

Ion chromatography of alkaline earth and heavy metal ions by on-column derivatization with bisazochromotropic acid

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(First received June 21st, 1993; revised manuscript received August 25th, 1993)

ABSTRACT

Two columns (50 mm × 4.6 mm I.D.) packed with sulphonated styrene–divinylbenzene (PS–DVB) gel (particle size 10 μm) and sulphonated silica gel (particle size 5 μm) were used for the separation of alkaline earth metal and several transition metal ions. The mobile phase contained 3-morpholinopropanesulphonic acid (MOPS)–NaOH buffer and a colour-forming chelating agent, and on-column derivatization and spectrophotometric detection were employed. Arsenazo III, Chlorophosphonazo III, Carboxyarsenazo, Sulphonazo III and Dimethylsulphonazo were examined as mobile phase components for good separation and highly sensitive detection. The PS–DVB gel column and Chlorophosphonazo III gave the highest sensitivity. Ca²⁺, Sr²⁺ and Ba²⁺ were separated in 5 min with a mobile phase containing 2 · 10⁻⁴ M Chlorophosphonazo III and 2.5 · 10⁻² M MOPS–NaOH buffer (pH 7.0), whereas Mg²⁺ was eluted after 30 min. An irregular retention sequence was observed, *i.e.*, Ca²⁺ < Sr²⁺ < Ba²⁺ << Mg²⁺; the capacity factors (*k'*) were 1.3, 3.3, 5.3 and 42.8, respectively. The separation of alkaline earth and several transition metal ions was also examined. Application to the determination of Ca²⁺ and Mg²⁺ in tap water is described.

INTRODUCTION

The separation and determination of alkaline earth metal and heavy metal ions have been carried out with ion-exchange columns mainly using conductimetric detection. On the other hand, reversed-phase high-performance liquid chromatography has been increasingly employed for the separation and determination of various metal ions with spectrophotometric detection.

However, spectrophotometric detection in the visible absorption region has not often been employed in ion-exchange chromatography.

Fritz and co-workers [1,2] reported the separation of alkaline earth metal ions on a low-capacity cation-exchange resin with postcolumn derivatization and spectrophotometric detection. However, with post-column derivatization the analytical precision is sometimes influenced by the pumping delivery system. Zenki [3] and Toei [4–7] employed mobile phases containing a colour-forming agent, which reacts with metal ions to form coloured complexes, and applied

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this method to the separation and determination of alkaline earth metal and some heavy metal ions. For higher sensitivity, better separation and better reproducibility, the selection of the column and chromogenic reagent and the eluent composition are important.

In this study, a sulphonated silica gel column and a sulphonated styrene–divinylbenzene (PS–DVB) gel column were examined for the separation of alkaline earth and some heavy metal ions. A mobile phase containing a chromogenic reagent and a buffer containing eluent cations, a double-plunger pump and a spectrophotometric detector were employed. The method was applied to the determination of Ca^{2+} and Mg^{2+} in tap waters. The separation of some heavy metal ions and alkaline earth metal ions was also studied.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an SRX-3600T double-plunger pump (Sanuki, Tokyo, Japan) or a CCPD double-plunger pump (Tosoh, Tokyo, Japan), a Model 860-CO column oven (JASCO, Tokyo, Japan), a JASCO Model 870 UV–Vis detector (wavelength range 190–700 nm; flow cell 8 μl ; optical path length 5 mm) and a JASCO CC-11 recorder. A Sanuki SVM-6M2L metal-free sample injector (dead volume 2 μl) was used.

A TSKgel IC-Cation column (Tosoh) packed with sulphonated porous PS–DVB copolymer gel (particle diameter 10 μm ; cation-exchange capacity 12 ± 2 $\mu\text{equiv./ml}$; pH range 1–12) and a TSK gel IC-Cation-SW column (Tosoh) packed with sulphonated silica gel (particle diameter 5 μm ; cation-exchange capacity 0.3 ± 0.1 mequiv./g ; pH range 2–8). Both columns were 50 mm \times 4.6 mm I.D. and were made of plastics.

PTFE tubing of 0.5 mm I.D. was used throughout except between the column and sample injector (0.25 mm I.D.).

A UV-250 double-beam spectrophotometer (Shimadzu, Kyoto, Japan) and an SAS-727 atomic absorption spectrophotometer (Seiko Denshi, Tokyo, Japan) were used.

Reagents

Five bisarylazochromotropic acids (Chlorophosphonazo III, Arsenazo III, Carboxyarsenazo, Sulphonazo III and Dimethylsulphonazo) were purchased from Dojindo (Kumamoto, Japan). Most of the Chlorophosphonazo III used in this work was kindly provided by Professor L. Sommer (Brno, Czech Republic). These reagents were used as received.

The solutions of alkaline earth metals were prepared by dissolving the analytical-reagent grade chlorides in water and were standardized against standard EDTA solution with the indicators. Calmagite for magnesium, Calcon carboxylic acid for calcium and magnesium Eriochrome Black T–EDTA for strontium and barium. Other metal ion solutions were prepared from the analytical-reagent grade nitrates or chlorides. These solutions were standardized against standard EDTA solution with the appropriate indicators.

Redistilled water or water purified by means of a Toray Pure LV-10T system (Toray, Tokyo, Japan) was used.

Mobile phases

Mobile phase A contained $2 \cdot 10^{-4}$ M Chlorophosphonazo III and $2.5 \cdot 10^{-2}$ M 3-morpholinopropanesulphonic acid (MOPS)–NaOH buffer. Mobile phase B contained $2 \cdot 10^{-4}$ M Chlorophosphonazo III, $2.5 \cdot 10^{-2}$ M MOPS–NaOH buffer and $6 \cdot 10^{-2}$ M KCl. Mobile phase C contained $2 \cdot 10^{-4}$ M Chlorophosphonazo III, $2.5 \cdot 10^{-2}$ M MOPS–NaOH buffer and $2 \cdot 10^{-2}$ M NaCl. The mobile phases, with the pH adjusted to 7.0, were filtered through a membrane filter (PTFE T050A047A; Toyoroshi, Tokyo, Japan).

Procedure

Mobile phase A was pumped at a flow-rate of 1.0 ml min^{-1} . After the column had been equilibrated with the mobile phase, an aliquot (10 μl) of sample solution was placed on the column via the injection valve. In order to prevent contamination with metal ions from a microsyringe, sample solution was introduced into the sample injector by suction. Elution of metal complexes was monitored at 655 nm. For

earlier elution of Mg^{2+} , mobile phase A was changed to mobile phase B after 10 min.

As city tap waters contained negligible concentrations of metal ions other than Ca^{2+} and Mg^{2+} , mobile phase C was employed for the determination of calcium and magnesium in tap water samples.

RESULTS AND DISCUSSION

Comparison of columns

Two kinds of columns, packed with sulphonated PS–DVB gel and sulphonated silica gel, were compared. With both columns the retention sequence of alkaline earth metal ions was the same, that is, $\text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+} \ll \text{Mg}^{2+}$. The strong retention of Mg^{2+} was unusual. According to the catalogue from Tosoh, Mg^{2+} elutes faster than Ca^{2+} when using 0.5 mM ethylenediamine solution (pH 6.0) as the mobile phase with the same columns. The sensitivity of detection was higher with the PS–DVB gel than with the silica gel column (Fig. 1). Therefore, the PS–DVB gel column was mainly used in subsequent work.

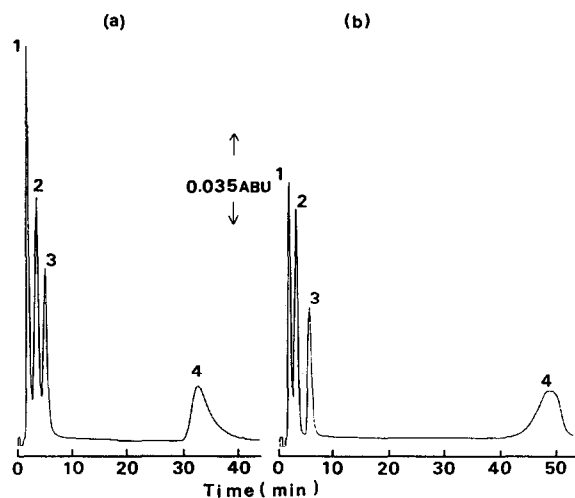


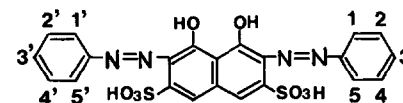
Fig. 1. Chromatograms of alkaline earth metal ions. (a) PS–DVB gel column (particle size 10 μm ; 50 mm \times 4.6 mm I.D.); (b) silica gel column (particle size 5 μm ; 50 mm \times 4.6 mm I.D.). Each metal ion concentration, $5 \cdot 10^{-4}$ M; injection volume, 10 μl ; mobile phase A, $2.5 \cdot 10^{-2}$ M MOPS–NaOH buffer containing $2 \cdot 10^{-4}$ M Chlorophosphonazo III (pH 7.0); flow-rate 1.0 ml min^{-1} ; detection wavelength 665 nm. Peaks: 1 = Ca; 2 = Sr; 3 = Ba; 4 = Mg.

Selection of chromogenic reagent

A chromogenic reagent as a component of the mobile phase may play the important role in the separation, elution and detection of metal ions. A chromogenic reagent that reacts with various metal ions to form stable and highly sensitive complexes must be selected. On the other hand, if the reagent forms inert complexes, good separations will not be obtained. Six water-soluble bisaryazo chromotropic acids, which are expected to form highly sensitive chelates with alkaline earth and heavy metal ions, were selected and examined. The structures of these reagents (Table I) are similar. Under almost identical conditions the absorption spectra of the reagents and the complexes with Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} were measured at ca. pH 7. Fairly large differences in absorbances between the free reagent and the complexes were observed with Chlorophosphonazo III and Arsenazo III. Chlorophosphonazo III was selected because the Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} complexes showed almost same absorbance at 665 nm. Chlorophosphonazo III, which gives the highest molar absorptivity of the complexes around pH 7, was employed as a component of mobile phase: $\epsilon = 2.7 \cdot 10^4$ (Mg), $3.8 \cdot 10^4$ (Ca), $4.1 \cdot 10^4$ (Sr) and $4.2 \cdot 10^4$ (Ba) $\text{l mol}^{-1} \text{cm}^{-1}$ at 660 nm. The absorption spectra are shown in Fig. 2. According to Budesinsky *et al.* [8], Chlorophosphonazo III forms complexes such as $\text{Mg}(\text{H}_4\text{L})^{2-}$, $\text{Ca}(\text{H}_8\text{L}_2)^{6-}$, $\text{Sr}(\text{H}_8\text{L}_2)^{6-}$ and $\text{Ba}(\text{H}_6\text{L}_2)^{8-}$ and the conditional stability constants were reported to be $10^{5.3}$, $10^{10.0}$, $10^{11.5}$ and $10^{12.5}$, respectively,

TABLE I

BISAZOCHROMOTROPIC ACIDS EXAMINED



| Compound | Substituents |
|-----------------------|---|
| Chlorophosphonazo III | 1,1'- PO_3H_2 , 3,3'-Cl |
| Arsenazo III | 1,1'- AsO_3H_2 |
| Carboxyarsenazo | 1-COOH, 1'- AsO_3H_2 |
| Sulphonazo III | 1,1'- SO_3H |
| Dimethylsulphonazo | 1,1'- SO_3H , 3,3'- CH_3 |

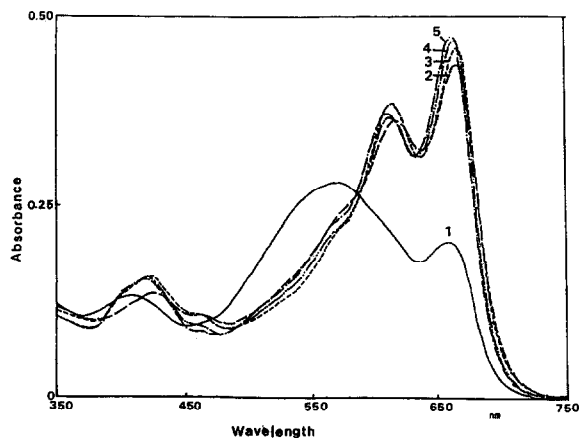


Fig. 2. Absorption spectra of Chlorophosphonazo III and its chelates at pH 7. 1 = Reagent; 2 = Mg; 3 = Ca; 4 = Sr; 5 = Ba. Reagent concentration, $1 \cdot 10^{-5}$ M; metal ion concentration, $8 \cdot 10^{-5}$ M.

at pH 7.5. The results of the continuous variation method in this work supported the ratio of metal to ligand in the complexes and the order of conditional stability constants estimated in the previous study [8].

Various heavy metal and rare earth metal ions also react with Chlorophosphonazo III to form similar coloured complexes.

Composition of mobile phase

MOPS-NaOH, $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$, ethylenediamine-HCl and tris(hydroxymethyl)aminomethane(Tris)-HCl were examined as components of the eluent. When ethylenediamine-HCl buffer was used the baseline was not stable and with Tris-HCl buffer Ba^{2+}

and Mg^{2+} were not detected. When MOPS-NaOH buffer or $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ buffer was used, better chromatograms were obtained. When the pH of mobile phase was varied from 6 to 8, the best chromatogram was obtained at pH 7. The MOPS-NaOH buffer was selected owing to the larger buffer capacity around pH 7 (Fig. 3a). The concentration of NaCl in the buffer affected the retention times of the metal ions. The retention times at various concentrations of Na^+ in the mobile phase are shown in Fig. 3b. The best separation of Ca^{2+} , Sr^{2+} and Ba^{2+} was obtained with a mobile phase containing $2.5 \cdot 10^{-2}$ M MOPS-NaOH buffer (pH 7), whereas the retention of Mg^{2+} was still very strong.

Concentration of Chlorophosphonazo III

With increasing concentration of Chlorophosphonazo III the retention time decreased and the peak height increased, but on increasing the concentration to $3 \cdot 10^{-4}$ M the separation deteriorated (Fig. 3c). To achieve a smaller background and higher sensitivity, the concentration of Chlorophosphonazo III in the mobile phase was selected as $2 \cdot 10^{-4}$ M. The capacity factors (k') of Ca, Sr, Ba and Mg were 1.3, 3.3, 5.3 and 42.8, respectively, with the PS-DVB column and 0.8, 2.3, 5.3 and 59.5, respectively, with the silica gel column. The conditional stability constants of Chlorophosphonazo III complexes at pH 7.5 have been reported to be $10^{5.3}$ (MgH_4L), $10^{10.0}$ (CaH_8L_2), $10^{11.5}$ (SrH_8L_2) and $10^{12.5}$ (BaH_6L_2) [8]. The con-

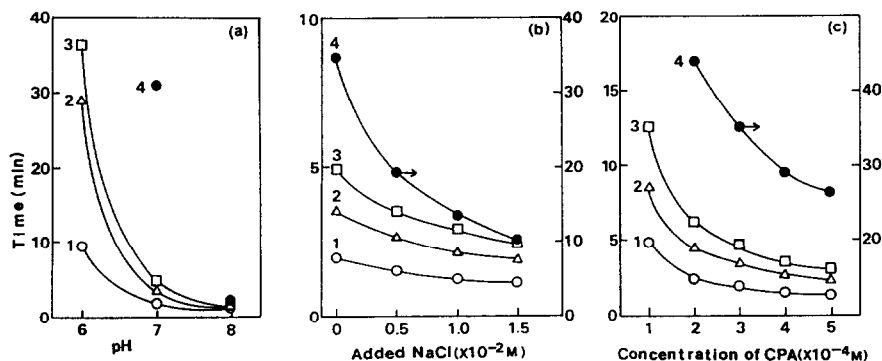


Fig. 3. Effects of (a) pH, (b) added NaCl concentration and (c) Chlorophosphonazo III concentration in the mobile phase on the retention time. 1 = Ca; 2 = Sr; 3 = Ba; 4 = Mg. The PS-DVB gel column was used.

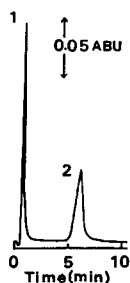


Fig. 4. Chromatogram of Ca and Mg. The PS-DVB gel column and mobile phase C were used. Peaks: 1 = Ca; 2 = Mg.

ditional stability constant of the magnesium complex is much smaller than those of the other complexes, whereas those of Ca, Sr and Ba complexes are almost same. The unusually strong retention of Mg^{2+} can be ascribed to the smaller interaction with Chlorophosphonazo III.

Effect of sodium ion concentration in mobile phase

The sodium ion concentration in the mobile phase was varied from $1.0 \cdot 10^{-2}$ to $2.0 \cdot 10^{-2}$ M. The retention of alkaline earth metals decreased with increasing Na^+ concentration. Using mobile phase A, Ca^{2+} , Sr^{2+} , Ba^{2+} and Mg^{2+} were separated, but the elution of Mg^{2+} was very slow (Fig. 1). For faster elution of Mg^{2+} , mobile phase A was changed to mobile phase B after 10 min. However, in this case the baseline raised and the reproducibility of Mg^{2+} determination deteriorated. When mobile phase C was used,

Ca^{2+} and Mg^{2+} were clearly separated within 7 min. The chromatogram is shown in Fig. 4.

Effect of column temperature

The temperature of the column oven was varied from 25 to 50°C. The retention times of Ca^{2+} , Sr^{2+} and Ba^{2+} increased with increasing temperature but the peaks became broad. As the separation was not improved, a temperature of ca. 25°C was employed.

Calibration output

The calibration output was linear from $0.5 \cdot 10^{-4}$ – $4.0 \cdot 10^{-4}$ M Ca^{2+} , Sr^{2+} and Ba^{2+} based on peak height (Fig. 5a) and from $0.5 \cdot 10^{-4}$ to $1.0 \cdot 10^{-3}$ M based on peak area (Fig. 5b). When only Ca^{2+} and Mg^{2+} were determined, the calibration outputs based on peak area were linear in the range $1.0 \cdot 10^{-5}$ – $8 \cdot 10^{-5}$ M or $1.0 \cdot 10^{-4}$ – $8 \cdot 10^{-4}$ M, as shown in Fig. 6.

Separation of alkaline earth metals and heavy metals

The retentions of Cr^{3+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , Al^{3+} and rare earth metal ions were examined. The peaks of Zn^{2+} , Cd^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} and Pb^{2+} were observed. Zn^{2+} , Cd^{2+} and Mn^{2+} were separated with mobile phase A (Fig. 7a), while Zn^{2+} and Cd^{2+} interfered with the determination of Ca^{2+} , Sr^{2+} and Ba^{2+} . By using an eluent containing $1.5 \cdot 10^{-2}$ M MOPS–NaOH buffer (pH 7) Ca^{2+} , Zn^{2+} , Sr^{2+} and Mn^{2+} were separated but Ba^{2+} and Cd^{2+} were not (Fig. 7b).

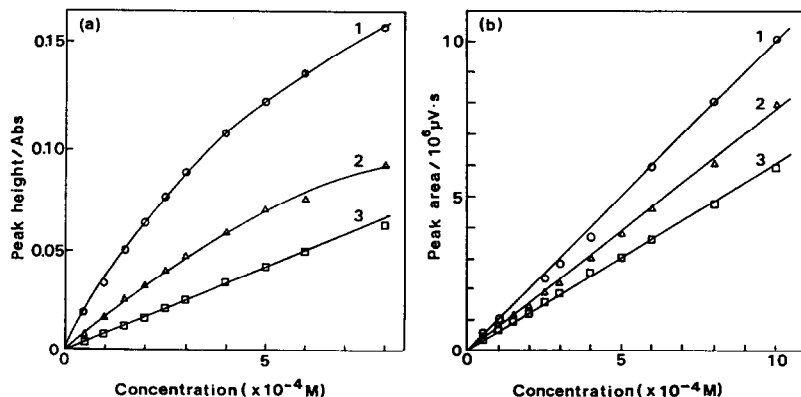


Fig. 5. Calibration outputs of Ca, Sr and Ba with mobile phase A. 1 = Ca; 2 = Sr; 3 = Ba. (a) Peak height; (b) peak area.

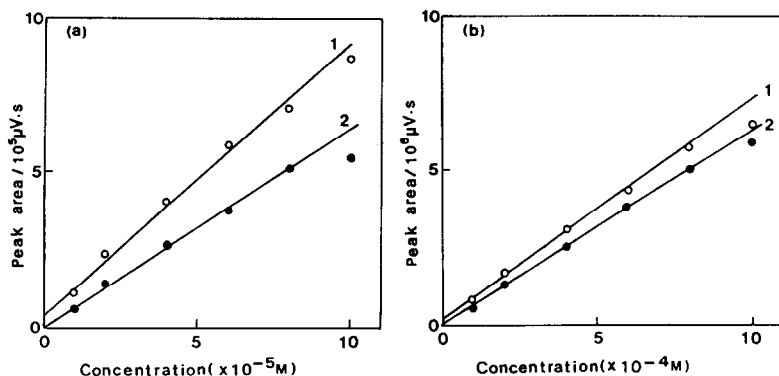


Fig. 6. Calibration outputs (peak area) of Ca and Mg with mobile phase C. 1 = Ca; 2 = Mg. Concentration range: (a) $(1.0-10) \cdot 10^{-5} M$; (b) $(1.0-10) \cdot 10^{-4} M$.

Co^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} and Mn^{2+} at $45^{\circ}C$ were separated but Zn^{2+} and Ca^{2+} were not, as shown in Fig. 7c.

Application

The method was applied to the determination of Ca^{2+} and Mg^{2+} in tap water samples. Using mobile phase A, Ca^{2+} was determined in several city tap waters. When strontium and barium ions ($2 \cdot 10^{-4} M$) were added to the water sample, they did not interfere with the determination of calcium.

Using mobile phase C, Ca^{2+} and Mg^{2+} were determined in the several tap water samples from Nagoya and other cities near Nagoya. Sr^{2+} , Ba^{2+} and heavy metal ions were not detected in

these sample waters. Selected results are given in Table II.

CONCLUSIONS

Sulphonated PS–DVB gel and sulphonated silica gel were compared for the separation of alkaline earth metal ions, and the former was found to be better with respect to separation and sensitivity. Chlorophosphonazo III was employed for on-column derivatization and detection. Metal ions in sample are exchanged with Na^{+} on the column and Chlorophosphonazo III in the mobile phase may accelerate the elution by forming the metal–Chlorophosphonazo III complexes. The larger retention of Mg^{2+} than those of other metal ions is considered to be due to the smaller stability constant of Mg–Chlorophosphonazo III complex.

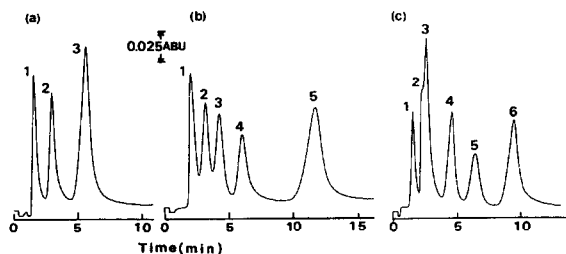


Fig. 7. Chromatograms of heavy metal and alkaline earth metal ions. Each metal ion concentration, $5 \cdot 10^{-4} M$; injection volume, $10 \mu l$. (a) Mobile phase A at room temperature; (b) mobile phase A containing $1.5 \cdot 10^{-2} M$ MOPS–NaOH buffer at room temperature; (c) mobile phase A at $45^{\circ}C$. Peaks: (a) 1 = Zn; 2 = Cd; 3 = Mn; (b) 1 = Ca; 2 = Zn; 3 = Sr; 4 = (Ba + Cd); 5 = Mn; (c) 1 = Co; 2 = Ca; 3 = Zn; 4 = Sr; 5 = Ba; 6 = Mn.

TABLE II

DETERMINATION OF CALCIUM AND MAGNESIUM IN TAP WATER SAMPLES ($mg l^{-1}$)

| Ion | Method | Sample No. | | | | |
|-----------|----------------|------------|------|------|------|------|
| | | 1 | 2 | 3 | 4 | 5 |
| Ca^{2+} | This work | 6.60 | 6.40 | 7.20 | 15.6 | 6.12 |
| | AAS | 6.80 | 6.24 | 7.20 | 15.3 | 5.84 |
| | EDTA titration | 6.72 | 6.32 | 7.20 | 15.2 | 6.04 |
| Mg^{2+} | This work | 1.62 | 0.70 | 1.14 | 2.64 | 0.91 |
| | AAS | 1.65 | 0.72 | 1.20 | 2.76 | 0.90 |
| | EDTA titration | 1.67 | 0.73 | 1.11 | 2.93 | 0.87 |

ACKNOWLEDGEMENT

We thank Professor L. Sommer of Masaryk University (Brno, Czech Republic) for valuable discussions and for providing Chlorophosphonazo III.

REFERENCES

- 1 J.S. Fritz and J.N. Story, *Anal. Chem.*, 46 (1974) 825.
- 2 D.L. Smith and J.S. Fritz, *Anal. Chim. Acta*, 204 (1988) 87.
- 3 M. Zenki, *Anal. Chem.*, 53 (1981) 968.
- 4 J. Toei and N. Baba, *J. Chromatogr.*, 361 (1986) 368.
- 5 J. Toei, *Chromatographia*, 23 (1987) 355.
- 6 J. Toei, *Chromatographia*, 23 (1987) 583.
- 7 J. Toei, *Analyst*, 113 (1988) 247.
- 8 B. Budesinsky, K. Haas and A. Bezdekova, *Collect. Czech. Chem. Commun.*, 32 (1967) 1528.