Monitoring of Buffer Exchange and Protein Concentration during UF/DF Using the Multi Attribute Method CLADE™ QA Scan

Introduction

In monoclonal antibody (mAb) manufacturing, ultrafiltration/diafiltration (UF/DF) is a vital step to both concentrate the product and exchange into the desired formulation buffer. Accurate monitoring of this step is critical for ensuring that final drug substance meets its quality attributes - especially protein concentration and excipient composition.

Traditional approaches rely on offline assays, estimations based on diavolumes, and assumptions about solute behaviour. However, these fail to account for non-idealities such as Donnan effect or volume exclusion effects, which are particularly pronounced at high mAb concentrations. This report presents how CLADE™ QA Scan, a mid-IR spectroscopic based analytics platform, enables near real-time, multi-attribute monitoring of protein concentration and formulation excipients throughout a UF/DF process, offering a new standard for in-process control and formulation precision.

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Methods

Experimental Setup

A Miniaturized tangential flow filtration (TFF) system (Formulatrix µPulse®) was used for UF/DF. Bovine Serum Albumin (BSA) at 10 g/L (Run A) and 20 g/L (Run B) in TRIS buffer (2.42 g/L) was used as starting Formulations. Buffer exchange (diafiltration) was performed prior to up-concentration, allowing 110 µL sample withdrawal from the retentate after each diavolume, to minimize BSA loss.

Run Parameters of Experiments:

Parameter	**Run A**	**Run B**
Membrane	RC 10 kDa	PES 10 kDa
Starting Volume	10 mL (Start A)	10 mL (Start B)
Step Size	2 mL	2 mL
Exchange Volume	10 mL (Formulation Buffer A)	10 mL (Formulation Buffer B)
Retentate Sampling	110 µL after each diavolume (8x)	110 µL after each diavolume (7x)
Permeate Sampling	110 µL after each diavolume	110 µL after each diavolume
Diavolumes	8	7
Up-Concentration	Sampling at 10, 8, 6, 4, 2 mL	Sampling at 10, 8, 6, 4, 2 mL

Buffer Compositions:

Component	**Buffer A [g/L]**	**Buffer B [g/L]**
Histidine	3.1	3.1
Methionine	1.5	1.5
Trehalose	65.0	61.7
Polysorbate 80	0.30	0.0

Run A uses Formulation Buffer A (contains Polysorbate 80). Whereas Run B uses Buffer B (no Polysorbate 80). For Run B PS80 was added post-UF/DF.

All concentrations were confirmed gravimetrically.

Analytical Methods

Spectral acquisition was performed using the CLADE™ MIRA Analyzer within 5 minutes and no sample preparation. Quadruplicate spectra were recorded for each calibration point to ensure data robustness. Due to sample volume restrictions, only a single measurement was conducted for other samples, utilizing the minimal required volume of 98 µL per measurement. For each run, a dedicated calibration model was created on the CLADE™ Sphere platform. For Run A QA Scan Model A. For Run B QA Scan Model B. QA Scan Models are generated using both the buffer and formulation buffer as calibration standards. For Run B, Buffer B was supplemented with 0.3 g/L PS80 to represent the finale formulated DP. Detailed QA Scan method calibration settings are provided in Annex Figures 7 and 8. In Run B, following the completion of UF/DF, Polysorbate 80 was titrated into the

concentrated product to achieve the target concentration of 0.3 g/L, with near real-time monitoring ensuring precise adjustment. For permeate samples, the CLADE™ Sphere data-driven method with zero-calibration was applied. The specific method parameters detailed in the Annex Figure 9.

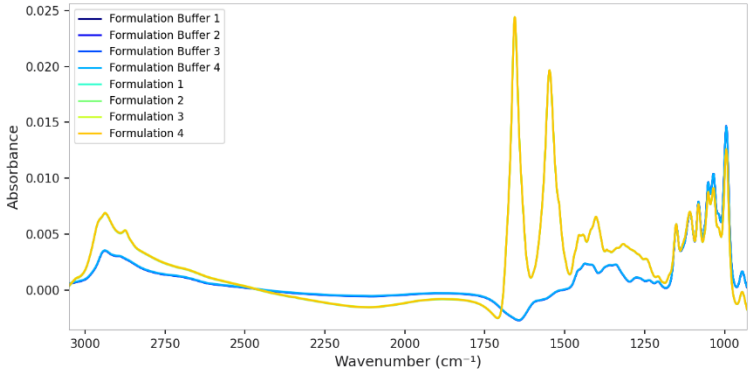

Figure 1 Calibration samples for QA Scan B. For both calibration samples (Formulation and Formulation Buffer) quadruplicates were generate. Here each spectrum is overlapped using the CALDE™ Spectral Workbench.

Results & Discussion

Run A: UF/DF with PS80 in Buffer

In Run A, the ultrafiltration/diafiltration process was performed using Formulation Buffer A, which contains Polysorbate 80 (PS80) as an excipient. Monitoring the buffer exchange and protein concentration in near real-time with the CLADE™ QA Scan revealed several important process characteristics.

During diafiltration (Figure 2), the TRIS buffer in the starting formulation was rapidly and efficiently exchanged. This was evident from the near-immediate decrease in TRIS concentration after just one diavolume, as measured by the QA Scan. The replacement by Buffer A proceeded as expected, demonstrating effective buffer exchange and supporting process robustness.

A key observation in Run A was the behaviour of Polysorbate 80. Unlike the other excipients, PS80 did not permeate the 10 kDa membrane during the diafiltration. Instead, it accumulated in the retentate throughout the process. The concentration of PS80 in the retentate increased steadily during diafiltration, reaching up to 2 g/L by the end of the DF. After UF, the concentration of PS80 further increased to approximately 8 g/L (Figure 2 magenta line). This accumulation was detected in near real-time and highlights the importance of monitoring excipient retention, as PS80 could easily reach levels above the target specification if not properly managed.

To assess whether any PS80 leakage occurred, permeate samples were analysed using the CLADE™ Sphere Data Driven method (Figure 3). No significant PS80 was detected in the permeate across all diavolumes and UF steps, confirming that the excipient was entirely retained by the membrane. All other excipients exhibited predictable behaviours. Excipient concentrations in the retentate and permeate were

mirroring each other, confirming both system performance and the analytical method's reliability.

Protein recovery and concentration were also closely tracked. BSA concentrations remained stable during the DF phase, with only minor decreases attributable to the required sample withdrawals. Upon entering the ultrafiltration step, the protein concentration increased sharply, ultimately achieving a ~4.6-fold up-concentration (from 9.25 g/L at the end of diafiltration to 43.18 g/L at the end of the process). The observed losses were minimal and can be explained by the cumulative sampling rather than process inefficiency.

These results confirm that the CLADE™ QA Scan offers a comprehensive, near real-time view of both protein and excipient concentrations simultaneously, allowing detection of potential process deviations, such as excipient accumulation, that could impact final drug product quality.

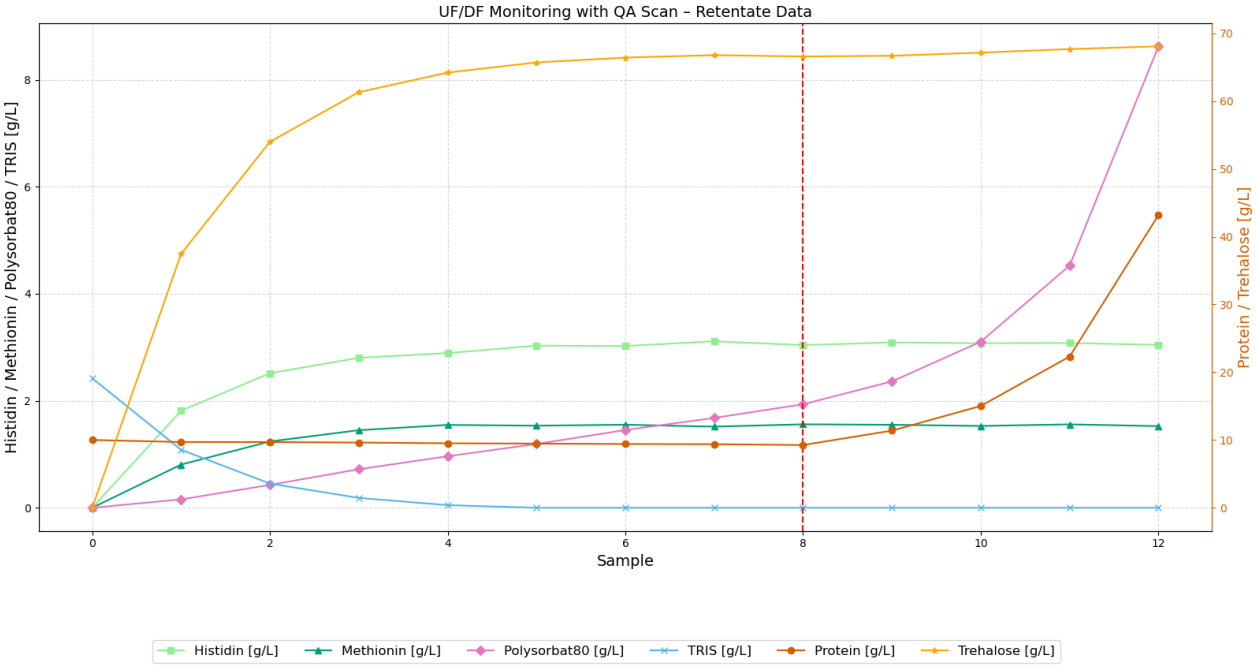

Figure 2 UF/DF monitoring of Run A. Red vertical line highlights the end of DF.

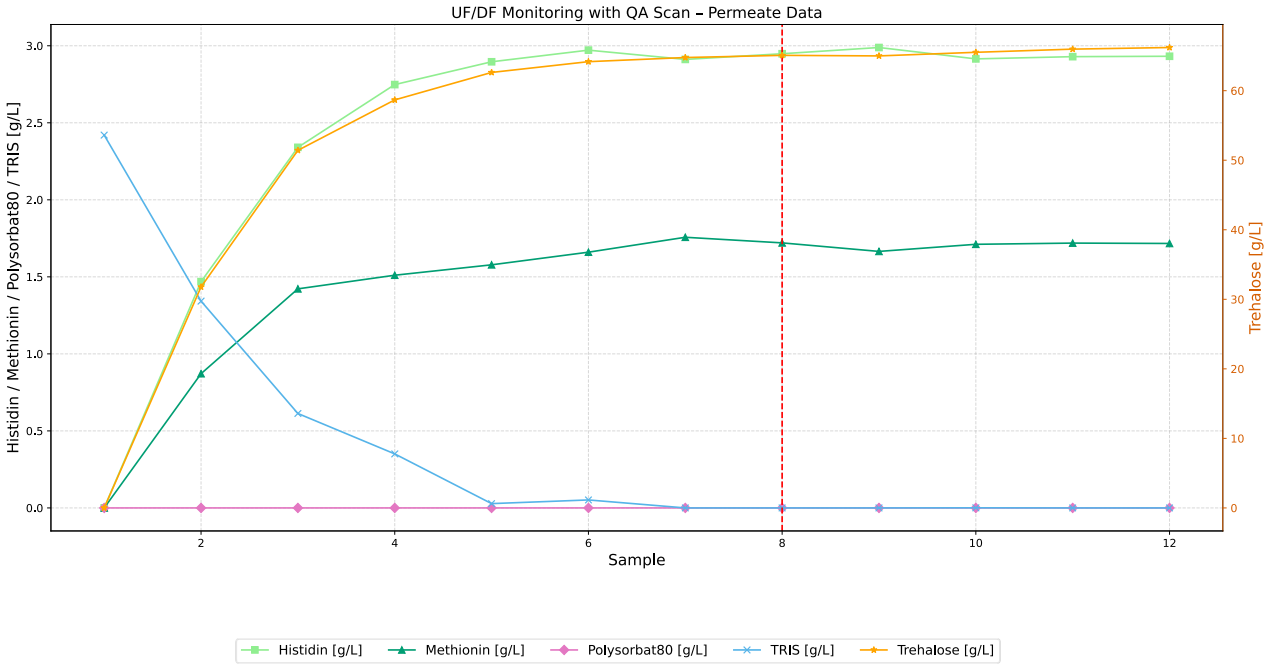

Figure 3 Permeate Data collected during the UF/DF process of Run A. Red vertical line highlights the end of DF.

Run B: UF/DF without PS80, with post-UF/DF PS80 addition

For Run B, the process was modified to use Buffer B, which does not contain Polysorbate 80 during the UF/DF steps. Instead, PS80 was only added after up-concentration, allowing a more conventional workflow and providing a direct comparison to the behaviour observed in Run A.

The near real-time monitoring of Run B showed similarly efficient buffer exchange. The TRIS buffer and other excipients were successfully replaced by Buffer B during DF, as indicated by the clear and immediate shift in their concentrations (Figure 4). Since no PS80 was present in the buffer during UF/DF, its measured concentration in both retentate and permeate remained at zero throughout the process. This finding was consistent with the model predictions and confirmed that the method could accurately detect the absence of this excipient, even when the calibration included a PS80 target concentration (see calibration details in Annex Figure 9).

Protein concentration behaved as expected: BSA was steadily up-concentrated, with the measured concentration increasing from 15.58 g/L before ultrafiltration to 76.31 g/L after the final concentration step, corresponding to a ~4.9-fold increase. The excipient concentrations in the permeate mirrored those observed in the retentate (Figure 5), reinforcing the reliability and reproducibility of the system.

After the UF/DF process was complete, Polysorbate 80 was added to the up-concentrated product by titrating a concentrated PS80 solution (15 g/L PS80 in Buffer B) into the sample. Each aliquot was again measured using the CLADE™ MIRA Analyzer and evaluated with QA Scan Method B, confirming that the target PS80 concentration (0.3 g/L) was achieved with precision. This stepwise approach provided tight control over the final excipient level, while direct at-line monitoring ensured that the formulation reached the required specifications.

These results demonstrate that excluding PS80 during UF/DF and adding it post-process can simplify process control and prevent unintended excipient accumulation. The CLADE™ QA Scan provided immediate feedback on both protein and excipient levels, reducing the risk of off-specification batches and supporting a robust manufacturing workflow.

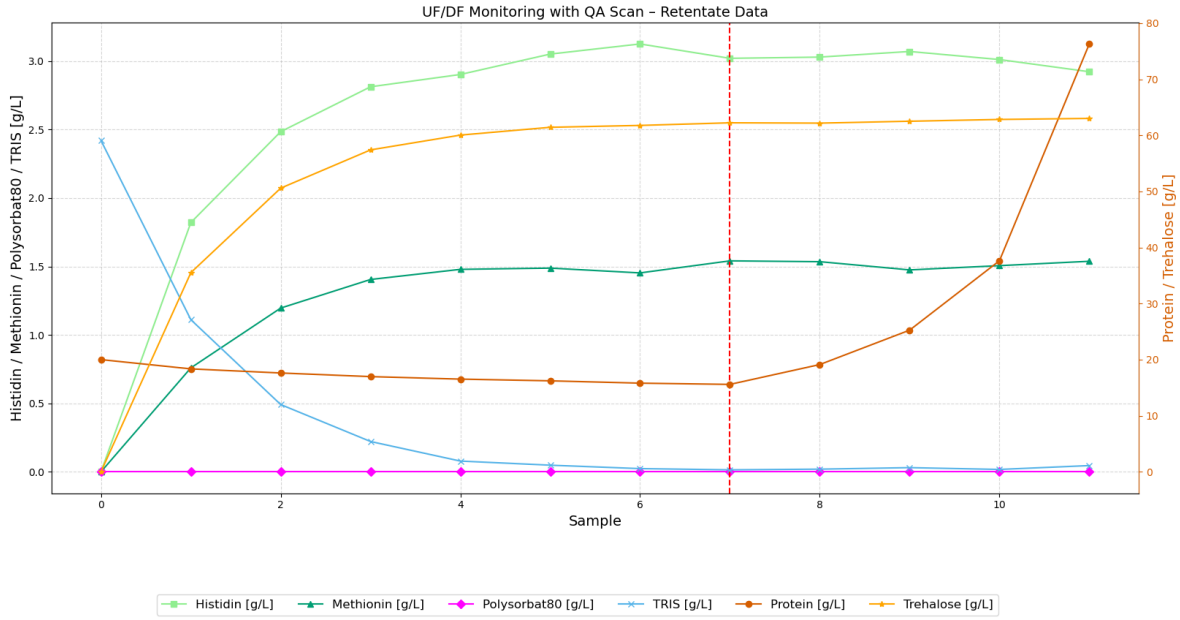

Figure 4 UF/DF monitoring of Run B. Red vertical line highlights the end of DF.

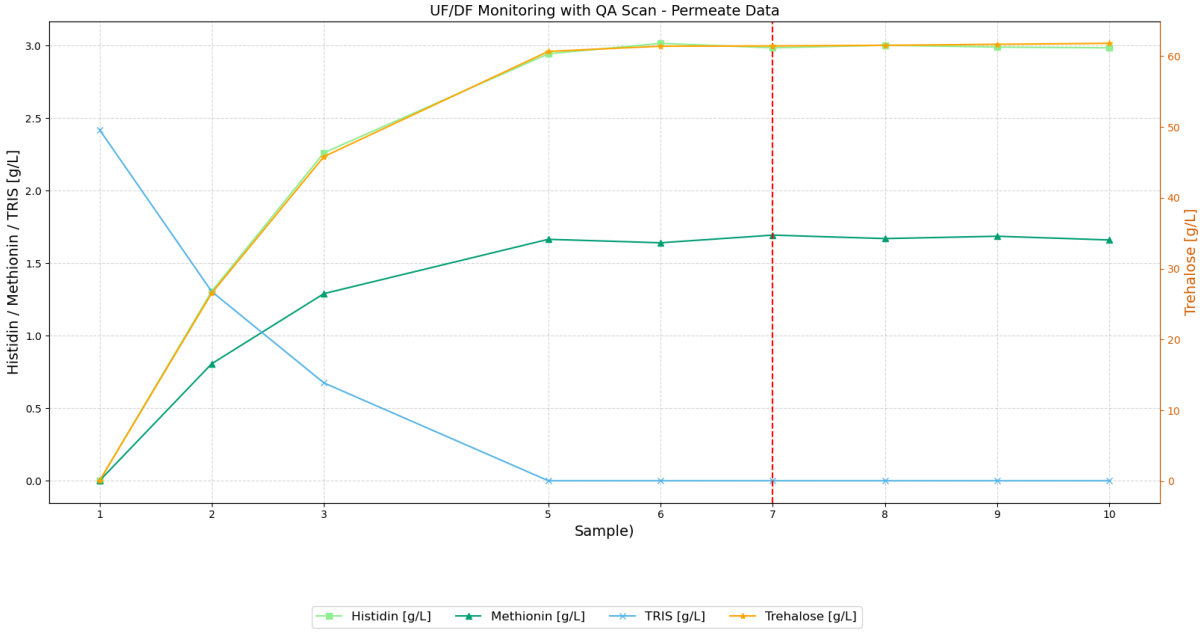

Figure 5 Permeate Data of Run B, quantified using the Data Driven method.

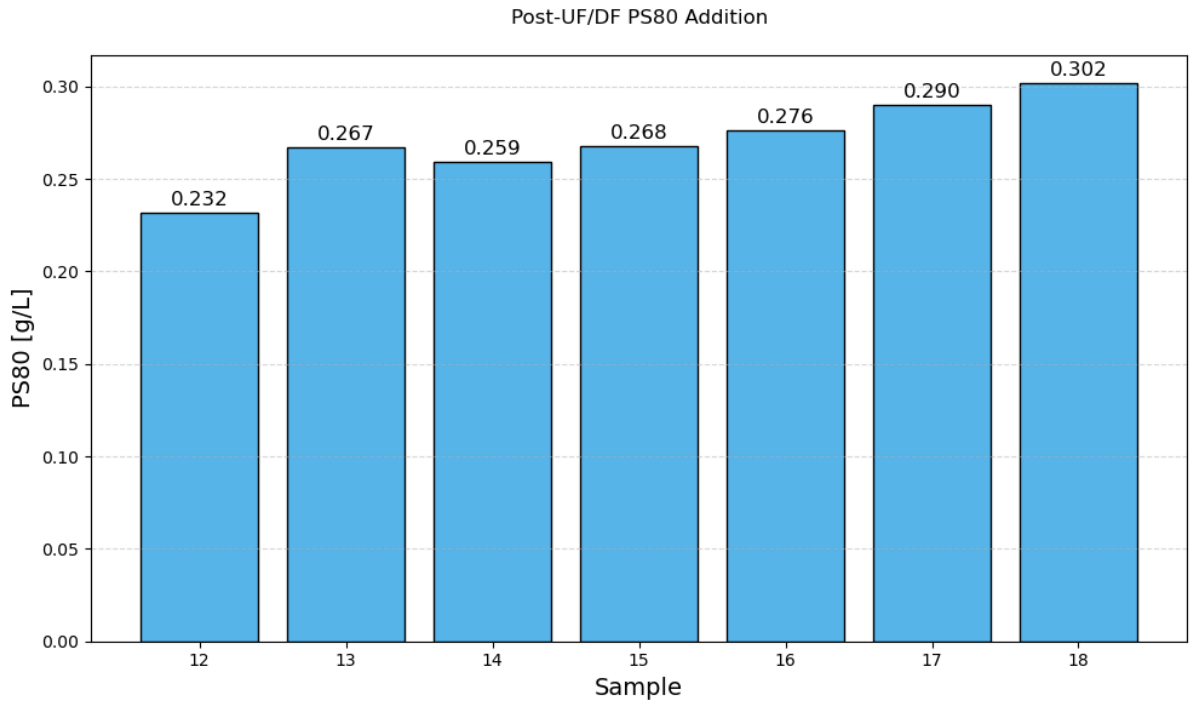

Figure 6 PS80 titration into the UF/DF product.

Conclusion

The data presented in this study clearly demonstrate that the CLADE™ QA Scan is a powerful and versatile tool for at-line in-process monitoring during ultrafiltration and diafiltration steps in protein formulation workflows. By enabling near real-time quantification of protein and excipient concentrations simultaneously, all with only one QA Scan method. The method offers significant advantages over traditional offline assays and theoretical calculations. In particular, the ability to detect excipient accumulation-such as Polysorbate 80 retention-and to confirm efficient buffer exchange supports a more controlled and predictable manufacturing process. The comparative results from Runs A and B further highlight the importance of monitoring excipient behaviour and provide actionable insight into process design choices, such as whether to include certain excipients during UF/DF or to add them post-process. Overall, the integration of CLADE™ QA Scan into UF/DF workflows enables a more robust, data-driven approach to bioprocessing, ultimately safeguarding drug product quality and process consistency.

Appendix

Table 1 Raw Data. Run A QA Scan evaluation results of retentate.

Sample	Alpha-helix [%]	Beta-sheet [%]	Histidine [g/L]	Methionine [g/L]	PS80 [g/L]	Protein [g/L]	TRIS [g/L]	Trehalose [g/L]	pH
1	58.01	1.57	1.813	0.807	0.156	9.697	1.084	37.451	7
2	58.14	1.43	2.515	1.239	0.427	9.692	0.452	53.99	7
3	58.38	1.36	2.803	1.45	0.720	9.62	0.184	61.313	7.1
4	58.37	1.35	2.891	1.548	0.963	9.510	0.049	64.207	7
5	58.37	1.31	3.031	1.534	1.192	9.464	0	65.704	7
6	58.65	1.37	3.023	1.552	1.453	9.406	0	66.414	7.1
7	58.82	1.36	3.112	1.516	1.679	9.375	0	66.777	7.1
8	58.80	1.25	3.04	1.560	1.935	9.254	0	66.584	7.1
9	58.72	1.39	3.091	1.551	2.361	11.375	0	66.683	7.1
10	58.79	1.29	3.075	1.529	3.104	15.016	0	67.151	7
11	58.80	1.29	3.08	1.559	4.526	22.308	0	67.669	7
12	58.73	1.33	3.044	1.526	8.625	43.183	0	68.074	7

Table 2 Raw Data. Run A Data Driven Method evaluation results of permeate.

Sample	Histidine [g/L]	Methionine [g/L]	PS80 [g/L]	TRIS [g/L]	Trehalose [g/L]
1	1.469	0.871	0	1.342	31.777
2	2.340	1.422	0	0.612	51.420
3	2.748	1.510	0	0.350	58.673
4	2.896	1.578	0	0.027	62.623
5	2.971	1.660	0	0.051	64.172
6	2.911	1.756	0	0	64.767
7	2.948	1.720	0	0	65.080
8	2.988	1.665	0	0	65.000
9	2.914	1.711	0	0	65.519
10	2.929	1.719	0	0	65.973
11	2.931	1.716	0	0	66.208

Table 3 Raw Data. Run B QA Scan evaluation results of retentate.

Sample	Alpha-helix [%]	Beta-sheet [%]	Histidine [g/L]	Methionine [g/L]	PS80 [g/L]	Protein [g/L]	TRIS [g/L]	Trehalose [g/L]	pH
1	57.84	1.7	1.821	0.759	0	18.35	1.111	35.512	7
2	58.04	1.72	2.485	1.196	0	17.616	0.491	50.586	7.1
3	58.04	1.69	2.813	1.405	0	16.971	0.221	57.409	7
4	58.14	1.7	2.903	1.479	0	16.523	0.078	60.049	6.9
5	58.12	1.67	3.052	1.488	0	16.218	0.048	61.439	6.9
6	58.14	1.68	3.126	1.453	0	15.803	0.023	61.776	6.9
7	58.05	1.84	3.021	1.541	0	15.581	0.014	62.240	6.9
8	58.2	1.73	3.03	1.535	0	19.120	0.019	62.184	7
9	58.14	1.75	3.071	1.475	0	25.255	0.03	62.520	6.9
10	58.09	1.74	3.012	1.506	0	37.634	0.017	62.840	6.9
11	58	1.77	2.923	1.538	0	76.306	0.045	63.021	6.9

Table 4 Raw Data. Run B Data Driven Method evaluation results of permeate.

Sample	Histidine [g/L]	Methionine [g/L]	TRIS [g/L]	Trehalose [g/L]
1	0.000	0.000	2.420	0.053
2	1.306	0.806	1.303	26.547
3	2.260	1.290	0.674	45.819
5	2.640	1.530	0.219	54.649
6	2.943	1.664	0	60.664
7	3.015	1.640	0	61.399
8	2.984	1.693	0	61.441
9	3.002	1.669	0	61.521
10	2.989	1.685	0	61.669
11	2.984	1.659	0	61.801

Table 5 Raw Data. Post UF/DF PS80 addition of Run B.

Sample	Alpha-helix [%]	Beta-sheet [%]	Histidine [g/L]	Methionine [g/L]	PS80 [g/L]	Protein [g/L]	TRIS [g/L]	Trehalose [g/L]	pH
12	58.16	1.6	3.110	1.575	0.064	98.912	0	61.489	7
13	58.17	1.59	1.554	1.612	0.2315	97.5805	0	61.363	6.95
14	58.19	1.575	3.109	1.605	0.267	97.583	0	61.520	6.9
15	58.21	1.575	3.130	1.571	0.259	97.412	0	61.319	6.95
16	58.19	1.575	3.114	1.598	0.2675	97.448	0	61.404	6.95
17	58.19	1.565	3.092	1.581	0.276	97.2255	0.011	61.285	6.95
18	58.19	1.57	3.120	1.6185	0.290	97.3265	0.006	61.382	6.95

Figure 7 Data Driven Method parameters for permeate analysis of Run A.

Methods ▸ Data Driven Method

Back

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Method Name	Method Type	Selected pH Range (min – max)	Method Creation Date	Created by
Goliath WasteV2 Available	Data Driven	6.5 – 7.5	17.6.2025, 10:21:28	lisa.reinold

Selected Components

Component	Min concentration [g/L]	Max concentration [g/L]	Derivative Used	Ranges of Interest [cm⁻¹]
Histidin	2.0004	5.0000	2	[950, 2250], [2450, 3020]
Methionin	1.9911	3.0000	2	[950, 2250], [2450, 3020]
Trehalose	4.9980	65.0000	2	[950, 2250], [2450, 3020]
TRIS	0.0000	3.0000	2	[950, 2250], [2450, 3020]

Figure 8 Data Driven Method parameters for permeate analysis of Run B

Method Calibration Data Set

Attribute	Calibration Point 1 - Formulation	Calibration Point 2 - Formulation Buffer
Histidin *	3.1	3.1
Methionin	1.5	1.5
Polysorbat80	0.3	0.3
Trehalose	65	65
TRIS	0	0
Protein Concentration	100	0
ph	7.1	7.1

* Histidin is selected as the main buffer.

Figure 9 Screenshot from the CLADE™ Sphere platform of the QA Scan method B parameter for Run B.