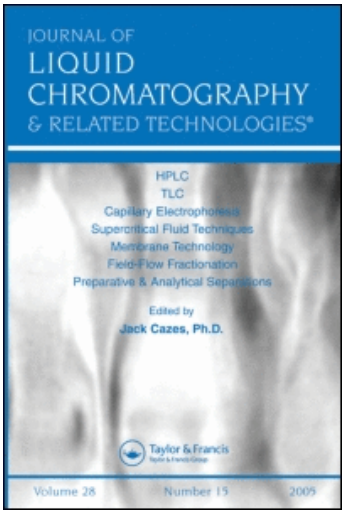

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Comparison of Microwave-Assisted and Ultrasound-Assisted Extraction for Determination of Main Water-Soluble Bioactive Constituents in Traditional Chinese Medicinal Preparation Tongmaichongji by HPLC-DAD

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ABSTRACT

Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) were compared as sample preparation methods for the determination of three main water-soluble bioactive constituents (danshensu, puerarin, and ferulic acid) in traditional Chinese medicinal preparation (TCMP) Tongmaichongji by HPLC-DAD. The optimal conditions of

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the two extraction methods were obtained. MAE could be completed in 2 min at 750 W of radiation power and 30% ethanol (pH 2.5) was used as extraction solvent. The time needed for UAE was 30 min, with 30% ethanol (pH 2.5) as extraction solvent. However, UAE allowed extraction at lower temperature. The present HPLC system used a Diamonsil C_{18} column ($150 \times 4.6\,mm^2$, I.D. 5 μm) connected with Zorbax Ext-C_{18} guard column ($12.5 \times 2.1\,mm^2$, 4-Pack) at 30°C, employing gradient elution with acetonitrile/water (pH 3) as mobile phase at a flow rate of 1 mL/min. The detection wavelength was set as: 0 min, 205 nm; 12 min, 254 nm; and 21 min, 320 nm. The linear calibration ranges were 6.8–81.9, 10.6–137.8, 4.9–77.8 μg/mL for danshensu, puerarin, and ferulic acid, respectively. The detection limits were 0.03, 0.10, 0.04 μg/mL for danshensu, puerarin, and ferulic acid, respectively. The contents of these three bioactive constituents in Tongmaichongji were successfully determined by the proposed method. The results indicated that there is a good agreement between the two extraction methods.

Key Words: Ultrasound-assisted extraction; Tongmaichongji; Microwave-assisted extraction; HPLC-DAD.

INTRODUCTION

Traditional Chinese medicinal preparations (TCMP) have been used with a long history throughout Southeast Asia, and can be found in the pharmacopoeias of numerous countries. In recent years, there have been a renaissance of interest in use of TCMP because of the realization that it can act in a synergistic manner in the human body, and can provide unique therapeutic properties with minimal or no undesirable side-effects.[1] A key factor in the widespread acceptance of TCMP involves its "modernization." In other words, the standardization and quality control of TCMP by use of modern science and technologies are critical; therefore, it is crucial to establish simple and reliable analytical technologies and methodologies for the chemical analysis of TCMP.

Sample preparation is the decisive first step in the analysis of TCMP, because it is necessary to extract the desired chemical constituents for further separation and determination. Microwave-assisted extraction (MAE) is a novel extraction technology, which combines microwave with traditional solvent extraction. Study shows that MAE has many advantages, such as shorter extraction time, less solvent consumption, higher extraction efficiency, better quality of products, and lower cost.[2] MAE has been widely applied for sample preparation of medicinal plants,[3–5] and environmental samples.[6–8]

Although, MAE has been widely applied to prepare medicinal plants samples, there is no report about the use of MAE in analysis of TCMP in any form, such as tablet, pill, capsule, and extraction granule up till now.

Ultrasound-assisted extraction (UAE) can be used for an extraction method with liquid solvents applied to analytes in solid matrices. The extraction process is fast in comparison with the traditional methods, due to the occurrence of particle disruption, because the contact surface area between solid and liquid phase is much greater.[9] At the same time, UAE is an inexpensive technology and has low instrumental requirements, and it also allows extraction in low temperature. UAE has been used for sample preparation of medicinal plants,[10,11] environmental samples,[12,13] and TCMP.[14,15]

Tongmaichongji preparation is a kind of TCMP used for the treatment of coronary heart disease, which is composed of three crude drugs, i.e., *Radix salviae miltiorrhizae*, *Rhizoma chuanxiong*, and *Radix puerariae*. Danshensu, puerarin, and ferulic acid, which play important roles in the clinic, are main water-soluble bioactive constituents in Tongmaichongji preparation. Pharmacological experiments showed that the biomedical effects of danshensu included improvement of blood circulation, prevention of blood loss in cardiac muscle, and resistance of blood coagulation.[16] Puerarin had the virtue of improvement of blood circulation, prevention of cardiovascular diseases, control of alcoholism, and treatment for arrhythmia.[17] Ferulic acid is a known antioxidant with various pharmacological properties, and generally effective as an antibiotic.[18] A literature search revealed that no satisfactory quality-control method for Tongmaichongji preparation has been established up till now, and only puerarin had been determined by HPLC,[19] and ferulic acid was only identified.[20] It is critical to establish a rapid and reliable quality-control means for Tongmaichongji preparation. An effective method for quality control is to determine main bioactive constituents in TCMP. Some other work has been done by our experimental group.[14,21]

In this study, the three main water-soluble bioactive constituents (danshensu, puerarin, and ferulic acid) in Tongmaichongji preparation were simultaneously analyzed by HPLC–DAD methods. To the sample preparation, MAE and UAE were applied and compared, and the conditions of MAE and UAE were both optimized. MAE resulted in a shorter extraction time; and UAE allowed extraction in lower temperatures. Determination result of MAE is in good agreement with that of UAE. The MAE was recommended for the sample preparation because of its celerity. The proposed method, as a rapid and reliable method, can be used for routine analysis of Tongmaichongji preparation for quality control. Furthermore, MAE is expected to be applicable for sample preparation of more TCMP.

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EXPERIMENTAL

Apparatus

HPLC was performed on a HP G1311A Quat Pump System, equipped with an HP G1315 diode array detector and HP G1328A manual injector (Hewlett Packard, USA). MAEs were performed with a Orient MDS-9000 Microwave Digestion System (Orient, Xi'an, China). UAEs were performed with a Transsonic SB3200 Apparatus (50 KHz, 120 W, Binengxin, Shanghai, China). An Avanti J-25 High-Speed Refrigerated Centrifuge (Beckman, USA) was used for centrifugation.

Reagents and Materials

Danshensu, puerarin, and ferulic acid were purchased from China National Institute of the Control of Pharmaceutical and Biological Products. Acetonitrile and methanol were HPLC grade. Ethanol was analytical grade. Redistilled water was used. *Radix salviae miltiorrhizae*, *Rhizoma chuanxiong*, and *Radix puerariae* were obtained from Tongrentang Pharmaceutical Group Company.

Two different Tongmaichongji preparations were analyzed. Tongmaichongji 1 (named T1) was prepared according to the Medicine Standards of Ministry of Health of P.R. China with *Radix salviae miltiorrhizae*, *Rhizoma chuanxiong*, and *Radix puerariae*.[22] Tongmaichongji 2 (named T2) was acquired from Beijing Kangdi Pharmaceutical Company (No. 20030102). Both sample T1 and T2 were ground into powder and dried for 4 hr at 50°C.

Ultrasound-Assisted Extraction Procedure

A 0.5 g of T1 was extracted with 10 mL 30% ethanol (pH 2.5) in an ultrasonic bath for 30 min. The supernatant liquid was separated from the solid phase by centrifugation for 10 min at a speed of 10,000 r/min. The solution was quantitatively transferred into a 10-mL volumetric flask and made up to volume with 30% ethanol (pH 2.5). The solutions were passed through 0.45-μm syringe filters before use.

Microwave-Assisted Extraction Procedure

A 0.5 g of T1 was weighed into the Teflon vessel, and then, 10 mL 30% ethanol (pH 2.5) was added. The Teflon vessel was closed and heated in the

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microwave oven for 2 min at 750 W. Once the digestion program was finished, the vessel was cooled to room temperature in an ice bath before being opened. The extract was centrifugated at a speed of 10,000 r/min for 10 min. The solution was quantitatively transferred into a 10-mL volumetric flask and made up to volume with 30% ethanol (pH 2.5). The solutions were passed through 0.45-μm syringe filters before use.

Chromatographic Conditions

Satisfactory separation of the three constituents was obtained with a Diamonsil C_{18} (150 × 4.6 mm^2, I.D. 5 μm, Dikma, Beijing, China) connected with Zorbax Ext-C_{18} guard column (12.5 × 2.1 mm^2, 4-Pack) at 30°C, eluted at a flow rate of 1 mL/min with gradient elution of A–B [A = acetonitrile; B = water (pH 3)], varying as follows: 0 min, 5 : 95; 10 min, 10 : 90; 12–19 min, 15 : 85, and 21 min, 20 : 80 (v/v). The detector was set as: 0 min, 205 nm; 12 min, 254 nm; and 21 min, 320 nm. Each solution of 20 μL was injected and chromatograms were recorded.

Standard Solutions

Stock solutions of puerarin and ferulic acid were prepared with methanol, and danshensu was directly prepared using water (pH 3), considering its stability. The various concentrations were within the range 6.8–81.9, 10.6–137.8, 4.9–77.8 μg/mL for danshensu, puerarin, and ferulic acid, respectively. Calibration graphs were plotted subsequently for linear regression analysis of the peak area with concentrations.

Sample Determination

A 0.5 g of T1 was extracted with the UAE procedure and MAE procedure, respectively; then it was analyzed in the chromatographic conditions as above. Calculations of the contents of danshensu, puerarin, and ferulic acid in T1 were based on calibration curves. At the same time, sample T2 was analyzed.

Recovery Studies

To study the accuracy and precision of the above methods, a recovery experiment was performed. Two different amounts of the stock solutions of

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danshensu, puerarin, and ferulic acid were added into the sample powder. Then the powder was dried, extracted with MAE and UAE procedures, respectively, and analyzed as above. The added concentration of analytes were 9.1 and 22.8 μg/mL for danshensu; 28.2 and 55.1 μg/mL for puerarin; and 8.5 and 19.5 μg/mL for ferulic acid, respectively. All samples were filtered through 0.45-μm syringe filters and injected for HPLC analysis.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

We chose optimal separation conditions involving a two phases system, acetonitrile/water (pH 3) as gradient elution solvent, which greatly reduced analysis time and allowed baseline separation for the three analytes' peaks. Maximally efficient detection was obtained by selecting the maximum absorption wavelength of every component (205 nm for danshensu, 254 nm for puerarin, and 320 nm for ferulic acid). The ultraviolet spectra of the three analytes were shown in Fig. 1. Satisfactory separation of the three constituents was obtained with a Diamonsil C_{18} (150 × 4.6 mm^2, I.D. 5 μm), connected with ZORBAX Ext-C_{18} guard column (12.5 × 2.1 mm^2, 4-Pack) at 30°C, and eluted at a flow rate of 1 mL/min.

The diode array detector facilitated the identification and confirmation of these three constituents. Figure 2(A) shows HPLC chromatograms of danshensu, puerarin, and ferulic acid with the retention times of 8.371 min for danshensu, 17.080 min for puerarin, and 25.503 min for ferulic acid. Figure 2(B) presents a chromatogram showing the separation of the constituents with sample preparation by ultrasonic extraction, and Fig. 2(C) described the chromatogram related to sample preparation by MAE.

Selection of Extraction Solvent for MAE and UAE

The selection of solvent influenced the yields of the analytes. Five different solvents, 30% ethanol, 50% ethanol, 80% ethanol, methanol, and 1% acetic acid, which were all adjusted to pH 2.5 with hydrochloric acid, were used for extraction of danshensu, puerarin, and ferulic acid in sample T1. The MAE and UAE procedures were applied, respectively. The microwave radiation was performed at 300 W for 5 min. The digestion vessel was taken out of the microwave oven at 2.5 min and cooled in an ice bath in order to avoid superheating, which will result in leakage of solution from the digestion vessel's valve. Then, again, it was put into the microwave oven to be radiated

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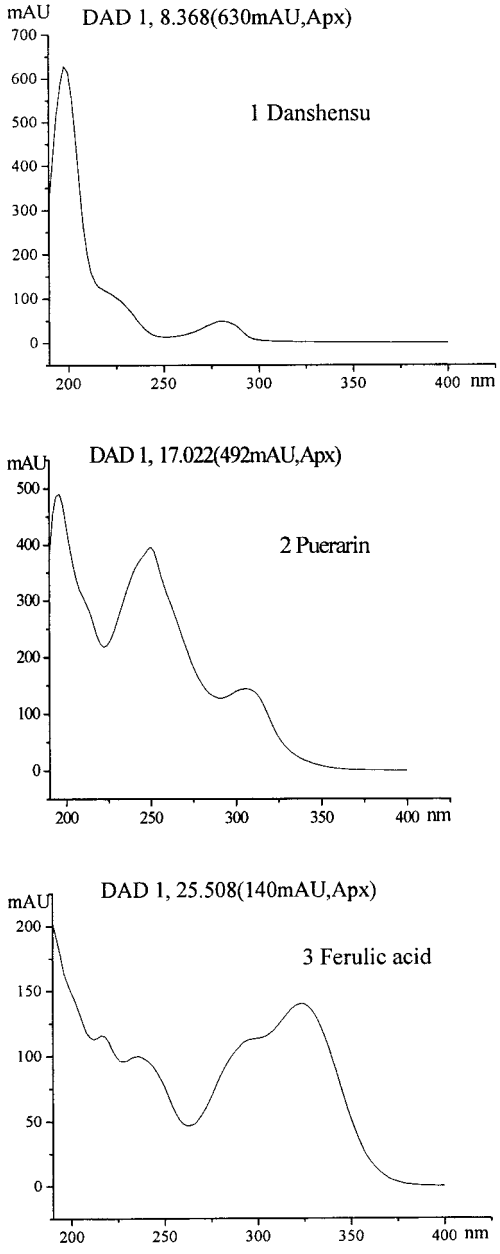

Figure 1. Ultraviolet spectra of danshensu, puerarin, and ferulic acid.

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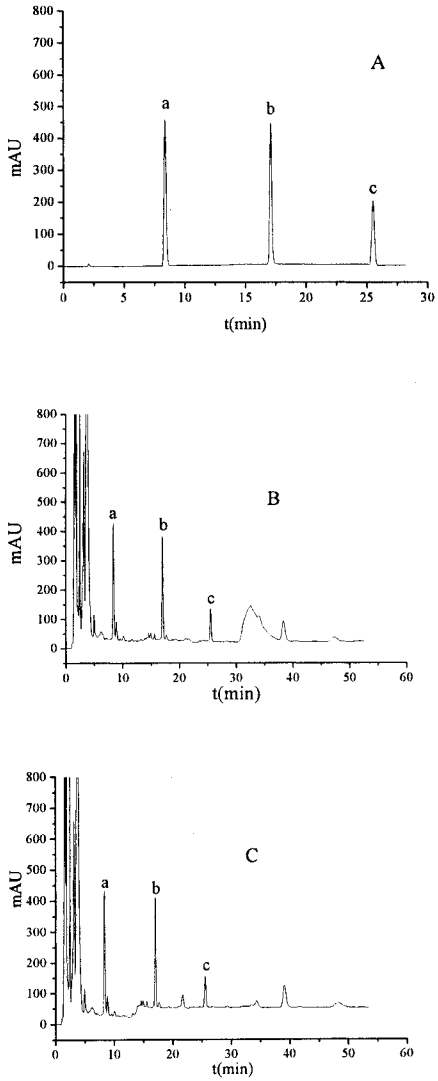

Figure 2. (A) HPLC chromatogram of danshensu (a), puerarin (b), and ferulic acid (c). (B) HPLC chromatogram of 30% ethanol (pH 2.5) extract of Tongmaichongji preparation with ultrasonic extraction. (C) HPLC chromatogram of ethanol (pH 2.5) extract of Tongmaichongji preparation with MAE. HPLC conditions, column: Diamonsil C_{18} column, $150 \times 4.6\,mm^2$ I.D., $5\,\mu m$; guard column: Zorbax Ext-C_{18} guard column ($12.5 \times 2.1\,mm^2$, 4-Pack); mobile phase: A–B [A = acetonitrile; B = water (pH 3)], 0 min, 5 : 95; 10 min, 10 : 90; 12–19 min, 15 : 85; and 21 min, 20 : 80 (v/v); flow rate: 1 mL/min; detector was set as: 0 min, 205 nm; 12 min, 254 nm; and 21 min, 320 nm.

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for the next 2.5 min. When the microwave radiation was finished, the digestion vessel was cooled to room temperature in an ice bath before being opened. The UAE lasted 30 min. All solutions were filtered through 0.45-μm syringe filters and injected for HPLC analysis. The yields of danshensu, puerarin, and ferulic acid were shown in Table 1. With 30% ethanol (pH 2.5), all of the three analytes yielded better extraction efficiency. Therefore, 30% ethanol (pH 2.5) was selected as the extraction solvent for both MAE and UAE throughout this work.

Selection of Ultrasound-Assisted Extraction Time

Temperature of the extraction medium increases with increasing sonication time. Usually, as the temperature increases up to 50°C, extraction efficiency is increased as a result of the larger number of cavitation nucleus formed.[9] The influence of sonication time on extraction efficiency of danshensu, puerarin, and ferulic acid was shown in Fig. 3. The three analytes could be extracted completely in 30 min. Therefore, a sonication time of 30 min seems to be suitable in order to shorten the analysis time.

Optimization of the Microwave-Assisted Extraction Conditions

Microwave radiation power and time were investigated at the same time. Radiation time was tested during 1, 2, 3, 5, 7.5, and 10 min; and radiation

Table 1. The yield (wt.%) of danshensu (Dan), puerarin (Pue), and ferulic acid (Fer) extracted from sample T1 by MAE and UAE methods.

	Yield (%)					
	Ultrasound-assisted extraction			Microwave-assisted extraction		
Extracting solvent	Dan	Pue	Fer	Dan	Pue	Fer
30% Ethanol	0.1039	0.1220	0.0423	0.1027	0.1216	0.0426
50% Ethanol	0.1036	0.1237	0.0414	0.1004	0.1176	0.0368
80% Ethanol	0.1040	0.1058	0.0421	0.0985	0.0941	0.0407
Methanol	0.0998	0.1214	0.0410	0.1023	0.110	0.0385
1% Acetic acid	0.1027	0.0963	0.0401	0.0993	0.1247	0.0379

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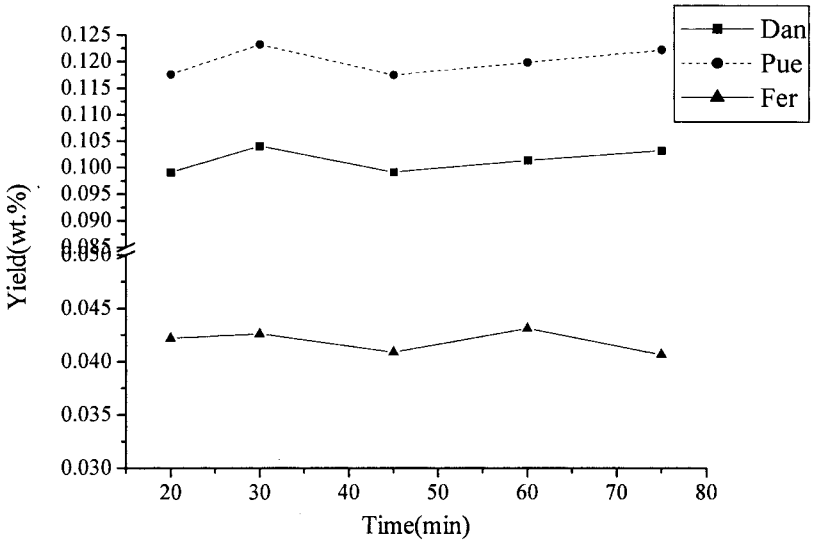

Figure 3. The effect of ultrasonic extraction time on yields of danshensu, puerarin, and ferulic acid.

power was studied at 300, 450, 600, and 750 W. A series of experiments was designed and the effect of radiation power and time was shown in Table 2. The digestion vessel was taken out of the microwave oven every 2 min when the radiation power was set at 750 or 600 W, and cooled in an ice bath in order to avoid superheating. Similarly, it was taken out every 3 min at 450 or 300 W and cooled. According to Table 2, yields of the three analytes have little improvement when the product of radiation power and time is above 1500 W min. Hence, 750 W was selected as microwave radiation power considering analysis time. Then, the radiation time was investigated again at 750 W. The effect of radiation time on the yields of the three analytes was described in Fig. 4. Two minutes was enough for the complete extraction of three analytes, danshensu, puerarin, and ferulic acid. We finally chose extraction for 2 min and set radiation power at 750 W.

Calibration Graphs and Reproducibility Test

Calibration graphs were plotted, based on linear regression analysis of the integrated peak areas (y, integration units) vs. concentrations (x, μg/mL) of the three constituents in the standard solution. Each standard solution was analyzed three times. The regression equations, correlation coefficients,

Table 2. The effect of microwave radiation power and time on yields of danshensu (Dan), puerarin (Pue) and ferulic acid (Fer).

No.	Radiation time (min)	Radiation power (W)	Yield (wt.%)		
			Dan	Pue	Fer
1	1	750	0.0877	0.0914	0.0403
2	2	450	0.1036	0.1038	0.0372
3	3	600	0.1003	0.1275	0.0394
4	5	300	0.1064	0.1237	0.0401
5	7.5	750	0.1078	0.1279	0.0400
6	10	450	0.1082	0.1249	0.0390
7	1	600	0.0719	0.0832	0.0386
8	2	300	0.0924	0.1031	0.0395
9	3	750	0.1094	0.1268	0.0413
10	5	450	0.1057	0.1197	0.0402
11	7.5	600	0.1043	0.1281	0.0395
12	10	300	0.1056	0.1260	0.0373

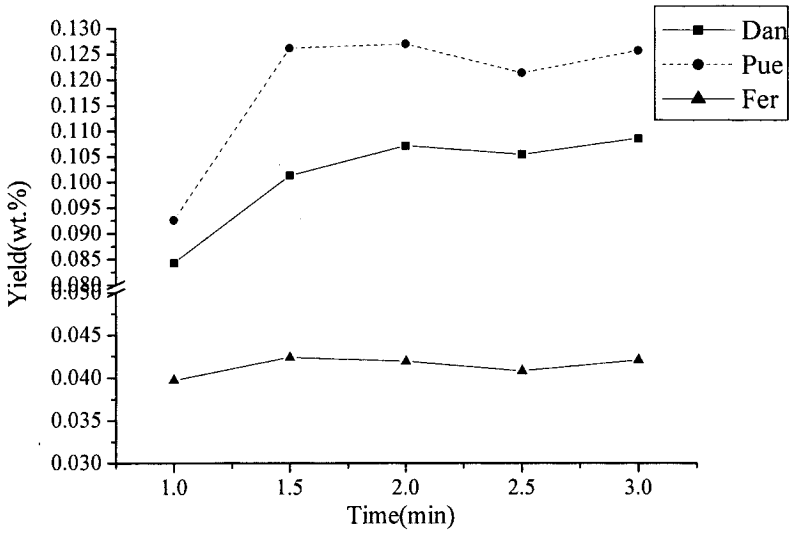

Figure 4. The effect of microwave-extraction time on yields of danshensu (Dan), puerarin (Pue), and ferulic acid (Fer) at 750 W of radiation power.

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Table 3. HPLC data for the calibration graphs.

Compound	Regression equation	Correlation coefficient	Linear range (μg/mL)	Detection limit (μg/mL)
Danshensu	$y = 49.117 + 108.236x$	0.99967	6.8–81.9	0.03
Puerarin	$y = -70.583 + 76.412x$	0.99995	10.6–137.8	0.10
Ferulic acid	$y = 29.231 + 81.823x$	0.99993	4.9–77.8	0.04

and linear ranges for the analysis of the three bioactive constituents are shown in Table 3. It showed good linear relationships between the peak areas and the concentrations. A signal to noise ratio of 3 was regarded as the detection limit. The detection limits of these three constituents are also shown in Table 3.

To assess the precision of these methods, we injected standard solutions of danshensu, puerarin, and ferulic acid, respectively, five times on the same day and in a 5-day period. The coefficient variations of intra-day and inter-day studies were less than 1.8 and 2.8%, respectively. The precision, as well as, accuracy of this assay was satisfactory (Table 4).

Comparison of Analytical Results Using MAE and UAE

MAE and UAE were both applied for sample preparation of T1 and T2 in their optimal conditions. Analytical results obtained by MAE and UAE,

Table 4. Intra-day and inter-day assay variations of danshensu, puerarin, and ferulic acid ($n = 5$).

Constituent	Concentration (μg/mL)	Intra-day RSD (%)	Inter-day RSD (%)
Danshensu	27.3	0.6	1.8
	54.6	0.4	1.4
	81.9	0.3	1.3
Puerarin	42.4	1.6	1.8
	74.2	0.4	0.5
	106.0	0.5	0.4
Ferulic acid	19.4	1.8	2.8
	38.9	1.4	1.5
	58.4	0.7	1.2

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Table 5. Contents of three bioactive constituents in T1 and T2 using MAE and UAE.

Sample	Extraction method	Content (mg/g)		
		Danshensu	Puerarin	Ferulic acid
T1	MAE	1.035 ± 0.017	1.244 ± 0.054	0.414 ± 0.002
	UAE	1.022 ± 0.008	1.234 ± 0.016	0.419 ± 0.003
T2	MAE	1.871 ± 0.057	7.277 ± 0.282	0.133 ± 0.004
	UAE	1.888 ± 0.011	7.193 ± 0.100	0.136 ± 0.003

corresponding to sample T1 and T2, are listed in Table 5. The following formula was used to investigate the analytical results with MAE and UAE.

$$\text{Relate recovery } (\%) = \left[\frac{(\text{content found with MAE})}{(\text{content found with UAE})}\right] \times 100$$

Relate recovery was 100.2 ± 1.6, 101.0 ± 0.3, and 98.3 ± 0.7 for danshensu, puerarin, and ferulic acid, respectively, thus indicating that there was good agreement between the two extraction methods.

Recoveries of the methods were studied through adding different amounts of the three analytes into the samples of known contents. The mixtures were extracted with MAE and UAE, respectively, and analyzed following the proposed procedures. The results of standard recovery study are given in Table 6.

Table 6. Recoveries of danshensu, puerarin, and ferulic acid in T1 with MAE and UAE ($n = 5$).

Constituent	Amount added (μg/mL)	Microwave-assisted extraction		Ultrasound-assisted extraction	
		Amount found (μg/mL)	Recovery (%)	Amount found (μg/mL)	Recovery (%)
Danshensu	9.1	9.4	103.3 ± 2.1	9.3	102.2 ± 0.6
	22.8	22.7	99.6 ± 3.8	21.7	95.2 ± 0.5
Puerarin	28.2	28.0	99.3 ± 3.0	27.2	96.5 ± 0.7
	55.1	54.3	98.5 ± 2.1	56.3	102.2 ± 0.6
Ferulic acid	8.5	8.6	101.2 ± 3.2	8.9	104.7 ± 1.1
	19.5	19.7	101.0 ± 2.0	19.1	97.9 ± 1.2

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CONCLUSION

The method described offers a rapid and efficient sample preparation for simultaneous analysis of three water-soluble bioactive constituents, i.e., danshensu, puerarin, and ferulic acid in Tongmaichongji preparation with HPLC-DAD. MAE was compared with UAE for sample preparation. MAE could be finished in 2 min, which is faster than UAE, which needs 30 min. But the procedure with UAE is safer than microwave radiation, as neither pressure nor high temperature is present during the extraction procedure, and UAE has lower instrumental requirements. Accurate and precise results were obtained with the two different extraction procedures; and the result of MAE showed good agreement with that of UAE. The proposed method can be applied for routine analysis of Tongmaichongji preparation for quality control.

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REFERENCES

1. Kaufman, P.B.; Csake, L.J.; Warber, S.; Duke, J.A.; Brielmann, H.L. *Natural Products from Plants*; CRC Press: Boca Raton, FL, 1999.
2. Ganzler, K.; Salgo, A.; Valko, K. Microwave extraction, a novel sample preparation method for chromatography. J. Chromatogr. **1986**, *371*, 299–306.
3. Wang, J.; Shen, P.N.; Shen, Y.J. Microwave-assisted extraction of effective compounds from traditional Chinese herbal medicine Ciwujia. Chinese J. Chem. Eng. **2003**, *11*, 231–233.
4. Hao, J.Y.; Han, W.; Huang, S.D.; Xue, B.Y.; Deng, X. Microwave-assisted extraction of artemisinin from *Artemisia anuna* L. Sep. Purif. Technol. **2002**, *28*, 191–196.
5. Guo, Z.K.; Jin, Q.H.; Fan, G.Q.; Duan, Y.P.; Qin, C.; Wen, M.J. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine *Radix puerariae*. Anal. Chim. Acta **2001**, *436*, 41–47.
6. Camel, V. Microwave-assisted solvent extraction of environmental samples. Trac-Trend Anal. Chem. **2000**, *19*, 229–248.
7. Wei, M.C.; Jen, J.F. Determination of chlorophenols in soil samples by microwave-assisted extraction coupled to headspace solid-phase

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microextraction and gas chromatography–electron-capture detection. J. Chromatogr. A **2003**, *1012*, 111–118.
8. Yang, J.S.; Lee, D.W.; Lim, H.B. Microwave-assisted extraction (MAE) of polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins from fly ash and sea sediments: effect of water and removal of interferences. J. Liq. Chromatogr. Rel. Technol. **2003**, *26*, 803–818.
9. Filgueiras, A.V.; Capelo, J.L.; Lavilla, I.; Bendicho, C. Comparison of ultrasound-assisted extraction and microwave-assisted digestion for determination of magnesium, manganese, and zinc in plant samples by flame atomic absorption spectrometry. Talanta **2000**, *53*, 433–441.
10. Hromadkova, Z.; Ebringerova, A.; Valachovic, P. Ultrasound-assisted extraction of water-soluble polysaccharides from the roots of valerian (*Valeriana officinalis L*). Ultrason. Sonochem. **2002**, *9*, 37–44.
11. Wu, J.Y.; Lin, L.D.; Chau, F.T. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. Ultrason. Sonochem. **2001**, *8*, 347–352.
12. Caballo-Lopez, A.; de Castro, M.D.L. Continuous ultrasound-assisted leaching of phenoxyacid herbicides in soil and sediment with in-situ sample treatment. Chromatographia **2003**, *58*, 257–262.
13. Yang, J.S.; Jeong, Y.H.; Lee, D.W.; Chang, Y.S. Effect of solvent and temperature on extraction of polychlorinated biphenyls and polychlorinated dibenzodioxins from fly ash. J. Liq. Chromatogr. Rel. Technol. **2001**, *24*, 2815–2825.
14. He, J.T.; Shi, Z.H.; Chang, W.B. Determination of puerarin and daidzein by RP-HPLC. Chinese J. Anal. Chem. **2004**, *32* (4).
15. Lay, H.L.; Chen, C.C. Simultaneous analysis of eight components in "ping-wei-san" by high performance liquid chromatography. J. Liq. Chromatogr. Rel. Technol. **2000**, *23*, 1439–1450.
16. Luo, H.J.; Bi, K.S.; Zhou, S.Y.; Wei, Q.H.; Zhang, R.H. Determination of danshensu, a major active compound of *Radix salvia miltiorrhiza* in dog plasma by HPLC with fluorescence detection. Biomed. Chromatogr. **2001**, *15*, 493–496.
17. Yu, B.S.; Yan, X.P.; Zhen, G.B.; Rao, Y.P. RP-HPLC determination of puerarin in Chinese traditional medicinal preparations containing pueraria. J. Pharm. Biomed. Anal. **2002**, *30*, 843–849.
18. Couteau, D.; Mathaly, P. Fixed-bed purification of ferulic acid from sugar-beet pulp using activated carbon: optimization studies. Bioresource Technol. **1998**, *64*, 17–25.
19. Yu, J.Q.; Lei, J.C.; Zhao, X.; Cai, H.S. Preparation of tongmaichongji and determination of puerarin. Acta Chinese Med. Pharm. **1998**, *3*, 53–54.
20. Su, Y.; Xiong, Y. Quality control of tongmai powder. China Pharmacist **2001**, *4*, 24–25.

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21. Shi, Z.H.; Chang, W.B. Simultaneous separation and determination, of five bioactive components in traditional Chinese medicinal formula, guanxin II, by HPLC. J. Liq. Chromatogr. Rel. Technol. **2003**, *26*, 469–482.
22. Pharmacopoeial Council of Ministry of Health of China. *Medicine Standards of Ministry of Health of China,* 1998 Ed.; Chemical Industry Press: Beijing, 1998; 25.

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