



Column Technology and Products



The world leader in serving science

Agenda

- Overview of LC column portfolio
- General-purpose LC columns
- Mixed-mode columns
- Application-specific columns
- Current challenges

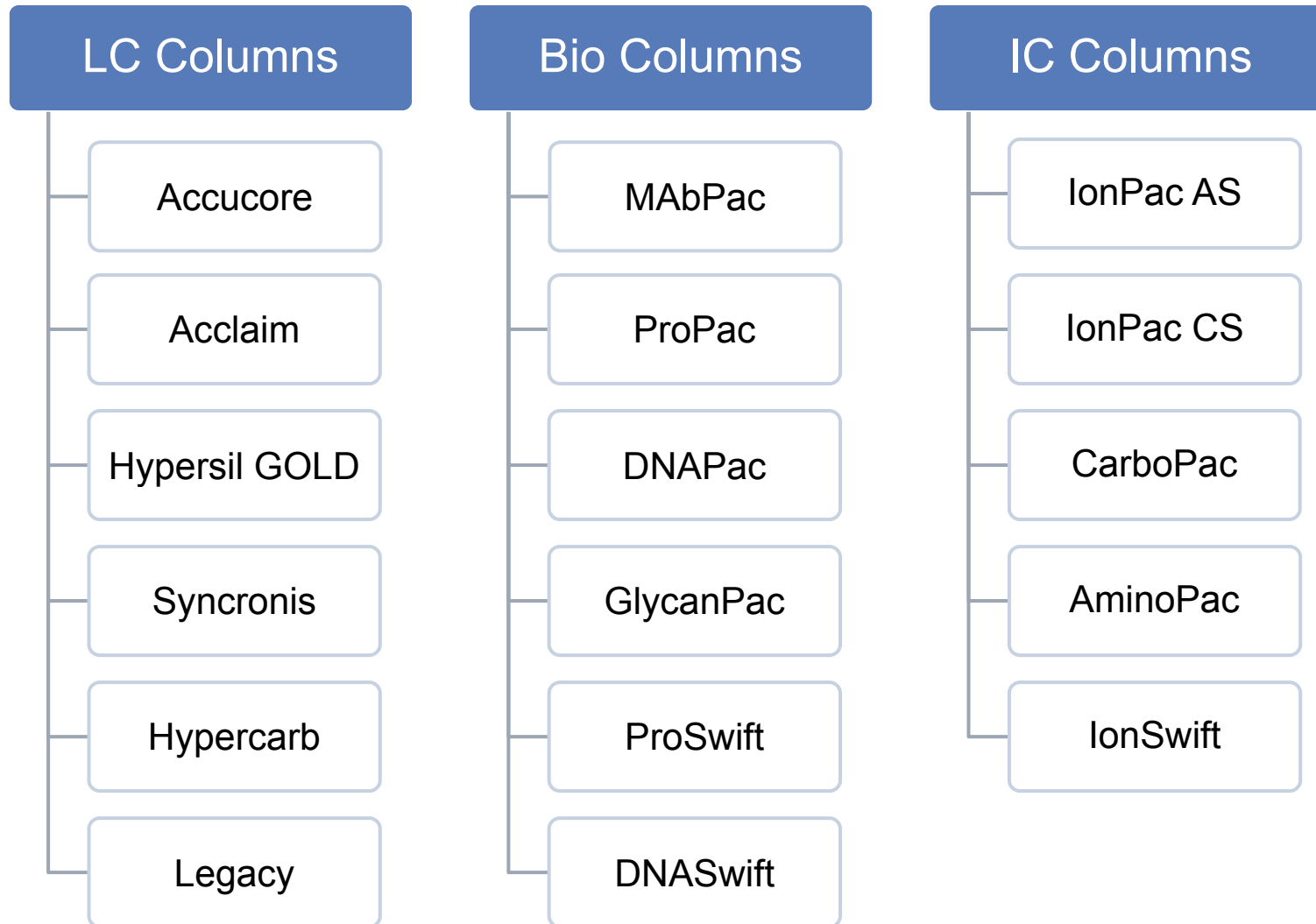


● Column Portfolio Overview

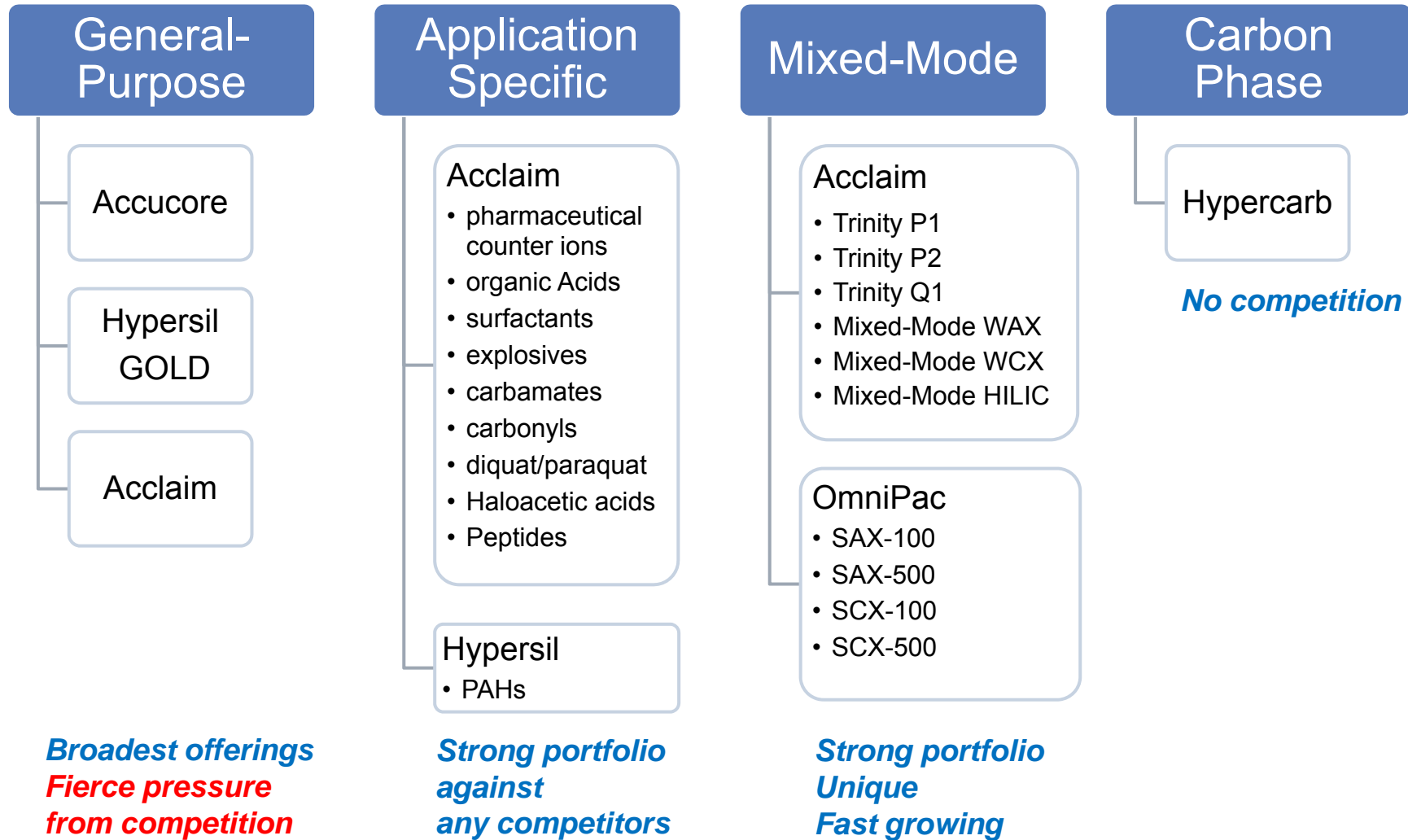


The world leader in serving science

Thermo Scientific Columns



Another Way to Look at Our HPLC Column Portfolio



The Focus of the Training

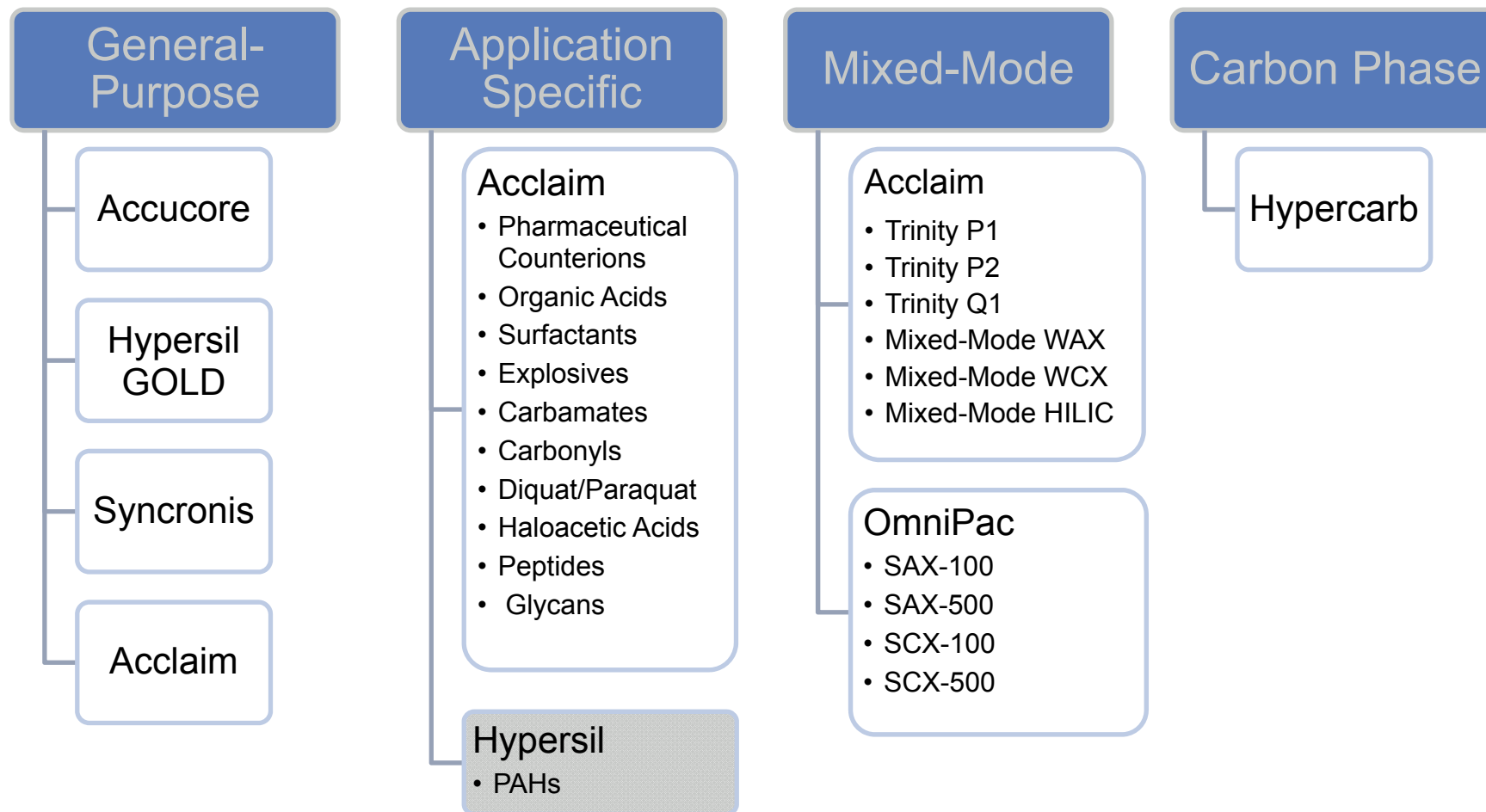
- Accucore family (general-purpose LC columns)
- Acclaim family (general-purpose/mixed-mode /application-specific columns)
- Latest bio-separation columns



● **General-Purpose Columns**

● **The world leader in serving science**

LC column portfolio



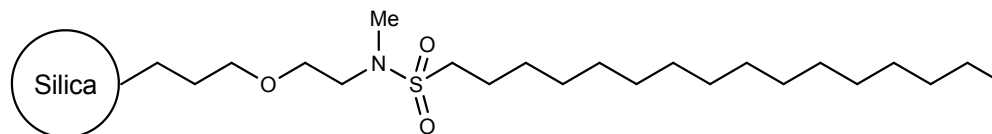
Reversed-Phase Columns

- Retention mechanism – hydrophobic interaction
- Separation mode
 - RP
 - on suppression/RP – needs highly aqueous mobile phase
 - Ion pairing/RP – MS compatibility, complex mobile phase, reproducibility
- Commonly used column types
 - C18
 - C8
 - Polar-embedded RP
 - C30

RP Columns

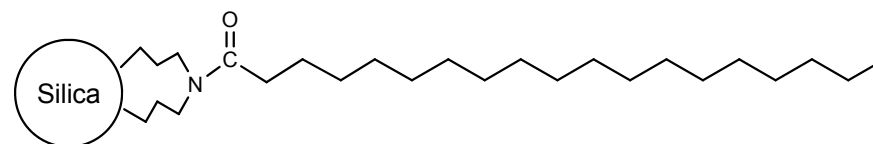
- Polar-embedded RP

- *Acclaim PolarAdvantage*



- *Acclaim PolarAdevanatge II C18 (PA2)*

- *Accucore Polar Premium*

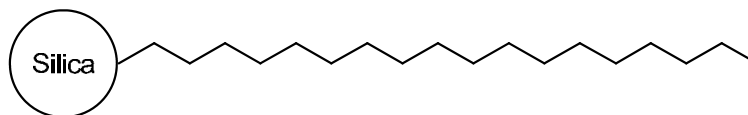


- Polar end-capped C18

- *Accucore AQ*

- *Acclaim C18*

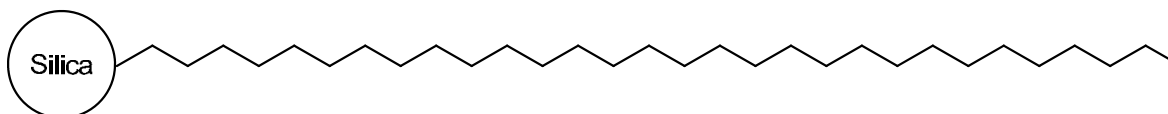
- *Hypersil GOLD*



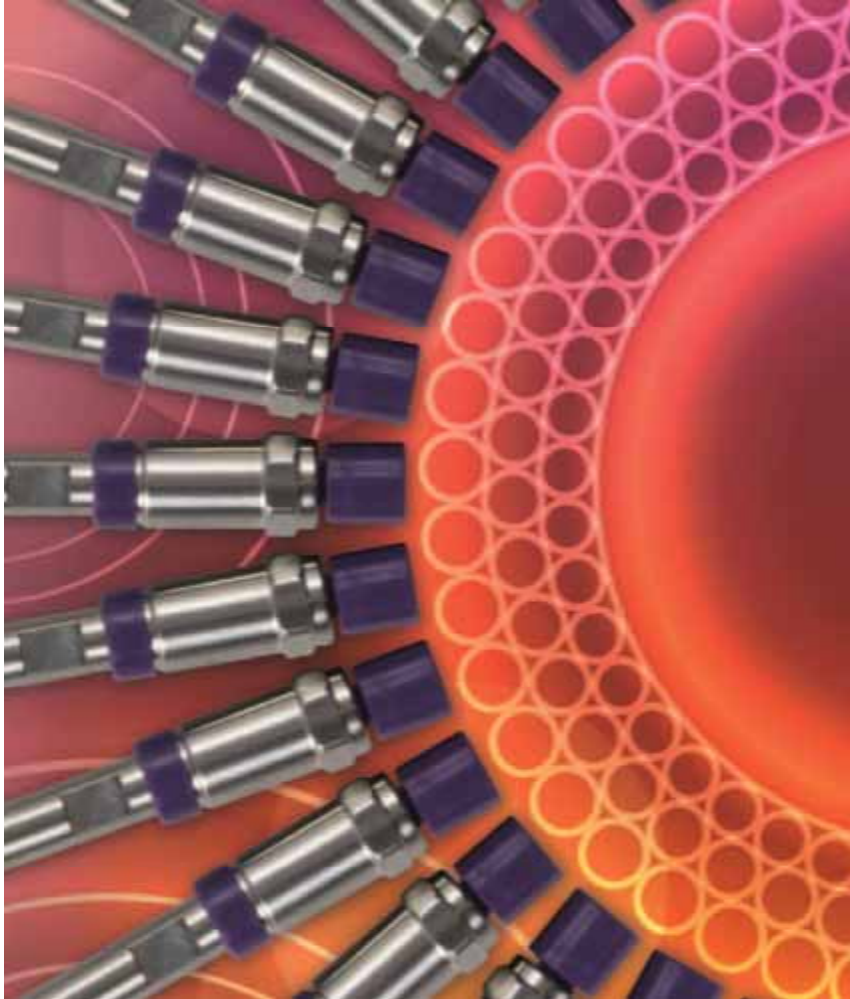
- C30

- *Acclaim C30*

- *Accucore C30*

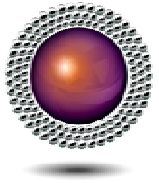
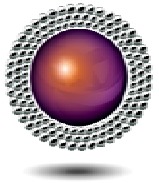
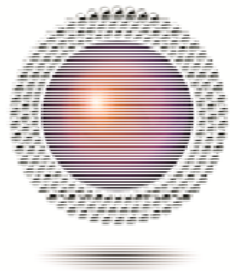


Accucore HPLC Columns



- Ultimate Core Performance – Speed and Selectivity Combined
- Solid core particles for fast separations with superb resolution
- Wide range of phases to meet all your separation needs
- Exceptionally rugged and easy to use columns

Accucore HPLC Column Family

Accucore HPLC Columns		<ul style="list-style-type: none">• Rugged and reproducible solid core particles• Fast separations with superb resolution• Low backpressures	<ul style="list-style-type: none">• 11 phases• 2.6 μm• HPLC & UHPLC• Optimized method
Accucore Columns for Biomolecules		<ul style="list-style-type: none">• 150 Å pore size for biomolecule separations• Superb resolution at low backpressures• Exceptionally rugged columns	<ul style="list-style-type: none">• 3 phases• 2.6 μm• HPLC & UHPLC• Optimized method
Accucore XL HPLC Columns		<ul style="list-style-type: none">• 4 μm solid core particles for all users• Same system, same method, better results• Robust, fast and easy to use	<ul style="list-style-type: none">• 2 phases• 4 μm• HPLC• Same method

5 New Accucore Phases for Selectivity

Accucore Polar Premium

Rugged amide embedded C18 phase that offers complementary selectivity to conventional C18

Accucore Phenyl-X

Unique reversed-phase shape selectivity with high aromatic selectivity

Accucore C30

High shape selectivity for hydrophobic, long chain, structurally related isomers

Accucore Urea-HILIC

Unique HILIC selectivity and low ion exchange activity

Accucore 150-Amide-HILIC

Designed for the separation of hydrophilic small molecules and bio-molecules in HILIC mode. An excellent choice for glycan separations

Why Core Enhanced Technology Works

$$H = A + \frac{B}{u} + Cu$$

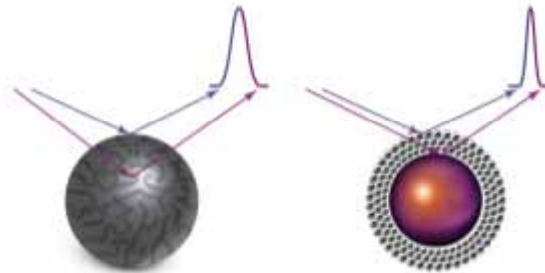
H Height equivalent to theoretical plate (column length/efficiency)

A Eddy diffusion

B Longitudinal diffusion

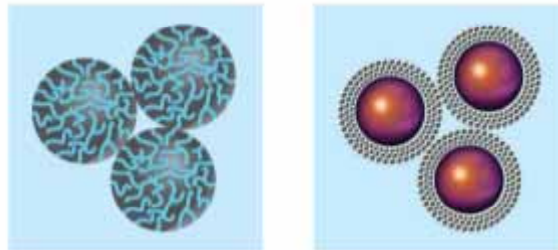
C Resistance to Mass Transfer

u Mobile phase linear velocity



Resistance to mass transfer is minimized by the solid core design of Core Enhanced Technology particles as the diffusional path of analytes is limited by the depth of the outer porous layer. The effect of this minimization is most noticeable for larger molecules.

The solid core design of the particles reduces the amount of mobile phase in the column resulting in a reduced void volume and less longitudinal diffusion. This effect can be seen in the lower t_r values obtained with Accucore HPLC columns compared to columns of the same dimensions packed with fully porous materials.



The tight control of Core Enhanced Technology particle diameter and automated packing process used for Accucore HPLC columns result in a tight, highly uniform packed bed that minimizes eddy diffusion.

- A term
 - Less **Eddy Diffusion**





- B term
 - Less **Longitudinal Diffusion**

- C term
 - Less **Resistance to Mass Transfer** (larger molecules)




- **More Efficiency / higher Peak Capacity**

Phase Characterization



T1: Hydrophobic Interactions

			Parameter	Term
	HR	Hydrophobic Retention	Retention of compounds based on their hydrophobicity	k'
	HS	Hydrophobic Selectivity	Separation of compounds that have similar structure, but differ slightly in hydrophobicity	α
	SS	Steric Selectivity	Separation of compounds that have similar structure, but differ in shape	α
	HBC	Hydrogen Bonding Capacity	Separation related to degree of end capping	α

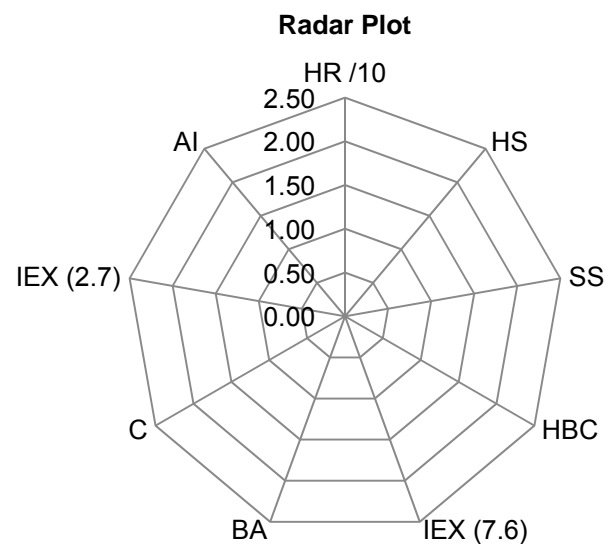
T2: Secondary Interactions Under Neutral pH

			Parameter	Term
	BA	Base Activity	Peak shape for basic analytes resulting from total silanol activity (all dissociated at pH 7.6)	t_r
	C	Chelation	Peak shapes for chelating analytes resulting from silica metal content	t_r
	IEX(7.6)	Ion Exchange Capacity (pH 7.6)	Separation between basic and neutral compounds resulting from total silanol activity (all dissociated at pH 7.6)	α

T3: Secondary Interactions Under Acidic pH

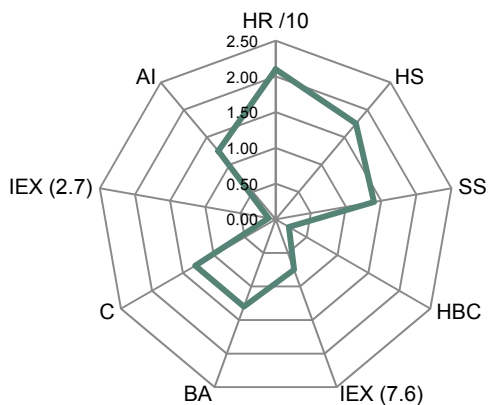
			Parameter	Term
	AI	Acid Interaction	Interactions resulting in poor peak shape for acidic analytes	t_r
	IEX(2.7)	Ion Exchange Capacity (pH 2.7)	Separation between basic and neutral compounds resulting from acidic silanol activity	α

HR	K' Pentylbenzene
HS	α Butylbenzene / Pentylbenzene
SS	α Triphenylene / o-Terphenyl
HBC	α Caffeine / Phenol
BA	tf Amitripyline
C	tf Quinizarin
IEX(7.6)	α Benzylamine / Phenol
AI	tf 4-Chlorocinnamic acid
IEX(2.7)	α Benzylamine / Phenol

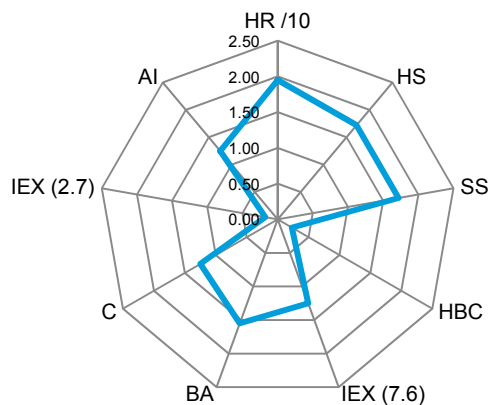


C18 Phase Characterisation

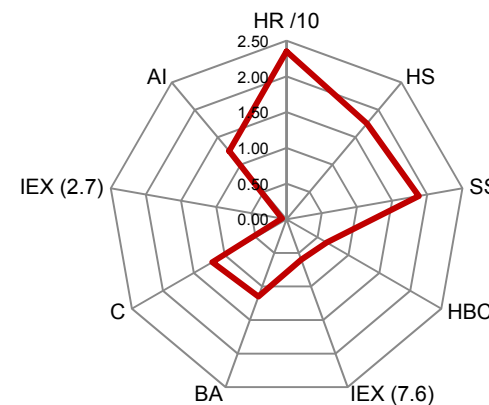
Accucore C18



Accucore aQ



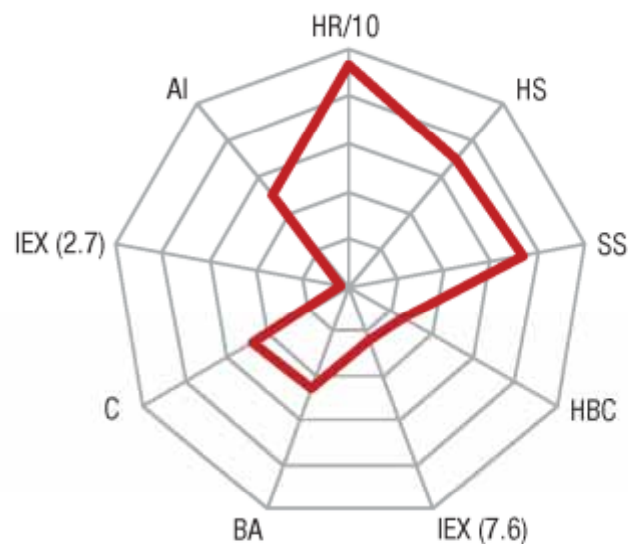
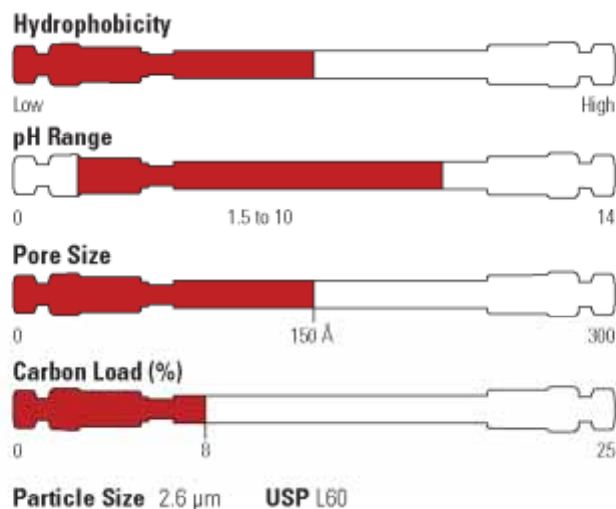
Accucore Polar Premium



Polar Premium shows different selectivity to both C18 and aQ

Polar Premium shows high hydrophobic retention

Accucore Polar Premium

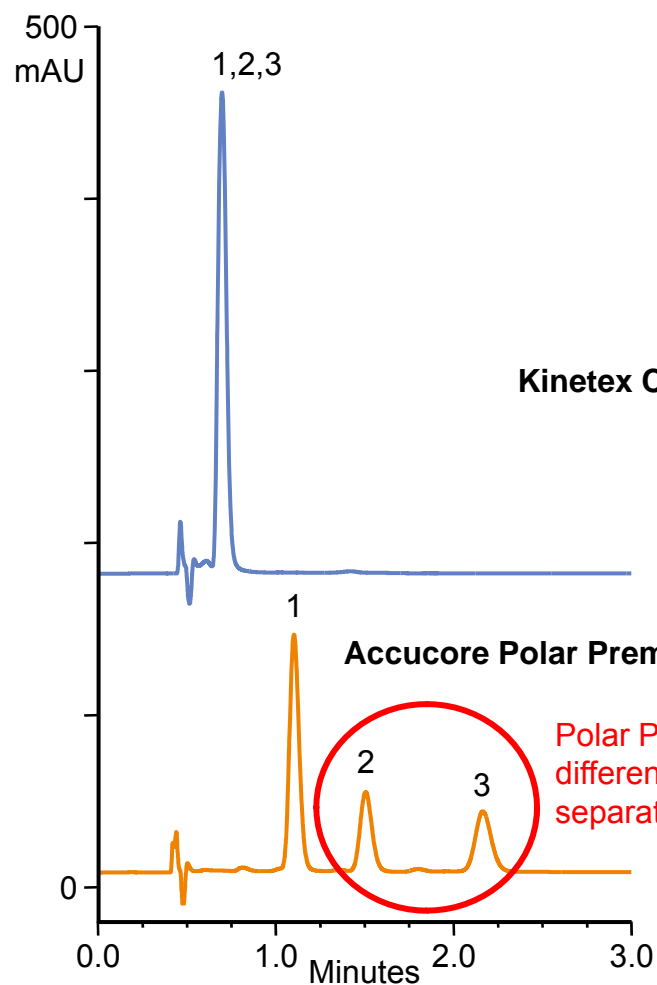


- Rugged amide-embedded C18 phase
- Selectivity complementary to conventional C18 phases
- Stable over a wide pH range and compatible with 100% aqueous mobile phase

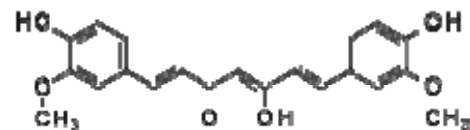
Accucore Polar Premium is an exceptionally rugged polar embedded reverse phase material that offers high efficiency, wider operating pH range and unique selectivity complementary to standard C18 phases.

The specially designed bonded phase is stable from pH 1.5 to 10.5 and will not undergo phase collapse in 100% aqueous mobile phase.

Curcuminoids (Turmeric)



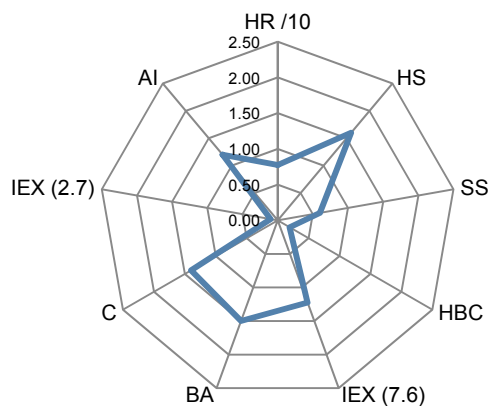
Columns:	Accucore Polar Premium Kinetex C18
Dimensions:	2.6 μ m, 100 x 3.0 mm
Mobile Phases:	A: Methanol B: 10 mM Phosphoric acid
Isocratic:	80% A, 20% B
Flow:	0.800 mL/min
Temperature:	40 $^{\circ}$ C
Injection:	6 μ L
Detector:	UV 428 nm
Sample:	Extract 100 mg ground turmeric with 20 mL of hot 70% ethanol; centrifuge, filter and dilute 10x in methanol.
Peaks:	1. Curcumin 2. Desmethoxycurcumin 3. Bis-desmethoxycurcumin



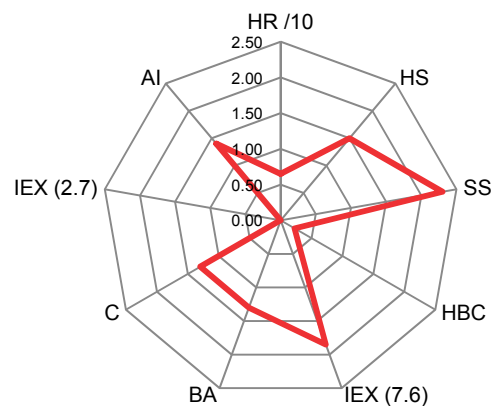
Kinetex® is a registered trademark of Phenomenex Inc., USA

Shape Selective Phase Characterisation

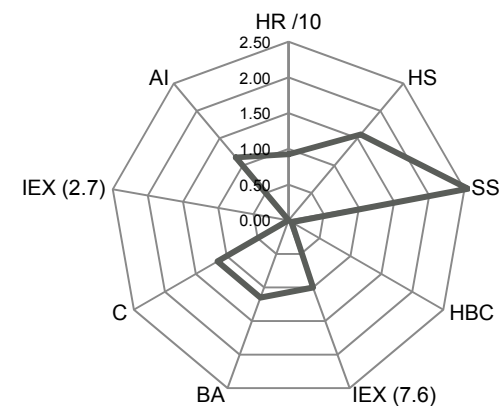
Accucore Phenyl-Hexyl



Accucore PFP



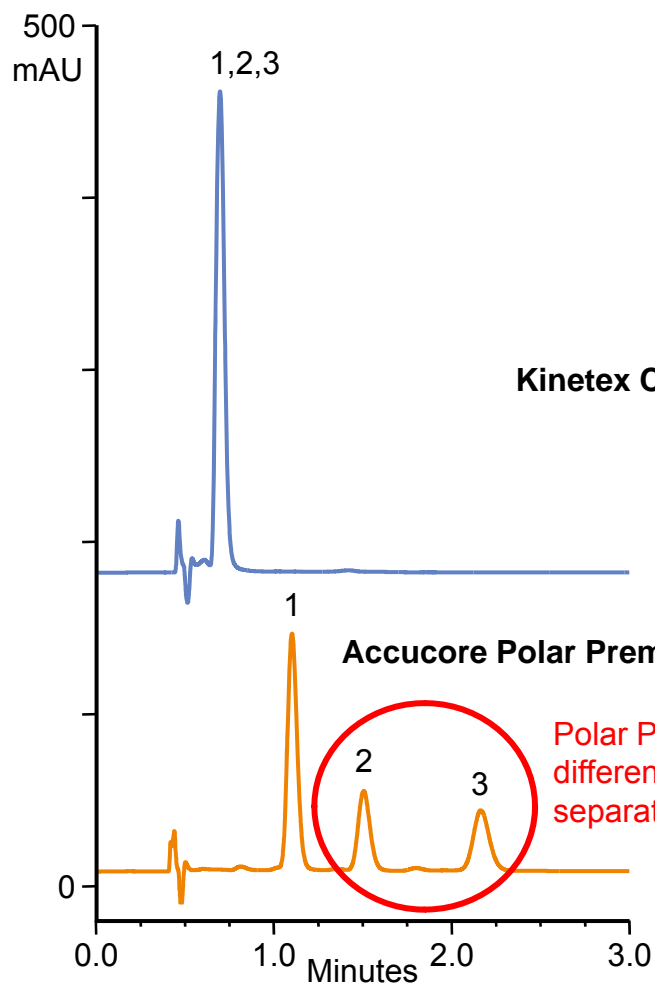
Accucore Phenyl-X



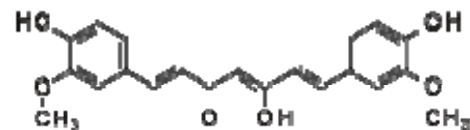
Phenyl-X shows highest shape (aromatic) selectivity

Phenyl-Hexyl shows opposite shape selectivity

Curcuminoids (Turmeric)

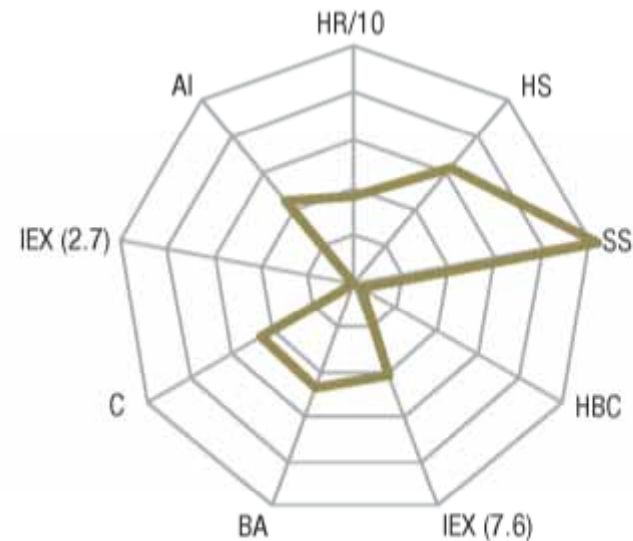
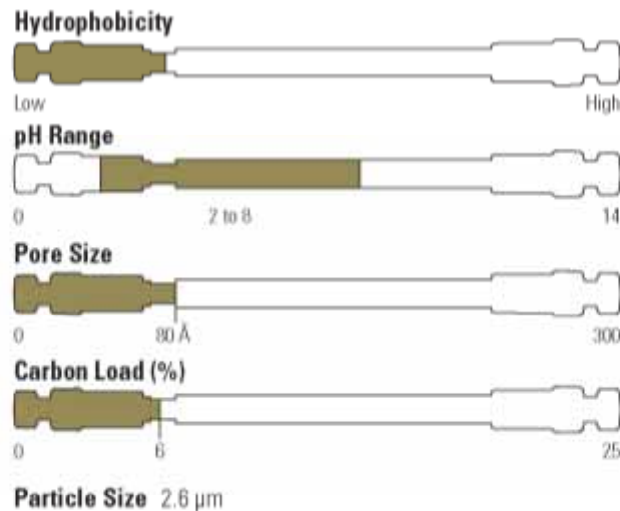


Columns:	Accucore Polar Premium Kinetex C18
Dimensions:	2.6 μ m, 100 x 3.0 mm
Mobile Phases:	A: Methanol B: 10 mM Phosphoric acid
Isocratic:	80% A, 20% B
Flow:	0.800 mL/min
Temperature:	40 $^{\circ}$ C
Injection:	6 μ L
Detector:	UV 428 nm
Sample:	Extract 100 mg ground turmeric with 20 mL of hot 70% ethanol; centrifuge, filter and dilute 10x in methanol.
Peaks:	1. Curcumin 2. Desmethoxycurcumin 3. Bis-desmethoxycurcumin



Kinetex® is a registered trademark of Phenomenex Inc., USA

Accucore Phenyl-X



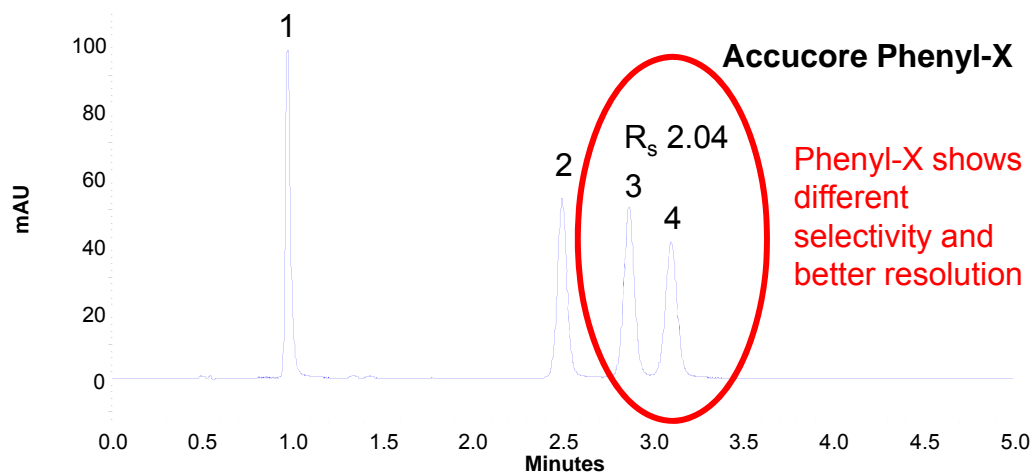
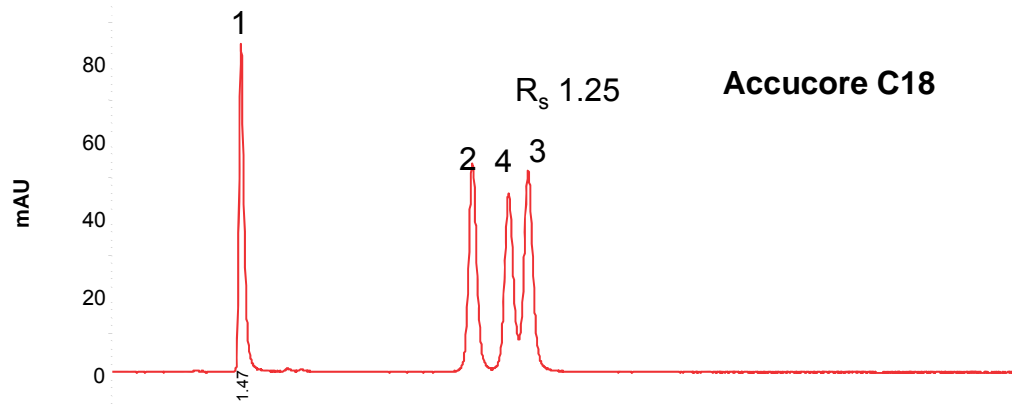
- Unique reversed-phase shape selectivity
- Enhanced selectivity for aromatic compounds
- Compatible with highly aqueous mobile phases
- Robust, high-efficiency, low column bleed

The proprietary Accucore Phenyl-X alkyl aromatic bonded phase provides a unique selectivity when compared to other reversed phase materials such as C18 or Phenyl.

Phenyl-X exhibits particularly high aromatic selectivity.

The advanced design of the bonded phase makes it compatible with highly aqueous mobile phases and robust, demonstrating very low bleed.

Estrogens



Columns: Accucore Phenyl-X
Accucore C18

Dimensions: 2.6 μ m, 100 x 2.1 mm

Mobile Phases: A: Acetonitrile
B: Methanol
C: Water

Isocratic: 15% A, 40% B, 45% C

Flow: 400 μ L/min

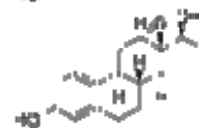
Temperature: 40 °C

Injection: 1 μ L

Detector: UV 220 nm



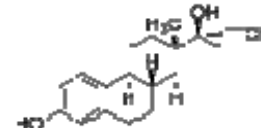
1. Estriol (E3)



2. Estradiol (E2)



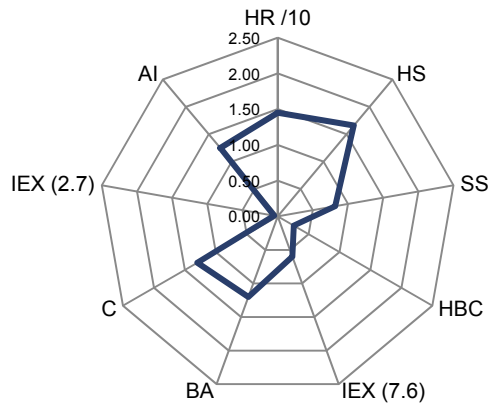
3. Estrone (E1)



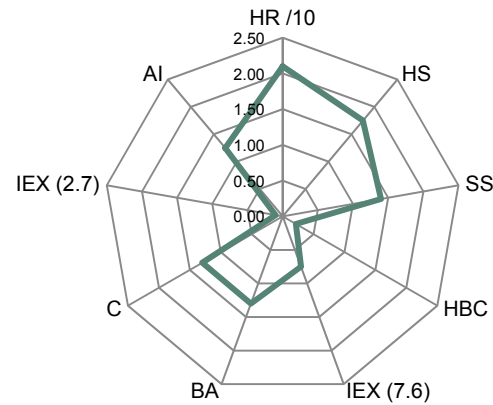
4. Ethynylestradiol

Carbon Chain Phase Characterisation

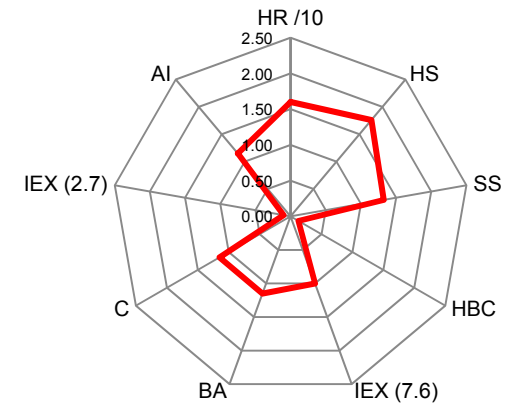
Accucore C8



Accucore C18



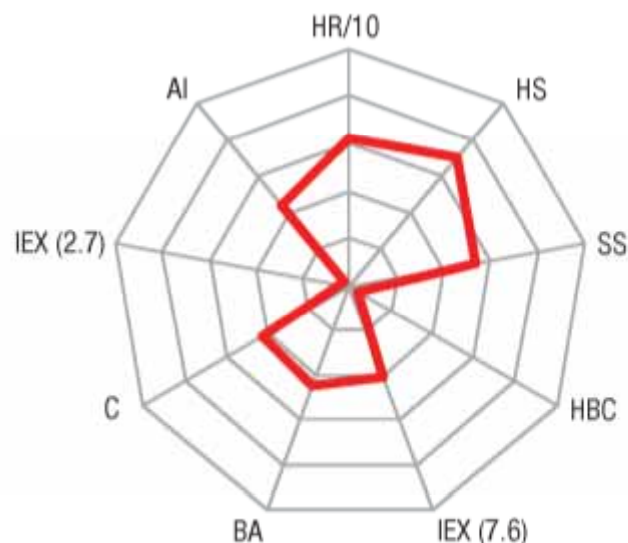
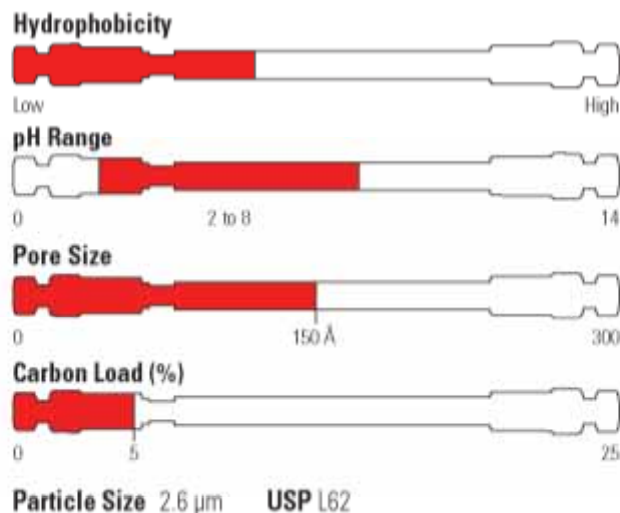
Accucore C30



C30 shows lower hydrophobic retention than C18

Shape selectivity appears similar to C18

Accucore C30



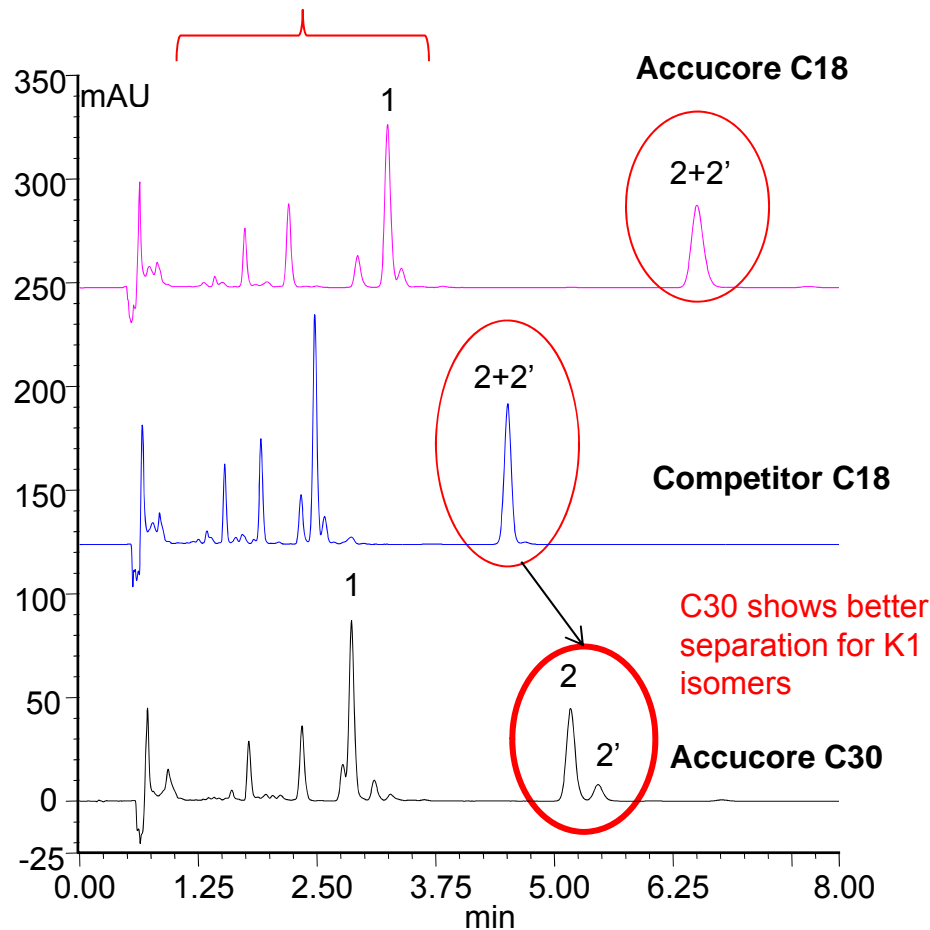
- Ideal for separation of hydrophobic, long alkyl chain compounds
- High shape selectivity for structurally related isomers
- Excellent aqueous-compatibility

Accucore C30 offers high shape selectivity for hydrophobic, long chain, structurally related isomers, for example carotenoids and steroids. This is a different form of shape selectivity from that measured in the SS phase characterisation test.

It is also an excellent alternative to normal-phase columns for lipid analysis. The optimized bonding density of the long alkyl chains facilitated by a wider pore diameter particle result in a phase that is stable even in highly aqueous mobile phases.

Vitamin K Isomers

Different selectivity for K2 isomers



Columns: Accucore C30
 Accucore C18
 Competitor C18

Dimensions: 2.6 μ m , 100 x 3.0 mm

Mobile Phases: Methanol:buffer, 98:2
 Buffer = 2 mM ammonium acetate

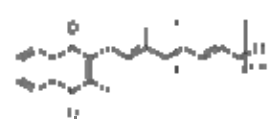
Flow: 650 μ L/min

Temperature: 20 $^{\circ}$ C

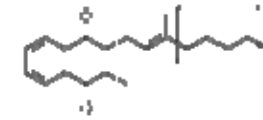
Injection: 5 μ L

Detector: UV 250 nm

Peaks: 1. Vitamin K2, 50 μ g/mL
 2. Vitamin K1, 50 μ g/mL
 Other peaks formed by UV irradiation



Vitamin K2



Vitamin K1

Kinetex $^{\circ}$ is a registered trademark of Phenomenex Inc., USA

Summary on Accucore Columns

- Represent state-of-the-art technology and offer high-resolution and high-throughput
- Cover a broad range of selectivity
- Compete against major competitors effectively

Acclaim HPLC Columns

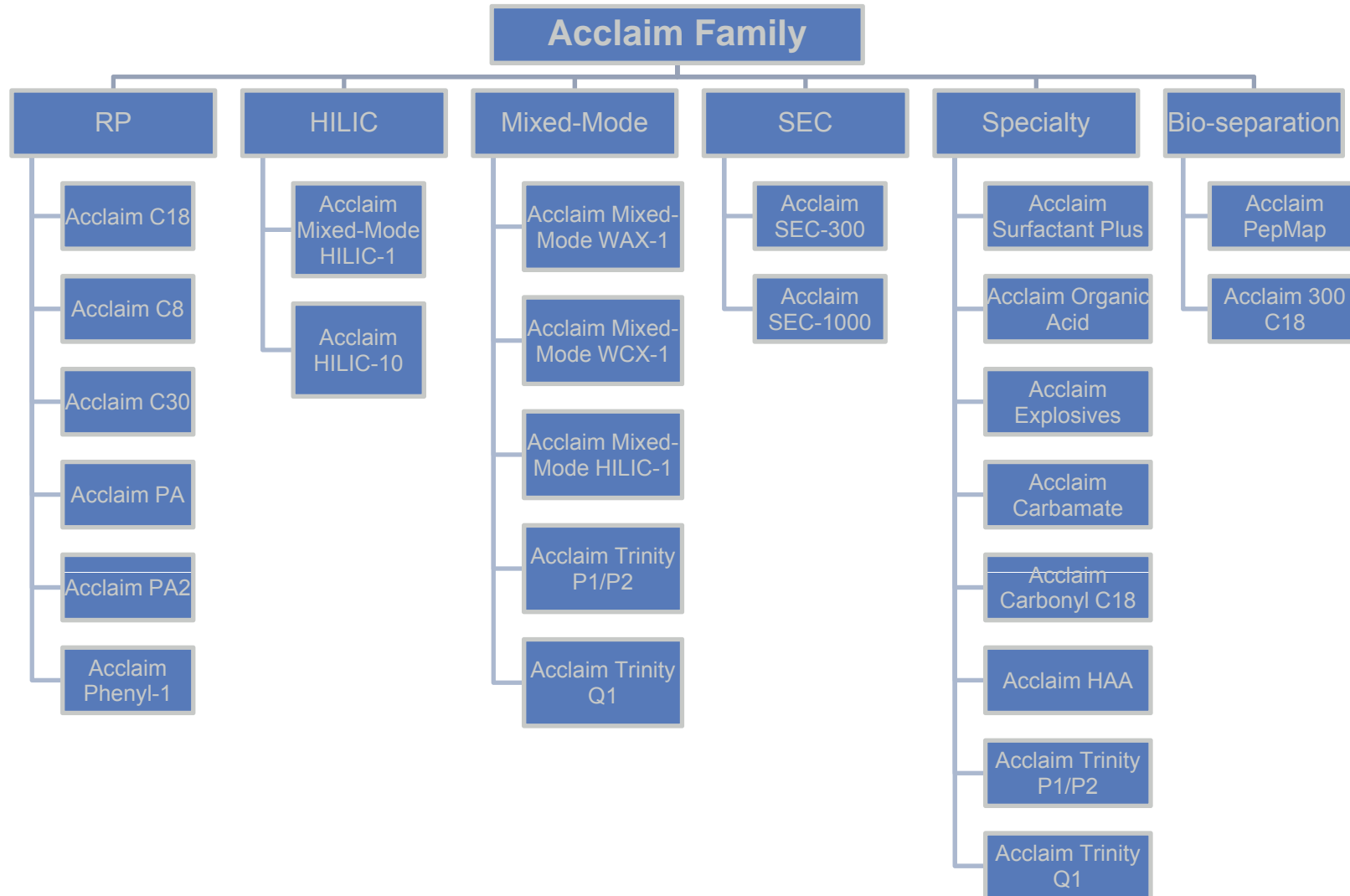


- Thermo Scientific™ Acclaim™ Columns provide UHPLC and HPLC users in markets such as pharmaceuticals, food safety, consumer products and environmental testing with high quality, easy to use methods through innovative column chemistry

Construction of Acclaim Bonded Phases

- High-purity silica substrate
 - High-purity: 99.99% (type B)
 - Pore size: 120 Å, 200 Å or 300 Å
 - Surface area: 300 m²/g, 200 m²/g or 100 m²/g
 - Particle size: 2.2, 3.0 or 4.5-µm
- Advanced surface technology
 - Many Acclaim columns use innovative silane ligands - designed and synthesized in-house, to ensure unique selectivity
- Quality control tested
 - Packing material tested
 - Each individual column tested

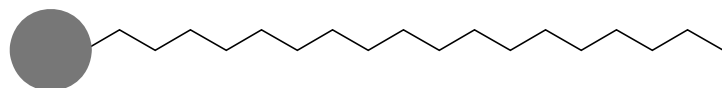
Acclaim Column Family



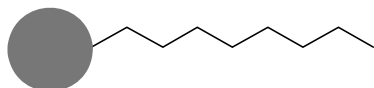
Acclaim Reversed-Phase Columns

Name	USP	Chemistry	Bonding	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	pH Range	Aqueous Compatibility
120 C18	L1	C18	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	90%
300 C18	L1	C18	Monomeric End-capped	3.0	300	100	2.0 – 8.0	95%
120 C8	L7	C8	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	90%
C30	L62	C30	Monomeric End-capped	3.0, 4.5	200	200	2.0 – 8.0	100%
PolarAdvantage (PA)	L60	Sulfonamide-embedded	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	100%
PolarAdvantage II (PA2)	L60	Amide-embedded	Multi-point bonding End-capped	2.2, 3.0, 4.5	120	300	1.5 – 10.5	100%
Phenyl-1	L11	Alkyl aromatic	Multi-point bonding End-capped	3.0, 4.5	120	300	2.0 – 8.0	100%

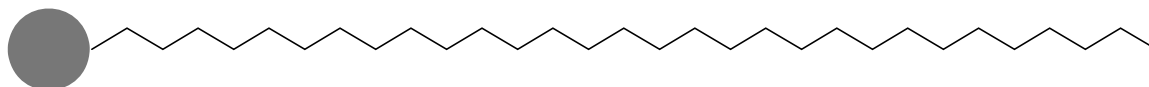
Acclaim Reversed-Phase Column Chemistries



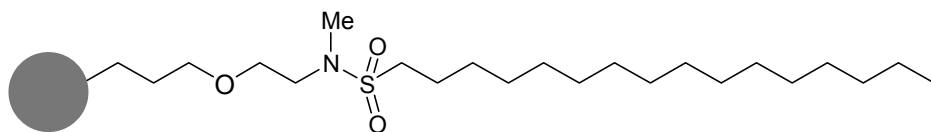
C18



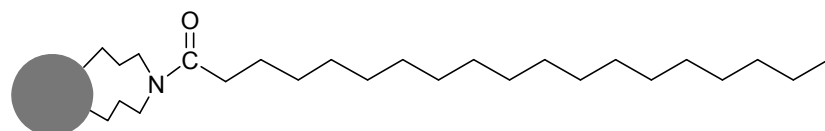
C8



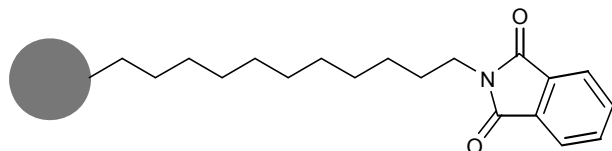
C30



PolarAdvantage (PA)

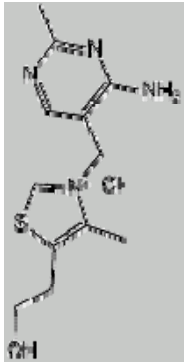


PolarAdvantage II (PA2)

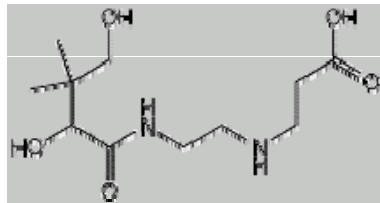


Phenyl-1

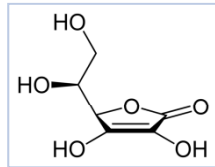
Water-Soluble Vitamins



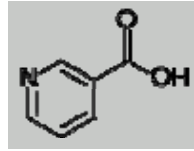
Thiamine (B1)



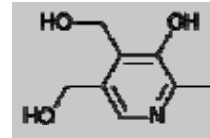
Pantothenic Acid



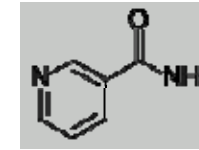
Ascorbic Acid (C)



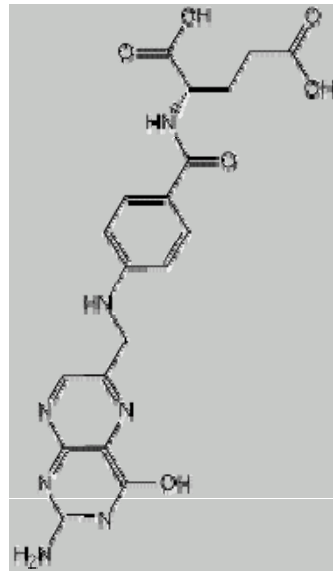
Niacin (B3)



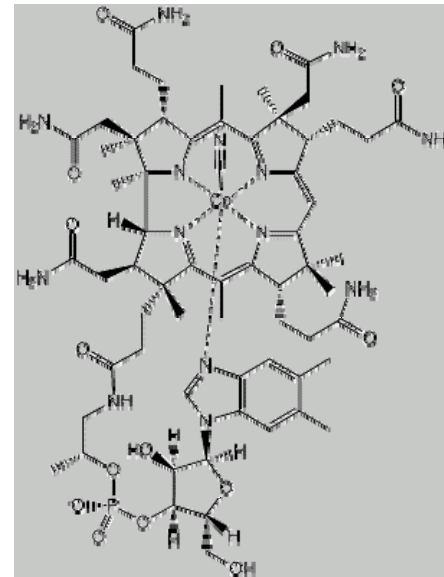
Pyridoxine (B6)



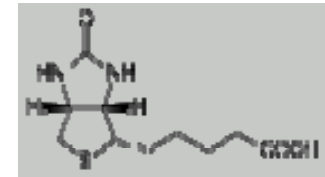
Niacinamide (B3)



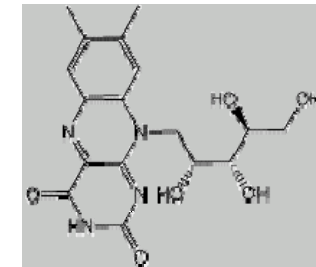
Folic Acid (Bc)



Cyanocobalamin (B12)

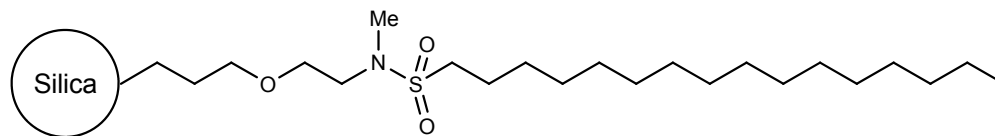
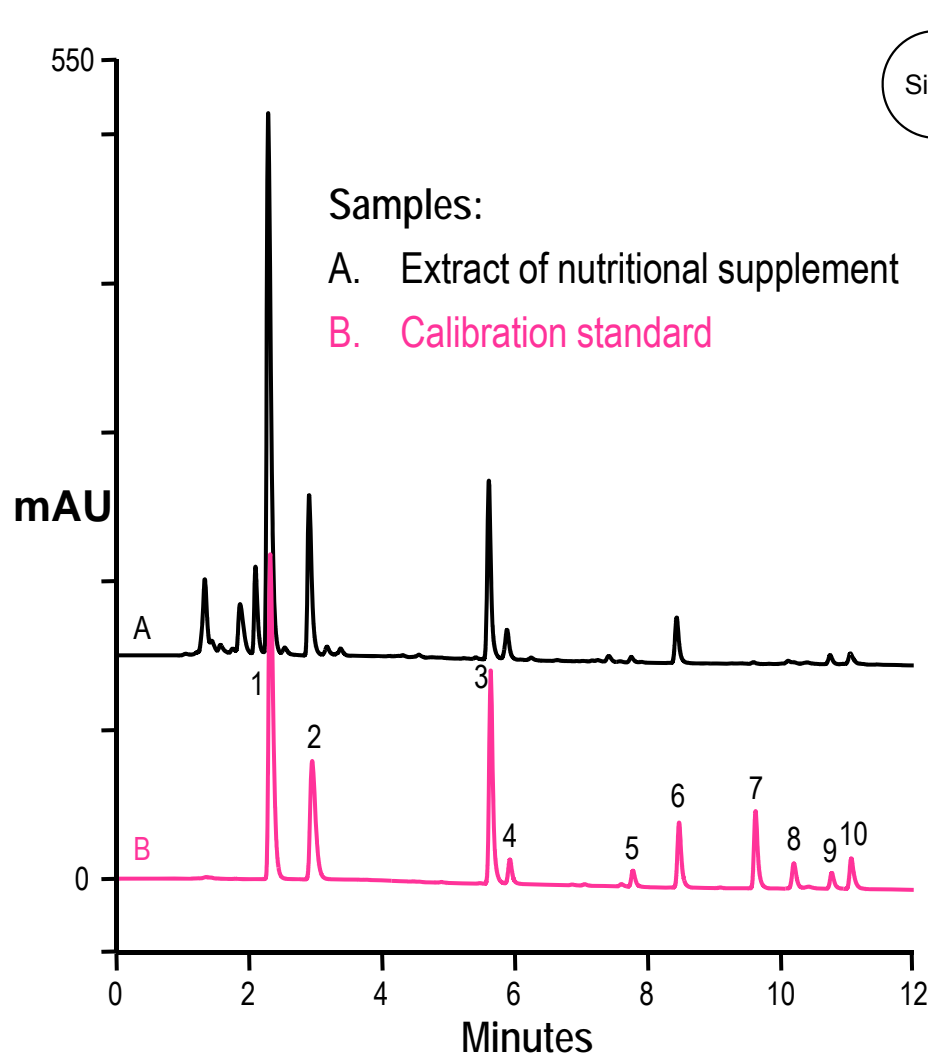


Biotin (H)



Riboflavin (B2)

Water-Soluble Vitamins in a Nutritional Supplement



Column: **Acclaim PolarAdvantage**, 3 μ m, 150x4.6 mm

Mobile Phases:

A: acetonitrile

B: water

C: 30 mM sodium hexanesulfonate, 30 mM H_3PO_4 , adjusted to pH 3.08 with NH_4OH

Gradient times:	-7	0	14	16
%A	0	0	30	30
%B	65	65	35	35
%C	35	35	35	35

Flow: 1.0 mL/min

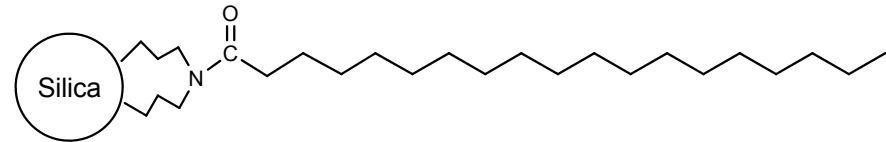
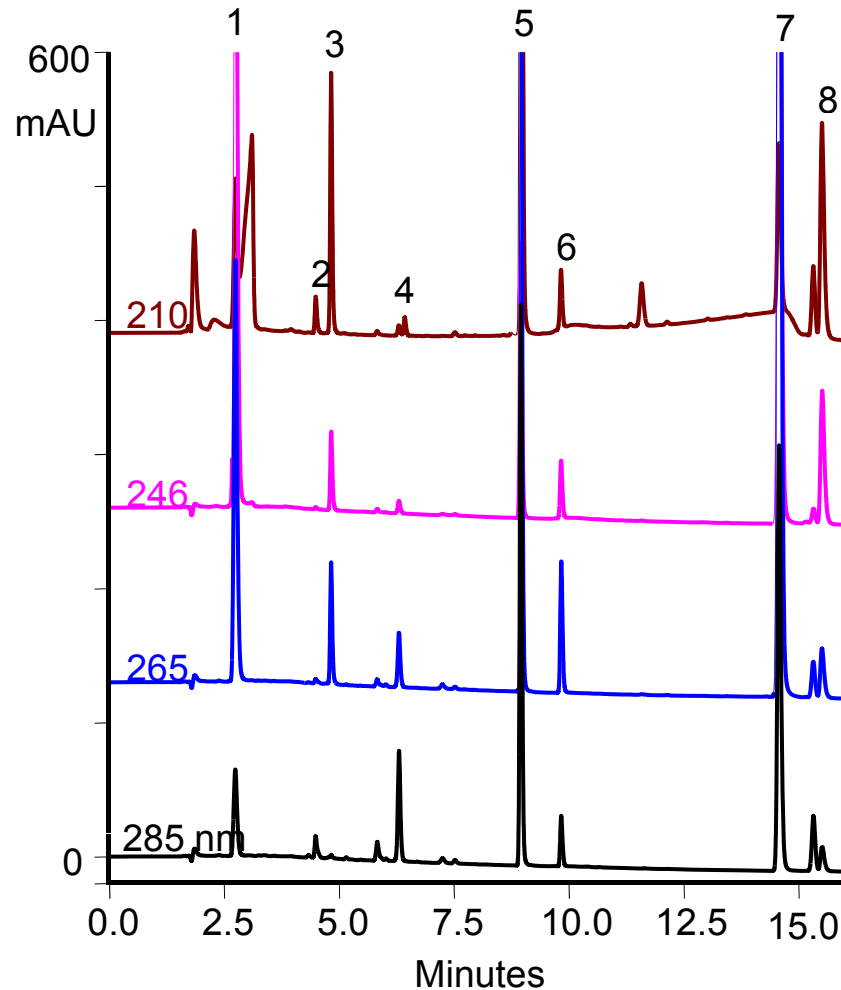
Temperature: 30 °C

Injection: 20 μ L

Detector: 210, 246, 265, and 285 nm

Peaks:	(μ g/mL)		
1. Ascorbic acid	120	6. Pyridoxine	2.5
2. Nicotinic acid	10	7. Folic acid	5.0
3. Niacinamide	10	8. Vitamin B12	1.5
4. Pantothenic acid	20	9. Thiamine	2.5
5. Riboflavin 5'-phosphate	2.5	10. Riboflavin	2.5

Ingredients in an Energy Drink



Column: **Acclaim PA2 C18**, 3 μ m

Dimension: 4.6 x 150 mm

Mobile Phases

A: Acetonitrile

B: 30 mM phosphate buffer, pH 3.28

C: 30 mM phosphate buffer, pH 2.54

Gradient:

Times (min): 0 14 16

%A: 0 40 40

%B: 100 0 0

%C: 0 60 60

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Injection: ASI-100 autosampler, 5 μ L

Detection: UV 210, 246, 265, 285 nm

Peaks:

1. Ascorbic acid

5. Caffeine

2. Pyridoxine

6. Riboflavin

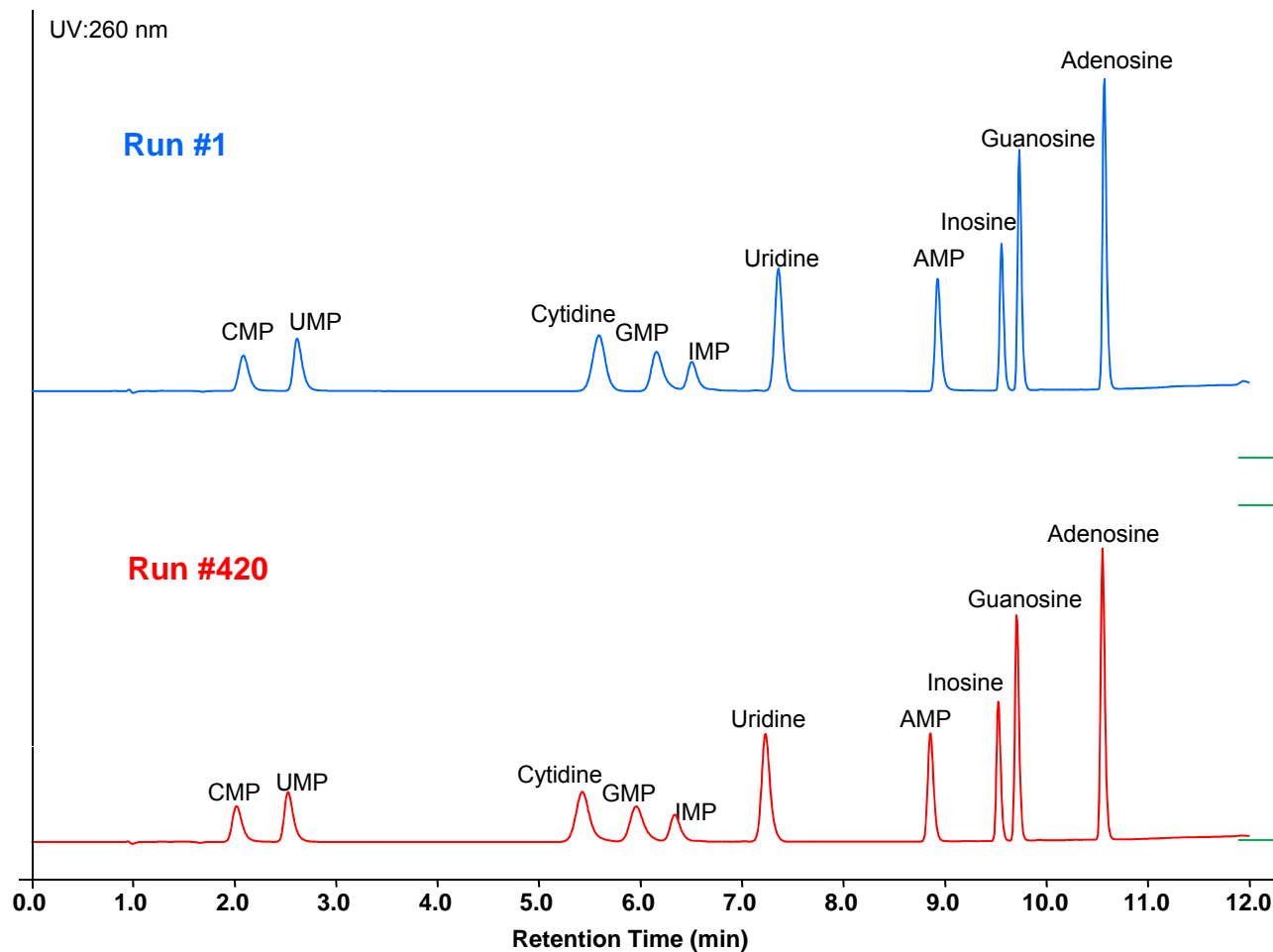
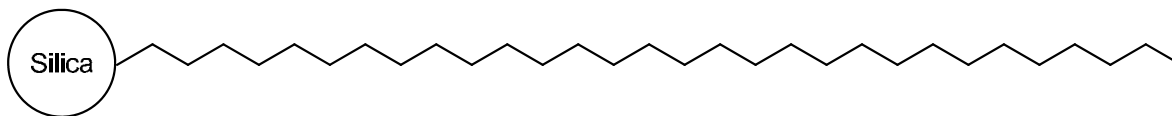
3. Niacinamide

7. Sorbic acid

4. Pantothenic acid

8. Benzoic acid

Nutritional Nucleotides & Nucleosides on **C30** phase

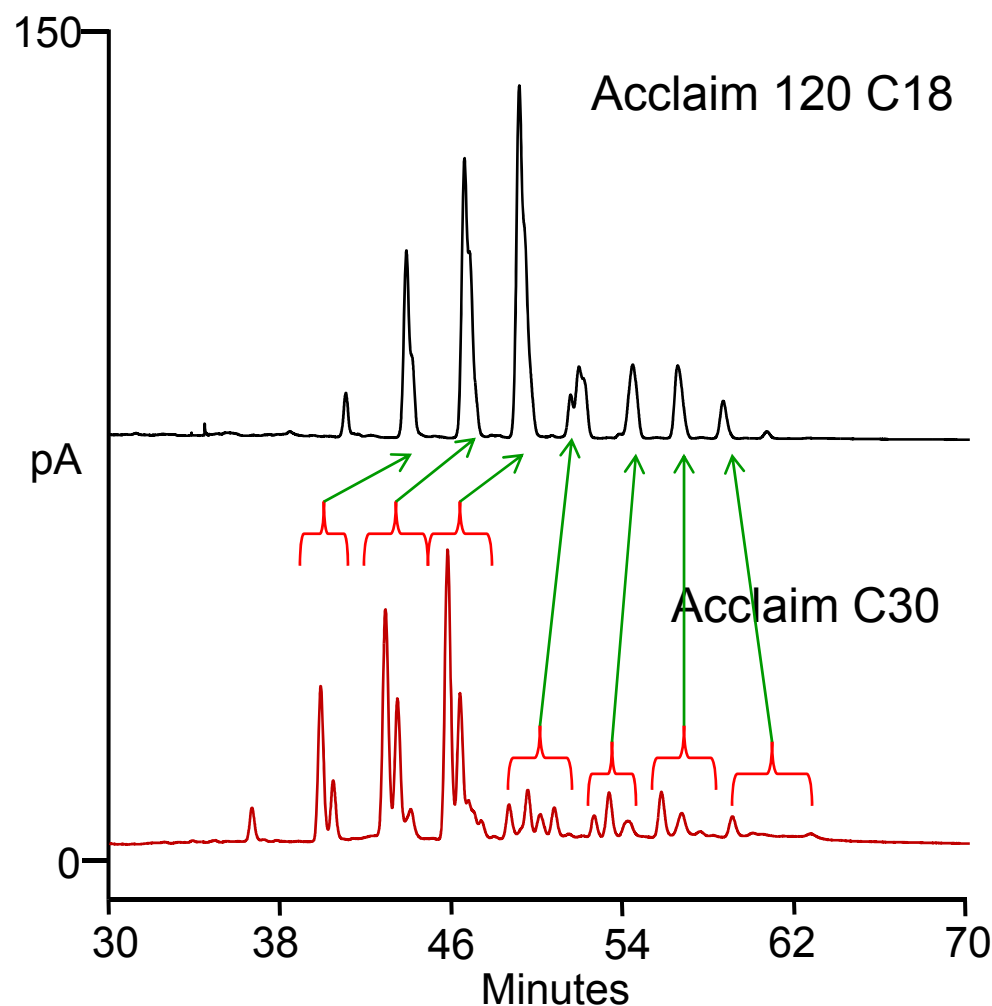


Column: **Acclaim C30**, 3 μ m
 Dimension: 2.1 \times 150 mm
 Mobile Phase: A) DI water
 B) NH₄OAc (100 mM, pH5)
 C) CH₃OH
 Temperature: 15 $^{\circ}$ C
 Flow Rate: 0.4 mL/min
 Injection: 10 μ L, 1 ppm of each analyte
 Detection: UV at 260 nm

Gradient:

Time	%A	%B	%C
-5.0	80	20	0
0	80	20	0
3.0	80	20	0
5.9	70	20	10
8.5	40	20	40
11.9	40	20	40
12.0	80	20	0

Analysis of Cooking Oil: C30 vs. C18

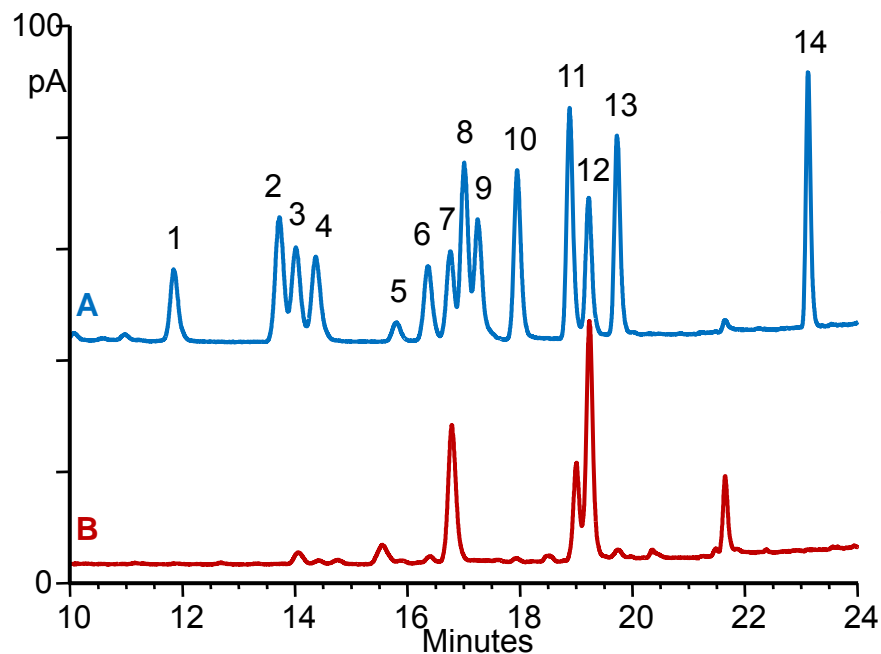


Column: **Acclaim C30** or Acclaim 120 C18, 5 μ m
 Dimension: 4.6x150 mm
 Mobile Phase: Acetonitrile (MeCN)/Iso-propanol (IPA)/
 Ammonium Acetate (0.1 M, pH5.0)
 (Buffer)
 Temperature: 40 ° C
 Flow Rate: 1.0 mL/min
 Inj. Volume: 2 μ L
 Detection: Corona *ultra* (Gain = 100 pA; Filter =
 medium; Neb. Temp = 25 ° C)
 Sample: Peanut oil (5 mg/mL in iso-propanol)

Gradient

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
70	0	95	5

Omega Fatty Acids

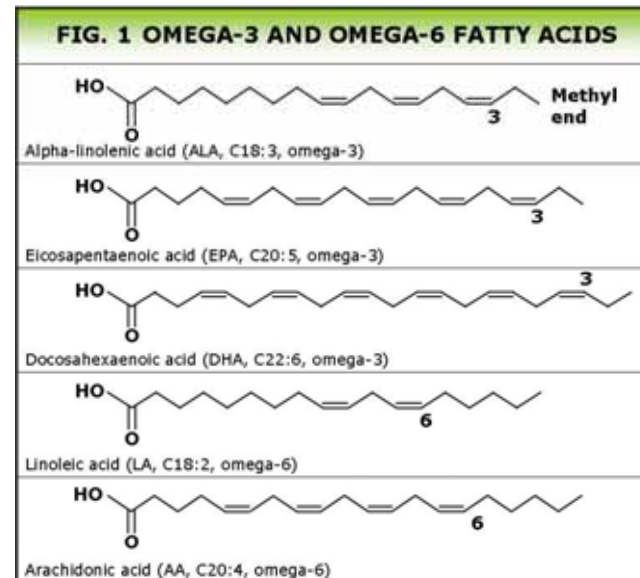


- Peaks:
- | | |
|-----------|----------------|
| 1. SDA | 8. DPA |
| 2. EPA | 9. 9E, 14Z-CLA |
| 3. ALA | 10. ETA |
| 4. GLA | 11. Adrenic |
| 5. DHA | 12. Oleic |
| 6. Arach. | 13. EDA |
| 7. LLA | 14. Erucic |

Column: **Acclaim C30**, 5 μ m, 4.6 x 150 mm
 LC System: UltiMate 3000 RS, Dual Gradient
 Mobile Phases:
 A) Water:Formic acid:Mobile phase B 900:3.6:100 (v/v)
 B) Acetone:Acetonitrile:THF:Formic acid 675:225:100:4(v/v)

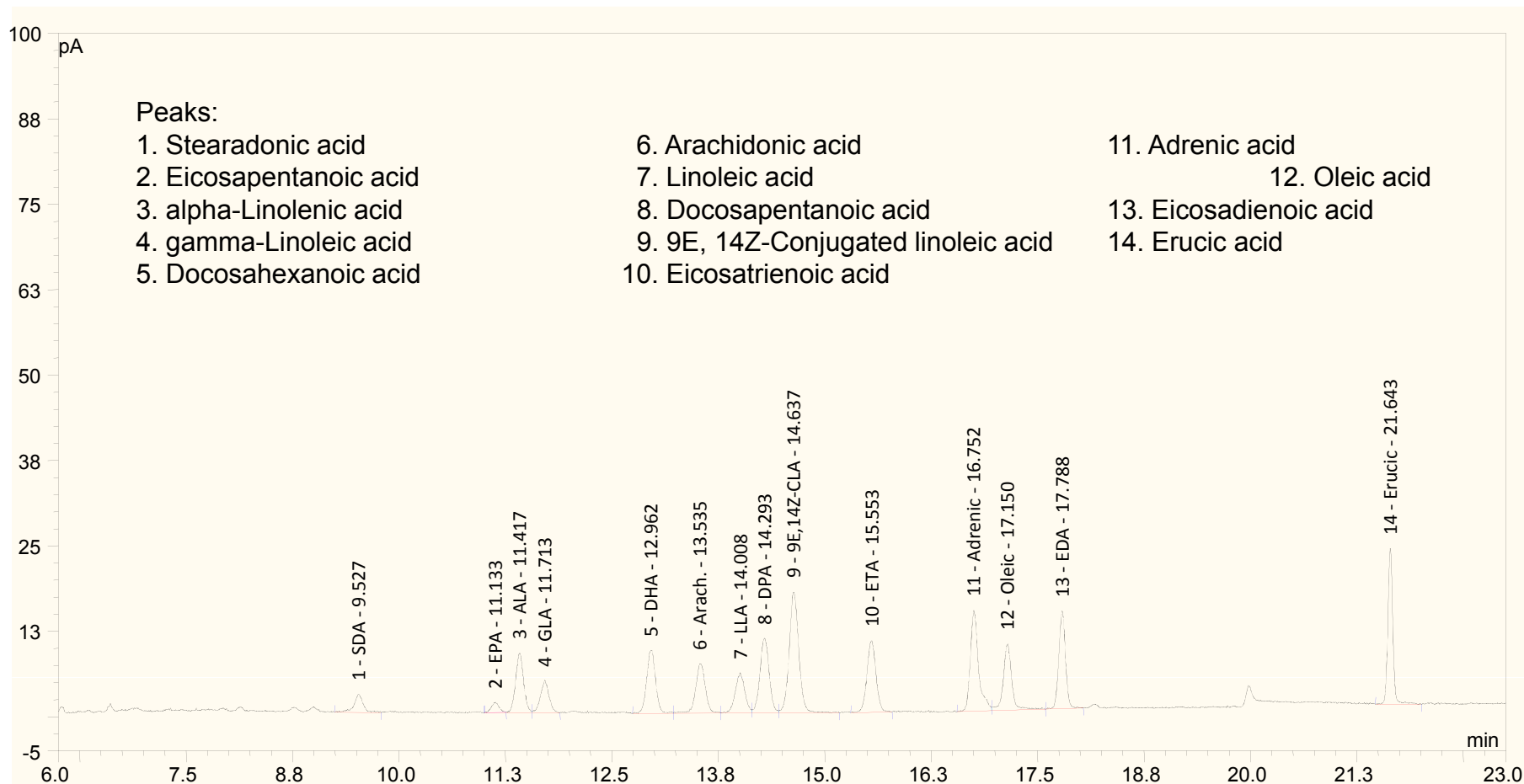
Gradient times (min):	0	1	13	22	24	29	32
%A	100	40	30	5	5	100	100
%B	0	60	70	95	95	0	0

Flow: 1.00 mL/min
 Temperature: 30 ° C
 Injection: 2 μ L
 Detection: Corona *ultra*, nebulizer 15 ° C, filter High
 Samples:
 A. Standards in isopropanol
 B. Saponified chicken fat



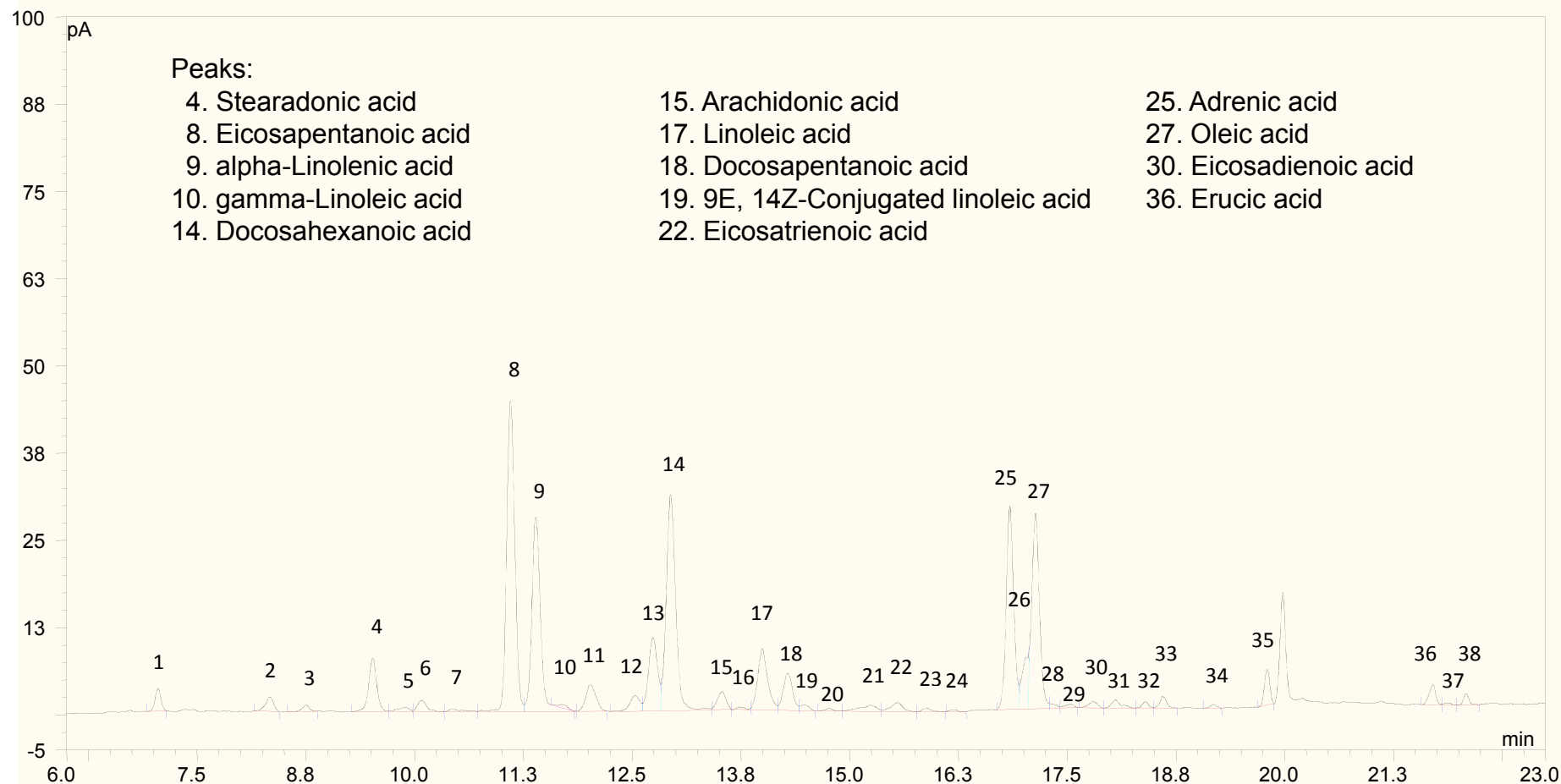
Omega fatty acids

3- μ m, 3x250-mm **Acclaim C30** column



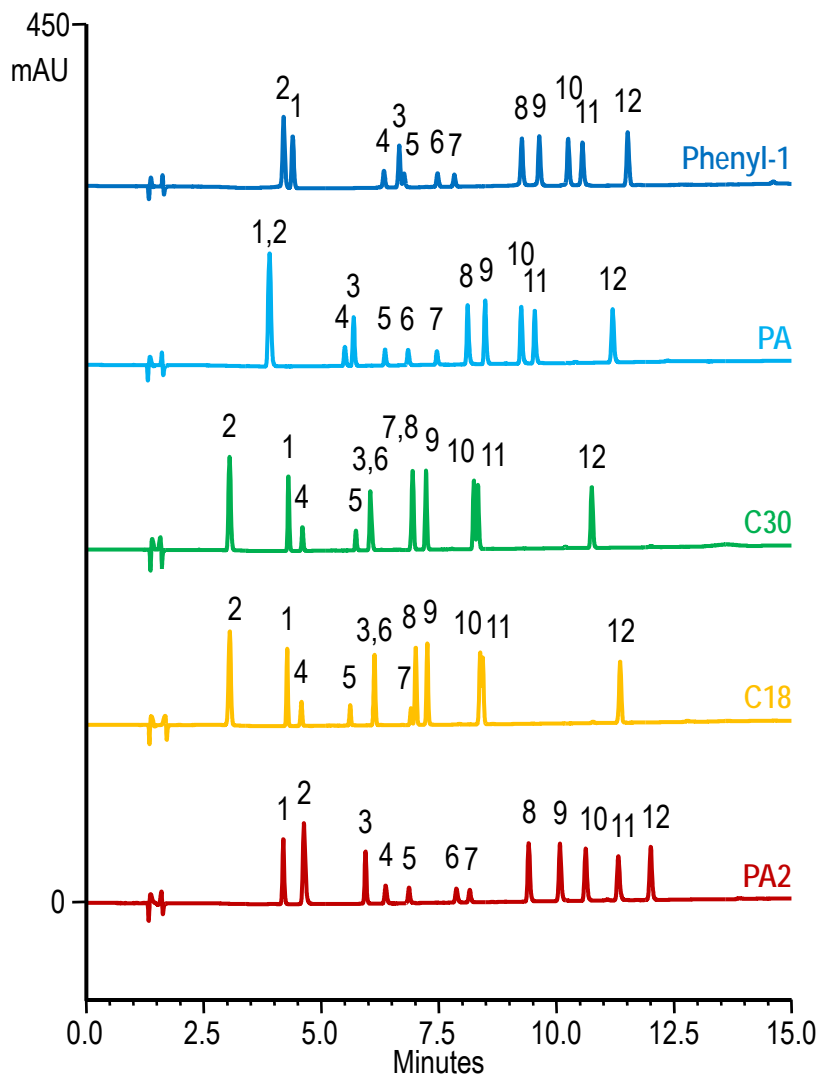
Fish Oil

3- μ m, 3x250-mm **Acclaim C30** column



HPLC-CAD chromatogram of 20 μ L Fish Oil, prepared as described with addition of 200 μ L isopropanol to aid in solubility. A total of 38 peaks were detected, with all 14 standards identified.

Selectivity Comparison: Catechins and Caffeine in Tea



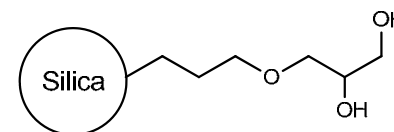
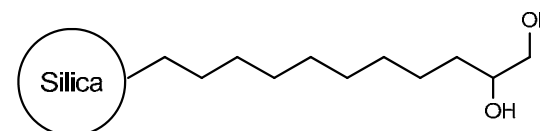
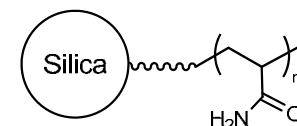
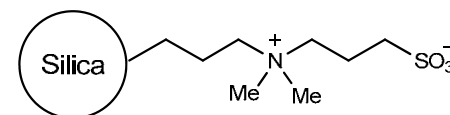
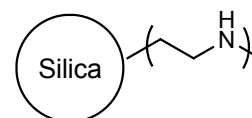
Columns:	Acclaim C18, C30, PA, PA2 and Phenyl-1, 3 μ m			
Dimension:	3 x 150 mm			
HPLC System:	UltiMate [®] 3000-RS			
Mobile Phases:	A: Acetonitrile B: 100 mM Formic acid + 20 mM ammonium formate C: water			
Gradient times, min:	-6	0	12	15
%A	5	5	50	50
%B	10	10	10	10
%C	85	85	40	40
Flow rate:	0.60 mL/min			
Injection:	5 μ L			
Temperature:	30 $^{\circ}$ C			
Detection:	UV at 254 nm, 5 Hz, 1 s resp. time			
Peaks:	1. theobromine	7. epicatechin		
	2. gallic acid	8. epigallocatechin gallate		
	3. caffeine	9. gallic acid gallate		
	4. gallic acid catechin	10. epicatechin gallate		
	5. epigallocatechin	11. catechin gallate		
	6. catechin	12. 3,4,5-trihydroxy cinnamic acid		

What is HILIC?

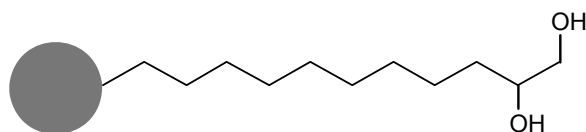
- HILIC – Hydrophilic Interaction Liquid Chromatography
 - Variation of normal-phase chromatography
 - Also called “reverse reversed-phase” or “aqueous normal phase” chromatography
 - Used to retain highly hydrophilic analytes
- Mobile phase
 - 60 – 95% solvent (e.g., acetonitrile) and 40 – 5% aqueous
- Stationary phase has polar/hydrophilic surface
 - Bare silica
 - Ionic bonded layer
 - Neutral bonded layer

HILIC Columns for Highly Hydrophilic Analytes

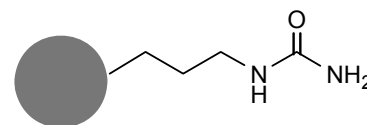
- Bare silica phases
 - *Accucore HILIC*
 - *Hypersil GOLD Silica*
 - *Synchronis Silica*
- Ionic bonded phases
 - *Hypersil GOLD HILIC*
 - *Synchronis HILIC*
- Neutral bonded phases
 - *Accucore 150-Amide-HILIC*
 - *Acclaim Mixed-Mode HILIC-1*
 - *BETASIL Diol*



Overview of Acclaim HILIC Columns



Acclaim Mixed-Mode HILIC-1



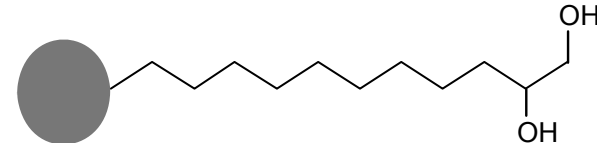
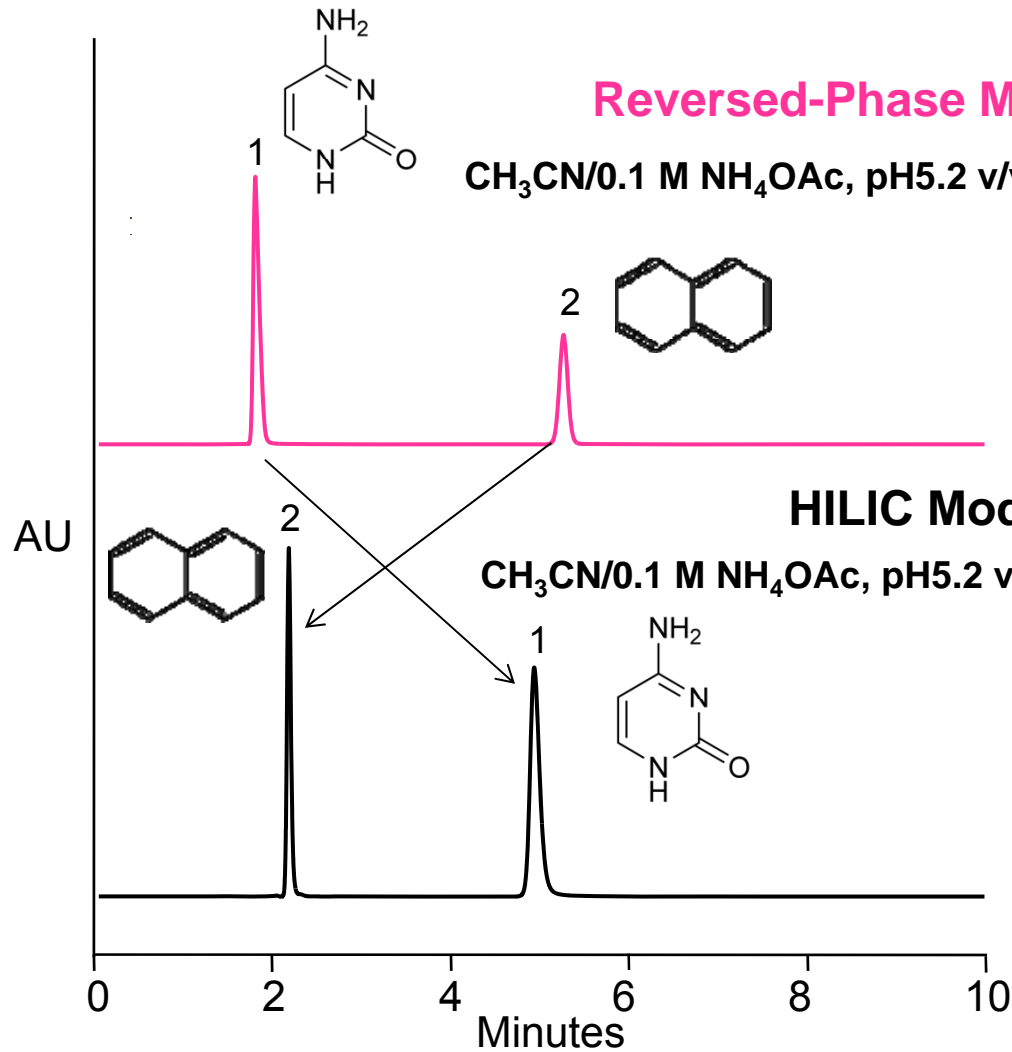
Acclaim HILIC-10

Name	Chemistry	Bonding	Particle Size (µm)	Pore Size (Å)	Surface Area (m ² /g)	pH Range	Aqueous Compatibility
Mixed-Mode HILIC-1	Alkyl Diol	Monomeric	3.0, 4.5	120	300	2.5 – 7.5	100%
HILIC-10	Urea	Multi-point bonding	3.0, 4.5	120	300	2.0 – 8.0	100%

Features

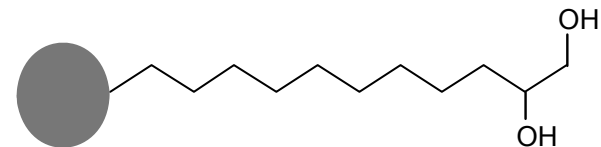
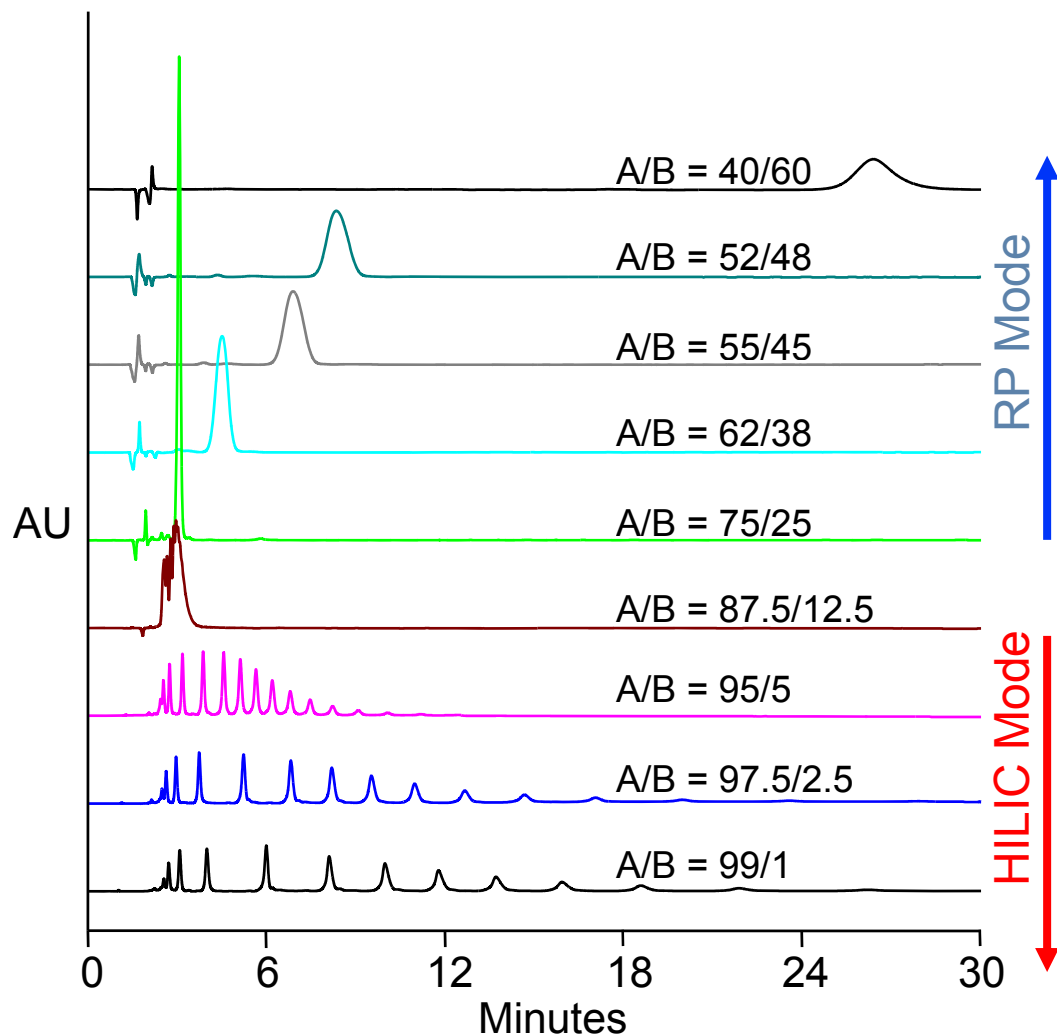
- Higher polarity for polar molecules that are not retained by reversed-phase chromatography
- Unique selectivity, complementary to reversed-phase columns
- Rugged column packing

Operation in Both RP and HILIC Modes

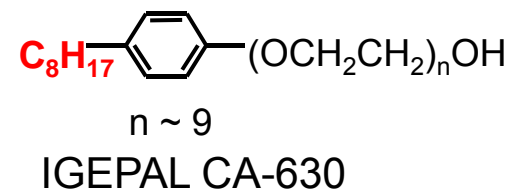


Column: Acclaim Mixed-Mode HILIC-1, 5 μm
Dimensions: 150x4.6 mm
Mobile Phase: CH₃CN/0.1 NH₄OAc, pH5.2
Temperature: 30 ° C
Flow Rate: 1 mL/min
Inj. Volume: 10 μL
Detection: UV @ 254 nm
Peaks: (100 ppm each)
1. Cytosine
2. Naphthalene

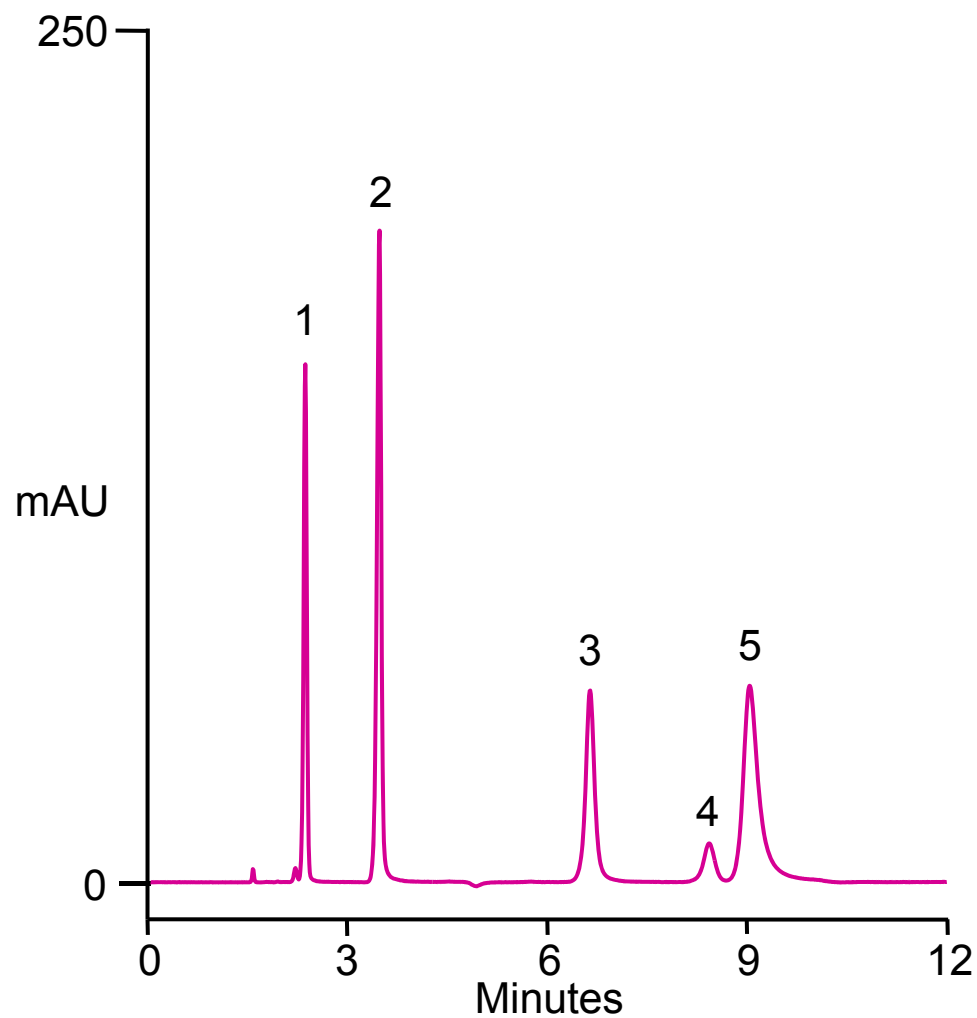
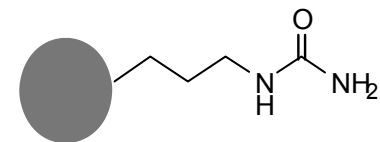
Separation Dependency of Organic Solvent



Column: Acclaim Mixed-Mode HILIC-1, 5 μ m
 Dimensions: 4.6 x 150 mm
 Mobile Phase: A – CH₃CN
 B – 0.1 M NH₄OAc, pH5.2
 Temperature: 30 °C
 Flow Rate: 1.0 mL/min
 Inj. Volume: 10 μ L
 Detection: UV @ 225 nm
 Sample: IGEPAL CA-630 (0.1%)



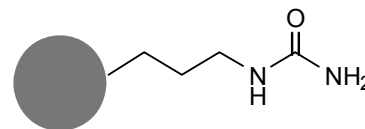
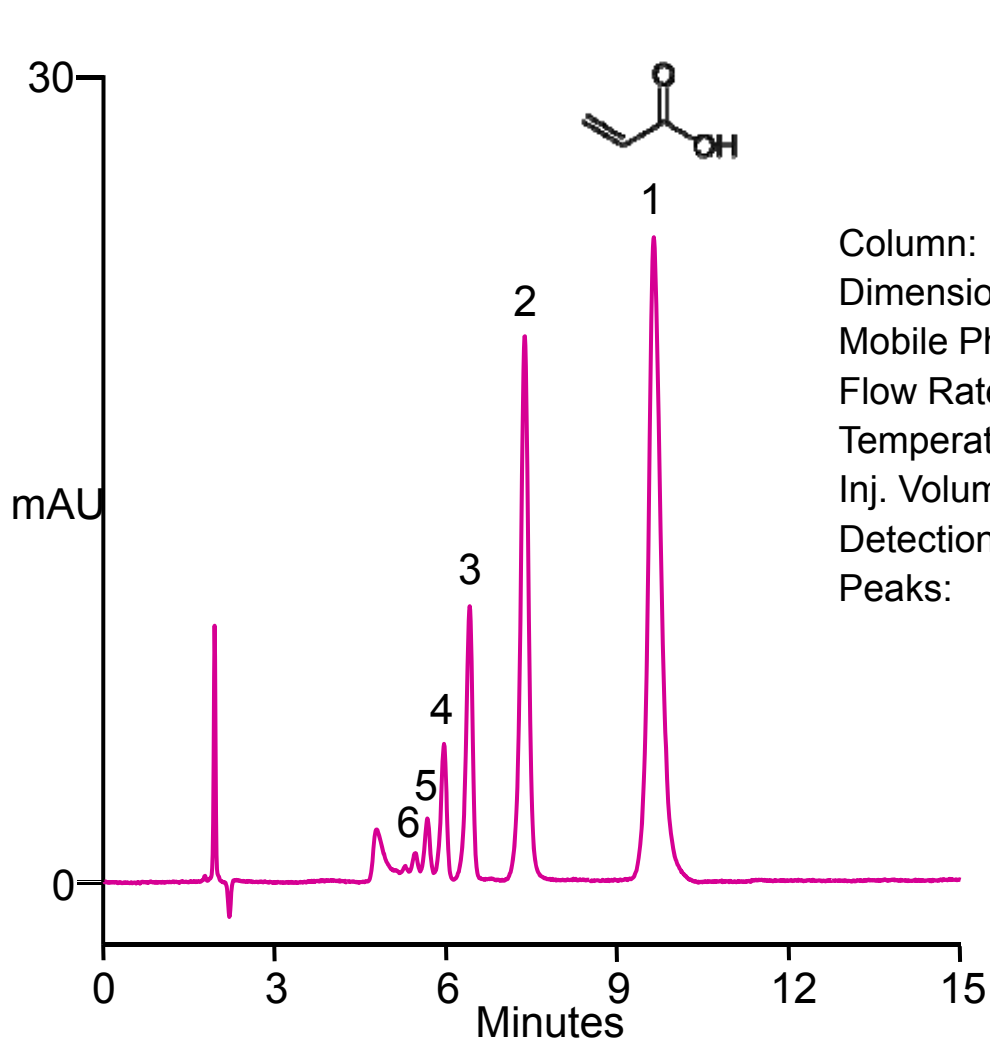
Hydrophilic Pharmaceuticals



Column: Acclaim HILIC-10, 3 μ m
Dimensions: 4.6 x 150 mm
Mobile Phase: 90/10 v/v CH₃CN/10 mM (total) NH₄OAc, pH5
Flow Rate: 1 mL/min
Temperature: 30 °C
Inj. Volume: 2 μ L
Detection: UV at 230 nm
Peaks:

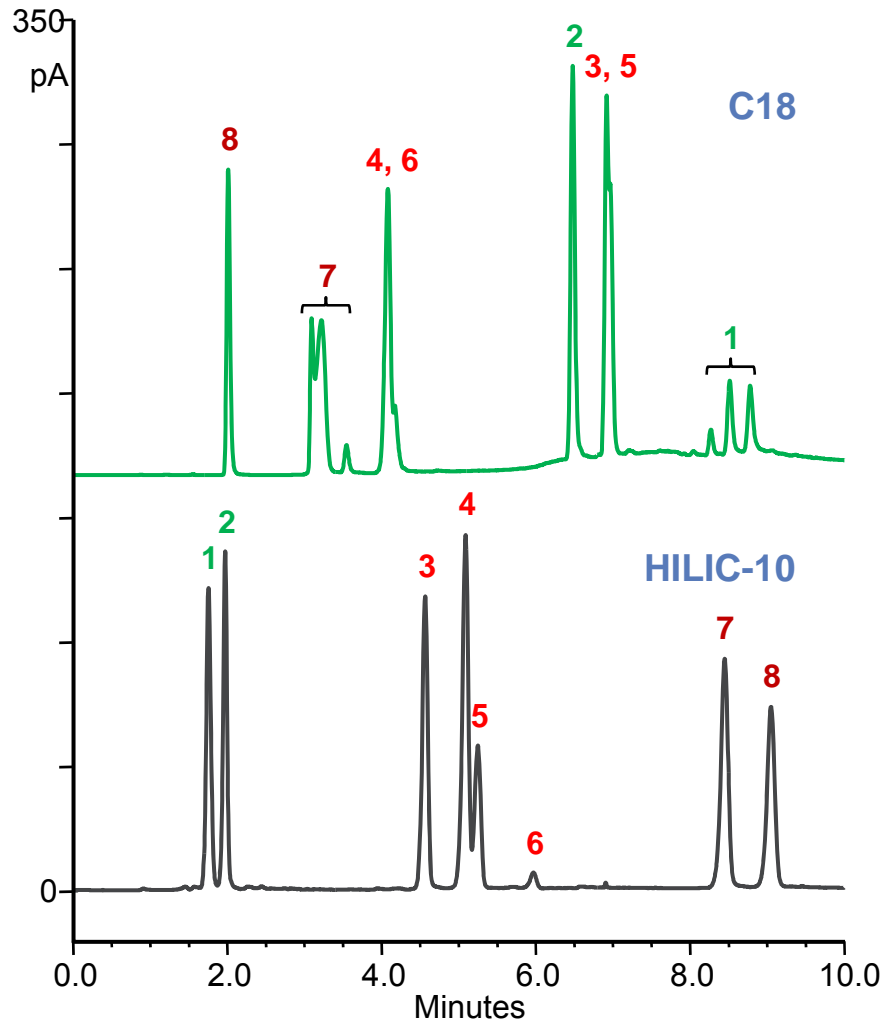
	mg/mL
1. Acetaminophen	0.1
2. Salicylic acid	0.1
3. Aspirin	0.2
4. Penicillin G	0.1
5. Metformin	0.1

Acrylic Acid and Oligomers



Column: Acclaim HILIC-10, 3 μm
Dimensions: 4.6 x 150 mm
Mobile Phase: 90/10 v/v $\text{CH}_3\text{CN}/10 \text{ mM (total) NH}_4\text{OAc, pH5}$
Flow Rate: 1 mL/min
Temperature: 30 $^\circ\text{C}$
Inj. Volume: 2 μL
Detection: UV at 230 nm
Peaks: (5 mg/mL in mobile phase)
1. Acrylic acid (monomer)
2. Dimer
3. Trimer
4. Tetramer
5. Pentamer
6. Hexamer

Glycerides: Acclaim HILIC-10 vs Acclaim 120 C18



NP Column:	Acclaim HILIC-10, 3 μ m				
RP Column:	Acclaim 120 C18, 3 μ m				
Dimensions:	3.0 x 150 mm				
HPLC System:	UltiMate 3000-RS				
NP Mobile Phases:	A: Heptane				
	B: 2-Propanol:Acetic acid 99.5:0.5 (v/v)				
Step Elution (min):	-6.0	0.0	0.5	4.0	10.0
%A	99	99	96	87	87
%B	1	1	4	13	13
RP Mobile Phases:	A: Acetonitrile				
	B: Ethyl Acetate				
Gradient times(min):	-6.0	0.0	0.5	5.5	10.0
%A	70	70	70	10	10
%B	30	30	30	90	90
Flow:	0.50 mL/min				
Temperature:	25 $^{\circ}$ C				
Injection:	4 μ L				
Detector:	Corona Ultra, nebulizer 15 $^{\circ}$ C				
Peaks:	1. Tristearin	5. Distearin 2			
	2. Trilaurin	6. Dilaurin 2			
	3. Distearin 1	7. Monostearin			
	4. Dilaurin 1	8. Monolaurin			



● **Mixed-Mode Columns**



● **The world leader in serving science**

Strategically Important Technological Platform

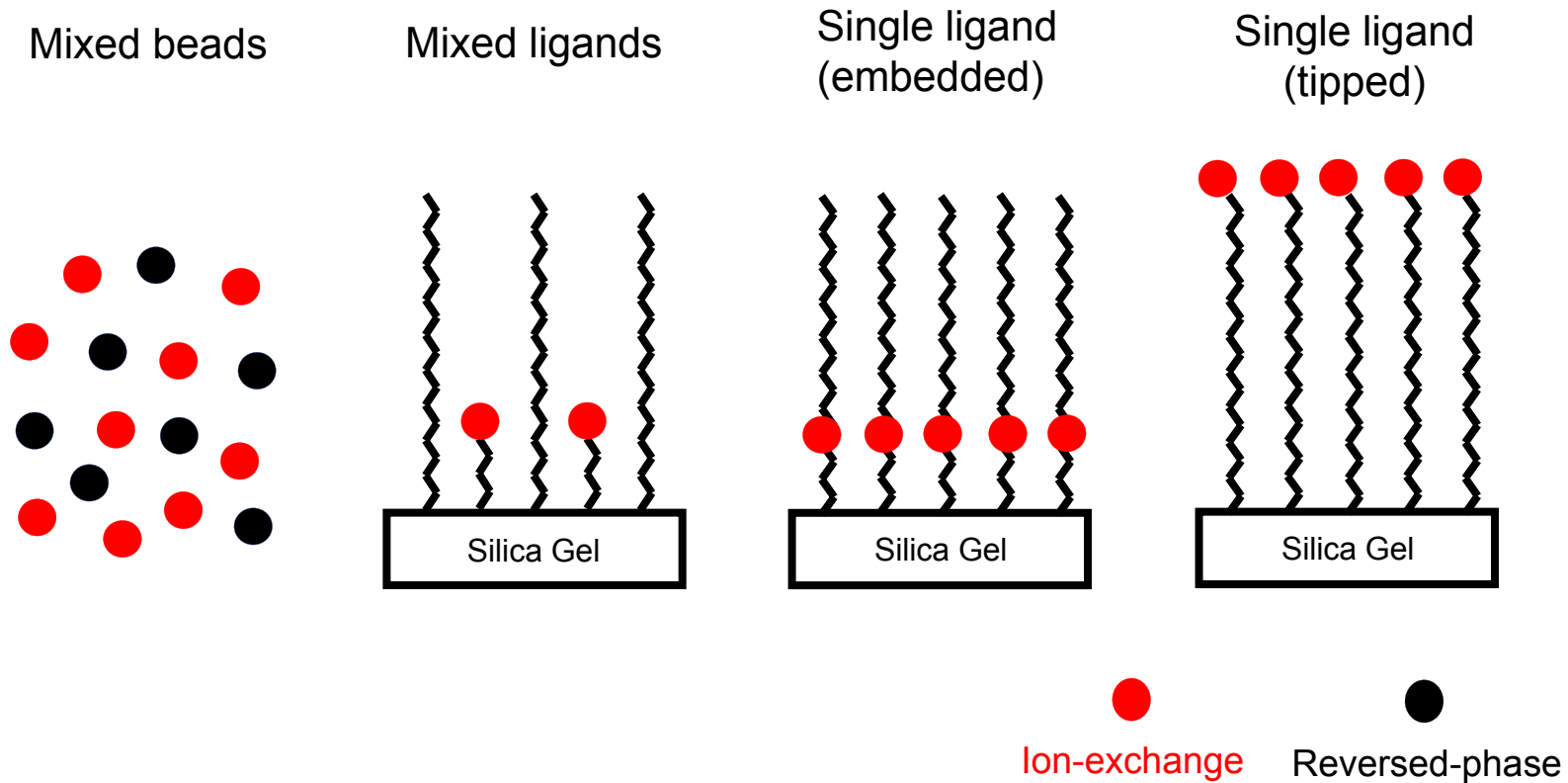
- Designed for specific, challenging and important applications
- Complement general-purpose column range
- Often as part of integrated solutions with HPLC and MS
- “Stepping stone” for more opportunities



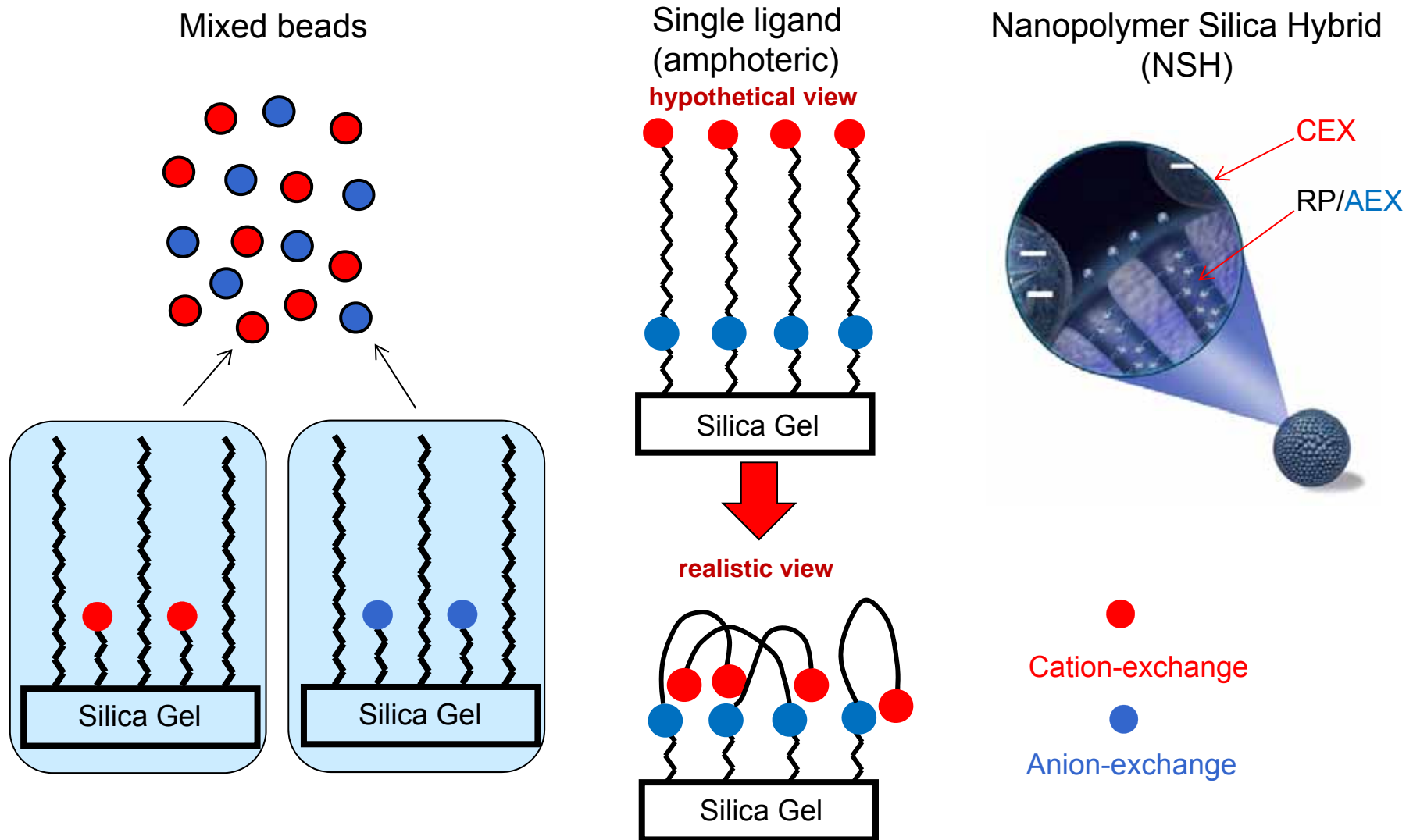
Application-Specific Columns

Name	Targeted Applications	Retention Mechanism	Particle Size (µm)	pH Range	Aqueous Compatibility
Acclaim Trinity P1	Pharmaceuticals and counterions	Mixed-mode	3.0	2.5 – 7.5	100%
Acclaim Trinity P2	Pharmaceuticals and counterions	Mixed-mode	3.0	2.5 – 7.5	100%
Acclaim Organic Acid	Organic acids	Reversed-phase	3.0, 4.5	2.0 – 8.0	100%
Acclaim Surfactant Plus	Various types of surfactants	Mixed-mode	3.0, 4.5	2.5 – 7.5	100%
Acclaim Carbamate	Carbamates pesticides/herbicides in EPA Method 531.2	Reversed-phase	2.2, 3.0, 4.5	2.0 – 8.0	90%
Acclaim Carbonyl C18	Ketones & aldehydes in U.S. EPA Methods 1667, 554, OT-11; CARB 1004	Reversed-phase	2.2	2.5 – 8.0	90%
Acclaim Explosives E2	Explosives related compounds in EPA Method 8330	Reversed-phase	2.2, 3.0, 4.5	2.5 – 8.0	90%
Acclaim Trinity Q1	Diquat and paraquat in EPA Method 549.2	Mixed-Mode	2.2	2.5 – 7.5	100%
Acclaim HAA	Haloacetic acids analysis in drinking water by LC/MS	Mixed-Mode	3.0	2.5 – 7.5	100%

RP/IEX Bimodal Mixed-Mode Phases

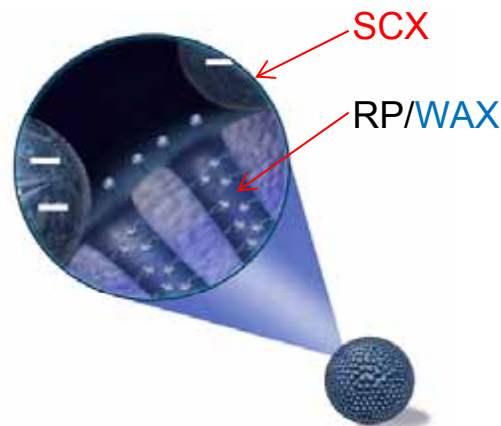


RP/AEX/CEX Trimodal Mixed-mode Phases

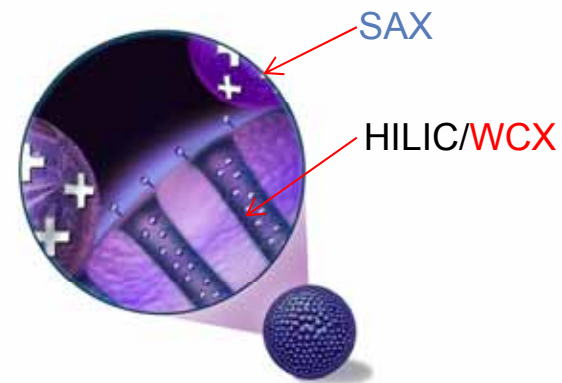


API and counterions

- Salt formation is important in drug development.
50% of all drugs are formulated as salt forms
- Challenges
 - RP: little or no retention for counterions; little to adequate retention for APIs
 - IEX: need both AEX and CEX columns; not for neutral API
 - HILIC: limited to hydrophilic analytes
- Solution
 - Trimodal phases designed for APIs and counterions

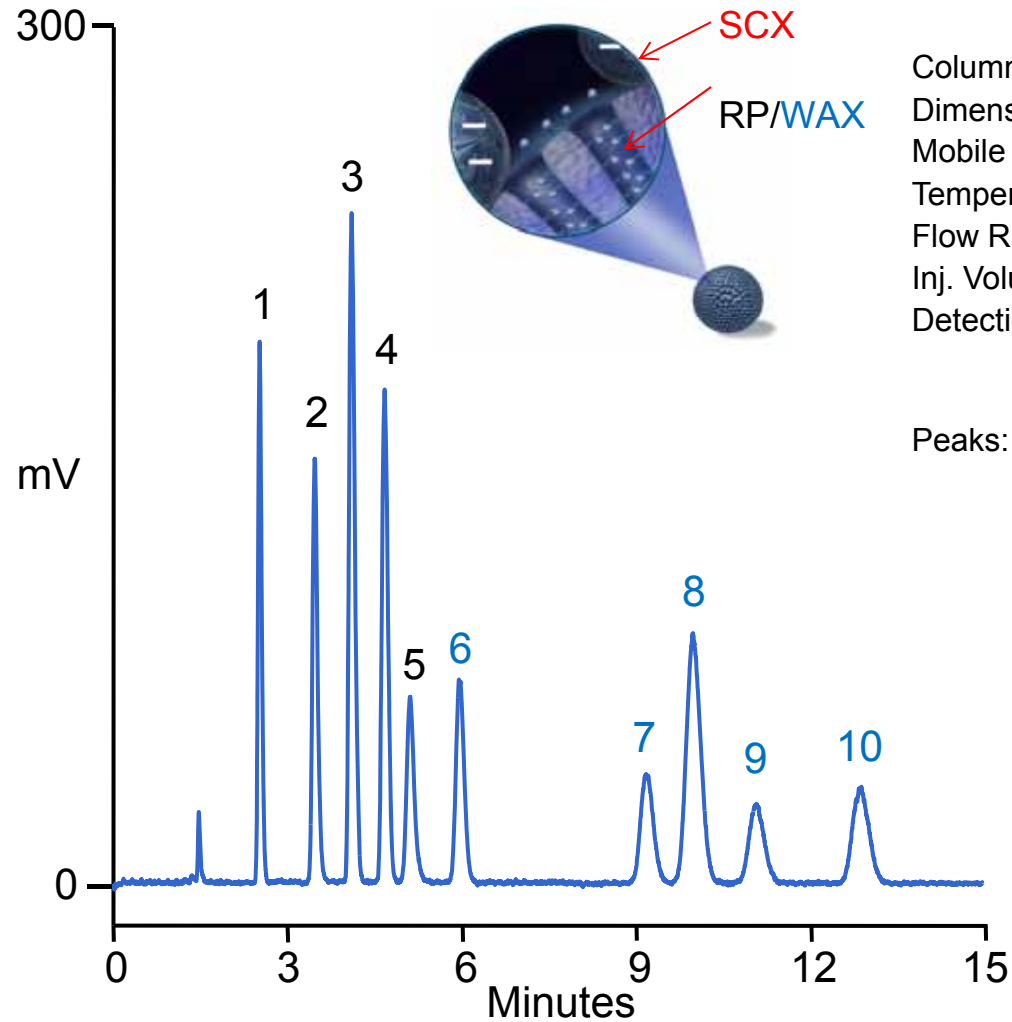


Acclaim Trinity P1



Acclaim Trinity P2

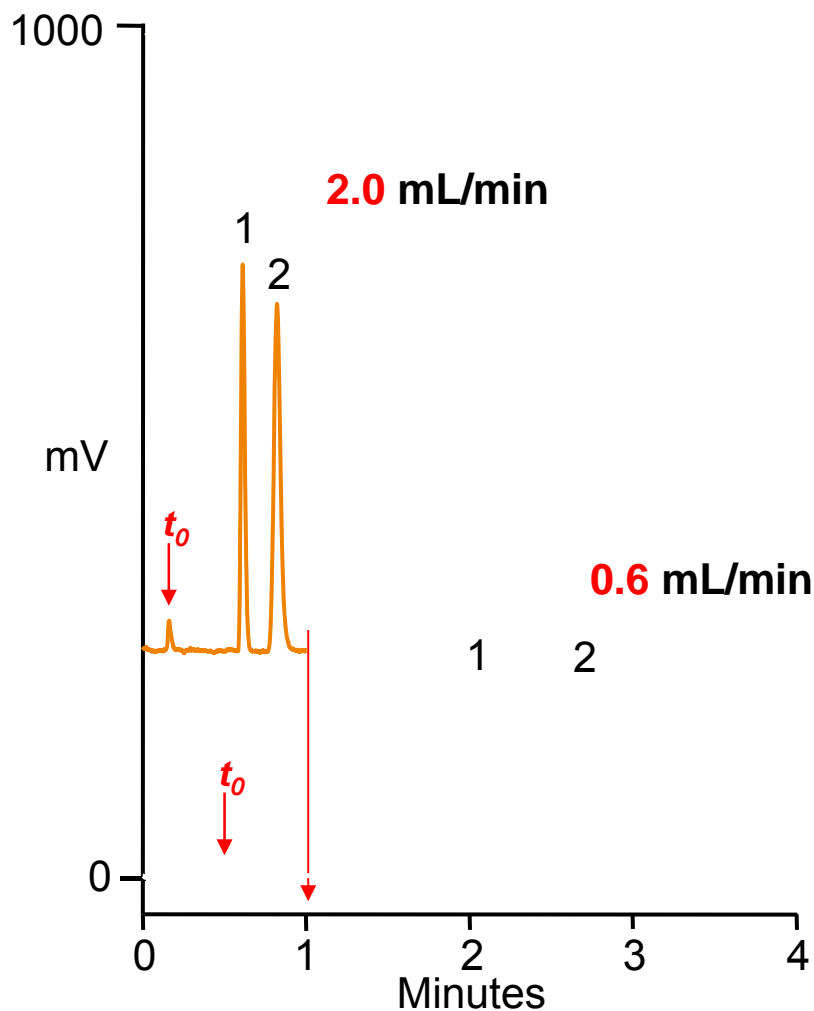
Pharmaceutical counterions



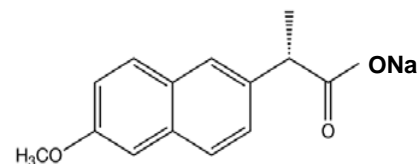
Column: **Acclaim Trinity P1**, 3 μ m
Dimensions: 3.0 \times 100 mm
Mobile Phase: 60/40 v/v CH₃CN/20 mM (total) NH₄OAc, pH5
Temperature: 30 $^{\circ}$ C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 μ L
Detection: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 30 $^{\circ}$ C)

Peaks: (50 to 100 ppm)
1. Choline
2. Tromethamine
3. Sodium
4. Potassium
5. Meglumine
6. Mesylate
7. Nitrate
8. Chloride
9. Bromide
10. Iodide

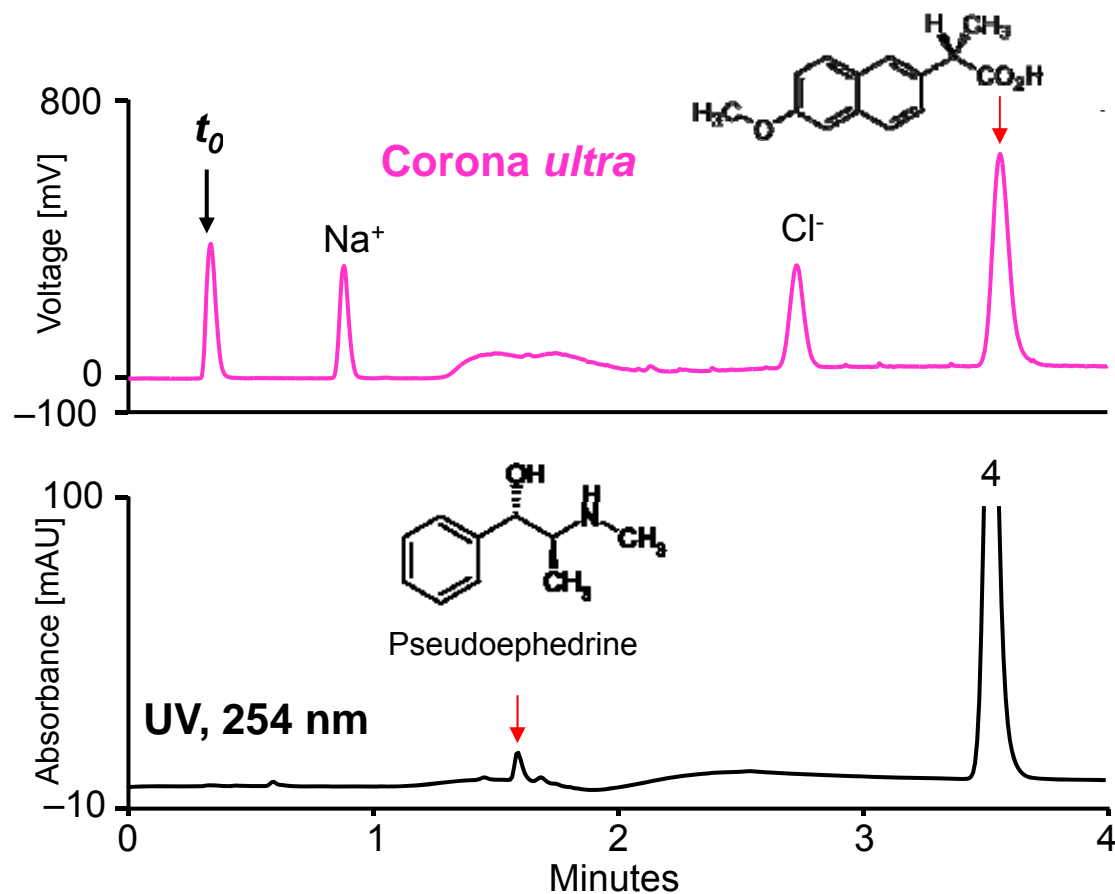
Hydrophobic drug & counterion (sodium naproxen)



Column: Acclaim Trinity P1, 3 μ m
Dimensions: 3.0 x 50 mm
Mobile Phase: 80/20 v/v CH₃CN/20 mM (total) NH₄OAc, pH5
Temperature: 30 °C
Flow Rate: 0.6 and 2.0 mL/min
Inj. Volume: 2.5 μ L
Detection: Corona *ultra*
(Gain = 100pA; Filter = med; Neb Temp = 30 °C)
Sample: Na, Naproxen (0.2 mg/mL in mobile phase)
Peaks:
1. Na⁺
2. Naproxen



Over-the-counter medicine for sinus & headache

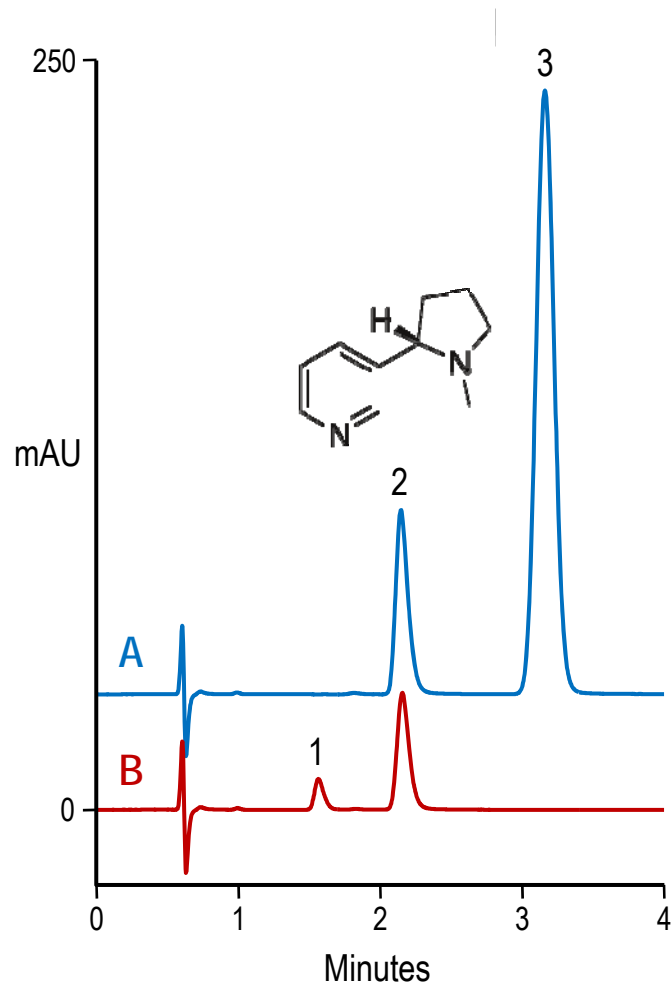


Column: Acclaim Trinity P1, 3 μ m
 Dimensions: 3.0 x 50 mm
 Mobile Phase: A: CH₃CN
 B: D.I. H₂O
 C: 0.1 M NH₄OAc, pH 5.2
 Temperature: 30 °C
 Flow Rate: 0.6 mL/min
 Inj. Volume: 1 μ L
 Detection: UV at 254 nm and a Corona *ultra* detector in series

Corona *ultra* settings:
 Gain = 100 pA; Filter = med; Neb. Temp = 30 °C
 Gradient:

Time (min)	% A	% B	% C	Curve
-4	20	65	15	5
0	20	65	15	5
0.1	20	65	15	5
1	80	0	20	5
4	80	0	20	5

Nicotine Salts



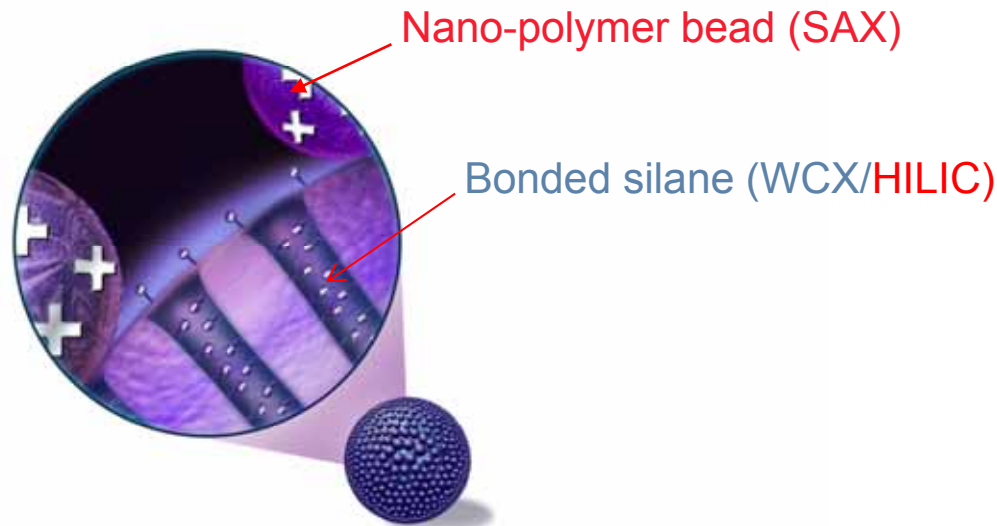
- *Ideal selectivity*
- *Simple method*
- *Not possible with any RP columns*

Column: Acclaim® Trinity P1, 3 µm
Dimension: 3.0 x 50 mm with 3.0 x 10 mm guard
HPLC System: UltiMate® 3000 RS
Mobile Phase: Dibasic sodium phosphate dodecahydrate 1.07 g + Monobasic sodium phosphate 1.80 g, Tetrasodium pyrophosphate decahydrate 27 mg + Acetonitrile 196 g + Water 750 g.

Flow rate: 0.60 mL/min
Injection: 5.0 µL
Temperature: 30 °C
Detection: UV at 210 nm
Samples: A. Nicotine salicylate
B. Nicotine bitartrate, 100 µg/mL

Peaks:
1. Tartrate
2. Nicotine
3. Salicylate

Nanopolymer Silica Hybrid (NSH) technology



Chemistry: HILIC/WCX/SAX

HILIC: Amide

WCX: Carboxylic

SAX: Quaternary amine

Silica Substrate:

High-purity, spherical, porous

Particle size: 3- μ m

Pore-size: 120 Å

Surface area: 300 m²/g

Formats:

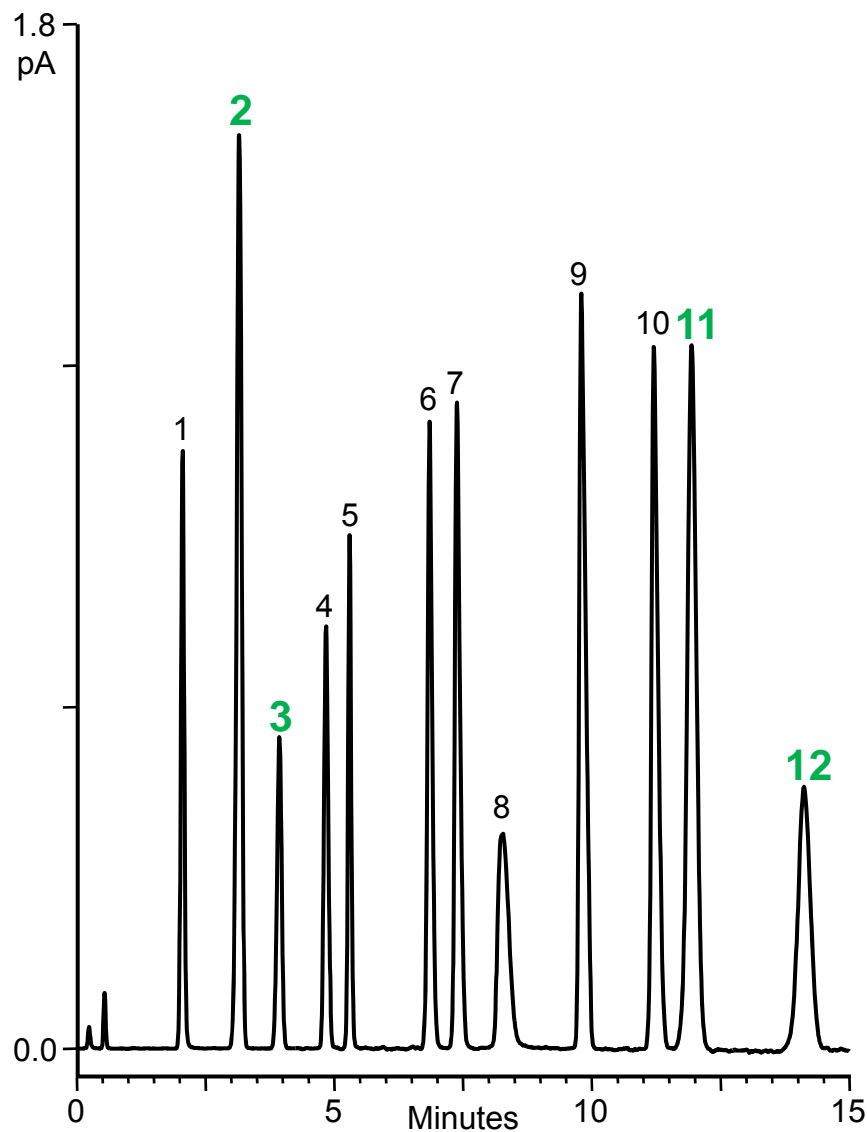
3.0x100-mm

3.0x50-mm

2.1x100-mm

2.1x50-mm

Pharmaceutical-Related Anions & Cations



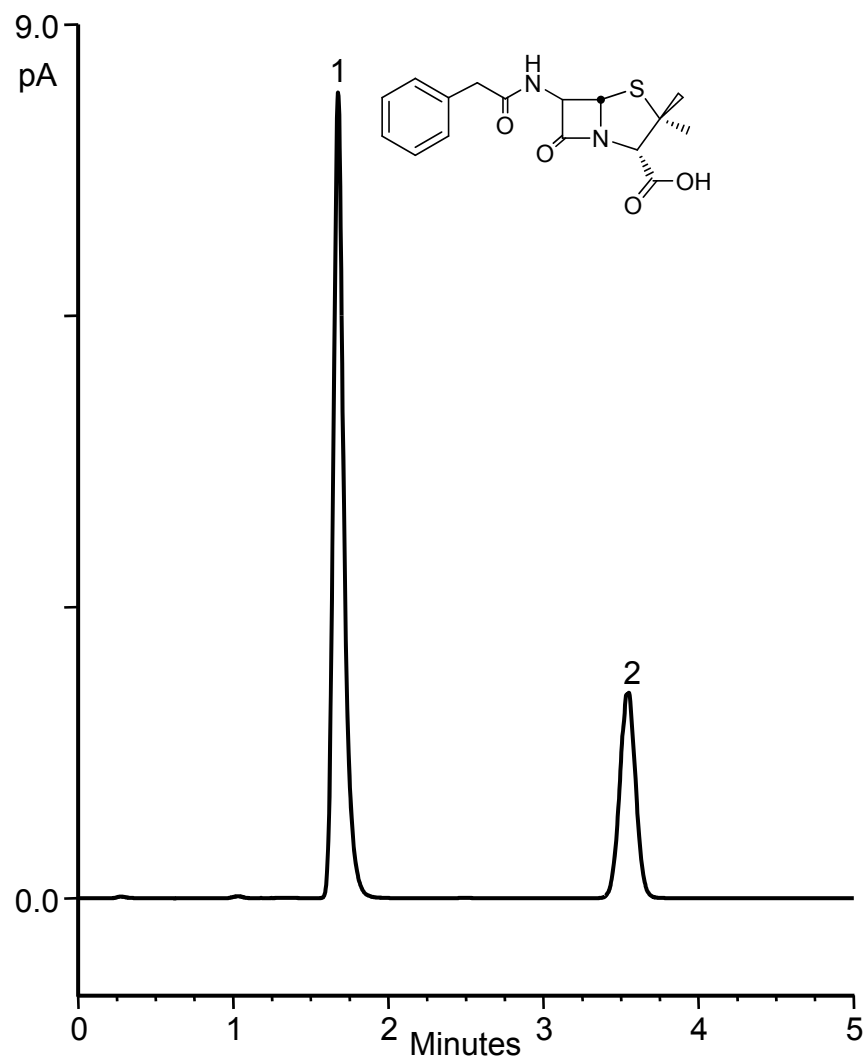
Column: **Acclaim Trinity P2**, 3 μ m
 Dimensions: 3.0 x 50 mm
 Mobile Phase: D.I. water and 100 mM NH_4OFm , pH 3.65
 Temperature: 30 $^\circ$ C
 Flow Rate: 0.60 mL/min
 Inj. Volume: 1 μ L
 Detection: Corona Veo
 Samples: 0.02 – 0.10 mg/mL each in D.I. water
 Peaks:

1. Phosphate
2. **Sodium**
3. **Potassium**
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. **Magnesium**
12. **Calcium**

Gradient:

Time (min)	H ₂ O	0.1 M Ammonium formate, pH3.65
-8	90	10
0	90	10
1	90	10
11	0	100
15	0	100

Penicillin G potassium



Column: **Acclaim Trinity P2**, 3 μ m

Dimensions: 3.0 x 50 mm

Mobile Phases: A: Acetonitrile

B: Water

C: 100 mM Ammonium formate, pH 3.65

Isocratic: 25% A / 50% B / 25% C

Temperature: 30 °C

Flow Rate: 0.50 mL/min

Inj. Volume: 1 μ L

Detection: Corona Veo: evaporator 55 °C, data rate 5 Hz,
filter 2 sec, power function 1.50

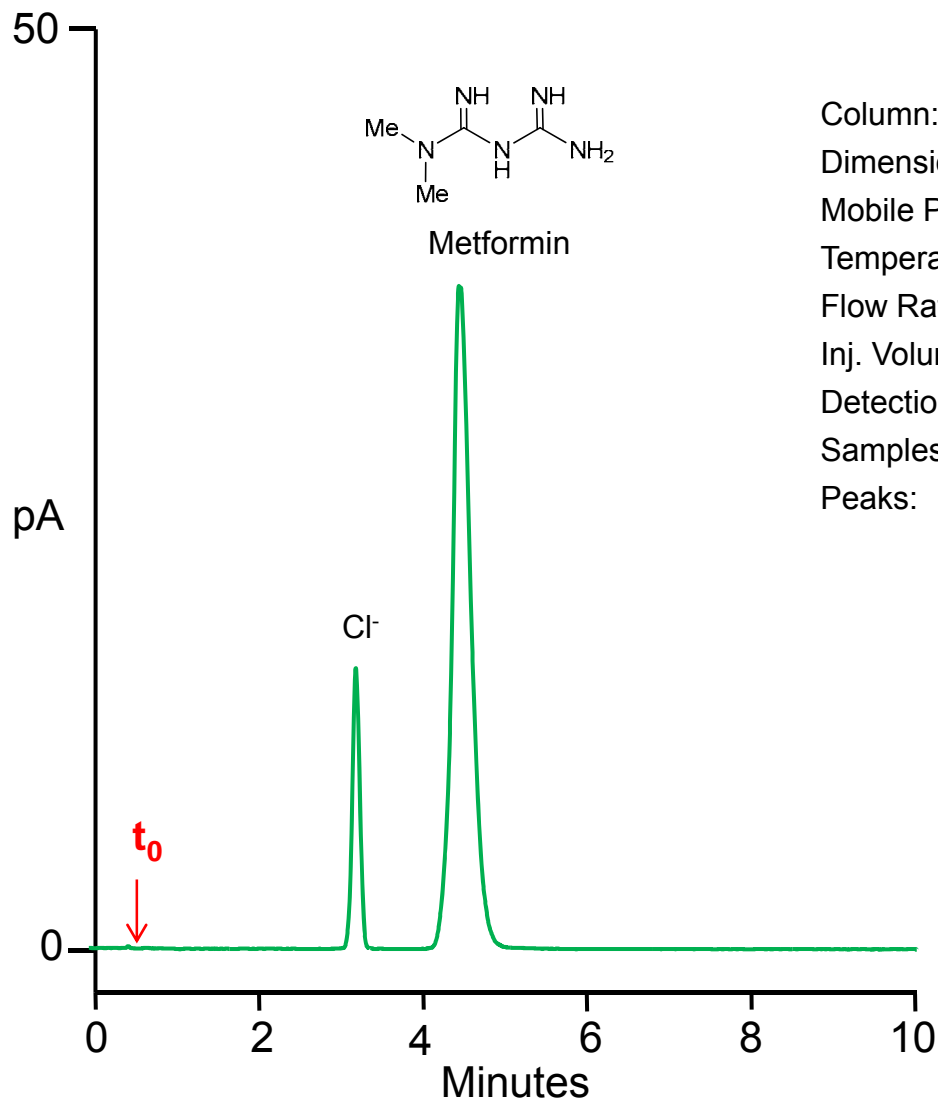
Sample: Potassium Penicillin G (0.1 mg/mL in D.I. water)

Peaks:

1. Penicillin G

2. Potassium

Metformin and its counterion, Cl⁻



Column: **Acclaim Trinity P2**, 3 μm

Dimensions: 3.0 x 50 mm

Mobile Phase: MeCN /100 mM NH₄OFm, pH3.65 v/v 80/20

Temperature: 30 ° C

Flow Rate: 0.50 mL/min

Inj. Volume: 1 μL

Detection: Corona Veo

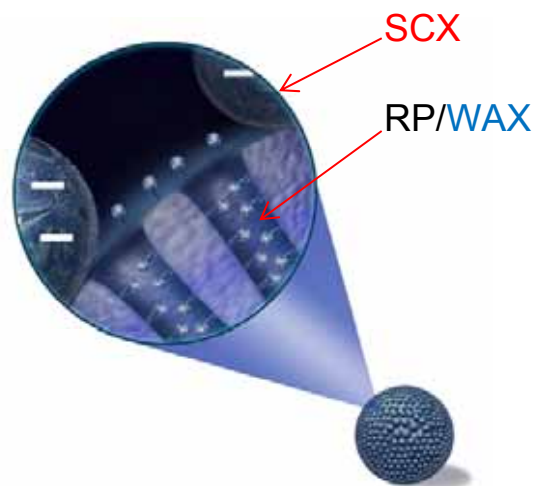
Samples: Metformin hydrogen chloride (0.1 mg/mL in D.I. water)

Peaks:

1. Chloride
2. Metformin

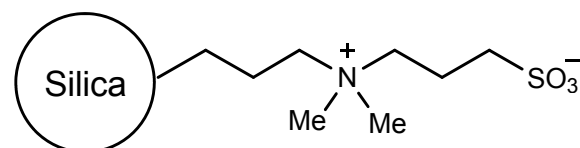
Acclaim Trinity P1 vs. ZIC-HILIC

Acclaim Trinity P1



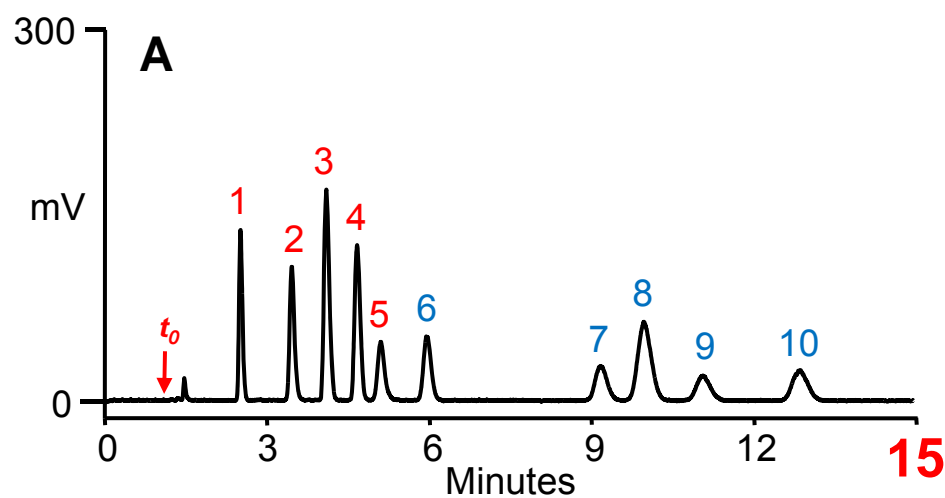
- Silica particles coated with nano-polymer beads
- Inner-pore: RP/WAX
- Outer surface: SCX

Zwitterionic HILIC



- Silica particles covalently modified with a highly polar zwitterionic layer

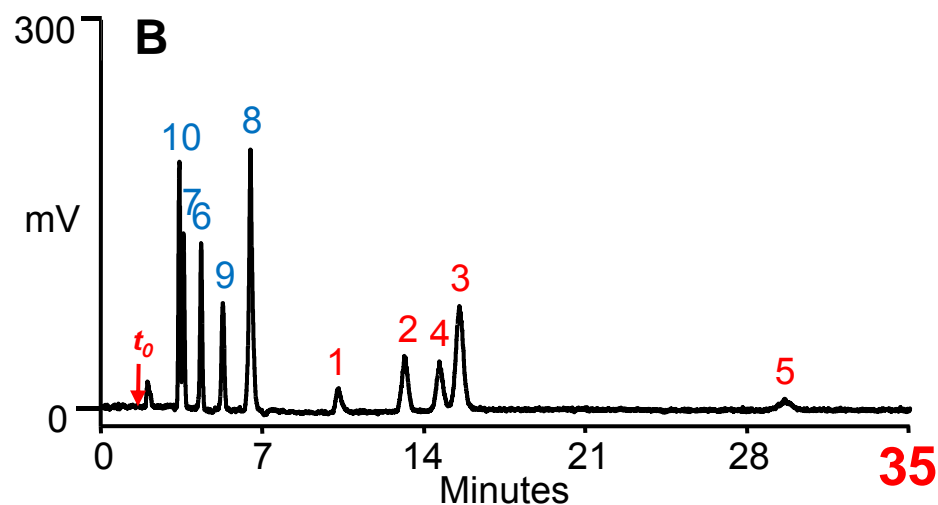
Separation of Pharmaceutical Counterions



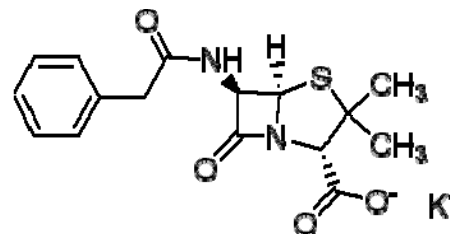
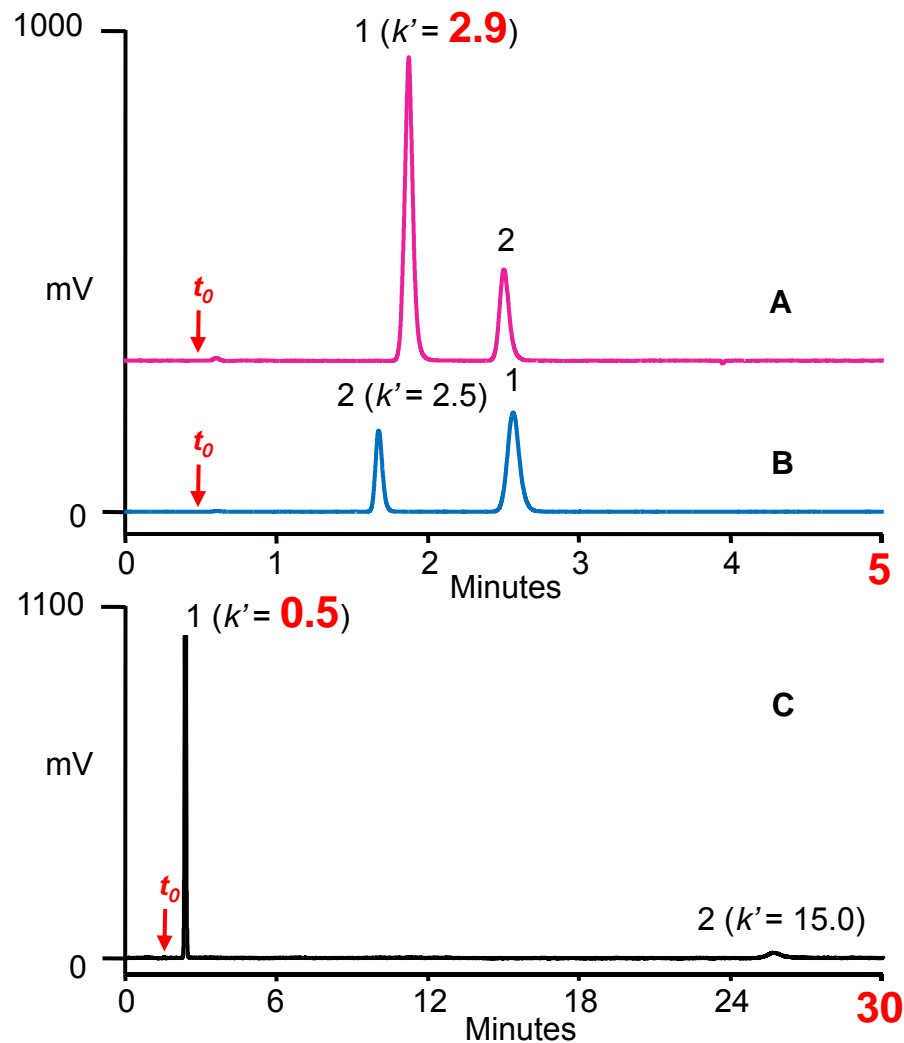
A:
 Column: Acclaim Trinity P1, 3 μm
 Dimensions: 3.0 x 100 mm
 Mobile Phase: 60/40 v/v $\text{CH}_3\text{CN}/20 \text{ mM}$ (total) NH_4OAc , pH5
 Temperature: 30 $^\circ\text{C}$
 Flow Rate: 0.5 mL/min
 Inj. Volume: 2 μL
 Detection: Aerosol based detector

B:
 Column: Zwitterionic HILIC, 5 μm
 Dimensions: 4.6 x 150 mm
 Mobile Phase: 80/20 v/v $\text{CH}_3\text{CN}/15 \text{ mM}$ (total) NH_4OAc , pH5
 Temperature: 30 $^\circ\text{C}$
 Flow Rate: 1 mL/min
 Inj. Volume: 5 μL
 Detection: Aerosol based detector

Peaks: (50 to 100 ppm)
 1. Choline
 2. Tromethamine
 3. Sodium
 4. Potassium
 5. Meglumine
 6. Mesylate
 7. Nitrate
 8. Chloride
 9. Bromide
 10. Iodide



Hydrophilic Acidic API & Counterion – Penicillin G Potassium



A & B
 Column: Acclaim Trinity P1, 3 μm
 Dimensions: 3.0 x 50 mm
 Mobile Phase: A: 80/20 v/v $\text{CH}_3\text{CN}/$ 20 mM (total) NH_4OAc , pH5.2
 B: 60/40 v/v $\text{CH}_3\text{CN}/$ 20 mM (total) NH_4OAc , pH5.2
 Temperature: 30 $^\circ\text{C}$
 Flow Rate: 0.6 mL/min
 Inj. Volume: 2 μL

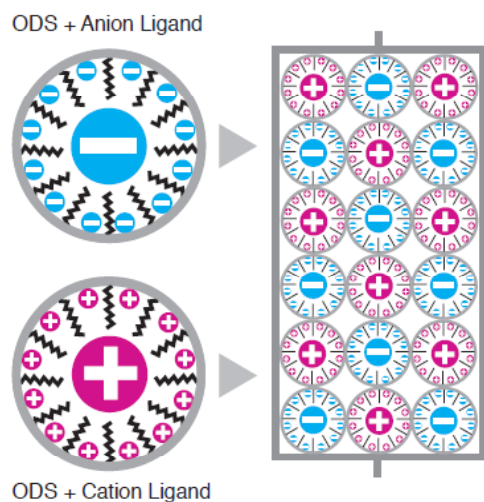
C
 Column: Zwitterionic HILIC, 5 μm
 Dimensions: 4.6 x 150 mm
 Mobile Phase: 85/15 v/v $\text{CH}_3\text{CN}/$ 15 mM (total) NH_4OAc , pH5.2
 Temperature: 30 $^\circ\text{C}$
 Flow Rate: 1.0 mL/min
 Inj. Volume: 5 μL

Detection: Aerosol based detector
 Sample: Penicillin G, K salt (0.2 mg/mL in mobile phase)
 Peaks:

1. Penicillin G
2. K^+

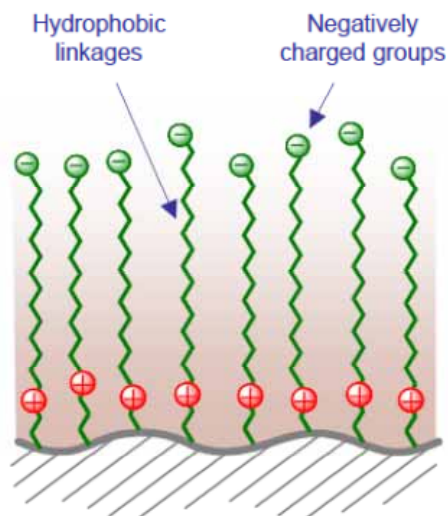
Trinity P1 vs. Competitors' Trimodal Columns

Type I



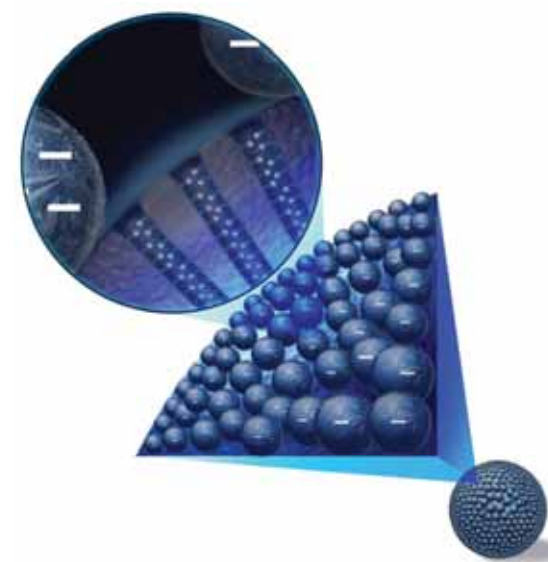
Scherzo SM-C18 (IMTAKT)

Type II



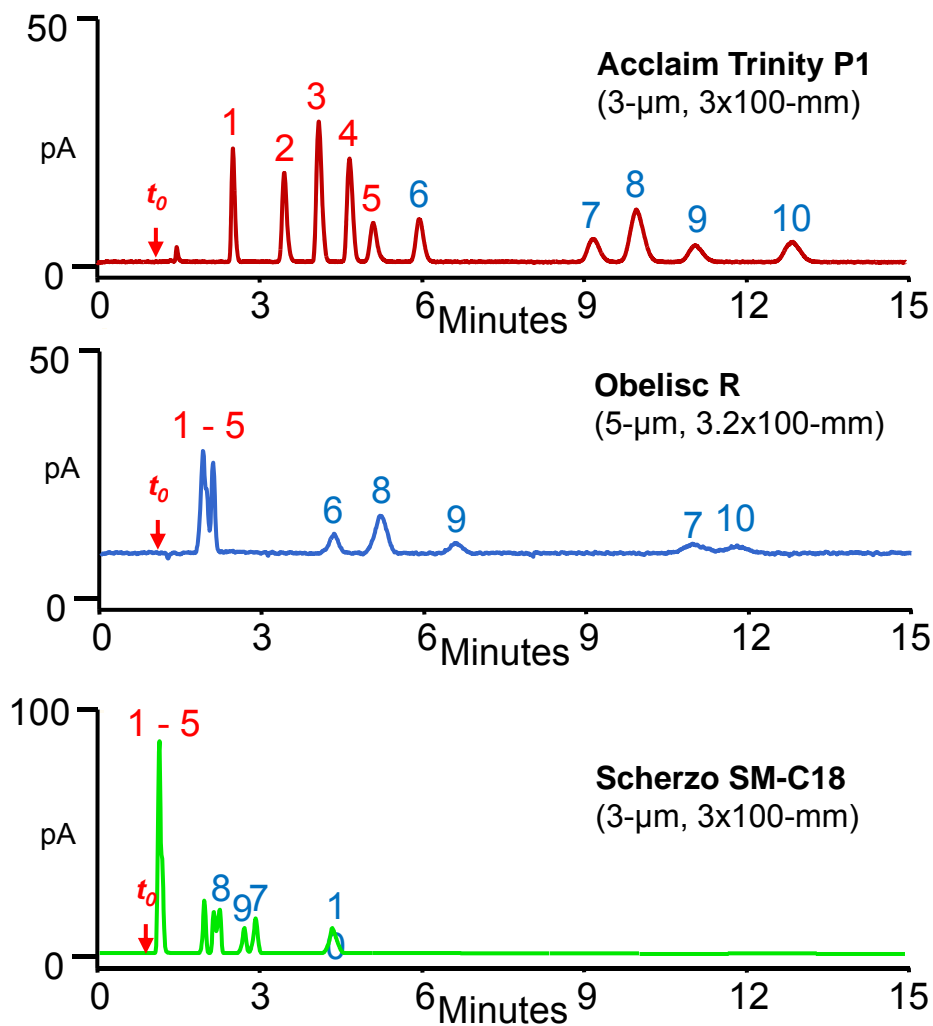
Obelisc R (SIELC)

Type III



Acclaim Trinity P1 (Thermo Fisher)

Pharmaceutical Counterions



Chromatographic Conditions:

Mobile Phase: Isocratic, optimized for each column

	MeCN	D.I. H ₂ O	100 mM NH ₄ OAc, pH5.0
Trinity P1	60%	25%	15%
Obelisc R	20%	70%	10%
Scherzo SM-C18	20%	70%	10%

Temperature: 30 ° C

Flow Rate: 0.5 mL/min

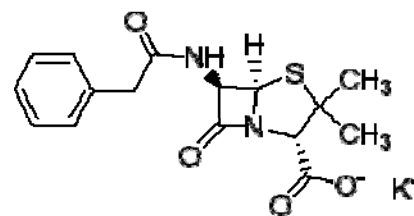
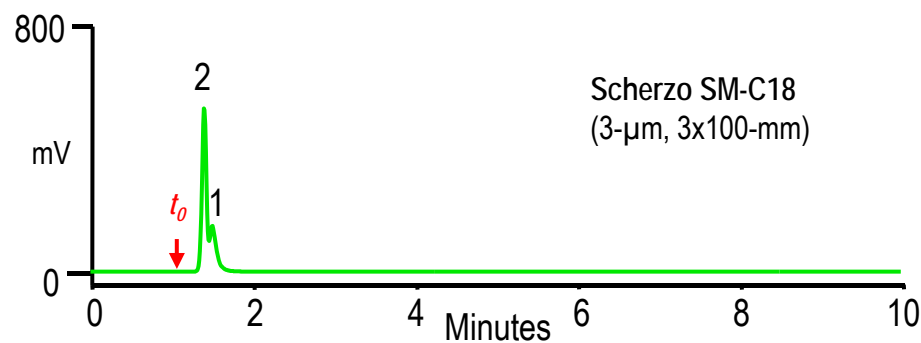
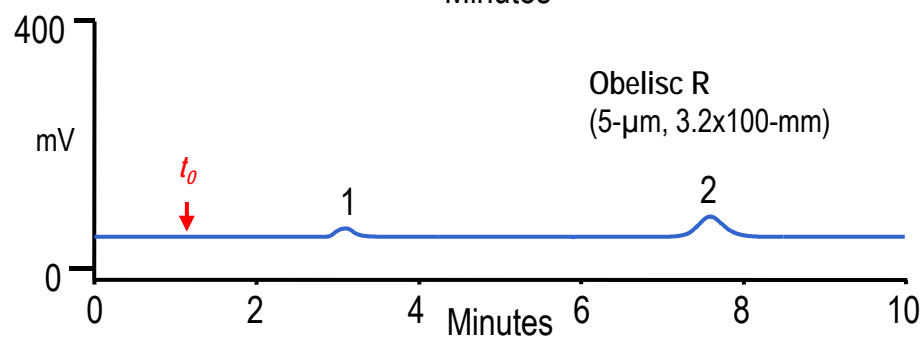
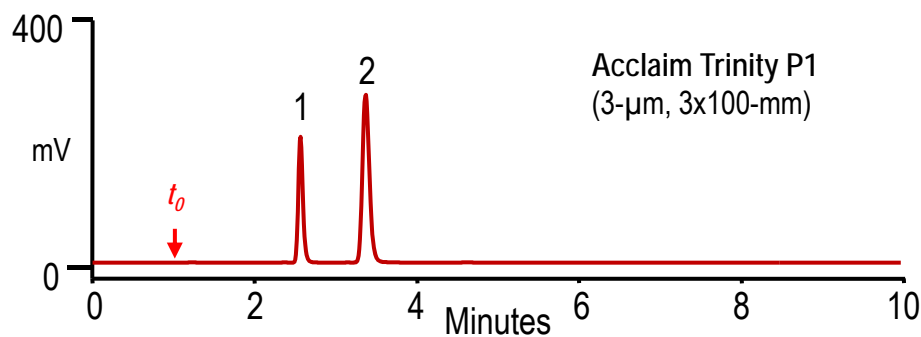
Inj. Volume: 2 μ L

Detection: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 30 ° C)

Peaks: (50 to 100 ppm)

- 1. Choline
- 2. Tromethamine
- 3. Sodium
- 4. Potassium
- 5. Meglumine
- 6. Mesylate
- 7. Nitrate
- 8. Chloride
- 9. Bromide
- 10. Iodide

Hydrophilic Acidic API & Counterion – Penicillin G Potassium



Chromatographic Conditions:

Mobile Phase: Isocratic, optimized for each column

	MeCN	D.I. H ₂ O	100 mM NH ₄ OAc, pH5.0
Trinity P1	60%	0%	40%
Obelisc R	50%	40%	10%
Scherzo SM-C18	70%	20%	10%

Temperature: 30 °C

Flow Rate: 0.5 mL/min

Inj. Volume: 1 μ L

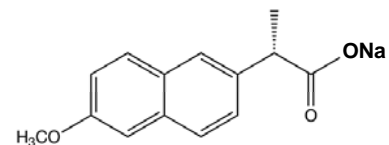
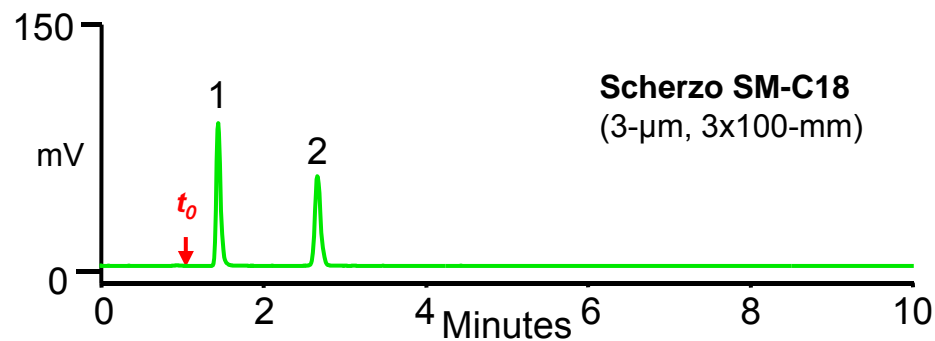
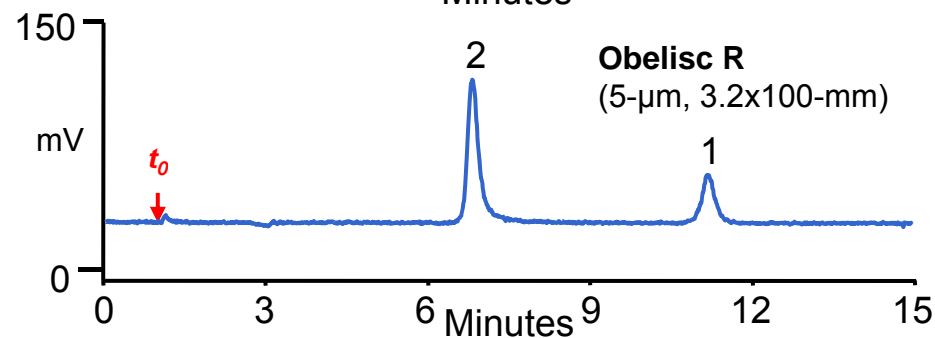
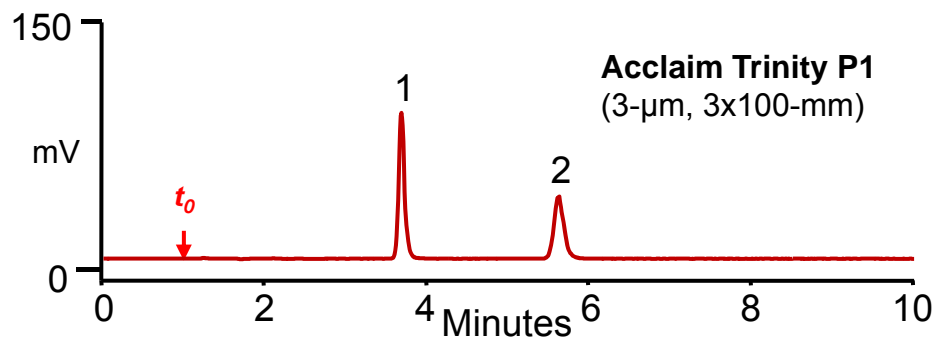
Detection: Aerosol based detector

Peaks: (0.2 mg/mL)

1. K⁺

2. Penicillin G

Hydrophobic Acidic API & counterion – Sodium Naproxen



Chromatographic Conditions:

Mobile Phase: Isocratic, optimized for each column

	MeCN	D.I. H ₂ O	100 mM NH ₄ OAc, pH5.0
Trinity P1	75%	0%	25%
Obelisc R	80%	0%	20%
Scherzo SM-C18	65%	30%	5%

Temperature: 30 °C

Flow Rate: 0.5 mL/min

Inj. Volume: 1 μ L

Detection: Aerosol based detector

Peaks: (0.2 mg/mL)

1. Na⁺

2. Naproxen