Determination of Asarinin in Xixin (*Asari Radix Et Rhizoma*)

Using Agilent InfinityLab Poroshell 120 EC-C18, 1.9 µm Columns

Application Note

Pharmaceutical

Author

Rongjie Fu

Agilent Technologies

Shanghai

Abstract

The active compound asarinin in Xixin (*Asari Radix Et Rhizoma*) was analyzed with both a sub-2 µm Agilent InfinityLab Poroshell 120 EC-C18 column and a traditional 5 µm column. Resolution was improved and the analysis time was shortened with the sub-2 µm column.

Introduction

To control the quality of Traditional Chinese Medicines (TCM), the levels of their main compounds are required to be determined using HPLC methods that are regulated in the China Pharmacopeia (CHP). The active compound in the TCM Xixin, asarinin, is required to be determined by HPLC with a traditional column according to CHP [1].

Superficially porous particle LC columns provide higher efficiency compared to totally porous particles of the same size without generating higher pressures. The newly developed sub-2 µm superficially porous particle columns give much higher efficiency and a shortened analysis time.

We transferred the CHP method from a traditional Agilent ZORBAX Eclipse Plus-C18, 4.6 × 250 mm, 5 µm column to InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm and 2.1 × 150 mm, 1.9 µm columns. The improved results included better resolution and a shorter analysis time.

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Materials and Methods

All reagents and solvents were HPLC or analytical grade. Acetonitrile was from JT Baker, USA. The TCM Xixin and asarinin were provided by a local pharmaceutical company in China. The standard solution was made by dissolving asarinin in methanol to achieve a concentration of 50 µg/mL.

HPLC and UHPLC analysis was performed with an Agilent 1290 Infinity LC including:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (DAD) (G4212A)

Table 1 shows the UHPLC system configuration details. Table 2 shows the LC method parameters.

Table 1. Agilent 1290 Infinity LC system configuration.

Parameter	Value
Agilent 1290 Infinity binary pump (G4220A):	35 µL solvent mixer: Jet weaver, 35 µL/100 µL (G4220–60006)
Agilent 1290 Infinity high performance autosampler (G4226A):	Seat assembly, low dispersion, for Agilent 1290 Infinity autosampler (G4226–87020) Autosampler and heater: capillary, stainless steel, 0.12 × 300 mm (G1316–87318) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182–0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182–0717)
Agilent 1290 Infinity thermostatted column compartment (G1316C):	Heat exchanger, 1.6 µL, L (G1316–80003) Heater and column: A-Line Quick Connect assembly, 105 mm, 0.12 mm (5067–5957) Column and flow cell: Capillary, red peak, 0.13 × 300 mm, 5 m/pk (5042–6461)
Agilent 1290 Infinity diode array detector (G4212A):	Max-Light cartridge flow cell, 10 mm, 1 µL (G4212–60008)
Agilent OpenLAB CDS ChemStation edition revision C.01.07 [27]:	G4226A: A.07.01 [001] G4220A: A.07.01 [0006] G1316C: A.07.01 [001] G4212A: B.07.01 [0005]

Table 2. HPLC/UHPLC Method Parameters.

Column	Mobile phase	Flow rate (mL/min)	Gradient	Inj. vol. (µL)	Sample preparation	TCC (°C)	DAD
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm (p/n 695675–902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775–902)	A) water B) acetonitrile	0.42	**Time (min) %B** 0 50 4 50 5.2 100 7.2 100 7.3 50 9 50	0.8	Weigh 0.5 g of Xixin powder and add 15 mL methanol. Extract by sonication for 45 minutes, then filter using a 0.2 µm filter (5190–5277)	40	287 nm, 40 Hz
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm (p/n 693675-902)	A) water B) acetonitrile	0.42	**Time (min) %B** 0 50 6 50 7.8 100 10.8 100 10.95 50 13.5 50	1.2		40	287 nm, 40 Hz
Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990–902)	A) water B) acetonitrile	1.0	**Time (min) %B** 0 50 20 50 26 100 36 100 36.5 50 45 50	10		40	287 nm, 10 Hz

Results and Discussion

This CHP-regulated method is used to determine the amount of asarinin in Xixin with a traditional 4.6 × 250 mm, 5 µm column. This method was run with an Agilent ZORBAX Plus C18, 4.6 × 250 mm, 5 µm column, then transferred to Agilent InfinityLab Poroshell 120, 1.9 µm, 150 mm, and 100 mm columns. The linear flow rate was doubled to achieve maximum efficiency for the sub-2 µm columns. The gradient time was adjusted according to the column length and flow rate.

The chromatogram in Figure 1 shows that the 5 µm column provided an acceptable resolution of asarinin for its quantitative analysis. Using an InfinityLab Poroshell 120, 2.1 × 100 mm, 1.9 µm column reduced the analysis time from 40 to 10 minutes with a slight improvement in the resolution of asarinin.

Normally, the minimum resolution required for quantitative analysis is 1.5. Both methods, using ZORBAX Eclipse Plus-C18, 4.6 × 250 mm, 5 µm and InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm columns meet the requirement of quantitative analysis. But the resolution was not good enough for quantitation. The advantages of using a longer sub-2 µm column include higher peak capacity with superior resolution, especially for critical peaks. However, longer columns also generate higher pressures. In this analysis, a longer InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column was used (Figure 1). The resolution of asarinin was improved significantly from 1.6 to 2.9, and there was a minor peak pair improvement from 1.3 to 1.6. The pressure was approximately 600 bar, which was far below the column pressure limit of 1,300 bar, and was suitable for use on an Agilent 1290 Infinity LC. A smaller superficially porous particle column, of 1.9 µm, provided higher efficiency and better resolution than the 2.7 µm column shown in Figure 2.

Figure 3 shows that the method run using the InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column is recommended for the quantitative analysis of asarinin in Xixin.

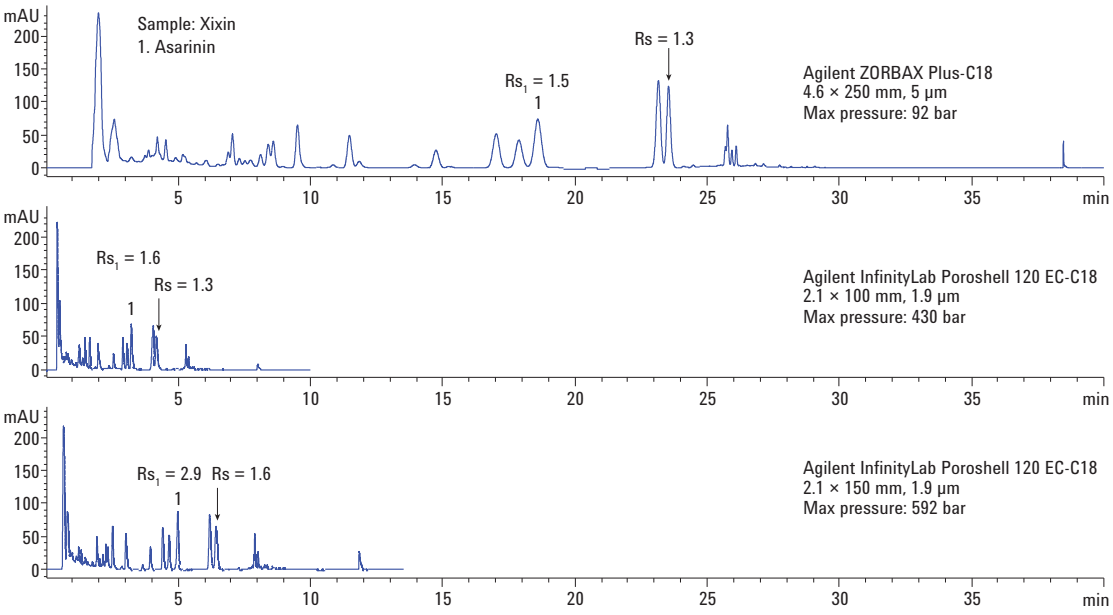

Figure 1. Chromatograms of Xixin analysis using an Agilent ZORBAX Eclipse Plus-C18, and Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm and 2.1 × 150 mm, 1.9 µm columns.

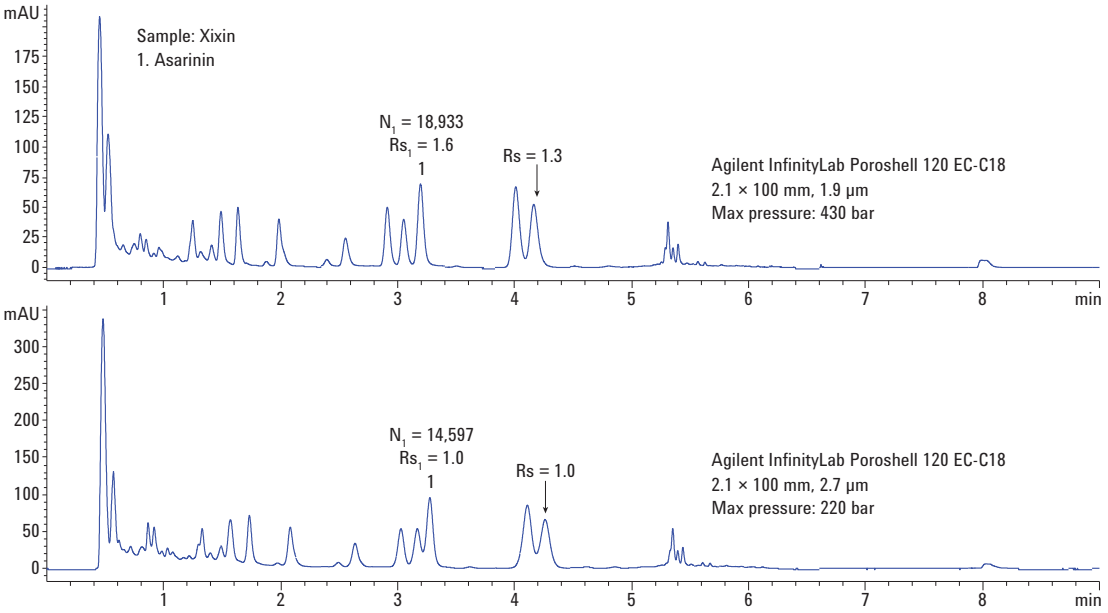

Figure 2. Comparison of chromatograms produced from the analysis of Xixin using an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm and an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm column.

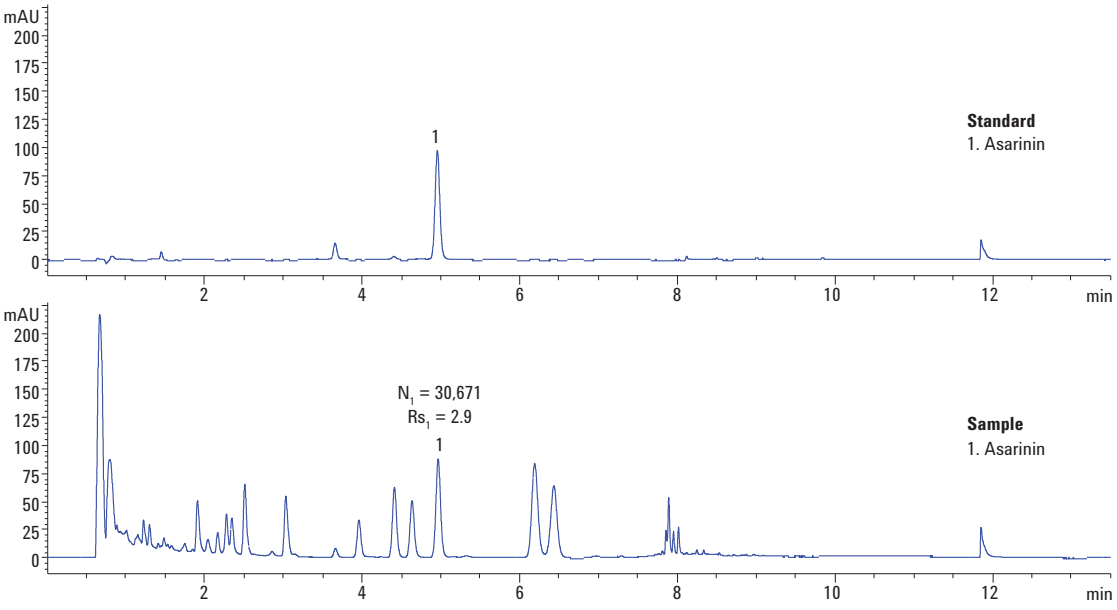

Figure 3. Quantitative analysis of asarinin in Xixin with an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column.

Conclusions

The sub-2 µm Agilent InfinityLab Poroshell 120 superficially porous particle columns provide both superior performance and fast analysis at UHPLC pressures. A long column gives much greater peak capacity than a short one, providing sufficient resolutions for target compound measurement.

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