Determination of 16 Polycyclic Aromatic Hydrocarbons in Water by Leaps On-line Solid Phase Extraction - High Performance Liquid Chromatography

Preface

Polycyclic aromatic hydrocarbons (PAHs) refer to compounds containing two or more benzene ring structures in their molecules. Due to their teratogenic, carcinogenic, mutagenic and biologically non-degradable properties, they are recognized as major organic pollutants affecting human health. PAHs are widely present in soil, air, water bodies and various foods, and most countries have regulations and restrictions on their contents. In order to strictly control the content of PAHs in daily drinking water, the Standards for Drinking Water Quality (GB 5749-2022) in China has set more stringent limit standards for PAHs.

At present, the most widely used detection methods for PAHs are mainly gas chromatography and liquid chromatography. High performance liquid chromatography (HPLC) is the most common due to its good selectivity and high sensitivity, and has become the preferred method for PAH analysis. As PAHs have low solubility in water, in order to determine trace PAHs in water, the pretreatment method specified in GB 5750.8-2023 Standard Test Methods for Drinking Water - Part 8: Organic Matter Indicators uses large-volume water samples for extraction and concentration. However, the pretreatment steps are cumbersome, prone to introduce measurement errors, time-consuming and require large sample volumes, making it difficult to meet the requirements of rapid analysis.

Aiming at the pretreatment challenges in the detection of PAHs in GB 5750.8-2023, Chromai has launched an on-line solid phase extraction system (online-SPE HPLC) combined with ultraviolet/fluorescence detectors, which can efficiently and accurately determine 16 PAHs in water. This solution features high automation, low solvent consumption, high sensitivity and good stability.

1. Experimental Section

1.1 Instrument System

1.1.1 Chromai Leaps on-line solid phase extraction system

1.1.2 D50Dual ternary gradient pump P60, large-volume automatic sampler A30 (standard 5000μL quantitative loop), column oven C10V1 (standard one 2-position six-way valve), ultraviolet detector D10, fluorescence detector D50.

1.2 Reagents and Materials

1.2.1 Reagents: Methanol (chromatographic grade), acetonitrile (chromatographic grade), water;

1.2.2 Reference substances: 16 PAHs mixed standard (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene) (200 mg/L in acetonitrile, 1 mL)

1.3 Preparation of Standard Solutions

1.3.1 Standard stock solution (10 μg/mL): The mixed standard of 16 PAHs was diluted with methanol to prepare a standard stock solution with a concentration of 10 μg/mL.

1.3.2 Preparation of standard working solutions: Mixed standard solutions with concentrations of 0.5ng/mL, 1ng/mL, 2ng/mL, 3ng/mL, 4ng/mL and 5ng/mL were prepared with 15% methanol aqueous solution.

2. Chromatographic Conditions

- Chromatographic columns: On-line solid phase extraction column: C18 4.0×10 mm, 5 μm.
- Analytical column: PAH special column 4.6×100 mm, 5 μm.
- Column temperature: 30 °C, injection volume: 5 mL. Gradient elution, time program Table 1.
- Detection wavelength: 16 PAHs were detected in series with ultraviolet and fluorescence detectors. Since acenaphthylene among the 16 PAHs has no fluorescence response, it was determined by an ultraviolet detector (wavelength 228 nm), and the other 15 compounds were detected by a fluorescence detector. The wavelength switching time of the fluorescence detector is shown in Table 2.

Table 1 Gradient Elution Time Program

On-line-SPE-1D					**Separation-2D**			
Time/min	Flow rate/mL/min	A%（Water）	B%（Methanol）	Valve position	Time/min	Flow rate/mL/min	A%（Water）	B%（Acetonitrile）
0.01	1.0	90	10	1-6	0.01	1.5	50	50
5	1.0	0	100		5	1.5	50	50
8	-	-	-	1-2	28	1.5	0	100
35	1.0	0	100		32	1.5	0	100
35.1	1.0	90	10		33	1.5	50	50
40	-	-	-	1-6	35	1.5	50	50
50	1.0	90	10					

Table 2 Fluorescence Detector Wavelength Switching Schedule

Time/min	Exitation wavelength/nm	Emission wavelength/nm	Compound
0.01	280	340	Naphthalene, acenaphthene, fluorene
9.9	300	400	Phenanthrene, anthracene

12.5	300	500	Fluoranthene
13.65	300	400	Pyrene, benzo[a]anthracene, chrysene
19.5	300	430	Benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene
24.1	300	400	Dibenzo[a,h]anthracene
25.3	300	430	Benzo[ghi]perylene
26	300	500	Indeno[1,2,3-cd]pyrene

3. On-line Solid Phase Extraction Process

The initial valve position is 1-6. A 5 mL sample is directly injected into the SPE column. After on-line extraction, enrichment and purification, the valve is switched to position 1-2, and the target analytes on the SPE column are flushed into the analytical flow path. After all analytes enter the analytical flow path, they are separated and determined on the analytical column. The valve is switched back to position 1-6, and the SPE column is cleaned to prepare for the next sample.

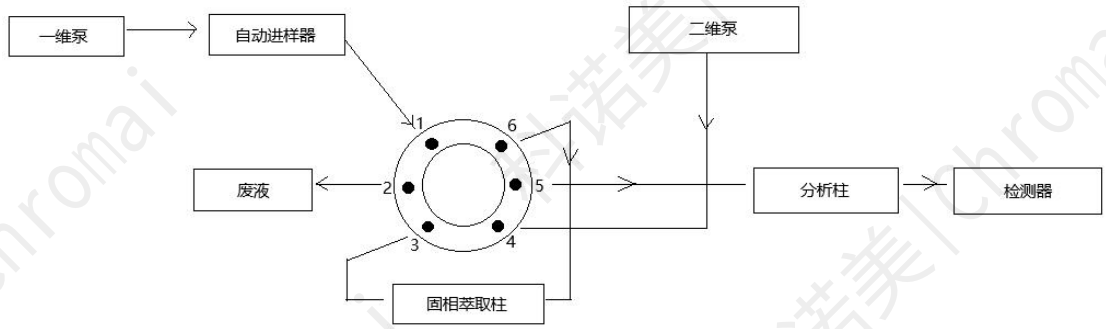

Figure 1 Schematic Diagram of On-line Solid Phase Extraction Process

4. Experimental Results

4.1 Standard Spectra

In this application, the 16 PAHs had good peak shapes, high resolution and stable retention times. The spectra of the ultraviolet and fluorescence detectors are shown in Figure 2.

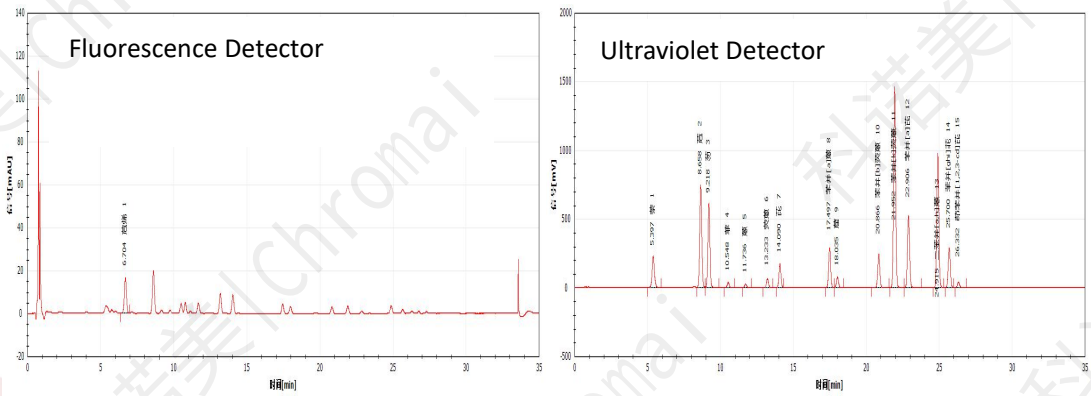

Figure 2 Chromatograms of 16 PAHs Standard Solution

4.2 Linear Relationship

The standard working solutions were injected in order of increasing concentration, and standard curves were plotted with different concentration points as the abscissa and the peak areas of PAHs at each concentration point as the ordinate. Each PAH had a good linear relationship within the linear range of 0.5-5.0 ng/mL, and the linear correlation coefficients were all greater than 0.997.

Table 3 Linear Results of PAHs

Peak	Compound	Linear range (ng/mL)	Linear equation	Correlation coefficient
1	Naphthalene	0.5-5.0	Y=383.95X+12.753	0.9971
2	Acenaphthylene	0.5-5.0	Y=37.339X+2.1973	0.9997
3	Acenaphthene	0.5-5.0	Y=1586.0X+201.18	0.9981
4	Fluorene	0.5-5.0	Y=1205.0X+81.143	0.9987
5	Phenanthrene	0.5-5.0	Y=72.053X-0.5169	0.9992

6	Anthracene	0.5-5.0	Y=45.626X-2.1648	0.9992
7	Fluoranthene	0.5-5.0	Y=123.37X-13.198	0.9997
8	Pyrene	0.5-5.0	Y=338.28X-32.967	0.9998
9	Benzo[a]anthracene	0.5-5.0	Y=576.50X-75.268	0.9998
10	Chrysene	0.5-5.0	Y=153.98X-22.300	0.9996
11	Benzo[b]fluoranthene	0.5-5.0	Y=497.49X-51.601	0.9997
12	Benzo[k]fluoranthene	0.5-5.0	Y=2941.1X-363.46	0.9991
13	Benzo[a]pyrene	0.5-5.0	Y=1085.3X-155.00	0.9988
14	Dibenzo[a,h]anthracene	0.5-2.0	Y=456.10X-26.749	0.9986
15	Benzo[ghi]perylene	0.5-3.0	Y=278.07X-22.364	0.9976
16	Indeno[1,2,3-cd]pyrene	0.5-5.0	Y=72.499X-3.8700	0.9990

4.3 Detection Limit, Quantification Limit, Recovery Rate

In this application, the detection limits of the 16 PAHs were between 0.2 and 7.9 ng/L, all lower than the detection limits specified in GB 5750.8-2023; the recovery rates of each compound at the spiked concentration of 1.0 ng/mL were between 90% and 120%, and the precision was less than 2.0%.

Detection Limit, Quantification Limit, Recovery Rate

Peak	Compound	Detection limit (ng/L)	Quatification limit (ng/L)	Spiked concentration 1.0 ng/mL		GB 5750.8-2023 Detection limit (ng/L)
				Recovery %	RSD%	
1	Naphthalene	0.24	0.79	96.81%-117.66%	1.97%	20.0
2	Acenaphthylene	7.9	26.7	105.91%-109.37%	1.33%	8.0
3	Acenaphthene	0.2	0.66	111.50%-115.47%	1.35%	8.0
4	Fluorene	0.3	1.0	103.51%-108.52%	1.82%	16.0
5	Phenanthrene	4.9	16.2	105.56%-106.93%	0.78%	20.0
6	Anthracene	8.2	27.1	102.42%-103.82%	0.56%	12.0

7	Fluoranthene	3.3	10.9	97.18%-99.80%	1.07%	16.0
8	Pyrene	1.2	4.0	98.61%-99.96%	0.57%	12.0
9	Benzo[a]anthracene	0.74	2.4	96.32%-97.18%	0.37%	5.0
10	Chrysene	2.8	9.2	97.96%-98.56%	0.28%	8.0
11	Benzo[b]fluoranthene	0.86	2.8	104.68%-107.38%	1.14%	5.0
12	Benzo[k]fluoranthene	0.14	0.46	97.41%-100.54%	1.72%	4.0
13	Benzo[a]pyrene	0.41	1.35	107.55%-110.01%	1.16%	2.0
14	Dibenzo[a,h]anthracene	0.9	2.97	107.79%-115.78%	1.67%	8.0
15	Benzo[ghi]perylene	1.5	4.95	110.33%-114.07%	1.48%	8.0
16	Indeno[1,2,3-cd]pyrene	5.4	17.8	107.95%-110.83%	1.11%	6.0

5. Conclusion

This study employs the Chromai Leaps on-line solid-phase extraction-high performance liquid chromatography (on-line SPE-HPLC) system combined with ultraviolet and fluorescence detectors to determine trace polycyclic aromatic hydrocarbons (PAHs) in water. Traditional solid-phase extraction involves cumbersome pretreatment steps, high consumption of reagents and consumables, and is prone to significant errors. In contrast, this method utilizes on-line sample concentration and purification, transferring enriched compounds to the analytical flow path via valve switching for quantitative analysis.

The process is simple and easy to operate, with high sensitivity and good stability. The linear correlation coefficients of all 16 PAHs are greater than 0.997, the spiked recovery rates range from 90% to 120%, and the detection limits of all compounds are lower than those specified in GB 5750.8-2023. This method is suitable for rapid and efficient detection of trace PAHs in water.