Waters

Improving LC Separations: Transferring Methods from HPLC to UPLC™

Eric Grumbach, Tom Wheat, Doug McCabe, Diane Diehl, and Jeff Mazzeo
Waters Corporation

An approach to improving separations by transferring methods from HPLC to UPLC™ is outlined.

Ultra performance liquid chromatography (UPLC™) is a technological breakthrough in separation science. Significant gains in speed, sensitivity, and resolution are enabled by UPLC™. To harness the power of UPLC™ in analytical laboratories, many HPLC methods can easily and routinely be transferred to UPLC™ by using readily available tools. In this application, we will transfer a method for the separation of caffeic acid derivatives found in echinacea purpurea from HPLC to UPLC™.

HPLC Conditions

Column: XTerra® MS C18 4.6 × 150 mm, 5 µm
Mobile Phase A: 0.1% TFA in water
Mobile Phase B: 0.08% TFA in acetonitrile
Flow Rate: 1.0 mL/min
Gradient Profile:

Time (min)	%A	%B	curve
0.0	92	8	
2.0	92	8	6
32.0	50	50	7
35.0	10	90	6
36.0	92	8	6
41.0	92	8	6

Injection Volume: 10 µL
Sample Concentration: 100 µg/mL
Column Temperature: 40 °C
Detection: UV @ 330 nm
Sampling Rate: 5 pts/s
Time Constant: 1.0
Instrument:Waters® Alliance® 2695 Separations Module with 2996 PDA

In this case, the HPLC column is an XTerra® MS C18 column. We can use the Waters Reversed-Phase Column Selectivity Chart to choose the ACQUITY UPLC™ chemistry that is closest in selectivity to the HPLC column. This tool is available at www.waters.com/selectivitychart. Using the tool, the ACQUITY UPLC™ BEH C18 column is closest in selectivity.

The HPLC column dimensions were 4.6 × 150 mm, 5 µm. To maintain the same resolution, we select a UPLC™ column that has a similar column length to particle size ratio (L/dp) as the HPLC column. Thus, we selected a 2.1 × 50 mm, 1.7 µm UPLC™ column.

To scale the separation from HPLC to UPLC™ a series of calculations are performed to scale the flow rate, injection volume and gradient table. To ensure accurate and quick scaling, the ACQUITY UPLC™ Columns Calculator handles all of the appropriate scaling calculations. We input the HPLC conditions into the calculator (as shown in Figure 1A) and the UPLC™ conditions are automatically displayed (as shown in Figure 1B).

(a)

Step	Time [min]	Flow [mL/min]	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	1.00	92	8	0.00	-
Init Hold	2.00	1.00	92	8	2.00	1.22
3	32.00	1.00	50	50	30.00	18.23
4	35.00	1.00	10	90	3.00	1.82
5	36.00	1.00	92	8	1.00	0.61
6	41.00	1.00	92	8	5.00	3.04
7						
8						
9						
10						

(b)

Geometrically Scaled Gradient: 2.1 X 50 mm

Step	Time [min]	Flow [mL/min]	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	0.613	92	8	0.00	-
Init Hold	0.36	0.613	92	8	0.36	1.93
3	3.76	0.613	50	50	3.40	18.23
4	4.10	0.613	10	90	0.34	1.82
5	4.22	0.613	92	8	0.12	0.64
6	4.78	0.613	92	8	0.56	3.00
7						
8						
9						
10						

Injection Volume: 0.7 µL
Pmax = 4681 PSI
Print Results ...
© 2005 Waters Corporation

Figure 1: Gradient tables from the ACQUITY UPLC™ Columns Calculator. A) Original HPLC gradient table as entered into the calculator. B) Geometrically scaled gradient at UPLC™ linear velocity as generated by the calculator.

Scaled UPLC™ Conditions

Column: ACQUITY UPLC™ BEH C18 2.1 × 50 mm, 1.7 µm
Mobile Phase A: 0.1% TFA in water
Mobile Phase B: 0.08% TFA in acetonitrile
Flow Rate: 0.613 mL/min
Gradient Profile:

Time (min)	%A	%B	curve
0.00	92	8	
0.36	92	8	6
3.76	50	50	7
4.10	10	90	6
4.22	92	8	6
4.78	92	8	6

Injection Volume: 0.7 µL

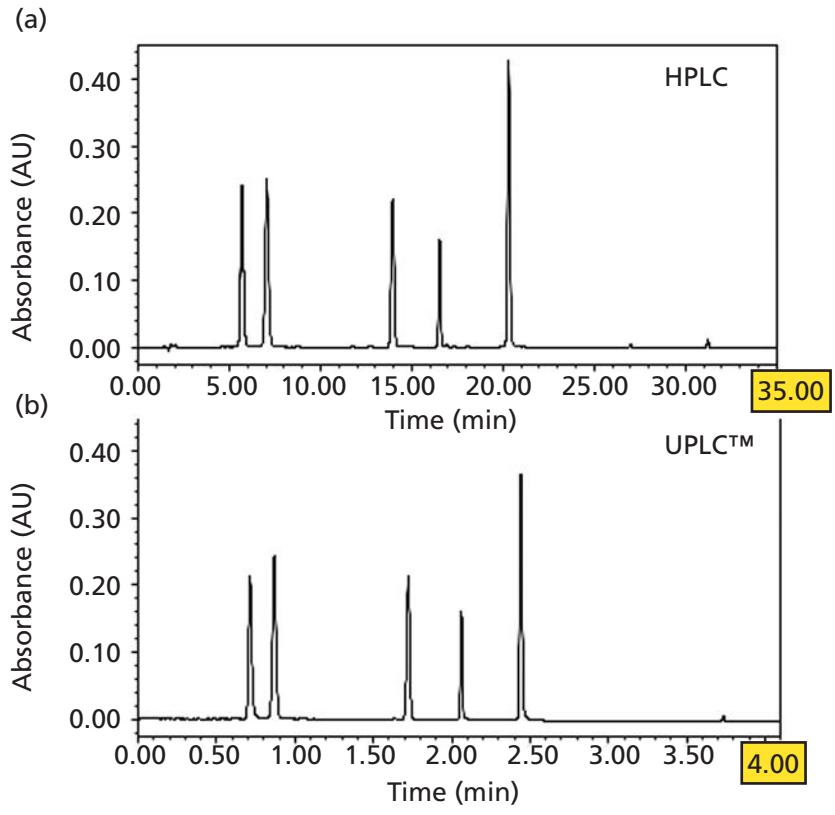

Figure 2: Separation of caffeic acid derivatives found in echinacea purpurea. A) HPLC separation. B) UPLC™ separation.

Sample Concentration: 100 µg/mL
Column Temperature: 40 °C
Detection: UV @ 330 nm
Sampling Rate: 20 pts/s
Time Constant: 0.2
Instrument: ACQUITY UPLC™ with ACQUITY 2996 PDA

Table I. Retention time and USP resolution data for the original HPLC method and the method transferred to UPLC™.

Name	Retention Time (min)	USP Resolution
HPLC Data		
1. Caftaric acid	5.71	
2. Chlorogenic acid	7.07	4.20
3. Cynarin	13.96	21.19
4. Echinacoside	16.54	10.16
5. Cichoic acid	20.32	17.14
UPLC™ Data		
1. Caftaric acid	0.71	
2. Chlorogenic acid	0.87	3.96
3. Cynarin	1.72	22.12
4. Echinacoside	2.06	11.40
5. Cichoic acid	2.44	14.28

Results

The original HPLC method had a cycle time of 41 min and resolution values as listed in Table 1. By using the Waters Reversed-Phase Column Selectivity Chart and the ACQUITY UPLC™ Columns Calculator, we successfully transferred the method to UPLC™. The new method has very similar resolution as listed in Table 1 and a cycle time of only 4.78 min – an 8.5× improvement in cycle time.

Conclusions

For improvements in speed, resolution, and sensitivity, HPLC methods can be successfully transferred to UPLC™ by using the available electronic tools. These separation improvements translate into significant cost reductions in the analytical laboratory.

Waters Corporation
34 Maple Street, Milford, MA 01757
tel. (508) 478-2000, fax (508) 478-1990
www.waters.com