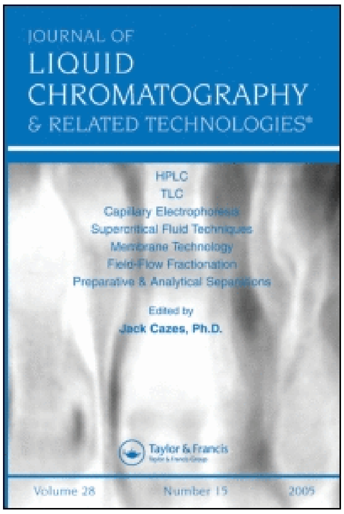

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CAPILLARY ION ELECTROPHORESIS OF INORGANIC CATIONS IN STANDARD REFERENCE MATERIALS FROM VEGETABLE SOURCE

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ABSTRACT

Taking into account the growing interest in applying capillary ion electrophoresis (CIE) for the determination of inorganic ions in real samples, this work focuses on CIE analysis of K^+, Na^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} in botanical and food reference materials.

Microwave-Assisted Digestion for sample preparation has been chosen ensuring a rapid, accurate, and reproducible treatment. A fused-silica capillary, 75 µm x 60 cm, a background electrolyte consisting of 6 mM imidazole and 10 mM α-hydroxyisobutyric acid (HIBA). and indirect UV detection at 214 nm were used.

Certified reference materials as Apple leaves SRM 1515 (NIST, USA), Beech leaves CRM 100, Rye flour CRM 381, Haricot beans CRM 383, and the green algae *Ulva lactuca* CRM 279 (BCR, Belgium) were selected. The optimal experimental conditions for microwave digestion and operational parameters in the electrophoresis system were studied.

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The calibration curves for K, Ca, Mg, and Mn ions were linear over the range of 0.6 ppm to 120 ppm and the detection limits (CLOD) were between 0.05 ppm and 0.20 ppm depending on the ions analyzed. Accuracy and precision within and between days were determined. The results were in good agreement with those reported in certified materials and they were also comparable with those obtained by atomic absorption and emission spectrometry.

INTRODUCTION

Capillary Ion Electrophoresis (CIE) is a simple and reliable technique that is becoming of common use in the analytical laboratory. Inorganic cations are determined with very high resolution in short run times[1-13,16] and the advantageous analytical performance for inorganic analysis[18,19] enables the consideration of CIE as an alternative to the well known methods of flame atomic absorption (FAAS) and emission spectrometry (FAES). CIE also shows a more simple operation, a lower reagent consumption, and a significant efficiency when it is compared to chromatographic methods.

The applicability of this technique in routine analysis of samples of different vegetable sources has not yet been considered very extensively but the proposal of a capillary electrophoretic method requires accurate and reliable results[5,9,11,14,17] according to international validation guidelines. It must be pointed out that the employment of standard reference materials is a key to take into account for ensuring the quality of analytical determinations.[9,14,15]

In this work, CIE analysis of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} in standard reference materials from vegetable source is performed after microwave digestion. The agreement of the results obtained by CIE with certified or recommended values of the reference materials under study is an important stage in the proposal of this technique as an alternative to conventional methods of inorganic ion analysis of these specimens.

EXPERIMENTAL

Instrumentation, Materials, and Methods

A Quanta 4000 Capillary Electrophoresis System (Waters, Milford, MA, USA) with Millenium™ Software (Waters) for data handling was used.

Sample pretreatment was performed on a programmable microwave digestion system, MDS 2000 (CEM Co., Matthews, NC, USA). The system is closed and the pressure conditions into the teflon vessels are automatically controlled.

Imidazole was supplied by Sigma (St. Louis, MO, USA) and α-hydroxyisobutyric acid (HIBA) was purchased from Waters (Millford, MA, USA). Suprapure nitric acid 65 % w/w (Merck, Darmstadt, Germany) was used. Deionized water was purified with a Purification System (Easy Pure, Barnstead, USA). All other reagents were of analytical grade.

Standard Reference Materials (SRM): Apple leaves SRM 1515 from National Institute of Standards & Technology (NIST, USA), Beech leaves CRM 100, Rye flour CRM 381, Haricot beans CRM 383, and Sea lettuce, *Ulva lactuca* CRM 279, provided by Bureau Community of Reference (BCR, Belgium), were used for analysis.

Solutions containing 1000 ppm of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} were purchased from Merck (Darmstadt, Germany).

Sample Preparation

Four samples of dried powder (400-500 mg), accurately weighted of each standard reference material were simultaneously treated with 10 mL of nitric acid in closed teflon vessels of a microwave digester. The treatment procedure was programmed in four steps at the power of 500 W applied for 8 min at each step and controlled pressure in the system was set as follows: 40, 85, 135, and 175 psi. Then, digestion vessels were cooled down to room temperature. Finally, the digests were quantitatively transferred to a beaker, dried at 150°C and the residues obtained were dissolved in 0.01% nitric acid. Simultaneously, duplicate digestion blanks were prepared.

Standard Solutions

Standard solutions were prepared by appropriate dilution of stock solutions of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} (1000 ppm) in 0.01% nitric acid to obtain ion concentrations similar to the samples of the reference standard materials.

Electrophoretic Conditions

Capillary electrophoretic analyses were performed using a fused silica capillary 60 cm (52 cm to detector), 75 μm i.d. (Waters, Milford, MA, USA). Samples were introduced by hydrostatic injection (10 cm height) for 10 sec. Applied voltage was +20 kV. Column temperature was maintained at 25°C and indirect detection was accomplished at 214 nm. The background electrolyte consisted of 6 mM imidazole and 10 mM HIBA, pH 4.0 adjusted with 1 M acetic acid.

Table 1

Limits of Detection (CLOD) and Quantitation (LOQ), Linearity, and Correlation Coefficients (r)

Cation	CLOD (ppm)	LOQ (ppm)	Regression Equation	r
K^+	0.20	0.60	$y = 25.2 + 57.0\ x$	0.9997
Ca^{2+}	0.20	0.60	$y = 4.40 + 143.3\ x$	0.9999
Na^+	0.05	0.15	$y = -11.8 + 126.9\ x$	0.9997
Mg^{2+}	0.05	0.15	$y = 95.3 + 288.0\ x$	0.9997
Mn^{2+}	0.20	0.60	$y = 16.6 + 134.9\ x$	0.9998

Table 2

Intra-Day and Inter-Day Precision of Electrophoretic System

		tm		A/tm	
Cation	ppm	x ± SD	RSD	x ± SD	RSD
K^+	40.0				
within day*		2.04 ± 0.01	0.04	2840.2 ± 9.4	0.3
between days**		3.13 ± 0.03	0.9	2939.8 ± 109.7	3.7
Ca^{2+}	20.0				
within day		3.88 ± 0.02	0.5	3378 ± 19.1	0.6
between days		4.01 ± 0.04	1.0	3133.2 ± 83.9	2.7
Na^+	1.0				
within day		3.96 ± 0.02	0.5	248.6 ±2.5	1.0
between days		4.08 ± 0.04	0.9	212.5 ± 4.7	2.2
Mg^{2+}	2.0				
within day		4.11 ± 0.02	0.5	607.4 ± 4.2	0.7
between days		4.25 ± 0.04	1.0	585.2 ± 23.0	3.9
Mn^{2+}	2.0				
within day		4.23 ± 0.02	0.5	216.1 ± 2.3	1.1
between days		4.38 ± 0.04	1.0	218.2 ± 8.7	4.0

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* Replicate injections (n = 3).

** Means values obtained in five different days.

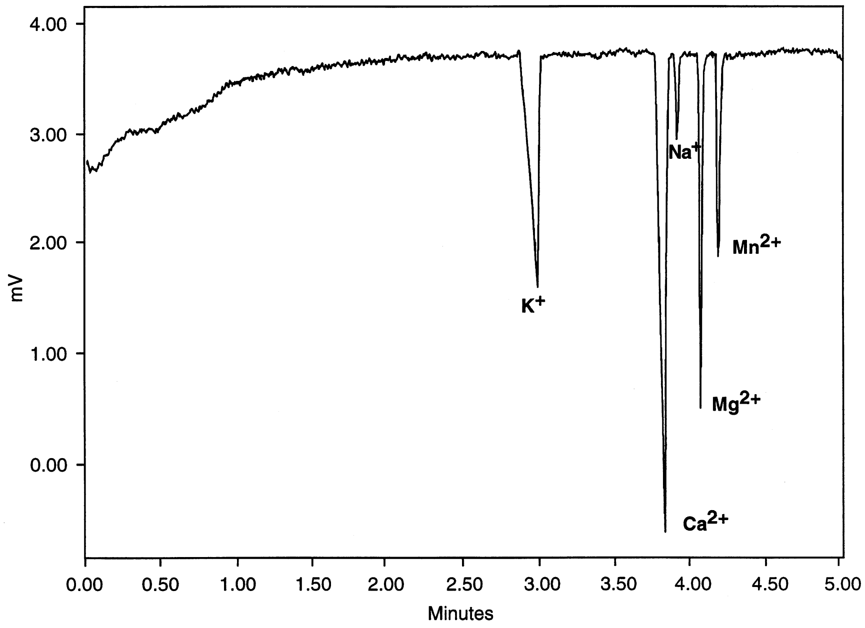

Figure 1. Electropherogram of the digested standard reference material (Beech leaves, CRM 100, BCR) in solution containing 40.0 ppm of K^+, 20.0 ppm of Ca^{2+}, 1.0 ppm of Na^+, 3.5 ppm of Mg^{2+} and 5.0 ppm of Mn^{2+}. Electrolyte buffer: 10 mM HIBA and 6 mM imidazole, pH=4.0. Hydrostatic injection was for 10 sec. Applied voltage was +20 kV. Indirect UV detection at 214 nm.

New capillaries were treated with 0.1 M sodium hydroxide for 10 min and then rinsed with water for 30 min. Conditioning of the capillary was performed by washing with running buffer for 10 min at the beginning of the day and 1.5 min between runs. At the end of each day the capillary was rinsed for 10 min with water.

RESULTS AND DISCUSSION

CIE analysis of Na^+, K^+, Ca^{2+}, Mg^{2+} and Mn^{2+} in standard reference materials from different vegetable sources was performed. A standard material from algal source, Sea lettuce was also studied.

All of the standard materials were processed by microwave acid digestion before CIE analysis. Most of the reports for the different SRMs indicate the classical acid wet digestion procedures for sample treatment.

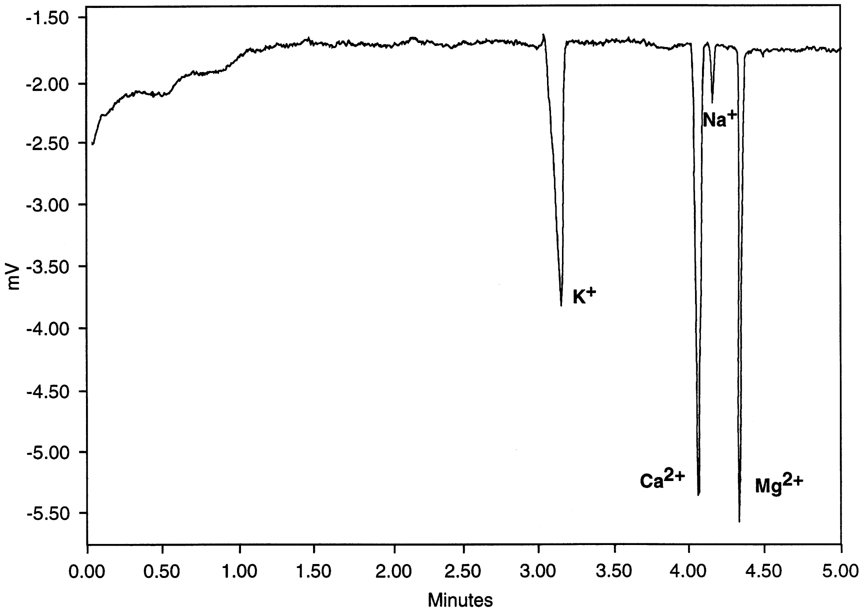

Figure 2. Electropherogram of the digested standard reference material (Haricot beans, CRM 383, BCR) in solution containing 40.7 ppm of K^+, 13.4 ppm of Ca^{2+}, 0.2 ppm of Na^+ and 6.3 ppm of Mg^{2+}. Experimental conditions are as noted in Figure 1.

However, microwave assisted digestion in closed vessels allows a rapid, uniform. complete, and reproducible sample treatment avoiding loss of volatiles and lower risk of contamination.[20-22] In the sample preparation step the employment of nitric acid proved to be a serious interference for the background electrolyte as other authors have just pointed out.[13,14]

The sample preparation procedure described in the experimental section avoided not only the nitric acid effect but also the presence of ammonium ion, although, 0.01% nitric acid was necessary to complete dissolution of the dried residue.

During the method development and optimization some chromophore co-ion agents were investigated, such as creatinine,[2,14] 4-methylbenzylamine,[4,5,8] or imidazole,[3,7,10,14] but a background electrolyte containing only imidazole and HIBA was the most suitable for the analysis of the real samples. Also, a sample loading of 10 s (12.7 nL) resulted in good peak shapes and the best resolution between calcium and sodium ion peaks.

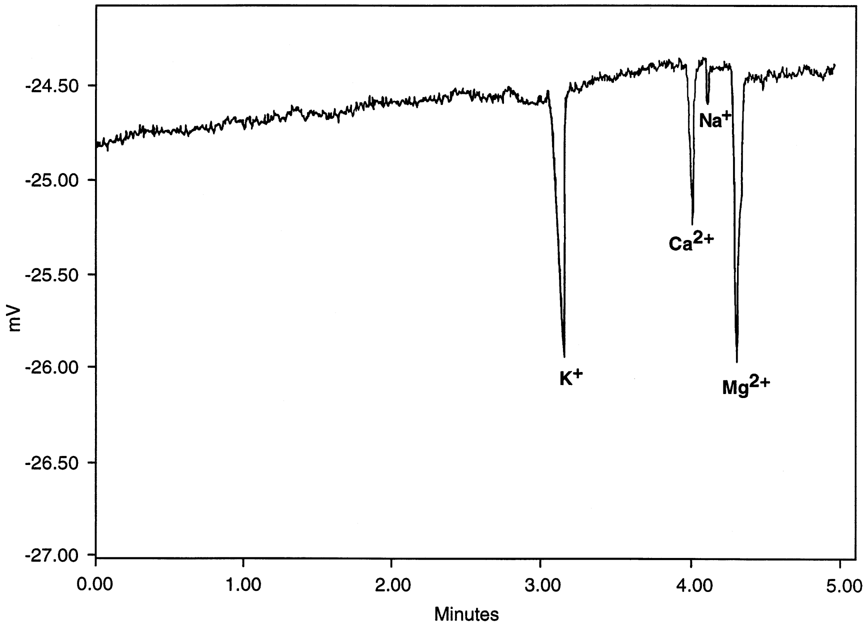

Figure 3. Electropherogram of the digested standard reference material (Rye flour, CRM 381, BCR) in solution containing 26.0 ppm of K^+, 2.0 ppm of Ca^{2+}, 0.2 ppm of Na^+, and 6.0 ppm of Mg^{2+}. Experimental conditions are as noted in Figure 1.

Validation of the methods was performed according to international guidelines. Selectivity, linearity, dynamic range, concentration limits of detection (CLOD), and quantitation (LOQ), accuracy, and precision were taken into account during method validation.

Calibration curves for potassium, calcium, magnesium, and manganese ions were determined analyzing duplicate samples of the standard solutions at five different concentrations over the range of 0.6 to 120 ppm and solutions of each concentration were injected twice.

For sodium ion this range was extended down to 0.15 ppm. An averaged value (n=2) of corrected peak areas (A/tm) for each point of the calibration curve was calculated and linearity was obtained by regression analysis. Equations and correlation coefficients (r) are shown in Table 1. Depending on the cations analyzed, CLOD ranged between 0.05 ppm and 0.20 ppm for a signal to noise ratio (S/N) equal to 3 and LOQ were between 0.15 ppm and 0.60 ppm for a S/N ratio equal to 10 (Table 1).

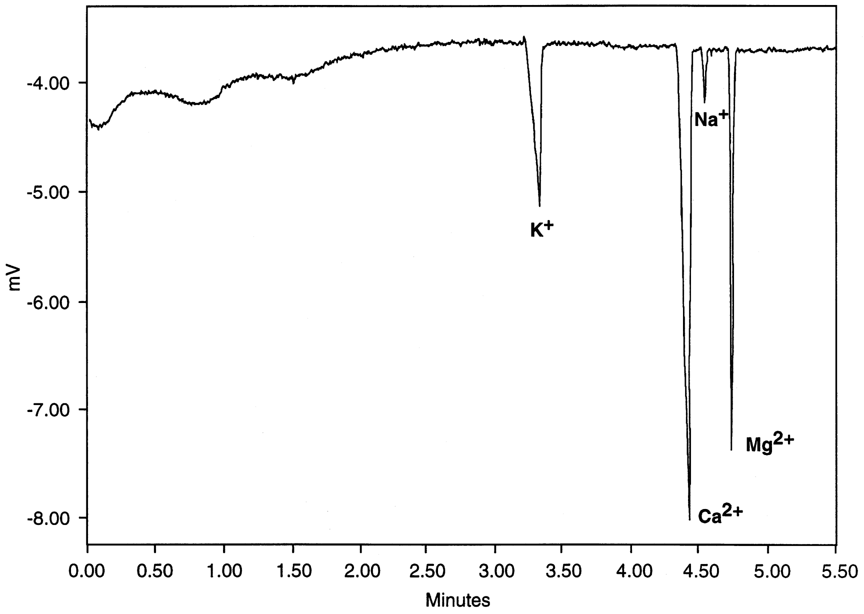

Figure 4. Electropherogram of the digested standard reference material (Apple leaves, SRM 1515, NIST) in solution containing 20.3 ppm of K^+, 22.1 ppm of Ca^{2+}, and 3.8 ppm of Mg^{2+}. Na^+ was not quantifiable. Experimental conditions are as noted in Figure 1.

Table 3

Values of K^+, Ca^{2+}, Mg^{2+}, and Mn^{2+} in Beech Leaves CRM 100 from BCR*

	Found			
Cation	**CIE**	**FAAS/FAES**	**Certified**	**Recommended**
K^+	10.18 ± 0.20	9.94 ± 0.14	9.94 ±0.20	
Ca^{2+}	5.48 ± 0.07	5.21 ± 0.07	5.30 ± 0.05	
Mg^{2+}	0.874 ± 0.018	0.923 ± 0.064	0.878 ± 0.017	
Mn^{2+}	1.32 ± 0.04	1.30 ± 0.04		1.27 - 1.36

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* mg/g Mean ± SD for four different experiments (n = 4).

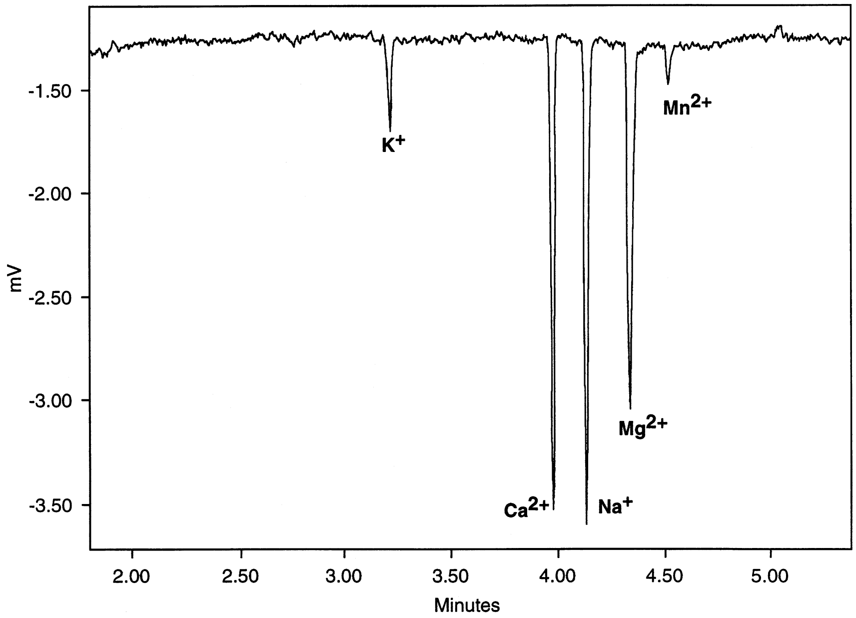

Figure 5. Electropherogram of the digested standard reference material (Sea lettuce, CRM 279, BCR) in solution containing 3.0 ppm of K^+, 5.0 ppm of Ca^{2+}, 5.3 ppm of Na^+, 7.0 ppm of Mg^{2+}, and 0.8 ppm of Mn^{2+}. Experimental conditions are as noted in Figure 1.

Table 4

Values of K^+, Ca^{2+}, Mg^{2+}, and Na^{2+} in Haricot Beans CRM 383 from BCR*

	Found			
Cation	CIE	FAAS/FAES	Certified	Recommended
K^+	7.3 ± 0.3	7.8 ± 0.3	7.8 ± 0.2	
Ca^{2+}	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.2	
Mg^{2+}	0.93 ± 0.04	0.94 ± 0.05		0.8 - 1.0
Na^+	0.083 ± 0.011	0.071 ± 0.006	0.075 ± 0.004	

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* mg/g, Mean ± SD for n = 4.

Table 5

Values of K^+, Na^+, Ca^+, and Mg^{2+}, in Rye Flour CRM 381 from BCR*

Cation	Found CIE	Found FAAS/FAES	Certified
K^+	2.97 ± 0.14	3.09 ± 0.18	2.85 ± 0.09
Ca^{2+}	0.207 ± 0.008	0.210 ± 0.010	0.221 ± 0.007
Mg^{2+}	0.429 ± 0.008	0.413 ± 0.007	0.425 ± 0.006
Na^+	0.020 ± 0.006	0.020 ± 0.004	0.019 ± 0.001

---

* mg/g, Mean ± SD for n = 4.

The RSD values obtained for the corrected peak areas (A/tm) ranged from 0.8 to 2.4% in within-day assays and from 2.2 to 4.0% in between-day assays. The RSD values for the migration times were lower than 1.0% in all cases. The data obtained for tm and A/ tm of the standard solutions tested under the operating conditions described in Experimental section are summarized in Table 2.

Standard Reference Materials

Electropherograms obtained from Beech leaves (CRM 100), Haricot beans (CRM 383), Rye flour (CRM 381), Apple leaves (SRM 1515), and *Ulva lactuca,* Sea lettuce (CRM 279) are shown in Figures 1-5, respectively.

A comparison of the results obtained by CIE, FAAS, and FAES is shown in Tables 3-6. No significant differences ($p>0.05$) between the mean values obtained for cations analyzed by electrophoretic and spectroscopic methods were found for all the certified materials studied. Agreement of the concentration values obtained by CIE with those certified or indicated in SRMs is shown in Tables 3-6 and the results demonstrated the accuracy of the method proposed for materials from vegetable source.

Manganese level in SRMs Rye flour (CRM 381), Haricot beans (CRM 383), and Apple leaves (SRM 1515) was below LOQ but reproducible results were obtained in Beech leaves (CRM 100) and Sea lettuce (CRM 279). Sodium was precisely quantified in SRMs Rye flour (CRM 381) and Haricot beans (CRM 383). In Apple leaves the quantitation of sodium was not possible due to the trace level content of this ion in the certified material.

Table 6

Values of K^+, $Ca^{2=}$, Na^+, Mg^{2+}, and Mn^{2+} in Apple Leaves SRM 1515 from NIST[a]

	Found		
Cation	**CIE**	**FAAS/FAES**	**Certified**
K^+	1.56 ± 0.14	1.55 ± 0.04	1.61 ± 0.02
Ca^{2+}	1.392 ± 0.134	1.580 ± 0.010	1.526 ± 0.015
Mg^{2+}	0.259 ± 0.033	0.283 ± 0.004	0.271 ± 0.008
Na^+	---[b]	---	0.00244 ± 0.00012
Mn^{2+}	---[c]	---	0.0054± 0.0003

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[a] g/%, Mean ± CI; Mean ± 95% confidence interval for n = 4. [b] Na^+ not quantifiable. [c] Mn^{2+} not detectable.

Table 7

Values of K^+, Ca^{2+}, Na^+, Mg^{2+}, and Mn^{2+} in *Ulva Lactuca* CRM 279 from BCR*

	K^+	**Ca^{2+}**	**Na^+**	**Mg^{2+}**	**Mn^{2+}**
CIE	10.8 ± 0.2 (11.1 ± 2.6)	21.1 ± 0.1 (22.1 ± 0.2)	19.7 ± 3.1 (19.9 ± 3.8)	12.8 ± 0.5 (13.1 ± 0.5)	1.67 ± 0.04 (1.82 ± 0.10)
FAAS/FAES	14.3 ± 0.3	20.01 ± 0.6	25.8 ± 1.0	15.1 ± 0.3	2.15 ± 010
Recommended	11.1 ± 14.6	25.3 - 28.9	26.0 - 26.5	13.1 - 14.4	2.03 - 2.15

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* mg/g, mean ± SD for n = 4. In brackets, values obtained by CIE using (20%, v/v) methanol added to the running buffer.

In Ulva lactuca (CRM 279) sodium could not be accurately quantified and addition of methanol (20%, v/v) to the background electrolyte[12,14] also produced erratic results. The employment of an electrolyte containing solely HIBA and imidazole gave similar results as those with methanol added to this buffer.

Reliable results for the cations under study were obtained in the matrices analyzed making unnecessary the addition of an organic solvent to the electrolyte as it is shown in Table 7 for CRM 279, *Ulva lactuca.* Sodium was the only analyte which coud not be accurately quantified. The high content of minerals in CRM 279 might be the cause of erratic values found for sodium that could not be overcome by diluting the digest and reducing the sample load. However, Table 7 shows comparable results between electrophoretic and spectrometric determinations of the rest of the cations studied in the algal reference material. Agreement with recommended (indicative) values for potassium, calcium, magnesium, and manganese were obtained by running the digest highly diluted.

CONCLUSIONS

In this work, CIE proved to be a suitable technique for the analysis of alkaline and alkaline-earth metals in certified reference materials from vegetable source and it may be proposed as an alternative to flame absorption or emission spectrometric methods.

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