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Isotachophoretic Analysis of Drugs. I. A Simultaneous Determination of Sulfite and Sulfate Ions[1]

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A rapid and precise method for the determination of sulfite ion by isotachophoresis was developed. The leading electrolyte used was 0.01 M L-histidine hydrochloride in 30% aqueous acetone (pH 4.0) and the terminating electrolyte was 0.01 M hexanoic acid (pH 3.5). This method also successfully detected sulfate, the oxidation product of sulfite, which may be formed in various pharmaceutical procedures including sterilization. The limits of detection were 0.2 mM as sulfite and 0.1 mM as sulfate. No pretreatment of the sample was required.

This procedure could be applied to the simultaneous determination of sulfite and sulfate and should be useful in pharmaceutical analysis.

Keywords——sulfite; sulfate determination; isotachophoresis; pharmaceutical analysis; antioxidant; pharmaceutical preparation

Sulfite salts, such as sodium bisulfite and sodium sulfite, are used as antioxidant and antibrowning agents in pharmaceutical preparations and their concentrations are often in the range of 0.01 to 1%.[2] Sulfite ion in liquid preparations is easily oxidized to sulfate by oxidants, or during formulation processes including sterilization. Therefore sulfate ion usually contaminates pharmaceutical preparations.

Many methods have been reported for the determination of sulfite ion, such as iodometric titration,[3] Conway and Monnier–Williams' microdiffusion analysis,[4] polarographic determination[5] and the pararosaniline method,[6] but few simple methods are available for the determination of sulfite and sulfate at the same time.

Recently it was reported that sulfite and sulfate ions were separated isotachophoretically by using chloride in aqueous 50% acetone as the leading electrolyte and hexanoic acid as the terminating electrolyte,[7] and this led us to develop the present isotachophoretic method. Extensive investigations were carried out to optimize the conditions, as has already been reported in part.[1]

Experimental

Apparatus——A Shimadzu IP-1B isotachophoretic analyzer was used to separate anions in a Teflon capillary tube, length 200 mm, inner diameter 0.5 mm. Detection of the ions was accomplished using a Shimadzu PGD-1, fitted with a potential gradient detector and a UV detector operating at 257 nm. Quantification of each sample was carried out by measuring zone lengths, which were calibrated from differential peaks by means of a digital integrator (Shimadzu ITG-4A). A solution of 0.01 M L-histidine hydrochloride in 30% aqueous acetone (pH 4.0) was used as the leading electrolyte, and 0.01 M hexanoic acid (pH 3.5) was used as the terminating electrolyte.

Procedure——A 5 μl aliquot of each sample was injected into the isotachophoresis apparatus. The migration current was maintained at 75 μA during the experiments. Chart speed was 10 cm/min.

Material——Various amino acid infusions were purchased: 5% ISPOL S (lot M105), 12% ISPOL (lot M165) and 3% ES POLYTAMIN (lot M101) from Daigo Nutritive Chemicals Co. Ltd., Osaka; MORIAMIN-2 (lot BD27A1), STRONG MORIAMIN S (lot JE-25A2) and STRONG MORIAMIN S-2 (lot KE18A1) from Morishita Pharmaceutical Co. Ltd., Osaka; 3% PROTEAMIN XT (lot 16004) from Tanabe Pharmaceutical Co. Ltd., Osaka and PANAMIN (lot 0691) from Otsuka Pharmaceutical Co. Ltd., Tokyo. Epinephrine injection, 0.1% (BOSMIN, lot 1597TCA), was purchased from Daiichi Pharmaceutical Co. Ltd., Tokyo,

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2% procaine hydrochloride injection (LOCAIN, lot 20914) from Fuso Pharmaceutical Co. Ltd., Osaka, and procaine hydrochloride (BANCAIN, lot IAA-32) from Banyu Pharmaceutical Co. Ltd., Tokyo. All other chemicals used were of guaranteed reagent grade.

Standard Solutions——Standard solutions A were prepared as follows: sodium sulfite was dissolved in distilled water to give six different concentrations between 0.2 and 12 mM, and the precise values were determined by iodometric titration. Standard solutions B were prepared as follows: accurately weighed portions of sodium sulfate were dissolved in distilled water to give five different concentrations between 0.1 and 10 mM.

Standard Mixtures——Standard mixtures of sulfite were prepared by mixing equal volumes of standard solution A and 4% procaine hydrochloride solution. Standard mixtures of sulfate were prepared by dissolving accurately weighed sodium sulfate in 2% procaine hydrochloride solution.

Results and Discussion

During electrophoresis in a capillary tube, ions form continuous zones having specific potential gradients in order of their mobility. The potential unit (P.U.) value, which is the ratio of the relative potential gradient value between leading and sample ions to that between leading and terminating ions, is expressed by the following equation proposed by Miyazaki and Katoh,[8]

$$\text{P. U. value} = \frac{PG_S - PG_L}{PG_T - PG_L}$$

where PG_S, PG_L and PG_T are the potential gradient of the sample ion, the potential gradient of the leading ion and the potential gradient of the terminating ion, respectively. Therefore identification of anion species can be carried out by comparison of P.U. values.

When aqueous 0.01 M L-histidine hydrochloride solution was used as a leading electrolyte, the difference of potential gradient between chloride and sulfate (P.U. value of sulfate: 0.055) was too small to be distinguished by a digital integrator. It was reported by Beckers and Everaerts[9] that the mobilities of ions in organic solvents or organic-water mixtures were fairly different from those in aqueous solvents with change of potential gradient. Several organic solvents such as methanol, acetone and dioxane were investigated as additives to the leading electrolyte, and acetone was found to be the most suitable to distinguish clearly between chloride and sulfate.

The effect of acetone in the leading electrolyte on the P.U. values of sulfite and sulfate ions is shown in Fig. 1. Increase of the acetone concentration in the leading electrolyte gave a larger P.U. value of sulfate ion. When the acetone concentration was raised above 30%, however, electric current breaks were frequently observed due to the generation of bubbles from the leading electrolyte, so approximately 30% acetone concentration in the leading electrolyte was found to be suitable for isotachophoresis.

The mobilities of the anions are also affected by the pH of the leading electrolyte.[10] The P.U. value of sulfite ion increased with increase of the pH in the range of 3.0 to 6.0, but that of sulfate ion was little affected (Fig. 2). On the basis of these results, a 0.01 M solution of L-histidine hydrochloride in 30% aqueous acetone (pH 4.0) and a 0.01 M solution of hexanoic acid (pH 3.5) were used as the leading and terminating electrolytes, respectively. No bubbles were generated in a Teflon tube under these conditions. The P.U. values of sulfite and sulfate ions were determined to be 0.46 and 0.10, respectively. An electropherogram illustrating the separation of sulfate ion from sulfite ion in this system is shown in Fig. 3.

Sulfite and sulfate ions in the standard solutions A and B were determined from their zone lengths by measuring the distances between the two differential peaks of the potential gradients with a digital integrator. The linear regression equations for calibration (concentration, x, *versus* retention time, y) were $y_i = 0.399x_i + 0.126$ (correlation coefficient of 0.999) for sulfite, and $y_{ii} = 0.609x_{ii} - 0.001$ (correlation coefficient of 1.000) for sulfate. The limits of detection were estimated to be 0.2 mM as sulfite and 0.1 mM as sulfate. The present method

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was demonstrated to be accurate enough for simultaneous quantitative determinations of sulfite and sulfate ions in pharmaceutical preparations.

The analytical results for sulfite and sulfate ions in standard mixtures showed a good correlation between the calculated and the found values for both ions, as shown in Table I.

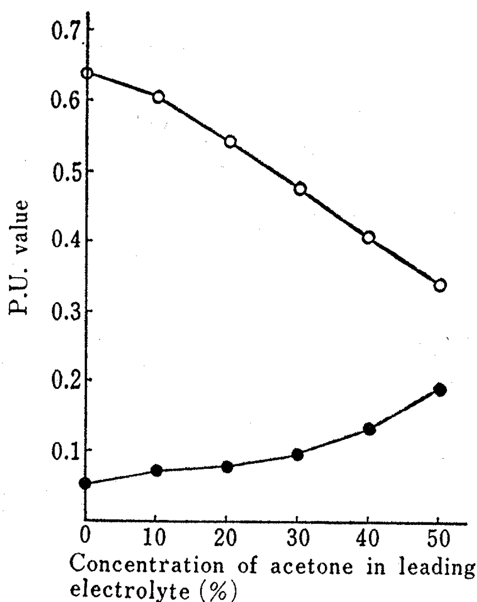

Fig. 1. Relationships between the P.U. Values of Sulfite and Sulfate and the Concentration of Acetone in the Leading Electrolyte

○, sulfite; ●, sulfate.
The leading electrolyte was 0.01 M L-histidine hydrochloride (pH 4.0) and terminating electrolyte was 0.01 M hexanoic acid (pH 3.5).

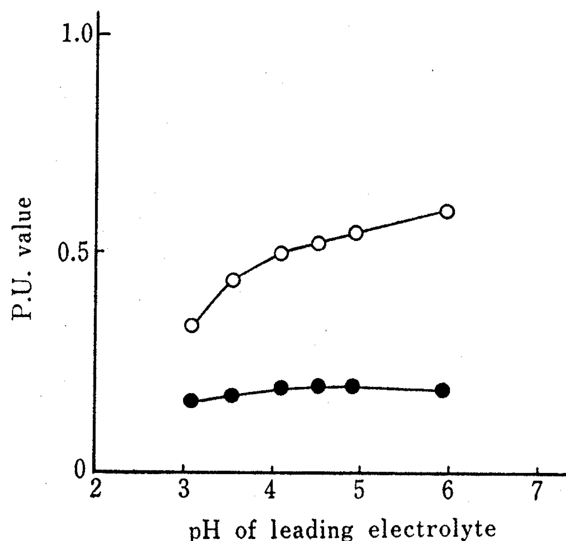

Fig. 2. Relationships between the P.U. Values of Sulfite and Sulfate and the pH of the Leading Electrolyte

○, sullfite; ●, sulfate.
The leading electrolyte was 0.01 M hydrochoric acid in 30% acetone and the terminating electrolyte was hexanoic acid (pH 3.5). The pH of the leading electrolyte was adjusted with L-histidine.

TABLE I. Recovery of Sulfite and Sulfate from Standard Mixtures[a] as determined by Isotachophoresis

Sulfite		Sulfate	
Added[b] (mM)	Found (mM)	Added (mM)	Found (mM)
3.69	3.86	0.50	0.48
4.51	4.50	1.00	0.95
8.34	8.57	2.00	2.00
11.78	11.86	5.00	5.10
		10.00	10.10

a) Standard mixtures contain 2% procaine hydrochloride.
b) Sulfite added was determined by iodometric titration.

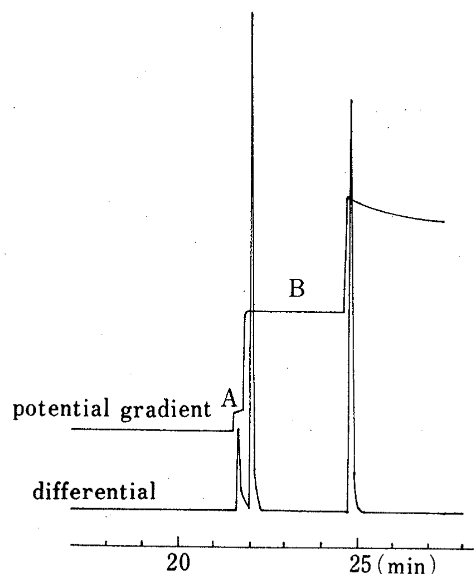

Fig. 3. Isotachopherogram for the Separation of Sulfite and Sulfate

Zone A, sulfate; zone B, sulfite.
The leading electrolyte was 0.01 M L-histidine hydrochloride (pH 4.0) and the terminating electrolyte was 0.01 M hexanoic acid (pH 3.5).

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The amounts of sulfite and sulfate in the commercial 0.1% epinephrine injections and 2% procaine hydrochloride injections were determined by the present method. Six samples taken from the same manufacturing lot number were examined. A 5 μl aliquot of the sample was injected directly into the isotachophoresis apparatus without pretreatment and analyzed. The determined values of sulfite and sulfate ions in these preparations are listed in Table II. The mean values of residual concentrations of sulfite in 0.1% epinephrine injection and 2% procaine hydrochloride injection were 4.15±0.04 mM and 8.45±0.11 mM, respectively. The variation coefficients of sulfite in 0.1% epinephrine injection and 2% procaine hydrochloride injection were calculated to be 0.96 and 1.30%, respectively. Since the initially added content of sulfite should be given by addition of the observed value of sulfate to that of sulfite, the initial contents of sulfite in 0.1% epinephrine injection and in 2% procaine hydrochloride injection could be estimated to be 4.50±0.11 mM and 8.77±0.10 mM, respectively. The initial sulfite values of both samples thus estimated by this method showed good reproducibility. The results indicated that more than 90% of the sulfite in these preparations remained intact.

TABLE II. Sulfite and Sulfate Contents in Commercial Injections[a] as determined by Isotachophoresis

Sample	n	Sulfite (mM)	Sulfate (mM)	Estimated initial sulfite[b] (mM)	C.V.[c] (%)
0.1% epinephrine injection	6	4.15±0.04	0.35±0.12	4.50±0.11	0.96
2% procaine hydrochroride injection	6	8.45±0.11	0.32±0.03	8.77±0.10	1.30
MORIAMIN-2	5	2.12±0.14	0.71±0.09	2.83±0.06	6.60

a) The same lot of commercial injection was tested in each case.
b) Initial content of sulfite was estimated by addition of the observed sulfate values to the observed sulfite value.
c) C.V.: coefficient of variation.

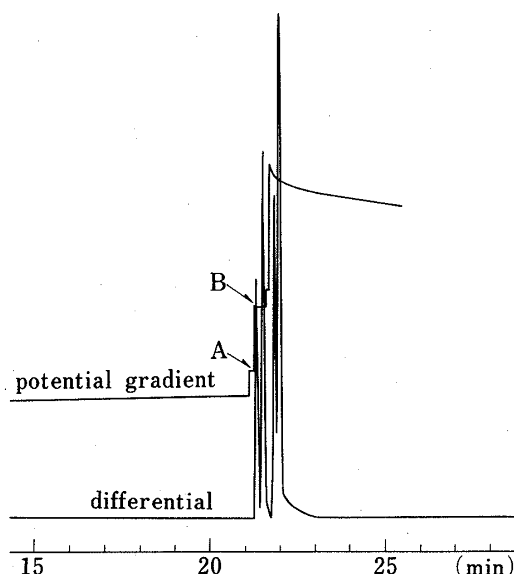

Fig. 4. Isotachopherogram for the Separation of Sulfite and Sulfate in Amino Acid Infusion

Zone A, sulfate: zone B, sulfite.
The amino acid infusion analysed was MORIAMIN-2. The leading electrolyte was 0.01 M L-histidine hydrochloride (pH 4.0) and the terminating electrolyte was 0.01 M hexanoic acid (pH 3.5).

TABLE III. Sulfite and Sulfate Contents in Commercial Amino Acid Infusions as determined by Isotachophoresis

Sample	Sulfite (mM)	Sulfate (mM)	Initial sulfite[a] (mM)	Residual amount of sulfite (%)
5% ISPOL S	1.78	0.51	2.29	77.7
12% ISPOL	1.33	0.31	1.64	81.1
3% ES POLYTAMIN	2.48	0.41	2.89	85.8
10% ES POLYTAMIN	2.38	0.28	2.66	89.5
STRONG MORIAMIN S	4.78	0.38	5.16	92.6
STRONG MORIAMIN S-2	3.05	0.61	3.66	83.3
3% PROTEAMIN XT	0.65	0.10	0.75	86.7
PANAMIN	1.48	0.23	1.71	86.5

a) Initial content of sulfite was estimated by addition of the observed sulfate values to the observed sulfite value.

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Sulfite and sulfate in commercial amino acid infusions were separated satisfactorily by this method. An isotachopherogram of MORIAMIN-2 is shown in Fig. 4. Amino acid and other coexistent anions in amino acid infusions were found not to interfere with the measurement of sulfite and sulfate. Five samples of the same commercial amino acid infusion, MORIAMIN-2, were then taken from the same manufacturing lot, and their values of sulfite and sulfate were determined by this method. The estimated initial content of sulfite and the coefficient of variation are shown in Table II. The amount of residual sulfite in the amino acid infusion was calculated to be approximately 75%.

Analytical results for sulfite and sulfate ions contained in the eight commercial amino acid infusions are shown in Table III;. Among these commercial preparations large differences in residual concentrations of sulfite were observed, and the initial sulfite contents added in the manufacturing procedure were also found to vary widely in the range of 0.75 to 5.16 mM.

As mentioned above, the present isotachophoretic method could be applied to the simultaneous determination of sulfite and sulfate ions by adding acetone to the leading mobile phase. The limits of detection were 0.2 mM as sulfite and 0.1 mM as sulfate. This detection method should be applicable to the determination of sulfite and sulfate in pharmaceutical preparations without pretreatment.

References and Notes

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