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Luna HPLC/UHPLC Columns Tips for Care and Use

General Information

Thank you for selecting the Phenomenex Luna or Luna Omega column. Every Phenomenex manufactured column is individually QC tested and issued a Certificate of Quality Assurance (CQA) which includes the column serial number, testing conditions, operating parameters shipping solvent, and other important column details. CQAs are available online at www.phenomenex.com/QD. You will need your column part number and serial number to download the CQA. We recommend that you enter your column details, including specifications and performance test results into your information management system for easy tracking and reference. If you have questions about your column's quality documentation contact: www.phenomenex.com/chat.

Inspection

Upon receipt of column:

1. Verify that the column is the one you ordered (i.e. dimension, particle size, media).
2. Check the column for any physical damage potentially caused during shipment.
3. Test the column immediately with a QC standard to verify performance.
4. Record the results of your test in your column information management system.

General Information

Luna Omega Phases	Description	Particle Sizes (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	pH Stability	Reversed Phase	Normal Phase	HILIC	IEX
C18	C18 ligand optimized for improved peak shape	1.6, 3, 5	100	260	11	1.5 - 8.5*	☾			
Polar C18	Enhanced selectivity/retention for polar analytes without diminishing useful non-polar retention	1.6, 3, 5	100	260	9	1.5 - 8.5*	☾			
PS C18	Mixed mode functionality offering enhanced retention of polar acids along with improved peak shape for strong bases	1.6, 3, 5	100	260	9	1.5 - 8.5*	☾			
SUGAR	Combined amide polyol /amino stationary phase with polar end-capping offers enhanced HILIC retention of sugars through multiple interaction mechanisms.	3	100	260	<2	2.0-7.0			☾	

* pH stability under gradient conditions. pH stability is 1.5 - 10 under isocratic conditions.

Luna Phases	Description	Particle Sizes (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	pH Stability	Reversed Phase	Normal Phase	HILIC	IEX
Silica(2)	Unbonded silica	3, 5, 10, 10-PREP, 15	100	400	—	2.0 - 7.5		☾	☾	
C5	5 Carbon ligand	5, 10	100	440	12.5	1.5 - 9.0*	☾			
C8(2)	C8 ligand optimized for improved peak shape	3, 5, 10, 10-PREP, 15	100	400	13.5	1.5 - 9.0*	☾			
C18(2)	C18 ligand optimized for improved peak shape	2.5, 3, 5, 10, 10-PREP, 15	100	400	17.5	1.5 - 9.0*	☾			
CN	Versatile CN phase	3, 5, 10	100	400	7.0	1.5 - 7.0	☾	☾		
NH_2	Rugged and reproducible NH_2	3, 5, 10	100	400	9.5	1.5 - 11	☾	☾	☾	☾
Phenyl-Hexyl	Phenyl phase attached to C6 (hexyl) ligand	3, 5, 10, 10-PREP, 15	100	400	17.5	1.5 - 9.0*	☾			
SCX	Benzene sulfonic acid	5, 10	100	400	Binding Capacity: 0.15 meq/g	2.0 - 7.0				☾
HILIC	Reproducible, cross-linked diol	3, 5	200	200	5.7	1.5 - 8.0			☾	
PFP(2)	Pentafluorophenyl with a C3 (propyl) linkage	3, 5	100	400	11.5	1.5 - 8.0	☾		☾	
Luna Polar Pesticides	Proprietary	3	100	380	8	2 - 8	☾		☾	

* pH stability under gradient conditions. pH stability is 1.5 - 10 under isocratic conditions.

Shipping Solvent

Usually, Luna and Luna Omega columns are shipped in a mixture of Acetonitrile/Water. The ratio of Acetonitrile to Water will vary depending on each stationary phase and column dimensions. The shipping solvent for Luna and Luna Omega columns is same as the column QC test solvent which is listed in the individual columns CQA (Certificate of Quality Assurance) provided online.

Unless otherwise noted on a column tag, Luna CN, NH_2 and Silica columns are shipped in Acetonitrile/Hexane (1:99 v/v). Luna SCX columns are shipped in 150 mM Ammonium Phosphate, pH 6.0, Luna HILIC columns are shipped in Acetonitrile/100 mM Ammonium Formate, pH 3.2 (90:10 v/v), and Luna Polar Pesticides columns are shipped in 100% Methanol.

Typical Flow Rate, Backpressure, and Temperature:

In the table below there are some typical values for common dimensions of Luna Omega and Luna HPLC/UHPLC columns for flow rates and backpressure. These values are for reference only and can differ significantly dependent upon LC system, running parameters, and sample analytes/matrix. The values have been obtained using a solvent system of Acetonitrile and Water; the ratio of Acetonitrile to Water varies depending upon the stationary phase and the column dimensions. The flow rate and solvent ratios are optimized to give the best efficiencies for the phase and dimensions.

Particle Size (µm)	Internal Diameter (ID)	Typical Flow (mL/min)	Typical Pressure (PSI)			
			50 mm	100 mm	150 mm	250 mm
1.6	2.1	0.5	4500	9500	11000	NA
3	2.1	0.2	750	2700	1500	2400
3	3.0	0.6	950	2700	1500	2400
3	4.6	1.25	812	3500	1500	2300
5	2.0	0.2	450	NA	650	1000
5	3.0	0.5	450	NA	900	1400
5	4.6	1.0	750	1350	850	1200
10	4.6	5.0	450	NA	350	500
10	21.2	15.0	203	NA	350	500

* Note: Backpressures from H2O/Methanol mixture for same column dimension and flow rate will be approximately 40% higher than H2O/Acetonitrile mixture.

Typical Flow Rates (Independent of particle size):

- 1.0 mL/min for 4.6 mm ID
- 0.2-0.6 mL/min for 2.1 mm ID

Max Backpressure:

- For 1.6 µm >15,000 psi (1,034 bar) may compromise column longevity.
- For Luna Omega 3 µm or 5 µm >5,000 psi (345 bar) may compromise column longevity.
- For Luna 3 µm or 5 µm >5000 psi (345 bar) may compromise column longevity.
- For Luna Polar Pesticides 3 µm >2900 psi (200 bar) may compromise column longevity. Even though Luna Polar Pesticides 3 µm columns can tolerate pressure up to 400 bar, the recommended normal operation pressure is 200 bar. Continuous use at extreme pressure may eventually damage the column and the pump.

Max Temperature:

- The suggested max temperature for Luna LC columns is 60°C. The temperature limits are dependent on the method's running conditions, specifically on the pH of mobile phase. Continuous use of Luna columns at the maximum temperature limit may compromise column longevity.
- The recommended maximum temperature for Luna Omega LC columns is 80°C, however temperature limits are dependent on running parameters such as mobile phase pH and composition. Continuous use of Luna Omega columns at the maximum temperature limit may compromise column longevity. Recommended temperature limits for Luna Omega phases based on common mobile phases are listed below:

	Temperature Limit (Degree Celsius °C)			
Phases	Formix Acid	TFA	Phosphate Buffer (PH 7.5)	Ammonium Bicarbonate Buffer (PH 8.5)
C18	90	80	50	50
Polar C18	90	80	30	30
PS C18	80	60	50	50

Mobile Phase Compatibility

When using any HPLC/UHPLC column, be sure to only use HPLC grade solvents and materials while also avoiding immiscible solvent/buffer combinations.

Additionally, use of solvent filtration is highly recommended to remove trace impurities from your mobile phase of choice. The following Luna phases are stable in 100% aqueous conditions: SUGAR, Polar C18, PS C18, PFP(2), Luna Polar Pestcides, and SCX. All Luna columns, except Luna Omega SUGAR, are stable in 100% organic conditions, but please ensure that mobile phase pH does not exceed individual stationary phase limits. See chart in column characteristics section (on previous page) for individual Luna stationary phase pH limits.

Column Installation

Initial set-up of your LC system is very important to ensure column performance:

- Check that your LC system is ready:
  1. Seals, lines, and injector are clean
  2. Lines primed (no dry lines or bubbles)
  3. Steady baseline
  4. 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:
  1. Ensure that HPLC grade mobile phase is well mixed, filtered, and degassed prior to use.
  2. 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 (2.1mm ID) or 1.0 mL/min (4.6 mm ID) for 5-10/min. 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.
  1. A steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system.
  2. 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.

Testing Column Performance

When testing column performance, please use the manufacturer approved test mix.

Reversed Phase	
Name:	Reversed Phase 2 Test Mix
Part No.:	AL0-3045
Contents:	Uracil, Acetophenone, Toluene, Naphthalene
Solvent:	Acetonitrile/Water (65:35 v/v)
Detection:	UV @ 254 nm
Injection Vol.:	Depends on dimension

Normal Phase	
Name:	Normal Phase Test Mix
Part No.:	AL0-3033
Contents:	meta-Xylene, Nitrobenzene
Solvent:	Hexane/Acetonitrile (99:1 v/v)
Detection:	UV @ 254 nm
Injection Vol.:	Depends on dimension

Column Cleaning

Reverse Phase:

- Clean with a gradient that is the 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, after the column has been flushed with Acetonitrile. When using IPA, ensure use of a low flow to prevent higher backpressures due to higher solvent viscosity.
- For materials that are very hydrophobic, try THF instead.

Normal Phase:

- Rinse with 10 column volumes of Chloroform, IPA, and/or Methylene Chloride.
- Then condition with mobile phase.

HILIC:

- To remove buffer, rinse with at least 10 column volumes of 95:5 Water:Acetonitrile. Repeat with 95:5 100 mM Ammonium Acetate (pH 5.8):Acetonitrile. Then finish cleaning by flushing the column with 95:5 Water:Acetonitrile.

Tips:

- When cleaning, set your flow rate lower than that of your method flow rate. Especially when attempting to clean using methanol or IPA.
- Cleaning for a longer period of time is more beneficial than adding more cycles.
- Working with very high amounts of THF is not recommended especially if system has plastic tubing. Cleaning with THF is fine if the tubing are metal.
- Try reverse flushing the column; slow flow against the direction of the arrow on the column label. Here are suggested reverse flush flow rates based on column ID:

  0.1 mL/min (2.1 mm ID)
  0.3 mL/min (3.0 mm ID)
  0.5 mL/min (4.6 mm ID)

Column Regeneration

Reversed Phase:

- Apply the same gradient flush as in the cleaning above, overnight at low flow.

Normal Phase:

- For water removal:

  1. Flush with 30 mL 2.5% 2,2 Dimethoxypropane and 2.5% Glacial Acetic Acid in Hexane.
  2. Then flush column with cleaning method from above, overnight at low flow rate.

For Luna Polar Pesticides:

Flush the analytical column using about 30 mL of each the solvent listed below:

1. 100% Methanol
2. 100% Acetonitrile
3. 75% Acetonitrile + 25% Isopropanol
4. 100% Isopropanol

If the column is flushed with hexane or dichloromethane, use isopropanol as a switching solvent before using any reverse-phase mobile phase.

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:

- Reversed phase: Acetonitrile/Water (65:35 v/v), or Methanol can be used in place of acetonitrile.
- Normal phase: 100% Hexane or IPA
- Ion-Exchange: 100% Methanol
- HILIC: Acetonitrile/Water (80:20 v/v)

Typical Loading Capacities

In the table below, typical values for loading capacities and injection volumes for different column IDs are shown for generic fully porous particle. These are general values and are provided for reference only, loading capacity of any column will change depending on the running conditions of your method.

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)

Tips for Extending Column Lifetime

- Routine cleaning of the column will help maintain the column life times.
- Always use the column within recommended pH and temperature limits.
- 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.
- Do not overload your column. Inject suitable sample concentrations and volumes. See chart above: Typical Loading Capacities
- Work in the appropriate separation mode for the column. Please see column characteristic chart for typical modes each stationary phase is used for.
- Store your column in appropriate solvent(s).
- Solvent switch correctly by slowly acclimating the phase from one miscible solvent to the other at a low flow: 0.1 mL/min for 2.1 mm ID and 0.5 mL/min for 4.6 mm ID.

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, reach out to our team of chromatography experts via live chat.

Live Chat: https://www.phenomenex.com/chat

For more information on Luna UHPLC, HPLC, and preparative columns, please visit www.phenomenex.com/Luna

www.Phenomenex.com/Luna

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