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# Development of an Assay Method for Natural Products Containing Cosmetics (II)–Licorice

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## ABSTRACT

This study established a quantification method for cosmetics containing licorice. During pretreatment, methanol was used as a solvent for extraction of lotion products and a salting-out method with a photodiode array detector for emulsions and creams. A gradient high performance liquid chromatographic method was established to determine liquiritin, glycyrrhizin and glycyrrhetinic acid in licorice-containing cosmetics. Samples were analyzed by gradient elution with a mobile phase containing acetonitrile and 1% phosphoric acid at a flow rate of 1.0 mL/min. Calibration curves of the three compounds were constructed in the range of 5.0 - 50.0 μg/mL in glycerin solution and 2.5 - 100.0 μg/mL in emulsion and cream. The correlation coefficients were all above 0.994. The average recoveries of these three compounds were 88.8 - 107.4%, 91.7 - 104.1% and 95.6 - 105.5% in glycerin solution, emulsion, and cream, respectively. The analytical method was further validated and employed in the assay of the commercial products. The results showed that glycyrrhizin could be detected in licorice extracts. With regard to the quantification of marker components in commercial products, glycyrrhizin (16.8 to 113.4 μg/mL) was detected in 3 out of 7 licorice-containing commercial products. Glycyrrhizin was the most abundant component in licorice, while other marker compounds were not detectable, probably due to low contents or extensive dilution. The method developed in this study can be applied to commercial cosmetics containing licorice for the assurance of product quality.

Key words: licorice, glycyrrhizin, liquiritin, glycyrrhetinic acid, cosmetics

## INTRODUCTION

Natural products, especially plant extracts, have been used as active constituents of drugs or cosmetics over human history. The contents of active components vary with origin, place of planting, cultivation, and collecting time; moreover, the active ingredients are sometimes degraded or lost during isolation or purification processes. It is important to develop methods for the quantification of the components of herb-containing cosmetics to ensure quality and customer safety. The constituents of an herb are complex and individual amount was little, therefore, it is more difficult to study herbal preparation than a single herb. The methodologies for the quantification of natural products in cosmetics are currently limited. We attempted to establish a series of simple and feasible analyses for Chinese herb-containing cosmetics in various dosage forms and formulas. In our previous study, a quantification method was established for aloe- and Scutellariae Radix-containing cosmetics.

Glycyrrhizae radix, one of the most popular herbal medicines, contains flavonoids, such as liquiritin and isoliquiritin, and triterpenoids such as glycyrrhizin, glycyrrhetinic acid and coumarins. Glycyrrhizae radix exerts various pharmacological actions involving neuroprotective[(1)], chemopreventive[(2)], anti-allergy, antioxidant, anti-inflammatory, anti-microbial and steroid-like effects[(3-5)]. These activities may be attributed to phenolic antioxidants involving isoflavan derivatives, coumarins and chalcones[(6)]. In dermatology, Glycyrrhizae radix also demonstrates various pharmacological benefits including treatment of atopic dermatitis[(7)], depigmentation, and reducing inflammation[(4,8,9)]. Their polyphenol compounds like glabrene and isoliquiritigenin exert strong antioxidant and tyrosinase-dependent melanin biosynthesis inhibition, suggesting that isoflavenes and chalcones may serve as candidates for skin-lightening agents[(10-12)]. Additionally, glabridin showed anti-inflammatory effects, inhibition of melanogenesis, and inhibition of UVB-induced pigmentation and erythema in the skins of guinea pigs when applied topically[(8)]. Licochalcone A, a chalcone compound in licorice, is a potent

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inhibitor of inflammatory facial redness[9] and UVB-induced irritated and damaged skin through the inhibition of COX-2-dependent $PGE_2$ production[4,13]. Based on its dermatological activities, Glycyrrhizae radix is frequently added to cosmetics. However, a method is needed to confirm that the actual components and amounts match what is listed on the labels of such products. A quantitative analysis system for natural products used in cosmetics is needed to ensure quality and consumer safety.

Although the literature reports the use of thin-layer chromatography and high-performance liquid chromatography (HPLC) for determination of licorice components in decoction and health foods[14-17], none has addressed licorice-containing cosmetic products. This study aimed to setup a pretreatment method and quantification analysis system for simultaneous determination of liquiritin, glycyrrhizin and glycyrrhetinic acid, the active and major components of Glycyrrhizae radix, in commercial cosmetic products.

## MATERIALS AND METHODS

### I. *Materials*

Liquiritin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Glycyrrhizin and glycyrrhetinic acid were from Sigma Chemical Co. (St. Louis, MO, USA), and 2-methylanthraquinone (2-MA) was from Aldrich Chemical Company (Milwaukee, WI, USA). Acetonitrile and methanol were obtained from J. T. Baker, Inc. (Phillipsburg, NJ, USA). Ortho-phosphoric acid (85%) was supplied by Riedel-deHaën AG (Seelze, Germany). Emulsifying ointment was obtained from Washington Pharmaceutical (Taiwan). The water used in this study was purified on a Milli-Q Waters Purification System (Millipore, Milford, MA, USA). Seven licorice containing cosmetics (CM03, CM04, CU01, CU02, CU03, CV01 and CW01) were purchased from local drug stores and supermarkets.

### II. *Liquid Chromatography*

HPLC was performed with a pump (LC-10AT vp, Shimadzu, Japan), an automatic injector (SPD-10AF, Shimadzu, Japan), a photodiode array detector (PDA; SIL-10AF, Shimadzu, Japan), and a degasser (ERC-3415, Japan). Separation was achieved on a Cosmosil 5C18 AR-II 5μ column (4.6 × 250 mm, Nacalai Tesque, Kyoto, Japan) at room temperature. The flow rate was 1.0 mL/min. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid, and the gradient program was set as follows: 20 : 80 (0 - 15 min), 45 : 55 (20 - 25 min), 65 : 35 (35 - 55 min) and 20 : 80 (60 - 70 min). Each injection volume was 40 μL.

### III. *Preparation of Blank Glycerin Solution, Eemulsion and Cream*

In order to exclude the matrix effect in the cosmetic products, blank cosmetic bases were prepared for the marker compounds spiked recovery assay.

#### (I) *Blank Glycerin Solution*

Ten mililiter of glycerin and 100 mL of deionized water were mixed to afford blank glycerin solution.

#### (II) *Blank Emulsion (o/w)*

Emulsifying ointment (15 g) was heated and mixed with deionized water (35 g). The mixture was stirred in the same direction until emulsified.

#### (III) *Blank Cream (w/o)*

The oil phase constituted with 62.5 g spermaceti, 60 g white wax and 280 g mineral oil was heated in a water bath at 70°C, and the water phase was sodium borate (2.5 g) dissolved in 95 g deionized water. The oil and water phase was then mixed by stirring in the same direction until emulsified at room temperature.

### IV. *Preparation of Standard Solutions and Calibration*

#### (I) *Standard Stock Solution*

Glycyrrhizin, liquiritin, and glycyrrhetinic acid were accurately weighed and dissolved in methanol as the stock standard solution. 2-MA was dissolved in methanol to afford a 50.0 μg/mL internal standard stock solution.

#### (II) *Standard Glycerin Solution*

The standard stock solutions were added to the blank glycerin solution, emulsion and cream as needed to prepare a series of standard solutions to 50.0, 25.0, 12.5, 10.0 and 5.0 μg/mL for calibrators.

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

#### (III) *Standard Emulsion and Cream*

The standard stock solutions were diluted individually with methanol in series to 100.0, 80.0, 50.0, 25.0, 20.0, 10.0, 5.0 and 2.5 μg/mL for calibrators. Calibration curves were generated using the above concentrations of standard solutions from least-squares regression of peak area in triplicate assays.

An 800-microliter standard solution was individually mixed with 80 mg of blank emulsion or cream and mixed well; a saturated NaCl solution (480 μL) was then added for salting out. The mixture was centrifuged at 9,860 ×*g* for 15 min, and 400 μL supernatant was spiked with 400 μL methanol containing 2.0 μg/mL of 2-MA as the internal

standard. After centrifugation, the supernatant was filtered through a 0.22 μm membrane filter (Millipore) for HPLC analysis.

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

V. *Preparation of Sample Solution and Quantification*

(I) *Lotion Containing Licorice Extract*

A 100-microliter lotion sample was spiked with 20 μL methanol containing 50.0 μg/mL of 2-MA as the internal standard. The solution was dried under nitrogen gas and dissolved with 60 μL methanol for HPLC analysis. The peaks were checked by photodiode array detector to confirm the composition.

Through comparison of 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 compound was obtained.

(II) *Emulsion and Cream Containing Licorice Extract*

About 80 mg of each emulsion/cream sample was weighed precisely and mixed with 800 μL methanol; saturated NaCl solution (480 μL) was then added for salting out. The mixture was centrifuged at 10,000 ×*g* for 15 min, and 400 μL supernantant was spiked with 400 μL methanol containing 2.0 μg/mL of 2-MA as the internal standard. After centrifugation, the supernatant was filtered through a 0.22 μm membrane filter (Millipore) for HPLC analysis. The peaks were identified by photodiode array detector to confirm the composition.

VI. *Validation*

(I) *Precision and Accuracy*

Within the standard calibration range, each standard of each concentration was analyzed by HPLC three times (once in the morning, afternoon and evening) per day (intraday) for three consecutive days (interday). The mean, standard deviation (SD), and coefficient of variation (CV) were calculated for an index of precision. The real concentrations were calculated from standard curves and used to calculate the relative error, which stands for accuracy.

(II) *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 peak height was three times that of the noise, and the lower limit of quantification (LLOQ) of the standards was achieved by measurement of the signal-to-noise peak height ratio of 10 : 1.

(III) *Recovery*

Three concentrations of the calibration standard were spiked into the blank glycerin solution, emulsion and cream preparation individually and assayed by HPLC-PDA. The recoveries were determined by the percentage of calculated concentration versus theoretical concentration.

## RESULTS

I. *Investigation of Cosmetic Sample Pretreatment*

Several extraction methods of commercial products have been examined, which included methanol, vacuum drying and solid phase extraction method. However, they were not suitable probably due to that some components in the preparations interacted with each other, or some chemical or physical reaction occurred during extraction process. Finally, a saturated NaCl solution was used for salting out the sample, followed by centrifugation at 10,000 ×*g* for 15 min. The supernatant was filtered through a 0.22 μm membrane filter to obtain clear filtrates for HPLC analysis. It is the optimum pretreatment for the cosmetic samples in this study.

II. *Validation of the Analysis Method*

In this study, a gradient elution was applied to separate glycyrrhizin, glycyrrhetinic acid and liquiritin in licorice. These three components and the internal standard (2-MA) were well resolved within 70 min by gradient elution. The chromatograms of standards and samples were shown in Figure 1. Calibration graphs for the constituents were obtained where Y was the peak-area ratio of components to the internal standard, and X was the concentration of the same components. The linear regression equation and correlation coefficient ($r$) in the analytical profile for glycyrrhizin, liquiritin and glycyrrhetinic acid in glycerin solution, emulsion and cream were listed in Table 1. A good linearity ($r > 0.994$) was achieved in the ranges of 5.0 - 50.0 μg/mL for glycyrrhizin, liquiritin and glycyrrhetinic acid in glycerin solution, 2.5 - 100.0 μg/mL and 5.0 - 50.0 μg/mL for the compounds in emulsion and cream. The lower limits of quantification (LLOQ) were 2.5 μg/mL for the three compounds, and the limits of detection (LOD), which represent the lowest detectable concentrations of analyte were 0.4, 0.3 and 0.1 μg/mL for liquiritin, glycyrrhizin and glycyrrhetinic acid, respectively.

Analytical accuracy, expressed as the percentage difference of the mean observed values compared with known concentration, varied from 2.5 to 100.0 μg/mL. The procedure was repeated three times daily for three consecutive days to evaluate the reproducibility for the analysis system. The results are listed in Table 2. The coefficients of variation

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(CV) of the intraday and interday ranged from 1.3% to 10.5% and 0.9% to 10.9%, which showed that the analysis result was acceptable when this quantitative methodology was applied to the assay of glycyrrhizin, liquiritin and glycyrrhetinic acid

**Table 1.** The regression equations, concentration ranges and regression coefficients of liquiritin, glycyrrhizin and glycyrrhetinic acid in licorice radix-containing cosmetic preparation

| Sample | Constituents | Regression equations | r | Linearity range (μg/mL) |
|---|---|---|---|---|
| Lotion | liquiritin | Y = 0.0852 X – 0.1734 | 0.999 | 5.0 - 50.0 |
| | glycyrrhizin | Y = 0.0628 X – 0.0299 | 0.999 | 5.0 - 50.0 |
| | glycyrrhetinic acid | Y = 0.1497 X – 0.0451 | 0.999 | 5.0 - 50.0 |
| Emulsion (o/w) | liquiritin | Y = 0.0486 X – 0.0048 | 0.998 | 5.0 - 100.0 |
| | glycyrrhizin | Y = 0.0426 X + 0.0046 | 0.998 | 2.5 - 100.0 |
| | glycyrrhetinic acid | Y = 0.1001 X + 0.0047 | 0.994 | 2.5 - 100.0 |
| Cream (w/o) | liquiritin | Y = 0.0538 X – 0.0074 | 0.999 | 2.5 - 100.0 |
| | glycyrrhizin | Y = 0.0376 X + 0.0134 | 0.999 | 5.0 - 100.0 |
| | glycyrrhetinic acid | Y = 0.1057 X – 0.0084 | 0.997 | 5.0 - 100.0 |

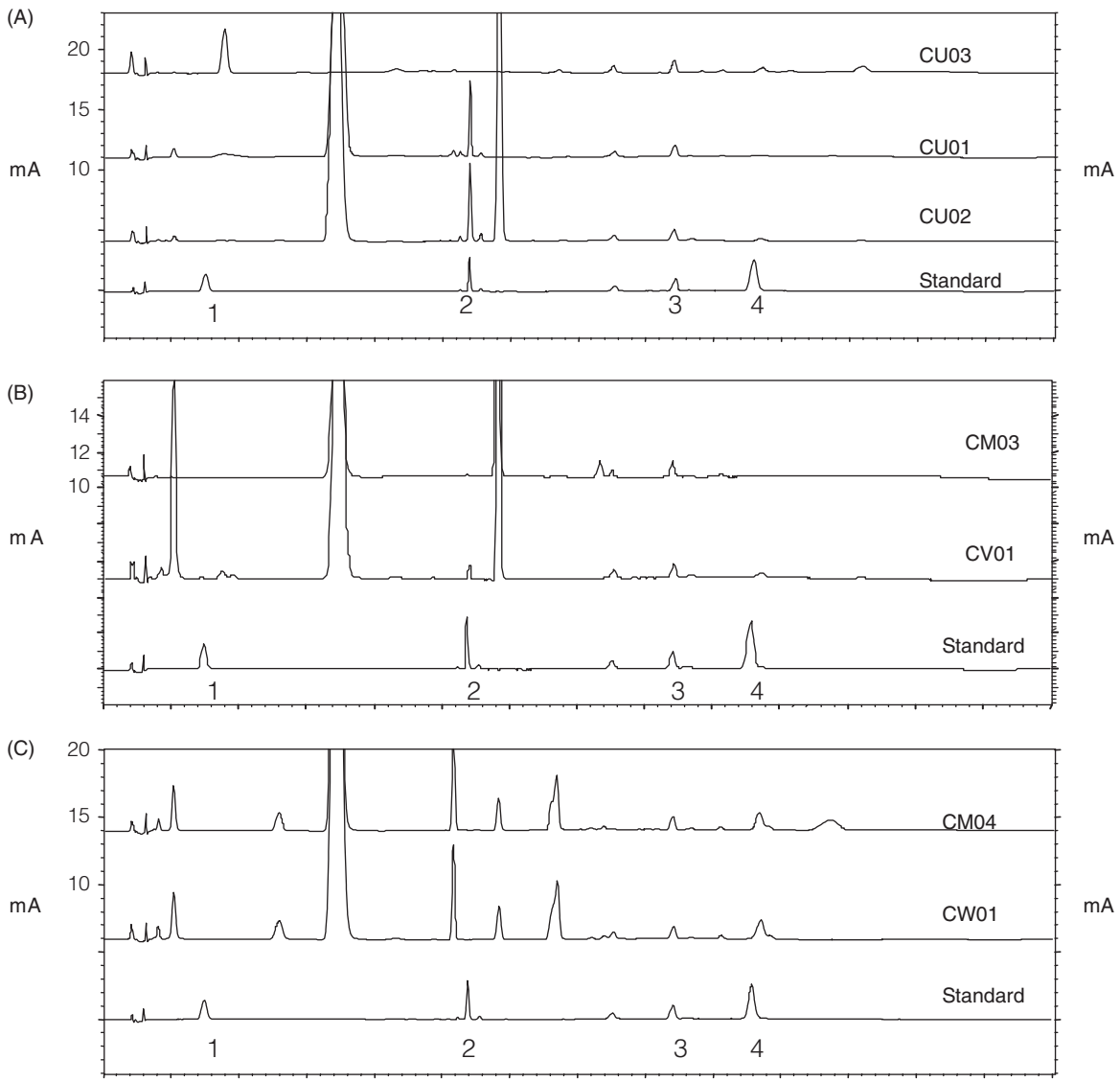


**Figure 1.** HPLC chromatograms of liquiritin, glycyrrhizin and glycyrrhetinic acid in licorice radix-containing emulsion (A: CU01-03, B: CM01 and CV01, and C: CM04 and CW01) and standards.
1. liquiritin, 2. glycyrrhizin, 3. 2-MA, 4. glycyrrhetinic acid (the final concentration of each standard was 50.0 μg/mL).

**Table 2.** Intraday and interday analytical precision and accuracy of liquiritin, glycyrrhizin and glycyrrhetinic acid in licorice radix-containing cosmetic preparation

| Sample | Compound | Conc (μg/mL) | Intraday | | Interday | |
|---|---|---|---|---|---|---|
| | | | Precision Mean ± SD (CV%) | Accuracy (%) | Precision Mean ± SD (CV%) | Accuracy (%) |
| Lotion | liquiritin | 50.0 | 49.9 ± 0.6 (1.3) | -0.2 | 51.8 ± 2.1 (4.1) | 3.6 |
| | | 25.0 | 25.5 ± 2.6 (10.3) | 1.8 | 20.2 ± 1.3 (6.4) | -19.4 |
| | | 12.5 | 11.7 ± 1.1 (9.1) | -6.7 | 13.6 ± 1.4 (9.9) | 8.7 |
| | | 10.0 | 10.1 ± 0.4 (3.6) | 1.4 | 11.4 ± 1.1 (8.6) | 13.7 |
| | | 5.0 | 5.3 ± 0.1 (1.9) | 6.3 | 5.6 ± 0.1 (2.0) | 11.3 |
| | glycyrrhizin | 50.0 | 50.6 ± 1.4 (2.7) | 1.2 | 49.7 ± 5.4 (10.9) | -0.5 |
| | | 25.0 | 23.8 ± 2.0 (8.4) | -4.6 | 25.8 ± 1.6 (6.0) | 3.2 |
| | | 12.5 | 12.0 ± 1.0 (8.7) | -4.3 | 12.6 ± 0.8 (6.3) | 0.6 |
| | | 10.0 | 10.2 ± 0.9 (8.4) | 2.1 | 9.0 ± 0.6 (6.4) | -9.7 |
| | | 5.0 | 5.9 ± 0.2 (3.9) | 18.2 | 5.35 ± 0.2 (3.4) | 6.9 |
| | glycyrrhetinic acid | 50.0 | 50.4 ± 1.5 (3.0) | 0.8 | 50.0 ± 1.1 (2.2) | 0.0 |
| | | 25.0 | 24.2 ± 2.5 (10.3) | -3.2 | 25.2 ± 1.7 (6.9) | 0.7 |
| | | 12.5 | 12.1 ± 1.0 (8.3) | -3.6 | 12.3 ± 0.2 (1.3) | -1.8 |
| | | 10.0 | 10.2 ± 0.7 (6.7) | 2.2 | 9.7 ± 0.3 (3.4) | -2.8 |
| | | 5.0 | 5.6 ± 0.3 (5.7) | 12.6 | 5.3 ± 0.3 (4.9) | 6.8 |
| Emulsion (o/w) | liquiritin | 100.0 | 109.0 ± 9.4 (8.6) | 9.0 | 110.3 ± 11.3 (10.3) | 10.3 |
| | | 80.0 | 82.1 ± 8.4 (10.2) | 2.6 | 79.1 ± 6.7 (8.5) | -1.2 |
| | | 50.0 | 48.9 ± 4.7 (9.6) | -2.3 | 45.0 ± 4.8 (10.8) | -9.9 |
| | | 25.0 | 24.0 ± 1.9 (7.9) | -4.0 | 24.6 ± 1.8 (7.5) | -1.8 |
| | | 20.0 | 18.5 ± 1.9 (10.5) | -7.7 | 20.5 ± 1.7 (8.1) | 2.3 |
| | | 5.0 | 5.1 ± 0.4 (8.1) | 2.3 | 5.0 ± 0.3(5.0) | 0.2 |
| | glycyrrhizin | 100.0 | 110.0 ± 7.0 (6.4) | 10.0 | 110.0 ± 6.6 (6.0) | 10.0 |
| | | 80.0 | 80.2 ± 7.1 (8.8) | 0.3 | 83.1 ± 4.0 (4.8) | 3.9 |
| | | 50.0 | 46.8 ± 3.3 (7.1) | -6.4 | 45.7 ± 1.4 (3.1) | -8.6 |
| | | 25.0 | 24.6 ± 2.1 (8.6) | -1.5 | 24.8 ± 0.5 (2.1) | -0.9 |
| | | 20.0 | 19.9 ± 2.1 (10.5) | -0.7 | 19.5 ± 0.4 (2.2) | -2.3 |
| | | 10.0 | 10.1 ± 0.8 (8.3) | 1.1 | 10.1 ± 0.2 (2.1) | 0.8 |
| | | 5.0 | 4.7 ± 0.4 (8.3) | -5.2 | 4.7 ± 0.2 (3.2) | -6.0 |
| | | 2.5 | 2.6 ± 0.1 (2.3) | 2.6 | 2.6 ± 0.2 (9.1) | 2.9 |
| | glycyrrhetinic acid | 100.0 | 112.9 ± 6.5 (5.8) | 12.9 | 110.7 ± 12.1 (10.9) | 10.7 |
| | | 80.0 | 85.2 ± 1.4 (1.7) | 6.4 | 85.0 ± 8.5 (10.0) | 6.3 |
| | | 50.0 | 46.9 ± 3.4 (7.3) | -6.3 | 45.3 ± 1.3 (2.9) | -9.3 |
| | | 20.0 | 18.4 ± 1.3 (7.0) | -8.3 | 18.0 ± 1.6 (8.8) | -9.8 |
| | | 10.0 | 10.1 ± 1.0 (9.8) | 0.7 | 11.4 ± 0.9 (8.2) | 14.4 |
| | | 5.0 | 4.4 ± 0.4 (9.3) | -12.4 | 4.1 ± 0.4 (9.2) | -17.6 |
| | | 2.5 | 2.7 ± 0.1 (2.0) | 6.8 | 2.7 ± 0.2 (7.1) | 8.6 |
| Cream (w/o) | liquiritin | 100.0 | 97.3 ± 5.9 (6.0) | -2.7 | 97.2 ± 3.3 (3.4) | -2.8 |
| | | 80.0 | 82.8 ± 5.6 (6.7) | 3.5 | 83.2 ± 3.2 (3.8) | 3.9 |
| | | 50.0 | 51.3 ± 2.3 (4.4) | 2.5 | 50.8 ± 2.0 (3.9) | 1.7 |
| | | 25.0 | 24.5 ± 1.7 (7.0) | -2.1 | 24.5 ± 0.6 (2.4) | -1.9 |
| | | 20.0 | 20.2 ± 1.3 (6.5) | 0.8 | 20.1 ± 0.5 (2.3) | 0.6 |
| | | 10.0 | 9.27 ± 0.67 (7.3) | -7.3 | 9.4 ± 0.2 (2.2) | -5.8 |
| | | 5.0 | 4.8 ± 0.2 (3.8) | -4.4 | 4.7 ± 0.1 (2.5) | -6.0 |
| | | 2.5 | 2.5 ± 0.1 (4.5) | -2.1 | 2.5 ± 0.0 (0.9) | 0.7 |

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**Table 2.** Continued

| Sample | Compound | Conc (μg/mL) | Intraday | | Interday | |
|---|---|---|---|---|---|---|
| | | | Precision Mean ± SD (CV%) | Accuracy (%) | Precision Mean ± SD (CV%) | Accuracy (%) |
| Cream (w/o) | glycyrrhizin | 100.0 | 97.4 ± 10.2 (10.4) | -2.6 | 97.1 ± 4.5 (4.6) | -2.9 |
| | | 80.0 | 82.7 ± 7.9 (9.6) | 3.4 | 83.3 ± 4.1 (4.9) | 4.1 |
| | | 50.0 | 51.4 ± 4.5 (8.7) | 2.7 | 50.8 ± 2.5 (5.0) | 1.6 |
| | | 25.0 | 24.3 ± 2.1 (8.6) | -3.0 | 24.0 ± 0.8 (3.4) | -4.1 |
| | | 20.0 | 19.9 ± 1.5 (7.7) | -0.4 | 21.3 ± 1.1 (5.3) | 6.6 |
| | | 10.0 | 9.3 ± 0.7 (7.4) | -7.4 | 8.9 ± 0.2 (2.7) | -11.0 |
| | | 5.0 | 5.1 ± 0.4 (7.9) | 1.4 | 4.6 ± 0.4 (8.4) | -7.8 |
| | glycyrrhetinic acid | 100.0 | 104.1 ± 6.3 (6.1) | 4.1 | 100.2 ± 9.6 (9.6) | 0.2 |
| | | 80.0 | 83.0 ± 7.1 (8.5) | 3.8 | 81.0 ± 1.6 (2.0) | 1.2 |
| | | 50.0 | 52.0 ± 4.9 (9.4) | 3.9 | 52.3 ± 3.4 (6.5) | 4.6 |
| | | 25.0 | 23.7 ± 1.3 (5.3) | -5.3 | 25.1 ± 2.5 (10.1) | 0.5 |
| | | 10.0 | 8.7 ± 0.3 (3.1) | -13.5 | 8.8 ± 0.6 (7.2) | -11.8 |
| | | 5.0 | 5.3 ± 0.2 (3.8) | 6.9 | 5.3 ± 0.2 (3.5) | 5.3 |

**Table 3.** The recoveries of liquiritin, glycyrrhizin and glycyrrhetinic acid in cosmetic preparation

| Dosage form | Conc. (μg/mL) | liquiritin | glycyrrhizin | glycyrrhetinic acid |
|---|---|---|---|---|
| Lotion | 25.0 | 100.2 ± 13.3 | 88.8 ± 10.0 | 93.3 ± 10.0 |
| | 12.5 | 98.9 ± 4.4 | 95.5 ± 4.8 | 90.5 ± 9.2 |
| | 5.0 | 107.4 ± 9.5 | 100.9 ± 8.7 | 104.8 ± 8.0 |
| Emulsion (o/w) | 80.0 | 99.4 ± 7.3 | 100.3 ± 8.8 | 106.5 ± 1.8 |
| | 20.0 | 92.3 ± 9.7 | 99.3 ± 10.4 | 91.7 ± 6.4 |
| | 5.0 | 99.8 ± 3.9 | 94.8 ± 7.8 | 104.1 ± 7.1 |
| Cream (w/o) | 80.0 | 99.8 ± 1.8 | 99.9 ± 4.7 | 100.6 ± 4.4 |
| | 20.0 | 100.8 ± 6.6 | 99.6 ± 7.7 | 97.6 ± 7.7 |
| | 5.0 | 95.6 ± 3.6 | 101.4 ± 8.0 | 105.5 ± 2.2 |

Data expressed by Mean ± SD (n = 3).

**Table 4.** The components in licorice-containing products (μg/mL)

| No | Dosage form | liquiritin | glycyrrhizin | glycyrrhetinic acid |
|---|---|---|---|---|
| CM03 | emulsion | ND | ND | ND |
| CM04 | emulsion | ND | ND | ND |
| CU01 | emulsion | ND | 113.4 | ND |
| CU02 | emulsion | ND | 98.3 | ND |
| CU03 | emulsion | ND | ND | ND |
| CV01 | emulsion | ND | 16.8 | ND |
| CW01 | emulsion | ND | ND | ND |

"ND" means not detected.

in cosmetics. The pretreatment method gave good recovery, and the average recoveries of the compounds were from 88.8% to 107.4% in glycerin solution at concentrations of 5.0, 12.5 and 25.0 μg/mL; from 91.7% to 106.4% in emulsion at concentrations of 5.0, 20.0 and 80.0 μg/mL; and from 95.6% to 105.5% in cream at concentrations of 5.0, 20.0 and 80.0 μg/mL (Table 3). For the herbal analysis, the recoveries indicated acceptable precision and accuracy.

III. *Quantification of Commercial Available Cosmetic Products*

The HPLC methodology was applied to measure the marker components in commercial cosmetic products. Seven licorice-containing emulsions were quantified. Table 4 gave the results, and the chromatograms were shown in Figure 1 - 3. The results indicated that licorice constituents were detectable in three emulsions, but only glycyrrhizin (16.8 - 113.4 μg/mL) was detected. These results indicated that the constituent contents of liquiritin and glycyrrhetinic acid in commercial cosmetics products were lower than LOD.

## DISCUSSION

Extraction of the bioactive components from cosmetics with various formulations is challenging for quantitative analysis. The formula and matrix is too complex to detect the components from cosmetics by single solvent or system. In previous studies, water, 70% ethanol, methanol and 5% Triton X-100 comprised the solvent, and heating or sonication was applied for crude drug extraction[14,17,18]. However, these methods were not efficient for the measurement of the components of cosmetics. In our previous study, commercial samples were dissolved in water, and sonication was used to extract α-hydroxyacids from cosmetics[19]. In another study, the $KH_2PO_4$ buffer solution was used for the preparation of a sample solution[20]. In this study, extraction with saturated NaCl solution as salting-out reagent was proved successful. This method was suitable for surfactant-rich products such as

the emulsion and cream samples in this study.

It is difficult and complex to investigate the chemical constituents from in herbs since the added amount and the extraction method of licorice-containing extracts, the constituent contents, and the origin of the Glycyrrhizae radix would affect the amount of target compounds in cosmetics. The results indicated that only 3 out of 7 licorice-containing emulsions could be detected by the above-mentioned method. For the exclusion of matrix effect, the standards of the marker components were spiked with blank glycerin solution, emulsion or cream, and all the marker components were detectable at the concentration of 5.5 - 50.0 μg/mL in lotion, 5.0 - 100.0 μg/mL in emulsion and 2.5 - 100.0 μg/mL in cream. The recoveries were 100 ± 10% and acceptable, thus, the matrix effect might be excluded. In Chinese Pharmacopoeia, *Glycyrrhiza glabra* and the other species of same genus are listed as licorice[21], in China Pharmacopoeia, three species of Glycyrrhizae radix (*Glycyrrhiza uralensis, G. glabra* and *G. inflate*) are listed as licorice[22], while in the Japanese Pharmacopoeia, two species are prescribed[23]. Different constituents might present in different species and sources of Glycyrrhizae radix. It has been reported that the variety of glycyrrhizin and liquritin contents in Glycyrrhizae radix ranged from 0.259 - 8.31% and 0.008 - 4.356%, respectively[24]. In addition, the glycyrrhetinic acid content in Glycyrrhizae radix was about 0.051%[25]. According to the formula of licorice-containing cosmetics, the percentage composition of licorice extract in formula generally was 0.05%, therefore, the individual amount of marker compound would be lower than the LODs of this method, especially liquiritin and glycyrrhetinic acid [26]. As glycyrrhizin is a major ingredient of various species of Glycyrrhizae radix and the most abundant of the three components detected in this study, it is a marker for the detection of Glycyrrhizae radix-containing products. It may explain why only glycyrrhizin was detected in the commercial samples. On the other hand, no official specification of licorice raw material for cosmetic is used to control its quality. The levels of these components may also be due to individual contents and their variety of raw materials. Furthermore, extensive dilution might be an issue owing to the yellowish-brown color of licorice extract would affect the appearance of finished cosmetic products.

Since the constituents of Chinese herbs are dependent on their species, sources, climate and process procedure, it is a challenge to impose quantitative control on products using these components, especially cosmetics. In addition, the quantitative assay of Chinese herb-containing cosmetics was deficient; the method described here is sufficiently sensitive and reliable to be used for the simultaneous determination of glycyrrhizin, glycyrrhetinic acid and liquiritin. It can be applied to evaluate the efficacy and to control the quality of licorice root-containing cosmetics. Due to the strong emphasis currently placed on consumer protection in the cosmetics market, it is important to establish a convenient and simple method for routine quality control of Chinese herb-containing cosmetics.

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