User Requirement Specification (URS) for 3 Column Continuous (3MCC) with In-line Buffer Stock Blending Chromatography System

VERSION 2

May 22, 2025

NIMBL

Revision History

Revision Number	Date	Description of Changes
0	May 31, 2022	Original Version
1	June 14, 2022	Modified to be more generic and match format
2	May 22, 2025	Updated per standard format / Final

Table of Contents

1 Purpose and Scope

The purpose of this User Requirements Specification (URS) is to specify an integrated three-column continuous (3MCC) capture chromatography system with integrated Inline Buffer Stock Blending (BSB) in accordance with its technical requirements and applicable current local and regulatory, environmental, health, and safety, and automation standards.

The intent is to define the critical aspects (CAs) and critical process parameters (CPPs) on which the system qualification will be based. This document also details performance requirements and various design criteria for the equipment to be used in the pharmaceutical environment under cGMP conditions. However, it is not the intent of this document to detail all mechanical, electrical and control requirements. The vendor shall supply all subordinate components necessary to meet the performance requirements established herein. Should the vendor find it necessary to deviate from the specific design and performance requirements detailed in this document, the vendor shall clearly state all the deviations in the proposal and give the reason(s) for each deviation.

The 3MCC with in-line BSB system will be referred to as “system” in this document. The system will be used in a continuous purification process for manufacture of biopharmaceutical products (e.g. monoclonal antibody production). It will also have the capability to make all required buffers from Buffer Stock Concentrates without the need for intermediate hold tanks. The system will operate continuously for twenty-eight (28) days. During this time a constant stream of feed material will be supplied to the system. The system will, through manual or pre-defined recipes, prepare columns to receive harvest feed followed by the capture and elution of target products from the feed, and each of the necessary buffers will be made inline by the system all from the same HMI. The equipment will be installed in a cGMP environment, specifically in non-hazardous Grade C or Grade D clean room with temperature control (15-25°C).

The system will be used for a 500-Liter, 2,000-Liter, and 4,000-Liter perfusion process over a range of titers. Multiple single-use flow paths may be required to cover this range.

The single-use, product contact flow path assembly is specified in the requirements section of this document. Feed product bags, final product collection bags, and buffer stock solution totes will be connected to this system to operate the Ultrafiltration Diafiltration (UFDF) steps. The requirements for the feed and final product bags and buffer stock totes are not within the scope of this URS.

2 Area of Application

This URS applies to the following systems:

Item	Description	Tag Number
1	3MCC System with BSB	TBD

3 Responsibilities

Function	Purpose of Signature
Local User	I am signing on behalf of the user and confirm that this document accurately reflects the technical user requirements.
Project Engineering	I am signing/authorizing this document and agree that the technologies specified in this document are correct and in line with current technical concepts and that each requirement is specific and measurable.
Automation	I am authorizing this document and agree that the automation requirements specified in this document are correct and in line with current technical concepts.
Health, Safety and Environment	I am authorizing this document and agree that the requirements specified in this document are in line with current health, safety and environmental standards.
Quality	I am signing on behalf on the Quality Unit and confirm that the content of this document is compliant with relevant internal and external cGMP standards.

4 Process

The three-column chromatography (3MCC) with in-line BSB system will be used for purification of the drug substance.

The overall diagram of the system is shown as P&ID drawings split into two parts, one that looks at the inlet portion (Figure 1) and the other looking at the column and outlet portion (Figure 2).

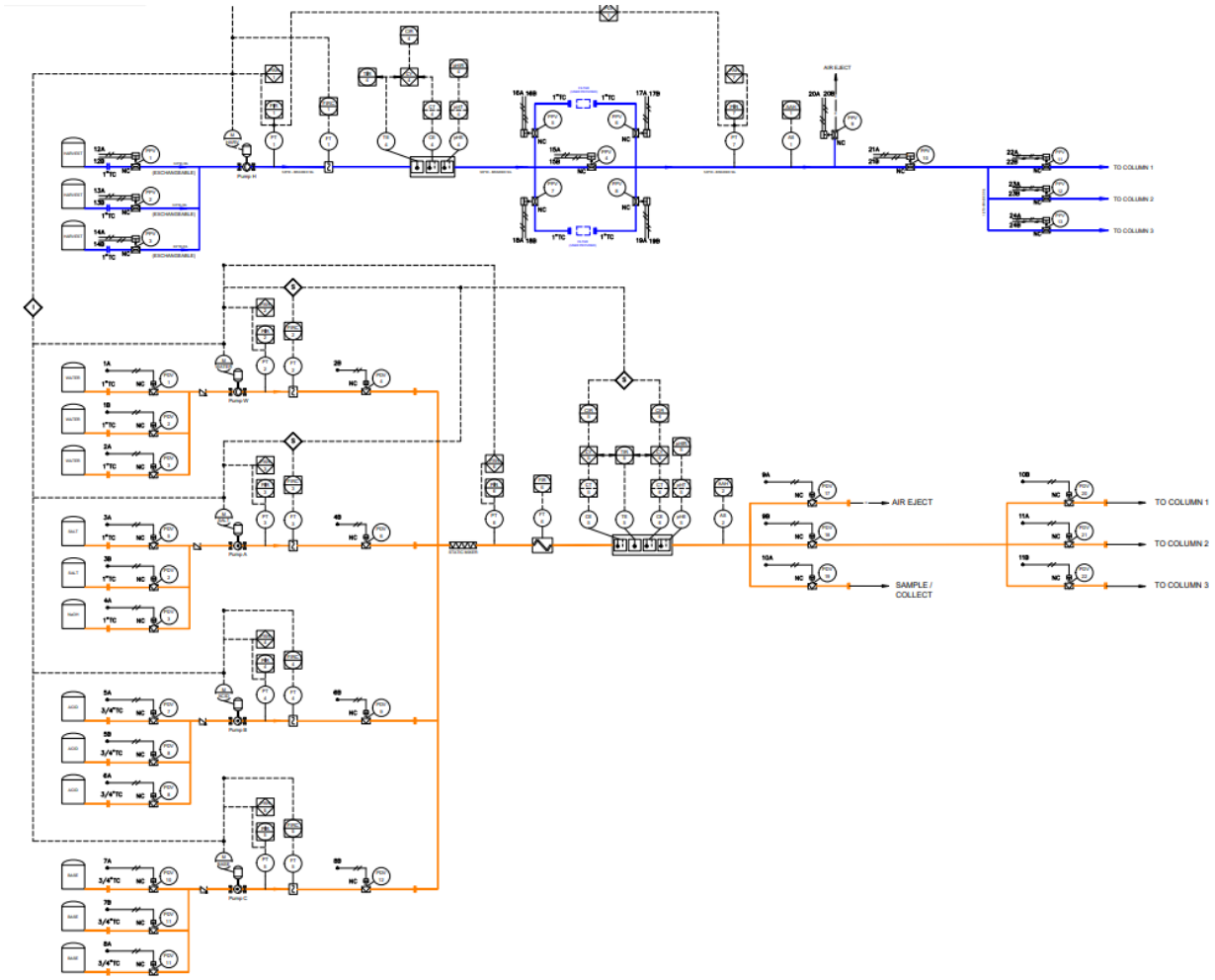

Figure 1. P&ID of the Buffer flow (orange) and Harvest flow (blue) lines of the 3CC/ BSB system.

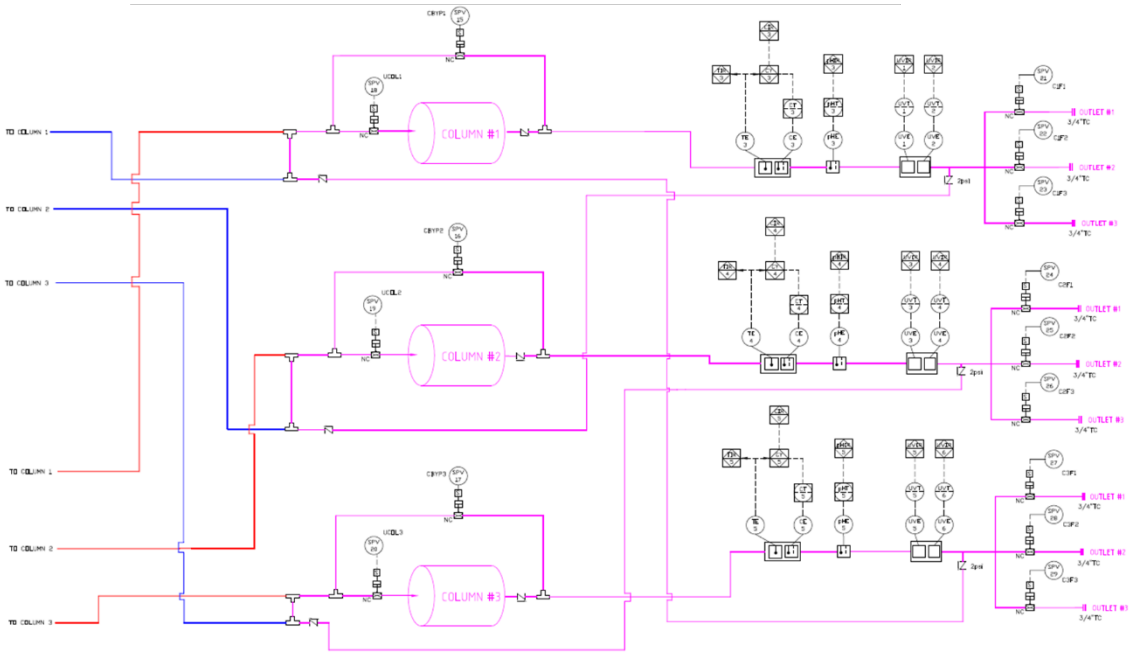

Figure 2. P&ID of the three-column continuous capture portion (pink), and connections to the Buffer flow (orange) and harvest flow (blue) lines of the 3CC/ BSB system.

The 3MCC System shall be an integrated process chromatography system capable of GMP manufacturing over a wide range of process conditions, as specified in Section 5 below. The system has capabilities of

(1) running chromatography functions on three sequential and alternating capture columns in such a way that a continuous feed is maintained. For each of the 3 columns, the chromatographic functions include loading clarified supernatant, buffer switching for wash elution, regeneration, and equilibration steps and concurrent fraction collection into flowthrough, product and waste receptables.
(2) Generating buffers inline through mixing of the individual liquid streams such as WFI, concentrated salt solution and acid and base constituents of the buffer which are pumped through their individual pumps and mixed inline to the desired molarity, pH and conductivity. Different buffers such as equilibration, wash, elution and regeneration buffers are produced continuously as needed per the continuous chromatography system program.

The system includes pre-column pressure sensor, air sensor and Bubble trap/auto air ejection, conductivity and pH sensing (pre- and post-column), UV sensing, column forward flow and bypass through the fraction collection along with post column sensors (UV, pH and conductivity) through to the fraction collection. Eluate switching between flowthrough product

collection and waste (for each column), based on process parameters such as time, volume, bed volume, UV, air, etc. with all three columns.

The system features 5 positive displacement, low pulsation Pumps of which one is a harvest pump and four are dedicated to buffer making typically consisting of a WFI pump, a salt pump and two for acid and base components of the constituent buffers that then merge via an inline mixer into the “buffer line”. The harvest pump has three inlets and each of the buffer pumps has 3 inlets presumably for different buffer components (monobasic/ dibasic phosphate, sodium acetate/ acetic acid, etc.) or more of the same type if larger volumes are needed. Each pump is followed by a pressure sensor, flowmeter and the harvest line connects to Temperature, conductivity & pH sensor (pre-column), dual switching precolumn filters (optional) and an air sensor and finally to the bank of 3 columns. The merged buffer line has a total buffer flow flowmeter along with pH, temperature and conductivity and air sensors as well before connecting to the column valving. Buffer Fluid can be ejected via a common waste (off spec buffer) line prior to the column or can be directly collected or distributed to the rest of the system (either column, product collection tank, sample or waste).

The three-column continuous capture process consists of the following:

- Three columns are operated simultaneously with harvest running through two in sequence and while simultaneously buffers are run through the third column wash, elution regeneration. As the 2nd column becomes the “leader” column in the load step and the 3rd column is the “follower” with the flowthrough from the 2nd column being directed to it for any residual product capture from flowthrough.
- First, both the first and second columns are loading, the third column is finishing the non-load wash, elute, regeneration and equilibrate steps, (the flow through from first column goes through the second column) for a short time and then entire load goes to the second column and flowthrough goes to the third column while the first column starts accepting buffers for execution of the wash, elute, regeneration and equilibration steps. The sequence is then repeated for the 1st column and 3rd columns.
- All columns run the same process recipe but are staggered in their operational sequences.
- Inline made buffers equilibration, washing, elution, and regeneration are used in each of the chromatography steps.
- Clarified feed is pumped via the pump in the system (controlled by flowmeter feedback for accuracy) to be delivered in an alternating fashion to the 3 columns but simultaneously to 2 columns as described earlier.
- Sensors monitor UV, Conductivity and pH of each column eluent prior to discharge from the system.
- Eluent flow is directed to either a common flowthrough, product or waste port.

5 Requirements

5.1 Product Quality Critical Assessment

An assessment has been performed to determine which requirements listed in this specification are critical to product quality. Where a requirement is deemed to be product quality critical, "Yes" is stated. If a requirement has been determined - as non-critical to product quality, one of the following justifications is provided as its rationale:

(1) **Health, safety, and environment critical** (for personnel and/or equipment only).
(2) **Business critical** (productivity, operations, process efficiency).
(3) **Technical requirement** with indirect or no impact on product quality, patient safety, and CGMP data integrity.
(4) **Non-product contact** (applies to equipment that is not in direct contact with the product).
(5) **Critical** but quality critical requirement is covered by another requirement in this URS. Reference is provided to the critical requirement.

The justifications (1 to 5) above are applicable for the entire document. At least one justification shall be stated per requirement.

5.2 General Requirements

Reference the General Equipment URS for details.

5.3 Functional Requirements

ID	Requirement	Product Quality Critical (Yes/No)	Comments / Questions
FNC-01	System shall have operating time of up to 28 days.	Y	
FNC-02	System dimensions shall not exceed 48'' x 60'' x 72'' (WxLxH).	N	2
FNC-03	The Skid shall be compatible with 1M NaOH and 20% alcohol for CIP operations, and is operable at 4 °C for cold room processing and up to 40°C.	Y	
FNC-04	The System must allow chromatography column operation in upflow and downflow.	Y	
FNC-05	System shall have a pressure range from 0-3.5 barg.	N	1
FNC-06	There shall be five low pulsation positive displacement metering pumps: Feed pump WFI pump Three Stock Solution Feed Pumps	Y	
FNC-07	The system shall have 15 inlets; with 3 inlets on all 5 pumps	Y	

FNC-08	The pumps shall be of a positive displacement, low-pulsation diaphragm or rotary type. Pressure attainable is 3 bar.	Y	
FNC-09	The Harvest loading flow path shall consist of 3 inlets with pneumatic diaphragm valves followed by the pump, flowmeter, pre-column pH and conductivity sensors, pressure and air sensor with auto air-bubble eject capability. The Harvest Pump shall have a flow range of 0.17 -10lpm.	Y	
FNC-10	The WFI and three Stock Solution Pumps shall have 3 inlets each. The WFI pump shall have a flow range of 0.17-10lpm One Stock solution pump shall have flow range of 0.04-2lpm Two Stock solution pumps shall have a flow range of 0.02-1lpm. Only one of the columns may accept flow buffer flow at a given time. I.e., while two columns are being loaded, the 3rd is being washed, eluted. Regenerated, and equilibrated. The purified antibody collected waste and flowthrough are collected from one of the 3 fractions.	Y	
FNC-11	The system shall have six flowmeters, one for each pump and one for the combined buffer. The Harvest, WFI and combined buffer flowmeters shall have an accuracy of +/15 ml/min at flowrates less than 1lpm and 1.5% of reading above 1lpm The stock solution pump flowmeters shall have an accuracy of +/-5 ml/min below 0.3 lpm and 1.5% of reading above 0.3 lpm Flow meter control loop for the pump can be disabled in the software UI for ±3% flow set point repeatability in the 0-1.2LPM range.	Y	
FNC-12	The pressure transducer shall measure process pressure for any system overpressure alarms (0-50 psi range, accuracy ±1.0 psi).	Y	
FNC-13	The system shall have an in-line mixer in the combined buffer line. The system shall measure the combined buffer pH and conductivity and reject it to waste if not within 0.1pH or 10% of the conductivity recipe set points. The system shall be able to meet the pH and conductivity recipe set points within 1 minute after starting to produce a given buffer.	Y	
FNC-14	The detection of bubbles shall be greater than or equal to 60% of the I.D. of the tubing.	Y	
FNC-15	pH sensors shall measure pH across the range 1-14, with an accuracy of ±0.1 pH.	Y	
FNC-16	Conductivity sensors shall measure conductivity across the range of 0-200mS ± 10mS.	Y	
FNC-17	UV sensors shall measure UV at the 280 nm & 260 nm wavelength (Range: 0-4.0 AU, Accuracy: ± 0.2 AU).	Y	

FNC-18	Temperature elements shall measure a range of 0-40°C, accuracy ±1°C.	Y	
FNC-19	Fluid shall be ejected via a common waste line, directly collected or distributed to the rest of the system (either column, product collection tank, sample or waste).	Y	
FNC-20	All valves shall be a standard size.	N	2
FNC-21	The flow path shall be smooth with low dead-volume and should be crevice free.	Y	
FNC-22	All connections shall be made to ensure closed aseptic operation.	Y	
FNC-23	The fluid contact materials shall be made of polymers that meet FDA, USP Class VI ADI free or similar standards.	Y	
FNC-24	UV, pH, and conductivity sensors shall continuously monitor each column's effluent. Flow through and non-elution steps are directed to a waste while the eluted mAb shall be collected.	Y	
FNC-25	After the post-column detectors, the process stream shall flow out to either a waste bag from each of the three columns or the product collection bag or flowthrough bag.	N	3
FNC-26	The entire flow path, i.e., pumps, flowmeters, electronic valves and sensors, filters and piping shall be housed in an open stainless-steel compartment with an intrinsic leak sensor.	N	4
FNC-27	All frames shall be made of stainless-steel tubing and brackets are made of anodized aluminum.	N	4
FNC-28	Standard Tri-clamp connections shall be used throughout the system.	N	3
FNC-29	Operator shall be able to view the following alarms: material flow, pressure, low pneumatic pressure, air, operational changes, communication, filter differential pressure, and leak.	N	2
FNC-30	Manual control (manual manipulation of valves and pumps) shall be available through an active Process Flow Diagram Screen. Software interlocks ensures that the pumps cannot run if any one of the inlet, column/bypass, or fraction valve is not open.	N	2, 3
FNC-31	The system shall be capable of unattended operation by programmed recipes of chromatography.	N	3
FNC-32	The system shall be able to display a chromatogram of the run.	Y	
FNC-33	The system shall be able to display conductivity peak resulting from a salt "slug" injection into column followed by elution with DI water. The system shall calculate Asymmetry and HETP of this peak to determine acceptable packing of column.	Y	
		Y	

5.4 Cleaning and Sanitization Requirements

See General Equipment URS for specifications.

5.5 Utility Requirements

ID	Requirement	Product Quality Critical (Yes/No)	Comments / Questions
UTL-01	System shall be able to connect to the following utilities: Instrument air (6 to 7 barg, dry and particulate free) Electrical Power (30A at 220VAC, 50/60 Hz)	N	3
UTL-02	The gas inlets of the system should be equipped with pneumatic quick connects.	N	3
UTL-03	Flow path to system inputs and from system outputs shall be (multi-use) hoses for the following fluids: -Instrument air	N	3
UTL-05	Vendor shall provide utility connection information, steady state and peak consumption demands.	N	

5.6 Automation Requirements

See General Equipment URS for specifications.

5.7 Metrology Requirements

See General Equipment URS for specifications.

5.8 Electrical Requirements

See General Equipment URS for specifications.

5.9 Health, Safety, and Environment

See General Equipment URS for specifications.

5.10 Maintenance requirements

See General Equipment URS for specifications.

5.11 Flexibility

See General Equipment URS for specifications.

5.12 Scope of required documentation

See General Equipment URS for specifications.

6 Abbreviations & Acronyms

Abbreviation / Acronym	Description
3MCC System	three-column continuous capture chromatography system.
A.U.	Absorbance units, the magnitude of a UV sensor signal
CFR	Code of Federal regulations
CIP	Clean in place
DI water	Deionized water
EPDM/PTFE	Ethylene propylene diene monomer / Polytetrafluoroethylene
FAT	Factory acceptance testing
FDA	Food and Drug Administration
FRS	Functional Requirement Specifications
GMP	Good manufacturing practices
HETP	Height equivalent to a theoretical plate
Hz	Hertz (1/s)
I/O	Input/Output
IQ	Installation qualification
LPM	Liters per minute
mS	Millisiemens
NEMA	National Electrical Manufacturers Association
OQ	Operational qualification
PLC	Programmable logic controller
PSI	Pressure per square inch
RTD	Resistance temperature detector
SAT	Site Acceptance Testing
SCADA	Supervisory control and data acquisition
USP	U.S. Pharmacopeia
UV	Ultraviolet sensor
VAC	AC voltage
VFD	Variable frequency drive