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Method development and validation for the determination of mineral elements in food and botanical materials by capillary electrophoresis

Q. Yang, C. Hartmann, J. Smeyers-Verbeke, D.L. Massart*

Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium

Abstract

A capillary electrophoresis (CE) procedure was developed and validated for the determination of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in solid natural products. Closed-vessel microwave acid digestion [HNO_3–H_2O_2 (2:0.5)] was used for the sample preparation. Digests of these samples were diluted with deionized water and the resulting solutions injected for CE. The excess of nitric acid in the samples was found to influence the analytical performance, so its effect was investigated in detail. Direct calibration with aqueous standard solutions was applicable for the analysis of all sample types. To evaluate the bias of the proposed procedure, reference materials were analysed. The results agreed well with the certified or recommended values. The precision of the procedure was evaluated with a two-level nested analysis of variance. This allows one to estimate separately the variance due to three factors (the CE measurement, the sample preparation and time) that are expected to contribute to the variability of the measurement results. For all the elements determined, the CE system repeatability (R.S.D.) was smaller than 4%, method repeatability smaller than 6% and the within-laboratory reproducibility smaller than 9%. The limit of detection (LOD) and limit of quantification (LOQ) in solution are below 600 μg/l, except for K^+, for which the LOQ is about 2 mg/l.

1. Introduction

Currently, metallic elements in natural products and biological materials are mostly determined using atomic absorption spectrometry (AAS) or inductively coupled plasma combined with atomic emission spectrometry (ICP-AES) or mass spectrometry (ICP-MS), as well as ion chromatography (IC). However, capillary electrophoresis (CE) seems to be an alternative multi-element technique. With this technique, inorganic ions are electrophoretically separated in a capillary and monitored on-column by direct or indirect UV detection. In case of indirect detection, a UV-absorbing substance is incorporated into the electrolyte buffer, providing a constant background absorbance. The zones of the ions are detected because they lead to a decrease in optical absorbance on passing through the detection window. The electrophoretic separation selectivity can be manipulated by complexation, changes in buffer pH [1], solvation of organic solvents [2,3] and addition of a surfactant to the electrolyte buffer [4]. For the separation of inorganic cations, we established a background electrolyte system composed of imidazole (as the absorbing probe), 2-hydroxyisobutyric acid (HIBA) and 18-crown-6 (as com-

* Corresponding author.

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plexing agents) and methanol, and described the mobility of the inorganic cations as a function of the important system parameters using an empirical [2] and a theoretical model [3], respectively. By applying the models, a selectivity optimization was carried out, resulting in a good separation of thirteen inorganic cations including ammonium and alkali, alkaline earth and transition metals. Separation of ions in tea infusion was conducted to examine the practical usefulness of the optimized CE method [2]. The experimental conditions derived earlier were applied here to evaluate the performance of the technique in the analysis of more complex samples.

There is growing interest in applying CE for the determination of metallic elements in real samples. Beck and Engelhardt [5] determined alkali and alkaline earth metals in mineral water and the results agreed with those obtained by using HPLC. A similar CE method [1] was applied to determine the elements in parenteral electrolyte solutions and beverages, and compared with flame atomic spectrometry (FAS). Most of the results obtained from both methods were in good agreement, except for Ca^{2+} in the parenteral solutions, showing slightly lower results than for FAS. This is probably due to the binding of the analyte ions to some sample components, such as proteins and amino acids [1,6]. It was suggested that a sample pretreatment was necessary [1]. Recently, Shi et al. [7] reported the determination of metallic elements in ocular lenses of animals and results comparable to those given by FAS were obtained. Oehrle et al. [8] determined metallic elements in waste water.

Solid materials have to be decomposed before performing CE. Wet acid digestion is commonly used to dissolve products such as foodstuffs and plants. The acid digestion is often carried out in a closed-vessel system and assisted by microwave heating, which ensures rapid sample preparation with minimized contamination and reduced loss of analytes [9,10]. Microwave-assisted digestion has been widely studied in the past and successful procedures are well documented. Recently, applying closed-vessel microwave-assisted digestion, Morawski et al. [11] determined Na^+, K^+, Mg^{2+} and Ca^{2+} in certain food products with CE. Most of the results were comparable to those given by AAS and ion chromatography. The results for K^+ were higher than expected, however. According to the authors, this was due to the co-migration of K^+ and NH_4^+. Kajiwara et al. [12] reported the determination of Ca^{2+} and Mg^{2+} in wheat flour, where EDTA extraction of these divalent elements was performed.

In our previous work on AAS [10], a rapid closed-vessel microwave-assisted acid digestion procedure was developed. A wide range of foodstuffs were decomposed using a digestion mixture of HNO_3 and H_2O_2. The sample preparation procedure was validated for the determination of trace aluminium, lead and cadmium by graphite furnace atomic absorption spectrometry. Here, we applied the same procedure to decompose food and botanical reference materials and various tea samples. The resulting solutions were diluted with Milli-Q-purified water and injected hydrostatically for the determination of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} with CE. It should be noted that the closed-vessel digestion results in an excess of acids in the sample solutions and therefore causes a high ionic strength, which can consequently affect the separation efficiency [13]. The effect of nitric acid was therefore investigated with synthetic solutions to understand better its influence on the analytical performance.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument was a Waters Quanta 4000 capillary electrophoresis system with a 20-sample carousel and a zinc lamp detector (214 nm). Accusep fused-silica capillaries (60 cm × 75 μm I.D.) were used in all analyses. A positive voltage of 20 kV was applied. The detector time constant was 0.3 s. Samples were introduced by hydrostatic injection from a 10-cm height for 20 s. The electrophero-

grams were recorded and treated with a Waters Model 810 data workstation equipped with a W51-Watch-Dog interface. Temperature control was carried out as described previously [1].

The digestions were performed on a programmable Milestone 1200 microwave digestion system with a maximum power supply of 1200 W and equipped with the ACM-100 automatic capping module. Teflon HPV 80 high-pressure vessels (80 ml) with safety shields which could withstand up to 120 bar of pressure and a temperature of 300°C were used.

2.2. Capillary preparation and cleaning

At the beginning of every working week, the capillary was washed successively with 0.1 *M* KOH, 0.1 *M* HCl, Milli-Q deionized water and background electrolyte, each for 5 min. Between each injection, the capillary was washed for 1 min with 0.1 *M* KOH and Milli-Q water and for 3 min with the electrolyte buffer. At the end of the day, the capillary was rinsed with Milli-Q water for 5 min and left filled with water.

2.3. Reagents and standards

Water used for the preparation of all solutions was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) and contained no detectable analyte cations.

Titrisol concentrates of 1000 μg/ml of Na^+, K^+, Mg^{2+}, Ca^{2+} and Mn^{2+} (Merck, Darmstadt, Germany) were used in this experiment. Standard solutions containing different concentrations of the above elements were prepared by mixing the appropriate amounts of the above concentrates.

Imidazole (99%, w/w) was of analytical-reagent grade (Merck). Methanol was of chromatographic grade (Merck). 2-Hydroxyisobutyric acid and 18-crown-6 were 99% pure reagents (Aldrich-Chemie, Steinheim, Germany). HNO_3 (65%, w/w) of high purity (Suprapur; Merck) was used for sample preparation. Hydrogen peroxide (30%, w/w) was of analytical-reagent grade (Merck). HCl (1 *M*) used for the buffer pH adjustment was obtained from Merck. 2-(Diethylamino)ethanol (99%, w/w) (zur Synthese; Merck) was used to neutralize samples.

2.4. Preparation of background electrolyte

First three stock solutions containing 500.0 m*M* imidazole, 130.6 m*M* HIBA and 50.0 m*M* 18-crown-6 were prepared. A CE background electrolyte buffer was then prepared by pipetting 5, 25 and 5.5 ml, respectively, of the above stock solutions and 100 ml methanol into a 500-ml plastic volumetric flask and diluting to volume with Milli-Q water. The pH of the buffer was adjusted to 4.5 ± 0.4 with 1 *M* HCl. The electrolyte solution was stored in a refrigerator. Just before use it was filtered through a 0.45-μm syringe filter (Millipore, Molsheim, France).

2.5. Standard reference materials, tea samples and their decomposition

NIST standard reference materials Bovine Liver (SRM 1577), Total Diet (SRM 1548), Oyster Tissue (SRM 1566a), Pine Needles (SRM 1575) and Citrus Leaves (SRM 1572) and IAEA reference material Fish Tissue (MA-B-3/TM) were used for the evaluation of the bias. From each type of reference sample, three portions of ca. 0.4 g were weighed. To each of the portions, 2 ml of HNO_3, 0.5 ml of H_2O_2 and 1 ml of Milli-Q water were added. Four samples were digested simultaneously using 180, 360 and 600 W power, each for 3 min, followed by 0 W for 3 min. The first three steps were then repeated. Finally, the system was operated for another 2 min at 0 W to remove possible acid vapour in the compartment of the oven. All of the digests were first diluted to 10 ml with Milli-Q water. The resulting solutions therefore contained at most 20% (v/v) nitric acid, which corresponds to 0.44 *M* (the consumption of nitric acid during digestion is not taken into account). The solutions were further diluted 25-, 50-, 125- or 250-fold to ensure that the element concentrations in the individual samples fell within the calibration range (0.5–10 μg/ml). Quantitative analysis was

performed with digests that had been diluted at least 50-fold.

Tea samples were bought at tea shops in China and Belgium. Two samples of ca. 0.4 g were digested and diluted. The digestion and dilution were described above.

Corresponding digestion blanks were also prepared according to the above procedure. The details about the contamination control and labware cleaning for the sample preparation can be found in Ref. [10].

3. Results and discussion

3.1. Use of the time-corrected peak area for calculation

Absolute peak areas for the standard solutions were observed to increase with time during the experiments. It is known that the peak area is inversely related to the migration velocity of the ions [1]. The slower an analyte zone moves, the larger is the absolute peak area. The velocity of the ion is influenced by many parameters, such as the temperature inside the capillary and capillary surface properties. The capillary surface influences the migration velocity, probably mainly through the change in electroosmotic flow (EOF). The EOF (V_{eof}) change causes variations in the effective migration velocity (V_{eff}) since $V_{eff} = V_{eof} + V_{ep} = (\mu_{eof} + \mu_{ep})E$, where μ_{eof} and μ_{ep} represent the electroosmotic mobility and electrophoretic mobility, respectively; E is the electrical field applied across the capillary. The ratio of the absolute peak area to migration time corrects for the change in migration caused by the variation in effective mobility of the analyte cations [14]. Therefore, the corrected area ($A_{corr.}$) was used in all calculations and computed as follows:

$$A_{corr.} = A/T_m$$

where A is the absolute peak area of an analyte cation zone and T_m is the migration time of the analyte cation.

3.2. Effect of nitric acid in samples on analytical performance

CE separation was found to be impossible if the sample solutions containing 20% (v/v) nitric acid (see Section 2.5) were injected directly. This is due to too high an ionic strength of the sample solutions, which lowers the separation efficiency [1,13]. The high ionic strength is caused by the excess of acid present after the sample digestion. In addition, the analytical performance can also be influenced by the high pH of these samples. Because of this, we first tried to neutralize the samples by titration with an organic base (2-diethylaminoethanol). However, this did not improve the results. Further, we investigated the effect of nitric acid using synthetic solutions containing 5 mg/l each of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} and 4, 8, 16 or 40 m*M* nitric acid. This corresponds to diluting a 20% (v/v) solution of nitric acid 100-, 50-, 25- and 10-fold, respectively. For comparison, a solution containing 5 mg/l of each of the five cations but without nitric acid was also run. It was found that the nitric acid decreased the peak heights, except for K^+, for which the peak height was observed to increase. This is due to the fact that nitric acid significantly decreases the mobilities of K^+ (see Fig. 1A) and consequently the mobility of K^+ is closer to that of the imidazole co-ion. As a result, the electromigration dispersion is reduced and the plate height increased. It is also seen from Fig. 1A that μ_{eof} decreases as the nitric acid concentration increases. At 16 m*M* nitric acid the electroosmosis flow marker (water peak) was no longer observed. This suggests that nitric acid affects the capillary surface.

As can be seen in Fig. 2, the separation of the five cations becomes worse with increasing nitric acid concentration, and finally is lost when the nitric acid concentration reaches 40 m*M*. However it follows from Fig. 1B that in the range 0–8 m*M* nitric acid the time-corrected peak areas are constant for Na^+, Ca^{2+} and Mg^{2+} while they show a slight decrease for K^+ and Mn^{2+}. They change in the presence of higher concentrations of nitric acid owing to insufficient separation causing inaccurate integration of peak areas.

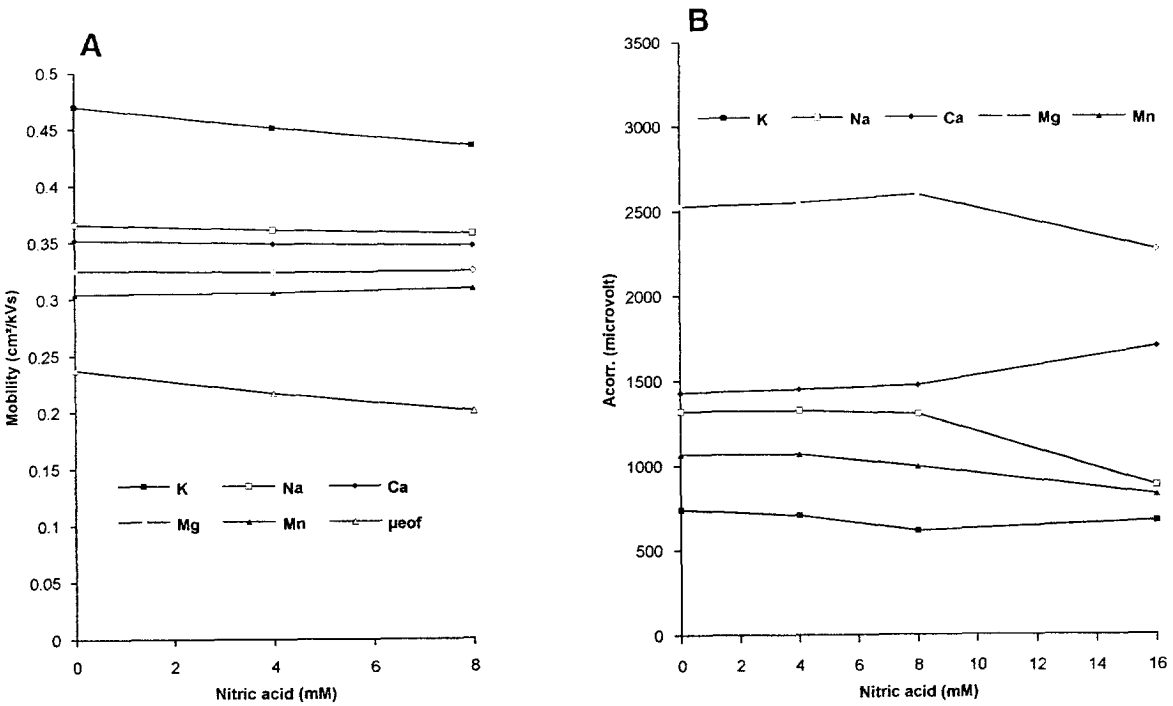

Fig. 1. (A) Change in mobilities of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} with increasing nitric acid concentration in samples. (B) Change in time-corrected peak areas with increasing nitric acid concentration in the measurement solution.

Therefore, direct determination using the corrected peak area as the response parameter appears to be possible in the presence of up to about 8 m*M* nitric acid in the samples.

The effect of sulphuric acid in the sample on the separation of some alkali and alkaline earth metal ions in CE was investigated by Riviello and Harrold [15]. Small amounts of sulphuric acid decreased the migration time for all analytes. As the acid concentration approached 25 m*M*, the migration times of these ions began to plateau, except for K^+ and NH_4^+, for which the migration times increased significantly. The authors concluded that as high as 25 m*M* sulphuric acid in the sample did not significantly degrade the separation.

3.3. Method validation

Test for linearity of the calibration line

Six independent series of standard solutions containing 0.5, 1, 2, 4, 6, 8 and 10 μg/ml of Na^+, K^+, Mg^{2+}, Ca^{2+} and Mn^{2+} were prepared. One injection was performed for each standard solution. The calibration lines were calculated using the time-corrected peak areas. It was found for Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} that the variances of the peak response increased with increase in the concentration of the analytes, in other words, the measurements are heteroscedastic. Therefore, weighted regression was applied for these cations [16]. The weights used are the reciprocal of the variances at each concentration level. Analysis of variance (ANOVA) for lack-of-fit to the ordinary or weighted regression lines [16,17] was performed to check the linearity of the calibration lines. In no case was lack of fit observed, which means that a straight-line model is adequate. The calibration lines are linear up to 10 mg/l.

Detection of matrix effects

The method of standard additions was used to detect matrix effects. It was performed on the

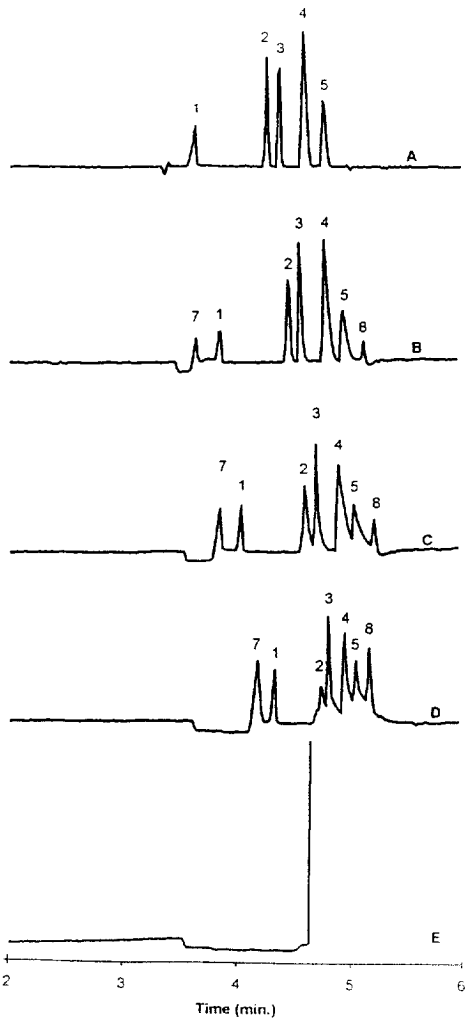

Fig. 2. Effect of nitric acid in the measurement solutions on the separation of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} (5 mg/l): (A) 0, (B) 4, (C) 8, (D) 16 and (E) 40 m*M* nitric acid. Peaks: 1 = K^+; 2 = Na^+; 3 = Ca^{2+}; 4 = Mg^{2+}; 5 = Mn^{2+}; 7 = H^+ and NH_4^+; 8 = unidentified.

total diet, bovine liver reference material and two tea samples. The standard addition lines were obtained from 50-fold dilution of their digests (see Section 2.5). Fig. 3 shows some electropherograms of the digested samples. Matrix effects were evaluated by comparing the slopes of the standard addition line and an aqueous calibration line. Their ratios range from 0.96 to 1.06 for K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+}, indicating the absence of important matrix effects that result in a relative systematic error. Moreover, a comparison of the slopes of the standard addition line and the calibration line for K^+ by means of a *t*-test [18] showed the difference not to be statistically significant (α = 5%). Consequently, direct calibration with aqueous solutions can be used for the sample analysis.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was evaluated from eight independent digestion blanks, which were spiked to produce a peak height, for each of the analyte cations, close to three times the baseline noise. It was estimated by taking three times the standard deviation of the peak areas obtained from these solutions and calculating the corresponding concentrations from the calibration lines. The LODs are 400 μg/l for K^+, 130 μg/l for Na^+, 170 μg/l for Ca^{2+}, 50 μg/l for Mg^{2+} and 220 μg/l for Mn^{2+}. By taking into account the dilution used (500 ml) and the dry sample mass (0.4 g), the LODs in the samples were obtained. They are 500 μg/g for K^+, 170 μg/g for Na^+, 220 μg/g for Ca^{2+}, 70 μg/g for Mg^{2+} and 280 μg/g for Mn^{2+}.

The LOQ is defined as the level at or above which the measurement precision is satisfactory for quantitative analysis. It was estimated by taking ten times [19] the standard deviation of the peak areas obtained form the eight blanks and subsequently calculating the corresponding concentrations from the calibration lines. The LOQs in solution are 2 mg/l for K^+, 410 μg/l for Na^+ and 580 μg/l for Ca^{2+}, 270 μg/l for Mg^{2+} and 560 μg/l for Mn^{2+}. These correspond to the following LOQs in the dry products: 2.5 mg/g for K^+, 520 μg/g for Na^+, 730 μg/g for Ca^{2+}, 340 μg/g for Mg^{2+} and 700 μg/g for Mn^{2+}.

Analysis of reference materials

Fig. 3 shows as an example the electropherograms of a total diet and an oyster tissue digest. Baseline separation of Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} is achieved, regardless of the difference in relative concentration of the metals in

the individual sample types. The peak that always appears before the K^+ peak is due to H^+, and to NH_4^+ when this ion is present in the sample or generated during the digestion. The unidentified peak coming later (peak No. 8) may be an artefact caused by the presence of nitric acid in the samples. As can be seen from Fig. 2, this peak becomes larger as the concentration of nitric acid increases.

The concentration of K^+, Na^+, Ca^{2+} and Mn^{2+} in the reference samples are given in Table 1. They were obtained from three independent digests, for each of which duplicate injections were performed. The standard deviation was estimated from the range of the three digestion means [20]. The results obtained with CE agree well with the certified values. It is concluded that the proposed method is sufficiently accurate. The relative standard deviation obtained for all the samples is less than 10%. It should be mentioned that although Na^+ was found in citrus leaves and pine needles digests, the concentrations of Na^+ in these samples was not determined because the concentration in the measurement solution is below the LOQ (520 μg/g for the dilution up to 500 ml and 260 μg/g for the dilution up to 250 ml). Of the reference materials analysed, only citrus leaves digest contains detectable Mn^{2+}.

Precision

The proposed procedure contains two main steps, the sample preparation and the CE measurement. The latter determines the system repeatability while both the sample preparation and the CE measurement contribute to the repeatability of the whole analytical procedure. For an estimation of the within-laboratory reproducibility, the between-day variation has also to be taken into account, which means that measurements on different days have to be performed. A two-level nested analysis of variance [21,22] was carried out to separately estimate the variance due to the CE measurement (s^2_{CE}), the sample preparation (s^2_{sample}) and time (s^2_{time}). The first variance component corresponds to the system repeatability variance. The sum of the first two variance components corresponds to the repeatability variance of the whole analytical procedure and the sum of all three variance components corresponds to the within-laboratory reproducibility variance. Commercial software, Statgraphics Plus [23], was used for performing the nested analysis of variance.

The precision was determined for the oyster tissue reference material and for a tea sample. The experiments were performed according to a fully nested design. Each day, during 7 days, two

Table 1
Comparison of CE results[a] and certified or recommended values

Reference material	Value	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (μg/g)
Total diet	CE	0.596 ± 0.046	0.592 ± 0.005	0.174 ± 0.006	0.0555 ± 0.0041	
(NIST SRM 1548)	Certified value	0.606 ± 0.028	0.625 ± 0.026	0.174 ± 0.007	0.0556 ± 0.0027	5.2 ± 0.4
Oyster tissue	CE	0.762 ± 0.016	0.398 ± 0.015	0.188 ± 0.006	0.109 ± 0.005	
(NIST SRM 1566a)	Certified value	0.790 ± 0.047	0.417 ± 0.013	0.196 ± 0.019	0.118 ± 0.017	12.3 ± 1.5
Fish tissue	CE	8.795 ± 0.346	1.905 ± 0.379	3.455 ± 0.070	1.227 ± 0.114	
(IAEA MA-B-3/TM)	Recommended value	9.00–10.0	2.00–2.31	3.18–3.60	1.04–1.20	2.22–3.03
Bovine liver	CE	1.096 ± 0.073	0.226 ± 0.006	0.0131 ± 0.0004	0.0589 ± 0.0036	
(NIST SRM 1577)	Certified value	0.97 ± 0.06	0.243 ± 0.013	0.0124 ± 0.0006	0.0604 ± 0.0009	10.3 ± 1.0
Pine needles	CE	0.346 ± 0.017		0.391 ± 0.012	0.108 ± 0.002	
(NIST SRM 1575)	Certified value	0.37 ± 0.02		0.41 ± 0.02		705.2 ± 24.7
Citrus leaves	CE	1.762 ± 0.026		3.123 ± 0.021	0.561 ± 0.005	675 ± 15
(NIST SRM 1572)	Certified value	1.82 ± 0.06	0.0160 ± 0.0020	3.15 ± 0.10	0.58 ± 0.03	23 ± 2

Experimental conditions: hydrostatic injection from 10 cm for 20 s; applied voltage, +20 kV; $I = \pm 5.4$ μA; background electrolyte, 5 m*M* imidazole–6.5 m*M* HIBA–0.55 m*M* 18-crown-6–20% (v/v) methanol (pH 4.5).

[a] The CE results were obtained from the analysis of three independent digests within one day. Each digest was injected twice. The S.D. was estimated as the range of the three digestion means divided by 1.91 [20].

independent samples were digested and for each digest duplicate injections were performed. The concentration of K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+} were calculated from the calibration lines measured on the same day. Table 2 shows the design and the results. The relative system repeatability standard deviations due only to the CE measurement are <3% for the oyster tissue and <4% for the tea sample. The relative method repeatability standard deviations are <6% for the oyster tissue and <4% for the tea. The relative within-laboratory reproducibility standard deviations are <9% for the oyster tissue and <5% for the tea. Therefore, it is concluded that the precision of the proposed procedure is acceptable.

Table 2
Precision obtained by a two-level nested analysis of variance

Nested design[a]			CE results (%, w/w)							
			Oyster tissue				Tea			
Day	Sample	Injection	K	Na	Ca	Mg	K	Ca	Mg	Mn
1	1	1	0.813	0.391	0.185	0.132	1.816	0.479	0.212	0.105
1	1	2	0.755	0.390	0.187	0.136	1.754	0.448	0.221	0.108
1	2	1	0.794	0.396	0.192	0.131	1.824	0.437	0.206	0.103
1	2	2	0.818	0.401	0.196	0.128	1.865	0.418	0.211	0.103
2	1	1	0.781	0.395	0.187	0.129	1.615	0.462	0.225	0.114
2	1	2	0.822	0.401	0.184	0.124	1.618	0.465	0.221	0.118
2	2	1	0.777	0.422	0.185	0.125	1.597	0.458	0.233	0.107
2	2	2	0.761	0.428	0.190	0.130	1.591	0.449	0.238	0.107
3	1	1	0.813	0.411	0.177	0.115	1.785	0.429	0.209	0.106
3	1	2	0.807	0.413	0.176	0.117	1.809	0.427	0.209	0.113
3	2	1	0.834	0.439	0.188	0.112	1.727	0.435	0.199	0.107
3	2	2	0.824	0.436	0.181	0.114	1.731	0.435	0.202	0.114
4	1	1	0.746	0.397	0.164	0.115	1.755	0.451	0.206	0.104
4	1	2	0.770	0.402	0.160	0.114	1.723	0.448	0.205	0.113
4	2	1	0.806	0.423	0.173	0.117	1.719	0.439	0.206	0.110
4	2	2	0.767	0.429	0.167	0.113	1.761	0.435	0.199	0.106
5	1	1	0.814	0.406	0.219	0.127	1.607	0.432	0.214	0.108
5	1	2	0.804	0.418	0.222	0.131	1.614	0.429	0.211	0.112
5	2	1	0.794	0.396	0.194	0.127	1.674	0.464	0.222	0.107
5	2	2	0.764	0.397	0.194	0.127	1.674	0.465	0.215	0.112
6	1	1	0.751	0.394	0.197	0.125	1.650	0.457	0.222	0.109
6	1	2	0.736	0.389	0.198	0.127	1.669	0.459	0.220	0.113
6	2	1	0.741	0.402	0.217	0.146	1.632	0.468	0.216	0.111
6	2	2	0.772	0.399	0.218	0.146	1.629	0.474	0.215	0.117
7	1	1	0.769	0.377	0.187	0.119	1.656	0.457	0.212	0.108
7	1	2	0.738	0.373	0.189	0.117	1.681	0.462	0.219	0.108
7	2	1	0.809	0.388	0.209	0.121	1.797	0.464	0.219	0.109
7	2	2	0.777	0.385	0.205	0.121	1.704	0.464	0.219	0.105
Mean			0.808	0.403	0.191	0.124	1.704	0.451	0.215	0.109
s^2_{CE}			$4.4 \cdot 10^{-4}$	$1.4 \cdot 10^{-5}$	$6.7 \cdot 10^{-6}$	$4.1 \cdot 10^{-6}$	$1.0 \cdot 10^{-3}$	$5.3 \cdot 10^{-5}$	$1.1 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$
s^2_{sample}			$1.7 \cdot 10^{-4}$	$1.8 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$	$2.9 \cdot 10^{-5}$	$1.0 \cdot 10^{-3}$	$1.8 \cdot 10^{-4}$	$2.3 \cdot 10^{-5}$	$1.5 \cdot 10^{-6}$
s^2_{time}			$2.9 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$	$1.6 \cdot 10^{-4}$	$5.7 \cdot 10^{-5}$	$5.0 \cdot 10^{-5}$	$3.6 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$	$3.4 \cdot 10^{-6}$
System repeatability, R.S.D. (%)			2.6	0.9	1.3	1.6	1.9	1.6	1.5	3.1
Method repeatability, R.S.D. (%)			3.1	3.4	5.9	4.6	2.6	3.4	2.7	3.3
Within-lab. reproducibility, R.S.D. (%)			3.7	4.4	8.9	7.6	4.9	3.6	4.5	3.7

[a] s^2_{CE} = variance due to CE system; s^2_{sample} = variance due to sample preparation; s^2_{time} = variance due to time.

3.4. Stability of the capillary

The capillary could be used for at least 400 injections without a significant change in performance.

3.5. Tea analysis

Fig. 3 shows an electropherogram of the 50-fold dilution of a tea digest. Baseline separation was achieved for K^+, Na^+, Ca^{2+}, Mg^{2+} and Mn^{2+}. Mn^{2+} was determined because tea is rich in this element [24,25]. The concentrations of the metals in teas with different geological origins (China, Sri Lanka, India and Japan) were determined by direct calibration with standard solutions and the results are given in Table 3. For each of the tea samples, the concentration was obtained by analysing two digestions, for each of which duplicate injections were performed. As can be seen from the results in Table 3 and Fig. 3, tea contains relatively large concentrations of micronutrients such as K^+, Mg^{2+}, Ca^{2+} and Mn^{2+} and a relatively small amount of Na^+. Tea is known to be a suitable beverage for people requiring a low-sodium diet [24].

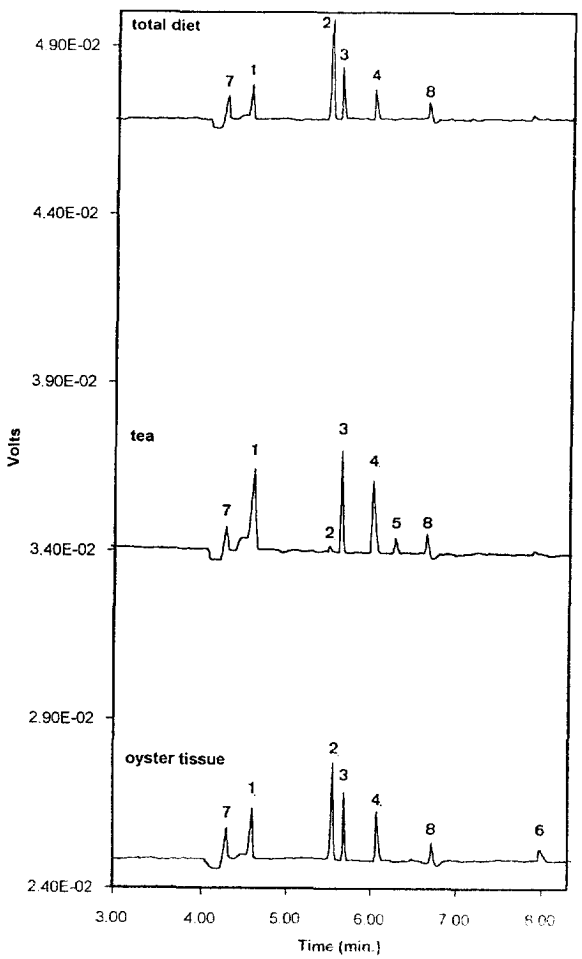

Fig. 3. Electropherograms of reference material and tea digests (50-fold dilution). Peaks: 1 = K^+; 2 = Na^+; 3 = Ca^{2+}; 4 = Mg^{2+}; 5 = Mn^{2+}; 6 = Zn^{2+}; 7 = H^+ and NH_4^+; 8 = unidentified. Experimental conditions: hydrostatic injection from 10 cm for 20 s, applied voltage, +20 kV; $I = \pm 5.4$ μA; background electrolyte, 5 m*M* imidazole–6.5 m*M* HIBA–0.55 m*M* 18-crown-6–20% methanol (pH 4.5).

4. Conclusions

The proposed method allows the rapid determination of some inorganic cations in natural products. Accurate results can be obtained by direct calibration with standard solutions. No further sample treatment is needed for the analysis of a variety of natural products. The time-corrected peak area is a suitable response parameter owing to its stability.

The high ionic strength of the sample, caused by the excess of acid in the samples, remains a limitation. Dilution is a solution to this problem, but it decreases the detectability. Analysis of low-concentration samples is therefore impossible with the present technique. The use of less nitric acid and more H_2O_2 in the digestion has been investigated, and showed some promise. However, the ratio of the amount of HNO_3 to the amount of H_2O_2 is limited because of safety considerations, especially in closed-vessel digestions. Membrane-based solid-phase extraction (SPE) [26–28] to eliminate the excess of NO_3^- from the measurement solution might be an alternative solution. It was recently demonstrated to be useful as a sample clean-up technique prior to CE of various anions [27]. It has also been shown to be an effective preconcentration technique for the determination of organic ions by capillary electrophoresis [28].

Table 3
Concentrations of K, Ca, Mg and Mn in teas of different geological origins obtained with CE

Tea	Origin	CE results (%, w/w)[a]			
		K	Ca	Mg	Mn (μg/g)
1	China	2.17 ± 0.03 (1.4)	0.339 ± 0.008 (2.4)	0.197 ± 0.004 (2.0)	741 ± 43.5 (5.9)
2	China	1.99 ± 0.10 (5.0)	0.468 ± 0.001 (0.2)	0.217 ± 0.008 (3.7)	1178 ± 21.5 (1.8)
3	Sri Lanka	2.36 ± 0.15 (6.4)	0.457 ± 0.009 (1.9)	0.191 ± 0.004 (2.1)	362 ± 9.9 (2.7)
4	Sri Lanka	2.17 ± 0.19 (8.8)	0.505 ± 0.002 (0.4)	0.193 ± 0.007 (3.7)	319 ± 24.3 (7.6)
5	India	1.62 ± 0.03 (1.9)	0.486 ± 0.006 (1.2)	0.160 ± 0.001 (0.9)	423 ± 53.5 (12.7)
6	India	2.16 ± 0.08 (3.7)	0.361 ± 0.000 (0.0)	0.206 ± 0.003 (1.4)	591 ± 4.6 (0.8)
7	Japan	1.89 ± 0.01 (0.5)	0.669 ± 0.028 (4.1)	0.238 ± 0.008 (3.2)	869 ± 18.3 (2.1)

[a] Mean ± S.D., with R.S.D. (%) in parentheses. The results were obtained from the analysis of two independent digests measured in duplicate injections within one day. The S.D. is equal to the range of the two digestion means divided by 1.42 [20]. Experimental conditions as in Table 1.

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