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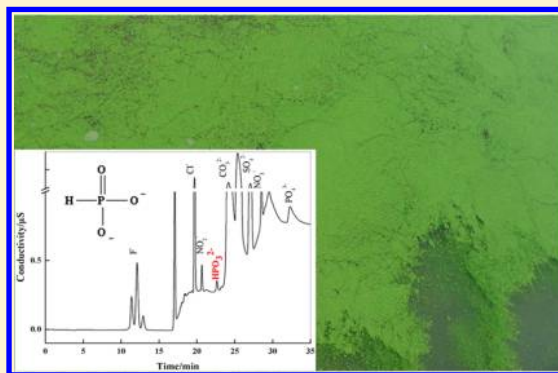
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S Supporting Information

ABSTRACT: The establishment of a sensitive and specific method for the detection of reduced phosphorus (P) is crucial for understanding P cycle. This paper presents the quantitative evidence of phosphite (P, +3) from the freshwater matrix correspondent to the typically eutrophic Lake Taihu in China. By ion chromatography coupled with gradient elution procedure, efficient separation of micromolar levels of phosphite is possible in the presence of millimolar levels of interfering ions, such as chloride, sulfate, and hydrogen carbonate in freshwater lakes. Optimal suppressed ion chromatography conditions include the use of 500 μ L injection volumes and an AS11 HC analytical column heated to 30 $^{\circ}$ C. The method detection limit of 0.002 μ M for phosphite was successfully applied for phosphite determination in natural water samples with recoveries ranging from 90.7 \pm 3.2% to 108 \pm 1.5%. Phosphite in the freshwater matrix was also verified using a two-dimensional capillary ion chromatography and ion chromatography coupled with mass spectrometry. Results confirmed the presence of phosphite in Lake Taihu ranging from 0.01 \pm 0.01 to 0.17 \pm 0.01 μ M, which correlated to 1–10% of the phosphate. Phosphite is an important component of P and may influence biogeochemical P cycle in lakes.



INTRODUCTION

It is well-known that phosphorus (P) plays an important role in plant and microbial nutrition, eutrophication, corrosion, and other geochemical processes.^{1–3} The determination of P species in environmental matrices is essential for the assessment of ecological health, research on biogeochemical processes, environmental monitoring and governmental management.¹ Phosphate (+5), the fully oxidized state, has been studied more thoroughly because it is assumed to be the dominant form in the environment. However, many other P species exist in natural environments, such as reduced P compounds (P with an oxidation state less than +5) including phosphide (–3), hypophosphite (+1), and phosphite (+3), which are important parts of the global P cycle.^{4–6} Among the reduced forms of P, phosphine (PH₃, –3) has been found worldwide and is assumed to be an important gaseous carrier of P.^{6–9} Unfortunately, little is known about the presence and transfer of other reduced P forms in nature.

Phosphite is widely marketed as a fungicide, and it is believed to be a more efficient P source in plants than phosphate because of its increased mobility and solubility (1000 times higher at similar pH and temperature) in the soil and also reduces P fixation costs to the plants.^{10–12} Other researchers have demonstrated that phosphite can be transformed to biologically available P through biological or abiotic pathways in various environments.^{13–15}

The detection of phosphite in aquatic ecosystems would be important for reevaluating the biogeochemical cycle and bioavailability of different P species in the natural environment. However, information on phosphite in the environment has been poor due to the lack of a standard speciation protocol for phosphite. This deficiency arose because this species was previously considered to be a non-natural compound. The traditional molybdate-reactive methods for P analysis cannot detect phosphite in aqueous samples; therefore, dissolved reactive P was believed to be restricted to phosphate. Recently, many analytical techniques have been established as excellent ways to identify phosphite in various matrices. These include paper chromatography,¹⁶ suppressed ion chromatography coupled with electrospray mass spectrometry (IC-ESI-MS),^{17,18} and nuclear magnetic resonance spectroscopy (NMR).^{4,15} Although each technique has its limitations, application of these approaches to identify the presence of phosphite has been successfully observed in several types of water matrices, including sediment, sewage treatment facilities, natural and synthetic geothermal pools. For example, Pech et al.¹⁷ reported the presence of 0.06 \pm 0.02 μ M phosphite in a

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geothermal pool, which was equal to the phosphate concentrations ($0.05 \pm 0.01 \mu\text{M}$). In addition, Morton^{1,19} and McDowell²⁰ confirmed that phosphite in representative matrices of natural waters could be detected at submicromolar levels. However, no research has been conducted to investigate the presence of phosphite in surface water, especially in P-limited eutrophicated lakes. Ion chromatography (IC) has been shown to be a reliable method for the determination of ionic species in the environmental and industrial samples.²¹ It also has been proven to be highly selective and sensitive for the detection of trace anions, such as phosphite,^{5,22} perchlorate,^{23,24} and bromate.²⁵ Determination of trace phosphite by IC in more complex sample matrices, such as surface water and groundwater, is more challenging because of the large excess of matrix interference. In addition, ions of the same retention times (RTs) will make it difficult to identify conclusively the compound of interest.²²

Eutrophication in various aquatic ecosystems has caused great social and scientific concern in China.^{3,9} Phosphate (+5) is traditionally associated with the P nutrient supply. However, reduced P could be of equal importance for phytoplankton.^{26,27} The potential source and distribution of PH_3 in eutrophic Lake Taihu has been widely reported.^{9,28} Additionally, arsenic (As) can form a variety of freshwater species.²⁹ Given As and P exhibit significant similarities in their overall chemistries and form a number of analogous compounds,³⁰ it is reasonable to assume that phosphite would exist, as well as arsenite, under the same conditions. The aim of this study is to develop a selective method for phosphite determination in freshwater samples.

EXPERIMENTAL SECTION

Materials. All chemicals were of high-purity, and nanopure water ($18.2 \text{ M}\Omega$) prepared by a Milli-Q Plus system (Millipore, Bedford, USA) was used throughout. The phosphite standard solution at a concentration of 10 mM was prepared by dissolving sodium phosphite-5-hydrate ($\text{Na}_2(\text{HPO}_3) \cdot 5\text{H}_2\text{O}$, Sigma) in nanopure water and was stored in the brown Nalgene high density polyethylene bottles (HDPE) and refrigerated at 4°C . The working solutions of phosphite were freshly diluted from their stocks. Standard solutions of chloride, nitrate, nitrite, sulfate, hydrogen carbonate, bromide, fluoride, and phosphate were prepared by dissolving the corresponding sodium salts (Certified ACS grade) purchased from Sigma. All solutions were stored in precleaned HDPE bottles and refrigerated at 4°C .

Ion Chromatography System (ICS) with Suppressed Conductivity. A Dionex Model 2000 ion chromatography system (ICS) with a suppressed conductivity detector (Sunnyvale, CA, USA) was employed and consisted of a GS 50 gradient pump, an EG40 electrolytic eluent generator configured with a potassium hydroxide (KOH) eluent cartridge, an anionic self-regenerating suppressor (ASRS), a Dionex IonPac AG11-HC (50 mm \times 4 mm) guard column, and a IonPac AS11 HC (250 mm \times 4 mm) analytical column. The suppressor current was set to 99 mA, and the detector cell temperature was held at 35°C . KOH solution was used as the eluent at a constant flow rate of 1 mL/min and was used in multistep gradient concentrations. The gradient process was as follows: 1 mM from 0 to 13.5 min; 1 to 18 mM from 13.5 to 15.5 min; 18 mM from 15.5 to 24 min; 18 to 40 mM from 24 to 27 min; 40 mM from 27 to 34 min; and 40 mM to 1 mM from 34.1 to 35 min. Data acquisition and instrument control were performed using the Dionex Chromeleon (6.50 SP2)

program. Data collection was performed in triplicate, and the results are given as mean value \pm standard deviation (S.D.).

Two-Dimensional Ion Chromatography System Coupled with Capillary Ion Chromatography (2D-CIC).

To verify the results of phosphite separated by the ICS system, the ICS-5000 ion chromatography system (Dionex; Thermo-Dionex China Application Center in Shanghai) was used. Differing from the conventional two-dimensional technique described by Lin et al.²⁴ and Wagner et al.,³¹ ICS-5000 is a two-dimensional ion chromatography system coupled with capillary ion chromatography (2D-CIC). The core technology is the use of a capillary column (0.4 mm \times 250 mm) in a 2D-CIC system, which allows for injection volumes as low as $0.4 \mu\text{L}$, while providing for a 100-fold improvement in sensitivity. The ICS 5000 system was configured for 2D-CIC applications using standard bore or microbore columns in the first dimension and a capillary column in the second dimension. Detailed information on the operating conditions of the 2D-CIC system used in the present study is listed in Supporting Information Table S1.

Ion Chromatography System Coupled with Suppressed Conductivity and Electrospray Ionization Mass Spectrometry (IC-ESI-MS). IC-ESI-MS was employed to provide the further phosphite confirmation of the preliminary peak assignment in ICS. The IC-ESI-MS system was a Dionex ICS-3000-MSQ Plus system (Dionex; Thermo-Dionex China Application Center in Beijing) consisting of a dual gradient pump unit (Dionex ICS-3000 DP), automation manager with two 10-port high pressure valves and two low-pressure 3-way valves, dual eluent generator unit (Dionex ICS-3000 EG), dual column and detector compartment (Dionex ICS-3000 DC), dual conductivity detectors and ion trap mass spectrometer (LCQ Deca, Thermo Finnigan). Detailed conditions of chromatography and mass spectrometer of ICS-3000-MSQ Plus system is summarized in Supporting Information Table S2.

Sampling and Sample Preparation. Lake Taihu, a typical shallow freshwater lake with an average depth of 1.9 m is located in one of the most populated and developed areas of China.³² Four representative samples from Lake Taihu, including surface, bottom and overlying water samples and interstitial water samples from sediments were taken on a research launch set out from Dapu Harbor at early morning (about 6:00 a.m.) for about two hours, on 8 July 2011 at four sites in the northern region of Lake Taihu. Details on description of the sampling sites, the corresponding physicochemical parameters of water and sediment at each site are shown in Supporting Information and Table S3.

Surface waters and bottom water samples (0.5 m above the water-sediment interface) were collected with a water sampler and then filled into the brown HDPE. The overlying waters (0.05 m above the column sediments) were siphoned off and then filled into the brown HDPE. All HDPE bottles were filled completely to minimize headspace and to exclude ambient air, so the probability for the conversion to phosphate during transport and storage was avoided. It is essential for the surface waters, bottom waters and overlying waters filtrate through the $0.22 \mu\text{m}$ filters to remove the majority of organisms (e.g., bacteria, alga) and particle minerals are carried out immediately after samples are collected to prevent short-term changes in P species.³³ Sediments were collected with a cylindrical sediment sampler (diameter 9.1 cm \times height 70 cm, purchased from Nanjing Institute of Geography and Limnology). The columnar sediments were immediately divided into three layers at 20 cm

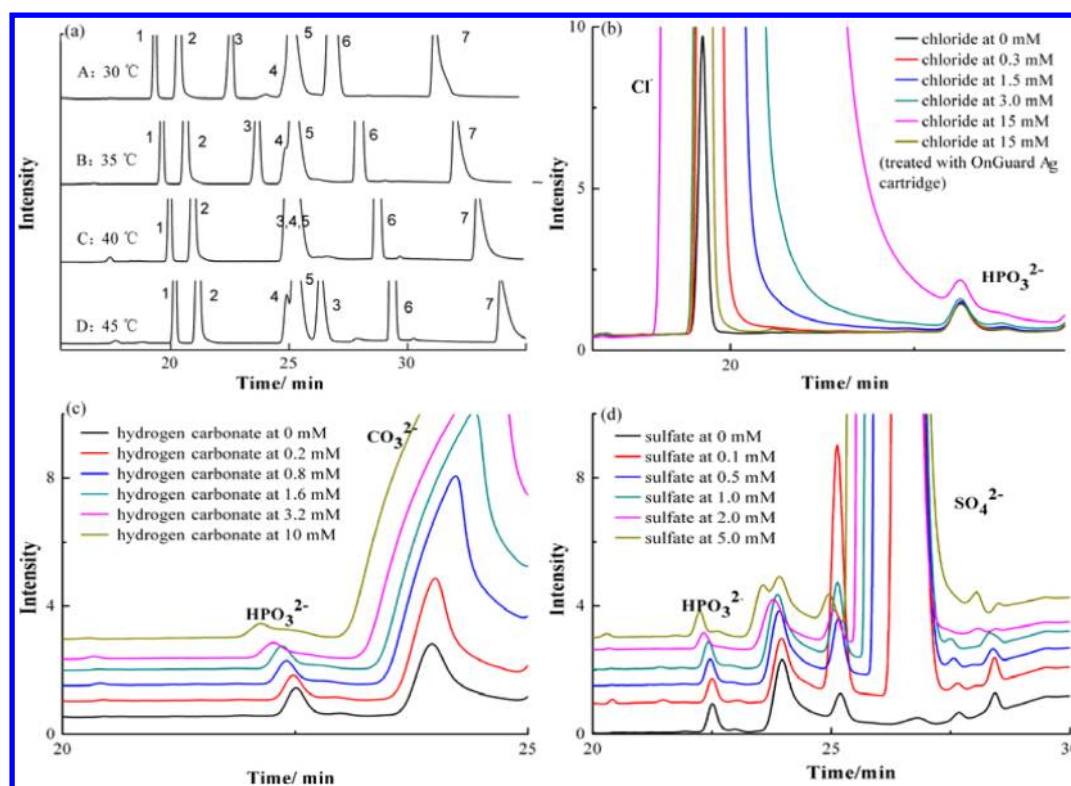


Figure 1. Performance of the modified chromatographic conditions. (a) Separation of phosphite from interfering anions at different column temperatures. Peak assignments: 1, chloride (0.17 mM); 2, nitrite (0.43 mM); 3, phosphite (10 μ M); 4, hydrogen carbonate (0.2 mM); 5, sulfate (0.31 mM); 6, nitrate (0.32 mM); 7, phosphate (0.32 mM). Detection of phosphite (1 μ M) in the presence of mM levels of interfering anions: chloride (b), hydrogen carbonate (c) and sulfate (d).

intervals and stored in black air-sealed plastic bags. Interstitial waters were extracted as soon as sediment samples were collected. Immediately after collection, all water samples were preserved at 0–4 °C in the dark and analyzed within two weeks. Our preliminary investigation has shown that phosphite was stable for at least 14 days under proposed preservation protocols.

Pretreatment Procedure. Bottom and overlying water samples were treated with an OnGuard Ag II cartridge (2.5 cc P/N 057090, Dionex) to reduce the high concentration of chloride and were directly injected into the IC system. Interstitial water samples were treated with OnGuard II RP columns (2.5 cc P/N 057084, Dionex) to remove organic compounds, hydrocarbons, and surfactants before injection into the IC system because of the presence of high concentrations of humus and organic macromolecules in the sediments that may interfere with IC detection.

Quality Assurance. For laboratory studies, synthetic lake water prepared from sodium salts was performed. Target anion concentrations and pH were determined in the preliminary investigation on Lake Taihu, and consequently the major background anions with measured concentrations and pH were as follows: chloride (0.81–4.21 mM), nitrate (0.05 to 0.28 mM), nitrite (0.002–0.03 mM), sulfate (0.51–1.31 mM), hydrogen carbonate (0.47–2.41 mM), bromide (0.001–0.003 mM), fluoride (0.02–0.07 mM), phosphate (0–0.003 mM), and pH (7.81–8.65). The final synthetic water included 2.1 chloride, 0.24 nitrate, 0.02 nitrite, 0.63 sulfate, 0.82 hydrogen carbonate, 0.002 bromide, 0.04 fluoride, and 0.002 mM phosphate, and the pH was 8.2. A linearity study was performed to ensure accurate quantification of phosphite in the synthetic

lake waters in the 0–1 μ M range. Calibration curves were accepted for quantitative analysis when its regression coefficients were over 0.999. To test the repeatability of the method described in the above procedure, three synthetic solutions containing 0.05 μ M phosphite were injected nine consecutive times. The method detection limit (MDL) for phosphite was defined as the peak height response which is three times the average background signals from blank samples. Furthermore, the recovery of the analytical method involved spiking the natural freshwater and interstitial water samples at a known amount of phosphite standard.

RESULTS AND DISCUSSION

Performance of the Modified Chromatographic Conditions. The separation of phosphite from other interfering anions in freshwater was attempted on a high-capacity AS11 HC column. μ M levels of phosphite in a freshwater lake can be separated completely on the AS11 HC column coupled with a multigradient program by potassium hydroxide (KOH) eluent. The inorganic P species including hypophosphite, phosphite, phosphate, and other common anions and the simple organic compounds can also be separated in an AS17 column.^{18,20,22} The AS11 HC column is specifically designed to resolve a large number of inorganic and organic acid anions in complex sample matrices, while AS17 column is a low-capacity column for fast, gradient separation of inorganic anions in high-purity sample matrices. The determination of trace anions by IC was always hampered by the presence of high concentrations of matrix interference, but an ICS system configured with a high-capacity IonPac AS11 HC hydroxide-selective anion-exchange column and run with a

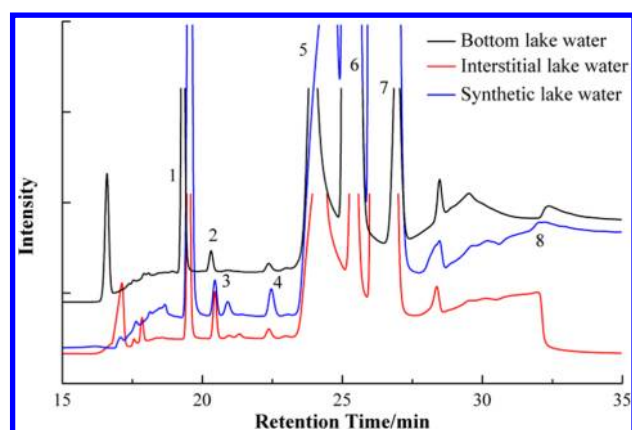


Figure 2. Representative ion chromatograms of the bottom lake water (black line), interstitial lake water (red line), and synthetic lake water spiked with 0.05 μM phosphite (blue line), from Lake Taihu. Peak assignments: (1) chloride; (2) nitrite; (3) bromide; (4) phosphite; (5) hydrogen carbonate; (6) nitrate; (7) sulfate; (8) phosphate.

Table 1. Results for Spiked Recoveries in Water Samples Collected from Lake Taihu

sample	Determination Result		Phosphite Spiked Recovery	
	phosphite (μM)	phosphate (μM)	added (μM)	recovery (%)
bottom water 1	0.012 ± 0.006	1.16 ± 0.01	0.05	90.7 ± 3.2
bottom water 2	0.069 ± 0.026	2.60 ± 0.05	0.05	93.6 ± 0.7
bottom water 3	<0.002	3.17 ± 0.07	0.03	108 ± 1.5
interstitial water (0–20 cm)	0.023 ± 0.025	2.10 ± 0.04	0.10	95.7 ± 5.8
interstitial water (20–40 cm)	0.081 ± 0.035	1.60 ± 0.02	0.10	98.6 ± 7.2
interstitial water (40–60 cm)	0.128 ± 0.086	1.91 ± 0.08	0.50	90.4 ± 4.9

multigradient program can overcome this problem. It has been verified that KOH eluent with higher pH values generated by an EG40 electrolytic eluent generator could ensure reproducible chromatograms. Meanwhile, the use of a suppressor (ASRS) regenerated with an external water source could also decrease the background noise and improve the sensitivity, which is critical for trace phosphite detection.

Two injection volumes (25 and 500 μL) were selected to compare the effect of injection volume on MDL. The estimated MDLs of phosphite for the 500 and 25 μL injection volumes are 0.002 and 0.146 μM , respectively. A lower MDL can be achieved with a larger injection volume.^{18,22} Increasing the injection volume to achieve optimal MDL, presented a far greater analytical challenge due to peak broadening and column overloading.¹⁸ This led to the coeluting of phosphite with the overfull interferences, such as chloride, sulfate, and hydrogen carbonate. However, by lowering the beginning (1 mM for the first 13.5 min) and middle plateau in the gradient, hydrogen carbonate and phosphite have enough time to separate on the column. It had been reported that the levels of phosphite in natural water samples were in the submicromolar range.^{5,18} It appears that the 500 μL injection volume falls within the level of phosphite previously observed in the environment.

Figure 1a shows a chromatographic separation of phosphite from interfering anions in the synthetic water sample at different column temperatures. Phosphite (peak 3) was completely separated from carbonate (peak 4) at 30 $^{\circ}\text{C}$.

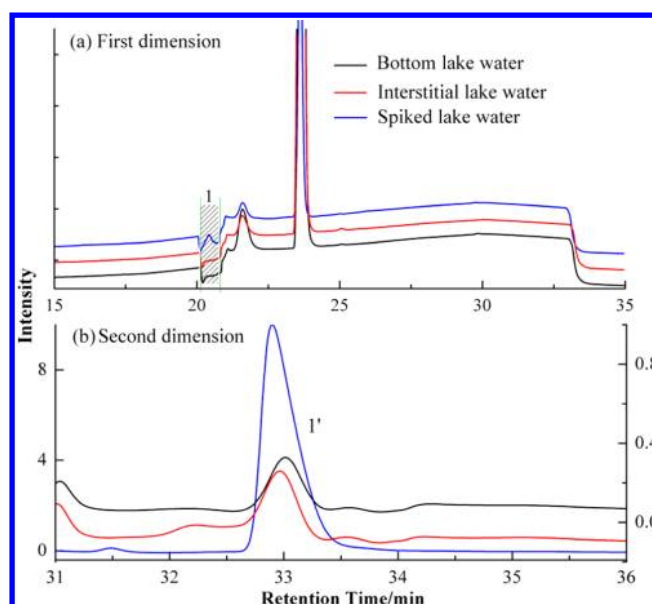


Figure 3. 2D-CIC chromatogram of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line). (a) Detection of phosphite in first dimension. (b) Further detection of phosphite in the second dimension, and data of spiked sample are scaled on the left axis. Both data of interstitial and bottom water were scaled on the right axis. Conditions were the same as in Supporting Information Table S1.

Increasing the column temperature caused the RTs of phosphite and other anions to increase, and the peaks for phosphite, hydrogen carbonate and sulfate were superimposed when the column temperature was 35 or 40 $^{\circ}\text{C}$. Column temperature controls the mobile phase viscosity, diffusivity, and sorption kinetics of the analytes, which consequently affects the separation between different anions.^{34,35} Considering the separation results and RTs of these profiles, the column temperature was set to 30 $^{\circ}\text{C}$ for the following experiments.

Effect of Major Interfering Anions, Chloride, Hydrogen Carbonate, and Sulfate, on the Separation of Phosphite. Considering the lake water samples' high concentrations of some common anions, particularly chloride, hydrogen carbonate, and sulfate, their effect on millimolar levels of phosphite separation was investigated. An overlay of chromatograms of 1 μM phosphite in the presence of 0–15 mM chloride are shown in Figure 1b. Similar plots were also obtained for phosphite separation in the presence of 0–10 mM hydrogen carbonate (Figure 1c) or 0–5 mM sulfate (Figure 1d). Efficient separation of μM levels of phosphite is possible in the presence of millimolar levels of interfering ions, such as chloride, sulfate, and hydrogen carbonate. When chloride concentrations range from 0 to 3 mM, phosphite recovery is above 90.2%. Increasing the chloride concentration to 15 mM caused the corresponding phosphite recovery to decrease by 82.7%. After pretreatment with the Ag II cartridge to remove chloride, sufficient baseline separation of phosphite can be achieved with 95.7% recovery. The detection of phosphite in natural freshwater is complicated by the presence of hydrogen carbonate because of its similar affinity toward the stationary.¹⁸ Under optimized conditions, 1 μM phosphite and hydrogen carbonate (below 3.2 mM) achieved baseline separation and more than 90% quantitative recoveries for phosphite. When hydrogen carbonate is set at higher concentration (10 mM), the wider peak with a leading edge of hydrogen carbonate can

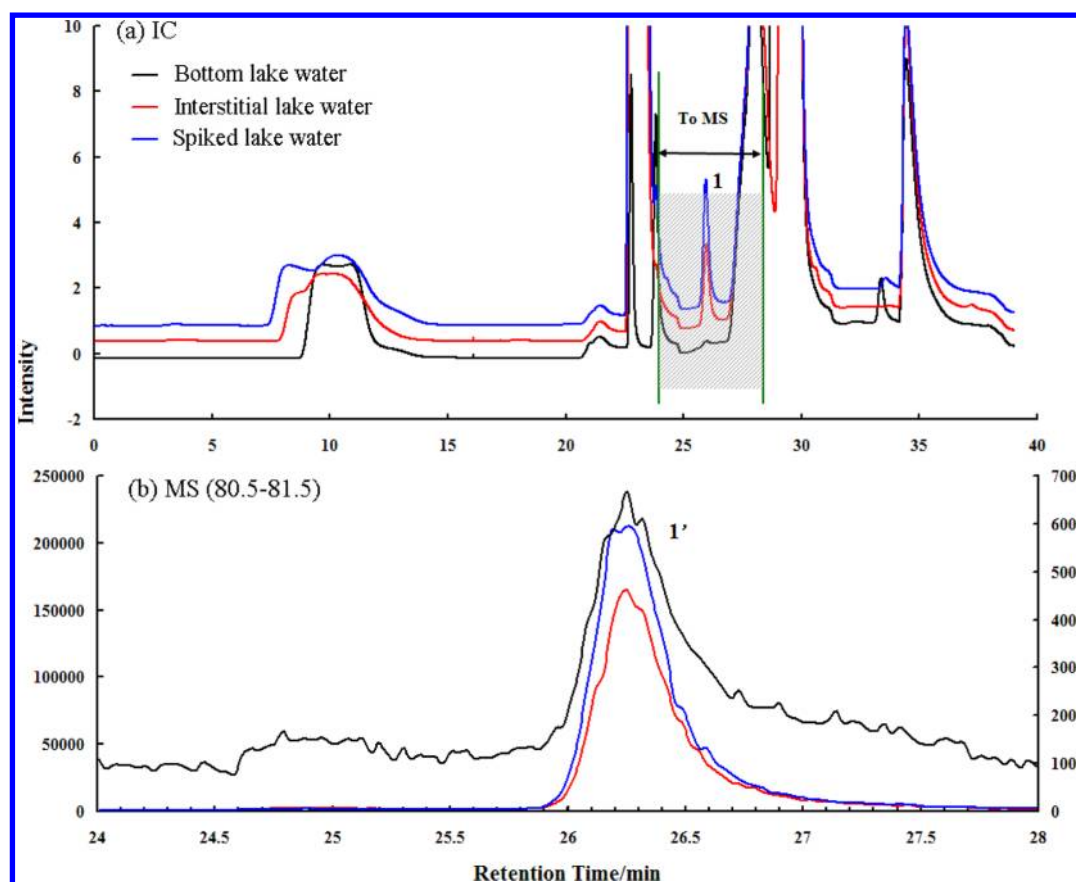


Figure 4. IC-ESI-MS chromatograms of phosphite in Lake Taihu bottom water (black line), interstitial water (red line), and spiked water (blue line). (a) Phosphite was analyzed with suppressed conductivity detector. (b) Phosphite was detected by electrospray mass spectrometry single ion monitoring experiments at $m/z = 80.5\text{--}81.5$, and ESI-MS data of bottom water are scaled on the right axis. Both ESI-MS data of interstitial and spiked water were scaled on the left axis. Conditions are shown in Supporting Information Table S2.

significantly affect the phosphite, and the recoveries decrease to 56.3%. However, it proved impossible to detect more than 5 mM hydrogen carbonate in Lake Taihu. The presence of 0–5 mM sulfate had little effect on phosphite with 83.4%–101.5% recoveries.

Peak Assignments and Spiked Recovery. Figure 2 presents representative ICS chromatograms of synthetic lake and natural freshwater samples. All peak assignments for the phosphite-containing synthetic matrix showed that 0.05 μM phosphite was well separated from other anions such as hydrogen carbonate and nitrite. The bottom lake water, interstitial lake water and synthetic water exhibit a well-defined revolution. The peak observed at a RT of 22.5 min was assigned to phosphite. Despite the fact that separation of phosphite and hydrogen carbonate would be challenging in natural waters, the resolution can be achieved by chromatography techniques, especially when a multigradient procedure is used to analyze the peaks.²⁰

The calibration graph constructed between peak areas (A) ($\mu\text{S}\cdot\text{min}$) and concentrations (C) (μM) were linear and fitted with the linear regression equation: $A = 0.1958C - 0.0005$, which had linear regression coefficients (R^2) between 0.9993 and 0.9995. These data show that quantification could be performed with good linearity and sensitivity. The relative standard deviation (RSD) of RT and peak area was 0.04% and 5.86%, respectively, which shows good reproducibility. Analytical results of recovery studies performed by adding phosphite standard solutions to original water samples are

listed in Table 1. It is clear that the phosphite recoveries from Lake Taihu water ranged from $90.7 \pm 3.2\%$ to $108 \pm 1.5\%$, suggesting that the method is efficient and could be applied to the analysis of phosphite in Lake Taihu water samples.

Demonstration of Method Performance Using 2D-CIC and IC-ESI-MS. Figure 3 shows the separation result obtained using two-dimensional capillary ion chromatography. The 2D-CIC technique, which is based on an ICS system, exhibits excellent matrix elimination and analyte enrichment without complex pretreatment thereby providing a correlation greater than 0.999 for the peak area. An injection volume of 10 μL was used in the first dimension and phosphite in the sample was roughly separated from interferences including hydrogen carbonate (Figure 3a). The majority of interferences can be excluded online with a column-switching technique utilizing 2D-CIC which differs from ICS. In this study, a time span of 20 to 20.8 min (shade area in Figure 3a) was used to transfer 25 μL effluent containing phosphite and minimized interferences from the first dimension into the MAX 100 capillary concentrator column in the second dimension where the analyte could achieve a further separation and concentration. The peak 1 and 1' are identified as phosphite by matching RT of the spiked sample. Compared to the traditional column, the MAX 100 capillary column is a hydroxide-selective monolithic anion-exchange column for fast separation of organic acids and inorganic anions, concentrating the analyte species and lowering detection limits by 2–5 orders of magnitude. It can be seen that the use of 2D-CIC system improves the separation

Table 2. Concentrations of Phosphite in Representative Water Samples from Lake Taihu

location ^a		phosphite/ μM	phosphate/ μM
Dapu Harbor	SW	0.023 ± 0.005	1.742 ± 0.000
	BW	0.038 ± 0.009	1.871 ± 0.002
	OW	0.098 ± 0.001	4.173 ± 0.010
	SIW	0.123 ± 0.007	3.106 ± 0.008
	MIW	0.167 ± 0.012	1.411 ± 0.013
	BIW	0.169 ± 0.006	2.541 ± 0.007
Gonghu Bay	SW	ND ^b	0.753 ± 0.000
	BW	0.026 ± 0.004	0.645 ± 0.002
	OW	0.068 ± 0.005	1.161 ± 0.012
	SIW	0.049 ± 0.005	1.686 ± 0.002
	MIW	0.113 ± 0.011	2.000 ± 0.007
	BIW	0.061 ± 0.007	1.726 ± 0.002
Meiliang Bay	SW	ND	0.968 ± 0.004
	BW	0.036 ± 0.005	0.839 ± 0.003
	OW	0.014 ± 0.006	3.169 ± 0.009
	SIW	0.039 ± 0.005	3.019 ± 0.005
	MIW	0.058 ± 0.018	1.686 ± 0.011
	BIW	0.006 ± 0.010	2.157 ± 0.005
Zhushan Bay	SW	ND	2.677 ± 0.012
	BW	0.037 ± 0.001	3.129 ± 0.010
	OW	0.074 ± 0.002	2.604 ± 0.008
	SIW	0.051 ± 0.003	4.298 ± 0.018
	MIW	0.113 ± 0.005	2.228 ± 0.009
	BIW	0.076 ± 0.009	1.976 ± 0.003

^aSW, surface water; BW, bottom water; OW, overlying water; SIW, interstitial water extracted from surface-layer sediment (0–20 cm); MIW, interstitial water extracted from interim-layer sediment (20–40 cm); BIW, interstitial water extracted from bottom-layer sediment (40–60 cm). ^bND = not detected.

for phosphite and also improves the MLD to 0.1 ng P/L (Figure 3b). The results demonstrate that there were micromolar levels of phosphite in the interstitial water and bottom water (Figure 3b), which was similar to the ICS detected results.

Figure 4 presents a typical ion chromatography of phosphite in Lake Taihu water samples, which was monitored using a suppressed conductivity detector (Figure 4a). Single ion monitoring experiments were conducted using mass spectrometer at $m/z = 80.5\text{--}81.5$ (Figure 4b), which corresponds to the single-charged ion of phosphite. Pretreated samples were separated by cation-exchange chromatography in IC, and the suppressed conductivity detector data was collected in the total runtime (40 min). The IC effluent of the time window from 24 to 28 min (shaded areas) was pulsed into the mass spectrometer. The tentative identity of peak 1 (RT = 25.9 min), separated well from adjacent peaks in Figure 4a, and was identified as phosphite by matching RT of the spiked sample. Chromatogram in Figure 4b contains a single and well-defined peak 1' eluted at 26.2 min at $m/z = 80.5\text{--}81.5$. The spiked samples were used to determine that phosphite elutes at 26.2 min in ESI-MS. It has been verified that the peak RT in ESI-MS is always 0.3 min later than that in IC due to the time interval between the two detectors. The peak 1' observed in Figure 4-b has a RT and a m/z in agreement with the assignment of peak 1 to phosphite, and the addition of 1 μM phosphite to water sample resulted in an increase in the peak 1', indicating that the observed single ion at $m/z = 80.5\text{--}81.5$ is phosphite. Results from 2D-CIC and IC-ESI-MS support the conclusion that

phosphite is present in Lake Taihu and agree with the ICS data presented in Figure 2 and Table 1.

Comparison of Different Analytical Methods Available for Phosphite. Here we present three potential methods (ICS, 2D-CIC and IC-ESI-MS) based on IC techniques for trace phosphite determination, which have been proven to be equivalent effective to some extent in determining phosphite in surface water samples. Compared to 2D-CIC and IC-ESI-MS, ICS is a relatively simple and speedy technique, but it is not highly specific. Determination of trace anions by ICS is always limited by the ability to resolve the targets from interferences. To overcome these problems, many approaches must be taken, such as passing through appropriate cartridges,^{18,20} the use of very high capacity columns capable of coping with the high ionic loading of the sample.¹⁷ Additionally, ICS method is also restricted to sample salinity. The analysis of samples with relatively higher salinity such as geothermal water samples, seawater samples or wastewater samples will be particularly challenging because the background ions affect heavily the resolution.²² Compared with the ICS on the trace anions which require determination in matrices containing excessive interference, the 2D-CIC system can provide the increased mass sensitivity, that is, the system would be more selective and sensitive without tedious pretreatment steps, which can separate and concentrate the interesting anions without matrix elimination. The 2D-CIC method may be suitable for phosphite measurements of more complicated environmental samples except for the relative high cost. IC-ESI-MS has been verified to be an excellent tool for trace inorganic compounds determination with various advantage features.³⁶ Moreover, it has been effectively applied to quantify and qualify phosphite in natural geothermal,^{17,20} synthetic schreibersite corrosion solution and termite extract samples.²² Drawbacks for IC-ESI-MS method derive from the interference with the same m/z and ion-adduct formation.

IC coupled with different detectors, for example, suppressed conductivity detector (ICS and 2D-CIC in this study), ICP-MS¹⁷ and ICP-ES³⁷ had been reported to be reliable for the determination of phosphite. Apart from that, many other analytical methods have been introduced for phosphite determination in different matrices to date, but have serious limitations. Some of these methods are not available in every analytical laboratory and require tedious pretreatment due to the complexity of environmental samples. For example, two such methods are ³¹P NMR and GC-MS developed by Pasek et al.¹⁵ and Glindemann et al.,³⁸ respectively. HPLC-ICP-ES³⁹ and HPIC⁴⁰ provided an interesting approach for phosphite determination, but they were not sensitive enough and lacked the stability needed for the micromole levels of phosphite. As for chemical analysis reported in previous literatures,^{41,42} the main difficulties were associated with either time and reagent consumption or the loss and combination of the analyte (Supporting Information Table S4).

Phosphite in Lake Taihu. Using the modified analytical method and protocols, phosphite presence was detected on a wide set of environmental samples (surface water, bottom water, overlying water and interstitial waters extracted from sediments at different depths). For the first time, phosphite was widely detected in Lake Taihu with concentrations ranging from $0.01 \pm 0.01 \mu\text{M}$ to $0.17 \pm 0.01 \mu\text{M}$ (Table 2). Interestingly, higher levels of phosphite were detected in the sediment than in the water. The amount of phosphite was about 1% to 10% of phosphate, indicating that phosphite was a

trace carrier of P in lake P cycles. However, this was likely only a small portion of the total phosphite content. For a more accurate analysis, it is also necessary to measure the consumption rate of phosphite by oxidation and possible microbial metabolism in the water column and in the oxic sediment–water boundary layer.²⁸ It has been reported that in P-limited eutrophic lakes, the dissolved reactive P concentration of 0.32 μM is enough to satisfy a request for phytoplankton, while at higher concentrations from 1 to 3.2 μM , algal blooms would likely be promoted.⁴³ It has been confirmed that phosphite could be involved in the biogeochemical P cycle and contribute to the eutrophication observed in Lake Taihu.²⁷ Given the bioavailability and high migration propensity of phosphite, further research is needed to investigate its fate in the environment especially in the eutrophic lake ecosystems and the associated risk to eutrophication.

■ ASSOCIATED CONTENT

■ Supporting Information

Further information on the operating conditions of 2D-CIC (Table S1) and IC-ESI-MS (Table S2); physicochemical properties of the investigated locations (Table S3); and comparison of different analytical methods available for phosphite determination in various environmental samples (Table S4). This material is available free of charge via the Internet at <http://pubs.acs.org/>

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Notes

The authors declare no competing financial interest.

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