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Performance Guide

Mobius® Chrom 2 System for Chromatography

How to Use This Guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® Chrom 2 system for Chromatography. This guide includes information from several applications and case studies that were designed and/or selected to provide a diverse overview of the system performance under a range of expected processing conditions.

The results included in this guide summarize outcomes and observations obtained in studies conducted using model feed streams and experimental conditions. Therefore, all test results should be confirmed by the end user using feed stream 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.

Introduction

The Mobius® Chrom 2 system with single use Flexware® assemblies is a fully automated system designed to enable the clinical and commercial-scale operation of chromatography processes for the downstream purification of MAbs, vaccines, viral vectors, and therapeutic proteins. The system has the same functionalities as conventional chromatography systems, and by incorporating a completely single-use flow path, it provides operational flexibility while eliminating concerns of carryover or cross contamination.

The Mobius® Chrom 2 system is composed of two separable units; a pump cart and a base cart holding the clamshell. The pump cart is equipped with two pumps individually linked to 5 inlets. Both pumps are followed by electromagnetic flowmeters for precise flow monitoring and totalization features. The validated maximal reachable flow rates during processing is 2.2 L/min per pump, with an overall flowrate range of 0.1 to 2.2 L/min for up to 2 cP solutions.

The base cart, containing the Flexware® within the clamshell, enables to select the flowpath and distribute the flow to the different system organs; – the bubble trap (BBT) enabling air removal and column protection with automatic level control – the pre-column filter – the pre-column instrumentation (conductivity and pH) – the column itself (upflow, downflow or bypass) – the post-column instrumentation (conductivity, pH and UV absorbance with dual wavelength) – the four different fraction outlets. Within the flowpath, a pre-column pressure control valve, located before the column, enables automatic pressure regulations. Two air sensors, one located on the product inlet and the second before the column, enable automatic end of product detection and secure the column against air bubbles.

Conductivity, and pH instrumentation is available before the column. Conductivity, UV absorbance and pH instrumentation is available after the column. The pH probe can be user-supplied and installed on site or pre-installed and irradiated in the fully closed Flexware® assembly.

The specific system configuration that was used to generate the performance data included in this guide will be noted in the methods section for each study.

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Summary of Studies

1. Hold-up volumes and drainability

Background and Objectives

The hold-up volumes correspond to the volumes contained within the flowpath when fully filled. It is determined section by section to enable hold up volume estimation according to the selected flowpath. This volume is particularly useful to monitor the progress of a process usually based on counted volume of solution and for specific application such as product buffer push etc.

System drainability is also assessed to estimate the remaining amount of liquid within the flowpath before dismantling.

Materials and Methods

The following tests have been performed on a Mobius® Chrom 2 system, equipped with single use open Flexware® assembly. According to the system design, open and closed flowpaths do not vary in flexware volumes nor drainability.

Hold up volumes

The entire Flexware® assemblies are dried and installed following the instructions provided in the user guide.

Pumps and all lines are primed with water ensuring all bubbles are removed. Valves are closed and/or clamps are placed to select the flowpath section to be measured. A draining point is opened, the fluid is recovered in a tared beaker and the weight is measured to define the section volume. Section selection and measurements are repeated to measure all main flowpath sections volume. Pressurized air push is used to force all the liquid out.

Note: Bubble trap volume will vary from one process to another according to level sensors placement and reached pressures, moreover, filter volume is not considered (refer to the specification sheet of the used filter for hold up volume quantification).

Drainability

The entire Flexware® assemblies are dried and installed following the instructions provided in the user guide. A water tank placed on a weight scale is connected to the system inlet and outlets. Pumps and all lines are primed with water ensuring all bubbles are removed. System inlets and outlets are closed and the lines used for water connection are drained within the weighted water tank. Exact volume of water contained within the system is assessed based on the water tank weight difference. An empty and tared tank is placed under the flowpath draining points. The system flowpath is drained using all available draining points and flowpaths. Additional air push is performed by running the pumps from unconnected inlets. Manually tubbing lining to help liquid displacement is also performed.

Drained water collected in the tank is weighted and compared to the initially calculated volume within the system flowpath to define the undrainable volume.

Note: Drainability measurement were performed with water (low viscosity). If the system isn't rinsed before dismantling, the remaining solution within the flowpath may have a higher viscosity and drainability results could vary.

Results

A summary of the measured hold up volumes is indicated in **Table 1** with descriptions of the corresponding flowpath section.

Table 1.
Summary of the hold up volumes measured.

All measured sections	
Flowpath section	**Measured volume (mL)**
H* (post-pump before BBT and Filter)	14.5
Post-pump 2 to XV012	21.7
Post-pump 1 to XV013	20.8
From XV018 to end of pre-column sensor	20.4
From BBT exit to XV016	32.9
From BBT entry (top of the tube) to XV014	31.4
From inlet 2E to pump entry	17.6
From inlet 1E to pump entry	9.4
From inlet 1E to pump entry	9.4
Column in reverse position, volume between column outlet and post-column instrumentation	66.6
Column in forward position, volume between column outlet and post-column instrumentation	78.7
Pump head internal volume when primed with connectors	9.8
Column bypass, volume between pre-column sensor to post-column sensor	29.8
Post-column sensor to waste valve	70
Post-column sensor to Fraction 1	64
Post-column sensor to Fraction 2	65
Post-column sensor to Fraction 3	68.5
BBT full without lines	1222
Column in reverse position, volume between pre-column sensor and column inlet	79.4
Column in forward position, volume between pre-column sensor and column inlet	75.5
Hold up for conductivity dilutions without BBT nor filter (Volume between both pumps lines gathering and pre-column sensor)	34.9
Hold up for conductivity dilutions with BBT and filter inline (Volume between both pumps lines gathering and pre-column sensor)	99.2 + BBT volume filling ratio* (1222) + Filter Hold-up volume
Volume between pump 1 outlet and column inlet (if column upflow, no BBT, no filter)	129.5
Volume between pump 2 outlet and column inlet (if column upflow, no BBT, no filter)	130.4

*H corresponds to the volume contain between XV012/XV013 and XV018 when the Bubble trap and the filter are bypassed.

Table 2.
Undrainable volume measured.

Undrainable volume	124 mL

2. Pressure drop against flow and flowpath configuration

Background and Objectives

Selecting an appropriate flow path line diameter for a chrom system involves ensuring that it is not so large as to result in excessive holdup volume, restricting in terms of HETP/As measurements and overall buffer consumption, while also making sure that it is not so small that it results in excessive pressure drop.

The measured system pressure drop is the line drop from the feed pump discharge through the feed lines, Bubble trap, filter line and column connection lines till the waste outlet. This pressure drop can be measured using the pumps pressure sensors (PIT001 and PIT002) and an additional external pressure sensor placed right after the system outlet. Pressure drop is calculated by subtracting outlet pressure to the pump pressure.

Since the Mobius® Chrom 2 system has a maximum pressure rating of 4 barg (58 psig), a high total system pressure drop could limit the flow rate that can be driven through the column to increase mass transfer and drive high flux. This is especially true at higher viscosities and for densely packed columns. In a worst-case, this could cause the flowrate setpoint for a particular column to be unachievable.

The objective of this test was to determine the pressure drop in the flow path as a function of flow rate and selected flow path configuration (single pump/dual pump, BBT, filter, column). Conditions from 0.1 to 2.2 L/min at a viscosity of 1 cP were evaluated.

Materials and Methods

All Flexware® assemblies were installed on the system as per the User Guide. Upper column connection line was connected to the column bottom connection line (no column in-between). A ¼ inch tubing was connected between the clamshell filter connections (thus, generated backpressure from a filter is not considered here as filter type/size will vary according to the process).

A calibrated manometer was placed at the waste outlet, directly after the clamshell outlet. A calibrated flowmeter was connected to the drain line after the manometer. A water tank was linked to inlets 1E and 2E, the drain outlet was redirected to the tank. The pumps and all lines were primed following the priming recommendations.

A selected flowpath and a selected flowrate were applied, pumps were started, once stability was reached (stable flowrate according to reference flowmeter and stable BBT volume), PIT001 and PIT002 readings were recorded from the HMI display as well as the pressure from the calibrated manometer at the waste outlet. Delta P between the pump exit pressure and the system outlet pressure was calculated and defined as the pressure drop for the selected flowpath and flowrate.

Measurements were done for 3 different flowpaths configurations described in **Figure 1**, **2** and **3**. Two pumps configuration were then tested for each flowpath; first with a single pump (P001) then with dual pump (each at 50% of the targeted flowrate), the highest resulting pressure was then selected to calculate the pressure drop. Flowrates were tested from 0.1 L/min to 2.2 L/min with 0.2 to 0.3 L/min increments. All tests were conducted at ambient temperature (19–24 °C).

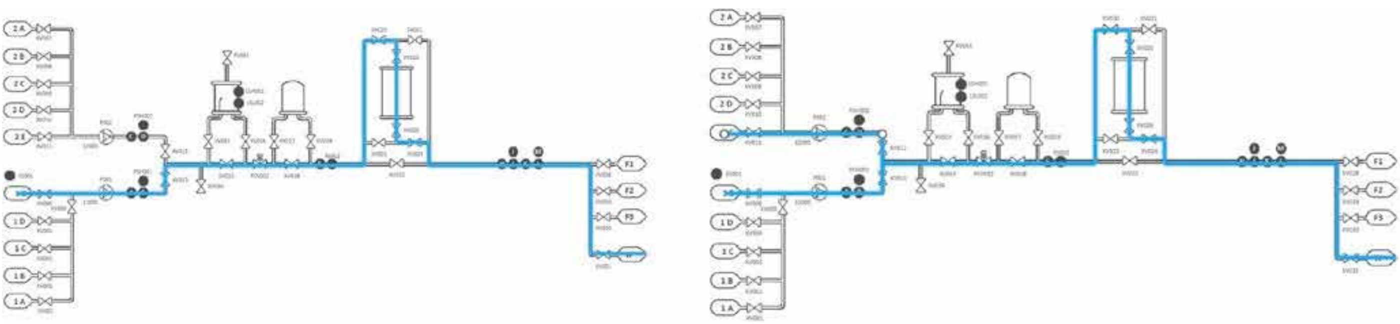


Figure 1.

Flowpath 1 – BBT bypassed, filter line bypassed, column forward.

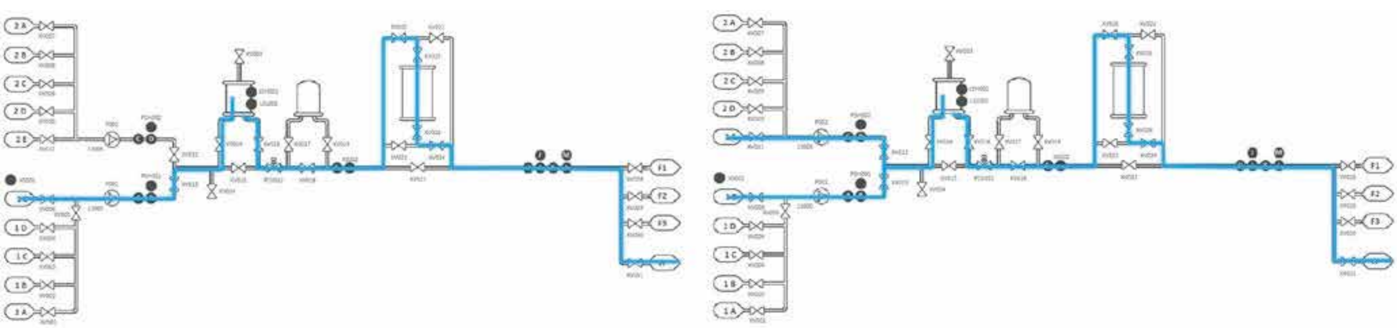


Figure 2.

Flowpath 2 – BBT inline, filter line bypassed, column forward.

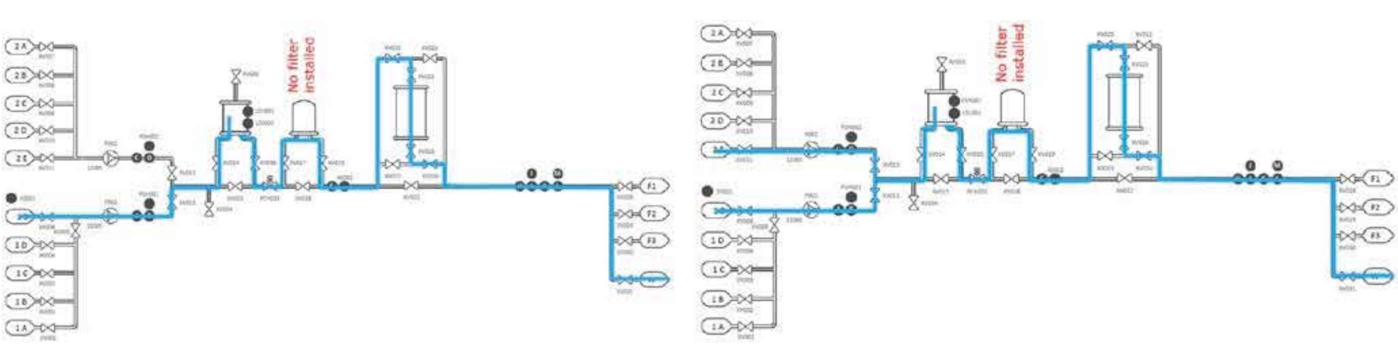


Figure 3.

Flowpath 3 – BBT inline, filter inline (no filter installed), column forward.

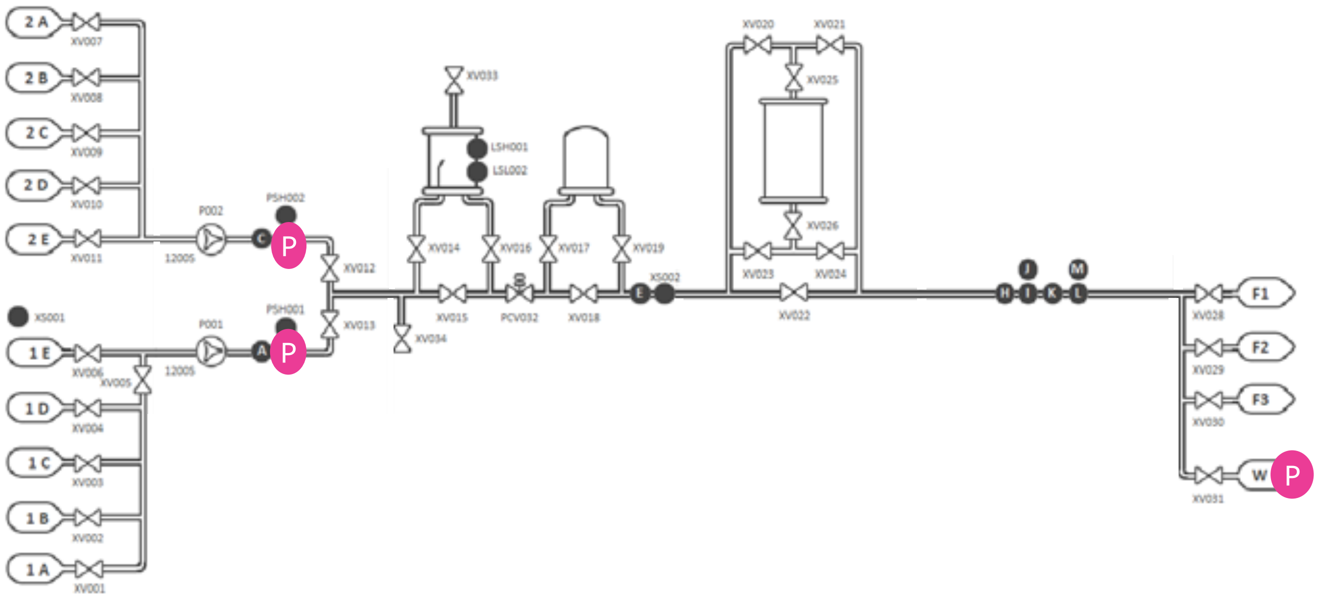


Figure 4.

Pressure sensor placement for the pressure drop assessment.

Results

The below Figure shows the pressure drop through the Mobius® Chrom 2 system across the full range of achievable flow rates and flow paths. Additional pressure drop created by the column, the filter or by additional tubing (connected to the outlet for example) is not considered and should be added to the below values to estimate processing capabilities under such conditions.

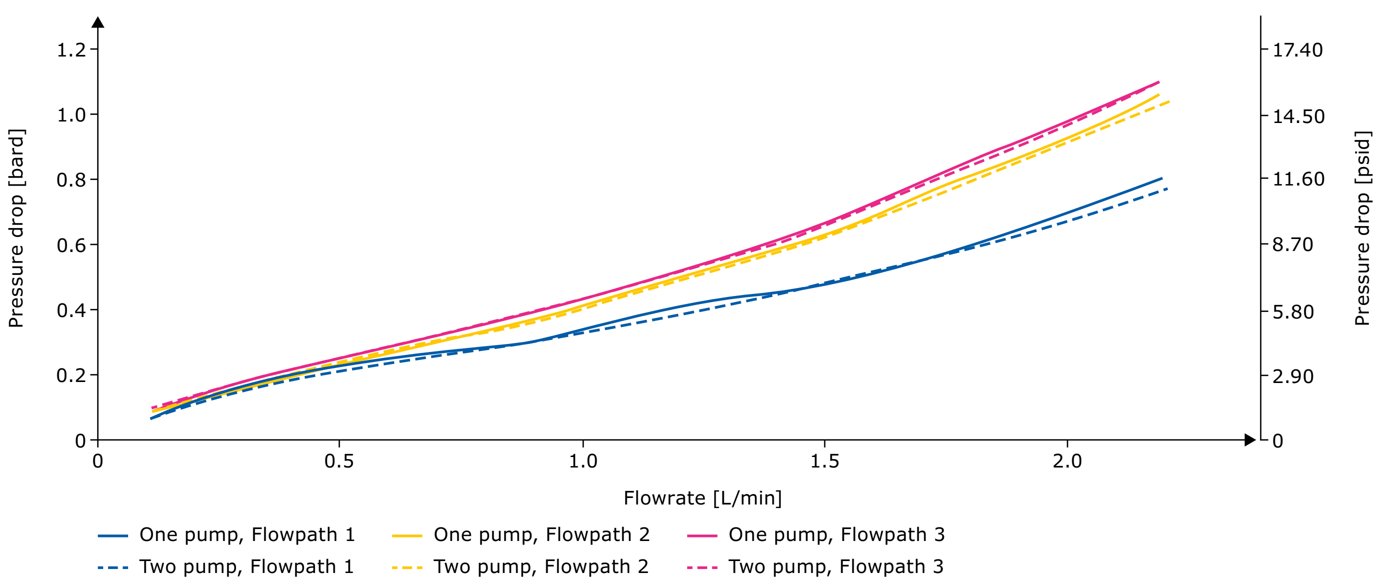


Figure 5.

Pressure drop against total flow according to the selected flowpath (at 1 cP).

3. Gradients linear, step, percentage and conductivity based

Background and Objectives

For some chromatography types, elution step may require performing gradients to enable better fractionation of specific components. The Chrom 2 system is equipped with two independent pumps that enable to perform linear or step gradients. These can be percentage-based gradients or, thanks to the pre-column conductivity sensor, conductivity-based gradients.

The linear gradient is a mixing where the proportion of each solution is constantly evolving to progressively increase the strength of the eluting solution and progressively elute components. Therefore, pumps must constantly adjust their speeds to follow the ramp of mixing ratio or conductivity over a certain time/volume, while maintaining the resulting total flowrate stable.

The step gradient is a series of isocratic steps, as stairs, where the proportion of both buffers to produce the eluting solution is maintained for a given component to elute before increasing to the next step eluting another component. To do so, pumps need to maintain a given mixing ratio or conductivity for a given amount of time/volume.

To demonstrate the system efficiency and precision on performed gradients, multiple conditions have been tested on the whole process flow range represented by three selected flowrates: 0.5, 1 & 2.2 L/min. Linear gradients percentage based, linear gradients conductivity based, and step gradients percentage based where performed.

Materials and Methods

All Flexware® assemblies were installed on the system as per the User Guide. Upper column connection line was connected to the column bottom connection line (no column in-between) with an additional hand valve for backpressure generation (to simulate a column). A reference flowmeter was connected to the waste outlet of the system. A concentrate solution of either known conductivity (NaCl solution) or known absorbance (Acetone solution) was connected to inlet 1E. A water tank was connected to the inlet 2E. Pumps were primed with their respective solutions. Lines were primed in water, BBT inline, filter bypassed column online and waste outlet. The water pump was started at the flowrate to be tested and a backpressure of ~2 bar was set by closing the installed manual hand valve, thus simulating a column backpressure. Pump was stopped and system set in a default flow path. Gradient to be tested was then started using an appropriate CCP® recipe. Conductivity based gradients were started with a pre-determined look up table (conductivity reference curve for NaCl). Step gradients were done percentage based with increments of 10% of primary pump.

The resulting reports were analyzed to evaluate total flow stability (based on the external flowmeter reading) and resulting errors on mixing (evaluated based on either the pre-column conductivity or absorbance readings).

Results

Below figures show an example of the resulting curves obtained from the different gradients performed and detail the resulting errors on total flowrate and errors on mixing for all tested gradient types and conditions. All gradients performed showed an accuracy within the validated range of +/-5% on total flowrate and +/-5% on mixing. Nonetheless most of the conducted gradients resulted in accuracies closer to +/-3% for total flow and mixing.

Linear gradient percentage-based

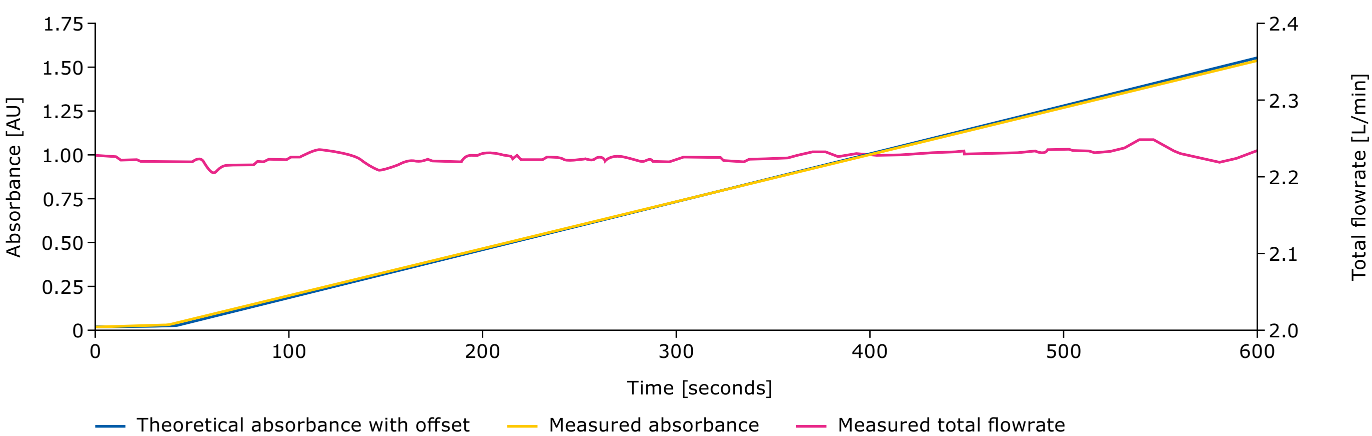


Figure 6.

Example of a percentage-based linear gradient resulting curve (gradient performed between an acetone solution at 1.650 AU and WFI, from 0% to 100% of primary pump over 10 min, 2.2 L/min total flowrate setpoint).

Linear gradient by percentage total flowrate error

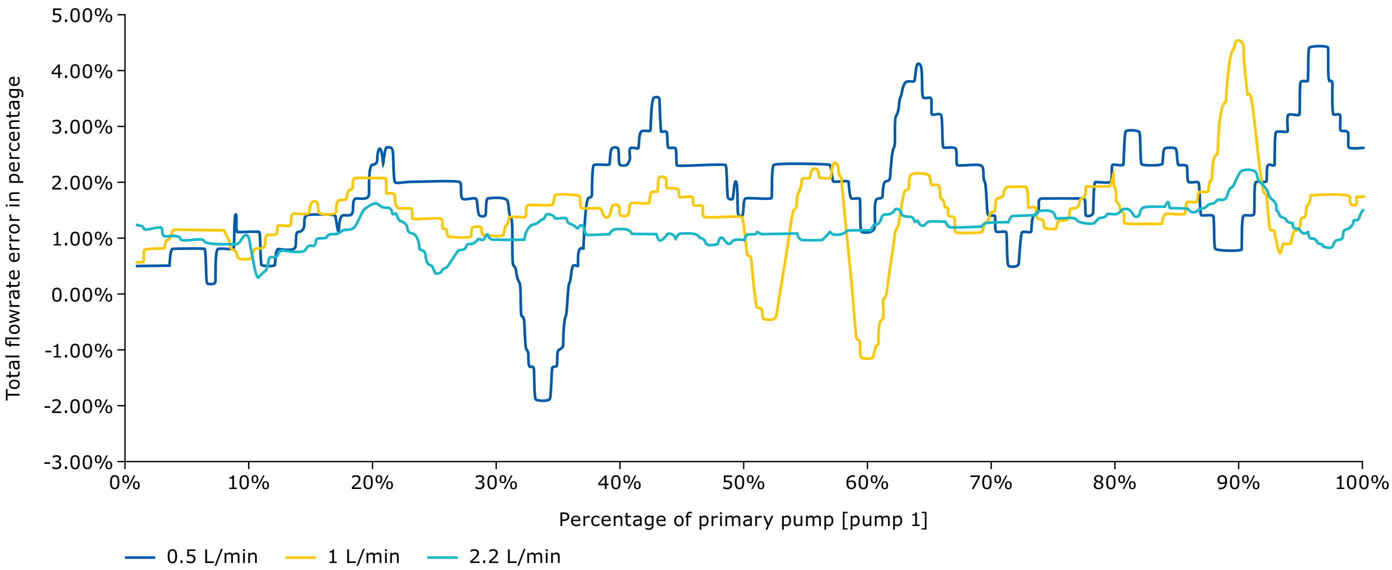


Figure 7.

Resulting total flowrate error for all linear gradients percentage based performed according to the percentage of primary pump.

Linear gradient by percentage error on mixing

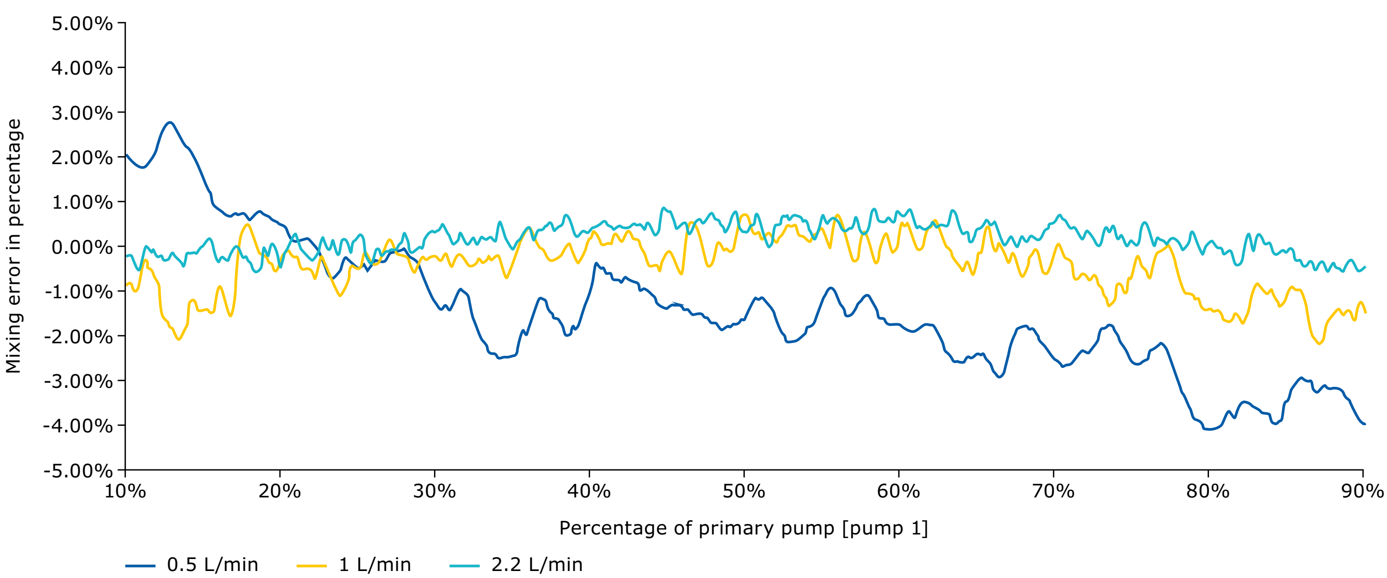


Figure 8.

Resulting mixing error for all linear gradients percentage based performed according to the percentage of primary pump.

Linear gradient conductivity-based

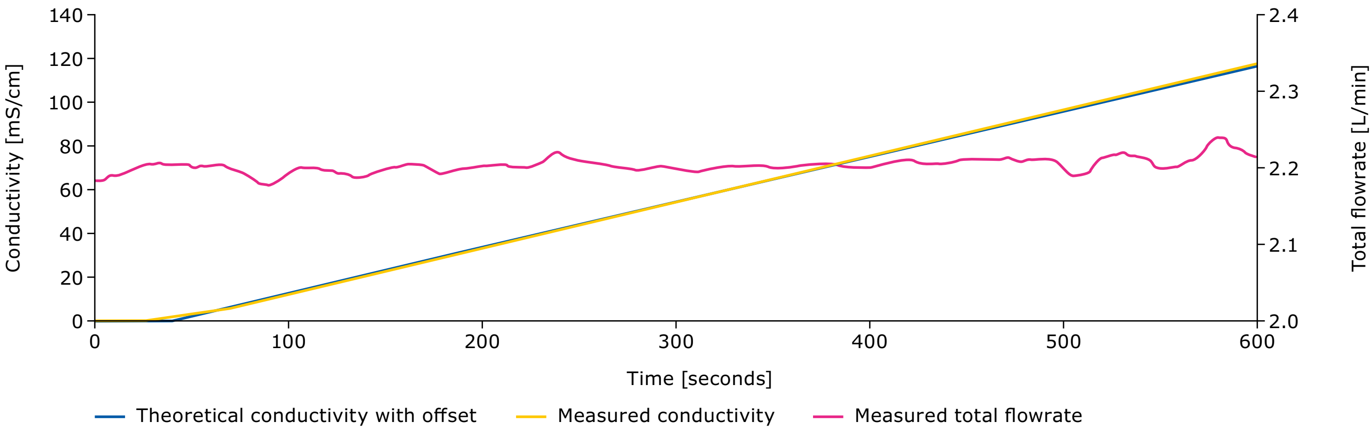


Figure 9.

Example of a conductivity-based linear gradient resulting curve (gradient performed between an NaCl solution at 125.7 mS/cm and WFI, from 0 mS/cm to 125.7 mS/cm over 10 min, 2.2 L/min total flowrate setpoint).

Linear gradient by conductivity error on total flowrate

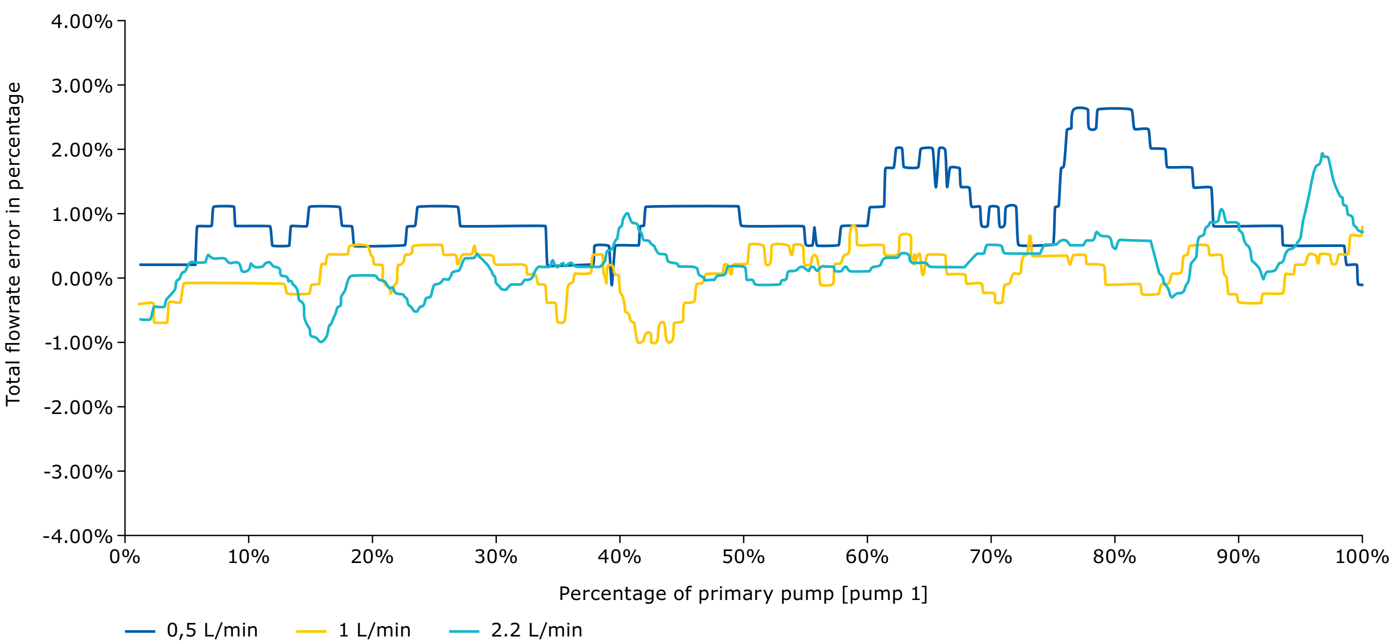


Figure 10.

Resulting total flowrate error for all linear gradients conductivity based performed according to the percentage of primary pump.

Linear gradient by conductivity error on mixing

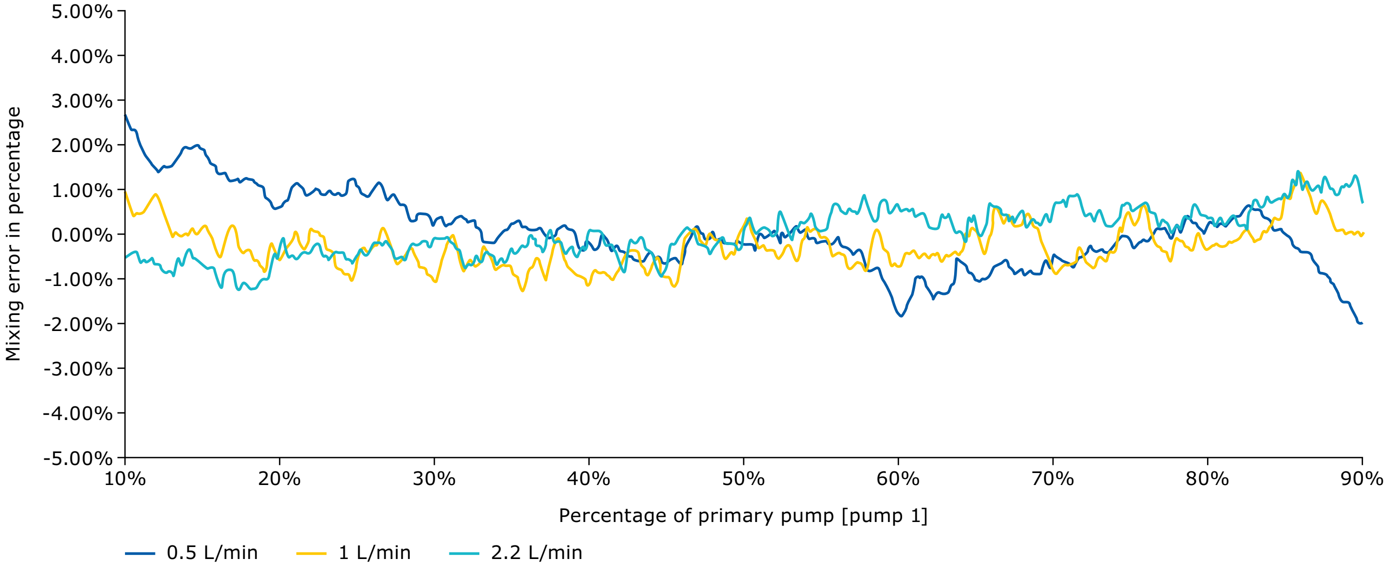


Figure 11.

Resulting mixing error for all linear gradients conductivity based performed according to the percentage of primary pump.

Step gradient percentage-based

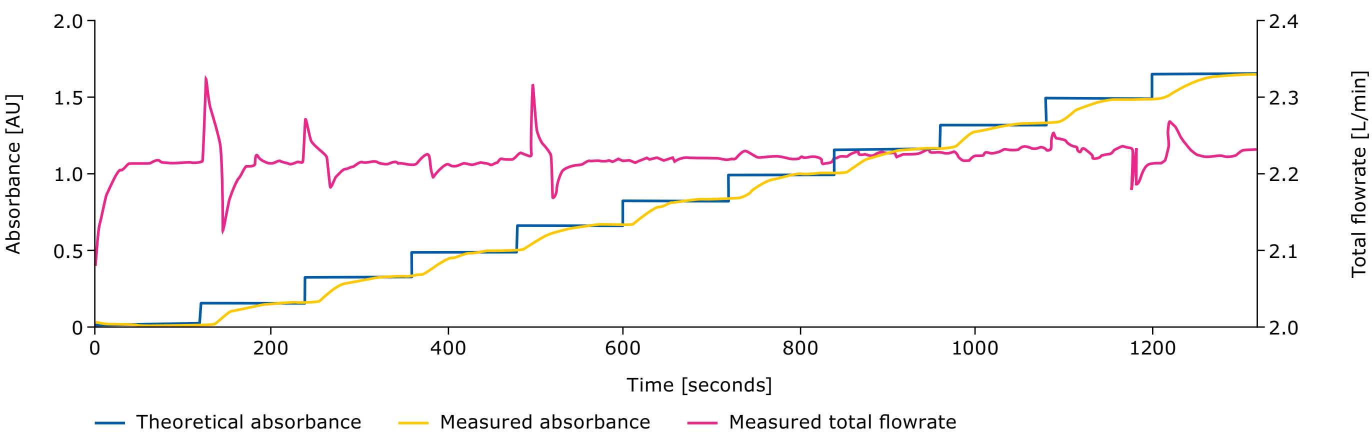


Figure 12.

Example of a percentage-based step gradient resulting curve (gradient performed between an acetone solution at 1.650 AU and WFI, from 0% to 100% over 11 increments of 120 seconds, 2.2 L/min total flowrate setpoint).

Step gradient by percentage error on total flowrate

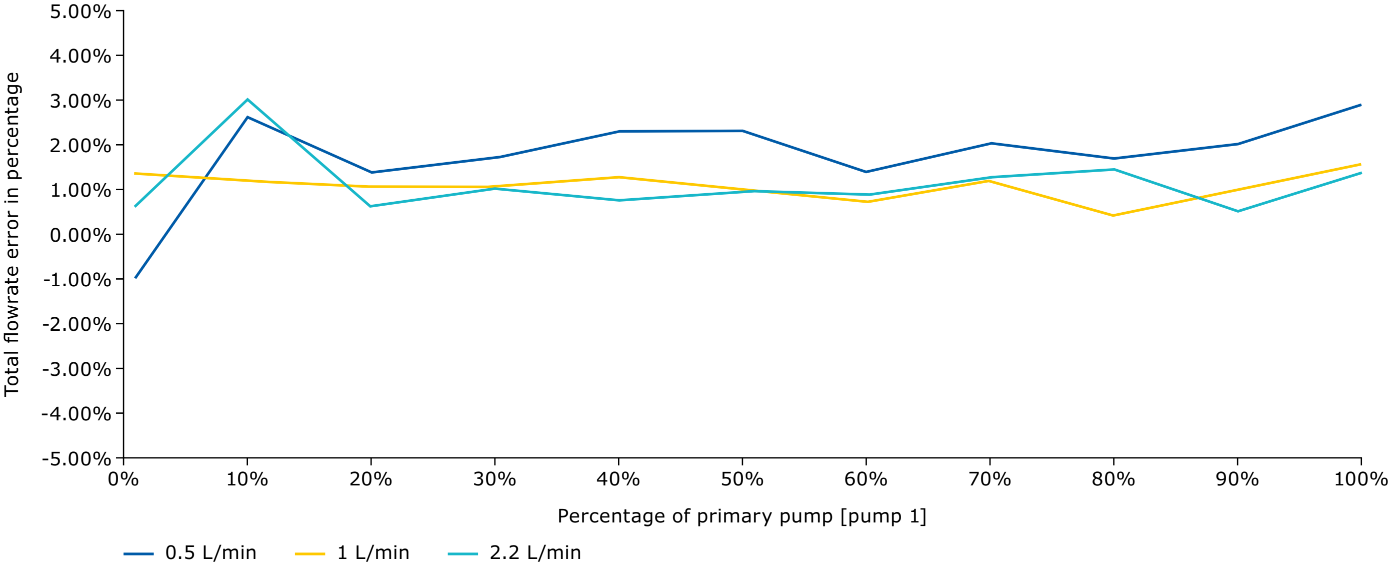


Figure 13.

Resulting total flowrate error for all step gradients percentage based performed according to the percentage of primary pump.

Step gradient by percentage error on mixing

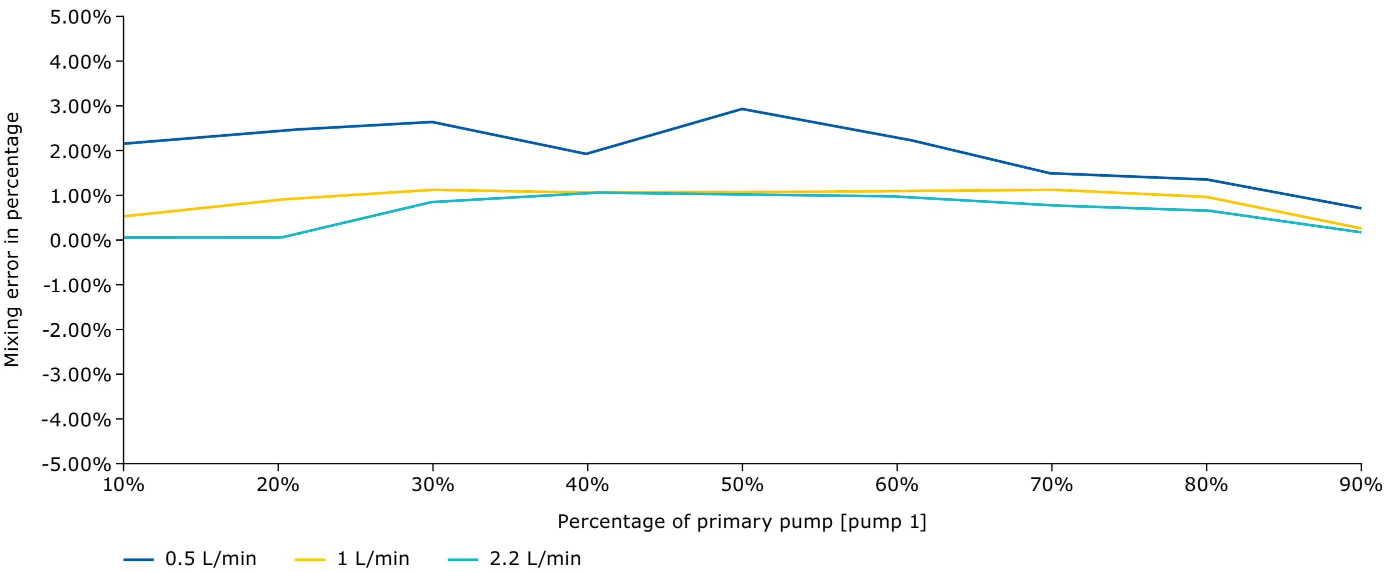


Figure 14.

Resulting mixing error for all step gradients percentage based performed according to the percentage of primary pump.

4. Column qualification

Background and Objectives

To validate the packed bed quality of a column and ensure its efficiency to perform the awaited process step, the HETP (Height of an Equivalent Theoretical Plate) and Asymmetry are usually assessed and compared to tolerances. To ease this assessment, the Mobius® Chrom 2 system can automatically measure these values and give a detailed summary of the measurement.

A standard recipe aiming at assessing these values is available as an example within the CCP® software.

Materials and Methods

All Flexware® assemblies were installed into the system as per the userguide. A 10 cm diameter QuikScale® column unproperly packed at 10 cm bed height (L) containing Eshmuno® A resin was connected to the system upper and lower column connections. An equilibration solution (water) was connected to inlet 1D and a pulse solution of 1% V/V acetone in water was connected to inlet 1E. Inlets were primed, and the column was equilibrated for 5 CVs using the equilibration solution from inlet 1D at 150 cm/h. A recipe was then used to perform the pulse, bypassing the Bubble trap, and switching for inlet 1E for a volume of 15 mL. Resulting absorbance curve was retrieved and automatic calculation performed by the system were compared to a manual calculation.

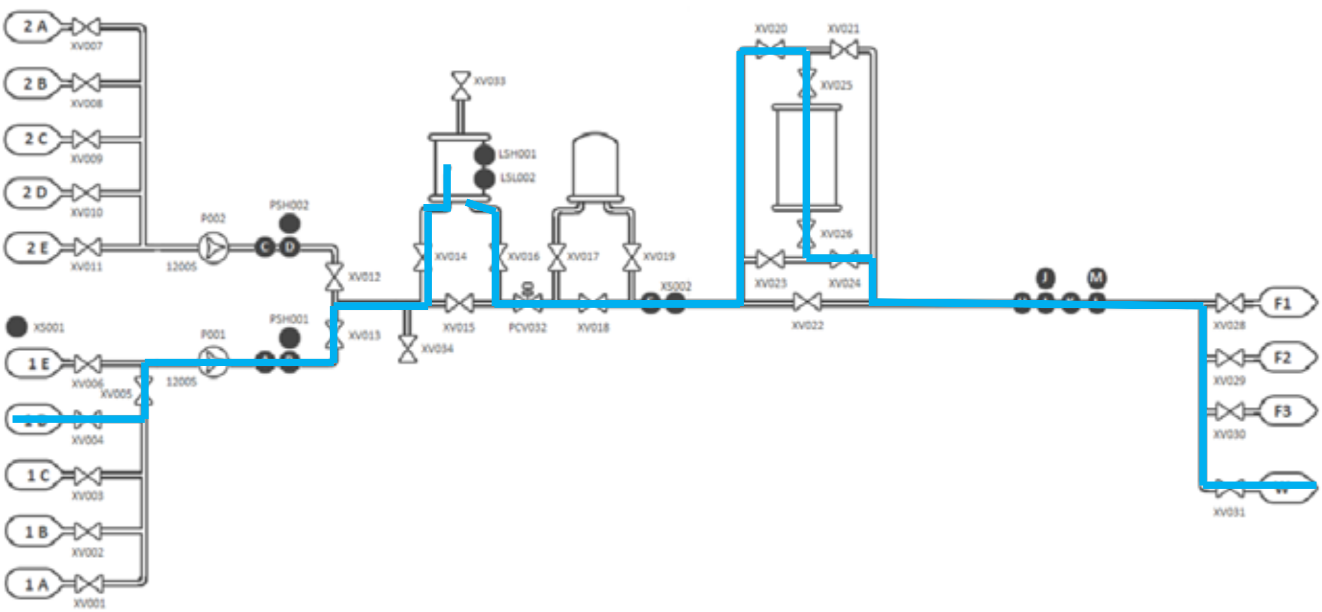


Figure 15.

Flowpath applied during equilibration and post pulse (BBT inline and inlet 1D).

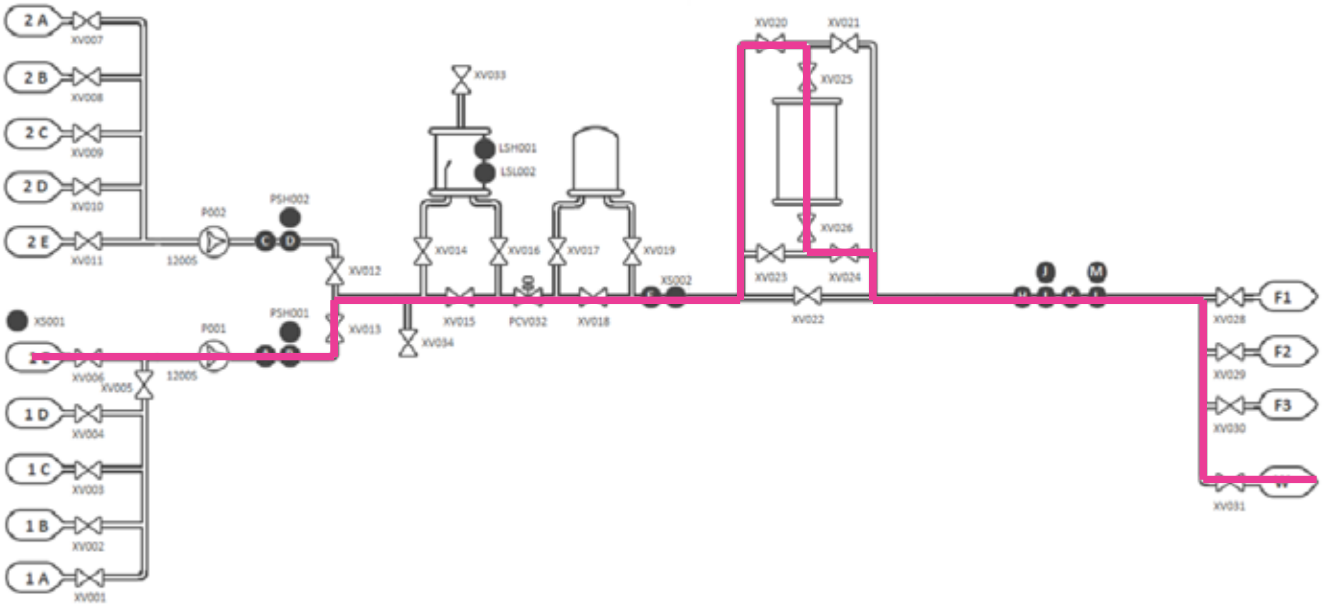
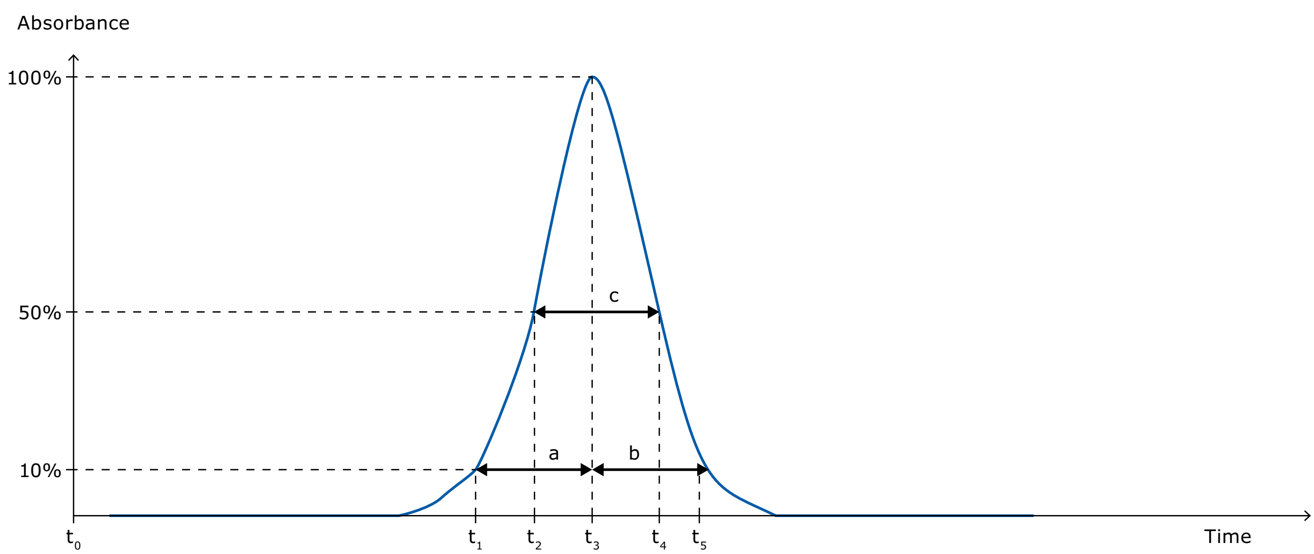


Figure 16.

Flowpath applied during pulse (BBT bypassed & inlet 1E).

Applied calculations for HETP and Asymmetry assessment:



Number of plates (N) = $5.54 * \left(\frac{c}{t_3}\right) = 5.54 * \left(\frac{t_2-t_4}{t_3}\right)$

HETP = $\left(\frac{L}{N}\right)$ with L the Columne bed height (cm)

Asymmetry = $\left(\frac{b}{a}\right) = \left(\frac{t_5-t_3}{t_1-t_3}\right)$

Results and conclusion

Results are detailed in the below table and graph, they demonstrate the capability of the system to detect the unproper packing of a column (As = 2.061 versus an expectancy of 0.8 < As < 1.8). Moreover, automatically calculated results compared to the manual calculation showed almost no deviation. A -3.17% error was calculated for the HETP and a 0.72% one for the Asymmetry value which represent unsignificant variations mainly linked to the frequency of historization of data points.

Table 3.

System results and manual calculations comparison.

Value measured	System calculation	Manual calculation	Resulting error on calculation
Number of plates (N)	160.77	166.03	-3.17%
HETP	0.0622	0.0602	-3.27%
Asymmetry	2.061	2.046	0.72%

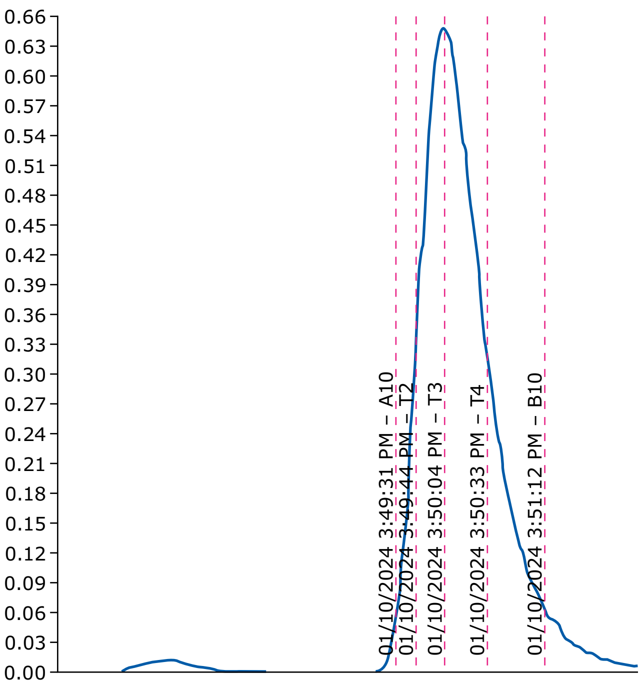


Figure 17.

HETP/AS test report generated in the CCP® report.

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