HPLC, LC/MS Columns

InertSustain AQ-C18

Future From Fukushima.

GL Sciences

Physical Properties

InertSustain® AQ-C18

✓ Silica	: ES Silica Gel	✓ Carbon Load	: 13.0 %
✓ Particle Size	: 1,9 µm, 3 µm, 5 µm	✓ End-capping	: Complete
✓ Surface Area	: 350 m^2/g	✓ pH Range	: 1 – 10
✓ Pore Size	: 100 Å (10 nm)	✓ USP Code	: L1
✓ Pore Volume	: 0.85 mL/g		
✓ Bonded Phase	: Octadecyl Groups		
✓ Bonded-Phase Structure	: * Monomeric		

**** Although it is classified as a monomeric type ODS, the bonding of ODS groups are optimized to retain highly polar compounds.***

Benefits of InertSustain AQ-C18

- Exceptional polar compound retention in reversed phase mode
- The usage of highly inert packing with superior column lifetime

✓ True polar compound retention without using any secondary interaction
✓ Improve separation between polar analytes and solvent peaks or sample matrices
✓ Enhanced retention can also prevent ion suppression in LC/MS methods

Method Development for Polar Compounds

First Choice Column
Conventional ODS Column

In case there were no retention of polar compounds…

1. Use of ion-pairing reagents

2. Use of 100% water mobile phase

Often experience shorter column lifetime, poor method reproducibility, increased instrument maintenance…

What other options are left?

Second Choice Columns

3. Use of different reversed phase columns

- Selecting different ODS columns having different carbon loading %
- Selecting polar embedded ODS columns
- Selecting different stationary phase columns such as Phenyl or PFP columns

- Really hard to tell if the polar analytes will be retained or not.
- Adsorbed peaks of target analytes occur due to secondary interaction.

Second Choice Columns

4. Use of different interactions

- Selecting mix mode columns (ion-exchange + ODS)
- Selecting HILIC columns (hydrophilic interaction)

▼

- Use of high concentration buffer (harsh mobile phase).
- Reproducibility issues.
- Takes time to equilibrate columns prior to the analysis.

Method Development for Polar Compounds

How will InertSustain AQ-C18 columns help with your polar samples?

- Easier to develop and optimize analytical conditions
- Offer strong polar compound retention even under 100% water or water rich mobile phases

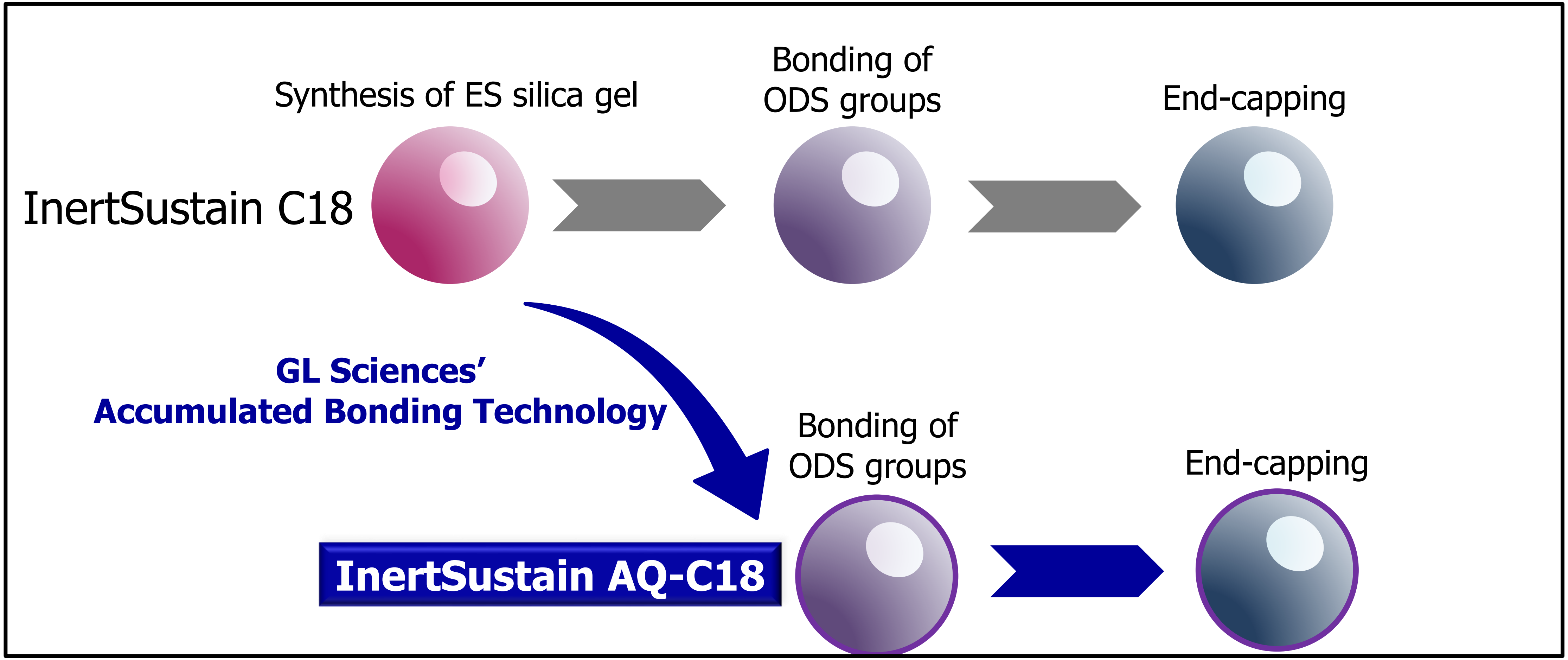
How will InertSustain AQ-C18 columns help with your polar samples?

- The usage of highly inert packing results in delivering symmetric peaks for virtually any type of compounds

- Eliminating the use of ion-pairing reagents improves method reproducibility, extends column lifetime and reduces instrument maintenance

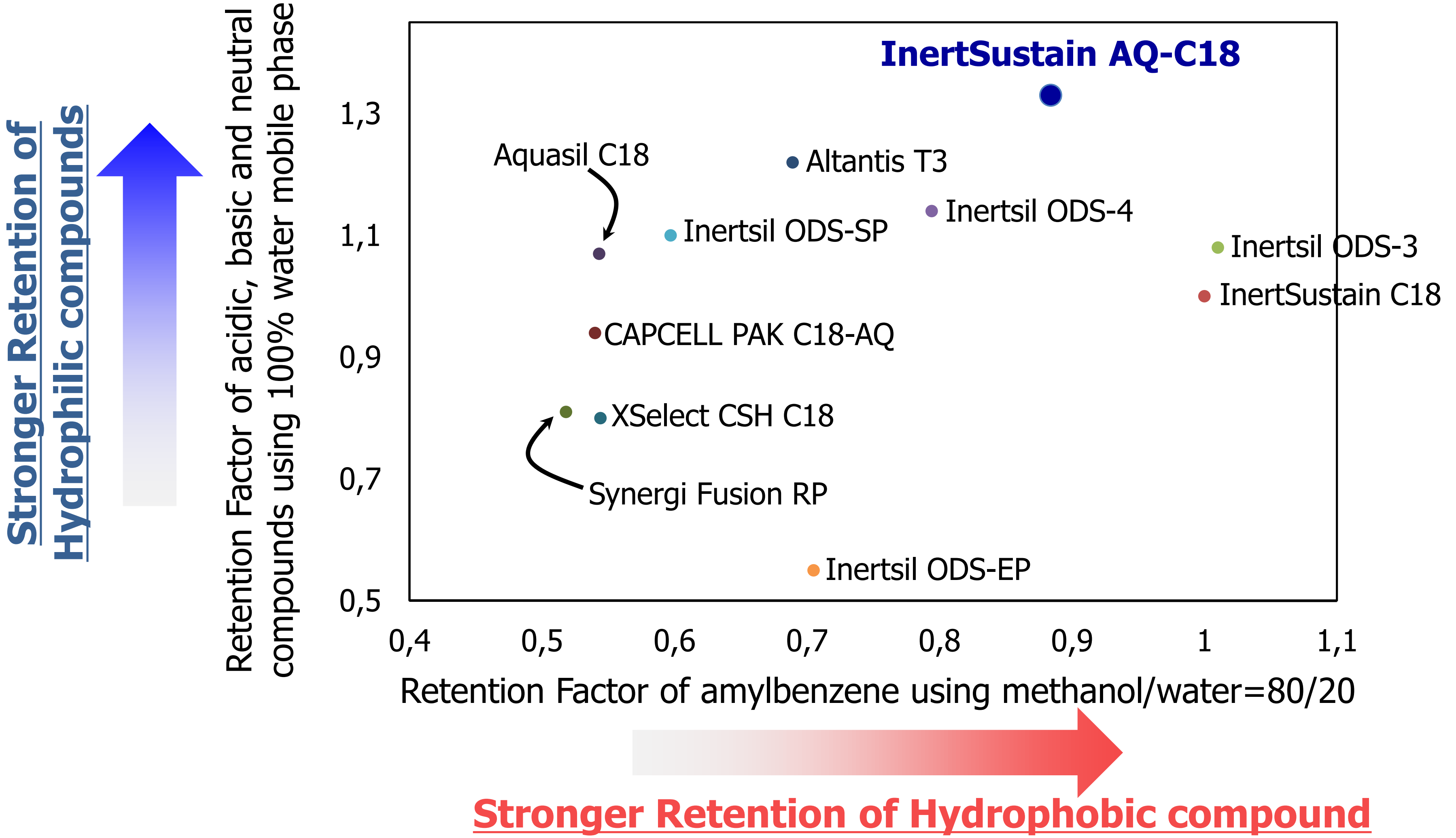
GL Sciences' Accumulated Bonding Technology

GL Sciences' accumulated bonding technology and know-how allows InertSustain AQ-C18 to provide exceptional polar compound retention. Specifically, optimization of bonding of the C18 groups at equal distance to the silica gel enable InertSustain AQ-C18 to offer significant retention for highly polar compounds even under water rich mobile phases.



Retention Properties of InertSustain AQ-C18

Comparison of Retentivity for Hydrophilic compounds between various ODS columns



Remarks:

1. The horizontal and vertical retention factor values were calculated with reference to the retention factor values obtained from InertSustain C18.
2. The horizontal values represents the retention factor of amylbenzene using methanol/water=80/20.
3. The vertical values represents the retention factor of acidic, basic and neutral compounds using 100% water mobile phase. (For further details, please see the next 2 slides)

Determination of Retentivity for Hydrophilic Compounds

Two different mobile phases were used in this testing, 0.1 % formic acid in H_2O and 0.1 % phosphoric acid in H_2O. And total of 6 compounds were used to determine the retention factor of these 6 compounds to confirm the retentivity of each column.

Testing Conditions 1.

Column : 5 μm, 150 × 4.6 mm I.D.

Eluent : 0.1 % formic acid in H_2O

Sample:

1. **Pyridoxamine (Basic)**
2. **Dopamine (Basic)**
3. **Acetic Acid (Acidic)**
4. **Succinic Acid (Acidic)**
5. **Uracil (Neutral)**
6. **5-methyluridine (Neutral)**

Testing Conditions 2.

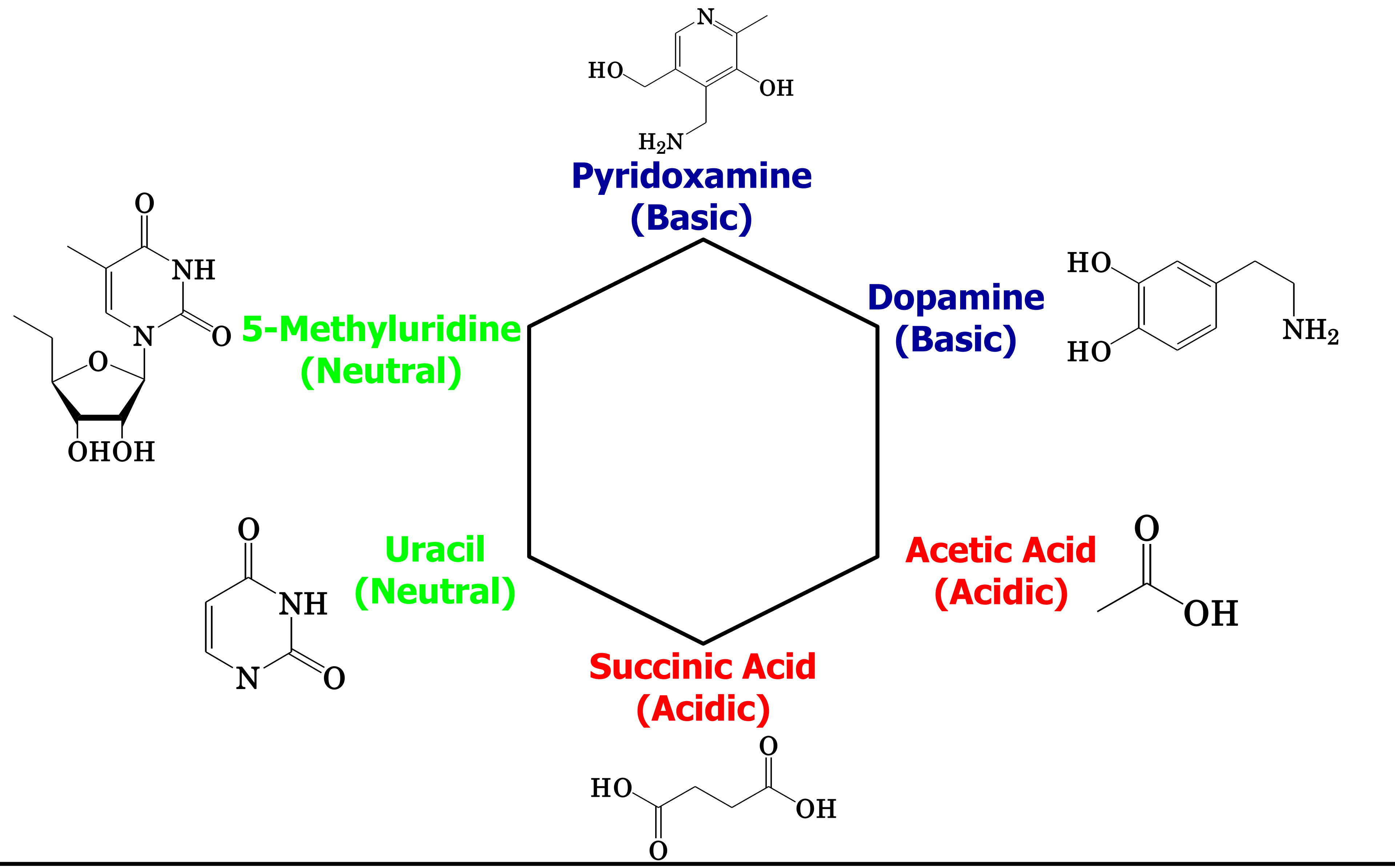
Column : 5 μm, 150 × 4.6 mm I.D.

Eluent : 0.1 % phosphoric acid in H_2O

Sample:

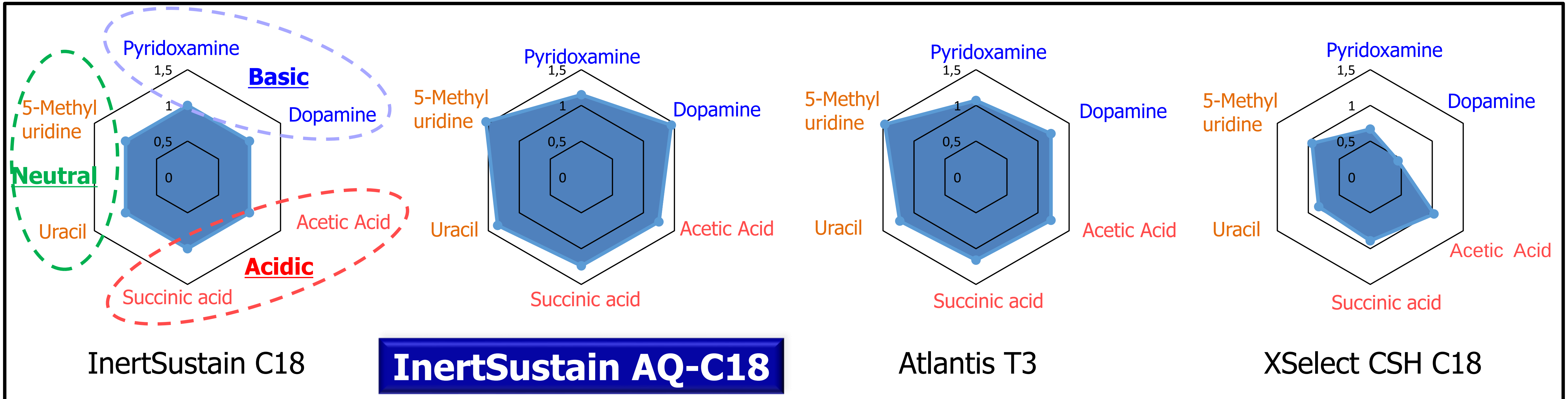
1. **Pyridoxamine (Basic)**
2. **Dopamine (Basic)**
3. **Acetic Acid (Acidic)**
4. **Succinic Acid (Acidic)**
5. **Uracil (Neutral)**
6. **5-methyluridine (Neutral)**

Determination of Retentivity for Hydrophilic Compounds



Determination of Retentivity for Hydrophilic Compounds

The following radar chart was created with reference to the retention factor values obtained from **InertSustain C18 (conventional ODS)**. **When the surface of silica is neutral and interactions between hydrophobic molecules are present under water rich mobile phases, the blue highlighted area will show larger area.**

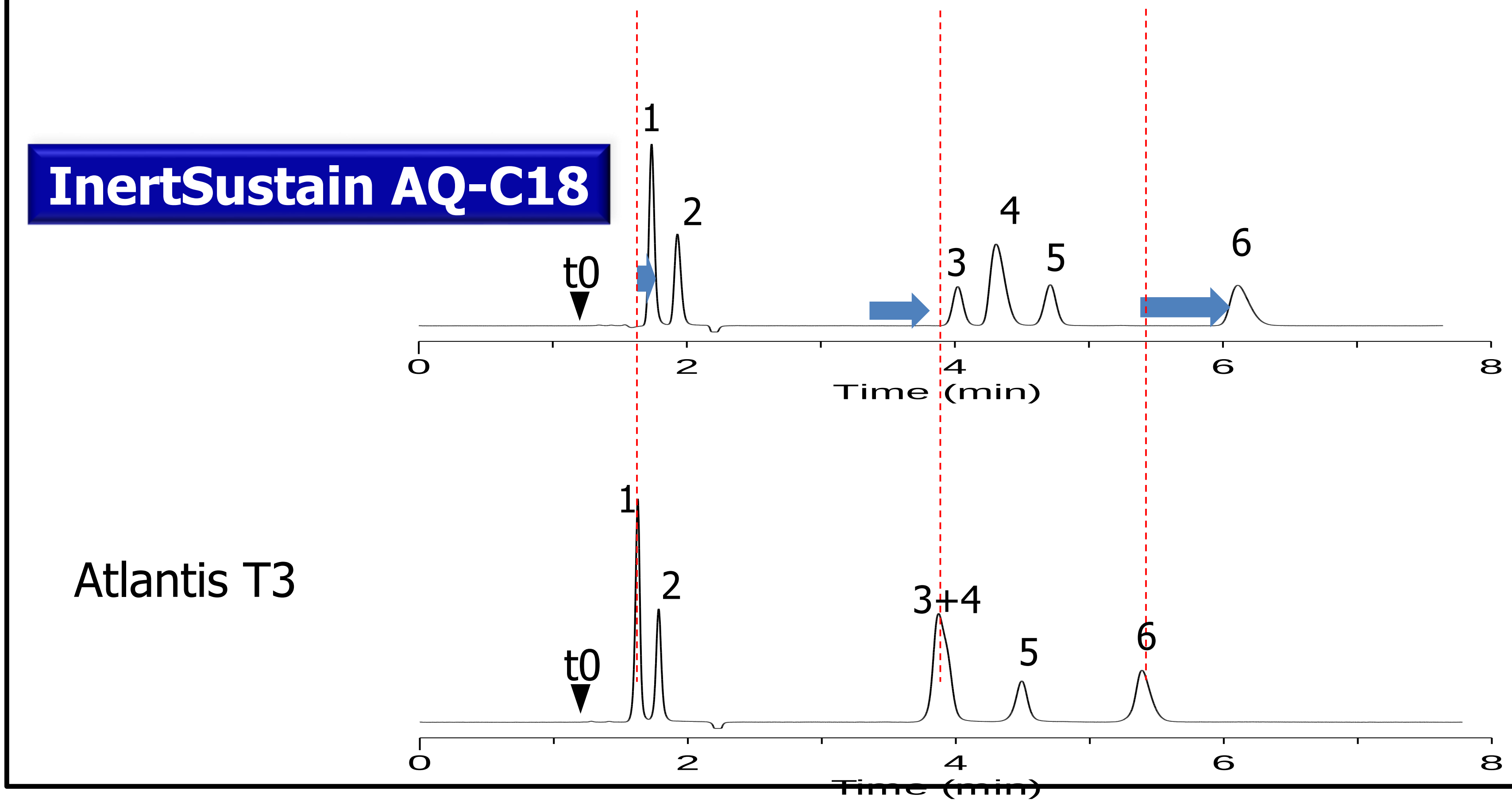


When the surface of the silica gel is basic: Retentivity = Acidic >> Basic (Neutral compounds are not influenced)

When the surface of the silica gel is acidic: Retentivity = Basic >> Acidic (Neutral compounds are not influenced)

Comparison of Retention for Water-Soluble Vitamin B

The use of ion-pairing reagents is a popular approach to retain water-soluble vitamins. Instead, 0.1% HCOOH in H_2O was used to demonstrate and compare the retention for water-soluble vitamins. As proven below, InertSustain AQ-C18 delivered significant retention without the need for such reagents.



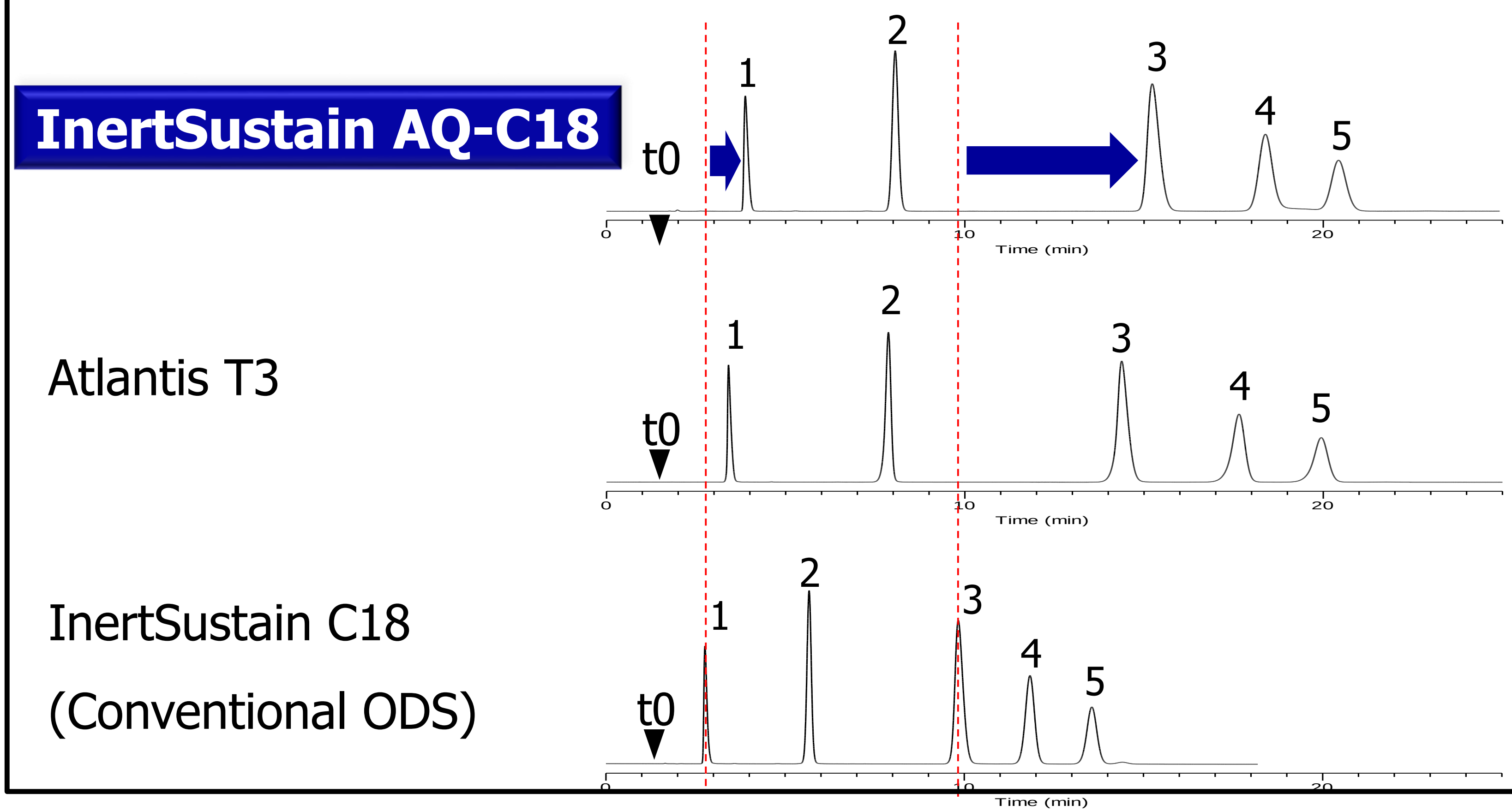
Conditions

Column : 5 µm, 150 × 4.6 mm I.D.
Eluent : 0.1% HCOOH in H_2O
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 210 nm

Sample
1. Pyridoxamine (B6)
2. Thiamin (B1)
3. Nicotinic Acid (B3)
4. Pyridoxal (B6)
5. Nicotinamide (B3)
6. Pyridoxine (B6)

Comparison of Retention for Nucleosides

Nucleosides are often analyzed in HILIC mode as they are highly hydrophilic . However, ODS columns also show some retention under 100% water mobile phase. In the following test, InertSustain AQ-C18 demonstrated to show simply stronger retention without the change of selectivity and analytical condition.



Conditions

Column : 5 μm, 150 × 4.6 mm I.D.
Eluent : 0.1% HCOOH in H_2O
Flow Rate : 1.0 mL / min
Col. Temp. : 40 ℃
Detection : UV 254 nm

Sample
1. Cytidine
2. Uridine
3. Adenosine
4. Guanosine
5. 5-Methyl uridine

Comparison of Retention for Catechins (Neutral)

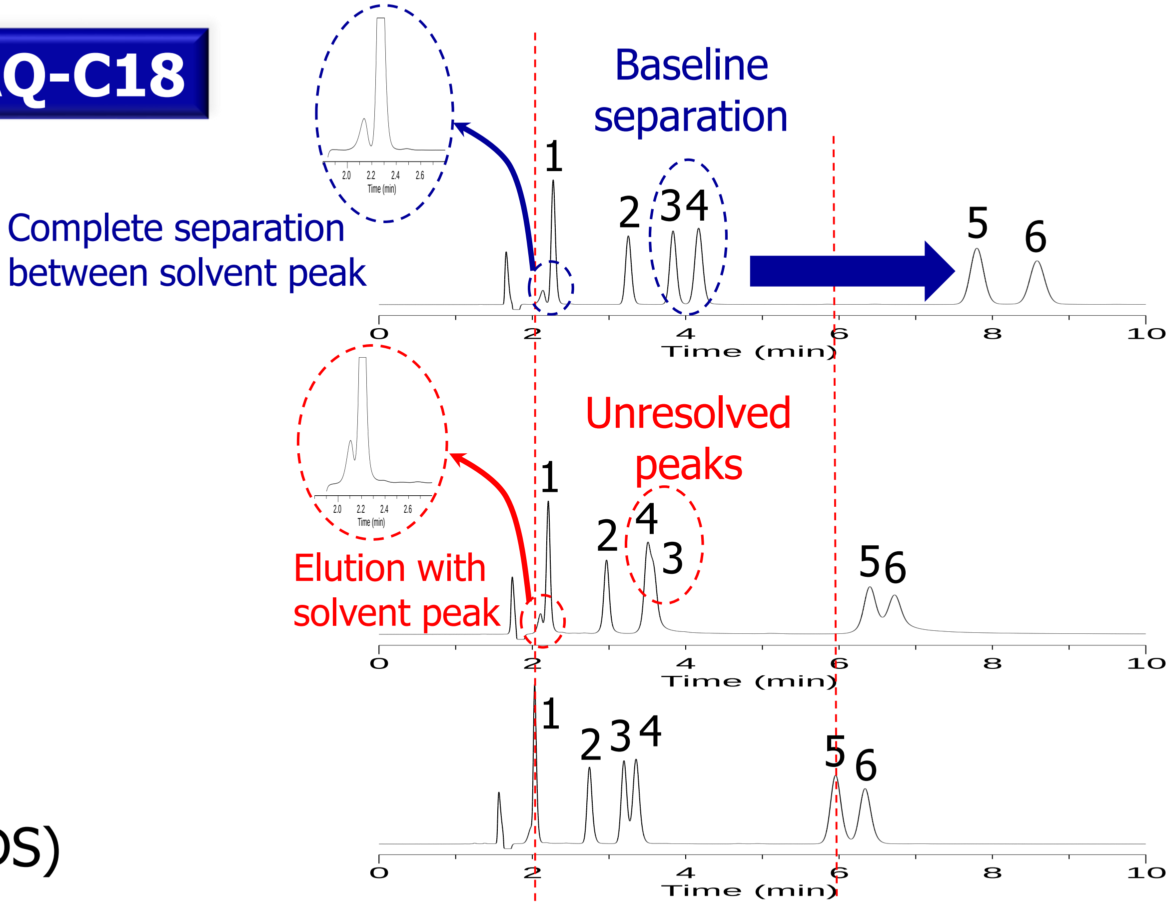
As shown below, InertSustain AQ-C18 delivered stronger retention of catechins even under 20% organic solvent mobile phase with exceptional peak shapes, while competitive column brand failed.
Furthermore, InertSustain AQ-C18 can prevent the co-elution between the targeted polar analytes and solvent peaks or sample matrices due to its enhanced retentivity.

InertSustain AQ-C18

Atlantis T3

InertSustain C18

(Convetnional ODS)



Conditions

Column : 5 µm, 150 × 4.6 mm I.D.

Eluent : **A) ACN**

B) 0.1% HCOOH in H_2O

A/B = 20 /80

Flow Rate : 1.0 mL / min

Col. Temp. : 40℃

Detection : UV 280 nm

Injection Vol. : 1 µL

Sample:

1. Gallocatechin(-) (GC(-))
2. Catechin(+) (C)
3. Epicatechin (-) (EC)
4. Epigallocatechin gallate(-) (EGCg)
5. Epicatechin gallate(-) (ECg)
6. Catechin gallate(-) (Cg)

100 µg/mL each

Comparison of Retention for Coffee Samples (Acidic, Basic)

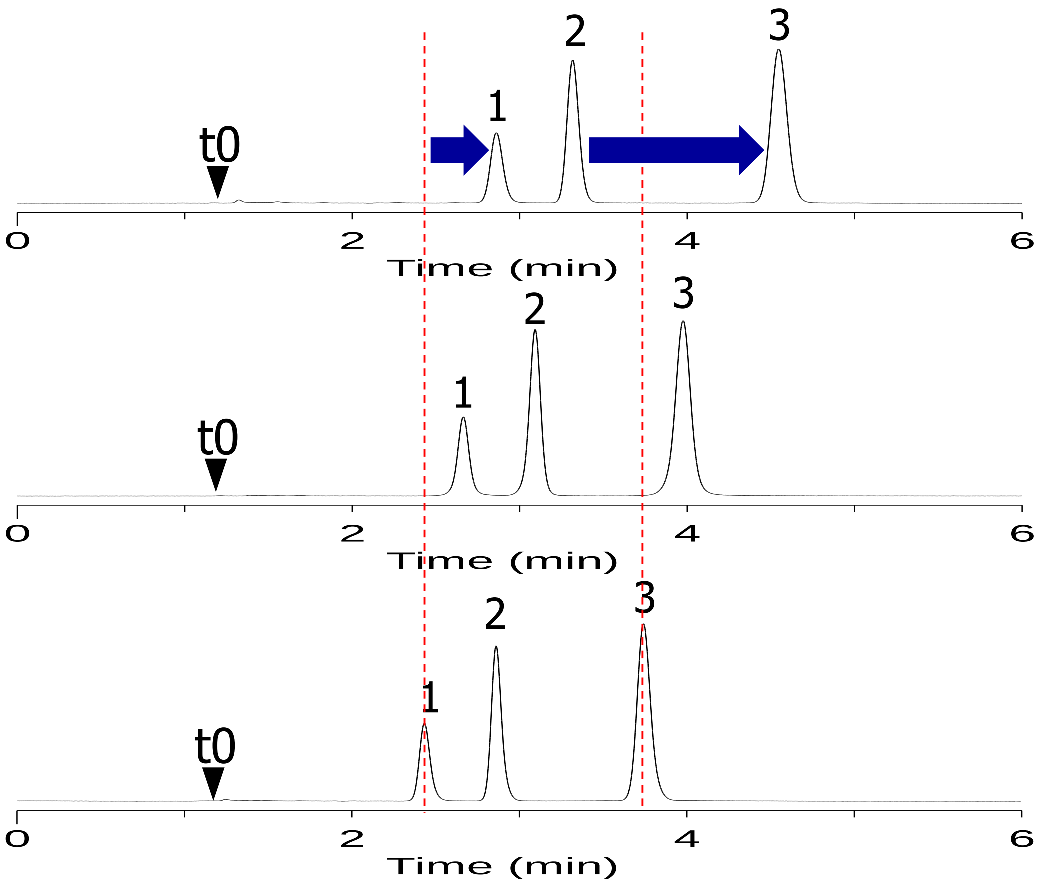
InertSustain AQ-C18 demonstrated stronger retention for hydrophilic coffee samples (acidic, basic) under 20% organic solvent mobile phase.

InertSustain AQ-C18

Atlantis T3

InertSustain C18

(Convetnional ODS)



Conditions

Column : 5 µm, 150 × 4.6 mm I.D.

Eluent : **A) ACN**

B) 0.1% HCOOH in H_2O

A/B = 20 /80

Flow Rate : 1.0 mL / min

Col. Temp. : 40 ℃

Detection : UV 270 nm

Injection Vol. : 1 µL

Sample

1. Chlorogenic acid 100 µg/mL (Acidic)
2. Caffeine 100 µg/mL (Basic)
3. Caffeic acid 100 µg/mL (Acidic)

Exceptional Peak Shapes + Retention for Polar Compounds

The structure of highly polar compounds contains ionic groups or metal complexes and they tend to be adsorbed to the packing material which results in tailing of peaks. In addition, polar embedded C18 columns or C18 columns with hydrophilic end-capping are available in the market for retaining highly polar compounds. However, they often show tailing or poor peak shapes due to the secondary interaction from their embedded functional groups.

InertSustain AQ-C18 not only provide strong retention for highly polar compounds, but also delivery symmetric peak shapes for virtually any type of analytes.

InertSustain AQ-C18

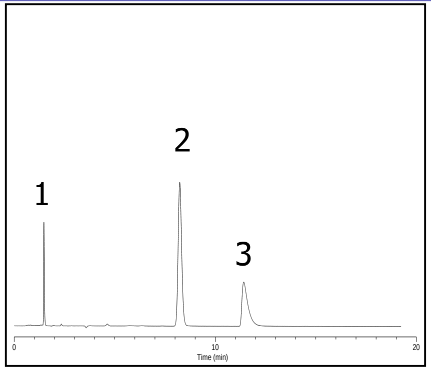
Offer Both Superior Benefits

Retention for Polar Compounds + Highly Inert Packing Material

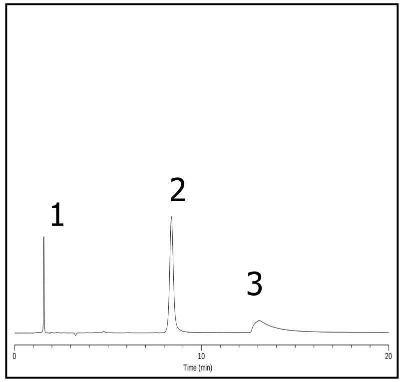
Comparison of Peak Shapes for Basic Compounds

Dextromethorphan is a strong basic compound. Severe tailing can be confirmed when the packing material contains residual silanol groups.

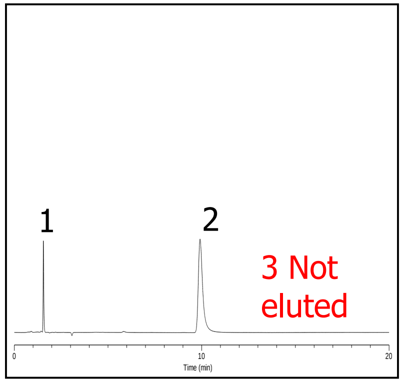
InertSustain AQ-C18



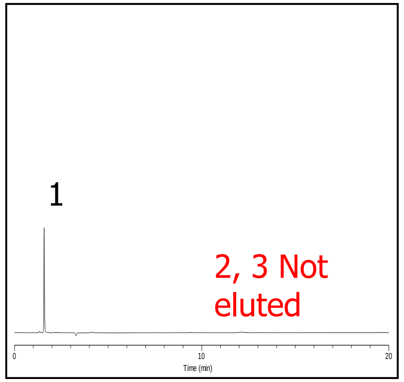
Atlantis T3



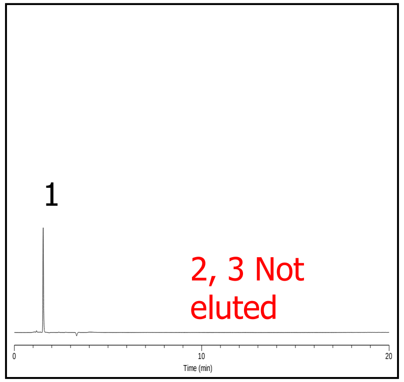
CAPCELL PAK C18-AQ



Synergi Fusion RP



Aquasil C18



Conditions

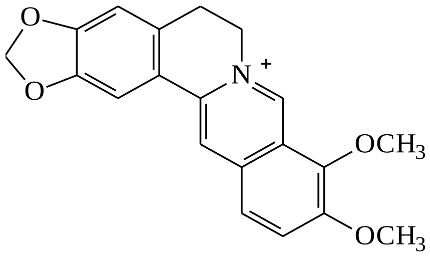
Eluent : A) CH_3CN B) 25 mM K_2HPO_4 (pH 7.0)
A/B = 30/70,v/v

Flow Rate : 1 mL/min

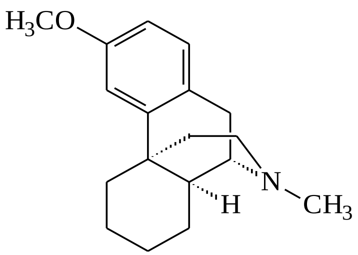
Col. Temp. : 40 ℃

Detection : UV 230 nm

Sample : 1. Uracil
2. Berberine chloride
3. Dextromethorphan



2. Berberine chloride

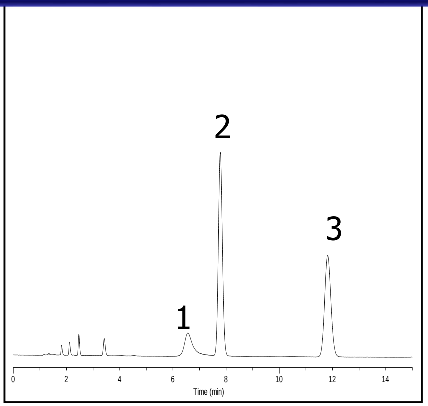


3. Dextromethorphan

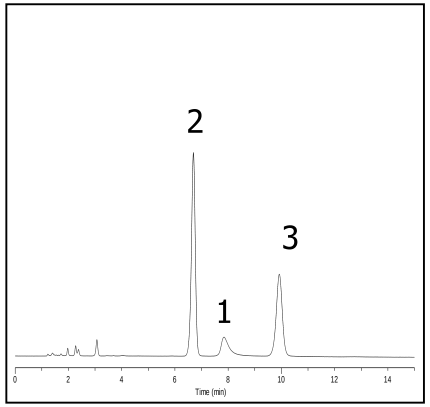
Comparison of Peak Shapes for Acidic Compounds

Sharp peaks can be obtained when analyzing Phenol or Salicylic Acid. However, as Brilliant Blue FCF has three sulfonic groups in its chemical structure, tailing will occur when the surface of the packing material is slightly basic.

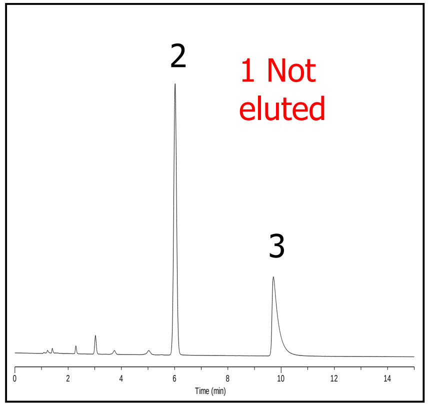
InertSustain AQ-C18



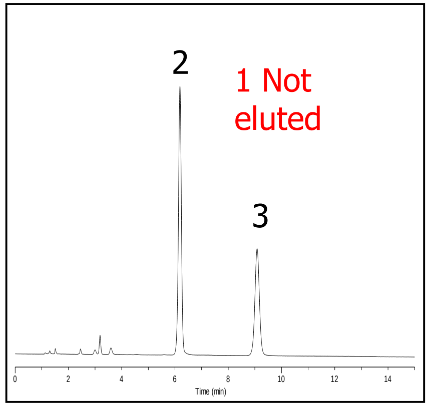
Atlantis T3



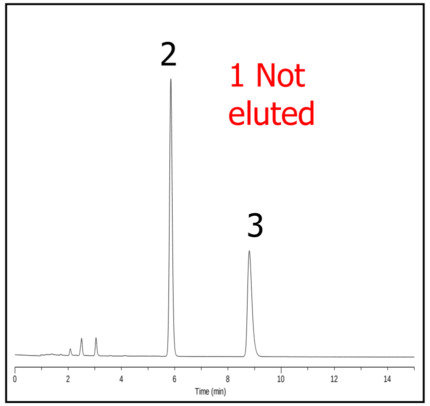
CAPCELL PAK C18-AQ



Synergi Fusion RP

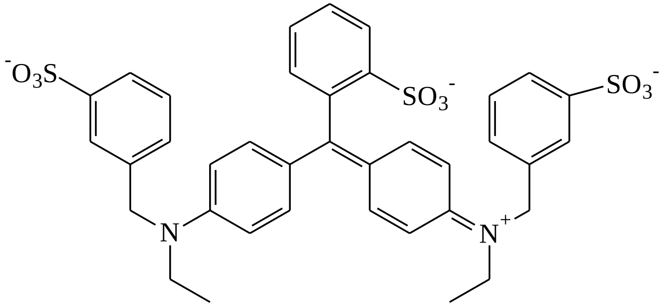


Aquasil C18



Conditions

Eluent : A) CH_3CN B) 0.1% H_3PO_4 in H_2O
A/B = 25/75,v/v
Flow Rate : 1 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm
Sample : **1. Brilliant Blue FCF**
2. Phenol
3. Salicylic acid

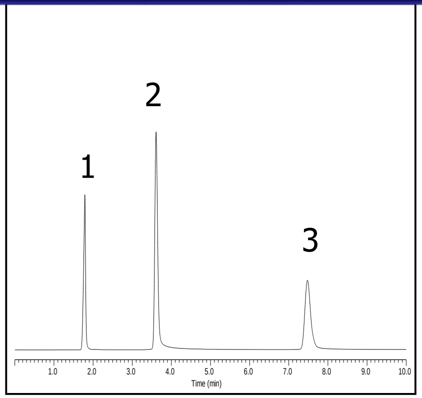


1. Brilliant Blue FCF

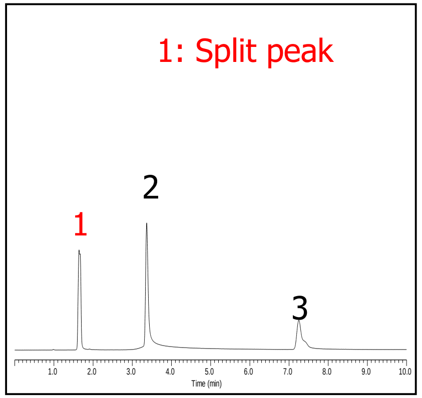
Comparison of Peak Shapes for Chelating Compounds

Hinokitiol and Piroctone Olamine are strong chelating compounds, which coordinately binds with the surface of residual trace metal impurities, resulting in severe tailing. However, the peak shape improves as the injection increases since the surface of the packing material of the adsorption active sites eventually become masked.

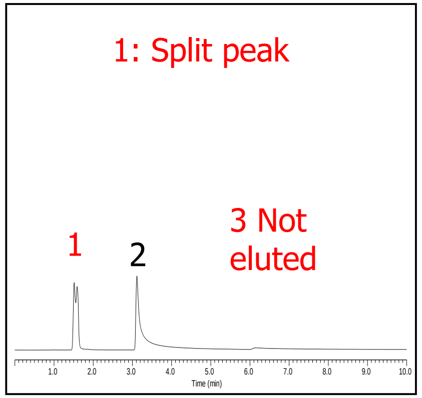
InertSustain AQ-C18



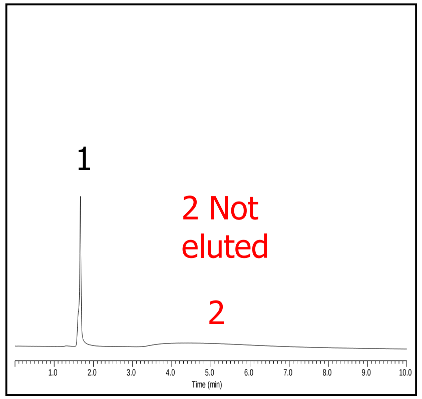
Atlantis T3



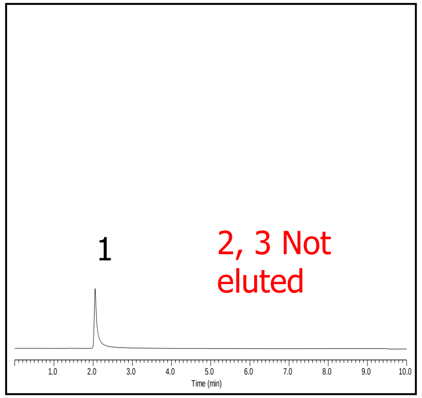
CAPCELL PAK C18-AQ



Synergi Fusion RP

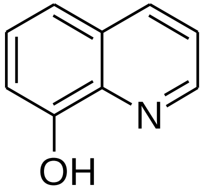


Aquasil C18

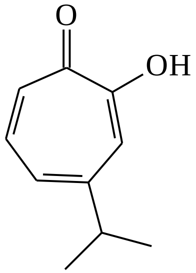


Conditions

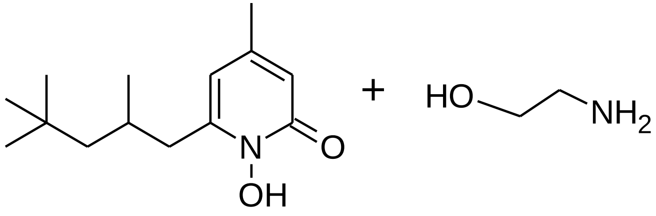
Eluent : A) CH_3CN B) 0.1%HCOOH in H_2O
A/B = 40/60
Flow Rate : 1mL/min
Col. Temp. : 40 ℃
Detection : UV 310 nm
Sample : 1. 8-Quinolinol
2. Hinokitiol
3. Piroctone Olamine



1. 8-Quinolino



2. Hinokitiol



3. Piroctone Olamine

Comparison of Compatibility with 100% Water Mobile Phase

When analyzing hydrophilic compounds under water rich mobile phase condition, once the pump is stopped, the hydrophobic bonded group pushes the aqueous mobile phase out off the pore in an irreversible fashion, in what has become known as the dewetting phenomenon. InertSustain AQ-C18 guarantees compatibility with 100% aqueous mobile phases without the risk of dewetting.

Testing Procedure:

1) 100 % water is introduced into column over 60 minutes.
2) Conduct Analysis (1^{st} injection).
3) Stop flow for 15 minutes.
4) 100 % water is introduced again into column over 30 minutes.
5) Conduct Analysis (2^{nd} injection).
6) Stop flow for 15 minutes again.
7) 100 % water is introduced again into column over 30 minutes.
8) Conduct Analysis (3^{rd} injection).
9) Disconnect the column from the system and stored for 24 hrs.
10) 100 % water is introduced into column over 30 minutes.
11) Conduct Analysis (4^{th} injection).

Conditions

Eluent : H_2O (100%)
Flow Rate : 1 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm

Sample :

1. Cytosine
2. Uracil
3. Guanine
4. Thymine
5. Adenine

Comparison of Compatibility with 100% Water Mobile Phase

InertSustain AQ-C18 | Atlantis T3

1st Injection

3rd Injection

4th Injection

Testing Procedure:

1) 100 % water is introduced into column over 60 minutes.
2) Conduct Analysis (1st injection).
3) Stop flow for 15 minutes.
4) 100 % water is introduced again into column over 30 minutes.
5) Conduct Analysis (2nd injection).
6) Stop flow for 15 minutes again.
7) 100 % water is introduced again into column over 30 minutes.
8) Conduct Analysis (3rd injection).
9) Disconnect the column from the system and stored for 24 hrs.
10) 100 % water is introduced into column over 30 minutes.
11) Conduct Analysis (4th injection).

Conditions

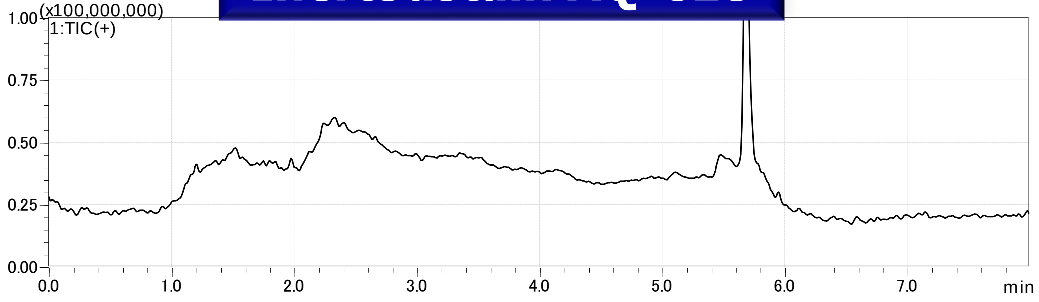
Eluent : H_2O (100%)
Flow Rate : 1 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm

Sample :

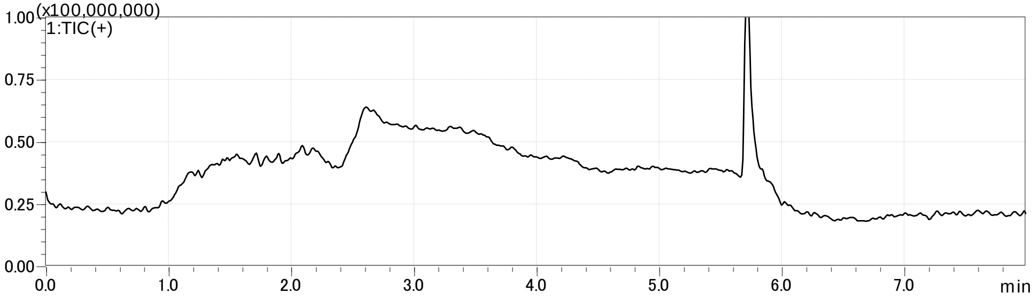
1. Cytosine
2. Uracil
3. Guanine
4. Thymine
5. Adenine

Comparison of Column Bleed for LC/MS Methods

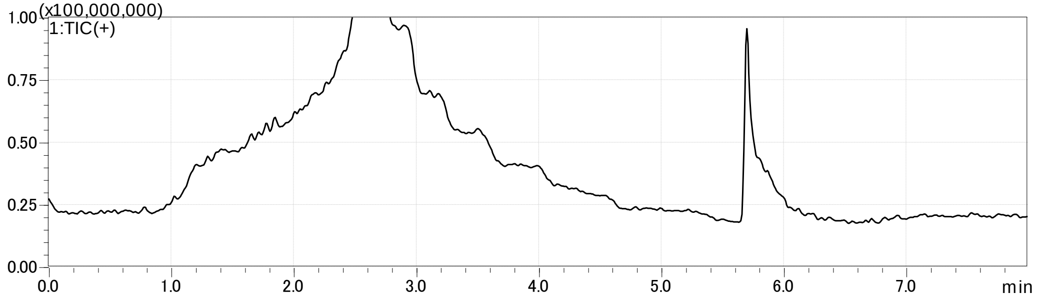
InertSustain AQ-C18



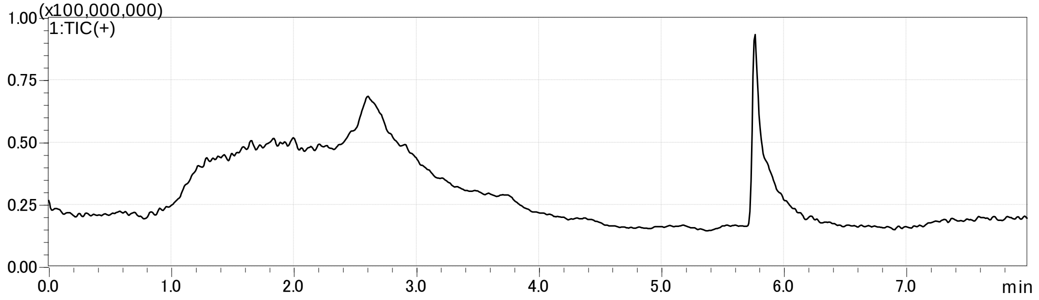
Atlantis T3



InertSustain C18



InertSustainSwift C18



Conditions

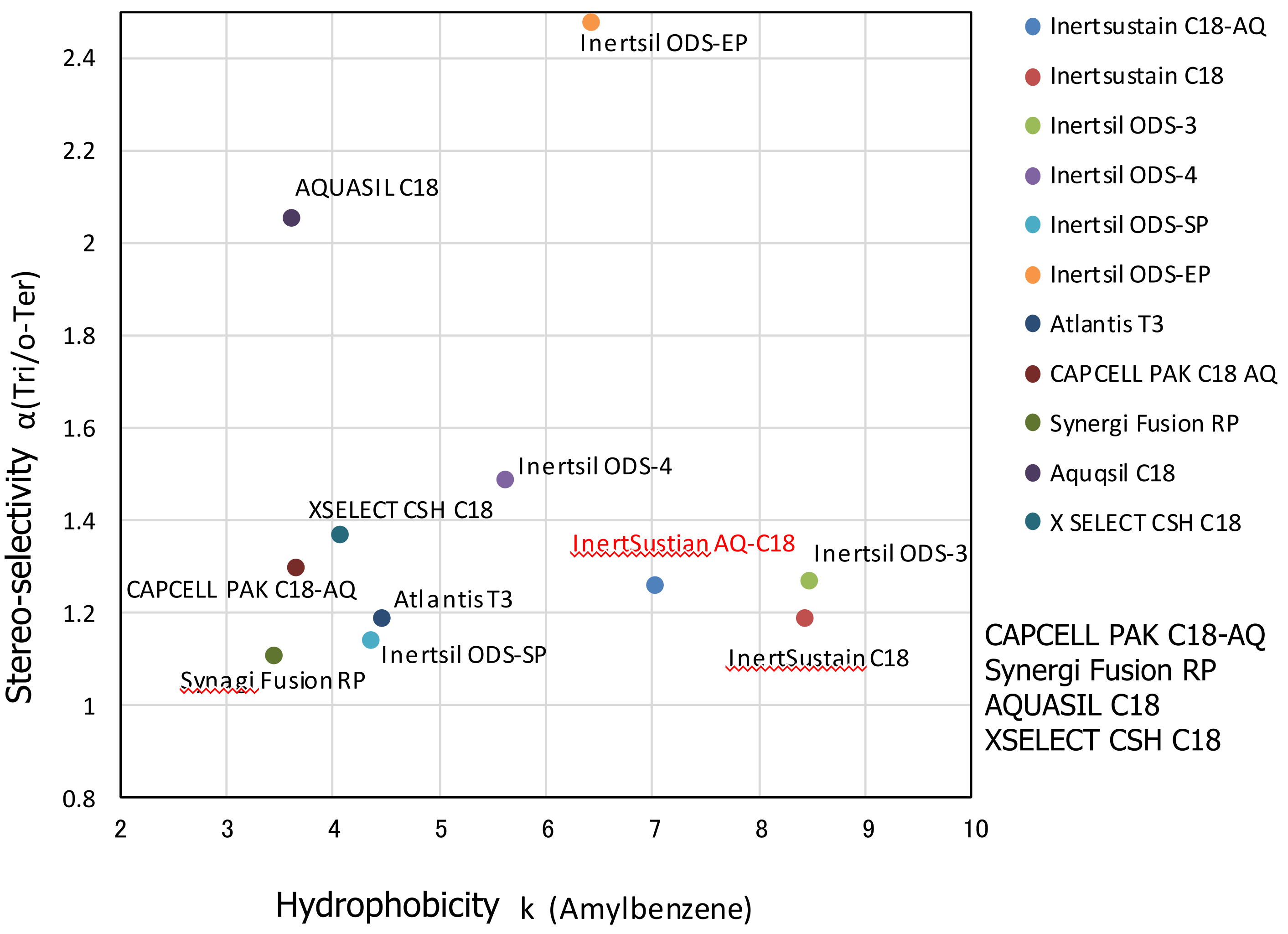
System : Nexera LCMS-8030 plus system
Column : 3 µm , 50 x 2.1 mm I.D
Eluent : A) 0.1% HCOOH in H_2O
B) 0.1% HCOOH in CH_3CN

Time (min)	B%
0	5
2	100
5	100
5.1	5
8	5

Flow Rate : 0.4 mL/min
Col. Temp. : 40 ℃
Detection : LC/MS/MS (ESI) , Positive
m/z 50 – 1,000

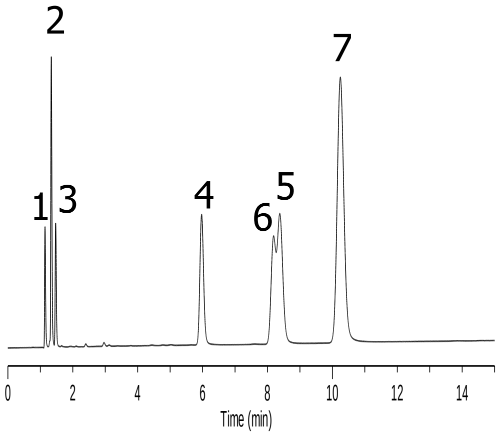
Comparison of Selectivity

The stereo-selectivity between InertSustain AQ-C18 and Inertsil ODS-3 are about the same.

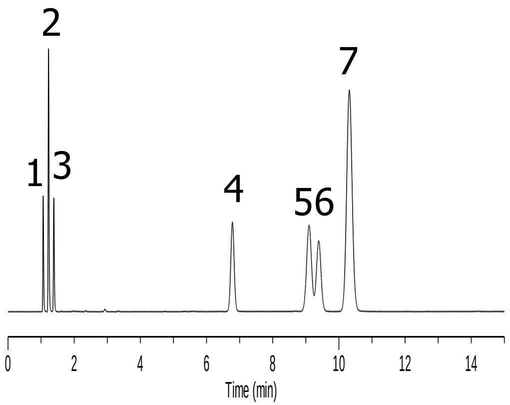


CAPCELL PAK C18-AQ	:Unknown, but probably isn't a polar embedded type
Synergi Fusion RP	:Polar embedded type
AQUASIL C18	:Hydrophilic end-capping type
XSELECT CSH C18	:Polar embedded (Surface of silica is positive charge)

Comparison of Selectivity



InertSustain AQ-C18

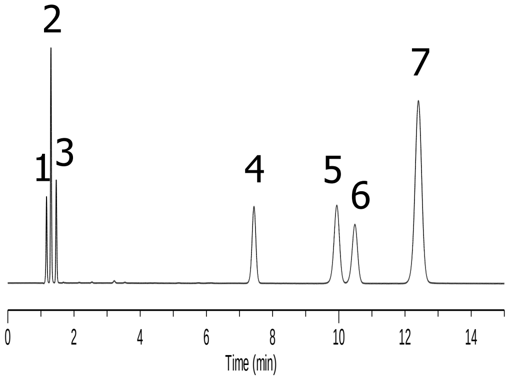


InertSustain C18

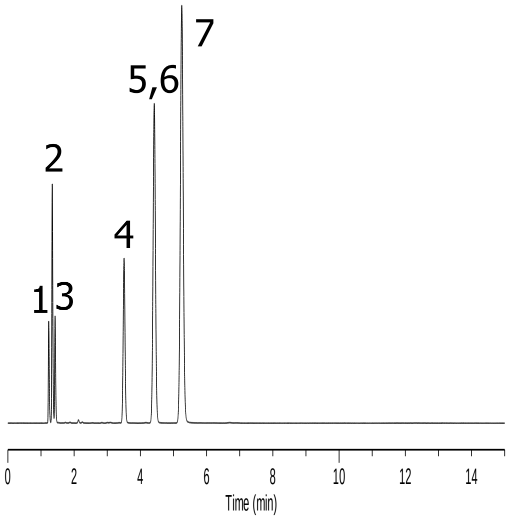
Conditions

Column :3 µm, 150 × 2.1 mm I.D.
Eluent : A) CH_3OH
B) H_2O
A/B = 80/20, v/v
Flow Rate :0.3 mL / min
Col. Temp. :40 ℃
Detection :UV 254 nm
Injection Vol. :1 µL

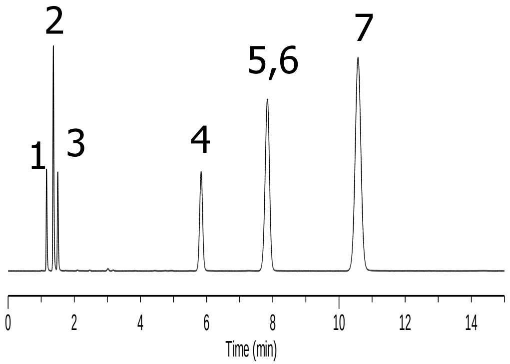
Sample:
1. Uracil 2. Caffeine 3. Phenol
4. Butylbenzene 5. *o*-Terphenyl
6. Amylbenzene 7. Triphenylene



Inertsil ODS-3



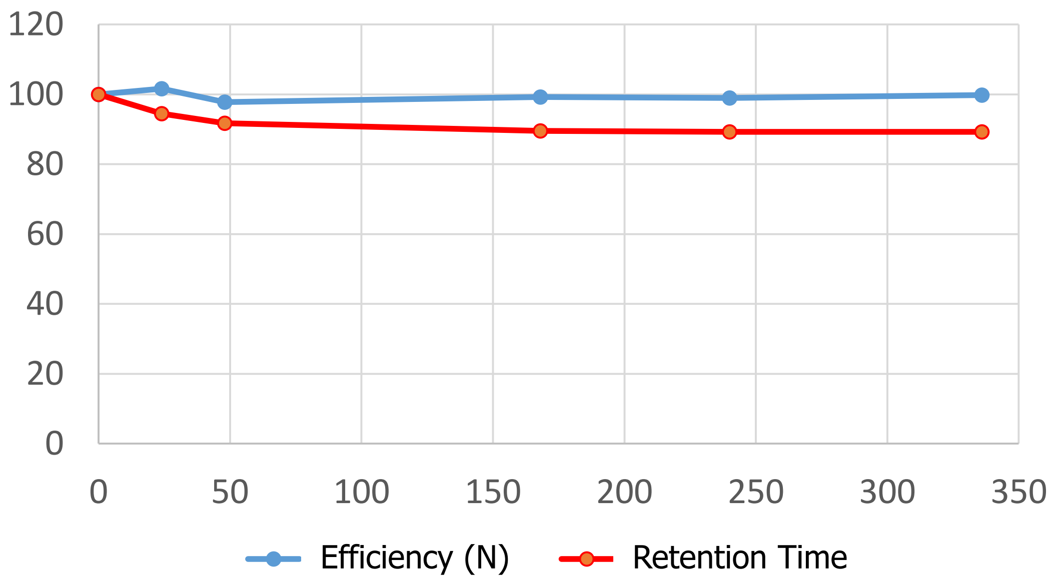
InertSustainSwift C18



Inertsil ODS-4

Extended Column Lifetime at Low pH

Durability Test at Low pH (pH 1.0)



Purging Conditions:

Column	:5 μm, 150 × 4.6 mm I.D.
Eluent	: CH_3CN/ H_2O/ TFA (10/90/1), v/v/v (pH 1)
Column Temp.	: 60 ℃

Analytical Conditions:

Eluent	: CH_3CN/H_2O = (65/35), v/v
Flow Rate	:1.0 mL / min
Col. Temp.	:40 ℃
Detection	:UV 254 nm
Sample	:Naphthalene

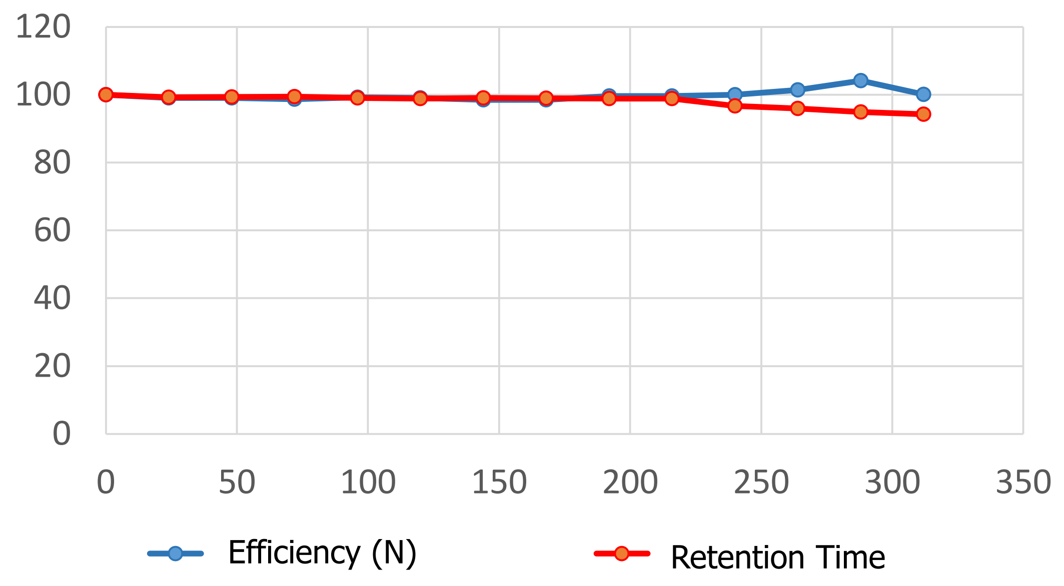
Results:

Retention Time :**Only 10.7% Retention Lost**

Efficiency (N) :**Only 0.2% Efficiency Lost**

Extended Column Lifetime at High pH

Durability Test at High pH (pH 9.5)



Purging Conditions:

Column	:5 µm, 150 × 4.6 mm I.D.
Eluent	:50 mM TEA (pH 9.5)/CH_3OH = 70/30, v/v
Flow Rate	:1.0 mL/min
Column Temp.	: 50 ℃

Analytical Conditions:

Eluent	:CH_3CN/H_2O = (65/35), v/v
Flow Rate	:1.0 mL / min
Col. Temp.	:40 ℃
Detection	:UV 254 nm
Sample	:Naphthalene

Results:

Retention Time :**Only 5.8% Retention Lost**

Efficiency (N) :**Only 0.6% Efficiency Lost**

Summary

First Choice Column
Conventional ODS Column

In case there were no retention of polar compounds…

Second Choice Column
InertSustain AQ-C18

*Easier to develop and optimize analytical conditions
*Offer strong polar compound retention even under 100% water or water rich mobile phases
*The usage of highly inert packing results in delivering symmetric peaks for virtually any type of compounds
*Eliminating the use of ion-pairing reagents improves method reproducibility, extends column lifetime and reduces instrument maintenance

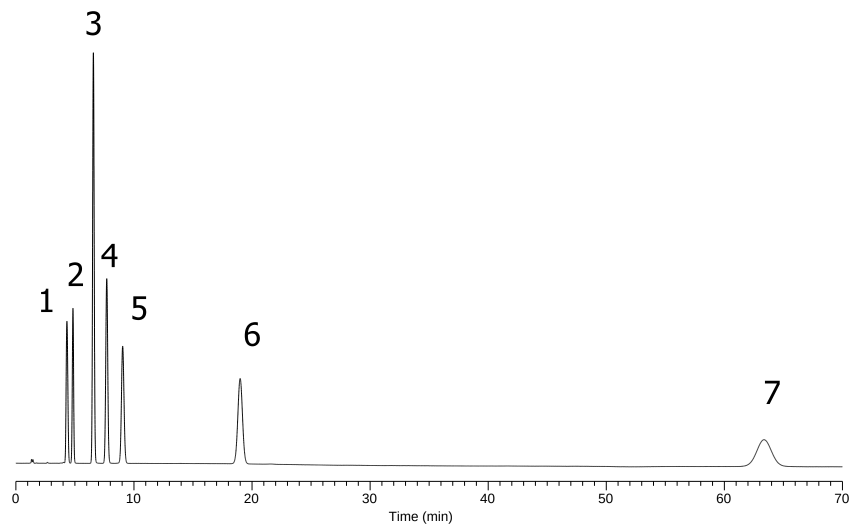
Column Selection Guide on InertSustain Series

- InertSustain C18 — First Choice Conventional ODS Column
- **InertSustain AQ-C18 — For Retaining Highly Polar Compounds**
- InertSustainSwift C18 — For Rapid Elution of samples
- InertSustain Phenyl
- InertSustain Phenylhexyl

(InertSustain Phenyl and InertSustain Phenylhexyl:) For Changing Elution Pattern and to Improve Separation via pi-pi- Interaction

Applications

Analysis of Metabolites of Purine Nucleosides

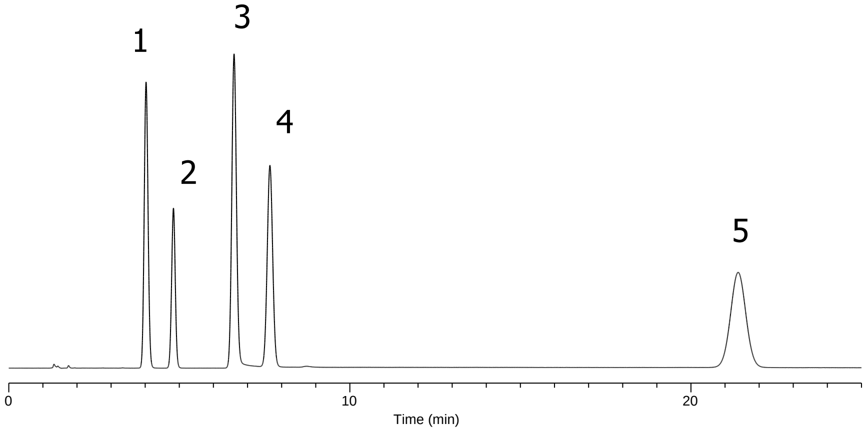


AMP Channel

Conditions

- Column : 5 µm, 150 × 4.6 mm I.D.
- Eluent : 20 mM NaH_2PO_4
- Flow Rate : 1.0 mL / min
- Col. Temp. : 40℃
- Detection : UV 254 nm
- Inj. Vol. : 2µL
- Sample : 100 ppm

1.IMP 2.Uric Acid 3. Hypoxanthine 4. Xanthine 5. AMP
6. Inosine 7. Adenosine



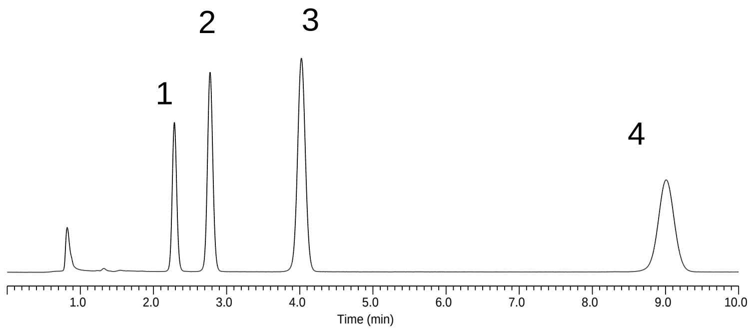
GMP Channel

Conditions

- Column : 5 µm, 150 × 4.6 mm I.D.
- Eluent : 20 mM NaH_2PO_4
- Flow Rate : 1.0 mL / min
- Col. Temp. : 40℃
- Detection : UV 254 nm
- Inj. Vol. : 2µL
- Sample : 100 ppm

1. GMP 2. Uric Acid 3. Guanine 4. Xanthine 5. Guanosine

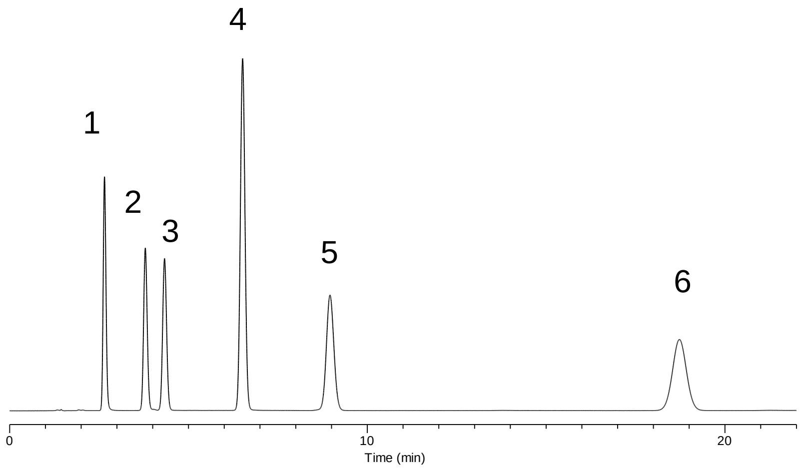
Analysis of Highly Hydrophilic Nucleotide Monophosphate



Conditions

Column	: 5 μm, 150 × 4.6 mm I.D.
Eluent	: 25mM NaH_2PO_4 in H_2O
Flow Rate	: 1.0 mL / min
Col. Temp.	: 40℃
Detection	: UV 254 nm
Inj. Vol.	: 1μL
Sample	: 500μg/mL
	1. CMP 2. UMP 3. GMP 4. AMP

Analysis of Fish Freshness, K-Value

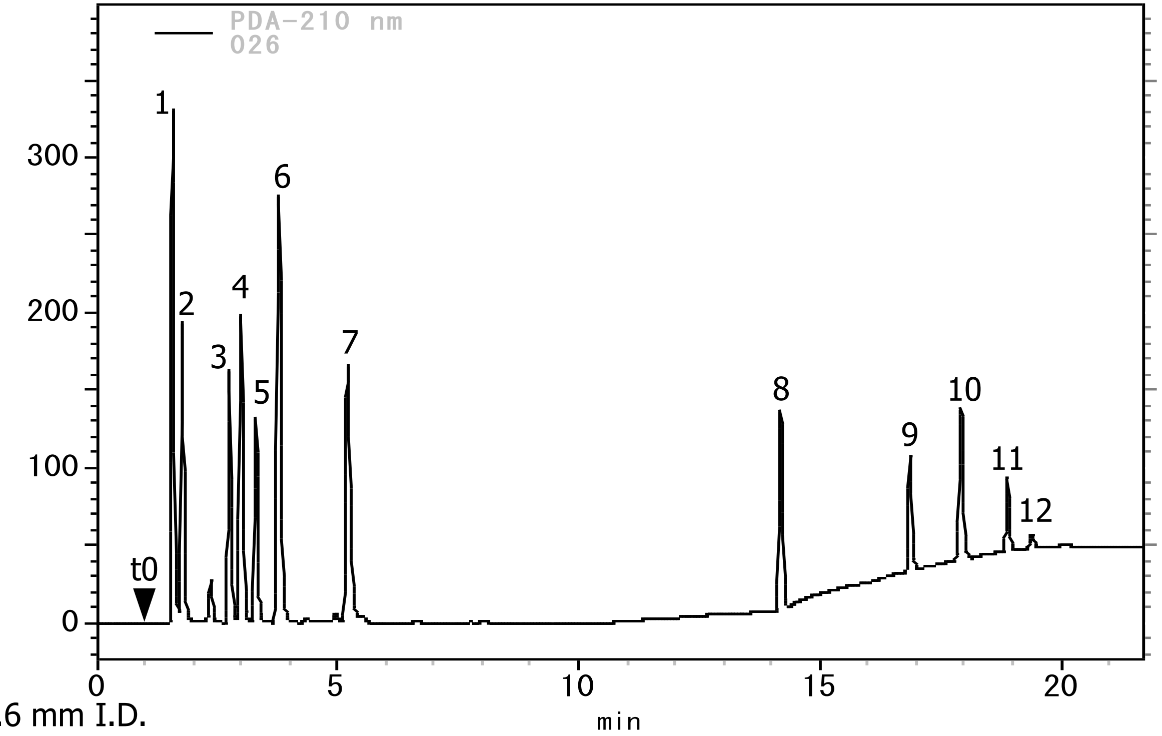


Conditions

Column : 5 µm, 150 × 4.6 mm I.D.
Eluent : 20 mM NaH_2PO_4
Flow Rate : 1.0 mL / min
Col. Temp. : 40℃
Detection : UV 254 nm
Inj. Vol. : 1 µL
Sample : 100 ppm
1. ATP 2. ADP 3. IMP 4. Hypoxanthine 5. AMP 6. Inosine

Analysis of Water-Soluble Vitamins

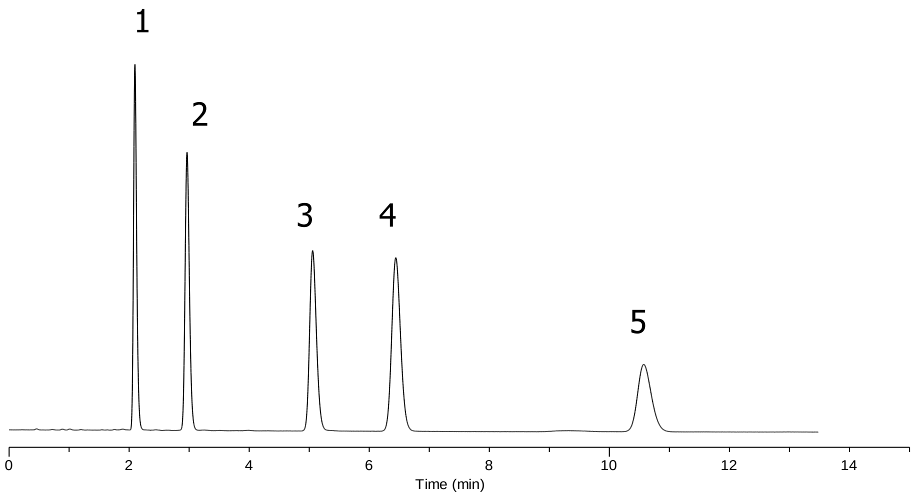
Without the use of ion-pairing reagents



Conditions

Column : 3 µm, 150 × 4.6 mm I.D.
Eluent : A: 0.1% H_3PO_4 in H_2O B: ACN A/B = 99/1 (5 min)- 99/1 – (20min) – 80/20 (5min), 99/1
Flow Rate : 1.0 mL / min
Col. Temp. : 40℃
Detection : UV 220 nm PDA
Inj. Vol. : 5µL
Sample : 1. Pyridoxamine 2. Thiamin 3. Nicotinic Acid 4. Ascorbic acid 5. Nicotinamide 6. Pyridoxal 7. Pyridoxine 8. Pantonenic Acid 9. Folic Acid 10. Cyanocobalamin 11. Riboflavin 12. Biotin

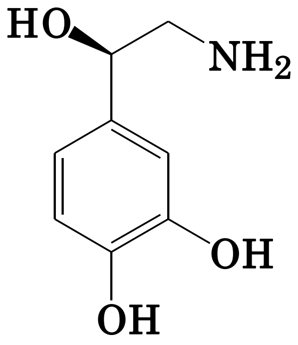
Analysis of Catecolamines



Conditions

Column	: 5 μm, 150 × 4.6 mm I.D.
Eluent	: 0.1% H_3PO_4 in H_2O
Flow Rate	: 1.0 mL / min
Col. Temp.	: 40℃
Detection	: UV 210 nm
Inj. Vol.	: 1 μL
Sample :	1. Norepinephrine 2. L-Adrenaline 3. Dopamine 4. L-DOPA 5. L-(-)-Tyrosine

1



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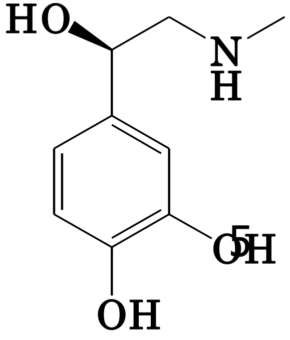

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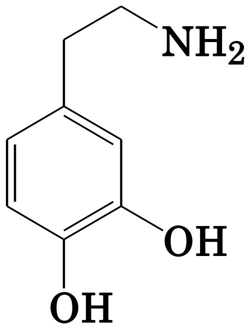
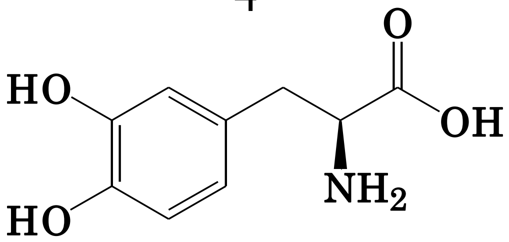
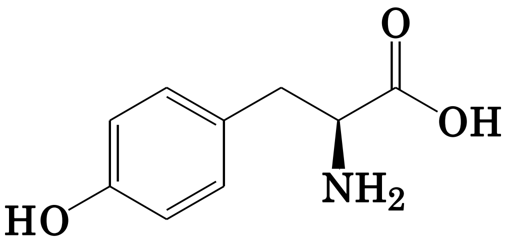


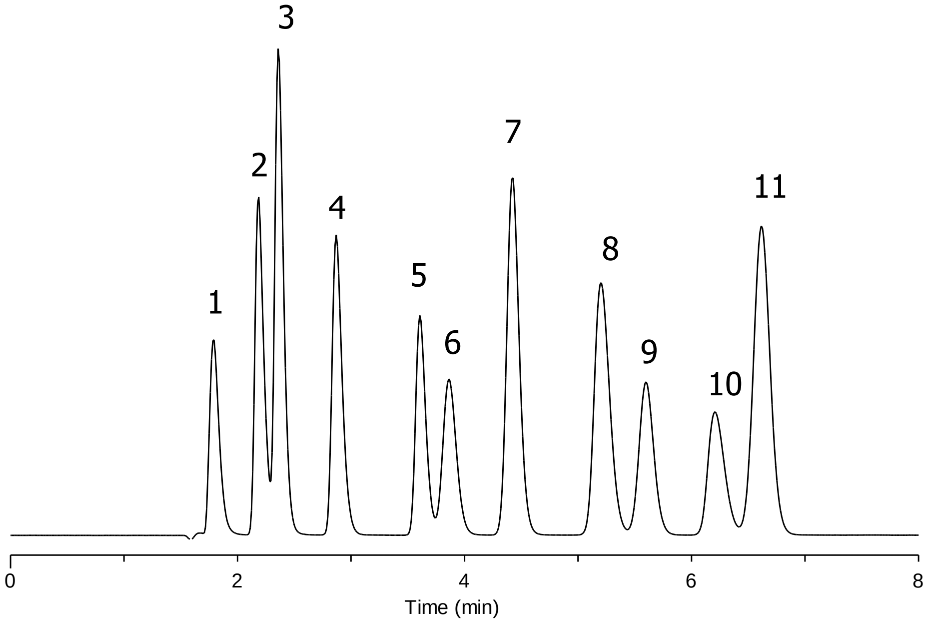
4



5



Analysis of Organic Acids



Conditions

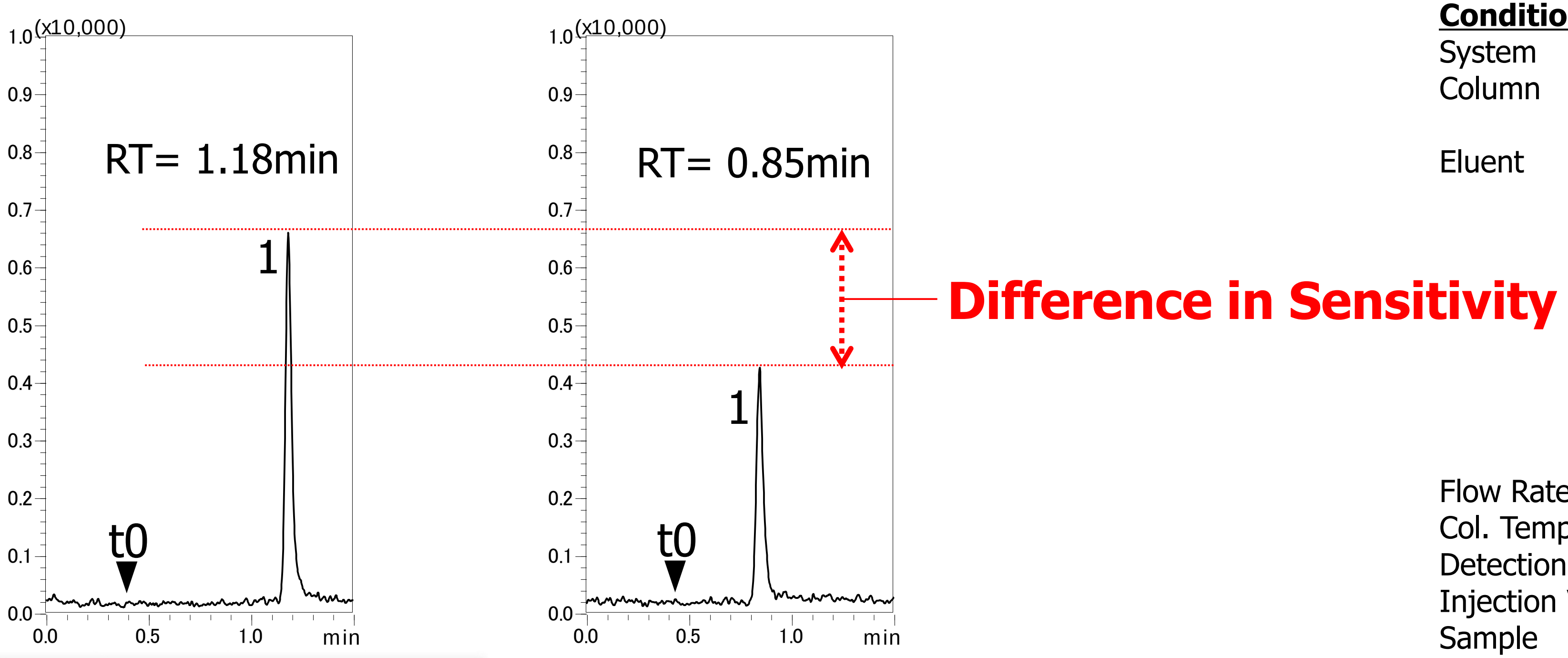
Column	: 5 µm, 150 × 4.6 mm I.D.
Eluent	: 50 mM NaH_2PO_4 in H_2O (pH 2.1, H_3PO_4)
Flow Rate	: 1.0 mL / min
Col. Temp.	: 40℃
Detection	: UV 210 nm
Inj. Vol.	: 10 µL

Sample :

1. Oxalic acid	50 mg/L
2. Tartaric acid	500 mg/L
3. Formic acid	1000 mg/L
4. Malic acid	1000 mg/L
5. Lactic acid	1000 mg/L
6. Acetic acid	1000 mg/L
7. Maleic acid	10 mg/L
8. Citric acid	1000 mg/L
9. Pyroglutamic acid	100 mg/L
10. Succinic acid	1000 mg/L
11. Fumaric acid	10 mg/L

Analysis of Water-Soluble Pesticides

Polar compounds coordinates to trace metal impurities in the packing resulting in providing poor peak shapes and low sensitivity. The same phenomenon is observed on water-soluble pesticides, however, InertSustain AQ-C18 delivers high sensitivity with exceptional peak shape, while other brand show adsorption of peak and lower sensitivity.



InertSustain AQ-C18　　Atlantis T3

Conditions

System : Nexera LCMS-8030 plus system
Column : InertSustain AQ-C18 (3 μm, 50 × 2.1 mm I.D)
Eluent : A) 0.1% HCOOH in H_2O
B) 0.1% HCOOH in CH_3CN

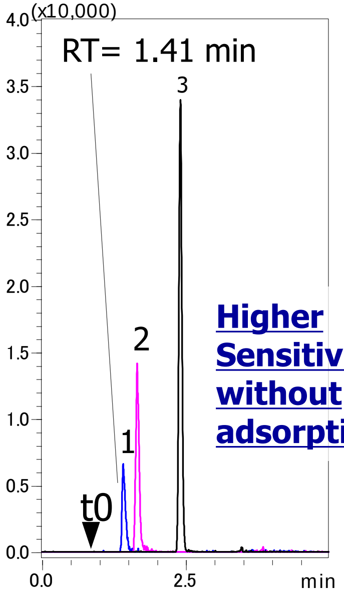
Time (min)	B%
0	5
1.50	30
1.51	5
3.00	5

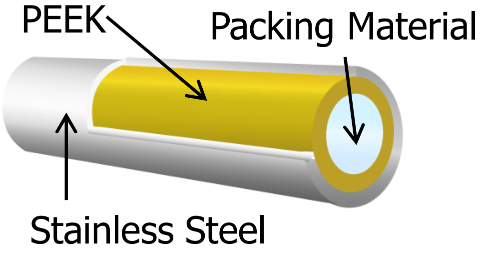
Flow Rate : 0.4 mL/min
Col. Temp. : 40 ℃
Detection : LC/MS/MS (ESI) , Positive
Injection Vol.: 2 μL
Sample : 1.Oxine Cu 200 μg/L

Analysis of Nucleotides via LC/MS/MS

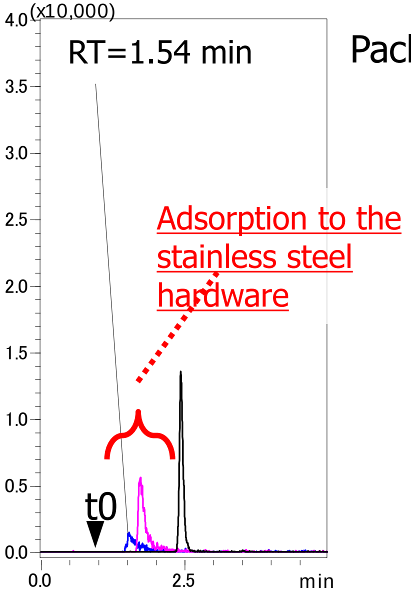
Samples such as nucleotides have several phosphate groups which is sensitive to stainless steel hardware. As shown below, the combination of highly inert packing material of InertSustain AQ-C18 and usage of a new Steel-Coated-PEEK hardware (metal-free) deliver excellent peak shapes with higher sensitivity for phosphate compounds WITHOUT the formation of phosphate-iron complexes found with stainless steel column hardwares.

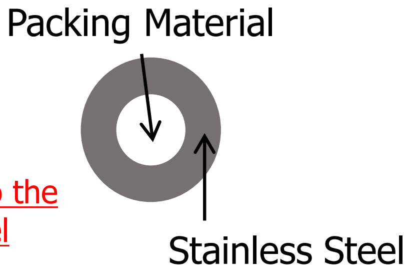
Steel-Coated-PEEK hardware Packed with InertSustain AQ-C18





Stainless Steel Hardware Packed with InertSustain AQ-C18





Conditions

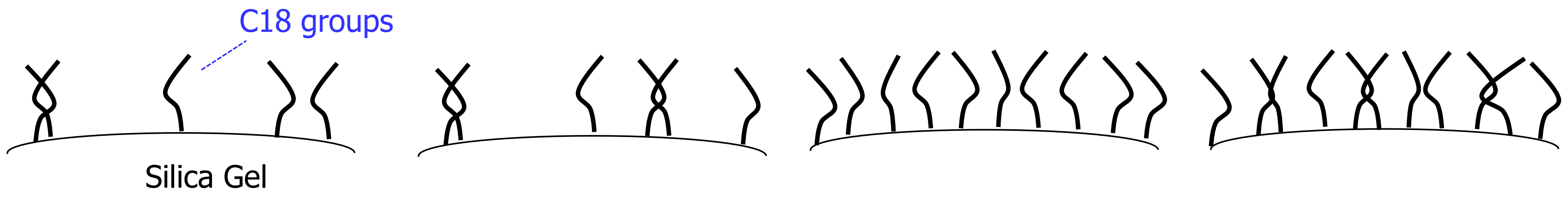
System : Nexera LCMS-8030 plus system
Column size : 3 μm HP, 150 x 2.1 mm I.D
Eluent : A) 5 mM Ammonium formate in H_2O
B) CH_3CN

Time (min)	B%
0	2
0.5	2
3.0	25
3.01	2
7.00	2

Flow Rate : 0.3 mL/min
Col. Temp. : 40 ℃
Detection : LC/MS/MS (ESI) , Positive
Injection Vol. : 2 μL
Sample : 1.ATP 500 μg/L
2.ADP 500 μg/L
3.AMP 500 μg/L

Retention Mechanism of InertSustain AQ-C18

InertSustain AQ-C18 columns are designed to achieve strong retention for highly polar compounds, which is the most challenging goals in developing reversed phase methods. **The optimization of bonding of the C18 groups at equal distance to the silica gel** enable InertSustain AQ-C18 to offer significant retention for highly polar compounds even under water rich mobile phases.



	Inertsil ODS-SP	Inertsil ODS-4	**InertSustain AQ-C18**	InertSustain C18
Carbon Loading	8.5 %	11 %	13 %	14 %
Silica Gel	3 Series Silica Gel	3 Series Silica Gel	**ES Silica Gel**	**ES Silica Gel**
Surface Area (m^2/g)	450	450	350	350
Bonding Distance of C18 Groups	Unadjusted	Unadjusted	**Optimized**	Unadjusted
Retention for Polar Analytes under Water Rich Mobile Phases	★★★★	★★★★	★★★★★	★★★

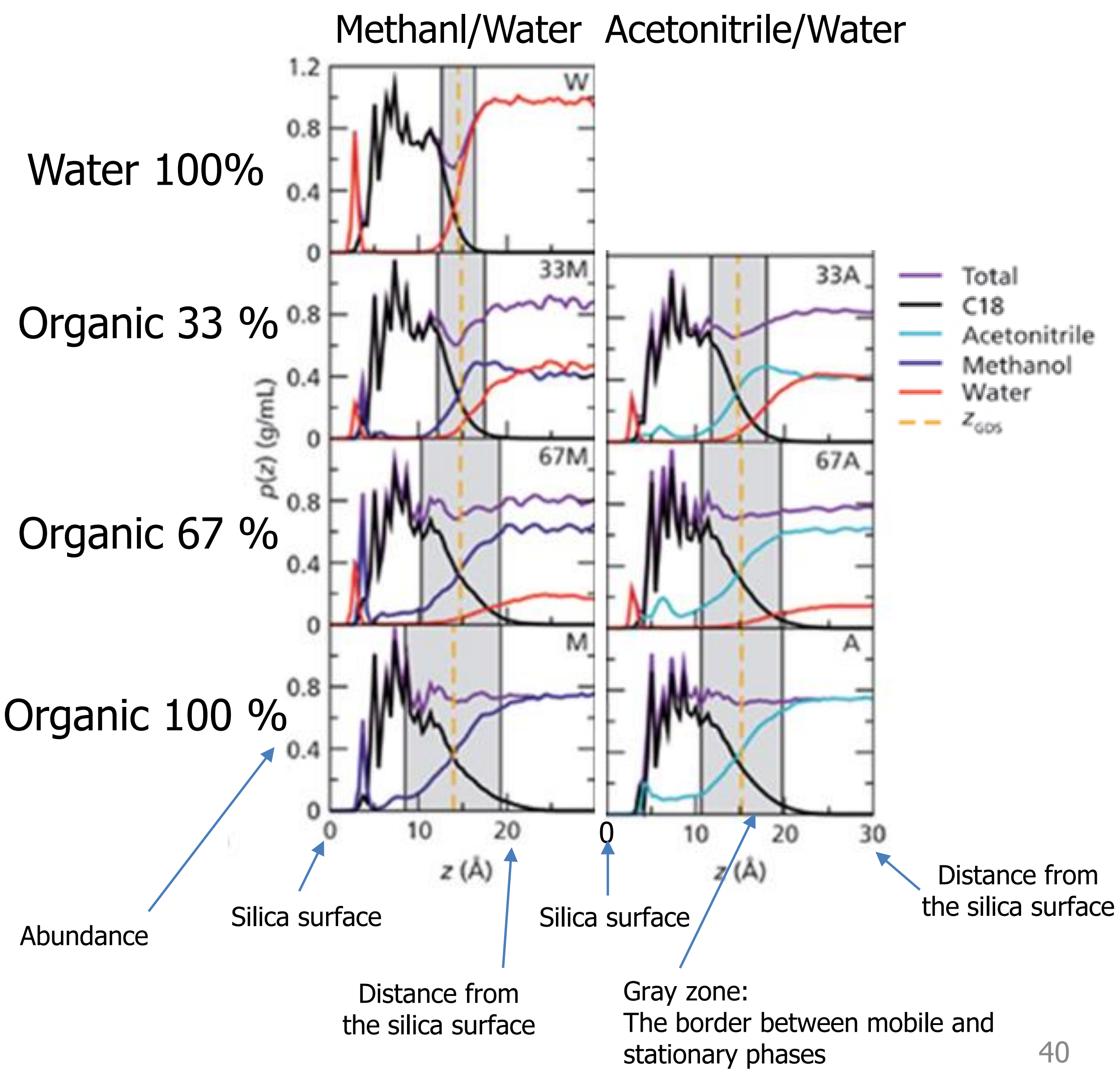
Cited Literature

Schure, Mark R., Jake L. Rafferty, Ling Zhang, and J. Ilja Siepmann. 2013. How reversed-phase liquid chromatography works. LCGC North America 31 (8) (JAN 01): 630-7.

http://www.chromatographyonline.com/how-reversed-phase-liquid-chromatography-works-0

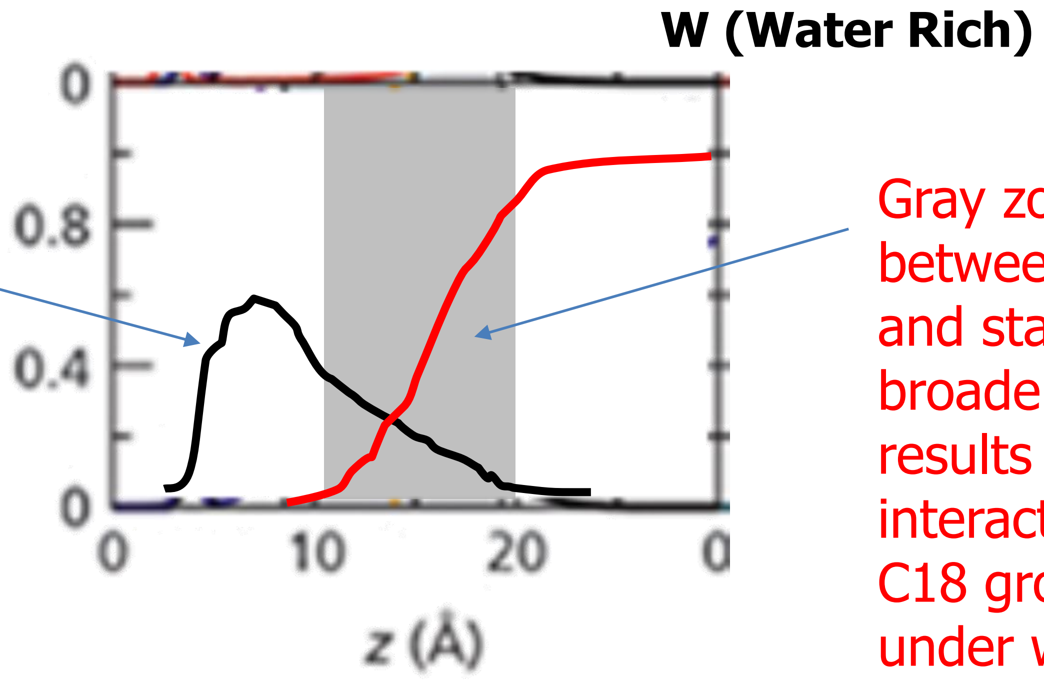
Density profiles of C18 chains, methanol, water, and acetonitrile for pure water (W), pure methanol (M), pure acetonitrile (A), and solvent mixtures given as mole percent.

It appears that very little solvent is present in the interior of the chain system for highly aqueous mobile phases and there are simply no solvent molecules to displace the solute. The solute, therefore, has little to no competition with the solvent for interaction sites within the chain interior region, resulting in less retention of analytes.



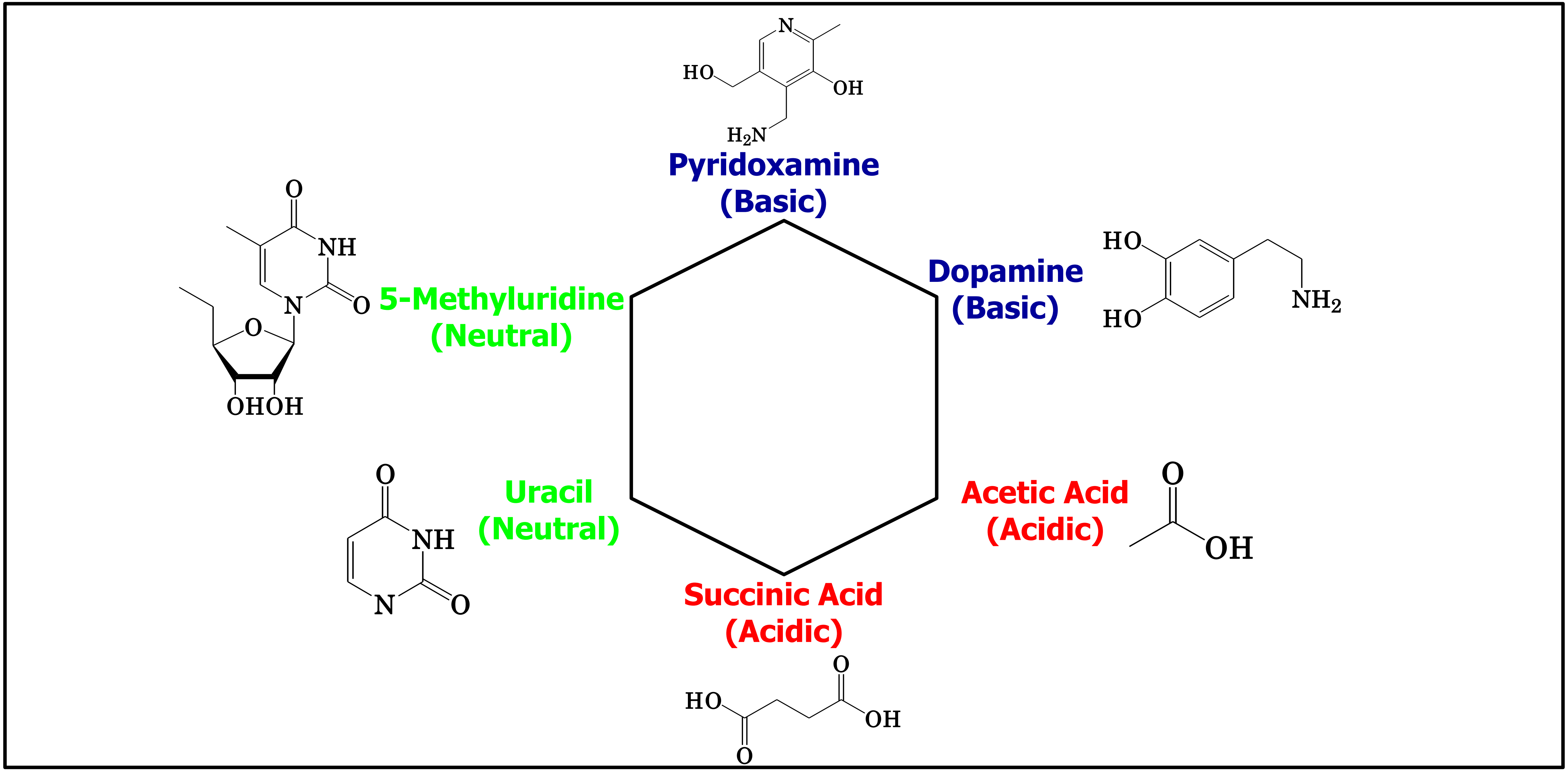
Estimated Model of **InertSustain AQ-C18** created by GL Sciences

The black C18 line is flattened, which indicates the C18 groups are straightened out instead of being tangled up at the surface of silica.



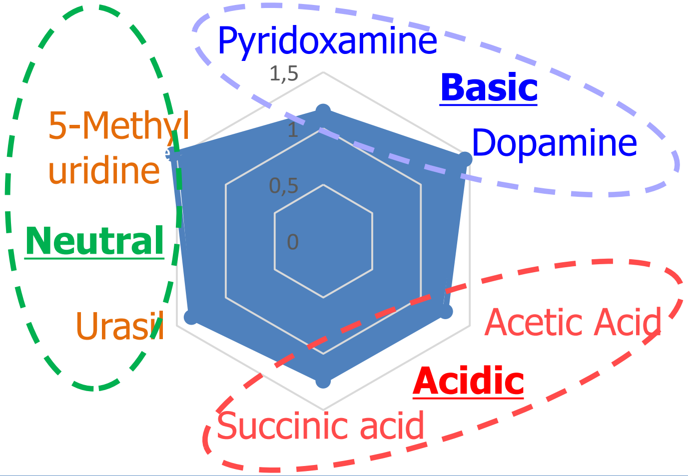
Gray zone, the border between mobile phase and stationary phase are broader/wider, which results in having more interaction between the C18 groups and analytes under water rich mobile phases.

Comparison of Retention Properties for Polar Compounds

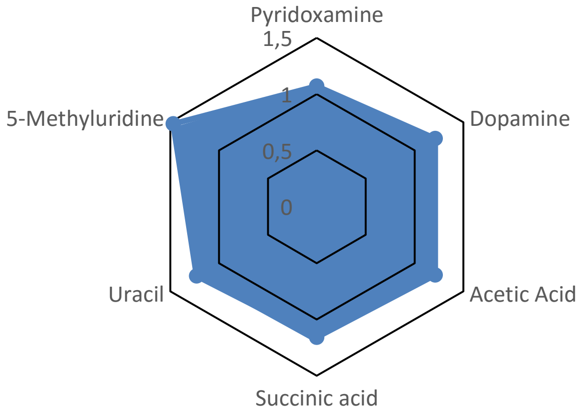


Comparison of Retention Properties for Polar Compounds

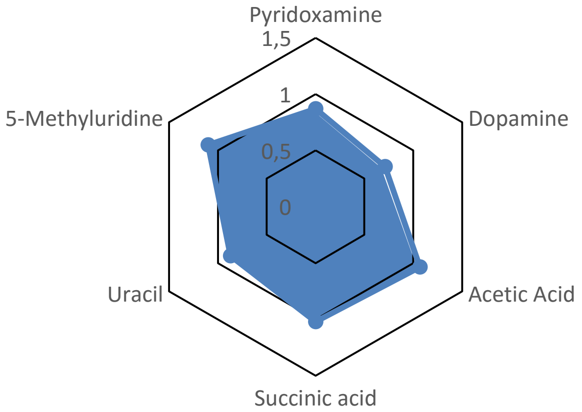
InertSustain AQ-C18



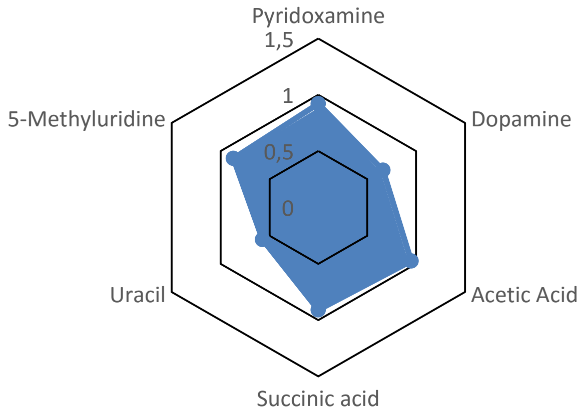
Atlantis T3



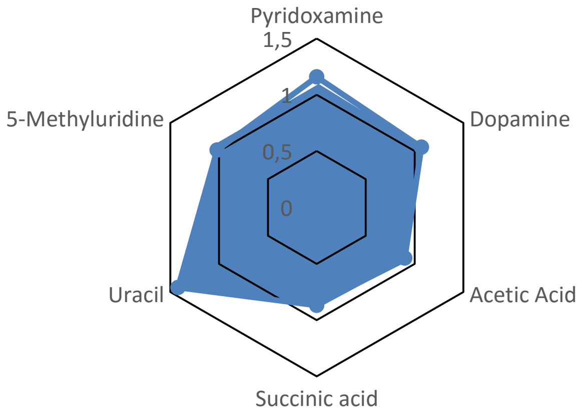
CAPCELL PAK C18-AQ



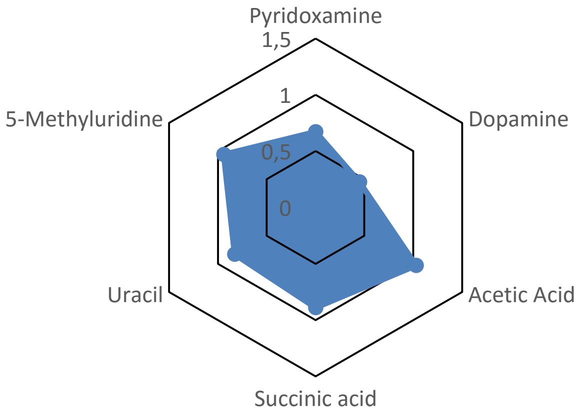
Synergi Fusion RP



AQUASIL C18

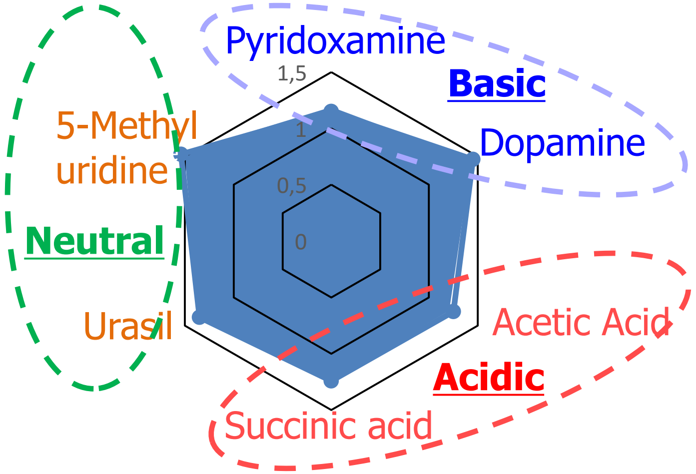


XSELECT CSH C18

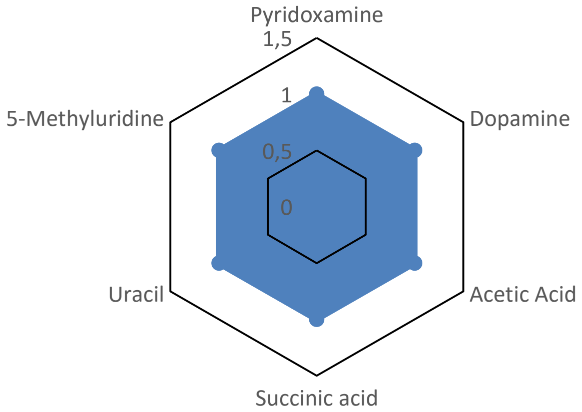


Comparison of Retention Properties for Polar Compounds

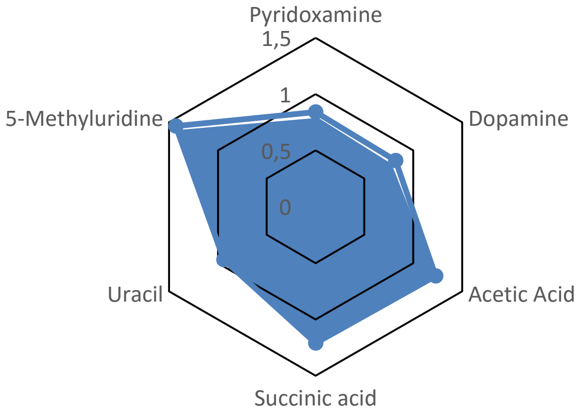
InertSustain AQ-C18



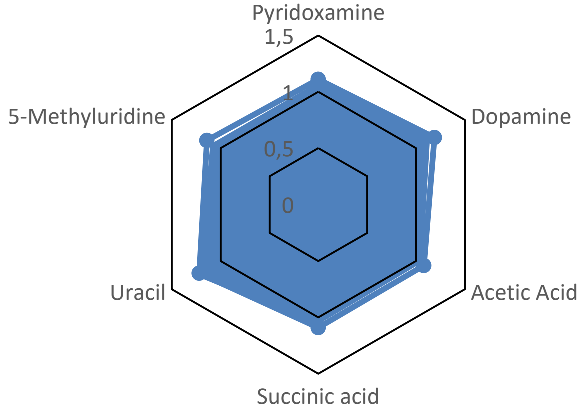
InertSustain C18



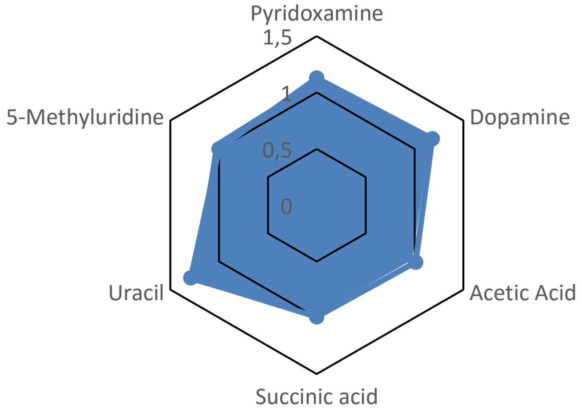
Inertsil ODS-3



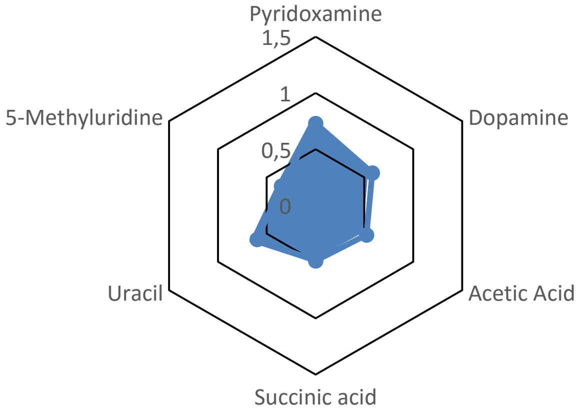
Inertsil ODS-4



Inertsil ODS-SP



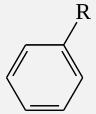
Inertsil ODS-EP



Comparison of Performance

Hydrophobic Property of Column

Sample No.6, n-Amylbenzene was used to determine the hydrophobic property of columns.



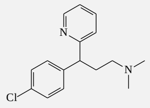
—CH_3
—CH_2CH_3
—$CH_2CH_2CH_3$
—$CH_2CH_2CH_2CH_3$
—$CH_2CH_2CH_2CH_2CH_3$

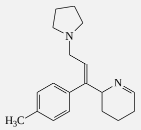
Conditions

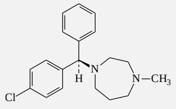
Column : 5 µm, 150 × 4.6 mm I.D.
Eluent : CH_3OH/H_2O = 80/20, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm
Injection Vol : 2.5 µL
Sample : 1. Uracil 2. Toluene 3. Ethylbenzene 4. Propylbenzene 5. n-Butylbenzene 6. n-Amylbenzene

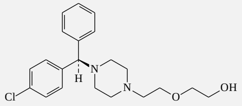
Basic Compound Test (1)

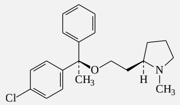
A basic compound test using antihistamine drugs and are highly basic, which can show tailing of peaks and different elution pattern on columns with insufficient end-capping. Column with insufficient end-capping will show later elution of sample 1, 2, 3 and 5.


1. Chlorpheniramine


2. Triprolidine


3. Homochlorcyclizine


4. Hydroxyzine

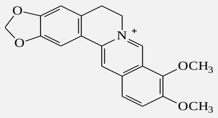

5. Clemastine

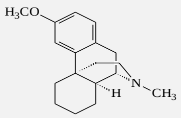
Conditions

Column : 5 µm, 150 × 4.6 mm I.D.
Eluent : : A) CH_3CN
B) 25mM K_2HPO_4 (pH 7.0, KH_2PO_4)
A/B = 60/40,v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 220 nm
Injection Vol : 1µL
Sample : 1. Chlorpheniramine 2. Triprolidine 3. Homochlorcyclizine 4. Hydroxyzine 5. Clemastine

Basic Compound Test (2)

Dextromethorphan is a strong basic compound. Severe tailing can be confirmed when the packing material contains residual silanol groups.


4. Berberine chloride


5. Dextromethorphan

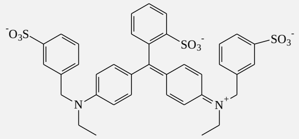
Conditions

Eluent : A) CH_3CN
B) 25 mM K_2HPO_4 (pH 7.0, KH_2PO_4)
A/B = 30/70,v/v
Flow Rate : 1mL/min
Col. Temp. : 40 ℃
Detection : UV 230 nm
Injection Vol : 2.5 µL
Sample : 1:Uracil 2:Berberine chloride 3:Dextromethorphan

Comparison of Performance

Acidic Compound Test

Sharp peaks can be obtained when analyzing Phenol or Salicylic Acid. However, as Brilliant Blue FCF has three sulfonic groups in its chemical structure, tailing will occur when the surface of the packing material is slightly basic.



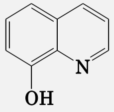
1. Brilliant Blue FCF

Conditions

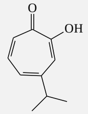
Column	: 5 μm, 150 × 4.6 mm I.D.
Eluent	: A) CH_3CN B) 0.1% H_3PO_4 A/B = 25/75,v/v
Flow Rate	: 1 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 254 nm
Injection Vol	: 2 μL
Sample	: 1.Brilliant Blue FCF 2. Phenol 3.Salicylic acid

Chelating Compound Test

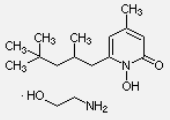
Traditional chelating compound test used phosphate buffer, which made it easy to elute these type of chelating compounds. In this test, formic acid mobile phase is used as it is regularly used in LC/MS methods and hard to detect in such condition. Piroctone Olamine is used in this test, which is now widely used as antimicrobial agents.



1. 8-Quinolinol



2. Hinokitiol



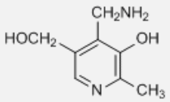
3. Piroctone Olamine

Conditions

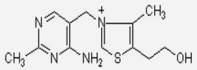
Column	: 5 μm, 150 × 4.6 mm I.D.
Eluent	: A) CH_3CN B) 0.1 %HCOOH A/B = 40/60,v/v
Flow Rate	: 1 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 310 nm
Injection Vol	: 2 μL
Sample	: 1. 8-Quinolinol 2. Hinokitiol 3. Piroctone Olamine

Polar Compound Test (Water-Soluble Vitamins)

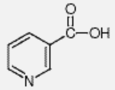
The use of ion-pairing reagents is a popular approach to retain water-soluble vitamins. Instead, 0.1% HCOOH in H_2O was used to demonstrate and compare the retention for water-soluble vitamins.



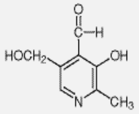
Pyridoxamine



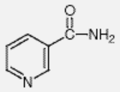
Thiamin



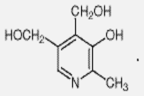
Nicotinic Acid



Pyridoxal



Nicotinamide



Pyridoxine

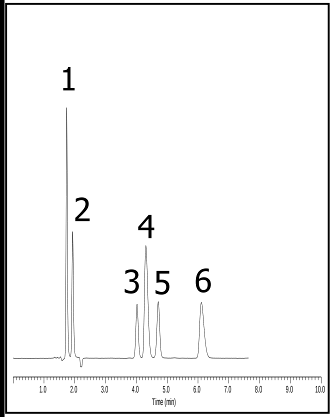
Conditions

Column	: 5 μm, 150 × 4.6 mm I.D.
Eluent	: 0.1 %HCOOH
Flow Rate	: 1 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 210 nm
Injection Vol	: 2 μL
Sample	: 1. Pyridoxamine (VB6) 2. Thiamin (VB1) 3. Nicotinic Acid (VB3) 4. Pyridoxal (VB6) 5. Nicotinamide (VB3) 6. Pyridoxine (VB6)

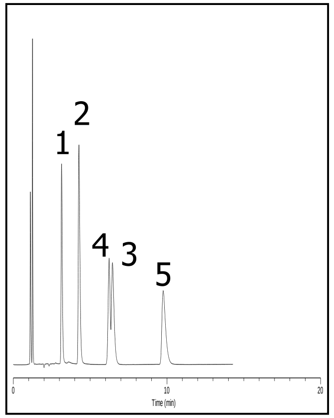
Comparison of Performance (1/4)

InertSustain AQ-C18

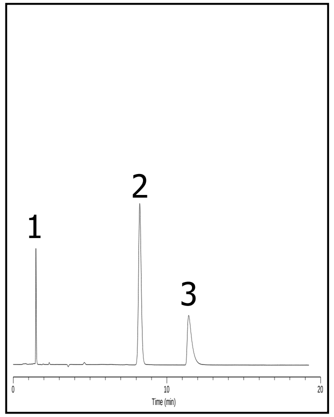
Polar Compound Test



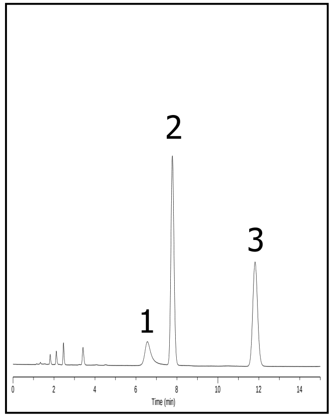
Basic Compound Test (1)



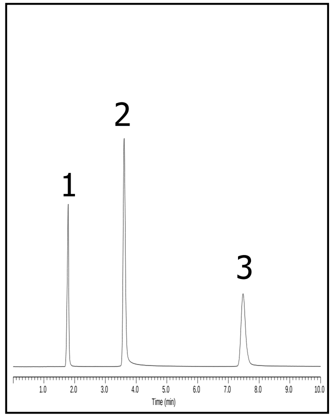
Basic Compound Test (2)



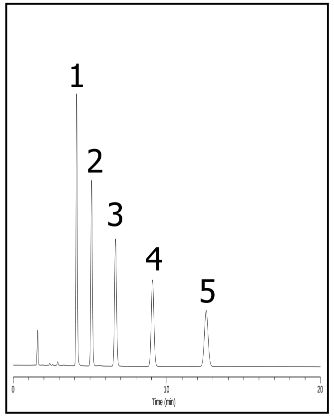
Acidic Compound Test



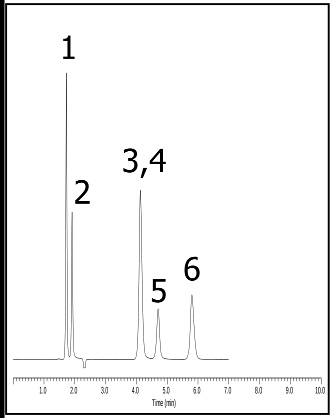
Chelating Compound Test

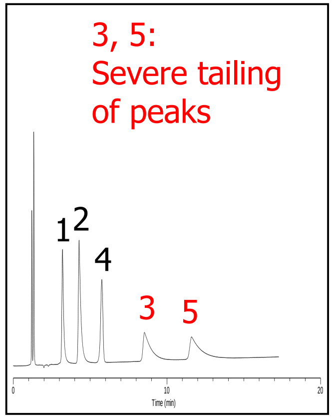


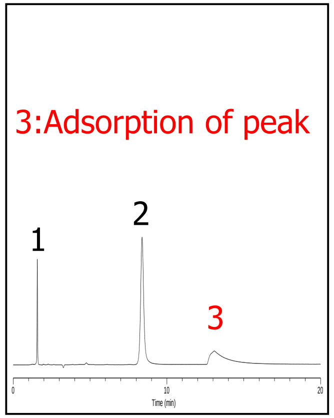
Hydrophobic Property

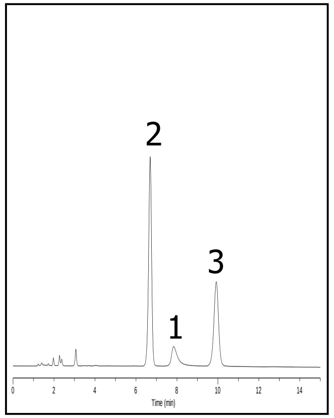


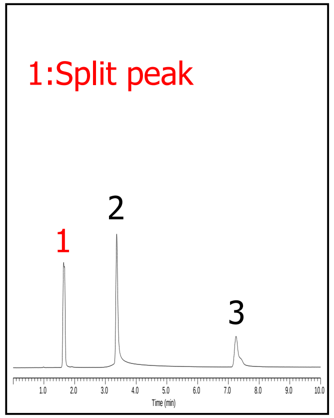
Atlantis T3

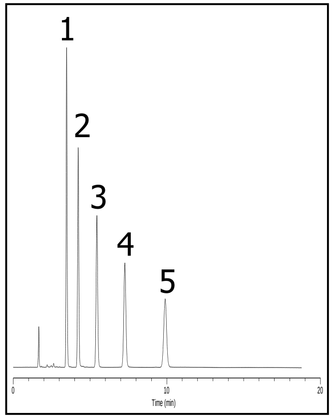








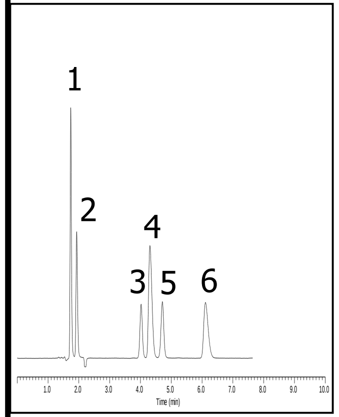


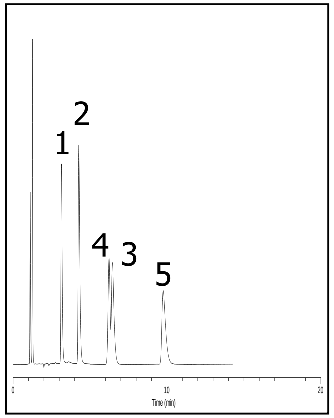


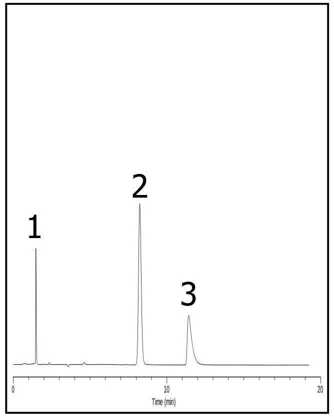
Comparison of Performance (2/4)

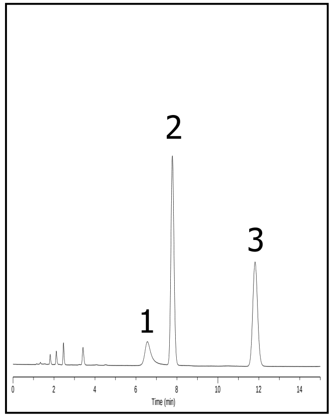
InertSustain AQ-C18

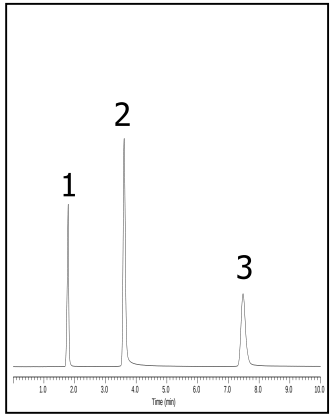
Polar Compound Test | Basic Compound Test (1) | Basic Compound Test (2) | Acidic Compound Test | Chelating Compound Test | Hydrophobic Property

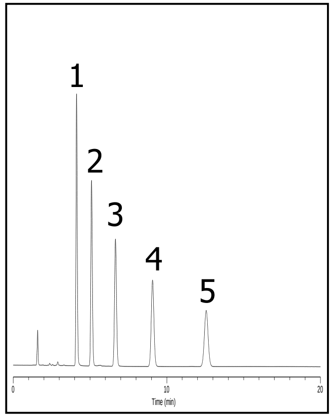




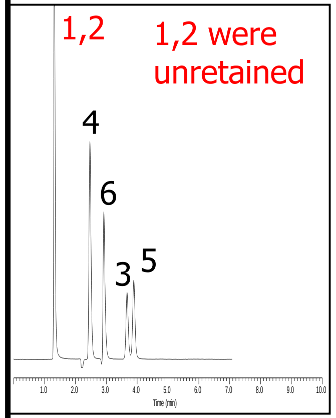


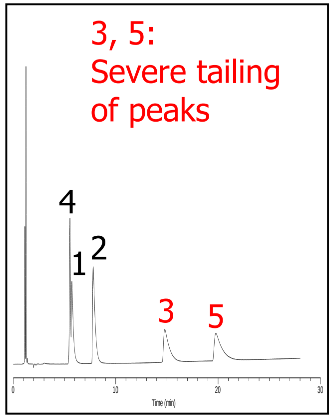


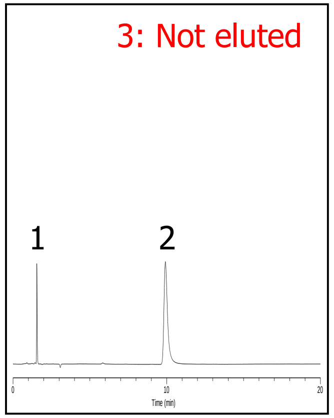


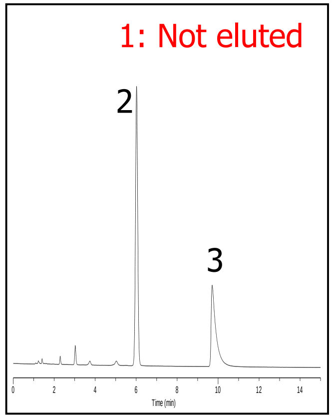


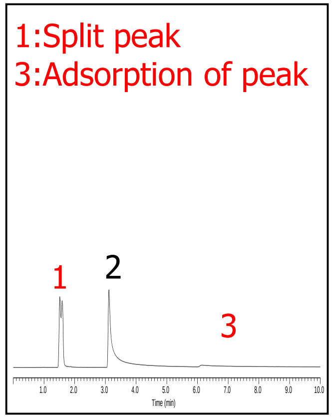
CAPCELL PAK C18-AQ

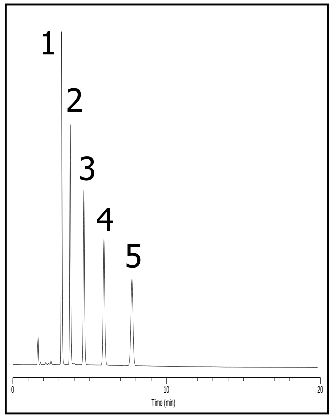








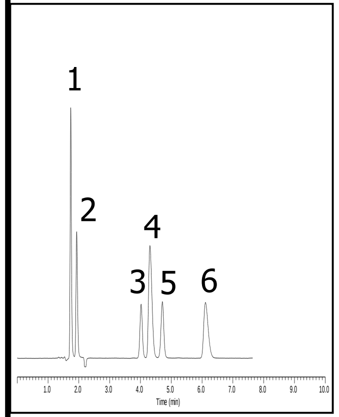




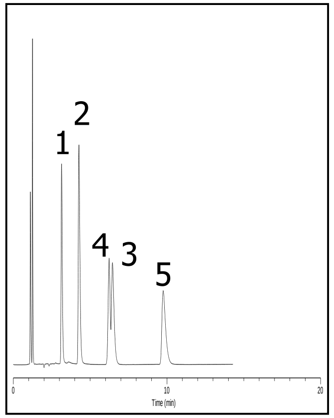
Comparison of Performance (3/4)

InertSustain AQ-C18

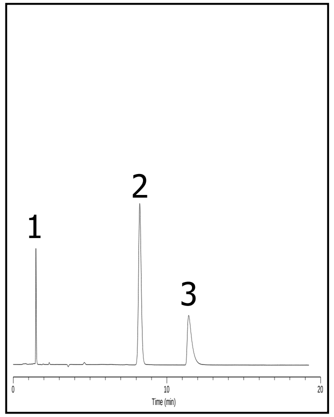
Polar Compound Test



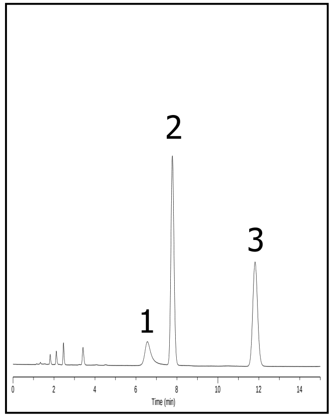
Basic Compound Test (1)



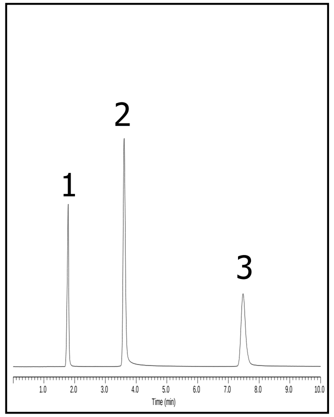
Basic Compound Test (2)



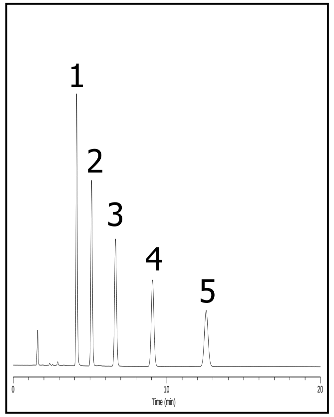
Acidic Compound Test



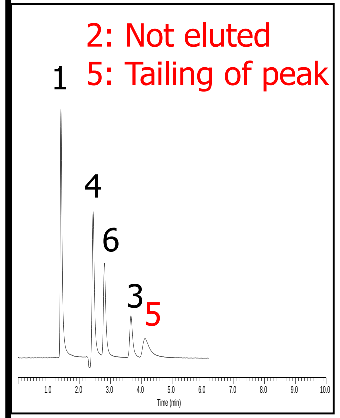
Chelating Compound Test

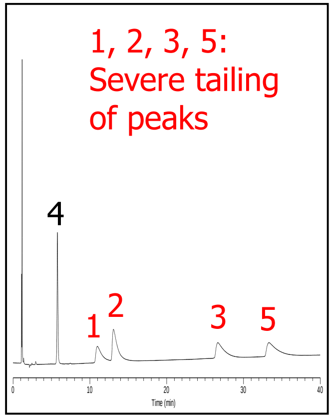


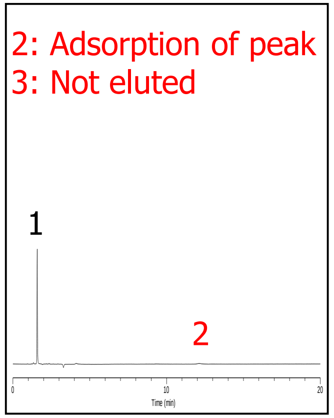
Hydrophobic Property

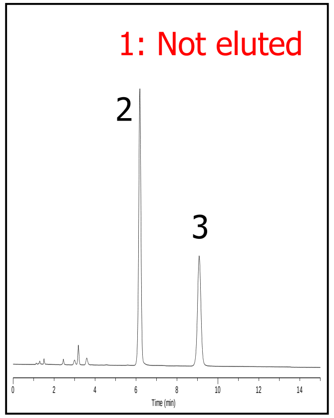


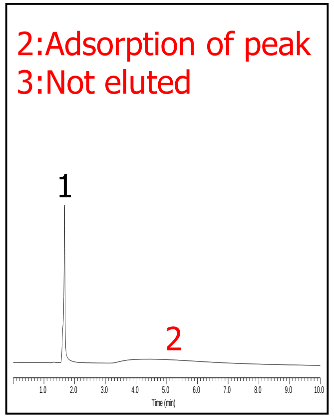
Synergi Fusion RP

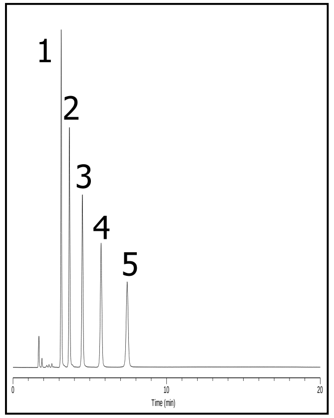








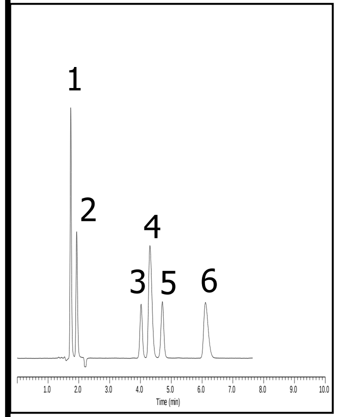




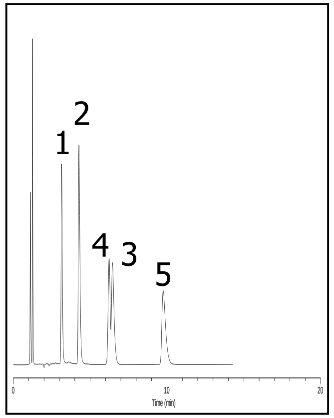
Comparison of Performance (4/4)

InertSustain AQ-C18

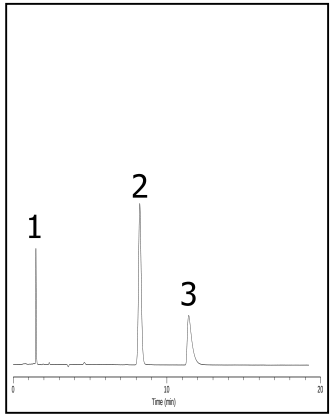
Polar Compound Test



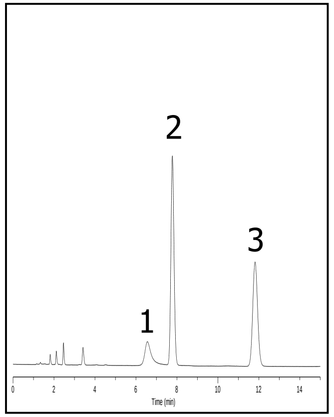
Basic Compound Test (1)



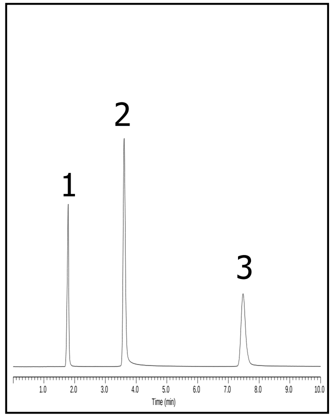
Basic Compound Test (2)



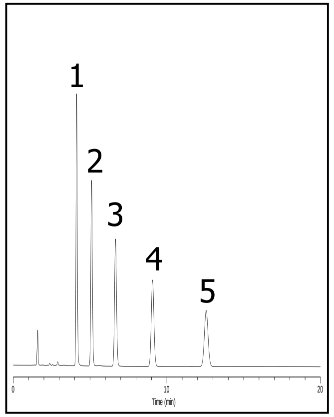
Acidic Compound Test



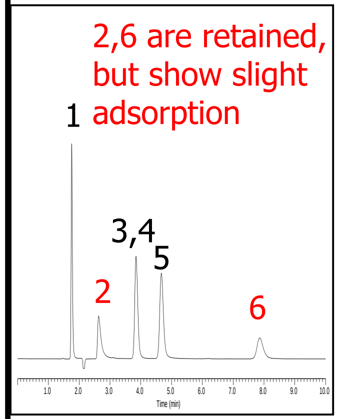
Chelating Compound Test

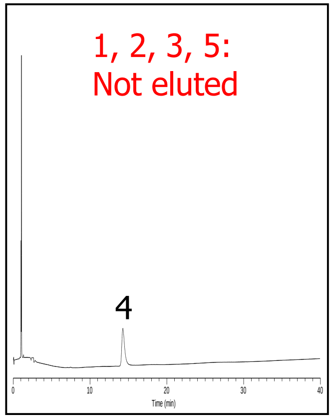


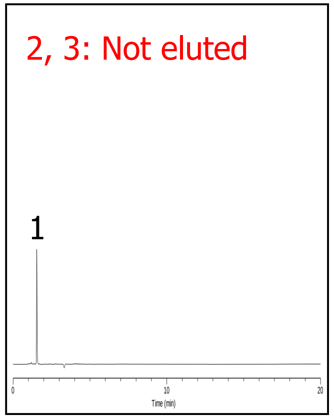
Hydrophobic Property

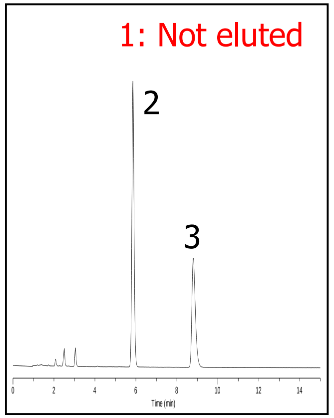


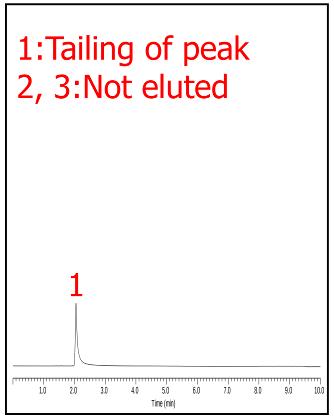
Aquasil C18

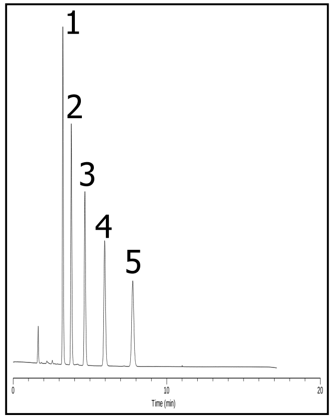








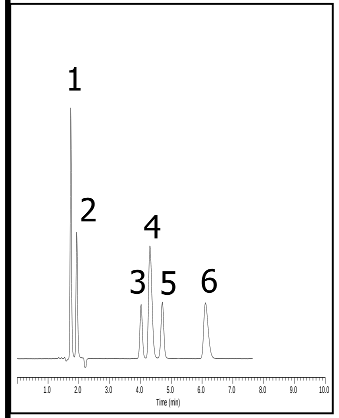




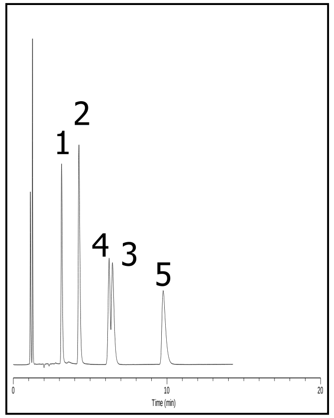
Comparison of Performance between GL Sciences' Columns (1/4)

InertSustain AQ-C18

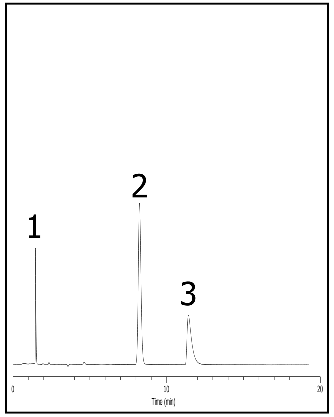
Polar Compound Test



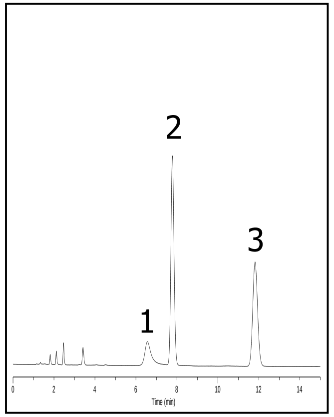
Basic Compound Test (1)



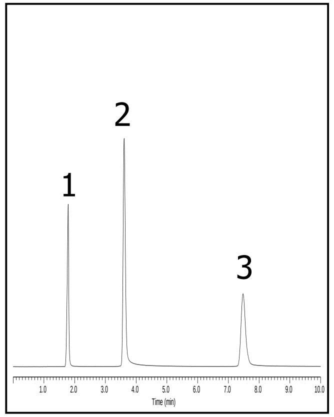
Basic Compound Test (2)



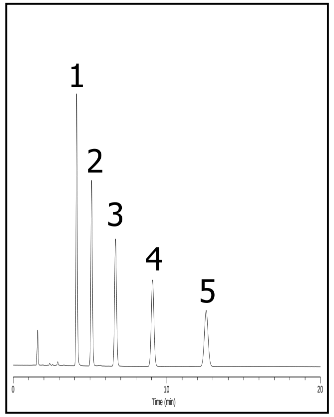
Acidic Compound Test



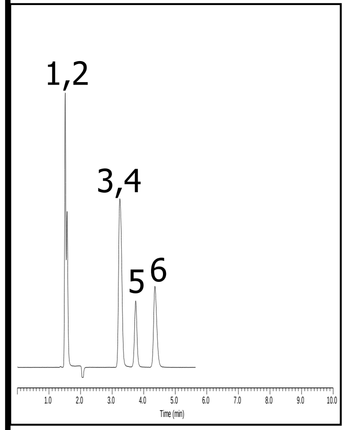
Chelating Compound Test

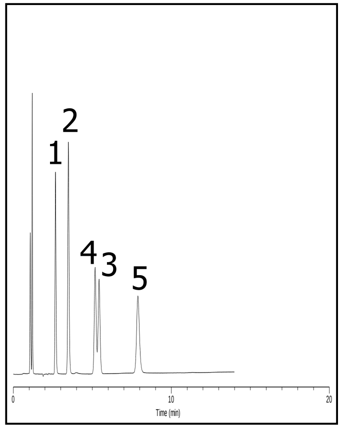


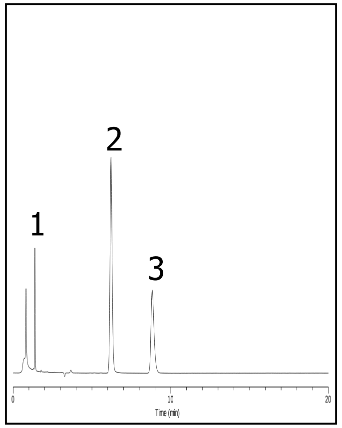
Hydrophobic Property

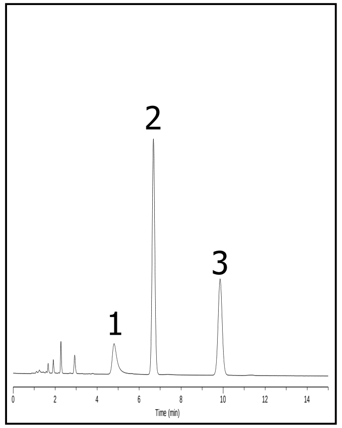


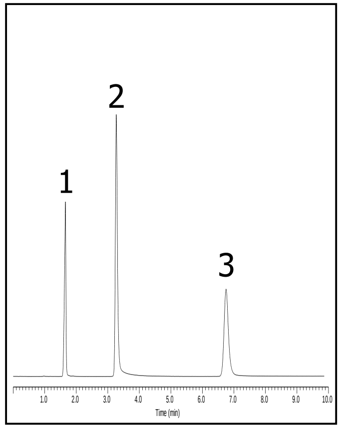
InerSustain C18

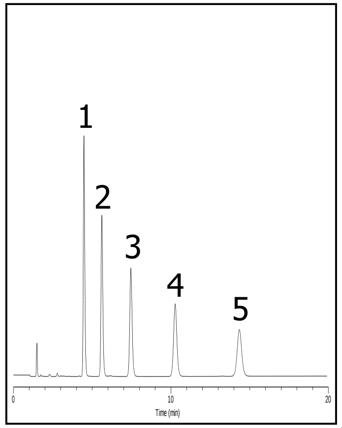








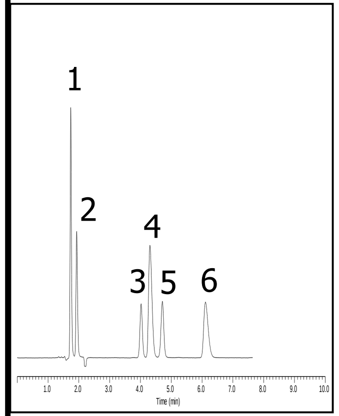


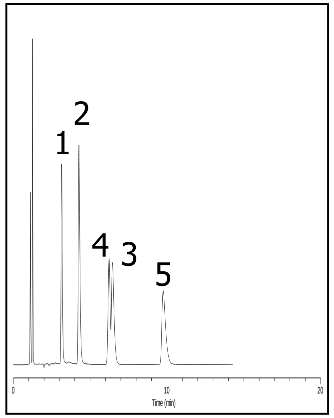


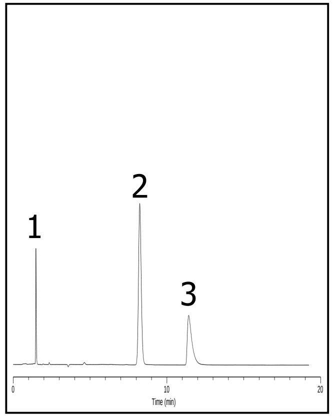
Comparison of Performance between GL Sciences' Columns (2/4)

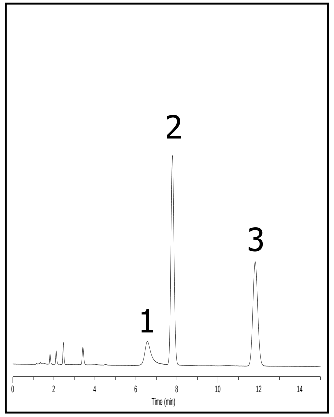
InertSustain AQ-C18

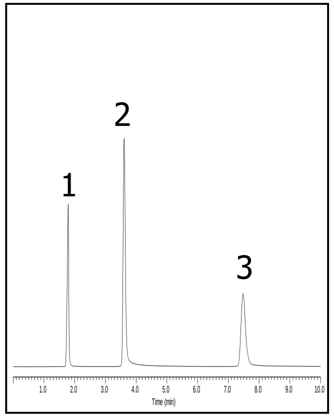
Polar Compound Test	Basic Compound Test (1)	Basic Compound Test (2)	Acidic Compound Test	Chelating Compound Test	Hydrophobic Property

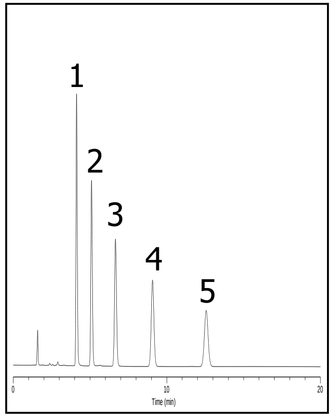




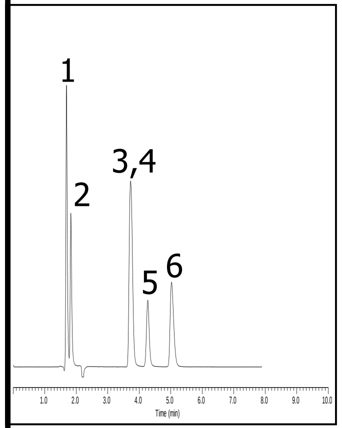


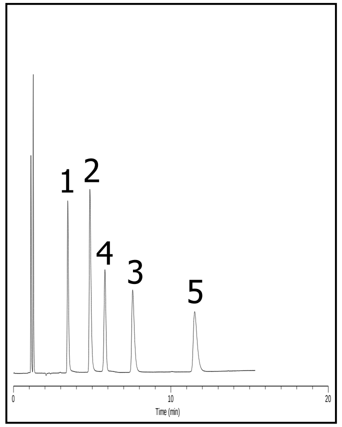


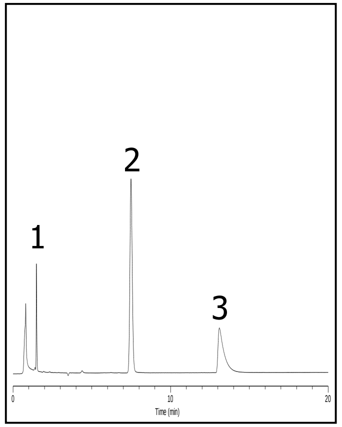


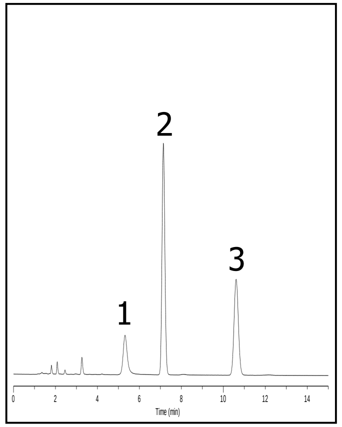


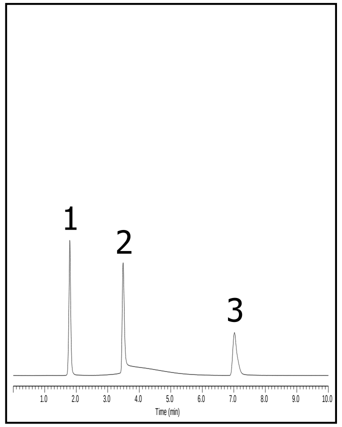
Inertsil ODS-4

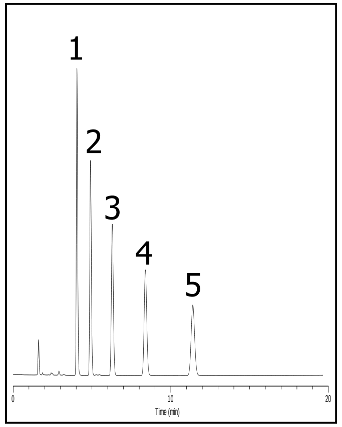








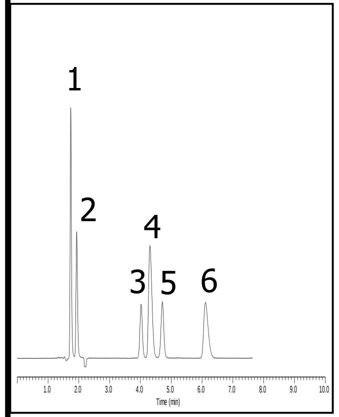




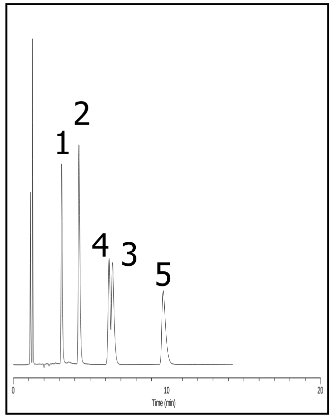
Comparison of Performance between GL Sciences' Columns (3/4)

InertSustain AQ-C18

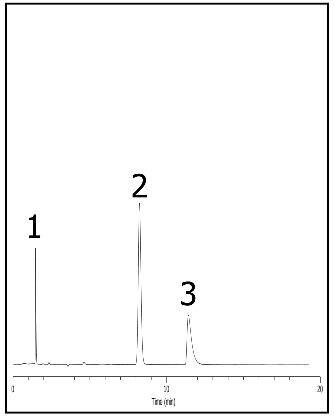
Polar Compound Test



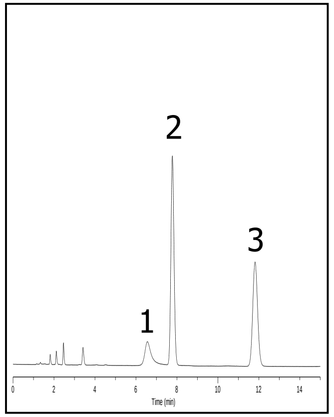
Basic Compound Test (1)



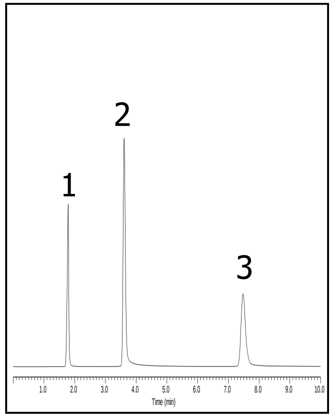
Basic Compound Test (2)



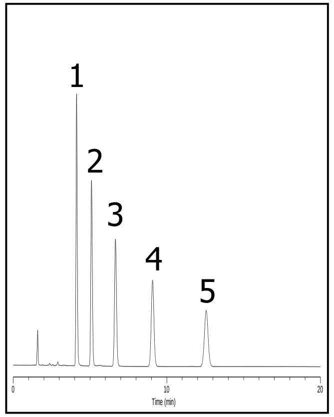
Acidic Compound Test



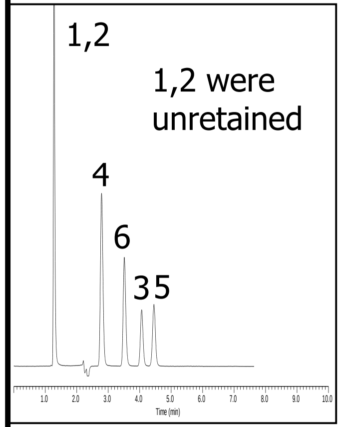
Chelating Compound Test

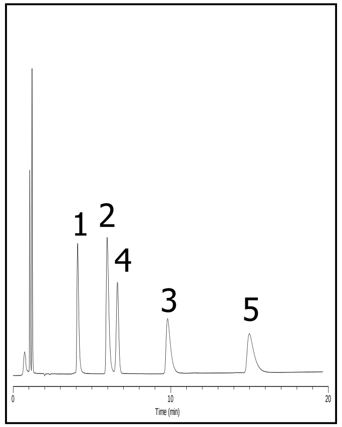


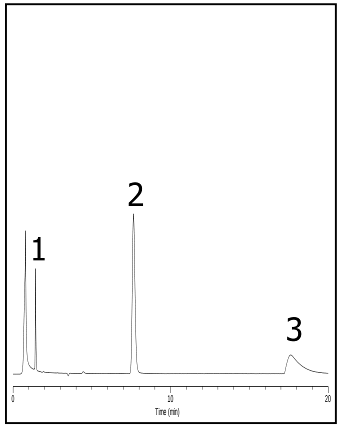
Hydrophobic Property

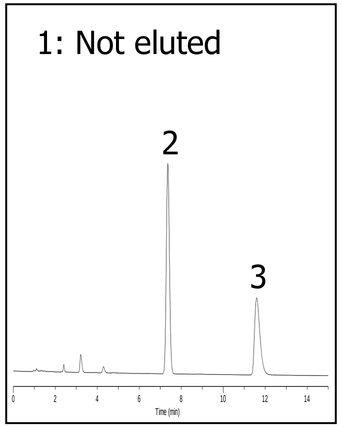


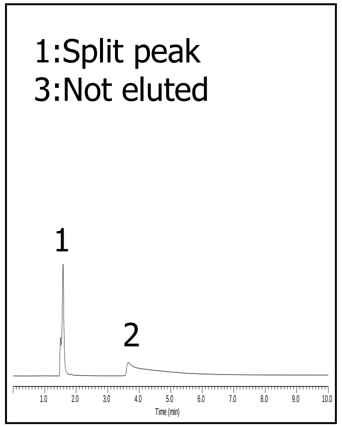
Inertsil ODS-3

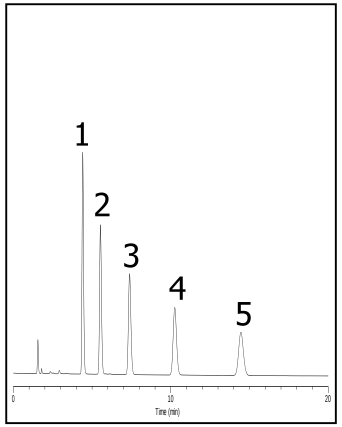








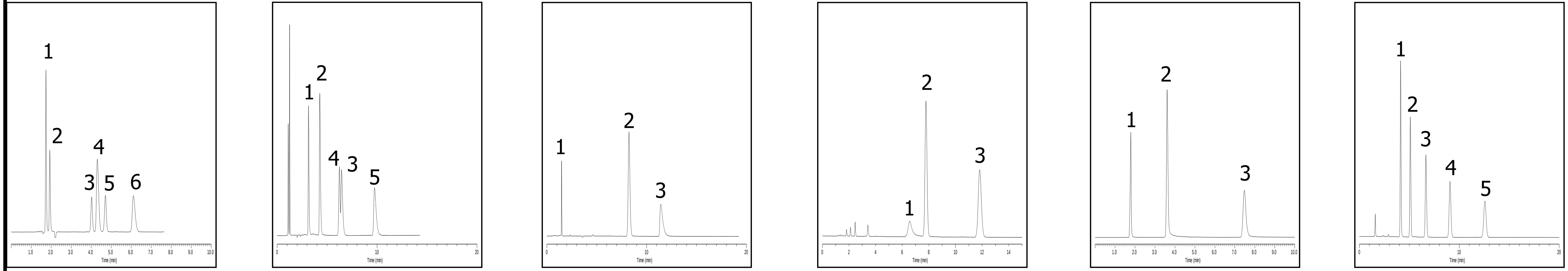




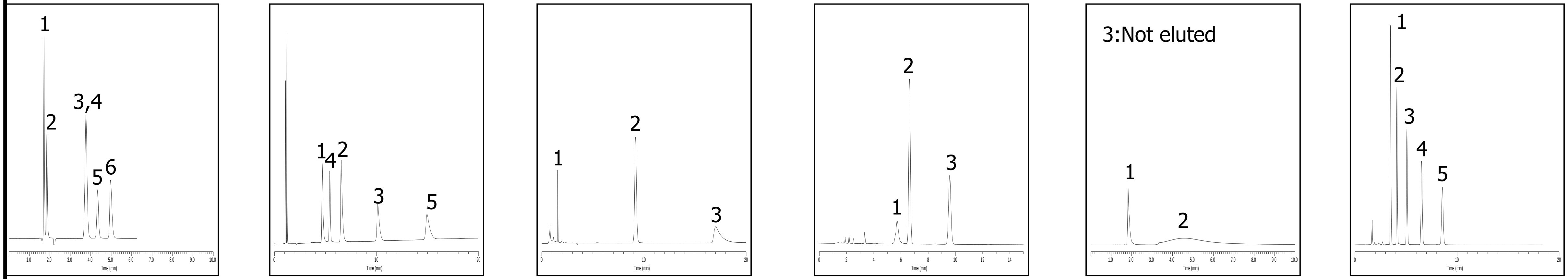
Comparison of Performance between GL Sciences' Columns (4/4)

InertSustain AQ-C18

Polar Compound Test | **Basic Compound Test (1)** | **Basic Compound Test (2)** | **Acidic Compound Test** | **Chelating Compound Test** | **Hydrophobic Property**



Inertsil ODS-SP

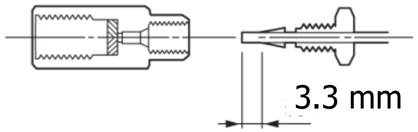


Ordering Information

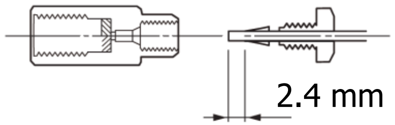
Analytical Columns

	Length/ I.D. (mm)	2.1	3.0	4.6
HP Series Particle Size：3 µm Max. Operating Pressure： 50 MPa (500 Bar)	30	5020-89920	5020-89926	5020-89932
	50	5020-89921	5020-89927	5020-89933
	75	5020-89922	5020-89928	5020-89934
	100	5020-89923	5020-89929	5020-89935
	150	5020-89924	5020-89930	5020-89936
	250	5020-89925	5020-89931	5020-89937

End-fitting Format



1/16” Waters End-fittings

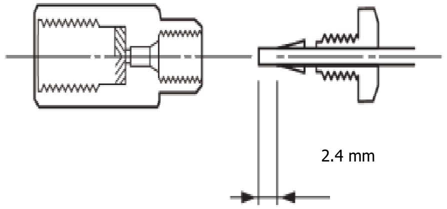


UP Type End-fittings

※ End-fittings are 1/16” Waters-compatible.
※ UHPLC compatible end-fittings are also available upon request for UHPLC systems (Ex: UPLC®) to avoid dead volume.
※ Indicate “UP Type end-fittings” when ordering. (Please note that UP type is not available for a 4.6 mm I.D. column)

UPLC® is a registered trademark of Waters Corporation.

	Length/ I.D. (mm)	2.1	3.0
Particle Size：1.9 µm Max. Operating Pressure： 80 MPa (800 Bar)	50	5020-89938	5020-89941
	100	5020-89939	5020-89942
	150	5020-89940	5020-89943



UP Type End-fittings

Ordering Information

Analytical Columns

	Length/I.D. (mm)	1.0	1.5		
Particle Size: 3 µm Max. Operating Pressure: 20 MPa (200 Bar)	30	5020-89871	5020-89877		
	50	5020-89872	5020-89878		
	75	5020-89873	5020-89879		
	100	5020-89874	5020-89880		
	150	5020-89875	5020-89881		
	250	5020-89876	5020-89882		
	Length/I.D. (mm)	2.1	3.0	4.0	4.6
	30	5020-89831	5020-89839	5020-89847	5020-89855
	50	5020-89832	5020-89840	5020-89848	5020-89856
	75	5020-89833	5020-89841	5020-89849	5020-89857
	100	5020-89834	5020-89842	5020-89850	5020-89858
	125	5020-89835	5020-89843	5020-89851	5020-89859
	150	5020-89836	5020-89844	5020-89852	5020-89860
	250	5020-89837	5020-89845	5020-89853	5020-89861

※ End-fittings are 1/16” Waters-compatible.
※ Other column sizes available upon request.

Ordering Information

Analytical Columns

	Length/I.D. (mm)	1.0	1.5		
Particle Size: 5 µm Max. Operating Pressure: 20 MPa (200 Bar)	30	5020-89741	5020-89747		
	50	5020-89742	5020-89748		
	75	5020-89743	5020-89749		
	100	5020-89744	5020-89750		
	150	5020-89745	5020-89751		
	250	5020-89746	5020-89752		
	Length/I.D. (mm)	2.1	3.0	4.0	4.6
	30	5020-89701	5020-89709	5020-89717	5020-89725
	50	5020-89702	5020-89710	5020-89718	5020-89726
	75	5020-89703	5020-89711	5020-89719	5020-89727
	100	5020-89704	5020-89712	5020-89720	5020-89728
	125	5020-89705	5020-89713	5020-89721	5020-89729
	150	5020-89706	5020-89714	5020-89722	5020-89730
	250	5020-89707	5020-89715	5020-89723	5020-89731

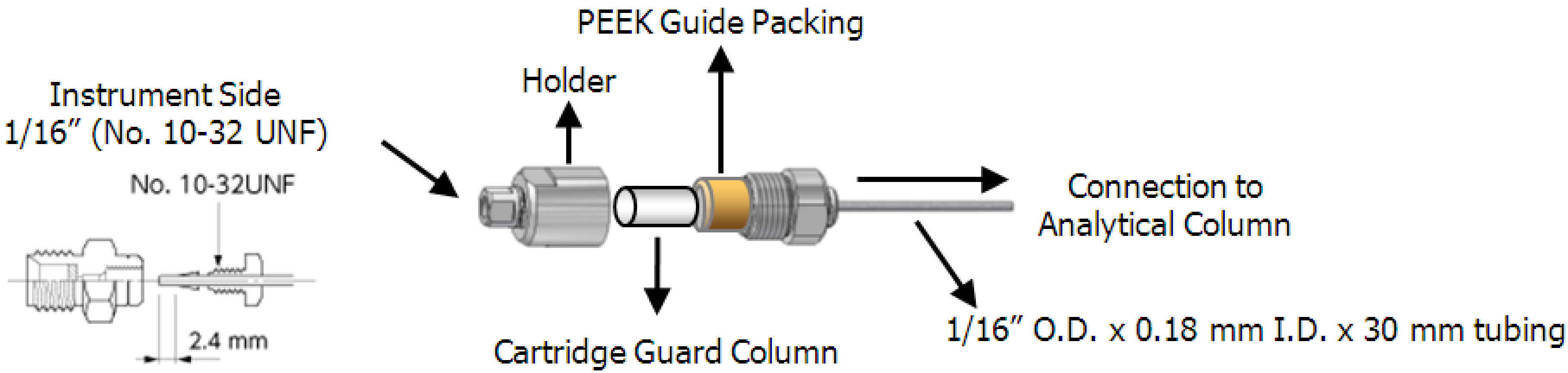
※ End-fittings are 1/16” Waters-compatible.
※ Other column sizes available upon request.

Ordering Information

Guard Column for UHPLC (Max. Operating Pressure 80 MPa, 800 Bar)

I.D. of the Analytical Column Applicable (mm)	Length (mm)	I.D. (mm)	Replacement Cartridge (2 pcs)	Cartridge (2 pcs) + Holder (1 pcs) Set
			Particle Size 3 µm	Particle Size 3 µm
1.0	10	1.5	5020-89824	5020-89827
1.5, 2.1		2.1	5020-89825	5020-89828
2.1, 3.0		3.0	5020-89826	5020-89829
Holder for Guard Column for UHPLC				5020-08630

※ For connection details, please refer to the diagram below.



Cartridge Guard Columns

I.D. of the Analytical Column Applicable (mm)	Length (mm)	I.D. (mm)	Replacement Cartridge E (2 pcs)		Cartridge E (2 pcs) + Holder (1 pcs) Set	
			Particle Size		Particle Size	
			3 µm	5 µm	3 µm	5 µm
1.0	10	1.0	5020-89910	5020-89808	5020-89911	5020-89809
1.5,2.1		1.5	5020-89912	5020-89810	5020-89913	5020-89811
2.1,3.0		3.0	5020-89908	5020-89806	5020-89909	5020-89807
4.0,4.6		4.0	5020-89906	5020-89804	5020-89907	5020-89805
2.1,3.0	20	3.0	5020-89916	5020-89814	5020-89917	5020-89815
4.0,4.6		4.0	5020-89914	5020-89812	5020-89915	5020-89813
Holder for Cartridge Guard Column E				For 10 mm Length		5020-08500
				For 20 mm Length		5020-08550

※ End-fittings are 1/16” Waters-compatible.
※ Maximum operating pressure is 20 MPa, 200 Bar.

Ordering Information

Preparative Columns

	Length/ I.D. (mm)	6.0	7.6	10	14	20
Particle Size:5 µm Max. Operating Pressure: 20 MPa (200 Bar)	50	5020-89757	5020-89761	5020-89765	5020-89769	5020-89773
	100	5020-89758	5020-89762	5020-89766	5020-89770	5020-89774
	150	5020-89759	5020-89763	5020-89767	5020-89771	5020-89775
	250	5020-89760	5020-89764	5020-89768	5020-89772	5020-89776

Preparative Columns

	Length x I.D. (mm)	6.0	7.6	10	14	20
Particle Size:5 µm Max. Operating Pressure: 20 MPa (200 Bar)	50	5020-89777	5020-89778	5020-89779	5020-89780	5020-89781

Ordering Information

Capillary Columns

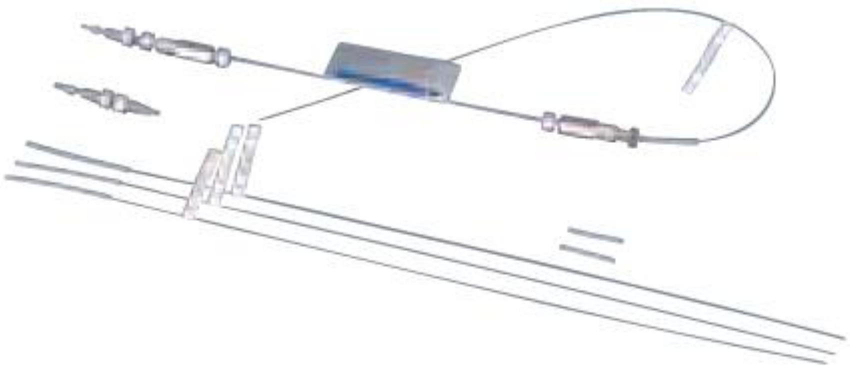
EX-Nano Columns Particle Size: 3 µm Max. Operating Pressure: 15 MPa (150 Bar)	Length/ I.D. (mm)	0.05	0.075	0.1	0.2
	50	5020-89894	5020-89897	5020-89900	5020-89903
	150	5020-89895	5020-89898	5020-89901	5020-89904
	250	5020-89896	5020-89899	5020-89902	5020-89905
EX Columns Particle Size: 3 µm Max. Operating Pressure: 20 MPa (200 Bar)	Length/ I.D. (mm)	0.3	0.5	0.7	
	50	5020-89887	5020-89889	5020-89891	
	150	5020-89888	5020-89890	5020-89892	
	250	-	-	-	
EX-Nano Columns Particle Size: 5 µm Max. Operating Pressure: 15 MPa (150 Bar)	Length/ I.D. (mm)	0.05	0.075	0.1	0.2
	50	5020-89792	5020-89795	5020-89798	5020-89801
	150	5020-89793	5020-89796	5020-89799	5020-89802
	250	5020-89794	5020-89797	5020-89800	5020-89803
EX Columns Particle Size: 5 µm Max. Operating Pressure: 20 MPa (200 Bar)	Length/ I.D. (mm)	0.3	0.5	0.7	
	50	5020-89784	5020-89786	5020-89788	
	150	5020-89785	5020-89787	5020-89789	
	250	-	-	-	

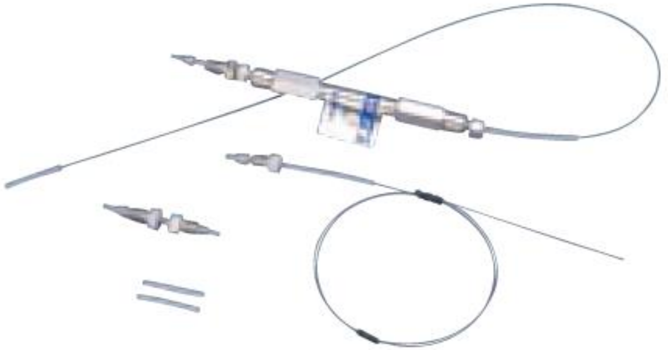
※ End-fittings are 1/16” Valco (10-32 UNF).
※ 1/32” Valco (6-40 UNF) end-fittings can also be arranged upon request.
※ Indicate “1/32” end-fittings” when ordering.

Ordering Information

Connection Kits for Capillary EX-Nano and EX Columns

·Column Coupler ·40 × 0.05 mm I.D. 1/16" O.D. Tubing (Both ends with male nuts including PEEK ferrules) ·Capillary Tubing Connector ·300 × 0.05 mm I.D. 0.375 mm O.D. Tubing ·300 × 0.03 mm I.D. 0.375 mm O.D. Tubing ·300 × 0.02 mm I.D. 0.375 mm O.D. Tubing (Male nut, PEEK ferrule, 1/16" O.D. PTFE with sleeve) ·PTFE Tubing 20 mm 2 pcs 1/16" O.D. (O.D. 0.375 mm Connection for Capillary Tubing)	For Capillary EX-Nano Columns 5020-01881
·Column Coupler ·40 × 0.1 mm I.D. 1/16" O.D.Tubing (Both ends with male nuts including PEEK ferrules) ·Capillary Tubing Connector 5020-01880 ·500 × 0.075 mm I.D. 0.375 mm O.D. Tubing (Male nut, PEEK ferrule, 1/16" O.D. PTFE with sleeve) ·PTFE Tubing 20 mm 2 pcs 1/16" O.D. (O.D. 0.375 mm Connection for Capillary Tubing)	For Capillary EX Columns 5020-01880



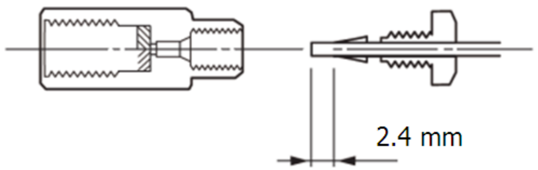


Ordering Information

Metal-Free PEEK Columns

	Length/ I.D. (mm)	2.1	4.6
PEEK Columns Particle Size: 5 µm Max. Operating Pressure: 20 MPa (200 Bar)	30	Please inquire	-
	33	Please inquire	Please inquire
	50	Please inquire	Please inquire
	75	-	Please inquire
	100	Please inquire	Please inquire
	150	Please inquire	Please inquire
	200	-	Please inquire
	250	Please inquire	Please inquire
	300	Please inquire	Please inquire
Steel-Coated PEEK Columns Particle Size: 3 µm Max. Operating Pressure: 50 MPa (500 Bar)	Length/ I.D. (mm)	2.1	4.6
	50	Please inquire	Please inquire
	100	Please inquire	Please inquire
	150	Please inquire	Please inquire
	250	Please inquire	Please inquire

End-fitting Format



UP Type End-fittings

※ End-fittings are UP type end-fittings.
※ For further product details, please contact your local GL Sciences' representative.

InertSearch Application Notes

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