Application Note

Pharmaceutical

Reproducing the TCM Method for the Characteristic Chromatogram of Guilin Xiguashuang

Using the Agilent 1290 Infinity II LC with ISET and Agilent InfinityLab Poroshell HPH-C18 column

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Abstract

A Chinese Pharmacopoeia (ChP) method of obtaining the characteristic chromatogram for Guilin Xiguashuang was reproduced using an Agilent 1290 Infinity II liquid chromatography (LC) system with 1290 Intelligent System Emulation Technology (ISET) and an Agilent InfinityLab Poroshell HPH-C18 column. With ISET, the difference between various LC systems can be minimized, enabling reproducible results across platforms. When emulating the Waters H-Class UPLC system, the InfinityLab Poroshell HPH-C18 met the ChP requirements for Guilin Xiguashuang[1], with the relative retention time (RRT) of all characteristic peaks within ± 10%.

Introduction

Characteristic chromatogram is a popular analysis method in traditional Chinese medicine (TCM). It has been used for quality control of TCM regulated in ChP. Many pharma companies are doing analysis of TCM following ChP methods. The methods included in the ChP were developed using specific LC systems. When a laboratory uses different system methods, the RRT may not be repeatable and may fail to meet ChP method requirements.

The 1290 Infinity II LC with ISET makes it possible to emulate non-Agilent instrumentation such as Waters Laboratory Equipment UPLC systems. Thus, with the help of the Agilent ISET and the flexibility and precision of the Agilent 1290 Infinity II system, the TCM characteristic chromatogram can be reproduced without changing the original method.

Agilent InfinityLab Poroshell HPH-C18 columns are packed with superficially porous particles and are a part of the Agilent InfinityLab Poroshell 120 family. The HPH-C18 column is designed to be stable when used with high-pH mobile phases, which is achieved by integrating organic material into the porous outer silica layer of the Poroshell particles. This chemistry differs from regular phases such as Poroshell 120 EC-C18 and Poroshell 120 SB-C18, providing a slightly different selectivity.

In this application note, the ChP method for characteristic chromatogram determination of Guilin Xiguashuang was run on a 1290 Infinity II LC, emulating Waters ACQUITY UPLC I-Class and H-Class systems. The seamless method transfer displayed excellent retention time correlation and generally showed better resolution.

Experimental

Instruments and materials

A 1290 Infinity II LC system was used including the following modules: a 1290 Infinity II binary pump (part number G7120A), 1290 Infinity II Multisampler (part number G7167B), 1290 Infinity II thermostatted column compartment (part number G7116B), and 1290 Infinity II diode array detector (part number G7117B), controlled and analyzed using Agilent OpenLab chromatography data system (CDS), Version 2.8 with ISET 4 Version 1.0.

Chromatographic conditions

Columns:

- Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 1.9 µm (part number 695675-902)
- Agilent InfinityLab Poroshell 120 SB-C18, 2.1 × 100 mm, 1.9 µm (part number 685675-902)
- Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 100 mm, 2.7 µm (part number 695775-942)
- Agilent InfinityLab Poroshell 120 Aq-C18, 2.1 × 100 mm, 2.7 µm (part number 695775-742)
- Agilent InfinityLab Poroshell HPH-C18, 2.1 × 100 mm, 1.9 µm (part number 695675-702)

Table 1. Chromatographic conditions.

Parameter	Value
Mobile Phase	A) 0.2% Phosphoric acid + 0.2% triethylamine B) Acetonitrile
Gradient	Time (min) B (%) 0.00 20.00 10.00 24.00 23.00 40.00 26.00 40.00 32.00 75.00 35.00 92.00 43.00 95.00 Stop: 45 min Post-run: 6 min
Flow Rate	0.2 mL/min
Column Temperature	30 °C
Injection Volume	1 µL
Detection	UV 254 nm

Regents and samples

All reagents and solvents were HPLC-grade. Acetonitrile, phosphoric acid, and triethylamine were purchased from Anpel Laboratory Technologies (Shanghai, China). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK).

The sample solution and reference standard solution were prepared according to the monograph Guilin Xiguashuang in the ChP method.

Results and discussion

Column screening with the Agilent 1290 Infinity II LC

The original method of characteristic chromatogram analysis for Guilin Xiguashuang in the ChP was developed on a Waters UPLC system with an ACQUITY UPLC BEH C18, 2.1 × 100 mm, 1.7 µm column. The same method was run using an Agilent 1290 Infinity II LC system with a binary pump and various chemistry columns. The LC conditions and column dimension were kept the same. The chromatograms are shown in Figure 1. The 12 peaks in the characteristic chromatogram were recorded according to the ChP method. The elution order of these target peaks was the same as the standard method in the ChP using the InfinityLab Poroshell HPH-C18 column. The RRTs of the peaks were calculated and are shown in Table 2. All RRTs of the peaks with InfinityLab Poroshell HPH-C18 were within the required range of ± 10%. This demonstrates that the selectivity of InfinityLab Poroshell HPC-C18 is similar to the Waters ACQUITY UPLC BEH C18, making it possible to replace the BEH C18 column with InfinityLab Poroshell HPH-C18 for the methods developed. Other phases show that the order of elution was reversed for several compounds. For example, peaks 7 and 8 eluted in reverse order with the Poroshell 120 Aq-C18 and SB-C18, both of which have the same C18 ligands. The two compounds coeluted with the Poroshell 120 EC-C18, which is another C18 ligand. The positively charged Poroshell 120 CS-C18 showed significantly different selectivity.

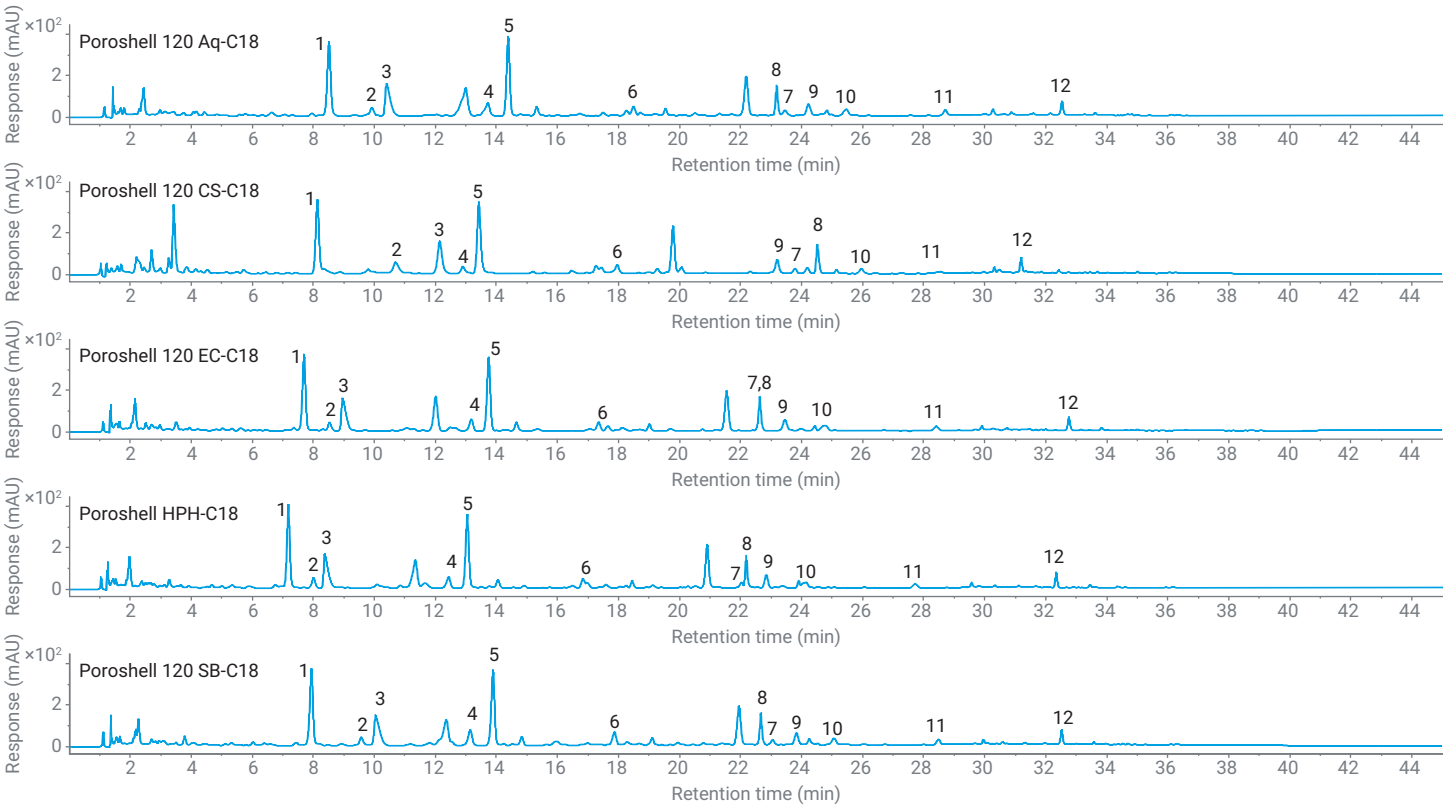

Figure 1. Characteristic chromatograms with various phases using an Agilent 1290 Infinity II LC.

Method emulating Waters UPLC systems

Because the original method was developed using a Waters UPLC system, both Waters ACQUITY UPLC I-Class and H-Class systems were emulated using the Agilent 1290 Infinity II LC system with ISET.

The chromatograms were similar, but with slightly stronger retention and better resolution of peaks 7 and 8 than the emulated ACQUITY UPLC H-Class system, as shown in Figure 2.

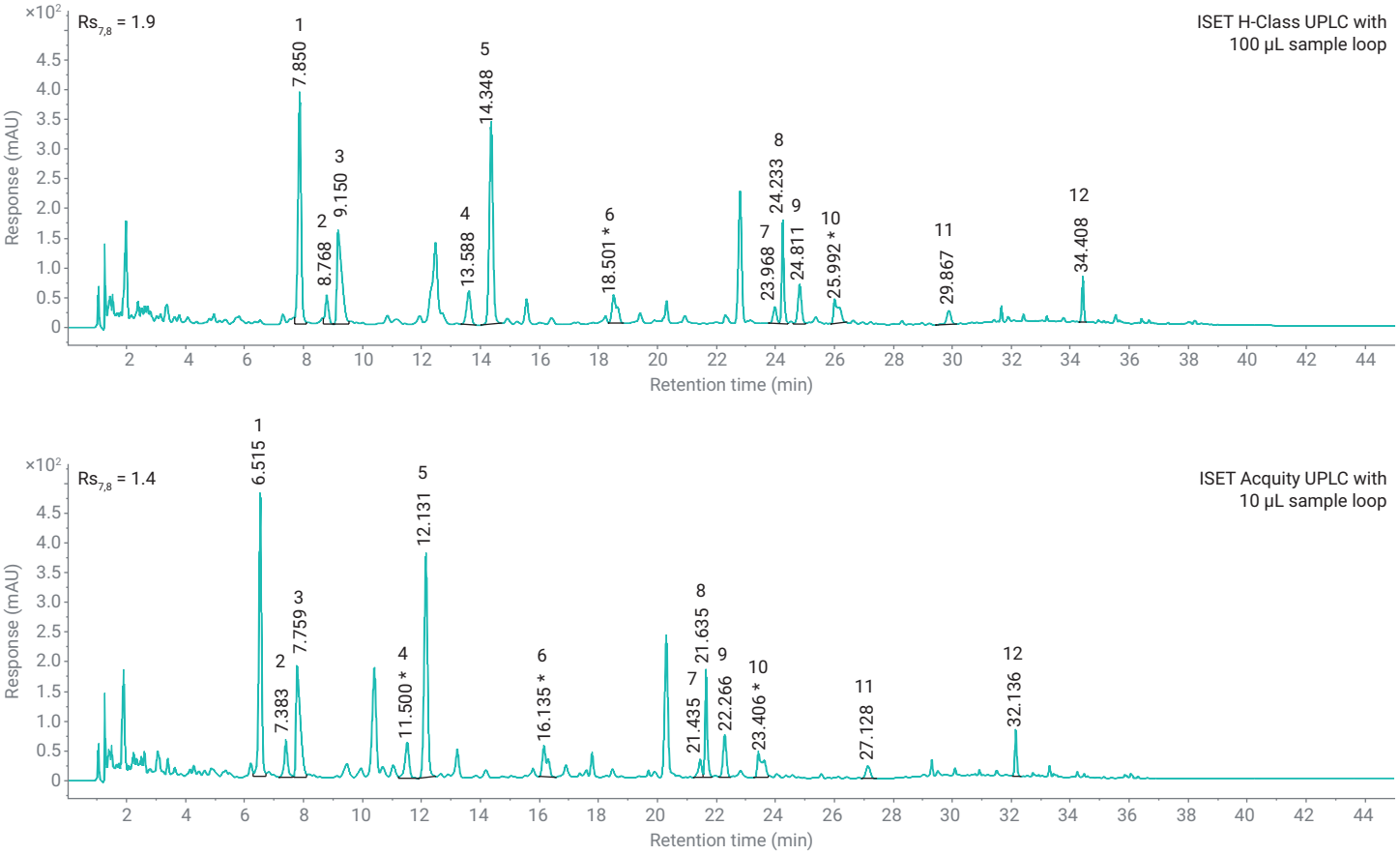

Figure 2. Chromatograms were achieved from two emulated Waters ACQUITY UPLC systems.

The RRTs achieved with two emulated instruments are shown in Tables 2 and 3. All RRTs met the ChP requirements when emulating the ACQUITY UPLC H-Class system, but the RRT of Peak 12 did not meet the RRT requirements when emulating the ACQUITY UPLC I-Class system. This may have been caused by the dwell volume of the two different systems.

Table 2. RRT results using an Agilent 1290 Infinity II binary pump with an Agilent InfinityLab Poroshell HPH-C18 column to emulate a Waters ACQUITY UPLC H-Class.

Peak No.	Required RRT	Specification (± 10%)	RT (min)	RRT	Status
1	0.55	0.495 ~ 0.605	7.85	0.54711458	Met
2	0.58	0.522 ~ 0.638	8.768	0.611095623	Met
3	0.62	0.558 ~ 0.682	9.15	0.637719543	Met
4	0.95	0.855 ~ 1.045	13.588	0.947030945	Met
5	1	1	14.348	1	/
6	1.22	1.098 ~ 1.342	18.501	1.289448007	Met
7	1.61	1.449 ~ 1.771	23.968	1.670476721	Met
8	1.64	1.476 ~ 1.804	24.233	1.688946195	Met
9	1.68	1.512 ~ 1.848	24.811	1.729230555	Met
10	1.78	1.602 ~ 1.958	25.992	1.811541678	Met
11	2.04	1.836 ~ 2.244	29.867	2.081614162	Met
12	2.37	2.133 ~ 2.607	34.408	2.398104265	Met

Table 3. RRT results using an Agilent 1290 Infinity II binary pump with an Agilent InfinityLab Poroshell HPH-C18 column to emulate a Waters ACQUITY UPLC I-Class.

Peak No.	Required RRT	Specification (± 10%)	RT (min)	RRT	Status
1	0.55	0.495 ~ 0.605	6.515	0.537053829	Met
2	0.58	0.522 ~ 0.638	7.383	0.608606051	Met
3	0.62	0.558 ~ 0.682	7.759	0.639601022	Met
4	0.95	0.855 ~ 1.045	11.5	0.947984503	Met
5	1	1	12.131	1	/
6	1.22	1.098 ~ 1.342	16.135	1.330063474	Met
7	1.61	1.449 ~ 1.771	21.435	1.766960679	Met
8	1.64	1.476 ~ 1.804	21.635	1.783447366	Met
9	1.68	1.512 ~ 1.848	22.266	1.835462864	Met
10	1.78	1.602 ~ 1.958	23.406	1.92943698	Met
11	2.04	1.836 ~ 2.244	27.128	2.236254225	Met
12	2.37	2.133 ~ 2.607	32.136	2.649080867	Did not meet

Dwell volume affects gradient elution significantly and may alter the resolution, retention time, and relative retentions. The instrument configurations differ from instrument to instrument, which leads to different dwell volumes. The dwell volume can be altered by changing the mixer in the pump and sample loop in the injector. The parameters of the emulated instruments, including mixer and sample loop volume, can be configured using OpenLab CDS Version 2.8 with ISET 4 Version 1.0, as shown in Figure 3. A larger sample loop of 100 µL gave better resolution for peaks 7 and 8 than with a theoretically selectable value of 0 µL loop within the ISET tool, as shown in Figure 4.

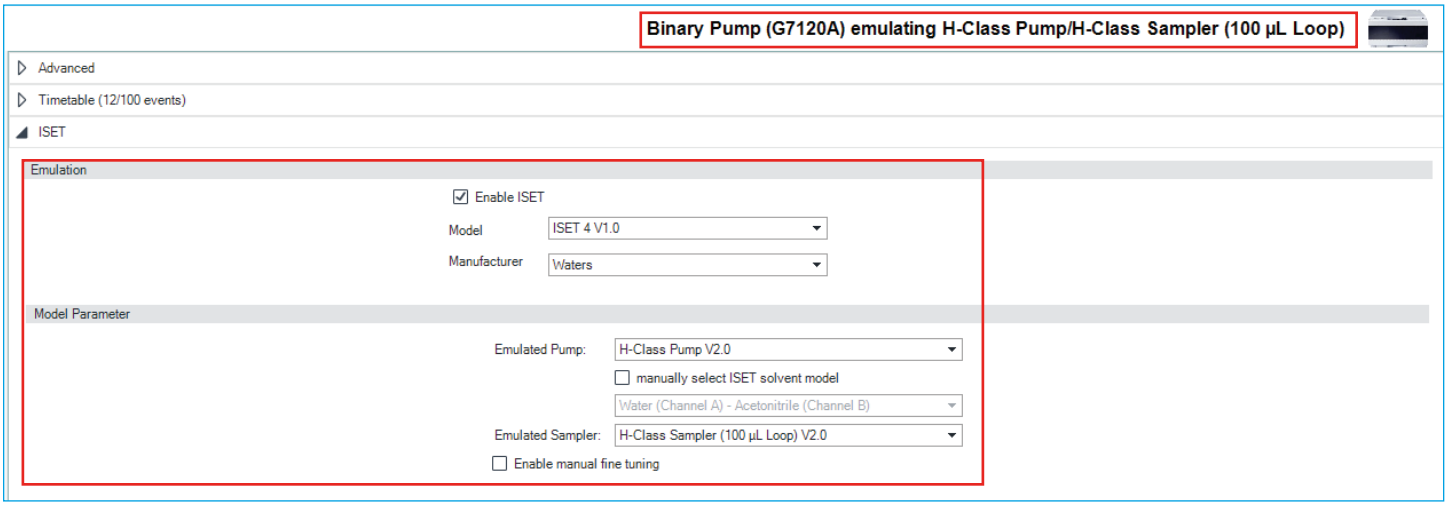

Figure 3. ISET setting in the Agilent OpenLab CDS.

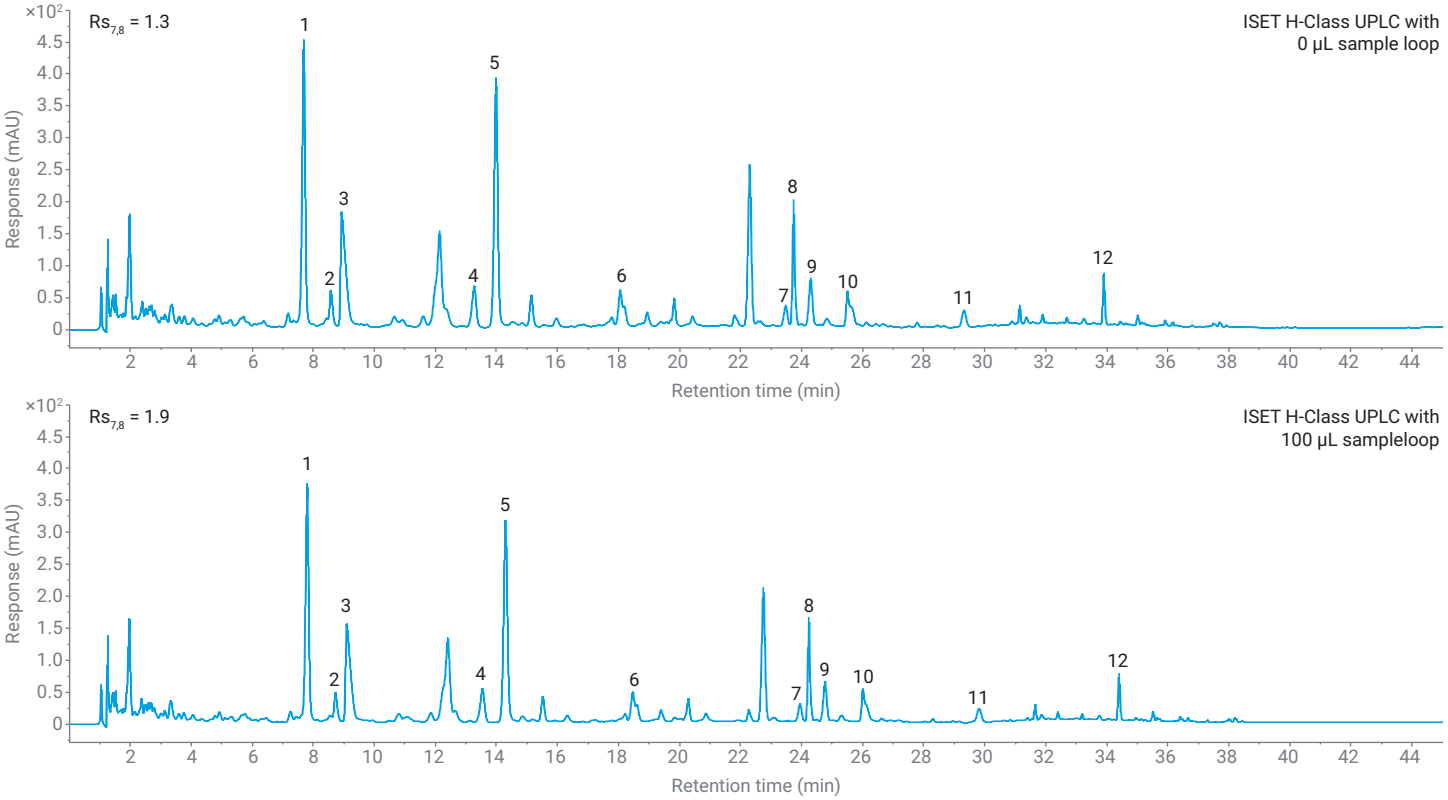

Figure 4. Chromatograms were achieved from an emulated Waters H-Class UPLC system with two different sample loops.

Conclusion

An Agilent 1290 Infinity II LC combined with ISET and an Agilent InfinityLab Poroshell HPH-C18 column reproduced the Chinese Pharmacopeia characteristic chromatogram for Guilin Xiguashuang. Emulating the Waters ACQUITY UPLC H-Class with selected mixer and sample loop settings improved resolution, and the RRTs of all target peaks were within ± 10% of the ChP specification. This configuration enables ChP-compliant analysis on Agilent LC systems without additional parameter adjustments, ensuring consistent quality control results across platforms.

Reference

1. China National Medical Products Administration and National Health Commission. Chinese Pharmacopeia. 2025 Edition, Vol 1: Guilin Xiguashuang.

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