DEVELOPING A SYSTEMATIC APPROACH TO METHOD TRANSFER FROM UPLC™ TO PREPARATIVE HPLC

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OVERVIEW

Ultra Performance LC (UPLC™) has been widely accepted by chromatographers because of the improvements over HPLC in the sensitivity, resolution and speed of separations. As scientists begin to use UPLC™ for impurity and metabolite profiling, they will need to transfer the methods to preparative LC to isolate and purify their compounds for further research. Therefore, it is necessary to systematically transfer UPLC™ assays not only to HPLC, but, more importantly, to preparative chromatography. In this presentation, we discuss a systematic approach with key guidelines and observations to expedite the method transfer from UPLC™ to preparative LC. Parameters that need to be considered include column chemistry and dimension, injection volume, temperature, mobile phase composition, flow rate, gradient method, cycle time, detector sensitivity, injector design and system volume. Step by step calculations are included for a better understanding of evaluating method transfer validity. In addition, observations on reducing total run time in preparative LC will be covered.

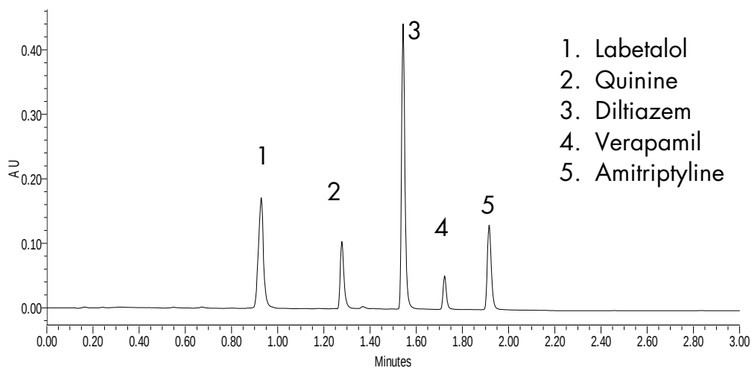
BEH Technology™

Acquity Ultra Performance LC™ XBridge™ COLUMNS

Ease of Migration from HPLC to UPLC™

- ACQUITY UPLC™ columns and XBridge™ columns are both based on BEH Technology™.
- Same column chemistry ensures the ease of scale-up from UPLC™ to preparative LC.

UPLC™ SEPARATION



Column: ACQUITY UPLC™ BEH C_{18}
2.1 x 50 mm, 1.7 µm
Mobile Phase A: 10 mM NH_4HCO_3, pH 10
Mobile Phase B: ACN:100 mM NH_4HCO_3, pH 10 (90:10) v/v
Flow Rate: 1.0 mL/min
Gradient: 2 min gradient from 5% B to 95% B, then hold 95% B for 1 min
Sample Concentration: 380 µg/mL total in water
Injection Volume: 2 µL
Temperature: Ambient
Detection: UV @ 245 nm
Instrument: Waters ACQUITY UPLC™ with PDA

GOALS

- Scale-up the separation from a 2.1 x 50 mm, 1.7 µm UPLC™ column to a 19 × 150 mm, 5 µm preparative column
- Scale-down to a 19 × 50 mm, 5 µm column
- Maximize the loading on the 19 × 50 mm, 5 µm preparative column

SCALING FLOW RATES

ACQUITY UPLC™: 1.0 mL/min
AutoPurification™: 30.6 mL/min

- 1.0 mL/min flow rate in UPLC™ was used for the fastest scouting separation.
- Geometrically scaled flow rate would be 81.9 mL/min on the prep column! But:

$$F_{opt} \propto \frac{1}{dp} \quad \text{Therefore:} \quad \frac{F_{opt\,Prep}}{F_{opt\,UPLC}} \propto \frac{dp_{UPLC}}{dp_{Prep}} = 27.8 \text{ mL/min}$$

- Flow rates of 27.8 ±10% mL/min were run and 30.6 mL/min resulted in the best separation.

SYSTEM VOLUME COMPENSATION

ACQUITY UPLC™: 0.105 mL
AutoPurification™: 2.60 mL

- Differences in system volumes can result in differences in retention times, selectivity and gradient formation.
- An initial hold time may be added to the prep run to compensate for this difference.
- The initial hold time is calculated using the following equation:

$$\left(\frac{SystemVolume_{UPLC}}{ColumnVolume_{UPLC}} - \frac{SystemVolume_{AutoPurification}}{ColumnVolume_{Prep}}\right) \times \frac{ColumnVolume_{Prep}}{FlowRate_{Prep}}$$

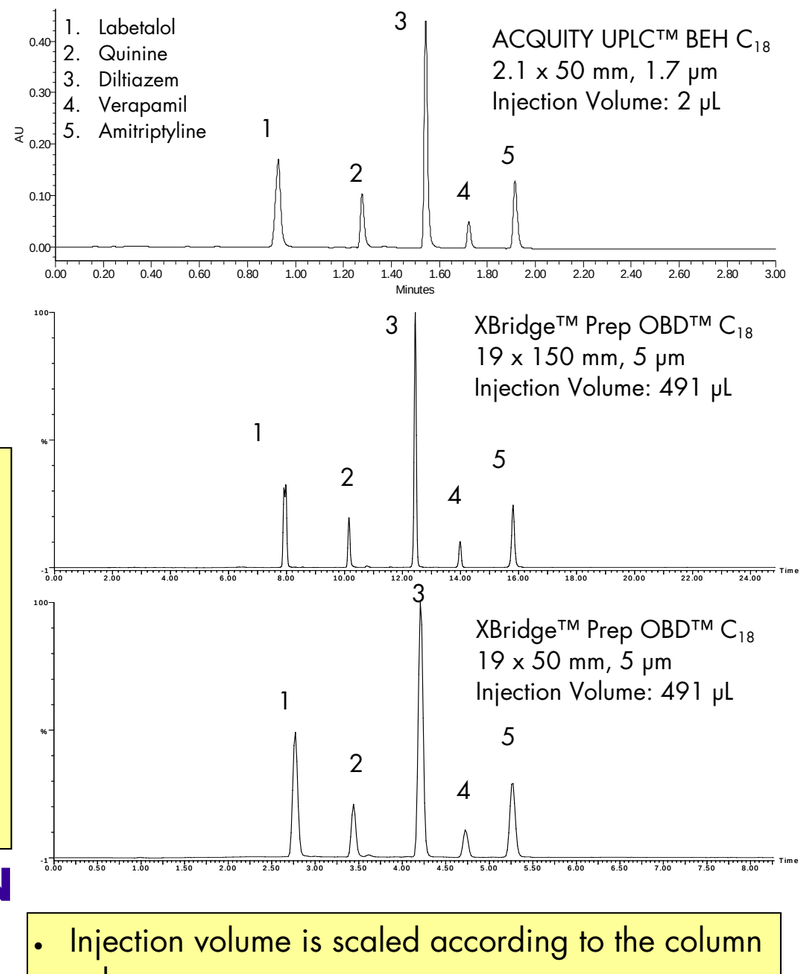
GRADIENT SCALING

- To maintain the same selectivity, segment durations in column volumes (c.v.) need to remain the same when scaling the gradient table from UPLC™ to Prep LC.

Table 1: Gradient Tables for Various Dimension Preparative Columns

	Steps	Time (min)	Flow Rate (mL/min)	%A	%B	Segment Duration Time (min)	Segment Duration (c.v.)
UPLC 2.1×50 mm, 1.7 µm	Initial hold	0	0.6	95	5	0	0
		2	0.6	5	95	2	11.55
		3	0.6	5	95	1	5.78
		3.1	0.6	95	5	0.1	0.58
		5.0	0.6	95	5	1.9	13.87
Prep LC 19×150 mm, 5 µm	Initial hold	0.78	30.6	95	5	0	0
		16.80	30.6	5	95	16.05	11.55
		24.83	30.6	5	95	8.03	5.78
		25.64	30.6	95	5	0.80	0.58
		44.90	30.6	95	5	19.26	13.87
Prep LC 19×50 mm, 5 µm	Initial hold	0.20	30.6	95	5	0	0
		5.55	30.6	5	95	5.35	11.55
		8.22	30.6	5	95	2.68	5.78
		8.49	30.6	95	5	0.27	0.58
		14.91	30.6	95	5	6.42	13.87

SCALING FROM UPLC™ TO PREP



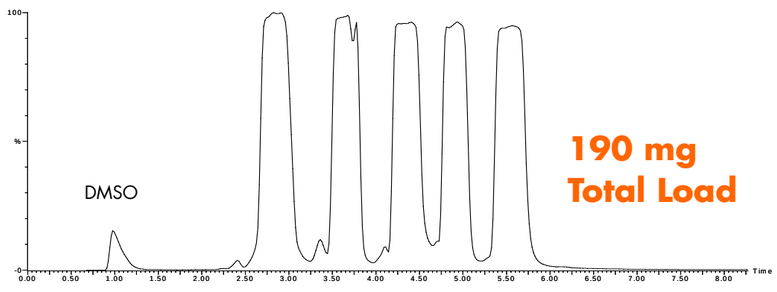
- Injection volume is scaled according to the column volumes

$$\text{Target injection volume} = \text{Original injection volume} \times \frac{\text{Target Column Volume}}{\text{Original Column Volume}}$$

- Excellent scaling is observed from UPLC™ to Prep

MAXIMIZE LOADING

XBridge™ Prep OBD™ C_{18}
19 x 50 mm, 5 µm



Sample Concentration: 190 mg/mL total in DMSO
Injection Volume: 1 mL
Temperature: Ambient
Detection: UV @ 245 nm
Instrument: Waters AutoPurification™ system

CONCLUSIONS

- Guidelines for successful method transfer
  - Scale and optimize the flow rates
  - Calculate initial hold time for Prep based on the two system volumes
  - Maintain the same segment duration (in column volumes) for geometric gradient scaling
  - Scale the injection volume according to the column volumes
  - Maximize loading on the prep column
- By following these simple guidelines, scaling from UPLC™ to Prep LC is readily achieved

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