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Short communication

Ion chromatographic determination of calcium and magnesium cations in human saliva and urine with a piezoelectric detector

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Abstract

A rapid sample extraction procedure for the determination of ascorbic acid (**AA**) by high performance liquid chromatography (HPLC) in multivitamin-mineral formulations containing interfering copper has been developed. The method takes special precautions to prevent degradation of **AA** in contact with high concentrations of interfering elements such as copper. Sample preparation involved addition of pyrogallol, citric acid solution and short time extraction (5 min.) under an atmosphere of nitrogen to prevent oxidation of **AA**. Another sample cleanup based on extraction of the multivitamin-mineral tablet with the extraction solution as described above, but with addition of strong cation exchange sorbent, was also developed. The copper and other minerals in the formula were retained on an ion exchange sorbent and thus represses the speed of oxidation of **AA**. Extracts of multivitamin-mineral tablets were analysed by ion-pair reversed phase HPLC and UV-diode array detection. Ten multivitamin formulations containing copper were analysed by the two proposed methods. The analytical results of **AA** obtained using the simple sample extraction method and the method involving addition of cation exchange were 89 - 115 % and 95 – 112 % respectively of declared concentrations. The parameters for the validation of the methods are given.

Keywords: Calcium; Magnesium; Ion chromatography; Piezoelectric quartz crystal (PQC); Saliva; Urine

1. Introduction

As important mineral elements for human life, calcium and magnesium play essential roles [1,2]. Recently, it was reported that calcium in blood and saliva was important for the evaluation of

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dental development [3,4]. Calcium and magnesium levels in human fluids are clinically determined using atomic adsorption spectroscopy (AAS) or colorimetry [1,2]. Titrition methods are gradually obsolete, and ion-selective electrode

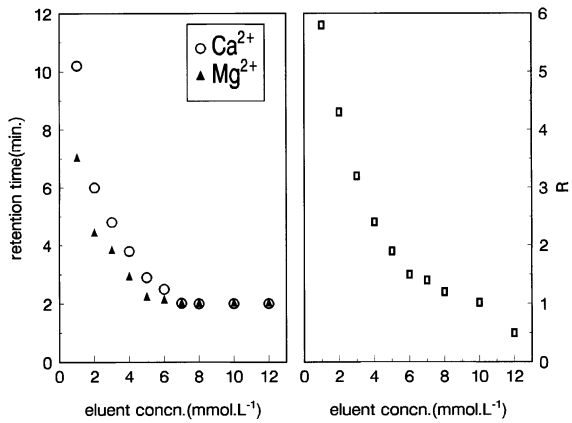

Fig. 1. Effects of total eluent concentration on resolution (R) and retention times of calcium and magnesium cations. Conditions: Shim-pack IC-CI column (5 × 150 mm), 40°C, mobile phase: eluent concentration = [tartaric acid] + [ethylenediamine] (1:1), 1.5 ml min^{-1}, detector: PQC detector.

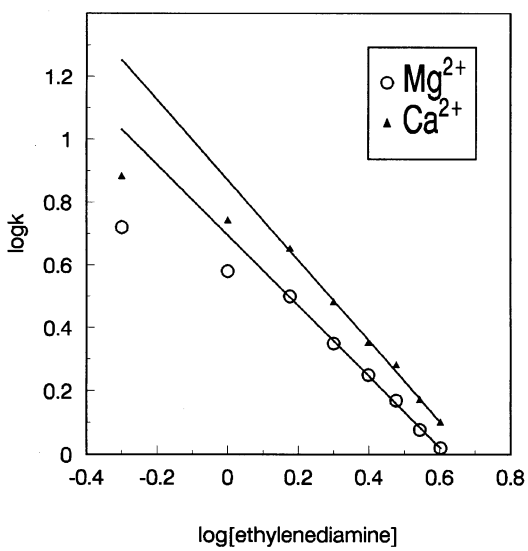

Fig. 2. Logarithmic plots of eluite retention factors versus ethylenediamine concentration (mmol l^{-1}). Conditions: Shim-pack IC-C1 column (5 × 150 mm), 40°C, mobile phase: [tartaric acid] + [ethylenediamine] = 6.0 mmol l^{-1}, 1.5 ml min^{-1}, detector: PQC detector.

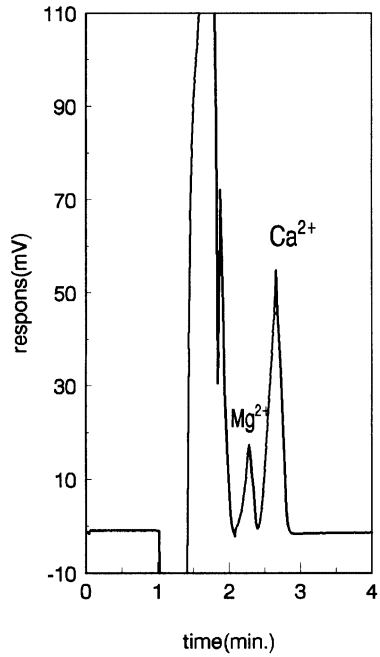

Fig. 3. Chromatogram of a urine sample. Conditions: Shim-pack IC-C1 column (5 × 150 mm), 40°C, mobile phase: tartaric acid (4 mmol l^{-1}) + ethylenediamine(2 mmol l^{-1}), pH 4.0, 1.5 ml min^{-1}, injection volume: 100 μl, detector: PQC detector.

(ISE) method is commonly used in case of determining calcium cation not complexed to proteins or other complexing agents, but interferences from accompanying ions, hydrogen ions and ionic strength difference are problematic for analysis. Buchley and Russell [5] reviewed those methods for assay of serum calcium cation, including non-potential methods (such as, spectrophotometry), potential techniques (such as, ISE method and other electrochemical methods), and some reference methods. Freaney and co-workers [6] described an automatic method for determining calcium in blood, serum and plasma. The development course of the reference methods for Ca and Mg determination was briefly outlined by Thienpont et al. [7]. AAS method was the first recommended reference procedure for determination of total Ca or total Mg.

Ion chromatography (IC), as a versatile separation technique, has achieved wide application in many areas since its development [8,9]. This tech-

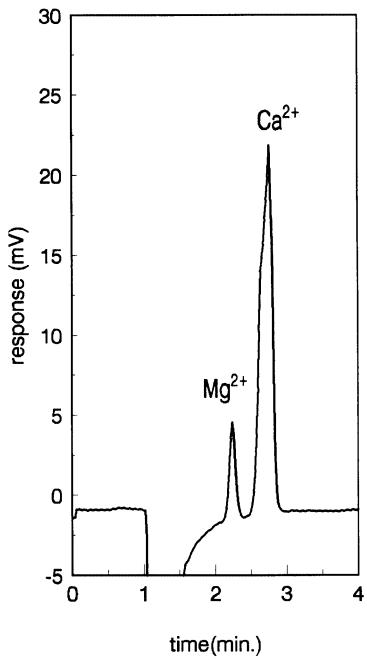

Fig. 4. Chromatogram of a saliva sample. Conditions were the same as that in the Fig. 3.

nique has also been used to determine calcium and magnesium in body fluids [4,7,10,11] and consequently was recommended as a candidate reference method for this purpose [7,11]. Among the several detection techniques for IC, conductometric detection is widely used because of its simplicity and specificity for ionized components [9,12]. In the present paper, a piezoelectric quartz crystal (PQC) detector was developed and used for ion chromatographic determination of Ca^{2+} and Mg^{2+} levels in human saliva and urine. In fact, PQC sensor has been heavily researched for its application in analytical chemistry since it was first proposed in 1964 by King [13]. Its feasibility as a gas chromatography detector was first demonstrated by the same author [14], and as liquid chromatography detector first by Konash [15] and then by Nomura [16,17]. PQC detector for IC was recently reported [18,19]. In principle, PQC detector responds conductivity and permittivity of a solution. However, it has striking advantages over the conventional conductivity detector with many respects, in particular, it's free of errors resulted from double electric layer and Faradaic impedance [18].

2. Experimental

2.1. Reagents and materials

The chemical used were of analytical grade or better. Distilled deionized water was used throughout. Mobile phase and sample solutions were filtered through a 0.45 μm filter membrane (Millipore, US) to protect the chromatography system.

2.2. Chromatographic system

Chromatographic separation was done using a Shimadzu IC-6A ion chromatography system (Kyoto, Japan), which consisted of an LP-6A liquid delivery pump, an SLC-6B system controller, an SIL-6B auto injector, and a CTO-6AS column oven. The column used was a Shimpack IC-C1 guard column (5.0 × 150 mm) packed with

Table 1
Calcium and magnesium levels in saliva and urine samples (mg l^{-1})[a]

Sample	Ca			Mg		
	Mean ($n = 5$)	CV (%)	AAS ($n = 3$)	Mean ($n = 5$)	CV (%)	AAS ($n = 3$)
Saliva 1 #	102	2.9		8.1	2.1	
Saliva 2 #	78.7	2.5	83.2 ($s = 1.8$)	8.0	2.4	8.5 ($s = 1.8$)
Urine 1 #	243	3.0		90.5	2.5	
Urine 2 #	399	3.5	412 ($s = 14$)	77.9	2.5	82.6 ($s = 1.8$)

[a] s is the standard deviation.

Table 2
Recoveries of Calcium and Magnesium by the IC-PQC

Sample	Ca			Mg		
	Added (μg)	Total found (μg)	Recovery (%)	Added (μg)	Total found (μg)	Recovery (%)
Saliva	0	102		0	8.1	
	80	181	99	8.0	15.7	95
	100	200	98	5.0	12.9	96
Urine	0	243		0	90.5	
	200	453	105	50	137.5	94
	250	510	107	100	180.5	90

a surface functional cation-exchange resin on polystyrenedivinylbenzene support incorporating a sulfonic acid base as a functional group. A Shim-pack IC-GC1 guard column (4.0 × 10 mm) was used. Mobile phase was a solution (pH 4.0) containing tartaric acid (4.0 mmol l^{-1}) and ethylenediamine (2 mmol l^{-1}) at a flow rate of 1.5 ml min^{-1}, which was degassed prior to use. The temperatures of the column and PQC detector were kept at the same temperature of 40°C so as to enable the best performance of the piezoelectric detector.

2.3. Piezoelectric detector

The piezoelectric detector was designed and constructed in this laboratory. It consisted of a serial piezoelectric quartz crystal (SPQC) oscillator and a conductivity detection cell. The SPQC device was assembled by connecting a 9 MHz quartz crystal to a TTL-IC oscillating circuit. Construction of the detection cell was made as the method described in the reference [18]. The cell constant of the detection cell was 3.2 cm^{-1}, which was experimentally proven to give best response of the piezoelectric detector.

The detector signal in PQC was primarily digital frequency. For convenience of automatic recording and processing of chromatogram in real time, a self-made frequency/voltage converter (F/V converter) [20] was used to transform the frequency signal into an analog signal of voltage, which was used by the chromatographic workstation Shimadzu CR-4A Chromatopac data processor (Kyoto, Japan).

2.4. Experimental procedure

The mobile phase was pumped into the chromatographic system to equilibrate it. When repetitive injections of a standard solution gave same retention times and peak areas, sample solution was then injected for analysis, chromatogram was recorded in real time as the aforementioned method.

2.5. Sample processing

The saliva sample was collected without apparent external stimuli (such as, chemical stimulatant, chewing, etc.) to the subjects. The salivary and urine samples were passed through a 0.45 μm filter membrane, and an aliquot of 100 μl filtrate was injected for analysis. The filtrate was diluted, if necessary, prior to injection.

3. Results and discussion

3.1. Influence of mobile phase concentration

The mobile phase concentration is an important affecting factor for ion chromatographic resolution and detection. Low concentration of eluent is usually used in IC, especially in the non-suppression mode, due to the necessity of low background conductivity for suitable detection sensitivity. But in many cases, a compromise has to be made between chromatographic resolution and detection sensitivity when an eluent concentration is chosen. For PQC detector, it responds

to both conductivity and permittivity of the solution. When a dilute solution was used as IC mobile phase, permittivity of the solution was kept constant, therefore, the conductivity change of the solution would be the only factor responded by SPQC sensor. It was demonstrated that the piezoelectric detector used in this work was rather sensitive over the range of 150–1200 μS cm^{-1} background conductivity.

Many different eluents were used for the separation of alkaline earth metals by IC. As the earliest used one, *o*-phenylenediamine had an awful drawback in that its oxide species were likely to be strongly retained on the inlet end of column [8]. Ethylenediammonium nitrate showed considerable merit for resolution of alkaline earth metal cations, but it was apt to influence of the pH value, because of a roving interference peak that likely occurred when it was used in pH 3.6–5.7. The mobile phase containing selective ligands usually reached an acceptable resolution of many cations, including alkaline earth metals. In this work, the solution containing tartaric acid and ethylenediamine was used as the mobile phase. Separation of calcium and magnesium was tested in case of different concentrations and various ratios of eluent ions and ligands. Experimental results (Fig. 1) showed that solution containing tartaric acid (4.0 mmol l^{-1}) and ethylenediamine (2.0 mmol l^{-1}) was an appropriate mobile phase (background conductivity about 870 μS cm^{-1}).

In IC theory, the logarithmic linearities between the eluite retention factor k and eluent concentration have been extensively demonstrated, provided the other conditions were kept consistent. In this work, logarithmic relationship between the eluites retention factor and eluent concentration was also investigated. When the total concentration of tartaric acid and ethylenediamine were kept unvaried, the logarithmic relation between the retention factor of calcium, magnesium cations and ethylenediamiane concentration was illustrated as Fig. 2, which showed linearities with slopes of -1.25 for Ca^{2+} and -1.12 for Mg^{2+} in region of higher concentration of ethylenediamine. The results could be explained as follows. According to Fritz's work [21], when a complexing agent was in presence with the eluent ion, the following equation was obvious,

$$\ln k = \frac{y}{x} \ln \alpha_M - \frac{y}{x} \ln [enH_2^{2+}] + C \quad (1)$$

wherein y and x were electric charges of the eluite M^{y+} and eluent ion ethylenediammonium enH_2^{2+}, respectively, and α_M was the fraction of metal existed as free cation in the solution, C was a constant depending upon the exchanging capacity of the resin and the equilibrium constant of the exchanging reaction between the metal cations and the exchanged ion on the resin surface. In Fig. 2, in the region of higher concentration of ethylenediamine, the mass action 'pulling' effect of the ethylenediammonium cation dominated the elution mechanism [21], the slopes were close to the theoretical value of -1 from Eq. (1); whereas in the region of lower concentration of ethylenediamine and hence higher concentration of tartaric acid, the complexing or 'pulling' effect of the tartrate anion could not be neglected in the elution mechanism, calcium and magnesium cations were eluted expeditiously by this complexing effect, their logarithmic retention factors deviated the linearities.

3.2. Sensitivity and precision of the method

Under the chosen conditions, in wide concentration ranges of cations (0.8–500 mg l^{-1} for Ca^{2+} and 1.0–500 mg l^{-1} for Mg^{2+}), there were good linearities between chromatographic peak area (A, $\times 10^3$μV s) and cation concentration (C, mg l^{-1}). The regression equations were listed as follows:

$$A = -0.988 + 1.35C_{Ca} \quad r = 0.9994$$

$$A = -0.819 + 2.68C_{Mg} \quad r = 0.9993$$

The detection limits, defined as signal-to-noise ratio of 3, for calcium and magnesium cations were 0.4 and 0.2 mg l^{-1}, respectively.

Repeatability of the methods was tested. Ca^{2+} and Mg^{2+} in the standard solution (20 mg l^{-1} for both cations) was repeatedly separated and determined for seven times by this developed method. The obtained coefficients of variation (CV) were 0.52% for Ca^{2+} and 0.47% for Mg^{2+}, respectively.

3.3. Analysis of human body fluids

The proposed IC-PQC method was used to determine Ca^{2+} and Mg^{2+} levels in human saliva and urine samples. Protein was isolated from the samples by filtering through a micropore filter membrane to protect the column. Typical chromatograms of urine and saliva sample were given as Figs. 3 and 4, respectively. The analytical results were summarized in Table 1. Recoveries of spiked calcium and magnesium were listed in Table 2, which showed recoveries of 90–107%. To validate the method, some saliva and urine samples were also subjected to AAS determination of calcium and magnesium using a GBC932AA atomic absorption spectrometer (GBC Scientific Equipment Pty Ltd., Australia), the results were also given in Table 1. The Student's t-test can be used to verify the agreement between both the methods. The t value at freedom degree of 6 (given by $n_1 + n_2 - 2 = 5 + 3 - 2 = 6$) and probability level of 99% is 3.707. The t values for the determination of Ca and Mg cations in salivary and urinary sample can be readily calculated as $t_{\text{saliva, Ca}} = 3.08$, $t_{\text{saliva, Mg}} = 3.42$, $t_{\text{urine, Ca}} = 1.78$, and $t_{\text{urine, Mg}} = 3.22$. The calculated t values were all smaller than 3.707 and hence showed no statistically significant difference between the IC-PQC and AAS for the determination of Ca^{2+} and Mg^{2+} cations.

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