

apHera™ NH₂ Series

Polyamine-Bonded Polymeric Gel Columns

Operating Instructions

NOTE: Although apHera columns are designed to operate at extremes of pH, the user should be aware that components of the HPLC system may be susceptible to acidic or basic conditions. Standard fittings on Astec HPLC Columns have deep Waters-type seats. Adjustable connecting hardware is highly recommended to ensure void-free connection.

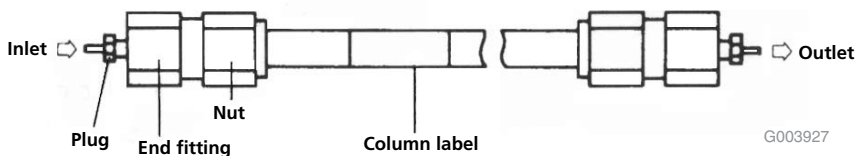
Be sure to read these instructions before using the apHera column for optimum performance and service life.

Introduction

All apHera NH₂ amino columns are packed with a hard polymeric gel containing chemically bonded polyamine groups, for unrivalled efficiency, stability, and reproducibility in the analysis of mono- and oligosaccharides by liquid chromatography with acetonitrile aqueous solution as the eluent and for the analysis of many other substances by ion-exchange chromatography with buffer solutions.

Saccharide analysis under the chromatographic conditions generally employed for conventional, polyamine-bonded silica-base amino columns, the NH₂ column provides equal resolution and far greater reproducibility. Because of its superior gel stability, the NH₂ also permits the selection of eluents ranging from pH 2 to pH 13 and a broad variety of buffers either singly or in combination, for eluent optimization and chromatographic efficiency otherwise unattainable in saccharide analysis.

Column Components



Specifications

Dimensions: As in Table 1 below
 Connecting fitting: Swagelok® type (inch)
 Column material: 316 SS

Table 1. Analytical and Preparative Columns

Type	Particle Size (mm)	Size (µm)	NTP*	Normal	Flow Rate Maximum	Max. Pres. (kg/cm ²)	pH range
NH ₂	250 x 4.6	5	7,500	0.5-1.0	1.5	150	
	250 x 10	5	10,000	2.0-3.0	5.0		
	300 x 21.5	5	10,000	3.0-5.0	8.0		
	300 x 21.5	9	9,000	5.0-10	12		
	300 x 28	9	9,000	10-20	25	100	2-13
	300 x 50	9	2,500	20-40	50		
	500 x 50	9	4,000	20-40	50		

*Number of theoretical plates, calculated as shown in "Measurement of NTP", on back page, for conditions as given in datasheet supplied with each column.

Column Installation

Direction of Flow

Install and use the NH₂ column with the flow through the column matching the flow direction arrow on the column label.

Connecting Tubing

It is important to use connecting lines which provide the smallest possible dead volume. For analytical columns, use tubes 0.2 - 0.3 mm inner diameter (for preparative columns, 0.2 - 0.5 mm) 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 wherever possible, as it may cause sample diffusion. Otherwise, use only a low dead volume tube union.

Pump Selection

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

Eluent pretreatment

Filtering

Always filter (0.5 mm 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.

Degassing

Always thoroughly degas the eluent before column injection. The use of an on-line degasser is recommended. Astec supplies a 4 channel degasser (Catalog No. 89704). Alternatively, degassing by helium aspiration under reduced pressure may be employed prior to injection, but it must be noted that the organic solvent content in aqueous solutions may be significantly affected by the degassing time.

Column Connection, Purging, and NTP Measurement

The column should always be installed in the following manner, for (a) safe, effective operation and (b) assessment of the column's functional integrity prior to initial and later use by standardized comparison of its NTP.

Purge all air from the LC system line, with solution of the composition described on the data-sheet -- aqueous 75% acetotriole.

Set the flow rate at 0.5 mL/min. for analytical columns (for preparative columns, at or lower than the normal flow rate) and start the pump. As the solution begins to emerge from the column input line, remove the column inlet and outlet stoppers and immediately connect the line to the inlet. After several drops of the solution emerge from the column outlet, connect the column outlet to the detector.

Set the flow rate at the rate given in the Column Assessment Parameters and purge the column with a solution flow totaling 10 to 20 times the volume of the column.

Determine the NTP under the conditions described in the Column Assessment Parameters, as a basic indicator of initial column performance and subsequent assessments of integrity. (See "Measurement of NTP").

Note: NTP determination at Astec, and in the above procedure, is performed with all amino bases in the free amino base state, to facilitate measurement and standardized comparison.

Column Equilibration for Consistent Retention Times

The NH₂ column, as shipped, is free of counter ions and thus contains only free amino bases. In initial use, its saccharide retention time will not stabilize until ionic equilibrium is attained between the gel and the eluent. Always follow the guidelines given in the following table for rapid attainment of equilibrium and consistent retention times.

		Eluent	
		Acetonitrile/water	Acetonitrile/buffer
Equilibration	Solution	100 mM ammonium acetate (pH 9.3-9.5).	100 mM tetrapropyl ammonium hydroxide ¹ acetic acid (pH 10), or 100 mM sodium acetate (pH 10.5).
	Quantity ²	10 to 20 times the column volume.	
	Flow rate	Normal (Table 1) or lower.	
Sample pretreatment ⁴	Purification ³	Remove polysaccharides, proteins, and nucleic acids, and other polymers.	
	Solid sample	Dissolve sample in water, add acetonitrile to obtain 50% or higher acetonitrile aq. solution.	Dissolve in buffer, add acetonitrile to obtain 50% or higher acetonitrile aq. solution.
	Aq. sample	Add acetonitrile to obtain 50% or higher acetonitrile aq. solution.	
Sample injection	Aq. acetonitrile sol.	Small quantity (100 mL or less for analytical columns) preferable.	
	Water solution	Smallest possible quantity (20 mL or less for analytical columns).	

¹ Alternatively, with equal effect, 100 mM tetrabutyl ammonium hydroxide.

² If at 30 °C; requirement halved at 40 °C.

³ The use of a guard column is recommended of either analytical or preparative columns, to minimize species adsorption on the main column.

⁴ Optimum separation efficiency is generally obtained with sample solutions which are similar to the eluent in composition. Use acetonitrile in the sample solution wherever possible.

Eluents

Saccharide Analysis

Water, acetonitrile, and ethanol, either singly or in mixtures of any ratio, may be used as eluent. With aqueous ethanol solutions, the flow rate should be lower than normal, because of their relatively high viscosity.

Various types of buffers soluble in acetonitrile or ethanol (such as tetrapropyl-ammonium acetate or sodium acetate) may be used together with the above components, so long as no observable precipitation occurs in the eluent.

Eluents of pH 2.0 to 13.0 may be used.

Ion-exchange chromatography

Buffer solutions such as phosphate, acetate, and tris, with or without NaCl, KCl, or Na₂SO₄, may be used.

Eluents of pH 2.0 to 13.0 may be used

Elution Modes

Isocratic, gradient, or stepwise elution.

Flow Rate

The flow rate should never, in any case, exceed the maximum flow rate given in Table 1. For frequent column usage the normal flow rate is recommended

Operating Temperatures

Operating temperatures should generally be within the range 4-50 °C. High-temperature operation may result in bubble generation, necessitating degassing or temperature reduction. Low-temperature operation may require reduced flow rates, because of increased eluent viscosity.

Column Cleaning

Long-term, repeated use of the column may cause considerable change in the elution characteristics of saccharides, due to accumulation of background adsorption from the sample solution. In these and other cases, the column may be cleaned in the following manner.

Pass aqueous 0.1 N nitric acid totaling 10 to 20 times the column volume through the column at the normal flow rate or lower. Purge all nitric acid from the column with distilled water, and pass aqueous 0.1 N sodium hydroxide totaling 10 to 20 times the column volume at the normal flow rate or lower.

For storage after cleaning, replace the alkali solution with 30-80% acetonitrile aqueous solution.

Column Handling and Storage

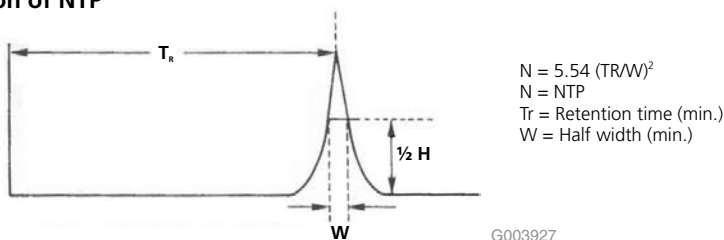
When not in use, the column may be left in the LC system without flushing for up to several days, so long as no corrosive agent or propagating bacteria are present. It is essential to ensure that no part of the flow line in the LC system or column becomes dry at any time during non-use. If any possibility of contamination or drying is present, thoroughly purge the column and LC system with aqueous 30-80% acetonitrile.

Disconnected columns should be stored in an area free from large temperature changes (preferably in a constant temperature room), with both ends tightly stoppered to prevent internal drying. Storage in an area exposed to direct sunlight or large temperature changes may cause column degradation.

Measurement of NTP

The measurement conditions for NTP determination are described in the datasheet.

Calculation of NTP



Notes:

1. The use of different solutes or eluents will result in different NTP values.
2. Any large dead volume in the LC system will result in lower NTP values.
3. For standardized comparison of the used column's performance with initial new-column performance, first thoroughly purge the column with aqueous sodium hydroxide, to remove all counter ions and thus obtain the free amino base gel form, and then proceed with the NTP determination as described in the "Column Installation Section".

Trademarks

Swagelok — Swagelok Co.
apHera — Sigma-Aldrich Co.