SHIMADZU
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ASPM-0037

High Performance Packed Column for HPLC

Shim-pack

Bio-IEX Series

INSTRUCTION MANUAL

■ Introduction

To maintain and maximize peak performance of Shim-pack Bio-IEX series columns, and to ensure the long life and stability of columns, please read the following instructions before use.

■ Specifications

The product specifications of this product are as follows.

Item	Shim-pack Bio IEX Q/Bio IEX SP		
Base material	Porous hydrophilic polymer		
Ion exchange group	- $CH_2N^+(CH_3)_3$ / -$CH_2CH_2CH_2SO_3^-$		
counter ion	Cl^- / Na^+		
Particle size (μm)	5		
Column sizes Length× ID (mm)	30×4.6	50×4.6	100×4.6
Recommended Flow rate (mL/min)	0.5-0.8	0.5-0.7	0.4-0.5
Max. Flow rate (mL/min)	1.0	0.8	0.6
Max. pressure (MPa)	2.5	3.0	3.5
Range of pH	2.0 - 12.0		
Temperature Range	4 - 60℃		
Column material	PEEK		

Item	Shim-pack Bio IEX Q-NP/Bio IEX SP-NP					
Base material	Non-porous hydrophilic polymer					
Ion exchange group	- $CH_2N^+(CH_3)_3$ / -$CH_2CH_2CH_2SO_3^-$					
counter ion	Cl^- / Na^+					
Particle size (μm)	5			3		
Column sizes Length× ID (mm)	30×4.6	50×4.6	100×4.6	30×4.6	50×4.6	100×4.6
Recommended Flow rate (mL/min)	1.0-1.5	1.0-1.2	0.2-0.8	0.7-1.0	0.5-1.0	0.2-0.5
Max. Flow rate (mL/min)	1.8	1.5	1.0	1.3	1.0	0.6
Max. pressure (MPa)	6.0	10.0	12.0	25.0		
Range of pH	2.0 - 12.0					
Temperature Range	4 - 60℃					
Column material	PEEK					

■ Operating Precautions

Check if anything is missing or damaged. If there are any signs of damage, notify your local Shimadzu representative at once.

Each of the Shim-pack Bio-IEX series columns is delivered with a Column Performance Report. The information supplied in the report include the column serial number, and chromatographic test conditions. Please keep the report for future reference.

■ Column performance

The Shim-pack Bio-IEX series have stable quality products for customers by QC tests. Shim-pack Scepter series columns are shipped with the solvent used for the final QC test of the column, as detailed in the Column Performance Report delivered with the column.

When switching between solvents with significantly, please take care of different polarities, the miscibility and precipitation of salts.

■ Column Installation

The flow direction of the column is shown on the column (→). When installing the column, ensure that the flow direction matches the mobile phase flow direction.

The presence of voids in the tubing connect part may cause leakage and result in poor column performance (theoretical plate, peak symmetry). Take care of the ferrule tip length or cut surface of the tubing to avoid creating a void.

Use the shortest possible tubing connection from the injector to the column to minimize peak broadening.

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The column should be connected with male nuts. Ensure that the fittings are connected properly to avoid creating dead volume between the tubing and the column interface. Male nuts can be ordered by referring to the part number below.

Item name	P/N	Remarks	Pressure
Male nut, PEEK	228-18565-84	5 pcs	20 MPa
Male nut 1.6 MN	228-16001	1 pc	130 MPa
Ferrule 1.6 F	228-16000-10	1 pc	130 MPa
UHPLC Fitting 2 S	228-56867-41	1 pc	130 MPa
Nexlock fittings	228-62544-90	1 pc	130 MPa

NOTE Stains or air in the flow line may deteriorate the column. Before connecting the column, be sure to flow the mobile phase to flush the flow line.

If peaks are tailing more on the early eluting compounds than later eluting compounds, there is a possibility that there is a dead volume. In such case, check that all column connections are properly connected.

Also, make sure to use appropriate internal diameter and length size of tubing at the injector and detector, especially when using semi-micro size columns, to avoid system dead volumes.

■ Sample

Samples should be dissolved in an eluent or solvent weaker than the mobile phase, which helps avoid sample precipitation at column inlet/head and inconsistent retention values.

In order to prevent the precipitation of salts contained in sample or solvent, check the miscibility of these with mobile phase before injection.

■ Clogging of column

The most common cause of the increase of column back pressure or split peaks is blockage of the inlet filter by sample particulates, or large quantities of lipophilic compounds adsorbing to the head of the column.

- Filtrate the mobile phase using a 0.45 μm membrane filter before using the column.
- Filtrate the sample using a syringe filter before injecting to the column.

■ Column Handling Precautions

Do not drop or bump the columns, to avoid a deterioration of the column performance. Please use below the maximum flow rate and maximum pressure shown in "Specification".

Avoiding using a column repeatedly near the pressure limit or sudden change in pressure, which may cause shortening of in the column life.

Column should be disconnected from the system after the pressure drop to "0".

Please note that operating the sample injection valve slowly or using an auto-sampler with slow valve switching speed will also generate a rapid pressure increase at the column inlet, which will cause premature column deterioration.

Generally, the target sample is adsorbed to column with 20 to 50 mM of buffer solution as the primary mobile phase, and eluted by salt concentration gradient (NaCl concentration is generally increased to about 0 to 0.5 M by gradient), or pH gradient. It is recommended that you wash the column with a buffer solution containing 1 M NaCl aq. for each run to remove impurities left on the column that are not eluted in final mobile phase.

Water-soluble organic solvent can be added to mobile phase up to about 30%. Check that the salts in the buffer solution do not precipitation prior to adding. In addition, ureas (≦8 M), guanidine hydrochloride (≦6 M), nonionic surfactants, cationic surfactants (Shim-pack Bio IEX Q/Q-NP only), and anionic surfactants (Shim-pack Bio IEX SP/SP-NP only) can be used as denaturants for proteins.

Do not use a solvent containing an oxidant as a mobile phase.

■ Column storage

Replace the mobile phase to the shipping fill solvent for long-term storage. Within one week, you can store with analytical mobile phase after high concentration salts can be removed.

The shipping solvent of column is shown below.

Shim pack Bio IEX Q/Q-NP:20 mM Tris-HCl buffer (pH 8.1)

Shim pack Bio IEX SP/SP-NP :20 mM sodium phosphate buffer (pH 6.8)

■ Washing the column

Lipid-soluble substances and small soluble substances may adsorb to column, resulting in changes in retention time and peak shapes and pressure. In these cases, washing the column by following procedures. If these rinse do not restore column performance, we recommend that you use the new column.

First, replace column with shipping fill solvent. Next, inject the rinse solvents 4~5 mL respectively of following solvent ((1) to (4)) with injector sending the shipping solvent (sample loop is conveniently larger such as ≧2 mL).

Rinse solvents

(1) 0.2 N NaOH aqueous solution /acetonitrile (80/20)

(2) 1 M acetic acid aqueous solution

(3) Mobile phase with added nonionic surfactant

(4) Mobile phase with 6 M guanidine hydrochloride

After cleaning by each rinse solvent injection, please check whether the retention time and peak shapes are recovering.

■ Technical Support

Shim-pack Bio-IEX Q/SP series columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact your local Shimadzu representative, who will ensure your complete satisfaction.

We regret that we cannot guarantee the lifetime of columns, also that we cannot accept any claim when performance has deteriorated due to noncompliance with the operation procedures elucidated above, or as a result of normal aging.