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# Optimization of automated solid-phase extraction for quantitation of polycyclic aromatic hydrocarbons in aqueous media by high-performance liquid chromatography–UV detection

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**Abstract**

A solid-phase extraction method for the quantitation of 16 regulated polycyclic aromatic hydrocarbons (PAHs) was developed and conditions were optimized to meet the recoveries stated in US Environmental Protection Agency Method 550.1. The best elution procedure soaked the extraction cartridge with acetonitrile followed by elution with methylene chloride. Rinsing the sample bottle with acetonitrile and combining the rinse with the sample extract was necessary to achieve the desired recoveries. The average recovery for a mixture of the sixteen regulated PAHs was 83%.

## 1. Introduction

One of the main concerns for the analysis of contaminates in an analytical support laboratory is how to accurately analyze samples without utilizing excessive amounts of time and laboratory space. Polycyclic aromatic hydrocarbons (PAHs) in ground water and other aqueous media are some of the contaminates which need to be analyzed for the regulatory testing and studies necessary for remediation work. To meet these needs, this analytical support laboratory chose to analyze PAHs in aqueous media by solid-phase extraction (SPE). We chose SPE since it is easily automated, uses less solvent, and uses less hood space than the more traditional liquid–liquid extraction methods. PAHs are potentially carcinogenic compounds 16 of which have been selected by the US Environmental Protection Agency (EPA) as Constant Decree priority pollutants for regulatory purposes [1].

The purpose of this study was to see if the procedure described in EPA Method 550.1 [2] could be automated and if higher concentrations of PAHs could successfully be used. EPA Method 550.1 is approved for the analysis of PAHs in drinking water by liquid–solid extraction. The calibration range for this method is in the low $\mu$g/l level. The concentration range need for our analyses ranged from this lower level up to the low mg/l levels.

Samples were initially analyzed according to the test procedures in Refs. [3] and [4]. These initial attempts to automate solid phase extraction of PAHs yielded recoveries of less than 50% for each compound. This led us to look at each step of the extraction process in order to get sample recoveries equivalent to those in ac-

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cepted EPA procedures such as EPA Method 550.1.

## 2. Materials and methods

Samples were prepared using equipment from Zymark (Hopkinton, MA, USA). The Zymark AutoTrace SPE workstation was used to automate the SPE of water samples. The AutoTrace operates with positive pressure liquid flow instead of the traditional vacuum manifold techniques. EnvirElute/PAH SPE columns from Varian (Sugarland, TX, USA) with 1 g sorbent mass and 6 ml column volume were used for sample concentration. The Zymark TurboVapII concentration workstation with nitrogen gas was used to further concentrate the extract from the AutoTrace to a final volume of 1 ml.

All PAH extracts were analyzed using equipment from Perkin-Elmer (PE, Norwalk, CT, USA), which was run according to the Quick-Turnaround HPLC method [5,6]. The HPLC system consisted of a Model 250 binary LC pump, an ISS-200 autosampler, and an LC235C diode array UV detector. A PE Chromspher-3 PAH column packed with 3 $\mu$m $d_p$ 120 Å polymeric $C_{18}$ material (100 mm × 4.6 mm with a 10-mm integral guard column) and a solvent gradient system with acetonitrile and water were used for separation of the PAHs. Turbochrom data acquisition program version 3.1 from PE Nelson (Cupertino, CA, USA) provided data acquisition and handling.

Optimization of the SPE procedure was carried out in three separate phases. The first phase was to set up the HPLC for analysis of PAHs. The second phase was to optimize the recovery of the concentration step. The final phase was to optimize the recovery of the automated SPE procedure.

The Zymark TurboVap II was used for concentrating the sample extract from the AutoTrace SPE workstation. To begin the second phase of the optimization procedure, the concentration step was optimized for operating conditions and physical handling techniques. A spiked solvent sample was initially concentrated on the TurboVap II. Recoveries of the lower-molecular-mass PAHs were less than 50%. Recoveries for the heavier PAHs were acceptable. The initial conditions for evaporation set the water bath at temperatures below the boiling point of the solvents: 40°C for methylene chloride (MeCl) and 60°C for acetonitrile (ACN).

The following steps were involved in optimizing the TurboVap for standard recovery. Different pressures for the nitrogen gas were evaluated, the temperature of the water bath was varied, and the amount and type of solvent was varied. The recommended operating pressure for the TurboVap is between 8 and 15 p.s.i. (1 p.s.i. = 6894.76 Pa). Varying the pressure in this range did not have an effect on the recoveries of the PAHs. A pressure of 11 p.s.i. of nitrogen was used for all subsequent concentrations via the TurboVap.

To determine the optimal operating conditions to concentrate MeCl, 10 ml of MeCl were added to a concentrator tube. Next, 12.5 mg/analyte of the PAH standard were injected into the concentrator tube. The TurboVap was then turned on and the solvent was evaporated down to 0.75 ml. This end point is detected automatically by sensors in the TurboVap II. At this point ACN was added to the concentrator tube. The ACN was thoroughly mixed with the remaining MeCl solution and concentrated. Finally, 0.25 ml of ACN were added drop-wise into the tube and used to rinse the slanted portion of it. This experiment was continued while varying the temperature, volume of ACN, and physical handling techniques. The optimal temperature for the water bath was determined to be 35°C for the elimination of MeCl. The optimal operating temperature to eliminate ACN was 40°C. These experiments also indicated that the optimal volume of ACN during the solvent exchange is 15 ml. This volume insured MeCl was evaporated and did not interfere with HPLC analysis. If MeCl was present in the sample injected onto the HPLC column, poor peak shape was observed.

When greater amounts of ACN were added during the solvent exchange residence time of the extract in the sample concentrator increased.

Experimentation showed that the residence time of the extract in the concentrator tubes should not be longer than 45 min. If the residence time was too long the more volatile PAHs were carried away by the nitrogen. Because of this, a lower temperature in the water bath did not necessarily insure higher recoveries.

For the analysis of PAHs by HPLC–UV, the UV detector was programmed using the following settings: 280 nm (peaks 1–5), 335 nm (peaks 6–10) and 360 nm (peaks 11–16). (For peak identification, see Table 1.) Interference could be seen on some of the peaks. These included acenaphthylene and anthracene. The interference caused the concentration of these peaks to be artificially high. These interferences are not the same ones noted in EPA Method 550.1 and may be dependent on the sorbent material used in the extraction or the HPLC column used. There were no problems getting the HPLC operational by this method. The only precaution that needed to be taken was to ensure that the wavelength changes on the diode array detector occurred after the appropriate peaks.

The Zymark AutoTrace SPE workstation was used to process the liquid samples. The samples were automatically pumped from the sample containers through EnvirElut/PAH extraction columns. The sample cartridges were then dried for 5 min with nitrogen to dry the sample cartridge before sample elution with ACN and MeCl chloride.

Zymark literature recommended soaking the extraction cartridge with solvent before elution to increase analyte recoveries. This procedure was implemented and did increase analyte recovery. Table 2 shows the AutoTrace extraction procedure.

## 3. Results

Sample recoveries for the overall process were determined from spiked tap water samples that were processed by the AutoTrace and TurboVap II and analyzed by HPLC. Initial standards processed in this manner did not have adequate analyte recoveries. To determine where the

Table 1
PAH Recoveries

| PAH | Recovery (%) | | | | |
|---|---|---|---|---|---|
| | First elution | Sample bottle | Sample bottle, adjusted | Second elution | Second elution, adjusted |
| (1) Naphthalene | 82.2 | 0.7 | 82.9 | 0.5 | 83.4 |
| (2) Acenaphthylene | 87.6 | 0.0 | 87.6 | 1.9 | 89.5 |
| (3) Acenaphthene | 83.3 | 0.0 | 83.3 | 3.5 | 86.8 |
| (4) Fluorene | 84.8 | 3.2 | 87.9 | 3.7 | 91.6 |
| (5) Phenathrene | 78.0 | 8.5 | 86.6 | 5.7 | 92.3 |
| (6) Anthracene | 63.1 | 70.3 | 133.5 | 5.1 | 138.5 |
| (7) Fluoranthene | 63.8 | 20.9 | 84.6 | 6.3 | 90.9 |
| (8) Pyrene | 55.5 | 20.1 | 75.7 | 6.8 | 82.5 |
| (9) Benz[*a*]anthracene | 34.8 | 52.5 | 87.3 | 3.2 | 90.5 |
| (10) Chrysene | 31.7 | 67.4 | 99.1 | 4.0 | 103.1 |
| (11) Benzo[*k*]fluoranthene | 33.5 | 56.3 | 89.8 | 0.0 | 89.8 |
| (12) Benzo[*a*]pyrene | 30.0 | 53.0 | 83.0 | 0.9 | 83.9 |
| (13) Dibenz[*a,h*]anthracene | 30.5 | 52.4 | 82.8 | 2.8 | 85.6 |
| (14) Benzo[*b*]fluoranthene | 36.5 | 58.9 | 95.4 | 24.9 | 120.3 |
| (15) Benzo[*ghi*]perylene | 31.8 | 55.3 | 87.2 | 1.2 | 88.4 |
| (16) Ideno[1,2,3-*cd*]pyrene | 28.7 | 55.9 | 84.6 | 3.2 | 87.7 |
| Average recovery (%) | 53.5 | | 89.4 | | 94.0 |

Table 2
AutoTrace extraction procedure

| Step | Autotrace command |
|---|---|
| 1 | Process six samples using the following procedure |
| 2 | Wash syringe with 7 ml of MeCl |
| 3 | Wash syringe with 7 ml of methanol |
| 4 | Wash syringe with 7 ml of water |
| 5 | Wash syringe with 3 ml of methanol |
| 6 | Condition column with 5 ml of MeCl into solvent waste |
| 7 | Dry column with nitrogen for 1 min |
| 8 | Condition column with 10 ml of methanol into solvent waste |
| 9 | Condition column with 5 ml of water into aqueous waste |
| 10 | Load 1000 ml of sample onto column |
| 11 | Rinse column with 10 ml of water into aqueous waste |
| 12 | Dry column with nitrogen gas for 5 min |
| 13 | Soak and collect 4.0-ml fraction using ACN |
| 14 | Pause for 3 min |
| 15 | Soak and collect 3.5-ml fraction using MeCl |
| 16 | Pause for 3 min |
| 17 | Collect 3.5-ml fraction into sample tube using MeCl |
| 18 | End |

The Zymark AutoTrace SPE workstation is used to extract and concentrate PAHs from aqueous media. Conditions for extraction: nitrogen pressure between 0.6 and 0.8 bar; solvents: acetonitrile, methanol, methylene chloride and water.

analytes were being lost in the sample preparation process, a number of experiments were performed. These experiments included elution of the extraction cartridge a second time, rinsing the sample bottle with ACN for concentration and analysis, and injecting the standard directly onto the SPE extraction cartridge instead of making an aqueous standard. Table 1 shows the results of these experiments. Fig. 1 shows the results when the standard was injected directly on to the SPE extraction column instead of loading an aqueous standard onto the SPE extraction cartridge. The recovery of PAHs from this experiment indicate that elution from the SPE extraction columns followed by concentration with the TurboVapII gives acceptable recoveries. Therefore, the only part of the extraction process that contributed to loss of analyte recovery was loading the sample onto the extraction cartridge and the loss of analyte in the sample bottle and the transfer process.

Table 1 shows that rinsing the sample bottle with ACN recovers a significant amount of the lost analyte. This indicates that much of the error in the SPE extraction process comes from making and loading the aqueous standard. To confirm this, a standard was placed directly on the SPE extraction cartridge. These cartridges were then eluted, concentrated and analyzed on

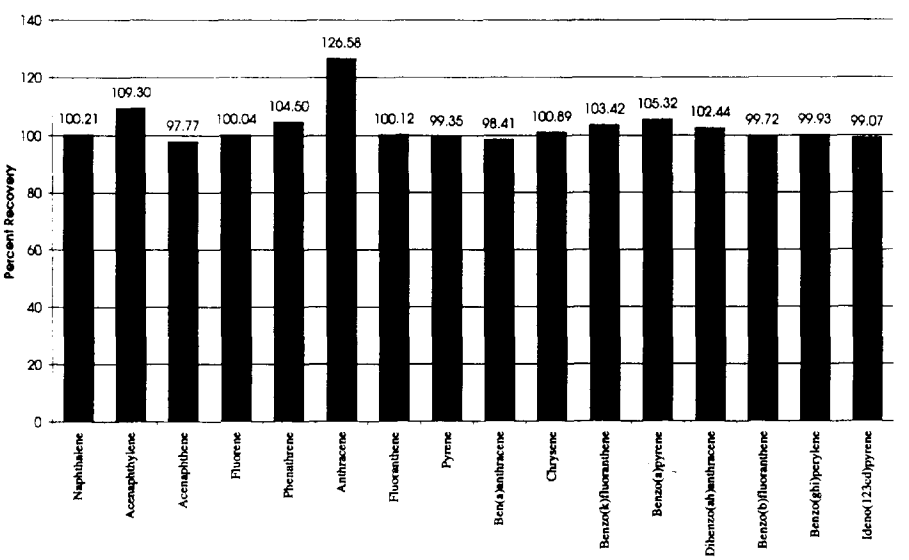


Fig. 1. Recovery with direct injection of standard onto SPE cartridge.

the HPLC. Fig. 1 shows excellent recoveries from this experiment.

Kerkdijk et al. [7] suggests rinsing the sample bottles with isopropanol (3 ×), 20% nitric acid and isopropanol respectively. The water sample is then to be diluted with isopropanol (3:1) to prevent PAH adsorption to the container wall. This process has not been tried in our laboratory, but looks promising.

A comparison of the variable-wavelength program and a program with a constant wavelength of 255 nm for the analysis of environmental samples was made. In addition to the variable-wavelength program used in the quick turnaround method, the second channel of the UV detector collected data at a constant wavelength of 255 nm. Fig. 2 shows a PAH standard run using the programmed UV detector, an extracted sample using the programmed UV detector, and the same extracted sample using the 255 nm wavelength only. These chromatograms show the benefit of the variable-wavelength program. Each of the 16 regulated PAHs is easily detected with the variable-wavelength program, but the detection of other contaminates is greatly reduced.

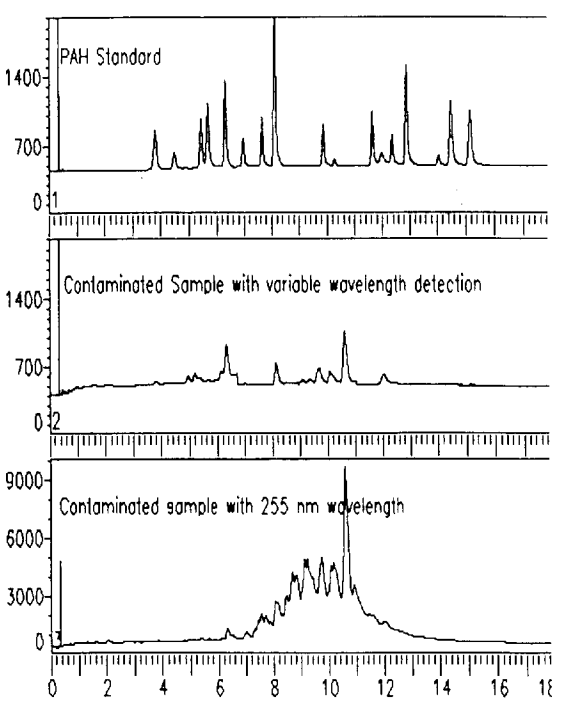


Fig. 2. PAH Standard run using programmed UV detection, an extracted sample using programmed UV detection, and the same extracted sample using detection at 255 nm only.

## 4. Discussion

SPE cartridges can successfully be used for the analysis of PAHs in aqueous media. In addition to the relatively low concentration of PAHs addressed in EPA Method 550.1, SPE can be used with concentrations of PAHs an order of magnitude higher. These higher concentrations are normally found at heavily contaminated sites or in enrichment cultures often used in laboratory studies.

While the automation of SPE would appear to be straightforward, each step of the sample preparation process had to be optimized separately. The preparation of aqueous PAH standards is difficult due to their low solubilities. Rinsing the sample container with ACN and adding the rinse to the solvent extract gives recoveries for this method which are equal to or better than those described in EPA Method 550.1. Rinsing the sample container is also part of the procedure described in EPA Method 550.1. Automation of the extraction process allows for efficient use of chemist time without giving up accuracy of data. The Perkin-Elmer Quick Turnaround method allows for detection of all 16 regulated PAHs without excessive interference from other compounds with a variable-wavelength UV detector.

A comparison between this method and EPA 550.1 is discussed below. A 3-$\mu$m particle diameter column was used instead of a 5-$\mu$m particle diameter column in the HPLC. This allowed for faster elution times (18 min instead of 30 min) without the loss of separation. Liquid–solid extraction cartridges were used instead of Empore extraction disks. The main disadvantage of the extraction cartridges has been the length of time necessary to load the sample onto the cartridge; this time is not reduced by the automated method, but six samples can be extracted

at the same time with no operator supervision. Interferences can be seen in both methods. This method uses only a UV detector instead of UV and fluorescence detectors. The same quality of data can be obtained due to the wavelength programming used by this method and the LC235C diode array UV detector allows for spectral confirmation of the peaks detected.

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