



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

Proof of Equivalency for the Analysis of Antibacterial Drugs with Penicillin-like Structures

Application Note

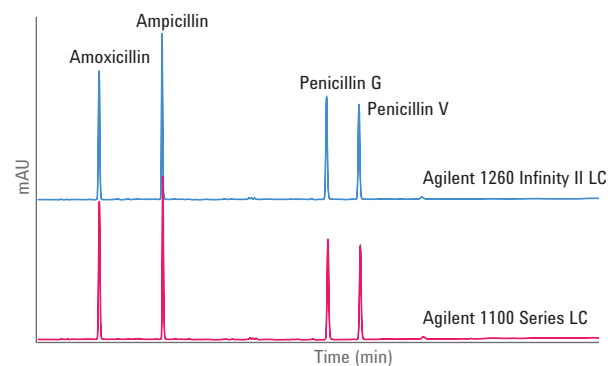
Small Molecule Pharmaceuticals

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Abstract

Instrument-to-instrument method transfer is an important topic, especially in the pharmaceutical industry, because method validation is time consuming. This Application Note shows the transfer of a conventional HPLC method for the analysis of antibacterial drugs with penicillin-like structures from an Agilent 1100 Series Binary LC to the Agilent 1260 Infinity II LC, and demonstrates that equivalent results in terms of retention time and resolution are obtained. In addition, the conventional HPLC method for the analysis of antibacterial drugs was transferred to UHPLC conditions on the Agilent 1260 Infinity II LC, providing time and solvent savings.



Agilent Technologies

Introduction

Penicillins are antibacterial drugs belonging to the group of β -lactam antibiotics, which have been used for more than 80 years¹. Penicillins are still considered one of the most important groups of antibiotics¹ with wide use in veterinary and human medicine². The basic structure of penicillins is 6-aminopenicillanic acid, which consists of a thiazolidine ring fused to a β -lactam ring with a side chain^{2,3}.

Instrument-to-instrument method transfer, for example, the transfer of conventional HPLC methods from older LC instruments to newer LC instruments, is an important topic for all laboratories throughout different industries⁴. Method transferability is compulsory for validated methods in the pharmaceutical industry, and is an important part of QA/QC in other industries. This Application Note shows the seamless transfer of a conventional HPLC method for the analysis of antibacterial drugs with penicillin-like structures from an Agilent 1100 Series Binary LC to an Agilent 1260 Infinity II LC, and demonstrates that equivalent results in terms of retention time and resolution are obtained. With its pressure range of up to 600 bar, the 1260 Infinity II LC allows laboratories to perform UHPLC analyses using Agilent InfinityLab Poroshell columns. The transfer of the conventional HPLC analysis of antibacterial drugs to UHPLC conditions is shown.

Experimental

Equipment

The Agilent 1260 Infinity II LC comprised the following modules:

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Vialsampler (G7129A) with integrated column compartment, 6.0 μ L heater, (Option #066) and sample cooler (Option #100)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with a standard 10-mm flow cell (G1315-60022)

The Agilent 1100 Series Binary LC comprised the following modules:

- Agilent 1100 Binary Pump (G1312A)
- Agilent 1100 Degasser (G1379A)
- Agilent 1100 Autosampler (G1313A)
- Agilent 1100 Thermostatted Column Compartment (G1316A)
- Agilent 1100 Diode Array Detector (G1315B) with a standard 10-mm flow cell (G1315-60022)

Software

Agilent OpenLAB CDS Version 2.1.

Columns

- Agilent ZORBAX Eclipse Plus C18, 4.6 \times 150 mm, 5 μ m (p/n 959993-902)
- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 \times 100 mm, 2.7 μ m (p/n 695975-902T)

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- μ m membrane point-of-use cartridge (Millipak, EMD Millipore, USA). Potassium dihydrogen phosphate and phosphoric acid were purchased from Merck (Darmstadt, Germany) and J. T. Baker (Deventer, Netherlands), respectively. Amoxicillin trihydrate, ampicillin, and penicillin G sodium salt were purchased from Sigma-Aldrich (Steinheim, Germany). Penicillin V potassium salt was obtained from Serva (Heidelberg, Germany).

Sample

A mixture of the antibacterial drugs amoxicillin, ampicillin, penicillin G, and penicillin V. A mixture of amoxicillin trihydrate, ampicillin, penicillin G sodium salt, and penicillin V potassium salt was prepared in water:acetonitrile (90:10, v:v) at a concentration of 100 μ g/mL each.

Methods

Table 1. Chromatographic conditions for conventional HPLC analysis.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm
Solvent	A) 25 mM KH ₂ PO ₄ in water pH 3 B) Acetonitrile
Gradient	5 %B at 0 minutes, 60 %B at 24 minutes
Stop time	24 minutes
Post time	10 minutes
Flow rate	1.500 mL/min
Temperature	40 °C
Injection volume	10.0 µL
Detection	204 nm/4 nm, Ref. 360 nm/100 nm, 10 Hz

Table 2. Chromatographic conditions for UHPLC analysis.

Parameter	Value
Column	Agilent InfinityLab Poroshell EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) 25 mM KH ₂ PO ₄ in water pH 3 B) Acetonitrile
Gradient	5 %B at 0 minutes, 60 %B at 8 minutes
Stop time	8 minutes
Post time	3.5 minutes
Flow rate	3.000 mL/min
Temperature	40 °C
Injection volume	10.0 µL
Detection	204 nm/4 nm, Ref. 360 nm/100 nm, 20 Hz

Results and Discussion

This Application Note shows the analysis of antibacterial drugs with penicillin-like structures using a conventional HPLC method on a 1100 Series Binary LC. The method was transferred to a 1260 Infinity II LC including a 1260 Infinity II Binary Pump for proof of equivalency. In addition, the conventional HPLC method was transferred to UHPLC conditions using the 1260 Infinity II LC, which provided time and solvent savings.

Figure 1 and Table 3 show the analysis of the antibacterial drugs amoxicillin, ampicillin, penicillin G, and penicillin V on the 1100 Series Binary LC, and the corresponding retention time and area precision as well as resolution.

The method for analysis of antibacterial drugs was transferred without any changes to the 1260 Infinity II LC. The resulting separation and the corresponding retention time and area precision as well as resolution are presented in Figure 2 and Table 4. Excellent retention time and area precision were obtained.

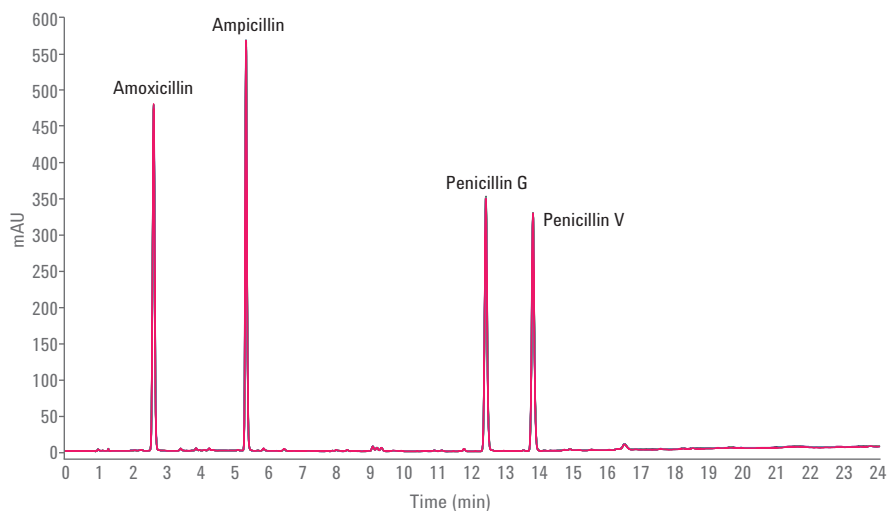


Table 3. Analysis of antibacterial drugs on an Agilent 1100 Series Binary LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Amoxicillin	2.63	0.04	2,139	0.09	–
Ampicillin	5.35	0.02	2,099	0.04	25.1
Penicillin G	12.40	0.01	1,842	0.35	60.2
Penicillin V	13.79	0.01	1,750	0.07	10.0

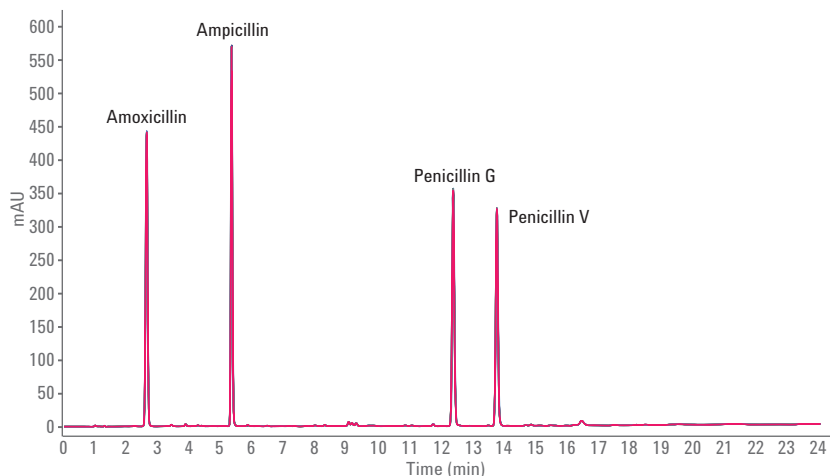


Figure 2. Analysis of antibacterial drugs on an Agilent 1260 Infinity II LC: overlay of 10 consecutive runs.

Table 4. Analysis of antibacterial drugs on an Agilent 1260 Infinity II LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Amoxicillin	2.62	0.06	1,997	0.13	–
Ampicillin	5.31	0.03	2,044	0.12	25.4
Penicillin G	12.35	0.02	1,813	0.31	62.7
Penicillin V	13.73	0.02	1,685	0.12	10.5

Figure 3 and Table 5 compare the retention times of the antibacterial drugs analyzed on the 1100 Series Binary LC and the 1260 Infinity II LC. With a maximum retention time deviation of 0.6 %, excellent agreement of retention times between the 1100 Series Binary LC and the 1260 Infinity II LC is observed. This demonstrates the potential for seamless method transfer from the 1100 Series Binary LC to the 1260 Infinity II LC for the analysis of antibacterial drugs.

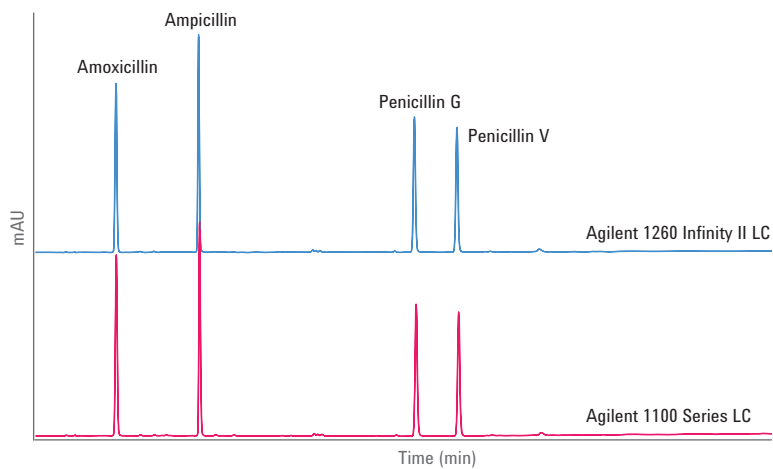


Figure 3. Analysis of antibacterial drugs on an Agilent 1100 Series Binary LC and an Agilent 1260 Infinity II LC.

Table 5. Analysis of antibacterial drugs on an Agilent 1100 Series Binary LC and an Agilent 1260 Infinity II LC; comparison of retention times.

Compound	RT Deviation (min)	RT Deviation (%)
Amoxicillin	-0.01	-0.4
Ampicillin	-0.03	-0.6
Penicillin G	-0.06	-0.5
Penicillin V	-0.06	-0.4

For this Application Note, instrument control of the 1100 Series Binary LC and the 1260 Infinity II LC as well as data analysis was performed using Agilent OpenLAB CDS version 2.1 software. This version of OpenLAB CDS offers a single software for liquid chromatography, gas chromatography, and mass spectrometry. It provides a flat user interface, and customized and interactive reporting with drag-and-drop template creation. Figure 4 shows an impression of the user-configurable layout of data analysis in OpenLAB CDS version 2.1.

Agilent InfinityLab columns and supplies work together perfectly with the 1260 Infinity II LC for maximum performance and efficiency of LC workflows. The Agilent InfinityLab Quick Connect fitting

(p/n 5067-6166, Quick Connect fitting with a 0.17×105 mm capillary) and Agilent InfinityLab 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.

Agilent InfinityLab Poroshell columns, in combination with the pressure range of up to 600 bar of the 1260 Infinity II LC, enable UHPLC analyses, offering time and solvent savings while maintaining or increasing peak resolution. When ordering a 1260 Infinity II LC, the customer has the choice between different InfinityLab Poroshell columns to be delivered with the system.

Using an InfinityLab Poroshell 120 EC-C18, 4.6×100 mm, $2.7 \mu\text{m}$ column (p/n 695975-902T) on the 1260 Infinity II LC, the conventional HPLC method for the analysis of antibacterial drugs was transferred to UHPLC conditions. Making use of the pressure range of the 1260 Infinity II LC, the InfinityLab Poroshell column can be operated at a relatively high flow rate of 3.0 mL/min (chromatographic conditions described in Table 2). Figure 5 and Table 6 show the analysis of the antibacterial drugs under UHPLC conditions. Under UHPLC conditions, the resolution could be increased, while the analysis time could be decreased by 67 %, and solvent use reduced by 33 %.

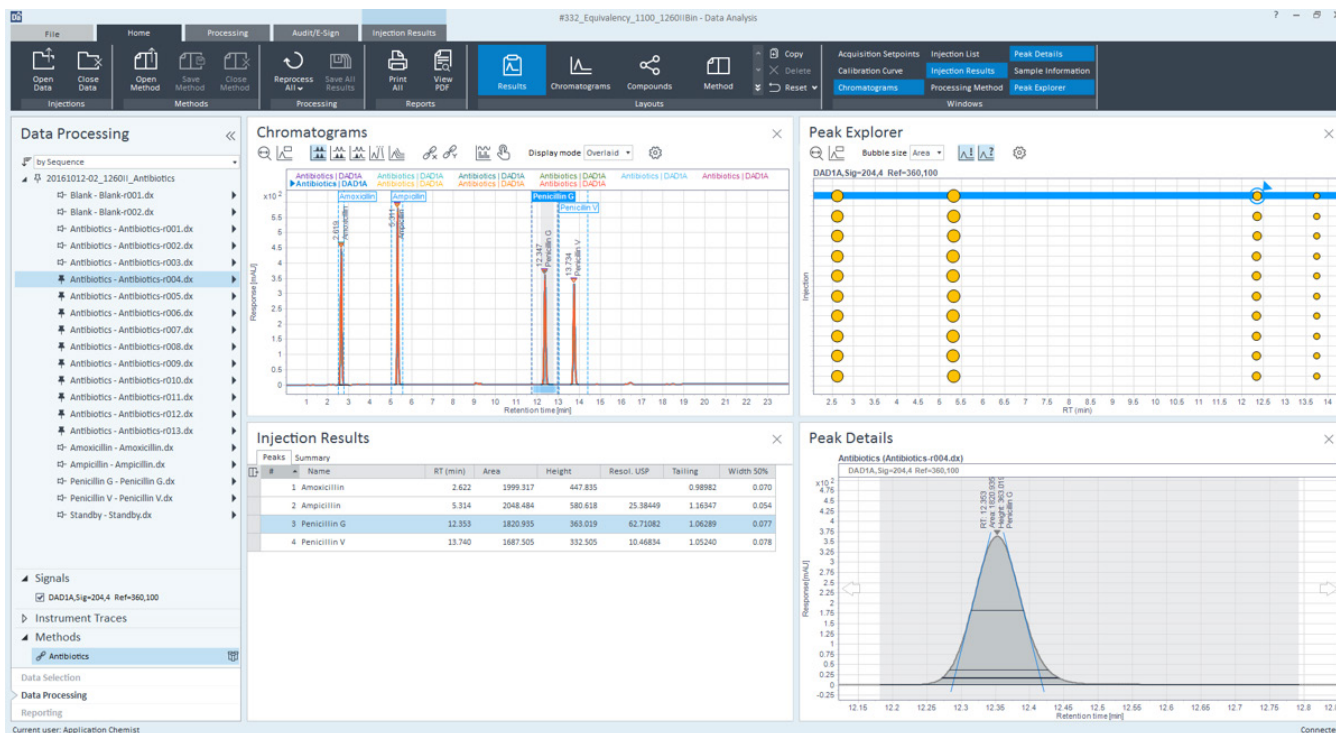


Figure 4. Impression of the data analysis in Agilent OpenLAB CDS version 2.1.

Conclusion

The transfer of the conventional HPLC method for the analysis of antibacterial drugs with penicillin-like structures from an Agilent 1100 Series Binary LC to an Agilent 1260 Infinity II LC showed a maximum retention time deviation of 0.6 %, which demonstrates seamless method transfer. Additionally, the transfer of the conventional HPLC method to UHPLC conditions on the 1260 Infinity II LC provided a decrease in analysis time of 67 %, and decreased solvent use of 33 %, while maintaining or increasing peak resolution.

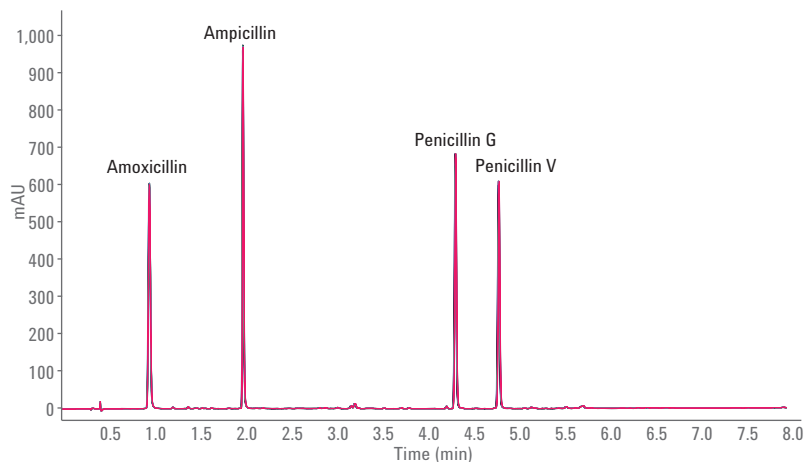


Figure 5. Analysis of antibacterial drugs under UHPLC conditions on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 6. Analysis of antibacterial drugs under UHPLC conditions on an Agilent 1260 Infinity II LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT [min]	RT RSD [%]	Area	Area RSD [%]	Resolution
Amoxicillin	0.963	0.16	1008	0.06	-
Ampicillin	1.995	0.08	1016	0.04	29.3
Penicillin G	4.343	0.07	919	0.04	75.6
Penicillin V	4.820	0.07	832	0.03	13.6

References

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© Agilent Technologies, Inc., 2016, 2020
Published in the USA, March 13, 2020
5991-7642EN



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