BioPhase 8800 system: Advancing capillary electrophoresis with native fluorescence detection

SCIEX CE team, 2025

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SCIEX
The power of precision

BioPhase 8800 system with NF

Year : **2025**

TAKE *CHARGE*

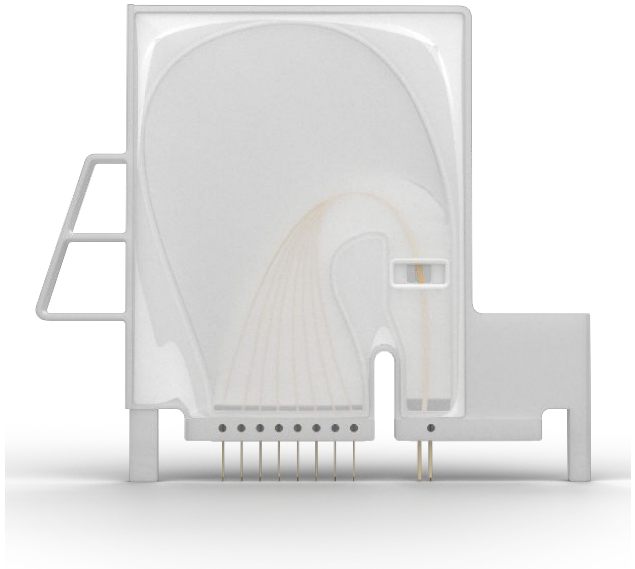
Maintain all existing capabilities on the BioPhase 8800 system.
Benefit from the addition of native fluorescence detection:

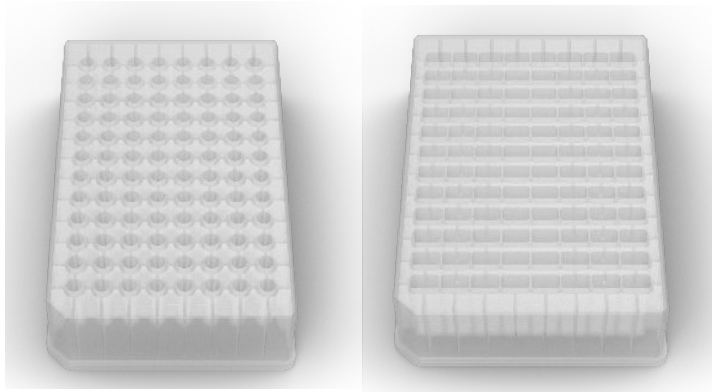
1. ***Protein detection with improved sensitivity compared to traditional UV detection.***
2. ***Faster, more confident peak integration than traditional UV due to significantly reduced noise and flattened baseline.***
3. ***No need for protein-dye conjugation during sample preparation, speeding up sample preparation.***

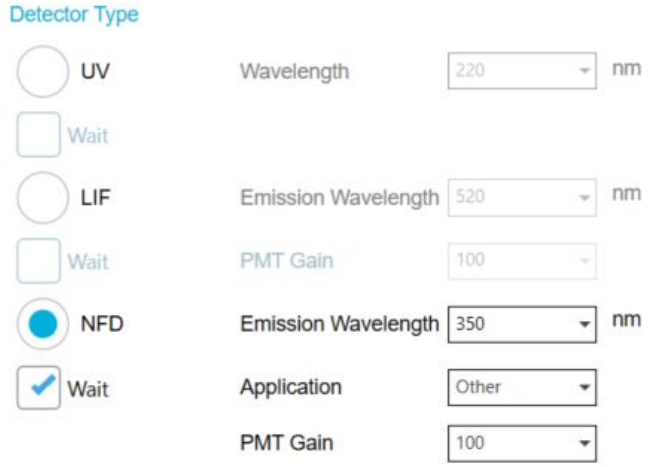
SCIEX

Features - hardware

- Factory-built cartridge with push-in load: Reduce user error
- 8 capillaries parallel processing: Speed up your sample analysis
- Built-in electrodes, interface block, and aperture: Reduce maintenance
- Smart chip with SN, usage tracking: Improve traceability
- Precise temperature capillary control: Maximize data reproducibility
- Plate-based sample/reagent: Automate processes
- Software-driven detector selection: Simplify detector changes and enable a single sequence with multiple detector types
- Reduced stray light and higher transmission: Gain higher signal intensity







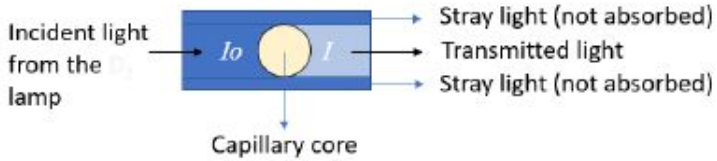


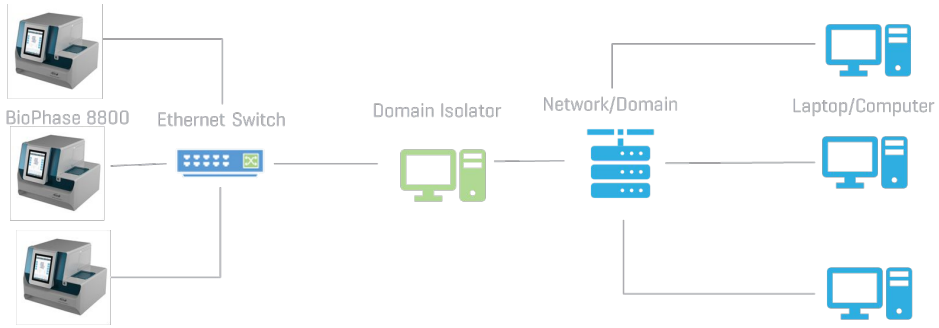
Figure 3. Schematic diagram of the stray light.

Features - software

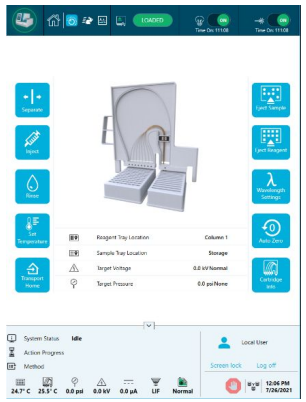
The user can choose to set up either in a local or a network configuration

Workstation computer
- Project Management SW
- BioPhase Standalone SW
  - Meth. Dev. / Data Analysis
- Log extractor

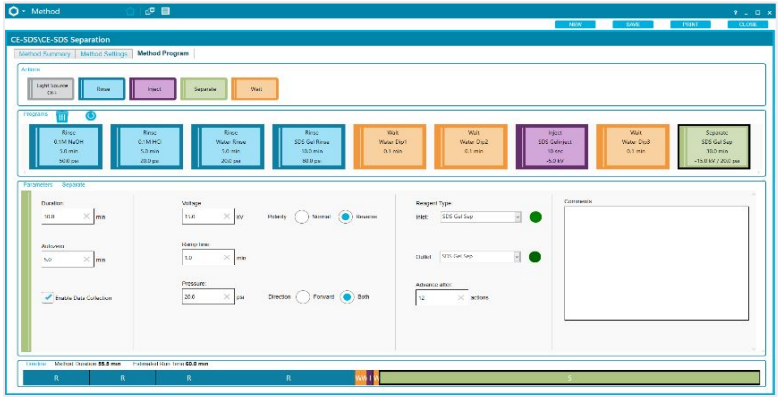
Instrument
- Instrument Configuration
  - PC Ethernet Address
- Login



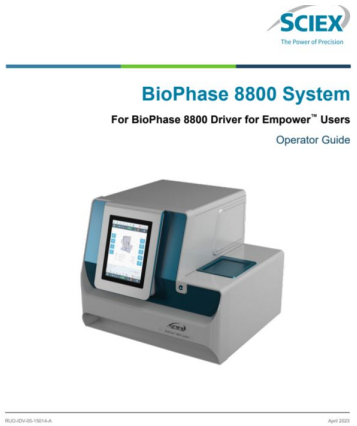
Intuitive touchscreen interface



Drag and drop method programming



BioPhase 8800 Driver for Empower software & documentation + OQs



Native fluorescence of proteins: A new way of detection

The native fluorescence of proteins originates from residues of three aromatic amino acids: **tryptophan** , tyrosine, and phenylalanine

Tryptophan : $\lambda_{ex}/\lambda_{em}$=280/350 nm

-- High emission intensity, the primary native fluorescence source in proteins

Tyrosine : $\lambda_{ex}/\lambda_{em}$=204/304 nm & $\lambda_{ex}/\lambda_{em}$=220/304 nm

-- High emission intensity, but quenched by its interaction with the peptide chain via energy transfer

Phenylalanine : $\lambda_{ex}/\lambda_{em}$=210/280 nm

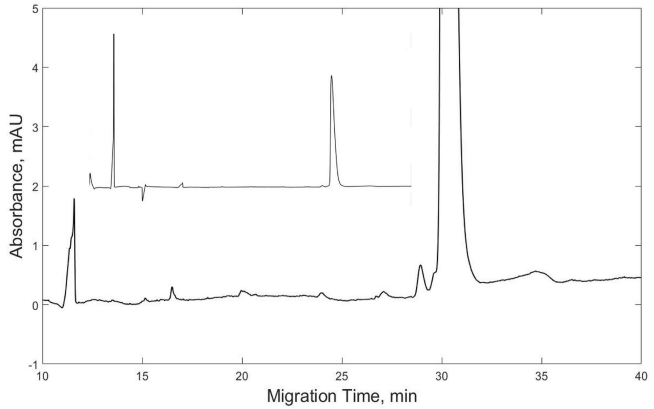
-- Low emission intensity

No Need to rely on low-wavelength amide bond or fluorescent tag labeling for protein detection

CE-SDS analysis for proteins:

BioPhase 8800 system with NF: non-reduced CE-SDS

Traditional UV 220 nm detection

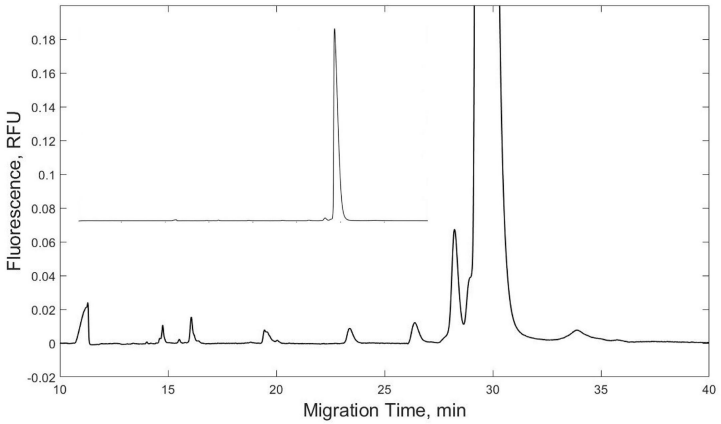


Protein labeling is not needed

Requires high sample concentration

NEW!

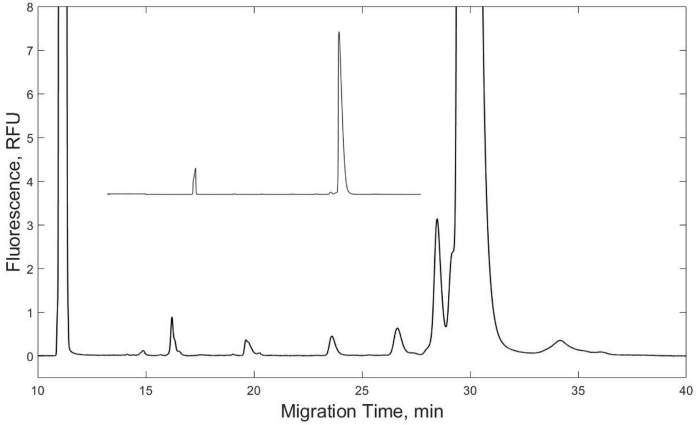
NF 350 nm detection



Protein labeling is not needed

Improved S/N ratio & flat baseline
compared to UV detection

LIF 600 nm detection



LIF dye conjugation to protein is **required**

Improved S/N ratio > 10X & flat baseline
compared to UV detection

Non-reduced CE-SDS

Sample: 1 mg/mL [1 mg/mL] non-reduced NIST IgG

Content is reprinted from the published manuscript Electrophoresis, https://doi.org/10.1002/elps.8154

BioPhase 8800 system with NF: non-reduced purity for mAb

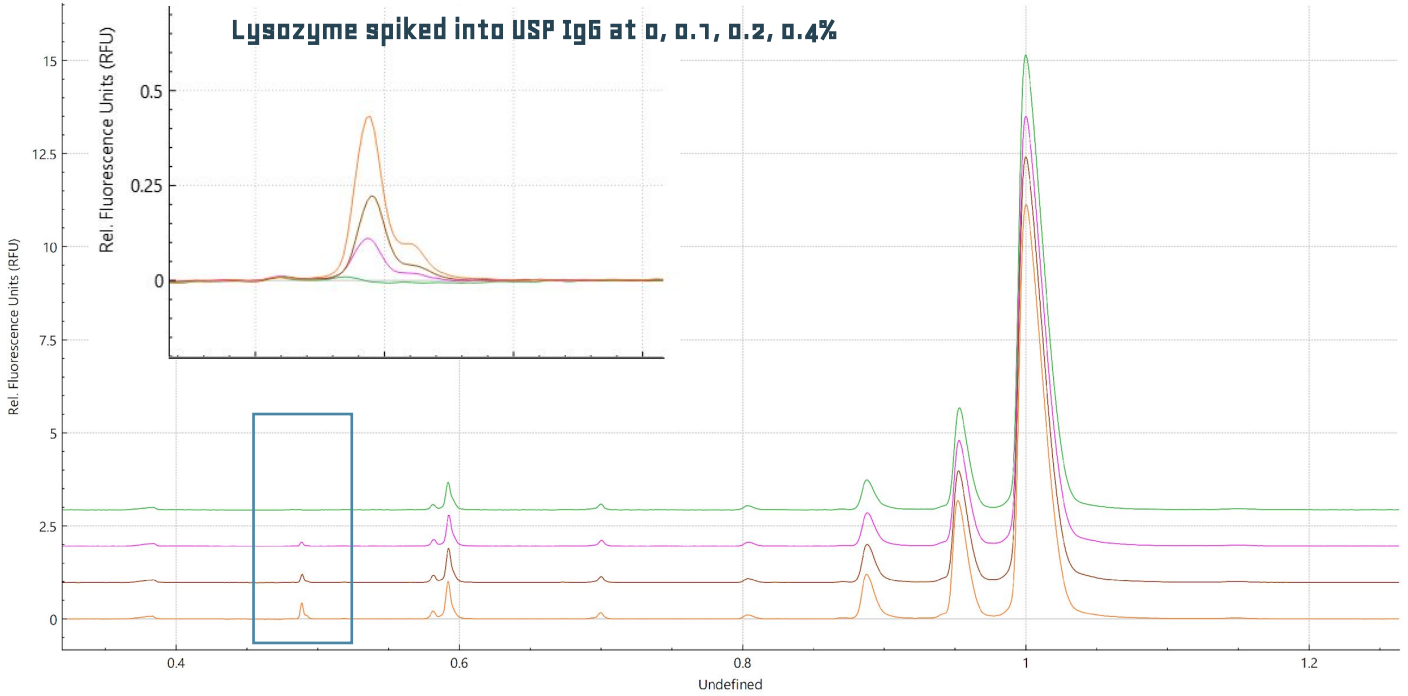
- The measured relative abundance of total fragments was comparable among the three detection approaches
- The measured relative abundance of aggregates with NFD is equivalent to that with LIF
- The aggregate peaks were not detectable with UV due to interference by the baseline anomalies

Sample Lot	Detection	Relative Abundance, %							
		L	H	HL	HH	HHL	Total	Intact mAb	Aggregates
1	UV	0.29	0.13	0.15	0.18	0.78	1.53	98.48	ND*
	NFD	0.24	0.14	0.15	0.24	1.00	1.77	97.84	0.40
	LIF	0.18	0.15	0.15	0.22	0.92	1.63	97.97	0.40
2	UV	0.29	0.14	0.11	0.17	1.07	1.78	98.22	ND*
	NFD	0.22	0.17	0.14	0.21	1.26	2.00	97.62	0.38
	LIF	0.24	0.14	0.14	0.25	1.09	1.86	97.77	0.37
3	UV	0.28	0.19	0.13	0.18	0.96	1.73	98.27	ND*
	NFD	0.21	0.20	0.15	0.22	1.11	1.88	97.74	0.38
	LIF	0.24	0.14	0.16	0.26	1.06	1.85	97.80	0.35

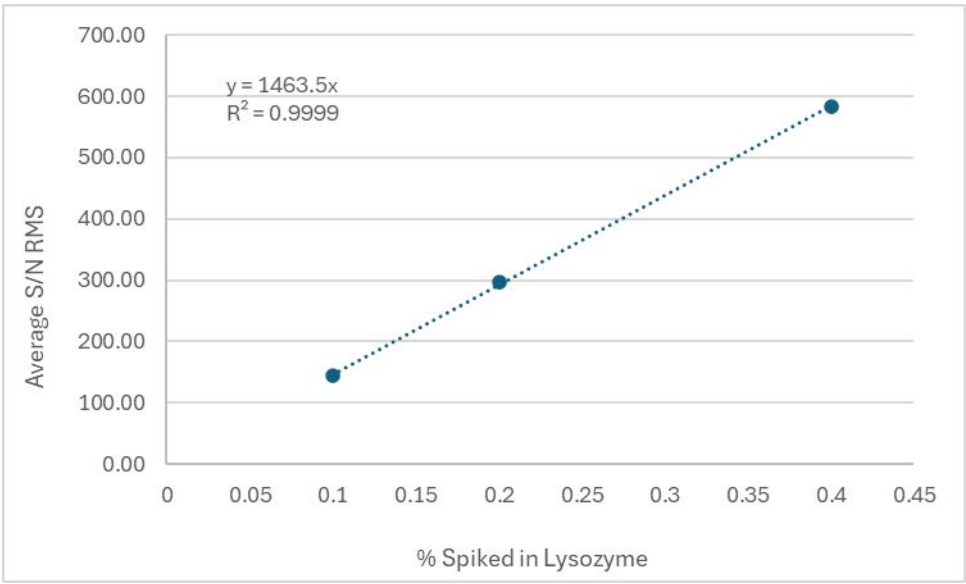
**ND – not detected. With UV absorbance detection, the aggregate peaks were not detected due to interference by the baseline anomalies.*

Content is reprinted from the published manuscript Electrophoresis, https://doi.org/10.1002/elps.8154

BioPhase 8800 system with NF: Quantitation of 0.01% spiked in lysozyme



NF 350 nm

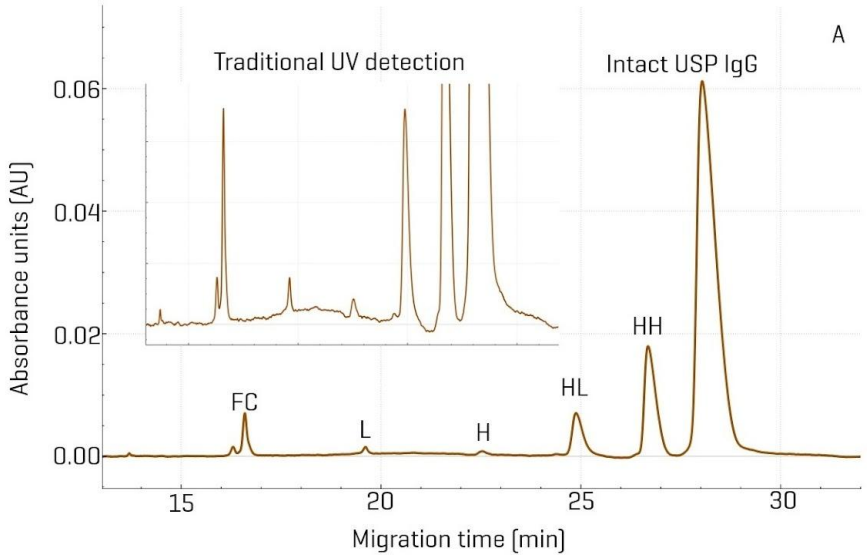


Sample: non-reduced USP IgG at 1 mg/mL.

- NFD 350 nm: S/N RMS at 0.1% spike in > 1000, linear extrapolation S/N RMS at 0.01% spike in > 10.
- UV 220 nm: S/N RMS at 0.1% spike in ~ 20

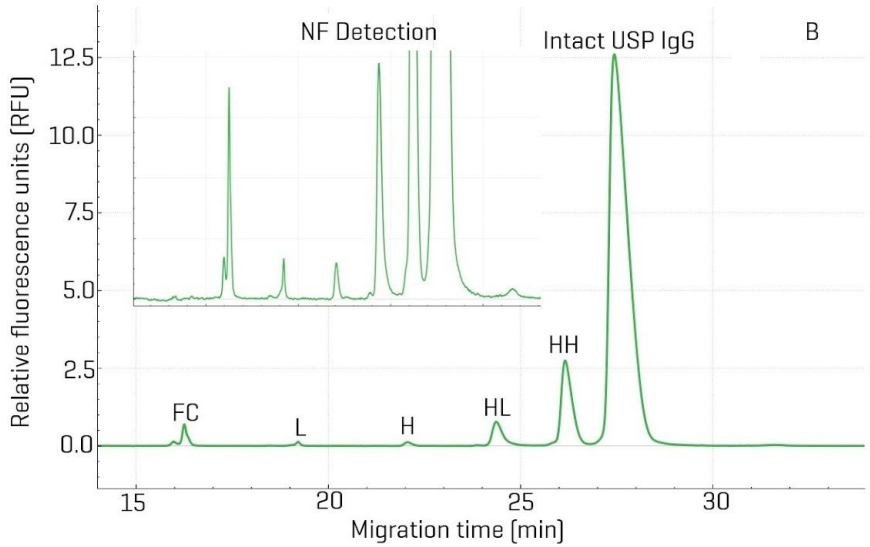
BioPhase 8800 system with NF: Quantification of protein fragments

Traditional UV detection: USP IgG



NF detection USP IgG

NEW!

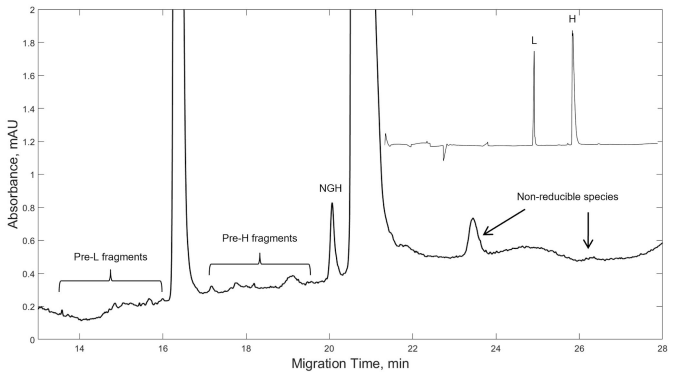


C

	UV detection		NF detection	
	CPA %	Average S/N RMS	CPA %	Average S/N RMS
USP IgG-HC	0.53	78	0.44	185
NIST mAb- HL	0.25	20	0.19	76
NIST mAb-HH	0.22	19	0.21	83

BioPhase 8800 system with NF: reduced CE-SDS

Traditional UV 220 nm detection

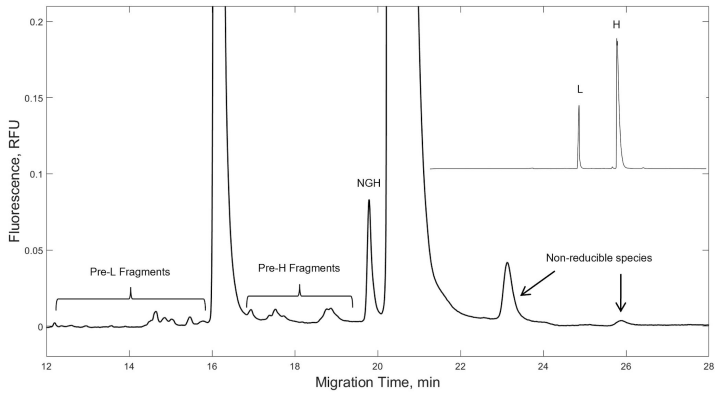


Protein labeling is not needed

Requires high sample concentration

NEW!

NFD 350 nm detection



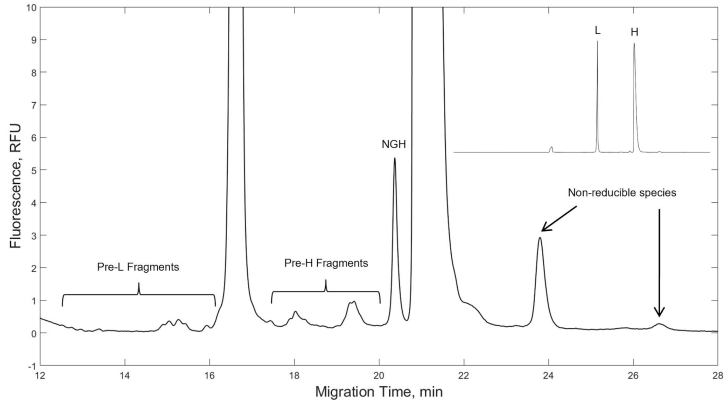
Protein labeling is not needed

and

Improved sensitivity & flat baseline

compared to UV detection

LIF-600 nm detection



LIF dye conjugation to protein is **required**

Improved sensitivity >10X & flat baseline

compared to UV detection

Reduced CE-SDS

Sample: 1000 µg/mL [1 mg/mL] reduced NIST IgG

Content is reprinted from the published manuscript Electrophoresis, https://doi.org/10.1002/elps.8154

BioPhase 8800 system with NF: glycan occupancy

Sample Lot	Glycosylation Site Occupancy on Heavy Chain, %		
	UV Absorbance	NFD	LIF
1	99.30	99.33	99.33
2	99.31	99.32	99.32
3	99.31	99.33	99.33

The determined glycosylation site occupancy was consistent, in the range of 99.30—99.33%, using the three detection approaches.

NIST mAb

Glycosylation site occupancy = 1- NGH/H %

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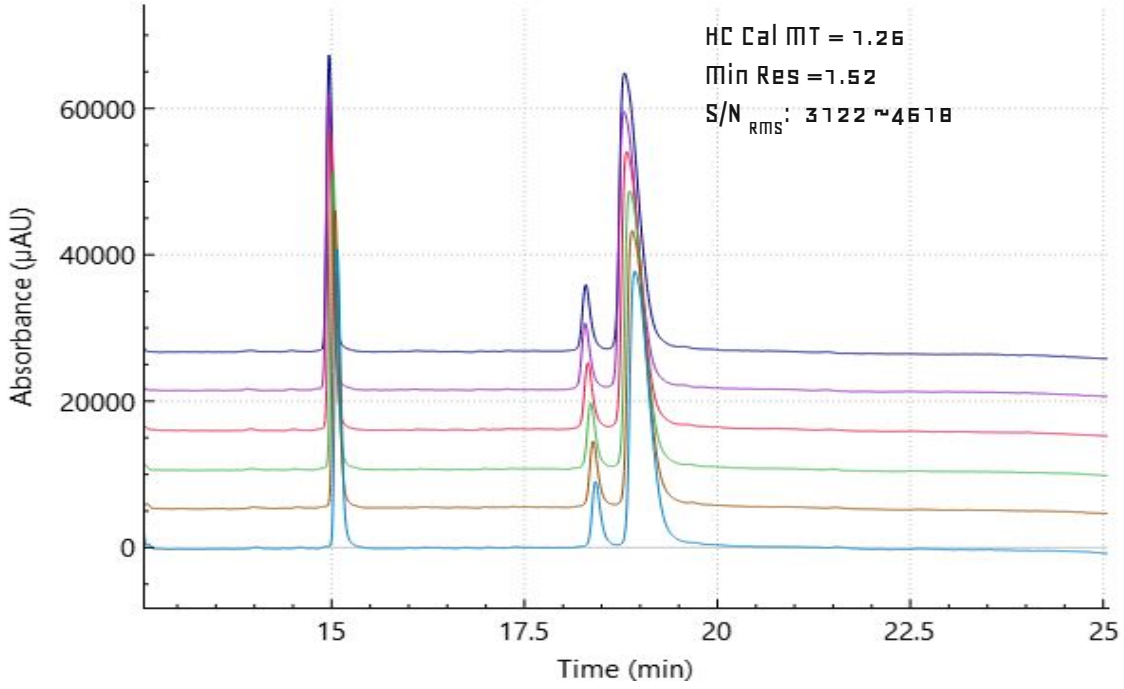
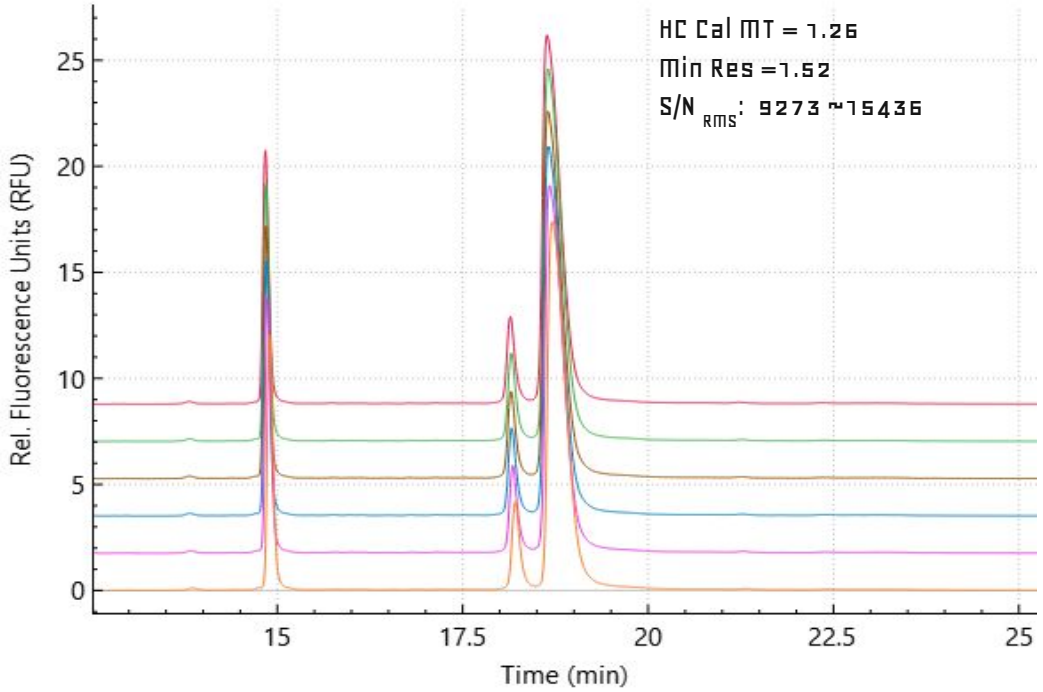
BioPhase 8800 system with NF: Consistency and repeatability

Traditional UV detection: IgG control



NF detection IgG Control

NEW!



Comparable profiles observed with the same level of repeatability

BioPhase 8800 system with NF: Consistency and repeatability

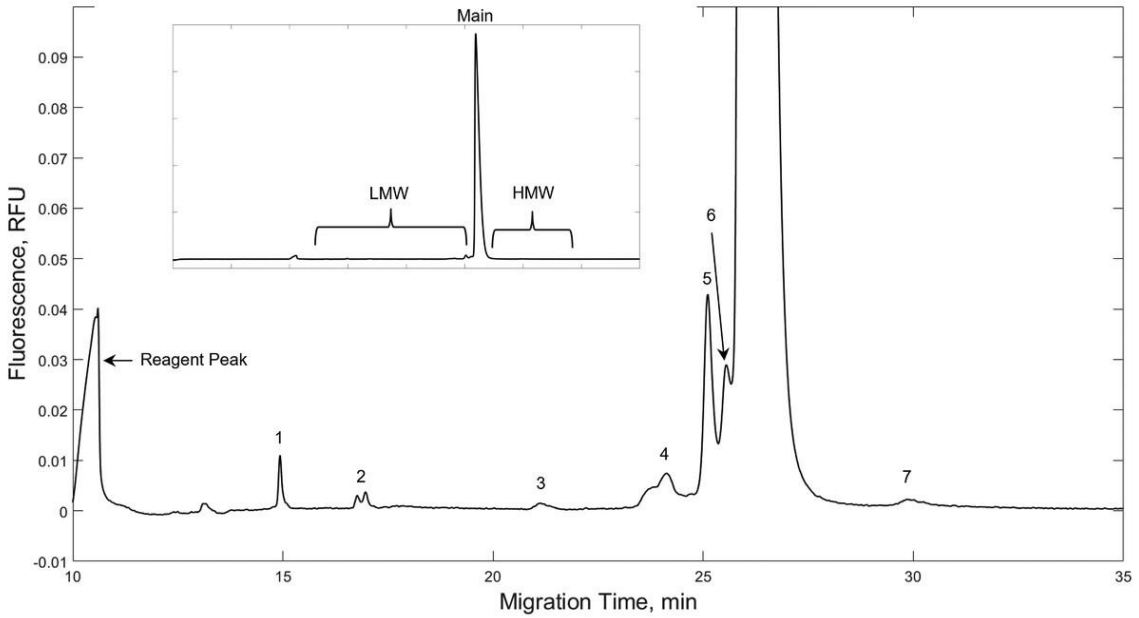
Intra-Capillary	(LC+HC)%- UV		(LC+HC)%- NFD		HC/(HGH_HC)% -UV		HC/(HGH_HC)% -NFD	
	Average	**% RSD**	**Average**	**% RSD**	**Average**	**% RSD**	**Average**	**% RSD**
A	92.55	0.07	91.63	0.04	89.86	0.11	89.77	0.04
B	92.50	0.05	91.63	0.03	89.79	0.07	89.76	0.02
C	92.50	0.06	91.63	0.01	89.80	0.07	89.75	0.02
D	92.52	0.04	91.63	0.02	89.80	0.06	89.76	0.02
E	92.56	0.06	91.62	0.01	89.84	0.10	89.74	0.02
F	92.51	0.04	91.62	0.03	89.79	0.06	89.75	0.03
G	92.53	0.07	91.61	0.03	89.84	0.11	89.73	0.03
H	92.52	0.07	91.64	0.05	89.81	0.11	89.76	0.06
Inter-capillary N = 48	***92.52***	***0.06***	***91.63***	***0.03***	***89.82***	***0.09***	***89.75***	***0.03***

Comparable sample purity with the same level of repeatability

Content is reprinted from the published manuscript Electrophoresis, https://doi.org/10.1002/elps.8154

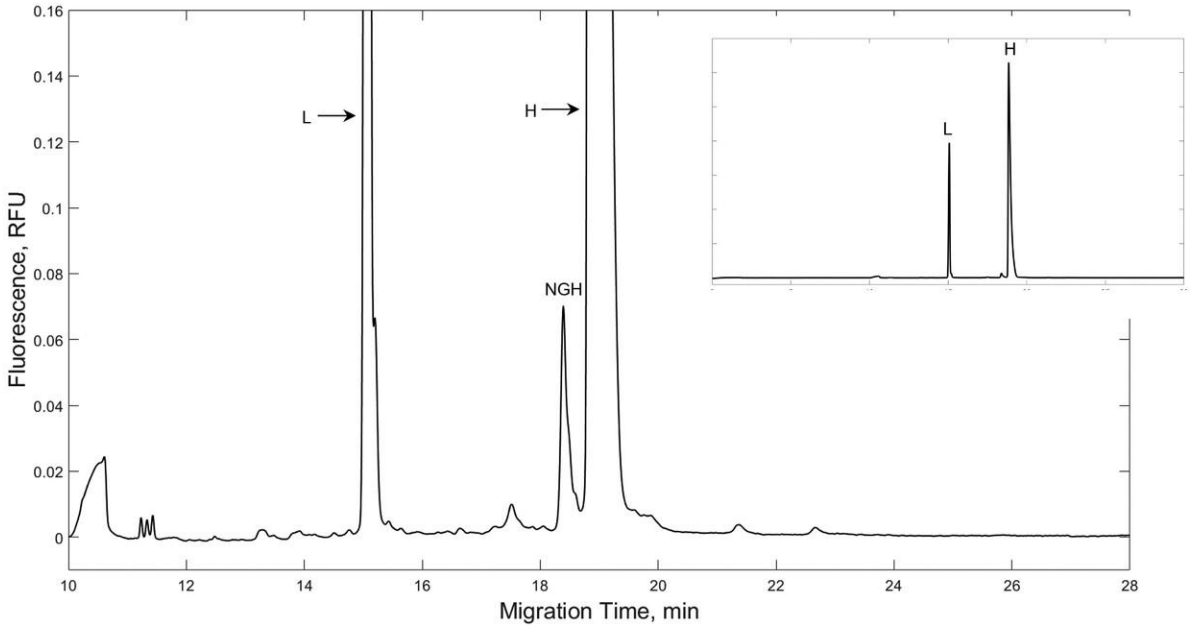
BioPhase 8800 system with NF: Emicizumab analyzed with CE-SDS

Non-reduced



LMW	Main	HMW
3.02%	96.85%	0.13%

Reduced



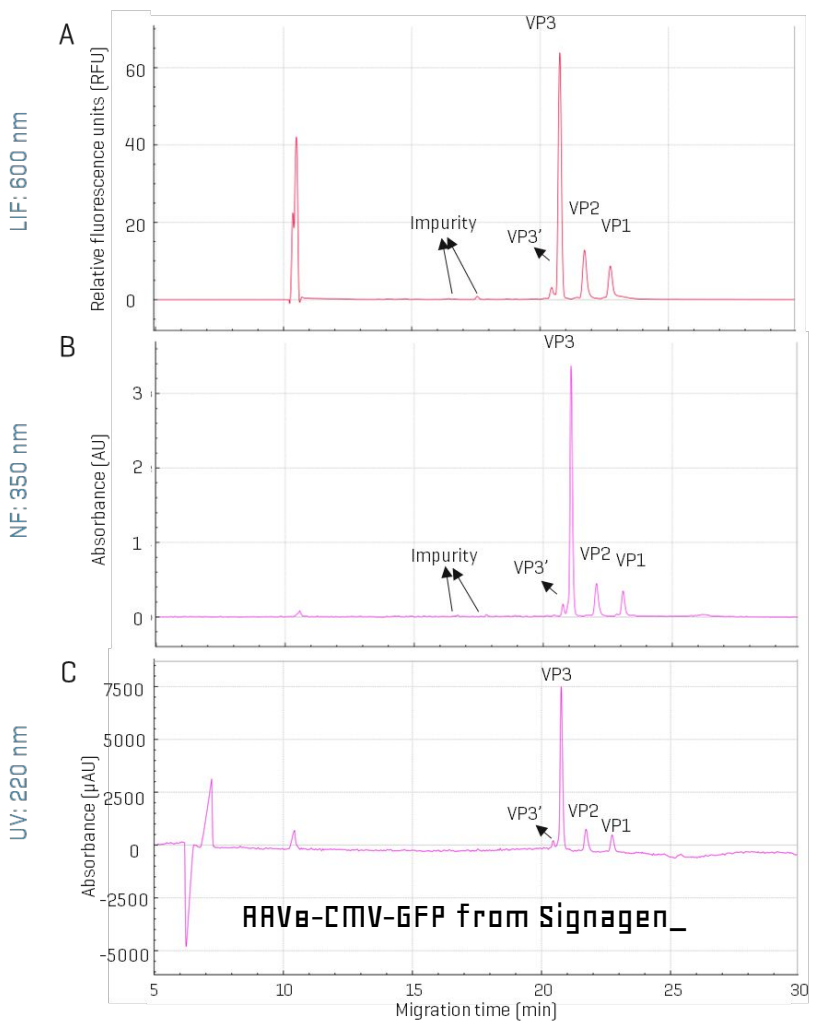
Glycosylation site occupancy: 98.01%

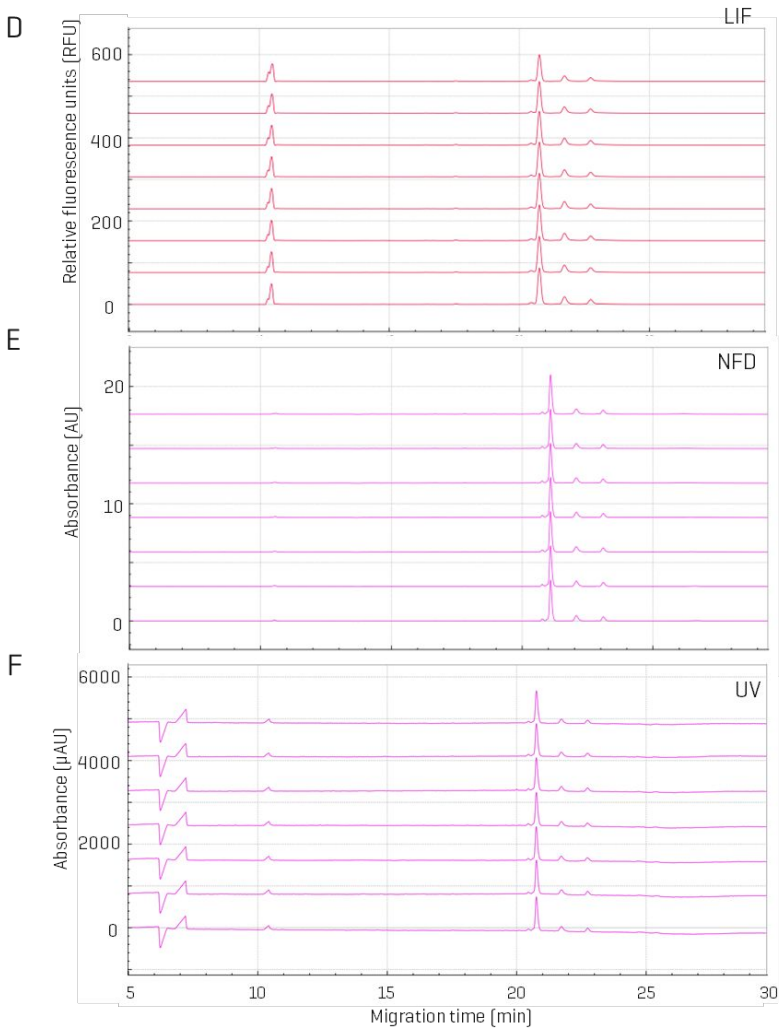
Sample: 0.5 mg/mL Emicizumab, Injection: 20 s @-5 kV

Content is reprinted from the published manuscript Electrophoresis, https://doi.org/10.1002/elps.8154

AAV capsid protein analysis

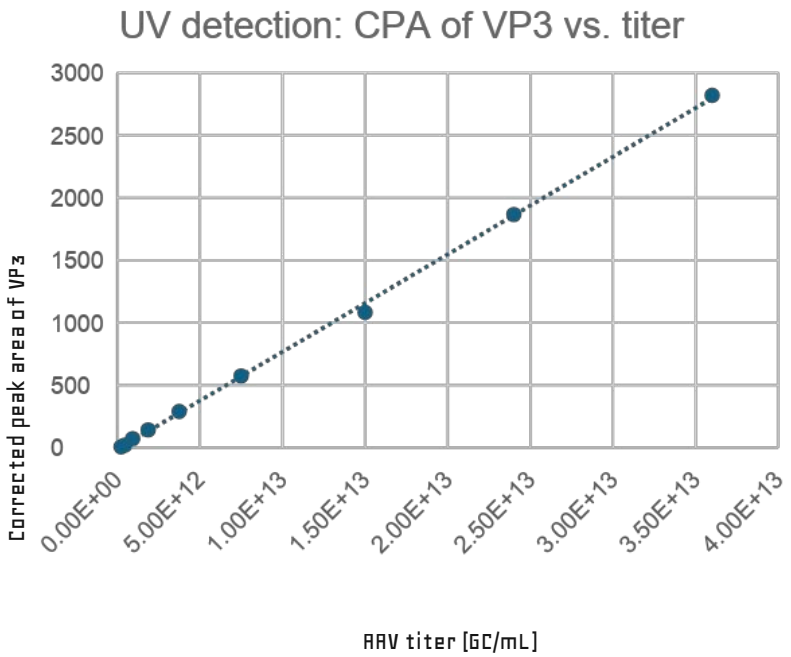
AAV8 capsid protein analysis on BioPhase 8800 system

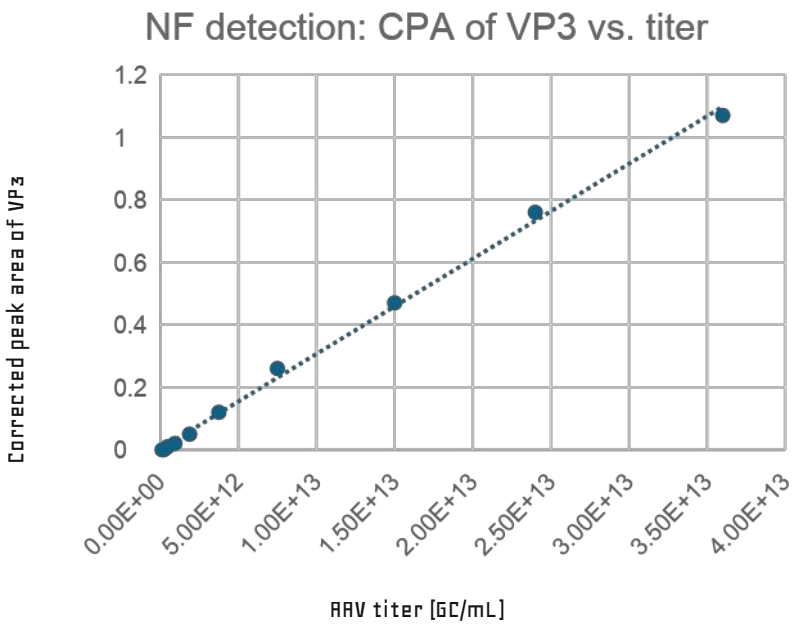


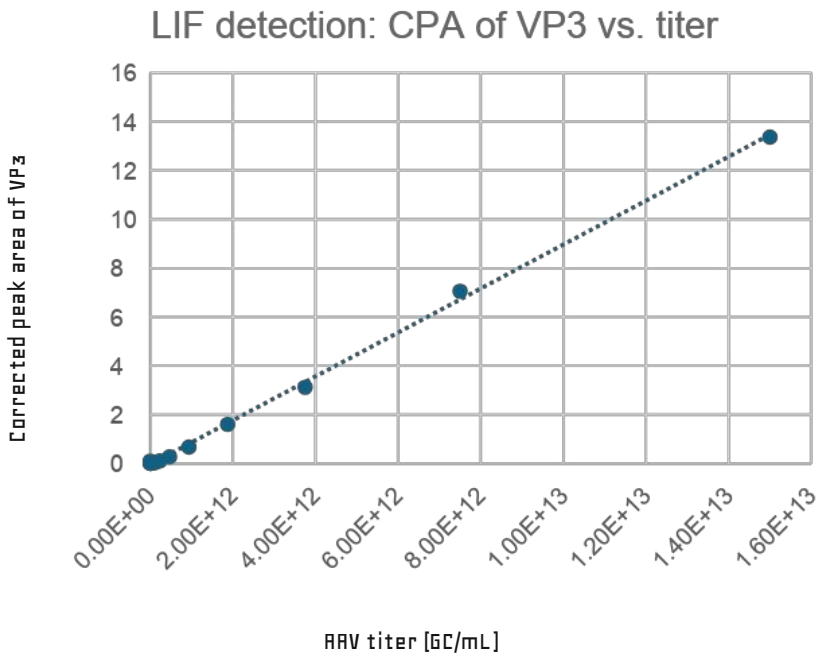


- Comparable capsid protein profiles across three different detections.
- Comparable repeatability across capillary, across injections was observed.

AAV8 capsid protein analysis on BioPhase 8800 system: dynamic range







The linearity plot of the CPA of VP3 versus the titer of an AAV8-CMP-GFP sample using the CE-SDS with UV 220nm, NF, LIF detection on the BioPhase 8800 system

	LDR [GC/mL]	LOQ [GC/mL]	LOD [GC/mL]	R^2
UV	4.7E+11 - 3.6E+13	9.4E+11	4.69E+11	0.999
NF	1.2 E+11 - 3.6E+13	4.7E+11	1.17E+11	0.999
LIF	9.2E+08 - 1.5E+13	9.16E+08	3.05E+08	0.999

AAV8 capsid protein analysis on BioPhase 8800 system: capsid ratio

Capsid Ratios

Experimental value			
	VP1	VP2	VP3
LIF	1.0	1.2	4.5
NFD	1.0	1.5	7.8
UV	1.0	1.4	7.5
Amino acid content			
Lys	32	22	17
Trp	15	12	12
aa	737	600	534
Normalized by amino acid content			
LIF	1.0	1.7	8.5
NFD	1.0	1.9	9.8
UV	1.0	1.7	10.4
Expected	**1.0**	**1.0**	**10**

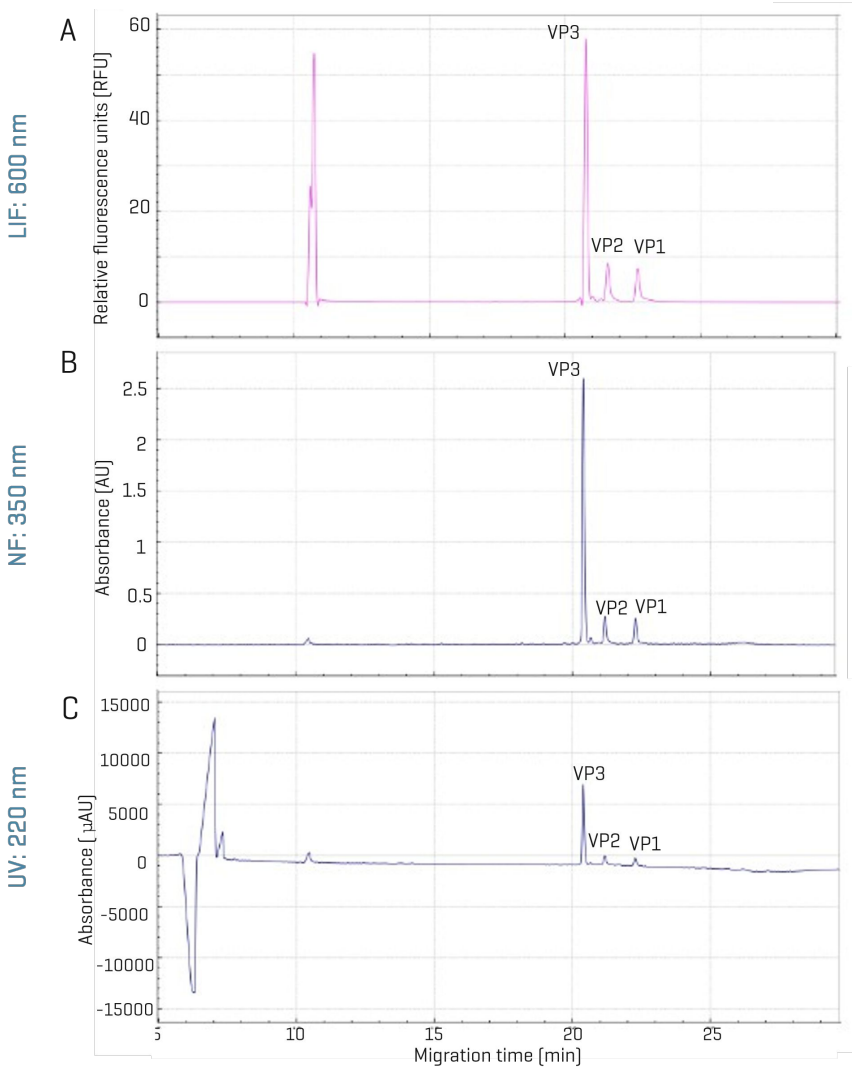
VP1
AADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADA
AALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKT

VP2
APGKKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTGDSESVPDPQPLGEPPAAPSGVGPNTM
AAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDN
TYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTI
QVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQ
FTYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQTTGGTANTQTLGFSQGGPNTMANQAKNWLP
GPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGILIFGKQNAAR
DNADYSDVMLTSEEEIKTTNPVATEEYGIVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDVYLQGPIWA
VP3
KIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQKEN
SKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

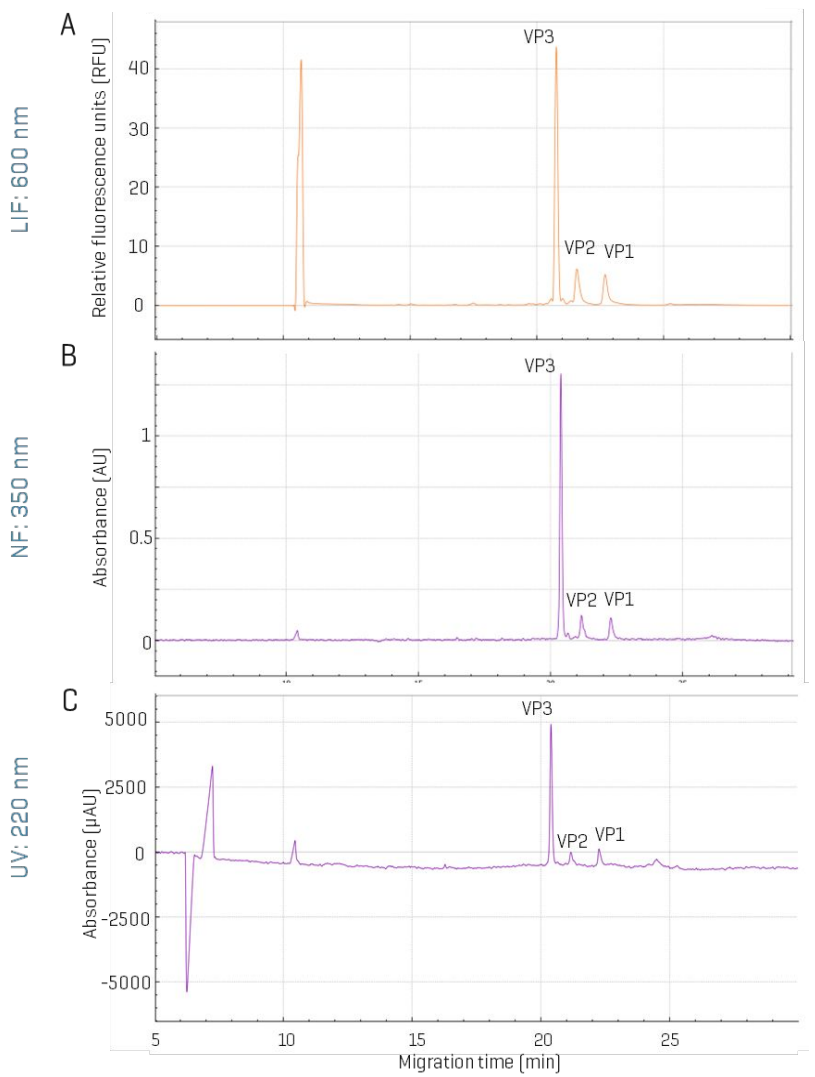
Capsid ratio using NF is similar to the values using UV, and closer to theoretical values

AAV9 capsid protein analysis on BioPhase 8800 system

scAAV9-CMV-LacZ



scAAV9-CMV-GFP



scAAV9-CMV-LacZ capsid ratio			
	VP1	VP2	VP3
LIF	1.0	1.0	4.5
NF	1.0	1.0	8.1
UV	1.0	1.0	8.2

scAAV9-CMV-GFP capsid ratio			
	VP1	VP2	VP3
LIF	1.0	1.2	5.2
NF	1.0	1.5	8.0
UV	1.0	1.4	8.6

Comparable trends for capsid protein ratios were observed when AAV9 is analyzed

AAV Capsid protein analysis using three different detection method

Signagen_AAV8-CMV-GFP	VP3				VP2		VP1	
	MT [min]	RSD% of MT	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%
LIF-600 nm	20.94	0.6	65.8	0.3	17.1	0.7	13.4	1.1
NF -360 nm	21.28	0.7	74.7	0.3	13.30	1.2	8.80	2.9
UV -220 nm	20.90	0.5	74.5	0.8	13.00	3.5	9.10	5.4

Signagen_AAV9-CMV-lacZ	VP3				VP2		VP1	
	MT [min]	RSD% of MT	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%
LIF-600 nm	20.50	0.4	70.30	1.2	14.00	1.90	13.40	1.1
NF -360 nm	20.89	0.8	81.34	0.6	9.48	1.27	9.18	4.0
UV -220 nm	20.38	0.6	80.85	1.1	10.06	5.76	9.09	6.6

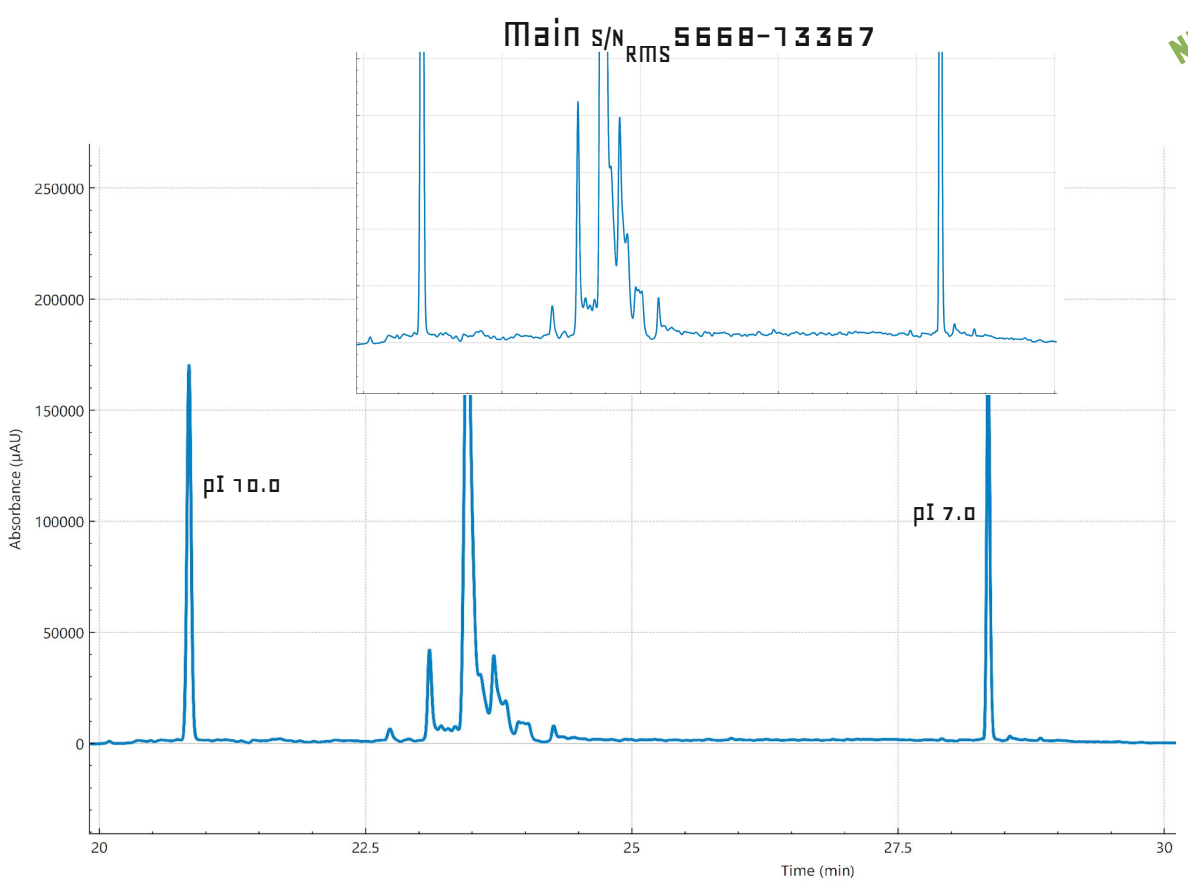
Signagen_scAAV9-CMV-GFP	VP3				VP2		VP1	
	MT [min]	RSD% of MT	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%	CPA%	RSD% of CPA%
LIF-600 nm	20.44	0.5	69.02	0.3	14.89	0.5	12.06	1.0
NF -360 nm	21.20	1.1	80.12	0.5	9.56	1.1	9.15	2.8
UV -220 nm	20.56	0.9	81.90	1.2	9.56	7.1	8.55	12.0

Comparable repeatability was observed between LIF, NF, and UV

cIEF analysis for proteins

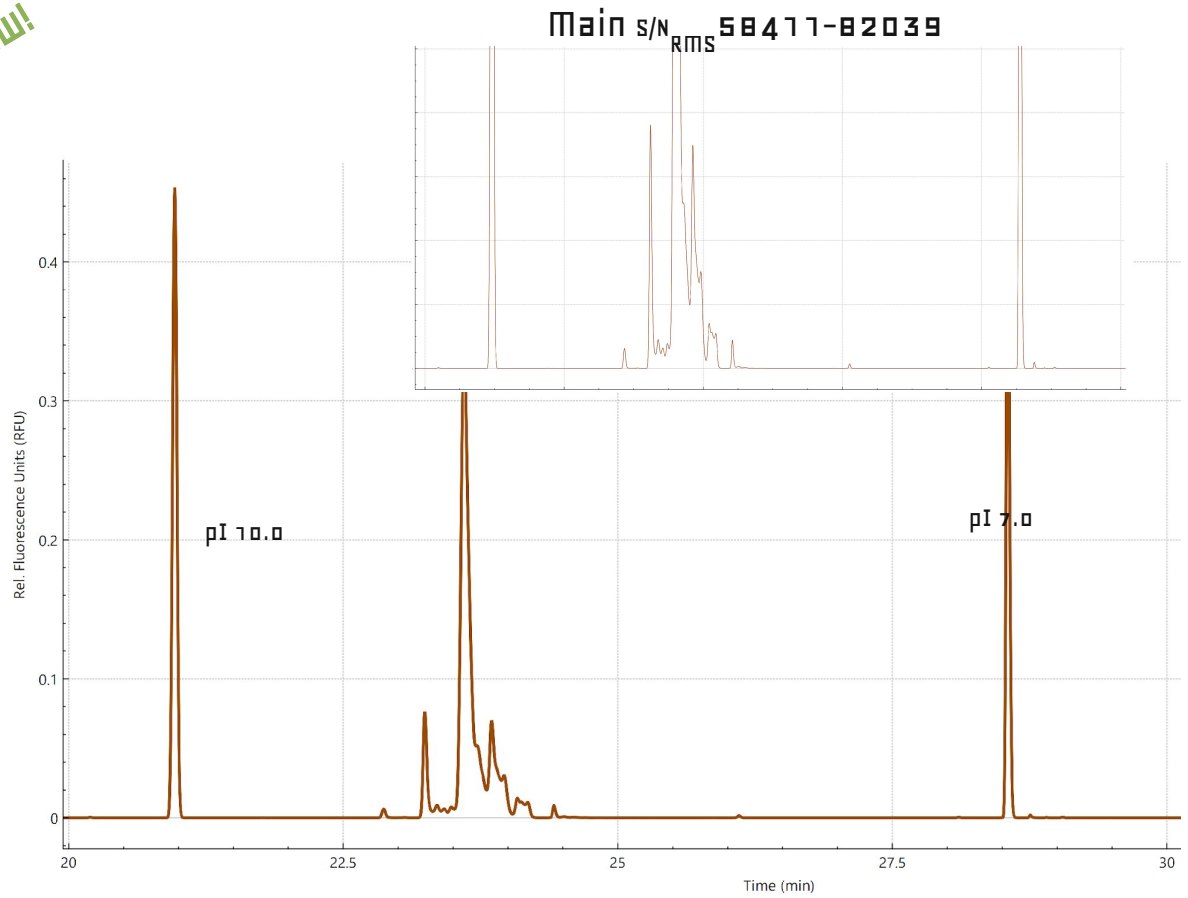
BioPhase 8800 system with NF: cIEF of NIST mAb

UV detection: NIST mAb



NEW!

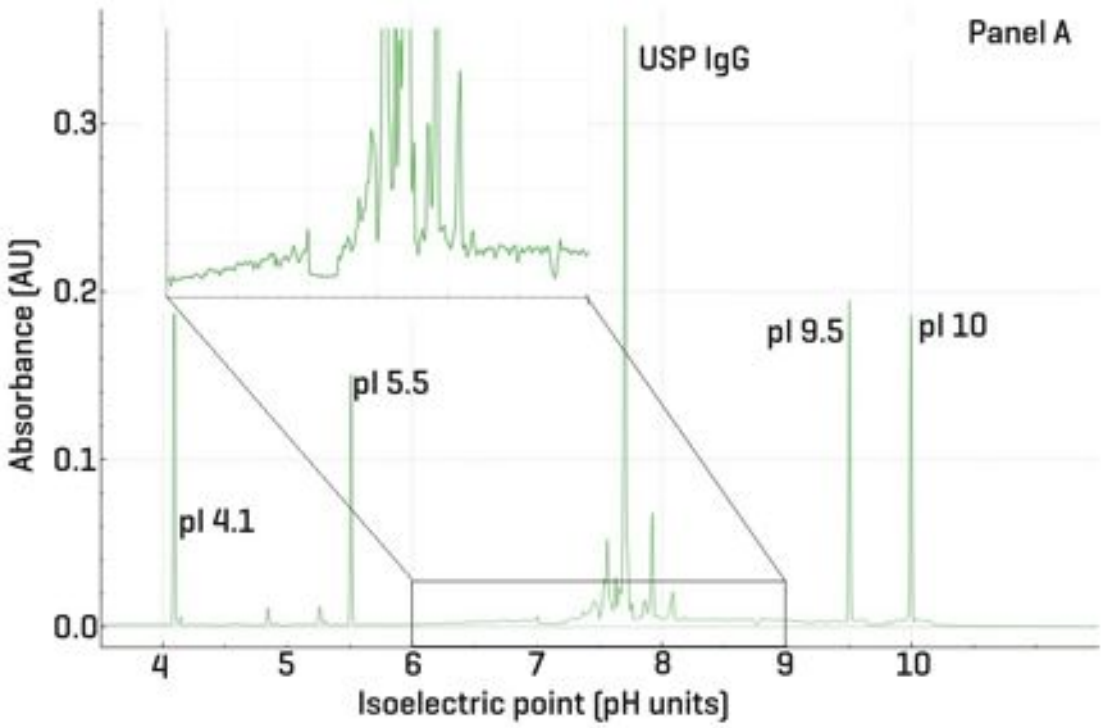
NF detection: NIST mAb



NF detection shows similar profiles for all charge variants with improved S/N ratios

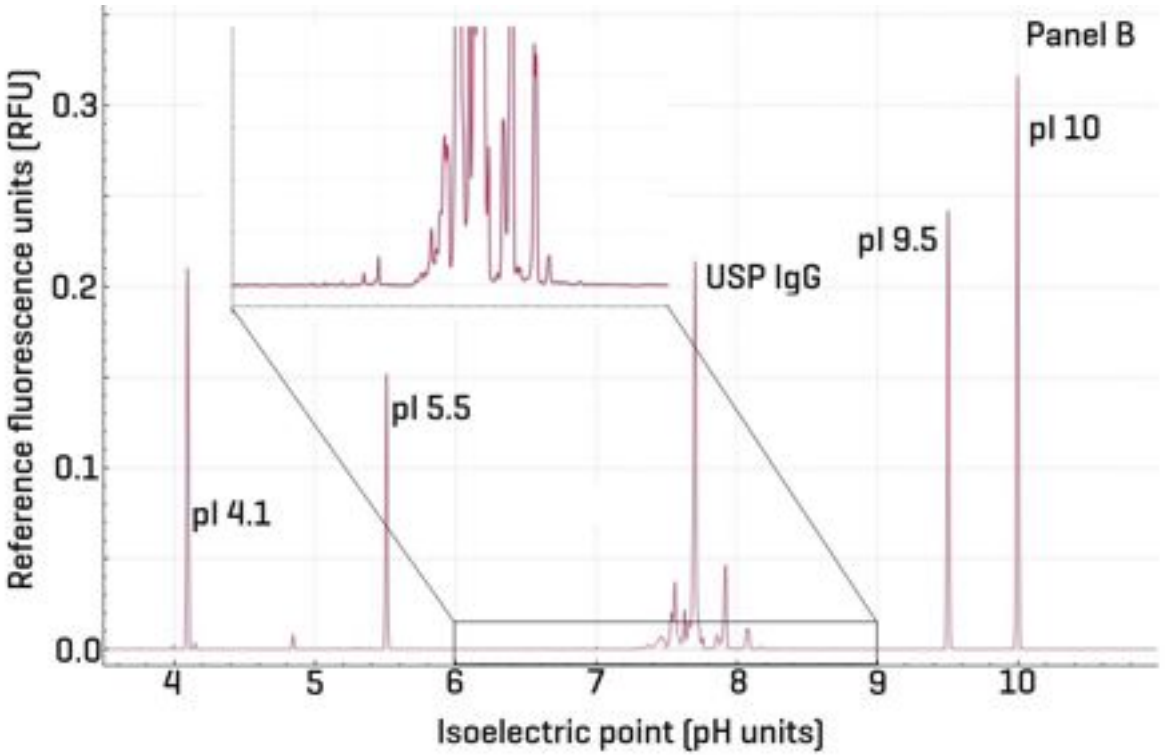
BioPhase 8800 system with NF: cIEF of USP IgG

UV detection: USP IgG



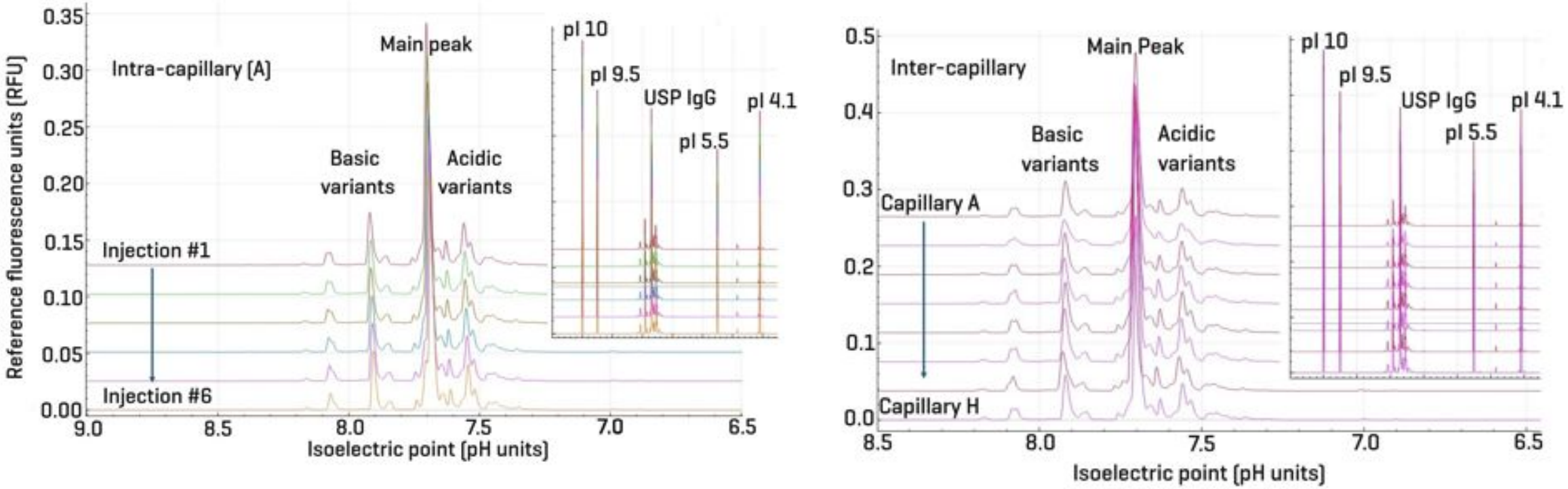
NF detection: USP IgG

NEW!



NF detection shows similar profiles for all charge variants with improved S/N ratios

BioPhase 8800 system with NF: repeatability



Intra and Inter-capillary reproducibility. The inset shows the zoomed-out electropherograms.

BioPhase 8800 system with NF: Consistency and repeatability

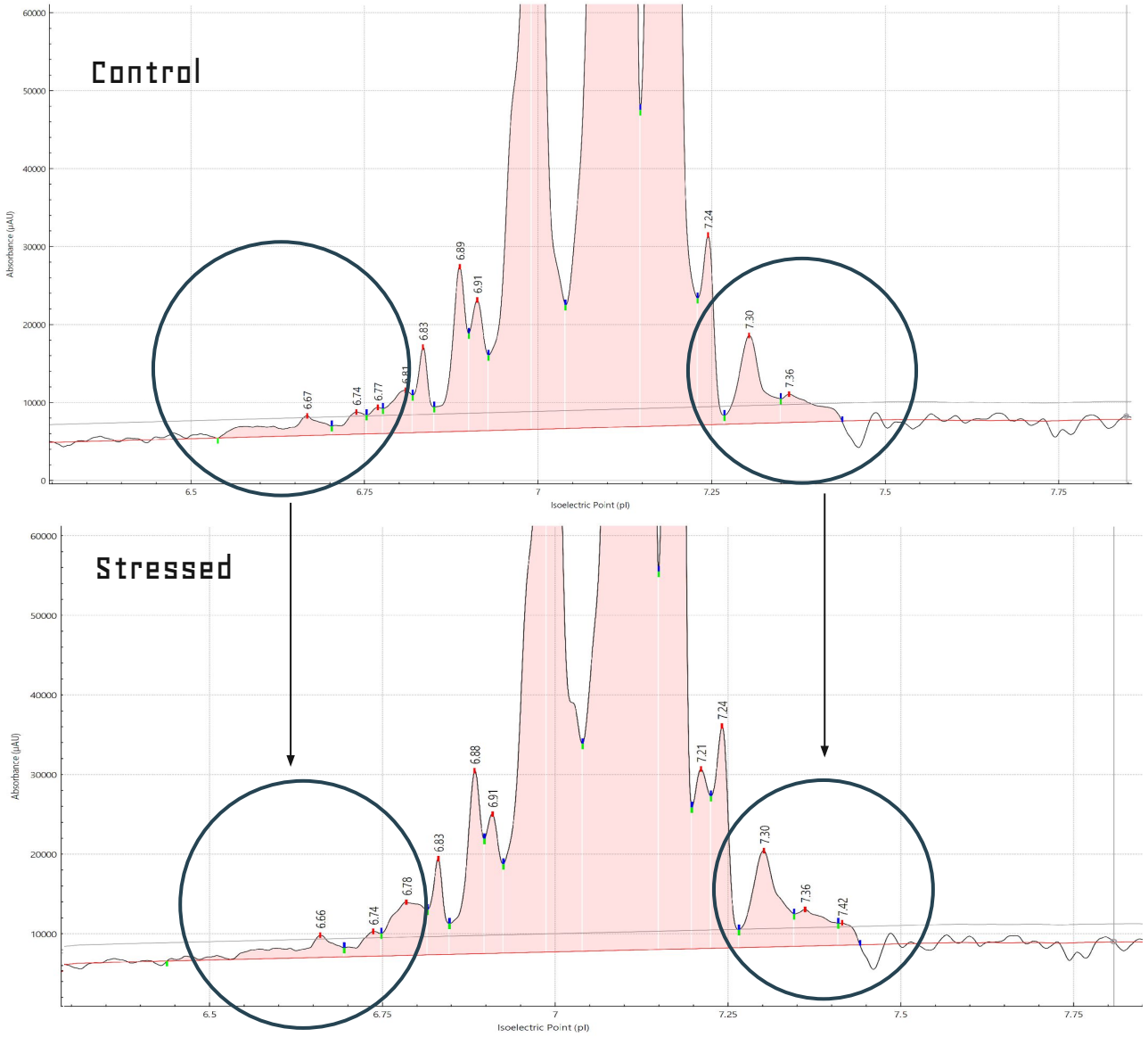
Intra-Capillary	Acidic %		Main %		Basic %		Main pI Values	
	Average	% RSD	Average	% RSD	Average	% RSD	Average	% RSD
A	15.72	0.4	63.81	0.5	20.46	1.18	7.66	0.1
B	16.07	1.2	61.80	0.6	22.13	0.95	7.66	0.1
C	16.25	0.6	61.25	0.5	22.50	0.91	7.66	0.2
D	16.30	0.6	61.36	0.7	22.35	1.47	7.66	0.1
E	15.94	0.8	62.43	0.8	21.63	1.82	7.66	0.1
F	15.93	0.6	62.75	0.8	21.32	1.94	7.65	0.2
G	15.75	0.4	63.03	0.5	21.22	1.27	7.66	0.1
H	15.46	1.1	64.46	0.7	20.08	1.49	7.66	0.1
Inter-capillary 48 injections	***15.93***	***1.8***	***62.61***	***1.8***	***21.46***	***2.0***	***7.66***	***0.1***

Comparable sample purity with the same level of repeatability

cIEF Profile comparison between control and stressed sample

UV detection

Baseline drifts make integration on the basic side more subjective/uncertain



The data were obtained using a prototype BioPhase 8800 system with NFD

cIEF Profile comparison between control and stressed sample

NEW!

NF detection

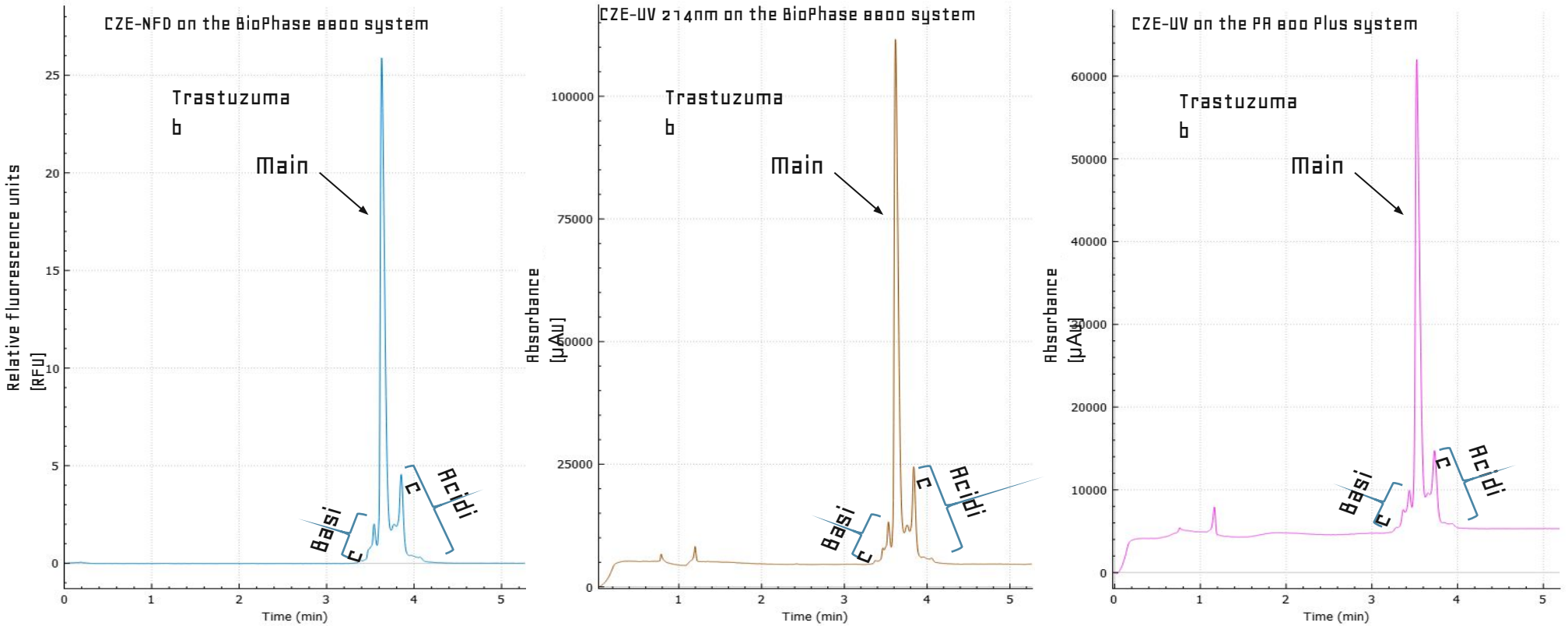
More confident to integrate the basic side and reflect the changes

		Basic %	Main%	Acidic%
Control	UV	3.39	78.20	18.12
NEW!	NFD	3.39	78.55	18.06
Stressed	UV	6.28	71.84	21.81
NEW!	NFD	8.84	69.57	21.55

The data were obtained using a prototype BioPhase 8800 system with NFD

CZE rapid charge variant analysis

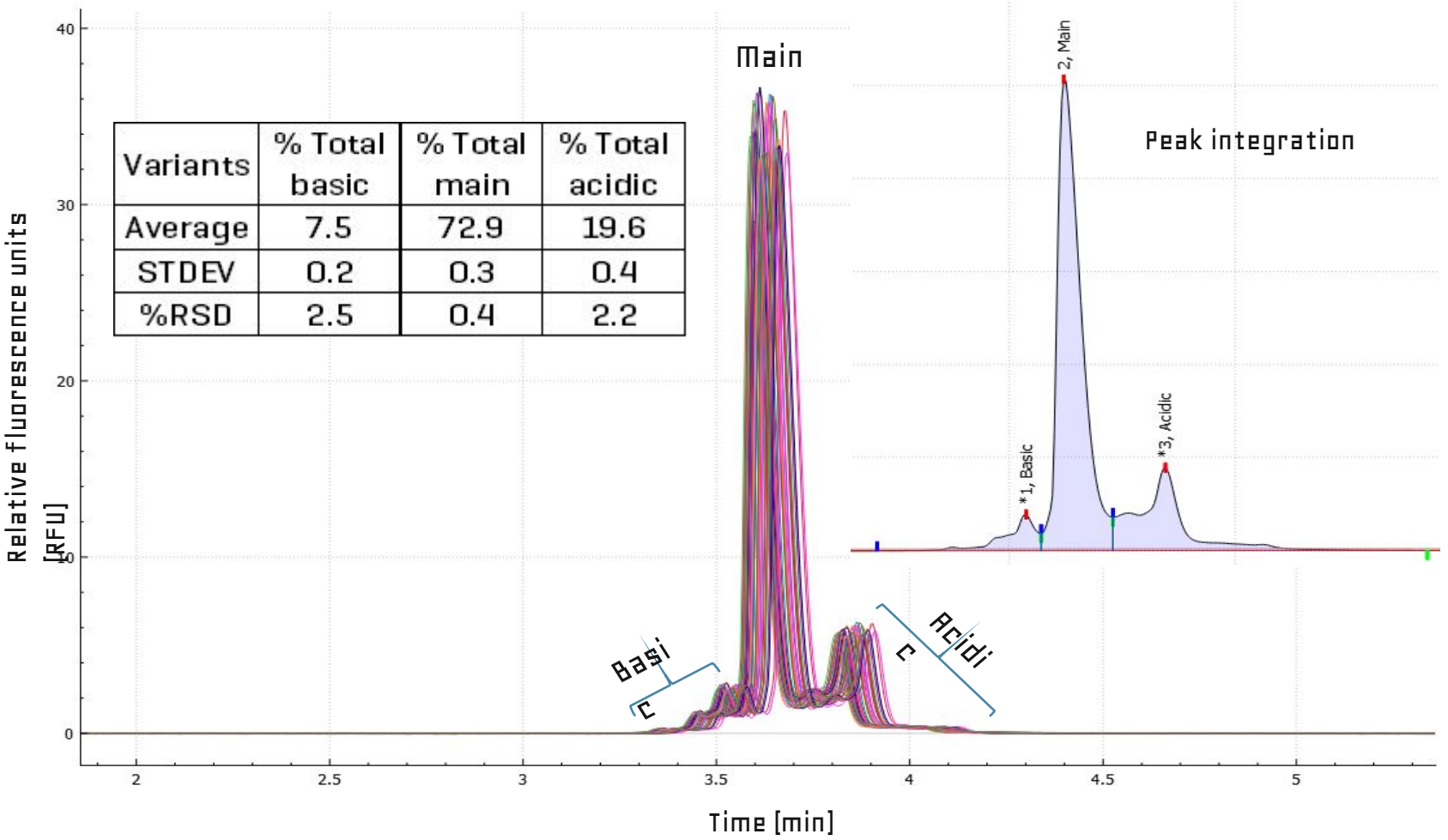
CZE analysis of Trastuzumab on the BioPhase 8800 system and the PA 800 Plus system.



	S/N ratio [RMS] for the main peak		
	Trastuzumab		
Detection	NFD on BP	UV on BP	UV on PA
# of injections	42	42	6
Average	10658	8856	5533

CZE analysis of Trastuzumab on the BioPhase 8800 system with NF: repeatability

Variants	% Total basic	% Total main	% Total acidic
Average	7.5	72.9	19.6
STDEV	0.2	0.3	0.4
%RSD	2.5	0.4	2.2



CZE analysis of Trastuzumab on the BioPhase 8800 system with NF: repeatability

	% Total basic variants			% Total main peak			% Total acidic variants		
Detection	NFD on BP	UV on BP	UV on PA 800	NFD on BP	UV on BP	UV on PA 800	NFD on BP	UV on BP	UV on PA800
# of injections	42	42	6	42	42	6	42	42	6
Average	7.5	8.3	9.2	72.9	71.3	71.2	19.6	20.4	19.6
STDEV	0.2	0.3	0.1	0.3	0.6	0.5	0.3	0.5	0.4
%RSD	2.5	3.1	1.6	0.4	0.8	0.7	1.7	2.3	2.2

Summary- NF detection for all protein workflows on BioPhase 8800 system

Native: signal relies on the naturally existing aromatic amino acid in the protein, eliminating the protein labeling process

Fluorescence: no direct transmission/absorbance interference from background media in the capillary, providing a clean background

BioPhase 8800 system: 8-capillary parallel processing and easy-to-integrate data, reducing the time to answer with high confidence

BioPhase 8800 system with NF : demonstrated to be suitable for all existing protein-related workflows, providing comparable results, faster and easier data processing.

Place holder for all the Sciex-related materials in CE Pharm.

Sciex Booth #8

Monday, Sep 8th

10:00-11:15 Monday, 8 September, 2025, Salon A-C

Session I - Application and Advancements in CE Innovations

Advancement of Capillary Gel Electrophoresis Through the Use of Native Fluorescence Detection

Jessica Taylor, *Teva Pharmaceutical Industries Ltd.*

11:45-12:45 Monday, 8 September, 2025, Foyer A-C

Lunch and Learn Technical Seminar Presented by SCIEX

Presentation type LS - Livestreamed

Session Speakers:

Advancing Capillary Electrophoresis Sensitivity and Repeatability with Native Fluorescence Detection on the BioPhase 8800 System

Fang Wang, *SCIEX*

Label-Free Native Fluorescence for High-Sensitivity Protein Analysis using a Multi-Capillary Electrophoresis System

Brian Wei, Sanofi

Tuesday and Wednesday, Sep 9-10th

11:45-12:45 Tuesday, 9 September, 2025, Foyer A-C

44 11:45-12:45

Enhancing protein purity analysis using capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) with native fluorescence detection (NFD) on the BioPhase 8800 system

Tingting Li[1], Jane Luo[1], Marcia Santos[1], Sahana Mollah[2]
[1] SCIEX, Brea, California, USA. [2] SCIEX, Redwood city, California, USA

45 11:45-12:45

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

Marcia Santos[1], Zoe Zhang[2], Sahana Mollah[2]
[1] SCIEX, Brea, California, USA. [2] SCIEX, Redwood city, California, USA

46 11:45-12:45

Characterization workflows for CRISPR-Cas9 gene editing products, plasmids, and mRNA drugs on a single capillary electrophoresis (CE) platform

Jane Luo[1], Tingting Li[1], Kerstin Pohl[2], Sahana Mollah[3]
[1] SCIEX, Brea, California, USA. [2] SCIEX, Marlborough, Massachusetts, USA. [3] SCIEX, Redwood City, California, USA

47 11:45-12:45

Advancing biopharmaceutical molecule charge heterogeneity analysis with native fluorescence detection on a multi-capillary electrophoresis platform

Jane Luo[1], Peter Holper[2], Marcia Santos[1], Tingting Li[1], Fang Wang[3], kerstin Pohl[3], Sahana Mollah[2]
[1] SCIEX, Brea, California, USA. [2] SCIEX, Redwood City, California, USA. [3] SCIEX, Marlborough, Massachusetts, USA

67 11:45-12:45

Advancing Purity Analysis of Protein Therapeutics: Evaluation of the Next-Generation BioPhase 8800 System with Native Fluorescence Detection

Vaidehi Bhagat, Maroly Gonzalez-Perez, Douglas Johnson
AstraZeneca, Gaithersburg, USA

49 10:00-11:00

Further Exploration of Native Fluorescence Detection in Capillary Electrophoresis

Matthew Myers, Jessica Taylor
Teva Pharmaceuticals, West Chester, Pennsylvania, USA

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