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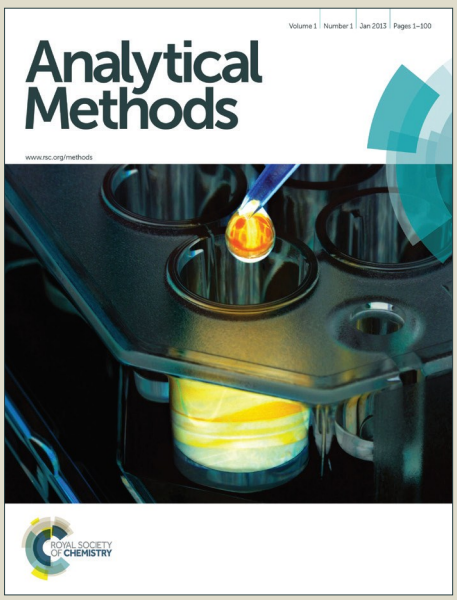

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Sensitive determination of glutaraldehyde in environmental water by derivatization and gas chromatography-mass spectrometry

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A highly sensitive gas chromatography mass spectrometric method (GC-MS) has been established for the determination of the level of glutaraldehyde in drinking water, surface water and waste water. The method is based on the derivatization of glutaraldehyde with *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine in water. The following optimum reaction conditions were established: reagent dosage, 10 mg L^{-1} of reagent; pH 4; reaction for 10 min at 70 ℃. The glutaraldehyde oxime derivative was detected by GC-MS after the micro liquid-liquid extraction with ethylacetate 0.1 mL. The limits of detection and the limits of quantification of glutaraldehyde were in a concentration range of 0.008-0.03 μg L^{-1} and 0.03-0.09 μg L^{-1} respectively in water matrices. The calibration curves showed good linearity with $r \geq 0.9995$; the accuracy was in a range of 90-107%; and the precision of the assay was better than 14% in all water matrices. This method is sensitive enough to permit reliable analysis of glutaraldehyde to the ng L^{-1} level in environmental water.

1. Introduction

Glutaraldehyde (GA) is a dialdehyde consisting of two formyl groups (Fig. 1) and a clear, colorless to pale straw-colored, pungent oily liquid that is soluble in all proportions in water and alcohol. It has found its widest application in various fields such as chemical sterilization and biomedical and pharmaceutical sciences;[1-4] it is mainly available as acidic aqueous solutions (pH 3.0–4.0), ranging in concentration from less than 2% to 70% (w/v).

The term Avian influenza (AI) refers to the disease caused by infection with the avian influenza type A viruses. These viruses occur naturally among wild aquatic birds worldwide, and can infect domestic poultry and other bird and animal species. In addition, serious outbreaks of foot-and-mouth disease often lead to the culling of enormous numbers of animals to halt outbreaks.[5] GA has been applied in the environment to disinfect humans and equipment near any outbreak area of AI or foot-and-mouth disease. The health effects of GA in humans and animals can be seen in irritation of the skin, eyes, and respiratory tract, and in skin sensitization. GA may be some risk to aquatic organisms, specifically algae.[1,4] These troubling effects are intensified by repeated exposure.[1,4] Until now, there is no regulation about maximum permitted concentration of GA in surface water and drinking water in any nation. But GA analysis is necessary to control the amount of the disinfectant in environmental water.

Several methods based on different principles have been proposed for the determination of GA. Spectrophotometry[4] and capillary electrophoresis (CE)[6] have been successfully introduced in the analysis of GA. High performance liquid chromatography (HPLC) methods[7-10] have also been reported as techniques for the determination of GA,

mainly in the air of work environments based on the derivatization with dinitrophenylhydrazine (DNPH).[7,8,10] Gas chromatography (GC) methods have also been reported for the determination of GA in the air of work environments using solid phase microextraction and electron-capture detection (ECD) after the on-fiber derivatization with *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA)[11] or in germicide products with flame ionization detection without derivatization.[12]

The choice of the derivatization reagent is important not only for the sensitive detection, but also for the stabilization of GA, improvement of chromatographic properties and responses (electrospray ionization-mass spectrometry). The best reagent reacts rapidly at weakly acidic pH to prevent side reaction of the analyte and specifically with GA to produce stable product. Until now, two reagents have been published for the GA derivatization. Generally, the reaction of GA with DNPH can yield three DNPhydrazones (E–E, E–Z and Z–Z) and three peaks; the presence of these isomers makes quantification difficult.[7] Moreover, the derivatization method using PFBHA is limited to the determination of GA in the air. Several simultaneous analytical methods of aldehydes in drinking water have been developed using solid-phase extraction,[13-15] and ultrasound-assisted dispersive liquid–liquid microextraction,[16] and micro liquid-liquid extraction[17] GC or headspace GC-mass spectrometry (HS-GC-MS)[18] after the derivatization with PFBHA, but GA was not only included as an analyte, but also most of their derivatives gave two isomer peaks. Therefore, the optimum reaction conditions of GA with PFBHA in water needs to be established because no studies to date have been reported on a GC-MS method for the determination of GA in environmental water.

This study focuses on determining the best reaction conditions of GA with PFBHA,

the best extraction conditions, and a GC-MS method to detect GA in water.

2. Materials and methods

2.1. Materials

PFBHA hydrochloride (≥99%), GA standard solution (70% in H_2O), acetone-d_6 (99.9 atom % D), methyl-*tert*-butyl ether (MTBE), ethyl ether, methylene chloride, pentane, hexane and ethyl acetate were obtained from Sigma-Aldrich (St. Louis, MO, USA). A stock standard solution of GA (1,000 mg L^{-1}) was freshly prepared before use by dissolving 10 mg of GA standard solution in 7.0 mL methanol or reagent water. The reagent water used in this study was purified using a Milli-Q-Reagent-Grade water system (ZD20) that had a resistivity of over 17 MΩ.

2.2. Derivatization and extraction

A water sample of 5 mL volume was placed into a 15 mL glass-stoppered test tube. 1.0 M HCl was added to control the pH 4 and the test tube was agitated for 1-2 min. 20 μL PFBHA (1% in water, w/v) and 50 μL of acetone-d_6 (0.1 mg L^{-1} in MeOH) as an internal standard (IS) were then added to the solution and the solution was reacted at 70 ℃. After 10 min, 0.1 mL of ethyl acetate and about 5 g of sodium sulfate were added to the solution for extraction by mechanical shaking for 5 min. The organic phase containing the PFBHA oxime derivative was carefully transferred to a V-shape insert in an auto vial, and a 1.0 μL sample of the solution was injected into the GC-MS system.

2.3. Water sampling

Ten surface water samples were collected from 10 basins in the Gum-River, Rep. of Korea, without headspace in 40 mL brown glass bottles that had been previously washed and then rinsed thoroughly with methanol, acetone, and distilled water before drying. The sampling sites were selected to uniformly represent all the streams of the river. Drinking water samples were collected after ozonation and/or chlorination from water treatment plants without headspace in 40 mL brown glass bottles. The waste water was collected from a pond beside the company offices, which is the point of discharge of a textile company situated in Daejeon in the Southwest Zone of the Rep. of Korea. The samples were preserved in a refrigerator in the laboratory, where they were analyzed within a period of 7 days after the time of collection.

2.4. Calibration and quantification

The calibration curve for the linearity test was established by adding 20, 50, and 100 μL of GA standard (0.005 mg L^{-1} in reagent water), 20, 50, 250, and 500 μL of GA standard (0.1 mg L^{-1} in reagent water), and 50 μL of IS (0.1 mg L^{-1} in MeOH) to 5 mL samples of surface water in which the analyte was not detected. The next procedures were performed according to the derivatization and extraction method. A calibration curve was obtained from the regression line of the peak area ratios of GA (m/z 181) to the IS (m/z 259) on concentration using a least-squares fit.

2.5. Gas chromatography-mass spectrometry

The analytical instruments used were an Agilent 7890 A gas chromatograph with a splitless injector (Agilent Technologies, Santa Clara, CA, USA). The analytical column was an HP-InnoWax capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness).

The oven temperature began at 40 ℃, was raised to 135 ℃ at 10 ℃ min^{-1}, and then was raised to 240 ℃ at 25 ℃ min^{-1} and held for 5 min. All mass spectra were obtained with an Agilent 5975 B instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230 ℃). Full-scan mass spectra (*m/z* 40-800) were recorded for the identification of the analyte at a high concentration. Confirmation of analytes was completed using four MS characteristic ions; the ratios of the three MS characteristic ions to quantification ion (m/z 181) and the GC-retention time matched those of the known standard compound. The ions selected by selected ion monitoring (SIM) were *m*/*z* 259, 181, 212, and 229 for acetone-d6-oxime derivative (internal standard) and *m*/*z* 490, 181, 293, and 278 for GA-oxime derivative.

3. Results and discussion

3.1. Derivatization

The most commonly used reaction to determine aldehydes and ketones is the reaction of an amino (NH_2) group of hydrazine or hydroxylamine with a carbonyl (C=O) group to form a stable Schiff base (C=N). The derivative has been very useful in analysis by HPLC or GC. GA has two formyl (CHO) groups attached to both end-carbon centers on a propane and also reacts with DNPH to form three DNPhydrazones, which are separated to three peaks by HPLC.[7]

We studied a derivatization method based on the reaction of GA with PFBHA. As depicted in Fig. 1, two carbonyl groups of GA react with two PFBHAs to make a GA bis-(*o*-pentafluorophenylmethyloxime) product, which appears a single peak by GC.

The derivative was tested in terms of reactivity with GA; the stability of the

derivative was analyzed to determine its utility in the determination of GA in water.

The reaction was performed at various pH values from 1.0–7.0 and showed high response as a mean value at a pH value of 4.0. No significant difference for the response was noted in the range of pH 1-5 and it declined slowly above pH 5.0, as shown in Fig. 2-a; therefore, the pH of 4.0 was selected. The pH value was correspond with an optimum pH condition proposed for dicarbonyl aldehydes in literature.[13,17] This weakly acidic pH may be the best condition to use to prevent side reactions because GA at strong acidic or alkaline pH can be polymerized by aldol condensation reaction to alpha, beta-unsaturated poly-glutaraldehyde.[1]

The derivatization was performed at various PFBHA concentrations (1.0, 2.0, 5.0, 10, 20, and 30 mg L^{-1}). The yield was continuously above 5.0 mg L^{-1} of PFBHA, as shown in Fig. 2-b. The optimum PFBHA amount was therefore set at 10 mg L^{-1}, considering the substances that consume the reagent in real samples. The other reaction conditions were set so as to have a reaction time of 10 min at a temperature of 70 ℃.

The optimum reaction temperature and time for GA with PFBHA were also studied. The reactivity of the derivative was analyzed at reaction temperatures of 40, 50, 60, 70, and 80 ℃; the reaction time was analyzed at values of 3, 5, 10, 20, 30, and 40 min. From the experiment, the optimal reaction condition was found to be 10 min at 70 ℃ (Fig. 3). The recovery declined slightly beyond the reaction temperature of 70 ℃. No significant variation in the area of the reaction product was noted over this time period.

As a result, the optimal reaction conditions of GA with PFBHA were found to be 10 mg L^{-1} PFBHA, pH 4, and reaction time of 10 min at 70 ℃.

To evaluate the stability of the derivatives, the experiment was repeated by analyzing

extracts stored in dark conditions at 4 ℃ and room temperature for 2 weeks. The % loss of the product after 2 weeks was within 2.0% and the derivative showed good stability.

The reaction has the following advantages in comparison with other derivatization reagents: this reaction makes only a single product, which makes the analysis quantitative. Also, this reaction is simple and rapid at mild pH condition, and very sensitive to GC-MS.

3.2. Extraction

For the GC-MS analysis, the derivative must be extracted from the water sample with an organic solvent. To study the optimum extraction pH and solvent of the derivative, the extraction was carried out over the range of pH 0-10. No significant difference in the area of the reaction product was noted in the range of pH 0-10. Therefore, the samples were extracted at a reaction pH of 4.0 without any pH adjustment. Ethyl acetate, MTBE, diethyl ether, pentane, hexane, and methylene chloride were tested as extraction solvents for the derivatives. Among the solvents tested, ethyl acetate was shown to have the highest peak area for extraction of the derivative and especially about two-fold higher peak area than that of hexane proposed as an extraction solvent in the literature.[17] Our purpose to find the optimum volume was to obtain the maximum signal of derivative without further concentration step.

The optimal volume of extraction solvent was evaluated after single extraction using 0.05, 0.1, 0.2, 0.3, and 0.4 mL ethyl acetate to obtain the maximum signal of derivative without concentration step, while the other parameters were kept constant. It is well known that the signal can be observed to decrease on increasing the volume of ethyl

acetate due to the dilution effect of the derivative. It is also very difficult to collect the upper layer using 0.05 mL volume of extraction solvent. Hence, a volume of 0.1 mL was selected as the optimal value for the extraction solvent to carry out the subsequent steps.

3.3. Chromatography and mass spectrometry

Drawback of PFBHA as a derivatizing agent is that their reaction products with aldehydes, except methanal, exist as two isomers, syn and anti, which are subject to analytical errors. To overcome this issue, a chromatographic method for a single peak from co-elution of two isomers was developed. When HP-1, HP-5 and DB-17 were used for the separation column, we cannot obtain a single peak. Otherwise, a sharp single peak could be achieved by the column selection of an HP-InnoWax capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness), and optimum oven temperature program proposed in the experimental part.

The column was stable over more than two hundred injections without notable retention time change within relative standard deviation (RSD) of 0.2 %. SIM chromatograms of m/z 181 for GA derivative and m/z 259 for IS are shown in Fig. 4. Separation of the derivatives and the internal standard from the background compounds of water was very good. There were no extraneous peaks observed in the chromatograms of blank water, tap water, surface water, or waste water at internal standard and GA retention times of 7.8 and 16.1 min, respectively.

A full-scan mass spectrum was obtained for the confirmation of the GA bis-(*o*-pentafluorophenylmethyloxime) during elution of the analyte peak at 16.1 min. The mass spectrum shows a molecular ion at *m/z* 490, and diagnostic ions at *m/z* 181, 293

and 278. The ions at *m/z* 293 and *m/z* 278 are from the loss of [$C_6F_5CH_2O$] and [$C_6F_5CH_2ONH$] from the molecular ion, respectively, and the ion at 181 is of pentafluorobenzyl group [$C_6F_5CH_2$] as shown in Fig. 5.

In order to perform the SIM analysis, the ion with higher abundance was chosen to achieve more sensitivity and that with higher m/z ratio was chosen to achieve more specificity. The ions m/z 293, 278 and 181 were evaluated to be ideal as quantification ion. We found that the selection of m/z 181 can give more high sensitivity without any interference at various matrices. For the confirmation of analyte in real samples, four MS characteristic ions (*m/z* 490, 181, 293, and 278) were simultaneously detected and the ratios of the three MS characteristic ions to quantification ion (m/z 181) were compared those of the standard compound (Fig. 5). The ratios showed no significant difference with those of the standard compound in spiked real samples. Moreover, quantification values of GA using m/z 181 showed no significant difference with those using m/z 293 or 278. Otherwise, analyst can choose m/z 293 or 278 as a quantification ion for the quantification of above μg/L.

3.4. Method validation

A calibration curve was obtained by the extraction and concentration after the derivatization of GA in surface water, in which no analyte was detected. The regression line of the peak area ratio of GA to the internal standard on concentration, obtained using a least-squares fit, demonstrated a linear relationship with correlation coefficients >0.9997. The lines of best fit for the analytes are shown in Table 1.

Using a 5.0 mL sample volume, the limit of detection (LOD) and limit of quantification (LOQ) in this study were calculated in a concentration range of 0.008-

0.03 μg L^{-1} and 0.028-0.09 μg L^{-1} in three water matrices. LOD and LOQ were estimated in each matric sample containing 0.02 μg L^{-1}, as the concentration resulting in a minimum signal-to-noise ratio of 3 and 10.[15] LOD and LOQ showed the similar values when they were estimated using tap water.

Accuracy and precision were assessed by determining the recovery in samples spiked in drinking water and surface water, respectively. Intra-day accuracy and precision were evaluated using five spiked samples at concentrations of 0.1, 1.0, and 10.0 μg L^{-1} for GA, and inter-day accuracy and precision were determined by their recovery in spiked samples on five different days. The reproducibility of the assay was very good, as shown in Table 1. The accuracy was in a range of 90-107% and the precision levels of the assay were better than 14%. To test the influence of the matrix on the determination of GA, the accuracy and precision of the method reported herein were assessed using waste water that contained dissolved organic compounds at a level of 23 mg L^{-1} as test samples for extreme organic compound contamination. Intra-day and inter-day accuracy and precision were determined using the same method as was used for drinking water and surface water. As a result, the accuracy was found to be in a range of 94-109%; precisions values of the assay were less than 9%. The method is thought to be reproducible enough to permit reliable analysis of GA without interference from severe contamination in surface water.

3.5. Application

We used the proposed method to analyze GA in ten surface water and drinking water samples. As a result, GA was not detected in any of the samples. GA can be discharged in surface water from waste water or can exist near outbreak areas of AI or foot-and-

mouth disease. However, its existence in natural water was not found in this study, perhaps because of the instability of GA in water and because the sampling time did not coincide with an outbreak time of AI or foot-and-mouth disease. Although GA was not detected in these specimen samples, continuous monitoring for GA in surface water and drinking water is necessary to manage GA in natural water.

4. Conclusions

A GC-MS analytical method based on the reaction of GA with PFBHA was developed. The major advantages of this method are as follows: (1) The chromatogram shows only a single peak of GA derivative and the reaction is simple and rapid at mild pH condition. (2) The proposed method can sensitively determine GA to LOD of 0.008-0.03 μg L^{-1} without interference from serious contaminants in environmental water. (3) This method offers a new GC-MS method and easier derivatization procedure. (4) This method requires 0.1 mL solvent for the sample extraction and is environmentally friendly.

The developed method provides an important tool for evaluating trace levels of GA in environmental samples.

Acknowledgments

This work was supported by the South Korean Ministry of Environment (MOE) as part of "the Environmental Health Action Program."

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Fig. 1 Derivatization of GA to its corresponding GA-oxime derivative.

Fig. 2 Effect of pH (A) and PFBHA concentration (B) on the reactivity of GA (reaction temperature = 70 ℃, reaction time = 10 min, area ratio of the derivative to phenanthrene-d10, which is used as an internal standard for calculation of the derivatization yield, Concentration of GA = 0.01 mg L^{-1}, monitoring ion = m/z 181 for GA, m/z 188 for phenanthrene-d10, monitoring retention time = 13.8 min for phenanthrene-d10 and 16.1 min for GA, reagent dosage = 10 mg L^{-1} in experiment A).

Fig. 3 Effect of the reaction temperature (pH = 4, reagent dosage = 10 mg L^{-1}, reaction time = 10 min) (A) and reaction time (pH =4, reagent dosage = 10 mg L^{-1}, reaction temperature = 70 ℃) (B) on the reactivity of GA with PFBHA (area ratio to phenanthrene-d10, concentration of GA = 0.01 mg L^{-1}, monitoring ion = m/z 181 for GA / m/z 188 for phenanthrene-d10, monitoring retention time = 13.8 min for phenanthrene-d10 and 16.1 min for GA).

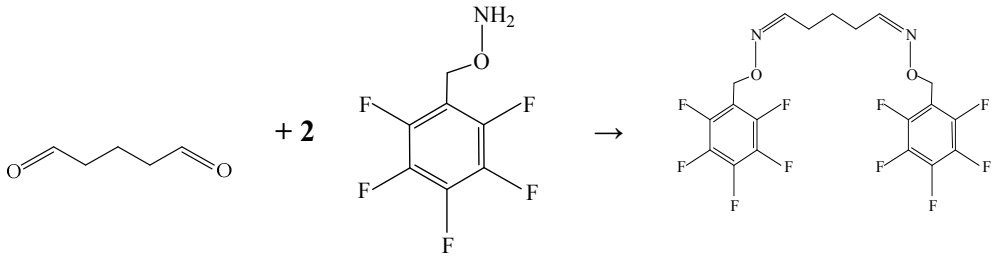
Fig. 4 GC-MS chromatograms of a surface water (A), in which the analyte was not detected, and the spiked sample with GA 1.0 μg L^{-1} in surface water (B), GA 1.0 μg L^{-1} in tap water (C) and GA 1.0 μg L^{-1} in waste water (D) (monitoring ion = m/z 181 for GA, m/z 259 for acetone-d6, monitoring retention time = 7.8 min for acetone-d6 and 16.1 min for GA).

Fig. 5 Mass spectrum of GA-oxime derivative (A) and ion composition of the peak (B).

(The concentration of GA = 0.01 mg L^{-1}, monitoring ion = m/z 181 for GA, m/z 259 for acetone-d6, monitoring retention time = 7.8 min for acetone-d6 and 16.1 min for GA).

Table 1 Intra and inter-day laboratory accuracy and precision results for the analysis of GA in water (*n*=5)

Matrix	Detection limit (μg L^{-1})		Calibration curve			Spiked Conc. (μg L^{-1})	Intra-day measured value			Inter-day measured value		
	LOD	LOQ	Conc Range (μg L^{-1})	Linear Equation	r		Mean ± SD (μg L^{-1})	Accuracy (%)	Precision (%)	Mean ± SD (μg L^{-1})	Accuracy (%)	Precision (%)
Tap water	0.008	0.03	0.03-100	y=0.0014x+ 0.0001	0.9998	0.1	0.1 ± 0.004	97.7	4.3	0.1 ± 0.006	98.4	5.7
						1	0.9 ± 0.041	90.2	4.5	0.9 ± 0.015	91.9	1.6
						10	9.7 ± 0.626	96.6	6.5	10.0 ± 0.739	99.5	7.4
Surface water	0.03	0.08	0.08-100	y=0.0064x+ 0.0034	0.9995	0.1	0.1 ± 0.001	100.5	1.4	0.1 ± 0.005	97.0	5.2
						1	1.0 ± 0.062	104.3	6.0	1.1 ± 0.097	107.1	9.0
						10	9.8 ± 0.711	98.2	7.2	10.1 ± 1.378	100.9	13.7
Waste water	0.03	0.09	0.1-100	y=0.0024x+ 0.0005	0.9996	0.1	0.1 ± 0.007	105.3	6.9	0.1 ± 0.003	108.8	3.1
						1	1.0 ± 0.074	98.9	7.5	0.9 ± 0.082	94.8	8.7
						10	10.1 ± 0.852	101.1	8.4	9.5 ± 0.494	95.4	5.2



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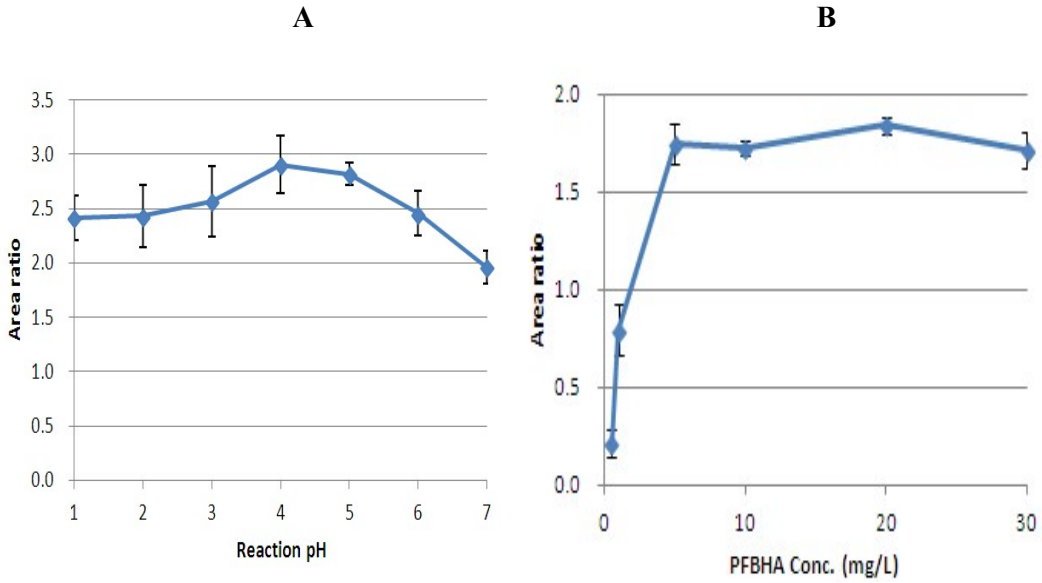

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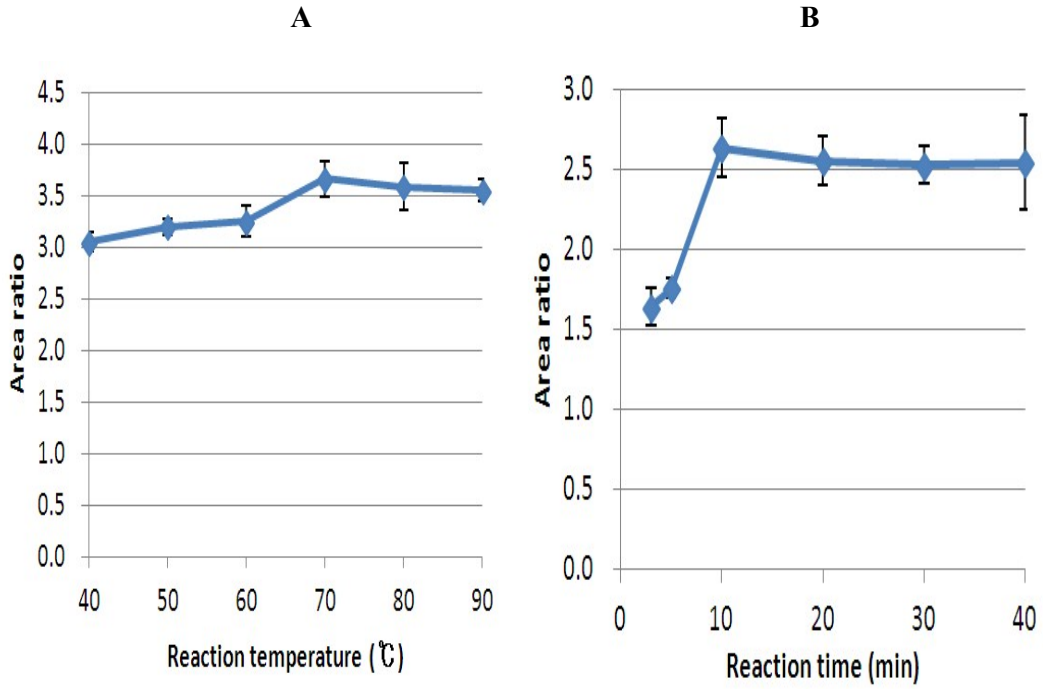

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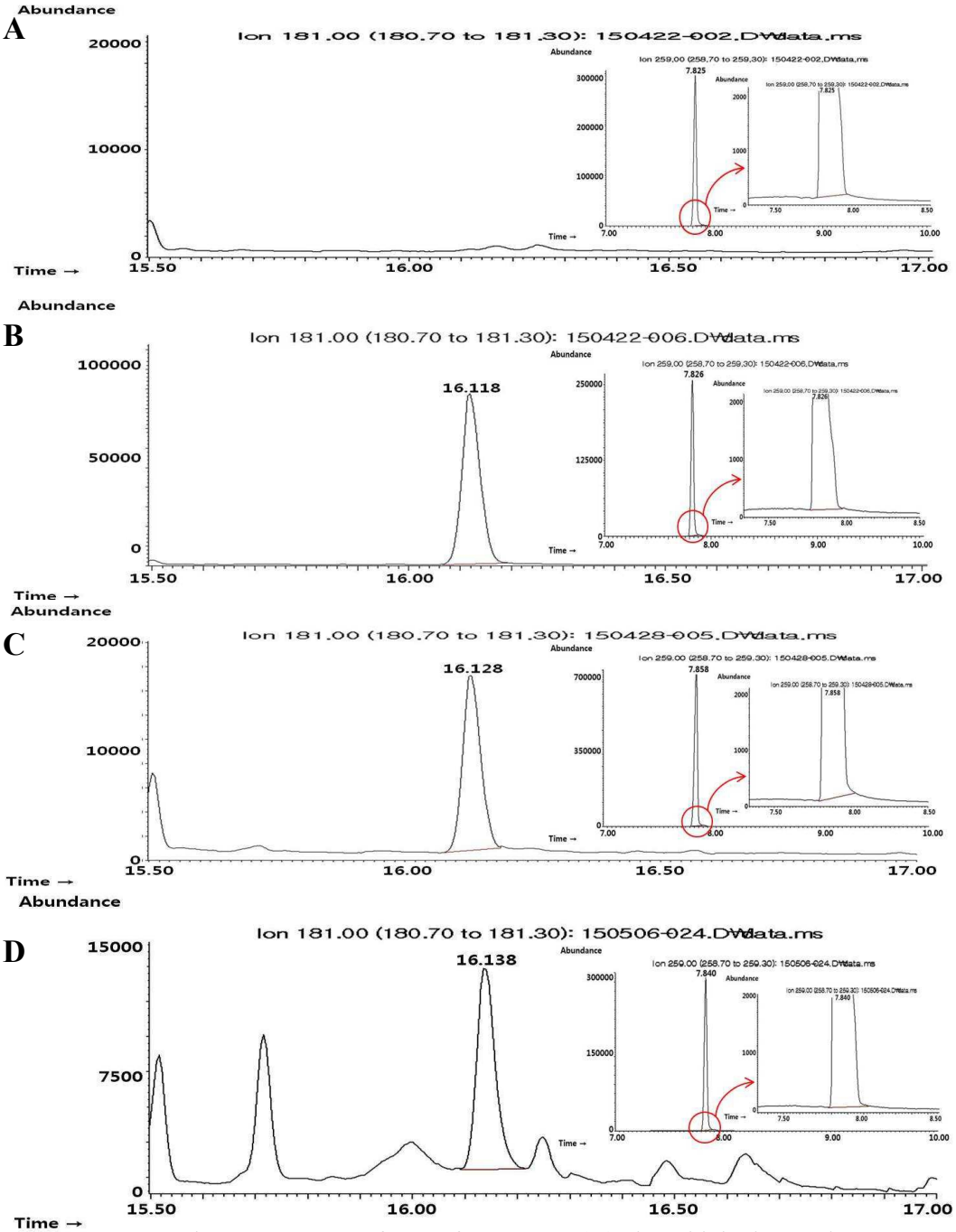

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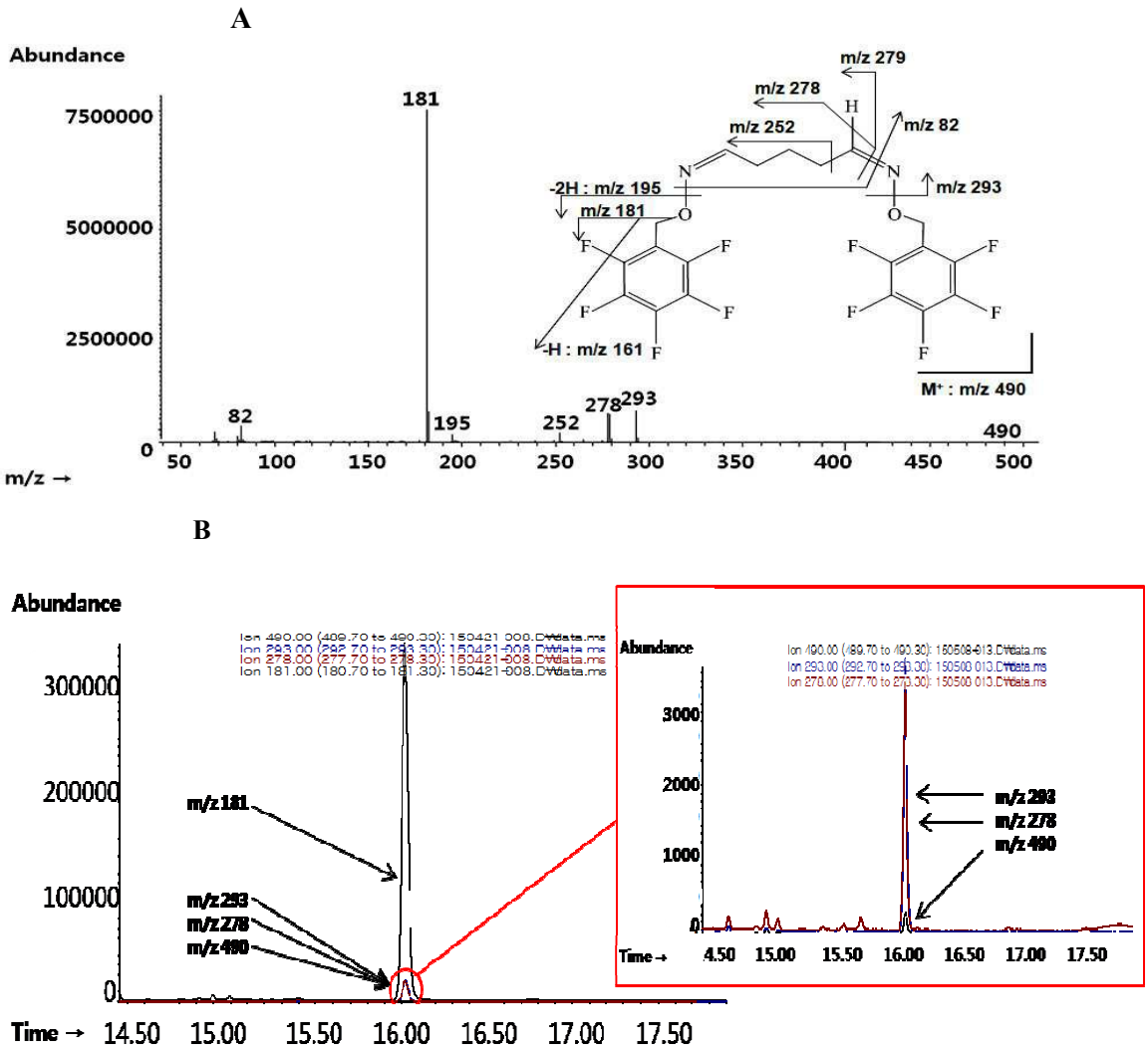

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