ThermoFisher SCIENTIFIC

HPLC columns

Hypersil GOLD Peptide columns

Product manual

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Contents

Introduction

Introduction to the Hypersil GOLD Peptide column

Thank you for choosing a Thermo Scientific™ Hypersil GOLD™ Peptide column. The Hypersil GOLD Peptide columns packing materials are specially designed to be used with HPLC and UHPLC systems.

Each batch of Hypersil GOLD Peptide column material is chromatographically tested with acidic, basic, and neutral analytes and has been qualified for peptide mapping.

The results are held to narrow specification ranges to ensure excellent and reproducible performance.

Every column is individually tested, and a Performance Chromatogram and Certificate of Batch Analysis are provided in the column box. Additionally, each batch of Hypersil GOLD Peptide media is QC tested with the separation of a protein digest.

Getting started

The Certificate of Analysis is specific to each batch of packing material contained in the Hypersil GOLD Peptide column. It includes the silica batch number, and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains important information such as the silica batch number, column serial number, plate count, tailing factor, capacity factor, and chromatographic conditions. It is recommended that these data are stored for future reference.

Column connectors

The Thermo Scientific™ Vanquish™ HPLC and UHPLC systems are equipped with Thermo Scientific™ Viper™ Fingertight Fittings of optimized length and inner diameter minimizing extra-column volume to maintain the resolution achieved by the column from sample injection to detector inlet by virtually dead-volume free connection.

The installation of Viper fingertight fittings is tool-free. Tighten the flow connection with the black knurl screw until a first resistance appears. Further tightening by approximate 45° will usually seal the connection. When installing the column, take the Viper fittings at both ends of the column and twist them simultaneously in opposite directions to an angle of 0° to 90° maximum on each side. Overtightening is to be avoided; no wrench is required. Please open and close all Viper connections at room temperature without flow rate applied.

For detailed information on installation and operation of Viper fingertight fittings, please refer to the Viper installation and operation guide. For detailed information on optimized standard capillary length, please refer to the Vanquish LC system manuals. All manuals and guides can be accessed via HPLC manuals and quick installation guides **website**.

System considerations

With 1.9 µm particles, analyses can be performed with a high linear velocity through the column without loss in performance, provided the LC system is optimized to operate under these conditions. To produce fast, efficient chromatography, all system components for the assay should also be considered. Modern UHPLC instruments, including the Vanquish UHPLC systems, will take account of these factors.

There are three major system considerations to remember when using short columns packed with 1.9 µm particles.

1. The system volume (connecting tubing I.D. and length, injection volume, UV detector flow cell volume) must be minimized.
2. The detector time constant and sampling rate need to be carefully selected.
3. When running fast gradients pump delay volume needs to be minimal.

Getting started (continued)

Column installation

1. Purge the solvent lines, and then connect the inlet end of the column to the injector outlet.
2. Next, flush the column with 100% organic mobile phase (either methanol or acetonitrile). To do this, set the pump flow rate to 0.1 mL/min and gradually increase it to 0.4 mL/min over a period of five minutes.
3. Once the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the detector. This will prevent air from entering the detection system and allow for faster baseline equilibration.
4. Gradually increase the flow rate as described in step two.
5. Finally, once a steady backpressure and baseline have been achieved, proceed to the next section.

Column equilibration/conditioning

Hypersil GOLD Peptide columns are shipped in a solution of 50/50 v/v ACN/H_2O. It is crucial to ensure that the new mobile phase system is compatible before switching from the current one. To do this, it is necessary to equilibrate the column with at least 10-column volumes of the new mobile phase. For more information on column volumes, please refer to Table 1.

Table 1. Empty column* volumes in mL (multiply by 10 for flush solvent volumes)*

Column length (mm)	Column volume (mL)
50	0.2 mL
100	0.4 mL
150	0.5 mL

* All columns are 2.1 mm I.D.

Table 2. Example column conditioning method for formic acid

Mobile phase	Flow rate (mL/min)	Column temp. (°C)	Time (hrs)	After conditioning
95/5 v/v H_2O/ACN + 0.1% formic acid	0.4	55	1	Continue to analysis or flush and store in 80/20 v/v ACN/H_2O

Before introducing a buffered mobile phase, it's important to flush the column with a water/organic solvent mixture that has the same or lower organic solvent content. This prevents the buffers from precipitating on your column or in your system. To do this, flush the column and system with five column volumes of the water/organic solvent mixture. For instance, if you use 60% methanol/40% buffer mobile phase, flush the column and system with 60% methanol in water first.

Please remember that if any additives in the mobile phase are at low concentrations, such as ion-pairing reagents, it may take around 50 column volumes to equilibrate completely.

Getting started (continued)

Procedure for using new, out-of-box columns

Before using a new column, it is crucial to confirm that it produces consistent chromatography and the desired level of chromatographic resolution. To achieve this, it is helpful to test the column's performance using a sample that represents the intended application. The number of injections required to achieve consistent performance may vary based on the sample characteristics and the LC system used. Method variables such as pH, mass load, ionic strength, and ion pairing can also influence the results.

Initial column efficiency determination

1. Before using the column, it is recommended that you perform an efficiency test. Thermo Fisher suggests analyzing the column upon receipt using a suitable solute mixture, as found in the "Performance Test Chromatogram".

2. Determine the number of theoretical plates (N) and use this value for periodic comparisons of performance over time.

3. To monitor the column's performance over time, repeat the test at pre-determined intervals. Note that slight variations may occur on two different HPLC systems due to factors such as the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

Column use

To ensure optimal performance of Hypersil GOLD Peptide columns, please follow these guidelines.

Temperature

For the optimal performance of Hypersil GOLD Peptide columns, operating them at temperatures ranging between 20–60 °C is recommended. This helps to improve selectivity, reduce solvent viscosity, and increase mass transfer rates. If you operate at high pH, it is advisable to use lower operating temperatures as this will prolong the column's lifetime. However, if you work at high temperatures (e.g. >70 °C), the column's lifetime may be shorter.

Solvents

For optimal column performance, it is recommended to use high-quality chromatography grade solvents. It is important to filter all aqueous buffers before use, as solvents that contain suspended particulate materials can clog the outside surface of the inlet distribution frit of the column. This can lead to an increase in operating pressure and reduced performance.

Sample preparation

1. The sample needs to be dissolved in a diluent that is compatible with the initial strength of the mobile phase.

2. The sample must be completely dissolved and free of any particles.

3. If the sample contains particles, it can be filtered using a 0.2 µm membrane. However, if the sample is dissolved in a solvent that contains an organic modifier such as acetonitrile or methanol, it is important to make sure that the membrane material does not dissolve in the solvent. You can reach out to the membrane manufacturer to confirm compatibility. Alternatively, you may consider centrifuging the sample for 20 minutes at 8000 rpm and transferring the supernatant liquid to an appropriate vial.

4. If your sample is protein based, please visit **thermofisher.com/smartdigest** for information on digestion protocols

Column use (continued)

Pressure

Hypersil GOLD Peptide columns can tolerate pressures of up to 21756 psi (1500 bar or 150 Mpa) for 100 and 150 mm length and 14503 psi (1000 bar or 100 Mpa) for 50 mm length.

pH range

The recommended operating pH range for Hypersil GOLD Peptide columns is 2 to 11. Table 3 provides a list of commonly used buffers and additives. It is important to note that the column lifetime may vary depending on the operating temperature and the type and concentration of buffer used. For example, using phosphate buffer at a pH of 8 or higher in combination with elevated temperatures can shorten the column's lifetime.

Table 3. Buffer recommendations for using Hypersil GOLD Peptide columns from pH 2 to 11

Additive/buffer	pKa	Buffer range	Volatility	Used for mass spec	Comments
TFA	0.3	–	Volatile	Yes	Ion pair additive, can suppress MS signal, used in the 0.02–0.1% range
Formic acid	3.75	–	Volatile	Yes	Maximum buffering obtained when used with ammonium formate salt. Used in 0.1–1.0% range
Acetic acid	4.76	3.76–5.76	Volatile	Yes	Maximum buffering obtained when used with ammonium acetate salt. Used in 0.1–1.0% range
Acetate (NH_4CH_2COOH)	4.76	8.2–10.2	Volatile	Yes	Used in 1–10 mM range. Note that sodium or potassium salts are not-volatile
Ammonium (Acetate)	9.2	8.2–10.2	Volatile	Yes	Used in 1–10 mM range
Ammonium (Formate)	9.2	2.75–4.75	Volatile	Yes	Used in 1–10 mM range
Formate (NH_4COOH)	3.75	1.15–3.15	Volatile	Yes	Used in 1–10 mM range. Note that sodium or potassium salts are not-volatile
Phosphate 1	2.15	6.2–8.2	Non-volatile	No	Traditional low pH buffer, good UV transparency
Phosphate 2	7.2	8.7–10.7	Non-volatile	No	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime
4-Methylmorpholine	~8.4	9.3–11.3	Volatile	Yes	Generally used 10 mM or less

Column use (continued)

Table 3. Buffer recommendations for using Hypersil GOLD Peptide columns from pH 2 to 11 (continued)

Additive/buffer	pKa	Buffer range	Volatility	Used for mass spec	Comments
Ammonium (Hydroxide) (NH_4OH)	9.2	6.8-11.3	Volatile	Yes	Keep concentration below 1 mM and temperature below 30 °C
CAPSO	9.7	9.5-11.5	Non-volatile	No	Zwitterionic buffer, compatible with acetonitrile, used in 1–10 mM range. Low odor
1-Methylpiperidine	10.2	9.7-11.7	Volatile	Yes	Used in 1–10 mM range
Ammonium bicarbonate	10.3 (NCO_3) 9.3 (NH_4^+) 6.3 (H_2CO_3)	6.8-11.3	Volatile	Yes	Used in 5–10 mM range (for MS work keep source > 150 °C). Adjust pH with ammonium hydroxide or acetic acid. Good buffering capacity at pH 10. Note: Use ammonium bicarbonate (NH_4HCO_3), not ammonium carbonate ($(NH_4)_2CO_3$)
CAPS	10.4	9.5-11.5	Non-volatile	No	Zwitterionic buffer, compatible with acetonitrile, used in 1–10 mM range. Low odor
Triethylamine	10.7	9.7-11.7	Volatile	Yes	Used in 1–10 mM range. Volatile only when titrated with acetic acid (not hydrochloric or phosphoric acid)
Pyrrolidine (as acetate salt)	11.3	10.3-12.3	Volatile	Yes	Used as ion-pair for DNA analysis at pH 7–9. Mild buffer, gives long lifetime
Glycine	2.4, 9.8	8.8-10.8	Non-volatile	No	Zwitterionic buffer, can give longer lifetime than borate buffer

Column care

Cleaning and regeneration

If you notice any changes in the peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution or increasing backpressure, it may indicate that there is contamination in the column. Flushing with a neat organic solvent is usually sufficient to remove the contaminant. However, if the flushing procedure does not solve the problem, you can flush the column using the following cleaning and regeneration procedures. Use the cleaning routine that matches the properties of the samples and/or what you believe is contaminating the column (refer to Table 4). Flush columns with 20-column volumes of HPLC-grade solvents with 0.2 mL/min flow rate. Increasing column oven temperature to 35–55 °C can also increase cleaning efficiency. Please contact your local Thermo Fisher Scientific office for additional support if the column performance remains poor after cleaning and regenerating.

Table 4. Column cleaning options

Polar samples	Proteinaceous digest samples
Water	**Option 1:** Repeatedly inject 100 µL aliquots of dimethyl sulfoxide (DMSO) using a reduced flow rate, delivering 50% Eluent A and 50% Eluent B
Methanol	**Option 2:** Gradient of 10% to 90% B where: A = 0.1% trifluoroacetic acid (TFA) in water; B = 0.1% trifluoroacetic acid (TFA) in acetonitrile (CH_3CN)
Isopropanol	**Option 3:** Flush column with 7 M guanidine hydrochloride, or 7 M urea

Note: To prevent precipitation in your column, flush it with 5–10 column volumes of water before using the suggested organic eluent column wash procedures, especially if your separation eluent contains phosphate buffer.

Storage

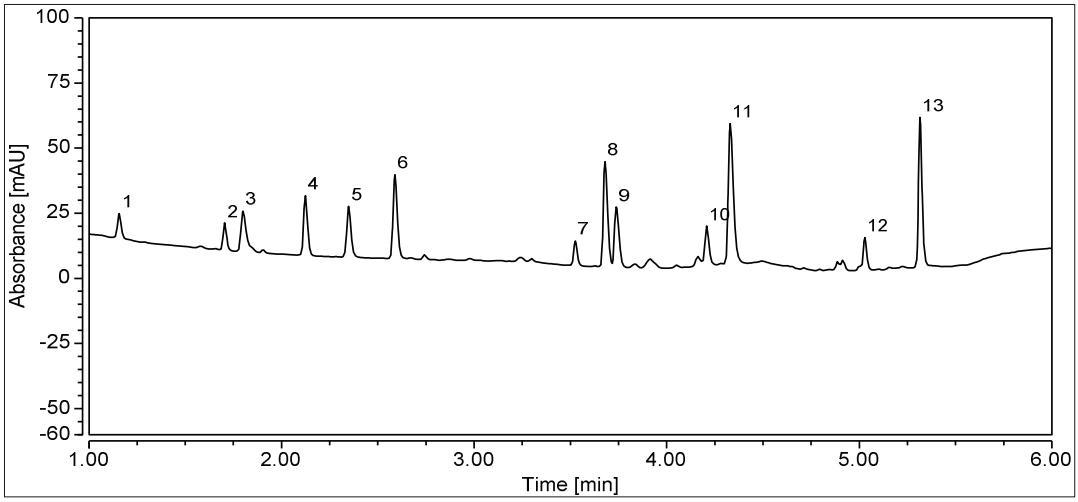
When storing the column for longer than four days at room temperature, storing it in 80/20 v/v ACN/H_2O is recommended. For applications that require elevated temperature, it is best to store the column immediately after use in 80/20 v/v ACN/H_2O to ensure the best column lifetime. Avoid storing the column in buffered eluents. If the mobile phase has buffer salt, flush the column with 10 column volumes of HPLC-grade water (refer to Table 1 for common column volumes) and replace it with 80/20 v/v ACN/H_2O for storage. Failure to perform this intermediate step could result in the precipitation of the buffer salt in the column when high % organic solvent is introduced. Ensure the column is completely sealed with the column plugs provided with each column to avoid evaporation and drying out of the bed. Please note that if a column has been run with a mobile phase that contains formate (e.g. ammonium formate, formic acid, etc.) and is then flushed with 80/20 v/v ACN/H_2O, it may require slightly longer equilibration times when re-installed and run again with a formate-containing mobile phase.

Chromatogram test and conditions

Batch Part Number: **26002-B**
Product Name: **Hypersil GOLD Peptide**
Batch Number: **21607**

Chromatography Test Conditions

Test Column Dimensions	50 x 2.1 mm		
Mobile Phase A	H2O + 0.1% FA		
Mobile Phase B	60/40 (v/v) ACN/H2O + 0.85% FA		
Flow Rate (mL/min)	0.4		
Injection Volume (µl)	5.0		
Wavelength (nm)	214		
Oven Temperature (°C)	40		
Sample	Cytochrome C Digest		
Peaks	1. Ac-GDVEK	6. TGQAPGFSYTDANK	11. IFVQKCAQCHTVEK
	2. KYIPGTK	7. GEREDLIAYLKK	12. GITWGEETLMEYLENPKK
	3. YIPGTK	8. TGPNLHGLFGR	13. GITWGEETLMEYLENPK
	4. IFVQK	9. MIFAGIK	
	5. KTGQAPGFSYTDANK	10. EDLIAYLK	
Eluent Profile	Time (min)	A (%)	B (%)
	0.00	95	5
	3.80	34.7	65.3
	5.50	56.5	43.5
	6.80	0	100
	7.50	0	100
	9.50	95	5



Chromatography Result

QC Parameter	Batch Result	Result
Asymmetry Peptide 1	1.2	PASS
Asymmetry Peptide 2	1.2	PASS
Asymmetry Peptide 6	1.3	PASS
Resolution 2/3	2.4	PASS
Resolution 8/9	1.4	PASS
Resolution 10/11	2.9	PASS

Cautionary note

1. These products may be hazardous after use, depending on the user's application. Therefore, it is recommended that they are only used by professional laboratory personnel who are trained in handling such materials. The purchaser and user are solely responsible for ensuring the safe use and disposal of the products.
2. All connection points in liquid chromatographic systems are potential sources of leaks. Users should be aware of the toxicity or flammability of their mobile phases.
3. Because of the small particle size, dry column packings are respirable. Thermo Fisher Scientific advises against removing the column end fittings and exposing the media. Columns should only be opened by trained personnel in a well-ventilated area.
4. Please adhere to the operating pressure limits noted for each column above. Exceeding these limits will compromise chromatographic performance and column lifetime and could be unsafe.

Ordering information

Ordering information

Description	Length × I.D. (mm)	Cat. no
Hypersil GOLD Peptide column, 1.9 µm, peptide separation	50 × 2.1 mm	26002-052130
Hypersil GOLD Peptide column, 1.9 µm, peptide separation	100 × 2.1 mm	26002-102130
Hypersil GOLD Peptide column, 1.9 µm, peptide separation	150 × 2.1 mm	26002-152130

Learn more at **thermofisher.com/hypersilgold**