Research Article

Determining the Levels of Four Phenylethanoid Glycosides and Five Triterpene Acids in Liuwei Dihuang Capsule Using Solid Phase Extraction with HPLC-UV

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In this study, we used quantitative high-performance liquid chromatography equipped with an ultraviolet detector (HPLC-UV) and solid phase extraction (SPE) to determine the levels of four phenylethanoid glycosides and five triterpene acids in Liuwei Dihuang capsules (LDCs). LDCs were methanol-extracted and purified using a 500mg/6mL silica-based C18 SPE cartridge. Two elutions were analyzed on a ChromCore C18 column under two HPLC conditions. To improve the pretreatment clean-up, an array of silica- and polymer-based SPE cartridges were compared. Both wash and elution steps were also optimized to achieve the highest removal of impurities. Under optimal chromatographic conditions, good linearity was achieved for all compounds (correlation coefficient of $r \geq 0.999$), with a quantification limit ranging from 0.0076 to 0.418 μg/mL. The method had satisfactory efficiency and reproducibility with recovery rates ranging from 91.6 to 99.3% with a relative standard deviation below 1.5%. Taken together, this demonstrated SPE as a suitable extension of HPLC-UV for the determination of phenylethanoid glycosides and triterpene acids in complex LDCs.

1. Introduction

Liuwei Dihuang (LD) is one of the most established traditional Chinese medicines that have been used for over a thousand years to treat backache, alopecia, menoxenia, and waist/knee pain [1]. LD consists of six crude herbs including Radix rehmannia, Fructus corni, Rhizoma dioscoreae, Rhizoma alismatis, Cortex moutan, and Poria cocos at a ratio of 8:4:4:3:3:3. Studies have shown that LD has therapeutic benefits for hypertension, diabetes, tuberculosis, neurosis, nephritis, neurasthenia, dementia, and Parkinson’s disease [2–4].

Traditionally, LD is prepared as pills or in decoctions. Modern pharmaceutical technologies now produce LD capsules (LDCs) that permit oral administration and ease of transportation/storage. Over 100 pharmaceutical manufacturers currently produce LDCs in China. As we know, the quality of herbal medicines correlates with the levels of their chemical constituents. These can differ according to environmental conditions, resulting in variable clinical effects. According to the Chinese Pharmacopoeia 2015, the significant specification for LDC is to determine the minimum content of paeonol and ursolic acid using HPLC and HPTLC for quality control (QC) [5], only Cortex moutan and Fructus corni are assessed during LDC preparation, but these do not represent the quality, safety, and efficacy of the specific LDC product totally.

Recent studies have shown that the bioactive components of LDC include various phenylethanoid glycosides and triterpene acids which originate from herbal Radix rehmannia and herbal Poria cocos, respectively. Both constituents possess antibacterial, anti-inflammatory, and antioxidative properties and can inhibit tyrosinase activity [6–12]. As such, phenylethanoid glycosides and triterpene acids should be considered during the QC of LDC. Previous QC approaches focus solely on the bioactive markers of morofficianloside, monoterpenes, and phenolics, originated
from herbal Cortex moutan and herbal Fructus corni in LD preparations. These studies used HPLC coupled to diode array detectors for the simultaneous determination of eight constituents in LD pills [13], employed micellar electrokinetic chromatography to measure bioactive constituents in LD pills [14], used HPLC-MS to detect bioactive compounds in LD pills [15], and employed HPLC-UV for the determination of specific constituents in LD preparations [16]. These methods, however, do not reflect the quality of Radix rehmannia and Poria cocos in LDC. To date, the determination of phenylethanoid glycosides and triterpene acids in LDC have not been reported due to poor sensitivity of the HPLC-UV method. It is now necessary to develop appropriate HPLC-UV-based methods that allow a consistent quality of product from different manufacturers. Recently, solid phase extraction (SPE) has emerged as an effective pretreatment technique that can enrich and purify samples for complex composition analysis [17, 18]. Thus, the combination of SPE and HPLC-UV can not only improve sensitivity but also eliminate interference during the analysis of herbal formulas.

In this study, we have performed the first quantification of four phenylethanoid glycosides and five triterpene acids in LDC samples through established SPE techniques. The methods were applied to 18 batches of LDC samples obtained from six independent manufacturers subsequently.

2. Experimental Procedures

2.1. Materials and Reagents. Standards of purpureaside C, jionoside A1, acteoside, isoacteoside, 3-O-acetyl-16α-hydroxytrametenolic acid, dehydropachymic acid, pachymic acid, trametenolic acid, and dehydrotrametenolic acid were obtained from the Shanghai R&D Center for the Standardization of Chinese Medicines. The purity of the chemicals was 99% or higher. The structures of four phenylethanoid glycosides and five triterpene acids are shown in Figure 1. SelectCore C18 (500 mg/6 mL), SelectCore HLB (200 mg/6 mL), Welchrom Alumina-N (500 mg/6 mL), and Welchrom PSA (500 mg/6 mL) were independently purchased from NanoChrom Technologies (Suzhou, China) and Welch Materials (Shanghai, China). They were conditioned through washing with 5 mL of methanol and 5 mL of ultrapure water prior to use. HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Whitby, Canada). Phosphoric acid and other analytical reagents were purchased from Aladdin (Shanghai, China). Ultrapure water was generated from the Milli-Q system (Millipore, MA, USA).

2.2. Preparation of Standard Solutions. Standard solutions (I) were prepared in methanol with the following concentrations: 15 μg/mL purpureaside C; 15 μg/mL jionoside A1; 20 μg/mL acteoside; and 12 μg/mL isoacteoside. Standard solutions (II) were prepared in methanol with the following concentrations: 5 μg/mL 3-O-acetyl 16α-hydroxytrametenolic acid; 20 μg/mL dehydropachymic acid; 80 μg/mL pachymic acid; 20 μg/mL trametenolic acid; and 20 μg/mL dehydrotrametenolic acid.

2.3. Sample Preparation. Eighteen batches of LDC (marked as samples LDC01–18) were collected from six Chinese medicinal manufacturers: T Pharmaceutical Co., Ltd. (samples LDC 01–03), X Pharmaceutical Co., Ltd. (samples LDC 04–06), Y Pharmaceutical Co., Ltd. (samples LDC 07–09), J Pharmaceutical Co., Ltd. (samples LDC10–12), R Pharmaceutical Co., Ltd. (samples LDC13–15), and P Pharmaceutical Co., Ltd. (samples LDC16–18). Negative samples (LDC lacking Radix rehmannia and LDC lacking Poria cocos) were produced in our laboratory.

2.4. Preparation of Sample Solutions. Fine LDC powder (1.5 g) was macerated in 50 mL of MeOH and extracted by ultrasonication for 30 min. After adjustment to its initial weight in MeOH, the mixture was centrifuged for 5 min at 6000 rpm. A total of 10 mL was then evaporated at 50°C, and the residue was dissolved in 10 mL of 10% MeOH. The mixture was loaded onto the SPE cartridge (SelectCore C18 500 mg/6 mL) at a controlled flow rate of 3 seconds per drop. The cartridge was washed with 20 mL of 10% MeOH, and the first components were eluted in 20 mL of 40% MeOH. Eluted samples were evaporated at 50°C and the residue was dissolved in 1 mL of MeOH (LDC solution A for the determination of the four phenylethanoids). The cartridge was then washed in 20 mL of 60% MeOH and the second components were eluted in 20 mL of 100% MeOH. The eluted solution was evaporated at 50°C and the residue was dissolved in 1 mL of MeOH (LDC solution B for the determination of the five triterpene acids).

The fine powder of the negative sample (without Radix rehmannia) (1.1 g) was macerated in 50 mL of MeOH and extracted with ultrasonification for 30 min. After adjustment to the initial weight in MeOH, samples were centrifuged for 5 min at 6000 rpm, 10 mL of the sample was evaporated at 50°C, and the residue was dissolved in 10 mL of 10% MeOH. The mixture was loaded onto the SPE cartridge (SelectCore C18 500 mg/6 mL). After washing the cartridge with 20 mL of 10% MeOH and 20 mL of 40% MeOH, the eluted solutions were collected and evaporated at 50°C. The residues were dissolved in 1 mL of MeOH (negative sample solution A).

The fine powder of the negative sample (without Poria cocos) (1.3 g) was prepared as above and loaded onto the SPE cartridge (SelectCore C18 500 mg/6 mL). The cartridge was washed in 20 mL of 10% MeOH, 20 mL of 40% MeOH, and 20 mL of 60% MeOH and eluted in 20 mL of 100% MeOH. The solution was evaporated at 50°C, and the residue was dissolved in 1 mL of MeOH (negative sample solution B).

The dried powder of Radix Rehmanniae and Poria cocos samples was pulverized and sifted through a 0.45 mm sieve. Approximately 1 g of dried powder was extracted in 50 mL of MeOH and sonicated for 30 min at 30°C. Supernatants were then combined and evaporated at 50°C. The residue was dissolved in 10 mL of methanol and used as the herbal Radix Rehmanniae and herbal Poria cocos test solutions.

2.5. Apparatus and Analytical Methods. HPLC analysis was performed on an Agilent 1260 liquid chromatography system that included a quaternary pump, thermostatic oven,
thermostatic autosampler, and a UV detector. Separation was performed on ChromCore™ C18 columns (250 mm × 4.6 mm, 5 μm). For the analysis of phenylethanoid glycosides, gradient program A was employed at a flow rate of 1 mL/min by combining solvent A (0.01% v/v phosphoric acid) and solvent B (acetonitrile) as follows: 0–40 min, 12–24% B. A pre-equilibration period of 10 min was established between individual runs. The detection wavelength was 334 nm and the column temperature was maintained at 30°C. The injection volume was 10 μL.

For the analysis of triterpene acid composition, gradient program B was employed at a flow rate of 1 mL/min by combining solvent A (0.01% v/v phosphoric acid) and solvent B (acetonitrile) as follows: 0–25 min, 70–95% B, 25–35 min, 95% B. A pre-equilibration period of 10 min was established between individual runs. The detection wavelength was 210 nm and the column temperature was maintained at 30°C. The injection volume was 10 μL.

3. Results and Discussion

3.1. Optimization of the Chromatographic Conditions. For gradient elutions, acetonitrile and methanol were tested as organic modifiers. Acetonitrile was selected as it produces a higher signal response and lower background noise compared to MeOH. A small volume of acid was added to the mobile phase to improve the peak shape and restrain peak tailing in the LDC extracts. Aqueous phosphoric acid solutions (0%, 0.1%, and 0.2%) were compared. The nine compounds could be separated at baseline in 0.1% phosphoric acid. To obtain optimal resolution of the C18 column and to shorten the analysis time, different gradient eluents of the mobile phase consisting of phosphoric acid and acetonitrile were studied. We tried to develop a gradient HPLC elution method to analyze nine compounds by switching the wavelength. However, due to the large differences in polarity of these nine target components, it must take more than 60 min to achieve
baseline separation and accompanied with sharp fluctuations in the baseline because of wavelength switching. Thus, two gradient elution HPLC methods must be developed. In this study, gradient program A was adjusted to ensure that the four phenylethanoid glycosides from Radix rehmannia could achieve baseline separation in the LDC sample without negative Radix rehmannia interference (Figure 2). Gradient program B was adjusted to ensure that the five triterpene acids originating from herbal Poria cocos could attain baseline separation in the LDC sample without Poria cocos sample interference (Figure 3).

3.2. Comparison of Different Solid Phases of Phenylethanoid Glycosides. Due to the complexity of LDC, sample pretreatment extraction methods with high levels of clean-up, enrichment, and recovery are necessary. To evaluate the effects of different SPE cartridges on the enrichment of phenylethanoid glycosides during LDC pretreatment, four SPE cartridges, namely, silica-based C18 (SelectCore C18 500 mg/6 mL), silica-based primary secondary amine (Welchrom PSA 500 mg/6 mL), polymer-based hydrophilic lipophilic balance (SelectCore HLB 200 mg/6 mL), and neutral alumina (Welchrom Alumina-N 500 mg/6 mL) were studied. The comparative chromatograms and recovery of the target compounds are indicated in Figure 4 and Table 1. C18 and HLB SPE cartridges produced high levels of phenylethanoid glycoside recovery, which exceeded 90%. However, LDC following HLB pretreatment led to high levels of impurity and interference compared to C18. These results indicate that C18 cartridges can be used for the quantitative analyses of phenylethanoid glycosides in LDC.

3.3. Optimization of the SPE Pretreatment Method for Phenylethanoid Glycosides. Due to the hydrophilic nature of phenylethanoid glycosides, the effects of 10%, 20%, 40%, and 50% MeOH on the enrichment efficiency of the four phenylethanoid glycosides were investigated. As shown in Figure 5, 20% MeOH led to loss of purpureaside C, while 40% MeOH produced high levels of recovery of the four phenylethanoid glycosides. No further increase was observed in 50% MeOH. From these results, 10% and 40% MeOH were selected for washing and elution steps, respectively.

3.4. Comparison of the Different Solid Phases of Triterpene Acids. To evaluate the effects of the different SPE cartridges on the enrichment of triterpene acids during LDC pretreatment, silica-based C18 (SelectCore C18 500 mg/6 mL), polymer-based hydrophilic lipophilic balance (SelectCore HLB 200 mg/6 mL), polymer-based strong anion exchange (Welchrom P-SAX 200 mg/6 mL), and neutral alumina (Welchrom Alumina-N 500 mg/6 mL) were assessed. The chromatograms and target compound recovery are shown in Figure 6 and Table 1. The C18 and HLB SPE cartridges produced over 90% recovery of the five triterpene acids. Considering the convenience and low costs of the SPE process, C18 cartridges were chosen for the quantitative analyses of triterpene acids in LDC.

3.5. Optimization of the SPE Methods for Triterpene Acids. Considering the hydrophobicity of triterpene acids, the effect of 40%, 60%, 80%, and 100% MeOH on the enrichment efficiency of the five triterpene acids was investigated. As shown in Figure 7, 80% MeOH led to loss of 3-O-acetyl-16α-hydroxytrametenolic acid and dehydropachymic acid, while 100% MeOH achieved high levels of recovery for all five triterpene acids. Thus, 60% and 100% MeOH were used for washing and elution steps, respectively.

3.6. Validation of the Phenylethanoid Glycosides and Triterpene Acids. For the analysis of triterpene acid and triterpene acid composition, the evaluated validation parameters including linearity, limits of detection (LODs), precision (repeatability), and recovery were examined (Table 2). The chromatographic method showed a favorable linearity in the concentration ranges assessed. The regression equation of purpureaside C was $Y = 9.8161X + 0.8779$ with a linear range of 2.24–56.00 μg/mL, and the regression equation of jionoside A1 was $Y = 8.9245X + 2.5501$ with a linear range of 2.21–55.25 μg/mL. The regression equation of aceteoside was $Y = 32.557X + 2.4327$ with a linear range of 2.31–57.75 μg/mL, and the regression equation of isacetoside was $Y = 13.012X − 4.4385$ with a linear range of 1.16–29.00 μg/mL. The regression equation of 3-O-acetyl-16α-hydroxytrametenolic acid was $Y = 6.6677X − 0.5066$ with a linear range of 1.08–43.2 μg/mL. The regression equation of dehydropachymic acid was $Y = 6.2417X + 0.0102$ with a linear range of 1.32–52.8 μg/mL. The regression equation of pachymic acid was $Y = 6.4675X − 1.3874$ with a linear range of 4.41–165.6 μg/mL. The regression equation of trametenolic acid was $Y = 6.4439X − 0.4147$ with a linear range of 3.78–151.2 μg/mL. The regression equation of dehydroramtenolic acid was $Y = 6.0755X + 1.626$ with a linear range of 2.7–108 μg/mL.

The reproducibility of the experimental procedure was evaluated by carried out seven replicate samples during the
same day. The RSDs of the experimental replicates were in the range of 0.35%–1.08%. The sensitivity of the method was verified by calculating 3 signal-to-noise ratios as LOD values and by calculating 10 signal-to-noise ratios as LOQ values.

3.7. Analysis of LDC Samples. The method reported here was utilized for the simultaneous determination of the four phenylethanoid glycosides and five triterpene acids in LDC samples purchased from the market with different

Table 1: Recoveries of the four phenylethanoid glycosides and five triterpene acids from spiked samples using different SPE cartridges.

| Compound                              | Recovery ± SDa (%) | C18  | Alumina-N | HLB  | PSA  | P-SAX |
|---------------------------------------|--------------------|------|-----------|------|------|-------|
| Purpureaside C                        | 91.7 ± 0.5         | 19.5 ± 2.8 | 96.7 ± 2.2 | 64.0 ± 2.7 |
| Jionoside A1                          | 92.2 ± 0.6         | 24.8 ± 4.1 | 97.3 ± 1.7 | 65.2 ± 2.3 |
| Acteoside                             | 95.5 ± 1.2         | 12.4 ± 3.1 | 90.4 ± 0.4 | 88.6 ± 0.4 |
| Isoacteoside                          | 90.3 ± 0.8         | 22.1 ± 3.3 | 93.6 ± 1.1 | 81.7 ± 1.8 |
| 3-O-Acetyl 16α-hydroxytrametenolic acid | 91.8 ± 0.2         | 15.6 ± 3.2 | 92.2 ± 2.5 | 62.0 ± 2.3 |
| Dehydropachymic acid                  | 96.6 ± 0.3         | 64.5 ± 2.4 | 96.8 ± 0.7 | NDb     |
| Pachymic acid                         | 97.8 ± 1.5         | 71.3 ± 2.1 | 90.6 ± 1.7 | 70.3 ± 0.9 |
| Trametenolic acid                     | 95.2 ± 0.6         | 56.1 ± 2.1 | 93.5 ± 2.1 | 88.6 ± 0.8 |
| Dehydrotrametenolic acid              | 94.5 ± 1.2         | 66.3 ± 2.2 | 90.2 ± 1.4 | 85.7 ± 1.5 |

aStandard deviation, n = 3. bBelow the detection limit.
manufacturers \( n = 18 \). As shown in Table 3, the average content of phenylethanoid glycosides and triterpene acids was 53.0 μg/0.3 g and 115.9 μg/0.3 g per capsule, respectively. The content of total triterpenoid acid is higher than that of total phenylethanolic glycoside in LDC; one of the reasons may be related to the manufacturing process of LDC that herbal *Poria cocos* is used as excipients after being crushed into powder, while other herbs are extracted by water.

**Figure 5:** Comparison of HPLC chromatograms obtained using different compositions of methanol for analysis four phenylethanoid glycosides in LDC. (1) Purpureaside C, (2) jionoside A1, (3) acteoside, and (4) isoacteoside.

**Figure 6:** Comparison of HPLC chromatograms obtained using different SPE methods for analysis five triterpene acids in LDC. (1) 3-O-Acetyl 16α-hydroxytrametenolic acid, (2) dehydropachymic acid, (3) pachymic acid, (4) trametenolic acid, and (5) dehydrotrametenolic acid.

**Figure 7:** Comparison of HPLC chromatograms obtained using different composition of methanol for analysis five triterpene acids in LDC. (1) 3-O-Acetyl 16α-hydroxytrametenolic acid, (2) dehydropachymic acid, (3) pachymic acid, (4) trametenolic acid, (5) dehydrotrametenolic acid.
Table 2: Method validation parameters of the four phenylethanoid glycosides and five triterpenes.

| Compound                        | Intraday precision (RSD%), \( n = 6 \) | Interday precision (RSD%), \( n = 3 \) | Regression equation \( Y = aX + b \) | Correlation coefficient (r) | Recovery ± SD\(^b\) (%) | LOD (μg/mL) | LOQ (μg/mL) |
|---------------------------------|----------------------------------------|--------------------------------------|-----------------------------------|-----------------------------|---------------------------|--------------|-------------|
| Purpureaside C                  | 0.72                                   | 0.98                                 | \( Y = 9.8164X + 0.8779 \)        | 0.9999                      | 93.7 ± 0.5                | 0.0044       | 0.0125      |
| Jionoside A1                    | 0.65                                   | 0.87                                 | \( Y = 8.9245X + 2.5501 \)        | 0.9998                      | 94.2 ± 0.6                | 0.0031       | 0.0124      |
| Acteoside                       | 0.58                                   | 0.64                                 | \( Y = 15.767X - 9.6178 \)        | 0.9995                      | 95.5 ± 1.2                | 0.0027       | 0.0081      |
| Isoacteoside                    | 0.82                                   | 1.02                                 | \( Y = 13.012X - 4.3485 \)        | 0.9996                      | 93.3 ± 0.8                | 0.0028       | 0.0076      |
| 3-O-Acetyl-16α-hydroxytrametenolic acid | 0.35                                   | 0.68                                 | \( Y = 6.6677X - 0.5066 \)        | 0.9998                      | 91.8 ± 0.2                | 0.052        | 0.186       |
| Dehydrodropachymic acid         | 0.62                                   | 1.05                                 | \( Y = 6.2417X + 0.0102 \)        | 0.9999                      | 96.6 ± 0.3                | 0.073        | 0.205       |
| Pachymic acid                   | 0.84                                   | 1.08                                 | \( Y = 6.4675X - 1.3874 \)        | 0.9998                      | 97.8 ± 1.5                | 0.136        | 0.418       |
| Trametenolic acid               | 0.46                                   | 0.85                                 | \( Y = 6.4439X - 0.4147 \)        | 0.9996                      | 95.2 ± 0.6                | 0.087        | 0.252       |
| Dehydrotrametenolic acid        | 0.55                                   | 0.92                                 | \( Y = 6.0755X + 1.626 \)         | 0.9997                      | 94.5 ± 1.2                | 0.064        | 0.198       |

\(^a\) Y is the peak area while \( X \) is the concentration (μg/mL). \(^b\) Standard deviation, \( n = 3 \).
| Sample | Purpureaside C | Jionoside A1 | Acteoside | Isoacteoside | Total phenylethanoid glycosides | 3-O-Acetyl-16α-hydroxytrametenolic acid | Dehydropachymic acid | Pachymic acid | Trametenolic acid | Dehydrotrametenolic acid | Total triterpene acids |
|--------|---------------|--------------|----------|--------------|---------------------------------|------------------------------------------|---------------------|--------------|----------------|-------------------------|------------------------|
| LDC01  | 10.8          | 14.3         | 18.2     | 8.1          | 51.4                            | 3.0                                      | 13.0                | 82.6         | 13.2          | 4.0                     | 115.8                  |
| LDC02  | 10.2          | 16.4         | 20.1     | 8.9          | 55.6                            | 3.0                                      | 12.7                | 74.8         | 11.3          | 3.8                     | 105.6                  |
| LDC03  | 12.0          | 15.3         | 19.5     | 8.6          | 55.4                            | 3.2                                      | 12.2                | 57.2         | 14.4          | 3.6                     | 90.6                   |
| LDC04  | 8.3           | 10.5         | 17.8     | 7.4          | 44.0                            | 4.5                                      | 20.1                | 72.2         | 29            | 23.2                    | 149                    |
| LDC05  | 8.8           | 11.4         | 18.3     | 7.5          | 46.0                            | 4.1                                      | 20.7                | 65.0         | 22.3          | 20.9                    | 133                    |
| LDC06  | 9.7           | 11.9         | 18.8     | 8.2          | 48.6                            | 3.4                                      | 20.2                | 63.6         | 21.8          | 20.1                    | 129.9                  |
| LDC07  | 13.2          | 15.6         | 23.4     | 10.1         | 62.3                            | 1.8                                      | 27.7                | 75.8         | 16.4          | 11.3                    | 133.0                  |
| LDC08  | 12.6          | 14.7         | 22.8     | 9.8          | 59.9                            | 1.8                                      | 27.6                | 74.6         | 15.6          | 10.8                    | 130.5                  |
| LDC09  | 14.1          | 16.9         | 24.5     | 9.7          | 65.2                            | 1.9                                      | 27.7                | 74.7         | 16.9          | 11.8                    | 132.9                  |
| LDC10  | 13.6          | 13.7         | 25.1     | 9.2          | 61.6                            | 1.6                                      | 34.0                | 58.5         | 29.9          | 13.9                    | 137.9                  |
| LDC11  | 12.8          | 13.1         | 24.8     | 9.4          | 60.1                            | 1.6                                      | 33.4                | 58.1         | 28.7          | 13.7                    | 135.4                  |
| LDC12  | 11.3          | 14.1         | 25.9     | 8.7          | 60.0                            | 1.6                                      | 33.6                | 57.0         | 30.6          | 13.7                    | 136.5                  |
| LDC13  | 10.7          | 13.7         | 20.5     | 8.5          | 53.4                            | 1.4                                      | 11.2                | 44.6         | 6.1           | 5.5                     | 68.7                   |
| LDC14  | 11.5          | 12.4         | 23.1     | 8.9          | 55.9                            | 1.3                                      | 11.7                | 46.5         | 6.5           | 5.4                     | 71.5                   |
| LDC15  | 9.7           | 10.1         | 19.3     | 7.8          | 46.9                            | 1.4                                      | 10.8                | 45.1         | 6.0           | 5.9                     | 69.3                   |
| LDC16  | 8.4           | 9.2          | 16.5     | 6.9          | 41.0                            | 3.4                                      | 19.0                | 53.5         | 23.0          | 17.7                    | 116.5                  |
| LDC17  | 7.9           | 8.6          | 16.9     | 6.2          | 39.6                            | 3.0                                      | 18.6                | 50.1         | 22.1          | 19.8                    | 113.6                  |
| LDC18  | 9.8           | 9.3          | 18.8     | 9.1          | 47.0                            | 3.5                                      | 18.5                | 54.5         | 23.0          | 17.3                    | 116.8                  |
mixed with herbal *Poria cocos* powder (excipients) evenly to make capsules [5].

4. Conclusions

In this study, two HPLC-UV methods were established to determine the content of four phenylethanoid glycosides and five triterpene acids in LDC based on the novel use of SPE. The results showed that the LOD, LOQ, accuracy, and precision of the two methods met the requirements for quantitative analysis. A total of 18 batches of LDCs from six manufacturers were analyzed, demonstrating the utility of our methods for the routine analysis of formula quality and safety.

**Abbreviations**

HPLC: High-performance liquid chromatography  
UV: equipped with ultraviolet detector  
SPE: Solid phase extraction  
LD: Liuwei Dihuang preparation  
LDC: Liuwei Dihuang capsule

**Data Availability**

The data used to support this study was obtained from the Shanghai University of Traditional Chinese Medicine, Shanghai, China, and are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Yong Zhang and Xian-Liang Zou authors contributed equally to this work.

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