LC columns

Column care guide and general method development information for Thermo Scientific HyperREZ XP columns

Applies to the Thermo Scientific™ HyperREZ™ XP (H+), HyperREZ™ XP (Pb2+), HyperREZ™ XP (Ca2+), HyperREZ™ (Na+), HyperREZ™ XP Organic Acid, HyperREZ™XP Sugar Alcohol, and HyperREZ™ XP RP columns

Before you get started

Manuals, specification sheets or technical guides for your column might be available to download from **thermofisher.com**. Type the P/N or product name in the search box. Helpful literature is near the bottom of the product page. Please read these before using the column.

Always start by investigating the Quality Assurance Report (QAR) accompanying your column. This document includes a lot of valuable information. For instance, investigate what solvent the column is shipped in. If the column is filled with something incompatible with your mobile phase, flush it out with a mutually compatible intermediate solvent. Condition the column before connecting it to the detector.

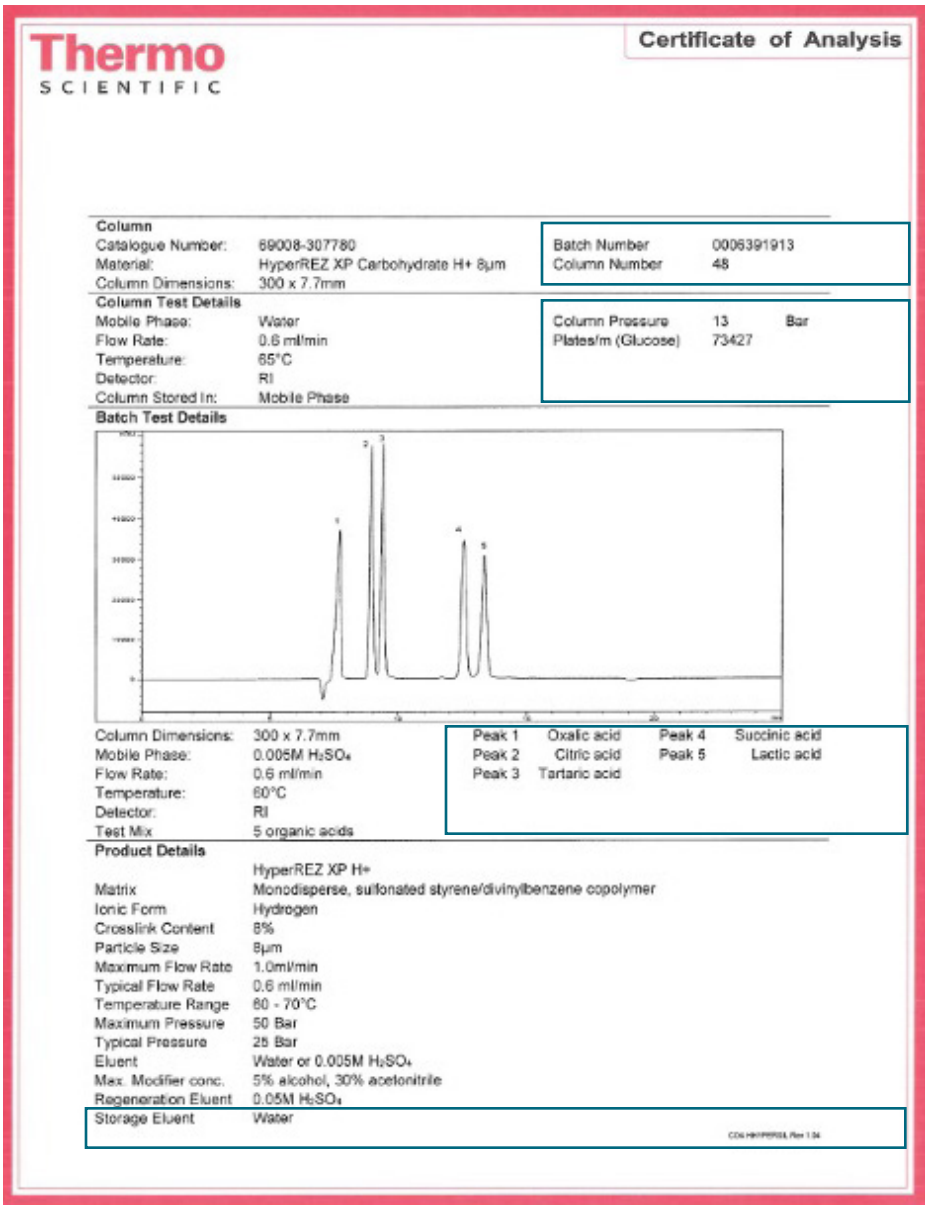
You should always strive to reproduce the chromatogram in your QAR when you receive the column into your lab. This way you can assure that the column is operating correctly when you start you method, and if you routinely repeat the column's CoA or QAR, you can notice column degradation early on and implement preventative measures if needed.

Always check for leaks before use.

Operational limits

Respect the limits for pressure, pH, temperature and solvent compatibility. The product manual, specification sheet or technical guide is the best reference for operational limits. If there is not a manual, see the online **catalog** or product web page on **thermofisher.com**

Operating near the extremes of the pH or temperature limits can reduce column life and increase column bleed.



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Certificate of Analysis

Column

Catalogue Number:	69008-307780
Material:	HyperREZ XP Carbohydrate H+ 8µm
Column Dimensions:	300 x 7.7mm

Batch Number	0006391913
Column Number	48

Column Test Details

Mobile Phase:	Water
Flow Rate:	0.6 ml/min
Temperature:	65°C
Detector:	RI
Column Stored In:	Mobile Phase

Column Pressure	13	Bar
Plates/m (Glucose)	73427	

Batch Test Details

Column Dimensions:	300 x 7.7mm
Mobile Phase:	0.005M H_2SO_4
Flow Rate:	0.6 ml/min
Temperature:	60°C
Detector:	RI
Test Mix	5 organic acids

Peak 1	Oxalic acid	Peak 4	Succinic acid
Peak 2	Citric acid	Peak 5	Lactic acid
Peak 3	Tartaric acid		

Product Details

	HyperREZ XP H+
Matrix	Monodisperse, sulfonated styrene/divinylbenzene copolymer
Ionic Form	Hydrogen
Crosslink Content	8%
Particle Size	8µm
Maximum Flow Rate	1.0ml/min
Typical Flow Rate	0.6 ml/min
Temperature Range	60 - 70°C
Maximum Pressure	50 Bar
Typical Pressure	25 Bar
Eluent	Water or 0.005M H_2SO_4
Max. Modifier conc.	5% alcohol, 30% acetonitrile
Regeneration Eluent	0.05M H_2SO_4
Storage Eluent	Water

Stationary phase as well as lot and serial number

Test conditions

Test results

Shipping solvent

This is an example of how you would read your CoA or QAR

Operational best practices

Clean samples make for robust methods and longer lifetime of your column. Always strive to clean you samples as much as possible to assure your best results. Filter samples to 1/10 of the particle size of the column. This in general means 5 µm, 8 µm or 10 µm, you can use 0.45 µm filters. Alternatively perform other sample preparation techniques such as Solid Phase-Extraction (SPE) to clean your sample for chemical as well as particulate contaminants. Always use a guard column or an inline filter to prolong the lifetime of your column. Exchange guard cartridges or filters regularly.

When considering the use of mobile phases, use appropriately high-quality ingredients. Ideally use factory-filtered HPLC-grade (or higher) solvents. Regularly maintain your water purifier to assure best quality. Do not “top up” buffer reservoirs. Always make a fresh batch in a clean bottle. Check buffers daily for microbial growth, especially if phosphate buffers are used. As much as practical, make solvent mixtures and buffers by weight. Check the pH before use. Filter buffers through a 0.2 µm membrane (0.1 µm for UHPLC).

Initial installation

The Thermo Scientific HyperRez XP carbohydrate columns are shipped in a ready to use condition but these steps should be followed prior to making your first injection.

All columns should be conditioned prior to use. The maximum pressure for these columns is 50 bar. If you exceed this pressure you can damage the column packing. Install the column only after you have flushed the entire HPLC system with DI water. Remember to make 2-3 false injections to clean out the sample loop. Connect the column and set the column heater/oven to the recommended temperature. Most columns will be operated at a temperature between 60 °C and 85 °C. Once the temperature is set, turn on the pump to 0.1 mL/min allow the column to equilibrate as the temperature is increasing. Once the column temperature reaches 45 °C you can increase the flow rate to 0.3 mL/min while carefully monitoring the pressure. Once the column has reached its optimum temperature (60-85 °C) you can increase the flow rate to 0.6 mL/min. In most cases this flow rate will provide the best separation.

It is **critical** to slowly bring the column from idle to operational conditions. Connect the column and set the column heater/oven to the recommended temperature. Most columns will be operated at a temperature between 60 °C and 85 °C. Once the temperature is set, turn on the pump to 0.1 mL/min allow the column to equilibrate as the temperature is increasing. Once the column temperature reaches 45 °C you can increase the flow rate to 0.3 mL/min while carefully monitoring the pressure. Once the column has reached its operational temperature (60-85 °C) you can increase the flow rate to 0.6 mL/min. In most cases this flow rate will provide the best separation.

Storage

The calcium, sodium and lead form columns can be stored in 100% DI water. Make sure the columns are flushed for at least 60 minutes prior to storage to remove and organic material. The columns can be stored in a laboratory refrigerator at 4°C for extended periods of time. **DO NOT FREEZE** the columns as this will destroy the packing material. You can add a small amount of acetonitrile (1%/V) to retard bacterial growth but this is not necessary if the column is stored at 4 °C. The hydrogen form column should be stored in dilute acid. The storage solvent should be 1/10th the concentration of the strength of the mobile phase. You can store the column in mobile phase overnight or for 2-3 days but for long term storage use a more dilute storage solvent. In all cases the columns should have the end caps securely tightened to prevent evaporation of the storage solvent.

Cleaning

Because of the nature of the samples injected on to the HyperRez columns they can become contaminated with strongly absorbed organic material. This is usually indicated by a loss of column performance and an increase in operating pressure. It is often possible to remove these organic contaminates by washing the column with small amounts of acetonitrile, ethanol or isopropanol. **Never use methanol to wash any HyperRez column. Do not inject sample in methanol unless it is first diluted with water.** Methanol will cause the packing to swell and can permanently damage the column. When washing the column care must be taken not to stress the column. Up to 30% acetonitrile can be used but only about 5% ethanol or isopropanol for washing. In the case of acetonitrile, start with a solution of 5% acetonitrile in water. Flush the column for at least 20 column volumes at a flow rate of 0.2 mL/min at 80C. Next use a 15% mixture of acetonitrile/water at the same conditions and finally wash with 30% acetonitrile/water using the same flow rate and temperature. You will then need to flush with water or dilute acid to remove the acetonitrile before re-equilibrating the column and resuming analysis.

Note: HyperREZ XP RP columns are an exception: they can tolerate 100% organic solvents

Mobile phase selection

Selecting the right mobile phase can be just as important as selecting the correct stationary phase. There are many considerations in making the selection. Choose mobile phases that are compatible with the column and LC equipment. UV detection requires that the mobile phase is transparent at the wavelengths of interest. Pay attention to the viscosity of the mobile phase so as not to exceed the pressure limit for the column or system. Use high-quality ingredients of the appropriate grade (HPLC) for the application.

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Typical mobile phases

Particle type	Monodisperse, sulfonated styrene/divinylbenzene						
Ionic form	Hydrogen	Lead	Calcium	Sodium	Organic acid	Sugar alcohol	Reversed-phase (no counterion and no alkyl ligand)
Max flow rate	1 mL/min	1 mL/min	1 mL/min	0.5 mL/min	1 mL/min	1 mL/min	5 mL/min
Typcial flow rate	0.6 mL/min	0.6 mL/min	0.6 mL/min	0.3 mL/min	0.6 mL/min	0.6 mL/min	1 mL/min
pH range	1 to 14						
Temperature	40-60° C (acid) 60-70° C (water)	70-90 °C	80-90 °C	80-90 °C	40-60 °C (acid) 60-70 °C (water)	80-90 °C	80 °C (max)
Typical pressure	20 bar	7 bar	12 bar	16 bar	20 bar	12 bar	200 bar (max)
Eluent	Water or 0.1 M H_2SO_4	Water	Water or 30% acetonitrile	Water	Dilute acid	Water	Unlimited buffer, 1–100% organic modifier
Regeneration	0.005 H_2SO_4	0.1 M $Pb(NO_3)_2$	0.1 M $Ca(NO_3)_2$	0.1 M $NaNO_3$	0.005 H_2SO_4	0.1 M $Ca(NO_3)_2$	High strength organic modifier
Storage eluent	Water	Water	Water	Water	Water	Water	7:1 acetonitrile/water
Tips and tricks					Strength of acid determines separation		

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