Seamless Method Transfer from UPLC® Technology to Preparative LC

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Scale-up from UPLC® Technology to preparative LC is readily achieved using Preparative OBD™ columns and typical scaling equations and principles.

UltraPerformance LC® (UPLC®) has been widely accepted by chromatographers because of improvements over HPLC in the sensitivity, resolution, and speed of separations. As scientists begin to use this technology 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 application, we provide information on how to scale a UPLC® impurity/degradant separation to a preparative LC separation.

UPLC® Conditions

Instrument:	ACQUITY UPLC® System with ACQUITY UPLC® PDA
Column:	ACQUITY UPLC® BEH C_{18}, 2.1 × 50 mm, 1.7 μm
Mobile Phase A:	10 mM NH_4HCO_3, pH 10
Mobile Phase B:	ACN
Flow Rate:	0.8 mL/min
Injection Volume:	5 μL (Full loop injection mode)
Sample:	Degraded ranitidine sample (Heated at 85 °C for 48 hr)
Gradient:	As indicated in the chromatogram
Detection:	UV @ 235 nm

Preparative LC Conditions

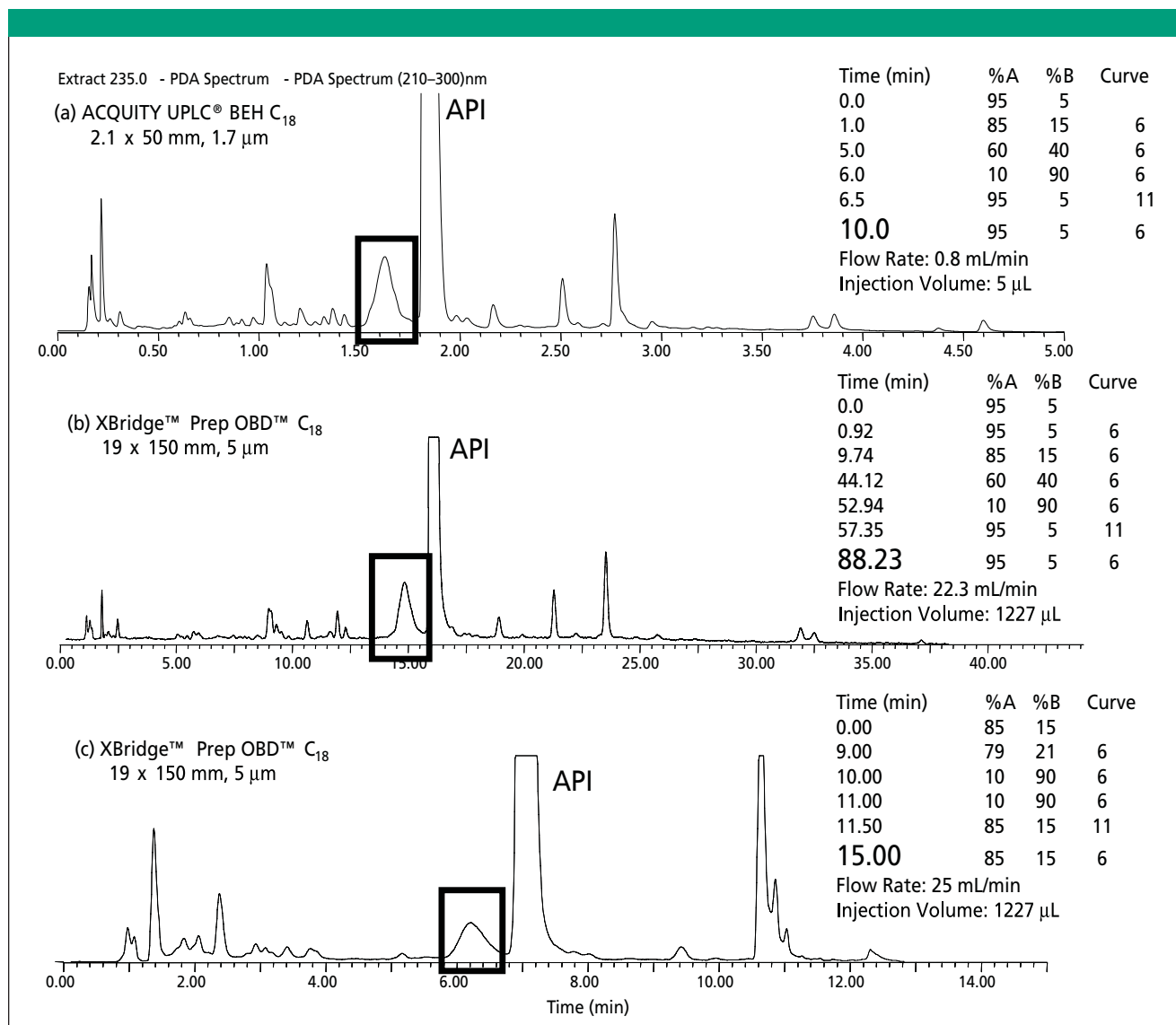|	
Instrument:	Waters AutoPurification™ system with 996 PDA detector
Column:	XBridge™ Prep OBD™ C_{18}, 19 × 150 mm, 5 μm
Mobile Phases:	Same as above
Flow Rate:	22.3 mL/min
Injection Volume:	1227 μL
Sample:	Degraded ranitidine sample (Heated at 85 °C for 48 hr)
Gradient:	As indicated in the chromatograms
Detection:	UV @ 235 nm

Results

The UPLC® separation of the ranitidine degradation sample is shown in Figure 1A. Ranitidine is clearly resolved from all other compounds in the mixture and the entire cycle time is only 10 min. The boxed peak is the analyte that needs to be collected for identification. The separation is first directly scaled to a 19 × 150 mm XBridge™ Prep OBD™ C_{18} column. This column dimension was chosen to maintain the same column length (L) to particle size ratio (dp) as in the UPLC® separation to ensure constant plate count and therefore maintain resolution. The XBridge™ chemistry is built on the same second generation Bridged Ethyl Hybrid (BEH) particle as the ACQUITY UPLC® BEH chemistry; therefore the same selectivity is maintained as we scale separations. As shown in Figure 1B, in order to maintain the selectivity and resolution, the overall cycle time needed to be increased to over 88 min. This long cycle time is not very practical for most separation scientists.

Therefore, modification of the gradient profile is necessary in order to help reduce the cycle time. We started the gradient at a higher % organic, maintained the same gradient slope to separate the component of interest from ranitidine, and quickly ramped to 90% organic to wash all other compounds off the column. The total cycle time has been reduced by 80%. Results are shown in Figure 1C.

Conclusions

UPLC® separations are seamlessly transferred to preparative LC by using traditional scaling principles. Further optimization is easily achieved by simple modification of the gradient profile. The use of the XBridge™ Prep OBD™ columns, built on the same base particle as ACQUITY UPLC® BEH columns, facilitates this transfer.

Extract 235.0   - PDA Spectrum   - PDA Spectrum (210–300)nm

(a) ACQUITY UPLC® BEH C$_{18}$
     2.1 x 50 mm, 1.7 µm

API

Time (min)	%A	%B	Curve
0.0	95	5	
1.0	85	15	6
5.0	60	40	6
6.0	10	90	6
6.5	95	5	11
10.0	95	5	6

Flow Rate: 0.8 mL/min
Injection Volume: 5 µL

(b) XBridge™ Prep OBD™ C$_{18}$
     19 x 150 mm, 5 µm

API

Time (min)	%A	%B	Curve
0.0	95	5	
0.92	95	5	6
9.74	85	15	6
44.12	60	40	6
52.94	10	90	6
57.35	95	5	11
88.23	95	5	6

Flow Rate: 22.3 mL/min
Injection Volume: 1227 µL

(c) XBridge™ Prep OBD™ C$_{18}$
     19 x 150 mm, 5 µm

API

Time (min)	%A	%B	Curve
0.00	85	15	
9.00	79	21	6
10.00	10	90	6
11.00	10	90	6
11.50	85	15	11
15.00	85	15	6

Flow Rate: 25 mL/min
Injection Volume: 1227 µL

Time (min)

Figure 1: Degradant/impurity profiles. A) ACQUITY UPLC® separation. B) Directly scaled preparative LC separation. C) Scaled preparative LC separation using a modified, focused gradient. Boxed peak is the degradant of interest for fraction collection. API is ranitidine.

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