Method Transfer from an Agilent 1200 Series LC to an Agilent 1260 Infinity II LC

Proof of Equivalency for the Analysis of Local Anesthetics

Application Note

Small Molecule Pharmaceuticals

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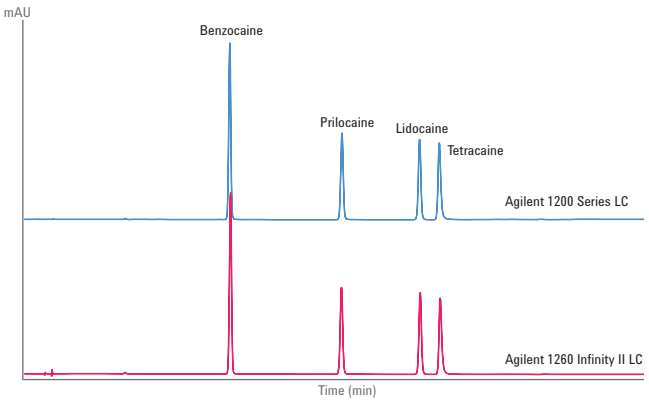
Abstract

Instrument-to-instrument method transfer is an important topic for all laboratories, and is especially important in the pharmaceutical industry. This Application Note shows the seamless transfer of a conventional LC method for the analysis of local anesthetics from an Agilent 1200 Series Binary LC to an Agilent 1260 Infinity II LC, and demonstrates that equivalent results in terms of retention time and resolution are obtained. In addition, the conventional LC method is transferred to UHPLC conditions on the 1260 Infinity II LC, which 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]. For validated methods in the pharmaceutical industry, instrument-to-instrument method transferability is compulsory. Method transferability is also important in QA/QC in other industries, as method validation is time-consuming. One example of the need for instrument-to-instrument method transfer is the method transfer to new LC instruments, such as the Agilent 1260 Infinity II LC.

This Application Note shows the seamless transfer of a conventional LC method for the analysis of the local anesthetics benzocaine, lidocaine, prilocaine, and tetracaine from an Agilent 1200 Series Binary LC to the 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 enables ultrahigh performance LC (UHPLC) analyses using Agilent InfinityLab Poroshell columns. The transfer of the conventional LC analysis of local anesthetics 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 µL heater, (Option #066) and sample cooler (Option #100)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with standard flow cell, 10 mm (G1315-60022)

The Agilent 1200 Series Binary LC comprised the following modules:

- Agilent 1200 Series Binary Pump (G1312A)
- Agilent 1260 Infinity High-Performance Degasser (G4225A)
- Agilent 1200 Series Autosampler (G1329A) with 1200 Series Thermostat (G1330B)
- Agilent 1200 Series Thermostatted Column Compartment (G1316A)
- Agilent 1200 Series Diode Array Detector (G1315D) with standard flow cell, 10 mm (G1315-60022)

Software

Agilent OpenLAB CDS Version 2.1.

Columns

- Agilent ZORBAX Extend-C18, 4.6 × 150 mm, 5 µm (p/n 773450-902)
- Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702T)
- Agilent InfinityLab Poroshell HPH-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-502T)

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). Potassium phosphate monobasic and potassium phosphate dibasic trihydrate were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany), respectively. Benzocaine, lidocaine, prilocaine hydrochloride, and tetracaine were purchased from Sigma-Aldrich (Steinheim, Germany).

Sample

A mixture of benzocaine, lidocaine, prilocaine hydrochloride, and tetracaine was prepared in water:acetonitrile (90:10, v:v) at a concentration of 100 µg/mL each.

Methods

Table 1. Chromatographic conditions for conventional LC analysis.

Parameter	Value
Column	Agilent ZORBAX Extend-C18, 4.6 × 150 mm, 5 µm
Solvent	A) 20 mM Potassium phosphate, pH 8 B) Acetonitrile
Gradient	5 %B at 0 minutes, 70 %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	225 nm/4 nm, reference 380 nm/40 nm, 10 Hz

Table 2. Chromatographic conditions for UHPLC analysis.

Parameter	Value
Column	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) 20 mM Potassium phosphate, pH 8 B) Acetonitrile
Gradient	5 %B at 0.00 minutes, 70 %B at 8.00 minutes
Stop time	8.00 minutes
Post time	3.33 minutes
Flow rate	3.000 mL/min
Temperature	40 °C
Injection volume	10 µL
Detection	225 nm/4 nm, reference 380 nm/40 nm, 20 Hz

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

Parameter	Value
Column	Agilent InfinityLab Poroshell HPH-C18, 3.0 × 50 mm, 2.7 µm
Solvent	A) 20 mM Potassium phosphate, pH 8 B) Acetonitrile
Gradient	5 %B at 0.00 minutes 70 %B at 2.67 minutes
Stop time	2.67 minutes
Post time	1.11 minutes
Flow rate	1.920 mL/min
Temperature	40 °C
Injection volume	4.25 µL
Detection	225 nm/4 nm, reference 380 nm/40 nm, 40 Hz

Results and Discussion

This Application Note shows the analysis of the local anesthetics benzocaine, lidocaine, prilocaine, and tetracaine using a conventional LC method on a 1200 Series Binary LC. The method is transferred to a 1260 Infinity II LC including a 1260 Infinity II Binary Pump for proof of equivalency. In addition, the conventional LC method is transferred to UHPLC conditions using the 1260 Infinity II LC.

Figure 1 and Table 4 show the analysis of the local anesthetics on the 1200 Series Binary LC and the corresponding retention time and area precision as well as resolution.

The method for the analysis of local anesthetics was transferred without any changes to the 1260 Infinity II LC. Figure 2 and Table 5 show the resulting separation and the corresponding retention time and area precision, as well as resolution. Excellent retention time and area precision were achieved. In terms of resolution, equivalent results were obtained on the 1200 Series Binary LC and the 1260 Infinity II LC.

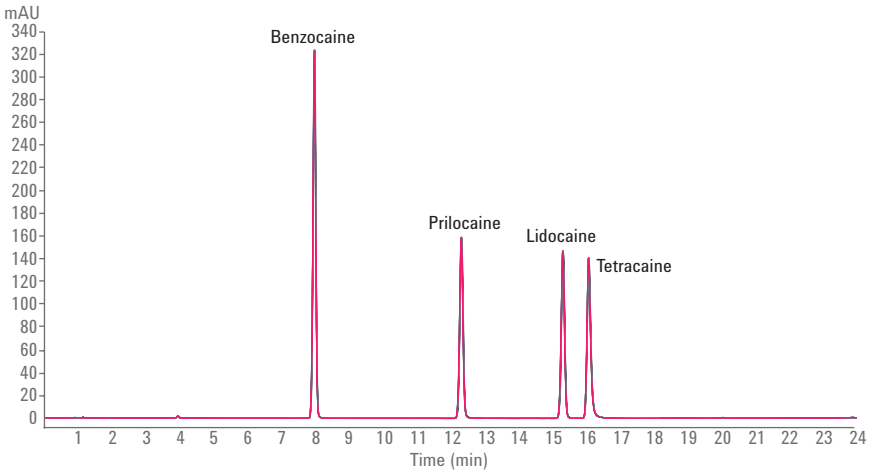

Figure 1. Analysis of local anesthetics on an Agilent 1200 Series Binary LC; overlay of 10 consecutive runs.

Table 4. Analysis of local anesthetics on an Agilent 1200 Series Binary LC; retention time and area precision were determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Benzocaine	7.96	0.02	1,887	0.07	–
Prilocaine	12.30	0.03	1,113	0.12	25.7
Lidocaine	15.31	0.02	1,039	0.10	16.2
Tetracaine	16.07	0.02	1,080	0.32	4.0

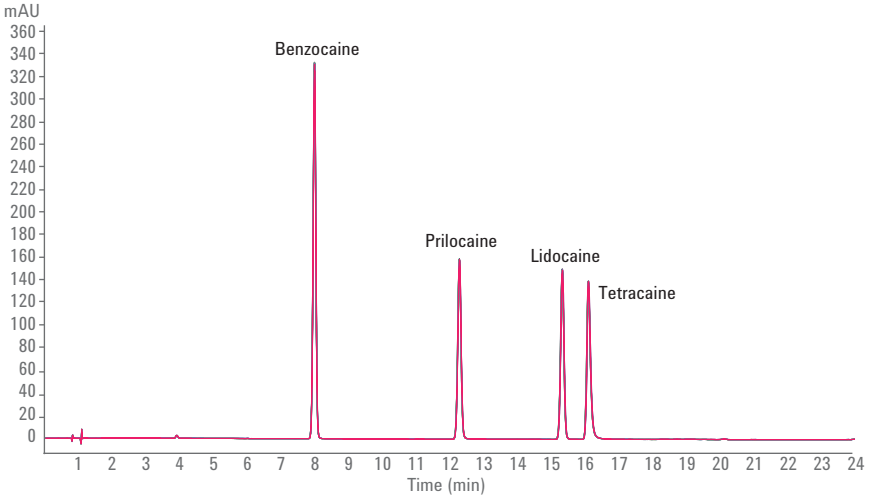

Figure 2. Analysis of local anesthetics on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 5. Analysis of local anesthetics 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
Benzocaine	7.98	0.02	1,907	0.17	–
Prilocaine	12.28	0.02	1,108	0.10	25.6
Lidocaine	15.33	0.03	1,057	0.12	16.5
Tetracaine	16.10	0.03	1,074	0.20	4.1

Figure 3 and Table 6 compare the retention times of the local anesthetics analyzed on the 1200 Series Binary LC and the 1260 Infinity II LC. With a maximum retention time deviation of 0.3 %, excellent agreement of retention times between the 1200 Series Binary LC and the 1260 Infinity II LC was observed. This proves the possibility for seamless method transfer from the 1200 Series Binary LC to the 1260 Infinity II LC for the conventional LC analysis of local anesthetics.

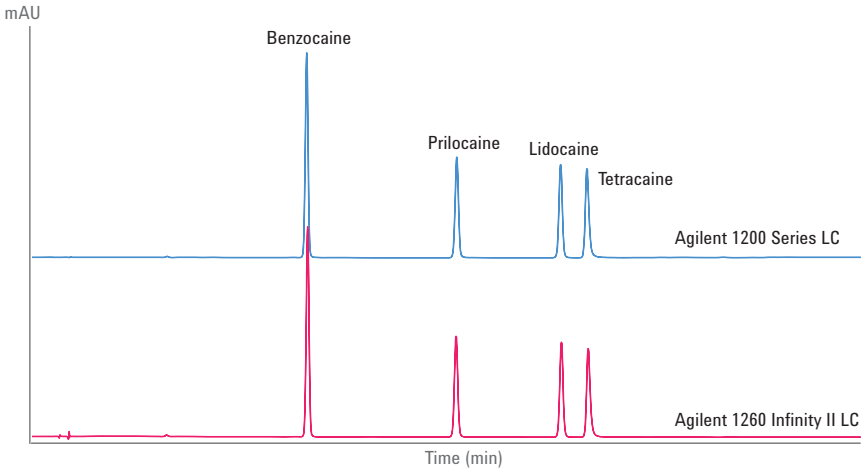

Figure 3. Analysis of local anesthetics on an Agilent 1200 Series Binary LC and an Agilent 1260 Infinity II LC.

Table 6. Analysis of local anesthetics on an Agilent 1200 Series Binary LC and an Agilent 1260 Infinity II LC; comparison of retention times.

Compound	RT Deviation (min)	RT Deviation (%)
Benzocaine	0.02	0.3
Prilocaine	-0.03	-0.2
Lidocaine	0.02	0.1
Tetracaine	0.03	0.2

For this Application Note, instrument control of the 1200 Series Binary LC and the 1260 Infinity II LC as well as data analysis was performed using Agilent OpenLAB CDS Version 2.1. This version of OpenLAB CDS offers a single software system 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 data analysis in OpenLAB CDS Version 2.1. The layout of the data analysis is user-configurable and enables, for example, the display of selected chromatograms, the peak explorer, injection results, and the UV spectrum of a selected peak at the same time.

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 (p/n 5067-6166, Quick Connect fitting with a 0.17 × 105 mm capillary) and 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 FlexBench (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 a choice between different InfinityLab Poroshell columns that can be delivered with the system.

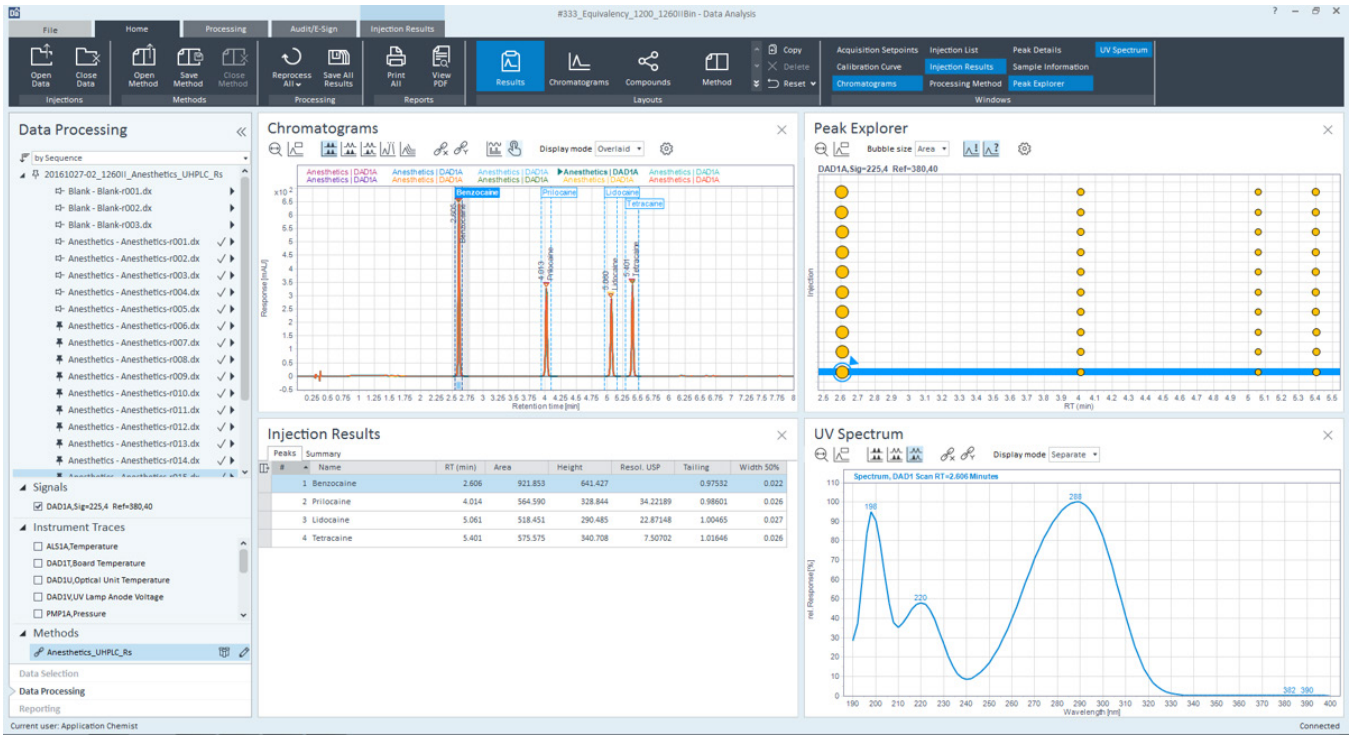

Figure 4. Impression of the data analysis in Agilent OpenLAB CDS Version 2.1.

Using an InfinityLab Poroshell HPH-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-702T) column, the conventional LC method for the analysis of local anesthetics was transferred to UHPLC conditions on the 1260 Infinity II LC (chromatographic conditions described in Table 2). Figure 5 and Table 7 show the analysis of the local anesthetics under UHPLC conditions. The resolution could be increased due to the increased efficiency of the InfinityLab Poroshell column. Meanwhile, the analysis time could be decreased by 67 %, and the solvent use reduced by 33 % due to the decreased column length and increased flow rate.

If further time and solvent savings are desired, the method for the analysis of local anesthetics can be transferred to UHPLC conditions optimized for speed using an InfinityLab Poroshell HPH-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-502T) column on the 1260 Infinity II LC (chromatographic conditions are described in Table 3). Figure 6 and Table 8 show the analysis of the local anesthetics under UHPLC conditions optimized for speed. Under these conditions, the analysis time could be decreased by almost 90 %, and the solvent use reduced by more than 85 % compared to the conventional LC analysis with only marginal loss in resolution.

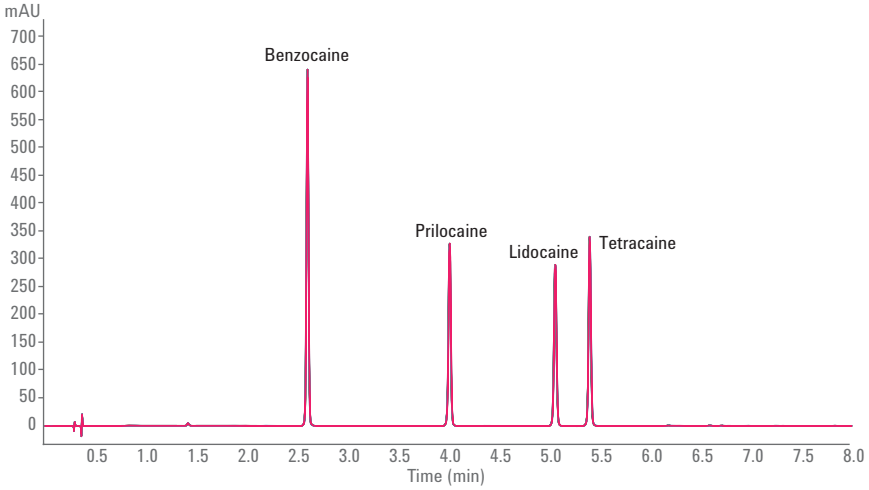

Figure 5. Analysis of local anesthetics under UHPLC conditions on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 7. Analysis of local anesthetics under UHPLC conditions 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
Benzocaine	2.61	0.02	923	0.06	–
Prilocaine	4.01	0.02	564	0.04	34.2
Lidocaine	5.06	0.02	519	0.04	22.9
Tetracaine	5.40	0.02	576	0.07	7.5

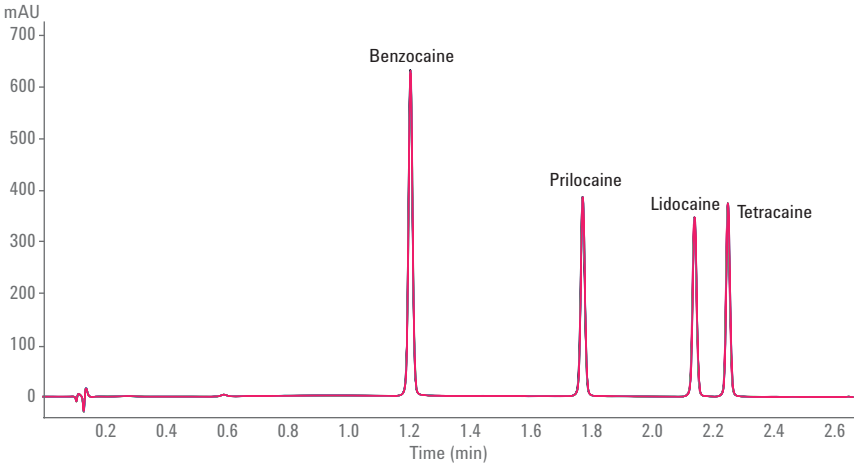

Figure 6. Analysis of local anesthetics under UHPLC conditions optimized for speed on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 8. Analysis of local anesthetics under UHPLC conditions optimized for speed 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
Benzocaine	1.21	0.04	619	0.20	–
Prilocaine	1.78	0.03	375	0.17	22.5
Lidocaine	2.15	0.03	338	0.17	14.7
Tetracaine	2.26	0.03	343	0.19	4.5

Conclusion

The transfer of the conventional LC method for the analysis of the local anesthetics benzocaine, lidocaine, prilocaine, and tetracaine from an Agilent 1200 Series Binary LC to an Agilent 1260 Infinity II LC showed a maximum retention time deviation of 0.3 %, while comparable peak resolution was obtained. This proves the equivalency of the 1260 Infinity II LC compared to the 1200 Series Binary LC for the conventional LC analysis of local anesthetics. With its pressure range of up to 600 bar, the 1260 Infinity II LC enables UHPLC analyses using Agilent InfinityLab Poroshell columns. By transfer of the conventional LC method for the analysis of local anesthetics to UHPLC conditions, the analysis time and solvent use were greatly reduced.

Reference

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

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