Application Note

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Analysis of Traditional Chinese Medicine Formula Granules for Quality Control

The Agilent InfinityLab Poroshell 120 Aq-C18 column matches standard method requirements

Author

Rongjie Fu
Agilent Technologies, Inc.

Abstract

A standard high-performance liquid chromatography (HPLC) method to obtain a characteristic chromatogram for a traditional Chinese medicine (TCM) formula granule from *Desmodium styracifolium (Osb.) Merr*. was reproduced with an Agilent InfinityLab Poroshell 120 Aq-C18 column. The results achieved with this column all meet the system suitability requirements in the standard method. Superficially porous HPLC columns such as the 2.7 µm Agilent InfinityLab Poroshell 120 show comparable efficiency to sub-2 µm particles with approximately half the backpressure.

Introduction

Characteristic HPLC chromatograms are considered a necessary test item for controlling the quality of TCM formula granules and are required by national drug standards in China.[1] For various reasons, quality control departments in pharmaceutical industries often choose similar columns instead of those used in the original method. An effective method is to screen the same type of column with similar selectivity to the original column, then reproduce the original method without other method adjustments. Reversed-phase columns such as C18 columns are the most popular for TCM quality control. Agilent provides many different C18 bonded phases, which makes it easier to screen a suitable column for TCM detection according to standard methods.

This study screened out an InfinityLab Poroshell 120 Aq-C18 column for measurement of a TCM formula granule from *Desmodium styracifolium (Osb.) Merr.* The InfinityLab Poroshell 120 Aq-C18 column has been developed based on superficially porous particles with an optimized C18 ligand with proprietary endcapping.[2] This design enables column use with a high aqueous mobile phase and less retention loss. The column also has stronger retention of polar compounds and different selectivity than current C18 columns.

Experimental

Instruments and materials

An Agilent 1290 Infinity II LC system with quaternary pump was used in this experiment. The system consisted of:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)

The LC column used was the Agilent InfinityLab Poroshell 120 Aq-C18, 2.1 × 150 mm, 2.7 µm (part number 693775-742).

All reagents and solvents were HPLC-grade. Acetonitrile and phosphoric acid were purchased from Anpel Laboratory Technologies (Shanghai, China). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). The sample was purchased from a local market. The sample was dissolved in 80% methanol, according to the national drug standard for formula granules from *Desmodium styracifolium (Osb.) Merr*.

HPLC method parameters

The method parameters used in the experiment are displayed in Table 1.

Table 1. Method parameters.

Parameter	Value
Mobile Phase	A) 0.1% Phosphoric acid in water B) Acetonitrile
Gradient	Time (min) B% 0 15 10 15 11 16 20 16
Stop Time	20 min Postrun: 5 min
Column Temperature	30 °C
Flow Rate	1.5 mL/min
Detector	UV 340 nm at 20 Hz
Injection Volume	1 µL

Results and discussion

The HPLC method was run on an original column from vendor W used in the standard and on different Agilent C18 columns, including an Agilent InfinityLab Poroshell 120 Aq-C18 column, without any change in parameters. Figure 1 shows the separation of the sample on a vendor W column and the InfinityLab Poroshell 120 Aq-C18 column. Both columns showed similar selectivity for the separation. Requirements for system suitability testing and the results tested with the InfinityLab Poroshell 120 Aq-C18 column are listed in the Table 2. The relative retention time, relative peak area, and theoretical plates of target peaks all met the requirements of the standard method.

Table 2. The system suitability requirements and measured values.

Peak	Relative Retention Time (RRT)	RRT on Agilent Aq-C18	Relative Peak Area (RA)	RA on Agilent Aq-C18	N (USP)	N of Peak 3
1	RT_1/RT_s: 0.68 ±10%	0.68	/	/	/	/
2	RT_2/RT_s: 0.87 ±10%	0.88	/	/	/	/
3 (S)	/	/	/	/	≥10,000	21,659
4	RT_4/RT_s: 1.06 ±10%	1.07	/	/	/	/
5	RT_5/RT_s: 1.25 ±10%	1.24	/	/	/	/
6	RT_6/RT_s: 1.41 ±10%	1.40	A_6/A_s ≥0.366	0.56	/	/
7	RT_7/RT_s: 1.92 ±10%	1.93	/	/	/	/

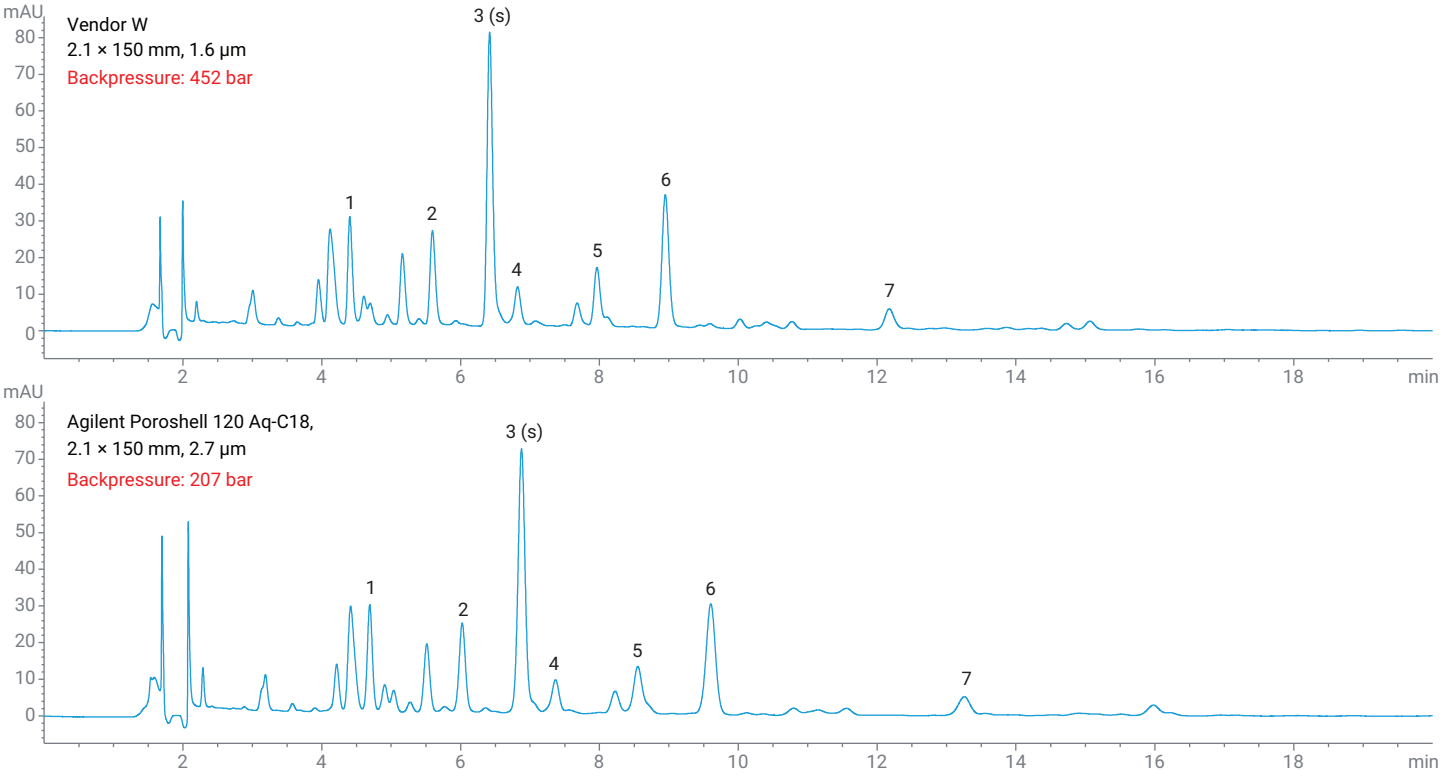

Figure 1. Characteristic chromatograms achieved on a vendor W column and an Agilent InfinityLab Poroshell 120 Aq-C18 column. Compounds identified: 3) Schaftoside, 4) Homoorientin, 6) Isoschaftoside, and 7) Isovitexin.

The standard method used a column with 1.6 µm particles. Although a column with larger particle sizes was used in this study, a close efficiency compared to the original column was obtained. The theoretical plates of peak 3 are much higher than those specified in the standard method. This result occurred because superficially porous Agilent HPLC columns such as the 2.7 µm InfinityLab Poroshell 120 provide comparable efficiency to sub-2 µm particles with approximately half the backpressure. Using a larger particle size such as the 2.7 µm column has the advantage of less sample preparation and less plugging with "dirty" samples while maintaining a high level of efficiency. Therefore, a longer column lifetime is expected with the 2.7 µm column than with a sub-2 µm column.

Similarity is important for the characteristic chromatogram detection of TCM formula granules. To achieve a separation similar to the standard chromatogram in the standard method, the retention time often needs to be adjusted to be consistent with the standard method. An appropriate flow rate adjustment is allowed without the need for revalidation. In this separation, when the flow rate on the InfinityLab Poroshell 120 Aq-C18 column was accelerated from 0.2 to 0.22 mL/min, the retention was consistent with the original method. Figure 2 shows a comparison between the accelerated separation on the InfinityLab Poroshell 120 Aq-C18 column and the original separation on the column of vendor W.

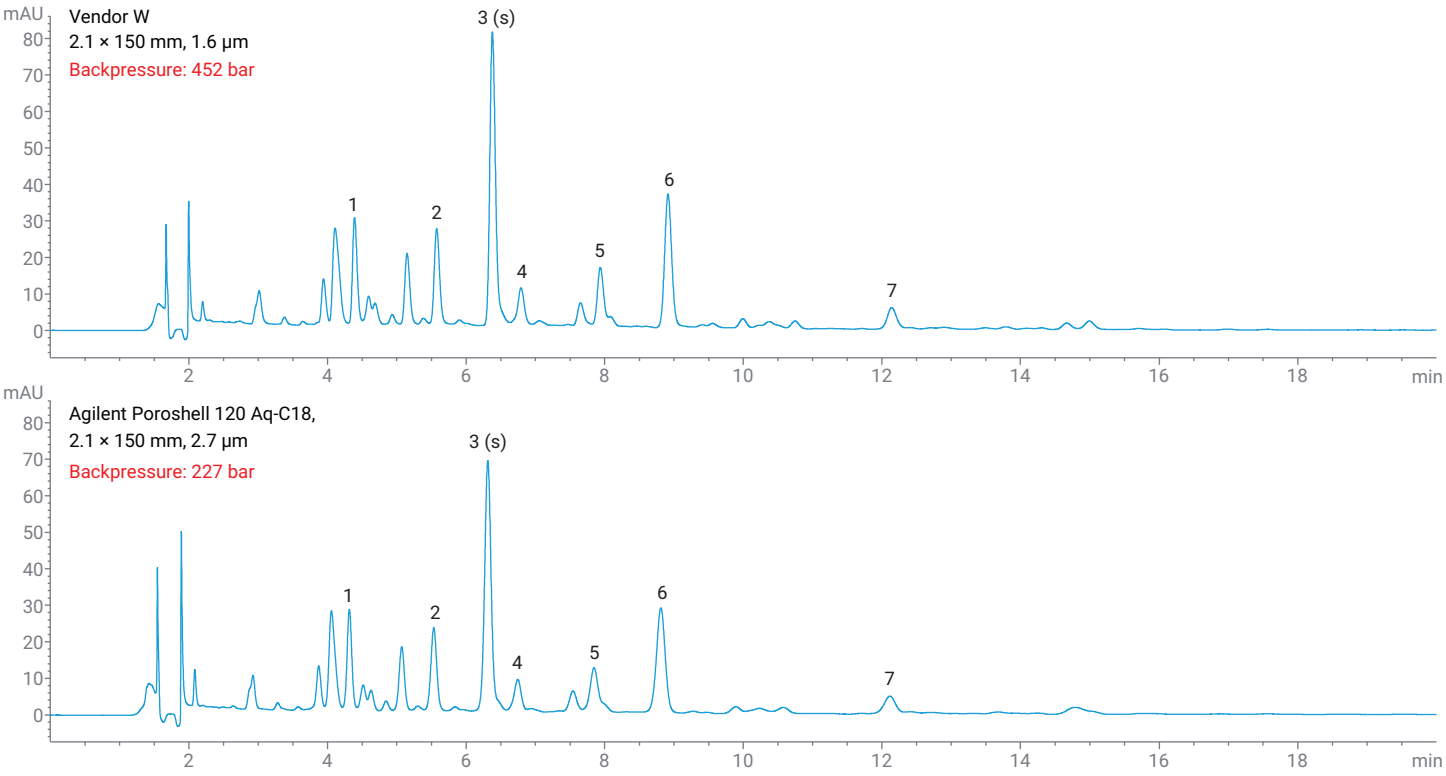

Figure 2. Comparison between the accelerated separation on the Agilent InfinityLab Poroshell 120 Aq-C18 column and the original separation on the column of vendor W.

Conclusion

The analysis of a characteristic chromatogram for a TCM formula granule from *Desmodium styracifolium (Osb.) Merr*. was performed with an Agilent InfinityLab Poroshell 120 Aq-C18 column. Requirements for system suitability testing all met the requirements of the original method. This column can be used for analysis of the characteristic chromatogram of a TCM formula granule from *Desmodium styracifolium (Osb.) Merr*.

References

1. National Drug Standard: YBZ-PFKL-2021054, *China State Food and Drug Administration*.
2. Fu, R.-J.; Wei, T.-C. Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance, *Agilent Technologies application note*, publication number 5994-5555EN, **2022**.

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