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An HPLC Method for the Simultaneous Determination of Marker Compounds of Aloe and Scutellariae Radix in Cosmetics

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ABSTRACT

The Department of Health in Taiwan has announced mandatory labeling of the whole composition of cosmetics since May 5, 2002. Chinese herbal extracts are commonly added to cosmetics to attract consumers. To follow the earlier-mentioned regulation, it is important to develop a quantitative method to examine the contents of products to which Chinese herbal extracts have been added. This study attempted to establish a quantitative method for aloe and *Scutellariae Radix* (SR) in cosmetics. An HPLC/UV method using gradient elution was established to analyze marker compounds in herbal extracts and spiked blank cosmetic bases. The calibration curves of the marker compounds were linear ($r > 0.99$) in the range of 1.6 to 50.0 μg/mL. The analytical method was validated and commercial products were assayed. The results indicated that aloin, aloe-emodin and chrysophanol were not detected in all four aloe extracts. Baicalin and wogonin were detected in the two SR extracts. However, marker compounds of these two herbs were not detectable in the 26 commercial product samples.

Key words: cosmetics, aloe, *Scutellariae Radix*, HPLC

INTRODUCTION

There is a global trend towards the development of natural ingredients like botanical extracts for skin care because of the adverse effects of artificial chemicals[(1)], including tumor, mutation and tetratogenesis. Natural products such as α-hydroxyacids are used for photoaging and whitening[(2)], whereas kojic acid, ellagic acid and arbutin are used for skin whitening. Many other plant extracts have been added to cosmetics for their bioactivities[(3)]. In recent years, Chinese herbs have attracted much interest and are increasingly used in cosmetics because of their beneficial biological effects. Aloe and S*cutellariae Radix* (SR) are common Chinese herbs added to cosmetics.

Aloe is one of the most important botanicals for dermatologic uses such as cosmeceuticals[(4)]. Compounds found in aloe include aloin, aloe-emodin, chrysophanol and aloinoside A and B[(5)]. The leaves are used to treat asthma, gastrointestinal ulcers, cardiovascular disease, tumors, burns and diabetes. Aloe is used in cosmetics for its anti-tyrosinase and anti-inflammatory effects[(5-7)]. Aloe leaf works as a moisturizing agent[(8)] and provides protection against UV degradation and chemical attacks[(9)]. Therefore, it might be useful in the topical treatment of inflammatory skin conditions such as UV-induced erythema[(10)]. On the other hand, SR, the roots of *Scutellaria baicalensis*, is widely used in clinical Chinese medicine as a treatment for inflammation, fever and allergic diseases[(11-12)]. In addition, it has anxiolytic, neuroprotective[(13)], and antioxidative effects on the central nervous system[(14)]. Flavonoids like baicalein and wogonin and their derivatives have been found to be the active ingredients of SR[(14-16)]. Both herbs have been used in commercial cosmetic products for their sun protection and antioxidant effects.

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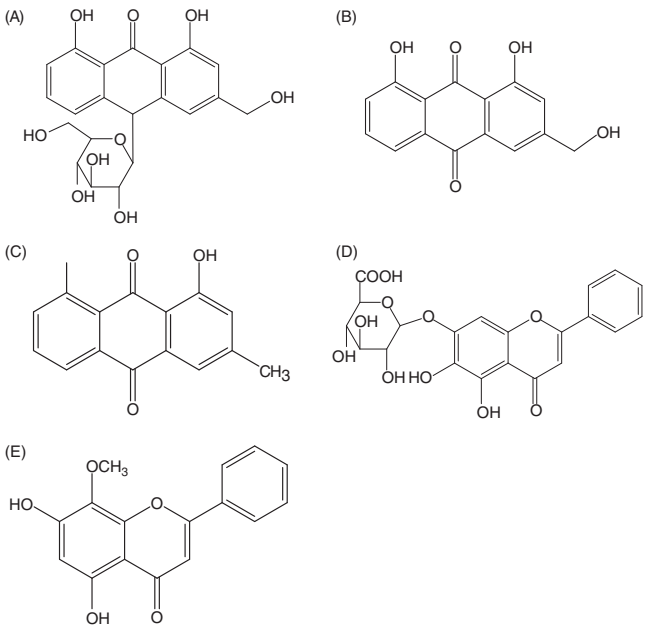

Figure 1. Chemical structures of polyphenols detected in this study. A: Aloin, B: Aloe-emodin, C: Chrysophanol, D: Baicalin and E: Wogonin.

Many countries have established regulations for cosmetics, including safety testing, product evaluation and chemical analyses to protect consumers. In addition, cosmetic ingredients, with the exception of flavors and fragrances, must be listed on the product label. Therefore, it becomes a concern whether the actual contents are consistent with those listed on product labels. However, it is not always clear whether herbal extracts have actually been added into Chinese herb-containing cosmetics in the market. This study used aloe and SR as a case study to employ a simple and accurate reversed-phase HPLC method for identification and quantification of the ingredients of Chinese herbs in cosmetics.

MATERIALS AND METHODS

I. *Materials and Chemicals*

Aloe and SR were purchased from a Chinese medicine retailer in Taichung, Taiwan. Analytical-grade reagents and solvents were used in all experiments. Aloe-emodin, aloin and mineral oil were obtained from Sigma (St. Louis, MO, U.S.A.). Chrysophanol and 2-methylanthraquinone (2-MA) were purchased from Aldrich (Milwaukee, WI, U.S.A.). Baicalin and wogonin were purchased from Wako (Osaka, Japan). Glycerin was purchased from Tong-Hwa Chemical (Taiwan). Spermaceti and white wax were purchased from First Chemical (Taiwan). Sodium borate was purchased from CFY Chemical (Taiwan). Emulsifying ointment was obtained from Washington Pharmaceutical (Taiwan). Acetonitrile and methanol were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Ortho-phosphoric acid (85%) was supplied by Riedel-deHaën AG (Seelze, Germany). Deionized water was obtained from Milli-Q Waters Purification System (Millipore, Milford, MA, U.S.A.).

II. *Instruments*

The HPLC apparatus was equipped with a pump (LC-10AT vp, Shimadzu, Japan), an automatic injector (SPD-10AF, Shimadzu, Japan), a UV-VIS detector (SPD-10A vp, Shimadzu, Japan), a degasser (ERC-3415, Japan) and a Cosmosil 5C18 AR-II 5μ column (4.6 × 250 mm, Nacalai Tesque, Kyoto, Japan).

III. *Methods*

(I) *HPLC Analysis Condition*

The mobile phase consisted of acetonitrile and 0.1% phosphoric acid. The gradient program was set as follows: 20 : 80 (0 - 15 min); 20 : 80 - 33 : 67 (15 - 20 min); 33 : 67 (20 - 25 min); 33 : 67 - 60 : 40 (25 - 50 min); 60 : 40 (50 - 60 min); 60 : 40 - 20 : 80 (60 - 70 min); 20 : 80 (70 - 80 min). The detection wavelength was 254 nm and the flow rate was 1.0 mL/min. Each injection volume was 40 μL.

(II) *Preparation of the Blank Cosmetic Bases*

For the exclusion of matrix effect in the cosmetic products, blank cosmetic bases were prepared for the spiked recovery assay of the marker compounds.

1. *Lotion*

Ten milliliter of glycerin was mixed with 100 mL of deionized water.

2. *Emulsion (o/w)*

Emulsifying ointment (15 g) was heated in a water bath, mixed with deionized water (35 g) and stirred in the same direction until emulsification occurred.

3. *Cream (w/o)*

Oil phase (62.5 g spermaceti, 60 g white wax and 280 g mineral oil) was heated in a water bath (70°C) until molten. Sodium borate (2.5 g) was dissolved in 95 g of deionized water (aqueous phase). The aqueous phase was added into the oil phase and stirred in the same direction until emulsification occurred at room temperature.

(III) *Preparation of Standard Solutions*

1. *Standard and Internal Standard Stock Solution*

Aloin, aloe-emodin, chrysophanol, baicalin and

wogonin were individually dissolved in methanol as the standard stock solution. The standard stock solutions were added to the blank lotion, emulsion and cream as needed to prepare a series of standard solutions (50.0, 25.0, 12.5, 6.3, 3.1 and 1.6 μg/mL) for calibration.

2-MA was dissolved in methanol to obtain 50.0 μg/mL (for lotion) and 2.0 μg/mL (for emulsion and cream) as the internal standard stock solution concentrations.

The peak area ratios of each standard to the internal standard versus the concentration of each standard were fitted to make the calibration curves. Based on the calibration curves, the linear regressions and correlative coefficients were determined.

2. *Standard Lotion*

A 100 μL standard solution was spiked with 20 μL of 2-MA stock solution. The mixture was dried by nitrogen gas and reconstituted with methanol (60 μL) for HPLC analysis.

3. *Standard Emulsion and Cream*

A 800 μL standard solution was individually added into 80 mg of blank emulsion/cream and mixed well. A saturated NaCl solution (480 μL) was used for salting out. The mixture was centrifuged at 10000 ×*g* for 15 min. Four hundred micromilliliter of the supernatant was spiked with 400 μL of methanol containing 2.0 μg/mL of 2-MA as an internal standard. After centrifugation, the supernatant was filtered through a 0.22-μm membrane filter (Millipore) for HPLC analysis.

(IV) *Preparation of Sample Solution and Quantification*

1. *Aloe-containing Products*

(1) *Lotion*

One hundred micromilliliter of lotion was spiked with 20 μL of methanol containing 50.0 μg/mL of 2-MA as an internal standard. The solution was dried under nitrogen gas and dissolved with 60 μL of methanol for HPLC analysis.

The concentration of each sample was obtained from the ratio of the peak area of the sample to the internal standard and the linear equation of the calibration curve.

(2) *Emulsion and Cream*

About 80 mg of each emulsion/cream sample was weighed precisely and mixed with 800 μL methanol, followed by addition of saturated NaCl solution (480 μL) for salting out. The mixture was centrifuged at 10000 ×*g* for 15 min, and 400 μL of the supernatant was spiked with 400 μL methanol containing 2.0 μg/mL of 2-MA as an internal standard. After centrifugation, the supernatant was filtered through a 0.22-μm membrane filter (Millipore) for HPLC analysis.

2. *Scutellariae Radix-containing Products*

(1) *Lotion*

Ten micromilliliter of SR extract (with known contents of bacalin and wogonin) was mixed with 90 μL of a blank cosmetic lotion base. 100 mL of the mixture was spiked with 20 μL of methanol containing 50.0 μg/mL of 2-MA as an internal standard. After centrifugation, the supernatant was filtered through a 0.22-μm membrane filter (Millipore) for HPLC analysis.

(2) *Emulsion and Cream*

About 72 mg of blank cosmetic emulsion/cream base was weighed precisely and mixed with 8 mL of SR extract (with known contents of baicalin and wogonin). 800 μL of methanol was then added. 480 μL of saturated NaCl solution was added to the mixture for salting out. The mixture was centrifuged at 10,000 ×*g* for 15 min, and 400 μL of supernatant was spiked with 400 μL of methanol containing 2.0 μg/mL of 2-MA as an internal standard. After centrifugation, the supernatant was filtered through a 0.22-μm membrane filter for HPLC analysis.

(V) *Validation*

1. *Precision and Accuracy*

Within the standard calibration range, the standard stock solution and the internal standard stock solution were quantified precisely. Each standard was analyzed by HPLC three times per day for three consecutive days. The mean, standard deviation (S.D.) and coefficient of variation (C.V.) were then calculated for an index of precision. The actual concentrations were calculated from standard curves and used to calculate the relative error, on which accuracy was based.

2. *Sensitivity*

The standard solutions with proper concentrations were prepared by dilution with methanol and analyzed by HPLC. The limit of detection (LOD) was obtained when the signal-to-noise ratio (in terms of peak height) was 3 : 1. The lower limit of quantification (LLOQ) of the standards was achieved when the signal-to-noise ratio was 10 : 1.

3. *Recovery*

Three concentrations of the calibration standard were spiked into the blank cosmetic lotion, emulsion and cream base, respectively, and assayed by HPLC. The recoveries were determined by the percentage of the calculated concentration versus the theoretical concentration.

RESULTS AND DISCUSSION

I. *Investigation of Cosmetic Sample Pre-treated Method*

The components of cosmetic formulae are complex

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and the pretreatment before quantitation is a key process for analysis. However, component interactions in the formulae leading to precipitation are not known. These interactions can affect the extraction yield of the active components. In addition, the formulae and matrices are too complex for complete extraction of the components from cosmetics by a single solvent or system. In literature, the solvent comprised of water, 70% ethanol, methanol and 5% Triton X-100, and heating or sonication was applied in crude drug extraction[17-19]. In our previous

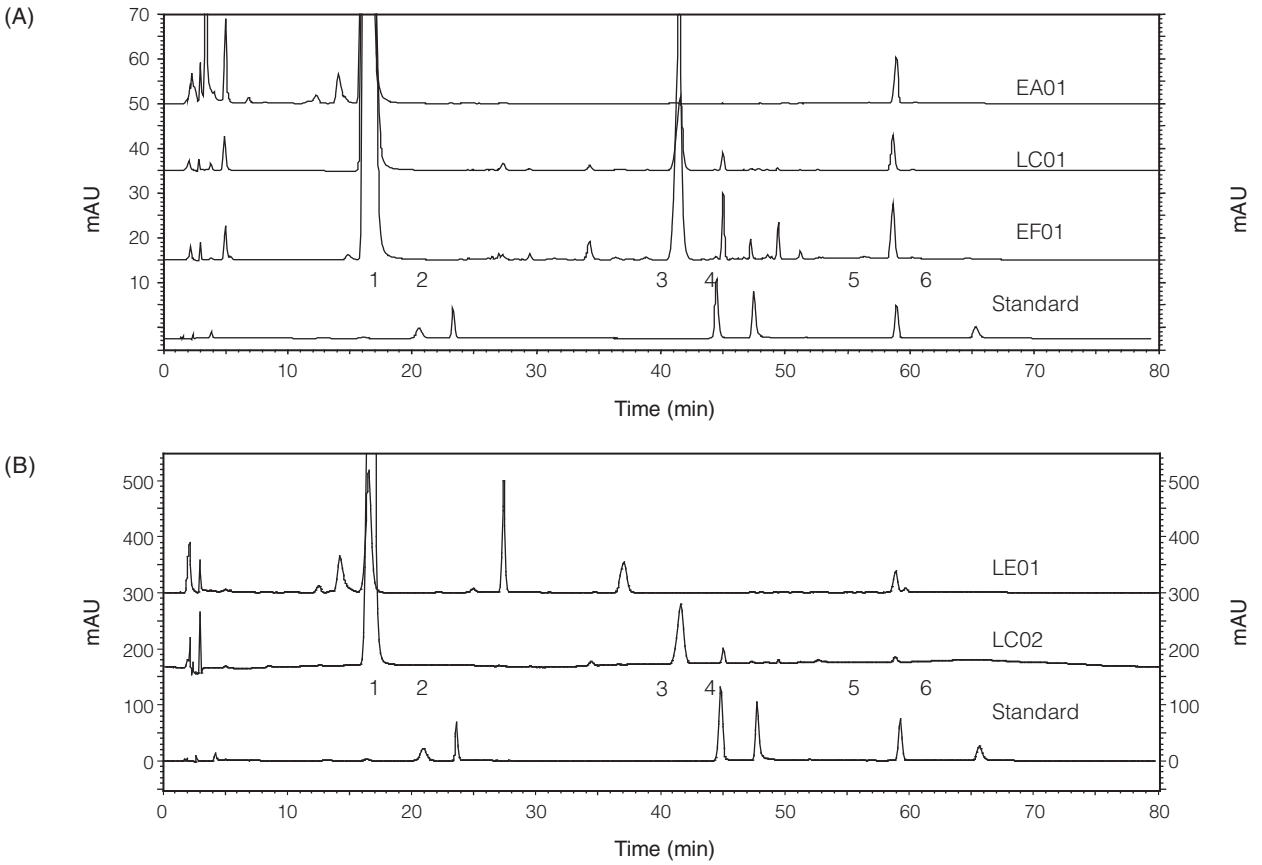

Figure 2. (A) HPLC chromatograms of aloin, aloe-emodin, chrysphanol, baicalin and wogonin in aloe-containing extracts (EA01 and EF01), aloe-containing lotion (LC01) and standards.
1. Aloin, 2. Baicalin, 3. Aloe-emodin, 4. Wogonin, 5. 2-Methylanthraquinone (internal standard) 6. Chrysophanol
(B) HPLC chromatograms of aloin, aloe-emodin and chrysophanol, in aloe-containing lotions (LC02 and LE01) and standards.

Table 1. The regression equations, concentration ranges and regression coefficients of aloin, aloe-emodin and chrysophanol in aloe-containing cosmetic preparations.

Sample	Constituent	Regression Equation	Conc. Range (μg/mL)	R
Lotion	Aloe-emodin	Y = 0.0850 X - 0.0059	3.1 - 50.0	0.997
	Chrysophanol	Y = 0.0624 X - 0.1402	3.1 - 50.0	0.999
Emulsion (o/w)	Aloin	Y = 0.01916 X - 0.0096	1.6 - 50.0	0.999
	Aloe-emodin	Y = 0.1714 X - 0.0928	1.6 - 50.0	0.999
	Chrysophanol	Y = 0.0463 X - 0.0278	1.6 - 50.0	0.986
Cream (w/o)	Aloin	Y = 0.01898 X + 0.0228	1.6 - 50.0	0.995
	Aloe-emodin	Y = 0.2999 X - 0.0454	1.6 - 50.0	0.993
	Chrysophanol	Y = 0.0623 X - 0.1579	3.1 - 50.0	0.996

study, sonication[20] and KH_2PO_4 buffer were used for cosmetic extraction[21]. However, they were not sufficient for cosmetics quantitation, especially for cosmetics containing Chinese herbs.

There are various methods for demulsification, including chemical, physical and biological methods. In chemical methods, organic solvent, acid and NaCl are commonly used to destroy the emulsion for extraction[22-23]. In this study, 40% methanol, vacuum-drying and solid phase extraction were used for the pretreatment of the sample. However, these treatments were not suitable. Subsequently, a saturated NaCl solution was used for salting out the sample, followed by centrifugation and filtration to obtain clear filtrates for HPLC analysis. It was found that salting out was the most appropriate pretreatment method for the cosmetic samples in this study.

II. *Quantitation of Aloe-containing Cosmetics*

Although some HPLC methods have been developed for the determination of the contents in aloe or SR decoction[24-26], this study attempted to establish an HPLC method for simultaneously determining the aloin, aloe-emodin and chrysophanol contents in aloe-containing cosmetics and the baicalin and wogonin contents in SR-containing cosmetics. HPLC chromatograms of aloin, aloe-emodin and chrysophanol in aloe-containing lotion products and standards are shown in Figures 2A and 2B. For simultaneous analysis of three ingredients with different polarities, a gradient elution analysis system was used. These three compounds and the internal standard (2-MA) were well resolved within 80 min.

The linear regressions and concentration ranges of the standard curves for aloe are shown in Table 1. In addition, the linear regression equation and correlation coefficient (r) in the analytical profile for aloe-emodin and chrysophanol in lotion and for aloin, aloe-emodin and chrysophanol in emulsion and cream are listed in Table 1. All calibration curves of aloe-emodin and chrysophanol were in good linear correlation with a correlation coefficient of 0.997-0.999 within a range of 3.1-50.0 μg/mL in lotion. Aloin, aloe-emodin and chrysophanol were in good linear correlation with a correlation coefficient of 0.986-0.999 within a range of 1.6-50.0 μg/mL in emulsion. The LLOQ were 1.6 μg/mL for the three compounds, and the LOD, which represented the lowest detectable concentration of the analytes (S/N > 3), were 0.04, 0.03, and 0.01 μg/mL for aloin, aloe-emodin and chrysophanol, respectively.

Validation of this analysis system is shown in Table 2. The relative standard deviations of the intra-day and inter-day results were 3.4 - 7.5% and 3.7 - 10.2% for aloe-emodin, and 3.2 - 9.2% and 2.2 - 7.1% for chrysophanol in lotion; 2.1 - 11.6% and 0.8 - 3.1% for aloe-emodin, 0.9 - 9.1% and 2.3 - 9.1% for aloin, and 0.9 - 9.5% and 2.6 - 9.4% for chrysophanol in emulsion (o/w); 2.4 - 9.7% and 3.1 - 7.6% for aloe-emodin, 2.2 - 8.5% and 6.8 - 11.8% for aloin, and 2.7 - 10.9% and 3.2 - 5.8% for chrysophanol in cream (w/o). Recoveries of these three anthraquinones were 86.9 - 102.9% for aloe-emodin and 95.6 - 102.4% for chrysophanol in lotion, respectively (Table 3). In emulsion, the recoveries were 94.7 - 106.2% for aloe-emodin, 95.3 - 107.8% for aloin, and 98.2 - 106.0% for chrysophanol. In addition, the recoveries were 95.3 - 97.2% for aloe-emodin, 98.7 - 99.8% for aloin, and 91.2 - 99.8% for chrysophanol in cream. The results showed acceptable precision, accuracy and recoveries.

Subsequently, this HPLC methodology was applied in the analysis of compounds in commercial cosmetic products. 4 aloe-containing extracts, 6 aloe-containing lotions and 17 aloe-containing emulsions were analyzed (the HPLC chromatograms are not shown). The results indicated that the contents of the active components were lower than the LOD, not only in all commercial products but also in the aloe raw material. However, these anthraquinones could be detected when spiked into the blank lotion and emulsion using the same analytical conditions. In addition, the recoveries ranged from 87 to 108%. The active ingredients may have been damaged or degraded during the extraction of the crude plants. Fresh aloe juices contain about 99.0 - 99.5% of water. Over 100 chemical ingredients exist in aloe. They can be divided into anthraquinones including aloin, aloe-emodin and chrysophanol, amino acids, organic acids and cellulose[5]. To determine the contents of anthraquinones in fresh aloe juices, the fresh aloe leaves were pulped and the juice was filtered through a 0.45-μm membrane filter and concentrated 4 times for HPLC analysis. The results indicated that aloe-emodin and chrysophanol were undetectable, and the aloin content was lower than LOD. There are no official specifications of aloe raw materials for cosmetic use. According to CTFA, the international cosmetic ingredient dictionary, aloe raw material is divided into 17 sources according to the extraction methods: 7 aloe-containing extracts, 5 aloe juices, 1 powder of aloe juice, 1 leaf protoplast, 1 leaf polysaccharide, 1 leaf water and 1 leaf[27]. However, there are no specifications for the aloin amount in those materials. For the product specification of the aloe vera gel spray dried powder 200X (cosmetic grade), aloin was less than 1.0 ppm from Concentrated Aloe Corporation[28]. Furthermore, it had been reported that, the contents of aloin-A and aloe-emodin were lower than the detectable levels in aloe-based products, especially liquid products, even when LC/MS was used[29]. It had been reported that aloin in aloe-based alcoholic beverages was not detected, owing to their instability and degradation in solution[30]. The low levels of these anthraquinones might be because more than 99% of the fresh aloe juice was water and these compounds were unstable in aqueous solutions. Furthermore, the contents of the marker compounds in fresh aloe juice were low, and the raw material might have been extensively diluted when added into the products[29]. Based on the above observations, the high water content, low content of anthraquinones in aloe and instability are the key contributing factors for detection.

Table 2. Validation of components in aloe-containing lotion, emulsion and cream.

Sample	Compound	Conc (μg/mL)	Intra-day		Inter-day	
			Precision Mean ± S.D. (C.V.%)	Accuracy (%)	Precision Mean ± S.D. (C.V.%)	Accuracy (%)
Lotion	Aloe-emodin	50.0	48.8 ± 1.7 (3.6)	-2.3	49.0 ± 2.2 (4.4)	-2.0
		25.0	27.6 ± 1.3 (4.8)	10.2	27.4 ± 1.9 (7.1)	9.6
		12.5	12.7 ± 0.7 (5.6)	1.7	12.2 ± 0.5 (3.7)	-2.6
		6.3	5.4 ± 0.4 (7.5)	-14.4	5.7 ± 0.6 (10.2)	-8.6
		3.1	2.4 ± 0.1 (3.4)	-21.9	2.6 ± 0.2 (7.0)	-16.6
	Chrysophanol	50.0	50.3 ± 4.0 (8.0)	0.5	49.2 ± 3.0 (6.0)	-1.6
		25.0	24.2 ± 1.7 (7.2)	-3.3	26.9 ± 0.6 (2.2)	7.6
		12.5	13.3 ± 1.2 (9.2)	6.7	12.5 ± 0.9 (7.1)	-0.1
		6.3	5.7 ± 0.2 (3.2)	-8.2	5.4 ± 0.2 (3.9)	-14.4
		3.1	3.4 ± 0.2 (6.6)	7.6	3.0 ± 0.1 (3.5)	-5.1
Emulsion (o/w)	Aloe-emodin	50.0	50.8 ± 3.3 (6.4)	1.5	50.6 ± 0.4 (0.8)	1.1
		25.0	23.4 ± 1.1 (4.6)	-6.6	23.7 ± 0.7 (3.1)	-5.3
		12.5	12.6 ± 0.8 (6.1)	0.5	12.7 ± 0.3 (2.1)	1.6
		6.3	6.5 ± 0.3 (4.7)	3.2	6.4 ± 0.1 (1.5)	2.5
		3.1	3.6 ± 0.4 (11.6)	15.9	3.5 ± 0.1 (1.7)	11.0
		1.6	$1.7 \pm 0.0_4$ (2.1)	9.1	$1.6 \pm 0.0_2$ (1.5)	3.7
	Aloin	50.0	50.3 ± 3.2 (6.3)	0.6	50.5 ± 1.2 (2.3)	1.0
		25.0	24.1 ± 1.3 (5.6)	-3.4	23.8 ± 1.1 (4.8)	-4.7
		12.5	12.8 ± 0.6 (4.6)	2.5	12.6 ± 0.5 (3.9)	0.7
		6.3	6.6 ± 0.6 (9.1)	5.2	6.5 ± 0.4 (6.7)	3.5
		3.1	3.3 ± 0.1 (2.8)	6.4	3.6 ± 0.3 (9.1)	14.6
		1.6	$1.3 \pm 0.0_1$ (0.9)	-18.4	1.5 ± 0.1 (5.5)	-6.3
	Chrysophanol	50.0	57.2 ± 3.2 (5.6)	14.3	60.1 ± 3.6 (6.0)	20.3
		25.0	20.8 ± 0.6 (3.1)	-17.0	22.6 ± 0.6 (2.6)	-9.4
		12.5	12.3 ± 0.3 (2.0)	-1.8	12.3 ± 1.2 (9.4)	-1.6
		6.3	5.8 ± 0.1 (1.8)	-6.7	5.0 ± 0.4 (7.7)	-20.1
		3.1	3.7 ± 0.4 (9.5)	17.1	3.5 ± 0.2 (5.2)	11.3
		1.6	$1.5 \pm 0.0_1$ (0.9)	-6.0	1.6 ± 0.1 (5.0)	0.1
Cream (w/o)	Aloe-emodin	50.0	58.3 ± 4.0 (6.8)	16.6	59.1 ± 2.9 (4.9)	18.2
		25.0	24.6 ± 2.4 (9.7)	-1.5	23.8 ± 1.2 (5.2)	-4.7
		12.5	11.8 ± 0.4 (3.0)	-5.3	11.9 ± 0.4 (3.1)	-4.7
		6.3	5.7 ± 0.4 (6.4)	-9.1	5.6 ± 0.4 (7.1)	-10.2
		3.1	2.9 ± 0.1 (2.4)	-6.5	3.0 ± 0.2 (4.9)	-2.8
		1.6	1.7 ± 0.1 (3.9)	5.7	1.6 ± 0.1 (7.6)	4.3

Table 2. Continued.

Sample	Compound	Conc (μg/mL)	Intra-day Precision Mean ± S.D. (C.V.%)	Intra-day Accuracy (%)	Inter-day Precision Mean ± S.D. (C.V.%)	Inter-day Accuracy (%)
Cream (w/o)	Aloin	50.0	52.8 ± 1.2 (2.2)	5.5	55.3 ± 6.5 (11.8)	10.7
		25.0	24.0 ± 1.7 (7.2)	-4.1	21.8 ± 1.5 (6.8)	-12.7
		12.5	11.1 ± 1.0 (8.5)	-11.1	11.4 ± 1.0 (8.9)	-8.8
		6.3	6.9 ± 0.5 (7.2)	10.4	7.1 ± 0.6 (8.6)	13.0
		1.6	1.6 ± 0.1 (5.3)	0.0	1.5 ± 0.1 (7.5)	-3.1
	Chrysophanol	50.0	54.5 ± 4.6 (8.4)	9.0	51.2 ± 1.6 (3.2)	2.4
		25.0	22.8 ± 0.8 (3.7)	-8.8	22.3 ± 0.7 (3.2)	-10.8
		12.5	12.1 ± 0.5 (4.5)	-3.3	12.4 ± 0.7 (5.6)	-1.0
		6.3	6.5 ± 0.7 (10.9)	3.7	7.4 ± 0.4 (5.8)	18.3
		3.1	3.1 ± 0.1 (2.7)	-0.2	3.6 ± 0.2 (5.4)	14.8

III. *Quantitation of Scutellariae Radix-containing Cosmetics*

HPLC chromatograms of baicalin and wogonin in cosmetic products and standards containing SR are shown in Figures 3A and 3B. The target compounds, baicalin and wogonin and the internal standard (2-MA) were well resolved within 80 min by gradient elution.

Linear regression analysis of the calibration curve between the response and the concentration of baicalin and wogonin demonstrated linearity over the range of 1.6-50.0 μg/mL, and the results of linear regression analysis showed that the correlation coefficients of the two flavonoids were 0.999 in lotion and 0.984-0.995 in emulsion (o/w) and cream (w/o), respectively (Table 4). The intra-day and inter-day precision and accuracy values are presented in Table 5. The overall mean precision, defined by the coefficient of variation, ranged from 1.1 - 10.1%. The pretreatment method yielded good recoveries, with the average recoveries of baicalin and wogonin from 91.1% to 106.8% at concentrations of 3.1, 12.5 and 25.0 μg/mL in three dosage forms (Table 6). The LLOQ and LOD were 1.6 and 0.2 μg/mL for baicalin, and 1.6 and 0.3 μg/mL for wogonin. The results of the validation indexes of this HPLC methodology indicated that all parameters were acceptable. To validate our methods, blank cosmetic bases were prepared in our laboratory, and extract containing 10% of SR was spiked (Table 7). The results indicated that this study established a feasible method for flavonoid detection in cosmetic products.

The HPLC methodology was applied to the determination of commercial cosmetic products. Two SR-containing extracts, one essential solution and two emulsions were quantified. The results indicated that baicalin and wogonin were detected only in SR-containing extracts,

Table 3. Recoveries of aloe-emodin, aloin and chrysophanol in cosmetic products.

Sample	Compound	Conc. (μg/mL)	Mean ± S.D.
Lotion	Aloe-emodin	25.0	102.9 ± 7.2
		12.5	92.5 ± 3.4
		3.1	86.9 ± 2.6
	Chrysophanol	25.0	100.8 ± 2.2
		12.5	95.6 ± 6. 6
		3.1	102.4 ± 3.0
Emulsion (o/w)	Aloe-emodin	25.0	94.7 ± 3.0
		12.5	101.6 ± 2.2
		3.1	106.7 ± 5.8
	Aloin	25.0	95.3 ± 4.6
		12.5	100.7 ± 4.0
		3.1	107.8 ± 1.6
	Chrysophanol	50.0	99.8 ± 4.4
		12.5	98.2 ± 2.0
		3.1	106.0 ± 1.6
Cream (w/o)	Aloe-emodin	25.0	95.3 ± 5.0
		12.5	95.3 ± 3.0
		3.1	97.2 ± 4.7
	Aloin	25.0	98.7 ± 1.6
		12.5	99.7 ± 3.0
		6.3	99.8 ± 1.8
	Chrysophanol	25.0	91.2 ± 3.4
		12.5	96.7 ± 4.4
		3.1	99.8 ± 2.7

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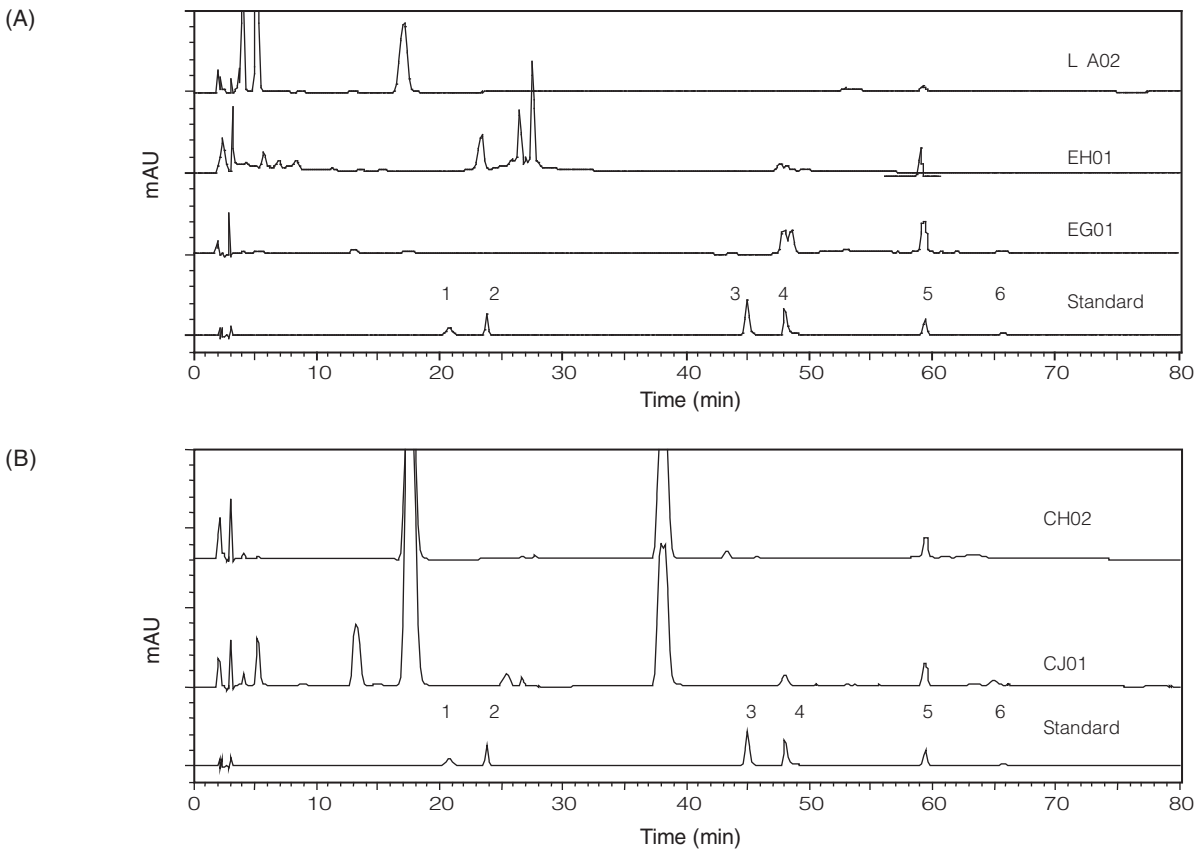

Figure 3. (A) HPLC chromatograms of baicalin and wogonin in Scutellariae Radix extract-containing extracts (EH01 and EG01), lotion (LA02) and standards.
(B) HPLC chromatograms of baicalin and wogonin in Scutellariae Radix extract-containing emulsions (CH02 and CJ01) and standards.

Table 4. Regression equations, concentration ranges and regression coefficients of baicalin and wogonin in Scutellariae Radix-containing cosmetic preparations.

Sample	Constituent	Regression Equation	Conc. Range (μg/mL)	R
Lotion	Baicalin	Y = 0.0207 X + 0.0284	1.6 - 50.0	0.999
	Wogonin	Y = 0.0444 X - 0.0075	1.6 - 50.0	0.999
Emulsion (o/w)	Baicalin	Y = 0.0282 X - 0.0505	3.1 - 50.0	0.993
	Wogonin	Y = 0.0819 X - 0.1209	3.1 - 50.0	0.995
Cream (w/o)	Baicalin	Y = 0.0291 X - 0.0231	3.1 - 50.0	0.993
	Wogonin	Y = 0.1445 X - 0.2009	3.1- 50.0	0.984

while others were undetectable (Figures 3A and 3B). The results suggested that the addition of SR-containing extracts was low or not added. The color of SR extract is yellowish-brown and would affect the appearance of finished cosmetic products. For this reason, SR extract required extensive dilution when added into the cosmetic products.

CONCLUSIONS

This study established a quantitative method for the simultaneous determination of 5 compounds, i.e. aloin, aloe-emodin and chrysophanol in aloe-containing cosmetic products and baicalin and wogonin in SR-containing cosmetic products. The methodology can

Table 5. Validation of components in Scutellariae Radix-containing lotion, emulsion and cream.

Sample	Compound	Conc (μg/mL)	Intra-day		Inter-day	
			Precision Mean ± S.D. (C.V.%)	Accuracy (%)	Precision Mean ± S.D. (C.V.%)	Accuracy (%)
Lotion	Baicalin	50.0	48.8 ± 2.2 (4.5)	-2.4	49.6 ± 3.2 (6.4)	-0.8
		25.0	27.8 ± 0.8 (2.8)	11.2	25.5 ± 1.7 (6.6)	2.1
		12.5	11.6 ± 0.5 (4.0)	-7.4	13.7 ± 1.2 (8.9)	9.2
		6.3	5.0 ± 0.2 (3.0)	-19.4	5.3 ± 0.2 (2.8)	-15.4
		1.6	1.3 ± 0.1 (8.3)	-16.9	1.3 ± 0.1 (4.2)	-18.6
	Wogonin	50.0	50.2 ± 4.4 (8.8)	0.5	48.7 ± 2.7 (5.5)	-2.5
		25.0	23.9 ± 1.8 (7.3)	-4.4	28.0 ± 1.3 (4.5)	11.9
		12.5	13.6 ± 1.4 (10.1)	9.1	12.1 ± 0.7 (5.7)	-3.2
		6.3	6.9 ± 0.5 (7.0)	9.7	5.6 ± 0.2 (4.2)	-10.6
		3.1	2.5 ± 0.2 (9.2)	-19.4	2.7 ± 0.2 (7.2)	-12.4
		1.6	1.3 ± 0.1 (9.7)	-17.6	1.3 ± 0.03 (2.4)	-15.6
Emulsion (o/w)	Baicalin	50.0	56.0 ± 3.5 (6.3)	12.1	56.6 ± 2.3 (4.0)	13.3
		25.0	23.3 ± 1.0 (4.5)	-6.7	24.3 ± 1.9 (7.9)	-2.8
		12.5	12.4 ± 0.3 (2.6)	-1.0	11.1 ± 0.5 (4.6)	-11.2
		6.3	5.7 ± 0.3 (4.9)	-8.8	6.1 ± 0.2 (4.0)	-3.1
		3.1	3.3 ± 0.1 (1.5)	4.7	3.3 ± 0.4 (11.4)	3.9
	Wogonin	50.0	56.1 ± 3.4 (6.1)	12.2	55.5 ± 2.3 (4.1)	10.9
		25.0	23.2 ± 1.3 (5.4)	-7.4	23.2 ± 0.9 (3.7)	-7.1
		12.5	12.2 ± 0.6 (4.9)	-2.2	12.2 ± 1.2 (9.6)	-2.3
		6.3	5.8 ± 0.6 (9.7)	-6.7	6.0 ± 0.4 (7.1)	-4.5
		3.1	3.3 ± 0.1 (1.6)	4.1	3.2 ± 0.2 (4.8)	3.2
Cream (w/o)	Baicalin	50.0	57.1 ± 2.5 (4.3)	14.2	55.3 ± 3.3 (5.9)	10.7
		25.0	22.8 ± 0.6 (2.5)	-8.9	22.4 ± 0.9 (3.9)	-10.2
		12.5	12.3 ± 0.6 (4.9)	-1.9	13.0 ± 0.9 (6.7)	3.8
		6.3	5.7 ± 0.2 (2.9)	-8.3	5.8 ± 0.1 (2.1)	-8.0
		3.1	3.3 ± 0.2 (4.7)	4.8	3.2 ± 0.1 (3.7)	3.7
	Wogonin	50.0	60.5 ± 1.8 (3.0)	21.0	60.4 ± 1.8 (3.0)	20.8
		25.0	22.6 ± 0.6 (2.6)	-9.4	23.0 ± 0.6 (2.7)	-8.1
		12.5	11.0 ± 0.5 (4.5)	-11.9	11.1 ± 0.4 (3.7)	-11.0
		6.3	5.8 ± 0.4 (7.3)	-7.5	5.7 ± 0.1 (2.2)	-8.5
		3.1	3.3 ± 0.1 (3.9)	6.3	3.3 ± 0.04 (1.1)	6.8

Table 6. Recoveries of baicalin and wogonin in cosmetic products.

Sample	Compound	Conc. (μg/mL)	Mean ± S.D.
Lotion	Baicalin	25.0	102.1 ± 6.7
		12.5	99.0 ± 3.6
		3.1	103.5 ± 3.9
	Wogonin	25.0	102.5 ± 5.5
		12.5	96.8 ± 5.5
		3.1	95.9 ± 8.2
Emulsion (o/w)	Baicalin	25.0	93.3 ± 4.2
		12.5	99.0 ± 2.6
		3.1	104.7 ± 1.6
	Wogonin	25.0	92.9 ± 3.4
		12.5	97.8 ± 9.4
		3.1	103.2 ± 5.0
Cream (w/o)	Baicalin	25.0	91.1 ± 2.3
		12.5	98.1 ± 4.8
		3.1	104.8 ± 4.9
	Wogonin	25.0	91.9 ± 2.5
		12.5	105.6 ± 6.2
		3.1	106.8 ± 1.2

Table 7. Regression equations and the content of baicalin and wogonin in lotion, emulsion and cream spiked with 10% Scutellariae Radix-containing cosmetic preparations (μg/mL).

Sample	Constituent	Concentration (μg/mL)*
Lotion	Baicalin	12.9 ± 0.09
	Wogonin	2.4 ± 0.03
Emulsion (o/w)	Baicalin	18.0 ± 0.46
	Wogonin	3.1 ± 0.21
Cream (w/o)	Baicalin	14.0 ± 0.26
	Wogonin	2.4 ± 0.07

*n = 3

be applied to the assay of cosmetics containing Chinese herbs. Most of the target compounds in the cosmetic products were not detectable, due to the low active constituents content, extensive dilution and degradation. Therefore, the establishment of a simple and feasible analytical method for Chinese herb-containing cosmetics to protect customers' right is an important issue in the dramatically growing cosmetic market.

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