SCIEX

Assessing method transfer and equivalency between the PA 800 Plus system and the BioPhase 8800 system

danaher

The power of precision

Introduction

Adopting a new analytical platform in the discovery, research, development, or quality control environments of biopharmaceutical development is a gradual process. This adoption requires thorough due diligence, including a comprehensive investigation of the platform's performance, reliability, and robustness. Equally important is assessing how the new platform compares to existing instrumentation in terms of data comparability and equivalency. These assessments are typically evaluated in protocols such as method transfers or bridging studies.

An analytical method transfer is a protocol used to establish a validated analytical test procedure in a new laboratory. The receiving lab must demonstrate that they can reliably and accurately perform the method, producing results equivalent to those in the original laboratory. While using the same instrument simplifies the process, it's not a requirement for a successful method transfer as the primary objective is ensuring equivalent results, regardless of the specific instrumentation used in the receiving laboratory.

Similarly, an analytical bridging study is a study designed to compare the performance of 2 different analytical methods or procedures, ensuring that they are comparable and can be used interchangeably. This is crucial when switching from one analytical method to another, for example, due to changes in technology, instrument availability, or regulatory requirements.

To facilitate method transfer, bridging, and ultimately platform adoption in biopharma, the BioPhase 8800 system was designed to use the same reagent and capillary as the PA 800 Plus system. This allows the user to achieve comparable results when performing CE-SDS, cIEF, glycan heterogeneity, RNA or DNA analyses. Each separation technique can use a designated kit to maximize reproducibility and robustness on either platform.

The bridging studies discussed in this paper are centered around the CE-SDS workflow. As CE-SDS is a widely used analytical technique for protein purity analysis, applied throughout the biopharmaceutical life cycle, it is an ideal technique to use as a case study to demonstrate method transfer strategies and evaluate data comparability between the PA 800 Plus system and BioPhase 8800 system.

High resolution CE-SDS method transfer from the PA 800 plus system to the BioPhase 8800 system

To successfully transfer the CE-SDS method from PA 800 Plus system to the BioPhase 8800 system one must attain the same separation profile, and migration time window for the peaks of interest. Demonstrating this data equivalency to current standard operating procedures (SOPs) that utilize the PA 800 Plus system is critical in order to gain acceptance for method transfer by regulatory agencies or technology adoption. Below we discuss some key considerations.

Addressing multiple capillaries

The BioPhase 8800 system enables 8 samples to be run in parallel, significantly increasing throughput and enabling faster turnaround times for multiple assays. The BioPhase cartridge is designed to accommodate 8 capillaries and the heat load generated during separation. The cartridge is available fully assembled, eliminating the need for assembly by the end-user. The cartridge design efficiently accommodates liquid cooling, operating within a temperature range of 15 to 40°C.

The innovative design of pre-assembled cartridge features 8 cannula-electrodes. Each cannula connects with the high-voltage power supply to deliver voltage for separation. The cannula design allows for concentric positioning of capillaries on the inlet side, enabling efficient interaction with the BioPhase 96 well plates. On the outlet side of the cartridge, capillaries are bundled together and exit parallel to the electrode. Inside the cartridge body, the capillaries are immersed in a liquid environment that effectively dissipates joule heating. The cartridges are equipped with a unique chip that tracks serial number, lot number, total number of runs, date in service, expiration date, manufacturing date, and capillary dimensions. This feature facilitates the electronic record-keeping of the cartridge's usage history.

This increase in capillaries changes the volume of reagents consumed during capillary conditioning. And so, in transferring the CE-SDS method there is a need to optimize the consumption of reagents during capillary conditioning, separations and capillary shutdown to achieve maximum throughput per sequence.

On the PA 800 Plus system the capillary rinse steps consist of a long rinse with NaOH, followed by HCl, water and finally the SDS separation gel. These rinses are essential to maintain run-to-run reproducibility, consistency, and capillary longevity.

This same strategy is used on the BioPhase 8800 system. However, keeping the same NaOH rinsing condition from the PA 800 plus system on the BioPhase 8800 system would limit the throughput because the system would run out of NaOH before the conclusion of a sequence.

Therefore, by taking advantage of the flexibility of the BioPhase 8800 system, the CE-SDS separation method now performs 2 NaOH rinses. The first rinse is performed at 80 psi to push the viscous SDS gel out of the capillary and the second rinse is performed at 20 psi for silica surface treatment. (Figure 1) shows a comparison between the pre-injection capillary rinsing steps between the BioPhase 8800 system and the PA 800 plus system.

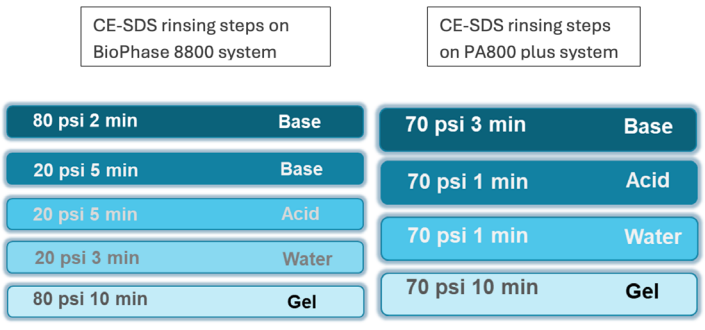


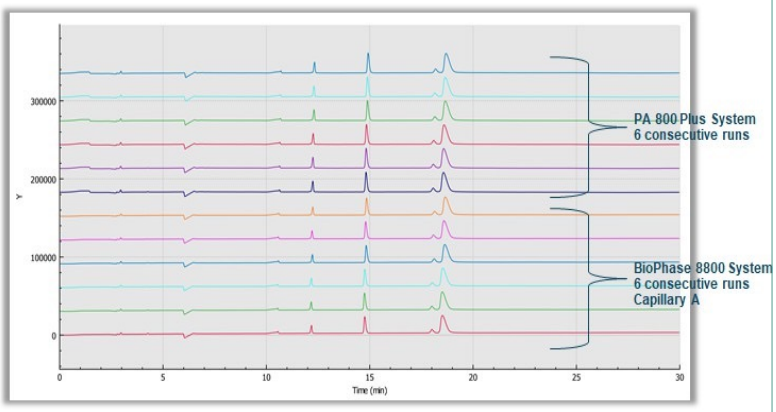
Figure 1. Shows a comparison between the pre-injection capillary rinsing steps between the BioPhase 8800 system and the PA 800 plus.

Evaluating rinsing procedure

To demonstrate the performance of the new rinsing procedure, Figure 2 shows an overlay of 6 consecutive runs from the PA 800 plus system (top) and BioPhase 8800 system (bottom). High resolution separation of all sample components, including the light chain, non-glycosylated heavy chain and the heavy chain, were obtained.

The 10 kDa protein was added to all samples as an internal standard and used for relative migration time calculation. The average relative migration time reproducibility of the BioPhase 8800 system for the heavy chain fragment was RSD=1.1%, as depicted in Table 1.

The average corrected peak area% reproducibility was RSD=0.85% for the non-glycosylated heavy chain, the smallest peak in the separation trace (Table 1). Please note that peak area reproducibility is usually lower for smaller peaks.



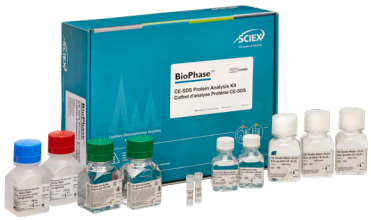


Figure 2. Comparative chart of 6 consecutive CE-SDS separations of the reduced IgG control standard sample on the BioPhase 8800 system and the PA 800 Plus system.

Table 1. Comparison of the relative migration times of the heavy chain (HC) peak in the multi- and single capillary electrophoresis instruments. Left panel: lane to lane (A-H) and run to run (1-16) migration times obtained by the BioPhase 8800 system. Right panel: run to run (1-15) migration times obtained by the PA 800 Plus single capillary system. The %RSD for the BioPhase 8800 system is 1.1% which correlates well with the 0.83 %RSD obtained with the PA 800 Plus system.

	Capillary A	Capillary B	Capillary C	Capillary D	Capillary E	Capillary F	Capillary G	Capillary H	Run Average	Run %RSD
Run 1	18.508	18.792	18.483	18.500	18.517	18.567	18.458	18.750	**18.572**	**0.68%**
Run 2	18.533	18.842	18.542	18.525	18.558	18.525	18.492	18.600	**18.577**	**0.60%**
Run 3	18.633	18.842	18.533	18.542	18.575	18.583	18.550	18.633	**18.611**	**0.54%**
Run 4	18.592	18.625	18.600	18.592	18.617	18.608	18.592	18.683	**18.614**	**0.17%**
Run 5	18.650	18.900	18.617	18.633	18.700	18.683	18.625	18.750	**18.695**	**0.50%**
Run 6	18.650	18.767	18.675	18.683	18.725	18.717	18.692	18.808	**18.715**	**0.28%**
Run 7	18.692	18.808	18.708	18.725	18.800	18.775	18.733	18.850	**18.761**	**0.30%**
Run 8	18.750	18.817	18.758	18.775	18.825	18.825	18.792	18.908	**18.806**	**0.27%**
Run 9	18.775	18.858	18.792	18.825	18.858	18.892	18.833	18.967	**18.850**	**0.32%**
Run 10	18.825	18.917	18.833	18.867	18.925	18.925	18.883	19.008	**18.898**	**0.31%**
Run 11	18.867	18.892	18.875	18.917	19.000	18.958	18.958	19.075	**18.943**	**0.37%**
Run 12	18.900	18.992	18.917	18.958	19.050	19.025	18.983	19.133	**18.995**	**0.40%**
Run 13	18.942	18.958	18.967	19.017	19.100	19.075	19.050	19.175	**19.035**	**0.42%**
Run 14	18.975	19.050	19.025	19.075	19.142	19.133	19.100	19.242	**19.093**	**0.43%**
Run 15	19.025	19.033	19.067	19.117	19.208	19.192	19.167	19.308	**19.140**	**0.51%**
Cap Average	**18.754**	**18.873**	**18.759**	**18.783**	**18.840**	**18.832**	**18.794**	**18.926**		
Cap %RSD	**0.87%**	**0.58%**	**0.99%**	**1.09%**	**1.20%**	**1.15%**	**1.20%**	**1.19%**		

Overall average (n=120)	**18.803**
Overall %RSD (n=120)	**1.10%**

Intra Max RSD	1.26%
Inter Max RSD	0.72%

	Migration Time
Run 1	18.575
Run 2	18.592
Run 3	18.658
Run 4	18.650
Run 5	18.692
Run 6	18.733
Run 7	18.767
Run 8	18.800
Run 9	18.992
Run 10	19.000
Run 11	18.900
Run 12	18.925
Run 13	18.933
Run 14	18.983
Run 15	19.000
Average	**18.813**
RSD	**0.83%**

Addressing improvements in sensitivity

The BioPhase 8800 system is equipped with both UV and LIF detection options. UV and LIF detection systems are integrated and method-driven, allowing users to take advantage of both detection modes in the same sequence.

The BioPhase 8800 system UV detection is equipped with 220 and 280 nm reflective filters. The reflective filter coupled with modern optics design allows for higher transmission efficiency and shows as an increased peak height compared to PA800 plus system (Figure 3).

Since heavy chain and light chain have slightly different absorbance at different wavelengths, we observed a shift in the HC/LC ratio while maintaining the %purity for a molecule due to the increased bandpass for the reflective filter. (Table 2) The LIF detection maintained high consistency between the 2 systems with 488 nm laser excitation and very similar detection technology. Multiple LIF detection filter options are also offered and the switch of LIF filter can be achieved within one minute following front panel guided steps.

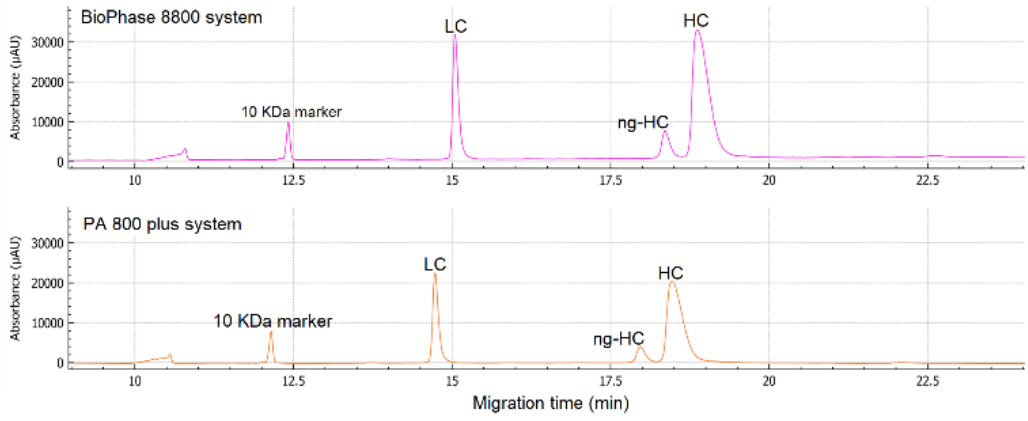


Figure 3.
The electropherograms of the IgG control on the BioPhase 8800 system (pink) and the PA800 Plus system (yellow).

*TCA= total time corrected peak area of all integrated peaks. The results shown here for PA800 plus system are an average of 3 injections and for BioPhase 8800 system, there is an average of 6 capillaries X 3 injections = 18 data files of the sample pooled sample preparation. The remaining 2 capillaries were buffer blanks with 10 kDa marker for baseline monitoring.

Table 2.
Time-corrected peak area of the main species in the reduced IgG control sample

Instrument	TCA*	%LC	%NG-HC	%HC	%Purity	HC/LC Ratio
BioPhase 8800 system	15913.0	27.92	7.40	63.77	99.07	2.28
PA 800 Plus system	10957.0	31.16	7.07	60.96	99.19	1.96
Relative % difference	37%	11%	5%	5%	0.12%	15%

The limit of detection (LOD), limit of quantification (LOQ) and linear range were first evaluated with UV detection. A serial dilution of the NIST monoclonal antibody standard was used for this study, starting from a 5 mg/mL solution all the way down to as low as 1.2 µg/mL. Figure 4 depicts the results showing excellent linearity for both linear-linear (panel A, r2=0.999) and log-log (panel C, r2=0.997) plots, the latter for the better visibility in the lower concentration range. The LOD with the signal-to-noise ratio value of S/N=3 was 2.4 µg/mL, while the LOQ with the signal-to-noise ratio value of 10 was S/N=4.9 µg/mL. As one can observe, the UV detection signal response was linear over 4 orders of magnitude. Panel B shows the relevant electropherogram sections with 300, 4.9 and 2.4 µg/mL sample injections. The detection linearity and limit (LOD and LOQ) data were similar to what was obtained earlier with the PA 800 Plus system.

The LOD, LOQ and detection range linearity were also evaluated with the higher sensitivity LIF detection. Similarly, a serial dilution of the standard NIST monoclonal antibody was used. However, in this instance, the range was from 38 µg/mL down to 4 ng/mL, considering the significantly greater detection sensitivity of the LIF system. Figure 5, shows the results with optimal linearity for both the linear-linear (panel A, r2=0.9999) and log-log (panel C, r2=0.9996) plots over the nearly 4 orders of magnitude concentration range. The LOD with the signal-to-noise ratio of S/N=3 was 4 ng/mL, while the LOQ with the S/N=8 was 10 ng/mL. Panel B, shows the relevant electropherogram sections from 38 µg/mL to 4 ng/mL concentration sample injections, all in individual capillaries of the multi-capillary system. This saved significant time as the entire concentration range can be evaluated in a single run. To increase the precision of the approach, the variance between the capillaries was normalized to the 10 kDa internal standard.

ICH Q3A provides recommendations to include information regarding specified impurities in certain new drug applications (NDAs), both for identified and unidentified impurities in new drug substance specifications. It also requires acquiring and evaluating data that establishes the biological safety of individual impurities, or a given impurity profile, at the levels defined.

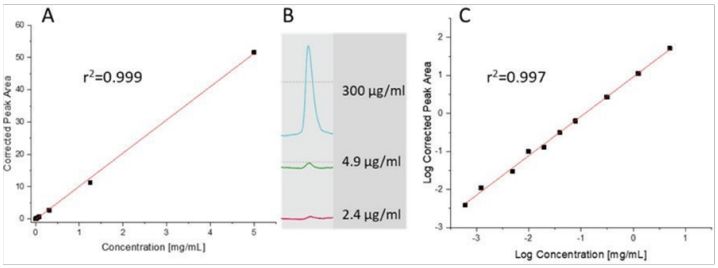


Figure 4. UV detection linearity, LOD and LOQ determination for the NIST monoclonal antibody standard with the BioPhase 8800 system using linear- linear (A) and log-log (C) interpretations. Panel B depicts the relevant electropherogram sections with 300, 4.9 and 2.4 µg/mL sample injections.

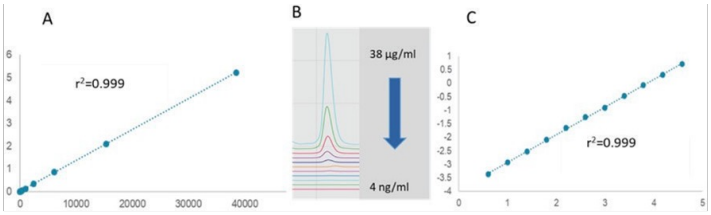
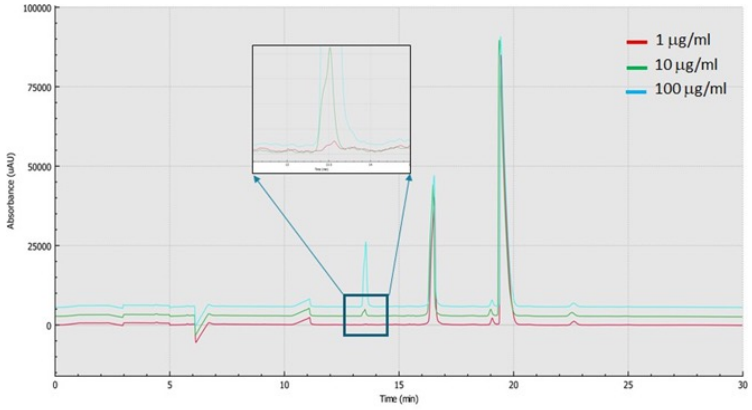


Figure 5. LIF detection linearity, LOD and LOQ for the NIST monoclonal antibody standard with the BioPhase 8800 system using linear-linear (A) and log-log (C) interpretations. Panel B depicts the relevant electropherogram sections from 38 µg/mL to 4 ng/mL sample injections.

Regulators specify 0.1% impurity level to the main drug product.

Figure 6, shows the analysis of 1000 mg/ml USP mAb 003, monoclonal IgG1 spiked with lysozyme at 100, 10 and 1 µg/ml levels, demonstrating the capability of the BioPhase 8800 system multi-capillary electrophoresis platform to readily detect the required 0.1% impurity level in the main product.



name	apex	ca	ca%	SNR RMS
Lysozyme 0.1%	13.57	29.68	0.09	24.79
mAb main product	19.44	32049.51	99.91	12535.09

Figure 6. Impurity analysis down to 0.1% of the main product (0.1% of lysozyme spiked into an IgG sample) with the BioPhase 8800 system. Conditions: -15kV with 1 minute ramp time and with 20 psi applied at both inlet and outlet reservoirs during separation. UV detection at 220 nm.

Addressing regulatory compliance on the BioPhase 8800 system

The Empower Chromatography Data System is a chromatography data system (CDS) widely used in the biopharmaceutical industry and allows instrument control and data processing from liquid chromatography, mass spectrometry, capillary electrophoresis and other analytical platforms. The BioPhase 8800 driver for Empower software minimizes the need to train users on multiple software platforms and ensures seamless workflow transfer in a compliant and secure manner.

The BioPhase 8800 driver for Empower software controls the system hardware and data collection, allows for method and sequence editing and stores multi-capillary data in the Empower CDS with linked sample information. After data acquisition, users can analyze the data directly in Empower CDS and apply any necessary electronic signatures on reports using sign-off capabilities.

Key features of the BioPhase 8800 driver for Empower software:

1. Seamless data acquisition: The driver allows for direct control of the BioPhase 8800 system hardware, including method and sequence editing capabilities
2. Data integrity: Direct transfer of data acquired on the BioPhase 8800 system with the ability to apply the 21 CFR Part 11 toolset in the Empower CDS to help satisfy GxP requirements
3. Streamlined data management: Facilitate seamless data management from analytical development to quality control

Workflow using the BioPhase 8800 driver for Empower software:

Create a CE method: There are 3 options to create a CE method for the BioPhase 8800 system. Users can download pre-made separation methods of interest from https://www.SCIEX.com. Alternatively, users can create or open a pre-loaded method using the BioPhase 8800 driver for Empower software. Finally, users can import a read-only copy of the method of interest into Empower CDS using the import function (Figure 7).

Create a sample set:

A sample set can be created directly in the BioPhase 8800 driver for Empower software or by using the sample set method editor. However, the plate layouts and sample set method for the BioPhase 8800 system must be defined or validated in the embedded method editor to avoid potential user errors (Figure X).

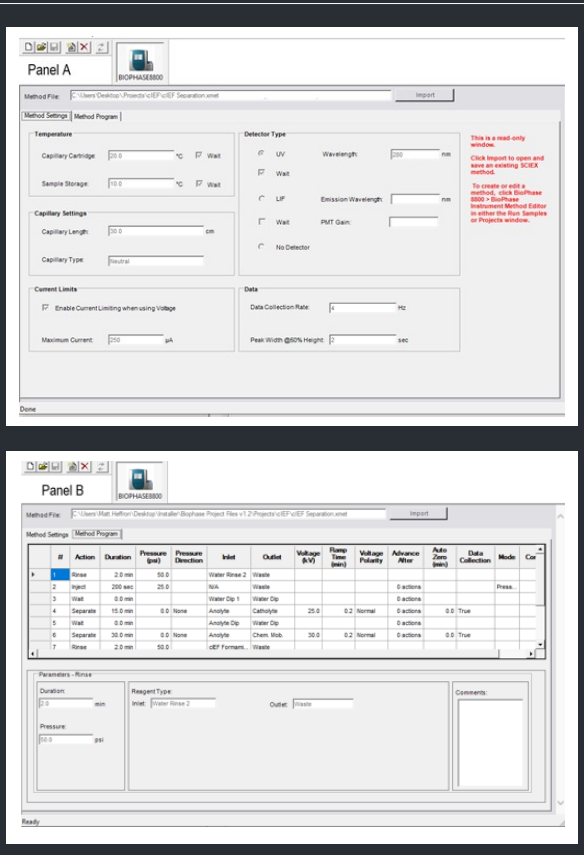


Figure 7. Embedded Empower CDS editor for method import (panel A) and BioPhase 8800 driver for Empower software (panel B). Panel A shows the method settings and Panel B the method time program.

Figure 8.

CE-SDS sample and reagent tray setup using the sample set method editor in the BioPhase 8800 driver for Empower software.

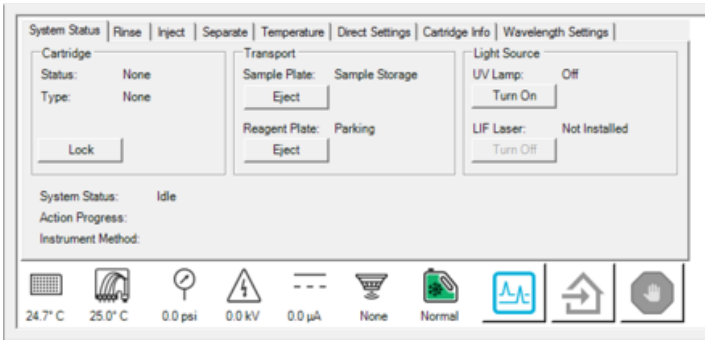


Figure 9.

System status pane from the direct control screen in the driver. The bar at the bottom (highlighted in blue) shows the instrument status in real-time.

Prepare the BioPhase 8800 system:

The next step is to set up the BioPhase 8800 system with reagent and sample trays to run a sequence. This task can be easily accomplished using the direct control functions in the driver (Figure 8). Manual operations are also available in the driver, such as setting rinses, injections and separations, changing wavelength settings, modifying cartridge and sample compartment temperature, and obtaining cartridge information. Additionally, the system status can be monitored in real-time (Figure 9).

Submit the sample set for separation:

The user can follow the functions in the Empower CDS to select an existing sample set method, as shown in Figure 10.

Monitor the separations via direct control:

The blue icon toward the bottom right of the direct control screen (Figure 11) allows users to monitor the separation status by opening the trace view option. The trace view can simultaneously display multiple electropherograms. The user can toggle between the optical, current, voltage and pressure traces. The user can also select which capillaries are displayed using the checkboxes at the bottom of the screen. Figure X shows the live view of UV absorption and current traces during a cIEF separation.

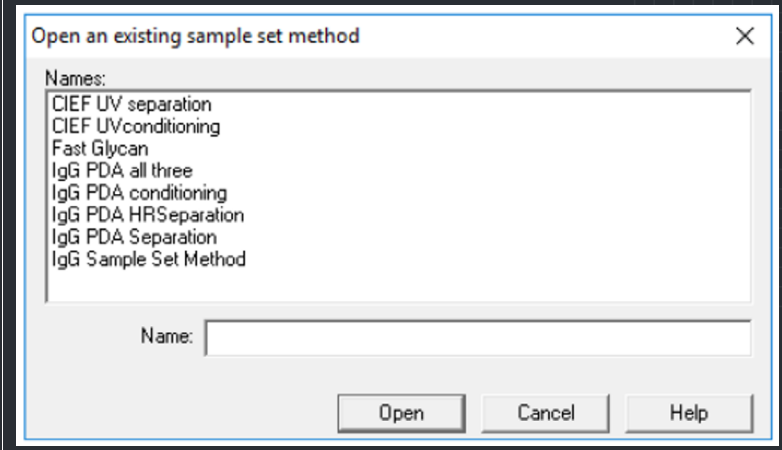


Figure 10.

Options available to load a sample set method.

Multi-channel CE-SDS and cIEF assays using the BioPhase 8800 driver for Empower software

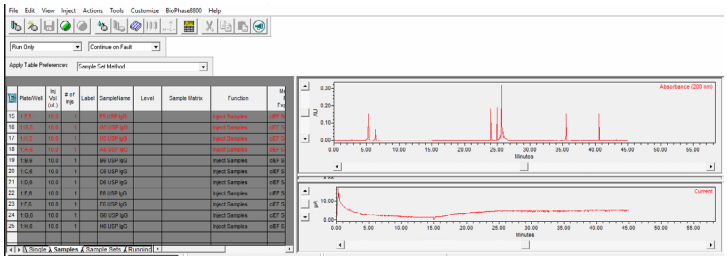


Figure 11. Sample set display on the BioPhase 8800 driver for Empower software showing the sample table and real-time separation profile with current trace.

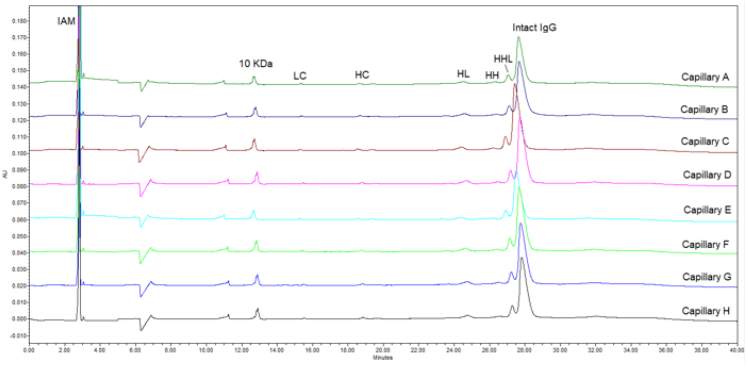
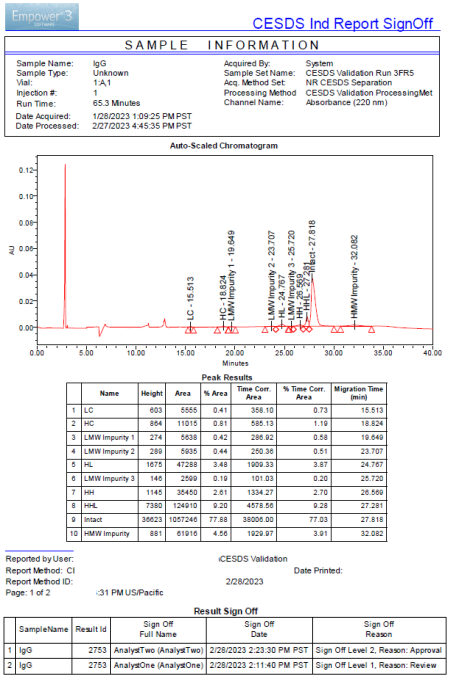


Figure 12. Overlay of 8 representative CE-SDS runs of IgG control standard (SCIEX) under non-reducing conditions. Peaks are labeled for the light chain (LC), heavy chain (HC), heavy-light (HL) chains, heavy-heavy (HH) chains and heavy-heavy-light (HHL) chains.

Analyzing the data, reporting and sign-off:

The BioPhase 8800 driver for Empower software allows users to access the resources currently available in the Empower CDS, from data analysis to sign-off.



CESDS Ind Report SignOff

SAMPLE INFORMATION

Sample Name:	IgG	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	CESDS Validation Run 3RS
Vial:	1:A1	Acq. Method Set:	NR CESDS Separation
Injection #:	1	Processing Method	CESDS Validation ProcessingMet
Run Time:	65.3 Minutes	Channel Name:	Absorbance (220 nm)
Date Acquired:	1/28/2023 1:09:25 PM PST		
Date Processed:	2/27/2023 4:45:35 PM PST		

Auto-Scaled Chromatogram

Peak Results

	Name	Height	Area	% Area	Time Corr. Area	% Time Corr. Area	Migration Time (min)
1	LC	603	5555	0.41	358.10	0.73	15.513
2	HC	864	11015	0.81	585.13	1.19	18.824
3	LMW Impurity 1	274	5638	0.42	286.92	0.58	19.649
4	LMW Impurity 2	289	5935	0.44	250.36	0.51	23.707
5	HL	1675	47288	3.48	1909.33	3.87	24.767
6	LMW Impurity 3	146	2599	0.19	101.03	0.20	25.720
7	HH	1145	35450	2.61	1334.27	2.70	26.569
8	HHL	7380	124910	9.20	4578.56	9.28	27.281
9	Intact	36623	1057246	77.88	38006.00	77.03	27.818
10	HMW Impurity	881	61916	4.56	1929.97	3.91	32.082

Reported by User: iCESDS Validation
Report Method: CI
Report Method ID: Date Printed: 2/28/2023
Page: 1 of 2 :31 PM US/Pacific

Result Sign Off

	SampleName	Result Id	Sign Off Full Name	Sign Off Date	Sign Off Reason
1	IgG	2753	AnalystTwo (AnalystTwo)	2/28/2023 2:23:30 PM PST	Sign Off Level 2, Reason: Approval
2	IgG	2753	AnalystOne (AnalystOne)	2/28/2023 2:11:40 PM PST	Sign Off Level 1, Reason: Review

Figure 13. Example of a report generated by the BioPhase 8800 driver for Empower software. This report is generated using compliance features available in the Empower CDS.

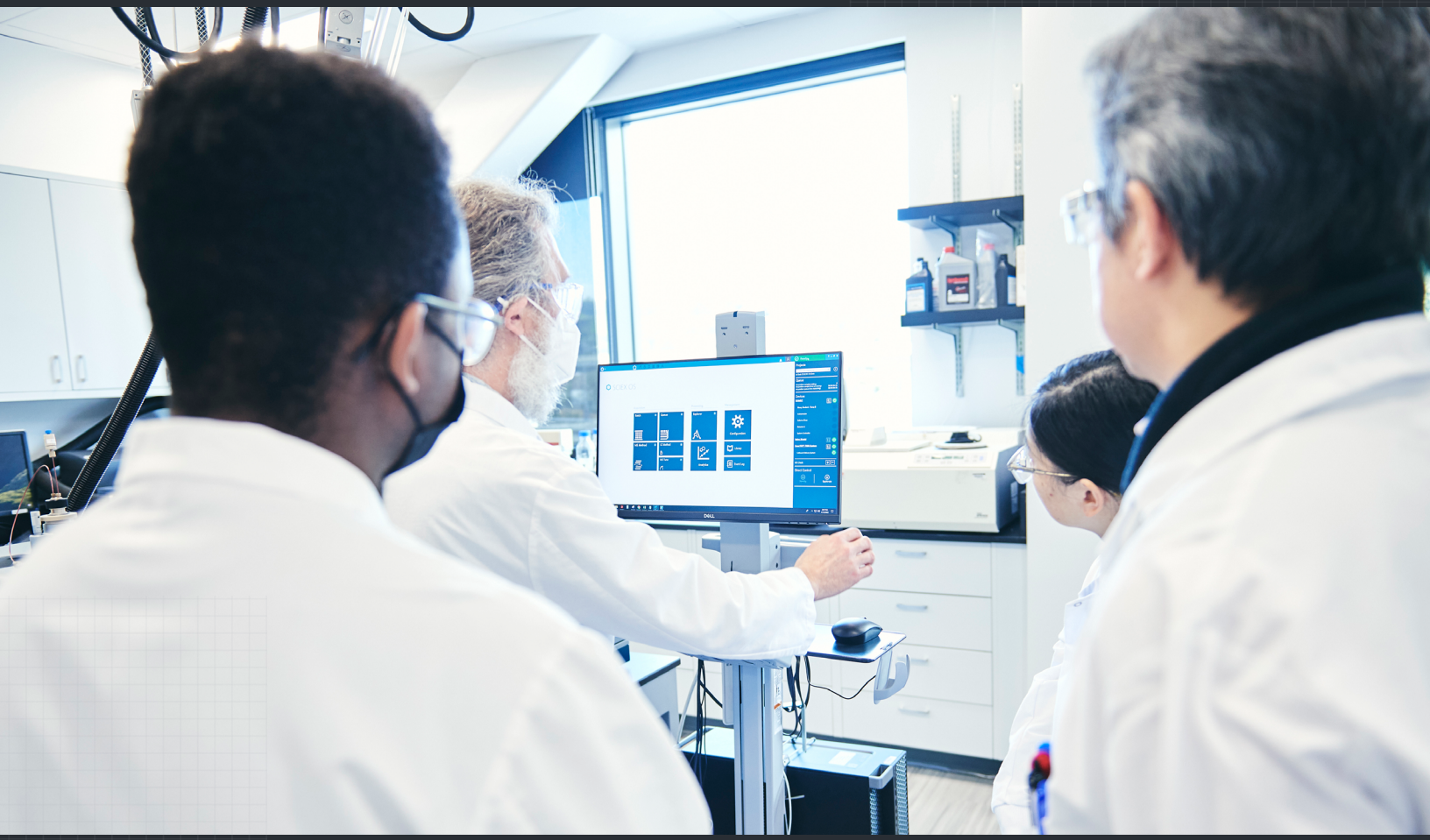
The BioPhase 8800 system is a multi-capillary system designed for high-throughput CE analysis. It effectively meets the extensive testing requirements of product and process development, while maintaining the quality of result and data comparability.

This paper discussed the considerations needed to demonstrate data equivalency and perform a bridging study between the BioPhase 8800 and the PA 800 Plus systems using the CE-SDS assay. The BioPhase 8800 system can consistently provide precise and equivalent results to the PA 800 Plus system for both high-resolution and high-speed CE-SDS methods, providing a higher throughput solution that operates within the parameters of current SOPs, enabling a smooth method transfer from early discovery to QC.

The BioPhase 8800 system can provide a higher throughput solution that operates within the parameters of current SOPs

Case studies

With the considerations of how improvements in hardware features on the BioPhase 8800 system can impact data equivalency, the following case studies discuss practical evaluations of the BioPhase 8000 system to the PA 800 Plus system and how to successfully transfer the CE-SDS method between the 2 platforms.



Case study 1

High-speed CE-SDS method transfer from the PA 800 Plus system to the BioPhase 8800 system

The IgG HS separation method on the PA 800 Plus system was created to reduce the total analysis time by 15 minutes, without compromising data quality compared to the IgG high-resolution (HR) method.

Unlike the PA 800 plus system the BioPhase 8800 system does not feature the CE-SDS separation using the 10 cm commonly known as the short side separation. The advantage of using the short side is the significant decrease in separation time. Even though the throughput of BioPhase 8800 system is much higher, the IgG high-speed (HS) method on the PA 800 plus system was validated and adopted by many biopharmaceutical companies

To support the demand for even higher throughput, the IgG HS method is successfully transferred from the PA 800 plus system to the 8-capillary BioPhase 8800 system as shown in Figure 14. The detailed evaluation is shown below:

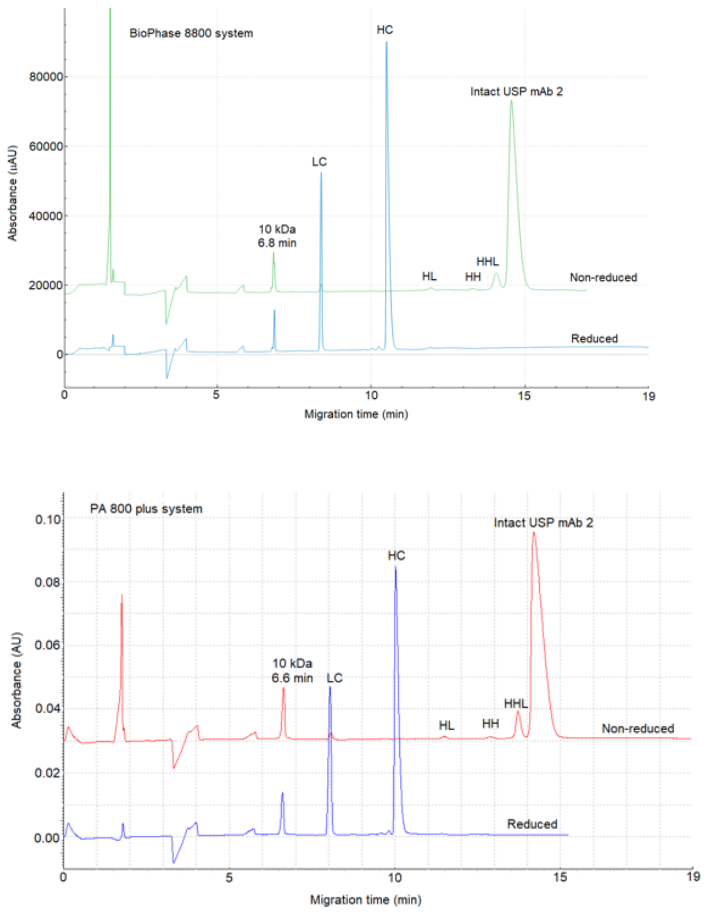
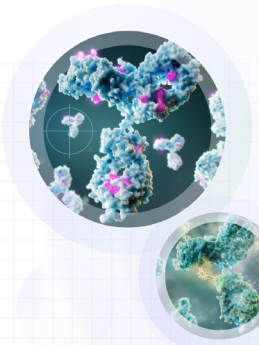


Figure 14.

Separation of USP mAb 002 under reducing and non-reducing conditions on the BioPhase 8000 system (left) and on PA 800 Plus system (right) using the HS CE-SDS method. Separation conditions are in the methods section. Migration time of 10 kDa marker on the BioPhase 8800 system using the HS CE-SDS method is within 1 min window compared to the migration time on the PA 800 Plus system.



Case study 1 *continued*

Selecting 10 kDa as the internal mobility marker and defining a migration time window for 10 kDa

In CE, using an internal mobility marker can be crucial. This is due to the consistent relative position between the marker and the sample peaks, which increases reproducibility between different runs, capillaries, chemistry lots, instruments, and users.

The first step in developing the method was to set a migration time requirement. Although any peak could be used as a marker, the migration time for a mAb molecule peak under reduced or non-reduced conditions can vary due to differences in the antibody hydrodynamic size and primary sequence. These differences can affect the hydrophobicity of the mAb. Consequently, using a mAb peak as a reference can impact assay accuracy and reproducibility, as it may change from molecule to molecule, making the method non-viable as a platform method.[1]

Figure 15 illustrates the difference between the migration time of the monomer peak for NISTmAb and SCIEX's IgG control standard mAbs.

Defining a migration time window for the 10 kDa internal marker is more reliable than using a mAb peak, as this marker exhibits a consistent migration pattern regardless of the antibody sample and the sample preparation conditions (reducing and non-reducing).

Figure 17 shows that the migration time of the 10 kDa internal marker using the IgG HS method was around 6.6 minutes on the PA 800 Plus system under reducing and non-reducing conditions. Therefore, the 10 kDa molecule was used as a marker to investigate other instrument settings in this study, with a requirement for the migration time set to 6.6 +/- 1 minute.

The migration time of the 10 kDa marker on the BioPhase 8800 system using the HS CE-SDS method was within a 1 min window compared to the migration time on the PA 800 Plus system, demonstrating data comparability between the two systems.

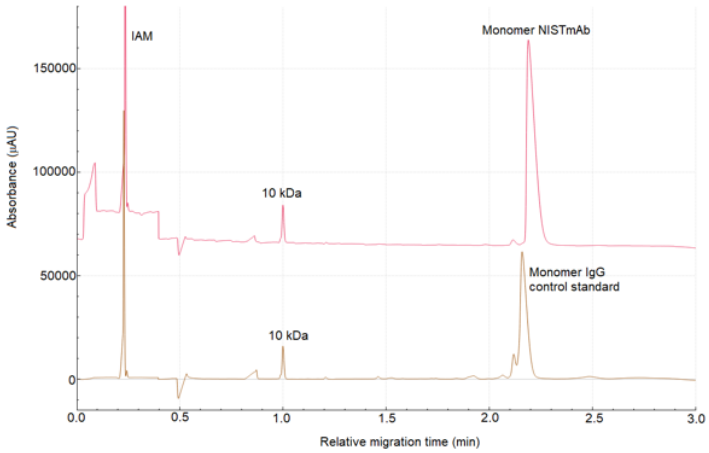
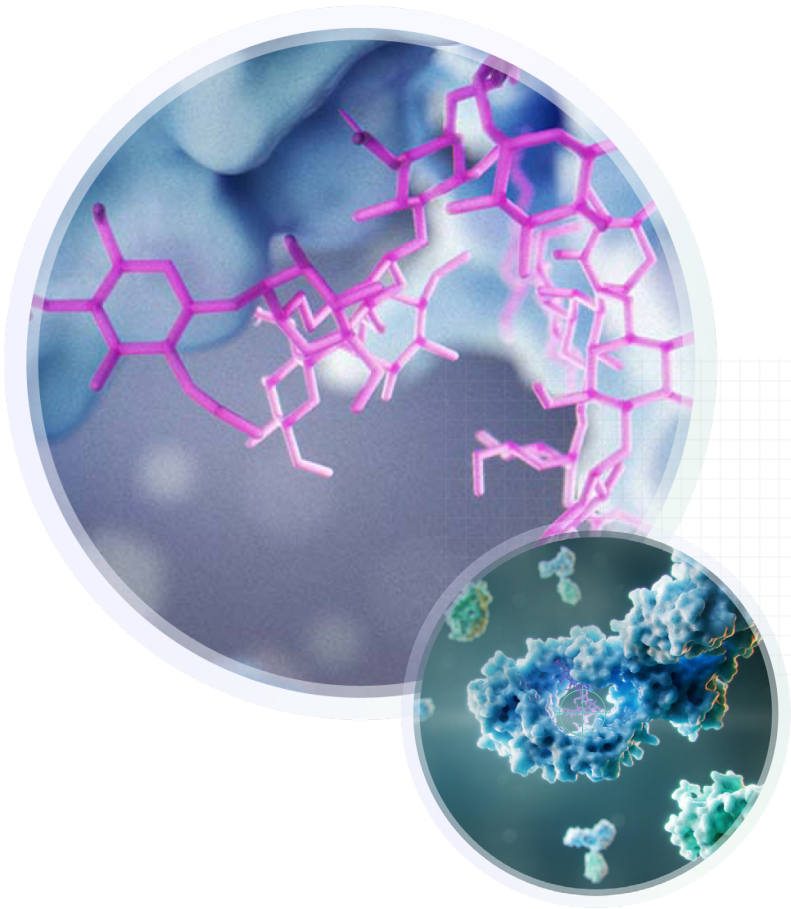


Figure 15.

Migration time difference of 2 different mAbs under non-reducing conditions. The top trace is from NISTmAb, and the bottom trace is from the IgG control standard under non-reducing conditions using the IgG standard method on the BioPhase 8800 system [-15 kV/25oC].



Investigating the separation temperature and electric field strength

It is well known that 3 main factors can impact the separation time: capillary length, electric field strength (voltage), and separation temperature[2].

On the BioPhase 8800 system, the total capillary length is fixed at 30 cm with 20 cm of effective length. Thus, the electric field and separation temperature are the only variables that can be adjusted in a method.

Figure 16 shows the separation of reduced USP mAb 002 under various voltages and temperature conditions.

As expected, as the electric field strength and temperature increased, peak migration time decreased. However, as the peaks of interest migrated faster, the baseline fluctuation from the separation gel buffer became more significant and could potentially interfere with peak integration.

The background signal from the separation gel buffer was found to be influenced by temperature and electric field strength.

Figure 16 shows that the baseline noise was less significant at lower temperatures, i.e., 18°C, even at higher electric fields.

Even though the baseline interference was mitigated at 18°C, the migration time of the 10 kDa marker at the highest electric field (-30 kV) was 7.69 min, which falls outside the target range of 6.5 +/- 1 min. Therefore, further method optimization was carried out as described below.

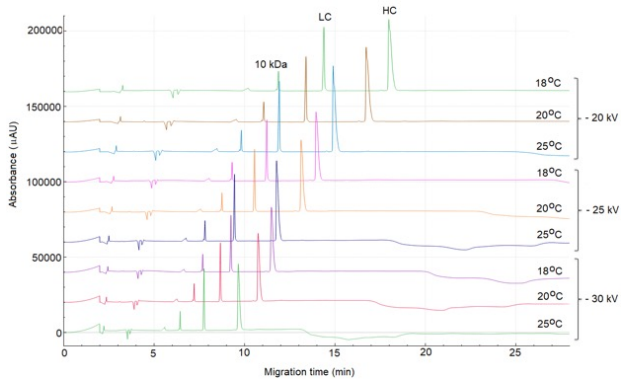


Figure 16. Overlay of the CE-SDS separation traces of USP mAb 002 at various separation voltages and temperatures.

Optimizing separation ramp time

Another method setting that is often overlooked is the ramp time. Ramp time is the time it takes the instrument's high voltage power supply to ramp from 0 kV to the set separation voltage.

The ramp time setting is crucial because it directly impacts the reproducibility of the separation.

When voltage is applied, the electrode generates a sudden heat wave that propagates to the surrounding areas, affecting the capillary.[3-4] The resulting temperature gradient may induce dispersion and reduce separation efficiency. However, theoretical models of the thermal gradient, as explained by Guiochon et al.,5 indicate that this effect is negligible for capillaries with an inner diameter of 100 mm. The inner diameter of the capillary used in this application was only 50 mm.

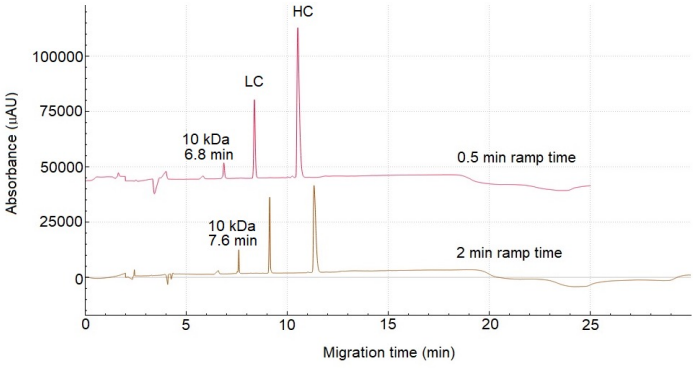


Figure 17. Overlay of electropherograms obtained with NISTmAb and USP mAb002 under non-reducing conditions using the IgG standard method from PA 800 Plus system [-30 kV/18oC].

Case study 2

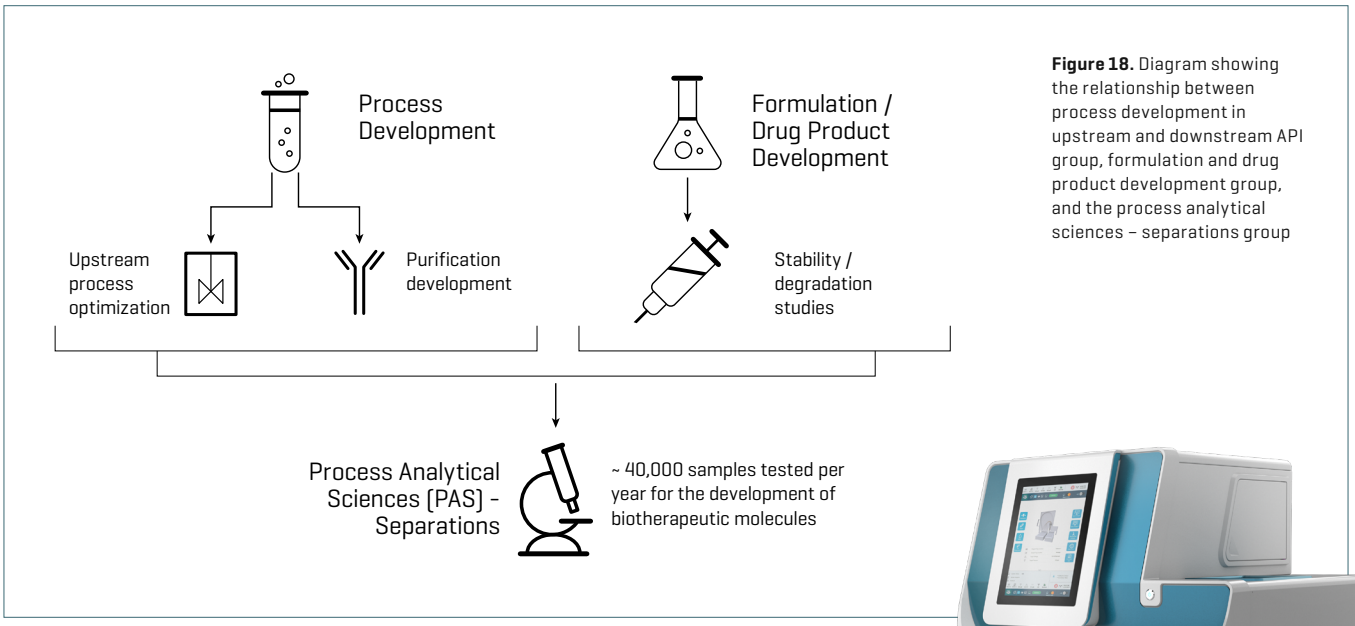


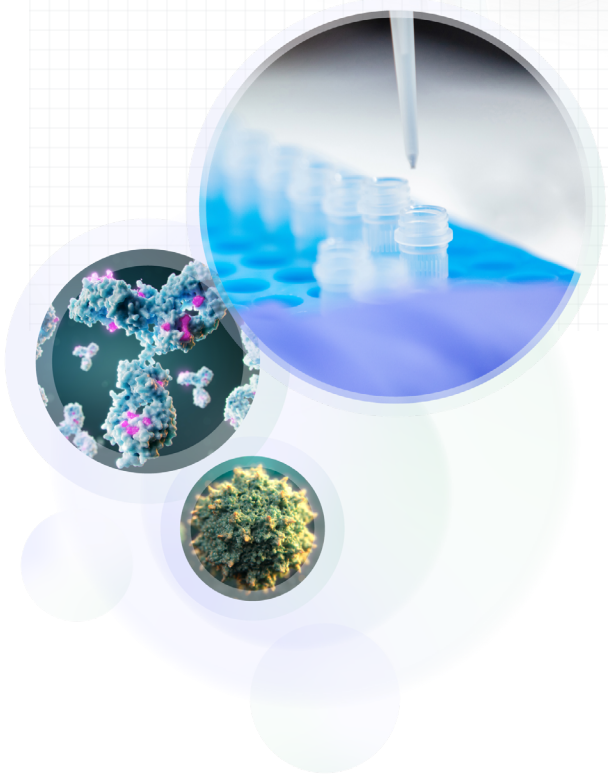
Figure 18. Diagram showing the relationship between process development in upstream and downstream API group, formulation and drug product development group, and the process analytical sciences – separations group

Increase throughput for the purity analysis of biotherapeutic proteins

In the biotherapeutic life cycle, the process analytical sciences (PAS) group at Johnson & Johnson is at the beginning of the development process of new biotherapeutics. This group receives test samples from both upstream and downstream API (active pharmaceutical ingredient) development groups as well as the formulations development group (Figure 18).

Per year, approximately 40,000 samples come to PAS for various analyses. The upstream samples come from process optimization for cell growth, such as bioreactor optimization. The downstream samples come from protein purification development.

The drug product development group conducts experiments and sends samples for buffer formulation, stability, and degradation studies.



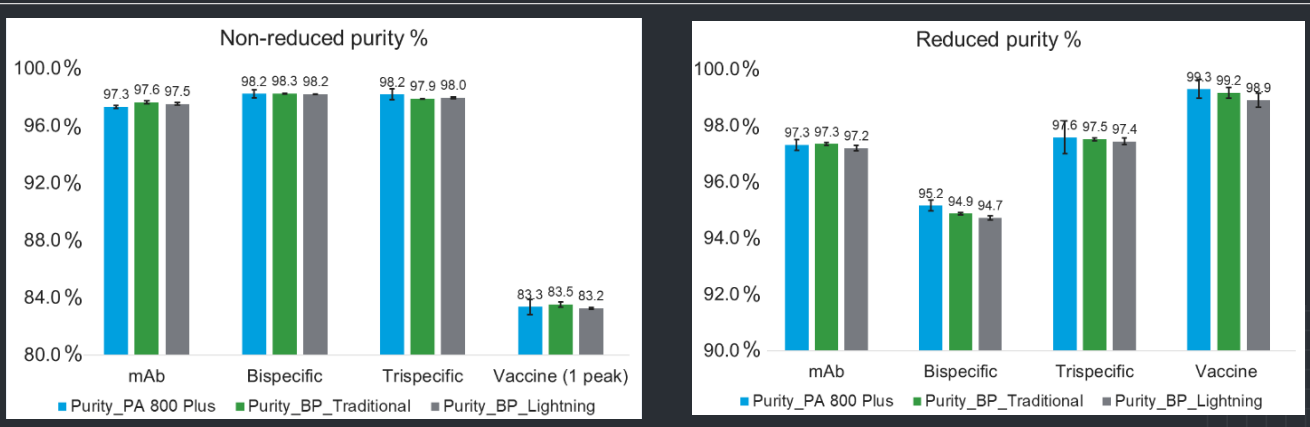


Figure 19.

Purity percentage comparison assessment between PA 800 Plus system and the BioPhase 8800 system of multiple molecule types under non-reduced (top) and reduced (bottom) conditions.

Evaluation of BioPhase 8800 system for early development

Four conditions were studied to assess the performance equivalence between the BioPhase 8800 system and the PA 800 Plus system for reduced and non-reduced CE-SDS samples:

- Comparison of purity assessment across multiple modalities
- Reproducibility and consistency assessment between the 8 capillaries on the BioPhase 8800 cartridge
- Linear dynamic range across multiple protein concentrations
- Comparison of purity assessment for various process development samples

Percentage purity assessment comparison across multiple modalities

A purified mAb molecule, bispecific, trispecific and a vaccine product were separated using the PA 800 Plus system and the BioPhase 8800 system for the purity assessment. On the BioPhase 8800 system, 2 separation methods were tested: the "traditional" and "lighting" methods. The significant difference between "traditional" and lightning CE-SDS methods is the pre-injection capillary rinse steps (table below).

Figure 19 shows purity values across different molecule types. The error bars on the PA 800 Plus system represent the standard deviation of n=6 runs across multiple instruments and analyst preparations. The error bars on the BioPhase 8000 system (BP) using traditional method represent the standard deviation of n=6 within a single sequence run. The error bars on the BP using lightning method also represents the standard deviation of n=6 within a single sequence run.

Pre-injection capillary conditioning steps:

Traditional	Lightning
Total time: 25 minutes	Total time: 10 minutes
Base: 80 PSI, 2 min	Base: 80 PSI, 3 min
Base: 20 PSI, 5 min	
Acid: 20 PSI, 5 min	Acid: 50 PSI, 2 min
Water: 20 PSI, 3 min	Water: 50 PSI, 1 min
Gel: 80 PSI, 10 min	Gel: 80 PSI, 4 min

Reproducibility and consistency assessment between the 8 capillaries on the BioPhase 8800 cartridge

A bispecific mAb sample was reduced and separated using the BioPhase 8800 system. Figure 19 shows the intra-capillary reproducibility of the 8 CE-SDS separations obtained in capillaries A through H.

Table 3 reveals the consistency between the capillaries in both total corrected peak area and purity percentage.

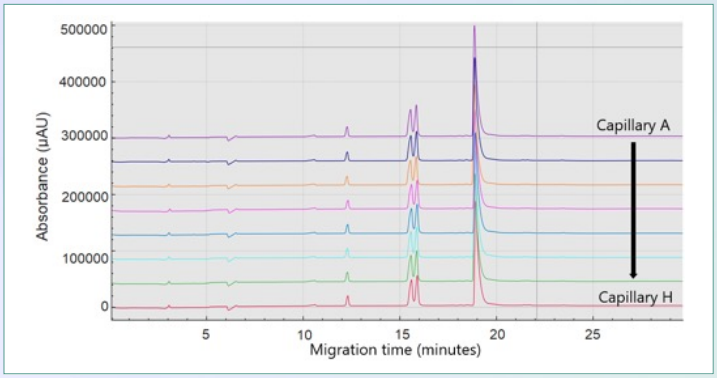


Figure 20. Overlay of 8 CE-SDS separations of a bispecific mAb under reducing conditions on the BioPhase 8800 system.

Table 3. Percentage of purity and total corrected peak area inter-capillary comparison on the BioPhase 8800 system.

Capillary	% Purity	Total corrected area
A	99.00	70536
B	98.94	67061
C	98.94	65531
D	98.87	65024
E	98.93	65769
F	98.98	69608
G	98.96	70238
H	98.94	67397

Linear dynamic range across multiple proteins at different concentrations on the BioPhase 8800 system

Three molecules were investigated, ranging from 0.375 to 3 mg/mL, to determine the linear response of the BioPhase 8800 system. Figure 21 shows the response between the total corrected peak area as a function of final protein concentration injected in the instrument. The R2 was > 0.99 for all 3 molecules, satisfying our requirements for linear response in the desired concentration range.

Figure 22 shows the consistency in the percentage purity obtained across the different protein concentrations for all 3 proteins demonstrating the consistency of the detector's response on the BioPhase 8800 system.

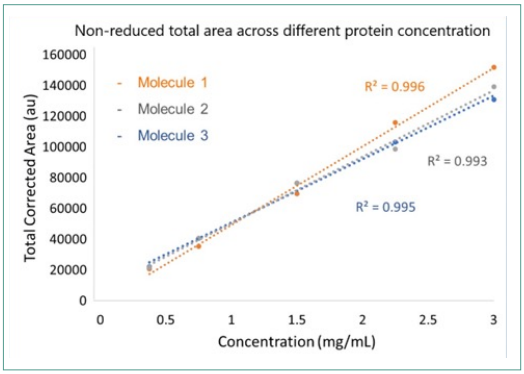


Figure 21. Linear curve of 3 molecules with different protein concentrations.

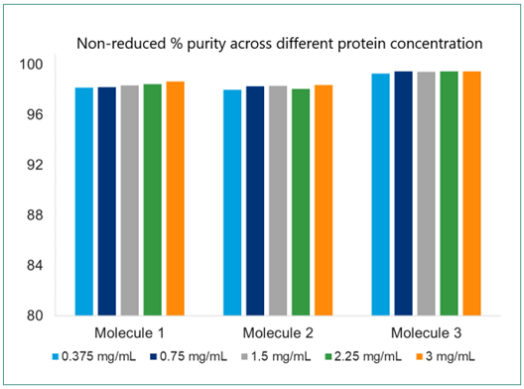


Figure 22. Consistency of percentage purity obtained for the 3 molecules studied at 5 different target concentrations.

Comparison of purity for various process development samples

The process development sample type is the most challenging sample type to determine the equivalency between PA 800 Plus system and the BioPhase 8800 system. Process development samples vary significantly depending on the excipients used, bioreactor conditions, and purification or formulation conditions.

Figure 6 shows a graph where the left Y-axis is the non-reduced percentage purity, and the right Y-axis is the difference in percentage purity of 21 process development samples. The green line shows the trend of the difference observed, and the red line across all graphs represents the passing threshold. The light blue bar is the data obtained using the BioPhase 8800 system, and the navy-blue bar is the PA 800 Plus system.

Most samples were observed to be within the passing threshold margin set between the two systems. However, samples 8 and 9 were deemed outliers due to the level of degradation observed.

Based on the data shown in Figure 23 it was determined that the BioPhase 8800 system performance is equivalent to the PA 800 Plus system.

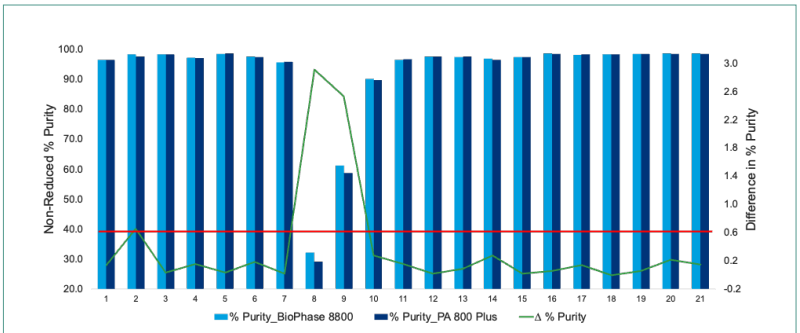


Figure 23. Twenty-One (21) Process development sample comparison between PA 800 Plus system and the BioPhase 8800 systems. Light blue bars represent the purity values observed using the BioPhase 8800 and the navy blue represents the values obtained using the PA 800 Plus system.

Case study 2 *continued*

Direct comparison between electrophoretic profiles

Figure 24 directly compares the PA 800 Plus and BioPhase 8800 systems. The data is a separation of a traditional mAb sample under non-reduced conditions. The electropherogram on the top shows the full view of the separation and circled is the difference in peak height between the IgG peaks on both platforms.

The peak height on the BioPhase 8800 system is considerably higher compared to the PA 800 Plus system. The difference in peak height and area is due to the innovative stray light control engineered into the BioPhase 8800 system, which increases the sensitivity of the system.

The zoomed-in view shows the overall peak shape for the smaller peaks to be comparable. Even though the total corrected area counts differ, the relative percentage and peak shapes are equivalent between the 2 instruments.

Similarly, Figure 25 shows the separation of the same molecule under reduced conditions. It is notable that the peaks are more intense on the BioPhase 8800 system compared to the PA 800 Plus system. Consequently, the ratio between the heavy and light chains differs between the 2 platforms, but the overall purities remained comparable. Thus, the results were determined to be equivalent.

Based on this extensive study, the BioPhase 8800 system was deemed a suitable alternative to PA 800 Plus system for early-phase CE-SDS testing.

To learn more about this case study please view this webinar.

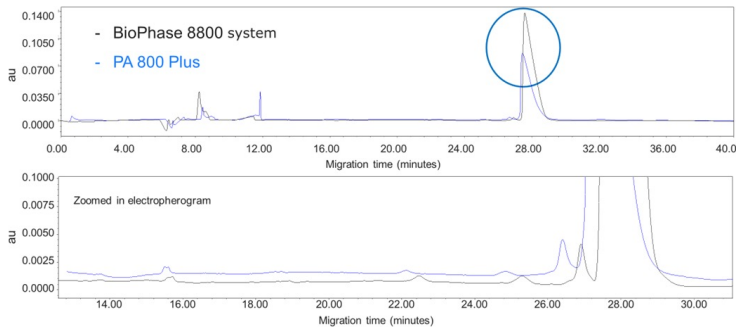


Figure 24. Direct comparison between PA 800 Plus and the BioPhase 8800 systems of a mAb sample under non-reduced conditions

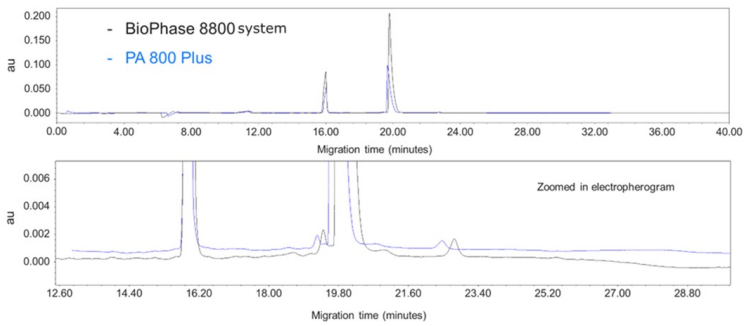


Figure 25. Direct comparison between PA 800 Plus and the BioPhase 8800 systems of a mAb sample under reduced conditions

Summary

The BioPhase 8800 system is a multi-capillary system designed for high-throughput CE analysis. It effectively meets the extensive testing requirements of product and process development, while maintaining quality of result and data equivalency.

This bridging study between the PA 800 Plus system and the BioPhase 8800 system demonstrated that the BioPhase 8800 system can consistently provide precise and equivalent results to PA 800 Plus system for both high-resolution and high-speed CE-SDS methods.

Furthermore, the BioPhase 8800 system comes with a compliant software package, enabling a smooth method transfer from early discoveries to QC labs.

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