InertSustain® Cyano Technical Data

October, 2017

GL Sciences

InertSustain Cyano Physical Properties

Silica	:	ES Silica Gel
Particle Size	:	3 μm, 5 μm
Surface Area	:	350 m^2/g
Pore Size	:	100 Å (10 nm)
Bonded Phase	:	Cyanopropyl Groups
End-capping	:	Yes
Carbon Loading	:	8 %
pH Range	:	2～7.5
USP Code	:	L10

Features of InertSustain Cyano

Cyano Column?

- Different selectivity compared to C18 and Phenyl phases.
  The rententivity is weaker than a Phenyl phase.
  (slide 4～14)

Comparison to Other Cyano Columns

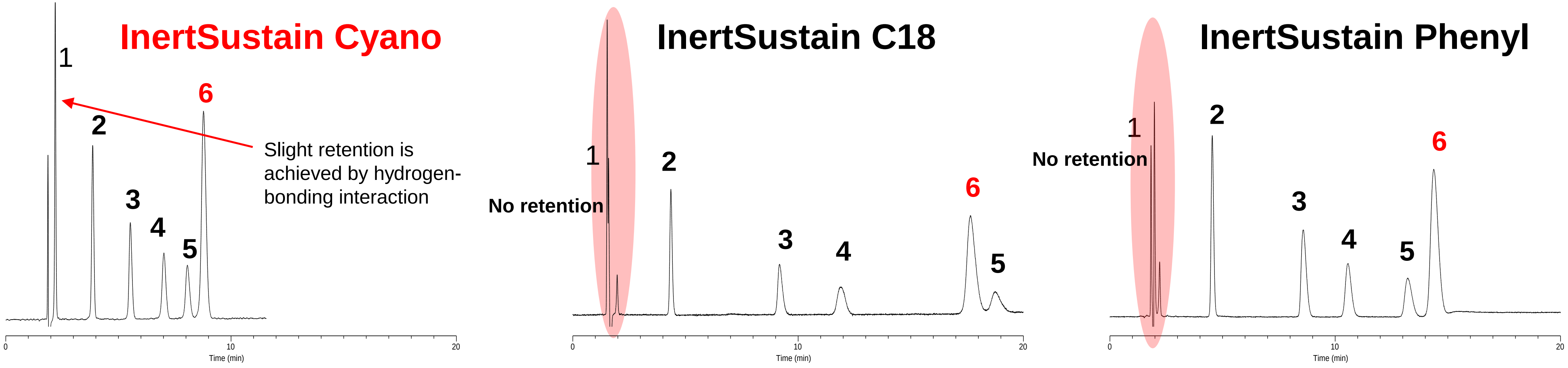
- Workable in both reversed and normal-phase modes.
  (slide 15～17)

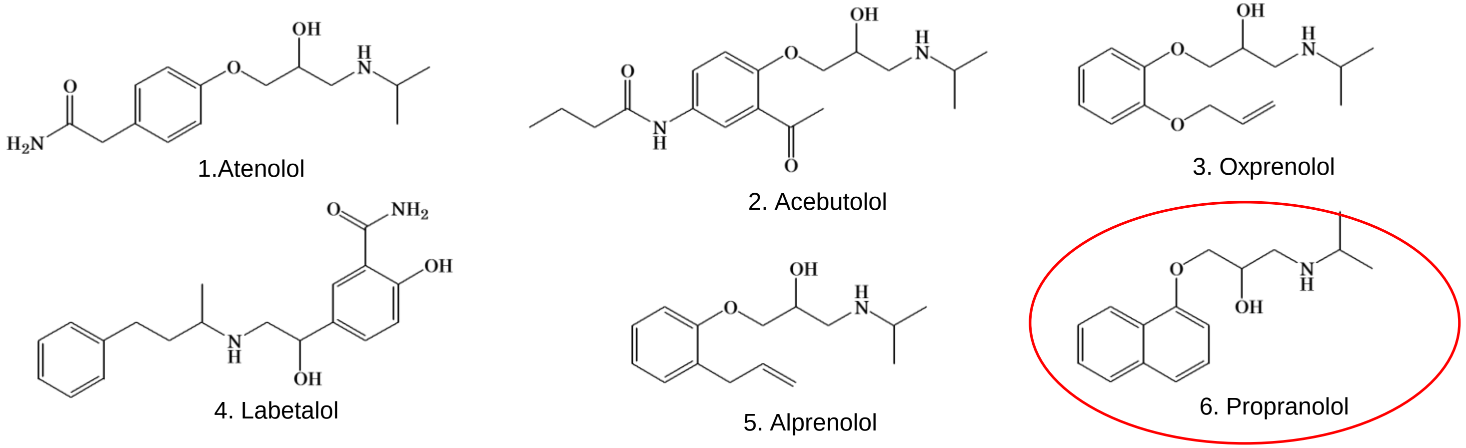
- Rigorous end-capped treatment provide better peak shapes
  (slide 18～19)

- Simply better reproducibility (slide 20)

Comparison between C18 and Phenyl phases (β Blockers)

The separation pattern between Alprenolol and Propranolol were different. Propranolol consist a naphthalene ring which was retained stronger on cyano and phenyl phases due to the pi electron interaction. As a result, the elution pattern was different to a C18 phase.





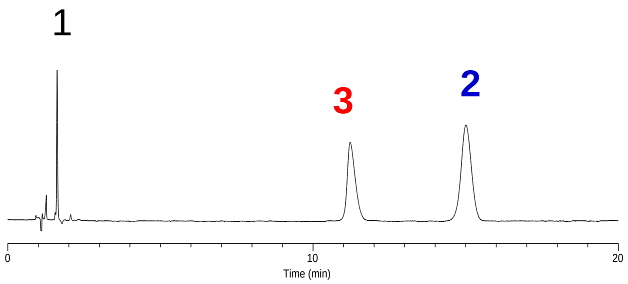
<u>Conditions</u>

Eluent	: A) 0.1 % HCOOH B) CH_3CN A/B = 80/20,v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 220 nm
Sample	: 1. Atenolol 2. Acebutolol 3. Oxprenolol 4. Labetalol 5. Alprenolol 6. Propranolol

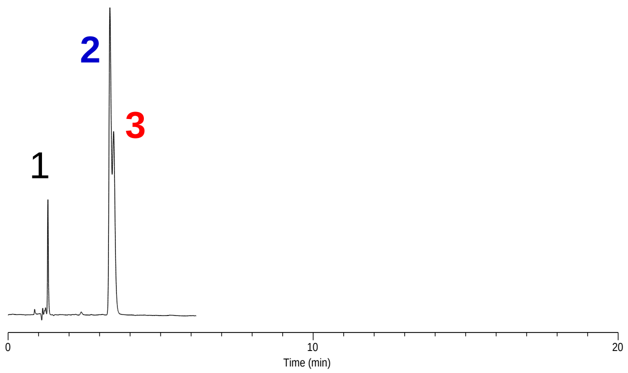
Comparison between C18 and Phenyl phases (Strong Bases)

As shown below, inertness to strong basic compounds were confirmed to be satisfactory. The elution pattern is different on a Cyano phase due to the difference in interaction. The interaction on a Cyano phase is mainly pi electron interaction.

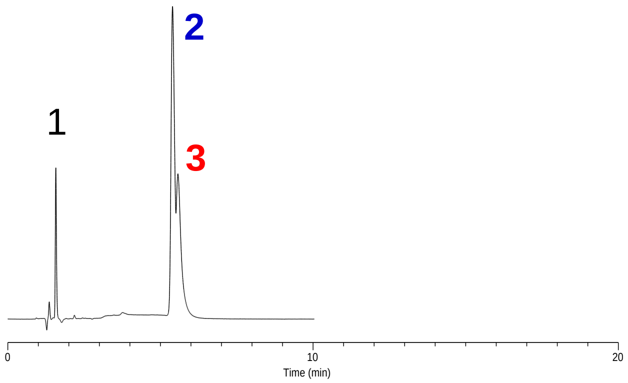
InertSustain Cyano



InertSustain C18

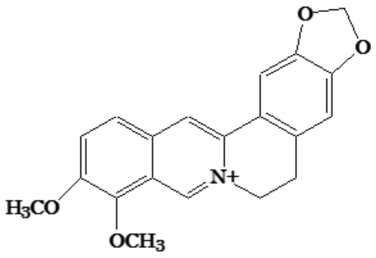


InertSustain Phenyl

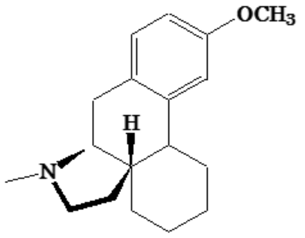


<u>Conditions</u>

Eluent : A) 5 mM $HCOONH_4$ B) CH_3CN
A/B＝60/40,v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 230 nm
Sample : 1.Uracil
2. Dextromethorphan
3. Berberine

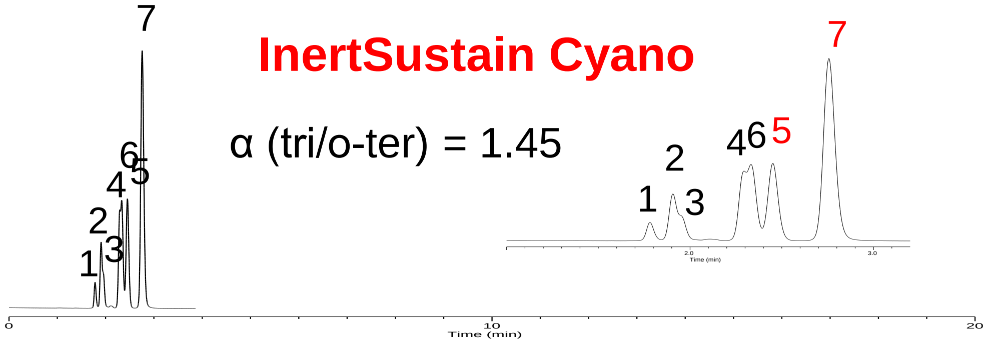


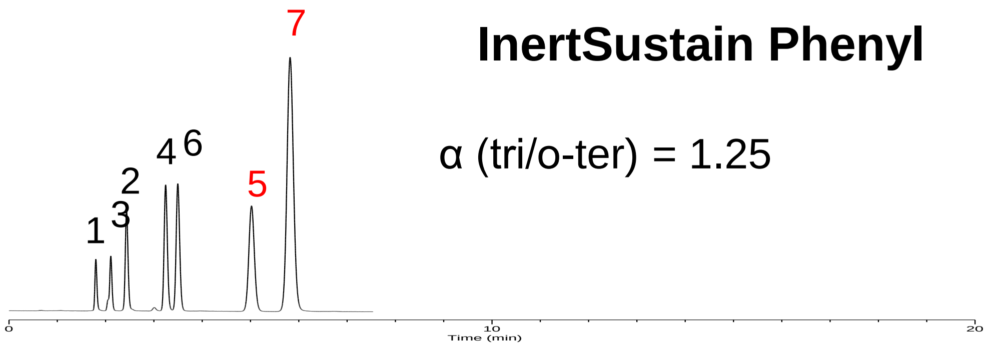
2. Berberine

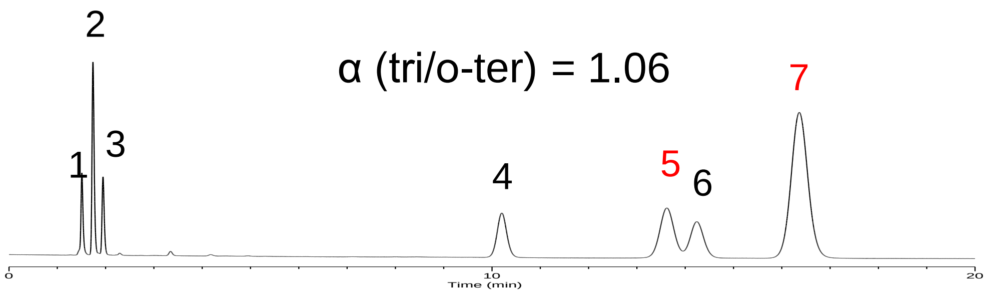


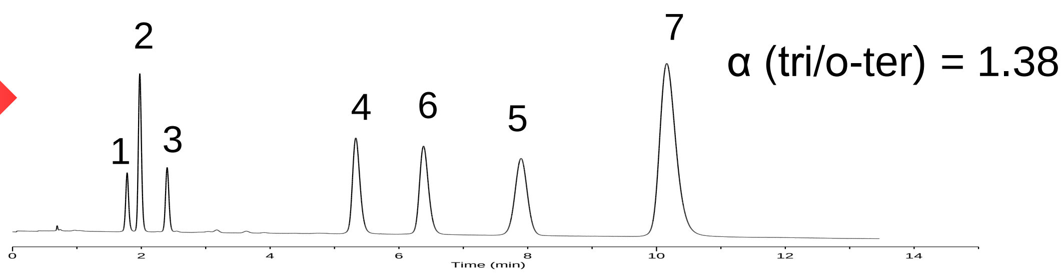
3. Dextromethorphan

Selectivity ① (Planar Molecule Recognition)





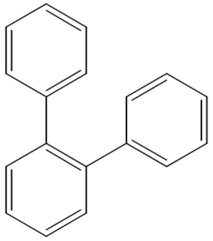




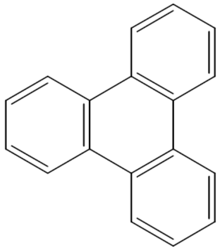
The retention of hydrophobic analytes are weaker on InertSustain Cyano when comparing to Phenyl phases. Analytes consisting benzene rings tend to be retained stronger due to the pi-pi interaction.

Conditions

Eluent : A) CH_3OH B) H_2O
A/B = 80/20, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm
Sample :
1. Uracil
2. Caffeine
3. Phenol
4. Butylbenzene
5. o-Terphenyl
6. Amylbenzene
7. Triphenylene



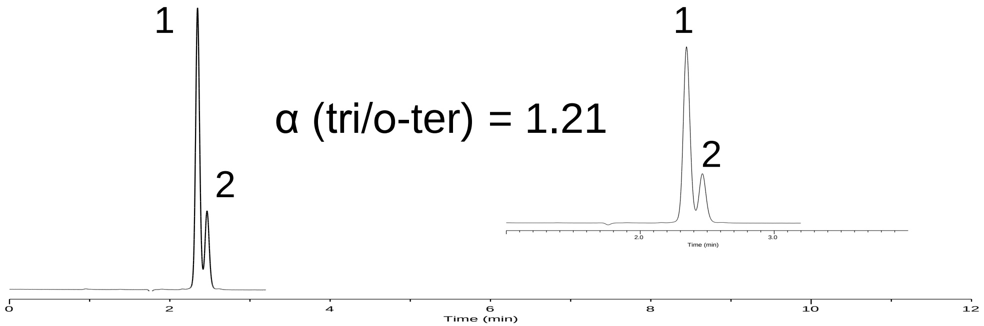
5. o-Terphenyl



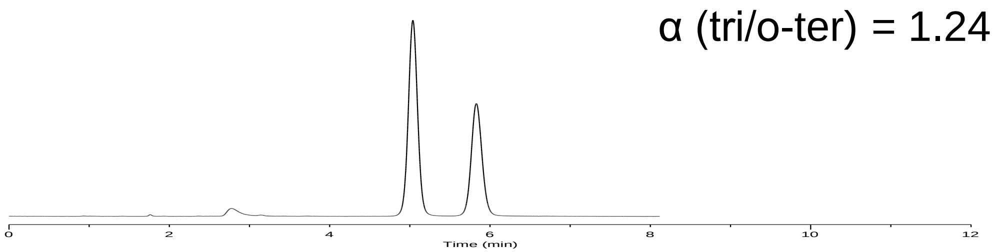
7. Triphenylene

Selectivity ② (Cis-, Trans-)

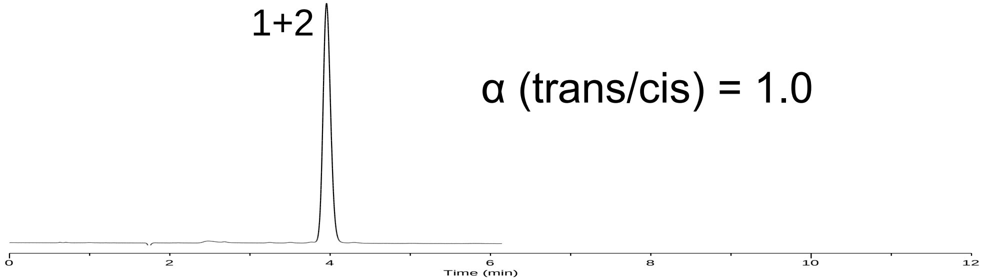
InertSustain Cyano



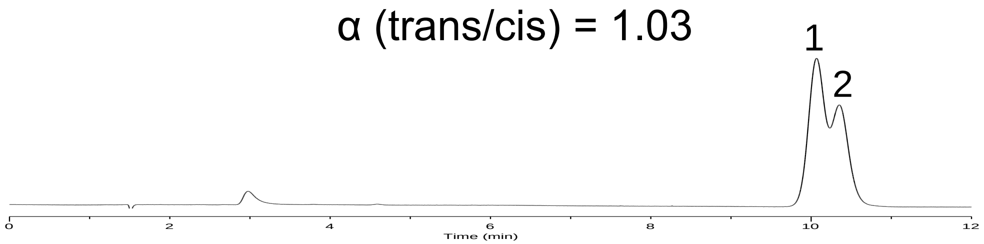
60 % Methanol



InertSustain Phenyl



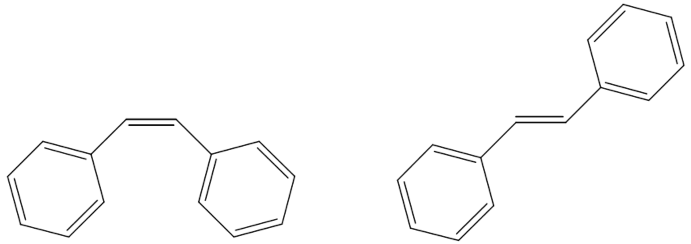
InertSustain C18



The separation of cis-, trans- were satisfactory under the following analytical conditions. However, the separation was improved further by decreasing the methanol content.

Conditions

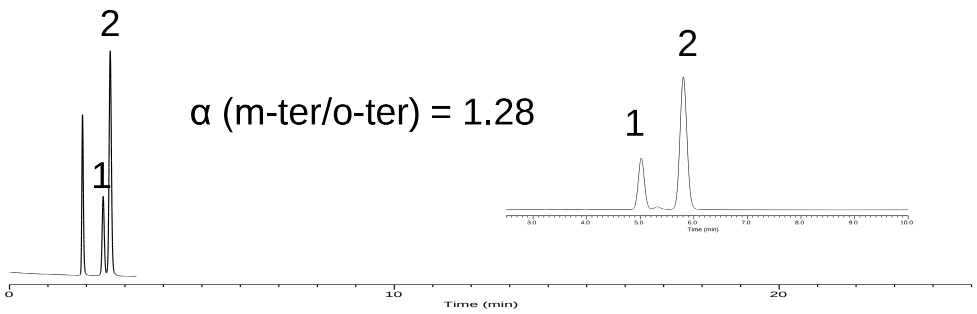
Eluent : A) CH_3OH B) H_2O
A/B = 80/20, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 210nm
Sample : 1. cis-Stilbene
2. trans-Stilbene



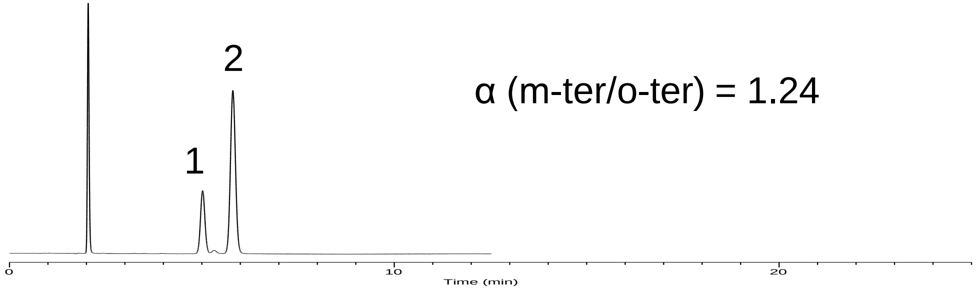
1. cis-Stilbene 2. trans-Stilbene

Selectivity ③ (Ortho-, Meta-)

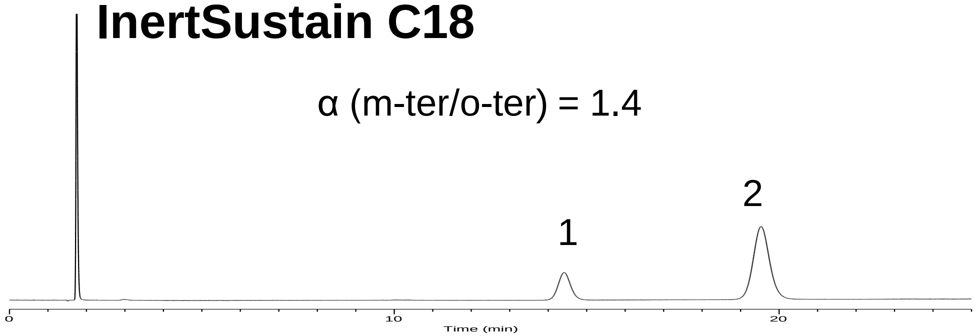
InertSustain Cyano



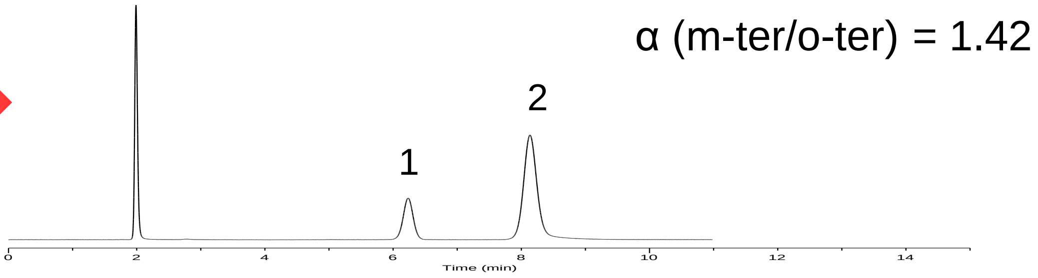
InertSustain Phenyl



InertSustain C18



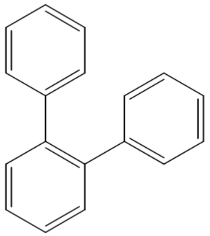
60 % Methanol



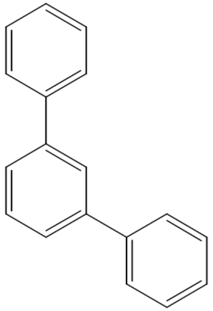
The separation of *o*-, *m*- were satisfactory under the following analytical conditions. However, the separation was improved further by decreasing the methanol content.

Conditions

Eluent	: A) CH_3OH B) H_2O
	A/B = 80/20, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 254 nm
Sample	: 1. *o*-Terphenyl
	2. *m*-Terphenyl

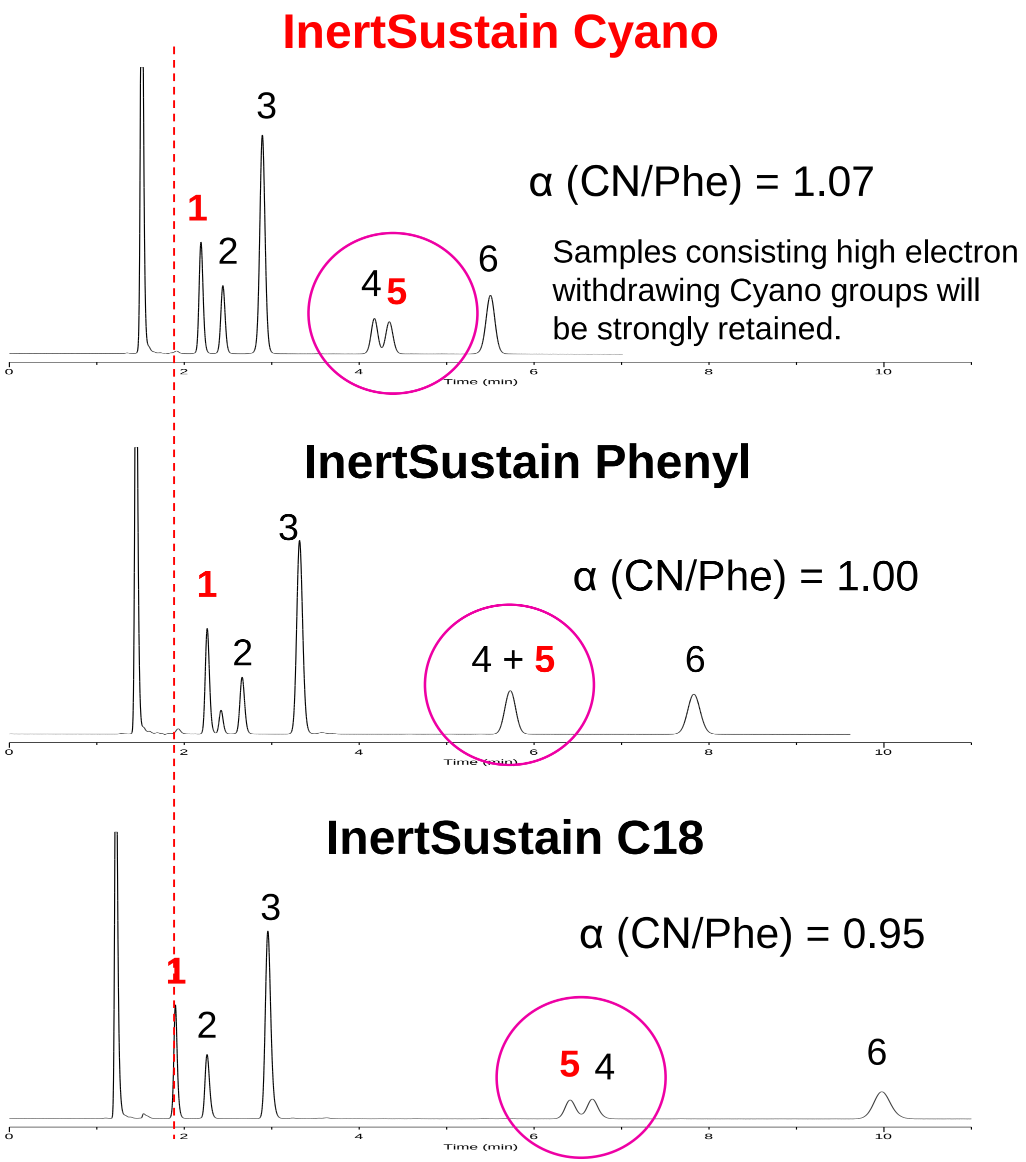


1. *o*-Terphenyl



2. *m*-Terphenyl

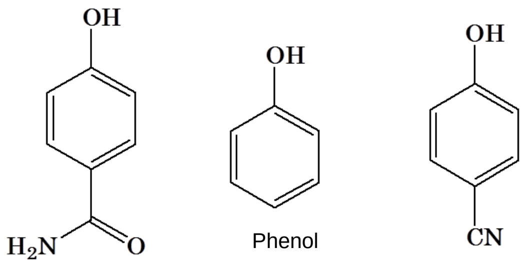
Hydrogen-Bonding, Electron Withdrawing ①



The retention of hydrophobic analytes will be low on InertSustain Cyano when comparing to a C18 phase, however, there is a retention for polar analytes.

Conditions

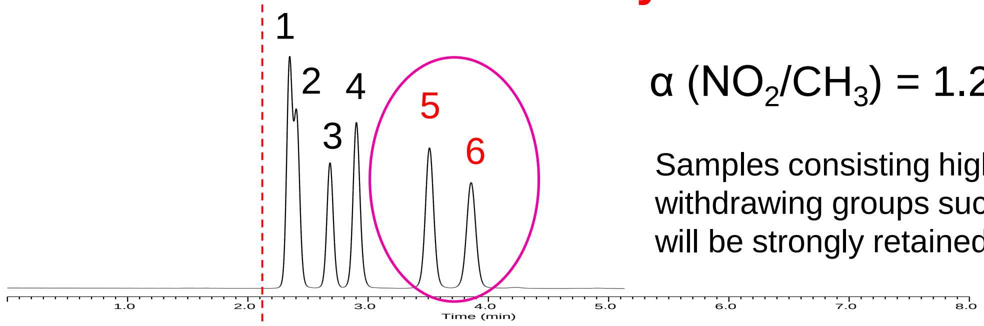
Eluent	: A)CH_3CN B) 0.1% H_3PO_4 A/B = 25/75, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 280 nm
Sample	: 1. 4-Hydroxybenzamide 2. Hydroquinone 3. 4-Hydoroxybenzoic acid 4. Phenol 5. 4-Hydoroxybenzonitoril 6. *p*-Nitrophenol



4-Hydroxybenzamide 4-Hydoroxybenzonitoril

Electron Withdrawing ②

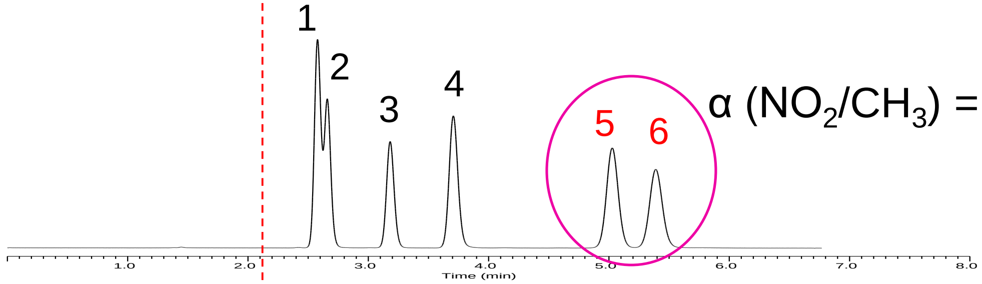
InertSustain Cyano



α (NO_2/CH_3) = 1.20

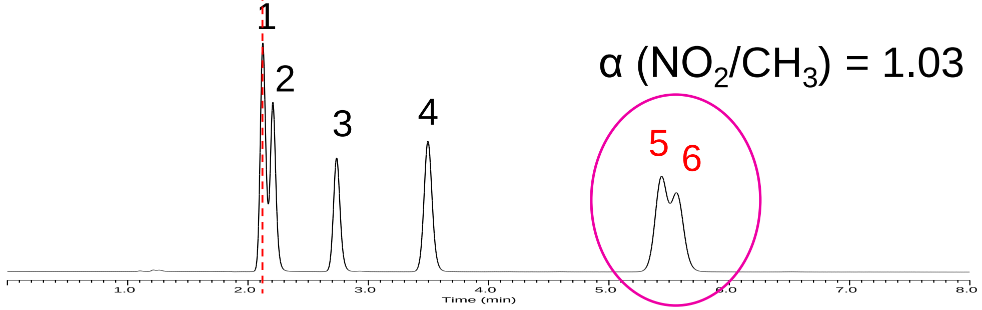
Samples consisting high electron withdrawing groups such as NO_2 will be strongly retained.

InertSustain Phenyl



α (NO_2/CH_3) = 1.11

InertSustain C18

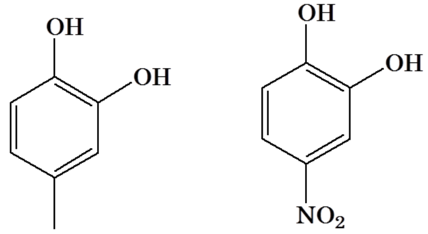


α (NO_2/CH_3) = 1.03

InertSustain Cyano retains samples consisting high electron withdrawing groups such as nitro groups much efficiently than Phenyl bonded phases.

<u>Conditions</u>

Eluent	: A) 0.1 % H_3PO_4 B) CH_3CN A/B = 75/25, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 280 nm
Sample	: 1. 3,4-Dihydroxy Benzoic Acid 2. Hydroquinone 3. Resorcinol 4. Catechol 5. 4-Methyl Catechol 6. 4-Nitrocatechol



5. 4-Methyl Catechol 6. 4-Nitrocatechol

Comparison of Performance

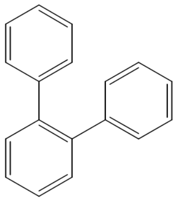
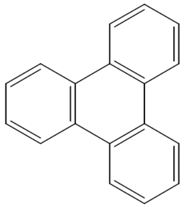
Planar Selectivity

Conditions

Eluent : A) CH_3OH B) H_2O
A/B = 80/20, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40℃
Detection : UV 254 nm

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

α= k(Tri)/k(Ter)

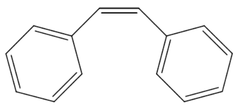
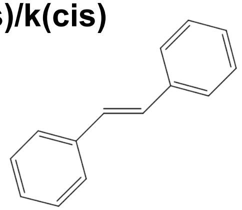
Separation of Cis-trans

Conditions

Eluent : A) CH_3OH B) H_2O
A/B = 80/20, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 210 nm

Sample :
1. **cis-Stilbene**
2. **trans-Stilbene**

α= k(trans)/k(cis)

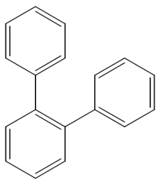
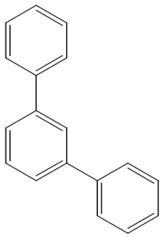
Separation of o, m-

Conditions

Eluent : A) CH_3OH B) H_2O
A/B = 80/20, v/v
Flow Rate : 1.0 mL / min
Col. Temp. : 40 ℃
Detection : UV 254 nm

α= k(m-)/k(o-)

Sample :
1. o-Terphenyl
2. m-Terphenyl

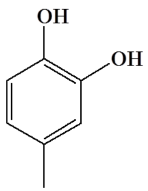
Electron Withdrawing using NO_2

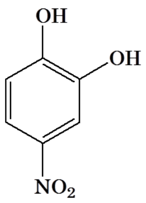
Conditions

Eluent : A) 0.1 % H_3PO_4 B) CH_3CN
A/B = 75/25, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 280 nm

α= k(NO_2)/k(CH_3)

Sample :
1. 3,4-Dihydroxy Benzoic Acid
2. Hydroquinone
3. Resorcinol
4. Catechol
5. **4-Methyl Catechol**
6. **4-Nitrocatechol**





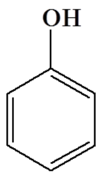
Electron Withdrawing using CN

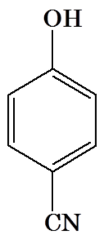
Conditions

Eluent : A) CH_3CN B) 0.1% H_3PO_4
A/B = 25/75, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 280 nm

α= k(CN)/k(OH)

Sample :
1. 4-Hydroxybenzamide
2. Hydroquinone
3. 4-Hydoroxybenzoic acid
4. **Phenol**
5. **4-Hydoroxybenzonitoril**
6. *p*-Nitrophenol





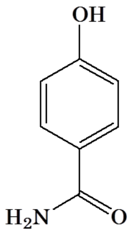
Hydrogen-Bonding using Amide

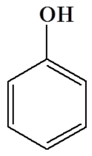
Conditions

Eluent : A) CH_3CN B) 0.1% H_3PO_4
A/B = 25/75, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 280 nm

α= k(Amide)/k(OH)

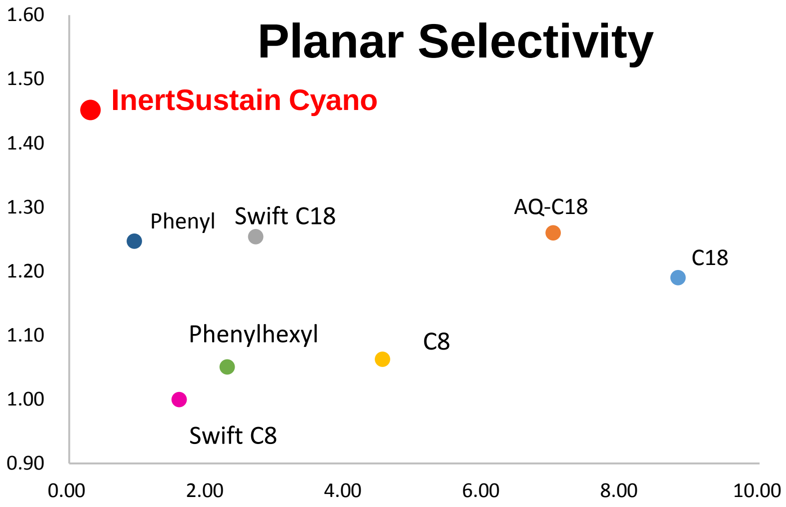
Sample :
1. **4-Hydroxybenzamide**
2. Hydroquinone
3. 4-Hydoroxybenzoic acid
4. **Phenol**
5. 4-Hydoroxybenzonitoril
6. p-Nitrophenol

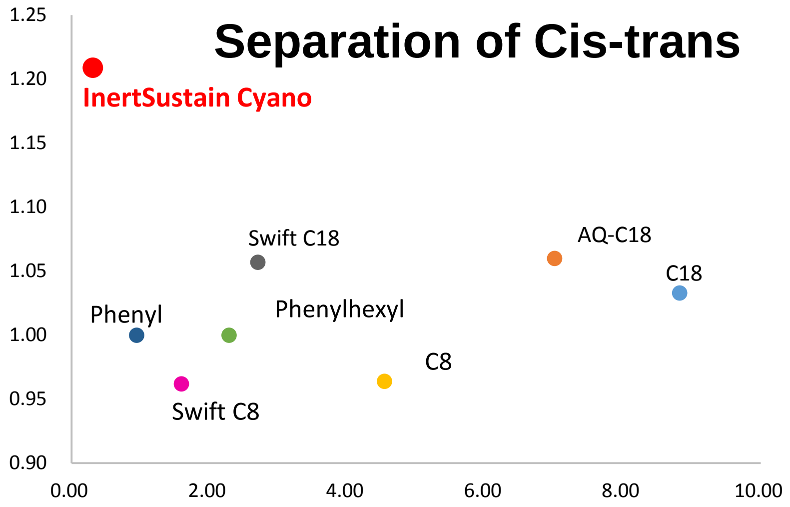


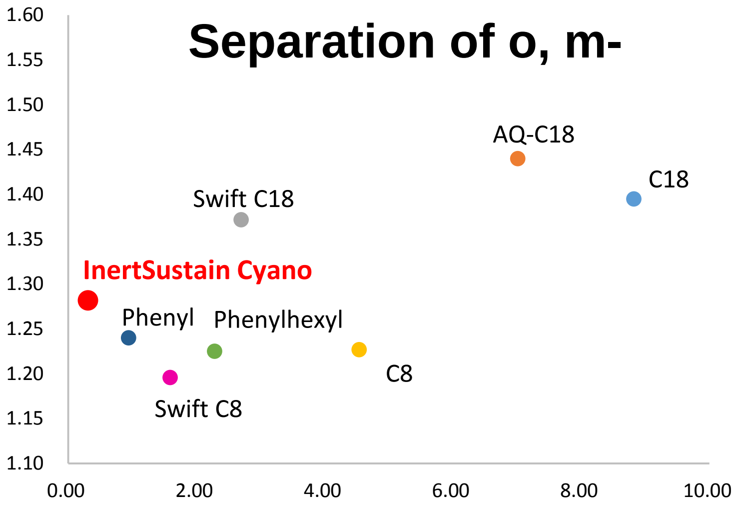


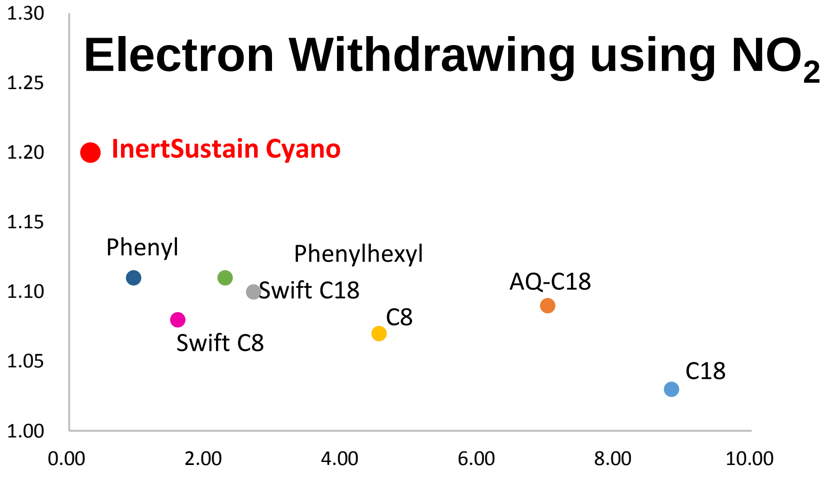
Selectivity of InertSustain Series

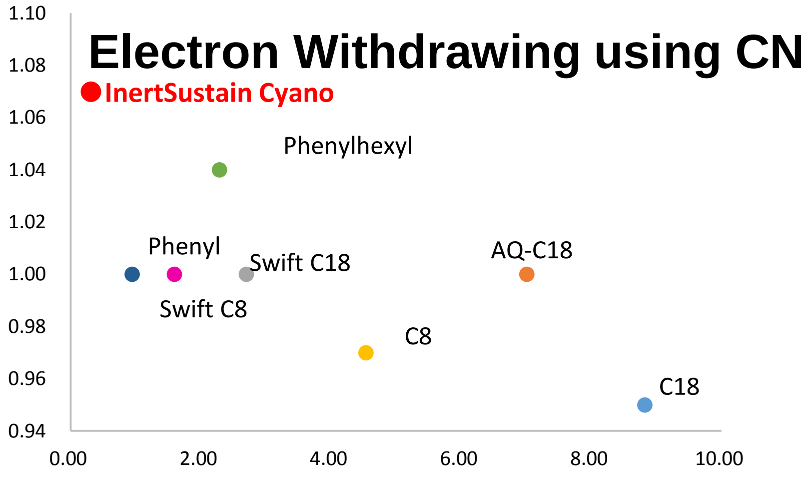
InertSustain Cyano provide low retention, but with unique selectivity

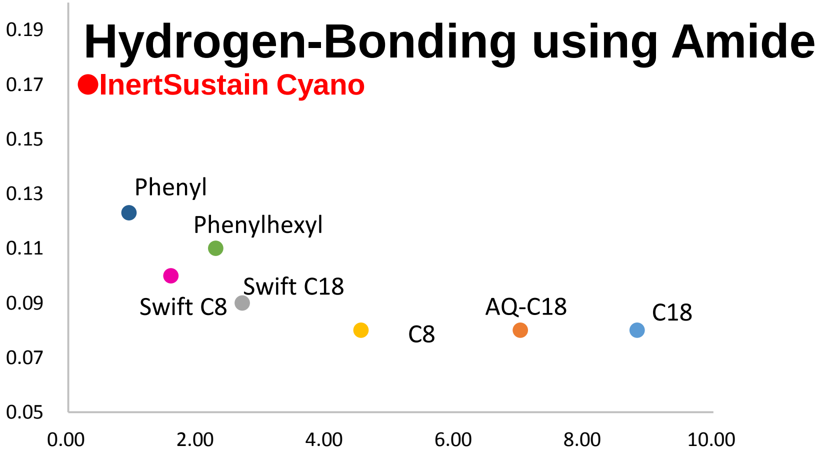










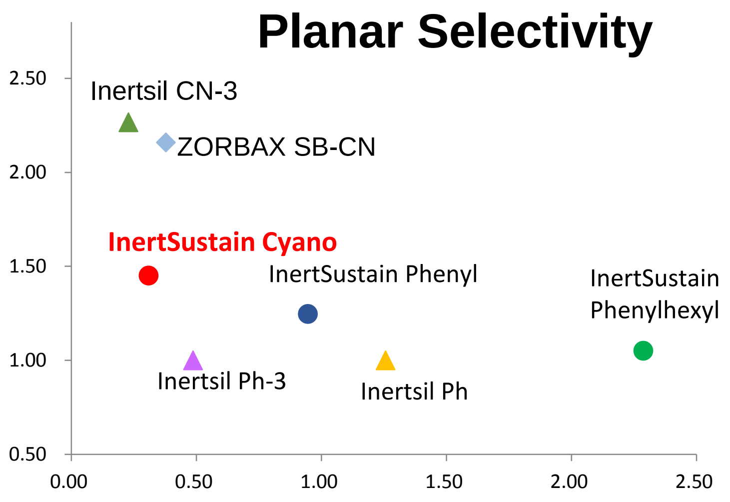


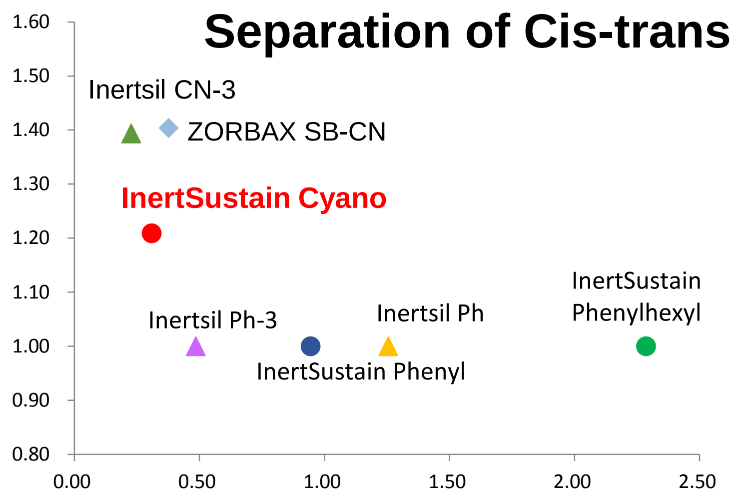
Horizontal axis : Hydrophobicity (Retention factor of butylbenzene k, CH_3OH : H_2O=80 : 20)
Vertical axis : Separation factor of each test

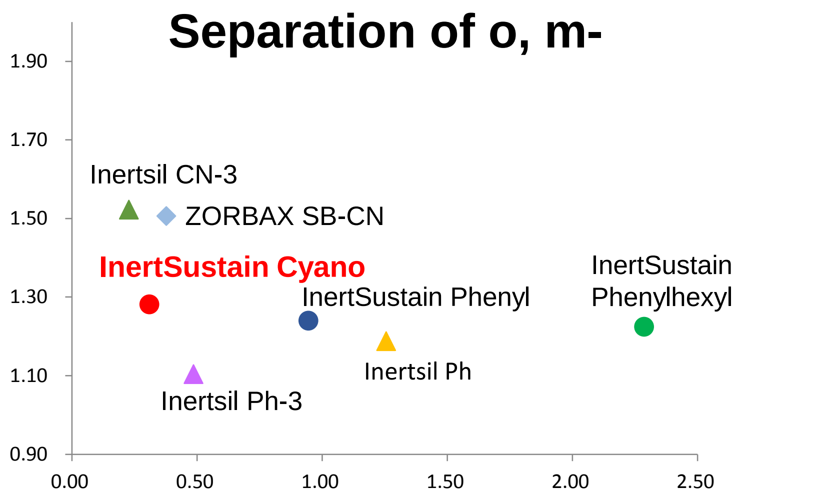
C18 : InertSustain C18
Swift C18 : InertSustainSwift C18
AQ-C18 : InertSustain AQ-C18
C8 : InertSustain C8
Swift C8 : InertSustainSwift C8
Phenyl : InertSustain Phenyl
Phenylhexyl : InertSustain Phenylhexyl

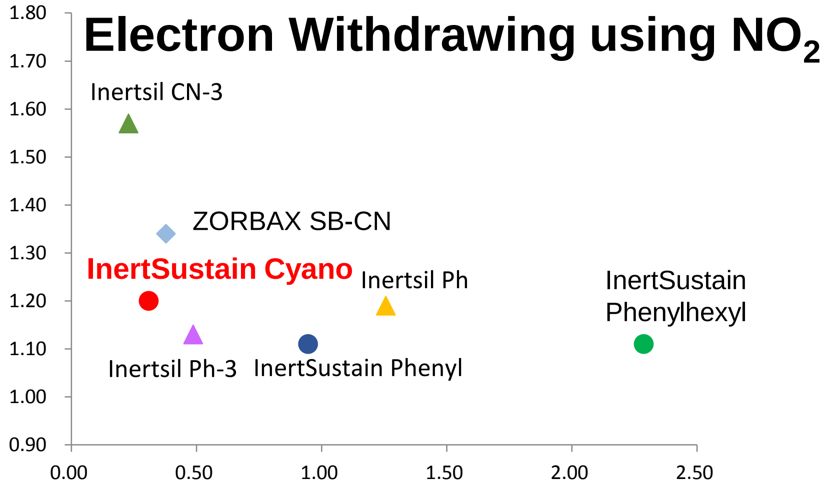
Selectivity of Cyano and Phenyl phases

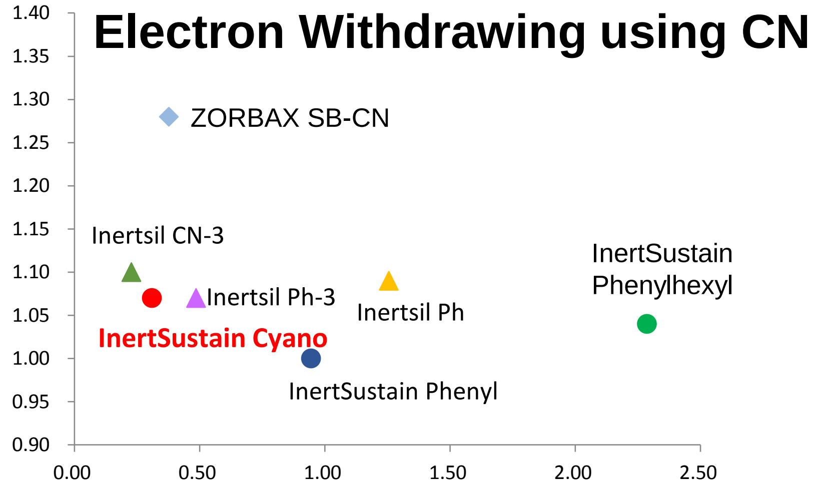
InertSustain Cyano offer better selectivity compared to Phenyl phases

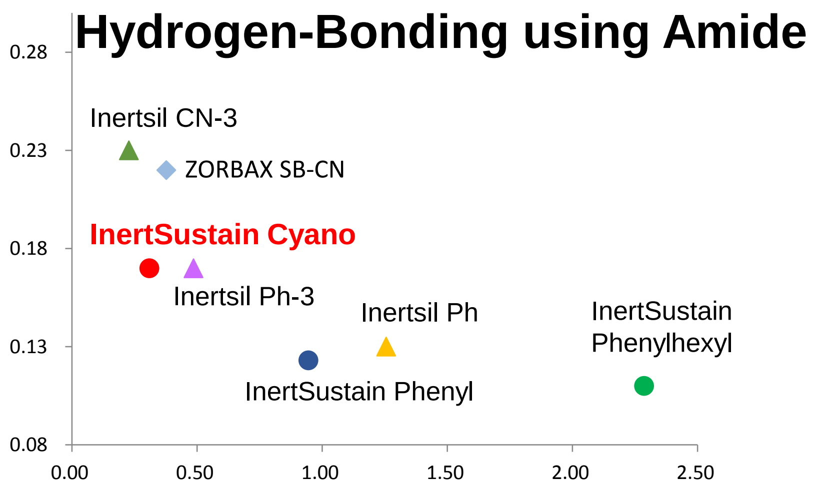








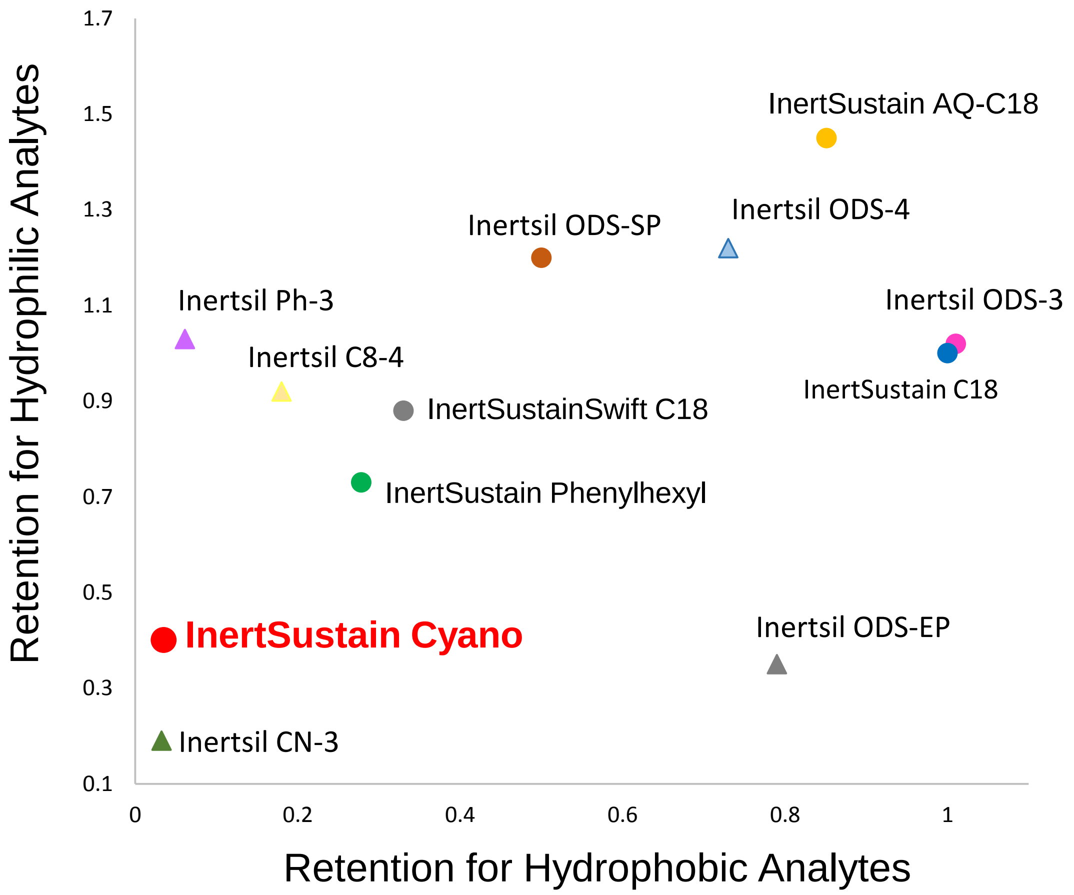




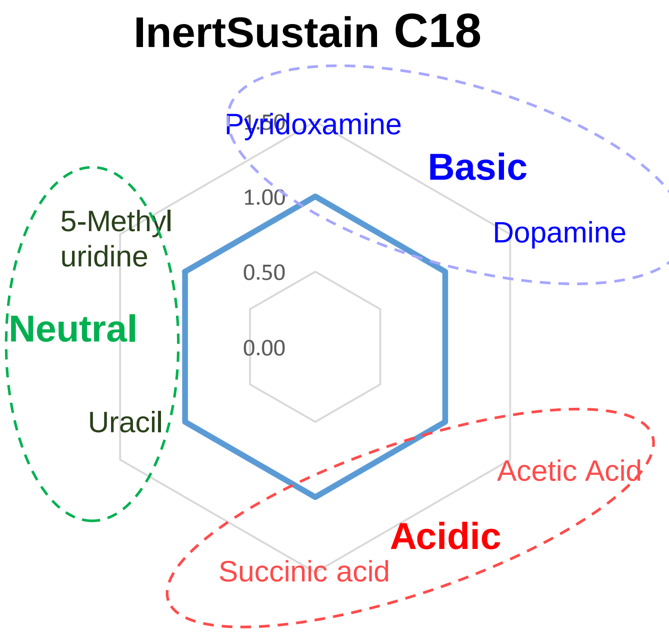
Horizontal axis : Hydrophobicity (Retention factor of butylbenzene k, CH_3OH:H_2O=80:20)
Vertical axis : Separation factor of each test

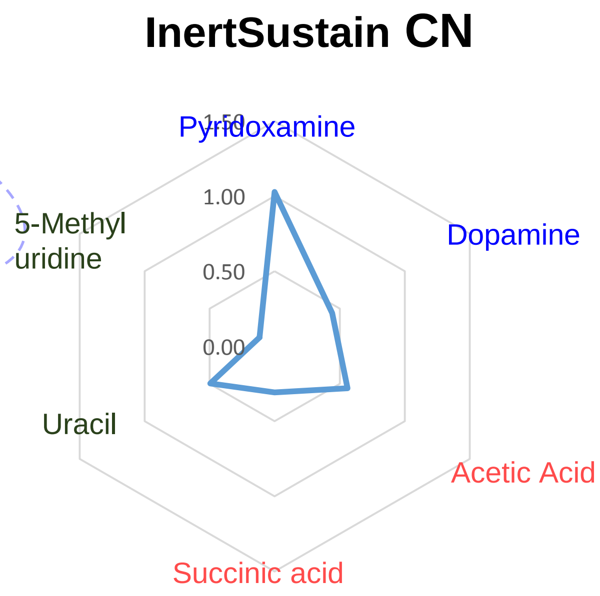
Retention for Polar Analytes

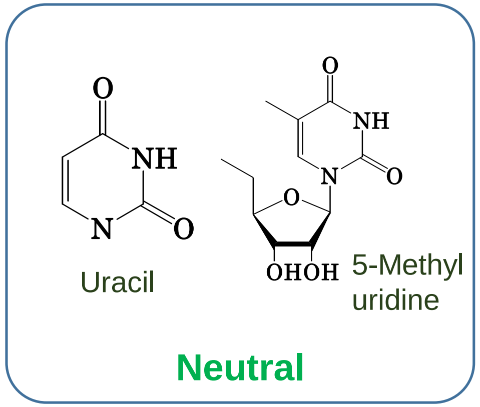
InertSustain Cyano do not offer retention for polar analytes under 100 % aqueous mobile phases

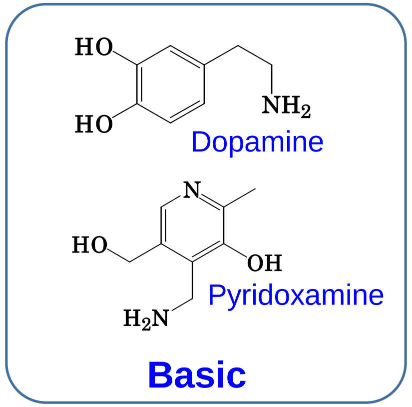


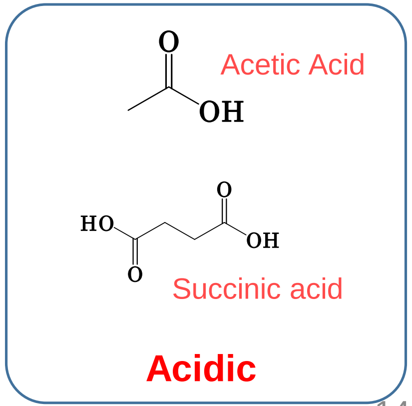
Horizontal axis : Retention factor of Amylbenzene k, CH_3OH : H_2O=80 : 20)
Vertical axis : Retention factor of neutral, basic and acidic analytes under 100 % aqueous mobile phase





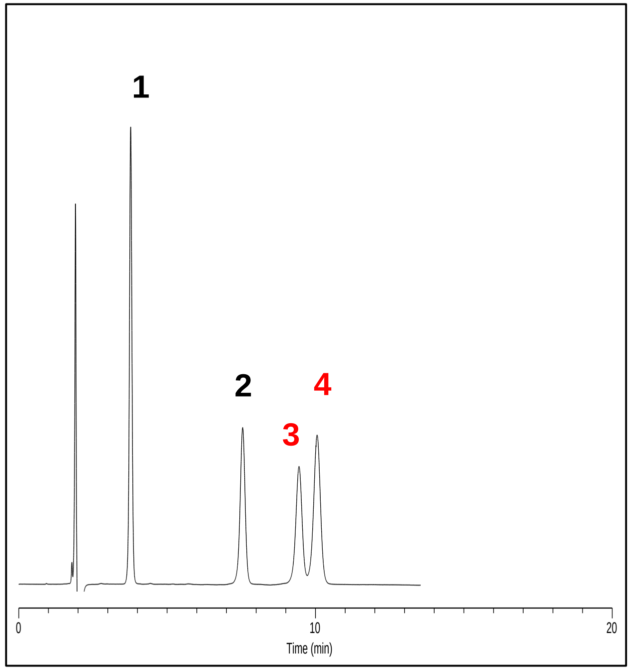






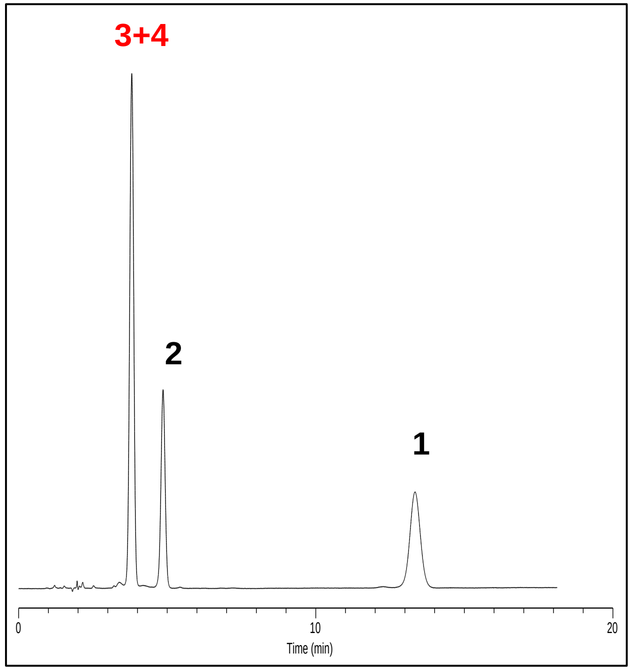
Comparison between Normal and Reversed-Phase Modes

Normal-Phase Mode



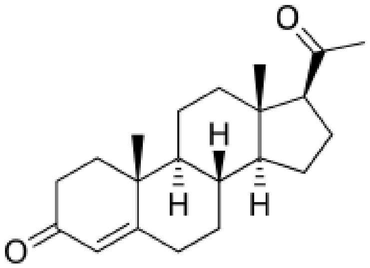
Conditions
Eluent : A) Hexane B) Ethanol
A/B = 90/10, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 220 nm

Reversed-Phase Mode

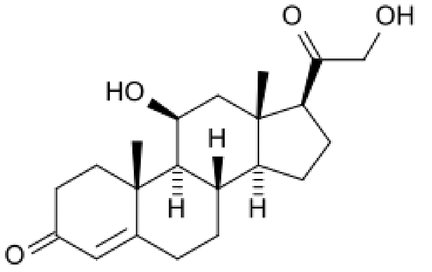


Conditions
Eluent : A) CH_3OH B) H_2O
A/B = 40/60, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 220 nm

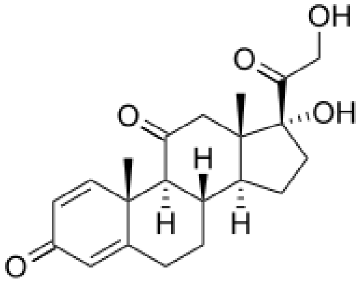
Separation may improve in normal-phase mode depending on the sample.



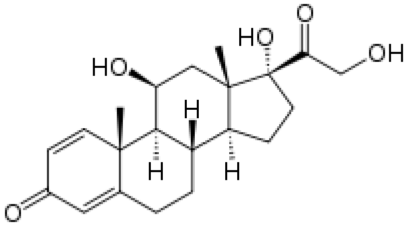
1. Progesterone



2. Corticosterone



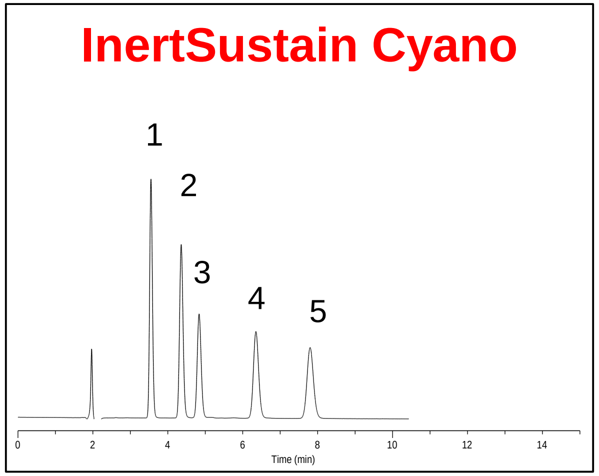
3. Prednisone

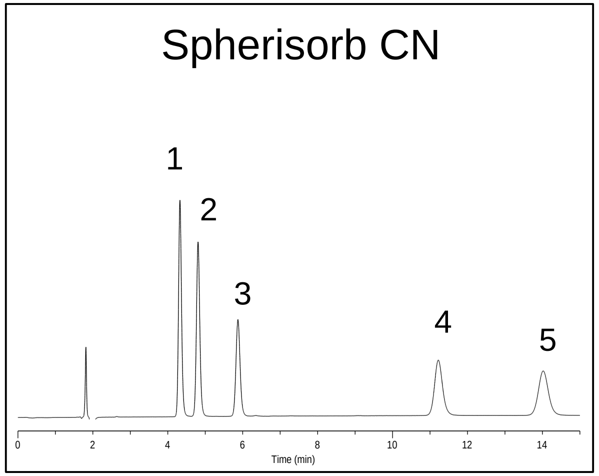


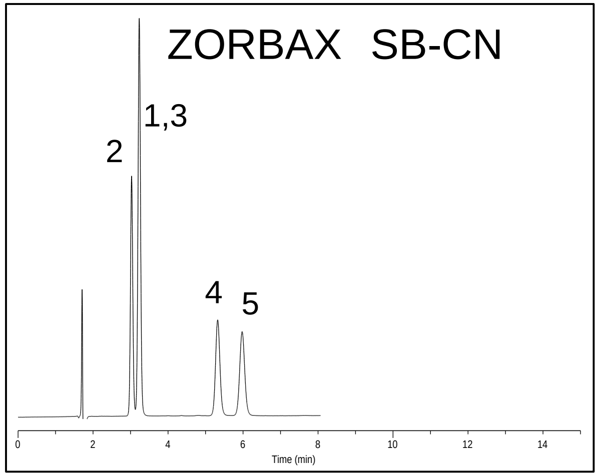
4. Prednisolone

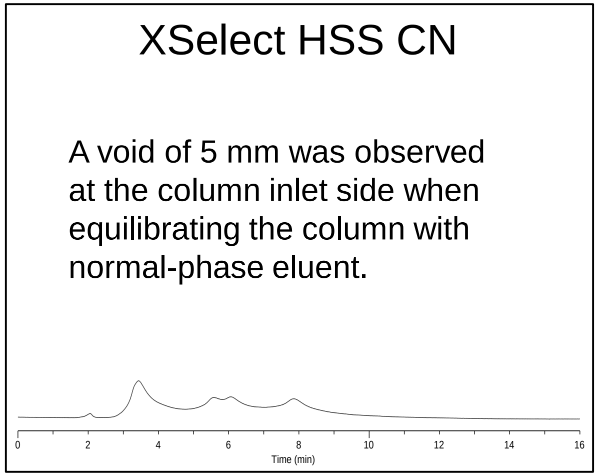
Analysis of Steroids by Normal-Phase Mode

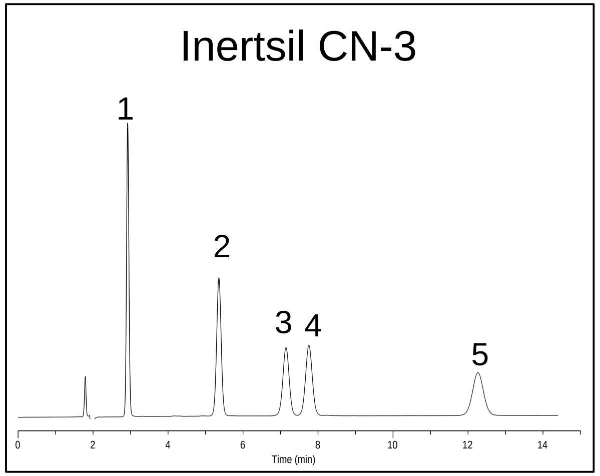
InertSustain Cyano columns can be used under Normal-Phase mode without any problem.











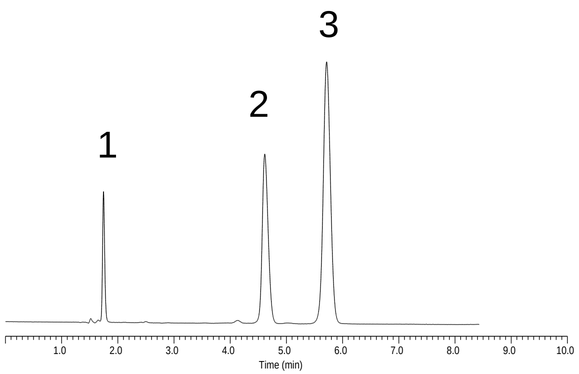
<u>Conditions</u>

Eluent	: A) Hexane B) Ethanol A/B = 90/10, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 220 nm
Sample	: 1. Progesterone 2. Estrone 3. β-Estradiol 4. Corticosterone 5. Hydrocortisone

Inertness to Strong Basic Compounds (Under Acidic Mobile Phase)

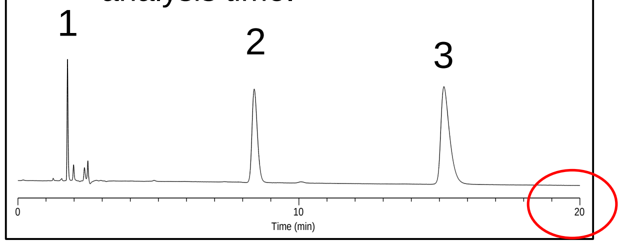
InertSustain Cyano columns are rigorously end-capped which provide symmetrical peak shapes for strong basic compounds.

InertSustain Cyano

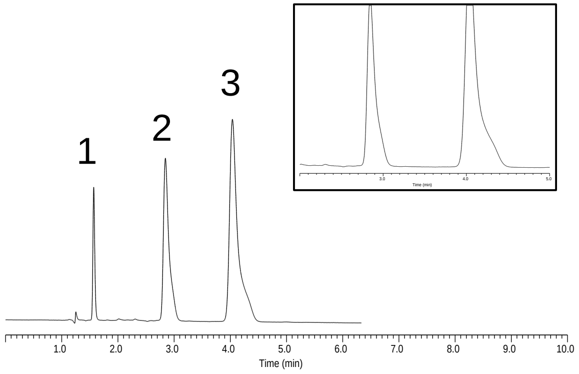


Spherisorb CN

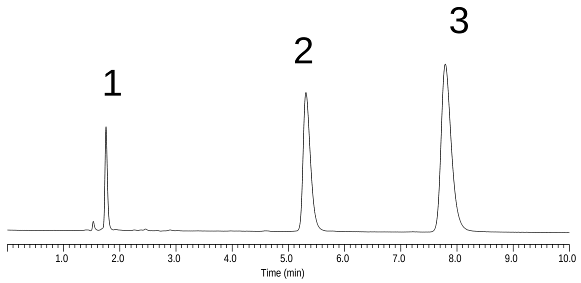
The residual silanols are strongly retaining the basic compounds, resulting in long analysis time.



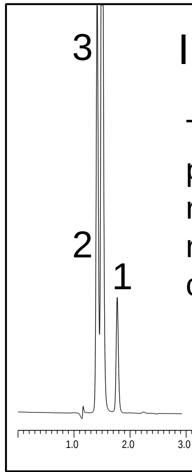
ZORBAX SB-CN



XSelect HSS CN



Inertsil CN-3



The surface of the silica is positively charged, resulting in showing no retention of basic compounds.

<u>Conditions</u>

Eluent : A) 0.1 % H_3PO_4 B) CH_3CN
A/B ＝75/25 , v/v

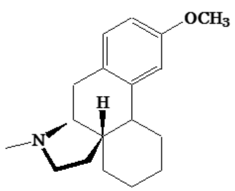
Flow Rate : 1.0 mL/min

Col. Temp. : 40 ℃

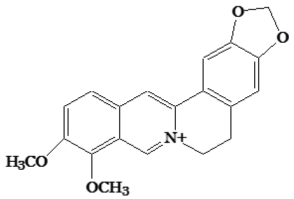
Detection : UV 230 nm

Sample :

1. Uracil 2. Dextromethorphan 3. Berberine



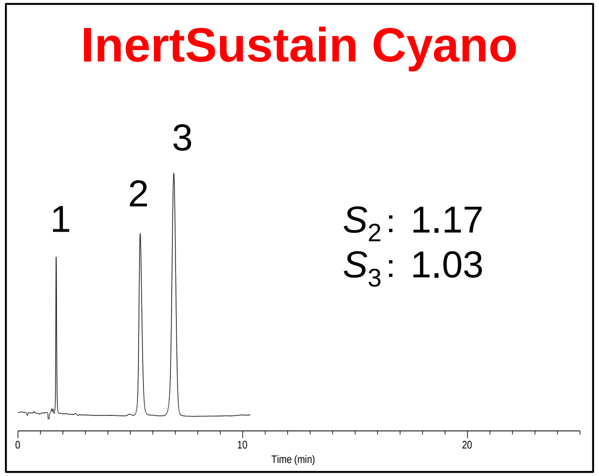
2. Dextromethorphan

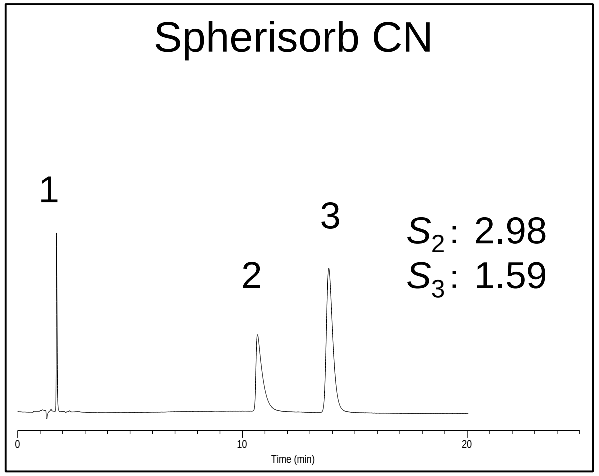


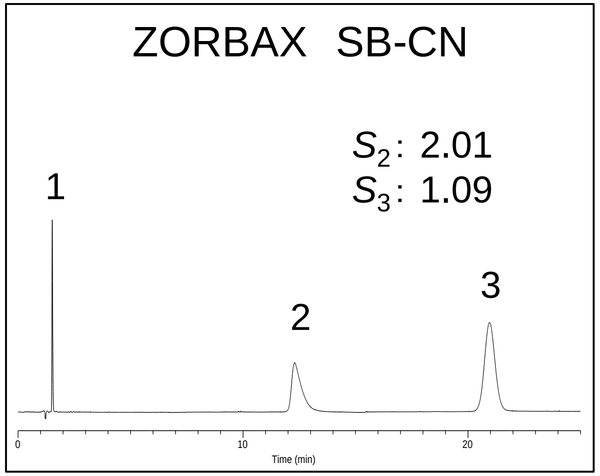
3. Berberine

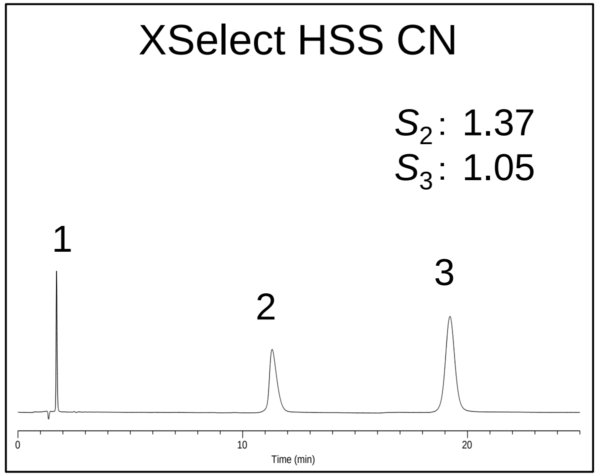
Inertness to Strong Basic Compounds (Under Neutral Mobile Phase)

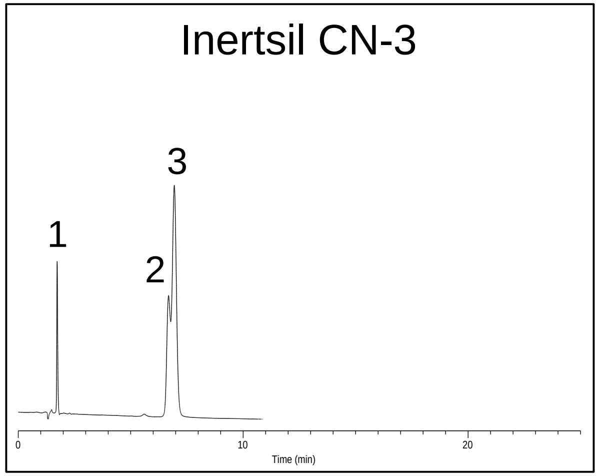
At neutral mobile phases, silanol groups present will act like an acidic moiety and will become ionized - increasing the tailing observed with analytes containing basic functional groups. As proven below, InertSustain Cyano does not show excessive tailing or retention of strong basic analytes.







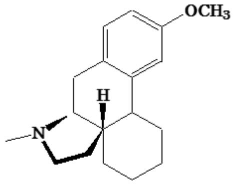




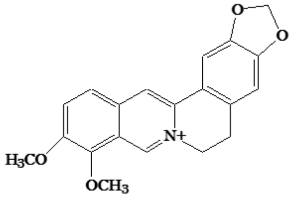
Conditions

Eluent	: A) 50 mM $HCOONH_4$ B) CH_3CN A/B = 70/30 , v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 230 nm

Sample :
1. Uracil 2. Dextromethorphan 3. Berberine



2. Dextromethorphan

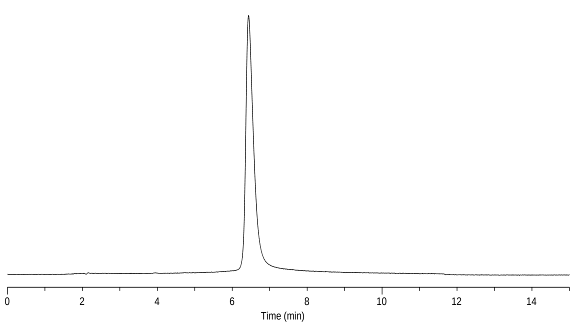


3. Berberine

Chelating Compound

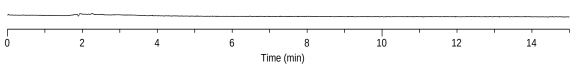
Hinokitiol is a strong chelating compound, which coordinately binds with the surface of residual trace metal impurities, resulting in severe tailing. As shown below, InertSustain Cyano provide superior peak shape for strong chelating compound.

InertSustain Cyano

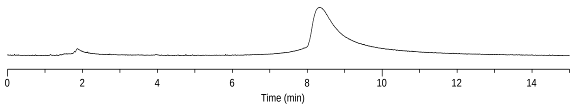


Spherisorb CN

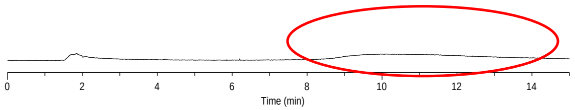
No elution



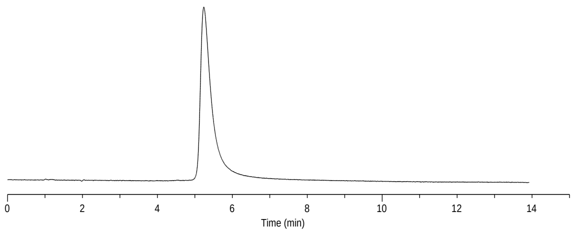
ZORBAX SB-CN



XSelect HSS CN



Inertsil CN-3



Conditions

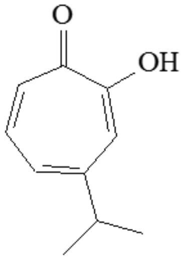
Eluent : A) 0.1 % H_3PO_4 B) CH_3CN
A/B ＝75/25, v/v

Flow Rate : 1.0 mL / min

Col. Temp. : 40 ℃

Detection : UV 310 nm

Sample : Hinokitiol

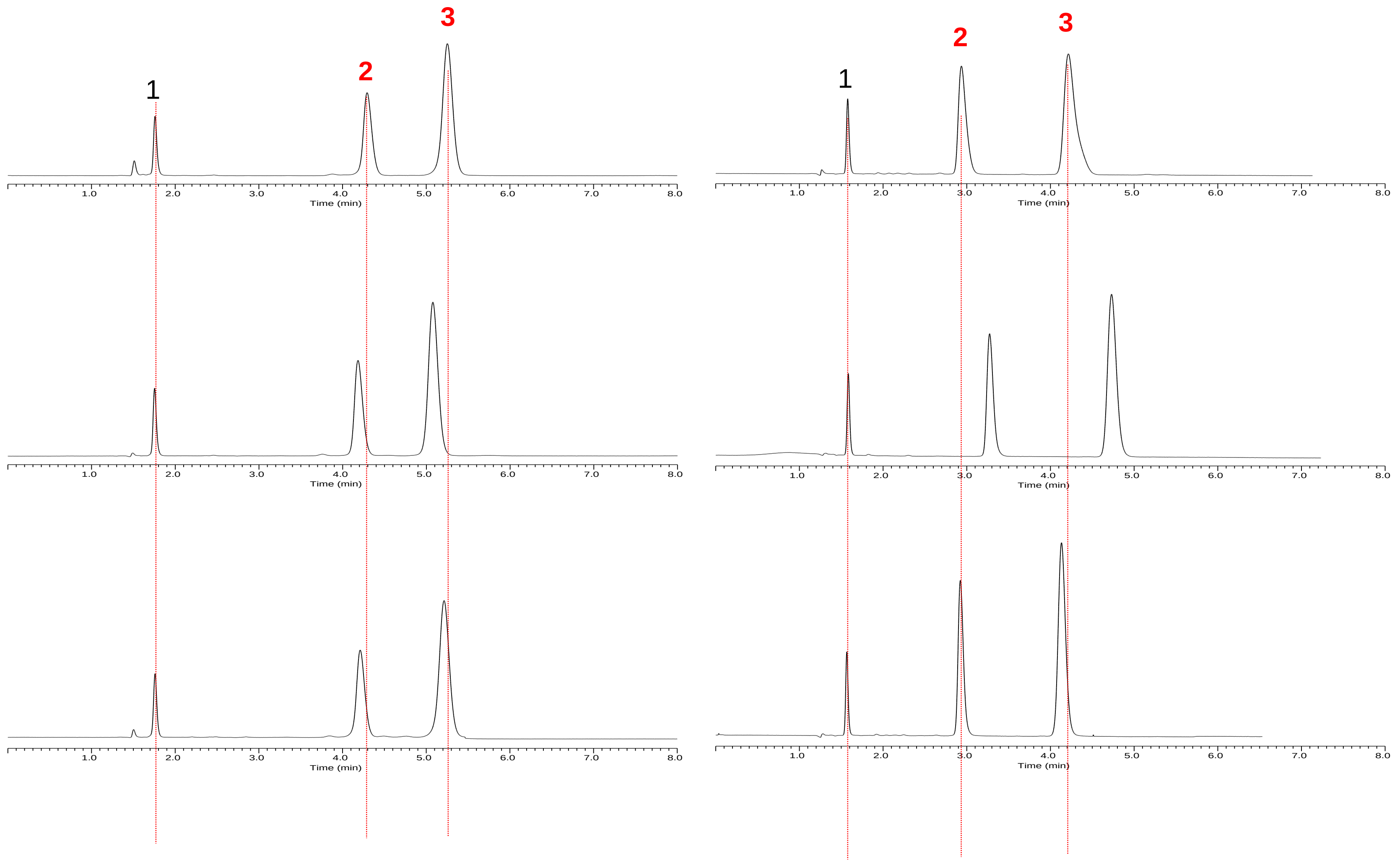


Reproducibility of Strong Basic Compounds

InertSustain Cyano

Zorbax SB-CN

Observed a variation of
inertness from lot-to-lot



Conditions

Eluent : A) CH_3CN B) 0.1% H_3PO_4
A/B = 25/75, v/v

Flow Rate : 1.0 mL/min

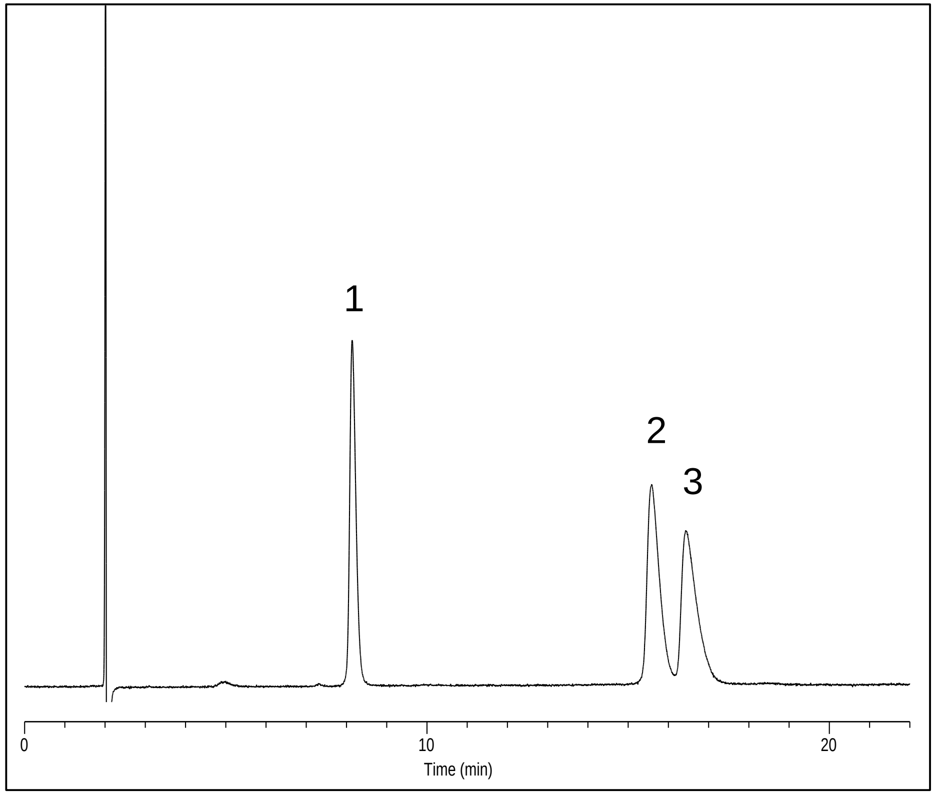
Col. Temp. : 40 ℃

Detection : UV 230 nm

Sample : 1. Uracil
2. Dextromethorphan
3. Berberine

Application　: *o*,*m*,*p*-Methylhippuric Acids

It is hard to separate m,p using a conventional C18 column. The InertSustain Cyano provided baseline separation due to its' unique selectivity.



Conditions

Column　: InertSustain Cyano,（3 μm, 150 × 4.6 mm I.D.）

Eluent　: A) CH_3CN　B) 0.1 % HCOOH

A/B = 1/99, v/v

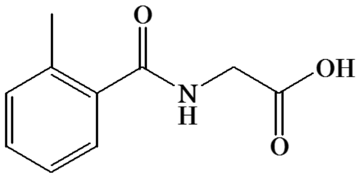
Flow Rate　: 1.0 mL/min

Col. Temp.　: 40 ℃

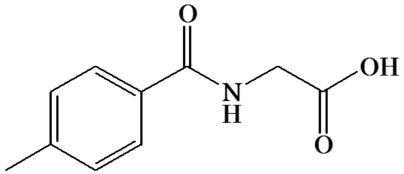
Detection　: UV 220 nm

Sample :

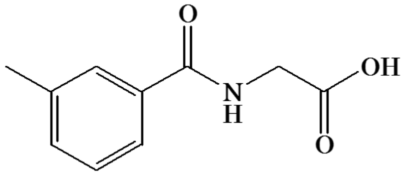
1. *o*-Methylhippuric Acid
2. *p*-Methylhippuric Acid
3. *m*-Methylhippuric Acid



o-Methylhippuric Acid

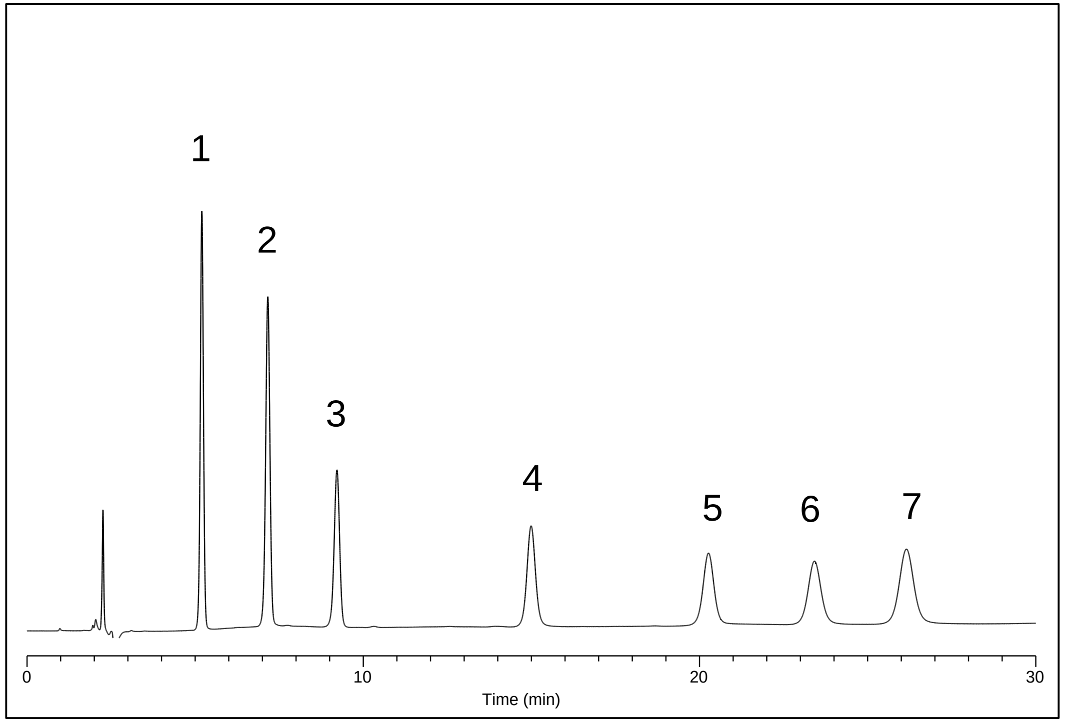


2. *p*-Methylhippuric Acid



3. *m*-Methylhippuric Acid

Application : Steroids by Normal-Phase Mode



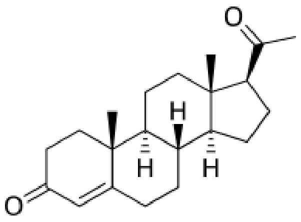
As the hydrophobicity of analytes are quite similar, failure in separation may be observed under reversed-phase mode. Separation may be improved by using normal-phase mode.

Conditions

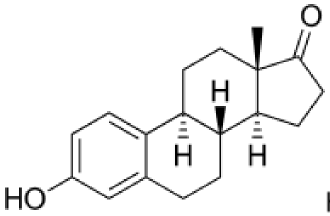
Column	: InertSustain Cyano, (3 μm, 150 × 4.6 mm I.D.)
Eluent	: A) Hexane B) Ethanol A/B = 95/5, v/v
Flow Rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: UV 220 nm

Sample :

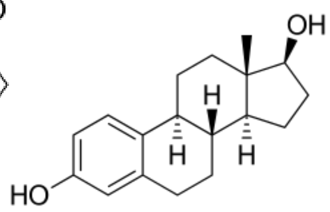
1. Progesterone 2. Estrone 3. β-Estradiol
4. Corticosterone 5. Hydrocortisone 6. Prednisone
7. Prednisolone



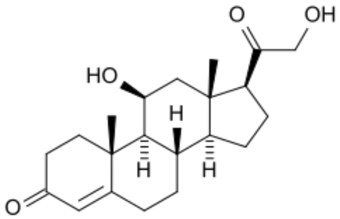
Progesterone



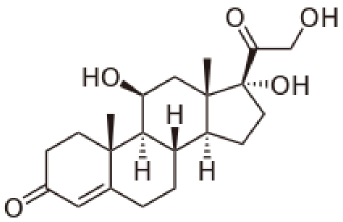
Estrone



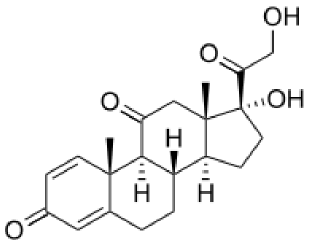
β-Estradiol



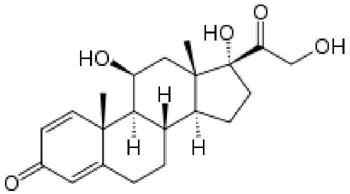
Corticosterone



Hydrocortisone

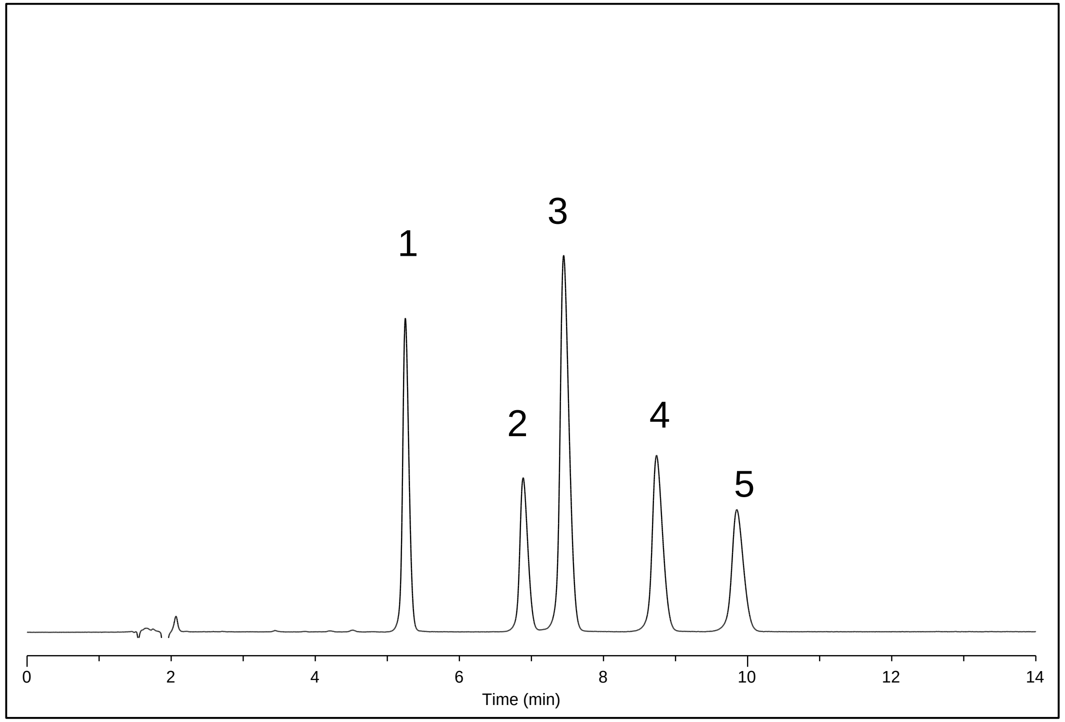


Prednisone



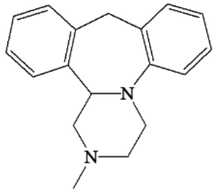
Prednisolone

Application : Antidepressants

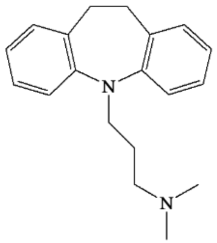


<u>Conditions</u>

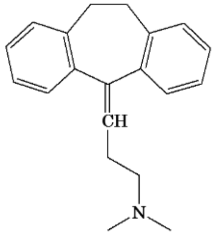
Column : InertSustain Cyano（3 µm, 150 × 4.6 mm I.D.）
Eluent : A) 10 mM $HCOONH_4$ (0.1% HCOOH) B) CH_3CN(0.1% HCOOH)
A/B = 70/30, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 230 nm
Sample :
1. Mianserine 2. Imipramine 3. Amitriptyline
4. Sertraline 5. Clomipramine



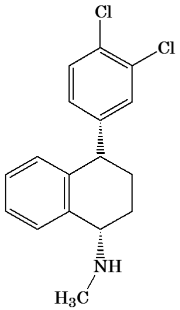
Mianserine



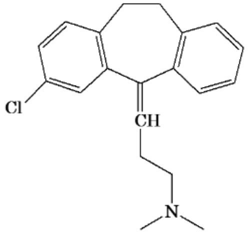
Imipramine



Amitriptyline

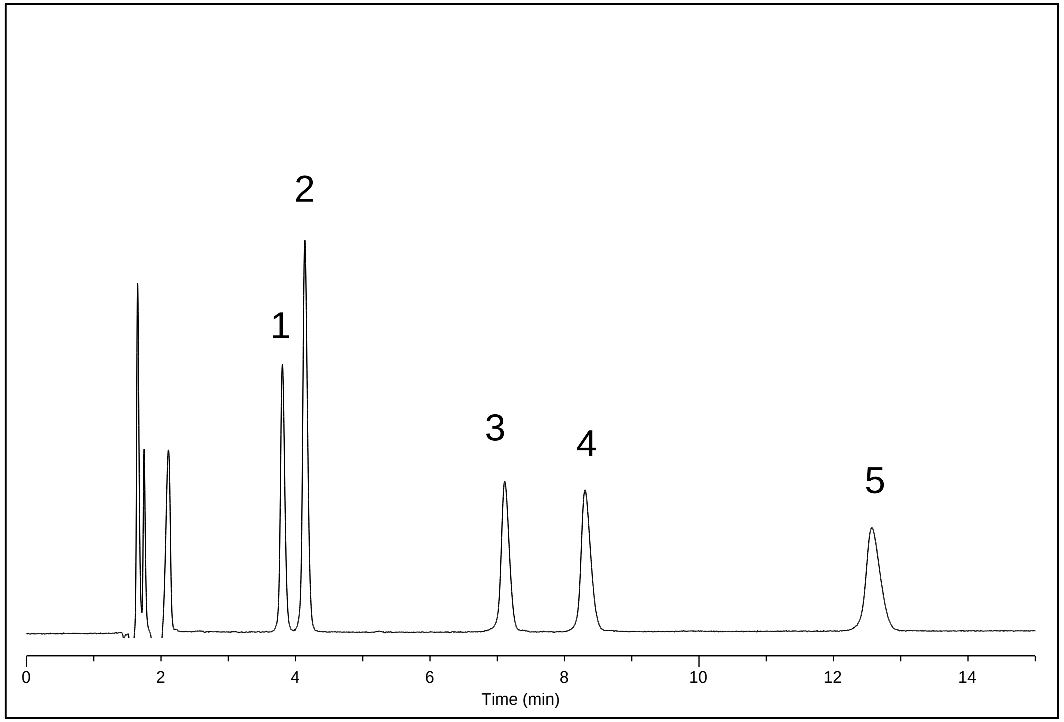


Sertraline



Clomipramine

Application : First-Generation Antihistamines



Conditions

Column : InertSustain Cyano（3 µm, 150 × 4.6 mm I.D.）

Eluent : A) 10 mM $HCOONH_4$(0.1%HCOOH) B) CH_3CN(0.1% HCOOH)
A/B = 70/30, v/v

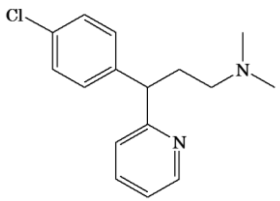
Flow Rate : 1.0 mL/min

Col. Temp. : 40 ℃

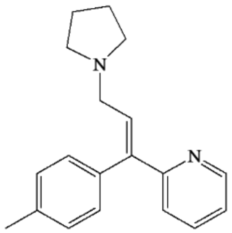
Detection : UV 220 nm

Sample :

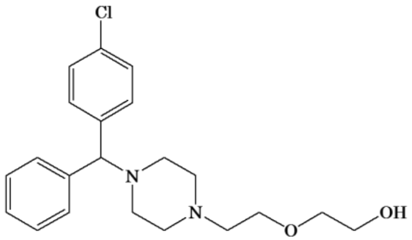
1. Chlorpheniramine 2. Triprolidine 3. Hydroxyzine
4. Homochlorcyclizine 5. Clemastine



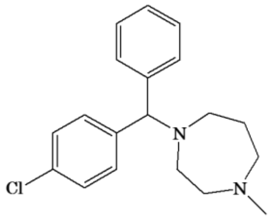
Chlorpheniramine



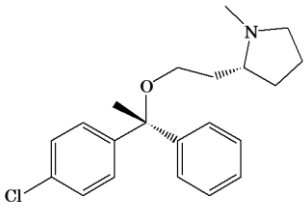
Triprolidine



Hydroxyzine

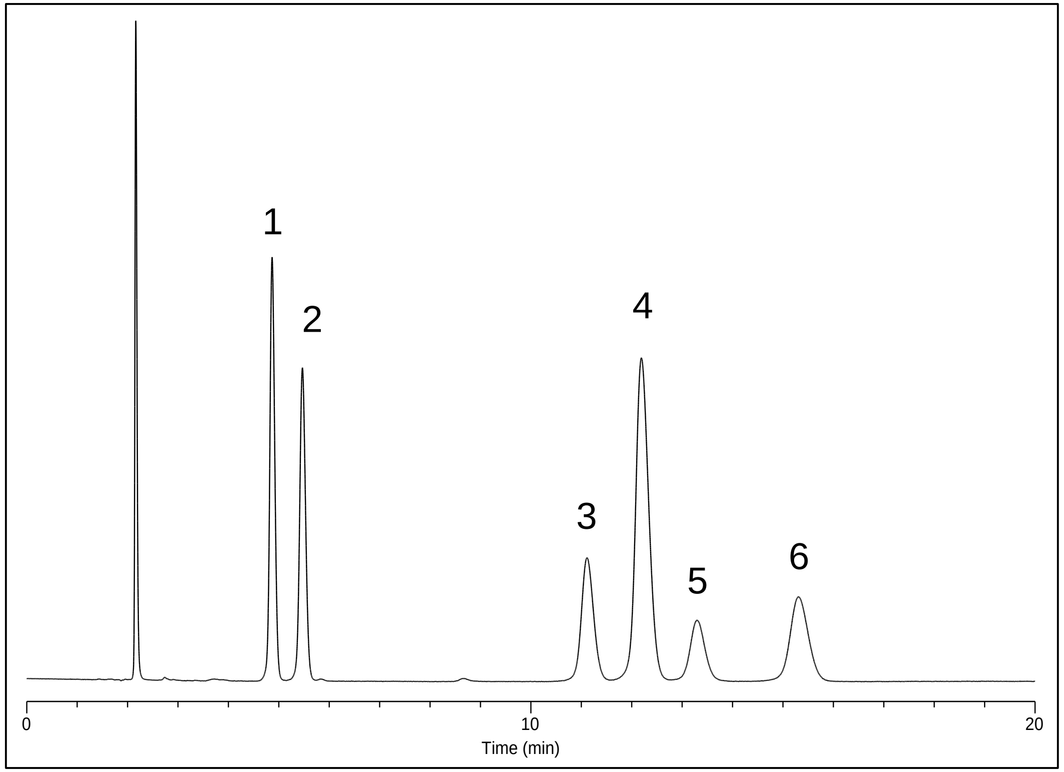


Homochlorcyclizine

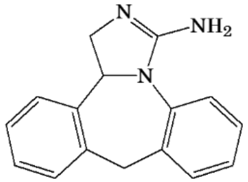


Clemastine

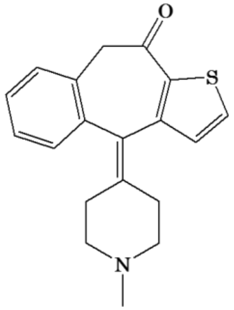
Application　: Second-Generation Antihistamines



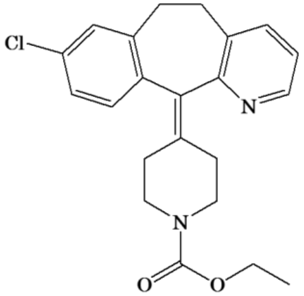
<u>Conditions</u>
Column　: InertSustain Cyano（3 μm, 150 × 4.6 mm I.D.）
Eluent　: A) 0.1% H_3PO_4　B) CH_3CN
A/B = 70/30, v/v
Flow Rate　: 1.0 mL/min
Col. Temp.　: 40 ℃
Detection　: UV 220 nm
Sample :
1. Epinastine　2. Ketotifen　3. Loratadine
4. Azelastine　5. Cetirizine　6. Fexofenadine



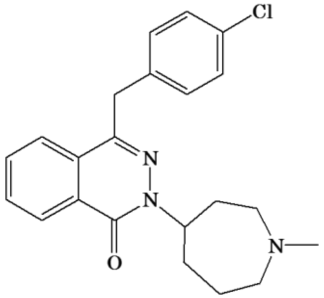
Epinastine



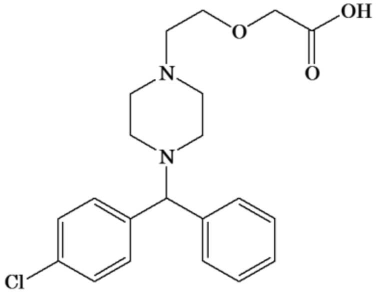
Ketotifen



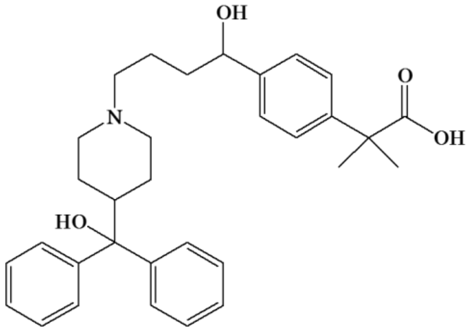
Loratadine



Azelastine

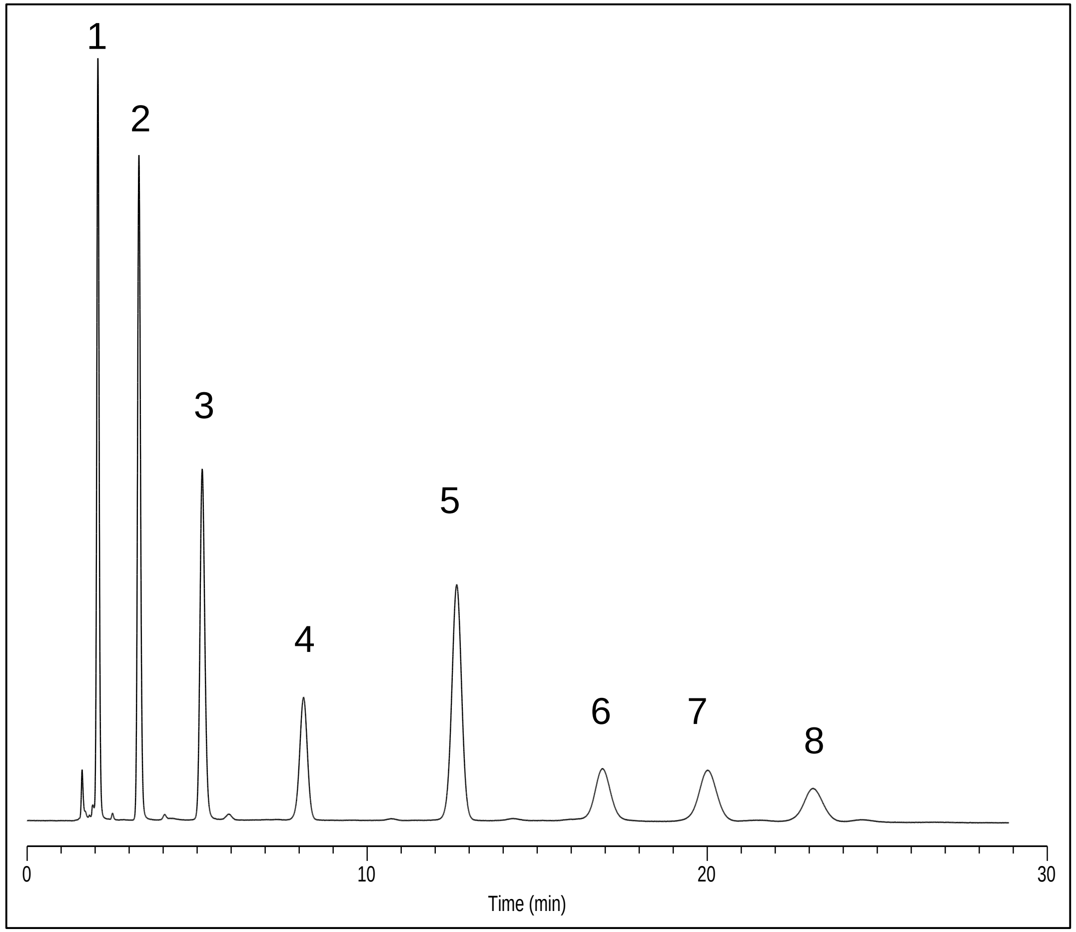


Cetirizine



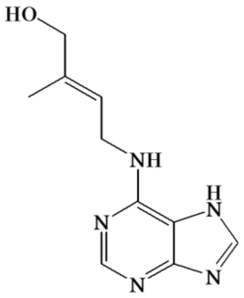
Fexofenadine

Application　: Phytohormones

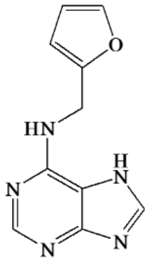


<u>Conditions</u>

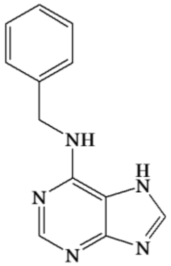
Column　: InertSustain Cyano (5 μm, 150 × 4.6 mm I.D.)
Eluent　: A) 0.1% H_3PO_4　B) CH_3OH
　A/B = 90/ 0, v/v
Flow Rate　: 1.0 mL/min
Col. Temp.　: 40℃
Detection　: UV 210 nm
Sample :
1. *t*-Zeatin　2. Kinetin　3. 6-Benzylaminopurine　4. Gibberellin A3
5. Indole-3-acetic acid　6. *t*-*t*-Abscsic acid　7. *c*-*t*-Abscsic acid　8. Jasmonic Acid



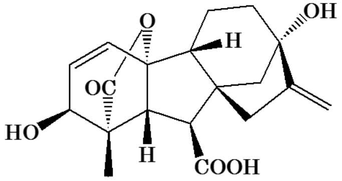
t-Zeatin



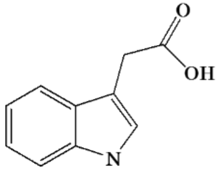
Kinetin



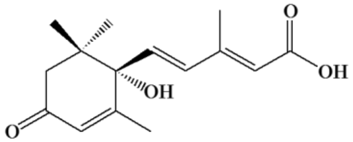
6-Benzylaminopurine



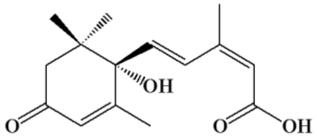
Gibberellin A3



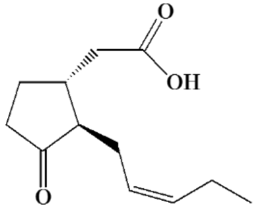
Indole-3-acetic acid



t-*t*-Abscisic Acid

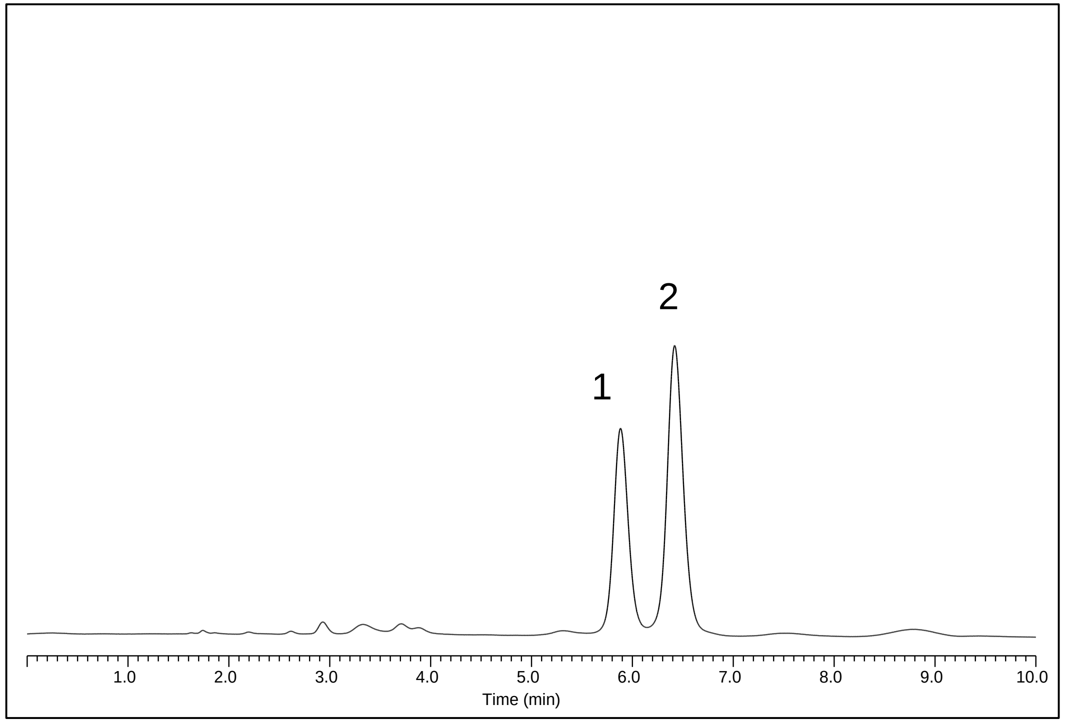


c-*t*-Abscisic Acid



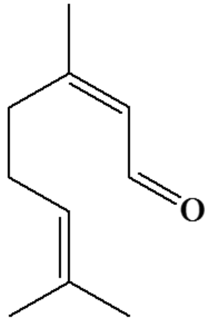
Jasmonic Acid

Application　: Citrals (geranial, neral)

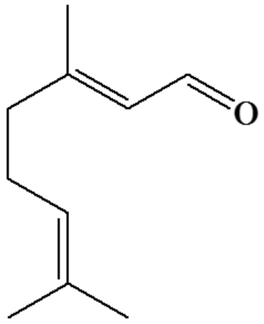


<u>Conditions</u>

Column : InertSustain Cyano (5 μm, 150 × 4.6 mm I.D.)
Eluent : A) 0.1% H_3PO_4 B) CH_3OH
A/B = 60/40, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 210 nm
Sample :
1. cis-Citral 2. Trans-Citral

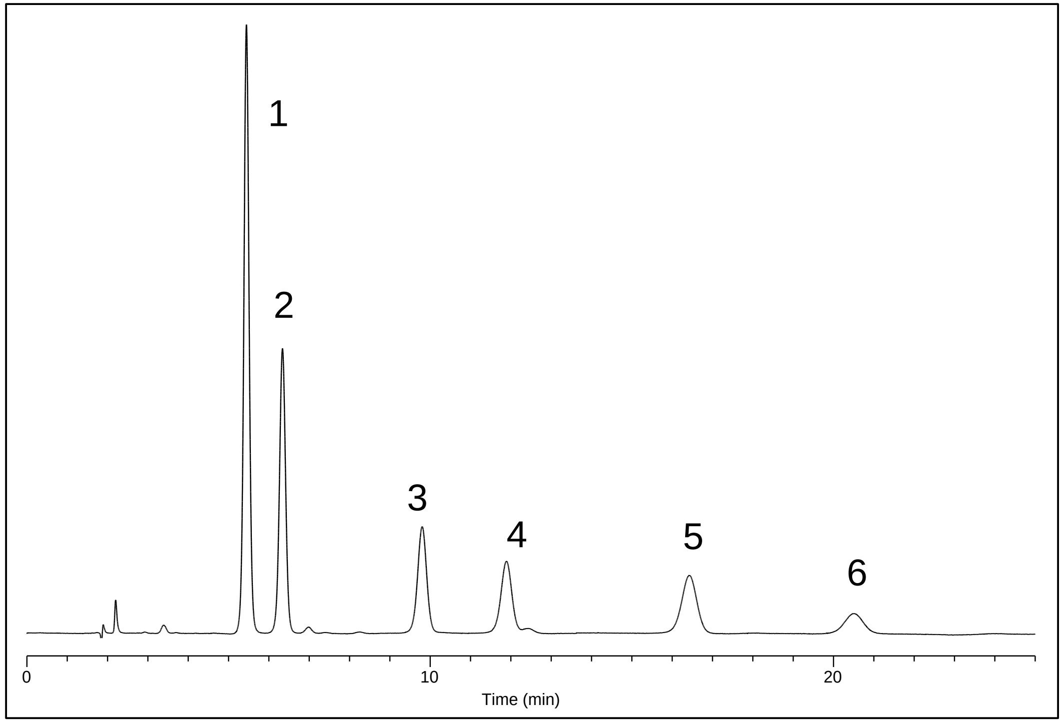


cis-Citral
(Neral)



trans-Citral
(Geranial)

Application　: Chlorophenols

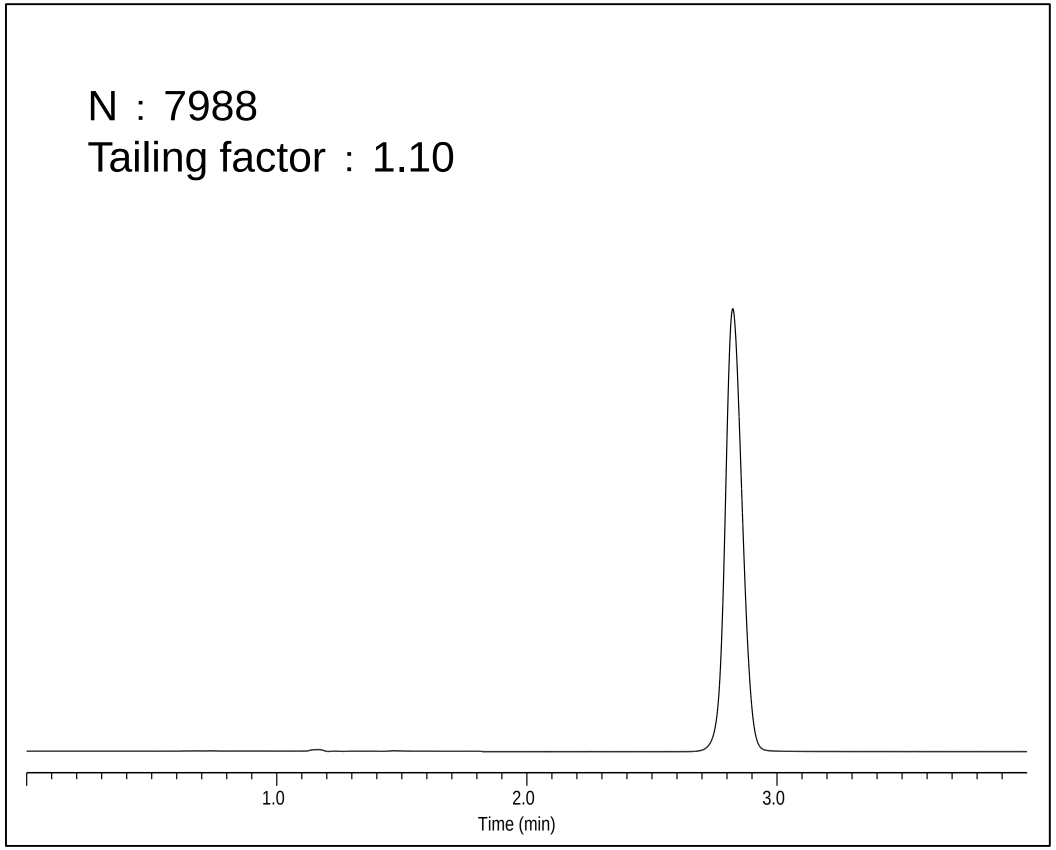


<u>Conditions</u>

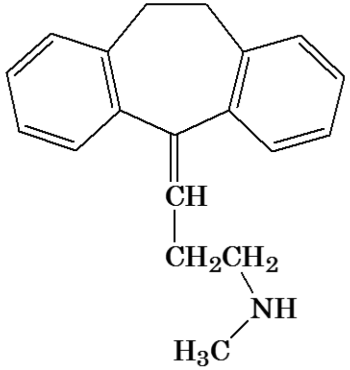
Column : InertSustain Cyano（5 μm, 150 ×4.6 mm I.D.）
Eluent : A) 0.1% H_3PO_4 B) CH_3OH
A/B = 60/40, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 280 nm
Sample :
1. 2,6-Dichlorophenol
2. 2,4-Dichloropehnol
3. 2,4,5-Trichlorophenol
4. 3,4,5-Trichlorophenol
5. 2,3,4,5-Tetrachlorophenol
6. Pentachlorophenol

Application　: Nortriptyline Hydrochloride Capseles

USP Method



Nortriptyline Hydrochloride Capseles

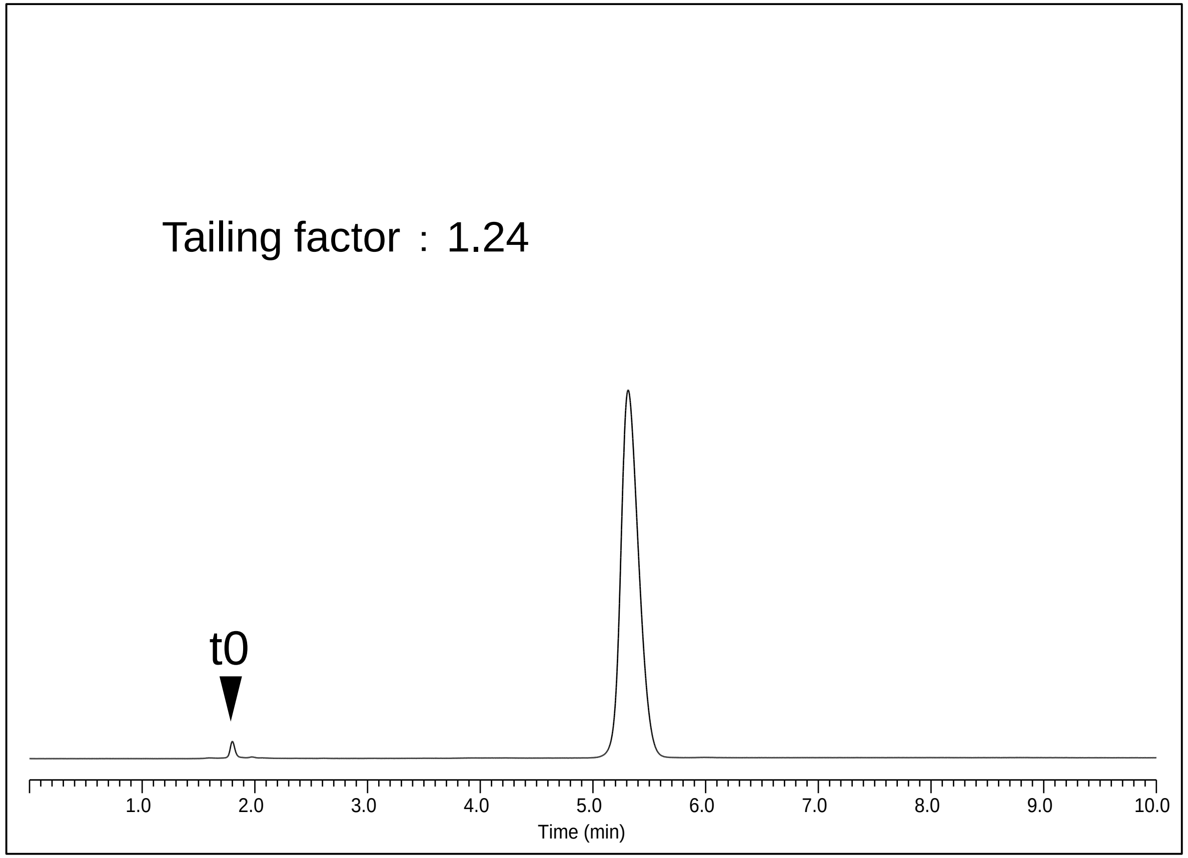


USP Column: 5 μm, 150 × 4.6 mm I.D.（L10）
System suitability requirements:
Efficiency (N) : ＞500
Tailineg factor : ＜ 3.0

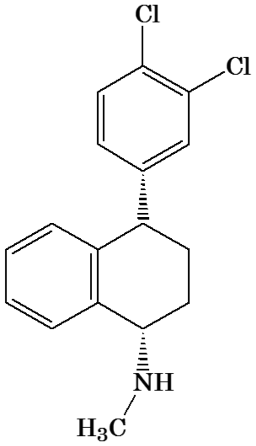
Sample Conc. : 0.38 mg/mL (in Methanol)
Mobile Phase : ACN : CH_3OH : 12 mM Potassium phosphate (pH 6.7) ＝ 40 : 43 : 17
Flow Rate : 2.5 mL
Detection : UV 239 nm
Injection : 5 μL

Application : Sertraline Hydrochloride

USP Method



Sertraline Hydrochloride

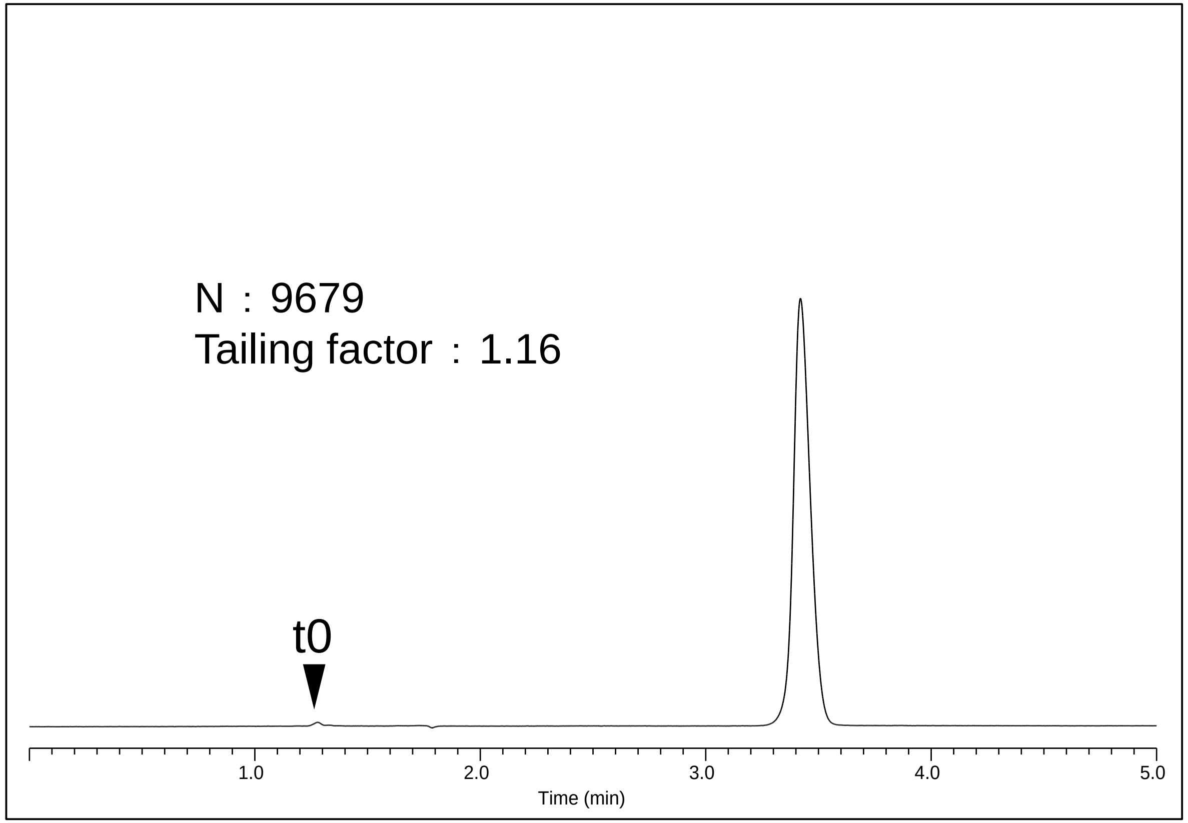


USP Column: 5 μm, 150 ×4.6 mm I.D.（L10）
System suitability requirements:
Tailineg factor : ＜ 2.0

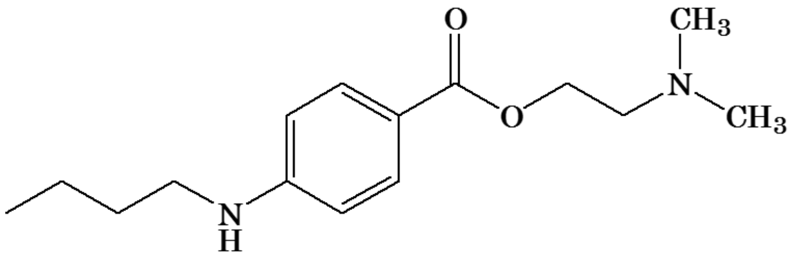
Sample Conc. : 0.050 mg/mL (in Mobile Phase)
Mobile Phase : CH_3OH : 0.1% (v//v) Phosphate acid
＝ 1 : 1
Flow Rate : 1.5 mL
Detection : UV 210 nm
Colum Temp. : 30 ℃
Injection : 10 μL

Application　: Tetracaine Hydrochloride Ophthalmic Solution

USP Method



Tetracaine Hydrochloride Ophthalmic Solution



USP Column：5 μm, 150 ×4.6 mm I.D.（L10）
System suitability requirements:
Efficiency (N)：＞500
Tailineg factor：＜2.0

Sample Conc.：0.1 mg/mL (in Water)
Mobile Phase：ACN：10 mM Ammonium phosphate (pH 3.0) ＝ 30：70
Flow Rate：2.0 mL
Detection：UV 280 nm
Injection：10 μL

GL Sciences' Reversed-Phase HPLC Column Selection Guide

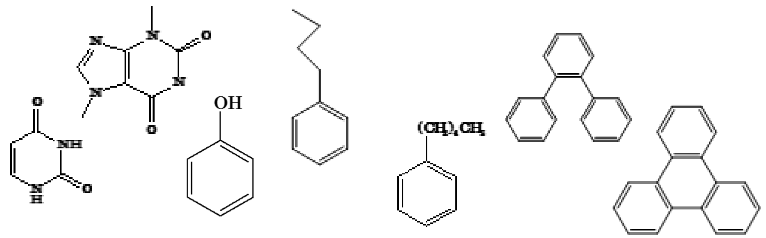
InertSustain C18	• First Choice C18 Column
InertSustain AQ-C18	• Ideal for Maximizing Retention for Highly Polar Compounds in Reversed Phase Methods with Highly Aqueous Mobile Phases
InertSustainSwift C18	• Rapid Elution of Samples in Isocratic Methods and Rapid Column Equilibration Time in Gradient Methods
Inertsil ODS-HL	• Ultra High Retentivity, High-Density Bonding of C18 Phase • Ideal for Separation of Basic Molecules & its Related Substances, Process Impurities
InertSustain Phenyl	• Provides not only pi-pi interactions, but also hydrogen bonding secondary interactions • Ideal for critical resolving compounds (e.g. metabolites) that could not be separated on a C18 phase
InertSustain Phenylhexyl	• Deliver complementary selectivity to straight alkyl-chain columns, but with slight pi-pi interactions • Industry leading inertness, lot-to-lot reproducibility and low back pressure
InertSustain Cyano	• First choice USP L10 column • The most Reliable and Reproducible Cyano column

GL Sciences' HILIC HPLC Column Selection Guide

InertSustain Amide	• Excellent for those hard to retain compounds using an ODS column • First choice HILIC column under HILIC Mode Separation
InertSustain NH2	• A Primary Amine Bonded column preventing anomer resolution with easy sugar analysis at low temperature • Highly reproducible results with exceptional stability and durability that will maintain performance over the lifetime of the method

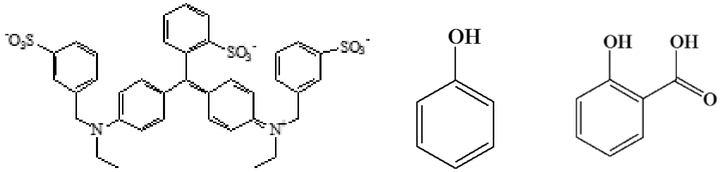
Explanation of Analytical Tests and Conditions

Selectivity Test



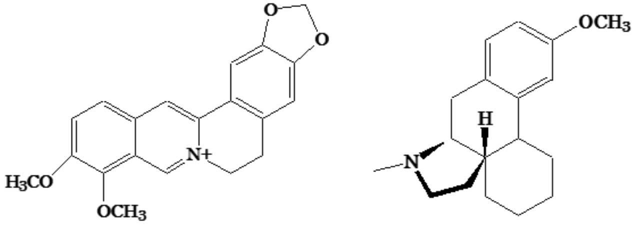
Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) CH_3OH B) H_2O A/B = 60/40, v/v
Flow Rate : 1.0 mL / min Col. Temp. : 40 ℃ Detection : UV 254 nm
Sample : 1. Uracil 2. Caffeine 3. Phenol 4; Butylbenzene
5. Amylbenzene 6. o-Tertphenyl 7. Triphenylene

Acidic Compound Test



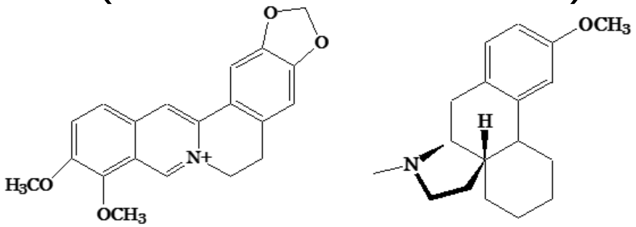
Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) ACN B) 0.1 % H_3PO_4 (H_2O) A/B = 75/25, v/v
Flow Rate : 1.0 mL / min Col. Temp. : 40℃
Detection : UV 254 nm
Sample : 1. Blue-FCF 2. Phenol 3. Salicylic Acid

Basic Compound Test 1 (Acidic mobile phase)



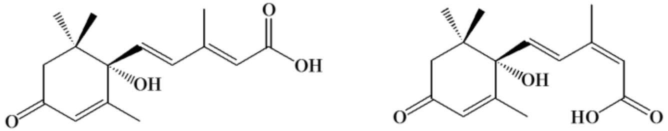
Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) ACN B) 0.1 % H_3PO_4 (H_2O) A/B = 75/25, v/v
Flow Rate : 1.0 mL / min Col. Temp. : 40 ℃
Detection : UV 230 nm
Sample : 1. Uracil 2. Dextromethorphan 3. Berberine

Basic Compound Test 2 (Neutral Mobile Phase)



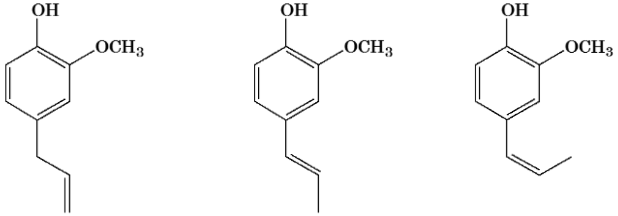
Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) ACN B) 50 mM $HCOONH_4$ A/B = 70/30, v/v
Flow Rate : 1.0 mL/min Col. Temp. : 40 ℃
Detection : UV 230 nm
Sample : 1. Uracil 2. Dextromethorphan 3. Berberine

Separation of Cis-trans Test 1



Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) ACN B) 0.1 % H_3PO_4 (H_2O) A/B = 75/25, v/v
Flow Rate : 1.0 mL/min Col. Temp. : 40 ℃
Detection : UV 210 nm
Sample : 1. (*S*)-2-*trans*-4-trans-abscisic acid
2. (*S*)-2-cis-4-trans-abscisic acid

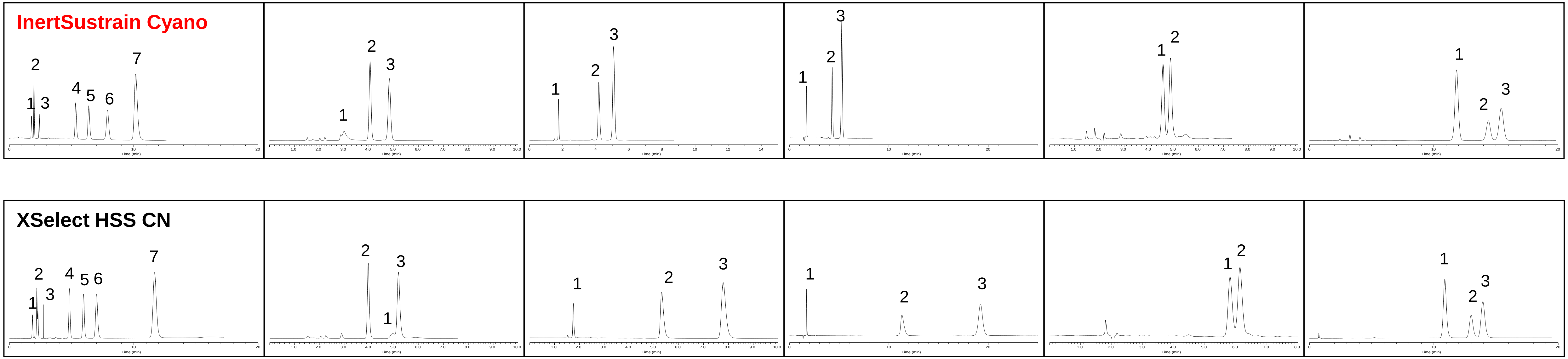
Separation of Cis-trans Test 2



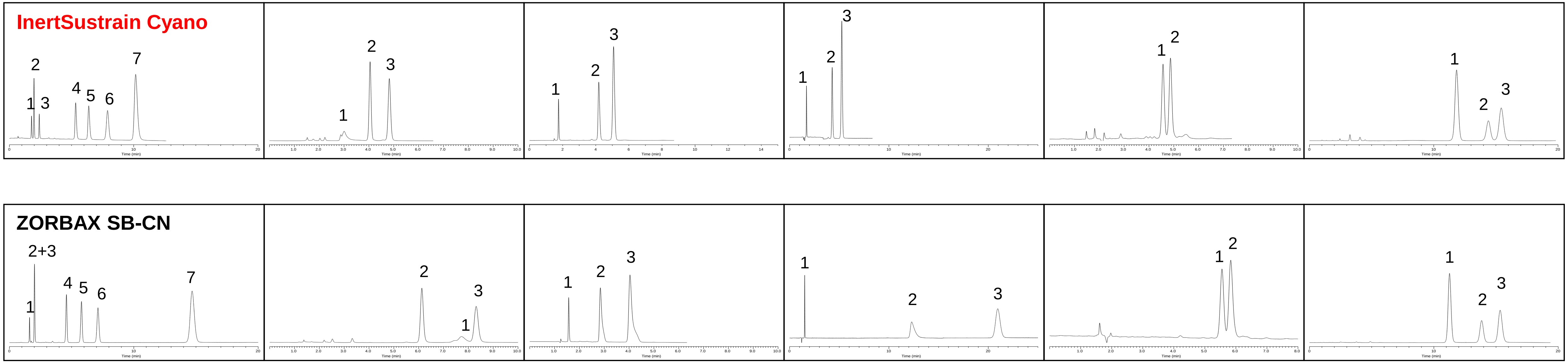
Column : 4.6 mm I.D × 150 mm, 5 μm
Eluent : A) CH_3OH B) H_2O A/B = 30/70, v/v
Flow Rate : 1.0 mL / min Col. Temp. : 40 ℃
Detection : UV 210 nm
Sample : 1. Eugenol 2. cis-Isoeugenol 3. trans- Isoeugenol

Comparison of Performance 1/3

Selectivity Test | Acidic Compound | Basic Compound 1 | Basic Compound 2 | Separation of Cis-trans 1 | Separation of Cis-trans 2



Selectivity Test | Acidic Compound | Basic Compound 1 | Basic Compound 2 | Separation of Cis-trans 1 | Separation of Cis-trans 2



Comparison of Performance 3/3