Agilent PL-SCX Cation-Exchange Media for Large Biomolecules

Technical Overview

Introduction

Agilent PL-SCX is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation and purification of a wide range of biomolecules, from small peptides to large proteins. The examples shown here demonstrate the capability of the media in the separation of proteins under harsh eluent conditions and the various column clean-up procedures that are used.

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Protein standards on PL-SCX

For globular protein analysis and purification, PL-SCX 1000Å material has the optimum pore size for maximum loading with low band broadening. Proteins that have a positive charge at the pH used for the separation will be retained by PL-SCX. The more basic the protein, such as lysozyme, the longer the elution time from the column under a typical NaCl gradient, as shown in Figure 1.

Conditions

Column: PL-SCX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1545-1802)
Eluent A: 0.02 M KH_2PO_4, pH 6
Eluent B: A + 0.5 M NaCl, pH 6
Gradient: Linear 0-100% B in 20 min
Detection: UV, 280 nm

Peak Identification

1. Myoglobin
2. α-chymotrypsinogen A
3. Cytochrome C
4. Lysozyme

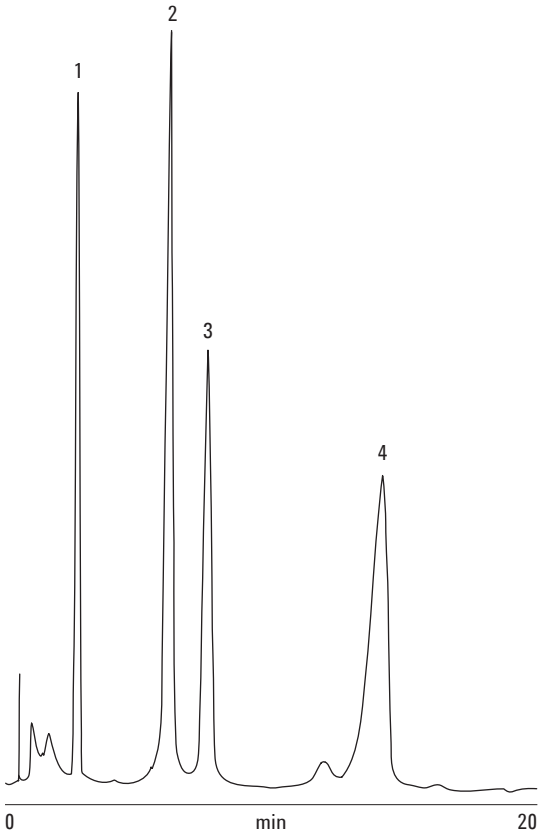

Figure 1. Raw data chromatogram of a protein on Agilent PL-SCX.

Chemical stability of PL-SCX

PL-SCX material is extremely stable when exposed to high pressure, strong acids and strong alkalis. The packing offers gradient elution with pH 1 to 14, 8 M buffer/salt concentration (limited only by solubility), 0 to 100% polar organics and 3000 psi (200 bar) pressures. PL-SCX adsorbent is stable to washes in acid, alkali, methanol and urea. A final wash in 0.2 to 0.5 M sodium chloride solution provides the sodium counter ion and restores full chromatographic performance, as shown in Figures 2 and 3, and Table 1.

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Table 1. Comparing protein separations on an Agilent PL-SCX column before and after washing with strong acids, alkalis and organics.

Wash solution (approx 40 column volumes)	R_2 Factor (peaks 2 and 3)	Protein capacity (mg lysozyme/mL CV)
Initial run	1.5	33
0.2 M HCl	1.7	31
0.2 M NaOH	2.0	33
Final run		
6 M Urea	1.6	33
1% TFA	1.6	31
10% Acetic acid	1.7	28
100% Methanol	1.6	31
0.5 M HCl	1.5	31
2 M NaOH	2.0	33

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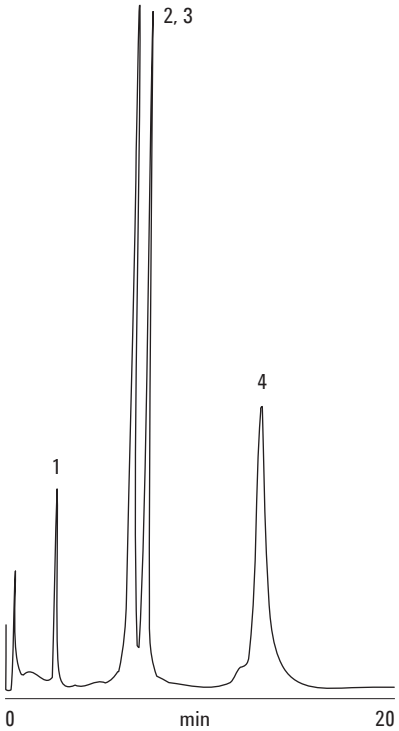

Figure 2. Initial chromatogram of a protein separation on Agilent PL-SCX.

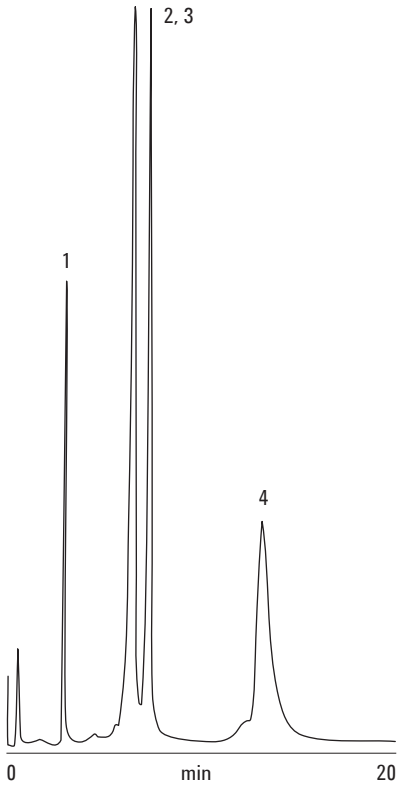

Figure 3. Final chromatogram of the same protein solution on the same column after washing with 40 column volumes of strong eluents.

Agilent PL-SCX Strong Cation-Exchange Columns

The very hydrophilic coating and strong cation-exchange functionality of the PS/DVB packing in PL-SCX delivers the optimum density of strong cation-exchange moieties required to analyze, separate and purify many biomolecules, from small peptides to large proteins. Two pore sizes are available, 1000Å and 4000Å, to provide good mass transfer characteristics for a range of solute sizes. The 5 µm media delivers separations at higher resolution with the 10 and 30 µm media used for medium and low pressure liquid chromatography.

These data represent typical results. For further information, contact your local Agilent Sales Office.

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