SeQuant

SeQuant AB – Innovators in Chemical Analysis™

APPLICATION NOTE 2700-01A

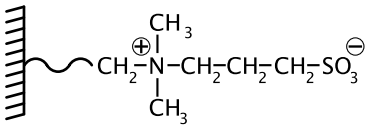
ZIC™ – HILIC

SEPARATION OF A TRYPTIC DIGEST OF CYTOCHROME C

ZIC™-HILIC takes advantage of weak electrostatic interactions between charged analytes and the zwitterionic stationary phase combined with the high efficiency and selectivity of hydrophilic interaction chromatography (HILIC). The ZIC™-HILIC column is suitable for analytes that are poorly retained on reversed phase columns,or as a tool to change selectivity and improve peak resolution. ZIC™-HILIC can be used for peptides, carbohydrates, proteins or digests, and various polar compounds.

INTRODUCTION

The ZIC™-Si stationary phase has covalently attached,highly polar zwitterionic functional groups of sulfobetaine type and is suitable for Zwitterion Chromatography (ZIC™)using aqueous eluents,or as a separation material for hydrophilic interaction chromatography (HILIC).

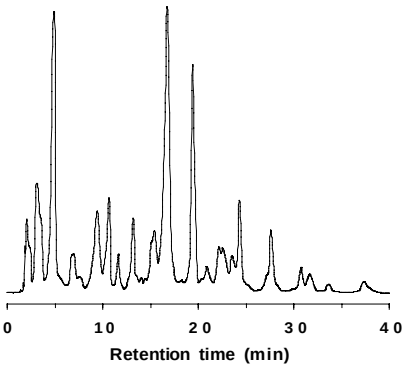


SEPARATION OF PEPTIDES USING A ZIC™-HILIC COLUMN

When operating the ZIC™-HILIC column,an eluent with high concentration of organic solvent (typically 80–85 % acetonitrile) is used to promote hydrophilic interactions between the analyte and the zwitterionic stationary phase. Elution can be accomplished using isocratic conditions,or by running a decreasing acetonitrile gradient.With the ZIC™-HILIC column,relatively low ionic strengths (10 –20 mM) are typically required in order to elute even highly charged peptides. Phosphate buffers are suitable for UV detection at 214 nm, while completely volatile buffers might be preferred in combination with mass spectrometric detection. The selectivity can also be changed by varying the pH of the buffer. The retention of peptides on ZIC™-HILIC columns generally increases with peptide hydrophilicity and with positive charge (presence of basic groups in the peptide side chain). Positive charges in the peptide side chain affect the retention both by increasing the general peptide hydrophilicity (HILIC retentivity)and by increasing the electrostatic interactions (ZIC™ retentivity).

RESULTS & DISCUSSION

The tryptic digest chromatographic map is presented to the right. According to the inherent general mechanism the retention of the peptides increases with their hydrophilicity,and positive charges that affect the retention both by increasing the hydrophilicity (HILIC retentivity) and the electrostatic interactions (ZIC™ retentivity).



Separation of a tryptic digest using gradient elution and a SeQuant ZIC™-HILIC column.

EXPERIMENTAL

Reagents and Materials Cytochrome c (from horse heart)was purchased from Sigma (St.Louis,MO),while the trypsin solution (0.25 %w/v)was obtained from GIBCO Invitrogen (Carlsbad,CA).Water used for eluent preparation was purified using a Milli-Q system (Millipore,MA)while all salts were of analytical grade.Acetonitrile (HPLC-grade)was from J.T.Baker (Deventer,The Netherlands).

Tryptic digestion of cytochrome c was achieved by dissolving 25 mg protein in 2.5 mL 0.1 M ammonium hydrogen carbonate buffer. Then 200 µL trypsin solution (0.25 %)was added to the protein solution and the digestion was allowed to proceed at 37 °C for three hours.The digestion was quenched by immediately freezing 500 µL aliquots of the digest in Eppendorf tubes.The digest was diluted (1:1) with acetonitrile before injection into the chromatographic system.

The chromatographic system comprised two compact pumps,a central processor,and a Lambda 1010 UV detector from Bischoff (Leonberg,Germany).The samples were injected through a 20 µL PEEK loop in a Rheodyne (Coati,CA)injector,and the UV detection was carried out at 214 nm.

The linear gradient was ranging from 0 % to 50 % of eluent B in 40 minutes at a flow rate of 0.8 mL/min.

Eluent A: 81 %(v/v)ACN and 19 %(v/v)15 mM KH_2PO_4 buffer,pH 4.5;

Eluent B: 30 %(v/v) ACN and 70 % (v/v)20 mM KH_2PO_4 buffer,pH 4.5.

The separation was achieved by a SeQuant ZIC ™ - HILIC, 5 µm,150 x 3 mm column.

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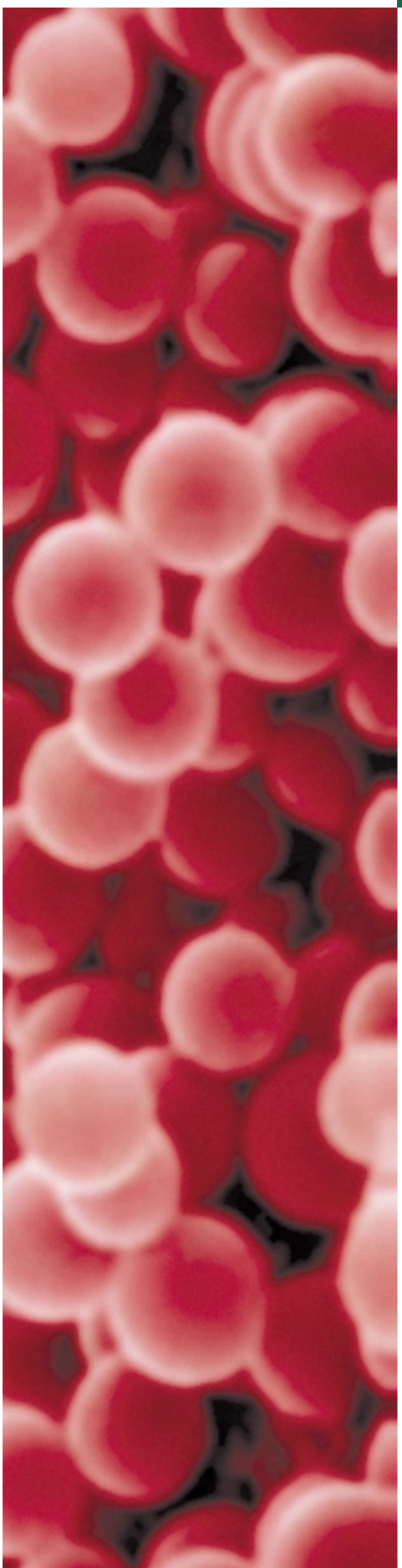

LITERATURE ON HILIC

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ORDERING INFORMATION

The SeQuant ZIC™-HILIC columns and frits are made from PEEK.This product is available with 5 µm or 10 µm particle size of porous silica.Other options available upon request. Solid phase extraction (SPE) syringes are available with ZIC™ – HILIC selectivity.

Product P/N	Length *mm*	ID *mm*	Particle size *µm*	Porosity Å	Price
Q2712-052	50	2.1	5	200	$774
Q2712-055	50	4.6	5	200	$774
Q2712-058	50	7.5	5	200	$878
Q2712-102	100	2.1	5	200	$878
Q2712-105	100	4.6	5	200	$878
Q2712-108	100	7.5	5	200	$1,034
Q2712-152	150	2.1	5	200	$976
Q2712-155	150	4.6	5	200	$976
Q2712-158	150	7.5	5	200	$1,268
Q2712-252	250	2.1	5	200	$1,164
Q2712-255	250	4.6	5	200	$1,164
Q2712-258	250	7.5	5	200	$1,982

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