

Multi-Capillary Gel Electrophoresis to Increase Throughput for the Purity Analysis of Biotherapeutic Proteins

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Antibody drug conjugate with four drug compounds linked to IgG immunoglobulin

Process Analytical Sciences (PAS), Analytical Development

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- 2 **Evaluating BioPhase 8800**
- 3 Early customer evaluation of full Empower control for the BioPhase 8800
- 4 **Final conclusions and future directions**





About Us



Quality driven, high throughput techniques are critical for the development of new drugs, meeting demanding timelines, and making quick informed decisions



Advancements in Technology for CE-SDS



- CE-SDS is frequently requested to determine the protein purity and fragmentation throughout development
 - >11,000 samples per year analyzed for early development CE-SDS testing
- To accommodate the increasing analytical demands, the BioPhase 8800 was evaluated as an alternative to the PA 800 Plus for early phase CE-SDS testing

PA 800 Plus

- Long running time could take multiple days to finish a run
 - 1 sample per hour
- Limited instrument availability due to workload
- Robust instrument with reproducible data

BioPhase 8800

- Parallel capillary format allows for 8 samples to be analyzed at once
 - Decrease in sample run time
 - Increase in instrument availability
- Reagents, capillaries, detection wavelength, and method of separation are the same as PA 800 Plus
- Automation friendly •









Agenda





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Evaluations of BioPhase 8800 for Early Development

Determining if the BioPhase 8800 could be used as an alternative to the PA 800 Plus for reduced and non-reduced CE-SDS samples

Compared traditional vs. lighting capillary conditioning

Capillary conditioning : 25 minutes (traditional)	Capillary conditioning : 10 minutes (lightning)
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

Assessed results across the parallel capillaries



Evaluated linearity across multiple protein concentrations



Assessed different modalities







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Similar Purities Across Different Molecules



BioPhase 8800 was determined to be equivalent comparing the lightning method vs traditional method as well as comparing to the PA 800 Plus

- Purity PA 800 Plus : Error bars show standard deviation for n=6 across multiple assays
- Purity BP Traditional: Error bars show standard deviation for n=6 within a single run
- Purity BP Lightning: Error bars show standard deviation for n=6 within a single run

Therapeutics Development & Supply (TDS)





Reproducibility Across Parallel Capillaries



• One molecule prepared across the 8 different capillaries provided reproducible results in terms of % purity and total corrected area



6 Purity	Total Corrected Area
99.00	70536
98.94	67061
98.94	65531
98.87	65024
98.93	65769
98.98	69608
98.96	70238
98.94	67397



Linearity with Different Protein Loads



Linearity and similar purities were observed for three different molecules prepared • at protein load concentrations 0.375 mg/mL to 3.000 mg/mL



Comparing Purities for Process Development Samples



- Different in-process samples were tested and compared (n=1) on the PA 800 Plus and BioPhase 8800 ۲
- Most samples were observed to be within 0.6% purity between BioPhase 8800 and PA 800 Plus ۲ – Observed a higher difference in % purity in degraded samples

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Similar Non-Reduced CE-SDS Profiles



- BioPhase 8800 has stray light correction whereas PA 800 Plus does not
- Similar relative area and peak shapes ٠



Similar Reduced CE-SDS Profiles



- Minutes Higher total area observed on BioPhase 8800 and different ratio of HC:LC between the instruments for the ٠ same molecule
 - BioPhase 8800 has stray light correction whereas PA 800 Plus does not _
- Similar relative area and peak shapes ٠



Summary of Results

- "Lighting" vs. "Traditional" conditioning

 Lighting method reduced cycle time by 40%
- Comparison of multiple modalities

 PA 800 Plus and BioPhase 8800 measured similar purity results
- Linearity
 - Observed linearity for the tested final protein concentrations ranging from 0.375 mg/mL to 3.000 mg/mL
- Reproducibility
 - Similar purities observed across parallel capillaries

Implemented testing on BioPhase 8800 as an alternative to PA 800 Plus for early phase CE-SDS testing in October 2022





Observations

- Higher total area observed on the BioPhase 8800
 Acceptance criteria adjusted to account for this difference
- Different ratio of HC:LC between the instruments
 - Differences in HC:LC ratio are not tracked at Janssen.
 - Acceptable based on similar purities

Lessons Learned

- If re-injection needed, new buffer rows must be made
- High humidity can cause condensation to form around the cartridge and on reagent plates, leading to current errors
- Steps in baseline occasionally occurred
 - Adjusted shutdown method to rinse in air and not in gel
 - Allowed the cartridge to be kept in the instrument until the end of its life

Collaboration with SCIEX was crucial to understanding differences and troubleshooting





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Early Customer Evaluation of the BioPhase Empower Driver

📱 Thunder in BioPhase\CE_SDS on PBED0448 as TMerlin1<u>/Superuser - Editing</u> SS Method: 10mgmL Sample Set Template - Run Samples

File Edit View Inject Actions Tools Customize BioPhase8800 Help





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Full Empower Control is Beneficial on the BioPhase 8800



- Empower is the preferred software to operate and analyze samples
 - Development version installed January 2023
- Enhancements to running in empower Ability for automation control - Same system used for PA 800 Plus • No disruption to the workflow • Running, analyzing, processing, and reporting done in
- Empower
 - Observe the run in real time from personal computer
- Provided feedback crucial to releasing commercial version
 - SCIEX optimized communication capabilities of the driver to better support 8 simultaneous separations Running Empower through CITRIX environment caused errors due to it being in a different location than the
- Empower database
 - SCIEX was able to improve installation and licensing of the Empower driver



Similar Profiles with Full Empower Control





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Final Conclusions and Future Directions





Future Directions

- Determine if the BioPhase 8800 will be considered for QC labs
 - Comparability studies
 - System suitability needs to be carefully designed
- Potential evaluation for different assays and modalities
 - Adeno-Associated Virus
 - Capillary Zone Electrophoresis
 - Glycan Analysis





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