InertSustain / Inertsil Series Care and Use Manual

Thank you for purchasing an InertSustain and Inertsil series column.

InertSustain and Inertsil series packing materials are subjected to a rigorous array of QC test in our ISO9001 compliant facility, with special emphasis on reagent purity, consistency and traceability in raw materials, and final products. A detailed analysis of the physical and chemical properties of InertSustain and Inertsil series columns, combined with tests for chromatographic selectivity and column packing material efficiency, ensure that each lot of InertSustain and Inertsil series columns are identical to all previous lots, and column-to column reproducibility is of the highest order.

To maintain and maximize peak performance of InertSustain and Inertsil series columns, and to ensure long life and stability of your column please read the following instructions before use.

(1) Checking contents

- ○ Check if anything is missing or damaged. If there are any signs of damage, notify your local GL representative at once.
- ○ Each InertSustain and Inertsil series column includes a Column Performance Report. The information included in the report is the lot number, column serial number, and chromatographic test conditions. Please retain this report for future reference.
- ○ InertSustain and Inertsil series columns are shipped in the solvent used for the final QC test of the column, as detailed in the Column Performance Report provided with the column.

(2) Specifications

SeparationType	Column	Shipping Solvent
Normal-Phase	InertSustain NH2 Inertsil SIL-100A, CN-3, NH2, Diol etc.	*n*-Hexane : Ethanol
Reversed-Phase	InertSustain C18, AQ-C18, Phenyl, PFP, Cyano etc. Inertsil ODS-4, ODS-3, C8-3, Ph-3 etc.	Water : Acetonitrile
HILIC-phase	InertSustain Amide Inertsil HILIC, Amide etc.	Water : Acetonitrile
	InertSustain NH2 with 100% CH_3CN etc.	Acetonitrile 100 %
Ion- exchange	Inertsil CX, AX etc.	Methanol 100%

(3) Handling

- ○ Do not drop or knock the column, to avoid a deterioration of the column performance.
- ○ To maximize column life, do not use the column at pressures greater than shown in the following table.

Particle Size	Column Dimensions	Recommended Operating Pressure
1.9 µm, 2 µm	All sizes	Under 80 MPa
HP 3 µm	All sizes	Under 50 MPa
3 µm, 5 µm	All sizes	Under 20 MPa

- ○ Avoid rapid pressure fluctuations.
  - ・ Make sure the column is at zero pressure before disconnecting it from the system.
- ○ Samples should be dissolved in the eluent or a solvent weaker than the eluent, which helps avoid sample precipitation at the column inlet/head and inconsistent retention values.
- ○ When switching between solvents with vastly different polarities, first purge the column with a mutually miscible solvent such as Isopropyl alcohol at a reduced flow rate (approximately 50% lower than the normal flow rate). Flush the column with at least five times the column volume (e.g. 10 mL for a 150 mm x 4.6 mm I.D. column).
- ○ If peaks tail more for early eluting compounds than later eluting compounds, there is a possibility that there is a dead volume. In this case, check all column connections are properly connected.
  Also, use appropriate internal diameter and length tubing between the injector and detector, especially when using semi-micro columns to avoid system dead volumes.
- ○ The most common cause of increased column back pressure or double peaks is blockage of the inlet filter by sample particulates, particles caused by aging pump seals, or large quantities of lipophilic compounds adsorbing onto the head of the column.
  - ・ Make sure the mobile phase is filtered using a 0.45 µm membrane filter before using the column.
  - ・ Make sure the sample is filtered using a “GL Chromatodisc” (syringe filter) before injecting onto the column.
  - ・ Installing a “Guard Column for UHPLC” or “Cartridge Guard Column E” can prevent column clogging problems.
- ○ Prior to the first injection, fully equilibrate the column with initial mobile phase conditions. Failure to do so will result in drifting retention times.
  (Columns used in HILIC mode or with ion-pair reagents present in mobile phases in reversed phased mode separations must be equilibrated thoroughly to avoid drift in retention times)
- ○ For maximum operating temperature and pH limits, please refer to the following table.
  (For pH range and maximum operating temperature information about columns not described in the table below, please visit the GL Sciences website)

Column	pH Range (From 20-40℃)	Maximum Operating Temperature	
InertSustain C18, Swift C18, Bio C18, AQ-C18	1-10 ※1,2,3	60℃ (pH 1-7)	50℃ (pH 1-10)
InertSustain C8, Swift C8, Phenylhexyl	1-10 ※1,2,3	60℃ (pH 2-7)	50℃ (pH 1-9)
InertSustain Amide	2-8.5 ※2	60℃ (pH 2-7)	50℃ (pH 2-8.5)
InertSustain Phenyl, NH2 , Cyano, PFP	2-7.5 ※2	60℃ (pH 2-7)	50℃ (pH 2-7.5)
Inertsil series	2-7.5 ※2	60℃ (pH 2-7)	50℃ (pH 2-7.5)

※1 If the column is used with eluents at pH1 - pH2 or pH9 - pH10, we recommend that they should be used at lower column temperature and with an eluent containing trifluoroacetic acid, formic acid, acetic acid, phosphate buffer or organic buffer, such as 5mM triethylamine. And if the column is used without organic solvent, it is recommended that the column is used within the pH range pH2 - pH8.

※2 To maximize the column lifetime, set the pH of the mobile phase within the range shown in the above table.

※3 To maximize the column lifetime, use a lower column temperature when the pH of the mobile phase is in the range 1～2 or 9～10. In addition, use a mixed mobile phase such as organic solvent and buffer rather than 100% buffer.

(4) Storing of Columns

- ○ After using reversed-phase columns with an eluent containing buffer or ion-pair reagent, wash the column thoroughly with a salt-free eluent before storing.
- ○ When storing reversed-phase columns, condition with 100% organic solvent such as acetonitrile or methanol.
- ○ After using a reversed-phase eluent with an NH2 column, wash the column with a mixed solvent of water/acetonitrile (50/50).
  Before storing reversed phase columns, replace the mobile phase with 100% acetonitrile.
- ○ For washing normal-phase columns, use ethanol or 2-propanol. In general, using alcohol eluents tends to increase the column backpressure.
  In this case, adjust the flow rate according to the maximum operating pressure.
  Before storing normal-phase columns, replace the mobile phase with 100% hexane.
- ○ After using HILIC columns with eluents containing buffer salts, replace the mobile phase with an eluent with high water content (50% water) in order to remove any hydrophilic substances, then replace the mobile phase with an eluent with high acetonitrile content (80% acetonitrile or higher) for storing the column.
- ○ When using Inertsil Amide columns, do not use any mobile phase containing less than 50% organic solvent.
- ○ Inertsil CX and Inertsil AX columns are shipped conditioned with 100% methanol. When using an elution buffer, replace the methanol mobile phase with pure water before use. After using a buffer mobile phase, wash the column with pure water, before storing in100% methanol.
- ○ When storing a column, close it with the included plugs and store it in a cool place with little temperature fluctuation.
- ○ The connecting parts of both UHPLC PEEK and regular PEEK columns are made from resin, making them extremely delicate and easy to damage. When connecting these columns to the system, make sure that PEEK compression fittings are used to prevent any damage to the connections.
- ○ The long term use of THF or chloroform with a UHPLC PEEK or regular PEEK column may cause early deterioration.

InertSustain and Inertsil series columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact your GL Sciences' supplier, who will ensure you complete satisfaction. We regret that GL Sciences cannot guarantee the lifetime of columns, nor can we accept any claim when their performance has deteriorated due to non-compliance with the above operating manual or as a result of normal aging.

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