



InertSustain Phenylhexyl™

GL Sciences Inc.

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Introduction

InertSustain Phenylhexyl

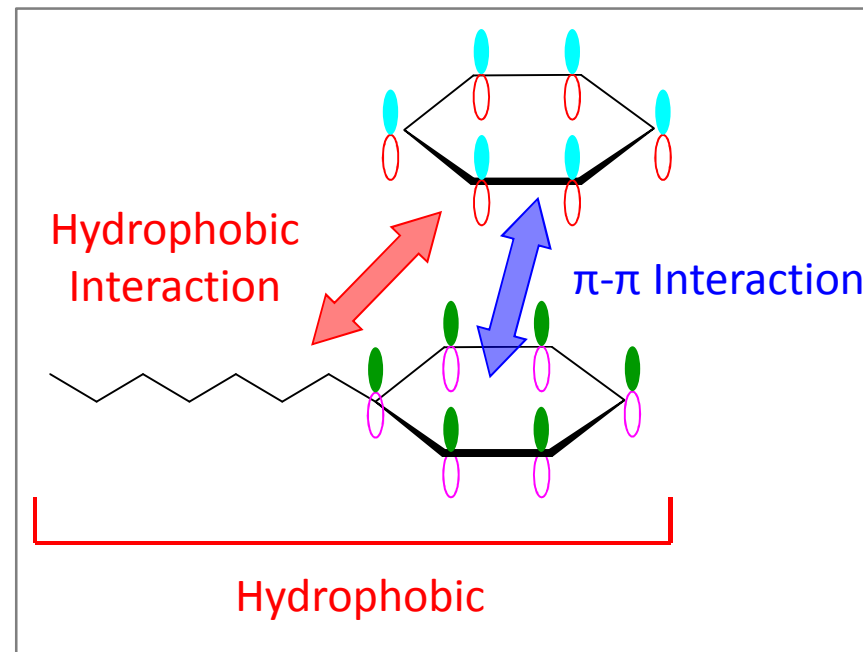
A New Second Choice Column to Change Chromatographic Behavior

InertSustain Phenylhexyl columns are bonded with phenylhexyl groups, which employs a phenyl ring with a hexyl (6-carbon) linker and is densely bonded to our newly developed ES silica gel delivering complementary selectivity to straight alkyl-chain columns, but with industry leading inertness, lot-to-lot reproducibility and low back pressure.



Physical Properties

Silica :	Newly Developed ES Silica Gel
Particle Size :	3 μm , 5 μm
Surface Area :	350 m^2/g
Pore Size :	100 \AA (10 nm)
Pore Volume :	0.85 mL/g
Bonded Phase :	Phenylhexyl Groups (C6-Ph)
End-capping :	Complete
Carbon Loading :	9.0 %
USP Code :	L11
pH Range :	1.0 to 10.0



Benefits of InertSustain Phenylhexyl

Advantages of InertSustain Phenylhexyl

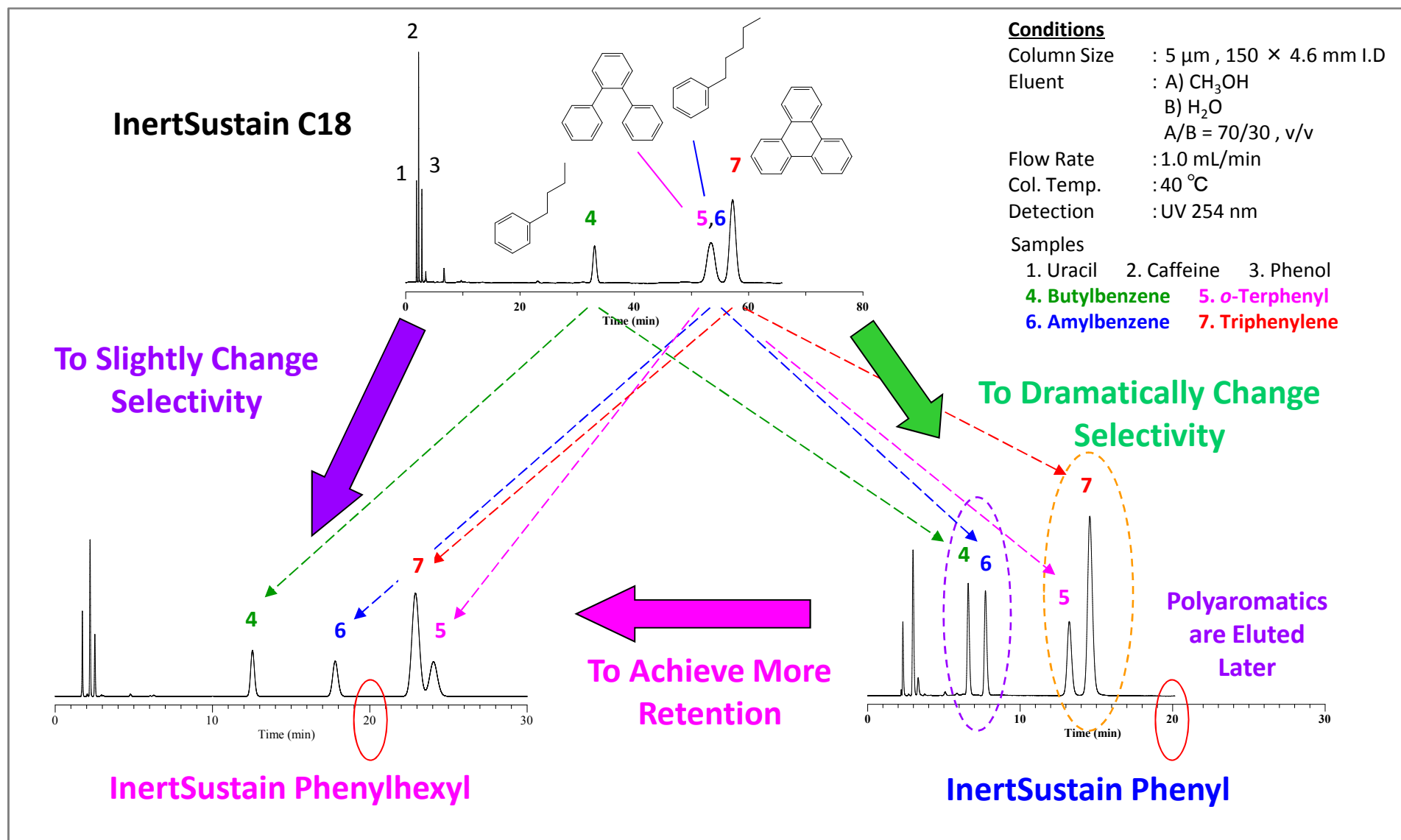
Different Selectivity to C18 Phases

Strong Retentivity

Highly Inert Packing Material
(Less Tailing of Peaks)

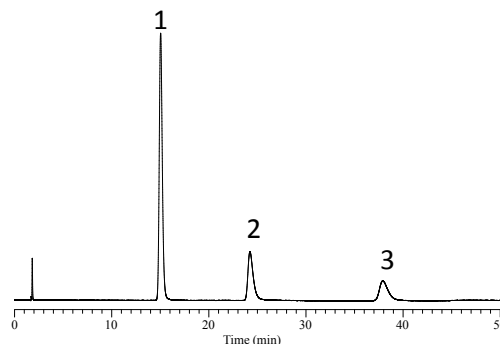
Extreme Durability = Longer Column Life

Different Selectivity to C18 Phases

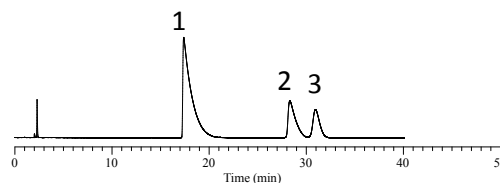


Different Selectivity to C18 Phases

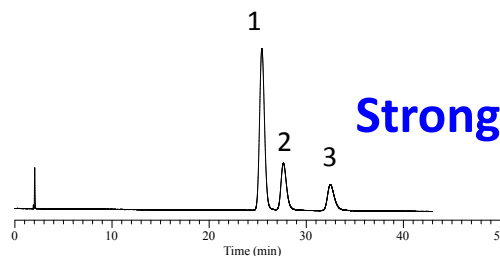
InertSustain C18



InertSustain Phenyl

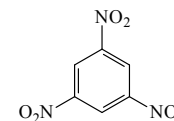


InertSustain Phenylhexyl

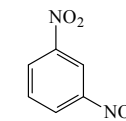


Conditions

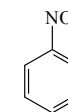
Column Size : 5 μm , 150 x 4.6 mm I.D
Eluent : A) CH_3OH
B) H_2O
A/B = 30/70, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 $^\circ\text{C}$
Detection : UV 210 nm
Sample : 1. 1,3,5-Trinitrobenzene
2. 1,3-Dinitrobenzene
3. Nitrobenzene



1. 1,3,5-Trinitrobenzene



2. 1,3-Dinitrobenzene

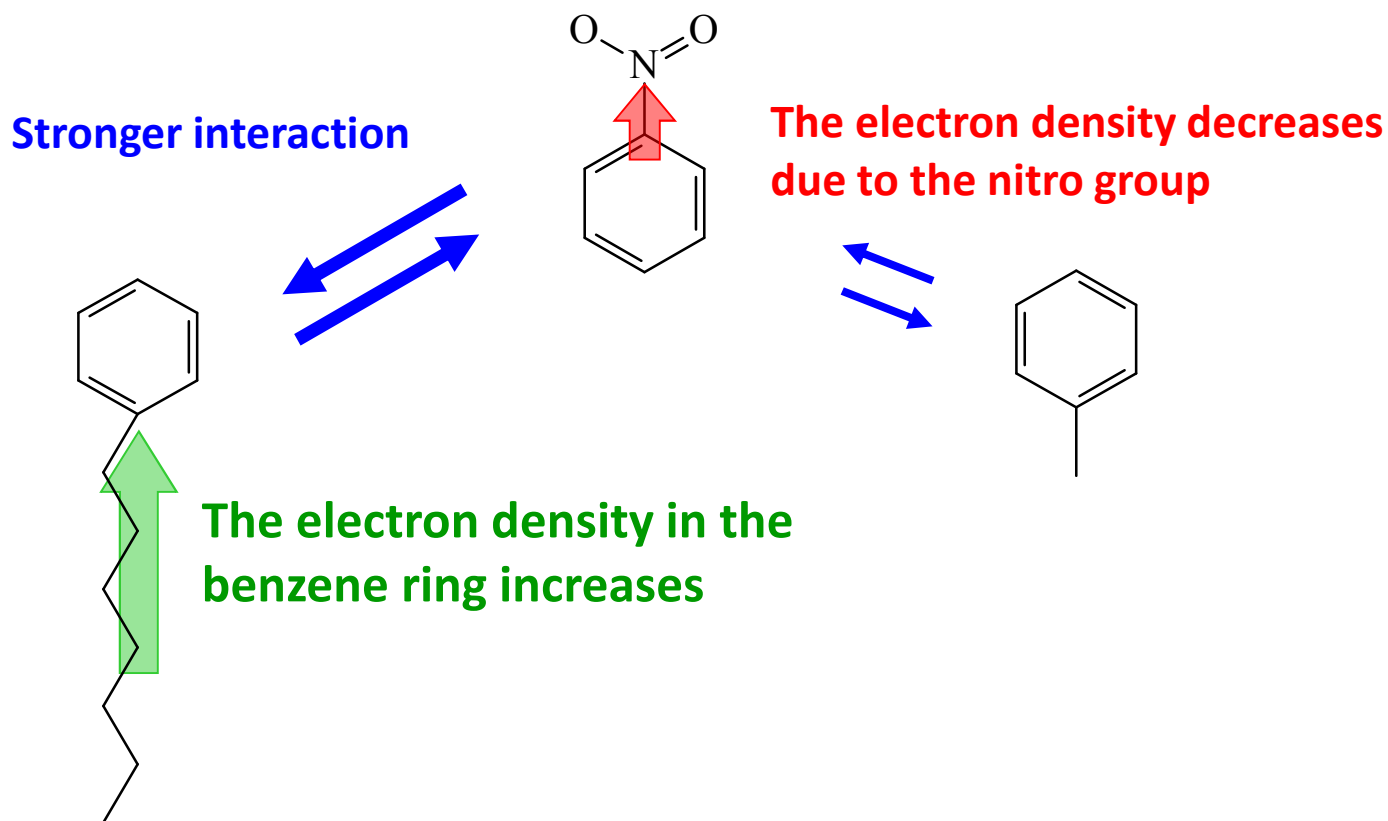


3. Nitrobenzene

InertSustain Phenylhexyl show strong retention for molecules containing nitro group.

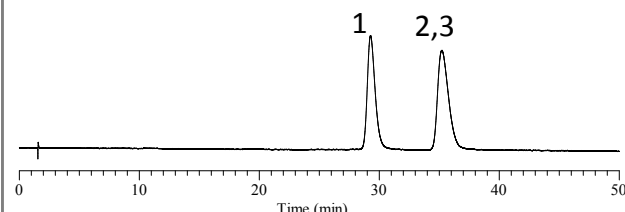
Different Selectivity to C18 Phases

Retention Mechanism of Nitro Compounds



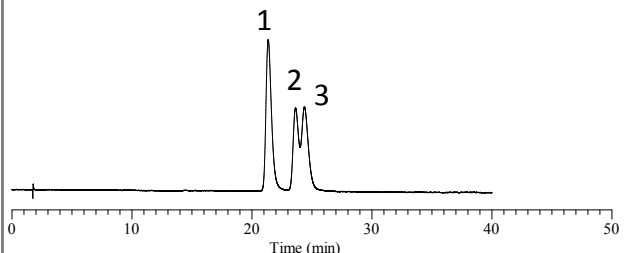
InertSustain Phenylhexyl creates high electron density in the benzene ring, resulting in strong retention of nitro compounds.

Different Selectivity to C18 Phases



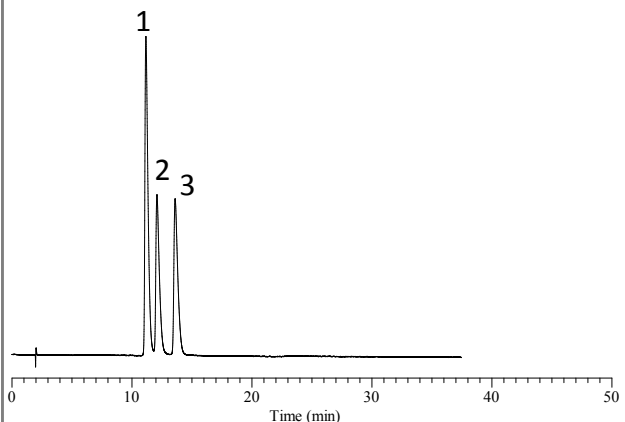
InertSustain C18

o- and m- cannot be separated



InertSustain Phenylhexyl

The selectivity of InertSustain Phenylhexyl is in between a C18 and Phenyl phase

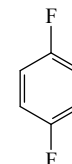


InertSustain Phenyl

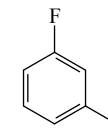
InertSustain Phenyl show good separation of fluorinated compounds

Conditions

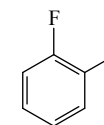
Column Size	: 5 μ m, 150 \times 4.6 mm I.D
Eluent	: A) CH ₃ OH B) H ₂ O A/B = 30/70, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 $^{\circ}$ C
Detection	: UV 254 nm
Sample	: 1. <i>p</i> -Difluorobenzene 2. <i>m</i> -Difluorobenzene 3. <i>o</i> -Difluorobenzene



p-Difluorobenzene



m-Difluorobenzene



o-Difluorobenzene

Benefits of InertSustain Phenylhexyl

Advantages of InertSustain Phenylhexyl

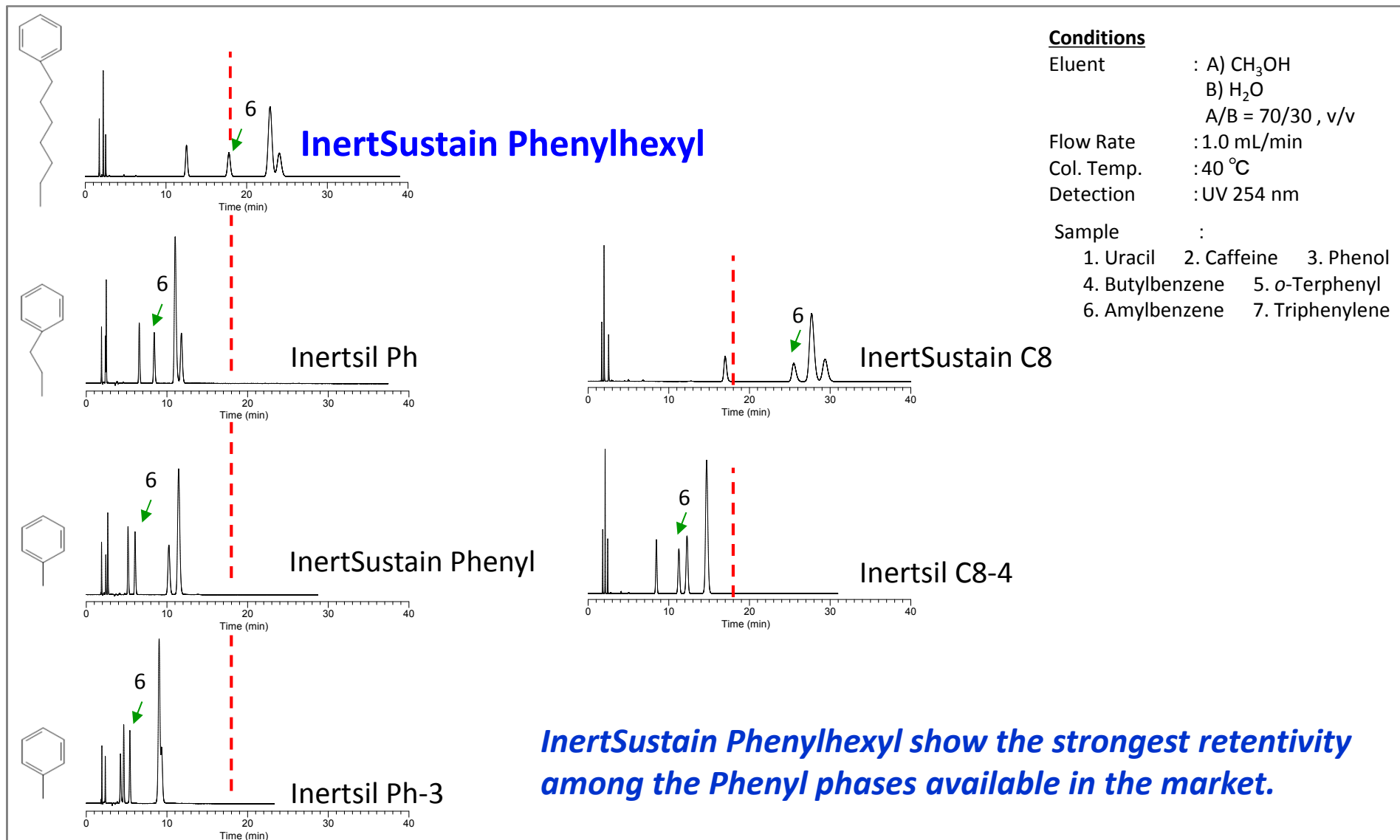
Different Selectivity to C18 Phases

Strong Retentivity

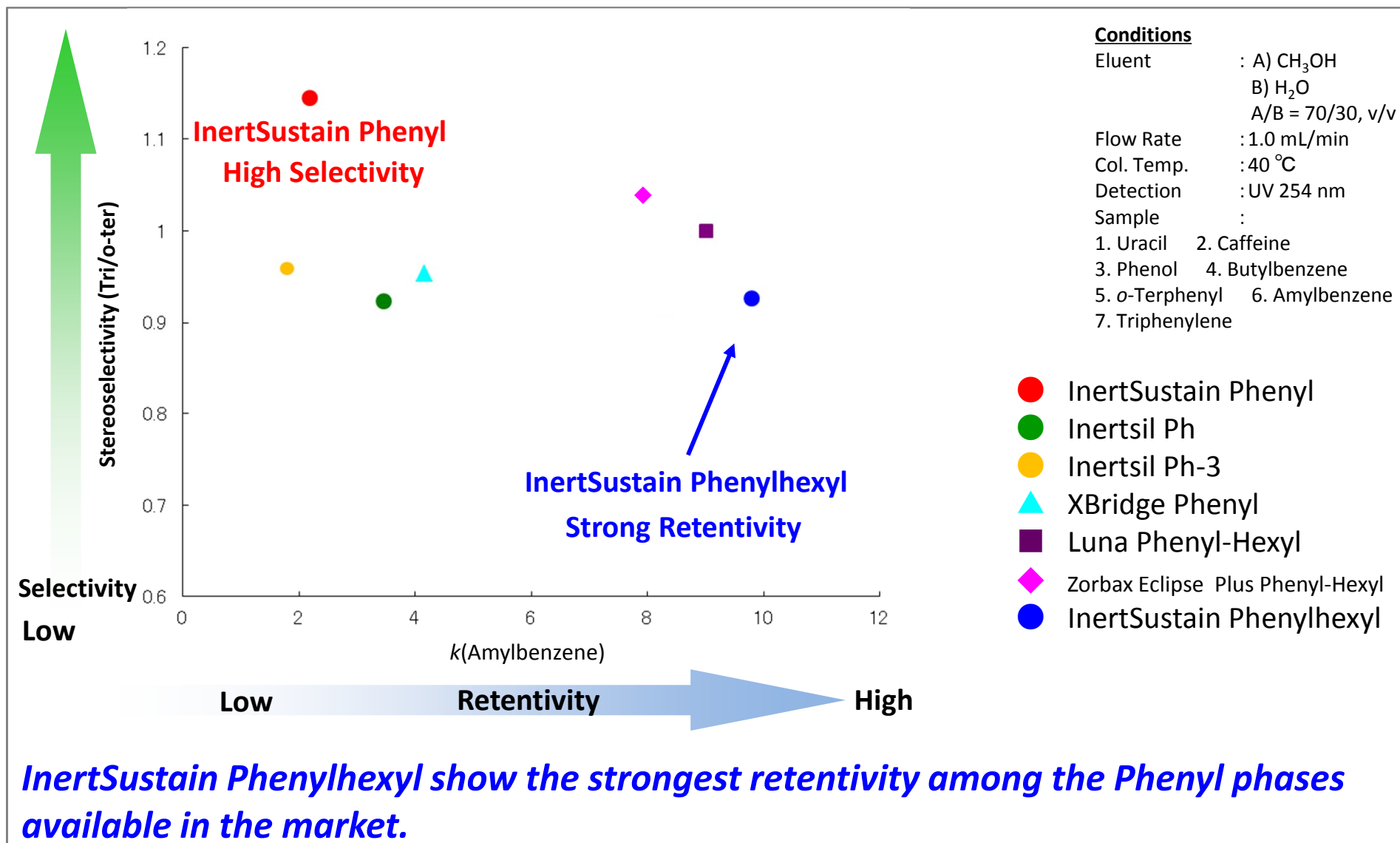
Highly Inert Packing Material
(Less Tailing of Peaks)

Extreme Durability = Longer Column Life

Strong Retentivity



Strong Retentivity



Benefits of InertSustain Phenylhexyl

Advantages of InertSustain Phenylhexyl

Different Selectivity to C18 Phases

Strong Retentivity

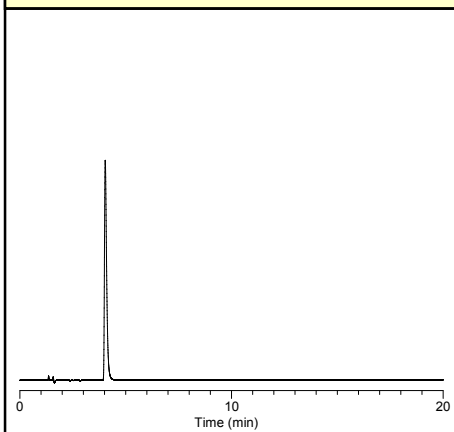
Highly Inert Packing Material
(Less Tailing of Peaks)

Extreme Durability = Longer Column Life

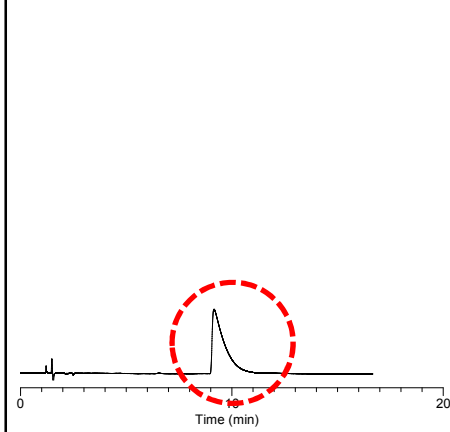
Benefits of Highly Inert Packing Material

Analysis of Strong Basic Compounds

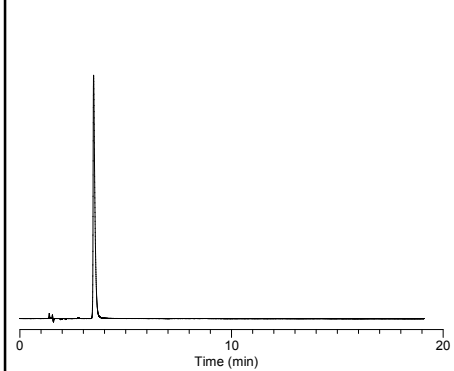
InertSustain Phenylhexyl



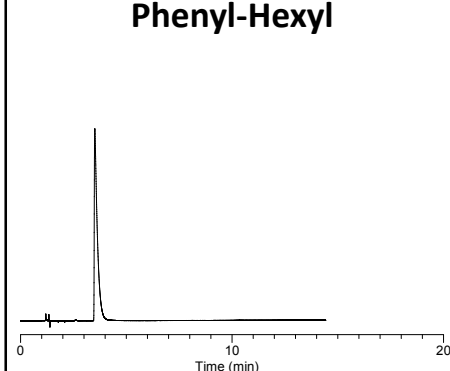
Luna Phenyl-Hexyl



XBridge Phenyl

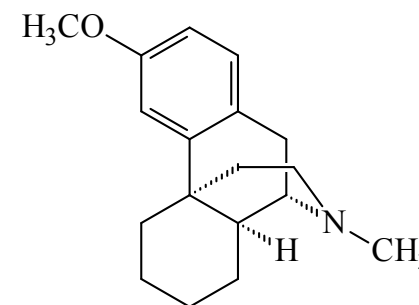


**Zorbax Eclipse Plus
Phenyl-Hexyl**



Conditions

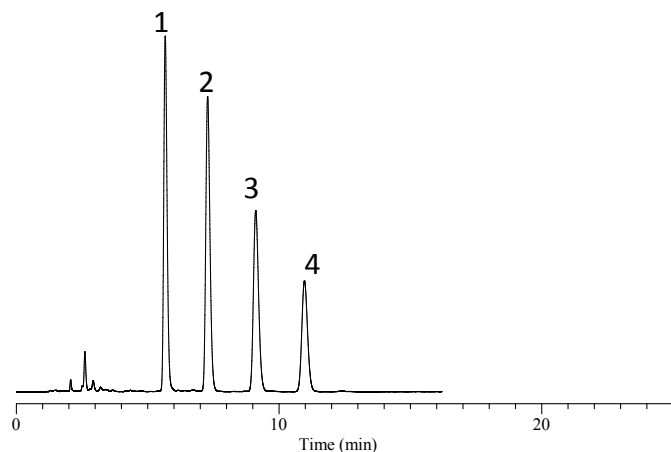
Eluent : A) CH₃CN
: B) 25 mM K₂HPO₄ (pH 7.0, KH₂PO₄)
: A/B = 40/60, v/v
Flow Rate : 1.0 mL / min
Col. Temp. : 40 °C
Detection : UV 230 nm
Sample : Dextromethorphan



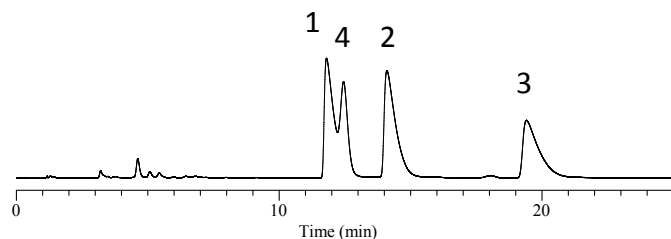
Benefits of Highly Inert Packing Material

Analysis of Antidepressants

InertSustain Phenylhexyl



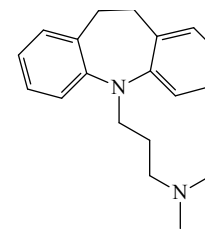
Luna Phenyl-Hexyl



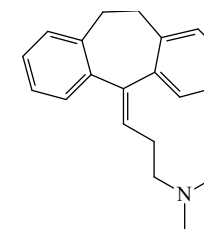
Conditions

Eluent : A) CH₃CN
 B) 25 mM KH₂PO₄ (pH 7.0, K₂HPO₄)
 A/B = 50/50, v/v

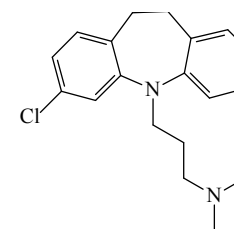
Flow Rate : 1.0 mL/min
 Col. Temp. : 40 °C
 Detection : UV 254 nm
 Sample : 1. Imipramine hydrochloride
 2. Amitriptyline hydrochloride
 3. Clomipramine hydrochloride
 4. Mianserin hydrochloride



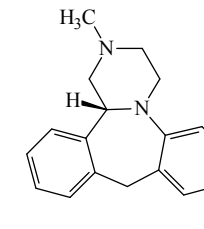
Imipramine hydrochloride



Amitriptyline hydrochloride



Clomipramine hydrochloride

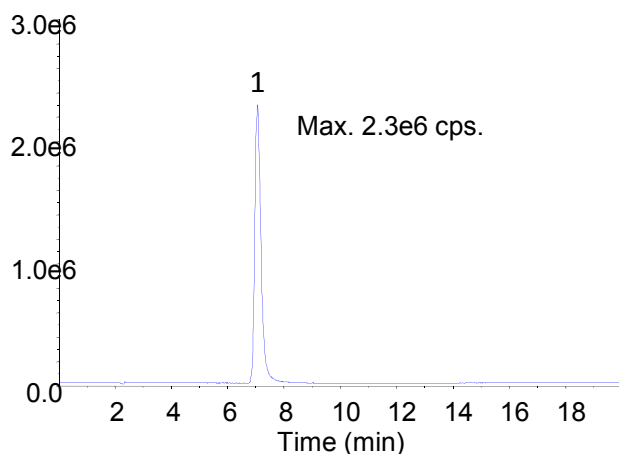


Mianserin hydrochloride

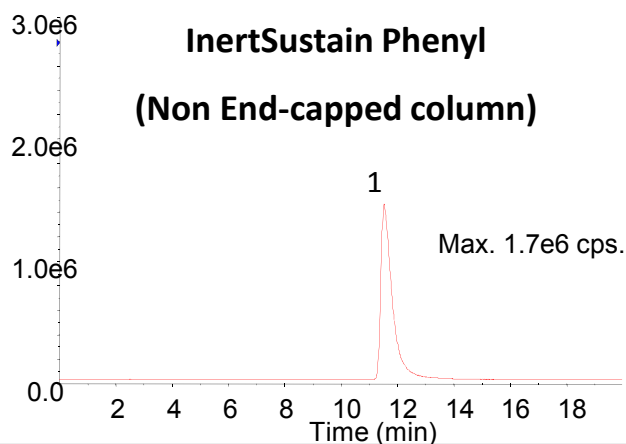
Benefits of Highly Inert Packing Material

Analysis of Strong Basic Compound via LC-MS/MS

InertSustain Phenylhexyl



InertSustain Phenyl (Non End-capped column)



Conditions

System : GL-7700

4000 QTRAP

Column : InertSustain Phenylhexyl (3 μ m, 150 x 2.1 mm I.D.)

InertSustain Phenyl (3 μ m, 150 x 2.1 mm I.D.)

Eluent : A) CH₃CN

B) 10 mM CH₃COONH₄ in H₂O

A/B = 30/70 (gradient mixer), v/v

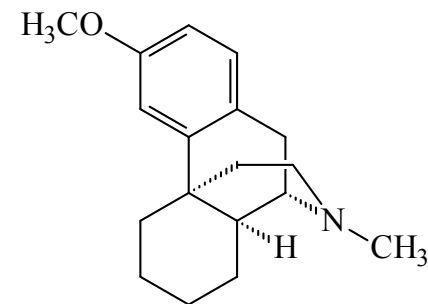
Flow rate : 200 μ L/min

Col. Temp. : 40 °C

Detection : SIM (ESI, Positive)

Injection Vol. : 1 μ L

Sample : Dextromethorphan 50 ng/mL

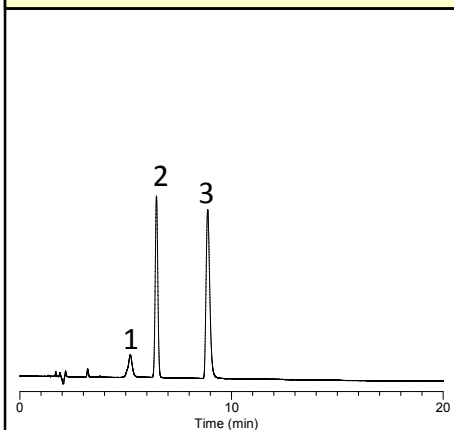


InertSustain Phenylhexyl employs a highly inert packing material which prevents secondary interaction between residual silanols delivering rapid results with higher sensitivity.

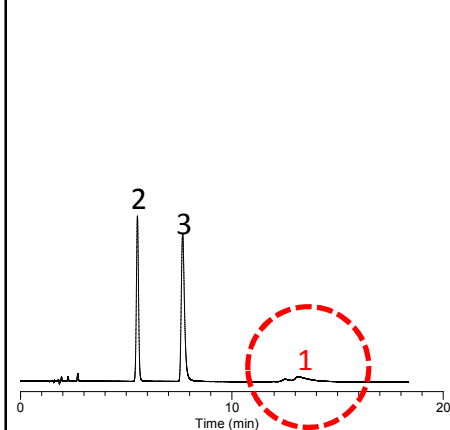
Benefits of Highly Inert Packing Material

Analysis of Strong Acidic Compounds

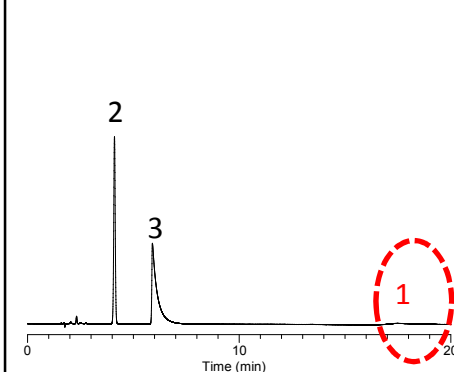
InertSustain Phenylhexyl



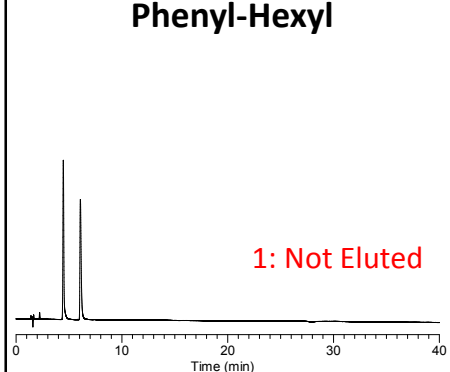
Luna Phenyl-Hexyl



XBridge Phenyl



Zorbax Eclipse Plus Phenyl-Hexyl



Conditions

Eluent : A) CH₃CN
: B) 0.1 % H₃PO₄
: A/B = 25/75, v/v

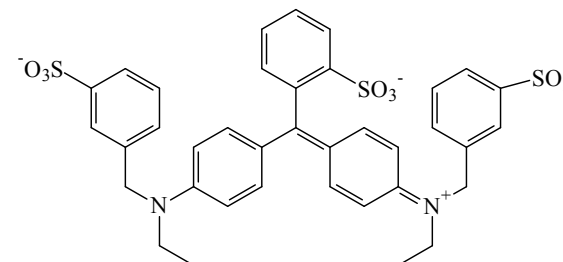
Flow rate : 1.0 mL/min

Col. Temp. : 40 °C

Detection : UV 254 nm

Sample :

1. Brilliant Blue FCF
2. Phenol
3. Salicylic acid



1. Brilliant Blue FCF

Benefits of InertSustain Phenylhexyl

Advantages of InertSustain Phenylhexyl

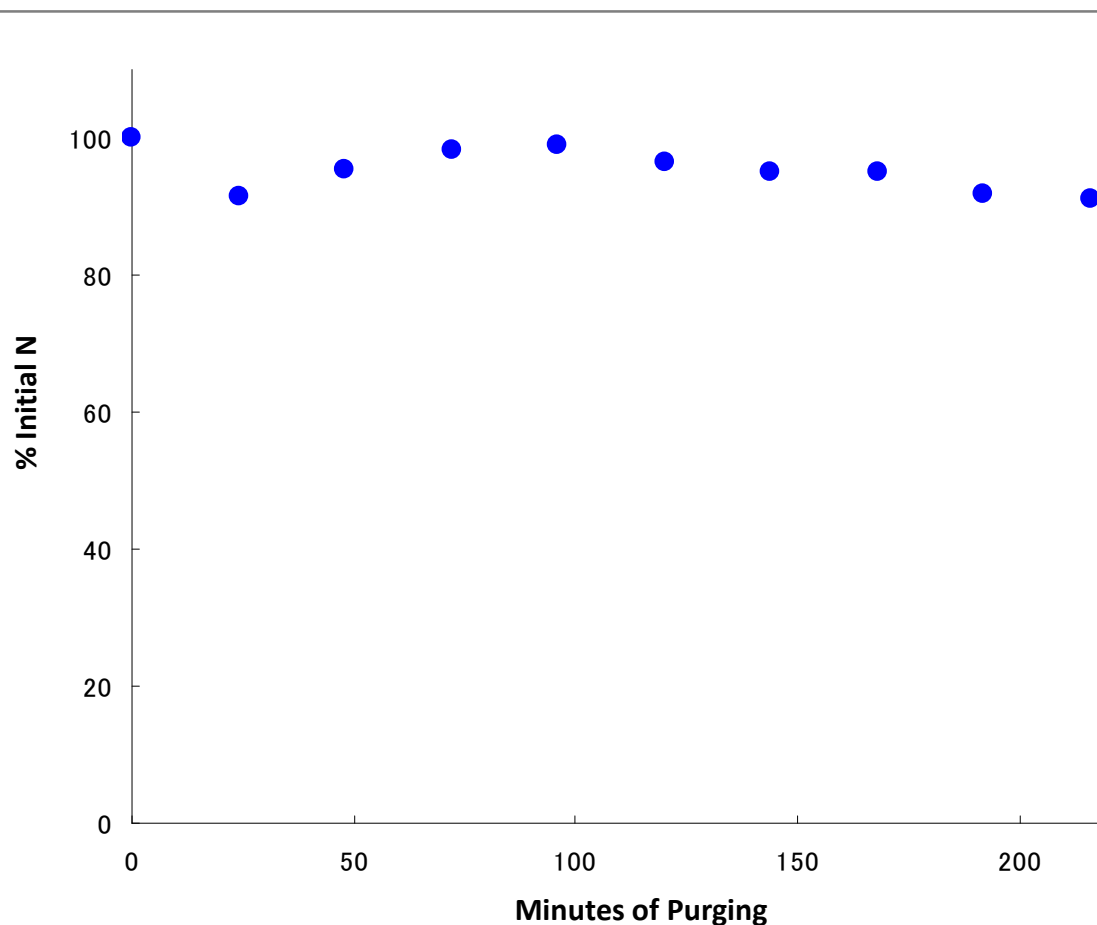
Different Selectivity to C18 Phases

Strong Retentivity

Highly Inert Packing Material
(Less Tailing of Peaks)

Extreme Durability = Longer Column Life

Extreme Durability



Purging Conditions

Column : 4.6 mm I.D. x 150 mm, 5 μ m
Eluent : 50 mM Triethylamine (pH 9.5) /
CH₃OH = 70/30, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 50 °C

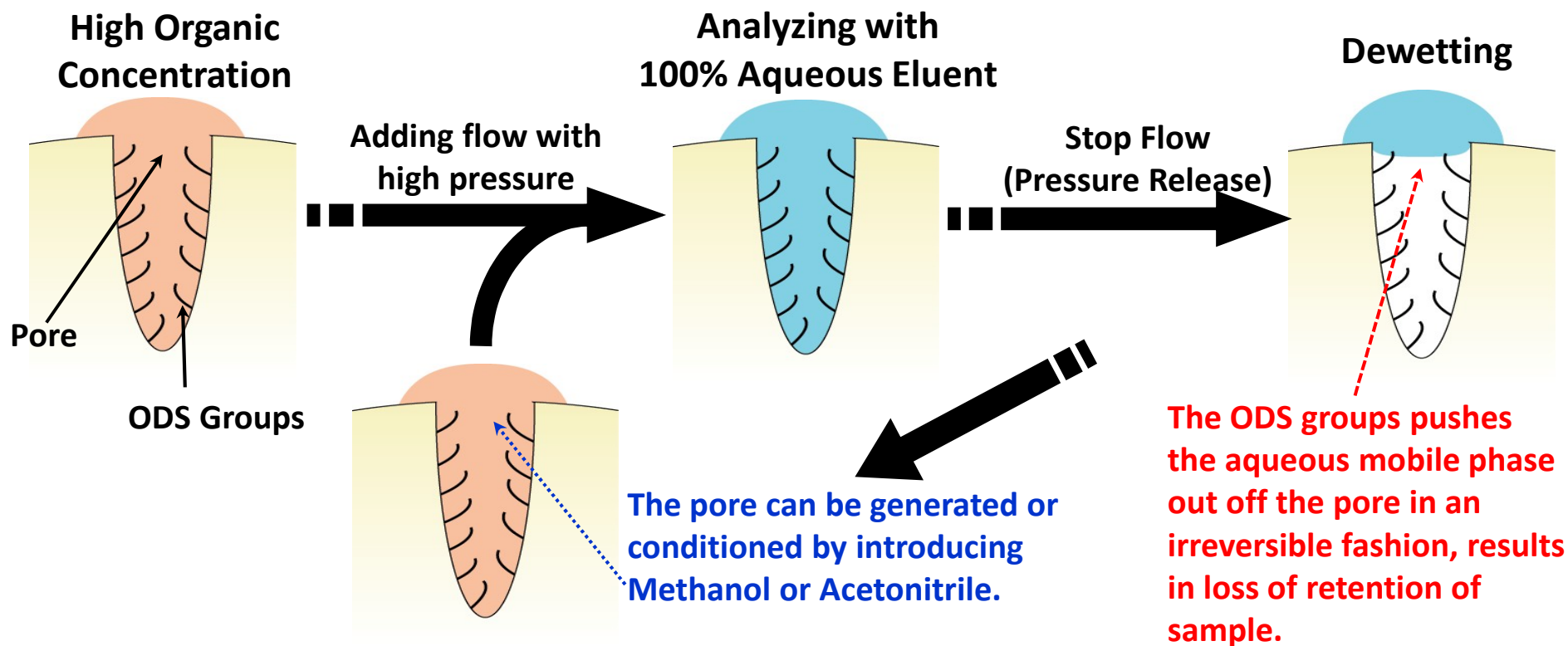
Analytical Conditions

Eluent : CH₃CN/H₂O = 65/35, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 °C
Detection : UV254 nm
Sample : Naphthalene

*Some samples require high pH for dissolution or to maintain stability.
InertSustainSwift C18 offers wide pH compatibility and high durability.*

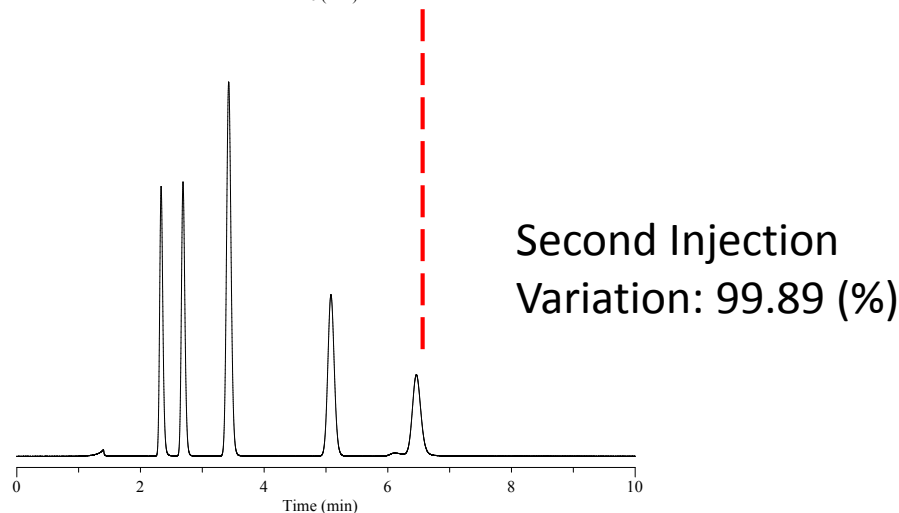
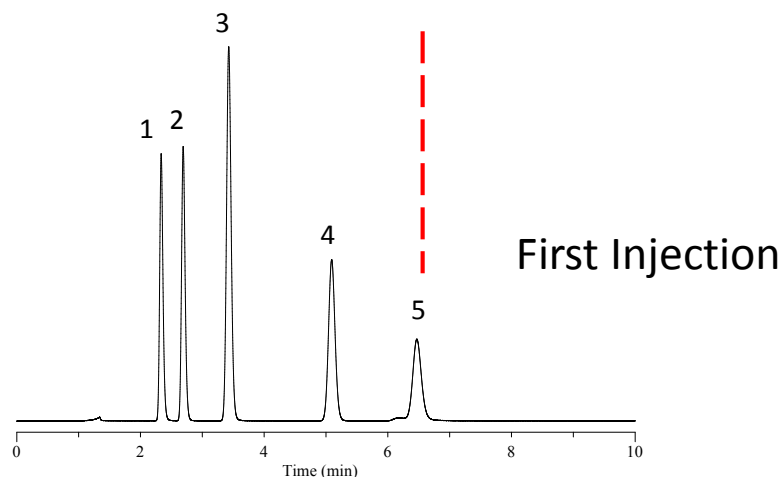
Extreme Durability + Stability

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.



Extreme Durability + Stability

InertSustain Phenylhexyl



Testing Conditions

- 1) 100 % water is introduced into column over 60 minutes.
- 2) Conduct analysis.
- 3) Stop flow for 15 minutes.
- 4) 100 % water is introduced again into column over 30 minutes.
- 5) Stop flow for 15 minutes again.
- 6) Conduct analysis

Conditions

Eluent	: 100 % H ₂ O
Flow rate	: 1.0 mL/min
Col. Temp.	: 40 °C
Detection	: UV 254 nm
Sample	: 1.Cytosine 2.Uracil
	3.Guanine 4.Thymine
	5.Adenine

InertSustain Phenylhexyl C18 demonstrates excellent resistance to dewetting guaranteeing highly stable, reliable and reproducible chromatograms.

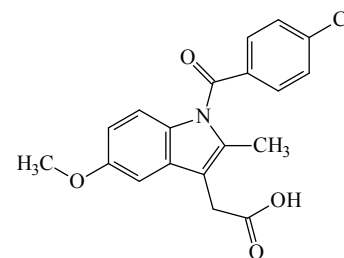
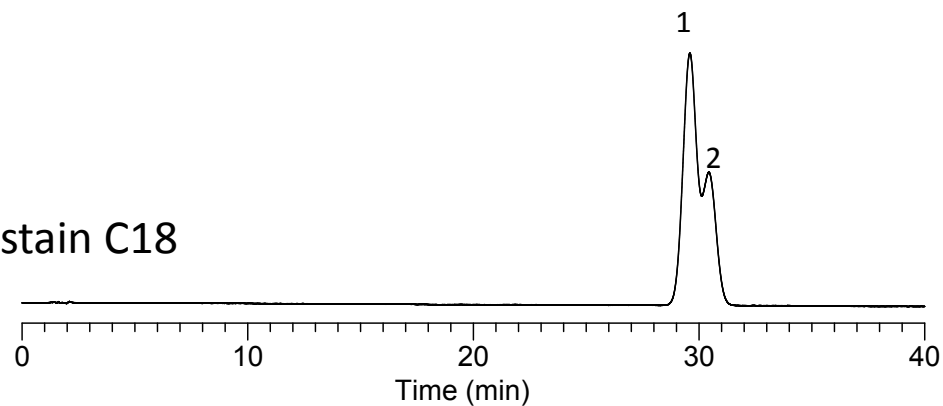
Applications

Analysis of Analgesic Drugs

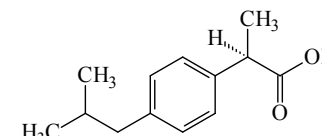
Conditions

Column Size	: 5 μ m , 150 \times 4.6 mm I.D.
Eluent	: A) CH ₃ CN B) 25 mM KH ₂ PO ₄ (pH 3.0, H ₃ PO ₄) A/B = 45/55, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 $^{\circ}$ C
Detection	: UV 230 nm
Sample	: 1. Indomethacin 2. Ibuprofen

InertSustain C18



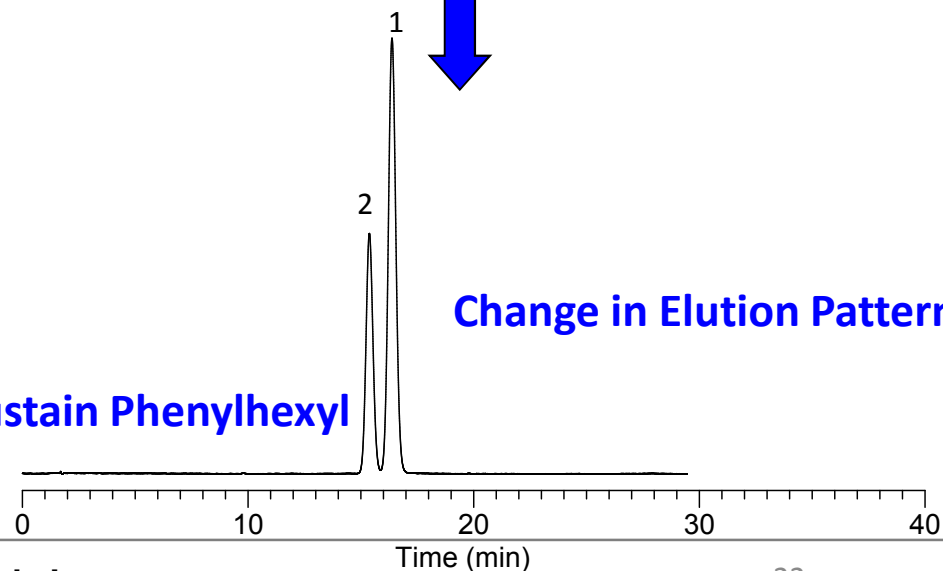
Indomethacin



Ibuprofen

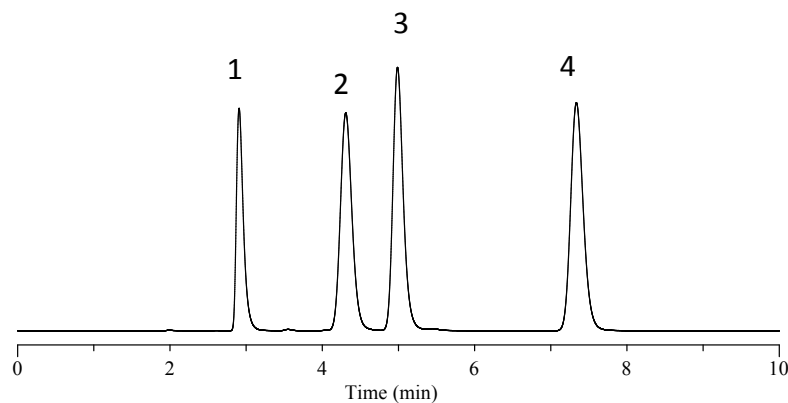
Change in Elution Pattern

InertSustain Phenylhexyl



Applications

Analysis of Vitamin B6



Conditions

Column Size : 5 μ m, 150 \times 4.6 mm I.D.

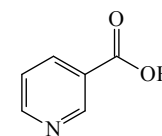
Eluent : 10 mM Ammonium acetate

Flow Rate : 1.0 mL/min

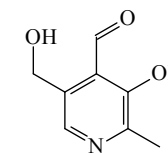
Col. Temp. : 40 $^{\circ}$ C

Detection : UV 230 nm

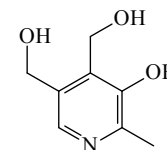
Sample : 1. Nicotinic acid 2. Pyridoxal
3. Pyridoxine 4. Nicotinamide



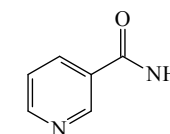
Nicotinic Acid



Pyridoxal



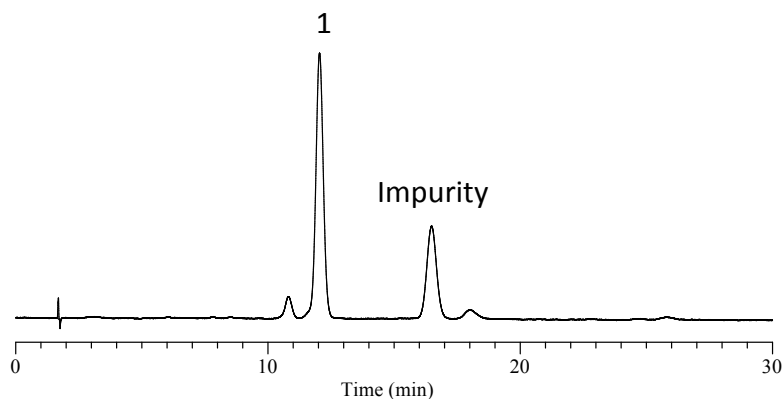
Pyridoxine



Nicotinamide

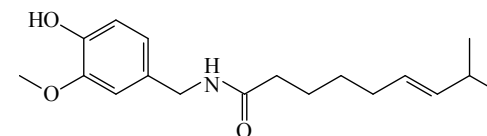
Applications

Analysis of Capsaicin



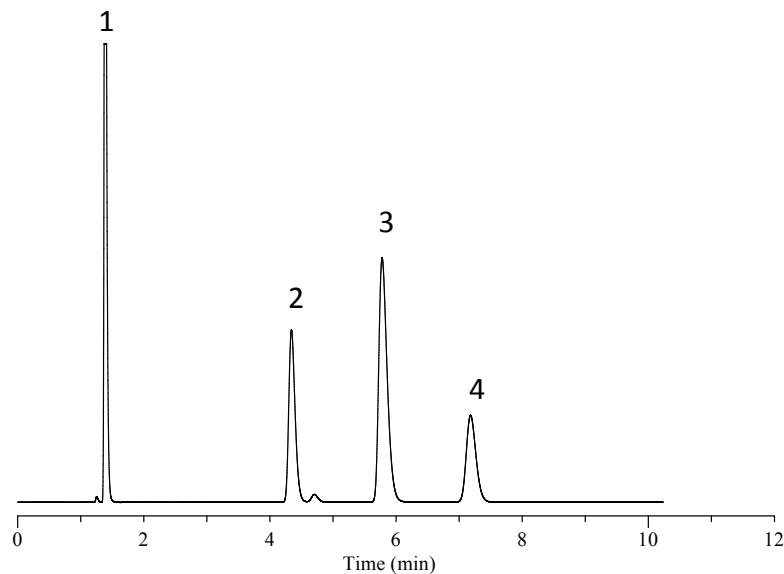
Conditions

Column Size : 5 μ m, 150 \times 4.6 mm I.D.
Eluent : A) CH₃OH
 B) H₂O
 A/B = 60/40, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 $^{\circ}$ C
Detection : UV 280 nm
Sample : 1. Capsaicin



Applications

Analysis of Antihistamine



Conditions

Column Size : 5 μ m, 150 \times 4.6 mm I.D.

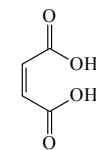
Eluent : A) CH₃CN
B) 25 mM KH₂PO₄ (pH 7.0, K₂HPO₄)
A/B = 40/60, v/v

Flow Rate : 1.0 mL/min

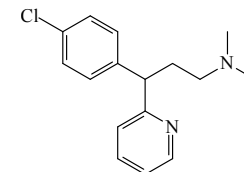
Col. Temp. : 40 °C

Detection : UV 230 nm

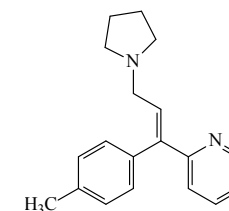
Sample : 1. Maleic acid 2. Chlorpheniramine
3. Triprololodine 4. Diphenhydramine



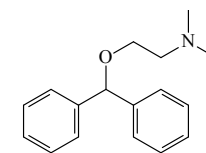
Maleic Acid



Chlorpheniramine



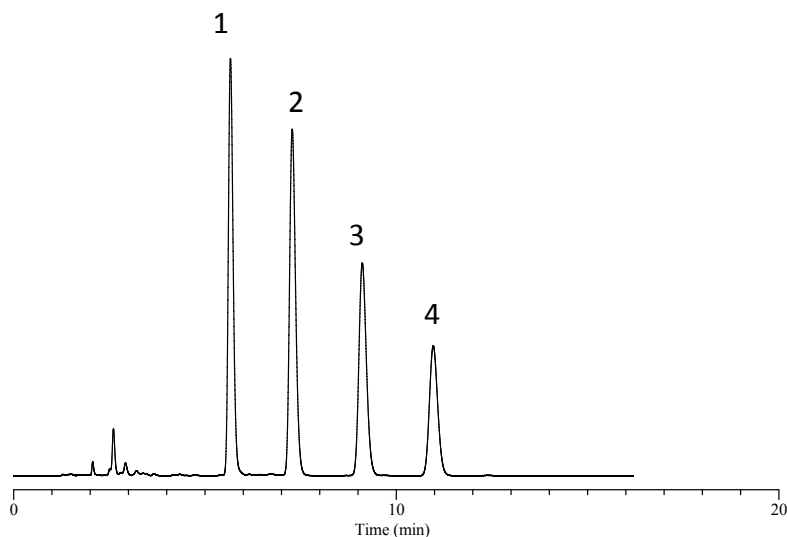
Triprololodine



Diphenhydramine

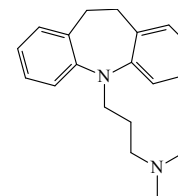
Applications

Analysis of Antidepressants

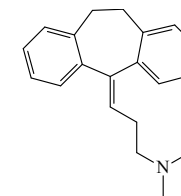


Conditions

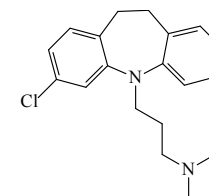
- Column Size : 5 μ m, 150 \times 4.6 mm I.D.
Eluent : A) CH₃CN
 B) 25 mM KH₂PO₄ (pH 7.0, K₂HPO₄)
 A/B = 50/50, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 $^{\circ}$ C
Detection : UV 254 nm
Sample : 1. Imipramine hydrochloride
 2. Amitriptyline hydrochloride
 3. Chlomipramine hydrochloride
 4. Mianserin hydrochloride



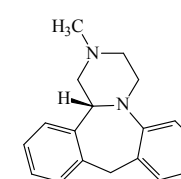
Imipramine hydrochloride



Amitriptyline hydrochloride



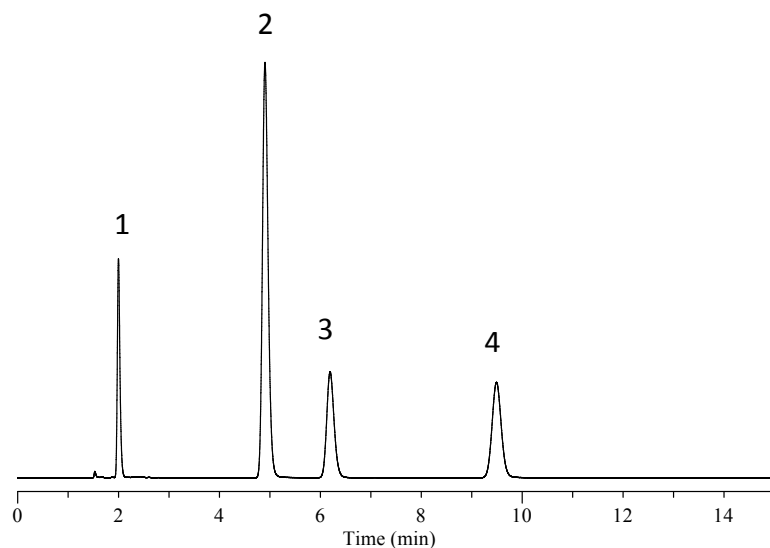
Chlomipramine hydrochloride



Mianserin hydrochloride

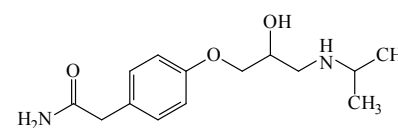
Applications

Analysis of Beta Blockers

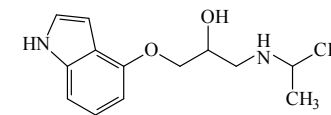


Conditions

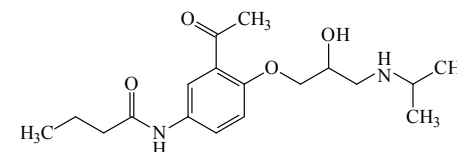
Column Size : 5 μ m, 150 \times 4.6 mm I.D.
Eluent : A) CH₃CN
 B) 25 mM KH₂PO₄ (pH 7.0, K₂HPO₄)
 A/B = 20/80, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 °C
Detection : UV 220 nm
Sample : 1. Atenolol 2. Pindolol
 3. Acebutolol 4. Phenol



Atenolol



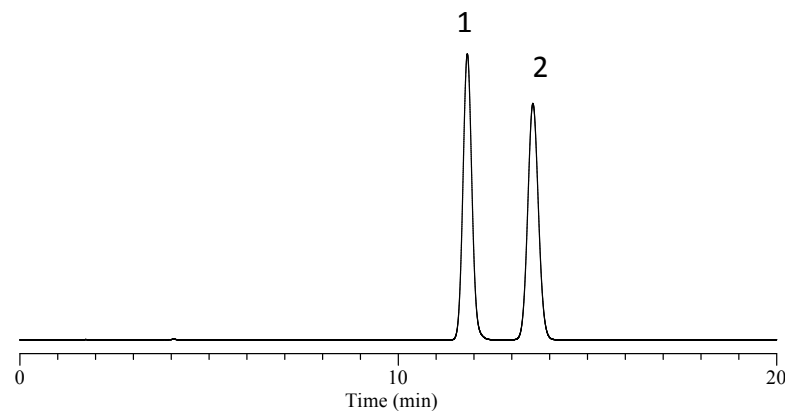
Pindolol



Acebutolol

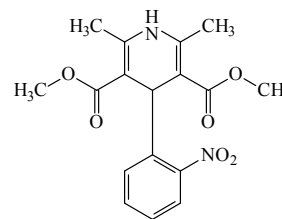
Applications

Analysis of Calcium Antagonist Agents

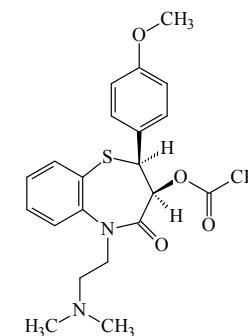


Conditions

Column Size : 5 μ m, 150 \times 4.6 mm I.D.
Eluent : A) CH₃CN
 B) 25 mM KH₂PO₄ (pH 7.0, K₂HPO₄)
 A/B = 40/60, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 °C
Detection : UV 235 nm
Sample : 1. Nifedipine
 2. Diltiazem



Nifedipine

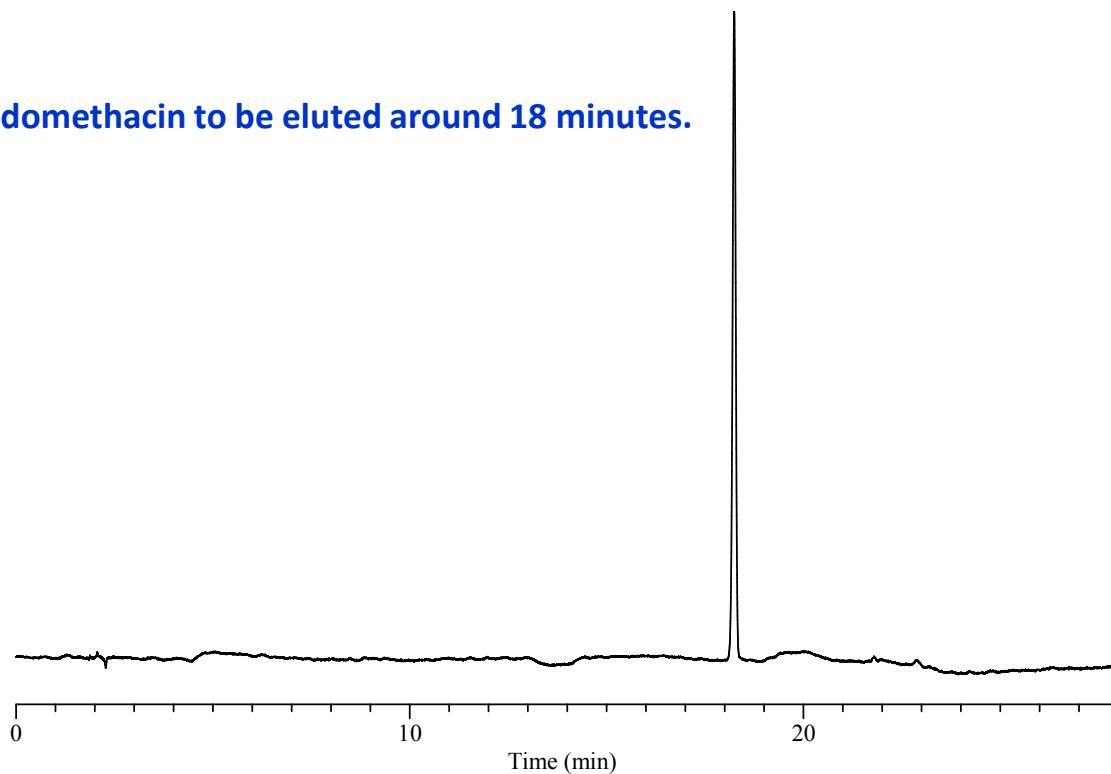


Diltiazem

Applications

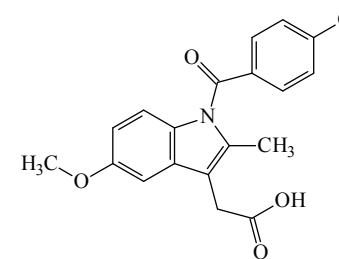
Analysis of Indomethacin by European Pharmacopeia

Indomethacin to be eluted around 18 minutes.



Conditions

Column	: InertSustain Phenylhexyl (3 μ m, 150 \times 4.6 mm I.D.)
Eluent	: A) 10 g/L CH ₃ COOH in H ₂ O B) CH ₃ CN A/B = 70/30 – 2 min - 70/30 – 9 min - 50/50 – 1 min - 50/50 - 0 min - 70/30 - 9 min - 30/70 – 6 min - 30/70, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 °C
Detection	: UV 254 nm
Injection Vol.	: 20 μ L



Indomethacin

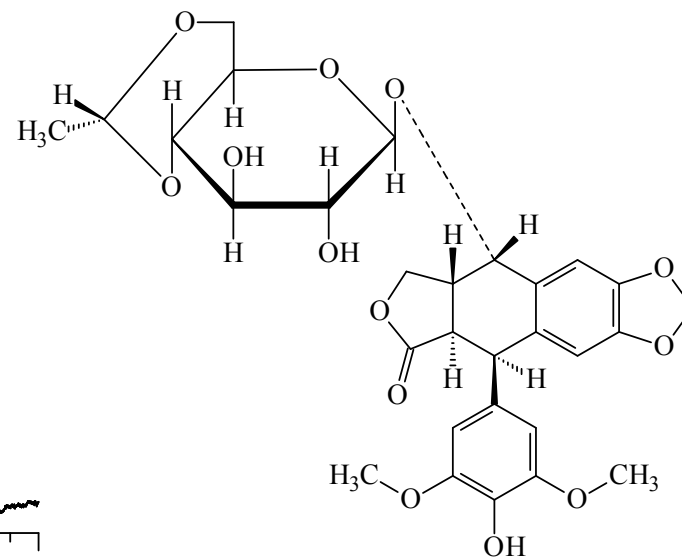
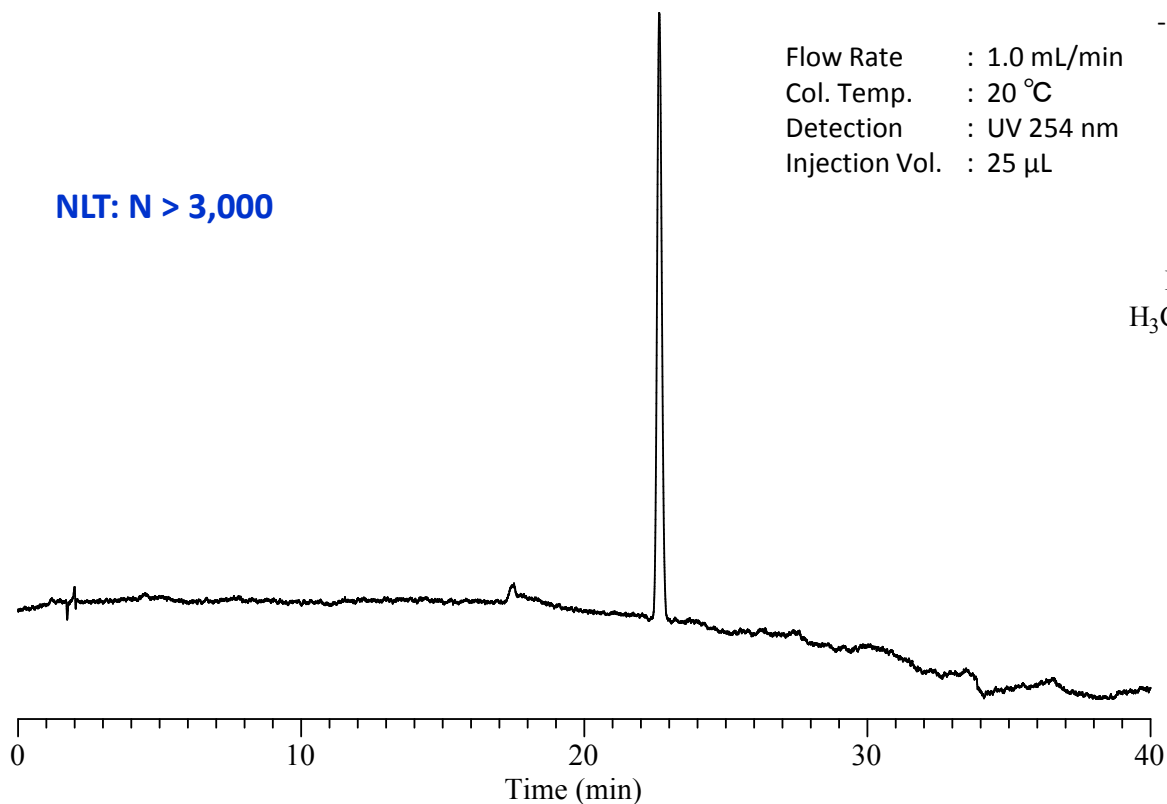
Applications

Analysis of Etopocide by Chinese Pharmacopeia

Conditions

Column : InertSustain Phenylhexyl (5 μ m, 250 \times 4.0 mm I.D.)
Eluent : A) CH₃CN/33 mM CH₃COONa (pH 4, CH₃COOH) = 20/80 v,v
B) CH₃CN/33 mM CH₃COONa (pH 4, CH₃COOH) = 60/40 v,v
A/B = 100/0 - 15 min - 100/0 - 15 min - 40/60 - 10 min - 40/60 - 2 min
- 0/100 - 3 min - 0/100 - 2 min - 100/0 - 3 min - 100/0, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 20 $^{\circ}$ C
Detection : UV 254 nm
Injection Vol. : 25 μ L

NLT: N > 3,000



Conclusion

Advantages of InertSustain Phenylhexyl

Different Selectivity to C18 Phases

Strong Retentivity

Highly Inert Packing Material
(Less Tailing of Peaks)

Extreme Durability = Longer Column Life

Conclusion

InertSustain Phenylhexyl is a perfect column for the following applications due to its benefits explained earlier.

- 1. To slightly change the elution pattern from a C18.**
- 2. Compatible to the European Pharmacopeia methods.**

Ordering Guide

Analytical Columns

	Length \ I.D.(mm)	2.1	3.0	4.6
HP Series Particle Size: 3 µm 50 Mpa(500 Bar)	30	5020-89209	5020-89215	5020-89221
	50	5020-89210	5020-89216	5020-89222
	75	5020-89211	5020-89217	5020-89223
	100	5020-89212	5020-89218	5020-89224
	150	5020-89213	5020-89219	5020-89225
	250	5020-89214	5020-89220	5020-89226

* 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)

* For maximum operating pressure information, please refer to page 46.

Ordering Guide

Particle Size: 3 µm	Length \ I.D. (mm)	1.0	1.5		
	30	5020-89160	5020-89166		
	50	5020-89161	5020-89167		
	75	5020-89162	5020-89168		
	100	5020-89163	5020-89169		
	150	5020-89164	5020-89170		
	250	5020-89165	5020-89171		
	Length \ I.D. (mm)	2.1	3.0	4.0	4.6
	30	5020-89124	5020-89131	5020-89138	5020-89145
	50	5020-89125	5020-89132	5020-89139	5020-89146
	75	5020-89126	5020-89133	5020-89140	5020-89147
	100	5020-89127	5020-89134	5020-89141	5020-89148
	150	5020-89128	5020-89135	5020-89142	5020-89149
	250	5020-89129	5020-89136	5020-89143	5020-89150
Particle Size: 5 µm	Length \ I.D. (mm)	1.0	1.5		
	30	5020-89038	5020-89044		
	50	5020-89039	5020-89045		
	75	5020-89040	5020-89046		
	100	5020-89041	5020-89047		
	150	5020-89042	5020-89048		
	250	5020-89043	5020-89049		
	Length \ I.D. (mm)	2.1	3.0	4.0	4.6
	30	5020-89001	5020-89008	5020-89015	5020-89022
	50	5020-89002	5020-89009	5020-89016	5020-89023
	75	5020-89003	5020-89010	5020-89017	5020-89024
	100	5020-89004	5020-89011	5020-89018	5020-89025
	150	5020-89005	5020-89012	5020-89019	5020-89026
	250	5020-89006	5020-89013	5020-89020	5020-89027

* End-fittings are 1/16" Waters-compatible.

* For maximum operating pressure information, please refer to page 48.

Ordering Guide

Cartridge Guard Column E

I.D. of the Analytical Column Applicable (mm)	Length (mm)	I.D. (mm)	Replacement Cartridge E Guard Column (2 EA.)		Cartridge E Holder / Cartridge Set (2 Cartridge E Guard Columns & 1 Holder)	
			Particle Size		Particle Size	
			3 µm	5 µm	3 µm	5 µm
1.0	10	1.0	5020-89199	5020-89105	5020-89200	5020-89106
1.5, 2.1		1.5	5020-89201	5020-89107	5020-89202	5020-89108
2.1, 3.0		3.0	5020-89197	5020-89103	5020-89198	5020-89104
4.0, 4.6		4.0	5020-89195	5020-89101	5020-89196	5020-89102
2.1, 3.0	20	3.0	5020-89205	5020-89111	5020-89206	5020-89112
4.0, 4.6		4.0	5020-89203	5020-89109	5020-89204	5020-89110
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.

* For maximum operating pressure information, please refer to page 46.

Comparison of Performance

Selectivity Test

Mobile Phase: A) CH₃CN
 B) H₂O
 A/B = 70/30, v/v
Flow Rate: 1.0 mL/min
Col. Temp.: 40 ° C
Detection: UV 254 nm
Injection Vol. : 5 µL

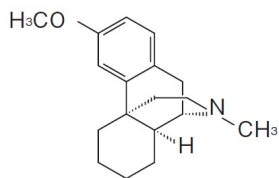
1. Uracil
2. Caffeine
3. Butylbenzene
4. Amylbenzene
5. Triphenylene
6. o-Terphenyl

Comparison of Performance

Strong Basic Compound Test

Dextromethorphan is a strong basic compound.

Severe tailing can be confirmed when the packing material contains residual silanol groups.



Mobile Phase: A) CH₃CN

B) 25 mM Phosphate buffer (pH 7.0, KH₂PO₄)

A/B = 40/60, v/v

Flow Rate: 1.0 mL/min

Col. Temp.: 40 ° C

Detection: UV 230 nm

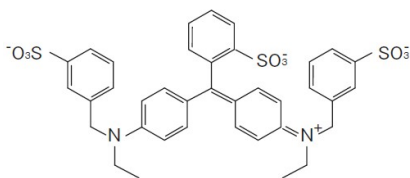
Injection Vol. : 3 µL

1. Dextromethorphan

Comparison of Performance

Strong 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.



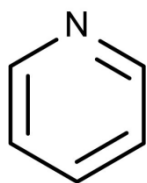
Mobile Phase: A) CH₃CN
B) 0.1 % H₃PO₄
A/B = 25/75
Flow Rate: 1.0 mL/min
Col. Temp.: 40 ° C
Detection: UV 254 nm
Injection Vol.: 5 µL

1. Brilliant Blue FCF
2. 4-Ethylbenzoic acid
3. Salicylic Acid

Comparison of Performance

Weak Basic Compound Test

Pyridine was widely used 20 years ago to check how well the columns are endcapped. Today, most column manufacturers are not using such sample due to the improved column technology. Tailing of Pyridine should NOT be observed today.



Mobile Phase: Methanol/Water = 30/70

Flow Rate: 1.0 mL/min

Col. Temp.: 40 ° C

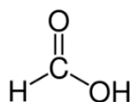
Detection: UV 254 nm

Injection Vol.: 3 µL

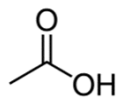
1. Pyridine
2. Phenol

Weak Acidic Compound Test

Formic and Acetic acids were used 20 years ago to check the inertness to acidic compounds. Today, most column manufacturers are not using such sample due to the improved column technology. Tailing of these acids should NOT be observed today.



1.



2.

Mobile Phase: 0.1%(v) H₃PO₄

Flow Rate: 1.0 mL/min

Col. Temp.: 40 ° C

Detection: UV 210 nm

Injection Vol.: 5 µL

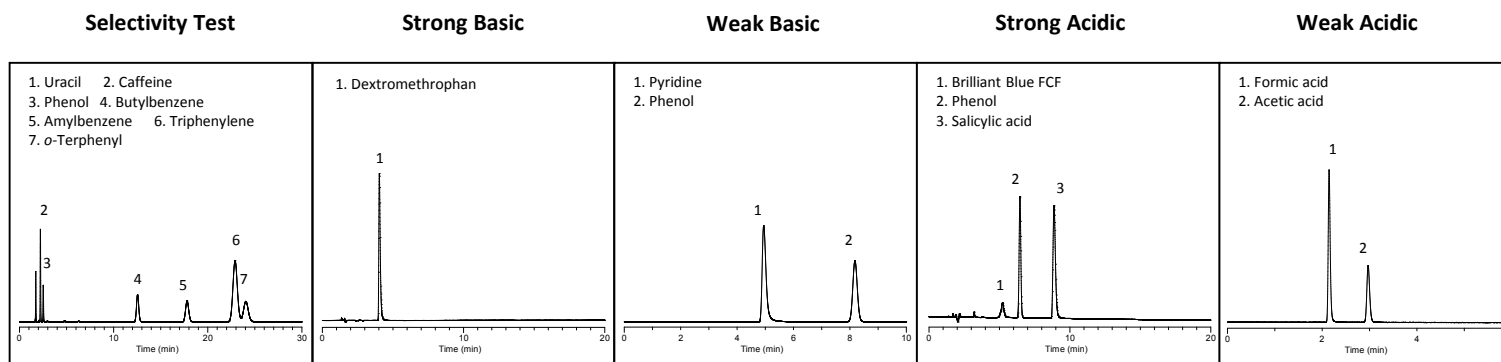
1. Formic Acid 0.1%
2. Acetic Acid 0.1%

Comparison of Performance

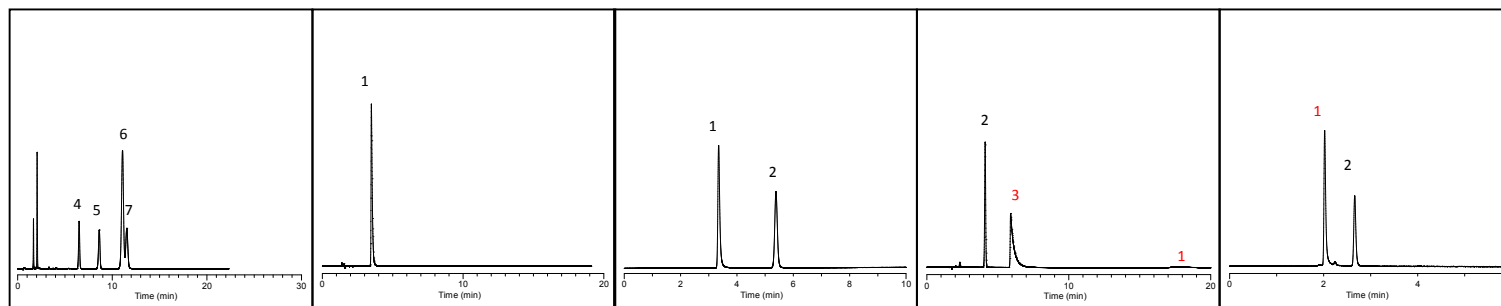
Brand Name	Bonded Phase	Size (mm)	Particle Size (μm)	Surface Area (m^2/g)	Pore Size (\AA)	Pore Volume (mL/g)	Carbon Loading (%)
InertSustain Phenylhexyl	Phenylhexyl Groups	4.6 × 150	5	350	100	0.85	9
XBridge Phenyl	Phenylhexyl Groups	4.6 × 150	5	185	130	0.74	15
Luna Phenyl-Hexyl	Phenylhexyl Groups	4.6 × 150	5	400	100	—	17.5
Zorbax Eclipse Plus Phenyl-Hexyl	Phenylhexyl Groups	4.6 × 150	5	160	95	—	

Comparison of Performance

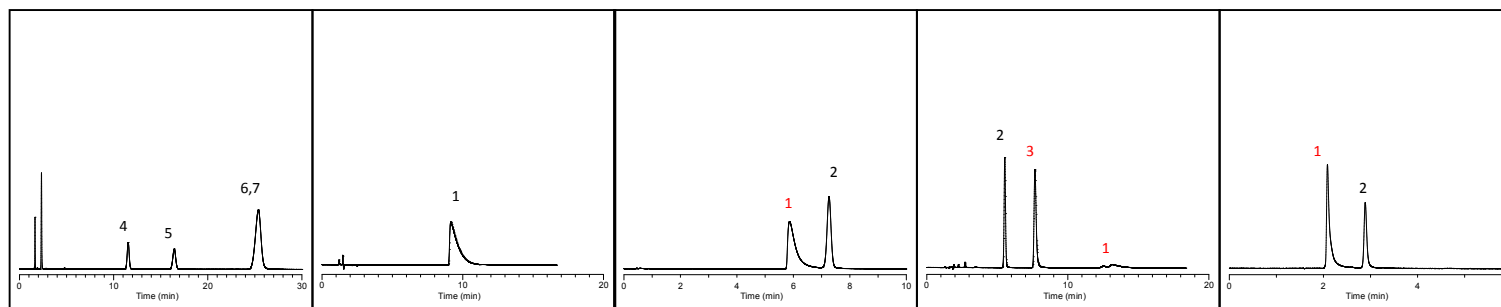
Inertsustain Phenylhexyl



XBridge Phenyl

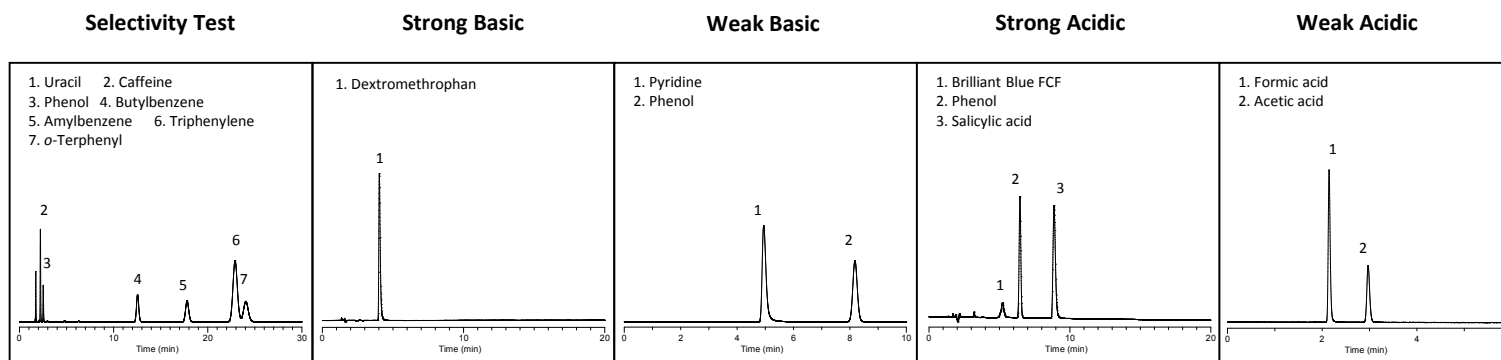


Luna Phenyl-Hexyl



Comparison of Performance

Inertsustain Phenylhexyl



Zorbax Phenyl-Hexyl

