Fluorescence detection of Trace Dimethylamine and Diethylamine in Natural Water by HPLC with O-phthalaldehyde and 7-chlore-4nitrobenzo-2-oax-1, 3-diazole

P.F. Gao*, J.S. Zheng, S.M. Zhang and X.J.Wen

College of Environmental Science and Engineering, Xiamen University of Technology, Xiamen 361024, China.

*E-mail: gaopf@xmut.edu.cn

Abstract: A method of detecting dimethylamine (DMA) and diethylamine (DEA) was developed by HPLC with pre-column derivatization and fluorescence detection in this paper. Two fluorescent derivatization reagents were chose to used in the method including o-phthalaldehyde (OPA) and 7-chlore-4-nitrobenzo-2-oax-1,3-diazole (NBD-Cl). Aliphatic amines in the natural water were labeled in two steps, the water samples were firstly reacted with OPA to eliminate aliphatic primary amines interferences, and then NBD-Cl could be used to selective detection of secondary amines, including dimethylamine and diethylamine. The detection limits of the method were 0.1 nmol/L and 10 nmol/L for DMA and DEA. Both of the linear ranges were 0.0005 -0.1mol/mL with correlation coefficients above 0.9978 and 0.9988. This method is repeatable, sensitive and accurate for the detection of DMA and DEA in natural water samples.

1. Introduce
As one of the most important disinfection by-products (DBPs), N-nitrosodimethylamine (NDMA) is a famous human carcinogen [1, 2]. It has become the research hotspot in water environment field gradually in recent years [3, 4]. Dimethylamine (DMA) is considered to be the most important precursor to the formation of NDMA [5]. It could be converted to NDMA by nitrosation and asymmetric dimethylhydrazine oxidation [6]. Therefore, it plays an important role in the detection of natural water quality [7].

At present, a series of mature methods were developed to detect dimethylamine and other short chain aliphatic amines, including high performance liquid chromatography (HPLC) [8, 9], capillary electrophoresis(CE) [10, 11], ion chromatography [12, 13]and gas chromatography (GC) [14, 15]. HPLC methods with fluorescent derivative reagents have been widely used to detect aliphatic amine, because of their simplicity and practically acceptable sensitivity. Due to the similar chromatographic behaviors of derivatives of dimethylamine, methylamine and ethylamine when they derivative reaction of diazomethane with acid herbicides occurred, the separation and determination of dimethylamine derivative are often interfered by the latter two aliphatic primary amine in HPLC under the condition of using the same kind of fluorescent derivative reagent. Therefore, Two fluorescent reagents with different maximum emission wavelengths were chose including OPA and NBD-Cl in the method. OPA can only react with primary amines. NBD-Cl can react with primary amines and secondary amines, and it was more feasible to achieve the reaction between NBD-Cl and secondary amines [11].
Based on the above characteristics, when OPA was first selected to react with the sample solution, the primary amines interference was eliminated. Interferences. The proposed method for the analysis of DMA is reproducible and sensitive, it will be helpful to study the relationship between secondary amines with NDMA in the natural water.

2. Methods and materials

2.1. Chemicals

Four aliphatic amines were purchased from Sigma (St. Louis, MO, USA). 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazol (NBD-Cl), 3-Mercaptopropionic acid, o-phthalaldehyde (OPA) were obtained from Afa Elsa (Tianjin) Chemical Co., Ltd. (Tianjin, China). Methanol (HPLC grade) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Water was purified using a Milli-Q RG system (Millipore, Bedford, MA, USA).

Standard solutions of different aliphatic amines were prepared by dissolving proper amount with water, and dilution of them to appropriate concentrations with water before use.

H3BO3-Na2B4O7 buffer was obtained by mixing 0.1 M Na2B4O7 solution with 0.1 M H3BO3 solution to the desired pH value. 13.4 mg OPA dissolved in 25mL methanol, take 5mL of the stock solution, and added 20 μL 3-mercaptopropionic acid, then diluted to 25 mL with borax buffer (pH10), the final solution were saved in 4 degrees refrigerator protected from light. All water samples were filtered through 0.45μm membrane filter.

2.2. Instrumentation and method

2.2.1 Fluorescence derivatization reaction with OPA and NBD-Cl. To a 1.5 mL vial containing 100 μL water sample under test, 200 μL OPA solution was added and reacted for 5 min at room temperature after mixture. Then the derivative solutions was heated at 40°C in the water bath for 60 min after the adding of 200 μL of NBD-Cl solution (10 mM in acetonitrile) and 100 μL borate buffer (100 mM at pH 7.0), 20 μL of derivative reaction mixture was injected to HPLC for analysing.

2.2.2 Chromatographic separation conditions of NBD-Cl derivative. As the mobile phase, methanol-water (40:60, v/v) was used at the flow rate of 0.8 mL min⁻¹. The detection wavelength of NBD-Cl derivatives was set at 470 nm in HPLC. The HPLC system consisted of an LC-20A chromatographic system (Shimadzu, Tokyo, Japan) with RF-10AXL fluorescence detector (Shimadzu, Tokyo, Japan). The separation and determination were achieved on a C18 column (5 μm, 250 mm × 4.6 mm i.d., Kromasil, Bohus, Sweden).

3. Results and discussion

3.1 Optimisation of the NBD-Cl derivatization conditions

The derivatization condition of OPA was similar to the report by Dugan et al [9]. When optimizing the derivatization condition of NBD-Cl, the effects of different dosage of NBD-Cl solution, pH values of reaction solutions, derivatization temperature and time were tested. The results could be seen in Figure 1. the dosage of NBD-Cl solution was set at 100μL. the optimum pH value of borate buffer solution was 7.0 with higher reaction yield. The best temperature and time of derivatization reaction were 40°C and 60 minutes, respectively.
3.2. The elimination of methylamine and ethylamine interferences

The experimental results show that the existences of methylamine and ethylamine had significant interference to the detection of DMA. Their derivates can not be effectively separated although the concentration of methanol was as low as 40 percent in the mobile phase. However, if the amines samples firstly reacted with OPA before NBD-Cl, the interferences of methylamine and ethylamine could be eliminated. All was shown in Figure 2.

3.3 Linear relationship and detection limit

It was evaluated that the linear relationship and detection limit of the method for DMA and DEA by HPLC. The experiment results were shown in Table 1. The method is simple and reproducible because it effectively solves the problems of primary amines interference.

| No.       | linear equations          | R²    | Detection range (M) | Detection limits (nM) |
|-----------|---------------------------|-------|---------------------|-----------------------|
| Dimethylamine | $y=2.05\times 10^3x+4.88\times 10^5$ | 0.9978 | 0.0005-0.1          | 0.1                   |
| Diethylamine  | $y=3.74\times 10^3x+1.29\times 10^4$ | 0.9987 | 0.0005-0.1          | 10                    |
3.4. Application of the method to lake water samples

Then this method was used to detect aliphatic secondary amines in the lake water sample. They were shown in Table 2. The experiment results of the standard addition method were shown in Figure 3.

Table 2. Analytical results for the lake water sample.

| Added (mg/L) | Found (mg/L) | R.S.D. (% n=6) | Recovery (%) |
|--------------|--------------|----------------|--------------|
| Dimethylamine | 0.025 | 0.316 | 2.8 | 93.7 |
| Diethylamine | 0 | 0.053 | 1.3 | |

Figure 3. Chromatograms obtained from lake water samples. Chromatograms obtained from (a) lake water sample; (b) the same water sample spiked with 5 μM of DMA and 0.5 μM of DEA. Peaks: (1) DMA; (2) DEA; Chromatographic conditions as in Figure 2.

4. Conclusion

OPA can block primary amines interference because it can only react primary amines but not secondary amines. And there is a significant difference in the different fluorescence detection wavelength between OPA derivative and NBD-Cl derivative. Therefore, a HPLC method with OPA and NBD-Cl as pre-column derivatization agent was developed for the determination of aliphatic secondary amines. it was well optimised including derivative conditions and HPLC conditions. Good performance was obtained with respect to selectivity, reproducible and sensitive. This method will be helpful to study the influence mechanism of trace DMA on the formation of NDMA. It will be a reliable research method for the study of disinfection by-products. At the same time, it is necessary to develop the novel highly sensitive fluorescent reagent for the determination of DMA.

Acknowledgments

This research was supported by funding from Science and Technology Planning Project of Xiamen City with Grant 3502ZZ20179042.

References

[1] Choi J., Valentine R.L. Formation of N-nitrosodimethylamine (NDMA) from reaction of monochloramine: a new disinfection by-product.[J]. Water Research, 2002, 36(4):817-824.
[2] Mitch W.A., Sedlak D.L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination[J]. Environmental Science & Technology, 2002, 36(4):588-595.

[3] Liu Y.D., Zhong R. Comparison of N-nitrosodimethylamine formation mechanisms from dimethylamine during chloramination and ozonation: A computational study.[J]. Journal of Hazardous Materials, 2017, 321:362-370.

[4] Spahr S., Cirpka O.A., Gunten U.V., et al. Formation of N-nitrosodimethylamine during chloramination of secondary and tertiary amines: Role of molecular oxygen and radical intermediates[J]. Environmental Science & Technology, 2017, 51(1):280-290.

[5] Andrzejewski P., Kasprzyk-Hordern B., Nawrocki J. N-nitrosodimethylamine (NDMA) formation during ozonation of dimethylamine-containing waters[J]. Water Research, 2008, 42(4):863-870.

[6] Andrzejewski P., Fijolek L., Nawrocki J. An influence of hypothetical products of dimethylamine ozonation on N-nitrosodimethylamine formation[J]. Journal of Hazardous Materials, 2012, 229-230(3):340-345.

[7] Przemysław A., Barbara K.H., Jacek N. The hazard of N-nitrosodimethylamine (NDMA) formation during water disinfection with strong oxidants[J]. Desalination, 2005, 176(1):37-45.

[8] Gao P.F., Zhang Z.X., Guo X.F., et al. Determination of primary and secondary aliphatic amines with high performance liquid chromatography based on the derivatization using 1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric ester)-difluoroboradiaza-s-indacene.[J]. Talanta, 2011, 84(4):1093-1098.

[9] Han L., Hou D., Ma C., et al. Simultaneous determination of eight short-chain aliphatic amines in drug substances by HPLC with diode array detection after derivatization with halonitrobenzenes[J]. Journal of Separation Science, 2017, 40:3074-3085.

[10] He L, Xu Z, Hirokawa T, et al. Simultaneous determination of aliphatic, aromatic and heterocyclic biogenic amines without derivatization by capillary electrophoresis and application in beer analysis[J]. Journal of Chromatography A, 2017, 1482:109-114.

[11] Dugan M E R, Thacker R D, Aalhus J L, et al. Analysis of 4-hydroxyproline using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol derivatization and micellar electrokinetic chromatography combined with laser-induced fluorescence detection[J]. Journal of Chromatography B Biomedical Sciences & Applications, 2000, 744(1):195-199.

[12] Zhou S., Lin J., Qin X., et al. Determination of atmospheric alkyamines by ion chromatography using 18-crown-6 as mobile phase additive.[J]. Journal of Chromatography A, 2018, 1563:154-161.

[13] Mori M, Helaleh M I, Xu Q, et al. Vacancy ion-exclusion/adsorption chromatography of aliphatic amines on a polymethacrylate-based weakly basic anion-exchange column.[J]. Journal of Chromatography A, 2004, 1039(1):129-133.

[14] Huang J., Ou C., Lv F., et al. Determination of aliphatic amines in food by on-fiber derivatization solid-phase microextraction with a novel zeolitic imidazolate framework 8-coated stainless steel fiber[J]. Talanta, 2017, 165:326-331.

[15] Liu F.X., Xin-Hui B.I., Ren Z.F., et al. Determination of Amines Associated with Particles by Gas Chromatography-mass Spectrometry[J]. Chinese Journal of Analytical Chemistry, 2017, 45(4):477-482.