

## Rapid analysis of benzo(a)pyrene in water at ppt level using online SPE coupled with HPLC with UV detection

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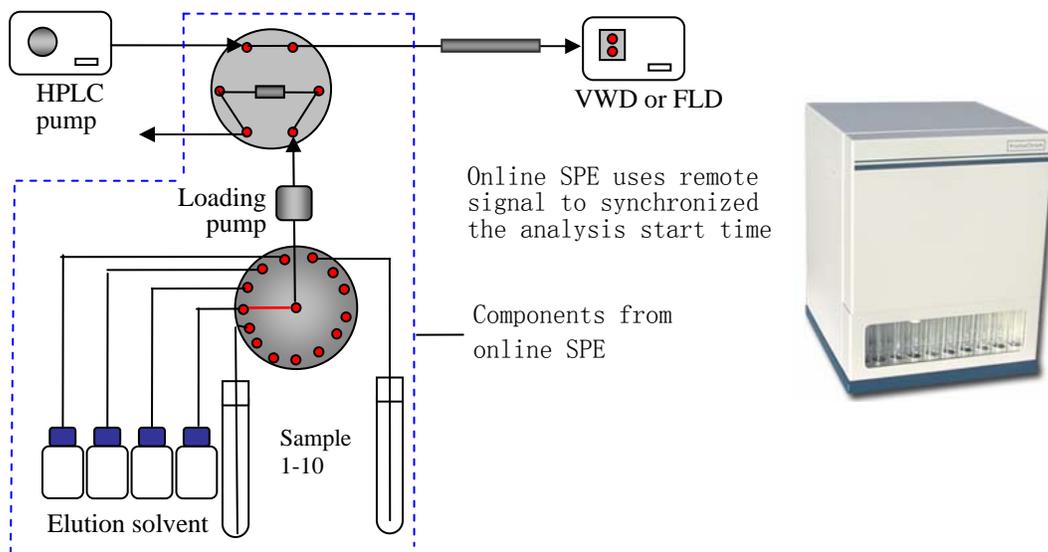
Benzo(a)pyrene is a carcinogenic environmental pollutant. Its analysis in water involves extraction of 500-1000 mL of sample using solid phase extraction or liquid/liquid partition, concentration of the extracts to 0.5-1 mL, and determination using GC-MS or HPLC. It normally takes 1-2 hours for analyzing one sample.

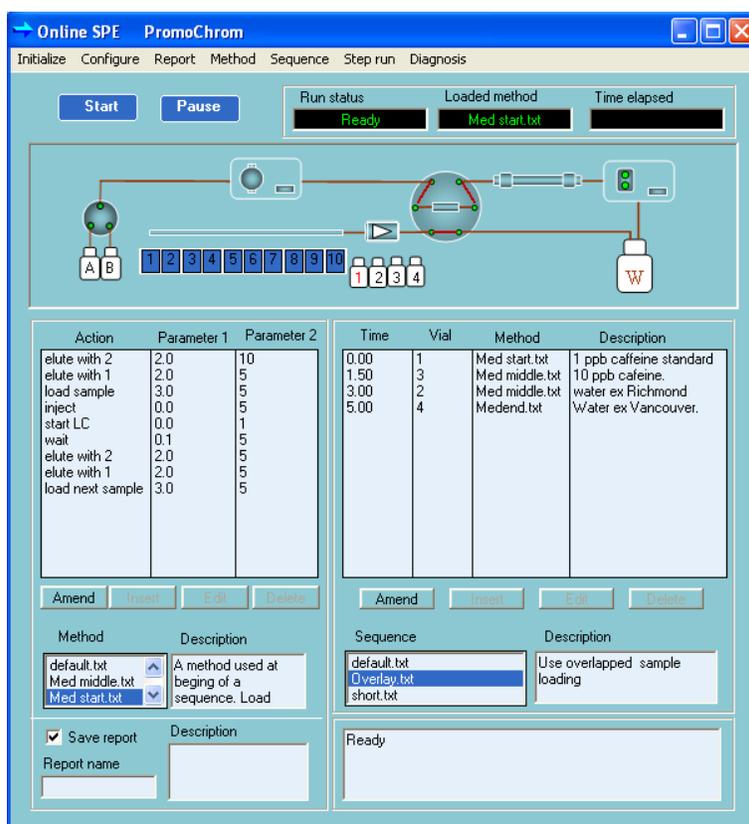
This application note describes a fast method for direct analysis of benzo(a)pyrene in water using online solid phase extraction coupled with HPLC with UV or fluorescence detection. By integrating sample extraction with HPLC analysis, the analysis time is reduced to 10 minutes and the sensitivity can reach sub ppt and even ppq level (part per quadrillion; pg/L).

### Instruments

1. HPLC: Agilent 1100 with a quaternary pump, an online degasser, and a variable wavelength detector.
2. Online solid phase extraction: Online SPE from PromoChrom Technologies

The working principle of the instruments is shown in the following picture:





User interface of the control software for online SPE

The structure of the software for Online SPE is similar to the Agilent ChemStation. It uses methods and sequences to achieve automation of the analysis. It has a function for overlapped injection. When the HPLC is analyzing a sample, the online SPE can start processing the next sample. For many cases, the sample preparation does not use any extra time at all and sample through put simply rely on the analysis of the HPLC.

## Methods

### 1. HPLC analysis

Column: PromSil C18, 4.6 x 250 mm, 5 µm; column temperature: ambient; mobile phase: methanol; flow rate: 1.5 mL/min; detection wavelength: 254 nm; analysis stop time: 9 minutes.

Under this condition, the retention time of penzo(a)pyrene is 5.5 minutes.

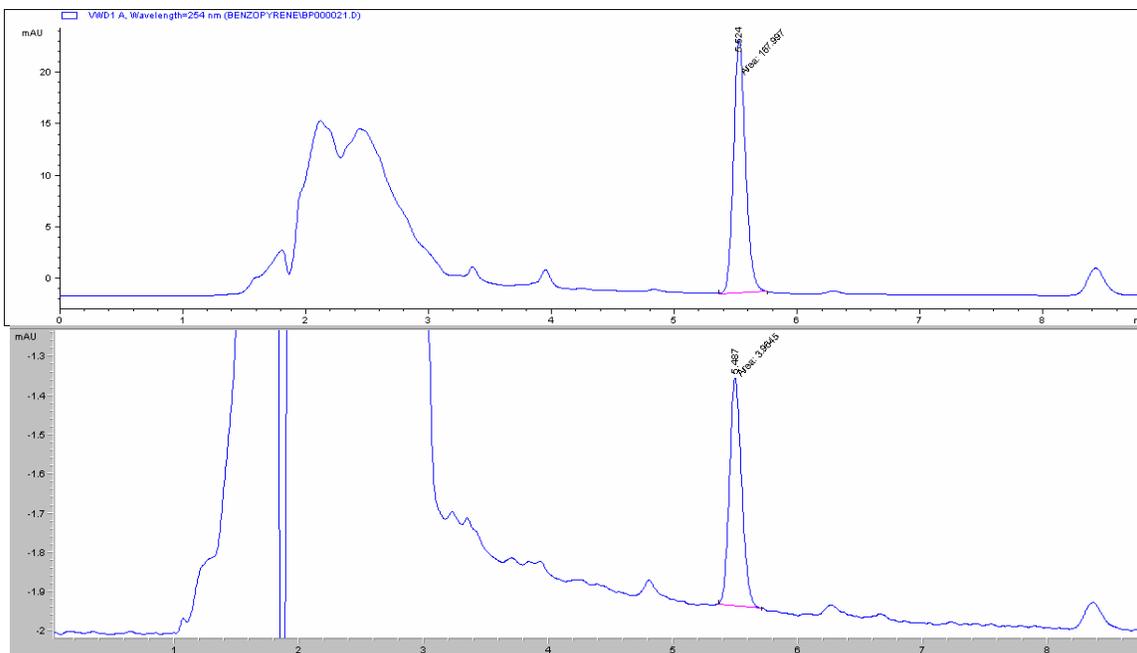
### 2. Online solid phase extraction

SPE column: Trap N, 4.6x10 mm; flow rate of loading pump: 6 mL/min; procedures:

load 20-40 mL of sample, elute SPE column using 2 mL IPA+water (10:90), inject and trigger HPLC start.

## Results

### 1. Sensitivity



Upper figure: tap water spiked with 2.5 ppb benzo(a)pyrene, inject 20 mL

Lower figure: tap water spiked with 25 ppt benzo(a)pyrene, inject 40 mL

The sample with 25 ppt benzo(a)pyrene gives a signal-to-noise ratio of 93. The quantitation limit of the method is estimated as 3 ppt. If a fluorescence detector is used, the sensitivity can increase 50-100 times. It is in part per quadrillion level. A fluorescence detector also offers better selectivity.

### 2. Repeatability

Analysis of tap water sample spiked with 2.5 ppb benzo(a)pyrene was analyzed was repeated 3 times. Below is the result:

Repeat	Peak Area(s.mAU)
1	166.6
2	169.5
3	168.2
Average	168.1
CV%	0.86

In case of analysis using offline SPE for extraction, the error is normally above 5% at this spike level. Since the online SPE approach does simplified procedure and does not involve human operation, the chance of error is considerably reduced.

**Comparison with methods based on offline SPE**

<b>Item</b>	<b>Offline SPE</b>	<b>Online SPE</b>
Efficiency	One sample needs 1 hour or more. This includes extraction of 500-1000 mL sample, reducing extracts to near dry, bringing back to 0.5-1 mL, and instrument determination.	One sample needs 10-15 minutes. By using overlapped injection, the sample extraction does not need extra time.
Material cost	Each sample needs one SPE column (\$2) and 30-50 mL high purity solvent (\$2).	One sample needs 3-5 mL high purity solvent (\$0.2). One SPE column can handle 50-100 samples (\$1 per sample).
Data quality	The error at ppb level is normally above 5%	Error is below 5% even at ppt level, due to simplified procedures and less human involvement.

Based on above comparison, if the analytes can be analyzed using HPLC, online SPE method is a better choice than offline SPE.

In spite of the advantages of online SPE, its application has been hindered by the following factors: 1) Expensive instrument; 2) complex operation and maintenance; 3) difficulties in method development.

To promote the use of online SPE, PromoChrom has made following improvements:

1) Reduce cost. The online SPE includes function of auto sampler and the cost is lower than an HPLC auto sampler. It also includes an elution pump, so that the HPLC does not need an auto sampler and a secondary pump. The cost of an online SPE-HPLC system is similar to a normal HPLC.

2) Simplified operation. Users only need to use simple commands (such as “load sample”, “elute with 1”, etc.). The instrument will look after the valve switch procedures.

3) Ready solution with guaranteed result. The ready solution includes SPE column, analytical column, sample handling procedures, and detailed methods. It can save users a lot of efforts in method development.