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Jupiter®
Protein and Peptide LC

Jupiter HPLC Columns Tips for Care and Use

General Information

Each Jupiter column manufactured by Phenomenex is individually prepared and tested. Every column is supplied with a Certificate of Quality Assurance (CQA) which indicates testing conditions, operating parameters, and column details. The column details, including specifications and performance test results should be entered into your information management system for easy tracking and reference. Electronic copies of your column's quality documentation can also be acquired at: www.phenomenex.com/mysupport.

Inspection

Upon receipt of column, please verify that the column you received is the one you ordered (i.e. dimension, particle size, media). Additionally, please check the column for any physical damage potentially caused during shipment. Test the column immediately to verify performance and record the result of your test in your column information management system.

Column Characteristics and Operating Recommendations

Phase	Base Material	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	End Capping	pH Range	USP Classification
Proteo	Fully Porous	90	475	15	yes	1.5-10.0	-
C18	Fully Porous	300	170	13.34	yes	1.5-10.0	L1
C5	Fully Porous	300	170	5.5	yes	1.5-10.0	-
C4	Fully Porous	300	170	5	yes	1.5-10.0	L26

Shipping Solvent

Unless otherwise noted on a column tag, Jupiter columns are shipped in 50:50 Acetonitrile: HPLC grade water. Acetonitrile and water ratios may differ based on dimension.

Backpressure limits

Typical Flow Rates (Independent of Particle Size)

- 0.02-0.1 mL/min for 1.0 mm ID
- 0.2-0.6 mL/min for 2.1 mm ID
- 1.0 mL/min for 4.6 mm ID
- 5.0-20 mL/min for 10.00 mm ID
- 10-200 mL/min for 21.2 mm ID

Max Backpressure

>5000 psi (345 bar) may compromise column longevity.

Max Temperature

- Suggested max temperature for Jupiter LC columns is 60 °C, however temperature limits are more so dependent on your running parameters.
- Continuous use of Jupiter columns at the maximum temperature limit may compromise column longevity.

Column Installation

Initial setup of your LC system is very important to ensure column performance:

Ensure That Your Lc System is Ready

- Seals, lines, injector clean
- Lines primed (no dry lines or bubbles)
- Steady baseline
- Consistent pressures

Flush LC system pump and line with mobile phase (HPLC grade and miscible with solvents that column is shipped in).

Mobile Phase Starting Conditions Check List

- Ensure that HPLC grade mobile phase is well mixed, filtered, and degassed prior to use.
- Ensure that column shipping solvent, remaining solvent in LC system, and mobile phase solvents are miscible.

Set flow rate to 0.1 mL/min (for 2.1-4.6 mm ID) and install the column making sure that the arrow is in the direction of flow. Then increase the flow rate to 0.2 mL/min (2.1 mm ID) or 1.0 mL/min (4.6 mm ID) for 5-10 minutes. Collect solvent in a small beaker.

Stop flow and wipe outlet end of column to remove any particulates before connecting to detector.

Install fitting/tubing into outlet end and run minimum 10 column volumes at low flow (~0.2 mL/min) while monitoring the backpressure.

- A steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system.
- Wide fluctuations in pressure may shock and damage the column so it's important to monitor the pressure. Monitor pressure as well as signal from the detector, when both are steady, the column is ready for use.

Column Cleaning

Clean with a gradient that is closest to the last solvent system on the system:

For example, if the last injection ended with Buffer/Acetonitrile (75:25), it's more appropriate to start with 95:5 Water/Acetonitrile and then move step by step as needed to increase organic content (i.e. 75:25 Water/Acetonitrile 50:50 Water/Acetonitrile 5:95 Water/Acetonitrile).

- For hydrophobic or oily materials, try flushing with IPA. When using IPA, ensure use of a low flow to prevent higher backpressures due to higher solvent viscosity.
- For materials that are very hydrophobic, you can try flushing with THF and water.

Tips

- If an increase in pressure is observed, you can try reverse flushing the column with the reduced flow rates indicated below:
  - 0.1 mL/min (2.1 mm diameter column)
  - 0.3 mL/min (3.0 mm diameter column)
  - 0.5 mL/min (4.6 mm diameter column)
- Flushing with THF requires stainless steel lines so please check your system configuration first before flushing with THF.
- Removing any endfitting or frits will void the warrantee so please contact your technical consultant if you have any questions concerning column cleaning.
- Other cleaning approaches you can try is to wash with several column volumes of 0.1 M Phosphate buffer, pH 3.0, 0.5 % SDS or 6 M guanidinium thiocyanate. Flush with water and then water and acetonitrile immediately afterwards.

Column Regeneration

There are several approaches to column regeneration. Here are some suggestions:

1. Flush the column at low flow, with the direction of flow reverse with 50:50 Acetonitrile/Water overnight without an oven temperature.
2. Flush the column at a gradient, at the direction of flow, starting with a ratio closest to that of the method running parameters and flush from that ratio to the inverse.
   For example, if the last injection ended with Buffer/Acetonitrile (75:25), it is more appropriate to start with 95:5 Water/Acetonitrile and then move step by step as needed to increase organic content (i.e. 75:25 Water/Acetonitrile 50:50 Water/Acetonitrile 5:95 Water/Acetonitrile) at ambient temperature.
3. After flushing with IPA or THF, flush the column at gradient with water and acetonitrile overnight at low flow.

Column Storage

It is very important to make sure that your column is clean before storage. This includes removal of buffer, salts, sample, and ion-pairing agents. The recommended storage conditions are:

Acetonitrile/Water (65:35 v/v), Methanol can be used in place of acetonitrile.

Tips

- Before store, make sure the column has been flushed with HPLC grade solvents.
- Store in HPLC grade or above solvents only.
- Avoid jostling and dropping the column as this might cause column shock.

Tips for Extending Column Lifetime

Sample Preparation

Check for sample solubility in mobile phase. Use mobile phase as diluent where possible. Trace impurities can dramatically degrade column life. Filter all samples using a 0.45 µm or 0.2 µm porosity filter prior to injection.

Tips

- Using a guard will also help reduce matrix by filtering out anionic species.
- Do not overload the column.

Matrix Cleanup

Utilize sample preparation techniques such as solid phase extraction (Strata-X SPE products) or accessories (Phenex™ Syringe Filters) to minimize the injection of unwanted contaminants onto your system and column.

Use the correct guard column or guard cartridge system (SecurityGuard™) to help remove particulates before they foul your column.

Typical Loading Capacities

Column Type	ID (mm)	Approx. Dead Volume (mL)*	Typical Flow Rate (mL)	Typical and (Max.) Injection Masses (mg)	Typical and (Max.) Injection Volumes (µL)**
Capillary (Fused Silica)	0.32	0.0075	0.001 - 0.02	0.001 (0.01)	1 (10)
Microbore	1.0	0.07	0.02 - 0.1	0.01 (0.1)	5 (25)
Analytical	4.6	1.5	0.5 - 2.0	0.1 (2.5)	10 (200)
Semi-Prep	10.0	7.3	5.0 - 20	1.0 (25)	50 (1000)
Preparative	20.0	29.2	10 - 200	5.0 (500)	200 (5000)

Testing Column Performance

When testing column performance please use the manufacturer approved test mix. Here's a simple procedure:

1. Clean or regenerate the column.
2. Condition with the mobile phase until steady pressure and baseline is achieved. Please use the running conditions to the right.
3. Inject a blank and observe if any unwanted peaks are still present.
4. Inject in triplicate the standard and compare to column CQA.
5. If good peak shape and other parameters are observed, inject your standard.
6. Compare the efficiency and tailing to previous injection to confirm if column cleaning and regeneration was successful.

Reversed Phase 2 Test Mix
Jupiter® C4, C5, C18; Jupiter Proteo

Part No.: AL0-3045

Unit quantity: 2 mL
Contains: Uracil; Acetophenone; Toluene; Naphthalene
Diluent: Acetonitrile / Water (75:25)

Test Conditions
For Jupiter C18 columns

Mobile Phase: Acetonitrile/Water (65:35)*
Flow Rate: 1.0 mL/min; 0.75 mL/min for 3 µm particles
Injection Volume: 1.0 µL
Detection: UV @ 254 nm

Test Conditions
For Jupiter C4 and C5 columns

Mobile Phase: Acetonitrile/Water (50:50)
Flow Rate: 1.0 mL/min
Injection Volume: 1.0 µL
Detection: UV @ 254 nm

*Columns with dimensions of 50 x 2.0 mm, 30 x 2.0 or 1.0 mm, the mobile phase ratio should be 50:50. Some 50 x 2.0 mm columns use 65:35. For other columns not listed above, see test chromatogram enclosed with column purchased.

Column Warranties

Phenomenex HPLC columns are warranted to meet the stated performance and quality and to be free of defects in material and workmanship. If you are unsatisfied for any reason, please give your Phenomenex Technical Representative a call. We'll do our best to solve the problem to your satisfaction. Should it become necessary to return the column, a Return Authorization Number must be obtained from Phenomenex first.

Column Shock

Handle columns with care. Do not drop or create physical shock. Do not start pump at high flow rates, instead ramp up gradually over a few minutes. Set your pump pressure limit to protect the column in event of blockage. This can create voids which will detrimentally affect the column's performance.

Disclaimers

New columns should be tested with the manufacturers recommended test mix, and previously used columns should be tested with the same or a suitable test mix for the analysis. Remember to re-equilibrate the system when changing solvents. Never change from one solvent to another which is immiscible, without going through an intermediate solvent which is miscible with both. This will damage the column. Never change to (or from) a buffer/salt solution where the buffer/salt is not soluble in the second solvent. Again this will damage the column. Never attempt to remove the column end fittings. This will void the warranty.

Column Questions and Support

If you have any additional questions, please reach out to our amazing technical team through:

Email: support@phxtechnical.zendesk.com

Live Chat: https://www.phenomenex.com/info/page/2015phenomchat

For more information on Jupiter HPLC and Preparative columns, please visit www.phenomenex.com/Jupiter