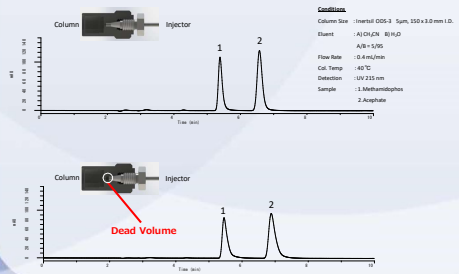
GL Sciences

Together, we can do more.

Column Cleaning and Storage

System Dead Volume : Tubing Connections

Influence of Dead Volume to the Peak Shape

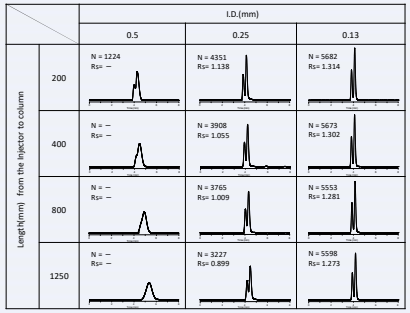


Conditions
Column Size : Inertsil ODS-3 5μm, 150 x 3.0 mm I.D.
Eluent : A) CH3CN B) H2O
A/B = 5/95
Flow Rate : 0.4 mL/min
Col. Temp : 40 ℃
Detection : UV 215 nm
Sample : 1.Methamidophos
2.Acephate

System Dead Volume

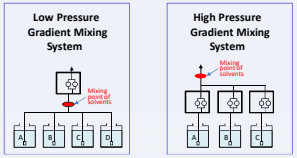
Tubing Connections from the Injector to Column

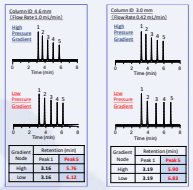
Influence of Dead Volume to the Peak Shape



Conditions
Column Size : InertSustain C18 3 μm, 150 x 2.1 mm I.D.
Eluent : A) CH3CN B) H2O
A/B = 50/50
Flow Rate : 0.2 mL/min
Col. Temp : 40 ℃
Detection : UV 254 nm
Sample : o,p-cresol

Gradient Delay

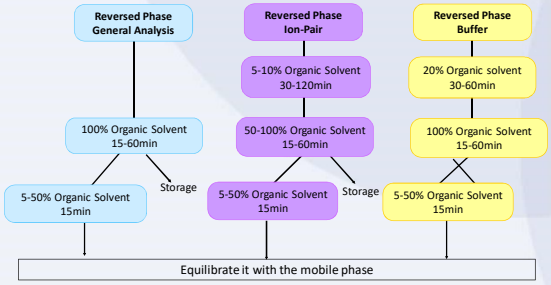




Conditions
Column Size : InertSustainSwift C18
Standard : 3 μm, 150 x 4.6 mm I.D.
Semi-micro : 3 μm, 150 x 3.0 mm I.D.
Eluent : A) CH3CN B) H2O
A/B = 50/50→6min→100/0, v/v
Col. Temp : 40 ℃
Detection : PDA 270 nm
Sample : o,p-cresol
Mixer Volume : Standard approx. 1.0 mL
Semi-micro approx. 0.4 mL
Sample : 1. 4-Methylphenol
2. 4-Ethylphenol
3. 4-Propylphenol
4. 4-Butylphenol
5. 4-Pentylphenol

General Method of Washing for Reversed Phase Column

The organic solvent can be either Acetonitrile or Methanol



●When the mobile phase does not contain any buffered mobile phases or ion-pairing reagents
Use high concentration organic solvent to remove the highly lipophilic contaminants. Increase the content of organic solvent up to 100%. Then, flush the column with 5 column volumes.
When observing excessive back pressure, reduce and adjust the flow rate.

Example Column Dimensions 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase acetonitrile/water = 65/35

Step 1 : Clean the column with 100 % acetonitrile at 1 mL/min for at least 30 minutes.

●When the mobile phase contain ion-pairing reagents
Depending on the ion-pairing reagent type, precipitation may occur when cleaning the column with 100 % water and extreme care is required. Clean the column with a water/organic solvent mixture, using the same content as in the mobile phase containing an ion-pairing reagent. For example, clean the column with 10% acetonitrile in water for at least 30 minutes. Then, clean it with water/acetonitrile = 50/50 for at least 30 minutes.
The content of organic solvent should be increased further when using ion-pairing reagents containing long alkyl chains to effectively remove out from the column.

Example Column Dimensions 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase 10 mM KH 2PO 4+ 2 mM *IPCC-09 (pH:2.5)/acetonitrile = 90/10

Step 1 : Clean the column with 10 % acetonitrile in water at 1 mL/min for at least 30 minutes.
Step 2 : Clean the column with acetonitrile/water = 50/50 for at least 30 minutes.
* IPCC-09 : Sodium 1-nonanesulfonate
* Please be aware that removal of 100% of the ion-pairing reagent may not be possible. Due to the fact that ion-pairing reagents can alter column selectivity, it is strongly recommended to dedicate columns to ion-pairing methods to avoid problems with reproducibility.

●When the mobile phase contain buffered mobile phases
Clean the column with a water/organic solvent mixture, using the same content as in th buffered mobile phase. For example, clean the column with 20% acetonitrile in water for at least 30 minutes. Then, clean it with 100 % acetonitrile.

Example Column Dimensions 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase 10 mM KH 2PO 4/acetonitrile =80/20
Step 1 : Clean the column with 20% acetonitrile in water at 1 mL/min for at least 30 minutes.
Step 2 : Clean the column with 100% acetonitrile at 1 mL/min for at least 30 minutes.
* When using the column again for the analysis, follow the procedures below to avoid precipitating mobile phase buffers on the column.
Step 1 : Equilibrate the column with 20% acetonitrile in water at 1 mL/min for at least 30 minutes.
Step 2 : Equilibrate the column with the buffered mobile phase to be used at 1 mL/min for at least 30 minutes.
Step 3 : The column may be considered fully equilibrated once a constant back pressure and stable baseline are observed.

General Method of Washing for Normal Phase Column

Normal-phase separations depend upon polar adsorptive interact ions, which the bonded phase is polar and the mobile phase is non-polar. Polar analytes will be more strongly retained than non-polar analytes in normal-phase chromatography. Clean the column with polar solvents to remove highly polar contaminants.

Hexane < Chloroform < Tetrahydrofuran < 2-Propanol < Ethanol
Weak solvents — Strong solvents

●Cleaning Inertsil SIL-100A, Inertsil SIL-150A, Inertsil WP 300 SIL, Inertsil NH2, Inertsil CN-3, Inertsil Diol, InertSustain NH2 Columns
Clean the column with ethanol or 2-propanol. Because alcohol solvents are quite viscous, adjust the flow rate to avoid excessive column back pressure.

Example Column Dimension 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase n-hexane/2-propanaol/acetic acid = 90 /10/0.1
Step 1 : Clean the column with 100 % 2-propanol at 0 .2 mL/min for at least 60 minutes.

General Method of Washing for HILIC Column

Under HILIC mode, polar analytes are retained with high organic mobile phases. The following describes the elution strength of solvents used in HILIC mode.

Tetrahydrofuran < Acetonitrile < 2-Propanol < Ethanol < Methanol < Water
Weak solvents — Strong solvents

●Cleaning InertSustain Amide Columns
To avoid precipitating mobile phases buffers within the column, clean the column with a water/organic solvent mixture, using the same content as in the buffered mobile phase. Clean the column with acetonitrile/water = 50/50 to remove highly polar contaminants.
If the column still shows shift in retention time or distorted peak shapes, clean the column with 100 % water for at least 30 minutes. After cleaning the column, make sure to thoroughly equilibrate the column with the mobile phase to be used in the analysis prior to use. Store the InertSustain Amide column in 100% acetonitrile.

Example Column Dimensions 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase 5 mM CH3COONH 4/acetonitrile = 10/90
Step 1 : Clean the column with acetonitrile/water = 90/10 at 1 mL/min for at least 30 minutes.
Step 2 : Clean the column with acetonitrile/water = 50/50 at 1 mL/min for at least 30 minutes.

●Cleaning Inertsil HILIC Columns
To avoid precipitating mobile phases buffers within the column, clean the column with a water/organic solvent mixture, using the same content as in the buffered mobile phase. Clean the column with 100 % water to remove highly polar contaminants.
Example Column Dimensions 4.6 mm I.D. x 250 mm

Method Flow Rate 1 mL/min
Method Mobile Phase 5 mM CH3COONH 4/acetonitrile = 10/90
Step 1 : Clean the column with acetonitrile/water = 90/10 at 1 mL/min for at least 30 minutes.
Step 2 : Clean the column with 100 % water in water at 1 mL/min for at least 30 minutes.

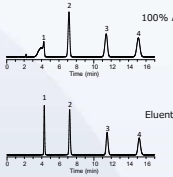
●Cleaning InertSustain NH2 Columns
To avoid precipitating mobile phases buffers within the column, clean the column with a water/organic solvent mixture, using the same content as in the buffered mobile phase. Clean the column with acetonitrile/water = 50/50 to remove highly polar contaminants.
If the column still shows shift in retention time or distorted peak shapes, clean the column with 50 mM ammonium formate (or ammonium acetate) aqueous solution/acetonitrile = 50/50 for at least 30 minutes. After cleaning the column, make sure t o thoroughly equilibrate the column with the mobile phase to be used in the analysis prior to use. Store the InertSustain NH2 column in 100% acetonitrile.

Example Column Dimensions 4.6 mm I.D. x 250 mm
Method Flow Rate 1 mL/min
Method Mobile Phase 5 mM CH3COONH4/acetonitrile = 10/90
Step 1 : Clean the column with acetonitrile/water = 90/10 at 1 mL/min for at least 30 minutes.
Step 2 : Clean the column with acetonitrile/water = 50/50 at 1 mL/min for at least 30 minutes.

Sample Diluent

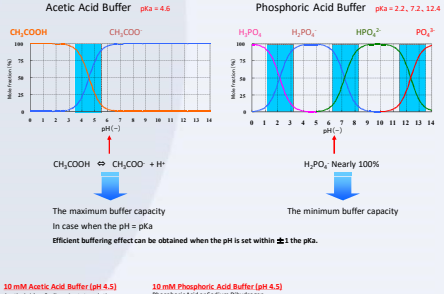
The effect of Sample Diluent

The sample was dissolved using...



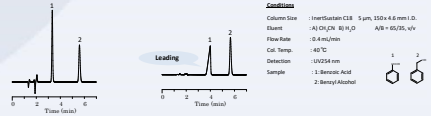
Conditions
Column Size : InertSustain AQ-C18 5μm, 150 x 4.6 mm I.D.
Eluent : A) CH3CN B) H2O
A/B = 10/90
Flow Rate : 1.0 mL/min
Col. Temp : 40 ℃
Detection : UV 280 nm
Sample : 1. 5-Hydroxymethyl-2-furaldehyde
2. 2-Furfural
3. 2-Acetylfuran
4. 5-Methyl-2-furfural
Injection : 10 μL

The Buffering Effect



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

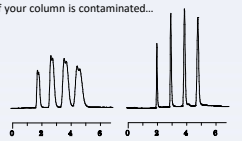
10 mM Phosphoric Acid Buffer (pH 4.5)
Phosphoric Acid or Sodium Dihydrogen Phosphate were prepared to 10 mM each and adjusted the pH at 4.5



Conditions
Column Size : InertSustain C18 5 μm, 150 x 4.6 mm I.D.
Eluent : A) CH3CN B) H2O 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 : InertsilODS-3 3 μm, 100 x 3.0 mm I.D.
Eluent : A) CH3CN B) H2O 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

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