Available online at www.sciencedirect.com
SCIENCE DIRECT®

Talanta 68 (2006) 1143–1147

Talanta

www.elsevier.com/locate/talanta

Rapid capillary zone electrophoresis method for the determination of metal cations in beverages

Silvia Suárez-Luque, Inés Mato, José F. Huidobro*,
Jesús Simal-Lozano

Facultad de Farmacia, Departamento de Química Analítica, Nutrición y Bromatología, Área de Nutrición y Bromatología,
Universidad de Santiago, 15782 Santiago de Compostela (Galicia), Spain

Received 24 February 2005; received in revised form 14 June 2005; accepted 14 July 2005
Available online 2 September 2005

Abstract

A rapid and reliable capillary zone electrophoresis method for the determination of inorganic cations was developed. The complete separation of K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Li^+ and Cu^{2+} can be achieved in 4 min with a simple electrolyte composed by 10 mM imidazole as the carrier buffer and background absorbance provider and acetic acid as the complexing agent (pH 3.60). Injection was performed hydrostatically by elevating the sample at 10 cm for 30 s. The running voltage was +25 kV at room temperature. Indirect UV-absorption detection was achieved at 185 nm. The detection limit was in the range between 0.06 mg/l (Mg^{2+}) and 0.57 mg/l (K^+) and the quantification limits ranged from 0.10 mg/l (Ni^{2+}) to 0.80 mg/l (Cu^{2+}). The calibration graphs were linear in the concentration range from the quantification limit till at least 1 g/l in K^+, 10 mg/l in Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Cd^{2+}, 40 mg/l in Na^+ and 12 mg/l in Li^+ and Cu^{2+}. The repeatability, intraday and interday analysis were $\leq$1.55% and $\leq$3.64% for migration time and $\leq$3.38% and $\leq$3.63% for peak area. The method developed has been applied to several beverage samples with only a simple dilution and filtration treatment of the sample. The proposed method is simple, fast, cheap and it is achieved with common products in either laboratory. For these reasons, it is a very useful method for routine analysis.

Keywords: Metal cations; Capillary zone electrophoresis; Beverages

1. Introduction

Capillary zone electrophoresis is one of the more powerful separation techniques for the analysis of a wide variety of sample matrices [1–3]. The advantages of ion analysis using this technique include resolution, speed, simplicity and reduced sample preparation. Hjertén [4] first reported the application of capillary electrophoresis with indirect detection for the analysis of inorganic and organic anions. From this work, a variety of application areas for capillary electrophoresis have been developed based on various separation principles.

Beck and Engelhardt [5] first proposed imidazole as a chromophore co-ion of the electrolyte for separation of metal cations. They investigated several background carrier electrolytes for indirect UV-absorption detection and found imidazole to be suitable for the separation of metal ions, amines and amino alcohols. Their results proved the applicability of this running electrolyte in routine analysis. Table 1 summarizes some electrophoretic methods published with imidazole as background electrolyte and indirect UV-absorption detection.

Some electrophoretic methods used imidazole as background electrolyte for the determination of cations in food samples. Beck and Engelhardt [5], Quang and Khaledi [6], Pantsar et al. [7] and Shakulashvili et al. [8] applied their methods to cation analysis in water samples and Yang et al. [9] developed a method for K^+, Ca^{2+}, Na^+, Mg^{2+} and

---

[*] Corresponding author. Tel.: +34 981 594626; fax: +34 981 594912.
E-mail address: qnhuidob@usc.es (J.F. Huidobro).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.07.016

Table 1
Some electrophoretic methods published with imidazole as background electrolyte and indirect UV-absorption detection

Metal cations analysed	Electrolyte composition	pH	Separation time (min)	Sample	Reference
K^+, Ca^{2+}, Na^+, Mg^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, NH_4^+, Zn^{2+}, Cu^{2+}	10 mM imidazole, 0.5% acetic acid	4.32	20	Water, human plasma	[2]
K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Li^+	5 mM imidazole, H_2SO_4	4.5	4	Water	[5]
K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Li^+, Zn^{2+}, Cu^+	6 mM imidazole, 12 mM α-hydroxyisobutyric acid, acetic acid	3.95	4	Water	[6]
K^+, Ca^{2+}, Na^+, Mg^{2+}	5 mM imidazole, 6.5 mM α-hydroxyisobutyric acid, 2 mM 18-crown-6 ether	4.1	5.5	Water	[7]
K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Li^+, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cr^{2+}	10 mM imidazole, 13 mM glycolic acid	4.32	20	Water	[8]
K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}	5 mM imidazole, 0.5 M H_2SO_4	4.5	3	Electrolyte solutions, apple juice, orange juice	[9]
NH_4^+, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Li^+, Zn^{2+}, Cu^+, Cr^{2+}	5 mM imidazole, 6.5 mM α-hydroxyisobutyric acid, 0.53 mM 18-crown-6 ether, MeOH 20%	4.5	12	Chinese tea infusions	[10]
Cs^{2+}, NH_4^+, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Li^+, Zn^{2+} Cu^{2+}, Pb^{2+}	10 mM imidazole, 5 mM lactic acid, 0.5 mM 18-crown-6 ether	4.5	5	No application	[19]
Cs^{2+}, K^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, Li^+, Zn^{2+}, Cu^{2+}, Pb^{2+}, Cr^{2+}	Pyridine or imidazole with glycolic acid	4.0	15	Water, food soil	[20]
K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Li^+	Imidazole with several complexing agents	Several pH values	Several times	No application	[21]

Cu^{2+} determination in apple juice and orange juice samples. Later, Yang et al. [10] proposed other method with a more complex electrolyte composition that was applied to Chinese tea infusions. Other background electrolytes were also used for the determination of cations in beverages. For instance, Weston and Brown [11] applied an electrophoretic method with background electrolyte based on 5 mM UVCat-1, 6.5 mM α-hydroxyisobutyric acid at pH 4.4 with indirect UV-absorption detection to determine K^+, Ca^{2+}, Na^+, Mg^{2+} and Cu^{2+} in a commercial orange juice. After that, Núñez et al. [12] analysed K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+} and Li^+ in wines with a background electrolyte composed by UVCat-1, α-hydroxyisobutyric acid and 18-crown-6 ether at pH 4.5 and indirect UV-absorption detection.

Apart from these references, several excellent reviews offer extensive lists of the capillary electrophoresis methods for cation analysis [1,3,13–16].

The determination of metal cations in beverages is important because they have influence on the organoleptic properties of foods (Núñez et al. [12]). This determination is usually made in a food quality control laboratory to study the nutritional value of some foodstuffs.

Then, this paper aim has been to optimize the parameters to obtain a simple and rapid capillary zone electrophoresis method for the determination of main metal cations in beverage samples for routine analysis.

2. Experimental

2.1. Chemicals

Analytical standard-grade K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Li^+, Cu^{2+} and imidazole were obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Stock standard solutions were obtained by dissolution of metal cations in Milli-Q water and stored at 4 °C. The Milli-Q water was purified by passage through a Compact Milli-RO and Milli-Q water system from Millipore, Milford, MA, USA.

Acetic acid and sodium hydroxide pellets were analytical-reagent grade and supplied by Merck KGaA, Darmstadt, Germany.

2.2. Buffers and pH adjustment

Running buffer contained 10 mM imidazole of background electrolyte. The pH was adjusted at 3.60 by adding 1 M acetic acid. The buffer solution was freshly prepared and filtered through a 0.45 μm membrane.

2.3. Apparatus

Separation was carried out on a Waters Capillary Ion Analyser (CIA System, 1.3 Version) equipped with a positive power supply and an UV detector with a 185 nm wavelength filter. Fused-silica capillaries with 75 μm i.d. and 60 cm in length were used (Waters Associates Inc., Milford, MA, USA). The distance from the point of injection to the window of on-column detection was 52.5 cm. Electropherograms were collected and plotted by the data acquisition system Millennium 2010 v. 2.15 with specific option CIA for capillary zone electrophoresis. A Crison micropH 2002 pH meter (Crison Instruments S.A., Alella, Barcelona, Spain) and a Selecta Agimatic-S magnetic stirrer (Selecta, Abrera, Barcelona, Spain) were also used. The electrolyte was filtered with membrane filters Phenomenex 0.45 μm, AFO-0504 (Phenomenex, CA, USA).

2.4. Electrophoretic procedure

Prior to use, new capillaries were treated with the following wash cycles: (i) 10 min with Milli-Q water, (ii) 10 min with 1 M NaOH, (iii) 10 min with 10 mM NaOH, (iv) 30 min with Milli-Q water and (v) 60 min with the background electrolyte. The (i) and (ii) steps were omitted for daily conditioning of the capillary and between injections, the capillary was washed 2 min with running electrolyte.

After the analysis of the day, the capillary was washed with 10 mM NaOH (10 min) and Milli-Q water (30 min).

Injection was performed hydrostatically by elevating the sample at 10 cm for 30 s. The separation run was carried out in 4 min at a constant voltage of +25 kV (current 25 μA) and at room temperature. The detection mode was indirect UV-absorption at 185 nm. Injections were made in triplicate.

2.5. Samples

The proposed method was applied to several beverages, as red wine, white wine, peach and apple juice, grape and pineapple juice, orange juice and orange soft drink. These samples were purchased at a local supermarket. 1 ml of sample was dissolved in 100 ml of Milli-Q water, filtered through 0.45 μm membrane and injected directly without any other sample treatment.

3. Results

The proposed method allowed the identification and quantification of K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Li^+ and Cu^{2+} in several beverage samples. These metal cations were identified by comparison of their migration times with those of standards and they were quantified by using an external standard calibration with peak area. Evaluation of peak area is recommended for analysis where high concentrations of analyte are expected and electromigration dispersion cannot be eliminated (Sustacek et al. [17]). Table 2 shows the migration times of cations analysed. In Fig. 1, an electropherogram with the complete separation of the cations studied in proposed conditions is shown.

Table 2
Migration times ($n = 3$), detection and quantification limits and correlation coefficients (r) of calibration plots of metal cations analysed in the proposed method

Cation	Migration time (min) ± S.D.	Detection limit (mg/l)	Quantification limit (mg/l)	r
K^+	2.40 ± 0.06	0.57	0.62	0.9993
Ba^{2+}	2.84 ± 0.08	0.37	0.50	0.9991
Ca^{2+}	2.99 ± 0.08	0.12	0.18	0.9996
Na^+	3.15 ± 0.09	0.12	0.17	0.9992
Mg^{2+}	3.23 ± 0.10	0.06	0.16	0.9992
Mn^{2+}	3.28 ± 0.10	0.11	0.13	0.9965
Ni^{2+}	3.31 ± 0.10	0.07	0.10	0.9993
Cd^{2+}	3.53 ± 0.12	0.16	0.25	0.9998
Li^+	3.75 ± 0.13	0.11	0.14	0.9988
Cu^{2+}	3.98 ± 0.15	0.53	0.80	0.9983

3.1. Detection and quantification limits

The detection limit (LOD) was calculated as $s_b + 3s$, where s_b is the average signal of 10 blank injections (absolute area value of each cation migration time ± 1%) and s the standard deviation. The quantification limit (LOQ) was calculated as $s_b + 10s$, where s_b is the average signal of 10 blank injections and s the standard deviation (ACS Committee on Environmental Improvement [18]). The LOD ranged from 0.06 mg/l for Mg^{2+} to 0.57 mg/l for K^+ and the LOQ ranged from 0.10 mg/l for Ni^{2+} to 0.80 mg/l for Cu^{2+} (Table 2).

3.2. Calibration curves

Calibration curves were determined for seven different concentrations of a mixture of cations standard solutions. Each calibration sample was injected in triplicate. Calibration graphs were obtained plotting concentration against peak area and applying the least squares method. Table 2 lists the correlation coefficients of these calibration plots which were linear in a wide interval from the quantification limit till at

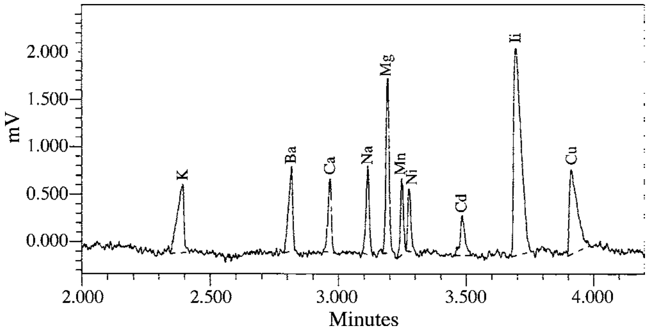

Fig. 1. Separation of metal cations of a standard mixture solution, when the developed electrophoretic method was applied (10 mM imidazole and acetic acid, pH 3.60, +25 kV, room temperature, indirect UV-absorption detection, 185 nm; K^+, Ba^{2+} and Cu^{2+}: 5 mg/l; Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+} and Li^+: 1 mg/l).

Table 3
Precision results in R.S.D. (%) of metal cations analysed by the proposed method

Cation	Intraday ($n = 5$)		Interday ($n = 3$)	
	Migration time	Peak area	Migration time	Peak area
K^+	0.79	1.07	2.27	1.82
Ba^{2+}	1.01	0.81	2.64	1.52
Ca^{2+}	1.08	1.05	2.77	1.03
Na^+	1.17	1.19	2.89	1.18
Mg^{2+}	1.19	1.30	2.97	1.16
Mn^{2+}	1.23	2.51	3.02	3.63
Ni^{2+}	1.24	1.43	3.04	1.34
Cd^{2+}	1.33	2.31	3.26	2.63
Li^+	1.46	1.73	3.43	1.35
Cu^{2+}	1.55	3.38	3.64	3.01

least 1 g/l in K^+, 10 mg/l in Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Cd^{2+}, 40 mg/l in Na^+ and 12 mg/l in Li^+ and Cu^{2+}.

3.3. Precision

Repeatability (interday and intraday) was studied to obtain the method precision. Intraday analysis was established by the injection of the standard mixture of cations five times at the same day. Interday repeatability was determined by analysing the standard mixture on 3 different days. Table 3 shows the results of the precision of migration time and peak area. The relative standard deviations (R.S.D., %) of the intraday and the interday studies are $\leq 1.55\%$ and $\leq 3.64\%$ for migration time and $\leq 3.38\%$ and $\leq 3.63\%$ for peak area, respectively. The variation of migration time could be reduced by the calculation of relative migration times with the addition of a reference compound.

3.4. Samples

The proposed method was applied to the analysis of cations in samples of red wine, white wine, peach and apple juice, grape and pineapple juice, orange juice and orange soft drink (Table 4). Fig. 2 shows an electropherogram of the separation of the cations analysed of grape and pineapple juice. K^+, Ca^{2+}, Na^+ and Mg^{2+} were found in all samples. Orange juice had the most number of quantified cations and peach and apple juice had the most content of quantified cations. Traces of Ni^{2+} were found in orange juice. So this method could be used for the detection of food contamination by heavy metals.

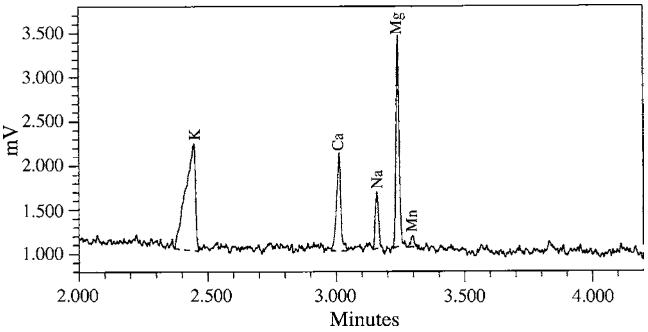

Fig. 2. Electropherogram of metal cations separated in a grape and pineapple juice sample with the proposed method.

4. Discussion

The most efficient background electrolyte in the separations of alkali and alkaline earth cations was imidazole because its electrophoretic mobility is close to those of metal cations. Imidazole was also a visualization agent in capillary zone electrophoresis with indirect UV-absorption detection (François et al. [19]). Lee and Lin [20] determined the mobility of imidazole, pyridine and benzyl amine as the background electrolyte. They concluded that the mobility of imidazole matched best with the alkali and alkaline earth metal ions and these ions were better separated in this buffer. Therefore, we selected imidazole as the best background electrolyte. The effect of imidazole concentration was studied in the 5–20 mM range by maintaining a constant pH value of 3.60. We have observed that if imidazole concentration was greater, the migration times of the cations were higher. A 10 mM imidazole concentration was selected as the optimum because the separation of the cations is the best in a short analysis time. Tacking into account the influence of background electrolyte complexing agent, Lin et al. [21] noted that the acid added to adjust the buffer pH affected the resolution and the migration order of the ions. If HCl was used to adjust the pH, Na^+ and Mg^{2+} overlapped. They also explain that acetic, glycolic, lactic acids and α-hydroxyisobutyric acid affected the cations separation in a similar way. Acetic acid is the most common acid so we selected it for pH adjustment.

On the other hand, the resolution at the baseline level and the cations separation were influenced by the pH (Lin et al. [21]). For this reason, the effect of the pH value was studied in a 3.50–5.00 range. We saw that the higher pH the faster migration times. Values up to 3.60 overlapping occurred and

Table 4
Metal cations concentration (mg/l) obtained when the proposed method was applied to beverage samples

	K^+	Ba^{2+}	Ca^{2+}	Na^+	Mg^{2+}	Mn^{2+}	Ni^{2+}
Red wine	16.11	–	1.54	0.78	1.12	–	–
White wine	14.34	–	0.98	0.63	0.96	–	–
Peach and apple juice	19.30	–	1.06	0.89	0.89	–	–
Grape and pineapple juice	18.11	–	2.20	1.15	1.69	0.23	–
Orange juice	21.82	0.94	0.89	0.39	1.11	–	0.14
Orange soft drink	7.01	0.55	1.23	0.30	–	–	–

also values up to 4.50 Cu^{2+} cannot be detected. Then, a 3.60 pH value was selected. Although, this pH value is out of the buffering region of both BGE components, we have selected anyway in order to improve cations sensibility and determine as much cations as possible.

Finally, the effect of voltage on separation and resolution has been studied. When voltage was increased, the analysis time was decreased but the complete separation of the analytes could be accomplished. Voltage of +25 kV was chosen because of the good separation of the cations in 4 min of analysis.

5. Conclusions

A rapid, simple and reliable capillary zone electrophoresis method for the determination of metal cations K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Li^+ and Cu^{2+} was developed in 4 min. The composition of background electrolyte was 10 mM imidazole as visualisation agent and acetic acid (1 M) as the complexing agent and to adjust the pH at 3.60. The running voltage was +25 kV at a room temperature. This method was applied to different beverage samples and only a simple treatment of dilution and filtration was necessary. The proposed method is simple, fast, cheap and it is achieved with common products in either laboratory. For these reasons, it is very useful method for routine analysis and for the detection of food contamination by heavy metals.

Acknowledgements

We thank Professor Rafael Cela Torrijos of the Analytical Chemistry Department of the Chemistry Faculty and Professor Juan Carlos García Monteagudo of the Chemistry-Physics Department of the Pharmacy Faculty both of University of Santiago de Compostela for their helpful comments.

References

[1] A.R. Timervaev, Electrophoresis 23 (2002) 3884.
[2] F. Qu, J.M. Lin, Z.L. Chen, J. Chromatogr. A 1022 (2004) 217.
[3] A.R. Timervaev, Electrophoresis 25 (2004) 4008.
[4] S. Hjertén, Chromatogr. Rev. 9 (1967) 122.
[5] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[6] C. Quang, M.G. Khaledi, J. Chromatogr. A 659 (1994) 459.
[7] M. Pantsar, P.K. Kallio, G. Manninen, Anal. Chim. Acta 314 (1995) 67.
[8] N. Shakulashvili, T. Faller, H. Engelhardt, J. Chromatogr. A 895 (2000) 205.
[9] Q. Yang, M. Jimidar, T.P. Hamoir, J. Smeyers-Verbeke, D.L. Massar, J. Chromatogr. A 673 (1994) 275.
[10] Q. Yang, J. Smeyers-Verbeke, W. Wu, M.S. Khots, D.L. Massart, J. Chromatogr. A 688 (1994) 339.
[11] A. Weston, P.R. Brown, J. Chromatogr. 602 (1992) 249.
[12] M. Nuñez, R.M. Peña, C. Herrero, S. García, J. AOAC Int. 83 (2000) 183.
[13] P. Blatny, F. Kvasnicka, J. Chromatogr. A 834 (1999) 419.
[14] J.S. Fritz, J. Chromatogr. A 884 (2000) 261.
[15] R. Timerbaev, O.A. Shpigun, Electrophoresis 21 (2000) 4179.
[16] J. Sadecka, J. Polonsky, J. Chromatogr. A 880 (2002) 243.
[17] V. Sustacek, F. Foret, P. Bocek, J. Chromatogr. 545 (1991) 239.
[18] ACS Committee on Environmental Improvement, Anal. Chem. 52 (1980) 2242.
[19] C. François, Ph. Morin, M. Dreux, J. Chromatogr. A 717 (1995) 393.
[20] Y.-H. Lee, T.-I. Lin, J. Chromatogr. A 675 (1994) 227.
[21] T.-I. Lin, Y.-H. Lee, Y.-C. Chen, J. Chromatogr. A 654 (1993) 167.