

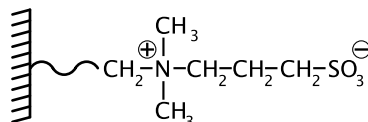
## ZIC™ – HILIC

### SEPARATION OF CATIONIC AND HYDROPHILIC PEPTIDES

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).



In contrast to mixed-mode cation-exchange HILIC applications, the zwitterionic stationary phase can provide a peptide selectivity benefiting from both hydrophilic- and electrostatic interactions, while maintaining a low eluent ionic strength.

The cationic and hydrophilic peptides studied are presented in the table below.

Model Peptide	Physical Properties		
	sequence	pI	Mw
Val-Gly-Ser-Glu	VGSE	4.6	390.4
Arg-Gly-Glu-Ser	RGES	6.0	447.4
Gly-Arg-Gly-Asp	GRGD	6.1	403.4
Angiotensin II	DRVYIHPF	6.7	1046.2
Gly-His-Lys	GHK	8.8	340.4
Bradykinin	RPPGFSPFR	12.0	1060.2

### RESULTS & DISCUSSION

The separation of three cationic model peptides (Angiotensin II, Bradykinin and the tripeptide Gly-His-Lys) is presented in Figure 2. The retention of the peptides increases with their hydrophobicity, and with positive charges that affect the retention both by increasing the hydrophobicity and the electrostatic interactions with the zwitterionic stationary phase. In HILIC water is the strong component of the mobile phase and the ionic strength can be modulated during the gradient to control the peak shape

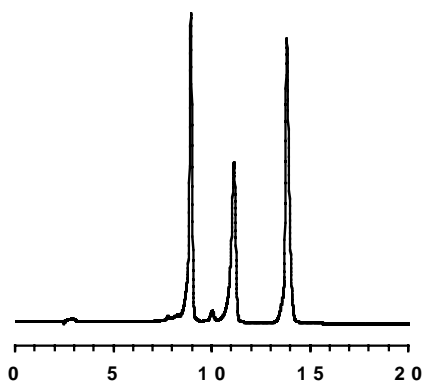


Figure 2. Separation of the cationic peptides Angiotensin II, Bradykinin and Gly-His-Lys (in elution order) on the SeQuant ZIC™-HILIC column using a gradient with increasing content of water and ion strength.

### EXPERIMENTAL

**Reagents and Materials** All peptides used were purchased from Sigma (St. Louis, MO). 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).

**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 flow rate was 0.8 mL/min in all experiments and a SeQuant ZIC™-HILIC, 5 µm, 150 x 4.6 mm column and a standard reversed phase column with identical dimensions were used.

For the separation of cationic peptides the linear gradient was 10 % to 100 % of eluent B in 30 minutes.

**Eluent A:** 85 % (v/v) ACN and 15 % (v/v) 10 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

**Eluent B:** 100 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

In the comparison between ZIC™-HILIC and reversed phase separation the linear gradient was 10 % to 40 % of eluent B in 25 minutes.

**Eluent A:** 85 % (v/v) ACN and 15 % (v/v) 10 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

**Eluent B:** 20 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

The experiments with reversed phase conditions was carried out using the linear gradient 0 % to 60 % of eluent B in 25 minutes. Experiments were carried out at pH 4.5.

**Eluent A:** 20 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

**Eluent B:** 85 % (v/v) ACN and 15 % (v/v) 10 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 4.5

and to some extent the retention. Acetate or formic acid buffers can be utilised when volatile constituents are required, while in these experiments phosphoric buffer was used due to its higher compatibility with UV-detection.

**Peptides poorly retained** under reversed phase conditions may be well retained and resolved by using ZIC™-HILIC conditions. Three model peptides were run in a reversed phase system and all of them eluted near the void volume although the initial eluent was just a buffer.

While using the orthogonal selectivity of the ZIC™-HILIC system all three peptides were nicely retained and resolved, as seen in Figure 3.

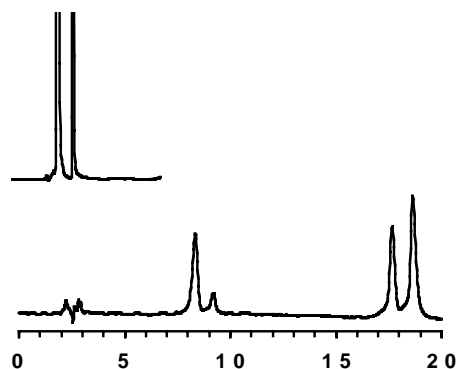


Figure 3. Comparison between a reversed phase separation (upper chromatograms) and a ZIC™-HILIC separation of three hydrophilic peptides (lower chromatogram). The elution order of the peaks in the latter is *Val-Gly-Ser-Glu*, *impurity*, *Arg-Gly-Glu-Ser*, and *Gly-Arg-Gly-Asp*.

## 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

Your local dealer of SeQuant columns: <http://www.nestgrp.com>

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