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Demonstrating Utility of the Phenomenex Online UHPLC/HPLC Method Transfer Tool Using the Luna® Omega C18 Columns

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Introduction

The analysis of an ABN7 test mix was used to assess the scalability for Luna Omega C18 columns across a range of particle sizes (1.6 µm, 3 µm, and 5 µm) in three different dimensions. In this technical note, we show how the Phenomenex online UHPLC/HPLC Method Transfer Tool (https://www.phenomenex.com/Tools/KinetexMethodTranslation) was utilized to determine method parameters when scaling from one particle and column size to another.

LC-UV Conditions

Column:	Luna Omega 1.6 µm C18
	Luna Omega 3 µm C18
	Luna Omega 5 µm C18
Dimension:	50 x 2.1 mm
	150 x 4.6 mm
	250 x 4.6 mm
Part No.:	00B-4742-AN
	00F-4784-E0
	00G-4785-E0
Mobile Phase:	A: Water
	B: Acetonitrile
Gradient:	See Figures
Flow Rate:	See Figures
Injection Volume:	1 µL, 50 x 2.1 mm column
	5 µL, 150 x 4.6 mm column
	5 µL, 250 x 4.6 mm column
Temperature:	30 °C
Detection:	UV @ 254 nm
LC System:	Agilent® 1260 Infinity
Analytes:	1. Uracil (1.0 mg/mL in Methanol)
	2. Pindolol (5.0 mg/mL in Methanol)
	3. Chlorpheniramine (5.0 mg/mL in Methanol)
	4. Nortriptyline (5.0 mg/mL in Methanol)
	5. 3-Methyl, 4-Nitrobenzoic acid (5.0 mg/mL in Methanol)
	6. 2-Hydroxy, 5-Methylbenzaldehyde (5.0 mg/mL in Methanol)
	7. Hexanophenone (5.0 mg/mL in Methanol)

Results and Discussion

Figure 1 shows the results of ABN7 run on a Luna Omega 1.6 µm C18, 50 x 2.1 mm column. The benefits of the smaller particle size and shorter column are apparent; baseline separation (resolution >2) of all analytes is achieved in less than 6 minutes. This highlights one of the advantages of UHPLC - the separation is achieved in a shorter time.

The Method Transfer Tool can be used to properly scale the gradient times and flow rate when scaling this separation from a Luna Omega 1.6 µm C18, 50 x 2.1 mm column to a Luna Omega 3 µm C18, 150 x 4.6 mm column (**Table 1**). While the analysis time has increased, the chromatographic resolution between the analytes is maintained due to the gradient and flow rate being scaled with the Method Transfer Tool. These new parameters generated the chromatogram displayed in **Figure 2**.

The Method Transfer Tool can now be used to further scale the gradient times and flow rate for a Luna Omega 5 µm C18, 250 x 4.6 mm column (**Table 2**). The resulting chromatogram is shown in **Figure 3**. Again, while the overall analysis time has increased, the chromatographic resolution between the analytes has been maintained due to the scaling of the gradient and flow rate.

The Method Transfer Tool can also be used for making parameter predictions when using the same column on two different instrument types. **Table 3** is an example of using a Luna Omega 5 µm C18, 250 x 4.6 mm column on a HPLC instrument with a larger delay volume but wanting to move it to a UHPLC instrument with a smaller delay volume. The red circle indicates the differences in the gradient table that results.

This tool can aid in the method transfer not only between different particle sizes and column dimensions, but also between new and older generation instruments having different system volumes. **Table 4** is an example of the same scaling that occurred for **Figure 2**; however, the instrument type was changed from a UHPLC to a HPLC. The red circle indicates the differences in the gradient table that results.

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Aside from the lab being his favorite place to be, Zeshan enjoys playing vintage videogames with his twin boys and loves every minute of reliving parenthood with his baby girl.

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Figure 1. Luna® Omega 1.6 µm C18, 50 x 2.1 mm

LC Column 1

Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (µm)	Porosity
50	2.1	0.4	1.6 FP ⓘ	0.660

Original Gradient Table

Time (min)	%A	%B	#CV	%B/CV
0	95	5	3.94	
0.5	95	5	1.75	
5.5	5	95	17.50	5.14
6.6	5	95	3.85	
7	95	5	1.40	
10	95	5		

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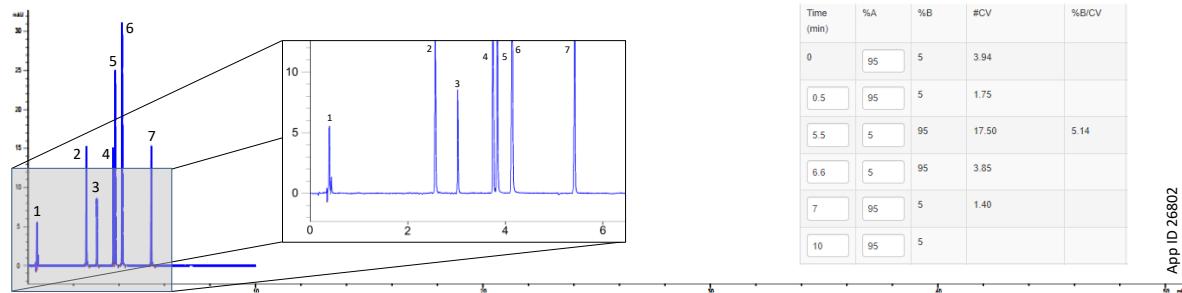
Peak No.	Analyte	Retention Time (min)	k'	Resolution
1	Uracil	0.39	-	-
2	Pindolol	2.56	5.55	62.1
3	Chlorpheniramine	3.02	6.73	14.5
4	Nortriptyline	3.74	8.57	21.4
5	3-Methyl, 4-Nitrobenzoic acid	3.84	8.81	2.7
6	2-Hydroxy, 5-Methylbenzaldehyde	4.14	9.59	8.6
7	Hexanophenone	5.42	12.86	33.6

Table 1. Scaling Calculator Predictions – 1.6 µm to 3 µm

LC Instrument 1		Predicted Total Pressure (bar)	LC Instrument 2		Predicted Total Pressure (bar)
Pressure Limit (bar) =	600	392	Pressure Limit (bar) =	600	519
Delay Volume (mL) =	0.75 ⓘ		Delay Volume (mL) =	0.75 ⓘ	
Instrument Type =	UHPLC		Instrument Type =	UHPLC	
Maximum Viscosity =	1.01E-03	ACN / H2O 25C	Separation Goal =	Geometric Scaling	

LC Column 1					LC Column 2				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (µm)	Porosity	Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (µm)	Porosity
50	2.1	0.4	1.6 FP ⓘ	0.660	150	4.6	1.92	3 FF ⓘ	0.660

Original Gradient Table					Scaled Gradient Table				
Time (min)	%A	%B	#CV	%B/CV	Time	%A	%B	#CV	%B/CV
0	95	5	6.56		0.00	95	5	0.46	
0.5	95	5	1.75		6.73	95	5	7.86	
5.5	5	95	17.50	5.14	21.73	5	95	17.50	5.14
6.6	5	95	3.85		25.03	5	95	3.85	
7	95	5	1.40		26.23	95	5	1.40	
10	95	5			30.05	95	5	4.46	

Figure 2. Luna® Omega 3 μm C18, 150 x 4.6 mm

LC Column 1				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity
150	4.6	1.92	3 FP ⓘ	0.660

Original Gradient Table

Time (min)	%A	%B	#CV	%B/CV
0	95	5	0.46	
6.73	95	5	7.85	
21.73	5	95		5.14
25.03	5	95	3.85	
26.23	95	5	1.40	
30.05	95	5	4.46	

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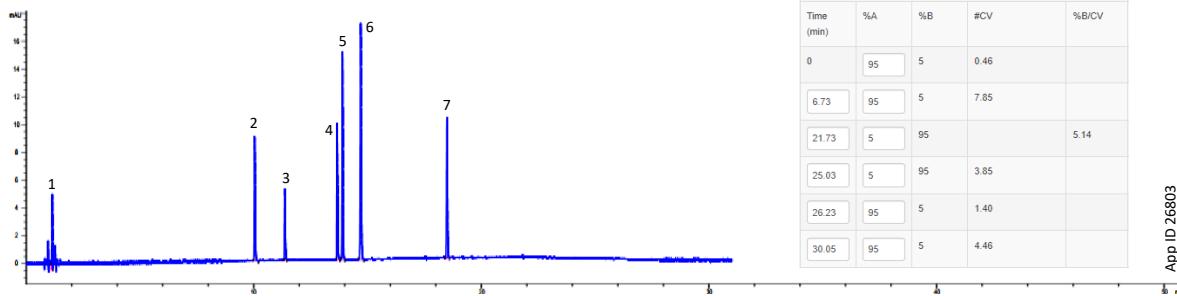
Peak No.	Analyte	Retention Time (min)	k'	Resolution
1	Uracil	1.15	-	-
2	Pindolol	10.04	7.72	200
3	Chlorpheniramine	11.36	8.87	19.89
4	Nortriptyline	13.66	10.87	34.68
5	3-Methyl, 4-Nitrobenzoic acid	13.90	11.07	3.37
6	2-Hydroxy, 5-Methylbenzaldehyde	14.70	11.77	10.45
7	Hexanophenone	18.49	15.06	49.25

Table 2. Scaling Calculator Predictions – 3 μm to 5 μm

LC Instrument 1		Predicted Total Pressure (bar)	LC Instrument 2		Predicted Total Pressure (bar)
Pressure Limit (bar) =	600	520	Pressure Limit (bar) =	1000	402
Delay Volume (mL) =	0.75 ⓘ		Delay Volume (mL) =	0.75 ⓘ	
Instrument Type =	UHPLC		Instrument Type =	UHPLC	
Maximum Viscosity =	1.01E-03	ACN / H2O 25C	Separation Goal =	Geometric Scaling	

LC Column 1					LC Column 2				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity	Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity
150	4.6	1.92	3 FP ⓘ	0.660	250	4.6	1.92	5 FF ⓘ	0.660

Original Gradient Table					Scaled Gradient Table				
Time (min)	%A	%B	#CV	%B/CV	Time	%A	%B	#CV	%B/CV
0	95	5	0.46		0.00	95	5	0.27	
6.73	95	5	7.85		11.48	95	5	8.04	
21.73	5	95		5.14	36.48	5	95	17.50	5.14
25.03	5	95	3.85		41.98	5	95	3.85	
26.23	95	5	1.40		43.98	95	5	1.40	
30.05	95	5	4.46		50.08	95	5	4.27	

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Figure 3. Luna® Omega 5 μm C18, 250 x 4.6 mm

LC Column 1				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity
250	4.6	1.92	5 FP	0.660

Original Gradient Table

Time (min)	%A	%B	#CV	%B/CV
0	95	5	0.27	
11.48	95	5	8.04	
36.48	5	95	17.50	5.14
41.98	5	95	3.85	
43.98	95	5	1.40	
50.08	95	5	4.27	

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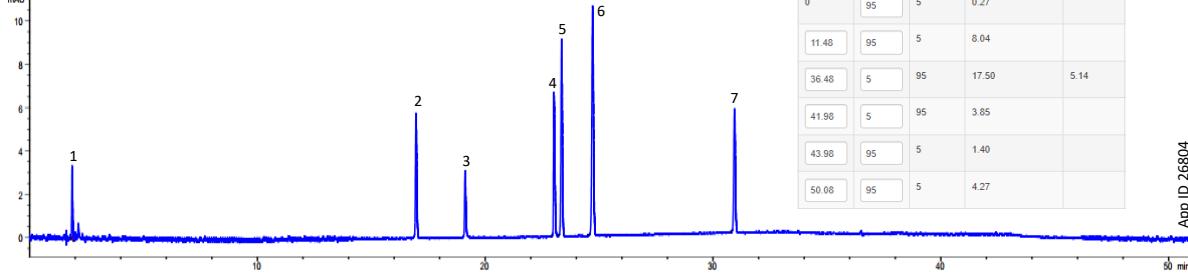
Peak No.	Analyte	Retention Time (min)	k'	Resolution
1	Uracil	1.87	-	-
2	Pindolol	16.96	8.08	200
3	Chlorpheniramine	19.11	9.24	21.01
4	Nortriptyline	23.02	11.33	38.12
5	3-Methyl, 4-Nitrobenzoic acid	23.35	11.51	3.02
6	2-Hydroxy, 5-Methylbenzaldehyde	24.71	12.24	10.83
7	Hexanophenone	30.94	15.57	47.35

Table 3. Scaling Calculator Predictions – HPLC to UHPLC, Same Column

LC Instrument 1		Predicted Total Pressure (bar)	LC Instrument 2		Predicted Total Pressure (bar)
Pressure Limit (bar) =	600	401	Pressure Limit (bar) =	600	520
Delay Volume (mL) =	1.45		Delay Volume (mL) =	0.75	
Instrument Type =	HPLC		Instrument Type =	UHPLC	
Maximum Viscosity =	1.01E-03	ACN / H2O 25C	Separation Goal =	Geometric Scaling	

LC Column 1					LC Column 2				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity	Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (μm)	Porosity
150	4.6	1.92	3 FP	0.660	150	4.6	1.92	3 FF	0.660

Original Gradient Table					Scaled Gradient Table				
Time (min)	%A	%B	#CV	%B/CV	Time	%A	%B	#CV	%B/CV
0	95	5	0.88		0.00	95	5	0.46	
6.73	95	5	7.85		7.09	95	5	8.28	
21.73	5	95		5.14	22.09	5	95	17.50	5.14
25.03	5	95	3.85		25.39	5	95	3.85	
26.23	95	5	1.40		26.59	95	5	1.40	
30.05	95	5	4.46		30.41	95	5	4.46	

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Table 4. Scaling Calculator Predictions – Scaling UHPLC to HPLC, 1.6 µm to 3 µm

LC Instrument 1			Predicted Total Pressure (bar)	LC Instrument 2			Predicted Total Pressure (bar)
Pressure Limit (bar) =	600		392	Pressure Limit (bar) =	600		401
Delay Volume (mL) =	0.75			Delay Volume (mL) =	1.45		
Instrument Type =	UHPLC			Instrument Type =	HPLC		
Maximum Viscosity =	1.01E-03	ACN / H2O 25C		Separation Goal =	Geometric Scaling		

LC Column 1					LC Column 2				
Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (µm)	Porosity	Length (mm)	Column ID (mm)	Flow Rate (mL/min)	Particle Type / Size (µm)	Porosity
50	2.1	0.4	1.6 FP	0.660	150	4.6	1.92	3 FF	0.660

Original Gradient Table					Scaled Gradient Table				
Time (min)	%A	%B	#CV	%B/CV	Time	%A	%B	#CV	%B/CV
0	95	5	6.56		0.00	95	5	0.88	
0.5	95	5	1.75		6.37	95	5	7.43	
5.5	5	95	17.50	5.14	21.37	5	95	17.50	5.14
6.6	5	95	3.85		24.67	5	95	3.85	
7	95	5	1.40		25.87	95	5	1.40	
10	95	5			30.05	95	5	4.88	

Table 5. Summary of Data From all Three Columns

Peak No.	Analyte	Luna® Omega 1.6 µm C18, 50 x 2.1 mm			Luna Omega 3 µm C18, 150 x 4.6 mm			Luna Omega 5 µm C18, 250 x 4.6 mm		
		Retention Time (min)	k'	Resolution	Retention Time (min)	k'	Resolution	Retention Time (min)	k'	Resolution
1	Uracil	0.39	-	-	1.15	-	-	1.87	-	-
2	Pindolol	2.56	5.55	62.1	10.04	7.72	200	16.96	8.08	200
3	Chlorpheniramine	3.02	6.73	14.5	11.36	8.87	19.89	19.11	9.24	21.01
4	Nortriptyline	3.74	8.57	21.4	13.66	10.87	34.68	23.02	11.33	38.12
5	3-Methyl, 4-Nitrobenzoic acid	3.84	8.81	2.7	13.90	11.07	3.37	23.35	11.51	3.02
6	2-Hydroxy, 5-Methylbenzaldehyde	4.14	9.59	8.6	14.70	11.77	10.45	24.71	12.24	10.83
7	Hexanophenone	5.42	12.86	33.6	18.49	15.06	49.25	30.94	15.57	47.35

Conclusion

The Method Transfer Tool illustrated in this technical note allows for scaling methods for transfer from HPLC to UHPLC, or vice versa, while preserving chromatographic resolution. The data summarized in **Table 5** demonstrate that scaling the gradient times and flow rate will result in an equivalent separation when there is a need or desire to change the particle size and dimensions of the column used. This tool can also be used when it is necessary to move from a UHPLC to a HPLC instrument. For example, when a method is developed on UHPLC but transferred to routine use on HPLC due to instrument or column constraints. Alternatively, when an existing HPLC method is desired to transfer to UHPLC to take advantage of reduced analysis times while maintaining overall chromatographic resolution. The data here represents the accuracy of geometric scaling between Luna® Omega C18 columns with different particle sizes and column dimensions using the UHPLC/HPLC Method Transfer Tool.

Luna Omega Ordering Information

1.6 μm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges‡ (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502

for 2.1 mm ID

3 μm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0* /10 pk**
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
C18	00B-4784-E0	00D-4784-E0	00F-4784-E0	00G-4784-E0	AJ0-7612
SUGAR	—	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495

for ID: 3.2-8.0 mm

5 μm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0* /10 pk**
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJ0-7612

for ID: 3.2-8.0 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282

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