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Simultaneous HPLC determination of hydrophilic whitening agents in cosmetic products

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Abstract

A high-performance liquid chromatographic method for quantifying four of the most common used hydrophilic whitening agent—glycolic acid (GA), ascorbic acid (AA), arbutin (ART) and Mg ascorbyl phosphate (MAP), has been developed. Isocratic separation was performed using a C18 column with ion-pair agent as mobile phase. The analytes were detected by ultraviolet light absorption at the wavelength of 220 and 240 nm, respectively. Calibration curves were found to be linear in the 8.0–36 mg/ml (GA), 10.0–300 μg/ml (AA and ART), and 5.6–451 μg/ml (MAP). The correlation coefficient of linear regression analysis were with the range 0.9974–0.9997. Recoveries of the four analytes were between 94.8 and 100.1% and the precision of this method was better than 6.9% relative standard deviation (R.S.D.) ($n = 3$). It was found that AA degraded in an aqueous solution. To be sure that AA was stable during the HPLC analysis, all analytes were dissolved in distilled water and these solutions were purged with nitrogen gas to remove oxygen and stored at 25 °C. The testing results show that the procedure is rapid, simple, selective method and it is suitable for routine analysis of commercial cosmetics.
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Keywords: Cosmetic product; Hydrophilic whitening agent; Glycolic acid; Ascorbic acid; Arbutin; Mg ascorbyl phophate; HPLC

1. Introduction

Westerners consider tanned skin healthy and beautiful. On the contrary, a light skin is considered beautiful in the oriental countries. Therefore, whitening lotions are very popular in the oriental. They are applied for whitening the skin. Chemical peeling is usually used in clinical treatment for the damaged facial skin arising from acne, melasma, common warts, and so on. In this technique, cosmetic products containing a chemotherapeutic agent are applied to the skin for stimulating renewal of the skin cells. The color of skin becomes lighter. Thus this chemo-therapeutic agent is also called a whitening agent [1–6]. It has been reported that a whitening agent like glycolic

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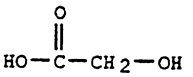
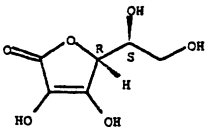
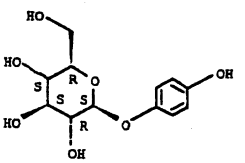
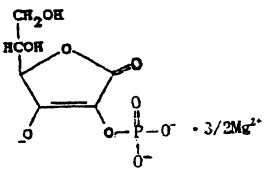
Table 1
Chemical structures of four hydrophilic whitening agents

Compound No.	Chemical name 【Common name】	Structure	Maximum authorized concentration (%)
1	hydroxyacetic acid 【glycolic acid】		----
2	Vitamin C 【L-(+)-ascorbic acid】		----
3	4-hydorxyphenyl-β-D-glucopyranoside 【arbutin】		7.0
4	L-ascorbic acid phosphate Magnesium salt 【Magnesium ascorbyl phosphate】		3.0

acid (GA), one of hydroxy acids, increases the hydration of the skin and, therefore, gives smoother appearance, resulting in less face lines and wrinkles [7,8]. There are two types of whitening agents: lipophilic whitening agents and hydrophilic agents. Examples of lipophilic whitening agents are kojic acid, salicylic acid, ascorbyl palmitate, azaleic acid, and adapalene. Examples of hydrophilic whitening agents are glycolic acid (GA), ascorbic acid (AA), arbutin (ART), and magnesium ascorbyl phosphate (MAP). The hydrophilic whitening agents are studied here because their polar groups have poor affinity to the C18 column used in HPLC and it is difficult to

separate them in HPLC. We used an ion-pair method to solve this problem.

The chemical structures and the maximum concentration of these hydrophilic whitening agents are given in Table 1. In Taiwan, there is no limit on the concentration of GA and AA used in the cosmetic products. ART, however, can be used up to 7 wt.% and MAP up to 3 wt.%. Although there is no limit on the concentration of GA used in the cosmetic products, it was reported by US Food and Drug Administration that this whitening agent can cause severe burns or swelling of the skin [9]. In generally, about 30–40 wt.% of GA is used in the cosmetics to be used in the beauty salons and higher concentration, 50–70 wt.%, is used in the cosmetic products to be used by physicians [10].

The purpose of this study is to develop a quantitative analysis method to accurately measure the level of the whitening agents in the cosmetic products. This work is of great importance for public safety. Although there are many publications describing the estimation of the whiteners in cosmetic formulations [11–16], the simultaneously quantitative analysis methods have not yet been reported in the literature. This study is related to an analytical method, which can detect and simultaneously quantify the hydrophilic whitening agents.

The hydrophilic whitening agents are not always stable in an aqueous solution. They are subjected to oxidation. Although several studies for the stability of AA had been reported [17–19], the environment of AA in these reports is not suitable for the HPLC for the simultaneous determination of the hydrophilic whiteners. It is important to make sure that the hydrophilic whitening agents are stable during the analysis for the accuracy of the results.

2. Experimental

2.1. Reagents and standards

GA, AA, ART and MAP and the commercial were purchased from Alfa Co. (American), Alfa Co. (American), Wako (Japanese), and Lipo. Co. (American), respectively. Methyl paraben and U-13 are obtained from Induchem. Co. (Switzerland). Citric acid, tetrabutylammonium hydroxide (TBAH), sorbitol, phosphoric acid, and potassium dihyrophosphate were purchased from Aldrich Co. (American). All chemicals were of analytical-reagent grade. Commercial cosmetic products, from various manufacturers, were purchased from retail stores and from a local pharmacy. Solvents and water were filtered through a 0.45 μm membrane and degassed.

2.2. Instrumentation

The HPLC apparatus (Hitachi Co., Japan) consisted of a modular chromatographic system. To which a pump (Model I-7100), an UV detector (Model I-7420; variable wavelength), and an injection valve with 25 μl sample loop (Model 7725, Rheodyne, Cotati, US) were attached. Data acquisition and processing were accomplished with a personal computer using SISC software (Taiwan). Sample injections were effected with a syringe of 25 μl (Hamilton, Switzerland). A 5 μm, 250 mm × 4.60 mm I.D. Stainless-steel Mightysil RP-18GP column was used.

2.3. Chromatographic conditions

Chromatographic analysis was carried out with a single column isocratic reverse phase method. In the ion-pairing method, the mobile phase was a 0.005 M potassium dihydrophosphate buffer solution including three chemicals: TBAH, methanol, and phosphoric acid. The amount of these three chemicals has great effect on the separation and the resolution of the spectrum of the HPLC. The concentration of TBAH was varied from 1 to 10 mM; methanol from 1 to 10 vol.%. Varied amount of phosphoric acid was added to the buffer solution to adjust the pH values from 2.5 to 5.0. The testing results will allow a suitable mobile phase to be chosen for a good resolution. All mobile phases were filtered through a Millipore filter, pore size 0.45 μm, and degassed by sonication before used. A flow rate of 1.1 ml/min was used. The analytes were detected by UV absorption at 220 or at 240 nm. The identity of the four

ingredients was assigned by chromatography with authentic standard. Quantification was carried out by the integration of the peak using external standardization method.

2.4. Calibration curve

Stock solutions were prepared by accurately weighting the agents and then dissolving them in water. Five working solutions of the four analytes were freshly prepared from their stock solutions by a ratio of 1–10 with the distilled water. Appropriate dilution of these working solutions gave concentrations of 10–300 μg/ml, except for GA and MAP, where the concentrations were 8.0–36.0 mg/ml and 5.6–451 μg/ml, respectively. Calibration curves were constructed by plotting the peak areas of each component versus concentration and gave the values of slope, along with the intercept and correlation coefficient for each calibration curve. The calibration curves were for the quantification of the four whitening agents in four samples of commercial cosmetics.

2.5. Stability study of ascorbic acid in aqueous solution

Effect of temperatures, oxygen, and pH values on the stability of AA in an aqueous solution was investigated. A working solution of 100 ppm AA was prepared by dissolving 10 mg of AA in 100 ml buffer solutions with pH values of 2 and in 100 ml distilled water with pH values of 6.9, respectively. For removing oxygen from the solutions, a portion of each buffer solution was collected and transferred to test-tube of Pyrex glass and bubbled with N_2 about 3 min, and then sealed with a Bakelite screw-cap and a silicone ring. In the other situation, a portion of each buffer solution was collected and stored in glass flasks with no N_2 bubbled and the temperature was preset at 10 and 25 °C, respectively. All the samples were collected in period of time and each sample was injected into the chromatograph for analysis using the conditions chosen in Section 2.3 above.

Table 2
Recovery of whitening compounds in cosmetic formulations

Compound	Amount added (μg/ml)	Recovery (%)	CV (%)
GA	10 000	99.6	0.6
	30 000	100.1	3.3
AA	30	95.6	3.7
	250	94.8	2.8
ART	30	97.8	3.6
	250	99.5	3.3
MAP	45	99.2	6.9
	360	97.7	1.3

Each value is the mean of triplicates.

2.6. Recovery study

A sample cream containing no whitening agents was used as a control in the recovery study. Varied amount of the four hydrophilic whitening agents, GA, AA, ART, and MAP, was added to this control cream. The concentration was given in Table 4. About 1 g of each of the prepared sample cream was weighed into a glass flask and 30 ml of distilled water was added and then the flask was immersed in an ultrasonic bath for 15 min thermostatted at 25 °C. The resulting solution was made up to volume 100 ml with distilled water and then was filtered and deoxygenated by N_2 for about 3 min and then sealed with a Bakelite screw-cap and a silicone ring.

3. Results and discussion

3.1. Chromatography and resolution

The structure of four whitening agents, GA, AA, ART, and MAP, is shown in Table 1. All of them are very polar molecules. For polar analytes, methanol or acetonitrile is usually used as a component of the mobile phase in C18 column or cyano-propyl column of HPLC. However, the co-elution of these polar analytes was found when methanol or acetonitrile was used. Therefore, it is impossible to separate these analytes. To solve this problem of the co-elution, ion-paring method was employed for reducing their polar character to obtain an accepted retention time. In addition, it is important to find a suitable separation conditions for the simultaneous analysis of four analytes in

cosmetic products. In order to obtain the optimum analytical condition, the influence of mobile phase and its pH values are considered here. The results obtained are illustrated in Fig. 1. The concentration of TBAH in the mobile phase was varied from 1 to 10 mM. The calculated capacity factor decreases as the concentration of the TBAH increases (see Fig. 1(a)). This drop in the values of the capacity factor is sharp for MAP but very mild for GA, ART, and AA. The significant difference for the capacity factor between MAP and the others can be due to the strong ionic character of MAP. A smaller difference in the capacity factor gives a reasonable retention time in HPLC. Therefore, the concentration of TBAH was chosen to be 10 mM for the mobile phase. The effect of the amount of methanol on capacity factor was shown in Fig. 1(b). The similar results were found as those for TBAH. A higher concentration of methanol, a smaller difference in the capacity factor, and a shorter retention time in

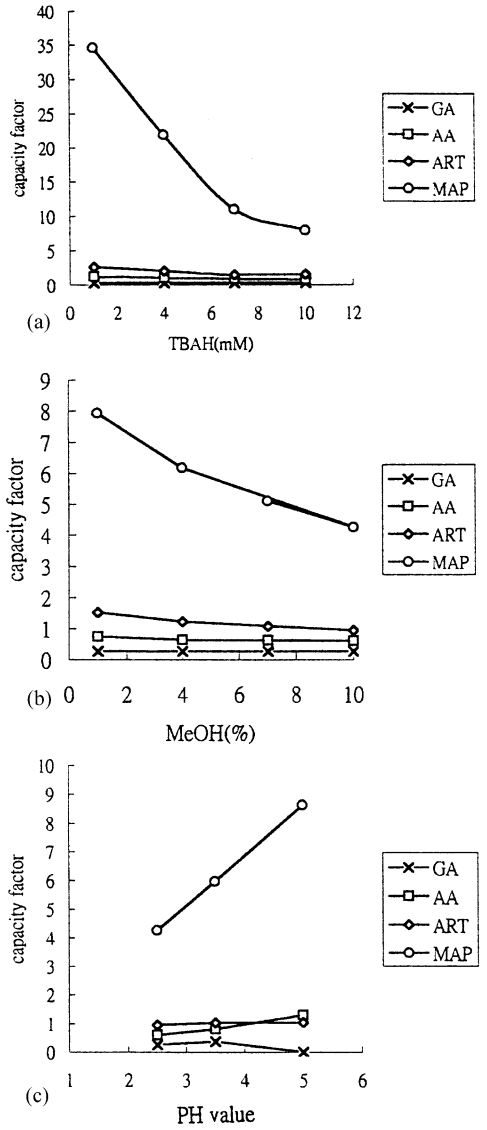


Fig. 1. (a) Effect of TBAH content on the values of the capacity factor in a 0.005 M KH_2PO_4 buffer solution. (b) Effect of methanol content on the values of the capacity factor in a 0.005 M KH_2PO_4 buffer solution containing 10 mM TBAH. (c) Effect of pH values of the solution on the values of the capacity factor in a 0.005 M KH_2PO_4 buffer solution containing 10 mM TBAH and 10 vol.% of methanol. The pH values were adjusted by adding phosphoric acid.

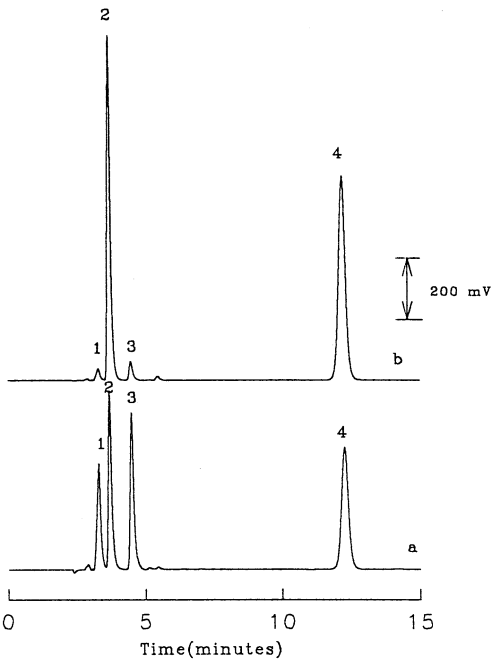


Fig. 2. Effect of the wavelength of the UV detector on the sensitivity of detection for a control cream containing 24 mg/ml of GA, 100 μg/ml of AA, 100 μg/ml of ART, and 180 μg/ml of MAP. (a) Chromatograph obtained at 220 nm. (b) Chromatograph obtained at 240 nm. The indicated peaks are the characteristic UV absorption peaks for the four hydrophilic whitening agents: peak 1 for GA, peak 2 for AA, peak 3 for ART, and peak 4 for MAP.

HPLC. Ten percent of methanol in volume was chosen in this case.

Varied amount of phosphoric acid was the added to the buffer solution mentioned above to alter the pH values from 2.5 to 5.0. At pH value of 5.0, broad and multiple peaks were observed in the chromatograph results. The chromatograph peaks for GA, AA, and ART are overlapped with each other. Unlike the previous results, the capacity factor increases with the increase of the pH values for MAP (see in Fig. 1(c)). The change in the capacity factor due to pH values is very small for GA, AA, and ART. It is essential to choose a pH value that gives enough differences in the values of the capacity factor for a good resolution in the HPLC. For these reasons, a pH value equal to 2.5 was chosen. As a result, a suitable mobile phase for HPLC analysis is a 0.005 M potassium dihydrophosphate buffer solution containing 10 mM TBAH, 10 vol.% of methanol, and phosphoric acid. The phosphoric acid was used to adjust a pH value of the buffer solution to 2.5.

3.2. Determination of wavelength for UV detector

The UV spectrum of these whitening agent dissolved in the mobile phase was obtained by using UV spectrophotometer. The maximum absorption peak is at 220 nm for GA, at 243 nm for AA, at 227 nm for ART, and at 238 nm for MAP.

At 240 nm, the UV detector has a high sensitivity in detecting AA and MAP, but very low sensitivity for GA and ART (see Fig. 2(b)). While at 220 nm, clear peaks in the HPLC chromatograph were shown for all the four whitening agents (see Fig. 2(a)). To obtain a better sensitivity for all of the analytes, the wavelength of the detector was adjusted to 220 nm for detection and quantification of four analytes. The procedure is relatively rapid with an analytical run time of 12.1 min at room temperature (around 26 °C).

3.3. Stability study for whitening agents

The AA undergoes decomposition to dehydroascorbic acid (DHA) when dissolved in aqueous. Therefore, it is of importance to keep the ascobic acid stable during analysis for the accuracy of the results. Fernandes et al. reported that the principal factors affecting the degradation are oxygen and temperature [19]. In their study, the degradation was significant in 1 h, and effect of pH values of the solution on the degradation was only mentioned at pH 5.0 and 5.6.

In our study, the stability of AA was assessed utilizing the concentration change of AA in a variety of different environmental conditions such as treated or untreated solution with N_2, pH value and temperature. The longer the time for a significant change, the more stable ascorbic is.

Table 3
Effect of temperatures, oxygen, and pH values on the stability of AA

Time (h)	No treatment				Time (h)	Oxygen was removed			
	10 °C		25 °C			10 °C		25 °C	
	pH 6.9	pH 2	pH 6.9	pH 2		pH 6.9	pH 2	pH 6.9	pH 2
0	100.3±1.5	99.7±1.5	99.3±1.5	99.8±3.1	0	99.3±1.5	99.8±1.1	99.8±1.9	100.1±1.9
0.5	100.7±1.2	100.0±2.6	96.8±0.8	99.7±2.1	12	99.7±1.6	99.6±0.7	100.1±0.9	99.7±1.5
1	100.6±1.2	99.7±1.5	94.3±1.5	99.1±2.2	16.8	99.3±2.5	99.0±1.0	100.0±1.7	94.3±1.5
1.5	99.0±1.9	100.3±1.0	91.0±1.7	97.0±1.7	24	100.0±2.0	98.0±1.0	99.4±0.7	92.0±1.2
2	100.7±2.5	100.4±1.2	89.0±2	97.2±1.8	48	99.5±2.3	91.0±0.6	99.8±2.0	88.1±1.8
2.5	97.3±1.2	99.8±2.0	85.3±1.5	95.0±1.0	72	99.4±1.6	86.7±1.5	99.7±2.1	83.0±2.0
5	85.0±0.7	99.8±2.3	63.7±1.5	89.7±2.1	96	99.2±1.7	83.5±1.6	99.5±2.0	77.0±2.2
7.5	67.3±2.1	97.2±3.9	26.3±2.1	83.7±1.2					
10	45.2±1.7	90.5±2.2	8.7±1.2	71.2±1.0					
23.3	5.8±0.2	81.0±1.0	1.0±0.2	53.8±1.3					

Mean of triplicate analysis±S.D.

The results, summarized in Table 3, demonstrate a strong correlation between stability and treated or untreated solution with N_2. The loss of AA in untreated solution is more rapid than treated solution. As showed in Table 3, at 10 °C and pH 2, the time required for 10% degradation of the AA was about 10 h for no treatment and about 48 h for treated solution. This may be due to the factor that much less oxygen is available for oxidation. It is noted that, in presence of oxygen and at 25 °C, the level of the AA of 8.7 and 71.2% after 10 h was observed for pH 6.9 and 2.0. This result can be explained by the higher amount of dissociated AA at pH 6.9 and this form should be less stable than undissociated form [19]. On the contrary, in the absence of oxygen and at 25 or 10 °C, the aqueous solution of AA is much better stable at pH 6.9. The reason for the AA to be more stable has not yet been further investigated. In relation of solution temperature, in untreated solutions the AA is less stable at higher temperature (25 °C) than at a lower temperature (10 °C). However, for treated solutions, the aqueous solution of AA is stable and was not affected by the temperature.

The study of the stability of other whitening agents, GA, ART, and MAP was also carried out in the same way. Unlike AA, these three whitening agents were very stable in aqueous solutions. No change in the concentration was observed in the solutions for 1 month. Accordingly, a best and most convenient condition for preparing sample solution was chosen to accurately quantify these four whitening agents at the same time. All the analyzed samples were prepared as distilled water with pH value of 6.9, followed by the treatment using nitrogen gas to remove oxygen and stored at 25 °C before these sample solutions were run in HPLC.

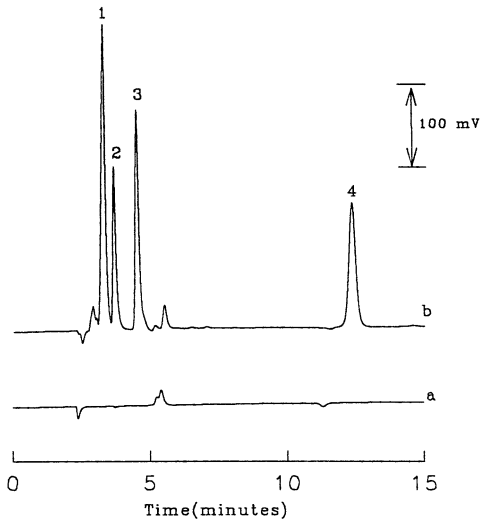


Fig. 3. (a) Chromatograph for a control cream (no whitening agents). Chromatograph for the same control cream containing 20.7 mg/g of GA, 65.0 μg/g of AA, 104.7 μg/g of ART, and 192.1 μg/g of MAP. Peak 1 is for GA; peak 2 for AA; peak 3 for ART; peak 4 for MAP.

3.4. Validation for linearity and assay for precision

Using the chromatographic conditions described, reasonable resolution was achieved be-

Table 4
Validation data from calibration curves of four hydrophilic whitening agents

Compounds	Concentration range	Regression equation	Correlation coefficient	Added (μg/ml)	Intra-day variation/ mean ± S.D. ($n = 5$)	CV (%)
GA	8.0–36.0 mg/ml	$Y = 1.79 \times 10^2X + 5.28 \times 10^5$	0.9974	10 000	10 000 ± 643	6.43
				30 000	30 000 ± 1550	5.17
AA	10.0–300 μg/ml	$Y = 2.16 \times 10^4X + 4.78 \times 10^5$	0.9996	20.0	20.0 ± 0.70	3.5
				250	250 ± 0.70	0.3
ART	10.2–304 μg/ml	$Y = 2.16 \times 10^4X + 7.91 \times 10^5$	0.9982	20.4	20.0 ± 0.77	3.9
				250	250 ± 0.93	0.4
MAP	5.6–451 μg/ml	$Y = 1045 \times 10^4X + 1.70 \times 10^5$	0.9997	22.0	22.4 ± 0.28	1.3
				400	400 ± 2.7	0.7

Y, peak area; X, concentration.

tween these analytes and the others compounds in the cosmetic product. The method was validated for linearity and precision. Linear curve fitting was applied to calculate the calibration curves for each whitening agent. The results are given in Table 4. Excellent linearity was obtained over the range from 5.6 to 451 μg/ml for all standards except for GA for which the range was from 8 to 36 mg/ml. The correlation coefficient is ranged from 0.9974 to 0.9997. The precision of the method was calculated as the relative standard deviation (R.S.D., $n = 5$) of assays containing the four hydrophilic whiteners in the same range of concentration. The R.S.D. range was found to be from 0.3 to 6.43%.

3.5. Recovery

A cream containing no whitening agents was used as a control in the recovery study. Small amounts of the four whitening agents were added to this control cream. The HPLC chromatograph shows that there is no UV absorption peak detected for a control cream (see Fig. 3(a)) and four absorption peaks was found for a sample cream containing these four whitening agent. In Fig. 3(b), peak 1 is the UV absorption peak for GA; peak 2 for AA; peak 3 for ART; peak 4 for MAP. Each whitening agent has its own retention time. This character will be used to identify these whitening agents later on. The results are summarized in Table 2. A range of 94–100% recovery for the four agents was obtained at both low and high concentration. The coefficients of variance calculated from three replicates were all less than 6.9%. The HPLC chromatograms of four analytes extracted from experimental formulation were shown in Fig. 3 and no interference was observed.

3.6. Application

Four commercial available cosmetic products were analyzed. They are whitening cream (cosmetic number 1), C20 whitening gel (cosmetic number 2), essence lotion (cosmetic number 3) and peel lotion (cosmetic number 4), respectively. These cosmetics were assayed using the procedure described in this study. The chromatographs for these four cosmetics are given in Fig. 4(a–d). Chromatograph 4 (a) gives a small absorption peak of peak 4, i.e. cosmetic number 1 contains MAP. Chromatograph 4 (b) exhibits a peak of peak 2, which is an indication that cosmetic number 2 contain AA. From chromatograph 4

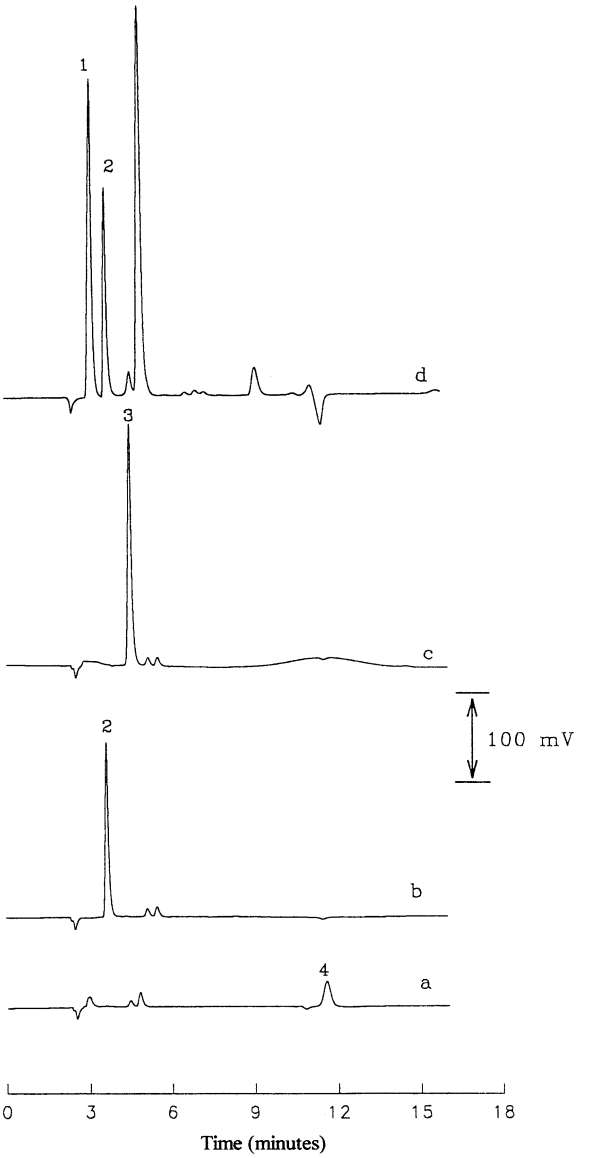


Fig. 4. Chromatograph for four commercial cosmetic products: (a) for cosmetic number 1, (b) for cosmetic number 2, (c) for cosmetic number 3, and for cosmetic number 4. Peak 1 is for GA, peak 2 for AA, peak 3 for ART, and peak 4 for MAP.

Table 5
Assay results for the HPLC analysis of the hydrophilic whitening agent in four commercial cosmetic products

Sample	Whitening agent	Label claim (%, w/w)	Found (%, w/w)	% of label	CV (%)
Cosmetic number 1	MAP	0.08	0.084	105.0	6.7
Cosmetic number 2	AA	20	19.77	98.9	1.9
Cosmetic number 3	ART	7	6.60	94.3	1.1
Cosmetic number 4	GA	50	50.23	100.0	2.2
	AA	0.2	0.19	95.0	3.6

Each value is the mean of triplicates.

(c) and (d), ART was identified in cosmetic number 3 and both GA and AA were identified in cosmetic number 4. There is an unmarked peak in chromatograph 4 (d). This UV absorption peak has a retention time longer than for peak 3 but much shorter than for peak 4. It is clear that this unmarked absorption peak is not due to the whitening agents but due to other ingredient in cosmetic number 4. Quantification of these whitening agents was carried out by the integration of the peaks in the chromatograph using external standardization method. The level of the whitening agents in these four cosmetics is given in Table 5. It is noted that the results obtained confirm the accuracy and show compliance with the labeled claim.

4. Conclusion

The HPLC assay developed is simple and rapid for the simultaneous analysis of the hydrophilic whitening agents. A capacity factor was used to choose a suitable mobile phase for HPLC. We need to choose a condition that gives a smaller difference in the capacity factor for a reasonable retention time. It is essential for the chosen condition to give enough differences in the values of the capacity factor for a good resolution in the chromatograph of HPLC. As a result, a suitable mobile phase for HPLC analysis is a 0.005 M potassium dihydrophosphate buffer solution containing 10 mM TBAH, 10 vol.% of methanol, and phosphoric acid. The phosphoric acid was used to adjust a pH value of the buffer solution to 2.5. A better detection wavelength for the whitening agents is at 220 nm for the HPLC. In addition, a convenient method for stabilizing AA in analytical procedure has been obtained. Which is important for the accuracy of the results.

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