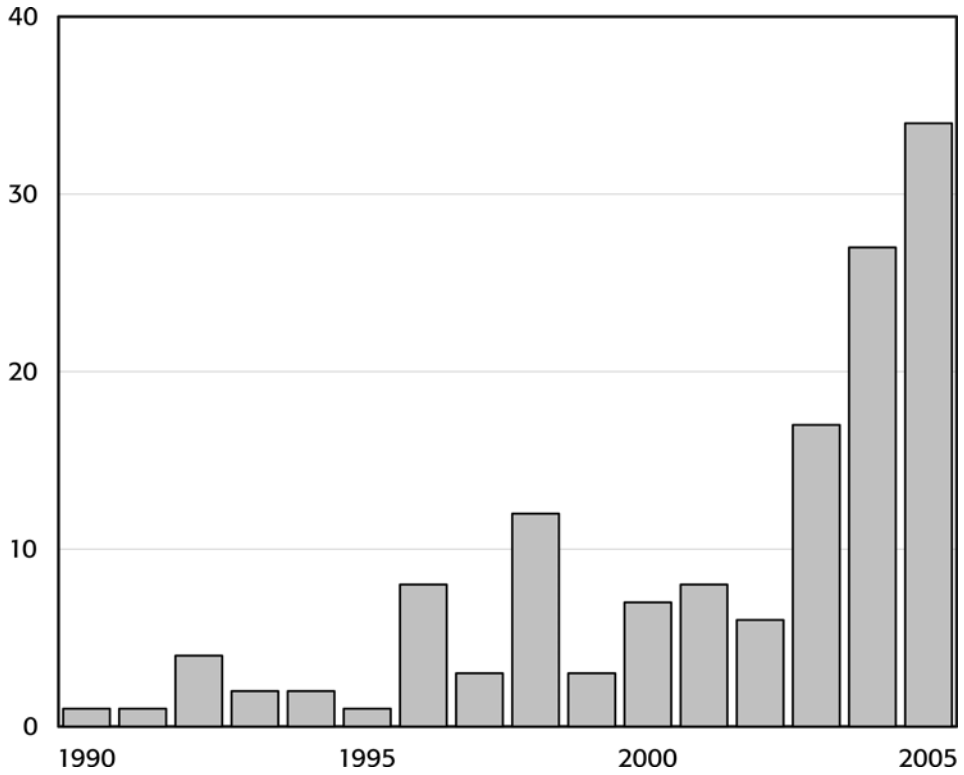
The novelty of zwitterionic stationary phases for hydrophilic interaction chromatography (HILIC)

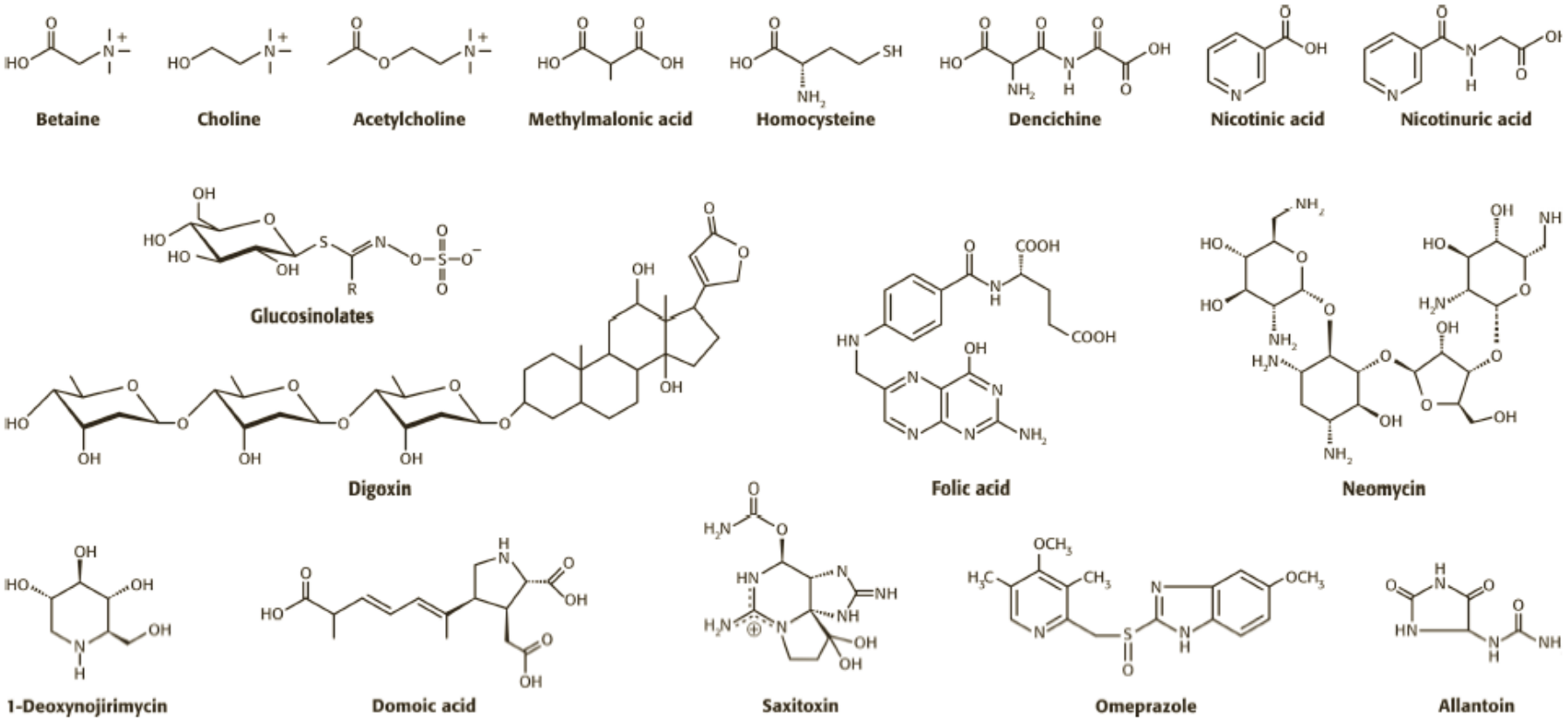
Increasing interest for HILIC



Annual number of *scientific* HILIC papers [1]

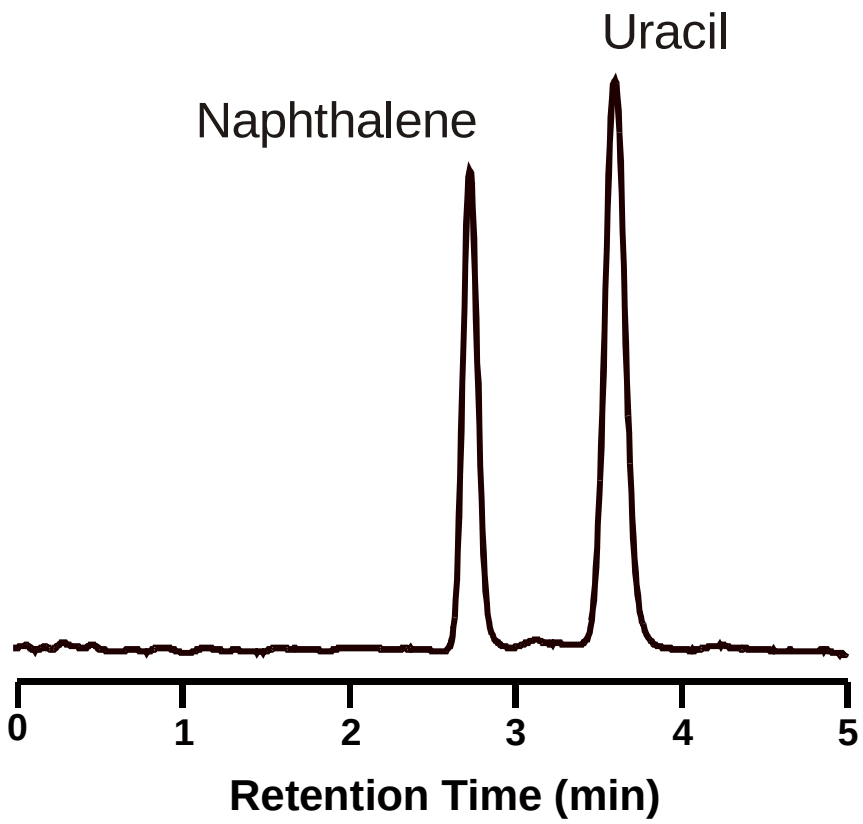
25% published year 2005, **ZIC®-HILIC** introduced 2002

Numerous of “HILIC compounds”…



Example of compounds separated by HILIC [1]

Why HILIC and how?



A hydrophilic stationary phase…

An eluent with high content of organic solvent

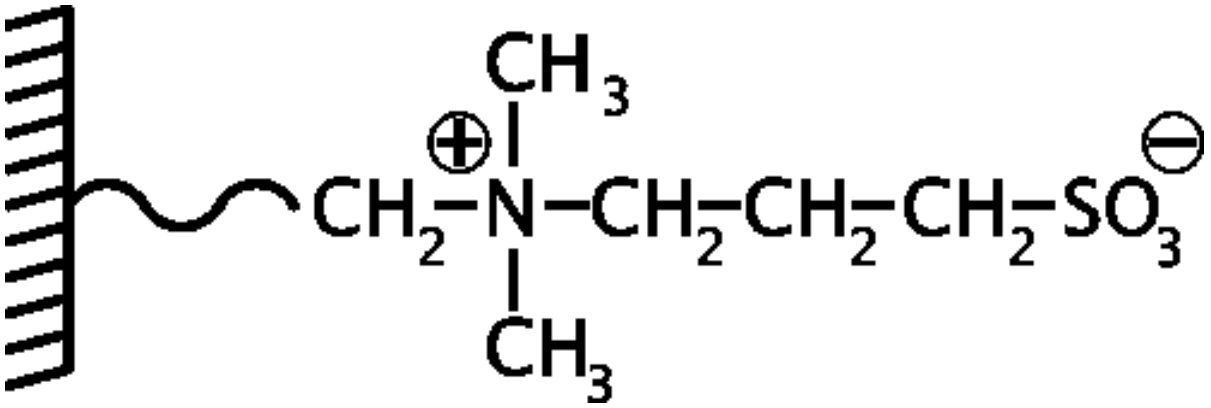
70 % (v/v) acetonitrile
30 % (v/v) water

ZIC®-HILIC capillary column

HILIC – The straightforward approach!

- Water strong solvent, easy controlled
- Higher solubility of polar compounds
- Increases ESI-MS sensitivity [2]
- Orthogonal to RP [3]

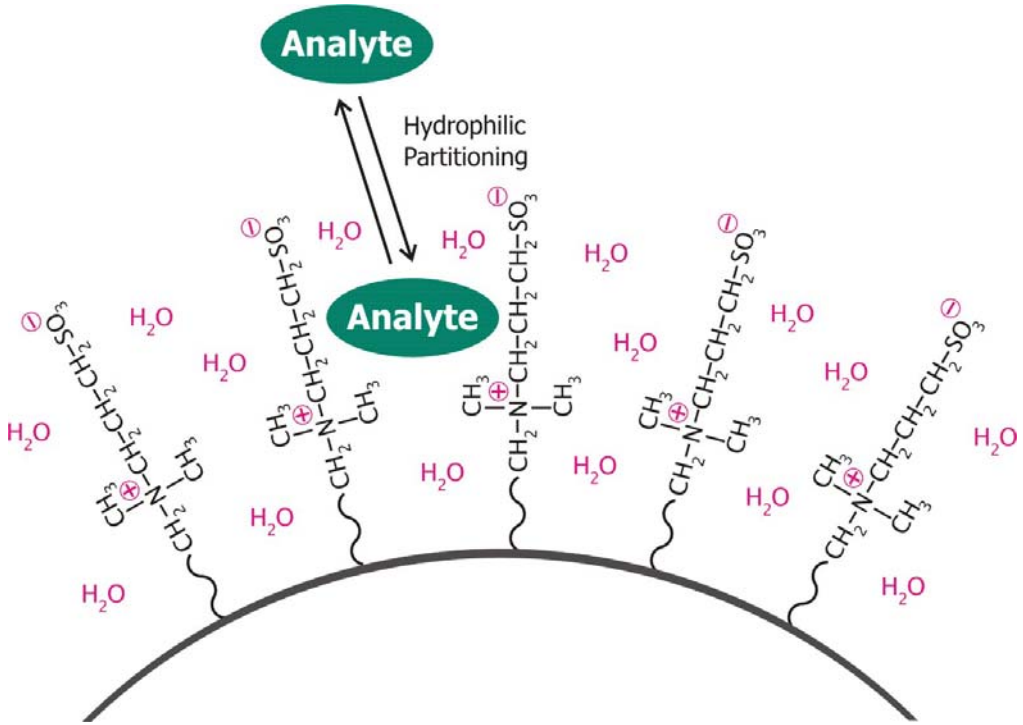
ZIC®-HILIC



The bonded stationary phase

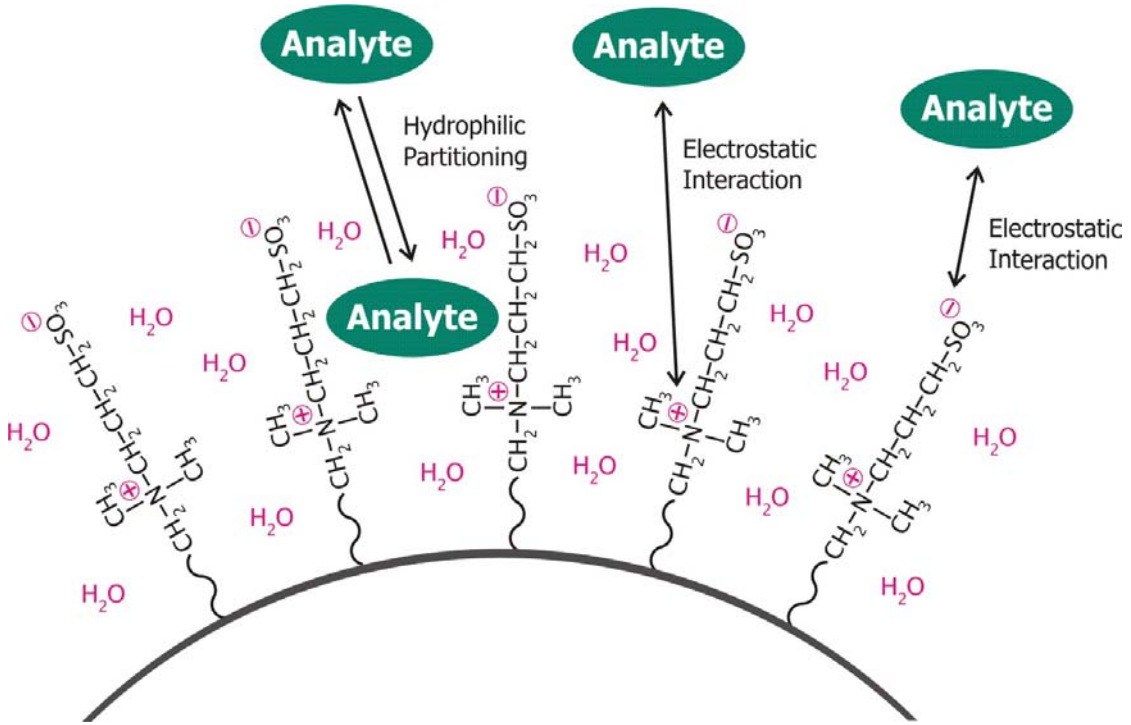
The HILIC retention process

Partitioning

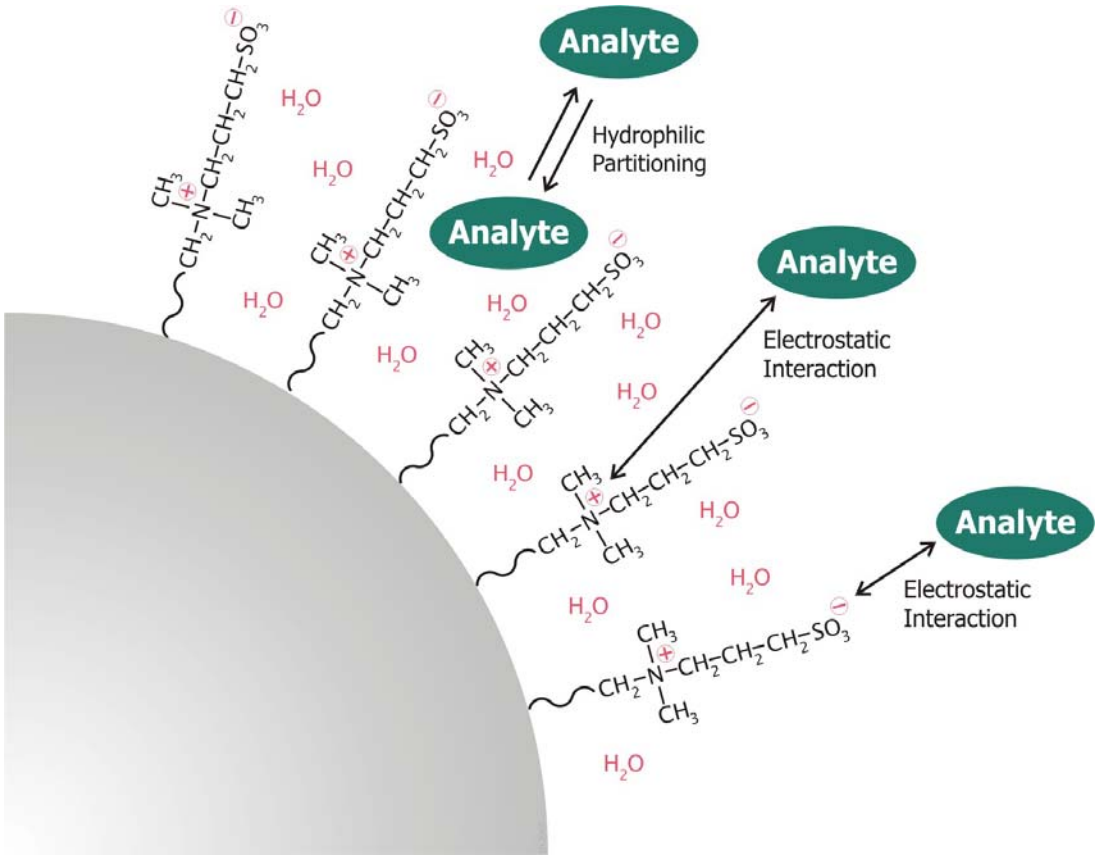


The HILIC retention process

Partitioning and electrostatic interaction



HILIC mechanism – still debated



"HILIC" shown by Samuelsson[4] in 1952 for monosaccarides on Amberlite IRA-400

Acronym "HILIC" suggested by Alpert[5] 1990

We need:

A water enriched layer in the stationary phase[6]

We use:

The zwitterion is acting as "immobilised bulk water"

Comparison of HILIC stationary phases

Neutral

- No ionic interactions
- Less selectivity
- pH-independent

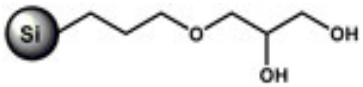
Charged

- Good selectivity
- Strong ionic interactions
- High [buffer] needed
- pH dependent

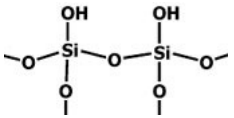
Zwitterionic

"Charged and neutral"

- Good selectivity
- Weak ionic interactions
- Stable aqueous layer
- pH-independent

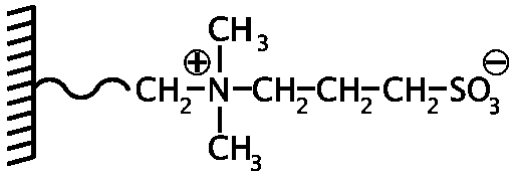


diol



silica

amino



ZIC®-HILIC & ZIC®-*p*HILIC

Orthogonal separation

Solid Phase Extraction (SPE) Selectivity

RP Solid Phase Extraction

- The sample is diluted in water and applied on SPE
- Elution by organic solvent

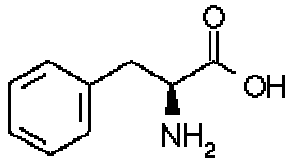
Peak compression on **ZIC®-HILIC** column

ZIC®-HILIC Solid Phase Extraction

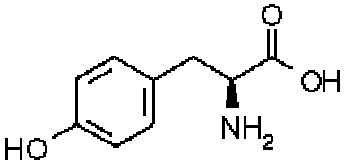
- The sample is diluted in acetonitrile and applied on SPE
- Elution by water or a buffer

Peak compression on RP column

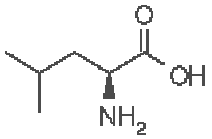
Separation of zwitterions



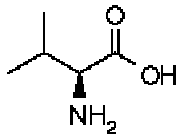
Phenylalanine **k´ = 1.0**



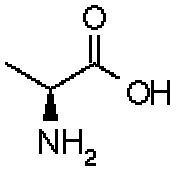
Tyrosine **k´ = 1.5**



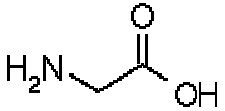
Isoleucine **k´ = 1.3**



Valine **k´ = 1.7**



Alanine **k´ = 2.5**



Glycine **k´ = 3.0**

Eluent:
70% (v/v) acetonitrile
30% (v/v) 20 mM NH_4Ac

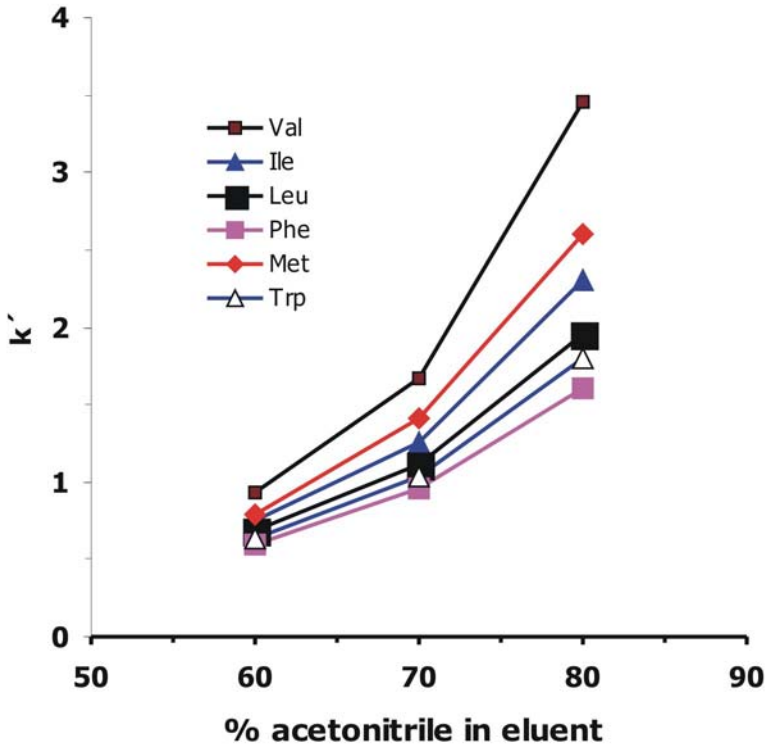
Flow rate: 0.5 mL/min

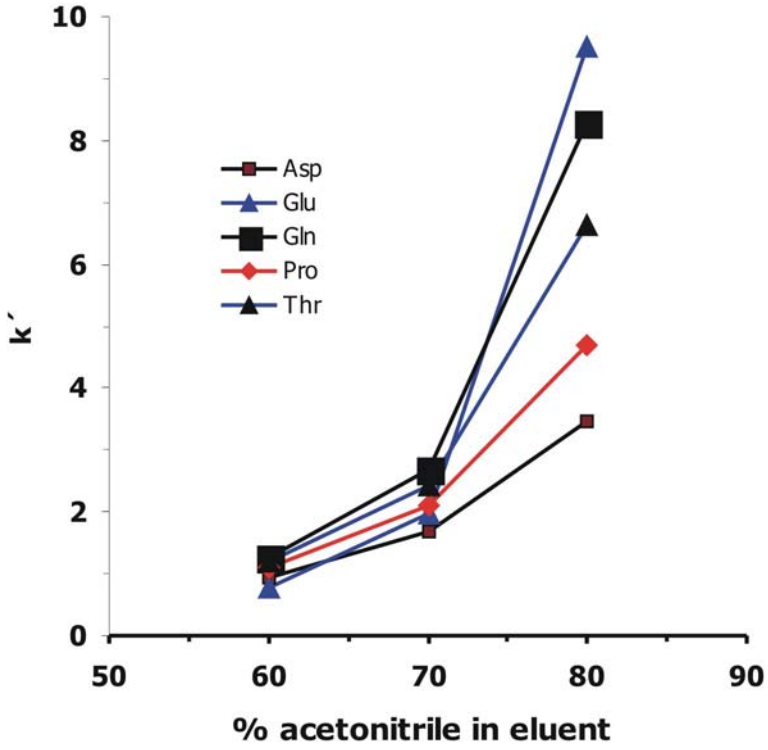
UV detection: 206 nm

Injection volume: 5 µL

ZIC®-HILIC column
(100 x 4.6 mm, 5 µm)

Amino Acid Retention





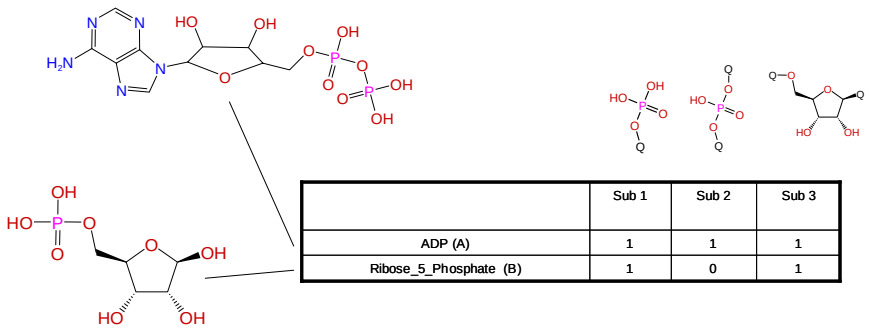
Less polar amino acids

More polar amino acids

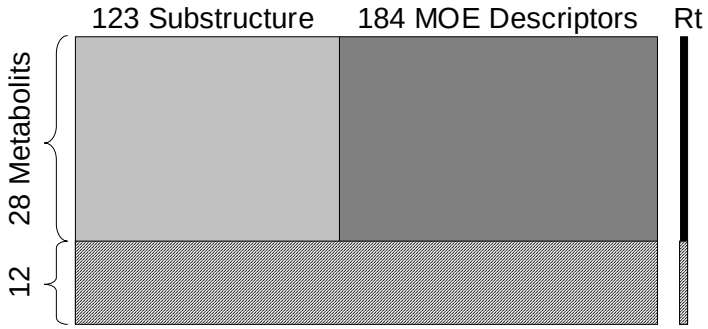
Chemical Descriptors for HILIC Retention?

Molecular volume, Hydrophobic regions, Hydrophilic regions, Log P (water/octanol partition coefficient), Hydrogen bonding, Polarizability, Amphiphilic moment etc

Is it possible to predict retention from chemical structure?

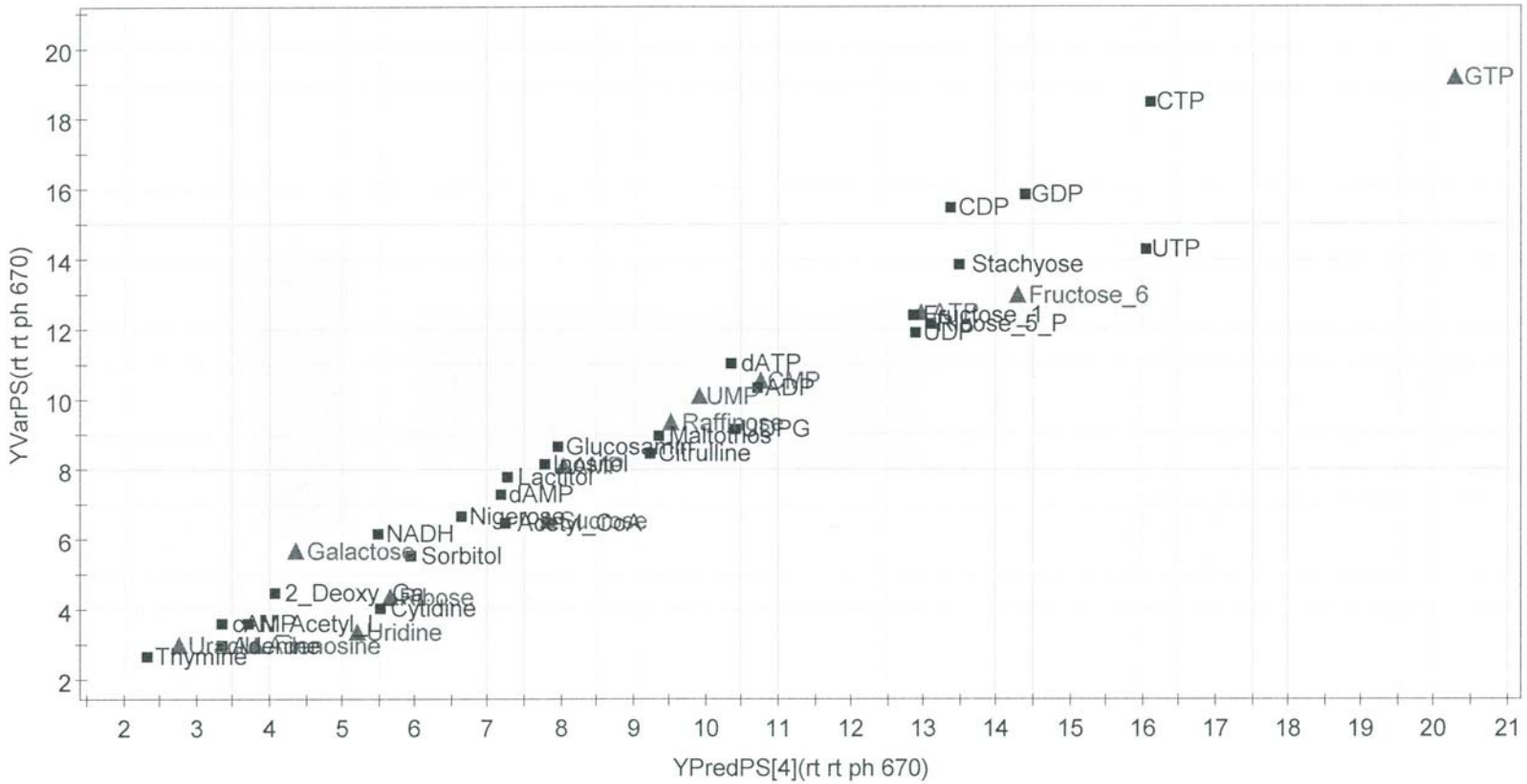


	Sub 1	Sub 2	Sub 3
ADP (A)	1	1	1
Ribose_5_Phosphate (B)	1	0	1

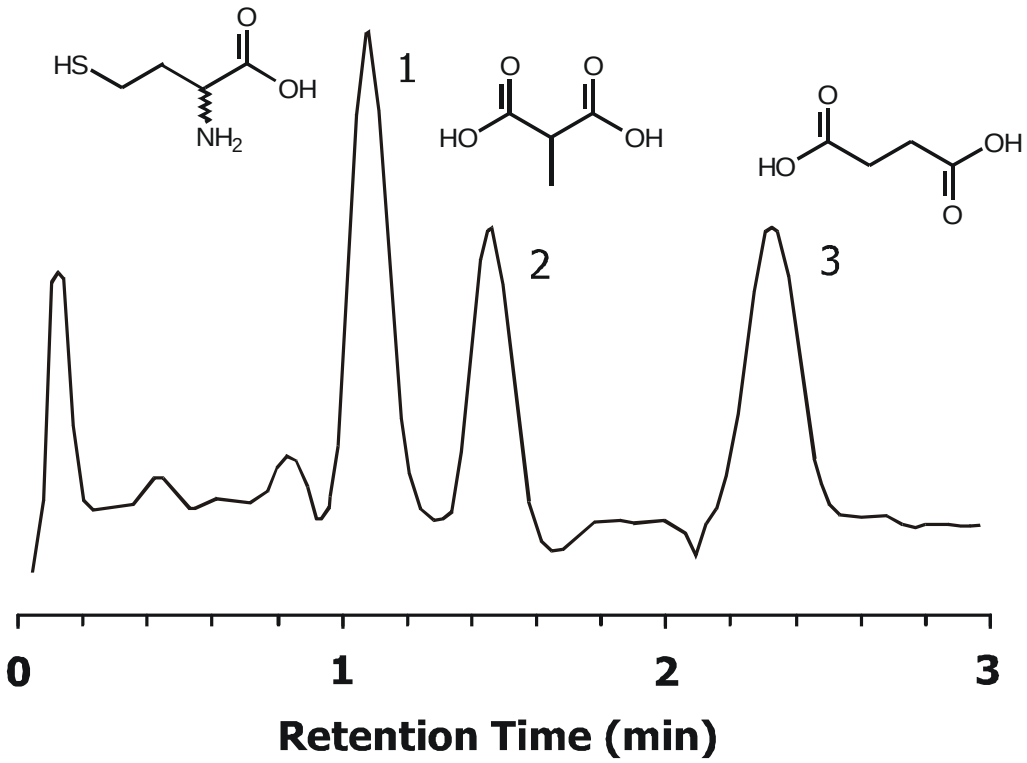


J. Gullberg *et al*, manuscript in prep

Predicted *vs* found ZIC®-HILIC retention



ZIC®-HILIC for biosamples



Homocysteine (1), Methylmalonic Acid (2) and Succinic Acid (3)

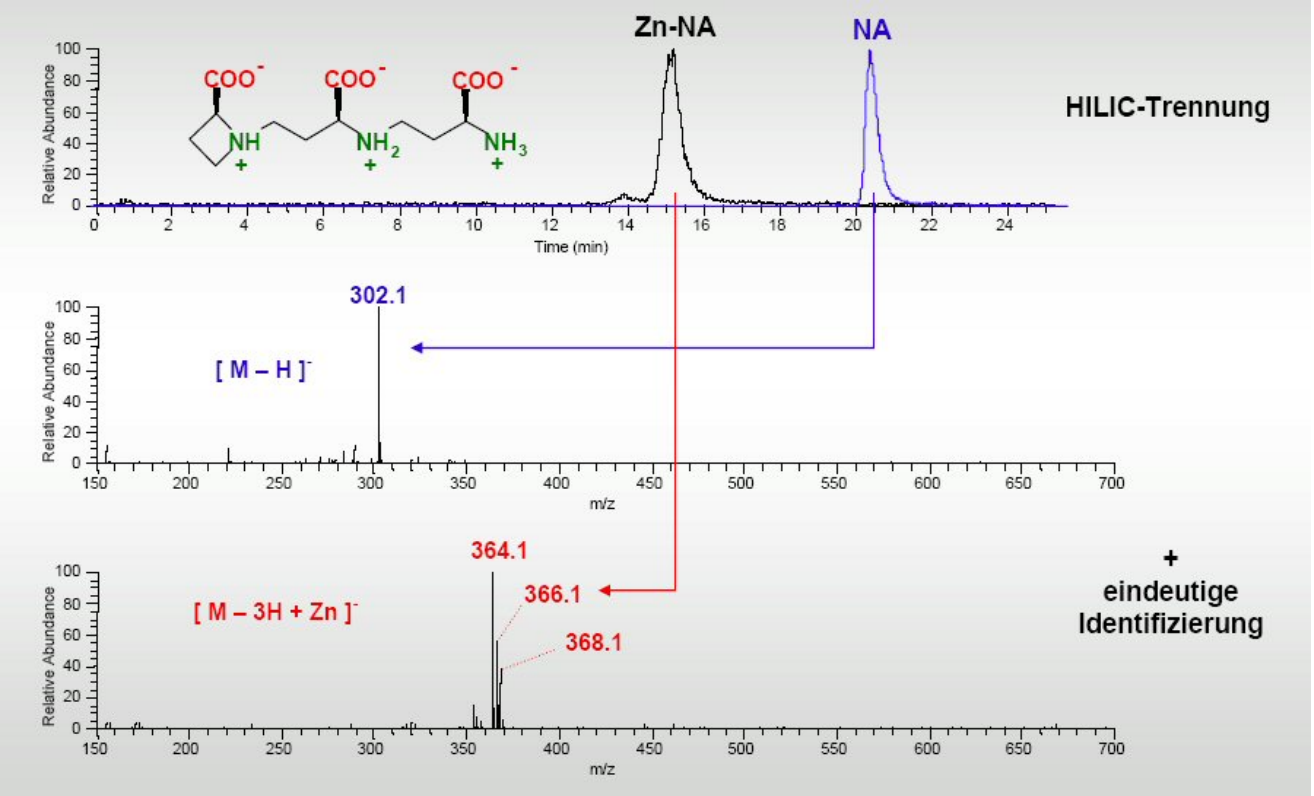
Precipitate plasma proteins in acetonitrile[7]

Centrifugate

Inject

ZIC®-HILIC gradients

Günther Weber, www.isas.de/



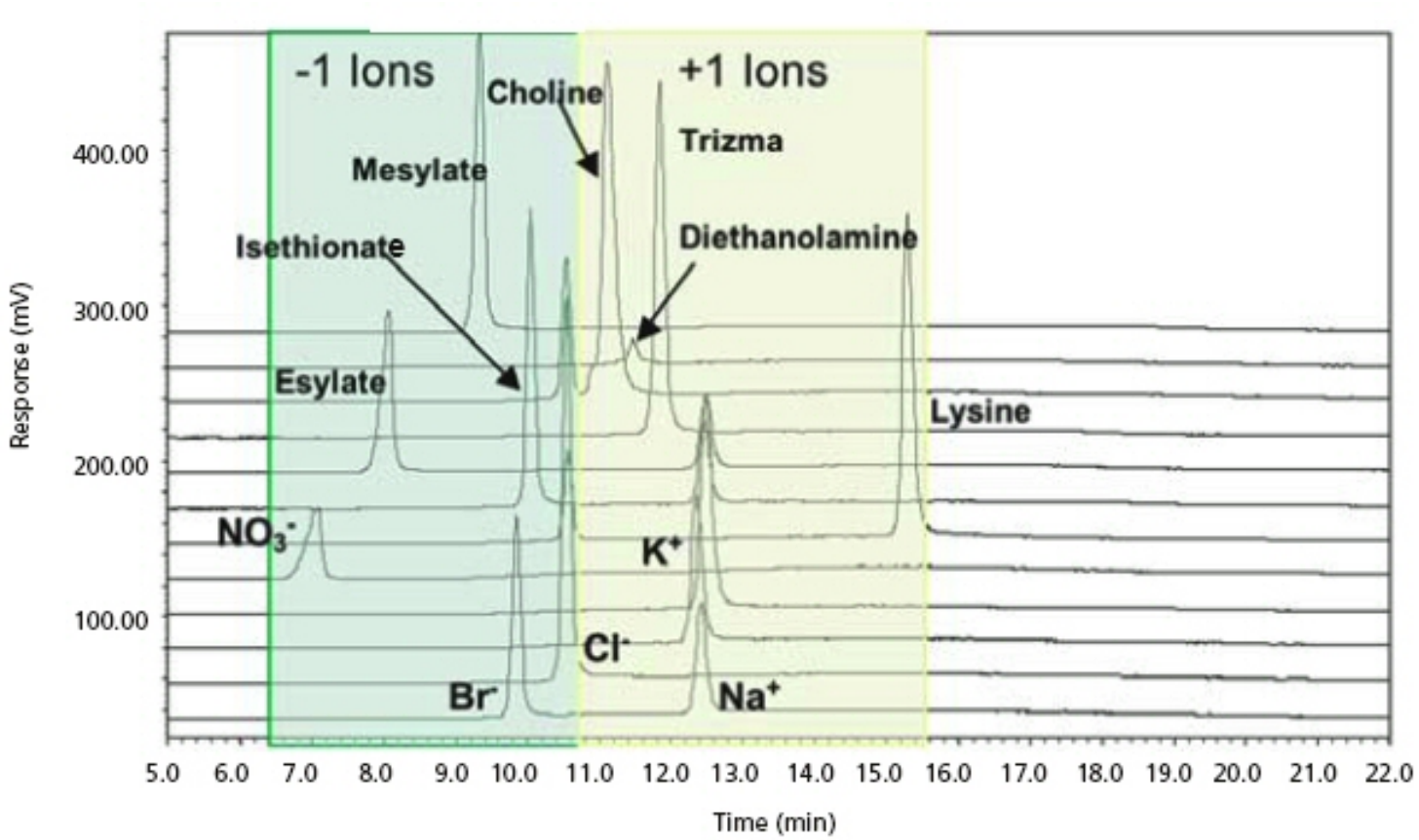
ZIC®-HILIC-Säule (150×1.0 mm) mit Vorsäule (14×1.0 mm) , 5µm, 5µL Injektionsvolumen

Binärer Gradient (0.15 mL / Min.):

0 – 3 Min.: 100% A
3 – 33 Min.: lin. Gradient auf 30% A + 70% B
33 – 40 Min.: 30% A + 70% B

A: 10 mM Ammoniumacetat+Acetonitril (10+90), pH 7.3
B: 30 mM Ammoniumacetat+Acetonitril (80+20), pH 7.3

Simultaneous separation of salts



ZIC®-HILIC gradient elution and ELSD detection [8]

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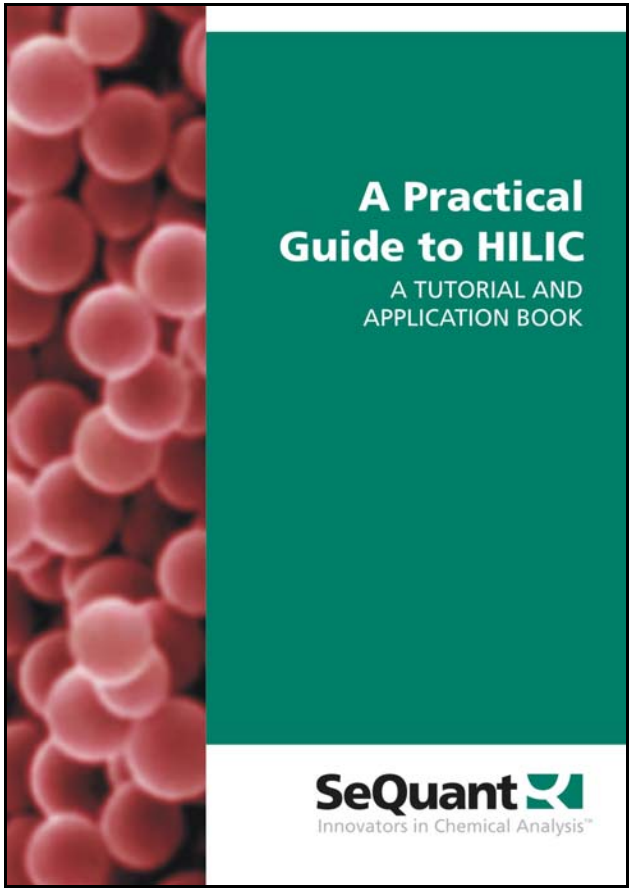

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