Asahipak® HPLC Column ES Series

Instructions on Handling and Use

Effective from February 1989

Be sure to read these instructions
before using the Asahipak column,
for optimum performance and service life

ASAHI CHEMICAL INDUSTRY CO.,LTD.

Contents

1. Introduction 1
2. Column components 1
3. Specifications 1
4. Column installation 2
5. Eluent and sample pretreatment 3
6. Compatible solvents 3
7. Flow rates 4
8. Operating temperatures 4
9. Column cleaning 4
10. Column handling and storage 4
11. Optimum analytical conditions 5
12. Measurement of NTP 5

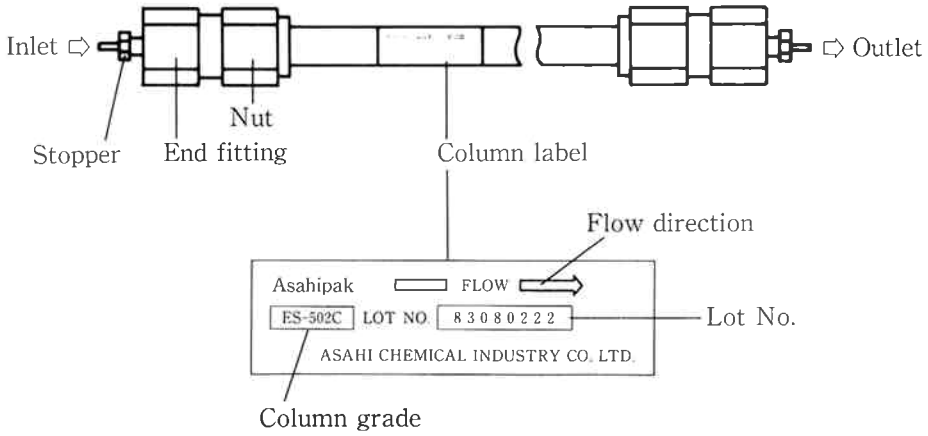
1. Introduction

The Asahipak ES series of HPLC columns, packed with porous gel particles composed of vinyl alcohol polymers with hydroxyl groups partially replaced by ion-exchange groups, are designed for high-speed ion-exchange chromatography.

They are particularly effective for analysis of proteins, peptides, and nucleic acid components, as the gel polymers themselves are hydrophilic and the gel is thus relatively free from hydrophobic adsorption.

It is important to read and observe the following instructions on their use and handling, for long service life with optimum performance.

2. Column components



3. Specifications

Dimensions : As in tables below
Connecting screws : Swagelock type (inch)
Column body : SUS316
Shipping charge : Solution described in INSPECTION DATA enclosed with column

3-1 Analytical grades

Standard-Size 7.6 mmID, 100 mmL

Grade	Particle size (μm)	Ion exchange group	Exchange capacity(meq/g)	NTP (per 100mm)	Flow rate (ml/min)		pH range
					Normal	Max	
ES-502C	9	$-COOH$	0.55±0.02	>3,000	1.0	1.5	2 ~12
ES-502N	9	$-N(C_2H_5)_2$	0.55±0.02	>3,000	1.0	1.5	2 ~12

3-2 Preparative grades

Standard size 21.5 mmID, 100 mmL

Grade	Particle size (μm)	Ion exchange group	Exchange capacity(meq/g)	NTP (per 100mm)	Flow rate (ml/min)		pH range
					Normal	Max	
ES-502CP	13	$-COOH$	0.55±0.02	2,500	4 ~ 6	8	2 ~12
ES-502NP	13	$-N(C_2H_5)_2$	0.55±0.02	2,500	4 ~ 6	8	2 ~12

3-3. Guard column for preparative columns

GS-20G, 7.6 mmID, 50 mmL

4. Column installation

Summary checklist.

As explained below, observation of the following installation conditions is important to effective stable performance.

Flow line, minimum dead volume	Inner diameter of 0.2−0.3 mm (anal. column) or 0.2−0.5 mm (prep. column) in injector-column and column-detector tubing. No use of inter-tube unions ; if unavoidable, then only low-dead-volume type. Shortest practicable tube length.
Pump	Selection of pump with minimum pulsation ; damper installation at pump outlet preferable.
Pre-connection line flushing	Eluent, fed through 0.5 μm filter.
Column connection and flushing	Prevention of air entry into column; warming of column to expand charged solution. Eluent, flow rate of 0.5 ml/min for prep. column or up to normal operating rate for anal. column. Start pump, connect column inlet then outlet, continue flushing for 60 min with eluent.

4-1. Flow line

It is important to use flow lines which provide the smallest possible dead volume and fluid diffusion. For analytical columns, use tubes 0.2−0.3 mm in inner diameter both from injector to column inlet and from column outlet to detector. The tubes should be as short as possible. Avoid the use of a tube union (inter-tube coupling) in the flow line, as it may cause sample diffusion. If inter-tube coupling is unavoidable, use only a low-dead-volume union.

The same consideration is necessary for preparative columns, even though they are less subject to the effects of flow line diffusion. Tubes up to 0.5 mm in innner diameter may be used, but utilization of analytical column lines is recommended wherever practicable.

4-2. Pump

Use a pump which provides minimum output flow pulsation. Strong pulsation will result in reduced resolution and shortened column life. Pulsation may be effectively reduced by using a damper at the pump outlet.

4-3. Preliminary purging of flow line

Before connecting the column in the LC system, completely purge the LC system flow line with filtered eluent. The total salt concentration in the eluent should generally be 20 to 600 mM. Do not use pure water alone for this purpose, as it would result in column degradation.

4-4. Connection of column

Remove the column inlet stopper, warm the column e.g. by rubbing the outside surface with a dry cloth for about 30 seconds until the shipping charge solution expands and begins to emerge from the inlet end fitting, and then remove the column outlet stopper. (This solution expansion may be omitted for preparative columns.)

Set the feed rate at 0.5 ml/min for the analytical column or 3.0 ml/min for the preparative column and start the pump. As eluent flows from the input line, connect it to the column inlet. After several drops of the shipping charge solution emerge from the outlet end fitting, connect the column outlet to the detector. Continue the eluent feed for about 60 minutes, to achieve complete purging of the shipping charge solution from the column.

5. Eluent and sample pretreatment

5-1. Filtering

Always filter (0.5 μm or smaller mesh) the eluent and the sample solution before column injection, as they may contain invisible undissolved particles which would cause column degradation or chromatographic "noise".

5-2 Degassing

Always thoroughly degas the eluent before column injection, most simply by stirring with ultrasonic wave or stirrer under reduced pressure. Commercial degassers may be conveniently employed.

6. Compatible solvents

6-1. Type and salt concentration

Total salt concentration should generally be 20−600 mM. Do not use pure water alone, as it would result in column degradation. Buffer solutions generally used in ion-exchange chromatography with the ES column are of the types and concentrations shown in the following tables.

ES-502C, ES-502CP

pH range	Buffer solution
3.8~4.3	Sodium formate
4.3~4.8	Sodium succinate
4.8~5.2	Sodium acetate
5.0~6.0	Sodium malonate
5.5~6.7	MES[1]
6.7~7.6	Sodium phosphate
7.6~8.2	HEPES[2]
8.2~8.7	BICINE[3]

ES-502N, ES-502NP

pH range	Buffer solution
4.8~5.0	N-methylpiperadine · HCl
5.0~6.0	Piperadine · HCl
5.8~6.4	Bis · tris · HCl[4]
6.4~7.3	Bis · tris · propane · HCl[5]
7.3~7.7	Triethanolamine · HCl
7.5~8.0	Tris · HCl
8.0~8.5	N-methyldiethanolamine · HCl
8.4~8.8	Diethanolamine · HCl
8.5~9.0	1,3-Diaminopropane · HCl
9.0~9.5	Ethanolamine · HCl

Salt solutions such as NaCl, KCl, Na_2SO_4, and K_2SO_4 may also be used either indepedently or in combination with these buffer solutions.

1) 2-(N-Morpholino)ethanesulfonic acid.
2) N-2-Hydroxyethylpiperadine-N-2-ethanesulphoric acid.
3) N, N-Bis(2-hydroxyl)glycine.
4) 2-Bis(2-hydroxylethyl)amino-2-(hydroxylmethyl)-1,3-propanediol.
5) 1,3-Bis[tris (hydroxylmethyl)-methylamine]propane.

6-2. pH range

The ES analytial and preparative columns are amenable to solutions of pH 2 to 12.

6-3. Organic solvents

Either methanol or acetonitrile can be used with the ES analytical and preparative columns in combination with salt or buffer solutions.

7. Flow rates

7-1. For eluent replacement

For analytical columns, 1 ml/min or lower and preferably 0.5 ml/min or lower ; for preparative columns, normal flow rate or lower.

7-2. For analysis

Generally as described in the column specifications of Section 3 above. With relatively viscous eluents or low operating temperatures, use lower flow rates.

8. Operating temperatures

Operating temperatures should generally be within the range 10 - 50℃.

In ion-exchange chromatography, separation efficiency 'may be influenced by the operating temperature. For highly reproducible chromatograms, a constant-temperature chamber or room is recommended.

9. Column cleaning

Elution characteristics of a column may change considerably after long, repeated usage, due to the accumulation of sample microadsorbents on the packing material surface. In such cases, cleaning the column with a solvent different from that generally used as an eluent may be effective for recovery of column efficiency.

Cleaning solutions. The following solutions are quite strong ; milder solutions may be employed as appropriate to the adsorbed sample.

Type of adsorption	Appropriate cleaning solutions	
	ES-502C, ES-502CP	ES-502N, ES-502NP
Ionic	● Aqueous solution of high salt concentration* ● 0.1 M acetic acid aqueous solution	● Aqueous solution of high salt concentration* ● 0.01 N sodium hydroxide aqueous solution
Hydrophobic	● 50 mM buffer solution containing 50% acetonitrile or methanol	● 50 mM buffer solution containing 50% acetonitrile or methanol

* Up to 600 mM, salt e.g. NaCl, KCl, Na_2SO_4, K_2SO_4

10. Column handling and storage

10-1. The column may be left in the LC system without flushing for up to several days between use, so long as no corrosive agent or propagating bacteria are present. If such sources of system contamination are present, the column and system should be thoroughly flushed with 50 mM buffer solution before they are left to stand. In either case, it is essential to ensure that no part of the flow line in the LC system or column becomes dry at any time during non-use.

10-2. Columns disconnected from the LC system should be tightly stoppered at both ends to prevent internal drying, and stored in an area free from large temperature changes and preferably in a constant-temperature room or chamber.

10-3. For prolonged storage and for transportation through districts subject to temperatures below 0℃, the column should be flushed with a 50 mM buffer solution and then charged with a 70/30 mixture of 50 mM buffer solution and methanol, to prevent corrosion and freezing, and tightly sealed with stoppers.

10-4. Never hold or store the column in areas exposed to direct sunlight or sharp changes in temperature.

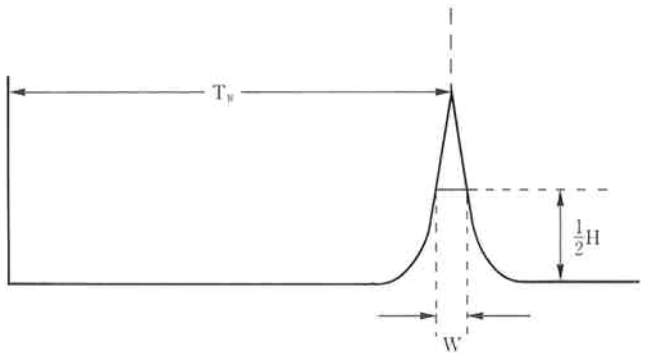
11. Optimum analytical conditions

For optimum analytical conditions in various applications, refer to Technical Data Sheet No. 10, "Guide to effective use of ES series".

12. Measurement of NTP

The measurement conditions employed in the determination of NTP are described in the INSTRUCTION DATA sheet enclosed with the ES column.

■Calculation of NTP



$$N = 5.54\left(\frac{T_R}{W}\right)^2$$

N = NTP
T_R = Retention time (min)
W = Half width (min)

Note that the use of different solutes or eluents will result in different NTP values. Any substantial dead volume in the LC system flow line will also result in lower NTP values.

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