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LIQUID CHROMATOGRAPHIC DETERMINATION OF BROMIDE AFTER PRE-COLUMN DERIVATIZATION TO 4-BROMOACETANILIDE

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SUMMARY

A pre-column reaction of bromide with 2-iodosobenzoic acid and acetanilide and reversed-phase high-performance liquid chromatography of the 4-bromo-acetanilide formed has permitted sensitive, selective and accurate determination of bromide with the use of an UV detector. Employing 4-N-acetylaminotoluene as an internal standard, the method has been applied to determine bromide in natural and seawater, pharmaceutical formulations and blood. As low as 6 μg l^{-1} of bromide were assayed. Large amounts of ions such as chloride, nitrate, phosphate, ammonium sulphide, thiocyanate, sulphate, iodide, thiosulphate, cyanide and nitrite can be tolerated. The method is rapid, simple and precise, and has a limit of detection of 0.2 ng of bromide.

INTRODUCTION

Bromide may occur in varying amounts in some fresh water streams due to industrial discharges or in well water and ground water supplies in coastal areas as a result of seawater intrusion. To determine possible contamination or the treatment level of bromide-containing biocides, there is a need for a simple and rapid procedure for the assay of trace amounts of bromide. Bromide determination in blood may also be required in the instances of intoxication from overdoses of bromide-containing sedatives.

Many different methods are available for determining bromide. Titrimetric procedures are based on oxidation[1-5] or silver(I) salt formation[6,7] and are subject to interference by the procedure of several other anions. Spectrophotometric procedures involve oxidation to bromine which is converted for its measurement either into triiodide ion by reaction with iodide[8], or to eosin[9] and bromophenol blue[10-12] by reaction with fluorescein and phenol red respectively. The oxidants used for this purpose include permanganate[8], peroxymonosulphate[10] and chloramine T[11,12]. The chloramine T concentration severely affects the absorbance of bromophenol blue and a secondary reaction with phenol red requires destruction of the excess of reagent or a very short reaction time. Though the peroxymonosulphate method is free from these shortcomings, a positive interference is caused by chloride and a negative interference

by ammonium ion. Anion-exchange minicolumns included a flow injection manifold have been applied to the spectrophotometric determination of anions (including bromide) based on displacement of an equivalent amount of thiocyanate and its evaluation as the iron(III) complex[13]. In another procedure, bromide was enriched on an ion exchanger, eluted with perchlorate, oxidized by peroxydisulphate to bromate, which was subsequently treated with iodide and the absorbance of the triiodide ion formed was measured[14,15]. This method has been adapted to flow injection analysis[16]. Ion chromatography with the use of a concentrator column is sometimes preferred over spectrophotometry[17–19]. Other methods are based on anion exchange[20–23], ion-pair formation[24,25] and liquid chromatography with UV detection at 190–200 nm[26,27].

The application of high-performance liquid chromatography (HPLC) to the analysis of inorganic anions with pre-column derivatization reaction and their sensitive UV detection is a new and as yet relatively unexplored field. The only report available is ostensibly that of Moss and Stephen[28] which concerned conversion of halide into phenylmercury(II) halide with the use of benzeneboronic acid and mercury(II) nitrate. After extraction into chloroform, the phenylmercury halides were determined by reversed-phase HPLC and detection at 220 nm. Improved separations and quantitative recoveries were obtained with the use of 4-bromobenzeneboronic acid (detection at 230 nm). However, the method may not be suitably disposed to analyse samples, *e.g.*, of seawater, which have one halide in very large excess over others. The following method is proposed.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Shimadzu LC-5A constant flow solvent delivery pump, a manual loop injector, a Zorbax C_{18} reversed-phase column (particle diameter 5 μm; 25 cm × 4.6 mm I.D.) and a Shimadzu C-R2AX integrator fitted with a printer-plotter. The peak area was used for quantitation.

Materials

2-Iodosobenzoic acid was synthesized by a modification of the method of Chinard and Hellerman[25].

In a 5-l Pyrex round-bottomed flask, equipped with a mechanical stirrer, 25 g of potassium permanganate were dissolved in 1 l of water. To this was added a solution of 45 ml of concentrated sulphuric acid in about 100 ml of water, followed by a slurry of 50 g of 2-iodobenzoic acid in 100 ml of water. The mixture was stirred, 500 ml of warm water were added and the solution was boiled for 15 min. About 500 ml more of boiling water were added and boiling was continued for 10 min. The hot solution was filtered. On cooling the filtrate afforded 42 g (80% yield) of 2-iodosobenzoic acid which was collected and recrystallized from hot water. The product was 99.8% pure as found by iodometric determination[29].

Acetanilide, 4-N-acetylaminotoluene and 4-bromoacetanilide were synthesized and purified by repeated recrystallization by using the reported methods[30].

A mixture of acetanilide and 2-iodosobenzoic acid was prepared by dissolving 150 mg of 2-iodosobenzoic acid and 100 mg of acetanilide in 100 ml of methanol in

a standard flask. It was filtered through a 0.45-μm membrane filter. A sulphuric acid solution was prepared by diluting 1.2 ml of analytical reagent grade concentrated sulphuric acid to 100 ml in methanol.

The mobile phase was prepared by mixing methanol and water, 65:35 (v/v).

Standards

Potassium bromide solution was prepared by dissolving 223.1 mg of AnalaR-grade potassium bromide (BDH) in 100 ml of water in a standard flask, and diluting 2 ml of this solution to 100 ml in a standard flask. This solution contained 30 μg ml^{-1} of bromide.

4-N-Acetylaminotoluene (N-acetyl-4-toluidine) as internal standard contained 25 mg of purified substance in 250 ml of methanol in a standard flask.

Procedures

Determination of bromide in standard solutions. A 200–1000-μl aliquot of a standard solution containing 6–30 μg of bromide was mixed with 200 μl of the internal standard solution of 4-N-acetylaminotoluene, 500 μl of the mixed reagent and 200 μl of sulphuric acid. The solution was diluted to 10 ml in mobile phase in a standard flask, shaken well for 1 min and a 10-μl aliquot was injected on the HPLC column. The solvent flow-rate was 1 ml min^{-1} and the column back pressure was approximated 50 kg cm^{-2}. The eluate was monitored by an UV detector set at 240 nm with a sensitivity of 0.04 a.u.f.s.

Determination of bromide in natural water. A 5-ml volume of filtered (0.45-μm membrane filter) natural water, containing 6–60 μg l^{-1} of bromide, was mixed with 20 μl of internal standard solution, 500 μl of the mixed reagent and 200 μl of sulphuric acid, and diluted to 10 ml in methanol in a standard flask. The solution was shaken well and an 100-μl aliquot was injected into the liquid chromatograph. The detector sensitivity was 0.005 a.u.f.s. and the other chromatographic conditions were as above.

Determination of bromide in seawater. A 50-ml volume of filtered (Whatman No. 42 paper) seawater was diluted to 100 ml in distilled water in a standard flask, and a 200–1000-μl portion of this solution was mixed with 200 μl of internal standard solution, 500 μl of mixed reagent and 200 μl of sulphuric acid, and made up to volume with mobile phase in a 10-ml standard flask. This solution was filtered through a 0.45-μm membrane filter and 10 μl of filtrate were injected in the HPLC column; the chromatographic conditions were as described before.

Determination of bromide in pharmaceutical injections. The contents of a known number of injection ampoules were mixed and diluted to known volume. A suitable portion was subjected to pre-column derivatization reaction and HPLC analysis as described for standard bromide solutions.

Determination of bromide in blood. A 2-ml volume of blood was mixed with 2 ml of water and 4 ml of acetonitrile in a 15-ml centrifuge tube and stirred for 10 min. The precipitated material was separated by centrifugation (*ca.* 5000 *g*) and the clear supernatant was transferred to another centrifuge tube. The solvent was evaporated to dryness at 50°C under a stream of nitrogen and the residue was reconstituted in mobile phase. The volume of mobile phase used depended on the anticipated concentration of bromide in the sample. A known aliquot of the reconstituted residue was subjected to pre-column derivatization and HPLC analysis as described for standard bromide solution.

RESULTS AND DISCUSSION

In the present work the determination of bromide is based on a sequence of pre-column reactions involving oxidation with 2-iodosobenzoic acid in an acidic medium to give bromine and the substitution reaction of bromine with acetanilide to form 4-bromoacetanilide.

$$2\ Br^- \ + \ 2\ H^+ \ + \ \text{[structure]} \longrightarrow Br_2 \ + \ \text{[structure]} \ + \ H_2O$$
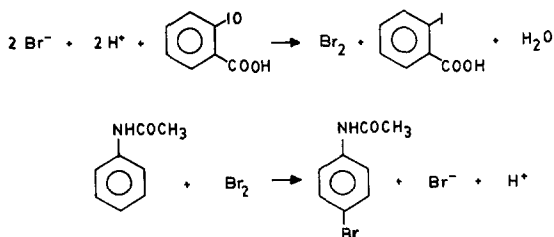
The bromide liberated from the substitution is again oxidized by 2-iodosobenzoic acid and this sequence of reactions continues until all the bromine is covalently bonded. Many compounds undergo substitution reaction with bromine but acetanilide was selected as a bromine scavenger because of its rapid reaction and the formation of a monobromo derivative as the sole product. The two positions *ortho* to the acetylamino group in acetanilide are sterically hindered and do not undergo electrophilic substitution. The 4-N-acetylaminotoluene internal standard does not react with bromine because the *para* position is already occupied by a methyl group. 2-Iodosobenzoic acid was chosen because it rapidly oxidizes bromide to bromine without any deleterious side reaction either with other reagents used or any organic matter present in the sample. The selectivity of 2-iodosobenzoic acid has already been demonstrated[5,31–34]. Its redox potential at 25°C was found to be 1.21 V at pH 1, 1.08 V at pH 2, 0.53 V at pH 4 and 0.48 V at pH 7[33].

Preliminary studies showed that the best separation of 4-bromoacetanilide from acetanilide, 2-iodo- and 2-iodosobenzoic acid was achieved on a C_{18} reversed-phase column by a mixture of methanol and distilled water (70:30, v/v). The internal standard was eluted near to the acetanilide peak but use of methanol–distilled water (65:35, v/v) avoided overlapping, and the analysis was complete in about 8 min (Fig. 1).

A response factor of 0.5930 for bromide (as 4-bromoacetanilide) *versus* 4-N-acetylaminotoluene was calculated by injecting pre-column reacted mixed standards in various concentration ratios. The bromide concentrations, C, were thus calculated from

$$C = 0.5930 \frac{A}{A_{IS}} \cdot C_{IS}$$

where A/A_{IS} is the ratio between the peak areas of 4-bromoacetanilide and the internal standard, and C_{IS} is the concentration of the internal standard.

The peaks in the HPLC chromatogram were identified by comparison of their HPLC retention times with those of the authentic substances under the same chromatographic conditions. These compounds of corresponding retention times had
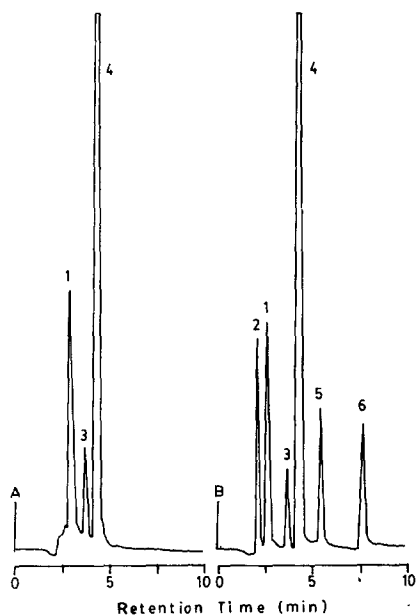
Fig. 1. Chromatograms of (A) reagents blank and (B) pre-column reacted bromide (31.07 ng) with the same reagents and 4-N-acetylaminotoluene (51.40 ng) used as the internal standard. Peaks: 1 = 2-iodosobenzoic acid; 2 = 2-iodobenzoic acid; 3 = impurity; 4 = acetanilide; 5 = 4-N-acetylaminotoluene and 6 = 4-bromoacetanilide. Mobile phase: methanol–water (65:35, v/v). Detection at 240 nm a.u.f.s. 0.04.

identical UV and IR spectra. Also, the agreement (within ± 1.5%) between the peak areas of equivalent molar masses of authentic 4-bromoacetanilide and of bromide (after derivatization to 4-bromoacetanilide) confirmed the quantitative nature of the pre-column reaction. The reaction mixture maintained the peak area for its 4-bromoacetanilide content for several days.

Calibration standard solutions of bromide were subjected to pre-column derivatization and the following quantities of bromide were injected: 6.4, 12.9, 19.4, 25.9, 32.4 and 38.9 ng; an UV detector (240 nm), sensitivity 0.04 a.u.f.s. A plot of peak

TABLE I

HPLC DETERMINATION OF BROMIDE IN PURE SOLUTIONS ($n = 6$)

R.S.D. = Relative standard deviation.

Solution No.	μg of bromide per 100 ml		
	Taken	Found	% R.S.D.
1	60.76	61.21	0.36
2	91.38	91.03	0.25
3	132.7	133.4	0.18
4	183.0	181.7	0.27
5	251.1	252.9	0.32
6	302.6	301.4	0.41

TABLE II

HPLC DETERMINATION OF TRACE LEVELS CONCENTRATION OF BROMIDE IN NATURAL WATER ($n = 5$)

The "standard composition, 0.005 N" contains (mequiv./l): Ca^{2+}, 3.175; Mg^{2+}, 0.870; Na^+, 0.785; K^+, 0.170; Cl^-, 0.5; SO_4^{2-}, 0.785 and HCO_3^-, 3.715[15].

Solution No.	Bromide added ($\mu g/1000$ ml)	% Recovery	% R.S.D.
1	6.1	95.4	3
2	9.9	96.3	2
3	20.4	96.8	2
4	31.7	97.7	1.5
5	42.0	98.9	0.7
6	50.1	99.7	0.3
7	62.3	100.8	0.4

area *versus* the amount (ng) injected was linear and gave a slope, intercept and correlation coefficient of 298, 12 and 0.9999 respectively.

Six solutions of bromide were prepared as the standards and analysed by the present method (Table I). The interference from a number of diverse ions was studied. Ions which do not vitiate the results when present up to 1000-fold w/w excess over bromide include chloride, nitrate, phosphate, calcium, barium, manganese, chromium(III), copper(II), zinc, cadmium, iron(III), cobalt(II), perchlorate, iodate and sulphate. Up to an 100-fold excess of ammonium ion can be tolerated. Reducing ions such as sulphide, thiocyanate, manganese(II), sulphite, thiosulphate, iron(II), thalium(I), iodide, cyanide and nitrite can be tolerated up to a 50-fold excess. Lead(II) and mercury(II) do not interfere when present up to a 20-fold excess over bromide. Sufficient excess of 2-iodosobenzoic acid should be added to compensate its consumption by any reducing agent or to speed up the oxidation of non-ionic bromide.

TABLE III

HPLC DETERMINATION OF BROMIDE IN SEAWATER ($n = 6$)

Sample*	mg of bromide/1000 ml				
	Taken	Present method	% R.S.D.	Comparison method	Ref.
Laboratory-made seawater:					
Sample No. 1[a]	25	25.83	0.3	24.36	10
Sample No. 2[b]	75	78.31	0.4	78.91	8
Sample No. 3[c]	150	152.5	0.2	153.7	14
Arabian Sea water	—	179.1	0.3	181.6	8

* The other substances present (mg/1000 ml) in the sample were (a) medium chloride (4000) and magnesium sulphate (200); (b) sodium chloride (2000), sodium hydrogencarbonate (500) and potassium chloride (200) and (c) sodium chloride (5000).

TABLE IV

HPLC ASSAY OF BROMIDE IN PHARMACEUTICAL INJECTIONS ($n = 6$)

Drug*	mg of total bromide/10 ml of injection					
	Label claim	Present method	% R.S.D.	Comparison method	% R.S.D.	Ref.
Calcibronat[a]	186.6	182.6	0.3	181.3	0.5	10
Calcibion[a]	186.6	195.0	0.3	195.7	0.4	14
Sedival[b]	310.8	301.4	0.4	302.8	0.5	8
Laboratory made						
Injection No. 1[c]	887.9	888.6	0.4	884.9	0.6	10
Injection No. 2[d]	788.7	790.2	0.4	790.7	0.5	6

    * Each 10 ml of injection included (a) calcium bromide/lactobionate (1240 mg); (b) sodium bromide (400 mg), chloral hydrate (200 mg) and phenobarbitone sodium (26.66 mg); (c) potassium bromide (500 mg), sodium bromide (500 mg) and ammonium bromide (200 mg) and (d) sodium bromide (600 mg), potassium bromide (200 mg), calcium bromide (7 mg), strontium bromide (3 mg), ammonium bromide (220 mg) and chloral hydrate (0.71 mg).

Any organic matter that also undergoes bromination, *e.g.*, phenol and aniline, interferes severely and should be removed, *e.g.*, by passing the test solution through a column packed with non-polar porous polystyrene–divinylbenzene resin (Amberlite XAD-2)[12].

    The present method has been applied to the determination of bromide in fresh water (at levels as low as 6 μg l^{-1}) and seawater. Seven laboratory-made synthetic fresh water samples of the relative ionic composition used previously[15] were analysed (Table II). Some laboratory made seawater samples and that collected from the Arabian Sea, Bombay, produced the results given in Table III. Due to a lower bromide content, a larger sample volume of river-water was taken for derivatization and diluted in methanol (instead of mobile phase as done in other analyses) to match the solvent composition approximately to that of the mobile phase. Results for the assay of bromide in pharmaceutical injections are given in Table IV, and those for blood samples doped with known amounts of bromide are given in Table V. In precision studies, the R.S.D. was determined by single injections of five or six samples

TABLE V

HPLC ASSAY OF BROMIDE IN BLOOD ($n = 5$)

Sample No.	μg of bromide per ml of blood		
	Added	Found by present method	% R.S.D.
1	3.18	3.12	0.5
2	10.81	10.89	0.3
3	16.36	16.20	0.3
4	21.74	21.82	0.3

TABLE VI

COMPARISON OF SENSITIVITY OF BROMIDE DETERMINATION BY DIVERSE METHODS

Serial No.	Method	Detector	LD*	Ref.
1	Ion chromatography; post-column reaction with chloramine T and 4,4'-bis(dimethylamino)-diphenylmethane	600 nm	15 ng	35
2	Liquid chromatography on Zipax SAX column	200 nm	10 ng	26
3	Reversed-phase ion-interaction chromatography	205–220 nm	24 ng	36
4	Liquid chromatography on aminopropyl bonded silica	214 nm	1 ng	37
5	Liquid chromatography on μBondapak NH$_2$	210 nm	10 ng	38
6	Ion-pair chromatography	205 nm	6 ppb	39
7	Ion-interaction liquid chromatography	205–220 nm	40 ng	40
8	Anion-exchange chromatography	Conductivity	1 ppm	41
9	Anion-exchange chromatography	Conductivity	20 ng	42
10	Ion-exchange chromatography	190 nm	1 ppb	27
11	Precolumn derivatization to 4-bromoacetanilide	240 nm	0.2 ng	Present work

* LD = Limit of detection; in ppb, the American billion (10^9) is meant.

derivatized separately. None of the samples of seawater, pharmaceutical injections and blood produced peaks closely eluted to those of the internal standard and 4-bromoacetanilide. The limit of detection is 0.2 ng of bromide (signal-to-noise ratio = 2).

It is clear that the proposed HPLC method for bromide involving precolumn derivatization to 4-bromoacetanilide is simple, rapid and precise, and free from interferences due to high level concentrations of diverse ions that adversely affect other methods. For the determination of bromide concentrations lower than 10 μg l^{-1} the present method should be a valuable asset. Table VI demonstrates the advantages of derivatization.

ACKNOWLEDGEMENT

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