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IMPROVEMENT OF ION CHROMATOGRAPHY WITH ULTRAVIOLET PHOTOMETRIC DETECTION AND COMPARISON WITH CONDUCTIVITY DETECTION FOR THE DETERMINATION OF SERUM CATIONS

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SUMMARY

Studies were made of the analytical conditions required for indirect photometric ion chromatography using ultraviolet photometric detection (UV method) for the determination of serum cations following a previously developed serum pre-treatment. The sensitivities of the conductivity detection (CD) and UV methods and the amounts of serum cations determined by both methods were compared. Attempts to improve the sensitivity of the conventional UV method are reported. It was found that the mobile phase previously reported by Small and Miller showed no quantitative response when more than 4 m*M* copper(II) sulphate pentahydrate was used. As a result, there was no significant difference in the amounts of serum cations shown by the CD and UV methods. However, by adding 0.5—5 m*M* cobalt(II) sulphate heptahydrate, nickel(II) sulphate hexahydrate, zinc(II) sulphate heptahydrate or cobalt(II) diammonium sulphate hexahydrate to 0.5—1.5 m*M* copper(II) sulphate pentahydrate, higher sensitivity and a quantitative response were attained.

INTRODUCTION

The author has previously reported an ion chromatographic method for the determintation of serum cations, used together with a conductivity detection (CD) method and a serum pre-treatment [1]. Recently, Small and Miller [2] reported an indirect photometric ion chromatographic method for the determination of ions using a UV detector (UV method) instead of a conductivity detector, in which the eluent salt with UV absorption in the mobile phase was replaced with sample ions without UV absorption, resulting in a reduction in

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UV absorption in the mobile phase. Serum cations were not determined by the UV method, nor were the CD and UV methods compared.

The author has now determined serum cations by the UV method after serum pre-treatment as previously described for the CD method [1]. Both sets of results were then compared. Various mobile phases other than those reported by Small and Miller [2] were studied in order to improve their UV method and, by using this improved UV method, a high sensitivity was attained.

EXPERIMENTAL

Reagents and materials

Reagents were as described previously [1], or were commercially available and chemically pure reagents. Serum was equine serum (Flow Labs., McLean, VA, U.S.A.) and the ultrafilter was an MPS-3 (Amicon, Danvers, MA, U.S.A.).

Equipment

The UV detector used was a Uvilog-8 (Oyo-Bunko, Tokyo, Japan). SGR-1A stepwise gradient elution and LC-3A high-performance liquid chromatographic (HPLC) equipment were obtained from Shimadzu (Tokyo, Japan). An ASC-4000 cation-exchange column (Oyo-Bunko) was used. All other instruments and equipment used were identical with those described previously [1].

Procedures

The CD method and the serum pre-treatment are shown in Table I. The

TABLE I

OPERATING CONDITIONS FOR ION CHROMATOGRAPHY WITH CONDUCTIVITY DETECTION

Parameter	Cation			
	Na	K	Mg	Ca
Injection volume (μl)	10	50	50	50
Column	Wescan cation-exchange column, 250 × 4.6 mm I.D. in all instances			
Temperature	Ambient	Ambient	Ambient	Ambient
Mobile phase	Aqueous HNO_3 (pH 2.1) for Na and K		0.07 ml of EDA*— 1.5 l of water (pH 6.1)	0.1 ml of EDA*— 1.5 l of water (pH 6.1)
Flow-rate (ml/min)	1.5	1.5	1.1	1.1
Detector	Wescan conductivity non-suppressor type detector, range × 10, course × 3 in all instances			
Serum treatment	Serum was diluted 100-fold with water for the determination of serum Na and 10-fold for the determination of serum K, Mg and Ca. Another treatment method was as follows: serum acidified to pH 3.0 with H_3PO_4 and non-acidified serum were ultrafiltered and the ultrafiltrate was neutralized and diluted for application to the HPLC system			

*Ethylenediamine hydrate.

**Acidified ultrafiltrate was neutralized with NaOH for divalent cation determination.

TABLE II

OPERATING CONDITIONS FOR ION CHROMATOGRAPHY WITH ULTRAVIOLET PHOTOMETRIC DETECTION

Parameter	Cation			
	Na	K	Mg	Ca
Injection volume (μl)	20	20	20	20
Column	ASC-4000 (250 × 4.6 mm I.D.), strong cation-exchange column in all instances			
Temperature	Ambient	Ambient	Ambient	Ambient
Mobile phase	1.28 m*M* $CuSO_4 \cdot 5H_2O$ for Na and K		3.2 m*M* $CuSO_4 \cdot 5H_2O$ for Mg and Ca	
Flow-rate (ml/min)	1.0	1.0	1.0	1.0
Detector	Uvilog-8 UV detector, detected at 218 nm, 0.02 a.u.f.s. in all instances			
Serum treatment	Non-acidified serum for the determination of free cations and serum acidified to pH 3.0 with the use of H_3PO_4 for the determination of free plus bound cations in serum were ultrafiltered. The ultrafiltrate was neutralized* and diluted 100-fold of serum with water for Na determination, 30-fold of serum with water for K determination, but was not diluted for Mg and Ca determinations. Sample treatment for the conducitivity detection method was the same as that used in the UV detection method in the comparison of the amounts of serum cations			
Retention time (min)	1.75	2.15	5.5	9.2

*Acidified ultrafiltrate was neutralized with NaOH (for divalent cation determination), with $Ca(OH)_2$ (for monovalent cation determination) and with $Sr(OH)_2$ (for simultaneous mono- and divalent cation determination).

analytical conditions for the conventional and the improved UV methods are shown in Tables II and Table VI, respectively.

RESULTS AND DISCUSSION

Small and Miller [2] have previously reported the simultaneous determination of mono- and divalent cations using a complicated system. This conventional UV method, using isocratic elution coupled with a single column, is unsatisfactory owing to incomplete separation of potassium from sodium and broadening of the calcium peak, none of which improved simultaneously. In this work, mono- and divalent cations have been determined separately by using different mobile phases to those used in the UV method.

Determination of monovalent cations

Analysis of standard aqueous solution. In the UV method, the logarithm of the retention time (t_R) of monovalent cations decreased linearly with increasing concentration of copper(II) sulphate pentahydrate (copper sulphate) in the mobile phase. A similar relationship was noted between the concentration of nitric acid in the mobile phase and log t_R of monovalent cations in the CD method.

The best separation of sodium from potassium and of sodium and potassium

from the vacant peak was obtained when the concentration of copper sulphate was around 1.28 m*M*. The vacant peak appeared around the void volume. The use of other copper sulphate concentrations was unsuccessful owing to poor separation. The highest sensitivity but with poor separation was attained when 0.5 m*M* copper sulphate was used, but concentrations lower than 0.5 m*M* showed decreased sensitivity with similarly poor separation.

Determination of free monovalent cations in serum. Non-acidified ultrafiltered serum was diluted 10—100-fold and injected into the HPLC system. The chromatogram shown in Fig. 1 was achieved when the injected ultrafiltrate was diluted 30-fold. It can be seen that the resolution of sodium from potassium was unsatisfactory owing to the concentration of serum sodium,

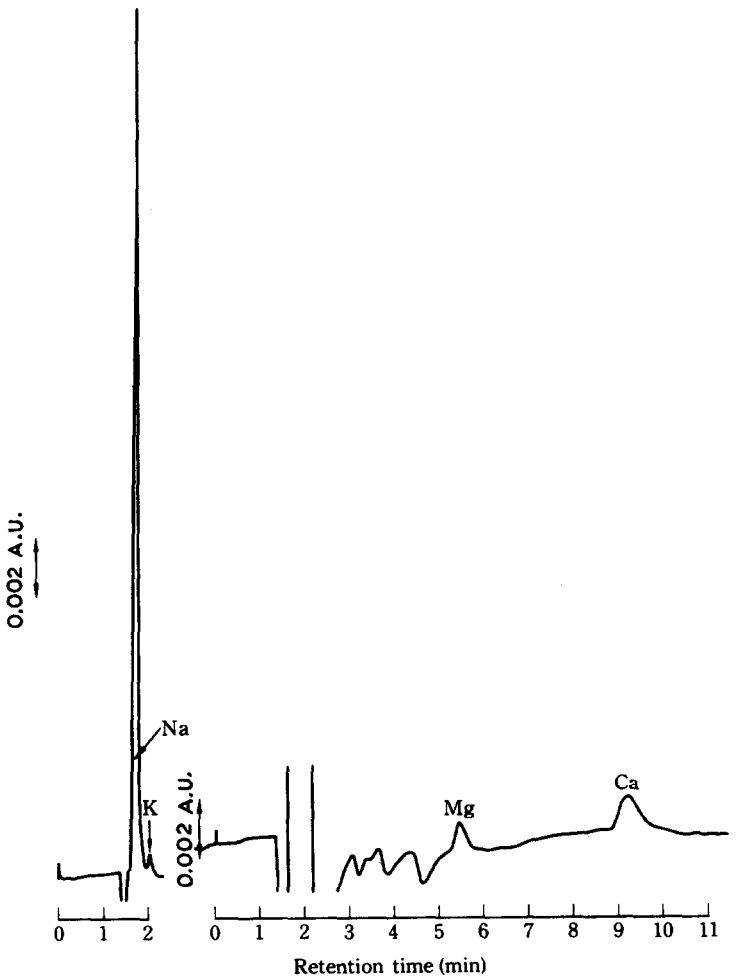

Fig. 1. Chromatograms of serum sodium, potassium, magnesium and calcium obtained by conventional ion chromatography with UV photometric detection. Volumes of 20 μl each of 30-fold diluted ultrafiltrate of serum for Na and K determination and of non-diluted ultrafiltrate of serum for Mg and Ca determination were applied. Concentrations of Na, K, Mg and Ca were 10.506, 0.506, 1.190 and 5.650 mg/dl, respectively. Other conditions as in Table II.

which was about 20 times that of serum potassium. In the present work using the conventional UV method, the ultrafiltrate of serum was diluted 100- and 30-fold for determining serum sodium and potassium, respectively.

The CD method (Fig. 2) is therefore superior to the conventional UV method for the determination of serum potassium, because potassium could not be clearly separated from sodium by the former method (Fig. 1) because the selectivity coefficient of copper(II) used in the mobile phase in the UV method is greater than that of the proton [3] used in the mobile phase of the CD method.

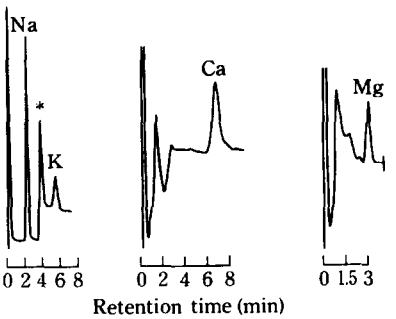

Fig. 2. Chromatograms of serum sodium, potassium, magnesium and calcium obtained by the conductivity detection method. A 100-μl volume of 100 fold diluted ultrafiltrate of serum for Na and K determination and 50 μl of 10-fold diluted ultrafiltrate of serum for Mg and Ca determination were applied. Concentrations of Na, K, Mg and Ca were 3.16, 0.15, 0.12 and 0.56 mg/dl, respectively. The asterisk indicates that the attenuation was changed from 3 for Na determination to 2 for K determination; the attenuation was 1 for Mg and Ca determinations. Retention times of Na, K, Mg and Ca were 2.50, 5.39, 2.95 and 6.40 min, respectively. Other conditions as in Table I.

Determination of the total amounts of free plus bound monovalent cations in serum. Serum was acidified to pH 3.0 with phosphoric acid and ultrafiltered. The ultrafiltrate was neutralized, diluted 10—100-fold with water and injected into the HPLC system. In the UV method, sodium and potassium could not be detected unless the ultrafiltrate was neutralized because the sample peaks were included in the vacant peak. In the CD method, the diluted ultrafiltrate was injected because the mobile phase was acidic (pH 2.1).

In comparing the total amounts with the free amounts of monovalent cations in serum, about 10% of serum sodium and potassium was found to be bound to serum protein.

Determination of divalent cations

Analysis of standard aqueous solution. Linearity was found between the concentration of ethylenediamine in the mobile phase in the CD method and log t_R of magnesium and calcium.

Magnesium and calcium could be quantified by the conventional UV method when the concentration of copper sulphate in the mobile phase was around 3.2 m*M*; the calibration graph for the determination of divalent cations was linear at 3.2 m*M*. The use of more than 4 m*M* of copper sulphate did not result in a quantitative response because the mobile phase at that concentration had an

excessive absorbance of more than 2, and therefore the absorbance difference between the eluent in the mobile phase and the sample ions was less than the detection limit. The use of concentrations other than ca. 3.2 m*M* was unsuccessful owing to the poor separation from the vacant peak and from serum admixtures and the difficulty in attaining a quantitative response.

The linearity range of mono- and divalent cations was from the detection limit (Table III) to around fifteen times the amount of each serum cation.

TABLE III

DETECTION LIMITS OF SODIUM, POTASSIUM, MAGNESIUM AND CALCIUM IN THE CONDUCTIVITY DETECTION AND UV PHOTOMETRIC DETECTION METHODS

Results in nanograms (mean of five determinations); coefficient of variation <0.5% in all instances. Signal-to-noise ratio = 2.

Cation	Conductivity detection method	Photometric detection method		
		Conventional	Improved	
			Stepwise	Isocratic
Na	3.8*	7.5§	7.5§	8.4§§
K	25.0*	11.7§	11.7§	12.8§§
Mg	15.0**	84.9§	10.6§§	9.8§§
Ca	28.0***	301.1§	34.0§§	34.0§§

*Attenuation 4; injection volume 10 µl.
**Attenuation 1; injection volume 50 µl.
***Attenuation 1; injection volume 20 µl.
§0.02 a.u.f.s.; injection volume 20 µl. Other conditions as in Table II.
§§0.02 a.u.f.s.; injection volume 20 µl. Other conditions as in Table VI.

Determination of free divalent cations in serum. The serum pre-treatment was the same as that used for free monovalent cations. Undiluted and ten-fold diluted ultrafiltrate were injected into the HPLC system for the determination by the conventional UV method and the CD method, respectively. The chromatograms of the free serum magnesium and calcium thus obtained by two methods are shown in Figs. 1 and 2, respectively, for comparison of UV and CD methods. The conventional UV method was less sensitive than the CD method, as shown in Table III. Taking into consideration this poor sensitivity to magnesium and calcium and broadening of calcium peak as shown in Fig. 1, the CD method was preferred for the determination of serum magnesium and calcium.

Determination of the total amounts of free plus bound divalent cations in serum. The serum pre-treatment was the same as that used for total monovalent cations. Undiluted and ten-fold diluted ultrafiltrate were injected into the HPLC system for the determination by the conventional UV method and the CD method, respectively. Comparing the total amounts with the free amounts of divalent cations in serum, about 30% of magnesium and about 50% of calcium in serum was bound to serum protein.

Comparison of the amounts of serum cations determined by the CD and the conventional UV methods

No substantial difference was found between the amounts determined using these two methods (Table IV). The within-run precisions of the amounts of free and total serum cations determined by the two methods were within 0.5% coefficient of variation (C.V., n = 10), the day-to-day precisions of both methods were within 1.1% C.V. (n = 10), and the month-to-month precisions were within 1.7% C.V. (n = 10) in all instances. The inter-group variation between these two methods was within 0.5% C.V. (n = 10).

TABLE IV

COMPARISON OF CONCENTRATIONS OF SERUM SODIUM, POTASSIUM, MAGNESIUM AND CALCIUM DETERMINED BY ION CHROMATOGRAPHY WITH UV AND CONDUCTIVITY DETECTION (CD)

Serum pre-treatment refers to Tables I and II. Amounts given are the means (mg/dl) (n = 3) ± coefficient of variation (%).

Cation	Free cations		Free plus bound cations	
	UV method	C.D. method	UV method	CD method
Na	316.0 ± 0.4	315.2 ± 0.5	350.0 ± 0.5	345.9 ± 0.4
K	15.4 ± 0.3	15.2 ± 0.3	18.2 ± 0.3	18.6 ± 0.4
Mg	1.3 ± 0.1	1.2 ± 0.0	1.8 ± 0.2	1.7 ± 0.1
Ca	5.5 ± 0.4	5.7 ± 0.2	11.0 ± 0.3	10.9 ± 0.4

Studies on the improvement of the use of copper sulphate only in the mobile phase in the conventional UV method

Small and Miller [2] used a complicated system for the determination of cations, in which the concentration of the eluent salt in the mobile phase, the column length, the exchange capacity and the detection wavelength were changed, and columns varying in length and exchange capacity were combined. Mono- and divalent cations were determined at 218 and 234 nm, respectively, with a mobile phase containing only copper sulphate in the first instance and copper(II) nitrate trihydrate (copper nitrate) in the second. When the concentration of copper sulphate exceeded 4 mM in order to shorten the t_R, a quantitative response was not attained. Although a quantitative response proportional to the concentration of cations was attained by reducing the concentration of the eluent in the mobile phase, for example to around 3.2 mM copper sulphate, the sensitivity was unsatisfactory (Table III). Accordingly, in order to establish a high sensitivity and rapid elution to avoid peak broadening at the same time as the simultaneous determination of mono- and divalent cations, eluent salts having little or no UV absorption (Table V) were added to the mobile phase, which contained either copper sulphate or copper nitrate at a low concentration of around 1 mM, and at that concentration of the eluent the UV absorbance was around 0.8. Several factors were considered for improving the mobile phase of the UV method reported by Small and Miller [2]. From the results shown in Tables III and V, the suitability of the molar

TABLE V

MOLAR ABSORPTIVITIES (ϵ) OF VARIOUS COMPOUNDS AT 220 nm

Compound	ϵ (l mol^{-1} cm^{-1})
$Zn(NO_3)_2 \cdot 6H_2O$	7000
$Ni(NO_3)_2 \cdot 6H_2O$	6925
$Co(NO_3)_2 \cdot 6H_2O$	6900
$Cu(NO_3)_2 \cdot 3H_2O$	7700
$ZnSO_4 \cdot 7H_2O$	6
$NiSO_4 \cdot 6H_2O$	3
$CoSO_4 \cdot 7H_2O$	10
$CuSO_4 \cdot 5H_2O$	798
$Co(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0
K_2CrO_4	1202

absorptivity of the eluent for attaining an adequate UV absorbance of around 0.7 at low concentration was considered to be one factor. Copper sulphate solution afforded a more sensitive method for the determination of monovalent cations than divalent cations, as its concentration in the former instance was lower. Copper sulphate has a suitable molar absorptivity, as shown in Table V, and therefore has a suitable absorbance at a given eluent concentration; for instance, at a low concentration of around 0.75 m*M*, which is a suitable concentration for the mobile phase (Fig. 4), it has a suitable absorbance of around 0.7. As discussed above, the use of potassium chromate (Table V) in place of copper sulphate was successful except that potassium analysis became impossible. Additionally, salts having a selectivity coefficient [3] for the proton of the sulphonic acid of the cation-exchange resin in the range of those of sodium, potassium, magnesium and calcium, and having little or no UV absorption at around 220 nm, was considered to be another factor for eluent salts to be added to copper sulphate or copper nitrate in the mobile phase. As a result, in the improved UV method, mono- and divalent cations were determined using a mixed solution containing 0.5—1.5 m*M* copper sulphate, the concentration of which was kept low in order to attain adequate absorbance in the mobile phase, combined with 0.5—5 m*M* cobalt(II) sulphate heptahydrate (cobalt sulphate), nickel(II) sulphate hexahydrate (nickel sulphate), zinc(II) sulphate hexahydrate (zinc sulphate) or cobalt(II) diammonium sulphate hexahydrate (cobalt diammonium sulphate) as the eluent salts; all of these salts except copper sulphate have little or no UV absorbance at around 220 nm (Table V) and their selectivity coefficients are intermediate between those of sodium and calcium [3].

Compared with the conventional UV method reported by Small and Miller [2], the determination of mono- and divalent cations described above was far more sensitive (Table III) and simpler, with a quantitative response. The detection limits were improved over those of the conventional UV method, as shown in Table III, with no significant difference among the types and concentrations of the eluent salts when added to 0.75—1.0 m*M* copper sulphate solution in the mobile phase. Log t_R decreased almost linearly with increasing concentration of cobalt sulphate, nickel sulphate, zinc sulphate or cobalt

diammonium sulphate when added to copper sulphate solution. The greater the t_R of the cations, the greater is the decrease in t_R.

Depending on the molar absorptivity (Table V) and the UV absorbance in the mobile phase, a mobile phase containing copper nitrate in combination with cobalt sulphate, nickel sulphate, zinc sulphate or cobalt diammonium sulphate was used to determine mono- and divalent cations at 234 instead of 218 nm. In this instance, the sensitivity was around one fifth of that obtained by the use of copper sulphate with no significant difference among the types and concentrations of the eluent salts when added to 1 m*M* copper nitrate in the mobile phase. Otherwise, the results obtained by the use of copper nitrate were virtually identical with those obtained by the use of copper sulphate in the mobile phase.

Simultaneous determination of mono- and divalent cations by the improved UV method

Using the improved UV method, step-wise gradient elution and isocratic elution procedures were conducted in order to determine mono- and divalent cations simultaneously. The conditions for the stepwise gradient elution and isocratic elution procedures are given in Table VI and the chromatograms obtained are shown in Figs. 3 and 4, respectively. In the improved UV method stepwise gradient elution was carried out in order simultaneously to achieve an adequate separation of potassium from sodium and to improve the broadening of the calcium peak. The stepwise gradient procedure was found to be superior to the isocratic elution procedure, even though there was little effect on the baseline when the mobile phase was exchanged. However, there was no substantial difference in the detection limits of the two procedures (Table III).

TABLE VI

CONDITIONS FOR STEPWISE GRADIENT AND ISOCRATIC ELUTION PROCEDURES IN THE IMPROVED UV METHOD FOR DETERMINATION OF MONO- AND DIVALENT CATIONS

Parameter	Stepwise gradient elution procedure*	Isocratic elution procedure
Injection volume (μl)	20	20
Column	ASC-4000 (250 × 4.6 mm I.D.), strong cation-exchange resin in both instances	
Temperature	Ambient	Ambient
Flow-rate (ml/min)	1.0	1.0
Detector	Uvilog-8 UV detector, detected at 218 nm, 0.02 a.u.f.s. in both instances	
Mobile phase	1.28 m*M* $CuSO_4 \cdot 5H_2O$ for Na and K and a mixed aqueous solution of 1 m*M* $CuSO_4 \cdot 5H_2O$ combined with 2 m*M* $CoSO_4 \cdot 7H_2O$ for Mg and Ca determination	Mixed aqueous solution of 0.75 m*M* $CuSO_4 \cdot 5H_2O$ combined with 0.75 m*M* $CoSO_4 \cdot 7H_2O$ for Na, K, Mg, and Ca determination

*The mobile phase for Na and K determination was changed to that for Mg and Ca determination 3 min after injection and again after a further 10 min, then returned to that for the determination of monovalent cations.

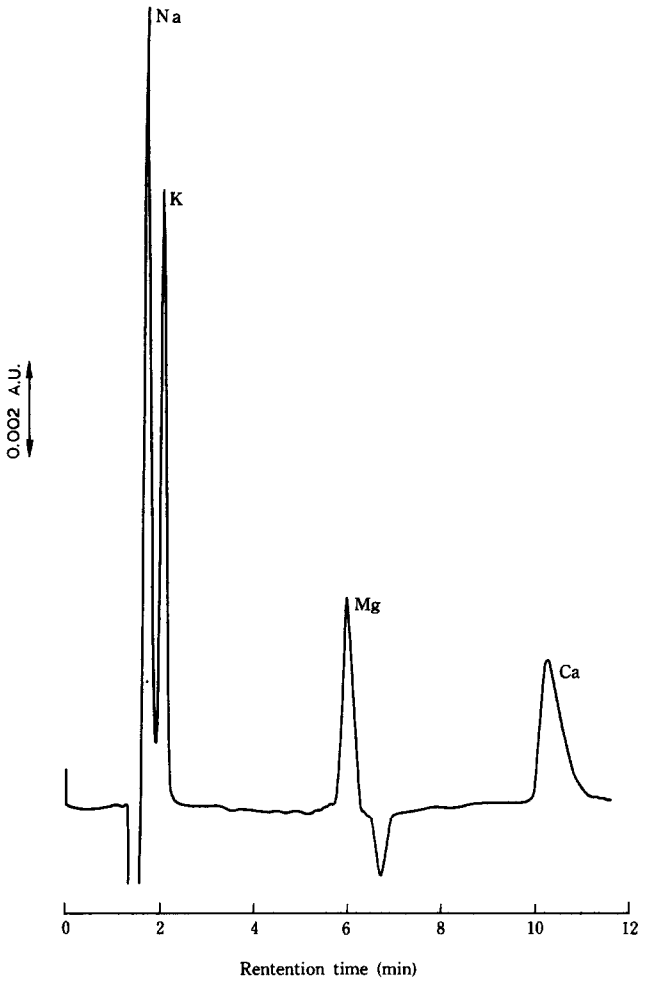

Fig. 3. Chromatogram of sodium, potassium, magnesium and calcium obtained by improved ion chromatography with UV photometric detection with the stepwise gradient elution procedure. Concentrations of Na, K, Mg and Ca were 1.814, 2.120, 0.976 and 2.108 mg/dl, respectively; 50 μl were applied. Other conditions as in Table VI.

Determination of free and total amounts of mono- and divalent cations in serum by the improved UV method

Free and total amounts of sodium, potassium, magnesium and calcium in serum were determined by using both the improved UV method with the isocratic elution and stepwise gradient elution procedures and the serum pretreatment described above. The results were almost the same as those determined by the conventional UV method, shown in Table IV. The C.V. of the intra- and inter-group analyses was less than 1.0% (n = 15) in both instances.

CONCLUSION

There was no substantial difference between the amounts of serum cations

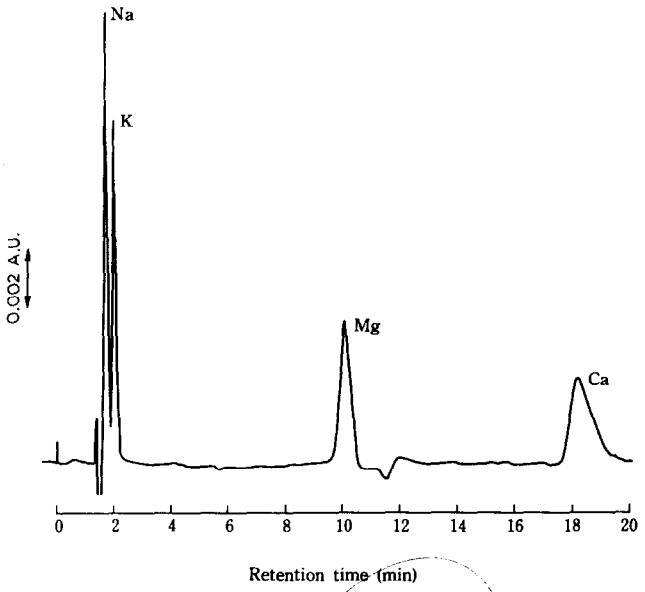

Fig. 4. Chromatogram of sodium, potassium, magnesium and calcium obtained by improved ion chromatography with UV photometric detection with the isocratic elution procedure. Concentrations of Na, K, Mg and Ca were 1.814, 2.120, 0.976 and 2.108 mg/dl, respectively; 50 μl were applied. Other analytical conditions as in Table VI.

determined by the CD and the indirect UV methods. The conventional UV method appears to be inferior to the CD method owing to the lack of a clear separation of potassium from sodium, low sensitivity and a limitation on the concentration of copper sulphate in the mobile phase. The sensitivity of the conventional UV method, however, could be improved by using an eluent containing copper sulphate at low concentration combined with either cobalt sulphate, zinc sulphate, nickel sulphate or cobalt diammonium sulphate.

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