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Application Note

Implementation of Multi-Column Chromatography Systems

1. Introduction

Before any manufacturing system is utilized in a Good Manufacturing Practice (GMP) environment for pharmaceutical drug production, it must be properly commissioned and qualified. The procedure for this qualification process is typically well documented and standardized, following an installation qualification/ operational qualification/process qualification/process validation (IQ/OQ/PQ/PV) workflow. The move to continuous processing has, however, complicated this process, primarily due to the long duration that the system is expected to remain in operation and in compliance. Multi-column chromatography systems are designed for such an operation.

The Mobius® Multi Column Capture System is designed to allow three column operation for primary capture using affinity resins. The cycling capture of the breakthrough on the second column whilst the non-loading steps are executed upon the third allows much greater utility of the resin and a semi-continuous elution stream to enable continuous bioprocessing with minimal surge tankage. As resin utilisation is increased with this technique, thereby reducing resin requirements, there is also utility for multi-column chromatography to intensify an existing fed batch process as well being a key operation within a contemporary continuous process. This provides flexibility for agile multi-product facilities like CDMOs and other facilities producing early clinical phase material.

There are several aspects of the system which require consideration for effective qualification at the manufacturing site. If employed within a continuous process the system could be active for over 20 days of constant operation, an order of magnitude longer than typical batch process operation would be. This increases the relative risks of certain process concerns, such as bioburden management, flow path integrity maintenance and even software platform robustness. These qualification concerns do not just apply to the Mobius® Multi Column Capture System and would eventually need to be considered for all single use systems used for long durations in a continuous bioprocessing facility.

This application note details a real-world approach to qualification of such a system, in collaboration with Transcenta.

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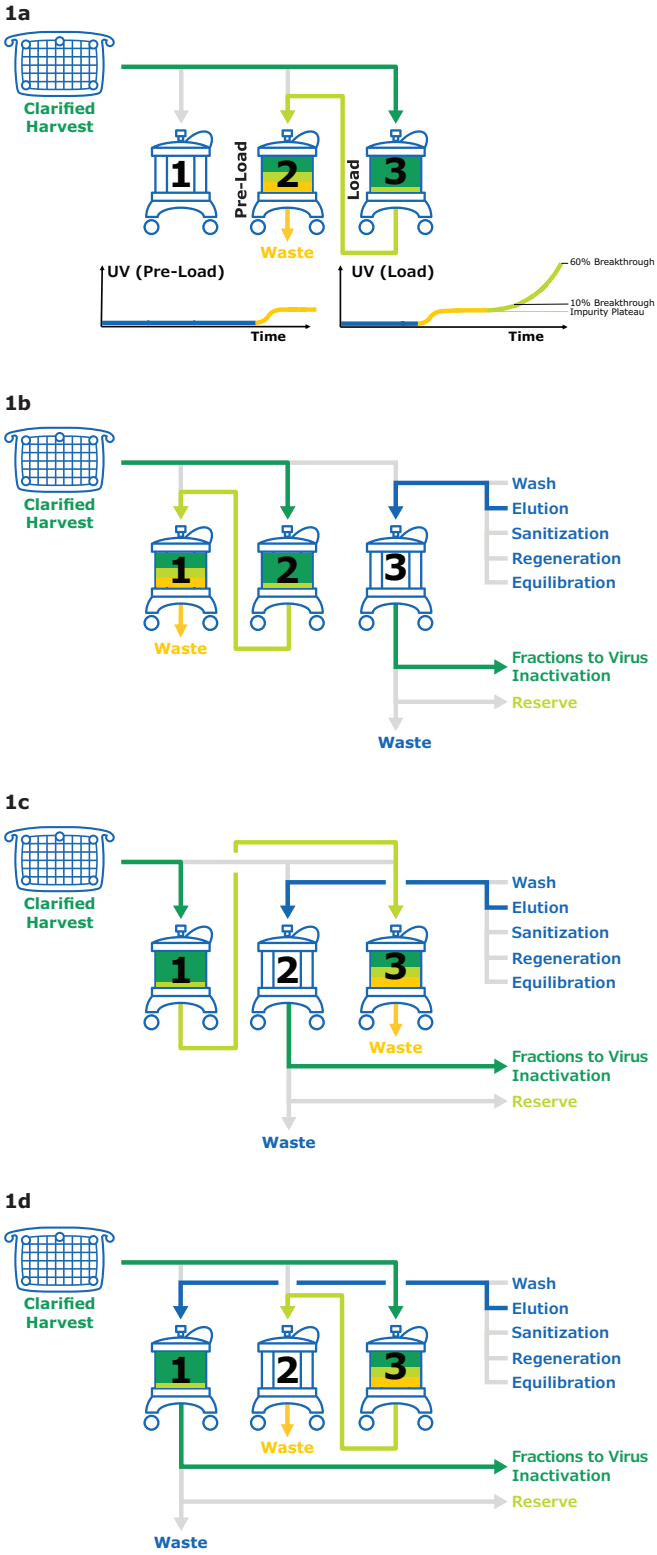

Figure 1.
Continuous cycle process.

2. How multi-column chromatography works

Multi-column chromatography enables a continuous loading of a feedstream. A typical flow path allows operation of three columns with two columns being loaded in series, and one set in parallel for non-loading steps.

As shown in **Figure 1**, three columns are operated simultaneously. Once column 3 is loaded (1a), it is disconnected from column 2 to be washed, eluted, and regenerated (1b). At the same time, column 2 (already partially loaded) is set in series with column 1 which remains unused and ready to accept product load. Once column 2 is loaded, it is disconnected from column 1 for wash, elution, and regeneration (1c). The same procedure is repeated with column 1. This complete sequence is called a cycle and can be repeated as many times as necessary to process all material or until the defined maximum number of cycles is reached.

Abbreviation table:

Abbreviation	Content
IQ/OQ	Installation Qualification/Operation Qualification
HCCF	Harvested Cell Culture Fluid
FAT	Factory Acceptance Test
EQ	Equilibration
MFG	Manufacturing
CV	Column Volume
PPQ	Process Performance Qualification
PAA	Peracetic acid
PV	Process Validation
HMI	Human-Machine Interface

3. Purpose of Qualification

The qualification and validation master plan outlines the work before the Mobius® Multi Column Capture System is ready to use in a GMP process. The purpose of the work is to ensure the system is installed correctly and each of the individual components is functioning to specification. The system is then qualified with product or a product mimic to test capabilities, and then validated to test robustness.

Scope

The scope of the studies below is for OQ/PQ, with IQ and water run part of OQ having been completed successfully. The confirmation run is that start of the PV stage.

Materials
Mobius® Multi Column Capture System
Mobius® Multi Column Capture Flexware® assemblies kit, with instrumentation
Peracetic acid 200mM
PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2% benzyl alcohol)
Clarified monoclonal antibody feed stream
IntuBio rapid bioburden detection system

3.1 Buffer qualification run summary

The buffer run is considered part of OQ and will allow testing certain sensors and other functionality that a water run cannot, such as magnetic flow meters and pH probes. The buffer test will simulate the process of conventional Protein A affinity capture, run with the same buffer, and load the sample with equilibration solution instead of HCCF during the loading step. The aims are:

- That the liquid handling parts of the system are within specifications, such as valves, pumps and mixers. This includes testing the inline buffer dilution functionality.
- That the probes and sensors are within specification, such as pH, conductivity, pressure and flow meters
- That the HMI and software components, including the alarm systems, are functioning as expected and reporting correctly.

Key conclusions

All buffer run qualification criteria were met.

The long duration requirements (18 days) of a continuous or hybrid batch process render full duration testing infeasible if n=3 datasets are required. As such a compromise shortening of the test whilst still challenging the system needs to be defined. In this case for buffer run OQ the minimum duration test was >18 hours.

3.2 Protein spike qualification run summary

One of the primary concerns with long duration processes is bioburden control, as the feed stream is cell culture media and is therefore highly conducive to microbial growth over the extended processing times the Mobius® Multi Column Capture System is expected to support. The use of a protein spike in buffer allows a quantitative assessment of bioburden levels during system operation. The protein spike also allows the testing of the more advanced functions of the Mobius® Multi Column Capture System, such as column switching, as these rely on a UV absorbance to control. The aims of the protein spike are therefore:

- Confirm bioburden and endotoxin level can be controlled at an acceptable level (<10 CFU/mL, <0.5 EU/mL). Samples to be taken post column (1-3) and also in the collected unified eluate collection bag.
- Confirm the UV breakthrough-based column switching is functioning properly.
- Confirm the online calculation function of column performance in long-term process works well and compare the result with offline results.
- Ensure the system is reliably integral after 18 days of operation.
- Reconfirm that the issues that occurs in previous run had been resolved.

The protein spike run required 3 consecutive runs to ensure that no issues occurred during the long continuous capture process and to demonstrate process robustness.

Key conclusions

All protein spike run qualification criteria were met.

As the chromatography columns were not delivered gamma irradiated, a column sanitisation step was required offline before installation It was demonstrated that despite PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2% benzyl alcohol) being known to deactivate spores[1] the sanitisation with this fluid was not effective. 1500 ppm peracetic acid (PAA) was implemented instead and provided 18 days of negative bioburden data. A gamma or X-ray irradiated pre-packed column or chromatography membrane would negate the requirement for this step.

3.3 Confirmation qualification run summary

After all functions and bioburden control strategies have been demonstrated, the Mobius® Multi Column Capture System needs to be tested with real HCCF/ protein samples to confirm that the system can run reproducibly over the long term. The use of cell culture media in this step is both representative of the expected process and a gold standard as regards microbial control. Qualification run was set as 38 cycles per column (114 total cycles) over 18 days. The aims of the confirmation run are therefore:

- Confirm bioburden can be controlled at an acceptable level in a rich media
- Confirm the UV breakthrough-based column switching is functioning correctly
- Ensure the system is reliably integral after 18 days of operation.
- Reconfirm that the issues that occurs in previous run had been resolved.

Key conclusions

All confirmation run qualification criteria were met and all previous issues have been resolved. The system is qualified and ready for the full scale engineering run.

4. Engineering process performance confirmation run

After the qualification process the system's performance needs to be verified. If this operation is fully compliant and meets all expectations then the material generated will be suitable for use in clinical development.

4.1 Bioburden testing

The bioburden level was sampled daily from the post column SmartSite™ sample port. As the flow paths are all supplied closed and gamma irradiated the bioburden risk is minimal. However, as demonstrated in the spike run qualification test the columns harbour Gram positive organisms that are not removed by NaOH.

The results of the bioburden study during GMP operation are shown in **table 1**.

Post column sample			Post column sample		
Day of operation	Rapid bioburden test CFU/10mL	Conventional bioburden test CFU/10mL	Day of operation	Rapid bioburden test CFU/10mL	Conventional bioburden test CFU/10mL
1	0	0	11	0	0
2	0	0	12	0	0
3	*	1	13	0	0
4	0	0	14	0	0
5	0	0	15	0	0
6	0	0	16	0	0
7	0	0	17	0	0
8	0	0	18	0	0
9	0	0	19	0	0
10	0	0			

Table 1: Daily bioburden load post column. The data collection for day 3 ran into an operational error and is not representative.

The data demonstrates that the PAA is a highly effective sanitant for these materials, even in a complex fluid flow path such as the internal channels of a packed chromatography column and where a Gram positive contaminant is known to exist.

In addition to the bioburden data, endotoxin levels were tested and found to be within the specification of <0.5 EU/ml.

4.2 Extended process duration performance

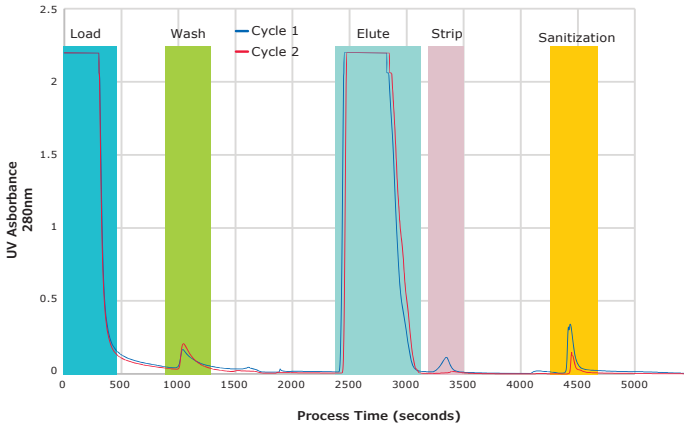

Figure 2: A comparison of the UV280nm traces from cycle 1 (red series) to cycle 34 (green series).

Figure 2 demonstrates an excellent reproducibility of performance between the 1st cycle of operation and very close to the last cycle on column 1, particularly as regards the critical elution peak. The increase in the strip peak for cycle 1 is most likely to be leached protein A. The traces for column 2 and 3 also demonstrate excellent comparability.

5. Conclusion

Qualification of a multi-column chromatography system to prepare for long duration GMP manufacture is more involved than the qualification of a system for short duration batch processing. Bioburden control is of particular concern for the primary capture step due to the richness of the feed and the length of time microbial contamination will have to propagate.

The qualification tests of buffer run, spike run and confirmation run demonstrated that the Mobius® Multi Column Capture System is reliable and robust in its performance and control for the expected duration. Sanitisation with PAA was demonstrated to be highly effective even with known Gram positive spore contamination present in complex flow paths. The full scale engineering run capably produced just over 10kg of clinical quality material.

References

[1] Development of a rapid sanitization solution for silica-based protein A affinity adsorbents, Marc Rogersa, Martha Hiraoka-Sutowb, Polly Makc, Fred Manna, Bénédicte Lebretonc,* a Millipore Corporation, Billerica, MA, USAb QC Microbiology, Genentech Inc., South San Francisco, CA, USAc Process Research & Development, Genentech Inc., South San Francisco, CA, USA' Journal of Chromatography A, 1216 (2009) 4589–4596

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Transcenta is a biologics drug developer and it manufactures biologics and provides CDMO service via its fully owned subsidiary HJB based in Hangzhou. Merck KGaA, Darmstadt, Germany and Transcenta have had an active collaboration regarding fully automated continuous bioprocess system development since 2020. The collaboration has been critical in guiding the development of the Mobius® Multi Column Capture System and the Flow-Through Polishing System.

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