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Determination of four phenolic endocrine disrupting chemicals in Dianchi Lake, China

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An efficient derivatization method using phenyltrimethylammonium (PTA-OH) has been developed to determine simultaneously four phenolic endocrine disrupting chemicals, 4-*n*-nonylphenol (4-*n*-NP), 4-*tert*-octylphenol (4-*t*-OP), bisphenol A (BPA) and 4-cumylphenol (4-CP) in surface water of Dianchi Lake (China) by solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS). Compared with silylation of target phenols using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS), methylation by PTA-OH displayed a higher response and stability based on the investigations of various derivatization conditions, including derivatization solvent, amount of derivatization reagent, reaction temperature and time. Experiments were carried out to examine the performance of the proposed method based on the correlation coefficient, the method quantification limit (MQL), mean recovery rate and relative standard deviation (RSD). Under optimum derivatization conditions, MQLs of the methylated target compounds were all below 1 ng L^{-1}. Results revealed that the proposed method exhibited a satisfactory precision and reproducibility for the separation and determination of target phenols. The proposed method had been applied to determine four phenols in surface water of Dianchi Lake located in southwest of China. The concentrations of 4-*n*-NP, 4-*t*-OP, BPA and 4-CP were determined to be 13.6-141.6 ng L^{-1}, N.D.-56.5 ng L^{-1}, N.D.- 4713.6 ng L^{-1} and 23.3-48.5 ng L^{-1}, respectively.

Keywords: derivatization; phenols; endocrine disrupting chemicals; gas chromatography-mass spectrometry; Dianchi Lake

1. Introduction

Surface water pollution caused by endocrine disrupting chemicals (EDCs), which include both natural and synthetic chemicals from food products, household products, pesticides, plastics, pharmaceuticals, industrial wastes and metals, has become an important international issue for its endocrine disrupting activity at trace level concentration and ubiquitous occurrence [1,2]. Four highly potent phenolic EDCs including 4-*n*-nonylphenol (4-*n*-NP), 4-*tert*-octylphenol (4-*t*-OP), bisphenol A (BPA) and 4-cumylphenol (4-CP) were selected as the target compounds in this study, all of which are used in household products, such as detergents, industry and in the production of plastics. These phenols have strong estrogenicity on human and wildlife, for example, causing birth defects, altering

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immune function, contributing to sexual dysfunction, even causing cancers and heart disease [1–5].

4-*n*-NP and 4-*t*-OP, as the major degradation products of alkylphenol polyethoxylate (anionic and non-ion surfactant) under aerobic or anaerobic condition, are widespread in the aquatic environment varying in concentration ranging from few percents of ng L^{-1} (low level) in drinking water resource to tens of thousands of ng L^{-1} in wastewater outfall [6–9]. BPA is mainly used as a monomer in the preparation of epoxide resins, polycarbonate plastics and as an antioxidant or stabilizer in polycarbonate plastics and polyvinylchloride. During manufactures of above-mentioned products, BPA is released directly or indirectly into the water environment. According to estimation, BPA consumption will increase to 5 million tons in 2010. 4-CP, which lacks a hydroxyl group on benzene rings compared with BPA, has the similar endocrine disrupting ability to BPA [10]. As a representing alkyated hindered phenols and an effective anti-oxidant, it is widely used in rubber, adhesive, plastic and cable industries.

High-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is usually a choice to determine low volatile and liquid soluble phenolic compounds simultaneously [11]. Owing to the relative higher cost and complex operation of HPLC, recently, GC-MS coupled with sample pretreatment has been widely reported and used to measure phenols because of its lower cost and higher separation and identification capabilities. In recent years, most studies were focused on instrumentation availability, operator expertise, and sample clean-up methods using effective liquid–liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME). Actually, derivatization plays a vital role for the determination of these phenols, which have poor volatility and low concentration in water, particularly when using GC-MS. So, many derivatization methods, such as silylation (TMS and TBS), methylation, trifluoroacetylation, diazomethane and pentafluorobenzyl [12–17], have been developed to enhance sensitivity and sharpen peak profile to determine phenolic compounds by GC-MS. Among the derivatization methods of phenols, silylation with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) is commonly used for its simple operation, mild conditions and good responses [18–20], although the derivation is influenced dramatically by amount of derivatization reagent, catalyst, heat condition and sample matrix influence. Anhydride is usually used as methylation reagent. Using acetic anhydride as derivatization reagent, Limam *et al*. recently developed a GC-MS method coupled with a headspace SPE to determine phenol, methylphenols, chlorophenols and BPA in water samples and industrial effluents [21]. The limits of detection (LOD) were reported to be in low ng L^{-1}, and linearity to be $r^2 > 0.9931$, and repeatability (RSD, $n = 5$) to be 4.8%–15.2%. Phenyltrimethylammonium (PTA-OH) is an efficient methylation reagent for some organic compounds with hydroxyl groups [22], but rarely used to derivatize phenols. Few studies were conducted to derivatize and determine 4-*n*-NP, 4-*t*-OP, BPA and 4-CP simultaneously [23]. Furthermore, most researches focused on plain lakes and rivers in China [1,15,24], studies are particularly rare on determination of these compounds in seriously polluted plateau lake. Dianchi Lake is located in Yunnan Province, southwestern China with an average elevation of 1887 m and watershed of 2920 km^2, in which there are 3.32 million population and many factories related with phenols. The EDC compounds like 4-*n*-NP, 4-*t*-OP, BPA and 4-CP have never been measured in this seriously polluted plateau lake.

In this paper, two derivatization methods, methylation with PTA-OH and silylation with BSTFA + 1%TMCS, were discussed and selected for the derivatization of 4-*n*-NP,

4-*t*-OP, BPA and 4-CP based on the investigations of various derivatization conditions, including derivatization solvent, amount of derivatization reagent, reaction temperature and time. Then the optimal derivatization method was applied to simultaneously determine the concentrations and distributions of target phenols in surface water from seriously polluted Dianchi Lake, China.

2. Experimental

2.1 *Reagents and materials*

All standards were of the highest purity and commercially available (purity >97%). Phenols (4-*n*-NP, 4-*t*-OP, BPA and 4-CP) and internal standard (bisphenol A-*d*16) were obtained from Sigma-Aldrich (USA). Stock solutions of individual compound were prepared in anhydrous methanol at 1 mg mL^{-1} and stored in a freezer at −20°C. The stock solutions were used to regularly prepare working standard solutions for spiking experiments. Derivatization reagent PTA-OH and BSTFA + 1%TMCS were supplied by Sigma-Aldrich (USA).

Organic solvents (methanol, dichloromethane, ethyl acetate, hexane, acetone and pyridine) used for sample treatment and analysis were HPLC grade. Methanol, ethyl acetate and acetone were purchased from Merck (Germany). Dichloromethane and hexane were supplied by J.T. Baker (USA). Pyridine was obtained from Sigma-Aldrich (USA). Anhydrous sodium sulfate (Na_2SO_4, AR) for removing moisture, HCl (AR) and NaOH (AR) for adjusting pH were supplied by Kunming Chemical Factory.

SPE cartridges Oasis HLB (6 mL, 500 mg) with N-vinylphrolidone copolymer were obtained from Waters (Milford, MA, USA). Deionized water was made by Millipore-Q ultrapure system (Millipore-S.A.S, Molsheim, elix5, France). All glassware was cleaned by SC 1160 automatic bottlewasher (SalvisLab, Switzerland) and then pyrolysed at 450°C for 4 h prior to use. GF/F filters (0.45 μm, Millipore, USA) were pyrolysed at 450°C for 4 h prior to use.

2.2 *Samples collection*

In this investigation, ten sampling sites (W1-W10) were selected at the state key monitoring sections for surface water quality control of Dianchi Lake in January, 2009. Water samples were collected in 4 L precleaned amber glass bottles. Methanol was added immediately into each sample (1:99, *v:v*) to suppress potential biodegradation. These samples were filtered through a pyrolysed GF/F filter (0.45 μm) to remove particle contaminants within 24 hours. Before SPE, the samples were stored at 4°C in a refrigerator.

2.3 *Sample preparation*

2.3.1 *Solid phase extraction*

SPE, which was used to remove matrix interference from sample extracts before derivatization and GC-MS analysis, was performed with the modified method of Liu *et al*. [13]. Before SPE, 1 mol L^{-1} HCl and 1 mol L^{-1} NaOH were used to adjust pH of surface water samples (1 L) and deionized water to 4–5. For SPE, Oasis HLB cartridges

were placed on 12-port Visiprep vacuum manifold (Supelco, USA), in which the cartridges were conditioned with 5 mL ethyl acetate, then followed by 5 mL methanol and 3×5 mL deionized water. After 1 L water samples passed through the cartridges at a flow rate of 4 mL min^{-1}, 10 mL deionized water-methanol (9:1, *v*/*v*) was used to wash the cartridges, which was then dried under vacuum for 30 min. Next, analytes were eluted to 20 mL vials from the cartridges with 10 mL of ethyl acetate at a flow rate of 1 mL min^{-1}, and the solvents were blown down to 0.5 mL under a gentle stream of nitrogen at room temperature (20°C). Finally, the extracts were quantitatively transferred into 2 mL reaction vials, and further evaporated to dryness under a gentle nitrogen stream for derivatization.

2.3.2 *Derivatization*

For analysis and optimization of derivatization methods, 0.1 mL of a prepared standard mixture solution, in which concentration of each phenol and internal standard was 100 ng mL^{-1}, was pipeted into a 2 mL amber vial and evaporated to dryness under a gentle nitrogen stream at room temperature (20°C). After adding 0.05 mL derivatization solvent and a certain amount of derivatization reagent PTA-OH or BSTFA + 1%TMCS, the vial was closed and mixed completely through vortex mixer (IKA, Germany). The derivatization reaction was carried out at a given temperature for a given time, followed by cooling derivatized samples to room temperature and then evaporating to 0.01 mL under a gentle nitrogen stream. At last, final solvent was added into vial to make final volume 0.1 mL for GC-MS analysis. In this study, the final solvent is the same as the derivatization solvent in each individual experiment to avoid the deviation caused by different solvents.

For real surface water sample, extracts of 1 L surface water sample through SPE were transferred into 2 mL vials. After adding 250 ng BPA-d_{16} as internal standard, it was derivatized with the optimized derivatization method using the same procedure as mentioned above.

2.4 *GC-MS analysis*

GC-MS analysis was performed on a Trace GC (Thermo Fisher Scientific, USA), equipped with a Trace DSQ quardrupole mass spectrometer and an autosampler Triplus AS. The DB-5 MS capillary column (Agilent, USA) with a 0.25 mm inner diameter, 30 m length, and 0.25 μm film thicknesses was used as a separation column. Helium carrier gas was maintained at a constant flow rate of 1 mL min^{-1}. Injector temperature was held at 280°C, and the injection volume was 1.0 μL in the splitless mode. The interface and the ion source temperature were set at 280°C and 250°C, respectively. Electron impact ionization energy was 70 eV. The column temperature was programmed as follows: the initial temperature was held at 80°C for 2 min, increased to 150°C at 15°C min^{-1}, then ramped at 8°C min^{-1} to 220°C and held for 1 min, finally increased to 300°C at 15°C min^{-1} and maintained at this temperature for 5 min. Mass spectra were scanned in full scan mode from 50-500 *m*/*z* mass range for qualitative analysis or selected ion monitoring (SIM) mode for quantitative analysis. The dwell time of each ion was set at 200 ms and the scan rate was 1.2 scan s^{-1}. Both GC and MS parameters were implemented using Xcalibur version 1.4 software. All target compounds were identified by their retention times and specific ions, and quantified using the internal standard method.

Table 1. Chromatogram information of derivatized target phenols and internal standard.

Compounds	Retention time (min)	Molecular ion	Characteristic ion
PTA-BPA-*d*16	16.51	270	252
PTA-4-*t*-OP	9.93	220	149
PTA-4-CP	13.25	226	211
PTA-4-*n*-NP	13.57	234	121
PTA-BPA	16.63	256	241
BST-BPA-*d*16	18.02	386	368
BST-4-*t*-OP	11.07	278	207
BST-4-CP	14.29	284	269
BST-4-*n*-NP	14.64	292	179
BST-BPA	18.11	372	357

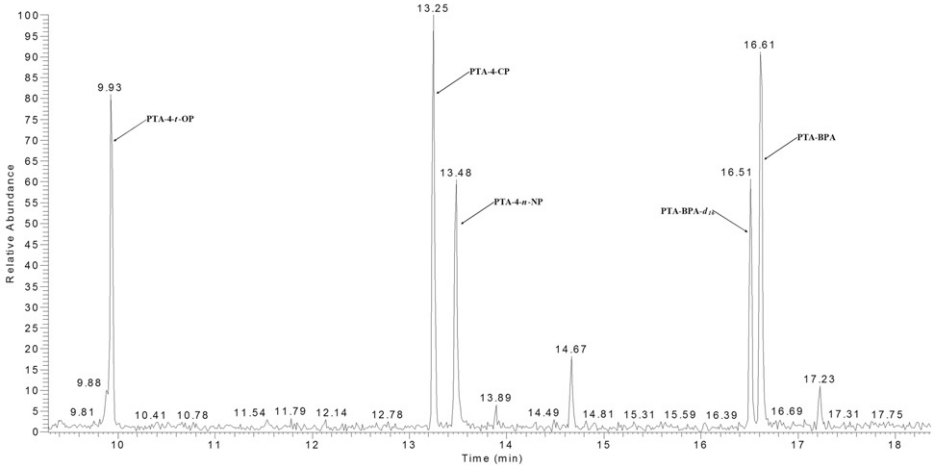

Figure 1. Total ion chromatogram of methylated target phenols. (Experimental conditions: derivatization reagent, 0.05 mL PTA-OH; derivatization solvent, ethyl acetate; T, 20°C; t, 5 min; C, 1000 ng mL^{-1}).

Chromatogram information of derivatized target chemicals and internal standard are listed in Table 1. Total ion chromatogram (TIC) for identifying methylated phenols and silylated phenols in standard solutions are shown in Figures 1 and 2, respectively.

3. Results and discussion

3.1 *Derivatization*

In this study, two derivatization methods (methylation with PTA-OH and silylation with BSTFA + 1%TMCS) were discussed to identify the optimum derivatization method and derivatization conditions of target phenols based on the investigations of derivatization solvent, amount of derivatization reagent, reaction temperature and time. Moreover, LOD determined based on the non-concentrated (0.1 mL) lowest standard concentration that

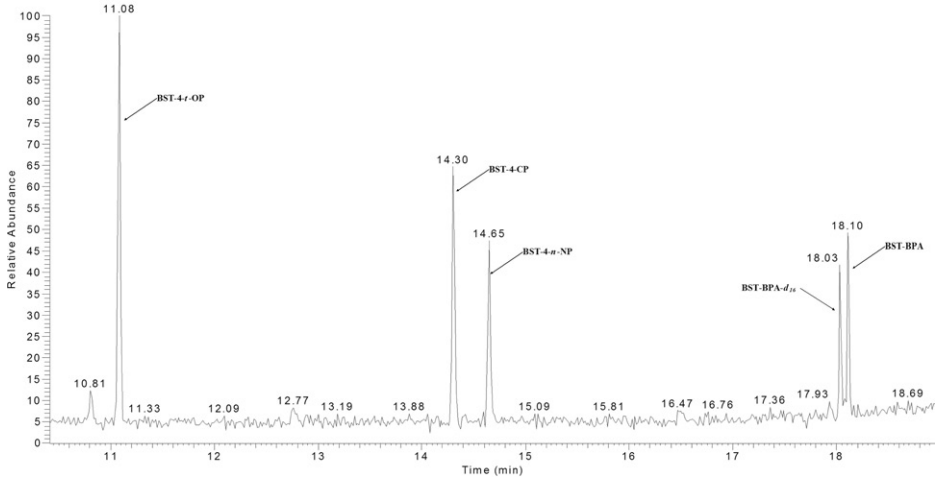

Figure 2. Total ion chromatogram of silylated target phenols. (Experimental conditions: derivatization reagent, 0.05 mL BSTFA + 1%TMCS; derivatization solvent, acetone; T, 20°C; t, 60 min; C, 1000 ng mL^{-1}).

produced a signal noise ratio equal to three, were obtained using the derivatization method under optimized conditions.

3.1.1 *Methylation*

Results of methylation derivatives of target phenols obtained with PTA-OH in different derivatization solvent (hexane, acetone, methanol and ethyl acetate) were presented in Figure 3. As shown in the figure, hexane produced poor responses for the methylated phenols especially for BPA and internal standard. This may be attributable to smaller solubility of methylated phenols in non-polar solvent, while for other derivatization solvents (acetone, methanol and ethyl acetate), the responses were reasonably good. Considering the final solvent is same as the derivatization solvent, it is necessary to make further choice from acetone, methanol and ethyl acetate. When acetone was used as final solvent, the peak area of methylated 4-*t*-OP was increased dramatically with the increase of storage time from 5 min to 12 h. This may be owing to some other products that have similar characteristic ion to methylated 4-*t*-OP and have been formed in the store process, which could be confirmed from the colour change of solution with the prolongation of storage time. Moreover, RSDs were calculated based on the RRFs of different storage time. Results revealed that RSD ranged from 3.29% of ethyl acetate to 29.67% of acetone, demonstrating that methylated phenols in ethyl acetate were more stable than that in methanol. Because the stability of target compounds after derivatization was very important for using auto-sample in GC-MS, ethyl acetate was finally used as derivatization solvent and final solvent.

For analysis and optimization of derivatization methods, effects of PTA-OH amount on derivatization were shown in Figure 4. Results indicated that if the amount of PTA-OH was not enough, the internal standard (BPA-d_{16}) will not be fully methylated, especially in cases of 4-*t*-OP and 4-*n*-NP, and therefore inducing the determination error. When the amount of PTA-OH was 0.001 mL, the methylated phenols achieved the largest peak

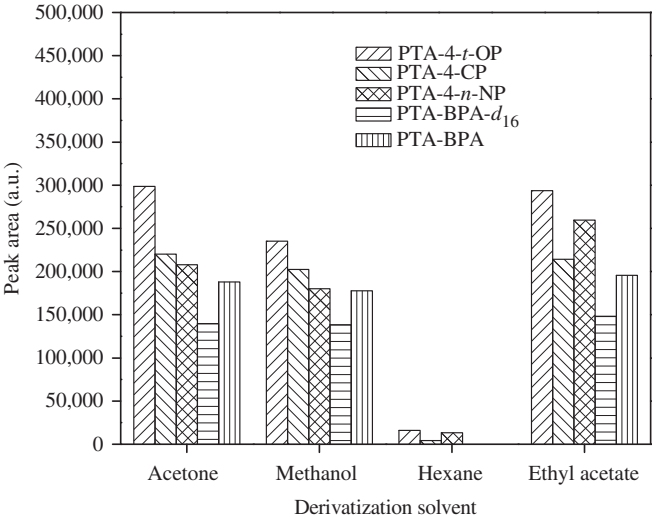

Figure 3. Methylation of target phenols and internal standard using different derivatization solvent. (Experimental conditions: derivatization reagent, 0.01 mL PTA-OH; T, 20°C; t, 30 min; C, 100 ng mL^{-1}).

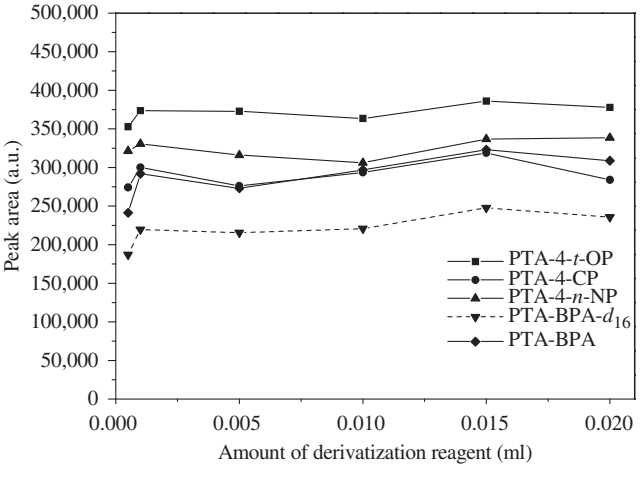

Figure 4. Methylation of target phenols and internal standard under different amount of PTA-OH. (Experimental conditions: derivatization reagent, PTA-OH; derivatization solvent, ethyl acetate; T, 20°C; t, 30 min; C, 100 ng mL^{-1}).

areas. If the amount of PTA-OH is excessive, there is no remarkable influence on methylation of target phenols. In real water sample, excess derivatization reagent must be added into water sample to compensate its consumption as result of complex contaminants in matrix and provide complete derivatization of target phenols. So, it is important to add excess derivatization reagent in derivatization of target phenols.

In order to further investigate the effects of excess amount of PTA-OH on derivatization of real surface water sample. 5000 ng of each target phenol and BPA-d_{16}

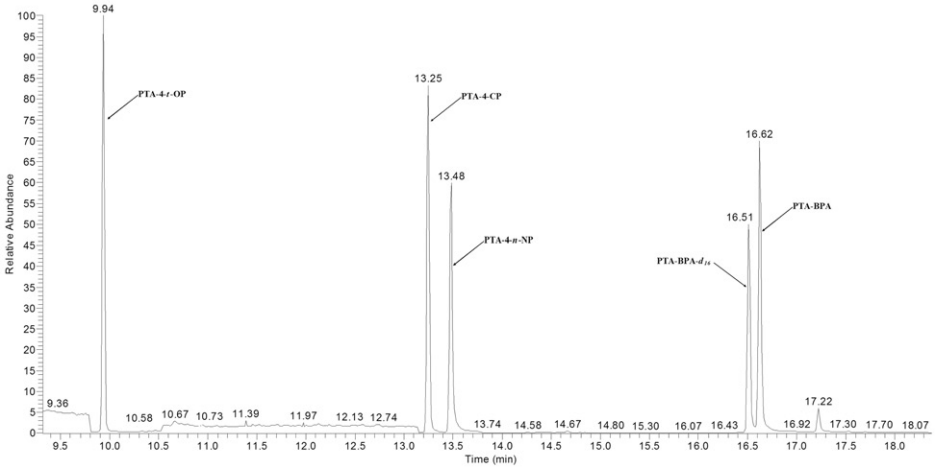

Figure 5. SIM chromatogram of non-derivatized and derivatized target phenols. (Experimental conditions: derivatization reagent, 0.05 mL PTA-OH; derivatization solvent, ethyl acetate; T, 20°C; t, 30 min).

were added into 1 L surface water sample (pH = 4–5) of Dianchi Lake. Then 0.05 mL PTA-OH was added into the extracts of the specially prepared 1 L surface water sample for derivatization. The derivatized sample was analysed using GC-MS in SIM mode including characteristic ions and molecular ions of non-derivatized and derivarized target phenols. As illustrated in Figure 5, there are not characteristic peaks of non-derivatized target phenols, and characteristic peaks of derivatized phenols are higher and sharpen. This indicates that four target phenols and internal standard have been completely derivatized. So 0.05 mL PTA-OH can be used securely to derivatize target phenols and added internal standard in 1 L surface water sample of Dianchi Lake.

Effect of reaction temperature on the derivatization of target phenols was investigated at 20, 30, 40, 50, 60 and 70°C. It demonstrated that the RRFs of methylated phenols had no remarkable difference with the increase of reaction temperature, except for 4-*t*-OP and 4-*n*-NP, which had better RRF at 60°C and 70°C, respectively. Taking boiling point of ethyl acetate (77.06°C), multicomponent analysis and simple operation into consideration, reaction temperature was set at 20°C in the following experiments.

Under the conditions mentioned above, the effects of reaction time were examined at 5, 30, 60, 90 and 120 min, respectively. It was revealed that the RRFs of most phenols achieved the highest values when the reaction time increased to 5 min except for 4-*t*-OP, which had the highest RRF at 120 min. However, there were no dramatic differences observed for RRF of methylated phenols in time ranging from 60 min to 120 min. By comprehensive consideration, 5 min was chosen as the optimum reaction time in the derivatization of phenols with PTA-OH. Under the optimal derivatization conditions of methylation, LOD of 4-*t*-OP, 4-CP, 4-*n*-NP and BPA was determined to be 0.1 ng mL^{-1}, 0.2 ng mL^{-1}, 0.1 ng mL^{-1}, 0.1 ng mL^{-1}, respectively.

3.1.2 *Silylation*

Effects of hexane, acetone, pyridine and ethyl acetate as solvent on silylation of phenols using BSTFA + 1%TMCS have been discussed separately in references [25–29]. In this

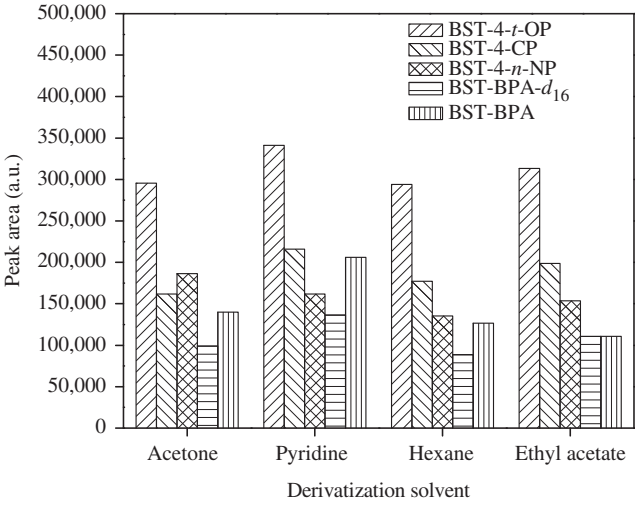

Figure 6. Silylation of target phenols and internal standard using different derivatization solvent . (Experimental conditions: derivatization reagent, 0.01 mL BSTFA + 1%TMCS; T, 20°C; t, 30 min; C, 100 ng mL^{-1}).

work, since applying to four different phenols and slight different pretreatment conditions, we have investigated the effects of the above mentioned four derivatization solvents on simultaneous silylation of four target phenols. As illustrated in Figure 6, four derivatization solvents have almost same peak areas of response. We then examined the stability by determining the RRFs of the derivatized products obtained using these four different solvents and stored for different time (ranging from 0 h to 12 h with time step of 2 h). RSD were calculated based on the RRFs of different storage time. Results revealed that RSD ranged from 0.79% of acetone to 6.64% of pyridine, and hexane and acetone were similar and more stable to be used as solvent than pyridine and ethyl acetate. Because of smaller solubility of target phenols in hexane, acetone was therefore chosen as derivatization solvent for the further study.

Effects of BSTFA + 1%TMCS amount on derivatization are shown in Figure 7. When the amount of BSTFA + 1%TMCS is 0.015 mL, the silylated phenols achieves the largest peak areas. However, when the amount exceeds 0.015 mL, the relatively smaller peak areas appeared for all the target compounds. Li *et al.* [25] also found that excess derivatization reagent had some negative influence on silylation of phenols, but did not provide detail information. When the amount of BSTFA + 1%TMCS was 0.02 mL and more, the peak areas are stable. Considering the application of BSTFA + 1%TMCS in real water sample, excess BSTFA + 1%TMCS is added for the further study.

Many researchers had reported influence of different temperature and time on silylation of phenols using BSTFA + 1%TMCS as derivatization reagent [28,30–33]. We inspected the influences of temperature and time using procedure as mentioned in section 2.3.2, which is similar to the procedure in references [30,34]. The results indicated that the optimum temperature and time were 20°C–70°C and 60 min, respectively. They agreed well with results reported in reference [35] that optimum temperature ranged from room temperature to 80°C, and optimum time ranged from several minutes

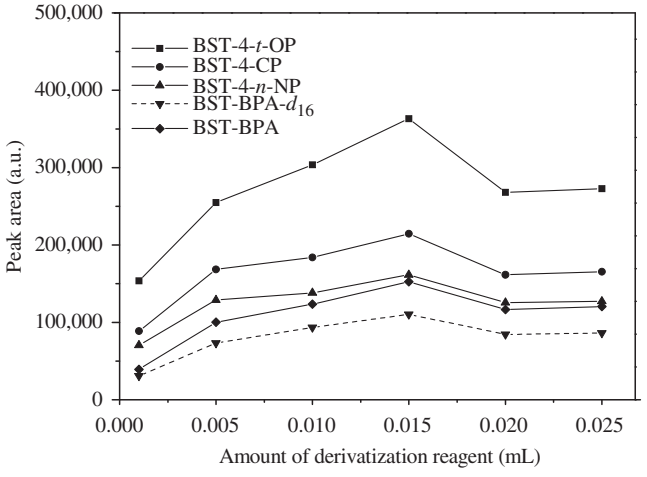

Figure 7. Silylation of target phenols and internal standard under different amount of BSTFA + 1%TMCS (Experimental conditions: derivatization reagent, BSTFA + 1%TMCS; derivatization solvent, acetone; T, 20°C; t, 30 min; C, 100 ng mL^{-1}).

to 3 h. Under the optimal derivatization conditions of silylation, LOD of 4-*t*-OP, 4-CP, 4-*n*-NP and BPA was determined to be 0.2 ng mL^{-1}, 0.1 ng mL^{-1}, 0.1 ng mL^{-1}, 0.1 ng mL^{-1}, respectively.

3.1.3 *Derivatization comparison*

As summary of the derivatization conditions of two derivatization methods, the optimum derivatization conditions of methylation were using ethyl acetate as derivatization solvent, adding excess PTA-OH, and reacting at 20°C for 5 min; and the optimum derivatization conditions of silylation were using acetone as derivatization solvent, adding excess BSTFA + 1%TMCS, and reacting at 20°C for 60 min.

Five features can be found out by comparing the derivatization conditions between methylation with PTA-OH and silylation with BSTFA + 1%TMCS. (1) Methylated phenols had relatively higher peak areas except for 4-*t*-OP, and the differences of peak areas among the four phenols were less than that of silylation. The LODs (estimated at $S/N = 3$) of methylated phenols were similar as that of silylated phenols, which were all bellow 1 ng mL^{-1}. (2) Within 12 hours methylated phenols in ethyl acetate has comparable stability as that of silylated phenols in acetone. (3) Excess BSTFA + 1%TMCS led to decrease of peak areas of silylated phenols, while that did not happen to methylated phenols when PTA-OH was in excess. (4) The contamination of chromatographic column caused by excess PTA-OH and ion source was less serious than that caused by excess BSTFA + 1%TMCS. (5) Both derivatization methods had comparable mild reaction temperature; while optimum reaction time of methylation was shorter than that of silylation.

Since the trace level of target phenols in nature water requires lower detection limit, higher stability, mild reaction conditions, and the higher efficiency of excess derivatization reagent, methylation using PTA-OH was suitable to be chosen for the derivatization of 4-*t*-OP, 4-CP, 4-*n*-NP and BPA. The optimum derivatization conditions were using ethyl

Table 2. Recoveries and precisions of target phenols.

Sample	Spiked level ($ng\,L^{-1}$)	Recovery (%)				RSD (%, $n=6$)			
		4-*t*-OP	4-CP	4-*n*-NP	BPA	4-*t*-OP	4-CP	4-*n*-NP	BPA
Utrapure water	10	59.6	100.3	89.7	73.4	2.3	3.4	8.8	4.9
	250	57.6	94.4	85.7	72.2	4.5	6.8	5.7	3.2
	1000	56.6	93.2	86.4	68.2	7.8	2.4	5.5	4.6

acetate as derivatization solvent, adding excess PTA-OH (0.05 mL for further studies in section 3.2), and reacting at 20°C for 5 min.

3.2 *Method application*

Under the optimal derivatization conditions, experiments were carried out to examine performance of the proposed method based on correlation coefficient, MQL, recovery rate and RSD. Quantification of the analytes was carried out through internal standard method using BPA-d_{16} as internal standard, by which the curve calibration, spike recovery and the concentration of these four phenols in water of Dianchi Lake were obtained. MQL were obtained using the PTA-OH derivatization method under optimized conditions. 1 L spiked sample was firstly extracted through SPE, then derivatized and concentrated to 0.1 mL. MQL was measured based on the concentrated lowest standard concentration that produced a signal noise ratio equal to ten.

The concentration of standards ranging from $1\,ng\,L^{-1}$ to $2000\,ng\,L^{-1}$ containing 250 ng BPA-d_{16} in 500 mL deionized water (pH = 4–5) were prepared and detected by the proposed method. The measured data were used to generate calibration curve and correlation coefficient. All correlation coefficients were greater than 0.99, and the MQLs were $0.3\,ng\,L^{-1}$, $0.5\,ng\,L^{-1}$, $0.2\,ng\,L^{-1}$ and $0.7\,ng\,L^{-1}$ for 4-*t*-OP, 4-CP, 4-*n*-NP and BPA (RSD < 10%, $n=3$), respectively.

For the recovery test, 500 mL mixed deionized water solution of four target phenols at concentrations of $20\,ng\,L^{-1}$, $500\,ng\,L^{-1}$ and $2000\,ng\,L^{-1}$, containing 250 ng BPA-d_{16}, were prepared and determined based on the proposed method. Table 2 lists the recoveries of target compounds at different concentrations. Results indicate that the mean recovery rates of the target compounds are 57.9%, 96.0%, 87.3% and 71.3% for 4-*n*-NP, 4-*t*-OP, BPA and 4-CP, respectively, and that all RSDs are less than 9.0%.

Ten surface water samples of Dianchi Lake were measured through the proposed method. Table 3 lists concentrations of 4-*n*-NP, 4-*t*-OP, BPA and 4-CP and its repeatability of the method detected in above mentioned sites. Among these samples, the four target phenols have been widely detected in Dianchi Lake and the highest concentrations were obtained at W1, which is the closest site to downtown of Kunming. Most of RSDs calculated based on RRFs of three parallel surface water samples was less than 20%. 4-*n*-NP and 4-*t*-OP, which were widely used in detergents and emulsifiers, ranged from 13.55 to $141.59\,ng\,L^{-1}$ and N.D. to $56.54\,ng\,L^{-1}$, respectively. The concentration ratio of 4-*n*-NP to 4-*t*-OP in every sampling site approximates 4 to 1, which is in accordance with quantities of nonylphenol ethoxylates and octylphenol

Table 3. Concentrations of target phenols and its RSD detected in surface water of Dianchi Lake ($ng\,L^{-1}$, $n=3$).

Sample No	4-*t*-OP	RSD	4-CP	RSD	4-*n*-NP	RSD	BPA	RSD
W1	56.5	14.8%	48.5	18.5%	141.6	19.4%	4713.6	1.5%
W2	30.3	5.6%	23.3	13.2%	120.3	17.1%	944.7	19.6%
W3	4.4	14.7%	31.2	12.5%	22.6	16.8%	339.7	14.4%
W4	2.9	8.8%	24.5	>20%	16.5	4.3%	216.6	7.3%
W5	<0.3	4.1%	30.6	17.4%	18.5	19.5%	997.9	>20%
W6	4.2	4.1%	32.8	6.4%	13.9	12.0%	821.8	>20%
W7	<0.3	11.0%	33.9	19.1%	13.6	6.6%	254.3	19.5%
W8	6.3	17.2%	45.1	5.4%	21.6	7.4%	165.5	7.4%
W9	5.4	15.9%	46.9	14.8%	19.6	5.5%	<0.7	6.2%
W10	<0.3	2.3%	33.9	7.2%	23.3	15.1%	44.0	13.6%

ethoxylates used in China. The concentration of 4-CP varied from 23.26 to 48.48 $ng\,L^{-1}$, did not change remarkably for different sampling points. It was probably owing to ubiquity of rubber, plastic and woodwork industries around Dianchi Lake. BPA had been widely detected in aquatic environments, usually at levels of several to hundreds nanograms per liter [1,36], but in Dianchi Lake, the highest concentration reached 4713.6 $ng\,L^{-1}$ at site W1, which was dramatically higher than those detected in other lakes [36,37] and only comparable with that of Hai River in China [15].

4. Conclusions

A method based on SPE and derivatization with PTA-OH followed by GC-MS has been developed for simultaneous determination of 4-*n*-NP, 4-*t*-OP, BPA and 4-CP in surface water of Dianchi Lake, China. Compared with silylation of target phenols using BSTFA + 1%TMCS, methylation by PTA-OH had five favorable features, which displayed a more effective derivatization for four phenols, and therefore suggested that PTA-OH was better than BSTFA + 1%TMCS to be chosen as derivatization reagent. The optimum derivatization conditions were using ethyl acetate as derivatization solvent, adding excess PTA-OH (e.g. 0.05 mL), and reacting at 20°C for 5 min. Experiments were carried out to examine performance of the proposed method based on correlation coefficients, MQLs, mean recovery rates and RSDs. Results revealed that the proposed method exhibited a satisfactory precision and reproducibility for the separation and determination of target phenols. The proposed method had been successfully applied to determine these phenols in Dianchi Lake, and provided the quantitative analysis of 4-*n*-NP, 4-*t*-OP, BPA and 4-CP with concentrations ranging from 13.6 to 141.6 $ng\,L^{-1}$, N.D. to 56.5 $ng\,L^{-1}$, N.D. to 4713.6 $ng\,L^{-1}$ and 23.3 to 48.5 $ng\,L^{-1}$, respectively.

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