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Multi-Selectivity Stationary Phases By Dr. Maisch HPLC GmbH

A State-of-the-Art Solution for HPLC, UPLC and preparative LC

Selectivity in HPLC

Selectivity are mainly driven by the interactions of sample molecule with the surface of the stationary phase supported by the mobile Phase

Possible Interactions in RP-HPLC

Phase / Sample interactions:

• Lipophilic	Non-polar
• Dipole – induced dipole • Dipole – Dipole • Hydrogen bonding • π complex bonding • cation exchange	polar

Multi-Selectivity in HPLC

Multi – Selectivity means that the Stationary Phase offers different possibilities for interactions to support and increase selectivity

Multi-Selectivity

Lipophilic – Polar Phases

- Not endcapped C18 / C8 / Phenyl
- AQ / HE
- EPS
- Cyano-Propyl
- Amino-Propyl
- Diol

Multi-Selectivity

Lipophilic – π-electron (Phenyl) Phases

- Propyl-Phenyl
- Hexyl-Phenyl
- Biphenyl
- Diphenyl
- C18-Phenyl
- C18-PFP
- PFP

Multi-Selectivity

Lipophilic – Ion exchange Phases

Mixed Mode Phases

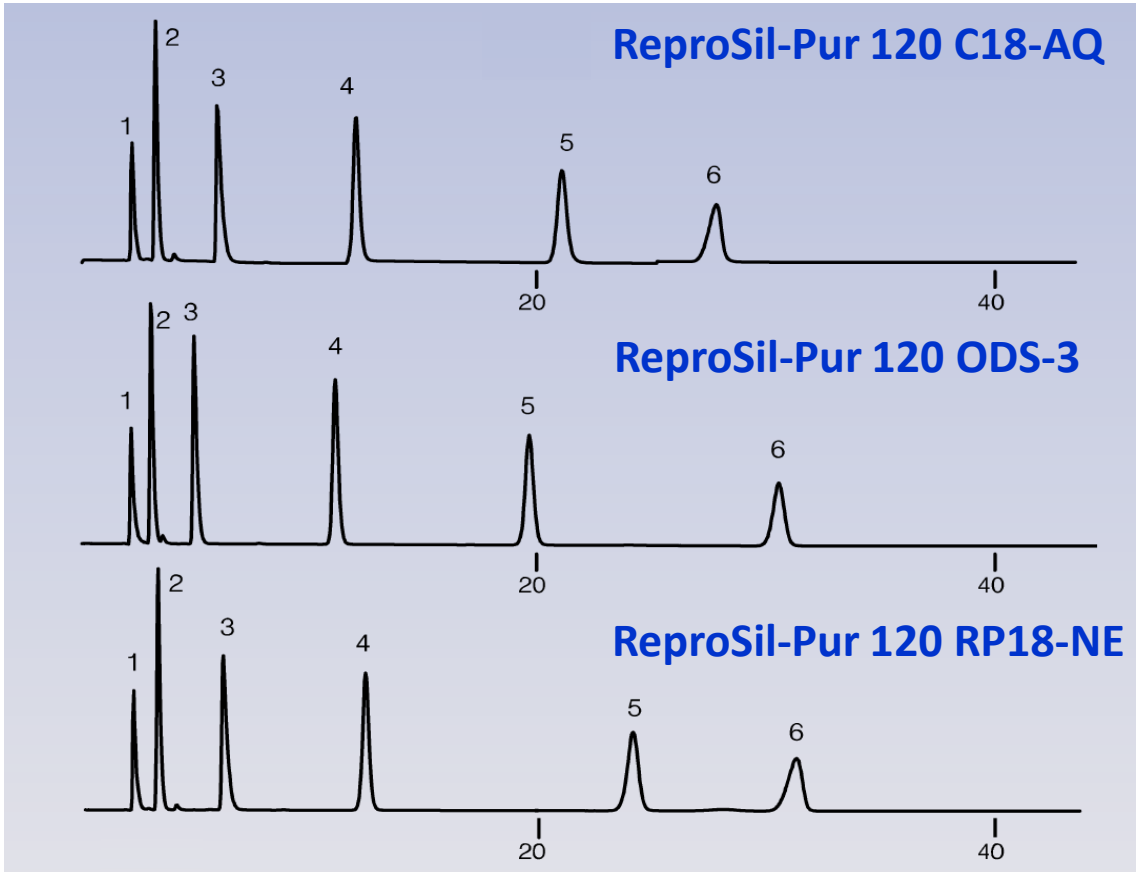
- C18 / SAX
- C8 / SAX
- C18 / SCX
- C8 / SCX
- Phenyl-SCX
- Propyl-SO_3H
- Propyl-Amino

Multi-Selectivity

Hilic - Phases

- Si
- EPS
- Cyano-Propyl
- Amino-Propyl
- Diol
- Phenyl-SCX
- SCX
- SAX
- Zwitter Ionic
- … special polar groups

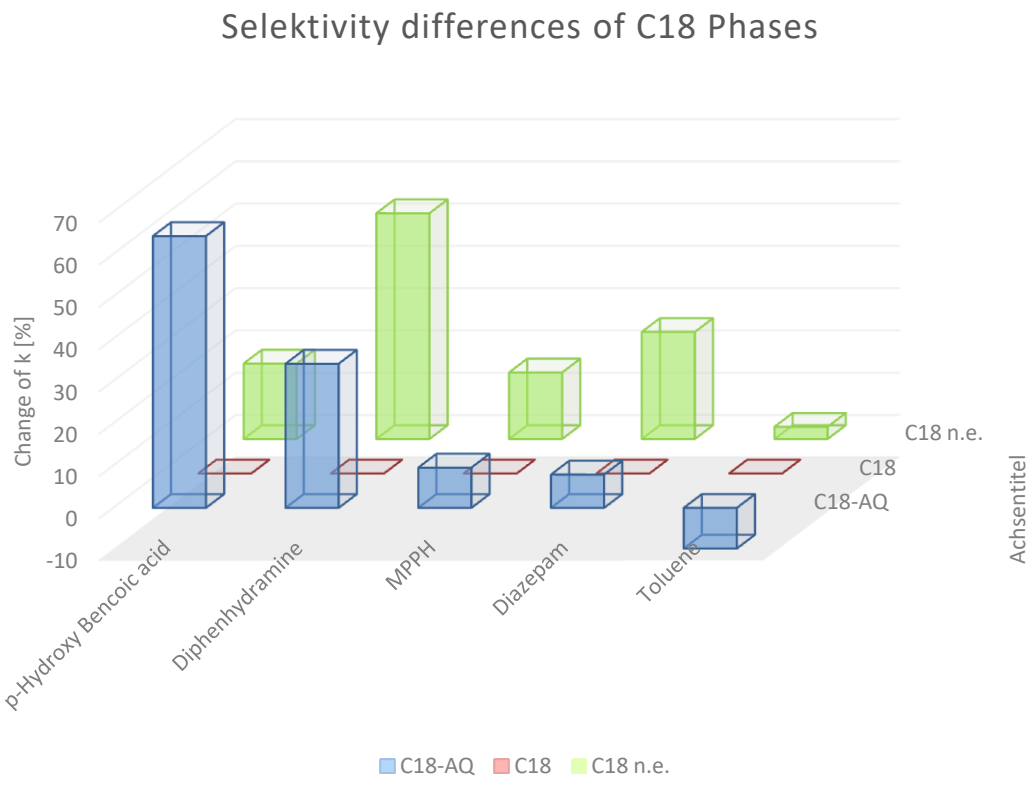
Lipophilic – Polar Phases



1) uracil
2) 4-hydroxybenzoic acid
3) diphenylhydramine
4) 5-(p-methylphenyl)-5-phenylhydantoin
5) diazepam
6) toluene

Flow rate: 1.0 ml/min
Eluent: Na-phosphate buffer, 50 mM, pH 2.3 / acetonitrile = 58 /42
Detection (UV):230 nm
NovoGrom 250 mm x 4.0 mm id.

Lipophilic – Polar Phases



Selectivity in HPLC Stationary Phases

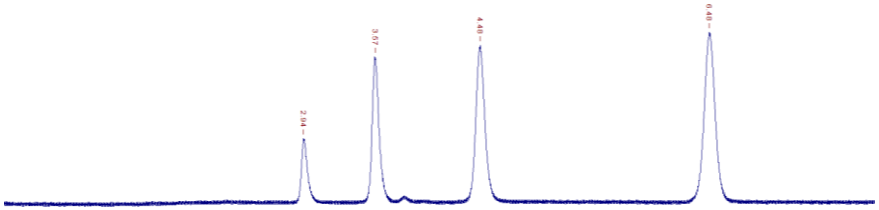
Phase	%C	Description
Reprospher 100 C30-DE	20%	double endc
Reprospher 100 C18	16%	endc.
Reprospher 100 C18-DE	16%	double endc
Reprospher 100 C18-Aqua	12%	pol.Gr.+ endc.
Reprospher 100 C18-Aqua-DE	12%	pol.Gr.+ double endc.
Reprospher 100 C18-NE	15%	not endc
Reprospher 100 C18-TDE	20%	polymer-C18-de
Reprospher 100 C18-TN	17%	polymer-C18, not endc.
Reprospher 100 C18-Phenyl		C18+Phenyl-Groups, endc.
Reprospher 100 C18/WCX		C18+carboxylic acids
Reprospher 100 C12	8%	endc.
Reprospher 100 C8-Aqua	8%	pol.Gr.+ endc
Reprospher 100 C8-Aqua-DE	8%	pol.Gr.+ d.endc.
Reprospher 100 C8	10%	endc.
Reprospher 100 C8-DE	10%	double endc.
Reprospher 100 C8-NE	9%	not endc.
Reprospher 100 C8-TN		polymer-C8, not endc.
Reprospher 100 Phenyl	9%	double endc.
Reprospher 100 Phenyl	9%	double endc.
Reprospher 100 Phenyl-NE	12%	not endc.
Reprospher 100 Phenyl-Hexyl	13%	not endc.
Reprospher 100 Phenyl-Hexyl-e	13%	endc.
Reprospher 100 Diphenyl (Phe-Si-Phe)		double endc.
Reprospher 100 Biphenyl (Si-Phe-Phe)		double endc.
Reprospher 100 PFP (Pentafluorphenyl)		double endc.

Selectivity in HPLC Stationary Phases - 2

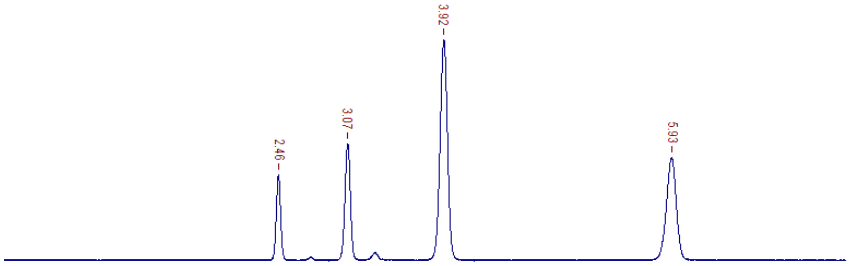
Reprospher 100 C6-TDE	8%	trifunct. endc.
Reprospher 100 C4-Aqua	6%	pol.Gr.+ endc.
Reprospher 100 C4	6%	endc.
Reprospher 100 C4-DE	7%	double endc.
Reprospher 100 CN	7%	
Reprospher 100 CN-DE	7%	double endc.
Reprospher 100 Diol-DE	7%	double endc.
Reprospher 100 Diol	7%	
Reprospher 100 2-EP		2-Ethylpyridin
Reprospher 100 4-EP		4-Ethylpyridin
Reprospher 100 NH2-DE	4%	endc.
Reprospher 100 NH2	4%	not endc.
Reprospher 100 DNH	5%	Diamin
Reprospher 100 PEI, Polyethylenimin		WAX for peptides / Nucleotide
Reprospher 100 Si		
Reprospher HILIC-P		HILIC for Peptides
Reprospher HILIC-A		HILIC forAcids
Reprospher HILIC-ARG		(with Arginin-Group)
Reprospher Acidosil-S		SO3H-Silica
Reprospher Acidosil-C		COOH-Silica

Selectivity in HPLC D4 Vergleich C18, Phenyl, C18-Phenyl

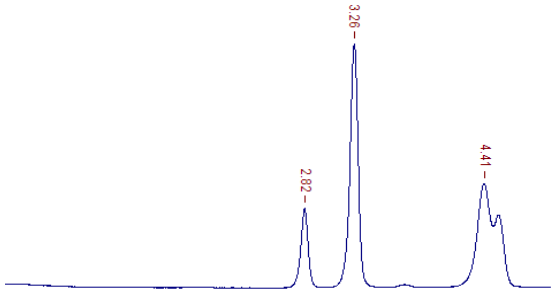
D4 Reprospher 100 C18



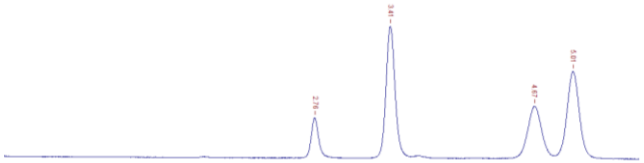
D4 Reprospher 100 C18-DE



D4 Reprospher 100 C18-Phenyl

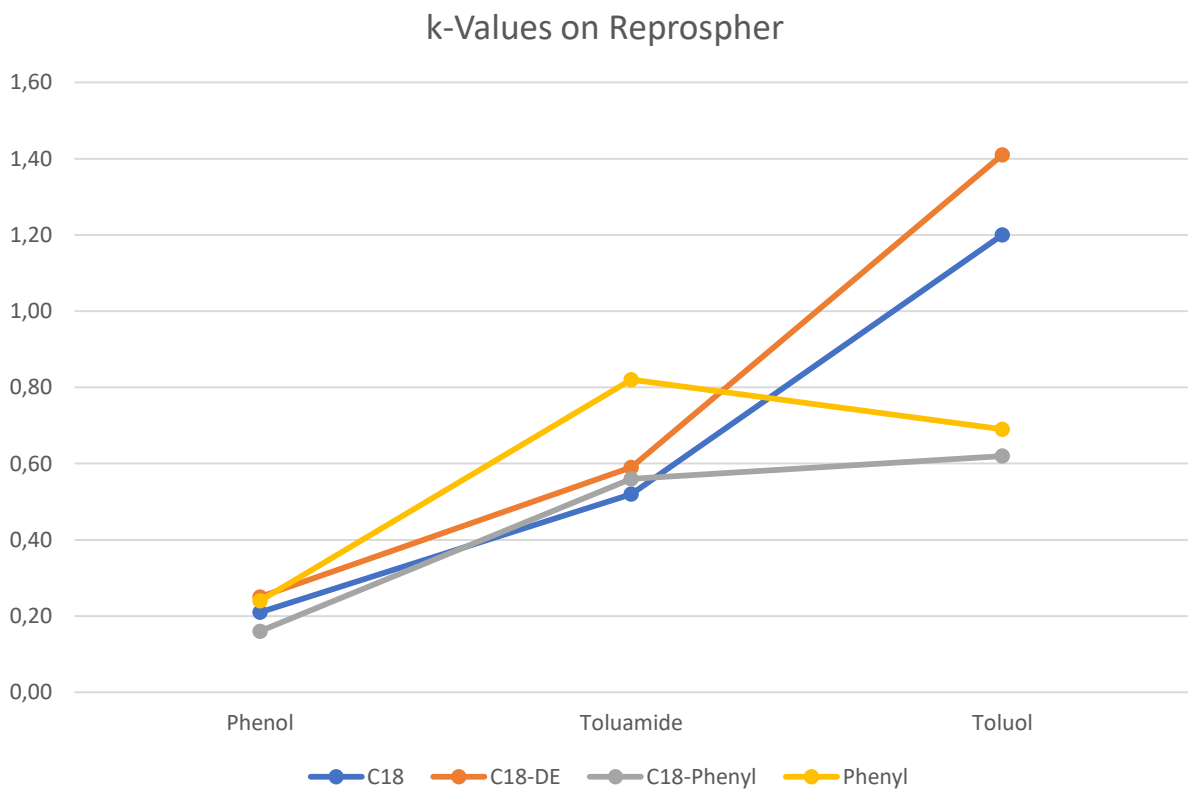


D4 Reprospher 100 Phenyl

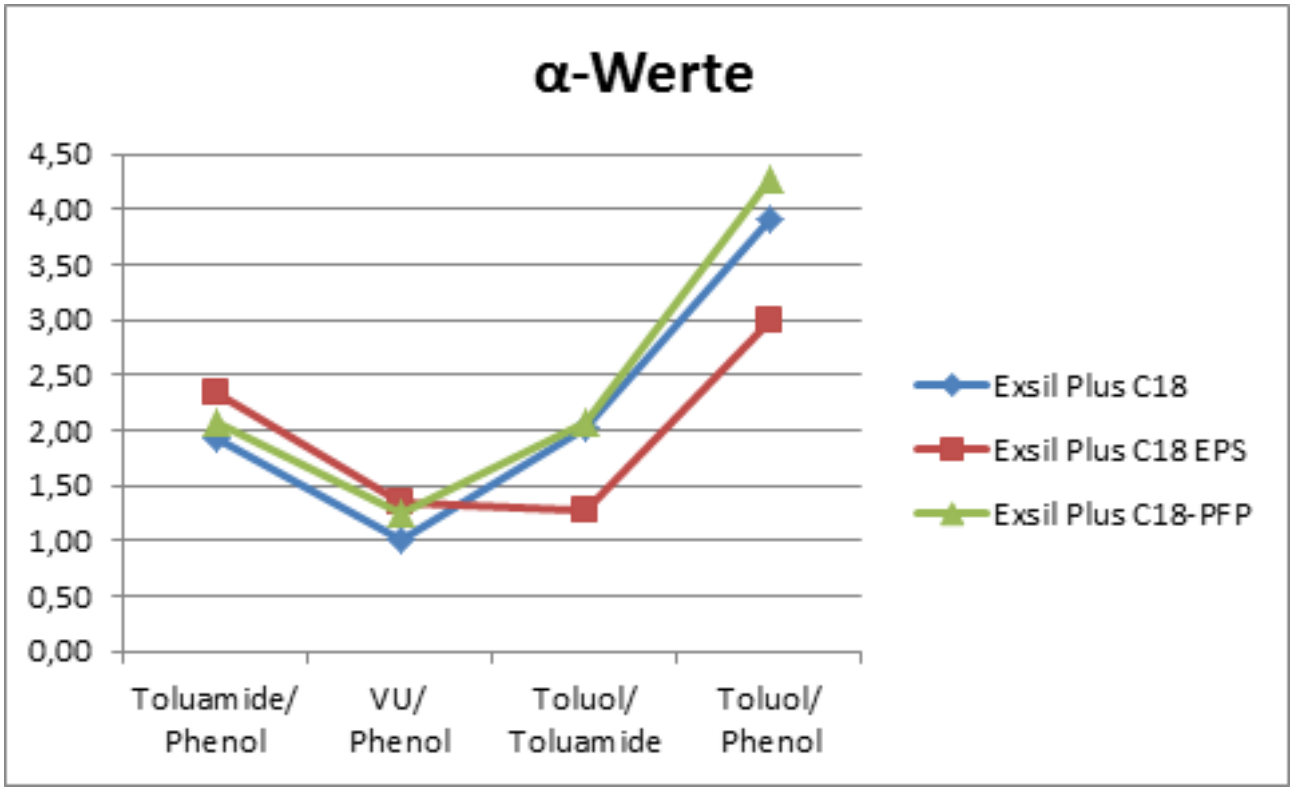


Selectivity in HPLC

D4-Test: C18, Phenyl, C18-Phenyl



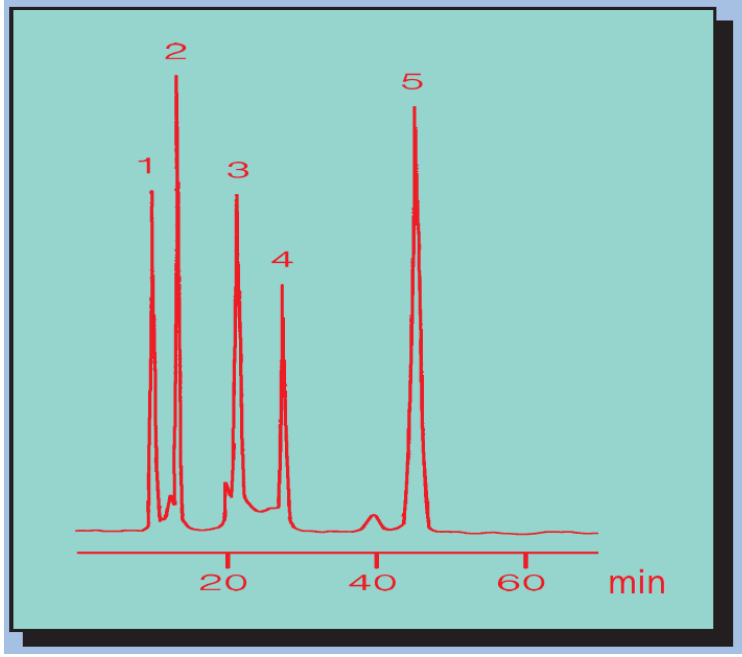
Selectivity in HPLC



Traditional Ion Exchange Chromatography

Salt gradient from low salt to high salt concentration

09 105 Separation of Proteins by Ion exchange HPLC



1) Trypsinogen
2) Ribonuclease A
3) Cytochrome C
4) Chymotrypsinogen
5) Lysozyme

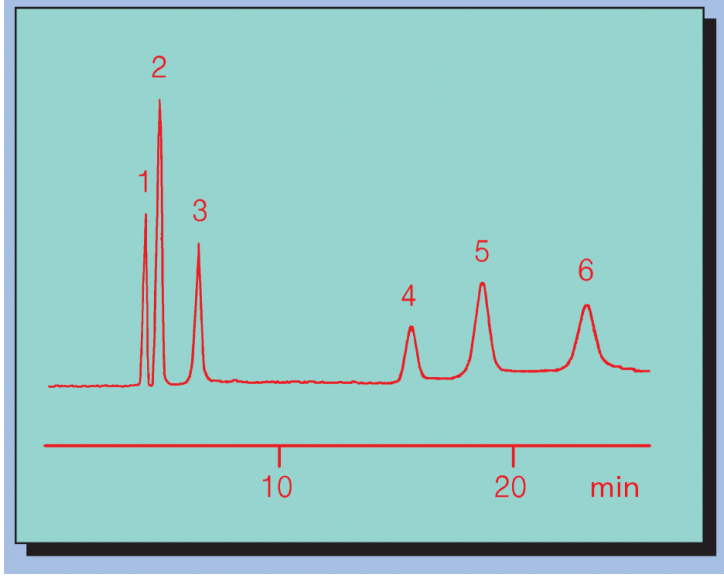
Column phase:		GROM-SIL 300 WCX, 7 µm
Column size:		250 x 4.6 mm
Eluent	**A:**	0.05 M NaH_2PO_4, pH 6.4
	B:	0.5 M NaH_2PO_4, pH 6.4
Gradient:		0-18% B (0-21 min), 18% B (21-26 min), 18-60% B (26-70 min)
Flow rate:		1.0 ml/min
Pressure:		7 MPa
Temperature:		RT
Detection (UV):		280 nm
Injection:		100 µl

Modern Ion Exchange Chromatography

3 possibilities for elution:

- Salt gradient from low salt to high salt concentration*
- pH shift / gradient
- ACN or MeOH gradient

07 127 Separation of Nucleotides



1) GMP
2) AMP
3) IMP
4) GDP
5) ADP
6) IDP

Column phase:		GROM-SIL 120 Amino-3 CP, 5 µm
Column size:		250 x 4.6 mm
Eluent	**A:**	5 mM KH_2PO_4, pH 2.9, 15% ACN
	B:	500 mM KH_2PO_4, pH 4.0, 15% ACN
Gradient:		40 - 100% B (0-20 min), 100% B (20-25 min)
Flow rate:		2 ml/min
Pressure:		14 MPa
Temperature:		RT
Detection (UV):		254 nm
Injection:		10 µl (160 µg/ml)

* For MS applications use volatile buffers up to 100 mM

Sterical - Multi Selectivity Effects

- Most of the RP/IEX phases are bonded with the RP and the IEX group: 2 different silanes are used
- The modern Mixed Mode Phases have the RP and the IEX group as one silane: the RP and IEX group has always the same distance and the sample molecule has two interaction points with a defined sterically orientation – goes a step ahead to Affinity Chromatography
- The similar situation we have with the Phenyl Phases:

  Propy-Phenyl: no mobility of the Phenyl group

  Hexyl-Phenyl: high mobility of the Phenyl group

  Biphenyl: two Phenyl groups in a chain with a defined orientation and distance

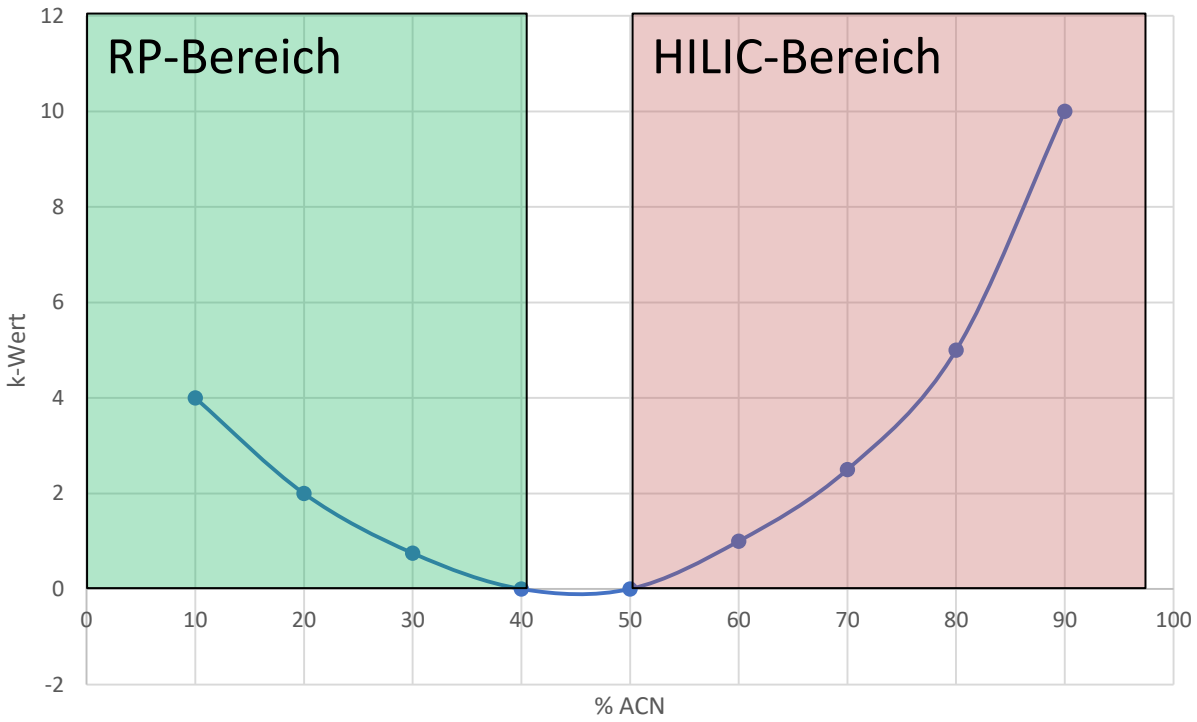
  Diphenyl: two Phenyl groups in tetraeder angle with a defined orientation and distance

Mobile Phase Strategies for RP / IEX

- Starting at low salt and low organic modifier:
  gives maximum retention for RP and IEX groups
- Keeping the salt concentration low and increase org. modifier
  IEX keeps longer active and RP elution starts
- Increasing salt concentration and org. modifier
  IEX and RP elution starts
- Using MeOH as org. modifier
  supports IEX / dissociation and leads to more retention
- Using ACN as org. modifier
  reduces IEX / dissociation and therefore retention

Selectivity in HPLC – charge situations

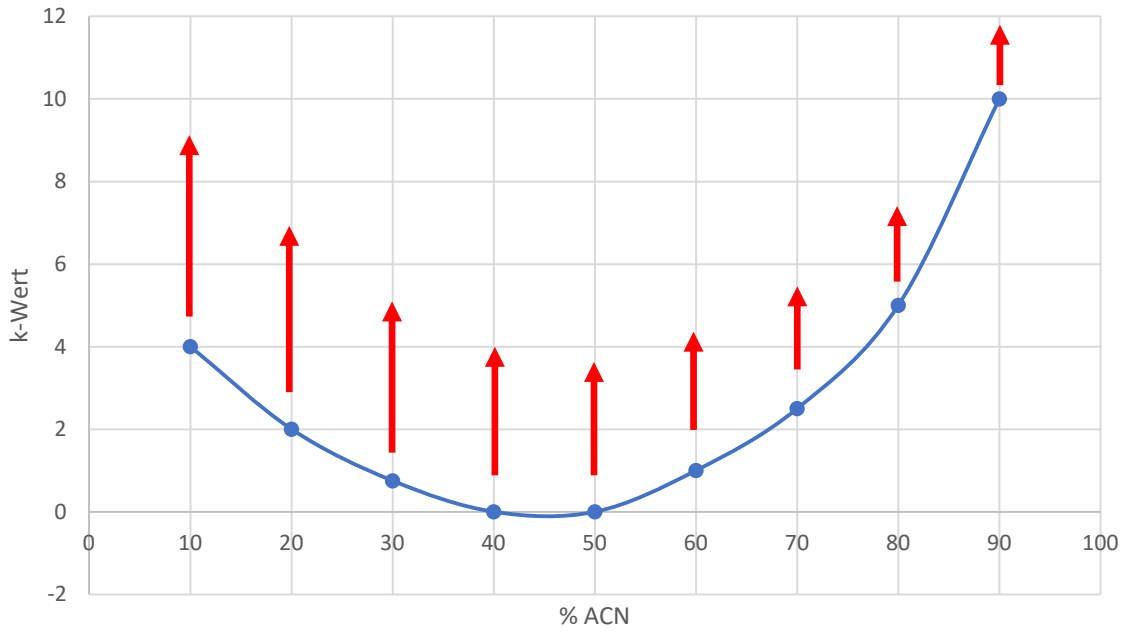
Amino Phase: Aminopropyl



Selectivity in HPLC – charge situations

Amino Phase:
Aminopropyl at a pH of 3-7 in the mobile Phase

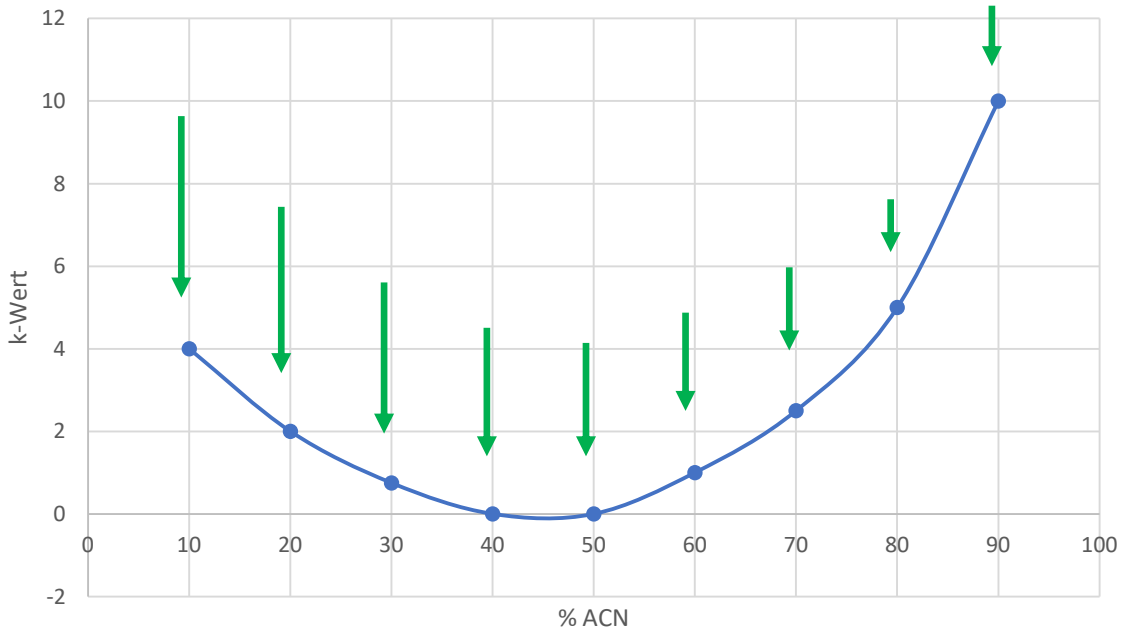
- Acidic Molecules: Increasing Retention – additional IEX
- More Acetonitrile – lower dissociation



Selectivity in HPLC – charge situations

Amino Phase:
Aminopropyl bei pH-Wert im Eluent 3-7

- basic Molecules: decreasing retention
- more Acetonitrile – lower Dissociation



Influence of Mobile Phase

Conversion of the eluent composition with the solubility parameter δ

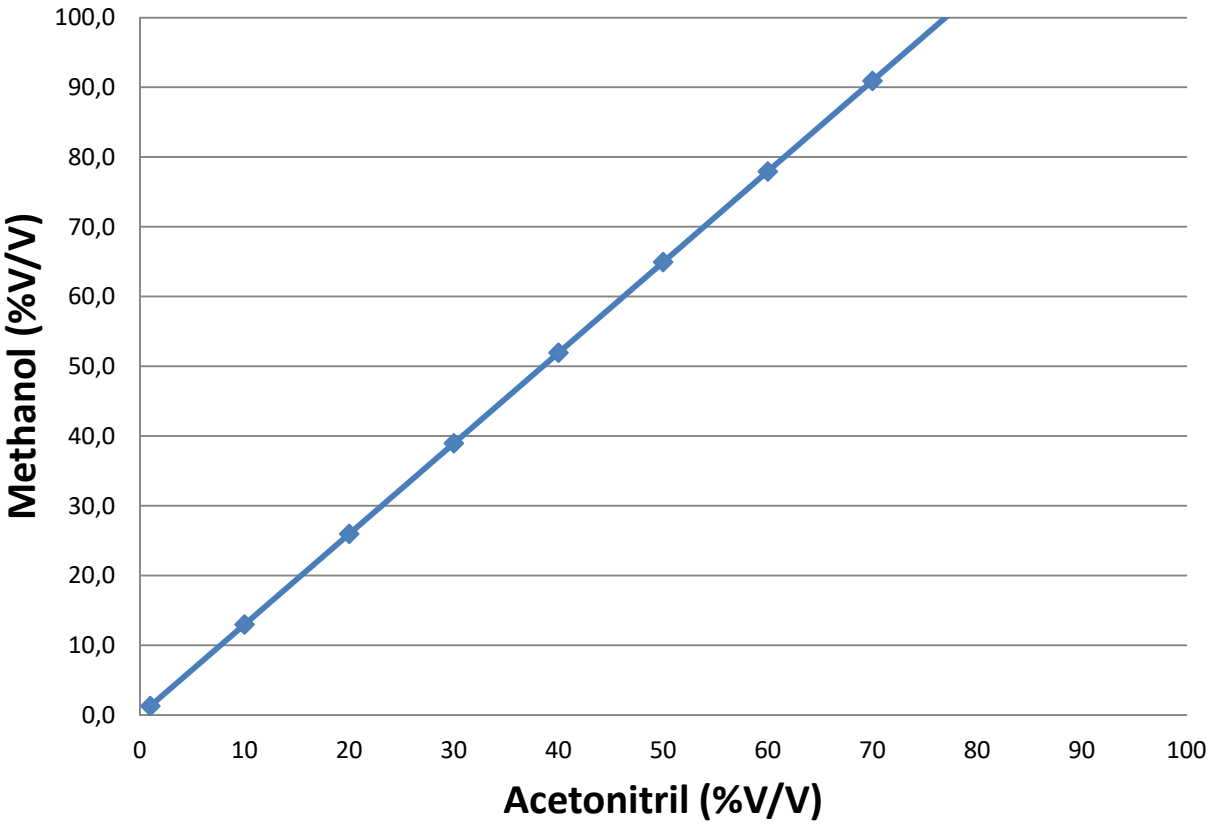
Acetonitril	δ_A	23,9
Water	δ_W	47,8
Methanol	δ_M	29,4
Mixture	δ_m	35,85

δ = Solubility parameter

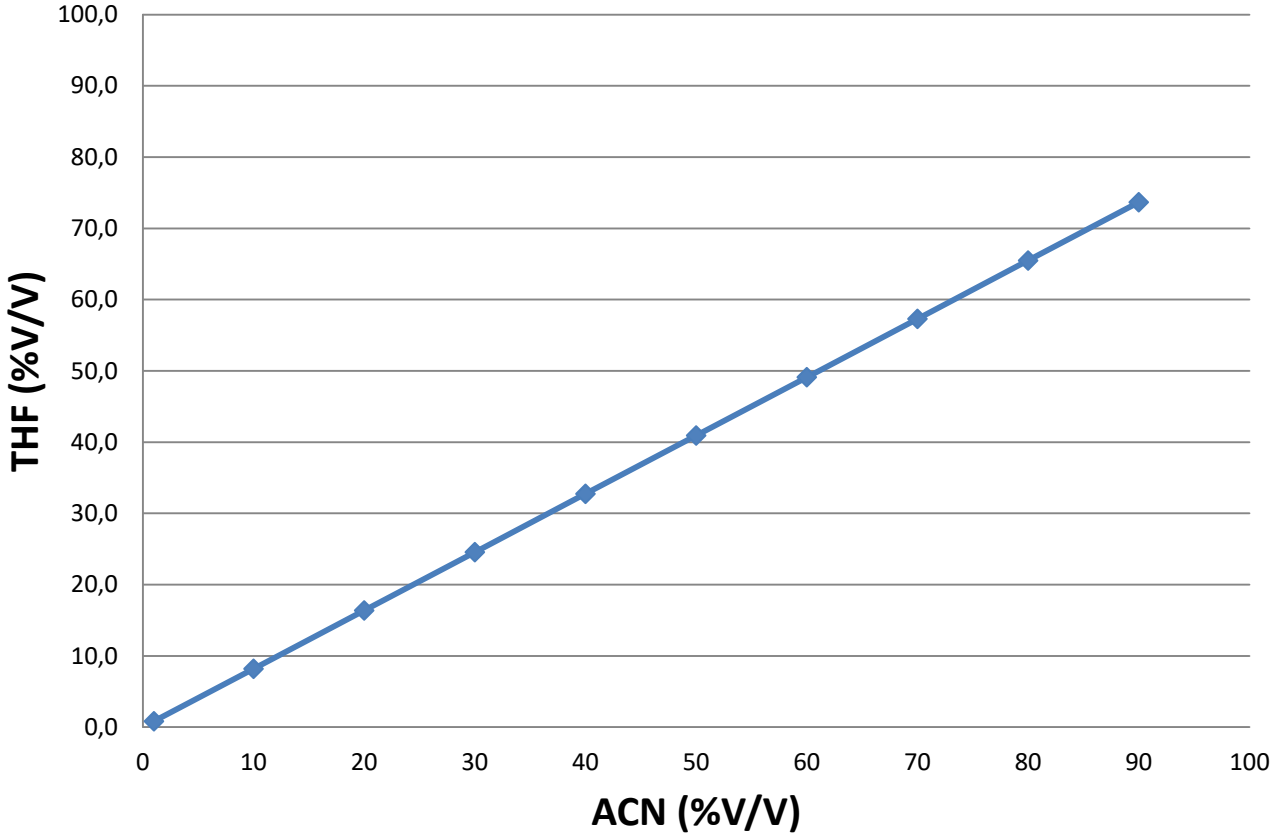
ϕ = Volume part

$$\delta_m = \Sigma_i \phi_i \delta_i$$

Influence of Mobile Phase



Influence of Mobile Phase



Influence of Mobile Phase

Column test mix:

Uracil
Phenol
N,N-Diethyl-M-Toluamide
Toluone

Standard eluent:
ACN : Water = 58 : 42

calculated:
MeOH : Water = 75 : 25

adjusted:
MeOH : Water = 65 : 35

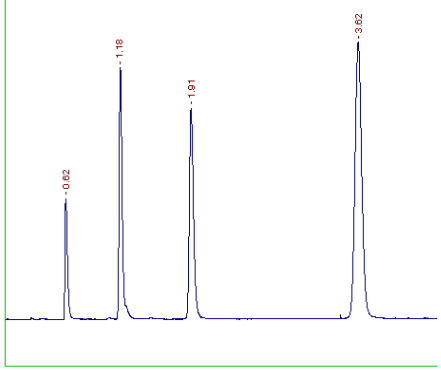
Influence of Mobile Phase

Standard C18

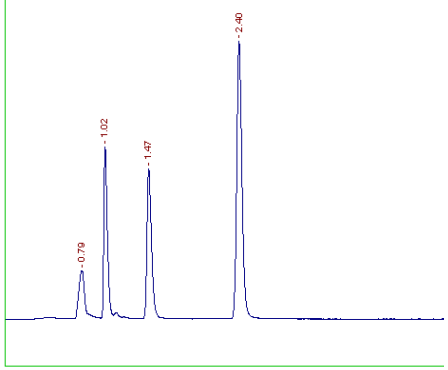
Reprospher C18, 3 µm;
100 x 2 mm

Standard C18

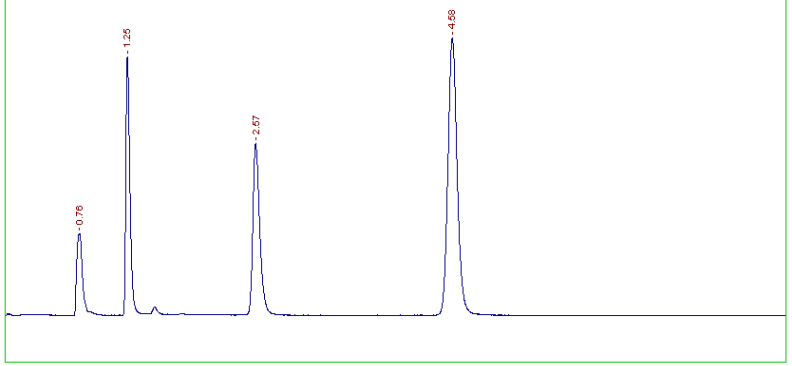
ACN : Wasser = 58 : 42



MeOH : Wasser = 75 : 25



MeOH : Wasser = 65 : 35



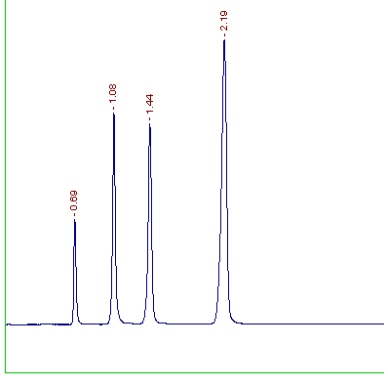
Influence of Mobile Phase

Polar C18

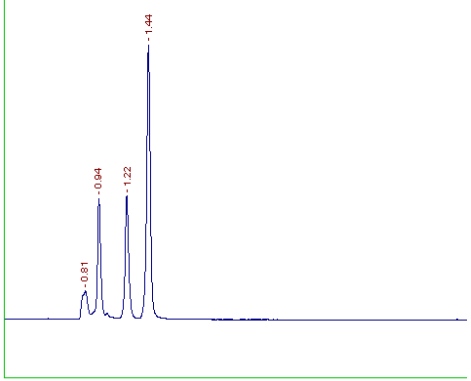
Platinum C18, 3 µm;
100 x 2 mm

Polare C18 Phase

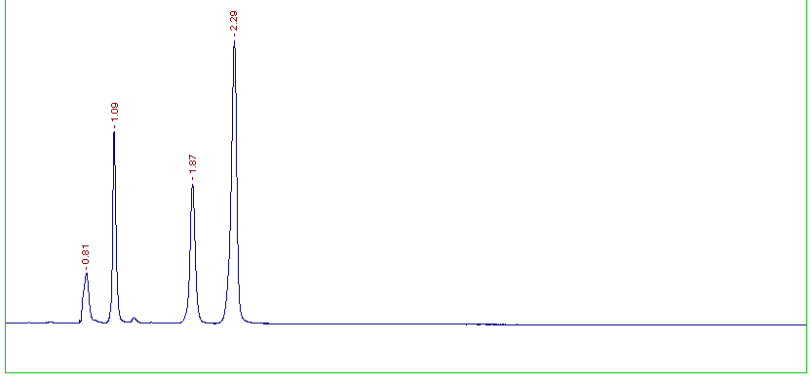
ACN : Wasser = 58 : 42



MeOH : Wasser = 75 : 25



MeOH : Wasser = 65 : 35



Influence of Mobile Phase

Phenyl - Phase

Reprosil Pur Phenyl, 4 µm;
100 x 2 mm