TRANSFERRING EXISTING HPLC METHODS TO UPLC: A SYSTEMATIC APPROACH

Waters

Eric S. Grumbach, Thomas E. Wheat and Jeffrey R. Mazzeo
Waters Corporation, Milford, MA

OVERVIEW

Ultra Performance LC™ is an analytical tool that has resulted from the evolution of column chemistry and instrumentation. This technique can be used to develop new analytical methods, and it also finds utility in improving the speed, sensitivity, and resolution of existing methods. Important considerations for illustrating the transfer of HPLC methods to UPLC are described.

EXPERIMENTAL APPROACH

- To transfer an existing HPLC method to a highly efficient UPLC method, the properties of the instrument, operating conditions and the column chemistry must be controlled and matched.
- A systematic procedure for controlling the variables to simplify the method transfer process is illustrated with a separation of *echinacea purpurea* and related products.
- Transferring an existing HPLC method to UPLC yields a 8.8X increase in throughput.
- The use of new electronic tools, streamlines the process to transfer methods to UPLC

EXPERIMENTAL CONDITIONS

Instruments:
Alliance 2695 Separations Module with 2996 PDA
ACQUITY UPLC™ with ACQUITY PDA

Chromatographic Conditions:
Column: XBridge™ C_{18} 4.6 x 150 mm, 5.0 µm
ACQUITY UPLC™ BEH C_{18} 2.1 x 50 mm, 1.7 µm
Mobile Phase A: 0.1% CF_3COOH in water
Mobile Phase B: 0.08% CF_3COOH in acetonitrile
Flow Rate: 1.0 mL/min (4.6 x 150 mm, 5 µm)
0.5 mL/min (2.1x 50 mm, 1.7 µm)
Gradient Profile: (as indicated)
Injection Volume: 10.0 µL (0.7 µL, 50 mm 1.7 µm scaled)
Sample Conc.: 100 µg/mL in 50% methanol with 0.05% CF_3COOH
Temperature: 40 °C
Detection: UV at 330 nm
Sampling Rate: 5 Hz (20 Hz)
Time Constant: 1.0 (0.1)

Analytes
1. Caftaric acid
2. Chlorgenic acid
3. Cynarin
4. Echinacoside
5. Cichoric acid

STEPS IN METHODS TRANSFER

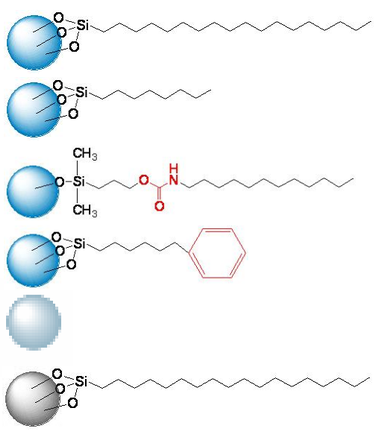
Column Chemistry
- Match column chemistry to provide similar selectivity and retentivity as original HPLC column
- Scale to appropriate column dimensions

Sample Considerations
- Scale injection amount; both mass and volume

Selection of Operating Conditions
- Scale flow rate to column cross-sectional area
- Adjust linear velocity appropriate to particle diameter
- Adjust all gradient segments, including equilibration
  - Express gradient duration in % change per column volume (cv) units
  - Calculate each segment as a number of column volumes
  - Calculate time required to deliver the same number of column volumes to the UPLC column at the chosen flow rate

UPLC COLUMN SELECTION

Different bonded phases provide distinct changes in hydrophobic character, silanol activity and ligand density, all of which can provide unique selectivity and retentivity. Method conversion is streamlined by choosing the UPLC column that most closely matches the selectivity of the original HPLC column



ACQUITY UPLC™ BEH C18
ACQUITY UPLC™ BEH C8
ACQUITY UPLC™ BEH Shield RP18
ACQUITY UPLC™ BEH Phenyl
ACQUITY UPLC™ BEH HILIC
ACQUITY UPLC™ **HSS T3**

SAMPLE CONSIDERATIONS

It is important to scale the injection volume and mass on column, relative to the column dimensions, to preserve chromatographic resolution and sensitivity. Sample diluent and concentration should be held constant.

Target Injection Volume = Original Injection Volume **X** $\frac{\text{Target Column Volume}}{\text{Original Column Volume}}$

Scaling a **10 µL** injection on 4.6x150 mm to 2.1x50 mm

Target Injection Volume = 10 µL x $\frac{3.14 \times 1.1^2 \times 50}{3.14 \times 2.3^2 \times 150}$

Target Injection Volume = 10 µL x $\frac{0.17}{2.49}$

Target Injection Volume = 0.7 µL

SYSTEM CONSIDERATIONS

Differences in volume between LC systems can lead to significantly different retention, selectivity, and gradient formation. Expressing the system volume as column volumes (cv) will determine the length of isocratic hold necessary in the UPLC method to replicate the peak profile of the HPLC method.

System Volume (cv) = $\frac{\text{System Volume}}{\text{Column Volume}}$

HPLC System Volume (cv) = $\frac{1.0 \text{ mL}}{2.49 \text{ mL}}$ **= 0.40 cv**

UPLC System Volume (cv) = $\frac{0.1 \text{ mL}}{0.17 \text{ mL}}$ **= 0.59 cv**

If the difference in system volume is less than 0.25 column volumes, the initial isocratic portion of the UPLC gradient method can be ignored.

HPLC TO UPLC METHOD TRANSFER

When converting a method from HPLC to UPLC, the replacement of both the instrumentation and column chemistry is necessary. Expressing the system volume and gradient segments in terms of column volumes, will reduce and control the number of variables in a method transfer.

Expressing Gradient Segments and System Volume in Terms of Column Volumes

For 30 min at 1.0 mL/min on a 4.6 x 150 mm column

Gradient Volume = Flow Rate x Time **=**
1.0 mL/min x 30 min **=** 30.0 mL

Column Volume = $\pi \times r^2 \times L$ **=**
$3.14 \times 0.23^2 \times 15$ **=** 2.49 mL

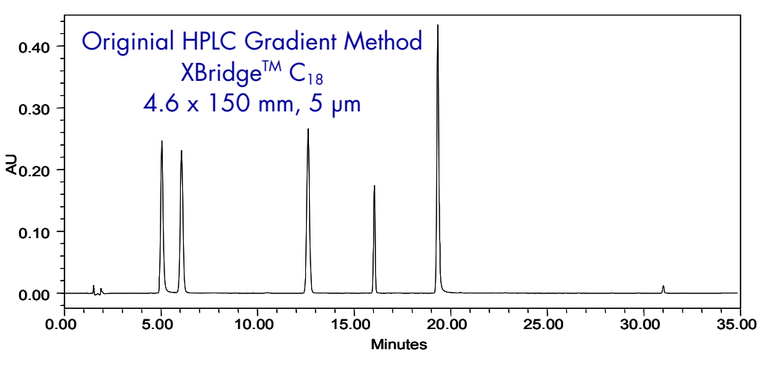
Gradient Duration **(cv) =** $\frac{\text{Gradient Volume}}{\text{Column Volume}}$

Gradient Duration **=** $\frac{30.0 \text{ mL}}{2.49 \text{ mL}}$ **= 12.03 cv**

The original HPLC method was performed on an LC with 1.0 mL system volume. Converting the system volume to column volumes will determine the length of isocratic hold necessary on the UPLC method to replicate the same peak profile.

HPLC system volume = 1.0 mL = 0.40 cv

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (Col. Vol.)
Initial	0	1	92	8			
Hold	2	1	92	8	6	2	0.8
1	32	1	50	50	7	30	12.03
2	35	1	10	90	6	3	1.203
3	36	1	92	8	6	1	0.4
4	41	1	92	8	6	5	2.01



Scaling to UPLC

Transferring a method to UPLC should take advantage of the uniquely useful characteristics of this technique. For scaling considerations, the most important property is the dependence of optimum flow rate or, more exactly, linear velocity, on particle size. Optimum linear velocity depends on the diffusion coefficient of the analytes. For small molecular weight compounds, optimal flow rate on a 2.1 mm i.d. UPLC column will be near 0.6 mL/min

Original Gradient Step 1: 30 min @ 1.0 mL/min is a duration of **12.03 cv**
Calculate Target Gradient Step1: (keeping duration @ **12.03 cv**)

Target Column Volume (2.1 x 50) = 0.17 mL

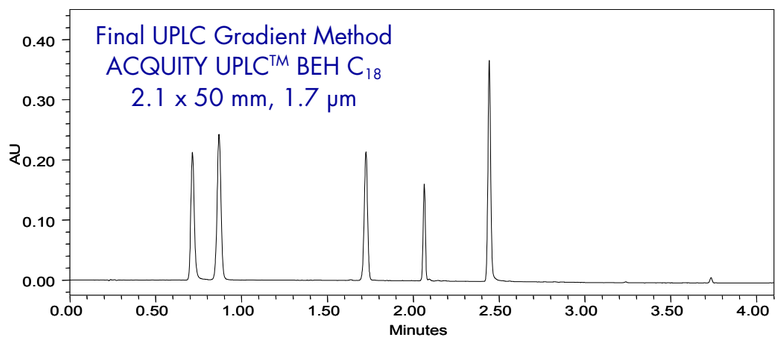
Gradient Step Volume = Duration (cv) x Target Column Volume
= 12.03 cv x 0.17 mL = 2.045 mL

Gradient Step Time = Gradient Step Volume / UPLC™ Flow Rate
= 2.045 mL / 0.613 mL/min. = 3.34 min.

Appropriately Scaled UPLC Method

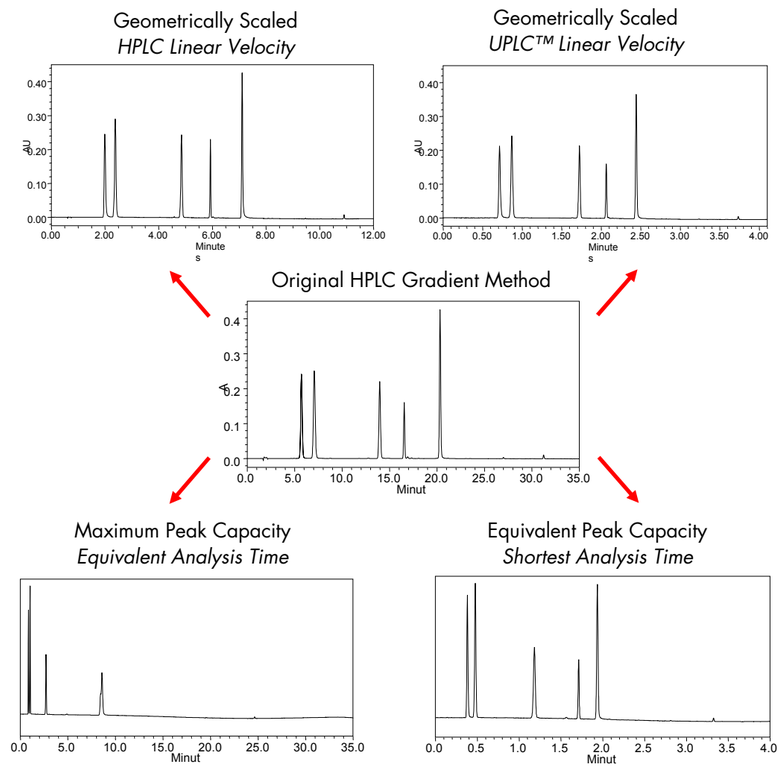
UPLC system volume = 0.1 mL = 0.59 cv

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (Col. Vol.)
Initial	0	0.613	92	8			
Hold	0.23	0.613	92	8	6	0.23	0.8
1	3.63	0.613	50	50	7	3.4	12.03
2	3.97	0.613	10	90	6	0.34	1.203
3	4.08	0.613	92	8	6	0.11	0.4
4	4.65	0.613	92	8	6	0.57	2.01



ELECTRONIC TOOLS

Electronic tools have been developed to assist in method transfer. Geometric scaling of flow rate, injection volume and gradient segments are automated by using The ACQUITY UPLC Columns Calculator that is included in the system software.



CONCLUSIONS

- Successful method transfer from HPLC—to—UPLC can be achieved
- Scale injection volume according to column dimensions
- Express system volume and gradient segments in terms of column volumes
- Speed of analysis improved 8.8X when transferred to UPLC
- The ACQUITY UPLC Columns Calculator can streamline the methods transfer process.
- Attention to detail will lead to successful method transfer

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