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Phenogel™
Organic GPC/SEC

Phenogel HPLC Columns Tips for Care and Use

General Information

Each Phenogel 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). Below is the column selection chart by molecular weight for each pore size. Please double check to make sure you ordered the appropriate pore size for your exclusion range. 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

Phenogel Column (Å)	Molecular Weight (Da)	Sample Type
50	100-3,000	Small Organics
100	500-6,000	
500	1,000-15,000	
10^3	1,000-75,000	Resins
10^4	5,000-500,000	
10^5	10,000-1,000,000	
10^6	60,000-10,000,000	High MW Polymers
Linear (2)	100-1,000,000	

Column Operating Recommendations

	Maximum Temperature (°C)	Maximum Pressure (psi)	Shipping Solvent	Mobile Phase Additives Max Tolerance (%)	Column Storage +++
Phenogel Phases	140+	1500	THF++	Water: 1 Low MW Amine: 0.5 Glacial Acetic Acid: 1	THF, Chloroform, Methylene Chloride, Toluene

+Temperature limits may vary depending on operating solvents and pore size, please see the chart below on more specific information.

++Phenogel columns are packed and shipped in Tetrahydrofuran (THF) but columns can also be shipped in solvents such as DMF, Methlyene Chloride, NMP, and o-CP

+++Store in solvent swell volume that is closest to that of the mobile phase to reduce conditioning for use again.

Solvent Compatibility Table

Mobile Phase Solvent	Phenogel Pore Size: (Å) 50	100	500	10^3	10^4	10^5	10^6	Linear & Mixed	Suggested Operating Temp.
Acetone	Y	Y	Y	Y	Y	Y	Y	Y	
Benzene	Y	Y	Y	Y	Y	Y	Y	Y	
Carbon Tetrachloride	Y	Y	Y	Y	Y	Y	Y	Y	
Chloroform	Y	Y	Y	Y	Y	Y	Y	Y	
30 % HFIP/Chloroform	Y	Y	Y	Y	Y	Y	Y	Y	
Diethyl Ether	Y	Y	Y	Y	Y	Y	Y	Y	
Dimethylacetamide (DMAC)	N	Y	Y	Y	Y	Y	Y	Y	60 °C
Dimethylformamide (DMF)	N	Y	Y	Y	Y	Y	Y	Y	60 °C
Dioxane	Y	Y	Y	Y	Y	Y	Y	Y	
DMSO	N	Y	Y	Y	Y	Y	Y	Y	60 °C
Ethyl Acetate	Y	Y	Y	Y	Y	Y	Y	Y	
Hexafluoroisopropanol (HFIP)	Y	Y	Y	Y	Y	Y	Y	Y	
Hexane	Y	Y	Y	Y	Y	Y	Y	Y	
M-Cresol	N	Y	Y	Y	Y	Y	Y	Y	100 °C
Methyl Ethyl Ketone	Y	Y	Y	Y	Y	Y	Y	Y	
Methylene Chloride	Y	Y	Y	Y	Y	Y	Y	Y	
0-Chlorophenol	N	Y	Y	Y	Y	Y	Y	Y	100 °C
0-Dichlorobenzene	N	Y	Y	Y	Y	Y	Y	Y	135 °C
Quinolin	N	Y	Y	Y	Y	Y	Y	Y	60 °C
Tetrahydrofuran	Y	Y	Y	Y	Y	Y	Y	Y	
Toluene	Y	Y	Y	Y	Y	Y	Y	Y	
Trichlorobenzene	N	Y	Y	Y	Y	Y	Y	Y	135 °C
Water	N	N	N	N	N	N	N	N	
Xylene	Y	Y	Y	Y	Y	Y	Y	Y	

N = Not Compatible
Y = Compatible

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 the LC system (pump and line) with HPLC grade mobile phase or solvent switching solvents, making sure all solvents in the system and column are miscible. Please make sure before installing the column that your running conditions are compatible with the shipping solvent, THF and that solvent switching is not necessary. You can find a chart of similar swell volumes below in the solvent switching section.

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 and compatible in terms of swell volume.

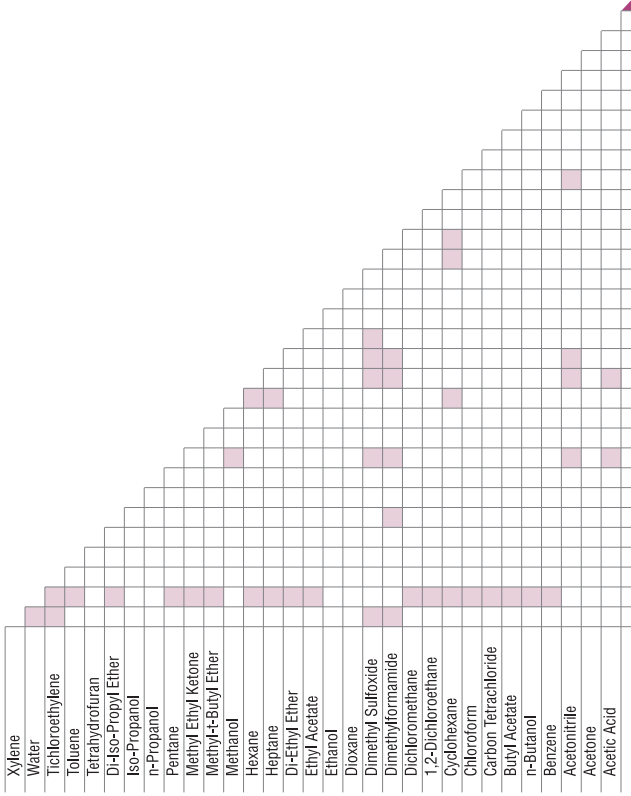
Installation Steps

1. Set the flow rate to 0.1 mL/min, oven temperature ambient.
2. Install the column in the direction of flow and condition the column for 10 minutes going to 1.0 mL/min.
3. Stop flow and wipe outlet end of column to remove any particulates before connecting to detector.
4. Install the column fully and flush for at least 10 column volumes.
5. Ramp flow to method flow and set oven temperature.
6. Monitor for pressure and baseline. When both are steady, the column is ready for use.
   a. A steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system.
   b. Wide fluctuations in pressure may shock and damage the column so it's important to observe the pressure.

Tips

- Always recondition the column when solvent changing, taking into account the solvents in the lines and the column.
- When working with a 30 % swell solvent, for example, do not switch to a 70-80 % swell volume solvent without using a 60 % or 50 % swell volume solvent. Please see the chart for solvent switching.

Solvent Miscibility Table



Solvent	Polarity Index	Refractive Index @ 20°C	UV(nm) Cutoff @ 1AU	Boiling Point (C°)	Viscosity (cPoise)	Solubility in Water (% w/w)
Acetic Acid	6.2	1.372	230	118	1.26	100
Acetone	5.1	1.359	330	56	0.32	100
Acetonitrile	5.8	1.344	190	82	0.37	100
Benzene	2.7	1.501	280	80	0.65	0.18
n-Butanol	4.0	1.394	254	125	0.73	0.43
Butyl Acetate	3.9	1.399	215	118	2.98	7.81
Carbon Tetrachloride	1.6	1.466	263	77	0.97	0.08
Chloroform	4.1	1.446	245	61	0.57	0.815
Cyclohexane	0.2	1.426	200	81	1.00	0.01
1,2-Dichloroethane[1]	3.5	1.444	225	84	0.79	0.81
Dichloromethane[2]	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide[3]	7.2	1.478	268	189	2.00	100
Dioxane	4.8	1.422	215	101	1.54	100
Ethanol	5.2	1.360	210	78	1.20	100
Ethyl Acetate	4.4	1.372	260	77	0.45	8.7
Di-Ethyl Ether	2.8	1.353	220	35	0.32	6.89
Heptane	0.0	1.387	200	98	0.39	0.0003
Hexane	0.0	1.375	200	69	0.33	0.001
Methanol	5.1	1.329	205	65	0.60	100
Methyl-t-Butyl Ether[4]	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone[5]	4.7	1.379	329	80	0.45	24
Pentane	0.0	1.358	200	36	0.23	0.004
n-Propanol	4.0	1.384	210	97	2.27	100
Iso-Propanol[6]	3.9	1.377	210	82	2.30	100
Di-Iso-Propyl Ether	2.2	1.368	220	68	0.37	
Tetrahydrofuran	4.0	1.407	215	65	0.55	100
Toluene	2.4	1.496	285	111	0.59	0.051
Trichloroethylene	1.0	1.477	273	87	0.57	0.11
Water	9.0	1.333	200	100	1.00	100
Xylene	2.5	1.500	290	139	0.61	0.018

Immiscible

Miscible

Immiscible means that in some proportions two phases will be produced

Synonym Table
[1] Ethylene Chloride
[2] Methylene Chloride
[3] Methyl Sulfoxide
[4] tert-Butyl Methyl Ether
[5] 2-Butanone
[6] 2-Propanol

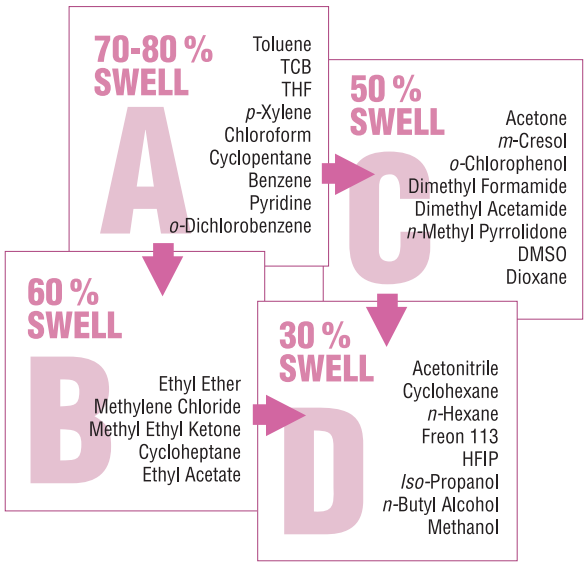
Solvent Switching

Although Phenogel™ columns are rugged and can withstand strong solvent changes, it is recommended that care be exercised when switching from high-swell solvents to low-swell solvents as improper solvent switching may result in a void. Here's our recommended solvent switching procedure:

1. First check that all solvents in the system and column are miscible and comparable in terms of swell volume (i.e. of the same swell volume). Below right is a chart for solvent miscibility.
2. Set the flow rate to 0.2 mL/min
3. Install the column and monitor the pressure, making sure that pressure does not exceed 1000 psi during the solvent switch.
4. Flush for at least 20 column volumes for each step to solvent switch form 70-80 % swell to 50 % swell to 30 % swell, for example.
5. When solvent switch is complete, condition at mobile phase conditions until steady baseline and pressure is observed
6. Slowly ramp up flow rate to method flow rate and temperature, keeping in mind temperature limits (see following chart).

Tips

- To help preserve column lifetime, dedicate one column to solvents within the same swell volume groups, keeping mind that having the same swell volume does not mean miscibility. If possible, dedicate one column to one solvent and always check your logs before use.
- If you accidentally went from a 70-80 % swell volume to that of a 30 % swell volume or vice verse, a void was likely created but you can try finding a solvent that is miscible with both in the 50 or 60 % swell volume category and try to regenerate the column.



Column Cleaning

Clean with mobile phase or a stronger organic solvent of comparable swell volume. Reverse flushing is acceptable though it is recommended to reduce the flow rate to half as to avoid shocking the column.

Tips

You can check whether the cleaning was successful by using the following procedure and comparing to the Certificate of Quality Assurance (CQA) found on our website.

- Sample: Acetone
- Mobile Phase: THF
- Flow Rate: 2 mL/min for 10 um Phenogel™ Columns
- 1 mL/min for 5 µm Phenogel Columns
- Injection: 1 µL

Column Regeneration

Flush the column at low flow overnight with your mobile phase to regenerate.

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.

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.

Tips

- Check before injecting your sample that the analyte and sample matrix is soluble in the mobile phase. Having low levels of mobile phase additives in your sample is fine as long as it is at the levels suggested in the preceding chart.
- Do not overload the column. See Typical Loading Capacities table below for loading parameters.

Typical Loading Capacities

Column Type	ID (mm)	Max Flow Rate (mL/min)	Typical and Max Injection Masses (mg)	Typical and Max Injection Volumes (µL)
Narrow Bore	4.6	0.35	0.1 (2.5)	10 (200)
Analytical	7.8	1.0		
Preparative	21.2	200	5(500)	200 (5000)

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 Phenogel HPLC columns, please visit www.phenomenex.com/Phenogel

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