The New Kid on the SCIEX Block:

An Assessment of the Higher-Throughput BioPhase 8800 Instrument for Purity and Fragment Analysis

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Presentation Summary

1. Background/Instrument Overview
2. Data Summary
3. Observations
4. Recommendations
5. Conclusion

Addressing Resource Demands

- Late-stage development places high resource demands for analytical support
  - Over a 5-month span, nearly 800 samples were tested to support one late-stage project
- Ideally the lot release methods should be used for process characterization to identify critical process parameters for the control strategy document
  - PA800 plus for fragment analysis is limited by throughput (~1 sample/hr) → the only way to increase throughput is increase instruments
  - Adds logistical problems of budget and space
  - Use of alternative higher throughput methods may result in data offsets and extra resource demands due to bridging exercises
- If you can't add more instruments, just **add more capillaries**!

BioPhase 8800 to the Rescue!

- "The SCIEX BioPhase 8800 system facilitates parallel processing of eight samples simultaneously, while retaining the capability to deliver sensitive CE-SDS, and cIEF analysis enabling uncompromised accuracy for large sample sets and faster time to answers." - https://sciex.com/products/capillary-electrophoresis/biophase-8800
- * The goal of our assessment is limited to monoclonal antibodies using our in-house platform CE-SDS method *
- The reagents, capillaries, and detection wavelength are the same as the PA 800 Plus
- All samples and buffers are prepared and tested in 96 well plates

- From August 2021 through July 2022, our department ran ~8000 samples on PA800 plus → nearly around the clock instrument use
- In an ideal world, the same testing on BioPhase would average to roughly 2 sequences per week!

Study Design

- Purpose: to understand instrument performance for assessing differences between the two platforms
  - ➢ Investigate any differences between capillaries or across the plate
  - ➢ BioPhase 8800 uses a UV beam which serves as the aperture (slightly larger than 200 um)
- Qualification-like studies were performed under reduced and non-reduced conditions to assess:
  - ➢ Repeatability
  - ➢ Linearity
  - ➢ LOD/LOQ

Columns

Capillaries	1	2	3	4	5	6	7	8	9	10	11	12
a	100	100	100	100	100	100	100	100	100	100	100	100
b	100	50	75	125	150	100	50	75	125	150	100	100
c	100	50	75	125	150	100	50	75	125	150	100	100
d	100	50	75	125	150	100	50	75	125	150	100	100
e	100	50	75	125	150	100	50	75	125	150	100	100
f	100	50	75	125	150	100	50	75	125	150	100	100
g	100	50	75	125	150	100	50	75	125	150	100	100
h	100	100	100	100	100	100	100	100	100	100	100	100

Orange: Non-reduced; Blue: Reduced

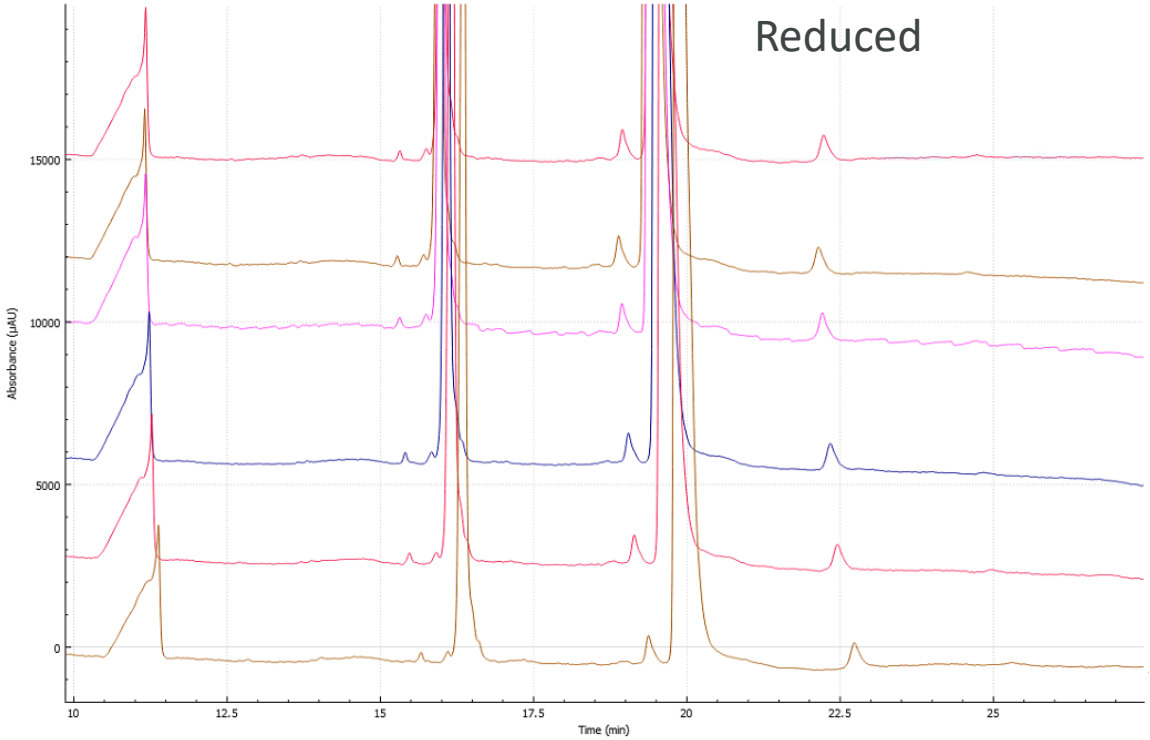
% of nominal concentration (1 mg/mL)

- Samples of each concentration were prepared in bulk → eliminates sample prep variations while assessing capillary and column differences
- Data is compared to PA800 plus method qualification results and historical Reference Standard data for each product

Molecule 1: Instrument E-gram Comparison

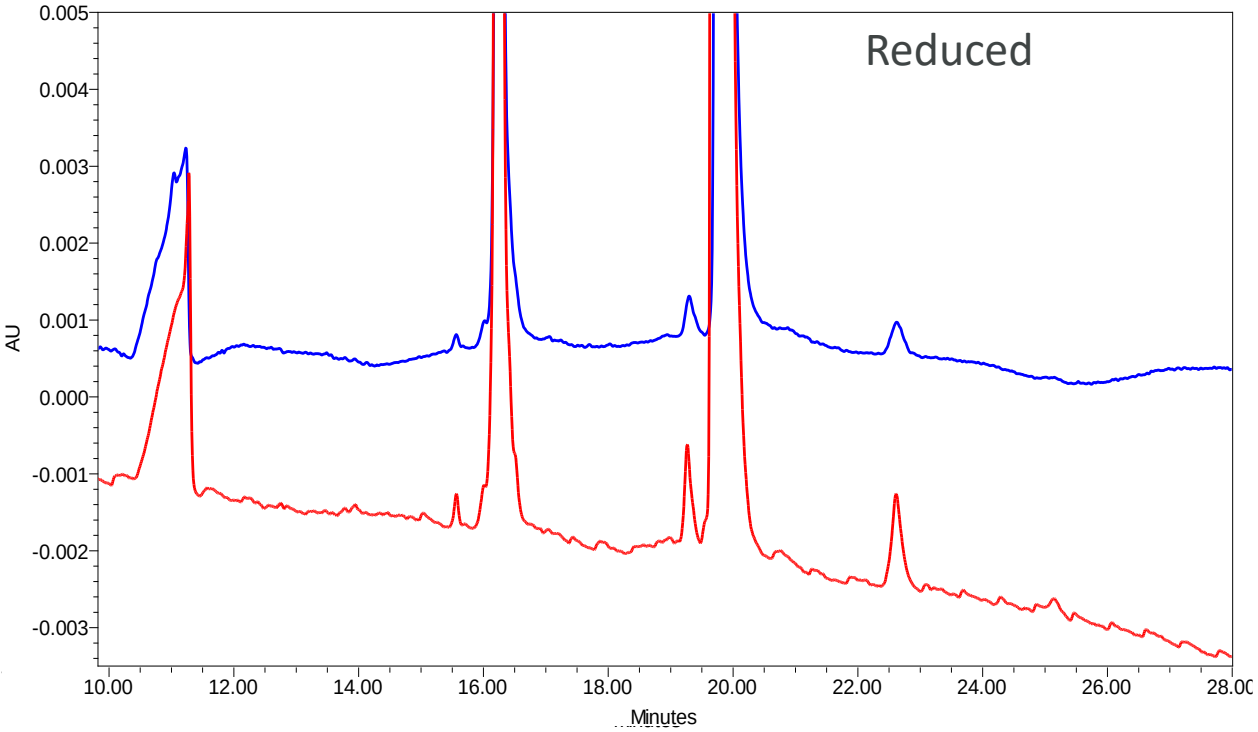
- Molecule 1 was chosen as it exhibits non-platform mAb fragmentation profiles

BioPhase 8800 was able to accurately and reproducibility generate consistent profiles



Electropherograms were comparable to PA800 plus

Blue: PA800 plus; Red: BioPhase 8800



Molecule 1: Method Qualification Summary

- Qualification data are consistent, and requirements are met across both instruments

Molecule 1	Parameter	BioPhase 8800	PA800 plus
Non-reduced	Repeatability (% CV)	0.4	0.2
	Linearity (R^2)	0.99 MPP TCA * 0.97 Impurities TCA	0.99 MPP TCA 0.99 Impurities TCA
Reduced	Repeatability (% CV)	0.1	0.1
	Linearity (R^2)	0.99 Purity TCA 0.99 Impurities TCA	0.99 Purity TCA 0.99 Impurities TCA

* Issue observed when first determining linearity (subsequent slide)

LOD and LOQ was calculated to be 0.1 and 0.3%. This is consistent with PA800 plus.

Plate Position Impacts Linearity Results

- Initial linearity calculations have R^2 = 0.97 for MMP and 0.90 for Impurities TCA, using all NR data points
  - ➢ Re-evaluation of 0.99 for MMP and 0.97 for Impurities, focused around area highlighted in red, rather than full 100% nominal load data

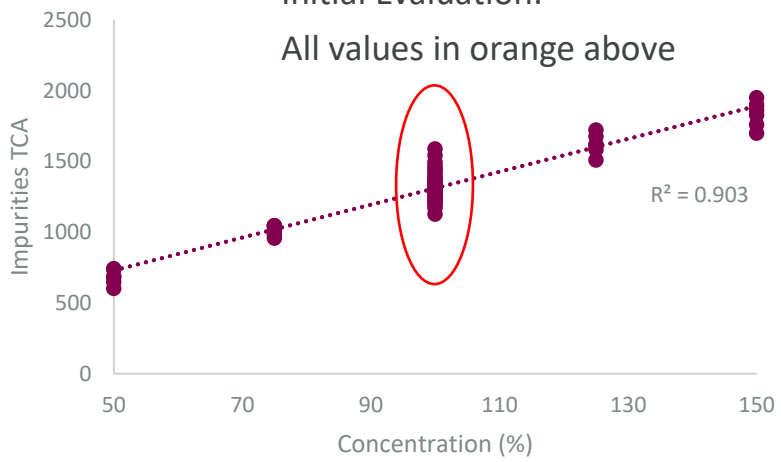
	1	2	3	4	5	6	7	8	9	10	11	12
a	100	100	100	100	100	100	100	100	100	100	100	100
b	100	50	75	125	150							100
c	100	50	75	125	150							100
d	100	50	75	125	150							100
e	100	50	75	125	150							100
f	100	50	75	125	150							100
g	100	50	75	125	150							100
h	100	100	100	100	100	100	100	100	100	100	100	100

Reduced samples

Where does the wide distribution of TCA come from?

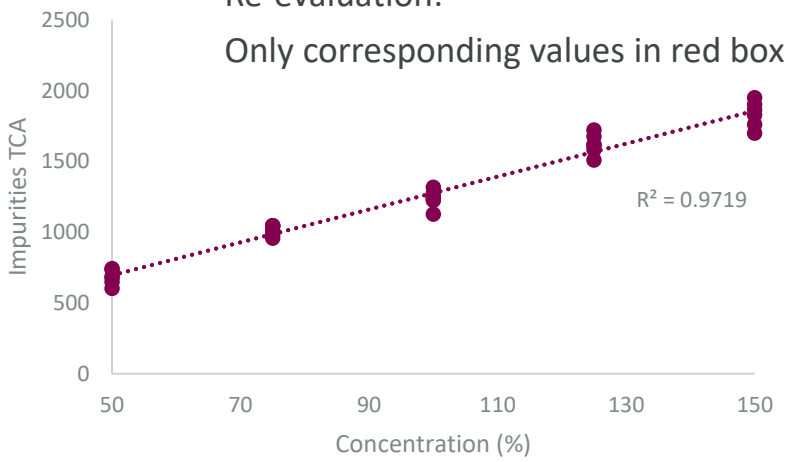
Initial Evaluation:

All values in orange above



Re-evaluation:

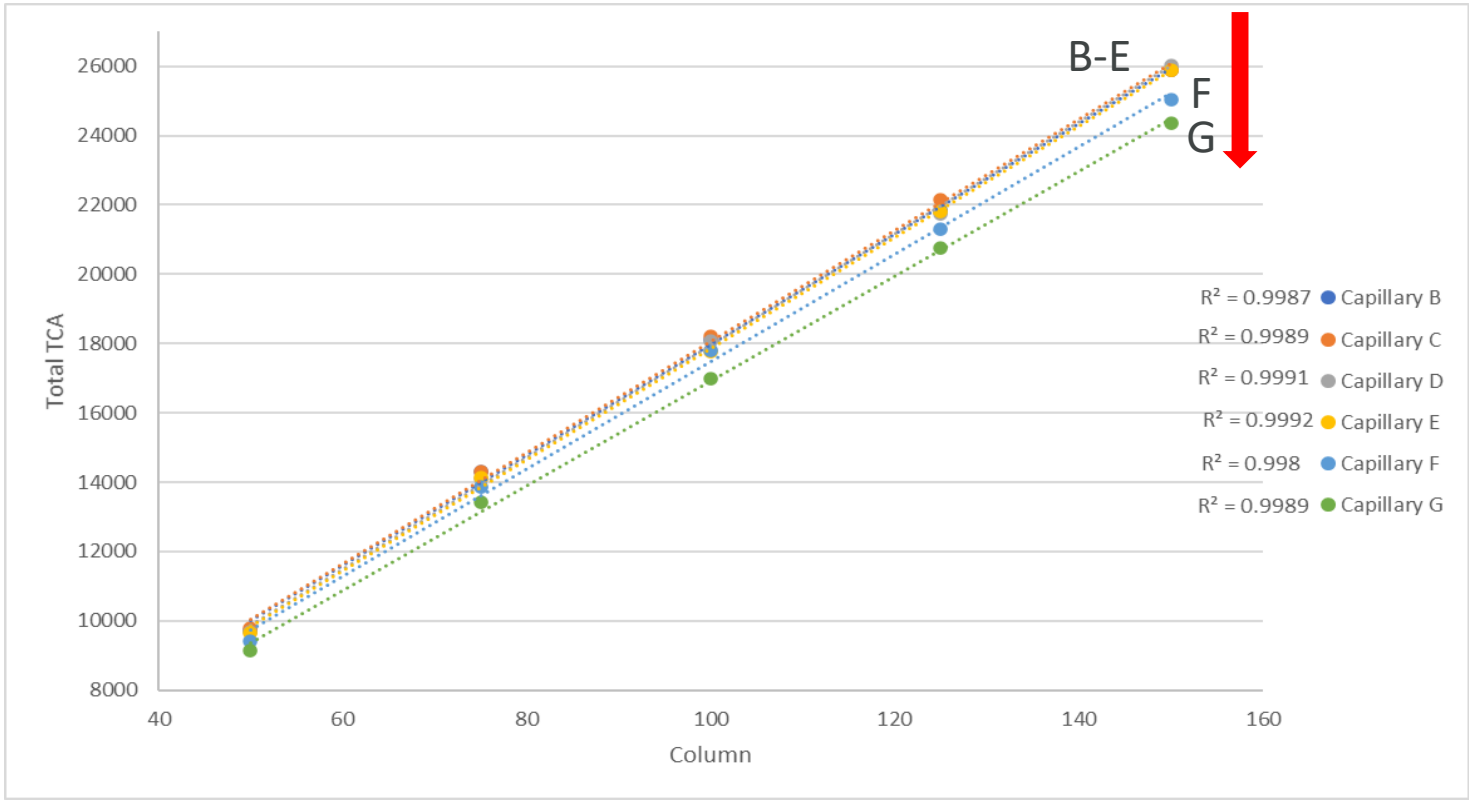
Only corresponding values in red box above



TCA Varies Depending on Capillary

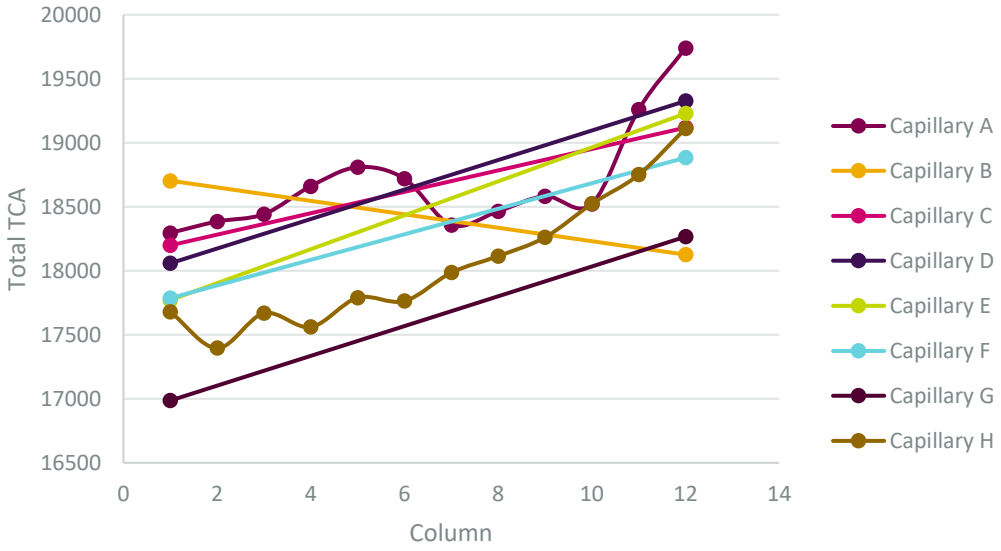
- Linearity of each individual capillary (except capillary C total impurities) was $R^2 > 0.99$
- There was a general trend of decreasing TCA moving down the capillaries

Capillary	Total TCA (50%)	Total TCA (100%)	Total TCA (150%)
B	9734	18127	25872
C	9798	18200	25897
D	9696	18059	26032
E	9652	17771	25877
F	9411	17788	25053
G	9136	16987	24367
% Difference B to G	7	7	6



TCA Varies Depending on Plate Position

- When looking at the border of full plate data the general trend is:
  - ➢ As you move down the plate (by capillaries), the area decreases
  - ➢ As you move across the plate (by columns), the area increases
  - ➢ Columns right next to each other show minimal change in TCA
- This does not affect overall % Purity results outside of expected variability of the molecule
- The effect was observed in other runs



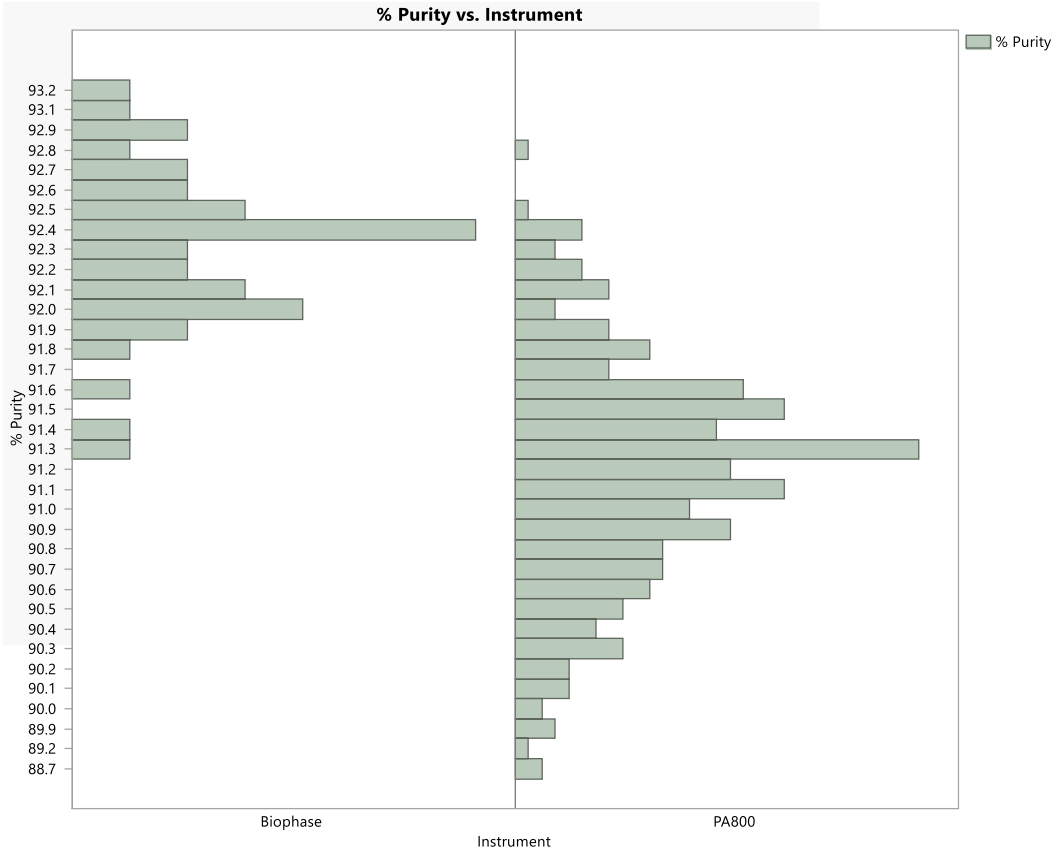
	Column 1	Column 12
Average	92.7	92.5
St. Dev	0.3	0.3
% CV	0.4	0.3

	Capillary A	Capillary H
Average	92.1	92.3
St. Dev	0.5	0.3
% CV	0.5	0.3

Offsets From Data Acquisition and Data Processing

- BioPhase 8800 acquired data was processed using both the BioPhase 8800 analysis software and Empower 3 and compared with historical PA800 plus acquired data (processed in Empower)
- Non-reduced differences:
  - BioPhase acquisition: BioPhase vs Empower processing software: 0.3% higher in BioPhase processed data
  - Empower processed: BioPhase vs historical PA800 acquired data: 0.8% higher in BioPhase acquired data
- Reduced differences:
  - BioPhase acquisition: BioPhase vs Empower processing software: 0.1% higher in Empower processed data
  - Empower processed: BioPhase vs historical PA800 acquired data: 0.6% higher in BioPhase acquired data

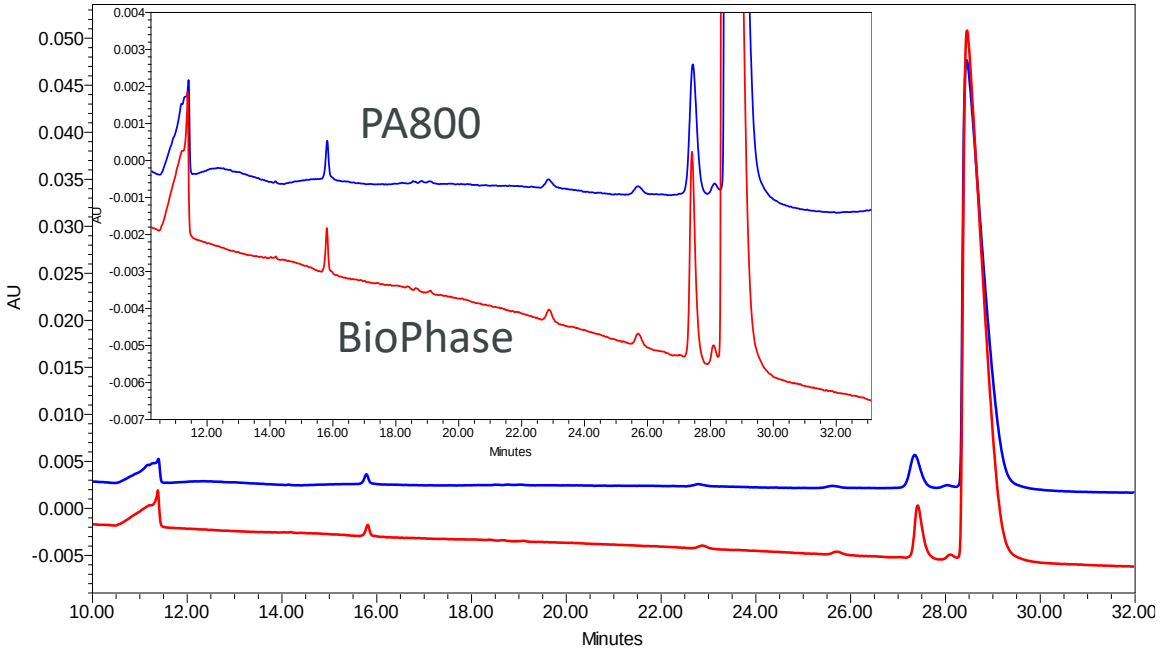
Non-reduced Data Summary



Historical PA800 plus data is more variable than BioPhase data, but unclear at this point whether it is true method variability or limited BioPhase data

Molecule 2 Summary

- Molecule 2 was chosen as it is prone to partial reduction and artificial fragmentation
- Minimal offset in %Purity was observed between the two instruments for both non-reduced and reduced analysis (most likely due to fewer fragments)
- Same observations for reduced data (not shown)



Parameter	BioPhase 8800	PA800 plus
Repeatability (% CV)	0.1	0.1
Linearity (R^2)	0.99 Purity TCA 0.99 Impurities TCA	0.99 Total TCA 0.99 Impurities TCA

	BioPhase 8800	PA800 plus
Average	93.7	93.5
St. Dev	0.1	0.1
% CV	0.1	0.1

Reproducibility of Results

- Both molecules were tested under non-reduced and reduced conditions to verify initial results
- Data trends are consistent with initial evaluation:
  - BioPhase trends slightly higher than PA800 plus for molecules with higher fragment
  - BioPhase variability is generally the same or less than PA800 plus

Molecule 1

Non-reduced	BioPhase 8800 Verification	PA800 plus historical data
Average	91.7	91.2
St. Dev	0.4	0.6
% CV	0.5	0.7

Reduced	BioPhase 8800 Verification	PA800 plus historical data
Average	97.2	97.3
St. Dev	0.5	0.3
% CV	0.5	0.3

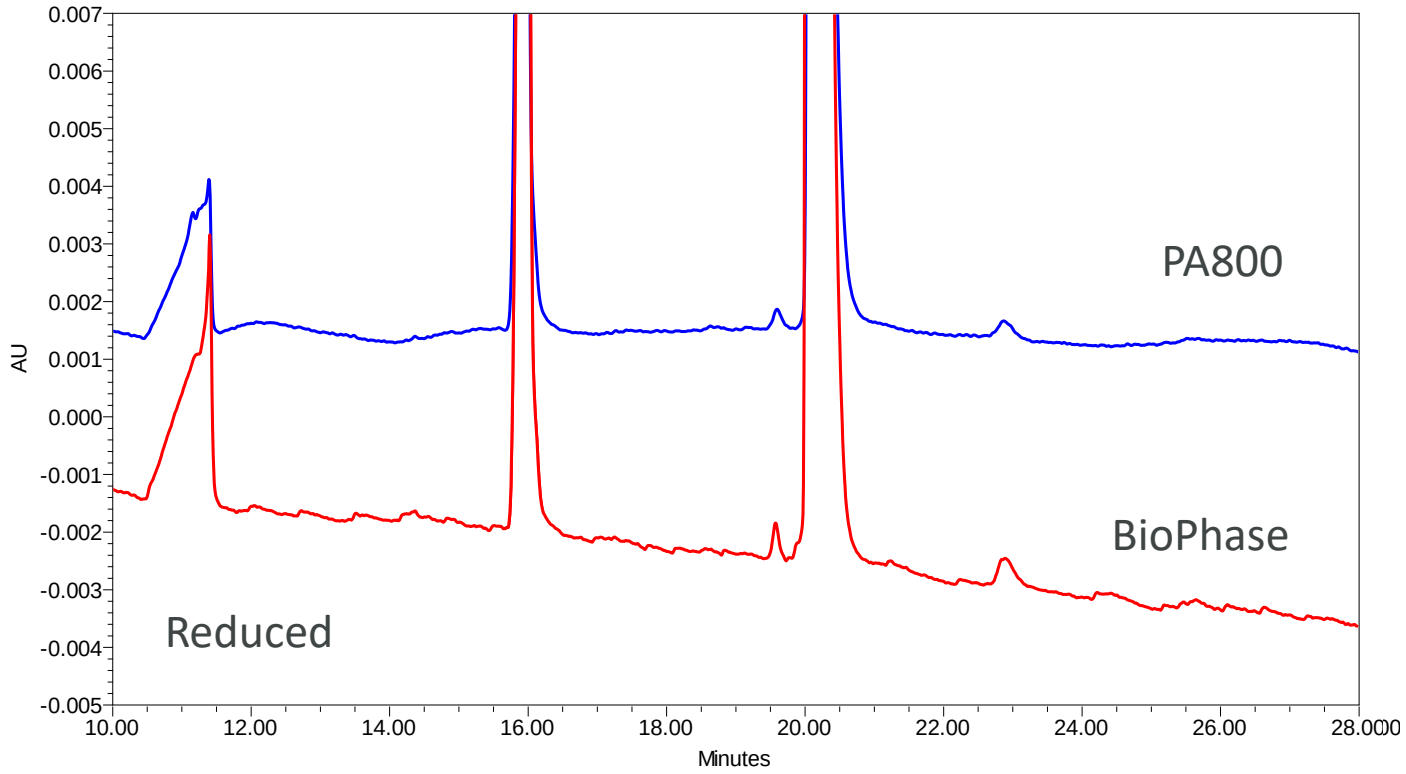
Molecule 2

Non-reduced	BioPhase 8800 Verification	PA800 plus historical data
Average	94.0	93.6
St. Dev	0.1	0.6
% CV	0.1	0.6

Reduced	BioPhase 8800 Verification	PA800 plus historical data
Average	99.4	99.3
St. Dev	0.1	0.3
% CV	0.1	0.3

Platform Separation Profiles Confirm Similar Instrument Performance

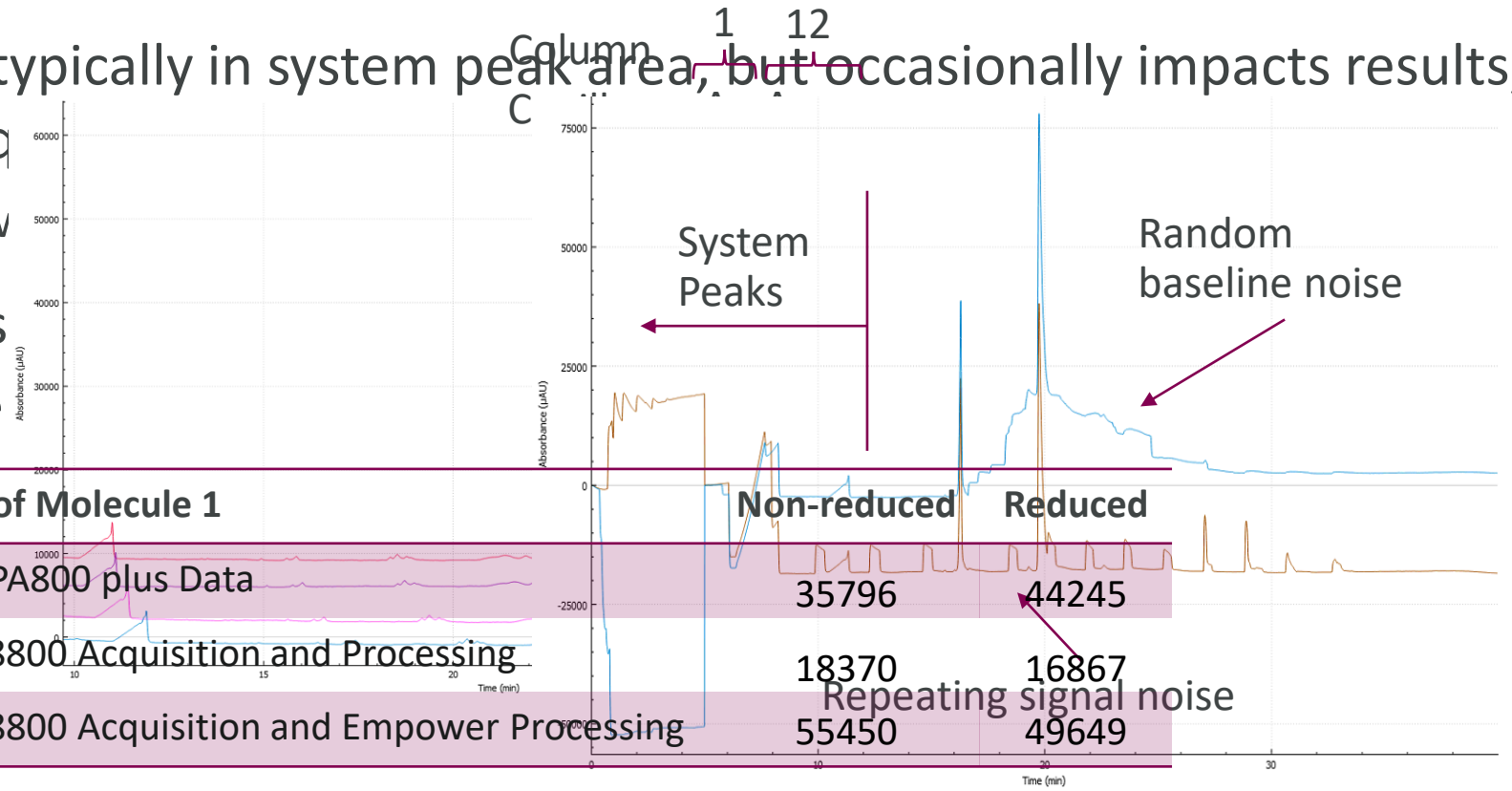
- A 3rd molecule with low impurity levels was tested under non-reduced and reduced conditions
- All data is consistent with historical PA800 plus data



Non-reduced reduced	BioPhase 8800 Verification	PA800 plus historical data
Average	97.8	97.5
St. Dev	0.1	0.2
% CV	0.1	0.2

General Observations

- Drift – seen in PA800 but each capillary in BioPhase drifts at different rates
  - ➢ Capillary A shows ~1 min drift; Capillary H shows >2.3 min drift
  - ➢ Does not impact purity results
- Noise – random, typically in system peak area, but occasionally impacts results, but will go away in subseq
  - ➢ Log files shared v
- TCA from Biophas
  - ➢ Cannot compare



Total TCA of Molecule 1	Non-reduced	Reduced
Historical PA800 plus Data	35796	44245
BioPhase 8800 Acquisition and Processing	18370	16867
BioPhase 8800 Acquisition and Empower Processing	55450	49649

Lessons Learned for Success

- Ensuring correct methods:
  - Application of pressure to inlet and outlet is required
  - After shutdown, capillaries must be in water
- Plate spinning is required – bubbles cause the capillaries to break
  - When capillaries break, the gel buffer gets into the coolant and needs to be drained
- Sample order matters!
  - Molecule 2 is prone to partial reduction – when running column 12 (non-reduced) after column 11 (reduced), the purity was on average 0.8% lower

Overall Biophase Impressions

- Pros
  - Sequence setup and sample testing steps are clear and intuitive
  - Minimal cleaning and maintenance required
  - Being able to import into Empower fits into our workflow
- Cons
  - Steep learning curve for using new BioPhase analysis tool
  - 96-well plate used has narrow wells and difficult to see bubbles
    - Air bubble in solution may induce capillary breakage, making the whole cartridge unusable
  - Unable to simply retest on the fly, like PA800 plus
- Per analyst feedback, Biophase sequences are time saving when there are more than 30 samples needing manual preparation

Conclusions

- BioPhase 8800 delivers quality results consistent with PA800 plus
  - No qualitative differences observed between both instruments
  - Slight purity offset between both instruments – increased when higher impurities were present
- The Biophase 8800 would fit into our development workflow
  - Cell line development, upstream and downstream development, and late-stage characterization work can be facilitated and expedited
  - Same day or next day turn-around of multiple samples
  - Adding automation to sample and buffer try prep would speed up the process and reduce possible sample error
- Overall: BioPhase 8800 fits nicely into development activities, but release methods will continue to be on PA800 plus

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