Gemini HPLC/UHPLC Columns Tips for Care and Use

General Information

Each Gemini 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. dimensions, 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

Gemini Phases	Shipping Solvent†	Particle Sizes (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	pH Stability	Reversed Phase	Normal Phase	HILIC	100% Aqueous Stable
C18	Acetonitrile/Water (65:35)	3,5,10	110	375	14	1.0-12.0*	✓			✓
NX-C18	Acetonitrile/Water (75:25)	3,5,10	110	375	14	1.0-12.0*	✓			✓
C6-Phenyl	Acetonitrile/Water (65:35)	3,5	110	375	12	1.0-12.0*	✓			

† Depending on column dimensions, organic to aqueous solvent ratios may vary slightly.
* pH stability under gradient conditions. pH stability is 1.5-10 under isocratic conditions.

Typical Flow Rate, Backpressure, Temperature:

Here are some typical values for common dimensions of Gemini HPLC and UHPLC columns. These numbers are not absolute values and can differ based on 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 (mm)	Typical Flow (mL/min)	Typical Pressure (PSI)		
			50 mm**	150 mm**	250 mm**
3	0.3	0.005	500	1000	NA
3	0.5	0.015	700	1400	NA
3	1.0	0.05	800	1000	NA
3	2.0	0.2	650	1550	NA
3	3.0	0.5	950	1900	2700
3	4.6	1	1000	1700	2600
5	2.0	0.2	400	650	850
5	3.0	0.5	550	1100	1550
5	4.6	1	1400	1500	1950
5	10.0	5	1400	1600	1800
5	21.2	20	500	800	1400
5	30.0	40	500	900	1200
10	4.6	1	NA	NA	1400
10	10.0	5	NA	NA	1400
10	21.2	20	NA	400	500
10	30.0	40	NA	400	600
10	50.0	50	NA	300	300

** Column Length

Typical Flow Rates (Independent of particle size):

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

Maximum Backpressure:

iii. > 5,000 psi (345 bar) may compromise column longevity.

Maximum Temperature:

iv. Recommended max temperature for Gemini LC columns is 60°C, however temperature limits are dependent on your running parameters. Running at a pH greater than 8 at 60°C will compromise column lifetime.
v. Continuous use of Gemini columns at the maximum temperature limit may compromise column longevity.

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. Gemini NX-C18 columns are stable in 100% aqueous conditions, but for all Gemini columns please ensure that mobile phase pH does not exceed individual stationary phase limits. See chart in column characteristics section for individual Gemini stationary phase pH limits.

Column Installation

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

Ensure that your LC system is ready:

1. Seals, lines, injector 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 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.

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 dimensions

* See next page for suggested loading capacities based on ID.

Column Cleaning

Reversed 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 (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 Tetrahydrofuran (THF) instead.

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 often more beneficial than adding more cycles.
- Working with very high amounts of THF is not recommended especially if system has PEEK tubing. Cleaning with THF is fine if the tubing are metal.
- Reverse flushing the column (against the direction of the arrow on the column), but reduce the flow. 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.
- Reverse flushing is acceptable.

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), Methanol can be used in place of acetonitrile.

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.
- Do not overload your column. Inject suitable sample concentrations and volumes.
- Work in the appropriate separation mode for the column. Please see Column Characteristics 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, 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 Gemini UHPLC, HPLC, and Preparative columns, please visit www.phenomenex.com/Gemini

Trademarks

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Gemini is patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362
CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

Strata-X is patented by Phenomenex. U.S. Patent Nos. 7,119,145

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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)

*The column Dead Volume (Vo) may be estimated from:
Column Dead Volume (mL) = Vo = 0.487 x d^2 x L

Where: L = column length (cm); 15 cm (150 mm) used for calculation.
d = column ID (cm, not mm)

**The maximum allowable Sample Injection Volume (Vi) can be estimated as follows: Maximum Injection Volume $= Vi = \frac{Vr}{2\sqrt{N}}$

Where: Vr = the retention volume of the first peak (mL)
N = number of theoretical plates per column

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