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An Evaluation of a Field-Based Method to Prepare Fresh Water Samples for Analysis of Sulfite and Thiosulfate by High-Performance Liquid Chromatography (HPLC)

By Anne L. Bates, William H. Orem, Harry E. Lerch, Margo D. Corum and Marisa Beck

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Abstract

This study provides an evauluation of a high-performance liquid chromatrograph method for analyzing sulfite and thiosulfate in fresh water field samples. Unlike other methods used for the analysis of sulfite and thiosulfate, this technique offers the advantage of being suitable for analysis of samples collected in the field far from a laboratory. Sulfite and thiosulfate are stabilized in the field by fixation with a derivatizing agent. The sulfite- and thiosulfate-derivative complexes are then stabilized on C-18 syringe cartridges. The cartridges are transported to the laboratory for analysis at a later date.

This study attempts to reproduce the results reported in the literature and to adapt this method to the equipment available in our laboratory and to the fresh surface water and pore water samples collected in south Florida.

Results of recovery experiments indicate that the thiosulfate-derivative complex is fully recovered and stable with a maximum error of ±15 % at the lowest concentrations for thiosulfate. The sulfite-derivative complex is fully recovered, however, the sulfite-derivative peak areas increase with time. In order to quantify sulfite, a peak enhancement correction factor must be applied, which increases the error to ±15 % to ±20 % at lower concentrations of sulfite.

Introduction

Field investigations are ongoing into the relationship between sulfur cycling and toxic methyl mercury production in the Florida Everglades. The purpose is to relate the content of methyl mercury in wetland sediment pore water to concentrations of sulfur species (sulfate, sulfide, sulfite and thiosulfate) in order to better understand the major factors involved in the processing of methyl mercury in sediment pore waters (Gilmour, 1991). Sulfite and/or thiosulfate may play an important role in methyl mercury production even though they typically occur at very low concentrations relative to sulfate and sulfide in wetland sediment.

Sulfate and sulfide are the sulfur species at the high and low ends of the sulfur oxidation-reduction range. Either of these two species can be analyzed by well-established methods. Sulfate is stable under oxic conditions and is analyzed by ion chromatography or by gravimetric methods after transfer of samples to the lab from the field. Sulfide is stable for a few hours under reducing conditions and is analyzed by selective ion electrode methods, either *in situ* or shortly after collection in the field. Sulfite and thiosulfate are sulfur species with intermediate oxidation states. Sulfite and thiosulfate occur at relatively low concentrations compared to sulfate and sulfide, and they are stable for only a few hours. The quantitative analysis of these two species has been a challenge because traditional methods for sulfite/thiosulfate analysis by photometric or iodometric titration determinations (Volkov and Zhabina, 1990) are not easy to perform under field conditions at remote locations.

Vairavamurthy and Mopper (1990) published a method for sulfite/thiosulfate analysis which calls for stabilization of sulfite and thiosulfate in the field for lab analysis at a later date by high-performance liquid chromatography (HPLC). Sulfite and thiosulfate are fixed in the field shortly after sample collection by derivatization with 2,2'-dithiobis(5-nitropyridine) ("DTNP"). The derivatization products are stabilized on C-18 cartridges. Vairavamurthy and Mopper (1990) state that the cartridges can be preserved under refrigeration (0-5°C) for at least two weeks prior to HPLC analysis. This method

has been adapted here for surface water and pore water samples collected in the Florida Everglades and the available laboratory equipment.

Analytical Methods

Chemicals and Solutions

Chemicals and solutions used in these procedures are listed in the Appendix.

Sample Collection in the Field: Surface Water and Pore Water collected from Mesocosms

Surface water was pumped from about midway between the sediment and the water surface through a 0.45 μm Sterivox[1] filter into cleaned and dry plastic containers. Pore water from "mesocosms", *in situ* experimental field cells of sediment isolated by inserting 1 m diameter polycarbonate cylinders into the marsh, was also pumped from various depths in the cell sediment through a 0.45 μm Sterivox filter into clean plastic containers. Aliquots (10.0 ml) of the collected water were placed in clean, dry scintillation vials containing the stabilizer mixture (0.5 ml acetate buffer and 0.5 ml DTNP derivatizer in acetonitrile; 0.05 ml of buffer and 0.05 ml of stabilizer per milliliter of standard/sample, as prescribed in Vairavamurthy and Mopper (1990)) within 10-15 minutes after collection. The derivatized porewater samples are kept in insulated coolers until arrival at the field lab. The derivatized samples were loaded onto prepared cartridges (see Sample Preparation in the Field, below) upon arrival at the field lab, normally within 4-8 hours after collection, dried with nitrogen gas, and then refrigerated pending HPLC analysis.

Sample Collection in the Field: Pore water from sediment cores

Porewater samples from sediment cores contained in polycarbonate cylinders were collected in the field, as described by Bates and others (2001), by squeezing the core at both ends to compress water through 0.45 μm filters into syringes inserted into lateral ports in the core cylinder. Aliquots (10.0 ml)

[1] Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

of pore water from the syringes were treated with stabilizer mixture within 10-15 minutes after collection. The derivatized samples were loaded onto prepared cartridges and then refrigerated pending HPLC analysis.

Sample Preparation in the Field

Syringe cartridges (C-18, Supelco, 6 ml, 500 mg capacity) are treated in the field lab by successive 5 ml rinses with HPLC-grade methanol, MilliQ water, and cartridge stabilizer solution. The solutions are drawn through the cartridges into a small vacuum chamber.

After collection and stabilization of the samples (as described above), the samples are transported to the field lab where they are processed by drawing them through the treated cartridges. The 500 mg capacity of the syringe cartridges is more than enough to absorb dissolved organic carbon and sulfide-derivative (DOC and sulfide occur at no more than 50 mg/liter and 4 mg/liter, respectively, in the Florida Everglades) as well as sulfite- and thiosulfate-derivatives from a 10 ml sample. The sample-loaded cartridges are dried by a stream of nitrogen gas for about 20 minutes. The cartridges are then sealed and transported to the lab for HPLC analysis by same-day air travel in ice-cold insulated boxes. Upon arrival in the laboratory, the cartridges are stored under refrigeration at a temperature not higher than 7°C, usually for no more than 3 days, before HPLC analysis.

HPLC equipment and running conditions

The HPLC system consists of a Waters Corporation 600 gradient pump and controller, a Rheodyne type injector with a 20ul loop, a Waters Nova-Pak C-18 3.9 x 150 mm reversed-phase column, and a Waters 966 Photodiode Array Detector. The system is controlled by Waters Millennium software programmed to record the detector absorbance at 320nm over a 30 minute gradient elution.

The eluents were TBA-HS buffer and HPLC-grade acetonitrile (see the Appendix for a description of chemicals and solutions). Both eluents were sparged with ultra-pure helium gas for 30 minutes prior to pumping. Sparging was continued during column equilibration and the sample runs.

The column was conditioned with a mixture of 90% TBA-HS buffer/10% acetonitrile for 30 minutes prior to the first gradient run. The eluent mix was varied at a constant ramp (Table 1) during the 30 minute gradient run.

Table 1. Eluent (TBA-HS buffer / acetronitrile) gradient for HPLC analysis of sulfite and thiosulfate.

Time (minutes)	Acetonitrile (%)
0 - 1	10
1 - 9	10 - 34
9 - 23	34 - 55
23 - 28	55 - 100
28 - 30	100

The eluent flow rate during the 30-minute analysis is 1.0 ml/minute. The 30-minute gradient analyses were followed by 10 minutes of conditioning/rinsing at 1.5 ml/min at the initial conditions (90% buffer and 10% acetonitrile) prior to injection of the next sample.

Standard Preparation and Analysis

Stock standard solutions consisted of 50mM sulfite and 50mM thiosulfate prepared daily in separate volumetric flasks from sodium thiosulfate and sodium sulfite chemicals diluted with deaerated (by sparging with nitrogen gas) MilliQ water. Working standards containing both sulfite and thiosulfate are prepared from the stock solutions by serial dilution with deaerated MilliQ water. Detector response is linear over the concentration range from 1.0 to at least 40µM. Standards are usually prepared in the concentration range from 1.0 to 30 µM, well within the concentration range of samples collected in the Florida Everglades.

About 10 minutes prior to injection, a 1.0 ml aliquot of working standard is transferred from the volumetric flask to a small glass vial containing 0.05 ml of 0.2 M acetate buffer and 0.05 ml of 10mM DTNP derivatizer in acetonitrile (0.05 ml of buffer and 0.05 ml of stabilizer per milliliter of standard/sample, as presecribed in Vairavamurthy and Mopper (1990)). After 10 minutes, 1.0 ml of HPLC grade methanol is added to the vial and mixed with the derivatized standard (addition of the methanol is essential to maintaining linearity of the calibration curves beyond 10μM for both sulfite and thiosulfate). An aliquot of the standard/stabilizer mixture is then loaded into the Rheodyne injector. The run begins upon injection of 20 µl of the standard or sample into the system.

Calibration

Waters Millennium software is used to calculate peak area responses for sulfite and thiosulfate derivatives. Retention times occur at about 16.0 minutes (sulfite standards) and 17.7 minutes (thiosulfate standards) (see Figure 1A). Standard peak areas vary by no more than ±10% for standards over concentrations ranging from 5.0 μM to 40 μM. The analytical precision for standard concentrations less than or equal to 2.5 μM are less than ±15 %. The limit of detection is about 0.5 μM for both sulfite and thiosulfate under our instrumental conditions, based on a signal to background ratio of at least 2. The linear range of the calibration curves extends to at least 40 μM for the sulfite and thiosulfate, beyond the upper limit normally encountered in our field samples.

Sample Elution and Analysis

About 10 minutes before injection, a sample cartridge is removed from refrigeration and attached to one of the ports of a small glass vacuum chamber. For field samples having a normal concentration of sulfite and thiosulfate (1.0—30.0 μM), 1.0 ml of HPLC grade methanol is added to the cartridge packing and is then drawn through a syringe and into a 4.0 ml glass vial. A second 1.0 ml of methanol is then drawn through the packing into the vial. The volume of methanol recovered is 1.6 ml, resulting in a 1.6 fold dilution of the samples relative to the standards. 1.0 ml of the methanol solution is

transferred from the vial into a second empty glass vial. This is then mixed with 1.0 ml of deaerated MilliQ water. The sample is then ready for injection, which occurs 10 minutes after the sample is eluted from the cartridge**.** Maintaining a consistent time between sample elution and injection (within ±5 minutes) is important because the sulfite-derivative chromatogram peak tends to increase over time. Sample results in μM units are then calculated from the calibration curves. Corrections for dilution and concentration effects on the samples relative to the standards are then applied (i.e., 10 ml of sample are processed and reduced to a volume of 1.6 ml, whereas only one ml of standard is processed).

Results and Discussion

The procedure typically yields discrete, well-resolved peaks for sulfite and thiosulfate for both standards and samples (Figs. 1A and 2A). A major derivatizer peak occurs at about 24 minutes. A sufide-derivative peak appears at 6.9 minutes, and a peak of unknown identity, possibly a thiol-derivatizer complex peak (Vairavamurthy and Mopper (1990), appears at a retention time of 19 minutes in chromatograms of standards (Fig. 1A) and samples (Fig. 2A). There are no interferences evident in the chromatograms of typical samples, as shown by the chromatogram of a typical pore water sample taken from the Florida Everglades (Fig. 2A).

Experiments were carried out in order to reproduce results reported in the literature (Vairavamurthy and Mopper, 1990) and to ascertain recoveries under our lab and instrumental conditions. The following were determined: recoveries of sulfite and thiosulfate standards from the cartridges, stabilities of derivatized sulfite and thiosulfate on cartridges under refrigeration, efficiency of methanol elution, and the stabilities of sulfite and thiosulfate in field samples before derivatization.

Figure 1. (A) Chromatogram of 30.0 µM Sulfite/Thiosulfate Standards and (B) Chromatogram of a Derivative Blank.

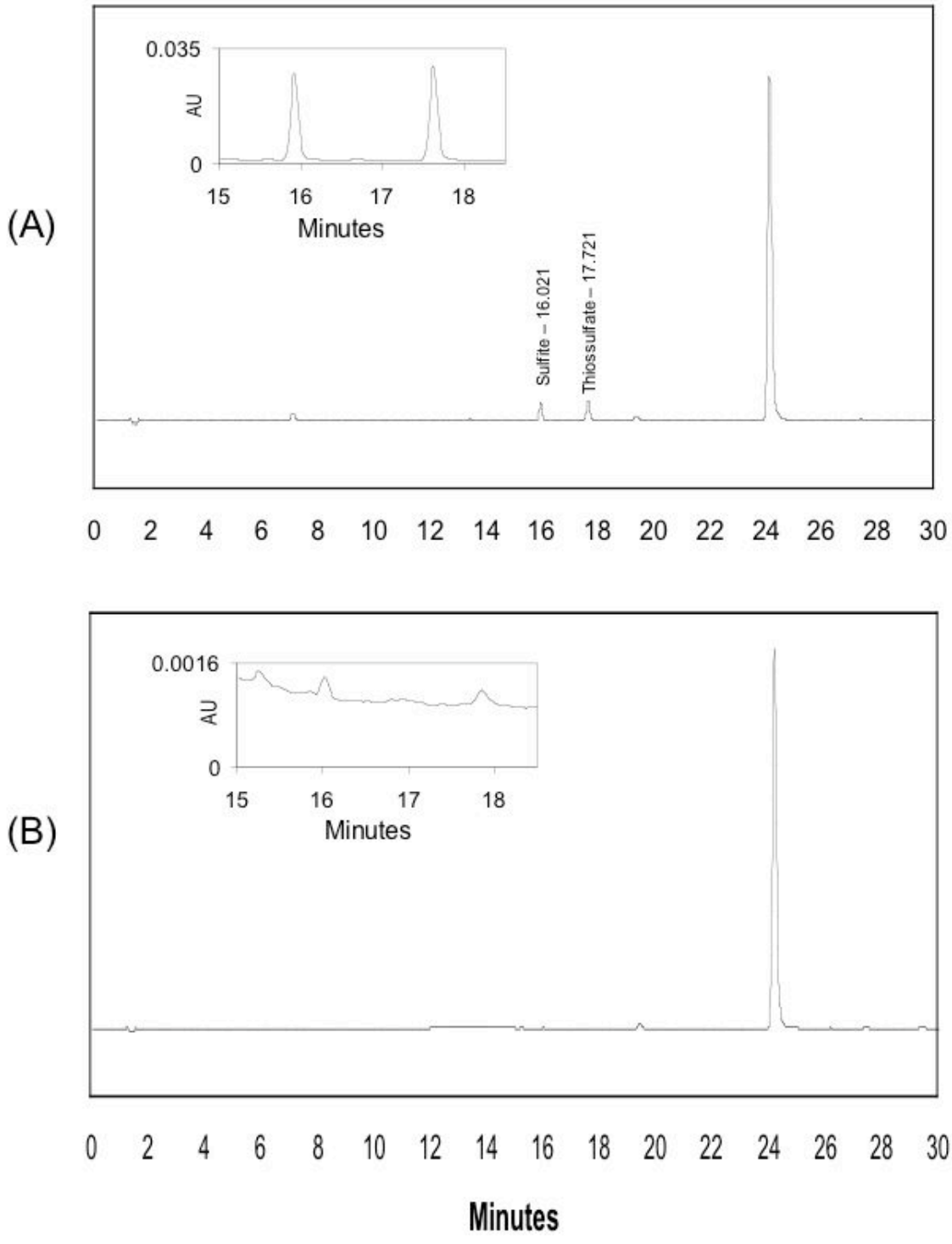
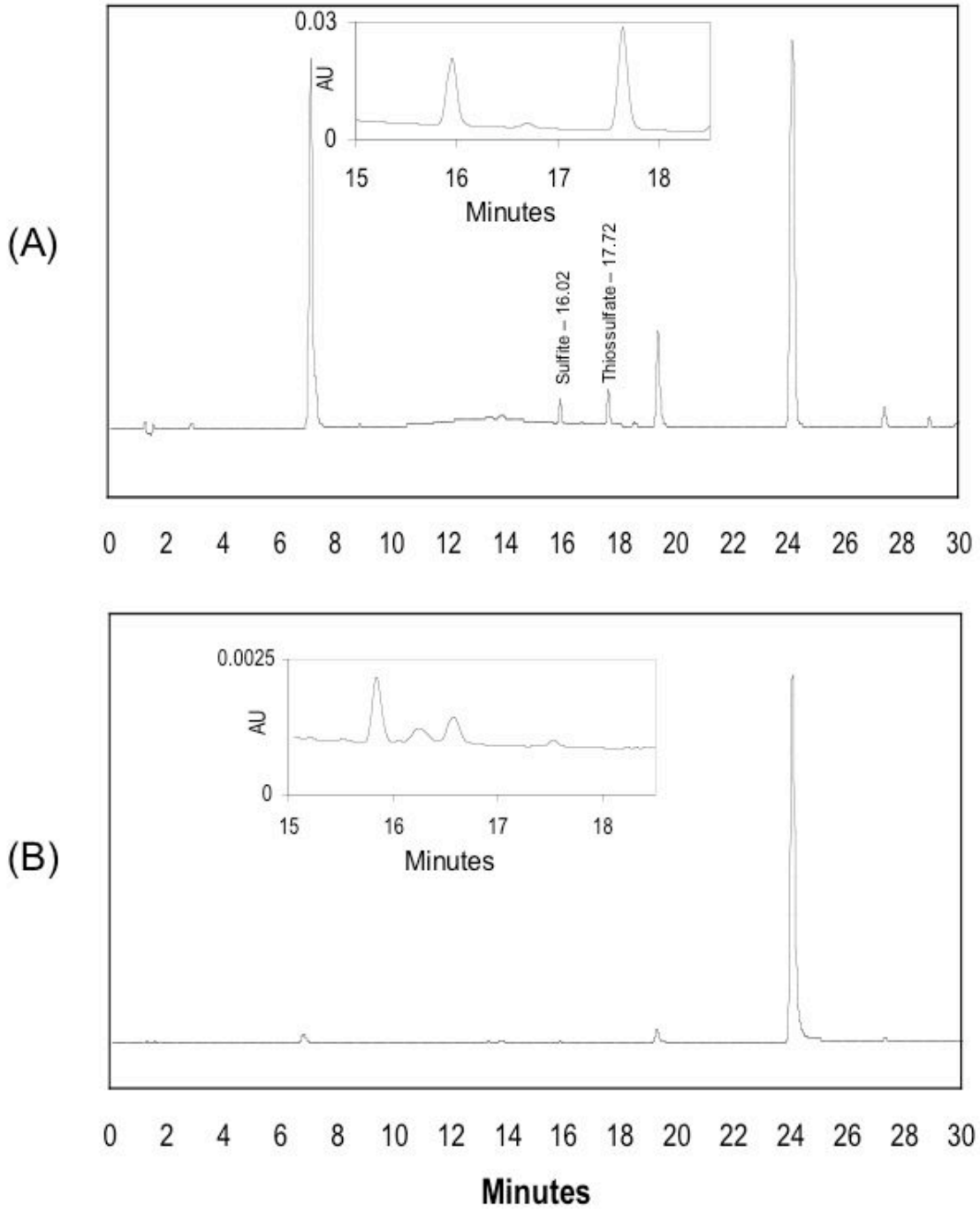


Figure 2. (A) Chromatogram of a Derivatized Pore Water Sample from Sediment in the Florida Everglades and (B) Chromatogram of a Derivative Blank Applied to a Cartridge.



Recovery of sulfite and thiosulfate from sample cartridges

Standards were loaded onto cartridges in order to determine recoveries. Working standards were prepared by serial dilution within 30 minutes prior to analysis by HPLC. One ml of working standard was prepared and run as described above (Standard Preparation and Analysis). This standard was not loaded onto a cartridge. Concurrently, another 1.0 ml aliquot of standard was prepared for application to a prepared cartridge in the same manner as the field samples: 1.0 ml of working standard was diluted in 9 ml of deaerated MilliQ water (10 ml of sample are processed in the field) and treated with stabilizer mixture (0.5ml of acetate buffer and 0.5ml of DTNP in acetonitrile solution, i.e., 0.05 ml of acetate buffer and 0.05 ml of DTNP in acetonitrile per ml of sample/standard). After mixing and waiting for 10 minutes, the derivatized standard was drawn through a treated cartridge. A stream of nitrogen gas dried the standard-loaded cartridge for 20 minutes. Immediately after drying, the standard was extracted from the cartridges with two 1.0 ml aliquots of HPLC grade methanol, as described above for the field samples (Sample Elution and Analysis), and then injected into the HPLC system 10 minutes later. HPLC results for the extracted samples were compared with results obtained from standards not loaded onto the cartridges after correcting for methanol dilution occurring during elution.

Comparison of HPLC peak areas for standards loaded and not loaded onto cartridges (Table 2) indicates that thiosulfate recovery is virtually 100 percent. However, sulfite recovery from the cartridges appears to be greater than 100 percent. Repeating the analysis 1.5 hours later on one of the 2.5 μM standards extracted from a cartridge showed that this elevation increased 57 % for the sulfite-derivative peak whereas the sulfite-derivative peak increased only slightly. For this reason, extracted samples must be injected into the HPLC system at a consistent time (about 10±5 minutes) after elution, and then the sulfite results must be multiplied by a correction factor. We calculate this correction factor to be 0.85±0.05, based on a comparison of the mean peak areas of standards loaded onto cartridges with mean peak areas obtained from standard calibration curves from many routine assays. There is an uncertainty of about ±15.0% for both sulfite and thiosulfate standards (not loaded onto cartridges) at the

Table 2. Effect of variation in sulfite and thiosulfate concentration on target analyte response (and recovery) both prior to and after charging to sample cartridges.

Concentration / Treatment	Sulfite-derivative Peak Areas Standard	Thiosulfate-derivative Peak Areas
1.0 µM / Not loaded onto cartridge	6386	6944
1.0 µM / Loaded onto cartridge, run 10 min after elution	7043	6066
1.0 µM / Loaded onto cartridge, run 10 min after elution	7280	6769
2.5 µM / Not loaded onto cartridge	14653	17592
2.5 µM / Loaded onto cartridge, run 10 min after elution	21562	17941
2.5 µM / Loaded onto cartridge, run 10 min after elution	24011	17027
2.5 µM / Loaded onto cartridge, run 10 min after elution	23827	18387
5.0 µM / Not loaded onto cartridge	27912	33097
5.0 µM / Loaded onto cartridge, run 10 min after elution	31902	33981
5.0 µM / Loaded onto cartridge, run 10 min after elution	36917	35546
5.0 µM / Loaded onto cartridge, run 10 min after elution	31418	34693
10.0 µM / Not loaded onto cartridge	58011	65716
10.0 µM / Loaded onto cartridge, run 10 min after elution	68490	63563

lower concentrations of 1.0 μM and 2.5 μM. This uncertainty is the standard deviation from the mean of peak areas obtained from standard calibration curves run for routine analyses. The uncertainty introduced by the correction factor for sulfite (0.85±0.05) increases the error for sulfite standards at low concentrations from ±15.0% to about ±20% for standards loaded onto cartridges.

It is important to note that storage or processing of field samples in HCl-washed containers greatly increases this peak enhancement, especially for sulfite but also for thiosulfate, possibly because of catalysis by residual acid. For this reason, storage containers for samples and standards used for this assay should not be acid-washed.

Stability of Sulfite and Thiosulfate on Refrigerated Cartridges

Nine standard-loaded cartridges were prepared within an hour of each other; each loaded with 10 ml of total sample containing 1.0 ml of 2.5 μM stds + 9.0 ml MilliQ water + 0.5 ml acetate buffer + 0.5 ml DTNP derivatizer. A concentration of 2.5 μM of each standard was selected because that

concentration approximates the concentrations found in most of the field samples, and because degradation of the samples over time would be most noticeable at the lower end of the concentration scale, represented by 2.5 μM. The loaded cartridges were nitrogen-dried and refrigerated at 7°C until ready for elution. Three of the cartridges were extracted and run on the day after preparation (Day 2). Three were run at 5 days after preparation, and three were run 14 days after preparation of the cartridges. Blanks ("derivatizer blanks") consisting of 10 ml of MilliQ water with 0.5 ml acetate buffer and 0.5 ml DTNP derivatizer were also prepared and loaded onto cartridges at the same time as the standard-loaded cartridges were prepared. On each day that the extracted standards and derivatizer blanks were run, a freshly prepared 2.5 μM standard, not loaded onto a cartridge, was also run. For comparison with the standards not loaded onto a cartridge, resulting peak areas of extracted standards and blanks were corrected for the dilution occurring during methanol elution from the cartridges. Results are summarized below (Table 3):

Table 3. An evaluation of the lenth of storage under refrigeration on subsequent analyte response.

	Sulfite-derivative Peak areas	Thiosulfate-derivative Peak areas
Day2		
2.5 μM standard, not loaded onto cartridge	13818	16954
Derivatizer blank, not loaded onto cartridge	351	0
Derivatizer blank, extracted from cartridge	933	1619
2.5 μM Standard, extracted from cartridge	24944	15224
2.5 μM Standard, extracted from cartridge	18612	15915
2.5 μM Standard, extracted from cartridge	17883	16915
Mean of standards extracted from cartridges	**20480**	**16351**
Day 5		
2.5 μM standard, not loaded onto cartridge	14496	17521
Derivatizer blank, not loaded onto cartridge	0	922
Derivatizer blank extracted from cartridge	8529	2455
2.5 μM Standard, extracted from cartridge	20923	14798
2.5 μM Standard, extracted from cartridge	23779	15510
2.5 μM Standard, extracted from cartridge	21381	15787
Mean of standards extracted from cartridges	**22028**	**15365**
Day 14		
2.5 μM standard, not loaded onto cartridge	13563	16728
Derivatizer blank, not loaded onto cartridge	30	660
Derivatizer blank extracted from cartridge	7405	1686
2.5 μM Standard, extracted from cartridge	28718	13352
2.5 μM Standard, extracted from cartridge	19405	16671
2.5 μM Standard, extracted from cartridge	22006	14731
Mean of standards extracted from cartridges	**23376**	**14918**

Average peak areas for the extracted standards appear to show a slight increase for sulfite and a slight decease for thiosulfate, over time from Day 2 to Day 14. However, the percent difference between the averages for day 2 and day 14 is 12% for sulfite and 8.8% for thiosulfate, both within the error of the method at 2.5 μM for sulfite (± 20%) or for thiosulfate (± 15%). These results are inconclusive, but do suggest that field samples should be run sooner rather than later. Note that the refrigerator temperature was 7°C, higher than the 0-5°C temperature range recommended in the literature (Vairavamurthy and Mopper, 1990).

When considering the derivatizer blank values in Table 3, it is apparent that the derivatizer blanks loaded onto cartridges are higher than those not loaded onto cartridges**.** (A prepared cartridge by itself does not produce this phenomenon: we have found that when prepared cartridges, with neither derivative blank nor sample added, were "extracted" with methanol and the methanol then mixed with MilliQ water and derivatizer, no signal was observed for either sulfite or thiosulfate on the chromatogram.) One key difference between standards loaded and not loaded onto the cartridges is that, although the amount of standard is the same, the amount of derivatizer is ten times higher in the extracted standard due to the concentration occurring during the elution. The reason for adding ten times the amount of derivatizer is to replicate field procedures for samples (where 10 ml of sample is processed) in the extracted standards; only 1.0 ml of unextracted standard is run in order to replicate laboratory preparation procedures for standards included in calibration curves. When a derivatizer blank (not loaded onto a cartridge) was prepared containing ten times the amount of derivitizer normally added to a blank (an amount equal to that extracted from a sample cartridge)**,** a signal was observed at about the same peak area as seen in extracted blanks in Table 2. Thus, the derivatizer, if present in high enough concentration, is raising blank values.

In an effort to ascertain if sulfite and/or thiosulfate play any role in enhancing this phenomenon, a controlled experiment was run with standards containing either sulfite or thiosulfate (but not both species), both loaded onto cartridges and not loaded onto cartridges. Derivatizer blanks were also run. Results, including sulfide-derivitive peak areas at 6.9 minutes retention time, as well as peak areas for the sultite- and thiosulfate-derivatives, are summarized below (Table 4).

Table 4. Comparison among peak areas of derivatizer blanks, sulfite standard, and thiosulfate standard, applied to cartridges and not applied to cartridges.

Standard concentration / Treatment	Sulfide-Derivative Peak Area @ 6.9 minutes	Sulfite-Derivative Peak Area @ 16.0 minutes	Thiosulfate-Derivative Peak Area @ 17.7 minutes
Derivatizer Blank / Not loaded onto a cartridge	2,424	0	0
Derivatizer Blank / Loaded onto cartridge	369,500	8,064	657
10 μM Sulfite	18,266	51,534	0
10 μM sulfite / Loaded onto cartridge	509,143	63,962	5,514
10 μM Thiosulfate	18,910	422	67,476
10 μM Thiosulfate / Loaded onto cartridge	258,255	10,322	65,483

Concentrating the blank by elution from a cartridge enhances the peak areas at all three retention times, especially at the sufide-derivative peak at 6.9 minutes, consistent with results for sulfite-derivative and thiosulfate-derivative peak areas for derivatizer blanks (Table 3) that were commented on above. The presence of either sulfite or thiosulfate enhances the peak at 6.9 minutes, with or without passing the sample through a cartridge. However, this peak is greatly enhanced in the samples loaded onto cartridges, particularly if sulfite is present. The sulfite-derivative peak at 16.0 minutes for a standard loaded onto a cartridge is also enhanced relative to a standard not loaded onto a cartridge. This enhancement does not occur at the thiosulfate-derivative peak at 17.7 minutes, and the sulfite-derivative peak for the thiosulfate standard loaded onto a cartridge is similar in area to that of a blank loaded onto a cartridge.

The peaks at retention times of 16.0 and 17.7 min increase to levels that cannot be accounted for by disproportionation between sulfite and thiosulfate. There are sulfur groups on both the 2,2'-dithiobis(5-nitropyridine) (DTNP) derivatizer and the tetrabutylammonium hydrogen sulfate (TBA-HS)

used to condition the cartridges (this method is not sensitive to sulfate, even at high concentrations). One possible explanation is that when the derivatizer is present at high enough concentrations, its breakdown products, which may include sulfide and sulfite, are being seen in their derivatized forms at retention times of 6.9 and 16.0 minutes.

Two changes to the procedure reduced, but did not eliminate, the problem of sulfite peak area increase. Centrifuging the samples to separate out the DTNP precipitate (which appears as a flocculent after addition to the aqueous samples) prior to application to a cartridge reduces both the amount and the rate of increase in peak size. Also, a 90 percent reduction in the amount of DTNP added was found to have an effect similar to centrifugation. A solution to this problem might be to simply reduce the amount of DTNP added to the samples, however this introduces another problem. The dilemma is that samples in the field can contain substantial amounts of sulfide, which also reacts with the derivatizer. As a result, the samples must be amended with enough derivatizer to react with sulfite and thiosulfate as well as sulfide. Centrifuging the samples is another possible solution to the problem. However, the problem of transporting a centrifuge in the field nullifies one of the principal advantages of this technique, which is ease of performance under field conditions. Because the error in the sulfite concentration is predictable when the assay is performed rigorously in the same way each time, the alternative is to carry out the procedure as described in the Analytical Methods section (above) and then apply the correction factor of 0.85±0.05 to the sulfite data. Yet another alternative would be to routinely load standards onto cartridges, and then to extract them just prior to standard calibration runs, thus canceling out the error. The drawback to this approach is that it is time consuming and thus impractical for a procedure that requires daily preparation of standards for the assay of a large number of samples that must be run under intense time pressure.

Efficiency of elution of high sulfite and thiosulfate concentrations from cartridges with methanol

A working standard with concentrations of 40 μM sulfite and thiosulfate (above the highest concentration observed in a small number of field samples from highly sulfidic field sites) was prepared in the same manner as a field sample (i.e., 10 ml of total sample containing 1.0 ml of 40 μM std + 9.0 ml MilliQ water + 0.5 ml acetate buffer + 0.5 ml DTNP derivatizer) was loaded onto a prepared cartridge, dried under nitrogen and then extracted with 2 successive 1.0 ml aliquots of HPLC-grade methanol. HPLC analysis results were then compared with those from 1.0 ml of the same working standard. Results indicated virtually 100 percent recovery from the cartridge (Table 5).

Table 5. Recoveries of 40 µM standard of sulfite and thiosulfate from cartridges using two 1.0 ml aliquots of methanol.

40.0 µM Standard	Sulfite Peak Areas	Thiosulfate Peak Areas
Not loaded onto cartridge	240805	269221
Extracted from cartridge	243030	265000

Thus, two 1.0 ml aliquots of methanol is sufficient for essentially 100 percent removal of sulfite and thiosulfate from the cartridges containing 40 μM of sulfite and 40 μM of thiosulfate. Using 3 ml of methanol to wash the cartridge would dilute many samples with low sulfite and thiosulfate concentrations below the limit of detection.

Stability of derivatized sulfite and thiosulfate before cartridge application

Laboratory experiments were conducted to determine the best field preparation protocol following sample collection. Using prepared standards, results were compared between three different field processing methods:

1. Collect, derivatize @ 10 minutes, apply to cartridge 10 minutes after derivatization

2. Collect, derivatize @ 10 minutes, apply to cartridge after 4 hours on ice.

3. Collect, keep on ice in an air-free vial for 4 hours, derivatize, apply to cartridge @ 10 minutes.

After loading the standards, all cartridges were dried using a flow of nitrogen gas and then refrigerated until ready for elution. After elution and HPLC analysis, results of the three protocols were compared to each other and to the results obtained from the same standard not loaded onto a cartridge. Results summarized in the Table 6 indicate that for thiosulfate there are no significant differences between any of the sample preparation conditions. Sulfite peak responses for the standards loaded onto cartridges were elevated relative to those standards not loaded onto a cartridge; however, there were no significant differences among the three standards loaded onto the cartridge. These results indicate that the samples should be stable if kept air-free on ice for 4 hours before derivatization, or if the samples are derivatized 10 minutes after collection and kept on ice for 4 hours.

Table 6. Evaluation of the effect of different simulated field sample collection/storage techniques on sulfite and thisulfate analytical responses.

Sample Treatment	Sulfite Peak Area	Thiosulfate Peak Area
2.5 μM Standard, Not loaded onto cartridge	15282	18568
2.5 μM Std, loaded onto cartridge 10 minutes after adding derivatizer	20038	18432
2.5 μM std, loaded onto cartridge 4 hours (on ice) after adding derivatizer	18654	18994
2.5 μM std, derivatizer added after 4 hours on ice in an air-free vial. Loaded onto cartridge after 10 minutes	21852	18387

Summary

The high performance liquid chromatography (HPLC) method described here has the major advantage of being a more feasible means for collecting and analyzing aqueous field samples for sulfite and thiosulfate in comparison to photometric or iodometric titration methods that do not lend themselves well to performance under field conditions. The results of laboratory recovery experiments indicate that this method works well with the caveat that there is a significant error (about ±20%) associated with the sulfite data. The error is due to the changes in the amount of sulfite-derivative that occur once the samples are extracted from the cartridges and to the correction that must be applied to the data to compensate for these changes. In contrast, thiosulfate peak areas appear to be quite stable over time, with a deviation of not more than ±15% at the lowest concentrations. These errors are calculated from the variability in the standards prepared and run in the laboratory. Field samples may have a larger, unknown cumulative error connected with variability in sample handling, shipping, and storage conditions. This error may be acceptable for studies where small differences between sulfite concentrations are not critical to an interpretation of the results.

Appendix

Chemicals:

2,2'-dithiobis(5-nitrophridine) ("DTNP"); Aldrich Chemicals

Sodium Acetate; Aldrich Chemicals

Sodium sulfite; Aldrich Chemicals

Sodium thiosulfate; Aldrich Chemicals

Tetrabutylammonium hydrogen sulfate ("TBA-HS"); Fluka Chemicals (chromatography grade)

Acetonitrile (HPLC grade); Fisher Chemicals

Methanol (HPLC grade); Fisher Chemicals

Ultra-pure MilliQ water (18.2 Mohms/cm^3)

Solutions:

HPLC eluent buffer (TBA-HS): 0.05 M NaAc, 7.5mM TBA-HS, pH = 3.50±0.05

Derivatizer solution: 10 mM DTNP in acetonitrile

Buffer: 0.2 M NaAc, pH = 6.00±0.05

C-18 cartridge stabilizer: 20 mM NaAc, 10 mM TBA-HS, pH = 6.00±0.05

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