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

Proof of Equivalency for the Analysis of Beta-Blockers

Application Note

Small Molecule Pharmaceuticals

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Abstract

Instrument-to-instrument method transfer is compulsory for validated methods in the pharmaceutical industry, but is also an important topic in other industries. This Application Note shows the seamless transfer of a method for the analysis of beta-blockers from an Agilent 1260 Infinity LC to an Agilent 1260 Infinity II LC, and proves that equivalent results in terms of retention time and resolution are obtained.
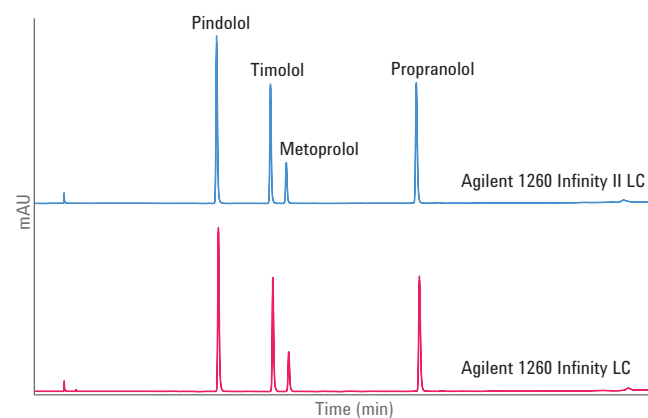
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Introduction

Method transferability from one instrument to another is important for laboratories throughout different industries[1]. In the pharmaceutical industry, instrument-to-instrument method transferability is compulsory for validated methods. One example for the need to transfer methods from one instrument to another is the method transfer to new LC instruments, such as the Agilent 1260 Infinity II LC. This Application Note shows the seamless method transfer from an Agilent 1260 Infinity LC to a 1260 Infinity II LC for the analysis of the beta-blockers metoprolol, pindolol, propranolol, and timolol. It demonstrates that equivalent results in terms of retention time and resolution are obtained. In addition, the LC method is transferred to UHPLC conditions optimized for speed that makes full use of the pressure range of the 1260 Infinity II LC, and enables a decrease in analysis time and solvent use.

Beta-blockers, also named β-adrenergic blocking agents or β-antagonists, are drugs used for the treatment of cardiovascular disorders, such as high blood pressure, coronary artery disease, and abnormal heart rhythms[2,3]. They work by blocking the effects of endogenous catecholamines (adrenaline and noradrenaline) on β-adrenergic receptors, which slows the nerve impulses to the heart, and thereby reduces its workload[2-4].

Experimental

Equipment

The Agilent 1260 Infinity II LC comprised the following modules:

- Agilent 1260 Infinity II Binary Pump (G7112B)

- Agilent 1260 Infinity II Multisampler (G7167A) with sample cooler (option #100)

- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)

- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with 10-mm Max-Light cartridge cell (G4212-60008)

The Agilent 1260 Infinity LC comprised the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B)

- Agilent 1260 Infinity Standard Degasser (G1322A)

- Agilent 1260 Infinity High Performance Autosampler (G1367E) with an Agilent 1290 Infinity Thermostat (G1330B)

- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)

- Agilent 1260 Infinity Diode Array Detector (G4212B) with 10-mm Max-Light cartridge cell (G4212-60008)

Software

Agilent OpenLAB CDS Version 2.1.

Columns

- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902T)

- 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, USA). Trifluoroacetic acid, metoprolol tartrate salt, pindolol, propranolol hydrochloride, and timolol maleate salt were purchased from Sigma-Aldrich (Steinheim, Germany).

Sample

A mixture of metoprolol, pindolol, propranolol, and timolol was prepared in water:acetonitrile (90:10, v:v) at a concentration of 10 µg/mL each.

Methods

Table 1. Chromatographic conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water + 0.1 % trifluoroacetic acid B) Acetonitrile + 0.09 % trifluoroacetic acid
Gradient	10 %B at 0 minutes, 50 %B at 16 minutes
Stop time	16 minutes
Post time	7 minutes
Flow rate	1.500 mL/min
Temperature	25 °C
Injection volume	10.0 µL
Detection	275 nm/4 nm Ref. 380 nm/40 nm, 20 Hz

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

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Solvent	A) Water + 0.1 % trifluoroacetic acid B) Acetonitrile + 0.09 % trifluoroacetic acid
Gradient	10 %B at 0.00 minutes, 50 %B at 2.67 minutes
Stop time	2.67 minutes
Post time	1.17 minutes
Flow rate	1.920 mL/min
Temperature	25 °C
Injection volume	4.25 µL
Detection	275 nm/4 nm Ref. 380 nm/40 nm, 40 Hz

Results and Discussion

This Application Note shows the analysis of the beta-blockers metoprolol, pindolol, propranolol, and timolol using a 1260 Infinity LC. The method was transferred to a 1260 Infinity II LC including an Agilent 1260 Infinity II Binary Pump for proof of equivalency. In addition, the method was transferred to UHPLC conditions optimized for speed using the 1260 Infinity II LC. Figure 1 and Table 3 show the analysis of the beta-blockers using the 1260 Infinity LC and the corresponding retention time and area precision as well as resolution.

The method for the analysis of beta-blockers was transferred without any changes to the 1260 Infinity II LC. Figure 2 and Table 4 show the resulting separation, the corresponding retention time, and area precision as well as resolution. Excellent precision of retention time and area was achieved.
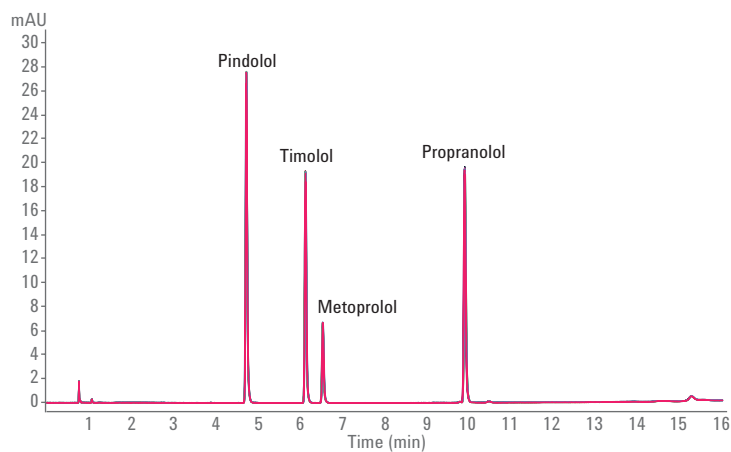

Figure 1. Analysis of beta-blockers using an Agilent 1260 Infinity LC; overlay of 10 consecutive runs.

Table 3. Analysis of beta-blockers using an Agilent 1260 Infinity LC; retention time and area precision determined from 10 consecutive runs.

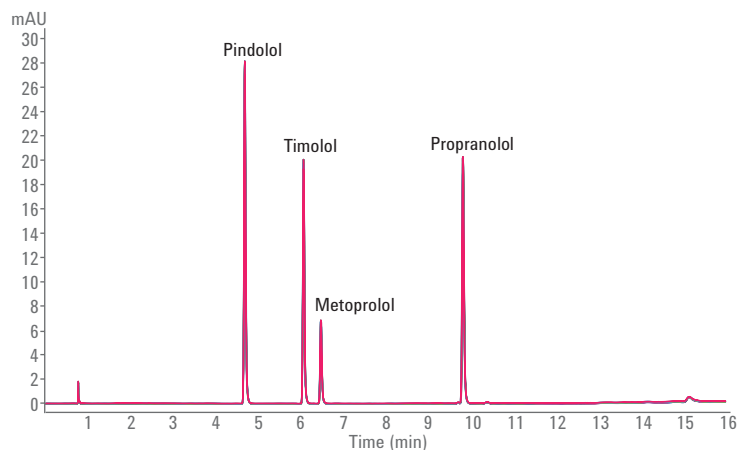| Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Pindolol	4.73	0.07	75.9	0.07	–
Timolol	6.13	0.07	52.2	0.10	20.1
Metoprolol	6.54	0.07	19.4	0.24	5.7
Propranolol	9.90	0.06	62.1	0.14	42.7

Figure 2. Analysis of beta-blockers on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 4. Analysis of beta-blockers 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
Pindolol	4.68	0.03	76.8	0.06	–
Timolol	6.07	0.02	53.7	0.12	20.0
Metoprolol	6.47	0.02	19.8	0.23	5.7
Propranolol	9.81	0.02	63.8	0.09	42.9

Figure 3 and Table 5 compare the retention times and resolution of the beta-blockers analyzed using the 1260 Infinity LC and the 1260 Infinity II LC. With a maximum retention time deviation of 1.0 %, excellent agreement of retention times between the 1260 Infinity LC and the 1260 Infinity II LC was observed. In terms of resolution, equivalent results were obtained on the 1260 Infinity LC and the 1260 Infinity II LC. This proves the possibility for seamless method transfer from the 1260 Infinity LC to the 1260 Infinity II LC for the analysis of beta-blockers.
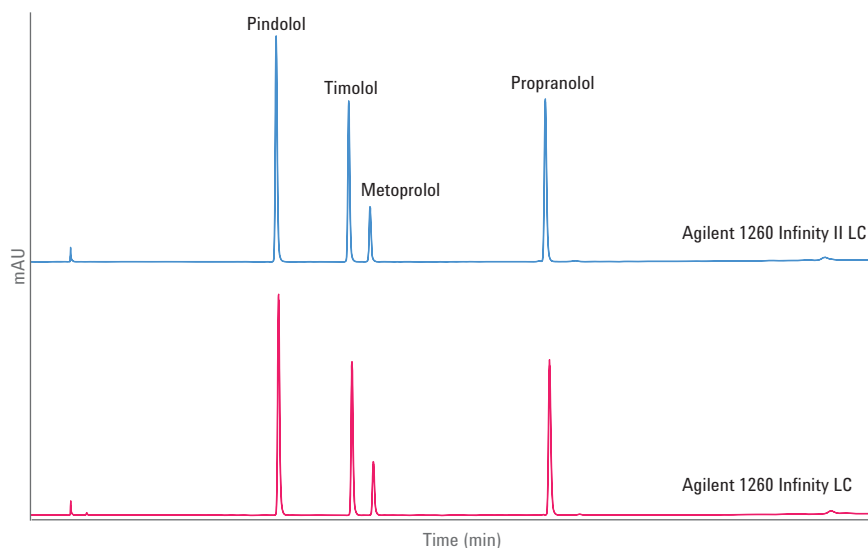

Figure 3. Analysis of beta-blockers on an Agilent 1260 Infinity LC and an Agilent 1260 Infinity II LC.

Table 5. Analysis of beta-blockers on an Agilent 1260 Infinity LC and an Agilent 1260 Infinity II LC; comparison of retention times and resolution.

Compound	RT Deviation (min)	RT Deviation (%)	Resolution deviation	Resolution deviation (%)
Pindolol	-0.05	-1.0	–	–
Timolol	-0.06	-1.0	-0.1	-0.5
Metoprolol	-0.06	-1.0	0.0	0.0
Propranolol	-0.08	-0.8	0.2	0.5

In this Application Note, Agilent OpenLAB CDS Version 2.1 was used for instrument control and data analysis. OpenLAB CDS Version 2.1 offers a single software for liquid chromatography, gas chromatography, and mass spectrometry. It provides a new, flat user interface and customized and interactive reporting with drag-and-drop template creation. Figure 4 shows an impression of the data analysis in OpenLAB CDS Version 2.1. The layout of the data analysis is user-configurable and enables, for example, the simultaneous display of sample information, selected chromatograms, injection results, and peak details for a selected peak.

Agilent InfinityLab columns and supplies work together perfectly with the 1260 Infinity II LC for maximum performance and efficiency of LC workflows. The InfinityLab Quick Connect (p/n 5067-6166, Quick Connect fitting with a 0.17 × 105 mm capillary) and Quick Turn fittings (p/n 5067-5966) enable a 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 (p/n 5043-1252) provides for efficient use of lab space and an ergonomic approach with easy access to the instrument.

Agilent InfinityLab Poroshell columns, 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 that can be delivered with the system, for example the InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column (p/n 695975-902T).
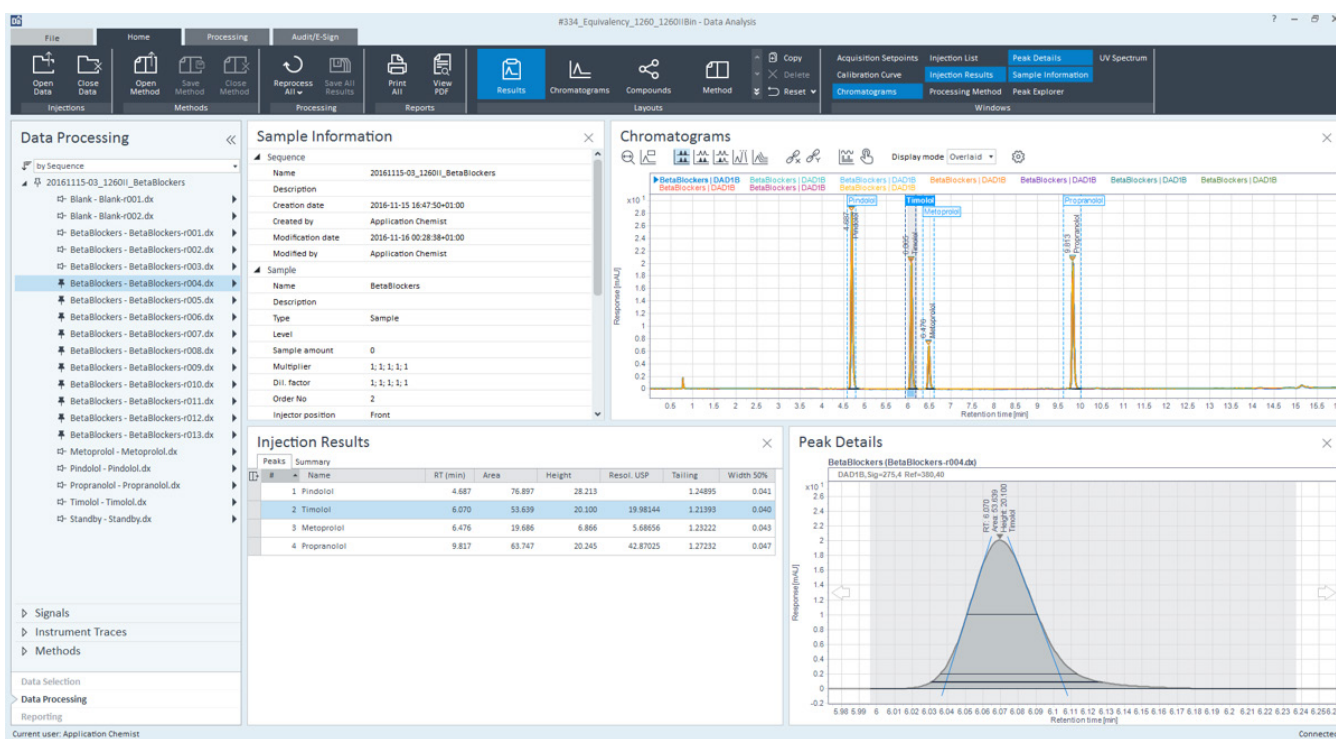

Figure 4. Impression of the data analysis in Agilent OpenLAB CDS Version 2.1.

Using an InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302T) column and making full use of the pressure range of the 1260 Infinity II LC, the method for the analysis of beta-blockers was transferred to UHPLC conditions optimized for speed (chromatographic conditions described in Table 2). Figure 5 and Table 6 show the analysis of the beta-blockers under these conditions. The use of UHPLC conditions optimized for speed enabled a decrease in the analysis time of 83 %, while the solvent use was reduced by 79 %.

Conclusion

The transfer of the method for the analysis of beta-blockers from an Agilent 1260 Infinity LC to the Agilent 1260 Infinity II LC showed a maximum retention time deviation of 1.0 %, and equivalent results in terms of peak resolution. This proves the equivalency of the 1260 Infinity II LC compared to the 1260 Infinity LC for the analysis of beta-blockers. By making full use of the pressure range of the 1260 Infinity II LC and transfer of the method for the analysis of beta-blockers to UHPLC conditions optimized for speed, the analysis time and solvent use were greatly reduced.
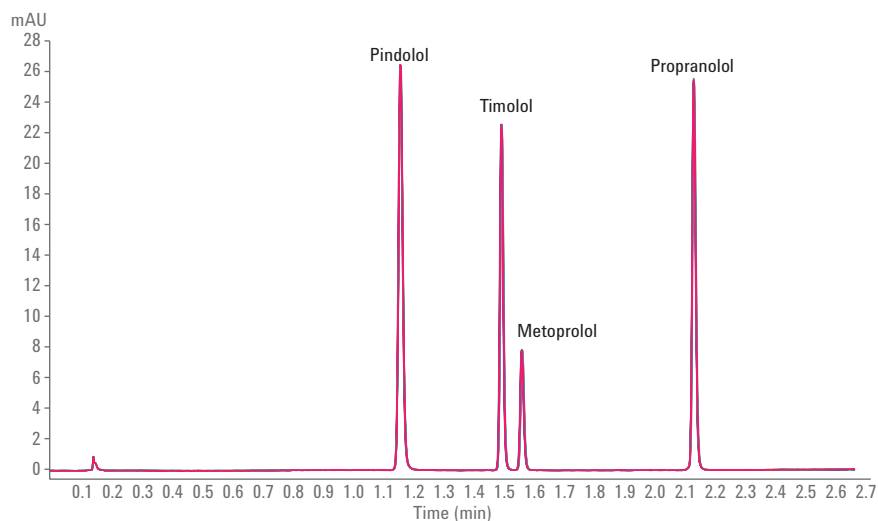

Figure 5. Analysis of beta-blockers under UHPLC conditions optimized for speed on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 6. Analysis of beta-blockers under UHPLC conditions optimized for speed 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
Pindolol	1.16	0.06	26.6	0.09	–
Timolol	1.50	0.07	17.7	0.09	14.4
Metoprolol	1.57	0.06	6.5	0.24	3.2
Propranolol	2.14	0.04	21.4	0.19	26.1

References

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

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