ACE® 1.7 µm Reversed-Phase and HILIC UHPLC Columns

Accelerating Method Development with Efficiency, Selectivity and Speed

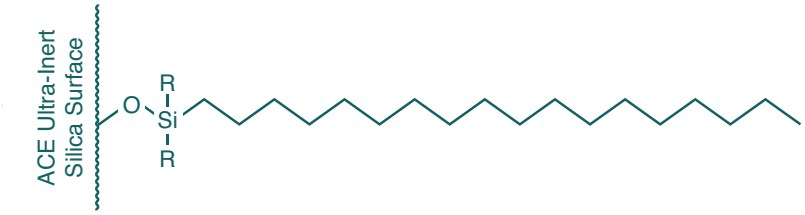
ACE®
HPLC and UHPLC Columns

ACE NOVEL REVERSED-PHASE DESIGN

Many of the newest ACE phases were developed because of shortcomings with existing phenyl, pentafluorophenyl, cyano, polar embedded and C18 columns on the market today. These problems include inadequate stability at low pH, unacceptable bleed (ligand cleavage), pore dewetting and poor peak shape for basic and acidic analytes. ACE's ability to design new stationary phase ligands is an effort to address these shortcomings.

ACE® C18

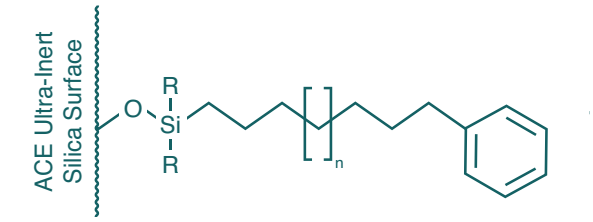
OCTADECYL SILANE LIGAND



- Optimized for maximum efficiency, superior peak shape and resolution
- Utilizes ultra-high purity silica for excellent peak shape and reproducibility

ACE® C18-AR

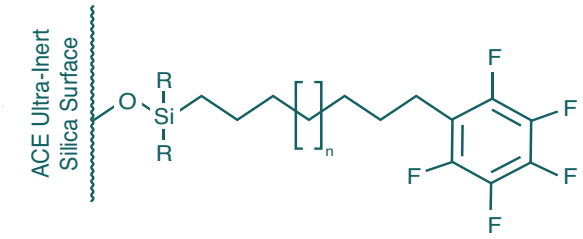
PROPRIETARY EXTENDED STABILITY LIGAND



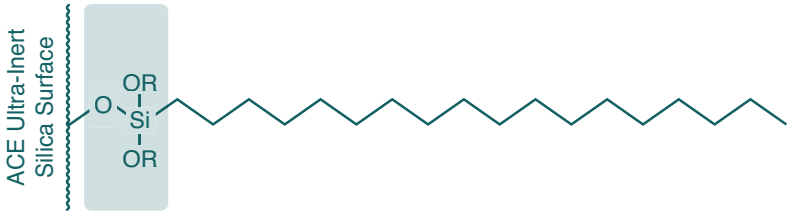
- A unique phase that has an extended alkyl chain with a terminal phenyl group
- Provides improved retention and stability compared to commercial phenylpropyl phases
- Usable in 100% aqueous eluents

ACE® C18-PFP

PROPRIETARY EXTENDED STABILITY LIGAND



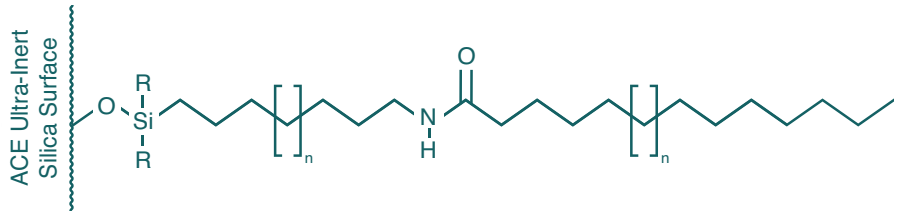
- A unique phase that has an extended alkyl chain with a terminal pentafluorophenyl group
- Provides improved retention and stability compared to commercial pentafluorophenylpropyl phases
- Usable in 100% aqueous eluents



ACE® SUPERC18

PROPRIETARY ENCAPSULATED BONDING TECHNOLOGY

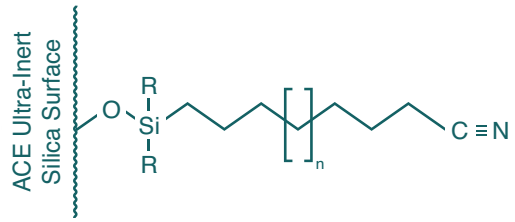
- A uniquely-bonded EBT™ (Encapsulated Bonding Technology) end-capped C18 phase, which provides unprecedented inertness and unparalleled efficiency over an extended pH range (pH 1.5–11.5)
- Excellent choice for low, mid and high pH work



ACE® C18-AMIDE

PROPRIETARY EXTENDED STABILITY LIGAND

- A uniquely-designed polar-embedded phase, which offers enhanced retention and resolution of polar acidic, phenolic, and hydroxy-substituted analytes
- Usable in 100% aqueous eluents



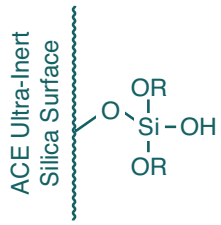
ACE® CN-ES

PROPRIETARY EXTENDED STABILITY LIGAND

- A unique phase that has an extended alkyl chain with a terminal cyano group
- Provides C18-like retention and stability compared to commercial cyanopropyl phases
- Usable with 100% aqueous mobile phases in RPLC mode, and also has excellent performance in normal-phase mode (NPLC)

ACE NOVEL HILIC PHASE DESIGN

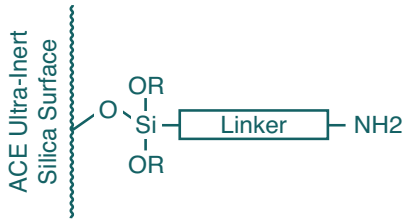
ACE HILIC columns provide alternative selectivity to ACE RPLC columns with excellent retention for polar analytes.



ACE® HILIC-A

PROPRIETARY ACIDIC PHASE

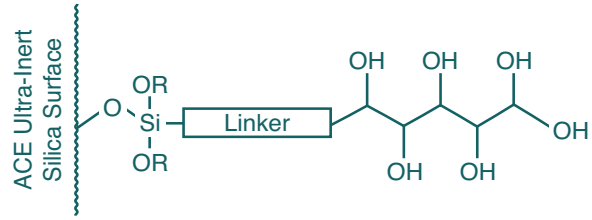
- An acidic character phase with an ionizable surface charge depending upon mobile phase pH



ACE® HILIC-B

PROPRIETARY BASIC PHASE

- A basic character phase with an ionizable positive surface charge depending upon mobile phase pH



ACE® HILIC-N

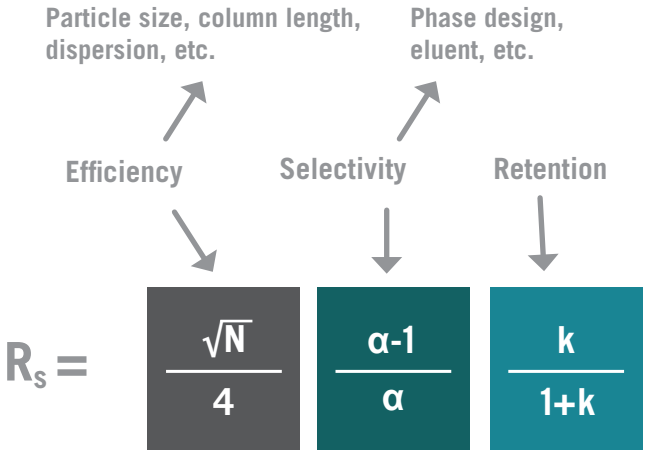
PROPRIETARY NEUTRAL PHASE

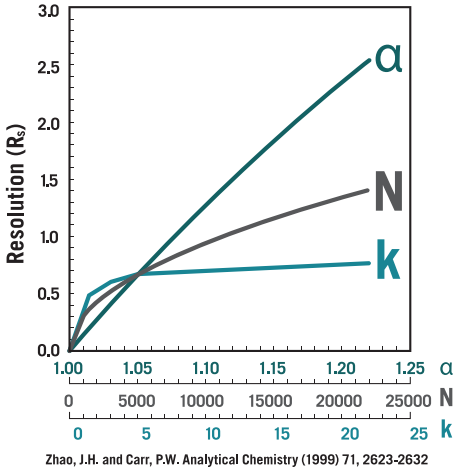
- A polar neutral character phase capable of H-bonding with other mechanisms of interaction

RESOLUTION, SELECTIVITY, EFFICIENCY AND RETENTION

Compared to Selectivity (α), Efficiency (N) and Retention (k) are much less powerful for improving Resolution (R_s).

When you apply unique bonded phases that have a variety of different mechanisms for retaining and separating analytes, and leverage the added efficiency of 1.7 µm particle size columns, Selectivity (α) and Efficiency (N) work together to make your separations easier to accomplish and your methods easier to develop.





Zhao, J.H. and Carr, P.W. Analytical Chemistry (1999) 71, 2623-2632

FACTORS THAT IMPACT SELECTIVITY FOR ISOCRATIC AND GRADIENT HPLC SEPARATIONS[1]

This is a list of parameters that affect reversed-phase selectivity (α or relative retention) for isocratic and gradient separations in HPLC and UHPLC. Column stationary phase is comparable to gradient time, percent organic modifier, and gradient steepness in its ability to affect relative retention, and thus, resolution.

Isocratic Separations

- **Mobile phase pH**
- **Column stationary phase**
- **Organic modifier choice**
- **Percent organic modifier**
- Column temperature
- Buffer choice
- Buffer concentration
- Additive concentration

Most Influence

Least Influence

Gradient Separations

- **All parameters for isocratic separations**
- **Gradient steepness (b=1/k*)**

$$k^* = \frac{85 \times t_G \times F}{\Delta\Phi \times V_m \times S}$$

- t_G = Gradient time
- F = Flow rate
- S = Constant
- Φ = Gradient range (%B final - %B initial)
- V_m = Column volume (length, diameter)
- Delay volume

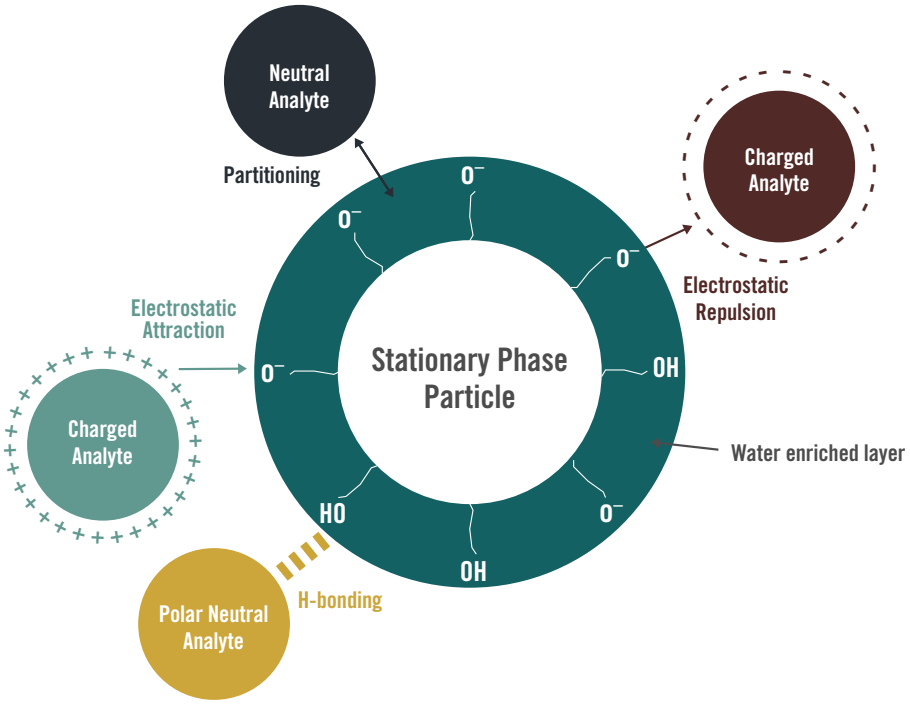
***For ionizable analytes, mobile phase pH is the most powerful factor for adjusting selectivity.**

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

ACE UHPLC HILIC COLUMNS

What is HILIC?

- HILIC is ideal for the separation and retention of polar species including polar neutral and polar ionised analytes
- HILIC separations typically include a polar stationary phase with high organic solvent containing mobile phases
- Mechanistically HILIC is complex and provides multiple modes of interaction between the analyte, stationary phase, eluent and water enriched layer at the stationary phase particle-eluent interface



When Should You Consider HILIC?

- HILIC provides the retention and separation of hydrophilic or polar to very polar analytes not well retained in RPLC
- Hydrophilic or polar to very polar analytes have log P values (measure of lipophilicity) of around zero or less
- Generally, polar analytes are suitable for HILIC if they elute before caffeine in gradient RPLC

ACE PHASES PROVIDE DIFFERENT SELECTIVITY DUE TO DIFFERING ANALYTE INTERACTIONS

Bonded Phases	Separations Mechanism and Relative Strength				
	Hydrophic Binding	π - π Interaction	Dipole-Dipole	Hydrogen Bonding	Shape Selectivity
C18	****	—	—	*	**
C18-AR	****	*** (DONOR)	*	**	***
C18-PFP	****	*** (ACCEPTOR)	****	***	****
SUPERC18	****	—	—	—	**
C18-AMIDE	****	—	**	****	** / ***
CN-ES	***	*	***	**	*

HILIC PHASE MECHANISMS

Phase	Partitioning	Anionic analyte interactions		Cationic analyte interactions		H-bonding
		Attraction	Repulsion	Attraction	Repulsion	
ACE HILIC-A	**	—	***	****	—	*
ACE HILIC-B	***	****	—	—	***	*
ACE HILIC-N	****	—	—	—	—	****

COLUMN SPECIFICATIONS

Packing Descriptions	USP Designations	Particle Size(s) (µm)	Pore Size (Å)	Carbon Load (%)	Surface Area (m^2/g)	Low pH Limit	High pH Limit	Max Temp Low pH Limit	Max Temp Upper pH Limit	Endcapped (Yes/No)	100% Aq Compatible (Yes/No)
C18	L1	1.7	100	15.5	300	2	8	60	40	YES	NO
C18-AR	L1	1.7	100	15.5	300	2	8	60	40	YES	YES
C18-PFP	L1	1.7	100	14.3	300	2	8	60	40	YES	YES
SUPERC18	L1	1.7	90	14.8	400	1.5	11.5	60	40	ENCAPSULATED	NO
C18-AMIDE	L1/L60	1.7	100	17.0	300	2	8	60	40	YES	YES
CN-ES	L10	1.7	100	12.6	300	2	8	60	40	YES	YES
HILIC-A	L3	1.7	100	NOT APPLICABLE	300	2	7	60	40	NO	NOT APPLICABLE
HILIC-B	L8	1.7	100	4.0	300	2	7	60	40	NO	NOT APPLICABLE
HILIC-N	PENDING	1.7	100	7.0	300	2	7	60	40	NO	NOT APPLICABLE

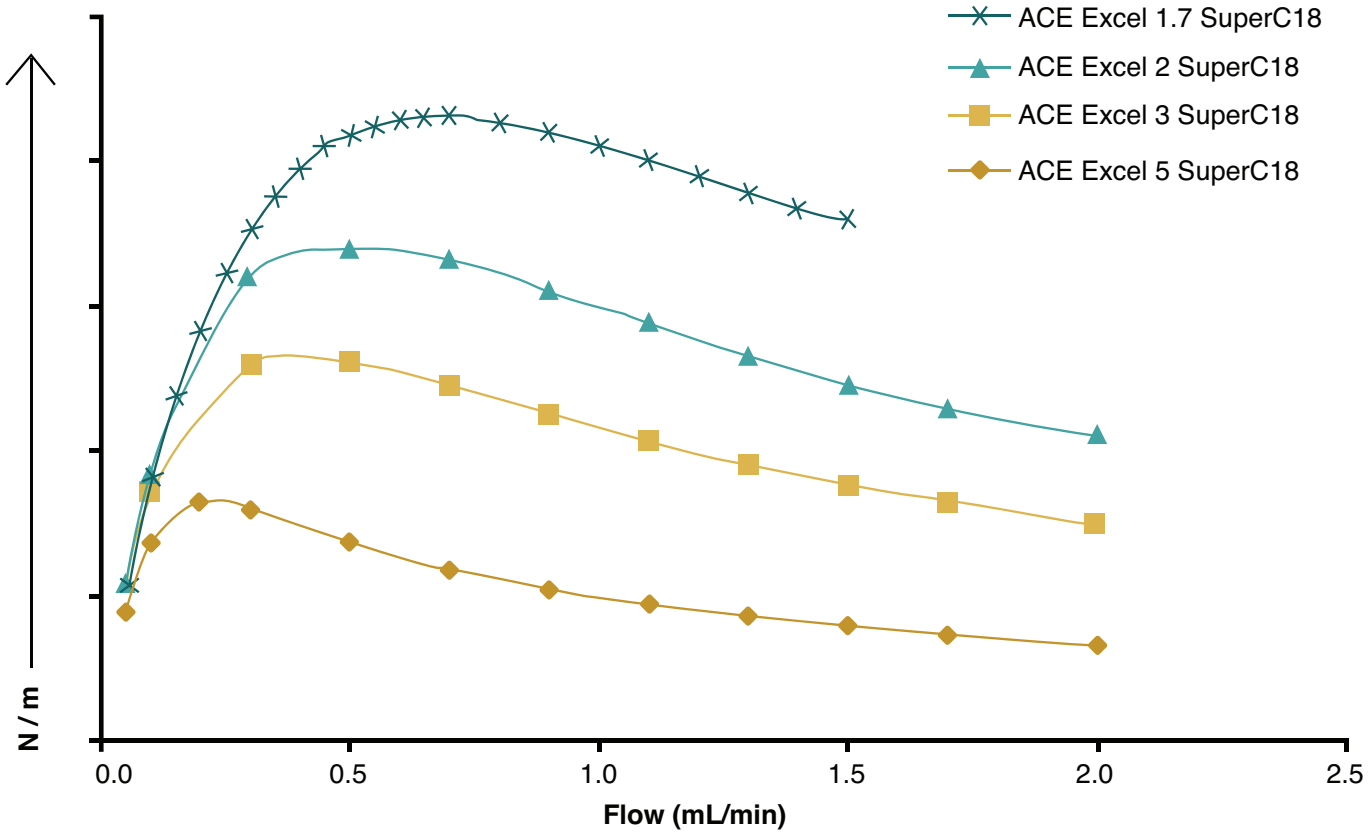
Note: At mid pH, longest column lifetime of silica and bonded silica columns is achieved by avoiding carbonate, phosphate and borate buffers, and by using concentrations in the 5-10 mM range with column temperatures at or below 40 °C.

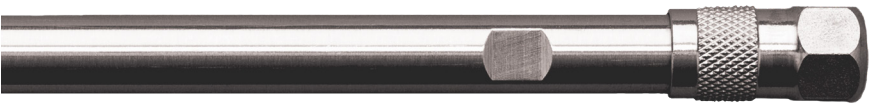
PLOTS OF EFFICIENCY

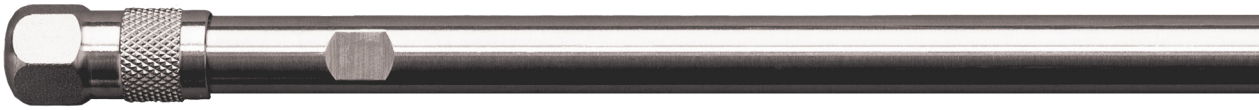
(N / m, PLATES PER METER) VERSUS FLOW RATE FOR DIFFERENT ACE PARTICLE SIZES

Note that each particle size has its own optimum flow rate (linear velocity), and that a smaller particle has an optimum flow rate at higher values. Smaller particles also have a broader optimum flow rate range.

Columns: 2.1 x 50 mm







ALL 6 ACE UHPLC PHASES PROVIDE DIFFERENT SELECTIVITY

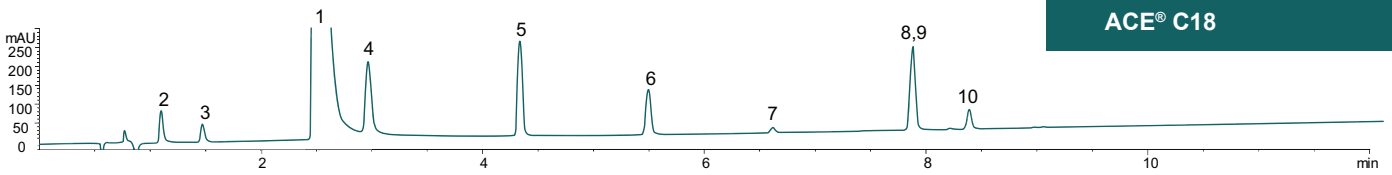
Ace Screening Platform: Acetaminophen and Related Substances – MeCN

Acetaminophen and related analytes at 0.5% (w/w)

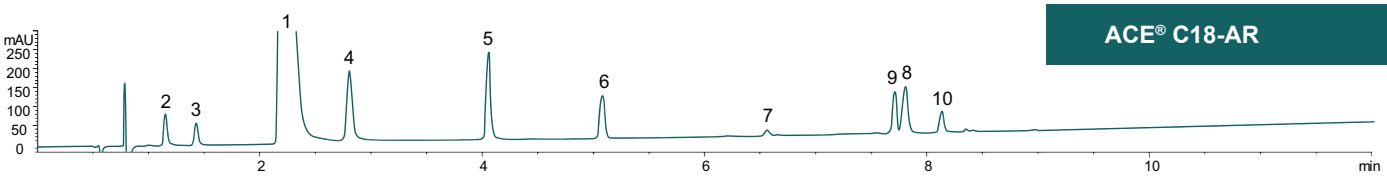
ACE® 1.7 µm 2.1 x 100 mm columns
A: 20 mM Ammonium acetate pH 5.8
B: 20 mM Ammonium acetate pH 5.8 in MeCN/$H_2$0 (9:1 v/v)
Gradient: 5 to 95%B in 15 mins
Temp: 40°C
Flow rate: 0.4 ml/min
Detection: 210 nm

1. Paracetamol
2. 4-Aminophenol
3. Hydroquinone
4. 2-Aminophenol
5. 2-Acetamidophenol
6. Phenol
7. 4-Nitrophenol
8. 2-Nitrophenol
9. 4-Chloroacetanilide
10. 4-Chlorophenol

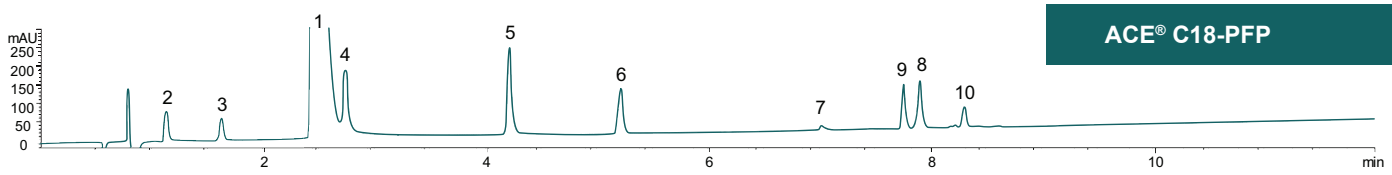
ACE® C18



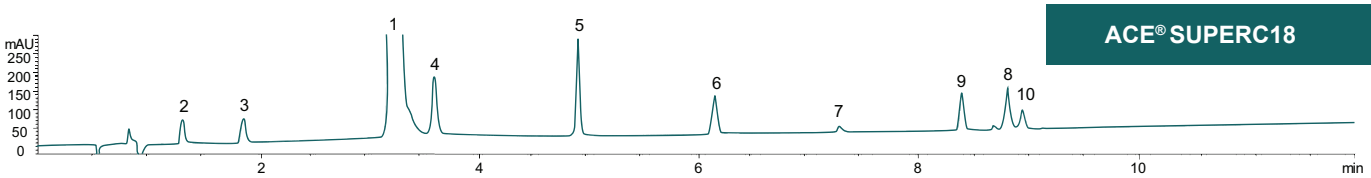
ACE® C18-AR



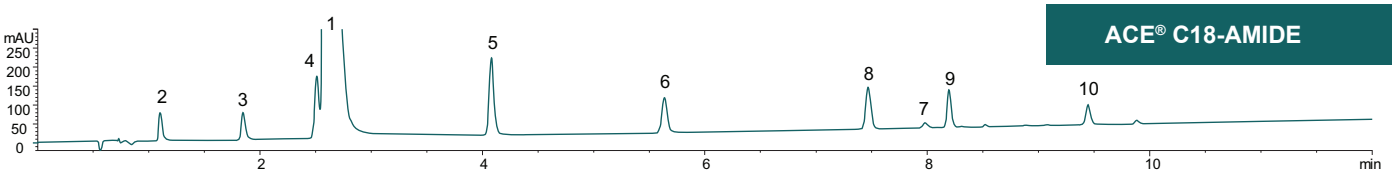
ACE® C18-PFP



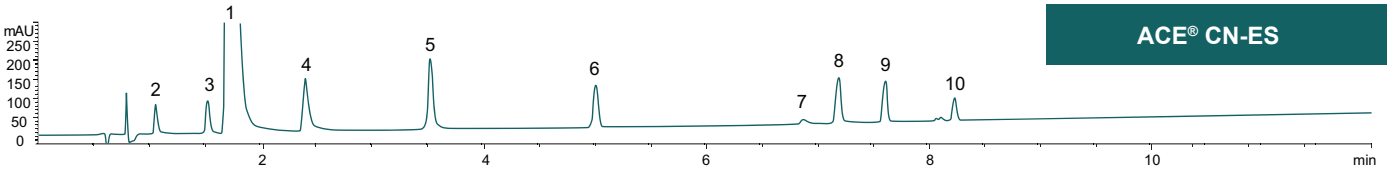
ACE® SUPERC18



ACE® C18-AMIDE



ACE® CN-ES



The above chromatograms demonstrate that using these novel stationary phases, either with one organic modifier, or with two, is a powerful screening approach to ensure that you can see and separate all of your analytes.

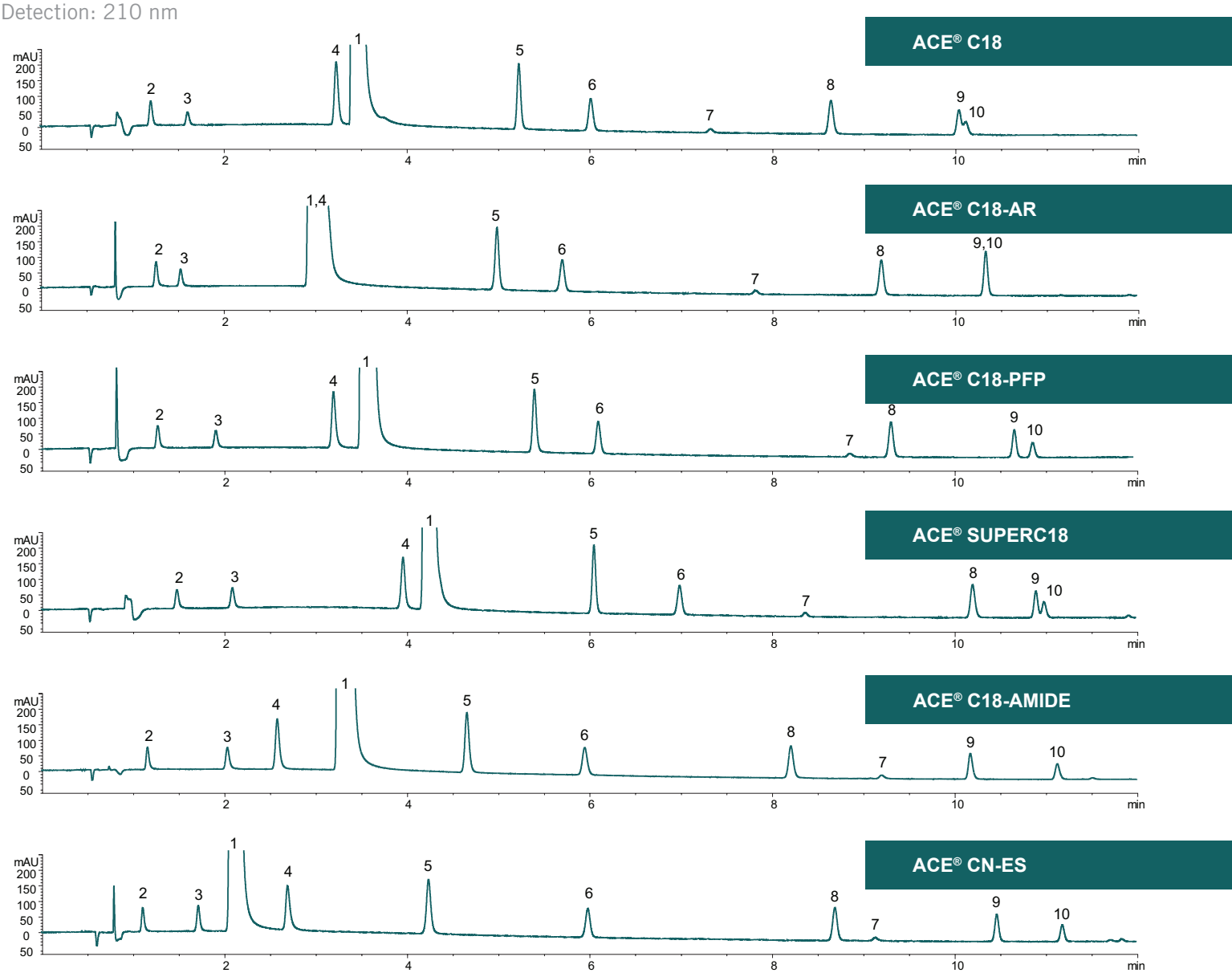
ALL 6 ACE UHPLC PHASES PROVIDE DIFFERENT SELECTIVITY

ACE Screening Platform: Acetaminophen and Related Substances – MeOH

Acetaminophen and related analytes at 0.5% (w/w)

ACE® 1.7 µm 2.1 x 100 mm columns
A: 20 mM Ammonium acetate pH 5.8
B: 20 mM Ammonium acetate pH 5.8 in MeOH/H_2O (9:1 v/v)
Gradient: 5 to 95%B in 15 mins
Temp: 40°C
Flow rate: 0.4 ml/min
Detection: 210 nm

1. Paracetamol
2. 4-Aminophenol
3. Hydroquinone
4. 2-Aminophenol
5. 2-Acetamidophenol
6. Phenol
7. 4-Nitrophenol
8. 2-Nitrophenol
9. 4-Chloroacetanilide
10. 4-Chlorophenol



The above chromatograms demonstrate that significant changes in retention and elution order are observed among the various ACE 1.7 µm novel phases but also between each phase in MeOH and MeCN.

FAST ANALYSIS OF ß-BLOCKERS AT HIGH pH

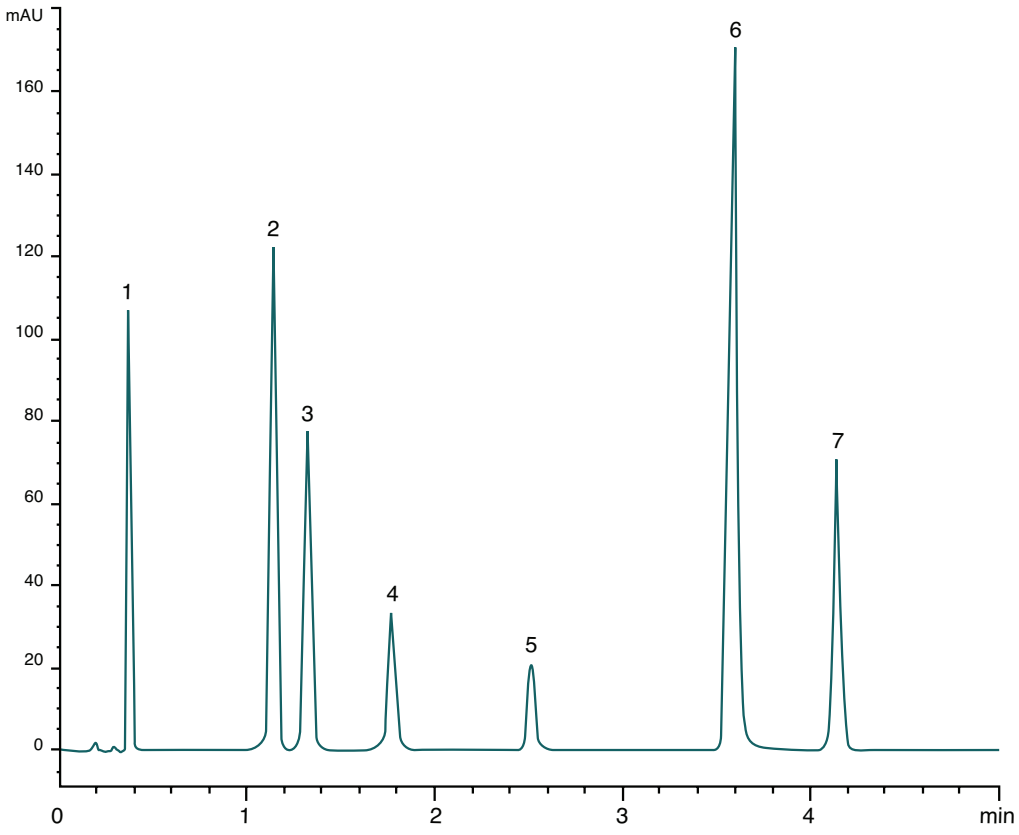
ACE 1.7 SuperC18 allows the fast separation of basic beta blockers at high pH using an LC-MS-compatible mobile phase.

Conditions

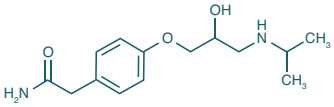
Column:	ACE 1.7 SuperC18
Dimensions:	3.0 X 50 mm
Part Number:	EXL-1711-0503U
Mobile Phase:	A: 0.1% ammonia in H_2O B: 0.1% ammonia in MeCN
Flow Rate:	1 mL/min
Injection:	0.7 µL
Temperature:	20°C
Detection:	UV, 230 nm

Time (mins)	%B
0.0	30
4.3	55
5.0	55
6.0	30

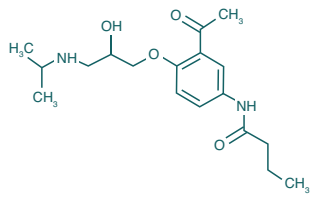
Post time 3 mins



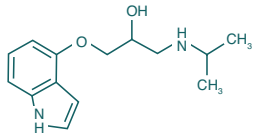
1. Atenolol



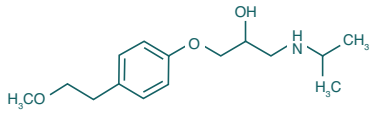
2. Acebutalol



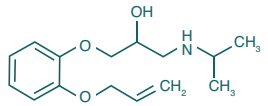
3. Pindolol



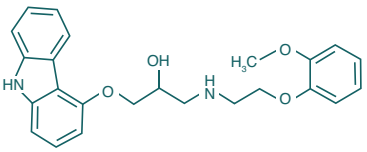
4. Metoprolol



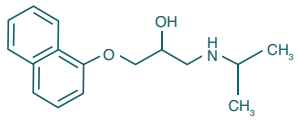
5. Oxprenolol



6. Carvedilol



7. Propranolol



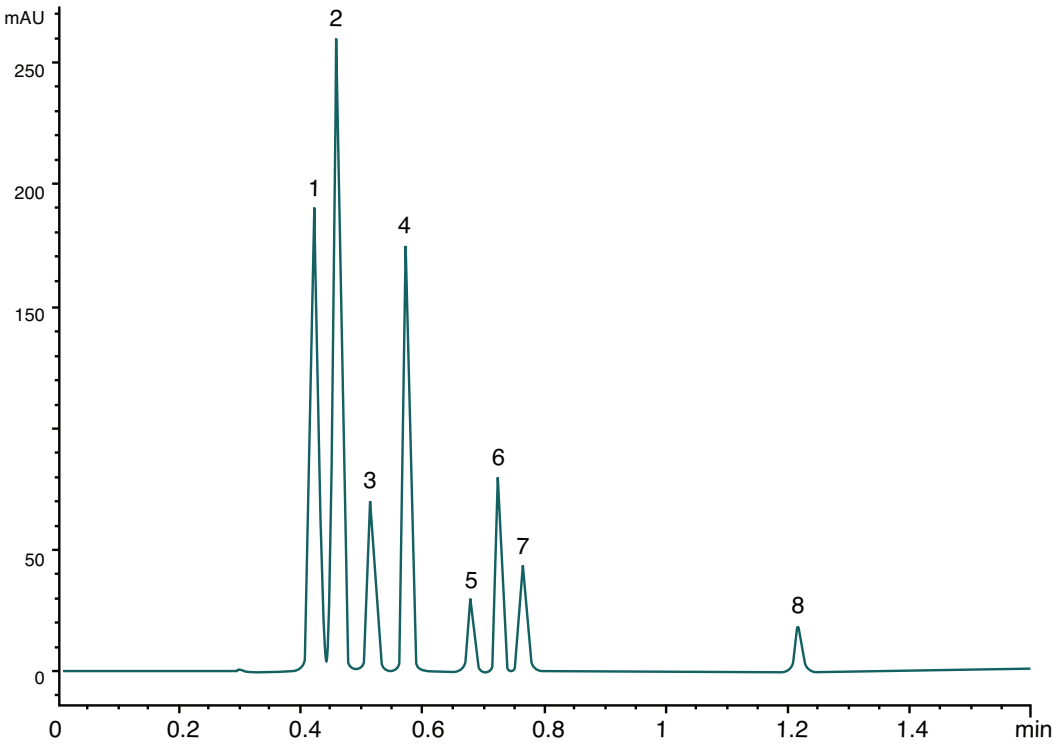
FAST SEPARATION OF VANILLIN COMPOUNDS

Polar hydroxy- and carboxy- substituted flavor ingredients, having very similar structures, are separated in less than 1.5 minutes using ACE 1.7 C18-Amide.

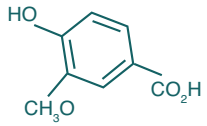
Conditions

Column:	ACE 1.7 C18-Amide	Flow Rate:	1.3 mL/min
Dimensions:	3.0 x 50 mm	Injection:	1 µL
Part Number:	EXL-1712-0503U	Temperature:	45°C
Mobile Phase:	A: 0.1% formic acid in H_2O	Detection:	UV 260 nm
	B: 0.1% formic acid in MeCN		

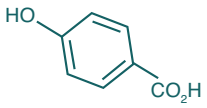
Time (mins)	%B
0.0	25
1.32	75
1.49	75
1.60	25



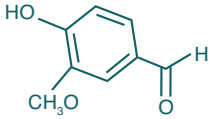
1. Vanillic acid



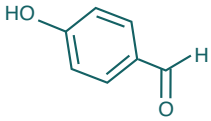
2. 4-Hydroxybenzoic acid



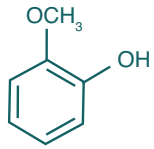
3. Vanillin



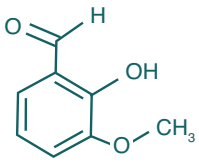
4. 4-Hydroxybenzaldehyde



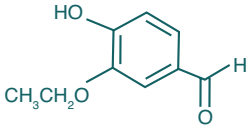
5. Guaicol



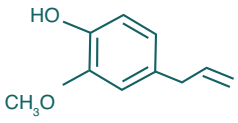
6. o-Vanillin



7. Ethyl Vanillin



8. Eugenol



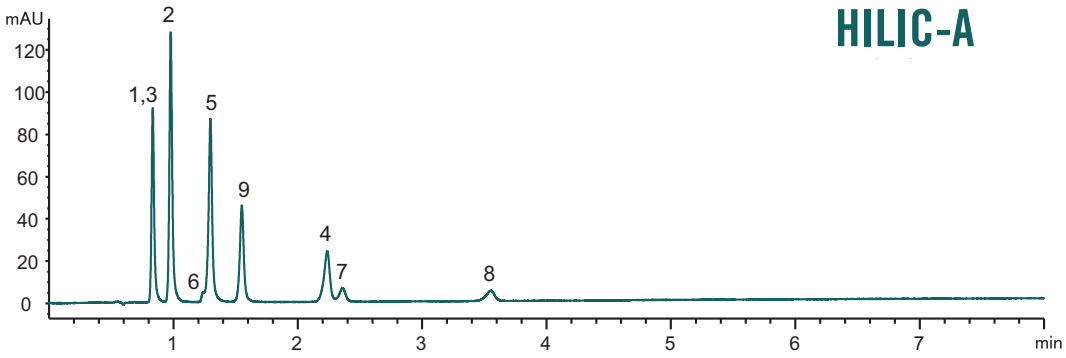
ACE HILIC COLUMNS SCREENING GRADIENTS

Columns: ACE 1.7 µm HILIC-A, HILIC-B, HILIC-N 2.1 x 100 mm UHPLC

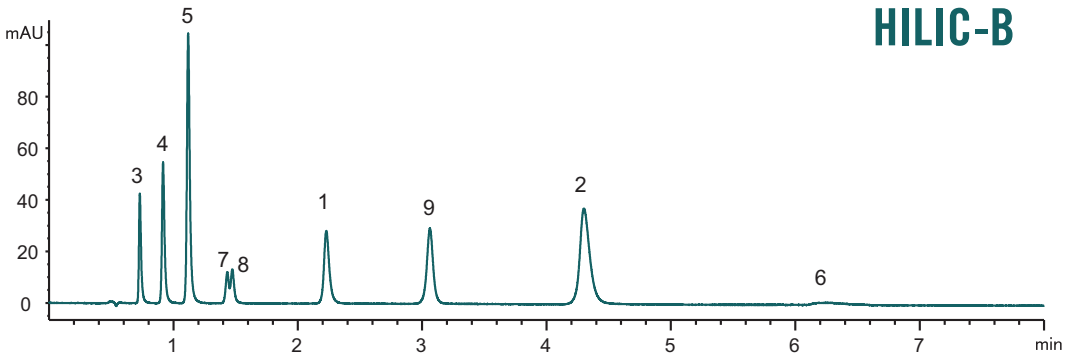
Mobile Phase: 10 mM ammonium formate pH 4.7 in 90:10 MeCN/H_2O, 0.5 mL/min, 25 °C, 254 nm, 1 µL injection

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

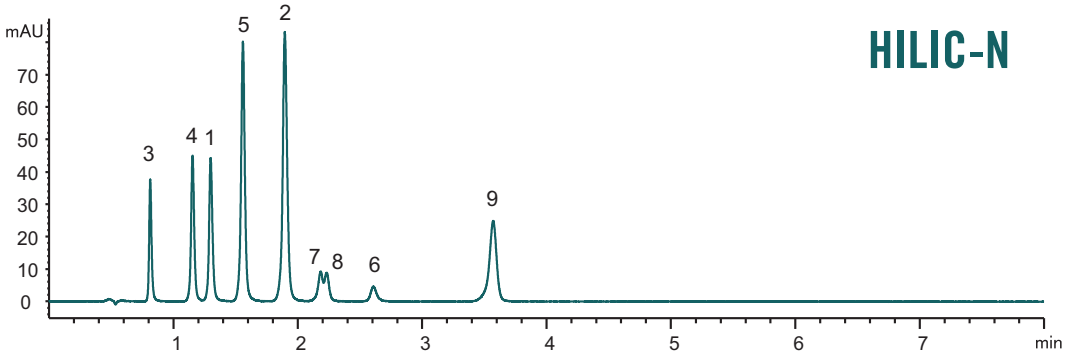
ACE® HILIC-A
2.1 x 100 mm



ACE® HILIC-B
2.1 x 100 mm



ACE® HILIC-N
2.1 x 100 mm



PART NUMBERS

	C18	C18-AR	C18-PFP	SuperC18	C18-Amide	CN-ES	HILIC-A	HILIC-B	HILIC-N
2.1 x 20 mm	EXL1710202U	EXL1790202U	EXL17100202U	EXL17110202U	EXL17120202U	EXL17130202U	HILA170202U	HILB170202U	HILN170202U
2.1 x 30 mm	EXL1710302U	EXL1790302U	EXL17100302U	EXL17110302U	EXL17120302U	EXL17130302U	HILA170302U	HILB170302U	HILN170302U
2.1 x 35 mm	EXL1713502U	EXL1793502U	EXL17103502U	EXL17113502U	EXL17123502U	EXL17133502U	HILA173502U	HILB173502U	HILN173502U
2.1 x 50 mm	EXL1710502U	EXL1790502U	EXL17100502U	EXL17110502U	EXL17120502U	EXL17130502U	HILA170502U	HILB170502U	HILN170502U
2.1 x 75 mm	EXL1717502U	EXL1797502U	EXL17107502U	EXL17117502U	EXL17127502U	EXL17137502U	HILA177502U	HILB177502U	HILN177502U
2.1 x 100 mm	EXL1711002U	EXL1791002U	EXL17101002U	EXL17111002U	EXL17121002U	EXL17131002U	HILA171002U	HILB171002U	HILN171002U
3.0 x 20 mm	EXL1710203U	EXL1790203U	EXL17100203U	EXL17110203U	EXL17120203U	EXL17130203U	HILA170203U	HILB170203U	HILN170203U
3.0 x 30 mm	EXL1710303U	EXL1790303U	EXL17100303U	EXL17110303U	EXL17120303U	EXL17130303U	HILA170303U	HILB170303U	HILN170303U
3.0 x 35 mm	EXL1713503U	EXL1793503U	EXL17103503U	EXL17113503U	EXL17123503U	EXL17133503U	HILA173503U	HILB173503U	HILN173503U
3.0 x 50 mm	EXL1710503U	EXL1790503U	EXL17100503U	EXL17110503U	EXL17120503U	EXL17130503U	HILA170503U	HILB170503U	HILN170503U
3.0 x 75 mm	EXL1717503U	EXL1797503U	EXL17107503U	EXL17117503U	EXL17127503U	EXL17137503U	HILA177503U	HILB177503U	HILN177503U
3.0 x 100 mm	EXL1711003U	EXL1791003U	EXL17101003U	EXL17111003U	EXL17121003U	EXL17131003U	HILA171003U	HILB171003U	HILN171003U

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