

apHera™ C18, C8 and C4 Polymeric Reversed Phase HPLC Columns

Operating Instructions

These Instructions Describe Procedures That Should Be Followed For Operator Safety As Well As Achieving The Optimum Performance And Service Of The Columns. These Instructions Should Be Read Before The Column Is Placed In Service.

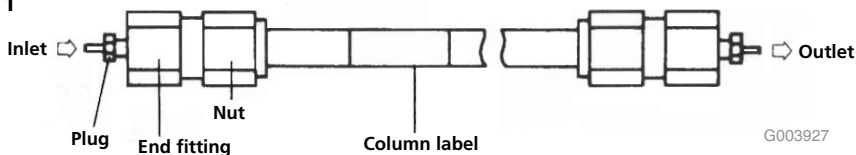
Introduction

Astec apHera columns for reversed phase chromatography are packed with spherical porous particles that have alkyl groups bonded to the surface. The underlying polymeric bead provides a stable polar surface. apHera C18 columns are compatible with the commonly used reversed-phase HPLC eluents between pH 2 to pH 13. The particles are available in two sizes. The smallest has a diameter of 5 μm . The small particles give the highest column efficiency and resolution, but at the expense of higher operating pressure. For preparative applications where lower operating pressure is important, we recommend the 9 μm particles.

apHera columns provide three different lengths of alkyl groups that modify the polar character of the rigid vinyl particle, giving a useful range of hydrophobicity. The least polar column is the C18, which has octadecyl groups bonded to the polymer. This is designed to give the strongest reversed phase retention, with selectivity similar to octadecyl silane. The most polar is the C4 series of columns. These have short butyl groups bonded to the polar polymeric surface. apHera C8 is intermediate in polarity with an octyl hydrocarbon covalently bonded to the particle. This packing is designed to have a hydrophobic character similar to the popular C8 or octyl columns. Thus, apHera columns for reversed phase chromatography combine a range of hydrophobic characteristics commonly found in silica packings with the high chemical and physical stability of polymer based particles.

Column

Figure 1



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Specifications

The specifications for each column are in Table 1, next page. The column tube and end fittings are constructed from passivated 316 stainless steel. The column end fitting is designed to minimize dead volume and band broadening. The depth of the fitting for the inlet and outlet tubes may be different than those used on columns from some other manufacturers. If you use a tube with a short nose extending from the fixed ferrule, there will be a cavity in the flow path that can lower the column efficiency (number of theoretical plates, NTP). For this reason users may want to reseal the ferrules to provide the correct depth or use adjustable nuts and ferrules.

Important 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 are highly recommended to insure void-free connection.

The mobile phase in the column at the time of shipment is listed on the datasheet enclosed with the column.

Table 1

Column Packing	Column Dimensions	Particle Size (μm)	C%	NTP*	Flow Rate (mL/min.)	
					Normal	Maximum
C18	250 x 4.6 mm	5	17	>14,000	0.5-0.8	1.5
	150 x 4.6 mm	5	17	>9,000	0.5-0.8	1.5
	250 x 6.0 mm	5	17	>14,000	0.5-1.0	2.5
	150 x 6.0 mm	5	17	>9,000	0.5-1.0	2.5
	250 x 10.0 mm	5	17	>10,000	1.5-2.0	3.0
C8	250 x 4.6 mm	5	10	>11,000	0.5-0.8	1.0
	150 x 4.6 mm	5	10	>7,000	0.5-0.8	1.0
	250 x 10.0 mm	5	10	>8,000	1.5-2.0	3.0
C4	250 x 4.6 mm	5	6	>9,000	0.5-0.8	1.0
	150 x 4.6 mm	5	6	>6,000	0.5-0.8	1.0
	250 x 10.0 mm	5	6	>7,000	1.5-2.0	3.0
C18	300 x 21.5 mm	9	17	>9,000	5-10	12
	300 x 28.0 mm	9	17	>9,000	10-20	25
C8	300 x 21.5 mm	9	10	>7,000	5-10	12
C4	300 x 21.5 mm	9	6	>6,000	5-10	12

*NTP, number of theoretical plates, is calculated using the formula and conditions given in the Column Assessment Parameters supplied with each column. Each column is individually tested to assure that it meets the high standards set for Astec columns. The maximum pressure limit is 150 kg/cm².

apHera C18, C8 and C4 columns are designed for use between pH 2 to pH 13.

Column Installation

Direction of Flow

Install and use the column with mobile phase flowing in the direction indicated on the column label. If the column shows very high resistance to flow, it may be partially plugged up. This is probably the end of the service life of the column. Some users have been successful in extending the life somewhat by back flushing the column. The outlet of the column is connected to the injector so the liquid flows into the normal outlet fitting. The other end, normally the column inlet, is placed into a beaker. The pump is then turned on at the minimum flow rate listed in Table 1. The column is back flushed for about one hour or overnight if the operating pressure shows signs of declining. The column then should be retested according to the procedure on the datasheet provided with each column. If the column is efficient, then one can restore the column to operation in the instrument in the normal manner.

Connection Tubes

For columns with diameters smaller than 10 mm I.D., use connection tubes from the injector and to the detector that have the shortest practical length and internal diameter no larger than 0.3 mm, (0.010 inch) internal diameter. The outer diameter must be 1/16 inch, (0.0625 inch). For preparative columns, (columns with internal diameters of 10 mm and larger), it is possible to use connection tubes with internal diameter up to 0.5 mm (0.020 inch). The length should be kept as short as practical.

Pump

The use of pumps with strong pulsations in the output flow or pressure may shorten column life and should be avoided. Pulsation can often be reduced by using a pulse dampener on the pump outlet.

Preliminary Purge

Before connecting the column to the system, purge the system and lines with liquid to remove any air or particles.

Connection of Column to HPLC

Connect the column inlet to the tube from the injector. Do not turn the fitting tight. Turn on the pump flow to one half the lower operating range in Table 1. Allow a small amount of the

mobile phase to bleed out in order to displace any air bubbles from the cavities in the column inlet. Tighten the inlet fitting. Then loosely connect the outlet fitting. After a few drops have formed, tighten the fitting. This should displace any air from the system.

Mobile Phase and Sample Preparation

Filtering

If particles are allowed to flow into the Astec column, the frit will trap the particles. Eventually, the column will become plugged. To avoid this, the mobile phase and sample should be passed through a 0.45 μm , or smaller, filter. For the mobile phase, the filter should be installed between the pump and injector. The sample should be passed through any one of the many different filters available for this purpose.

Filtering is not always effective in preventing plugging of the column. Experience shows that if column plugging is a frequent problem, the plug is often located at the very top of the column. Guard columns are short sacrificial columns that are installed ahead of the main column to protect it. When a plug occurs, the guard column is replaced.

Degassing

Numerous practical problems are avoided when one uses mobile phases that have been treated to remove, or at least lower their content of dissolved gases, especially air. The most convenient way to remove dissolved air is to gently bubble helium through the mobile phase reservoirs. If one is using mobile phases containing a mixture with volatile components, then the helium may slowly remove the volatile components, which can cause a systematic shift in the retention times of the analyte. In this case, the use of a commercial on-line degasser is recommended. Astec offers a 4 channel HPLC eluent degasser (Catalog No. 89704).

Compatible Mobile Phases

apHera C18, C8 and C4 columns can be used with mobile phases of eluents containing water, methanol and acetonitrile. In general, water/acetonitrile combinations provide greater efficiency, especially for compounds containing an aromatic ring.

For control of pH within the range of pH 2 and pH 13, phosphate, acetate, tris-HCl, NaCl, KCl, Na_2SO_4 , and trifluoroacetic acid buffers can be used. It is important to note that the mobile phase should be filtered before use as described in **Mobile Phase and Sample Preparation** - Filtering.

CAUTION! Adding organic solvents to salt and buffer solutions can lead to precipitation of the salt. Precipitation can occur in the column, which will often cause a catastrophic rise in the pressure across the column. If this does occur, try washing out the precipitate by reversing the flow on the column with distilled water. Use a slow flow rate initially to keep the back pressure low, below 150 kg/cm^2 .

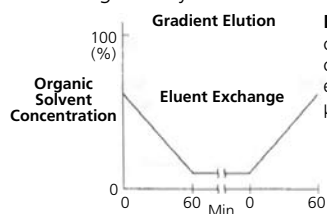
It is important to check the solubility of mixed solvent buffers before running a solvent program. This can be done by making a quick scouting run in a test tube. Slowly add the strong eluent to a small quantity of the starting eluent. If the solution turns cloudy or white, this means precipitate is forming. Other buffer combinations should be tried.

Elution Modes

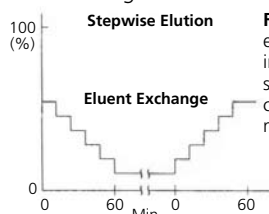
All apHera columns can be used in isocratic and gradient elution modes. Take care to avoid precipitation of buffer components during the run.

apHera 9 μm C18, C8 and C4

With the 9 μm columns, avoid sharp changes in mobile phase composition at high flow rates. Rapid changes can cause settling of the column bed. To minimize the risk, changes in composition should be made at flow rates of 5 mL/min . or lower. Large changes in solvent composition should be made gradually as shown in Figures 2 and 3 for both increasing and decreasing concentration.



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apHera 5 μm C18, C8 and C4

There are no restrictions on the rate of change of mobile phase composition provided that the column is operating within the specific range of flow rate and pressure.

Flow Rate

Operation of the column at flow rates higher than specified as normal in Table 1 will probably shorten the useful life of the column. Exceeding the maximum flow rate specification in Table 1 will probably immediately destroy the column. For this reason, the pressure limit on your pump should be adjusted to about 10% to 20% above the pressure normally found for your particular column and mobile phase. This should provide quick protection should a high flow excursion occur. Never allow the pressure limit to be set above 150 kg/cm².

Operating Temperature

apHera C18, C8 and C4 columns can be operated at temperatures from 4 °C to 60 °C. At elevated temperatures, degassing may be required to prevent formation of bubbles in the detector. At low temperature, the viscosity of the mobile phase will increase. Care should be taken to avoid exceeding the maximum pressure specification of the column. This may require decreasing the flow rate. Flow rate is proportional to pressure, so cutting the flow in half will decrease the pressure on the column by half.

Column Handling and Storage

The column bed should not be allowed to dry out. Retain the plugs used in shipping the column and reinstall them tightly when the column is not being used.

During storage the column should not be exposed to strong swings in temperature, vibration or direct sunlight.

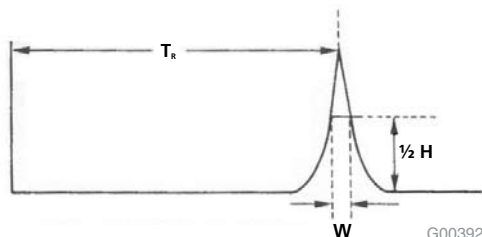
apHera C18, C8 and C4 columns may be left connected in the HPLC system for several days as long as the instrument is free from corrosive agents, buffers and organisms that could support growth of material that plugs the column. If such agents are a potential problem, the column can be flushed first with water, then 50% methanol, and then left standing in the instrument. Washing the column with water is important to remove buffer components that could precipitate during standing or by adding the 50% methanol (see section on **Compatible Mobile Phases**). Also it is important that the column not be permitted to dry out.

Sample Preparation

In general, the sample should be dissolved in the mobile phase. Dissolving the sample in the mobile phase often avoids large "system peaks" in the beginning of the chromatogram. For gradient elution the sample should be in a solution corresponding to the initial mobile phase composition. In some cases it is desirable to prepare the sample in a solution with an elution strength slightly weaker than the mobile phase. Upon injection, the sample components will tend to focus in a narrow band on top of the column. This can improve detection limits.

Measurement of NTP

The measurement conditions employed in the determination of NTP are described in the data-sheet enclosed with the column.



$$N = 5.54 (TR/W)^2$$

$N = \text{NTP}$
 $Tr = \text{Retention time (min.)}$
 $W = \text{Half width (min.)}$

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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