

## Inertsil Column Check upon receipt of Column

**Step 1 :** Confirm Genuine supply of Inertsil C8-3 /Inertsil ODS-3 & Inertsil ODS-3V by checking **LASER ETCHED** serial number on the column.



**Step 2 :** Check the column for signs of physical damage which may have occurred during shipping. Contact **LCGC** immediately to report any problems.

**Step 3 :** Make sure that the column you received is the column that you ordered.

**Step 4 :** Take note of the solvent contained in the column during shipping solvent. (The solvent used for shipping is the same as that used as the mobile phase on the QC test chromatogram ). Before attempting to change solvents, make certain that the eluent you will be introducing into the column is **COMPLETELY** miscible with the eluent contained in the column, to avoid precipitation of buffer salts or other mobile phase additives.

**Step 5 :** Test the column to verify column efficiency and back pressure (using one of the later-eluting components of the QC test sample).Contacts **LCGC** immediately to report any problems. This is important because the **MOST COMMON** complaint about HPLC columns is high back pressure. Given that the columns are all tested during QA/QC, high back pressure is almost **ALWAYS** the result of particulates introduced during "equilibration" or the first few sample injections.

## In-Situ Column Cleaning

Columns that become fouled over time can sometimes be rejuvenated with an aggressive rinsing sequence, as shown below. In all cases, reverse the column (e.g. attach the outlet end of the column to the pump, and pump the eluent directly into a waste reservoir) and flush the column with 50ml volumes of the indicated solvents in the indicated sequences.

*\* If mobile phase contains a buffer, flush the column with the mobile phase MINUS the buffer first, to avoid precipitation of the buffer in the pure MeOH remaining in the column.*

**In-Situ Column Cleaning (Recommended Column Temperature is at 40C)**

Steps	Reverse Phase	HILIC Phase	Normal Phase	IonExchange Phase	Protein Removal
1	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90	CH <sub>3</sub> CN / 0.1% TFA = 10/90	**Isopropanol	***High concentration buffer	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90
2	100% CH <sub>3</sub> CN or 100% CH <sub>3</sub> OH	0.1% TFA in 100% CH <sub>3</sub> CN	Methylene Chloride	Distilled Water	0.1% TFA in 100% CH <sub>3</sub> CN
3	*(0.1% TFA in 100% CH <sub>3</sub> CN)	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90	Hexane	100% CH <sub>3</sub> CN or 100% CH <sub>3</sub> OH	**Isopropanol
4	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90	Mobile Phase	Mobile Phase	Distilled Water	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90
5	Mobile Phase			Mobile Phase	Mobile Phase

\* In case the column is heavily deteriorated.

\*\* Please be careful with the column back pressure.

\*\*\* Use the same buffer used in the mobile phase, but with higher concentration. However, the limit of the concentration shall be upto 100mM

## Appendix 1 -1

**Inertsil Series Shipping Solvent Information (Mobile Phase used in Column Performance Test)**

As of May, 2011

**Note:**

\* The injection volume of standard sample used in the Column Performance Test is all 1  $\mu$ L for a 4.6mm I.D. column.

\* For other column internal diameter sizes, injection volumes or concentration of standard samples were adjusted proportional to column cross section

Packings Inertsil	Column Size	Mobile Phase used in Column Performance Test	Analyte used in Column Performance Test	Sample Diluting Solvent
ODS-2 ODS-3	5um ;150 x 4.6mm I.D. 5um ;250 x 4.6mm I.D.	CH <sub>3</sub> OH / H <sub>2</sub> O = 85 / 15	n-Propylbenzene (0.01 mg/mL)	CH <sub>3</sub> OH / H <sub>2</sub> O = 85/15
ODS-4	ODS-SP; 20mm,30mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 50 / 50		
ODS-SP	other than those above	CH <sub>3</sub> CN / H <sub>2</sub> O = 65 / 35	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
InertSustain C18 ODS-3V ODS-4V ODS-P ODS-EP Peptides C18 Sulfa C18 ODS-80A ODS PREP ODS PREP C8	ALL	CH <sub>3</sub> CN / H <sub>2</sub> O = 65 / 35	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
C8-3	$\geq$ 100mm < 100mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 65 / 35 CH <sub>3</sub> CN / H <sub>2</sub> O = 50 / 50	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
C8-4	$\geq$ 75mm < 75mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 65 / 35 CH <sub>3</sub> CN / H <sub>2</sub> O = 50 / 50	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
C8	$\geq$ 200mm < 200mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 65 / 35 CH <sub>3</sub> CN / H <sub>2</sub> O = 50 / 50	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35

## Things to Follow –For Better Life of column

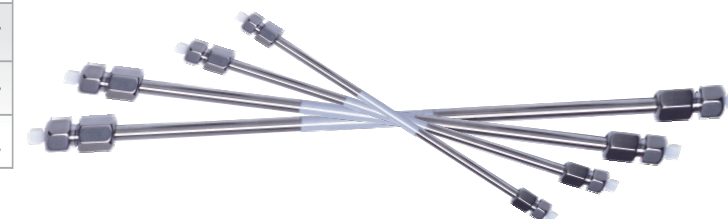
✓ **Filter Sample :** Samples should be filtered through a 0.45 $\mu$  for columns having particle size 3 $\mu$ m & above. Sample must be filtered through 0.2 $\mu$  syringe filter while using with 2 $\mu$ m (UPLC ) Use of Guard column will improve life of the column.

✓ **Filter Solvent & Buffers :** Organic solvents & buffer used in the mobile phase should be filtered through 0.45 $\mu$  for the columns having particle size 3 $\mu$ m & above . Solvents & buffers must be filtered through 0.2 $\mu$  syringe filter while using with 2 $\mu$ m (UPLC ) columns.

✓ Use of Guard column will improve life of the column.

✓ Wash column thoroughly after use to remove buffers. [ Ion apir buffers requires longer washing time compare to other inorganic buffers ]

✓ Store the column in appropriate storage solvents



## pH Stability

### Solvent Selection

Columns last longest when they are used with benign eluents. Using eluents of high pH or low pH can dissolve silica or catalyze hydrolysis of the bonded phase. Try to stay within the pH range of 2 - 7.5 on Inertsil columns except Inertsil Sustain C18/C8/Phenyl (Which is stable within pH range 1-10.5). If you use a pH outside this range, column life might be reduced

### Temperature Stability

**Column Oven Temperature :**

Columns last longest when they are used with Temp of below 60 C

## Inertsil Column Storage

COLUMN STORAGE conditions can have a profound effect on column lifetime and performance -after-storage. Before extended storage (e.g. greater than 2 days), rinse the column **COMPLETELY** free of eluents containing buffers, ion-pair reagents, or inorganic solutes, by flushing with 20-50 column volumes of the eluent **WITHOUT** the dissolved additives. Then flush the column with 5-10 column volumes of water (reverse phase columns only). Then flush the column with 20 volumes of storage solvent .

### INERTSIL® HPLC Column Storage Instructions

Inertsil Phase	Short term Storage Solvent (Within a Month)	Long term Storage Solvent (More than a Month)	Shipping Solvent
Reverse Phase C18, C8, C4, Ph-3, Ph...	Same Organic % as Mobile Phase without additives	100% CH <sub>3</sub> OH or 100% CH <sub>3</sub> CN	See Appendix 1-1 &
HILIC Phase Hilic (Diol), NH2, Amide...			
Normal Phase SIL, Diol, NH2, CN...		100% Hexane	
Ionexchange Phase CX, AX, NH2...	CH <sub>3</sub> CN / H <sub>2</sub> O = 10/90	100% CH <sub>3</sub> OH or 100% CH <sub>3</sub> CN	Appendix 1-2

## Appendix 1 -2

Packings	Column Size	Mobile Phase used in Column Performance Test	Analyte used in Column Performance Test	Sample Diluting Solvent
Ph-3 Ph C4 WP 300C18 WP 300C8 WP 300C4 300C8	ALL	CH <sub>3</sub> CN / H <sub>2</sub> O = 50 / 50	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
SIL-100A SIL-150A Diol WP 300SIL WP 300Diol PREP SIL	ALL	Hexane / Etanol = 95 / 5	o-nitroanisole (0.07 v/v %)	n-Hexane 100%
CN-3 NH2	ALL	Hexane / Etanol = 98 / 2	o-nitroanisole (0.07 v/v %)	n-Hexane 100%
HILIC	150mm , 250mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 95 / 5	Caffeine (0.2 mg/mL)	CH <sub>3</sub> CN 100%
	100mm , 75mm	CH <sub>3</sub> CN / H <sub>2</sub> O = 98 / 2		
	$\leq$ 50mm	CH <sub>3</sub> CN 100%		
AX	ALL	60mM KH <sub>2</sub> PO <sub>4</sub> (pH 3.0 , H <sub>3</sub> PO <sub>4</sub> )	UMP (0.1 w/v %)	H <sub>2</sub> O
CX	ALL	200mM HCOONH <sub>4</sub> (pH 5.0 , HCOOH)	Cytosine (0.15 mg/mL)	H <sub>2</sub> O
MonoClad C18- HS	ALL	CH <sub>3</sub> CN / H <sub>2</sub> O = 60 / 40	Naphthalene (1 mg/mL)	CH <sub>3</sub> CN / H <sub>2</sub> O = 65/35
Inertsphere Sugar-1	150 x 4.6 mm I.D.	100mM NaOH	Glucose (10 $\mu$ g/mL) with ECD Detector	2% Ethanol aq.

## Things to Avoid –For Better Life of column

✓ Dropping or otherwise "shocking" columns can disrupt the column bed and cause peak splitting.


✓ Use of eluents in the pH range of conventional LC Columns 2 - 7.5 will maximize column life, though higher pH eluents have been used successfully.

✓ Opening the column

✓ Using Extreme temperatures with extreme pH\*.

✓ Using corrosive buffers /mobile phase

✓ Inertsustain C18 is stable for order pH range of 1-10.5

 The lower the operating pressure, the longer the operating life. Though higher pressures are possible, the lower the operating pressure, typically, the longer the operating life of the column.

**\*Inertsil Columns offers lower pressure columns in the market**