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Determination of α-hydroxy acids in cosmetic products by capillary electrophoresis

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Abstract

In this work, a simple and reliable method for the simultaneous analysis of α-hydroxy acids such as tartaric, glycolic and lactic acids in cosmetic products was developed using capillary electrophoresis with indirect UV detection at 254 nm. A buffer solution containing 10 mmol l^{-1} potassium phthalate (pH 4.1) and 0.5 mmol l^{-1} cetyltrimethylammonium bromide as electroosmotic flow modifier allowed baseline resolution of the analytes in approximately 3 min. A few validation parameters of the proposed method include: good linearity for all compounds in the range from 10 to 100 mg l^{-1} with coefficients of correlation larger than 0.9999. The average recoveries of tartaric, glycolic and lactic acids in commercial samples were 99.12, 99.41 and 99.43%, respectively. The method presented acceptable precision with average relative standard deviation of 0.54% (assay of commercial samples), 0.44% (peak area) and 0.16% (migration time).

Keywords: α-Hydroxy acids; Cosmetics; Capillary electrophoresis; Indirect detection; Validation

1. Introduction

The α-hydroxy acids (AHAs) are organic acids with a hydroxyl group on the carbon adjacent to the carboxylic acid group (alpha carbon), naturally occurring in most fruits, milk, sugar cane juice, wines and beer [1–3].

In recent years, the use of AHAs in cosmetic products has greatly expanded. AHAs are used in many cosmetic products such as exfoliants, moisturizers and emollients to correct skin disorders, to increase skin hydration, to induce the removal of the outer layers of the skin and to improve some of the visible effects of ageing by reducing lines and wrinkles, and stimulating skin cell renewal. The most often used AHAs in cosmetic preparations are glycolic, lactic, tartaric, mandelic and citric acids, and many are used in combination [2].

The Food and Drug Administration (FDA) concluded that certain formulations containing AHAs can affect the skin, since AHAs seem to be capable of penetrating the skin barrier, increasing skin sensitivity to UV radiation during application. Some undesirable effects include facial redness, burning, rash formation, itching and skin discoloration [1,3]. These effects depend on the product's pH level, the vehicle cream, the frequency of use and in which part of the skin is applied, as well as the AHAs concentration. In order to AHAs to produce the maximum "anti-ageing" efficacy, the concentration of the AHAs must be greater than 10%, however, this is the condition in which they present the most irritating properties, provoking undesirable side-effects to consumers. The recommended daily range concentration of AHAs in cosmetic products is 1–10% [2–5]. Hence, for both quality control purposes and to prevent excessive concentrations, there is a need for development and validation of analytical methods for quantitative determination of AHAs.

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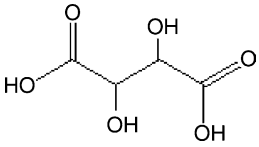
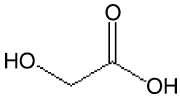
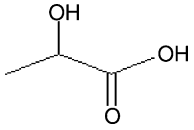
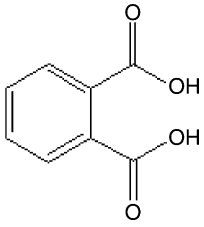
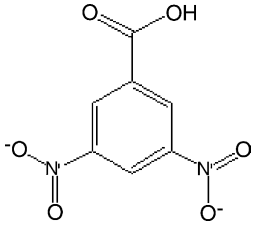
Table 1
Chemical structures, pK_a values and ionic mobilities (μ_1 and μ_2) of tartaric, glycolic, lactic, phthalic and 3,5-dinitrobenzoic acids [23]

Name	Structure	pK_{a1}	pK_{a2}	μ_1 ($\times 10^{-5}$ cm^2 V^{-1} s^{-1})	μ_2 ($\times 10^{-5}$ cm^2 V^{-1} s^{-1})
Tartaric acid		3.04	4.37	−32.6	−60.7
Glycolic acid		3.89	–	−42.4	–
Lactic acid		3.86	–	−36.5	–
Phthalic acid		2.95	5.41	−28.1	−52.9
3,5-Dinitrobenzoic acid		2.82	–	−29.3	–

Capillary electrophoresis (CE) is a separation technique based on the differential migration of charged molecules through a small capillary under the influence of an electric field [6]. CE has been applied to several important analytical problems including the assessment of active principles and impurities of pharmaceuticals and biopharmaceuticals [7]. The various advantages offered by CE include high resolution, short analysis times, the need for a small amount of sample and low maintenance costs.

The detection of analytes in CE is usually performed by direct photometric detection. Most CE separations of low-molecular-mass organic acids are carried out using indirect UV detection because of the low UV absorptivities of these analytes in the region above 220 nm [8,9–12].

Many authors reported the use of capillary electrophoresis (CE) for the determination of AHAs in beverages, foods and fruits [8,13–21]. However, only a paper was found in the literature using CE for the quantitative determination of lactic acid in cosmetic products [22].

In this research, a CE method with indirect UV detection was developed, optimized and validated for the simultaneous quantitative determination of tartaric, glycolic and lactic acids in cosmetic cream. The structures of AHAs can be observed in Table 1.

2. Experimental

2.1. Instrumentation

All experiments were performed on a CE system, model P/ACE 5510 from Beckman Instruments (Fullerton, CA, USA) equipped with a filter-carrousel UV detector.

Uncoated fused-silica capillary (Polymicron Technologies, Phoenix, AZ, USA) was used in all experiments with dimensions of 57 cm total length, 50 cm effective length, and 75 μm i.d.

2.2. Reagents, standards and samples

All reagents and solvents were of analytical grade and were used without further purification.

Water was purified by deionization through a Milli-Q® system (Millipore, Bedford, MA, USA) and was used to prepare all solutions.

Cetyltrimethylammonium bromide (CTAB) was obtained from Aldrich® (Sigma–Aldrich, St. Louis, MO, USA); potassium hydrogen phthalate, sodium hydroxide and ethanol were obtained from Merck® (Darmstadt, Germany).

The standards of tartaric acid, glycolic acid and lactic acid were obtained from Aldrich®. The cosmetic creams were purchased from local market stores.

2.3. Preparation of background electrolyte (BGE)

All experiments were performed with 10 mmol l^{-1} potassium phthalate solution containing 0.5 mmol l^{-1} CTAB (final pH = 4.1).

2.4. Standard and sample solutions

2.4.1. Standard solutions

Standard stock solutions of tartaric, glycolic and lactic acids were prepared in deionized water in a concentration of 500 μg ml^{-1}. Aliquots of standard stock solutions of tartaric, glycolic and lactic acids were diluted to give solutions of final concentrations of 10, 20, 40, 60, 80 and 100 μg ml^{-1}.

2.4.2. Sample solutions

An amount close to 1 g of each sample was accurately weighed and transferred to 250 ml volumetric flasks and diluted with ethanol, followed by an ultrasonic treatment for 15 min and stirred with a magnetic stirrer for 10 min till complete dissolution. The volume was completed with ethanol. Then, the solutions were filtered through filter paper, rejecting the first 10 ml. A 5 ml aliquot was transferred to a 25 ml volumetric flask and diluted to volume with deionized water.

2.5. Electrophoretic procedure

The capillary was preconditioned by rinsing with 1 mol l^{-1} NaOH solution (20 psi, 5 min), followed by deionized water (5 min) and electrolyte (30 min).

Sample was injected hydrodynamically during 5 s at 0.5 psi. The separation was carried out at 20 kV to the anode, constant voltage conditions (≈21 μA). The system temperature was maintained at 35 °C. In between runs, the capillary was usually reconditioned by rinsing with of the electrolyte solution (20 psi, 2 min). The indirect UV detection signal was recorded at 254 nm. Data acquisition and treatment was controlled by the software supplied by the manufacturer (Beckman P/ACE System Gold® Software, v1.1). The migration order was established by injecting the AHAs solutions individually.

3. Results and discussion

3.1. Method optimization

In the electrophoretic separation of compounds that present weak acid character, at least two species should be considered: the non-ionized acid molecule (with zero mobility) and its conjugated base, an anion. The concept of effective mobility is used to describe the migration behavior of such weak electrolytes. By definition, any substance (*i*) presented in solution as different species (*j*), related each other by a rapid acid–base equilibrium, migrates in the electric field as a single solute, exhibiting an effective mobility (μ_i^{eff}) calculated by the following equation:

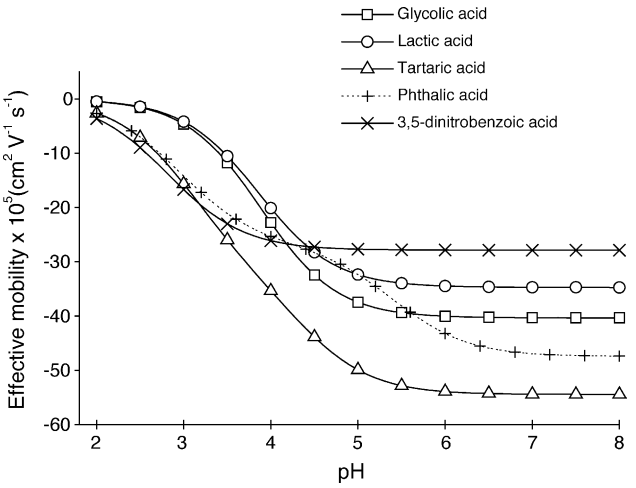


Fig. 1. Effective mobility vs. pH curves.

$$\mu_i^{\text{eff}} = \sum \mu_j \alpha_j \quad (1)$$

where α_j is the molar fraction or distribution function and μ_j is the ionic mobility of each individual species (*j*).

For monoprotic acids, the distribution function can be expressed in terms of the dissociation constant and the medium acidity:

$$\alpha_j = \frac{K_a}{K_a + [H^+]} \quad (2)$$

The ionic mobility (μ_j) and pK_a values for each analyte under consideration and the two anionic chromophores that are commonly used for indirect detection in capillary electrophoresis are shown in Table 1 [23]. From these data, effective mobility versus pH curves could be constructed (Fig. 1). The inspection of such curves gives a preliminary assessment of an appropriate pH range where the separation is likely to occur, i.e. where relative differences of mobility are maximized. By considering that good peak symmetry is usually obtained when the analyte mobility matches the chromophore mobility, an appropriate chromophore can also be selected from the inspection of the mobility versus pH curves. In addition, for high sensitivity during indirect detection, a chromophore should also exhibit high molar absorptivity.

As it can be inferred from inspection of Fig. 1, above pH 3.7 the separation of all analytes can be approached experimentally. Likewise, both chromophores present mobility close to that of the analytes and could possibly generated equally well-defined peaks. However, pH 4.1 was selected in conjunction with phthalate as chromophore due to two factors. Firstly, at pH 4.1, the phthalic system presents good buffering capacity. Secondly, the experimental preparation

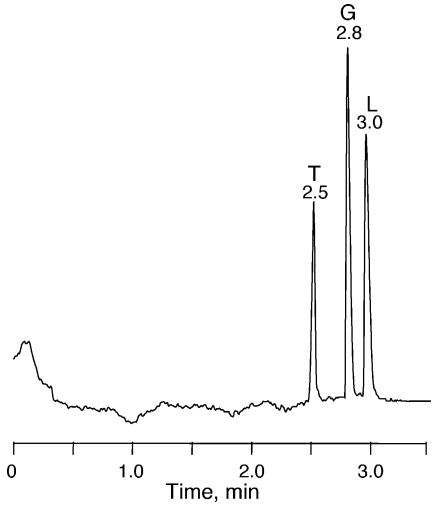


Fig. 2. Electropherogram of AHAs standard solution (60 μg ml^{-1} of each AHA): (T) tartaric acid, (G) glycolic acid and (L) lactic acid. Conditions: BGE 10 mmol l^{-1} potassium hydrogen phthalate, 0.5 mmol l^{-1} CTAB, pH 4.1. Separation voltage 20 kV to the anode, detection UV 254 nm, temperature 35 °C.

of the buffer is straightforward: a simple dissolution of potassium hydrogen phthalate salt in water already gives a solution at the selected pH. The easy of buffer preparation will certainly add to the method improved ruggedness. And finally, since at the selected pH, the electroosmotic flow (eof) presents small magnitude, a buffer modifier, CTAB at 0.5 mmol l^{-1} concentration, was then added to the electrolyte, to promote a fast analysis time. CTAB speeds up the separation because electrophoretic and electroosmotic velocities are in the same direction. Above 0.1 mmol l^{-1} concentration, eof is already reversed, but 0.5 mmol l^{-1} CTAB is commonly used because the eof is stable and constant [24].

The separation of tartaric, glycolic and lactic acids in standard solution is shown in Fig. 2. The peaks are well resolved within a 3 min run time.

3.2. Quantitative test of the analytical method

International organizations, such United States Pharmacopeia (USP), the International Conference on Harmonization (ICH) Guidelines and AOAC INTERNATIONAL provide recommendations on the various parameters to be determined for validation of analytical methodologies [25–28]. In general the criteria for validation applied to CE methods are similar to those applied to HPLC assays [29,30]. In this research were evaluated the parameters of linearity, precision, accuracy, limit of quantitation and specificity.

Table 2
Results obtained in the validation of the CE method regarding linearity and LOQ

	AHAs		
	T	G	L
Linearity			
Range of concentration (μg ml^{-1})	10–100	10–100	10–100
Number of solutions	6	6	6
Slope	99.38	158.55	139.12
Standard error of slope	0.16	0.12	0.09
Intercept	20.34	10.32	5.82
Standard error of intercept	9.79	7.02	5.23
Correlation coefficient (r)	0.99990	0.99990	0.99998
Limit of quantitation (μg ml^{-1})	0.99	0.44	0.38

Average of 10 determinations. T: tartaric acid; G: glycolic acid; L: lactic acid.

3.2.1. Linearity

The linearity of an analytical method is its ability to produce test results that are directly proportional to the concentration of the analytes in the sample within a given range [25–30].

The linearity of the present method was investigated by analyzing standard solutions containing a mixture of the three AHAs with six different levels of known concentrations in the range of 10–100 μg ml^{-1}. Each solution was injected in duplicate. In order to obtain the calibration curve for each analyte, the respective peak areas in the electropherograms were plotted against concentration (μg ml^{-1}). The calibration curves were obtained using linear least squares regression procedures. The slope, intercept and the correlation coefficient (r) for each AHA are shown in Table 2.

The coefficients of correlation were close to unity (0.99993), hence, there was a linear relationship between the amount of AHAs and the detection response.

Table 3
Statistical data and results obtained in the determination of AHAs contained in commercially available samples analyzed by CE

Statistical data	Sample A (face cream)			Sample B (face cream)	Sample C (face cream)	Sample D (hand cream)	Sample E (foot cream)
	T	G	L	G	G	L	L
Migration time R.S.D. (%)[a]	0.24	0.21	0.13	0.12	0.12	0.19	0.13
Peak area R.S.D. (%)[a]	0.51	0.65	0.31	0.59	0.63	0.22	0.19
Assay R.S.D. (%)[a]	0.98	0.33	0.82	0.25	0.49	0.57	0.34
Confidence limit (P = 95%)	99.28 ± 0.17	99.02 ± 0.14	100.17 ± 0.23	99.70 ± 0.14	100.25 ± 0.11	100.90 ± 0.33	100.84 ± 0.12
Amount declared (%, w/w)	3.00	7.50	5.00	10.00	4.00	10.00	6.00
Amount found (%, w/w)	2.98	7.43	5.01	9.97	4.01	10.09	6.05

T: tartaric acid; G: glycolic acid; L: lactic acid. A, B, C, D and E: commercial samples.
[a] Average of 10 determinations.

Table 4
Recovery of standard AHAs solutions added to commercially available samples (A, B, C, D and E) analyzed by CE

	Sample A (face cream)			Sample B (face cream)	Sample C (face cream)	Sample D (hand cream)	Sample E (foot cream)
	T	G	L	G	G	L	L
Amount added ($\mu g\,ml^{-1}$)	2.40	6.00	4.00	8.00	6.00	8.00	4.80
	4.80	12.00	8.00	16.00	12.00	16.00	9.60
	7.20	18.00	12.00	24.00	18.00	24.00	14.40
Amount found[a] ($\mu g\,ml^{-1}$)	2.38 ± 0.26	5.95 ± 0.05	3.98 ± 0.58	7.95 ± 0.15	5.99 ± 0.19	7.91 ± 0.13	4.76 ± 0.38
	4.72 ± 0.39	12.03 ± 0.21	7.98 ± 0.65	15.87 ± 0.16	11.79 ± 0.11	15.88 ± 0.35	9.68 ± 0.12
	7.19 ± 0.25	17.94 ± 0.54	11.84 ± 0.41	23.89 ± 0.51	17.90 ± 0.51	23.92 ± 0.20	14.28 ± 0.97
Recovery (%)	99.17	99.17	99.50	99.38	99.83	98.88	99.17
	98.33	100.25	99.75	99.19	98.25	99.25	100.83
	99.86	99.67	98.67	99.54	99.44	99.67	99.17
Average recovery (%)	99.12	99.70	99.30	99.37	99.17	99.27	99.72

T: tartaric acid; G: glycolic acid; L: lactic acid. A, B, C, D and E: commercial samples.
[a] Average of three determinations.

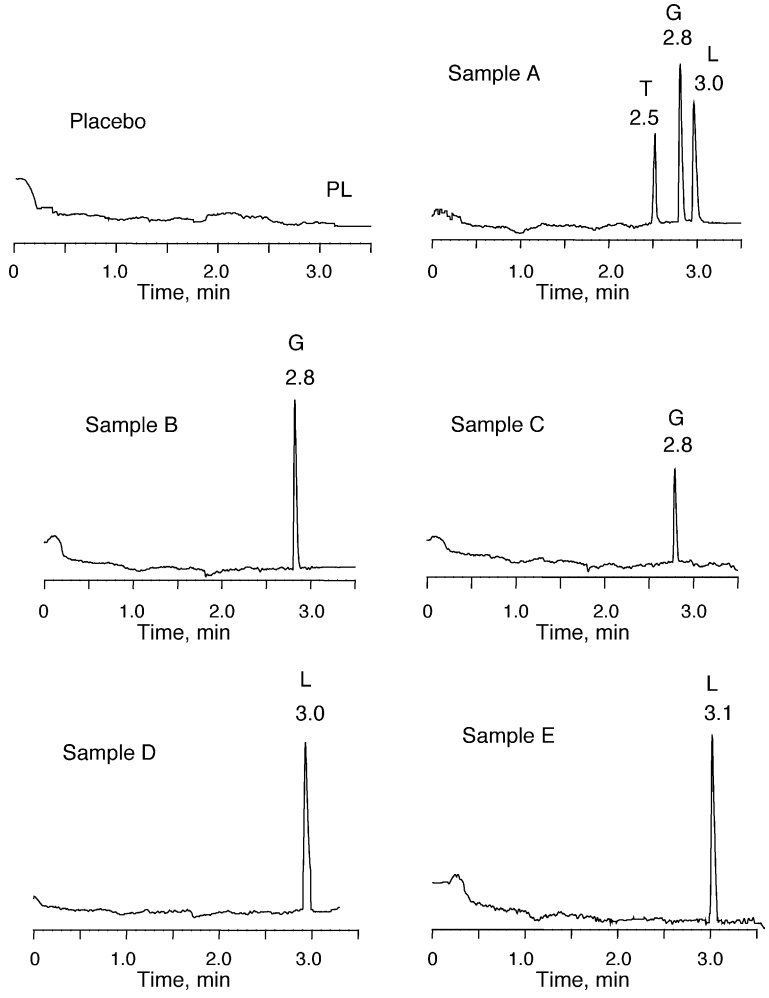


Fig. 3. Electropherograms of the placebo (PL) and commercial samples A, B, C, D, and E containing AHAs: (T) tartaric acid, (G) glycolic acid and (L) lactic acid. Conditions: BGE 10 mmol l^{-1} potassium hydrogen phthalate, 0.5 mmol l^{-1} CTAB, pH 4.1. Separation voltage 20 kV to the anode, detection UV 254 nm, temperature 35 °C.

3.2.2. Precision

Precision is defined as the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision may be measured as repeatability, reproducibility and intermediate precision [25–30]. In this research, the precision was studied as the repeatability level, which is obtained from the analysis of identical test materials in the same conditions over a short period of time, carried out by the same analyst with the same equipment.

Precision was expressed in terms of relative standard deviation (R.S.D.) and was obtained by injecting in the capillary electrophoresis equipment, 10 individual sample solutions in duplicate, prepared as previously described, and three replicate injections of standard solutions. The results for each sample are summarized in Table 3.

The average R.S.D. was better than 0.98% indicating that the proposed CE method presents acceptable repeatability.

3.2.3. Accuracy

Accuracy is the closeness of agreement between the value obtained by the method and the value that is accepted as a reference value [25–30].

The accuracy was determined by recovery test performed according to ICH guidelines [25,26]. Three known concentration levels of tartaric acid (2.4–7.2 $\mu g\,ml^{-1}$), glycolic acid (6–18 $\mu g\,ml^{-1}$) and lactic acid (4–12 $\mu g\,ml^{-1}$) standard solutions were added to samples and the resulting spiked samples were submitted to the assay procedure. All samples were injected in three replicates for each concentration.

Table 4 shows the accuracy results, expressed as percent recovery. The method showed excellent recovery, with values close to 100%.

3.2.4. Limit of quantitation (LOQ)

The LOQ is defined as the smallest concentration that can be determined with suitable precision and accuracy. LOQ was defined according to the United States Pharmacopeia 26.ed [27] and was determined based on the standard deviation (S.D.) of *y*-intercepts of regression linear and the slope of the calibration curve (*S*), according to the formula $LOQ = 10 \times (S.D./S)$. Results were presented in Table 2.

3.2.5. Specificity

The specificity was determinated by comparing the results obtained in the analysis of placebo formulation, containing only the excipients, with those obtained in the analysis of AHAs standard solution. Fig. 3 shows that there is no interference from excipients.

3.3. Analyses of commercial cosmetic samples

The proposed CE method was applied in the quantitative determination of AHAs in commercially available cosmetic products. The data in Table 3 shows that the assay results were in good agreement with the labeled content. Fig. 3 shows electropherograms obtained during analyses of the samples using the proposed method.

4. Conclusion

A CE method for the analysis of AHAs in cosmetic products has been developed and validated. The preparation of samples was easy and efficient. The migration time observed (3 min) allowed a rapid determination of the AHAs, which is important for routine analysis. The validation results concerning linearity, accuracy, precision and limit of quantitation were satisfactory. The proposed method offers a highly efficient separation, and can be applied for quantitative determination of AHAs in commercially available cosmetic products.

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