A comparison of the chemical composition in Salvia miltiorrhiza Bunge from 5 different regions in Shaanxi province by direct injection ESI-Q-TOF-MS (Postprint)

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Abstract

ESI-Q-TOF-MS, owing to its advantages of high scanning speed and sensitivity, is widely applied in the identification of chemical constituents in traditional Chinese medicine. It can provide accurate mass measurements of fragment ions and possible elemental compositions, is less susceptible to factors such as the growing environment and extraction conditions of medicinal materials, and enables relatively accurate evaluation of the quality of Salvia miltiorrhiza (danshen) from different regions of Shaanxi. This study employed an ESI-Q-TOF-MS direct injection analysis method to conduct comparative analysis of the chemical constituents of danshen from five regions in Shaanxi, namely Shangzhou, Luonan, Dali, Danfeng, and Tongchuan. By evaluating variations in the mass spectrometric abundance of water-soluble and lipid-soluble components of danshen, the chemical constituent content differences among different regions were assessed, the chemical constituents of danshen from various producing areas were identified, and the optimal cultivation region for danshen was selected through comprehensive analysis. The results demonstrated that danshen from all five regions contained nine lipid-soluble chemical constituents including tanshinone IIA, cryptotanshinone, tanshinone I, dihydrotanshinone, tanshinol, miltirone, and tanshinone II, as well as nine water-soluble chemical constituents including danshensu, caffeic acid, ferulic acid, rosmarinic acid, protocatechuic acid, lithospermic acid, protocatechualdehyde, salvianolic acid A, and salvianolic acid B. Among these, the contents of sodium danshensu, lithospermic acid, salvianolic acid B, and cryptotanshinone were generally high, with mass spectrometric abundances exceeding 30%. However, due to differences in cultivation environments, the chemical constituent content of danshen varied significantly among regions. For instance, danshen produced in Shangzhou contained higher amounts of biologically active tanshinones, with the content of tanshinone I being far higher than that in the other four producing regions, reaching a mass spectrometric abundance of 72.6%, whereas the mass spectrometric abundance of tanshinone I in other regions was only 1.8-11.3%. The study indicated that the quality of danshen, ranked by region, was Shangzhou > Tongchuan > Dali > Luonan > Danfeng. This method provides a scientific, reliable, and convenient approach for the quality evaluation of traditional Chinese medicinal materials, offers a new pathway for the establishment of medicinal material specifications and grades, and furnishes important information for the selection of cultivation regions for danshen in Shaanxi.

Full Text

A Comparison of the Chemical Composition in *Salvia miltiorrhiza* Bunge from Five Different Regions in Shaanxi Province by Direct Injection ESI-Q-TOF-MS

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Abstract

ESI-Q-TOF-MS is widely applied for identifying chemical constituents in traditional Chinese medicine due to its high scanning speed and sensitivity, providing precise mass numbers and possible elemental compositions of fragment ions. This method is less affected by growth environment and extraction conditions, enabling accurate evaluation of Danshen (*Salvia miltiorrhiza*) quality across different regions of Shaanxi Province. We developed a direct injection ESI-Q-TOF-MS method to analyze Danshen samples from Shangzhou, Luonan, Dali, Danfeng, and Tongchuan. By comparing MS abundances of water-soluble and lipid-soluble extracts, significant variations in chemical composition were observed. All five regional samples contained nine lipophilic constituents (Tanshinone I, Tanshinone IIA, Tanshinone IIB, Cryptotanshinone, Dihydrotanshinone, Danshenxinkun A, Danshenxinkun D, 2-Isopropyl-8-methylphenanthrene-3,4-dione, and 7-beta-hydroxy-8-13-abietadiene-11,12-dione) and nine hydrophilic constituents (Danshensu, Caffeic acid, Ferulic acid, Rosmarinic acid, Prolithospermic acid, Lithospermic acid, Protocatechuic acid, Salvianolic acid A, and Salvianolic acid B). Danshensu, Lithospermic acid, Salvianolic acid B, and Cryptotanshinone showed particularly high content, with MS abundances exceeding 30%. However, the content of identical chemical constituents varied substantially across regions. Danshen from Shangzhou contained significantly higher levels of biologically active tanshinones, with Tanshinone I abundance reaching 72.6%—far exceeding the 1.8-11.3% observed in the other four regions. Comprehensive comparison revealed the quality ranking by region as: Shangzhou >

Tongchuan > Dali > Luonan > Danfeng. This direct injection ESI-Q-TOF-MS method provides a scientific, reliable, and convenient approach for evaluating medicinal material quality and offers a new pathway for establishing specifications and grades of traditional Chinese medicine. The system is not only suitable for analyzing Danshen composition in Shaanxi Province but can also be readily adapted and applied to other Danshen cultivation regions.

Keywords: Danshen; ESI-Q-TOF-MS; chemical composition; abundance; medicinal quality

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Introduction

The dry root and rhizome of *Salvia miltiorrhiza* Bunge (family Labiatae), known as "Danshen" in Chinese, has been widely used medicinally in China and neighboring countries for centuries. Its extracts are clinically important, particularly for treating cardiovascular diseases, and demonstrate therapeutic effects against atherosclerosis, myocardial ischemia, and hepatic fibrosis. Danshen's remarkable free radical scavenging capacity stems from its secondary metabolites, including the well-studied active constituents salvianolic acids and tanshinones. However, Danshen composition varies significantly by region, necessitating scientifically robust and convenient identification methods to compare chemical constituents across different areas and ensure material quality and clinical efficacy.

Danshen is widely cultivated across Shaanxi Province, including in Tongchuan, Dali, Danfeng, Luonan, and Shangzhou. Soil, climate, planting patterns, and extraction methods can all affect component content. While various methods have been investigated for qualitative and quantitative analysis—including HPLC, LC-MS, UPLC-MS, CE, NMR, and LC-DAD-MS—few studies have compared the chemical characteristics of Danshen specifically from Shaanxi Province. This study employs direct injection ESI-Q-TOF-MS technology to detect 18 chemical compounds present across all five regional samples, most of which contain primary medicinal benefits. The abundance values in MS spectra differ considerably among both components and locations under identical conditions. Through comprehensive analysis, four components emerge as potential markers for quality control using this direct injection method. This system is not only applicable to Danshen from Shaanxi Province but can also be easily adapted for other cultivation regions.

1.1 Materials and Reagents

HPLC-grade methanol was purchased from E. Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Ethanol was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Annual Danshen roots were collected from Tongchuan, Dali, Danfeng, Luonan, and Shangzhou in Shaanxi Province in September 2016.

1.2 Sample Preparation

Alcohol Extract: Roots were thoroughly dried in warm air (50 °C), ground, and sieved through a No. 60 mesh. Accurately weighed fine powder (1.0000 g) was extracted with 50 mL ethanol for 30 minutes using microwave extraction. After cooling, the solution was filtered through paper, and ethanol was removed by reduced pressure distillation. The residue was dissolved in methanol and diluted to 5.0 mL in a volumetric flask. Solutions were filtered through a 0.45 m membrane filter before direct injection into the ESI-Q-TOF-MS system.

Water Extract: Roots were processed as above (dried at 50 °C, ground, and sieved). Accurately weighed powder (1.0000 g) was extracted with 50 mL water for 3 hours in a 90 °C water bath. After cooling, the solution was centrifuged at 12,000 rpm for 15 minutes, then freeze-dried to remove water. The crude extract was reconstituted in water at 0.5 mg · mL ^{1} concentration and filtered through a 0.45 m membrane filter.

1.3 Instrumentation and Analytical Conditions

All analyses were performed on a Bruker micrOTOF-Q II ESI-Q-TOF-MS system in tune-low mode. Sodium formate solution served as calibration standard in Enhanced Quadratic model. Sample injection volume was 10.0 L, with detection from 50 to 3000 m/z in tune-low mode. Capillary voltage was set to 4000 V for alcohol extracts and 3500 V for water extracts. Nebulizer pressure was 0.4 Bar. Carrier gas (nitrogen and helium) had a dry gas flow rate of 4.0 L · min ^{1} and dry heater temperature of 180 °C. Prepared samples from each location were injected (10.0 L) and analyzed in triplicate.

1.4 Data Analysis

Chromatographic data were recorded and processed using Bruker Compass DataAnalysis 4.0 software. Ion abundance refers to ion signal intensity used as the vertical coordinate in standard spectra. The ion abundance at the peak of maximum ionic strength was set as 100% for a specified charge ratio, with other peaks expressed as percentages relative to this maximum.

The abundance (A) is calculated as: A = (I / I_max) × 100%, where I is ion strength and I_max is the maximum peak strength.

2. Results and Discussion

ESI-Q-TOF-MS parameters including fragmentor voltage, spray pressure, and capillary voltage were optimized to enhance molecular ion peaks and relative abundances for both alcohol and water extracts. A typical direct injection ESI-Q-TOF-MS spectrum is shown in [Figure 1: see original paper].

Eighteen known chemical constituents were identified from fragment ion information that effectively reflects Danshen composition structures [Figure 2: see original paper]. The spectra provided detailed MS analysis results, with compound identification summarized in . In positive ion mode, [M+Na] adduct ions were predominantly observed—for example, the peak at m/z 317.1128 corresponded to Tanshinone IIA as its [M+Na] adduct. In negative ion mode, [M-H] adduct ions were commonly observed; for instance, the peak at m/z 717.1540 represented salvianolic acid B. While compound classification was nearly uniform across the five regional samples, significant distinctions existed for specific constituents.

Based on previous work, the characteristic fragmentation of Tanshinone IIA (representative lipophilic component) is shown in [Figure 3: see original paper]. Tanshinone IIA loses neutral fragments (-CH , H O, and -CO) to form peaks at m/z 303.1237, 300.1032, and 289.1124. The fragment at m/z 285.1383 results from H O loss from m/z 303.1237 or -CH cleavage from m/z 300.1032, which can further fragment to m/z 195.0441 and 272.1162. Overall, Tanshinone IIA fragmentation occurs through loss of small neutral molecules such as -CH , -CO, and H O.

Abundance analysis revealed drastic variation among the 18 chemical constituents across the five locations under identical conditions, likely attributable to different planting environments. For lipophilic components , [Figure 4: see original paper], certain compounds—including Tanshinone I, Cryptotanshinone, Danshenxinkun A, and Danshenxinkun D—showed particularly large variation by region. Shangzhou Danshen demonstrated overwhelming superiority in Tanshinone I, Danshenxinkun A, and Danshenxinkun D content, with abundance values of 72.6%, 100%, and 100%, respectively. The differences between maximum and minimum values for these compounds were 70.8%, 96.0%, and 96.1%. Meanwhile, Tongchuan Danshen contained the most Cryptotanshinone (100%), followed by Danfeng (63.8%). The remaining five lipophilic components showed smaller variation: Tanshinone IIA, Tanshinone IIB, Dihydrotanshinone, 7- -hydroxy-8-13-abietadiene-11,12-dione, and 2-isopropyl-8-methylphenanthrene-3,4-dione reached maximum abundances of 20.7% (Dali), 7.5% (Dali), 32.1% (Dali), 6.2% (Shangzhou), and 16.7% (Tongchuan), respectively. Overall, Cryptotanshinone showed the highest abundance across all samples, followed by Danshenxinkun A (range: 4.0–100%). The regional ranking for lipophilic component content was: Shangzhou > Dali > Tongchuan > Luonan > Danfeng.

For hydrophilic components , [Figure 5: see original paper], abundance variation followed similar trends. All samples contained higher levels of Danshensu, Lithospermic acid, Prolithospermic acid, and Salvianolic acid B compared to other constituents. These four components reached maximum abundances of 100.0% (Shangzhou & Tongchuan for Danshensu), 100.0% (Luonan for Lithospermic acid), 51.9% (Shangzhou for Prolithospermic acid), and 65.1% (Luonan for Salvianolic acid B). Notably, Danshensu and Lithospermic acid showed strong signal responses (>45.0% abundance) with clear gaps between maximum and minimum values (55.2% and 41.6%, respectively). Ferulic acid, Rosmarinic acid, and Salvianolic acid A showed low abundance values with similar variation patterns, reaching maxima of 6.6%, 6.5%, and 15.9% in Shangzhou Danshen, without obvious regional distribution patterns. Salvianolic acid B, an important clinical active component, was highest in Luonan Danshen (65.1%) and lowest in Shangzhou (34.1%). Overall, Danshensu and Lithospermic acid showed the highest abundances, followed by Salvianolic acid B (range: 34.1–65.1%). The regional ranking for hydrophilic component content was: Shangzhou > Tongchuan > Luonan > Dali > Danfeng.

3. Conclusion

This study developed a scientific, reliable, and convenient method using direct injection ESI-Q-TOF-MS to compare chemical composition in Danshen from different regions. Eighteen constituents were identified across all samples, with composition strongly correlated to geographical origin and showing drastic variation under identical conditions. The comprehensive quality ranking by region was: Shangzhou > Tongchuan > Dali > Luonan > Danfeng. Cryptotanshinone, Danshensu, Lithospermic acid, and Salvianolic acid B can serve as potential markers for quality control evaluation. These methods and results provide guidance for effective Danshen cultivation and medicinal collection in Shaanxi Province.

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Note: Figure translations are in progress. See original paper for figures.