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

Proof of Equivalency for the Analysis of Polyphenols

Technical Overview

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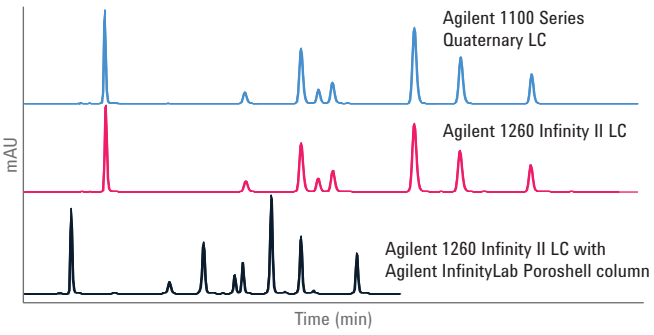
Abstract

The transfer of analytical methods from instrument to instrument is an important topic in analytical laboratories. This Technical Overview demonstrates method transfer from a legacy LC system (Agilent 1100 Series Quaternary LC) to an Agilent 1260 Infinity II LC. A sample comprising eight polyphenols is separated and evaluated regarding resolution, retention time, and area precision. Both the 1260 Infinity II LC and its predecessor produce highly precise, equivalent results without significant retention time differences. Method transfer to UHPLC conditions using an Agilent InfinityLab Poroshell column largely reduces analysis time and solvent consumption.



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Introduction

Transferring analytical methods from existing to new instruments can be a time-consuming task. In controlled environments such as the pharmaceutical industry, food analysis, and QA/QC laboratories, analytical methods have to be validated to comply with national and international standards. Whenever methods are transferred from one instrument to another, the performance of the method has to be proven to be equivalent on the new instrument, for example, by system suitability tests.

This Technical Overview describes the transfer of a method from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity II LC. Resolution, retention time (RT), and area precision are evaluated, as well as RT shifts between the different systems. A similar method transfer was used for the analysis of antihistaminic drugs, proving equivalency of the 1100 Series Quaternary LC and the 1260 Infinity II LC using an acidic phosphate buffer in the mobile phase[1].

This Technical Overview focuses on the transfer of a separation method for polyphenols under acidic conditions, and compares the performance of different heat exchangers of the 1260 Infinity II LC. Unlike the 1100 Series Quaternary LC, the 1260 Infinity II LC features UHPLC capabilities with a pressure stability up to 600 bar. To benefit from this feature, the method was transferred to UHPLC conditions using an Agilent InfinityLab Poroshell column. An optimized UHPLC method significantly reduces both analysis time and solvent consumption.

Accessories from the Agilent InfinityLab family complement and facilitate the transfer from HPLC to UHPLC methods. Agilent InfinityLab Quick Turn and Quick Connect column fittings, for example, enable a tool-free column connection that is fit for UHPLC conditions. Column ID tags of InfinityLab Poroshell columns provide details such as column dimensions and pressure/temperature maximum, and keep track of the number of injections, thereby giving extra confidence and traceability in routine analyses.

Instrument control, method transfer, and data analysis were conducted using Agilent OpenLAB CDS software version 2.1, which features a clearer yet familiar design with an intuitive interface.

Experimental

Instrumentation

The method for the separation and detection of the polyphenols sample was developed on an Agilent 1100 Series Quaternary LC comprising 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), equipped with a 10 mm standard flow cell (G1315-60022)

The Agilent 1260 Infinity II LC to which the method was transferred consisted of the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Vialsampler (G7129A) with integrated column compartment featuring a 6 µL heat exchanger (Option #66) and sample cooler (Option #100)
- Agilent 1260 Infinity II Variable Wavelength Detector (G7114A), equipped with a 10 mm standard flow cell (G1314-60186)

Chromatographic conditions

HPLC separation with an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm column	
Mobile phase	A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile
Flow rate	1.5 mL/min
Gradient	0 minutes – 10 %B 1 minutes – 10 %B 12 minutes – 30 %B 13 minutes – 95 %B
Stop time	15 minutes
Post time	10 minutes
Injection volume	5 µL
Column temperature	30 °C
Detection	280/4 nm, no reference Data rate 10 Hz
UHPLC separation with an Agilent InfinityLab Poroshell 120 SB-C18, 2.1 × 150 mm, 2.7 µm column	
Mobile phase	A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile
Flow rate	0.6 mL/min
Gradient	0 minutes – 10 %B 0.42 minutes – 10 %B 5.00 minutes – 30 %B 5.42 minutes – 95 %B
Stop time	7 minutes
Post time	5 minutes
Injection volume	1 µL
Column temperature	30 °C
Detection	280/4 nm, no reference Data rate 80 Hz

In a third system setup, the Agilent 1260 Infinity II Vialsampler (G7129A) was used with an integrated column compartment featuring a 3 µL heat exchanger (Option #063).

Columns

- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 7995218-595)
- Agilent InfinityLab Poroshell 120 SB-C18, 2.1 × 150 mm, 2.7 µm (p/n 683775-902T)

Software

Agilent OpenLAB CDS Version 2.1

Solvents and samples

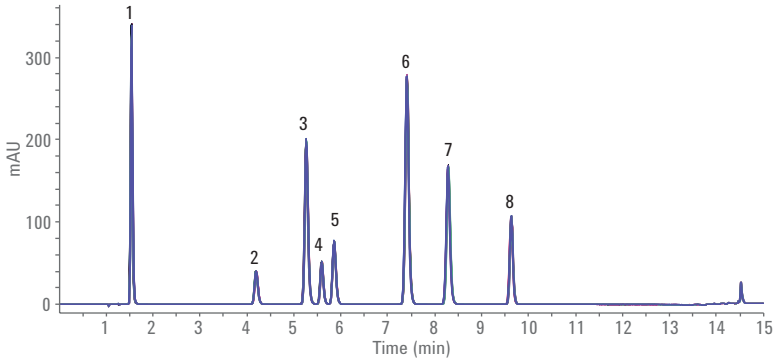
All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid, gallic acid, (+)-catechin, caffeic acid, (–)-epicatechin, (–)-epigallocatechin gallate, *p*-coumaric acid, ferulic acid, and naringin were purchased from Sigma-Aldrich (Steinheim, Germany).

Samples were dissolved in methanol:water (1:1 by volume), then mixed and diluted with the mobile phase at starting conditions (10 %B) to a final concentration of 100 ng/µL.

Results and Discussion

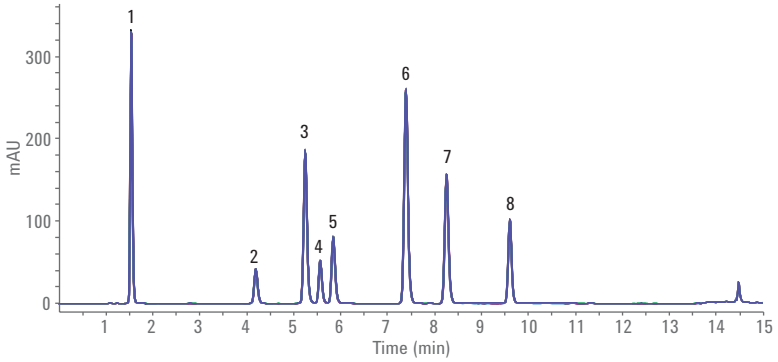
Figure 1 shows a chromatogram overlay of six consecutive runs conducted on a 1100 Series Quaternary LC. All peaks were separated to baseline with high reproducibility. Relative standard deviations (RSD) of retention time (RT) and area were below 0.14 and 0.10 %, respectively.

The method was first transferred to a 1260 Infinity II LC that was equipped with an integrated column compartment with a 6 µL heat exchanger. This heat exchanger features a 0.17 mm internal diameter capillary providing a robust performance over the entire flow range of the 1260 Infinity II Quaternary Pump, and is therefore suitable for any kind of routine analysis. The acquired data on the 1260 Infinity II LC showed similar separation and resolution to the previous system (Figure 2). RT and area RSDs were excellent, below 0.08 and 0.12 %, respectively, demonstrating a performance equivalent to the 1100 Series Quaternary LC.



No.	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Gallic acid	1.541	0.139	0.074	—
2	Catechin	4.199	0.066	0.085	27.7
3	Caffeic acid	5.266	0.050	0.086	8.4
4	Epicatechin	5.595	0.045	0.049	2.7
5	Epigallocatechin gallate	5.859	0.029	0.075	2.1
6	*p*-Coumaric acid	7.408	0.035	0.060	11.5
7	Ferulic acid	8.287	0.044	0.072	6.3
8	Naringin	9.630	0.041	0.090	10.1

Figure 1. Chromatogram overlay and peak properties of six consecutive samples separated on an Agilent 1100 Series Quaternary LC.



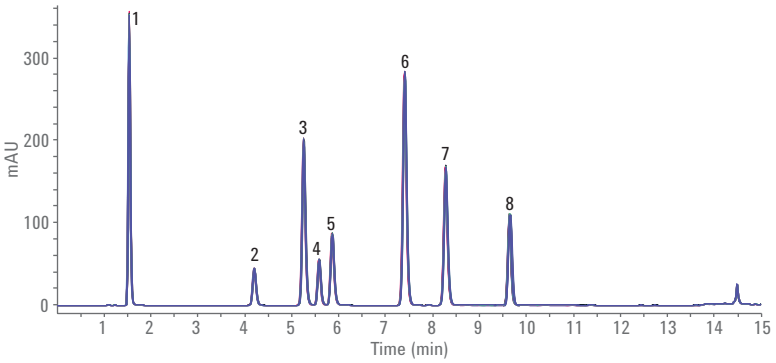
No.	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Gallic acid	1.559	0.078	0.119	—
2	Catechin	4.213	0.046	0.104	26.0
3	Caffeic acid	5.264	0.015	0.095	8.0
4	Epicatechin	5.584	0.027	0.081	2.5
5	Epigallocatechin gallate	5.861	0.036	0.124	2.1
6	*p*-Coumaric acid	7.408	0.016	0.078	11.0
7	Ferulic acid	8.272	0.023	0.074	5.9
8	Naringin	9.618	0.004	0.072	9.7

Figure 2. Chromatogram overlay and peak properties of six consecutive samples separated on an Agilent 1260 Infinity II LC equipped with a 6 µL heat exchanger.

If method development demands the lowest dispersion, the Agilent 1260 Infinity II Vialsampler can be equipped with an integrated column compartment featuring a 3 µL heat exchanger. This heat exchanger has built-in capillaries with an internal diameter of 0.12 mm, and is suitable only for flow rates below 3 mL/min. Due to the small internal diameter, it is crucial to filter samples thoroughly before analysis to avoid clogging the internal capillary. When the sample was analyzed on the system with the 3 µL heat exchanger, all compounds were separated with similar resolution to the system with the 6 µL heat exchanger (Figure 3). Evaluation of six consecutive separations produced RT and area RSDs below 0.11 and 0.10 %, respectively, proving that performance was equivalent in both the 1100 Series Quaternary LC and the 1260 Infinity II LC with a 6 µL heat exchanger.

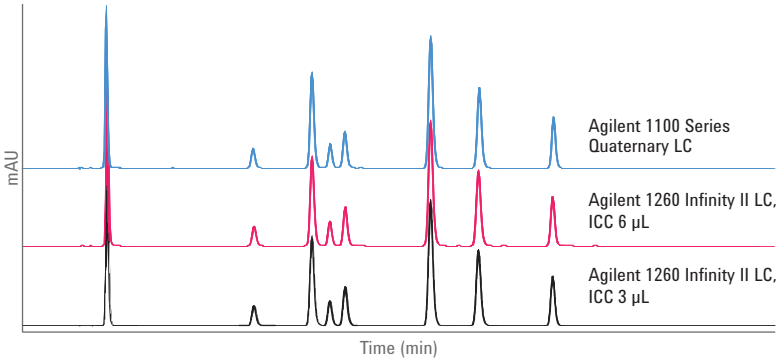
Figure 4 gives an overview of absolute and relative shifts in RT of the eight compounds after method transfer. Regardless of the 1260 Infinity II heat exchanger internal volume, the transfer from the 1100 Series Quaternary LC to the 1260 Infinity II LC produced an absolute shift in RT of ± 0.02 minutes or less, equaling less than ±1.2 %.

Between the two different heat exchangers available for the integrated column compartment of the 1260 Infinity II LC, retention time shifts were less than ± 0.7 % (absolute shifts of ± 0.03 minutes or less). Specifications of the Agilent Intelligent System Emulation Technology (ISET), a software that emulates pump characteristics of legacy and non-Agilent instruments, restrict the shift in RTs to a maximum of ± 0.3 minutes or ± 5 %[2]. The deviations found after the method transfer, as described in this Technical Overview, are well within these boundaries. This demonstrates that methods can be transferred not only from a 1100 Series Quaternary LC to a 1260 Infinity II LC, but also between two different heat exchanger configurations of the latter system without significantly impairing RT stability.



No.	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Gallic acid	1.549	0.107	0.071	—
2	Catechin	4.213	0.066	0.091	26.0
3	Caffeic acid	5.264	0.057	0.086	8.0
4	Epicatechin	5.594	0.054	0.087	2.6
5	Epigallocatechin gallate	5.874	0.047	0.083	2.2
6	*p*-Coumaric acid	7.415	0.045	0.083	11.0
7	Ferulic acid	8.288	0.042	0.080	6.0
8	Naringin	9.648	0.038	0.068	9.8

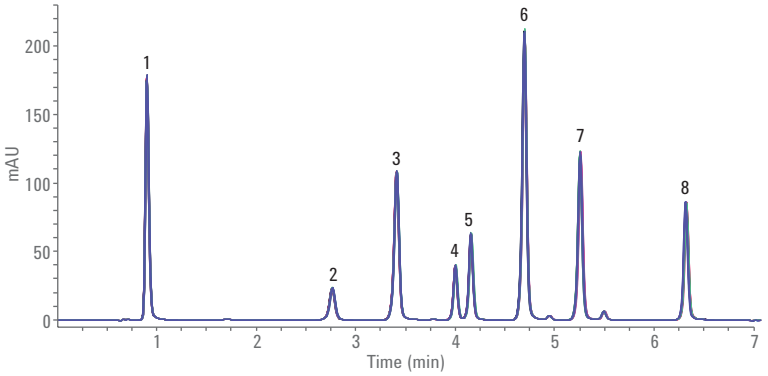
Figure 3. Chromatogram overlay and peak properties of six consecutive samples separated on an Agilent 1260 Infinity II LC equipped with a 3 µL heat exchanger.



	Agilent 1100 versus ICC 6 µL		Agilent 1100 versus ICC 3 µL		ICC 3 µL versus ICC 6 µL	
Compound	ΔRT (min)	ΔRT (%)	ΔRT (min)	ΔRT (%)	ΔRT (min)	ΔRT (%)
Gallic acid	0.02	1.17	0.01	0.52	0.01	0.65
Catechin	0.01	0.33	0.01	0.33	0.00	0.00
Caffeic acid	0.00	–0.04	0.00	–0.04	0.00	0.00
Epicatechin	–0.01	–0.20	0.00	–0.02	–0.01	–0.18
Epigallocatechin gallate	0.00	0.03	0.01	0.26	–0.01	–0.22
p-Coumaric acid	0.00	0.00	0.01	0.09	–0.01	–0.09
Ferulic acid	–0.02	–0.18	0.00	0.01	–0.02	–0.19
Naringin	–0.01	–0.12	0.02	0.19	–0.03	–0.31

Figure 4. Absolute and relative retention time shifts comparing the column compartment heat exchangers of the Agilent 1100 Series Quaternary LC (blue) and the Agilent 1260 Infinity II LC with 6 µL (red, ICC 6 µL) and 3 µL (black, ICC 3 µL) internal volume.

To exploit the UHPLC capabilities of the 1260 Infinity II LC, the method was transferred from standard HPLC to UHPLC conditions using an Agilent InfinityLab Poroshell column. These columns feature smaller, superficially porous particles, and a pressure stability up to 600 bar, enabling adjustments of gradient and flow parameters to work at higher system pressure while simultaneously reducing analysis time and solvent consumption. Optional column ID tags hold important details such as dimensions, serial and batch number, maximum temperature and pressure, and keep track of the number of injections, thus providing a plus in traceability and security in routine analyses. With an Agilent InfinityLab Poroshell 120 SB-C18, 2.1 × 150 mm, 2.7 µm column operated at a flow rate of 0.6 mL/min, the analysis time was reduced to 12 minutes, corresponding to a total time and solvent savings of 52 and 80.8 %, respectively. Baseline separation of all compounds could be maintained. RT and area precision were on a high level, yielding RSDs below 0.07 and 0.23 %, respectively, over a series of six consecutive runs (Figure 5).



No.	Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
1	Gallic acid	0.902	0.041	0.133	—
2	Catechin	2.765	0.069	0.125	22.7
3	Caffeic acid	3.412	0.037	0.183	6.8
4	Epicatechin	4.003	0.041	0.223	7.4
5	Epigallocatechin gallate	4.159	0.047	0.124	2.1
6	*p*-Coumaric acid	4.698	0.033	0.194	7.0
7	Ferulic acid	5.258	0.058	0.210	7.1
8	Naringin	6.321	0.055	0.128	13.9

Figure 5. Chromatogram overlay and peak properties of six consecutive samples separated on an Agilent 1260 Infinity II LC with an UHPLC method optimized for speed using an Agilent InfinityLab Poroshell column.

Conclusion

This Technical Overview describes the method transfer from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity II LC. The latter system was used in two configurations, using different heat exchangers for high robustness and low dispersion, respectively. The separation of a sample containing eight polyphenols produced equivalent results regarding RT and area precision on all system setups used. Over six consecutive runs, RSDs of RT were below 0.14 % on the 1100 Series Quaternary LC, and below 0.11 % on the 1260 Infinity II LC. Area RSDs were below 0.13 % on all systems. All compounds were separated to baseline. After method transfer from the 1100 Series Quaternary LC to the 1260 Infinity II LC, RT shifts of 0.02 minutes or less were observed. The two different heat exchangers used in the latter system produced an RT deviation of less than 0.7 %. This data proves the high performance of the 1260 Infinity II LC and its equivalency compared with the preceding 1100 Series Quaternary LC, enabling seamless transfer of existing methods to new instruments. In addition, method transfer to UHPLC conditions using an InfinityLab Poroshell column cuts analysis time in half and reduces solvent consumption by more than 80 %, while resolution and precision are maintained on a high level.

References

1. Krieger, S. 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, *Agilent Technologies Technical Overview*, publication number 5991-6914EN, **2016**.

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

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