Process Chromatography Column Qualification

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General

The purification of bio-sourced molecules such as enzymes, monoclonal antibodies or vaccines usually requires one or several process steps using Low Pressure Liquid Chromatography (LPLC). This technique is intended to separate molecules from co-products of the same size, which is not possible with filtration techniques. Regardless of the variability of the bio-sourced product and neglecting the variability of the chromatography media, the reproducibility of the LPLC process from batch to batch can simply be summarized with the following:

- Reproducible mobile phase: same composition of the mobile phases used (equilibration/elution/regeneration solutions) and same flow rate. In terms of installation, these points essentially concern the buffer preparation installation as well as the inline dilution and flow control of the chromatography process system. This is not the topic of the present technical note.
- Good and even arrangement of the bed of chromatography media in the column from run to run. This results essentially from the media packing technique, the column design and the media integrity.

A well packed bed in a column equipped with efficient distributors ensures an even distribution of the mobile phase through the section of the column, and a plug flow transfer of the mobile phase along the columns vertical axis. For evaluating the column performance after packing, a column qualification test, often called "HETP and Asymetry test", is usually performed.

Principle of the Chromatography Column qualification test

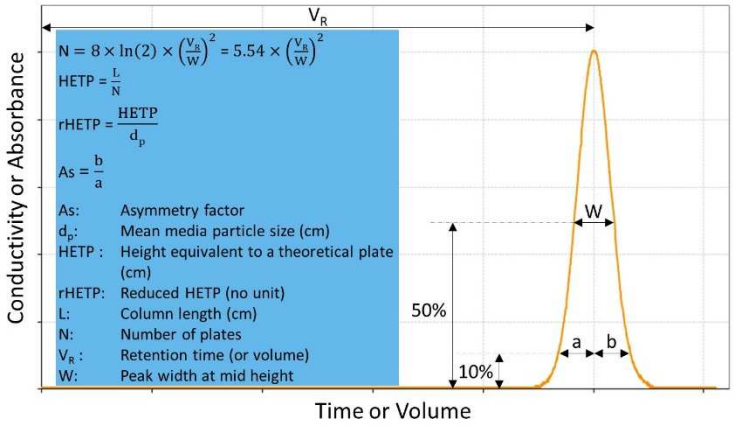
This test consists of evaluating the distortion of a marker pulse injected after its residence time through the column. Three criteria are assessed:

1. Normality. It is expected to obtain one peak with a Gaussian-like curve. A shoulder, double peak or peak shape very different from a Gaussian curve can indicate a bed heterogeneity or an equipment defect. If the peak is abnormal, no need to calculate the further criteria: the column needs to be repacked. If repacking provides the same result, the column condition must be checked, especially the watertightness of the distributors and the filter integrity.
2. Peak spreading, evaluated through the Height Equivalent to a Theoretical Plate (HETP), which is a concept providing a measure of the peaks spreading relatively to the distance performed along the column axis.
3. Peak asymmetry, that compares the two half sides of the peak.

Acceptance levels

The acceptance criteria depends very much on the chromatography mode, the type of media and the application:

- Chromatography mode: "Isocratic" mode where molecules migrate at different speed through the column is more demanding in terms of packing performance than "bind & elute" mode where the target molecules are adsorbed by the media, or "polishing" mode where the co-products are adsorbed.



- Type of media: it is expected that the smaller the particle is, the higher is the number of plates. The use of rHETP as acceptance criteria instead of N provides a packing performance less dependent on the media size. Pharma grade media, which have smaller and less disperse size, provides lower rHETP than food grade media, which have larger and more disperse size. Used media also provides lower result in terms of N compared to new media.
- The application may require more or less packing performance depending on the difficulty of the separation, i.e.: the difference of affinity between the target molecules and the other molecules. The acceptance level for As and rHETP are thus evaluated during performance qualification. Too much tolerance may result in purification variability while too stringent acceptance level may induce frequent non-conform packings, hence repacking exercises, which means waste of resources and time.

Isocratic mode (for instance: size exclusion) requires a high N, thus a low rHETP, and a good symmetry to ensure the best peaks separation. Acceptance level may for instance require $rHETP \leq 3$ and $0.8 < As < 1.2$.

For "bind & elute" mode, N influences the volume of the elution, so the concentration of the purified product, rather than the purity. For instance, if N is reduced by half, the elution volume may increase up to 40%. Acceptance level may for instance be $rHETP \leq 6$ and $0.7 < As < 1.8$. This may also stand for the "polishing" mode.

Conditions for making a representative Chromatography Column qualification test

Installation

The qualification test requires an equipment with

- A pump capable of injecting 1-2 CV of mobile phase at constant flow with a discharge pressure sufficient to handle the packed bed, the piping and instrument pressure drops;
- A sensor, usually conductivity or UV probe, for measuring the sample concentration with a decent precision. The sensor must be placed as close as possible to the column outlet;

- A sample injection system to minimize the dilution or distortion of the sample peak and the change of flow through the column. The table on the right details the usual solutions;
- Set of piping and valves for handling the fluid and the different fluid paths. The sizing of the piping should be sufficiently large to avoid pressure drop greater than 1 bar in addition to the column pressure drop. It shall also not be too large, ie: more than 4% of the column volume, as larger void volume would alter the N and As values. If the system has a bubble trap, it should be bypassed during the performance test;
- The chromatography media used shall be in good condition, free of fines and large contaminants.

Choice of the test conditions

The test conditions depend on the kind of media used.

Base buffer and marker set

The set depends on the kind of media used. The base buffer is usually of the same chemical composition as the equilibration buffer. The marker should be a small molecule capable of diffusing in all the pores of the media.

- For ion exchange, water can be used as base buffer if non-ionic marker is used, such as acetone 1-2%, with detection in UV at 280nm. If salt sample is used, combined with conductivity probe, the base buffer must be ionic as well with sufficient concentration to avoid interaction between media and marker. For instance: 0.2 to 0.4 M NaCl base buffer with 2M NaCl marker. Use of salt marker and water as baseline induces low asymmetry!
- For normal phase or reverse phase chromatography, or hydrophobic media, 20% ethanol can be used as base buffer and 2-3% acetone in 20% Ethanol can used as marker.
- For size exclusion chromatography, water can be used as base buffer and acetone 1-2% or 1M NaCl can be used as marker.

Linear speed

Low speed, around 20-70cm/h, usually provides the best results. However, in case of routine test for qualifying a packed bed before a production run, linear speed corresponding to the process condition can be used, such as 100-200cm/h.

Volume of marker

A volume corresponding to 1 to 3% of column volume shall be used. It must be noticed that 1% column volume provides higher number of plates compared to 3%.

Equilibration

Before injecting the test sample, the column bed should be stabilized with at least 1 to 3 column volume of equilibration buffer. If the purification process uses steps in upflow and downflow direction through the column, it is advisory to run the equilibration for 3 CV in each direction. Finish with the direction in which the performance test will be performed. If the equilibration buffer is not the base buffer for the performance test, the equilibration step should be followed with at least 1 to 2 CV of the base buffer, until the concentration measured with the instrument after the column shows a stable value.

Injection Method	Pros/Cons
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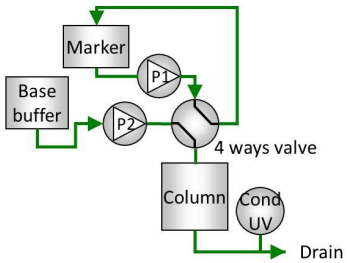
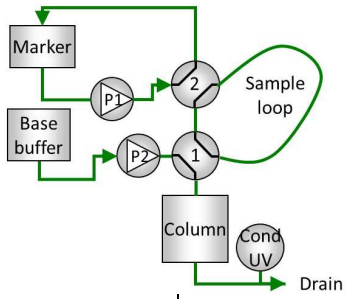
1- After equilibration, system is primed with marker with column and air trap bypassed; 2- Marker sample is injected in column; 3- System is primed again with base buffer with column bypassed; 4- Base buffer is injected through the column.	Pro: low investment as process systems have all necessary hardware. Cons: stop of flow through the column induces some sample diffusion in the bed. Piping void volume induces also broadening of the marker peak. N is thus reduced. Large volume of marker used.
Dedicated sample pump with 4-way valve at column inlet	
	
1- During equilibration with P2, the pump P1 and 4 ways valve are primed with marker; 2- After equilibration, the system switch to pump P1 and 4 ways valve to inject the marker in the column; 3- After marker injection, the system switch to pump P2 and 4 ways valve to inject base buffer in the column.	Pro: Minimize marker peak distortion before injection. Can work with large sample volume. Cons: May induce flow rate variation when switching valve and pump, thus sample diffusion in column. Requires more hardware.
Sample loop with two 4-way valves	
	
1- During equilibration with pump P2, pump P1 primes the loop with marker (valve 2 configured as valve 1 on picture); 2- The loop is isolated by switching valve 2 and is ready for load (as seen in picture); 3- After equilibration, the valve 1 is switched (as valve 2 on picture) to inject sample in the column.	Pro: No flow change and sharp marker peak = ideal configuration recommended by VERDOT. Minimal marker consumption. Cons: Difficult with very large columns (requires long sample loop). Requires more hardware.