Analysis of Organic Pollutants by Micro Scale Liquid-Liquid Extraction and On-column Large Volume Injection Gas Chromatography

by

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Abstract:

The analysis of organic pollutants in water is traditionally done following EPA procedures which commonly use liquid-liquid extraction. One liter of water is extracted three times with 60 mL of an organic solvent. The extract is concentrated and analyzed by gas chromatography. This procedure is time consuming and can cause losses of semi-volatile components, in addition to requiring a relatively large amount of organic solvent (180 mL). By performing the extraction directly in a GC autosampler vial using one milliliter of sample water and one milliliter of organic solvent, then injecting a large volume (~150 μL) of the organic layer taken directly from the vial by an autosampler, the same analysis can be done simpler, quicker, and with much less organic solvent (1 mL). This thesis describes this technique and the results obtained for semivolatiles, PAHs, pesticides, PCBs and phenols.

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1. Introduction

The presence of trace amounts of organic pollutants in water is both an environmental and a health concern. Those substances considered to pose a health risk are defined by the United States Environmental Protection Agency in its list of priority pollutants, which contains 113 compounds (1). These pollutants constitute a wide spectrum of substances including aliphatic and aromatic hydrocarbons, aldehydes, alcohols, phenols, chlorinated solvents, polychlorinated biphenyls (PCBs), pesticides, phthalates, metals, anions, and organometallic complexes. They reach the aquatic environment via mining and ore processing, fuel consumption, industrial processing, agricultural practices, urban runoff, etc.(1)

Federal regulations and standards for water quality are based on the water's intended use. Drinking water is obviously the most stringently controlled. Compounds are routinely monitored at levels from mg/L (ppm) down to ng/L (ppt) (2). The EPA 500 methods describe procedures for the analysis of drinking water while the EPA 600 series describes methods for the analysis of waste water. EPA methods categorize the pollutants into 13 classes and provide protocols for both their extraction and detection.

1.1 Water Analysis

The determination of organic pollutants in water consists of three steps: sample collection, sample preparation, and analysis. Of these steps, sample preparation is often the most complex and time consuming (2). Typically, pollutants require both extraction and concentration. Often, the extract requires additional cleanup since organic interferents are extracted along with the analytes of interest.

The traditional technique used for sample preparation in water analysis is liquid-liquid extraction (LLE). It can be performed either by shaking water samples with organic solvent or through a continuous liquid-liquid extractor. Both of these procedures use relatively large amounts of organic solvent, and are time consuming. In addition, it is usually necessary to concentrate the extract as it is often too dilute for direct analysis. The EPA prescribed concentration step requires the use of a Kuderna-Danish concentrator to evaporate the solvent (2). During the process, volatile and semi-volatile analytes may be lost along with the solvent (2).

After extraction, the most common analytical technique is gas chromatography. In fact, EPA methods 500 and 600 specifically cite gas chromatography as the recommended analysis technique for volatile and semi-volatile compounds (2). A small fraction (1-2 μL) of the extract is

injected into the gas chromatograph and the analytes are detected with either a flame ionization detector (FID) or mass spectrometer (MS).

1.2 Research objective

The sample preparation procedure as prescribed by the EPA exhibits the potential for both analyte loss and contamination. The official methods are both tedious and slow. As noted, the liquid-liquid extraction process uses a large amount of organic solvent. Although GC separation has become extensively automated, sample preparation has remained primarily a task of manual labor by skilled technicians. As a result, automated on-line extraction procedures which use only small amounts of solvent are very desirable.

In 1983, Fogelqvist and Larsson (3) injected a 250 μL pentane extract of sea water on-column for the temperature programmed GC analysis of low molecular weight halocarbons. Rather than follow the traditional liquid-liquid extraction procedure with concentration and analysis, they injected a large volume directly on-column to prevent the loss of low concentration volatiles. By doing so, they were able to detect low levels (0.8-3 μg/L) with good precision. To improve this process, microscale liquid-liquid extraction in autosampler vials with large volume on-column injection was proposed.

The limitation was expected to be based on the efficiency of the simple extraction process.

It was the objective of this work to demonstrate the feasibility of an integrated liquid-liquid extraction and GC analysis technique using cold on-column large volume injection. The technique investigated was to use one milliliter of sample water along with one milliliter of organic solvent placed into a gas chromatograph autosampler vial. The vial was shaken, and then a large volume (typically 150 μL) of the organic layer was injected into the GC. This technique has the advantage over the prescribed procedures because it is simple, automated, avoids sample loss and contamination, and uses only one milliliter of organic solvent.

2. Background

The method used for this work combines liquid-liquid extraction and large volume injection. Large volume injection is necessary since the sample is directly injected from a simple one step liquid-liquid extraction without prior concentration. Without it, the sensitivity required for water analysis would not be achieved.

2.1 Liquid-Liquid Extraction

The liquid-liquid extraction sample preparation procedure is based on analyte equilibrium between the aqueous and organic phases as controlled by the distribution coefficient. When the distribution coefficient is favorable towards the organic solvent, the extraction from water can be simple, rapid, and quantitative (4). To achieve high extraction efficiencies multi-step extractions are generally performed. The EPA prescribes three 60 mL extractions of a one liter water sample.

Traditionally, sensitivity is provided by extracting from a large sample of water since it contains a larger amount of analyte. Hence the use of one liter of water sample in official EPA procedures. The method developed in our laboratory used only one milliliter of water and only one extraction. This simple procedure allows integration of extraction and analysis into a

continous process, but requires a large volume injection to achieve the necessary sensitivity.

In our proposed procedure, equal volumes of organic and water phase are used, and 15% of the total organic extract is injected into the GC. In the official EPA procedure 1 liter of water is extracted three times with 60 mL of organic solvent. The extract is concentrated to 1 mL and only 1 μL injected. Figure 1 compares three extraction procedures, showing the proposed method to be the simplest and the most efficient.

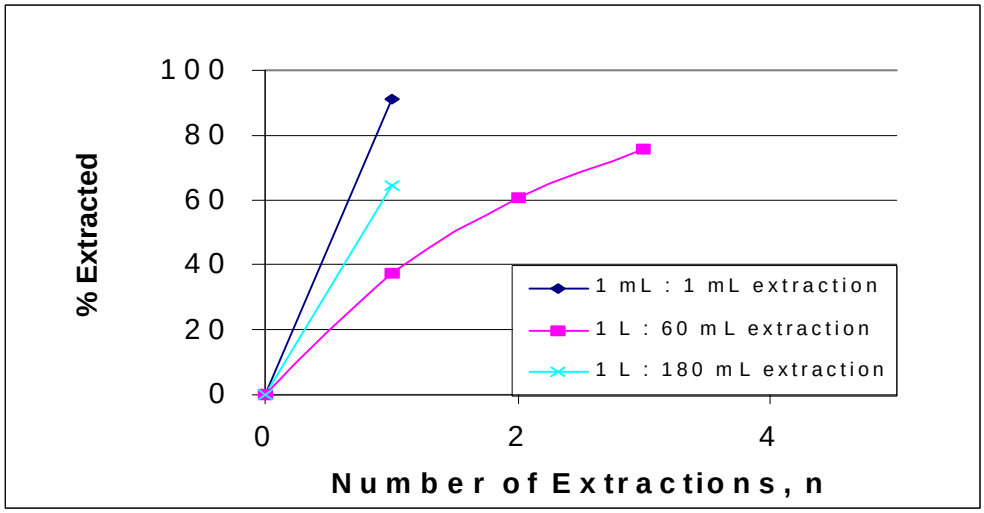

Figure 1 – Comparison of EPA and Microscale Extractions
(assumes a distribution coefficient of 10)

Assume a water sample containing 1 microgram of analyte per liter (1 ppb), and a distribution coefficient of 10/1, organic/aqueous phase. The weight of analyte injected into the GC can then be predicted for the EPA and proposed procedures. As shown in Appendix A, the EPA procedure will introduce 0.756 pg of analyte into the GC. The in-vial extraction procedure

will introduce 0.14 pg as calculated in Appendix B. The two procedures studied here inject roughly the same amount of analyte, with the EPA extraction injecting approximately 5 times more analyte.

2.2 Sample Introduction

In order to achieve the sensitivity required in this analysis, a 150 μL sample was introduced into the GC. There are two methods available for large volume GC injection: (1) programmed temperature vaporizing (PTV) injectors (5); and (2) cold on-column injection systems (6). The PTV is a modification of the conventional split/splitless injector while cold on-column is a modification of on-column injection.

2.2.1 Split/Splitless injectors

The traditional method of sample introduction in gas chromatography is through a split/splitless type injector. In split injection, a small sample (typically 1 μL) is introduced into a hot (250°C) injector by a syringe. The sample is flash vaporized in a glass liner and only a fraction is taken into the chromatographic column. Most of the sample leaves the GC through the split vent to prevent overloading the column. The total amount of sample which reaches the detector is typically 1-2% of 1 μL.

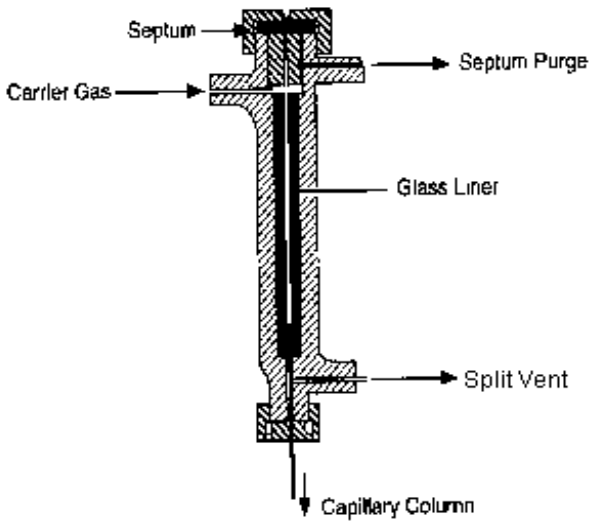

Figure 2 – Split/Splitless Injector Crossection (reprinted from 7)

In the splitless mode, the same injector is used but the sample is injected without any splitting. The chromatographic column must be at a cool temperature which allows the sample solvent to condense after entering the column. The split vent is opened after a short period of time (~45 sec) to flush out any sample remaining in the liner. The column is then slowly heated so only the volatile solvent is evaporated. The solvent vaporizes, beginning from the inlet side, and the analytes are condensed into a concentrated band in the remaining solvent. As the column temperature increases, the analytes are themselves vaporized and carried through the column. This was termed the "solvent effect" and was first described by Grob (8) in 1974. This mode is used for trace analysis when column overloading is not a problem and high sensitivity is required.

2.2.2 Sample Introduction – On-Column Injectors

The technique of placing the sample directly in the chromatographic column was introduced in the early stages of gas chromatography. However, successful on-column injection into capillary columns was not achieved until 1977 when Schoemburg *et al* (9) accomplished on-column injection into standard glass capillaries. On-column type injectors operate by inserting the syringe needle directly into a wide diameter column.

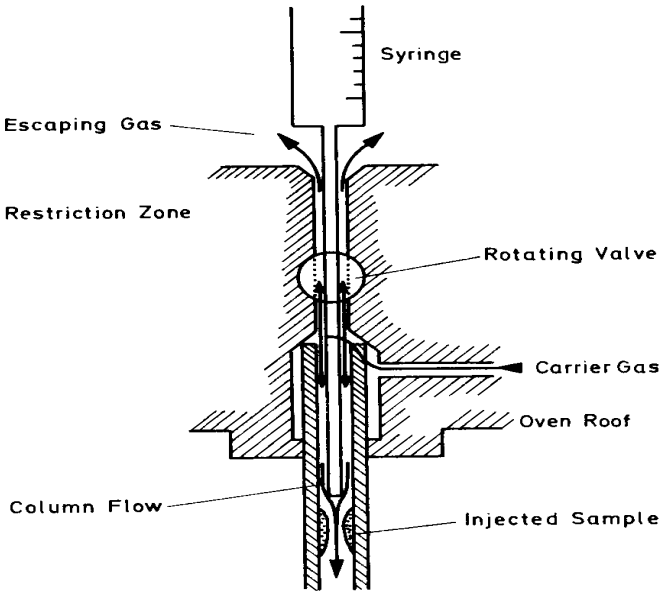

Figure 3 – On-column Injector Crossection (reprinted from 10)

On-column sample introduction is typically done with the column "cold" as in splitless injection. The column is initially at a temperature close to the boiling point of the sample solvent. The sample is slowly injected as a

liquid into the column, forming a liquid flooded zone. The column is then temperature programmed to achieve a "solvent effect". On-column injection is a high sensitivity sample introduction technique since all of the injected sample reaches the detector.

2.2.3 Sample Introduction – Large Volume Injection

In split/splitless injection and on-column injection, sample volumes of 1-5 μL are typically introduced. By injecting a larger volume of sample, sensitivity can be improved. In 1979 Vogt *et al* (11) constructed a new type of injector, taking advantage of the difference in boiling points between the solvent and analytes. They accomplished analysis with a modified split/splitless injector by using a liner packed with silica gel and the injector temperature cooled close to the solvent boiling point. The sample was injected into and retained in the liner. The solvent was evaporated with a high split flow through the split vent. The split vent was then closed and the injector was rapidly heated to vaporize the remaining material. This technique was later called programmed temperature vaporizing (PTV) injection, and is now commonly used. The application range of this technique is limited by co-evaporization of volatile analytes with the vented solvent (12).

In 1984 Zlatkis *et al* (13) used an on-column technique in which they retained the volatiles with a two column setup and cold trap. The cold trap was necessary to prevent what Grob (14) had previously described as “band broadening in space”. It is a peak distortion problem which occurs in both on-column injection and splitless injection. If the solvent is not strong enough to retain the analytes or analyte-stationary phase interaction in the liquid flooded zone is too strong, the analytes will remain spread out over a large section of column. Thus, a proper solvent effect is not achieved and band broadening occurs.

Grob proposed the “retention gap” as a method for eliminating this peak distortion. A “retention gap” is a short (5 to 10 m) capillary tube that is uncoated and deactivated. It is placed between the injector and regular capillary column. The retention gap must provide minimal retention to allow easy sample migration and reconcentration by the solvent effect (14). The retention gap must contain the entire liquid flooded zone of the column. Components are reconcentrated at the edge of the stationary phase in the standard capillary column. Such a procedure is necessary for less volatile analytes, because they do not easily migrate with the solvent and are often left spread throughout the initial liquid flooded zone. This results in poor chromatography.

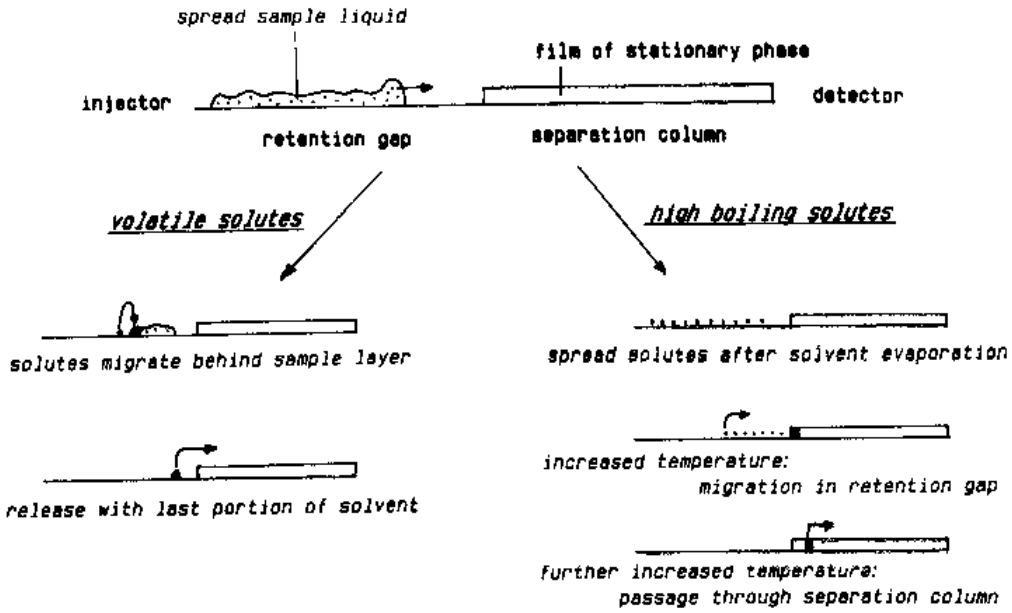

Figure 4 – Solvent Effect in a Retention Gap (reprinted from 10)

This retention gap technology has subsequently been exploited for the use of large volume, cold on-column injection. Grob *et al* (12) were the first to describe this procedure in 1985. A large internal diameter (0.53 mm ID), 40 meter long capillary column was deactivated for use as the retention gap. A 15 meter portion of that column was coated with liquid stationary phase. The specially prepared column was connected to a standard capillary column.

Grob *et al* (12) were able to analyze 100 µL samples without band broadening or loss of volatiles. To further advance this technique a T-connection was used to replace the joint between the retention gap and separation column. The T was connected to a solvent vent exit (SVE).

Now, a large portion of solvent was vented out of the instrument producing a much smaller solvent peak. By optimizing the solvent vent time, all of the analytes were retained.

Figure 5 shows a schematic of the Carlo Erba large volume injection system, which was based on Grob's work and used in our analysis. The primary components for cold on-column large volume injection are the desolvation precolumn, T-connection, and solvent vent exit (SVE). Their configuration is represented in Figure 5.

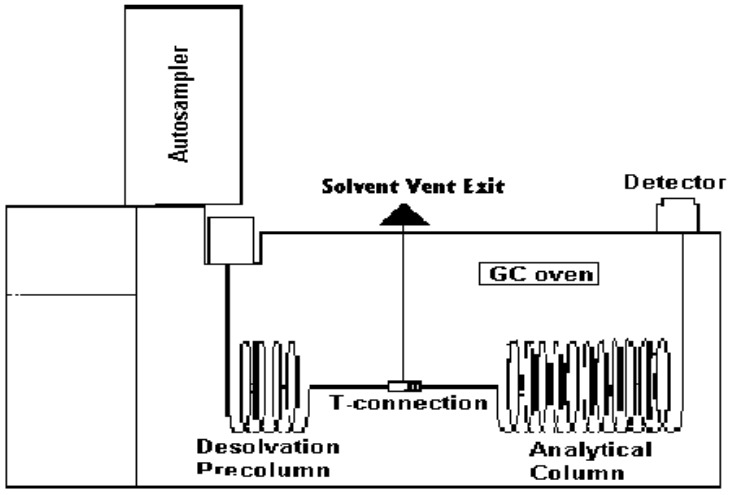

Figure 5 – Schematic of Carlo Erba Large Volume Injection System

3. Experimental

The objective of this work was to determine the advantages (if any) of the in-vial extraction procedure for typical organic pollutants found in water at routinely monitored concentrations. Hexane was selected as the extraction solvent because of its GC compatibility and low miscibility with water. HPLC grade water was spiked with standard compounds in acetone and the extract compared with the same standards in hexane.

3.1 Instrumental Conditions

The instrument used was a Carlo Erba (Milano, Italy) model 8000 gas chromatograph with Chromcard software, a flame ionization detector, and CS model 800 autosampler. A cold on-column large volume injection system was used with a 250 μL syringe and accompanying software. The flame ionization detector was supported by a Packard Instruments (Downers Grove, IL) model 9200 hydrogen generator, and GC grade compressed air. The FID was maintained at 300°C.

The desolvation precolumn used was a Mega (Milano, Italy) 15 m x 0.53 mm ID column with the first 12 m deactivated and uncoated, and the remaining 3 meters coated with SE-54 (95% Dimethylpolysiloxane, 5% Phenyl) stationary phase, 0.45 μm film thickness. The precolumn was

connected to the analytical column by a steel T-connection. The analytical column was a Mega (Milano, Italy) SE-54, 25 m x 0.32 mm ID x 0.25 μm film thickness column. The GC conditions are summarized in Table 1.

Table 1 –GC Analysis Conditions	
Solvent	n-hexane
Injection volume	150 μL
Injection speed	5 μL/sec
Vented solvent	142 μL
Solvent delay time	21 sec
Secondary cooling	70 sec
Oven program	60°C for 0.85 min, 10°C/min to 300°C, hold 5 min.
Head pressure	100 kPa

The extractions were performed by putting one milliliter of spiked water in a standard 2.5 mL Chromacole (Milano, Italy) autosampler vial followed by one milliliter of hexane. The vial was capped, shaken for 30 seconds and placed in the autosampler tray. The depth of the autosampler needle was adjusted to sample only the organic layer.

Standards were initially diluted in GC grade hexane. They were then further diluted either in hexane or acetone. HPLC grade water was spiked with the compounds dissolved in acetone. Standards were run in triplicate and extractions were analyzed six times with one injection made from each vial. A solvent blank and extraction blank were included with each sequence.

3.2.1 Preparation of Test Samples - Semivolatiles

Six test compounds were initially selected: 2,4-dinitrotoluene, naphthalene, di-isooctylphthalate, 1,2-dichlorobenzene, acenaphthene, and fluoranthene. These were individually purchased from Supelco (Bellefonte, PA). Standards were prepared as a mixture in hexane at approximately 10, 20, 30, 40, 50, 70, and 80 ppb. An acetone solution was used for spiking water at three concentrations with the six test compounds: 20 ppb, 40 ppb, and 50 ppb.

A 44 compound base/neutral extractable mixture containing the six compounds in methylene chloride was purchased from Hewlett-Packard (Wilmington, DE). The composition is listed in Appendix C. This mixture was divided into two solutions; one in hexane and the other in acetone for water spiking. These solutions were analyzed at 100 ppb levels. The temperature programming rate was altered to 6°C/min.

To improve reproducibility tetradecane was explored as an internal standard. The stock solution was prepared by dilution in hexane. This was then added to the standard solutions.

3.2.2 Preparation of Test Samples - PAH Mixture

A sixteen component polynuclear aromatic mixture (catalog #8500-6035) in acetonitrile was purchased from Hewlett-Packard (Wilmington,

DE). This was diluted two fold in hexane; first to 10 ppm and then to 20 and 40 ppb. Water was spiked with the PAH solution in acetone. The GC oven temperature program was altered; 60°C to 300°C at 6°C/min. The mixture contained acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, benzo(a)pyrene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

3.2.3 Preparation of Test Samples - Pesticide Mixture

Four pesticides were selected: lindane®, diazinon®, chlorpyrifos®, methoxychlor®. These were purchased from Sigma-Aldrich (Milwaukee, WI) in solid form. They were first individually dissolved in hexane and then diluted as a mixture in acetone. The extraction was performed on water spiked at 80 ppb with the pesticide solution in acetone.

3.2.4 Preparation of Test Samples - PCB Mixture

A polychlorinated biphenyl mixture was purchased from Supelco (Bellefonte, PA) containing eight PCBs in hexane: 2-chlorobiphenyl, 2,3-dichlorobiphenyl, 2,4,5-trichlorobiphenyl, 2,2’,4,4’-tetrachlorobiphenyl, 2,2’,3’,4,6-pentachlorobiphenyl, 2,2’,4,4’,5,6’-hexachlorobiphenyl, 2,2’,3,3’,4,4’,6-heptachlorobiphenyl, 2,2’,3,3’,4,5’,6,6’-octachlorobiphenyl.

This mixture was diluted in hexane and acetone twice to achieve solutions of 80 ppb.

The extraction was also performed by adding 200 mg of salt (KCl) to the water in an attempt to improve recoveries. Methylene chloride was also tried as an extraction solvent.

3.2.5 Preparation of Test Samples - Phenol Mixture

A phenol mixture (catalog #4-8904) containing 11 phenols in methylene chloride was purchased from Supelco (Bellefonte, PA). It was diluted to 10 ppm and then to 200 ppb in hexane. It was analyzed in the large volume injection mode and by manually injecting 1 μL on-column with the large volume injection system offline. No extractions were performed on this mixture. The mixture contained phenol, 2-chlorophenol, 2-nitrophenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 4-chloro-3-methyphenol, 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol.

4. Results and Discussion

The primary focus of this work was the trace analysis of semivolatile compounds in water. This topic was chosen for several reasons. First, the semivolatiles have the greatest chance of being lost during the concentration step in the official EPA procedures. They also constitute a large spectrum of the toxic organic compounds routinely analyzed by GC. Additionally, the EPA extraction procedure for semivolatiles does not require any special steps, such as derivatization or salting out and is a "simple" extraction. Once the technique was proven with semivolatiles, it was expanded to include PAH's, pesticides, PCB's, and phenols.

The EPA considers 30% relative standard deviation and 0.99 R^2 values acceptable for these trace levels (1). R^2 values describe how well a linear regression curve fits the experimental data. The extraction technique was evaluated with these standards in mind.

4.1 Contamination

When injecting a large volume (150 μL), solvent impurities are significantly magnified. As a result it is difficult to obtain "clean" solvent. After the analysis of several hexane grades, it was decided that a pure solvent would not be attainable. The analysis conditions were adjusted to compensate for the contamination by lengthening the initial isothermal hold

time to allow for better separation of the analytes from the solvent. Figure 6 is a chromatogram of the hexane used in the analysis and demonstrates its contamination. The contamination is believed to be higher molecular weight hydrocarbons, such as heptanes, octanes and nonanes, but it was not studied in this analysis.

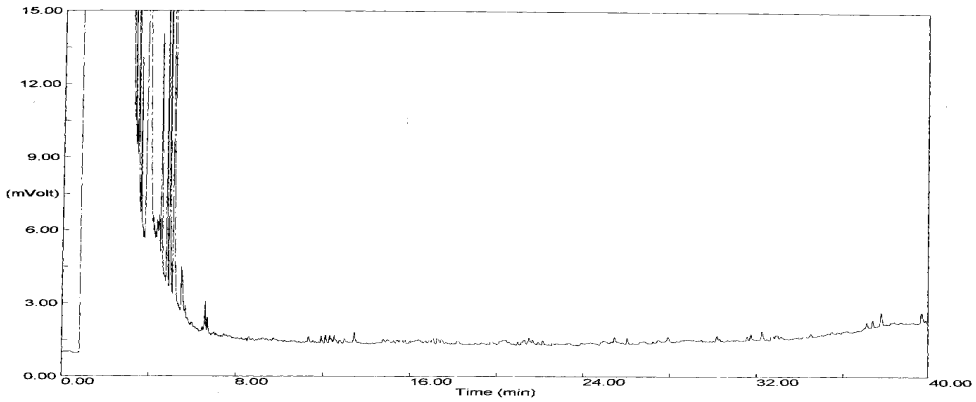

Figure 6 – Hexane Blank

To ensure that the water used for spiking was “clean”, an extraction was performed on the pure water using the same conditions as used for the true samples. Figure 7 is the chromatogram of the blank water extraction.

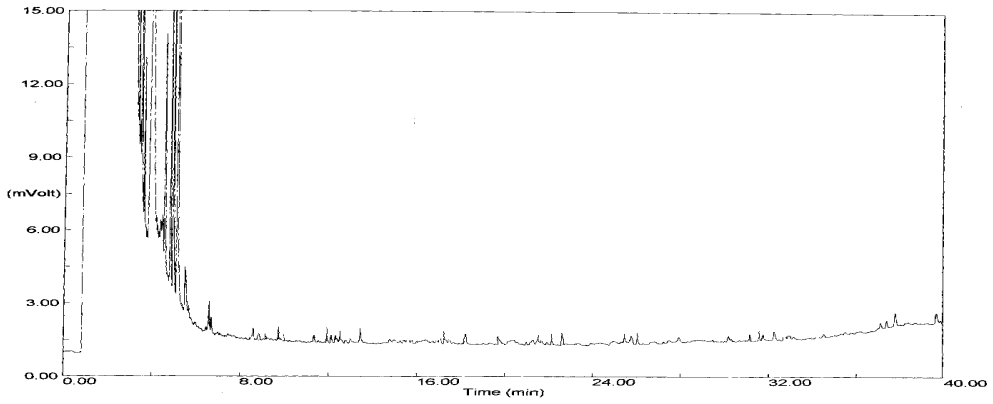

Figure 7 – Blank Water Extraction

A second problem resulted from sample contamination after multiple injections from the same vial. A series of unknown peaks appeared as indicated in Figure 8. With each consecutive injection, the response increased. The solution was analyzed using mass spectroscopy (MS), however the solution was too dilute for identification of the contaminate peaks. Concentration during the large volume injection process made the contamination quickly apparent in relation to the low level of analytes. The problem was resolved by making one injection per vial when working at low concentrations (ppb levels).

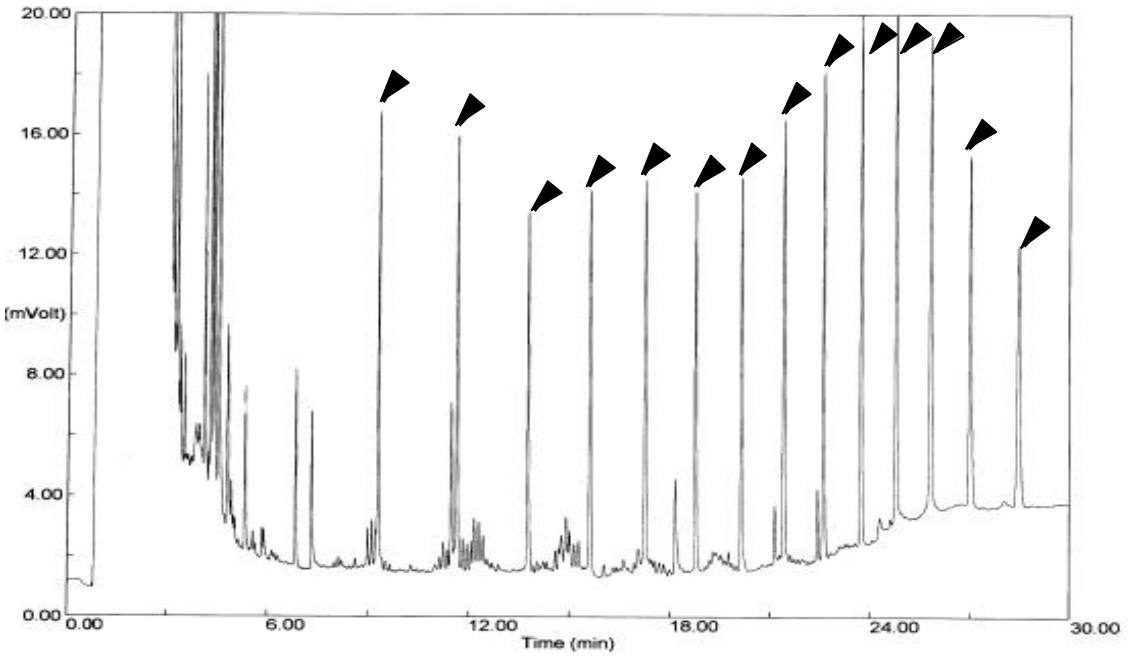

Figure 8 –Contamination of Semivolatile Solution – GC/FID
(three injections – arrows indicate contamination)

4.2.1 Semivolatile Analysis - Standards

The six component semivolatile standard was analyzed three times at each concentration, covering the range of 10 to 80 ppb. Figure 9 is an example standard chromatogram.

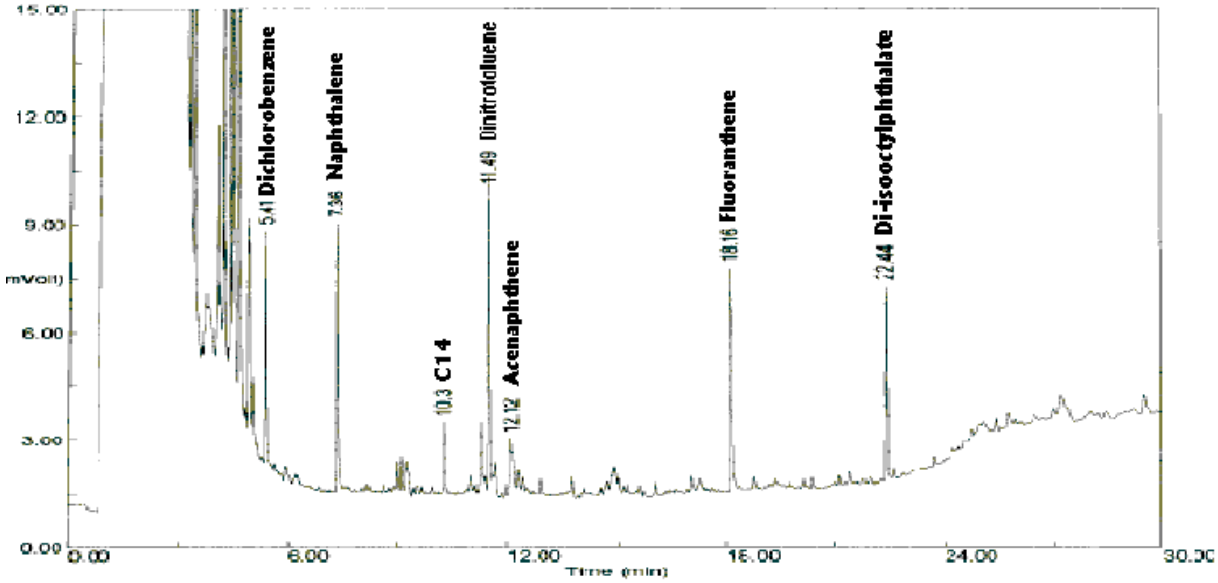

Figure 9 - Semivolatile Standard – GC/FID (40 ppb)

The detector response was graphed versus actual concentration and a calibration curve was produced for each analyte based on average peak area for the three runs. Good linearity was found for each analyte based on R^2 values for the curve fit. The same analysis was performed using tetradecane as an internal standard. No significant improvement was found by using an internal standard (See Table 2). As a result, the external standard technique, which is simpler, was used for the remainder of the analysis.

Table 2 –Linearity Comparison (R^2 values)		
Compound	External Standard	Internal Standard
1,2-dichlorobenzene	0.9964	0.9953
naphthalene	0.9931	0.9973
2,6-dinitrotoluene	0.9927	0.9912
Acenaphthene	0.9958	0.9726
Fluoranthene	0.9919	0.9994
Di-isooctylphthalate	0.9912	0.9983

A student T-test performed on the data using the 99% confidence interval concluded that there is no significant difference between the external and internal standard methods for these six compounds.

4.2.2 Semivolatile Analysis – Extractions

The extractions were performed six times at 20, 40, and 50 ppb levels. A set of extractions was also performed on the 44 compound base/neutral mixture which contained the six analytes at 100 ppb. Recoveries were good (see Table 3). Figure 10 is a representative chromatogram of the extraction. Di-isooctylphthalate had a secondary source contributing to its response and produced inaccurate results. Also 2,6-dinitrotoluene had problems with a co-eluting peak resulting from contamination.

Table 3 – Semivolatile Extraction Efficiencies (n=6)				
Compound	% Recovered ± σ			
	20 ppb	40 ppb	50 ppb	100 ppb
Dichlorobenzene	52.0 ± 2.3	60.6 ± 3.8	80.3 ± 0.7	83.2 ± 6.9
Naphthalene	65.1 ± 5.2	72.6 ± 2.2	84.4 ± 2.9	90.1 ± 6.2
Acenaphthene	62.5 ± 2.4	76.0 ± 8.1	81.7 ± 2.8	95.5 ± 4.9
Dinitrotoluene	---	151.2 ± 13.7	105.1 ± 9.5	100.1 ± 15.1
Fluoranthene	74.2 ± 8.4	78.2 ± 3.9	74.2 ± 5.3	85.6 ± 5.5
Di-isooctylphthalate	---	74.4 ± 4.5	26.2 ± 20.1	24.2 ± 3.0

Calibration curves were created for the analytes based on average peak area for the six extractions (See Figures 11-16). Good linearity was achieved for the four concentrations based on R^2 values from the calibration curve fits. Extraction efficiencies were calculated by comparison of average peak areas of the six extractions and three standards.

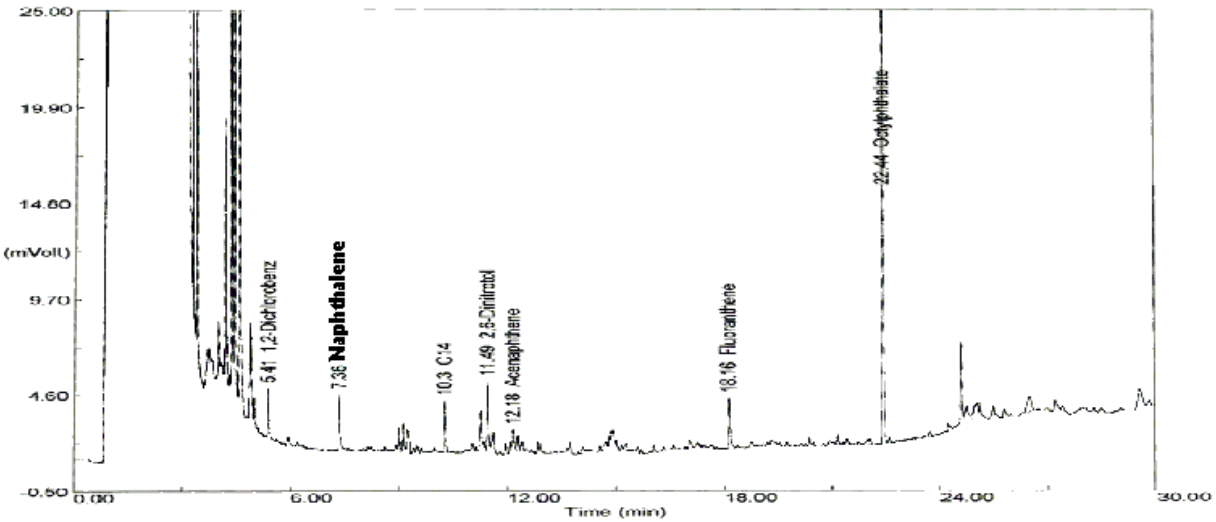

Figure 10 – Semivolatile Extraction Chromatogram (20 ppb)

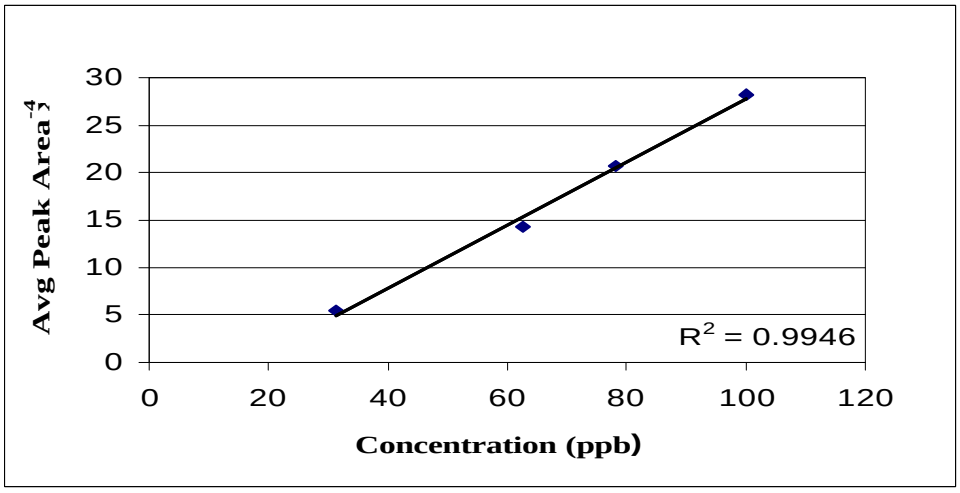

Figure 11 – 1,2-Dichlrorbenzene Calibration Curve

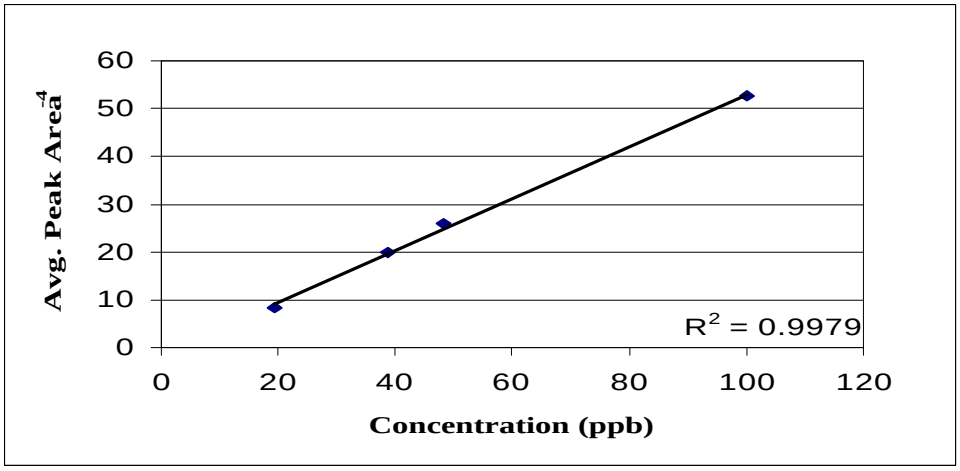

Figure 12 – Naphthalene Calibration Curve

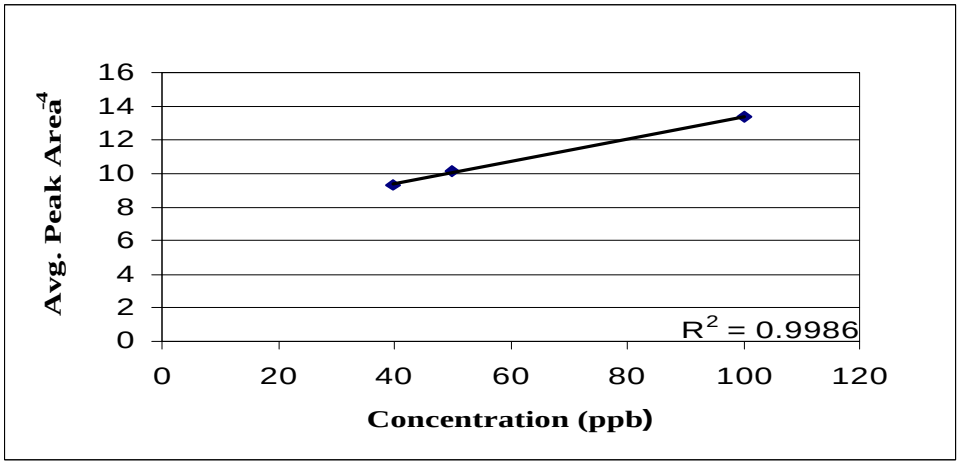

Figure 13 – 2,6-Dinitrotoluene Calibration Curve

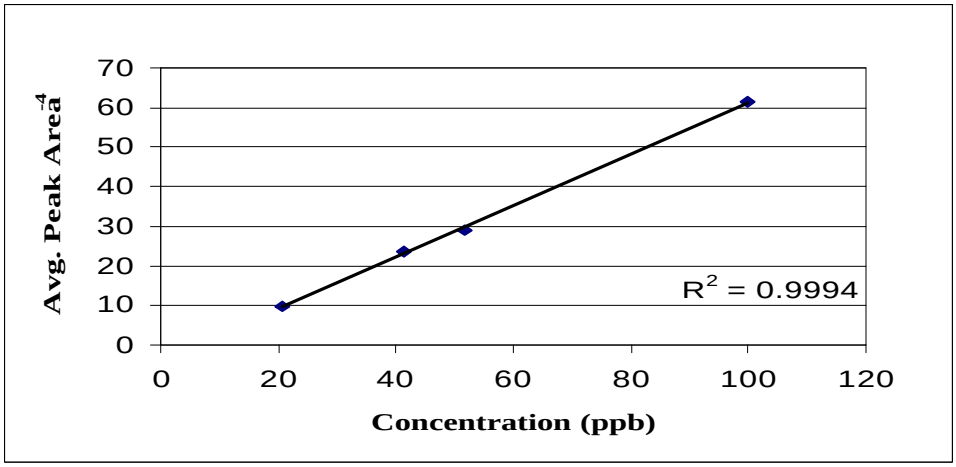

Figure 14 – Acenaphthene Calibration Curve

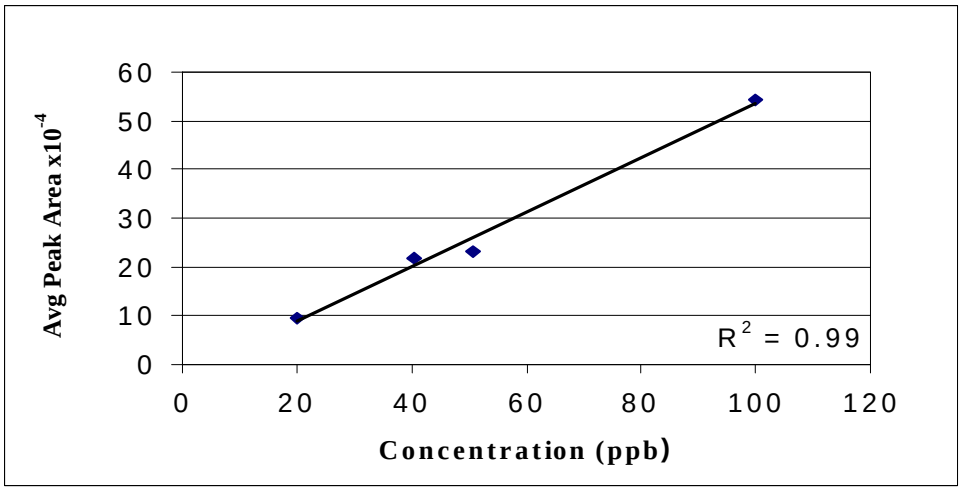

Figure 15 – Fluoranthene Calibration Curve

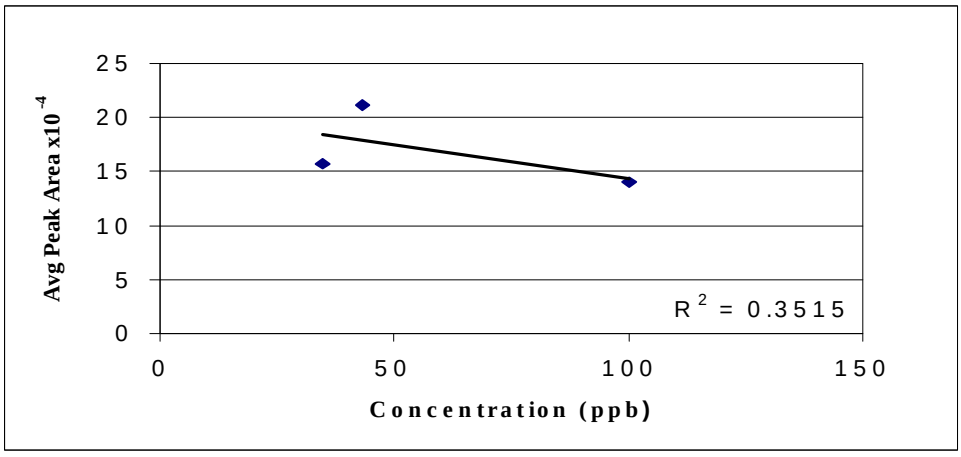

Figure 16 – Di-Isooctylphthalate Calibration Curve

4.3 PAH Analysis

The majority of compounds in the sixteen component polynuclear aromatic hydrocarbon mixture are considered semivolatiles. The purpose in analyzing this mixture was not only to extend the range of tested compounds but also to compare large volume injection systems. The same solution had been analyzed previously in our laboratory on a programmed temperature vaporizing (PTV) injector for an earlier thesis.

4.3.1 PAH Analysis - Recovery

Eight of the compounds were selected for analysis because of their high resolution. The extraction was performed at 40 ppb and provided high efficiencies and good reproducibility (comparable with those of the previous analysis). However, benzoperylene yielded poor results due in part to coelution with another compound.

Table 4 – PAH Recovery Data (n=6)

Compound	% Recovery ± σ
Napthalene	70.2 ± 2.3
Acenaphthylene	80.1 ± 2.4
Acenapthene	77.5 ± 3.7
Fluorene	82.1 ± 3.4
Flouranthene	71.9 ± 2.1
Pyrene	69.7 ± 4.3
Benzo(a)pyrene	75.2 ± 7.1
Benzoperylene	50.2 ± 11.9

4.3.2 PAH Analysis – Large Volume Injection

One of the advantages of the cold on-column injection technique is the capability of analyzing compounds that are close in boiling point to the extraction solvent. Figure 17 compares PTV and cold on-column large volume injection. Of particular interest is the first peak, napthalene. In the PTV analysis (Fig 17b), napthalene coelutes with the solvent and is partially vented. In the cold on-column technique (Fig 17a) the peak is resolved from the solvent and completely recovered, allowing sensitive, quantitative analysis.

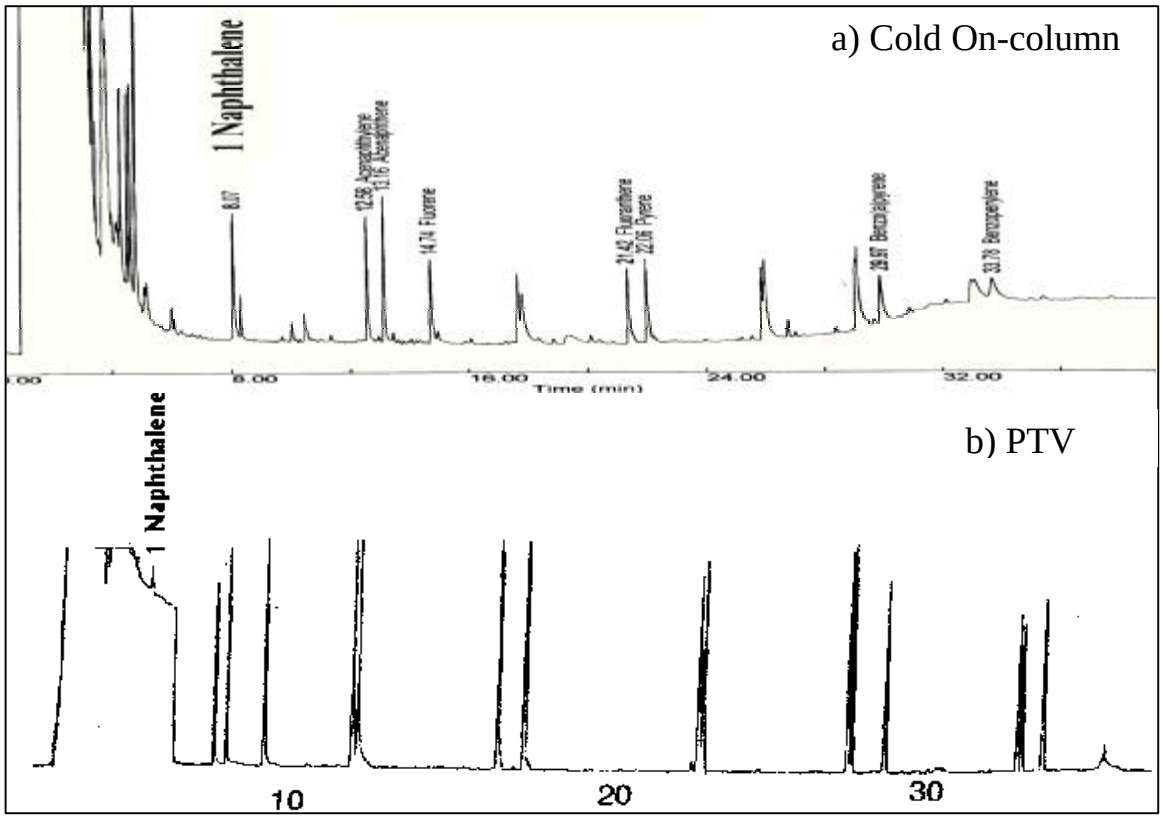

Figure 17 – PAH Analysis (a) Cold On-column Large Volume Injection of Extract – 40 ppb (b) PTV Injection – 0.4 ppm (compliments of Dr. Y. Wang)

4.4 Pesticide Analysis

The pesticide extraction was performed at 80 ppb six times and provided high efficiencies and good reproducibility. The extraction data is summarized in Table 6 and Figure 18 is a typical chromatogram.

Table 6 – Pesticide Recovery Data (n=6)	
Compound	% Recovery ± σ
Lindane®	91.7 ± 11.9
Diazinon®	89.2 ± 0.7
Chlorpyrifos®	79.4 ± 2.1
Methoxychlor®	79.6 ± 4.2

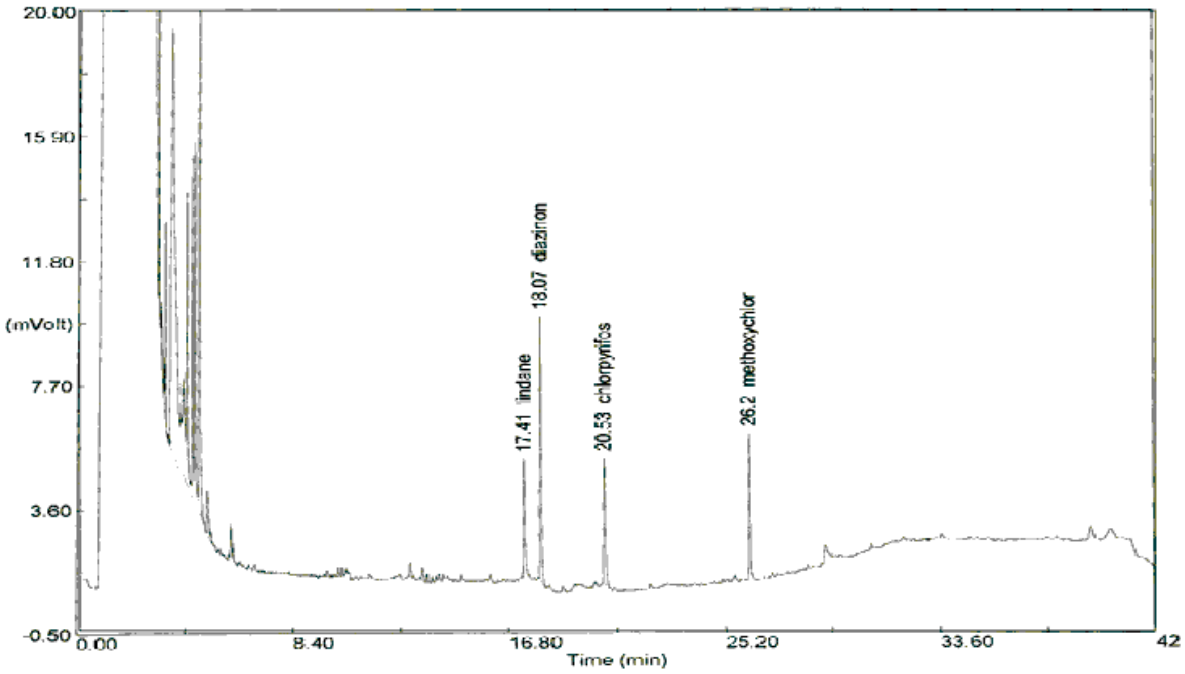

Figure 18 – In Vial Extraction and Large Volume Injection of Pesticide Solution (80 ppb extraction)

4.5 PCB Analysis

The PCB solution was analyzed at the 80 ppb level. The extraction had poor recoveries ranging from 61% to 16%. The solutions were remade and analyzed again. Recoveries were similar. Salt (200 mg of KCl) was added to the extraction vial, but had no effect on improving recoveries. Methylene chloride was tried as an extraction solvent without a significant improvement in recoveries. The results are summarized in Table 7. Figure 19 is an example of the extraction using hexane as the extraction solvent. At this time no reason for the poor results is known but adsorption on the glassware may account for some of the losses.

Table 7 – PCB Recovery Data (n=6)

Compound	% Recovered		
	Hexane	Salt	Methylene Chloride
Chlorobiphenyl	61	62	70
Dichlorobiphenyl	40	52	59
Trichlorobiphenyl	26	37	37
Tetrachlorobiphenyl	26	25	29
Pentachlorobiphenyl	24	19	25
Hexachlorobiphenyl	21	18	23
Heptachlorobiphenyl	16	73	68
Octachlorobiphenyl	20	126	108

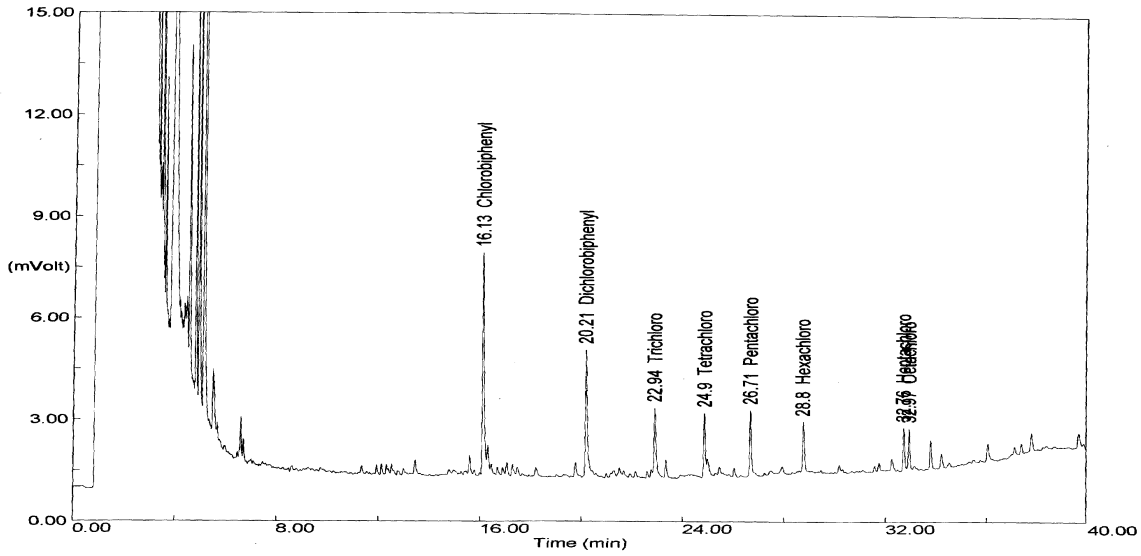

Figure 19– PCB Extraction Chromatogram (hexane extraction solvent)

4.6 Phenol Analysis

The phenol standard was analyzed at the 200 ppb level. The chromatogram had no recognizable peaks (see Figure 20). The solution was remade and produced the same results.

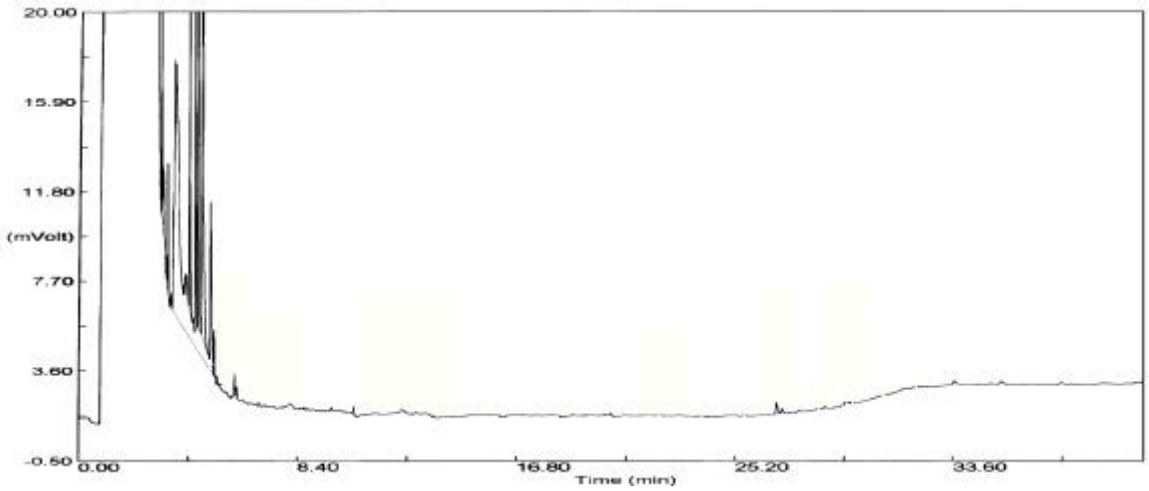

Figure 20 – Phenol Standard (200 ppb)

The 10 ppm standard was analyzed with an identical analytical column on an HP6890 with a model 5973 mass spectrometer. This analysis provided identification of nine of the eleven phenols (see Figure 21). The large volume injection system was disengaged on the Carlo Erba system, and the 10 ppm standard was analyzed in on-column mode. No peaks were distinguishable (see Figure 22).

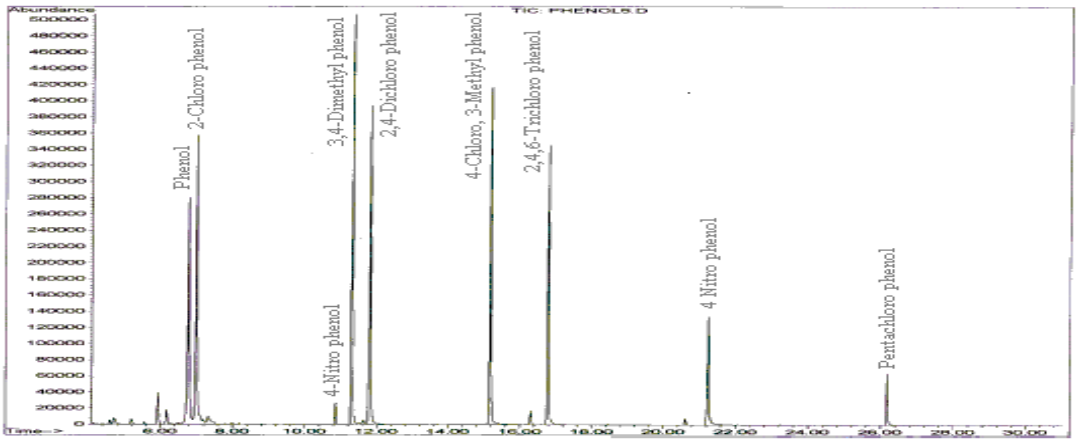

Figure 21 – Phenol Standard on HP 5973 MS
(10 ppm, 1 µL injection, splitless)

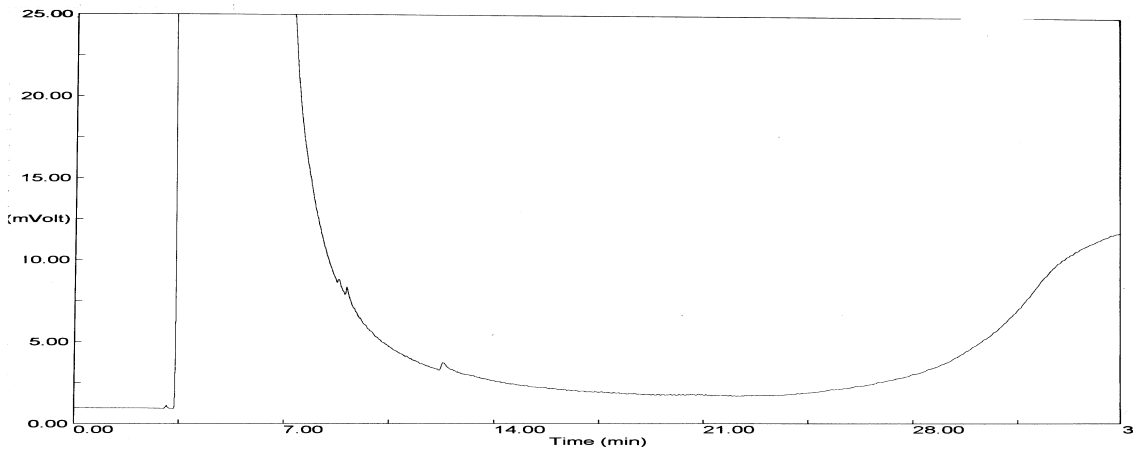

Figure 22 – Phenol Standard on Carlo Erba System
(10 ppm, 1 µL injection, on-column)

4.7 Precolumn Adsorption

The phenol analysis indicates that irreversible adsorption was occurring in the Carlo Erba system. This appears to result from activation of the precolumn. Fused silica columns are sensitive to moisture and are externally coated with polyimide to protect them from the atmosphere. However, the precolumn, which has no stationary phase, has no internal protection. Since the samples are in contact with the water layer in the vial before injection, each injection introduces a small portion of water. This water may cause activation of the precolumn by hydrolysis of inert siloxane bridges to active silanol groups.

Water sensitivity was one of the reasons for selecting hexane as the extraction solvent. However, this level of activation was not predicted when the analysis began. It is believed that the use of methylene chloride promoted reactivation. It is recommended that the precolumn activity be monitored with a test mixture after installation and that the precolumn be replaced before significant activation occurs**.**

4.7.1 Precolumn Adsorption - Semivolatile Analysis

The extent of precolumn activation depends on the particular analytes and solvents under investigation. The phenols were analyzed last, when the

precolumn was most active. They were completely adsorbed. The semivolatiles were the first mixture to be evaluated.

Although the semivolatile extractions provided good linearity for most of the compounds, as analyte concentration decreased, percent recovery decreased. This occurred in a linear fashion for dichlorobenzene, naphthalene, acenaphthene, and fluoranthene as shown in Figures 23-26. It is believed this partially resulted from loss of analyte through adsorption in the precolumn. The low concentration analytes were affected more since a larger percentage was lost for them. However, losses may also have occurred by adsorption on glassware external to the GC system.

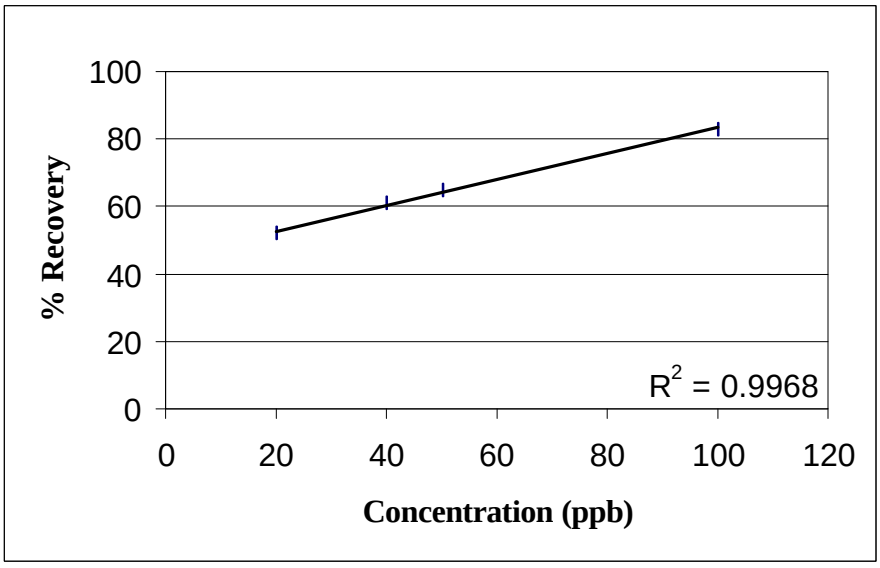

Figure 23 – 1,2-Dichlorobenzene Recovery Curve

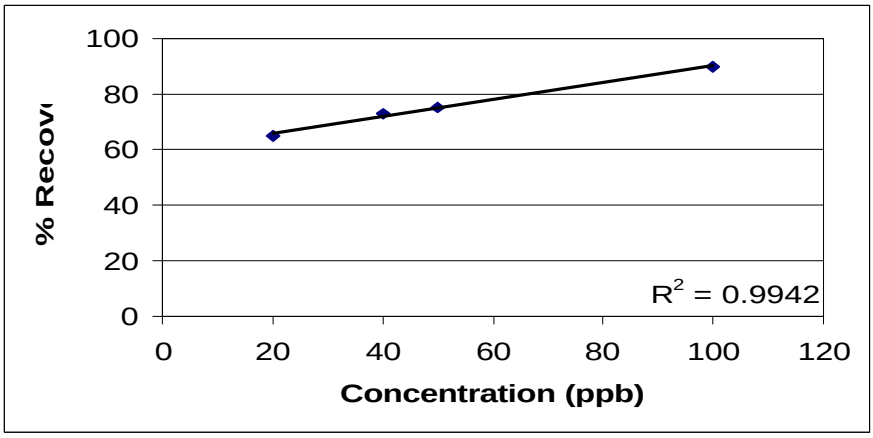

Figure 24 – Naphthalene Recovery Curve

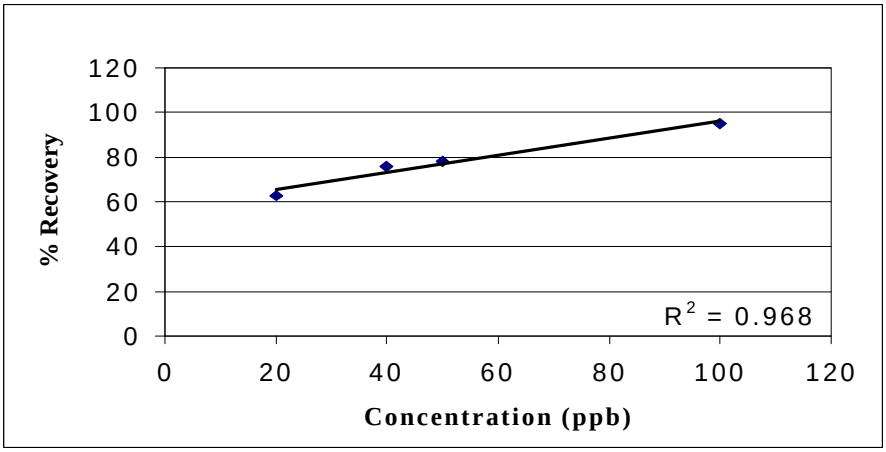

Figure 25 – Acenaphthene Recovery Curve

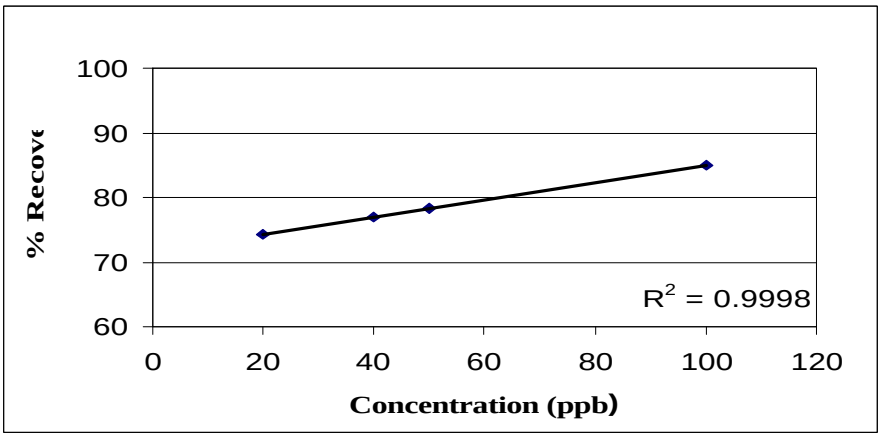

Figure 26 – Fluoranthene Recovery Curve

5. Conclusions

The in-vial extraction procedure provided high efficiency and good precision for the analysis of the semivolatile, PAH, and pesticide mixtures. The on-column large volume injection technique with retention gap allowed quantitative analysis for low boiling point analytes down to 20 ppb levels. Large volume injection allows for a 150 fold improvement in sensitivity and makes this integrated extraction, analysis procedure simple and accurate. However, there are significant limitations.

Contamination becomes a problem when working with low concentration analytes due to extraction and concentration of interferents. Extraction efficiency of the simple in-vial procedure also limits the range of analytes analyzable by the procedure. The PCB analysis was unsuccessful. Additionally, the influence of precolumn activation must be monitored. Complete or partial adsorption of analytes can occur, as is believed to have taken place in the phenol analysis.

The procedure is a promising technique, but requires a more in depth investigation into the effects of precolumn activation to be practical.

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Appendix A

Calculation of analyte weight injected into gas chromatograph for EPA extraction.

Assume: water sample containing 1 μg/L
distribution coefficient of 10/1 organic/aqueous phase
EPA procedure: three 60 mL extractions from 1 L water

First Extraction

1L water contains 1 μg of analyte
Total Analyte = 1 μg = Weight in Organic Phase +Weight in Aqueous Phase

$$Kd = 10 = \frac{\text{Concentration in Organic Phase}}{\text{Concentration in Aqueous Phase}}$$

$$10 = \frac{\text{Weight in Organic Phase /Volume Organic Phase}}{\text{Weight in Aqueous Phase/Volume Aqueous Phase}}$$

$$10 = \frac{\text{Wt org/60 mL}}{\text{Wt aq/1000 mL}} \quad \Rightarrow \quad 0.6 \text{ x Wt aq} = \text{Wt org}$$

Substitute: 1 μg = 0.6 x Wt aq + Wt aq
Wt aq = 0.625 μg
Wt org = 0.375 μg

Second Extraction

Total Analyte left in Water = 0.625 μg
0.625 μg = Wt in Org Phase + Wt Aq Phase

$$10 = \frac{\text{Wt org/60 mL}}{\text{Wt aq/1000 mL}} \quad \Rightarrow \quad 0.6 \text{ x Wt aq} = \text{Wt org}$$

0.625 μg = 0.6 x Wt aq + Wt aq
Wt aq = 0.391 μg
Wt org = 0.234 μg

Third Extraction

Total Analyte left in Water = 0.391 μg

0.391 μg = Wt in Org Phase + Wt Aq Phase

$$10 = \frac{\text{Wt org/60 mL}}{\text{Wt aq/1000 mL}} \quad \Rightarrow \quad 0.6 \times \text{Wt aq} = \text{Wt org}$$

0.391 μg = 0.6xWt aq + Wt aq

Wt aq = 0.244 μg

Wt org = 0.147 μg

Total Extracted Weight = 0.375 + 0.234 + 0.147 = 0.756 μg

Concentrate 180 mL extract to 1 mL

Concentration of Extract = 0.756 μg/1 mL

Inject 1 μL into GC; this introduces **0.756 pg**

Appendix B

Calculation of analyte weight injected into gas chromatograph for large volume injection microscale extraction.

Assume: water sample containing 1 μg/L
distribution coefficient of 10/1 organic/aqueous phase
one 1 mL extraction from 1 mL water

1 mL water contains 1 pg of analyte
Total Analyte = 1 pg = Weight in Organic Phase + Weight in Aqueous Phase

$$Kd = 10 = \frac{\text{Concentration in Organic Phase}}{\text{Concentration in Aqueous Phase}}$$

$$10 = \frac{\text{Weight in Organic Phase /Volume Organic Phase}}{\text{Weight in Aqueous Phase/Volume Aqueous Phase}}$$

$$10 = \frac{\text{Wt org/1 mL}}{\text{Wt aq/1 mL}} \quad \Rightarrow \quad 10 \times \text{Wt aq} = \text{Wt org}$$

Substitute: $0.001\ \mu g = 10 \times \text{Wt aq} + \text{Wt aq}$
$\text{Wt aq} = 9.09 \times 10^{-5}\ \mu g$
$\text{Wt org} = 9.09 \times 10^{-4}\ \mu g$

Total Extracted Weight = 9.09×10^{-4} μg
Concentration of Extract = 9.09×10^{-4} μg/1 mL

Inject 150 μL into GC; this introduces **0.136 pg**

Appendix C

Base/Neutral Extractables Mixture in methylene chloride part # HP 8500-5998

Acenaphthene
Acenaphthylene
Anthracene
Azobenzene
Benzo(a)anthracene
Benzo(k)fluoranthene
Benzo(ghi)perylene
Benzo(a)pyrene
Bis(2-chloroethyl) ether
Bis(2-chloroisopropyl) ether
4-Bromophenyl phenyl ether
Butyl benzyl phthalate
2-Chloronapthalene
4-Chlorophenyl phenyl ether
Chrysene
Dibenz(a,h) anthracene
Di-n-butyl phthalate
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
Diethyl phthalate
Dimethyl phthalate
2,6-Dinitrotoluene
2,4-Dinitrotoluene
Di-isooctylphthalate
Fluoranthene
Fluorene
Hexachlorobenzene
Hexachlorobutadiene
Hexachlorocyclopentadiene
Hexanchlrorethane
Indeno(1,2,3-cd)pyrene
Isophorone
Naphthalene
Nitrobenzene
Benzo(b)fluoranthene
N-nitrosodimethylamine
N-nitrosodi-n-propylamine
N-nitrosodiphenylamine
Phenathrene
Pyrene
Bis(2-ethylhexl) phthalate
Bis(2-chloroethoxy) methane
1,2,4-Trichlorobenzene

Vita

Mark Schneider was born on October 5, 1971 to the German immigrant parents Elfriede and Manfred Schneider in Passaic New Jersey. He received his public schooling in Wayne NJ, after which he entered Virginia Tech. He graduated with an engineering degree in 1994. He reentered the university in 1997 to pursue an advanced degree in analytical chemistry. This work is one of several projects that he participated in during his time in the laboratory.