

# Agilent FlowTrap Columns

## Data Sheet

### Installation

#### Analytical and semi-preparative columns up to 21.2 mm id

A 1/16 in stainless steel tubing is recommended for column connections, 0.002 in id for microbore, 0.010 in id for analytical work and 0.020 in id for preparative work. Connecting tubing lengths should be minimized to avoid excessive dead volume which will diminish system performance. Column connections should be made using Parker compatible 1/16 in nuts and ferrules with special reference to compatibility of column connectors as illustrated in Figure 1. Connect the HPLC column in the flow direction indicated. The nut and ferrule should be tightened 1/4 of a turn past finger tight by applying the wrenches as shown in Figure 2.

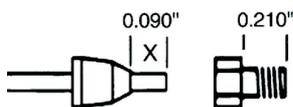


Figure 1. Compatible connectors.

The distance “x” for the standard column end fitting is 0.090 in and a minimum male nut length of 0.210 in is required. Some fittings from other manufacturers may not be compatible, such as Waters and Rheodyne. If unsure, please contact Agilent Technologies.

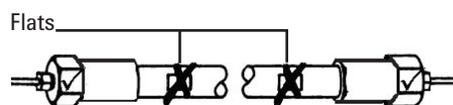


Figure 2. Don't use wrenches on the flats.

To avoid loosening the end fittings and causing leaks, wrenches must be used on the endfitting adjacent to the connecting nut and NOT on the column barrel or the opposite endfitting.

### Flow Rate/Column Pressure

The 4.6 mm, 7.5 mm, 10.0 mm, and 21.2 mm id columns available in the FlowTrap range are all pressure rated to 1500 psi (100 bar). Under no circumstances should the pressure rating of the column be exceeded. The relatively large particle size of the FlowTrap

(40 micron) means that higher flow rates are tolerated and sample loading in most solvents can be achieved without significant back pressure issues.

### Shipping Eluent

FlowTrap columns are supplied containing 7:1 (w/w) acetonitrile/water. Columns are securely sealed with end caps which must ALWAYS be replaced when the column is disconnected from the system to prevent the column from drying out.

### Column Conditioning

In order to maximize the performance and capacity of the FlowTrap column a first use column activation step is required. This step introduces the sorbent to the eluent system which is intended for use during the back flush elution component of the Flow Trapping process.

For most applications, Agilent recommends that the column is washed in 100% organic solvent for at least 15 minutes followed by a return back to aqueous prior to first sample loading. The recommended eluents for this step are 100% acetonitrile or 4:1 (w/w) dichloromethane/methanol.

Agilent also recommends that if the column has not been used for a reasonable length of time then this activation procedure should be repeated. This will ensure that all of the available surface area within the sorbent is conditioned and available for compound trapping.

### Effective Loading Capacities

Dimensions	Capacity Range*
150 × 4.6 mm	0–50 mg
150 × 7.5 mm	50–150 mg
150 × 10.0 mm	150–200 mg
150 × 21.2 mm	250–500 mg

\*This quoted capacity is a recommendation only based on representative loading studies of several pharmaceutical drugs with a molecular weight < 500. Observed capacities will vary according to compound type and eluent constitution. The column should never be overloaded as this significantly increases the risk of sample precipitation.

## Loading Eluent Modifications

If the loading eluent contains a higher percentage of organic modifier, then dilution with water will significantly improve retention and capacity. For compounds that have very high or very low  $pK_a$  values, other eluent modifications can be made. The trapping capacity for acidic compounds can be increased by lowering the pH of the loading solution and trapping capacity for basic compounds can be improved by increasing the pH. Care should be taken while conducting such dilutions so as not to cause sample precipitation.

## Mobile Phases

All eluents should be HPLC grade. Mobile phases should be filtered (< 0.5  $\mu\text{m}$  filter) and thoroughly degassed before use. The physical and chemical stability of FlowTrap adsorbents allows buffered mobile phases across the complete pH range to be used. The concentration of buffer salt should be selected carefully so as to minimize potential salt crystallization on the stationary phase.

## Column Regeneration

Post compound trapping the column needs to be cleaned and regenerated prior to the addition of the next compound. An effective wash protocol must be followed to reduce the incidence of carryover. The recommended generic protocol is shown in Table 1. The organic modifier can be changed depending on the compound and application in use.

Table 1. Generic Protocol for Column Regeneration

5 min	100% $\text{CH}_3\text{CN}$	Reverse elute
2 min	RO water	Forward
5 min	2M $\text{NH}_3$ aqueous solution	Forward
5 min	0–100% ACN gradient	Forward
5 min	RO water	Forward

If acetonitrile is not suitable for a particular application as the initial eluting solvent, then a 4:1 (v/v) dichloromethane/ methanol system can be used in replacement. The ammonia wash and organic gradient are essential components of the clean up process.

## Column Efficiency Testing

Each column is provided with its own individual test certificate. Agilent recommends that the column be re-checked from time to time to monitor its performance. A 7:1 (w/w) acetonitrile/water is

used, with acetone as the unretained test probe. System factors, such as dead volume, flow rate, and temperature can significantly affect the results obtained. It is important that these factors are taken into account when comparing the results to those contained in the test certificate.

## Long Term Cleaning

If the column begins to exhibit signs of deterioration, the following clean up procedures may be beneficial. In all cleaning operations, the direction of flow should be reversed. Particulate matter blocking the inlet frit and causing excessive back pressure may sometimes be removed when gently flushed in this direction. The use of a higher strength organic modifier (such as acetone or tetrahydrofuran) may remove heavily hydrophobically bound contaminants. The FlowTrap column will also tolerate strong bases and a wash with 2M ammonia solution is an effective method of cleaning in place.

## Storage

On removing the column from the system, the end plugs must be replaced to prevent the column from drying out, as this would disrupt the packed bed. The end plugs need only be applied finger tight. Long term storage in buffer should be avoided to prevent the risk of crystallization of buffer salts. A high organic composition is recommended to inhibit bacterial growth.

## Agilent Ordering Information

For more information on our products, visit our web site at [www.agilent.com/chem/columns](http://www.agilent.com/chem/columns).

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