http://dx.doi.org/10.5012/bkcs.2013.34.6.1783

Simultaneous Fluorimetric Determination of On-line Preconcentrated HANs, DCAD and TCAD by Using RPLC with a Postcolumn Derivatization System

Sung-woon Jung and Yong-wook Choi†,*

Department of Environmental Science, Jeonju University, Jeonju 560-759, Korea
*†Specialized Institute of Environmental Health Science. *E-mail: cyw411@jj.ac.kr*
Received March 4, 2013, Accepted March 20, 2013

A simultaneous analytical method has been developed for the fluorimetric determination of haloacetonitriles (HANs) [dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), dibromoacetonitrile (DBAN), haloacetamides [dichloroacetamide (DCAD), and trichloroacetamde (TCAD)] in drinking water by using the combined on-line perconcentration/reversed phase liquid chromatography (RPLC)-postcolumn detection system. This on-line perconcentration system was achieved by employing a precolumn packed with a commercial solid phase extraction (SPE) sorbent for the enrichment and purification of the target analytes. The haloacetonitriles and haloacetamides were separated on CN analytical column in a 7.5% methanol-0.02 M phosphate buffered mobile phase at pH 3. The column effluents were reacted with postcolumn reagents of o-phthaldialdehyde (OPA) and sulfite ion at pH 11.5, to produce a highly fluorescent isoindole fluorophore, which were measured with a fluorescence detector. Under the optimized conditions for RPLC and the postcolumn derivatization system all of the coefficient of determination of the standard calibration curves for the target analytes were over 0.99 and had a linear range from 5 to 100 μg/L. The detection limits showed 1.6 μg/L for DCAD, 0.1 μg/L for TCAD, 0.6 μg/L for DCAN, 1.6 μg/L for TCAN and 1 μg/L for DBAN, and the recoveries were ranged from 64 to 99% except for DCAD with precisions less than 4.9% in distilled water, and from 72(± 4%) to 116%(± 2%) in tap water.

Key Words : HANs, DCAD, TCAD, RPLC-postcolumn detection, On-line preconcentration system

Introduction

For human, water is the origin of life and the indispensible component for the maintenance of life. However, countless water-borne diseases like typhoid and cholera, and various pollutants had emerged by the early 20th century due to rapid industrialization, populational growth and urbanization. These days emphasis is placed on the importance of filtered water along with an effort to obtain clean water. Currently, in most countries purification processing facilities are operated to remove various pollutants from raw water through physical, chemical, and biological processes, and strive to supply clean tap water to its citizens.

Among these processes, dating back 100 years, the chemical method of using chlorine was introduced along with filtration for the disinfection of drinking water. Due to the advantages in disinfection and economic efficiency, chlorine has continued to be used more widely in comparison to other disinfectants.[1] However, the disadvantages of chlorine disinfection is the major occurrence of disinfection by-products (DBPs) as it reacts with artificial organic pollutants, natural organic matters (NOMs), or algae in the water. The most common DBPs are trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloketones (HKs), halopicrins (HPs), *etc*.[2-7] Dichloroacetic acid (DCAA), a type of HAAs, is a probable human carcinogen (B_2), and TCAA (trichloroacetic acid) has been classified as a possible human carcinogen (C) by the US EPA. Because these substances are nonvolatile, they have potentially higher hazardous levels than that of volatile organic pollutants like THMs.[8-10] Therefore, various papers on the occurrence of the disinfection by-products, content status, and other relative research papers are being published within Korea.[11-20] Although the domestic regulation regarding the drinking-water disinfection by-products was set as 0.1 mg/L, which is the only guideline for THMs, since January of 2003, the drinking-water guidelines for HAAs were regulated to 0.1 mg/L in every water treatment plant of more than 100,000 tons. The guidelines of other types of HANs such as DCAN, TCAN, DBAN and chloral hydrate were newly regulated at 0.09 mg/L, 0.004 mg/L, 0.1 mg/L and 0.03 mg/L, respectively, and from July of 2004, these guidelines were extendedly applied in all domestic water treatment plants.

The final products of HANs during chlorination in drinking water are known as HAAs, and haloamides (HADs) as intermediates are found in the middle of reaction of HANs with chlorine.[21] The pK_a of DCAD and TCAD, the representative substances of haloamides, are measured to be 13.55 and 12.42 at the temperature of 25 °C, respectively,[22] and each are hydrolyzed within the pH range between 9.5 and 12 to generate ammonia.[23] In general, HANs according to the analytical method EPA 551.1 are extracted using a liquid-liquid extraction, and analyzed by gas chromatrograph-electron capture detector (GC-ECD).[24] As for HAAs, including DCAA and TCAA, methylation with diazomethane is carried out according to the analytical method EPA 552,[25] or

methylation with acidic methanol, as described in the analytical method EPA 552.1[26] or 552.2,[27] is followed by extraction with methyltertiarybutylether (MTBE) and followed by analysis with GC-ECD. Furthermore, the recent research on the analysis of HAAs by using ion chromatography after its enrichment with SPE or by using capillary electrophoresis has been published as well.[28-32] However, the analytical method regarding haloamides is not well worked. Richardson[33] measured TCAD in drinking water by using GC with mass spectrometry (GC-MS method), and Rapp[34] measured DCAD and TCAD by the same method as that used for the HANs. On the other hand, if DCAD and DCAA coexist, the analysis of DCAA by the analytical method EPA 552.2 could result in more positive errors than expected because DCAD is transformed into DCAA during the previous process and this produces positive errors in the analysis of DCAA.[36] Therefore, a selective analytical method, which analyzes haloamides selectively and quantifies HANs more easily at the same time needs further research. As haloamides and HANs possess both the carbonyl and amine group, the analysis by using derivatizing reagents that react to each functional group selectively can be considered. In this research, the study suggests a simultaneous analytical method of on-line preconcetrated two types of haloamides (DCAD and TCAD) and three types of HANs (DCAN, TCAN and DBAN) by using HPLC with postcolumn derivatization system as a modified method including an additional on-line concentration equipment from the analytical method published by Choi and Reckhow.[35] By establishing a method for selectively measuring haloamides and HANs quickly, this particular method can be suggested as an alternative method for HANs and as a new method for HADs, and also contribute to a rapid analytical method for work regarding the occurrence and the removal of disinfection by-products in chlorinated drinking water.

Experimental Section

Preparation of Reagents and Samples. As a mobile phase, a 0.02 M phosphate buffer solution with a pH range from 3 to 7 was prepared by using NaH_2PO_4 (Aldrich) and Na_2HPO_4 (Aldrich), and a following fine pH adjustment was done by using 50% phosphoric acid or 3 M NaOH (Aldrich). All standard samples of DCAD (2,2-Dichloroacetamide, MERCK, 98%), TCAD (2,2,2-Trichloroacetamide, MERCK, 98%), DCAN (Aldrich, 98%), TCAN (Aldrich, 98%) and DBAN (Aldrich 95%) were prepared from solids at 1000 mg/L in MeOH (Fisher HPLC) weight, and if necessary, the samples were diluted using ultrapure water. KOH (Aldrich Chemical Company) used for the postcolumn reaction was prepared into 1 M concentration and was diluted into adequate concentrations for usage, while a 1 L buffer solution for the derivatization reaction was prepared by diluting 0.1 mol of $Na_3PO_4 \cdot 12H_2O$ into 900 mL of ultrapure water by adjusting the buffer solution to pH 11.5. A 0.02 M reagent of Na_2SO_3 (Aldrich, 98+%), a nucleophile, was prepared using 0.1 M phosphate buffer solution before use. OPA (Aldrich) was prepared by completely dissolving 0.025 mol of OPA into 25 mL of MeOH and diluting up to 250 mL in ultrapure water before use. For the on-line concentration column of HANs and HADs, STARATA-X (surface modified styrene-divinylbenzen polymer phase) from Phenomenex, Lichrolut EN (Ethyl vinyl benzen divinyl benzene polymer phase) from MERCK, HLB (N-vinylpyrolidone + divinylbenzene phase) from Oasis, and C_{18} (strong hydrophobicity) and CN (polar bonded phase) from Waters were used.

Measuring Devices and Apparatus. The HPLC instrument was a LC-10AD system including two serial connected 6-port valves (M 7125, Rheodyne, CA, USA) with preconcentration column (2.1 × 20 mm, 25 μm) and with a 100 μL loop, respectively, and a RF-535 Fluorescence detector and a UV-VIS detector (SPD-10A) from Shimadzu. SB-C_{18} (4.6 × 150 mm, 5 μm), SB-phenyl (4.6 × 150 mm, 5 μm) and SB-CN (4.6 × 150 mm, 5 μm) from Agilent were used as analytical columns and the incubated water bath used Digital Water Bath SB-1000 from EYELA. Consisting of an 8-roller/4-channel, the peristaltic pump used for the reaction of the postcolumn derivatization was Minipuls-3 from Gilson. Tubes and reaction coils used were made of teflon with dimension of 0.5 mm ID × 1/16" OD. The Delta 350 from Mettler was used as a pH meter, and the weight of samples was measured by an Explorer chemical balance from Ohaus while a Autochro-2000 software from Younglin Instrument was used for data acquisition.

Experimental Procedure. A 7.5% MeOH-0.02 M phosphate buffer solution (at pH 3) with a flow rate of 0.8 mL/min was eluted through the polar CN analytical column. At the same time, from the postcolumn detection system, a 0.1 M phosphate buffer solution which included KOH, OPA and sulfite was flowed with the flow rate of 0.625 mL/min to stabilize the baseline of the Fluorescence detector. The standard materials of HANs (DCAN, TCAN and DBAN) and HADs (DCAD and TCAD) on the on-line concentration column packed with SPE resin were injected in a rear valve. Desorption was assessed by an 100 μL of elution solvent (50% THF) in the loop of a front valve by switching from

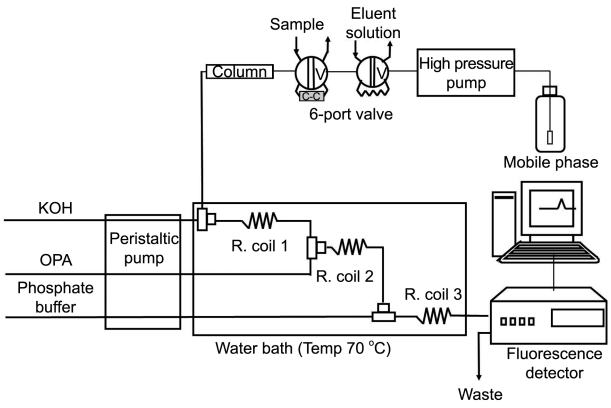

Figure 1. Schematic diagram of RPLC-postcolumn detection for the determination of DCAN, TCAN, DBAN, DCAD, and TCAD. Column: SB-CN, fluorescence detector, λ_{ex} = 363 nm, λ_{em} = 425 nm; C-C: pre-concentration column.

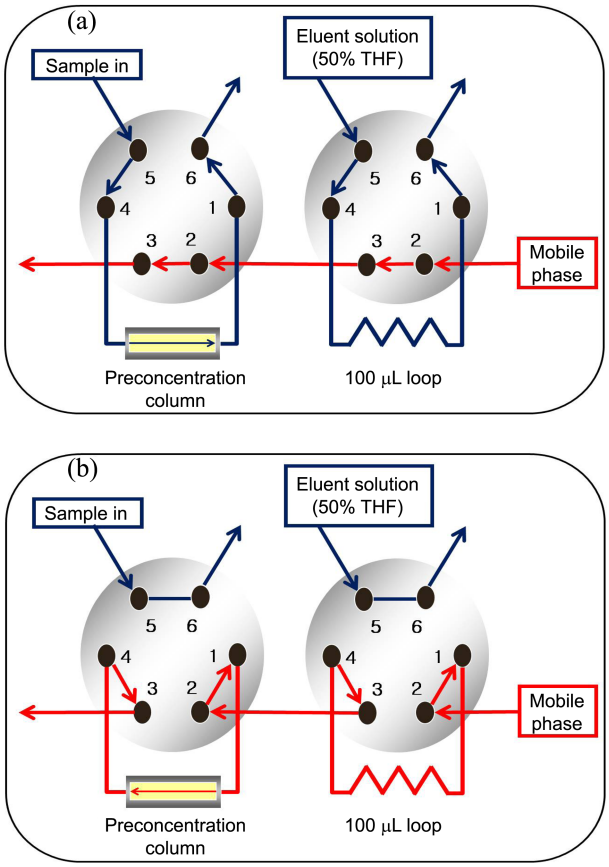

Figure 2. Schematic flow diagram of two 6-port injection valves connected with a 100 μL loop (a front valve) and with a pre-concentration column (a rear valve), respectively. (a) load position for pre-concentration of samples (HANs and HADs); (b) inject position for eluting samples (HANs and HADs).

load position to inject position, followed by transportation of those standard materials toward the analytical column for the determination by the postcolumn detection system.

When DCAD, DCAN, TCAD, DBAN and TCAN are eluted, they are mixed with KOH at the first T-union, and are hydrolyzed into ammonia. At the second stage of T-union, they are mixed with OPA while at the third stage of T-union, a 0.1 M phosphate buffer solution, including sulfite is mixed with them, followed by the measurement of isoindole, which was generated within the reaction coil, adjusted to a pH of 11.5, under 363 nm and 425 nm of the excitation and emission wavelength from the fluorescence detector. The RPLC-post-column detection system was schematically discribed in Figure 1 and Figure 2.

Results and Discussion

Optimal Conditions in the Postcolumn Derivatization Reaction. The optimal condition for the HPLC postcolumn derivatization reaction using the three reagent solutions of KOH, OPA and sulfite was investigated. Each sample of HANs (DCAN, TCAN, DBAN) and HADs (DCAD, TCAD) is mixed with KOH through the first T-union to be hydrolyzed into ammonia. To obtain the optimal concentration for

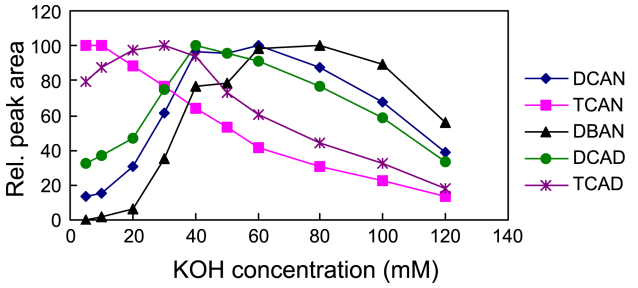

Figure 3. Effect of KOH concentration on fluorescence intensity. Conditions: HANs, HADs concentration 10 ppm; KOH coil 5 m; OPA, 15 mM, coil, 0.5 m; 0.1 M phosphate buffer (pH 11.5); sulfite, 15 mM, coil, 6 m; temperature 70 °C; mobile phase, 7.5% MeOH-0.02 M phosphate buffer (pH 3), flowrate, 1 mL/min; 50 μL injection.

KOH to decompose HANs and HADs into ammonia, based on the conditions suggested by Choi and Reckhow,[35] the lengths of the reaction coils 1, 2 and 3 were set at 5 m, 0.5 m and 6 m, respectively. Concentrations of KOH, OPA and sulfite were 50 mM, 15 mM and 15 mM, respectively, while the concentration of a phosphate buffer solution was 0.1 M at pH 11, while a temperature of the water bath was fixed at 70 °C. The change of the peak areas of the derivatives of HANs and HADs with KOH concentration from 5 to 120 mM were studied. As shown in Figure 3, the 40 mM of KOH with its maximum peak area was selected as the optimal condition among the five samples. Furthermore, the average relative peak area of derivatives of HANs and HADs was the highest at 7 m when the length of the KOH decomposition coil was changed from 3 to 9 m while keeping the concentrate of KOH at 40 mM (Figure 4). Thus, the optimal concentration of KOH and the length of the decomposition coil were fixed into 40 mM and 7 m, respectively, and then those were used as optimal.

To obtain the optimal concentration for the reaction between OPA and HANs and HADs hydrolyzed by KOH, the concentration of OPA was changed from 10 to 60 mM. As a result, it was shown that the peak area of TCAD, which showed the lowest reaction efficiency among the five types of HANs and HADs at 25 mM, occupied 96.1% of the maximum peak area (Figure 5). After studying the peak area of the derivatives of HANs and HADs by changing the length of the reaction coil from 0.5 to 3 m to determine the

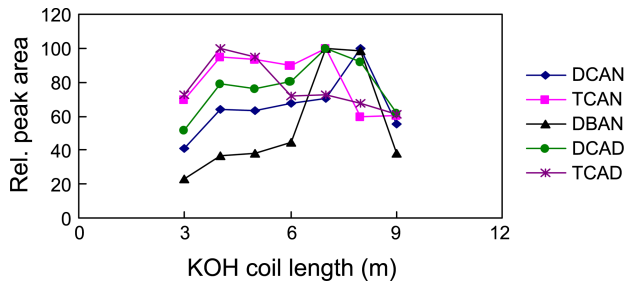

Figure 4. Effect of KOH coil length on fluorescence intensity. Conditions: the same conditions as Figure 3 except for 40 mM of KOH.

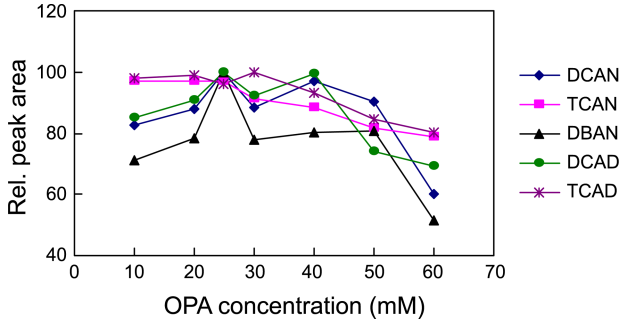

Figure 5. Effect of OPA concentration on fluorescence intensity. Conditions: the same conditions as Figure 4 except for a 7 m coil length of 40 mM KOH.

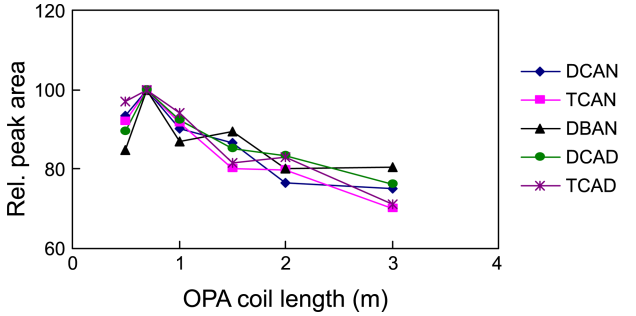

Figure 6. Effect of OPA coil length on fluorescence intensity. Conditions: the same conditions as Figure 5 except for the OPA concentration was at 25 mM.

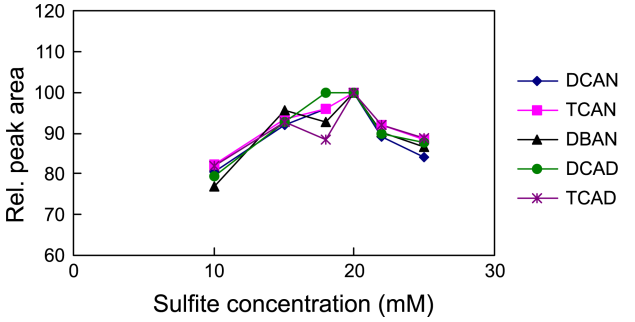

Figure 7. Effect of sulfite concentration on fluorescence intensity. Conditions: the same conditions as Figure 6 except the reaction coil length of 25 mM OPA was 0.7 m.

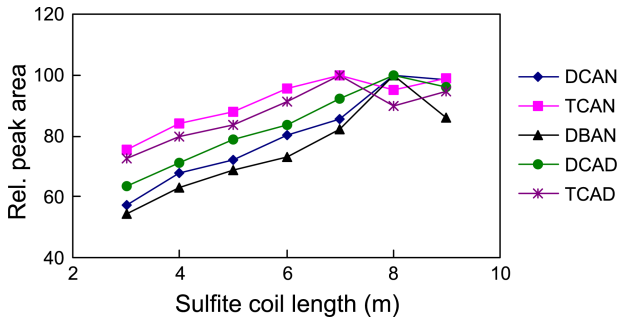

Figure 8. Effect of sulfite coil length on fluorescence intensity. Conditions: the same conditions as Figure 7 except the sulfite concentration was 20 mM.

optimal length of the reaction coil of OPA, as shown in Figure 6, the maximum value occurred when the length of the OPA reaction coil was 0.7 m. From here, the value continued to be reduced until the length reached 3 m. Therefore, the optimal OPA concentration and the length of the reaction coil were fixed at 25 mM and 0.7 m, respectively.

Sulfite was dissolved into the 0.1 M phosphate buffer solution to be used, and to obtain the optimal concentration toward the derivatized reaction of HANs and HADs, as the concentration of sulfite was changed from 10 to 25 mM, while the maximum sensitivity of all was shown at 20 mM (Figure 7). Moreover, when the length of sulfite reaction coil was changed from 3 to 9 m, the peak area of derivatives of HANs and HADs, as shown in Figure 8, displayed a maximum value in the range of 7-8 m. As more samples showed the maximum peak area value at 8 m rather than at 7 m, the sulfite concentration and the length of the reaction coil were fixed at 20 mM and 8 m, respectively.

Optimization of MeOH Composition in the Mobile Phase. Based on the optimal conditions of the coil temperature, pH and the reagent solution of postcolumn derivatization, SB-C_{18}, SB-Phenyl and SB-CN analytical columns were used to obtain the optimal resolution for the five types of HANs and HADs. The flow rate of the mobile phase was set at 1 mL/min. A 0.02 M phosphate buffer solution, used for peptides analysis by Kai *et al.*,[36] that is independent from the pH of the postcolumn derivatization reaction with its concentration and type of salt, was selected as a mobile phase for separating HANs and HADs, and the change of resolution was studied on each column with composition of MeOH.

Analytical Column with the Stationary Phase of C_{18}. At the stationary phase nonpolar C_{18} analytical column, the reaction condition of postcolumn derivatization was set as the optimal condition above. As a result of studying the elution time of HANs and HADs, by changing the composition of MeOH from 7.5 to 20% under the mobile phase of a 0.02 M phosphate buffer solution at pH 3, the higher the MeOH

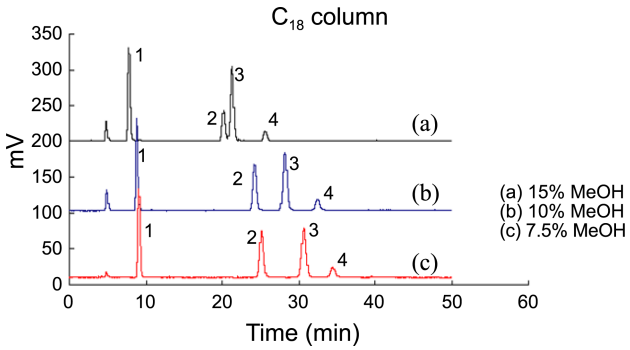

Figure 9. Effect of MeOH composition (%) on the separation of three HANs and two HADs mixture by C_{18} Column. Conditions : stationary phase; C_{18} column (15 cm × 4.6 mm ID), mobile phase; MeOH (0.02 M phosphate buffer at pH 3), injection volume; 50 μL, room temperature, detector; Fluorescence (λ_{ex} = 363 nm, λ_{em} = 425 nm), samples; (1) DCAD (2) DCAN (3) TCAD (4) DBAN.

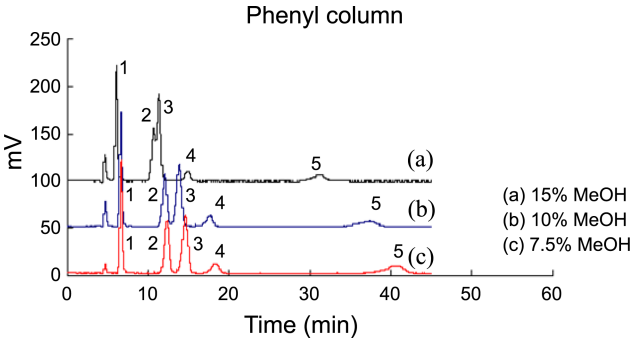

Figure 10. Effect of MeOH composition (%) on the separation of three HANs and two HADs mixture by phenyl column. Conditions: the same conditions as Figure 9 except for (5) TCAN.

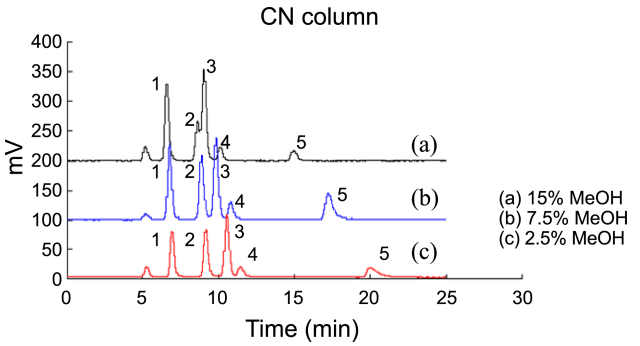

Figure 11. Effect of MeOH composition (%) on the separation of three HANs and two HADs mixture by CN column. Conditions: the same conditions as Figure 9.

composition, the lower the elution time for all samples, as shown in Figure 9. Furthermore, it is confirmed that until a 15% MeOH composition, all samples except for TCAN, were eluted to complete a baseline separation. However, TCAN was not detected within 50 minutes even under the 20% of MeOH composition, and TCAD and DCAN did not have complete baseline separations. For detecting HANs and HADs under the C_{18} column, it is considered that the MeOH composition should be more than 20%, and if the MeOH composition is over 20%, all samples are eluted to prevent the baseline separation.

Analytical Column with the Stationary Phase of Phenyl. Similar to the experiments of the C_{18} analytical column, the experiment of the postcolumn derivatization reaction condition and mobile phase on the phenyl analytical column changed the MeOH composition from 7.5 to 20% to observe the elution time of HANs and HADs. As a result, as shown in Figure 10, as the MeOH composition is increased, the elution time is shortened. Within 40 minutes, until a 10% of the MeOH composition, all samples of HANs and HADs are eluted to complete the baseline separation and it was confirmed that the elution time was shortened to 30 minutes or less in 15% MeOH compositions, however, the baseline separation of DCAN and TCAN was not completed. Therefore, although the 10% MeOH composition was the optimal elution condition for the phenyl column, it is evaluated to be inadequate, because it requires more time to have eluted all samples (40 min).

Analytical Column with the Stationary Phase of CN. Similar to the experiments of the Phenyl and C_{18} analytical column, the experiment of the postcolumn derivatization reaction conditions and mobile phase under the CN analytical column changed the MeOH composition from 2.5 to 15%, and the elution time of HANs and HADs were observed. As a result, Similar to the other column experiments, it is confirmed that the elution times of HANs and HADs decreases as the MeOH composition increases (Figure 11). Under the 10% MeOH composition, all samples of HANs and HADs were eluted within 16 minutes to complete the baseline separation. Although the elution time was shortened to 15 minutes under the 15% MeOH composition, the baseline separation of DCAN and TCAD was not completed properly. Therefore, considering the resolution and the elution time for HANs and HADs on the CN analytical column, a 7.5% MeOH was selected to be optimal. In this study, the CN analytical column (7.5% MeOH) which all samples were allowed to elute within 17 minutes with the complete baseline separation was selected to be the best optimal column. The repeatability of the peak area five time replication analyses for the five types of HANs and HADs under the same conditions, provided positive results in the range from 0.7 to 2.4% of relative standard deviation (Table 1).

SPE for Preconcentration. To establish the on-line preconcentration of HANs and HADs, the SPE cartridges with the best absorbing efficiency were selected through the batch method, using five types of Solid Phase Extractions (SPE) : STARATA-X (Phenomenex), Lichrolut EN (MERCK), HLB (Oasis), C_{18} (Waters) and CN (Waters). The five types of 1000 mg/L stock solutions – three types of HANs and two types of HADs were diluted into 10 mg/L with ultrapure water. After washing the cartridges with 2 mL of MeOH through the 10 mL syringe, stabilized them with 4 mL of

Table 1. Repeatability of HANs and HADs in mobile phase of 0.02 M phosphate buffer at pH 3 on CN stationary phase

Compound	n = 5					Average	SD	RSD%
	1	2	3	4	5			
DCAD	2820.4	2812.7	2739.1	2739.9	2772.0	2750.3	18.8	0.7
DCAN	2647.2	2754.5	2681.7	2758.7	2768.1	2736.2	47.4	1.7
TCAD	2937.9	2936.6	2895.2	2847.8	2760.6	2834.5	68.3	2.4
DBAN	1092.6	1048.6	966.9	1013.8	993.2	991.3	23.5	2.4
TCAN	927.2	943.9	907.3	933.8	938.5	926.6	16.8	1.8

Table 2. Adsorption efficiency for preconcentration of HANs and HADs by batch method using Sep-Pak cartridges packed with five different packing materials

SPE Cartridges	Compounds	Standard concentration (mg/L)	Adsorption efficiency
STARATA-X (surface modified styrene-divinylbenzen polymer phase)	DCAD	10	100.0
	DCAN	10	100.0
	TCAD	10	100.0
	DBAN	10	100.0
	TCAN	10	100.0
Lichrolut EN (Ethyl vinyl benzen divinyl benzene polymer phase)	DCAD	10	100.0
	DCAN	10	100.0
	TCAD	10	100.0
	DBAN	10	100.0
	TCAN	10	100.0
HLB (*N*-vinylprrolidone + divinylbenzene phase)	DCAD	10	71.7
	DCAN	10	100.0
	TCAD	10	100.0
	DBAN	10	100.0
	TCAN	10	100.0
C_{18} (octadecyl bonded, endcapped silica)	DCAD	10	2.8
	DCAN	10	40.9
	TCAD	10	5.7
	DBAN	10	36.3
	TCAN	10	100.0
CN (cyanopropyl bonded, endcapped silica)	DCAD	10	16.0
	DCAN	10	32.3
	TCAD	10	24.3
	DBAN	10	30.4
	TCAN	10	79.8

ultrapure water, and injected 2 mL of sample, and then the solution eluted through each cartridge was measured twice on the CN column using the postcolumn derivatization reaction. As a result, as shown in Table 2, the adsorption levels of the following STARATA-X (Phenomenex), Lichrolut EN (MERCK) and HLB (Oasis) were relatively high among the six SPE candidates. Two types of SPE cartridges, STARATA-X and Lichrolut EN, showed all the 100% of adsorption rate for five samples of HANs and HADs, Therefore, STARATA-X and Lichrolut EN were selected to be used as packing materials in the on-line concentration column.

Desorbing Eluent. For packing the two types of SPE cartridges, STARATA-X and Lichrolut EN, on the on-line preconcentration column, and concentrating and detecting HANs and HADs, a 7.5% MeOH – 0.02 M phosphate buffer at pH 3 - in the mobile phase was used as an elution solvent, but the elution was not fulfilled. Therefore, using the system as shown in Figure 1, four types of organic solvents such as methanol, ethanol, acetonitrile and tetrahydrofuran (THF) were used to determine the optimal desorption condition. When methanol, ethanol or acetonitrile was used, as shown in Figure 12, the bands of HANs and HADs were eluted such that the baseline separation was not completed, and

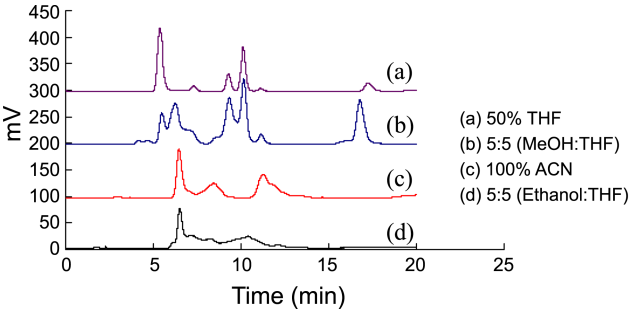

Figure 12. Effect of desorption solvents on desorption efficiency of preconcentrated analytes.

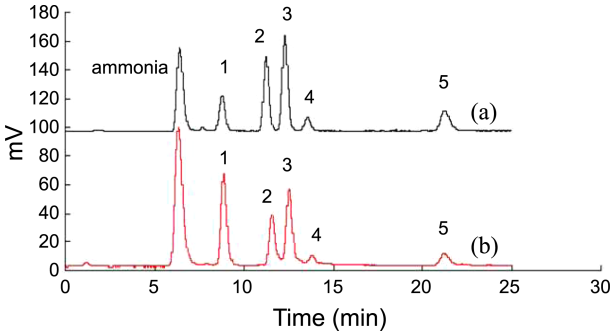

Figure 13. Liquid chromatogram of three HANs and two HADs mixture by on-line preconcentration – postcolumn determination system. Conditions: (a) STARATA SPE resin (b) Lichrolut EN SPE resin; Column, CN (15 cm × 4.6 mm ID); 7.5% MeOH-0.02 M phosphate buffer (pH 3), flowrate, 0.8 mL/min; Load 1 mL; Elute 100 μL Fluorescence detector, λ_{ex} = 363 nm, λ_{em} = 425 nm. Samples, (1) DCAD (2) DCAN (3) TCAD (4) DBAN (5) TCAN.

also band tailing was generated. On the other hand, such phenomena did not happen under THF, and all samples were able to be eluted. Therefore, 50% of THF was used to measure the desorption efficiency on the STARATA-X and Lichrolut EN SPE cartridges. The result described that STARATA-X showed a better efficiency for all samples, except for DCAD, while in case of Lichrolut EN the baseline separation was not proceeded properly (Figure 13). After the STARATA-X, surface modified styrene-divinylbenzen polymer phase, was packed in the on-line concentration column which connected to the rear 6-port valve, while 100 μL of 50% THF was injected into the loop of the front 6-port valve, the analysis of HANs and HADs were carried out. As a result, under the same conditions, recoveries of HANs and HADs were 84% for DCAN, 89% for TCAN, 98% for DBAN and 64% for DCAD and 99% for TCAD, respectively, as shown in Table 3. It is considered that the reason for the low recovery of DCAD was the decomposition of DCAD into ammonia during the adsorption and desorption on preconcentration column in judgment from the increase of ammonia band area in the liquid chromatogram as shown in Figure 13 and 14.

Calibration Curve and Detection Limit. The Quantitative behaviors of HANs and HADs, under the optimal conditions obtained above for all samples, as shown in Table 4, showed linearity with the coefficient of determination to be over

Table 3. Recovery test for three HANs and two HADs by RPLC-postcolumn detection with on-line preconcentration system packed by STARATA-X as a packing material

Compounds	Standard concentration (mg/L)	Load volume (mL)	RSD% (n=3)	Recovery
DCAD	0.1	1	0.6	64.4
DCAN	0.1	1	0.6	84.3
TCAD	0.1	1	0.5	99.5
DBAN	0.1	1	4.9	98.1
TCAN	0.1	1	4.3	89.4

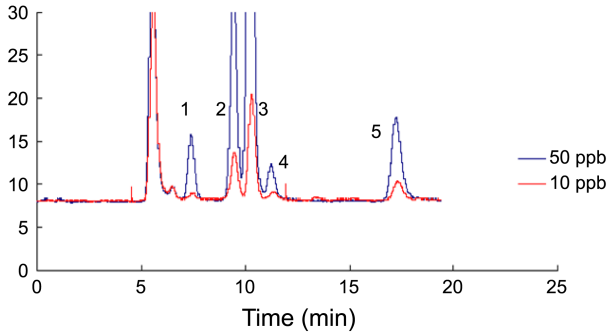

Figure 14. Liquid chromatogram of three HANs and two HADs mixture by RPLC-postcolumn derivatization. Conditions: column, CN (15 cm × 4.6 mm ID); 7.5% MeOH-0.02 M phosphate buffer (pH 3), flowrate, 0.8 mL/min; load 1 mL; desorption solvent 100 μL, Fluorescence detector, λ_{ex} = 363 nm, λ_{em} = 425 nm. samples; (1) DCAD (2) DCAN (3) TCAD (4) DBAN (5) TCAN.

R^2=0.99 on the calibration curve generated by varying each concentration from 5 to 100 μg/L. The detection limits (3) were 1.6 μg/L for DCAD, 0.1 μg/L for TCAD, 0.6 μg/L for DCAN, 1.6 μg/L for TCAN and 1 μg/L for DBAN, and when repetitively measured five times, the relative standard deviations of the peak area ranged from 0.2 to 4.6% as shown in Table 4. Considering 100 μg/L for DBAN, 90 μg/L for DCAN, and 4 μg/L for TCAN among the guideline for water quality of drinking water in Korea, the result represents that the method suggested in this work is suitable to be accepted as an alternative method regarding the existing analytical method US EPA 551.1 or the Korean standard method ES 05551 because the on-line concentration-RPLC with postcolumn detection method is simple, safe and efficient, in comparison to the liquid-liquid extraction followed by GC-ECD or GC-MS.

Table 4. Regression coefficients of standard calibration curves for determination of three HANs and two HADs by using RPLC-postcolumn detection with on-line preconcentration system

Compounds	Linear range (μg/L)	Coefficient of determination (R^2)	$Y = aX + b$	
			a	*b*
DCAD	5-100	0.992	3.21	-16.87
DCAN	5-100	0.999	10.52	14.90
TCAD	5-100	0.999	27.72	-6.28
DBAN	5-100	0.999	1.88	-0.06
TCAN	5-100	0.993	5.98	13.10

Recovery. To determine the recoveries for three HANs and two HADs, each were spiked into tab water to prepare the concentration to be 0.1 mg/L, and after a 0.45 μm Teflon membrane filtration, 1 mL of the filtrates were preconcentrated in the on-line concentration column, and eluted on the CN column under the same conditions as above. The fluorescence detector (λ_{ex} = 363 nm, λ_{em} = 425 nm) was used and quantified through the postcolumn derivatization reaction. The percent recoveries varied from 97 (± 2%) to 116% (± 2%) for HANs, from 72 (± 4%) for DCAD to 115% (± 1%) for TCAD.

Conclusion

The developed method established the possibility for an on-line preconcentration–RPLC-postcolumn detection analysis for ultra-trace HADs and HANs in water through a direct injection with no sample pretreatment steps other than membrane filtration.

As the optimal condition to determine HADs and HANs with a fluorescence detector through the RPLC with a postcolumn derivatization reaction, the concentration of KOH and the length of the decomposition coil were 40 mM and 7 m, respectively, while the concentration of OPA and the length of the reaction coil were 25 mM and 0.7 m, respectively, and the concentration of sulfite and the length of the reaction coil were 20 mM and 8 m, respectively. Furthermore, the reaction temperature and reaction pH of 0.1 M phosphate buffer solution with sulfite were 70 °C and 11.5, respectively. The baseline separation for HANs and HADs was completed when the 7.5% MeOH-0.02 M phosphate (pH 3) was in the mobile phase on the CN analytical column. The described method represents good repeatability, sensitivity and recovery except for DCAD, due to its degradation into ammonia during the on-line desorption. Also, this system shows much lower detection limit in sub ppb level so that this method can be suggested as an alternating analytical method for HANs and a new method for HADs in place of the well-known analytical methods of LLE-GC/ECD or GC/MS.

References

1. Kim, H. S.; Yoon, J. Y.; Yeom, C. M. *Kor. Soc. Wat. Wast.* **2000**, *16*, 585.
2. Glatz, B. A. *J. AWWA* **1978**, *70*(8), 465.
3. Chen, A. M. *Science* **1980**, *207*(4), 90.
4. Oliver, B. G. *J. AWWA* **1983**, *17*(2), 80.
5. Krasner, S. W.; McGuire, M. J.; Jacangelo, J. G. *et al*., *J. AWWA* **1989**, *80*(8), 41.
6. Singer, P. C. *J. Environ. Engineer.* **1993**, *120*, 727.
7. Reckhow, D. A.; Singer, P. C. *J. AWWA* 1984, *76*(4), 151.
8. US EPA. Federal register, US EPA 1994.
9. Ozawa, H. *J. Chromatogr.* **1993**, *644*, 375.
10. Mueller, M. G.; Wagner E. D.; Mccalla, K.; Richardson, S. D.; Woo, Y. K.; Plewa, M. J *Environ. Sci. Technol.* **2007**, *41*, 645-651.

11. Park, H.; Kim, C. M.; Chang, H. S.; Kim, H. S.; Park, C. M.; Yu, B. J. *J. of KSEE* **2006**, *28*(10), 1031.
12. Chung, Y.; Shin, D. C.; Lim, Y. W.; Kim, J. S.; Park, Y. S. *Kor. J. Environ. Tox.* **1997**, *12*(3), 23.
13. Son, H. J.; Roh, J. S.; Bae, S. D.; Choi, Y. I.; Jung, C. W. *J. of KSEE* **2007**, *29*(4), 412.
14. Choi, S. N.; Kwak, S. Y.; Pyo, H. S.; Kim, J. H.; Moon, M. H. *J. of KSEE* **2007**, *29*(6), 630.
15. Yeom, C. M.; Choi, Y. S.; Beon, S. J.; Cho, S. H.; Yoon, J. Y. *Kor. Soc. Wat. Wast.* **2002**, *16*(2), 169.
16. Park, S. J.; Pyo, H. S.; Park, S. S. *A Study on the Analytical Method and National Surveys of Trace Hazardous Compounds in Drinking Water*, 5th ed.; KAIST Report: 1997.
17. Yeom, C. M.; Choi, Y. S.; Cho, S. H.; Yoon, J. Y. *J. Kor. Soc. Water Qual.* **2003**, *19*(1), 127-133.
18. Lee, K. J.; Hong, J. E.; Pyo, H. S.; Park, S. J.; You, J. G.; Lee, D. W. *Anal. Sci. Tech.* **2003**, *16*(3), 249.
19. Park, Y. S. M.D. Thesis, Yonsei University, 1996.
20. Kim, J. S. M.D. Thesis, Yonsei University, 1996.
21. Alouini, Z.; Seux, R. *Wat. Res.* **1987**, *21*(3), 335-343.
22. Glezer, V.; Harris, B.; Tal, N.; Iosefzon, B.; Lev, O. *Wat. Res.* **1999**, *33*(8), 1938.
23. Kezdy, F.; Bruylants, A. *Bull. Soc. Chim. Belg.* **1960**, *69*, 602.
24. Mersaar, U.; Bratt, L. *Acta Chem. Scand.* **1974**, *A28*(7), 715.
25. U.S. EPA Method 552, 1990.
26. U.S. EPA Method 552.1, 1992.
27. U.S. EPA Method 552.2, 1992.
28. Barron, L.; Paull, B. *Anal. Chem.* **2004**, *522*, 153.
29. Martinez, D.; Borrull, F.; Calull, M. *J. Chromatogr.* **1999**, *853*, 187.
30. Martinez, D.; Borrull, F.; Calull, M. *J. Chromatogr.* **1998**, *827*, 105.
31. Sun, Y. X.; Gu, P. J. *Environ. Sci. Technol.* **2007**, *19*, 885.
32. Lopez-Avila, V.; Goor, T.; Gas, B.; Coufal, P. *J. Chromatogr.* **2003**, *993*, 143.
33. Richardson, S. D.; Thruston, A. D.; Caughran, T. V.; Chen, P. H.; Colette, T. W.; Floyd, T. L.; Schenck, K. M.; Lykins, B. W.; Sun, G.-R.; Majetich, G. *Environ. Sci. Technol.* **1999**, *33*(19), 3378.
34. Rapp, T. M.D. Thesis, University of Massachusetts, 2000.
35. Choi, Y. W.; Rekhow, D. A. *Bull. Korean Chem. Soc*. **2004**, *25*(6), 900.
36. Kai, M.; Kojima, E.; Ohkura, Y. *J. Chromatogr. A* **1993**, *653*, 235.