Application Note

Small Molecule Pharmaceuticals

Agilent Trusted Answers

Agilent 1260 Infinity II Prime LC with ISET

Emulation of the Agilent 1200 Series Quaternary LC for the Analysis of Antihistaminic Drugs

Suitable for Agilent 1260 Infinity III LC

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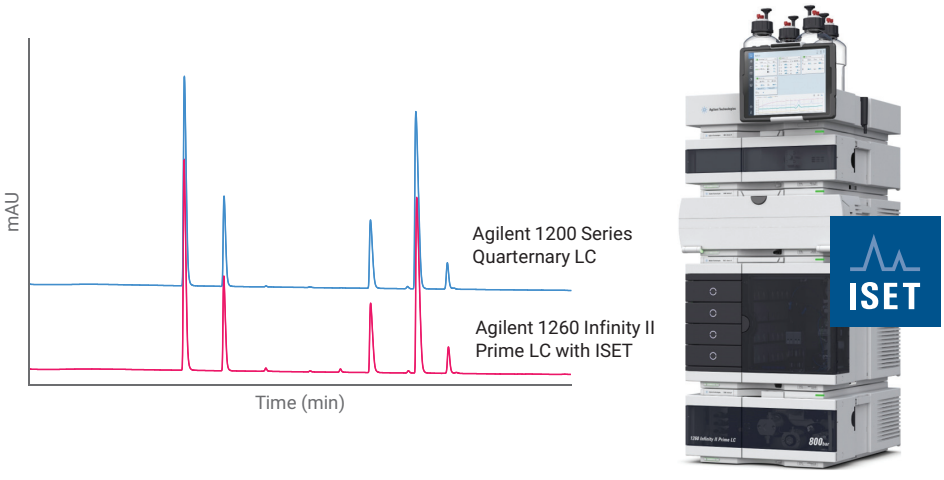
Abstract

Agilent Intelligent System Emulation technology (ISET) facilitates seamless transfer of analytical methods from conventional LC systems such as the Agilent 1200 Series Quaternary LC to the Agilent 1260 Infinity II Prime LC. This Application Note demonstrates the benefit of ISET for the analysis of five antihistaminic drugs. Retention times and resolution of the different experiments were evaluated and compared to the original data obtained by the 1200 Series Quaternary LC. By enabling ISET, excellent agreement on both systems was achieved.



Introduction

The transfer of an analytical method from a conventional HPLC system to an UHPLC system such as the Agilent 1260 Infinity II Prime LC is an important and often critical topic for various laboratories, especially in highly regulated environments. UHPLC systems have lower system delay volumes than conventional HPLC systems, and usually exhibit different pump mixing behaviors. These characteristics might result in different retention times (RTs) and resolution during instrument-to-instrument method transfer. To avoid time-consuming and expensive revalidation for legacy analytical methods, an isocratic hold at the beginning of the run or the installation of additional delay volume can be deployed[1]. However, with this approach, only the differences in delay volume will be considered, and not the differences in mixing behavior of the two analytical pumps.

Agilent Intelligent System Emulation technology (ISET) provides seamless method transfer and delivers similar chromatographic results[2]. By enabling ISET, a compensation of the different delay volumes and pump mixing behaviors is possible without any change of the analytical method or any modifications to the LC system.

This Application Note describes the separation of a mixture of five antihistaminic drugs. The drug mixture was analyzed first on an Agilent 1200 Series Quaternary LC. The analytical method was then transferred to an Agilent 1260 Infinity II Prime LC, and the experiments were conducted with and without ISET functionality. The fine-tuning option of ISET was used in an additional experiment to demonstrate how analytical method transfer can be further optimized. Retention times and resolution were evaluated and compared to the results of the 1200 Series Quaternary LC.

Experimental

Instrumentation

For the analysis of the antihistaminic drugs, the following instruments were used.

Agilent 1200 Series Quaternary LC:

- Agilent 1200 Series Quaternary Pump (G1311A)
- Agilent 1200 Series Autosampler (G1329A)
- Agilent 1200 Series Thermostatted Column Compartment (G1316A)
- Agilent 1200 Series Diode Array Detector (G1315D)

Agilent 1260 Infinity II Prime LC:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Multisampler (G7167A), equipped with an integrated sample cooler (Option #100)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 II Diode Array Detector (G7117C)

Table 1. Chromatographic parameters for analysis of the antihistaminic drug mixture.

Parameter	Value
Column	Agilent ZORBAX SB C18, 4.6 × 75 mm, 3.5 µm (p/n 866953-902)
Mobile phase	A) 0.1 % TFA in water B) acetonitrile
Flow rate	0.8 mL/min
Gradient	10 to 55 %B in 10 minutes
Stop time	12 minutes
Post time	5 minutes
Injection volume	5 µL with 3 seconds needle wash (50 % acetonitrile in water)
Column temperature	25 °C
Detection	232/8 nm, Ref. Off, 20 Hz

Software

Agilent OpenLAB CDS 2.2 (M8413A)

Sample

Mixture of five antihistaminic drugs, each with a concentration of 150 ng/µL (in order of elution): tripelenamine, chlorpheniramine, tetracaine, promethazine, and cetirizine.

Chemicals

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Antihistaminic drugs were bought from Sigma-Aldrich Corp., St. Louis, USA.

Results and Discussion

The five antihistaminic drugs were analyzed with a conventional analytical method on a 1200 Series Quaternary LC using a 4.6 × 75 mm column packed with 3.5-µm particles. First, the analytical method was transferred to the 1260 Infinity II Prime LC without ISET enabled. As expected, all five analytes clearly shifted to earlier retention times. Next, the ISET functionality was enabled. Figure 1 shows an overlay of the original chromatogram acquired with the 1200 Series Quaternary LC, and the two chromatograms measured with the 1260 Infinity II Prime LC with and without ISET.

Using ISET, the RTs of the antihistaminic drugs were in agreement with the original chromatogram, and showed a deviation in RT below 3 %, which is well within the ISET specification of 5 %[2]. Conversely, the RTs without using ISET shifted between 5 and approximately 11 %.

To further optimize the agreement of the retention times, the fine-tuning function within ISET was activated. A Delay Volume Offset of −50 µL shifted all peaks to slightly earlier retention times. Figure 2 shows an overlay of the original chromatogram and two chromatograms acquired with the 1260 Infinity II Prime LC using ISET with and without fine tuning.

As a result of deploying the fine-tuning function, the retention times of the five antihistaminic drugs were in agreement with the original chromatogram showing RT shifts below 0.5 %. Table 2 shows a retention time overview of the conducted experiments.

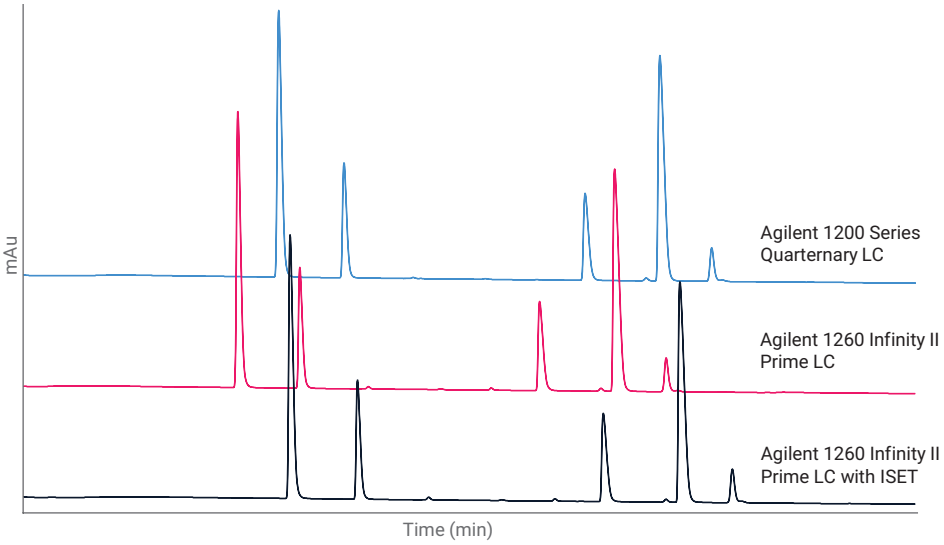


Figure 1. Analysis of antihistaminic drugs using the Agilent 1200 Series Quaternary LC (blue), followed by transferring the analytical method to the Agilent 1260 Infinity II Prime LC without ISET (red) and with ISET (black).

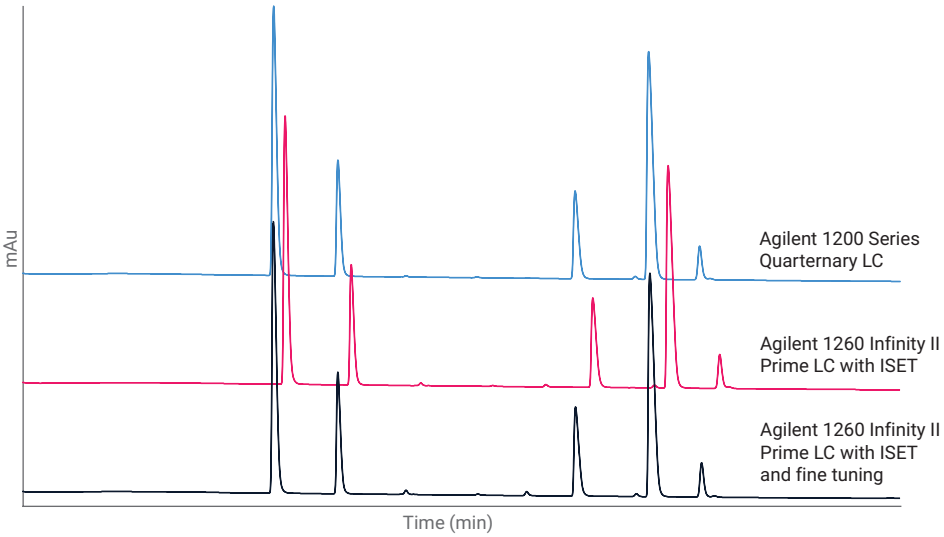


Figure 2. Overlay of the chromatogram of the analysis of antihistaminic drugs on an Agilent 1200 Series Quaternary LC (blue), an Agilent 1260 Infinity II Prime LC using ISET (red), and an Agilent 1260 Infinity II Prime LC using ISET with fine tuning (black).

Table 2. Retention times of the different antihistaminic drugs analyzed on an Agilent 1200 Series Quaternary LC and Agilent 1260 Infinity II Prime LC without ISET, with ISET, and ISET with fine tuning.

Agilent 1200 Series Quarternary LC	Agilent 1260 Infinity II Prime LC		
RT (min)	RT (min)	RT with ISET (min)	RT with ISET and fine tuning (min)
4.51	4.02	4.64	4.50
5.28	4.75	5.43	5.27
8.11	7.58	8.33	8.13
8.99	8.46	9.23	9.02
9.60	9.06	9.84	9.64

Figure 3 shows the evaluated RT shifts.

The precision of retention times and areas, as well as the resolution, were compared between the 1200 Series Quaternary LC and the 1260 Infinity II Prime LC using ISET and fine-tuning. To evaluate these values, 10 consecutive runs were conducted on both LC systems (Table 3). Both systems showed excellent precision vales, whereas the 1260 Infinity II Prime LC performed slightly better regarding RT and area precision.

Conclusion

Agilent Intelligent System Emulation technology (ISET) facilitates seamless analytical method transfer from a conventional HPLC to the Agilent 1260 Infinity II Prime LC. The ISET functionality is easy and straightforward to use, and does not require any changes of the instrument or modifications of the analytical method to achieve nearly identical chromatograms. A conventional analytical method for the analysis of antihistaminic drugs was transferred from the Agilent 1200 Series Quaternary LC to the 1260 Infinity II Prime LC. By enabling ISET and using the fine-tuning option for the 1260 Infinity II Prime LC, excellent agreement of retention times with a deviation below 0.5 % was obtained. In addition, by transferring the analytical method, the precision of RTs and areas was improved, and the 1260 Infinity Prime LC demonstrated excellent performance.

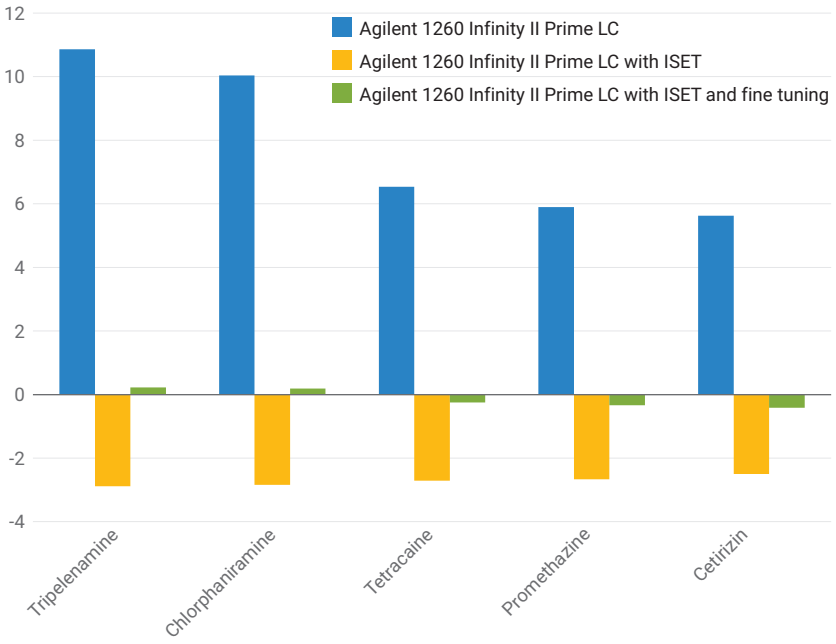


Figure 3. Deviation of retention time from an Agilent 1200 Series LC system to an Agilent 1260 Infinity II Prime LC with and without ISET and additional fine tuning.

Table 3. Comparison of retention time and area precision, and resolution between an Agilent 1200 Series Quaternary LC and an Agilent 1260 Infinity II Prime LC.

	RSD RT (%)		RSD Area (%)		Resolution	
	Agilent 1200 Series Quarternary LC	Agilent 1260 Infinity II Prime LC	Agilent 1200 Series Quarternary LC	Agilent 1260 Infinity II Prime LC	Agilent 1200 Series Quarternary LC	Agilent 1260 Infinity II Prime LC
Tripelenamine	0.06	0.03	0.091	0.052	–	–
Chlorphaniramine	0.05	0.03	0.111	0.052	8.06	8.34
Tetracaine	0.02	0.03	0.139	0.052	27.78	28.56
Promethazine	0.02	0.02	0.095	0.074	7.05	7.24
Cetirizin	0.02	0.01	0.135	0.072	5.47	5.59

References

1. Gratzfeld-Huesgen, A. Transferring methods to the Agilent 1290 Infinity LC using Intelligent System Emulation Technology (ISET) – Analysis of metoclopramide hydrochloride and its impurities, *Agilent Technologies Application Note*, publication number 5990-9692EN, **2014**.
2. Agilent InfinityLab LC with ISET, *Agilent Technologies User Manual*, part number G7120-90310, **2017**.

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