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Automated precolumn concentration and high-performance liquid chromatographic analysis of polynuclear aromatic hydrocarbons in water using a single pump and a single valve

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Abstract

The analysis of polynuclear aromatic hydrocarbons (PAHs) has been widely practiced using liquid–liquid extraction as a method of sample clean-up and sample enrichment. For the analysis of PAHs by HPLC, alternative sample preparation methods using solid-phase extraction or precolumn concentration have been reported. These methods normally required a switching valve and/or pump in addition to the HPLC system. This study reports a precolumn concentration method using no additional valve or pump to the HPLC system. The method is evaluated for recoveries, reproducibilities, linearity and minimum detection limits.

1. Introduction

A major current environmental concern is the presence of polynuclear aromatic hydrocarbons in soil, water, petroleum products, seafood etc. Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous pollutants introduced into the environment by the pyrolysis or combustion of organic material. The presence of these compounds in the environment is a health concern due to their carcinogenicity. The US Environmental Protection Agency (EPA) has classified sixteen PAHs as priority pollutants and is requiring monitoring of drinking water, industrial waste water and groundwater from waste disposal sites.

Methods for PAH analyses have been developed using HPLC with UV–Vis and fluorescence detection [1–6].

The current EPA Method 550 for determination of PAHs in drinking water uses liquid–liquid extraction for sample preparation. Liquid–liquid extraction is tedious, time-consuming and produces large amounts of waste organic solvent.

As an alternative or improved method over liquid–liquid extraction for sample clean-up and enrichment, solid-phase extraction (SPE) has been developed over the last fifteen years. Manual off-line SPE has been demonstrated in the analysis of PAHs in drinking water [3,7] and in seafood [8]. Further, SPE performed on-line with HPLC has been reported in the analysis of biological fluids [9,10]. The precolumn used for SPE was placed on-line between the autosampler and the analytical column. It was controlled by a switching valve and swept by one or more external pumps [3,7–10]. Another simplified (and less expensive) configuration was reported [11] where the autosampler, switching valve and external pump were replaced by a manual sy-

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ringe-loading injector. On-line precolumn concentration has also been reported in the analysis of drinking water [12] in which an autosampler specially adapted for precolumn switching was used.

In this study, we describe a new automated method of precolumn concentration for the analysis of drinking water. This method uses an autosampler controlled by a customized "Method Development Language" and a precolumn which replaces the sample loop in the autosampler. The only switching valve used is the one in the autosampler and the only pump used is the HPLC pump. No extra switching valve or external pump is required. A small aliquot of the sample (typically 1.5 ml or multiples of it) is concentrated or enriched on a precolumn using the technique of SPE, and then eluted on-line onto the HPLC system. In the HPLC analysis, the sensitivity for the different PAHs was optimized by using a variable-wavelength program for both fluorescence and UV detection.

This precolumn concentration process is fast and simultaneous with the HPLC analysis of the previous sample. Compared to liquid–liquid extraction, it saves time and reduces solvent waste significantly. The system configuration is the same as a regular HPLC system except for the unique autosampler.

2. Experimental

2.1. Instrumentation

The HPLC system, consisting of P4000 pump with solvent-conditioning module, AS3000 autosampler, FL2000 fluorescence detector, FOCUS UV–Vis detector, PC1000 software and Method Development Language software, was from Thermo Separation Products (Fremont, CA, USA). The PAH column, 15 cm × 4.6 mm with built-in guard column was from Keystone Scientific (Bellefonte, PA, USA). The precolumn used was an Aquapore RP-18 cartridge, 1 cm × 3.2 mm from Perkin-Elmer, Applied Biosystems Division (Foster City, CA, USA).

2.2. Materials

PAH standard mixture was obtained from Supelco (Bellefonte, PA, USA). Acetone, HPLC grade, and acetonitrile, HPLC grade were from J.T. Baker (Phillipsburg, NJ, USA).

2.3. Methods

An automated precolumn concentration (PCC) system was set up as follows: the sample loop in the injection valve of the AS3000 was replaced with an Aquapore precolumn. The AS3000 was controlled by a customized "Method Development Language" which manipulated the syringe movements, injection valve, flush valve and solvent valve. For conditioning of the precolumn, four different solvents could be selected to pass through the precolumn in increments of up to 1.5 ml (preparative syringe capacity in the autosampler), with coordination of the syringe and the valve positions. The system also picked up sample from a vial, up to 1.5 ml each time (autosampler vial capacity) and loaded it onto the precolumn, thus concentrating the sample on the precolumn. Finally a rinse step was performed using the same mechanism as the conditioning step. The waste in each PCC step went directly to a waste line in the autosampler. The injection valve was then switched and the mobile phase eluted the precolumn concentrated analytes onto the analytical column. While the HPLC was analyzing a sample, the precolumn concentration of the next sample was performed simultaneously.

The PCC method used in this study consists of the following steps: (1) the precolumn was conditioned with 1.5 ml methanol, followed by 1.3 ml water (Fig. 1a); (2) 1.5 ml sample was loaded on the precolumn (Fig. 1b and c); (3) the precolumn was rinsed with 1 ml water (Fig. 1a); (4) the concentrated sample was injected onto the HPLC system (Fig. 1d).

HPLC conditions are shown in Table 1.

Calibration standard was prepared by diluting the standard mixture (concentrations shown in Table 2) 1:100 with acetone. A 5-μl volume was injected (using Push Loop) for calibration.

Table 1
HPLC conditions

Column	Keystone PAH, 15 cm × 4.6 mm with built-in guard column			
Mobile phase	Time (min)	Acetonitrile (%)	Water (%)	
	0.00	50	50	
	3.00	50	50	
	23.00	95	5	
	30.00	95	5	
	30.01	50	50	
	33.00	50	50	
Mobile phase	Time (min)	Acetonitrile (%)	Water (%)	
modification	0.00	51	49	
for 577-μl	0.10	51	49	
loop	0.11	50	50	
injection	3.00	50	50	
	Remainder of gradient same as above			
Flow-rate	1.5 ml/min			
Detection:	Fluorescence with timed wavelength program:			
	Time (min)	Excitation (nm)	Emission (nm)	
	0.00	220	340	Auto zero
	8.50	266	324	Auto zero
	10.70	250	380	Auto zero
	13.60	230	420	Auto zero
	17.00	270	388	Auto zero
	22.00	250	420	Auto zero
	27.50	290	420	Auto zero
	31.00	300	482	Auto zero
	33.00	300	482	
	UV with timed wavelength program:			
	Time (min)	Wavelength (nm)		
	0.00	270	Auto zero	
	10.80	254	Auto zero	
	13.60	240	Auto zero	
	17.50	260	Auto zero	
	21.50	254	Auto zero	
	27.50	300	Auto zero	
	33.00	300		

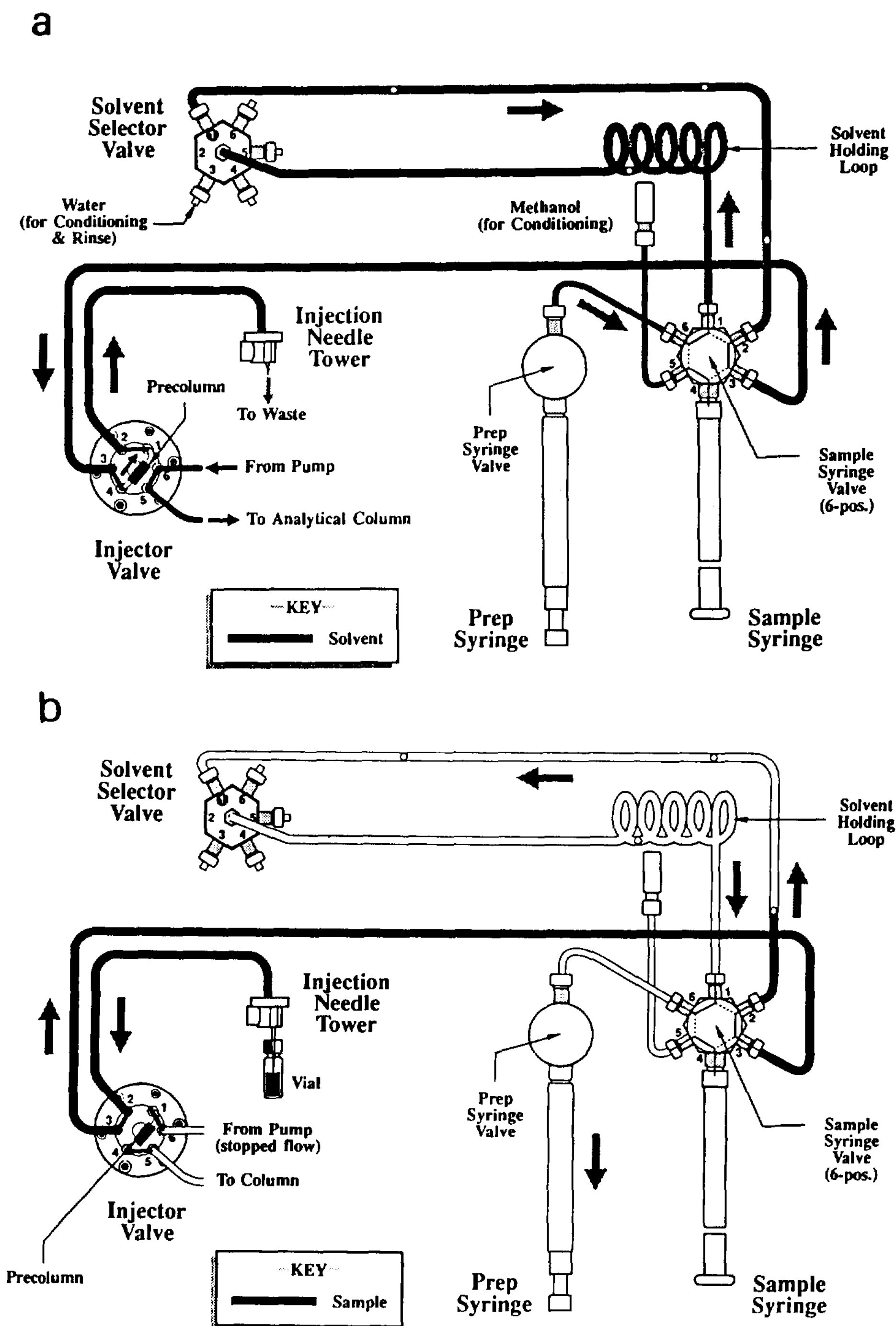

Fig. 1. (a) Precolumn conditioning and rinsing flow path (methanol passes through solvent holding loop and precolumn to waste). (b) Sampling flow path (sample drawn from sample vial to holding loop). (c) Precolumn loading flow path (sample pushed from holding loop through precolumn to waste). (d) Injection flow path (mobile phase flows through precolumn, eluting sample onto HPLC column). Prep = Preparative.

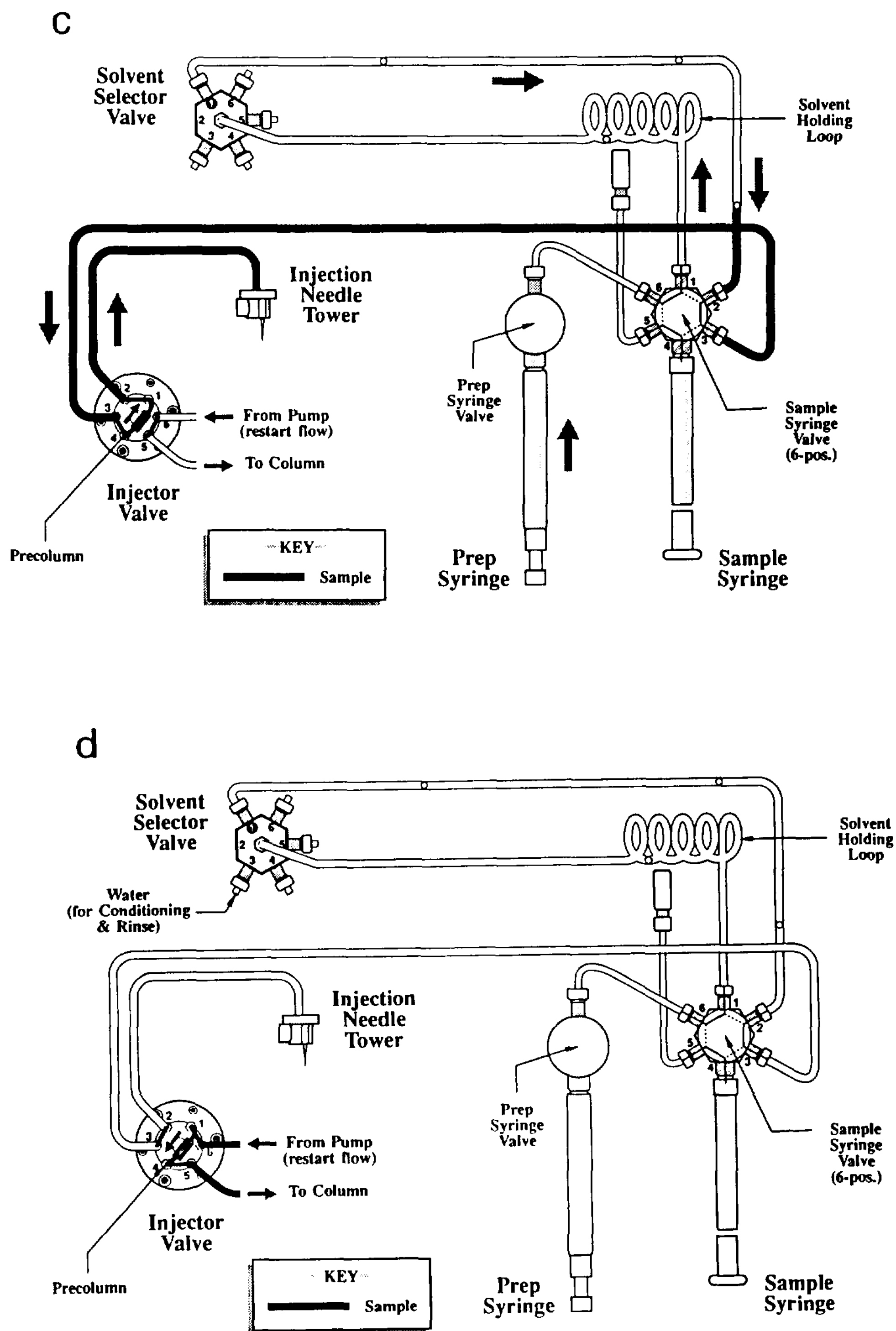

Fig. 1 (*Continued*).

Table 2
Concentrations of PAH standard mixture from Supelco

Component	μg/ml
(1) Naphthalene	1000
(2) Acenaphthylene	2000
(3) Acenaphthene	1000
(4) Fluorene	200
(5) Phenanthrene	100
(6) Anthracene	100
(7) Fluoranthene	200
(8) Pyrene	100
(9) Benz[*a*]anthracene	100
(10) Chrysene	100
(11) Benzo[*b*]fluoranthene	200
(12) Benzo[*k*]fluoranthene	100
(13) Benzo[*a*]pyrene	100
(14) Dibenz[*a,h*]anthracene	200
(15) Benzo[*ghi*]perylene	200
(16) Indeno[1,2,3-*cd*]pyrene	100

Spiked blank was prepared by spiking 1 l of HPLC-grade water with 1 ml of the calibration standard and used to determine recovery, reproducibility and linearity of the PCC method.

To determine recovery, a full-loop injection of the spiked blank was injected as the *reference standard*. The loop was pre-calibrated to be 577 μl. To compensate for the retention time shifting effect of the large volume of weak solvent injected, the gradient was modified by using an initial mobile phase of acetonitrile–water (51:49) for 0.1 min. The concentration of this reference standard was calculated using the calibration standard above. Then 1500 μl of the spiked blank were analyzed using the PCC method.

To determine reproducibility of multiple analyses of the same spiked blank, six consecutive PCC–HPLC runs of 1500 μl of the spiked blank were performed. To determine reproducibility of recoveries among different spiked blanks, 3 l of water in three different flasks were spiked, and the recoveries of six samples from each spiked blank were averaged. The relative standard deviation of the results of the three spiked blanks were calculated.

To determine linearity (of this method, not of the calibration standard) a 1500-μl blank was run, followed by 300, 600, 900, 1200, 1500 and 1800 μl of spiked blank. Due to the volume limitation of the sample vial, the 1800-μl spiked blank was composed of two consecutive samplings, 900 μl each, from two consecutive vials.

To determine the minimum detection limits, 50, 30 and 20 μl of the spiked blank were run. The detection limit is defined as the amount of sample which yields a peak at a signal-to-noise ratio equal to 3.

3. Results

The chromatogram of 1.5 ml of spiked blank using UV detection shows 16 peaks at a very low signal-to-noise level (Fig. 2a). The chromatogram using simultaneous fluorescence detection shows 14 peaks with significantly higher sensitivity compared to UV while acenaphthylene and indeno[1,2,3-*cd*]pyrene are undetected (Fig. 2b). In this study, UV data are used for the calculations for acenaphthalene, but are too close to the detection limit for the other peaks. Therefore only fluorescence data are used for the calculations for the other peaks, except indeno[1,2,3-*cd*]pyrene, for which neither fluorescence nor UV has sufficient sensitivity for this application.

3.1. Recovery

Normally, recoveries are calculated based on the calibration standard. However, in this study, the reference standard was found to have a significant loss in concentration from the theoretical values based on dilution of the calibration standard. The experimental and theoretical values of the reference standard are compared in Table 3. The recoveries of the spiked blank based on the reference standard (assumed to be 100%) are also shown in Table 3.

3.2. Reproducibility

Reproducibility of retention times (Table 4) and areas (Table 5) of six consecutive PCC-injection sequences of 1500 μl of a spiked blank is shown as relative standard deviation (R.S.D.)

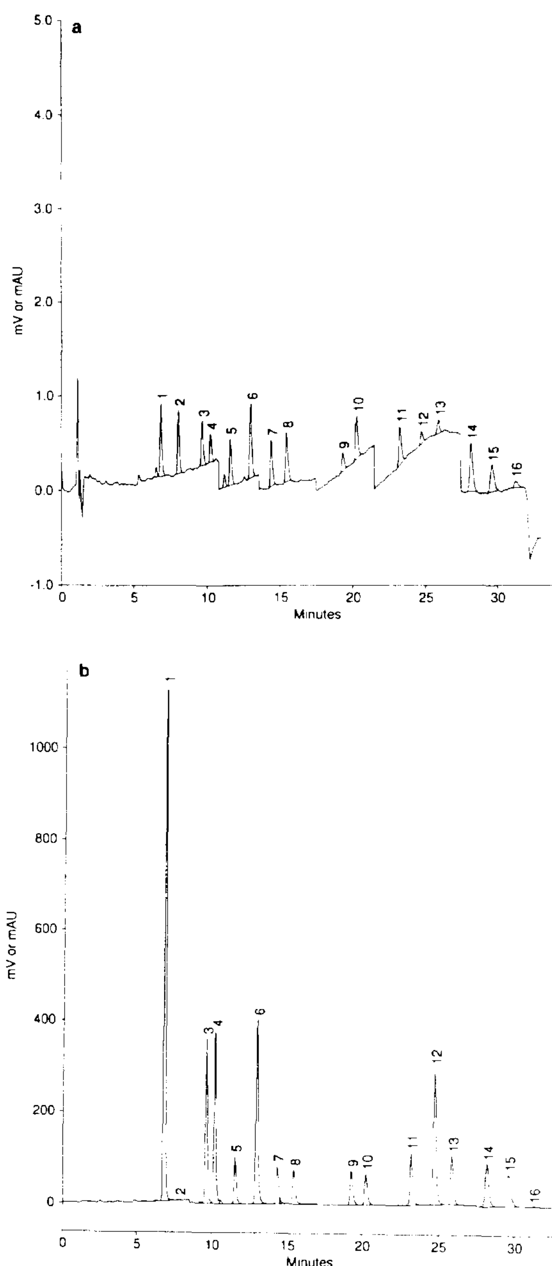

Fig. 2. Chromatograms of PAH priority pollutants in 1.5 ml of spiked blank using PCC–HPLC and (a) UV detection or (b) fluorescence detection. Peaks: 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a,h*]anthracene; 15 = benzo[*ghi*]perylene; 16 = indeno[1,2,3-*cd*]pyrene.

for each peak as automatically calculated using the PC1000 software.

Reproducibilities of recoveries of three different spiked blanks are also shown in Table 5.

3.3. Linearity

A six-point calibration was obtained for peaks 1 and 3–15 using fluorescence. A five-point calibration was obtained for peak 2 using UV since the 300-μl data point fell below the detection limit.

The correlation coefficients determined by fluorescence for these 14 peaks range from 0.9733 to 0.9991. The correlation coefficient determined for acenaphthylene by UV was 0.9965 (Table 6).

3.4. Minimum detection limits

The minimum detection limits for the 15 PAHs in μg/l (of the spiked sample) with a signal-to-noise ratio of 3 are reported in Table 6. Of the 15 compounds, 14 meet the minimum detection limit spiking level in EPA Method 550 with the exception of fluoranthene. Doubling the load volume lowered the detection limit for fluoranthene to 0.025 μg/l, which meets the minimum detection limit spiking level.

4. Discussion

The most significant advantage of the PCC method presented is its efficiency compared to liquid–liquid extraction. The PCC process takes only 10 to 15 min (vs. hours in liquid–liquid extraction) and can be performed simultaneously with the previous HPLC analysis. The amount of organic waste per sample is reduced by approximately 200 ml per extraction, or several liters per week for the average environmental laboratory.

While off-line SPE has the advantage of using a new cartridge for each sample and allows a large volume of sample to pass through, the PCC method here has shown clean blanks between samples.

A major difference between this method and

Table 3
Concentration of reference standard and recoveries of spiked blank (fluorescence detection, except peak 2, UV detection)

Peak	Theoretical concentration (μg/l)	% Of theoretical concentration found in reference standard	Recovery (%) with PCC based on reference standard
(1) Naphthalene	10	117	85
(2) Acenaphthylene	20	94	81
(3) Acenaphthene	10	97	81
(4) Fluorene	2	99	75
(5) Phenanthrene	1	97	65
(6) Anthracene	1	100	59
(7) Fluoranthene	2	92	58
(8) Pyrene	1	89	61
(9) Benz[*a*]anthracene	1	58	35
(10) Chrysene	1	60	39
(11) Benzo[*b*]fluoranthene	2	53	32
(12) Benzo[*k*]fluoranthene	1	65	38
(13) Benzo[*a*]pyrene	1	49	36
(14) Dibenz[*a,h*]anthracene	2	128	59
(15) Benzo[*ghi*]perylene	2	94	65
(16) Indeno[1,2,3-*cd*]pyrene	1	–	–

EPA Method 550 is that an aliquot (1.5 ml) of the spiked blank is analyzed here while the whole spiked blank (1 l) is analyzed in the EPA method. In this study, the spiked blank was found, by direct loop injection, to have a significantly lower concentration than the theoretical value assuming complete solution. For that reason, relative (rather than absolute) recoveries were calculated based on the reference standard.

Table 4
Reproducibility of retention times for precolumn concentrated PAH priority pollutants

Peak	R.S.D. of retention time (n = 6)
(1) Naphthalene	0.84
(2) Acenaphthylene[a]	0.78
(3) Acenaphthene	0.65
(4) Fluorene	0.68
(5) Phenanthrene	0.69
(6) Anthracene	0.69
(7) Fluoranthene	0.64
(8) Pyrene	0.60
(9) Benz[*a*]anthracene	0.54
(10) Chrysene	0.56
(11) Benzo[*b*]fluoranthene	0.53
(12) Benzo[*k*]fluoranthene	0.53
(13) Benzo[*a*]pyrene	0.52
(14) Dibenz[*a,h*]anthracene	0.63
(15) Benzo[*ghi*]perylene	0.67

[a] By UV detection. All other peaks by fluorescence detection.

By using the technique of precolumn concentration and fluorescence detection, this method is able to meet the spiking-level requirement for 14 of the 16 PAHs analyzed when only 1.5 ml (from one autosampler vial) was loaded. When two vials of the sample are loaded, increasing the sample size to 3 ml, the detection sensitivity for fluoranthene can be increased to meet the EPA-required spiking level. The detection limit study was not performed by actual dilution to the EPA-required spiking level and analyzing 1500 μl because of the non-linearity of the dilution of the spike. For the same reason, linearity was not performed by analyzing 1500 μl of different dilutions, but rather by different volumes of the same dilution.

This PCC method produced good results for linearity. Peak area R.S.D.s are less than 10% in 40 out of the 48 instances and recoveries are higher than 50% in 9 out of 15 analytes in Table 5. Calculations of recoveries were dependent on the peak areas of the reference standard, which

Table 5
Reproducibility of areas and recoveries for precolumn concentrated PAH priority pollutants for different spiked blanks

Peak	R.S.D. of peak areas (%)			Recoveries	
	Spiked blank 1 ($n=6$)	Spiked blank 2 ($n=6$)	Spiked blank 3 ($n=6$)	Average recovery (%) ($n=3$)	R.S.D. (%) ($n=3$)
(1) Naphthalene	4.78	1.95	6.47	80.3	9.01
(2) Acenaphthylene[a]	1.35	3.48	2.84	72.0	11.0
(3) Acenaphthene	3.41	2.11	2.57	85.0	12.4
(4) Fluorene	3.25	2.29	5.34	71.7	6.9
(5) Phenanthrene	3.77	4.38	7.09	63.0	11.4
(6) Anthracene	3.25	5.20	4.02	56.3	11.4
(7) Fluoranthene	3.58	7.56	5.04	56.7	9.7
(8) Pyrene	5.44	7.60	7.47	61.2	16.2
(9) Benz[*a*]anthracene	4.17	11.11	5.60	34.3	14.7
(10) Chrysene	3.83	10.83	4.31	36.7	21.2
(11) Benzo[*b*]fluoranthene	22.13	18.92	2.05	26.0	25.2
(12) Benzo[*k*]fluoranthene	11.69	20.01	4.56	40.7	32.5
(13) Benzo[*a*]pyrene	10.86	3.90	5.15	30.3	18.6
(14) Dibenz[*a,h*]anthracene	3.40	10.27	1.53	49.0	28.9
(15) Benzo[*ghi*]perylene	4.29	7.65	1.47	73.0	15.5

[a] By UV detection. All other peaks by fluorescence detection.

was a 577-μl injection. No doubt, that had a volume overloading effect causing a certain amount of peak broadening, but this reference standard eliminates the variable of PAH solubility in the spike blank for the purpose of evaluating the PCC method.

Table 6
Correlation coefficients and minimum detection limits of precolumn concentrated PAH priority pollutants

Peak	Correlation coefficient	Minimum detection limit (μg/l)
(1) Naphthalene	0.9950	0.29
(2) Acenaphthylene[a]	0.9965	4.44
(3) Acenaphthene	0.9994	0.25
(4) Fluorene	0.9908	0.05
(5) Phenanthrene	0.9987	0.025
(6) Anthracene	0.9962	0.026
(7) Fluoranthene[b]	0.9977	0.05
(8) Pyrene	0.9733	0.022
(9) Benz[*a*]anthracene	0.9816	0.007
(10) Chrysene	0.9841	0.012
(11) Benzo[*b*]fluoranthene	0.9841	0.01
(12) Benzo[*k*]fluoranthene	0.9845	0.009
(13) Benzo[*a*]pyrene	0.9889	0.01
(14) Dibenz[*a,h*]anthracene	0.9854	0.02
(15) Benzo[*ghi*]perylene	0.9991	0.018

[a] By UV detection. All other peaks by fluorescence detection.
[b] Using twice the sample volume lowers the minimum detection limit to 0.025 μg/l.

The above finding of a "loss" of PAHs when an aliquot of a spiked blank was analyzed is consistent with some reports on spike recovery studies of PAHs. PAHs are classified as hydrophobic organic compounds which are relatively insoluble in water and tend to absorb onto other non-aqueous phases, either through hydrophobic interaction when the non-aqueous phase is a non-polar compound [13] or through conjugate π bonding when the non-aqueous phase is a polar compound [14,15]. It has been speculated that since spiked analytes may often be less retained on or in the environmental matrices than the native analytes, the use of spike recovery studies may overestimate the efficiencies of extraction methods [16,17]. The results in this study support this statement for extraction of the whole spiked sample. This raises the question as to whether spiked blanks are currently being treated properly using liquid–liquid extraction if the analytes of interest are not homogeneous in the matrix. Further study would be required to resolve this issue.

5. Conclusions

A new method of precolumn concentration has been presented using a single pump and a single valve controlled by a customized "Method Development Language". Analysis of PAHs in water has been performed using this method with as little as 1.5 ml of sample resulting in good reproducibility, linearity and sensitivity. This method also addresses a difference in treatment (compared to the liquid–liquid extraction method) of PAH-spiked water samples which may have a problem of inhomogeneity in the spiking. This method is potentially useful for fast screening of drinking water samples.

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