Thermo Fisher SCIENTIFIC

LC columns

Column care guide and general method development information for mixed-mode columns

Applies to Acclaim Mixed Mode WAX-1, Acclaim Mixed Mode WCX-1, Acclaim Mixed Mode HILIC-1, Acclaim Trinity and Acclaim Surfactant 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.

For UHPLC columns operating at high pressure > 400 bar, it can take the column 20 – 30 minutes of extra time to come to thermal steady-state after the column oven is ready. Continue equilibration until the pressure and detector baselines are stable.

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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Part Number: 25002-052130
Column: Hypersil GOLD™
Serial Number: 20110299
Lot Number: 17047
Column Dimensions: 50 mm x 2.1 mm

Stationary phase as well as lot and serial number

Chromatographic Parameters

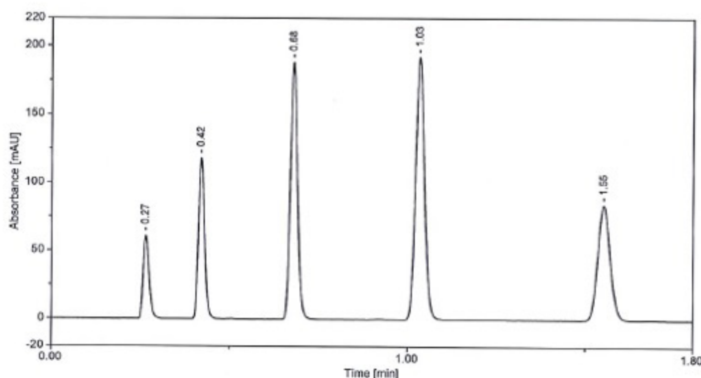
Mobile Phase: 50/50 Acetonitrile/Water
Flow Rate: 0.5 mL/min
Sample Volume: 1 µL
Wavelength: UV @ 254 nm
Particle Size: 1.9 µm
Pore Size: 175 Å

Test conditions

Temperature: Ambient

Column Storage: Mobile Phase
Column Back Pressure: 3976 psi

Shipping solvent



Peak No.	Component	RT(min)	N plates/meter	Tailing Factor (EP)	Capacity
1	Theophylline	0.27	24780	1.34	0.00
2	p-Nitroaniline	0.42	59240	1.28	0.58
3	Methyl Benzoate	0.68	113720	1.21	1.56
4	Phenetole	1.03	161840	1.14	2.90
5	o-Xylene	1.55	186940	1.11	4.85

Test results

QC Approval: R.S.

Legacy Production\LTVIL-LEGPROD18\ID5024\2020-04\09

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 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).

Initial installation

1. Check the QAR or CoA for the storage solution. Flush the column with a compatible buffer, for example 100 mM ammonium acetate (pH ~5).
2. Condition with repeated gradients from high salt to high organic until the baseline is consistent.
3. Equilibrate with the initial mobile phase.
4. Mobile phases should always be buffered within the pH limits of the column. Avoid unbuffered mobile phases, especially for columns with amine functional groups.
5. Weak cation exchange (WCX) columns should not be used with alcohol solvents. The alcohol can esterify the carboxylic acid groups and cause retention time shift. This includes, amongst others, Thermo Scientific™ Acclaim™ Mixed-Mode WCX-1, Thermo Scientific™ Trinity™ P2 and Thermo Scientific™ Trinity™ Q1 columns.

Storage

For short-term (< 3 days), it is acceptable to leave the column installed on the LC and filled with mobile phase at room temperature.

For long-term storage:

1. Flush out buffers with 0.01 M ammonium acetate buffer (pH ~5). Raise your gradient to 70% or more compatible organic solvent. For weak anion exchange (WAX) columns with amine groups (Thermo Scientific™ Hypersil GOLD™ Amino, Thermo Scientific™ Acclaim™ Mixed-Mode WAX-1, Thermo Scientific™ Acclaim™ Surfactant Plus columns), 100% MeCN is the best organic solvent.
2. Consult the CoA or QAR and adjust gradient to optimal storage conditions. Remove the column from the instrumentation and attach the solid end fitting. Store the column at room temperature.

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 a run elevated strong solvent 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.

Particulate matter: If particle size of the column is >2 µm reverse the direction of the column and perform a back flush of the column at half normal velocity. Flush directly to waste. For sub 2 µm columns do not backflush.

General cleaning:

1. Flush out incompatible buffers with 1 M ammonium acetate (pH ~5).
2. Run repeated gradients with 5% to 95% compatible organic solvent.

Ionic polymers or proteins: Run repeated gradients of 0.02 to 1 M NaCl at pH 6.

Metal ions:

1. Start by flushing with pH ~4 ammonium acetate or ammonium formate.
2. Then slowly change the buffer to a chelating buffer (buffer + 20 mM EDTA, pyrophosphate or oxalate at pH ~4).
3. Monitor UV for stable baseline.
4. Then slowly change the buffer from a chelating buffer back to a normal buffer. Continue until all the chelating agent is flushed from the column and LC system.
5. Equilibrate to mobile phase conditions.

WCX columns that have been esterified by alcohol solvents should be treated differently: Treat with 10 mM methanesulfonic acid at 45 °C for 30 minutes. Test to see if normal retention has been restored. Repeat if necessary.

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 organic solvents, used as a weak or strong solvent depending on mode solvent, with buffer as the strong or weak solvent depending on the mode.

Selecting the right mobile phase can be just as important as selecting the correct stationary phase. When operating mixed more columns, especially mixed mode columns that can be run in multiple modes, such as reversed phase and HILIC, the composition of the mobile phase is important in terms of what retention you can expect from your compound. Two factors that play a large role when using mixed mode columns are the pH and the ionic strength. Mixed mode columns are traditionally used for compounds that are not easily resolved by traditional chromatography, based on hydrophobicity, but rather have various functional groups prone to negative or positive charge. These compounds will change their retention to the stationary phase based on the pH and ionic strength in the mobile phase, as well as the organic and mode used to separate the molecules. Methods on mixed mode columns will require more attention to detail and a higher degree of robustness compared to for example reversed phase methods, due to the nature of the complexity of the interactions.

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. When control at a lower pH (2-3) is desired, phosphate, or stronger organic acids such as TFA or formic 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 precipitate which could cause blockages and system contamination.

Often the best guides to buffer and mobile phase design are found in the product manuals and application notes.

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

Expect reproducible results with sample prep, columns and vials

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