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Capillary zone electrophoresis method for the simultaneous determination of cations in honey

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Abstract

A capillary electrophoresis system for the simultaneous determination of cations in honey samples has been developed. The complete separation and quantification of K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+} and Li^+, which represent more than 99% of the total content of cations in honey, can be achieved in 4 min with only a dilution and filtration of the honey sample. Electrolyte solution was composed by 10 mM imidazole as the carrier buffer and background absorbance provider and acetic acid as the complexing agent (pH 3.60). The running voltage was + 25 kV at 25 °C. Indirect UV detection was achieved at 185 nm. Under the optimum conditions the detection limits ranged from 0.02 to 48.2 mg/kg and the quantification limits have ranged from 0.41 to 48.7 mg/kg. Precision data in honey samples analysed have shown repeatability and reproducibility RSD (%) lower than 2.84 and 6.62%, respectively. Recoveries of cations in honey samples analysed have ranged from 88.5 to 101.8%. These cations were identified by their relative migration times with regard to Ba^{2+} migration time used as reference standard and they were quantified by using an external standard calibration. Twenty-five honey samples were analysed to test the proposed method. Mean contents of 1.22×10^3, 93, 85, 54, 11, 1.9 and 2.3 mg/kg were found, respectively, for K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+} and Li^+ cations in analysed honeys. These results were similar than the obtained by other authors.

Keywords: Cations; Capillary electrophoresis; Honey

1. Introduction

Honey is a complex matrix which minerals are minority components. The mineral content in floral honeys ranged from 0.020 to 1.028% with an average content of 0.169% [1]. Taking into account cations present in honey, potassium is the predominant and in minor quantity there are calcium, sodium and magnesium. Manganese, copper, iron, nickel, lithium or cadmium are also in honey as trace elements. The mineral and trace element content in honey samples could give an indication of environmental pollution and herewith also an indication of the geographical origin of honey [2]. Latorre et al. [3] determined nine metals in honey samples and they concluded that the metal profile provided enough information to enable a classification rule to identify honeys, according to their geographical origin. Finally, Terrab et al. [4] also studied minerals of honeys and they obtained good results in the classification of the samples, according to their geographical origin.

Some authors have determined cations in honey by different procedures, like atomic absorption and emission spectroscopy [3,5–10], X-ray fluorescence [11], flame emission photometry [9] or inductively coupled plasma atomic emission spectrometry [4,12–17].

Tacking into account these references, all procedures carried out a mineralization and/or a digestion of the samples previously to their determination except the method of Barisic et al. [11]. Nevertheless, in this method, majority minerals as K^+, Na^+ or Mg^{2+}, were not determined. These previous treatments of the samples made analytical determinations longs and tedious.

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For these reasons the aim of this work is the investigation of the capillary electrophoresis technique for the development of a rapid and simple method for the determination of cations in honey without any treatment of the sample. It was the first time that a capillary electrophoresis method was applied to honey.

2. Experimental

2.1. Chemicals

Analytical standard-grade K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Li^+ and imidazole were obtained from Sigma. Stock standard solutions were obtained by dissolution of cations in purified water and stored at 4 °C. The water was purified by passage through a Ultra Pure water system Milli-Q plus from Millipore.

Acetic acid and sodium hydroxide pellets were analytical-reagent grade and supplied by Merck.

2.2. Buffers and pH adjustment

Running buffer contained 10 mM imidazole of background electrolyte. The pH was adjusted at a 3.60 value by adding 1 M acetic acid, which is also a complexing agent. 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 a UV detector with a 185 nm wavelength filter. Fused-silica capillaries with 75 μm I.D. and 60 cm in length were used. 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 electrophoresis (Waters Associates, Inc. Milford, MA, USA). 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 procedures

Prior to use, new capillaries were treated with the following wash cycle: (i) 10 min with purified water, (ii) 10 min with 1 M NaOH, (iii) 10 min with 10 mM NaOH, (iv) 30 min with purified 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 with 2 min running electrolyte.

After all analysis of the day, the capillary was also washed with 10 mM NaOH (10 min) and purified water (30 min).

Sample injection was carried out in a hydrodynamic mode during 30 s. The separation run was at a constant voltage of + 25 kV at 25 °C and it is achieved in 4 min. Indirect UV detection was achieved at 185 nm.

2.5. Samples

This work was carried out on 25 samples from Galicia (Northwestern Spain) labelled as "Indicación Xeográfica Protexida-Mel de Galicia". The samples were stored in darkness at room temperature until analysis. These samples were classified according to their colour determined by the method of Brice et al. [18], in light and dark honeys. Light honeys were considered water white, extra white and white honeys and extra light amber, light amber and amber of Brice et al. classification had correspondence with dark honeys. This method is Official in the AOAC [19]. The samples were also classified according to their geographical origin in the four Galician regions.

For the electrophoretic analysis 1 g of honey was dissolved in 10 ml of purified water, filtered through a 0.45 μm membrane and injected directly without any other sample treatment. Each sample was injected in triplicate. This amount of honey (1 g/10 ml) was the greatest quantity to obtain a good response without interferences.

All standars and samples were injected in triplicate. All statistical analysis were made with Statgraphics [20].

3. Results and discussion

First of all, working with standard solutions, 10 cations (K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Li^+ and Cu^{2+}) were separated and identified by the present electrophoretic method. When this method was applied to honey samples K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+} and Li^+ cations were identified and quantified (Fig. 1), which represent more than 99% of the total content of total cations [1]. These cations were identified by comparison of the relative migration times of their peaks with the Ba^{2+} migration time used as reference compound. These cations were quantified by using a external standard calibration.

3.1. Detection and quantification limits

The detection limit 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 was calculated as $s_b + 10s$, where s_b is the average signal of 10 blank injections and s the standard deviation [21]. The detection limits ranged from 0.02 mg/kg for Li^+ to 48.2 mg/kg for K^+ and the quantification limits ranged from 0.41 mg/kg for Ni^{2+} to 48.7 mg/kg for K^+.

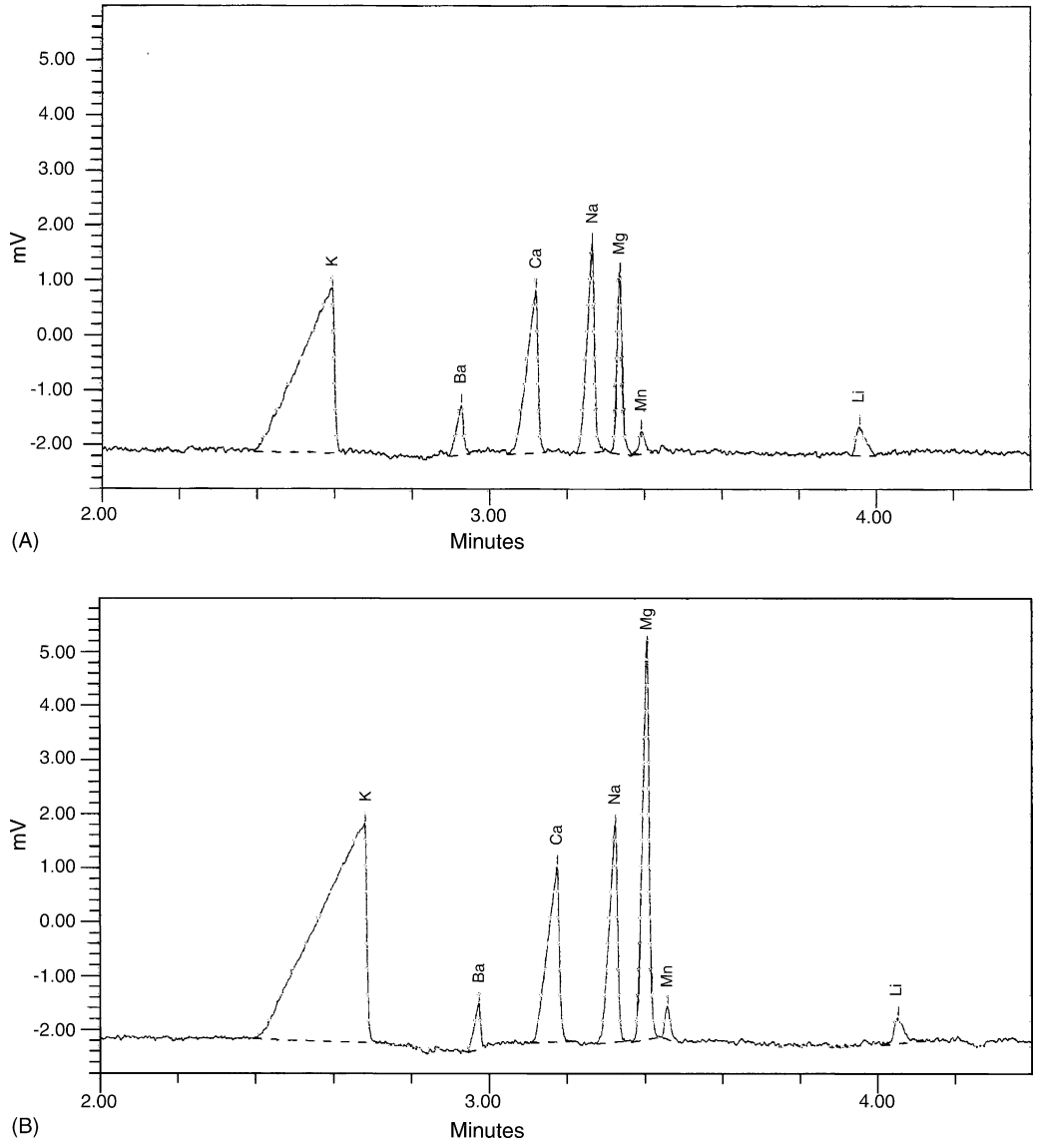

Fig. 1. Electropherograms of analysed cations by the proposed method in a light honey (A) and a dark honey (B). Ba^{2+} was added as reference compound for the calculation of the relative migration times.

3.2. Calibration curves

Calibration curves were determined for several different concentrations of a mixture of cations standard solutions. Each calibration sample was injected in triplicate. Plotting concentration against peak area and applying the least squares method obtained calibration graphs for each compound. Each plot was linear in a wide interval from quantification limits to 10 g/kg for K^+, 200 mg/kg for Ca^{2+}, 400 mg/kg for Na^+ and Mg^{2+}, 100 mg/kg for Mn^{2+} and Ni^{2+} and to 50 mg/kg for Li^+. A regression analysis with each cation was made. A *p*-value in the ANOVA test of 0.0000 was obtained for all cations. Since the *p*-value is less than 0.01, there is a statistically significant relationship between area and concentration at the 99% confidence level.

Table 1
Recoveries (%) obtained with the method of standard additions for analysed cations and *p*-value from ANOVA test for the comparison of the slopes of regression lines obtained when matrix effect was studied

Cation	Recovery (%) ± SD	*p*-Value
K^+	96.7 ± 2.5	0.8209
Ca^{2+}	100.4 ± 1.2	0.5368
Na^+	100.5 ± 1.1	0.2223
Mg^{2+}	100.9 ± 1.8	0.1535
Mn^{2+}	101.8 ± 3.3	0.2603
Ni^{2+}	88.5 ± 2.9	0.7322
Li^+	99.7 ± 1.7	0.2423

Table 2
Cations content (mg/kg) of analysed honeys

No. of sample	Geographical origin	Colour	Cation (mg/kg)						
			K^+ ($\times 10^3$)	Ca^{2+}	Na^+	Mg^{2+}	Mn^{2+}	Ni^{2+}	Li^+
1	A Coruña	Light	0.68	109	102	21	12	ND	1.6
2	A Coruña	Light	0.70	92	64	22	10	ND	2.0
3	A Coruña	Light	0.76	88	75	22	8.0	ND	2.8
4	A Coruña	Light	0.62	91	154	22	5.5	ND	NQ
5	A Coruña	Dark	0.78	89	45	17	11	ND	1.3
6	A Coruña	Dark	0.80	90	81	20	6.4	ND	2.0
7	Lugo	Dark	0.95	62	64	27	6.0	ND	3.2
8	Lugo	Dark	0.81	179	219	25	8.1	ND	1.4
9	Lugo	Dark	0.86	60	166	20	ND	ND	2.4
10	Lugo	Dark	0.50	50	90	36	7.0	ND	ND
11	Lugo	Dark	0.79	75	221	19	3.8	0.3	NQ
12	Lugo	Dark	0.61	56	68	18	2.3	1.5	1.8
13	Lugo	Dark	0.99	76	91	35	6.7	ND	1.8
14	Lugo	Dark	1.20	87	53	56	11	ND	1.8
15	Lugo	Dark	1.40	101	67	52	11	ND	2.8
16	Ourense	Dark	2.33	146	26	209	7.9	ND	3.2
17	Ourense	Dark	2.53	125	31	204	6.3	ND	2.9
18	Ourense	Dark	1.87	88	40	130	8.1	ND	0.93
19	Ourense	Dark	1.31	62	87	27	7.8	ND	3.6
20	Ourense	Dark	4.38	106	39	69	29	ND	2.2
21	Ourense	Dark	1.89	136	47	205	51	ND	2.1
22	Pontevedra	Light	0.66	116	56	18	13	ND	NQ
23	Pontevedra	Dark	0.97	77	101	20	4.5	ND	2.1
24	Pontevedra	Dark	1.47	76	67	41	12	4.7	3.6
25	Pontevedra	Dark	0.74	89	72	22	18	1.1	ND
Mean			1.22	93	85	54	11	1.9	2.3
SD			0.85	30	52	62	10	2.0	0.77
V_{min}			0.50	50	26	17	2.3	0.25	0.93
V_{max}			4.38	179	221	209	51	4.7	3.6

ND: not detectable. NQ: not quantificable.

3.3. Precision

The precision study has comprised repeatability and reproducibility assays. They were developed in three different honeys, which contained low, medium and high cations levels. The repeatability was established by injecting five times the same honey. The reproducibility was determined by analysing each sample of honey on three different days over about one month.

3.3.1. Migration times

When a capillary electrophoresis analytical method is developed, a common problem is the great variation in the absolute migration times [22]. A possible solution was the

Table 3
Cations content (mg/kg) obtained by other authors

Author	Cation (mg/kg)						
	K^+ ($\times 10^3$)	Ca^{2+}	Na^+	Mg^{2+}	Mn^{2+}	Ni^{2+}	Li^+
[1]	0.94 (0.10–4.7)	50 (5–266)	47 (6–400)	27 (7–126)	2.2 (0.2–9.5)	–	–
[5]	0.65 (0.19–1.12)	88 (56–120)	98 (63–133)	38 (3–73)	21 (19–23)	–	–
[6]	1.34 (0.68–2.01)	–	115 (62–168)	77 (34–120)	5 (2–8)	<0.2	0.05 (0.02–0.08)
[7]	0.47 (0.44–0.50)	48 (41–54)	96 (90–102)	37 (33–41)	3.0 (2.6–3.4)	–	–
[8]	0.68 (0.05–6.78)	120 (51–420)	87 (22–476)	55 (7–173)	3.9 (0.4–45)	0.23 (0.01–3.37)	–
[10]	–	192 (15–654)	–	71 (7–547)	11 (0–214)	0.99 (ND–1.70)	0.27 (0.15–1.20)
[11]	–	66 (22–110)	–	–	0.8 (0.1–1.4)	0.05 (0–0.10)	–
[12]	0.66 (0.13–2.16)	–	–	76 (36–220)	0.7 (0.1–2.0)	–	–
[13]	1.03 (0.15–3.89)	–	–	130 (68–166)	30 (4–58)	–	–
[14]	0.43 (0.07–1.81)	–	–	105 (24–324)	2.0 (0.3–5.0)	–	–
[15]	0.22 (0.21–0.89)	124 (61–280)	–	35 (11–90)	0.8 (0.2–2.0)	–	–
[17]	0.68 (0.14–2.61)	181 (110–248)	389 (256–501)	77 (37–139)	–	–	–

Mean ($V_{Min} - V_{Max}$).

use of relative migration times respect to a reference compound [23,24]. Ba^{2+} was used as reference compound for the identification of cations in honey samples because it was not present in honey samples at the detection limit of this method. In the study of precision of migration times better results were obtained for relative migration times. The relative standard deviations (RSD%) of the repeatability and the reproducibility were ≤0.44 and ≤0.56% for relative migration times and ≤1.55 and ≤2.27% for absolute migration times.

3.3.2. Honey content

The relative standard deviations (RSD%) of the repeatability and the reproducibility were ≤2.84 and ≤6.62%. For areas, a one-way analysis of variance was performed to test whether there were significantly differences between duplicates of injection triplicates and repetitions (five for repeatability and three for reproducibility). If the *p*-value of the *F*-test was greater than 0.05, there was not a statistically significant difference at 95% confidence level. All *p*-values studied were greater than 0.05 so we can conclude that precision is good for the determination of cations in honey samples by the proposed method.

3.4. Recovery

We have established the accuracy of the cation analysis by using the method of standard additions. Different amounts of each cation standards were added to equal volumes of the sample and then diluted to the same volume. Table 1 lists the percentage of recoveries obtained for each cation. Furthermore, to test whether there was a matrix effect, recovery assay must be analysed with different concentrations of sample. If regression lines obtained from the comparison of recoveries were parallel, we can conclude that there was not a matrix effect. Honey samples at two different concentrations were analysed and parallel lines were tested with the analysis of the slopes of the regression lines with an ANOVA test. If the *p*-value for the slopes was greater than 0.10 (Table 1), statistically significant differences among the slopes for the various volumes of sample at the 90% or higher confidence level were not found. Therefore, there was not a matrix effect for the determination of cations in honey samples by the proposed method.

3.5. Content of cations of honeys analysed

The cations content of 25 honey samples analysed is shown in Table 2. Table 3 summarizes results obtained by other authors [1,5–8,10–15,17]. As you can see these results are close to those obtained in this work in all cases except for Li^{+}, which we obtained higher results. However, two authors only determined this cation and they have obtained very different results between them.

Fig. 1 shows electropherograms of a light and a dark honey. A variability in the quantitative composition of cations was found in honeys. This variability could have any relation with the colour of the honeys because the content of K^{+}, Na^{+}, Mg^{2+} and Li^{+} cations were greater in dark honeys and Ni^{2+} cation was only detected in dark samples. Results obtained for Ca^{2+} and Mn^{2+} were similar in both honey types.

Tacking into account the geographical origin of the samples an important variability in honey cations content was found too. To test whether this variability was significantly an one-way ANOVA test was made with each component and results different statistically significant were found for K^{+}, Mg^{2+} and Mn^{2+}. We have made then a multiple range test (Fisher's least significant difference procedure) for these

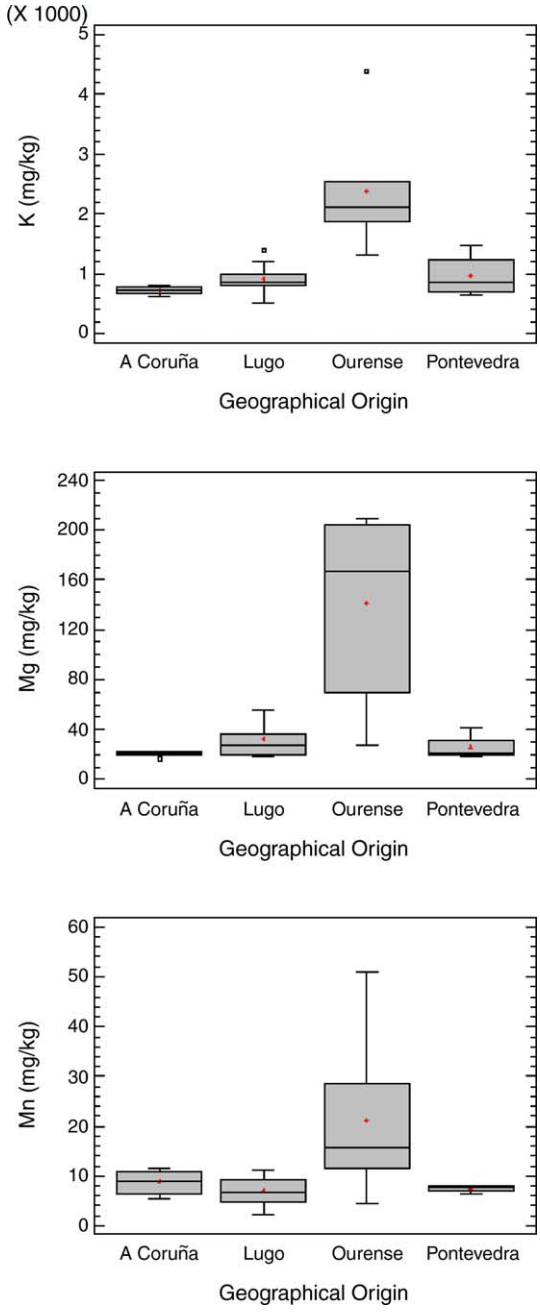

Fig. 2. Box and Whiskers plots of the content of K^{+}, Mg^{2+} and Mn^{2+} cations in honey samples analysed classified, according to their geographical origin (95% confidence level).

cations in order to know which regions could be differentiated and results showed that honey samples from Orense region could be identified at the 95% confidence level. Fig. 2 shows the Box and Whiskers plots of these three cations contents classified, according to their geographical origin.

4. Conclusions

The proposed method allows the separation and quantification of cations K^+, Ca^{2+}, Na^+, Mg^{2+}, Mn^{2+}, Ni^{2+} and Li^+ in honey samples by capillary electrophoresis in 4 min of analysis for the first time. These cations represent more than 99% of the total content of cations in honey. The electrophoretic analysis was simple, rapid and did not require any other preparation of sample than dilution and filtration. It means a great improvement in the determination of cations in honey samples because a calcination of the samples is not necessary and it could be useful for routine analysis. The cation contents of honey could be used to establish the geographical origin of samples.

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