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# Establishment and Validation of Quantitative Analysis of Multi-Components by Single-Marker for Quality Assessment of Compound Danshen Preparations

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**Summary.** Compound danshen preparations (CDPs) are used clinically for the treatment of cardiovascular and cerebrovascular diseases. By using the quantitative analysis of multi-components by single-marker (QAMS) method, sixteen compounds (danshensu, protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, notoginsenoside R1, salvianolic acid B, ginsenoside Rg1, ginsenoside Re, salvianolic acid A, salvianolic acid C, ginsenoside Rb1, ginsenoside Rd, cryptotanshinone, and tanshinone IIA were quantified on an ACQUITY ultraperformance liquid chromatography (UPLC) HSS T3 column (2.1 × 100 mm, 1.8 μm) with the mobile phase consisting of 0.1% formic acid aqueous solution (A) and acetonitrile (B) using a gradient elution at the flow rate of 0.30 mL/min in 30 min at 30°C, which was also validated by UPLC-diode array detection (DAD) and UPLC-electrospray ionization multistage/mass spectrometry (ESI-MS/MS) for assuring the feasibility and accuracy. Tested by robustness experiment under slightly changeable conditions, the stability of relative correction factor (RCF) proved to be stable, with RSDs below 5.69%, except for notoginsenoside R1 with relative standard deviation (RSD) 7.83%. This reliable and convenient QAMS method resolved the problem of standard substance insufficiency and improved the quality assessment of preparations consisting of complex compounds with different chemical structures, such as CDPs.

**Key Words:** compound danshen preparation, QAMS, robustness, quantification

## Introduction

Compound danshen preparations (CDPs) are used clinically for the treatment of coronary heart disease, cardiac angina and atherosclerosis [1–3] and have gained excellent reputation among patients with cardiovascular and cerebrovascular diseases. In recent years, some extra clinical uses of CDP have been found, including the treatment of avascular necrosis of femoral head, arterial intima-media thickness, and thalidomide-related venous

thromboembolism [4–6]. Several preparations have been introduced by the Pharmacopoeia of the People's Republic of China, such as Compound Danshen Dripping Pill (CDDP), Compound Danshen Tablet (CDT), Danqi Tablet, Xinkeshu Tablet, Guanxin Danshen Tablet, etc. Among them, CDDP and CDT are the most commonly used preparations. These two CDPs also contain, besides *Radix Salvia miltiorrhizae* (Danshen) and *Radix Notoginseng* (Sanqi), a third ingredient *Borneolum Syntheticum* (Borneol). CDDP is prepared from the water extract of Danshen and Sanqi together with polyglycol and borneol, while CDT is prepared from both ethanolic and water extract of Danshen mixed with the powder of Sanqi and Borneol [7]. This explains the insufficiency of the lipid-soluble compounds in CDDP.

Currently, many analytical methods have been established for the quality assessment of CDP, such as high-performance liquid chromatography (HPLC) [8–10], high-performance capillary electrophoresis (HPCE) [11], high-performance liquid chromatography coupled with evaporative light scattering detector (HPLC-ELSD) [8]. With the development of analytical methods, LC-MS [12–15] and LC-TOF-MS [16–18] have become popular owing to their high sensitivity and accuracy. The simultaneous determination of multi-components shows suitability for the quality control of CDPs; however, some of the standard substances required are expensive and not readily available in the desired quantities. Quantitative analysis of multi-components by single-marker (QAMS) effectively addresses this problem as it requires only a readily available compound to serve as internal reference standard for each class of related compounds. Some research papers have validated the feasibility of QAMS in quality control of TCM [19–26], proving that this method is easy, convenient, and reliable. So far, QAMS has been applied in the quality assessment of Danshen and Sanqi raw herbs and some preparations composed of the two herbs. In Danshen, five phenolic acids [20] and four diterpenoids [21, 25] have been determined, and in Sanqi, three saponins [22]. In Danshen Injection, seven phenolic acids [23] have also been determined, and in CDT, four diterpenoids [24]. However, previous research determined only one group of chemically similar compounds per analytical procedure.

In this study, a comprehensive QAMS method was established for the quality assessment of CDPs. Danshensu, ginsenoside Rg1, and tanshinone IIA were selected as internal reference substances for the quantitative analysis of the other 13 compounds in CDDP and CDT. The quantitative results acquired by QAMS method were validated by ultraperformance liquid chromatography–diode array detection (UPLC–DAD) and UPLC-electrospray ionization multistage/mass spectrometry (ESI-MS/MS) methods. The robustness test also revealed that the relative correction factors (RCFs) were nearly stable in variable environments. The validated method was

further successfully applied to various CDPs produced by different manufacturers, which proved that QAMS method was satisfactory for the quality assessment of CDPs.

# Experimental

## Chemicals and Materials

Formic acid of HPLC grade was bought from Meridian Medical Technologies (MREDA, USA). Acetonitrile and methanol of HPLC grade were bought from Fisher Scientific (USA). Water was purified by a Milli-Q water purification system (Millipore, USA).

Reference standards including danshensu, protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid A, salvianolic acid C, ginsenoside Rg1, ginsenoside Rd, ginsenoside Re, ginsenoside Rb1 and notoginsenoside R1, licochalcone A as internal standard were purchased from Tianjin Zhongxin Pharmaceutical Group Co. Ltd (Tianjin, China). Cryptotanshinone and tanshinone IIA were obtained from DELTA pharmaceutical standards manufacturer (Anhui, China). The purities of all the standard substances were higher than 98% except for cryptotanshinone whose purity was about 94%. The nine batches of CDDP were produced by Tasly Group (Tianjin, China), while six batches of CDT were bought from Guangdong Dint Pharmaceutical Group Co. Ltd (Guangdong, China) and Beijing Tongrentang Pharmaceutical Group Co. Ltd (Beijing, China).

## Preparation of Standard Solutions

Stock solutions were prepared by dissolving each reference standard, which was weighted accurately, in methanol to the final concentrations of 2.054 mg/mL for danshensu, 1.042 mg/mL for protocatechuic acid, 1.098 mg/mL for protocatechuicaldehyde, 1.036 mg/mL for caffeic acid, 1.236 mg/mL for rosmarinic acid, 1.278 mg/mL for lithospermic acid, 0.976 mg/mL for salvianolic acid B, 2.142 mg/mL for salvianolic acid A, 1.214 mg/mL for salvianolic acid C, 1.192 mg/mL for notoginsenoside R1, 2.140 mg/mL for ginsenoside Rg1, 1.144 mg/mL for ginsenoside Re, 1.898 mg/mL for ginsenoside Rb1, 1.542 mg/mL for ginsenoside Rd, 1.124 mg/mL for cryptotanshinone, 1.050 mg/mL for tanshinone IIA and 1.022 mg/mL for licochalcone A. The concentration of licochalcone A was diluted with methanol to 10.220 μg/mL. A mixed solution composed of the sixteen standard compounds was diluted with 70% methanol to obtain a series of con-

centrations in order to construct the calibration curves. The solutions were kept in 4 °C refrigerator when not in use.

## Preparation of Sample Solutions

CDDP and CDT were ground into powder with the purpose of obtaining the homogeneous samples. Each sample (0.110 g) was sonicated for 30 min with 10 mL 70% methanol, cooled at room temperature, and then centrifuged at 14000 rpm for 10 min to get the supernatant solution. Since the contents of some compounds in CDT are apparently higher than those in CDDP, the supernatant solution of CDT was diluted to one-tenth of the original concentration before analysis. Each analytical sample was composed of 450 μL supernatant and 50 μL internal standard (IS) solution. The concentration of IS solution reached 1.022 μg/mL in sample solution. After vortex-mixing for 1 min, 2 μL of the resultant solution was injected into UPLC-DAD-MS/MS for analysis.

## UPLC-DAD Analysis

The experiment was performed on Waters ACQUITY UPLC-DAD system, equipped with binary solvent manager, sample manager, thermostatically controlled column compartment, diode array detector (DAD), using the software MassLynx V4.1 version for the analysis. The separation was carried on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) at 30°C. Mobile phase was composed of 0.1% (*v/v*) formic acid–water (A) and acetonitrile (B) using a gradient elution of 5–16% B at 0–4 min, 16–24% B at 4–15 min, 24% B at 15–16 min, 24–48% B at 16–20 min, 48–55% B at 20–22 min, 55%–60% B at 22–27 min, 60%–100% B at 27–29 min, 100% B at 29–30 min. The flow rate was 0.30 mL/min, and the injection volume was 2 μL. The detection wavelength was switched as follows: 280 nm at 0.00–3.00 min, 258 nm at 3.01–3.50 min, 280 nm at 3.51–30.00 min. Another wavelength channel was set at 203 nm to detect the saponins. The optimized UPLC-DAD chromatograms of the standards and the CDDP sample solution are shown in *Fig. 1*.

## UPLC-ESI-MS/MS Analysis

For the MS analysis, Waters ACQUITY UPLC system tandem Waters Quattro Premier XE triple-quadrupole mass spectrometer was used for the quantitative analysis. The temperatures of source and desolvation were 120°C and 300°C, respectively. The capillary voltage was set at 3.2 kV in positive

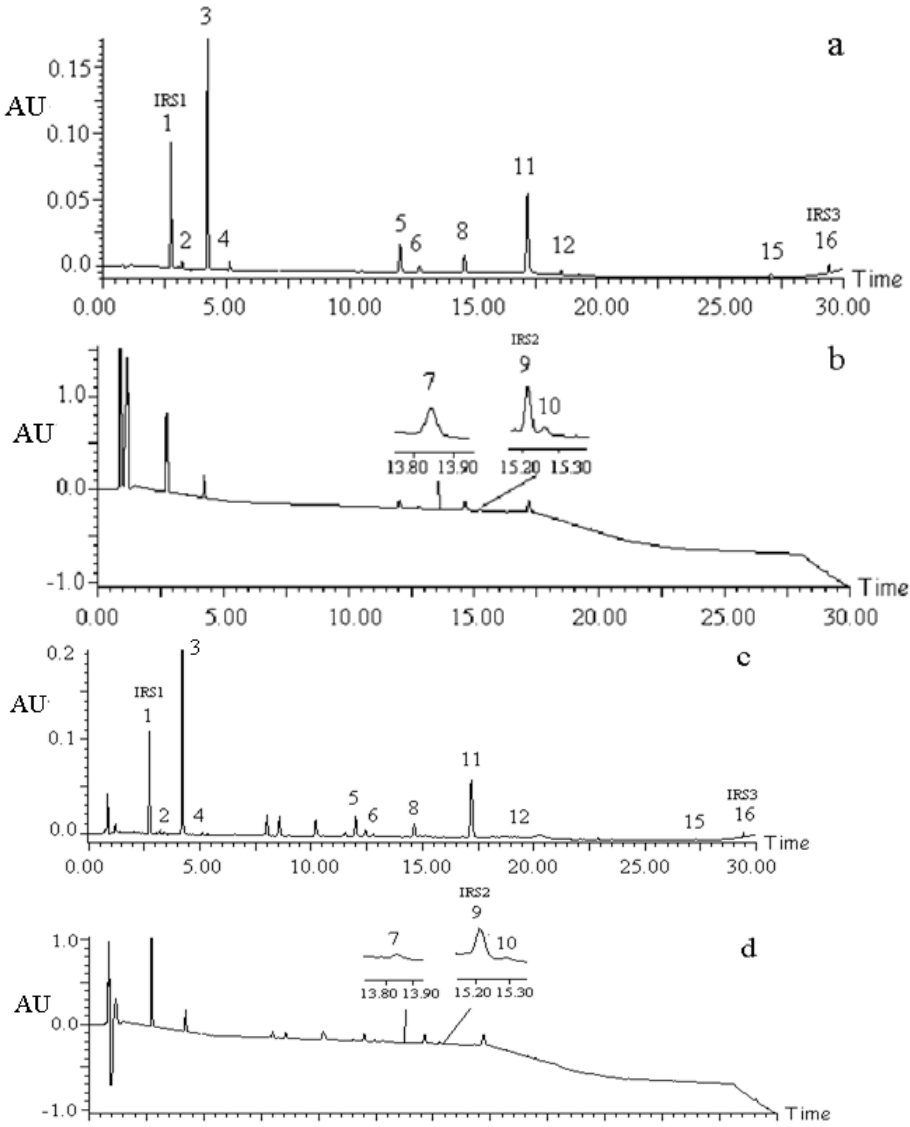


*Fig. 1.* UPLC-DAD chromatograms of (a) mixed standard solution in 280 nm, (b) mixed standard solution in 203 nm, (c) the sample solution of CDDP in 280 nm, (d) the sample solution of CDDP in 203 nm are shown. IRS1, IRS2, IRS3 represent the three internal reference standards (danshensu, ginsenoside Rg1 and tanshinone IIA). 1–12 represent danshensu, protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, notoginsenoside R1, salvianolic acid B, ginsenoside Rg1, ginsenoside Re, salvianolic acid A and salvianolic acid C. 15–16 represent cryptotanshinone and tanshinone IIA.

ion mode and −2.8 kV in negative ion mode. The flow rates of desolvation gas (nitrogen) and cone gas (nitrogen) were set at 600 L/h and 50 L/h while that of collision activation dissociation gas (argon) was set at 0.20 mL/min. The extractor volt was 3 V. Quadrupoles 1 and 3 were maintained at unit mass resolution and the dwell time was set at 50 ms. The mass range was set at *m/z* 100–1150 using full-scan mode. Twenty percent eluent containing the sample was allowed to flow into the mass spectrometer by solvent splitting. The multiple-reaction monitoring (MRM) chromatograms are shown in *Fig. 2*.

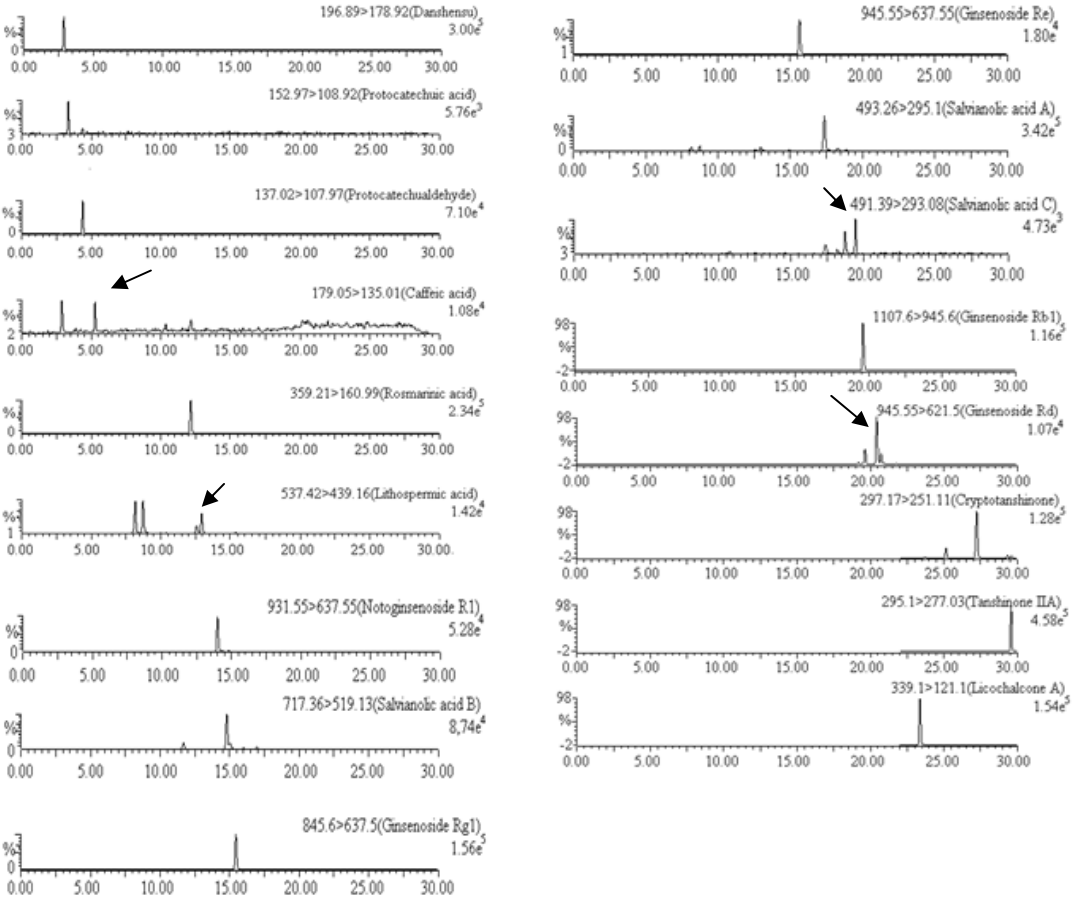


*Fig. 2.* Representative MRM chromatograms of 16 compounds and IS (licochalcone A) in CDDP sample solution are shown. The isomerides of some analytes existed in the sample solution, the marker analytes are distinguished from their isomerides with the arrow

# Results and Discussion

## Method Validation of UPLC-DAD

Method validations were performed under the optimized UPLC conditions described above. The methods were validated in terms of the calibration curves, the limits of detection and quantification (LOD and LOQ), intra-day and inter-day precisions, repeatability, stability, and recovery test. The result listed in *Table I* showed that all the calibration curves indicated good linearity with the correlation coefficient $r \geq 0.9994$. The intra-day precision was obtained by analyzing the sample six consecutive times a day and the inter-day precision by analyzing the sample for three consecutive days. From the results obtained, the relative standard deviation (RSD) values of intra-day precision were between 0.54% and 6.29%. The RSD values of inter-day precision were between 1.97% and 7.42%, while the RSD values of repeatability were between 0.50% and 7.73%. The stability test results (RSD values were below 5.32%) proved that compounds in the sample solution were stable within 16 h. The recovery was evaluated by adding known amounts of standard compounds into a certain amount (0.055 g) of CDDP. The mixture was subjected to the same sample extraction method mentioned above. Sample detection was done in duplicate. The recoveries of the compounds ranged from 98.34% to 105.26%.

## The Calculation of Relative Correction Factor (RCF) in QAMS Method

Wang et al. have clarified the theory of QAMS method. In their study, a cheap and easily available compound was chosen as the internal reference substance, and its content was determined by external standard method. The RCFs were obtained according to eq. (1)

$$fsi = \frac{fs}{fi} = \frac{Cs / As}{Ci / Ai} (i = 1, 2, 3, ..., n). \quad (1)$$

($A_s$ represents the peak area of internal reference substance; $C_s$ represents the concentration of internal reference substance; $A_i$ represents the peak area of remaining substance; $C_i$ represents the concentration of remaining substance.)

Then, the contents of the other compounds were calculated by the RCFs, which were validated by the robustness test. Up to now, this method was only applied to determine the content of substances with similar chemical

*Table I.* The method validation data of 16 compounds with UPLC-DAD

| Compound | Regressive equation[a] (*n*=3) | *r* | Linear range (μg/mL) | LOD[b] (μg/mL) | LOQ[c] (μg/mL) | Precision RSD (%) | | Repeatability RSD (%) (*n*=6) | Stability RSD (%) | Average recovery (*n*=6) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | intra-day (*n*=6) | inter-day (*n*=3) | | | Recovery (%) | RSD (%) |
| Danshensu | $Y = 64.28X - 24.94$ | 0.9998 | 5.14–328.64 | 0.154 | 0.513 | 0.57 | 2.37 | 0.50 | 4.03 | 101.22 | 3.08 |
| Protocatechuic acid | $Y = 453.02X - 0.22$ | 0.9998 | 0.04–2.50 | 0.012 | 0.040 | 0.92 | 2.24 | 1.16 | 3.86 | 98.34 | 2.88 |
| Protocatechuical-dehyde | $Y = 454.00X + 44.69$ | 0.9999 | 1.37–87.84 | 0.021 | 0.069 | 0.62 | 2.20 | 0.88 | 3.66 | 104.31 | 1.74 |
| Caffeic acid | $Y = 407.95X - 1.07$ | 0.9999 | 0.06–3.73 | 0.018 | 0.060 | 2.43 | 3.04 | 3.03 | 5.32 | 100.41 | 4.11 |
| Rosmarinic acid | $Y = 204.68X + 10.31$ | 0.9999 | 0.58–37.08 | 0.035 | 0.116 | 0.60 | 2.17 | 0.78 | 3.66 | 102.77 | 3.69 |
| Lithospermic acid | $Y = 123.59X + 1.42$ | 0.9999 | 0.24–15.34 | 0.014 | 0.048 | 0.88 | 2.89 | 1.24 | 3.93 | 98.83 | 2.49 |
| Notoginsenoside R1 | $Y = 31.39X + 16.92$ | 0.9997 | 1.19–38.14 | 0.179 | 0.596 | 2.79 | 3.61 | 5.77 | 2.16 | 105.26 | 8.14 |
| Salvianolic acid B | $Y = 108.65X - 3.79$ | 0.9998 | 0.73–46.85 | 0.219 | 0.730 | 0.64 | 2.24 | 0.94 | 3.54 | 104.95 | 1.88 |
| Ginsenoside Rg1 | $Y = 33.84X + 52.63$ | 0.9998 | 5.35–171.20 | 0.803 | 2.675 | 1.42 | 3.99 | 3.23 | 3.31 | 104.00 | 5.63 |
| Ginsenoside Re | $Y = 27.10X + 3.46$ | 0.9998 | 0.86–27.46 | 0.258 | 0.860 | 4.30 | 7.42 | 7.73 | 2.49 | 99.61 | 8.94 |
| Salvianolic acid A | $Y = 312.80X + 87.89$ | 0.9999 | 1.34–85.68 | 0.080 | 0.268 | 0.54 | 1.97 | 0.96 | 3.22 | 99.66 | 2.14 |
| Salvianolic acid C | $Y = 456.99X + 0.54$ | 0.9994 | 0.01–0.63 | 0.003 | 0.010 | 6.29 | 5.66 | 6.77 | 4.13 | 104.69 | 5.10 |
| Ginsenoside Rb1[d] | – | – | – | – | – | – | – | – | – | – | – |
| Ginsenoside Rd[d] | – | – | – | – | – | – | – | – | – | – | – |
| Cryptotanshinone | $Y = 278.84X$ | 0.9995 | 0.04–2.70 | 0.012 | 0.040 | 2.78 | 2.62 | 2.92 | 3.51 | 99.68 | 2.94 |
| Tanshinone IIA | $Y = 394.63X - 0.09$ | 0.9999 | 0.11–3.36 | 0.016 | 0.052 | 0.85 | 1.97 | 0.97 | 3.12 | 100.50 | 2.27 |

[a] The regressive equations were presented as $Y = aX + b$ . $Y$ and $X$ were defined as peak area and concentration of compound, respectively.
[b] LOD refers to the limits of detection, S/N = 3
[c] LOQ refers to the limits of quantification, S/N = 10
[d] Ginsenoside Rb1 and ginsenoside Rd have no method validation data of UPLC-DAD for their weak response.

structure in every analytical procedure. In this study, due to the high content in CDPs and relative low price in commercial aspect, three internal reference substances were selected to simultaneously determine all the three sorts of compounds in CDPs which were as follows: danshensu was selected as internal reference substance for the determination of phenolic acid, ginsenoside Rg1 for saponin, and tanshinone IIA for diterpenoid, respectively. Ginsenoside Rb1 and ginsenoside Rd showed weak ultraviolet response, which affected the accuracy of detection. So it was unsuitable to determine the contents of these two substances using QAMS method. The RCFs of danshensu to protocatechuic acid, protocatechuicaldehyde, caffeic acid, rosmarinic acid, lithospermic acid, salvianolic acid B, salvianolic acid A and salvianolic acid C were 7.118, 7.187, 6.316, 3.250, 1.977, 1.702, 5.055, and 7.107, respectively. The RCFs of ginsenoside Rg1 to notoginsenoside R1 and ginsenoside *Re* were 0.947 and 0.770. The RCF of tanshinone IIA to cryptotanshinone was 0.741. All the RCFs were calculated by a series of standard solutions with different concentrations, with the RSDs below 5.86%. Among them, the RCFs of danshensu to protocatechuicaldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B were similar to the reference data [20]. The same situation occurred in the RCF of tanshinone IIA to cryptotanshinone as well [21, 24]. According to the literature [22], ginsenoside Rb1 was selected as the internal reference substance. However, the response of ginsenoside Rb1 in CDPs was much weaker than that in raw material herbs. Hence, ginsenoside Rg1 was chosen as the internal reference substance in our study.

## Validation of QAMS Method

### Robustness Test

RCFs were affected by variable factors in the whole analytical procedure. Environmental factors and operational factors were mainly investigated in robustness test [25]. In our study, the concentration of formic acid, the column temperature, and the flow rate of mobile phase were tested in ACQUITY UPLC HSS T3 column. In addition, columns with different inner diameters and filling materials were also investigated. The detailed results are shown in *Table II*, which suggested that the RCFs were generally stable under the slightly changeable conditions, with the RSDs below 5.69%. Among them, the RCF of ginsenoside Rg1 to notoginsenoside R1 were relatively unstable, with the RSDs above 6.00%.

*Table II.* The fluctuation of RCFs in the robustness test for QAMS method

| Compound | RCFs (Mean±SD) | | | |
|---|---|---|---|---|
| | F1[c]-concentration of formic acid (0.05% Formic acid, 0.10% Formic acid, 0.20% Formic acid) | F2-column temperature (29°C, 30°C, 31°C) | F3-flow rate of mobile phase (0.25 mL/min, 0.30 mL/min, 0.35 mL/min) | F4-columns (Column 1[b] and Column 2[b] ) |
| Danshensu [a] | – | – | – | – |
| Protocatechuic acid | 7.231±0.156 | 7.184±0.198 | 7.151±0.162 | 7.237±0.211 |
| Protocatechuicaldehyde | 7.254±0.103 | 7.173±0.059 | 7.182±0.066 | 7.119±0.120 |
| Caffeic acid | 6.376±0.094 | 6.275±0.197 | 6.381±0.090 | 6.272±0.099 |
| Rosmarinic acid | 3.180±0.092 | 3.244±0.035 | 3.228±0.044 | 3.272±0.066 |
| Lithospermic acid | 1.928±0.061 | 1.965±0.066 | 1.936±0.053 | 1.917±0.079 |
| Notoginsenoside R1 | 1.047±0.078 | 1.039±0.081 | 1.030±0.072 | 0.980±0.063 |
| Salvianolic acid B | 1.706±0.035 | 1.703±0.032 | 1.720±0.034 | 1.694±0.027 |
| Ginsenoside Rg1 [a] | – | – | – | – |
| Ginsenoside Re | 0.781±0.041 | 0.821±0.047 | 0.803±0.046 | 0.763±0.026 |
| Salvianolic acid A | 4.958±0.090 | 5.011±0.185 | 4.989±0.081 | 5.013±0.095 |
| Salvianolic acid C | 7.217±0.297 | 7.439±0.321 | 7.362±0.279 | 7.215±0.259 |
| Ginsenoside Rb1 [d] | – | – | – | – |
| Ginsenoside Rd [d] | – | – | – | – |
| Cryptotanshinone | 0.721±0.036 | 0.751±0.027 | 0.740±0.026 | 0.745±0.031 |
| Tanshinone IIA [a] | – | – | – | – |

[a] Danshensu, ginsenoside Rg1, tanshinone IIA were internal reference substances and had no RCFs.

[b] Column 1 = ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm); Column 2 = ACQUITY UPLC BEH C18 column (3.0 × 100 mm, 1.7 μm)

[c] The test of F1 was performed on Column 1 with the column temperature (30°C) and the flow rate of mobile phase (0.30 mL/min). The test of F2 was performed on Column 1 with the concentration of formic acid (0.10% Formic acid) and the flow rate of mobile phase (0.30 mL/min). The test of F3 was performed on Column 1 with the concentration of formic acid (0.10% Formic acid) and the column temperature (30°C). The test of F4 was performed on both Column 1 and Column 2 with the concentration of formic acid (0.10% Formic acid), the column temperature (30 °C) and the flow rate of mobile phase (0.30 mL/min).

[d] Ginsenoside Rb1 and ginsenoside Rd have no RCFs for their weak response.

## Comparison of the Results Obtained by UPLC-DAD, UPLC-ESI-MS/MS and QAMS

In order to assure the accuracy of this newly established QAMS method, three batches of CDDP with the number CDDP-1 to CDDP-3 were each determined by three methods, that is, UPLC-DAD, UPLC-ESI-MS/MS, and QAMS.

The UPLC-ESI-MS/MS method was validated by a series of experiments, such as the calibration curves, LOD and LOQ, intra-day and inter-day precisions, repeatability, stability, and recovery tests. From the results obtained, the RSD values of intra-day precision were between 1.37% and 6.88%, and the RSD values of inter-day precision were between 1.72% and 7.93%, respectively. The RSDs of repeatability ranged from 2.21% to 12.73%, while the recoveries ranged from 90.73% to 109.91%. Except forginsenoside Rb1 and ginsenoside Rd only determined by UPLC-ESI-MS/MS method, the remaining fourteen compounds were determined by the three analytical methods simultaneously.

For each batch of CDDP, the contents of each of the 14 compounds obtained from the three different analytical methods were in accord with RSDs below 6.09%. The detailed results are shown in *Table III*. This suggested that the QAMS method was accurate enough to evaluate the quality of CDPs. The previous studies only compare the results obtained by QAMS method and external standard method with UV detector [19–24], whereas this study adds the MS method to assure the feasibility and accuracy of QAMS.

### The Location of Compounds of Interest in the QAMS Method

The most crucial parts in this study were the stability of the RCFs and the location of corresponding compounds on the chromatographic column. The previous studies usually focused on using the difference of retention time obtained for different compounds or on using the relative retention time when locating the corresponding compounds. In fact, the retention time of the individual compounds varied slightly with different columns and instruments, especially on the columns with different inner diameters and filling materials. Nevertheless, the mass spectral data for each compound in the different conditions were the same. By analyzing how well the mass spectra obtained from certain compounds in different retention time matched those obtained from the pure substances, the accurate positions of certain compounds were identified for the different chromatographic columns. In this study, the MRM mode of MS detector was applied in the location of corresponding compounds. For UPLC-ESI-MS/MS, three groups of constituents show different responses when identified by mass spectrometry

*Table III.* The content of 14 compounds in 3 batches of CDDP determined by UPLC-DAD, UPLC-ESI-MS/MS and QAMS

| No. | Compound | Method | Content (μg/g) | | | No. | Compound | Method | Content (μg/g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CDDP-1 | CDDP-2 | CDDP-3 | | | | CDDP-1 | CDDP-2 | CDDP-3 |
| 1 | Danshensu (internal reference substance) | DAD | 8600.74 | 8149.71 | 8696.58 | 8 | Salvianolic acid A | DAD | 2378.09 | 1659.83 | 1773.90 |
| | | MS | 8645.54 | 8171.51 | 8707.89 | | | MS | 2318.80 | 1676.41 | 1755.12 |
| | | QAMS[a] | 8600.74 | 8149.71 | 8696.58 | | | QAMS | 2322.53 | 1628.20 | 1739.13 |
| | | RSD (%) | 0.30 | 0.15 | 0.07 | | | RSD (%) | 1.42 | 1.48 | 0.99 |
| 2 | Protocatechuic acid | DAD | 33.67 | 48.30 | 56.18 | 9 | Salvianolic acid C | DAD | 7.64 | 6.38 | 5.26 |
| | | MS | 33.95 | 48.32 | 56.55 | | | MS | 7.00 | 5.80 | 4.91 |
| | | QAMS | 33.42 | 47.98 | 55.80 | | | QAMS | 7.78 | 6.51 | 5.39 |
| | | RSD (%) | 0.79 | 0.40 | 0.67 | | | RSD (%) | 5.55 | 6.09 | 4.77 |
| 3 | Protocatechuicaldehyde | DAD | 2545.63 | 2002.35 | 2244.97 | 10 | Ginsenoside Rg1 (internal reference substance) | DAD | 6047.12 | 5383.12 | 5019.16 |
| | | MS | 2525.10 | 2010.04 | 2268.87 | | | MS | 6051.35 | 5341.87 | 5027.49 |
| | | QAMS | 2520.37 | 1984.28 | 2223.92 | | | QAMS[a] | 6047.12 | 5383.12 | 5019.16 |
| | | RSD (%) | 0.53 | 0.66 | 1.00 | | | RSD (%) | 0.04 | 0.44 | 0.10 |
| 4 | Caffeic acid | DAD | 41.99 | 33.07 | 35.00 | 11 | Notoginsenoside R1 | DAD | 1162.82 | 930.71 | 965.81 |
| | | MS | 40.80 | 33.75 | 36.04 | | | MS | 1139.09 | 922.03 | 946.06 |
| | | QAMS | 42.13 | 33.13 | 35.07 | | | QAMS | 1159.16 | 934.44 | 966.27 |
| | | RSD (%) | 1.75 | 1.12 | 1.64 | | | RSD (%) | 1.11 | 0.69 | 1.20 |
| 5 | Rosmarinic acid | DAD | 1039.20 | 788.44 | 826.95 | 12 | Ginsenoside Re | DAD | 593.83 | 503.18 | 401.85 |
| | | MS | 1016.03 | 793.46 | 826.24 | | | MS | 587.56 | 501.39 | 400.57 |
| | | QAMS | 1026.66 | 779.96 | 817.97 | | | QAMS | 615.32 | 521.72 | 418.21 |
| | | RSD (%) | 1.13 | 0.87 | 0.61 | | | RSD (%) | 2.43 | 2.21 | 2.42 |
| 6 | Lithospermic acid | DAD | 238.02 | 226.96 | 256.32 | 13 | Tanshinone IIA (internal reference substance) | DAD | 52.16 | 38.71 | 34.71 |
| | | MS | 241.81 | 228.30 | 260.73 | | | MS | 52.22 | 39.65 | 36.23 |
| | | QAMS | 233.42 | 222.61 | 251.31 | | | QAMS[a] | 52.16 | 38.71 | 34.71 |
| | | RSD (%) | 1.77 | 1.32 | 1.84 | | | RSD (%) | 0.07 | 1.39 | 2.48 |
| 7 | Salvianolic acid B | DAD | 1978.59 | 965.75 | 887.71 | 14 | Cryptotanshinone | DAD | 32.24 | 21.35 | 16.26 |
| | | MS | 1963.12 | 950.48 | 869.75 | | | MS | 33.29 | 21.39 | 15.69 |
| | | QAMS | 1969.84 | 959.92 | 882.02 | | | QAMS | 30.75 | 20.36 | 15.51 |
| | | RSD (%) | 0.39 | 0.80 | 1.04 | | | RSD (%) | 3.97 | 2.76 | 2.47 |

[a] The contents of three internal reference substances determined by QAMS were calculated by the same calibration curves with the DAD method.

*Table IV*. Optimized multiple-reaction-monitoring (MRM) parameters for 16 compounds and the IS

| Compound | Retention time | Negative mode | | Positive mode | MRM | Product ion [b] | Cone voltage (V) | Collision voltage (eV) | Dwell time (ms) |
|---|---|---|---|---|---|---|---|---|---|
| | $t_R$(min) | $[M-H]^-$ | $[M+HCOO]^-$ | $[M+H]^+$ | | | | | |
| Danshensu | 2.90 | 196.89 | – | – | 196.89→178.92 | 178.92[M–H–$H_2O$]$^-$ | 20 | 12 | 0.05 |
| Protocatechuic acid | 3.36 | 152.97 | – | – | 152.97→108.92 | 108.92[M–H–$CO_2$]$^-$ | 20 | 15 | 0.05 |
| Protocatechuicaldehyde | 4.39 | 137.02 | – | – | 137.02→107.97 | 107.97[M–H–CHO]$^-$ | 25 | 22 | 0.05 |
| Caffeic acid | 5.29 | 179.05 | – | – | 179.05→135.01 | 135.01[M–H–$CO_2$]$^-$ | 20 | 15 | 0.05 |
| Rosmarinic acid | 12.18 | 359.21 | – | – | 359.21→160.99 | 160.99[M–H–DSU]$^-$ | 20 | 15 | 0.05 |
| Lithospermic acid | 12.98 | 537.42 | – | – | 537.42→493.16 | 493.16[M–H–$CO_2$]$^-$ | 12 | 10 | 0.05 |
| Notoginsenoside R1 | 13.97 | 931.93 | – | – | 931.93→637.55 | 637.55[M–H–Xyl–Glc]$^-$ | 52 | 36 | 0.05 |
| Salvianolic acid B | 14.78 | 717.36 | – | – | 717.36→519.13 | 519.13[M–H–DSU]$^-$ | 28 | 20 | 0.05 |
| Ginsenoside Rg1 | 15.40 | 799.69 | 845.90 | – | 845.90→637.50 | 637.50[M–H–Glc]$^-$ | 40 | 28 | 0.05 |
| Ginsenoside Re | 15.60 | 945.80 | 991.92 | – | 945.80→637.55 | 637.55[M–H–Rham–Glc]$^-$ | 56 | 36 | 0.05 |
| Salvianolic acid A | 17.35 | 493.26 | – | – | 493.26→295.10 | 295.10[M–H–DSU]$^-$ | 25 | 18 | 0.05 |
| Salvianolic acid C | 19.47 | 491.39 | – | – | 491.39→293.08 | 293.08[M–H–DSU]$^-$ | 28 | 22 | 0.05 |
| Ginsenoside Rb1 | 19.61 | 1107.98 | – | – | 1107.98→945.60 | 945.60[M–H–Glc]$^-$ | 70 | 40 | 0.05 |
| Ginsenoside Rd | 20.41 | 945.83 | 991.94 | – | 945.83→621.50 | 621.50[M–H–2Glc]$^-$ | 60 | 35 | 0.05 |
| Cryptotanshinone | 27.23 | – | – | 297.17 | 297.17→251.11 | 251.11[M+H–$H_2O$–CO]$^+$ | 35 | 30 | 0.05 |
| Tanshinone IIA | 29.59 | – | – | 295.10 | 295.10→277.03 | 277.03[M+H–$H_2O$]$^+$ | 30 | 25 | 0.05 |
| Licochalcone A (IS) | 23.37 | – | – | 339.50 | 339.50→121.08 | 121.08[M+H–$C_{14}H_{18}O_2$]$^+$ | 28 | 28 | 0.05 |

[a] "–" in the "Positive ions" or "Negative ions" column means no mass spectrum signals.

[b] Abbreviations used in this column: DSU = danshensu, Xyl = xylose, Glc = glucose, Rham = rhamnose, $C_{14}H_{18}O_2$ = 5-methoxy-2-(2-methylbut- 3-en-2-yl)-4-vinylphenol

*Table V*. The contents of 16 compounds in 6 batches of CDDP and 6 batches of CDT with QAMS

| Compound | Content (μg/g) (Mean±SD) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CDT-1 | CDT-2 | CDT-3 | CDT-4 | CDT-5 | CDT-6 | CDDP-1 | CDDP-2 | CDDP-3 | CDDP-4 | CDDP-5 | CDDP-6 |
| Danshensu | 2692.47±17.50 | 3106.28±32.08 | 2573.16±42.01 | 1731.47±19.39 | 856.64±24.04 | 1726.51±14.29 | 8127.43±48.39 | 8126.82±57.14 | 8203.50±68.27 | 9093.12±28.02 | 8930.70±81.18 | 9231.92±91.29 |
| Protocatechuic acid | 11.57±0.04 | 14.79±0.24 | 13.81±0.35 | 9.23±0.42 | 8.01±0.33 | 8.14±0.18 | 44.96±3.51 | 41.88±1.20 | 45.07±1.73 | 37.39±1.15 | 50.52±0.25 | 38.00±2.31 |
| Protocatechuicaldehyde[a] | tr | tr | tr | tr | tr | tr | 1834.08±11.48 | 1975.31±26.73 | 2041.78±17.94 | 2549.99±8.26 | 2268.19±20.73 | 2643.23±22.72 |
| Caffeic acid | 33.21±1.09 | 32.06±0.81 | 32.02±1.16 | 29.69±2.65 | 23.31±0.34 | 26.67±0.67 | 29.10±1.82 | 40.21±0.26 | 35.89±0.14 | 34.08±0.30 | 35.14±1.42 | 34.15±1.28 |
| Rosmarinic acid | 1000.85±5.44 | 1095.69±10.07 | 954.41±4.54 | 1212.04±11.19 | 984.24±19.59 | 1246.07±10.96 | 773.31±4.13 | 843.31±4.39 | 800.82±8.17 | 992.84±5.54 | 973.25±8.92 | 1037.09±10.22 |
| Lithospermic acid | 1587.68±15.67 | 1961.60±29.19 | 1649.27±37.48 | 950.95±12.48 | 994.74±21.30 | 954.55±4.88 | 254.37±2.19 | 294.00±2.58 | 269.53±2.54 | 179.37±3.56 | 246.57±2.59 | 166.66±0.79 |
| Notoginsenoside R1 | 654.31±96.05 | 592.12±44.63 | 618.95±17.37 | 1611.87±57.59 | 1860.99±32.04 | 1522.85±42.24 | 929.08±3.63 | 932.83±52.89 | 956.90±13.69 | 931.58±12.24 | 940.40±60.20 | 1076.21±32.64 |
| Salvianolic acid B | 20465.36±118.93 | 21382.60±166.90 | 20791.15±79.00 | 21848.02±78.89 | 19894.09±15.91 | 21833.81±86.59 | 977.76±5.97 | 1245.82±4.21 | 1012.37±9.61 | 1562.51±4.90 | 1088.27±11.12 | 1468.88±13.48 |
| Ginsenoside Rg1 | 2844.65±192.04 | 3017.10±217.69 | 3119.02±296.43 | 7867.23±156.98 | 7967.16±483.29 | 7964.74±82.04 | 5736.70±88.70 | 5027.66±95.68 | 5652.80±113.70 | 4948.31±82.79 | 4947.71±162.87 | 5296.43±80.24 |
| Ginsenoside Re[a] | tr | tr | tr | 1059.13±40.60 | 1066.14±30.62 | 1113.60±58.69 | 554.45±16.21 | 520.42±14.18 | 573.14±20.95 | 547.08±6.87 | 540.18±10.15 | 528.50±37.69 |
| Salvianolic acid A | 1762.45±10.62 | 2029.43±13.98 | 1598.09±17.85 | 1136.06±17.13 | 467.69±10.23 | 1058.24±8.54 | 1779.55±4.31 | 1879.28±14.67 | 1813.72±17.38 | 2266.26±10.70 | 1978.93±8.68 | 2298.74±10.04 |
| Salvianolic acid C | 13.78±0.58 | 13.63±0.84 | 13.20±1.18 | 20.68±0.45 | 16.90±0.25 | 24.31±0.54 | 6.14±0.05 | 6.17±0.21 | 6.01±0.21 | 5.53±0.08 | 7.41±0.24 | 6.12±0.10 |
| Ginsenoside Rb1[6] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Ginsenoside Rd[b] | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Cryptotanshinone | 707.99±1.39 | 678.96±7.93 | 728.65±6.10 | 643.43±3.00 | 229.59±1.92 | 681.33±15.07 | 19.44±0.50 | 19.92±0.95 | 17.25±0.29 | 33.28±0.32 | 21.49±0.68 | 39.94±0.54 |
| Tanshinone IIA | 1005.37±3.63 | 940.38±2.03 | 1027.04±15.39 | 946.56±0.30 | 676.84±8.77 | 986.23±3.89 | 38.82±0.49 | 34.15±1.11 | 33.23±0.21 | 57.09±0.74 | 42.19±0.41 | 67.03±0.27 |

[a] Protocatechuicaldehyde and ginsenoside Re in some batches of CDTs are below the limits of quantification. tr = trace
[b] Ginsenoside Rb1 and ginsenoside Rd can not be determined by QAMS for their weak response. nd = not detected

with an ESI source. The phenolic acids exhibited high response ions of $[M–H]^-$ and the saponins exhibited high response ions of $[M–H]^-$ or $[M+HCOO]^-$ in negative mode, while the diterpenoid quinones exhibited high response ions of $[M+H]^+$ in positive mode. The characteristics of the fragment ions of the compounds match those described in the literature [12, 15]. The detailed information is given in *Table IV*. Every compound has its own precursor ion and product ion, which supplied the precise identification of the same compound with different retention time.

### Application of QAMS Method

The contents of sixteen compounds in six remaining batches of CDDP and six batches of CDT were determined using the QAMS method. From the results, it was obvious that the contents varied between different dosage forms as well as between different batches of the same product. The disparity may be attributed to the difference in the manufacturing procedure. The differences were that the contents of danshensu and protocatechuicaldehyde in CDDP were higher than those in CDT. Danshensu was the most abundant compound in CDDP with content range of 8126.82 μg/g–9231.92 μg/g, protocatechuicaldehyde with the range of 1834.08 μg/g–2643.23 μg/g. These explain that danshensu is selected as marker substance for CDDP in the Pharmacopoeia of the Peoples Republic of China. The contents of lithospermic acid, salvianolic acid B, tanshinone IIA, and cryptotanshinone in CDT were much higher than those in CDDP. The contents of salvianolic acid B with highest amount and tanshinone IIA were more stable than other compounds in CDT. These results explain the reason for the use of salvianolic acid B as marker substance for water-soluble compounds and tanshinone IIA for lipid-soluble compounds in CDT in the Pharmacopoeia of the Peoples Republic of China. The detailed information is given in *Table V*.

## Conclusion

A new analytical method was established in this study for the assessment of compound danshen preparations. Sixteen compounds were then quantified with the new QAMS method. The established QAMS method can simultaneously determine the contents of three groups of compounds with different chemical structures. It can resolve the problem caused by insufficiency of the standard compounds. Consequently, it will become the new trend in quantitative analysis of herbal medicines and their preparations.

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