Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Performance Guide

Mobius® Multi Column Capture System

MERCK

How to Use this Guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® Multi Column Capture system. The results included in this guide summarize outcomes and observations obtained in studies conducted using particular model feedstreams and experimental conditions run on a system equipped with a closed-enabled flowpath. Therefore, all test results should be confirmed by the end user using feedstream and process conditions representative of the user's application. It is important to note that results are intended as general examples and should not be construed as product claims or specifications.

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1 Introduction

The purpose of this document is to describe the performance of the Mobius® Multi Column Capture system and highlight the working principles of its innovative features. The performance behavior of the system has been validated but cannot be precisely represented with figures and graphs for every situation.

The Mobius® Multi Column Capture system enables a continuous loading of a feedstream. The 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.

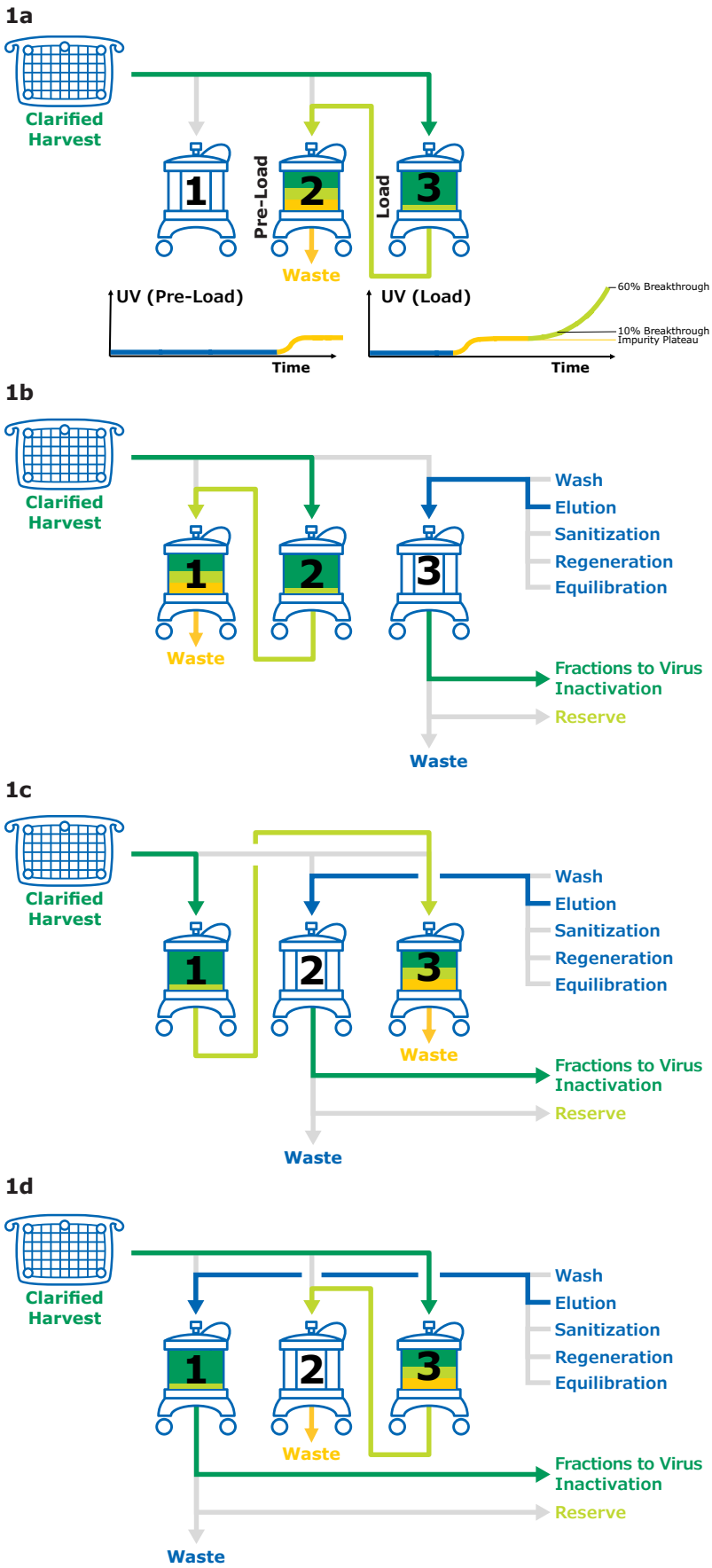

Figure 1.
Continuous cycle process.

2 Continuous Cycle

2.1 Fixed CV as Trigger for Column Switch

2.1.1 Functional Description and Test Objective

The multi column capture process can be controlled by different triggers to switch from one process phase to another (from loading to non-loading). One of these triggers is the column volume (CV) trigger, which bases the phase switch on a pre-selected CV per phase. The system converts the measured flow into CV according to the column parameters saved and the flowmeter readings (volume totalizer).

The purpose of this test was to confirm that the switch from one column to the other is triggered as expected in the continuous cycle settings when the targeted CV is reached.

2.1.2 Materials and Methods

A complete Flexware® assembly is installed following instructions provided in the user guide. Three 10 cm diameter columns (two Repligen columns and one QuikScale® QS100 column), packed at 10 cm bed height with Fractogel® SO_3-resin, are connected to the system columns ports. Buffer A, 2.5M NaCl, is connected to inlet S6 and to the product inlet (Figure 2). Buffer B, 0.25M NaCl, is connected to inlet S5. The system is primed entirely with the bubble traps and the filter is bypassed.

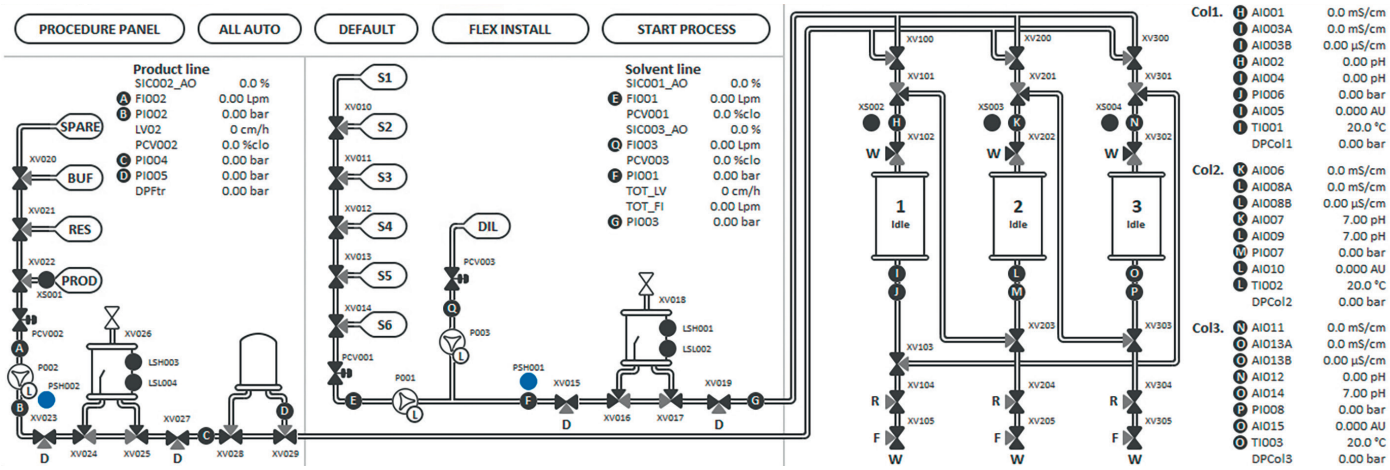

Figure 2.
Screenshot of a full option Mobius® Multi Column Capture system interface P&ID.

The continuous cycle settings are shown in Table 1. Peak detection and breakthrough detection are disabled, and no dilution or priority is set. Column parameters are set at 10 cm bed height and 0.79 L CV. Flow velocities are set at 150 cm/h for all the steps (minimum, nominal, and maximal flow velocities are set at the same value). The continuous cycle is started for four cycles using a recipe.

Step	Buffer	Inlet	Flow velocity (cm/h)	Trigger
Loading	A	Product	150	5 CV
Wash	A	S6	150	0.01 CV
Regeneration	A	S6	150	2 CV
Equilibration	B	S5	150	2 CV

Table 1.
Continuous cycle settings.

2.1.3 Results

As shown in the following tables, the system made the switch between phases based on the CV trigger set for each. During equilibration, ~1% variation in CV values was observed and can be attributed to the time required for the system to switch from using the solvent line to the product line when passing through the column.

Column 1 Behavior								
Date Time	Phase	Cycle	Inlet	Outlet	CV	Volume (L)	Duration (min)	LV (cm/h)
11/30/2021 7:50:40 PM	Preload	1	Product	Waste	5.00	3.95	20.00	150.00
11/30/2021 8:10:43 PM	Loading	1	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 8:10:54 PM	Wash A	1	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 8:18:46 PM	Regeneration	1	S6	Waste	2.00	1.58	7.87	150.00
11/30/2021 8:26:50 PM	Equilibration	1	S5	Waste	2.02	1.59	8.05	150.00
11/30/2021 8:50:48 PM	Preload	2	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 9:10:50 PM	Loading	2	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 9:11:02 PM	Wash A	2	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 9:18:57 PM	Regeneration	2	S6	Waste	2.00	1.58	7.92	150.00
11/30/2021 9:26:59 PM	Equilibration	2	S5	Waste	2.02	1.59	8.03	150.00
11/30/2021 9:50:57 PM	Preload	3	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 10:11:00 PM	Loading	3	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 10:11:11 PM	Wash A	3	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 10:19:04 PM	Regeneration	3	S6	Waste	2.00	1.58	7.88	150.00
11/30/2021 10:27:05 PM	Equilibration	3	S5	Waste	2.01	1.59	8.02	150.00
11/30/2021 10:51:06 PM	Preload	4	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 11:11:08 PM	Loading	4	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 11:11:21 PM	Wash A	4	S6	Waste	0.01	0.01	0.17	150.00
11/30/2021 11:19:12 PM	Regeneration	4	S6	Waste	2.00	1.58	7.85	150.00
11/30/2021 11:27:18 PM	Equilibration	4	S5	Waste	2.02	1.59	8.05	150.00

Table 2.
Column 1 process report.

Column 2 Behavior								
Date Time	Phase	Cycle	Inlet	Outlet	CV	Volume (L)	Duration (min)	LV (cm/h)
11/30/2021 7:30:38 PM	Preload	1	Product	Waste	5.00	3.95	20.17	150.00
11/30/2021 7:50:40 PM	Loading	1	Product	Next column	5.00	3.95	20.00	150.00
11/30/2021 7:50:51 PM	Wash A	1	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 7:58:45 PM	Regeneration	1	S6	Waste	2.00	1.58	7.90	150.00
11/30/2021 8:06:48 PM	Equilibration	1	S5	Waste	2.02	1.59	8.05	150.00
11/30/2021 8:30:45 PM	Preload	2	Product	Waste	5.00	3.95	20.00	150.00
11/30/2021 8:50:48 PM	Loading	2	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 8:50:59 PM	Wash A	2	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 8:58:47 PM	Regeneration	2	S6	Waste	2.00	1.58	7.82	150.00
11/30/2021 9:06:51 PM	Equilibration	2	S5	Waste	2.01	1.59	8.05	150.00
11/30/2021 9:30:54 PM	Preload	3	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 9:50:57 PM	Loading	3	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 9:51:08 PM	Wash A	3	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 9:59:01 PM	Regeneration	3	S6	Waste	2.00	1.58	7.88	150.00
11/30/2021 10:07:04 PM	Equilibration	3	S5	Waste	2.01	1.59	8.05	150.00
11/30/2021 10:31:03 PM	Preload	4	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 10:51:06 PM	Loading	4	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 10:51:18 PM	Wash A	4	S6	Waste	0.01	0.01	0.17	150.00
11/30/2021 10:59:10 PM	Regeneration	4	S6	Waste	2.00	1.58	7.87	150.00
11/30/2021 11:07:13 PM	Equilibration	4	S5	Waste	2.01	1.59	8.05	150.00

Table 3.
Column 2 process report.

Column 3 Behavior								
Date Time	Phase	Cycle	Inlet	Outlet	CV	Volume (L)	Duration (min)	LV (cm/h)
11/30/2021 7:23:52 PM	Loading	1	Product	Next column	3.65	2.89	14.68	150.00
11/30/2021 7:25:10 PM	Loading	1	Spare	Next column	3.65	2.89	14.70	150.00
11/30/2021 7:30:38 PM	Loading	1	Product	Next column	5.00	3.95	20.17	150.00
11/30/2021 7:30:50 PM	Wash A	1	S6	Waste	0.01	0.01	0.17	150.00
11/30/2021 7:38:40 PM	Regeneration	1	S6	Waste	2.00	1.58	7.83	150.00
11/30/2021 7:46:44 PM	Equilibration	1	S5	Waste	2.01	1.59	8.07	150.00
11/30/2021 8:10:43 PM	Preload	2	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 8:30:45 PM	Loading	2	Product	Next column	5.00	3.95	20.00	150.00
11/30/2021 8:30:56 PM	Wash A	2	S6	Waste	0.01	0.01	0.15	150.00
11/30/2021 8:38:50 PM	Regeneration	2	S6	Waste	2.00	1.58	7.90	150.00
11/30/2021 8:46:53 PM	Equilibration	2	S5	Waste	2.01	1.59	8.05	150.00
11/30/2021 9.10:50 PM	Preload	3	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 9:30:54 PM	Loading	3	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 9:31:04 PM	Wash A	3	S6	Waste	0.01	0.01	0.13	150.00
11/30/2021 9:38:56 PM	Regeneration	3	S6	Waste	2.00	1.58	7.87	150.00
11/30/2021 9:46:59 FM	Equilibration	3	S5	Waste	2.01	1.59	8.05	150.00
11/30/2021 10:11:00 PM	Preload	4	Product	Waste	5.00	3.95	20.02	150.00
11/30/2021 10:31:03 PM	Loading	4	Product	Next column	5.00	3.95	20.02	150.00
11/30/2021 10:31:12 PM	Wash A	4	S6	Waste	0.01	0.01	0.12	150.00
11/30/2021 10:39:04 PM	Regeneration	4	S6	Waste	2.00	1.58	7.87	150.00
11/30/2021 10:47:07 PM	Equilibration	4	S5	Waste	2.01	1.59	3.05	150.00
11/30/2021 11:11:08 PM	Preload	5	Product	Waste	5.00	3.95	20.02	150.00

Table 4.
Column 3 process report.

2.2 Absolute UV as Trigger for Column Switch

2.2.1 Functional Description and Test Objective

The main advantage of continuous chromatography is the ability to approach the theoretical maximal binding capacity of the resin by loading two columns in series and allowing resin saturation without concern of product loss via breakthrough. This reduces the amount of resin needed to process a given amount of product and the associated resin cost.

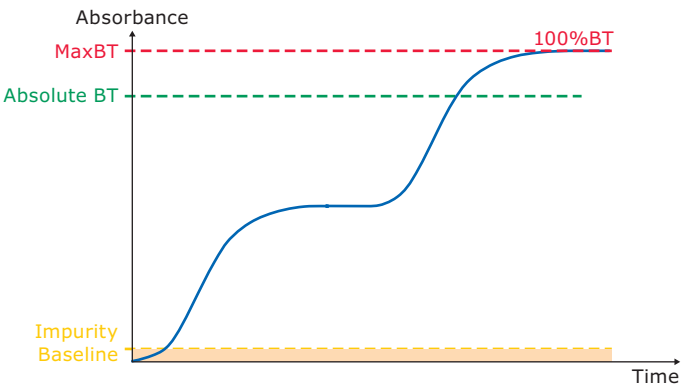

Figure 3.
Breakthrough detection through absolute UV detail.

The system offers different techniques to monitor the breakthrough of the loaded column including absolute UV trigger (Figure 3). Made for steady product concentration loadings, the absolute UV trigger is based on the post column UV sensor. A fixed and pre-selected UV value is set and the switch from one column to the following column is made once the post column UV value reaches the target. Compared to a CV trigger, this methodology adds a control on the outflow of the column and prevents, for example, product loss due to deterioration of the media capacity over the process while ensuring maximum use.

2.2.2 Materials and Methods

A complete Flexware® assembly is installed following the instructions provided in the user guide. Three QuikScale® QS200 columns, 20 cm in diameter, packed at 10 cm bed height with Fractogel® SO_3-resin, are connected to the system columns ports. An IgG solution (4.5 g/L) is connected to the product inlet. Wash, elution, sanitization, regeneration, and equilibration buffers are connected to the solvent line. The system is primed, and a continuous cycle is started for one cycle with a breakthrough detection based on absolute UV set at 1.200 AU. After two column loading phases, the absolute UV trigger is changed to 1.280 AU to check the system response.

2.2.3 Results

The first column to be loaded when starting a continuous cycle is column 3, followed by column 2 and column 1. Columns 2 and 3 were loaded with absolute absorbance detection criteria set at 1.2 AU whereas column 1 had a setpoint of 1.280 AU. Outcomes show that all breakthroughs were detected at the set value or above: 1.2 for column 3, 1.201 for column 2, and 1.284 for column 1 (Table 5). An overshoot of 0.001 AU and 0.004 AU visible for column 2 and 1 respectively, can be explained by the jumps in absorbance readings of the UV sensors, with the recorded value being the first value read above the targeted UV value. The system has shown the capability to trigger column switch based on absolute UV values as expected.

Cycle	Column	Breakthrough detection (AU)
1	Column 3	1.200
	Column 2	1.201
	Column 1	1.284

Table 5.
Breakthrough detection values (AU).

For more process data, please consult our Application. Note: Multi Column Capture Prototype Tests for Monoclonal Antibody Capture During Project nextBioPharmDSP (Horizon 2020) – MK_AN7549EN – available on our website sigmaaldrich.com.

2.3 Relative UV as Trigger for Column Switch

2.3.1 Functional Description and Test Objective

To further control and refine the breakthrough detection and column phase switch on the Mobius® Multi Column Capture system, relative UV can be used as a trigger. Like the absolute UV trigger, control is based on the post-column UV sensor but considers the impurity plateau observable in capture chromatography and allows the trigger to be set as a percentage of breakthrough.

To do so, a fixed value of the maximal absorbance of the solution is set in the process parameters. This value corresponds to the absorbance of the feed solution, which corresponds to the solution flowing out of the column if 100% of breakthrough is attained (%MaxBT). Additionally, a target percentage of breakthrough (Δ%DetectB) must be set, as well as impurity plateau detection criteria (minimum value and stability criteria).

In process, the system automatically searches for the impurity plateau and once detected, considers this value as the 0% breakthrough value (0%BT; Figure 4). By subtracting this UV value from the maximal absorbance (%MaxBT), a breakthrough range from 0% to 100% can be defined. A linear regression is generated between these two points and the percentage of breakthrough set is converted into an absorbance value. The calculated value is set automatically as a trigger, and the loading stops once the post column UV reaches this value. The advantage of this technique is the ability to consider impurity variations in the bulk; maximal absorbance remains the same if not changed manually.

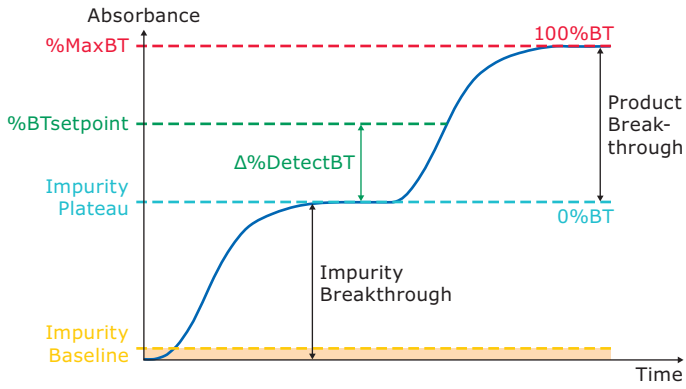

Figure 4.
Breakthrough detection through relative UV detail.

The purpose of this test is to confirm that the system efficiently and automatically detects the impurity plateau and trigger the switch of columns being loaded following the relative UV criteria setpoint.

2.3.2 Materials and Methods

A complete Flexware® assembly is installed following the instructions provided in the user guide. Three QuikScale® QS200 columns, 20 cm in diameter, packed at 10 cm bed height with Fractogel® SO_3-resin, are connected to the system columns ports. An IgG solution (4.5 g/L) is connected to the product inlet. The absorbance of the feed solution is measured at 1.5 AU. Wash, elution, sanitization, regeneration, and equilibration buffers are connected to the solvent line. The system is primed in equilibration buffer, and a continuous cycle is started for three cycles with a breakthrough detection based on relative UV. Parameters for the breakthrough detection are shown in Table 6.

Loading parameters	
Loading linear velocity	380 cm/h
Corresponding flowrate	2 L/min
Resulting residence time	1.58 min
Breakthrough detection setpoints	
Start detection	0.05 AU
% Max AU	60%
Max AU	1.5 AU
Plateau detection setpoints	
Plateau stable deadband	0.01 AU
Stability time	20 s

Table 6.
Breakthrough detection parameters and plateau detection parameters.

2.3.3 Results

Trial outcomes are summarized in Table 7, which compares observed breakthrough levels to the theoretical breakthrough levels and provides the impurity plateau detection value. From these results, errors are significant for the first cycle (*) and then decrease from the second cycle. The explanation for this is that when a continuous cycle is started, the first cycle does not reach stability and UV breakthrough setpoints are not attained. The first column loading is made on a column that has not been preloaded and the product line filter is filled in equilibration buffer. Due to the dilution occurring in the filter and the lack of product in the column at the start of the loading phase, the trigger stopping the loading is the loading column volume trigger set as a security and not the relative UV trigger which was programmed. Consequently, the second column is not preloaded as expected, and the same effect is occurring on that column. The effect diminishes over the three loadings of the first cycle.

From the second cycle, the continuous process reaches its stable state, and the breakthrough trigger is effective. The system can trigger column loadings based on relative UV with no significant deviation on calculated setpoints when continuous process stability is reached.

Column	Impurity Plateau level (AU)	Breakthrough level (AU)	Theoretical breakthrough level (AU)	Error on Breakthrough (%)
Cycle 1				
3	0.6331	0.94	1.15	–18%*
2	0.1549	0.495	0.96	–49%*
1	0.0697	0.64	0.93	–31%*
Cycle 2				
3	0.1759	0.971	0.97	0.07%
2	0.1421	0.93	0.96	–2.81%
1	0.100	0.94	0.94	0.00%
Cycle 3				
3	0.1343	0.954	0.95	0.03%
2	0.1377	0.955	0.96	–0.01%
1	0.1339	0.954	0.95	0.05%

Table 7.
Summary of outcomes per column and cycle including impurity plateau level, breakthrough level, theoretical breakthrough level and calculated error on the breakthrough.

Please consult our application note to see more process data: Prototype Tests for Monoclonal Antibody Capture During Project nextBioPharmDSP (Horizon 2020) – MK_AN7549EN – available on our website sigmaaldrich.com.

2.4 Area Detection as Trigger for Column Switch

2.4.1 Functional Description and Test Objective

Meant for perfusion processes with slight concentration changes of the feed, area detection still enables steady breakthrough detection. Area detection is based on post column UV sensors like other detection methodologies. It is not only based on the UV value but on the whole UV curve; by integrating the UV curve from the detected impurity plateau, the system can calculate the area and breakthrough can be triggered by an area setpoint (Figure 5). To simplify the area setpoint determination, it is possible to ask the system to automatically determine this setpoint value. Therefore, a relative UV trigger must be set, and the corresponding area based on the three first cycles averages are calculated for each column independently. From the fourth cycle, the system triggers the loading based on this automatically set average value.

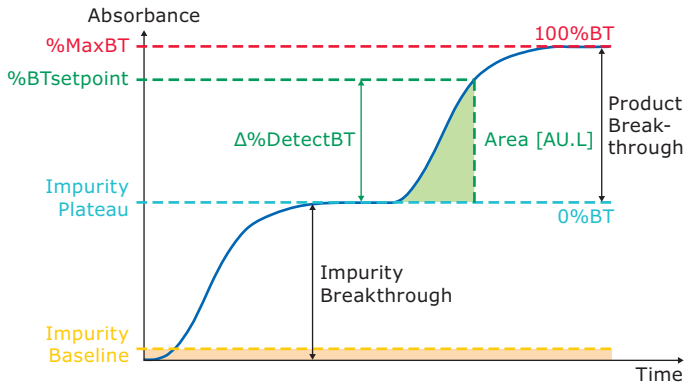

Figure 5.
Area detection as a breakthrough trigger (full loading phase).

The objectives of this test are to determine if the system can automatically calculate the area, determine the area average of three cycles for each column, set the calculated value as the new trigger and respect this trigger for the following cycles.

2.4.2 Materials and Methods

A complete Flexware® assembly is installed following the instructions provided in the user guide. Three QuikScale® QS200 columns, 20 cm in diameter, packed at 10 cm bed height with Fractogel® SO_3-resin, are connected to the system columns ports. A cooled IgG solution (4.5 g/L) is connected to the product inlet. The absorbance of the IgG solution is measured at 1.5 AU. Wash, elution, sanitization, regeneration, and equilibration buffers are connected to the solvent line. The system is fully primed in equilibration buffer and a continuous cycle is started with breakthrough detection based on relative UV with automatic area detection implementation. Parameters for the breakthrough detection are shown in Table 8. After five cycles, the continuous cycle is paused overnight and restarted the following day. Measured areas and averages by the system are compared to manually calculated ones.

Breakthrough detection setpoints	
Start detection	0.05 AU
% Max AU	60%
Max AU	1.5 AU
Plateau detection setpoints	
Plateau stable deadband	0.01 AU
Stability time	20 s

Table 8.
Breakthrough detection parameters and plateau detection parameters.

2.4.3 Results

Column switch has been triggered by relative UV over the first three cycles (Table 9). The breakthrough area measured over these three first cycles was considered, and the average value calculated by the system was automatically set as a setpoint for each column without significant error. Starting at cycle 4, cycles were triggered by areas respecting the setpoints calculated by the system with no significant deviation. The first column loaded at cycle 6* underwent an overnight hold during the breakthrough and during the area calculation, thus showing significant error in the area calculation and an unrealistic outcome.

Results demonstrate that when used as a breakthrough trigger for column switching, area detection is functional. The column switch was properly triggered for multiple cycles according to the calculated setpoint. This allows for reproducibility in the quantity of protein being loaded.

Cycle	Column 3		Column 2		Column 1	
	Breakthrough area (AU.L)	Breakthrough level (AU)	Breakthrough area (AU.L)	Breakthrough level (AU)	Breakthrough area (AU.L)	Breakthrough level (AU)
1	7.52	0.94	9.90	0.495	11.10	0.64
2	15.22	0.971	10.04	0.93	16.50	0.94
3	18.05	0.954	16.26	0.955	14.37	0.954
Breakthrough area (AU.L)						
Manually calculated average	13.60		12.07		13.99	
System setpoint	13.56		11.92		13.88	
4	13.57		11.93		13.92	
5	13.64		11.93		13.92	
6	-2.48*		11.96		13.92	
7	13.57		11.96		13.89	
8	13.60		11.94		13.93	

Table 9.
Breakthrough area according to cycle and column.

2.5 Step Prioritization

2.5.1 Overview

One of the challenges of continuous capture processing with three columns is the capacity of the system to adapt to processing timing. The non-loading step (counting all other chromatography steps except loading) should always be faster than the loading step to allow a continuous loading with no interruption, ensuring the readiness of a column when breakthrough target is reached on the loaded column. For the loading phase, the concentration of the product, capacity of the columns, breakthrough detection, and elution peak detection may vary and influence the required time. For the non-loading phases, dilution and special triggers may affect the time needed to fulfill a step. These timing changes are potential causes of shifts in process synchronization between loading and non-loading phases which could lead to interruptions in the continuous process. To prevent any desynchronization leading to a nonviable process, the multi-column capture system has been programmed to automatically change the non-loading steps duration according to predefined setpoints. The following section provides a description of this function.

2.5.2 Functional Description

When setting the parameters for the continuous cycle, the non-loading phase duration must be set as a percentage of the loading phase (Figure 6). This ratio value will be the system synchronization target to reach and maintain during the complete continuous cycle. Non-loading steps should always remain shorter than the loading one, thus ensuring the readiness of the column for the following column switch. Therefore, this value is usually below 80% to 90%, as a minimum of 10% to 20% margin is recommended to ensure that any unpredictable external phenomenon is not totally desynchronizing the system.

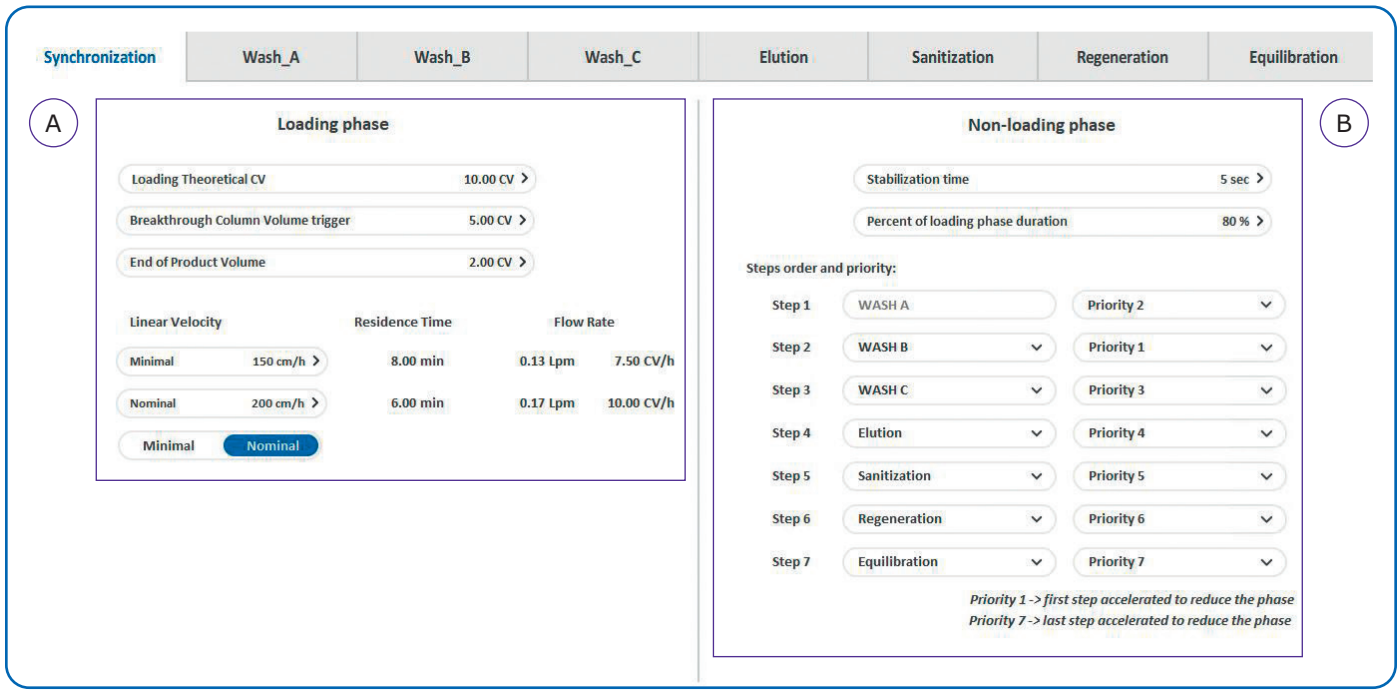

Figure 6.
Screenshot of the Loading phase setting menu (A) and Non-loading phase synchronization setting menu with step prioritization (B).

For processes involving uneven, non-loading step triggers, where the duration of a step is not only based on flow velocity and column volume, but also another trigger is selected as “and” (for example, conductivity or pH) a larger margin is advised (Figure 7; Reference B).

After having set the “percent of loading phase” setpoint, a priority setpoint can be assigned to each non-loading step (Figure 7). Steps that do not have a limitation on flow velocity or duration may be prioritized. Steps that are more sensitive to flow variations or are based on a running time are set with a lower priority. It is also possible to not set a priority and fix the velocity. The intent is to give an order to all steps to define which can be accelerated or decelerated first to accommodate the loading time while respecting the time and flow requirements of each step. For each non-loading step, a maximal and minimal flow can be defined to limit the acceleration or deceleration.

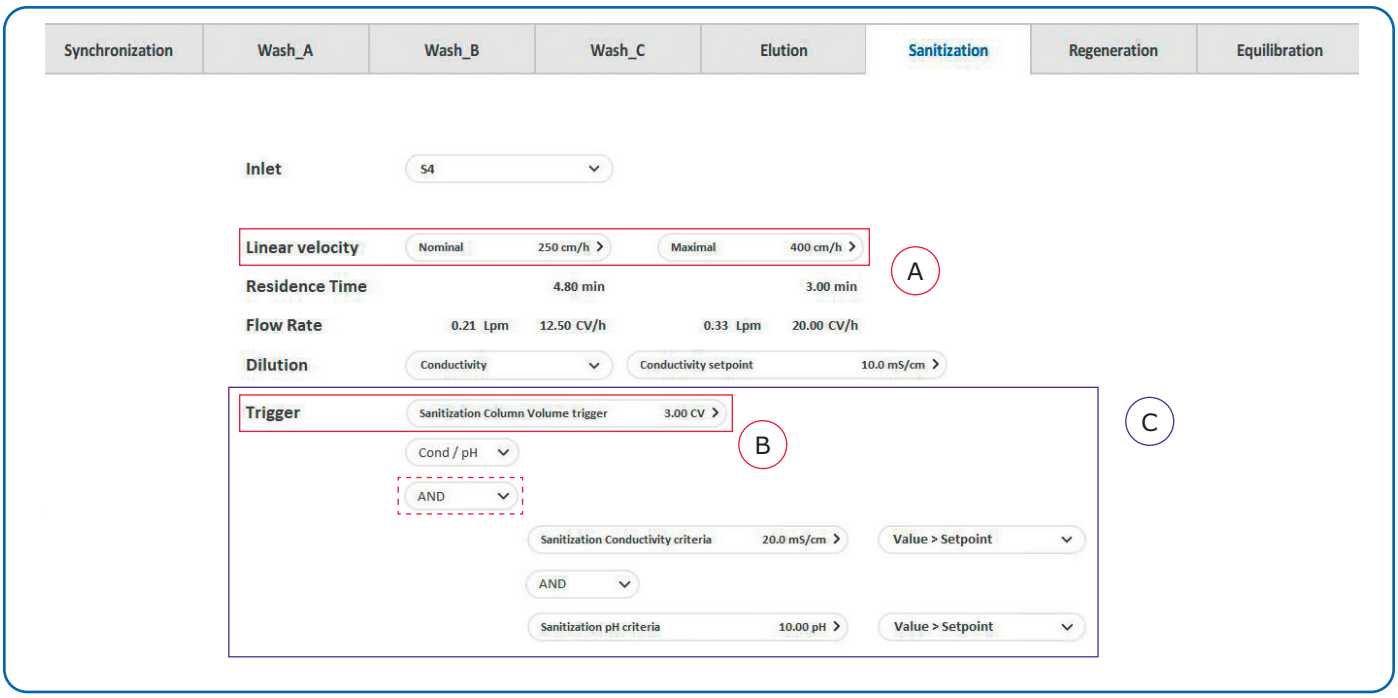

Figure 7.
Screenshot of the speed setpoints menu for sanitization. Nominal and maximal speed selection (A), CV trigger for this step (B), and complete triggers for this step, CV, conductivity, and pH (C).

Once all setpoints are entered, including "percent of loading phase", step priorities, and step maximal and minimal flowrates, the system can calculate the time required by the non-loading phase and adapt non-loading velocities to reach the time ratio targeted. To do so, the system automatically compares the necessary loading time of the previous cycle to the estimated non-loading phase duration. Loading and non-loading phases are compared according to the column order. To respect the process order, non-loading of column 3 is compared to loading of column 2, non-loading of column 2 is compared to loading of column 1, and the non-loading of column 1 is compared to the loading of column 3 (Figure 8). If the previous loading cycle was shorter, the system increases non-loading step speeds so that it matches back the ratio set. This is where step priorities are essential. Steps are adjusted according to their priority; if the step put in priority one already reached its maximal flow, the next step in the priority order is accelerated, and so on. Several steps can be accelerated in a single adjustment and the system automatically finds the optimal configuration to match the targeted proportions. After every cycle, the calculation restarts, and non-loading steps flow velocities are redefined. An alarm is triggered in the case where all steps are at maximal velocity already and the ratio is still not achievable.

For the phases that have durations that can vary disproportionally to the flow velocity (elution based on peak detection), sanitization if conductivity and/or pH triggers, regeneration if conductivity criteria triggers, equilibration if conductivity and/or pH criteria trigger, the system bases itself on the trigger CVs entered for these stages. In the case where another trigger is added to the criteria by an "and" symbol (conductivity or pH related), the trigger CV can be exceeded, and the synchronization would then be distorted. The selected ratio must take this possibility into account.

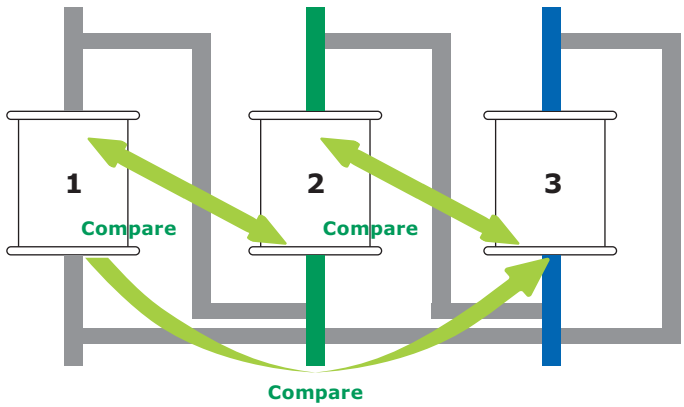

Figure 8.
Scheme of the comparison order for step prioritization and continuous process synchronization.

3 Long Process Duration Test

3.1 Twenty Day Duration Test

3.1.1 Functional Description and Test Objective

All flowpath components including sensors (flowmeter, pressure sensors, pH sensors, conductivity sensors, UV sensors), valves, pumps, tubing including bubble trap bags, and connectors have been tested for extended usage (20 days at 4 bar and 2 L/min; fluid at 20°C) for their performance (range and accuracy). In this section we present the overall flowpath tested on the system for 20 days and the components that can impact flowpath integrity. All duration tests presented in this section were performed on gamma irradiated components/tubing.

One part of the Process Qualification (PQ) consists of performing two 20-day runs to validate the three parts of the system and to detect any other unexpected issue:

- Flexware® assemblies: Every single-use component can withstand the process conditions without showing defects (leaks) between 3.5 and 4 bar on an active flowpath, applied for 22 days (20 days + 10%). All flowpath configurations were tested (AseptiQuick®, TC and MPC connectors, multi-use and single-use pH probe).
- Hardware: Robust and steady hardware (no loose parts, no shutdown, no issue).
- Software: No bugs along the process, no issue with data recording and export.

3.1.2 Materials and Methods

The test is performed by connecting all inlets and outlets to a water tank to create a recirculation loop. All steps are triggered by volume (no use of UV for breakthrough and peak detections since water is used). All non-loading steps are configured from wash A to equilibration and all inlets are used from S1 to S6. Dilution is performed at one out of two non-loading step, and both solvent and product lines are running at maximum flowrate (2 L/min). The pressure is adjusted to 3.8 bar for both lines by using a back pressure regulator. The average temperature of the water is 22°C.

3.1.3 Results

The pressure fluctuates between 3.0 and 3.8 bar on the product line and from 0.0 to 3.8 bar on the solvent line due to the cycle sequence. An example of pressure recording is provided in Figure 9. At the end of the 22 days, no leaks occurred on the complete flowpath, no issue was detected on the hardware and no bugs on the software, either during the process or during the export of the data. These two runs confirmed that the system can operate a process over a period of 20 days at room temperature.

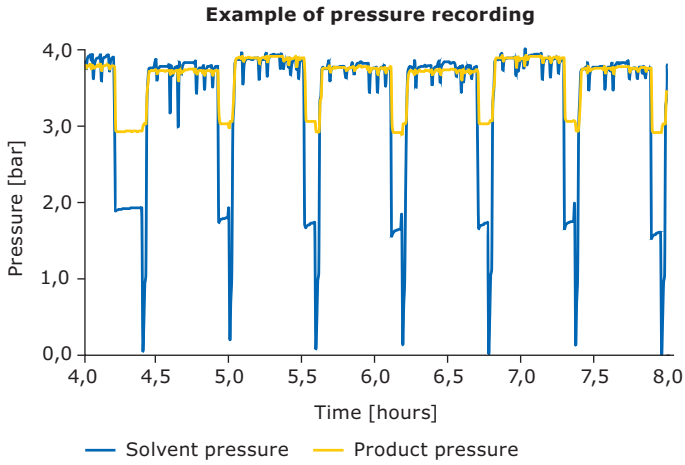

Figure 9.
Column 1 process report.

3.2 Maximum Valve Actuations

3.2.1 Functional Description and Objective

Continuous processes over 20 days require a high number of actuations of the pinch valves. The purpose of this study is to ensure that valve actuations do not damage the tubing.

3.2.2 Materials and Methods

The three different types of valves were tested in triplicate:

- 3 way-valves for 10,000 actuations with Pharma 80 tubing
- Pressure control valve for 2,000 actuations with Pharma 65 tubing
- Bubble trap venting valve for 100 actuations with Pharma 80 tubing

The number of actuations tested represents a worst-case scenario for a 20-day process duration representing more than 500 cycles.

3.2.3 Results

For all kind of valves, tubing can show some marks at the end of the test but no damage to the silicone and no leaks were observed (Figure 10).

Figure 10.
Impact of pinch valves on the tubing.

3.3 Pump Operating Time

3.3.1 Functional Description and Objective

The pump was tested during the process qualification (PQ) duration test (cf. 2.1) which is most representative of a real process; the pressure, however, was fluctuating due to cycle sequences and does not represent the worst-case parameter. The purpose of this study is to define the lifetime of the pump chamber when applying consistent maximum pressure.

3.3.2 Materials and Methods

This study was performed on a bench with a consistent pressure of 3.8 bar along the test while PQ varied from 3.0 to 3.8 depending on the process step (and valve switch; Figure 11). The flow was set at 2 L/min, the highest value of the pump range. The test was performed by using a chiller set at 20°C and a recirculation loop including the pump, a flowmeter and a pressure sensor. The pressure is adjusted by a manual pinch clamp.

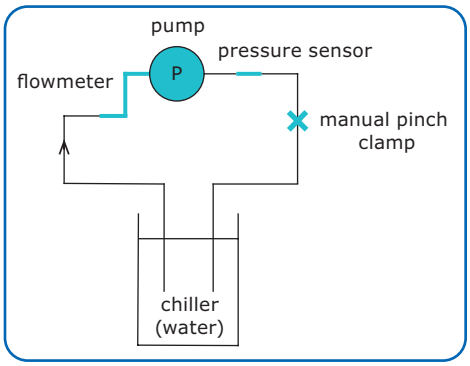

Figure 11.
Bench for pump chamber testing.

3.3.3 Results

No leaks were observed on the pump chambers after 22 days on nine different pump chambers (Table 10). The first leak was observed after 30 days and was located on one of the four diaphragms/membranes (Figure 12).

Parameters	Starting date	Duration (days)
3.8 bars, 2 L/min, 20°C	2-Dec-2021	51*
	2-Dec-2021	51*
	12-Jan-2022	38
	12-Jan-2022	38
	01-Feb-2022	35*
	01-Feb-2022	35*
	21-Feb-2022	31
	8-Mar-2022	27*
	8-Mar-2022	27*

**Test stopped before failure*

Table 10.
Pump chamber duration test at 20°C.

Figure 12.
Pump chamber diaphragm failure.

Based on these results (section 2.1, 2.2 and 2.3), the Mobius® Multi Column Capture system allows continuous processing without interruption for 20 days at 2 L/min, 3.8 bars and with a fluid temperature of 20°C.

3.4 Batch Report for Continuous Process

3.4.1 Overview

Continuous chromatography systems can run for up to 20 days without interruption. The amount of process data gathered is significant and includes several redundant steps. To facilitate reading the report and summarizing the main information regarding column cycles and step timing and volumes, two simplified report tables have been designed in addition to those for common batch reporting. The first table groups the main step information by columns, whereas the second one groups the breakthrough detection information per column.

When generating a report on the Mobius® Multi Column Capture system, it is possible to select the sections to save and display in the report. In addition to pre-run setpoints, event reports and more, it is now possible to select a column report (Figure 13). This specific part of the report will create multiple tables that group all major information regarding columns and breakthrough values in an organized classification per time, cycle, and column.

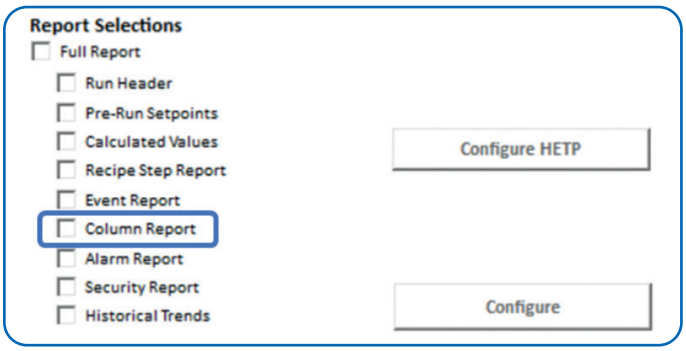

Figure 13.
Report sections selection menu.

The first type of table generated (column behavior), is created independently for each single column (Table 11). It groups all the information regarding the distinct phases occurring on the column including start time, definition, cycle number, inlet selected, outlet selected, number of CV, corresponding volume, duration, and flow velocity of the phase. This summary is generated from the events which makes the cycle analyses easier by pre-treating the data.

Column 1 Behavior								
Date Time	**Phase**	**Cycle**	**Inlet**	**Outlet**	**CV**	**Volume (L)**	**Duration (min)**	**LV (cm/h)**
09/22/2021 4:48:25 PM	Preload	1	Product	Waste	0.40	0.40	0.52	121.00
09/22/2021 4:49:52 PM	Loading	1	Product	Next column	1.10	1.10	1.42	121.00
09/22/2021 4:49:57 PM	Wash A	1	S1	Reserve	0.10	0.10	0.07	123.00
09/22/2021 4:50:01 PM	Wash A	1	S1	Waste	0.21	0.21	0.13	123.00
09/22/2021 4:50:05 PM	Wash B	1	S2	Waste	0.10	0.10	0.07	131.00
09/22/2021 4:50:09 PM	Wash C	1	S3	Waste	0.10	0.10	0.07	141.00

Table 11.
Column behavior table example.

The second table, also generated from the events, groups the breakthrough detection information from each column at each cycle including time, column number, cycle number, breakthrough UV value, plateau UV value and calculated area (Table 12).

Column Breakthrough					
Date Time	**Breakthrough**	**Cycle**	**BT (AU)**	**Plateau (AU)**	**Area (AU.L)**
09/22/2021 4:47:54 PM	Column 3	1	0.940	0.154	13.60
09/22/2021 4:51:17 PM	Column 3	2	0.971	0.162	13.57
09/22/2021 4:54:41 PM	Column 3	3	0.954	0.149	13.64
09/22/2021 4:58:05 PM	Column 3	4	0.930	0.156	13.60

Table 12.
Column breakthrough table example.

4 Dilution

The Mobius® Multi Column Capture system is equipped with inline buffer dilution capabilities on the solvent line. Two types of dilution control can be selected: Percentage (volumetric) or Conductivity. Both dilution types are linked to a regulation loop, based on pre-pump flowmeters for the percentage control and based on pre-column conductivity sensors for the conductivity control. System capabilities and dilution type performances are described below.

4.1 Dilution with Conductivity Control

4.1.1 Materials and Methods

For dilution by conductivity, Flexware® assemblies are installed on the system following the User Guide instructions. Draining line is connected to waste.

A tank filled with WFI is connected to the dilution inlet and a second tank containing a 3M NaCl solution is connected to the solvent entry S6. The NaCl line is primed by setting the flow path with the bubble trap (BBT) bypassed, the filter bypassed, and the solvent line directed to column 1 in a bypass to drain configuration. The solvent pump is started at 1 L/min until the conductivity read by AI001 is stable. Read conductivity (= 173 mS/cm) is reported as the conductivity of the initial NaCl solution and will be considered as the reference conductivity for this inlet. The same priming process is completed for the water line to remove air and salt residues in the line, conductivity read by AI001 once stable is set as the reference conductivity for this line (0 mS/cm).

Before starting the dilution, the conductivity regulation mode is selected and the total flowrate setpoint, the conductivity setpoint and the holdup volume between pump and conductivity sensor are set. Eventually, these setpoints are set using a recipe. Once all setpoints are set, dilution is initiated in the dilution menu on the Common Control Platform® (CCP®) software or using a recipe action. If stabilization is not reached within 30 minutes, the dilution is aborted and defined as not achievable. All data is recorded and saved.

Prior to restarting any dilution rate trial, the entire line is rinsed with WFI until the conductivity read by the pre-column conductivity sensor equals the set reference value of this line. Dilutions with the in-line bubble trap were performed to assess the effect of the added dilution chamber on the line. In this case, the dilution is started with the bubble trap filled with water, and the hold up volume is adjusted accordingly.

An additional test has been performed to assess time gain to reach conductivity setpoint after the first cycle. Two non-loading steps were configured at a flowrate of 0.4 L/min and a conductivity setpoint of 30 mS/cm for the first step and 60 mS/cm for the second step. Two cycles have been performed to compare the time to reach the dilution stability.

4.1.2 Performance, Stabilization Times

Different effects need to be considered when performing conductivity-based dilutions on the Mobius® Multi Column Capture system to ensure the compatibility of the dilution with the synchronization of the continuous process, bubble trap online or not, stability criteria, and total flow. The main variable to play with is time. Dilution stabilization should not be too time consuming, or the synchronization calculation would be out of range. The following results highlight the time required to reach stabilization according to the stability criteria and the dilution rate set (Table 13 and Table 14).

Dilution rate	x2	x5		x10		x20	x40	
Conductivity setpoint (mS/cm)	103.3	46.8		25.5		14.5	8.8	
Total flowrate Flow (L/min)								
1	Not reached							
2	00:11:16							
6		00:08:10	00:06:16	Not reached	00:05:37			
10				00:04:02		00:05:23		
20						00:06:10	00:14:19	
40							00:08:02	00:01:34
120							00:01:19	

Table 13.
Required time (hh:mm:ss) to reach stability according to total flow, dilution rate and stability criteria.

Legend					
Stability criteria used	Flow deadband (%)	20	20	10	20
	Flow stable time (s)	30	30	30	30
	Conductivity deadband (%)	5	6.5	5	35
	Conductivity stable time (s)	30	30	30	30

Table 14.
Stability criteria set according to color.

Results show that the deadband should be set carefully when selecting the stability criteria. As this parameter is set in percentage of the targeted value, the smaller the target, the greater the deadband; otherwise, stability may never be reached.

To reduce the stabilization time of a dilution, dilution rate from the same step of the previous loading phase is saved and set as the new setpoint at the start of the next dilution. Thus, if no solution change is made between two loading phases, concentrate and/or water source, the system starts the regulation at the perfect pump ratio. Regulation loop corrections are minimized, and stability of the dilution is reached more rapidly (Figure 14). Measurements performed have shown a time gain of 42 and 52 seconds from dilution start to entering the deadband, translating in the same time advantage to reach stability.

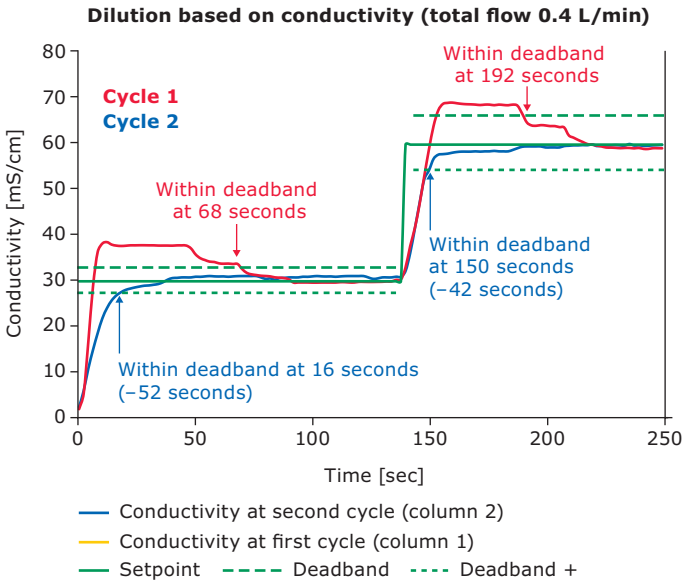

Figure 14.
Example of time gain to reach conductivity setpoint after the first cycle.

Dilution precision has been assessed by calculating the maximal mixing error of stable dilutions. Results are summarized in Table 15 and show that all dilution remained below 4% for all tested dilutions.

Dilution	x2	x5	x10	x20	x40
Conductivity setpoint (mS/cm)	103.3	46.8	25.5	14.5	8.8
Flow (L/min)					
1	Not reached				
2	3.82%				
6		0.49%	3%		
10			2.27%	0.44%	
20				1.38%	0.58%
40					0.73%
80					0.61%
120					0.96%

Table 15.
Mixing error in % according to the dilution criteria in conductivity without BBT. Grey area symbolizes the theoretically unreachable dilutions due to pump limitations (concentrate flow rate is below pump theoretical capacity).

4.1.3 Concentrate Consumption

During the dilution stabilization, the flow path is diverted to the drain, bypassing the column, preventing unwanted solution mix to flow through the column. In this regard, concentrate solution flowed during the stabilization is lost to the drain. Values presented in Table 15 provide an estimate on concentrate consumption when running dilutions based on conductivity. Consumed concentrate must be put in relation with the stability criteria set; the stricter the stability criteria, the harder it is for the system to reach stability and concentrate consumption is higher. Therefore, results are presented with the corresponding stability criteria set during measurement (Table 16).

Dilution rate	x2	x5		x10		x20	x40	
Conductivity setpoint (mS/cm)	103.3	46.8		25.5		14.5	8.8	
Flow (L/min)								
1	Not reached							
2	0.339							
6		0.163	0.090	Not reached, conductivity deadband too low	0.112			
10				0.067		0.045		
20						0.103	0.119	
40							0.134	0.026
80							Not reached, conductivity deadband to low	
120							Not reached, conductivity deadband too low	0.066
150							Not reached, conductivity deadband too low	

Table 15.
Volume (in L) needed to reach stability for conductivity-based dilutions without BBT.

Legend					
Stability criteria used	Flow deadband (%)	20	20	10	20
	Flow stable time (s)	30	30	30	30
	Conductivity deadband (%)	5	6.5	5	35
	Conductivity stable time (s)	30	30	30	30

Table 16.
Stability criteria setpoints.

4.1.4 Bubble Trap Considerations

Use of the bubble trap (BBT) when performing in-line dilution should be assessed on a case-by-case basis, depending on the flow rate and the number of buffers being diluted to ensure synchronization of the continuous process remains within the defined duration. The main reasons for BBT usage restriction for dilutions are linked to dilution chamber creation, holdup volume increases and difficulty defining, and mixing of the diluted solution with previous step solution. These factors increase the time required to stabilize the dilution which significantly slows down the non-loading steps duration.

Dilution chamber creation and mixing effect:

The BBT is a filled 3D bag that removes any air bubbles from the feedstream. During use, it is filled up to a maximum level of 1.4 L of solution and acts as a surge container adding volume to the line. The added volume inevitably creates a pocket where dilution is occurring between the remaining previous step solution in the BBT and the new solution. When a dilution is started with the BBT inline, the BBT is filled with the previous step solution. If densities between the two solutions are significantly different, mixing occurring in the BBT is not ideal. Depending on the flow at which the dilution is conducted, part of the less dense solution may remain at the top part of the BBT.

Holdup volume increase:

The holdup volume to enter into the dilution per conductivity menu corresponds to the volume between the exit of the pumps and the conductivity sensor. This volume is used to calculate, according to the flow applied, the time needed for the solution to travel between the exit of the pump and the conductivity sensor. This time is then defined as the minimal time between two corrections of the regulation loop. This prevents the loop from acting on the pump flow setpoints before having a precise reading of the previous modification. According to the flow path selected upstream to the conductivity sensor (BBT bypassed/in-line), the holdup volume is different and must be adapted. If the holdup volume is set too high, it increases the stabilization time and lowers the reactivity of the system. In contrast, when the holdup volume is set too low, it can make the system unstable which also increases the stabilization time and the error throughout the entire dilution. When placing the BBT online for dilutions, the holdup volume is increased (Table 17) which makes the regulation loop slower. The time to reach stability is slow, and dilution may become unusable in a continuous processing as it may impact synchronization between loading and non-loading duration. This effect is less pronounced for near maximal flows.

Bubble trap	Holdup Volume (L)
BBT bypassed: from mixing junction point to conductivity sensor pre-column	0.07
With BBT Inline	1.4 (when bubble trap full)

Table 17.
Dilution holdup volume setpoint according to the upstream flowpath configuration.

The sensor controlling the dilution is the conductivity sensor placed before the column. The BBT volume is therefore already considered as it is placed upstream to the sensor. Adjusting the holdup volume to take the bubble trap volume into account is not sufficient since the dilution effect occurring inside the bubble trap is not considered. The system is correcting pumps speeds relying on a measurement that is not representative of the actual pump outputs because the dilution effect delays the adjustment; this leads to an unstable regulation. Theoretically, when the BBT is inline, the holdup volume should be overestimated to include this dilution effect.

4.2 Dilution with Volumetric Control

4.2.1 Materials and Methods

For dilution by percentage, all Flexware® assemblies are installed on the system following the User Guide instructions. Draining line is connected to waste.

A tank filled with WFI is connected to the dilution inlet and a second tank containing an acetone solution (absorbance ≈1.8 AU) is connected to the solvent entry S6. The WFI line is primed by setting the flow path with the BBT bypassed, the filter bypassed, and the dilution line directed to column 1 in a forward position to waste. The line is flushed with the dilution pump at 1 L/min for at least 2 L after having removed all the air from the inlet. A UV zero is performed on the AI005 sensor. The dilution pump is then stopped, and the acetone solution inlet/line is primed the same way by keeping the system in the same flowpath configuration. Solvent pump flow is set at 1 L/min until no air is remaining in the line and the absorbance read by the UV sensor is stable for more than 1 minute. The stable absorbance value is reported as the absorbance of the acetone solution and is considered as the reference absorbance of the initial solution. The solvent pump is stopped and the flowpath is flushed using the dilution pump at 1 L/min until the absorbance read by AI005 decreases to 0 AU (absorbance of WFI).

The dilution is set in percentage mode and the total flowrate setpoint and the dilution percentage setpoints are fixed. Eventually, these setpoints are set using a recipe.

Dilution is started in the dilution menu on CCP® software or using a recipe while keeping the flow path as is (BBT bypassed, solvent line directed to column 1 and column 1 to waste). If stabilization is not reached within 30 minutes, the dilution is aborted and defined as not achievable. All data is recorded and saved.

Between dilutions, the line is flushed with WFI from the dilution pump to bring the absorbance back to 0 AU. The system is then ready to start another dilution with different setpoints. Dilutions with the in-line BBT were performed to assess the effect of the added dilution chamber on the line. In this case, the dilution is started with the bubble trap filled with water, and the hold up volume is adjusted accordingly.

4.2.2 Performance, Stabilization Times

Complete curves and data will not be detailed in this document. Examples of obtained curves, with and without the BBT, are shown in Figure 15 and Figure 16.

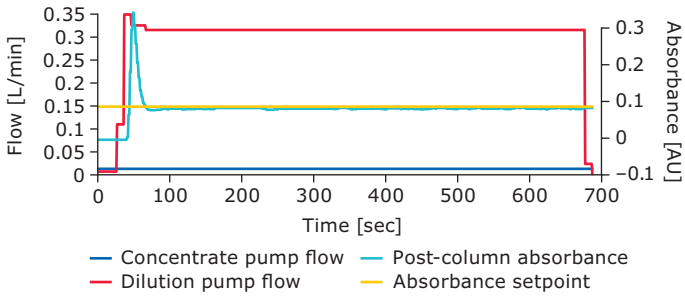

Figure 15.
Obtained curves for the dilution without BBT at 20 L/h and x20 dilution (setpoint 0.089 AU).

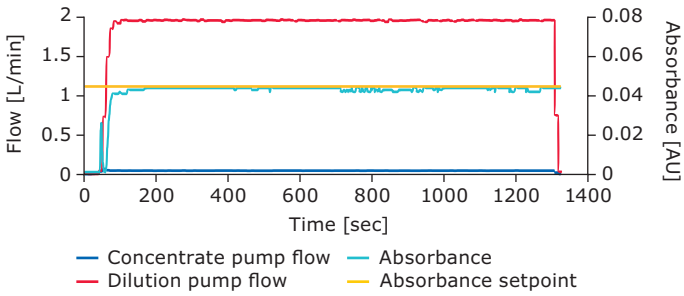

Figure 16.
Obtained curves for the dilution with BBT at 120 L/h and x40 dilution (setpoint 0.045 AU).

Data generated during this trial were used to qualify the capabilities of the system to control dilutions by percentage. On the system, this control is allowed by the flowmeters placed upstream to the pumps. Thus, the regulation loop is short and effective in comparison to a conductivity based dilution.

Results obtained by performing dilutions by percentage with the BBT bypassed are presented in Tables 18–21 are representative of the capabilities of the system. Once stability was reached, mixing errors were never above 4.5% for all tested rates, even theoretically unreachable dilutions. However, the theoretically unreachable dilutions tested at 1 L/h and 2 L/h with dilution rates of x2 and x5, respectively, never reached the targeted flowrate. This does not influence the outcome of the trial as these dilutions were only tested for information. For all measurements, during the first step of stabilization, an overshoot of concentration was always observed. This is linked to the start of both pumps where the concentrate and dilution pumps jump from stopped to the first flow increment; the pumps are not starting at the right increment which is linked to the motor capabilities that require a certain torque to start.

This torque is too high at the beginning and the pump is already at too high of a flow; as such, the ratio between both pumps is not correct and this generates an overshoot at the start of a dilution. Stability criteria (deadband and stability time) ensure that the dilution reaches stability prior to being sent to the column. The stabilization time and deadband fixed for all measurements were set to integrate the time needed to flow the holdup volume at the tested total flowrate. Measured errors showed that it was sufficient to ensure a stable dilution. Globally, pumps reached the total flowrate and pump percentages in approximately 30 seconds; the remaining time needed for stabilization corresponds to the stability criteria set which are proper to a configuration or process.

Results without and with BBT are summarized in the following tables:

- Stabilization time required for each dilution in seconds. (Table 18 and Table 20)
- Maximal error once stability criteria reached measured in %. (Table 19 and Table 21)

Without BBT:

Dilution						
Flow (L/min)	x2	x5	x10		x20	x40
1	Not reached					
2	00:03:39	Not reached				
6		00:01:26				
10			00:00:57	00:00:58	00:00:47	
20					00:00:58	00:00:43
40		00:00:40				00:00:42
80		00:00:46				00:00:50
120			00:00:58			00:00:59

Legend						
Stability criteria used	Flow deadband (%)	10	10	10	10	10
	Flow stable time (s)	360	180	60	45	30

Table 18.
Average time to reach stability (hh:mm:ss) for percentage dilution without BBT.

Dilution					
Flow (L/min)	x2	x5	x10	x20	x40
1	Not reached				
2	3.13%	Not reached			
6		1.82%	Not tested		
10			4.36%	3.80%	
20				1.21%	0.44%
40		0.54%			0.39%
80		0.24%			0.51%
120			0.16%		0.03%

Table 19.
Max. error (in %) for dilution in percentage without BBT. Grey cells correspond to theoretically unreachable dilutions.

With BBT:

Dilution rate target	
Flow (L/h)	X40
120	00:00:58

Table 20.
Average time to reach stability (hh:mm:ss) for dilution in percentage with BBT.

Dilution rate target	
Flow (L/h)	X40
120	0.22%

Table 21.
Max. error (in %) for dilution in percentage with BBT.

In comparison to dilutions based on conductivity, for percentage based dilutions, the BBT effect is reduced. To illustrate it, a dilution with inline BBT is performed, based on a 2.5% primary pump ratio at 120 L/h and started with the same stability criteria (stability deadband 10% and stability time 30 s) as for the corresponding dilution performed without the BBT. As expected, results have shown that the stabilization time was the same (58 s vs. 59 s) without BBT. This can be explained by the fact that the control loop is made through the flowmeters and not via a sensor placed downstream to the BBT. Thus, the downstream flow path modification is not considered when performing a dilution by percentage. To counteract this phenomenon in continuous processing, once a percentage dilution is stable, a holdup volume is flowed prior to switching the column online. This ensures that only the correctly diluted solution is sent to the column.

According to the flow at which the dilution is performed, this could represent a considerable amount of time; for example, at 0.1 L/min, it takes 14 minutes to flow the 1.4 L of holdup volume. The additional holdup volume flush is not taken into account for the previously shown results.

4.2.3 Concentrate Consumption

As with a conductivity-based dilution, when a percentage-based dilution is started, the system regulation loops require some time to properly stabilize the dilution to the desired setpoints. For such dilutions, this includes the time to ramp both pumps to the desired total flow and adjust proportions of both pumps. Additionally, once flows are stable with correct proportions, a holdup volume (volume between the pumps outlet and the column inlet) is flowed to ensure the complete line is filled with the correct solution before sending it to the column. During this stabilization, the flow is directed to the pre-column waste and the concentrate used during dilution stabilization is lost to the drain.

Values presented in Tables 22 and 23 provide an estimate on concentrate consumption when running dilutions based on volumetric control. Consumed concentrate must be put in relation with the stability criteria set; the stricter the stability criteria, the harder it is for the system to reach stability and concentrate consumption is higher. Therefore, results are presented with the corresponding stability criteria set during measurement (Table 24).

Dilution						
Flow (L/min)	x2	x5	x10		x20	x40
1	Not reached					
2	0.0610	Not reached				
6		0.0287				
10			0.0159	0.0161	0.0261	
20					0.0160	0.0595
40		0.0887				0.0117
80		0.0680				0.0273
120			0.1933			0.0492

Table 22.
Volume (in L) needed to reach stability for percentage dilution without BBT.

Dilution rate target	
Flow (L/h)	X40
120	0.049

Table 23.
Volume (in L) needed to reach stability for dilution in percentage with online BBT.

Legend						
Stability criteria used	Flow deadband (%)	10	10	10	10	10
	Flow stable time (s)	360	180	60	45	30

Table 24.
Stability criteria setpoints (and color code associated to Table 23).

4.2.4 BBT Considerations

Controlling a dilution by percentage is made possible by acting on the pump speed according to the reading of the flowmeter placed right before the pump. The volume in the BBT is, therefore, not considered as it is placed downstream.

Once the system reaches the correct total flow and the correct mixing rate (total flow stable + percentage of each pump correct) which confirms the dilution is correct, the system waits for an entire holdup volume to consider the dilution as stable. This situation is ideal for a flow path that does not contain any dilution chamber and ensures that the correct dilution rate reached the column entry line before sending any buffer in the column. When the flow path contains a downstream dilution chamber, such as a BBT, waiting for a single holdup volume is not enough since dilution effects occurring in this chamber are not considered. This does not ensure that the solution sent into the bubble trap exactly corresponds to the solution flowing out of it. Moreover, the volume required to ensure this steady state depends on the mixing occurring in the BBT which itself depends on the flow, the density of the two solutions, and the volume contained in the BBT. The volume contained in the BBT is also depending on the flow, the downstream pressure, and the placement of the LSH. It is therefore extremely difficult to predict the holdup volume and the effect of the dilution chamber. To counter this effect, the holdup volume set for the dilution should be highly overestimated, thus, reaching the dilution stability is much slower.

BBT effect is less notable compared to the conductivity dilution, due to the placement of the bubble trap not within the regulation loop flowpath. BBT effect cannot be measured as easily in volumetric dilution control vs. conductivity dilution control.

5 Test HETP and Asymmetry

5.1 Overview

The Mobius® Multi Column Capture system provides a method for monitoring column chromatography without having to stop the production process, solving the prior art problem of monitoring column quality and efficiency (column performance). The monitoring functions without the need to stop the process, the need to add additional test specific steps to the process flow, or the need to add reagents specific for column testing purposes (such as pulse chemicals). In other words, column performance is monitored without interrupting the process or adding more complexity.

The quality of the packed bed is assessed by determining a change in conductivity, a measurable parameter (Figures 17–19). Thus, the present method of height equivalent to theoretical plates (HETP) frontal analysis consists of suddenly changing the solution flowing in the column to generate a step of the measured value. By using the frontal analysis on existing chromatography operations that generate those steps and repeating this analysis cycle after cycle, it is possible to monitor the quality of the packed bed without interrupting the process. Moreover, repetition of this analysis cycle after cycle allows one to ascertain changes in column quality during the continuous process.

On the Mobius® Multi Column Capture system, the HETP and asymmetry measurement are made between the regeneration step and the equilibration step. The system collects the conductivity values from the post column sensor and applies a Savitzky-Golay smoothing filter to it. The first derivate is applied to the smoothen data to obtain a Gaussian-like curve. HETP and asymmetry values are then extracted from this curve using conventional measurement techniques also used in pulse analyses. All calculated data cycle after cycle and for each column are saved and visible on the system screen (last calculated value) and in the report as well. Alarms can be configured to automatically monitor the output value (HETP and Asymmetry) and secure the process.

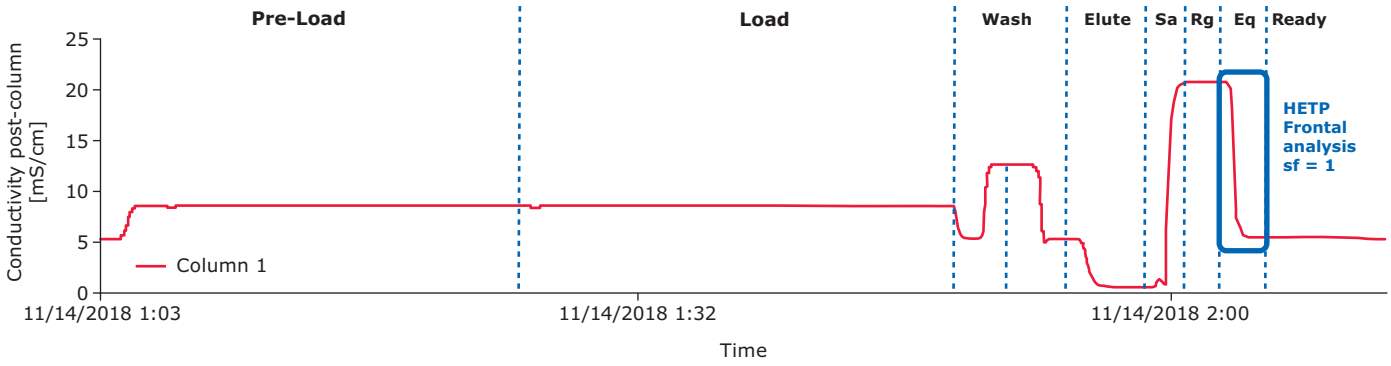

Figure 17.
Example of chromatogram (conductivity against time) highlighting the moment where the analysis is made.

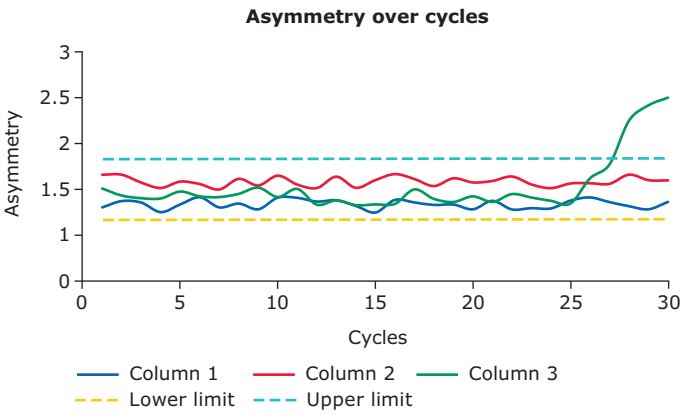

Figure 18.
Example of obtainable curves/data for asymmetry, highlighting the possibility to see a column asymmetry drift.

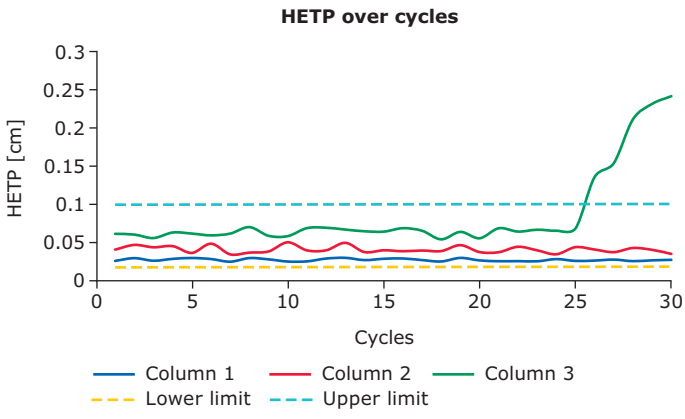

Figure 19.
Example of obtainable curves/data for HETP, highlighting the possibility to see a column HETP drift.

6 NaOH Impact on Flexware® Assemblies During a Continuous Process

For general chemical compatibility information please refer to document RF1025EN00 “Chemical Compatibility of Mobius® Components”.

6.1 Test Objective

The Mobius® Multi Column Capture system can be used for continuous chromatography running over a long period of time. At each cycle during this process, regular sanitization/regeneration steps with NaOH are applied. Assuming a continuous process of 20 days, the cumulative time of exposure is considered to be a maximum of 3.5 days; therefore, all components used on the system must withstand the time contact of 3.5 days with NaOH 0.5M.

A study was performed to ensure there is no impact neither on sensor accuracy or on the Flexware® assemblies (tubing and components) after extended exposure to NaOH solution.

6.2 Materials and Methods

An assembly was built with all Mobius® Multi Column Capture system components in line and both inlet and outlet were put in a recirculation tank containing NaOH 0.5M. The following cycle was repeated three times:

- 8 hours at 2 L/min, no back pressure
- 16 hours filled with NaOH but without flow (static conditions)

For the last half day:

- 4 additional hours at 2 L/min without back pressure
- 8 hours filled with NaOH but without flow (static conditions)

This process results in 3.5 days in contact with NaOH including 28 hours in dynamic conditions (2 L/min).

At the end of the test the following acceptance criteria were evaluated:

- No leakage on the loop at 4 bar water pressure
- Sensor accuracy still within the accepted range (please check the datasheet “Data Sheet: Mobius® Multi Column Capture System”, available at sigmaaldrich.com/chrom-systems for specification and ranges of accuracy)
- Tubing should not stick where valves are pinched
- Opening and closing of the drain valve still works as intended (functional test)

The assembly was gamma irradiated before the test which was performed at ambient temperature.

Components tested:

- Pump chamber
- Flowmeter
- Single-use cell (Conductivity and UV)
- Tubing
- Connectors
- Fittings
- Sampling valve
- Pressure sensor

The pH sensor was tested in the frame of pH drift study (see section 7.3).

6.3 Results

No leaks were observed on the complete Flexware® assembly, and the accuracy of all sensors remained within specifications at the end of the test. This test shows that the flowpath of the Mobius® Multi Column Capture system can be used for a continuous process of 20 days with repeated exposure to NaOH.

7 pH Drift and Recalibration During a Continuous Process

7.1 Accuracy of Single-Use (SU) pH Probes after In-Process Calibration

This study evaluated the accuracy of the single-use pH probe after initial calibration followed by in-process calibration on the pH range [2-12].

7.1.1 Test Objective

Single-use pH probes are delivered with pre-calibration coefficients. To improve the accuracy after dry storage, a 1-point calibration can be performed after a wetting-time of 30 minutes following the priming of the system Flexware® assemblies. The objective of the test is to confirm and characterize the accuracy of the pH range [2-12] after in-process calibration, and to characterize measurement shift and trends after which the shift starts to appear.

7.1.2 Material and Methods

Standard solutions from 2.00 to 12.00 pH in increments of 1.00 pH unit were used. Six single-use pH probes were 1-point (process) calibrated in the pH 7.00 standard solution. The probes have been tested in beakers with the specified various standard solutions.

Acceptance criteria:

- +/-0.10 pH in the pH range [6-8]
- +/-0.15 pH in the pH range [2-9]
- +/-0.30 pH if pH >10

7.1.3 Results

All the measurements in standard solutions are within the accuracy claims (Table 25).

Buffer solutions pH	2	3	4	5	6	7	8	9	10
SN 1178	0.03	-0.02	0.06	0.06	0.00	0.00	0.02	0.14	0.21
SN 1183	0.01	0.02	0.05	0.10	0.10	0.00	0.10	0.12	0.21
SN 1193	-0.06	-0.07	-0.02	0.02	0.07	0.00	0.07	0.11	0.19
SN 1187	-0.08	-0.07	-0.08	-0.05	-0.09	0.00	-0.07	0.13	0.12
SN 1185	0.00	0.00	0.02	0.03	0.00	0.00	-0.01	0.05	0.07
SN 1194	-0.05	0.00	0.02	0.03	0.05	0.00	0.03	0.15	0.00

Table 25.
pH reading relative error vs. buffer pH.

7.2 Process Duration Impact on pH Measurement Drift

7.2.1 Test Objective

pH sensors can show drift due to a long and continuous use in buffers with reduced salt molarity. To characterize this drift, single-use pH sensors have been compared to multi-use pH sensors as a reference.

7.2.2 Materials and Methods

This study was performed on a bench using the following materials:

Solutions

- NaCl solution of 2 mS/cm as for conductivity
- IgG proteins at 1 mg/mL

Hardware

- QF 30 SU Pump – Quattroflow®
- 2 single-use pH probe Oneferm – Hamilton in flow through measurement chambers
- 1 multi-use pH probe Polylite plus as reference – Hamilton
- Temperature regulation system

Software

- pH readings with ArcAir software from Hamilton

Methods

The system was in continuous operation over 35 days and run at 50 mL/min at 37°C. Controls in standard pH 4 and pH 7 solutions of the reference multi-use probe were compared before each measurement and recalibrated if required (Figure 19). The reference multi-use pH probe was tested before each measurement of the single-use pH probe and was recalibrated if required.

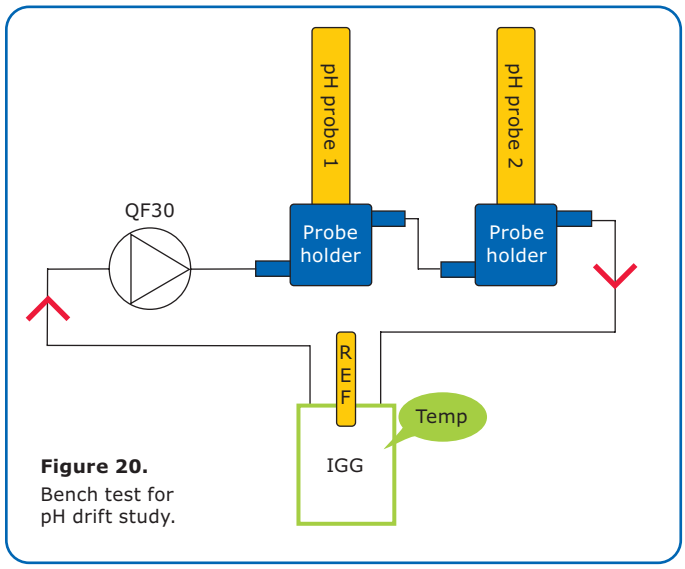

Figure 20.
Bench test for pH drift study.

7.2.3 Results

The characterized drift of single-use probes was less than 0.05 pH unit over 35 days (Figure 20). Under this pH condition, no specific daily process calibration is required to keep the calibration accurate (see following section for recalibration recommendation under process conditions considering cycles and sanitization).

7.3 NaOH Impact on pH Measurement Accuracy

7.3.1 Test Objective

pH sensors can show a drift due to prolonged use and harsh environments such as an NaOH solution used during sanitization. To ensure the accuracy of measurement throughout the sensor lifecycle, sanitization cycles have been reproduced at bench scale during the minimal operating time (Figure 21).

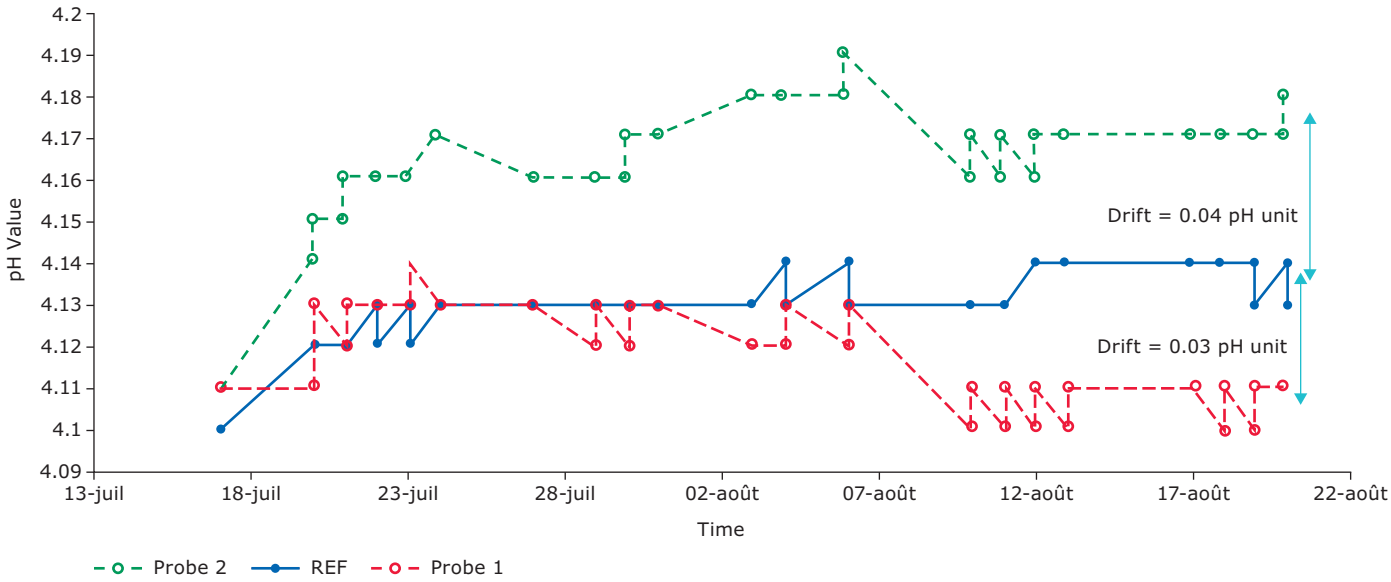

Figure 21.
Drift results of single-use (SU) pH probes compared to multi-use (MU) pH probe as reference. pH probes 1 and 2 are the SU probes; "REF" is the MU probe.

7.3.2 Material and Methods

This study was performed using the following materials:

Solutions

- 0.5M NaOH
- 0.04M HEPES

Hardware

- QF 150 SU Pump – Quattroflow®
- 1 single-use pH probe Oneferm – Hamilton in Optek SUC measurement chamber
- 1 multi-use pH probe Easyferm Bio – Hamilton in Optek SUC measurement chamber
- Pressure sensor
- Temperature regulation system

Software

- pH readings with ArcAir software from Hamilton

Methods

The study was run over a 20-day period. Each day:

- 4 bars of pressure, 4 hours/day in contact with NaOH with a flowrate of 500 mL/min at 30°C (80 hours in contact with NaOH over 20 days)
- 20 hours in HEPES solution, 500 mL/min, no pressure, 30°C
- Control in standard pH 3 and pH 7 solutions each day after sanitization

7.3.3 Results

Drift increases for the single-use and the multi-use probes with the number of cycles/days of a 0.5M NaOH 4-hour sanitization according to a power law (Figures 22 and 23). The drift is about 0.30 pH unit at the end of the experiment. A process calibration every three days is thus required to keep the measurement within the claims of accuracy. These tests demonstrated that the behavior of single- and multi-use pH probes is similar under similar exposure to buffer and NaOH.

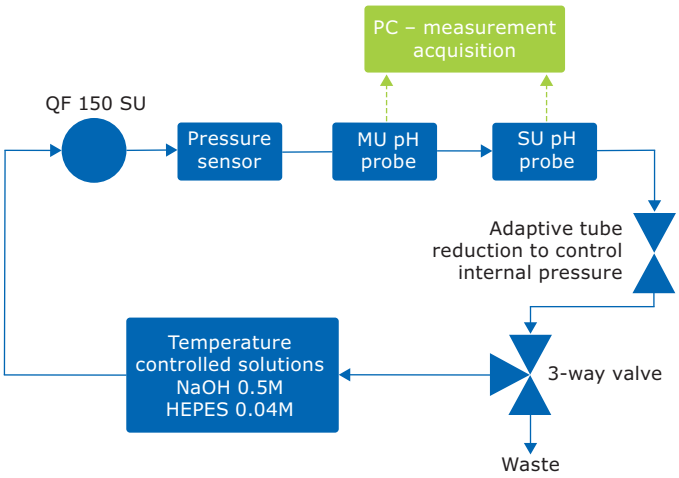

Figure 22.
Bench test for NaOH impact on single-use and multi-use pH probes.

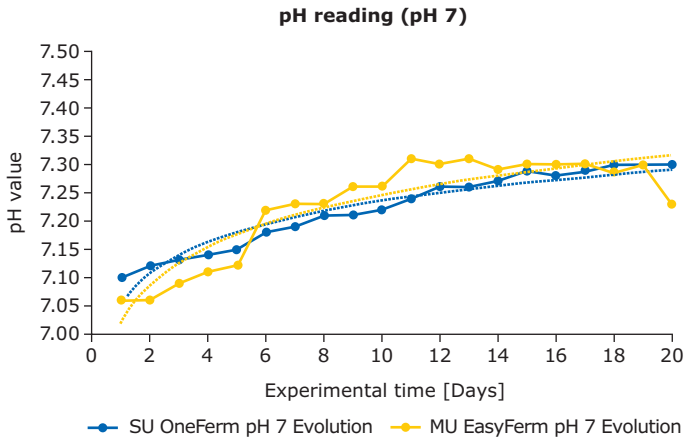

Figure 23.
Results of pH drift of single-use and multi-use pH probe (pH 7).

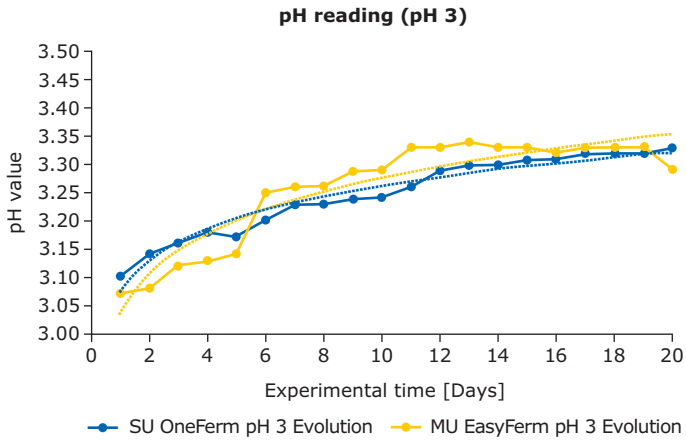

Figure 24.
Results of pH drift of single-use and multi-use pH probe (pH 3).

For more information, please visit:

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