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Simultaneous Determination of Magnesium Ascorbyl Phosphate, Ascorbyl Glucoside, Kojic Acid, Arbutin and Hydroquinone in Skin Whitening Cosmetics by HPLC

SHOU-CHIEH HUANG[1], CHENG-CHIN LIN[1], MING-CHUAN HUANG[1]* AND KUO-CHING WEN[2]

[1] Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, 161-2 Kunyang St., Nangang District, Taipei City 115, Taiwan (R.O.C.)

[2] Department of Cosmeceutics, China Medical University, 91 Hsueh-Shih Rd., North Section, Taichung City 404, Taiwan (R.O.C.)

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ABSTRACT

A high performance liquid chromatographic method was developed for simultaneous determination of 5 whitening ingredients: magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone decomposed from arbutin in cosmetics. Samples were extracted with 0.05 M KH_2PO_4 buffer solution (pH 2.5) and analyzed using a Cosmosil 5 C18-AR-II column. A mixture of 0.05 M KH_2PO_4 buffer solution (pH 2.5) and methanol (99:1, v/v) was used as mobile phase. The UV detector was set at 280 nm. Pyridoxine was used as an internal standard. The related coefficients, R^2, of regression equations of the 5 standard curves were 0.9998-1.0000. The relative standard deviations of the 5 ingredients for intraday and interday analysis were less than 2.4%. The average recoveries of these 5 ingredients spiked in sample ranged from 93.5% to 103.3%. The relative standard deviations of average recoveries were less than 1.3%. The limits of quantitation in cosmetics were 80.0, 20.0, 3.0, 15.0 and 10.0 μg/mL for magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone, respectively.

Key words: magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin, hydroquinone, whitening cosmetics, HPLC

INTRODUCTION

Magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid and arbutin were admitted as whitening ingredients for cosmetics by the Department of Health, Executive Yuan, R.O.C. in 2000[(1)]. The allowable amounts are 3%, 2%, 2% and 7%, respectively. Both magnesium ascorbyl phosphate and ascorbyl glucoside have been recognized in reducing the aging of facial skin. They also provide a range of benefits, such as inhibition of biosynthesis of melanogenesis, promotion of collagen synthesis and prevention of free radical formation[(2)]. Kojic acid is derived from a fungus that is able to chelate the copper-containing enzyme tyrosinase in the formation of melanin. Kojic acid is thus claimed to have skin-lightening property, because of the capability of inactivating tyrosinase. Arbutin can be isolated from the leaves of common bearberry. Its chemical structure is similar to hydroquinone with a combination of β-D-glucopyranose and hydroquinone. The hydroquinone component released can be regarded as an impurity[(3)] and the maximum allowable amount is 20 ppm[(1)]. Both arbutin and hydroquinone inhibit the conversion of tyrosine to melanin by inhibiting tyrosinase[(4)]. The Department of Health announced that hydroquinone is used only as drug[(5)] because of its side effects including dermatitis, erythema, burning and hyperpigmentation.

* Author for correspondence. Tel: 886-2-26531212; Fax: 886-2-26531213; E-mail: mchuang@nlfd.gov.tw

According to the reports in literature, the 5 ingredients are determined mostly by reversed-phase high performance liquid chromatography (RP-HPLC)[(6-12)]. The purpose of this study was to develop a rapid and simple quantitative assay for simultaneous determination of above five ingredients using reversed-phase HPLC with photodiode array detection. By applying the developed HPLC method, the quantifications of these 5 whitening ingredients from markets were also determined.

MATERIALS AND METHODS

I. *Materials*

Hydroquinone, arbutin (98%) and kojic acid were purchased from Sigma (St. Louis, MO, USA). Magnesium ascorbyl phosphate (71.4%) was purchased from Nikkol (Tokyo, Japan). Ascorbyl glucoside (98%) and cream blank were given by Shiseido Co., Ltd. (Chung-Li, Taiwan). Pyridoxine (99%) was purchased from Chem Service (West Chester, PA, USA). Methanol (LC grade), Phosphoric acid (85%, reagent grade) and Potassium dihydrogen phosphate (reagent grade) were purchased from Merck (Darmstadt, Germany).

II. *Instruments*

HPLC consisted of Waters (Milford, MA, USA) Model

515 Pump, Waters 717 plus Autosampler and Waters 996 Photodiode Array Detector. Milli-Q Waters Purification System (Millipore, Milford, MA, USA) was used for filtration (deionization) of water.

III. *Methods*

(I) *Analysis condition*

The chromatography column was Cosmosil 5 C18-AR-II (4.6 mm i.d. × 250 mm). Mobile phase consisted of the mixtures of 0.05M KH_2PO_4 buffer (the pH was adjusted with phosphoric acid to 2.5) and methanol with 99:1 ratio. The flow rate was 0.9 mL/min and the detecting wavelength was set at 280 nm. The volume for each injection was 20 μL.

(II) *Preparation of standard solutions*

1. Pyridoxine with a concentration of 1.0 mg/mL was prepared as the internal standard stock solution.

2. Magnesium ascorbyl phosphate (20.0 mg/mL), ascorbyl glucoside (10.0 mg/mL), arbutin (10.0 mg/mL), hydroquinone (5.0 mg/mL) and kojic acid (2.0 mg/mL) were prepared as the standard stock solutions. The stock solutions were diluted by adding the de-ionized water as needed to prepare a series of standard solutions.

(III) *Preparation of sample solution and quantification*

About 1 g of each cosmetic sample was weighed precisely, mixed with an appropriate amount of the internal standard stock solution and diluted with twenty-fold of 0.05M KH_2PO_4 buffer (pH 2.5). A homogeneous suspension was obtained after 30 min of sonification. The suspension was filtered and the filtrate was further diluted with 0.05M KH_2PO_4 buffer (pH 2.5) until the final concentrations of the whitening ingredients were within the standard calibration range and the internal standard 50 μg/mL before HPLC analysis. By comparing the ratio of the peak area of the sample to the internal standard from the linear equation of the calibration curve, the concentration of each sample was obtained.

(IV) *Calibration curve*

Five different concentrations of standard solutions (Table 1) were prepared from the stock solutions and 50 μg/mL of pyridoxine as an internal standard was added and analyzed, respectively. Linear regression equations and correlation coefficients were obtained from the plots of concentration versus peak area ratio of standard to internal standard solutions.

Table 1. Calibration curves and detection limits of 5 whitening ingredients

Compound	Concentration (mg/mL)	Regression equation	R^2	Limit of detection (μg/mL)
Magnesium ascorbyl phosphate	200, 400, 600, 800, 1000	Y=0.015231+0.000336X	0.9998	2
Ascorbyl glucoside	100, 200, 300, 400, 500	Y=0.007738+0.001947X	0.9999	1
Kojic acid	20, 40, 60, 80, 100	Y=-0.016252+0.026719X	1.0000	0.2
Arbutin	100, 200, 300, 400, 500	Y=0.006412+0.004782X	0.9999	1
Hydroquinone	50, 100, 150, 200, 250	Y=-0.00133+0.012228X	1.0000	0.5

Y: Ratio of peak area of whitening ingredient to internal standard.
X: amount of whitening ingredient analyzed by HPLC.

Table 2. Relative standard deviations of intraday and interday analysis of 5 whitening ingredients

Compound	Concentration (μg/mL)	Mean ± S.D. (R.S.D.%)	
		Intraday[a]	Interday[b]
Magnesium ascorbyl phosphate	300	299.84 ± 2.61 (0.87)	296.12 ± 6.91 (2.33)
	500	507.07 ± 2.51 (0.49)	500.47 ± 11.99 (2.40)
	700	718.54 ± 5.32 (0.74)	699.15 ± 13.97 (2.00)
Ascorbyl glucoside	150	151.64 ± 1.16 (0.77)	150.22 ± 1.78 (1.19)
	250	256.45 ± 1.19 (0.46)	250.43 ± 3.78 (1.51)
	350	357.65 ± 0.96 (0.27)	349.62 ± 5.04 (1.44)
Kojic acid	30	30.08 ± 0.12 (0.40)	29.77 ± 0.21 (0.69)
	50	50.19 ± 0.07 (0.15)	49.70 ± 0.89 (1.79)
	70	69.98 ± 0.13 (0.19)	69.50 ± 1.36 (1.96)
Arbutin	150	149.07 ± 0.83 (0.55)	149.68 ± 0.78 (0.52)
	250	248.97 ± 1.50 (0.60)	247.22 ± 2.38 (0.96)
	350	343.82 ± 2.37 (0.69)	343.48 ± 5.13 (1.49)
Hydroquinone	75	74.92 ± 0.26 (0.35)	75.18 ± 0.37 (0.49)
	125	125.35 ± 0.11 (0.09)	125.00 ± 1.46 (1.16)
	175	173.43 ± 0.40 (0.23)	173.55 ± 2.24 (1.29)

[a]n = 3.
[b]n = 18, triplicate injection each day for 6 consecutive days.

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(V) *Validation*

1. Precision

Within the standard calibration range, the standard stock solution and the internal standard stock solution were quantified precisely and diluted with de-ionized water to three different concentrations (Table 2). Fifty μg/mL of pyridoxine was added to each standard solution as an internal standard. The samples were injected into HPLC for analysis in triplicates on the same day and the analysis was continued for 6 consecutive days. The standard deviation (S.D.) and relative standard deviation (R.S.D.) were then calculated.

2. Accuracy

Ingredients with known concentrations (Table 3) were added to the blank cream and injected into HPLC for analysis after the same preparation as described in "III. (III) Preparation of sample solution and quantification". Each concentration was analyzed in triplicates. The recovery (%) was calculated from average peak area ratio of sample to internal standard by the obtained linear equation.

3. Limit of detection

Five standard solutions with proper concentrations were prepared by dilution with de-ionized water and analyzed by HPLC. The limit of detection was obtained when the signal peak height was three times the noise.

4. Limit of quantification

Quantification was determined after spiking blank cream with 5 standard solutions of various concentrations, followed by HPLC analysis. The limit of quantification of the 5 samples was achieved by measurement of the signal-to-noise peak height ratio of 10:1.

(VI) *Stability study*

Four test samples including a blank cream, 0.05 M KH_2PO_4 buffer (pH 2.5) solution, commercial arbutin cream and lotion were studied. Samples were stored at room temperature (25˚C) and periodically assayed for one month.

RESULTS AND DISSCUSSION

I. *Analysis Method*

Magnesium ascorbyl phosphate and ascorbyl glucoside are classified as polar compounds, while kojic acid, arbutin and hydroquinone are less polar and easier to analyze by using simple organic solvent and water mixture as mobile phase[11]. On the contrary, analysis of magnesium ascorbyl phosphate is relatively more complicated because of its high polarity. As a result, the amino-packing column instead of octylsilicated C18 column was used[6-9]. In order to analyze 5 ingredients simultaneously, the mobile phase changed into phosphate buffer under acidic condition with high acid resistant reverse-phase C18 column named Cosmosil 5 μm-AR-II. In a preliminary test, we found that the retention times of magnesium ascorbyl phosphate and ascorbyl glucoside are dependent on pH value of phosphate buffer. When pH decreased to 2.5, there was an optimum resolution between them. However, arbutin and hydroquinone were eluted at the same time with mobile phase as described above. Therefore, different amounts of methanol (1%, 2% and 5%, v/v) in mobile phase were tested. The best condition can be obtained when methanol/phosphate buffer (1/99, v/v) was used as the mobile phase. At the same time, thiamine, riboflavin and pyridoxine were tried as internal standards. Pyridoxine was capable of yielding a satisfactory retention time in the mobile phase system. Figure 1 shows well-resolved peaks of the 5 whitening ingredients and internal standard within 17 min. Magnesium ascorbyl phosphate, ascorbyl glucoside, pyridoxine, kojic acid, arbutin, and hydroquinone were eluted at 4.6, 5.5, 10.8, 13.6, 14.9, and 16.5 min, and the maximum absorption wavelength appeared at 236, 242, 290, 269, 282, and 289 nm, respectively. To upgrade the sensitivity for the detection of the impurity of arbutin in cosmetic product, 280 nm was chosen as detection wavelength in our developed HPLC quantitation method.

The linear regression equation, correlation coefficient (R^2) and the limit of the detection in the analytical profile for magnesium ascorbyl phosphate, ascorbyl glucoside, kojic acid, arbutin and hydroquinone are listed in Table 1. All calibration curves of the 5 ingredients were in good linear correlation with correlation coefficient of 0.9998~1.0000. The limits of detection were 2 μg/mL for

Table 3. Recoveries of 5 whitening ingredients in spiked samples

Compound	Spiked concentration (μg/mL)	Recovery (%) Mean ± S.D. (R.S.D.%)[a]
Magnesium ascorbyl phosphate	200	94.2 ± 0.17 (0.18)
	400	102.3 ± 0.88 (0.86)
	600	97.5 ± 0.51 (0.53)
Ascorbyl glucoside	100	101.4 ± 0.33 (0.32)
	200	102.4 ± 0.94 (0.91)
	300	102.4 ± 0.49 (0.48)
Kojic acid	20	103.3 ± 0.17 (0.17)
	40	103.0 ± 1.26 (1.22)
	60	97.5 ± 0.64 (0.66)
Arbutin	100	102.2 ± 0.29 (0.29)
	200	101.8 ± 0.95 (0.94)
	300	98.2 ± 0.43 (0.44)
Hydroquinone	50	94.2 ± 0.71 (0.75)
	100	94.2 ± 0.70 (0.74)
	150	93.5 ± 0.18 (0.19)

[a]n = 3.

magnesium ascorbyl phosphate, 1 μg/mL for ascorbyl glucoside, 0.2 μg/mL for kojic acid, 1 μg/mL for arbutin, and 0.5 μg/mL for hydroquinone, respectively.

II. *Sample Preparation*

All 6 components including internal standard have good solubility in de-ionized water, so the procedure employed is based on sample dilution with de-ionized water, followed by direct RP-HPLC. However, de-ionized water was not suitable for the determination of hydroquinone because of its poor recoveries ranged 65-75%. We switched to ethanol and 0.05 M KH_2PO_4 buffer (pH 2.5) for improvement in solubility. But serious peak tailing was found with ethanol in HPLC chromatogram of hydroquinone, while good recoveries up to 93% were obtained by using 0.05 M KH_2PO_4 buffer (pH 2.5) solution.

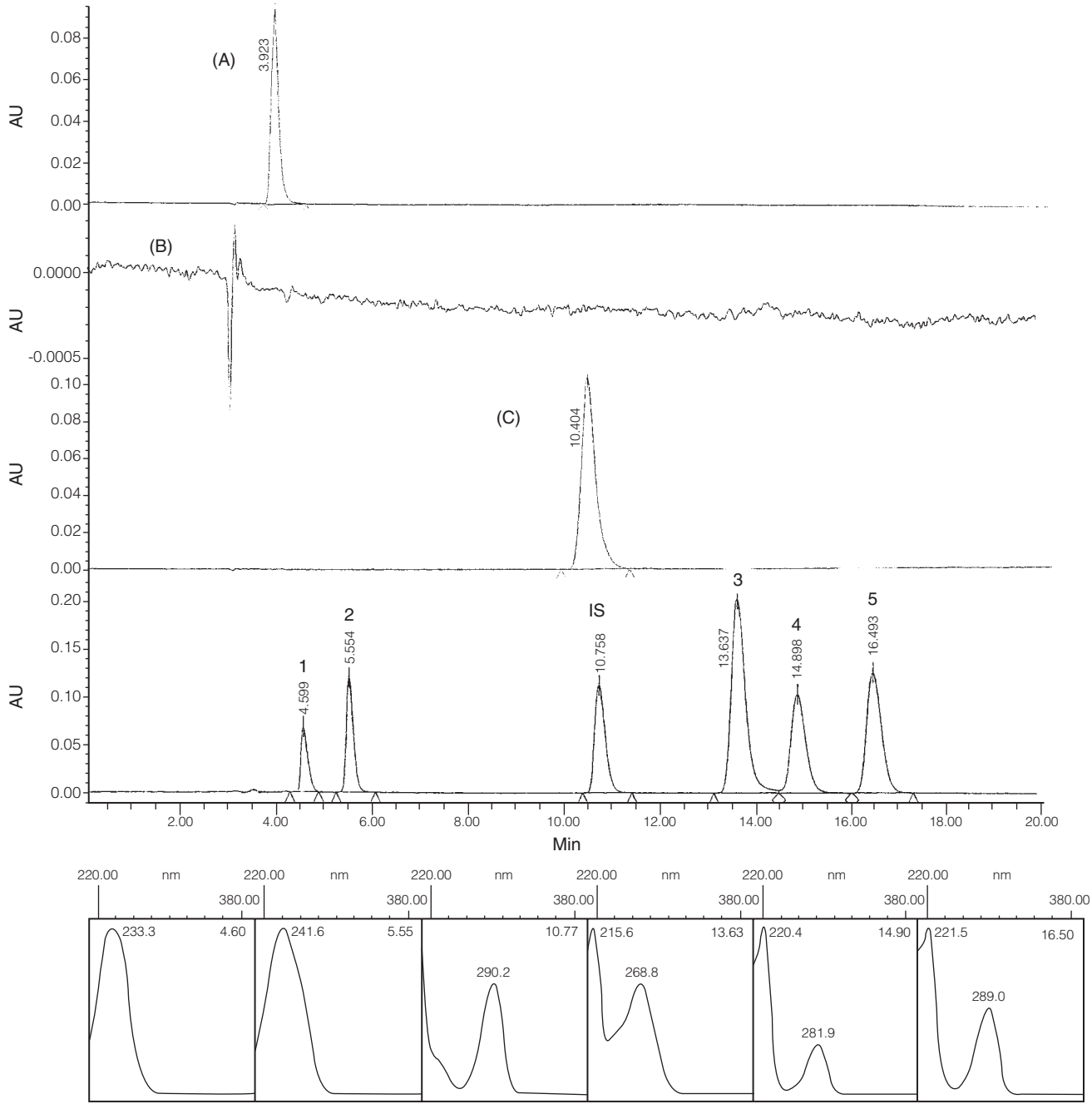

Figure 1. Retention time of thiamine, riboflavin, pyridoxine and 5 whitening ingredients.
(A) thiamine (conc. = 50 μg/mL); (B) riboflavin (conc. = 50 μg/mL); (C) pyridoxine (conc. = 50μg/mL);
1. magnesium ascorbyl phosphate (conc. = 400 μg/mL); 2. ascorbyl glucoside (conc. = 200 μg/mL); 3. kojic acid (conc. = 40 μg/mL);
4. arbutin (conc. = 200 μg/mL); 5. hydroquinone (conc. = 100 μg/mL); IS. Pyridoxine (conc. = 50 μg/mL) as the internal standard.

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III. *Stability Study*

Arbutin is easily hydrolyzed under dilute acid and in emulsion[3]. A stability of arbutin in 0.05M KH_2PO_4 buffer (pH 2.5) was performed in order to verify its compatibility with the buffer as dilute solutions. Four different test samples including blank cream, 0.05 M KH_2PO_4 buffer (pH 2.5), commercial arbutin cream and lotion were studied. The samples were stored at room temperature (25˚C) and periodically analyzed under "III. (III) Preparation of sample solution and quantification" for 1 month. Figure 2 shows that there was no significant degradation of arbutin in blank cream after 1 month of storage. However, arbutin in commercial cream within 8 storage days began to hydrolyze in 0.05 M KH_2PO_4 buffer (pH 2.5) solution. After 1 month of storage, the recovery of arbutin was 73.4%. Contemporaneously, the amounts of hydroquinone released were determined and demonstrated in Figure 3 all of which comply within 20 ppm of the allowable content within 1 month. The result of this study showed that arbutin was stable using the developed sample preparation within 4 storage days.

Table 4. Quantification of whitening ingredients in marketing cosmetics

Samples	Whitening ingredient	Labeled amount in sample (%)	Amount detected[a]
Cream 1	Magnesium ascorbyl phosphate	1.0	0.94
Cream 2	Magnesium ascorbyl phosphate	1.0	0.93
Lotion 1	Ascorbyl glucoside	2.0	1.89
Lotion 2	Ascorbyl glucoside	2.0	1.98
Lotion 3	Kojic acid	1.0	1.00
Lotion 4	Kojic acid	2.0	2.06
Essence	Arbutin	2.0	2.07
Cream 3	Arbutin	1.0	0.98
Cream 4	Hydroquinone	4.0	3.96
Cream 5	Hydroquinone	4.0	3.85

[a]n = 3.

IV. *Validation*

Test results of the 5 whitening ingredients for the intraday and interday are listed in Table 2. The relative standard deviations of the intraday and interday were between 0.09~0.87% and 0.49~2.40%. The average recov-

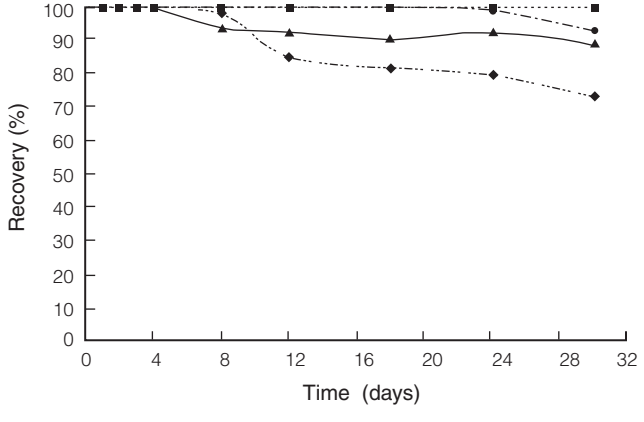

Figure 2. Content of arbutin after 1 month of storage at room temperature.
- blank cream spiked with 200 ppm arbutin
- 0.05 M KH_2PO_4 (pH2.5) spiked with 200 ppm arbutin
- commercial cosmetics product (cream)
- commercial cosmetics product (lotion)

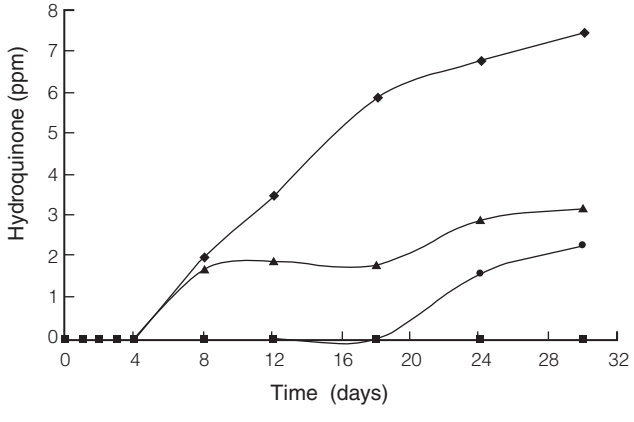

Figure 3. Content of Hydroquinone after 1 month of storage at room temperature.
- blank cream spiked with 200 ppm arbutin
- 0.05 M KH_2PO_4 (pH2.5) spiked with 200 ppm arbutin
- commercial cosmetics product (cream)
- commercial cosmetics product (lotion)

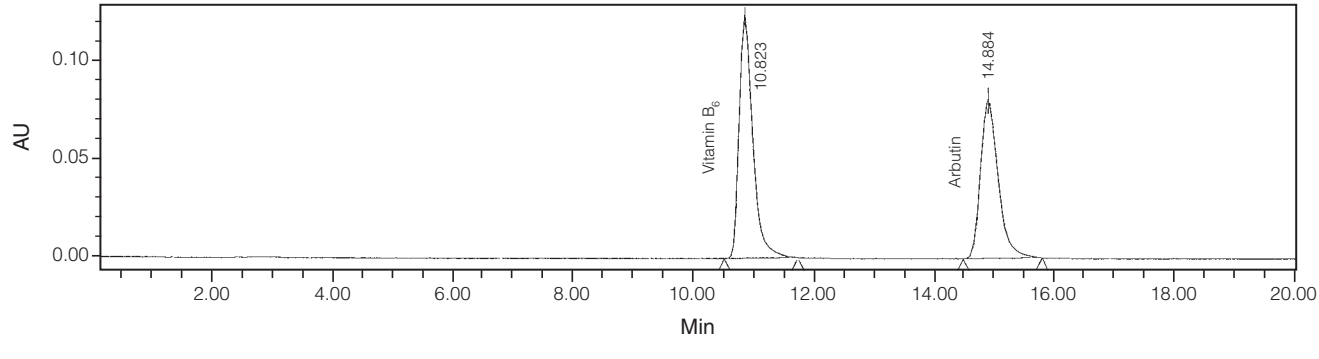

Figure 4. HPLC chromatogram of cosmetic product - cream 3.

eries of the 5 ingredients spiked in sample ranged from 93.5% to 103.3% (Table 3). The R.S.D. of recovery rate in these 5 compounds was 0.17~1.22%. The detection of quantitation was also determined using the same procedure as above, and the results were 80.0 μg/mL for magnesium ascorbyl phosphate, 20.0 μg/mL for ascorbyl glucoside, 3.0 μg/mL for kojic acid, 15.0 μg/mL for arbutin and 10 μg/mL for hydroquinone, respectively.

V. *Contents of Whitening Ingredients in Commercial Products*

In order to assess suitability of the developed HPLC method, 10 different commercial products were tested. The contents of the whitening ingredients in commercially available samples were all within 90~110% of the labeled amount as shown in Table 4. A typical HPLC chromatogram of sample cream 3 is shown in Figure 4.

In this report, we had established a simple, fast, and feasible RP-HPLC method for the simultaneous determination of 5 whitening ingredients in cosmetics. The method can be utilized successfully for routine analysis in the laboratory.

ACKNOWLEDGMENTS

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