Application Note

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HPLC Method Modernization for Tanshinone Analysis Using Sub-2 µm Columns

Rapid analysis of traditional Chinese medicine components with significant time reduction and solvent savings

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Abstract

Traditional high-performance liquid chromatography (HPLC) methods for traditional Chinese medicine (TCM) analysis require lengthy analysis times and high solvent consumption. This application note demonstrates the modernization of a Chinese Pharmacopeia (ChP) method for tanshinone analysis using the Agilent 1290 Infinity III LC system with Agilent ZORBAX RRHD Extend C-18 column. The updated method significantly reduced analysis time and solvent consumption, helping lower laboratory costs while maintaining analytical performance and supporting sustainability.

Introduction

TCM analysis represents a significant analytical challenge in pharmaceutical quality control, requiring extended chromatographic run times that limit laboratory throughput and increase operational costs. The ChP monograph for tanshinones—the bioactive constituents of *Salvia miltiorrhiza*—necessitates rigorous fingerprint and assay methods to ensure product consistency and efficacy. However, conventional HPLC approaches employing larger-particle-size columns (5 µm) demand prolonged analysis times and substantial solvent consumption, creating both economic and environmental burdens for analytical laboratories.

Recent advances in chromatographic technology have enabled the development of sub-2 µm stationary phases capable of operating at elevated pressures while maintaining or improving chromatographic resolution. The ChP General Chapter <0512>, aligned with established guidelines for HPLC method modernization, provides a systematic framework for transitioning traditional methods to contemporary sub-2 µm column formats. This transition offers the potential to substantially reduce analysis time and resource consumption without compromising analytical performance—a critical consideration for high-throughput pharmaceutical analysis.

A particular challenge in tanshinone analysis involves achieving baseline resolution of structurally similar compounds, specifically cryptotanshinone and tanshinone I, which require selective stationary phase chemistry to achieve adequate separation. The unique selectivity of extended C18 phases designed for high-pH conditions presents an opportunity to address this analytical challenge while simultaneously modernizing existing methods.

This work demonstrates the successful modernization of ChP tanshinone fingerprint and assay methods by transitioning from conventional 5 µm columns to sub-2 µm columns, consequently reducing analysis time by 86% while maintaining system suitability and analytical resolution. This approach addresses the dual imperatives of improving laboratory efficiency and reducing environmental impact in pharmaceutical quality control.

Experimental

Chemicals and reagents

Cryptotanshinone and tanshinone IIA reference substances with purity > 98% were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing China). HPLC-grade methanol, acetonitrile, and phosphoric acid (H_3PO_4) were purchased from J&K Scientific (Beijing, China). Ultrapure water was prepared using an ELGA water purification system (ELGA LabWater, High Wycombe, UK). All solvents and reagents were used without further purification.

HPLC method adjustment for tanshinone analysis

Two separate methods were run for tanshinone analysis: 1) an assay method for quantification of cryptotanshinone and tanshinone IIA and 2) a fingerprint method for comprehensive characterization of the tanshinone profile. Both methods were modernized from conventional 5 µm columns to sub-2 µm columns following ChP General Chapter <0512> guidelines. The detailed method parameters are outlined in Tables 1 and 2.

Table 1. Original assay HPLC method and modernized assay UHPLC method.

Parameter	Original Method	Adjusted Method
Instrument	Agilent 1260 Infinity III LC with quaternary pump	Agilent 1290 Infinity III LC with binary pump
Column	Agilent ZORBAX Extend-C18, 4.6 × 250 mm, 5 µm (p/n 770450-902)	Agilent ZORBAX RRHD Extend-C18, 2.1 × 100 mm, 1.8 µm (p/n 758700-902)
Mobile Phase	A: 0.026% H_3PO_4 in water B: acetonitrile	A: 0.026% H_3PO_4 in water B: acetonitrile
Gradient	0 min, 60% B; 20 min, 90% B; 30 min, 90% B; post-run, 5 min	0 min, 60% B; 2.88 min, 90% B; 4.32 min, 90% B; post-run, 1.5 min
Flow Rate	1.2 mL/min	0.695 mL/min
Column Temperature	25 °C	25 °C
Injection Volume	10 µL	1 µL
Detection	270 nm (2.5 Hz)	270 nm (40 Hz)

Table 2. Original HPLC method and modernized UHPLC method for fingerprint analysis.

Parameter	Original Method	Adjusted Method
Instrument	Agilent 1260 Infinity III LC with quaternary pump	Agilent 1290 Infinity III LC with binary pump
Column	Agilent ZORBAX Extend-C18, 4.6 × 250 mm, 5 µm	Agilent ZORBAX RRHD Extend-C18, 2.1 × 100 mm, 1.8 µm
Mobile Phase	A) 0.026% H_3PO_4 in water B) Acetonitrile	A) 0.026% H_3PO_4 in water B) Acetonitrile
Gradient	0 min, 20% B; 20 min, 60% B; 50 min, 80% B; 51 min, 20% B; 60 min, 20% B	0 min, 20% B; 2.88 min, 90% B; 7.2 min, 90% B; 7.34 min, 20% B; 8.64 min, 20% B
Flow Rate	0.8 mL/min	0.463 mL/min
Column Temperature	25 °C	25 °C
Injection Volume	10 µL	1 µL
Detection	270 nm (2.5 Hz)	270 nm (40 Hz)

Preparation of standard solution

An appropriate amount of cryptotanshinone and tanshinone IIA reference substances was accurately weighed and dissolved in methanol to prepare a solution containing 10 µg of cryptotanshinone and 60 µg of tanshinone IIA per mL for assay analysis. For fingerprint analysis, a solution of methanol containing 30 µg of cryptotanshinone and 130 µg of tanshinone IIA per mL was prepared.

Preparation of sample solution

A 5 mg sample was accurately weighed and transferred to a 10 mL volumetric flask. Methanol was added to dissolve and dilute to the mark, and the solution was mixed well and filtered prior to analysis. For fingerprint analysis, a 10 mg sample in 10 mL methanol solution was prepared.

Method adjustment parameters

Method adjustments were performed according to ChP General Chapter <0512>. The adjustment strategy is also consistent with the modernization principles described in USP General Chapter <621> Chromatography, which permits defined adjustments to chromatographic conditions, including column dimensions and particle size, gradient time, flow rate, and injection volume—if system suitability requirements are met. The column length to particle diameter ratio (L/dp) was maintained within the range of −25 to +50%. Gradient time, flow rate, and injection volume were adjusted using Equations 1, 2, and 3, respectively.

Equation 1.

$$tG2 = tG1 \times (F1/F2) \times ([L2 \times dc2^2] / [L1 \times dc1^2])$$

Equation 2.

$$F2 = F1 \times [(dc2^2 \times dp1)/(dc1^2 \times dp2)]$$

Equation 3.

$$V2 = V1 \times [(L2 \times dc2^2)/(L1 \times dc1^2)]$$

Where:

tG1 = Gradient time indicated in the monograph (min)

tG2 = Adjusted gradient time (min)

F1 = Flow rate indicated in the monograph (mL/min)

F2 = Adjusted flow rate (mL/min)

dc1 = Internal diameter of the column indicated in the monograph (mm)

dc2 = Internal diameter of the column used (mm)

dp1 = Particle size indicated in the monograph (µm)

dp2 = Particle size of the column used (µm)

V1 = Injection volume indicated in the monograph (µL)

V2 = Adjusted injection volume (µL)

L1 = Column length indicated in the monograph (mm)

L2 = Adjusted column length (mm)

All other parameters remained unchanged.

Results and discussion

The modernization of TCM methods for tanshinone analysis from conventional 5 µm columns to sub-2 µm columns was successfully achieved using the ChP General Chapter <0512> adjustment guidelines. Two separate HPLC methods—one for fingerprint analysis and one for assay analysis—were optimized and transferred to sub-2 µm ZORBAX RRHD Extend-C18 columns while maintaining analytical performance and resolution.

Assay method adjustment

The original assay method employed a ZORBAX Extend-C18 column (4.6 × 250 mm, 5 µm) with a 30-minute total run time. Following the ChP General Chapter <0512> guidelines, the method was adjusted to a ZORBAX RRHD Extend-C18 column (2.1 × 100 mm, 1.8 µm) with a total run time of 4.32 minutes. The gradient time was recalculated using Equation 1, resulting in a reduction from 20 to 2.88 minutes for the main gradient phase. The flow rate was adjusted from 1.2 to 0.463 mL/min, and the injection volume was reduced from 10 to 1 µL according to the volume adjustment formula (Equation 3). This modernization achieved an 86% reduction in analysis time while maintaining baseline separation of cryptotanshinone and tanshinone IIA with a resolution (Rs) of 2.0 in both methods.

System suitability parameters confirmed the robustness of the adjusted assay method. The plate number (N) for cryptotanshinone increased from 30,162 to 33,528, exceeding the requirement of greater than 20,000 theoretical plates. The resolution between cryptotanshinone and adjacent peaks remained at 2.0, meeting the pharmacopoeia requirement of greater than 1.5. Relative standard deviation (RSD) of peak areas from five injections was 0.4% for cryptotanshinone and 0.3% for tanshinone IIA—both well below the 2.0% limit. Tailing factors (Tf) for both peaks remained within the acceptable range of 0.8 to 1.8, with values of 1.04 for cryptotanshinone and 1.04 and 1.00 for tanshinone IIA in the original and adjusted methods, respectively (Figure 1).

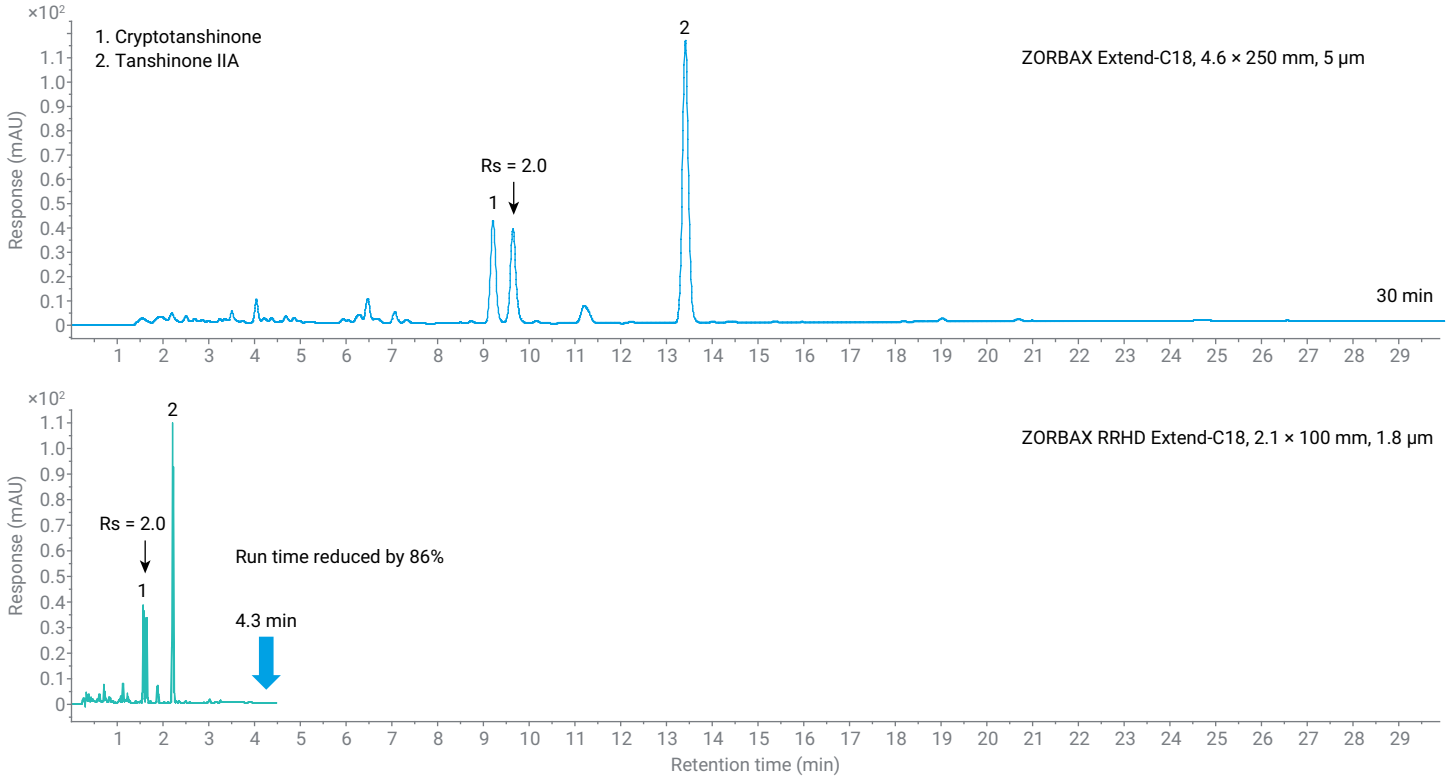


Figure 1. Overlaid chromatograms comparing assay method performance on Agilent ZORBAX Extend-C18 (4.6 × 250 mm, 5 µm) and Agilent ZORBAX RRHD Extend-C18 (2.1 × 100 mm, 1.8 µm) columns showing baseline resolution (Rs = 2.0).

Fingerprint method adjustment

The fingerprint analysis method was similarly modernized from a 60-minute total run time on the 5 µm column to 8.64 minutes on the 1.8 µm column. The gradient was adjusted as described in Table 2. The flow rate was reduced from 0.8 to 0.463 mL/min, and the injection volume was reduced from 10 to 1 µL. The fingerprint chromatograms demonstrated excellent repeatability following the method transfer, with the characteristic peak pattern preserved and all major components baseline resolved (Figure 2). The analysis time was reduced by 86%, from 50 to 7.2 minutes, while maintaining the separation quality required for fingerprint identification.

Column selection and performance

The Agilent ZORBAX Extend-C18 phase was selected from multiple C18 stationary phases screened during method development due to its unique selectivity for the critical compound pair of cryptotanshinone and tanshinone I. This selectivity enabled the achievement of high resolution between these compounds. The sub-2 µm ZORBAX RRHD Extend-C18 column demonstrated superior durability under elevated pressure conditions, with a pressure limit of 1200 bar, accommodating the increased backpressure associated with smaller particle sizes and faster flow rates.

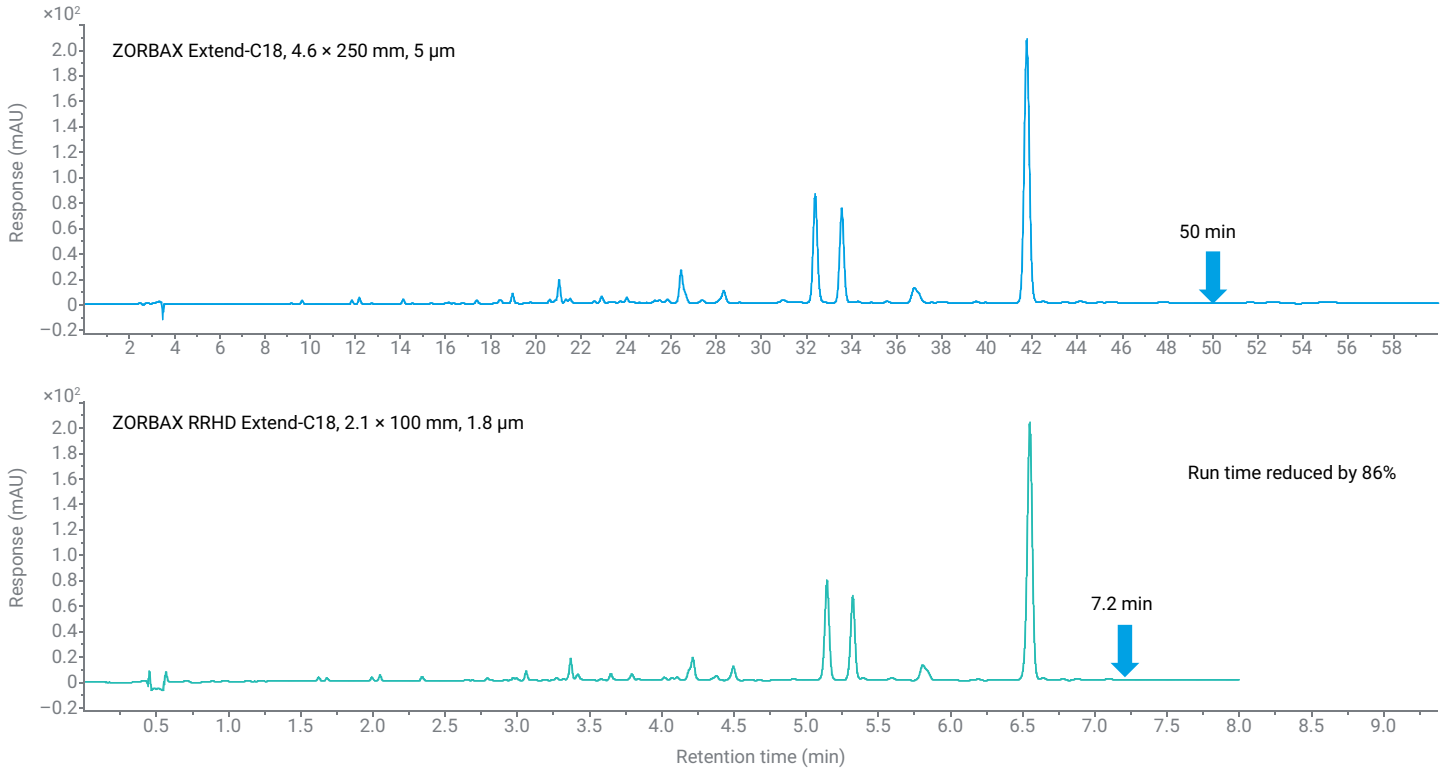


Figure 2. Fingerprint chromatograms of tanshinone extract obtained on the original 5 µm column (50-minute run time) and the modernized 1.8 µm column (7.2-minute run time), demonstrating preservation of peak pattern.

System suitability testing

System suitability was evaluated for the adjusted assay method. The results are shown in Table 3. The plate number (N) for cryptotanshinone was determined to be greater than 20,000. The resolution (Rs) between cryptotanshinone and adjacent peaks was maintained at greater than 1.5. The RSD of peak areas from five injections was less than 2.0%. The tailing factor (Tf) for cryptotanshinone and tanshinone IIA was maintained within the range of 0.8 to 1.8.

The successful modernization of both assay and fingerprint methods demonstrates that sub-2 µm columns could effectively replace conventional 5 µm columns for tanshinone analysis while providing substantial improvements in throughput and solvent consumption. The 86% reduction in analysis time and 92% solvent saving translates to significant cost savings and environmental benefits for analytical laboratories performing routine tanshinone quantification and fingerprint analysis.

Conclusion

This work successfully modernized ChP monograph HPLC methods for tanshinone analysis by transitioning from traditional 5 µm columns to sub-2 µm columns. Using the Agilent ZORBAX RRHD Extend-C18 (2.1 × 100 mm, 1.8 µm) column with an Agilent 1290 Infinity III LC system, both assay and fingerprint methods achieved significant improvements in analysis time while maintaining baseline resolution for cryptotanshinone and tanshinone IIA. System suitability parameters were met with plate numbers exceeding 33,528 and RSD values below 0.4%. The method adjustment adhered to ChP General Chapter <0512> guidelines, demonstrating that sub-2 µm columns provide enhanced throughput, improved cost efficiency, and a notable 92% reduction in solvent consumption, significantly reducing environmental impact while maintaining excellent analytical performance.

References

1. HPLC method, Chromatography, Chinese Pharmacopeia General Chapter. *Chinese Pharmacopeia* vol 4, **2025**.
2. Chinese Pharmacopeia Monograph: Tanshinones, *China Pharmacopeia*, **2025**.
3. United States Pharmacopeia. General Chapter <621> Chromatography. USP–NF, official from December 1, 2022; revised harmonized standard describing allowable chromatographic system adjustments for method transfer and modernization.

Table 3. System suitability results for assay method.

Column	N1 (Plate Number of Cryptotanshinone)(> 20,000)	The Rs of Peak 1 and Adjacent Peaks (> 1.5)	RSD (%) of 5 Injections (< 2.0%)	Tf (Cryptotanshinone and Tanshinone IIA) (0.8–1.8)
ZORBAX Extend-C18, 4.6 × 250 mm, 5 µm	30,162	2.0	Area of peak 1: 0.4% Area of peak 2: 0.3%	Tf of peak 1: 1.04 Tf of peak 2: 1.04
ZORBAX RRHD Extend-C18, 2.1 × 100 mm, 1.8 µm	33,528	2.0	Area of peak 1: 0.4% Area of peak 2: 0.3%	Tf of peak 1: 1.04 Tf of peak 2: 1.00

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DE-017169

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Printed in the USA, September 4, 2026
5994-9445EN

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