Determination of Benzo[a]pyrene in Municipal Drinking Water Using Automated Solid-Phase Extraction and Liquid Chromatography with Fluorescence Detection

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Keywords
Dionex AutoTrace 280 SPE, HPLC-FLD, Polyaromatic hydrocarbons, Acclaim C18; GB5749-2006

Introduction

Benzo[a]pyrene is a polycyclic aromatic hydrocarbon (PAH) formed by the fusion of benzene and pyrene, the most common sources of which are emissions from the burning of fuels, barbecuing and deep-frying foods, and car exhaust fumes. It is a highly active indirect carcinogen prone to causing lung cancer and heart disease. For this reason, environmental agencies and organizations around the world have set strict limits on the amount of benzo[a]pyrene in water, with the British HPA setting a limit of 0.01 µg/L, the US Environmental and Protection Agency (EPA) setting a limit of 0.2 µg/L, and the World Health Organization setting a limit of 0.7 µg/L. Similarly, China's Ministry of Environmental Protection (MEP) promulgated Environmental Quality Standards for Surface Water GB3838-2002[1] mandating a benzo[a]pyrene content limit of 2.8×10^{-6} mg/L in environmental water samples.

The recommended extraction and analysis method for benzo[a]pyrene in environmental waters is Chinese national standard GB 13198-91. This method starts with liquid-liquid extraction using n-hexane to enrich benzo[a]pyrene from water samples, then drying and purifying the liquid extract using Florisil and anhydrous sodium sulfate, concentrating the extract and bringing it to a defined volume, and measuring the benzo[a]pyrene content using HPLC with fluorescence detection (FLD).[2]

The Chinese Ministry of Environmental Protection also passed the GB5749-2006,[3] a Domestic Drinking Water Health Standard mandating a benzo[a]pyrene content limit of 1.0×10^{-5} mg/L in drinking water. This Standard also recommends the use of liquid-liquid extraction with cyclohexane to enrich benzo[a]pyrene from water samples, followed by purifying and drying the liquid extract using aluminum oxide and anhydrous sodium sulfate, concentrating the extract to a defined volume, and measuring the benzo[a]pyrene content using HPLC-FLD.[4] These methods both use liquid-liquid extraction to separate benzo[a]pyrene from the water substrate; they are non-selective and cumbersome processes.

Solid-phase extraction (SPE) technology uses a selective solid phase to achieve the selective absorption and elution of the target analyte. Compared to liquid-liquid extraction technology, it is simpler, has better selectivity, reduces the use of organic solvents, creates less environmental pollution, and is less harmful to the health of the personnel conducting the experiment. Therefore SPE extraction is gradually becoming the main sample preparation method for water analysis.

Recently the new Chinese National Standard HJ 478-2009 replaced GB13198-91, adding methods for the SPE enrichment of benzo[a]pyrene, further confirm the benefits of SPE. A Thermo Scientific™ Dionex™ AutoTrace 280 Solid-Phase extraction instrument was used for this study. It reduced analyst intervention and automatically completed SPE column activation, sample loading, and the elution and collection of the target compound, therefore conserving manpower for the laboratory. The eluted samples were solvent exchanged and concentrated to a defined volume, and then measured the trace benzo[a]pyrene in municipal water using HPLC-FLD.

Equipments

- Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system
- Dionex AutoTrace 280 SPE instrument

Test Conditions	
Column:	Thermo Scientific™ Acclaim™ C18 Reversed-Phase Analytical HPLC, 5 µm, 150 × 4.6 mm (P/N 059148)
Column Temp.:	30 °C
Mobile Phase:	Methanol: water=90:10
Flow Rate:	2.00 mL/min
Injection Vol:	20 µL
Detection:	Fluorescence detector (FLD), Ex=290 nm, Em=410 nm
SPE Column:	Cleanert C18, 200 mg/6 mL or equivalent

SPE Steps

Sample Preparation

Tap water was collected from the Thermo Scientific laboratory in Beijing. The water samples were prepared for extraction by adding 100 mL of isopropanol into 500 mL of municipal water and shake well.

Table 1. SPE process.

No.	Process Six Samples Using the Following Procedure
1	Condition cartridge with 5.0 mL methanol (MeOH) into solvent waste
2	Condition cartridge with 5.0 mL ethyl acetate (EtOAc) into solvent waste
3	Condition cartridge with 5.0 mL dichloromethane (DCM) into solvent waste
4	Condition cartridge with 5.0 mL ethyl acetate (EtOAc) into solvent waste
5	Condition cartridge with 5.0 mL MeOH into solvent waste
6	Condition cartridge with 5.0 mL 20% Isopropanol (IPA) into solvent waste
7	Load 650.0 mL of sample onto cartridge
8	Rinse cartridge with 5.0 mL of 20% IPA into aqueous waste
9	Dry cartridge with gas for 10 min
10	Collect 2.0 mL Fraction into sample tube using ethyl acetate
11	Collect 4.0 mL Fraction into sample tube using DCM
12	Collect 4.0 mL Fraction into sample tube using DCM
13	Collect 4.0 mL Fraction into sample tube using DCM

Solvents	
Solvent 1:	Water (reagent grade)
Solvent 2:	CH_3OH (methanol)
Solvent 3:	EtOAc (ethyl acetate)
Solvent 4:	DCM (dichloromethane)
Solvent 5:	20% Isopropanol (IPA)
Flow Conditions	
Condition Flow:	15.0 mL/min
Load Flow:	10.0 mL/min
Rinse Flow:	10.0 mL/min
Elute Flow:	3.0 mL/min
Condition Air Flow:	15.0 mL/min
Rinse Air Flow:	10.0 mL/min
Elute Air Flow:	3.0 mL/min

Use pesticide or HPLC grade solvents

Drying, Solvent Exchanging and Concentrating to a Defined Volume

Place the eluent sample in a nitrogen blowdown unit, concentrate by purging with nitrogen at a constant temperature of 50 °C, perform solvent exchange with ethyl acetate and reduce to 1 mL; then inject 20 µL into the liquid chromatograph, and carry out separation and measurement.

Results and Discussion

Selection of SPE Conditions

Benzo[a]pyrene is hydrophobic and tends to be absorbed onto stationary phase, and therefore the resin base SPE columns are not suitable for Benzo[a]pyrene. Therefore, this study compared extraction effects of different C18 SPE columns and proved that 200 mg/6 mL C18 SPE columns required the lowest volume of eluent and produced the best recovery results.

The relatively strong absorbability of benzo[a]pyrene causes it to leave a certain amount of residue within the sample bottles and instrument sample loading tubes, and decrease the recovery rate for the sample. For this reason, it is necessary to thoroughly wash the sample bottle with an organic solvent after fully transferring the sample into the SPE column. 100 mL of isopropanol was added to every 500 mL sample to increase the solubility of benzo[a]pyrene and reduce the absorption onto the sample bottles and instrument sample loading tubes, therefore eliminating the complicated rinsing process and simplifying the procedure.

The final experiment concentrated trace benzo[a]pyrene in high-volume water samples the Dionex Ultimate 3000 HPLC system a Dionex AutoTrace 280 instrument and then analyzing using HPLC-FLD. A Cleanert C18 (200 mg/6 mL)column was used for the SPE step, sample loading rate is 10 mL/min; ethyl acetate and dichloromethane were used to elute the target compound.

The collected eluent samples were dried by nitrogen blow down, solvent exchanged, concentrated and brought to a defined volume, and then directly injected into an Dionex UltiMate 3000 LC system. Figure 1 shows the chromatogram of municipal drinking water that is not spiked with benzo[a]pyrene. Figure 2 shows the chromatogram of municipal drinking water that is spiked with 0.002 ug/L benzo[a]pyrene.

Spiked Results and Recovery

The several batches of tap water samples were analyzed according to the method (enriched by Dionex AutoTrace 280 instrument and analyzed using HPLC) and conditions, and no benzo[a]pyrene was detected in any of the water samples. The good recovery rates of spiked water samples indicates that this method is accurate and reliable. See Table 1 for selected results.

Table 1. Actual samples and spiked sample recovery rates.[a]

	Measurement µg/L	Addition µg/L	Recovery Rate %
BaP	ND	1.0	98
		2.0	88
		5.0	83
		10.0	90

[a]All test results have been converted to a final constant volume of 1 mL.
ND means not detected.

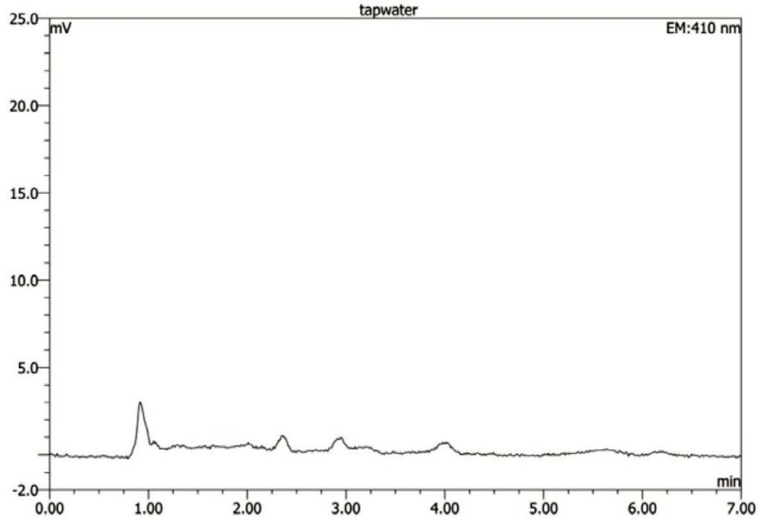

Figure 1. The chromatograms of Municipal water samples (no benzo[a]pyrene detected).

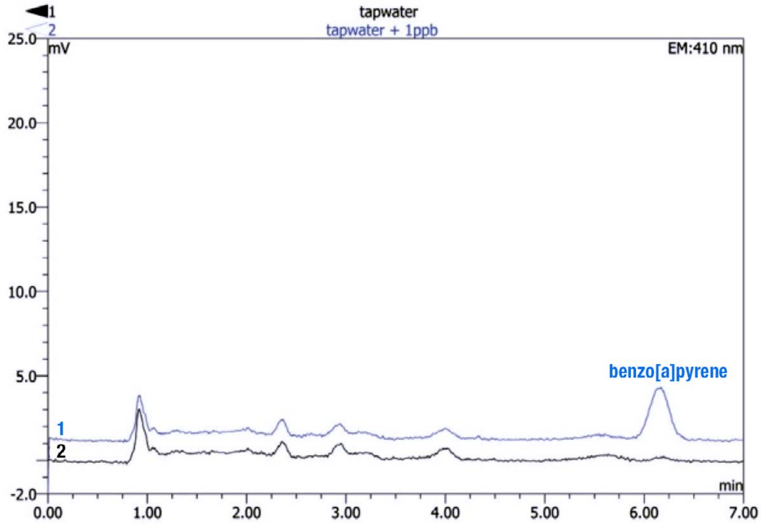

Figure 2. The chromatograms of Municipal water samples and spiked samples.
(1) Municipal water sample); (2) Spiked with 0.002 µg/L of benzo[a]pyrene)

Conclusions

Using this method with the conditions selected, it is possible to accurately quantify the benzo[a]pyrene content in municipal water at less than 0.002 µg/L, which is in full compliance with the limits required by Chinese National Standards GB 5749-2006 and GB 3838-2002. Using a fully automated SPE instrument reduces the use of organic solvents and eases concerns regarding environmental pollution, while reducing the number of labor-intensive manual interventions and increasing the efficiency of the experiment.

References

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