SUPERCRITICAL FLUID EXTRACTION OF ORGANICS IN ROCKS AND URBAN DUST AND IDENTIFICATION OF ORGANIC COMPOUNDS IN SEWAGE SLUDGE PRODUCTS AND SOILS

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Advisor: Professor David C. Locke

A supercritical fluid extraction (SFE)/chromatography method was developed for the analysis of hydrocarbons and nitrated polycyclic aromatic hydrocarbons in solid environmental samples. A systemic study for the extraction conditions was performed including extraction pressure, temperature, time, effect of the addition of modifiers, and collection efficiencies. Quantitative extraction of C_{16} to C_{32} alkanes (recovery $\geq$ 90%) was obtained at 8000 psi, 99°C for 20 min extraction. The addition of 10 mol% hexane or toluene improved the extraction efficiency. Six rock samples were extracted at optimum SFE conditions and C_{14} to C_{24} were identified. The optimum extraction conditions for nitrated polycyclic aromatic hydrocarbons were at 8500 psi, 99°C for 25 min. The addition of 10 mol% of methanol or acetone improved the extraction efficiency. 1-Nitro-pyrene, 6-, and 7-nitro-benzocoumarin were identified in NIST SRM 1649 urban dust sample. SFE was compared with Soxhlet extraction. Compared with Soxhlet extraction, SFE is a quicker, simpler and safer extraction method.

The identification of organic compounds in the sewage sludge products is important for the evaluation of sludge utility. GC-MS and HPLC were used for the identification of organic compounds in complex New York sewage sludge products and soil samples. The most numerous compounds identified were petroleum hydrocarbons. PAHs, phenols, phthalate esters, fatty acids and some cholesterol derivatives were also identified. The sources of the compounds were discussed. Semiquantitative results and total organic carbon in the samples were tested.

ACKNOWLEDGMENTS

Several years diligent study and work resulted in completion of this thesis. I would like to take this opportunity to express my grateful thanks to all who supported and encouraged me to make this thesis a success.

First and foremost, I am sincerely grateful to my advisor Dr. David C. Locke, for his numerous valuable advisements and support which gave me a deep effect for my future career. It is pleasure to work with him and no words can sufficiently express my hearty thanks to him. Next, I would like to express my warmest hearty thanks to my parents, without their love, encouragement and help I could not have got today's achievement and come so far in my career.

I gratefully acknowledge my committee members, Dr. Arthur D. Baker and Dr. Ronald L. Birke for their valuable guidance and suggestion. I would like to express my thanks to my fellow graduate students, especially to Guang Li, for their assistant and cooperation. I am very grateful to the Chemistry Department of Queens College for the teaching assistantship, to the Graduate School of the City University of New York for the university fellowship.

At last and most, my hearty thanks to my husband, Yuejin Liu, for his love, understanding, and help.

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1.1 Introduction

The initial extraction and complete recovery of organic analytes from solid samples are very important steps for quantitative analysis. Soxhlet and sonication extractions are EPA approved methods [1] for the extraction of organic compounds from solids. However, they need large amounts of liquid solvent and long extraction times, which can range from several hours to days. The long extraction times and hot solvents can cause the degradation of some compounds.

Microwave assisted extraction has been developed since 1985 [2] to satisfy the requirements of speed and efficient extraction for today's analytical chemistry laboratory. It is the process of heating solvents in contact with a sample with microwave energy to facilitate partition of compounds of analytical interest from the sample matrix into the solvent. During microwave assisted extraction, the solvent and sample are put into a closed vessel and rapidly heated by microwave energy and the temperature then held for a set time. On completion of the heating cycle, removal of the microwave energy enables the vessel to cool quickly and the extract can be removed for analysis. Microwave assisted extraction has several advantages in comparison with Soxhlet extraction. Firstly, it reduces the amount of solvent used in Soxhlet extraction by a factor of 10 or more; typically, 30 ml solvent is used for microwave extraction. Second, it decreases the extraction time from hours to minutes. The closed-vessel microwave heating extraction

approach can reduce exposure of solvent vapors to the laboratory personnel and to the environment. Ganzler and co-workers **[3]** in 1986 published the first paper exploring the use of microwave energy to partition vicine and convicine from fava beans. The result was comparable to that of traditional Soxhlet extraction. Since then, it has been applied to the analysis of biological **[4]**, polymer **[5]** and environmental **[6-7]** samples. Microwave assisted extraction is a relatively new method and there are relatively few publications. A commercial instrument, which can handle 12 samples simultaneously, is available now from CEM Corp. (Matthews, NC) as the MES 100.

Even though microwave assisted extraction has significantly decreased the extraction time and the amount of solvent used, it is not applicable to the extraction of thermally sensitive compounds. Recent concerns about the hazardous nature of many commonly used solvents, the costs and environmental dangers of waste solvent disposal, and the emission of hazardous solvent vapors into the atmosphere during sample concentration, all support the development of alternative sample extraction methods. Supercritical fluid extraction (SFE) has become a sample extraction method of increasing interest and been developing rapidly in recent years. SFE is a technique that exploits the solvent power of supercritical fluids. It operates at relatively low temperatures and pressures. Although Hannay and Hogarth **[8]** reported the high solvent power of supercritical fluids over a century ago, the rapid development of theory and practical application of SFE has occurred only in the recent decade. The attention this techniques is getting comes from its advantages over traditional solvent extraction methods.

- SFE is fast. Because supercritical fluids have diffusivities an order of magnitude higher and viscosities an order of magnitude lower than liquid solvents, they have much better mass transfer characteristics. Quantitative SFE generally is completed in 10-60 minutes, whereas liquid extraction can range from several hours to days.

- SFE is friendly to the environment. The most common supercritical fluid used for extractions is CO_2, which is inert, nontoxic, nonflammable, and available pure and inexpensive. The generation of liquid waste solvents and exposure of laboratory personnel to toxic solvents is greatly reduced or eliminated by SFE.

- SFE is applicable to the extraction of thermally unstable compounds. SFE can be performed at low temperature because CO_2 has a low critical temperature (31.1°C). SFE is also safe for the extraction of foods or pharmaceutical samples.

The solvent strength of a liquid is essentially constant regardless of extraction conditions, but the solvent strength of a supercritical fluid depends on the extraction temperature and pressure. The solvent strength of a supercritical fluid can thus easily be controlled. At a constant temperature, extraction at lower pressures will favor less polar analytes, and extraction at higher pressure will favor more polar and higher molecular weight analytes. SFE makes class-selective separation available. The selective separation of groups or compound classes can simplify a mixture of analytes before analysis. In some cases, selective fractionation allows easier resolution of the compounds of interest.

Most of the supercritical fluids used for extraction are gases at ambient conditions. Liquid solvent extracts need to be concentrated prior to the determination of trace organic analytes, which requires additional time, can result in the loss of more volatile analytes, and releases organic vapors to the environment. In contrast, concentration steps after SFE are greatly simplified and direct coupling of the SFE step to chromatographic techniques is available.

New extraction methods such as SFE need to be tested by application to the analysis of a variety of compounds in different matrices. The purpose of this work is to study the SFE efficiency of hydrocarbons in rock samples. The identification of hydrocarbons in rock samples is basic to search for petroleum fields. SFE may be applicable to geoprospecting studies.

1. 2 Supercritical Fluid Extraction

1.2.1 Principle of SFE

A supercritical fluid (SF) is a substance that is heated and pressurized to a temperature and pressure above its critical temperature, T_c, and critical pressure, P_c. Supercritical fluids have unique physicochemical properties which make them attractive as solvents for extraction. The most important and useful property of a SF is its density, which exceeds that of its parent gas and is close to that of normal liquid **[9]** (Table 1.1). This gives a SF the solvating power close to that of a liquid. For a given SF, the solvent strength is directly related to its density, ρ, as described by Giddings' equation **[10]**:

$$\delta = 1.25\ P_c^{1/2}(\rho/\rho_l)$$

where δ is the Hildebrand solubility parameter, and ρ_l is the density of the SF gas in its liquid state. Figure 1.1 shows how the density of a SF changes with extraction pressure and temperature **[11]**. Various combinations of temperature and pressure yield the same density value.

When the concentrations of the target analytes are at minor or trace levels, the analytes need only be soluble enough in the SF to be transported out of the matrix. The diffusivity of solutes in a SF is about one order of magnitude greater than in a normal liquid, so dissolved analytes move through the SF more quickly. The viscosity of a SF is about one order of magnitude lower than a liquid, which means it can move easily to penetrate into a porous solid material and have a faster mass transport rate. In addition, the zero surface tension of the SF allows for efficient penetration into microporous materials. These properties of supercritical fluids, liquid-like density, gas-like viscosity and diffusivity, and zero surface tension make them "super solvents" with potential wide use for extractions.

Supercritical CO_2 has been chosen for most SFE studies, primarily because of its attractive physical characteristics: relatively low critical temperature and pressure, non-toxicity, inertness and high purity at low cost. In general, SF CO_2 is an excellent extraction solvent for the extraction of non-polar species. However, it is less efficient for the extraction of polar compounds, and other polar solvents (additives or entrainers) may have to be added to the CO_2 . The characteristics of some supercritical fluids are listed in Table 1.2.

Perhaps because of the historical development of SFE in process engineering, most of the available theories of SFE are borrowed from

engineering studies. Two SFE mechanisms, one a thermodynamic mechanism **[12]** considering solubilities and matrix effects, and the other a kinetic mechanism **[13]** considering desorption kinetics, have been presented. King **[14]** applied thermodynamic principles to predict solubilization, extraction, and fractionation conditions. Erkey and Akgerman **[15]** used a dynamic tracer pulse technique to measure adsorption equilibrium constants for naphthalene on alumina in the presence of CO_2. Giddings' solubility parameter theory was used to develop a method which permitted the quantitative estimation of solute solubility levels in dense and liquefied gases over a range of pressures and temperatures **[16]**. Because SFE is relatively new in the analytical area, further theoretical studies will be necessary to understand the mechanisms and interactions among the matrix surface, the analytes, and the SF, and the functional mechanism of solvent modifiers.

Table 1.1 Comparison of Properties of Supercritical Fluid with Liquid and Gas [a]

	ρ (g/cm^3)[b]	η (g/cm.s)	D (cm^2/s)
gas (STP)	(0.6-2)x10^{-3}	(1-3)x10^{-4}	(1-4)x10^{-1}
SF[c]	0.2-0.5	(1-3)x10^{-4}	10^{-3}-10^{-4}
liquid	0.6-2	(0.2-3)x10^{-2}	(0.2-2)x10^{-5}

[a] : from reference 9.

[b] : ρ = density, η = viscosity, D = diffusion coefficient

[c] : properties at T_c & P_c

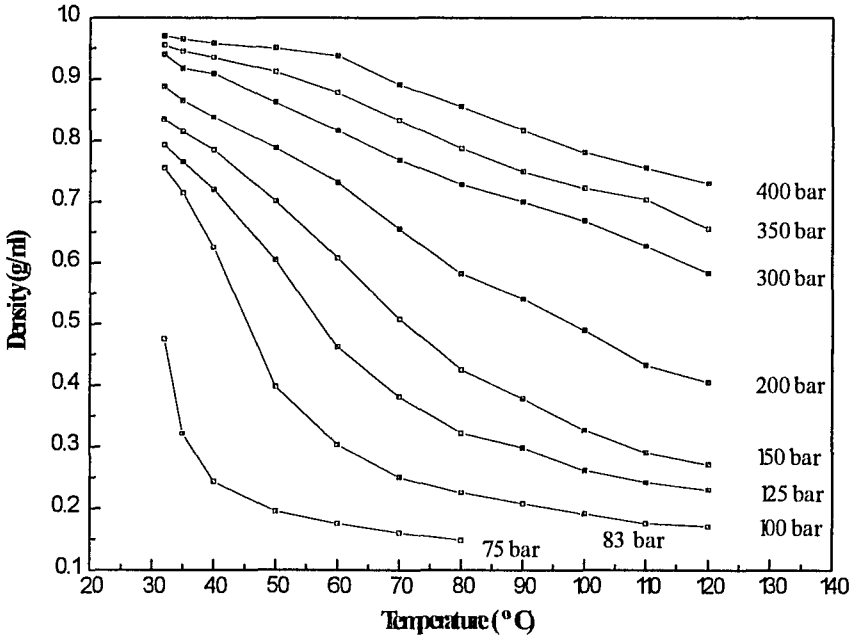


Figure 1.1 Plots of Carbon Dioxide Density Versus Temperature at Various Pressures [a]

[a] : from reference 11.

Table 1.2 Characteristics of Selected Fluids [a]

Fluid	T_c (°C)	P_c (atm)	ρ_c (g/ml)	Solubility parameter, δ
CO_2	31.1	74.8	0.472	10.7
N_2O	36.6	73.4	0.445	10.6
NH_3	132.4	115.0	0.211	13.2
MeOH	240.1	82.0	0.246	14.4
CH_3CH_2OH	243.2	62.2	0.275	-----
CH_3COCH_3	235.1	47.6	0.242	-----
$CHCl_3$	263.4	54.4	0.525	-----
$CHClF_2$	96.1	49.1	------	-----
H_2O	374.4	224.1	0.281	-----
methane	-82.4	46.6	0.160	-----
ethane	32.4	49.5	0.219	6.6
propane	96.8	43.1	0.219	-----
butane	155.2	38.5	0.225	-----
hexane	234.4	30.5	0.230	-----
toluene	318.8	41.4	------	-----
MeOH-CO_2(10:90)	51.5	74.2	------	-----
$CHClF_2$-CO_2(10:90)	38.2	69.6	------	-----
toluene-CO_2(10:90)	67.2	67.9	------	-----

[a] : from references: 26, 27 & 28.

1.2.2 Static and Dynamic Extraction

SFE can be performed in either the static or dynamic mode. Static extraction is carried out by placing the sample in contact with the SF in a closed container. After a set period of time, a valve is opened to allow the analytes to be swept into the collection device. In dynamic extraction, the SF is constantly flowing through the cell, and a flow restrictor is used to maintain pressure in the extraction vessel and to allow the SF to depressurize into the collection device. The static mode allows better penetration of the matrix by the fluid, and the dynamic extraction avoids saturation of the extraction fluid, leading to shorter extraction time. The two extraction modes can be combined to provide better and quicker extraction **[17]**.

1.2.3 Factors Affecting Extraction

SFE is controlled by a complex relationship between many experimental variables, including the pressure, temperature, addition of modifiers, fluid velocity, cell geometry and matrix effects.

1.2.3.1 Influence of Pressure

The fluid pressure is one of the main parameters that influence the extraction recovery. The choice of extraction pressure should not only be one higher than the critical pressure of the fluid, the threshold pressure of the target analytes should also be considered **[18]**. The threshold pressure corresponds to the pressure at which the solute partitions into the SF. Each compound must pass its threshold pressure in order for its solubility in the SF to increase to a maximum value with increasing extraction pressure **[19]**. The solubilities of the analytes increase with increasing pressure because at a given temperature the density of CO_2 increases with pressure.

Consequently, the higher the extraction pressure, the smaller the volume of fluid necessary for a given extraction **[20]**. However, class-selective separations from complex matrices can be obtained by programming the extraction pressure. For example, alkanes can be extracted from urban air particulates with CO_2 at 75 atm (45°C) whereas the PAHs remained unextracted until the pressure is raised to 300 atm **[21]**. David and co-workers **[22]** successfully obtained three separation fractions in the analysis of flavor and fragrance samples by SFE. They stepped the pressure to different densities of CO_2 at a constant temperature (50°C). In first fraction, the essential oils were extracted at a density of 0.3 g/ml. The bitter acids in the second fraction were extracted at a density of 0.70 g/ml. The majority of the lipids were extracted at a density of 0.9 g/ml.

1.2.3.2 Influence of Temperature

Temperature is an important but complex parameter in SFE. At a constant pressure, as the temperature increases the density of CO_2 decreases nonlinearly, which reduces solubilities of solutes. However, higher temperature also increases the volatilities of the analytes and helps the escaping tendency of the extract from the matrix in terms of enthalpy and phase transfer kinetics. A lower temperature-higher density procedure is useful for thermally sensitive compounds. Higher temperature favors the extraction of volatile compounds. For example, when the temperature is increased from 80°C to 120°C, the extraction recovery of diuron from soil with methanol-modified CO_2 is increased from 75% to 99% **[23]**. However, the benefits of high extraction temperature have not received much attention until recently. For example, John and co-workers **[24]** found that the effect of extraction temperature was more important than extraction pressure for

the extraction of PCBs from sediment and PAHs from air particulate matter, which were efficiently extracted only at 200°C. PCBs were effectively extracted at any pressure (150-650 atm), while both higher temperature and pressure increased the recovery of PAHs from air particulate matter.

1.2.3.3 Addition of Modifiers

The addition of modifiers can be achieved either by means of a separate pump via a mixing device, or from a pre-mixed cylinder. However, it should be remembered that if using a cylinder, the modifier composition changes slightly as the contents are consumed. Alternatively, modifier may be added directly to the matrix before extraction. The mechanism by which the addition of modifiers improves the extraction efficiency is not very clear. Generally, it is thought that addition of modifiers aids extraction efficiency in two ways: by aiding in the displacement of the analytes from the matrix, and by increasing the solubilities of the analytes in the mixed solvent **[25]**.

In general, highly polar analytes can not be quantitatively extracted by supercritical CO_2 alone, and a few percent of a polar solvent modifier must be added to the CO_2 for efficient extraction. Methanol has been the most widely used modifier and in some cases, the extraction efficiency has increased dramatically. For example, the extraction efficiency of dibenzo[a,l]carbazole from XAD-2 sorbent resin **[29]** and 2,3,7,8-tetrachlorodibenzo-p-dioxin from soil samples **[30]** was doubled by the addition of methanol to CO_2. The extraction efficiency of diuron from soil **[23]** was increased from less than 10% to more than 90% by the addition of methanol to CO_2. Besides methanol, a variety of organic compounds have been used, such as methylene chloride **[25]**, toluene and $CHClF_2$ **[28]**. The

selection of modifiers and their concentration has been largely empirical because very little analyte solubility data exist for modified supercritical fluids. In addition, the competitive interactions between the modified SF and the target analytes with the sorption sites on the bulk matrix are poorly understood. Recently, a SF-Solver software was produced by Isco, Inc. **[26],** which can be used to calculate the critical parameters for co-solvent and it will be helpful for the choosing of suitable modifiers.

As an alternative to the addition of modifiers, chemical derivatization during SFE also can improve the extraction efficiency of reactive compounds **[31]**. The enhanced yield is attributed to the interaction of the derivatization reagents, not with the analyte molecules themselves, but rather with the adsorptive sites to which the analyte is adsorbed **[32]**. Hills and Hill **[33]** added hexamethyldisilane and trimethylchlorosilane (2:1 v/v) derivatization reagents, which were used to reacted with a wide variety of functional groups including carboxylic acids, alcohols, phenols, and amines, to NIST standard reference material (SRM) 1649 and the National Research Council of Canada standard reference material HS-3, prior to SFE with CO_2 for the extraction of PAHs. They compared these results with those obtained by the addition of 10% methanol to the CO_2. The data showed that the addition of derivatization reagents was better than the addition of 10% methanol modifier for the extraction.

1.2.3.4 Influence of Flow Rate and Cell Geometry

In the dynamic extraction mode, the flow rate of the SF through the cell has a strong influence on the extraction efficiency. The slower the fluid speed, the deeper it penetrates the matrix and the higher the extraction efficiency.

For example, ^{14}C-labeled linear alkylbenzene sulphonates were better extracted by supercritical CO_2 modified with 40% methanol from a sludge-amended soil with a fluid CO_2 flow rate of 0.45 ml/min (mean recovery 91± 1%) instead of 1.2 ml/min (mean recovery 76±1%), the same volume of fluid being used in each instance **[34]**. However, the slower flow rate results in longer extraction times. On the other hand, high flow rates can result in a decrease in the recovery either because of an elevated pressure drop through the extraction cell or by increasing analyte loss during decompression of the fluid. For a given extraction cell, the flow rate can be easily changed by using a new restrictor with a different inside diameter.

For a given flow rate, the fluid linear velocity can be changed by using several cells having the same volume but different inside diameters. Higher extraction efficiencies are expected with short, broad cells because the fluid linear velocity decreases as the cell diameter increases. This has been observed for the extraction of PCBs spiked into C18 sorbents by supercritical CO_2 with a flow rate of 0.075 ml/min and a total fluid volume of 1.5 ml. The use of a short, broad cell with dimension 9.9 mm i.d. x 9.9 mm instead of a long, narrow cell with dimension 4.4 mm i.d. x 50.0 m, increased the extraction recovery nearly 50% **[35]**.

1.2.3.5 Influence of the Nature of the Matrix

Factors such as the particle size, shape, surface area, porosity, moisture, and level of extractable solutes all affect the extraction efficiency. In addition, the interactions between solutes and active sites of the matrix must be considered.

1.2.4 Collection of Extracts

The collection of extracts is a very important step for successful SFE. Even if all the target analytes of interest have been extracted from the sample under optimum extraction conditions, if some are lost during the collection step, high extraction efficiency still can't be obtained. Thus the quantitative efficacy of the collection device must be determined using appropriate spike recovery studies prior to further development of a SFE method. Generally, the collection of extracts can be performed either by on-line or off-line collection mode. In on-line collection, the supercritical extract is depressurized and the dissolved analytes directly transferred to a chromatographic system. In off-line SFE, the dissolved analytes are depressurized into a collection system such as a solid adsorbent trap or a solvent-filled or empty vial, and the collected analytes determined by appropriate methods. The advantages of on-line SFE include the elimination of sample handling between extraction and chromatographic analysis and the potential to achieve maximum sensitivity by quantitatively transferring the extracted analytes into the chromatographic column. The disadvantages are that the sample loadability of the extraction vessel is relatively small, due to the small analyte capacity of the analytical chromatographic column, and that the extracts are not available for analysis by a different method. In comparison, off-line SFE can load more samples to the extraction vessel and collected extracts can be analyzed by any appropriate method. However, different variables need to be considered during off-line collection, such as restrictor plugging, SF flow during depressurization, collection temperature, choice of solvent for the collection, and the possibility of the loss of volatiles during collection and concentration.

1.2.4.1 Solid Adsorbent Trap

To collect the extracts by a solid adsorbent trap, a suitable adsorbent must be chosen which can quantitatively retain all of the analytes of interest as well as allow them to be quantitatively recovered after collection. After collection, a suitable solvent is used to elute the collected compounds from the solid trap, which is then concentrated and chromatographically analyzed. Silanized glass beads, C18 pellicular packing material, XAD-2, silica gel and Tenax **[36-40]** have all been used for the solid traps. Ashraf-Khorassani and co-workers **[36]** packed silanized glass beads into the trap and used methylene chloride as wash solvent; the collection efficiency for PAHs was greater than 90%. Miles et al. **[37]** used 30-μm C18 pellicular packing material to adsorb the extracted compounds from fresh garlic and onion matrices.

The decompression velocity of CO_2 should be controlled during collection. Otherwise, volatiles and even semivolatiles can be lost because of the formation of aerosols by fast decompression of CO_2 **[36]**. The addition of an organic modifier can also affect the collection efficiency. Mulcahey **[41]** reported that quantitative retention on adsorbent traps was difficult when methanol was added to CO_2 for the extraction because methanol became a liquid solvent upon depressurization and could itself elute the target analytes from the adsorbent resin during the SFE step, resulting in low recoveries.

1.2.4.2 Liquid Solvent Trap

Liquid solvent traps have been widely used because they use a few ml of organic solvent less than that used to elute solid adsorbent traps, and are immediately ready for chromatographic analysis using conventional

injection techniques. However, various parameters affect the collection efficiency and have to be considered, such as collection temperature, flow rate of extraction fluid, the use of restrictor heaters and the type of solvent used. Restrictor plugging can often occur during extraction when the sample matrix contains high concentrations of extractable material or water since the depressurization occurs at the restrictor tip and inside the restrictor **[42]**. The reduction of the extraction fluid density within the restrictor can cause a decrease in the solubility of the analytes. The decrease in analyte solubility, combined with the Joule-Thomson cooling effect of the expanding extraction fluid at the restrictor exit, produces a supercritical solvent which may lead to analytes precipitating and ultimately plugging the inside of the restrictor. The restrictor plugging problem can be solved by heating the restrictor during the extraction **[43-44]**. The flow rate of CO_2 also can affect the collection efficiency of a liquid solvent trap. Trapped molecules can be purged from the collection solvent by the high gas flow of the depressurized extraction fluid **[45]**.

Langenfeld and co-workers **[46]** systematically studied collection solvent polarity, volume, depth and collection solvent temperature. They examined the collection efficiency of 66 compounds in different solvents with a range of volatility and polarity. The results showed that collection solvent polarity and temperature were more important factors than collection solvent volume and depth for efficient collection. Greater than 90% collection recoveries were attained for all test analytes by controlling the solvent temperature at 5°C.

1.2.4.3 Solventless Collection

Solventless collection means the extracted analytes are depressurized into an empty vial and rinsed out with a few ml of solvent. Poor recoveries using empty vials have been reported **[29][36][47]**, especially for trace organics. But when large quantities of bulk matrix are extracted, such as fat from meats, direct depressurization into an empty vial has been successful **[48]**. Recently, Miller and Hawthorne **[49]** reported high collection efficiencies (>90%) for PCBs, PAHs, gasoline and diesel fuel by solventless collection. Following static SFE, the analytes were collected by rapidly (3-30s) depressurizing the CO_2 effluent through a 178 μm i.d. stainless tube into an empty capped screwtop vial. The use of wider stainless steel tube instead of flow restrictor avoided the restrictor plugging even for the extraction of wet samples. The concentrations of test solutions were in the range of 4.0 mg/ml to 100 mg/ml, and the spiked amounts were 5 mg for n-alkanes, 0.04 mg for PCBs and 0.07 mg for PAHs. Collection time had a significant effect on the recoveries of more volatile compounds. When the collection time was 30 s, just 40-70% of C_6-C_7 were recovered, but when the collection time was reduced to 3 s, the collection efficiency was increased to greater than 90%.

1.2.4.4 On-Line SFE

On-line SFE has most often been coupled with gas chromatography (SFE-GC) or supercritical fluid chromatography(SFE-SFC). SFE can be coupled with capillary GC by inserting the extraction cell restrictor through an on-line column injection port into the capillary column itself **[50]**. This gives the best sensitivity with dilute samples. For concentrated or larger sized samples, the supercritical fluid extract is depressurized into a conventional split-splitless injection port, via a heated transfer line **[51]**. Other interfaces,

such as a thermodesorption-cold trap injection system **[52]**, a programmed-temperature vaporizer injector **[53]** and a six-port valve **[54]** have also been used to couple SFE with GC. Hawthorne et al. **[55]** used SFE-GC to analyze terpenes and oxyterpenes from tree needles, leaves and wood. SFE/SFC coupling was achieved by flowing the extract through an injection loop and collecting the analytes at the head of the SFC column. Once SFE is completed, the extracted organics are swept from the accumulating device into the SFC column using the SFC mobile phase, and the analysis is carried out under appropriate chromatographic conditions. SFE/SFC was used to analyze pesticide residues from soil **[56]** and drugs from kidney tissue **[57]**.

SFE has also been coupled with other chromatographic techniques. On-line SFE/HPLC **[58]** and SFE/SFC/FTIR **[59]** were reported. Cortes et al. **[60]** developed an on-line multidimensional SFE/micro column LC/open-tubular GC for the quantitative determination of ppb levels of the pesticide chlorpyrifos in grass sample. Barber et al. **[61]** combined SFE with a quadrupole MS to determine the solubility of solid CCl_4 in supercritical CF_4.

1.2.5 Applications of SFE

SFE has been widely used to extract various types of compounds from different matrices. Most often it is used to extract compounds from solids, but it also maybe used to extract analytes from gases or liquids. To extract organic compounds from gas samples, the gas is first trapped on a solid phase sorbent, which is then SFEd. A few studies have shown the efficiency of supercritical fluids in extracting compounds with different volatilities from solid phase-trapped gas samples **[62-64]**. For aqueous samples, direct SFE is difficult because of the relatively high solubility of water in

supercritical CO_2 (approximately 0.3%) **[65]**. Only a few studies have dealt with the direct SFE of liquids, such as that of Roop et al. **[66]** who directly SFEd phenol from contaminated water, and John and Wendawiak **[67],** who directly extracted PAHs by supercritical CO_2 from viscous engine oil. Liquid samples are usually first adsorbed on a solid material such as Tenax, XAD resin, charcoal, polyurethane foam or a solid phase extraction disc, and then are placed in the extraction chamber to be SFEd. For example, sulphonyl urea herbicides can be isolated from water samples by solid phase extraction **[68]**. The analytes were then eluted from the extraction disk by SFE using CO_2 + 5% methanol. Recoveries of the herbicides were mostly higher than 80% and were measured for 1 dm^3 water sample and concentration levels of 50 $\mu g/dm^3$. More information about the application of SFE for gases and liquids can be found in Camel's review paper **[69]**.

SFE has been widely used for the analysis of various organic compounds from environmental, pharmaceutical, food, and polymer samples. Chester **[70]** and Janda **[71]** reviewed the application of SFE by the type of compounds analyzed. The use of supercritical fluids for the study of chemical reactions will be a interesting research subject and more publications will appear in the future **[82]**.

1. 3 Hydrocarbons

Petroleum hydrocarbons are found in soil, rock and marine sediments **[72-74]**. The hydrocarbons can be of biogenic origin or contamination from diesel oil spills or leaks from underground storage tanks, for example, hydrocarbons have been used as bio-markers by geochemists and their

identification is important in the exploration for fossil fuels deposited within the Earth.

The extraction of hydrocarbons from soil and rock can be performed by conventional liquid extraction **[74]**. Recently, SFE has been used to extract them from environmental samples **[75-76]**. SFE can selectively extract hydrocarbons from soil in shorter time than liquid extraction. The conventional liquid extraction of hydrocarbons from rock typically takes 48 hr and requires extract cleanup by preparative TLC to separate the alkanes from aromatic hydrocarbons and nitrogen-, and sulfur-containing species. However, SFE can selectively extract only the alkanes from rock sample. Extraction times were just 21 min. C_{12}-C_{35} hydrocarbons were identified in rock samples **[76]**.

The analysis of hydrocarbons usually is carried out by GC with flame ionization detector (FID). The combination of high resolution capillary columns with the FID allows the determination of hydrocarbons at the sub-ppb level **[74]**. Ashraf-Khorassani and co-workers **[77]** used SFC to analyze saturated hydrocarbons and aromatics in petroleum. The hydrocarbons and aromatics in petroleum streams were selectively separated by Petropak-S column combined with FID.

1.4 Experimental

1.4.1 Chemicals and Supplies

Reference hydrocarbons were obtained from following sources: dodecane (C_{12}) , 99%, was purchased from Alfa Products (Danvers, MA), tetradecane (C_{14}), 99% and hexadecane (C_{16}), 99%, from Metro Scientific, Inc. (Farmingdale, NY), Octadecane (C_{18}), 99%, from Sigma Chemical Company (St. Louis, MO), tetracosane (C_{24}), 99%, octacosane (C_{28}), 97%, and dotriacontane (C_{32}), 97%, from Aldrich Chemical Company Inc. (Milwaukee, WI). Hexane was of HPLC grade (Fisher Scientific). A stock solution of n-C_{12}-C_{32} was prepared containing around 1 mg/ml of each in hexane. The standard solution of n-C_{12}-C_{32} was prepared by transferring a suitable amount of the stock solution to a volumetric flask and diluting with hexane. The concentrations of the stock solution and the standard solution used to spike filter paper for the SFE study are listed in Table 1.3. The stock solution and the standard solution of n-C_{12}-C_{32} were stored in a -18°C refrigerator until used.

Adsorbent materials used for the trap study were C18 and Tenax TA (60-80 mesh) from Alltech Associates Inc. (Deerfield, IL), XAD-2 from Supelco Inc. (Bellefonte, PA), Chromosorb W (60-80 mesh) from Applied Science Laboratories Inc. (State College, PA) and silica gel (60-200 mesh) from J. T. Baker Inc. (Philipsburg, NJ). Except C_{18}, all of the adsorbent materials were cleaned by Soxhlet extraction with suitable solvent for 8 hr.

Table 1.3 Stock and Standard Solutions of n-C_{12}-C_{32} Hydrocarbons

Compound	Stock Solution (mg/ml)	Standard Solution (ng/μl)
C_{12}	1.00	10.0
C_{14}	1.00	10.0
C_{16}	1.00	10.0
C_{18}	0.993	9.93
C_{24}	0.991	9.91
C_{28}	0.998	9.98
C_{32}	0.999	9.99

1.4.2 Rock Samples

Six rock samples: BE-1, BIT-2, ULA, ULB, MO2 and MO3 were obtained from Geology Department of Queens College (grateful to Professor Charlotte Schreiber for providing the samples). They were collected from Lorca Basin in the southeast of Spain. The age of rocks was upper Niocene ($5.3\text{-}23.7 \times 10^6$ years).

1.4.3 SFE Apparatus

A SFE-703 was briefly lent by Dionex Corp. The instrument is equipped with a 703E co-solvent delivery system and can extract eight samples simultaneously. The extractions using the addition of modifiers were performed using the 703.

All other extractions were performed using a lab-made SFE apparatus depicted in Figure 1.2. The apparatus consisted of a gas tank (A) to supply the SFE fluid; a regulator (B) to control the pressure of the gas tank; a motor-driven single-ended diaphragm compressor (C) (Newport Scientific, Jessup, MD) capable of producing up to 10,000 psi; an extraction chamber (D) to load samples; a heating jacket (E) (Scientific Systems Inc., State College, PA), which can control temperatures to a maximum of 99°C ± 0.1°C; an exit valve (F) to control the flow rate of the supercritical fluid and to depressurize the system to atmospheric pressure in a controlled fashion; and a stainless steel trap (G) which was packed with solid adsorbents to collect extracted compounds. The extraction chamber was cut from 5/16" i.d. stainless steel tubing purchased from Alltech Associates Inc. (Deerfield, IL) and cut into 11" lengths. This tubing can endure the pressures up to

10,000 psi. A smaller stainless steel tube (7" x 3/16") was used to pack solid adsorbent materials and collect extracts.

1.4.4 SFE Procedure

The general procedure using the lab-made SFE apparatus was as follows. First, the extraction chamber, valve, elbow connection tube, and trap were rinsed with solvents, such as hexane, methylene chloride (HPLC grade, Fisher Scientific) and acetone (spectrophotometric grade, Aldrich Chemical Company Inc.), and dried by N_2 gas. The sample was packed into the extraction chamber. Both sides of the extraction chamber were supported with glass wool. The trap was loaded with a solid adsorbent material and supported with glass wool plugs on both sides. To remove the air in the extraction system before SFE, the CO_2 (SFC grade) gas tank was turned on and the regulator adjusted to let a small amount of CO_2 pass through the system for about 2 min. Then the valve was closed and the oven temperature set to the desired temperature. After the temperature was balanced, the compressor was turned on and controlled via the regulator of the CO_2 gas tank until the desired pressure was reached. Then the extraction time was set which did not include the time for equilibration of oven temperature and pressure. After the extraction was complete, the compressor and gas tank were turned off, the valve slowly opened and the extraction chamber depressurized to atmospheric pressure. Meanwhile, the extracted compounds were carried into the collection trap. The SFE system was disconnected and the valve, elbow tube and trap were eluted with about 30 ml hexane. This hexane solution was concentrated to 1.0 ml in a Buchi rotary evaporator (Brinkmann Instruments Inc., Westbury, NY) and analyzed using GC.

1.4.5 Trap Study

The quantitative collection ability of several solid adsorbent materials; the adsorbent trap plus a solvent back-up trap; and a cold solvent back-up trap were investigated. In each case, 1.0 ml of a standard solution containing about 10 μg each of C_{12}-C_{32} n-paraffins was spiked onto a piece of filter paper (Fisher brand, 9 cm i.d.), which was prewashed ultrasonically with hexane and methylene chloride (50/50 (v/v)) for 1 hr. After the solvent was evaporated from the filter paper it was cut into small pieces and loaded into the extraction chamber. The solid adsorbents tested were C18, Tenax TA, XAD-2, Chromosorb W and silica gel. All static extractions were performed at 7000 psi and 99°C for 20 min.

To evaluate the solvent and the cold back-up traps, a piece of Teflon tubing was used to connect the adsorbent trap to volumetric flask containing 25 ml of hexane. During depressurization, CO_2 plus the extracted compounds first passed through the solid trap, and then entered the solvent back-up trap. In order to increase the collection efficiency of SFE for low boiling point compounds, a cold back-up trap was added. The configuration is shown in Figure 1.3. A piece of Teflon tube was used to connect the end of the solid trap and inlet of the cooled glass tube, which was contained in a dry ice+CCl_4 Dewar flask, and a second piece of Teflon tubing was used to connect the outlet of the cooled glass tube to a volumetric flask containing 25 ml hexane.

The elution efficiency of hexane for the paraffins from the adsorbent materials was investigated. 1.0 ml of the standard solution of n-C_{12}-C_{32} was spiked onto the same amount of adsorbent materials packed into the SFE

trap. 30 ml of hexane was used to elute the trap. The solution was concentrated to 1.0 ml in a rotary evaporator and analyzed using GC to determine the recovery of the n-C_{12}-C_{32} eluted from the adsorbent materials.

1.4.6 SFE Optimum Conditions

Extraction pressure, temperature and equilibrium extraction time are the three important parameters affecting the extraction efficiency of SFE. Five different pressures: 3000 psi, 5000 psi, 7000 psi, 8000 psi, 9000 psi were studied at 99°C for 20 min. Four different extraction times: 10 min, 15 min, 20 min, 30 min, and five different temperatures: 25°C, 35°C, 55°C, 75°C and 99°C were studied at both 7000 psi and 8000 psi. 1.0 ml standard solution of n-C_{12}-C_{32} was spiked onto a piece of the filter paper for all of extractions.

In order to study the effect of a modifier, 10 % toluene, 10% hexane and 10% methylene chloride by molar were added to CO_2 respectively by 703E co-solvent delivery system, and the spiked filter papers were extracted with SFE-703 instrument at 7000 psi , 100°C for 20 min.

1.4.7 Soxhlet Extraction

To compare with SFE, Soxhlet extraction of spiked filter paper with 1.0 ml standard solution of n-C_{12}-C_{32} was performed. The spiked filter paper was cut into small pieces and put in a Pyrex glass thimble (25 x 85 mm, Fisher Scientific). The Soxhlet extraction was carried out with about 60 ml hexane for 20 hr. The extraction was repeated five times.

To Soxhlet extract the rock samples, about 2 g (exactly weighed) of each rock sample was extracted with 60 ml hexane for 24 hr.

1.4.8 GC Determination

Extracts were analyzed using a Hewlett Packard model 5880 GC equipped with a flame ionization detector (FID) and a crosslinked methyl silicone gum (12 m x 0.2 mm x 0.33 μm film thickness) capillary column (Hewlett Packard). Helium was the carrier gas. A 5890A GC integrator was used to measure peak areas and heights for the quantitation. To obtain higher sensitivity and more repeatable quantitative results, a splitless mode was employed with a split valve closed time of 0.75 min. Generally, exactly 2.0 μl sample was injected for the analysis.

Gas chromatographic conditions were: oven temperature was held initially at 50°C for 2 min and programmed to 280°C at 10 °C/min and held for 10 min. Injector and detector temperature were maintained at 340°C and 320°C, respectively. For the analysis of rock sample extracts, the oven temperature program rate was changed to 3 °C/min to get better separation because the rock samples were complicated and a lot of peaks appeared on the chromatogram.

For quantitative analysis, different concentrations of n-C_{12}-C_{32} standard solutions were prepared and a calibration curve was plotted. The concentrations of n-C_{12}-C_{32} in the rock samples were calculated as the following formula:

$$C_i = \frac{A_{sample}}{A_{st.}} \times C_{i\,st.} \times V_{inj.\,st.} \times \frac{V_{conc.sample}}{V_{inj.\,sample}} \times \frac{1}{W_{sample}}$$

where,

A_{sample} : peak area of the sample

$A_{st.}$: peak area of the standard solution

$C_{i\ st.}$: concentration of the standard solution

$V_{inj.\ st.}$: injection volume of the standard solution

$V_{conc.sample}$: the volume of the sample solution after extraction and concentration

$V_{inj.\ sample}$: the injection volume of the sample

W_{sample} : the weight of the sample extracted

All of the standard solutions and the solutions concentrated after the extraction were injected into GC at least five times to examine the reproducibility of GC. To reduce variance of the GC-FID determination, the standard n-C_{12}-C_{32} solution was injected before, between and after the analysis of extracted solutions or sample solutions every day.

1.5 Results and Discussion

The separation and determination of precision in the GC analysis of hydrocarbons was the first important work needed to be done. To optimize SFE conditions for the hydrocarbons, different extraction temperatures, pressures and times were studied. To distinguish low recovery caused by the extraction step vs the collection step, a experiment was performed to evaluate the collection efficiency of the adsorbent trap. To test whether there was contamination from the filter paper matrix, solid adsorbent, or solvent, a blank test for whole process of SFE was performed. The recoveries of SFE were determined by comparing the GC peak areas of SFEd n-C_{12}-C_{32} with those of directly injected standard solution of n-C_{12}-C_{32}, calculated by

$$\text{Recovery (R)} = \frac{A_e}{A_S}$$

Where A_e is the peak area of a SFEd hydrocarbon and A_S is the peak area of standard hydrocarbon.

Six rock samples collected from Lorca Basin were analyzed for hydrocarbons. This is basic information in the study of the form and maturation mechanism of organic material present in sediments and is also a signal of the source for petroleum. The results of SFE and Soxhlet extraction are discussed later in this section.

1.5.1 GC Study to Analyze n-C_{12}-C_{32} Standard Solution

The n-C_{12}-C_{32} standard solution was totally separated by GC. A high resolution chromatogram is shown in Figure 1.4. The retention time and peak area are listed in Table 1.4. The chromatogram and the values of the peak areas indicate there was discrimination against higher hydrocarbons. The discrimination was caused by the low volatility of the higher hydrocarbons, which did not evaporate immediately in the injection port. Table 1.5 shows that as the injection port temperature is increased, the discrimination against higher hydrocarbons is decreased. However, to avoid thermal decomposition of the compounds, the injection port temperature was set at 340°C. Wang et al. **[78]** studied injection discrimination against higher hydrocarbons by comparison with on-column injection. The cold on-column injection technique may eliminate the discrimination, but the column can easily be damaged by direct on-column injection.

To increase the sensitivity of the GC, a splitless injection mode was employed. The initial temperature of the column was set at 50°C which is lower than the boiling point of solvent hexane to recondense the sample components at the top of the chromatographic column and avoid the peaks broadening or splitting by solvent flooding **[79-80]**.

Calibration of n-C_{12}-C_{32} was carried out over the concentration range 0.5-10 ppm which was the concentration range in the sample rock extracts. Each solution was repeatedly injected three times and the averages of the peak areas were used for plotting. Figure 1.5 shows that the plots are linear and the correlation coefficients are better than 0.99.

To check the repeatability of the GC for the analysis of n-C_{12}-C_{32}, the 10 ppm n-C_{12}-C_{32} standard solution was injected 5 times and the relative standard deviation (RSD) results are listed in Table 1.6. The results show that the higher hydrocarbons have the higher RSD. The detection limit for the determination of n-C_{12}-C_{32} at S/N of 3 and attenuation at -2 , by GC with a FID ranged from 20 pg for n-C_{12} to 50 pg for n-C_{32} (Table 1.6).

Table 1.4 Retention Times and Peak Areas of n-C_{12}-C_{32} [a]

Compound	tr (min)	Peak Area (integrator units)
C_{12}	8.18	108.7
C_{14}	10.99	110.5
C_{16}	13.45	111.4
C_{18}	15.65	92.80
C_{24}	21.10	68.03
C_{28}	24.03	52.70
C_{32}	26.92	43.79

[a] : GC conditions: initial column temperature: 50°C, kept for 2 min, then increased to 280°C at a temperature program rate of 10°C/min and held for 5 min. Injection Port temperature: 340°C. Injection volume: 2.0 µl. Standard solution: 10 ppm of each compound.

Table 1.5 Injection Port Temperature and Relative Peak Area

Temp.	310°C	320°C	340°C	375°C
C_{12}	1.000*	1.000	1.000	1.000
C_{14}	1.009	1.010	1.017	1.018
C_{16}	1.021	1.023	1.025	1.028
C_{18}	0.838	0.838	0.854	0.852
C_{24}	0.609	0.623	0.626	0.766
C_{28}	0.417	0.454	0.485	0.537
C_{32}	0.312	0.363	0.403	0.469

* normalized areas relative to peak area of C_{12}.

Table 1.6 Detection Limit and Precision for n-C_{12}-C_{32} Analysis

Compound	Detection Limit (pg)	Precision (RSD)
C_{12}	20	2.0%
C_{14}	22	2.3%
C_{16}	20	2.1%
C_{18}	24	2.1%
C_{24}	26	14%
C_{28}	34	16%
C_{32}	50	21%

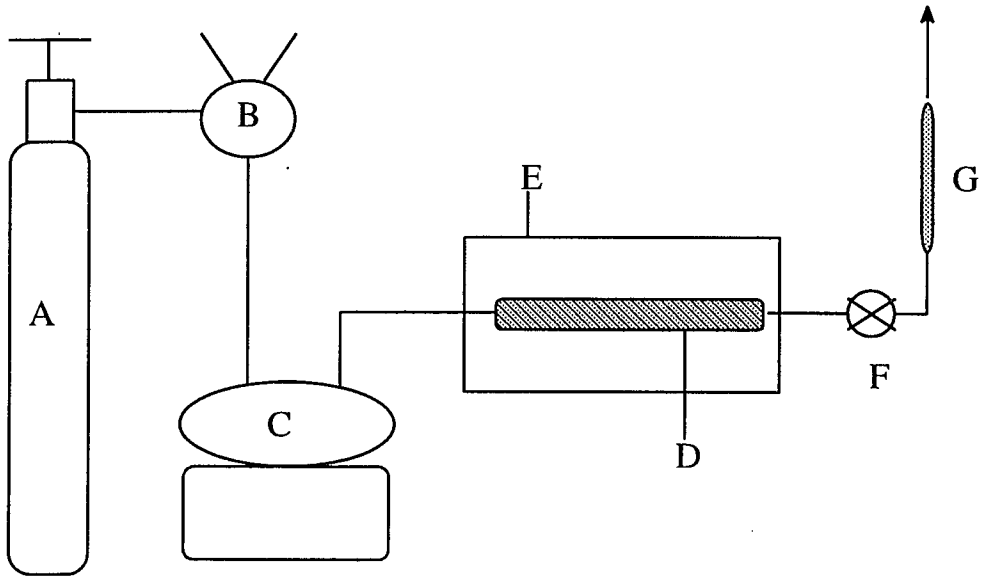


Figure 1.2 Schematic Diagram of SFE

A: Gas tank B: Regulator C: Compressor D: Extraction chamber
E: Heating jacket F: Valve G: Trap

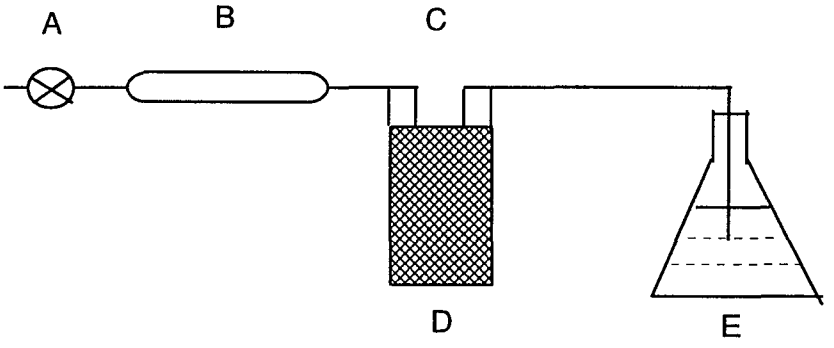


Figure 1.3 Schematic Diagram of the Cold Trap

A: Valve B: Trap C: Cold trap

D: Dewar containing dry ice+CCl_4 E: Solvent back trap

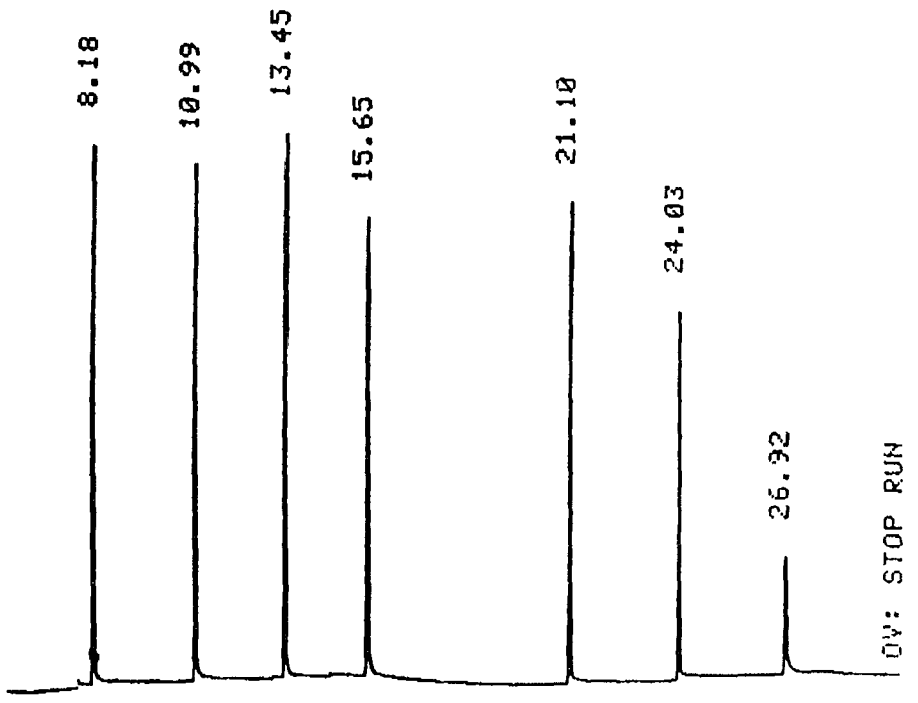


Figure 1.4 GC Chromatogram of Standard Solution of n-C_{12}-C_{32}

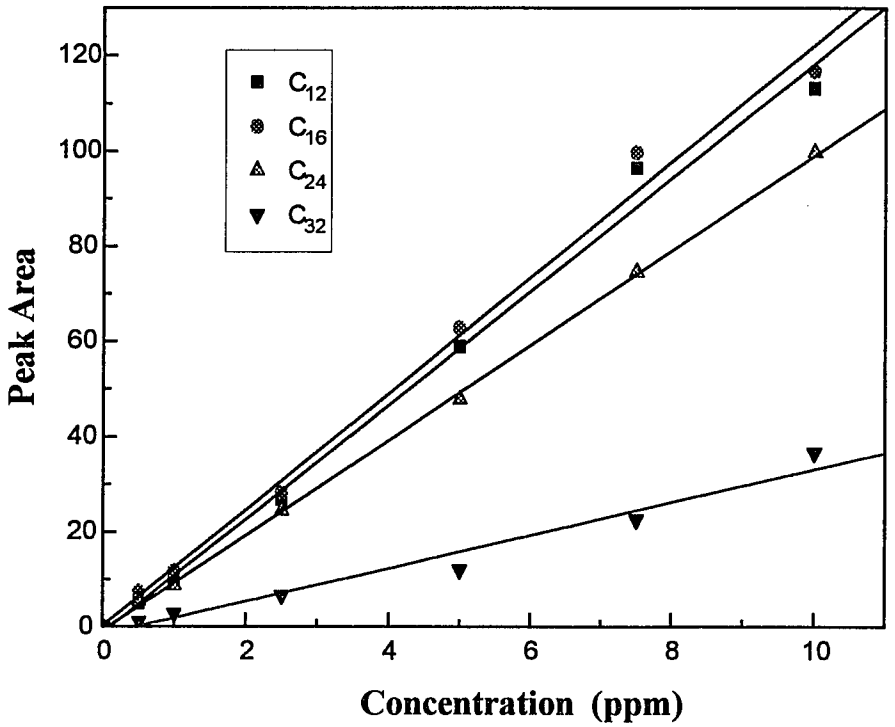


Figure 1.5 Calibration Curve for Standard Solution of n-C_{12}-C_{32}

1.5.2 Evaluation of Traps

The development of quantitative SFE methods for the recovery of organics from the sample requires three steps: quantitative partitioning of the analytes from the sample into the extraction fluid, quantitative removal from the extraction chamber, and quantitative collection of the extracted analytes. Spike recovery studies are often not valid for determination of extraction efficiency from complex real samples because spiked compounds may not be exposed to the same active sites as the native compounds. However, spike studies are an excellent method to evaluate the collection efficiency. One ml of a standard solution of n-C_{12}-C_{32} containing about 10 μg of each compound was spiked onto a piece of prewashed filter paper. Several solid adsorbent materials were tested as traps for the compounds after extraction and depressurization. To evaluate the collection effect of a solid trap, there are two critical steps. First, the extracted compounds must be totally carried into the solid trap and adsorbed on the solid adsorbent material, and second, the compounds must be quantitatively eluted from the solid adsorbent substance using a suitable solvent.

Chromosorb W, silica gel, XAD-2, Tenax TA and C18 were the five adsorbent materials evaluated for hydrocarbons. Table 1.7 shows that C18 is a best solid adsorbent material for hydrocarbons. Except n-C_{12}, most of other compounds showed good recoveries. The flow rate of CO_2 or the rate of depressurization also affected the efficiency of collection. For higher rates of depressurization, the compounds tend to form aerosols and evaporate which causes loss of analytes and yields a lower collection recovery **[36]**. During the experiments, the valve was always carefully

controlled to slow down the rate of decompression and get the same collection efficiency.

In an attempt to increase the collection efficiency for lower hydrocarbons, a cold trap was used to trap the analytes. The cold trap may improve the collection efficiency for the more volatile compounds **[81]**, but Table 1.8 shows it did not improve recovery much for n-C_{12} and the recoveries of n-C_{14}-C_{18} almost kept at the same level.

To determine whether the lower recovery of n-C_{12} was caused by the adsorbing step or eluting step including concentration after elution, 1.0 ml n-C_{12}-C_{32} standard solution was spiked onto similar amount of C18 adsorbent substance as filled the collection trap, eluted with 30 ml of hexane and concentrated to 1.0 ml in a rotary evaporator and GC analyzed. Table 1.9 shows that all of the compounds were quantitatively recovered. So the lower recovery of n-C_{12} must be due to loss during the depressurization step.

Table 1.7 Recovery by SFE of n-C_{12}-C_{32} with Various Adsorbent Materials (7000 psi, 99°C, 20 min) [a]

	Chromosorb W	Silica Gel	XAD-2	Tenax TA	C18
C_{12}	1.0%	9.0%	1.4%	5.3%	10%
C_{14}	47%	50%	46%	62%	77%
C_{16}	80%	54%	64%	66%	80%
C_{18}	75%	53%	67%	69%	87%
C_{24}	44%	28%	64%	67%	98%
C_{28}	40%	29%	64%	67%	95%
C_{32}	53%	41%	70%	71%	93%

[a] : the extractions were carried out using the lab-made SFE apparatus.

Table 1.8 Recovery by SFE of n-C_{12}-C_{32} with Different Traps (7000 psi, 99°C, 20 min) [a]

	Tenax TA	Tenax TA+25mlC_6H_{14}	Tenax TA+dryice+CCl_4
C_{12}	5.7%	5.2%	7.1%
C_{14}	62%	65%	63%
C_{16}	66%	64%	64%
C_{18}	69%	71%	69%
C_{24}	67%	65%	67%
C_{28}	67%	66%	66%
C_{32}	71%	70%	71%

[a] : the extractions were carried out using the lab-made SFE apparatus.

Table 1.9 Elution Efficiency from C18 Solid Adsorbent

Compound	Recovery
C_{12}	96%
C_{14}	100%
C_{16}	99%
C_{18}	100%
C_{24}	99%
C_{28}	99%
C_{32}	97%

1.5.3 Evaluation of Optimum SFE Conditions for Hydrocarbons

Extraction pressure, temperature and time, the three basic parameters affecting the recovery in SFE, were all tested. In addition, several modifiers were added to CO_2 to determine their effect on the extraction of hydrocarbons by SFE.

1.5.3.1 Effect of Pressure

To determine the effect of pressure on the SFE of hydrocarbons, 3000 psi, 5000 psi, 7000 psi, 8000 psi and 9000 psi, 99°C for 20 min were evaluated. The experiments were run in duplicate and the results (Table 1.10) show that as pressure increases, the recovery of the hydrocarbons increases. Except for n-C_{12} and n-C_{14}, all hydrocarbons were quantitatively recovered (>90%) at 8000 psi and 9000 psi. For n-C_{14}, and especially for n-C_{12}, even at 9000 psi, the recovery of SFE was very low, because the compounds escaped during depressurization step. To compare Soxhlet extraction, 1.0 ml standard solution of n-C_{12}-C_{32} was spiked onto a piece of prewashed filter paper and extracted with 60 ml hexane solvent for 20 hr. The extraction was repeated five times and the extraction efficiency was comparable to SFE at higher pressure (see Table 1.10). Table 1. 10 shows that the recovery of n-C_{12} of Soxhlet extraction is very low (9.2%). To understand the reason of such lower recovery, 1.0 ml standard solution of n-C_{12}-C_{32} was added to 60 ml hexane and the mixture solution was refluxed for 20 hr. Then the solution was concentrated to 1.0 ml by a KD condenser and analyzed by GC. The average recovery of n-C_{12} of two runs was 92%, so n-C_{12} was not lost in the refluxing or condensing steps.

1.5.3.2 Effect of Extraction Time and Temperature

The hydrocarbons were SFEd at 99°C and 7000 psi for 10 min, 15 min, 20 min and 30 min. The experiments were run in duplicate. Table 1.11 shows that an equilibrium extraction time of 20 min is sufficient.

Hydrocarbons were SFEd at 25°C, 35°C, 55°C, 75°C and 99°C, at 8000 psi for 20 min. Table 1.12 shows that when the temperature was increased from 25°C to 35°C, the recovery of SFE for all of hydrocarbons was increased, because at 25°C the CO_2 is subcritical while at 35°C it is supercritical (the critical temperature of CO_2 is 31.1°C). When the temperature was increased from 35°C to 99°C, for n-C_{12} to n-C_{16} the recoveries of SFE increased, but for n-C_{18} to n-C_{32} the recoveries remained constant.

1.5.3.3 Effect of the Addition of Modifiers

CO_2 was modified by the addition of 10% n-C_6H_{14}, 10% $C_6H_5CH_3$, and 10% CH_2Cl_2 by molar respectively. The experiments were run in duplicate. Table 1.13 shows that addition of n-C_6H_{14} and $C_6H_5CH_3$ increase the recoveries of hydrocarbons. The addition of CH_2Cl_2 did not obviously improve the efficiency of SFE and for some compounds, the extraction efficiency was decreased. n-C_6H_{14} was chosen as a modifier because it has properties similar to the extracted hydrocarbons so the addition to CO_2 increased the solubilities of hydrocarbons and resulted in higher extraction recovery. This result is consistent with Dobbs **[83]** who found the solubility of hydrocarbons in supercritical CO_2 increased significantly with the addition of small amount of alkane modifiers. CH_2Cl_2 is a somewhat polar solvent which does not increase the solubility of hydrocarbons or improve the extraction efficiency. $C_6H_5CH_3$, although it has aromatic character, did

not increase the solubility of hydrocarbons. Analyte solubility in the supercritical extracting fluid is not the only factor influencing the SFE process. Diffusion and matrix effects are also very important factors. The diffusion process involved a combination of analyte diffusion from the filter paper matrix, diffusion of the supercritical fluid into the matrix, and replacement of analyte molecules by solvent molecules on active sites within the filter paper matrix.

Table 1.10 Recovery by SFE of n-C_{12}-C_{32} at 99°C, 20 min and Different Pressures [a]

Compound	3000psi	5000psi	7000psi	8000psi	9000psi	Soxhlet
C_{12}	2.0	2.7	5.2	5.2	5.0	9.2
C_{14}	24	38	60	62	64	73
C_{16}	34	56	79	91	92	98
C_{18}	40	62	85	95	99	98
C_{24}	53	63	90	93	100	97
C_{28}	51	56	87	94	100	94
C_{32}	54	62	89	90	100	93

[a] : the extractions were carried out using the lab-made SFE apparatus.

Table 1.11 Recovery by SFE of n-C_{12}-C_{32} at 7000 psi, 99°C and Different Times [a]

Compound	10 min	15 min	20 min	30 min
C_{12}	2.3	1.4	5.2	5.2
C_{14}	46	44	60	61
C_{16}	58	59	79	79
C_{18}	70	72	85	85
C_{24}	72	74	90	90
C_{28}	64	73	87	88
C_{32}	59	76	89	88

[a] : the extractions were carried out using the lab-made SFE apparatus.

Table 1.12 Recovery by SFE of n-C_{12}-C_{32} at 8000 psi, 20 min and Different Temperatures [a]

Compound	25°C	35°C	55°C	75°C	99°C
C_{12}	0.6	1.4	1.6	2.0	5.2
C_{14}	34	36	41	45	62
C_{16}	64	84	85	85	91
C_{18}	77	92	93	92	95
C_{24}	79	95	96	93	93
C_{28}	78	91	93	95	94
C_{32}	57	94	90	91	90

[a] : the extractions were carried out using the lab-made SFE apparatus.

Table 1.13 Effect of Addition of Modifiers
(Recovery by SFE at 7000 psi, 100°C for 20 min [a])

Compound	CO_2	CO_2+10%C_6H_{14}	CO_2+10%$C_6H_5CH_3$	CO_2+10%CH_2Cl_2
C_{14}	46	48	71	20
C_{16}	73	84	92	70
C_{18}	83	95	100	81
C_{24}	82	92	99	85
C_{28}	81	100	100	84
C_{32}	86	100	100	89

[a] : the extractions were performed using the SFE-703 instrument.

1.5.4 Analysis of Rock Samples

Six rock samples collected from the Lorca Basin, in Southeast Spain were SFEd under the optimum SFE conditions. After SFE the solution was concentrated to 1.0 ml and analyzed directly by GC. To compare with SFE, each sample (about 2.0 g, exactly weighed) was Soxhlet extracted with 60 ml hexane for 24 hr. The quantitative results are listed in Table 1.14. Generally, the results of SFE are comparable with those of Soxhlet extraction. However, in some samples and for some compounds, the concentrations determined by SFE are a little higher or lower than those determined by Soxhlet extraction, which may due to relative experiment error from the repeatability of GC, extraction and condensation. For six samples, no n-C_{12} was found, which does not necessarily mean there was none this compound in the samples, but rather the recovery of this compound was very low. Within the detection limit, no n-C_{28} and n-C_{32} compounds were found in the rock samples.

In conclusion, (1) n-C_{14}-C_{24} have been detected in the range of 0.14 to 9.44 μg/g from the rock samples. SFE is a reliable extraction method for the analysis of hydrocarbons from the rock samples and saves much time comparing with Soxhlet extraction. (2) n-C_{16}-C_{32} can be quantitative extracted (>90%) by SFE at 99^{0}C, 8000 psi for 20 min. The extraction efficiency increases with increasing pressure. The effect of temperature in the range of 35^{0}C to 99^{0}C to the extraction is small. The extraction efficiency can be improved by the addition of n-C_6H_{14} or $C_6H_5CH_3$ to CO_2.

Table 1.14 Quantitative Results for Rock Samples (μg/g)

Compound	SFE	Soxhlet
	BE-1 Sample	
C_{14}	0.45	1.04
C_{16}	0.90	1.16
C_{18}	0.80	0.68
C_{24}	0.47	nd[a]
	BIT-2 Sample	
C_{14}	5.95	4.60
C_{16}	9.44	8.31
C_{18}	7.67	7.51
C_{24}	0.41	nd
	ULA Sample	
C_{14}	0.86	1.19
C_{16}	1.33	0.84
C_{18}	0.56	0.42
C_{24}	0.26	nd

Table 1.14 continued

	ULB Sample	
C_{14}	0.98	0.81
C_{16}	1.38	0.96
C_{18}	0.90	0.33
C_{24}	0.26	0.14
	MO2 Sample	
C_{14}	0.54	0.83
C_{16}	1.47	1.32
C_{18}	0.79	0.59
C_{24}	0.68	0.67
	MO3 Sample	
C_{14}	0.80	0.92
C_{16}	1.57	1.15
C_{18}	0.84	0.52
C_{24}	0.39	nd

[a] : nd = not detected.

Chapter 2 Supercritical Fluid Extraction and Chromatographic Analysis of Nitrated Polycyclic Aromatic Hydrocarbons (NO_2-PAHs)

2.1 Introduction

It is well known that ambient atmospheric particles contain adsorbed organics such as PAHs and NO_2-PAHs **[1-3]**, which are mutagenic and carcinogenic. Some NO_2-PAHs are much strongly mutagenic and carcinogenic than PAHs. PAHs are mutagenic in the Ames salmonella assay only in the process of tissue homogenate, or by addition of metabolizing enzymes; however, most NO_2-PAHs are direct-acting mutagens **[4]**. Work from several laboratories has showed that 25-50% of the direct-acting mutagenicity observed for atmospheric particle extracts was due to mono- and dinitropyrene **[5-7]**. Furthermore, some NO_2-PAHs exhibited mutagenic properties during tests with mammalian cells **[8]**, as well as carcinogenic properties during animal tests **[9]**. At least nine NO_2-PAHs identified in diesel exhaust (1-nitropyrene, 2-nitrofluorene, 3-nitrofluorenthane, 2-nitronaphthalene, 4-nitrobiphenyl,5-nitroacenaphthene, and 1,3-, 1,6-, and 1,8-dinitropyrene) produce cancer in laboratory animals **[10-11]**. This information has led both the International Agency for Research on Cancer and U.S. National Toxicology Program to evaluate the toxicity of NO_2-PAHs.

Most often, strains TA 98 and TA 100 are used in the Ames salmonella assay to test the mutagenicity of the compounds. For 1-NO_2-pyrene, there were 469 revertants/nmole for TA 98, and 148 revertants/nmole for TA 100 **[12]**. Ball and co-workers **[13]** investigated the use of TA 102, a strain of

bacteria sensitive to compounds that can oxidize DNA, to detect a new class of mutagenic compounds in diesel particle extracts. The contribution of mono- and dinitroarenes to the total TA 102 mutagenicity of the diesel particle extract was less than 1% of the total mutagenicity, so there must be a new class of the compounds contributing to TA 102 mutagenicity. However, no qualitative results were reported for this new type of compound.

A few studies have been done to investigate the relationship between the mutagenicity of NO_2-PAHs and their molecular structures or the energies of their molecular orbitals. For example, Kalopman et al. **[14]** demonstrated that the mutagenicity of a series of NO_2-PAHs in the Ames salmonella assay is correlated with the ease of their nitroreduction, as calculated from the energy of their lowest unoccupied molecular orbitals. Fu et al. **[15]** studied the use of orientation of NO_2- group in NO_2-PAHs to predict their direct-acting bacterial mutagenicity. They reported that NO_2-PAHs with perpendicularly oriented nitro groups exhibited either very little or no direct-acting bacterial mutagenicity, but nitro-substituents which are coplanar or nearly coplanar with the aromatic rings were strong direct-acting mutagens.

The sources of NO_2-PAHs in ambient atmospheric particles include the incomplete combustion of organic materials emitted from diesel exhaust **[16]**, gasoline exhaust **[17]**, airplane engines **[18]**, coal-burning power plants **[19]**, and cigarette smoke **[20]**. In addition, the chemical reactions and conversion of gaseous two to four ring parent PAHs with OH radicals and NO_x are more important sources of the NO_2-PAHs in the atmosphere. For example, 1-NO_2-pyrene in the atmosphere is directly emitted from combustion sources **[21]**, but 2-NO_2-pyrene and 2-NO_2-fluoranthene are

formed in the atmosphere from OH radical-initiated reactions of pyrene and fluoranthene **[22]-[23]** and both are more abundant in ambient particles. In addition to atmospheric particles, NO_2-PAHs were also detected in food such as grilled chicken, grilled corn, mackerel, pork, beef and tea **[24]**. Levels of 1-NO_2-pyrene in grilled chicken were found to range from 0.4 to 11 ng/g.

2.1.1 Analysis of NO_2-PAHs

Although NO_2-PAHs are apparently widespread, they are generally found in trace amounts in a complex organic matrix. To detect trace amounts of NO_2-PAHs in complex mixtures, highly sensitive and selective analytical methods are required. Gas chromatography (GC) with universal and selective detectors has been applied to the analysis. Oehme et al. **[25]** have evaluated the simultaneous use of flame ionization detector (FID) and electron capture detector (ECD) for the analysis of NO_2-PAHs. The FID is less sensitive to NO_2-PAHs than the ECD, with detection limits of 0.1-0.2 ng at best, and is also nonselective relative to other detectors. The ECD has very good sensitivity to NO_2-PAHs, on the order of 1-2 pg, and its selectivity also fairly good due to the electron affinity of the NO_2-PAHs. However, the response of the ECD varies by up to a factor of 60 between nitro compounds. Several researchers have found alkali-flame nitrogen-phosphorous detectors (NPD) have detection limits similar to the FID and a selectivity toward nitrogen compounds in general that is better than the ECD **[26-27]**. White and co-workers **[28]** evaluated the use of a thermionic detector operated in a nitrogen atmosphere (TID-1-N_2) for the detection of NO_2-PAHs. The TID-1-N_2 showed a very high degree of sensitivity and specificity for compounds containing electronegative functionality, particularly nitrated compounds. 45 NO_2-PAHs were separated on a 30 m x 0.20 m fused-silica column

coated with a 0.25 μm film of SE-52, and the detector responded linearly to 2,2'-dinitrobiphenyl and 9-nitroanthracene within the approximate concentration ranges of 110 ng to 3 pg per compound. The minimum detectable quantity for these two compounds was around 3 pg. TID-1-N_2 was shown to respond selectively to low levels of NO_2-PAHs in a coal extract aromatic fraction and gave no response to PAHs.

A chemiluminescent detector (CD) was evaluated for the analysis of NO_2-PAHs via GC by Robbat and co-workers **[28]**. The selective detection of NO_2-PAHs over other hydrocarbons was compared to the FID and MS detectors. The CD responded linearly to most of the 54 NO_2-PAHs studied from 100 ng to 50 pg. The detection limit was 50 pg for most to the compounds at a S/N of 3. In addition to the detector, the solvent is also one of most important factors in improving sensitivity in GC. Galceran and Moyano **[29]** studied the effect of solvent polarity on the determination of NO_2-PAHs using capillary GC with splitless injection. Peak splitting with two or more maxima were observed when methanol and acetonitrile were used as solvents to prepare standard solutions of NO_2-PAHs, and there was no peak splitting when methylene chloride and acetone were chosen as solvents. The splitting can be eliminated using a retention gap or by increasing the initial column temperature.

Mass spectrometry (MS) detectors combined with GC have been also used for the analysis of NO_2-PAHs. The MS provides an abundance of information on the structure of the molecule and therefore can allow for tentative identification of a compound when no standard is available. Electron impact ionization (EI) and negative ion chemical ionization (NICI)

spectrometries combined with GC have been used extensively for the identification of NO_2-PAHs **[30-31]**. The sensitivity of EIMS to NO_2-PAHs is relatively low, but the detection limit for 1-NO_2-2-methylnaphthalene was 1 pg at S/N of 3 by EICIMS **[31]**. EICIMS also gives a strong molecular ion for NO_2-PAHs, but is limited by the lack of reference spectra.

Tungeln and Fun **[32]** successfully separated 28 NO_2-PAHs and their derivatives by the combination of reverse phase and normal phase high performance liquid chromatography (HPLC) with UV detector. They found that the polarity of the molecule is a principle factor in determining the HPLC retention time. In the reverse phase system, the general elution order of the compounds is: parent NO_2-PAHs > phenolic derivatives > epoxides > dihydrodiols > tetrahydrotetrols. Normal phase HPLC gave opposite retention order but with different separability among some of the compounds.

2.1.2 Extraction of NO_2-PAHs from Diesel Exhaust Particles

To analyze the NO_2-PAHs from diesel exhaust particles, usually the diesel exhaust particles are collected on a glass fiber filter, and the filter is extracted by Soxhlet or sonication with organic solvent. After extraction, the extracts are fractionated using a bioassay-directed column fractionation scheme to separate the complex mixture into substantially less complex fractions for the analysis. Bioassay-directed column fractionation is a sequential fractionation method and was originally used for the identification of mutagenic activity of each fraction in the sample. The extract is chromatographied on a normal-phase silica gel column and the column is eluted with different solvents from non-polar to polar. Salmeen et al. **[4]**

Soxhlet-extracted with methylene chloride the filter collecting the diesel exhaust from a passenger car, then fractionated the extracts on a silica gel column, and eluted with hexane, dichloromethane and acetonitrile. Six NO_2-PAHs, 1-NO_2-pyrene, 3-NO_2-fluoranthene, 8-NO_2-fluoranthene, 1,3-, 1,6- and 1,8-dinitropyrene were identified. Concentrations were in the range of 0.3-75 ppm. Xu et al. **[33]** Soxhlet-extracted diesel exhaust particles with 6 L of methylene chloride for 24 hr, then fractionated the extracts on a HPLC silica column. More than fifty NO_2-PAHs were identified but no quantitative results were reported. Savard and co-workers **[34]** sequentially ultrasonically-extracted Standard Reference Material (SRM) 1650 diesel exhaust particles with hexane, diethyl ether and methanol. 2-NO_2-fluorene and 1-NO_2-pyrene were identified at concentrations of 35.0 μg/g and 4.2 μg/g, respectively. Paschka and co-workers **[35]** SFEd SRM 1650 and 1-NO_2-pyrene recovery was 117±7% using $CHClF_2$ for the extraction.

2.1.3 Extraction of NO_2-PAHs from Airborne Particulates

SRM 1649 urban dust was ultrasonically-extracted with methylene chloride; 2-NO_2-fluorene at a concentration of 0.72 μg/g was found **[34]**. Nishioka et al. **[36]** Soxhlet-extracted air particulates for 24 hr with methylene chloride and then 24 hr with methanol. After extraction, the extracts were bioassay-directed multiply fractionated. First, the extract was acid/base partitioned and fractionated on a open-bed silica gel column. Then the organic acid and methylene chloride silica gel effluent was further fractionated on a normal phase HPLC column, eluted with hexane, methylene chloride and methanol at different ratios. Seven hydroxylated NO_2-PAHs at a concentration range of 5-700 μg/g were identified.

The purpose of this work was to develop qualitative and quantitative methods with SFE and chromatography for the analysis of strong mutagenic and carcinogenic NO_2-PAHs, including 1-NO_2-pyrene, and 6- and 7-NO_2-3,4-benzocoumarin. These compounds are of interest because 1-NO_2-pyrene has been identified in diesel exhaust particles, and contributed up to 20% of the mutagenic response of the extracts **[6]**. 6-NO_2-3,4-benzocoumarin is the reaction product of gas phase phenanthrene with OH radical in the presence of NO_x **[37]** and has been identified from the ambient air samples collected in southern California **[38]**. The use of a supercritical fluid can significantly reduce the extraction time and decrease the amount of liquid solvent required. Few researchers have used SFE to extract NO_2-PAHs **[35][39]** and there are no publications using SFE for the extraction of 6- and 7-NO_2-3,4-benzocoumarin. This work is applied to the analysis of trace NO_2-PAHs in environmental samples.

2.2 Experimental

2.2.1 Chemicals and Supplies

The compounds studied were obtained from following sources: 1-NO_2-pyrene, 97%, 6-NO_2-3,4-benzocoumarin, 7-NO_2-3,4-benzocoumarin, 98%, and 9-xanthenone were all purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). 9-Fluorenone was obtained from Eastman Organic Chemicals, (Rochester, NY). The National Institute of Standards and Technology (NIST) SRM 1649 urban dust was purchased from NIST. All of solvents used were HPLC or spectrophotometric grade.

A stock solution of 1-NO_2-pyrene, 6-NO_2-3,4-benzocoumarin, 7-NO_2-3,4-benzocoumarin, 9-fluorenone and 9-xanthenone was prepared by dissolving the appropriate amount of each compound in methylene chloride. The solution was stored in a -18°C refrigerator until used. A standard solution of the mixture used in GC, HPLC and SFE studies was prepared by transferring a suitable amount of the stock solution to a volumetric flask and diluting with acetonitrile. The concentrations of the stock solution and standard solution are listed in Table 2.1.

Table 2.1 Stock and Standard Solutions

Compound	Stock Solution (mg/ml)	Standard Solution (ng/μl)
9-fluorenone	1.06	10.6
9-xanthenone	1.09	10.9
6-NO_2-3,4-benzocoumarin	0.980	9.80
7-NO_2-3,4-benzocoumarin	1.01	10.1
1-NO_2-pyrene	0.996	9.96

2.2.2 Supercritical Fluid Extraction

The instruments used for this part of the SFE study were (a) a SFE-703 equipped with a co-solvent delivery system 703E, loaned by the Dionex Company, which has eight extraction chambers and can extract eight samples simultaneously, and (b) the lab-made SFE apparatus which was described in section 1.4.3.

The procedure for SFE is the same as described in the SFE study of hydrocarbons. A 1.0 ml standard solution was spiked onto a piece of prewashed filter paper, cut into small pieces, and loaded into the extraction chamber. Silica gel was chosen as the solid adsorbent material for the collection trap and methylene chloride was used to elute collected extracts from the adsorbent. The SFE-703 instrument was designed to collect extracted compounds in solvent directly, using a steel capillary column to connect the extraction chamber and collection bottle. Before extraction, 10 ml of methylene chloride was loaded into the collection bottle. After extraction, the solution was concentrated to 1.0 ml by a Kuderna-Danish (KD) condenser (Supelco).

To study the effect of pressure, temperature and solvent modifier on the recovery of NO_2-PAHs, 1.0 ml of the standard solution was spiked onto a piece of filter paper and SFE was carried out at 5000 psi, 7000 psi, and 8500 psi at 60°C for 25 min and at 60°C, 99°C, and 200°C at 7000 psi for 25 min. Acetone and methanol were chosen to modify the CO_2 solvent. All of extractions were run in duplicate.

To simulate the extraction efficiency of NO_2-PAHs from real samples and to study the matrix effect, 1.0 ml of the standard solution was spiked onto 1.0 g of SRM 1649 urban dust which had been Soxhlet extracted for 24 hr, and for which GC showed there were no NO_2-PAHs left. SFE was carried out at 7000 psi and 150°C for 25 min.

To identify the NO_2-PAHs in the SRM 1649 urban dust, 0.9640 g sample was SFEd at 7000 psi and 150°C for 25 min.

2.2.3 Soxhlet Extraction

To compare with Soxhlet extraction, 1.0 ml of the standard solution was spiked onto a piece of filter paper and Soxhlet extracted with 60 ml methylene chloride for 24 hr. The experiment was run in triplicate.

A sample of SRM 1649 urban dust (0.9985 g) was Soxhlet-extracted for 24 hr with 60 ml methylene chloride. The solution was concentrated to 1.0 ml by a KD condenser and then carefully transferred to a silica gel column for clean up and prefractionation. The column fractions were concentrated and analyzed by GC.

2.2.4 GC Determination

Aliquots (2.0 μl) of the standard solutions and extracts were analyzed on a Hewlett-Packard model 5880 GC equipped with a FID. Helium was used as a carrier gas and splitless injection was employed to get higher sensitivity and more repeatable quantitative results. A 29 m x 0.32 mm, 0.25 μm film thickness DB-5 bonded phase (crosslinked 95% dimethyl-5% diphenyl polysiloxane) column was chosen for analysis. The oven temperature was

held at 40°C for 2 min after injection and was then increased at 14 °C/min to 260°C where it was held for 5 min. Injection port and detector temperatures were 300°C.

2.2.5 HPLC Determination

A Perkin-Elmer LC-400 HPLC equipped with a LC-90 UV detector and a LCI-100 computing integrator was used. 6.0 µl solution was injected for each analysis by means of a 6.0 µl sample loop. Three columns: Lichrosorb SI 100, 25 cm x 4.6 mm (Aldrich); Zorbax CN 25 cm x 4.6 mm (Du Pont Instrument); and Partisil 5 C18 25 cm x 4.6 mm (Whatman) were evaluated to separate the standard solution.

To find a suitable wavelength for the UV detector, a UV scan of a solution of 6- and 7-NO_2-3,4-benzocoumarin was made. The UV instrument used was HP 8452A Diode Array Spectrophotometer.

2.2.6 Column Fractionation

A concentrated methylene chloride solution (1.0 ml) of Soxhlet-extracted SRM 1649 urban dust sample was fractionated on a 12 cm x 1.0 cm column of silica gel which had been Soxhlet extracted before packing with methylene chloride and hexane for 24 hr. To check whether the column was really clean and to avoid contamination from the column, the column was eluted with 20 ml each of hexane, methylene chloride, acetonitrile, and methanol. Each fraction collected was concentrated to 1.0 ml and GC-analyzed before fractionating the sample. No non-solvent peaks were observed. After transferring 1.0 ml of sample extraction solution to the column, the column was eluted with solvents as following: solvent 1, 20 ml

C_6H_{14}; solvent 2, 20 ml C_6H_{14}+CH_2Cl_2 (60:40); solvent 3, 20 ml C_6H_{14}+CH_2Cl_2 (30:70); solvent 4, 20 ml CH_2Cl_2; solvent 5, 20 ml CH_2Cl_2+ACN (50:50); solvent 6, 20 ml ACN; solvent 7, 20 ml MeOH. Each fraction was collected and concentrated to 1.0 ml and GC analyzed.

To check when NO_2-PAHs eluted in which fraction and to study the recovery of the column, 1.0 ml of the standard solution was transferred to the column and fractionated, and then analyzed by GC.

2.3 Results and Discussion

To compare with the behavior of NO_2-substituted PAHs, two non-NO_2 group-substituted PAHs, 9-fluorenone and 9-xanthenone were chosen for study. The chemical structures of the compounds studied are listed in Table 2.2.

2.3.1 GC of NO_2-PAHs

The separation of NO_2-PAHs on the DB-5 column using a FID is shown in Figure 2.1. The five compounds were completely separated. The order of elution of the compounds from the column was determined by their boiling points and strength of interaction with the column. Because NO_2-PAHs are strongly adsorbed, they easily contaminate the injection line, leading to poor, nonrepeatable quantitative results. Thus the injection line was cleaned up by soaking in chromic acid and rinsing with methylene chloride periodically during the study .

The detection limit of each compound at S/N of 3 is listed in Table 2.3. Compared with hydrocarbons studied, for which the detection limits may be as low as 20 pg, NO_2-PAHs have a somewhat higher detection limit, because of their smaller FID response factor and their adsorbing tendency.

For quantitative analysis and to check the linear dynamic range of the detector for these compounds, a series of standard solutions ranging from 0.5 ppm to 100 ppm were prepared from the stock solution at 10 mg/ml and diluted with acetonitrile. All of standard solutions were analyzed under the same conditions and the calibration curve is shown in Figure 2.2.

2.3.2 HPLC Separation

Considering that the SRM 1649 urban dust is a very complicated sample, containing many hydrocarbons, HPLC determination of NO_2-PAHs was studied because the HPLC UV detector is much more selective than the FID in GC. A UV scan was performed to determine a suitable wavelength. The UV spectrum of the solution of 6- and 7-NO_2-3,4-benzocoumarin is shown in Figure 2.3. At 212 nm they have an absorption maximum, but at this wavelength some solvents such as methanol and methylene chloride also absorb, which would disturb the analysis, so the UV detector was set at 260 nm for the analysis of NO_2-PAHs.

A Partisil 5 C18 reversed-phase column (25 cm x 4.6 mm) was used and the chromatogram of the NO_2-PAHs separated at 20% H_2O and 80% ACN mobile phase is shown in Figure 2.4. 6- and 7-NO_2-3,4-benzocoumarin compounds could not be separated. Different mobile phases such as MeOH and different ratios of the mobile phases were tested, but all of these failed to separate these two compounds. Two other normal phase columns, a silica column and a cyano column were tried, but neither separated these two compounds. It appears that a special column for isomer compound separation would be needed to separate these two compounds.

Table 2.2 Chemical Structure of the Compounds

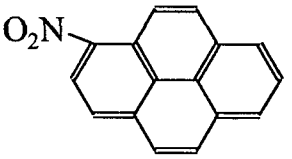
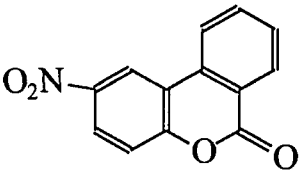
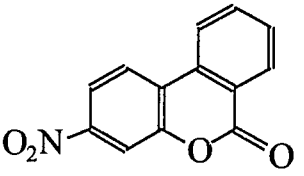
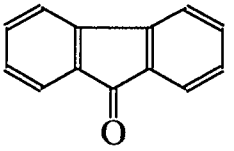
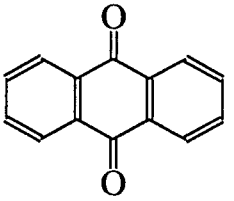
No	Compounds	Structures
1	1-NO_2-Pyrene	
2	6-NO_2-3,4-benzocoumarin	
3	7-NO_2-3,4-benzocoumarin	
4	9-Fluorenone	
5	9-Xanthenone	

Table 2.3 Detection Limits and GC Retention Times

Compound	tr (min)	Detection limit (ng)
9-fluorenone	12.06	0.12
9-xanthenone	13.04	0.12
6-NO_2-3,4-benzocoumarin	16.52	0.20
7-NO_2-3,4-benzocoumarin	16.89	0.27
1-NO_2-pyrene	18.44	0.10

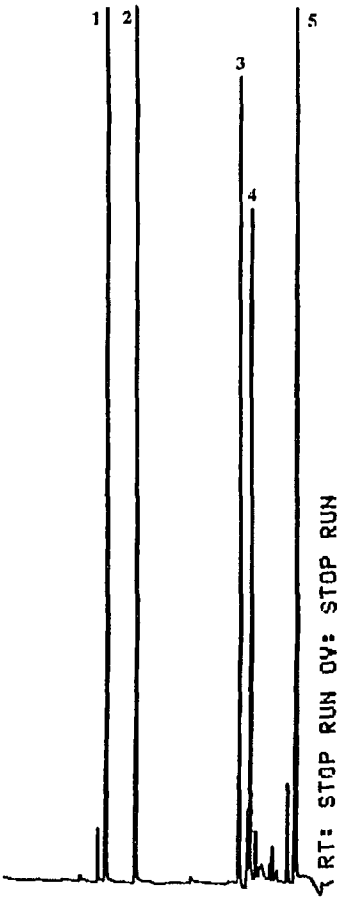


Figure 2. 1 Gas Chromatogram of the Standard Mixture of NO_2-PAHs

1. 9-fluorenone 2. 9-xanthenone 3. 6-NO_2-3,4-benzocoumarin
4. 7- NO_2-3,4-benzocoumarin 5. 1-NO_2-pyrene

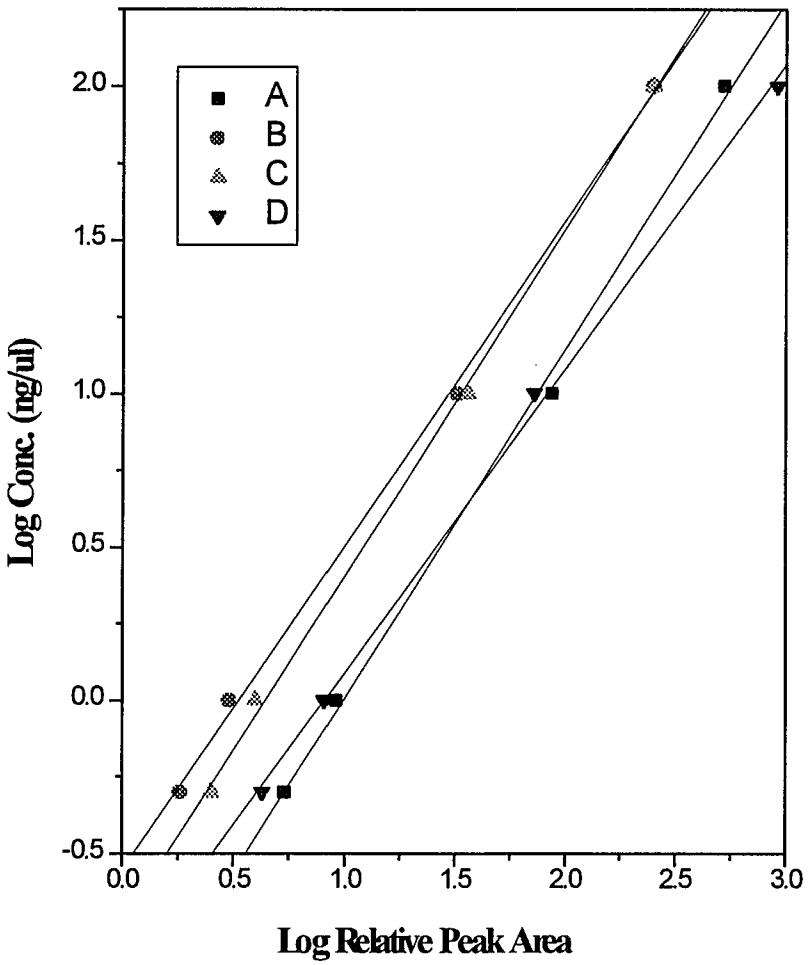
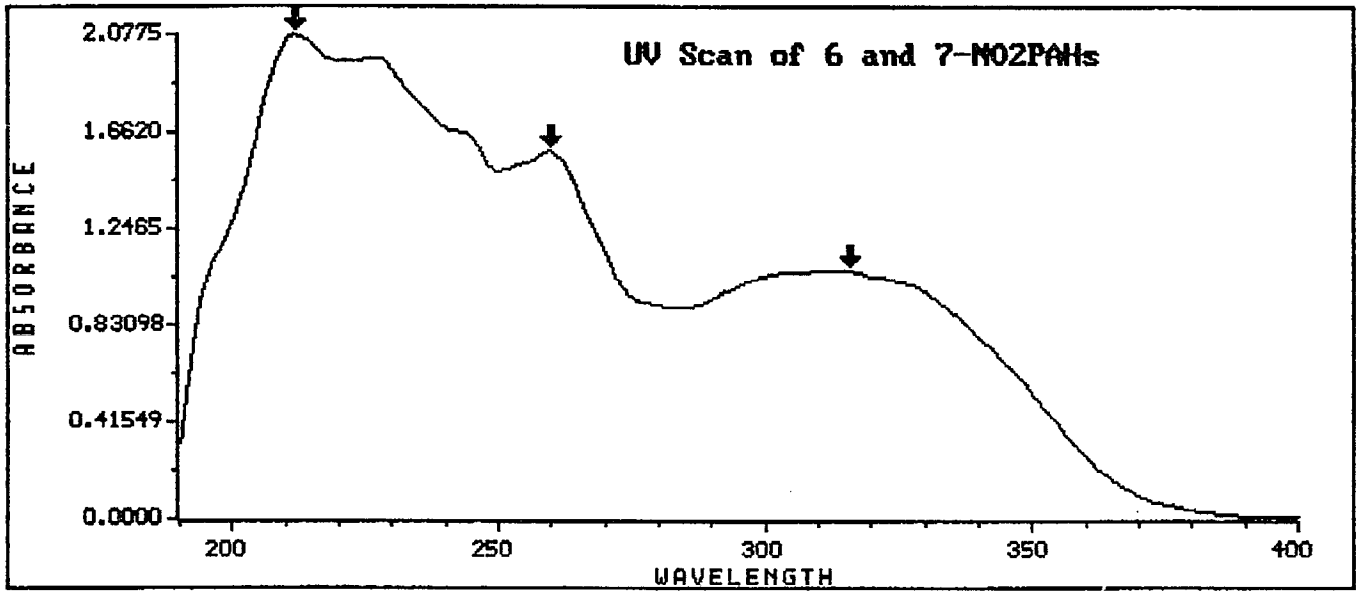


Figure 2.2 Calibration Curve for NO_2-PAHs

A: 1-NO_2-pyrene B: 7-NO_2-3,4-benzocoumarin

C: 6-NO_2-3,4-benzocoumarin D: 9-fluorenone



Annotated Wavelengths:

1	: Wavelength =	212	Result =	2.077454
2	: Wavelength =	260	Result =	1.580322
3	: Wavelength =	316	Result =	1.063675

Figure 2.3 UV Spectrum of 6- and 7-NO_2-3,4-benzocoumarin (10 ppm)

Figure 2.4 HPLC Chromatogram of NO_2-PAHs

1. 6- and 7- NO_2-3,4-benzocoumarin
2. 9-xanthenone 3. 1-NO_2-pyrene

2.3.3 Effect of Pressure, Temperature and Solvent Modifiers on the Efficiency of SFE for NO_2-PAHs

The extraction of a specified solute from a matrix necessitates the optimization of several parameters, i.e. the pressure, temperature and the possible addition of an organic modifier to the extraction fluid. Each of them will be discussed in the following.

2.3.3.1 Effect of Pressure

The effect of pressure on the SFE of NO_2-PAHs compounds is shown in Table 2.4. The results show that as the pressure is increased from 5000 psi to 8500 psi, the recovery of each compound increases. At a given temperature, the density of the extraction fluid (CO_2) increases with pressure so a higher solubility of the solutes results. The recoveries of each compound at 5000 psi and 7000 psi are almost the same, but increase slightly at the higher pressure.

To compare Soxhlet extraction with SFE, the standard solution of NO_2-PAHs was spiked onto a piece of prewashed filter paper and Soxhlet extracted with 60 ml methylene chloride for 24 hr. The results (Table 2.4) show almost the same recovery as SFE at 8500 psi, but SFE took much shorter time than Soxhlet extraction.

Table 2.4 shows that no compound is recovered 100%. The efficiency of SFE includes two important steps. The first one is whether all the compounds are totally extracted from the matrix and the second step is whether all of extracts are collected. To study the collection efficiency of SFE to NO_2-PAHs, 1.0 ml of the standard solution was spiked onto the same

amount of silica gel as packed into the collection trap in SFE. It was then eluted with 30 ml methylene chloride and the solution was concentrated to 1.0 ml by a KD condenser and analyzed by GC. To determine whether the compounds evaporated during concentrating step, 1.0 ml of standard solution was added to 30 ml methylene chloride and the solution was concentrated to 1.0 ml by a KD condenser directly and analyzed. Table 2.5 and Table 2.6 indicate that none of compounds was lost in the concentrating step and almost all of them were recovered from silica gel column. So less than 100% recoveries of NO_2-PAHs may be due to incomplete extraction from the filter paper or that some of them were lost during depressurization step by the formation of aerosol.

Table 2.4 Recoveries Compared with Soxhlet Extraction at 60°C, 25 min and Different Pressures [a]

Compound	5000psi	7000psi	8500psi	Soxhlet
9-Fluorenone	51	53	76	87
9-xanthenone	39	40	74	85
6-NO_2-3,4-benzocoumarin	51	52	85	84
7-NO_2-3,4-benzocoumarin	45	48	82	81
1-NO_2-pyrene	61	62	90	80

[a]: SFE was carried out using the lab-made apparatus.

Table 2.5 Recovery after Concentrating with a KD Condenser

Compound	Recovery
9-fluorenone	98%
9-xanthenone	99%
6-NO_2-3,4-benzocoumarin	99%
7-NO_2-3,4-benzocoumarin	97%
1-NO_2-pyrene	99%

Table 2.6 Elution Efficiency from Silica Gel

Compound	Recovery
9-fluorenone	92%
9-xanthenone	93%
6-NO_2-3,4-benzocoumarin	97%
7-NO_2-3,4-benzocoumarin	94%
1-NO_2-pyrene	96%

2.3.3.2 Effect of Temperature

The effect of temperature on the SFE of NO_2-PAHs is shown in Table 2.7. The recovery of 9-fluorenone and 9-xanthenone increased as the temperature was increased. The recovery of three NO_2-PAHs compounds was almost independent of temperature. At a constant pressure, the density of CO_2 decreases when the temperature rises, but the volatility of the solute increases as the temperature rises. The SFE recoveries of 9-fluorenone and 9-xanthenone increased as the temperature was increased. This can be attributed to their volatilities increasing faster with temperature than their solubility in CO_2 decreasing. However, for three NO_2-PAHs, the effect of their solubility decreasing and the volatility increasing with temperature was almost the same, so their SFE recoveries were almost independent of the temperature over the range tested.

2.3.3.3 Influence of Solvent Modifier

Because the three NO_2-PAHs are all polar compounds but the polarity of the CO_2 SFE solvent is very low, MeOH and CH_3COCH_3 were studied as modifiers. 10 mol% of each was added to CO_2 using the SFE-703E co-solvent delivery system. The results (Table 2.8) show that the addition of MeOH and CH_3COCH_3 to CO_2 increases the recovery of three NO_2-PAHs compounds. The reason for higher recovery is that their solubility is higher in the mixed solvent than in CO_2, probably because of the hydrogen bonding between NO_2-PAHs and MeOH.

Table 2.7 Recovery by SFE at 7000 psi, 25 min and Different Temperatures [a]

Compound	60°C	99°C	200°C
9-fluorenone	53	59	74
9-xanthenone	40	50	61
6-NO_2-3,4-benzocoumarin	52	56	56
7-NO_2-3,4-benzocoumarin	46	43	44
1-NO_2-pyrene	62	64	64

[a]: SFE was carried out by the lab-made apparatus.

Table 2.8 Effect of Addition of Modifiers [a]

Solvent	6-NO_2-BC[b]	7-NO_2-BC[b]	1-NO_2-pyrene
CO_2	56	42	64
CO_2+10%CH_3COCH_3	72	65	68
CO_2+10%MeOH	85	70	80

[a] : % Recovery, 7000 psi, 100°C, 25 min. SFE was carried out using the SFE-703 instrument.

[b] : 6-NO_2-BC = 6-NO_2-3,4-benzocoumarin

7-NO_2-BC = 7-NO_2-3,4-benzocoumarin

2.3.4 Analysis of NIST SRM 1649, Urban Dust

2.3.4.1 Determination of NO_2-PAHs in SRM 1649 Urban Dust Sample by Soxhlet Extraction, Column Fractionation and GC Analysis

The SRM 1649 urban dust sample (0.9985 g) was Soxhlet extracted with 60 ml methylene chloride for 24 hr. The extract was a dark brown solution. Since the solution was too complex for direct GC analysis, the solution was concentrated to 1.0 ml and then fractionated on a silica gel column to clean up and preseparate the sample. Each of the seven fractions was concentrated to 1.0 ml and analyzed by GC. To study which NO_2-PAHs were eluted in each solvent fraction and what the efficiency of the column fractionation was, 1.0 ml of the standard solution was transferred to the column and fractionated. All of standard compounds were eluted in fraction No.2. The recoveries of these compounds from the column are listed in Table 2.9. 9-Fluorenone and 9-xanthenone were quantitatively eluted from the column, but the three NO_2-PAHs compounds were not. Even with the more polar solvents, ACN and MeOH, these NO_2-PAHs were not eluted quantitatively. This is probably because of the strong adsorption properties of these compounds on silica gel.

The identification of the compounds in SRM 1649 urban dust was made by retention time comparisons. To check the identification, 10 μl of the stock solution was added to fraction No.2 of SRM 1649 and analyzed by GC. The chromatogram showed that the identified peaks were higher after the addition of the stock solution, which confirm the peak identity. Quantitative results were obtained by comparing the peak areas with these of the standard compounds. The results are shown in Table 2.11.

2.3.4.2 Determination of NO_2-PAHs in SRM 1649 Urban Dust Sample by SFE and GC Analysis

1.0 ml of standard solution was spiked onto 1.0 g of a clean SRM 1649 urban dust sample which had been Soxhlet extracted with 60 ml methylene chloride for 24 hr. After spiking, the sample was allowed to stand 2 hr to evaporate the solvent in the standard solution. The sample was then packed into the extraction chamber and SFEd at 7000 psi, 150°C for 25 min. The recovery of SFE is listed in Table 2.10. The results show that 9-fluorenone and 9-xanthenone had higher recovery than three NO_2-PAHs. The solubilities of these are higher than those of the NO_2-PAHs in CO_2, and the interaction between these and the SRM 1649 matrix was smaller than that of NO_2-PAHs.

SRM 1649 urban dust sample (0.9640 g) was SFEd at 7000 psi, 150°C for 25 min. After extraction, the collected methylene chloride solution was concentrated to 1.0 ml. The color of concentrated solution was lighter than that of Soxhlet extraction so it was directly analyzed by GC. From this point of view, SFE was more selective than Soxhlet extraction. The concentrations of NO_2-PAHs in SRM 1649 urban dust sample are listed in Table 2.11. These concentrations are comparable with the results obtained from Soxhlet extraction. SFE is a reliable sample extraction method for the analysis of NO_2-PAHs in urban dust which takes less time and uses less organic solvent than the traditional Soxhlet extraction method.

Table 2.9 Elution Efficiency from Silica Gel Column

Compound	Recovery
9-fluorenone	100
9-xanthenone	99
6-NO_2-3,4-benzocoumarin	58
7-NO_2-3,4-benzocoumarin	45
1-NO_2-pyrene	71

Table 2.10 Recovery of Spiked SRM 1649 Urban Dust Sample (SFE at 7000 psi, 150°C for 25 min)

Compound	Recovery
9-fluorenone	65%
9-xanthenone	63%
6-NO_2-3,4-benzocoumarin	48%
7-NO_2-3,4-benzocoumarin	44%
1-NO_2-pyrene	59%

Table 2.11 Concentrations of NO_2-PAHs in SRM 1649 Sample

Compound	Conc.(μg/g)[a]	Conc.(μg/g)[b]
6-NO_2-3,4-benzocoumarin	2.02	2.49
7-NO_2-3,4-benzocoumarin	1.67	1.17
1-NO_2-pyrene	1.78	1.44

[a] : The sample was SFEd at 7000 psi, 150 °C for 25 min.

[b] : The sample was Soxhlet extracted with 60 ml methylene chloride for 24 hr.

Chapter 3 The Analysis of Sewage Sludge and Soil for Organic Compounds

3.1 Introduction

Since the early 1900's, New York City along with other municipalities in the region have disposed of sewage sludge in the ocean. In 1924, an ocean disposal site called the 12-mile site was selected for sewage sludge disposal as an alternative to discharging sludge into the upper New York Bay. In 1986, a new site, the 106 mile deepwater municipal sludge dump site was designated by the U.S. Environmental Protection Agency (EPA) to replace the 12 mile site. During the same period, the Ocean Dumping Ban Act of 1988 was issued by the Congress which required New York City to develop a land-based sludge management program to replace ocean disposal. The New York Department of Environmental Protection (DEP) designed immediate, intermediate and long range plans to be in compliance with this requirement. The immediate plan involved constructing dewatering facilities at 8 of the 14 water pollution control plants in New York City, distributed as shown in Figure 3.1 [1]. All dewatering facilities were to have been fully constructed and operable in 1992. Under the intermediate range plan, sludge from the water pollution control plants would be handled by contracted private firms. The private firms would thermally dry or chemically stabilize the dewatered sludge, and land-apply or landfill the city's sludge out-of-state. This plan is effective until June 1998 when the long range plan is to be implemented. Under the long range plan, the city will implement its own system of sludge processing and use. By 2020, 50% of sludge will be

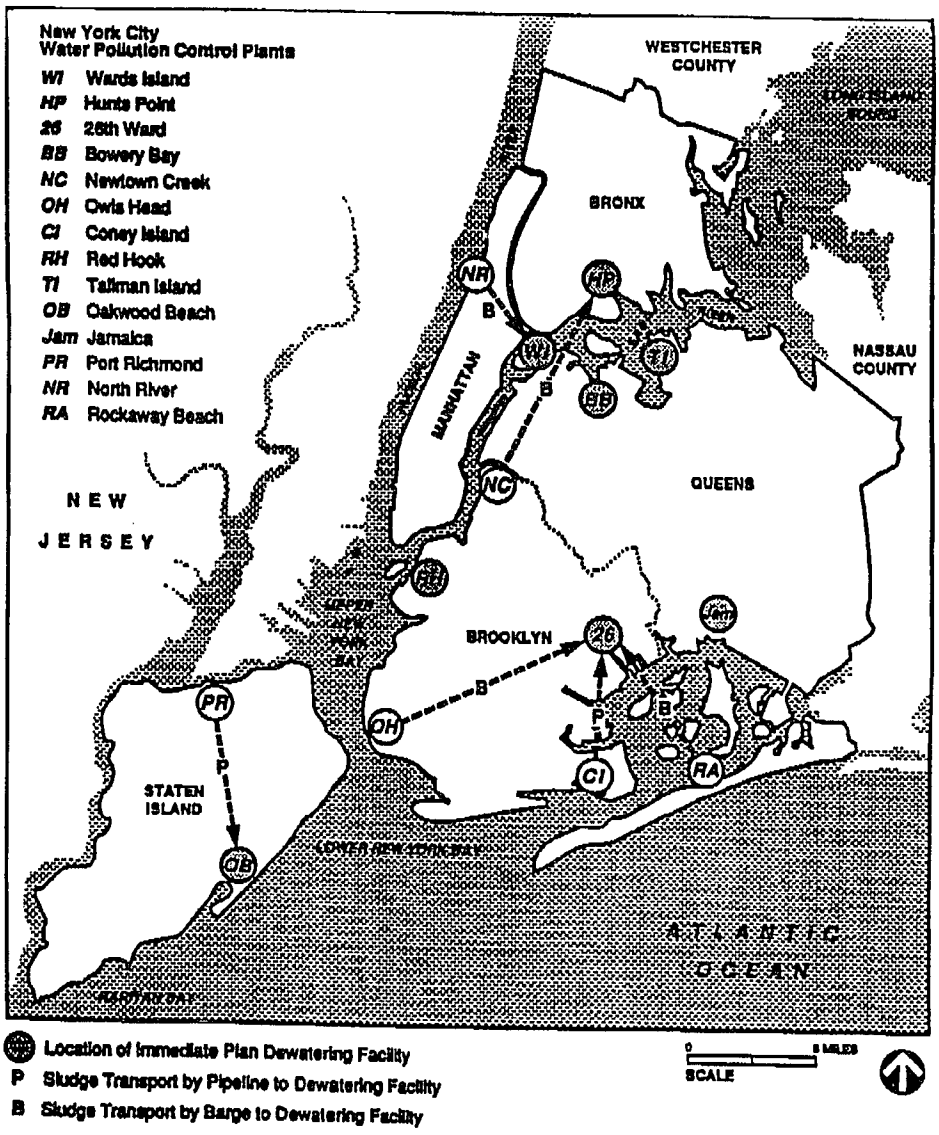


Figure 3.1 New York City Water Pollution Control Plants (from ref. 1)

dewatered and composted, 30% of sludge thermally dried and the rest one chemically stabilized. The chemically stabilized sludge is intended for use as cover material at landfills in the area or for agriculture purpose. The thermally dried product is intended for market distribution as a fertilizer amendment. Composted sludge maybe used as soil conditioner to improve the soil conditions for plant growth, and for landfill cover.

How do we monitor the composition change of the sludge after treatment? What toxic organics are still present? What are the health effects associated with sludge treatment? It is necessary to develop reliable methods for the characterization of the sludge samples. The purpose of this work is to develop analytical methods for the identification and determination of organic compounds in representative sludge products and soil samples. These methods are applied to the study of the characteristics of New York City sludge.

3.1.1 Sludge Treatment

Sludge treatment processes include dewatering, composting, thermally drying, chemical and aerobic stabilization.

Centrifugal dewatering technologies use physical force or pressure to separate water from solids in liquid sludge. Generally, mechanical dewatering employs initial polymer conditioning (flocculation) of sludge to enhance the separation of solids and water. The big advantage of sludge dewatering is the significant reduction in volume of material, which facilitates transportation or further processing. Kawasaki **[2-3]** reviewed

centrifugal settling for dewatering sludge. Lipke [4] compared the dewatering efficiency of different dewatering equipments.

Although not a process used by NYC, Sludge dewatering capability is usually greatly improved by slow freezing, then thawing **[5]**. Colder temperatures, longer freezing times, and slower freezing rates have beneficial effects. Sludge dewatering efficiency decreases with increasing freezing speed; however, a long freezing time will cause the process to be uneconomical when compared with other conditioning/dewatering processes **[6]**. Lee **[7]** studied the effect of a fast freeze/thaw treatment (with freezing speed about 40 mm/h) on sludge dewaterability. After a freeze/thaw treatment, the moisture movement resistance and the bound water content of the sludge cake both decreased, the floc volume and sludge compressibility were reduced, and the sludge filterability was improved greatly.

The purpose of composting sludge is to decompose volatile organic matter into a stable organic residue using micro-organisms present in the sludge. During the process, water and carbon dioxide are released and heat is generated, which reduces the number of pathogens in the sludge and promotes the evaporation of water. Pathogens are destroyed to below detection limits within 1 year of storage of composted dewatered sludge **[8]**. To achieve successful composting, sufficient air must be available for aerobic decomposition; the composting mass must be porous to allow transfer of air to the sludge mass and the dewatered sludge must be maintained at moisture levels between 40 and 60 percent. To maintain the proper moisture level and increase the porosity of the material, the dewatered sludge is first mixed with a carbonaceous bulking agent such as

wood chips, and then composted in a static pile or vessel. Johnston [9] compared eight in-vessel composting sites for product quality and marketability, stability and retention time, aeration, process monitoring and control, mixing characteristics, and odor control. The development of municipal sludge composting from the early 1960s to the present was studied from the first extended pile system to more sophisticated in-vessel systems and odor control system [10].

For thermally drying sludge, the temperature reached in the process must be high enough to destroy pathogens, but not so high as to change its chemical composition [11-13]. The dried product is significantly reduced in volume and weight. Dewatered sludge can be thermally dried either by direct contact or indirect contact drying. These two drying systems and their cost were compared and reviewed by Gruter [14]. Direct contact drying involves bringing hot gases in direct contact with the dewatered sludge, indirect contact drying involves using a contact medium, such as a hollow paddle or metal disc, to transfer heat from the hot gas to the dewatered sludge. Usually, the thermal drying/pelletization system consists of three distinct processes. First, sludge is mixed with recycled, nonuniform sludge pellets in an effort to bring the feed sludge to a proper moisture content. Second, feed sludge is passed through a thermal dryer, where hot air is passed over the material to drive off moisture and produce multiple-sized dry granular sludge particles. Then the material is sent through a series of separators, where the properly sized pellets are removed and conveyed to a storage silo, while the oversized pellets are crushed and, along with the undersized pellets, are returned to the head of the process.

Aerobic stabilization is a biological stabilization method to reduce pathogens in the sludge. Oxidation-reduction potential must be measured during aerobic sludge digestion **[15]**. The oxidation-reduction potential was related to level of oxygen and nitrate. Venturi aeration provided oxygen and mixing for thermophilic aerobic sludge digestion **[16]**. Beginning in 1988, three full-scale autothermal thermophilic aerobic digestion facilities were constructed or upgraded to demonstrate high-temperature aerobic digestion of municipal sludges in British Columbia, Canada **[17]**. Sludges from six waste water treatment plants were evaluated for reduction in pathogen indicator bacteria by aerobic digestion **[18]**.

Chemical stabilization involves mixing a chemical agent with the sludge to produce either a temporarily or a permanently stabilized product. The purpose of this treatment process is to reduce pathogens, eliminate odors, control the potential for putrefaction of organic matter within the sludge, and bind the heavy metals within the sludge. Addition of lime, the N-VIRO soil process and the ChemFix process are all chemical stabilization methods.

Micro-organisms in the sludge work to decompose the organic material. This decomposition subsequently produces offensive odors. The addition of lime to the sludge may raise the pH value to 11 or greater, at high pH levels, the microorganisms are inactived and decomposition does not occur. Hence, offensive odors are not generated. Phosphorus fixation is also improved with lime addition **[19]**. Christy **[20]** reviewed the addition of lime to sludges from the point of view off regulatory requirements, basic chemistry, design factors, and operating cost. The use of lime-stabilized sludge resulted

in increases in pH, available nutrients, and decreases in neutralizable acidity and exchangeable metal levels **[21]**.

The N-VIRO soil process involves mixing dewatered sludge with cement kiln dust to raise the pH to a level of 12 or greater. The mixture is then held at this pH for a period of 72 hr. During this time, chemical reaction between the alkaline cement kiln dust material and the sludge produces a significant amount of heat which raises the temperature of the mixture to 52°C or greater, thus reducing the pathogen population. This temperature is maintained for a period of 12 hr. The mixture is then windrowed and air-dried until a solids concentration of 50% is attained, resulting in a soil-like product.

The ChemFix process combines the dewatered sludge with a dry reagent (Portland cement) and a liquid reagent (soluble silicate product) to fix, stabilize, and solidify sludge. When these reagents are combined with the sludge, a silicate complex is formed. The resulting chemical matrix formed by these silicates renders the product physically and chemical stable. Most heavy metals and other constituents that appear in the raw or digested sludge become part of the silicate complexes and are chemically fixed.

3.1.2 Sludge Treatment in Other Cities and Countries

A liquid sludge management plan using subsurface injection was successfully implemented in Ohio **[22]**. A new sludge dewatering facility in Dallas successfully uses high pressure pumps, evolved from concrete pumps, to move 20% solids sludge 400 m to a pug mill facility **[23]**. An in-vessel system to compost sludge was built to serve Bristol, Tennessee, and Bristol,

Virginia [24]. Regional sludge management strategies using a variety of disposal options were successfully developed for Vermont [25] and Los Angeles [26]. Oklahoma City cut sludge management and disposal cost in half by switching from a predewatered lime stabilization process to postdewatered lime stabilization of their sludge [27]. In New Jersey, a sludge incinerator was constructed and its excess capacity was marketed to neighboring communities for disposal of their sludge, with benefits to all parties [28].

A process tested in Canada successfully used low temperatures to convert sludge to liquid oil and solid char fuel products in an oxygen-free environment [29]. The Swiss developed a high temperature drying and pelletizing process that produced a highly marketable fertilizer product. In the United Kingdom, one new sludge incinerator was operated by Yorkshire Water with competitive operating costs [31]. Five different sludge utilization technologies were developed for the city of Tokyo, Japan: conversion of sludge to compost, conversion of sludge to fuel, conversion of sludge to melted slag, production of artificial aggregate from ashes, and press burning of incineration ashes [32]. A system to melt wastewater sludge following carbonization was developed in Japan [33]. The process used a swirling flow melting furnace and produced a molten slag. Sludge slag occupied less volume than incinerated ash and appeared durable and suitable for use in a variety of construction application [34]. Process design characteristics of the sludge melting process such as melting point and pour point, were presented by Murakami [35]. Sludge management practices at small wastewater plants in Norway, usually employing gravity thickening and aerobic digestion, were reviewed by Paulsrud [36].

3.1.3 Organics Identified in Sludge

Humic acids and fulvic substances were extracted from sludge samples and were analyzed using fluorescence spectroscopy **[37-38]**. Chlorobenzene was identified from waste water sludge using capillary gas chromatography **[39]**. Chlorinated dioxins/furans were identified in raw and treated sludge and hepta- and octa-chlorodibenzodioxin were most frequently detected **[40]**. PAHs, aromatic surfactants, PCBs were detected in sludge samples by HPLC **[41-42]**. Wild **[43]** measured PAHs in sludge samples; the mean PAHs concentration for 29 sludge samples was 50 mg/kg. Di-(2-ethylhexyl) phthalate **[44]** and trialkylamines **[45]** were also identified in sludge samples. Organotin compounds such as monobutyltin, dibutyltin and tributyltin were monitored in sludge **[46]** and the toxicological impact of species of organotins in sludge were investigated **[47]**.

3.2 Experimental

3.2.1 Soxhlet Extraction of Sludge and Soil Samples and Solid Phase Extraction (SPE) to Clean up the Extracts

Typical sludge and soil samples including thermally dried pellets, dewatered sludge, and composted sludge, were supplied by NYC DEP, and top soil and garden soil were collected locally. About 10 g of each were Soxhlet extracted with 250 ml methylene chloride for 20 hr.

The extracts were dried by passing through an anhydrous sodium sulfate column and concentrated to about 1.0 ml using a rotary evaporator. The solvent was exchanged to hexane by the addition of 5.0 ml hexane and evaporation to 1.0 ml, twice. This solution was carefully transferred to a 500 mg (2.8 ml) silica column purchased from Alltech Associates Inc. (Deerfield, IL) and eluted with 5.6 ml each of C_6H_{14}, CH_2Cl_2 and MeOH (all HPLC grade), respectively. Each collected fraction was filtered with a Anotop 2.0μm disposable microfilter (Alltech Associates Inc., Deerfield, IL) and concentrated to 0.3 ml using a Kuderna-Danish condenser, then analyzed by GC/MS and HPLC.

To measure the recovery of this method, the sludge and soil samples were spiked with 0.1 mg/g each of n-hexadecane-d_{34} (99%), pyrene-d_{10} (98%), and di-n-octyl phthalate-d_4 (98%) purchased from Cambridge Isotope Laboratories (Andover, MA) before Soxhlet extraction, by suspending about 1 g sample in 10 ml of methylene chloride and adding 100 μl of 1000 ppm deuterated standard solution. The solvent was allowed to evaporate over night. The spiked samples were treated as above.

3.2.2 The Identification of Organic Compounds by GC/MS and HPLC

A Hewlett Packard 5988A GC/quadrupole MS coupled to a HP1000 data system was used to identify the organic compounds in the samples. Generally, a 2.0 μl sample was injected into a splitless line and carried into a 30 m x 0.25 mm, film thickness 0.33 μm, DB-5 column (J&W Scientific, Folsom, CA). The initial oven temperature was set at 40°C and held for 2 min. Then the temperature was increased at 8 °C/min programming rate to 280°C where it was held for about 30 min, depending on the sample. To measure the retention times and obtain reference mass spectra, standard solutions of n-C_{12}-C_{32} hydrocarbons and dialkyl phthalates were analyzed. The identification of normal hydrocarbons and phthalates was achieved by comparing retention time and mass spectra with those of the standards. The identification of other compounds was made by comparing mass spectra with those of standards in the computer data system which includes about 70,000 mass spectra of organic compounds.

A Hewlett Packard 1090 series II HPLC equipped with a diode array detector and a 486/66U Vectra computer, with which the HPLC instrument was controlled by the Chemstation software installed in it, was used to identify PAHs in the samples. The introduction of the sample solution (25 μl) was achieved using the autosampler system. A 25 cm x 4.6 mm C18 reversed-phase, W/Hastalloy Frits column (Vydac, Hesperia, CA) was used for all of separations. Initial eluent composition was 50% acetonitrile/water, which was kept for 3 min, then 50% acetonitrile was increased to 100% acetonitrile at a linear gradient in 15 min. Generally, a flow rate of 1.5 ml/min was applied and separations were carried out at 40°C. To measure the retention times and UV spectra of the standard compounds, a 20 ppm

standard solution of sixteen EPA priority pollutant PAHs was injected. The PAHs in the sample were identified by comparing the retention times and UV spectra with those of standards.

3.2.3 Quantitation

Semiquantitative estimates of the concentration of each component were obtained by comparing peak areas obtained by GC-MS with those of the n-hexadecane-d_{34}, pyrene-d_{10} and di-n-octyl phthalate-d_4 external standards to the peak areas of the analytes. For PAHs, the concentrations were calculated by the comparison of sample peak areas obtained by HPLC with those of the standard solution of PAHs.

3.2.4 The Analysis of Total Organic Carbon in Sludge and Soil Samples

The total organic carbon (TOC) in sludge and soil samples was determined by a chemical oxidization-reduction titration method (Walkley-Black method) **[48]**. In this method, it is assumed all of the organic compounds in the samples are oxidized completely by chromic acid. An excess of a standard solution of chromic acid is added to the sample, allowed to react, and the excess amount of the chromic acid is back titrated with a standard solution of $Fe(NH_4)_2(SO_4)_2.6H_2O$. The TOC determined by this method represents chromic acid-oxidizable materials in the sludge and soil samples.

A typical analytical procedure is as following: an accurately weighed sludge or soil sample (around 0.1 g) was placed in a 250 ml Erlenmeyer flask and exactly 10.0 ml of 1.00 N $K_2Cr_2O_7$ standard solution was added to the sample. Concentrated sulfuric acid (20 ml) was added to the flask and was mixed by gentle rotation. After about 20 min standing, the solution was

diluted to about 175 ml with distilled water followed by a sequential addition of 10 ml of 85% H_3PO_4, 0.2 g NaF, and 30 drops of diphenylamine indicator which was prepared by dissolving approximately 0.25 g of diphenylamine in 10 ml of distilled water and 50 ml of concentrated sulfuric acid. The contents were then back-titrated with a 0.50 N standard solution of $Fe(NH_4)_2(SO_4)_2.6H_2O$ delivered from a 50 ml burette. The color of the solution in the mixture was dull green at the beginning and shifted to a turbid blue as the titration proceeded. At the end point, the color sharply changed to a bright green. Control experiments (the blank system without samples) were also performed using the identical procedure. The percentage of TOC in the samples was calculated from the following equation:

$$TOC\ \% = 10 \times (1\text{-}T/S) \times f$$

where S is the volume in ml of $Fe(NH_4)_2(SO_4)_2.6H_2O$ solution used in the blank titration without the samples, T is the volume in ml of $Fe(NH_4)_2(SO_4)_2.6H_2O$ solution used in the sample titration, and f is a factor expressed as following:

$$f = (1.00N) \times (12/4000) \times 1.30 \times (100/W)$$

where 1.00 N is the concentration of $K_2Cr_2O_7$, 12/4000 is the meq weight of carbon in g , 1.30 is a correction factor to account for unrecovered organic C by this method **[65]**, and W is the weight of the sample in g.

For comparison with this method, a dry ashing procedure was used. Around 1.0 g of sample (accurately weighed) was put into a crucible which had been heated twice to constant weight, and heated at 105°C for 1 hr, and then cooled and weighed. The percentage of water and volatiles in the sample was determined by the difference in weight of the sample before and after heating. Then the sample in the crucible was ashed at 800°C for 3 hr, cooled

and weighed. The percentage of organic in the sample was calculated by the weight of the sample dividing the loss in weight of the sample after ashing.

3.3 Results and Discussion

Samples of two soils, a "top soil" for lawns or trees and a garden soil, and three representative treated sludge samples obtained from the DEP: thermally dried pellets, dewatered sludge and composted sludge were extracted and analyzed. The most numerous components identified were saturated hydrocarbons. PAHs, phenols, phthalates, some cholesterol derivatives and fatty acids were also identified in these samples. The concentrations of the compounds were in the range of 0.1 to 130 μg/g.

3.3.1 Identification of Organic Compounds in the Samples by GC/MS

A blank test from Soxhlet extraction to silica gel column clean up was performed to identify components coming from extraction solvent or the silica gel column. Only dioctyl phthalate was detected in the blank test.

The identification of the components was based on comparison of mass spectra of the GC peaks with those of the standards in the computer data system. Standard solutions of C_{12} to C_{32} (30 ppm) hydrocarbons and dimethyl, diethyl, dibutyl, dioctyl phthalates (30 ppm) were injected and separated by GC-MS at the same analytical conditions as for the samples. Figure 3.2 and Figure 3.3 show the relationship between the retention time t_r and carbon number for normal hydrocarbons, and retention time to alkyl carbon number of phthalates. The identification of the normal hydrocarbons and phthalates in the samples was confirmed by comparing the retention times with the plots.

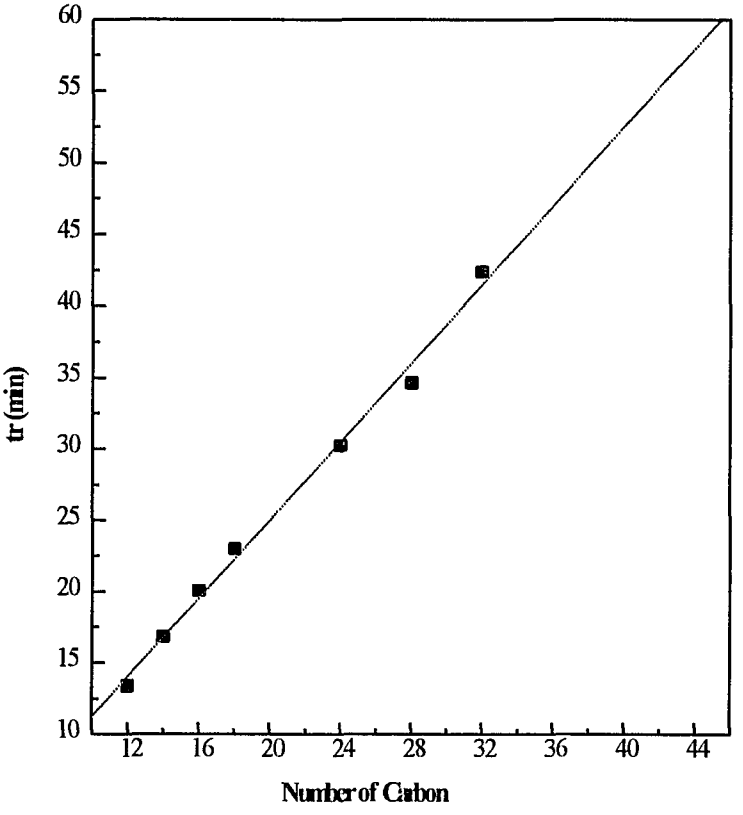


Figure 3.2 Retention Time tr vs Number of Carbons for Normal Hydrocarbons

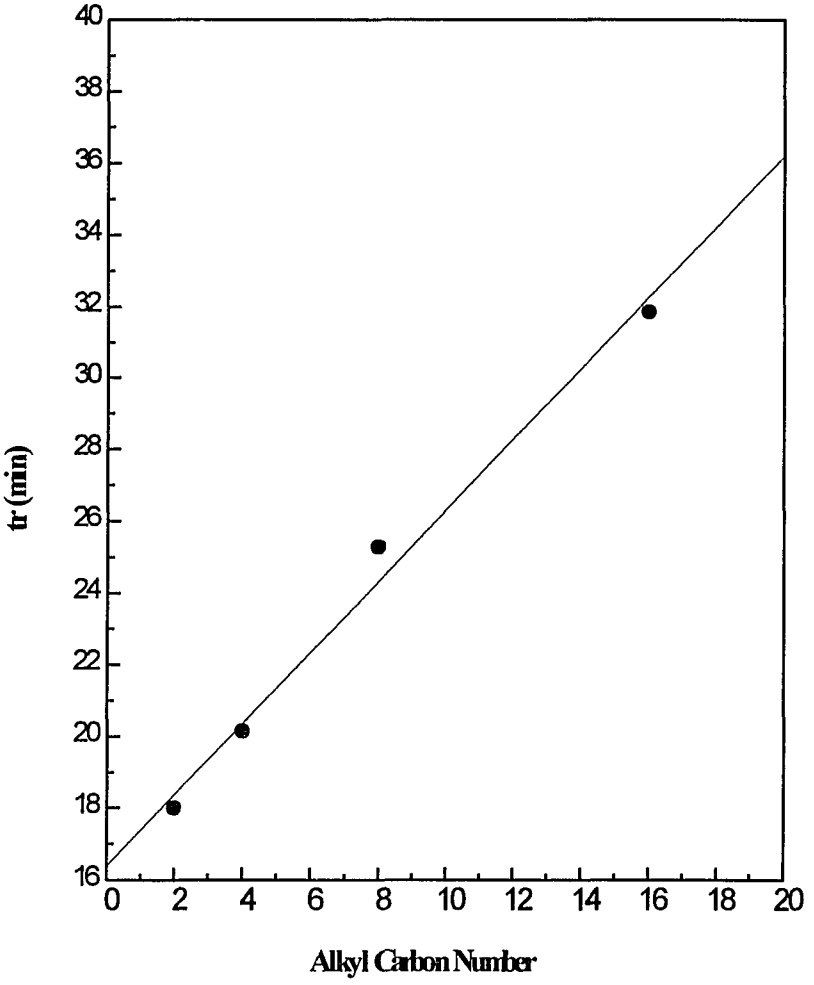


Figure 3.3 Retention Time tr vs Alkyl Carbon Number for Di-alkyl Phthalates

Figure 3.5-3.16 show there are many organic compounds in the samples. Most of the peaks in the chromatograms were identified and the results are listed in Table 3.1 to 3.9. To confirm the identification of N,N-dimethyl-alkylamines, the retention time of standards was plotted vs. the alkyl carbon number (Figure 3.4). N,N-dimethyl-alkylamines and alkyl-phenols may come from washing machine effluents of fabric softeners in household laundry detergents **[49]**. Nonyl phenol isomers, degradation products of the antioxidant tris-(nonyl phenol)-phosphite **[50]** were found in thermally treated and dewatered sludges. The results show that the most numerous constituents are the saturated hydrocarbons, and most of these eluted in the first column fraction, the hexane fraction. The source of these compounds is probably petroleum released by vehicles **[51]** washed into storm sewers, and illegal dumping into sewers. These petroleum-type hydrocarbons have also been found in marine sediments **[52]**. Branched and cyclic alkanes in the samples may result from a complex combination of physical and biological influences **[53]**. The widely used plastic additive, the plasticizer bis-2-ethylhexyl phthalate, was found in all of samples. The high disposal rates of plastics may be producing an environmental reservoir of phthalates from which these compounds are slowly leached; the environmental levels will not decrease readily even if phthalate use were to be stopped **[54]**. Studies of this compound with mammals have generally indicated a low order of active toxicity **[55]**, but it affects lipid biosynthesis in rats **[56]**. Fatty acids and cholesterol derivatives have biological sources **[57]**. Cholest-5-en-3-ol, coprostanol, is often used as a chemical marker of urban sewage contamination **[58]**.

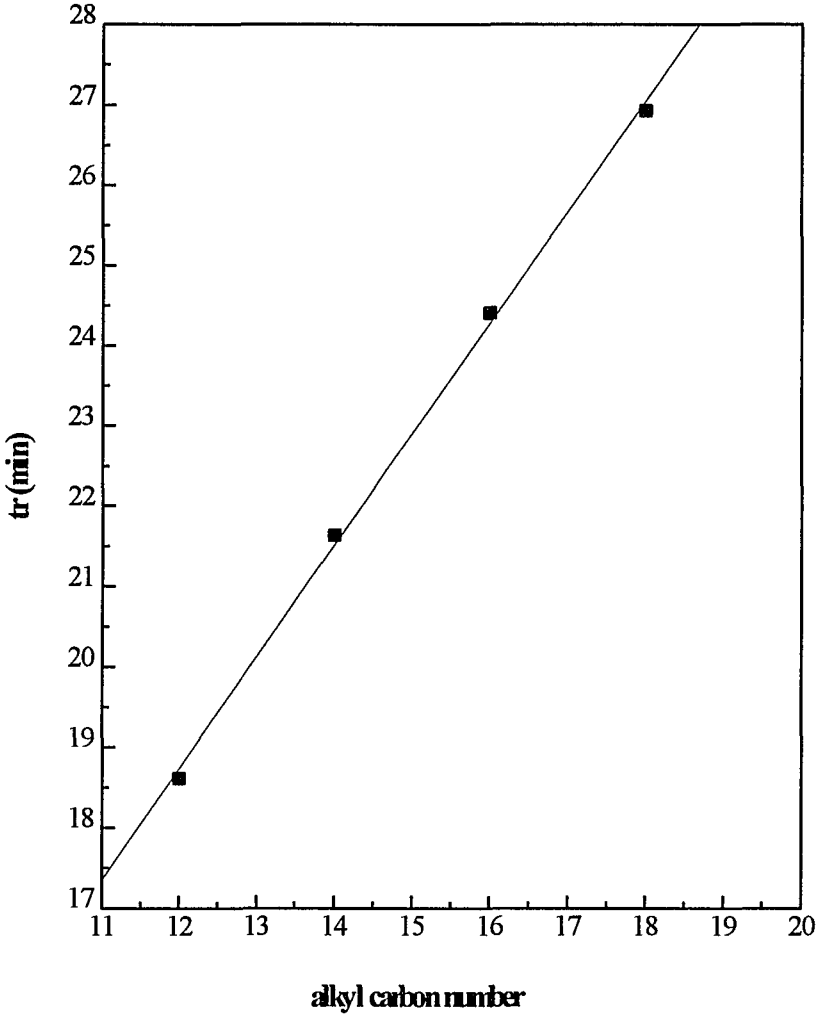


Figure 3.4 Retention Time tr vs Alkyl Carbon Number for DiMe-alkyl Amines

The compounds range in concentration from 0.1 to 130 μg/g. The compound at highest concentration (127 μg/g) is dihydrocholestrol in the thermally treated pellets.

The GC-MS results showed that pyrene-d_{10} and most of n-hexadecane-d_{34} were eluted in the hexane fraction, the residual n-hexadecane-d_{34} in the methylene chloride fraction, and all of di-n-octyl phthalate-d_4 in methylene chloride fraction. The recoveries of deuterated compounds were calculated by dividing the sum of the peak areas of fractions of each by that of 100 ppm deuterated standard solution. The recoveries are listed in Table 3.10.

Table 3.1 Compounds Identified in Thermally Treated Pellets
C_6H_{14} fraction

tr	Compound	Conc. (μg/g)
9.45	4-methyl pentanoic acid	31
10.89	benzene methane thiol	7.0
11.12	4-methyl phenol	78
12.30	i-$C_{12}H_{26}$	23
12.41	i-$C_{12}H_{26}$	9.7
12.51	i-$C_{12}H_{26}$	7.7
12.64	i-$C_{12}H_{26}$	6.8
12.95	i-$C_{12}H_{26}$	27
13.10	i-$C_{12}H_{26}$	8.8
13.23	i-$C_{12}H_{26}$	7.2
13.49	i-$C_{13}H_{28}$	63
14.24	i-$C_{13}H_{28}$	12
14.37	i-$C_{13}H_{28}$	31
14.62	i-$C_{14}H_{30}$	114
15.04	i-$C_{13}H_{28}$	20
15.11	n-$C_{13}H_{28}$	17
15.32	i-$C_{14}H_{30}$	11
15.94	i-$C_{14}H_{30}$	21
16.15	i-$C_{14}H_{30}$	14
16.25	i-$C_{14}H_{30}$	16
16.38	i-$C_{15}H_{32}$	25
16.48	i-$C_{15}H_{32}$	17
16.77	i-$C_{14}H_{30}$	30
16.90	n-$C_{14}H_{30}$	16
17.00	2-methyl-1-H-indole	7.9

Table 3.1 continued

17.88	i-$C_{16}H_{34}$	26
17.93	i-$C_{16}H_{34}$	13
18.11	i-$C_{16}H_{34}$	10
18.45	i-$C_{15}H_{32}$	34
18.58	n-$C_{15}H_{32}$	29
19.22	trimethyl naphthalene	30
19.46	i-$C_{17}H_{36}$	22
19.71	i-$C_{17}H_{36}$ + trimethyl naphthalene	6.0
20.05	i-$C_{16}H_{34}$	26
20.18	n-$C_{16}H_{34}$	34
20.78	i-$C_{18}H_{38}$	26
20.93	i-$C_{18}H_{38}$	24
21.09	i-$C_{18}H_{38}$	14
21.27	i-$C_{18}H_{38}$	8.2
21.73	n-$C_{17}H_{36}$	19
21.81	i-$C_{18}H_{38}$	23
22.00-22.72	nonyl phenol isomers	60
22.85	dipropyl phthalate	28
23.16	i-$C_{18}H_{38}$	76
23.29	i-$C_{20}H_{42}$	30
23.71	i-$C_{20}H_{42}$	18
27.08	i-$C_{21}H_{44}$	17
28.69	i-$C_{23}H_{48}$	9.5
31.47	n-$C_{25}H_{52}$	35

Table 3.2 Compounds Identified in Thermally Treated Pellets

tr	Compound	Conc. (μg/g)
	CH_2Cl_2 fraction	
11.19	4-methyl phenol	44
15.44	1-H-indole	13
18.11	tridecanol	16
25.34	hexadecanoic acid	19
31.86	bis-2-ethylhexyl phthalate	15
40.22	dihydrocholesterol	127
41.44	cholest-5-en-3-ol	53
	MeOH fraction	
9.38	chloromethyl benzene	3.8
13.04	1-methyl-2-pyrrolidinone	12
18.62	N,N-dimethyl dodecanamine	12
21.63	N,N-dimethyl tetradecanamine	19
24.41	N,N-dimethyl hexadecanamine	17
26.93	N,N-dimethyl octadecanamine	32

Table 3.3 Compounds Identified in Dewatered Sludge, C_6H_{14} fraction

tr	Compound	Conc. (μg/g)
9.06	i-$C_{10}H_{22}$	6.7
9.27	i-$C_{10}H_{22}$	1.1
9.57	4-isopropyl toluene + 2,2-dimethyl hexanone	3.1
11.25	i-$C_{11}H_{24}$	5.1
11.56	decahydro-methyl naphthalene	15
11.89	decahydro-methyl naphthalene	17
12.48	i-$C_{12}H_{26}$	34
12.67	i-$C_{12}H_{26}$	11
13.70	i-$C_{13}H_{28}$	36
14.16	hexyl-cyclohexane	9.5
14.39	i-$C_{13}H_{28}$	22
14.52	i-$C_{13}H_{28}$	9.6
14.63	i-$C_{13}H_{28}$	19
14.78	i-$C_{14}H_{30}$	46
15.22	i-$C_{13}H_{28}$	26
15.45	i-$C_{14}H_{30}$	13
16.00	octyl-cyclohexane	13
16.51	i-$C_{15}H_{32}$	7.4
16.90	n-$C_{14}H_{30}$	8.7
17.93	i-$C_{16}H_{34}$	5.1
18.58	i-$C_{16}H_{34}$	8.4

Table 3.3 continued

20.15	i-$C_{16}H_{34}$	5.2
20.87	1-chlorotetradecane	4.7
21.67	i-$C_{17}H_{36}$	9.3
21.75	i-$C_{19}H_{40}$	4.0
23.09	n-$C_{18}H_{38}$	4.4
24.33	i-$C_{19}H_{40}$	2.5
24.44	n-$C_{19}H_{40}$	2.8
25.49	hexadecanoic acid	4.3
25.73	n-$C_{20}H_{42}$	3.4
26.97	n-$C_{21}H_{44}$	2.4
28.15	n-$C_{22}H_{46}$	1.4
29.29	n-$C_{23}H_{48}$	1.2
30.37	n-$C_{24}H_{50}$	3.7
31.98	bis-2-ethylhexyl phthalate	5.9
41.41	cholest-5-en-3-ol	3.3

Table 3.4 Compounds Identified in Dewatered Sludge, CH_2Cl_2 fraction

tr	Compound	Conc. (μg/g)
8.72	butanoic acid	11
9.30	phenol	34
10.49	4-methyl pentanoic acid	8.9
10.60	hexanoic acid	3.0
11.18	4-methyl phenol	45
12.30	i-$C_{12}H_{26}$	4.7
12.43	i-$C_{12}H_{26}$	3.4
12.50	i-$C_{12}H_{26}$	4.0
12.63	i-$C_{12}H_{26}$	3.5
12.68	i-$C_{12}H_{26}$	5.3
12.93	i-$C_{12}H_{26}$	5.3
13.21	i-$C_{12}H_{26}$	3.8
13.47	i-$C_{13}H_{28}$	9.9
13.62	i-$C_{13}H_{28}$	2.1
13.72	i-$C_{13}H_{28}$	4.1
13.98	hexyl-cyclohexane	5.0
14.21	i-$C_{13}H_{28}$	6.2
14.33	i-$C_{14}H_{30}$	2.7
14.41	i-$C_{13}H_{28}$	4.5
14.59	i-$C_{14}H_{30}$	12
15.89	heptyl cyclohexane	3.0

Table 3.4 continued

16.19	i-$C_{15}H_{32}$	1.3
16.40	i-$C_{15}H_{32}$	1.5
16.80	n-$C_{14}H_{30}$	2.8
17.18	benzenepropanic acid	4.4
17.85	i-$C_{15}H_{32}$	1.3
18.48	i-$C_{15}H_{32}$	5.3
20.82	i-$C_{16}H_{34}$	1.7
21.89-22.68	nonyl phenol isomers	10
22.81	dipropyl phthalate	4.4
23.01	n-$C_{18}H_{38}$	2.9
23.98	pentadecanoic acid	2.7
25.69	hexadecanoic acid	19
28.08	octadecanoic acid	7.1
30.37	dioctyl adipate	12
31.98	bis-2-ethylhexyl phthalate	19

Table 3.5 Compounds Identified in Dewatered Sludge MeOH fraction

tr	Compound	Conc. (μg/g)
9.45	chloromethyl benzene	1.8
18.69	N,N-dimethyl dodecanamine	1.8
21.73	N,N-dimethyl tetradecanamine	3.1
24.48	N,N-dimethyl hexadecanamine	2.1
27.11	N,N-dimethyl octadecanamine	3.7

Table 3.6 Compounds Identified in Composted Sludge

tr	Compound	Conc. (μg/g)
	CH_2Cl_2 fraction	
18.08	1-dodecanol	5.7
30.35	dioctyl adipate	38
	MeOH fraction	
21.71	N,N-dimethyl tetradecanamine	0.67
26.70	1-chloro octadecane	3.3
27.04	N,N-dimethyl octadecanamine	8.4

Table 3.7 Compounds Identified in Top Soil

tr	Compound	Conc.(μg/g)
	C_6H_{14} fraction	
7.38	ethyl benzene	14
7.48	dimethyl benzene	24
8.88	benzaldehyde	49
10.17	benzyl alcohol	8.2
10.28	i-$C_{12}H_{26}$	1.9
18.67	2,6-bis(1,1-dimethylethyl)-4-methyl phenol, (butylated hydroxy toluene)	5.9
26.79	i-$C_{21}H_{44}$	2.2
29.12	i-$C_{25}H_{52}$	5.0
30.12	i-$C_{25}H_{52}$	1.7
31.25	i-$C_{26}H_{54}$	8.3
33.36	i-$C_{28}H_{58}$	7.6
36.03	i-$C_{30}H_{62}$	7.5
45.48	i-$C_{38}H_{78}$	4.8
	CH_2Cl_2 fraction	
18.66	2,6-bis(1,1-dimethyl ethyl)-4-methyl phenol, (butylated hydroxy toluene)	2.2
25.28	hexadecanoic acid	1.5
28.98	1-octadecanol	5.0
31.20	1-docosanol	13
31.82	bis-2-ethylhexyl phthalate	30
33.35	1-hexacosanol	11

Table 3.8 Compounds Identified in Garden Soil ,C_6H_{14} fraction

tr	Compound	Conc.(μg/g)
18.45	n-$C_{15}H_{32}$	0.47
18.72	2,6-bis(1,2-dimethyl)-4-methyl phenol	1.5
20.03	n-$C_{16}H_{34}$	0.32
23.27	phenanthrene or anthracene	4.2
24.82	methyl phenanthrene	0.64
24.92	methyl phenanthrene	0.66
25.19	methyl phenanthrene	1.6
25.78	1-phenyl naphthalene	0.28
26.61	dimethyl phenanthrene	0.51
26.85	i-$C_{22}H_{46}$	0.80
27.06	fluoranthrene	4.0
27.79	pyrene	5.1
28.03	i-$C_{23}H_{48}$	0.29
28.62	methyl pyrene	0.52
28.94	methyl pyrene	0.69
29.15	i-$C_{23}H_{48}$	1.3
29.55	methyl pyrene	1.0
30.25	n-$C_{24}H_{50}$	0.38
30.78	i-$C_{25}H_{52}$	1.5
30.94	benzo[b]naphtho[2,1,d]thiophene	0.89

Table 3.8 continued

31.29	i-$C_{26}H_{54}$	1.0
31.69	benz[a]anthracene	2.0
31.80	chrysene	2.9
32.31	i-$C_{27}H_{56}$	0.94
32.44	n-$C_{26}H_{54}$	0.44
33.41	i-$C_{28}H_{58}$	1.2
33.89-34.64	dinonyl phthalate isomers	3.7
35.71	benz[e]pyrene	1.0
36.08	i-$C_{30}H_{62}$	2.5
36.94	benzo[e]pyrene	1.3
37.24	benzo[k]fluoranthene	1.6
39.91	i-$C_{31}H_{64}$	2.8
45.64	i-$C_{34}H_{70}$	0.73

Table 3.9 Compounds Identified in Garden Soil, CH_2Cl_2 fraction

tr	Compound	Conc. (μg/g)
18.17	i-$C_{16}H_{34}$	0.58
18.44	n-$C_{15}H_{32}$	0.54
20.02	n-$C_{16}H_{34}$	0.40
31.65	diheptyl phthalate	4.6
31.92	bis-2-ethylhexyl phthalate	28

Table 3. 10 Recovery of Deuterated Compounds

Sample	hexadecane-d_{34}	pyrene-d_{10}	di-octyl phthalate-d_4
thermally treated pellets	93	94	99
dewatered sludge	90	101	86
composted sludge	99	90	102
garden soil	96	82	84

3.3.2 Identification of PAHs in the Samples by HPLC

The retention times and UV spectra of the standard compounds were measured by running a standard solution of sixteen EPA priority pollutant PAHs by HPLC. The chromatogram (Figure 3.17) shows that all of the compounds were well separated. The identification of PAHs in the samples was achieved by the comparison of retention times and UV spectra with those of standards. The UV detector in HPLC is more selective and sensitive than GC-MS for PAHs. Thus only a few PAHs were detected by GC-MS, but a total of 18 PAHs (Table 3.11) were identified in different samples by HPLC at concentrations in the range of 0.05 to 4.5 μg/g. The PAHs may come from industrial operations, power and heat generation, and residential heating **[59-60]**, as well as diesel and gasoline-engine vehicles **[61-62]**. PAHs from various sources associated with particulate matter are also found in fly ash, urban dust and soils **[63]**.

PAHs are strong carcinogens and are one of main classes of chemical carcinogens in the human environment that have consistently been shown to induce mammary tumors in rodents. Human PAHs exposure results from a wide variety of occupational, environmental and dietary sources, and may be a factor in the increasing incidence of breast cancer in American women **[64]**. Benzo(a)pyrene is a relative strongly carcinogenic PAH to which particular attention should be paid.

3.3.3 Total Organic Carbon(TOC) in the Samples

The TOC of the samples was measured by oxidization-reduction titration and ashing methods. The results are listed in Table 3.12. The results show that the TOC determined by the ashing method is higher than that by the

titration method, which is consistent with what Walkley stated **[48]**. The higher oxides of minerals such as MnO_2, Mn_3O_4 which probably exist in the samples may lead to low TOC results by the titration method, and any mineral weight lost in ashing may result in a higher apparent TOC. The percentage of water and volatiles in thermally treated pellets is much lower than that in dewatered sludge and TOC% is almost the same for both of the dried solids, which indicates that the amounts of water or the volume of the sludge were greatly decreased by further thermally drying treatment and that benefits for the transportation of the sludge. The amount of water in the sludge was also decreased by composting, but the amount of water lost is not as much as by thermally drying.

Table 3.11 Concentrations of PAHs in Sludge Products (μg/g)

Compound	TTP *	DS*	CS*	GS*
naphthalene		4.5		
acenaphthene		2.9		
phenanthrene	0.15		1.6	1.5
anthracene			0.04	0.26
fluoranthene			0.53	3.6
methyl phenanthrene				0.19
pyrene			0.72	3.2
benz[a]anthracene				1.7
chrysene				2.2
benzo[e]pyrene			2.7	1.8
benzo[b]fluoranthene			2.8	1.7
benzo[k]fluoranthene				1.1
benzo[a]pyrene				1.4
dibenz[a,h]anthracene				0.65
benzo[ghi]perylene				1.1
perylene	1.0			
indeno[1,2,3,-cd]pyrene				0.94
benzo[b]chrysene				0.18

* TTP = thermally treated pellets
DS = dewatered sludge
CS = composted sludge
GS = garden soil

Table 3.12 Total Organic Carbon in the Samples

Sample	TOC, %[a]	TOC, %[b]	H_2O+volatiles,%
thermally treated pellets	13.3 ± 2.3[c]	59.0 ± 0.2	5.1 ± 0.1
garden soil	4.9 ± 0.3	7.5 ±0.5	17.4±0.4
compost pile	15.6 ± 0.0	19.9 ± 0.3	47.6±0.5
dewatered sludge	15.2 ± 0.4	18.5 ±1.3	72.3±1.1

[a] : TOC, % determined by the titration method

[b] : TOC, % determined by the ashing method

[c] : standard deviation of three trials

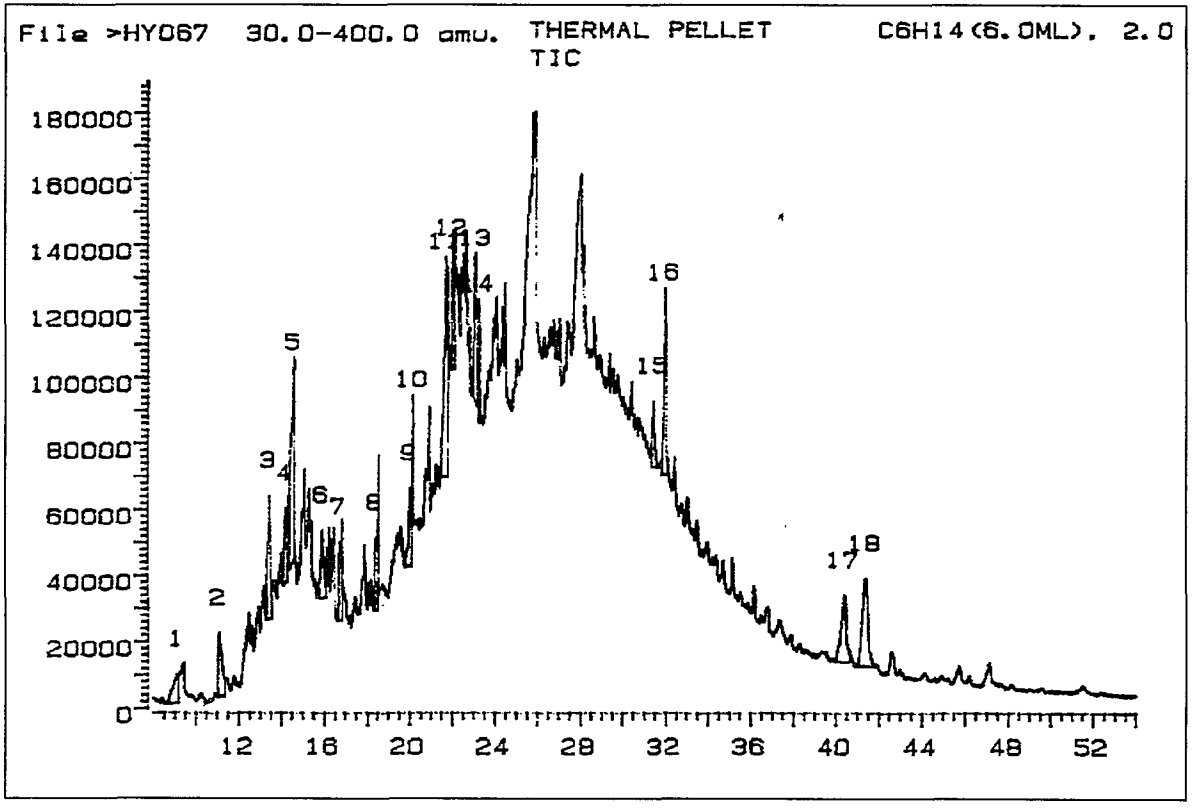


Figure 3.5 Total Ion Chromatogram of Thermally Treated Pellets (C_6H_{14} fraction)

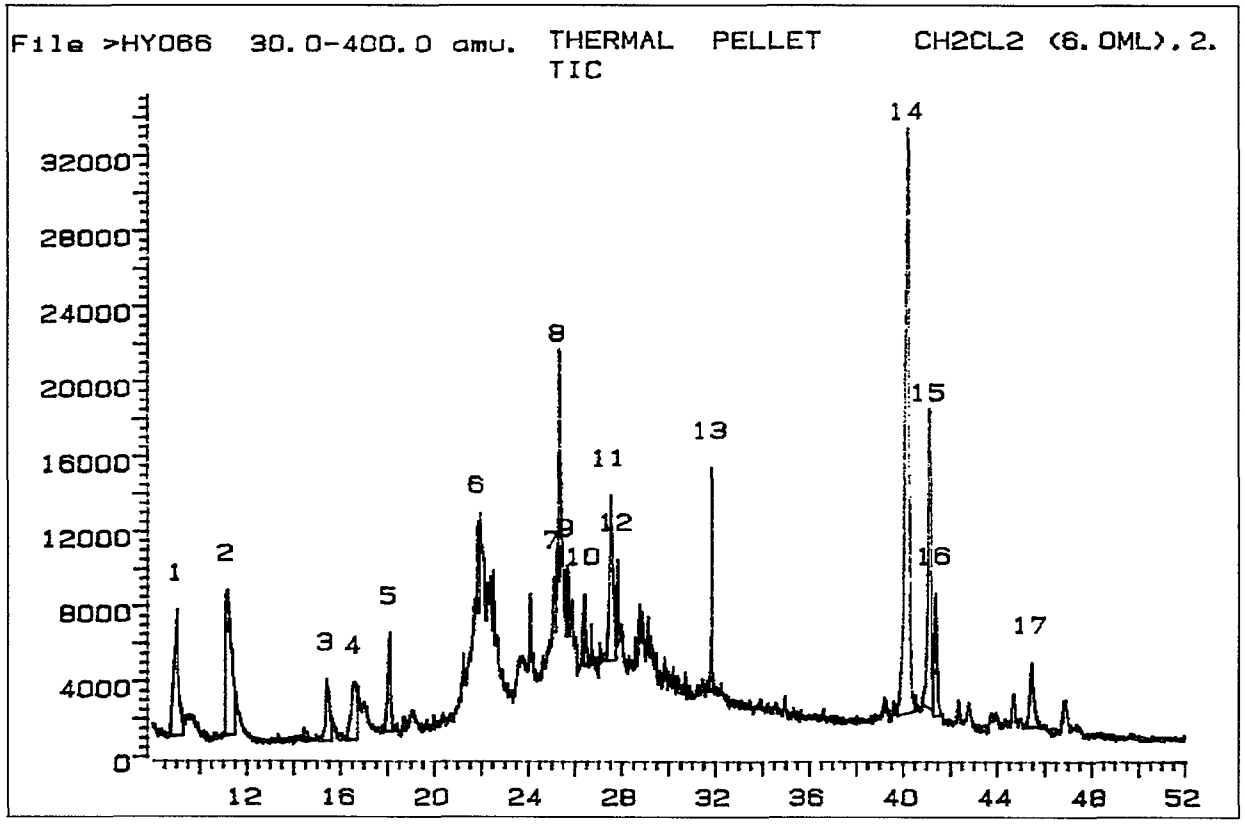


Figure 3.6 Total Ion Chromatogram of Thermally Treated Pellets (CH_2Cl_2 fraction)

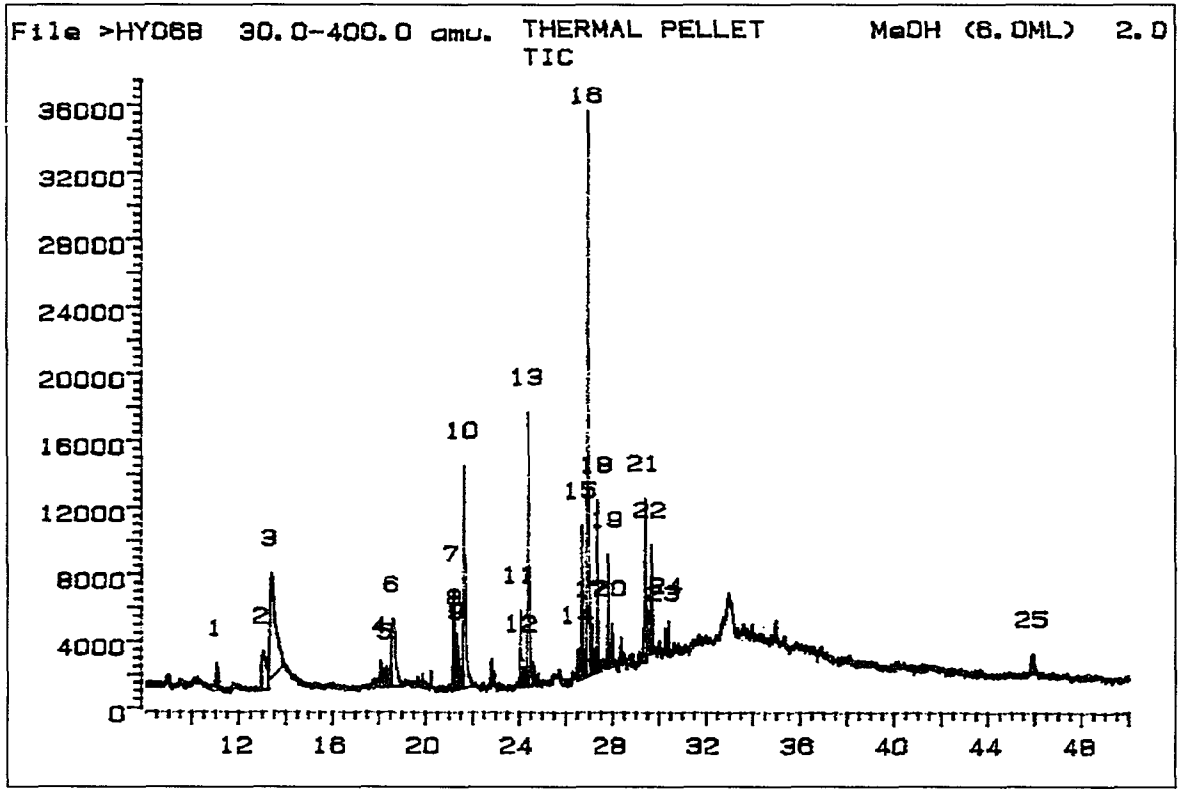


Figure 3.7 Total Ion Chromatogram of Thermally Treated Pellets (MeOH fraction)

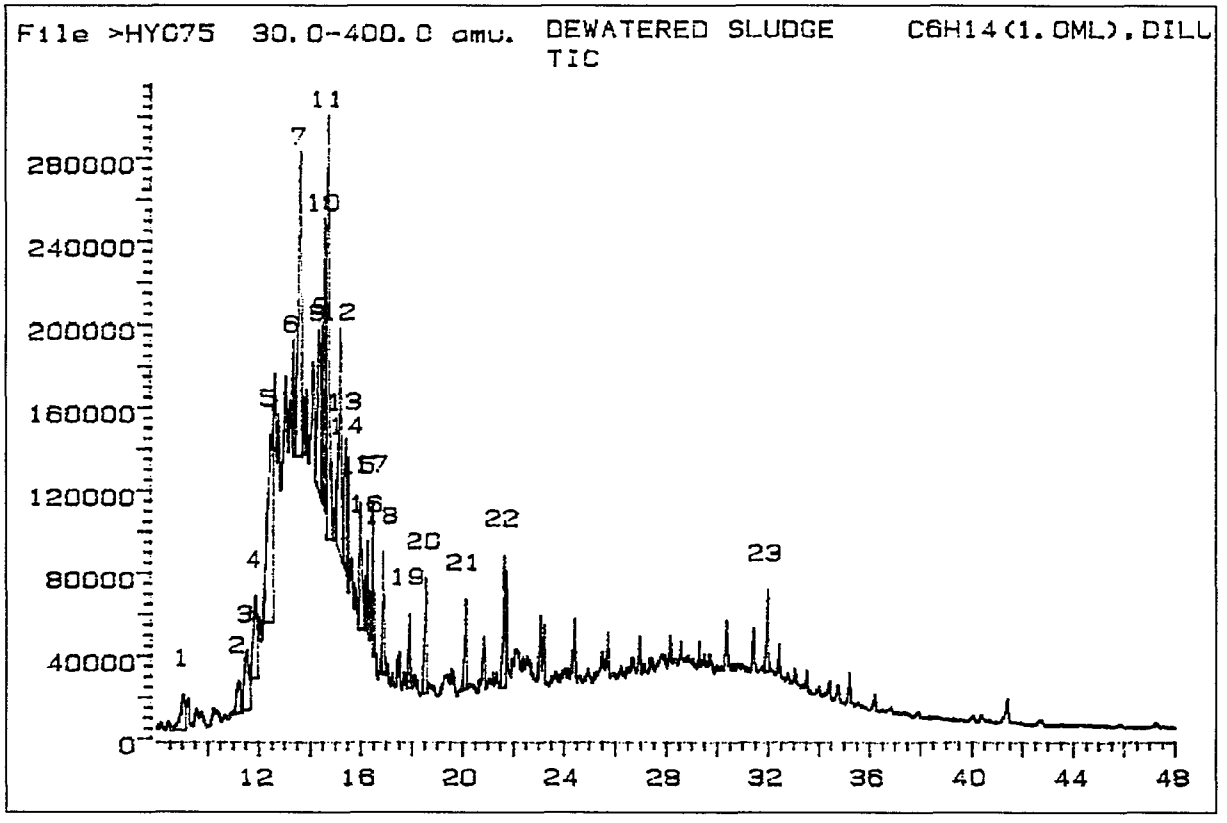


Figure 3.8 Total Ion Chromatogram of Dewatered Sludge (C_6H_{14} fraction)

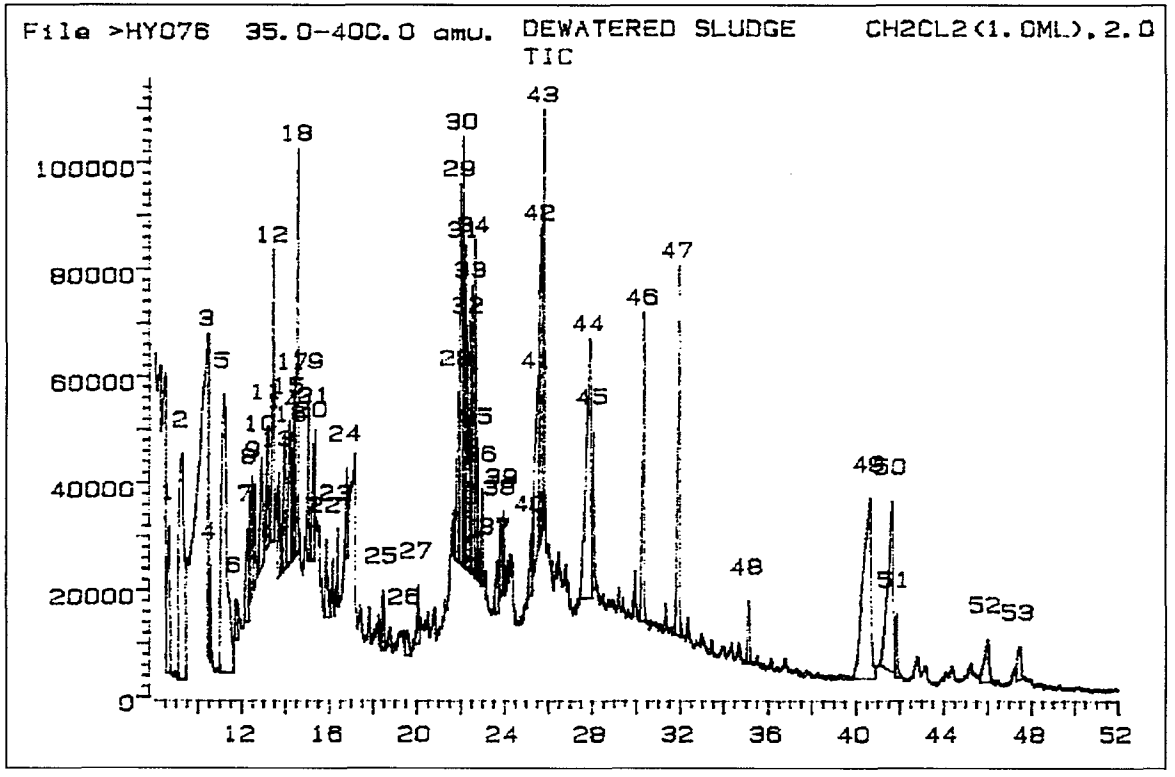


Figure 3.9 Total Ion Chromatogram of Dewatered Sludge (CH_2Cl_2 fraction)

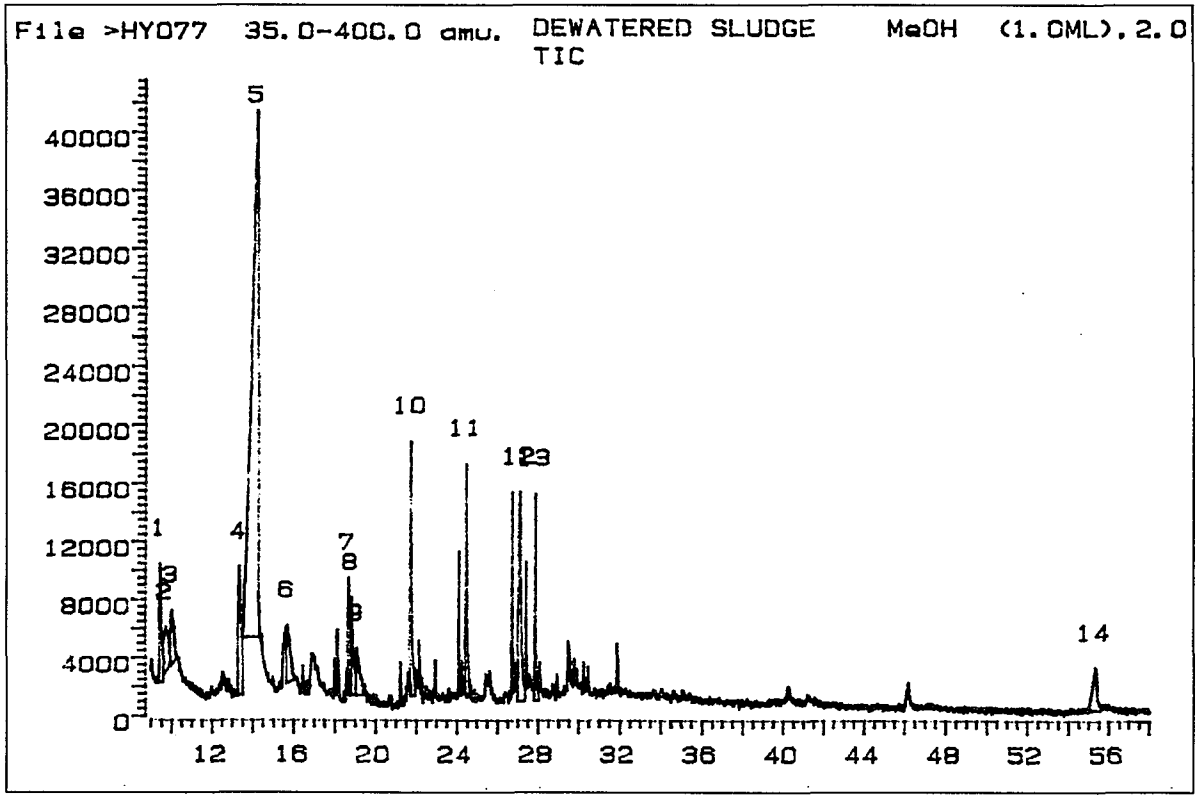


Figure 3.10 Total Ion Chromatogram of Dewatered Sludge (MeOH fraction)

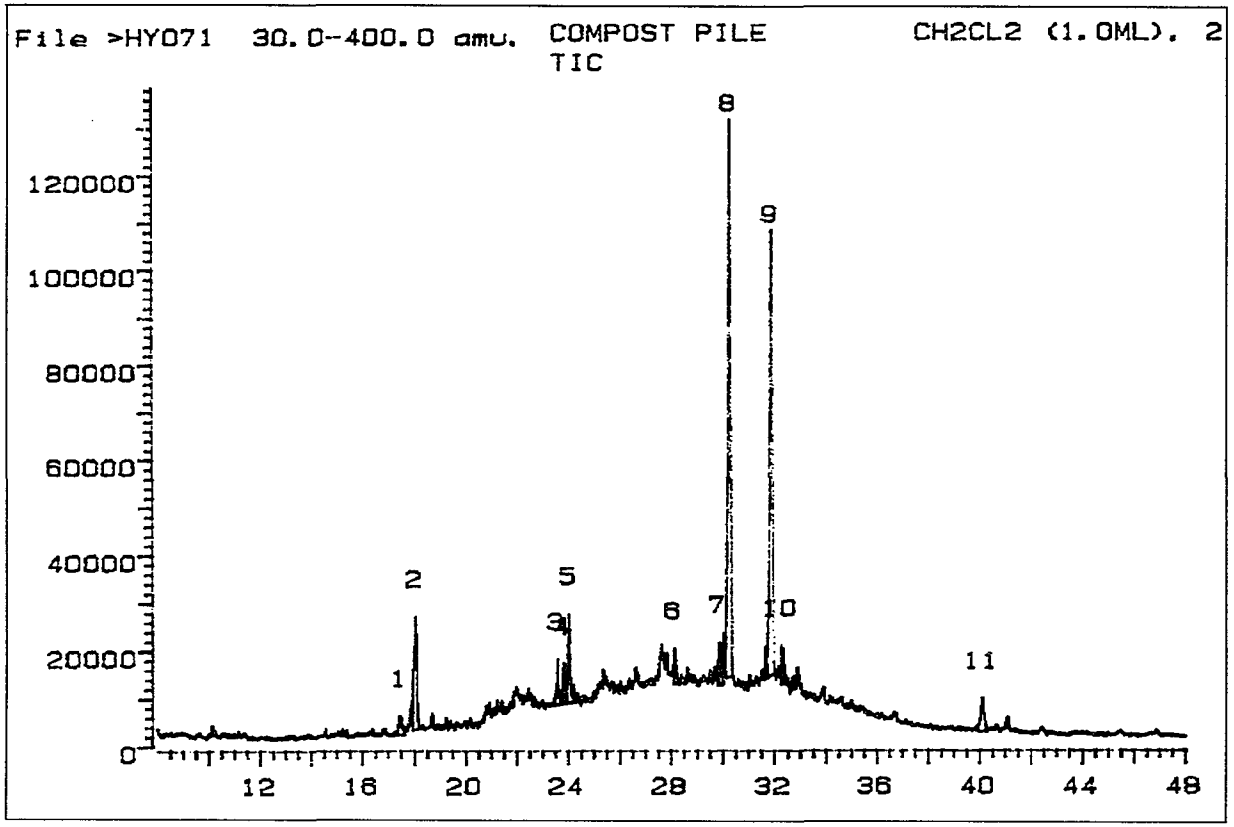


Figure 3.11 Total Ion Chromatogram of Compost Pile (CH_2Cl_2 fraction)

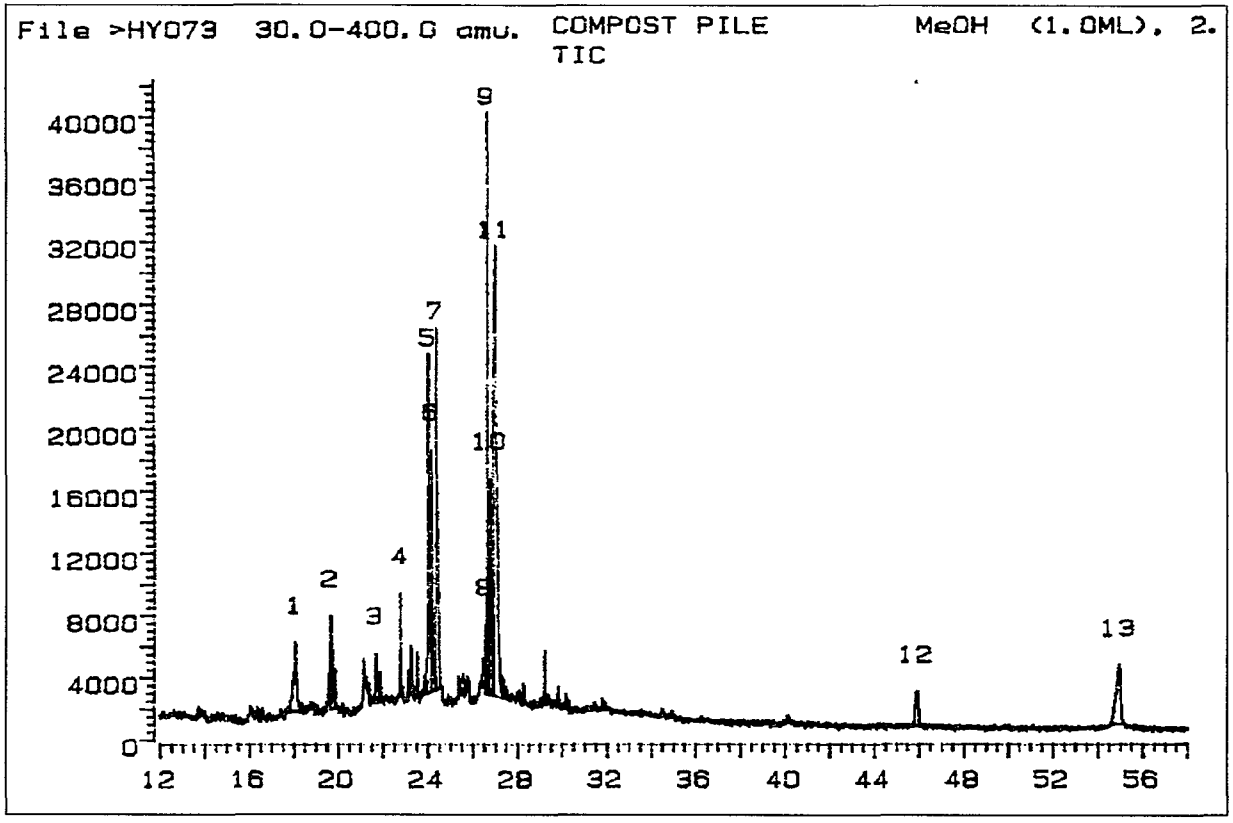


Figure 3.12 Total Ion Chromatogram of Compost Pile (MeOH fraction)

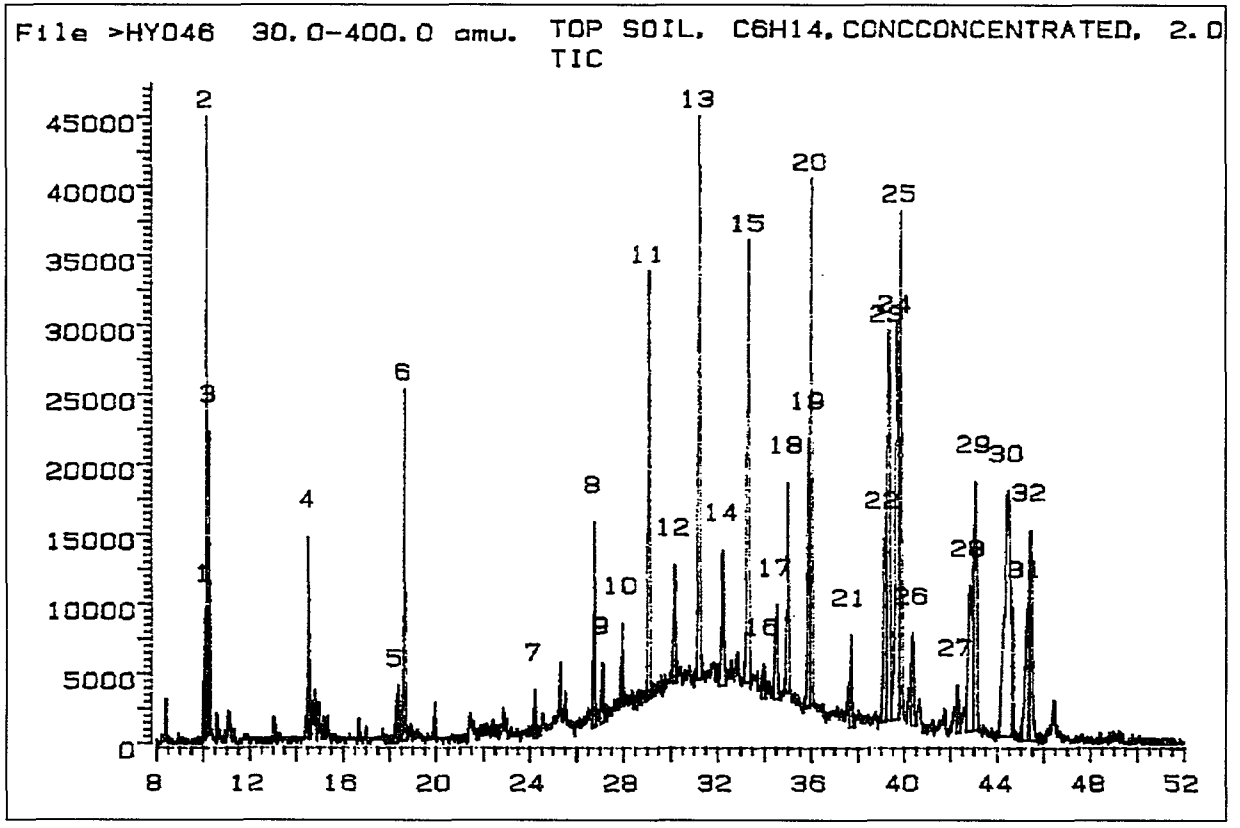


Figure 3.13 Total Ion Chromatogram of Top Soil (C_6H_{14} fraction)

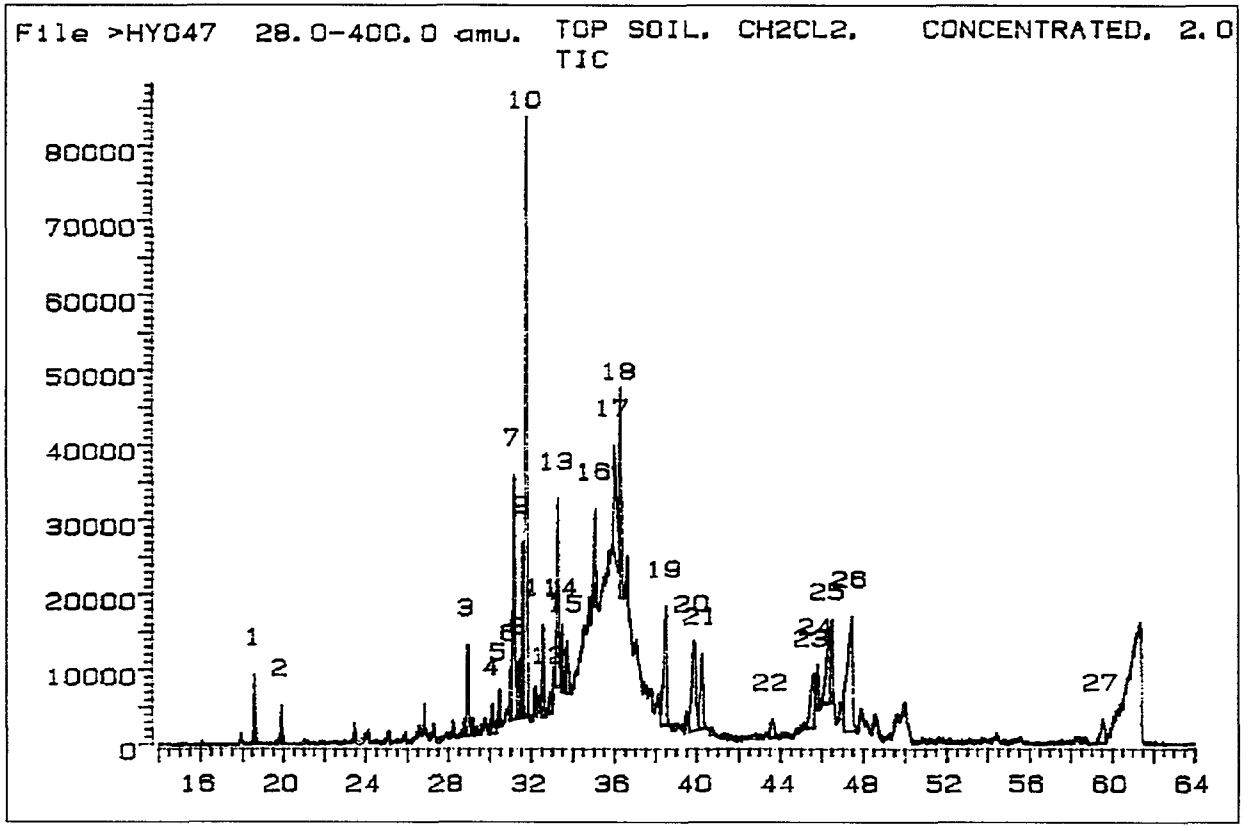


Figure 3.14 Total Ion Chromatogram of Top Soil (CH_2Cl_2 fraction)

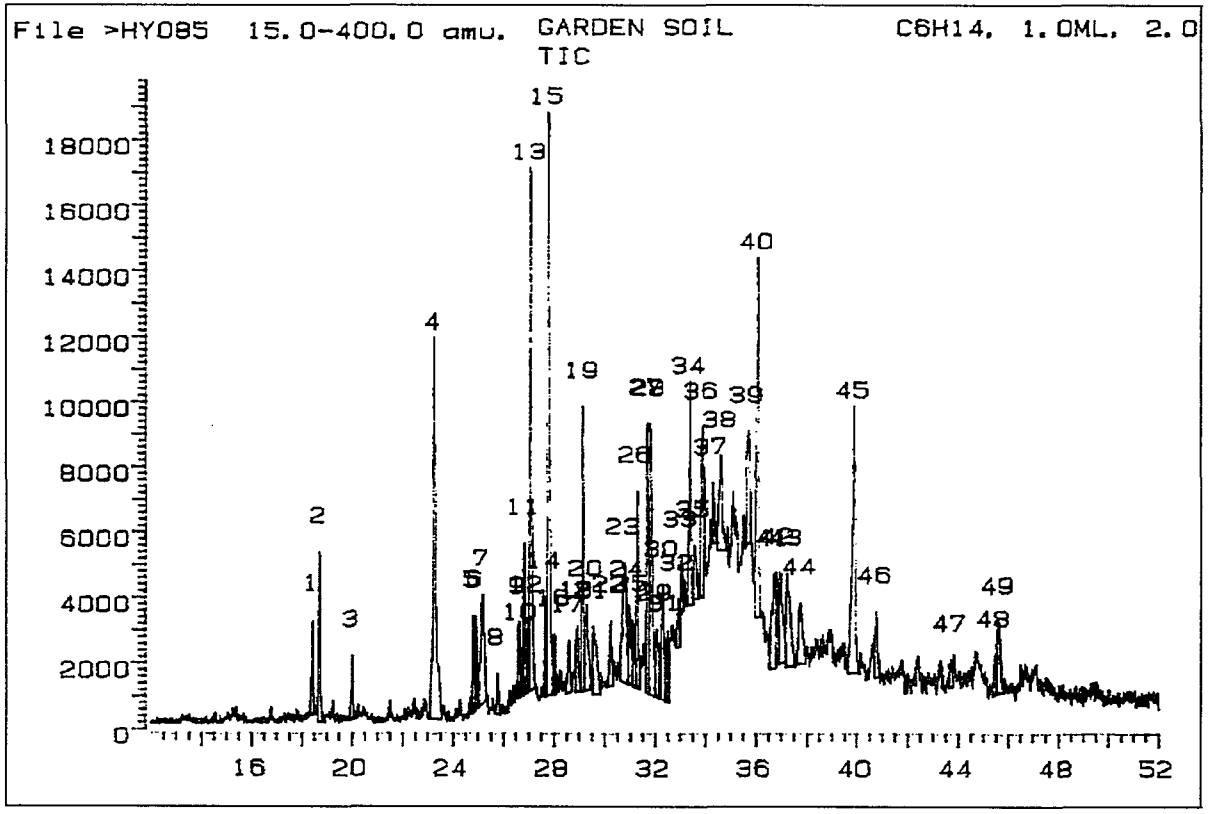


Figure 3.15 Total Ion Chromatogram of Garden Soil (C_6H_{14} fraction)

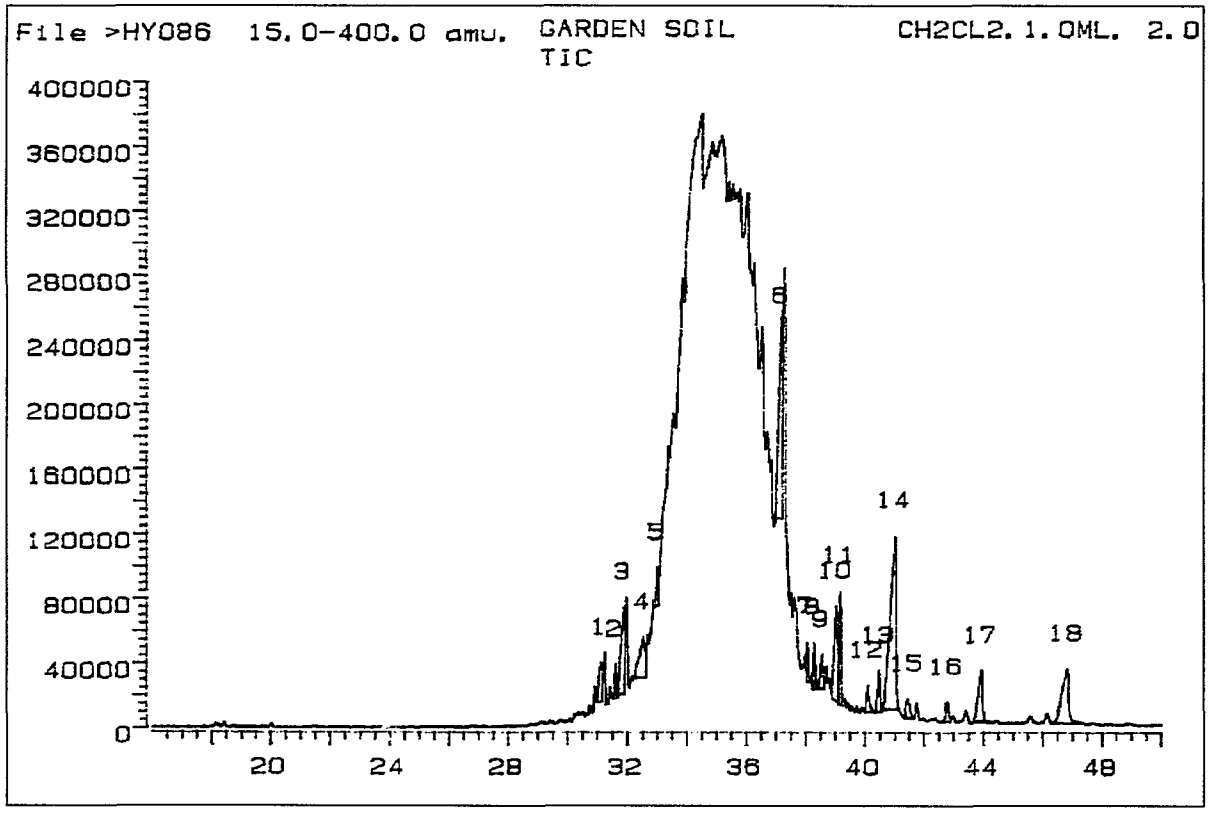


Figure 3.16 Total Ion Chromatogram of Garden Soil (CH_2Cl_2 fraction)

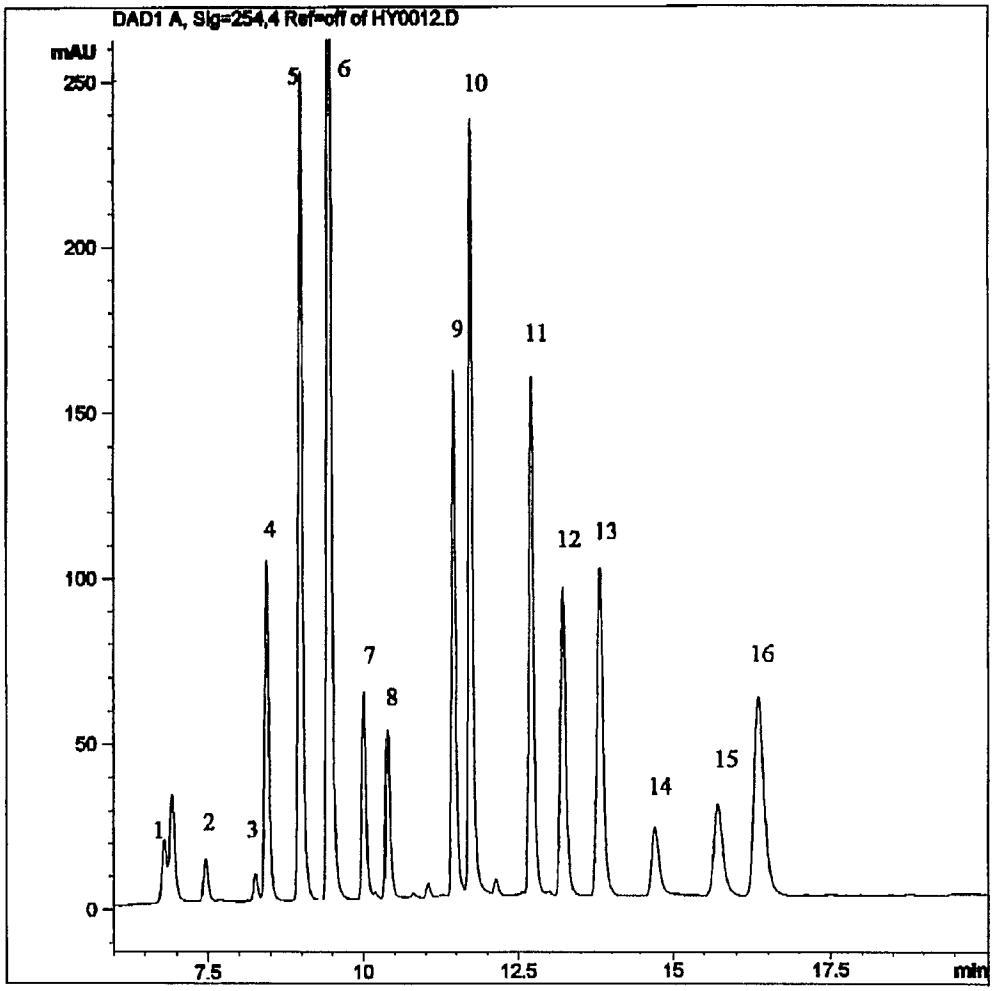


Figure 3.17 HPLC Chromatogram of PAH Standards

1. naphthalene 2. acenaphthylene 3. acenaphthene 4. fluorene
5. phenanthrene 6. anthracene 7. fluoranthene 8. pyrene
9. benz(a)anthracene 10. chrysene
11. benzo(b)fluoranthene 12. benzo(k)fluoranthene
13. benzo(a)pyrene 14. dibenzo(a,h)anthracene
15. benzo(ghi)perylene 16. indeno(1,2,3-cd)pyrene

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