GL Sciences

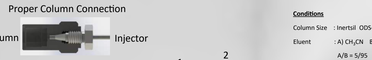
Everything you need for Critical Separation

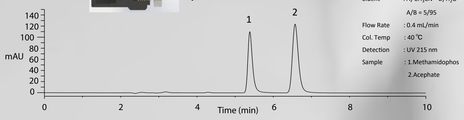
History of Inertsil / InertSustain

System Dead Volume
Tubing Connections from Injector to Column

Dead volume causes peaks tailing and broadening.

Proper Column Connection

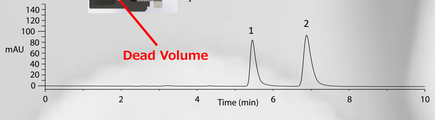




Conditions

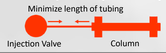
Column Size : Inertsil ODS-3 5μm, 150 x 3.0 mm I.D.
Eluent : A) CH_3CN B) H_3O
A/B = 5/95
Flow Rate : 0.4 mL/min
Col. Temp : 40 ℃
Detection : UV 215 nm
Sample : 1.Methamidophos
2.Acephate

Improper Column Connection



The Influence of Tubing Length and Diameter

Minimizing length of tubing and tubing I.D. between injection valve and column improves peak shapes.



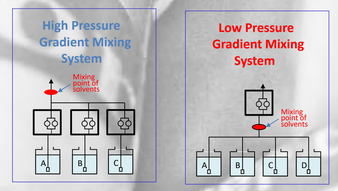
Length(mm) from the Injection Valve to Column	Tubing I.D. (mm) 0.5	0.25	0.13
200	N = 1224 Rs= —	N = 4351 Rs= 1.138	N = 5682 Rs= 1.314
400	N = — Rs= —	N = 3908 Rs= 1.055	N = 5673 Rs= 1.302
800	N = — Rs= —	N = 3765 Rs= 1.009	N = 5553 Rs= 1.281
1250	N = — Rs= —	N = 3227 Rs= 0.899	N = 5598 Rs= 1.273

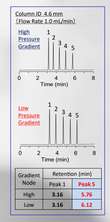
Conditions

Column Size : InertSustain C18 3 μm, 150 x 2.1 mm I.D.
Eluent : A) CH_3CN B) H_3O
A/B = 50/50
Flow Rate : 0.2 mL/min
Col. Temp : 40 ℃
Detection : UV 254 nm
Sample : o,p-cresol

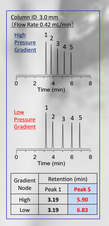
Gradient Delay

The gradient delay can be observed more on low pressure gradient system when using smaller I.D. size at low flow rates.





Gradient	Retention (min)
Node	Peak 1 / Peak 5
High	3.16 / 5.76
Low	3.16 / 6.12

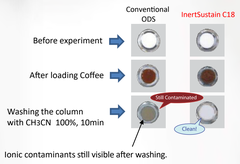


Gradient	Retention (min)
Node	Peak 1 / Peak 5
High	3.19 / 5.90
Low	3.19 / 6.83

Conditions

Column Size : InertSustainSwift C18
Standard : 3 μm, 150 x 4.6 mm I.D.
Semi-micro : 3 μm, 150 x 3.0 mm I.D.
Eluent : A) CH_3CN B) H_3O
A/B = 50/50 – 6min – 100/0, v/v
Col. Temp : 40 ℃
Detection : PDA 270 nm
Mixer Volume : Standard approx. 1.0 mL
Semi-micro approx. 0.4 mL
Sample : 1. 4-Methylphenol
2. 4-Ethylphenol
3. 4-Propylphenol
4. 4-Butylphenol
5. 4-Pentylphenol

『Inertness and Sustainability』



『Sophisticated self-manufacturing technology for the base silica』

InertSustain C18

First Choice Column for Various Applications

- Strong Retention
- High Inertness
- Wide pH range（pH1-10）
- Low back pressure

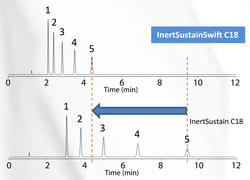
Inertsil ODS-3

A Milestone for Chromatography

- GL Sciences' most popular phase
- Strong retention
- Low back pressure

InertSustainSwift C18

For High-Throughput Analysis

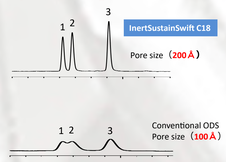


200 Å (20 nm) 100 Å (10 nm)

For Peptides and Oligonucleotides

Conditions

Column Size : 3 μm, 100 x 3.0 mm I.D.
Eluent : A: 5 mM TEAA in H_2O (pH 6.5)
B: 5 mM TEAA in H_2O (pH 6.5) /CH_3CN
A/B = 80/20
Flow Rate : 0.8 mL/min
Col. Temp : 40 ℃
Detection : UV 260 nm
Sample 1. catgacgttcctgatgct (**18 mer**, M.W. 5465.61)
2. ccatgacgttccatgatgct (**19 mer**, M.W. 5754.79)
3. tccatgacgttccatgatgct (**20 mer**, M.W. 6058.99)

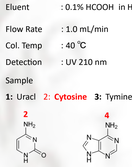


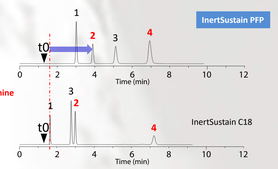
InertSustain PFP

Strong Retention for Highly Polar and Basic Analytes

Conditions

Column Size : 5 μm, 150 x 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: Uracil **2:** Cytosine **3:** Tymine **4:** Adenine

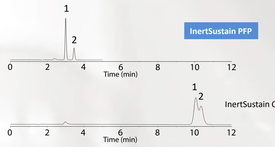




For Isomers and Impurities

Conditions

Column Size : 5 μm, 150 x 4.6 mm I.D.
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



1 2

1986 **Inertsil ODS**

1987 **Inertsil ODS-2**
- Inertsil C8
- Inertsil SIL-150A

1988
- Inertsil PREP-ODS
- Inertsil Ph
- Inertsil C4

1992
- Inertsil ODS-80A

1994 **Inertsil ODS-3**
- Inertsil SIL-100A
- Inertsil C8-3
- Inertsil Ph-3
- Inertsil CN-3
- Inertsil ODS-3V

1999
- Inertsil ODS-P
- Inertsil NH2
- Inertsil WP300 SIL
- Inertsil WP300 C8
- Inertsil Diol

2002 **Inertsil WP300 C18**
- Inertsil ODS-EP
- Inertsil WP300 Diol
- Inertsil Peptides C18
- Inertsil WP300 C4

2004
- Inertsil CX
- Inertsil AX

2005
- Inertsil ODS-SP

2008 **Inertsil ODS-4**
- Inertsil HILIC

2009
- Inertsil C8-4

2010 **InertSustain C18**
- Inertsil Amide
- Inertsil ODS-4V

2012 **InertSustain C8**
InertSustain NH2
- InertSustain Phenyl

2014 **InertSustainSwift C18**
- InertSustain Phenylhexyl
- InertSustainBio

2015 **InertSustain AQ-C18**

2016 **Inertsil ODS-HL**
InertSustainSwift C8
- InertSustain Amide

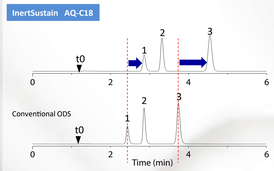
2017
- InertSustain Cyano

2018 **InertSustain PFP**

History continues

InertSustain AQ-C18

- **The Best Suited Column for Polar Analytes**

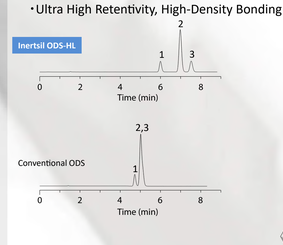


Conditions

Column Size : 5 μm, 150 x 4.6 mm I.D.
Eluent : A) CH_3CN B) 0.1% HCOOH in H_2O
A/B = 20/80, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 270 nm
Sample : 1. Chlorogenic acid (Acidic)
2. Caffeine (Basic)
3. Caffeic acid (Acidic)

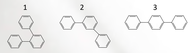
Inertsil ODS-HL

- **For Isomers and Process Impurities**
- Ultra High Retentivity, High-Density Bonding of C18 Phase



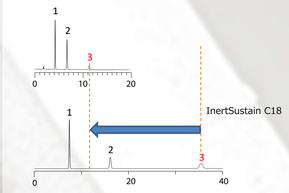
Conditions

Column Size : 5 μm, 150 x 4.6 mm I.D.
Eluent : A) CH_3CN B) H_2O
A/B = 85/15, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 254 nm
Sample : 1. *o*-Terphenyl
2. *m*-Terphenyl
3. *p*-Terphenyl



InertSustain C8

- **First Choice for C8 Column**
- **Faster Analysis for Highly Hydrophobic Analytes**

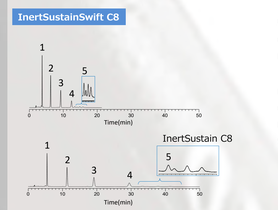


Conditions

Column Size : 5 μm, 150 x 4.6 mm I.D.
Eluent : CH_3CN
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 270 nm
Sample : 1. Vitamin K2(MK-4)
2. Vitamin K1
3. Vitamin K2(MK-7)

InertSustainSwift C8

- **InertSustainSwift C8 Offers Rapid Elution Comparing from InertSustain C8.**

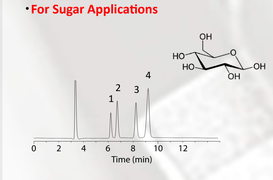


Conditions

Column Size : 5 μm, 150 x 4.6 mm I.D.
Eluent : A) CH_3CN B) H_2O
A/B = 90/10, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : UV 300 nm
Sample : 1. Retinol （Vitamin A）
2. Cholecalciferol （Vitamin D3）
3. α-tocopherol （Vitamin E）
4. Phylloquinone （Vitamin K1）
5. Impurities of 1

InertSustain NH2

- **For Sugar Applications**

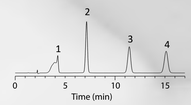


Conditions

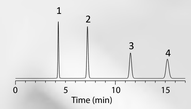
Column Size : 5 μm, 250 x 4.6 mm I.D.
Eluent : A) CH_3CN B) H_2O
A/B = 75/25, v/v
Flow Rate : 1.0 mL/min
Col. Temp. : 40 ℃
Detection : RI
Sample : 1. Fructose
2. Glucose
3. Sucrose
4. Maltose

Sample Diluent

Ideally, the sample diluent should have a composition as close as possible to the mobile phase.



Dissolved with 100% Acetonitrile



Dissolved with Mobile phase

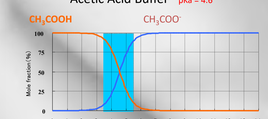
Conditions

Column : InertSustain AQ-C18 5μm, 150 x 4.6 mm I.D.
Eluent : A) CH_3CN B) H_2O
A/B = 10/90
Flow Rate : 1.0 mL/min
Col. Temp : 40 ℃
Detection : UV 280 nm
Sample : 1. 5-Hydroxymethyl-2-furaldehyde
2. 2-Furfural
3. 2-Acetylfuran
4. 5-Methyl-2-furfural
Injection : 10 μL

Buffering Effect

The selection of proper buffer depends on analyte's functional group and pKa Values.

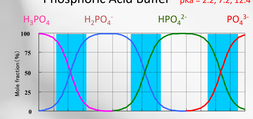
Acetic Acid Buffer pKa = 4.6



$CH_3COOH \Leftrightarrow CH_3COO^- + H^+$

Maximum buffer capacity

Phosphoric Acid Buffer pKa = 2.2, 7.2, 12.4

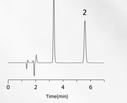


$H_2PO_4^-$ Nearly 100%

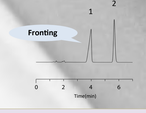
Minimum buffer capacity

In case when the pH is same as pKa
Efficient buffering effect can be obtained when the pH is set within ±1 the pKa.

10 mM Acetic Acid Buffer (pH 4.5)
Acetic Acid or Sodium Acetate solutions were prepared to 10 mM each and adjusted the pH at 4.5

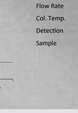


10 mM Phosphoric Acid Buffer (pH 4.5)
Phosphoric Acid or Sodium Dihydrogen Phosphate were prepared to 10 mM each and adjusted the pH at 4.5



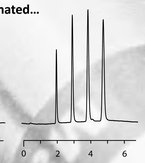
Conditions

Column Size : InertSustain C18 5 μm, 150 x 4.6 mm I.D.
Eluent : A) CH_3CN B) H_2O A/B = 65/35, v/v
Flow Rate : 0.4 mL/min
Col. Temp. : 40 ℃
Detection : UV254 nm
Sample : 1: Benzoic Acid
2: Benzyl Alcohol



Column Contamination

If your column is contaminated...

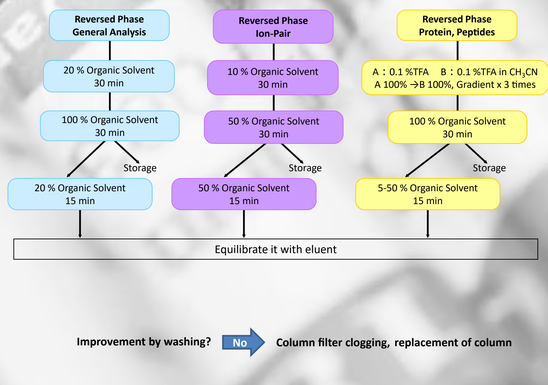


Conditions

Column Size : Inertsil ODS-3
3 μm, 100 x 3.0 mm I.D.
Eluent : A) CH_3CN B) H_2O
A/B = 65/35, v/v
Flow Rate : 0.4 mL/min
Col. Temp. : 40 ℃
Detection : UV254 nm
Sample : 1: Acetophenone
2: Benzene
3: Toluene
4: Naphthalene

General Method of Washing a Reversed Phase Column

The organic solvent can be either Acetonitrile or Methanol



Improvement by washing? No → **Column filter clogging, replacement of column**