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Research Article

Determination of bisphenol A with effective pretreatment medium using automated column-switching HPLC with fluorescence detection

A practical way for reducing contaminants, such as humic acids, and solving column-clogging problem in environmental water analysis with liquid chromatography is proposed. Detection interference by contamination is one of the most important issues of the environmental analyses. Moreover, due to the recent smaller diameter and fine particle size of an analytical column for HPLC system, a column-clogging problem is another practical difficulty as well. We found it possible to solve these problems by employing column-switching HPLC, which consists of a pretreatment column containing surface-modified polymer particles and flow changeover valves for cleaning the remaining matrices in the pretreatment column prior to analysis. This method was successfully applied to actual HPLC-fluorescence detection of bisphenol A. Limit of detection (LOD) in real sample was <0.7 ng/L. Repeatability was around 1.4% and recovery was around 97% or more. A particular pressure increase was not observed in 150 repeated analyses of real river water samples.

Keywords: Column clogging / Environmental samples / Fluorescence / Pretreatment medium
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1 Introduction

For the purpose of environmental analysis of trace amounts of chemical substances existing in water samples, various analytical methods are employed. For a comprehensive survey, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) has been employed because of the ease of use, relatively simple protocol and fairly good sensitivity despite relatively low reproducibility [1]. On the other hand, a chromatographic determination affords highly reliable results compared with the other alternative biological methods mentioned. The concerning issue is that even low concentrations of chemical substances, such as endocrine disrupters, in our water resources may have some impacts. Consequently, a highly reliable analytical method affording superior recovery, repeatability as well as sensitivity is strongly required.

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Abbreviations: **BPA**, bisphenol A; **EDMA**, ethylene dimethacrylate; **FLD**, fluorescence detection; **MASK**, methacrylic acid 3-sulfopropyl potassium salt; **NOM**, natural organic matter

An alternative and efficient approach for the analysis to meet these current demands would be to provide a fully automated chromatographic procedure. Among several chromatographic approaches, high-performance liquid chromatographic (HPLC) system has its obvious benefits over other methods [2, 3], such as gas chromatography (GC). HPLC analysis requires simple pretreatment because the target compounds are already contained in liquid phase, whereas GC would require a complicated vaporizing procedure for sample pretreatment [4–8]. Especially, the effective removal of contaminants contributes to sensitive detection with commonly used detectors.

To overcome the potential contamination problems associated with real environmental sample analysis, manual sample pretreatment procedures should be excluded as much as possible. Therefore, an online auto-pretreatment system is the effective alternative. In this study, the online column-switching pretreatment procedure was employed. It can meet the demands of preventing the loss of small amount of sample and large amount of sample concentration as well [9, 10]. An ordinary HPLC column-switching system configuration consists of a pretreatment column connected to an analytical column via a six-port flow changeover valve (high-pressure valve). A volume of 10–100 mL of water sample could be injected onto the pretreatment column by utilizing the column-switching method [11]. Additionally, the column-clogging problem becomes serious when the sample solution contains complex matrices [12–14]. To achieve highly reliable

analytical results of the trace amounts of chemical substances in environmental water samples, a selective concentration is essential.

Selective concentration can be attained by two different approaches. One is using the specific strong interaction between target compounds and the adsorbent. The other is using the weak or no interaction between unwanted co-existing compounds and the adsorbent. One of the former examples is a molecular imprinting technique and one of the latter examples is a surface modification of the adsorbent. Our research group has already reported that those two approaches become compatible techniques in the pretreatment procedures for HPLC and LC–MS analyses [15–17]. Surface modification with methacrylic acid 3-sulfopropyl potassium salt (MASK) onto the pretreatment medium is one of the solutions for interference of detection [16], which is another challenge in real sample analyses with HPLC.

In this work, we added one more high-pressure valve into flow line to wash pretreatment column without applying the existing sample solution in the flow line including suction pipe onto pretreatment column. To confirm the ability of this column-switching HPLC, we took an example of the determination of bisphenol A (BPA), which is known for its endocrine disrupting activities, with fluorescence detection (FLD). Due to the harmful effects on the ecosystem, ultra-low level of detection, such as 1 ng/L, is required [18–23]. Ultra-low level of BPA is commonly detected with MS or MS/MS detector. Effective removal of interference from real water sample provides a possibility to do nanogram per litre level of BPA determination with cheaper and more universal fluorescence detector, which has significant meaning to provide ultra-low level of determination at low-cost facilities.

After optimization of the analytical conditions, we evaluated the fundamental performance of two-valve column-switching HPLC-FLD coupled with MASK-modified pretreatment column using BPA as an analyte. We estimated the repeatability and detection limit with real environmental water samples or natural organic matters (NOM) solution. Furthermore, 180 repeated analyses including auto-pretreatment procedures of real river water were performed and the column pressure change was recorded to confirm the practical robustness of the method.

2 Materials and methods

2.1 Apparatus

The column-switching HPLC-FLD system from Shimadzu (Kyoto, Japan) consisted of three LC-20A solvent delivery pumps (one for sample delivery), a CTO-20A column oven, two FCV-12AH two-position flow changeover valves, an FCV-13AL six-port flow selection valve, an RF-20Axs fluorescent detector, a CBM-20A system controller, and LC solution work station software (Fig. 1).

2.2 Chemicals and reagents

Ethylene dimethacrylate (EDMA) (monomer) was used as the cross-linking agent purchased from Wako Pure Chemicals (Osaka, Japan) and then purified by vacuum distillation techniques to remove polymerization inhibitor [24]. MASK was purchased from Tokyo Kasei Chemicals (Tokyo, Japan) and used without further purification. A polymerization initiator, 2,2′-azobis-(2,4-dimethylvaleronitrile) (ADVN), and benzoyl peroxide were purchased from Wako Pure Chemicals. Toluene (porogenic solvent) was purchased from Nacalai Tesque and was of the highest grade. Acetonitrile and methanol for preparing HPLC mobile phase was purchased from Wako Pure Chemicals. BPA was purchased from Sigma-Aldrich (St. Louis, MO, USA). Suwannee river NOM were purchased from International Humic Substances Society (St. Paul, MN, USA). Water for preparing BPA standard solution was obtained from Milli-Q water purification system of Millipore (Bedford, MA, USA) and then

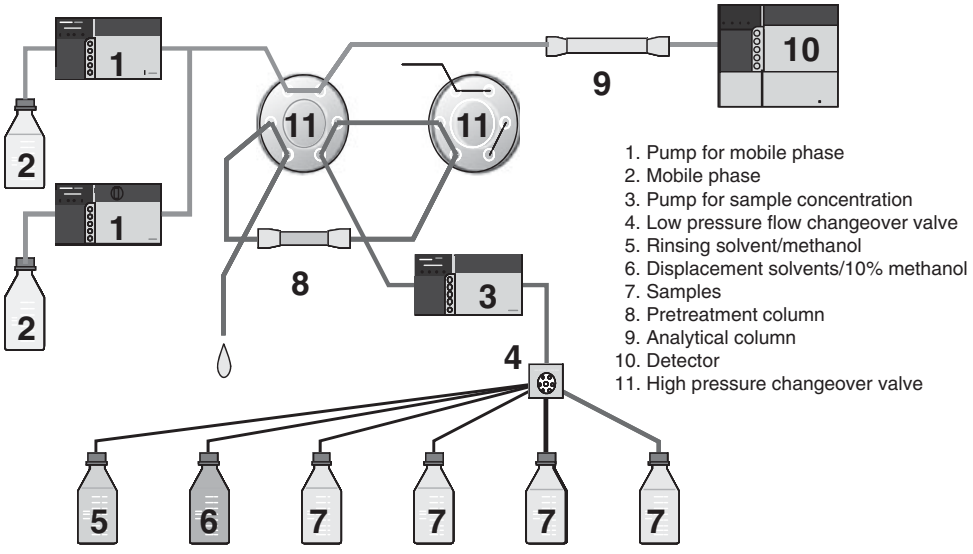

Figure 1. Flow diagram of the column-switching HPLC system. (1) Pumps for mobile phase, (2) mobile phase, (3) pump for water samples, (4) low-pressure flow selection valve, (5) rinsing solvent, (6) washing solvent, (7) samples, (8) pretreatment column, (9) analytical column, (10) FL detector, (11) high-pressure two-position flow changeover valve.

purified with an empore disk (47 mm od of SDB-XD type) from 3M (St. Paul). Real water sample was taken from *Yura* river located in the northern part of Kyoto city.

2.3 Preparation of MASK-modified panicles

Seed polymer particles were prepared with styrene monomer [25]. By using the seed polymer, uniformly sized macroporous EDMA-based polymer particles were prepared by a multi-step swelling and polymerization method [26]. Obtained EDMA particles were modified with MASK followed by our previous study [17]. Also, in order to know the possibility of commercialization of the product, we prepared 50 g of MASK-modified particles (particle diameter: 5 μm) as one batch. The obtained particles were packed into a stainless-steel column (30 or 100 mm × 4.6 mm id).

2.4 HPLC analyses

2.4.1 Removal of humic acids

In order to confirm the effect of chemical modification with MASK, the NOM water (10 mg/L) was concentrated with the prepared pretreatment column on column-switching HPLC by UV detection. The HPLC conditions are as follows: analytical column, Shim-pack VP-ODS (150 mm × 4.6 mm id); pretreatment columns, EDMA- or MASK–EDMA-packed column (100 mm × 4.6 mm id); mobile phase, 10 mM phosphate buffer (pH 2.6)/acetonitrile = 65:35; flow rate (analytical), 0.8 mL/min; (pretreatment), 2.0 mL/min; sample load, 50.0 mL; detection, UV 275 nm; Temp., 40°C; sample, river water.

2.4.2 Practical detection with HPLC-FLD column-switching system

Analytical conditions were as follows: analytical column, Shim-pack VP-ODS (150 mm × 4.6 mm id); pretreatment columns, MASK–EDMA-packed column (30 mm × 4.6 mm id); mobile phase, 10 mM phosphate buffer (pH 2.6)/acetonitrile = 65:35; flow rate (analytical), 0.8 mL/min; (pretreatment), 2.0 mL/min; sample load, 50.0 mL; detection, FL Ex. at 230 nm, Em. at 310 nm; Temp., 40°C; washing time for flow line, 1 min (2 mL); washing time for pretreatment column, 4 min (8 mL); re-equilibration time, 5 min after rinsing with just 70% acetonitrile for 2 min.

A flow diagram of the column-switching HPLC-FLD is shown in Fig. 1. The system contained two high-pressure two-position switching valves and one low-pressure six-position switching valve. Real samples were filtered with a 0.22-μm membrane filter prior to use. Valve sequence is shown in Fig. 2 and as follows (caption numbers correspond to sequence numbers in Fig. 2):

(i) The sample delivery pump and pretreatment column were connected via the left high-pressure valve and then sample solution was delivered directly into the pretreatment column.
(ii) After applying 50 mL of the sample solution, the right high-pressure valve was changed to isolate the pretreatment column and the sample delivery pump. Simultaneously, the low-pressure valve was changed to select the washing solvent. Consequently, the water sample that remained in the flow line (around 2 mL) was washed with a clean washing solvent (10% methanol). Thus, there was no unwanted additional concentration of the water sample that remained in the flow line.
(iii) The right high-pressure valve was changed again and the pretreatment column was washed with the washing solvent (10% methanol) to remove the remaining water sample. Here, the contaminants weakly retained on the pretreatment column can be removed.
(iv) The left high-pressure valve was changed and then the mobile phase was provided through the pretreatment column. Retained compounds in the pretreatment column were led to the analytical column. Simultaneously, data acquisition was started. After five minutes, the left valve was changed again to prevent excess apply of unwanted strongly retained compounds in the pretreatment column.
(v) During the analysis, the low-pressure valve was changed to rinsing solvent (methanol) to wash the pretreatment column and then the low-pressure valve was changed to the washing solvent to fulfill the pretreatment column with proper solvent for preparing the next pretreatment. Remaining methanol in the pretreatment column could cause worse recovery and peak broadening due to its strong eluting power. Pretreatment and analysis can be preformed simultaneously for saving run time.

2.4.3 Other practical parameters

(i) Recovery was calculated by peak area of concentrated 50 mL of 10–200 ng/L BPA in river water compared with the direct injection of 1 μL of 0.5–10 mg/L BPA.
(ii) Repeatability was estimated by six times repeated analyses with 10 ng/L BPA in river water.
(iii) The limit of detection (LOD) for each compound was calculated as corresponding response of 3.3 times as baseline noise. The baseline noise was estimated by the workstation software with subjecting the ASTM (the American Society for Testing and Materials) method described in ASTM E-1657-94. Spiked real river water (5 ng/L) was analyzed for LOD.
(iv) Linearity for each compound was evaluated by using spiked river water (2.5, 5, 10, 100, 1000 ng/L for BPA) thrice.

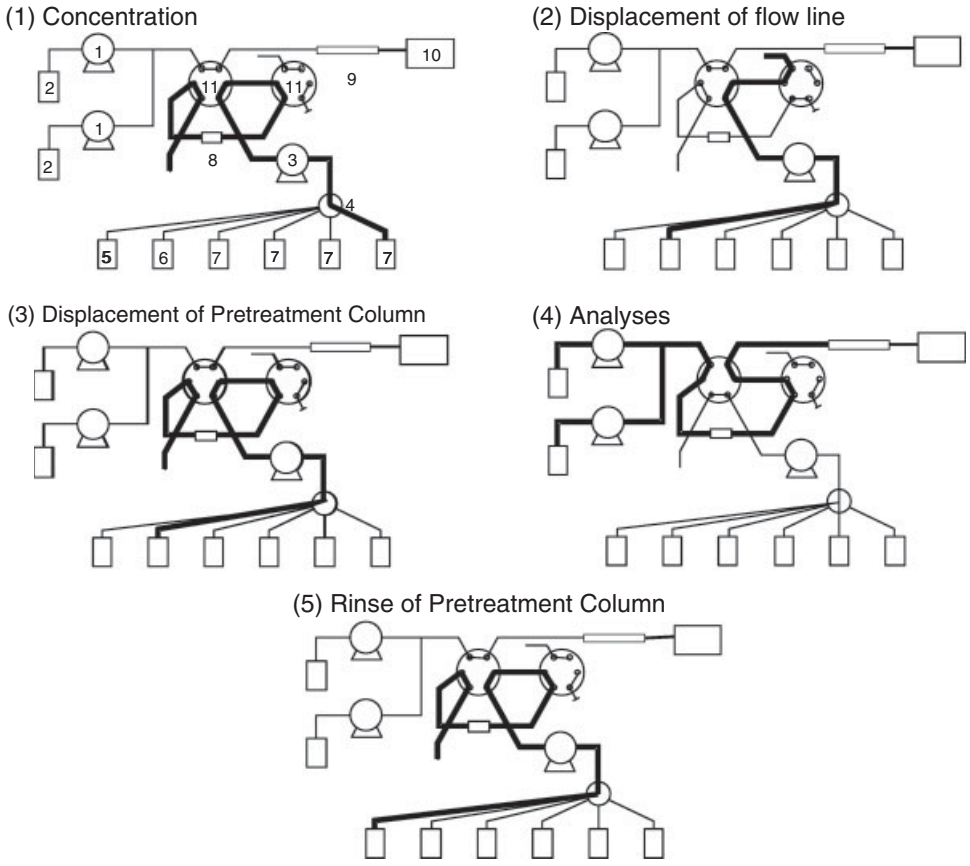

Figure 2. Valve sequence of the method. Analysis and pretreatment can be performed simultaneously. Consequently, one analysis including 50 mL of sample concentration took around 30 min. It is possible to execute the pretreatment procedure (1–3) and analysis (4) simultaneously for saving analysis time.

(v) One hundred eighty repeated analyses of real river water samples spiked with each 10 ng/L of BPA were performed and the pressure at the beginning of every analysis was recorded.

3 Results and discussions

3.1 Removal of humic acid

The effect of MASK-modification was already reported for BPA and β-estradiol (E2) separately [16, 17]. Here, we confirmed the same effect of MASK-modified particles obtained by large-scale synthesis. The effect could be caused by the repulsive force between sulfonic groups and humic substances. Besides sulfonic groups, other cation exchange resin can be used for the same purpose [27]. Contamination interference causes uncertainties of sensitivity, repeatability, robustness, etc. in trace amounts of determination. Therefore, the interference should be controlled for the evaluation of the method performance. Comparative chromatograms for pretreatment columns that were packed with MASK-modified EDMA or EDMA particles on real river water sample are shown in Fig. 3. The chromatogram obtained by the MASK-modified pretreatment column showed clearly reduction in peaks based on humic acids. In this case, the surface modification with polar group may decrease hydrophobic retention ability of original particles but the removal of interference can surpass it. Consequently, the MASK-modified particles absolutely promise the effective removal of humic acids in the environmental water samples.

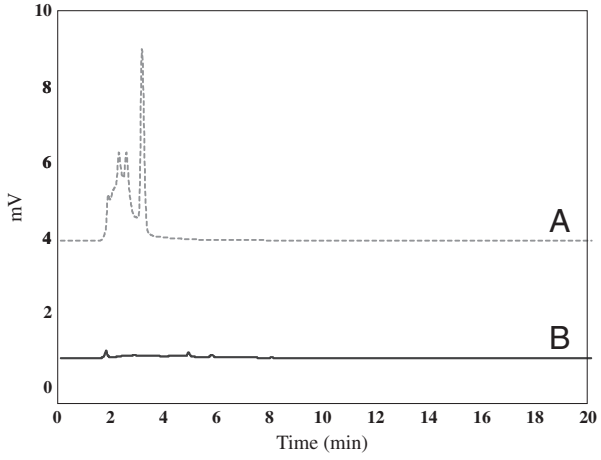

Figure 3. Removal of humic acids in river water sample: (A) dotted line: EDMA packed, (B) solid line: MASK-EDMA packed; analytical column, Shim-pack VP-ODS (150 mm × 4.6 mm id); pretreatment columns, EDMA- or MASK–EDMA-packed column (100 mm × 4.6 mm id); mobile phase, 10 mM phosphate buffer (pH 2.6)/acetonitrile = 65:35; flow rate (analytical), 0.8 mL/min; (pretreatment), 2.0 mL/min; sample load, 50.0 mL; detection, UV 275 nm; Temp., 40°C; sample, river water.

3.2 Effect of washing pretreatment and analytical column

There is no doubt that the cause of such trouble comes from sample solution. So, it is crucially effective to remove the sample solution that remains in the flow line and the pretreatment column before the introduction onto the analytical column. In step (2) of Fig. 2, sample solution that remained in the flow line was removed to prevent excess additions onto the pretreatment column. This can be performed due to the additional valve. Then, the pretreatment column was washed with clean washing solvent in step (3). Consequently, the remaining sample solution was excluded from HPLC system as well. Furthermore, when certain level of eluting power can be added to the washing solvent, weakly retained compounds can be washed out before the analysis. We employed 10% methanol aqueous solution as the washing solvent. The eluting power of the solvent was stronger than that of just water but caused no particular peak broadening in the resulted chromatograms. Just for solving column-clogging problem, to employ water, which has no hydrophobic eluting power, as washing solvent is effective as well. The composition of washing solvent can be selected according to the strength of the retention of the target compounds in the pretreatment column. As shown in our previous study [28], multi-different valve operations provided almost same peak response but the baseline stability was different. Considering the results of the 180 repeated analyses described later, it may be suggested that the introduction of the co-existing substance in the water sample could cause the column clogging during repeated analyses and emerging baseline drifting.

3.3 Quantitative evaluations

The recovery, repeatability, LOD and linearity are summarized in Table 1. As shown in this table, the quantitative recovery was confirmed. Additionally, the superposed chromatograms concerning RSD evaluation for pseudoenvironmental water (10 ng/L BPA) are shown in Fig. 4. These chromatograms also suggested that the HPLC-FLD column-switching system satisfied the quantitative analysis for low concentration BPA in environment. Totally, this system can be utilized for the practical analyses.

With regard to LOD, we confirmed that 0.09 ng/L could be detected when we utilized the standard solution of which the water was purified with Empore™ disk. About the environmental water, the LOD of BPA was estimated at 0.7 ng/L based on Fig. 5. Here, BPA was not detected from original river water sample. In this point, it is not easy to achieve this degree of LOD by mass spectrometer and/or electrochemical detector. However, these results show that the newly developed system including effective pretreatment and two-valve washing enabled the quantitative nanogram per liter level of detection with commonly used FLD. We believe that the effects were caused by only pretreatment procedures and washing process.

Table 1. Fundamental parameter of BPA analyses

Recovery	> 97%
Reproducibility	1.4%RSD
Limit of detection	0.09 ng/L
Linearity	$R^2 = 0.9999$

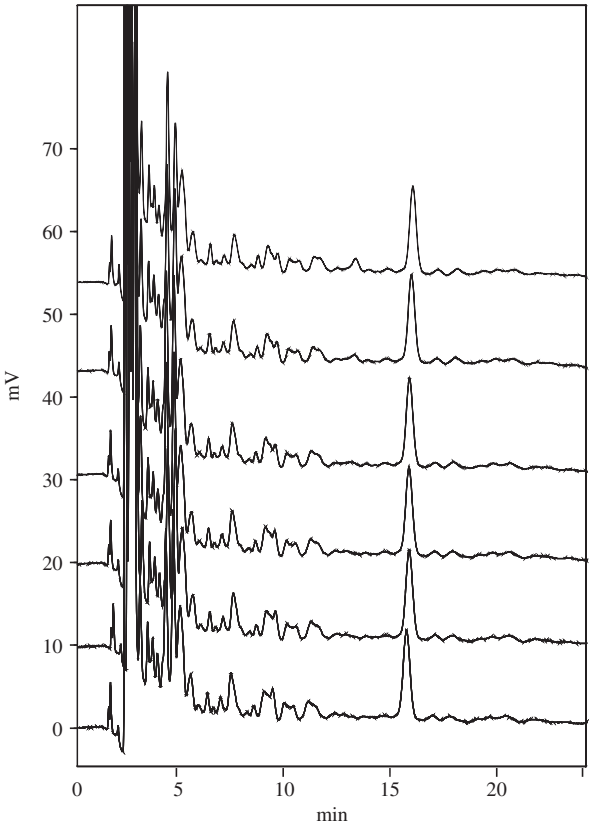

Figure 4. Repetitive analyses of river water including BPA analytical column, Shim-pack VP-ODS (150 mm × 4.6 mm id); pretreatment columns, MASK–EDMA-packed column (30 mm × 4.6 mm id); mobile phase, 10 mM phosphate buffer (pH 2.6)/acetonitrile = 65:35; flow rate (analytical), 0.8 mL/min; (pretreatment), 2.0 mL/min; sample load, 50.0 mL; detection, FL Ex. at 230 nm, Em. at 310 nm; Temp., 40°C; washing time for flow line, 1 min (2 mL); washing time for pretreatment column, 4 min (8 mL); re-equilibration time, 5 min after rinsing with just 70% acetonitrile for 2 min.

3.4 Robustness evaluation

Variations of both analytical and pretreatment column pressures are shown in Fig. 6. Increase in pressure was observed but the overall pressure increase was 2.5 MPa in the analytical column and 0.5 MPa in the pretreatment column until 150 times analyses. On the other hand,

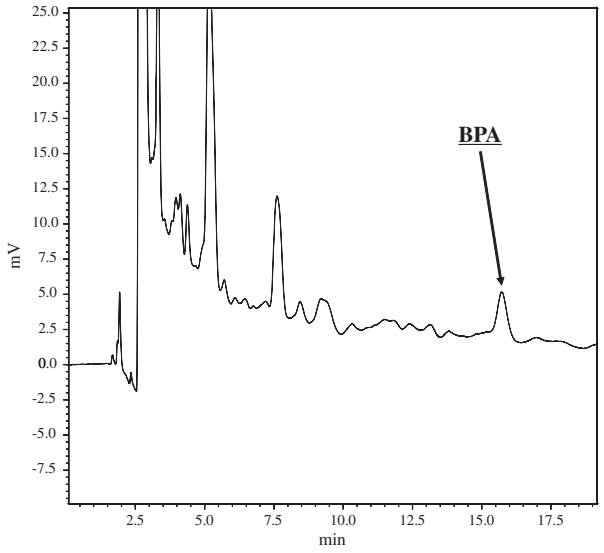

Figure 5. Chromatogram of 2.5 ppt BPA in river water LC condition was the same as in Fig. 4.

Figure 6. Column pressure increase in over 100 repeated analyses: (A) squares: analytical column; (B) triangles: pretreatment column. Washing of pretreatment column was effective for suppressing column-clogging problem. No significant pressure increase was observed.

increase in the rate of pressure in the analytical column used with the ordinary column-switching HPLC was much higher and the upper limitation was met after 30 repeated analyses. In general, 150 times analyses are permissible for environmental water. Especially, the stability of pretreatment column was satisfied in this study.

One of the causes of the clogging problem may be very small water-soluble substance that cannot be trapped by a 0.22-μm membrane filter. The remaining substance might be stuck in the frit or inlet of analytical column due to hydrophobic mobile phase.

Based on the results, it is essential that the real water sample that remains after concentration has to be removed from the LC system. Most possible and reliable solution is two-valve HPLC system, which can contribute to improve the reliability of column-switching HPLC analysis.

4 Concluding remarks

A new dedicated column-switching HPLC-FLD for environmental water analysis has been established and evaluated. It can detect trace level of chemical compounds in real sample matrix by using universal fluorescence detector. The anionic surface modification for the pretreatment column has been confirmed effective to reduce interference of environmental water samples. Column-clogging problem in the column has been effectively improved by using the two-valve column-switching HPLC system.

Fundamental performance, such as recovery, repeatability, and linearity, were satisfactory and good robustness was confirmed as well. Significant pressure increase was not observed during 150 repeated real sample analyses, while sudden pressure increase was observed after 150 injections and the pressure reached column limitation before 200 injections. The column-switching HPLC system can be operated simultaneously for the analysis and the pretreatment in repeated analyses. Consequently, total analysis time can be greatly saved. Trace amounts of hydrophobic chemical compounds in surface water can be reliably determined with the two-valve HPLC method. In the future works, this procedure using column-switching HPLC-FLD will be useful for quantitative detection of fluorescent compounds, such as polyaromatic hydrocarbons (PAHs).

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The authors have declared no conflict of interest.

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