Method Transfer from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity II LC

Proof of Equivalency for the Analysis of Antihistaminic Drugs

Suitable for Agilent
1260 Infinity III LC

Application Note

Small Molecule Pharmaceuticals

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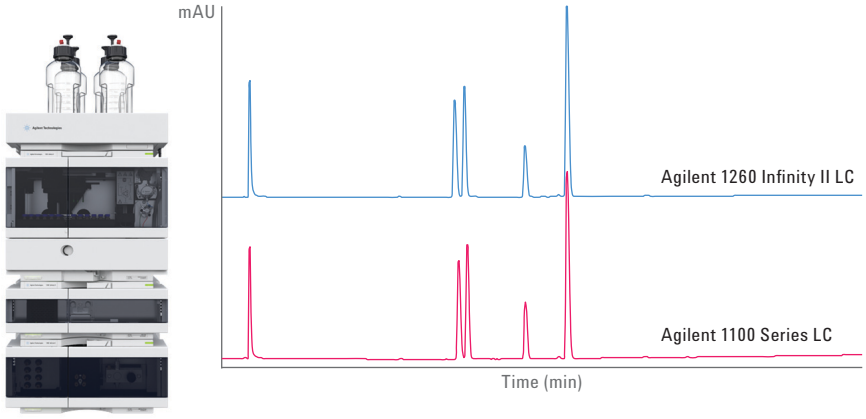
Abstract

The transfer of conventional LC methods from legacy equipment to new instrumentation is an important topic in all laboratories. This Application Note shows the transfer of a conventional LC method for the analysis of antihistaminic drugs from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity II LC, and proves that equivalent results in terms of retention times and resolution are obtained. Also, the conventional LC method is transferred to UHPLC conditions optimized for resolution as well as for speed using the 1260 Infinity II LC. This offers the possibility of increasing peak resolution, while enabling time and solvent savings.



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Introduction

Instrument-to-instrument method transfer is an important topic for all laboratories throughout different industries[1]. Especially for validated methods in the pharmaceutical industry, instrument-to-instrument method transfer is compulsory, but it is also important in QA/QC in other industries. One example of instrument-to-instrument method transfer is the transfer of conventional LC methods from older equipment such as the Agilent 1100 Series Quaternary LC, to new instruments such as the Agilent 1260 Infinity II LC. This Application Note shows the analysis of antihistaminic drugs using a 1100 Series Quaternary LC. The method is transferred to the 1260 Infinity II LC, and equivalent results in terms of retention time and resolution are obtained. With a pressure range of up to 600 bar, the 1260 Infinity II LC enables performing UHPLC analyses using Agilent InfinityLab Poroshell columns. The transfer of the conventional LC analysis of antihistaminic drugs to UHPLC conditions, optimized for resolution as well as for speed, will be shown.

Histamine is an endogenous biogenic amine that acts as a neurotransmitter in the nervous system and as a local mediator in the gut, skin, and immune system[2]. It evokes complex physiological changes that occur through four receptors; the first histamine receptor (H1 receptor) is widely expressed throughout the body, and is responsible for most of the symptoms caused by histamine in allergies[2]. Antihistaminic drugs (H1 receptor antagonists) have long been used in the treatment of nausea, vomiting, and allergies[2].

Experimental

Instrumentation

The Agilent 1260 Infinity II LC comprised the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Vialsampler (G7129A) with integrated column compartment, 3.0 µL heater (Option #063) and sample cooler (Option #100)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with standard 10-mm flow cell, (G1315-60022)

The Agilent 1100 Series Quaternary LC comprised the following modules:

- Agilent 1100 Quaternary Pump (G1311A)
- Agilent 1100 Degasser (G1379A)
- Agilent 1100 Autosampler (G1313A)
- Agilent 1100 Thermostatted Column Compartment (G1316A)
- Agilent 1100 Diode Array Detector (G1315B) with standard flow cell, 10 mm (G1315-60022)

Software

Agilent OpenLAB CDS Version 2.1 (availability planned for September 2016)

Columns

- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
- Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm (p/n 693975-302T)
- Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302T)

Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Potassium dihydrogen phosphate, tripelenamine hydrochloride, chlorpheniramine maleate, tetracaine, and promethazine hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany).

Sample

A mixture of the antihistaminic drugs tripelenamine, chlorpheniramine, tetracaine, and promethazine was prepared in 25 mM potassium dihydrogen phosphate in water, pH 3/acetonitrile (90/10; v/v) at a concentration of 50 µg/mL.

Methods

Table 1. Chromatographic conditions for conventional LC analysis.

Parameter	Description
Column	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm
Solvent	A) 25 mM Potassium dihydrogen phosphate in water, pH 3 B) Acetonitrile
Gradient	10 %B at 0 minutes, 55 %B at 25 minutes
Stop time	25 minutes
Post time	10 minutes
Flow rate	1.5 mL/min
Temperature	40 °C
Injection volume	10.0 µL
Detection	204/4 nm, reference 360/100 nm, data rate 10 Hz

Table 2. Chromatographic conditions for UHPLC analysis optimized for resolution.

Parameter	Description
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm
Solvent	A) 25 mM Potassium dihydrogen phosphate in water, pH 3 B) Acetonitrile
Gradient	10 %B at 0 minutes, 55 %B at 13.3 minutes
Stop time	13.3 minutes
Post time	6 minutes
Flow rate	1.2 mL/min
Temperature	40 °C
Injection volume	4.25 µL
Detection	204/4 nm, reference 360/100 nm, data rate 40 Hz

Table 3. Chromatographic conditions for UHPLC analysis optimized for speed.

Parameter	Description
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Solvent	A) 25 mM Potassium dihydrogen phosphate in water, pH 3 B) Acetonitrile
Gradient	10 %B at 0 minutes, 55 %B at 2.13 minutes
Stop time	2.13 minutes
Post time	1 minute
Flow rate	2.5 mL/min
Temperature	40 °C
Injection volume	4.25 µL
Detection	204/4 nm, reference 360/100 nm, data rate 80 Hz

Results and Discussion

This Application Note shows the analysis of antihistaminic drugs using a conventional LC method on a 1100 Series Quaternary LC. This method is transferred to a 1260 Infinity II LC for proof of equivalency. Additionally, the conventional LC method for the analysis of antihistaminic drugs is transferred to UHPLC conditions optimized for resolution as well as for speed using the 1260 Infinity II LC.

Figure 1 shows the conventional LC analysis of the four antihistaminic drugs tripelenamine, chlorpheniramine, tetracaine, and promethazine on the 1100 Series Quaternary LC. Excellent retention time and area precision was obtained (Table 4).

Figure 2 shows the conventional LC analysis of the antihistaminic drugs run on the 1260 Infinity II LC, and Table 5 presents the corresponding retention time and area precision. In terms of retention time and area precision, the 1260 Infinity II LC outperforms the excellent 1100 Series Quaternary LC. In addition, a slight increase in resolution was observed.

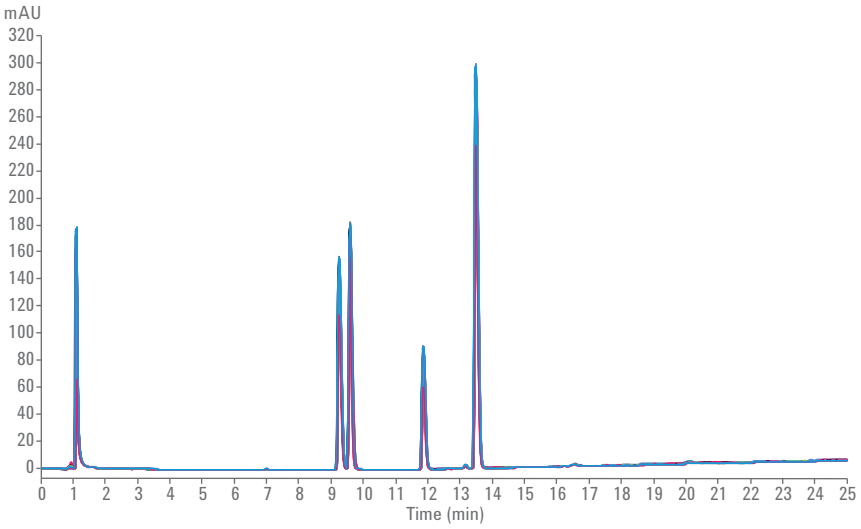

Figure 1. Conventional LC analysis of antihistaminic drugs on an Agilent 1100 Series Quaternary LC; overlay of 10 consecutive runs.

Table 4. Conventional LC analysis of antihistaminic drugs on an Agilent 1100 Series Quaternary LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Tripelenamine	9.26	0.05	1,033.5	0.26	64.6
Chlorpheniramine	9.60	0.05	1,048.3	0.28	2.0
Tetracaine	11.86	0.05	577.2	0.26	13.8
Promethazine	13.49	0.03	2,014.5	0.25	9.1

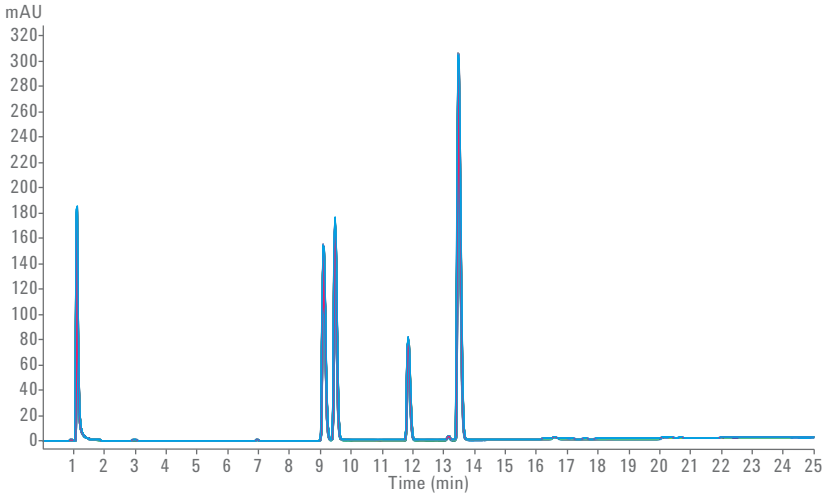

Figure 2. Conventional LC analysis of antihistaminic drugs on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Figure 3 and Table 6 compare the retention times of the antihistaminic drugs analyzed on the 1100 Series Quaternary LC and the 1260 Infinity II LC. With a maximum deviation of –1.7 %, excellent agreement of retention times was observed. This proves the equivalency of the 1260 Infinity II LC compared to the 1100 Series Quaternary LC for the analysis of the antihistaminic drugs.

Table 5. Conventional LC analysis of antihistaminic drugs on an Agilent 1260 Infinity II LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Tripelenamine	9.10	0.01	1,018.9	0.21	63.3
Chlorpheniramine	9.48	0.02	1,007.0	0.21	2.3
Tetracaine	11.84	0.02	508.9	0.17	14.5
Promethazine	13.48	0.01	2,017.1	0.14	9.2

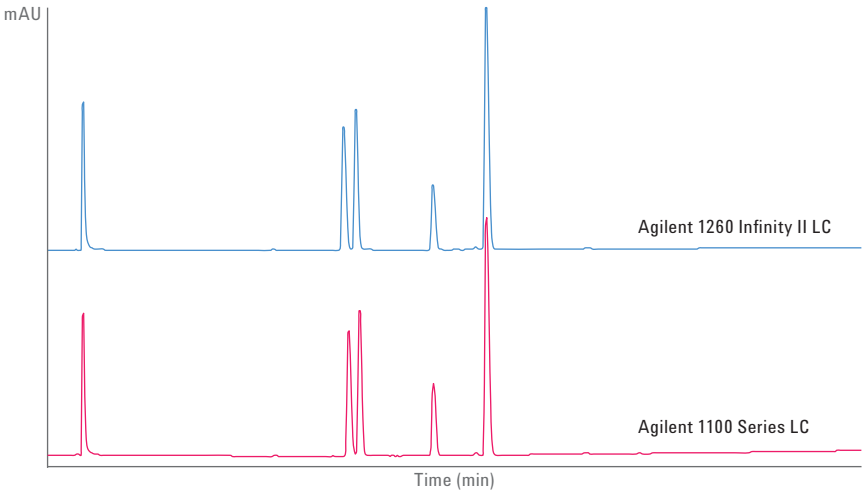

Figure 3. Conventional LC analysis of antihistaminic drugs on an Agilent 1100 Series Quaternary LC and an Agilent 1260 Infinity II LC.

Table 6. Conventional LC analysis of antihistaminic drugs on an Agilent 1100 Series Quaternary LC and an Agilent 1260 Infinity II LC; comparison of retention times.

Compound	RT Deviation (min)	RT Deviation (%)
Tripelenamine	–0.16	–1.7
Chlorpheniramine	–0.12	–1.3
Tetracaine	–0.02	–0.2
Promethazine	–0.02	–0.1

Instrument control and data analysis was performed using Agilent OpenLAB CDS Version 2.1. Figure 4 shows an impression of the data analysis in OpenLAB CDS Version 2.1. OpenLAB CDS Version 2.1 offers a single software system for liquid chromatography, gas chromatography, and mass spectrometry. It provides a new, flat user interface, and enables advanced technical support for compliance reporting. It also provides customized and interactive reporting with drag and drop template creation, and enables review of relevant data only.

In combination with the 1260 Infinity II LC, Agilent InfinityLab supplies were used. The Agilent InfinityLab Quick Connect fittings (p/n 5067-6166, with a 0.17 × 105 mm capillary) and Agilent Quick Turn fittings (p/n 5067-5966) enable tool-free, fast, and easy column installation, ensuring a perfect column connection independent of the user. The setup of the 1260 Infinity II LC on the Agilent InfinityLab Flex Bench rack (p/n 5043-1252) enables efficient use of lab space and an ergonomic approach with easy access to the instrument.

The pressure range of up to 600 bar of the 1260 Infinity II LC allows UHPLC analyses using InfinityLab Poroshell columns. When ordering a 1260 Infinity II LC, the customer has the choice between different InfinityLab Poroshell columns that can be delivered with the system, for example, the Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm column (p/n 693975-302T) or the Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm column (p/n 699975-302T). For the analysis of antihistaminic drugs, those columns allow the method transfer to UHPLC conditions optimized for resolution and speed, respectively.

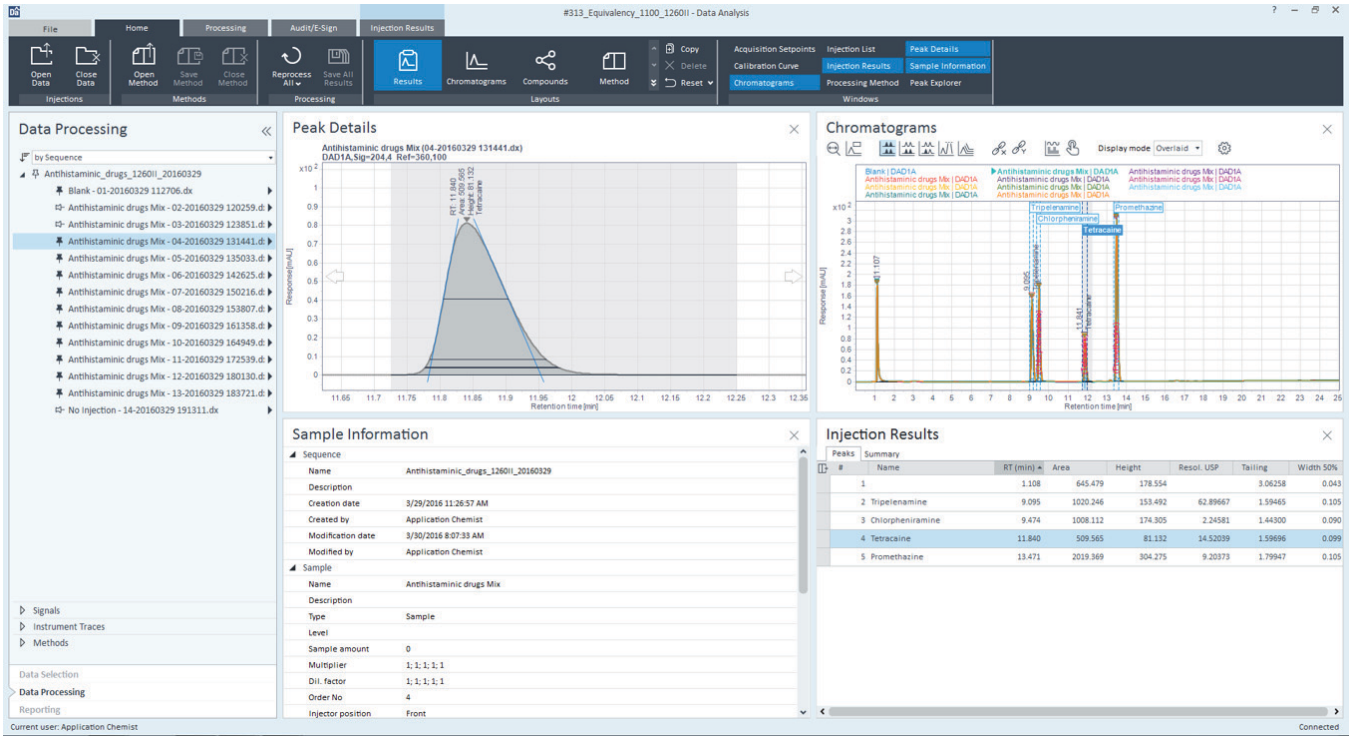

Figure 4. Impression of the data analysis in Agilent OpenLAB CDS Version 2.1.

Figure 5 and Table 7 show the analysis of the antihistaminic drugs under UHPLC conditions optimized for resolution. Making full use of the pressure range of the 1260 Infinity II LC, the InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm column can be operated at a relatively high flow rate of 1.2 mL/min. Under these conditions, the resolution of tripelenamine and chlorpheniramine was increased from 2.3 with the conventional LC method to 5.4 with the UHPLC method optimized for resolution. Also, the analysis time could be decreased by 47 %, and the solvent use reduced by 57 %.

Deploying the InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm column, the analysis of antihistaminic drugs can be transferred to UHPLC conditions optimized for speed, as shown in Figure 6 and Table 8. Compared to the conventional LC method, the resolution of tripelenamine and chlorpheniramine was still increased from 2.3 to 2.9. At the same time, the analysis time and solvent use could be greatly reduced by 91 and 86 %, respectively.

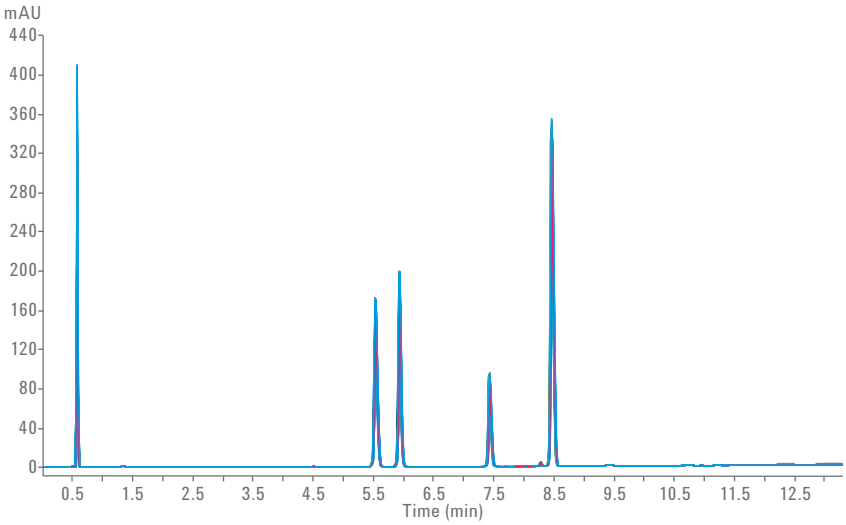

Figure 5. UHPLC analysis optimized for resolution of antihistaminic drugs on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 7. UHPLC analysis optimized for resolution of antihistaminic drugs on an Agilent 1260 Infinity II LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Tripelenamine	5.53	0.05	523.7	0.05	97.2
Chlorpheniramine	5.93	0.03	515.5	0.10	5.4
Tetracaine	7.43	0.04	260.6	0.09	21.0
Promethazine	8.46	0.03	1,028.2	0.04	13.6

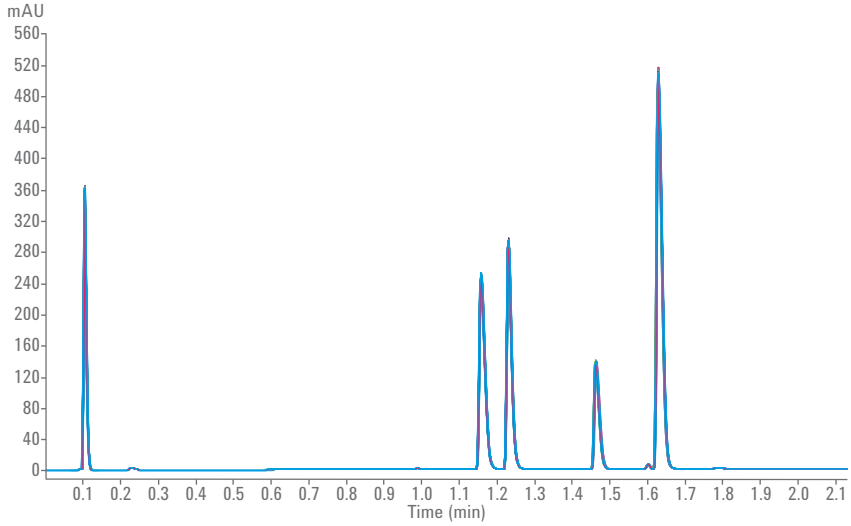

Figure 6. UHPLC analysis optimized for speed of antihistaminic drugs on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Conclusion

The transfer of a conventional LC method for the analysis of antihistaminic drugs from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity II LC showed a maximum retention time deviation of –1.7 % as well as a slight increase in resolution, and thereby proves the equivalency of the 1260 Infinity II LC compared to the 1100 Series Quaternary LC for the analysis of the antihistaminic drugs. With a pressure range of up to 600 bar, the 1260 Infinity II LC enables UHPLC analysis using Agilent InfinityLab Poroshell columns. By transfer of the conventional LC method to UHPLC conditions optimized for resolution as well as for speed, peak resolution was increased, and at the same time analysis time and solvent use were reduced.

References

1. Agilent 1290 Infinity with ISET, *Agilent Technologies User Manual*, part number G4220-90314, **2015**.

2. Panula*, et al.,* International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors, *Pharmacological Reviews* **2015**, *67*, 601–655.

Table 8. UHPLC analysis optimized for speed of antihistaminic drugs on an Agilent 1260 Infinity II LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Tripelenamine	1.16	0.00	254.4	0.19	53.0
Chlorpheniramine	1.23	0.00	252.1	0.23	2.9
Tetracaine	1.46	0.05	126.7	0.25	9.8
Promethazine	1.63	0.04	500.0	0.19	6.4

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