

Agilent FlowTrap Columns

Agilent FlowTrap is a groundbreaking technology that revolutionizes the way in which compounds are isolated from preparative HPLC fractions and other highly aqueous solutions.

Available in standard HPLC column hardware, FlowTrap columns will help dramatically reduce dry-down times and increase throughput for compound recovery.

Key benefits of the FlowTrap accelerated compound enrichment technique

- **Reduce dry-down times.** Solvent-switch your compound from a high volume of water-based HPLC eluent to a low volume of volatile solvent, thus dramatically reducing the evaporation times needed for compound isolation.
- **Desalt in-situ.** Ion-pairing reagents, such as trifluoroacetic acid (TFA), can be removed from the compound during trapping, allowing the isolation of freebase compounds. This reduces the risk of hydrolysis, which can cause compound degradation.
- **Application diversity.** The strongly retentive sorbent will retain compounds within broad LogP and pKa ranges – covering a wide range of products.
- **Highly versatile.** The standard HPLC column format can be used in conjunction with most HPLC instruments and pumps. A range of column sizes allows for seamless scale-up as compound batch sizes increase.

An effective way to isolate compounds

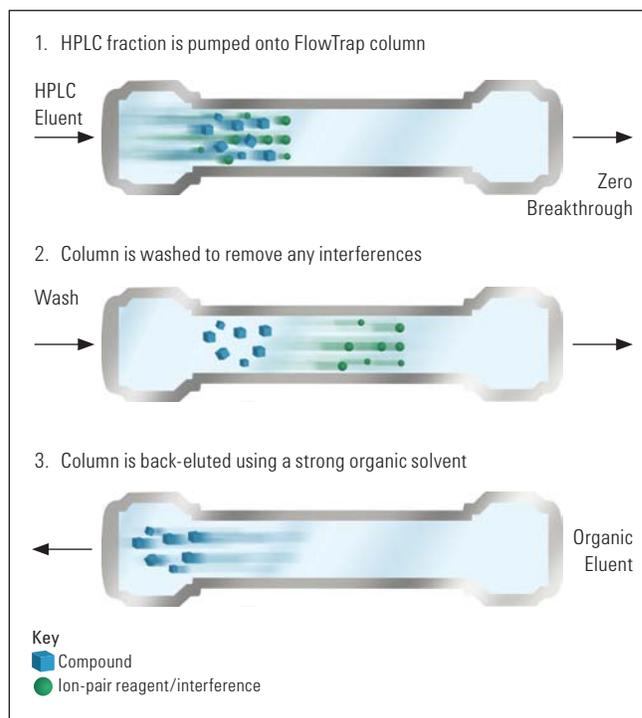
Existing preparative HPLC workflow relies heavily on the evaporation of HPLC eluents in order to isolate final purified compounds. Eluents that contain a large percentage of water often need long evaporation times at elevated temperatures. This slows down laboratory throughput and may be detrimental to compound integrity.



How FlowTrap works

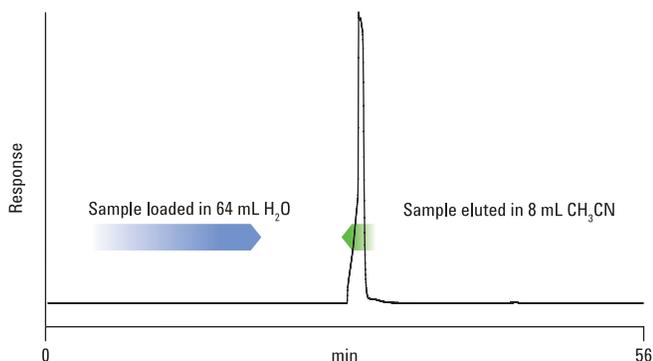
FlowTrap columns are ultra-retentive, high-capacity, hydrophobic columns that capture and concentrate small molecules. Once trapped, the desired analyte can be back-eluted using a small volume of a volatile organic solvent, such as CH₃CN or MeOH, affording simpler compound isolation.

Schematic showing how FlowTrap columns can be used to concentrate small molecules.

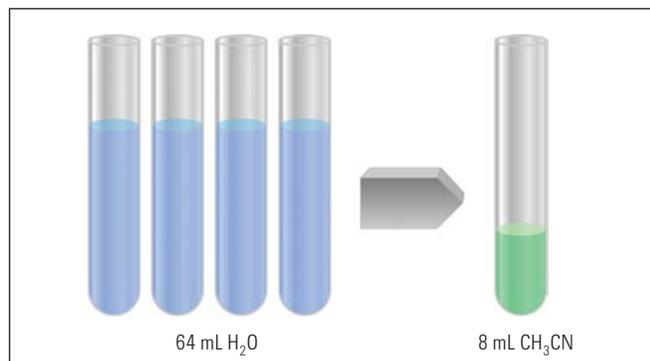


Excellent capacity and reproducibility

FlowTrap columns contain a novel polymeric sorbent that allows for excellent retention of compounds and facile elution with suitable organic solvents. The efficiency of the packed bed allows for excellent reproducibility over many cycles.



Two mg/mL aqueous caffeine solution is loaded dynamically into the FlowTrap column until 128 mg has been loaded (i.e., 64 mL) with no breakthrough. Following an aqueous wash, the loaded caffeine is eluted in just 8 mL CH₃CN.



In one cycle, 64 mL of aqueous caffeine solution was effectively solvent-switched into 8 mL of CH₃CN.

Eluent considerations

If the loading eluent contains a higher percentage of organic modifier, 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; trapping capacity for basic compounds can be improved by increasing the pH.

Column regeneration without carry-over

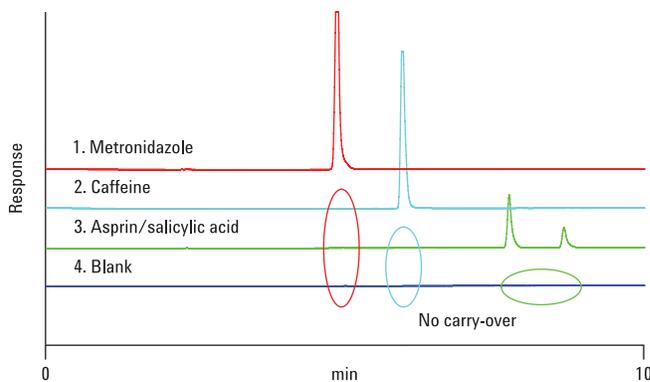
The FlowTrap column is a multiple-use device that may be regenerated and used in up to 500 cycles when run under optimized conditions. Effective regeneration can be achieved by a strong basic wash, followed by a 0-100% organic gradient. In this example, CH₃CN is used, but other solvents can be used depending on compound solubility. These vigorous washing conditions are not detrimental to column performance.

Time	Eluent	Flow Direction
0	R.O. water	Forward →
2	100% CH ₃ CN	← Reverse
7	R.O. water	Forward →
9.5	2 M NH ₃	Forward →
14.5	0-100% CH ₃ CN gradient	Forward →
18.5	R.O. water	Forward →
30.5	R.O. water	Forward →

The generic elution and regeneration procedure used in the carry-over studies.

The following overlaid chromatograms clearly show no residual carry-over between individual trapping runs of 1) metronidazole, 2) caffeine, and 3) an aspirin/salicylic acid mix.

Conditions
 Column Polaris C18-A 4.6 x 150 mm, 5 μm (P/N: A2000150X046)
 Eluent A: H₂O; B: CH₃CN
 Flow rate 1 mL/min
 Temperature Ambient
 Gradient 0-100% B over 10 min
 UV detection 260 nm
 Instrument Agilent Prep LC



Removal of an ion-pairing reagent: TFA

The use of acidic mobile phase additives, such as TFA, in preparative chromatography is common. When such eluents are evaporated, residual acid will increase in concentration and may form salts with the purified compound. Using the FlowTrap accelerated compound enrichment technique, residual TFA can be washed out of the sample without eluting the target compound. This method can also be used to remove other ion-pair reagents, such as formic and acetic acids.

As show in the following elution chromatograms of a 26-mg metronidazole load after the A) water-washing method (green) and B) base-washing method (blue), the elution peak of the freebase metronidazole is significantly sharper.

Conditions

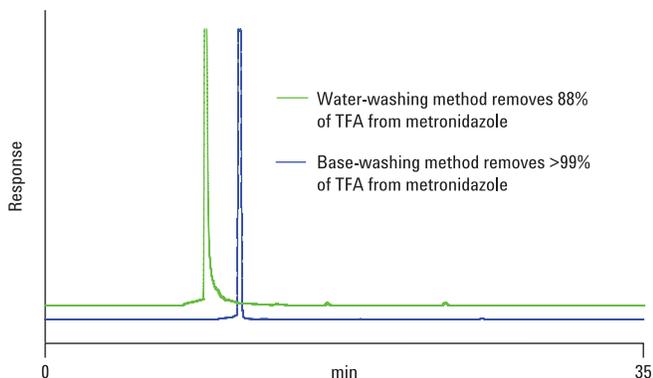
Loading eluent 12 mL - metronidazole @ 2 mg/mL in water + 0.1% TFA

Wash conditions

A) Water (R.O. water)
Flow rate: (4 mL/min) for 4 min

B) Base
2 M NH₃; Flow rate: (4 mL/min) for 2 min then
R.O. water; Flow rate: (4 mL/min) for 4 min

Elution 100% CH₃CN over 5 min; Flow rate: (4 mL/min)



Compound	Washing Protocol	Amount of TFA Removed*
Metronidazole	A) Water	88%
Metronidazole	B) Base	>99%

* Calculated using 19F NMR analysis

Instrument compatibility

FlowTrap columns can be used for flow or fraction trapping applications on any HPLC equipment configured to operate in forward and reverse flow using appropriate switching valves.

Ordering information

FlowTrap Columns

Dimensions	Part Number	Capacity Range*
4.6 x 150 mm (pair)	PL1560-3M07	50 mg
7.5 x 150 mm (pair)	PL1160-3M07	50-150 mg
10.0 x 150 mm (pair)	PL1060-3M07	150-200 mg
21.2 x 150 mm (pair)	PL1E60-3M07	200-400 mg

* This quoted capacity is a recommendation only, based on representative loading studies, and may vary according to compound type and eluent composition.

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