Waters™

Application Note

Selectivity Differences Between Three C_{18} Columns and the Impact Upon Preparative Separations

Jo-Ann M. Jablonski, Kim Haynes, Kathy Lawrence

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates that there are differences between C_{18} columns and illustrates how the selectivity differences between three different C_{18} columns impact preparative separations. Additionally, the use of Optimum Bed Density (OBD™) preparative columns is shown to provide preparative separations that, when scaled up, are highly similar to analytical separations carried out using the same stationary phase.[1]

Benefits

- Different C_{18} stationary phases provide additional selectivity choices (*i.e.*, impacting the resolution and order of peak elution) for separating target compounds.

- OBD Prep columns are packed with uniform column bed density, which effectively reduces prep column failure and increases column lifetime.

- The highly controlled OBD Column packing process ensures preparative columns are of similar bed density to the analytical column of the same chemistry, thus making preparative chromatographic profiles analogous to

the analytical run. Similar chromatography at the analytical and preparative scales simplifies scale-up and compound isolation.

· Predictable scale-up takes the uncertainty out of isolating compounds from complex crude sample mixtures and improves the efficiency of the purification process.

Introduction

Chemists routinely isolate compounds from crude compound mixtures in their quest to develop synthetic intermediates and purified products. Although some purification scientists may routinely screen columns with different stationary phases to find the one which provides the best separation for isolating the target compound, other chemists prefer to simplify the process by using a C_{18} column exclusively. C_{18} stationary phases are often considered universal, but all C_{18} columns are not the same. Stationary phase characteristics and column attributes influence a separation in distinctive ways, therefore particular C_{18} brands may provide different chromatographic results. Once a stationary phase is selected, and before purification proceeds, an analytical HPLC separation of the sample provides a chromatographic profile of the crude product. The isolation of the target compounds is simplified when the preparative chromatography mirrors the analytical run. Following the basic rules of scaling and using OBD Prep columns are instrumental for effectively and predictably scaling the separation and isolating the target compounds with ease.[2]

This study illustrates the differences between three C_{18} stationary phases and shows how, with proper scaling and optimized column packing, preparative chromatography mirrors the analytical result. Peak identification and target compound isolation are simplified when the separation is preserved during scaleup.

Experimental

Sample Description

A sample mixture, which included two acids (benzoic acid, diclofenac), three bases (benzamide, clomipramine, diphenhydramine) and three neutrals (hydrocortisone, estradiol, flavone) was prepared by combining different masses of each of the components in a 20 mL scintillation vial and dissolving in 20 mL of dimethyl sulfoxide

(DMSO). The final sample mixture concentration was 78.5 mg/mL.

LC Conditions

LC system:	Waters™ AutoPurification System
Detection:	2998 Photodiode Array Detector
Columns:	XBridge™ BEH™ C_{18}, 5 µm Column, 4.6 x 50 mm, p/n: 186003113
	XBridge BEH C_{18} OBD Prep Column, 5 µm, 30 x 50 mm, p/n: 186002980
	XSelect™ CSH™ C_{18} Column, 5 µm, 4.6 x 50 mm, p/n: 186005287
	XSelect CSH C_{18} OBD Prep Column, 5 µm, 30 x 50 mm, p/n: 186005423
	Vendor Y C_{18}, 4.6 x 50 mm, 5 µm Column
	Vendor Y C_{18}, 30 x 50 mm, 5 µm Column
Column temperature:	Ambient
Sample temperature:	Ambient
Injection volumes:	Analytical 2 µL; Prep 85.1 µL
Flow rates:	Analytical 0.7 mL/min; Prep 29.8 mL/min
Mobile phase A:	Water with 0.1% Trifluoroacetic acid
Mobile phase B:	Acetonitrile with 0.1% Trifluoroacetic acid

Gradient Table: Analytical Method

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.7	95	5	6
7.14	0.7	5	95	6
8.57	0.7	5	95	6
9.29	0.7	95	5	6
14.29	0.7	95	5	6

Gradient Table: Preparative Method

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	29.8	95	5	6
0.96	29.8	95	5	6
8.10	29.8	5	95	6
9.53	29.8	5	95	6
10.25	29.8	95	5	6
15.25	29.8	95	5	6

Data Management

Chromatography Software: MassLynx™ version 4.2

Application Manager: FractionLynx

Results and Discussion

The isolation and purification of target compounds from crude sample mixtures is often considered challenging because of the perceived uncertainty that accompanies the process of scaling a separation from analytical to

prep. While there are many reasons for the unpredictability that many chemists encounter with scale-up, one of the simplest rules to employ relates to the column chemistry -- always choose the same column chemistry (particle type, ligand bonding) and brand when scaling from analytical to prep. Although C_{18} columns are considered the "universal" chemistry for many separations of molecularly diverse mixtures, all C_{18} columns are not the same. Specific stationary phase attributes such as particle size and type, bonding density, carbon load, surface area, pore volume, pore size, and whether a column is end-capped all impact the chromatographic separation. But understandably, it is common practice to scale up to preparative LC using a larger particle size stationary phase in the preparative column. Consequently, it is unnecessary to utilize the identical particle size of the stationary phase in a preparative column as was used in the scouting or analytical column as long as the two columns have a similar ratio of column length to particle size (L/d_p).

Because purification scientists want preparative chromatograms to look like their analytical profiles, preparative columns packed with the same bed density as their analytical counterparts help to ensure predictable scaleup. OBD columns are produced with a highly controlled process that ensures the column bed is uniform throughout the entire length and width of the column. These strict controls in column packing and manufacturing lead to longer column lifetimes and, ultimately, to improvements in the purification process.

To illustrate the differences between different C_{18} columns, the separations obtained from three unique chemistries were compared at both the analytical and preparative scales. XBridge BEH C_{18}, XSelect CSH C_{18} and a C_{18} column from another vendor were compared under the same experimental conditions. A sample mixture composed of acidic, basic, and neutral compounds was prepared and was used throughout the study.

Figure 1 illustrates the separation of the sample mixture using XBridge BEH C_{18} Columns. Clomipramine, flavone, and diclofenac are well-resolved analytically, even in the slightly overloaded condition (which is acceptable for discerning the maximum sample load on the analytical column), prior to scale up. The separation is maintained at the larger, semi-prep scale.
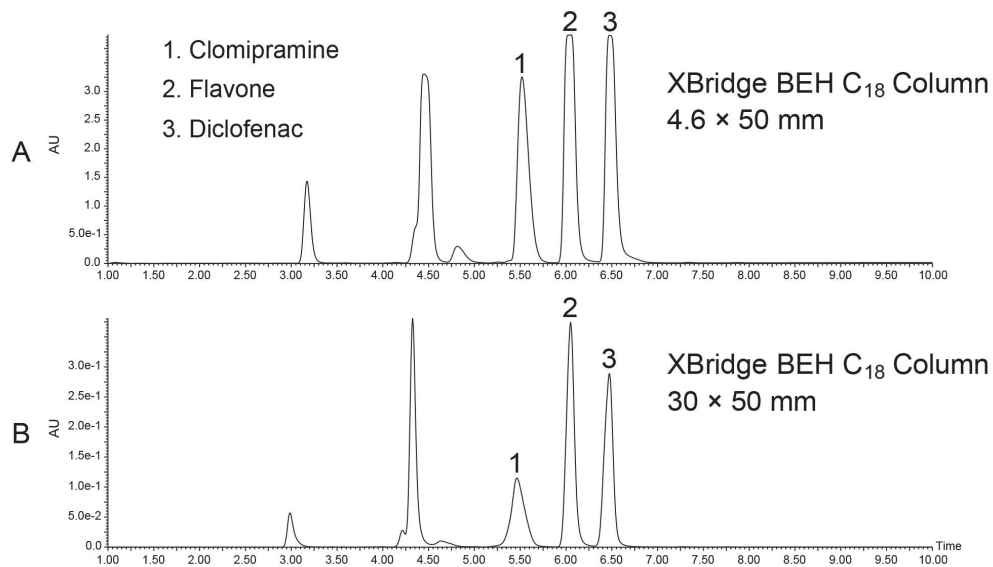
Figure 1. Direct scaleup of the sample mixture on XBridge BEH C$_{18}$ Column.

Figure 2 shows the separation of the sample mixture using XSelect CSH C$_{18}$ Columns. The base, clomipramine, elutes earlier in the chromatogram as compared to the separation obtained using XBridge BEH C$_{18}$ Columns due to the positively charged surface on the CSH particle.[3,4] For the same reason the acid, diclofenac, elutes later in the chromatogram. The peaks of interest are well-resolved and exhibit good peak shape, which will promote straightforward fraction triggering and target compound collection.
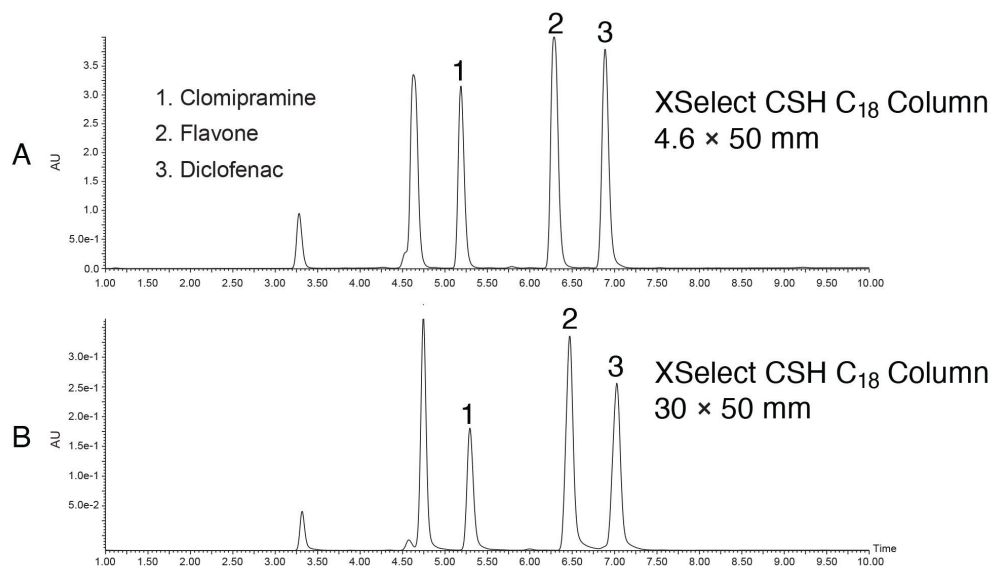
Figure 2. Direct scaleup of the sample mixture on XSelect CSH C_{18} Column.

Figure 3 demonstrates the separation of the sample mixture using C_{18} columns from a different vendor. Although direct scaleup is possible with Vendor Y's C_{18} columns, they are not suitable for separating flavone and diclofenac under these conditions, as shown by the low resolution for these compounds. Clomipramine, though well separated from the other peaks, has a wider peak as compared to the clomipramine peak obtained using the XBridge C_{18} and XSelect C_{18} Columns. A wider peak leads to a larger fraction volume and a longer drying time for the final stages of compound recovery.
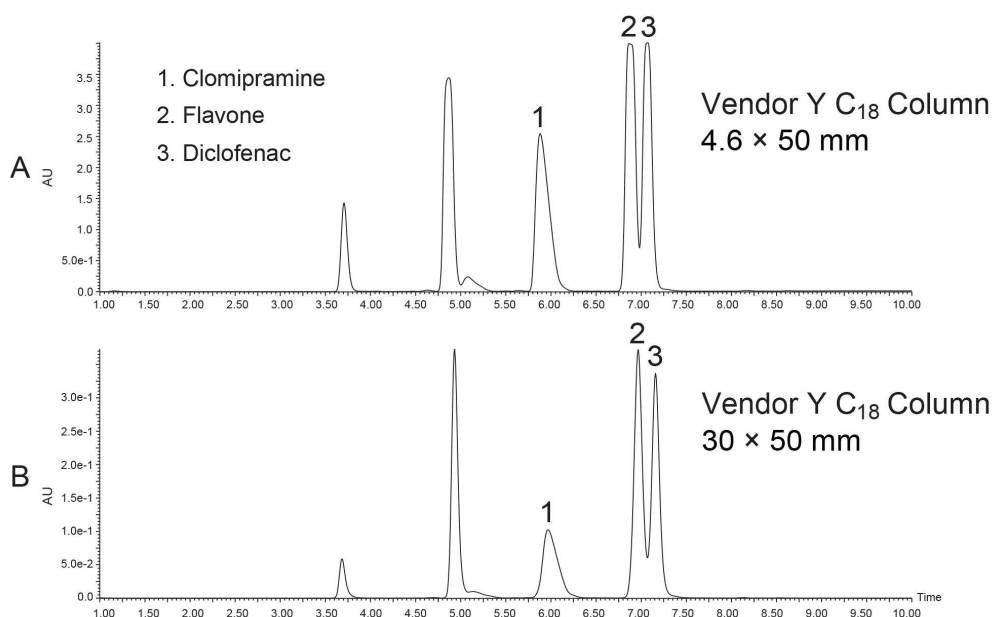
Figure 3. Direct scaleup of the sample mixture on Vendor Y C$_{18}$ Column.

A comparison of the preparative chromatograms from all three columns highlighted in this study indicates that the XSelect CSH C$_{18}$ Column is the best choice for this sample mixture, as shown in Figure 4. The clomipramine peak is narrower on the XSelect CSH C$_{18}$ Column and the flavone and diclofenac are completely resolved. Table 1 shows the peak widths calculated at 5% of the peak height for each of the three target compounds on each of the three preparative columns. The peak widths for flavone and diclofenac are quite similar on all three columns. The base, clomipramine, has a distinctly narrower peak on the CSH column, most likely due to the positively charged surface on the CSH particle.
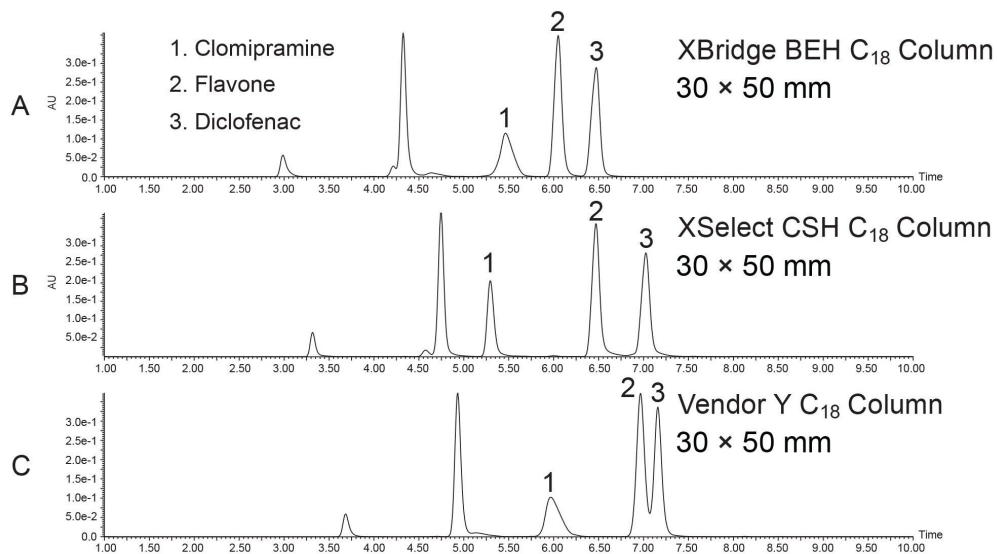
Figure 4. Comparison of the separations obtained on the 30 x 50 mm preparative columns.

Peak width at 5% of peak height (min)			
	BEH C$_{18}$	CSH C$_{18}$	Vendor Y C$_{18}$
Clomipramine	0.36	0.21	0.40
Flavone	0.21	0.22	0.23
Diclofenac	0.22	0.24	0.23

Table 1. Peak width at 5% of Peak Height (min).

Conclusion

Although C$_{18}$ columns are considered "universal", different C$_{18}$ stationary phases exhibit disparate separation profiles and selectivities when compared under the same experimental conditions. Screening several C$_{18}$ stationary phases is recommended to find the best one for isolating a particular target or for diverse and complex sample mixtures. Choosing the stationary phase with the best selectivity for the target compound and using an

OBD preparative column are instrumental for providing predictable scaleup when isolating and purifying target compounds from sample mixtures. OBD preparative columns are engineered for longevity and exceptional performance in the purification laboratory, which ultimately leads to process efficiency.

References

1. Waters Corporation, Topics in Liquid Chromatography, Part 2: Optimum Bed Density [OBD] Columns: Enabling Technology for Laboratory-Scale Isolation and Purification, White Paper, 720001939 < https://www.waters.com/webassets/cms/library/docs/720001939en.pdf> . 2012.

2. Aubin A, Jablonski J. Prep 150 LC System: Considerations for Analytical to Preparative Scaling. www.waters.com <http://www.waters.com/> . Waters Corporation; Waters Application Note. 720005458. 2015.

3. Lucie N, Hana V, Solich P. *Talanta* 2012, *93*, 99.

4. Iraneta P, Wyndham K, McCabe D, Walter T. A Review of Waters Hybrid Particle Technology, Part 3. Charged Surface Hybrid (CSH) Technology and Its Use in Liquid Chromatography, White Paper, 720003929 < https://www.waters.com/webassets/cms/library/docs/720003929en.pdf> 2011.

Featured Products

AutoPurification System <https://www.waters.com/10007147>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

MassLynx MS Software <https://www.waters.com/513662>

FractionLynx <https://www.waters.com/513795>

720008334, May 2024

Terms of Use Privacy Policy Trademarks Careers Legal and Privacy Notices Cookies
Cookie Preferences