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# Quantification of sulfur and sulfur-containing compounds in wastewaters by means of a combination of liquid chromatographic methods

T.J. Hurse[a,b,*], W.P.P. Abeydeera[b]

[a]*Advanced Wastewater Management Centre, University of Queensland, Brisbane, Qld 4072, Australia*
[b]*Department of Chemical Engineering, University of Queensland, Brisbane, Qld 4072, Australia*

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**Abstract**

Low-micromolar concentrations of sulfite, thiosulfate and sulfide, present in synthetic wastewater or anaerobic digester effluent, were quantified by means of derivatization with monobromobimane, followed by HPLC separation with fluorescence detection. The concentration of elemental sulfur was determined, after its extraction with chloroform from the derivatized sample, by HPLC with UV detection. Recoveries of sulfide (both matrices), and of thiosulfate and sulfite (synthetic wastewater) were between 98 and 103%. The in-run RSDs on separate derivatizations were 13 and 19% for sulfite (two tests), between 1.5 and 6.6% for thiosulfate (two tests) and between 4.1 and 7.7% for sulfide (three tests). Response factors for derivatives of sulfide and thiosulfate, but not sulfite, were steady over a 13-month period during which 730 samples were analysed. Dithionate and tetrathionate did not seem to be detectable with this method. The distinctness of the elemental sulfur and the derivatizing-agent peaks was improved considerably by detecting elution at 297 instead of 263 nm.


*Keywords:* Wastewater analysis; Derivatization, LC; Anaerobic treatment; Sulfide; Thiosulfate; Sulfite; Monobromobimane

## 1. Introduction

Those overseeing the safe handling and treatment of wastewater have frequently to deal with the issue of sulfide, which causes corrosion and unpleasant odours, can endanger the safety of sewer-workers, and may hinder further treatment of the wastewater. Many simple sulfur-containing anions are convertible to sulfide — and vice-versa — under conditions encountered by the wastewater as it flows from its source, through the sewers and treatment plants, into the environment. Therefore, when assessing whether sulfide-related problems may arise, one must consider sulfide, and the potential sources and sinks of sulfide. For this purpose, it will usually be sufficient to quantify those sulfur-containing compounds accounting for most of the sulfur. The overall picture that has emerged from research into sulfide oxidation in aqueous systems is that sulfide, elemental sulfur, thiosulfate, sulfate, or some combination of these usually account for most of the sulfur [1–12]. Given

*Corresponding author. Present address: Advanced Wastewater Management Centre, University of Queensland, Brisbane, Qld 4072, Australia. Tel.: +61-7-3365-4729; fax: +61-7-3365-4726.
*E-mail address:* timothyh@awmc.uq.edu.au (T.J. Hurse).

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the variety of sulfur-containing compounds, and the reactivity of some of them, methods that allow several such compounds to be preserved in a single sample, and to be separated and quantified later, are especially valued [13]. One such example is a chromatographic method described by Rethmeier et al. (1997) that enables sulfide, elemental sulfur, thiosulfate, sulfite and sulfate concentrations to be measured — and the presence of polysulfide to be detected — in a single 50-μl sample of seawater [14]. The sample is added to a reagent containing a fluorophore, monobromobimane (mBrB), which reacts with the aforementioned anions, except sulfate. The resulting bimane derivatives are fluorescent, and are separated and detected with an HPLC system comprising a reversed-phase column and a fluorescence detector. The sulfur and sulfate, which remain unreacted, are separately quantified with two other HPLC techniques.

The overall method has yet to be tried in wastewater studies. The report of Rethmeier et al. provided some indicative chromatograms, some calibration data, and the retention times of the relevant peaks, but no quantitative information about the accuracy, precision and reliability of the method. Other workers have reported the detection levels and the precision of the bimane protocol, or a variant thereof [15,16], but the matrices in those studies were not wastewater, and the elution buffers in one case were quite different. The effect of storage time on response has been described [15,17], but its effect on precision has not. The variation of retention times has yet to be quantified. Despite the application of the method in cases where the matrix resembles the synthetic medium used in our work [18], recoveries of thiosulfate and sulfide have only been reported [17,19] for a quite different bimane protocol. In this paper we report: recoveries of sulfide, thiosulfate and sulfite in synthetic wastewater; the precision with which these anions are measured; recovery of sulfide in industrial wastewater; and the variability of retention times. As regards the elemental sulfur protocol a practical way is advanced for reducing the degree to which the sulfur and extracted-derivatizing-agent peaks overlap. Finally, the responses to dithionite, dithionate, and tetrathionate are described for the first time.

## 2. Methods

### 2.1. Chemicals for chromatography

Standard solutions of sulfur-containing anions were prepared from the corresponding AR-grade sodium salts ($Na_2S{\cdot}9H_2O$, S-4766, Sigma, St. Louis, MO; anhydrous $Na_2SO_3$, S-4672, Sigma; $Na_2S_2O_3{\cdot}5H_2O$, 10268-4G, BDH Kilsyth, Vic). Sodium sulfide crystals were rinsed in ultrapure water and patted dry with lint-free tissues just prior to being weighed, as is the usual practice [15,20]. The elemental sulfur used for sulfur standards (BDH, Poole, UK) was found by analysis with an elemental analyser (Carlo Erba 1106) to be not significantly less pure than AR-grade sulfur flakes (A 21,329-2, Aldrich, Milwaukee, WI), which dissolved too slowly in the solvent (chloroform, HPLC-grade, Burdick and Jackson, Muskegon, MI) to be of use. Chemicals used for the derivatization were *N*-2-hydroxyethyl 1-piperazine-*N′*-2-ethanesulfonic acid (HEPES) (101926.83, ICN Biomedicals, Aurora, OH), EDTA (AR D/0700/53, Fisons, Loughborough, UK), acetonitrile (HPLC-grade, EM Science, Gibbstown, NJ), methane sulfonic acid (M-6391, Sigma) and mBrB (B-4380, Sigma). The mBrB was stored at −20°C in the dark. Chemicals used in the eluents were methanol (HPLC-grade, EM Science) and acetic acid (AnalR 10001, BDH, Kilsyth, Vic). Water used was, unless otherwise stated, ultrapure (17 MΩ) water passed through a filter having a pore size of 0.2 μm. Buffered acetic acid solution was passed through the same type of filter, and degassed with helium before use, as was any ultrapure water for standard solutions. Solutions of dithionite, dithionate, and tetrathionate were prepared from the corresponding sodium salts ($Na_2S_2O_4$, S-1256, Sigma; $Na_2S_2O_6{\cdot}2H_2O$, 30135, BDH, Poole, UK; $Na_2S_4O_6{\cdot}2H_2O$, 72028, Fluka, Buchs, Switzerland).

### 2.2. Wastewater

The synthetic wastewater was obtained by passing an anaerobic sulfide-containing medium through a reactor in which green sulfur bacteria were oxidizing the sulfide. This medium was prepared according to the protocol for Pfennig's medium [21] for green

sulfur bacteria, except that: trace elements were added in ''solution 1'' and the $KH_2PO_4$ was autoclaved separately; the calcium concentration was reduced by 80%; the autoclaved ingredients except sulfide were combined and sparged with $CO_2$; and this sparged sulfide-free solution was mixed with a sulfide solution of predetermined concentration, in the volumetric ratio of 3.5:1, respectively, just ahead of the reactor. The sulfide-free and sulfide solutions were maintained under a nitrogen atmosphere. All water used in the medium was deionized, and all chemicals used were of AR-grade. A synthetic wastewater matrix used for the anion standards (Section 2.3.1) was made by combining the sulfide-free part of the medium with autoclaved, deionized water in the ratio of 3.5 parts to 1.

The industrial wastewater was collected in clean, sterile, 500-ml bottles from a hose connected to an anaerobic digester that was treating wastewater from a paper-recycling plant. The bottles were filled completely, capped, and kept on ice until transferred to a 4°C cold-room.

### *2.3. Quantification of sulfur-containing species*

The analysis protocols applied were essentially those described by Rethmeier et al., with some minor modifications that are described below. The protocol for sulfate quantification was not utilized owing to there being another, satisfactory technique already in use in our laboratory.

#### *2.3.1. Quantification of sulfide, thiosulfate and sulfite (reduced-S protocol)*

The 50-μl sample of wastewater was added directly to 110 μl of derivatizing agent comprising 50 μl of buffer (50 m*M* HEPES, 5 m*M* EDTA, pH 8.0), 50 μl of acetonitrile and 10 μl of mBrB (48 m*M* in acetonitrile). The methane sulfonic acid solution (100 μl of 65 m*M*) was added 30 min later. Prior to analysis solids were removed by centrifugation (10 000 *g* for 2 min). The derivatives were separated on an HPLC system comprising a Merck LiChrospher 60 RP-select B column (length, diameter and particle size of 125 mm, 4 mm and 5 μm, respectively), an autosampler (Waters WISP710B), an automated gradient controller (Waters 680), pumps (Waters 501), a fluorescence detector (Shimadzu RF-10AXL), and an integrator-printer (Waters 740 data module). The injection volume was 20 μl. Eluent flow-rate, eluent system (A, aqueous acetic acid solution, 0.25%, adjusted to pH of 4.0 with 10 *M* NaOH solution: B, 100% methanol), elution protocol (see below), and the excitation and detection wavelengths (380 and 480 nm) were as specified by Rethmeier et al., but the column was operated at ambient temperature (~23°C) instead of at 35°C. The column was equilibrated by the passage of solution B for at least 30 min, then solution A for 30 min, and then one whole gradient. The elution protocol was: 0–7 min, 12% B, isocratic; 7–15 min, 12–30% B, linear gradient; 15–19 min, 30% B, isocratic; 19–23 min, 30–50% B, linear gradient; 23–30 min, 50–100% B, linear gradient; 30–33 min, 100% B, isocratic; 33–33.1 min, 100–12% B, linear gradient; and 33.1–40 min, 12% B, isocratic (column regeneration).

System response was calibrated with derivatized standards, made by derivatizing freshly-prepared standard solutions of a single sulfur-containing anion made up in synthetic wastewater matrix. Owing to the restricted capability of the 740 data module to integrate certain overlapping peaks accurately, peak height was used as the measure of system response.

#### *2.3.2. Quantification of elemental sulfur*

Each chloroform extraction was performed in a 250-μl glass limited-volume-insert (Waters, cat. no. 15199), which was then centrifuged (7000 *g* for 15 min at 4°C) to provide a solid-free extract for analysis. The HPLC system comprised a Merck LiChrospher 100 RP-18 column (length, diameter and particle size of 125 mm, 4 mm and 5 μm, respectively), a manual injector (Waters U6K) fitted with a 50-μl sample loop, a pump (Waters 501), a column heater module (Waters), a temperature control module (Waters), a tunable absorbance detector (Waters 484), and an integrator-printer (Waters 740 data module). Column temperature, eluent flow-rate, and eluent composition were as specified in Rethmeier et al. Detection wavelength was either 263 or 297 nm (Section 3.4). The column was allowed to equilibrate by passage of the eluent for 1 h. Standard solutions of sulfur dissolved in chloroform were used

for calibrating the system. Spectral absorbances of standards and of chloroform extracts were measured as necessary with a scanning spectrophotometer (Hitachi U-3000), operated with a slit width of 2.0 nm. The solution, held in a quartz cuvette, was scanned at a rate of 300 nm/min. The matrix absorbance was taken to be zero.

### *2.4. Procedures for assessing recoveries and the precision of the analysis protocols*

The procedures used for establishing recoveries and the precision of the reduced-S analysis protocol are set out below. Sulfur concentrations in our wastewater samples were below the limit of detection (LOD), so meaningful estimates of precision could not be obtained for the sulfur protocol.

Each precision study entailed the analysis of at least six replicate samples, and the calculation of the SD of the results. To quantify the random variation arising throughout the process beginning with sampling, the replicates were separate derivatizations of a common wastewater sample (termed wastewater replicates). To estimate the variation entering just the chromatographic process, the replicates were drawn from a common pool of derivatized wastewater (called analysis replicates).

Owing to the rapid reaction between oxygen and both sulfite and sulfide [12] the procedure for assessing recovery had to be augmented with measures to limit the exposure of both wastewater and spiking solutions to air. Spiking solutions were prepared in helium-sparged, ultrapure water and were used within 30 min of their preparation. In the studies of sulfide, the spikes were drawn directly from the volumetric flask in which the spiking solution had been prepared. The procedure for spiking was: to pipette, gently and with the pipette tip submerged, 1800 (2000, in one study) μl of wastewater into a 2-ml centrifuge tube; to add the known amount of analyte (a volume of between 0 and 200 μl) keeping the tip submerged; to close the tube and to invert it twice, gently, so as to mix its contents; and to draw and add the sample to the derivatizing agent without further delay. The synthetic wastewater was drawn directly from the reactor, and the industrial wastewater from the sample-collection bottle, while the contents were being gently stirred. So as to detect any shift in background concentration, the first and the last — and often the median — recovery samples were prepared according to the protocol, but without a spike. The observed concentrations were plotted against the corresponding expected concentrations, and the data points were fitted with a straight line by the method of least squares. The slope of the line was taken to be the recovery.

## 3. Results and discussion

### *3.1. Calibrations*

The characteristics of typical full-suite calibration curves, each obtained by a linear regression of the responses to a suite of different derivatized standards analysed consecutively, are summarized in Table 1. These straight lines fit the data very closely, as had been found by Rethmeier et al. [14] (matrix unspecified) and Zopfi et al. [16] (seawater and ultrapure water). The excellent linearity showed that the peak height was a satisfactory measure of response. The full-suite calibration data for sulfide, thiosulfate and sulfur were consistently well-fitted by straight lines, whereas some of the sulfite calibration data were anomalous. Responses to successive injections

Table 1
Characteristics of the typical full-suite calibration curves

| Analyte | Concentration range (μ*M*) | Slope (units/μ*M*) | Response-axis intercept: slope (μ*M*) | $R^2$ |
|---|---|---|---|---|
| Sulfide | 0–1070 | 689 | 4.5 | 0.9998 |
| Thiosulfate | 0–29 | 657 | 0.04 | 0.9992 |
| Sulfite | 0–50 | 682 | −0.9 | 0.9984 |
| Sulfur | 0–125 | 398 | −1.8 | 0.9994 |

of the same derivatized sulfite standard could differ considerably.

During a 13-month period in which 730 samples were run, 83 derivatized sulfide standards were analysed. The 83 responses are plotted against the corresponding sulfide concentrations (Fig. 1a), and the points are well-fitted by a straight line passing through the origin. The responses to derivatized thiosulfate standards from this period were similarly well-fitted (data not shown), but the responses to derivatized sulfite standards, plotted in Fig. 1b, were not. This erratic pattern of response to the sulfite derivative, which is consistent with the occurrence of anomalous sulfite calibration data, does not seem to have been caused by malfunction of the HPLC system, insufficient column equilibration, or systematic errors in the compositions of the standards.

### 3.2. Recovery

Recovery studies were carried out with both synthetic and industrial wastewater. Three studies concerned sulfide, whereas the recoveries of sulfite and thiosulfate were each studied once. Results of these studies are set out in Table 2. The recoveries of sulfide in all matrices and of thiosulfate lay between 99 and 103%. The recovery of sulfite, 98%, should be interpreted cautiously given the variation of the sulfite-derivative response factor.

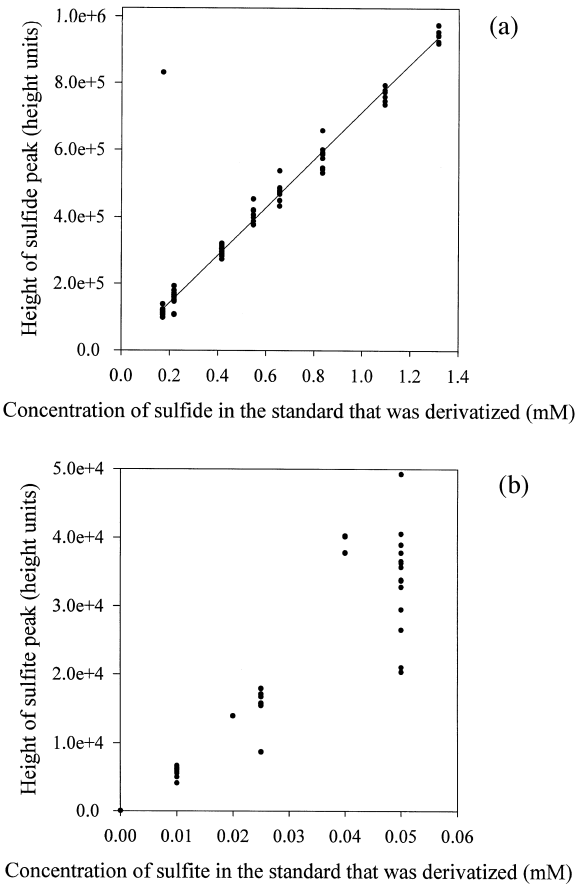


Fig. 1. Long-term system responses to derivatized standards of sulfide and sulfite.

### 3.3. Precision studies

Six investigations were carried out into the precision of the protocol for reduced-S compounds, and five of these involved wastewater replicates. The results of all precision studies are presented in Table 3. The fourth and fifth wastewater replicate studies were conducted with the samples used in the third, but after a further 8 and 13 months of storage, respectively. As observed by other workers [15,17] storage time had practically no effect on the average inferred concentrations of thiosulfate and sulfide. The mean inferred sulfite concentrations did vary, however, probably because, in these replicates, the sulfite responses were very close to the minimum recognized by the integrator. The RSDs for sulfide measurements were similar (6.0, 5.2 and 7.0%), but those for thiosulfate measurements were not (1.5, 1.6 and 9.0%). A more comprehensive study would be needed to establish whether the precision of thiosulfate measurements is truly affected by storage, however.

Some of the precision data reported by other workers are set out in Table 4. Their tests and ours, when conducted with similar types of replicates, produced similar RSDs. The SD estimated from the unweighted regression of the recovery data (the error sum of squares divided by the number of degrees of freedom, all to the power of one half [22]) is a useful check of the SD obtained from the replicate studies. The two methods yielded similar SDs if the concentration of the analyte in the replicate study was similar to the mid-range concentration in the recovery study, e.g. in the first and third sulfide

Table 2
Results and details of recovery studies

| Species and study number | Matrix (type of wastewater) | Conc. range in the study (μ*M*) | Number of samples analysed | Gradient of regression line (recovery)± SD of gradient | Vertical-axis intercept of regression line ±SD of intercept (μ*M*) | Standard deviation (μ*M*)[a] | Standard deviation as % of mid-range |
|---|---|---|---|---|---|---|---|
| Sulfide (1) | Synthetic | 510–990 | 6 | 1.02±0.08 | −20±56 | 34 | 4.5 |
| Sulfide (2) | Synthetic | 82–620 | 6 | 1.03±0.05 | −3±19 | 26 | 7.3 |
| Sulfide (3) | Industrial | 600–980 | 9 | 1.02±0.07 | −26±51 | 29 | 3.6 |
| Thiosulfate | Synthetic | 2.9–37 | 7 | 0.99±0.01 | −0.05±0.24 | 0.41 | 2.1 |
| Sulfite | Synthetic | 1.4–85 | 7 | 0.98±0.06 | −0.6±2.7 | 4.9 | 11 |

[a] Defined in text.

Table 3
Results and details of replicate tests

| Nature of replicates and study number | Number of replicates | Sulfite | | Thiosulfate | | Sulfide | |
|---|---|---|---|---|---|---|---|
| | | Conc. (μ*M*) | RSD (%) | Conc. (μ*M*) | RSD (%) | Conc. (μ*M*) | RSD (%) |
| Analysis | 10 | 76 | 2.1 | 61 | 1.7 | 570 | 3.5 |
| Wastewater (1) | 8 | ND[a] | ND | ND | ND | 880 | 4.1 |
| Wastewater (2) | 6 | 11 | 19 | 46 | 6.6 | 340 | 7.7 |
| Wastewater (3) | 7 | ND | ND | 61 | 1.5 | 380 | 6.0 |
| Wastewater (4) | 7 | 3.2 | 13 | 64 | 1.6 | 380 | 5.2 |
| Wastewater (5) | 7 | ND | ND | 64 | 9.0 | 370 | 7.0 |

[a] Not detected.

recovery studies and the first wastewater replicate study. If these concentrations were dissimilar, as for thiosulfate and sulfite, the RSDs were, nevertheless, similar.

### *3.4. Recognition and attribution of peaks*

Typical reduced-S chromatograms for a matrix blank and a derivatized wastewater sample are included in Fig. 2a,b. The attribution of each peak is indicated. The scale was set so that the detail of the smallest of the reduced-S peaks would be clear. From a random selection of 206 of the 970 chromatograms obtained to date with the system, the arithmetic mean and SD of the retention time were determined to be, respectively, 3.19 and 0.18 min for the sulfite derivative ($n=119$), 7.12 and 0.12 min for the thiosulfate derivative ($n=82$), and 26.56 and 0.08 min for the sulfide derivative ($n=206$).

Rethmeier et al. had tested their protocol down to

Table 4
Precision data reported by other workers

| Ref. | Sulfite | | Thiosulfate | | Sulfide | | Matrix |
|---|---|---|---|---|---|---|---|
| | Conc. (μ*M*) | RSD (%) | Conc. (μ*M*) | RSD (%) | Conc. (μ*M*) | RSD (%) | |
| [15] | 100 | 2.5 | 100 | 1.1 | 100 | 1.1 | Seawater |
| [15] | 100 | 4.6 | 100 | 3.5 | 100 | 4.7 | Seawater |
| [15] | NR[a] | NR | 100 | 4.4 | 100 | 5.0 | Coelomic fluid |
| [16] | 10 | 3 | 10 | 3 | NR | NR | Milli-Q or seawater |

[a] Not reported.

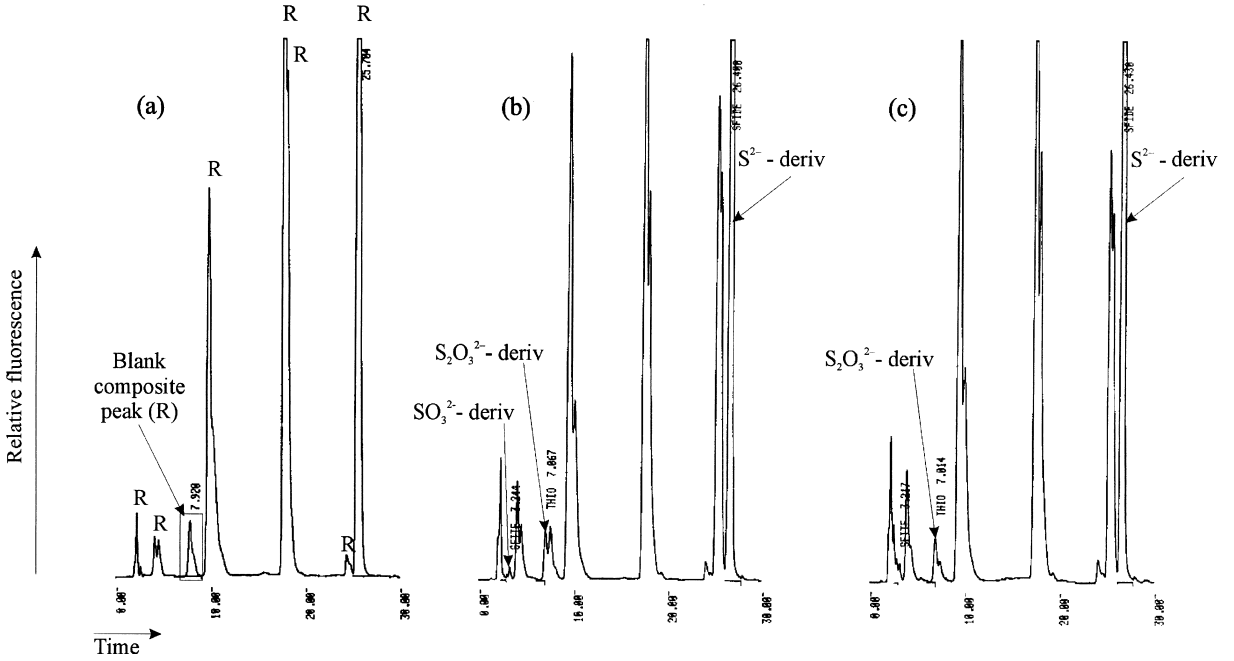


Fig. 2. Typical chromatograms (reduced-S protocol) obtained from: (a) derivatized matrix to which MSA solutin was added; (b) derivatized wastewater to which MSA solution was added; (c) derivatized wastewater to which $H_2O$ was added instead of MSA solution. Estimated concentrations: sulfite (2.5 μ*M*), thiosulfate (14.9 μ*M*) and sulfide (602 μ*M*). Peaks present in the reagent blanks are indicated with the symbol ''R''.

levels of 5, 1 and 5 μ*M*, respectively, for sulfite, thiosulfate and sulfide. As the method is far more sensitive than needed for wastewater studies, we did not attempt a rigorous evaluation of the LODs for sulfide and sulfite. The lower limits tested by Rethmeier et al. were achievable with the wastewater matrices, but occasionally the background would become noisy 20 min after sample injection, and remain so until the end of the run, causing the inferred sulfide concentration to be overestimated by up to 15 μ*M*. We were unable to elucidate the origins of this noise, which had not occurred with any other separation protocol tried on the same equipment.

It is clear from Fig. 2b that the thiosulfate derivative (''$S_2O_3^{2-}$-deriv'' in the figure) is not well separated from other components, whose elution results in the blank composite peak. The height of this peak is greatly reduced if methane sulfonic acid (MSA) solution is omitted (Fig. 2c). Attempts to eliminate this peak by changing the source of the MSA and the ultrapure water used for its dilution were unsuccessful. So were our attempts to improve the separation of the peaks by altering the starting composition of the first gradient step (95:5, 80:20 and 84:16 instead of 88:12) and the form of the gradient evolution (linear, non-linear). To assess the effect of MSA solution on the inferred thiosulfate concentration, wastewater duplicates, one of which received 110 μl of ultrapure water instead of the 110 μl of MSA solution, were prepared, and their chromatograms compared (Fig. 2b,c). The addition of MSA raised the inferred thiosulfate concentration by ~0.8 μ*M*, and prevented detection of thiosulfate at concentrations under 0.8 μ*M*. The omission of the MSA solution lowered the LOD for thiosulfate, and had no noticeable effect on the sulfide-derivative response, but it did make the peaks eluting in the first 5 min after injection more complex (Fig. 2c).

Typical elemental-S chromatograms corresponding to a standard solution of sulfur prepared in chloroform, and to a chloroform extract of a derivatized sample, spiked with a sulfur standard, are presented in Fig. 3a,b. The matrix and the sulfur peaks are indicated. Within any particular run, the SD of the

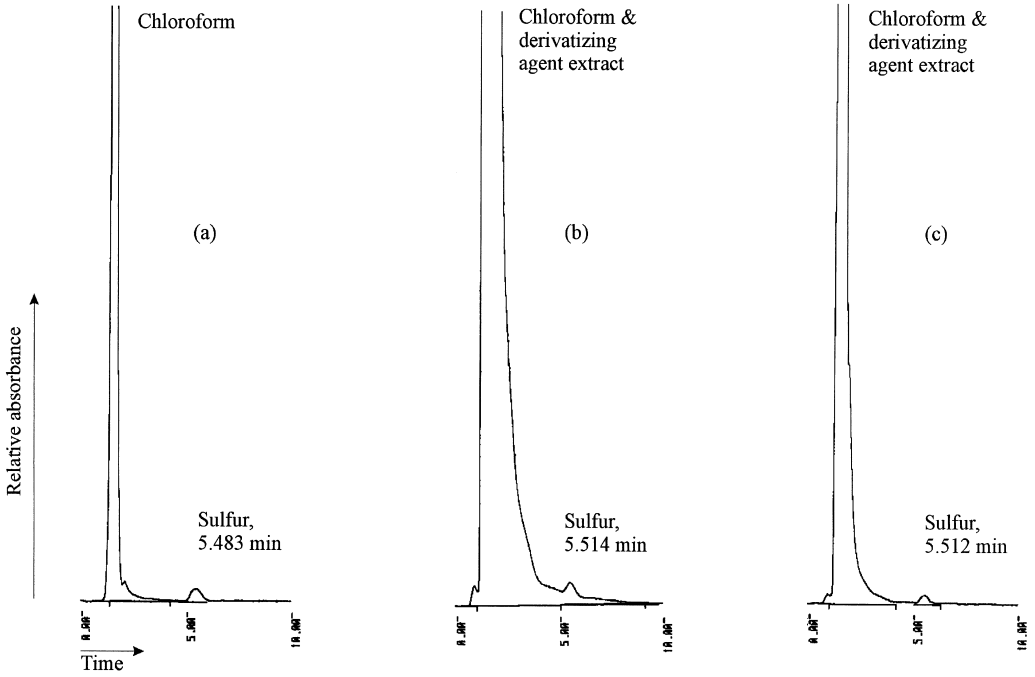


Fig. 3. Chromatograms (elemental sulfur protocol) illustrating the influence of detection wavelength on peak distinctness. (a) Sulfur in chloroform, elution detected at 263 nm; (b) sulfur-standard-spiked chloroform-extract of a derivatized sample, elution detected at 263 nm; and (c), as for (b) but with elution detected at 297 nm. The sulfur concentration was 0.2 mg/l in all three cases.

retention time was less than 0.1 min. The protocol enabled detection of sulfur in the wastewater at concentrations above 33 μ*M*. As can be seen in Fig. 3b, baseline separation was not observed in the chromatograms for the samples that contained components of the derivatizing agent. We tried to minimize the tailing effect of the derivatizing agent extract by selecting a detection wavelength that would suppress this peak substantially while still allowing the sulfur to be recognized. The spectral absorbances of the chloroform extract of derivatizing agent, and of sulfur standards prepared in chloroform were determined over the wavelength range from 250 to 400 nm (Fig. 4). The spectral absorbance of sulfur relative to that of the extracted derivatizing agent, depicted in that figure, was found to rise with increasing wavelength to a maximum value at 297 nm. As expected, an increase in detection wavelength to 297 nm greatly improves the distinctness of the two peaks, but reduces the sulfur response by 40% (Fig. 3b,c). It also makes the response factor more sensitive to variations in the detection wavelength.

### *3.5. Response to other sulfur oxyanions*

The chromatograms obtained by subjecting a 30.6 μ*M* tetrathionate solution and a 41.2 μ*M* dithionate solution to the reduced-S protocol were not noticeably different from the chromatogram of a reagent blank. The chromatogram for a 51.1 μ*M* dithionite solution contained no unfamiliar peaks but featured what appeared to be a large sulfite-derivative peak and a small thiosulfate-derivative peak. This attribution of the peaks is consistent with reports that in basic aqueous solutions dithionite can decompose rapidly, yielding sulfite [23] and, sometimes, thiosulfate also [24]. Furthermore, the inferred concentration of sulfur as sulfite or thiosulfate (99.3 and 11.2 μ*M*, respectively) tallies well with the original

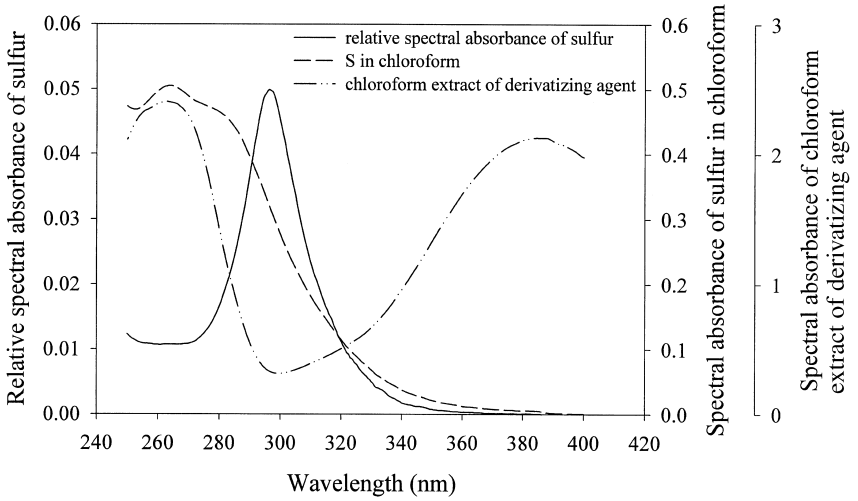


Fig. 4. Spectral absorbances of a solution of sulfur in chloroform and of a chloroform extract of derivatizing agent. The spectral absorbance of chloroform was the zero reference.

concentration of sulfur as dithionite (102.2 μ*M*) which was based on the stated 88% minimum purity.

### *3.6. Relevance of the method to wastewater studies*

The analysis of the industrial wastewater revealed the presence of thiosulfate at a concentration of 80 μ*M* (9 mg/l). This represented ~15% of the sulfur present as sulfide, a proportion that ought not to be overlooked. The ability to quantify thiosulfate — which is often not quantified in wastewater — as well as sulfide is therefore a useful feature of the protocol.

MBrB can react with compounds like carboxylates, amines and phosphates — albeit more slowly than with thiols. The resulting derivatives are fluorescent and apparently lead to sizeable peaks in the chromatogram if the substrates are present in high millimolar or molar concentrations [17,25]. As such concentrations of amines and various carboxylates may sometimes be anticipated in effluents from anaerobic digesters and in sewage, there may in such cases be interference from these compounds. These interferences might best be identified by applying the protocol to a sample prepared from wastewater that has been treated so as either to remove the analytes one wishes to quantify, or to render them unreactive with the bimane, such as by reacting them with 2-pyridyl disulfide (PDS) [13,15].

## 4. Conclusions

The protocol for quantifying sulfide, sulfite and thiosulfate was applied to the case of wastewater. The recoveries were within the range from 98 to 103%. The in-run RSDs, assessed with a common derivatized sample, were 2.1% for sulfite, 1.7% for thiosulfate and 3.5% for sulfide. The in-run RSDs, assessed with separate derivatizations of a common wastewater sample, lay in the ranges 13–19% for sulfite (two tests), 1.5–6.6% for thiosulfate (three tests), and 4.1–7.7% (three tests) for sulfide. The response factors for sulfide and thiosulfate derivatives varied little over a 13-month period, but that for the sulfite derivative varied appreciably. The retention times of the derivatives were steady, with that of the sulfide derivative having a SD of 0.08 min. The LOD for thiosulfate in wastewater was 0.8 μ*M* and the protocol caused thiosulfate concentrations to be overestimated by 0.8 μ*M*. The LOD for thiosulfate can be lowered by omitting the MSA

solution, but the detection and quantification of the sulfite derivative at very low levels will be made difficult owing to additional peak complexity.

Concerning the sulfur protocol, we found that the sulfur peak and the peak associated with extracted components of the derivatizing agent do overlap. The distinctness of the two peaks is greatly improved by detecting elution at 297 nm, but the response to sulfur is reduced by 40%.

The presence of either dithionate or tetrathionate in the sample did not produce an observable response in the chromatogram. The chromatogram obtained from a freshly-prepared dithionite solution featured a large peak believed to correspond to the sulfite derivative, and of a size that suggested that the dithionite had degraded almost completely into sulfite during the derivatization.

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