Tips for Column Care and Use

LUNA™ Polar Pesticides

Congratulations on your purchase of the Luna™ Polar Pesticides HPLC Column! Below are recommended instructions for the care and use of this column.

General Information

Each Luna Polar Pesticides HPLC 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.

Inspection

Upon receipt of the column, please verify that the column you received matches your order (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

Phases	Ligand	Particle Size (µm)	Pore Size (Å)	Surface Area (m2/g)	Carbon Load (%)	pH stability	Recommended Mode
Luna Polar Pesticides	Proprietary	3	100	380	8	2-8	Reverse Phase Hilic

Shipping Solvent

Luna Polar Pesticides Columns are shipped in 100% Methanol.

Typical Flow Rate, Backpressure, and Temperature

Here are some typical values for common dimensions of Luna Polar Pesticides HPLC column. These numbers are not absolute values and can differ based on the LC system, running parameters, and sample analytes/matrix. The values below have been created using a solvent system of Acetonitrile and Water.

Particle Size (µm)	Internal Diameter (ID)	Typical Flow (ml/min)	Typical Pressure (PSI)		
			50 mm	150 mm	250 mm
3	2.0	0.2	750	1500	2400
3	3.0	0.6	950	1500	2400
3	4.6	1.0	812	1500	2300

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

Even though Luna Polar Pesticides 3 µm columns can tolerate pressure up to 400 bar, the recommended normal operation pressure is 200 bars. 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; however, temperature limits are dependent on your running parameters.
- Continuous use of Luna Polar Pesticides columns at the maximum temperature limit may compromise the column's longevity.

Mobile Phase Compatibility

When using any HPLC column, be sure to only use HPLC grade solvents and materials while also avoiding immiscible solvent/buffer combinations. Additionally, the use of solvent 0.2-0.45µm membrane filtration is highly recommended to remove trace impurities from your mobile phase of choice.

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

Mobile phase starting conditions check list:

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

- Set the 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 minutes. Collect the solvent in a small beaker.
- Stop the flow and wipe the outlet end of column to remove any particulates before connecting to the detector.
- Install the fitting/tubing into the outlet end and run a 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 the pressure as well as the signal from the detector. When both are steady, the column is ready for use.

Column Cleaning

Reverse Phase

- 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 Isopropyl alcohol 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.

HILIC

- To remove buffer, rinse with at least 10 column volumes of 95:5 Water/ Acetronitile.

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 (~30 min)
- 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)

Tips for Extending Column Lifetime

- 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. For Luna Polar Pesticides HPLC columns, the optimal column guard is SecurityGuard ULTRA (part No. 000-4798-AN)
- Do not overload your column. Inject suitable sample concentrations and volumes. See chart in previous page.
- 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 Shock

Handle your HPLC 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.

Regeneration

Over time, it is expected that the column backpressure may increase slightly. However, sudden increase in backpressure suggests that the column inlet frit may be clogged by physical particulates or large molecules. This will result in packed bed collapsing and increased dead volume. When this occurs, the column efficiency decreases, and peaks become distorted and may come out at different retention times. In this case, column back flushing can be applied by allowing the flow entering the column in a reversed direction at very low flow rate, such as 0.1 mL/min using appropriate solvents (high percentage of aqueous in many cases). Below is a generic approach for different types of column regeneration.

Regeneration of a reverse-phase column

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

- 100% Methanol
- 100% Acetonitrile
- 75% Acetonitrile + 25% Isopropanol
- 100% Isopropanol

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

Have more questions about your column care, installation, method development, or any other analytical challenge?

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