Review

Analysis of *Salvia miltiorrhiza* (Danshen)

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Danshen is one of the most popular traditional Chinese medicines (TCMs) in China. In recent years, people become increasingly aware of the value of Danshen, which is attracting global attention. Modern researches indicate that phenolic acids and tanshinones are the main chemical components related to the bioactivities and pharmacological properties of Danshen. Because of the diversity of the chemical structures and characters of these components, an analytical method of Danshen varies, including GC–MS, HPLC–DAD–MS, and so on. Besides that, the development of the analytical technology makes the quality control of Danshen more effective and reliable. Quality evaluation is from single or several components analysis to fingerprint, or in combination. In this paper, much attention was paid to introducing the methods developed for the isolation of major compounds from Danshen and identification of the chemical components of Danshen.

Key words: *Salvia miltiorrhiza* (Danshen), water-soluble compounds, lipophilic compounds, isolation, quantitative analysis, fingerprint.

INTRODUCTION

Nowadays, screening of bioactive compounds in traditional Chinese medicines (TCMs) has become one of the excellent ways to discover new drugs, which is attracting more global attention (Bradbury et al., 2005). *Salvia miltiorrhiza* root (Labiatae, Laminaceae) is slightly cold and bitter, which has the functions of promoting blood circulation, nourishing the nerves, and restoring menstrual flow to stop pain. Modern pharmacological studies have proven its widespread pharmacological activities. It has been widely used as anticoagulation, vasodilators, antibacterial and anti-inflammatory (He et al., 2010; Liu et al., 2010). Salvia has been widely applied for several decades in clinics in China, Korea, Japan and other Asian countries, for the treatment of heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, dysmenorrheal, chronic renal failure, neuroasthenic insomnia and anticancer. It also has cytotoxicity against human tumor cell lines. Salvia has the special role of anti-tumor. Its application is expected to become a clinical adjuvant treatment of cancer (Li et al., 2008; Wu et al., 2003; Cheng, 2006; Zhang et al., 1999; Lee et al., 2008; Wen et al., 2010). In virtue of its good treatment and few side effects, Danshen has been officially listed in the Chinese Pharmacopoeia and used widely and successfully in clinics in China.

Over the past 50 years, comprehensive researches on its chemical components and biological activities were carried out by people. Up to now, over 20 phenolic acids have been isolated and identified from Danshen, including caffeic acid monomers and oligomers, and the latter are also called depsides or salvianolic acids (Jiang et al., 2005).

Danshen has attracted increasing attentions in recent years because of its widespread pharmacological activities. Modern researches indicated that many biological effects of Danshen were related to two kinds of chemical components, phenolic acids and tanshinones.

Chemical constituents from *S. miltiorrhiza* root extract (SMRE) are classified into 2 major categories which are water-soluble compounds (WSC) and lipophilic diterpenoid quinines (LDQ), both of the compounds have been mostly identified and purified (Gu et al., 2004; Hu et
Figure 1. Major water-soluble compounds derived from SMRE. Major lipophilic compounds of SMRE are shown in Figure 2 including tanshinone I (TsI), tanshinone IIA (TsIIA), tanshinone IIB (TsIIB), cryptotanshinone (CTs), tanshindiol C (TsC), 15, 16-Dihydrotanshinone I (15, 16-DTsI), isotanshinone I (ITsI) and isotanshinone II (ITsII).

The WSCs of SMRE are mainly phenolic acid compounds, including single phenolic acids and polyphenolic acids. Single phenolic acids include protocatechuic aldehyde (PAI), protocatechuic acid (PA), caffeic acid (CA) and 3, 4-dihydroxyphenyl lactic acid (DLA, also called danshensu), whereas polyphenolic acids include rosmarinic acid (RA), lithospermic acid (LSA), salvianolic acid A (Sal A), salvianolic acid B (Sal B), and other salvianolic acids which are shown in Figure 1. At present, the methods of determining the content of total phenolic can be categorized into two groups, which are FeCl₃-KFeCN Color-display method and NaNO₂-Al(NO₃)₃ complexation Color-display method. There are many interference factors by using FeCl₃-KFeCN Color-display method to determine total phenolic. In addition, the specificity is worse, which makes the result to be inaccurate. However, compared with FeCl₃-KFeCN Color-display method, the specificity of NaNO₂-Al(NO₃)₃ complexation Color-display method is better and the result is more accurate. Therefore, NaNO₂-Al(NO₃)₃ complexation Color-display method is often used to determine total phenolic (Wang et al., 2010).

The major LDQs of SMRE are tanshinone I (TsI), tanshinone IIA (TsIIA), tanshinone IIB (TsIIB), cryptotanshinone (CTs), tanshindiol C (TsC), 15, 16-Dihydrotanshinone I (15, 16-DTsI), isotanshinone I (ITsI), isotanshinone II (ITsII) and other tanshinones which are shown in Figure 2. Tanshinone IIA is a kind of heat sensitive substance which is unstable when heating is applied. Therefore, it is preferred to control the temperature exactly, but not too high. Moreover, it should be kept in dark to prevent tanshinone IIA degradation by light or oxygen in the air. HPLC is usually used to determine the content of tanshinone IIA (Zhang et al., 2010).

The metabolic pathways in vivo available for some constituents of WSC have been elucidated (Zhang et al., 2005; Wu et al., 2006). The biological actions of compounds isolated from SMRE had been clarified over the last few years, and several lines of evidence have been accumulated which indicated the diversity of the potentials of SMRE in attenuating microcirculatory disturbance, including antioxidation, suppression of the adhesion molecules expression, inhibition of platelet aggregation, inhibition of mast cell degranulation, inhibition of apoptosis and amelioration of the target organs injury, such as the heart, brain, liver, kidneys and lungs. Therefore, SMRE has emerged as a candidate for improving microcirculatory disturbance by acting on multiple targets. There have been some reviews concerning the major ingredients of SMRE and preclinical results. This review will focus on the ameliorating effects of compounds derived from SMRE of microcirculatory disturbance and target organ injury induced by I/R.

As to quality control of Danshen apart from macroscopic and microscopic authentication, chemical identification is an important and useful means as it directly associates with the medicinal functions. Phenolic...
acids B and tanshinones II A are usually chosen as marker compounds to assess the quality of Danshen. Quantitative analysis of the two components is reviewed in this paper. However, phenolic acids B and tanshinones II A not only exist in Danshen, but also in other plants, such as Salvia przewalskii Maxim, Salvia scarea L and Salvia genus. Therefore, chemical identification of Danshen by simply use of Ferulic acid and Z-ligustilide as marker compounds seems to be insufficient.

The official drug of Danshen is the roots of *Salvia miltiorrhiza*. The quality of this kind of Danshen is guaranteed and has been proven by the clinical application of thousands of years. In China, Danshen cultivated in Fangcheng County of Henan Province is regarded as the authentic herb according to traditional experience. However, several other substitute herbs have also been used in clinical trials, such as *S. przewalskii maxim* (Gansu), *S. yunnanensis* C. H. Wight (Yunnan), *T. bowleyane* Dun (Zhejiang, Jiangxi), and *S. trijuga* Diels(Dail); the presence of these substitutions makes the quality control of Danshen more difficult.

In light of this, chromatographic fingerprint analysis was proposed to perform the quality control of Danshen. Chromatographic fingerprint analysis of TCMs not only represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products, but also forms a comprehensive chemical base for further research on their possible synergistic working mechanism. The development of chromatographic fingerprint of Danshen is also discussed in this review (Zeng et al., 2006; Liang et al., 1992; Wang et al., 2006; Manne et al., 1999; Hu et al., 2004; Yi et al., 2010). Some methods are developed for the isolation and identification of the main classes of compounds and the chemical components isolated from Danshen.

Major water-soluble compounds derived from SMRE are shown in Figure 1 including protocatechuic aldehyde (Pal), protocatechuic acid (PA), caffeic acid (CA), 3, 4-dihydroxyphenyl lacticacid (named as danshensu, DLA), lithospermic acid (LSA), salvianolic acid A (SalA), and salvianolic acid B (SalB).

### CHEMICAL COMPOUNDS

#### Major water-soluble compounds

#### Chemical structures

*S. miltiorrhiza* effective components include fat-soluble and water-soluble ingredients. Water-soluble ingredients mainly consist of adjacent catechol structure of phenolic acids compounds, which are one kind of active compounds in Danshen with parent nucleus of water-soluble compounds. Over the past 50 years, the chemical constituents and biological activities of Danshen have been well studied. Since the 1980s, Chinese and Japanese scientists have studied the water soluble constituents from Danshen and isolated more than 20 phenolic acids from this plant. Chemical structures of phenolic acids in Danshen are shown in Figure 1, containing proto-catechuic aldehyde, protocatechuic acid, coffee acid, rp-hplc, rosemary acid, the purple oxalic acid, Dan phenolic acids A~I, Danshen acid ethyl, etc (Li et al., 2008). It does not only have an effect on increasing coronary flow and myocardial ischemia, but also has the function of resistance of lipid peroxidation, scavenging free radicals, and treatment of chronic renal transplantation (Wu et al., 2003). The current market mainly includes two kinds of red-rooted salvia preparations: one kind is *S. miltiorrhiza* radices, including injection, danshen mixture, danshen particles, etc; and the other kind is gentleman medicine by *S. miltiorrhiza*, notoginseng, such as Chinese medicine (Cheng, 2006), including compound radices, compound danshen particles, compound danshen drop pill, compound danshen tablet, injection, *salvia miltiorrhiza* capsule, etc.

### Isolation and sample pretreatment

For about 10 years, the fat-soluble constituents of *S. miltiorrhiza*, in which only two were separated from *S. miltiorrhiza* monoterpenes compounds have been proven by pharmacological studies (Zhang et al., 1999). Yet, Chinese traditionally use the water decoction of Danshen, so the study of water-soluble parts of *S. miltiorrhiza* is more meaningful. In recent years, Danshen water-soluble phenolic compounds have become increasingly important in the field of medicine. Modern pharmacology research shows that Danshen has a unique effect on the cardiovascular and cerebrovascular diseases. Research shows that the main reason is the water-soluble ingredients used and proto-catechuic aldehyde including rosemary acid, purple oxalic acid, and Dan phenolic acids (A, B, C, D, E, F, G, etc.) which are the main effective components of Danshen (Lee et al., 2008). However, the content of phenolic acids is the highest which accounts for about 70% of the total Dan phenolic acids. So extraction rate and cream rate of Dan phenolic acids as the index are determined by choice of the optimum technological conditions of *S. miltiorrhiza* extraction (Cao et al., 2005, 1996).

Since the 1980s, a large proportion of the currently known water-soluble compounds have been isolated and many papers began to concentrate on rapid analysis of compounds in plant material and medicinal herbal products. Water-soluble components are considered as the important components and quantified by HPLC to optimize the extracted process. However, water-soluble compounds are thermally labile and may easily be isomerized in high temperature. So HPLC is the main
analytical methods for quantification of water-soluble compounds which refer to content determination method of gardenia Dan phenolic acids B in 2005 edition of the Chinese Pharmacopoeia. Chromatography and system applicability tests are as follows: silica was used as filler, acetonitrile - methanol - formic acid - water (10:30:1.59) was used as a mobile phase, and 286 nm detected wavelength were for plates. According to the theory, peak calculation of Dan phenolic acids B shall not be lower than 40. Solution preparation is as follows: The reference solution concentration is 60 µg/ml made by 60% methanol. An amount of 0.030 g Dan phenolic acids B was exactly taken into a volumetric flask filled with about 60% of methanol, after which ultrasonic treatment was applied at a frequency of 50 kHz 300 W 30 min, diluted with 60% of methanol to scale, filtrated with microfiltration membrane (0.45 µm) (Zhao et al., 1996; Jiang et al., 2006), and 10 µl of it was exactly injected into the reference solution and the content of Dan phenolic acids was determined by HPLC.

Quantitative analysis

Effective component of water-soluble compounds are phenolic acids. HPLC was used for quantification of the water-soluble compounds. HPLC is so accurate that it has become a prominent method for detecting and standardizing the water-soluble compounds. Agilent 1100 (a high-performance liquid chromatography), methanol (chromatography pure), water (filtered with 0.45 um membrane) (Fan et al., 2009), Dan phenolic acids B (batch number 1115622200302) and coffee acid (batch number 1108852200102) were purchased from Chinese pharmaceutical and biological products. All sorts of S. miltiorrhiza agents respectively were from Shanghai (Wang et al., 2005), Nanjing, Guangzhou, etc. Eight manufacturers and source are shown in the tables. Hence, the sample preparation and HPLC analytical conditions should be strictly controlled to avoid any detectable degradation of the water-soluble compounds and unacceptable fluctuation during the quantification analysis (Table 1).

Major lipophilic compounds

Chemical structures

Danshen contains a series of hydrophobic compounds, though the main Lipophilic components of Danshen which have various bioactive effects are shown in Figure 2. These lipophilic components contain tanshinone I (Tsl), tanshinone IIA (TslIIA), tanshinone IIB (TslIIB), cryptotanshinone (CTs), tanshindiol C(Tsc), 15, 16-dihydrotanshinone I (15,16-DTsI), isotanshinone I (ITsl), isotanshinone II (ITslII) and other tanshinones. It was not until the late 1989s that more information on lipophilic components of Danshen from this herb began to appear. Some new ones were isolated from Danshen, such as Tanshinone, methylenetanshinquinone, Salvia methylene-dihydro-quinone 7 - β. Hydroxy - 8, 13 - Rosin diene, 11, 12-Dione, 4-methylene-miltirone, 1, 2, 5, 6-tetrahy-drotanshiquinone I. Of all the components, tanshinone IIA is the main ingredient of Danshen, as shown in Figure 2, which has been isolated and identified from S. miltiorrhiza, most of which are diterpenoids involving tanshinones of o-quinonoids, royleanones of p-quinonoids, and other types. Some of these tanshinones are related to the medicinal functions of Danshen.

Nowadays, more than 70 hydrophobic compounds have been isolated and identified from S. miltiorrhiza, in succession, such as tanshinones of o-quinonoids, royleanones of p-quinonoids, and other types. Some of these diterpenoid compounds are believed to be the major bioactive ingredients in S. miltiorrhiza lipophilic diterpenoid quinines (LDQ), which are mostly the combined quinonoids, ketone compounds, with characteristics of orange. Terpenoids relevant S. miltiorrhiza participating in Danshen are particularly rich. People began to research the two broad ingredients in 1934, until 1987. At least, 30 new compounds have been separated.

Isolation and sample pretreatment

Because of their potent biological activities, there is an increasing interest in the isolation and purification of tanshinones including Tanshinone I and Tanshinone IIA from S. miltiorrhiza Bunge. Tanshinones IIA is unstable under the condition of moisture and high temperature. In order to reduce the decomposition of the products and prove the manufacturing strength, we sought for the concerned industry. Huie et al. (2002) reviewed sample preparation techniques for extracting tanshinones from medicinal plants. Several methods have been reported for the separation of some of these tanshinones, including TLC, HPLC, CCC and SFE immobilized isosome chromatography.

The extraction of lipophilics components with ethanol from S. miltiorrhiza was done by the traditional method. Usually Tanshinone IIA, as the content index, is affected by different ethanol concentration, extraction time, extraction times and solvent dosage during the extraction; orthogonal test was used. Guo et al. (2002) proved that the extraction solvent of the dissolution of tanshinone was the significant impact indicator. The three major factors are ethanol consumption, extraction time and temperature. The optimum extraction was selected by orthogonal S. miltiorrhiza Bunge adding 8 times of 70% ethanol to water and extracted for 2 h for each extraction. The kinds of solvent and models of extraction have a great influence upon the extraction. Even now,
Table 1. Manufacturers and batch numbers of eight Danshen formulations.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Formulation</th>
<th>Manufacturer</th>
<th>Batch No.</th>
<th>Content of <em>Salvia miltiorrhiza</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Compound Danshen tablets</td>
<td>Beijing Tongren tang Zhiyao</td>
<td>6120558</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td>Compound Danshen tablets</td>
<td>Shanhai Leiyun shang Yaoye</td>
<td>070305</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>Compound Danshen tablets</td>
<td>Guangzhou Baiyunshan Heijhuangpu Zhongyao</td>
<td>D7A031</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>Compound Danshen tablets</td>
<td>Shanghai Huanghai Zhiyao</td>
<td>070106</td>
<td>0.45</td>
</tr>
<tr>
<td>5</td>
<td>Compound Danshen buccal tablets</td>
<td>Hebei Anguo Yaoye</td>
<td>05PJ30</td>
<td>0.45</td>
</tr>
<tr>
<td>6</td>
<td>Danshen tablets</td>
<td>Shanghai Leiyunshang Yaoye</td>
<td>070204</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Danshen tablets</td>
<td>Shanghai Huanghai Zhiyao</td>
<td>070202</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Danshen tablets</td>
<td>Nanjing Housheng Yaoye</td>
<td>070107</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 2.** Major lipophilic compounds of SMRE.

These common and effective extraction methods are still widely used with some small variations of the solvent type, which were preferred to be extracted.

In the past years, a series of separation techniques, such as TLC, silica gel column chromatography and preparative HPLC (Jung et al., 2009; Yoon et al., 1999; Mosaddik, 2003; Fang et al., 2007), have been successfully applied for the purification of various compounds of *S. miltiorrhiza* Bunge. However, these traditional chromatographic methods are often time-consuming and suffer from sample loss because some minor samples are easily absorbed onto the solid support matrix. Several methods have been successfully applied to separate and purify tanshinones including tanshinone I and tanshinone II A from *S. miltiorrhiza* Bunge (Tian et al., 2002, 2000; Li et al., 2001). SFE is an environmentally-benign, efficient extraction technique; it is considered a well-established, idly-accepted and increasingly-used sample-preparation technique based on solid-phase adsorption. Chromatography/Mass spectrometry (HPLC/MS) is one of the most powerful techniques for the rapid identification of constituents of plant extracts (Ye et al., 2005; Liu et al., 2005). Another factor that added to this situation is the need to apply quality control to traditional medicines, such as TCMs quality control, which is becoming more prominent in Western scientific literatures (Chen and Chen, 1992; Naito et al., 1992). Up to now, most literatures about
lipophilic compounds focus on the analysis of tanshinones publications, while literatures about other compounds are very few.

**Quantitative analysis**

According to traditional experience, the distinctive root of *Salvia* species with red colour is used as an indicator of Danshen quality evaluation and grading. Thus the red colored diterpenoidal tanshinones have been chosen as the marker compounds for quality evaluation of Danshen. For quantitation of tanshinones in an herbal matrix, the reversed phase high performance liquid chromatography (HPLC) aided with diode array detector (DAD) or photodiode array detector (PDA) has become a routine method in most of the laboratories. Tanshinones display strong ultra-violet (UV) absorption at 254–280 nm and could be measured with good selectivity and sensitivity when HPLC–UV or DAD detector was used (Shi et al., 2005). The capillary electro chromatography (CEC) method (Li et al., 2007) was also applied for the quantitative determination of four tanshinones (dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone IIA) in Danshen.

A high extraction efficiency (>98.5%) was achieved using an optimized pressure liquid extraction (PLE) for sample preparation procedure and a good separation obtained on a hypersil C18 capillary column. CEC is superior to HPLC in high resolution, high sensitivity, short analysis time and low organic solvent consumption while its disadvantage lies in its poor repeatability. A liquid chromatography with electro spray ionization tandem mass spectrometry (LC–ESI–MS/MS) method was reported for quantitation of four diterpenoids (Dihydrotanshinone I, Cryptotanshinone, Tanshinone I, and Tanshinone IIA) in Danshen. Although the established LC–ESI–MS/MS method demonstrated higher selectivity, sensitivity and shorter running time, the precision, repeatability and recovery appeared not so ideal and need to be further improved (Hu et al., 2005). Recently, an ultra-high-pressure liquid chromatographic method was developed for simultaneous quantitation of 10 diterpenoidal quinones in Danshen, and a proper separation was achieved within 8 min of run time (Li et al., 2008).

**Fingerprint**

In the area of TCMs quality control, simple, rapid and sensitive detection capabilities are very necessary for the investigation of chemical differences in plant anatomy and geographical variations (Thappa et al., 2005), or seasonal changes of plant metabolites (Bylaite et al., 1998). Nowadays, chromatographic fingerprints are being widely used in quality control of Danshen. In particular, a HPLC fingerprint method was developed which was used for the isolation and identification of not only phenolic acids, but also tanshinones, etc. However, chromatographic fingerprints are very sensitive to distinguish the compounds in the same herbs.

**Chromatographic conditions**

The following were observed for the chromatographic conditions of the herbs: chromatographic column: Lichrospher ODS 22 (250 mm x 4.6mm x 5µm); methanol mobile phase A (Zeng et al., 2006): methanol; methanol mobile phase B: 1% glacial acetic acid solution; flow rate: 1 mL/min, column temperature: 30 degrees; column detected wavelength: 254 nm; injection volume: 20 µL; and linear gradient elution program: 0 min 25% A – 70 min 75% A.

**Ms detection conditions**

For Ms detection conditions, electro spray ionization, nitrogen and auxiliary pressure were atomized respectively for 211 x 10⁵ Pa and 315 x 10⁵ Pa. The capillary temperature is 35°C, with positive ions scanning (Brody et al., 2006), and the scanning spray voltage is 5 kV. The scanning quality range is from 100 ~ 9 m/z; for secondary ion mass spectrometry, air pressure is 1 Pa and CID energy is 30 ev.

**Liquid preparation**

The solution: Ten pieces of Danshen tablets were taken, with an average amount of sugar (Janus et al., 2004), and an adequate amount of *S. miltiorrhiza* medicine (211.015 g) was applied into a 25 mL volumetric flask and dissolved with some water. Ultrasonic treatment was performed for 30 min, and the flask was allowed to cool down, after which water was added to the scale. The filtrate was filtered with Millipore filter, and was then renewed as the solution.

**Fingerprint reference solution:** Control medicinal powder (0.15 g) was precisely put into 25 mL volumetric flask and dissolved with some water. Ultrasonic treatment for carried out for 30 min. The flask was left to cool, after which water was added to the scale. The filtrate was filtered with Millipore filter, and was then renewed as the solution (Gandhi et al., 2000).

**Reference solution:** Dan phenolic acids B and coffee acid were precisely taken respectively, and dissolved with methanol and glacial acetic acid solution (50:50) (Xu et al., 2006). A density of 200 mg/L Dan phenolic acids B and coffee acid solution was obtained.
Table 2. Fingerprint similarity of model samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (%)</th>
<th>Cosθ</th>
<th>RED (Theoretical value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.9998</td>
<td>0.799(0.800)</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.9999</td>
<td>0.514(0.500)</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>1.00</td>
<td>0.200(0.200)</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1.00</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0.9999</td>
<td>0.234(0.200)</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>0.9998</td>
<td>0.545(0.500)</td>
</tr>
<tr>
<td>7</td>
<td>200</td>
<td>0.9998</td>
<td>1.096(1.000)</td>
</tr>
</tbody>
</table>

*Fingerprint reference material* b: the weight ratio of the model samples to the fingerprint reference material; c: relative Euclidean distance.

Common peak

According to the “preparation method” in the study’s chromatographic conditions, *Salvia miltiorrhiza* tablets from 8 manufacturers were separately determined. Common peak was used to control *salvia miltiorrhiza* tablet and the 16 corresponding *salvia miltiorrhiza* medicinal products. During its determination, LC2 MS/MS technology combined with chemical (Jiang et al., 2006) was used.

Precision

The pharmaceutical production of Shanghai LeiYunshang pharmaceutical companies was taken, and the sample solution was repeated five times (Alcala et al., 2003). Each has a relative retention time, and the relative peak area of the reserved RSD is 0.02, 0.14 and 4.96%, which showed good precision instruments.

Repeatability

The pharmaceutical production of Shanghai LeiYunshang pharmaceutical companies was taken, and the selected solution was used for 5 preparations. The results of the common peak relative retention time and the relative retention of the peak area of RSD are 0.15 ~ 0.66% and 1.23 ~ 4.71%, which shows that this method has good repeatability.

Recovery

Samples of phenolic acids and coffee acid were taken and examined for recovery. The pharmaceutical productions of Shanghai LeiYunshang pharmaceutical companies (coffee acid content of 0.090 mg/g) were precisely taken at 0.15 g, and coffee acid was added to it. According to the “Reference solution” the solution preparation was selected, and the recovery results show that the average recovery rate and the RSD of six times is 101.2%.

Similarity evaluation index analysis

The pharmaceutical production of Shanghai LeiYunshang pharmaceutical companies was taken according to the “preparation methods” sample respectively, in addition to the specified amount of 20, 50, 80, 100, 120, 150 and 200% of the model. The solution and sample were processed with HPLC fingerprint. The model of high performance liquid chromatographic fingerprint samples of the relative retention time and the relative peak area is almost identical (Giacobini et al., 2004), due to the fact that the characteristics of fingerprints lie in the differences of content composition. By 100% reference, the calculation model of the different samples with the reference model sample has qualitative similarity (Cos theta) and quantitative similarity (Ep-red). For the planning and control of cosine value (0-1), it was observed that the qualitative level was closer to the higher level of 1, while for the Relative Euclidean distance value (0 ~ theta), it was observed that the quantitative level was closer to the higher level of 1. Results in Table 2 show that even distribution of chemical composition was completely consistent (Cos theta = 1.0) in the samples (Mesulam et al., 2004), due to the fact that they may exist between the quality of significant differences; Ep-red can accurately reflect the fingerprint characteristics between samples diversity with the whole difference degree content component. At the same time, the qualitative indexes of Cos theta is similar with the quantitative index of ep-red accurately, using the fingerprint criteria for more effective and reasonable quality of herbal complex evaluation and control.

Sample

According to the “preparation method”, each Danshen preparation product solution (n = 3) was determined by
Table 3. Contents of common peaks in Danshen tablets (mg/g).

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Sample no.</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>x±s</td>
<td>RSD(%)</td>
<td>Component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.14</td>
<td>0.48</td>
<td>0.35</td>
<td>0.08</td>
<td>0.83</td>
<td>0.28</td>
<td>0.05</td>
<td>0.44</td>
<td>0.15</td>
<td>0.33±0.26</td>
<td>77.6</td>
<td>Tanshinol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>0.12</td>
<td>0.14</td>
<td>0.02</td>
<td>0.1</td>
<td>0.05</td>
<td>0.00</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06±0.05</td>
<td>77.1</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.00</td>
<td>0.15</td>
<td>0.06</td>
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<td>0.50</td>
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<td>0.68</td>
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<td>Isomer of Salvianolic acid A</td>
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SalvianolicacidB/(mg/pertablet) 6.65 6.12 5.96 5.52 5.63 11.3 14.4 12.7 ...

Referring to a chromatographic condition of the determination method, and the content of the Dan phenolic acids B was calculated with every piece of it (Kuwai et al., 1989). The equivalent conversion showing the original medicinal content of the Dan phenolic acids per gram, in relation to other contents of water-soluble ingredients were estimated with Dan phenolic acids B. A qualitative and quantitative similarity was observed for the mutual water-soluble ingredients and the original medicinal content per gram. In accordance with the provisions of the pharmaceutical standards, the content of Dan phenolic acids B in compound Danshen tablet was not less than 5 mg/g, and Tanshinone II A was not less than 0.2 mg/slices (Watkins et al., 1994). Dan phenolic acids B in S. miltiorrhiza tablet was not less than 11 mg for each. The results in Table 3 indicated that the content of each tablet, calculation content of Dan phenolic acids B of compound Danshen tablet and S. miltiorrhiza tablet is in accordance with the drug standards. Different manufacturers of S. miltiorrhiza preparations are with relative content (Ibebunjo et al., 1997), especially the difference between relatively lower levels of composition. Each tablet in common composition of RSD is of 8.8 ~ 221.6% among proto-catechuc aldehyde, purple oxalic acid, rosemary acid, and Dan phenolic acids isomers with a percentage greater than 100% in Hebei province (Lev-Lehman et al., 1996).

The RSD of AnGuo pharmaceutical production of compound Danshen tablet was used for detecting the relative content of 0.83 mg/g, while Shanghai yellow pharmaceutical production of compound Danshen tablet was used for detecting the relative contents of 0.08 mg/g.

**Stability**

The pharmaceutical production of Shanghai LeiYunshang pharmaceutical companies was taken with the solution preparations of 0 and 1.5 g respectively, and the determination was at 4, 8, 12, and 24 h. The results of the common peak relative retention time and the relative retention peak area of RSD were 0.07 ~ 0.66%, and 0.6 ~
4.44%. It was observed that the phenolic acids composition was stable in room temperature within 24 h.

CONCLUSION

Numerous analytical techniques dealing with various constituents in Danshen were summarised. As to the quality control of Danshen, there is much eagerness to obtain as much information as possible from a chromatographic fingerprint. HPLC is the most commonly used established chromatographic technology and fast developed in recent years. Quality control of Danshen is developed from single or several components of fingerprint analysis, which is a big progress for quality control of this herb. This technology can help us to distinguish the adulteration from authentic herbs and to evaluate the quality of substitute herbs. However, because its mechanism of action is not clear, the criterion used for the quality evaluation of Danshen from traditional experience is without modern scientific confirmation hence, it is difficult to guarantee the authenticity, safety and effectiveness of this herb.

Acknowledgements

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REFERENCES


