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Abstract

A new gas chromatographic method for the determination of sulfate was developed. In this method, sulfate was quantitatively converted to a volatile derivative, dimethyl sulfate, by a two-step procedure. First, sulfate was converted to silver sulfate by reaction with silver oxide, and then to dimethyl sulfate by reaction with methyl iodide. The derivative was analyzed by gas chromatography. Methyl methanesulfonate was used as an internal standard. The method was applied to the determination of total urinary sulfate. Phosphate and chloride ions, which interfered with the present method, were eliminated with the use of basic magnesium carbonate and an excess of silver oxide, respectively. Recovery was over 96% when 5 to 40 mumol/ml of sulfate was added to human urine samples.

KEYWORDS: gas chromatography, sulfate determination, dimethy1 sulfate, sulfuric acid, urinary sulfate

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A new gas chromatographic method for the determination of sulfate was developed. In this method, sulfate was quantitatively converted to a volatile derivative, dimethyl sulfate, by a two-step procedure. First, sulfate was converted to silver sulfate by reaction with silver oxide, and then to dimethyl sulfate by reaction with methyl iodide. The derivative was analyzed by gas chromatography. Methyl methanesulfonate was used as an internal standard. The method was applied to the determination of total urinary sulfate. Phosphate and chloride ions, which interfered with the present method, were eliminated with the use of basic magnesium carbonate and an excess of silver oxide, respectively. Recovery was over 96% when 5 to 40 μmol/ml of sulfate was added to human urine samples.

Key words : **gas chromatography, sulfate determination, dimethyl sulfate, sulfuric acid, urinary sulfate**

Sulfate is excreted in the urine of higher animals as the major end product of sulfur metabolism (1). Many methods have been described for the determination of sulfate in biological materials. However, the gas chromatographic determination of sulfuric acid has not been studied extensively because of the lack of a derivative which is suitable for gas chromatographic analysis. Butts and Rainey (2) detected trimethylsilyl sulfate by gas chromatographic analysis of inorganic oxyanions, but the compound decomposed rapidly. Mawhinney (3) reported an improved procedure for separating a stable derivative, *t*-butyldimethylsilyl sulfate. However, this method has not been used for the analysis of sulfate in biological materials because it results in the production of many volatile derivatives. Thus, the quantitative analysis of sulfuric acid in biological materials by gas chromatography has been difficult.

In the present paper, we describe a new procedure for the gas chromatographic determination of inorganic sulfate after its conversion to dimethyl sulfate and report the application of the method to the determination of sulfate in human urine.

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Materials and Methods

Reagents. Magnesium hydroxide carbonate (GR) was obtained from E. Merck, Darmastadt, West Germany. Methyl methanesulfonate (purity,

99%) was purchased from Aldrich Chemical Company, Milwaukee, WI, USA. All other chemicals used were of analytical grade and were purchased from Wako Pure Chemical Ind., Ltd., Osaka, Japan. Standard sulfate solutions were prepared from potassium sulfate.

Instruments. A Shimadzu gas chromatograph (4CM, Shimadzu Seisakusyo, Ltd., Kyoto, Japan) equipped with a hydrogen flame ionization detector was used. A column of 2% OV-17 and 0.1% Unisole 400 on Gas Chrom Q (80-100 mesh) (Gasukuro Kogyo, Ltd., Tokyo, Japan) packed in a silanized glass tube (3 mm i.d.×2 m) was used. The column was conditioned at 250°C for 28 h with nitrogen gas at a flow rate of 30 ml/min. The operating conditions were as follows: oven temperature, 60°C; injection temperature, 120°C; flow rate of nitrogen gas as the carrier gas, 55 ml/min.

The identification of dimethyl sulfate was performed using a gas chromatograph-mass spectrometer (GC-MS) (Shimadzu 9020-FD, Shimadzu Seisakusyo, Ltd.). The GC-MS was equipped with the same type of column as described above and was operated to obtain an electron impact mass spectrum under the following conditions: trap current, 60 μA; ionizing voltage, 70 eV; accelerating voltage, 3.0 kV; ion source temperature, 250°C.

Derivatization procedure. Dimethyl sulfate was formed from sulfuric acid according to the scheme shown in Fig. 1. One ml of a solution containing 2-40 μmol of potassium sulfate was applied to a column of Dowex 50 W, ×4 (200-400 mesh, 0.7×8 cm, H^+ form) in order to remove potassium ion. The column was washed with deionized water, and the initial 10 ml of the effluent was collected. The solution was placed in a test tube with a Teflon-lined screw cap, and 0.1 g of silver oxide was added. The mixture was shaken vigorously using a mechanical shaker at room temperature for 90 min. The mixture was then centrifuged at 1200×*g* for 5 min. Three ml of the resulting supernatant was placed in a test tube and evaporated to dryness using a centrifugal evaporator at 40°C under a reduced pressure. Five-tenths ml of methyl iodide and 0.1 g of sea sand were added to the test tube, and the mixture was shaken vigorously for 1 min. Then the mixture was incubated at 55°C for 1 h. After shaking the mixture vigorously for 1 min, it was incubated for one additional hour. After the above derivatization reaction, 0.5 ml of 6 mM methyl methanesulfonate in chloroform was added as an internal standard, and 6 μl of the mixture was analyzed by gas chromatography under the conditions described above.

Determination of sulfate was performed by measuring the peak area on the chromatogram.

Determination of urinary sulfate. Major anions other than sulfate ion present in urine are chloride and phosphate ions. These two anions were removed as silver chloride and magnesium phosphate as follows. To 2.0 ml of human urine placed in a test tube with a Teflon-lined screw cap, 0.2 ml of 2 M hydrogen chloride was added and the mixture was heated at 80°C for 2 h in order to hydrolyze ethereal sulfate. After neutralization with 2 M sodium hydroxide, the volume was adjusted to 2.5 ml with water. Basic magnesium carbonate (0.25 g, solid) was added, and the mixture was heated in a boiling water bath for 3 min. Then it was chilled and centrifuged at 1200×*g* for 5 min. One ml of the resulting supernatant was applied to a column of Dowex 50 W, × 4 (200-400 mesh, 0.7×8 cm, H^+ form), and the column was washed with water. Ten ml of the initial effluent was collected and shaken with 0.2 g of silver oxide as above for 30 min. After the addition of 0.1 g of silver oxide, the mixture was shaken for one additional hour. The mixture was centrifuged, and the resulting supernatant was processed as above for the determination of authentic sulfuric acid.

Creatinine was determined by the Jaffe reaction (4).

Results

Derivatization and recovery of sulfuric acid. Sulfuric acid did not react with methyl iodide under usual conditions. Therefore, sulfuric acid was converted to dimethyl sulfate in two steps as shown in Fig. 1. The formation of dimethyl sulfate was confirmed by comparison of the mass spectrum with that of authentic dimethyl sulfate (data not shown). As shown in Table 1, the yield of derivatization was over 98%.

Methyl methanesulfonate was used as an internal standard in the present study. Fig. 2A illustrates a chromatogram of dimethyl sulfate and methyl methanesulfonate. Both peaks show no tailing and are separated well from each other.

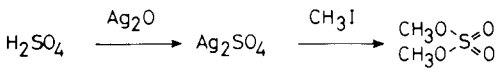

Fig. 1 Scheme of the conversion of sulfate to dimethyl sulfate.

Table 1 Yield of dimethyl sulfate formed from potassium sulfate[a]

Sulfate added (μmol/ml)	Dimethyl sulfate formed[b] (μmol/ml)	Yield[b] (%)
2.0	1.97 ± 0.13	98.3 ± 6.3
5.0	5.06 ± 0.18	101.1 ± 3.6
10.0	9.88 ± 0.39	98.8 ± 3.9
20.0	20.10 ± 0.50	100.5 ± 2.5
40.0	40.16 ± 1.26	100.4 ± 3.1

a: Sulfate was converted to dimenthyl sulfate, and the latter was determined by gas chromatography. Experimental details are described under Materials and Methods.

b: Mean ± SD of 5 determinations.

Effect of anions on the recovery of sulfate as dimethyl sulfate. The effect of chloride and phosphate ions on the recovery of sulfate as dimethyl sulfate was studied. Chloride ion inhibited the formation of silver sulfate because the solubility of silver chloride is lower than that of silver sulfate. However, chloride ion was effectively eliminated with the use of an excess amount of silver oxide.

As shown in Table 2, phosphate ion at a concentration of 150 μmol/ml in the sample solution reduced the recovery of dimethyl sulfate by about 13%. However, the interference was eliminated by treating the sample with basic magnesium carbonate (5).

Methanesulfonate at the same concentration as that of sulfate interfered with the derivatization of sulfate by 10%, indicating

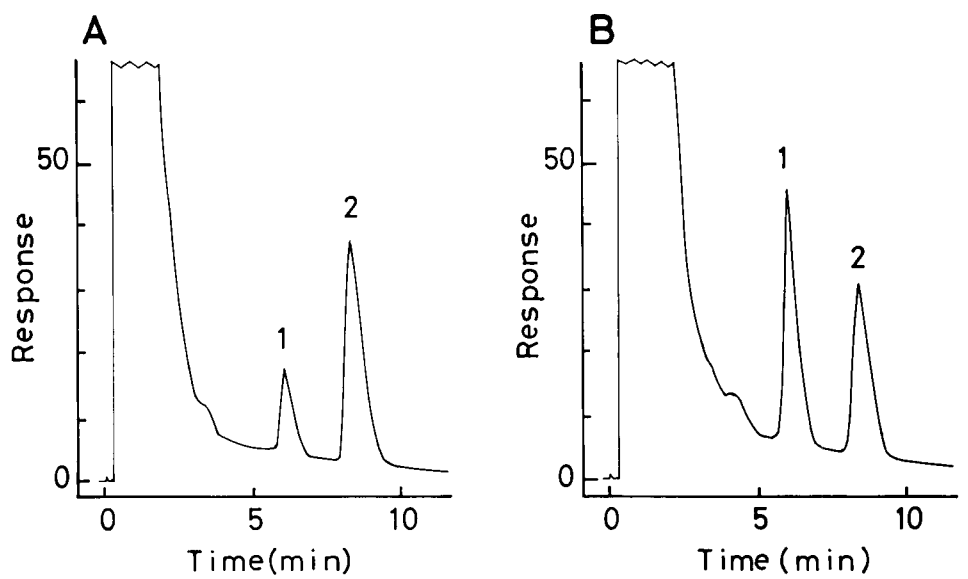

Fig. 2 Gas chromatograms of dimethyl sulfate (derived from sulfate) and methyl methanesulfonate (internal standard). Sulfate was converted to dimethyl sulfate by reaction with silver oxide and then with methyl iodide. Dimethyl sulfate was analyzed by gas chromatography. Chromatogram A was obtained with a solution containing 10 μmol/ml of potassium sulfate and B with human urine. Experimental details are described under Materials and Methods. Peaks: 1, dimethyl sulfate; 2, internal standard.

that methanesulfonate should not be added before the derivatization procedure.

Determination of urinary sulfate. The present method was applied to human urine, and a chromatogram obtained was illustrated in Fig. 2B. Peaks of dimethyl sulfate and methyl methanesulfonate (the internal standard) were well separated as when authentic samples were analyzed as shown in Fig. 2A. The figure also shows that no interfering peaks were present in the region of these peaks. The identity of these two peaks was confirmed by GC-MS as for the peaks of authentic samples (data not shown). Table 3 shows the recovery of various amounts of sulfate, as dimethyl sulfate, added to the urine. The recovery was over 95.8%.

The present method was applied to the urine of five healthy male Japanese ranging from 27 to 54 years of age. The amounts of total sulfate were around 10 μmol/mg of creatinine as shown in Table 4.

Discussion

Sulfate was quantitatively converted to dimethyl sulfate under the conditions described in this paper. The present results show that this method could be applied to the determination of total sulfate in the urine.

Methanesulfonate was converted to methyl methanesulfonate by the present method, as tosylates have been converted to alkyl tosylates (6). The yield of the formation of methyl methanesulfonate from methanesulfonic acid

Table 2 Effect of some anions on the recovery of sulfate[a]

Anion added (μmol/ml)	Sulfate added (μmol/ml)	Sulfate determined[b] (μmol/ml)	Recovery[b] (%)
None	10.0	10.05 ± 0.35	100.5 ± 3.5
Chloride (800)	10.0	9.98 ± 0.28	99.8 ± 2.8
Phosphate (150)	10.0	8.69 ± 0.31	86.9 ± 3.1
Phosphate[c] (150)	10.0	9.99 ± 0.30	99.9 ± 3.0
Methanesulfonate (10)	10.0	9.03 ± 0.41	90.3 ± 4.1

a: Sulfate was determined after derivatization to dimethyl sulfate. Experimental details are described under Materials and Methods.
b: Mean ± SD of 6 determinations.
c: Phosphate ion was removed by treatment with magnesium hydroxide carbonate before derivatization.

Table 3 Recovery of sulfate added to human urine[a]

Sulfate added (μmol/ml)	Sulfate determined[b] (μmol/ml)	Recovery[b] (%)
0.0	6.88 ± 0.15	
5.0	11.87 ± 0.34	99.8 ± 6.8
10.0	16.69 ± 0.42	98.1 ± 4.2
20.0	26.03 ± 1.02	95.8 ± 5.1
40.0	45.99 ± 1.36	97.8 ± 3.4

a: Sulfate was determined by gas chromatography after it was converted to dimethyl sulfate as described under Materials and Methods.
b: Mean ± SD of 4 determinations.

Table 4 Total sulfate excreted in human urine[a]

Sample No.	Total sulfate[b] (μmol/mg of creatinine)
1	12.14 ± 0.41
2	11.38 ± 0.40
3	10.46 ± 0.38
4	10.35 ± 0.26
5	6.22 ± 0.16

a: Total sulfate was determined by the present gas chromatographic method. Details are described under Materials and Methods.
b: Mean ± SD of 5 determinations.

solution under the present conditions was 98.5±2.9%. However, the addition of methanesulfonic acid to sulfate solutions (Table 2) or urine samples interfered with the formation of dimethyl sulfate, resulting in a decrease in its recovery. These results indicate that methanesulfonic acid should not be added before the derivatization procedure. Therefore, methyl methanesulfonate was added after the derivatization procedure as the internal standard.

The interference of phosphate ion with the present method was effectively eliminated by precipitating the ion as magnesium phosphate according to the method of Fritz and Yamamura (5).

Chloride ion interfered with the present method by converting silver sulfate to insoluble silver chloride. As sulfate ion did not react directly with methyl iodide, chloride must be eliminated before sulfate ion is converted to silver sulfate. The elimination of chloride ion as silver chloride was performed with the use of excess silver oxide as reported by Koita (7). In the present method, the amount of silver oxide used was about five equivalents of the chloride ion present in the urine sample.

When the present method was applied to urine samples without the hydrolysis step, the recovery of sulfate was about 80%, and values varied from sample to sample. This result indicates possible interference of dimethyl sulfate formation by ethereal sulfate. Similar interference was observed when methanesulfonate was added to a sulfate solution as shown in Table 2. These substances, which have a sulfonic group, seem to reduce the formation of silver sulfate.

As shown in Table 3, the recovery of sulfate was high when the sample was hydrolyzed before the derivatization was performed. Thus, these results indicate that the present method can be applied to the determination of total sulfate in the urine.

The value of total sulfate in the urine determined by the present method (Table 4) is somewhat lower than that of other reports (1, 8). The difference might indicate a difference in protein intake. Further study about this has to be performed.

Many methods for the determination of sulfate have been reported. The method involving the precipitation of benzidine sulfate (9) has been widely used. However, it is not recommended because of the toxicity of benzidine. Colorimetric determination using barium chloranilate (10), a turbidimetric method involving the production of barium sulfate (11, 12) and a titration method with barium perchlorate (5) have also been used. Small *et al.* developed an ion-exchange chromatographic method using a conductimetric detector for the analysis of ionic compounds (13), and sulfate and other anions in urine were easily analyzed by the method (14). The ion chromatographic method is advantageous since derivatization is not required. The present gas chromatographic method converts sulfate ion to a volatile derivative. Therefore, this method seems to be applicable to the isotopic analysis of sulfate by GC-MS.

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