Transfer of a Heart Disease Treatment Analysis from an Agilent 1100 System to an UltiMate 3000 HPLC System

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Technical Note 162

Key Words
HPLC Method Transfer, Agilent 1100 System, UltiMate 3000 System, Gradient Application, Gradient Delay Volume Adjustment, Peak Dispersion, Peak Resolution

Goal
The goal of this Technical Note is to demonstrate a seamless transfer of a gradient HPLC method from an Agilent® 1100 HPLC system to a Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system.

Introduction
Transfer of high performance liquid chromatography (HPLC) methods is common practice in analytical laboratories. Because an identical column format and chemistry are employed, users often expect the same chromatographic result; however, this is not always the case.[1] The transfer can involve different instruments, module generations,[2] laboratories, and companies, and the challenge related to it can therefore vary largely.

As instruments age and are no longer supported by vendors, like the 1100 Series from Agilent, which became obsolete at the end of May 2015, a situation can arise where an existing method needs to be transferred to a different instrument. Very often, one requirement for the method transfer is the best match to the previous chromatographic results. Many adverse effects encountered during the analytical method transfer can be traced to the instrument. One significant issue involves gradient separations that are in much more common use today than in the past.[3] Hence, this Technical Note focuses on comparative testing of a gradient HPLC application on a Quaternary Agilent 1100 system and a Quaternary UltiMate 3000 system.

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Experimental

Instrumentation

Agilent 1100 System	
Degasser:	G1322A Degasser
Pump:	G1311A QuatPump with standard mixer
Sampler:	G1367A WPALS
Sampler thermostat:	G1330B (in stack but not operated)
Column thermostat:	G1316A ColComp with 6 µL preheater
Detector:	G1315A DAD with analytical flow cell, 13 µL

Default capillaries were used for flow connections of the devices.

UltiMate 3000 SD System	
Degasser:	SRD-3400 (P/N 5035.9245)
Pump:	LPG-3400SD (P/N 5040.0031)
Sampler:	WPS-3000TSL (P/N 5822.0020)
Column thermostat:	TCC-3000SD (P/N 5730.0010) with 7 µL preheater
Detector:	DAD-3000 (P/N 5082.0010) with analytical flow cell, 13 µL
Mixer:	350 µL + 50 µL or 750 µL + 50 µL

Default Thermo Scientific™ Dionex™ Viper™ capillaries were used for flow connections of the devices.

Chromatographic Conditions and Settings	
Column:	Thermo Scientific™ Accucore™ XL column, C18, 4.6 × 150 mm, 4 µm, P/N 74104-154630
Mobile phase:	A: Water with 0.1% formic acid B: Methanol with 0.07% formic acid

Gradient:

t [min]	%A	%B
0	90	10
10	20	80
11.5	20	80
12	90	10
17	90	10

Flow rate:	1.2 mL/min
Column temperature:	50 °C
Injection volume:	25 µL
UV detection wavelength:	214 nm
Data rate:	10 Hz
Response time:	0.5 s
Bandwidth:	4 nm
Slit width:	4 nm

Peak Identification and Concentration		
1.	Hydrochlorothiazide	10 µg/mL
2.	Chlorthalidone	20 µg/mL
3.	Enalapril	60 µg/mL
4.	Impurity	
5.	Ramipril	60 µg/mL
6.	Telmisartan	20 µg/mL
7.	Azilsartan	20 µg/mL
8.	Valsartan	20 µg/mL

Data Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software version 7.2

Results and Discussion

The same method parameters and the same column were used to separate the sample on the two instruments in the default configuration. Figure 1 shows a comparison of the obtained data. The red rectangles in the Agilent 1100 system data mark baseline artifacts resulting from the injection (left) and the gradient step at 12 min (right). These artifacts look very similar between the systems. More importantly, the chromatograms also look very similar, however peaks elute slightly earlier with the UltiMate 3000 system. This is a consequence of the optimized fluidics and the smaller gradient delay volume (GDV) of this system. Another consequence of the improved fluidics is that all peaks are higher and narrower.

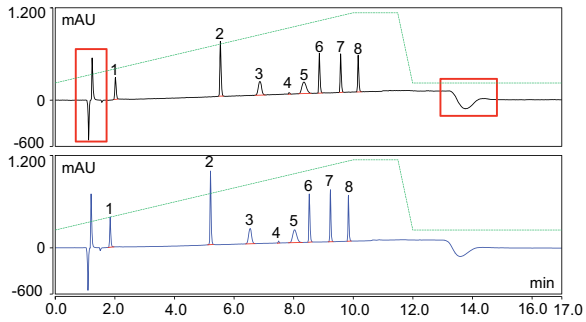

Figure 1. Gradient separation of heart disease treatment drugs performed on an Agilent 1100 system (black) and an UltiMate 3000 system (blue), both with default flow connections. The red rectangles indicate baseline artifacts caused by the injection and the final gradient step.

To increase the GDV and to shift the peaks closer toward the Agilent 1100 retention times, a larger mixer can be installed in the UltiMate 3000 pump. The UltiMate 3000 pump uses a flexible two-stage SpinFlow™ mixer with a radial and a longitudinal mixing part. Changing the mixing volume is both easy and fast. Different mixers covering a wide range of mixing volumes are available as shown in Table 1.

Table 1. Available combinations of mixers and resulting mixing volume.

Description	SD Pumps* P/N	RS Pumps* P/N
Mixer for 35 µL mixing volume	6040.5000	6042.5000
Mixer for 100 µL mixing volume	6040.5100	6042.5100
Mixer for 200 µL mixing volume	6040.5110	
Mixer for 400 µL mixing volume	6040.5310	
Mixer for 800 µL mixing volume	6040.5750	
Mixer for 1550 µL mixing volume	6040.5450	

*except ISO-3100SD

We replaced the default 350 µL longitudinal mixer with a 750 µL mixer for a total mixing volume of 800 µL (P/N 6040.5750) to be more comparable with the Agilent 1100 system retention times. The overlay in Figure 2 shows how similar the peaks elute with this setup. Table 2 compares retention times of the peaks. Peaks 2-8 in Figure 2 have a maximum deviation of only 0.06 min; Peak 1 deviates by 0.19 min. The slightly pronounced retention time difference is likely to be caused by more efficient mobile phase pre-heating of the UltiMate 3000 system impacting the isocratic elution mechanism of the peak. If wanted, this difference could be reduced by a smaller volume pre-heater and by adding more extra column volume (ECV). However, this additional ECV would create more dispersion, reducing the improvements of the chromatography obtained with the UltiMate 3000 system (Table 3).

Table 2. Retention times obtained with Agilent 1100 and UltiMate 3000 systems (with 800 µL mixer).

Peak	Peak Name	Agilent 1100 System	UltiMate 3000 System	Retention Time Difference [min]
		Retention Time [min]	Retention Time [min]	
1	Hydrochlorothiazide	2.02	1.84	0.19
2	Chlortalidone	5.54	5.49	0.06
3	Enalapril	6.86	6.87	0.00
4	Impurity	7.85	7.78	0.07
5	Ramipril	8.35	8.41	-0.06
6	Telmisartan	8.86	8.92	-0.06
7	Azilsartan	9.58	9.55	0.03
8	Valsartan	10.17	10.15	-0.02

Table 3. Improvements on peak height, width, and resolution obtained with the UltiMate 3000 System (800 µL mixer) compared to the Agilent 1100 System.

Peak	Peak Name	Peak Height Improvement [%]	Peak Width Reduction (at 50% Peak Height) [%]	Resolution Improvement to Next Peak [%]
1	Hydrochlorothiazide	37%	12%	17%
2	Chlortalidone	31%	15%	23%
3	Enalapril	32%	19%	13%
4	Impurity	98%	19%	36%
5	Ramipril	22%	10%	11%
6	Telmisartan	3%	10%	-1%
7	Azilsartan	24%	19%	22%
8	Valsartan	30%	18%	n.a.

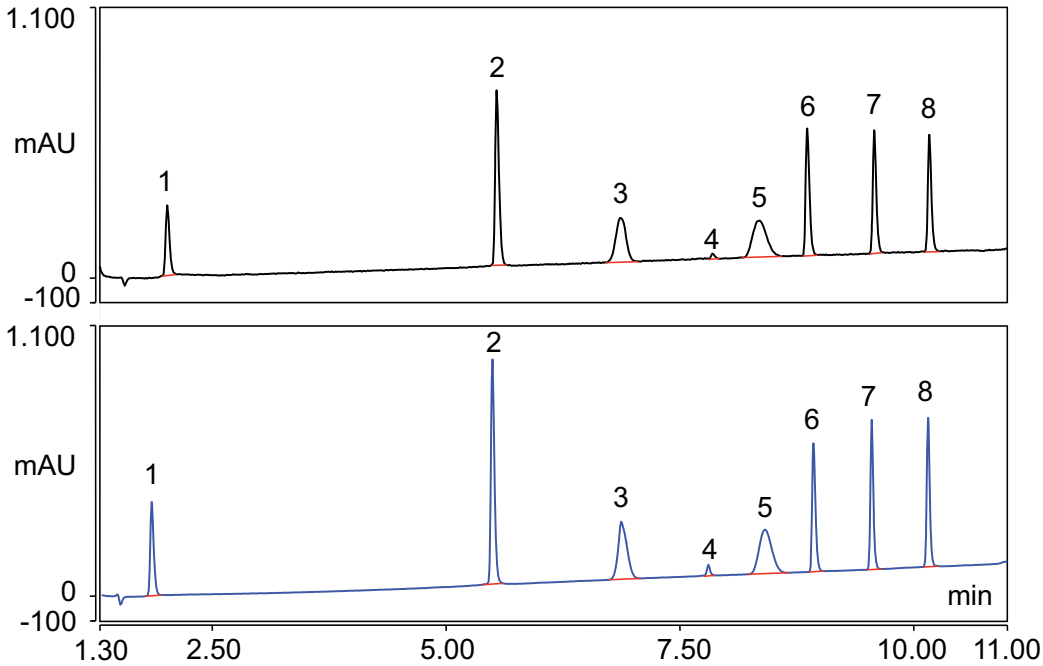

Figure 2. Gradient separation of heart disease treatment drugs performed on an Agilent 1100 system (black) and an UltiMate 3000 system (blue) with 800 µL mixer. The retention times obtained with both instruments match very well.

Conclusion

The method transfer of a heart disease treatment gradient separation from an Agilent 1100 to an UltiMate 3000 system is exceptionally easy. After the installation of an 800 µL mixer, the peak retention times and the elution profiles are almost identical. At the same time, the UltiMate 3000 system creates less peak dispersion for higher and better resolved peaks.

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