

Analysis of Inorganic Cations in Honey by Capillary Zone Electrophoresis with Indirect UV Detection

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An indirect UV detection method based on capillary electrophoresis was developed to separate eleven metal cations completely, including alkali, alkaline earth and transition metal, which are related to evaluate the quality of honey. The background electrolyte contains 15 mmol/L chromophore imidazole, and acetic acid (pH = 3.7), which functioned as pH adjustor and complexing reagent. The selected cations can be completely separated within 8 min under hydrodynamic mode injection with a running voltage of 20 kV at 25°C. Limit of detection, linearity, reproducibility relative standard deviation of migration time and recoveries are in the range of 0.01–0.21 mg/L, 0.06–60.0 mg/L, 2.1–3.4% and 95.4–104.1%, respectively. The applicability of the method is shown by the analysis of honey samples, including a comparison with results of pretreatment and unpretreatment of eight samples. The results demonstrate that the developed method can conveniently be used in routine analysis of honey.

Introduction

Honey is an important secondary metabolism product of plants produced by honeybees. It is a remarkable complex natural liquid or semiliquid made up of more than a hundred substances (1), most of which are various carbohydrates, proteins, amino acids and vitamins (2). Thus, honey is widely used as a sugar substitute, an ingredient or a natural preservative due to its flavor and sweet taste. Apart from various nutritional effects related to the simple carbohydrates (1, 3, 4), minerals also present in honey in relative low levels and normally account approximately 0.1–0.2% for nectar honeys and 1% for honeydew honeys (5, 6), which can be appreciated as a supplementary source of metals in human diet. These metals in honey primarily come from soil and their concentrations greatly depend on the soil composition, which is decided by geochemical and geological features, such as volcanic and hydrothermal activity, regional conditions and climatic changes in the forage area of the bees (6). Metal ions in honey are stable over a long time in a certain collected site, hence, the analysis of the metal content is respected as a useful way for recognizing and discriminating honey, assessing the quality and safety of honey products and detecting potential fraud in its stated origin and quality (7–9). In addition, the content of metals in honey can also be affected by anthropogenic factors attributed to improperly releasing containments into the environment. Mines and steelworks, industrial and urban areas or highways in or near the bees' forage area can result in an increase in the concentrations of certain elements in honey as an effect of pollution by chemical wastes and exhaust fumes (10–13). If the contents of

elements, especially the heavy metals such as Cu, Co, Ba, Cd and Cr, exceed the certain safely levels, a wide range of ecotoxicological effects can arise (2). Accordingly, honey could be one of the suitable biomarkers to indicate the levels of air, water and soil contamination.

In recent years, some works have been devoted to the analysis of cations in honey by electrochemical, spectroscopic and chromatographic methods. Many electrochemical methods have been applied in the determination of heavy metals. For example, Muñoz (12) simultaneously determined Pb, Cd and Zn using differential stripping potentiometry. With differential pulse anodic stripping voltammetry (DPASV), Sanna (13) developed a method to detect Cu, Pb, Cd and Zn at a quite low limit of detection (LOD). However, because most of the electrochemical methods have been based on the cathodic reduction of these cations, it almost impossible to detect alkali and alkali-earth metal ions. Inductivity coupled plasma with atomic emission spectrometry (ICP–AES) (14) and cold vapour-inductively coupled plasma emission spectrometry (CV-ICP–OES) (15) can also be applied in cation analysis. Additionally, metallic elements and nonmetallic ions can be detected. These methods are especially preferred in the determination of trace Hg. Regarded as a powerful and convenient instrumental analytical method, ion chromatography has also widely been used in the analysis of Cd, Co, Cu, Fe, Ni, Pb and Zn with post-column derivatization by 4-(2-pyridylazo)resorcinol (PAR) (16). Some methods mentioned previously need extensive sample preparation, large amounts of reagents and samples and consume a great deal of time, which perhaps leads to decreases in efficiency and precision or even cause the loss of some trace analytes (17–20). Comparing with these methods, capillary zone electrophoresis (CZE) has higher efficiency, consumes fewer reagents and samples and is faster (20), which makes CZE an alternative or complement to other analytical methods. However, research on the separation and determination of metal cations in honey by CZE is limited so far.

Ultraviolet (UV) detectors are normally the first choice for the CZE systems. Unfortunately, most cations lack UV absorption properties. This problem can be solved by two kinds of techniques if a UV detector is used: (i) derivatization by the addition of a good UV absorbing tag to enhance the detection; and (ii) employing an indirect detection method in which a UV-absorbing co-ion (the so-called chromophore or probe) is added into the background electrolyte (BGE) and the detection is accomplished by displacement of this co-ion by UV-transparent analytes. Derivatization procedures to improve separation and detection are chosen reluctantly because the

enhanced results often justify the extra work in sample preparation and method validation (20, 21). Furthermore, the appropriate derivatization chemistry for some cations like Na^+ and K^+ is unavailable, which makes the indirect detection method preferred.

Chromophores, such as benzylamine (BA), *N,N*-dimethylbenzylamine (DBA), imidazole and its derivants have widely been employed in the BGE of indirect methods (22–25). Among them, the electrophoretic mobility of imidazole perfectly matches alkali, alkaline earth and even some heavy metal ions. This property makes it a suitable chromophore for indirect UV detection of common metals, and it has been successfully applied in the environment (26–28), food (29, 30, 31) and beverage (21) inspection since it was recommended by Beck (25). In some previous works (29), the concentrations they used were relatively low and it seems that the influence of the concentration has not been taken into account. According to the principle of separation, the ionic strength of BGE and the strength of the sample could significantly affect separation efficiency and resolution. This suggests that better results might be obtained at higher BGE, i.e., imidazole concentration.

To eliminate the surface Gibbs free energy, the inner wall of capillary might adsorb the Pb^{2+} to form insoluble lead silicate (32), which not only influences the quantitative analysis of Pb^{2+} , but also the separation effect of other cations. Fe and Zn are considered two of the basic elements for human beings, and their negative effects are usually negligible. For instance, Fe affects the taste, color and smell of honeys. A median excess of Zn will retard the absorption of Cu. Therefore, Fe and Zn are also important for honey, and should also be added to the metal ions detection for quality control. Considering the importance of identification and qualification of metal cations in honey, in this work, we developed a convenient CZE method with indirect UV detection for the determination of eleven metal ions, including K^+ , Ba^{2+} , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} , Li^+ and Cu^{2+} , which commonly appear in honeys. As shown in the following, no pretreatment was required and these selected cations could be completely separated in a relatively short run time.

Experimental

Chemicals

The reagents, including hydrochloric acid, acetic acid, sodium hydroxide, imidazole and nitrates of K^+ , Ba^{2+} , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} , Li^+ and Cu^{2+} , are analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd.

Standards and BGE

The reserved cation standard solutions were prepared from the corresponding nitrate salts, whose concentrations were 500 mg/kg. The calibration standards were prepared by diluted from the reserved solutions. Ultra-pure water used in this work was obtained from a Milli-Q system (18.2 M Ω ·cm, Millipore, Milford, MA). The solutions were stored at 4°C.

The BGE consisted of certain concentrations of imidazole, whose pH was adjusted to required values by adding 10 mol/L acetic acid. The solutions were filtered through 0.45 μm membranes and degassed before use.

Apparatus

The separation procedure was performed on a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System equipped with a diode array detector (DAD) and interfaced with the 32 Karat software for data acquisition (Beckman, Danvers, MA). An uncoated fused-silica capillary with 75 μm i.d. and 57 cm length was used. The distance from the point of injection to on-column detecting window was 50 cm.

Electrophoretic procedure

The new capillary was rinsed at 20 psi (1 psi = 6,894.76 Pa) with the following steps: Milli-Q water for 10 min, methanol for 20 min, 1 mol/L NaOH for 20 min, 1 mol/L HCl for 20 min and finally the Milli-Q water for 10 min. Milli-Q water rinsing for 5 min and BGE for 20 min were required for daily conditioning of the capillary; the capillary was washed for 2 min with BGE between injections. The capillary was rinsed with Milli-Q water for 10 min after daily analysis.

Injection was performed under hydrodynamic mode at an overpressure of 0.5 psi for 5 s. The electrophoretic procedures were conducted at 25°C.

Samples

In this work, eight honey samples from different origins in China were assayed by the developed method. Honeys were purchased from a local supermarket and prepared with two approaches. The samples, weighed for 2.000 g, were diluted 10-fold and filtered by 0.45 μm membranes to remove the solid particles, then analyzed directly by the developed CE method without further pretreatment. In the other method, honey samples were handled by means of hot decomposition to remove carbohydrates in the matrix. Initially, the same samples were weighed equally to unpretreated samples into porcelain crucibles and carbonized at 200°C for 0.5 h and then incinerated at 600°C until they reached a constant mass (approximately 2 h). After cooling to room temperature, 1.0 mol/L HCl was added to the resulting ashes. Next, the solutions were heated to dryness. Finally the residues were re-dissolved in 10 mL Milli-Q water and filtered by 0.45 μm membranes. Each standard solution and sample was injected five times to testify the repeatability and precision.

Results and Discussion

Effects of separation

BA, DBA and imidazole are regularly employed as chromophores for the indirect UV detection method. Imidazole was chosen by consideration of solubility (BA, 36.0 g/L; DBA, 1.2 g/L; imidazole, 560 g/L), pK_b (BA, 9.40; DBA, 5.15; imidazole, 6.95) and the ionization degree. Furthermore, one of the general principles that can lead to higher separation efficiency is a BGE with relatively high ionic concentration and samples with relative low ionic concentration. Thus, the concentration of imidazole is especially critical. Figure 1 illustrates the effect of imidazole concentration on the resolutions of the cations, in which the pH of the BGE was kept at 4.0. When the imidazole concentration is 7 mmol/L, cations pass through the detecting

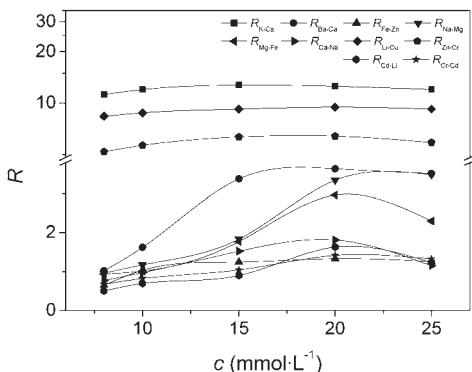


Figure 1. Effect of imidazole concentration on resolution of 11 cations. The pH of BGE was held at 4.0 by acetic acid and the separation temperature was 25°C.

window in the order of K^+ , Ba^{2+} , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} , Li^+ and Cu^{2+} within a few minutes. However, most of the adjacent peaks (so-called critical band pair), except peak pairs K^+/Ba^{2+} , Zn^{2+}/Cr^{3+} and Li^+/Cu^{2+} , are so close that they appear to separate poorly, which is attributed to the small differences among their mobilities. With the increase of imidazole concentration, resolutions improve until they almost reach their maximum at imidazole concentration of 20 mmol/L. If the concentration is increased further, 25 mmol/L, for instance, the resolutions decrease gradually because the apparent mobilities of some cations become close again. At pH 4.0, the best separation can be obtained at 20 mmol/L, at which cations except Fe^{2+}/Zn^{2+} can be separated completely ($R > 1.5$); however, asymmetric peaks and stronger noise emerge, probably caused by elevated BGE conductivity, which is not beneficial for accurate quantitation of sample components. This result strongly suggests that a lower concentration, i.e., 15 mmol/L, should be considered. Under this condition, a shorter run time was achieved; however, pairs Ca^{2+}/Na^+ , Fe^{2+}/Zn^{2+} and Cd^{2+}/Li^+ did not satisfy the requirement of complete separation. Therefore, further optimization procedures are required to fulfil the goals of separations.

Another parameter impacted on resolution is the mobility of electroosmotic flow (EOF) in fused silica capillary, the changes of which strongly depend on the pH value of BGE, because it affects the degree of dissociation of the silanol group and consequently the charge density of inner capillary surface, and then ultimately influences the EOF velocity. The separation cannot be fulfilled if the EOF mobility is too fast, however, the separation efficiency and peak shape will be worse if EOF velocity is too slow. With the purpose of separating overlapped peak groups, i.e., Ca^{2+}/Na^+ , Fe^{2+}/Zn^{2+} and Cd^{2+}/Li^+ , acetic acid, which also functions as a weak complexing reagent, was used to adjust the pH to alter the EOF velocity; thus, the separation could be improved. For clarity, only the resolution of two closest adjacent peaks (R_{min}), was plotted with systematical variations in pH from 3.2 to 5.2 at the presence of 15 mmol/L imidazole. The results are shown in Figure 2. At the initial pH (3.2), band pair Na^+/Mg^{2+} is critical and its resolution ($R = 1.04$) should be improved. When the pH was increased to 3.5, Na^+/Mg^{2+} pair was greatly resolved ($R = 1.23$), but there was only a small improvement in the separation of Fe^{2+}/Zn^{2+} . As a result, peak pair

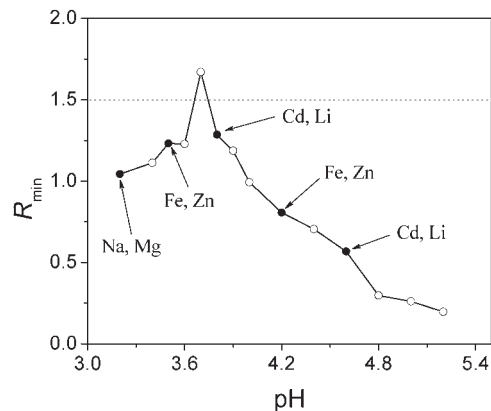


Figure 2. The dependence of minimum resolution (R_{min}) on pH. The concentration of imidazole is 15 mmol/L and the separation temperature is 25°C.

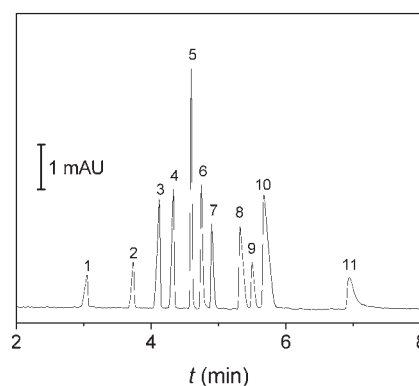


Figure 3. Electropherogram of reference standard mixture. Peaks on the electropherogram: 1, K^+ ; 2, Ba^{2+} ; 3, Ca^{2+} ; 4, Na^+ ; 5, Mg^{2+} ; 6, Fe^{2+} ; 7, Zn^{2+} ; 8, Cr^{3+} ; 9, Cd^{2+} ; 10, Li^+ ; and 11, Cu^{2+} , respectively. Their concentrations were 2 mg/kg. The separation conditions are: 15 mmol/L imidazole and acetic acid (pH 3.70), 25°C, 20 kV, indirect UV detection at 214 nm, hydrodynamic mode injection by overpressure at anodic end with 0.5 psi for 5 s. The following conditions are the same except otherwise stated.

Fe^{2+}/Zn^{2+} is critical under such conditions. If the pH was adjusted to 3.7, Fe^{2+}/Zn^{2+} could be adequately separated ($R = 1.67$), which suggested that total components could complete the baseline separation. Further increases in pH generated a decrease in resolution for these band pairs. It was concluded that pH 3.7 should be the best acidic condition for separation.

In previous works, 185 nm (29) was selected for data acquisition when imidazole served as the probe of BGE to separate alkaline and alkaline earth metals; however, the concentration of imidazole here is slightly higher than others at 5–10 mmol/L, so that UV absorption is greatly enhanced at that wavelength (cutoff wavelength = 231 nm) and the signal-to-noise ratio (S/N) thus decreases significantly in the far-UV region. Based on this consideration, a wavelength of 214 nm, which is available for almost all UV detectors, was chosen for signal recording in this work. High voltage is another stratagem to obtain better resolution and peak shape. However, excess voltage can lead to elevated current and the undesirable Joule heating effect that results in distortion peaks. The separation voltage was settled at 20 kV after a batch of experiments was verified from 15 to 25 kV.

Table I

Linearity, LOD, Correlation Coefficients and Repeatability of the Cations Analyzed Using the Developed Method

Cation	Migration time (t^R)(min)	LOD (mg/kg)	Linearity	Correlation coefficient (r)	RSD (% , n = 5)
K ⁺	4.033	0.21	$y = 1236.77 + 359.248x$ (0.43–50.0 mg/kg)	0.9996	3.2
Ba ²⁺	5.025	0.15	$y = 390.475 + 301.495x$ (0.20–40.0 mg/kg)	0.9998	2.4
Ca ²⁺	5.400	0.08	$y = 1503.36 + 1138.20x$ (0.16–60.0 mg/kg)	0.9995	2.1
Na ⁺	5.721	0.07	$y = 215.89 + 967.90x$ (0.15–60.0 mg/kg)	0.9998	3.1
Mg ²⁺	5.892	0.01	$y = 1161.94 + 2282.31x$ (0.06–50.0 mg/kg)	0.9997	2.0
Fe ²⁺	6.027	0.06	$y = 219.90 + 1009.5x$ (0.12–45.0 mg/kg)	0.9997	3.4
Zn ²⁺	6.148	0.12	$y = 73.39 + 438.21x$ (0.25–30.0 mg/kg)	0.9995	2.4
Cr ³⁺	6.482	0.15	$y = -102.26 + 548.03x$ (0.30–45.0 mg/kg)	0.9998	2.5
Cd ²⁺	6.796	0.16	$y = 126.970 + 300.820x$ (0.28–35.0 mg/kg)	0.9997	3.0
Li ⁺	6.988	0.07	$y = 917.117 + 1246.82x$ (0.15–35.0 mg/kg)	0.9995	2.6
Cu ²⁺	8.229	0.21	$y = 1519.57 + 606.449x$ (0.47–40.0 mg/kg)	0.9997	3.2

Table II

Standard Addition Recovery of 11 Cations (n = 5)

	Spiked 3.00 mg/kg		Spiked 5.00 mg/kg		Spiked 7.00 mg/kg	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
K ⁺	96.3	5.5	97.5	4.8	96.8	5.1
Ba ²⁺	98.3	1.5	97.4	2.1	97.5	2.4
Ca ²⁺	95.7	2.2	96.8	2.5	96.5	2.5
Na ⁺	99.1	1.4	98.1	2.4	98.7	1.9
Mg ²⁺	98.4	0.9	98.2	1.6	97.9	1.8
Fe ²⁺	99.2	2.1	99.5	2.4	99.2	2.3
Zn ²⁺	101.5	2.0	100.6	1.9	101.3	2.6
Cr ³⁺	99.3	1.4	100.3	1.5	100.5	1.7
Cd ²⁺	96.9	3.9	97.7	3.0	96.2	3.8
Li ⁺	95.4	5.2	95.4	4.8	96.1	4.4
Cu ²⁺	104.1	5.8	103.4	4.6	103.6	5.0

Detection conditions

Reference standards of selected metals were injected both individually and as a mixture with the developed method to measure their migration time (t_m) by which the analytes were identified. Figure 3 shows a representative electrophoregram containing 11 cations with a concentration of 2 mg/kg for each. The 11 components can be separated within 8 min. Band pair 6/7 (Fe²⁺/Zn²⁺) is the critical pair with smallest resolution ($R \approx 1.7 > 1.5$). The degree of hydrolysis decided the migration order of 11 cations. Compared to the alkali metals, Li⁺, Na⁺ and K⁺, the hydrolysis degree of Li⁺ is the most serious, and Na⁺ lists second, then Li⁺ owns the biggest diameter of hydrolysis. Therefore, the migration orders should be K⁺, Na⁺ and Li⁺. The same situation happens to alkaline earth metals, which list as Ba²⁺, Ca²⁺ and Mg²⁺. The hydrolysis degree of Cr³⁺ and Cu²⁺ is much more than Fe²⁺ and Zn²⁺, and consequently the migration times of Cr³⁺ and Cu²⁺ are longer than Fe²⁺ and Zn²⁺, respectively. As baseline separation was achieved, both peak-height and peak-area calibrations can be used for quantitative measurement. Considering that the interference of neighboring peaks can be ignored and intrinsically decreases the noise on signal, peak-area calibration is much preferred in this work. The linear dependence of peak area (y) on concentration (x) is presented as $y = ax + b$ with a correlation coefficient r, where a and b are the coefficients of regression equations. The sensitivity of the method is expressed in term of LOD, calculated by three times of S/N ratio. The validation results, including the linear ranges, linear equations, LODs, correlation coefficients and relative standard deviations (RSDs) of migration time are presented in Table I.

Table III

Concentration of Metal Ions in Honey Samples After their Direct Dissolution in Water (Unpretreated) and With Hot Decomposition Pretreatment (Pretreated)

Samples		K ⁺	Ca ²⁺	Na ⁺	Mg ²⁺	Fe ²⁺	Cr ³⁺	Cu ²⁺
		(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
1	Pretreated	69.7	8.51	6.33	15.6	—*	—	—
	Unpretreated	122	14.1	11.6	18.0	—	—	—
2	Pretreated	82.1	52.2	78.4	6.82	—	—	—
	Unpretreated	131	77.2	90.2	9.20	—	—	NQ [†]
3	Pretreated	332	42.0	47.0	62.1	—	—	—
	Unpretreated	352	67.1	81.0	85.1	NQ	—	—
4	Pretreated	34.8	—	NQ	4.20	—	—	—
	Unpretreated	55.5	—	6.4	6.40	—	—	—
5	Pretreated	34.6	—	9.13	—	—	—	—
	Unpretreated	57.0	—	16.5	—	—	—	—
6	Pretreated	61.8	11.6	NQ	5.36	—	—	—
	Unpretreated	127	14.9	7.22	8.16	—	—	NQ
7	Pretreated	18.5	22.9	6.35	2.73	—	—	—
	Unpretreated	57.8	28.6	24.9	8.95	NQ	4.60	4.82
8	Pretreated	6.72	NQ	21.4	1.70	NQ	NQ	—
	Unpretreated	9.95	1.70	35.3	2.50	2.83	NQ	NQ

*Cations were undetectable.

†Cations could be detected but not quantified.

The correlation coefficients range from 0.9995 for Li⁺ to 0.9998 for Ba²⁺, Mg²⁺ and Cr³⁺, and the LODs were distributed from 0.01 mg/kg to 0.21 mg/kg. The RSD, ranging from 2.0 to 3.4%, manifests that the precision of the method was acceptable.

To testify the accuracy of the method, standard addition recovery and RSD were calculated after attaining peak-areas of adding 1 mL standard solution with the concentration of 3.00, 5.00 and 7.00 mg/kg, respectively, into 5-mL blank samples, as listed in Table II. The recoveries range from 95.4 to 104.1%, indicating that the method was repeatable and accurate.

Analytical application

In the traditional method, sample preparation is necessary to remove carbohydrates and other interferences from the honey matrix. However, the developed method can determine cations directly in honey without other pretreatment except dilution and filtration. Therefore, a comparison test was carried for eight honey samples to evaluate the influence of the hot

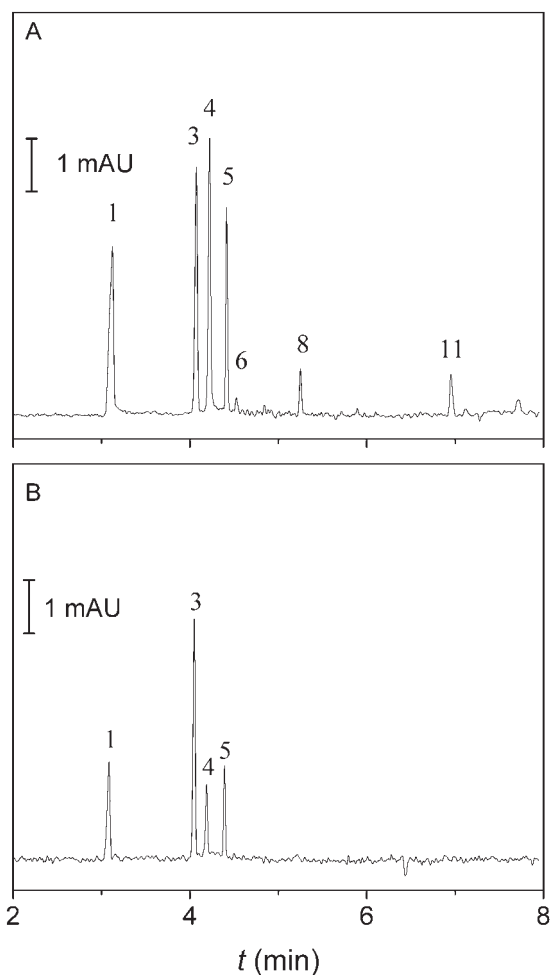


Figure 4. Representative electropherogram profile of various metal cations obtained in Sample 7: unpretreated (A); pretreated (B).

decomposition treatment on the detection before the CE procedure and the results are listed in Table II, with RSDs ranging from 2.8 to 8.9%. Typical electropherograms of Sample 7 are shown in Figure 4, where Figures 4A and 4B represent unpretreated and pretreated sample, respectively. The data Table II and the electropherogram of Figure 4 clearly show that pretreatment like hot decomposition can decrease the concentration of cations in honey, and even cause the disappearance of some components, as shown in Figure 4. The contents of massive constituents such as K^+ , Ca^{2+} , Na^+ and Mg^{2+} drop dramatically after pretreatment. The detectable Fe^{2+} and quantitative Cu^{2+} in Figure 4A finally disappear in Figure 4B. Because the accuracy could be significantly decreased with the intricate pretreatment and the determination is not interfered by the matrix in honeys, unpretreatment is selected for the developed method.

Ba^{2+} , Cd^{2+} and Zn^{2+} , which are of special interest in food safety, as well as Li^+ , are found in very low levels and not detected in given samples. However, their corresponding peaks can appear in the electropherograms with anticipant peak-areas if reference standards were deliberately added in the samples, which manifested the capability of the developed method.

Conclusions

A simple CZE method with indirect UV detection was successfully developed to determine eleven common cations, including K^+ , Ba^{2+} , Ca^{2+} , Na^+ , Mg^{2+} , Fe^{2+} , Zn^{2+} , Cr^{3+} , Cd^{2+} , Li^+ and Cu^{2+} . The optimized parameters include 15 mmol/L chromophore imidazole and pH adjustment acetic acid (pH 3.7), which also acts as complexing reagent. The separation was performed at a voltage of 20 kV and a wavelength of 214 nm was selected for the data acquisition. The developed method was demonstrated to be linear, precise and acceptable.

Eight real samples from different botanical origins in China were examined, both with and without pretreatment, before CZE analysis. None presented excessive toxic metals ions with both approaches. When hot decomposition was conducted, significant loss in metal contents could be observed, which suggested that such pretreatment could decrease the accuracy in the determination of cations in honey and should not be recommended for the developed method. Furthermore, sample preparation steps other than dilution and filtration are not necessary, which makes it suitable for routine analysis.

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