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Stationary Phase Selectivity: A Powerful Tool for Challenging Separations

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In Partnership with our authorized channel partner in the USA



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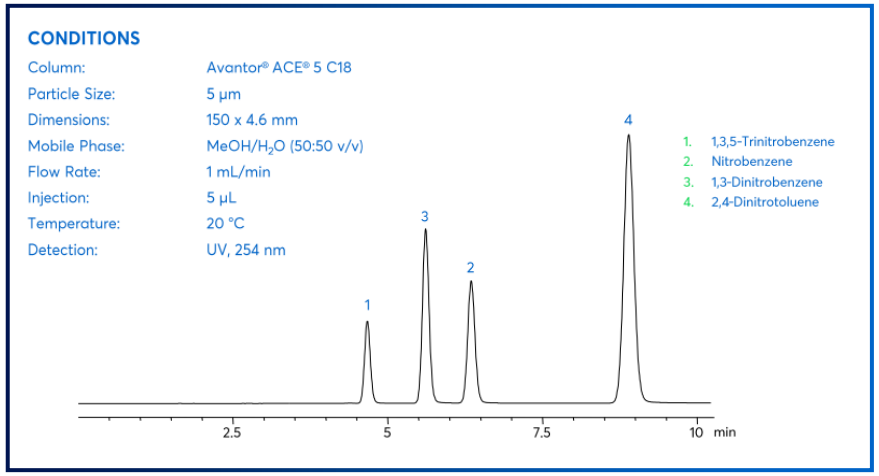
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Chromatography: what is the goal?

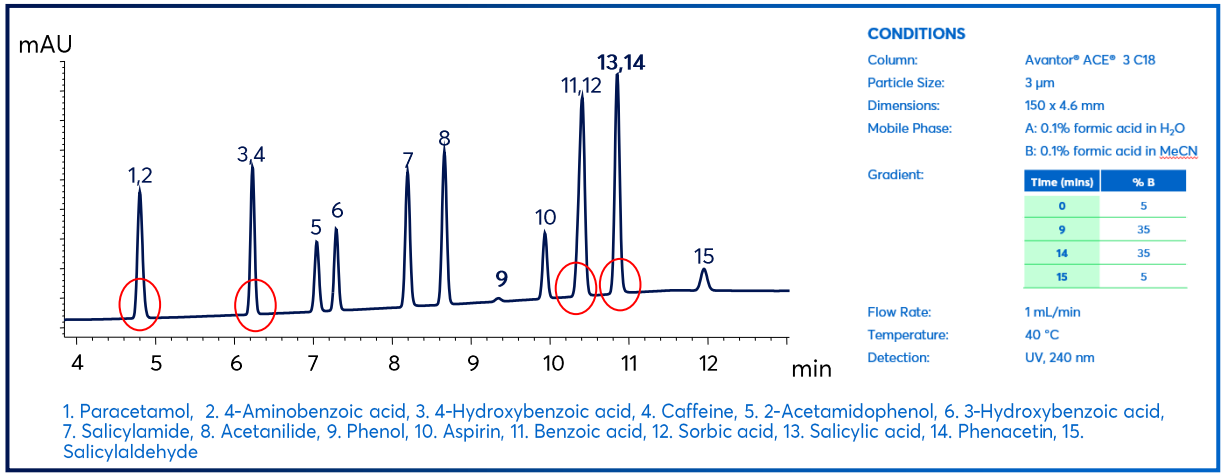
The primary goal of chromatography is to separate the components of a sample based on their differential interactions with a stationary phase and a mobile phase, resulting in different migration rates. This separation enables:

- Identification of individual components
- Quantification of components
- Purification/isolation of target compounds

Some separations are simple….



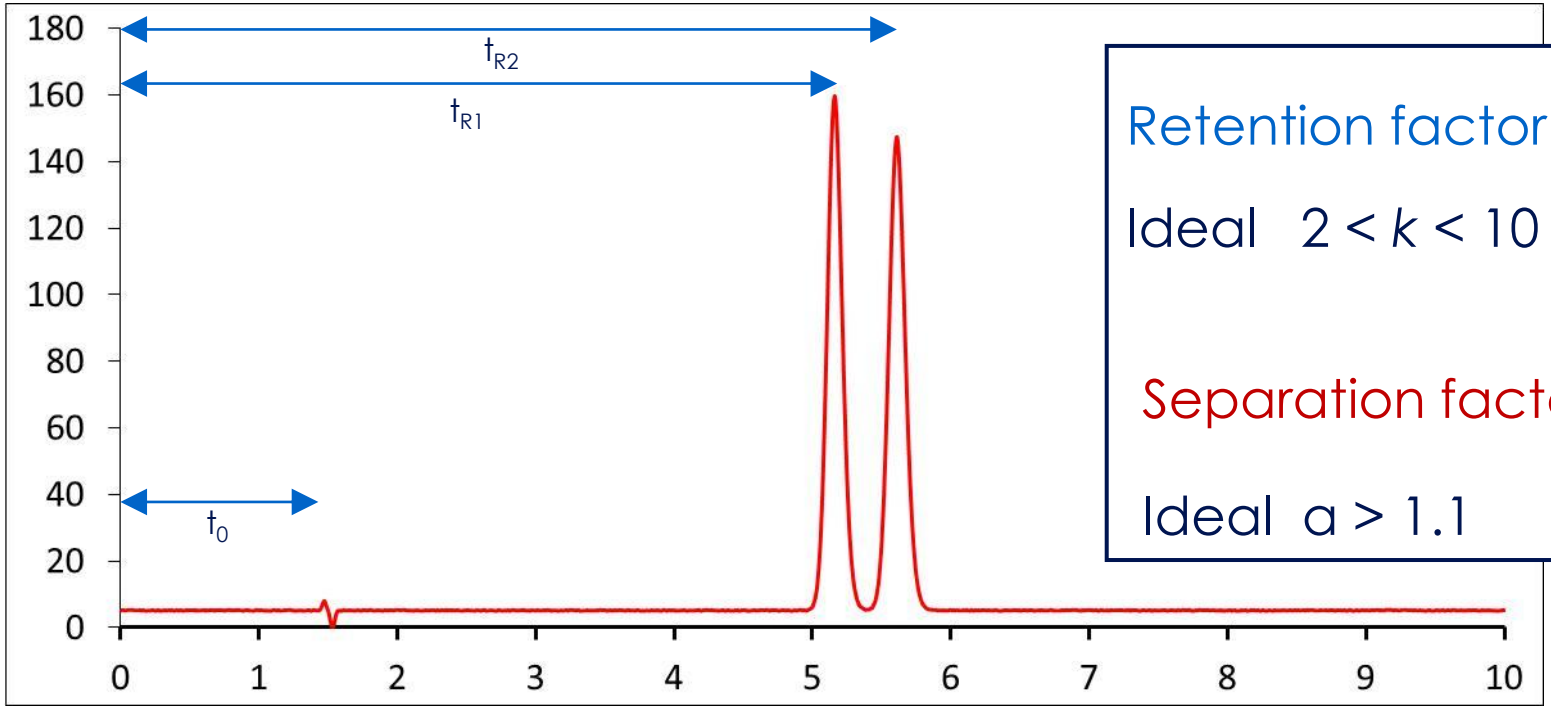
Others are more challenging….



We need to optimize separation selectivity

What is selectivity?

- Alpha (α) is the symbol used to denote the separation factor or separation selectivity between 2 adjacent peaks



Retention factor $k = \frac{t_R - t_0}{t_0}$

Ideal $2 < k < 10$

Separation factor $\alpha = \frac{k_2}{k_1}$

Ideal $a > 1.1$

- Selectivity can be thought of as 'peak spacing'
- Selectivity values should be > 1.0

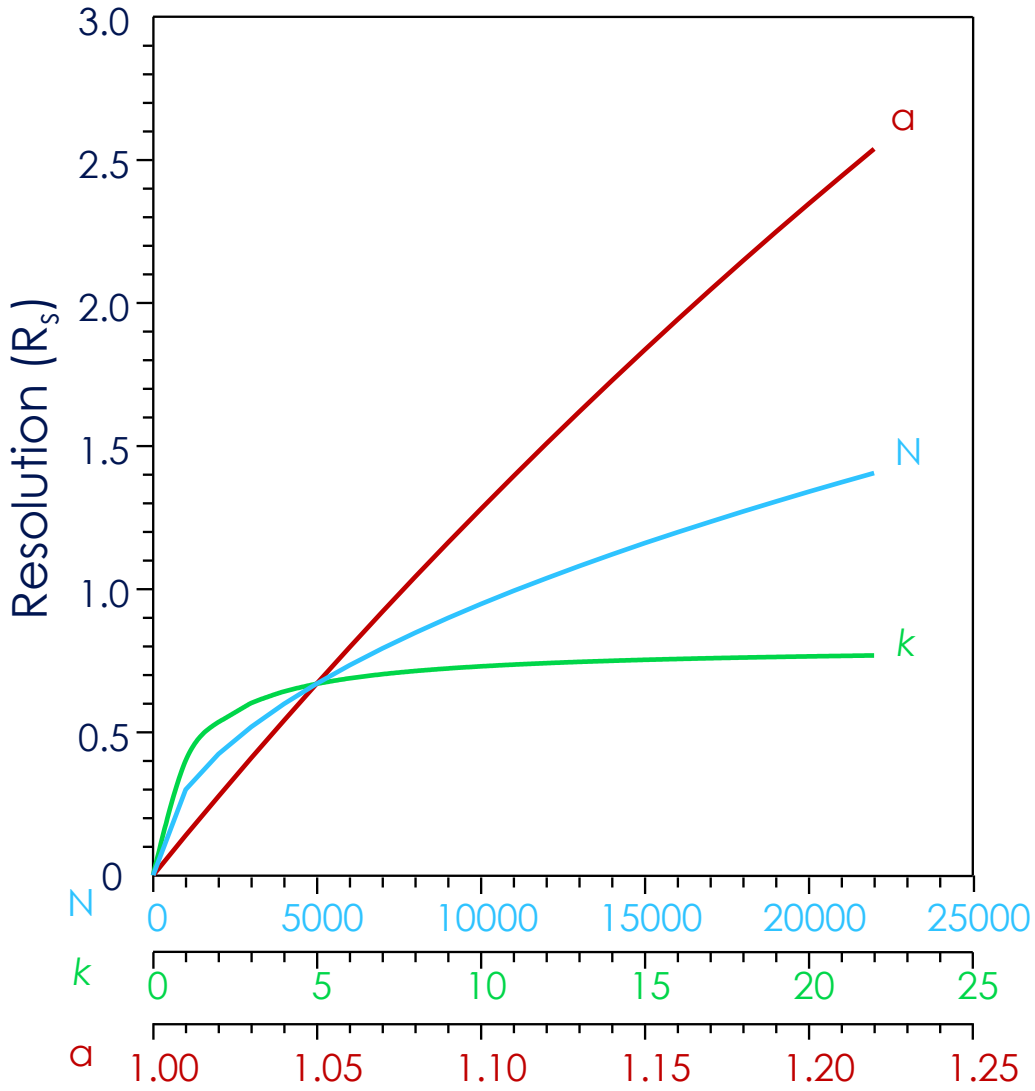
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Resolution equation (Purnell equation)

$$R_s = \frac{1}{4}\sqrt{N_2}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$$

Efficiency | Selectivity | Retention factor

Selectivity (a) is the key to resolution and efficiency (N) boosts performance



Purnell, J.H. (1960). "The correlation of separating power and efficiency of gas-chromatographic columns". *J. Chem. Soc.*: 1268–1274.

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Which factors[1] affect selectivity?

- Strongly influenced by physicochemical properties of the analyte, stationary phase, eluent etc.
- From a practical perspective:

Isocratic Separations	MOST Influence ↑ LEAST Influence	Gradient Separations
– Column stationary phase type – pH (ionizable analytes only) – Organic modifier type – % Organic modifier – Buffer selection – Column temperature – Buffer concentration		– All parameters for isocratic PLUS – Gradient steepness – k* (t_G, F, V_m, ΔΦ, M) $$k^* = \frac{t_G F}{\Delta\Phi V_m M}$$ – Dwell volume – Column dimensions

Stationary phase chemistry is powerful!

[1] Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, Dolan, 2010, p.29, Wiley & sons

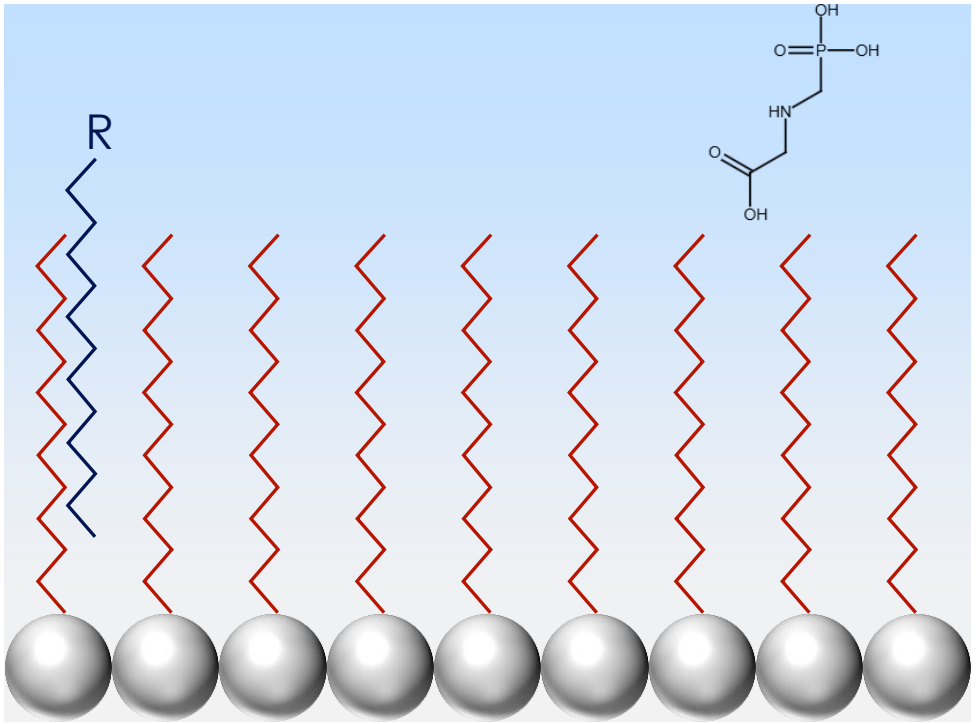
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What interactions drive retention?

Hydrophobic (dispersive) interactions

Non-polar/hydrophobic regions of the analyte interact with the stationary phase ligand via van der Waals and London dispersion forces

For alkyl phases, longer chains are more hydrophobic (C4<C8<C18…)



Hydrogen bonding

A non-covalent interaction between a hydrogen atom bonded to an electronegative atom and a nearby electronegative atom.



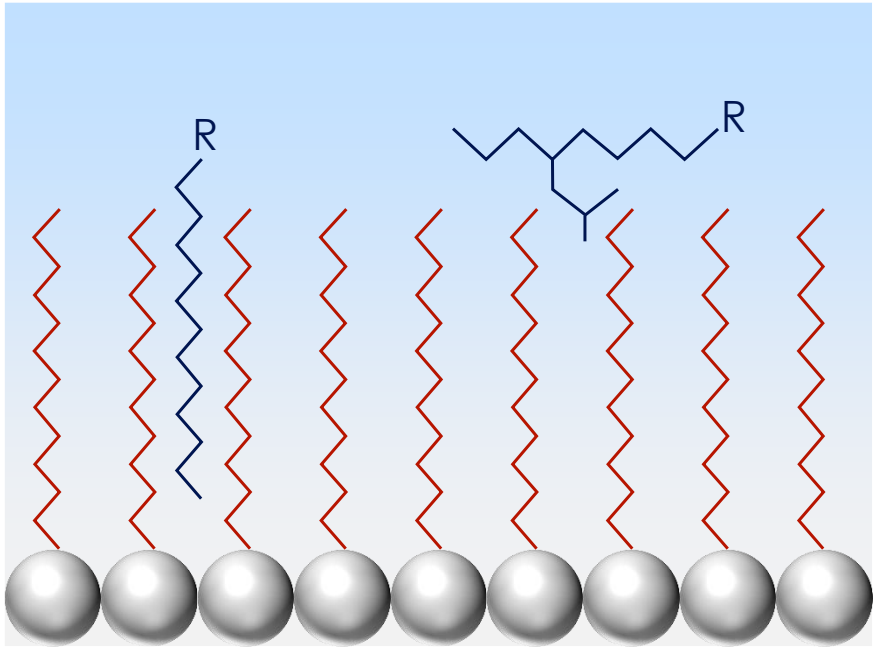
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What interactions drive retention?

Steric effects (shape selectivity)

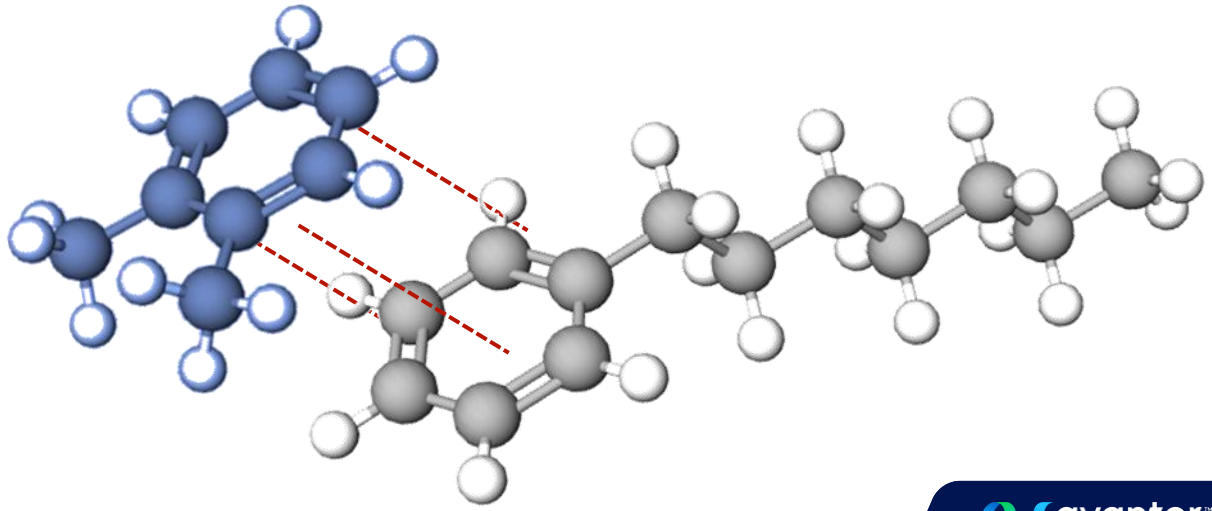
Differences in analyte molecular size, shape, and conformational flexibility.

Impacts how effectively analytes can penetrate, orient within, and interact with the stationary phase



π - π interactions

Interactions occur due to intermolecular overlapping of p-orbitals in p-conjugated systems



Images created using https://molview.org/

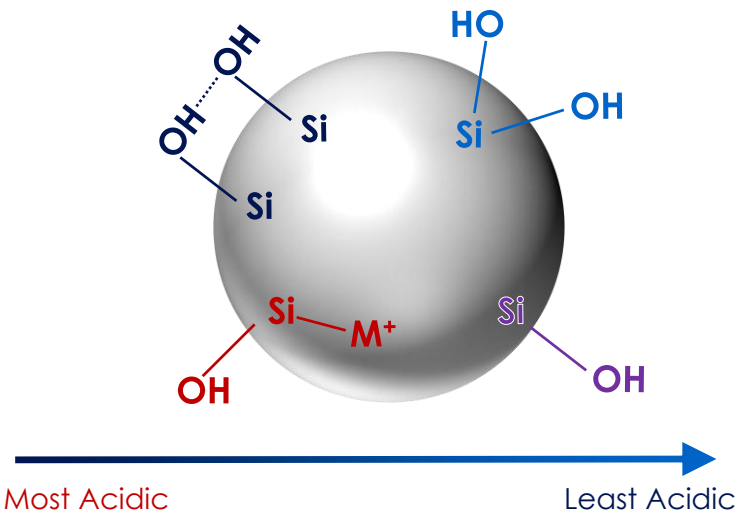
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What interactions drive retention?

Ionic Interactions

Strong interaction between positively and negatively charged groups, e.g. IEX phases.

Can form between analyte and stationary phase surface



Dipole-dipole

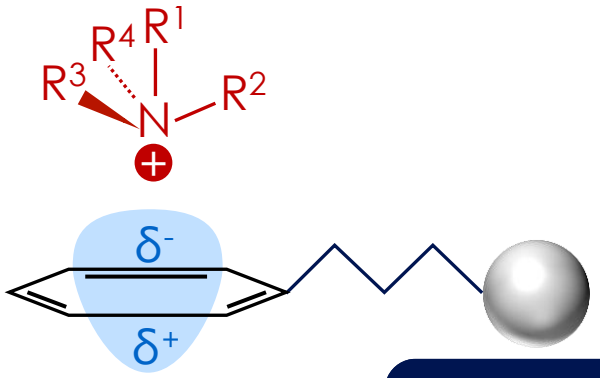
Electrostatic attraction between molecules or functional groups that possess permanent dipoles

Weaker than hydrogen bonding



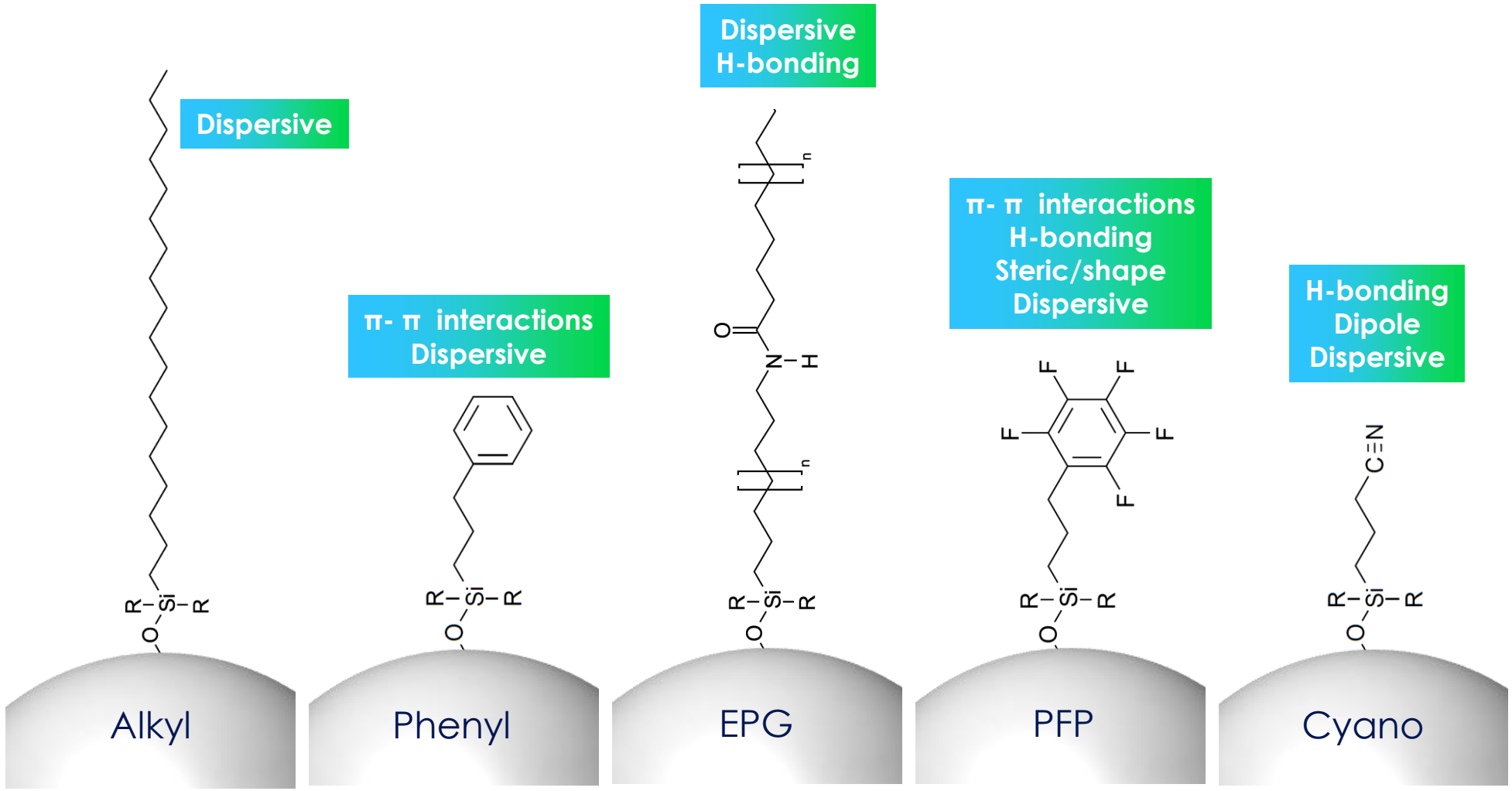
Induced dipole

Result when an ion or permanent dipole distorts the electron cloud on a non-polar species and induces a dipole. (Weak forces).



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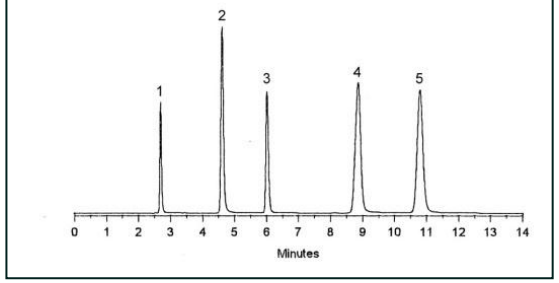
RP stationary phase interactions



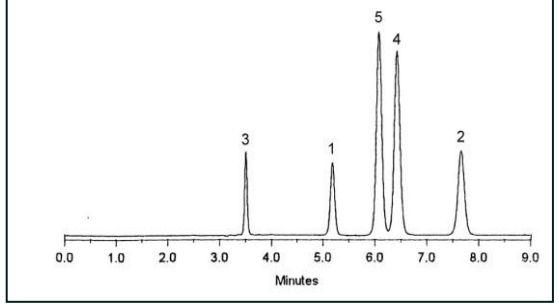
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Influence of retention mechanism on selectivity

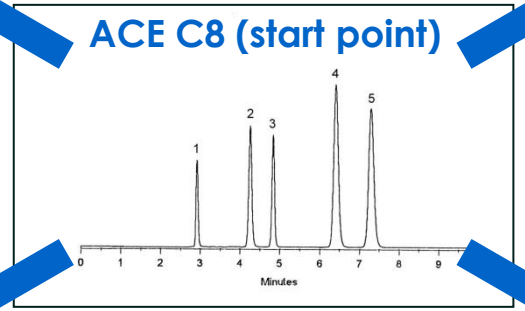
ACE C18 – Increase retention



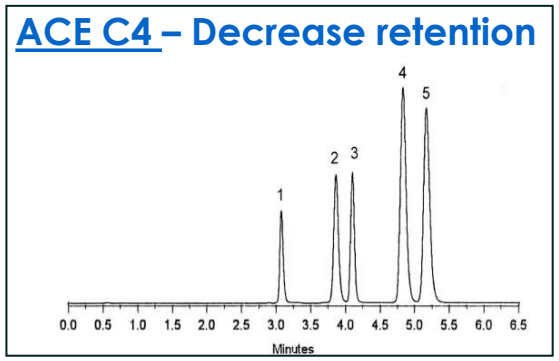
ACE CN – Elution order



ACE C8 (start point)

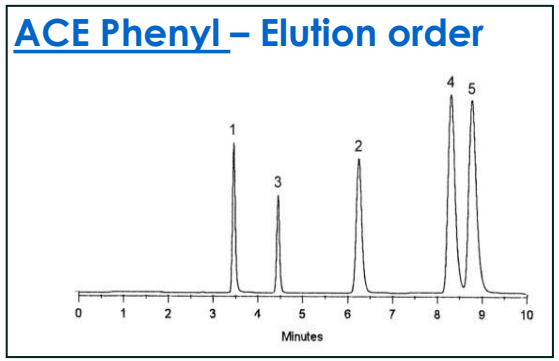


ACE C4 – Decrease retention



Hydrophobicity dominated

ACE Phenyl – Elution order

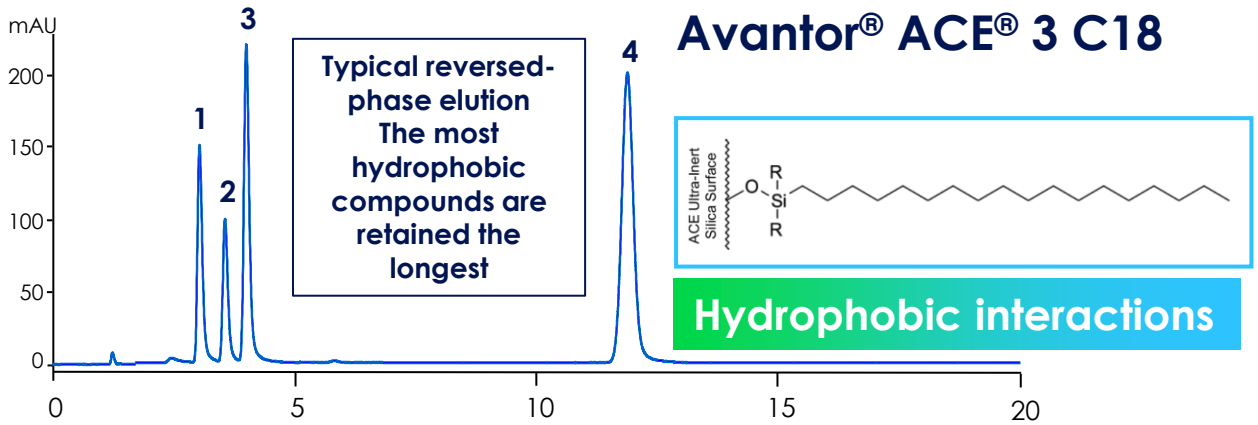


Dipole-dipole, pi-pi mechanisms etc

Column: 250 x 4.6 mm 5 µm Mobile phase: 80:20 MeOH/25 mM KH_2PO_4 (pH 6.0) Flow: 1.00 ml/min
Components; 1: Norephedrine, 2: Nortriptyline, 3: Toluene, 4: Imipramine, 5: Amitriptyline Wavelength: 215nm

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Reversed-phase separations: stationary phase selectivity

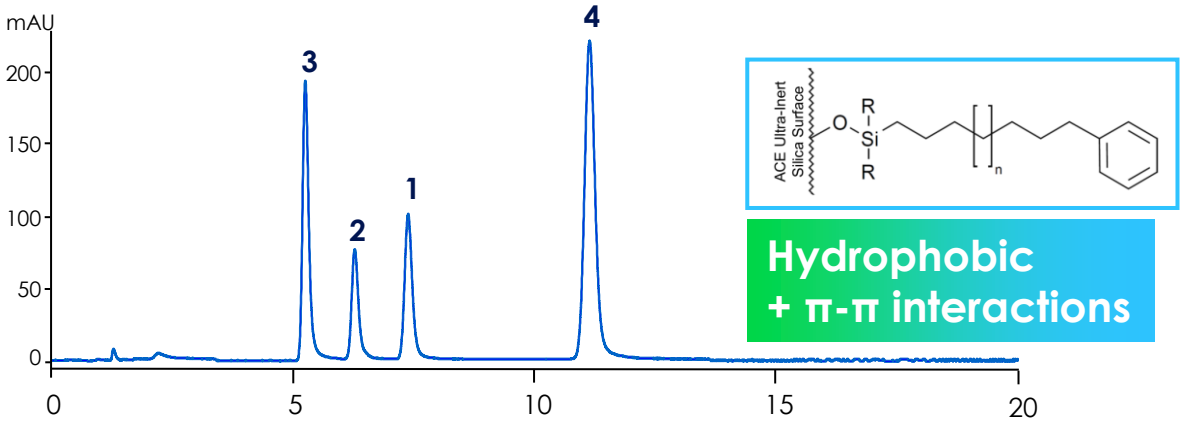


Typical reversed-phase elution
The most hydrophobic compounds are retained the longest

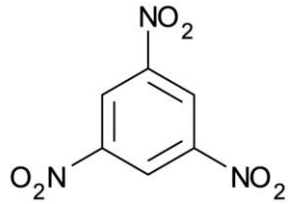
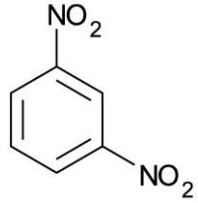
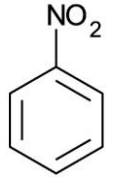
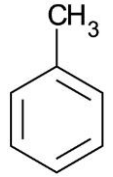
Avantor® ACE® 3 C18

Hydrophobic interactions

Avantor® ACE® 3 C18-AR



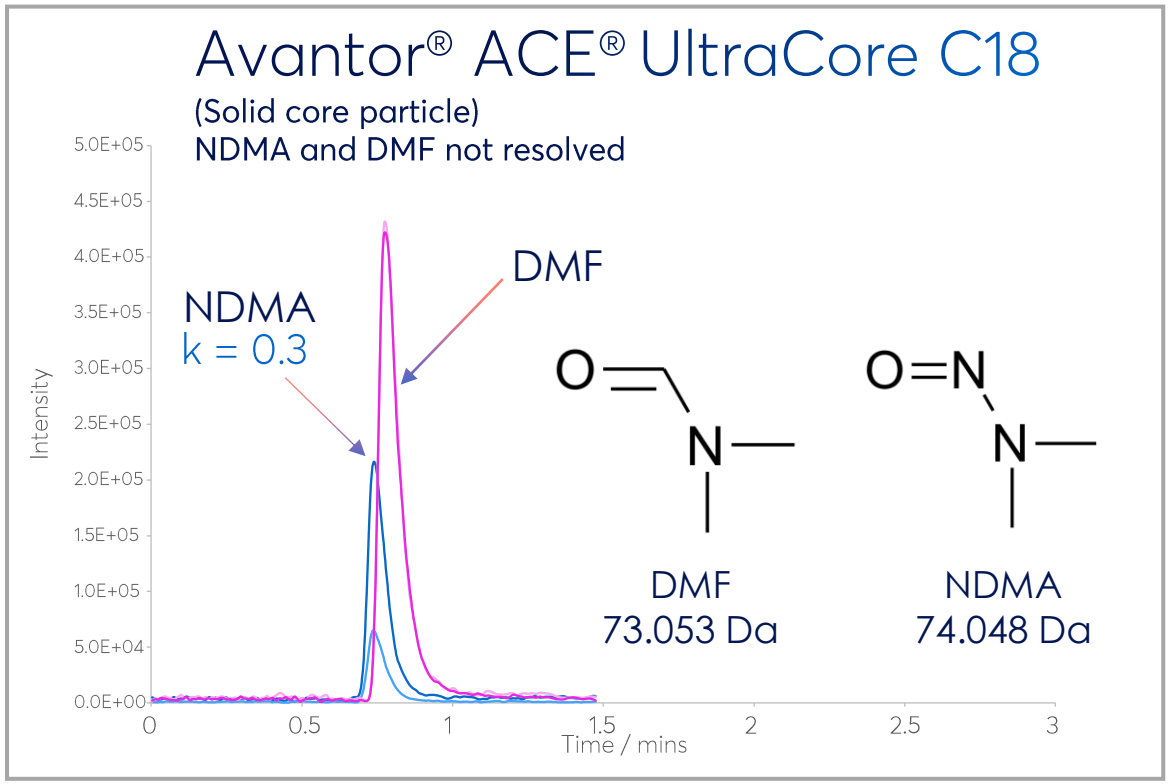
Hydrophobic + π-π interactions

		logP
1. 1,3,5-trinitrobenzene		1.79
2. 1,3-dinitrobenzene		1.85
3. nitrobenzene		1.91
4. toluene		2.49

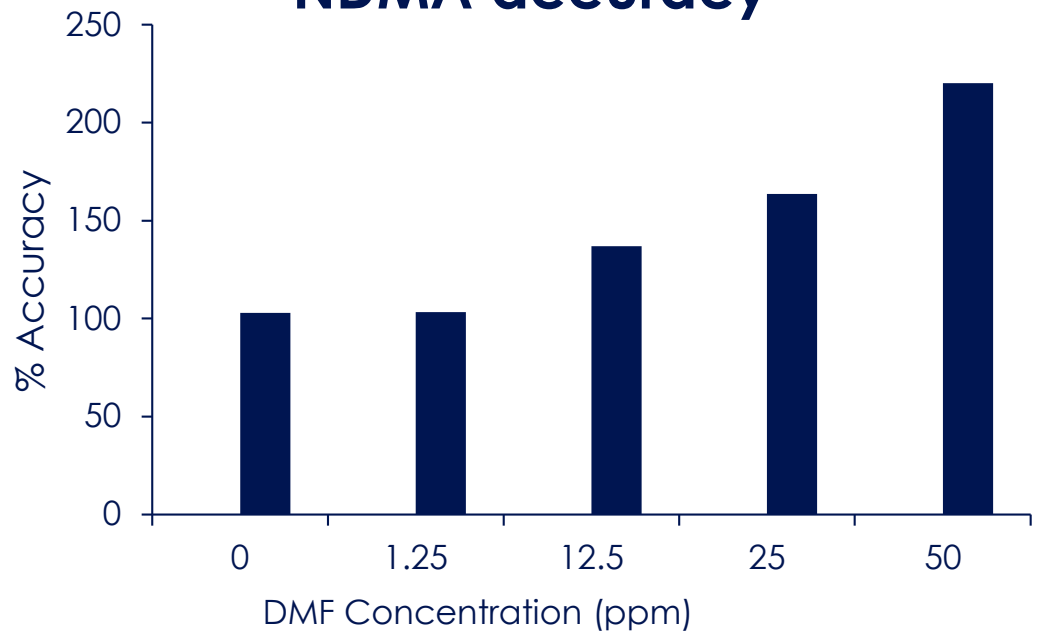
Column dimensions: 100 x 3.0 mm
Mobile phase: Isocratic, MeOH:H_2O 50:50 (v/v)
Flow rate: 0.43 ml/min
Column temperature: 40 °C
Injection vol: 2 µl
Detection: UV, 210 nm

Stationary phase chemistry helps solve tough challenges

- NDMA is a highly genotoxic impurity
- NDMA limits as low as 0.12 ppm, LOQ 2 ppb
- DMF residual solvent limit 880 ppm



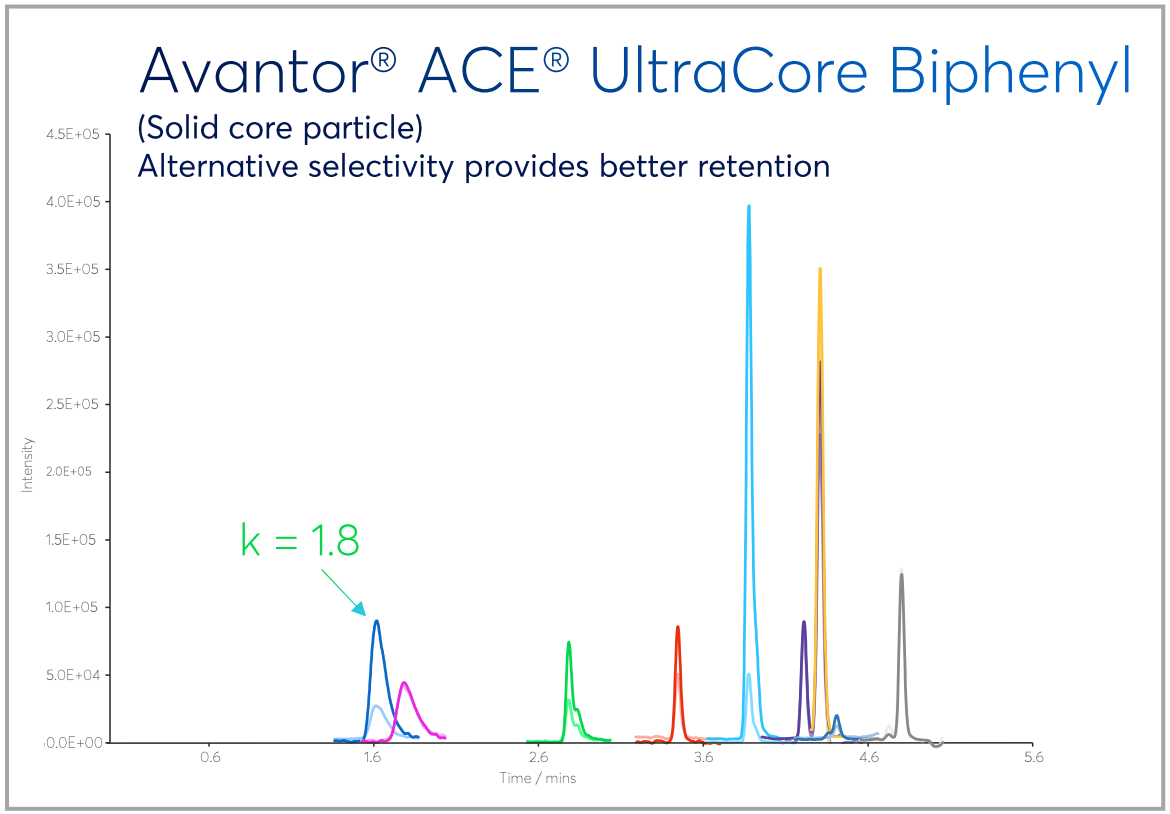
DMF spiking experiment: NDMA accuracy



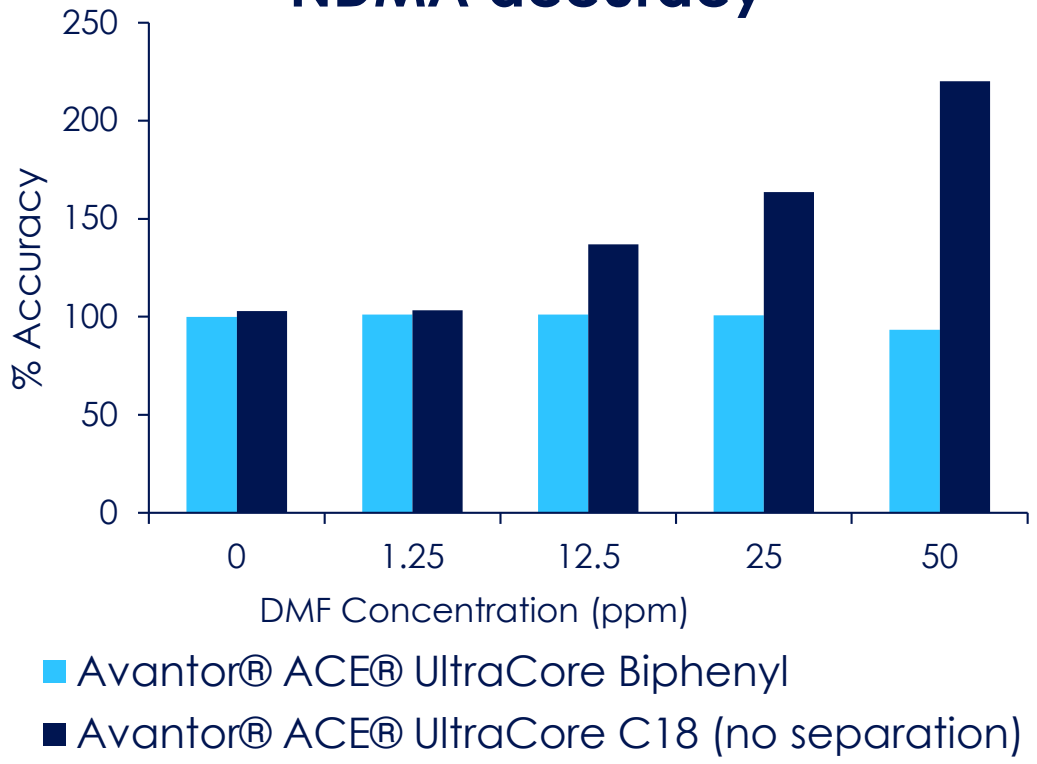
■ Avantor® ACE® UltraCore C18 (no separation)

Stationary phase chemistry helps solve tough challenges

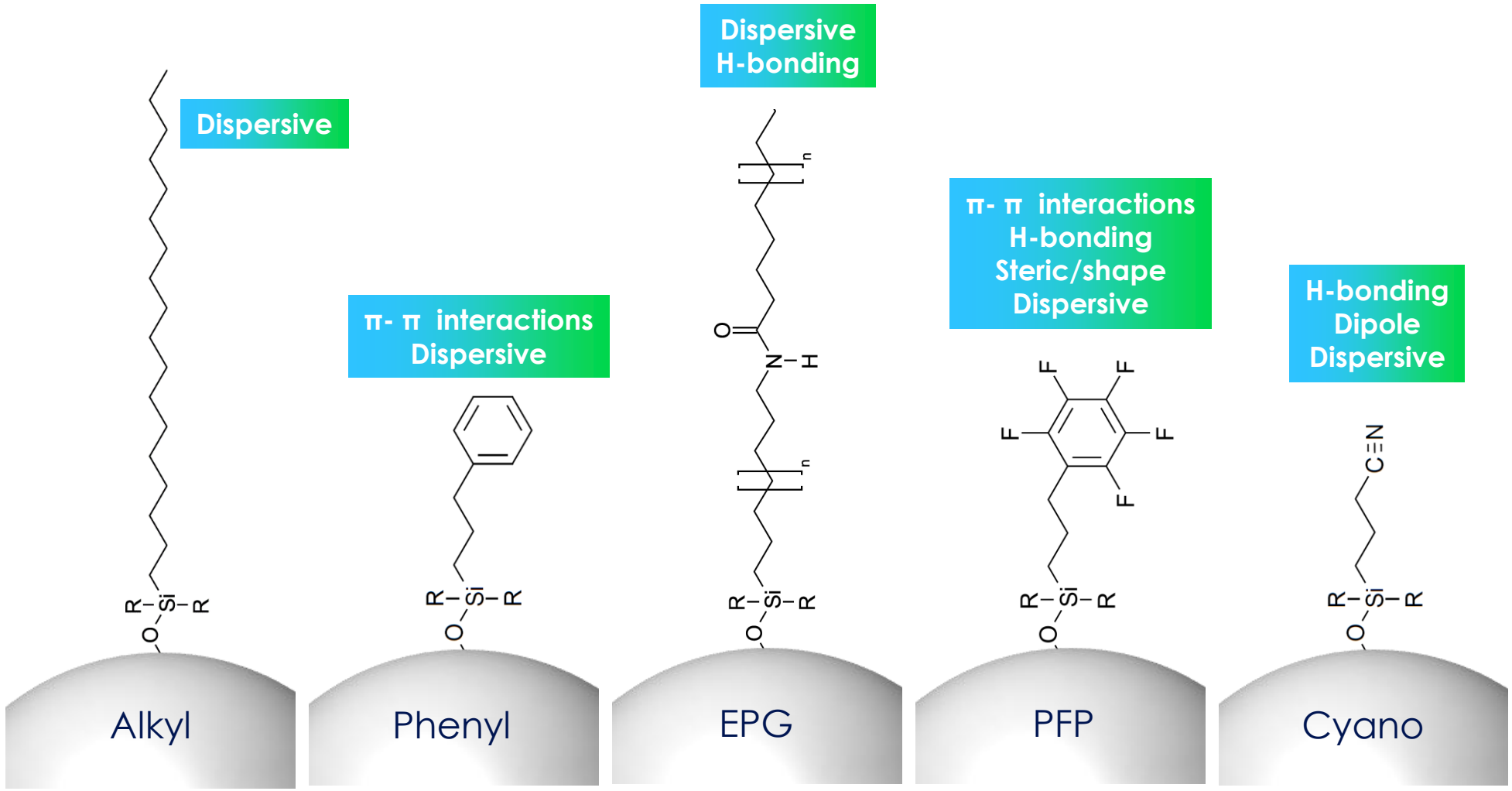
- NDMA is a highly genotoxic impurity
- NDMA limits as low as 0.12 ppm, LOQ 2 ppb
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DMF spiking experiment: NDMA accuracy

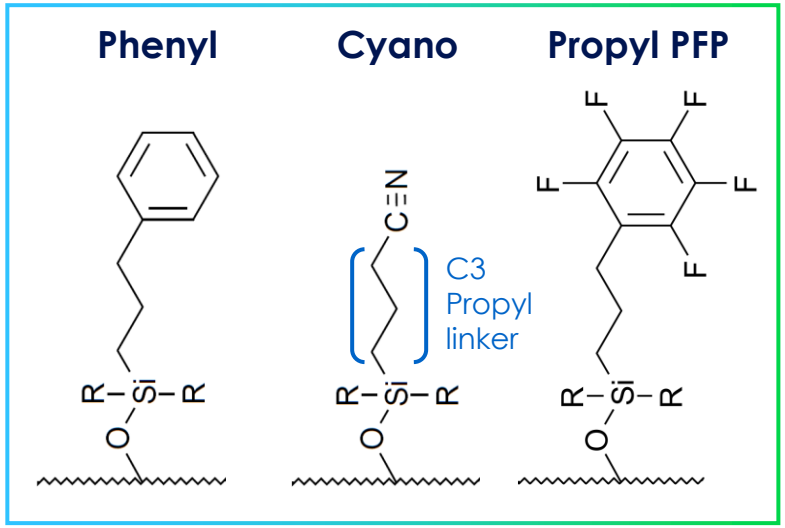


RP stationary phase interactions

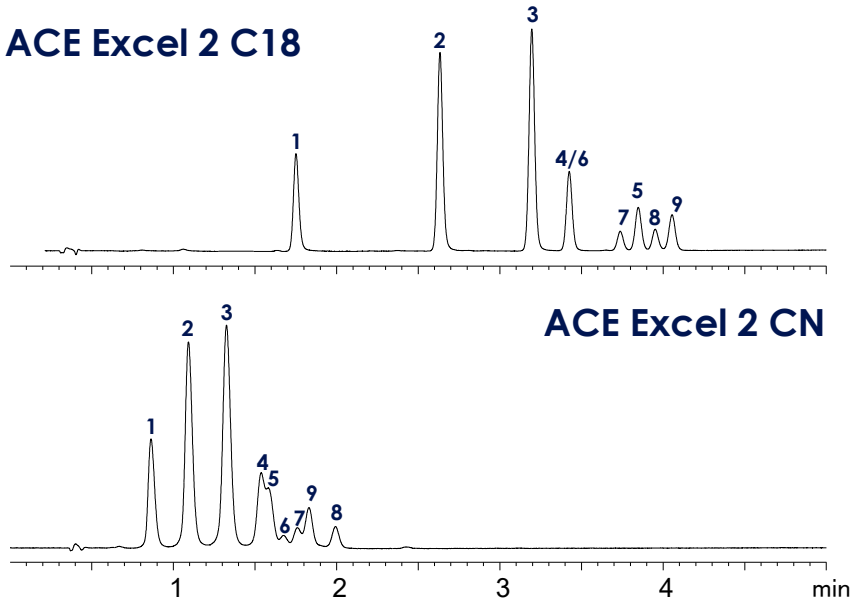


Stationary phase design

- Many alternative selectivity phases tend to be bonded to the stationary phase by short (often propyl, C3) linkers.
- These phases can offer very different selectivity compared to a C18 but have some key disadvantages:
- Reduced hydrophobicity
  – not ideal for reversed phase

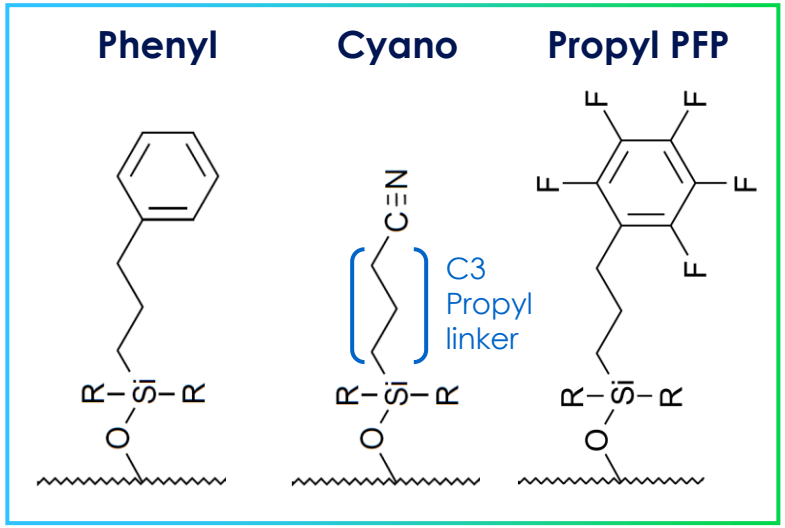


Steroid separation



Stationary phase design

- Many alternative selectivity phases tend to be bonded to the stationary phase by short (often propyl, C3) linkers.
- These phases can offer very different selectivity compared to a C18 but have some key disadvantages:
- Reduced hydrophobicity
  – not ideal for reversed phase
- Lower stability and lifetime
- Higher phase bleed



Accelerated low pH stability studies at high temperature

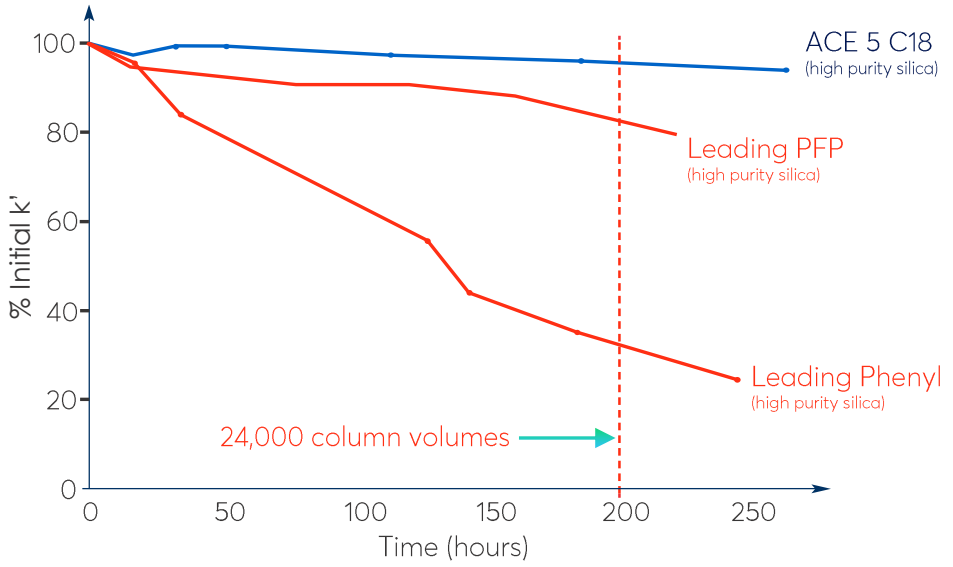
Conditions:

Column Dimensions: 50 x 2.1 mm

Mobile Phase: 5:95 (v/v) MeOH:0.1% TFA in H_2O pH 1.9

Flow Rate: 0.20 mL/min

Temperature: 80 °C

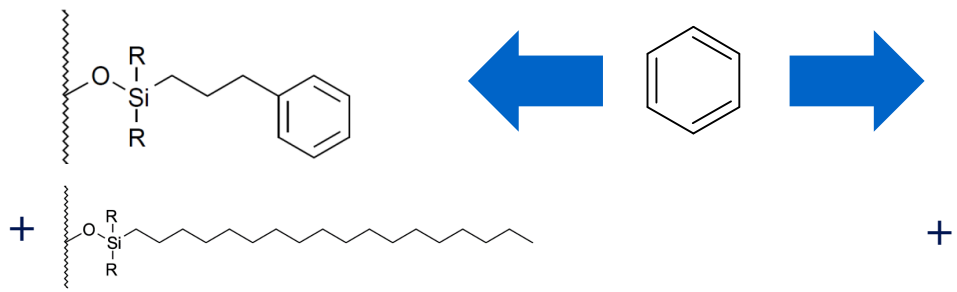


New stationary phase design

Can we introduce additional modes of retention into a C18 platform?

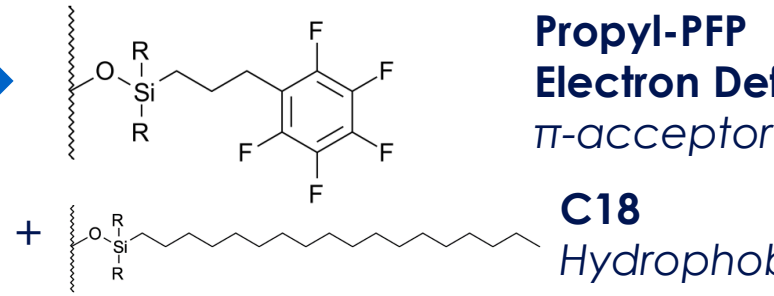
Propyl-Phenyl
Electron Rich Ring
π-donor (Lewis-base)

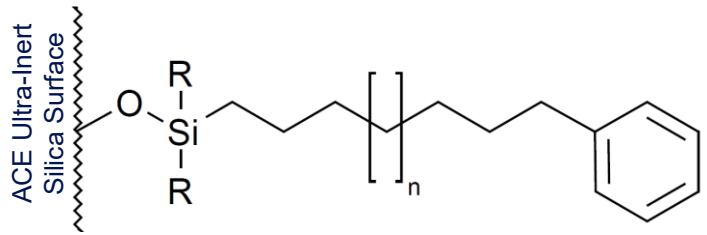
C18
Hydrophobic



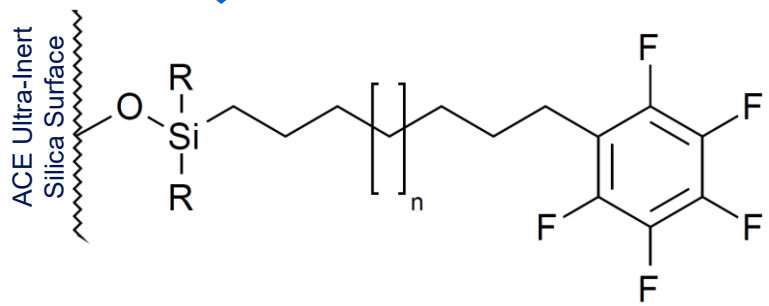
Propyl-PFP
Electron Deficient Ring
π-acceptor (Lewis-acid)

C18
Hydrophobic





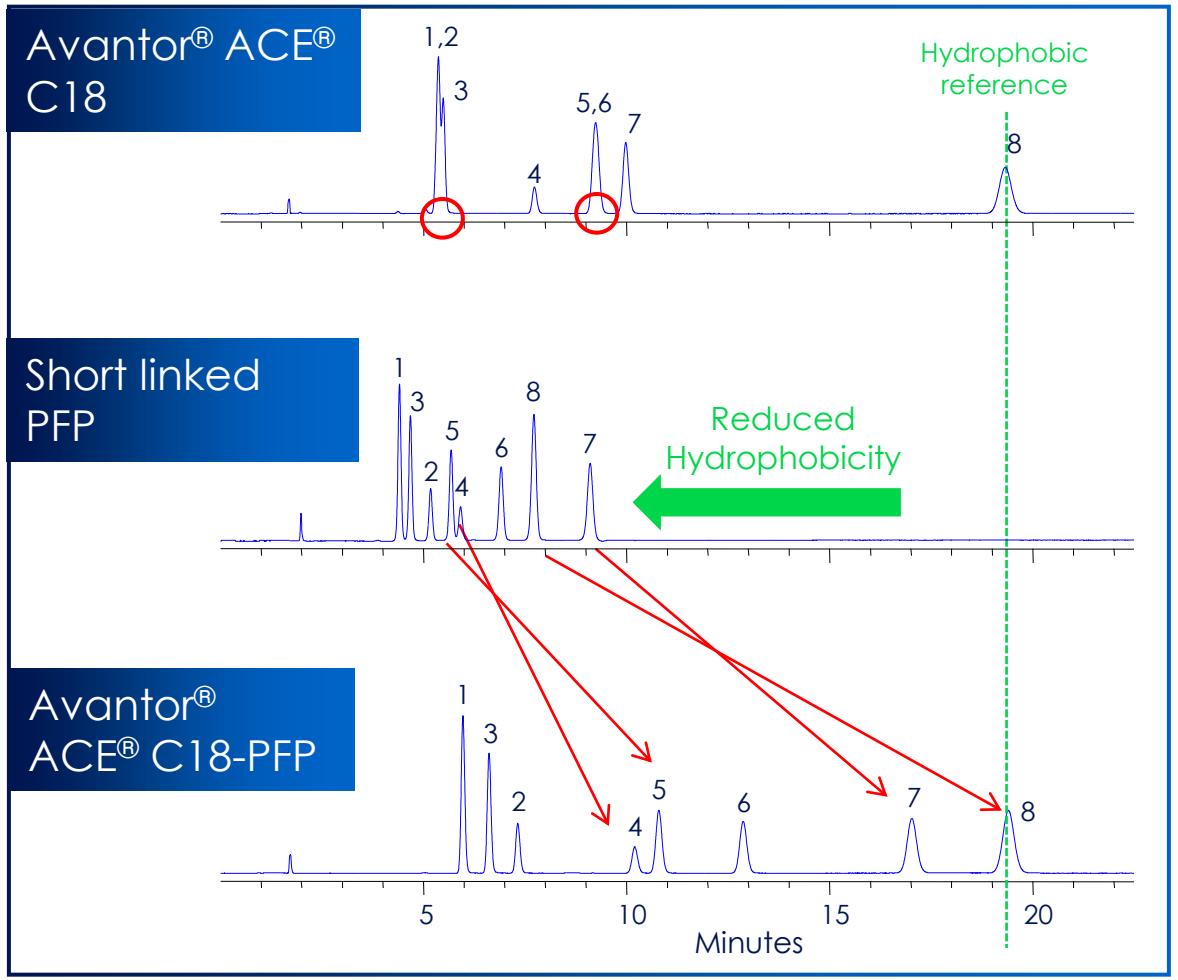
Avantor® ACE® C18-AR



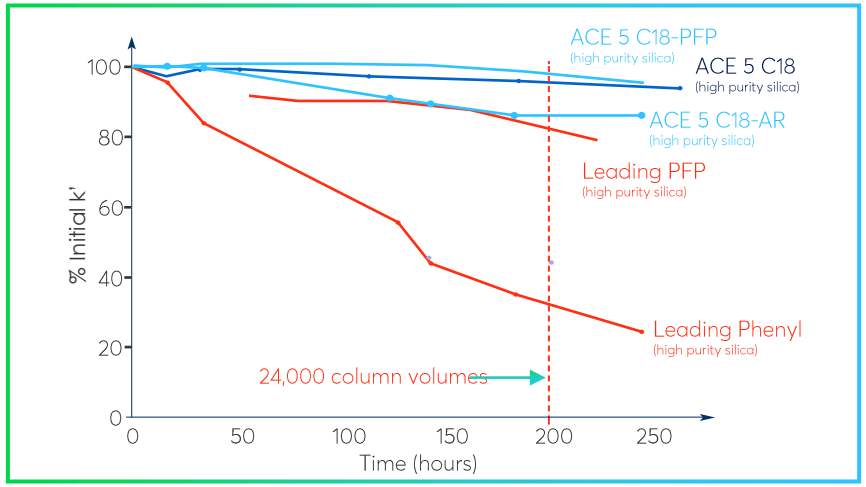
Avantor® ACE® C18-PFP

Phases designed to have C18-like retention, stability and robustness, whilst providing multiple modes of retention <u>i.e. maximize selectivity</u>

Avantor® ACE® C18-PFP: methoxybenzene isomers



- ACE C18-PFP mechanism combines hydrophobicity, shape selectivity, dipole-dipole and π-π interactions



Accelerated low pH stability studies at high temperature

Conditions:

Column Dimensions: 50 x 2.1 mm

Mobile Phase: 5:95 (v/v) MeOH:0.1% TFA in H_2O pH 1.9

Flow Rate: 0.20 mL/min

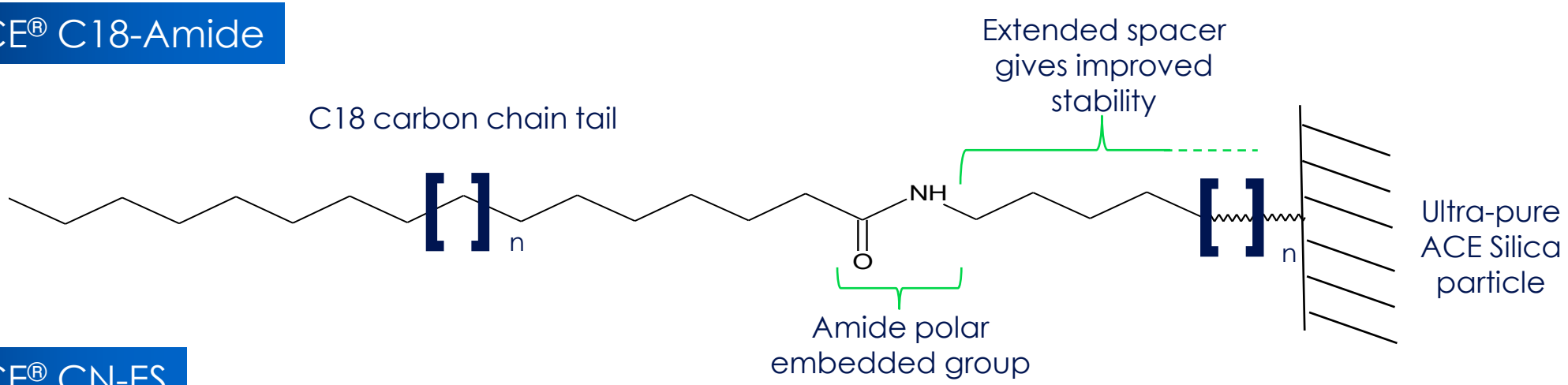
Temperature: 80 °C

Mobile phase 50:50 v/v MeOH / H_2O; Column= 150 x 4.6 mm id; 1.00 ml/min; 40 °C; 254 nm
Sample: 1) 1,2,3-trimethoxybenzene, 2) 1,2,4-trimethoxybenzene, 3) 1,2-dimethoxybenzene, 4) 1,4-dimethoxybenzene 5) methoxybenzene, 6) 1,3-dimethoxybenzene, 7) 1,3,5-trimethoxybenzene, 8) toluene (ref)

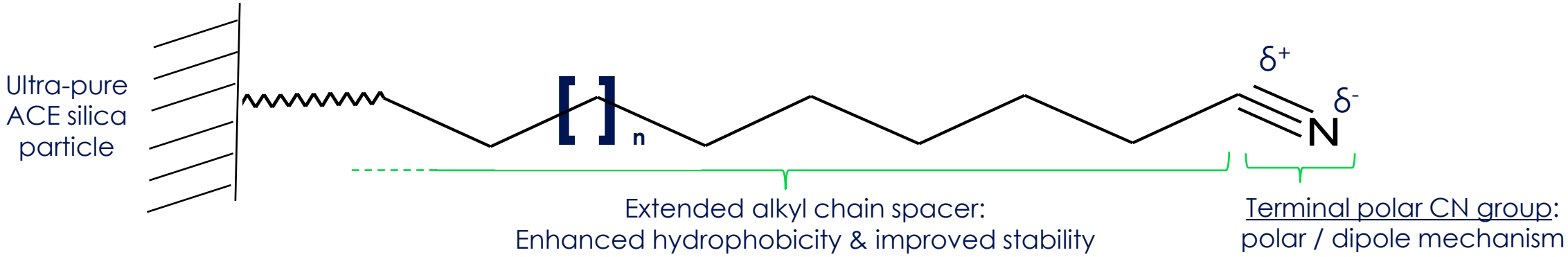
Comparative separations may not be representative of all applications. Phenomenex columns were not used in this comparison.

Scientific led stationary phase design: other phases

Avantor® ACE® C18-Amide



Avantor® ACE® CN-ES



Multiple mechanisms of interaction, low bleed and are 100% wettable: i.e. maximise selectivity

How do we characterise selectivity?



- It is useful to characterize stationary phase chemistry:
  - Design new stationary phases
  - Help guide stationary phase selection
  - Design structured and rational method development protocols

- Several approaches have been published that are designed to probe/characterise chromatographic selectivity, for example:
  - Tanaka approach[1]
  - Snyder-Dolan Hydrophobic subtraction model (HSM)[2]
  - Neue selectivity approach[3]

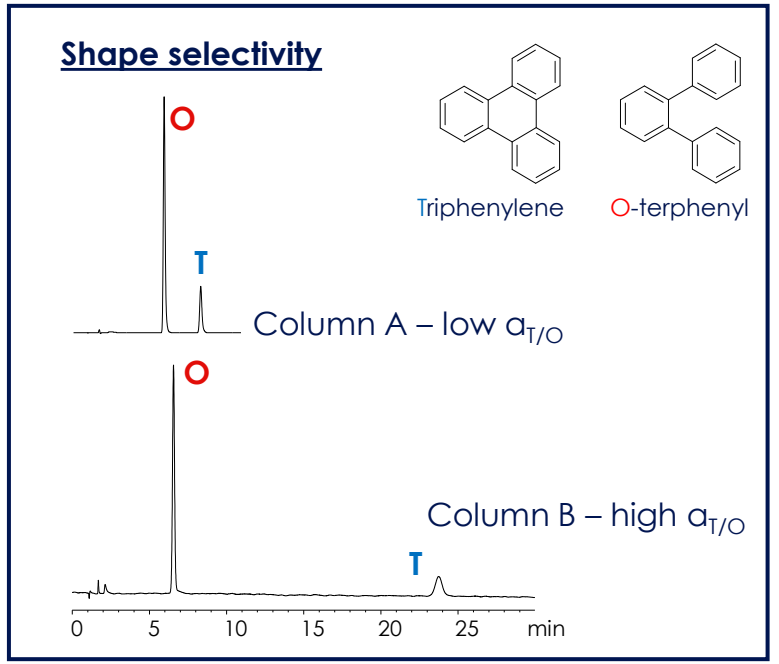
1. K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, *J. Chromatogr. Sci.* (1989) **27** 721
2. Wilson, NS. Nelson, MD. Dolan, JW. Snyder, LR, Wilcott, RG. Carr, PW. J. chromatogr. A (2002) **961** 171-193
3. Neue, O'Gara, Méndez "Selectivity in Reversed-Phase Separations: Influence of the Stationary Phase", *J. Chromatogr. A* (2006) **1127** 161-174

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Tanaka characterization

- Developed by Tanaka to characterize and compare LC stationary phases
- Standard probe analytes are used to assess specific chromatographic interactions between the analyte and stationary phase

Type of interaction	Analyte(s)	Parameter
Hydrophobic	Amylbenzene, butylbenzene	k_{PB}, a_{CH2}
Hydrogen bonding	Caffeine, phenol	$a_{C/P}$
Steric/shape	Triphenylene, *O*-terphenyl	$a_{T/O}$
Phenolic	Phenol, benzylalcohol	$a_{P/BnOH\ pH\ 2.7}$
Acidic cation exchange capacity	Benzylamine, phenol	$a_{B/P\ pH2.7}$
Total cation exchange capacity	Benzylamine, phenol	$a_{B/P\ pH7.6}$

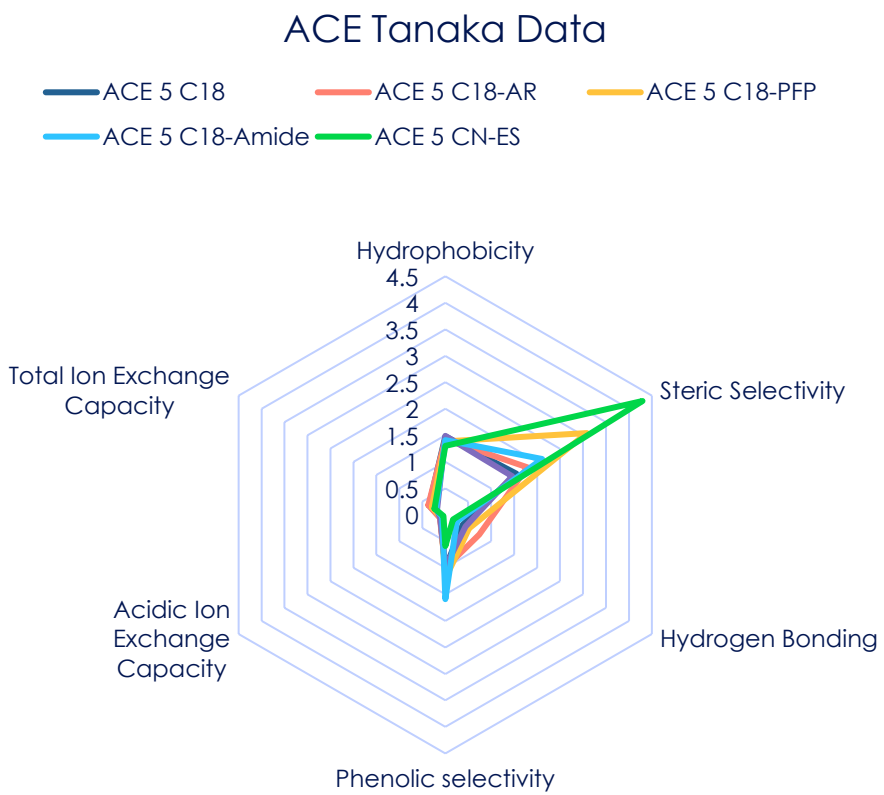


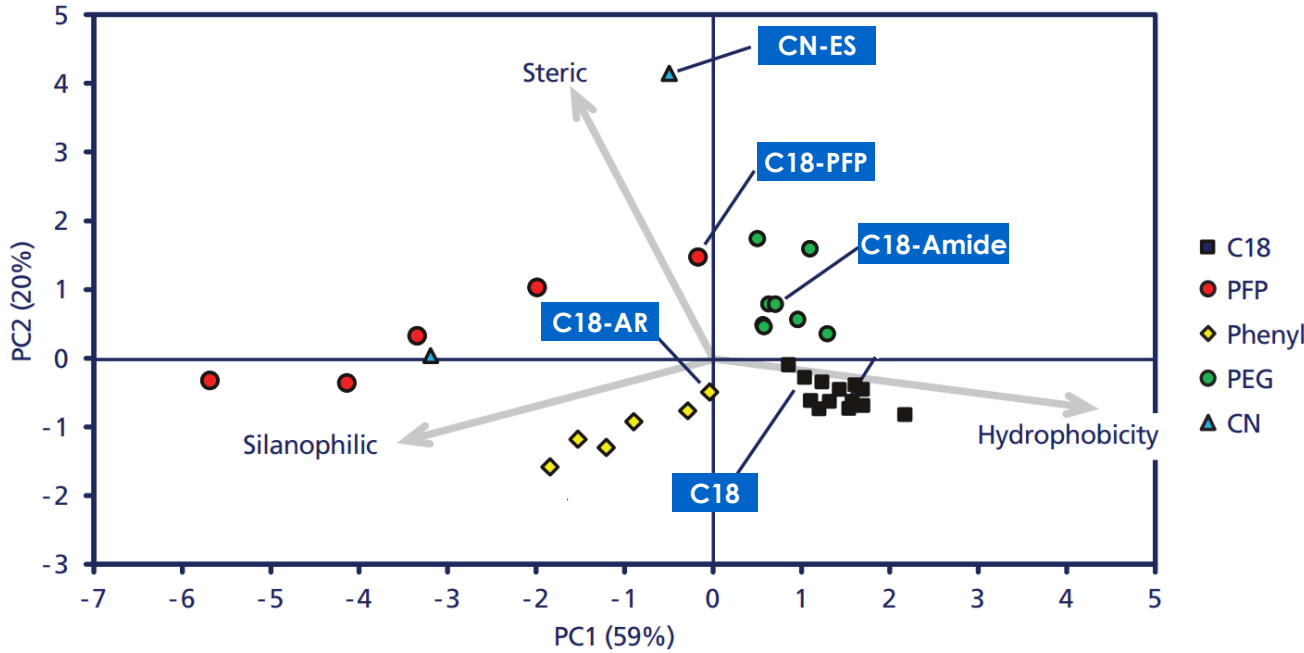
- Can be extended to include compounds to probe aromatic and dipole interactions[2,3]

1. [1] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, *J. Chromatogr. Sci.* 27 (1989) 721
2. Merkopoulou C *et al, Chromatographia* (2009) **10** 705-715
3. Euerby, M. James, M. Axelsson, B-O. Rosen, O. Petersson, P. *J. Sep. Sci* (2012) **35** 2592-2698

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Tanaka characterization of Avantor® ACE® novel phases

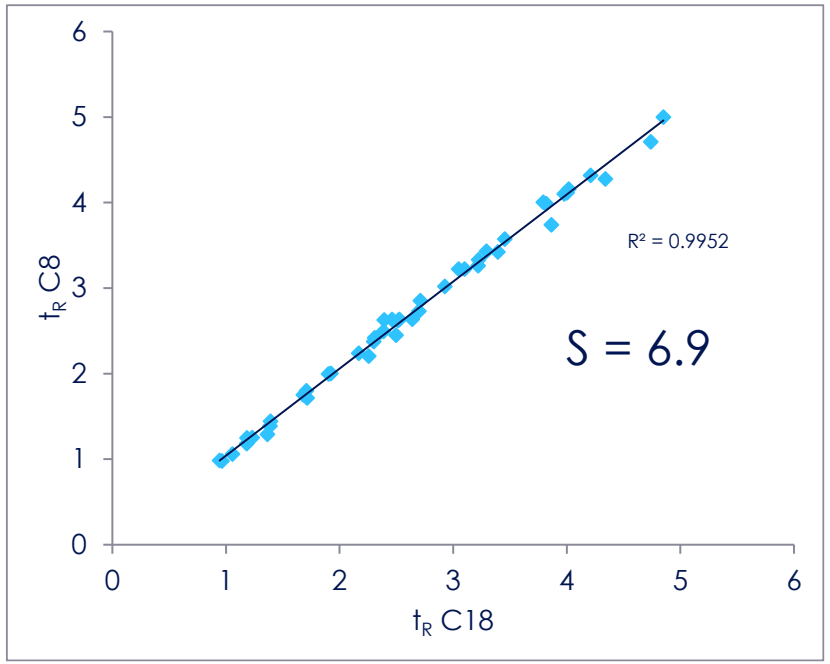




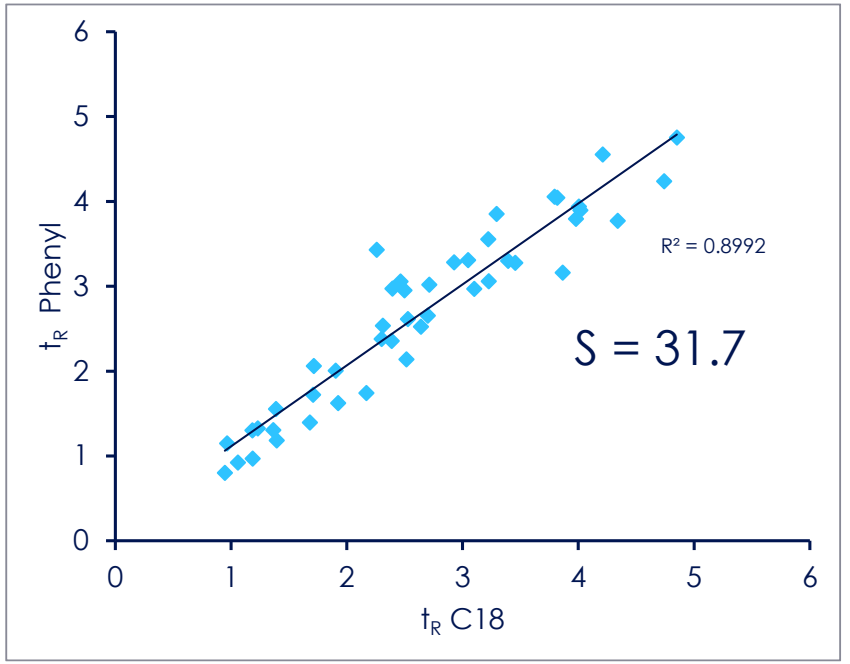
Adapted from Euerby, MR, Fever, M, Hulse, J, James, M, Petersson, P, Pipe, C, *LCGC Europe* (2016) **29** 8-21

Selectivity value (S) approach

- Developed by Neue, O'Gara and Méndez to obtain a numerical value for how similar/different two columns are in terms of their selectivity.[1]
- A large number of analytes are screened under identical gradient conditions on two columns.
- The retention times are plotted for both columns and the selectivity correlations determined (r^2)
- Selectivity values S are calculated
- Can compare selectivity under different mobile phase conditions



$$S = 100\sqrt{1 - r^2}$$



1. Neue, O'Gara, Méndez "Selectivity in Reversed-Phase Separations: Influence of the Stationary Phase", *J. Chromatogr. A* (2006) **1127** 161-174

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Selectivity values for Avantor® ACE® novel phases

41 structurally diverse analytes (basic, acidic, neutrals, and phenolic) we screened under gradient conditions to obtain selectivity values.

Chemistry	C18	C18-AR	C18-PFP	C18-Amide	CN-ES
C18	0	19	15	18	25
C18-AR		0	15	28	27
C18-PFP			0	19	20
C18-Amide				0	19
CN-ES					0

5 min gradient (3-100% B) on 50 x 2.1 mm columns
A : 20 mM Ammonium formate pH 3.0 (aq)
B: 20 mM Ammonium formate pH 3.0 in MeOH:H_2O (9:1 v/v)
Flow rate 0.4 mL/min
Temperature: 40 °C

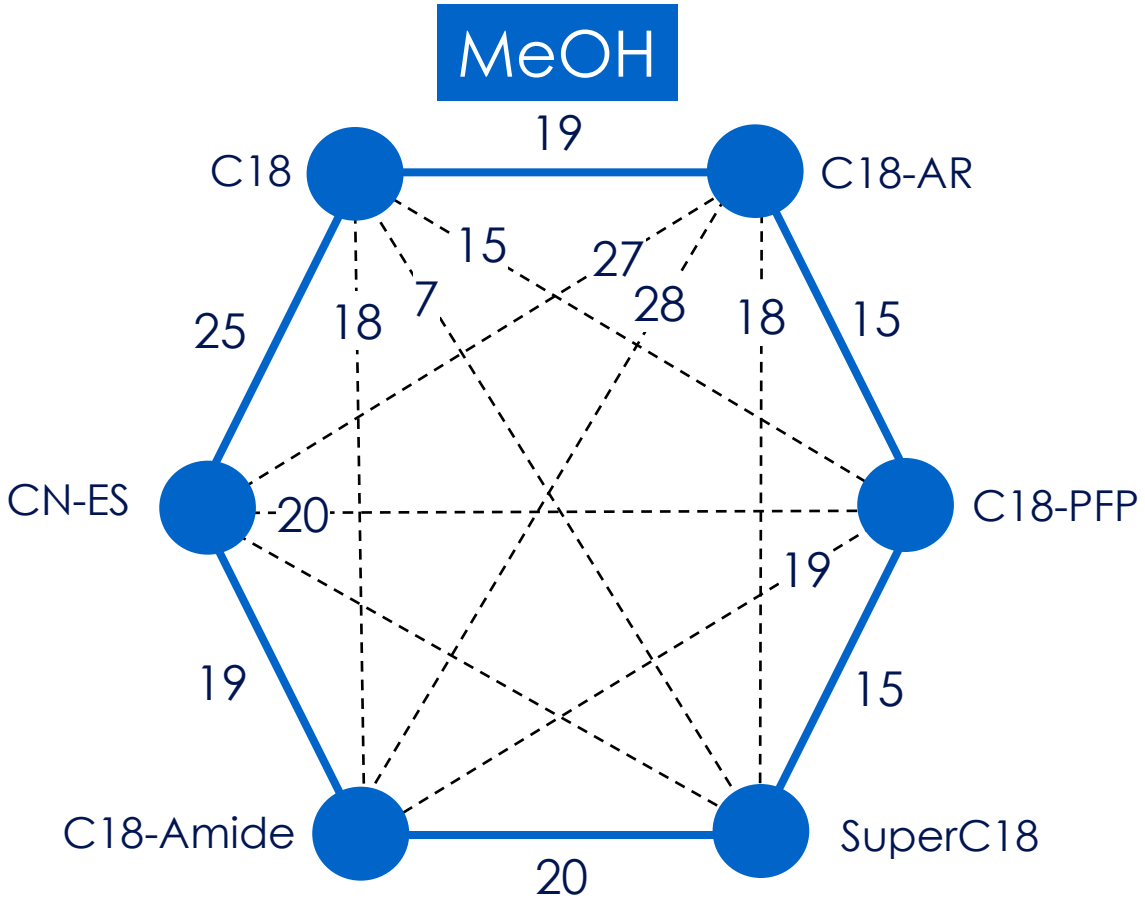
$$S = 100\sqrt{1 - r^2}$$

Euerby, MR, Fever, M, Hulse, J, James, M, Petersson, P, Pipe, C, *LCGC Europe* (2016) **29** 8-21

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Building a method development platform

6 stationary phases, 1 solvent, 1 low pH





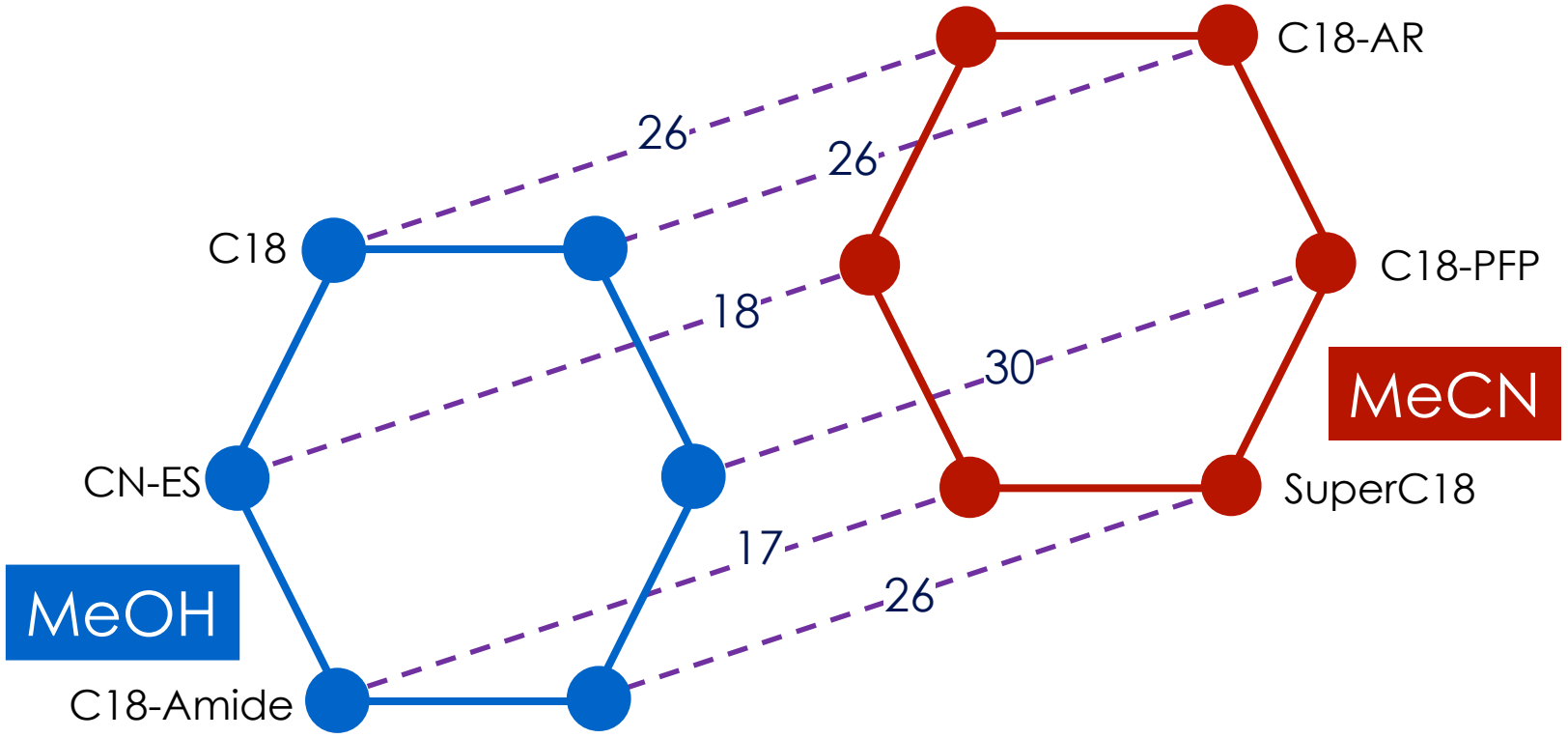
6 Column Method Development Platform Based Upon The Power of Phase Chemistry / Selectivity

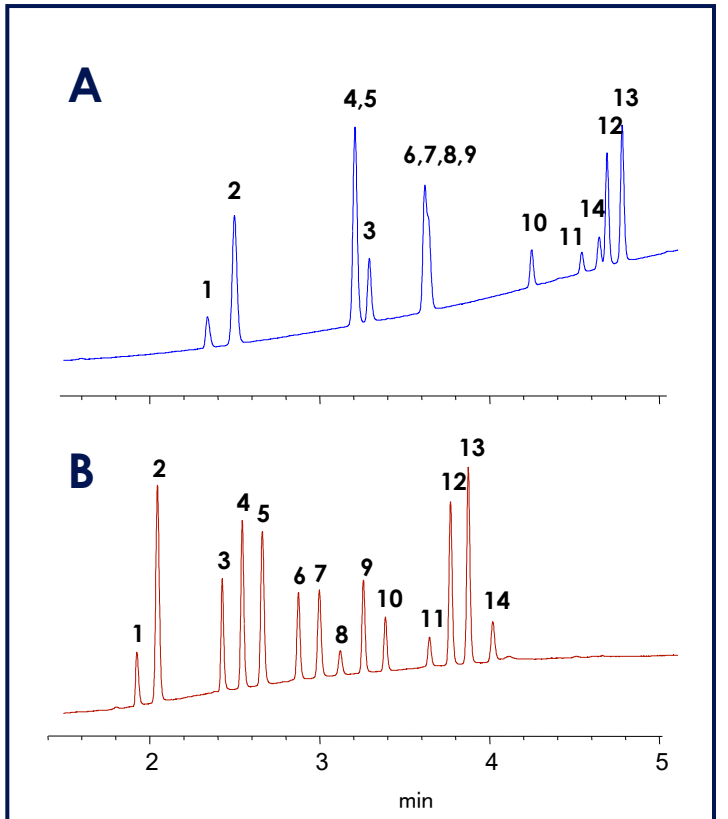
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Building a method development platform

The organic modifier has a big impact on selectivity too
Great for method development!

6 stationary phases, 2 solvents, 1 low pH





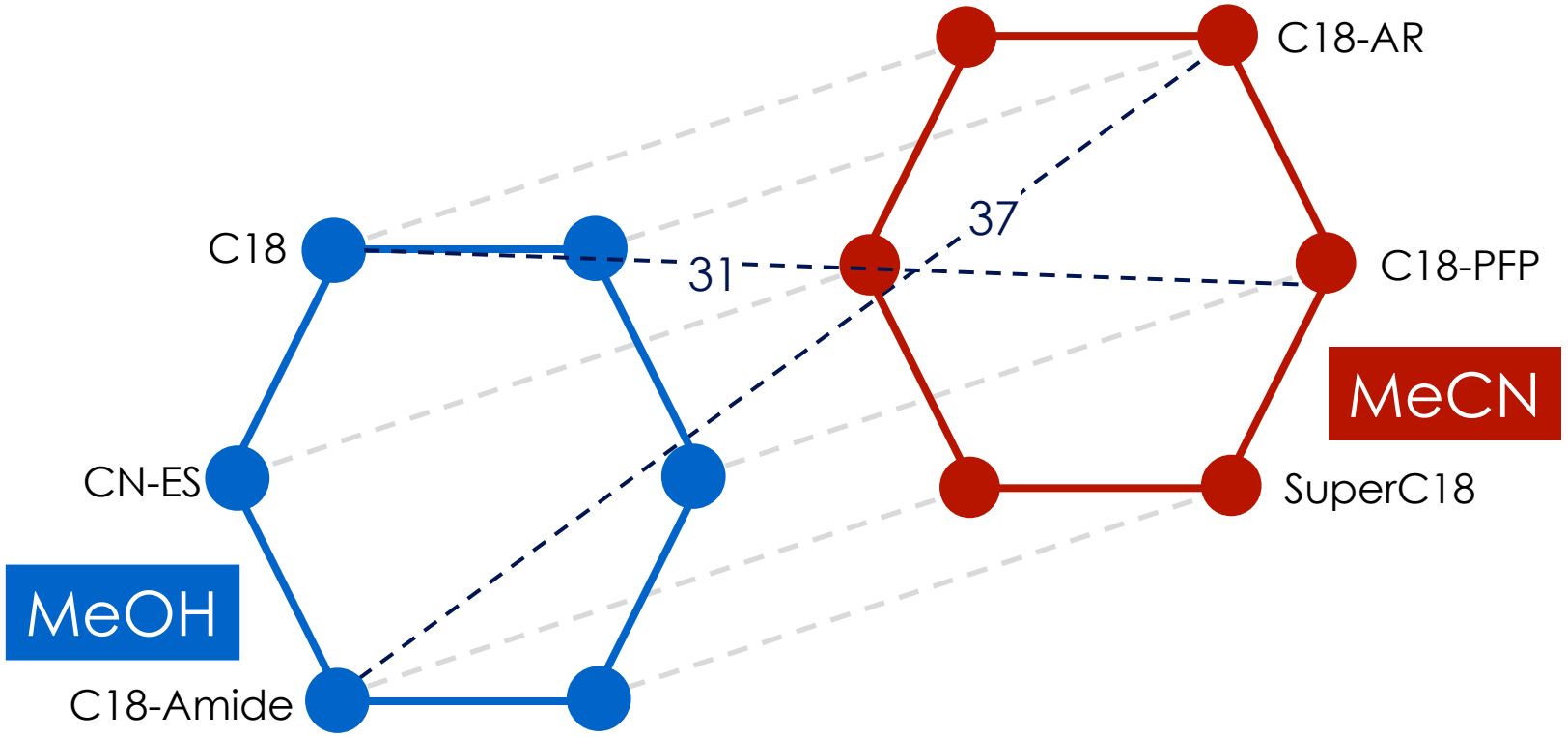
ACE 3 C18 50 x 2.1 mm
A: MeOH as modifier
B: MeCN as modifier

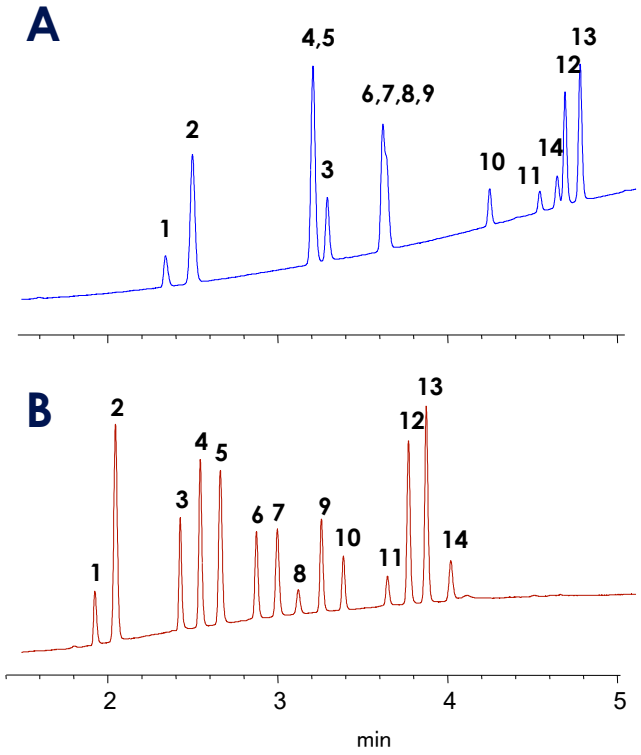
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Building a method development platform

The organic modifier has a big impact on selectivity too
Great for method development!

6 stationary phases, 2 solvents, 1 low pH





ACE 3 C18 50 x 2.1 mm
A: MeOH as modifier
B: MeCN as modifier

Method development workflow

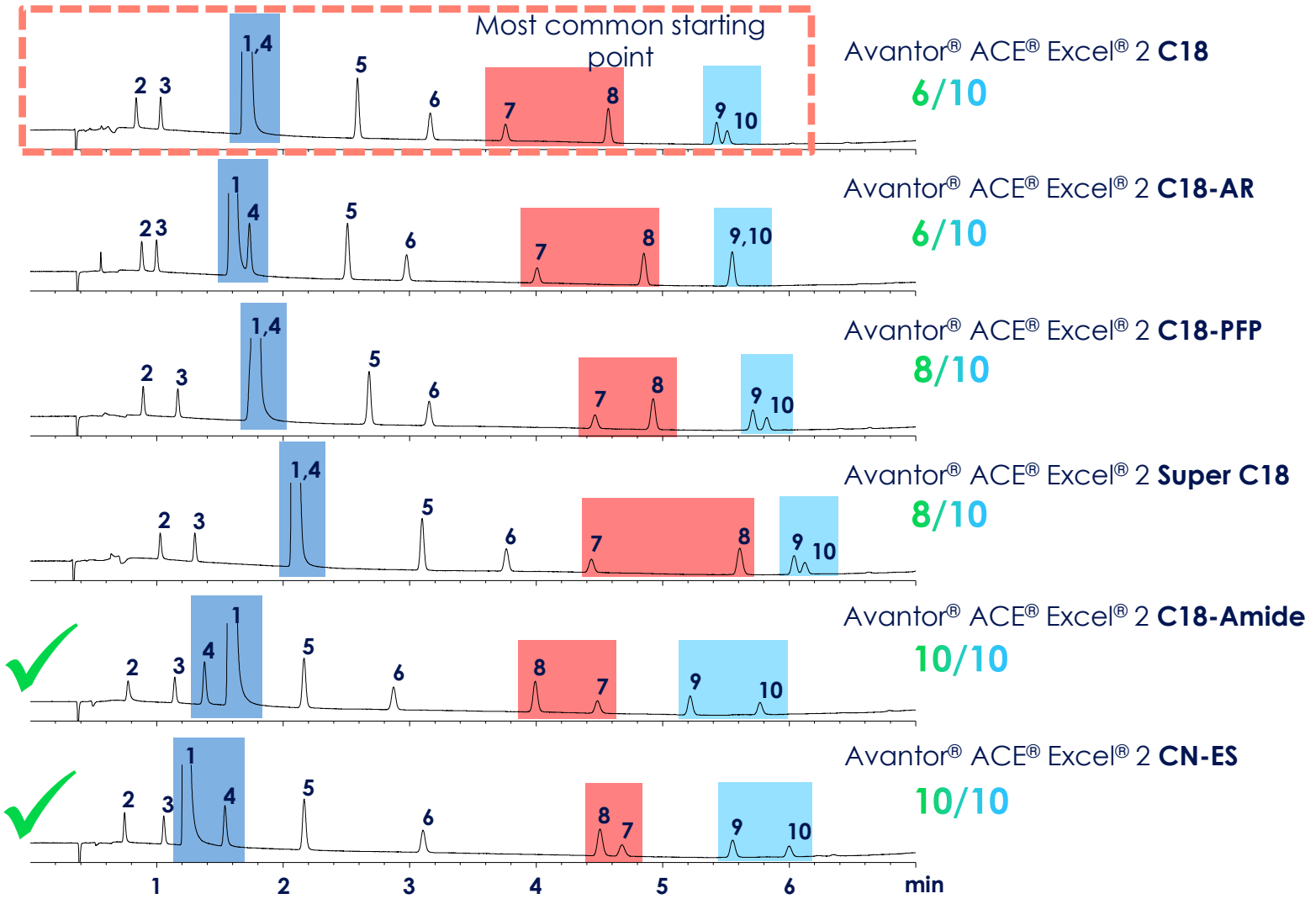


- The screening data is used to select a column/organic modifier combination for method optimization.
- Method optimization requires investigation of other parameters:
  - Gradient time and %B range
  - Temperature
  - pH
- **Retention modelling software (ChromSword Offline 5.2 Lite)** can be used to streamline method optimization.

ChromSword

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Total selectivity, method development: screening platform



Avantor® ACE® Excel® 2 **C18**

6/10

Avantor® ACE® Excel® 2 **C18-AR**

6/10

Avantor® ACE® Excel® 2 **C18-PFP**

8/10

Avantor® ACE® Excel® 2 **Super C18**

8/10

Avantor® ACE® Excel® 2 **C18-Amide**

10/10

Avantor® ACE® Excel® 2 **CN-ES**

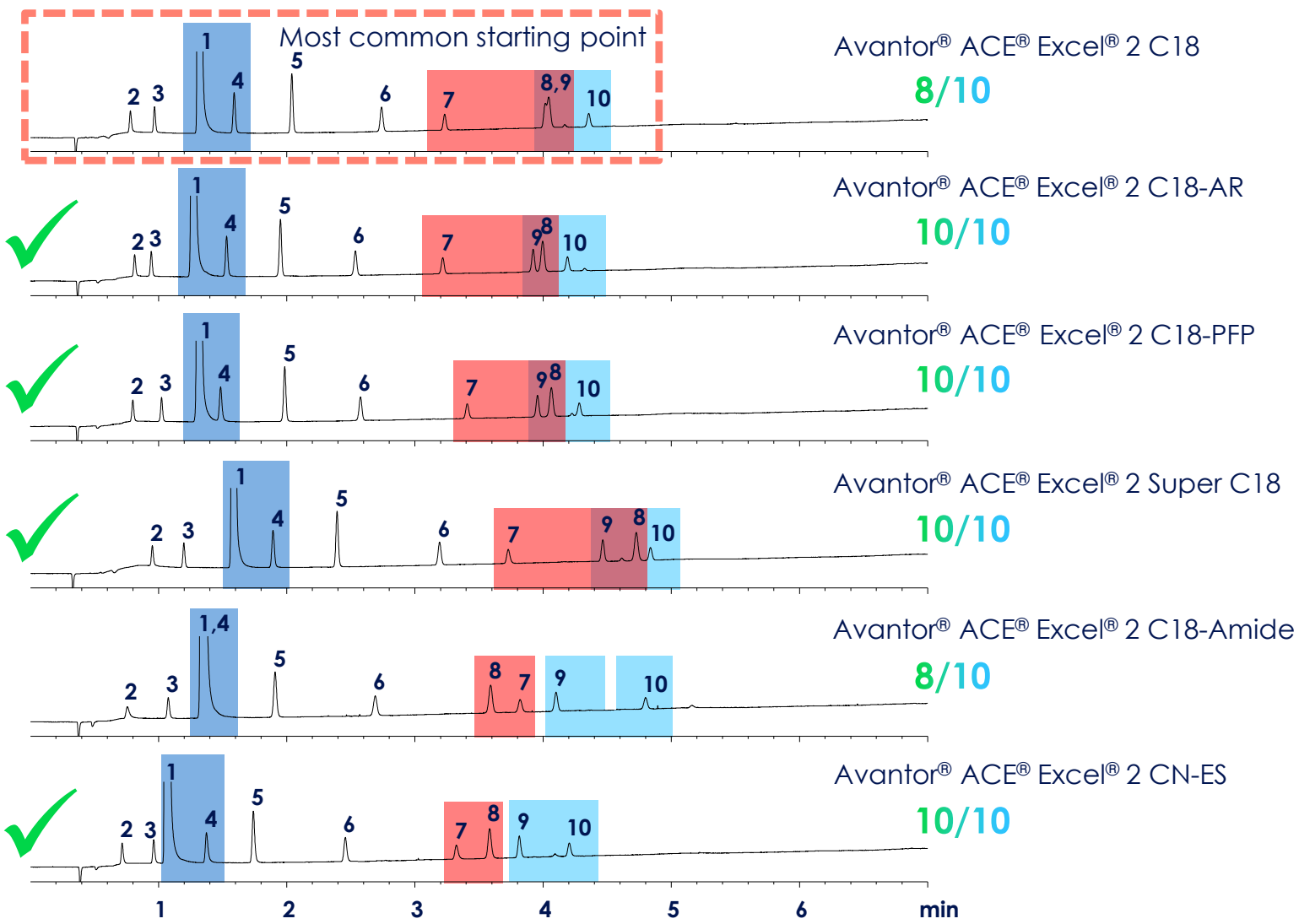
10/10

MeOH

General Scouting Conditions:

Columns: 100 x 3.0 mm, 2 µm
A: 20 mM ammonium acetate pH 6.0
B: 20 mM ammonium acetate pH 6.0 in MeOH or MeCN:H_2O (9:1 v/v)
Gradient: 5 to 95 %B in 10 mins
Temp: 40 °C, 2 µl injection,
Flow rate: 1.2 ml/min

Total selectivity, method development: screening platform

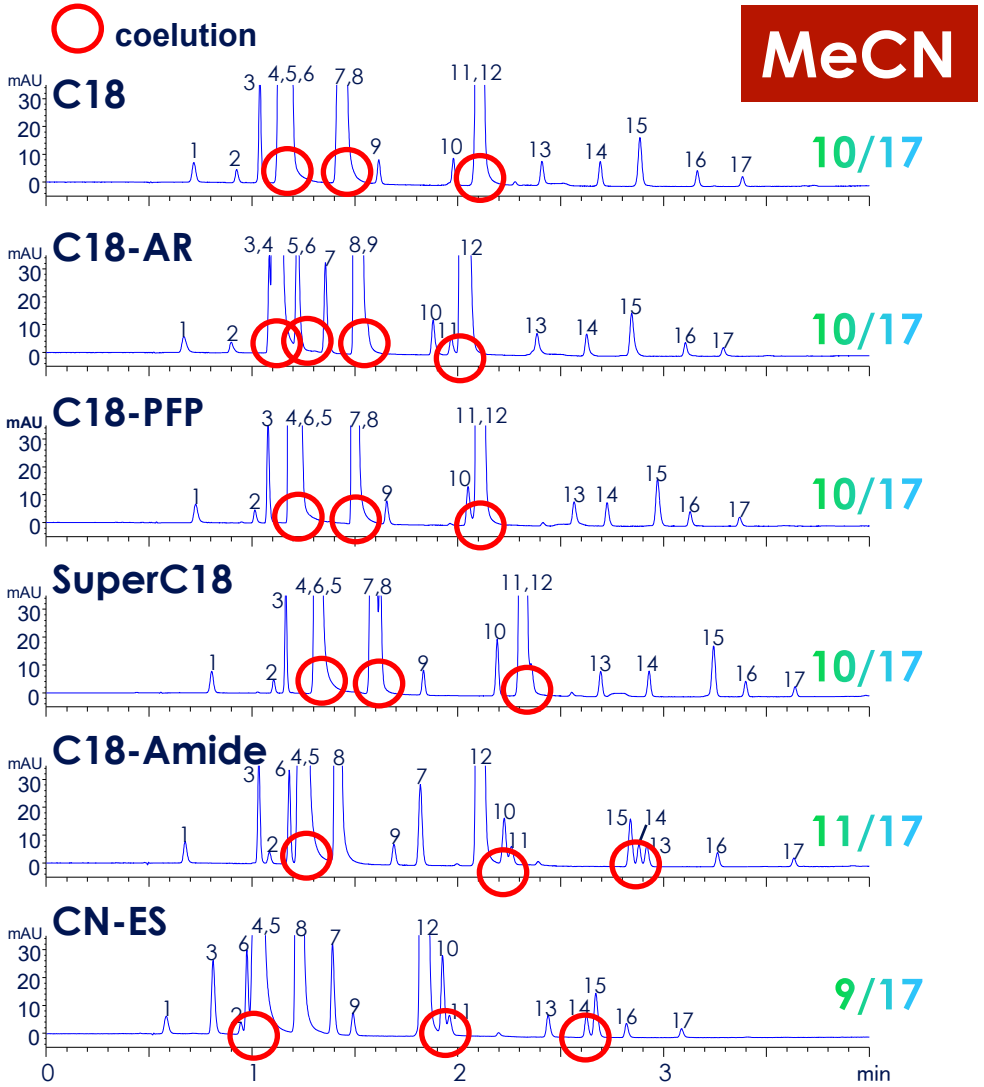


MeCN

General Scouting Conditions:

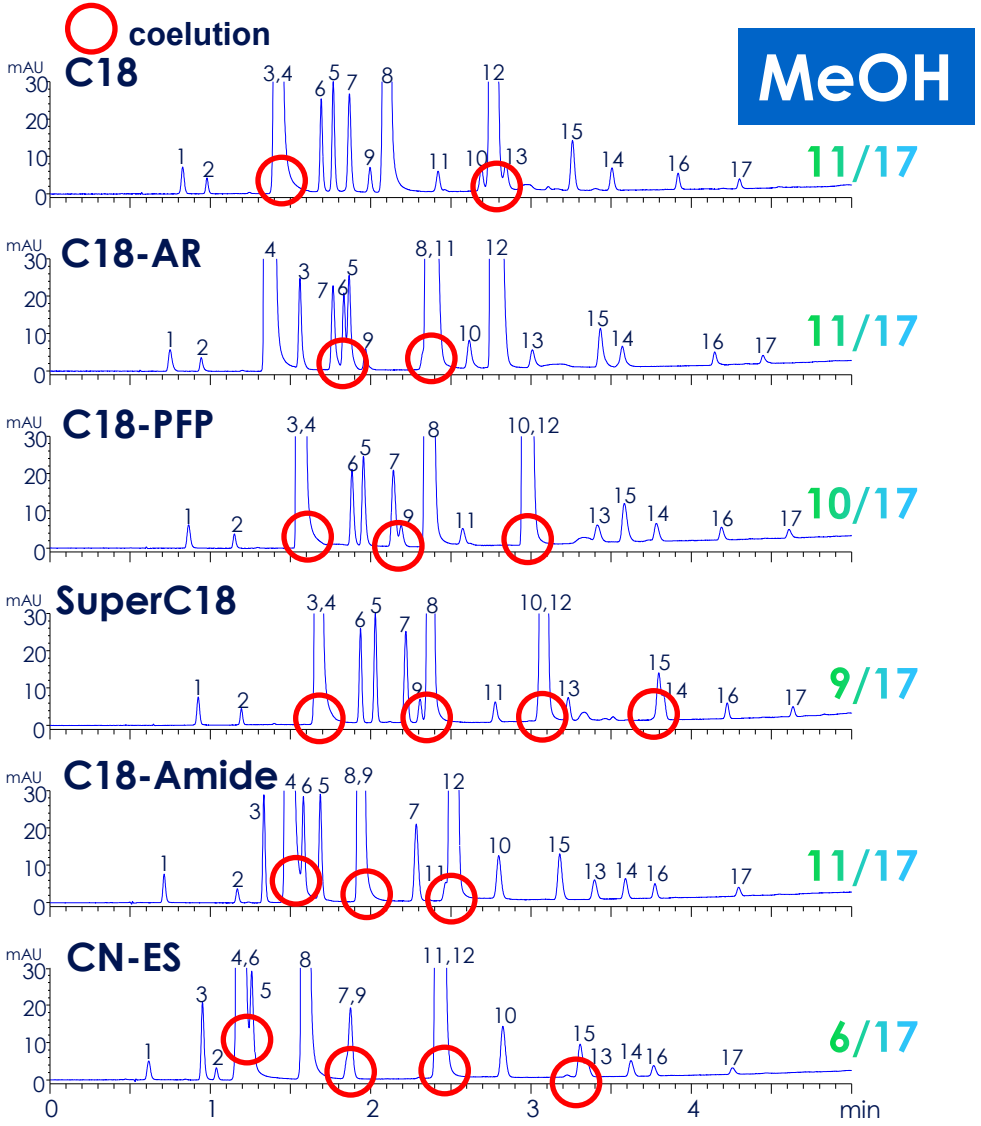
Columns: 100 x 3.0 mm, 2 µm
A: 20 mM ammonium acetate pH 6.0
B: 20 mM ammonium acetate pH 6.0 in MeOH or MeCN:H_2O (9:1 v/v)
Gradient: 5 to 95 %B in 10 mins
Temp: 40 °C, 2 µl injection,
Flow rate: 1.2 ml/min

6 Column screen of triple API sample & impurities (17 peaks)



Columns:	Avantor® ACE® Excel 2, 100 x 3.0 mm
System:	VWR Hitachi ChromasterUltra Rs
Mobile phases:	
	A: 10 mM ammonium formate pH 3.0
	B1: 10 mM ammonium formate pH 3.0 in MeCN:H_2O 9:1 v/v
	B2: 10 mM ammonium formate pH 3.0 in MeOH:H_2O 9:1 v/v
Gradient:	5-95% B in 5 minutes
Temp:	40 °C
Flow rate:	1.2 ml/min
Detection:	UV, 270 nm
Sample:	Aspirin: 5 mg/ml, Paracetamol: 3.3 mg/ml, Caffeine: 0.75 mg/ml, Impurities spiked at 0.1% (wrt aspirin)
	1. 2-Aminophenol, 2. Hydroquinone, 3. Theobromine, **4. Paracetamol**, 5. Theophylline, 6. Paraxanthine, 7. 4- Hydroxybenzoic acid, **8. Caffeine**, 9. 2-Acetamidophenol, 10. 2-Hydroxybenzoic acid, 11. Phenol, **12. Aspirin**, 13. 4-Nitrophenol, 14. 4-Chloroacetanilide, 15. 2-Nitrophenol, 16. Acetylsalicylsalicylic acid, 17. Salsalate

6 Column screen of triple API sample & impurities (17 peaks)

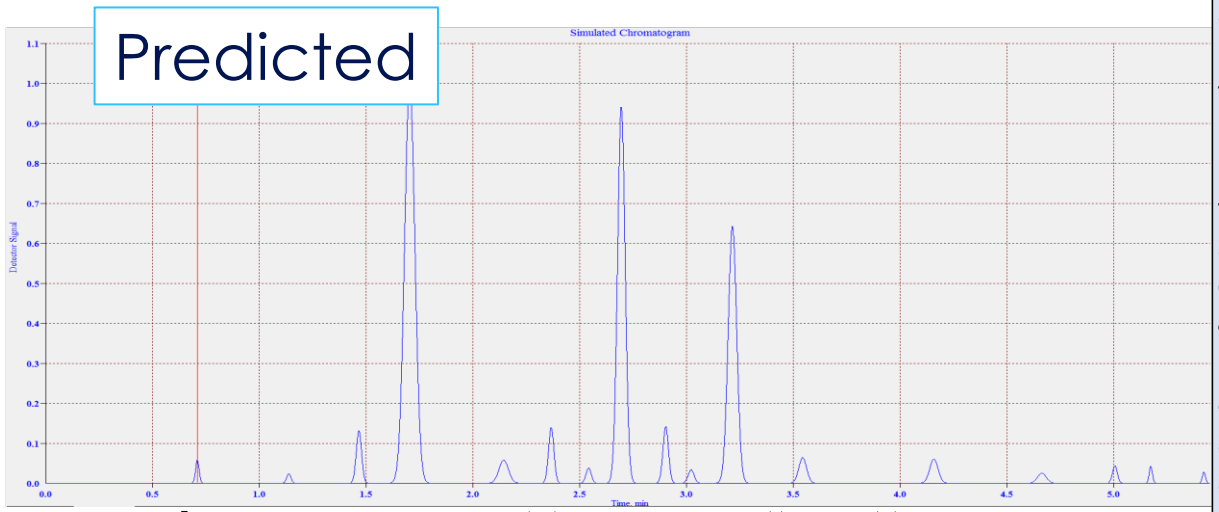


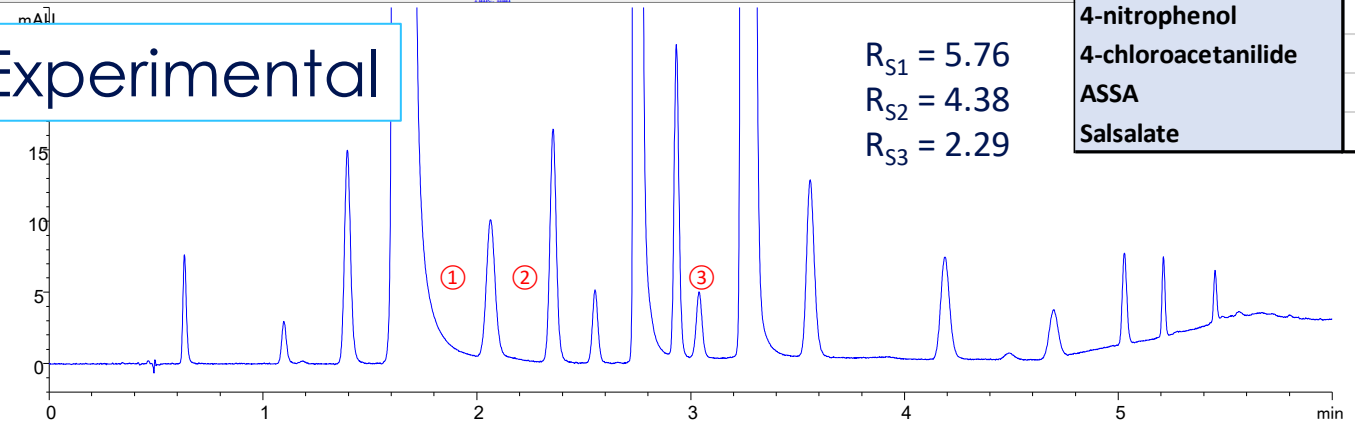
- Methanol provides better retention and selectivity for the more hydrophilic analytes.
- The screening approach provides multiple options to pursue for obtaining a full separation.
- The Avantor® ACE® C18, C18-AR and C18-Amide all resolve 11 of the 14 impurity peaks.
- Further optimization required after the initial 6 column, 2 solvent screen.

1. 2-Aminophenol, 2. Hydroquinone, 3. Theobromine, **4. Paracetamol**, 5. Theophylline, 6. Paraxanthine, 7. 4-Hydroxybenzoic acid, **8. Caffeine**, 9. 2-Acetamidophenol, 10. 2-Hydroxybenzoic acid, 11. Phenol, **12. Aspirin**, 13. 4-Nitrophenol, 14. 4-Chloroacetanilide, 15. 2-Nitrophenol, 16. Acetylsalicylsalicylic acid, 17. Salsalate

Optimising the separation with ChromSword 2 / ChromSword Lite 5.1

Final separation (Avantor® ACE® Excel 2 C18-Amide)

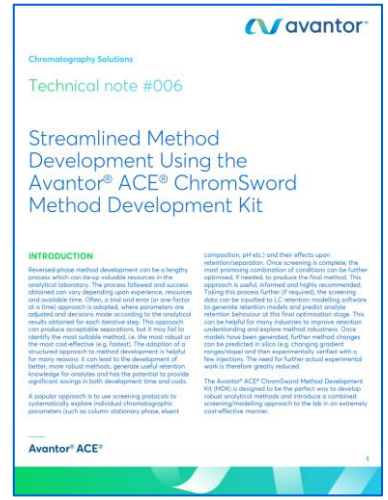




Compound	Predicted t_R	Experimental t_R	% error
2-aminophenol	0.71	0.633	10.8
hydroquinone	1.14	1.098	3.7
theobromine	1.47	1.395	5.1
paracetamol	1.70	1.633	3.9
paraxanthine	2.14	2.064	3.6
theophylline	2.37	2.357	0.5
2-acetamidophenol	2.54	2.554	-0.6
caffeine	2.69	2.747	-2.1
4-hydroxybenzoic acid	2.90	2.933	-1.1
phenol	3.02	3.039	-0.6
aspirin	3.21	3.26	-1.6
2-hydroxybenzoic acid	3.54	3.559	-0.5
2-nitrophenol	4.16	4.189	-0.7
4-nitrophenol	4.66	4.697	-0.8
4-chloroacetanilide	5.01	5.027	-0.3
ASSA	5.17	5.21	-0.8
Salsalate	5.42	5.452	-0.6

Gradient Profile

t (mins.)	%B
0.0	10
1.6	10
2.2	34
4.0	40
5.0	90
6.0	90



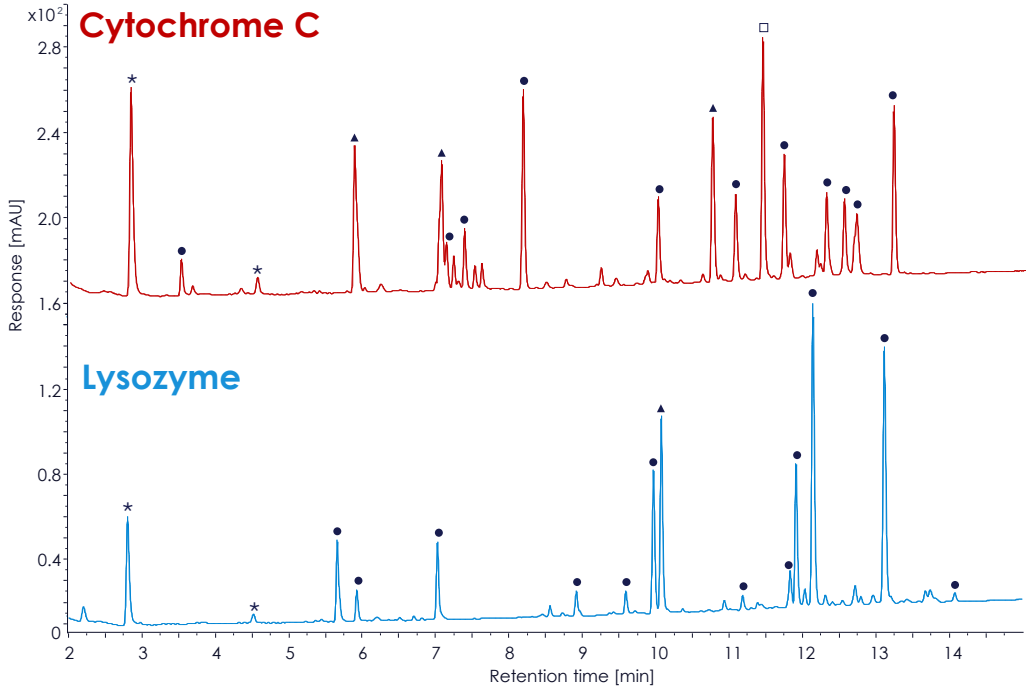
ChromSword avantor™

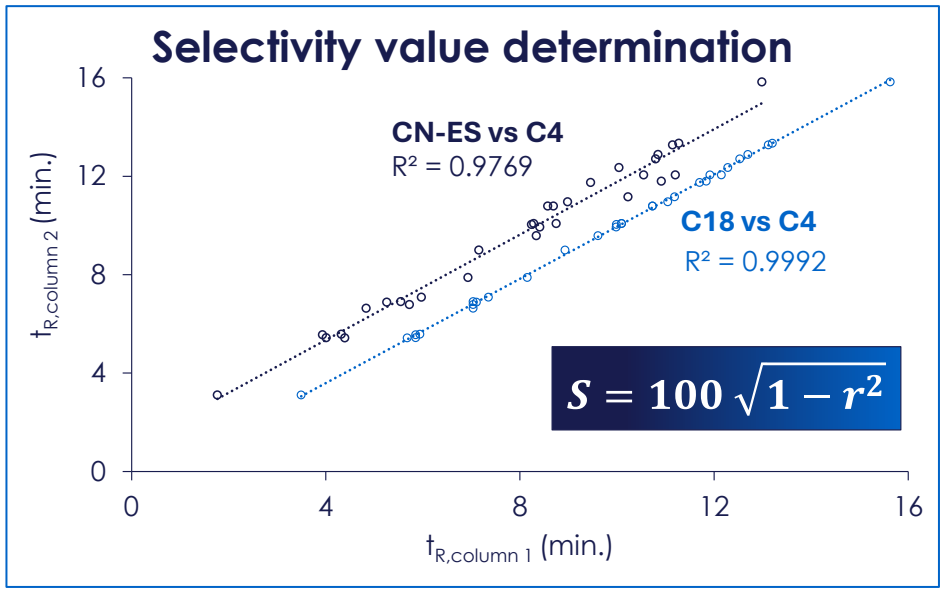
Application to other analyte classes and separation modes

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Exploring peptide selectivity

- Peptide digests used to generate S values for a range of prototype ACE phases bonded to 150 Å silica

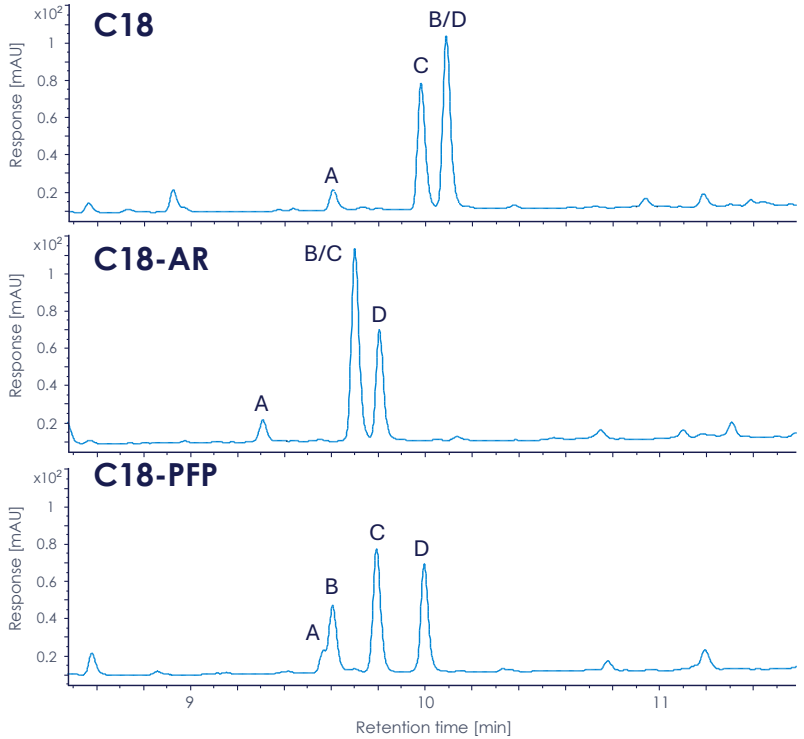


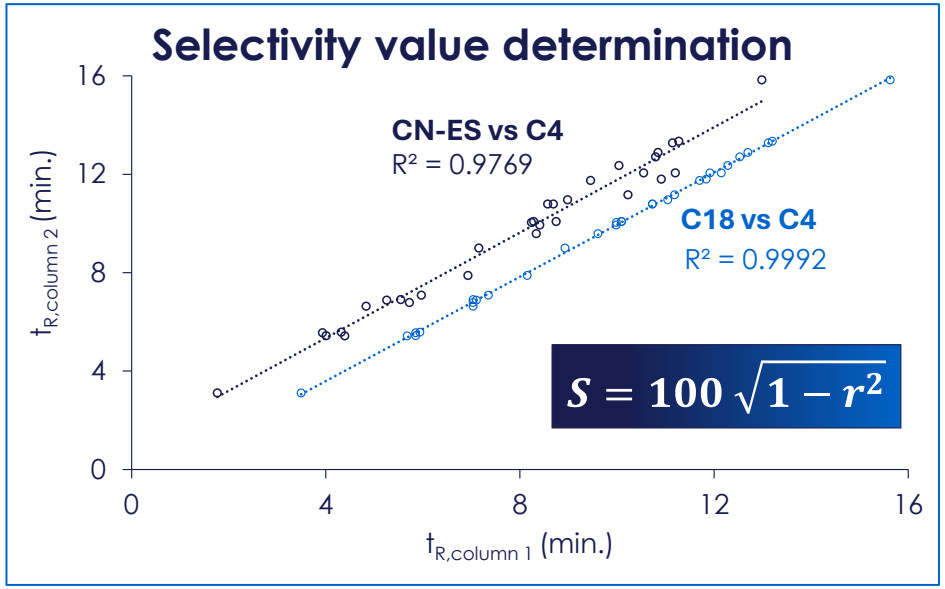


	C18	C18-AR	C18-PFP	CN-ES	Super C18	C4	C8
C18	0	7.8	7.3	14.7	3.6	2.8	2.2
C18-AR		0	4.8	9.6	7.3	8.9	8.9
C18-PFP			0	8.0	6.9	8.2	8.1
CN-ES				0	14.0	15.2	15.3
SuperC18					0	5.4	4.0
C4						0	2.0
C8							0

Exploring peptide selectivity

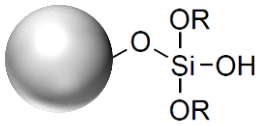
- Peptide digests used to generate S values for a range of prototype ACE phases bonded to 150 Å silica
- Alkyl phase S values very low – little selectivity variation
- Lower S values than small molecules, but significant selectivity differences can be observed



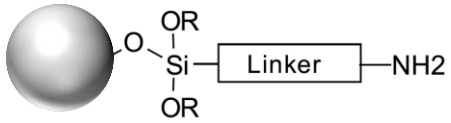


	C18	C18-AR	C18-PFP	CN-ES	Super C18	C4	C8
C18	0	7.8	7.3	14.7	3.6	2.8	2.2
C18-AR		0	4.8	9.6	7.3	8.9	8.9
C18-PFP			0	8.0	6.9	8.2	8.1
CN-ES				0	14.0	15.2	15.3
SuperC18					0	5.4	4.0
C4						0	2.0
C8							0

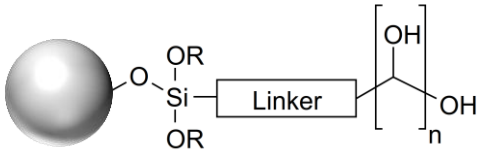
Tanaka characterization results for Avantor ACE HILIC columns



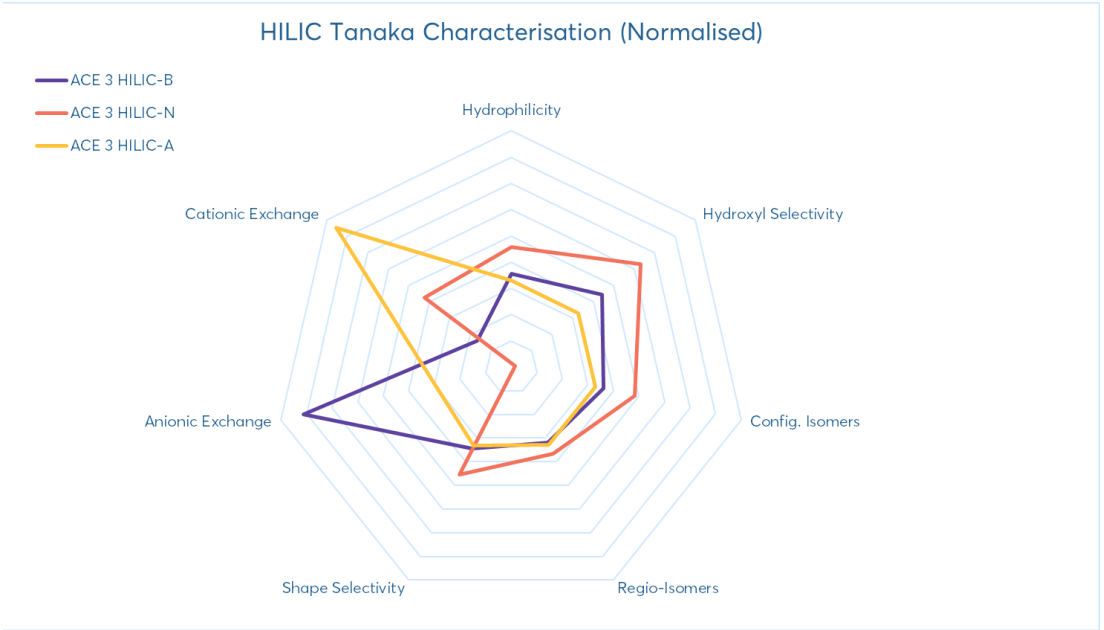
Avantor® ACE® HILIC-A
Proprietary Acidic Phase



Avantor® ACE® HILIC-B
Proprietary Basic Phase



Avantor® ACE® HILIC-N
Proprietary Neutral Phase

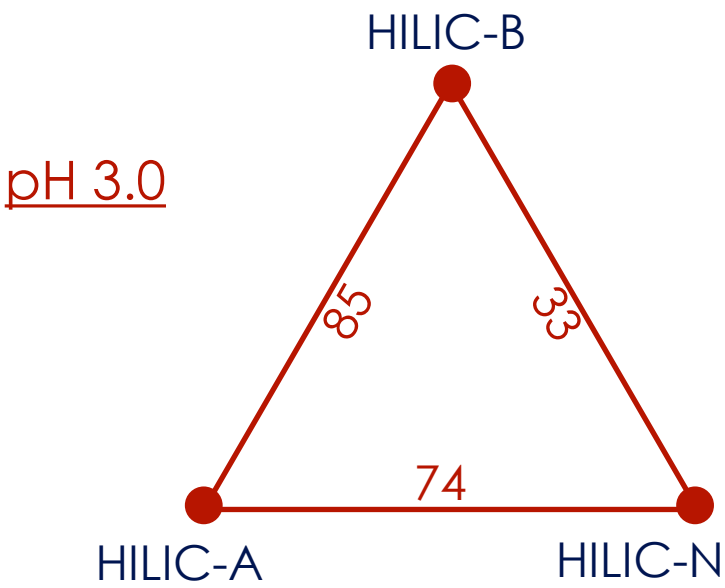


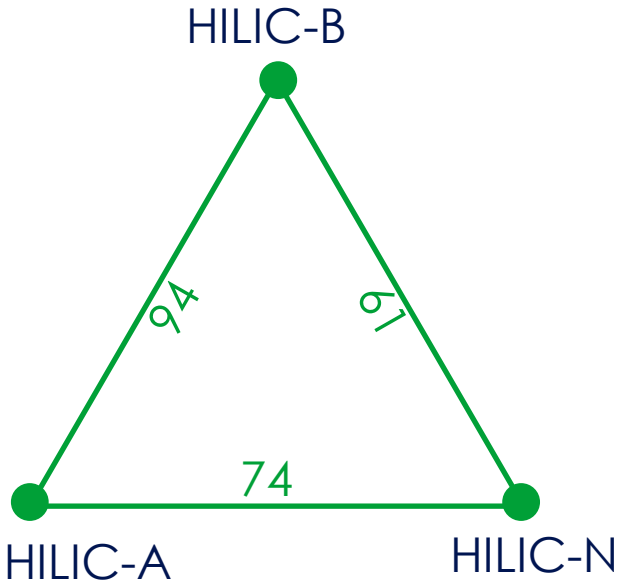
Parameter	Calculation
α(pH)	Retention factor ratio (Tb/Tp) between theobromine (Tb) and theophylline (Tp)
α(CH2)	Retention factor ratio (U/5mU) between uridine (U) and 5-methyluridine (5mU)
α(OH)	Retention factor ratio (U/2dU) between uridine (U) and 2'-deoxyuridine (2dU)
α(V/A)	Retention factor ratio between vidarabine (V) and adenosine (A)

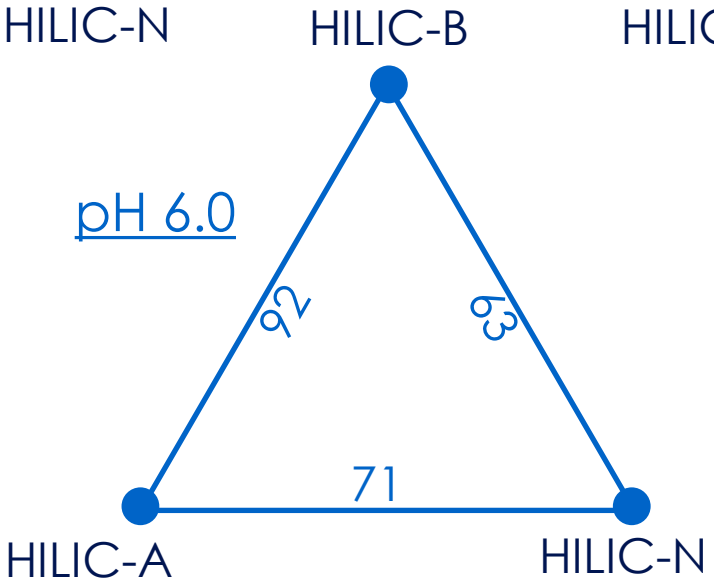
Parameter	Calculation
α(2dG/3dG)	Retention factor ratio between 2'-deoxyguanosine (2dG) and 3' deoxyguanosine (3dG)
α(α/β)	Retention factor ratio between 4-nitrophenyl-α-D-glucopyranoside (α) and 4-nitrophenyl-β-D-glucopyranoside (β)
α(AX)	Retention factor ratio (SpTS/U) between sodium p-toluenesulfonate (SpTS) and uridine (U)
α(CX)	Retention factor ratio (TMPAC/U) between N,N,N-trimethylphenyl ammonium chloride (TMPAC) and uridine (U)

Y. Kawachi, T. Ikegami, H. Takubo, Y. Ikegami, M. Miyamoto, N. Tanaka, *J. Chrom. A*, (2011) **1218** 5903-19

HILIC method development - influence of eluent pH





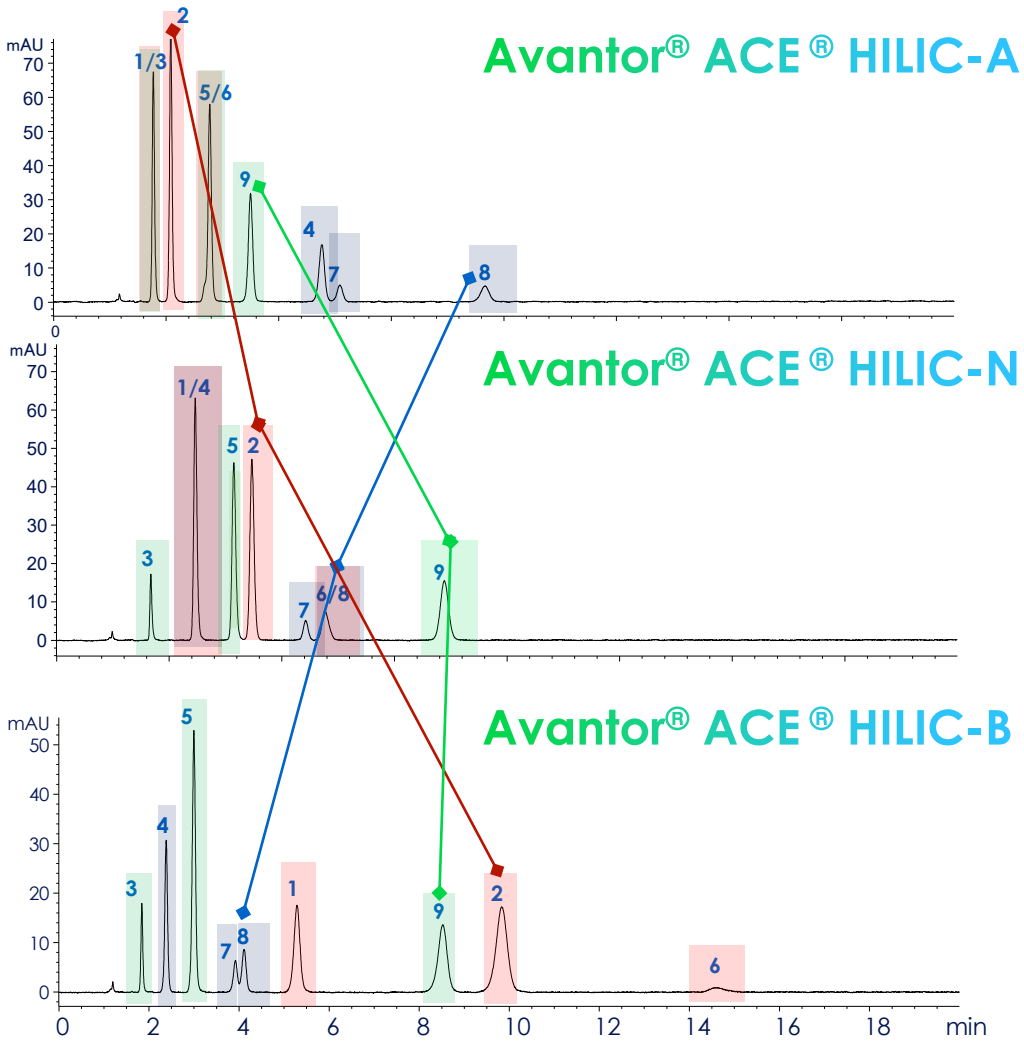


- Using a HILIC characterisation protocol with 54 polar analytes, Selectivity values in HILIC mode were determined:

- These data indicate that phases of acidic, neutral and basic character offer suitable differences for HILIC method development activities

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HILIC stationary phases with different selectivity



Elution order

1/3, 2, 5/6, 9, 4, 7, 8

3, 1/4, 5, 2, 7, 6/8, 9

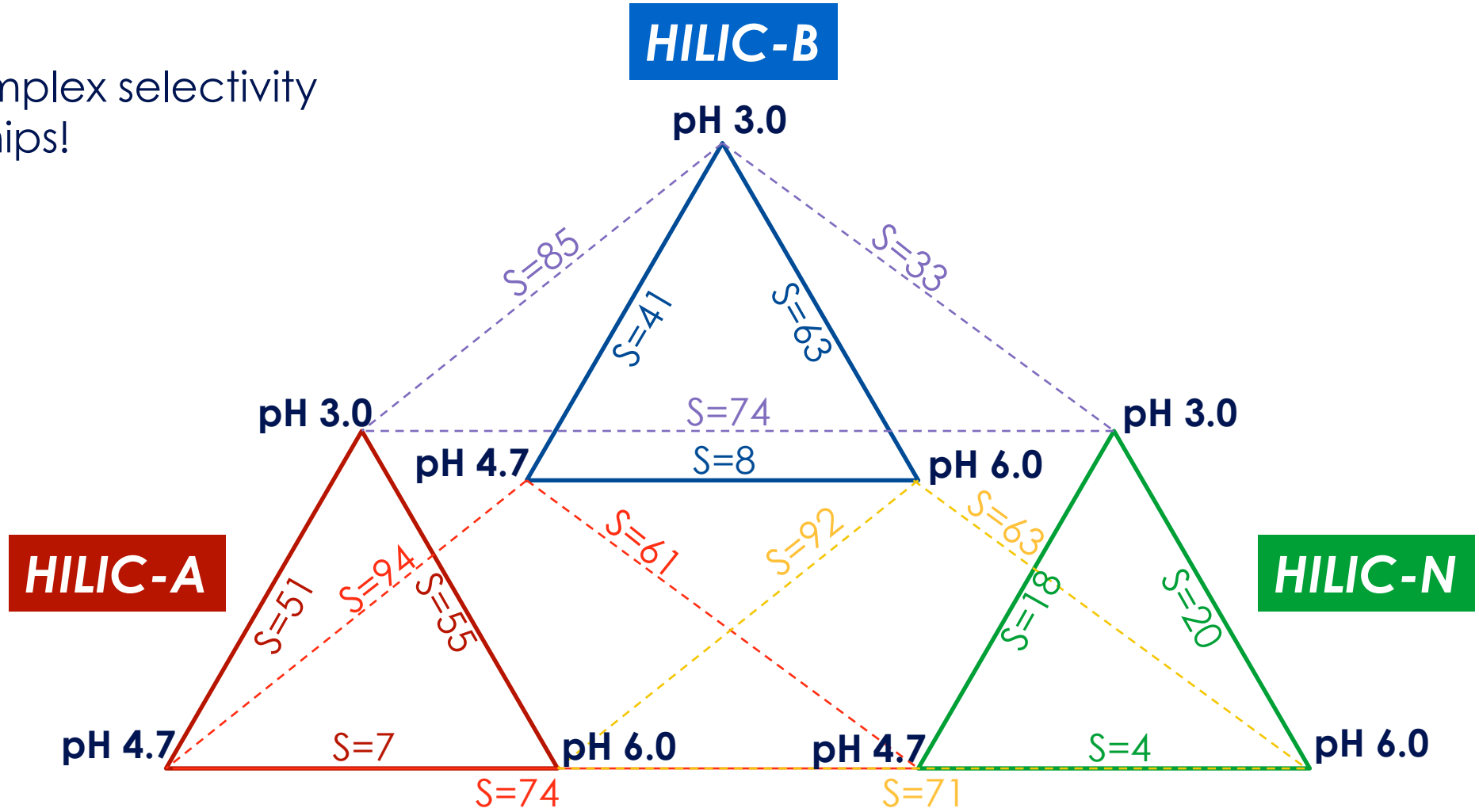
3, 4, 5, 7/8, 1, 9, 2, 6

Mobile phase:	10 mM ammonium formate pH 4.7 in MeCN/H_2O (90:10 v/v)
Flow:	1.5 ml/min
Temperature:	25 °C
Detection:	254 nm
Injection:	5 µl

1. **p-Aminobenzoic acid**
2. **4-Hydroxybenzoic acid**
3. **Nicotinamide**
4. **Acebutolol**
5. **Adenine**
6. **Mandelic acid**
7. **Tyramine**
8. **Atenolol**
9. **2-Deoxyguanosine**

HILIC method development

More complex selectivity relationships!



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HILIC method development flow chart

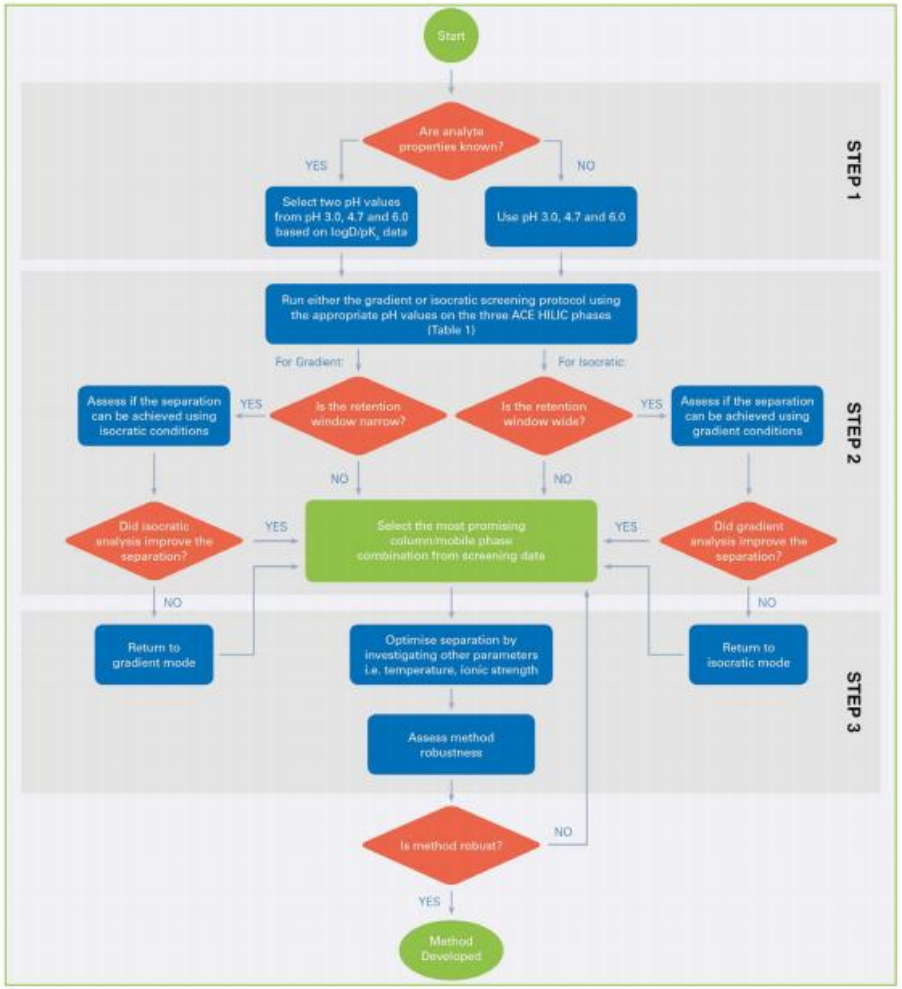


FIGURE 1: HILIC method development flow chart.

Column	ACE HILIC-A, ACE HILIC-B and ACE HILIC-N, 150 x 4.6, 5 µm
Isocratic screening	10 mM ammonium formate in MeCN/H_2O (9:1 v/v) ammonium formate at pH 3.0, 4.7 or 6.0
Gradient screening	Line A: 10 mM ammonium formate in MeCN/H_2O (94:6 v/v) Line B: 10 mM ammonium formate in MeCN/H_2O (50:50 v/v) Ammonium formate at pH 3.0, 4.7 or 6.0

Time (min)	%B
0	0
15	100
20	100
21	0
41	0

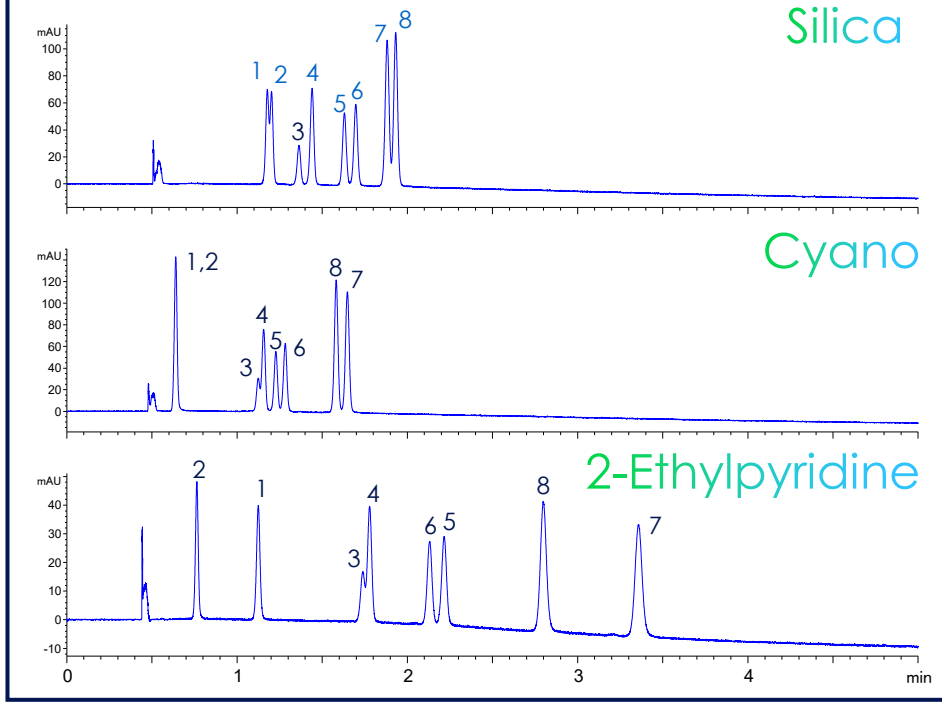
Flow rate	1.5 ml/min
Temperature	25 °C
Detection	Dependent on sample

TABLE 1: Recommended isocratic and gradient HILIC screening conditions.

Measuring the selectivity range of prototype SFC phases

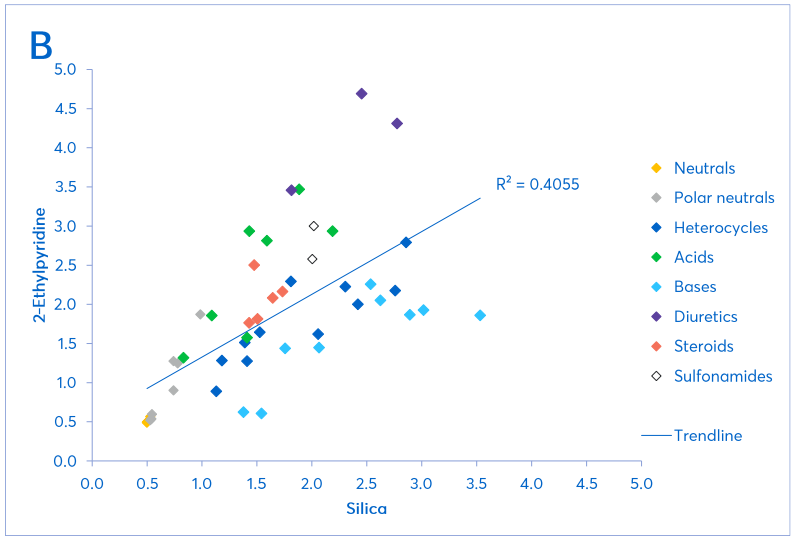
	Silica	Cyano	2-Ethyl pyridine
Silica	0	51	77
Cyano		0	59
2-Ethyl Pyridine			0

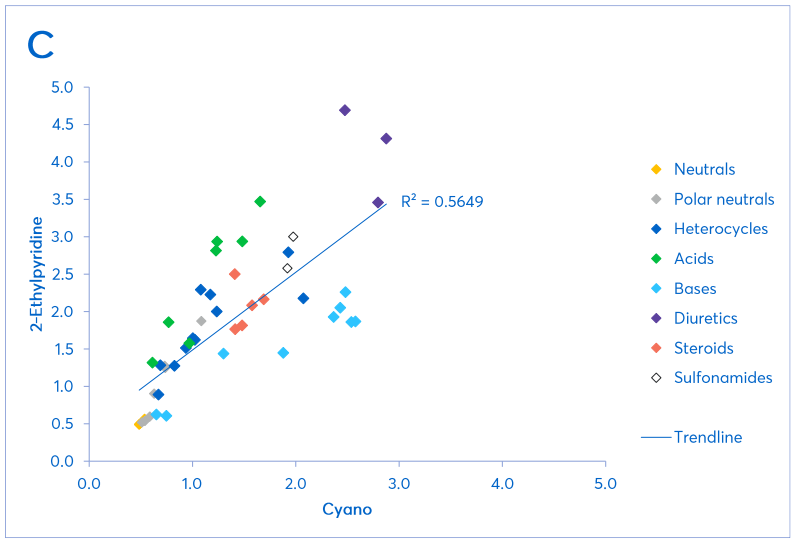
- High S values indicating that selectivity differences are significant



Columns: 150 x 4.6 mm, 5 µm
Mobile phases: A: CO_2, B: MeOH
Gradient: 10 to 55% B in 5 mins
Flow rate: 4 mL/min
BPR Pressure: 150 bar
Injection volume: 5 µL
Temperature: 40 °C
Detection: UV, 254 nm

Sample:
1. Theophylline
2. Caffeine
3. Cortisone
4. Prednisone
5. Hydrocortisone
6. Prednisolone
7. Sulfaquinoxaline
8. Sulfamerazine.





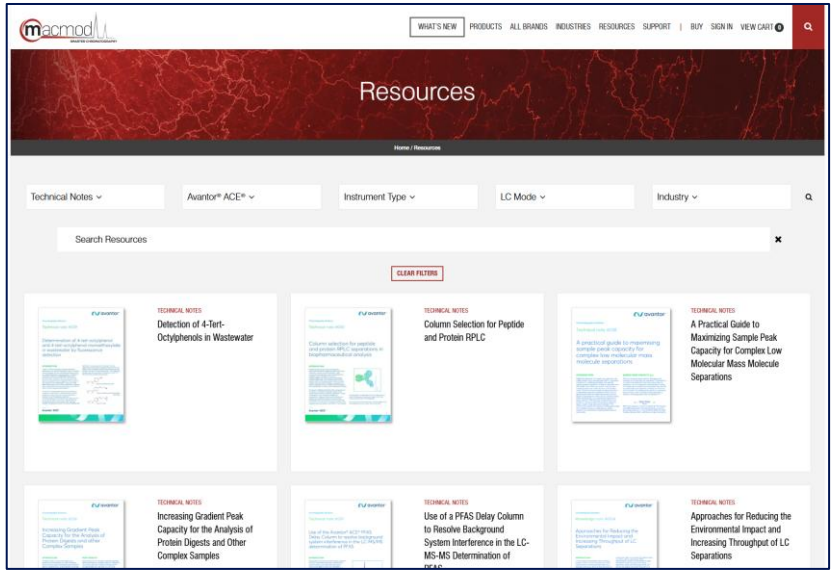
Edge, T, James, M, Pipe, Bylikin, S, Field, J, Euerby, M, *LCGC Supplements* (2022) **40** (s6) 9-22

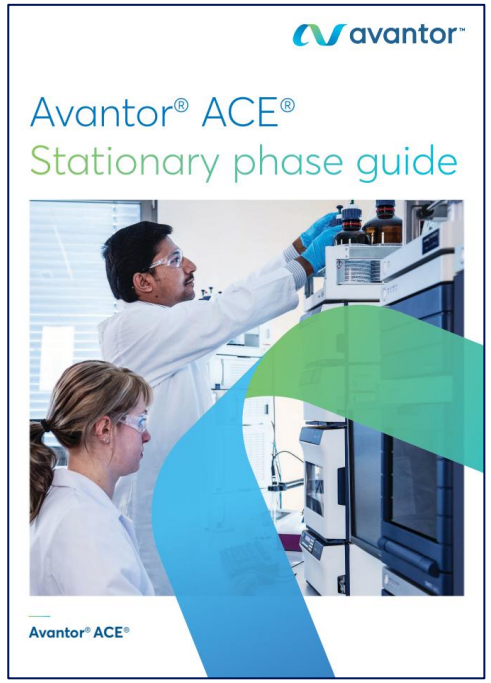
Conclusions

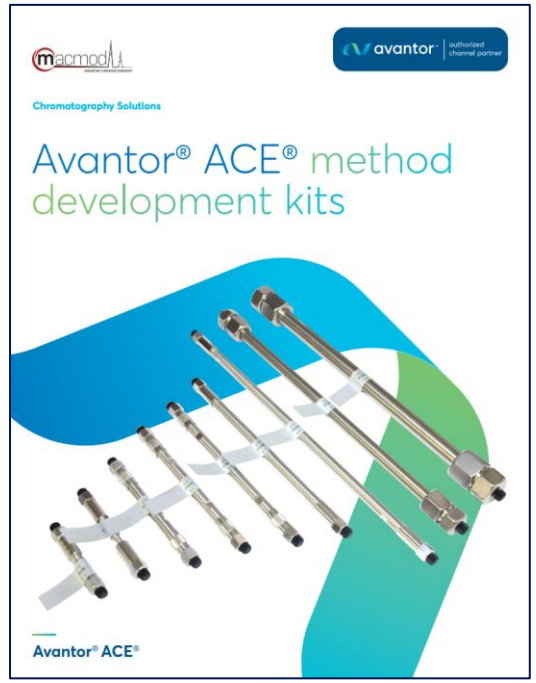
- Selectivity is powerful in chromatography
- Understanding selectivity aids method development by focussing efforts on high impact variables
- It is possible to design stationary phases to maximize selectivity
- Screening columns with differing retention mechanisms is useful for method development – explore ‘selectivity space’
- An optimised method development platform based on selectivity has been described
- Novel stationary phases show potential advantages for peptide separations
- Stationary phase selectivity is also powerful in HILIC and SFC

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Thank you for your attention

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