



Automated Liquid-Liquid Extraction of PAH Compounds in Water

Application Note

Environmental

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Abstract

Extraction of Poly Aromatic Hydrocarbon (PAH) compounds from water involves using a large volume of solvent. The advent of more sensitive Mass Spectrometers (MS) coupled with Large Volume Injection (LVI) onto the Gas Chromatograph (GC) has aided in better detection of PAH compounds. In consequence, micro-extraction of PAH compounds from water has become a viable solution for sample preparation. Micro-extraction reduces sample size, solvent usage, container and freight cost thus decreasing laboratory costs and sample preparation time. This application will investigate an automated liquid-liquid extraction technique for the preparation of PAH water samples.

Introduction:

In 2002, the United States Environmental Protection Agency (USEPA) published Method 3511, Organic Compounds in Water by Microextraction. This method is used for the extraction of semi-volatile and volatile compounds out of a water matrix. The method calls for a sample filled to volume in a 40ml vial, remove 5mls, and then add surrogate standard, 2mls of methylene chloride and sodium chloride. Next, the sample is shaken and centrifuged. Finally, the methylene chloride extract is removed and dried for sampling and analysis. This method was developed in order to limit solvent waste and streamline sample extraction.

In order to achieve the detection limits required for semi-volatile analysis using micro-extraction a Large Volume Injection (LVI) is required. During LVI a large amount of sample is introduced into the Gas Chromatograph (GC), the solvent is eliminated using Programmable Temperature Vaporization (PTV) and the analytes are transferred to the GC column for separation. The analysis of the analytes is then done by a detector.

The introduction of microextraction coupled with LVI has led many environmental labs to explore more efficient methods of extraction. Semi-volatile water samples are often very contaminated with Poly Aromatic Hydrocarbons (PAHs) and performing a full extraction only to have to make dilutions to the sample can be frustrating. This application will investigate an automated liquid-liquid microextraction technique using EST Analytical's FLEX Autosampling system.

Experimental:

For this application, a Titan XL Large Volume Injection (LVI) port was installed into an Agilent 7890GC. Analyte separation was done using a Restek Rxi-5 Sil MS 30m x 250mm x 0.25 μ m column that was mounted in the GC. An Agilent 5975 Inert XL Mass Selective Detector (MSD) was used for compound analysis. Finally, EST Analytical's FLEX Autosampler was used for automated sample extraction employing the FLEX liquid mode and a 250 μ l syringe. See Tables 1 and 2 for sampling and analysis parameters.

Autosampler	Flex
General	
Method Type	Liquid
Sample Preparation (Run Twice)	
Rinse Volume	100% (250 μ l)
Rinse Fill Rate	100%
Rinse Cycles	1
Rinse Dispense Rate	100%
Solvent Pump Cycles	1
Solvent Pump Volume	100% (250 μ l)
Solvent Volume	100% (250 μ l)
Solvent Fill Rate	2%
Solvent Fill Delay	0 sec
Sample Vial Needle Depth	75%
Incubate/Agitate	
Incubation Temperature	25°C
Incubation Time	0.1 min
Agitate	Yes
Agitation Time	30.0 min
Agitation Delay	0.1 min
Agitation Speed	100% (750rpm)
Ambient Equilibration Time	2.0 min
Rinse	
Rinse Volume	8% (20 μ l)
Rinse Fill Rate	100%
Rinse Cycles	2
Sample	
Sample Volume	4% (10 μ l)
Sample Depth	100%
Sample Depth Speed	10%
Sample Fill Rate	1%
Sample Fill Delay	5 sec
Sample Rinse Volume	8% (20 μ l)
Sample Rinse Cycles	1%
Sample Pump Volume	8% (20 μ l)
Sample Pump Cycles	2
Dispense Rate	50%
Air Volume Gap	
Air Fill Volume	4% (10 μ l)
Single Injection Port	
Injection Rate	5%
Injection Volume	8% (20 μ l)
Pre-Injection Delay	1 sec
Post-Injection Delay	1 sec
Rinse	
Rinse Volume	8% (20 μ l)
Rinse Fill Rate	100%
Rinse Cycles	2

Table 1: FLEX Autosampler Experimental Parameters

GC/MS	Agilent 7890/5975 (10 μ l)
Inlet	PTV Solvent Vent
Inlet Temp.	45°C for 0.2 min, 500°C/min to 300°C for 34.5 min
Inlet Head Pressure	14.442 psi
Split	NA
Purge Flow to Split Vent	50ml/min at 1.5 min
Vent Flow	100ml/min
Vent pressure	5psi until 0.75min
Cryo	On at 50°C
Liner	TITAN XL SB Deactivated Liner with Glass Wool
Column	Rxi-5Sil MS 30m x 0.25mm I.D. x 0.25 μ m film thickness
Oven Temp. Program	35°C hold for 4.0 min, ramp 10°C/min to 320°C hold for 2.0 min, 34.5 min run time
Column Flow Rate	1.0ml/min.
Gas	Helium
Total Flow	53ml/min.
Source Temp.	230°C
Quad Temp.	150°C
MS Transfer Line Temp.	280°C
Solvent Delay	5.0 min
Acquisition Mode	Scan
Scan Range	m/z 35-500
Sampling Rate	3.12 scans/sec

Table 2: GC/MS Experimental Parameters

Poly Aromatic Hydrocarbon standards were procured from Restek. The standards were in either methanol or acetone so as to be soluble in water and reflect the extraction process accurately. A half gram of sodium chloride was added to each sample in order to aid the extraction and each sample was extracted with 500 μ l dichloromethane. The calibration range of the study was 0.5ng to 200ng on column injection. Table 3 describes the PAH curve preparation.

On Column Concentration	Standard Concentration	Standard Amount	Final Vol.	Vol. Inj.
0.5ng	50ng/ μ l	2 μ l	2ml	10 μ l
1ng	50ng/ μ l	4 μ l	2ml	10 μ l
5ng	50ng/ μ l	20 μ l	2ml	10 μ l
25ng	50ng/ μ l	100 μ l	2ml	10 μ l
50ng	50ng/ μ l	200 μ l	2ml	10 μ l
100ng	50ng/ μ l	400 μ l	2ml	10 μ l
200ng	50ng/ μ l	800 μ l	2ml	10 μ l

Table 3: PAH Curve Preparation

Seven replicate samples were run at the lowest calibration level in order to establish method detection limits. Furthermore, seven replicate standards were prepared at the 50ng level in order to determine the precision and accuracy of the extraction. After the curve, detection limits and precision and accuracy were ascertained, a standard was prepared at the 50ng level for direct

injection. Four replicate injections were run of the direct injection and the average area count of each compound was compared to the average area count of the extracted compound. This was done in order to evaluate the extraction process. The experimental results are listed in Tables 4 and 5 while the chromatogram of the 50ng standard is displayed in Figure 1.

Compound	Curve Linearity	MDL 0.5ng	%RSD 50ng	% Recovery 50ng
naphthalene	0.999	0.21	3.78	109.09
acenaphthene	0.998	0.25	3.41	109.92
fluorene	0.999	0.19	4.27	112.93
phenanthrene	0.998	0.18	4.67	116.38
anthracene	0.998	0.23	4.95	114.19
fluoranthene	0.999	0.24	4.58	116.27
pyrene	0.998	0.25	4.48	118.03
benz(a)anthracene	0.998	0.19	4.76	118.77
chrysene	0.998	0.25	5.26	117.88
benzo(b)fluoranthene	0.998	0.21	5.23	117.16
benzo(k)fluoranthene	0.999	0.25	5.76	113.49
benzo(a)pyrene	0.998	0.18	4.93	115.99

Table 4: Experimental Results

Compound	Ave. Area Count Extraction	Ave. Area Count Direct Injection	% Recovery
naphthalene	2563576	2722563	94.16
acenaphthene	2264637	2367991	95.64
fluorene	2816625	3174128	88.74
phenanthrene	3536678	4423869	79.95
anthracene	3580077	4394524	81.47
fluoranthene	3272881	4325039	75.67
pyrene	3399881	4417938	76.96
benz(a)anthracene	2402066	2626281	91.46
chrysene	2312371	2404596	96.16
benzo(b)fluoranthene	2201559	2029099	108.50
benzo(k)fluoranthene	2260008	2020863	111.83
benzo(a)pyrene	2183809	1895852	115.19

Table 5: Extraction versus Direct Injection Recoveries

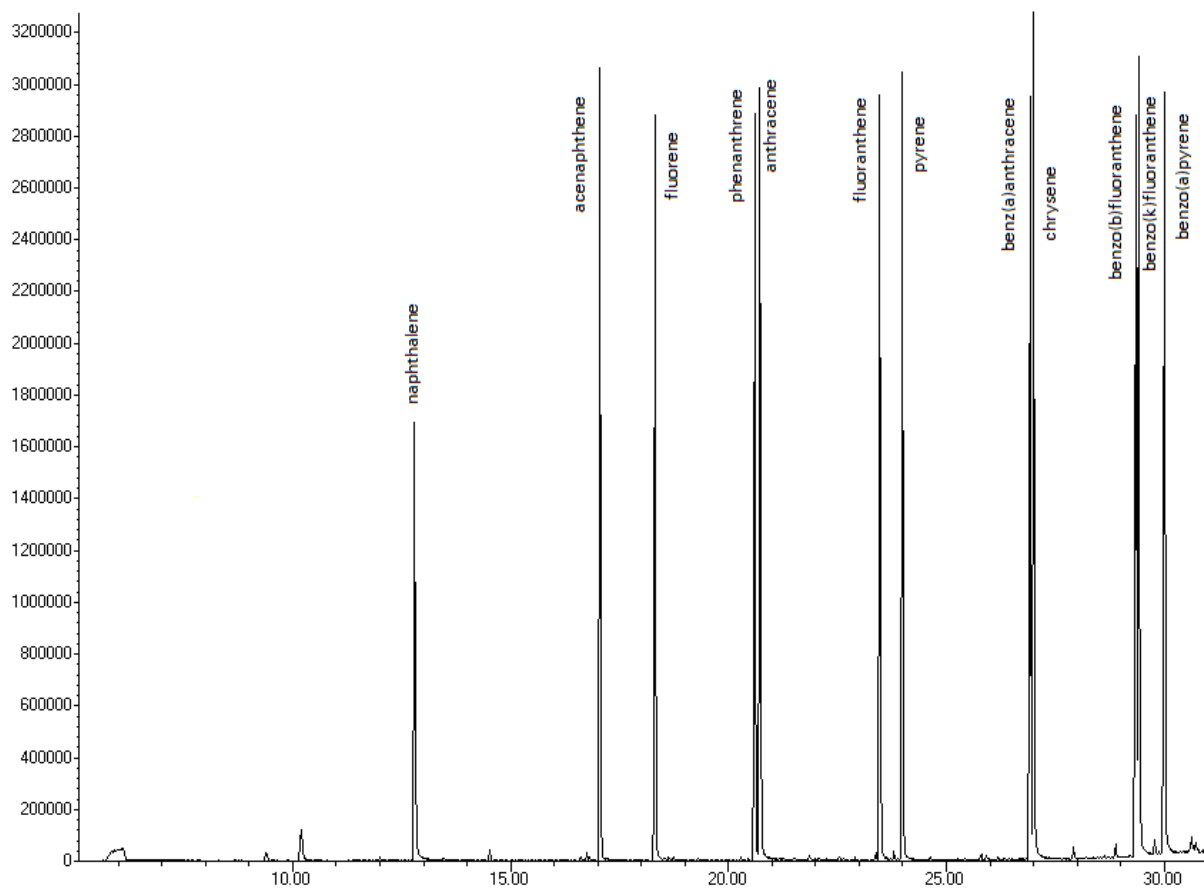


Figure 1: Chromatogram of 50ng Standard

Conclusions:

Automated extraction of PAH compounds proved to be a reliable process. The calibration was linear from 0.5ng to 200ng on column. While the precision of the analyte recoveries were less than 6% relative standard deviation with over 100% recovery. Contrasting the extraction process with a direct injection of the analytes showed better than 75% recoveries of all of the compounds of interest. This procedure would be an excellent technique for the screening of PAH water samples. The sampling is automated and solvent use for each extraction is limited to 500µl.

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