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## Short Communication

# Simple method for the determination of inorganic sulfate in human serum and urine using single-column ion chromatography

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ABSTRACT

A single-column ion chromatographic assay with conductivity detection was developed to determine inorganic sulfate concentrations in human plasma and urine samples. Plasma samples were ultrafiltered to remove proteins. Plasma ultrafiltrate and urine samples were diluted prior to injection onto the anion-exchange column. The described method is simple, fast, sensitive and reproducible and was used to study the effect of subchronic administration of acetaminophen on the plasma concentrations and urinary excretion of inorganic sulfate in healthy volunteers.

## INTRODUCTION

Sulfate conjugation is an important biotransformation pathway for many endogenous compounds and xenobiotics [1,2]. The sulfoconjugation process is considered to be influenced by the supply of inorganic sulfate, the concentration of adenosine-3′-phosphate-5′-sulfatophosphate (PAPS) and the activity of sulfotransferase enzymes [1]. Measurement of inorganic sulfate in biological fluids is essential for studying the factors controlling the rate of sulfoconjugation [3].

A large number of assays, including turbidimetric and spectrophotometric methods, have been developed to determine the concentration of inorganic sulfate in serum and urine [2]. In recent years, several ion chromatographic techniques for the determination of anions including sulfate, usually with electrical conductivity detection, have been developed [4–8]. Ion chromatographic assays are simpler, more sensitive and more accurate than previous methods. Initially the procedure involved the use of two columns, a separator column and a sup-

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pressor column (suppressed or dual-column technique) [4–6]. Non-suppressed or single-column ion chromatographic techniques have also been developed and are considerably less expensive [7,8].

We describe here a simple single-column ion chromatographic assay for the determination of inorganic sulfate in human serum and urine. Ultrafiltration rather than precipitation was used to remove proteins from the serum samples.

## EXPERIMENTAL

### *Chemicals*

Potassium hydrogenphthalate, sodium hydroxide and sodium sulfate (anhydrous) were of analytical-reagent grade (Fisher Scientific, Fairlawn, NJ, U.S.A.).

### *Biological samples*

Serum and urine samples were obtained from seven healthy male volunteers who participated in a study investigating the circadian variation in serum inorganic sulfate concentrations. Details of this study are published elsewhere [9].

### *Preparation of serum samples*

A 500-$\mu$l aliquot of high-performance liquid chromatographic (HPLC)-grade water (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) was transferred to an Amicon (Danvers, MA, U.S.A.) MPS-1 micropartition system fitted with an amicon YMT cellulose membrane filter and centrifuged for 17 min (2000 *g*, ambient temperature) using a fixed-angle rotor (IEC HN S11 centrifuge; International Equipment, Needham Heights, MA, U.S.A.). The PTFE filtrate cup of the ultrafiltration device was changed and a 500-$\mu$l aliquot of serum was transferred to the sample reservoir and centrifuged using the same conditions as described above. The ultrafiltrate was diluted appropriately (usually a dilution factor of 8) with HPLC-grade water and a 50-$\mu$l aliquot was injected into the HPLC system. The integrity of the membrane filters was routinely checked by testing the ultrafiltrate for protein with urinary dipsticks (Labstix-R; Miles Labs., Ames Company Division, Rexdale, Canada).

### *Preparation of urine samples*

A sample of frozen urine (3–4 ml) was allowed to thaw at room temperature, vortex-mixed (Canadian Labs. Supplies, Toronto, Canada) and placed in a glass tube (100 × 16 mm I.D.; Kimble Products, Toledo, OH, U.S.A.). The sample was then centrifuged at 2000 *g* at 4°C for 10 min (Accuspin-FR centrifuge; Beckman, Palo Alto, CA, U.S.A.). An aliquot of the centrifuged urine was diluted appropriately (80–100 fold) with HPLC grade water and a 50-$\mu$l sample was injected into the HPLC system.

*Calibration graphs*

Various amounts of sodium sulfate (0, 25, 50, 75, 100, 125, 187.5 and 250 $\mu$mol) in aquous solution (diluted from a 1 m*M* stock solution in HPLC-grade water) were placed in glass tubes (100 × 16 mm I.D.) and HPLC-grade water was added to give a total volume of 1.0 ml. These standard solutions were mixed and 50-$\mu$l aliquots of the ultrafiltrate were injected into the HPLC system. Ultrafiltration (using prewashed membrane filters) of aqueous sulfate standards yielded ultrafiltrates with inorganic sulfate concentrations not different from those obtained following direct injection of the sulfate standard solutions. Calibration graphs were constructed by plotting peak height *versus* concentration of inorganic sulfate. A quality control standard (100 $\mu M$) was injected at the end of each day of analysis.

*Ion chromatography*

Diluted serum ultrafiltrates and urine samples were analyzed on an isocratic HPLC system consisting of a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injection valve, an LKB (Bromma, Sweden) Model 2150 pump and a Waters–Millipore (Milford, MA, U.S.A.) Model 430 conductivity detector. Samples (50 $\mu$l) were injected using a Hamilton (Reno, NV, U.S.A.) 100-$\mu$l injection syringe. The temperature of the detector cell was maintained at 35°C. The signal from the conductivity detector (set at 2.5 $\mu$S full-scale) was monitored by a strip-chart recorder (Linear Instruments, Irvine, CA, U.S.A.).

The anion-exchange column (Waters Anion IC-Pak, 50 mm × 4.5 mm I.D.) was protected by an IC-Pak anion guard column insert (Waters–Millipore) and a Rheodyne Model 7302 column inlet filter. The mobile phase (4 m*M* potassium hydrogenphthalate adjusted to pH 4.5 with 1 *M* sodium hydroxide solution) was pumped through the system at a flow-rate of 1 ml/min. The mobile phase was degassed prior to use.

## RESULTS AND DISCUSSION

The proposed method is fast, reliable, reproducible and sensitive. The retention time for the sulfate ion peak was 6.8 min (Fig. 1). A system peak always eluted at 10.5 min, making the overall run time *ca.* 12 min. As this system peak did not interfere with the quantification of the sulfate ion, it was considered of no major consequence. No other interfering peaks showed up in the serum ultrafiltrate and urine chromatograms of samples from over twenty healthy volunteers studied so far.

The calibration graphs were linear and reproducible over the concentration range utilized. Although no internal standard was used, there was an excellent correlation between the peak height of standards and their respective concentrations ($r > 0.99$). The inter-day relative standard deviation (R.S.D.) for the slopes of the calibration lines was 4.8% ($n = 22$) and the $y$-intercept was only slightly

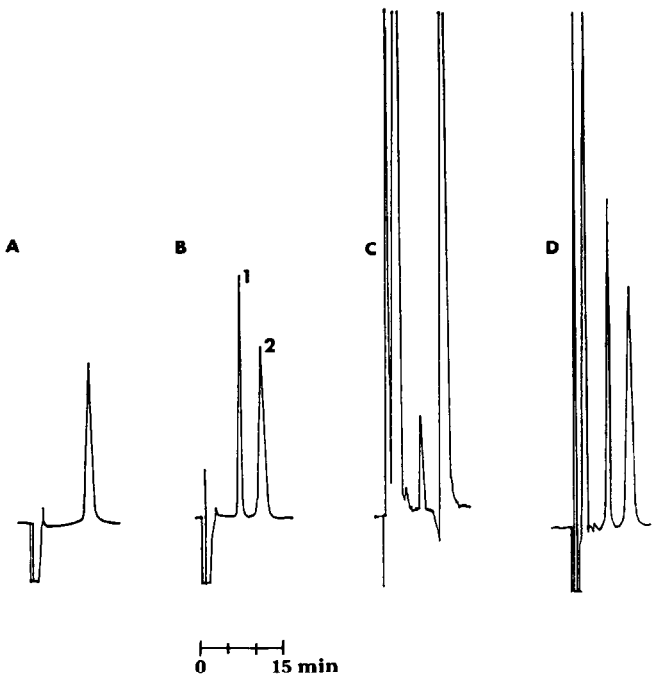


Fig. 1. Representative chromatograms for the determination of inorganic sulfate in serum and urine. Peaks: 1 = $SO_4^{2-}$; 2 = system peak. (A) Blank (HPLC-grade water); (B) aqueous standard (100 $\mu M$); (C) serum sample from a healthy volunteer; (D) urine sample from a healthy volunteer.

different from zero (0.167 ± 1.186, mean ± S.D.). The quality control standard (100 $\mu M$) injected at the end of each day of analysis gave a value of 100.8 ± 2.6 $\mu M$ ($n = 22$). The R.S.D.s for the peak heights of inorganic sulfate, calculated from the standards used to construct the calibration graphs ($n = 22$), were less than 6.1% (Table I).

TABLE I

RELATIVE STANDARD DEVIATIONS OF PEAK HEIGHTS FOR INORGANIC SULFATE IN AQUEOUS STANDARDS

Aqueous standards were ultrafiltered and an aliquot of the ultrafiltrate was injected into the HPLC system. Aqueous standards injected without prior ultrafiltration gave identical peak heights and similar R.S.D.s.

| Inorganic sulfate concentration ($\mu M$) | R.S.D. (%) | Inorganic sulfate concentration ($\mu M$) | R.S.D. (%) |
|---|---|---|---|
| 25 | 4.54 | 125 | 2.79 |
| 50 | 4.37 | 187.5 | 2.38 |
| 75 | 3.10 | 250 | 6.10 |
| 100 | 2.42 | | |

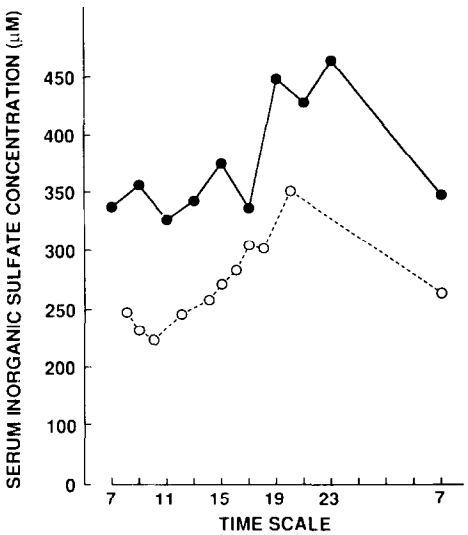


Fig. 2. Serum inorganic sulfate concentrations in a healthy volunteer over a 24-h period. (●) Control; (○) following subchronic acetaminophen administration (650 mg four times daily for four days). *x*-Axis in h.

Ultrafiltration to clean up biological samples prior to quantification of ions has been reported previously [10–14]. For example, Matsushita [13] used ultrafiltration prior to ion chromatography to determine unbound calcium and magnesium in blood serum, milk and egg white. As inorganic sulfate does not bind to serum proteins [10], and sulfate concentrations are therefore the same in serum and serum ultrafiltrate [15], ultrafiltration would be a valid procedure for removing proteins from serum samples prior to quantification of the sulfate ion. We found ultrafiltration of the serum samples to be a better method than acetonitrile precipitation for protein removal prior to analysis. The chromatograms showed less interferences, sharper sulfate ion peaks and a prolonged column lifetime (well over 1500 injections without any decrease in column performance). However, the cellulosic membrane filters we used contain some inorganic sulfate, which can easily be removed by a single wash with HPLC-grade water.

## CONCLUSION

The proposed method has the advantage of using a single anion-exchange column, which is a considerably simpler and less expensive procedure than the dual-column techniques frequently used to determine sulfate in biological samples. Ultrafiltration rather than protein precipitation was used to remove proteins from the serum samples. Fig. 2 shows the application of the method to study the effect of subchronic acetaminophen administration on the serum inorganic sulfate levels of healthy volunteers [9].

REFERENCES

1 G. J. Mulder, in G. J. Mulder (Editor), *Sulfation of Drugs and Related Compounds*, CRC Press, Boca Raton, FL, 1981, pp. 131-185.
2 L. G. Tallgren, *Acta Med. Scand., Suppl.*, 640 (1980) 1.
3 G. Levy, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 45 (1986) 2235.
4 H. Small, T. S. Stevens and W. C. Bauman, *Anal. Chem.*, 47 (1975) 1801.
5 C. Anderson, *Clin. Chem.*, 22 (1976) 1424.
6 D. E. C. Cole and C. R. Scriver, *J. Chromatogr.*, 225 (1981) 359.
7 M. E. Morris, *Doctoral Dissertation*, University of New York, Buffalo, NY, 1984, pp. 40–54.
8 G. Schmuckler, A. L. Jagoe, J. E. Girarg and P. E. Buell, *J. Chromatogr.*, 356 (1986) 413.
9 D. A. Hoffman, S. M. Wallace and R. K. Verbeeck, *Eur. J. Clin. Pharmacol.*, 39 (1990) 143.
10 W. Buchberger and K. Winsauer, *Mikrochim. Acta*, III (1985) 347.
11 D. E. C. Cole and D. A. Landry, *J. Chromatogr.*, 337 (1985) 267.
12 H. Shintani, *J. Chromatogr.*, 344 (1985) 145.
13 S. Matsushita, *Anal. Chim. Acta*, 172 (1985) 249.
14 W. Buchberger, *J. Chromatogr.*, 439 (1988) 129.
15 J. B. Whitlam and K. F. Brown, *J. Pharm. Sci.*, 70 (1981) 146.