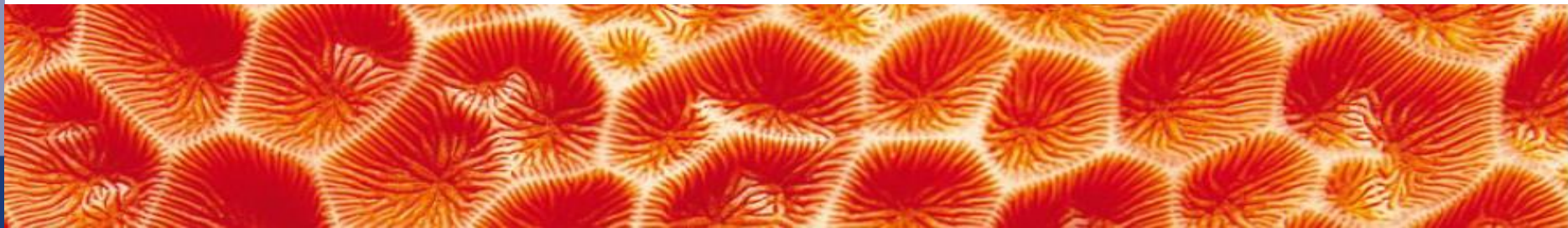
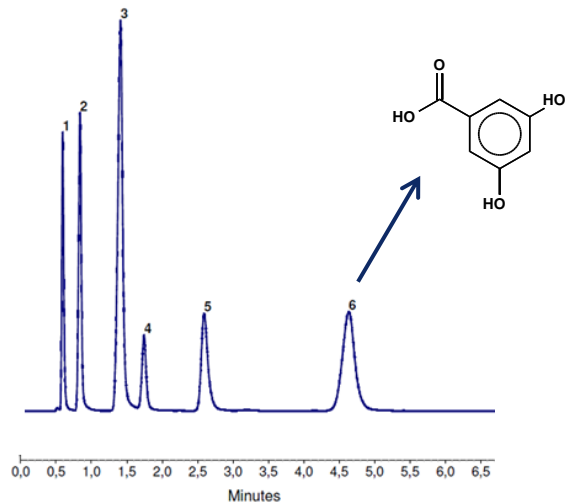


## Eurospher II HILIC

- ▶ *How do you analyze very polar and hydrophilic compounds?*



# Eurospher II HILIC

## ► *What is the principle of HILIC?*

**HILIC** or **H**ydrophilic Interaction **L**iquid **C**hromatography combines characteristics of the 3 major modes:

**Stationary phase:**

Polar modifications of silica gel or polymers - like NP-mode

**Mobile phase:**

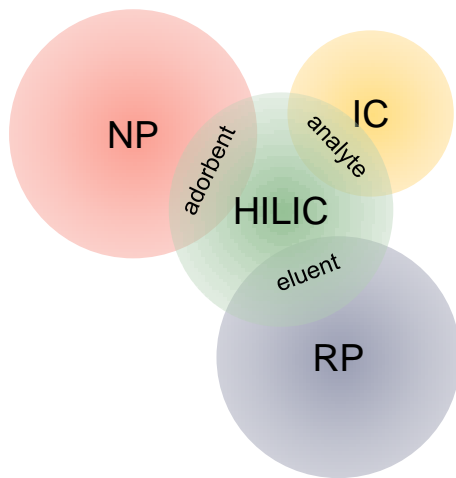
Mixtures of aqueous buffer systems and organic modifiers – like RP-mode

**Fields of application:**

Quite polar compounds, organic and inorganic ions – like IC

**Partition chromatography between mobile and stationary phase:**

Distribution of analytes between water-poor layer of mobile phase and water-rich layer on surface of polar stationary phase  
Weak electrostatic mechanisms and hydrogen donor interactions between neutral polar molecules under high organic elution conditions



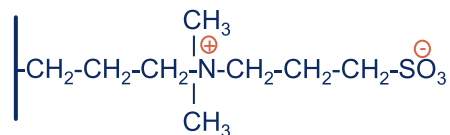
”HILIC is NP chromatography of polar and ionic compounds under RP conditions”

# Eurospher II HILIC

## ► *What are the characteristics?*

Modification	% Carbon	pH range	Column code
Eurospher II HILIC	7 %	2 – 8.5	xxxE120E2J

<b>Silica gel:</b>	ultra pure, > 99.99 %
<b>Modification:</b>	Ammonium – sulfonic acid
<b>Metal content:</b>	< 10 ppm
<b>Particle size:</b>	2 µm, 3 µm, 5 µm
<b>Particle shape:</b>	spherical
<b>Pore size:</b>	100 Å
<b>Specific surface:</b>	320 ± 20 m <sup>2</sup> /g
<b>Pore volume:</b>	0.8 ml/g
<b>Density:</b>	430 g/l

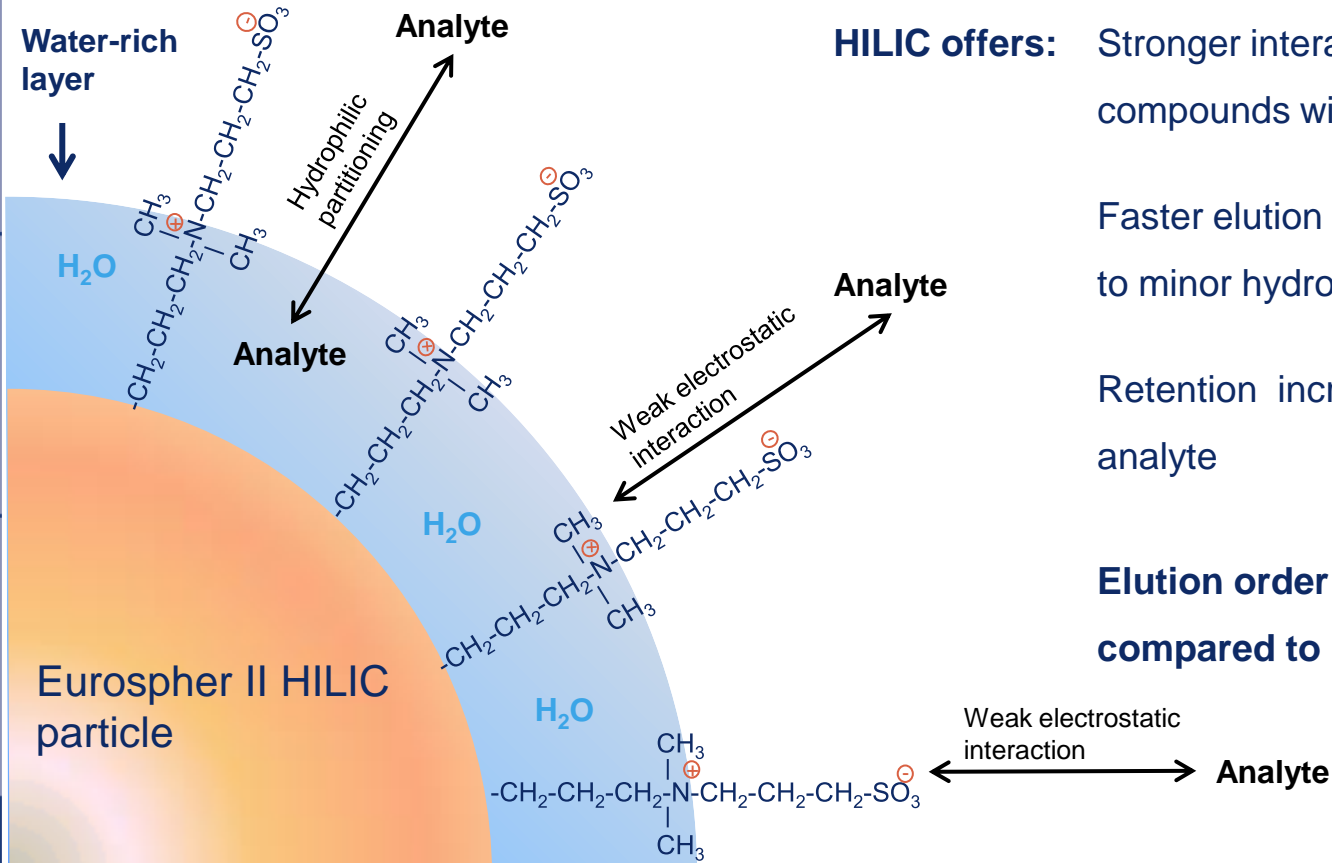


**Special zwitterionic modification:**  
Neutrally charged but highly polar surface



# Eurospher II HILIC

## ▶ Suffering from poor retention of polar analytes?



**HILIC offers:** Stronger interaction of more polar compounds with stationary aqueous layer

Faster elution of nonpolar compounds due to minor hydrophobic interactions

Retention increases with charge of the analyte

**Elution order is often inverse on HILIC compared to RP columns**



## Eurospher II HILIC

### ▶ *When are the new HILIC phases recommended?*

Recommended for the analysis of hydrophilic compounds like:

- Polar organic acids and bases
  - Natural polar compounds
  - Nucleosides
  - Oligonucleotides
  - Amino acids
  - Peptides
  - Water soluble vitamins
- Completely MS compatible
  - Ultra fast equilibration
  - Suitable in Analytical and Preparative scale



check [www.knauer.net](http://www.knauer.net) for Applications!

# Application with Eurospher II HILIC

## ► *Hydroxybenzoic acids*

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

**Eluent:** A: 5 mM NH<sub>4</sub>-acetate pH 4.78

B: Acetonitrile

**Gradient:** Isocratic 90 % B

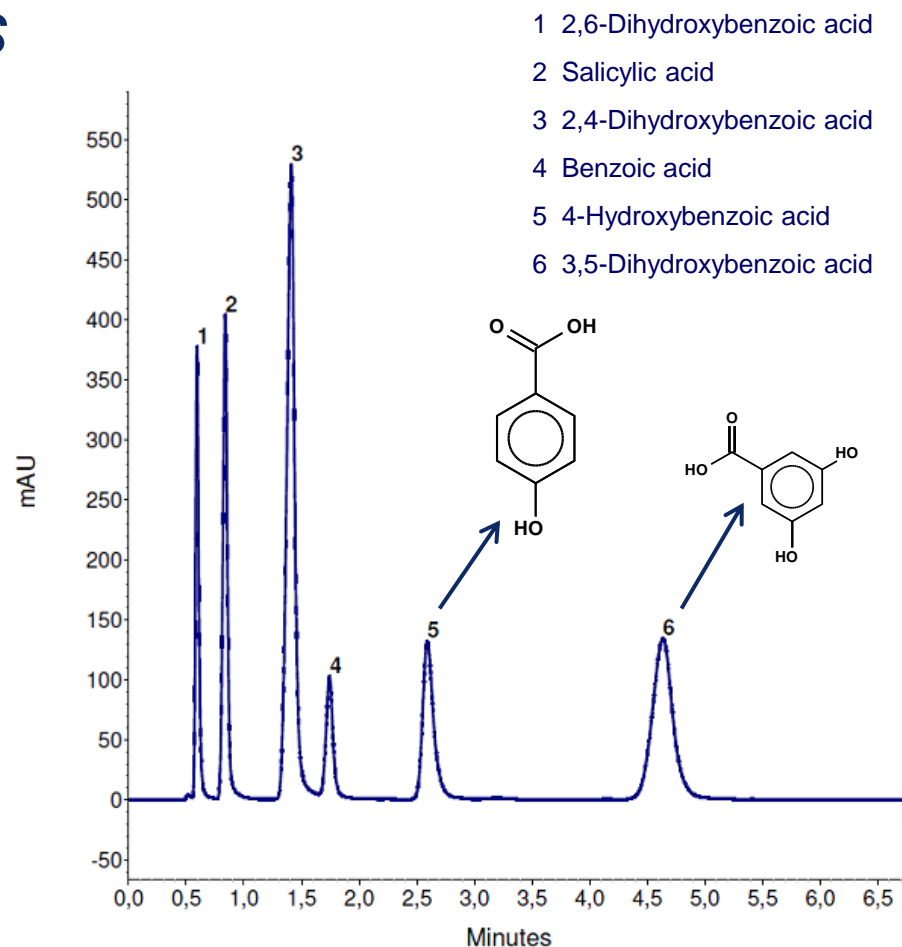
**Flow rate:** 1.0 ml/min

**Temperature:** 25 ° C

**Volume:** 1 µl

**Detection:** UVD 2.1L, 10 mm flow cell

210 nm, 20 Hz, 0,05 s



# Application with Eurospher II HILIC

## ► *Water-soluble Vitamins*

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

**Eluent:** A: 25 mM NH<sub>4</sub>-acetate pH 4  
B: Acetonitrile

**Gradient:** 0.0 – 0.7 min 20 % A  
0.7 – 1.4 min 20 – 30 % A  
1.4 – 5.0 min 30 % A

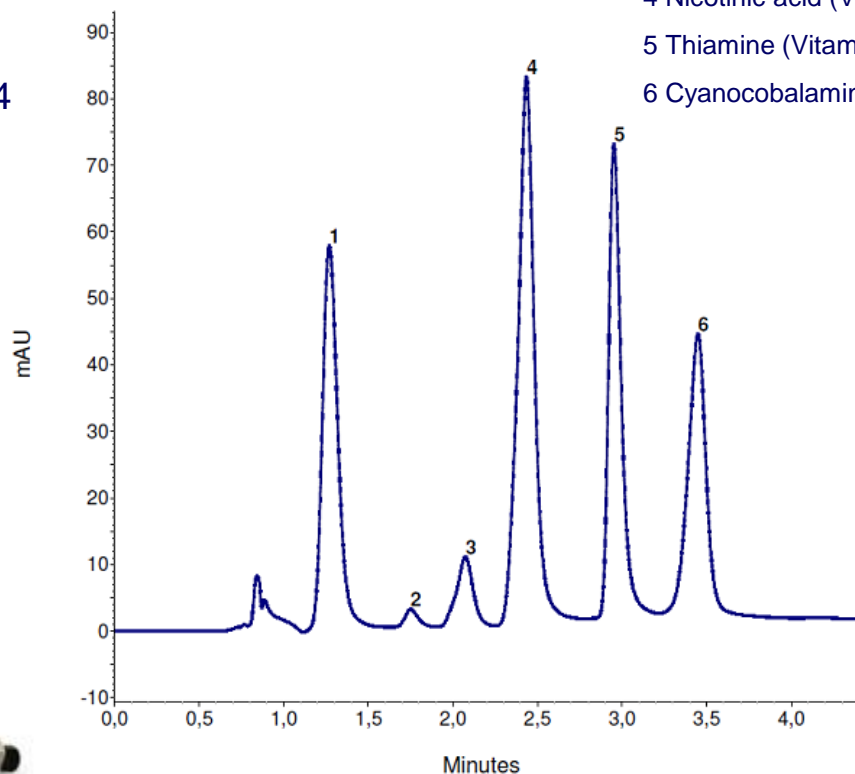
**Flow rate:** 1.0 ml/min

**Temperature:** 25 ° C

**Volume:** 10 µl

**Detection:** UVD 2.1L, 10 mm flow cell  
254 nm, 20 Hz, 0,5s

- 1 Pyridoxal (Vitamin B<sub>6</sub>)
- 2 Folic acid (Vitamin B<sub>9</sub>)
- 3 Ascorbic acid (Vitamin C)
- 4 Nicotinic acid (Vitamin B<sub>3</sub>)
- 5 Thiamine (Vitamin B<sub>1</sub>)
- 6 Cyanocobalamine (Vitamin B<sub>12</sub>)



# Application with Eurospher II HILIC

## ► Organic acids

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

**Eluent:** A: 200 mM NH<sub>4</sub>-acetate pH 6.8

B: Acetonitrile

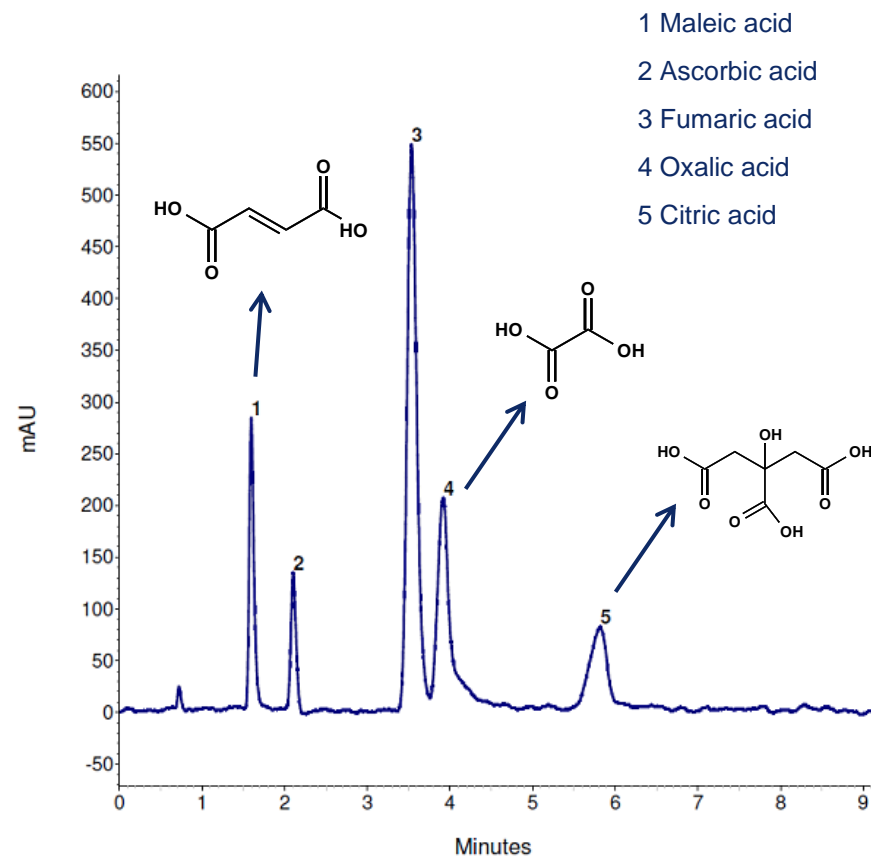
**Gradient:** Isocratic 70 % B

**Flow rate:** 1.0 ml/min

**Temperature:** 25 ° C

**Volume:** 1 µl

**Detection:** UVD 2.1L, 10 mm flow cell  
210 nm, 10 Hz, 0,2s





# Eurospher II HILIC

## ► Ordering Information

The last 7 digits of the Order No. comprise the stationary phase

**Eurospher II 100-2 HILIC**

Order No. ...E120E2F

**Eurospher II 100-3 HILIC**

Order No. ...E120E2G

**Eurospher II 100-5 HILIC**

Order No. ...E120E2J

The first 3 digits of the Order No. comprise the column dimensions

analytical column ID	3 mm ID	4 mm ID	4.6 mm ID
5 mm (pre column)	P5C...	P5D...	P5D...
30 mm length	03C...	03D...	03D...
50 mm length	05C...	05D...	05D...
100 mm length	10C...	10D...	10E...
125 mm length	12C...	12D...	12E...
150 mm length	15C...	15D...	15E...
250 mm length	25C...	25D...	25E...
300 mm length	n.a.	30D...	n.a.

