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Original Article

HPLC combined with chemometrics for quality control of Huamoyan Granules or Capsules

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ABSTRACT

Objective: Huamaoyan Granules (HMYG) and Huamaoyan Capsules (HMYC) are Chinese patent medicines with different dosage forms of the same prescription. Due to the different preparation process, the chemical composition of these Chinese patent medicines varies greatly among different forms, but there were few studies on the difference comparison and quality control of them. In order to improve the effectiveness and safety in its clinical application, an idea combining high performance liquid chromatography (HPLC) and chemometrics was put forward to study the quality control of Chinese patent medicines in different dosage forms of the same prescription.

Methods: The differential markers of HMYG and HMYC were explored based on HPLC fingerprint and chemometrics including orthogonal projections to latent structures-discriminant analysis (OPLS-DA), principal component analysis (PCA), and hierarchical cluster analysis (HCA). Finally, the quantitative analysis method of related components was established by HPLC.

Results: A quality control method for HMYG and HMYC was established. Firstly, the chemical components of HMYG and HMYC were systematically analyzed by HPLC fingerprinting. Further exploration showed that there were 20 characteristic peaks and 57 common peaks. Then, the potential differential markers between HMYG and HMYC were explored by chemometrics, and the differential markers were screened after intersection with the 20 characteristic peaks. Finally, HPLC quantitative analysis methods for nine components were established, including seven differential markers (neochlorogenic acid, protocatechualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, rosmarinic acid and salvianolic acid A). The results of HPLC quantitative analysis showed that the contents of eight components in HMYG and HMYC samples were significantly different. According to the above results, the differential markers between HMYG and HMYC screened based on HPLC fingerprint and chemometrics can effectively characterize the differences between the two dosage forms.

Conclusion: The present work provides a rapid and effective method for routine quality evaluation and control of HMYG and HMYC. This work also provides feasible methods for the quality evaluation and control of Chinese patent medicines with different dosage forms of the same prescription.

1. Introduction

Chinese patent medicine is composed of a single or several herbs or their active ingredients, which plays a therapeutic role through mutual coordination, and is prepared by modern advanced pharmaceutical technology. There are many dosage forms, including granule, tablet, pill, capsule and mixture, etc. (Chinese Pharmacopoeia Commission, 2020). Chinese patent medicine is an important part of modern traditional Chinese medicine, which is widely used to treat diseases in China because of its convenient application. The prescription is an important content of traditional Chinese medicine (TCM). Its composition is based on the basic principles of TCM theory system and dialectical treatment. Its curative effect and quality control have the characteristics of integrity, complexity and diversification (Zhang, 2015).

In the 2020 edition of *Chinese Pharmacopoeia*, *Volume I*, the number of Chinese patent medicines has reached 1607, involving 26 dosage forms. It contains a large number of Chinese patent medicines with different dosage forms of the same prescription, such as Reyanning Heji (mixture), Reyanning Pian (tablet) and Reyanning Keli (granule); Qufeng Zhitong Wan (pill), Qufeng Zhitong Pian (tablet) and Qufeng Zhitong Jiaonang (capsule), etc.

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Chinese patent medicine of different dosage forms with the same name, the same prescription and the same indications may have differences in composition and content. However, in actual clinical application, they may be applied to the treatment of the same disease, and the differences in composition may lead to differences in clinical efficacy.

Research shows that Guanxin Suhe Jiaonang (capsule) was better than Guanxin Suhe Wan (pill) in anti-myocardial ischemia, antihypoxia and other pharmacological effects (Zhang, Cui, Sun, Cheng, & Tang, 2000). Chen et al. (2010) studied and compared the antidiarrheal, antiemetic and antispasmodic effects of different dosage forms of Huoxiang Zhengqi. And the results showed that the effect of Huoxiang Zhengqi Jiaonang (capsule) was the strongest, and Huoxiang Zhengqi Ruanjiaonang (soft capsule) was stronger than other formulations.

Huamoyan variety series are the first batch of innovative drugs that synovium serve as therapeutic targets for joint diseases in China, including Huamaoyan granules, Huamaoyan capsules and Huamaoyan tablets, which are composed of 13 botanical drugs: *Prunellae Spica* (Xiakucao in Chinese), *Ligustri Lucidi Fructus* (Nvzhenzi in Chinese), *Ilicis Cornutae Folium* (Gouguye in Chinese), *Astragali Radix* (Mengguhuangqi in Chinese), *Stephaniae Tetrandrae Radix* (Fangji in Chinese), *Coicis Semen* (Yiyiren in Chinese), *Smilacis Glabrae Rhizoma* (Tufuling in Chinese), *Luffae Fructus Retinervus* (Sigualuo in Chinese), *Lycopi Herba* (Zelan in Chinese), *Salviae Miltiorrhizae Radix* et *Rhizoma* (Danshen in Chinese), *Angelicae Sinensis Radix* (Danggui in Chinese), *Cyathulae Radix* (Chuanniuxi in Chinese), *Siegesbeckiae Herba* (Xixiancao in Chinese). The composition of Huamoyan variety series prescription is complex, and there are only a few related studies on the material basis and quality control of Huamoyan variety series, which mainly involve tetrandrine, fangchinoline (Kang, Zhang, Liu, & Li, 2020), astragaloside IV, calycosin 7-*O*-*β*-*D*-glucopyranoside (Jiang, Yan, Liu, & Yu, 2020), salidroside, specnuezhenide (Zhang et al., 2019), salvianolic acid B, protocatechuic aldehyde (Zhao, Wang, & Xia, 2009) and ursolic acid (Li, Bai, & Zhang, 2011). Moreover, in the 2020 edition of the *Chinese Pharmacopoeia*, the content control ingredient of Huamoyan variety series is only salvianolic acid B, which has certain limitations. Huamoyan variety series were used to treat acute and chronic synovitis or post knee operation with symptoms such as swelling, distending, and painful joints with fixed location, and inhibit joint bending and stretching. Studies have shown that Huamaoyan Granules (HMYG) is widely used in the clinic to treat various joint synovitis, such as acute synovitis (Cao, Jia, & Wang, 2015), chronic synovitis (Chen et al., 2014), knee osteoarthritis synovitis (Zhang & Guo, 2016), gouty arthritis (Zhang et al., 2020), etc., with significant effects and fewer side effects (Li et al., 2019).

Most of the prescriptions of Chinese patent medicine are multiple herbs, which have various active ingredients. High performance liquid chromatography (HPLC) can be used for fingerprint analysis to separate and detect the compounds present in the sample, and obtain the chromatograms of various chemical components and their characteristics in the sample (Sabir, Rafi, & Darusman, 2017). In addition, the fingerprint similarity results can be used to further compare the differences of different samples through the multi-component spectrogram information, but the key component information cannot be screened out from the complex component data. Chemometrics analysis can extract effective information of interest from a large number of data through mathematical and statistical methods, which has a unique advantage in finding key component information from a large amount of spectrogram information brought by fingerprint analysis (Chen et al., 2008).

In this study, HPLC was used to establish the fingerprint for HMYG and Huamaoyan Capsules (HMYC) with same manufacturer to reveal the differences among different dosage forms, and the fingerprint data was further processed by orthogonal projections to latent structures-discriminant analysis (OPLS-DA), principal component analysis (PCA) and hierarchical cluster analysis (HCA) to explore the difference markers among different dosage forms. Finally, a quantitative analysis method suitable for the quality control of Huamoyan variety series was established to provide an idea and basis for solving the above problems.

2. Materials and methods

2.1. Samples and chemicals

A total of 20 batches of samples (HMYG and HMYC) were acquired from Shineway Pharmaceutical Group Ltd. (Shijiazhuang, China), with 10 batches of each dosage form. The samples were preserved in tightly closed containers at room temperature prior to analysis.

Neochlorogenic acid (purity ≥ 98% by HPLC, Batch No. P20A11L121936), cryptochlorogenic acid (purity ≥ 98% by HPLC, Batch No. P16A10U95423), neoastilbin (purity ≥ 98% by HPLC, Batch No. P08N9S74591), salvianolic acid A (purity ≥ 98% by HPLC, Batch No. Z23D10X106625), neoisoastilbin (purity ≥ 98% by HPLC, Batch No. 218J11B118982) and isoastilbin (purity ≥ 98% by HPLC, Batch No.P22J9FS3283) were supplied by Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Reference substances guanosine (93.6%, Batch No. 111977–201501), danshensu (97.8%, Batch No. 110855–201915), ferulic acid (99.0%, Batch No. 1110773–201614), calycosin 7-*O*-*β*-*D*-glucopyranoside (96.8%, Batch No. 111920–201907), protocatechualdehyde (99.6%, Batch No. 110810–201909), caffeic acid (99.7%, Batch No. 110885–201703), chlorogenic acid (96.1%, Batch No. 110753–202018), astilbin (93.6%, Batch No. 111798–201805), nuezhenide (95.0%, Batch No. 111926–201906), rosmarinic acid (Ros A, 98.1%, Batch No. 111871–202007), and salvianolic acid B (Sal B, 96.6%, Batch No. 111562–201917) were acquired from the China Food and Drug Testing Institute (Beijing, China).

Acetonitrile (HPLC grade) and phosphoric acid (HPLC grade) were obtained from Thermo Fisher Scientific (Shanghai, China). Other reagents used in the experiment were purchased from commercial reagent companies and were of analytical grade.

2.2. HPLC fingerprints and quantitative analysis

2.2.1. Preparation of references and sample solutions

HMYG were mixed, grounded, and weighed to prepare exactly 2 g samples. After adding 75% methanol (25 mL), the weight was accurately measured, and the sample was sonicated for 20 min. After centrifugation at 10 000 rpm for 10 min, samples were filtered through a 0.22 μm microporous filter membrane, and the filtrate was analyzed.

HMYC was weighed to prepare exactly 0.5 g samples. The rest of the operation was the same as the above method of HMYG. An appropriate amount of reference substances were accurately weighed and dissolved with 75% methanol. All standard solutions were further filtered by a 0.22 μm membrane before injection.

2.2.2. Chromatographic conditions

HPLC analysis was performed on Thermo Scientific Dionex Utimate 3000 HPLC equipped with diode array detector (Thermo Scientific, Germering, Germany). The chromatographic separation was carried out on Xselect® HSS T3 C_{18} column (250 mm × 4.6 m m, 5 μm) (Waters, Milford, MA, USA) and the column temperature was maintained at 30 °C. The detection wavelength of HPLC fingerprints was 287 nm, and the detection wavelength of HPLC quantitative assay method was 280 nm (danshensu, protocatechualdehyde), 287 nm (salvianolic acid A, salvianolic acid

B), 327 nm (neochlorogenic acid, cryptochlorogenic acid, caffeic acid, chlorogenic acid, rosmarinic acid). The injection volume was 10 μL. The mobile phase consisted of a gradient mixture of acetonitrile (A) and 0.1% phosphoric acid–water solution (volume percent, B). The flow rate and gradient elution process are shown in Table 1.

2.2.3. Method validation

The precision, stability and repeatability were used to validate the method of HPLC fingerprint. The linearity, range, specificity, stability, repeatability, precision and accuracy were used to validate the HPLC quantitative assay method.

2.3. Data analysis

2.3.1. HPLC fingerprints and similarity evaluation

The software named Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2012A, National Committee of Pharmacopoeia, China) was used to establish HPLC fingerprints and perform similarity analysis. And the results of similarity evaluation can assess the similarities of samples.

2.3.2. Chemometrics analysis

OPLS-DA, PCA, HCA were employed to analyze the HPLC fingerprint data using the SIMCA software (Version 14.1, Umetrics, Sweden). Variable importance in projection (VIP) was calculated by OPLS-DA model. VIP $\geq$ 1 was the condition to screen the difference components that could characterize the difference in dosage forms. PCA and HCA were used to establish a Huamoyan dosage forms discrimination model to verify whether the screened differential components could characterize the differences between HMYG and HMYC.

2.3.3. Statistical analysis

Statistical analysis of content determination results was performed using GraphPad Prism 9.0 (GraphPad Sofware, San Diego, California, USA). Multiple unpaired *t*-test was used for significance analysis, *P*-value of < 0.01 was considered to be statistically significant.

3. Results

3.1. Results of HPLC fingerprints

3.1.1. HPLC fingerprints and similarity analysis of HMYG and HMYC

A total of 20 batches of HMYG and HMYC samples were analyzed by the established HPLC fingerprint method. The chromatographic data of 0–100 min were imported into the software Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2012A, National Committee of Pharmacopoeia, China) to obtain HPLC fingerprint and similarity results. The HPLC fingerprint results of HMYG and HMYC were shown in Fig. 1, and the similarity results were presented in Tables S1 and S2 in the supplemental file. A total of 57 common peaks and 20 characteristic peaks were extracted from the fingerprint results of HMYG and HMYC.

Table 1
Gradient elution process of HPLC.

Time (min)	Acetonitrile (%)	0.1% Phosphoric acid–water solution (%)	Flow rate (mL/min)
0–3	2	98	1
3–12	2–10	98–90	1
12–19	10–12	90–88	1
19–28	12–14	88–86	1
28–36	14–17	86–83	1
36–42	17–19	83–81	1
42–72	19–23	81–77	1
72–82	23–25	77–75	1
82–100	25–35	75–65	1

As shown in Fig. 2, based on the 20 characteristic peaks, a total of 17 characteristic components were identified by comparing them with references, including guanosine, danshensu, neochlorogenic acid, protocatechualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, ferulic acid, calycosin 7-*O*-*β*-*D*-glucopyranoside, neoastilbin, astilbin, nuezhenide, neoisoastilbin, isoastilbin, rosmarinic acid, salvianolic acid B and salvianolic acid A.

3.1.2. Validation of HPLC fingerprints method

The same sample solution was injected for six consecutive times. The similarity evaluation of HPLC fingerprint method was > 0.900, indicating that the precision of the instrument is good. Six samples of the same batch of test solution were injected for analysis. The similarity evaluation of HPLC fingerprint method was > 0.900, indicating the repeatability of the method is good. The same sample solution was injected at 0, 2, 4, 8, 12, and 24 h after sample preparation. The similarity evaluation of HPLC fingerprint method was > 0.900, indicating the stability of the sample preparation method was good.

3.2. Results of chemometrics analysis

3.2.1. Potential differential markers screened by OPLS-DA

OPLS-DA analysis was performed on the 57 common peak data in the fingerprint of HMYG and HMYC, and the OPLS-DA score plot was shown in Fig. 3A after the outlier batch of S10 was eliminated. The 19 batches of samples were all fell within a 95% confidence interval (within the oval circle), and could be clearly divided into two groups according to dosage form (granule, capsule). HMYC samples with the positive scores of t1 were located in the right quadrant, whereas HMYG samples were distributed in the left quadrant and showed the negative scores of t1.

A model of OPLS-DA with R^2X of 0.802, R^2Y of 0.987 and Q^2 of 0.962 was established with good predictive ability. The results of OPLS-DA model validity was shown in Fig. 3B, after 200 permutation tests, the R^2 and Q^2 values of the original OPLS-DA models were still higher than the corresponding values of the permuted models. Moreover, the Q^2Y intercept for the established OPLS-DA models was < 0, indicating that there is no overfitting in the model. This OPLS-DA model can be used to screen and analyze the differential markers of HMYG and HMYC.

The VIP plot produced by OPLS-DA provides is usually used to explain the contribution of variables to classification. As shown in Fig. 3C, a total of 12 components were selected with VIP > 1 as the standard.

Combined with the 17 characteristic components of HPLC fingerprint, seven differential markers, including neochlorogenic acid, protocatechualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, rosmarinic acid and salvianolic acid A, were selected from 12 components with VIP > 1.

3.2.2. PCA and HCA analysis

PCA is an unsupervised method for multivariate analysis by reducing data dimensionality while preserving data covariance. It is commonly used for visual analysis of classification trends (Lubes & Goodarzi, 2017). Fig. 4A showed the PCA score plot obtained by using the peak area data of seven differential markers (neochlorogenic acid, protocatechualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, rosmarinic acid and salvianolic acid A) screened by OPLS-DA and HPLC fingerprint. The principal component 1 explained 78.3% of the total variance in the original information, and it is considered that PC1 has a good reliability

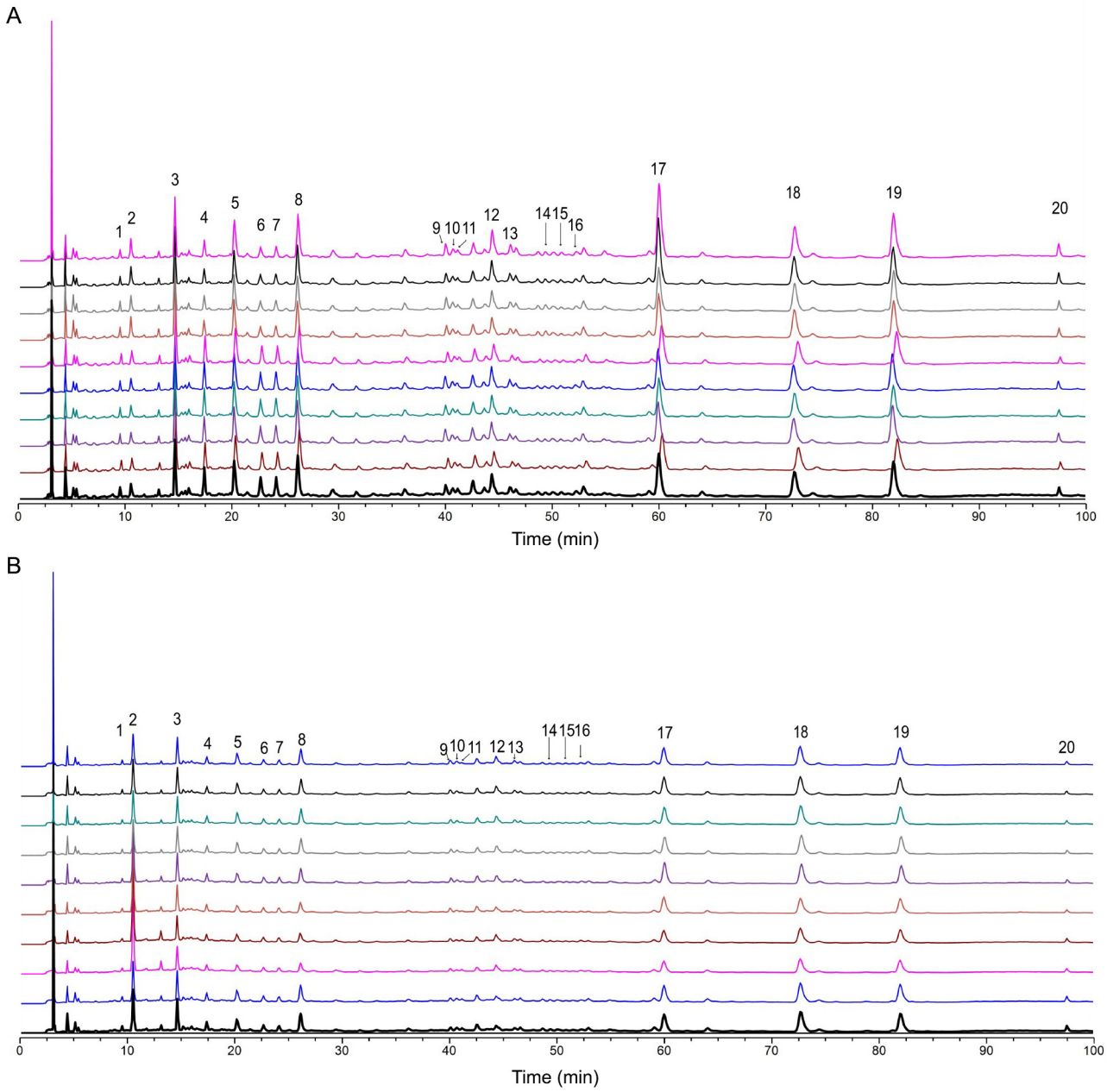

Fig. 1. Reference fingerprints of HMYG (A) and HMYC (B).

to analyze samples. HMYG and HMYC were clearly separated according to PC1, with HMYC mainly distributed in the negative interval and HMYG mainly distributed in the positive interval.

Further, peak area data of seven selected differential markers were imported into HCA for analysis. Ward's method was used to construct clusters and calculate the distance between clusters. As shown in the Fig. 4B, all HMYG clustered in the left group of the dendrogram, and all HMYC clustered in the right group of the dendrogram, which verified the result of PCA.

The results of PCA and HCA showed that seven differential markers previously selected could well characterize the differences between HMYG and HMYC.

3.3. Determination of nine components in HMYG and HMYC by HPLC

According to the results of OPLS-DA, seven differential markers were selected from 17 characteristic components identified by fingerprint results as indicator components for content determination. In *Chinese Pharmacopoeia*, HMYG and HMYC were previously determined only for salvianolic acid B content. Some studies have shown that danshensu has obvious anti-inflammatory effects, such as obvious anti-inflammatory and protective effects in the treatment of osteoarthritis (Xu et al., 2021). Therefore, in this study, we established a HPLC quantitative method for the simultaneous determination of nine components in HMYG and HMYC for the first time. The structures of the nine compounds were shown in Fig. 5.

The HPLC quantitative method was validated for linearity, range, specificity, stability, repeatability, precision, accuracy. As shown in Table 2, the concentration of nine components showed a good linear relationship with peak areas ($r > 0.999$). Furthermore, the relative standard deviation (RSD) values of repeatability, stability, precision, accuracy were all lower than 3%. Results of the validation showed that the established method met the requirements of quantitative analysis.

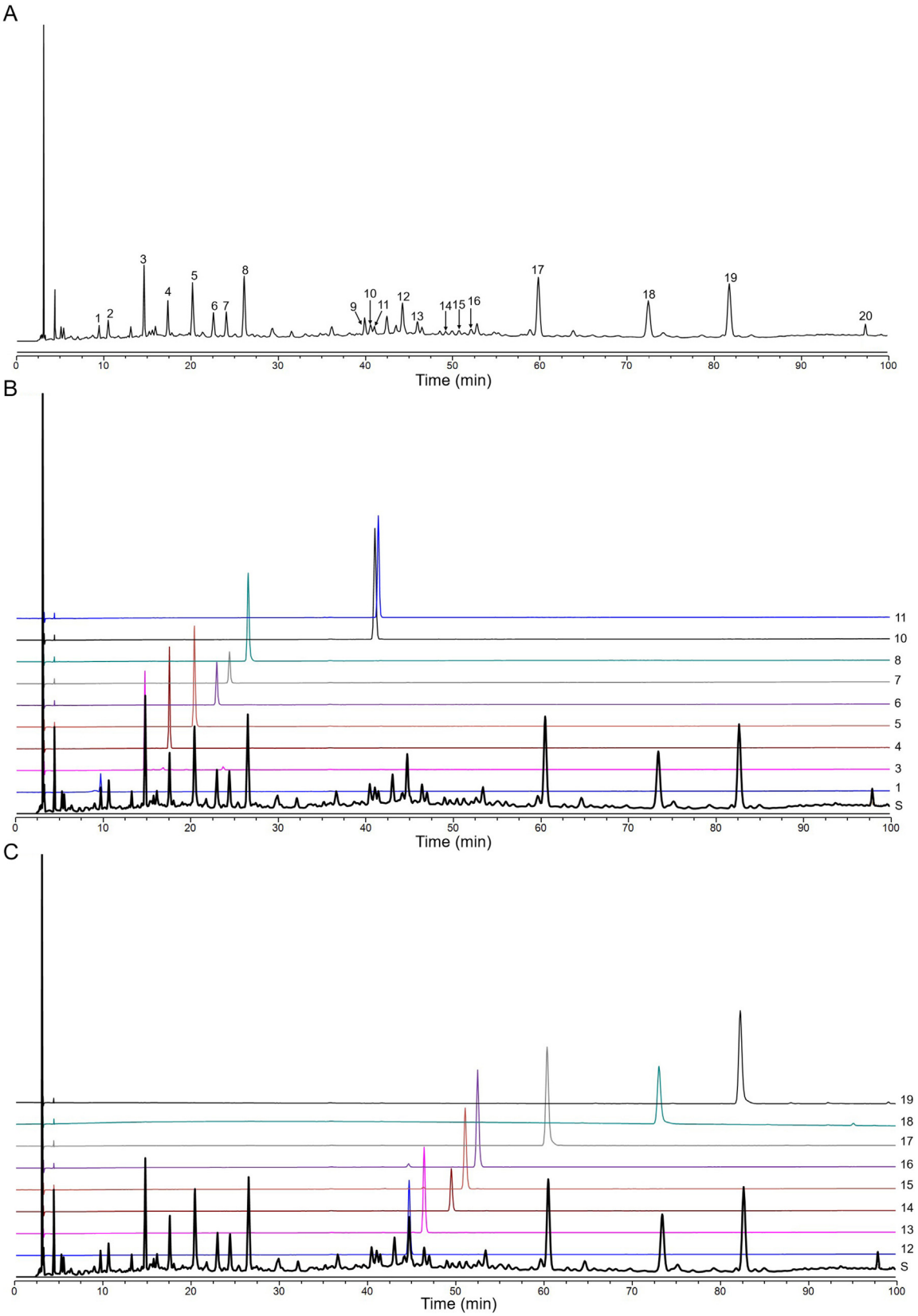

Fig. 2. Huamoyan Granules fingerprint and characteristic peak identification. (A) Sample chromatogram; (B and C) Comparison chromatogram between sample and 17 reference substances: 1-guanosine, 3-danshensu, 4-neochlorogenic acid, 5-protocatechualdehyde, 6-chlorogenic acid, 7-cryptochlorogenic acid, 8-caffeic acid, 10-ferulic acid, 11-calycosin 7-*O*-*β*-*D*-glucopyranoside, 12-neoastilbin, 13-astilbin, 14-nuezhenide, 15-neoisoastilbin, 16-isoastilbin, 17-rosmarinic acid, 18-salvianolic acid B, 19-salvianolic acid A.

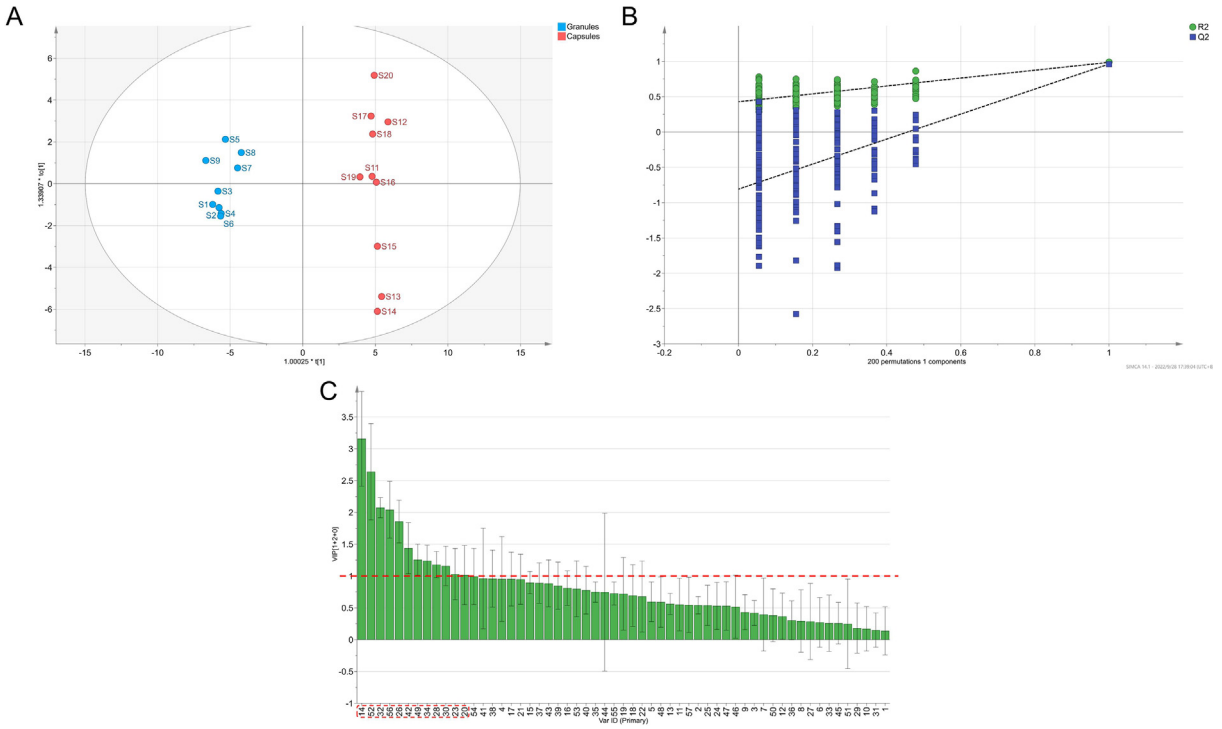

Fig. 3. Results of OPLS-DA and variable importance for projection (VIP) plot. (A) OPLS-DA score plot of HMYG and HMYC [R^2X = 0.802, R^2Y = 0.987 and Q^2 = 0.962]; (B) Permutations test (n = 200) [R^2Y = (0.0, 0.428), Q^2Y = (0.0, −0.808)]; (C) Variable importance for projection (VIP) plot.

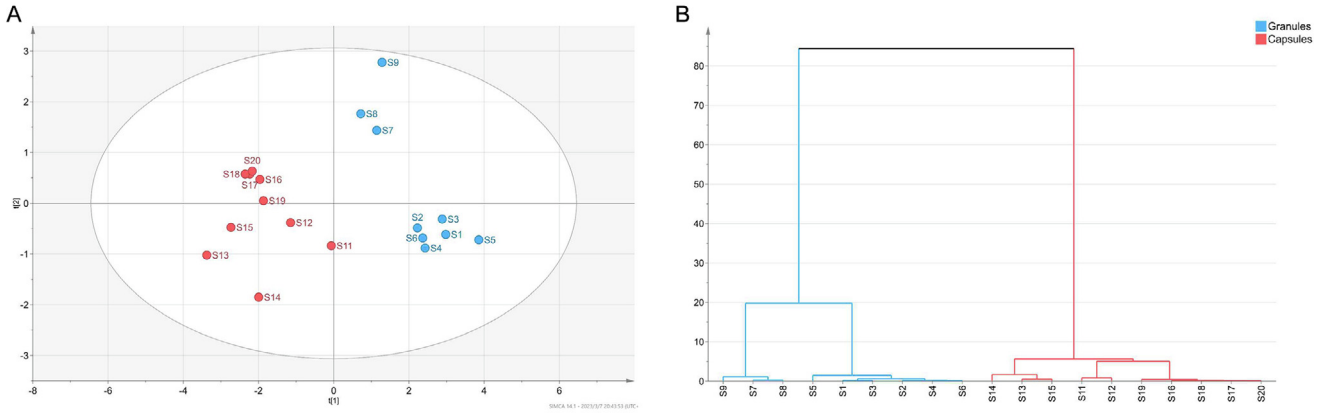

Fig. 4. Results of PCA (A, R^2X = 0.959 and Q^2 = 0.891) and HCA (B).

The contents determination results of nine components in HMYC and HMYG were shown in Figs. 6 and 7 after the conversion of the same raw drug amount (17.04 g). As shown in Fig. 7, the contents of eight components including neochlorogenic acid, protocatechualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, rosmarinic acid, danshensu, salvianolic acid A were significantly higher in HMYG than in HMYC.

The results of HPLC quantitative analysis above indicated that the contents of eight components in HMYG and HMYC samples were significantly different. Therefore, it is necessary and important to screen and control the differences between different dosage forms of Huamoyan.

4. Conclusion

In this study, we put forward a quality control idea for Chinese patent medicine with different dosage forms in the same prescription. Due to the influence of production process and other factors, the contents of some components in Chinese patent medicine with different dosage forms in the same prescription are quite different, which may affect the clinical efficacy. Firstly, we started with HMYG and HMYC, and established their HPLC fingerprint method. The fingerprint similarity results showed that there were differences between HMYG and HMYC, and further 57 common peaks and 20 characteristic peaks from the fingerprint results were

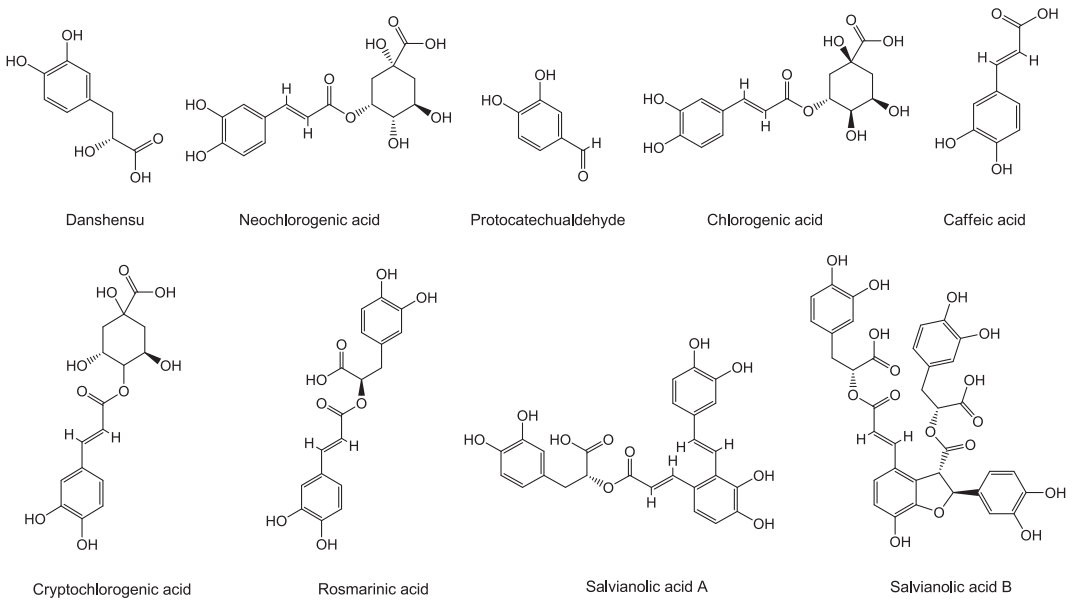

Fig. 5. Nine components in HMYG and HMYC.

Table 2
Validation of HPLC quantitative assay method.

Compounds	Calibration curves	Linear ranges (mg/mL)	Stability RSD (%)	Repeatability RSD (%)	Precision RSD (%)	Accuracy RSD (%)
Danshensu	$y = 126.37x + 0.084\ 2$ ($r = 0.999\ 7$)	0.000 8–0.413 9	1.24	1.44	1.51	1.40
Neochlorogenic acid	$y = 543.64x - 0.029\ 8$ ($r = 0.999\ 8$)	0.000 9–0.055 1	0.63	1.63	0.73	2.59
Protocatechualdehyde	$y = 796.62x - 0.199\ 3$ ($r = 0.999\ 6$)	0.000 8–0.049 7	1.78	2.49	0.58	2.39
Chlorogenic acid	$y = 561.43x - 0.028\ 9$ ($r = 0.999\ 9$)	0.000 8–0.047 0	1.24	1.54	0.64	1.25
Caffeic acid	$y = 931.50x + 0.010\ 6$ ($r = 0.999\ 8$)	0.000 8–0.082 1	1.25	0.97	0.73	1.41
Cryptochlorogenic acid	$y = 349.27x - 0.006\ 4$ ($r = 0.999\ 6$)	0.000 8–0.077 2	1.34	2.27	0.99	1.37
Rosmarinic acid	$y = 514.22x + 0.174\ 6$ ($r = 0.999\ 9$)	0.000 9–0.455 6	1.25	1.17	0.66	0.91
Salvianolic acid A	$y = 483.41x + 0.186\ 6$ ($r = 0.999\ 8$)	0.000 8–0.397 2	1.69	0.94	0.21	1.54
Salvianolic acid B	$y = 225.25x + 0.132\ 6$ ($r = 0.999\ 8$)	0.000 8–0.390 7	0.87	1.58	0.6	1.50

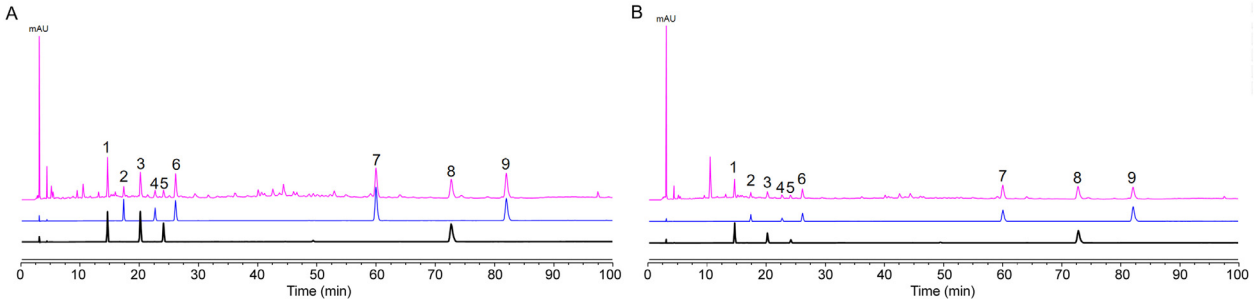

Fig. 6. HPLC chromatogram of nine components in HMYG (A) and HMYC (B). 1-danshensu; 2-neochlorogenic acid; 3-protocatechualdehyde; 4-chlorogenic acid; 5-cryptochlorogenic acid; 6-caffeic acid; 7-rosmarinic acid; 8-salvianolic acid B; 9-salvianolic acid A.

obtained. A total of 17 components were identified by comparing the 20 characteristic peaks with reference compounds. Then, in order to find the difference markers between HMYG and HMYC, the difference model of them was constructed by OPLS-DA using 57 common peak data, and the potential difference markers were screened by VIP value. The intersection of the screened potential difference markers and the previous qualitative 17 components was taken to obtain the difference markers being used for quantitative analysis, and the difference markers were tested by PCA and HCA. The results showed that the differential markers obtained by screening could effectively distinguish HMYC from HMYG in PCA and HCA. Finally, combining with the quality control standards of *Pharmacopoeia of the People's Republic of China* and other factors, a HPLC method was established for the determination of nine selected components, including seven differential markers. The content determination results showed that the content of dan-

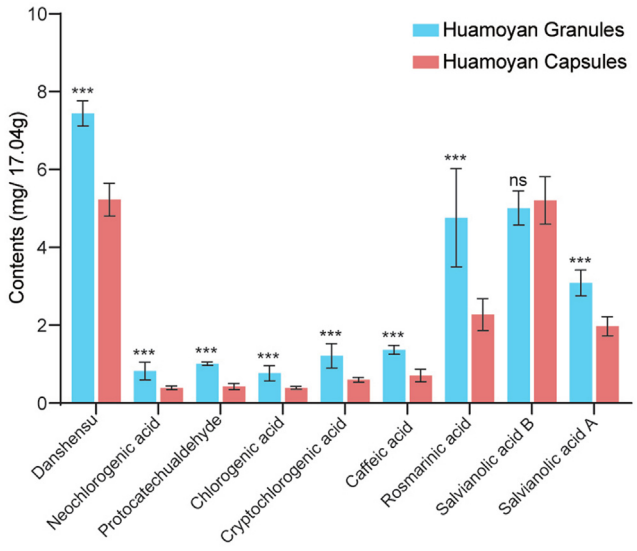

Fig. 7. Determination results and comparison of nine components in HMYG and HMYC. ($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ *vs* HMYC).

shensu, neochlorogenic acid, protocathualdehyde, chlorogenic acid, cryptochlorogenic acid, caffeic acid, rosmarinic acid and salvianolic acid A in HMYG was significantly higher than that in HMYC, which may be related to the concentration process of Chinese medical extract in the preparation process of HMYG and HMYC. The heating time of HMYG during the concentration process is longer than that of HMYC. According to some reports, phenolic acids such as salvianolic acid A, caffeic acid, danshensu, and protocathualdehyde are unstable, which are prone to degradation and interconversion especially under heating conditions or in aqueous solution (Pan, 2019; Li, Zhang, Zhou, Zhang, & Liu, 2020). However, the effect of the composition difference on the clinical pharmacological action of HMYG and HMYC is still unclear. This study provides material basis for further exploring the difference of clinical curative effect between HMYG and HMYC. In addition, this method can be used as a rapid and useful routine quality evaluation and control method, which is of great help to improve the quality standard and optimize the clinical use of Chinese patent medicines with different dosage forms of the same prescription.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2023.03.005.

References

Cao, Y. Y., Jia, P., & Wang, C. H. (2015). Clinical study of oral synovitis particles combined the herbal penetration therapy on acute traumatic knee synovitis. *Journal of Emergency in Traditional Chinese Medicine, 24*(3), 413–414.

Chen, Y., Zhu, S. B., Xie, M. Y., Nie, S. P., Liu, W., Li, C., ... Wang, Y. X. (2008). Quality control and original discrimination of *Ganoderma lucidum* based on high-performance liquid chromatographic fingerprints and combined chemometrics methods. *Analytica Chimica Acta, 623*(2), 146–156.

Chen, F. R., Liu, T., Yue, N., Bao, P., Zhi, D. G., & Huang, L. H. (2010). Comparative study on efficacy of different formulations of Huoxiang Zhengqi products. *Drugs & Clinic, 25*(6), 434–439.

Chen, B. C., Wang, F., Sun, R., Dong, J. T., Wang, X. F., & Chen, J. Q. (2014). Clinical efficacy of Hua-mo-yan granule in treating acute and chronic knee synovitis. *Chinese Journal of Bone and Joint Surgery, 7*(3), 226–230.

Chinese Pharmacopoeia Commission (2020). *Pharmacopoeia of the People's Republic of China 2020*. Beijing: China Medical Science Press.

Jiang, F. C., Yan, C. Y., Liu, S. L., & Yu, Y. Y. (2020). Determination of calycosin7-*O*-*β*-*D*-glucopyranoside for preparation of Huamoyan by HPLC. *Chinese Journal of Drug Evaluation, 37*(2), 111–114.

Kang, K., Zhang, J. J., Liu, S. L., & Li, N. (2020). Simultaneous determination of tetrandrine and fangchinoline in Huamoyan Tablets/Granules by solid phase extraction-HPLC method. *Chinese Journal of Drug Evaluation, 37*(3), 196–200.

Li, J. S., Bai, X. T., & Zhang, B. (2011). Assay of ursolic acid in Huamoyan Granules by HPLC. *Asia-Pacific Traditional Medicine, 7*(5), 30–31.

Lubes, G., & Goodarzi, M. (2017). Analysis of volatile compounds by advanced analytical techniques and multivariate chemometrics. *Chemical Reviews, 117*(9), 6399–6422.

Li, Z., Zheng, G. C., Liu, T. J., Yang, S. S., Wang, J., Gao, J., & Li, F. (2019). Clinical application of Huamoyan granule. *Asia-Pacific Traditional Medicine, 15*(12), 187–189.

Li, S. Q., Zhang, S. N., Zhou, L. H., Zhang, F. L., & Liu, W. Y. (2020). Research progress on salvianolic acids' degradation and transformation in aqueous solution from *Salvia Miltiorrhiza*. *Journal of Liaoning University of Traditional Chinese Medicine, 22*(4), 109–117.

Pan, J. Y. (2019). *Study on degradation of salvianolic acids*. Thesis of Doctor Degree: Zhejiang University.

Sabir, A., Rafi, M., & Darusman, L. K. (2017). Discrimination of red and white rice bran from Indonesia using HPLC fingerprint analysis combined with chemometrics. *Food Chemistry, 221*, 1717–1722.

Xu, Z. X., Ke, T., Zhang, Y. F., Guo, L. C., Chen, F., & He, W. B. (2021). Danshensu inhibits the IL-1 beta-induced inflammatory response in chondrocytes and osteoarthritis possibly via suppressing NF-kappa B signaling pathway. *Molecular Medicine, 27*(1), 80.

Zhang, Y. Y., Cui, L. J., Sun, W. J., Cheng, J. Y., & Tang, X. (2000). Comparison of pharmacological effects between Guanxin Suhe Wan and Guanxin Suhe Ruanjiaonang. *Pharmacology and Clinics of Chinese Materia Medica, 5*, 6–7.

Zhao, X. J., Wang, S. J., & Xia, H. L. (2009). Study on quality standard for Huamoyan Tablet. *Traditional Chinese Drug Research and Clinical Pharmacology, 20*(4), 366–368.

Zhang, L. Q. (2015). Elementary analysis of quality standards of traditional Chinese patent medicines in the *Chinese Pharmacopoeia* 2015 Edition. *Chinese Pharmaceutical Journal*, *50*(20), 1754-1756.

Zhang, K. L., & Guo, Y. X. (2016). Clinical observation on the curative effect of external application of Chinese medicine combined with Huamoyan Keli and functional exercise on knee osteoarthritis synovitis. *Rheumatism and Arthritis, 5*(6), 18–20.

Zhang, J. J., Kang, K., Yan, C. Y., Zhou, L., Hu, J. L., & Zhao, Y. R. (2019). Determination of specnuezhenide in Synovitis Granules and Synovitis Tablets by RP-HPLC. *Chinese Journal of Drug Evaluation, 36*(6), 420–422.

Zhang, Y. T., Xie, Q. F., Liang, H., Xie, J. H., Liu, X. Y., Wang, G. J., ... Li, Q. (2020). The clinical study on Huamoyan Granule for treatment of damp heat accumulation type gouty arthritis. *Research and Practice on Chinese Medicines, 34*(4), 70–73.