Preparative HPLC Scale-Up of Antibiotics

Application

Biochemistry

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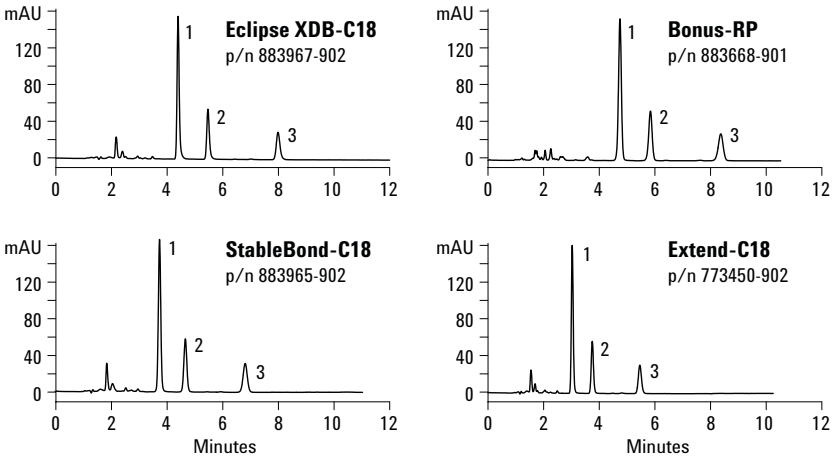
Introduction

Many labs need to purify small amounts of materials for structure elucidation, standards and/or for use in early toxicology studies. Fast, convenient, and cost-effective preparative method development is easily achieved by scaling up from analytical HPLC separation conditions. Plus, exploring different bonded-phase options for optimum peak shape and maximum selectivity is practically investigated on an analytical scale without the major investment in the purchase of preparative columns. In this investigation, the peak shape and separation of three antibiotics–dicloxacillan, cloxacillan and oxacillan–are evaluated on four different ZORBAX bonded-phase selectivities, and the best separation is scaled up in a linear manner to a 21.2 × 150 mm, 5 µm, ZORBAX PrepHT Eclipse XDB-C18 preparative column. When following the suggested easy scale-up procedure below, the preparative separation results provide high yields with excellent throughput and purity.



Conditions

Column size	4.6 × 150-mm, 5-µm
Temperature	Ambient
Flow rate	1 mL/min
Detection	UV (254 nm)
Injection size	10-µL (30-µL) each

Figure 1. Analytical column separations of three antibiotics on four different bonded-phase selectivities.

Highlights

- ZORBAX HPLC columns provide easy scale-up using analytical columns for method development.
- ZORBAX HPLC scalable columns are available in a wide variety of bonded phases for rapid optimization of sample peak shape and separation selectivity.
- ZORBAX scalable analytical and PrepHT columns minimize time and effort in separation scale-up work with resulting excellent yield, purity, and throughput.

Mobile phase

65% 0.1% Trifluroacetic acid (TFA)

35% Acetonitrile (ACN) +0.1% TFA

Peak identification

Peak	Compound
1	Dicloxacillan
2	Cloxacillan
3	Oxacillan

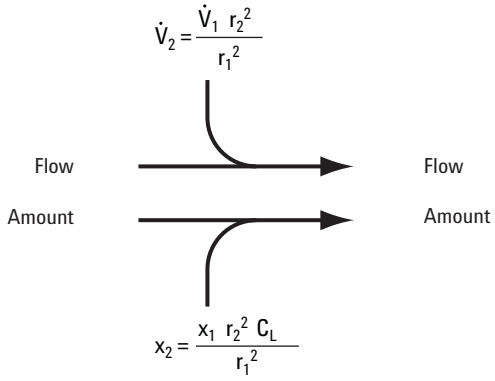
Analtyical Method Development

Figure 1 shows the analytical, low pH, isocratic separation of three antibiotics dicloxacillan, cloxacillan, and oxacillan on four different ZORBAX bonded-phase selectivities: ZORBAX Eclipse XDB-C18, Bonus RP, StableBond-C18, and Extend-C18, each having identical column configurations, 4.6 × 150 mm, 5 µm. Since all four bonded phases provided good resolution and peak shape for the compounds under study, any of these columns would be a good choice for scale-up. For this investigation in particular, the ZORBAX Eclipse XDB-C18 bonded phase, designed for its excellent chromatographic performance at both low and high pH and short separation time, is selected.

General Guidance for Scale-Up Procedure

The first rule that guides an analyst through scaling up an HPLC method developed on an analytical column assumes that the surface chemistry, the bonded phases together with the stationary phase, of the analytical and preparative packing materials are the same. The second rule is that the sample load should be scaled in proportion to the column volume in both mass load and volume load. And finally, for the most efficient scale-up, the analytical and preparative column lengths should be identical, that is, the ratio of the column lengths should be equal to one. Only column diameter between the analytical and preparative columns should change. Using the formulas in Figure 2, you can determine the flow rate and injection volume for your preparative separation, leaving other chromatographic parameters unchanged, and make your injection.

Analytical column 1 **Preparative column 2**



$\dot{V}_1$ = Flow column 1
X_1 = Max. amount column 1
r_1 = Radius column 1*

$\dot{V}_2$ = Flow column 2
X_2 = Max. amount column 2
r_2 = Radius column 2*

*Remember r = d/2 where d is column diameter
C_L = Ratio lengths of columns = 1 (in this case)

Figure 2. Method development scale-up calculations.

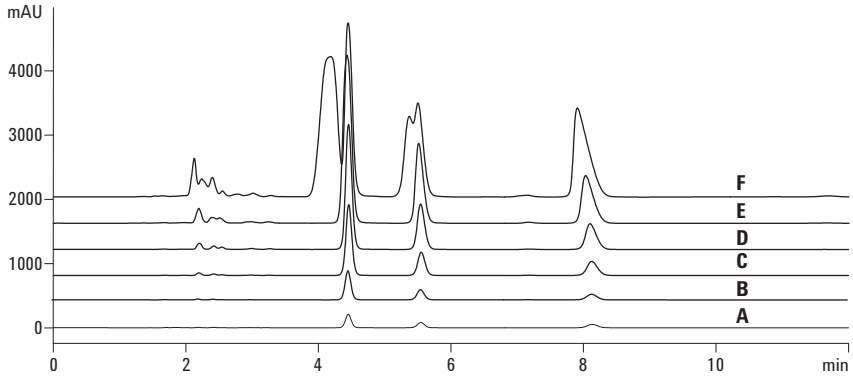
Scale-Up on Analytical Column Dimensions

Scaling up on an analytical size column rather than a preparative column saves sample, solvent, and time. By injecting increasing amounts of sample onto the analytical column, one can quickly determine the point where the overload begins to affect peak shape and resolution. Figure 3 shows the injection of increasing amounts of the three antibiotics onto a 4.6 × 150-mm analytical

column. Note that as the mass increases, there is a point where peak overload begins to occur. At the overload point, peaks begin to become nonsymmetrical and can eventually become very distorted as evidenced by the upper chromatogram of Figure 3. Therefore, when scaling up to a larger column, one may want to scale back somewhat to maintain good peak shape, resolution and peak purity. On the other hand, to improve sample load and throughput, one may want to increase the sample size and actually overload the column until peaks start to overlap and purity is compromised. In Figure 3, there is a nonlinear response of the UV detector signal at the highest sample loadings. Nonlinearity occurs because we are saturating the detector electronics. Even with a nonlinear detector response, we can still perform preparative scale-up.

Preparative Separation

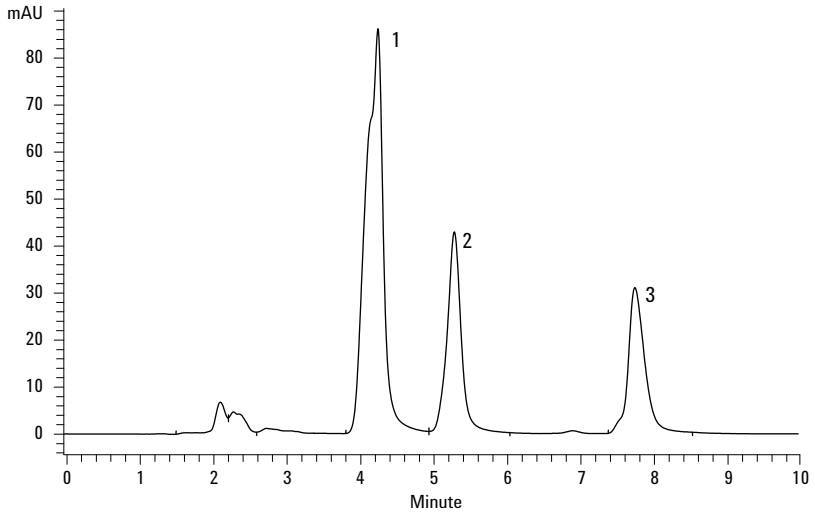
In the case of the preparative scale-up, we used the preparative scale-up factors equivalent to chromatogram E (Figure 3) which was just before overloading occurred on the analytical column. The resulting scaled preparative separation on a 21.2 × 150 mm, 5-µm ZORBAX Eclipse XDB-C18 of the three antibiotics is shown in Figure 4. The flow rate used on this preparative column is 21.2 times faster than the flow rate used on the analytical column and the amount injected was also increased by the same amount, resulting in the identical separation and analysis time of less than 9 minutes.



Conditions

Column size	4.6- × 150-mm, 5-µm
Temperature	Ambient
Flow rate	1 mL/min
Detection	UV (254 nm)
Injection size	30-µL of each mass shown
Sample:	peak 1) dicloxacillan, peak 2) cloxacillan, peak 3) oxacillan
Chromatogram A:	5.1-µg each
Chromatogram B:	11-µg each
Chromatogram C:	24-µg each
Chromatogram D:	51-µg each
Chromatogram E:	102-µg each
Chromatogram F:	253-µg each

Figure 3. Scale-up of three antibiotics on a Zorbax Eclipse XDB-C18 analytical column.



Mobile phase
65% 0.1% TFA
35% ACN +0.1% TFA

Peak identification	
Peak	**Compound**
1	Dicloxacillan
2	Cloxacillan
3	Oxacillan

Conditions	
Columns	**ZORBAX Prep HT Eclipse XDB-C18 21.2 × 150 mm, 5 µm**
Temperature	RT
Flow rate	21.2 mL/min
Detection	UV (254 nm)
Injection size	636-µL (2.2-mg)

Figure 4. High resolution separation of three antibiotics on the ZORBAX PrepHT Eclipse XDB-C18.

Conclusions

This investigation shows that ZORBAX scalable analytical and PrepHT columns can save significant time and effort in separations scale-up. By applying a simple set of scale-up guidelines, one can develop a preparative separation that effectively collects and isolates purified products with excellent yield, purity, and throughput. In addition, ZORBAX columns provide many selectivity options for early optimization of solute peak shape and separation selectivity.

Instrumentation

All analytical work (flow rate: 1 mL/min) was performed using an Agilent 1100 LC equipped with a solvent degasser, binary pump, auto-sampler, heated column compartment, and an ultraviolet detector. All preparative work (flow rate: 21.2 mL/min) was performed on an Agilent purification system.

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