

Chromatography Solutions

Avantor® ACE®, method development kits



Avantor manufactures a range of cutting edge U/HPLC chromatography products from its ISO 9001/ISO 14001 production facility.

The Avantor® ACE® portfolio provides a premium quality product with unique phases that separates what other columns cannot. The range includes novel and traditional stationary phases based on ultra inert silica for excellent reproducibility.



Avantor® ACE® method development kits

INTELLIGENT SOLUTIONS FOR METHOD DEVELOPMENT

- 5 different ACE Method Development Kits available in a wide range of dimensions for rapid, systematic method development
- Each kit contains carefully selected ACE phases which enables the power of selectivity to be fully exploited
- Each ACE phase provides different selectivity due to differing interactions

FREE

METHOD DEVELOPMENT SUPPORT!

- Not sure which ACE phase or kit will work best for your application?
- FREE Application Support and FREE Method Support Service
- Trust your method development to our experts and free up time for your other projects!

Contact our expert method development team via chromsupport@avantorsciences.com

	Bonded Phase	SEPARATION MECHANISM AND RELATIVE STRENGTH ¹				
		Hydrophobic Binding	π - π Interaction	Dipole-Dipole	Hydrogen Bonding	Shape Selectivity
ACE Advanced Method Development Kit (see page 5)	ACE C18	****	-	-	*	**
	ACE C18-AR	****	*** (donor)	*	**	***
	ACE C18-PFP	****	*** (acceptor)	****	***	****
ACE Extended Method Development Kit (see page 9)	ACE SuperC18	****	-	-	-	**
	ACE C18-Amide	****	-	**	****	**/**
	ACE CN-ES	***	*	***	**	*
ACE UltraCore Method Development Kit (see page 13)	ACE UltraCore SuperC18	***	-	-	-	**
	ACE UltraCore SuperPhenylHexyl	**	*** (donor)	*	**	***
ACE Bioanalytical 300Å Method Development Kit (see page 16)	ACE C18-300	**	-	-	*	*
	ACE C4-300	*	-	-	-	-
	ACE Phenyl-300	*	** (donor)	*	**	**

¹ Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using >100 characterising analytes.

	Bonded Phase	SEPARATION MECHANISM AND RELATIVE STRENGTH ²					
		Partitioning	Anionic Analyte Interactions		Cationic Analyte Interactions		H-bonding
			Attraction	Repulsion	Attraction	Repulsion	
ACE HILIC Method Development Kit (see page 19)	ACE HILIC-A	**	-	***	****	-	*
	ACE HILIC-B	***	****	-	-	***	*
	ACE HILIC-N	****	-	-	-	-	****

² Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using >50 characterising analytes.

Why use ACE method development kits?

USING ACE METHOD DEVELOPMENT KITS TO IMPROVE SEPARATIONS

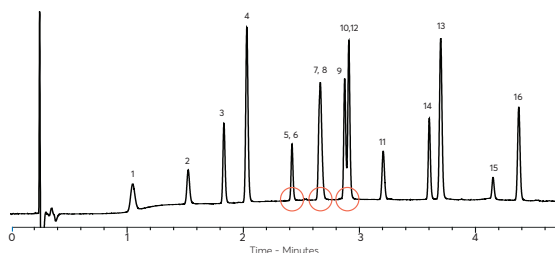
- ACE HPLC/UHPLC columns have earned a well deserved reputation for delivering excellent efficiency, reproducibility and lifetime.
- ACE Method Development Kits group together columns with different mechanisms of interaction to maximise selectivity and improve the likelihood of separating difficult or closely related analytes in mixtures.
- Screening columns containing different bonded phases under the same mobile phase conditions can help you achieve your desired separation more quickly, therefore increasing productivity.

ACE®
Stationary Phases
Virtually Eliminate
the Negative Effects of
Silanols on UHPLC &
HPLC Separations

Si-OH

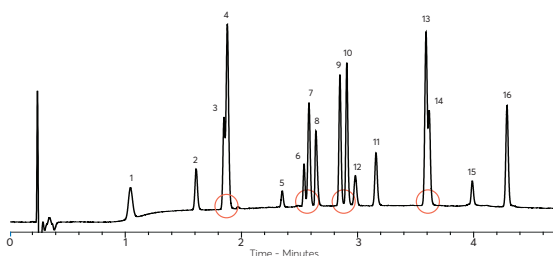
COLUMNS WITHIN ACE METHOD DEVELOPMENT KITS PROVIDE ALTERNATIVE SELECTIVITY

ACE C18



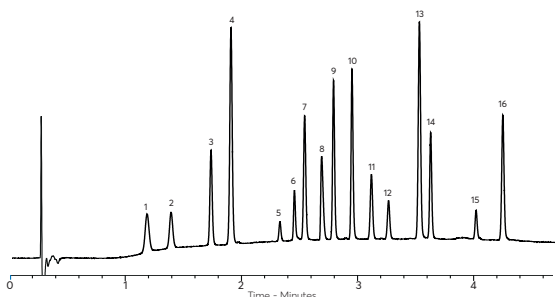
ACE C18 provides excellent peak shape, but here the essentially “hydrophobic-only” interaction results in co-elution. Investigation of alternative bonded phases using the same test conditions is recommended.

ACE C18-PFP



ACE C18-PFP provides additional interactions compared to alkyl C18 phases. Whilst these change selectivity, in this instance co-elution of different analyte pairs is observed.

ACE C18-AR



The ACE C18-AR phase provides a further change in selectivity due to different interaction contributions – ultimately enabling a successful separation. Screening alternative phases can maximise selectivity and reduce method development time.

Sample: 1) metronidazole 2) 4-hydroxybenzoic acid 3) 3-hydroxybenzoic acid 4) benzyl alcohol 5) benzoic acid 6) myrecetin 7) p-cresol 8) propranolol 9) ethyl paraben 10) furosemide 11) anisole 12) 1,3,5-trinitrobenzene 13) toluene 14) nimesulide 15) mefenamic acid 16) 1,2,3-trichlorobenzene
Mobile Phase: A = 0.1% formic acid in H₂O B = 0.1% formic acid in MeCN - **Gradient:** 3 – 100% B in 5 minutes
Column Dimensions: 50 x 2.1mm - **Flow Rate:** 0.60ml/min - **Temperature:** 40°C - **Detection:** 210nm

Application # 1901

ACE Advanced method development kit

- Contains ACE C18, ACE C18-AR and ACE C18-PFP phases
- Ideal starting point for routine method development
- Available in a wide range of dimensions
- Particularly recommended for compounds containing aromatic rings

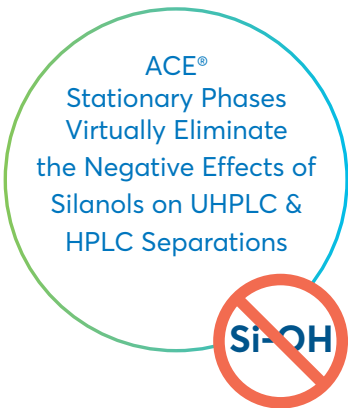
Phase	Functional Group	Endcapped	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Recommended pH Range	100% Aqueous Compatible	USP Listing
ACE C18	Octadecyl (C18)	Yes	1.7, 2, 3, 5	100	300	15.5	2.0-8.0 ^a	No	L1
ACE C18-AR	C18 with integral Phenyl	Yes	1.7, 2, 3, 5, 10	100	300	15.5	2.0-8.0 ^a	Yes	L1
ACE C18-PFP	C18 with integral PFP	Yes	1.7, 2, 3, 5, 10	100	300	14.3	2.0-8.0 ^a	Yes	L1

^a For optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at higher pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC/MS Buffer Selection" by John Dolan – please contact your distributor to request your FREE copy.

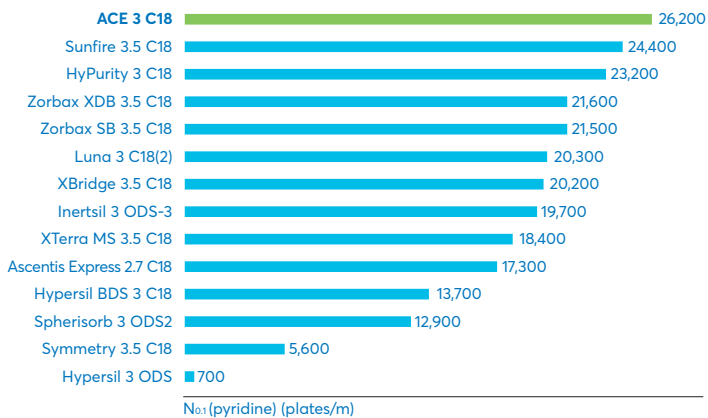
ACE C18	ACE C18-AR	ACE C18-PFP
<p>ACE C18 remains the "go-to" column of choice for HPLC and UHPLC separations. With an excellent reputation for performance, reproducibility and lifetime, ACE C18 provides a rugged, reproducible starting point for method development.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Analytes differing in hydrophobicity – Polar, moderately polar and non-polar analytes – Uncharged acids and bases – Ionized acids or bases using ion-pairing – Ideal starting point for method development 	<p>ACE C18-AR combines the excellent performance and advantages of the ACE C18 phase with the added selectivity of an integral phenyl group.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Analytes with π-bonding and conjugated systems – Analytes with electron delocalization and electron withdrawing groups, such as halogens, nitro groups, ketones, esters and acids – Analytes with different dipole moments – Analytes differing in hydrophobicity – Stereoisomers, steroids, substituted aromatics and sulphur containing compounds – Fully wettable - 100% aqueous buffer compatible – Applications where C18 does not provide adequate separation – Applications where conventional phenyl phases provide insufficient retention, poor stability, or significant bleed 	<p>ACE C18-PFP brings together the stability, reproducibility and low bleed of the ACE C18 phase with the additional selectivity of an integral pentafluorophenyl (PFP) group.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Analytes with π-bonding – Analytes with electron donating groups, such as phenols, aromatic ethers and amines – Analytes with proton donor groups – Analytes with different dipole moments – Analytes differing in hydrophobicity – Structural isomers, steroids, substituted aromatics and taxanes – Fully wettable - 100% aqueous buffer compatible – Applications where C18 does not provide adequate separation – Applications where conventional PFP phases provide insufficient retention, poor stability or significant bleed

ACE C18 - COMPARISON OF COLUMN INERTNESS

- Column brands from major manufacturers investigated
- Comparison of column efficiency for pyridine – a basic molecule



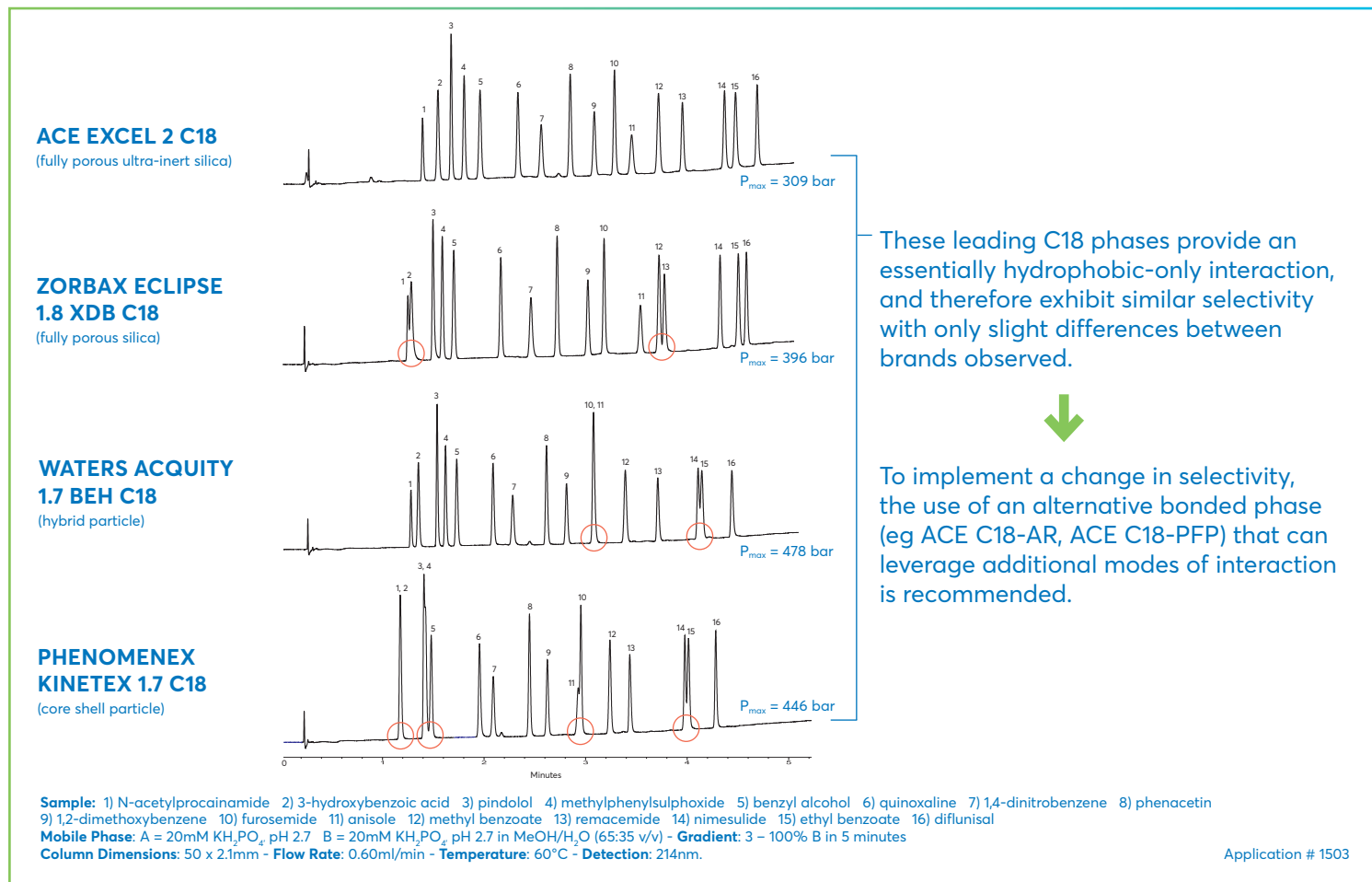
PEAK EFFICIENCY COMPARISON



Column Dimensions: 50 x 2.1mm - Sample: 1) uracil 2) pyridine 3) phenol - Mobile Phase: 40:60 (v/v) MeOH/H₂O - Flow Rate: 0.20ml/min - Temperature: 22°C - Detection: 254nm
Comparative data may not be representative of all applications.
Please see back page for acknowledgement of trademarks. Application # 1911

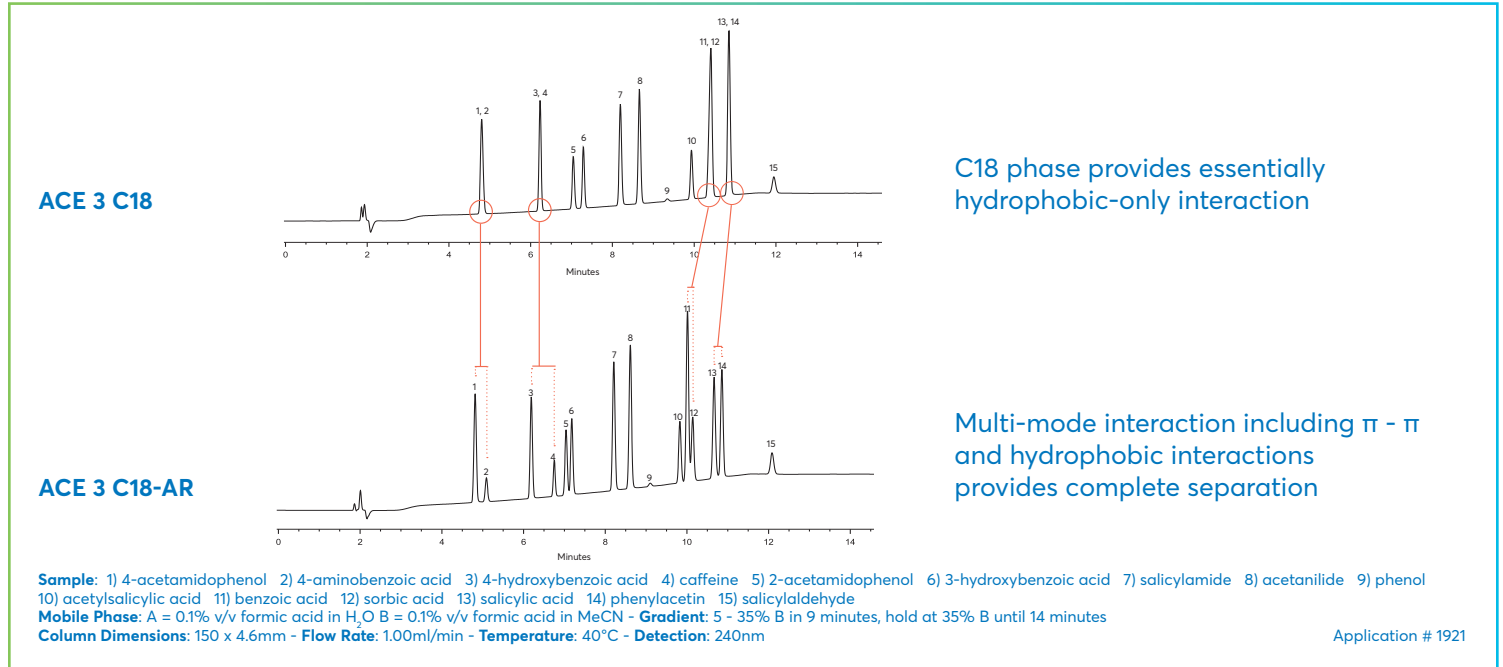
ACE C18 DELIVERS EXCELLENT PERFORMANCE

RAPID UHPLC SCREENING OF 16 PHARMACEUTICALS AND RELATED COMPOUNDS



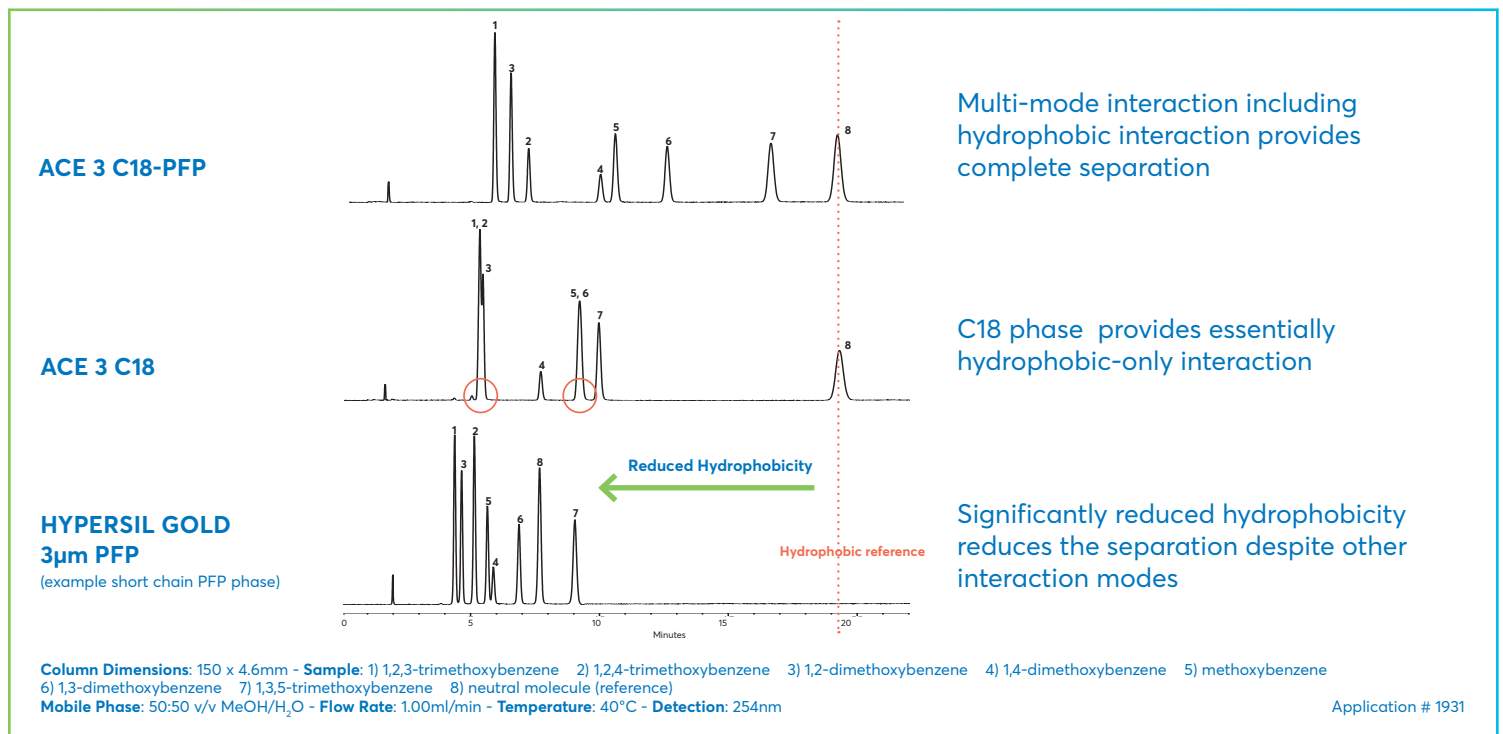
LEVERAGING THE UNIQUE SELECTIVITY OF ACE C18-AR

IMPROVING AN ANALGESICS SEPARATION BY CHANGING PHASE



ACE C18-PFP PROVIDES A SEPARATION THAT A C18 OR PFP COLUMN ALONE CANNOT ACHIEVE

THE IMPORTANCE OF MAINTAINING HYDROPHOBICITY DURING MULTI-MODE INTERACTIONS



METHOD DEVELOPMENT KITS

ACE ADVANCED METHOD DEVELOPMENT KITS

Contains 3 columns: ACE C18, ACE C18-AR and ACE C18-PFP of specified dimensions

Column Dimensions	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)			
	1.7µm	2µm	3µm	5µm
2.1 x 50mm	MDKA-17-0502U	MDKA-2-0502U	MDKA-3-0502U	MDKA-5-0502U
2.1 x 100mm	MDKA-17-1002U	MDKA-2-1002U	MDKA-3-1002U	MDKA-5-1002U
2.1 x 150mm	-	MDKA-2-1502U	MDKA-3-1502U	MDKA-5-1502U
2.1 x 250mm	-	-	MDKA-3-2502U	MDKA-5-2502U
3.0 x 50mm	MDKA-17-0503U	MDKA-2-0503U	MDKA-3-0503U	MDKA-5-0503U
3.0 x 100mm	MDKA-17-1003U	MDKA-2-1003U	MDKA-3-1003U	MDKA-5-1003U
3.0 x 150mm	-	MDKA-2-1503U	MDKA-3-1503U	MDKA-5-1503U
3.0 x 250mm	-	-	MDKA-3-2503U	MDKA-5-2503U
4.6 x 50mm	-	MDKA-2-0546U	MDKA-3-0546U	MDKA-5-0546U
4.6 x 100mm	-	MDKA-2-1046U	MDKA-3-1046U	MDKA-5-1046U
4.6 x 150mm	-	MDKA-2-1546U	MDKA-3-1546U	MDKA-5-1546U
4.6 x 250mm	-	-	MDKA-3-2546U	MDKA-5-2546U

Guard columns
are available
for all of these
phases

ACE Extended method development kit

- Contains ACE SuperC18, ACE C18-Amide and ACE CN-ES phases
- Use ACE SuperC18 to exploit selectivity changes at low, intermediate and high pH
- Available in a wide range of dimensions
- ACE C18-Amide and ACE CN-ES phases both offer alternative selectivity, especially for polar molecules

Phase	Functional Group	Endcapped	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Recommended pH Range	100% Aqueous Compatible	USP Listing
ACE SuperC18	Octadecyl (C18)	Encapsulated bonding	1.7, 2, 3, 5, 10	90	400	14.8	1.5-11.5 ^a	No	L1
ACE C18-Amide	C18 with integral amide polar group	Yes	1.7, 2, 3, 5, 10	100	300	16.4	2.0-8.0 ^b	Yes	L1/L60
ACE CN-ES	CN with proprietary extended alkyl spacer	Yes	1.7, 2, 3, 5, 10	100	300	12.6	2.0-8.0 ^b	Yes	L10

^a ACE SuperC18 is designed for use with LC/MS compatible buffers. Further information is contained within "ACE SuperC18 - A Guide to Buffer Selection" – please contact your distributor to request your FREE copy.

^b For optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at higher pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC/MS Buffer Selection" by John Dolan – please contact your distributor to request your FREE copy.

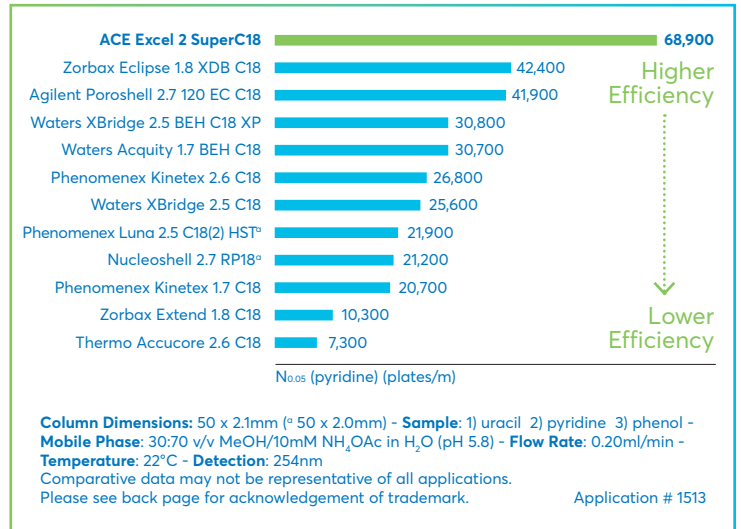
ACE SUPERC18	ACE C18-AMIDE	ACE CN-ES
<p>ACE SuperC18 is a uniquely bonded, EBT endcapped C18 phase which offers unprecedented inertness, excellent efficiency and uncompromising durability over an extended pH range of 1.5 – 11.5.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Analytes differing in hydrophobicity – Polar, moderately polar and non-polar analytes – Uncharged acids and bases – Ionized acids or bases using ion-pairing – Recommended starting point for developing methods at intermediate and high pH to exploit selectivity changes 	<p>ACE C18-Amide is a uniquely designed polar-embedded phase that offers enhanced retention and resolution of polar acidic, phenolic and hydroxy-substituted analytes. The extended spacer ligand technology provides extended column lifetime.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Small water soluble analytes and polar molecules - especially acidic species – Analytes with H bond donors, acids, bases and phenolic compounds – Small peptides – Analytes differing in hydrophobicity – Fully wettable - 100% aqueous buffer compatible – Applications where C18 does not provide adequate separation – Applications where conventional amide/polar embedded phases provide insufficient retention, poor stability, or significant bleed 	<p>ACE CN-ES is a unique phase having an extended alkyl chain with a terminal cyano group. It provides C18 levels of retention and stability compared to commercial cyano propyl phases which typically exhibit low retentivity and poor stability.</p> <p>Recommended Applications</p> <ul style="list-style-type: none"> – Mixtures of very polar, polar and non-polar analytes – Analytes with double and triple bonds – Analytes differing in hydrophobicity – Suitable for NP and RP separations – Fully wettable - 100% aqueous buffer compatible – Applications where a typical C18 column does not provide adequate separation – Applications where traditional CN bonded phases provide insufficient retention, poor stability or significant bleed – An orthogonal phase for method development

ACE SUPERC18 PROVIDES EXCELLENT COLUMN INERTNESS

- Leading column brands in 50 x 2.1mm LC/MS compatible dimensions at intermediate pH 5.8
- Silica, Hybrid and Superficially Porous particle technologies compared
- Comparison of column efficiency for pyridine – a basic molecule
- Efficiency measured at 5% peak height to account for peak tailing effects

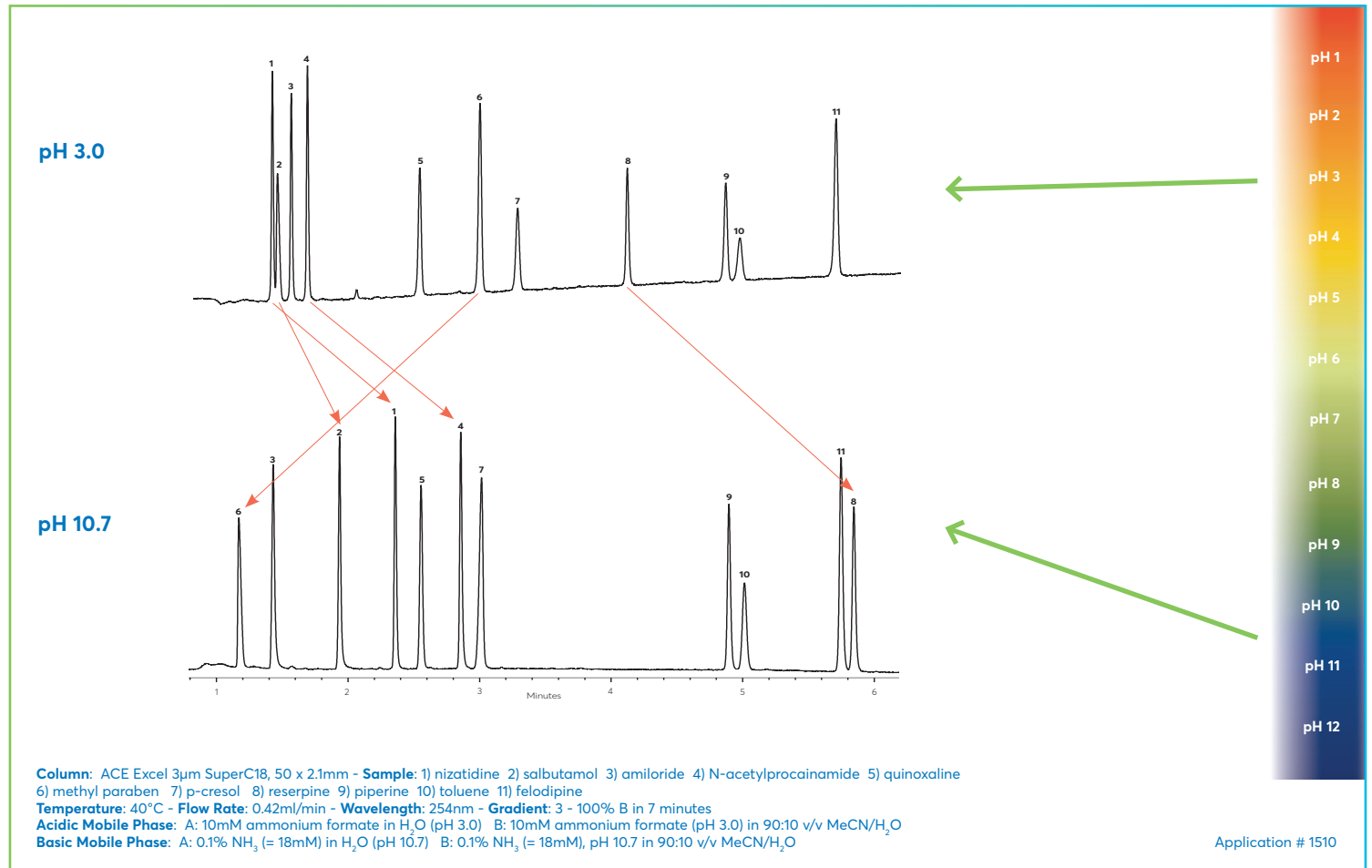
PEAK EFFICIENCY COMPARISON

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USE ACE SUPERC18 TO INVESTIGATE PH EFFECTS

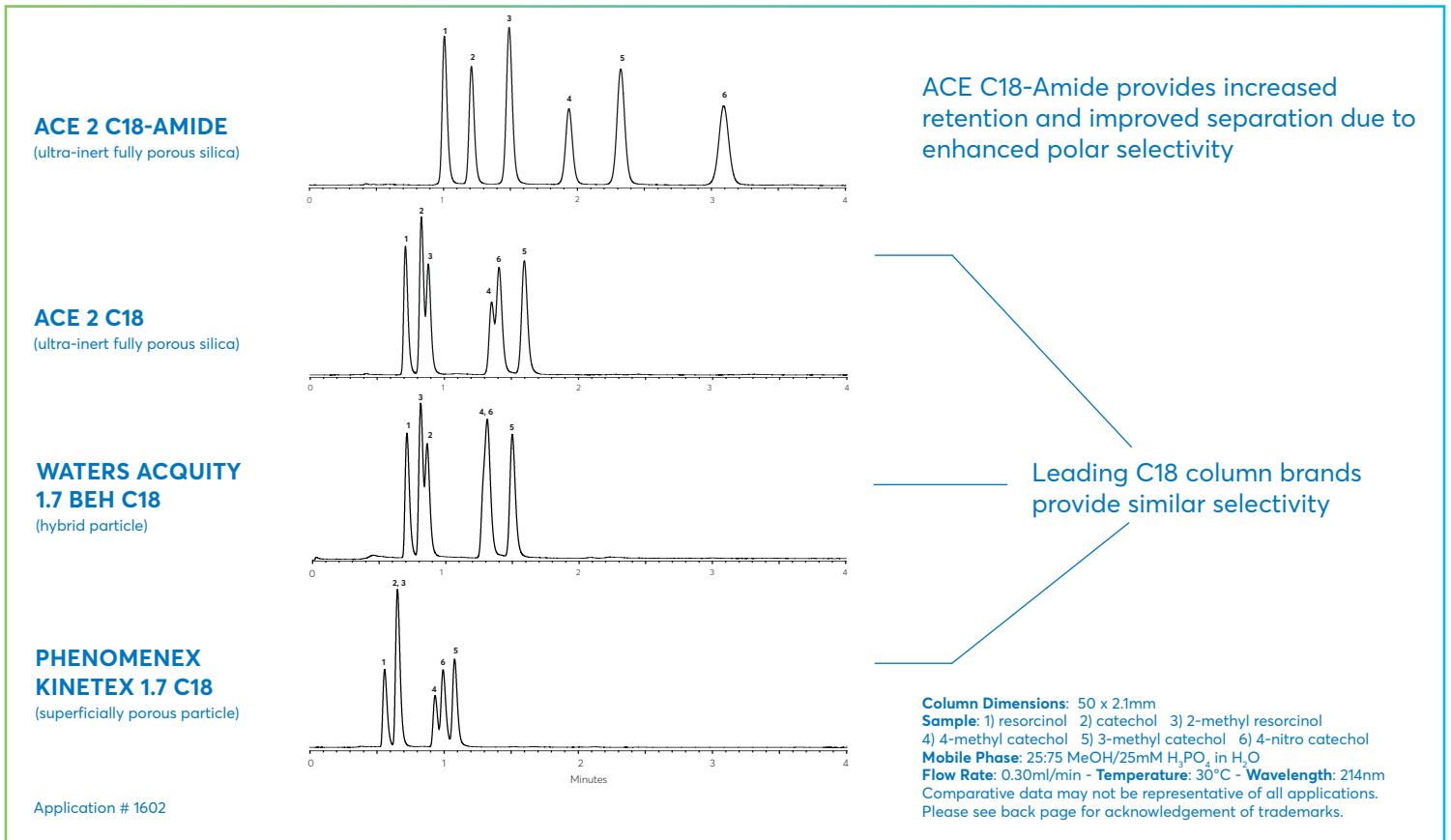
EXPLOIT SELECTIVITY BY ADJUSTING PH



ACE C18-AMIDE PROVIDES ENHANCED POLAR SELECTIVITY

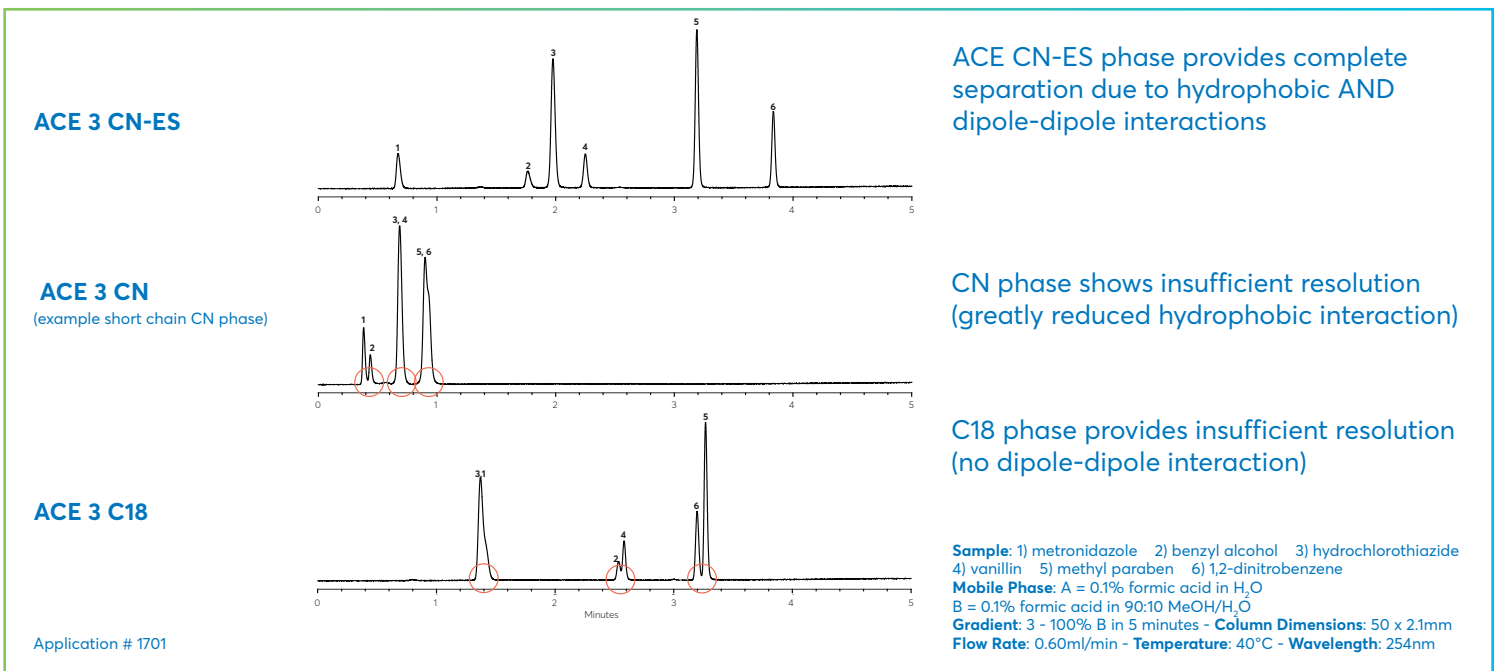
ADVANTAGES OF MULTI-MODE INTERACTIONS FOR HPLC SEPARATIONS

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ACE CN-ES PROVIDES ALTERNATIVE SELECTIVITY

ACE CN-ES PROVIDES A SEPARATION THAT A C18 OR CN COLUMN ALONE CANNOT ACHIEVE



ACE EXTENDED METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

Contains 3 columns: ACE SuperC18, ACE C18-Amide and ACE CN-ES of specified dimensions

Column Dimensions	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)			
	1.7µm	2µm	3µm	5µm
2.1 x 50mm	MDKE-17-0502U	MDKE-2-0502U	MDKE-3-0502U	MDKE-5-0502U
2.1 x 100mm	MDKE-17-1002U	MDKE-2-1002U	MDKE-3-1002U	MDKE-5-1002U
2.1 x 150mm	-	MDKE-2-1502U	MDKE-3-1502U	MDKE-5-1502U
2.1 x 250mm	-	-	MDKE-3-2502U	MDKE-5-2502U
3.0 x 50mm	MDKE-17-0503U	MDKE-2-0503U	MDKE-3-0503U	MDKE-5-0503U
3.0 x 100mm	MDKE-17-1003U	MDKE-2-1003U	MDKE-3-1003U	MDKE-5-1003U
3.0 x 150mm	-	MDKE-2-1503U	MDKE-3-1503U	MDKE-5-1503U
3.0 x 250mm	-	-	MDKE-3-2503U	MDKE-5-2503U
4.6 x 50mm	-	MDKE-2-0546U	MDKE-3-0546U	MDKE-5-0546U
4.6 x 100mm	-	MDKE-2-1046U	MDKE-3-1046U	MDKE-5-1046U
4.6 x 150mm	-	MDKE-2-1546U	MDKE-3-1546U	MDKE-5-1546U
4.6 x 250mm	-	-	MDKE-3-2546U	MDKE-5-2546U

Guard columns are available for all of these phases

ACE UltraCore method development kit

- Contains ACE UltraCore SuperC18 and SuperPhenylHexyl phases
- Use to exploit selectivity changes at low, intermediate and high pH
- Available in a wide range of dimensions
- Ultra inert core-shell particles and Encapsulated Bonding Technology (EBT™) provide excellent peak shape

Phase	Functional Group	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Maximum pH Range	USP Listing
ACE UltraCore 2.5 SuperC18	Octadecyl encapsulated	2.5	95	130	7.0	1.5-11.0 ^a	L1
ACE UltraCore 2.5 SuperPhenylHexyl	Phenyl-Hexyl encapsulated	2.5	95	130	4.6	1.5-11.0 ^a	L11
ACE UltraCore 5 SuperC18	Octadecyl encapsulated	5	95	100	5.4	1.5-11.0 ^a	L1
ACE UltraCore 5 SuperPhenylHexyl	Phenyl-Hexyl encapsulated	5	95	100	3.6	1.5-11.0 ^a	L11

^a ACE UltraCore columns are designed for use with LC/MS compatible buffers. Further information is contained within "ACE UltraCore – A Guide to Buffer Selection" - please contact your distributor to request your FREE copy.

ACE EXTENDED METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

- ACE UltraCore SuperC18 and SuperPhenylHexyl phases are manufactured using our unique Encapsulated Bonding Technology (EBT™)
- This technology dramatically increases ligand coverage of the silica surface and effectively eliminates the negative effects of unbonded silanol groups
- The higher ligand coverage results in improved inertness, chromatographic performance and stability

ACE®
Stationary Phases
Virtually Eliminate
the Negative Effects of
Silanols on UHPLC &
HPLC Separations

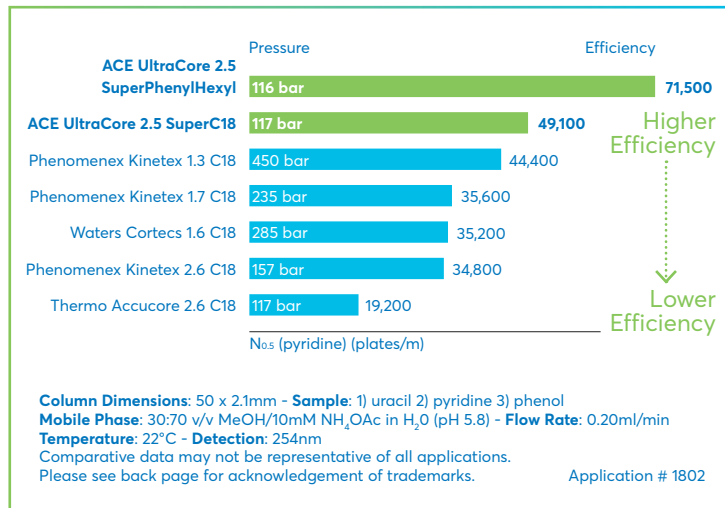
~~Si-OH~~

ACE ULTRACORE COLUMNS ARE HIGHLY INERT

- Solid-core columns from leading manufacturers investigated
- Comparison of column efficiency for pyridine – a basic molecule

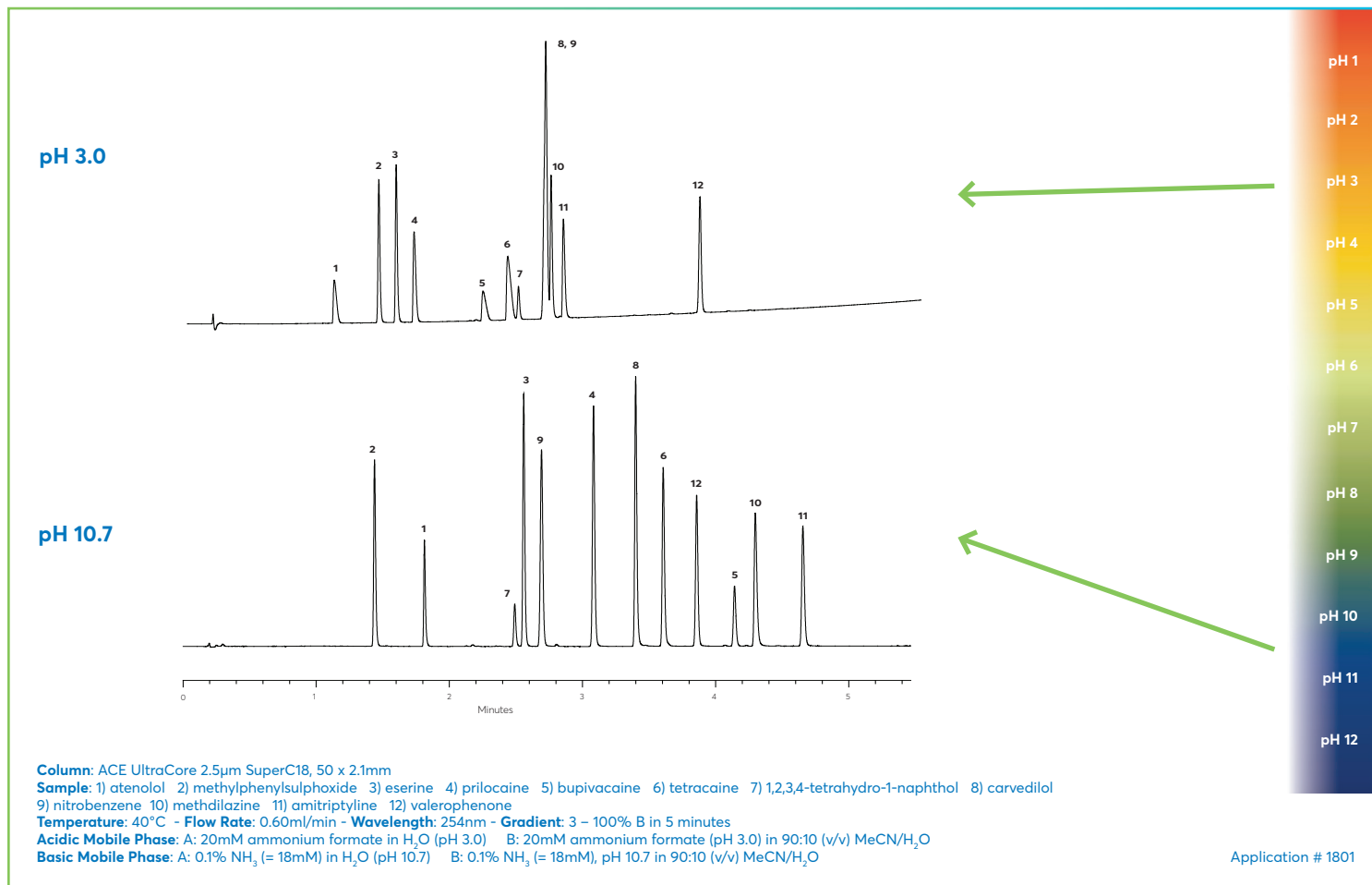
PEAK EFFICIENCY COMPARISON

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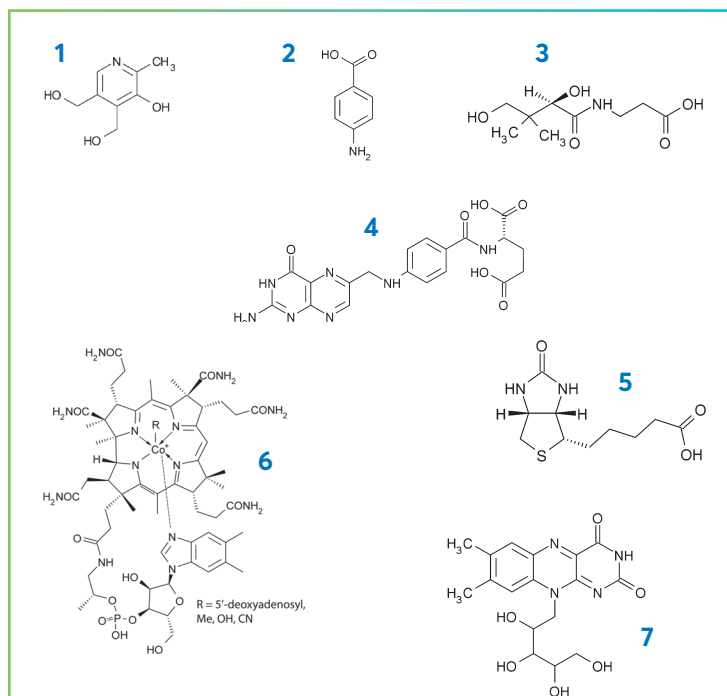
USE ACE SUPERC18 TO INVESTIGATE PH EFFECTS

EXPLOIT SELECTIVITY BY ADJUSTING PH



INTRODUCING SELECTIVITY CHANGES USING ACE ULTRACORE METHOD DEVELOPMENT KITS

VITAMIN SEPARATION

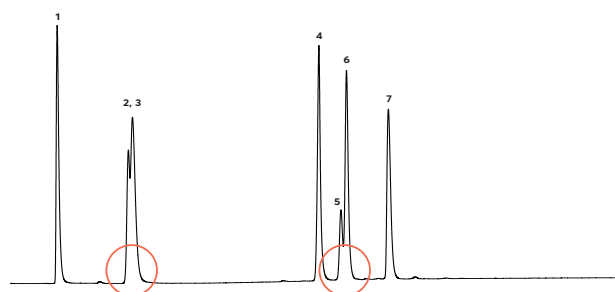


ACE ULTRACORE METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

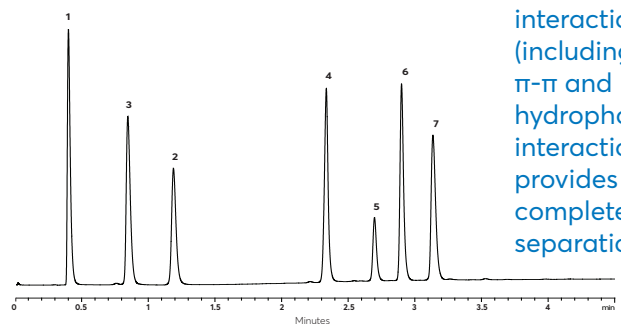
Contains 2 columns: ACE UltraCore SuperC18 and ACE UltraCore SuperPhenylHexyl of specified dimensions

Column Dimensions	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)	
	2.5 μ m	5 μ m
2.1 x 50mm	MDKU-25-0502U	MDKU-5-0502U
2.1 x 100mm	MDKU-25-1002U	MDKU-5-1002U
2.1 x 150mm	MDKU-25-1502U	MDKU-5-1502U
2.1 x 250mm	-	MDKU-5-2502U
3.0 x 50mm	MDKU-25-0503U	MDKU-5-0503U
3.0 x 100mm	MDKU-25-1003U	MDKU-5-1003U
3.0 x 150mm	MDKU-25-1503U	MDKU-5-1503U
3.0 x 250mm	-	MDKU-5-2503U
4.6 x 50mm	MDKU-25-0546U	MDKU-5-0546U
4.6 x 100mm	MDKU-25-1046U	MDKU-5-1046U
4.6 x 150mm	MDKU-25-1546U	MDKU-5-1546U
4.6 x 250mm	-	MDKU-5-2546U

ACE ULTRACORE SUPERC18



ACE ULTRACORE SUPERPHENYLHEXYL



Multi-mode interaction (including π - π and hydrophobic interactions) provides complete separation.

Column: 50 x 2.1mm, 2.5 μ m

Sample: 1) pyridoxine 2) p-aminobenzoic acid 3) pantoic acid 4) folic acid 5) d-biotin 6) cyanocobalamin 7) riboflavin

Mobile Phase A: 20 mM KH_2PO_4 pH 2.7 (aq) B: 20 mM KH_2PO_4 pH 2.7 in MeOH:H₂O 50:50 (v/v)

Gradient: Time (mins) 0 1.50 3.00 3.75 4.50
%B 20 60 70 70 20

Flow Rate: 0.40 ml/min

Temperature: 40°C - Wavelength: 205nm

Application # 1941

Guard columns are available for all of these phases

ACE Bioanalytical 300Å method development kit

- Contain ACE C18-300, ACE C4-300 and ACE Phenyl-300 phases
- Ideal starting point for protein and peptide method development
- Available in a wide range of dimensions
- Ultra-inert 300Å phases provide excellent peak shape and reproducibility

Phase	Functional Group	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Recommended pH Range	USP Listing
ACE C18-300	Octadecyl (C18)	3, 5, 10	300	100	9.0	2.0-8.0 ^a	L1
ACE C4-300	Butyl (C4)	3, 5, 10	300	100	2.6	2.0-8.0 ^a	L26
ACE Phenyl-300	Phenyl	3, 5, 10	300	100	5.3	2.0-8.0 ^a	L11

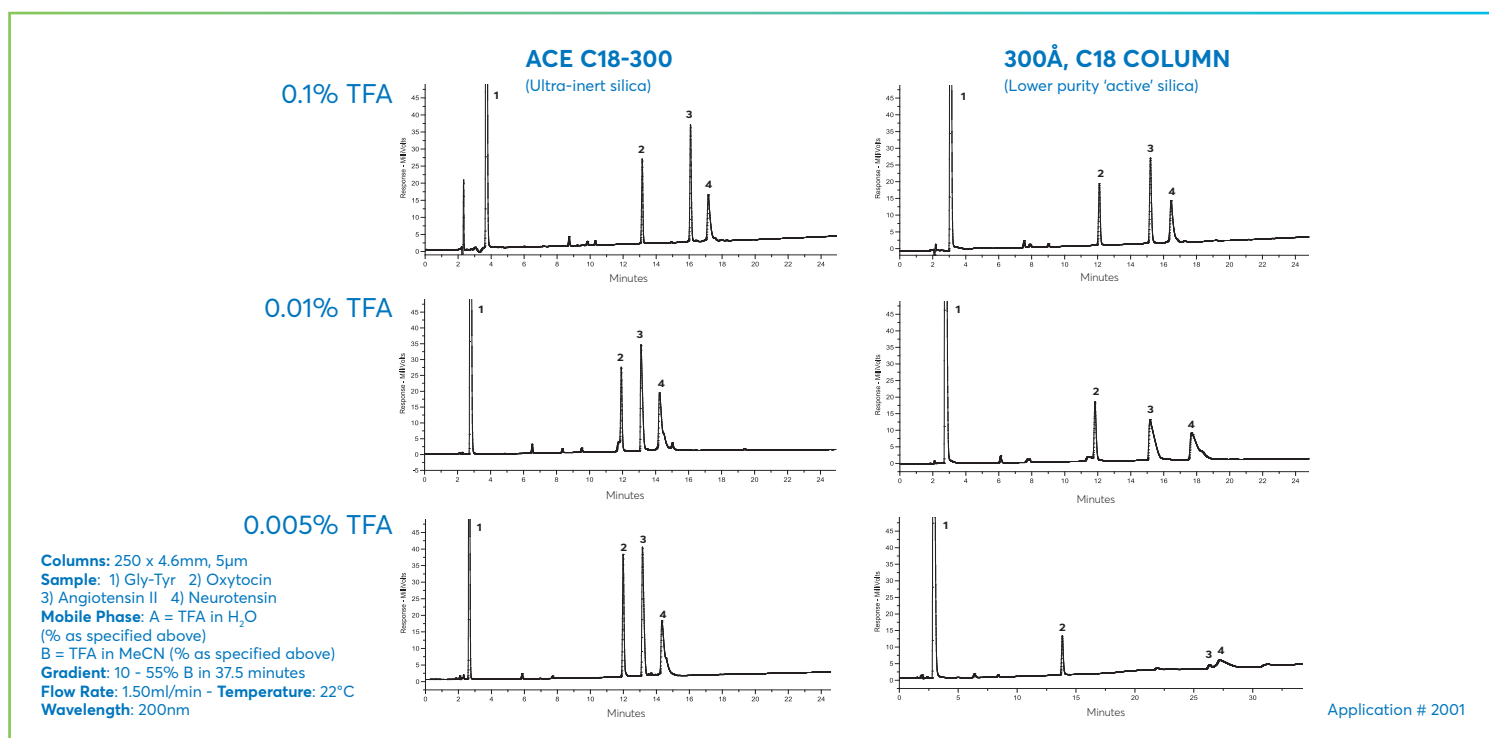
^aFor optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at higher pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC/MS Buffer Selection" by John Dolan – please contact your distributor to request your FREE copy.

ACE 300Å ULTRA-INERT COLUMNS PROVIDE IMPROVED PEAK SHAPE

ACE 300Å ultra-inert HPLC columns are manufactured using advanced technology that virtually eliminates the negative effects of silanols and metal contamination for the separation of peptides, proteins and other high molecular weight biomolecules.

The ultra-inert characteristics of ACE 300Å columns permit the use of as little as 0.005% TFA in the mobile phase. Lower purity columns show unacceptable peak tailing even when using as much as 0.01% TFA. The ability to run at reduced TFA concentrations results in increased sensitivity.

ACE 300Å BIOANALYTICAL COLUMNS PROVIDE EXCELLENT PEAK SHAPE



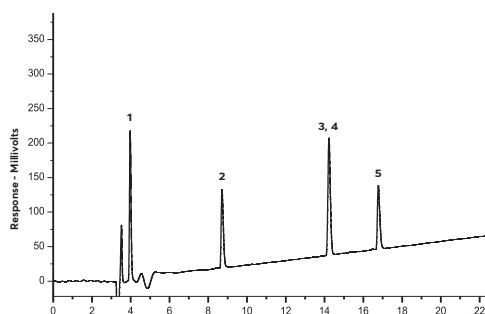
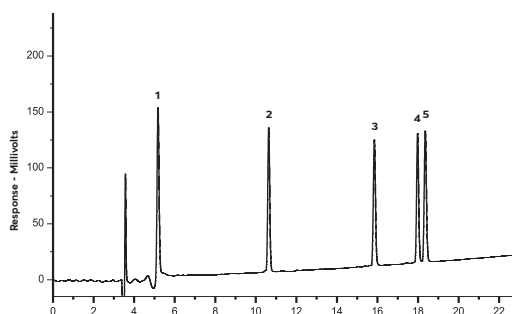
USING ACE BIOANALYTICAL 300Å METHOD DEVELOPMENT KITS TO OPTIMISE SELECTIVITY

INTRODUCING SELECTIVITY CHANGES BY CAREFUL CONSIDERATION OF BONDED PHASE AND MOBILE PHASE ADDITIVE

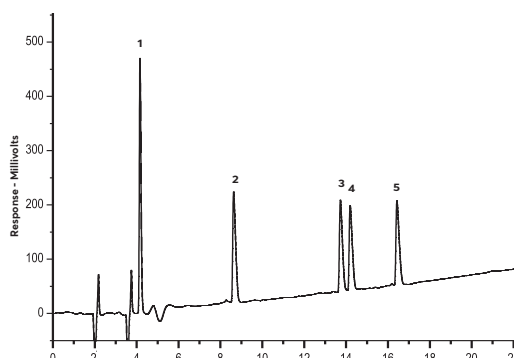
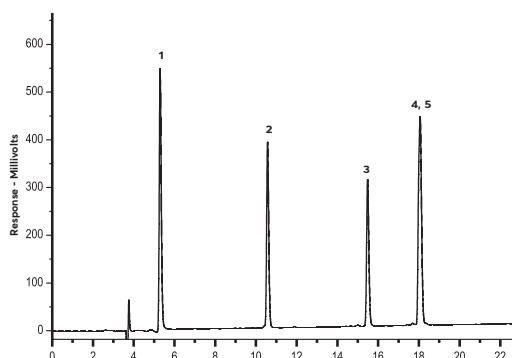
0.1% TFA

0.1% Formic Acid

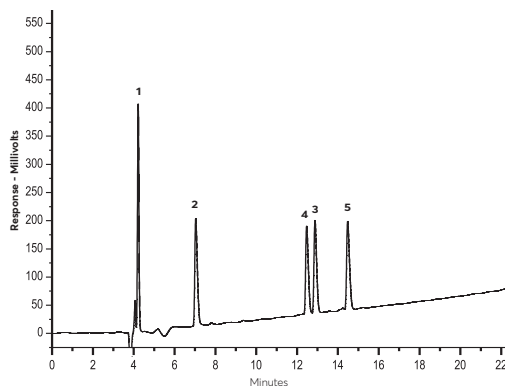
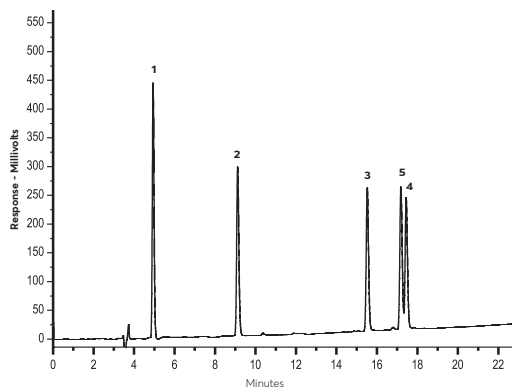
ACE C18-300



ACE C4-300



ACE Phenyl-300



The ACE 300Å C18, C4 and Phenyl chemistries contained within ACE Bioanalytical 300Å Method Development Kits enable the analyst to investigate selectivity effects due to phase variations.

The ultra-inert characteristics of the ACE 300Å silica enable different mobile phase additives to be investigated without a deterioration in peak shape or sensitivity.

Columns: 250 x 4.6mm, 5µm

Sample: 1) Gly-Tyr 2) Val-Tyr-Val 3) Methionine enkephalin 4) Angiotensin II 5) Leucine enkephalin

Mobile Phase: A = 0.1% TFA in H₂O or 0.1% Formic Acid in H₂O (as specified above) B = MeCN Gradient: 10 – 40% B in 25 minutes

Flow Rate: 1.00ml/min - Temperature: 22°C - Wavelength: 220nm

Application # 2002

ACE BIOANALYTICAL 300Å METHOD DEVELOPMENT HPLC COLUMN KITS

Contains 3 columns: ACE C18-300, ACE C4-300 and ACE Phenyl-300 of specified dimensions

Column Dimensions	(HPLC hardware format with 275bar/4000psi pressure limit)	
	3µm	5µm
2.1 x 50mm	MDKB-3-0502	MDKB-5-0502
2.1 x 100mm	MDKB-3-1002	MDKB-5-1002
2.1 x 150mm	MDKB-3-1502	MDKB-5-1502
2.1 x 250mm	-	MDKB-5-2502
3.0 x 50mm	MDKB-3-0503	MDKB-5-0503
3.0 x 100mm	MDKB-3-1003	MDKB-5-1003
3.0 x 150mm	MDKB-3-1503	MDKB-5-1503
3.0 x 250mm	-	MDKB-5-2503
4.6 x 50mm	MDKB-3-0546	MDKB-5-0546
4.6 x 100mm	MDKB-3-1046	MDKB-5-1046
4.6 x 150mm	MDKB-3-1546	MDKB-5-1546
4.6 x 250mm	-	MDKB-5-2546

Guard columns are available for all of these phases

ACE HILIC method development kit

- Contains ACE HILIC-A, ACE HILIC-B and ACE HILIC-N phases
- Alternative and improved selectivity to reversed-phase for polar and very polar analytes
- Available in a wide range of dimensions
- ACE HILIC-A, ACE HILIC-B and ACE HILIC-N provide alternative selectivity to each other

Phase	Functional Group	Endcapped	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	Recommended pH Range	USP Listing
ACE HILIC-A	Proprietary SIL	No	1.7, 3, 5	100	300	-	2.0-7.0	L3
ACE HILIC-B	Proprietary Aminopropyl	No	1.7, 3, 5	100	300	4.0	2.0-7.0	L8
ACE HILIC-N	Proprietary Polyhydroxy	No	1.7, 3, 5	100	300	7.0	2.0-7.0	Pending

WHAT IS HILIC?

- Hydrophilic Interaction Liquid Chromatography (HILIC) was first described by Alpert*
- 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 (Fig 1) and provides multiple modes of interaction between the analyte, stationary phase, eluent and water enriched layer at the stationary phase particle-eluent interface**

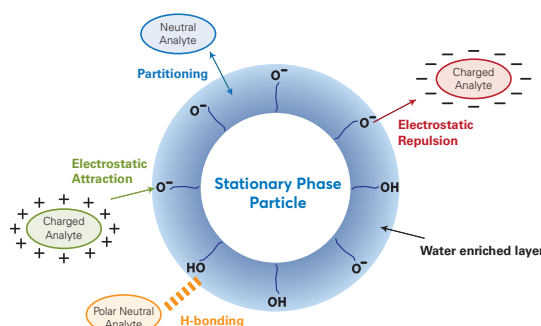


Figure 1. Schematic of interactions between different types of polar analytes and a stationary phase in HILIC mode

* A. J. Alpert, J. Chromatogr., 499 (1990) 177.

** See the FREE ACE guide to reproducible HILIC method development for more information

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 (Fig 2A)
- Generally, polar analytes are suitable for HILIC if they elute before caffeine in gradient RPLC (Fig 2B)

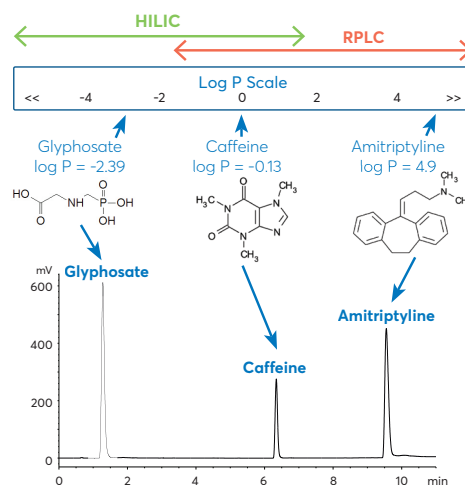


Figure 2A. Analyte suitability for HILIC from Log P

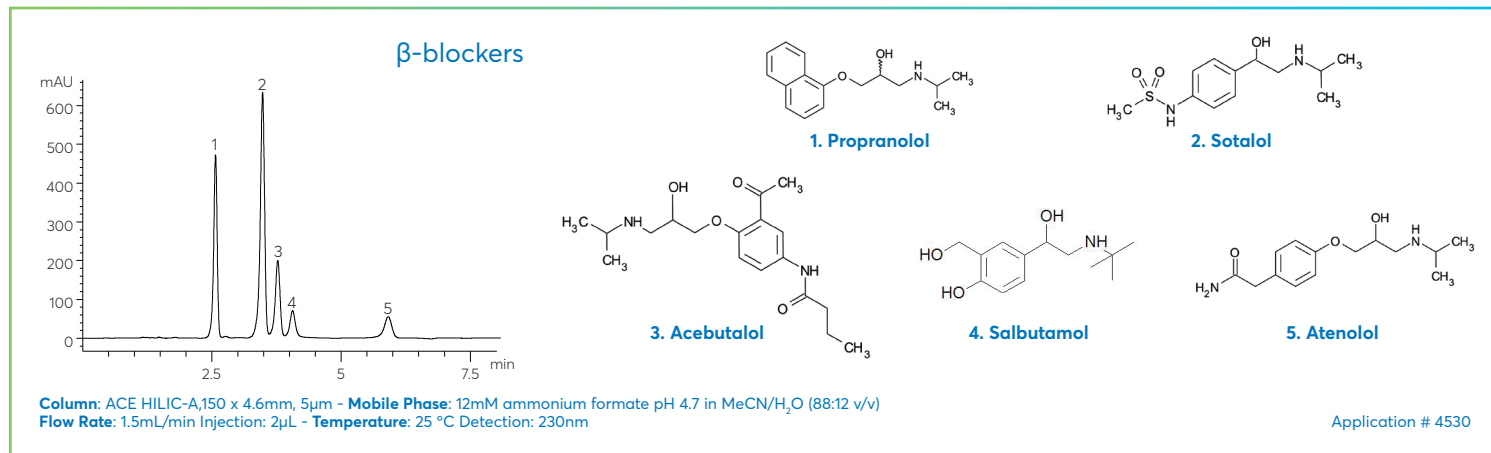
Figure 2B. Analyte suitability for HILIC from gradient RPLC

Column: ACE Excel C18, 100 x 3.0mm, 2µm - Mobile Phase: A = 10mM ammonium formate, pH 3.0 (aq), B = 10mM ammonium formate, pH 3.0 in 90:10 v/v MeCN:H₂O - Gradient: 5-100% B in 10 minutes - Detection: ELSD - Flow Rate: 0.4mL min - Temperature: 30 °C - Injection: 10µL
 Analysed using VWR-Hitachi Chromaster with VWR ELSD90.
 Figure reproduced with permission and adapted from work first published in Chromatography Today, Volume 8, Issue 4, November/December 2015

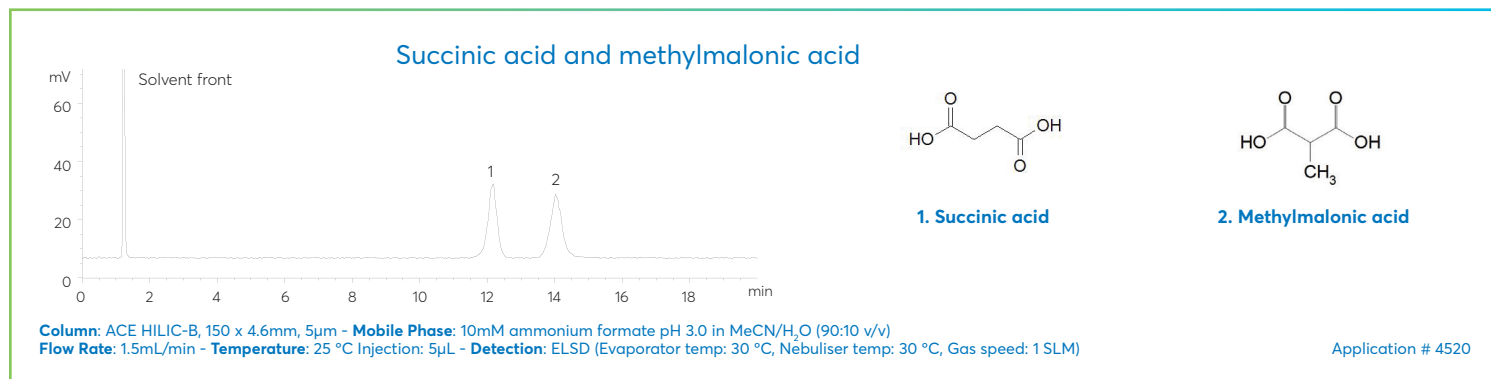
ACE HILIC COLUMNS – 3 ALTERNATIVE SELECTIVITIES

ACE HILIC-A	ACE HILIC-B	ACE HILIC-N
An acidic character phase with an ionisable negative surface charge depending on mobile phase pH	A basic character phase with an ionisable positive surface charge depending on mobile phase pH	A neutral character phase capable of H-bonding amongst other mechanisms of interaction

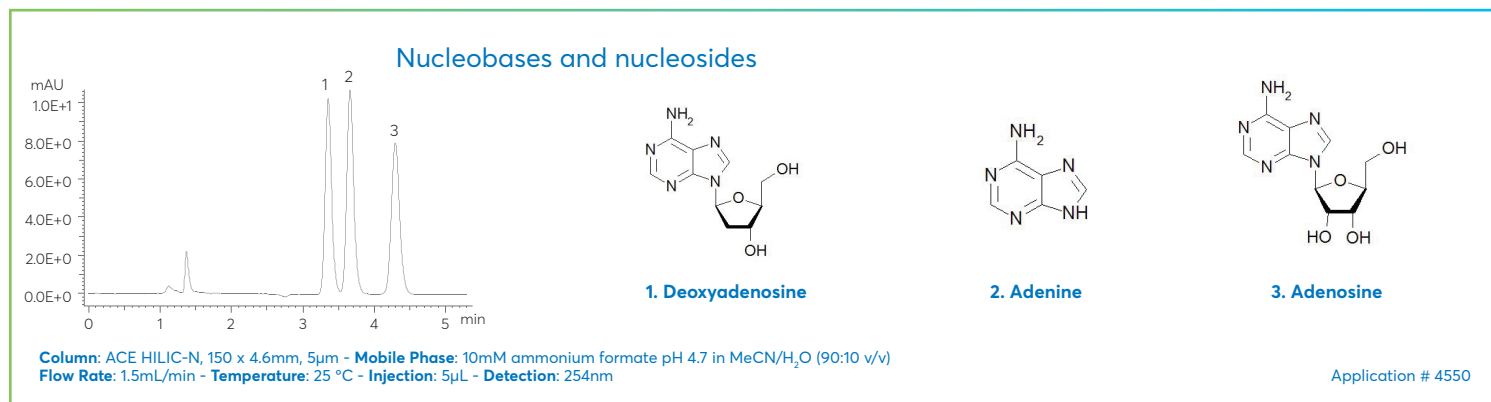
ACE HILIC-A - An acidic character phase



ACE HILIC-B - A basic character phase



ACE HILIC-N - A polar neutral character phase



ACE HILIC METHOD DEVELOPMENT

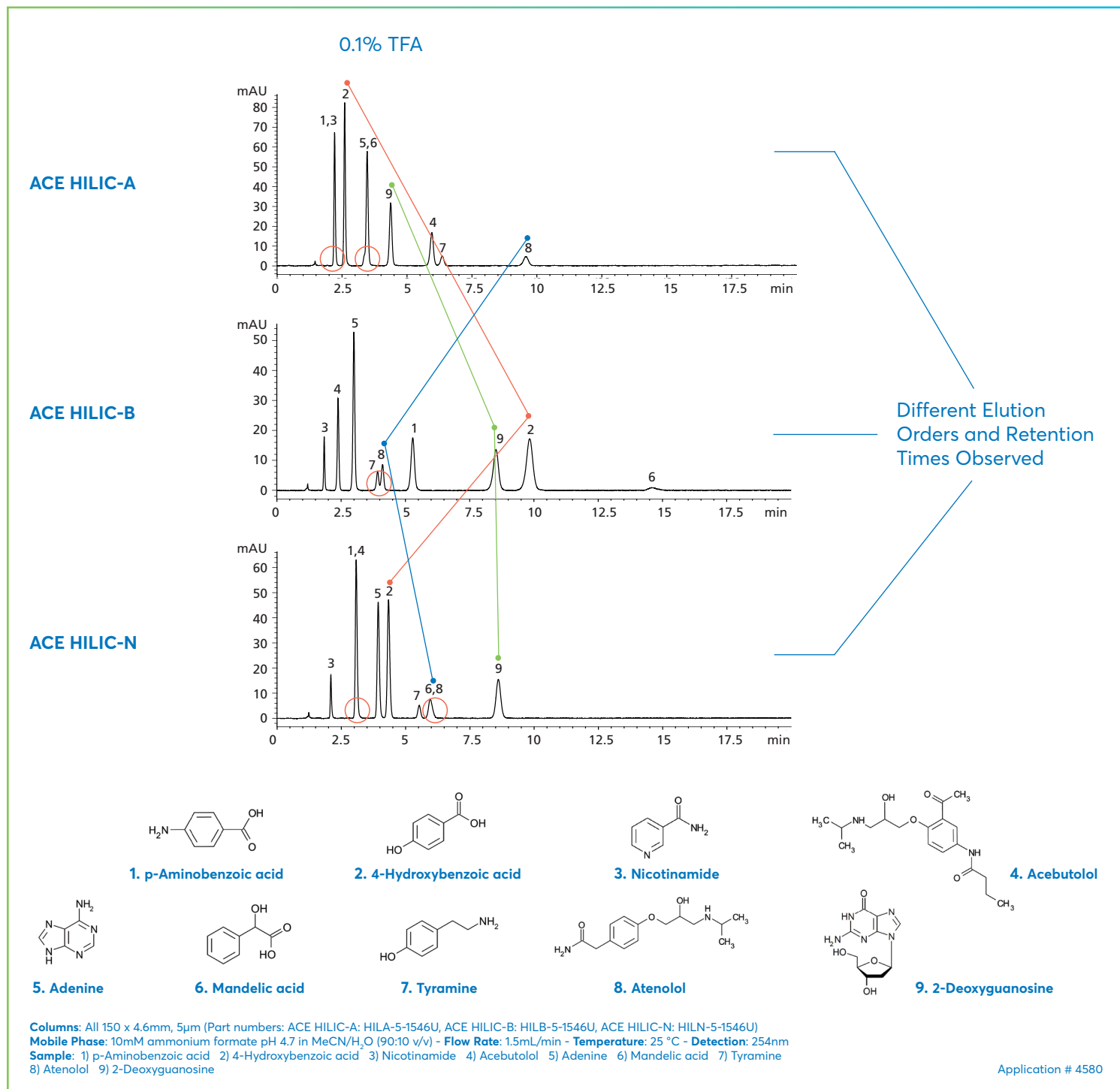
- ACE HILIC columns provide alternative selectivity to each other.
- The power of systematic screening of different phase chemistries for HILIC method development is seen below.
- Maximise your HILIC method development success by following the ACE HILIC method development protocol using

three optimised ACE HILIC column chemistries – protocol available in the FREE HILIC Method Development guide.

CONCLUSIONS

ACE HILIC columns provide alternative selectivity to each other – ideal for HILIC method development.

ADVANTAGES OF USING ACE HILIC METHOD DEVELOPMENT KITS



Application # 4580

METHOD DEVELOPMENT KITS

FREE HILIC METHOD DEVELOPMENT TECHNICAL GUIDE

A 38 page HILIC Method Development Technical Guide illustrating a tried and tested approach to HILIC method development is available. Request your copy today and learn how to develop reproducible and robust HILIC methods simply and efficiently.

Alternatively, please contact our technical support team via chromsupport@avantorsciences.com

ACE HILIC METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

Contains 3 columns: ACE HILIC-A, ACE HILIC-B and ACE HILIC-N of specified dimensions

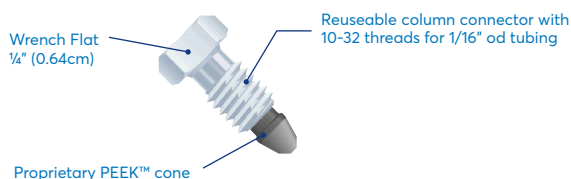
Column Dimensions	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)		
	1.7µm	3µm	5µm
2.1 x 50mm	MDKH-17-0502U	MDKH-3-0502U	MDKH-5-0502U
2.1 x 100mm	MDKH-17-1002U	MDKH-3-1002U	MDKH-5-1002U
2.1 x 150mm	-	MDKH-3-1502U	MDKH-5-1502U
2.1 x 250mm	-	MDKH-3-2502U	MDKH-5-2502U
3.0 x 50mm	MDKH-17-0503U	MDKH-3-0503U	MDKH-5-0503U
3.0 x 100mm	MDKH-17-1003U	MDKH-3-1003U	MDKH-5-1003U
3.0 x 150mm	-	MDKH-3-1503U	MDKH-5-1503U
3.0 x 250mm	-	MDKH-3-2503U	MDKH-5-2503U
4.6 x 50mm	-	MDKH-3-0546U	MDKH-5-0546U
4.6 x 100mm	-	MDKH-3-1046U	MDKH-5-1046U
4.6 x 150mm	-	MDKH-3-1546U	MDKH-5-1546U
4.6 x 250mm	-	MDKH-3-2546U	MDKH-5-2546U

UHPLC and HPLC column accessories

UHPLC COLUMN CONNECTORS

- Pressure rating >1700 bar (>25000 psi)
- Compatible with all UHPLC systems¹
- Compatible with all UHPLC column brands
- Eliminates poor connections
- Innovative reusable design

ACE Excel UHPLC Column Connector
(p/n EXL-CC10, 10 pack)



All UHPLC column brands require correct installation in order to realise maximum column efficiency. To avoid connection problems, permanently swaged fittings are not recommended as they do not allow free movement between the tubing, fitting and column inlet on installation. This can result in a poorly connected column that shows unexpected peak tailing due to the introduction of extra column volume (dead volume) to the system. Alternatively, a leak at the inlet fitting connection may be observed.

ACE Excel UHPLC Column Connectors (p/n EXL-CC10, 10 pack) enable the inlet end of UHPLC columns to be correctly installed every time. Their unique reusable design ensures that they maintain a 1700 bar (25000 psi) pressure rating with repeated use, yet do not permanently swage onto the inlet tubing. To maximise the lifetime of the fitting, the use of a torque wrench (p/n EXL-TW) is required.

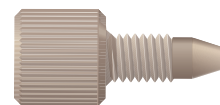
At the outlet end of the UHPLC column (where pressure demands are lower but a correct connection remains important), ACE Fingertight HPLC Column Connectors (p/n ACE-CC10, 10 pack, see below) may alternatively be used.

¹Note: For inlet connections onto a Waters Acquity system (containing a Waters Acquity 1/16" fitting and ferrule on the inlet tubing) the use of a pre-column filter incorporating the unique Waters Acquity column port profile is alternatively recommended (p/n EXL-PCF10/ACQ - 10 pack) to ensure maximum compatibility with the Waters Acquity system fittings.

HPLC COLUMN CONNECTORS

- Fingertight to 350 bar (5000 psi)
- Reuseable and simple to install
- Eliminates poor connections
- Compatible with all HPLC column brands and instruments

ACE Fingertight HPLC Column Connector
(p/n ACE-CC10, 10 pack)



ACE Fingertight HPLC Column Connectors (p/n ACE-CC10, 10 pack) are recommended for the connection of both the inlet and outlet ends of HPLC columns.

Manufactured from premium quality PEEK™, the fittings simply hand tighten to provide a perfect column connection, and are pressure rated to 350 bar/5000 psi.

ACE Fingertight HPLC Column Connectors may additionally be used at the outlet end of UHPLC columns, where pressure demands are lower but a correct connection remains important.

Setting science in motion to create a better world

Avantor® is a leading global provider of mission critical products and services to customers in the biopharma, healthcare, education & government, and advanced technologies & applied materials industries. We operate in more than 30 countries and deliver an extensive portfolio of products and services.