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ONYX™ Monolithic LC

Onyx HPLC Columns Tips for Care and Use

General Information

Each Onyx 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	Macropore (µm) & Mesopore (Å)	Surface Area (m^2/g)	Carbon Load (%)	End Capping	pH Range
Silica	Monolithic	2, 130	300	0	no	2.0-7.5
C8	Monolithic	2, 130	300	11	yes	2.0-7.5
C18	Monolithic	2, 130	300	18	yes	2.0-7.5
C18*	Monolithic	1.5, 130	300	18	Yes	2.0-7.5
HD-C18	Monolithic	1, 130	300	18	Yes	2.0-7.5

Shipping Solvent

Unless otherwise noted on a column tag, Onyx Silica is shipped in 95:5 n-heptane dioxane and Onyx C18 phases are shipped in 80:20 Methanol: HPLC grade water for the 0.1 x 150 mm format. All other formats of the C18 phase is shipped in 60:40 Acetonitrile: HPLC grade water.

Backpressure limits

Typical Flow Rates (Independent of particle size):

- 1.0-9.0 mL/min for 4.6 mm ID
- 1-3 µL/min for 0.1 mm ID

Max Backpressure:

- For 4.6 mm ID > 3000 psi (206 bar) may compromise column longevity.
- For 0.1 mm ID > 4500 psi (310 bar) may compromise column longevity.

Max Temperature:

- Suggested max temperature for Onyx LC columns is 45 °C, however temperature limits are more so dependent on your running parameters.
- Continuous use of Onyx columns at the maximum temperature limit may compromise column longevity.

Column Installation

Make sure that the mobile phase and system is ready prior to installing the column:

1. Use HPLC grade and above solvents only. Make sure that all solvents used are miscible: in the system, in the sample/standard, and in the mobile phase reservoirs.
2. Mobile phase is properly mixed and degassed prior to putting on the system.
3. Flush the lines with mobile phase. Make sure there are no bubbles in system. You can check by setting the pump at 0.1 mL/min and increase the flow to normal flow rate over 5 minutes, while monitoring the flow from the line to see if there's a steady flow.
4. Make sure all valves, seals and the injection needle is clean.

Capillary Scale (0.1 mm ID)

Onyx capillary columns are equipped with PEEK 1/16" fittings and green sleeves. The fittings and sleeves are compatible with any 360 µm OD fused silica tubing. Onyx capillary columns can be connected directly onto nano/capillary-HPLCs with UV or MS detection with fingertight fitting or PTFE tubing.

1. Connect the column with the direction of flow, as indicated on the column.
2. Connect a 360 µm OD fused silica tubing with the PEEK 1/16" fittings and the green sleeves to tighten the tubing.
3. Make sure that the tubing is seated all the way down into the fittings and that the tubing enters all the way to the bottom of the injector port. Optimize the tubing to minimize the dead volume.
4. Take care to not over bend the capillary.
5. Condition the column for at least 10 column volumes until a steady baseline is achieved.

Analytical Scale (2-4.6 mm ID)

1. Connect the column with the direction of flow, as indicated on the column.
2. Flush column for 5 minutes with 100 % acetonitrile at a flow rate of 4 mL/min.
3. Condition the column until a steady baseline is achieved.

Tips

- Columns can dry out during shipping and stocking. You can use the above procedure to regenerate the column.
- It is recommended to use PEEK fittings to connect 1/16" tubing to Onyx columns.
- Maximum tubing length at end must not exceed 2 mm to avoid damaging of the monolithic column material.
- Change guard cartridge regularly (within 500 injections).

Column Cleaning

Capillary Scale (0.1 mm ID)

Reverse Phase

Flush at 1 µm/min with 95:5 acetonitrile: water.

Analytical Scale (2-4.6 mm ID)

Normal Phase

Flush at 3 mL/min for 5 mins with the following. Do not flush with chlorinated hydrocarbons, DMSO or THF at greater than 50 %. Do not reverse flush.

1. N-heptane
2. (50/50) v/v, N-heptane/dioxane
3. Dioxane
4. (50/50) v/v, N-heptane/dioxane

Analytical Scale (2-4.6 mm ID)

Reverse Phase

Flush at 3 mL/min for 5 column volumes with the following.

1. Water
2. Acetonitrile
3. Isopropanol
4. Heptane
5. Isopropanol
6. Acetonitrile
7. Water

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

Column Regeneration

Normal Phase

Flush at 3 mL/min for 5 column volumes with the following. Do not flush with chlorinated hydrocarbons, DMSO or THF at greater than 50 %. Do not reverse flush.

1. N-heptane
2. (50/50) v/v, N-heptane/dioxane
3. Dioxane
4. (50/50) v/v, N-heptane/dioxane

Reverse Phase

Flush at 3 mL/min for 5 column volumes with the following.

1. 95:5 Acetonitrile: water
2. THF can be used to remove very oily samples but do not exceed 50 % when flushing.

Column Storage Tips

Capillary Scale (0.1 mm ID)

1. Stop the flow and let the column decompress for 5-15 mins.
2. Do not remove the column from the system until the column inlet pressure is less than 70 psi.
3. Make sure the column is clean before storage (i.e. No buffer, ion pairs, acids etc).
4. Flush with water first and then acetonitrile before storage.
5. Make sure the plugs are firmly in place.

Analytical Scale (2-4.6 mm ID)

1. Make sure column pressure is less than 70 psi before taking the column off the system.
2. Make sure the column is clean before storing (i.e. No buffer, ion pairs, acids, etc).
3. Flush with water first and then acetonitrile before storage.
4. Make sure the plugs are firmly in place.

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.

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.

Onyx Monolithic Reversed Phase Test Mix
(For Onyx C8, C18, and HD-C18)

Part No.: AL0-7836

Unit quantity:	2 mL
Contains:	Thiourea 10 µg/mL; Progesterone 100 µg/mL; Anthracene 10 µg/mL
Diluent:	Acetonitrile/Water (60:40)

Test Conditions

Mobile Phase:	Acetonitrile/Water (60:40)
Flow Rate:	2.0 mL/min*
Injection Volume:	1.0 µL
Detection:	UV @ 254 nm
Storage Conditions:	Refrigerate @ 4 °C

* For a 50 x 4.6 mm column

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.

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 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.

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 Onyx HPLC and Preparative columns, please visit www.phenomenex.com/Onyx