Choosing the Right UHPLC Column for Analyzing Strongly Basic Molecules

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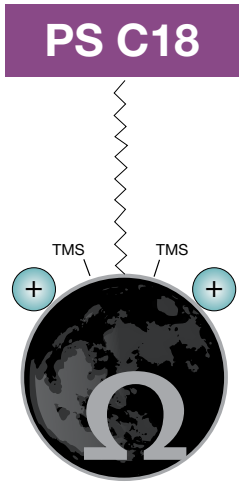
Overview

One of the biggest challenges in reversed phase chromatography has always been maintaining acceptable peak shape for strongly basic analytes. With typical silica-based reversed phase media, it is impossible to completely eliminate unbonded silanol groups, even with extensive end-capping. These residual silanols can interact via ion-exchange mechanisms with the charged basic functional groups on analyte molecules, leading to peak tailing and, in some cases, adsorption that can confound accurate quantitation. These unwanted secondary interactions can be overcome through the use of ion-pairing or ion-masking agents, such as alkyl sulfonic acid additives (e.g. hexane sulfonic acid) or triethylamine (TEA), but these sorts of additives are notoriously finicky and can lead to method robustness problems as subtle alterations in the concentrations of these additives can have dramatic effects on retention, as well as peak shape. Peak tailing can be especially problematic when using typical LC-MS compatible mobile phases, such as 0.1 % formic acid or ammonium formate, as these volatile buffers do not seem to be as effective as UV compatible mobile phases like potassium phosphate or trifluoroacetic acid (TFA) at blocking or minimizing silanol interactions.

To overcome this challenge, we have designed the Luna® Omega 1.6 µm PS C18 phase which contains a proprietary positively charged functional group on the surface, in addition to the standard C18 bonding and trimethylsilane (TMS) end-capping. Although the exact mechanisms are not fully defined, the presence of this positive charge on the surface results in a dramatic improvement in peak shape for basic analytes, even when using weak buffer systems such as 0.1 % formic acid. In the example below, we are attempting to isolate and quantify a small impurity eluting immediately before the peak for the basic antihistamine diphenhydramine (p*K*a ~8.98).

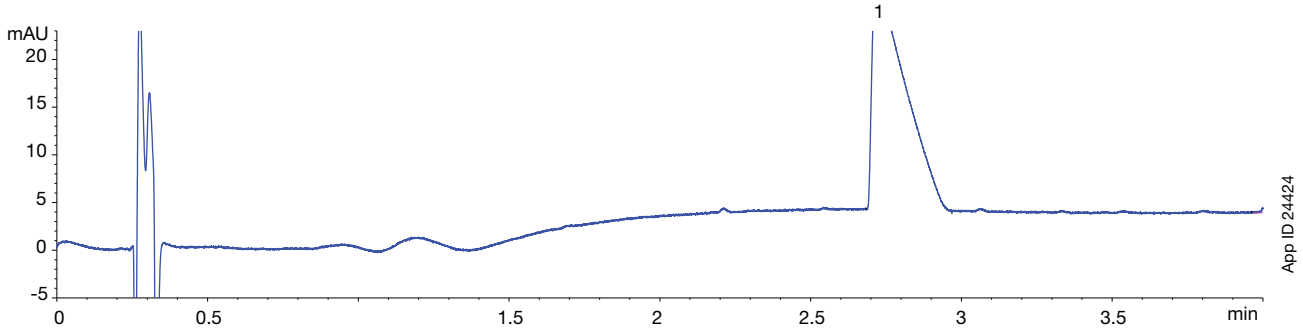
Using an LC-MS compatible mobile phase of 0.1 % formic acid in water and acetonitrile under a simple linear gradient, a hybrid particle C18 UHPLC column (Waters® ACQUITY® BEH 1.7 µm C18; **Figure 1**) displays an exceptionally broad and tailing peak shape, and the early eluting impurity is not observed (either absorbed or buried under the main peak). However, when analyzed using the Luna® Omega 1.6 µm PS C18 column under identical running conditions (**Figure 2**), you can see the vast improvement in peak width and reduced peak tailing, allowing full visualization and separation of the early eluting impurity, marked with the asterisk (*).



Luna Omega PS C18

Unique, 100 % aqueous stable mixed-mode phase that provides both polar and non-polar retention. The surface contains a positive charged ligand which aids in the retention of acidic compounds through ionic interactions, while the C18 ligand promotes general reversed phase retention. The positively charged surface also improves basic compound peaks shape through ionic repulsion.

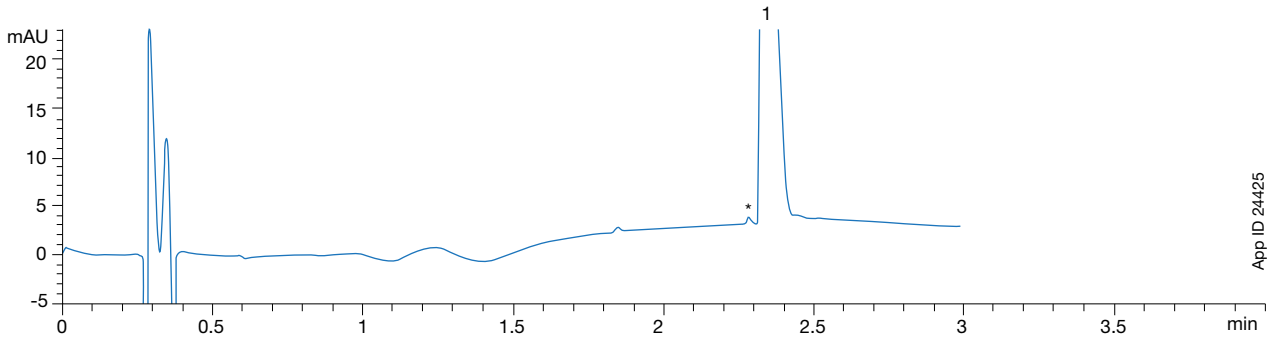
Figure 1.
Waters® ACQUITY® BEH 1.7 µm C18



HPLC Conditions

Column:	As specified
Dimension:	50 x 2.1 mm
Mobile Phase:	A: 0.1 % Formic acid in Water B: 0.1 % Formic acid in Acetonitrile
Gradient:	**Time (min) % B** 0 5 5 95
Flow Rate:	0.5 mL/min
Temperature:	40 °C
Detection:	UV @ 254 nm
Sample:	1. Diphenhydramine

Figure 2.
Luna® Omega PS C18



Phenomenex is not affiliated with Waters Technologies Corporation. Comparative separations may not be representative of all applications.

Luna® Omega Ordering Information

1.6 µm Microbore Columns (mm)			
Phases	**50 x 1.0**	**100 x 1.0**	**150 x 1.0**
Polar C18	00B-4748-A0	00D-4748-A0	00F-4748-A0
C18	00B-4742-A0	00D-4742-A0	00F-4742-A0

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Polar C18	00A-4748-AN	00B-4748-AN	00D-4748-AN	00F-4748-AN	AJ0-9505
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJ0-9502
					for 2.1 mm ID

3 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**4 x 2.0***
Polar C18	00A-4760-AN	00B-4760-AN	00D-4760-AN	00F-4760-AN	AJ0-7600
PS C18	00A-4758-AN	00B-4758-AN	00D-4758-AN	00F-4758-AN	AJ0-7605
				for ID:	2.0 - 3.0 mm

3 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
Polar C18	00B-4760-Y0	00D-4760-Y0	00F-4760-Y0	AJ0-7600
PS C18	00B-4758-Y0	00D-4758-Y0	00F-4758-Y0	AJ0-7605
			for ID:	2.0 - 3.0 mm

3 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0***
Polar C18	00B-4760-E0	00D-4760-E0	00F-4760-E0	00G-4760-E0	AJ0-7601
PS C18	00B-4758-E0	00D-4758-E0	00F-4758-E0	00G-4758-E0	AJ0-7606
				for ID:	3.2-8.0 mm

5 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**30 x 2.1**	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**4 x 2.0***
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	AJ0-7600
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJ0-7605
				for ID:	2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0***
Polar C18	00B-4754-Y0	00D-4754-Y0	00F-4754-Y0	AJ0-7600
PS C18	00B-4753-Y0	00D-4753-Y0	00F-4753-Y0	AJ0-7605
			for ID:	2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 3.0***
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
				for ID:	3.2-8.0 mm

5 µm Axia™ Packed Preparative Columns (mm)			SecurityGuard Cartridges (mm)
Phases	**150 x 21.2**	**250 x 21.2**	**15 x 21.2****
Polar C18	00F-4754-P0-AX	00G-4754-P0-AX	AJ0-7603
PS C18	00F-4753-P0-AX	00G-4753-P0-AX	AJ0-7608
			for ID: 18-29 mm

5 µm Axia™ Packed Preparative Columns (mm)				SecurityGuard Cartridges (mm)
Phases	**150 x 30**	**250 x 30**	**250 x 50**	**15 x 30.0♦**
Polar C18	00F-4754-U0-AX	00G-4754-U0-AX	00G-4754-V0-AX	AJ0-7604
PS C18	00F-4753-U0-AX	00G-4753-U0-AX	00G-4753-V0-AX	AJ0-7609
				for ID: 30-49 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
♦ PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277

guarantee

If Luna analytical columns do not provide at least an equivalent separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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