## Support

Heparin-5PW was prepared by introducing heparin into G5000PW.
Amount of immobilized ligand : 4-6 mg/ml
Particle size : $10 \mu \mathrm{~m}$
Pore size : ca. $1000 \AA$

## Column

Size : $75 \times 7.5 \mathrm{~mm}$ I.D.
Solvent : distilled water

## pH range for separation

5-10

## Salt concentration range

less than 3 M

## Flow rate range

less than $1.2 \mathrm{ml} / \mathrm{min}$
Flow rates of $0.5-1.0 \mathrm{ml} / \mathrm{min}$ are recommended in general.

## Column washing

$0.1-0.2 \mathrm{~N} \mathrm{NaOH}$ are very effective to wash or regenerate columns.
Usually, columns can be regenerated by injecting $0.1-0.2 \mathrm{~N} \mathrm{NaOH}$ of $1-2 \mathrm{ml}$ several times using sample injector. When this procedure did not help. wash the column by injecting 20-40\% acetic acid of $1-2 \mathrm{ml}$ several times.

## Guard column

The use of guard column ( $10 \times 6 \mathrm{~mm}$ I.D.) is recommended for a long service life of analytical columns.
The change of both inlet side filter and support once a week is recommended for every day use.
The guard column can be packed by pouring the concentrated slurry of Heparin-5PW guard gel into it and then sucking with aspirator.
The old filter can be re-used after washing by exposing it to ultrasonic in $0.1-0.2 \mathrm{~N} \mathrm{NaOH}$ for about 30 min .

## Heparin-5PW guard gel

Support prepared by introducing heparin into G5000PW of 20-30 $\mu \mathrm{m}$ in particle diameter for use in guard column

## Storage

When Heparin-5PW columns are stored for more than several days, replace the solvent in columns with distilled water.
Store the columns at around $4^{\circ} \mathrm{C}$.

## Test for theoretical plate number

Following conditions are recommended to test columns for their theoretical plate numbers and As (asymmetry factor).

Eluent : 10 mM sodium acetate buffer ( pH 5.0 )*
Flow rate : $1.0 \mathrm{ml} / \mathrm{min}$
Sample : cytidine ( $0.05 \%, 20 \mu \mathrm{l}$ )
Heparin-5PW columns have theoretical plate numbers more than 1,300 plates per column and As of 0.8-1.6 at a time of delivery.

* Prepare 10 mM sodium acetate aqueous solution and 10 mM acetic acid aqueous solution.
Add the two aqueous solutions properly so that the pH of the mixture becomes 5.0.

