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Zwitterion Chromatography – ZIC™

A novel technique, with unique selectivity, suitable for preparative scale separations?

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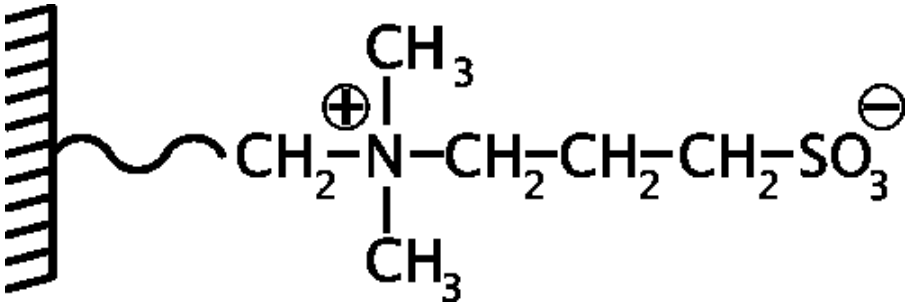
What is Zwitterion Chromatography?



Our definition:

Liquid chromatography on a stationary phase having covalently bound zwitterionic functional groups.

The ZIC™ stationary phase

- Balanced stoichiometry and a zero net charge
- Weak electrostatic interactions with charged analytes
- Separation may be performed in totally aqueous buffers
- High recovery – absence of hydrophobic interactions
- Selectivity benefit by charge and hydrophilicity
- Suitable for *Hydrophilic Interaction Chromatography*
  → ZIC™-HILIC Columns

The ZIC™-HILIC stationary phase

- Permanent zwitterion in the practical pH range
- Stoichiometric molar balance sulfur:nitrogen (~1:1)
- Spherical porous silica particles (typically 5 µm, 200Å)
- Carbon content; ~10 %

Hydrophilic Interaction Chromatography (HILIC)

Isocratic or gradient elution using a mobile phase having an initial high content (>70%) of acetonitrile or low alcohols on a hydrophilic stationary phase, e.g., anion exchangers.

How do I use these columns?

The ZIC™ mode

- Separation by weak electrostatic interactions in aqueous eluents without organic solvents.
- Separation of proteins using gradient elution and typically lower electrolyte concentration than for traditional ion-exchange phases.
- Protein selectivity comparable to cation-exchange chromatography.

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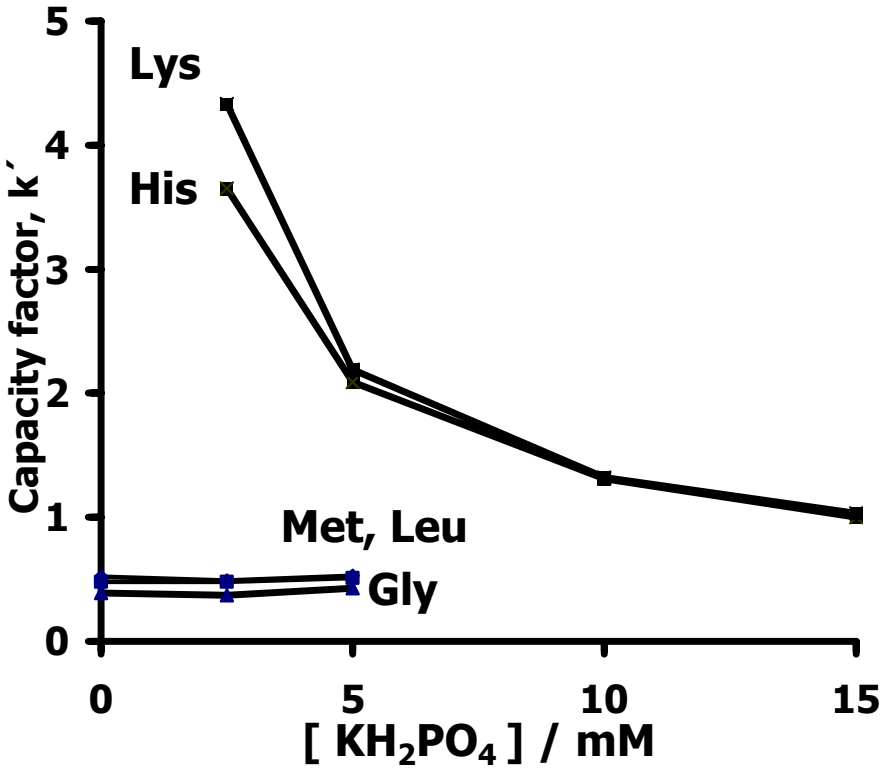
Amino acid retention in ZIC™ mode



- Low retention of AA with hydrophobic side groups (Leu)
- Low retention of hydrophilic AA without charged side groups (Gly, Met)
- Retention of basic AA due to electrostatic interaction with the zwitterionic group (Lys, His)

How do I use these columns?

The ZIC™-HILIC mode

- An initially high content of organic solvent (e.g., alcohol, acetonitrile) in the eluent. Gradient elution by increasing the content of water and eventually raising electrolyte concentration. Volatile buffers may be used.
- Unique selectivity from hydrophilic interactions superimposed onto *weak* electrostatic interactions.
- Suitable for peptides, carbohydrates, natural products, plant hormones, amino acids, and numerous more or less polar analytes.

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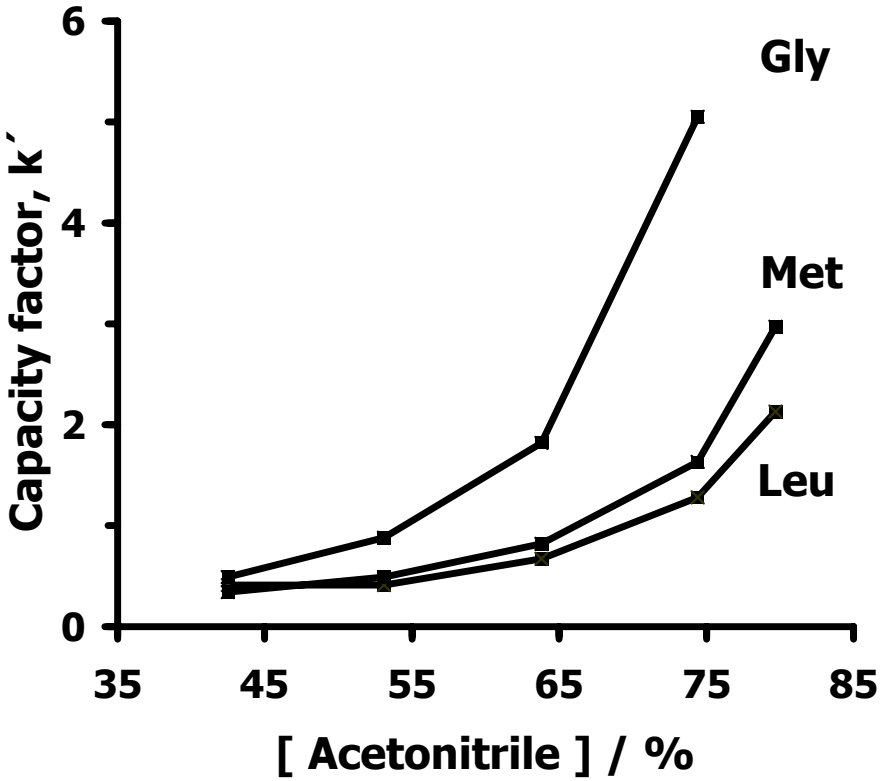
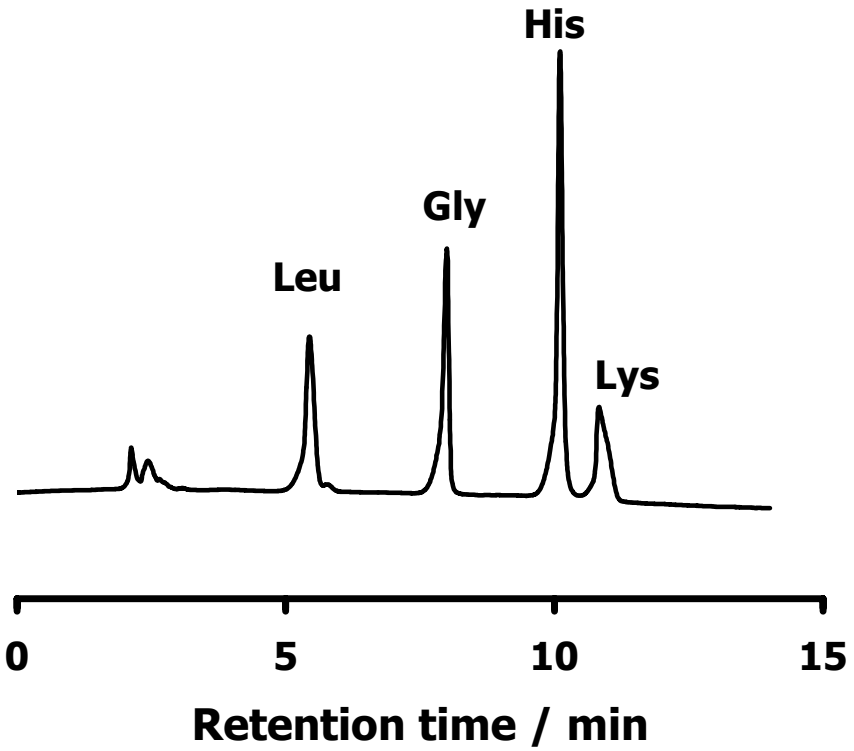
Amino acid retention in ZIC™-HILIC mode



- Retention of AA increases with the concentration of acetonitrile in the eluent
- Retention increases with AA hydrophilicity
  Gly > Met > Leu

ZIC™-HILIC separation of amino acids



Retention determined by:

- Hydrophilicity
- Charge

Linear gradient 4 %/min of Eluent B

Eluent A: 85 %(v/v) acetonitrile, 15 %(v/v) 10 mM KH_2PO_4 pH 4.5

Eluent B: 40 mM KH_2PO_4 pH 4.5

Flow rate: 0.8 mL/min, UV detection 214 nm
Injection volume: 20 µL (2.5 mg/mL each of gly, leu and lys; 0.15 mg/mL His).

ZIC™-HILIC column (4.6×150 mm, 200Å, 5 µm)

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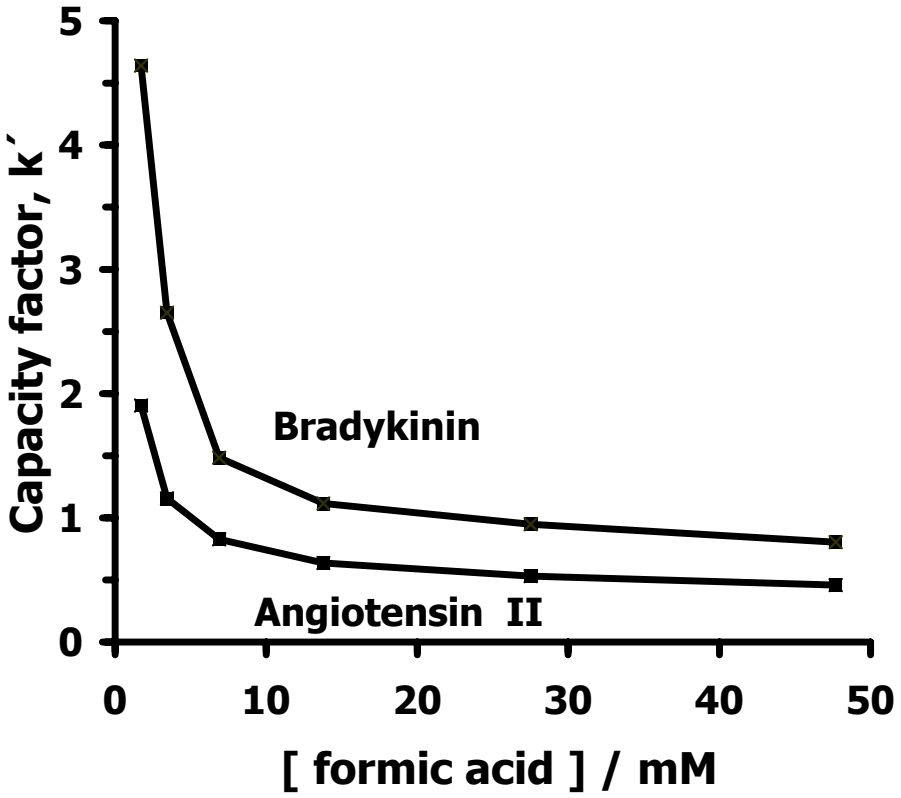
ZIC™ separation of peptides



Isocratic separation by:

- Charge

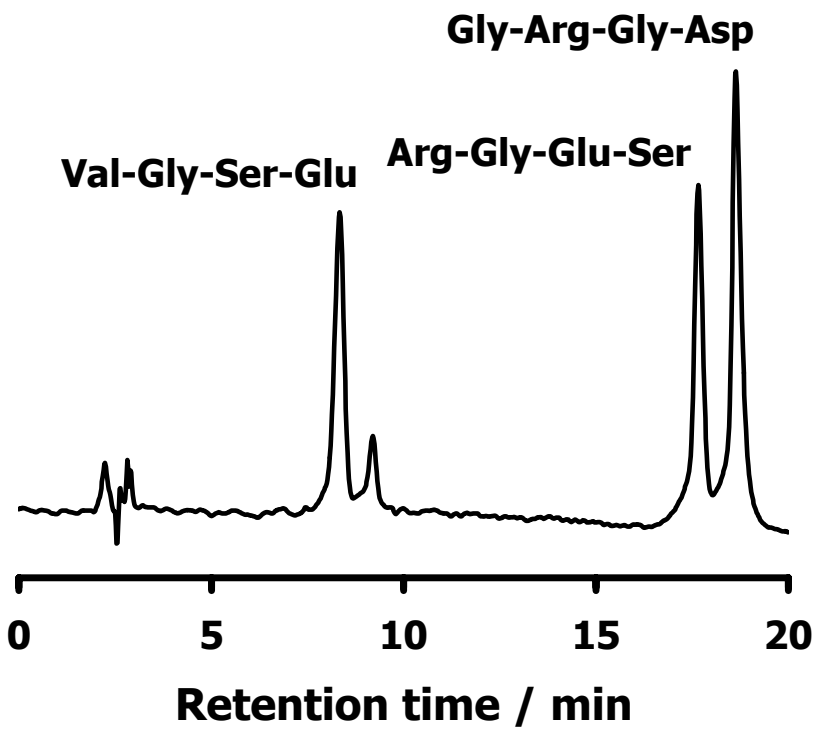
Bradykinin:
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Angiotensin II:
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

Eluent: formic acid at pH 2.6

Flow rate: 0.8 mL/min, UV detection 214 nm
Injection volume: 20 µL.

ZIC™-Si column (3 × 150 mm, 200Å, 10 µm).

Separation of peptides by ZIC™-HILIC



Retention determined by:

- Hydrophilicity
- Charge

Linear gradient 10-40 % of Eluent B in 25 minutes

Eluent A: 85 %(v/v) acetonitrile, 15 %(v/v) 10 mM KH_2PO_4 pH 4.5

Eluent B: 20 mM KH_2PO_4 pH 4.5

Flow rate: 0.8 mL/min, UV detection 214 nm
Injection volume: 20 µL

ZIC™-HILIC column (4.6×150 mm, 5 µm)

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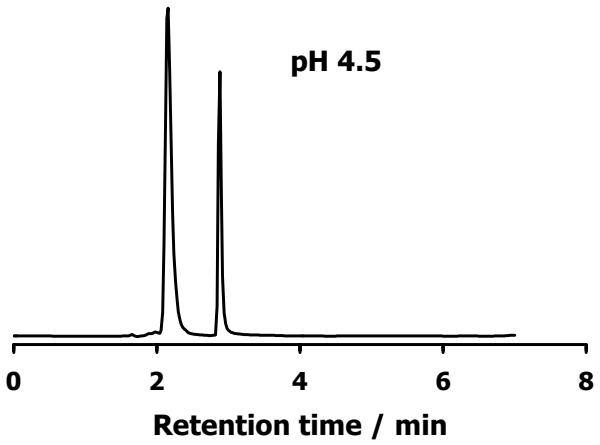

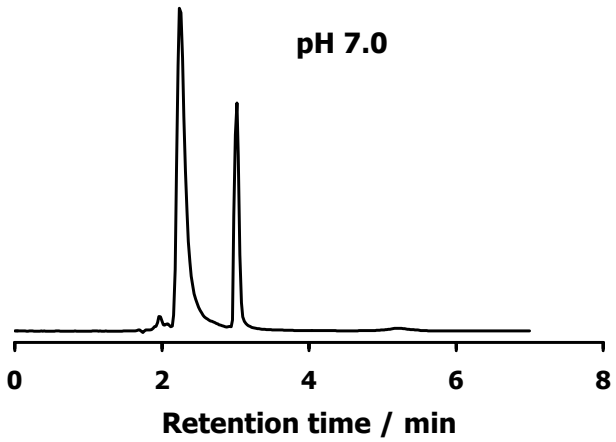

.....or by RPLC

Less successful due to peptide hydrophilicity and/or charge

Linear gradient 0-60 % of Eluent B in 25 minutes

Eluent A: 20 mM KH_2PO_4 pH 4.5 *or* 7.0
Eluent B: 85 %(v/v) acetonitrile, 15 %(v/v) 10 mM KH_2PO_4 pH 4.5 *or* 7.0

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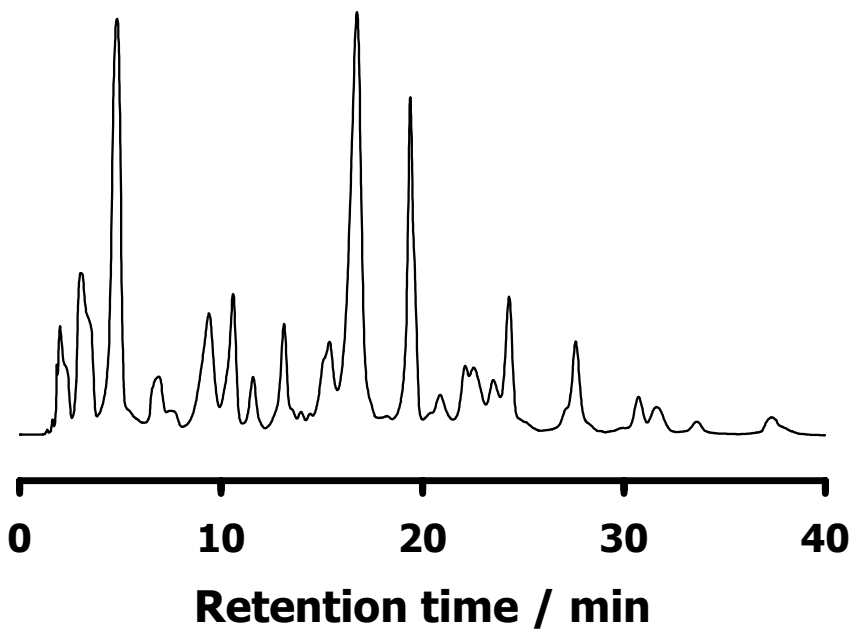
Tryptic digest separation using ZIC™-HILIC



Retention ortogonal to a reversed phase separation

Linear gradient 0-50 % of Eluent B in 40 minutes

Eluent A: 81 %(v/v) acetonitrile, 19 %(v/v) 15 mM KH_2PO_4 pH 4.5

Eluent B: 30 %(v/v) acetonitrile, 70 %(v/v) 20 mM KH_2PO_4 pH 4.5

Flow rate: 0.8 mL/min, UV detection 214 nm
Injection volume: 20 µL

ZIC™-HILIC column (4.6×150 mm, 5 µm)

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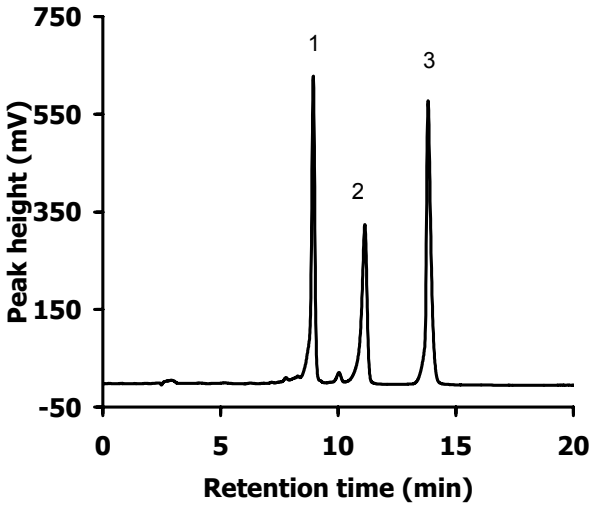
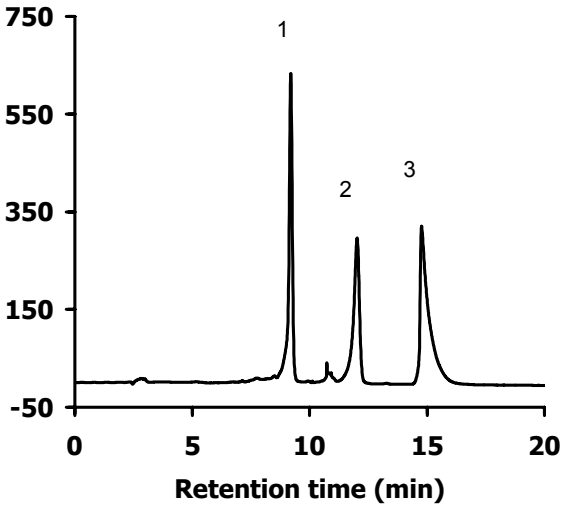
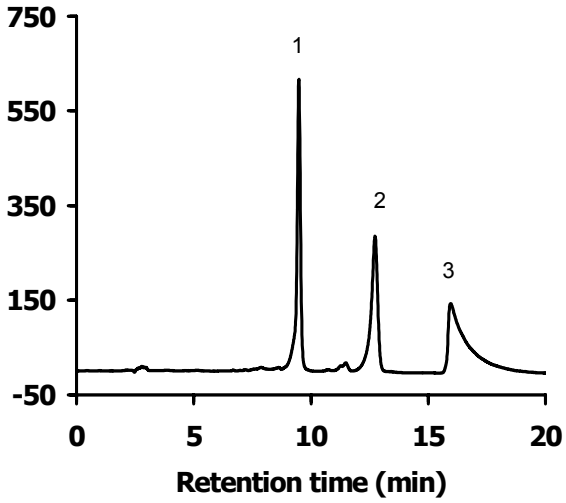
Effect of ionic strength on cationic peptides



100 mM



50 mM



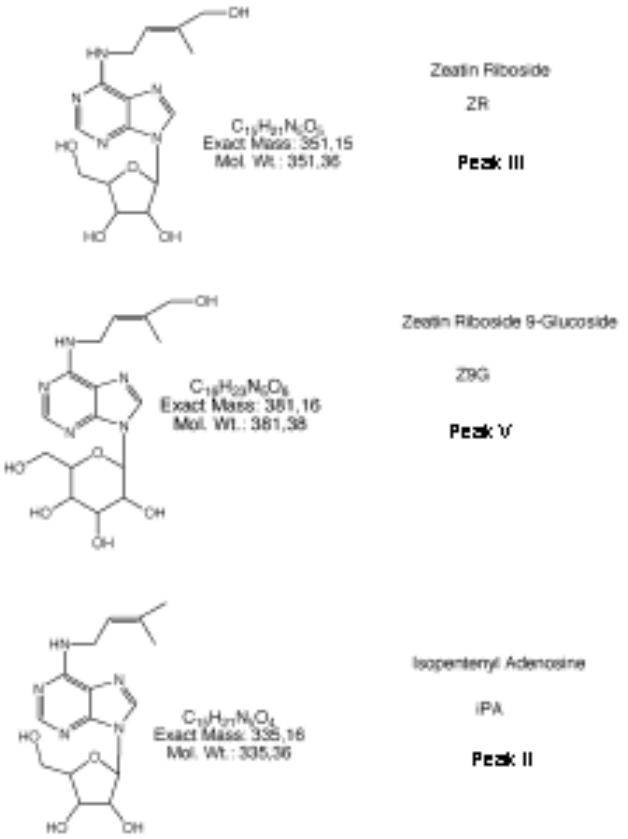
30 mM

Peptides
(**1**) Asp-Arg-Val-Tyr-Ile-His-Pro-Phe; (**2**) Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg; (**3**) Gly-His-Lys.

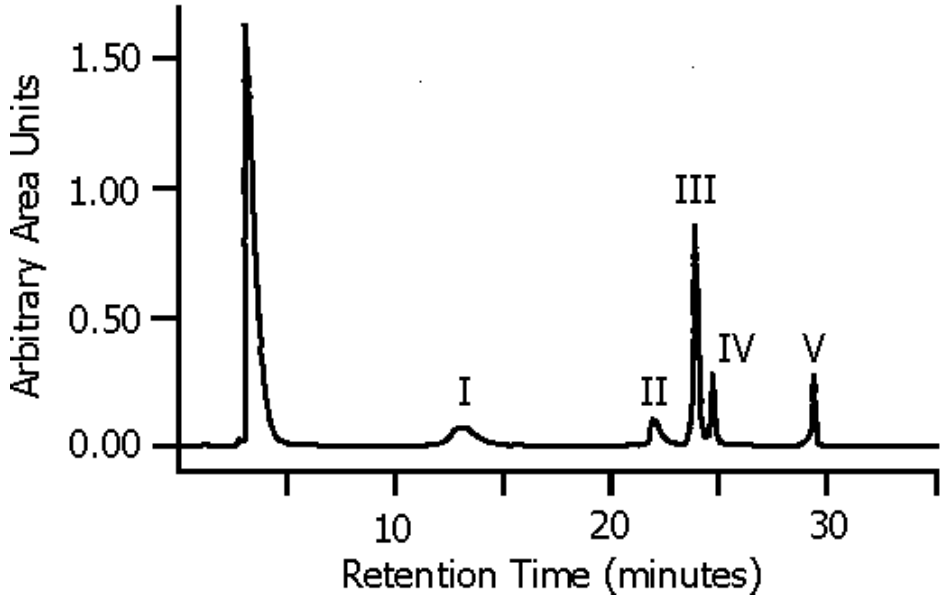
Linear gradient ranging from 10-70 % eluent B in 20 minutes
Eluent A: 85 % (v/v) ACN, 15 % (v/v) 10 mM KH_2PO_4 pH 4.5.
Eluent B: 100 mM *or* 50 mM *or* 30 mM KH_2PO_4 pH 4.5;

ZIC™-HILIC column (4.6×150 mm, 200Å, 5 µm)

ZIC™-HILIC separation of plant hormones



With kind permission from Anders Nordström (Dept. of Forest Genetics and Plant Physiology, SLU, Umeå, Sweden)



Linear gradient 0-15 % in 10 minutes and 15-27 % in 30 minutes of Eluent B

Eluent A: 100 %(v/v) acetonitrile
Eluent B: 6.5 mM ammonium acetate pH 5.5

ZIC™-HILIC column (3.0×150 mm, 200Å, 5 µm)

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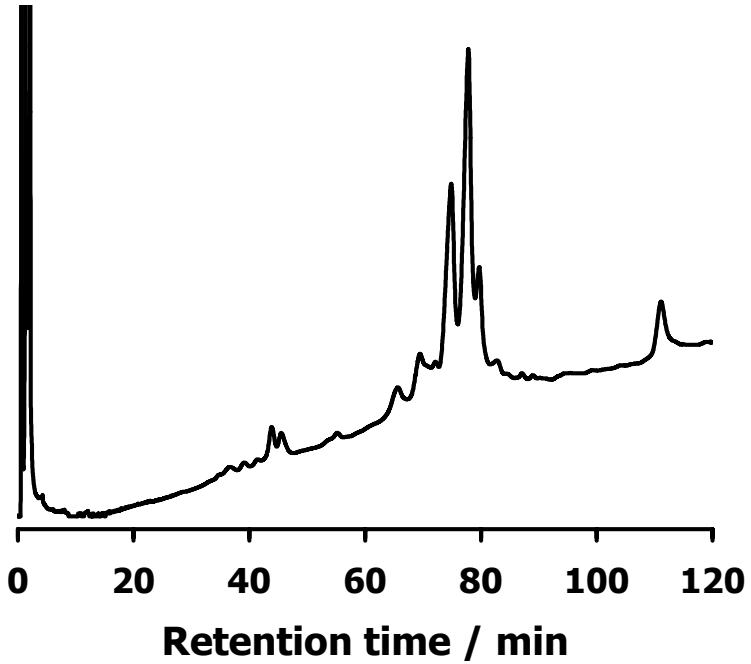
ZIC™-HILIC separation of a plant extract

General structure of sapogenin glycoside, i.e., steroidal saponin

R = sugar moiety; glucose, galactose, pentose etc.

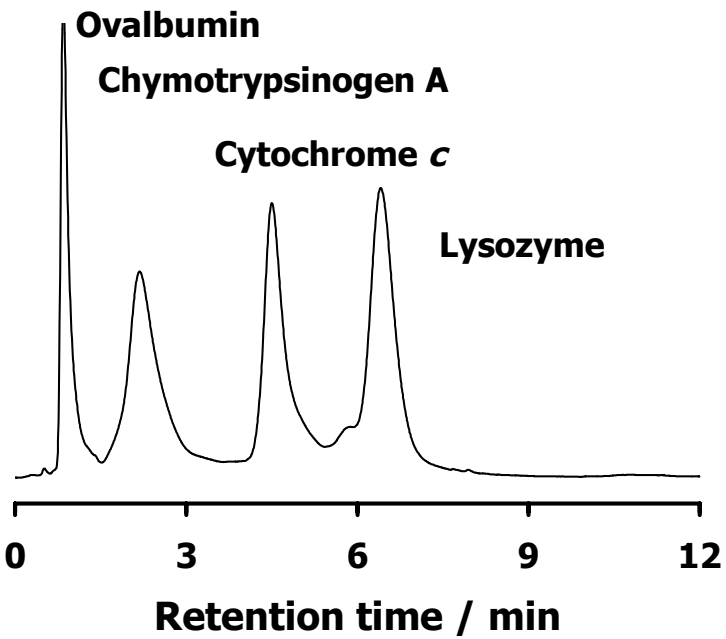


Linear gradient 10-100 % in 120 minutes

Eluent A: 96 %(v/v) acetonitrile 4 % (v/v)
10 mM ammonium acetate pH 5.6

Eluent B: 75 %(v/v) acetonitrile 4 % (v/v)
10 mM ammonium acetate pH 5.6

ZIC™ separation of proteins



- Loading capacity of lysozyme ≈42 mg/mL column volume
- Recovery of lysozyme >95%

Gradient: 30 % of Eluent B for 3 minutes
30-100 % of Eluent B in 4 minutes

Eluent A: 5 mM phosphate buffer pH 6
Eluent B: 5 mM phosphate buffer pH 6
250 mM NaCl

ZIC™-Si column (4.6×50 mm, 200Å, 10 µm)

Generally milder conditions than for SCX columns

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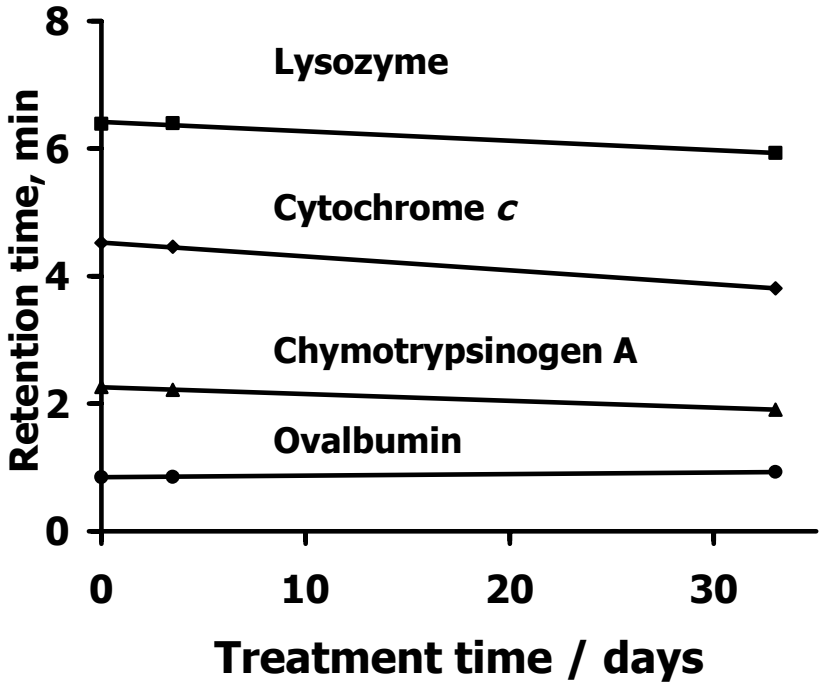
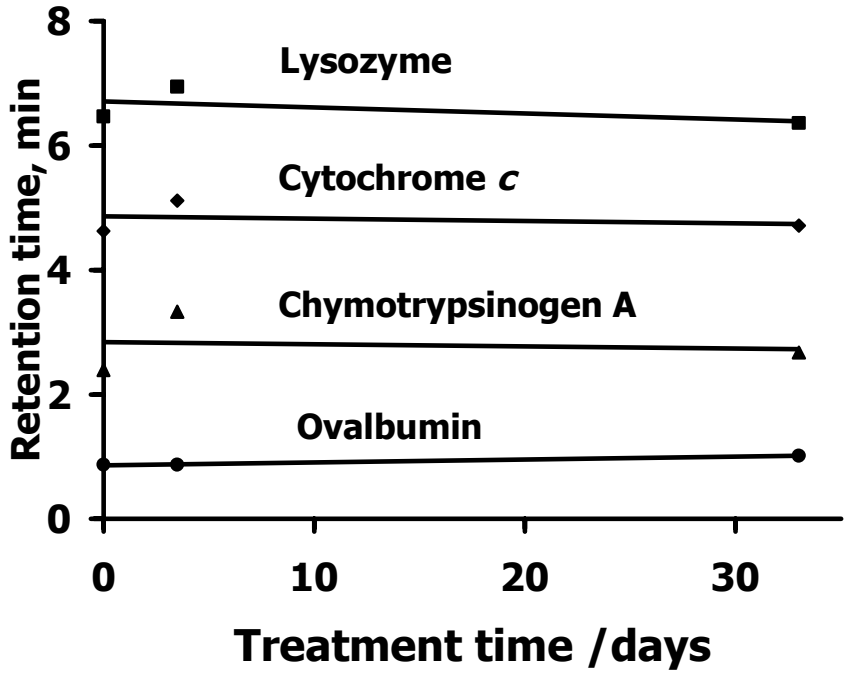
Stability of the ZIC™ stationary phase



pH 2 at 50 °C



pH 8 at 50 °C

Conclusions

- The ZIC™-HILIC column is suitable for peptides and polar compounds poorly retained on RPLC phases.
- ZIC™ can be employed as a complement to cation-exchange chromatography of charged peptides.
- Volatile buffers can be employed.
- The electrostatic effect may be utilized to improve the selectivity and resolution in the ZIC™-HILIC mode
- ZIC™ is a useful tool, soon in everyone's hand… (*sic!*)

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