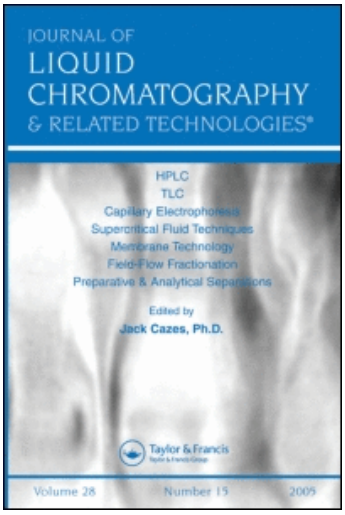


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# DEVELOPMENT OF A SOLID PHASE EXTRACTION PROTOCOL FOR THE SIMULTANEOUS DETERMINATION OF ANTHRACENE AND ITS OXIDATION PRODUCTS IN SURFACE WATERS BY REVERSED-PHASE HPLC

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# DEVELOPMENT OF A SOLID PHASE EXTRACTION PROTOCOL FOR THE SIMULTANEOUS DETERMINATION OF ANTHRACENE AND ITS OXIDATION PRODUCTS IN SURFACE WATERS BY REVERSED-PHASE HPLC

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## ABSTRACT

A gradient reversed-phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of anthracene, anthraquinone, and 1-hydroxyanthraquinone, with photodiode array detection at 250 nm, has been developed. This method of the determination of anthracene and its oxidation products appears in the literature for the first time. The separation has been achieved on a Kromasil 100 $C_8$ 5 μm 250 × 4 mm column, applying a 10 min linear gradient elution starting with 85% methanol and 15% 0.05 M ammonium acetate and ending up with 95% of methanol and 5% 0.05 M ammonium acetate, at a flow-rate 0.7 mL/min, using 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine (lamotrigine) as internal standard. Calibration

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curves were rectilinear for 0.1 to 3.0 ng of anthracene, for 0.1 to 10.0 ng of anthraquinone, and for 0.5 to 20.0 ng of 1-hydroxyanthraquinone, when 10 μL was injected. The detection limits were found to be 0.05 ng injected on-column for anthracene and anthraquinone and 0.3 ng on-column for 1-hydroxyanthraquinone. The average intra- and inter-day R.S.Ds for injection precision (in terms of peak area) were 1.95% and 3.62%, respectively. The method was applied to the analysis of river and lake waters. A protocol, combining solid phase extraction (SPE) with sonication of matrix with sorbent, was developed for enhancement of recovery. The proposed protocol was chosen among other studied, after optimization of each step. Mean recoveries were 50% for anthracene, 71% for anthraquinone, and 105% for 1-hydroxy anthraquinone.

*Key Words*: Solid phase extraction; Anthracene; Anthraquinone; 1-Hydroxyanthraquinone; Surface waters



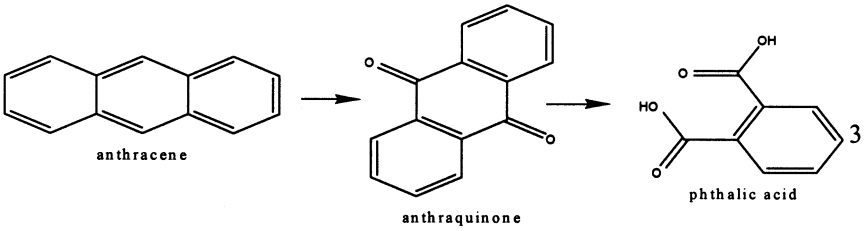
## INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous and persistent throughout the environment. They are generally distributed from both natural and man-made sources, emitted from forest fires, internal combustion, and diesel engines, power plants, refuse incinerators, and industrial processes. They are also formed as byproducts of the incomplete combustion of organic materials, such as wood, fossil fuels, and refuse. The toxicity of PAHs is known to be enhanced by light, via photosensitization reactions and oxidation to more toxic compounds. Many PAHs undergo photochemical oxidation while adsorbed onto atmospheric particles. Polynuclear aromatic hydrocarbons occurrence in natural waters or sediments results from deposition of atmospheric particulate matter or through discharge from oil spills and municipal and industrial effluents. Polynuclear aromatic hydrocarbons associated with sediments or the water body may be degraded by abiotic processes (chemical oxidation and photooxidation) or biological transformations, such as microbial degradation and uptake and subsequent metabolism by aquatic organisms.[1]

Quinones are intermediates in the environmental oxidation of PAHs. One species of PAHs is anthracene, obtained in the distillation of coal tar. Anthracene is an important intermediate in the manufacture of various dyestuffs, while also being used as a catalyst in the isomerization of vegetable oils and in various coating applications. Anthracene readily oxidizes with formation of 9,10-anthracenedione (anthraquinone) as the primary product, thus, resulting in a predominance of the

latter on atmospheric particles. It has been shown that 12–19% of anthracene emitted on particulate matter is oxidized to anthraquinone and 2–5% to 1-hydroxyanthraquinone. Anthraquinone and its derivatives are used by modern wood pulping processes to catalytically enhance delignification.[2,3]

The biodegradation of anthracene has the following pathway:




The biodegradability of anthraquinone is confirmed to be much more rapid than that of anthracene, i.e., biodegradability of 90 vs. 30% over 30 days.[4]

Because of the strong carcinogenic activity of several PAHs, their determination in various matrices has gained considerable attention. However, their levels in natural waters are extremely low due to their low water solubility, therefore, their determination in environmental samples by conventional analytical methods is laborious, with poor recoveries. The levels of quinones in natural waters and urban air are in the order of $10^{-8}$–$10^{-11}$ g/L and $10^{-8}$–$10^{-10}$ g/m$^3$, respectively.[2]

Several high performance liquid chromatography (HPLC) methods for the determination of anthracene along with other PAHs,[2,3,5–9] or for the determination of anthraquinone in environmental pollutants and in a variety of matrices, have been published.[9–15] However, to our knowledge, none of them deals with the simultaneous quantification of anthracene and its oxidation products, anthraquinone and 1-hydroxyanthraquinone in environmental samples or other matrices.

In this paper, a simple, rapid, and highly sensitive reversed-phase high performance liquid chromatography (RP-HPLC) method for the simultaneous determination of anthracene, anthraquinone, and 1-hydroxyanthraquinone is reported, using a simple linear methanol–0.05 M ammonium acetate gradient with UV detection.

The developed method is applied to surface waters, river, and lake water, after solid phase extraction (SPE) for analytes isolation and pre-concentration. Various protocols were studied to optimize analytes' recovery. Solid phase extraction was investigated in its conventional form, as well as in combination with LLE and in direct contact of sorbent material with sample matrix. Optimum conditions include sonication of sorbent with sample matrix for 20 min, to enhance analytes' recovery.

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## EXPERIMENTAL

### Reagents and Materials

Anthracene and anthraquinone were obtained from Fluka Chemie AG (Switzerland) and 1-hydroxyanthraquinone was obtained from Aldrich Chemicals (Aldrich, Europe). The internal standard 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine (lamotrigine) was obtained from Wellcome Foundation (London, UK). Acetone, hexane, cyclohexane, tetrahydrofuran, and ammonium acetate (pro analysi) were obtained from Merck (Darmstadt, Germany). HPLC-grade 2-propanol, acetonitrile, and methanol were purchased from Riedel-de Haën AG (Seelze, Germany).

The solid phase extraction study was performed using the following cartrigdes: Bond Elut 2OH (200 mg/3 mL) and Abselut Nexus (100 mg/3 mL) obtained from Varian Chrompack (Middelburg, The Netherlands), and $C_{18}$-MERCK (400 mg/3 mL) obtained from Merck (Darmstadt, Germany). Empore discs (8 μ SDB-RPS, 60 AÅ) were supplied from 3 M (Minneapolis, Minnesota, USA).



### Instrumentation

A Shimadzu (Kyoto, Japan) quaternary low-pressure gradient system was used for the chromatographic determination of examined analytes. The solvent lines were mixed in an FCV-9AL mixer and an LC-9A pump was used to deliver the mobile phase to the analytical column. Sample injection was performed by a SIL-9A autosampler and detection was achieved by an SPD-M6A Photodiode Array Detector, which complied with Data acquisition software Class-M10A. Chromatograms were stored on the hard disk of a Function 486 PC and printed ona Hewlett-Packard LaserJet 4L Printer. Degassing of solvents was achieved bycontinuous helium sparking in the solvent flasks through a DGU-2A degassing unit.

The analytical column was a Kromasil 100 $C_8$ 5 μm (250 × 4 mm ID) obtained from MZ Analysentechnik (Mainz, Germany).

A glass vacuum solvent-filtration apparatus obtained from Alltech Associates Inc. (Deerfield, IL, USA) was employed for the filtration of ammonium acetate, using 0.2 μm membrane filters obtained from Schleicher & Schuell (Dassel, Germany). A Glass-col, Terre Haute 47802 small vortexer and an ultrasonic bath Transsonic 460/H (ELMA) were used for sample preparation.

The SPE cartridges were placed in a Vac-Elut ten-position manifold vacuum system, obtained from Analytichem International, a division of Varian (Harbor City, CA, USA).

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## Chromatographic Conditions

The linear gradient elution was carried out at ambient temperature, with a system of 0.05 M ammonium acetate and methanol, starting with 85% and ending up to 95% of methanol, over a period of 10 min. The flow rate was 0.7 mL/min. The analytes were monitored at 250 nm. The internal standard was used at a concentration of 5 ng/μL in $CH_3OH-CH_3COONH_4$ (85 : 15 v/v).

## Preparation of Standard Solutions and Samples

Stock methanolic solutions of anthracene, anthraquinone, 1-hydroxy-anthraquinone, and lamotrigine (the internal standard) were prepared separately at the 100 ng/μL concentration level. The analytes were serially diluted with

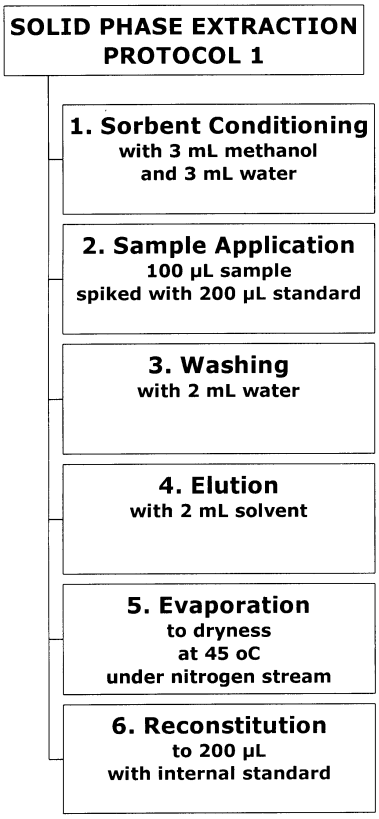


*Figure 1.* Solid phase extraction protocol 1.

methanol and appropriate volumes of these dilute solutions of each analyte, were added as a mixture in 50 mL volumetric flasks for the preparation of the working standard solutions. A 2.5 mL volume of the 100 ng/μL solution of lamotrigine was added to each working standard solution, and the final volumes were made up with an 85 : 15% v/v mixture of methanol–0.05 M ammonium acetate. All solutions were kept refrigerated and protected from light.

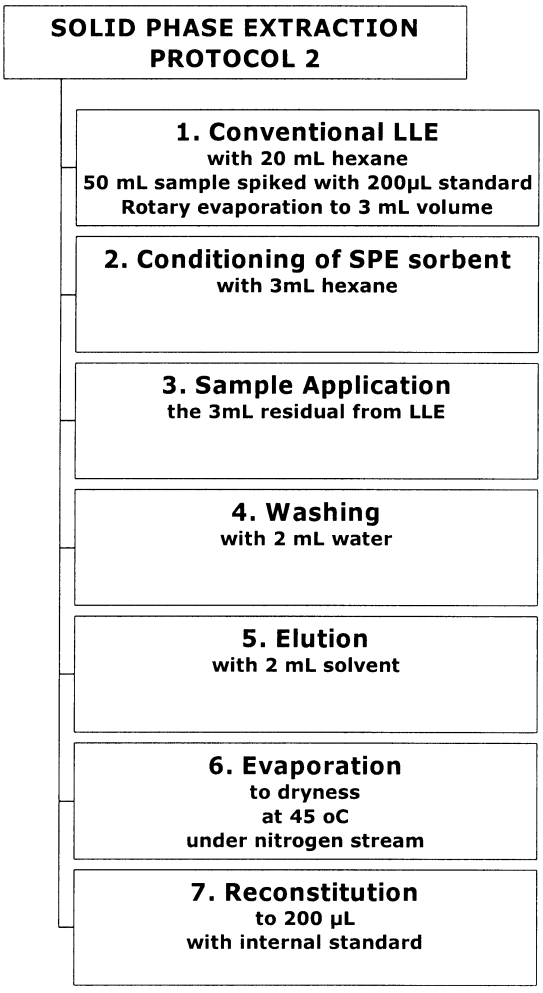


***Figure 2.*** Solid phase extraction protocol 2.

The resulting concentrations in the working solutions ranged from 0.01 to 0.3 ng/μL with respect to anthracene, from 0.01 to 1.0 ng/μL with respect to anthraquinone, and from 0.05 to 2.0 ng/μL with respect to 1-hydroxyanthraquinone, all containing 5 ng/μL of the internal standard.

Three replicate injections of each standard solution were made and the means of the peak area ratios of the analytes relative to internal standard were plotted as a function of the concentration, thus, defining the linear working range of each compound.

## Sample Preparation

Various extraction schemes were examined in order to establish the optimum protocol for anthracene and its oxidation products in surface waters.



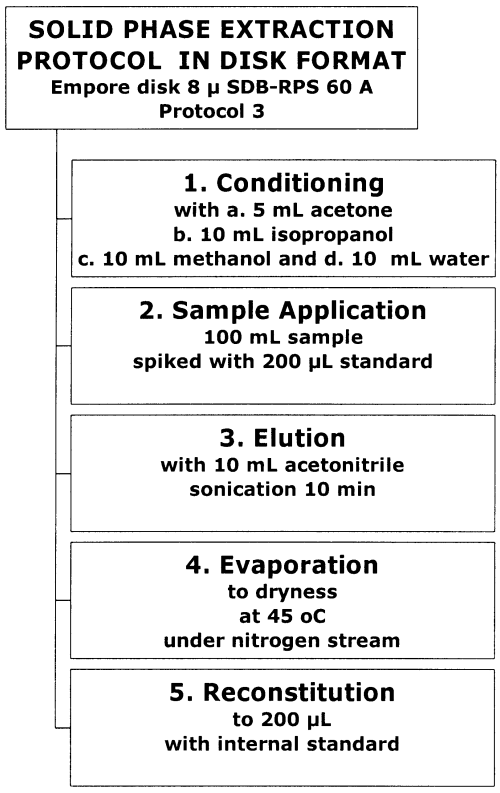


*Figure 3.* Solid phase extraction protocol 3 with disc format.

The protocols used in this assay include conventional liquid–liquid extraction in combination with SPE, SPE using cartridge and disc format, as well as the combination of SPE and sonication at two steps, i.e., the sample application step and the elution step. In the latter method, different extraction conditions were studied concerning elution solvents and sonication time. The sorbent material was poured out from the cartridge and transferred into a glass beaker with the sample. The direct contact of sorbent with the sample matrix or the eluent solvent, combined with sonication, facilitated analytes interactions with sorbent and eluent solvent, respectively, thus, yielding higher recovery rates.

The examined protocols are illustrated in Figs. 1–4.

## Application to River and Lake Water Samples

Pooled river and lake water samples were filtered through 0.2 μm filters and spiked with different concentrations of the analytes as follows: To 25 mL volumetric flasks, a 200 μL volume of standard mixtures of the analytes, at

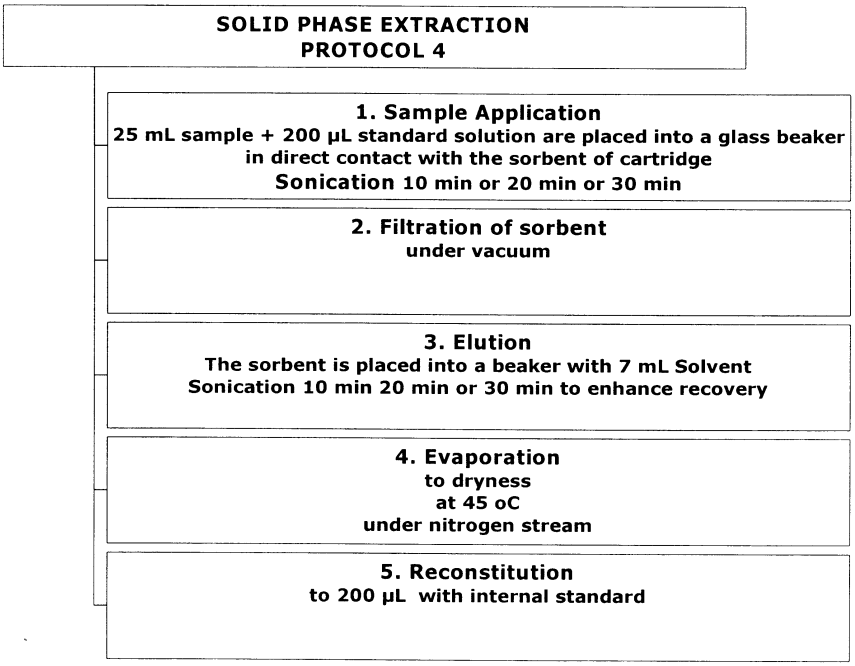


*Figure 4.* Solid phase extraction protocol 4.

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different concentrations each time, was added and the volumes were made up with the river or lake waters' pooled sample. The spiked samples were subsequently extracted under sonication, as described in protocol 4 as shown in Fig. 4.

The resulting concentrations of the analytes in the spiked solutions were in the range of 0.05 to 0.25 ng/μL with respect to anthracene, and 0.1 to 2.0 ng/μL with respect to anthraquinone and 1-hydroxyanthrauinone.

Three replicate injections of each solution were made and the means of peak areas vs. concentration were finally employed for quantitation.

## RESULTS AND DISCUSSION

### Chromatography

With the selected elution system and flow rate, the analysis was completed within 10 min and the separation between the analytes and the internal standard was very satisfactory, as can be seen in Fig. 5. The resolution factors, $R_s$, were found to be 3.4 between lamotrigine and anthraquinone, 1.9 between anthraquinone and 1-hydroxyanthraquinone, and 1.2 between 1-hydroxyanthraquinone and anthracene. The retention times of the compounds were 3.397 min for lamotrigine (the internal standard), 5.902 min for anthraquinone, 7.066 min for 1-hydroxyanthraquinone, and 7.865 min for anthracene.

### Method Validation

The reliability of the proposed method in terms of precision, accuracy and linearity was studied. The linear regression equations and correlation coefficients are given in Table 1.

The linear working range for standard solutions extended from 0.01 to 0.3 ng/μL for anthracene, from 0.01 to 1.0 ng/μL for anthraquinone, and from 0.05 to 2.0 ng/μL for 1-hydroxyanthraquinone. The detection limits of the analytes, defined as those quantities producing a signal of a peak height twice the size of background noise, were found to be 0.05 ng on-column for anthracene and anthraquinone and 0.3 ng on-column for 1-hydroxyanthraquinone.

The linearity for spiked river and lake water samples held from 0.03 to 0.3 ng/μL for anthracene, from 0.03 to 1.0 ng/μL for anthraquinone and from 0.05 to 2.0 ng/μL for 1-hydroxyanthraquinone.

In order to assess the precision and accuracy of the method, the repeatability of replicate injections within-day and the reproducibility of repeated injections from day-to-day were tested. For this purpose, standard mixtures of the analytes at three concentration levels, in the presence of internal standard, were injected six

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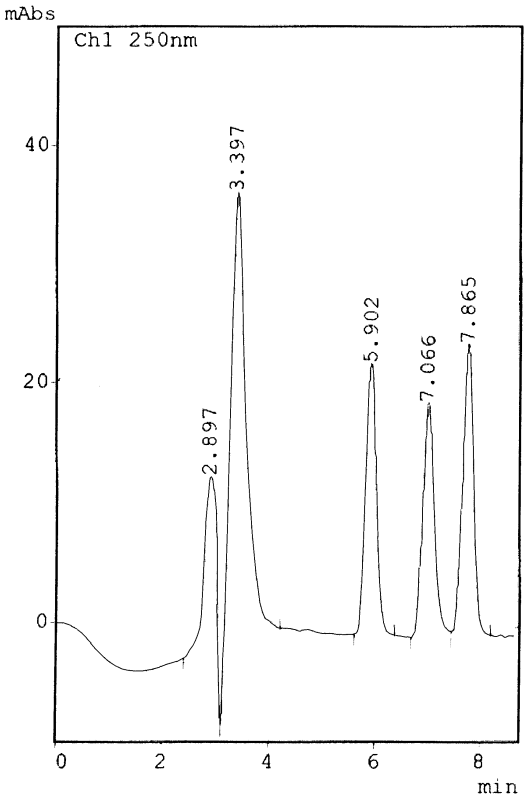


***Figure 5.*** Chromatogram of a standard solution containing 0.1 ng/μL of each analyte and 5 ng/μL of internal standard; chromatographic conditions as described under experimental; peaks: 3.397 min = internal standard, 5.902 min = anthraquinone, 7.066 min = 1-hydroxyanthraquinone, and 7.865 min = anthracene.

times each within the same day and three times/day over a period of six consecutive days, respectively. In the latter case, the six measured mean values of three determinations/day/standard represent the overall between-day (inter-day) precision of the method. The accuracy was assessed by the following equation:

$$\text{Accuracy (\%)} = \frac{(\text{Mean determined value} - \text{Theoretical value})}{\text{Theoretical value}} \times 100$$

As can be seen in Table 2, the R.S.Ds ranged from 0.97 to 3.79% for the intra-day and from 1.23 to 5.48% for the inter-day measurements. Results presented in Table 2 indicate satisfactory precision and accuracy of the method.

***Table 1.*** Statistical Evaluation of Regression Equations

| Samples | Regression Equations $Y=(a\pm S_a)+(b\pm S_b)X$ | Correlation Coefficients |
|---|---|---|
| | Standard solutions | |
| ANC | $Y=(0.04656\pm0.01257)+(4.803\pm0.0995)X$ | 0.9991 |
| ANQ | $Y=(0.03076\pm0.01410)+(1.665\pm0.0362)X$ | 0.9988 |
| OH-ANQ | $Y=(0.00657\pm0.00844)+(0.8404\pm0.00946)X$ | 0.9997 |
| ANC | $Y^*=(-4627.5\pm12918.08)+(1{,}488{,}098\pm77898.95)X$ | 0.9918 |
| ANQ | $Y^*=(20153.19\pm7376.695)+(164179.2\pm7131.32)X$ | 0.9944 |
| OH-ANQ | $Y^*=(20179.78\pm8127.566)+(156004.4\pm7857.215)X$ | 0.9924 |
| | River water | |
| ANC | $Y^*=(-14{,}646\pm13677.84)+(630{,}022\pm99888.86)X$ | 0.9911 |
| ANQ | $Y^*=(29344.51\pm16398.69)+(104920.5\pm14192.81)X$ | 0.9947 |
| OH-ANQ | $Y^*=(35517.39\pm17863.69)+(118817.2\pm17269.48)X$ | 0.9904 |
| | Lake water | |
| ANC | $Y^*=(269654.13\pm5943.08)\ X+(10021.4\pm1011.5)$ | 0.9963 |
| ANQ | $Y^*=(58636.15\pm16191.26)X+(61278.58\pm9406.274)$ | 0.9977 |
| OH-ANQ | $Y^*=(47322.4\pm1838.723)X+(27580.26\pm1917.48)$ | 0.9970 |

$Y$ = Peak area ratio of analyte to internal standard. $Y^*$ = Peak area. $X$ = Concentration in ng/μL. $a$ = Intercept. $b$ = Slope. $S_a$, $S_b$ = Standard deviations of intercept and slope respectively.
ANC = Anthracene.
ANQ = Anthraquinone.
OH-ANQ = 1-hydroxy anthraquinone.

***Table 2.*** Within-Day and Between-Day Precision and Accuracy of the Assay for the Simultaneous Determination of Anthracene, Anthraquinone, and 1-Hydroxyanthraquinone in Standard Solutions

| | | Within-Day | | | Between-Day | | |
|---|---|---|---|---|---|---|---|
| Compound | Injected Quantity (ng) | Mean Measured Quantity, $x^a \pm$ SD | RSD (%) | Accuracy[c] (%) | Mean Measured Quantity, $x^b \pm$ SD | RSD (%) | Accuracy (%) |
| ANC | 0.470 | $0.433 \pm 0.0083$ | 1.92 | −7.9 | $0.438 \pm 0.024$ | 5.48 | −6.8 |
| | 0.940 | $0.964 \pm 0.0094$ | 0.97 | 2.6 | $0.962 \pm 0.018$ | 1.90 | 2.3 |
| | 2.82 | $2.88 \pm 0.071$ | 2.48 | 2.1 | $2.88 \pm 0.035$ | 1.23 | 2.1 |
| ANQ | 0.490 | $0.463 \pm 0.015$ | 3.24 | −5.5 | $0.464 \pm 0.014$ | 3.01 | −5.3 |
| | 0.980 | $0.996 \pm 0.019$ | 1.91 | 1.6 | $0.987 \pm 0.035$ | 3.55 | 0.7 |
| | 2.94 | $3.14 \pm 0.034$ | 1.08 | 6.8 | $3.13 \pm 0.15$ | 4.73 | 6.5 |
| OH-ANQ | 0.510 | $0.475 \pm 0.018$ | 3.79 | −6.9 | $0.481 \pm 0.021$ | 4.37 | −5.7 |
| | 1.02 | $0.965 \pm 0.0096$ | 0.99 | −5.4 | $1.06 \pm 0.055$ | 5.19 | 3.9 |
| | 3.06 | $2.86 \pm 0.033$ | 1.15 | −6.5 | $2.95 \pm 0.095$ | 3.22 | −3.6 |

[a]Mean values obtained from the linear regression equations for $n = 6$ determinations;
[b]Overall mean values obtained from the linear regression equations for $n = 3$ determinations/day over a period of 6 days.
[c]Accuracy (%) $= \dfrac{(\text{Mean determined value} - \text{Theoretical value})}{\text{Theoretical value}} \times 100$

## Sample Preparation

For the analysis of river and lake waters an SPE study using different cartridges and different extraction conditions was carried out, in order to establish the best procedure for removing matrix interference and obtain high analyte recoveries. All trials were performed with surface water samples spiked with a standard containing 0.5 ng/μL in anthraquinone and 1-OH anthraquinone and 0.1 ng/μL in anthracene. Three determinations were performed with each protocol.

The extraction of anthracene was difficult. As it can be noticed from results in Table 3, it was not extracted in many cases, remaining strongly bound to the sorbent. However, recovery was enhanced when the sorbent was sonicated with the sample and during elution step with the eluent solvent. Optimization of eluent solvent lead to the use of acetonitrile, that was found to give higher extraction rates from Nexus cartridges used without sorbent conditioning. Extraction was



***Table 3.*** Recovery of Anthracene and Its Derivatives from Surface Water Using Protocols 1 and 2 with Different Eluent Solvents

| | Recovery (%) | | |
|---|---|---|---|
| Analyte | $CH_3OH$ | $CH_3CN$ | $CH_3CH(OH)CH_3$ |
| | Protocol 1-NEXUS | | |
| ANC | NE[a] | NE | NE |
| ANQ | NE | 21.3 | NE |
| OH-ANQ | NE | 5.4 | NE |
| | Protocol 1-$C_{18}$ | | |
| ANC | NE | NE | NE |
| ANQ | 14.0 | 12.5 | 3.2 |
| OH-ANQ | NE | 8.4 | NE |
| | Protocol 2 (Diol) | | |
| ANC | NE | NE | NE |
| ANQ | 14.4 | 66.3 | 36.5 |
| OH-ANQ | 17.9 | 55.4 | 49.8 |

| Analyte | Protocol 2 (Diol) LLE Cyclohexane $CH_3CN$ Elution | Protocol 3 Empore Disc |
|---|---|---|
| ANC | NE | 5.7 |
| ANQ | 52.3 | 37.9 |
| OH-ANQ | 33.0 | 40.0 |

[a]NE = not extracted.

***Table 4.*** Optimization of Sonication Time for Anthracene and Derivatives Extraction from Surface Water Using Protocol 4 (Raster Shows the Higher Obtained Values)

| | Recovery (%) | | |
|---|---|---|---|
| | Sonication Time | | |
| Analyte | 10 min | 20 min | 10 min[a] |
| A. Without conditioning + $CH_3CN$ as eluent solvent | | | |
| ANC | 20.2 | 50.4 | 10.3 |
| ANQ | 30.3 | 70.9 | 25.1 |
| OH-ANQ | 32.9 | 105.4 | 33.0 |
| | Sonication Time | | |
| Analyte | 10 min | 20 min | 20 min[b] |
| B. Without conditioning + $CH_3CH(OH)CH_3$ as eluent solvent | | | |
| ANC | 25.3 | 32.9 | 34.4 |
| ANQ | 26.0 | 40.5 | 44.0 |
| OH-ANQ | 36.8 | 45.4 | 50.3 |

[a]Sorbent from two cartridges.
[b]With pre-conditioning of sorbent.



improved by increasing sonication time, however, this should not exceed the 20 min period as acetonitrile was evaporated due to temperature increase in the ultrasonic bath.

A pooled river water sample derived from four different river samples collected in Northern Greece and a pooled lake water sample from two samplings at

***Table 5.*** Optimization of Eluent Solvent for Anthracene and Derivatives Extraction from Surface Water Using Protocol 4 with 20 min Sonication

| Analyte | Acetone | THF Recovery % | Isopropanol–$CH_3COONH_4$ (95 : 5) | MeOH[a] | $CH_3CN$ |
|---|---|---|---|---|---|
| ANC | NE | NE | NE | 13.3 | 25.0 |
| ANQ | 40.5 | 58.9 | 50.4 | 21.3 | 73.4 |
| OH-ANQ | 45.4 | 50.5 | 40.5 | 28.5 | 67.0 |

[a]10 min sonication.

***Table 6.*** Recovery of Anthracene and Derivatives from Spiked River and Lake Water

| Pooled River Water | | | | Pooled Lake Water | | | |
|---|---|---|---|---|---|---|---|
| Added (ng/L) | Found* ± SD (ng/μL) | Relative Recovery | Absolute Recovery (%) | Added (ng/μL) | Found* ± SD (ng/μL) | Relative Recovery | Absolute Recovery (%) |
| | | | | ANC | | | |
| 1 | 1.1 ± 0.1 | 110.0 | 47.0 | 1 | 1.0 ± 0.05 | 100.0 | 45.0 |
| 2 | 2.2 ± 0.1 | 110.0 | 47.0 | 2 | 2.1 ± 0.1 | 105.0 | 46.0 |
| 3 | 3.2 ± 0.2 | 106.7 | 41.0 | 4 | 4.4 ± 0.2 | 110.0 | 47.5 |
| 4 | 4.4 ± 0.3 | 110.0 | 47.5 | 5 | 5.4 ± 0.2 | 108.0 | 47.0 |
| | | | | ANQ | | | |
| 6 | 5.8 ± 0.2 | 96.7 | 68.3 | 2 | 2.2 ± 0.1 | 110.0 | 75.0 |
| 10 | 10.2 ± 0.3 | 102.0 | 73.0 | 6 | 6.8 ± 0.3 | 113.3 | 76.7 |
| 20 | 23.1 ± 0.5 | 115.5 | 77.5 | 10 | 11.4 ± 0.3 | 114.0 | 77.0 |
| 40 | 38.4 ± 0.8 | 96.0 | 68.0 | 20 | 21.9 ± 0.7 | 109.5 | 74.5 |
| | | | | OH-ANQ | | | |
| 6 | 5.8 ± 0.4 | 96.7 | 100.7 | 2 | 2.0 ± 0.1 | 100.0 | 105.0 |
| 10 | 10.5 ± 0.4 | 105.0 | 109.0 | 6 | 5.9 ± 0.3 | 98.3 | 103.3 |
| 20 | 20.0 ± 0.7 | 100.0 | 104.5 | 10 | 9.4 ± 0.7 | 94.0 | 98.0 |
| 40 | 37.3 ± 1.1 | 93.2 | 97.0 | 40 | 38.4 ± 1.6 | 96.0 | 99.5 |

Absolute Recovery (%) = (Peak area of extracted standard vs. Peak area of non-extracted standard solution of identical concentration) × 100.
Relative Recovery (%) = (Peak area of extracted sample vs. Peak area of extracted standard solution of identical concentration) × 100.

a lake in Northern Greece, were used. Aliquots of 25 mL of the pooled sample spiked with 200 μL of a standard mixture, were treated as described under protocol 4 (Fig. 4), using the following conditions: Abselut Nexus sorbent without preconditioning was poured out in a glass beaker, sonicated for 20 min with the water sample, and eluted with acetonitrile. A non spiked sample was also prepared by adding a 200 μL volume of methanol to 25 mL aliquots of the sample.

The eluates from the SPE sorbent were evaporated to dryness in a water bath at 45°C under nitrogen and the residues were reconstituted with a 200 μL volume of internal standard solution.

The eluates from the sample application and washing steps were also evaporated to dryness and analyzed after reconstitution to 200 μL with internal standard solution. No analyte peaks were detected in the respective chromatograms, indicating that eventual low recoveries should not be attributed to losses during these steps.



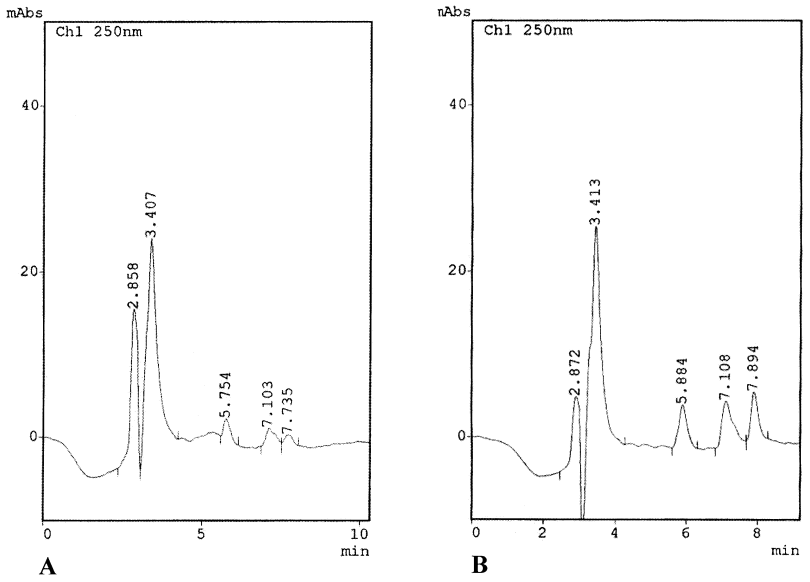


***Figure 6.*** A. Chromatogram of a non-spiked pooled river water sample. Peaks: 3.407 min = internal standard, 5.754 min = anthraquinone, 7.103 min = 1-hydroxyanthraquinone, and 7.735 min = anthracene. B. Chromatogram of a pooled river water sample spiked with a standard containing 0.5 ng/μL anthraquinone and 1-OH anthraquinone and 0.1 ng/μL in anthracene and 5 ng/μL of internal standard; chromatographic conditions as described under experimental; peaks: 3.413 min = internal standard, 5.884 min = anthraquinone, 7.108 min = 1-hydroxyanthraquinone, and 7.894 min = anthracene.

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During the sample preparation study applied to the river and lake waters, a matrix interference with the internal standard peak was noticed; therefore, the calibration curves for the surface water samples were constructed using peak areas (instead of peak area ratios).

The absolute extraction recovery was high for 1-hydroxyanthraquinone, satisfactory for anthraquinone, but only 50% for anthracene. The latter could not be improved with any of the testing conditions, as can be seen in Tables 3 to 5. In most cases, anthracene is either slightly extracted or not extracted at all. The absolute recovery was estimated as the ratio of peak areas of an extracted vs. a non-extracted standard solution of an identical concentration. Relative recovery of analytes from samples vs. extracted standard solution of identical concentration was in the range from 93.2% to 110%, as can be seen in Table 6.



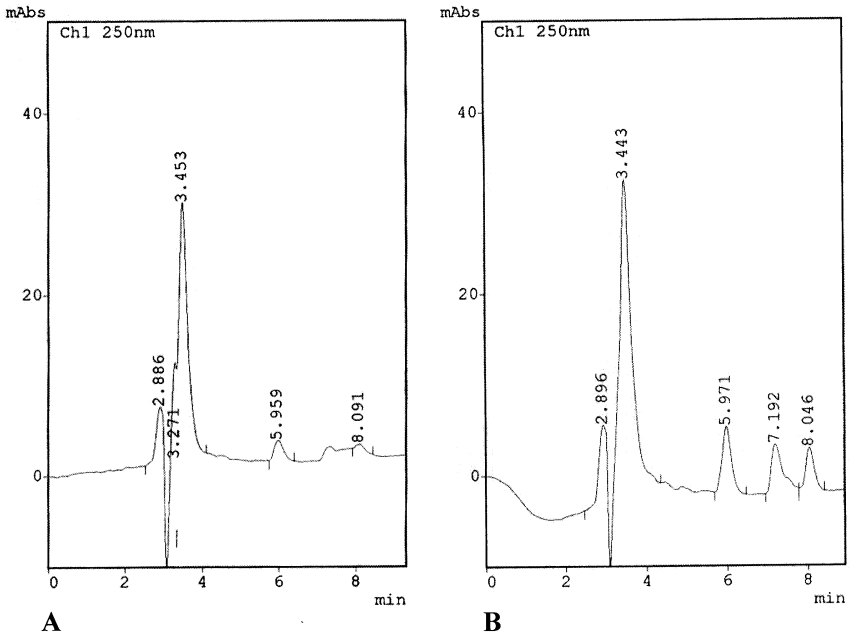


***Figure 7.*** A. Chromatogram of a non-spiked pooled lake water sample. Peaks: 3.453 min = internal standard, 5.969 min = anthraquinone, and 8.091 min = anthracene. B. Chromatogram of a pooled lake water sample spiked with a standard containing 0.5 ng/μL anthraquinone and 1-OH anthraquinone and 0.1 ng/μL in anthracene and 5 ng/μL of internal standard; chromatographic conditions as described under experimental; peaks: 3.443 min = internal standard, 5.971 min = anthraquinone, 7.192 min = 1-hydroxyanthraquinone, and 8.046 min = anthracene.

### Application to Real Samples

Typical chromatograms from spiked and non-spiked river and lake water samples are presented in Figs. 6 and 7. River samples were found to contain 1.21 ng/μL of anthracene, 1.6 ng/μL 1- hydroxy anthraquinone, and 0.74 ng/μL anthraquinone. Lake samples contained 1.29 ng/μL anthraquinone and 0.05 ng/μL of anthracene.

## CONCLUSION

The proposed RP-HPLC method presents a precise and accurate simultaneous determination of anthracene and its oxidation products, anthraquinone and 1-hydroxyanthraquinone, appearing in the literature for the first time. The method makes use of a simple linear methanol–ammonium acetate gradient, over ten-minutes' time and is characterized by high sensitivity, which makes it suitable for environmental monitoring of anthracene and its derivatives. The method has been applied to the analysis of river and lake waters with satisfactory precision and accuracy. Solid phase extraction combined with sonication was proven to yield the higher recovery rates.



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