CE Pharm 2025

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

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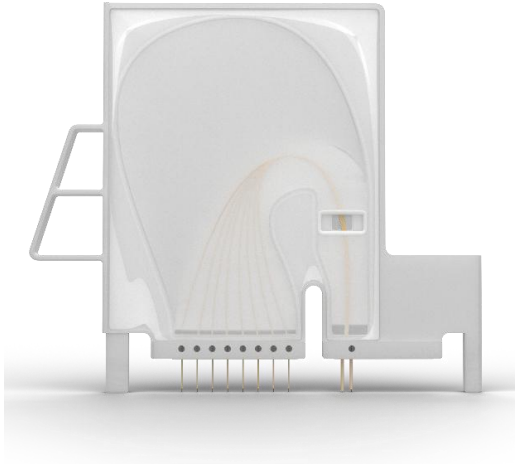
Agenda

1. Introduction
2. Peak-to-Peak Comparison
3. Stressed and Enzyme Digested Samples
4. Loading Linearity and LOQ
5. Low Concentration Evaluation
6. Summary and Conclusions

Teva- Analytical Development

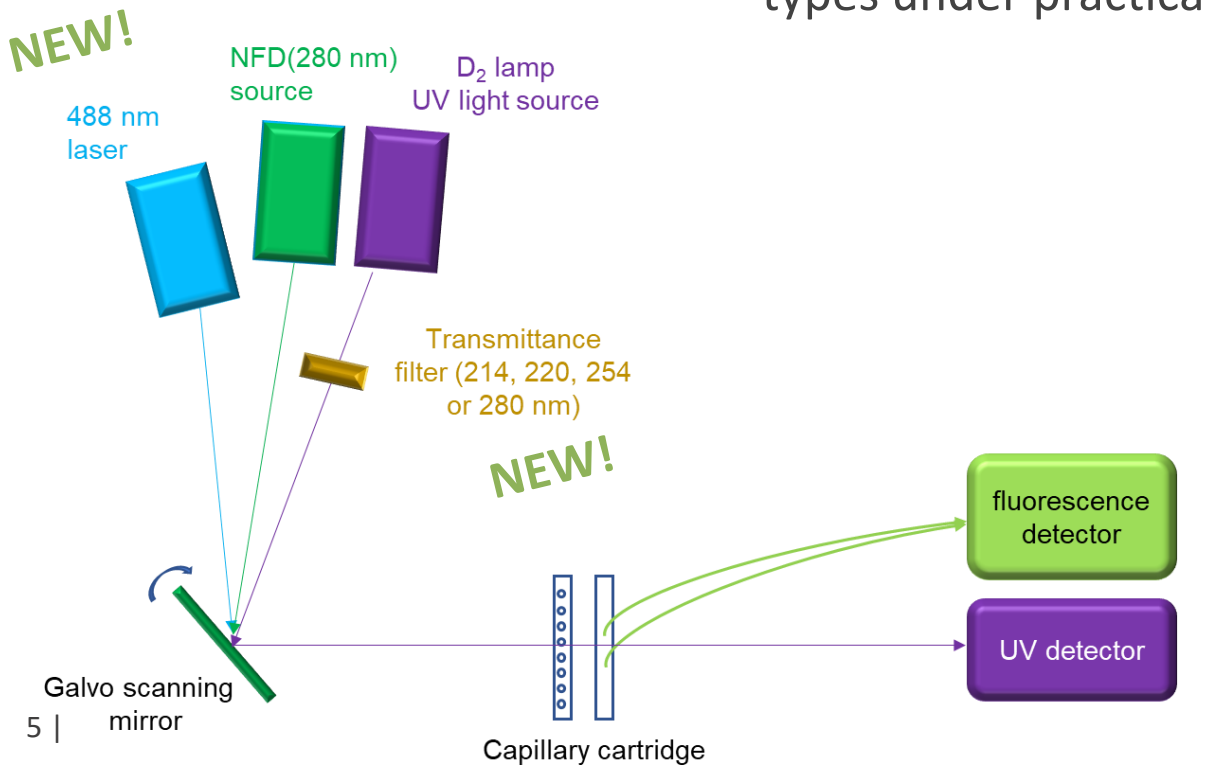
- Analytical Development (AD) team is part of Biologics CMC in West Chester, PA
- Our team supports non-GMP release and stability studies
- Qualified methods are transferred to our QC groups for formal release and stability testing
- Reduced and Non-Reduced CGE sample analyses are routinely performed to support process development and formulation development
- In 2023, the AD lab brought the 8-capillary Sciex BioPhase 8800 online to support high throughput studies
  - The BioPhase has offered a significant improvement in throughput for early phase studies. However, data processing still presents a hurdle due to the amount of manual integration that is typically required for processing CGE data

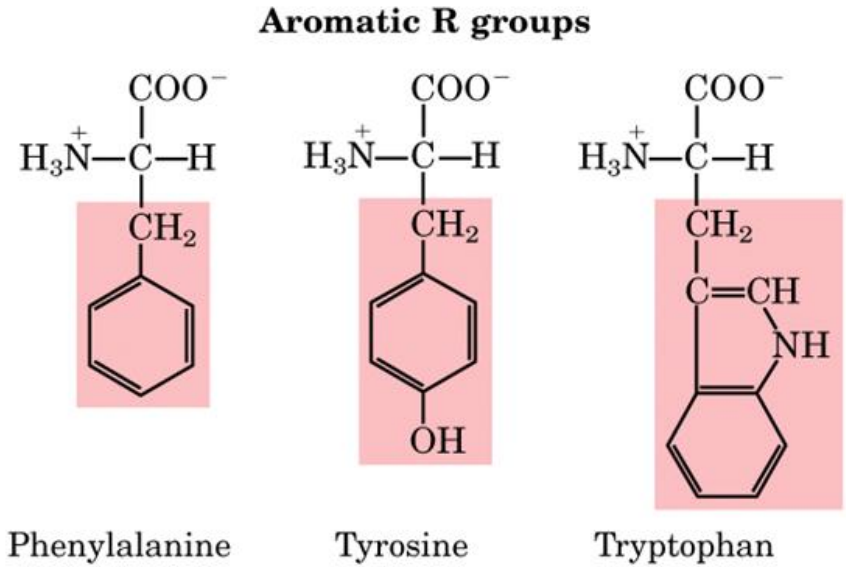


Native Fluorescence Detection

- Integration of CGE data can be significantly improved using native fluorescence detection (NFD)
- NFD employs a molecule's native fluorescence capabilities from aromatic amino acids
- NFD eliminates baseline noise/interference that can interfere in UV spectra and does not require any additional labeling that is typically used in LIF methods

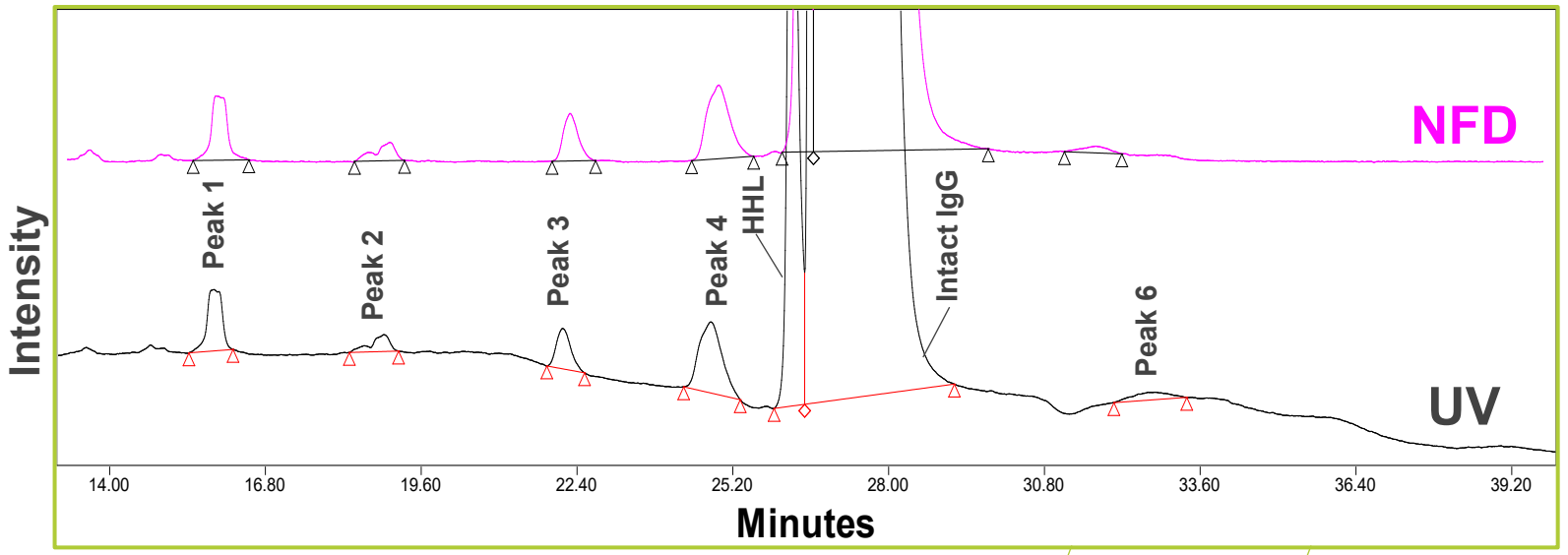
Pre-commercial evaluation of the Sciex BioPhase with NF was conducted to examine the analytical distinctions between NF and UV detection across a range of sample types under practical laboratory conditions.

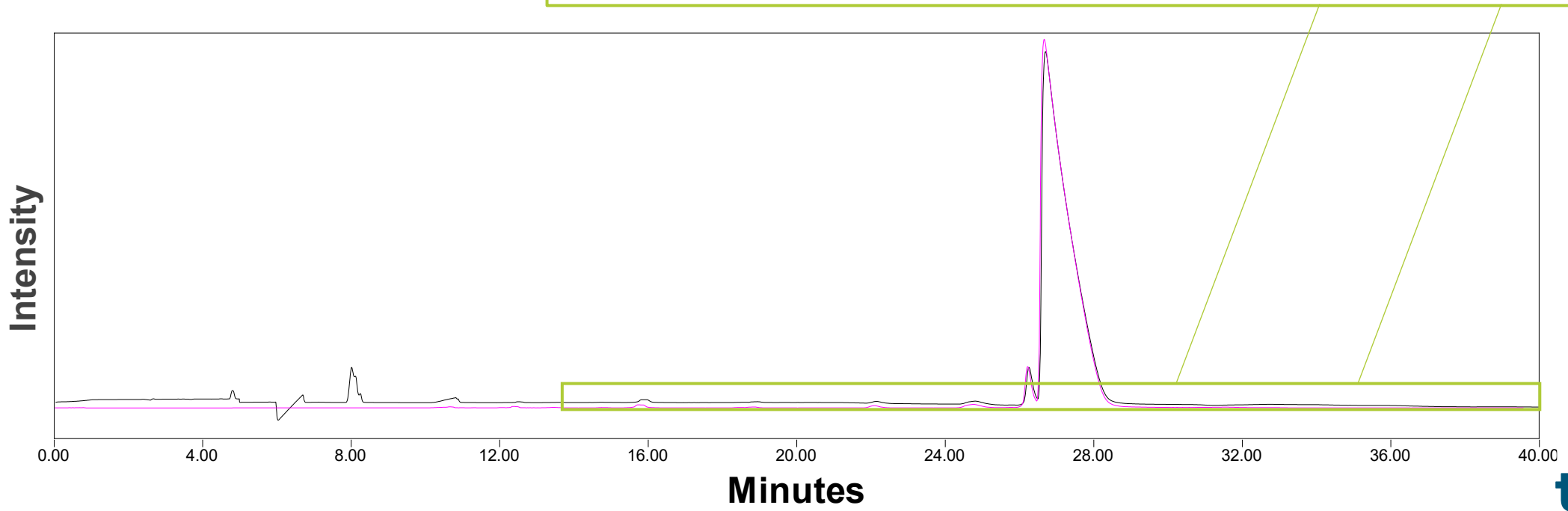




Non-Reduced CGE

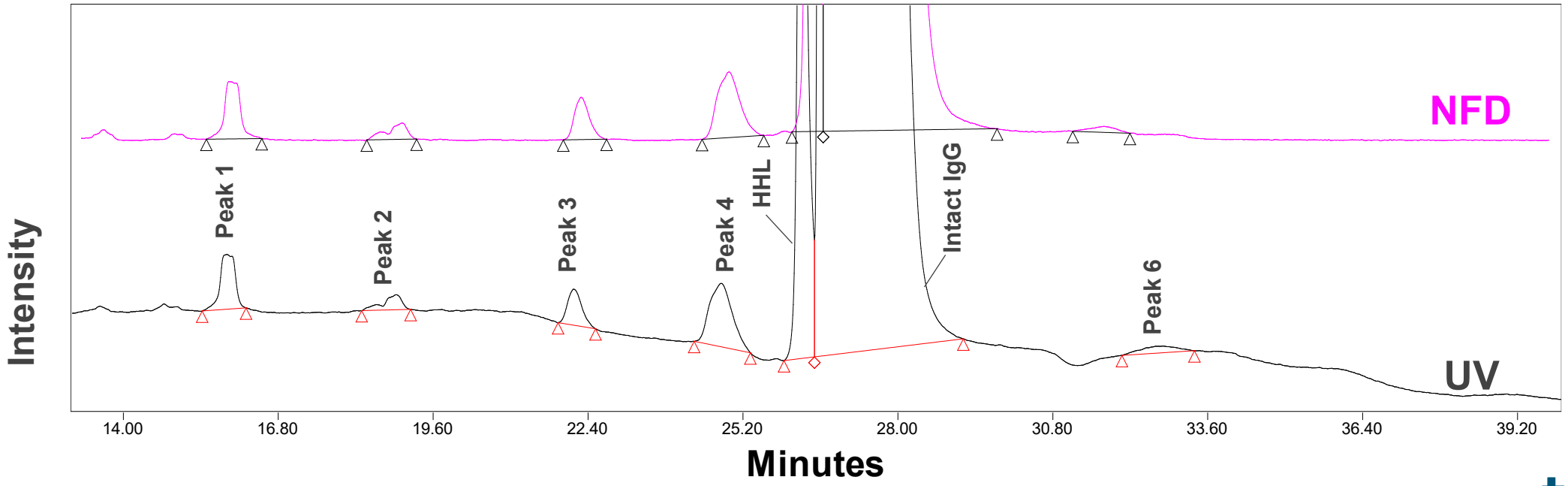
- Peaks are consistent between UV and NFD
- Detection of HMW species is significantly improved with the flat baseline in NFD detection





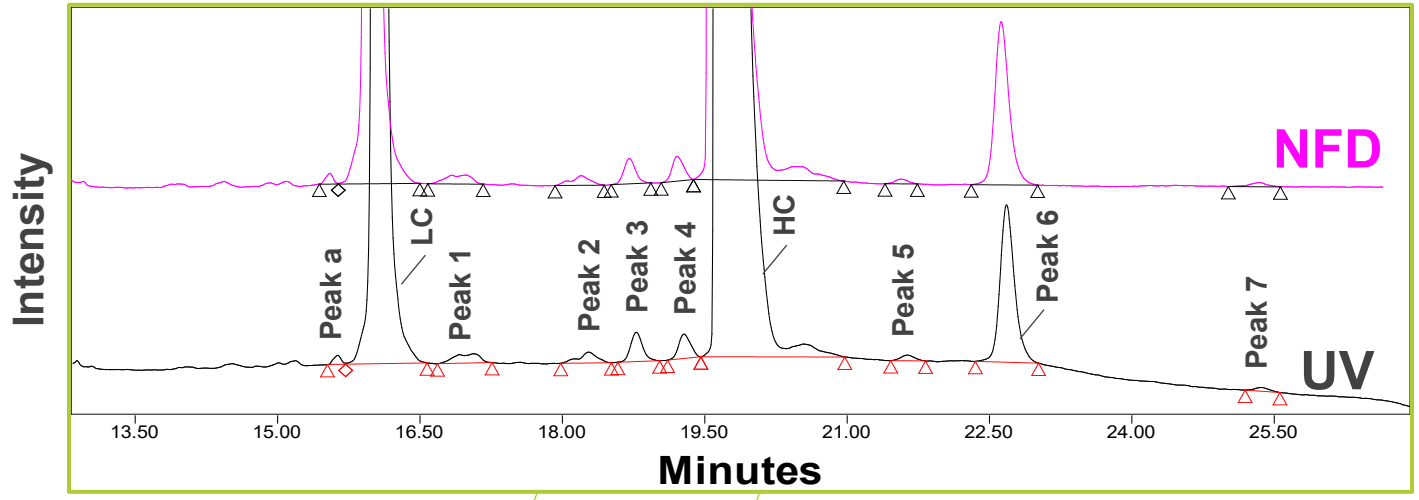
Non-Reduced CGE

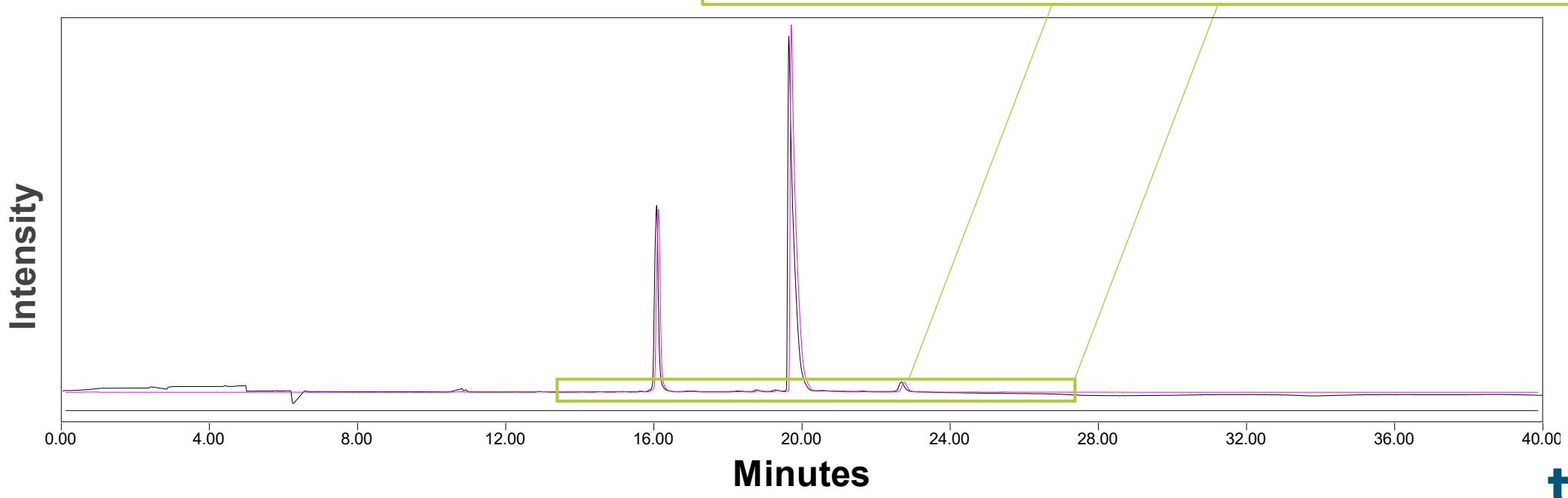
Detection Method		Reported Results				Individual Peak %TCA						
		%Fragment	%HHL	%Purity	%HMW	Peak 1	Peak 2	Peak 3	Peak 4	HHL	IgG	Peak 6
UV	$Average_{n=3}$	1.4	2.6	96.0	0.1	0.53	0.15	0.22	0.55	2.59	95.92	0.06
	$\%RSD_{n=3}$	4.0	0.0	0.1	86.6	6.6	7.3	1.6	3.7	0.8	0.1	24.9
NFD	$Average_{n=3}$	1.5	2.7	95.7	0.1	0.54	0.15	0.26	0.54	2.73	95.74	0.05
	$\%RSD_{n=3}$	0.0	2.1	0.1	0.0	2.0	3.4	2.4	0.6	1.7	0.1	15.3



Reduced CGE

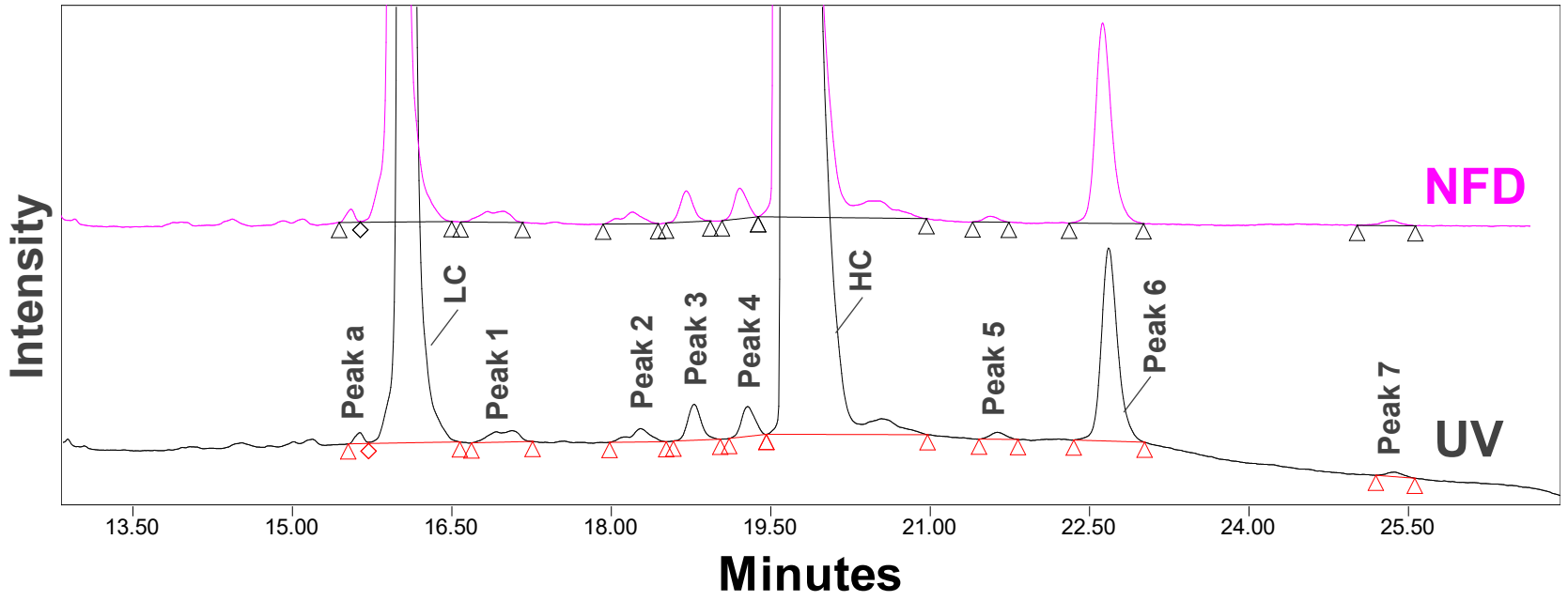
- Peaks are consistent between UV and NFD





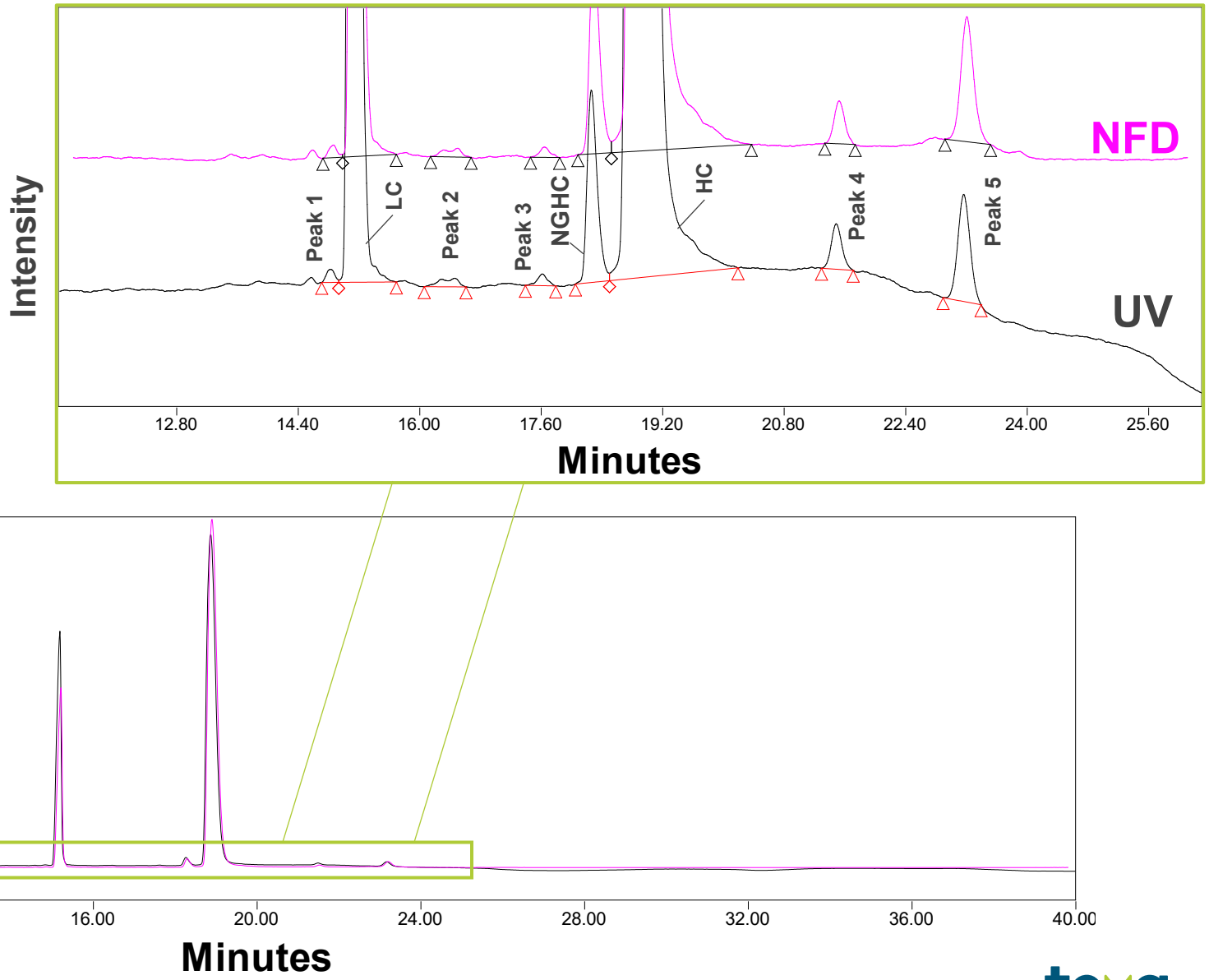
Reduced CGE

Detection Method		Reported Results			Individual Peak %TCA									
		%Fragment	%Purity (LC+HC)	%Other	Peak a	LC	Peak 1	Peak 2	Peak 3	Peak 4	HC	Peak 5	Peak 6	Peak 7
UV	$Average_{n=3}$	1.0	97.4	1.7	0.08	29.86	0.22	0.17	0.29	0.23	67.49	0.06	1.56	0.04
	$\%RSD_{n=3}$	0.0	0.1	3.5	12.0	0.1	5.0	5.2	1.6	0.1	0.0	3.6	1.3	14.2
NFD	$Average_{n=3}$	0.9	97.4	1.7	0.08	28.65	0.22	0.15	0.25	0.23	68.74	0.05	1.6	0.04
	$\%RSD_{n=3}$	6.2	0.0	0.0	11.9	0.0	2.4	2.8	2.7	1.4	0.0	5.9	0.2	18.3



Reduced CGE

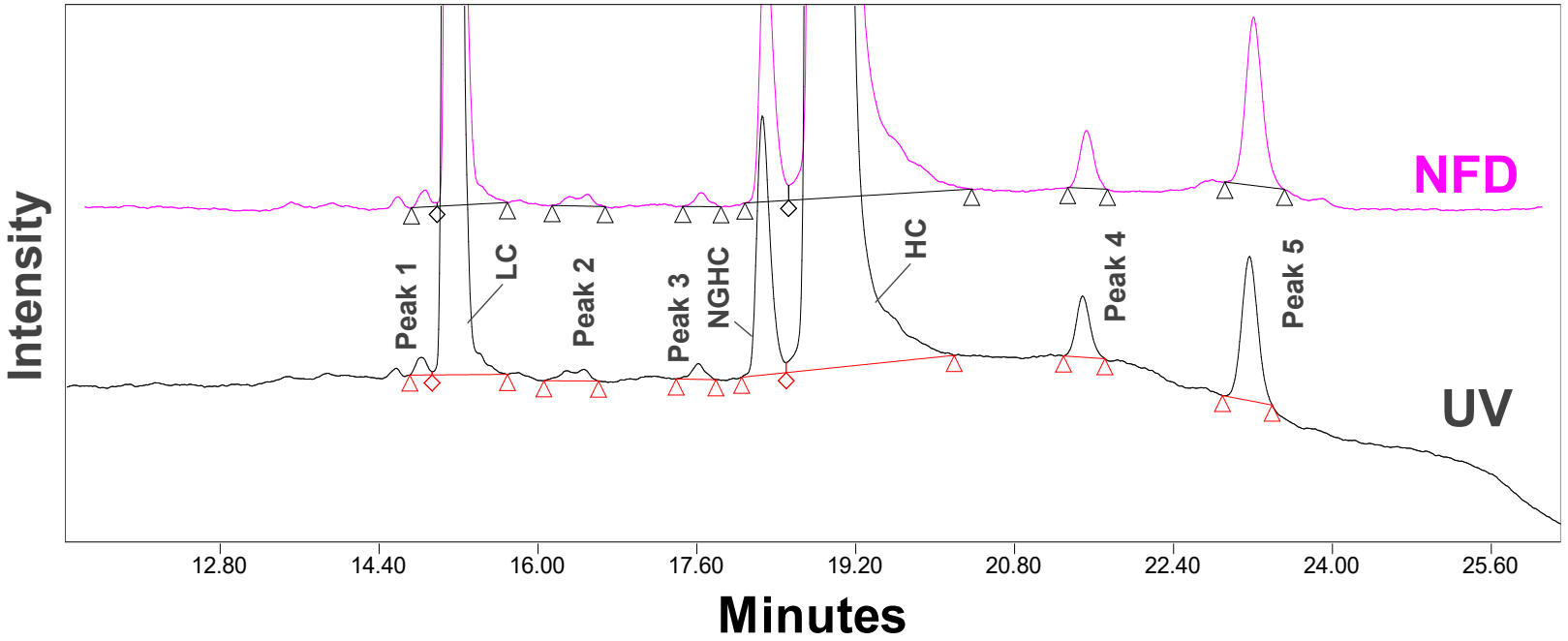
- All peaks detectable in both UV and NFD
- It was noted that some molecules show differences in the HC:LC between NFD and UV, although the reported results remain comparable



Reduced CGE

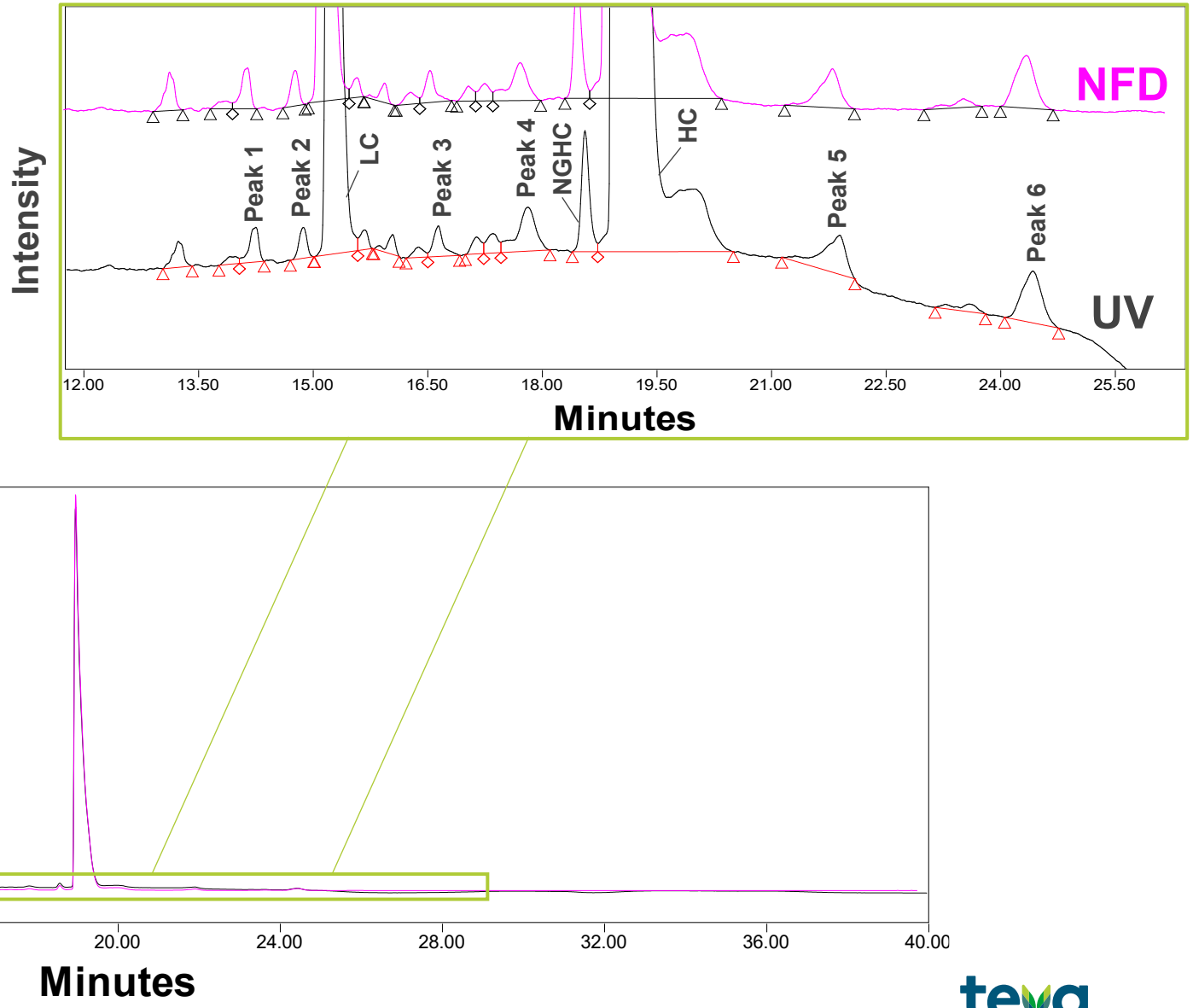
Detection Method		Reported Results				Individual Peak %TCA							
		%Fragment	%Purity (LC+HC)	%Other	%NGHC	Peak 1	LC	Peak 2	Peak 3	NGHC	HC	Peak 4	Peak 5
UV	$Average_{n=3}$	0.2	98.0	0.8	1.4	0.08	29.39	0.09	0.08	1.00	68.58	0.21	0.58
	$\%RSD_{n=3}$	24.7	0.0	0.0	4.0	8.9	0.1	13.3	16.9	2.1	0.1	3.1	5.9
NFD	$Average_{n=3}$	0.2	97.7	0.9	1.5	0.07	23.04	0.09	0.06	1.14	74.65	0.22	0.73
	$\%RSD_{n=3}$	0.0	0.1	6.2	0.0	2.3	0.2	7.3	1.7	0.9	0.1	5.5	7.6

There is roughly a 6% increase in %HC and a corresponding 6% decrease in %LC using NFD detection



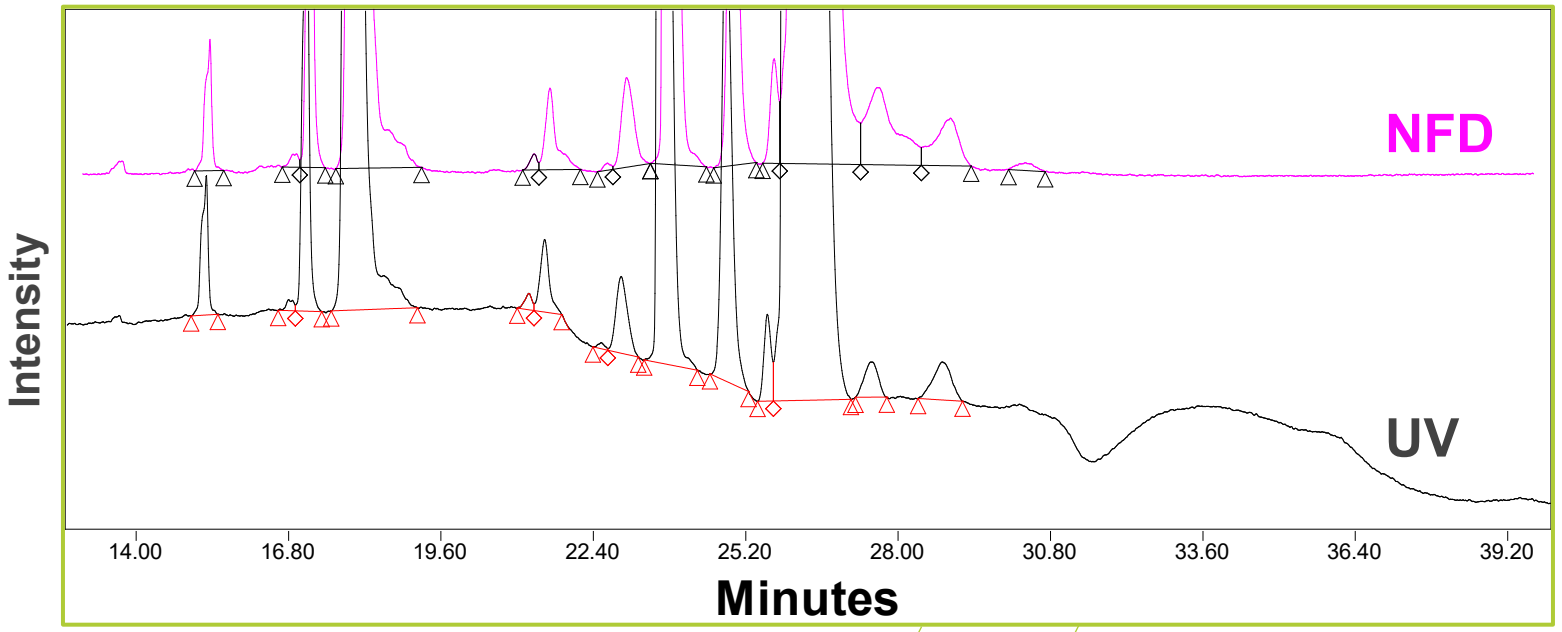
Reduced CGE: Stressed Sample

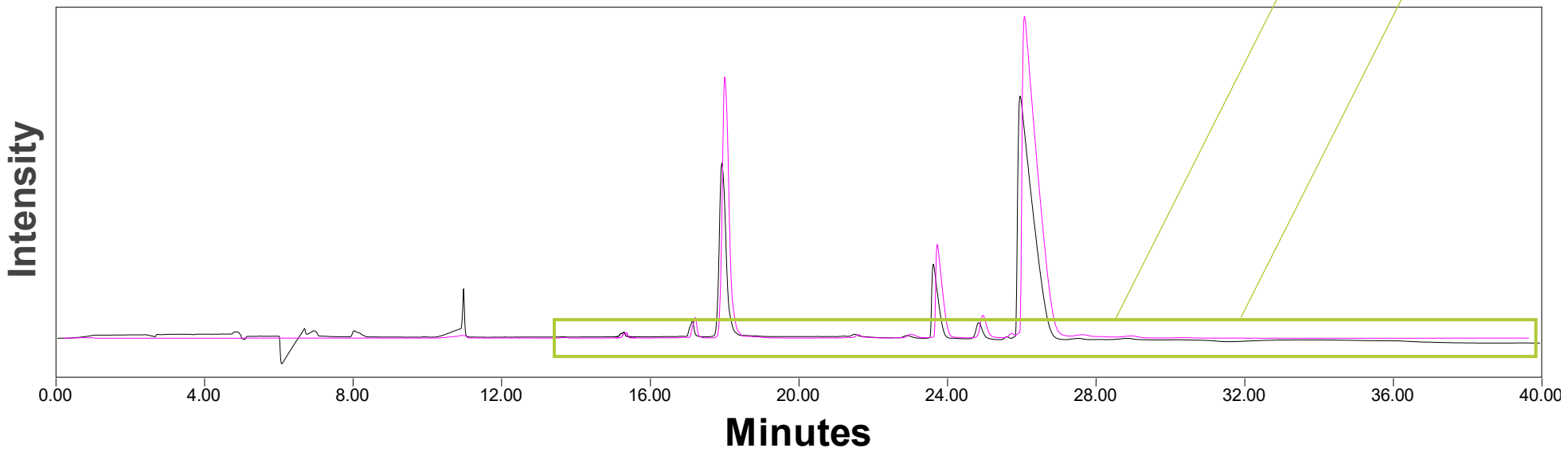
- Material was stressed at 40°C for 8 weeks
- All additional peaks that were detected with stress under UV were also detectable with NFD at similar levels
- Differences in HC:LC seen in stressed material as well



Non-Reduced CGE: Digested Sample

- A standard sample was digested using the IdeS enzyme
- mAb is cleaved at the hinge region to create Fc and F(ab)2 fragments
- Some differences in major peak %TCA values, but overall results are comparable

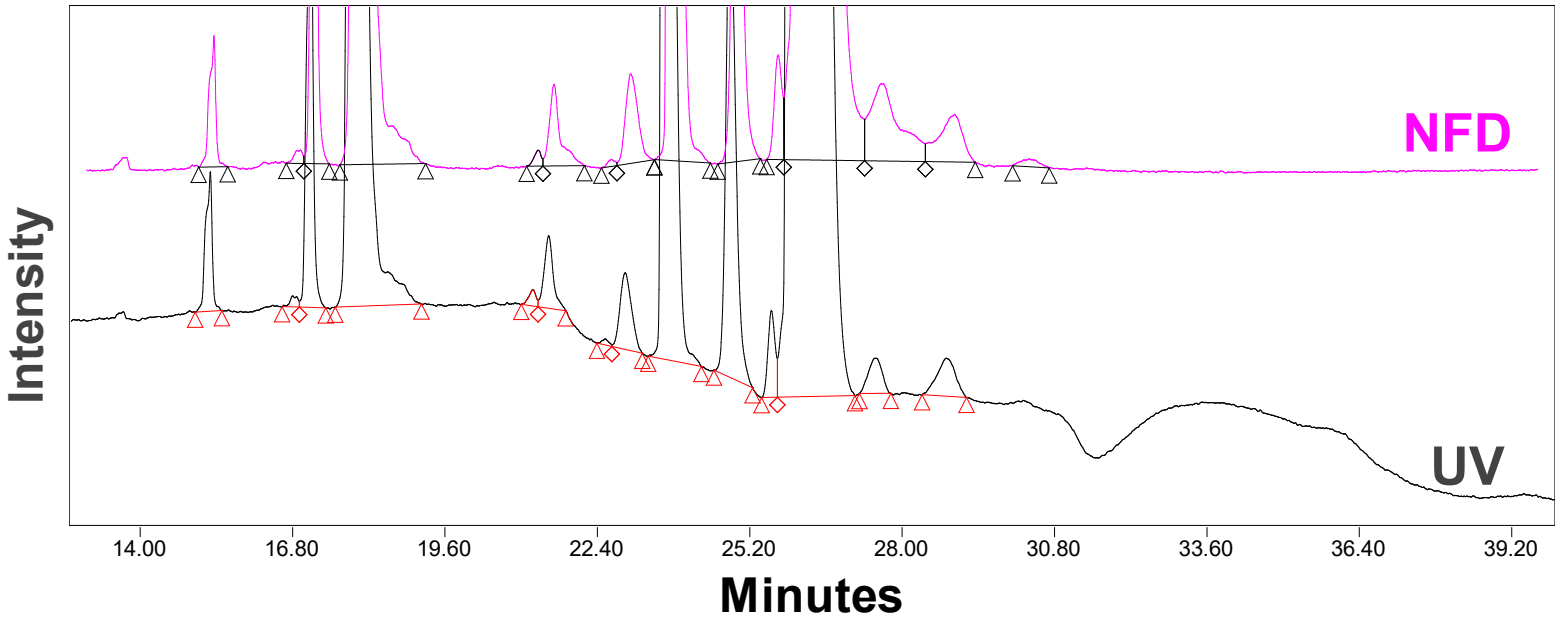




Non-Reduced CGE: Digested Sample

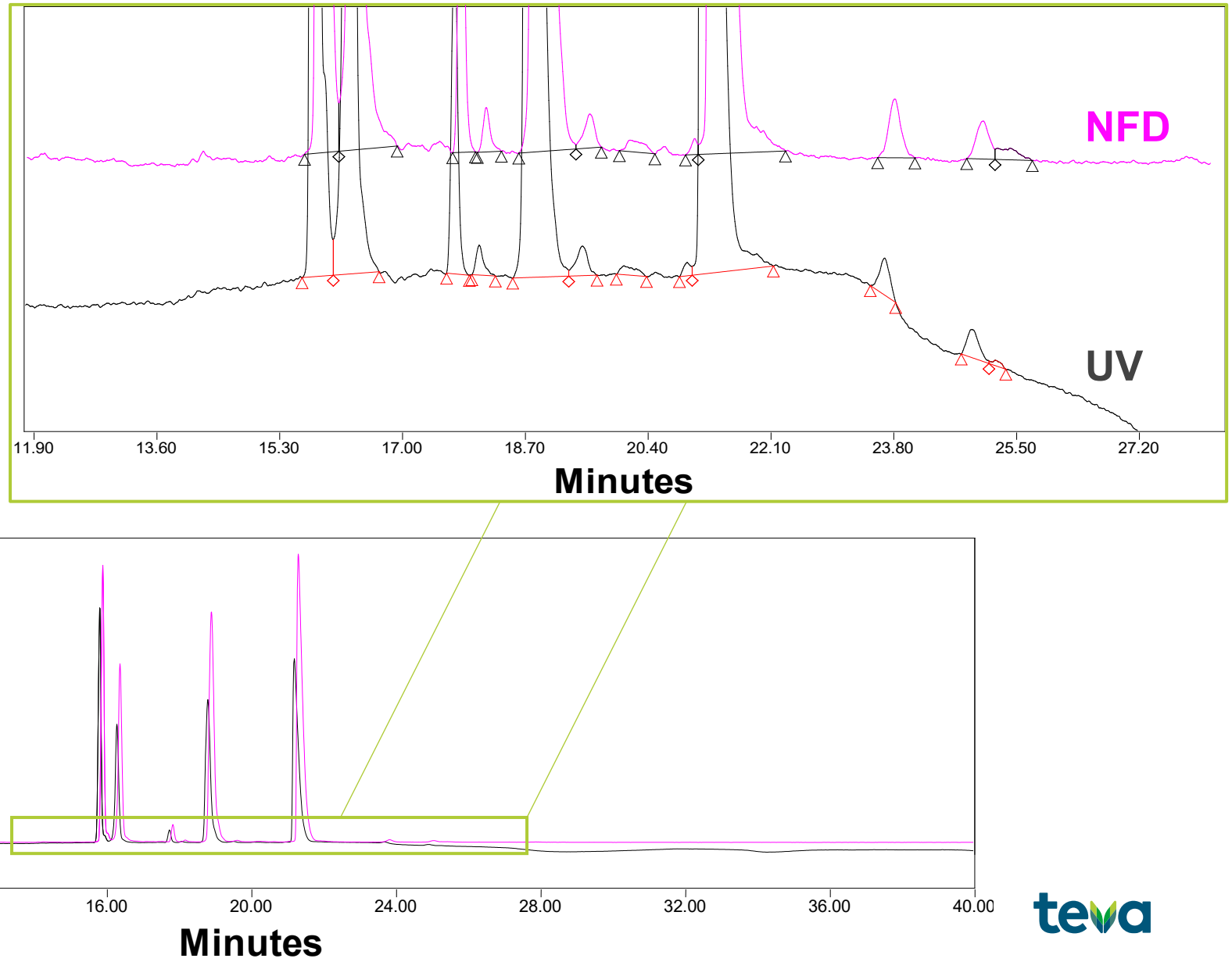
Detection Method		Results	Individual Peak %TCA													
		%Sum of Major Peaks	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13	Peak 14	New Peak 1
UV	$Average_{n=3}$	86.1	0.5685	0.0566	1.6547	27.8632	0.0524	0.3759	0.0151	0.3615	8.3736	1.8148	0.2557	58.2235	0.1723	0.2124	ND
	$\%RSD_{n=3}$	0.1	1.4	14.2	0.3	0.9	5.9	20.2	23.0	2.4	0.9	0.5	8.0	0.5	4.3	10.4	N/A
NFD	$Average_{n=3}$	86.3	0.4621	0.0579	1.5409	30.1519	0.0471	0.3484	0.0181	0.4083	7.6845	1.7856	0.2600	56.1620	0.7124	0.3190	0.0420
	$\%RSD_{n=3}$	0.1	0.6	1.6	0.6	1.0	5.0	3.8	9.1	1.2	0.5	0.8	1.3	0.6	2.6	4.9	13.5

Similar to R-CGE results, the digested sample shows differences in the %TCA values for the largest peaks between UV and NFD



Reduced CGE: Digested Sample

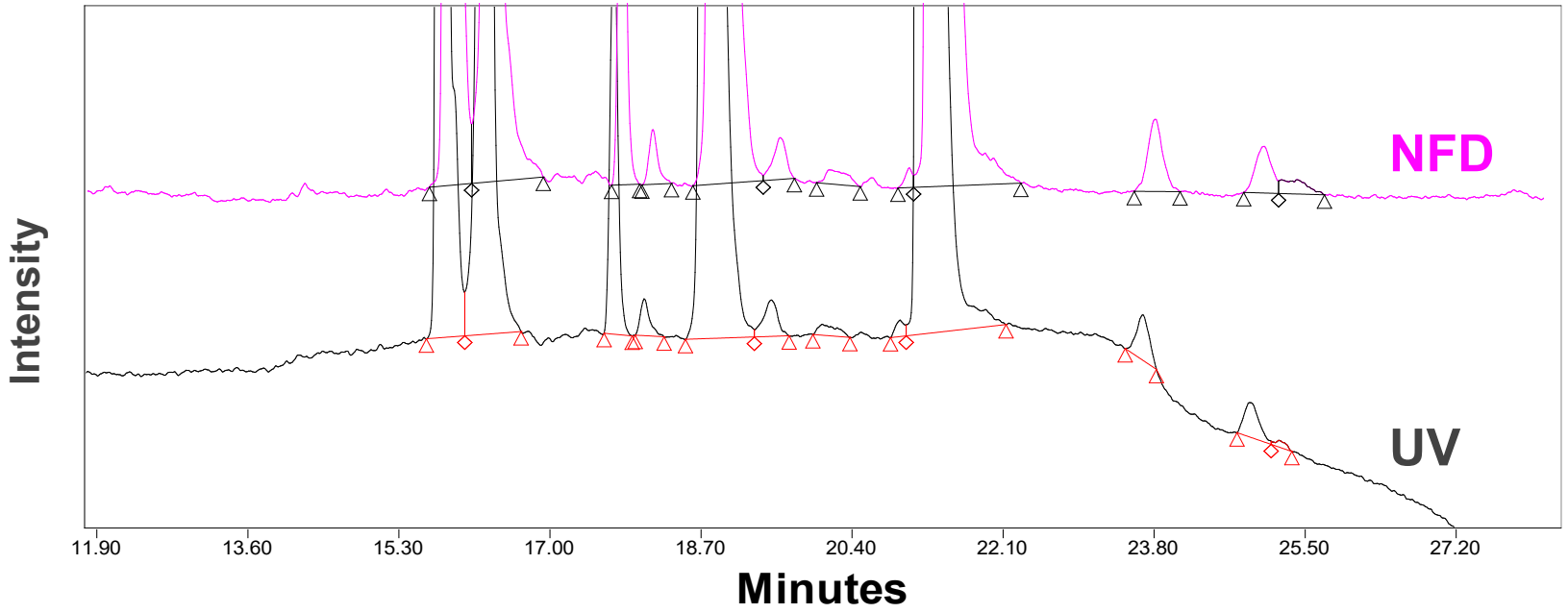
- All peaks detected in UV were also detected in NFD
- Some differences in major peak %TCA values, but overall results are comparable



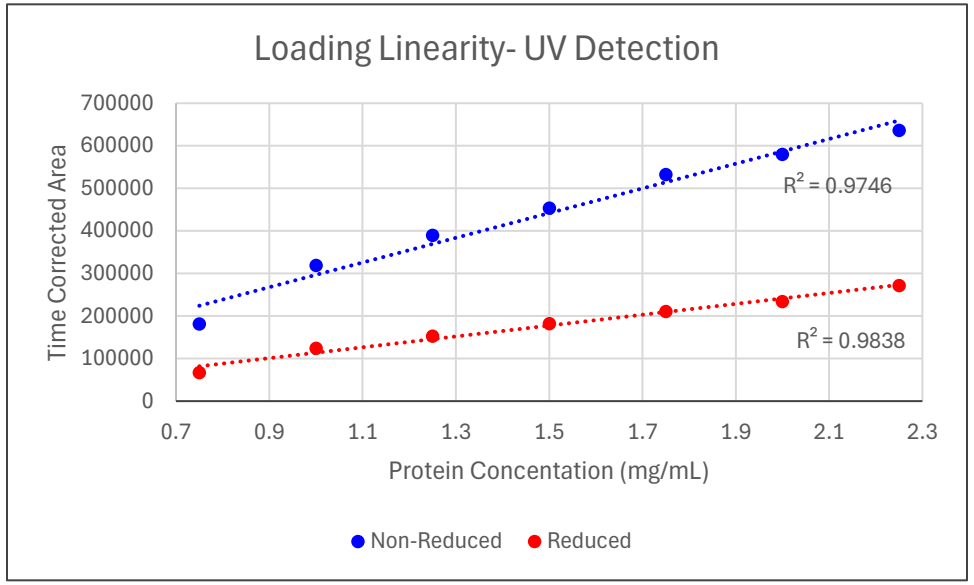
Reduced CGE: Digested Sample

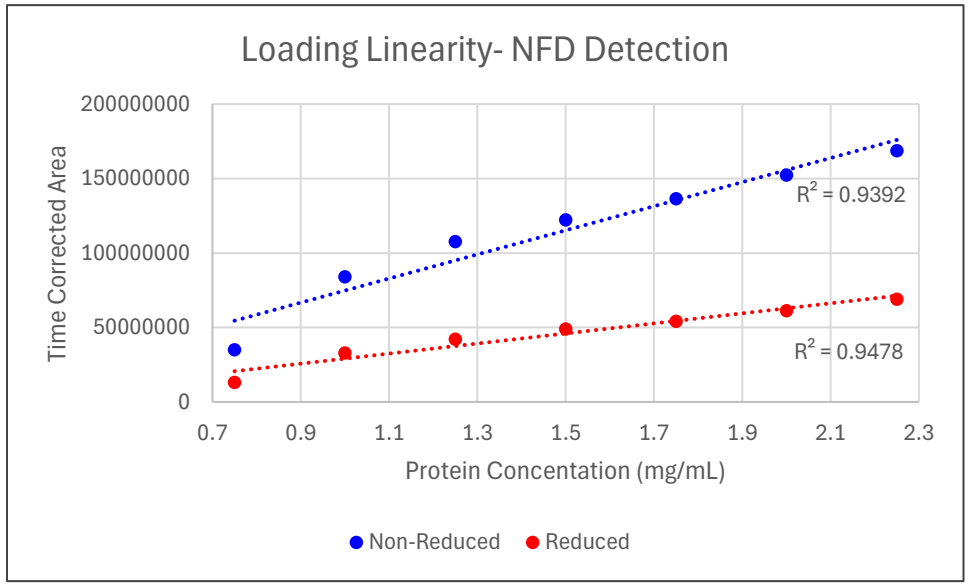
Detection Method		Results	Individual Peak %TCA											
		%Sum of Major Peaks	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12
UV	$Average_{n=3}$	97.9	26.6813	16.6956	1.3306	0.1232	23.9512	0.1784	0.0857	0.0435	30.5209	0.2387	0.1436	0.0224
	$\%RSD_{n=3}$	0.1	0.2	0.7	0.7	11.2	0.2	11.1	6.5	22.1	0.2	35.2	7.2	N/A
NFD	$Average_{n=3}$	97.8	21.5679	17.2239	1.2367	0.1558	26.3929	0.1629	0.0816	0.0458	32.6087	0.2641	0.1862	0.0734
	$\%RSD_{n=3}$	0.0	0.2	0.0	0.2	5.7	0.1	6.4	6.2	17.0	0.1	5.7	9.6	7.8

Similar to R-CGE results, the digested sample shows differences in the %TCA values for the largest peaks between UV and NFD



Loading Linearity



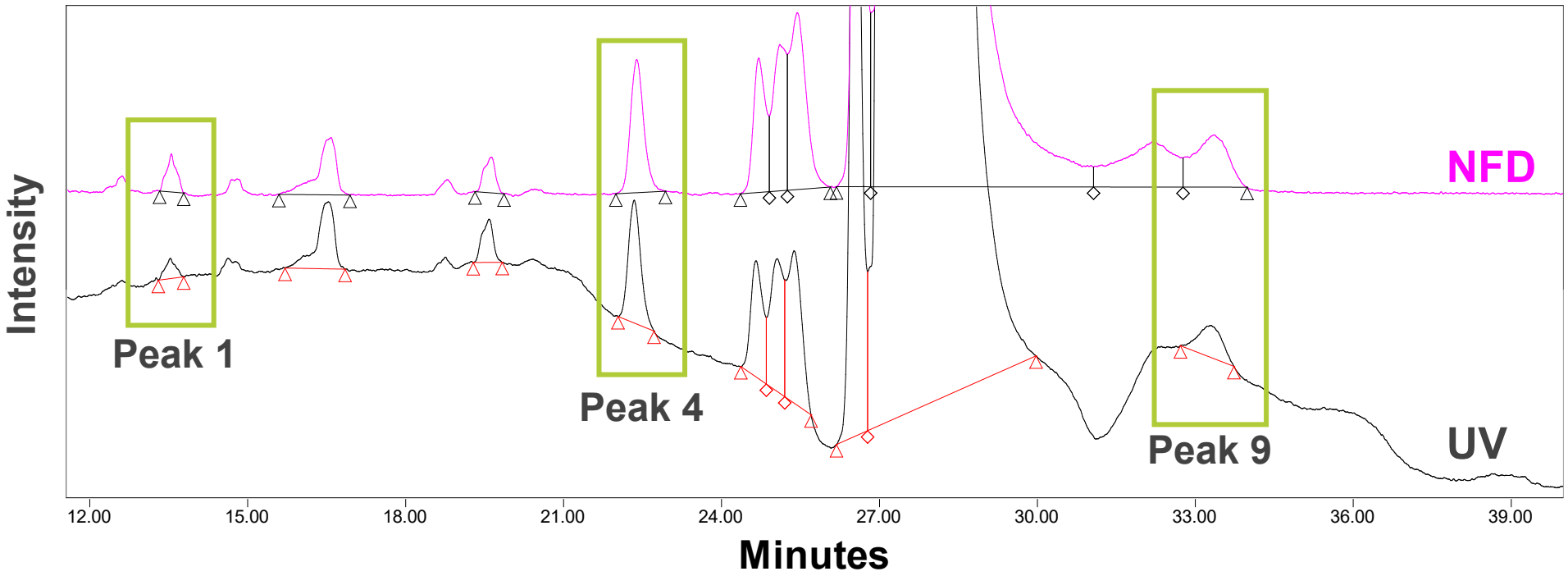


- A test molecule was prepared at 7 final protein concentrations under reduced and non-reduced conditions
- Each concentration was injected 3 times and detected using both UV and NFD
- The 3 injections were averaged and used to determine the linearity of each detection method

LOQ

- The data from the loading linearity study was then employed to investigate any differences in LOQ that may exist between UV and NFD
- Three minor peaks were selected based on the baseline interference present in the UV trace
- Precision and Accuracy was calculated throughout the tested range of 0.75-2.25 mg/mL (50-150% of the target concentration of 1.5 mg/mL) for each selected peak
- Based on passing Precision and Accuracy results, LOQ was calculated using the following equation:

$$\text{LOQ} = \left(\frac{\text{Avg}_{n=3}\text{TCA of minor peak at lowest passing level of the range}}{\text{Avg}_{n=3}\text{ Total TCA at the target level of the range}}\right) \times 100$$



LOQ

UV

Peak 1: 0.07 %TCA

- Range= 50-150%
- Precision at 50%= 18.7%
- Precision at 100%= 11.3%
- Accuracy at 50%= 118%
- Calculated LOQ= 0.03%

Peak 4: 0.25 %TCA

- Range= 50-150%
- Precision at 50%= 5.1%
- Precision at 100%= 2.4%
- Accuracy at 50%= 102%
- Calculated LOQ= 0.10%

Peak 9: 0.10 %TCA

- Range= 133-150%
- Precision at 50%= 12.1%
- Precision at 100%= 20.6%
- Accuracy at 50%= 200%
- Calculated LOQ= 0.11%

NFD

Peak 1: 0.10 %TCA

- Range= 50-150%
- Precision at 50%= 6.1%
- Precision at 100%= 3.7%
- Accuracy at 50%= 104%
- Calculated LOQ= 0.03%

Peak 4: 0.29 %TCA

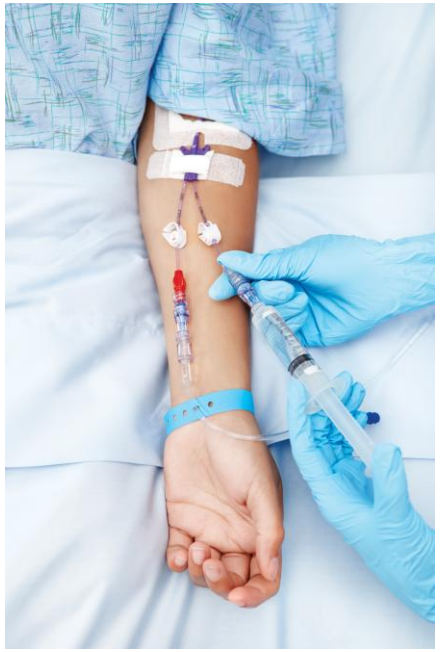
- Range= 50-150%
- Precision at 50%= 0.8%
- Precision at 100%= 6.7%
- Accuracy at 50%= 96%
- Calculated LOQ= 0.08%

Peak 9: 0.19 %TCA

- Range= 50-150%
- Precision at 50%= 11.3%
- Precision at 100%= 3.7%
- Accuracy at 50%= 128%
- Calculated LOQ= 0.07%

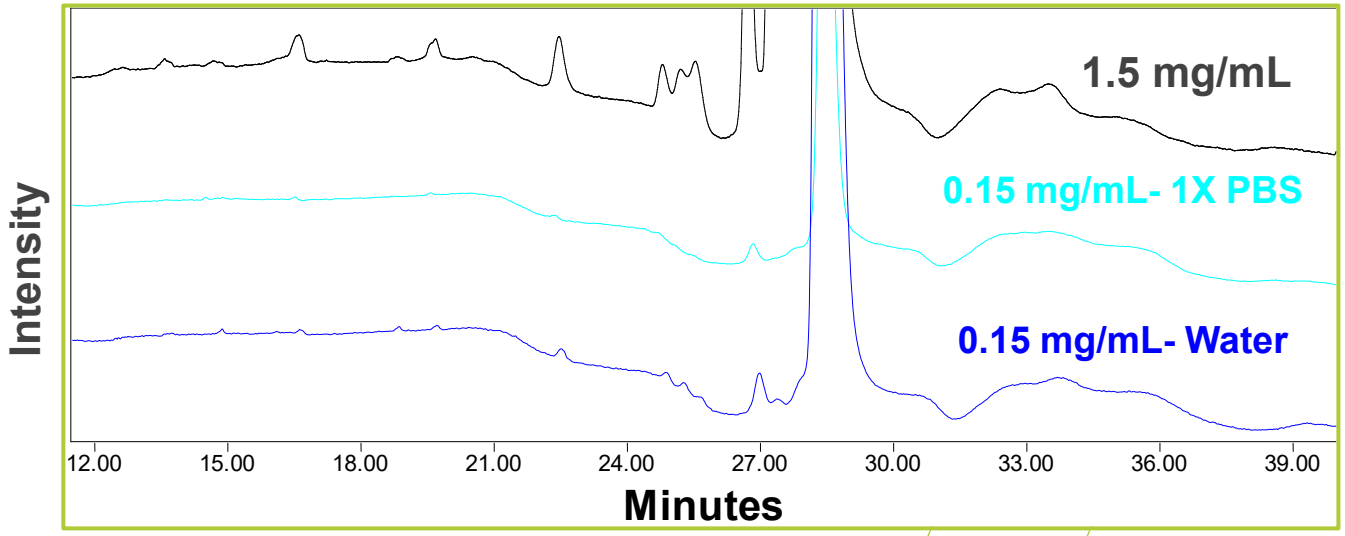
Applications in Low Concentration Samples

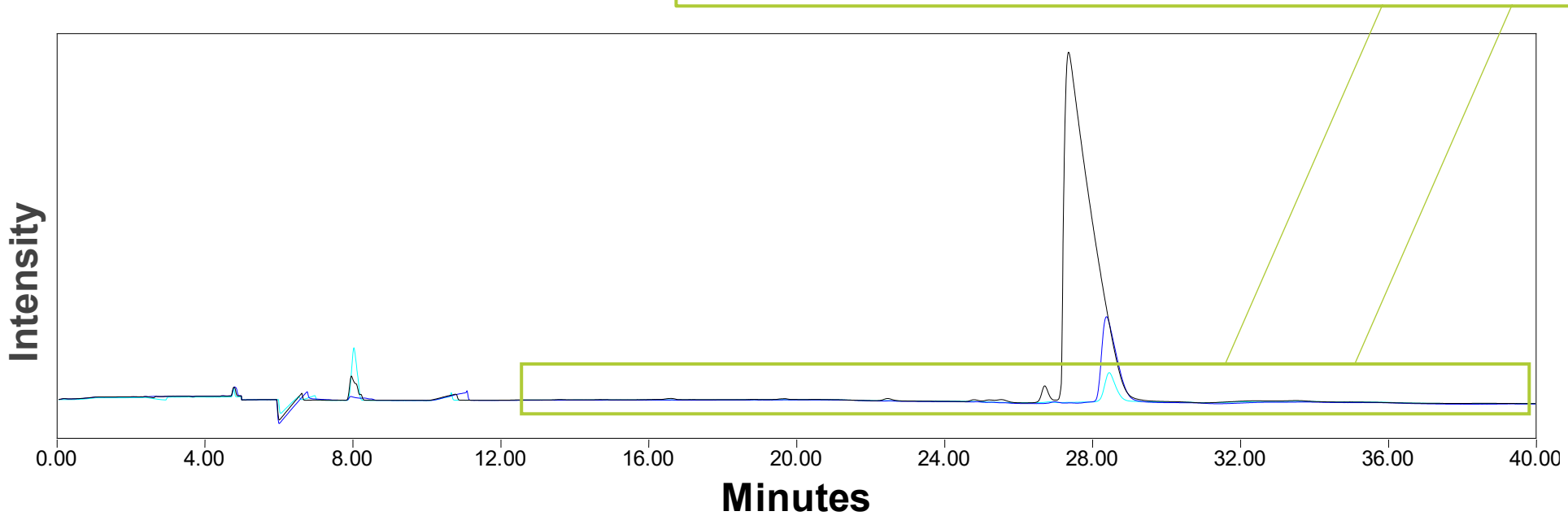
- Low concentration samples are common:
  - Highly potent drugs to be delivered at lower doses require low DP concentrations
  - Downstream processing is often done at low concentrations and in non-formulated matrices
  - Clinical in-use studies mock protein delivery in intravenous delivery systems at low concentrations and in different matrices (dextrose, saline, etc.)
- Typical approach to testing low concentration samples:
  - Modify sample injection
  - Modify sample preparation
  - Implement buffer exchange
  - Utilize orthogonal chip-based method



Low Concentration Samples: UV Detection

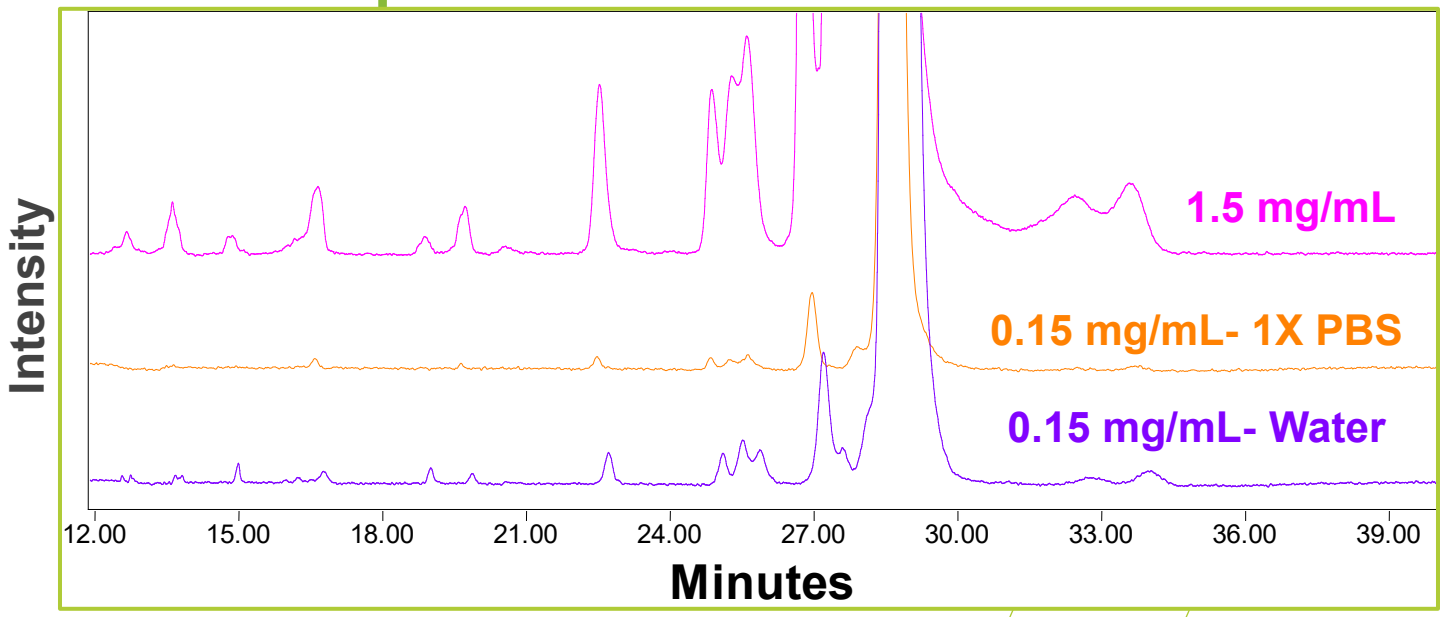
- In low concentration samples, only main peaks are detectable under standard injection and preparation conditions
- Modification of sample preparation and/or injection is **required** for sample analysis

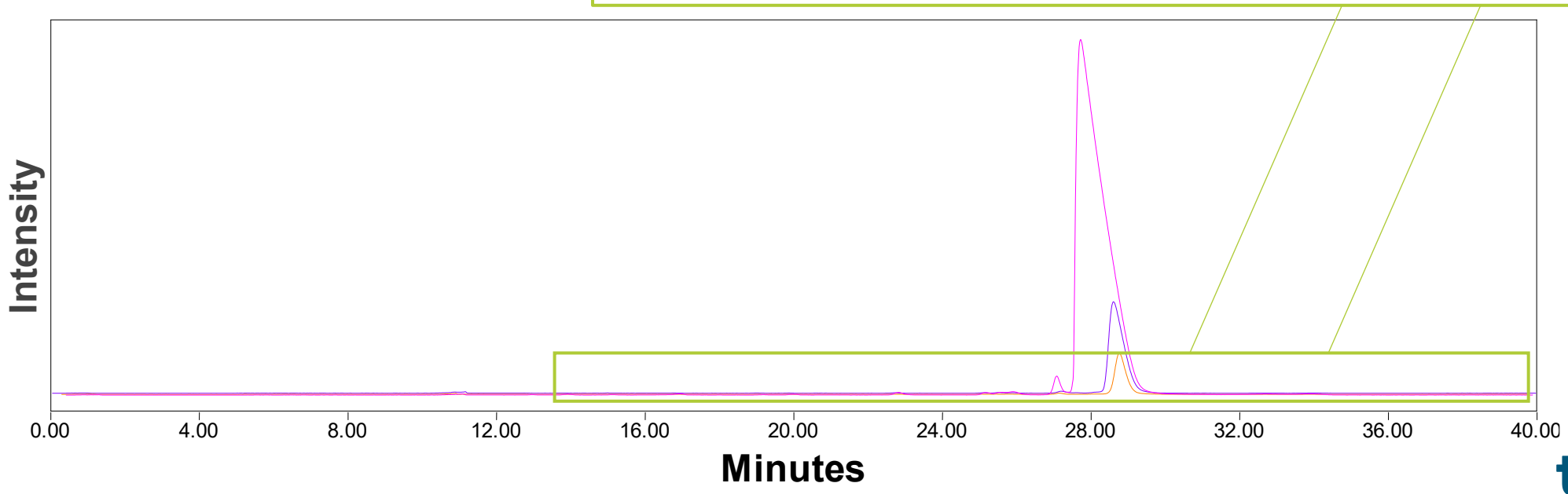




Low Concentration Samples: NFD Detection

- In low concentration samples, all integrated minor peaks are detectable
- Modification of sample preparation and/or injection may be employed for ease of analysis particularly with interfering matrices





Final Thoughts: QC Applications

- Standard GMP practices currently discourage manual integration
  - Current practice in QC labs often involves tailoring the processing method to each sample set, to properly integrate samples and account for baseline changes without the need for manual intervention
- NFD offers the potential for significant improvements due to lack of baseline interference
  - Automatic integration is applicable without processing method modification
  - Notable time savings in data processing for analyst and reviewers
  - Improved precision due to integration consistency
  - Provides confidence when identifying new peaks
- However, to best fit GMP workflow needs (particularly QC labs), NFD would need to be paired to a single capillary system

Thoughts from Pre-Commercial Evaluation

- Thus far, all peaks present in UV have been detectable using NFD
  - Assessments may have to be done on a molecule-by-molecule basis prior to routine use
- Similar reported results between UV and NFD
  - Increase in HC:LC in NFD for some molecules, but reported results remain consistent between UV and NFD
- Potential for improvements in Range due to increased signal intensity in NFD
  - Range can potentially be extended to include lower concentrations
  - Increased signal intensity helps to decrease the complications of salt interference

- Further optimization possible for NFD application:
  - Unable to normalize UV and NFD signals in BioPhase Software to overlay traces
  - Single capillary instrument equipped with NFD
  - Simultaneous collection of UV and NFD

- Benefits of NFD can be applied to analysis of charge variants as well
  - Check out our poster, Further Exploration of Native Fluorescence Detection in Capillary Electrophoresis, at the poster sessions

Acknowledgments

Teva

- Diane Gingrich, *Senior Director, CMC Biologics*
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- AD Separations Team, *CMC Biologics*

Sciex

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- Kerstin Pohl

Thank You!

Questions?