avantor™

LC-MS Solutions

Avantor® ACE®, method development kits

Avantor® ACE®

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
Learn more: vwr.com/ace

	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.

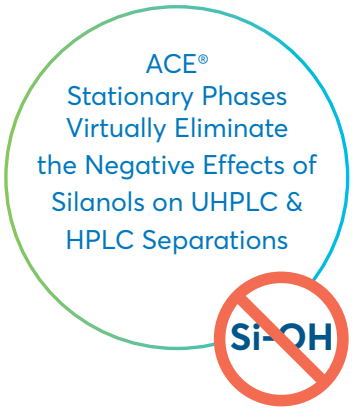
	Bonded Phase	SEPARATION MECHANISM AND RELATIVE STRENGTH [2]					
		Partitioning	Anionic Analyte Interactions		Cationic Analyte Interactions		H-bonding
			Attraction	Repulsion	Attraction	Repulsion	
ACE HILIC Method Development Kit (see page 19)	ACE HILIC-A	**	-	***	****	-	*
	ACE HILIC-B	***	****	-	-	***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

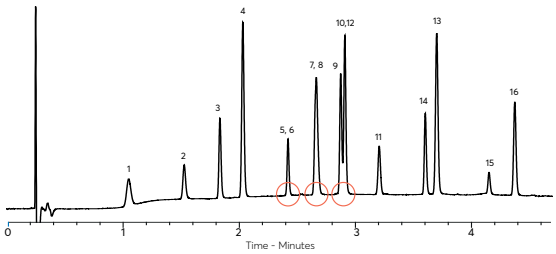
USING ACE METHOD DEVELOPMENT KITS TO IMPROVE SEPARATIONS

- 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.



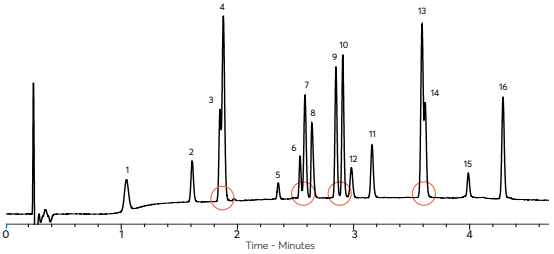
COLUMNS WITHIN ACE METHOD DEVELOPMENT KITS PROVIDE ALTERNATIVE SELECTIVITY

ACE C18



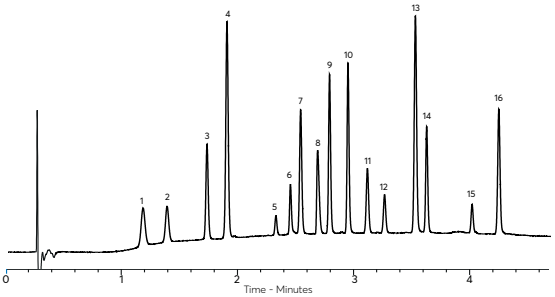
ACE C18 provides excellent peak shape, but here the essentially "hydrophobic-only" interaction results in co-elution.
Investigation of alternative bonded phases using the same test conditions is recommended.

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 or visit **vwr.com/ace**.

ACE C18

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.

Recommended Applications

- 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

ACE C18-AR

ACE C18-AR combines the excellent performance and advantages of the ACE C18 phase with the added selectivity of an integral phenyl group.

Recommended Applications

- 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

ACE C18-PFP

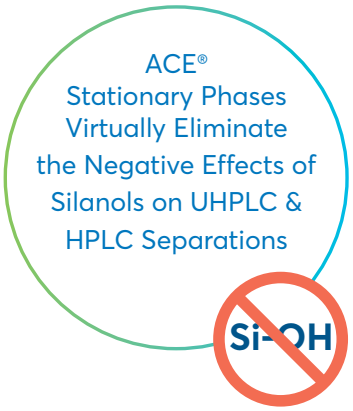
ACE C18-PFP brings together the stability, reproducibility and low bleed of the ACE C18 phase with the additional selectivity of an integral pentafluorophenyl (PFP) group.

Recommended Applications

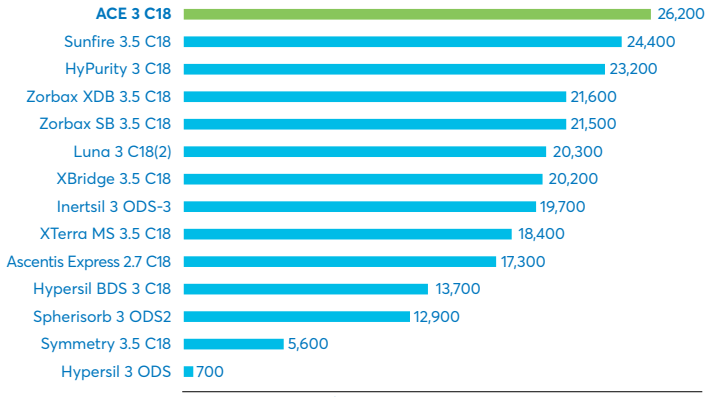
- Analytes with π-bonding
- Analytes with electron donating groups, such as phenols, aromatic ethers and amines
- Analytes with proton donor groups
- Analytes with different dipole moments
- Analytes differing in hydrophobicity
- Stuctural isomers, steroids, substituted aromatics and taxanes
- Fully 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

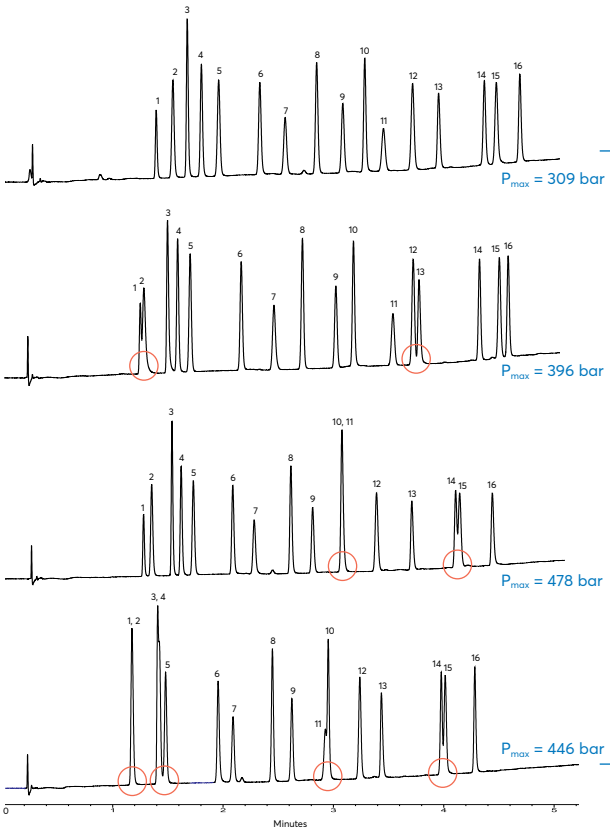
RAPID UHPLC SCREENING OF 16 PHARMACEUTICALS AND RELATED COMPOUNDS

ACE EXCEL 2 C18
(fully porous ultra-inert silica)

ZORBAX ECLIPSE 1.8 XDB C18
(fully porous silica)

WATERS ACQUITY 1.7 BEH C18
(hybrid particle)

PHENOMENEX KINETEX 1.7 C18
(core shell particle)



These leading C18 phases provide an essentially hydrophobic-only interaction, and therefore exhibit similar selectivity with only slight differences between brands observed.

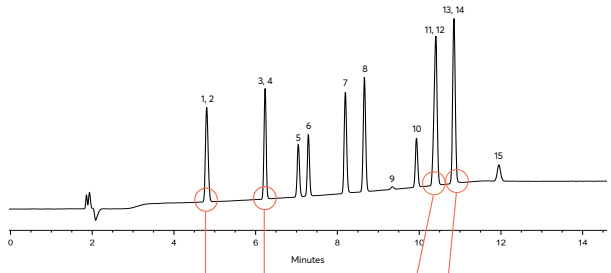
To implement a change in selectivity, the use of an alternative bonded phase (eg ACE C18-AR, ACE C18-PFP) that can leverage additional modes of interaction is recommended.

Sample: 1) N-acetylprocainamide 2) 3-hydroxybenzoic acid 3) pindolol 4) methylphenylsulphoxide 5) benzyl alcohol 6) quinoxaline 7) 1,4-dinitrobenzene 8) phenacetin 9) 1,2-dimethoxybenzene 10) furosemide 11) anisole 12) methyl benzoate 13) remacemide 14) nimesulide 15) ethyl benzoate 16) diflunisal
Mobile Phase: A = 20mM KH_2PO_4 pH 2.7 B = 20mM KH_2PO_4 pH 2.7 in MeOH/H_2O (65:35 v/v) - **Gradient**: 3 – 100% B in 5 minutes
Column Dimensions: 50 x 2.1mm - **Flow Rate**: 0.60ml/min - **Temperature**: 60°C - **Detection**: 214nm. Application # 1503

LEVERAGING THE UNIQUE SELECTIVITY OF ACE C18-AR

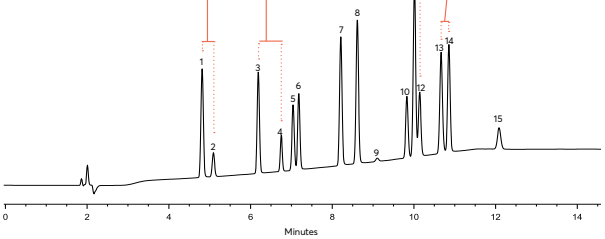
IMPROVING AN ANALGESICS SEPARATION BY CHANGING PHASE

ACE 3 C18



C18 phase provides essentially hydrophobic-only interaction

ACE 3 C18-AR



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

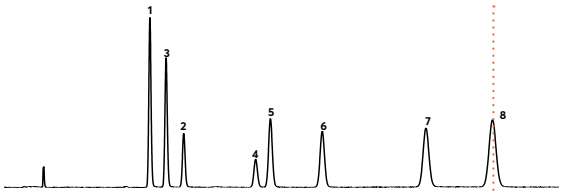
Sample: 1) 4-acetamidophenol 2) 4-aminobenzoic acid 3) 4-hydroxybenzoic acid 4) caffeine 5) 2-acetamidophenol 6) 3-hydroxybenzoic acid 7) salicylamide 8) acetanilide 9) phenol 10) acetylsalicylic acid 11) benzoic acid 12) sorbic acid 13) salicylic acid 14) phenylacetin 15) salicylaldehyde
Mobile Phase: A = 0.1% v/v formic acid in H_2O B = 0.1% v/v formic acid in MeCN - **Gradient**: 5 - 35% B in 9 minutes, hold at 35% B until 14 minutes
Column Dimensions: 150 x 4.6mm - **Flow Rate**: 1.00ml/min - **Temperature**: 40°C - **Detection**: 240nm

Application # 1921

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

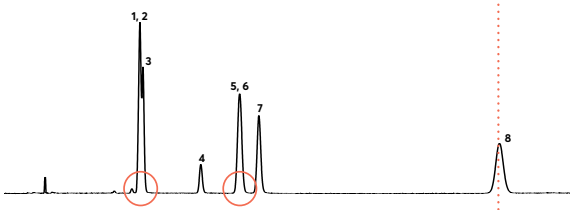
THE IMPORTANCE OF MAINTAINING HYDROPHOBICITY DURING MULTI-MODE INTERACTIONS

ACE 3 C18-PFP



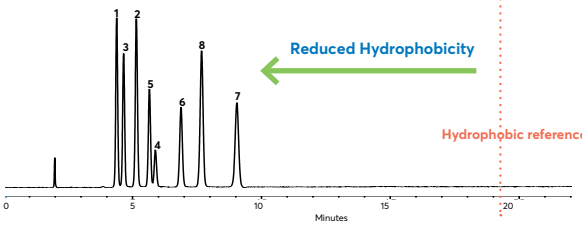
Multi-mode interaction including hydrophobic interaction provides complete separation

ACE 3 C18



C18 phase provides essentially hydrophobic-only interaction

HYPERSIL GOLD 3µm PFP
(example short chain PFP phase)



Significantly reduced hydrophobicity reduces the separation despite other interaction modes

Column Dimensions: 150 x 4.6mm - **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) neutral molecule (reference)
Mobile Phase: 50:50 v/v MeOH/H_2O - **Flow Rate**: 1.00ml/min - **Temperature**: 40°C - **Detection**: 254nm

Application # 1931

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	76396-154	76396-198	76396-294	76396-394
2.1 x 100mm	76396-158	76396-212	76396-308	76396-408
2.1 x 150mm	-	76396-240	76396-336	76396-436
2.1 x 250mm	-	-	76396-340	76396-450
3.0 x 50mm	76396-156	76396-200	76396-296	76396-396
3.0 x 100mm	76396-160	76396-214	76396-310	76396-410
3.0 x 150mm	-	76396-242	76396-338	76396-438
3.0 x 250mm	-	-	76396-342	76396-452
4.6 x 50mm	-	76396-202	76396-298	76396-398
4.6 x 100mm	-	76396-216	76396-312	76396-412
4.6 x 150mm	-	76396-244	76382-658	76396-440
4.6 x 250mm	-	-	76396-344	76396-454

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 or visit **vwr.com/ace**.

[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 or visit **vwr.com/ace**

ACE SUPERC18

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.

Recommended Applications

- 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

ACE C18-AMIDE

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.

Recommended Applications

- 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

ACE CN-ES

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.

Recommended Applications

- 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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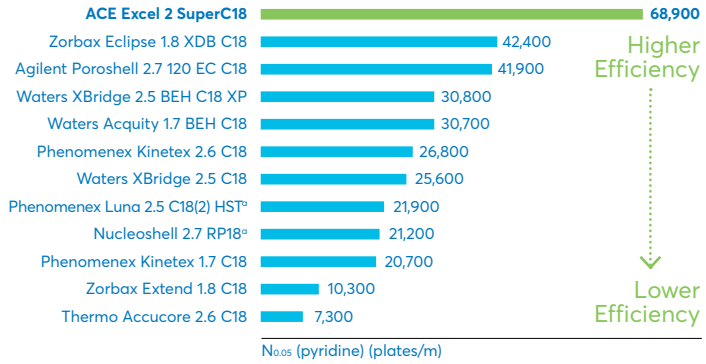
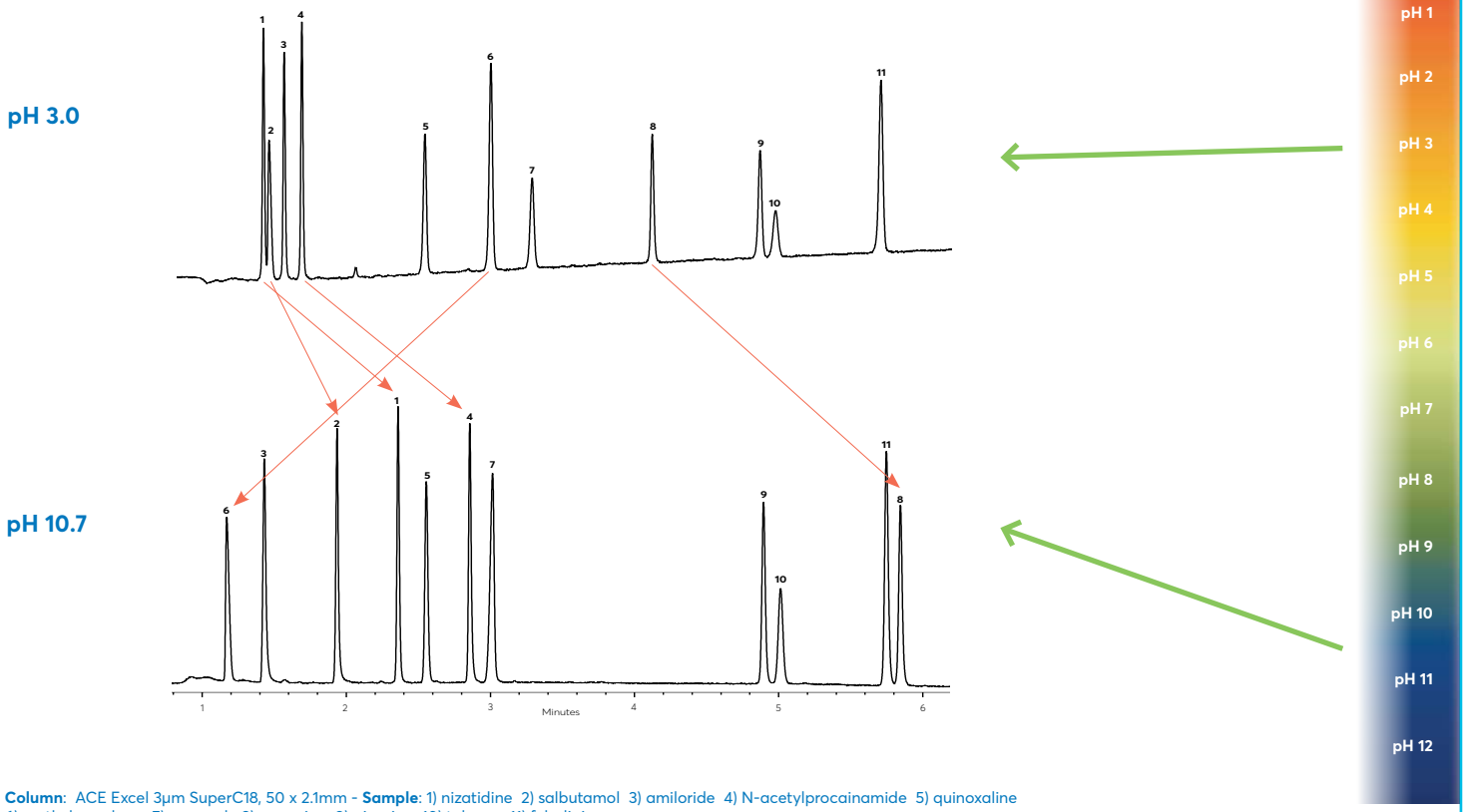


Column Dimensions: 50 x 2.1mm ([a] 50 x 2.0mm) - **Sample:** 1) uracil 2) pyridine 3) phenol - **Mobile Phase:** 30:70 v/v MeOH/10mM NH_4OAc in H_2O (pH 5.8) - **Flow Rate:** 0.20ml/min - **Temperature:** 22°C - **Detection:** 254nm
Comparative data may not be representative of all applications.
Please see back page for acknowledgement of trademark. Application # 1513

USE ACE SUPERC18 TO INVESTIGATE PH EFFECTS

EXPLOIT SELECTIVITY BY ADJUSTING PH



Column: ACE Excel 3µm SuperC18, 50 x 2.1mm - **Sample:** 1) nizatidine 2) salbutamol 3) amiloride 4) N-acetylprocainamide 5) quinoxaline 6) methyl paraben 7) p-cresol 8) reserpine 9) piperine 10) toluene 11) felodipine
Temperature: 40°C - **Flow Rate:** 0.42ml/min - **Wavelength:** 254nm - **Gradient:** 3 - 100% B in 7 minutes
Acidic Mobile Phase: A: 10mM ammonium formate in H_2O (pH 3.0) B: 10mM ammonium formate (pH 3.0) in 90:10 v/v MeCN/H_2O
Basic Mobile Phase: A: 0.1% NH_3 (= 18mM) in H_2O (pH 10.7) B: 0.1% NH_3 (= 18mM), pH 10.7 in 90:10 v/v MeCN/H_2O Application # 1510

ACE C18-AMIDE PROVIDES ENHANCED POLAR SELECTIVITY

ADVANTAGES OF MULTI-MODE INTERACTIONS FOR HPLC SEPARATIONS

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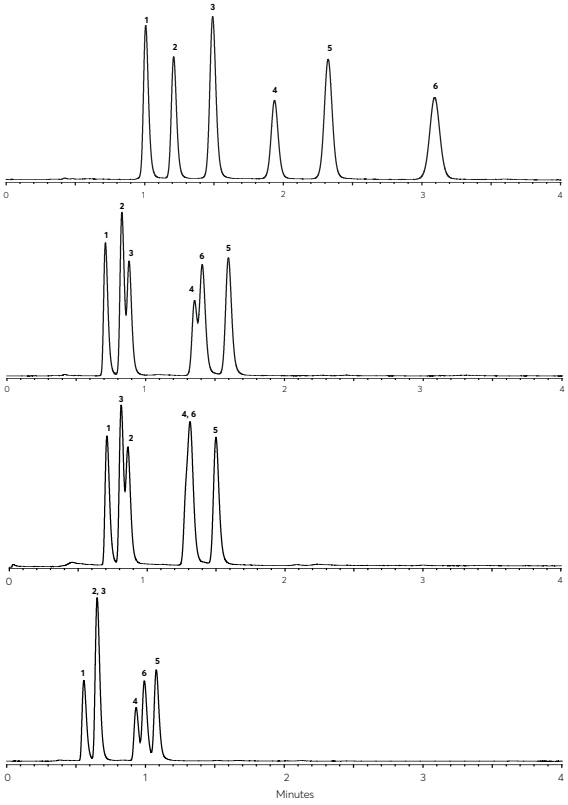
ACE 2 C18-AMIDE
(ultra-inert fully porous silica)

ACE 2 C18
(ultra-inert fully porous silica)

WATERS ACQUITY 1.7 BEH C18
(hybrid particle)

PHENOMENEX KINETEX 1.7 C18
(superficially porous particle)



ACE C18-Amide provides increased retention and improved separation due to enhanced polar selectivity

Leading C18 column brands provide similar selectivity

Column Dimensions: 50 x 2.1mm
Sample: 1) resorcinol 2) catechol 3) 2-methyl resorcinol 4) 4-methyl catechol 5) 3-methyl catechol 6) 4-nitro catechol
Mobile Phase: 25:75 MeOH/25mM H_3PO_4 in H_2O
Flow Rate: 0.30ml/min - **Temperature**: 30°C - **Wavelength**: 214nm
Comparative data may not be representative of all applications.
Please see back page for acknowledgement of trademarks.

Application # 1602

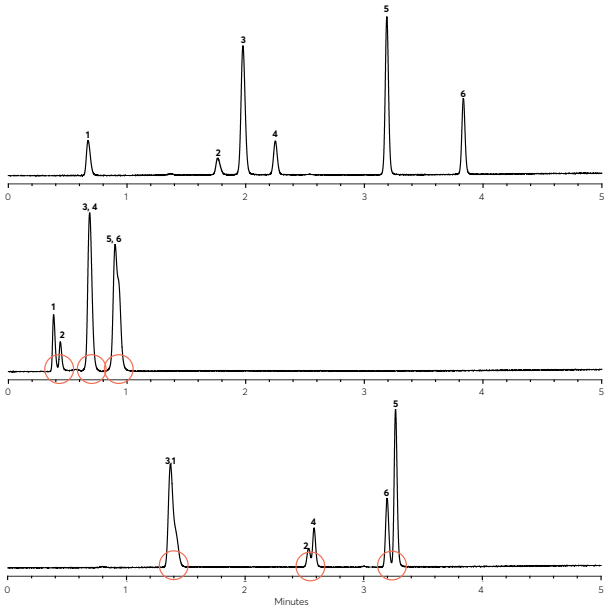
ACE CN-ES PROVIDES ALTERNATIVE SELECTIVITY

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

ACE 3 CN-ES

ACE 3 CN
(example short chain CN phase)

ACE 3 C18



ACE CN-ES phase provides complete separation due to hydrophobic AND dipole-dipole interactions

CN phase shows insufficient resolution (greatly reduced hydrophobic interaction)

C18 phase provides insufficient resolution (no dipole-dipole interaction)

Sample: 1) metronidazole 2) benzyl alcohol 3) hydrochlorothiazide 4) vanillin 5) methyl paraben 6) 1,2-dinitrobenzene
Mobile Phase: A = 0.1% formic acid in H_2O
B = 0.1% formic acid in 90:10 MeOH/H_2O
Gradient: 3 - 100% B in 5 minutes - **Column Dimensions**: 50 x 2.1mm
Flow Rate: 0.60ml/min - **Temperature**: 40°C - **Wavelength**: 254nm

Application # 1701

ACE EXTENDED METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

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

	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)			
Column Dimensions	1.7µm	2µm	3µm	5µm
2.1 x 50mm	76396-804	76396-848	76396-942	76397-044
2.1 x 100mm	76396-808	76396-862	76396-956	76397-058
2.1 x 150mm	-	76396-888	76396-984	76397-086
2.1 x 250mm	-	-	76396-990	76397-100
3.0 x 50mm	76396-806	76396-850	76396-944	76397-046
3.0 x 100mm	76396-810	76396-864	76396-958	76397-060
3.0 x 150mm	-	76396-890	76396-986	76397-088
3.0 x 250mm	-	-	76396-992	76397-102
4.6 x 50mm	-	76396-852	76396-946	76397-048
4.6 x 100mm	-	76396-866	76396-960	76397-062
4.6 x 150mm	-	76396-892	76396-988	76397-090
4.6 x 250mm	-	-	76396-994	76397-104

Guard columns are available for all of these phases

ACE UltraCore method development kit

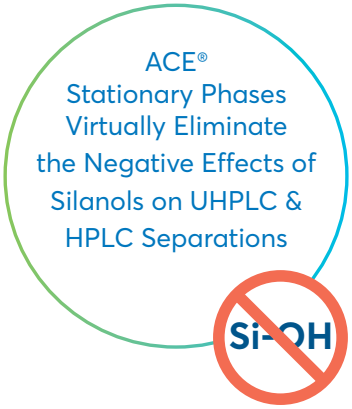
- Contains ACE UltraCore SuperC18 and SuperPhenylHexyl phases
- Use to exploit selectivity changes at low, intermediate and high pH
- Available in a wide range of dimensions
- Ultra inert core-shell particles and Encapsulated Bonding Technology (EBT™) provide excellent peak shape

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 or visit **vwr.com/ace**.

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

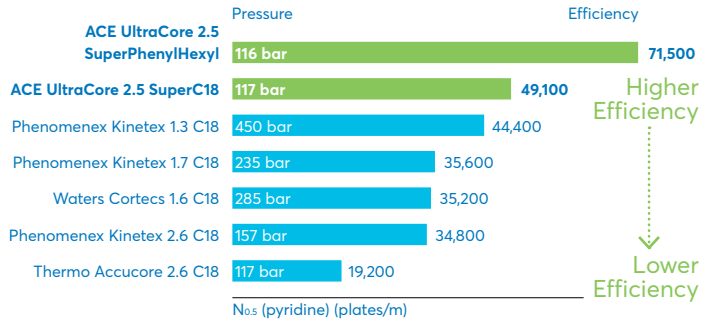


ACE ULTRACORE COLUMNS ARE HIGHLY INERT

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

PEAK EFFICIENCY COMPARISON

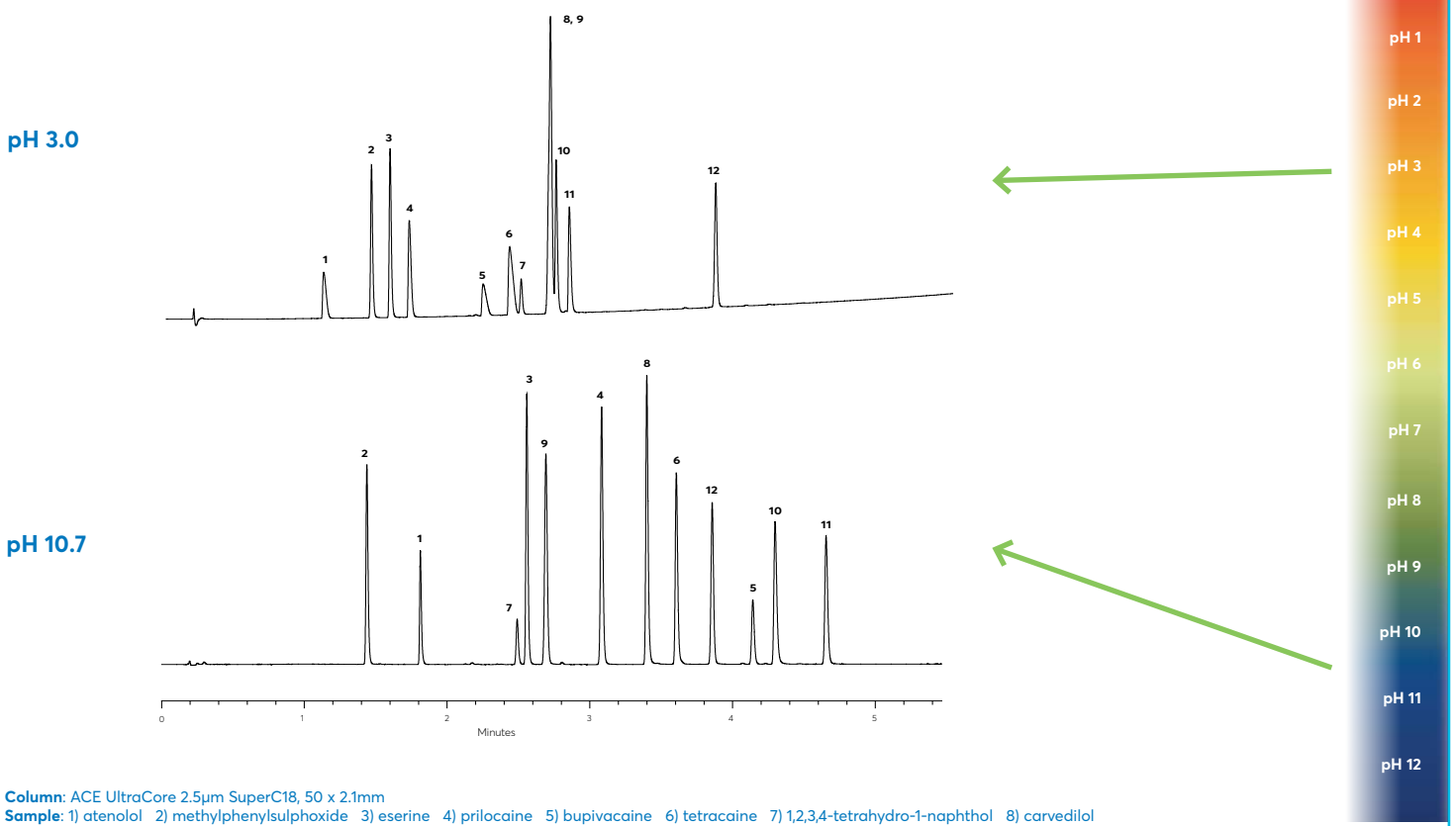
Reproduced with kind permission of The Open University, UK



Column Dimensions: 50 x 2.1mm - **Sample**: 1) uracil 2) pyridine 3) phenol
Mobile Phase: 30:70 v/v MeOH/10mM NH_4OAc in H_20 (pH 5.8) - **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 # 1802

USE ACE SUPERC18 TO INVESTIGATE PH EFFECTS

EXPLOIT SELECTIVITY BY ADJUSTING PH

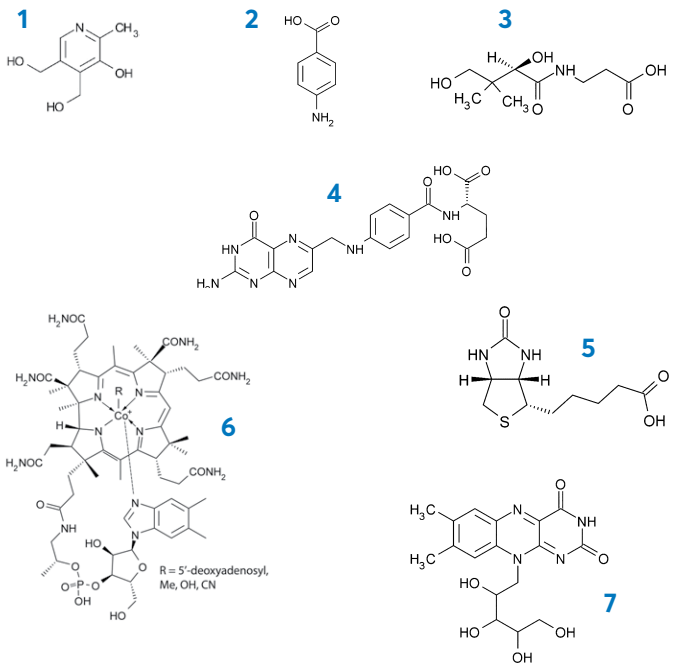


Column: ACE UltraCore 2.5µm SuperC18, 50 x 2.1mm
Sample: 1) atenolol 2) methylphenylsulphoxide 3) eserine 4) prilocaine 5) bupivacaine 6) tetracaine 7) 1,2,3,4-tetrahydro-1-naphthol 8) carvedilol 9) nitrobenzene 10) methdilazine 11) amitriptyline 12) valerophenone
Temperature: 40°C - **Flow Rate**: 0.60ml/min - **Wavelength**: 254nm - **Gradient**: 3 – 100% B in 5 minutes
Acidic Mobile Phase: A: 20mM ammonium formate in H_2O (pH 3.0) B: 20mM ammonium formate (pH 3.0) in 90:10 (v/v) MeCN/H_2O
Basic Mobile Phase: A: 0.1% NH_3 (= 18mM) in H_2O (pH 10.7) B: 0.1% NH_3 (= 18mM), pH 10.7 in 90:10 (v/v) MeCN/H_2O

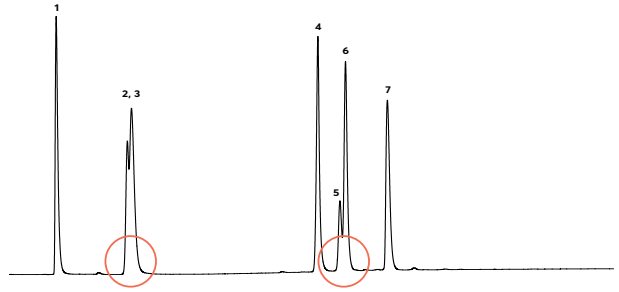
Application # 1801

INTRODUCING SELECTIVITY CHANGES USING ACE ULTRACORE METHOD DEVELOPMENT KITS

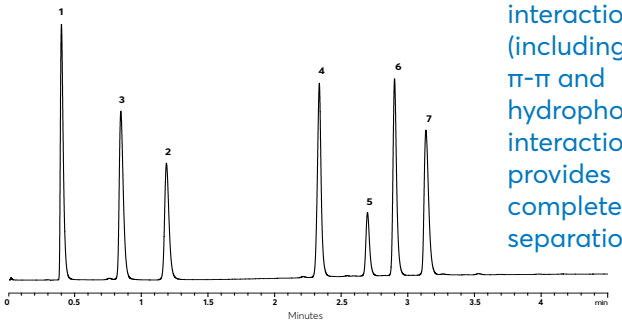
VITAMIN SEPARATION



ACE ULTRACORE SUPERC18



ACE ULTRACORE SUPERPHENYLHEXYL



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

Column: 50 x 2.1mm, 2.5µm
Sample: 1) pyridoxine 2) p-aminobenzoic acid 3) pantothenic 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:H_2O 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

ACE ULTRACORE METHOD DEVELOPMENT UHPLC/HPLC COLUMN KITS

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

	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)	
Column Dimensions	**2.5µm**	**5µm**
2.1 x 50mm	76397-390	76397-486
2.1 x 100mm	76397-404	76397-500
2.1 x 150mm	76397-432	76397-528
2.1 x 250mm	-	76397-542
3.0 x 50mm	76397-392	76397-488
3.0 x 100mm	76397-406	76397-502
3.0 x 150mm	76397-434	76397-530
3.0 x 250mm	-	76397-544
4.6 x 50mm	76397-394	76397-490
4.6 x 100mm	76397-408	76397-504
4.6 x 150mm	76397-436	76397-532
4.6 x 250mm	-	76397-546

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

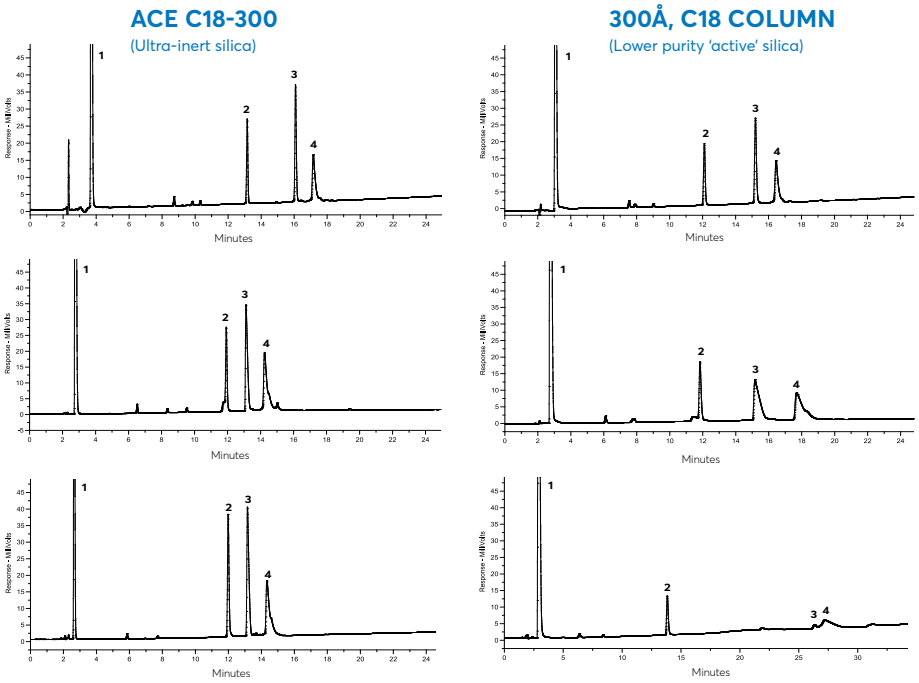
[a] For optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at higher pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC/MS Buffer Selection"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

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

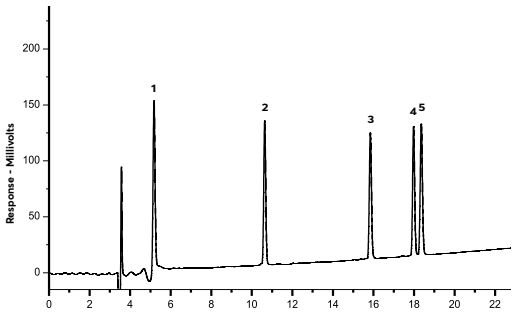
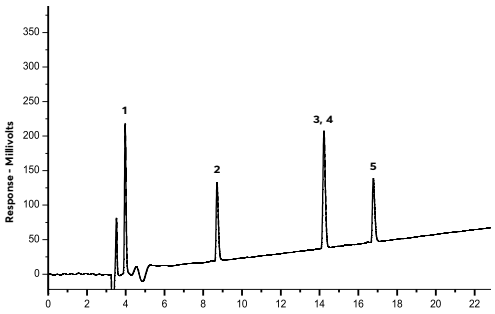
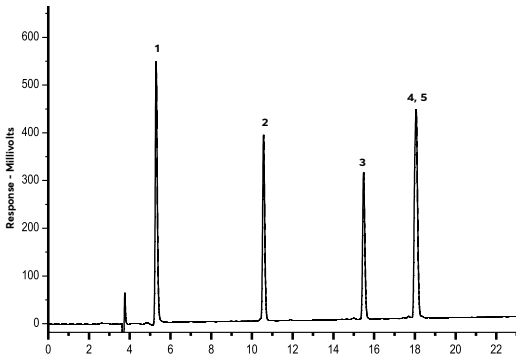
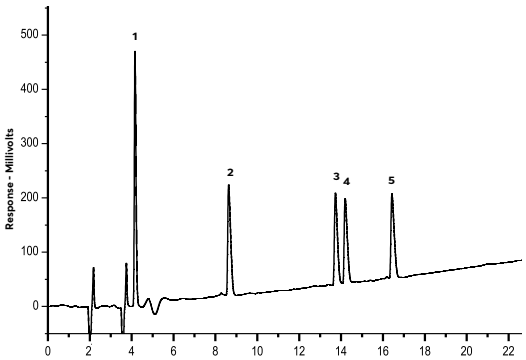
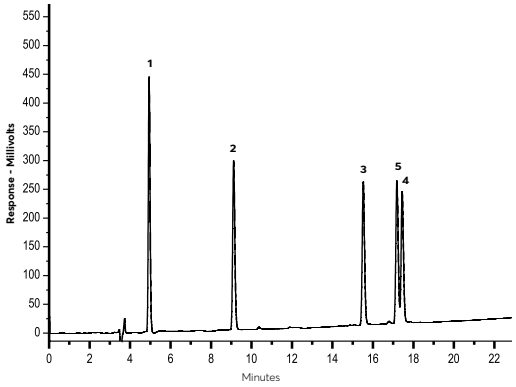
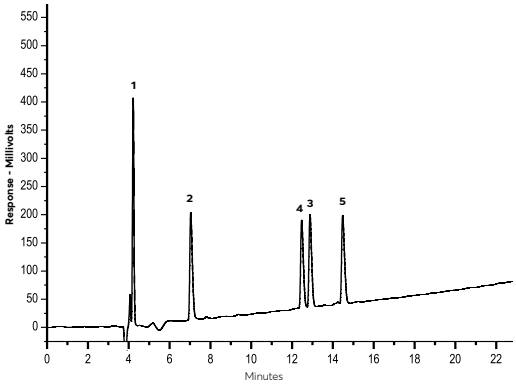


Columns: 250 x 4.6mm, 5µm
Sample: 1) Gly-Tyr 2) Oxytocin 3) Angiotensin II 4) Neurotensin
Mobile Phase: A = TFA in H_2O (% as specified above)
B = TFA in MeCN (% as specified above)
Gradient: 10 - 55% B in 37.5 minutes
Flow Rate: 1.50ml/min - **Temperature**: 22°C
Wavelength: 200nm

Application # 2001

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 H_2O or 0.1% Formic Acid in H_2O (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	76396-504	76396-600
2.1 x 100mm	76396-518	76396-614
2.1 x 150mm	76396-546	76396-642
2.1 x 250mm	-	76396-656
3.0 x 50mm	76396-506	76396-602
3.0 x 100mm	76396-520	76396-616
3.0 x 150mm	76396-548	76396-644
3.0 x 250mm	-	76396-658
4.6 x 50mm	76396-508	76396-604
4.6 x 100mm	76396-522	76396-618
4.6 x 150mm	76396-550	76396-646
4.6 x 250mm	-	76396-660

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**

* 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)

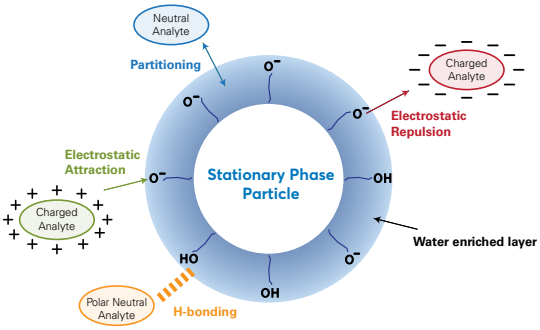


Figure 1. Schematic of interactions between different types of polar analytes and a stationary phase in HILIC mode

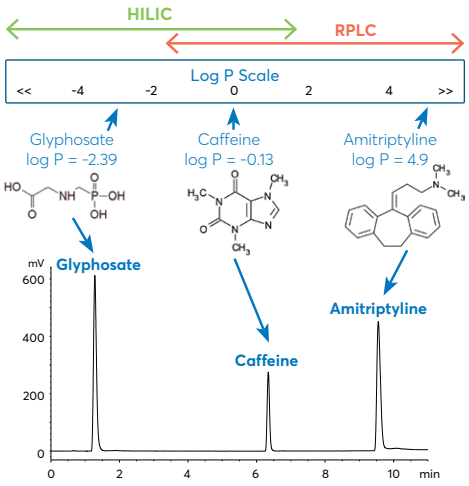


Figure 2A. Analyte suitability for HILIC from Log P

Figure 2B. Analyte suitability for HILIC from gradient RPLC

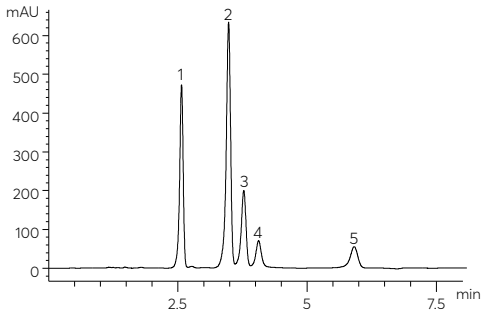
Column: ACE Excel C18, 100 x 3.0mm, 2µm - **Mobile Phase**: A = 10mM ammonium formate, pH 3.0 (aq) B = 10mM ammonium formate, pH 3.0 in 90:10 v/v MeCN:H_2O - **Gradient**: 5-100% B in 10 minutes - **Detection**: ELSD - **Flow Rate**: 0.4mL min - **Temperature**: 30 °C - **Injection**: 10µL **Analysed 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

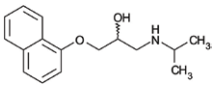
ACE HILIC COLUMNS – 3 ALTERNATIVE SELECTIVITIES

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

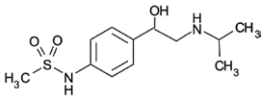
ACE HILIC-A - An **acidic character** phase

β-blockers

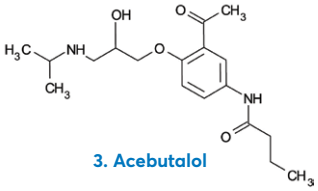




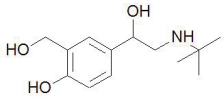
1. Propranolol



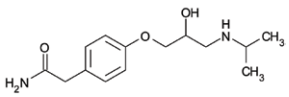
2. Sotalol



3. Acebutalol



4. Salbutamol



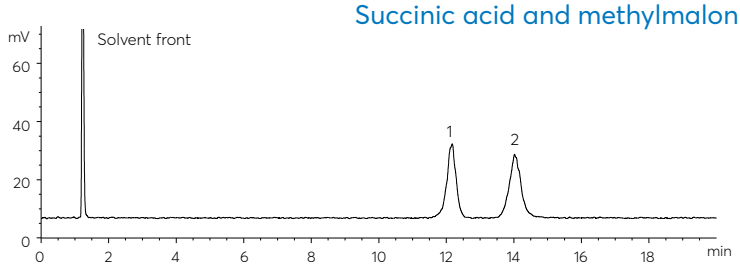
5. Atenolol

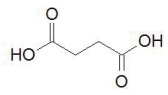
Column: ACE HILIC-A,150 x 4.6mm, 5µm - **Mobile Phase**: 12mM ammonium formate pH 4.7 in MeCN/H_2O (88:12 v/v)
Flow Rate: 1.5mL/min Injection: 2µL - **Temperature**: 25 °C Detection: 230nm

Application # 4530

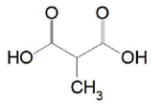
ACE HILIC-B - A **basic character** phase

Succinic acid and methylmalonic acid





1. Succinic acid



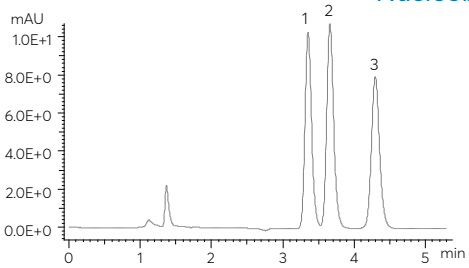
2. Methylmalonic acid

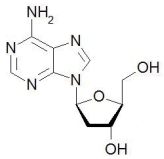
Column: ACE HILIC-B, 150 x 4.6mm, 5µm - **Mobile Phase**: 10mM ammonium formate pH 3.0 in MeCN/H_2O (90:10 v/v)
Flow Rate: 1.5mL/min - **Temperature**: 25 °C Injection: 5µL - **Detection**: ELSD (Evaporator temp: 30 °C, Nebuliser temp: 30 °C, Gas speed: 1 SLM)

Application # 4520

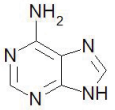
ACE HILIC-N - A polar **neutral character** phase

Nucleobases and nucleosides

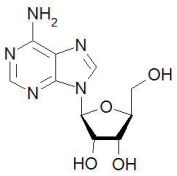




1. Deoxyadenosine



2. Adenine



3. Adenosine

Column: ACE HILIC-N, 150 x 4.6mm, 5µm - **Mobile Phase**: 10mM ammonium formate pH 4.7 in MeCN/H_2O (90:10 v/v)
Flow Rate: 1.5mL/min - **Temperature**: 25 °C - **Injection**: 5µL - **Detection**: 254nm

Application # 4550

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.

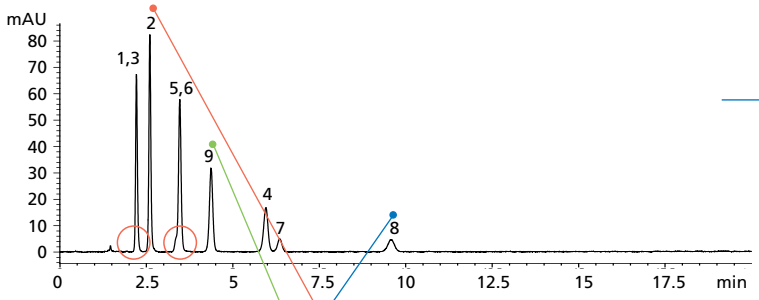
CONCLUSIONS

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

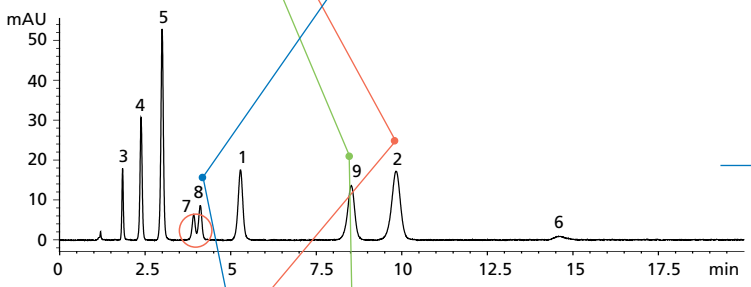
ADVANTAGES OF USING ACE HILIC METHOD DEVELOPMENT KITS

0.1% TFA

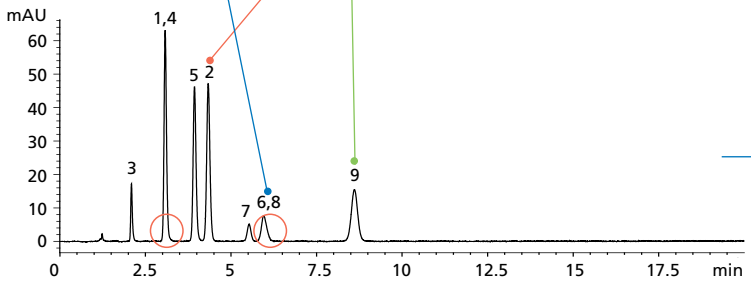
ACE HILIC-A



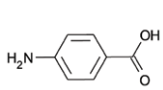
ACE HILIC-B



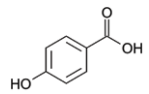
ACE HILIC-N



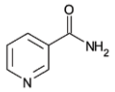
Different Elution Orders and Retention Times Observed



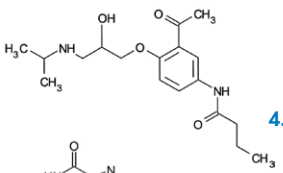
1. p-Aminobenzoic acid



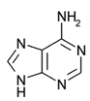
2. 4-Hydroxybenzoic acid



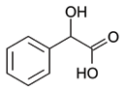
3. Nicotinamide



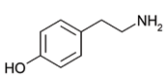
4. Acebutolol



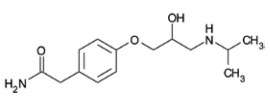
5. Adenine



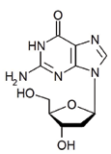
6. Mandelic acid



7. Tyramine



8. Atenolol



9. 2-Deoxyguanosine

Columns: All 150 x 4.6mm, 5µm (Part numbers: ACE HILIC-A: HILA-5-1546U, ACE HILIC-B: HILB-5-1546U, ACE HILIC-N: HILN-5-1546U)
Mobile Phase: 10mM ammonium formate pH 4.7 in MeCN/H_2O (90:10 v/v) - **Flow Rate**: 1.5mL/min - **Temperature**: 25 °C - **Detection**: 254nm
Sample: 1) p-Aminobenzoic acid 2) 4-Hydroxybenzoic acid 3) Nicotinamide 4) Acebutolol 5) Adenine 6) Mandelic acid 7) Tyramine 8) Atenolol 9) 2-Deoxyguanosine

Application # 4580

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. Available at **vwr.com/ace**
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

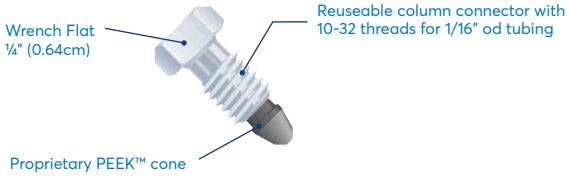
Column Dimensions	(UHPLC/HPLC hardware format with 1000bar/15000psi pressure limit)		
	1.7µm	3µm	5µm
2.1 x 50mm	76397-134	76397-178	76397-280
2.1 x 100mm	76397-138	76397-192	76397-294
2.1 x 150mm	-	76397-220	76397-322
2.1 x 250mm	-	76397-226	76397-336
3.0 x 50mm	76397-136	76397-180	76397-282
3.0 x 100mm	76397-140	76397-194	76397-296
3.0 x 150mm	-	76397-222	76397-324
3.0 x 250mm	-	76397-228	76397-338
4.6 x 50mm	-	76397-182	76397-284
4.6 x 100mm	-	76397-196	76397-298
4.6 x 150mm	-	76397-224	76397-326
4.6 x 250mm	-	76397-230	76397-340

UHPLC and HPLC column accessories

UHPLC COLUMN CONNECTORS

- Pressure rating >1700 bar (>25000 psi)
- Compatible with all UHPLC systems[1]
- 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 reuseable 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.

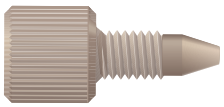
At the outlet end of the UHPLC column (where pressure demands are lower but a correct connection remains important), ACE Fingertight HPLC Column Connectors (p/n ACE-CC10, 10 pack, see below) may alternatively be used.

[1] **Note**: 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.

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