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Letter to the Editor

Determination of phosphate, sulfate and oxalate in urine by ion chromatography

Sir,

Quantification of typical urinary anions such as oxalate, phosphate and sulfate is important in the study of urolithiasis [1]. Although Anderson [2] has demonstrated that ion chromatography (IC) can be used for the simultaneous analysis of ions in biological fluids, their method, when used for chloride, nitrate, phosphate and sulfate ion determination in human urine requires approximately 35 min with standard IC operating conditions. The method described herein allows for the simultaneous determination of urinary phosphate, sulfate and oxalate concentrations in about 16 min with mean recoveries of about 103, 102 and 102%, respectively. Phosphate and oxalate concentrations in different urines as determined by IC compared well with those obtained using colorimetric methods.

EXPERIMENTAL

Instrumentation and chromatographic conditions

A Dionex QIC analyzer (Dionex, Sunnyvale, CA, U.S.A.) incorporating a fiber suppressor (or anion micromembrane suppressor) and conductivity detector together with a Dionex fast anion separator column (HPIC-AS3) and guard column (HPIC-AG3) was used. The conductivity detector, adjusted to a sensitivity of 30 μS to resolve phosphate and sulfate peaks, was changed to 3 μS before the appearance of the oxalate peak. Chromatograms were obtained on an Omniscribe chart recorder (Houston Instruments, Graphics Division of Bausch and Lomb, Austin, TX, U.S.A.) at 1 V full scale sensitivity. The mobile phase, a mixture of $2.42 \cdot 10^{-4}$ mol l^{-1} sodium bicarbonate and $1.81 \cdot 10^{-4}$ mol l^{-1} sodium carbonate, was run isocratically at 132 ml h^{-1}. Regenerant, $5.0 \cdot 10^{-2}$ mol l^{-1} sulfuric acid, was also maintained at a flow-rate of approximately 120 ml h^{-1} for an uninterrupted use of the chromatograph.

Sample preparation

Overnight urines collected from normal and stone-forming male subjects were acidified by the addition of 1% (v/v) concentrated hydrochloric acid and were kept frozen in closed vials until analyzed. Just prior to analysis, the samples were

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thawed and brought to 37°C. Aliquots for analysis were diluted forty-fold with triple-distilled, deionized water and filtered (0.22 μm) (Millipore, Bedford, MA, U.S.A.).

RESULTS AND DISCUSSION

Under the IC conditions described, the phosphate, sulfate and oxalate peaks eluted at 6, 10 and 15 min, respectively (Fig. 1). Peak heights obtained for urine samples were compared with mixed standards of phosphate (25–100 mg l^{-1}), sulfate (25–100 mg l^{-1}) and oxalate (0.25–2 mg l^{-1}) prepared fresh daily. Linear plots of peak height against analyte concentration were obtained over these concentration ranges of the three anions, with regression correlation coefficients of 0.99995, 0.99982 and 0.99907 for phosphate, sulfate and oxalate, respectively. Recovery, following standard additions of phosphate ($1.65 \cdot 10^{-4}$ mol l^{-1}), sulfate ($1.67 \cdot 10^{-4}$ mol l^{-1}) and oxalate ($1.82 \cdot 10^{-5}$ mol l^{-1}) to several urines was good, yielding a relative standard deviation (R.S.D.) of less than 6% in all cases ($n = 11$). Urinary sulfate and phosphate concentrations are relatively high, so that their precise determination could be achieved with little difficulty. Since oxalate is present at relatively low levels in urine, however, its recovery and precision were further tested by the addition of varying amounts of oxalate to a urine containing $2.7 \cdot 10^{-4}$ mol l^{-1} oxalate. The results, shown in Table I, indicate that limit of detection for oxalate was $5.0 \cdot 10^{-6}$ mol l^{-1} in urine (prior to dilution), with an R.S.D. of less than 10%. Since most urines having low oxalate levels also contain low concentrations of sulfate and phosphate, the oxalate could be determined in less dilute aliquots, typically 10–20%. One sample which contained $1.4 \cdot 10^{-6}$ mol l^{-1} oxalate was reliably analyzed using only a five-fold dilution.

Because ascorbic acid undergoes transformation to oxalate at high pH, it has been suggested that the Dionex method may be subject to uncertainties due to the relatively high pH of the mobile phase [3]. However, chromatograms obtained for urine samples with up to $1.14 \cdot 10^{-4}$ mol l^{-1} added ascorbate showed no interference despite the fact that this concentration is nearly ten times that excreted by normal adults [4].

The phosphate concentrations of nine normal and two stone-former urines as determined by IC compared especially well with a colorimetric assay [5] yielding the regression equation y (IC) $= 0.95x$ (colorimetry) $+ 0.043$, with a correlation coefficient (r) of 0.996. The oxalate concentrations of ten normal and three stone-former urine samples were in good accord with the enzymatic procedure of Sigma (St. Louis, MO, U.S.A.) [6] yielding the regression equation y (IC) $= 1.21x$ (Sigma) $- 0.079$ ($r = 0.952$). Since an IC method for sulfate analysis in other biological fluids such as human saliva and sweat has been established [7], method comparison studies for sulfate analysis in urine were not made. However, in five different urines, diluted forty-fold and treated with barium chloride to precipitate the sulfate as barium sulfate, the sulfate peak height in the filtrates was reduced to the solubility of barium sulfate, thus confirming the specificity of the sulfate peak.

In conclusion, a rapid and accurate method for the simultaneous determination

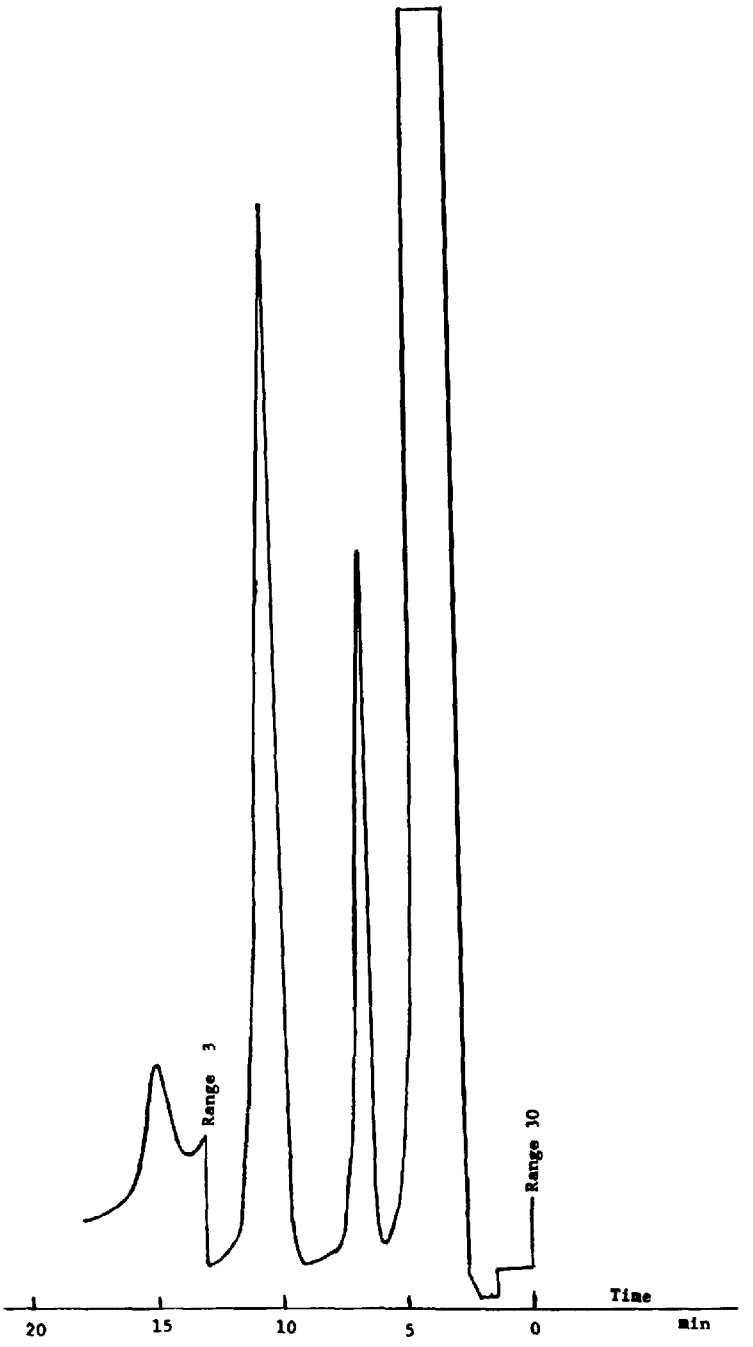

Fig. 1. Chromatogram of 50 mg l^{-1} phosphate, 50 mg l^{-1} sulfate and 1 mg l^{-1} oxalate in presence of 1% (v/v) concentrated hydrochloric acid. Chart speed: 5 mm min^{-1}.

TABLE I

URINARY OXALATE ANALYSIS WITH STANDARD OXALATE ADDITION

Added ($mol\,l^{-1} \times 10^{-3}$)	Analyzed ($mol\,l^{-1} \times 10^{-3}$)	R.S.D. (%)	Recovery (%)
0.050	0.053 ± 0.005	9.4	106.0
0.100	0.105 ± 0.005	4.8	105.0
0.142	0.143 ± 0.007	4.9	100.7
0.284	0.277 ± 0.010	3.6	97.5
0.473	0.467 ± 0.007	1.5	98.5
		Mean	101.6

of urinary oxalate, sulfate and phosphate has been developed using a relatively inexpensive ion chromatograph. The method may readily be used for routine screening purposes as well as for the in vitro modelling of mineralization processes carried out in whole urine [8,9].

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