Scanning Electron Microscopy and Liquid Chromatography for Physical and Chemical Inspection of Industrial Pharmaceutical Traditional Chinese Herbal Medicine

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ABSTRACT: Multiherbal preparation of *Coptidis rhizoma*, *Scutellariae radix*, and *Rhei rhizoma* is a well-known herbal formula, which is widely used in the prescription for relieving heat toxicity, inflammation of the intestine, and eczema. However, little is known about the characteristics of the physical and chemical qualities of industrial pharmaceutical products. The aim of the study is to develop a liquid chromatography system to examine the quality and quantity of pharmaceutical products. Besides scanning electron microscopy, light microscopy photographs with Congo red staining and iodine–KI staining were used for physical examination of the quality of the pharmaceutical products. A reverse-phase C18 column was used to separate the analytes of baicalin, berberine, rhein, and p-hydroxybenzoate (internal standard) with a gradient eluent mobile phase of acetonitrile and 10 mM NaH₂PO₄ (pH 3.0, adjusted by orthophosphoric acid). The results demonstrated that a large variety of content range presents among the testing herbal pharmaceutical products. The contents of rhein, baicalin, and berberine were around 0.22 mg/g, respectively. The physical examination data demonstrated that different brands of industrial pharmaceutical products have different shapes of granules or rods. In summary, to ensure the clinical efficacy of complicated herbal medicine, both quality and quantity controls are all very important. This study provides a reference standard operating procedure guide for the quality control (QC) with chemical and physical examination for the Chinese herbal pharmaceutical products of San–Huang–Xie–Xin–Tang (SHXXT).

1. INTRODUCTION

According to the Chinese ancient medicinal literature, the herbal formula San–Huang–Xie–Xin–Tang (SHXXT) consists of *Rhei Rhizoma* (*Rheum officinale* Bail.), *Scutellariae Radix* (*Scutellaria baicalensis* Georgi), and *Coptidis Rhizoma* (*Coptis chinensis* Franch.) with a ratio of 2:1:1 or 1:1:1 as the dose of the composition. SHXXT is used for relieving heat toxicity, inflammation of the intestine, ulceration of tongue and mouth, eczema, and relieving the liver and toothache. Additionally, in contemporary scientific reports, this herbal preparation can be used to protect gastric mucosa⁵ and treat gastric inflammatory symptoms.⁶ A vast amount of literature demonstrated that the herbal formula emerges in various bioactivities, including antioxidation,⁵ anti-inflammatory,⁴ anti-hypertension⁵, anti-hepatitis C virus effects,⁹ anti-atherogenicity,¹⁰ and cardioprotection,¹¹ and the compound coptisine blocks NLRP3 inflammasome activation.¹² A previous report indicated that SHXXT protects against lipopolysaccharide (LPS)-activated microglia and 6-OHDA-induced neurotoxicity by reducing inflammation and oxidative stress.¹³ In the experiment of the Parkinson’s disease model, SHXXT provides protection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity through antioxidation and anti-apoptosis effects.¹⁴ Clinical studies have shown that SHXXT has an anti-atherosclerosis effect on human aortic smooth muscle cells.¹⁵ In animal experiments, SHXXT has protective effects on the heart of model rats with acute myocardial apoptosis induced by ischemia and reperfusion.¹²,¹⁶ Recently, SHXXT increasingly engaged in the treatment of dyslipidemia in Taiwan.

Traditionally, the preparation of traditional Chinese herbal formulas is an elaborative process. Following the processing of ancient Chinese medicinal books are important steps in decocting traditional Chinese herbal prescriptions.¹⁶ However, this time-consuming process may hardly be suitable for the
current fast-paced life. Therefore, commercial traditional Chinese formula products have developed over five decades for replacing the original and has become a modern trend. The numerous excipients, such as starch, gelatin, lactose, carboxymethyl cellulose, and crude herb powder, were added for granulation, which may affect the concentration of herbal ingredients. Therefore, to inspect the quality of active components of these products, a validated analytical method was required. A chromatographic method was applied to examine the quality of SHXXT previously. Besides physical inspection such as the shape of granules, the morphology of the traditional Chinese medicine powder or the additives of pharmaceutical herbal materials by scanning electron microscopy was used. Besides, due to the complicated herbal ingredients, it is not possible to measure all compounds from the herbs. Based on the guidance of quality control (QC), quality assurance (QA), and standard operation procedure (SOP) by the Department of Chinese Medicine and Pharmacy, Ministry of Health and Welfare, Taiwan, some potential bioactive ingredients or major compounds should be analyzed for the herbal preparation. Therefore, the potential bioactive compounds of baicalin, berberine, and rhein were selected for SHXXT from this study.

Based on the description above, our hypothesis is that the pharmaceutical formulation of granules may affect the chemical and physical qualities of the herbal medicine. To investigate this hypothesis, the aim of the study is to develop a validated chromatographic method to monitor the herbal ingredient of various pharmaceutical herbal products. To examine the physical quality of various brands of pharmaceutical products, scanning electron microscopy and light microscopy with Congo red and potassium iodide staining were applied to inspect the particle appearance and the crude fibers of herbal materials of SHXXT.

2. RESULTS AND DISCUSSION

2.1. Optimization of Separation. To achieve a suitable separation, adjusting the combination of the mobile phase, the type of column, and the pH value are the paths to reach our goal. Thus, not only different ratios of water and organic phases were tested but phosphate solutions with different pH values were also evaluated. The data suggested that the combination of acetonitrile and sodium dihydrogen phosphate adjusted with phosphoric acid was most suitable for the analysis due to the shape, separation, and retention times of the analyte peaks. Additionally, various reverse-phase columns were tested, but the Agilent HC-C18 reverse-phase column is the only one approaching our goal. Therefore, the eluent mobile phase used consists of acetonitrile and 10 mM NaH2PO4, and a gradient system was used according to the following program: 0.01–60 min, 5–95% A; 60–65 min, 95% A; 65–67 min, 95–5% A; and 67–87 min, 5% A. Finally, analyzing the pharmaceutical products with this optimized method was performed in this study. The high-performance liquid chromatography (HPLC) chromatograms of baicalin, berberine, and rhein in Figure 1 were detected at wavelengths of 276, 238, and 228 nm, respectively. The retention times of the standards are as follows: 21.6 min for baicalin, 24.9 min for berberine, and 32.8 min for rhein. The three compounds were detected under different wavelengths, which were (A) 276 nm for baicalin, (B) 238 nm for berberine, and (C) 228 nm for rhein. 1: baicalin, 2: berberine, and 3: rhein.

Figure 1. HPLC chromatograms of the three components, baicalin, berberine, and rhein. The three compounds were detected under individual wavelengths, which were (A) 276 nm for baicalin, (B) 238 nm for berberine, and (C) 228 nm for rhein. 1: baicalin, 2: berberine, and 3: rhein.

addition, this pattern of brand E was similar to those of the other brands.

2.2. Method Validation. Method validations of baicalin, berberine, and rhein were analyzed, including evaluation of the method’s lower limit of quantification (LLOQ), accuracy, and intraday as well as interday precision. The validation curves in the concentration range of 0.1–10 μg/mL baicalin, berberine, and rhein showed good linearity and acceptable correlation coefficients. Tables 1 and 2 show that the LLOQ for baicalin, berberine, and rhein was 0.1 μg/mL, and the intraday and interday precisions of baicalin, berberine, and rhein ranged from 1.98 to 13.36% and 2.44 to 14.32%, respectively. Compared with a previous report, this developed protocol has enhanced the sensitivity of the previous research (limit of detection (LOD): 0.17–2.06 μg/mL). Additionally, the intraday and interday accuracy were respectively in the range of −13.31 to 14.31 and −15.77 to 14.42%. The precision and accuracy values ranged less than ±15%, and the LLOQ values were within ±20%. Hence, it is acceptable in accordance with the FDA guidelines of the bioanalytical validation method, so this analytical method emerged to be stable and efficient. The selected HPLC wavelengths used to determine biologically active compounds in commercial drugs are consistent with previous reports on baicalin, berberine, and rhein. The HPLC method used to quantify the drug SHXXT product was well validated.
Figure 2. HPLC chromatograms of brand E extracts after 100-fold dilution. (A) Baicalin (8.27 μg/mL), retention time: 21.4 min, detection wavelength: 276 nm; (B) berberine (0.81 μg/mL), retention time: 24.4 min, detection wavelength: 238 nm; and (C) rhizin (0.60 μg/mL), retention time: 32.7 min, detection wavelength: 228 nm. 1: baicalin, 2: berberine, and 3: rhizin.

Table 1. Intraday Precision and Accuracy of Baicalin, Berberine, and Rhein

| nominal concentration (μg/mL) | observed concentration (μg/mL) | precision (%) | accuracy (%) |
|-------------------------------|-------------------------------|---------------|--------------|
| Baicalin                      |                               |               |              |
| 0.1                           | 0.11 ± 0.01                   | 7.08          | 14.31        |
| 0.5                           | 0.42 ± 0.04                   | 8.17          | −13.31       |
| 1                             | 0.98 ± 1.05                   | 9.07          | −2.43        |
| 5                             | 5.16 ± 0.39                   | 12.45         | 3.19         |
| 10                            | 9.93 ± 0.47                   | 4.78          | −0.74        |
| Berberine                     |                               |               |              |
| 0.1                           | 0.10 ± 0.01                   | 8.36          | −0.56        |
| 0.5                           | 0.46 ± 0.03                   | 8.80          | −8.08        |
| 1                             | 1.05 ± 0.12                   | 11.14         | 5.28         |
| 5                             | 4.98 ± 0.18                   | 3.54          | −0.35        |
| 10                            | 10.01 ± 0.34                  | 3.44          | 0.05         |
| Rhein                         |                               |               |              |
| 0.1                           | 0.09 ± 0.01                   | 5.93          | −12.93       |
| 0.5                           | 0.45 ± 0.04                   | 9.42          | −10.06       |
| 1                             | 0.97 ± 0.13                   | 13.36         | −3.35        |
| 5                             | 5.18 ± 0.54                   | 10.90         | 3.63         |
| 10                            | 9.92 ± 0.20                   | 1.98          | −0.85        |

Table 2. Interday Precision and Accuracy of Baicalin, Berberine, and Rhein

| nominal concentration (μg/mL) | observed concentration (μg/mL) | precision (%) | accuracy (%) |
|-------------------------------|-------------------------------|---------------|--------------|
| Baicalin                      |                               |               |              |
| 0.1                           | 0.11 ± 0.01                   | 4.82          | 14.42        |
| 0.5                           | 0.45 ± 0.06                   | 12.62         | −9.91        |
| 1                             | 0.87 ± 0.07                   | 8.14          | −12.82       |
| 5                             | 5.09 ± 0.36                   | 12.45         | 1.81         |
| 10                            | 9.95 ± 0.44                   | 4.41          | −0.47        |
| Berberine                     |                               |               |              |
| 0.1                           | 0.10 ± 0.01                   | 13.77         | 1.45         |
| 0.5                           | 0.48 ± 0.03                   | 14.32         | −3.63        |
| 1                             | 1.11 ± 0.12                   | 10.78         | 10.74        |
| 5                             | 5.59 ± 0.70                   | 12.36         | 13.76        |
| 10                            | 9.85 ± 0.44                   | 10.33         | −1.53        |
| Rhein                         |                               |               |              |
| 0.1                           | 0.08 ± 0.01                   | 3.76          | −15.77       |
| 0.5                           | 0.46 ± 0.04                   | 8.78          | −7.95        |
| 1                             | 0.98 ± 0.10                   | 10.59         | −2.21        |
| 5                             | 5.20 ± 0.14                   | 2.44          | 3.95         |
| 10                            | 9.90 ± 0.33                   | 3.32          | −0.96        |

Data are expressed as the mean ± S.D. (n = 3). Precision: RSD (%) = [standard deviation/Cobs] × 100. Accuracy: Bias (%) = [(Cobs − Cnom)/Cnom] × 100.

2.3. Contents of SHXXT Commercial Products. The different formulations of 12 samples, including powder forms, tablet forms, and one SHXXT water decoction, were evaluated using the optimized parameters. All of the examined products contained three active ingredients, as shown in Table 3. As is shown, among the herbal pharmaceutical products, the contents of rhein, baicalin, and berberine were approximately 0.22–22.46, 0.44–50.79, and 0.41–2.48 mg/g, respectively. The results suggest that the three major ingredients are detectable in each sample. Baicalin is the most abundant flavonoid in SHXXT, the result of which is similar to previous reports. However, the percentages of the three compounds in tested samples are significantly varied. According to the chart in Figure 3, brand D contains the most abundantly analyzed compositions than that of others. Excluding brand I, the baicalin level of SHXXT products and water decoction is more abundant than the other two compositions. Nonetheless,

Table 3. Quantitation of the Bioactive Compounds in Different Brands of Commercial San–Huang–Xie–Xin–Tang Products

| brands | baicalin (mg/g) | berberine (mg/g) | rhein (mg/g) |
|--------|----------------|-----------------|--------------|
| A      | 15.44 ± 3.40   | 1.26 ± 0.11     | 0.49 ± 0.08  |
| B      | 5.18 ± 0.20    | 0.07 ± 0.06     | 0.22 ± 0.04  |
| C      | 23.06 ± 3.76   | 0.41 ± 0.08     | 1.26 ± 0.20  |
| D      | 50.79 ± 3.69   | 1.36 ± 0.14     | 1.13 ± 0.07  |
| E      | 6.81 ± 2.90    | 0.73 ± 0.19     | 0.56 ± 0.12  |
| F      | 28.64 ± 1.30   | 0.49 ± 0.04     | 0.78 ± 0.24  |
| G      | 3.54 ± 0.19    | 1.20 ± 0.03     | 3.79 ± 0.51  |
| H      | 14.30 ± 1.18   | 1.92 ± 0.14     | 4.35 ± 0.57  |
| I      | 0.44 ± 0.21    | 1.33 ± 0.19     | 22.46 ± 1.67 |
| J      | 7.07 ± 2.28    | 1.66 ± 0.52     | 0.92 ± 0.29  |
| K      | 21.76 ± 0.81   | 2.48 ± 0.13     | 9.19 ± 0.41  |
| L      | 9.09 ± 0.08    | 1.03 ± 0.01     | 1.94 ± 0.12  |

Data are expressed as the mean ± SD (n = 3).
berberine is the minor ingredient of SHXXT products. The proportions of baicalin levels in brands C, D, and F are over 90, and brand F is particularly nearby 96 percent. Furthermore, brand D has the most abundant ingredient in baicalin of all tested samples, and the majority of the composition in brand I is rhein. The influences of the difference in the ratio of three chemical compositions between each sample are collecting herbal origins, herbal growth environments, the periods of cultivation, decoction processes, and granulation processes. As we mentioned before, the composition ratio of brand I is extremely different from others; hence, tracking some achievable information and analyzing them on the basis of our knowledge, the probable impact comes from the decoction processes. We hypothesized that the different decoction processes may affect the ability of extraction for certain compounds becoming more easily; thus, the specific component would be a higher amount than others. According to our findings, the underground parts of Rhei Rhizoma were baked with ethanol before decoction that causes the rhein level in brand I dramatic higher than other brands because of its physical properties. Additionally, baicalin, a flavonoid glycoside, is slightly water-soluble. As a result, the baicalin level of tested brands, excepting brand I, is the most abundant compound of the three due to the fact that the herbs were decocted with boiling water. Consequently, our finding supports our prior hypothesis, the decoction process will impact the ratio of extracted ingredients.

2.4. Microscopic Application for Determining the Additives of SHXXT Commercial Products. Poly sacchar-
ides are stained with Congo red through strong noncovalent interactions. The identification of the cellulose fibers via Congo red staining was achieved by an Aperio ScanScope slide scanner. The results revealed that SHXXT products made from different manufacturers (Figure 4a−k) and raw herbal powder (Figure 4m−o) exhibited red or pink masses, which indicated that samples A−K contain fiber components, suggesting the possible use of raw herbal powder or cellulose fiber as additives. The starch testing solution usually consists of iodine and potassium iodine. The complex of amylose and triiodide ions is responsible for the formation of a deep blue color.18,19 The identification of starch was evaluated by light microscopy using iodine−KI reagent staining. As shown by the photograph in Figure 5p, cornstarch turned blue or violet when stained with iodine−KI reagent, which was taken as a positive control for assessing the other samples. Furthermore, brands C, E, F, H, and K visibly indicate that they contain a certain amount of cornstarch, particularly brand K.

3. CONCLUSIONS

This study developed chemical and physical methods for evaluating the quality of the herbal pharmaceutical product SHXXT. A rapid and sensitive validated HPLC method was designed for the determination of the contents of rhein, baicalin, and berberine in eleven commercial traditional Chinese products. Among the tested herbal pharmaceutical products, in samples D and H, the baicalin level of powders and granulations was in the range of 50.79−14.3 mg/g, while the content of berberine was in the range of 1.36−1.92 mg/g and the rhein level was in the range of 1.13−4.35 mg/g. According to our investigation, the ratios of the three compounds in SHXXT products were extremely different because of different processing techniques. Congo Red staining was used to evaluate the amount of cellulose, and Iodine−KI staining was used to examine the amount of starch. According to our study, not only quality but also quantity controls for the bioactive compounds in different brands of pharmaceutics are
all very important to ensure the efficacy of herbal medicine. In addition, a standard method to examine the quality and quantity of various brands of commercial herbal formulas should be very important to ensure the clinical efficacy of herbal medicine.

4. MATERIALS AND METHODS

4.1. Chemical and Reagents. Rhein, baicalin, berberine, and p-hydroxybenzoate were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). HPLC-grade methanol, sodium dihydrogen phosphate (NaH2PO4), and orthophosphoric acid (H3PO4, 85%) were purchased from E. Merck (Darmstadt, Germany). Triple-deionized water (Millipore, Bedford, MA) was used in the study. The root and stem parts of Rhei Rhizoma, Scutellariae Radix, and Coptidis Rhizoma were purchased from the Lu–An Chinese Medicine Pharmacy (Taipei, Taiwan). The obtained herbs were identified after comparison with the specimens in the National Research Institute of Chinese Medicine of Taiwan. The manufacturers of commercial pharmaceutical products of SHXXT included SunTen Pharmaceutical Co., Ltd. (Taipei, Taiwan), Kaiser Pharmaceutical Co., Ltd. (Taiwan, Taiwan), KODA Pharmaceutical Co., Ltd. (Taoyuan, Taiwan), Sheng Chang Pharmaceutical Co. (Tainan, Taiwan), Chuan Feng Tang Pharmaceutical Co., Ltd. (Taoyuan, Taiwan), Chuang-Song-Zong Pharmaceutical Co., Ltd. (Kaohsiung, Taiwan), Tong-Yang Pharmaceutical Co., Ltd. (Tainan, Taiwan), and Fu Tain Pharmaceutical Co., Ltd. (Changhua, Taiwan). Eleven different testing samples from these manufacturers are presented with numeral codes when showing the analysis results. None of the manufacturers of the herbal products funded this investigation.

4.2. Extraction. According to the ancient Chinese medical literature, Synopsis of Golden Chamber, we prepared the decoction of SHXXT with Rhei Rhizoma, Scutellariae Radix and Coptidis Rhizoma in a ratio of 2:1:1, which was 18 g of Rhei Rhizoma, 9 g of Scutellariae Radix, and 9 g of Coptidis Rhizoma. First, these herbs were placed in a stainless-steel pot with 600 mL of cold water and then boiled to obtain the decoction. The decoction was covered and kept boiling for 20 min with low heat. Next, the concoction was filtered out. In the second step, we added 600 mL of cold water to the residue, and the first process was repeated after the concoction was filtered off. Finally, all of the samples were mixed together.

4.3. HPLC Conditions. Baicalin, berberine, and rhein were determined using a Shimadzu HPLC instrument (LC-20AT, A SPD-M20A UV-vis detector. Baicalin, berberine, rhein, and p-hydroxybenzoate (internal standard) were separated by an HPLC column (Agilent HC-C18 column: 100 × 4.6 mm² i.d., 5 µm). All analytes were monitored at 276 ± 4, 238 ± 4, and 228 ± 4 nm for the peak area determinations of baicalin, berberine, and rhein, respectively. The eluent mobile phase consisted of acetonitrile (A) and 10 mM NaH2PO4 (B) (pH = 3.0, adjusted by orthophosphoric acid). The gradient system was employed as the following program: 0.01−60 min, 5−95% A; 60−65 min, 95% A; 65−67 min, 95−5% A; and 67−87 min, 5% A.

4.4. Method Validation. Five concentrations ranging from 0.1 to 10 µg/mL of three QC standards were evaluated for calibrating the instruments. Linear calibration curves of all QC standards were evaluated based on the standard error of the slope and correlation coefficient (r² > 0.995). The limits of detection were estimated by a signal-to-noise ratio (S/N) of 3:1. The interday and intraday precision and accuracy were assessed using the percentage of relative standard deviation and bias. Six replicates of linear calibration curves on the same day and on consecutive days were estimated for the intraday and interday variation, respectively. The formulas RSD (%) = (standard deviation (SD)/Cobs) × 100 and bias (%) = [(Cobs − Cnom)/Cnom] × 100 were employed for calculating the percentage of relative standard deviation and bias. Cobs and Cnom denote the mean value of the observed concentrations and the nominal concentration, respectively. The accuracy and precision were less than ±15% and ±20%, respectively, at the LOQ for all of the analytes and thus were acceptable in this study.

4.5. Light Microscopy Photographs of Congo Red and Iodine−KI Stained Samples. Light microscopy images were acquired using an Aperio ScanScope CS scanner. Sample suspensions were placed on the microslides, and then, before being covered with coverslips, the samples were stained with 0.1% Congo red or 2% iodine−KI solution. Under a 100× microscope magnification, pink staining and purple staining were observed in the Congo red-stained slides and iodine solution stained slides, respectively.18

4.6. Statistical Analysis. SigmaPlot software (version 13.0) was used for statistical analyses. Aperio ScanScope CS (Aperio Technologies, Vista, CA) with the Aperio ImageScope (version 10.0) software was used to perform the microscopy photographic analysis.

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**Author Contributions**

W.-Y.P. performed the experiments, analyzed the data, and prepared the manuscript; T.-H.T. edited the paper and supervised the project.

**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Lin, W. C.; Tan, T. W. The role of gastric muscle relaxation in cytoprotection induced by San−Huang−Xie−Xin−Tang in rats. *J. Ethnopharmacol.* 1994, 44, 171−179.

(2) Saegusa, Y.; Sugiyama, A.; Takahara, A.; Nagasawa, Y.; Hashimoto, K. Relationship between phosphiesterase inhibition induced by several Kampo medicines and smooth muscle relaxation of gastrointestinal tract tissues of rats. *J. Pharmacol. Sci.* 2003, 93, 62−68.

(3) Iijima, O. T.; Takeda, H.; Matsumiya, T. Effects of San′o−shasitō on the antioxidative mechanism in spontaneous familial hypercholesterolaemic rabbits. *Pharmacol. Res.* 2000, 41, 137−141.

(4) Lo, Y. C.; Lin, Y. L.; Yu, K. L.; Lai, Y. H.; Wu, Y. C.; Ann, L. M.; Chen, I. J. San−Huang−Xie−Xin−Tang inhibits Helicobacter pylori-induced inflammation in human gastric epithelial AGS cells. *J. Ethnopharmacol.* 2007, 112, 537−544.

(5) Shih, Y. T.; Wu, D. C.; Liu, C. M.; Yang, Y. C.; Chen, I. J.; Lo, Y. C. San−Huang−Xie−Xin−Tang inhibits cytoprotection induced by lipopolysaccharide-exposed rat lungs. *J. Ethnopharmacol.* 2005, 101, 68−74.

(6) Chen, H. C.; Hsieh, M. T. Two-year experience with “San-Huang-Hsieh-Hsin-Tang” in essential hypertension. *Am. J. Chin. Med.* 1986, 14, 51−58.

(7) Chen, H. C.; Hsieh, M. T.; Tsai, H. Y.; Chang, H. H.; Wang, T. F.; Shibuya, T. Studies on the “San-Huang-Hsieh-Hsin-Tang” in the treatment of essential hypertension. *Taiwan Yi Xue Hui Za Zhi* 1984, 83, 340−346.

(8) Tsai, H. H.; Chen, I. J.; Lo, Y. C. Effects of San−Huang−Xie−Xin−Tang on U46619-induced increase in pulmonary arterial blood pressure. *J. Ethnopharmacol.* 2008, 117, 457−462.

(9) Lee, J. C.; Tseng, C. K.; Wu, S. F.; Chang, F. R.; Chiu, C. C.; Wu, Y. C. San−Huang−Xie−Xin−Tang extract suppresses hepatitis C virus replication and virus-induced cyclooxygenase-2 expression. *J. Viral Hepatitis* 2011, 18, e315−424.

(10) Wang, Y. S.; Lin, R. T.; Cheng, H. Y.; Yang, S. F.; Chou, W. W.; Juo, S. H. Anti-atherogenic effect of San−Huang−Xie−Xin−Tang, a traditional Chinese medicine, in cultured human aortic smooth muscle cells. *J. Ethnopharmacol.* 2011, 133, 442−457.

(11) Liou, S. F.; Ho, J. H.; Liang, J. C.; Ke, H. J.; Chen, I. J.; Wu, J. R.; Yeh, J. L. San−Huang−Xie−Xin−Tang protects cardiomyocytes against hypoxia/reoxygenation injury via inhibition of oxidative stress-induced apoptosis. *J. Nat. Med.* 2012, 66, 311−320.

(12) Wu, J. L.; Luo, Y.; Jiang, Q.; Li, S.; Huang, W.; Xiang, L.; Liu, D.; Hu, Y.; Wang, P.; Lu, X.; Zhang, G.; Wang, F.; Meng, X. Coptisine from Coptis chinensis blocks NLRP3 inflammasome activation through eNOS and MAPK pathways. *Evidence-Based Complementary Altern. Med.* 2011, 2011, No. 915051.

(13) Lo, Y. C.; Shih, Y. T.; Tseng, Y. T.; Hsu, H. T. Neuroprotective Effects of San−Huang−Xie−Xin−Tang in the MPP (+)/MPTP Models of Parkinson’s Disease In Vitro and In Vivo. *Evidence-Based Complementary Altern. Med.* 2012, 2012, 1−10.

(14) Li, C. Y.; Hou, Y. C.; Lee Chao, P. D.; Shia, C. S.; Hsu, I. C.; Fang, S. H. Potential ex vivo immunomodulatory effects of San−Huang−Xie−Xin−Tang and its component herbs on mice and humans. *J. Ethnopharmacol.* 2010, 127, 292−298.

(15) Lo, V.; Barrett, P. Cooking up Fine Remedies: On the Culinary Aesthetic in a Sixteenth-Century Chinese Materia Medica. *Med. Hist.* 2005, 49, 395−422.

(16) Trivedi, N. R.; Rajan, M. G.; Johnson, J. R.; Shukla, A. J. Pharmaceutical approaches to preparing pelletized dosage forms using the extrusion-spheronization process. *Crit. Rev. Ther. Drug Carrier Syst.* 2007, 24, 1−40.

(17) Cheng, Y. Y.; Tsai, T. H. Analysis of Sheng-Mai-San, a Ginseng-Containing Multiple Components Traditional Chinese Herbal Medicine Using Liquid Chromatography Tandem Mass Spectrometry and Physical Examination by Electron and Light Microscopes. *Molecules* 2016, 21, 1159−1175.

(18) Wu, T. Y.; Chang, F. R.; Liou, J. R.; Lo, I. W.; Chung, T. C.; Lee, L. Y.; Chi, C. C.; Du, Y. C.; Wong, M. H.; Juo, S. H.; Lee, C. C.; Wu, Y. C. Rapid HPLC Quantification Approach for Detection of Active Constituents in Modern Combinatorial Formula, San−Huang−Xie−Xin−Tang (SHXXT). *Front. Pharmacol.* 2016, 7, No. 374.

(19) Liu, J. H.; Cheng, Y. Y.; Hsieh, C. H.; Tsai, T. H. Identification of a Multicomponent Traditional Herbal Medicine by HPLC−MS and Electron and Light Microscopy. *Molecules* 2017, 22, No. 2242.

(20) Li, S. L.; Song, J. Z.; Qiao, C. F.; Zhou, Y.; Xu, H. X. UPLC−PDA−TOFMS based chemical profiling approach to rapidly evaluate chemical consistency between traditional and dispensing granule decoctions of traditional medicine combinatorial formulae. *J. Pharm. Biomed. Anal.* 2010, 52, 468−478.

(21) Chang, L. C.; Sun, S. W. Micellar electrokinetic chromatography for separation of a mixture of coticlal alkanoids, scute flavonoids, and rhubarb anthraquinones and bianthrones. *J. Pharm. Biomed. Anal.* 2006, 40, 62−67.

(22) Lai, M. Y.; Chen, C. C.; Hsu, S. L.; Chao, P. D. I. Analysis and Comparison of Baicalin, Baicalein and Wogonin Contents in Traditional Decoctions and Commercial Extracts of Scutellariae Radix. *J. Food Drug Anal.* 2001, 9, 145−149.

(23) Shih, Y. T.; Wu, D. C.; Liu, C. M.; Yang, Y. C.; Chen, I. J.; Lo, Y. C. San−Huang−Xie−Xin−Tang inhibits Helicobacter pylori-induced inflammation in human gastric epithelial AGS cells. *J. Ethnopharmacol.* 2007, 112, 537−544.

(24) Samim, M.; Sandkuijl, D.; Tretyakov, I.; Cisek, R.; Barzda, V. Differential polarization nonlinear optical microscopy with adaptive optics controlled multiplexed beams. *Int. J. Mol. Sci.* 2013, 14, 18520−18534.

(25) Bailey, J. M.; Whelan, W. J. Physical properties of starch. I. Relationship between iodine stain and chain length. *J. Biol. Chem.* 1961, 236, 969−973.

(26) Cheng, Y. Y.; Tsai, T. H. Analysis of Sheng-Mai-San, a Ginseng-Containing Multiple Components Traditional Chinese Herbal Medicine Using Liquid Chromatography Tandem Mass Spectrometry and Physical Examination by Electron and Light Microscopes. *Molecules* 2016, 21, 1159−1175.