LC columns

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

Applies to all Thermo Scientific™ Hypercarb™ porous graphitic carbon 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. Some columns include a Quick-Start Guide in the box and/or a yellow caution tag on the column. Please read these before using the column.

Always start by investigating the Certificate of Analysis (CoA) or 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. Some detectors such as charged aerosol and mass spectrometers are highly sensitive to column bleed. Condition the column before connecting it to the detector.

You should always strive to reproduce the chromatogram in your CoA or 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.

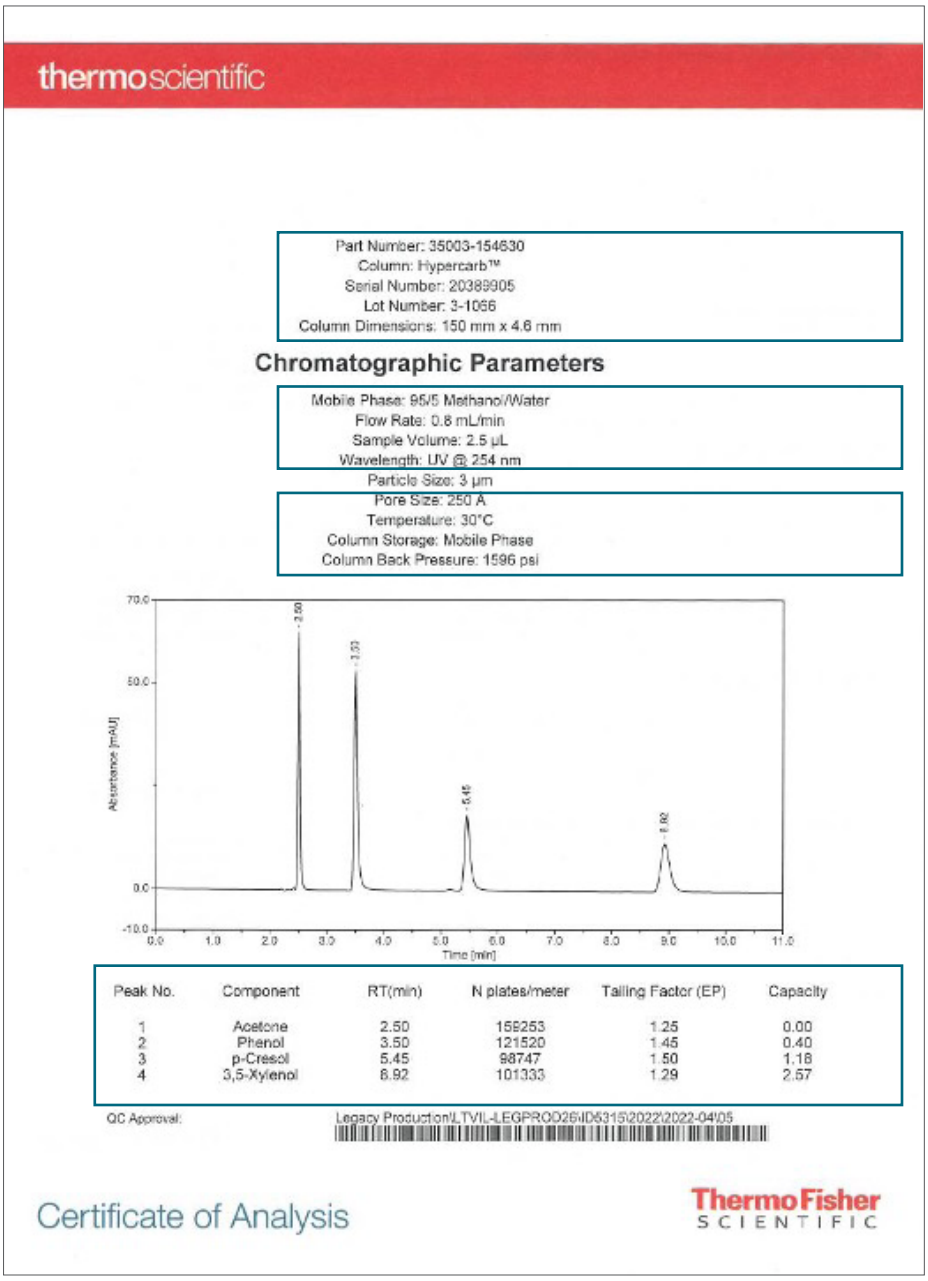
Operational limits

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**

Operational best practices

Clean samples make for robust methods and longer lifetime of your column. Always strive to clean your 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 for sub 2 µm or near 2 µm particle column—use a 0.2 µm filter. For larger particle sizes, such as 5 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).



Stationary phase as well as lot and serial number

Test conditions

Shipping solvent

Test results

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

Initial installation

The Thermo Scientific Hypercarb columns are limited to 350 bar (5000 psi) because the carbon is more brittle than traditional silica materials. Set the pressure limits on the LC before installing the column. If temperatures above ambient +10 °C will be used, a pre-column mobile-phase heater is recommended to improve the chromatography. If temperatures above 50 °C will be used, a post-column mobile-phase cooler is recommended to protect the detector cell and reduce noise.

Storage

For short-term storage, it is acceptable to leave the column installed in the LC with mobile phase at room temperature.

For long-term storage, flush with a solvent mixture similar to the mobile phase without acid or base additives. If you are not sure of its future use, we recommend 95:5 MeOH:H_2O since this is the initial shipping solvent.

Cleaning

It is always advised to have regular cleaning of your column. If you run gradients this could include a few minutes longer run at the top of your gradient. For isocratic runs, this could be an elevated organic run in-between samples to assure that the column is cleaned for the next run. Alternatively, a cleaning procedure at the end of a sequence may also suffice, depending on the method and sample cleanliness.

However, situations may occur where you will have to clean your column more extensively. Before using any cleaning solvent outside your usual mobile phases, check that it is compatible with the column and LC system. Below follows a series of various contaminants and how to clean these from the column.

To remove TFA from the column flush with THF for 70 column volumes. To remove amines flush with MeCN for 120 column volumes at 75 °C.

General washes

Acid-base wash:

1. Start with 50% THF and 50% H_2O + 1% TFA.
2. Gradient to 50% THF and 50% H_2O + 1% NaOH over 1 hour.
3. Gradient back to 50% THF and 50% H_2O + 1% TFA over 1 hour.
4. Repeat for 8-16 hours

Be sure to re-equilibrate with mobile phase.

This wash can be performed in reverse flush.

Strong organic wash:

1. Flush the column for 1 hour with 100% acetone.
2. Flush the column for 2 hours with 100% dibutylether.
3. Flush the column for 1 hour with 100% acetone.
4. Re-equilibrate column with aqueous mobile phase.

Remember to check if all cleaning solvents are compatible with your LC.

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. Mass spectrometers and charged aerosol detectors require that all ingredients are volatile. 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, UHPLC, LC-MS, UHPLC-MS) for the application.

Typical mobile phases would be methanol or acetonitrile, used as a strong organic solvent with buffer or clean water as the weak solvent.

Hypercarb is susceptible to “memory” effects. Used columns might not perform the same as new ones. Therefore, new method development projects should start with a new Hypercarb column. Some methods may involve specific conditioning steps to assure method robustness.

Oxidizing agents and reducing agents can react with the carbon surface and alter its retention properties. Properly controlled, this is a useful feature of Hypercarb, but should not be used lightly.

A more detailed description about method development on Hypercarb, can be found **here**.

Buffer selection

By controlling the pH of the mobile phase buffers control the retention of analytes and improve peak shape. Remember that a true buffer should have the ability to resist pH change when a sample is introduced at a different pH, and that buffer capacity is only 100% at the pK value of the acid or base. At pH 4, phosphate is a poor buffer and would change rapidly toward one of its pKa values if a more acidic or basic sample were introduced. As a rule, one should work within ±1 pH unit of the buffer pKa value for good pH control of the mobile phase. Adequate buffer concentrations for HPLC tend to be in the 10-100 millimolar level depending on the size and nature of the sample, as well as the column packing material. Phases based on highly pure silica with robust bondings such as the Thermo Scientic™ Hypersil GOLD™ range, are often more compatible with dilute buffers than traditional packings. When control at a lower pH (2-3) is desired, phosphate, or stronger organic acids such as TFA or acetic acid, are commonly used. If control at pH 4-5 is desired, an organic acid buffer such as acetate or citrate should be considered in place of phosphate. The figure to the right shows the importance of choosing the correct pH for a separation. Even slight changes in pH, either from measuring errors, mixing complications with the pump, or atmospheric water adsorption into the mobile phase, can alter any method if not properly buffered. Care should be taken when choosing a buffer and organic modifier mixture to ensure that a solution of the two does not produce a solid salt which could cause blockages and system contamination.

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Common buffer systems

Buffer		pK_a	Useful pH range	MS-compatible
TFA		0.30		Yes
Phosphate	pK_1	2.1	1.1 – 3.1	No
	pK_2	7.2	6.2 – 8.2	No
	pK_3	12.3	11.3 – 13.3	No
Citrate	pK_1	3.1	2.1 – 4.1	No
	pK_2	4.7	3.7 – 5.7	No
	pK_3	5.4	4.4 – 6.4	No
Formate		3.8	2.8 – 4.8	Yes
Acetate		4.8	3.8 – 5.8	Yes
Tris base (Trizma, THAM)		8.3	7.3 – 9.3	Yes
Ammonia		9.2	8.2 – 10.2	Yes
Borate		9.2	8.2 – 10.2	No
Diethylamine		10.5	9.5 – 11.5	Yes
Carbonate	pK_1	6.4	5.4 – 7.4	Yes
	pK_2	10.3	9.3 – 11.3	Yes
Triethanolamine	—	7.80	—	Yes

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