Waters™

5 Rules of Scaling LC Purification

Rule #4: Leverage identical column attributes and similar LDP

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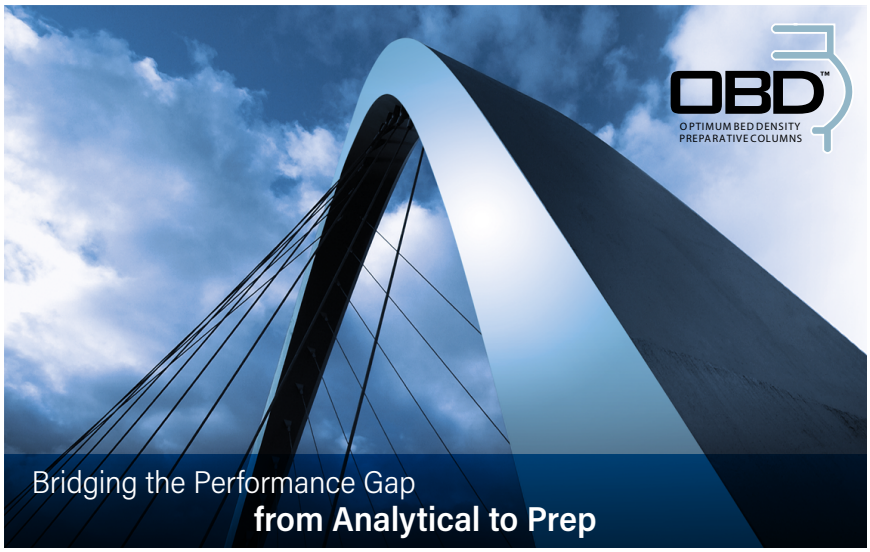
LC Column attributes may include several column specifications that a manufacturer provides for a given column product. These column attributes can be specific particle features, such as base particle type and size, surface area, ligand type and density, carbon load percentage, and the presence or absence of end-capping. Although column producers put a great deal of energy into designing columns with precision to possess defined characteristics, users are often more interested in getting favorable chromatographic results.

Chemists that use LC Columns to determine target purity in mixtures, or to purify compound targets, are focused on results, including the return of a purified target. When evaluating the outcome, chemists want to know two things: whether the target is pure enough and whether there is enough to submit for the next experiment or synthesis step. Therefore, the purification process is merely a means to an end for many. Equally so, the attributes of systems and columns may seem irrelevant until the process leads to a favorable outcome.

Consequently, many chemists employ LC Columns adequate for general use, such as one with a lengthy alkyl chain bonded to a silica base particle. Due to its retention properties, the C_{18} bonded columns are often considered the universal column packing of choice for reversed-phase applications, including reaction monitoring, crude sample analysis, target isolation, and target purity assessment.

Although it is assumed that a C_{18} column will separate a broad spectrum of new molecular entities with simple linear gradient methods, all C_{18} LC Columns are not alike. Since C_{18} columns (whether obtained from various vendors or selected from different brands from the same vendor) reflect different attributes and are manufactured to diverse specifications, performance results will vary.



The disparity in results from different C_{18} columns poses a risk of indirect scaling, and the pain of target loss when scaling-up to PREP, if the chemist is unaware of the variance in column attributes.

Therefore, column attributes affect separations and can impact the success of a separation. To ensure identical scale up to PREP, use the same column brand, particle type, and ligand. Waters manufactures several column brands which have C_{18} bondings, including XBridge™, XSelect™, Sunfire™, Atlantis™, XTerra™, and others. As a leader in particle innovation and in column packing, Waters has LC Column particle types that are quite diverse in terms of their attributes and are specifically designed to be fit-for-purpose and scale.

Particle types include: 1) the bridged ethylene hybrid (BEH) particle technology, 2) the charged surface hybrid (CSH) technology, 3) the high-strength silica (HSS) technology, 4) spherical silica and 5) irregular silica types. Particles for PREP range in size from 2.5 µm to 105 µm and are manufactured for the demands of purification.

To scale-up a separation predictably, use preparative columns with identical attributes, considering all column characteristics in addition to ligand bonding (C_{18}). This practice helps to ensure success when scaling up to isolate targets using liquid chromatography.

Equally important, to maintain resolution when scaling from UPLC™, UHPLC, or analytical HPLC to Prep, keep the L/dp ratio (also known as LDP) the same, or very close in value. In this ratio expression, L is defined as the length of the column, and dp is the diameter of the particle in the column packing (often referred to as "particle size").

The following describe examples of L/dp calculations. If a 1.7 µm size packing is used to perform a target purity assessment on a 2.1 x 50 mm UPLC Column, then the L/dp is 29,412 (see Calculation 1).

If a column with a 5 µm particle size is to be used for PREP, to maintain the resolution in the analytical scouting run used for target purity assessment, using a PREP Column with a 150 mm length will yield a consistent L/dp (see Calculation 2).

Calculation 1 (analytical column)

L/dp = 50mm/1.7 µm

Convert 50 mm to µm: *50 mm x 1000 µm/mm*

L/dp = 50,000 µm/1.7 µm

L/dp = 29,412

Calculation 2 (preparative column)

L/dp = 150mm/5 µm

Convert 150 mm to µm: 150 mm x 1000 µm/mm

L/dp = 150,000 µm/5 µm

L/dp = 30,000

Since the L/dp calculations for the analytical and PREP columns are similar (determined as 29,412 and 30,000, respectively), the separations will be similar. Choose the column diameter based on the amount of crude material to be purified. Use the Estimated Mass Loading Chart as a guide for selecting the most appropriately sized column diameter for the purification. Doing the L/dp math to guide your column and particle size choices will ensure nearly identical resolution when scaling up for purification.

		L/d_p	Column length (mm)						
			20	30	50	75	100	150	250
Particle size (µm)	Fully porous	1.7		17,600	29,400	44,100	58,800	88,200	
		1.8		16,700	27,800	41,700	55,600	83,300	
		2.5	8,000	12,000	20,000	30,000	40,000	60,000	
		3.5	5,700	8,600	14,300	21,400	28,600	42,900	71,400
		5.0	4,000	6,000	10,000	15,000	20,000	30,000	50,000
	Solid-Core*	1.6		25,000	41,700	62,500	83,300	125,000	
		2.7		14,800	24,700	37,000	49,400	74,100	

**L/dp based on the increased efficiency of CORTECS® Solid-Core particles*

Learn more at waters.com/prep, where one can find the downloadable Prep Wall Chart, which includes the column mass loading reference table for reference.

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