Improving the Analysis of Herbal Medicinal Products by Fused Core™ particle technology and evaluation of different Liquid Chromatography Stationary Phase Chemistries

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Content

- Herbal Medicinal Products – what are the challenges?
- Fused Core technology
- Selectivity as best approach to solve separation challenges

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Challenges in QC, in-process control and stability testing of Herbal Medicinal Products

Constituent with known therapeutic activity
Constituents which are generally accepted to contribute substantially to the therapeutic activity
Active Marker
Generally accepted to contribute to the therapeutic activity but are not responsible for the full therapeutic effect
Analytical Marker
Characteristic constituents that serve sole analytical purposes

Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003370.pdf).
Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003272.pdf).

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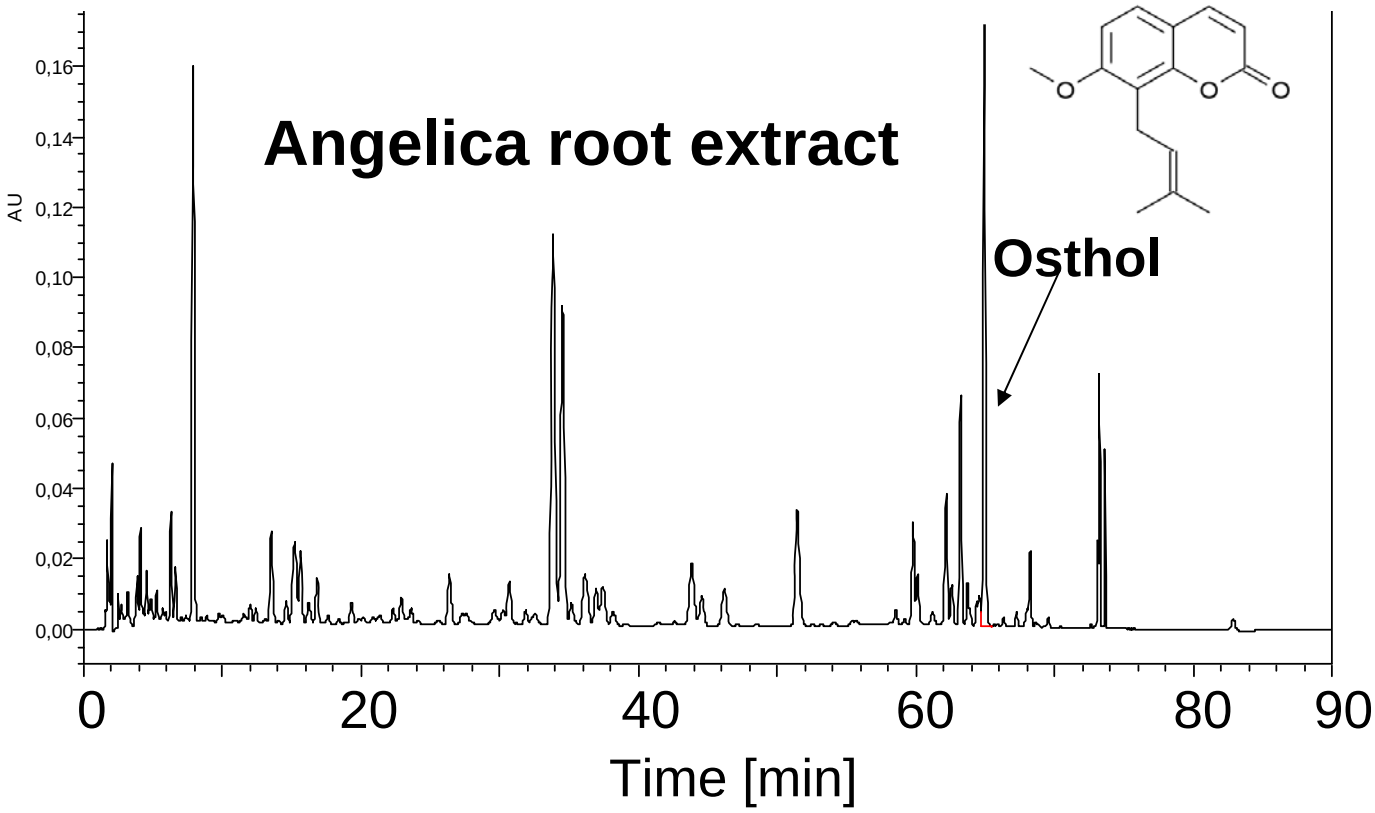
Challenges in QC, in-process control and stability testing of Herbal Medicinal Products



Stat. Phase:
Reprosil-Pur ODS-3, 150 x 4,6 mm, 3 µm
Mob. Phase:
Wasser/TFA/ACN/MeOH, Gradient
Flow rate:
1,0 ml/min
Temperature:
45 ° C
Detection:
323 nm
HPLC system:
Waters Alliance 2695

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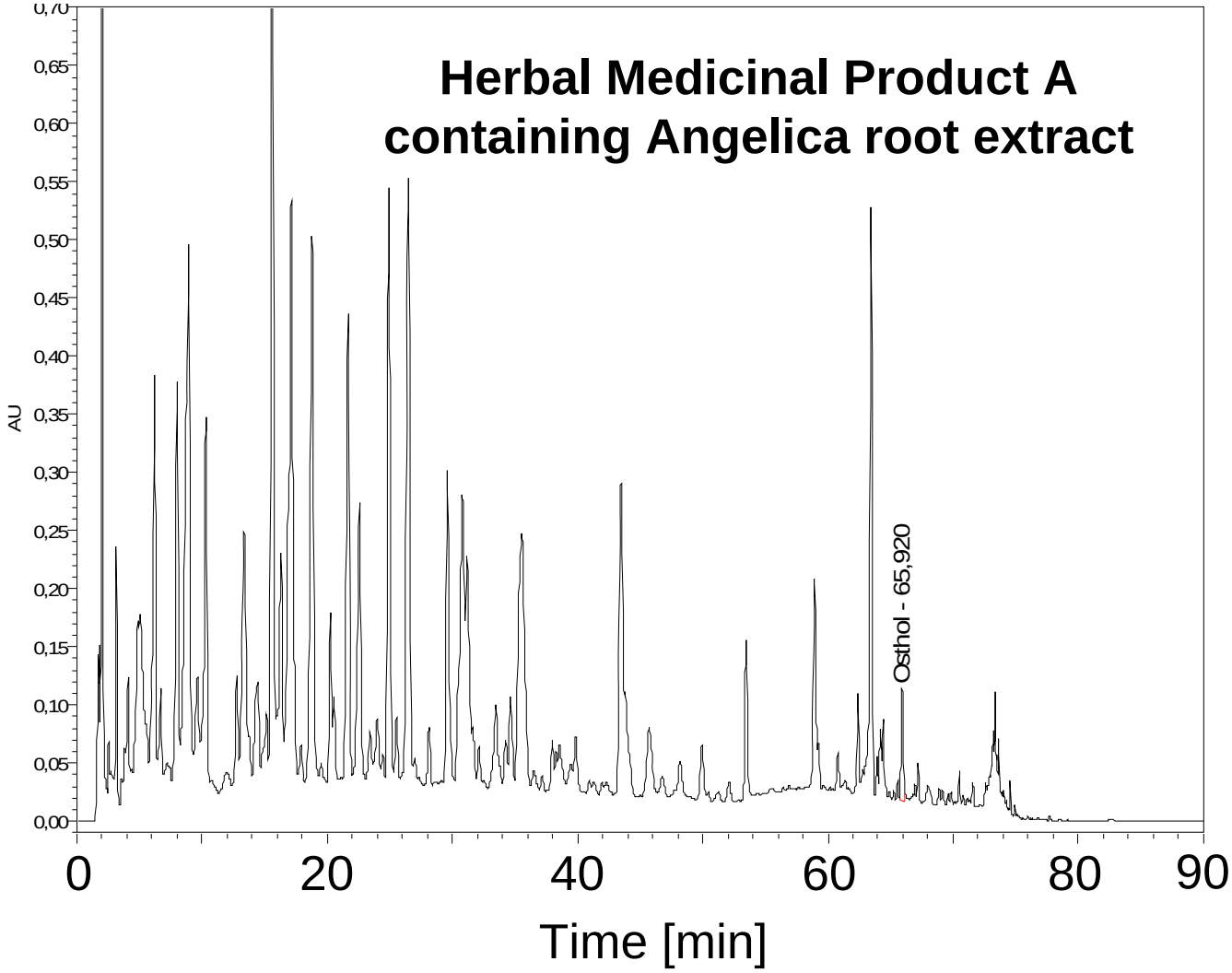
Challenges in QC, in-process control and stability testing of Herbal Medicinal Products



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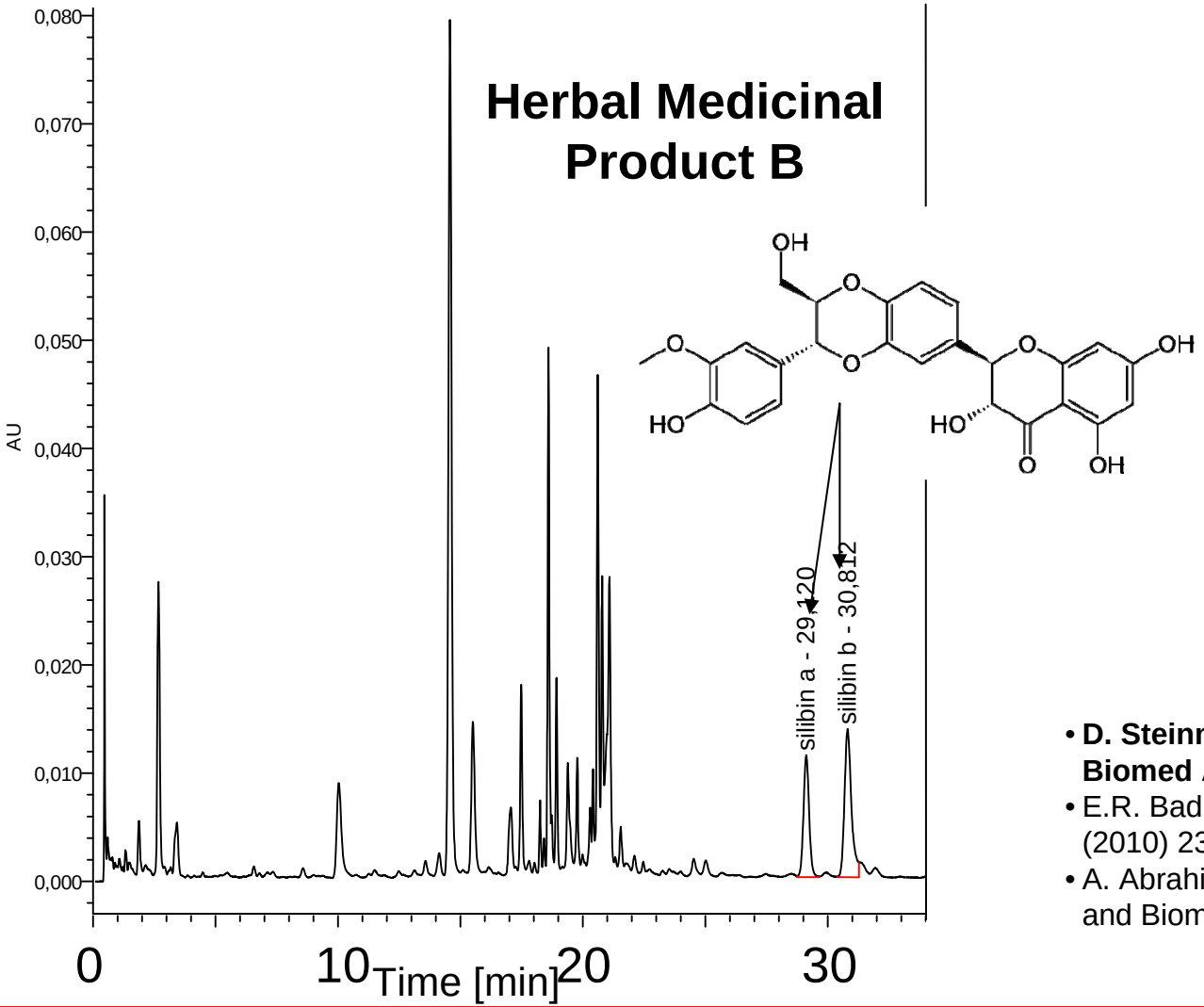
Improvements in analysis of Herbal Medicinal Products by Fused Core™ technology



Stat. Phase:
Ascentis Express C18, 100 x 2.1 mm (2.7 µm)
Mob. Phase:
Water/TFA/ACN/MeOH, Gradient
Flow rate:
0,4 ml/min
Temp.: **Det.:**
30 ° C 287 nm
HPLC system:
Waters Acquity UPLC

- **D. Steinmann, M. Ganzera, J Pharm Biomed Analysis, 55/4 (2011) 744-757**
- E.R. Badman et al., J. Chromatogr. B 878 (2010) 2307–2313
- A. Abrahim et al., Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 131–137

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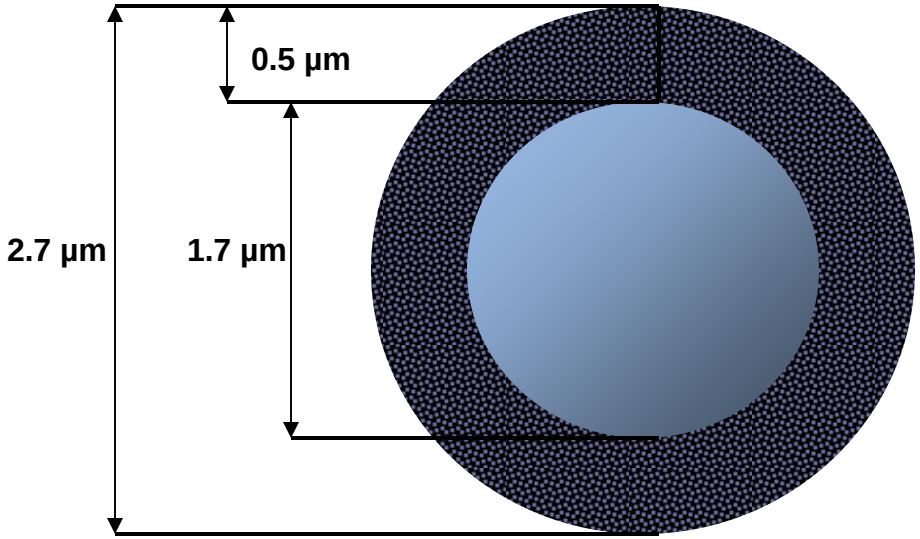
Fused-Core technology

- Innovative approach in HPLC by Dr. J. J. Kirkland in 2006
- Porous silica with high capacity on solid, non-porous core
- Highly pure silica



- **2.7 µm silica particle**
- **1.7 µm solid core**
- **0.5 µm porous SiO_2 layer**
- **90 Å pore size**
- **Very narrow particle size distribution**
- **C18, C8, HILIC, RP-Amide, Phenyl-Hexyl, Penta-fluorophenyl, Peptide**

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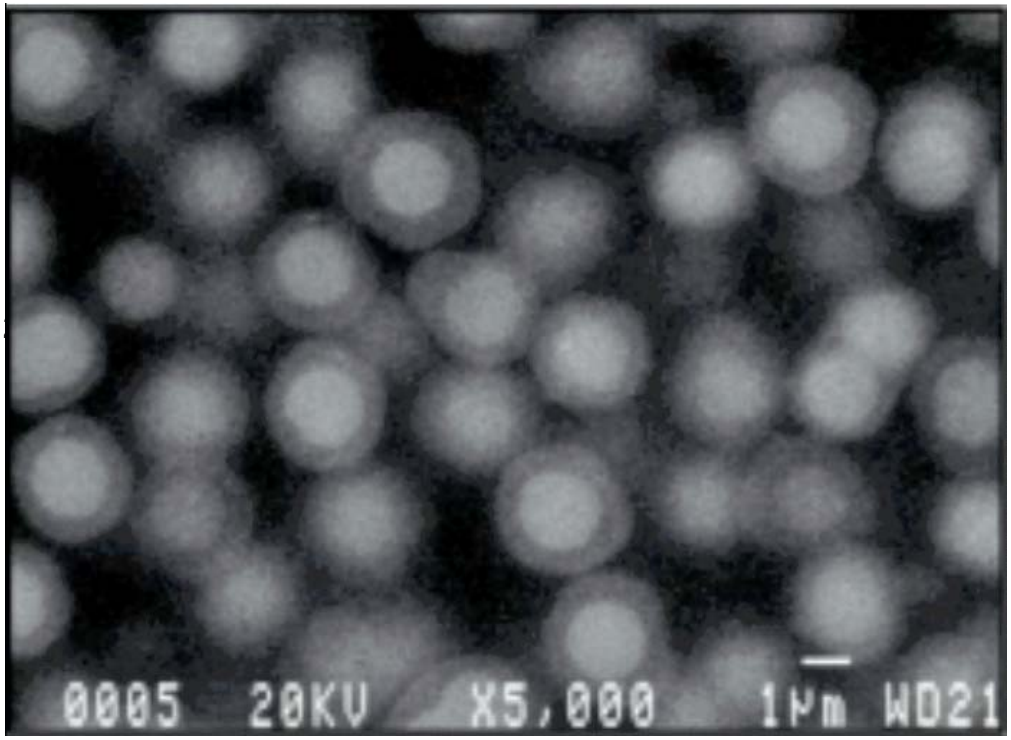

- **2.7 µm silica particle**
- **1.7 µm solid core**
- **0.5 µm porous SiO_2 layer**
- **90 Å pore size**
- **Very narrow particle size distribution**
- **C18, C8, HILIC, RP-Amide, Phenyl-Hexyl, Penta-fluorophenyl, Peptide**

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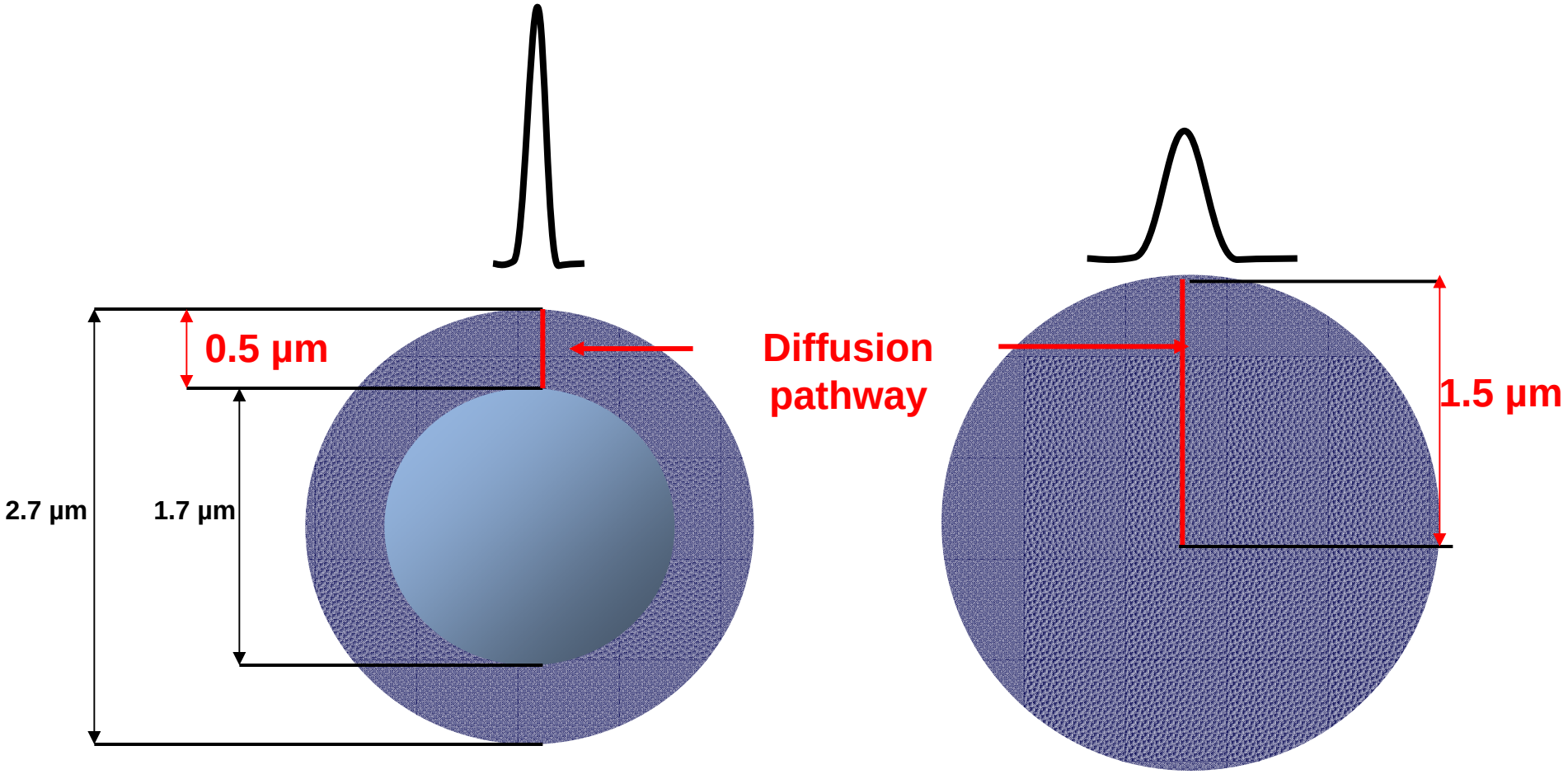
Fused-Core technology provides higher efficiency



The shorter diffusion pathway facilitates the mass transfer!

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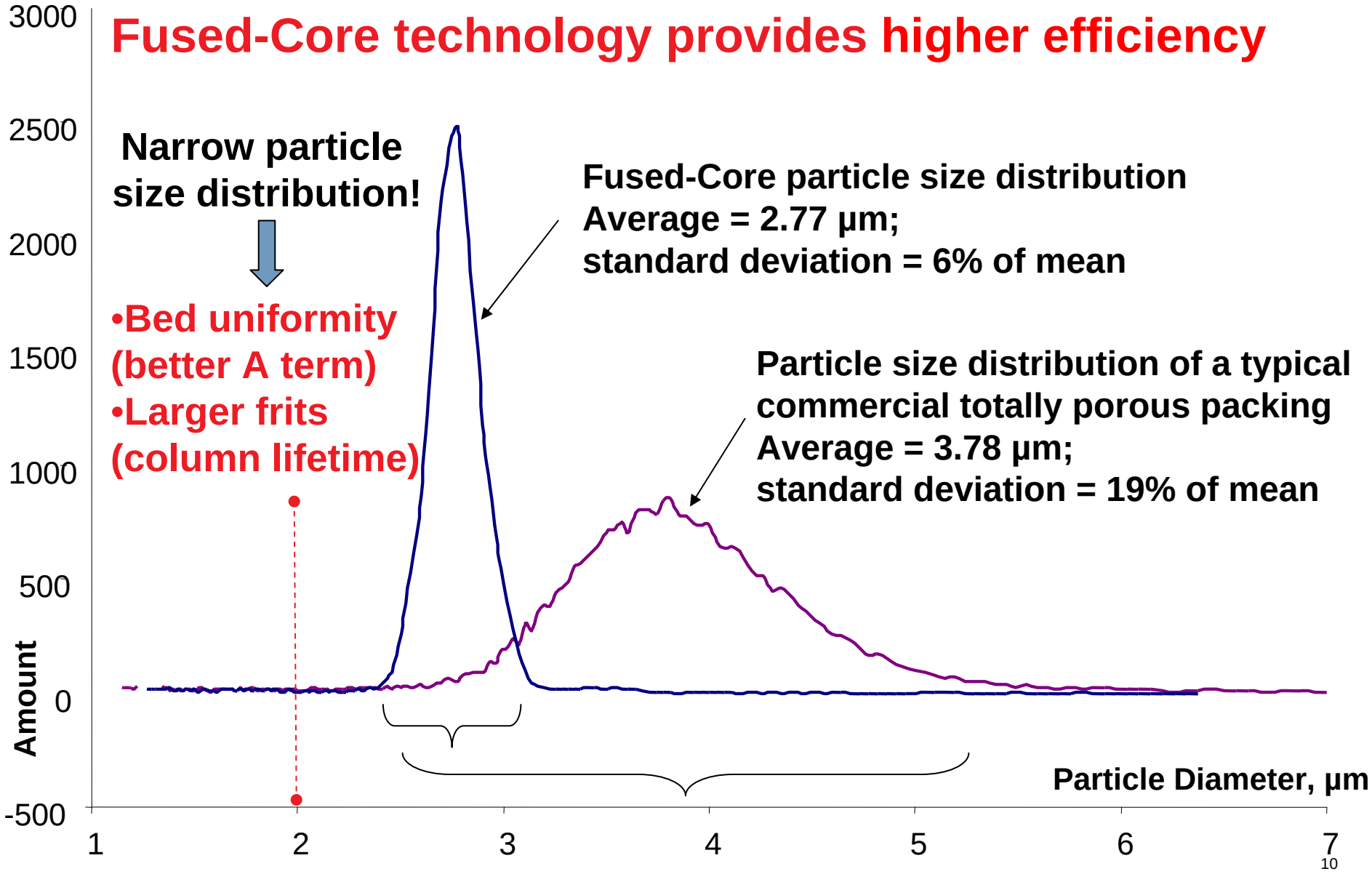
Fused-Core technology provides higher efficiency



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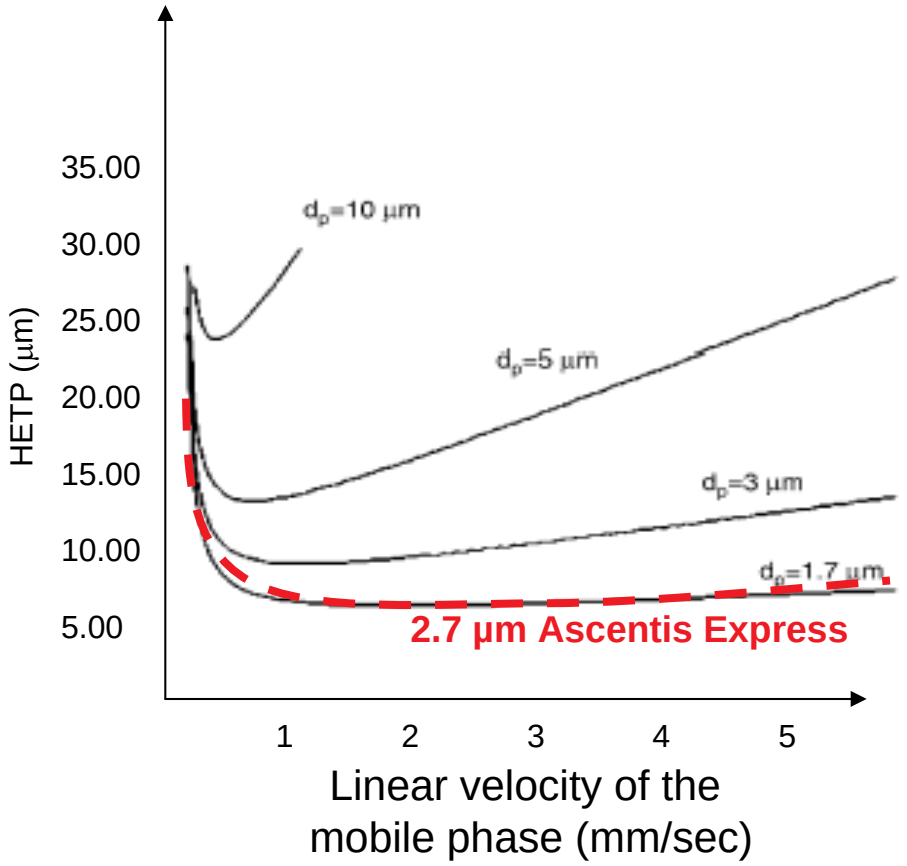
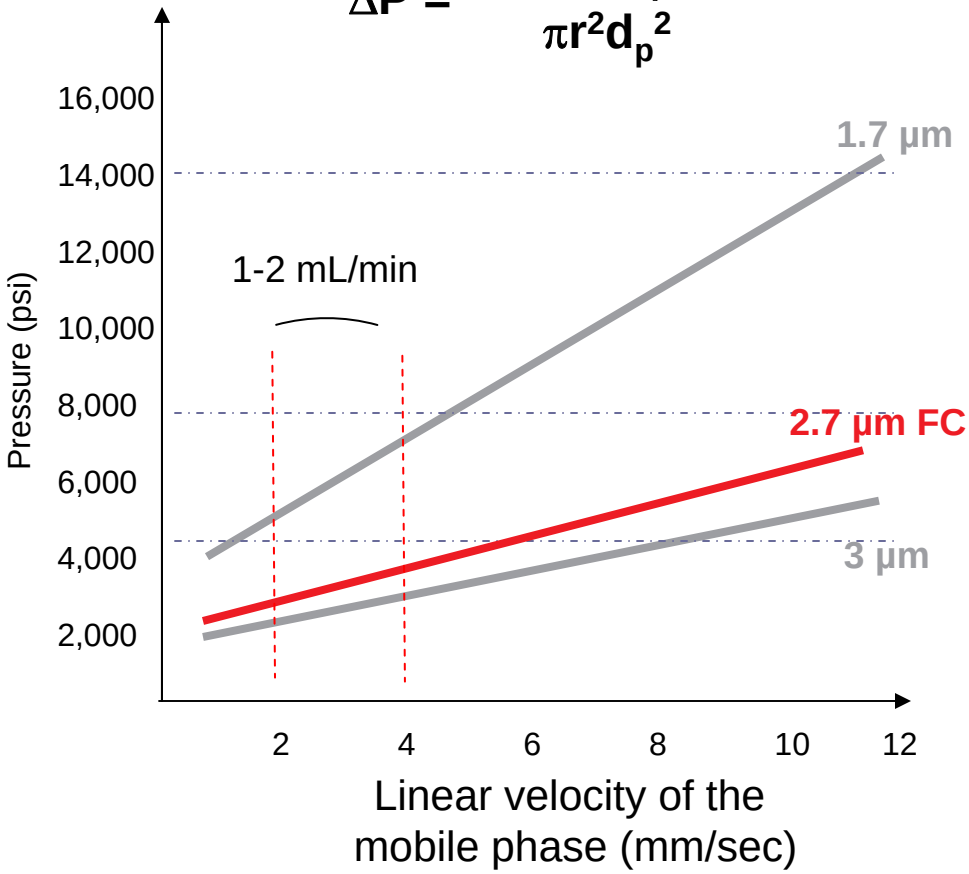
Comparison of pressure and efficiency

$$H = A + B/u + Cu$$



$$\Delta P = \frac{1000F\eta L}{\pi r^2 d_p^2}$$



*50x4.6 mm columns, 55/45 ACN/water

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Three Factors Control HPLC and UHPLC Resolution

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{\alpha - 1}{\alpha}$$

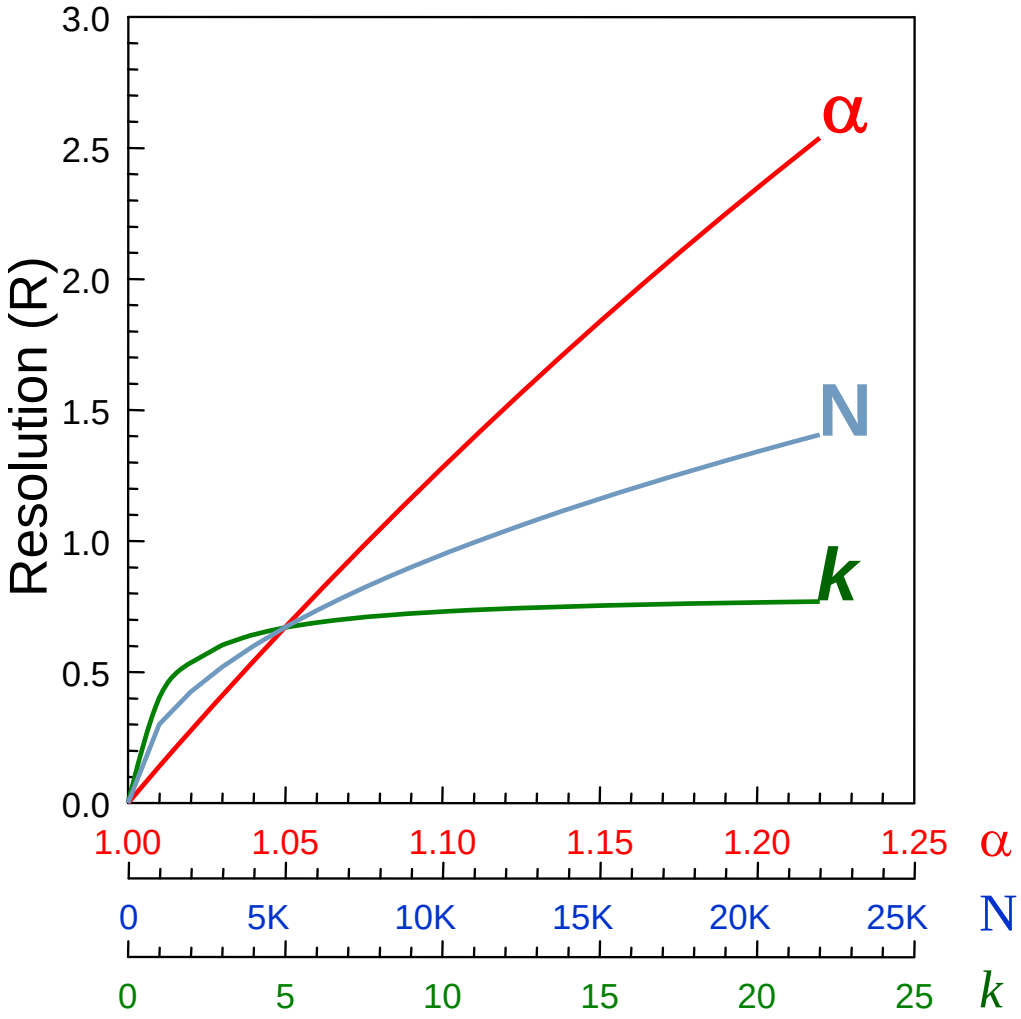
$N = 16\,(t_R/w)^2$ or

$N = 5.5\,(t_R/w)^2$

$k = (t_R - t_0)/t_0$

$\alpha = k_2/k_1$

All factors are important, but selectivity is considered the most powerful term.



Zhao, J.H., P.W. Carr., Anal. Chem. 71(14) (1999) 2623-2632

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Selectivity Variables in Reversed-Phase *

- Continuous variables (solvent):
  - type (organic, water)
  - pH (especially ionizable solutes)
  - additives (type and concentration)
  - temperature
  - solvent strength
- Discontinuous variable (column):
  - Stat. type (phase and substrate)

More predictable
(modeling software available)

Some analysts may spend too much time here "force feeding" C18 columns.

Less predictable
(screening required)

* Excerpted with permission from John Dolan, 2009 Minnesota Chromatography Forum Spring Symposium; adapted by R. Henry.

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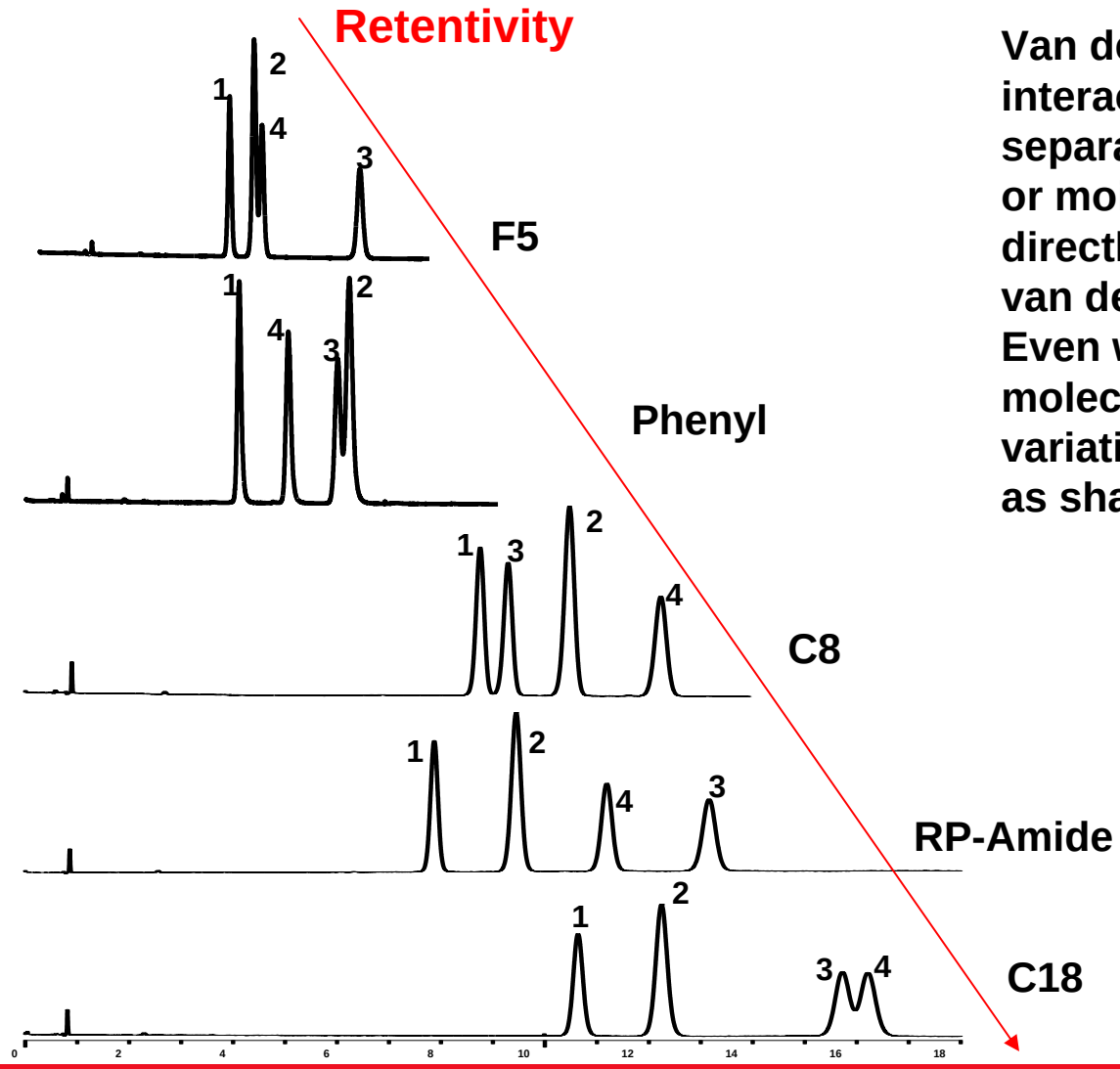
Selectivity by different stationary phases



Van der Waals interactions are the main interaction responsible for the separation of hydrophobic molecules or moieties. Amylbenzene retention is directly related to the strength of the van der waals interactions of a phase. Even with strictly hydrocarbon molecules, there are selectivity variations based on such phenomena as shape selectivity.

1. Butylbenzene
2. o-Terphenyl
3. Triphenylene,
4. Amylbenzene

Col. Dim.: 15x4.6 5um
65% ACN/35% Water
1.5mL/min, 220nm, 5uL

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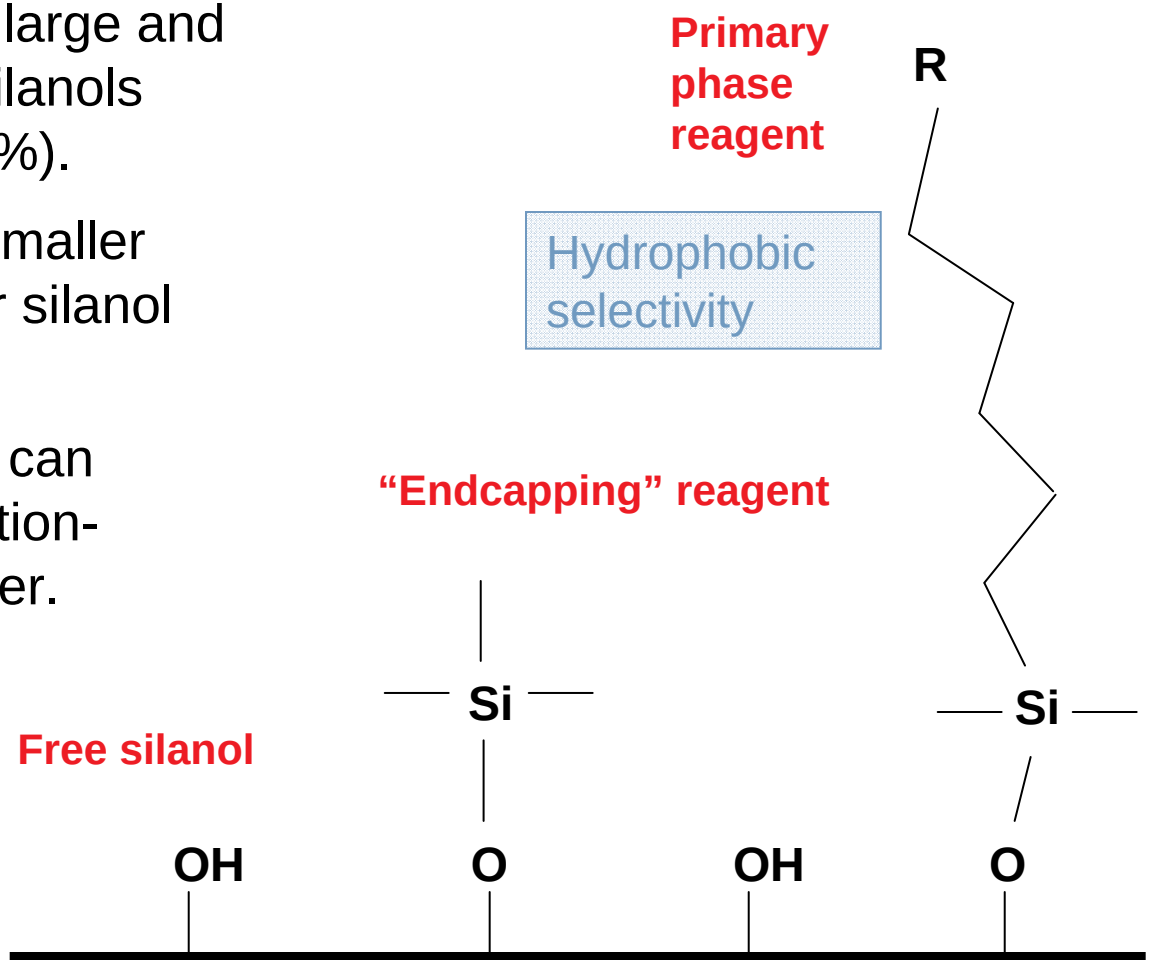
Alkyl Bonded Phases (C18 and C8)

- C18 reagents are large and can leave some silanols unreacted (ca. 50%).
- C8 reagents are smaller and provide better silanol coverage
- At pH >4, silanols can ionize and add cation-exchange character.



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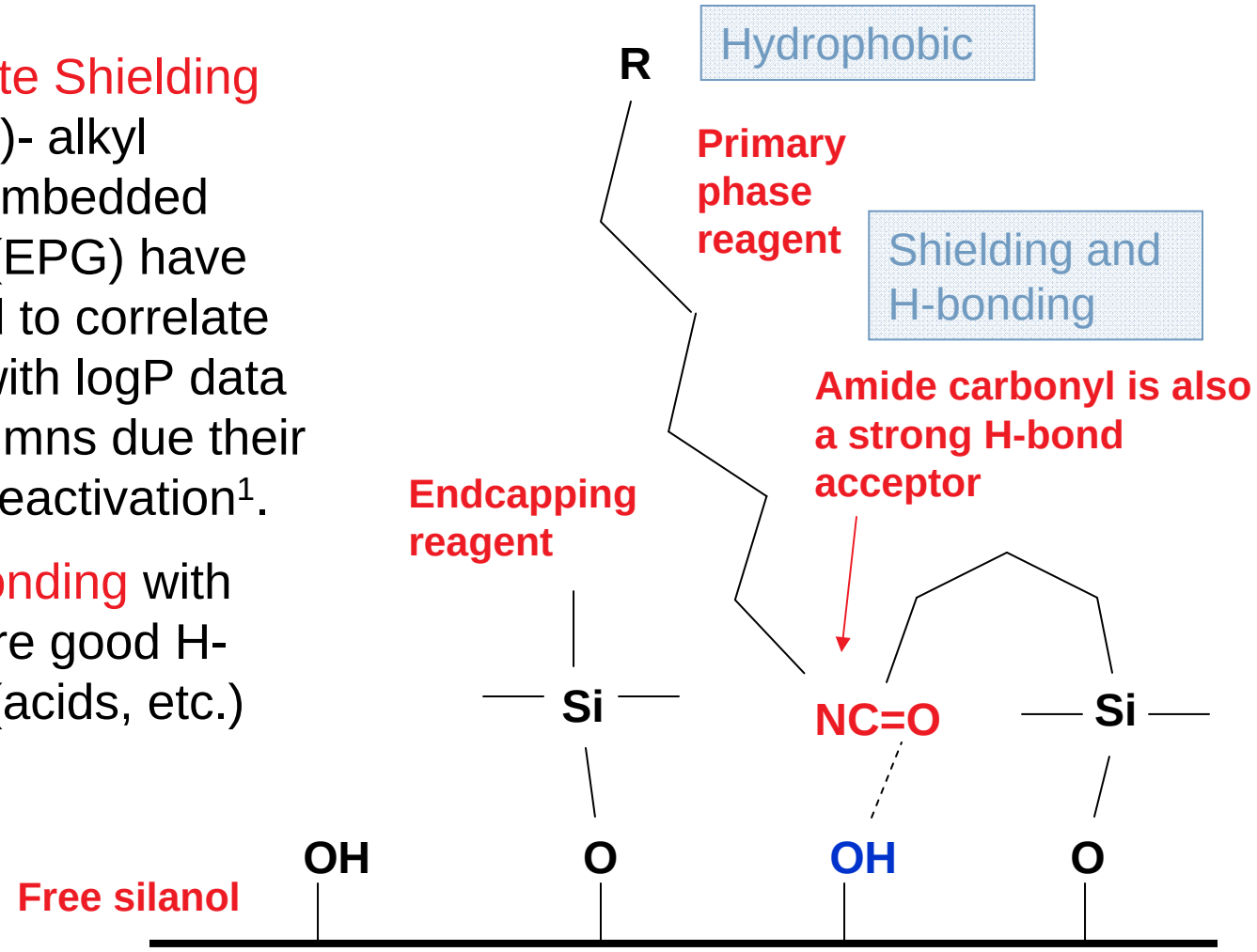
Alkyl Amide Bonded Phases (abbreviated: RP-Amide)

- Possible Solute Shielding (basic solutes)- alkyl phases with embedded polar groups (EPG) have been reported to correlate much better with logP data than C18 columns due their higher base deactivation[1].
- Possible H-bonding with solutes that are good H-bond donors (acids, etc.)



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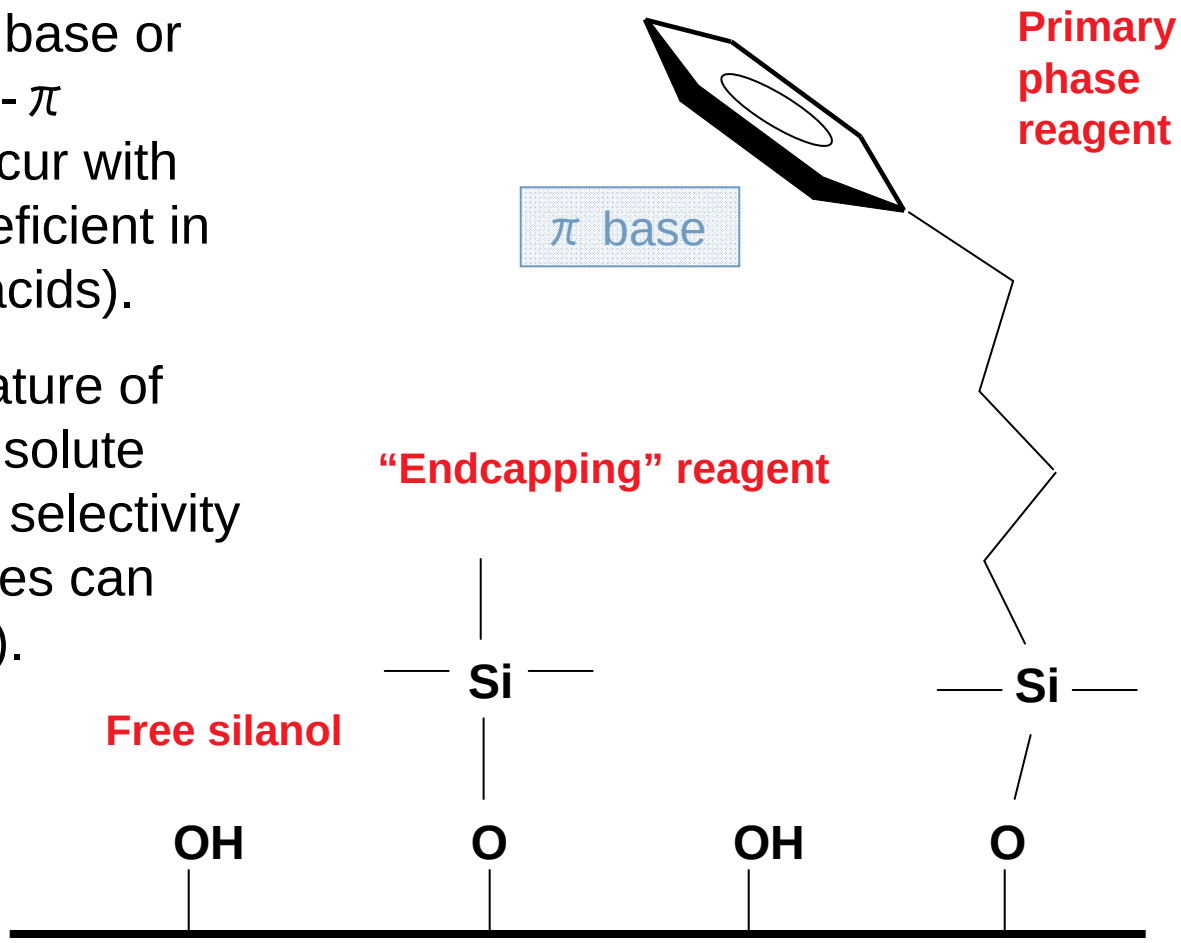
Phenyl Bonded Phases

- Phenyl is a Lewis base or electron donor; π - π interaction can occur with solutes that are deficient in electrons (Lewis acids).
- Due to the rigid nature of the aromatic ring, solute shape can dictate selectivity (how closely solutes can approach the ring).



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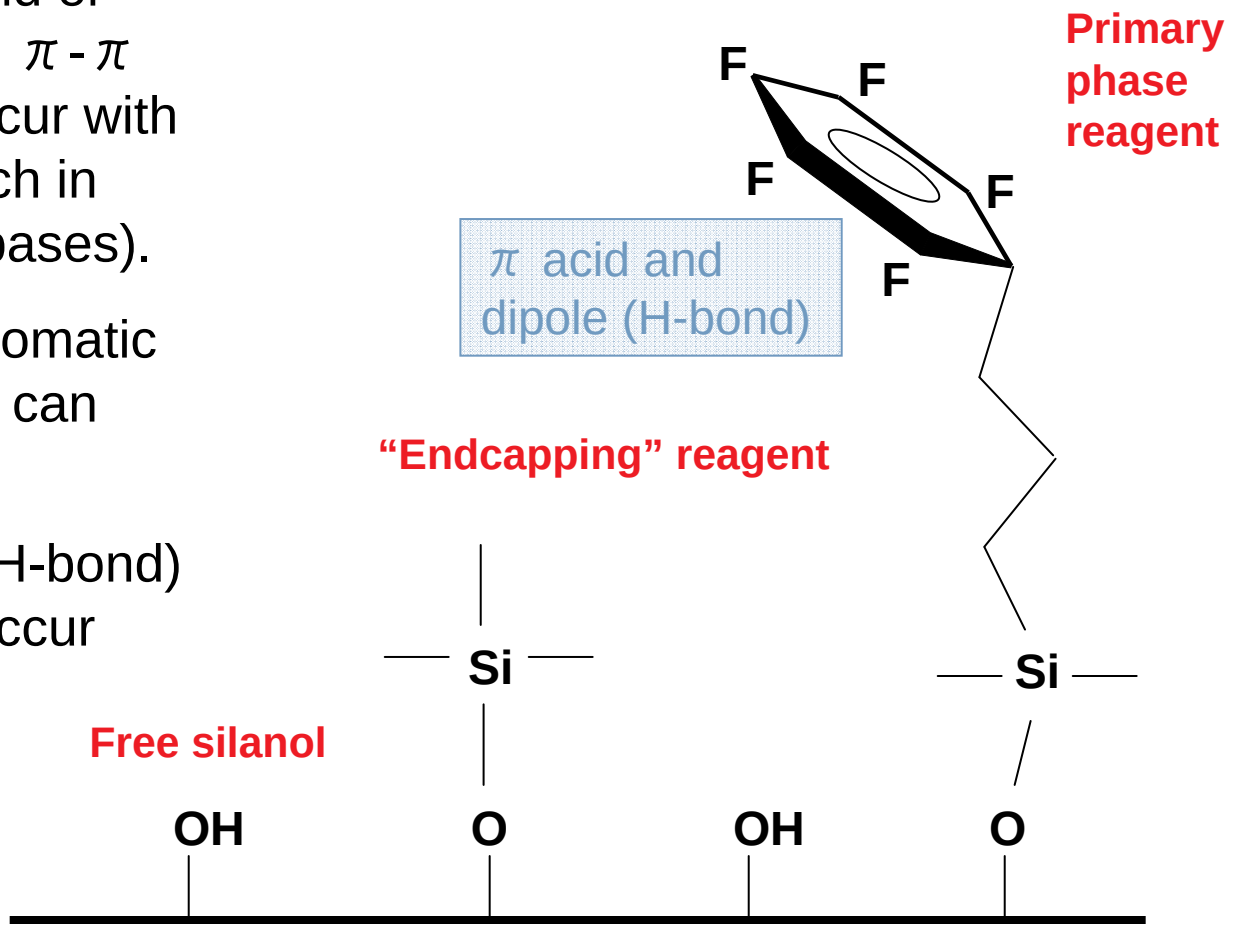
Pentafluorophenyl (F5) Bonded Phases

- PFP is a Lewis acid or electron acceptor; π - π interaction can occur with solutes that are rich in electrons (Lewis bases).
- Due to the rigid aromatic ring, solute shape can dictate selectivity.
- Dipolar (possible H-bond) interactions can occur



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Classification by Possible Chemical Interactions[a]

Bonded Phase	Hydrophobic	H-Bonding	Dipolar	π - π	Steric[b]	Ionic[b]
C18	Very Strong	Weak	No	No	No	Moderate
C8	Strong	Weak	No	No	No	Weak
RP-Amide	Strong	Strong Acceptor	Moderate	No	Weak	Very weak
Phenyl	Strong	Weak Acceptor	Weak	Strong Donor	Strong (Rigid)	Weak
F5 or PFP	Moderate	Moderate Acceptor	Strong	Strong Acceptor	Strong (Rigid)	Moderate
Cyano	Light to Moderate	Weak Acceptor	Strong	Weak	No	Moderate

a. Using Euerby[2] variation of Snyder-Dolan-Carr Hydrophobic Subtraction Model[3].

b. Steric and Ionic probe data are not very helpful in predicting or interpreting steroid selectivity results; however, they are always underlying factors with silica bonded phases.

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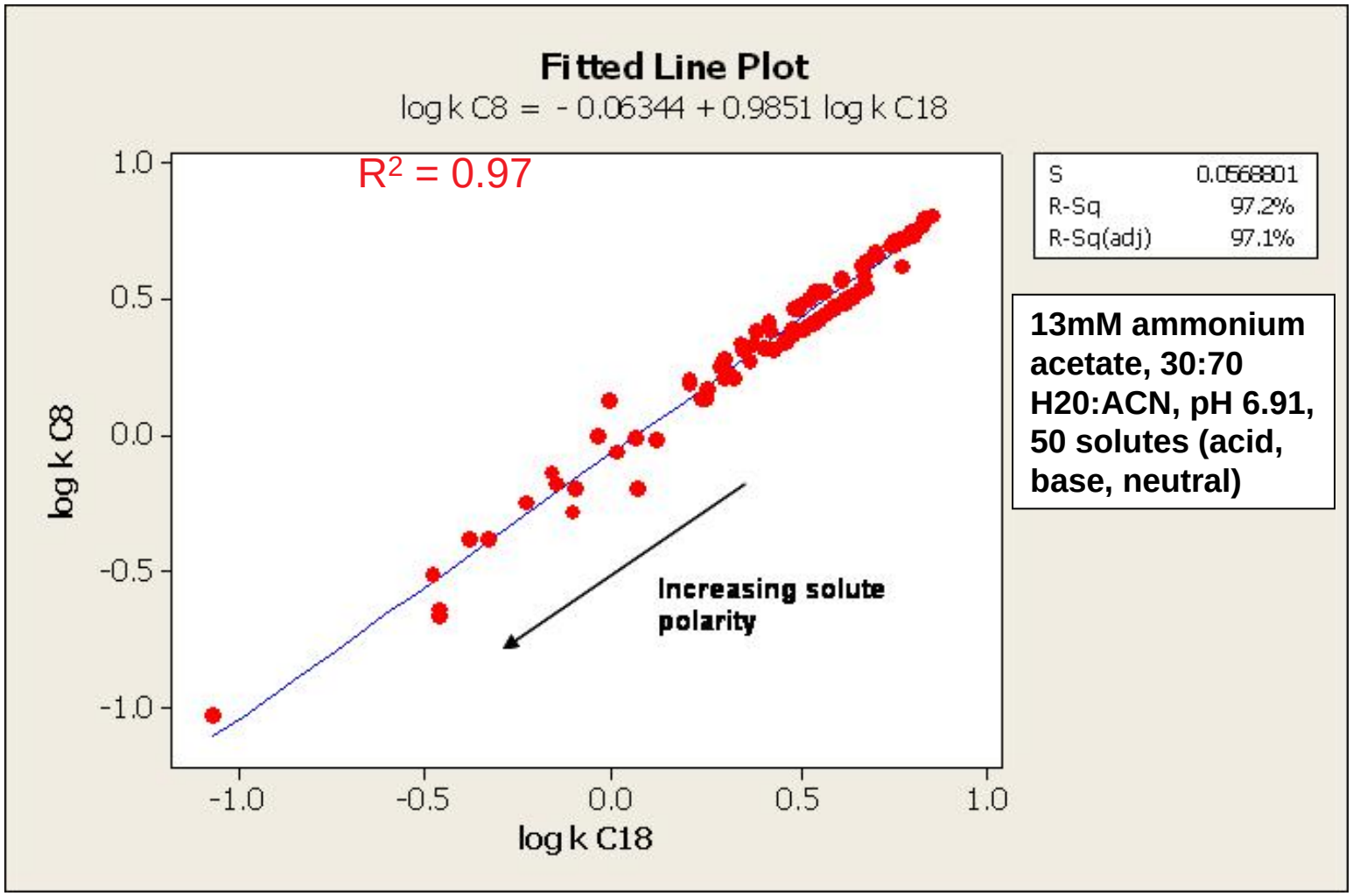
Columns with same or similar phases should show high correlation: C18 vs C8



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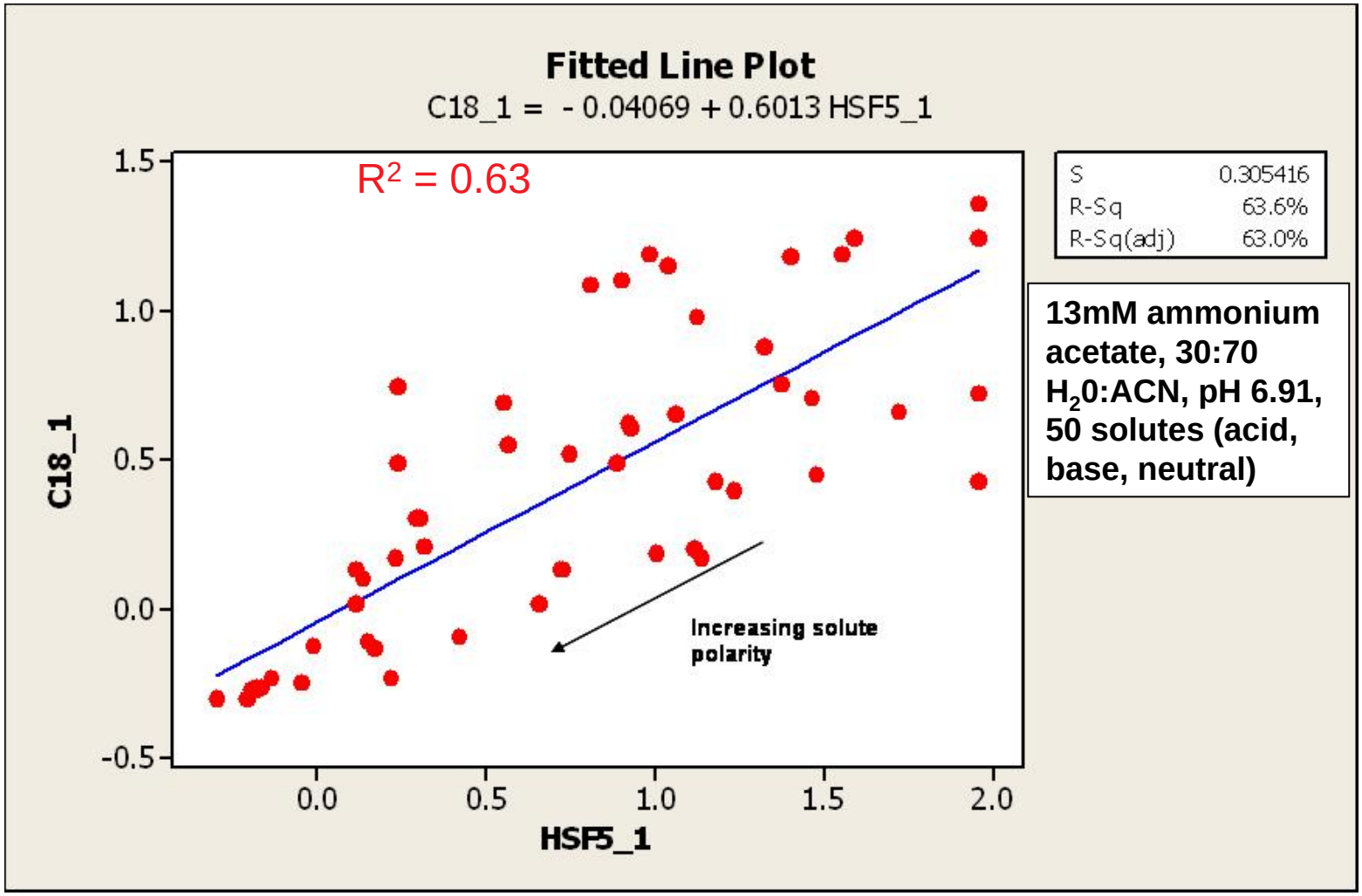
Columns with different phases should show orthogonality: C18 vs F5 (PFP)



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Experimental Design

- Five different stationary phases on Fused Core particles
  - Ascentis Express C18
  - Ascentis Express C8
  - Ascentis Express RP-Amide
  - Ascentis Express F5 (Pentafluorophenyl)
  - Ascentis Express Phenyl-Hexyl
- have been tested with two mobile phases consisting of either
  - Methanol/water
  - Acetonitrile/water
- as organic modifier on different herbal drugs such as Withania, Panax Ginseng and Milk Thistle.

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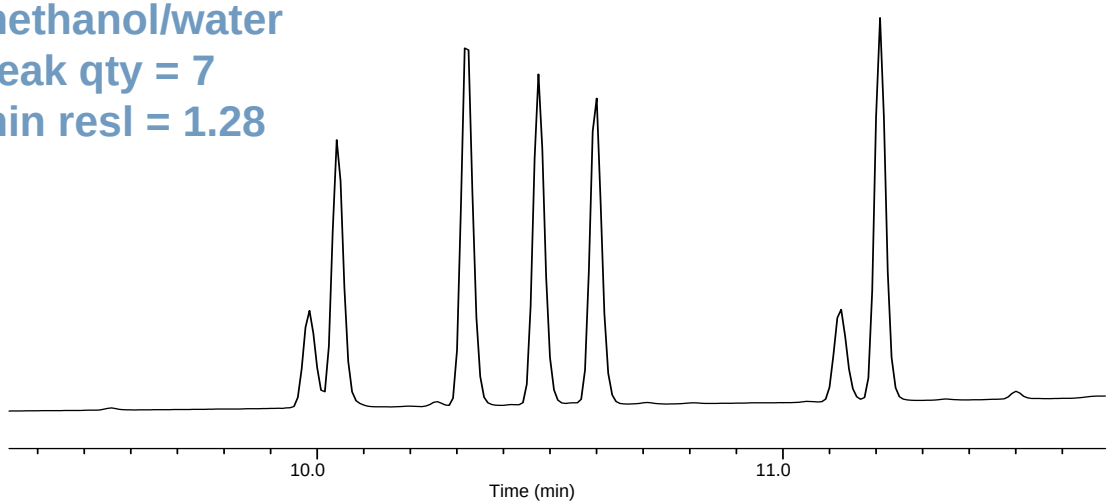
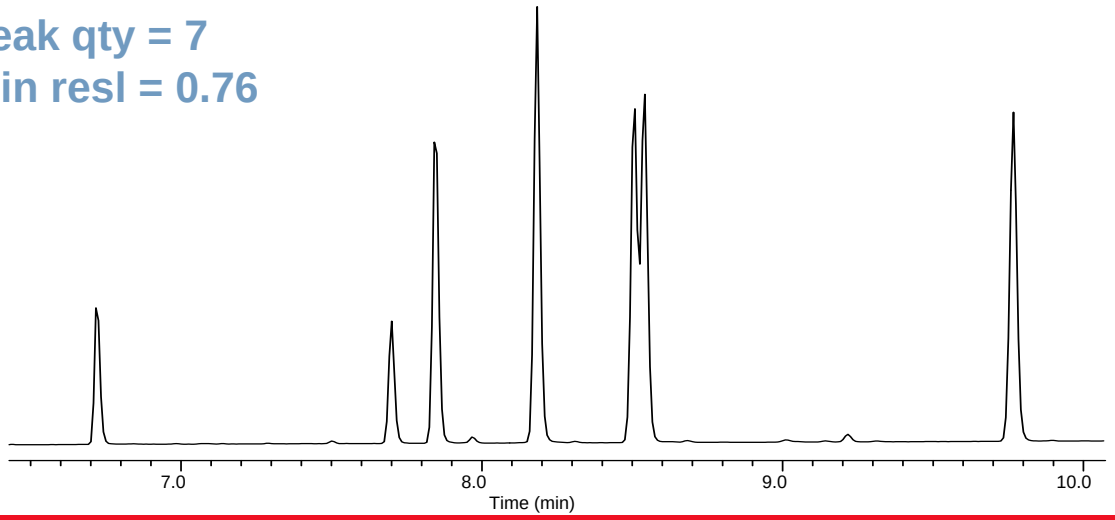
Whitania potential marker compounds, C18

methanol/water
peak qty = 7
min resl = 1.28



acetonitrile/water
peak qty = 7
min resl = 0.76



- **Withanone**
- **Withanolide A**
- **Withanolide B**
- **Withaferin A**
- **12-Deoxywithastra-monolide**
- **Withanoside IV**
- **Withanoside V**

Ascentis Express C18

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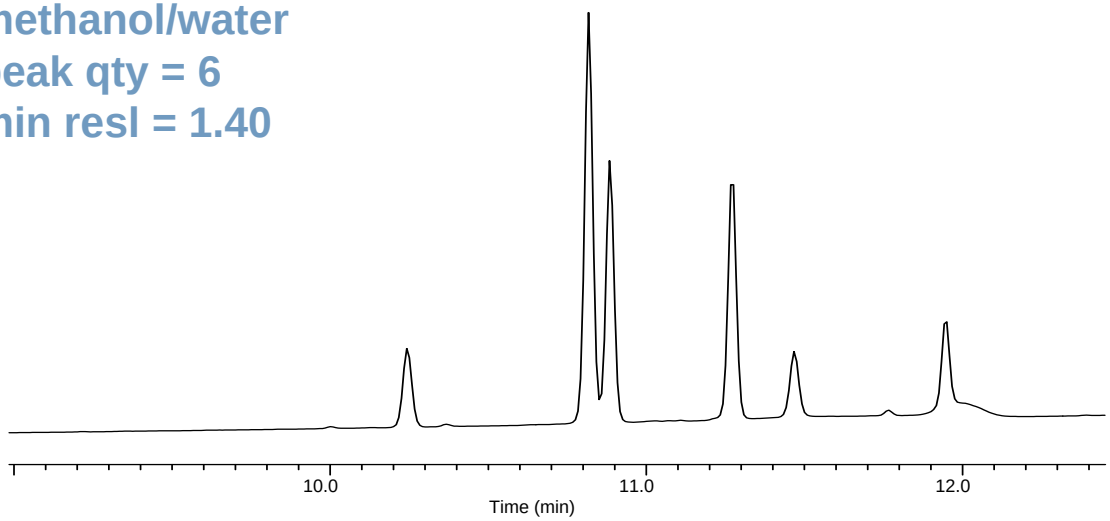
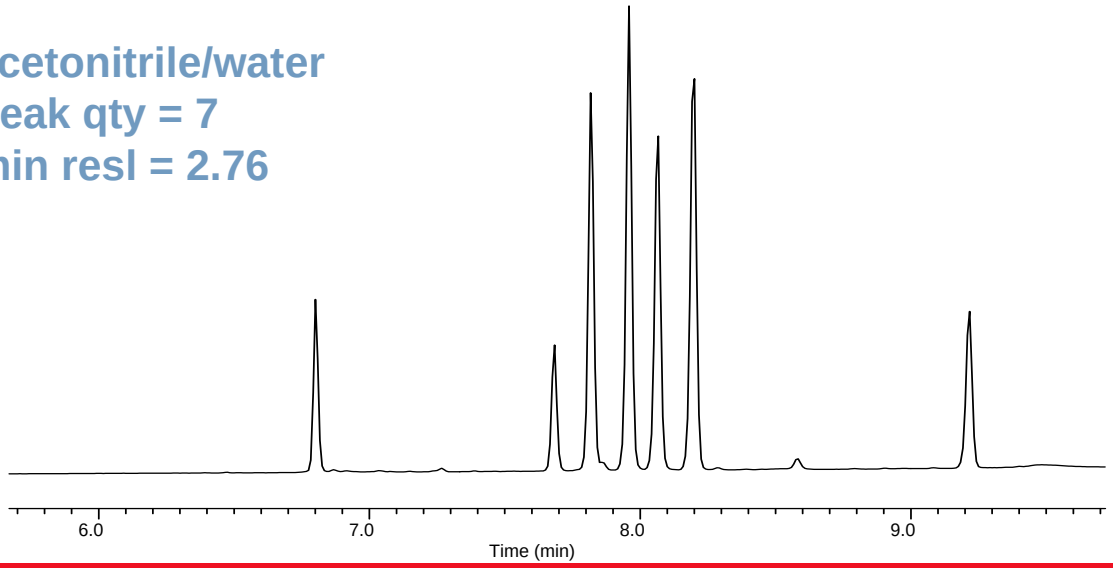
Whitania potential marker compounds, PFP

methanol/water
peak qty = 6
min resl = 1.40



acetonitrile/water
peak qty = 7
min resl = 2.76



- **Withanone**
- **Withanolide A**
- **Withanolide B**
- **Withaferin A**
- **12-Deoxywithastra-monolide**
- **Withanoside IV**
- **Withanoside V**

Ascentis Express F5

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Withania results –
Sorted by peak quantity then min. resolution

phase	organic	peaks	min resl
F5	acetonitrile	7	2,76
Phenyl-Hexyl	acetonitrile	7	2,01
Phenyl-Hexyl	methanol	7	1,36
C18	methanol	7	1,28
C8	acetonitrile	7	0,98
C18	acetonitrile	7	0,76
RP-Amide	acetonitrile	7	0,46
C8	methanol	6	2,55
F5	methanol	6	1,40
RP-Amide	methanol	5	0,90

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Panax ginseng results – Sorted by peak quantity then min. resolution

phase	organic	peak qty	min resl
C18	methanol	7	0,99
C8	methanol	7	0,94
Phenyl-Hexyl	methanol	7	0,77
F5	methanol	7	0,49
RP-Amide	methanol	6	1,63
RP-Amide	acetonitrile	6	1,39
C8	acetonitrile	6	1,24
C18	acetonitrile	6	0,71
F5	acetonitrile	6	0,58
Phenyl-Hexyl	acetonitrile	5	1,94

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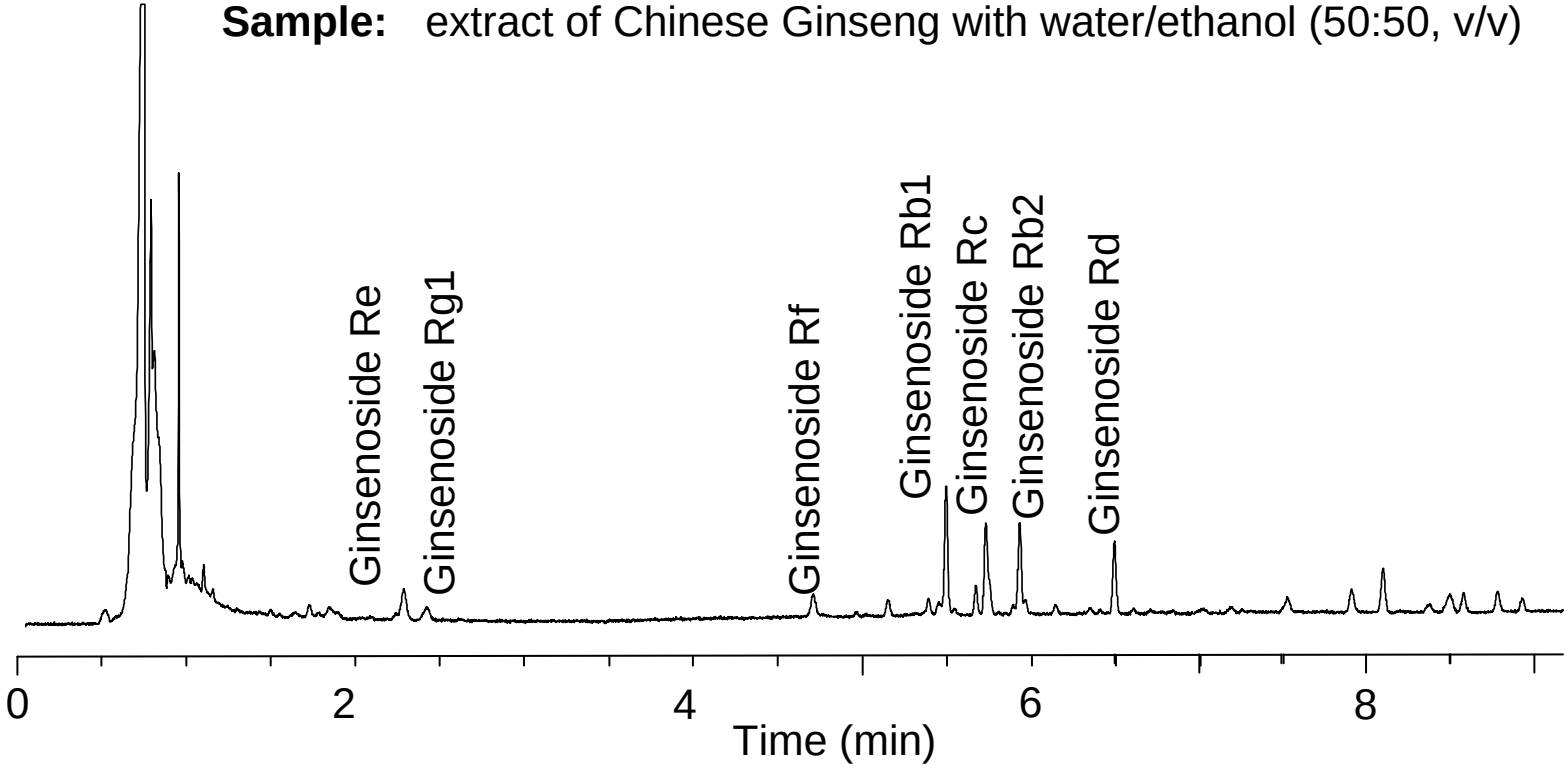
Extract of Chinese Panax Ginseng

Column: Ascentis Express C18, 150 x 4.6 mm
Mob. Phase A: water
Mob. Phase B: acetonitrile
Gradient: 0-1.5 min at 25% B, 1.5-13.5 min to 85% B, 13.5-14.5 min at 85% B
Flow rate: 1.5 mL/min **Temp.:** 60 oC
Det.: 205 nm **Injection:** 10 µL
Sample: extract of Chinese Ginseng with water/ethanol (50:50, v/v)



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Milk Thistle (Silybum marianum) results – Sorted by peak quantity then min. resolution

phase	organic	peak qty	min resl
C18	methanol	9	-
F5	methanol	8	-
C18	acetonitrile	8	-
Phenyl-Hexyl	methanol	7	-
Phenyl-Hexyl	acetonitrile	7	-
F5	acetonitrile	7	-
C8	methanol	7	-
C8	acetonitrile	7	-
RP-Amide	methanol	6	-
RP-Amide	acetonitrile	6	-

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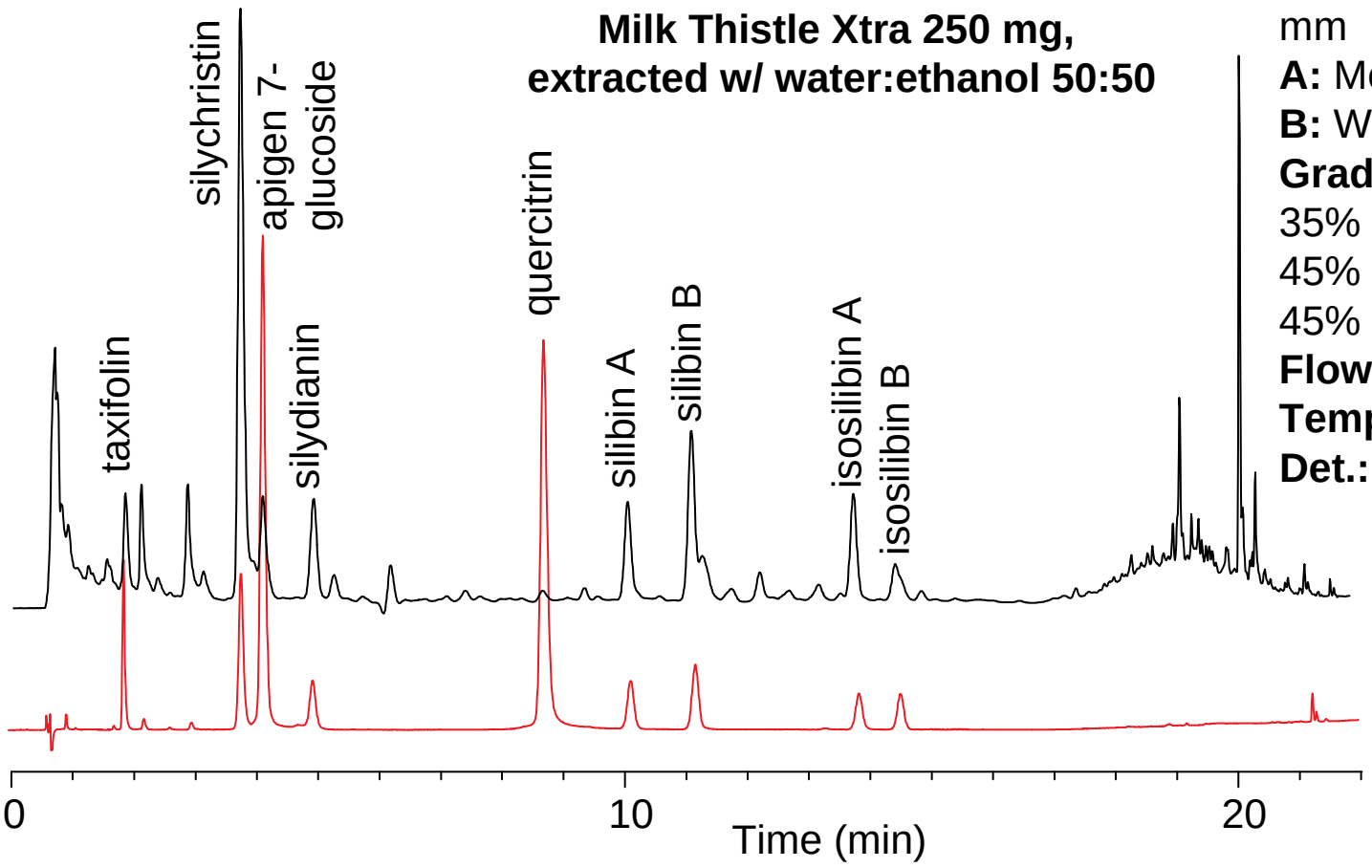
Milk Thistle (Silybum marianum) – Markers (red) and Herbal Medicinal Product (black)



Column: Ascentis Express C18, 100 x 3 mm
A: MeOH
B: Water + 0,1 % FA
Gradient: 0-3 min at 35% B, 3-13 min to 45% B, 13-15 min at 45% B
Flow rate: 0.6 mL/min.
Temp.: 35 °C
Det.: 254 nm

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Conclusions

- Fused Core particle technology provides a good base for separation of highly complex mixtures
- Selectivity by initial screening of orthogonal stationary and mobile phases results highest resolution
  - Selectivity is most strongly impacted by different stationary phases
- The approach of initial column screening provides fast and reliable separations

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Thank you!

- Dave S. Bell
- Hugh Cramer
- Richard A. Henry

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