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

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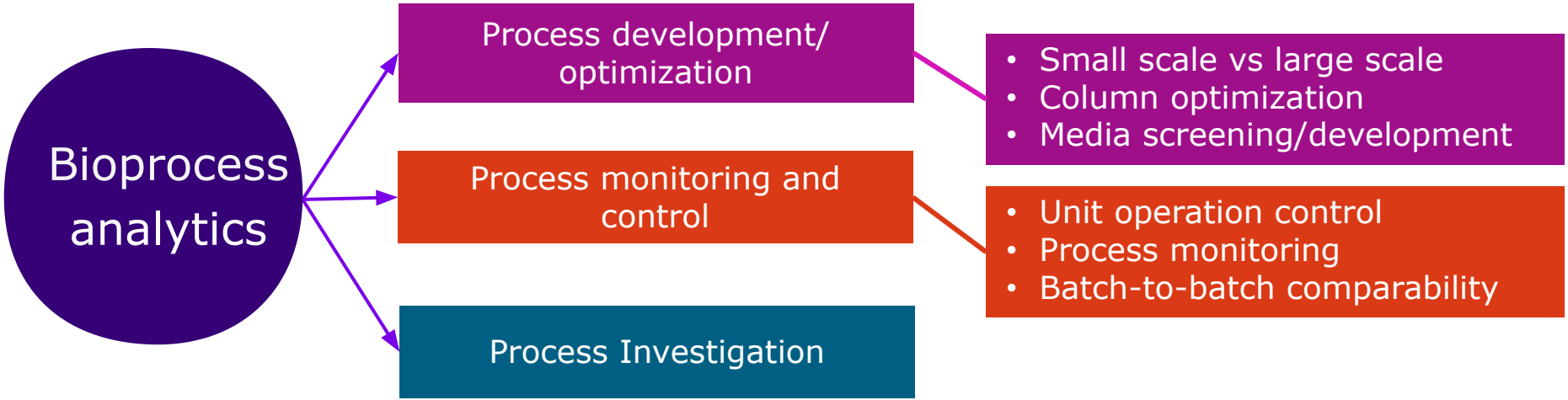
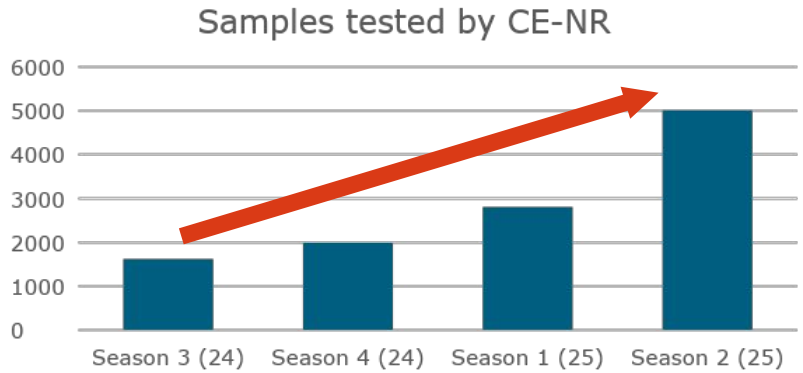
A high throughput analysis is needed to support screening large numbers of samples



- Supports the analytical needs of different functions and aspects.
- Sample throughput and fast data turnaround are key enablers for process development and optimization.

Sensitivity is key for speed-up the early-stage bioprocess development



Early-stage bioprocess analytics requires high sensitivity assays.

High throughput purity analysis requires chemical labeling or dyes for improved sensitivity.

- Inconsistent label
- Dye peaks
- dye artifacts
- Extra works

Ambr 250

Monitoring over time

Purity analysis

CE-SDS

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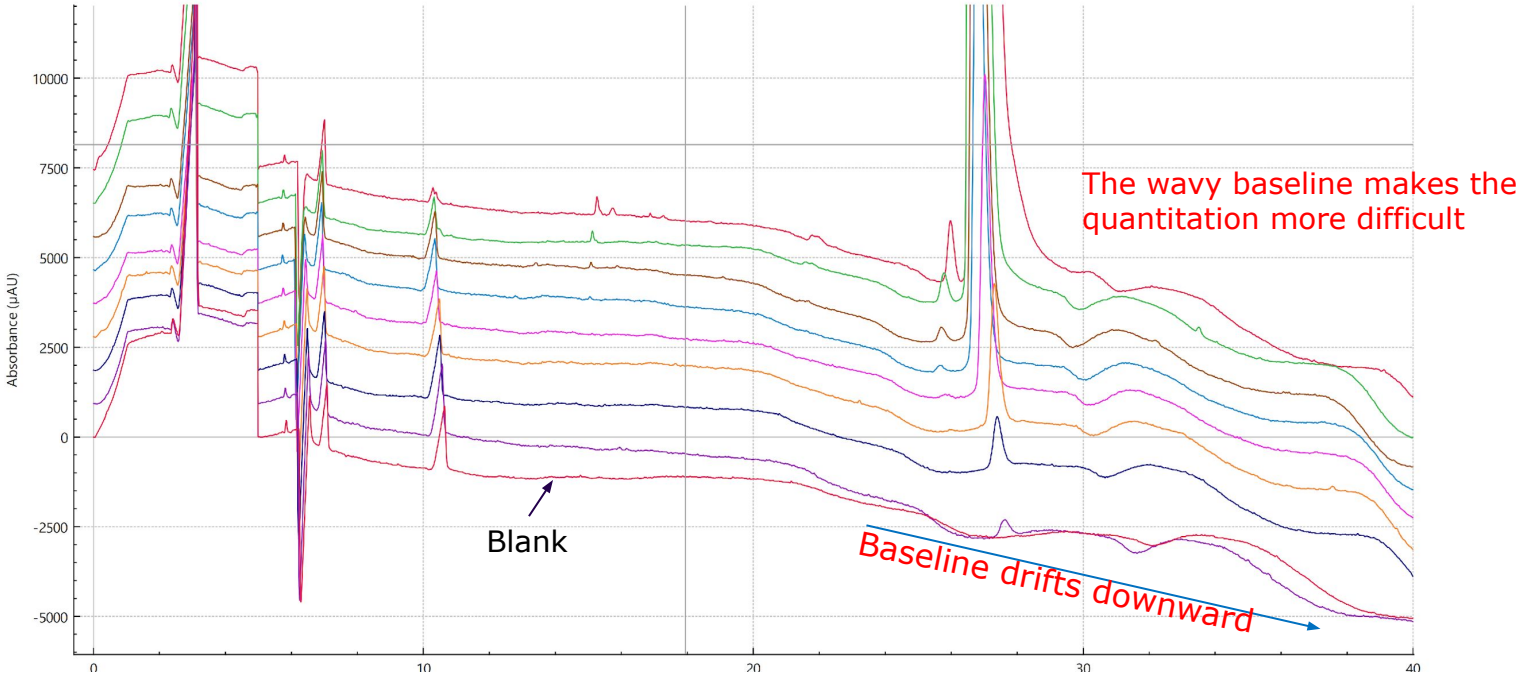
UV Detection is not sensitive enough for testing the low abundance bioprocess samples

Eight dilutions were tested as 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04, 0.02 (mg/mL)



High-throughput BioPhase 8800 system with Native Fluorescence (NF) Detection

PA 800 Plus

Next generation

BioPhase 8800 system with NF Detection

The BioPhase 8800 system
- **Running 8 capillaries in parallel**
- **Native fluorescence detection added**
- **Enhanced sensitivity with NF**

About Native Fluorescence: **Signals generated from aromatic amino acids exist in most proteins**

- Arises naturally from the protein's own amino acids in their excited state.
- Requires no external reagents or chemical modifications.
- Tryptophan, tyrosine, and phenylalanine are the primary contributors to protein fluorescence.
  - mAbs have multiple conserved tryptophan and tyrosine residues in the Fab region. In addition, the Fc region also contains multiple conserved tryptophan and tyrosine residues[1].
- These amino acids absorb UV light (typically around 280 nm) and emit fluorescence at longer wavelengths—around 350 nm for tryptophan.

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[1] Stephen R. Campion, Conserved aromatic residues as determinants in the folding and assembly of immunoglobulin variable domains, Molecular Immunology, Volume 70, 2016, Pages 63-71

Three monoclonal antibodies were selected for Native Fluorescence (NF) Detection study

Three monoclonal antibodies- mAb1, mAb2 and NIST IgG as examples

Eight dilutions were tested as 2.50, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04, 0.02 (mg/mL)

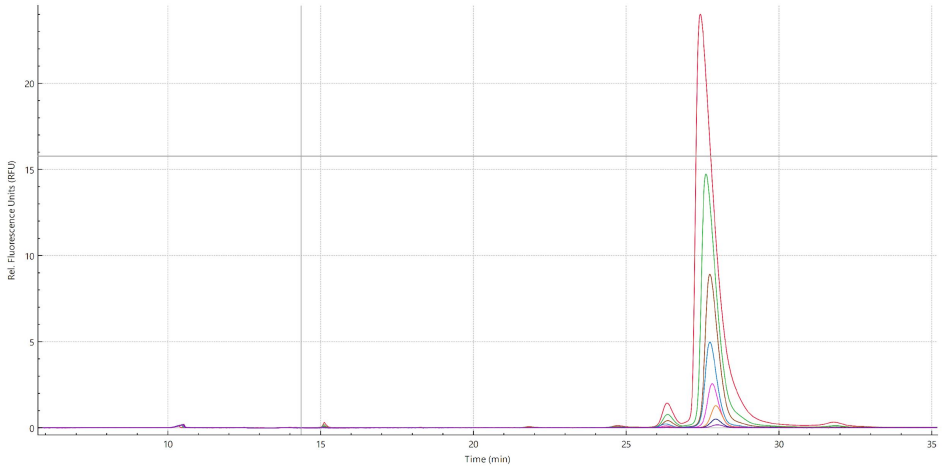
- Three mAbs prepared with eight dilutions and blank were tested on two different detectors (UV, NF)
- Limit of detection (LOD) were determined
  - The detection limit (DL) can be expressed as: $DL = 3.3\sigma / S$
  - where σ = the standard deviation of the response
  - S = the slope of the calibration curve

The minimum detectable response was determined by 3.3 * σ of baseline signal response + baseline signal mean.

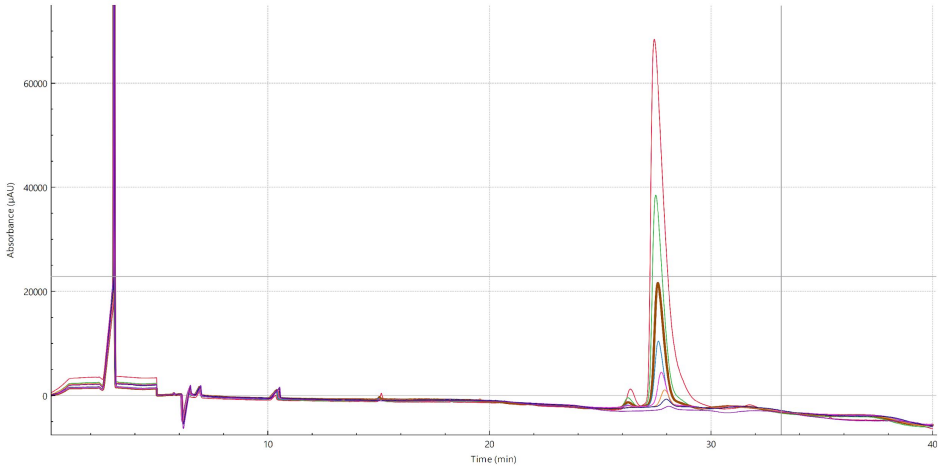
The LOD were calculated by using the minimum detectable signal as Y and solve X (concentration)

Overlay of NF and UV e-grams for eight mAb1 dilutions: NF shows cleaner profiles

NF Detection



UV Detection



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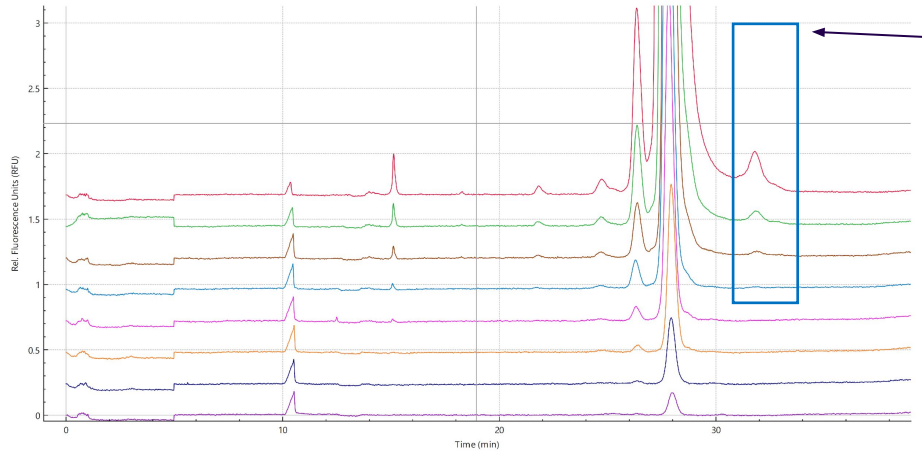
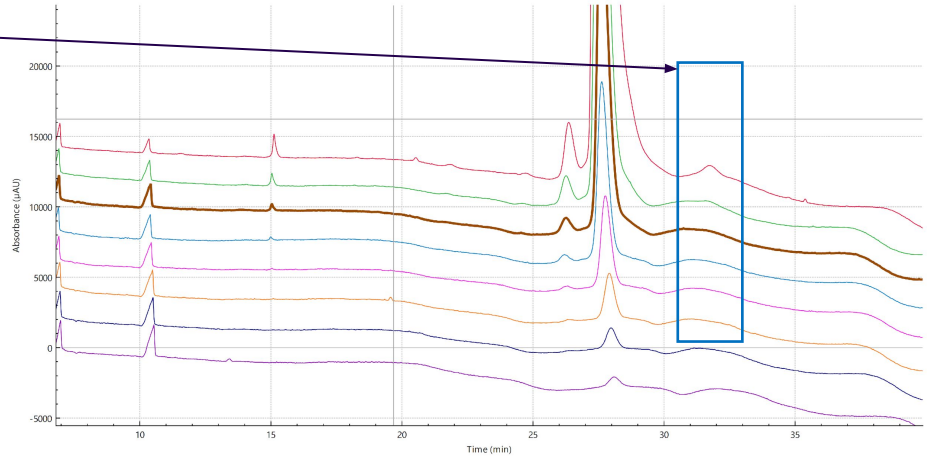
Overlay of NF and UV e-grams for eight mAb1 dilutions (Baseline zoom-in): NF's straight baseline simplifies analysis

NF Detection



UV Detection

The wavy baseline makes the peak assignment more difficult



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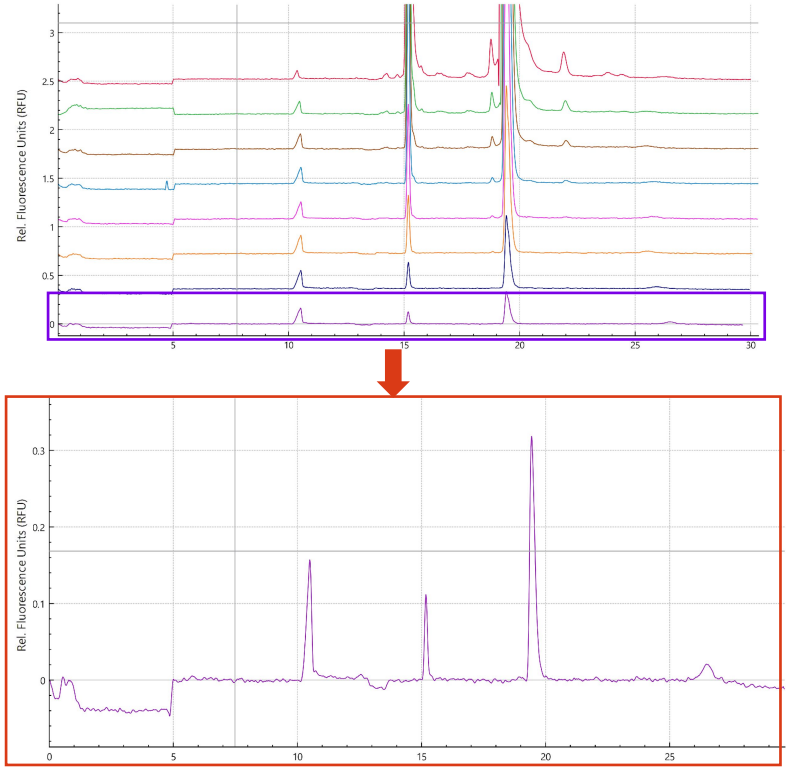
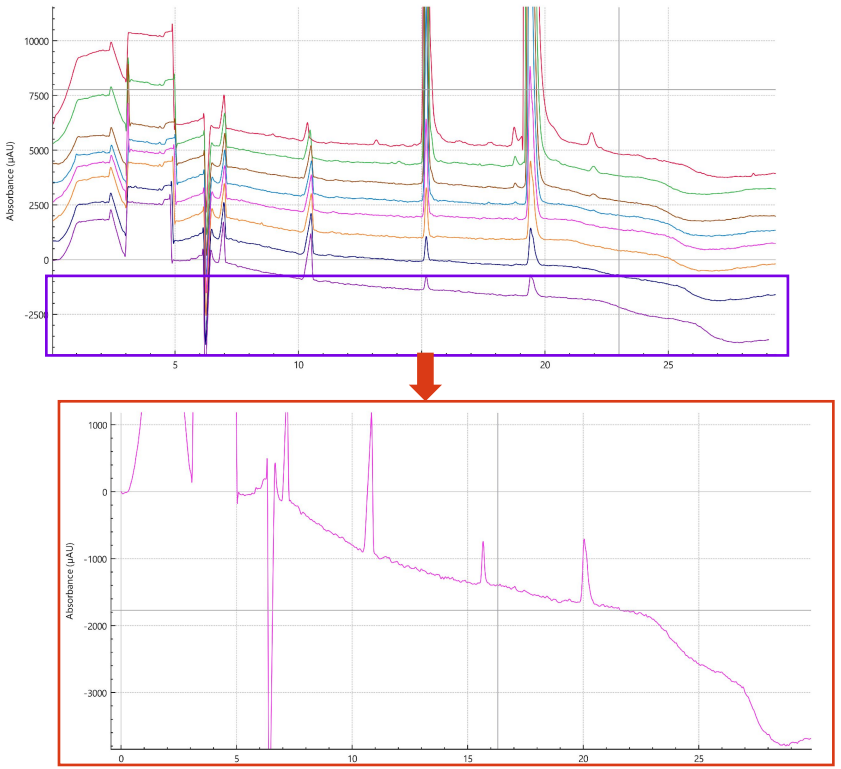
Comparison of NF and UV e-grams for eight mAb1 dilutions (Baseline zoom-in, reduced method): NF shows better baseline

NF Detection



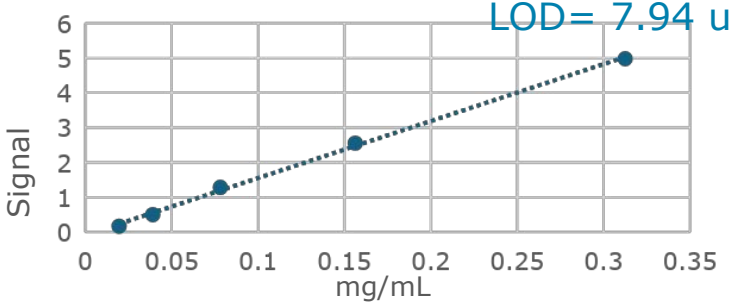
UV Detection



The NF detection of mAb1 shows >10× higher sensitivity than UV detection

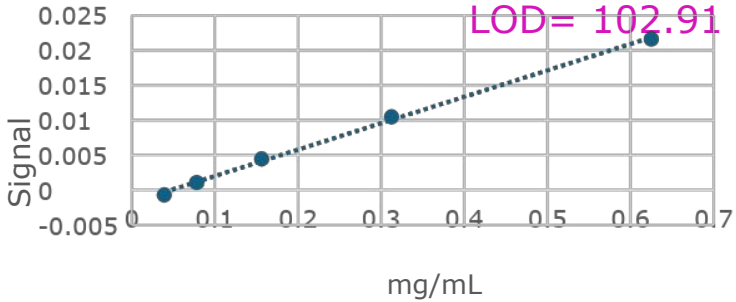
NF

LOD= 7.94 ug/mL



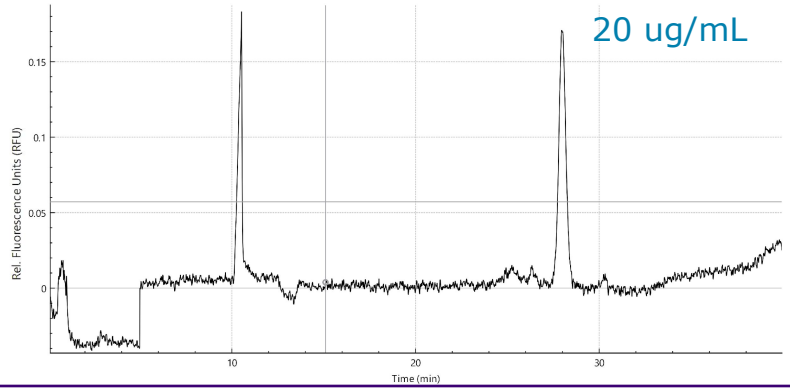
UV

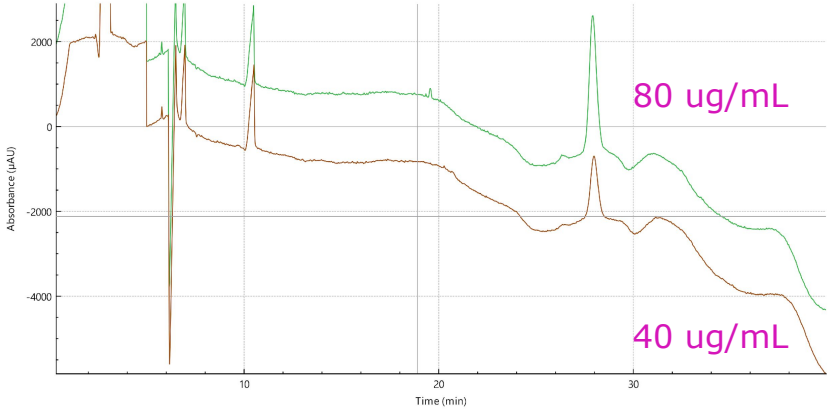
LOD= 102.91 ug/mL



E-gram example

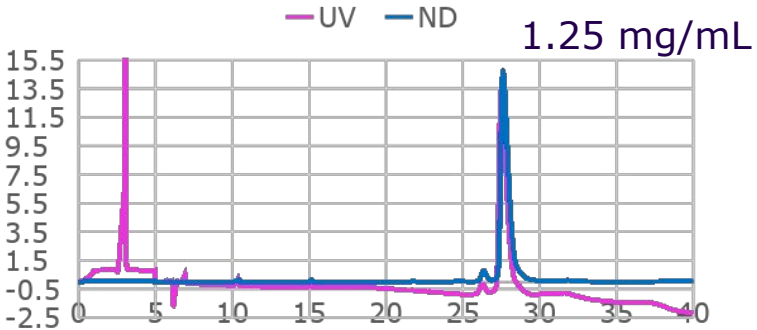
20 ug/mL

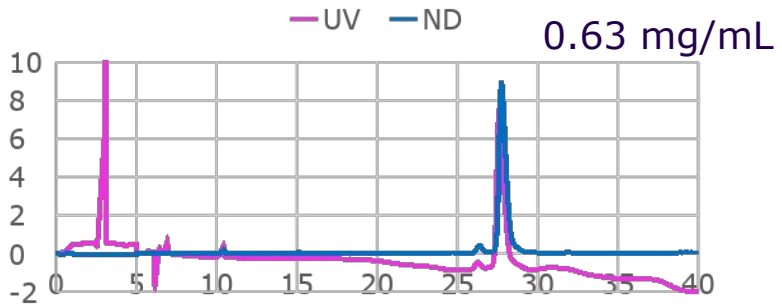


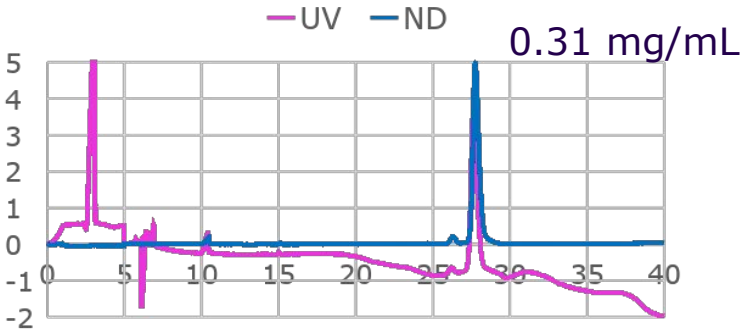


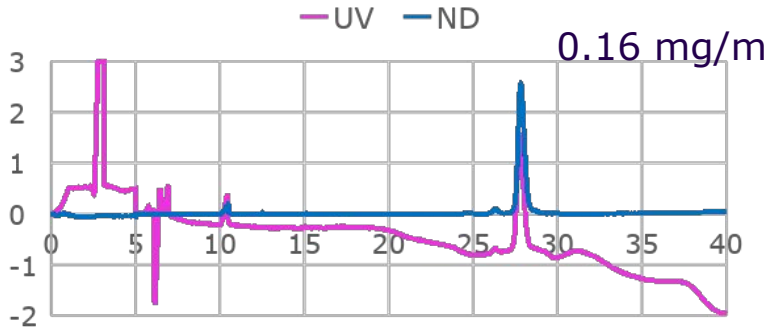
UV peak integration becomes more complex at lower sample levels

Raw data was re-scale to preform an overlay comparison use mAb1 for example



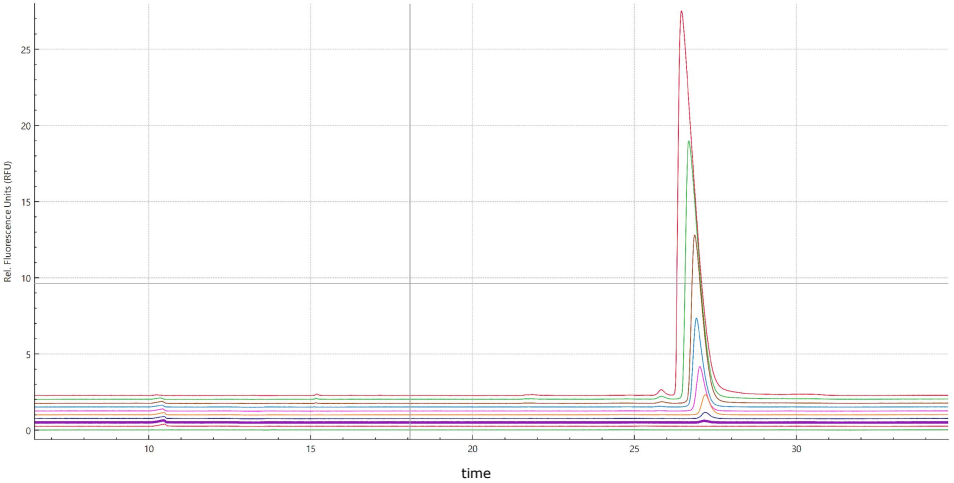




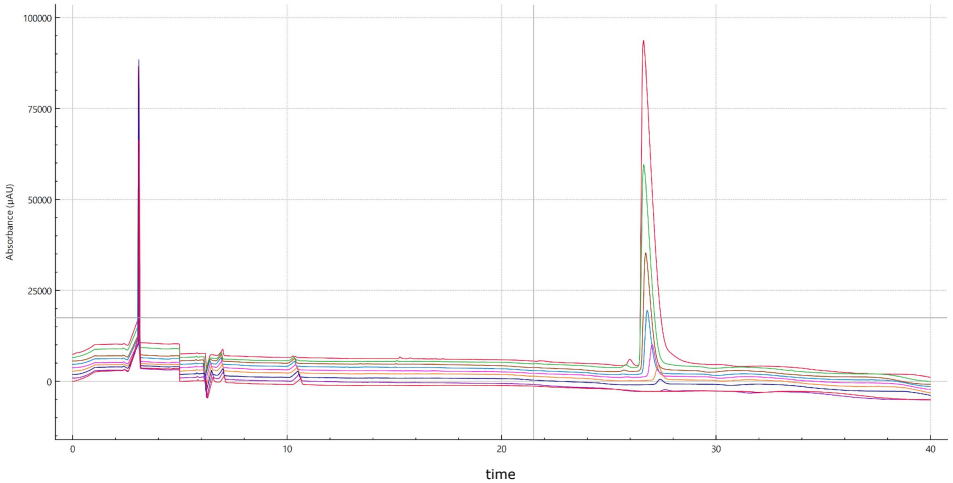


Overlay of NF and UV e-grams for eight mAb2 dilutions: NF shows cleaner profiles

NF Detection



UV Detection



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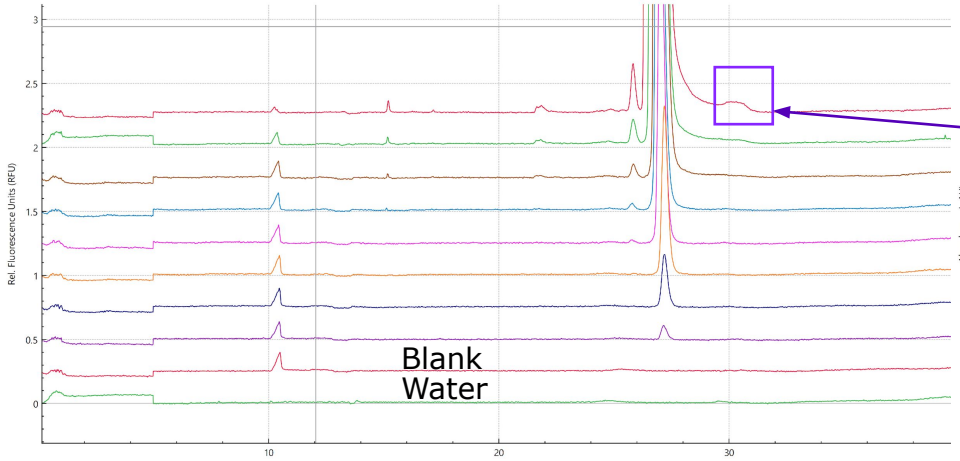
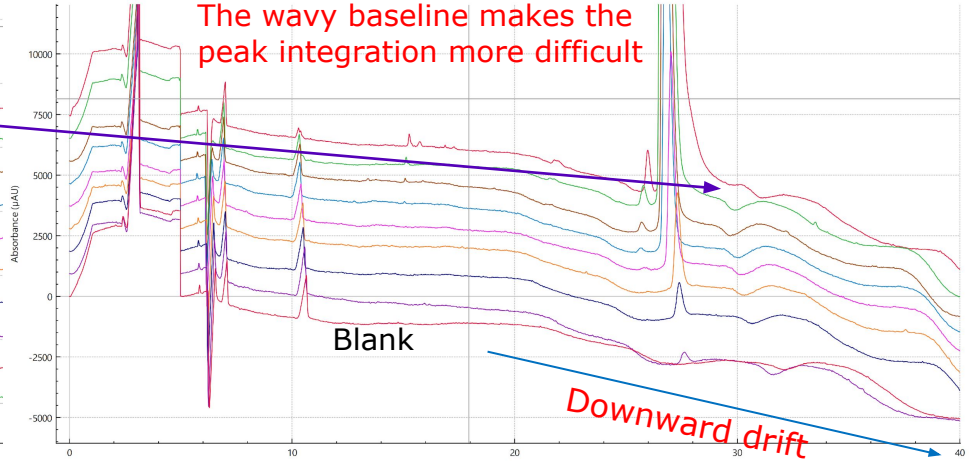
Overlay of NF and UV e-grams for eight mAb2 dilutions (Baseline zoom-in): NF's straight baseline simplifies analysis

NF Detection

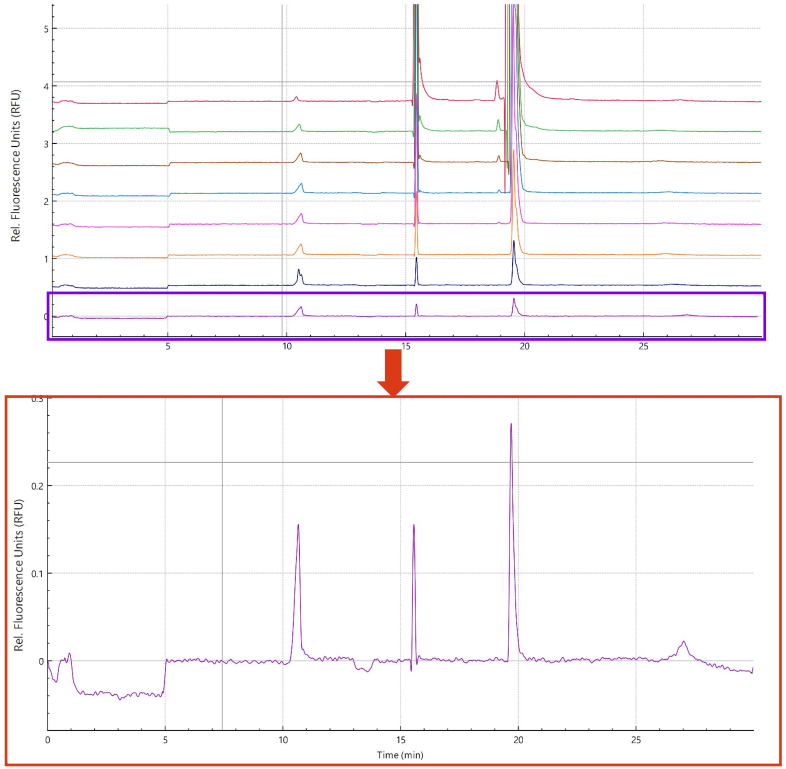


UV Detection

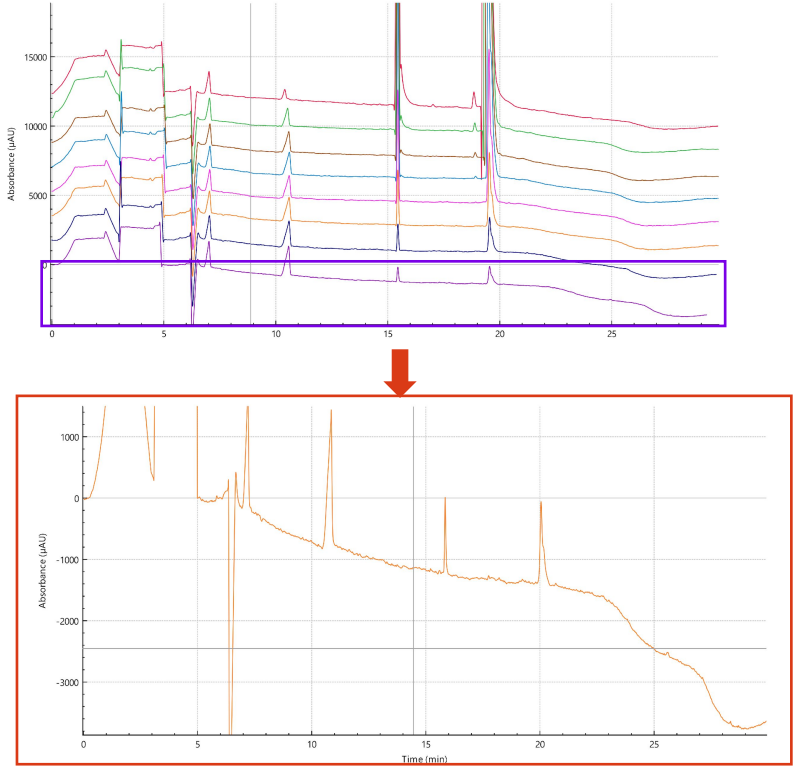


Comparison of NF and UV e-grams for eight mAb2 dilutions (Baseline zoom-in, reduced method): NF shows better baseline

NF Detection

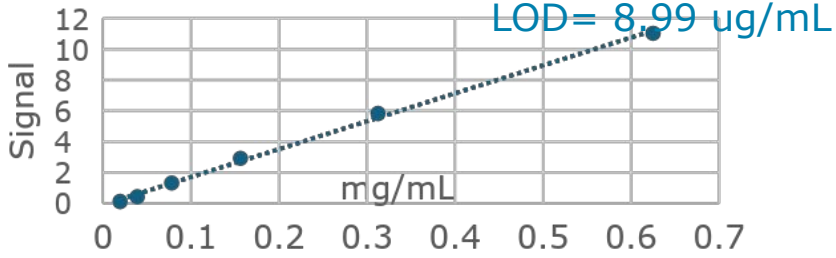


UV Detection

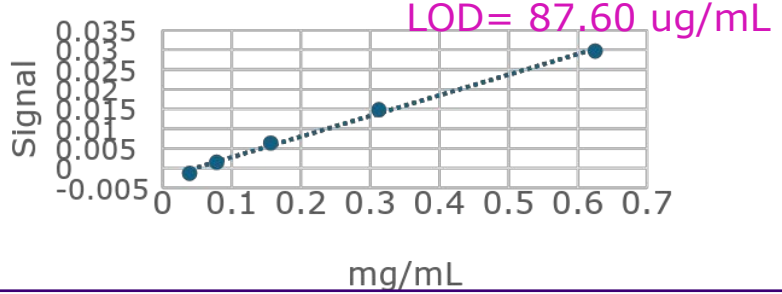


The NF detection of mAb2 shows ~10× higher sensitivity than UV detection

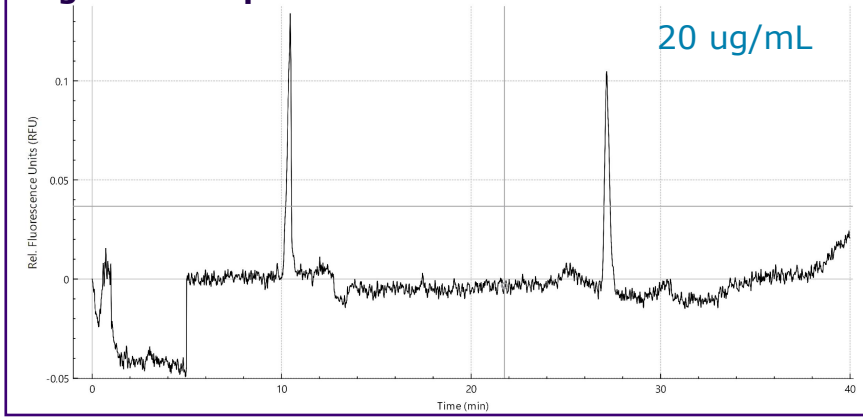
NF

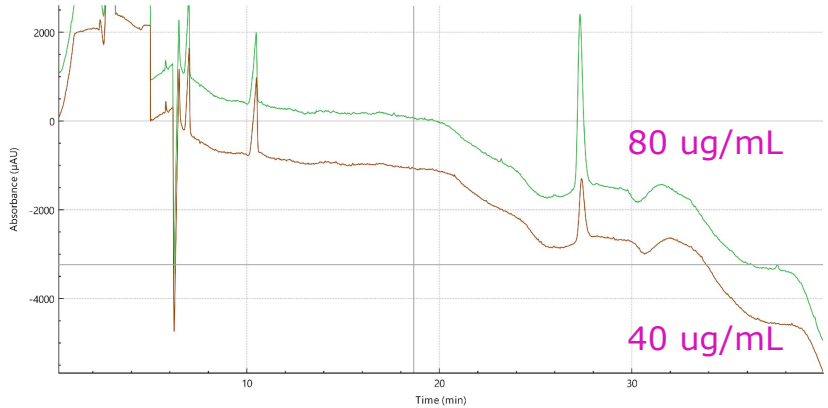


UV



E-gram example

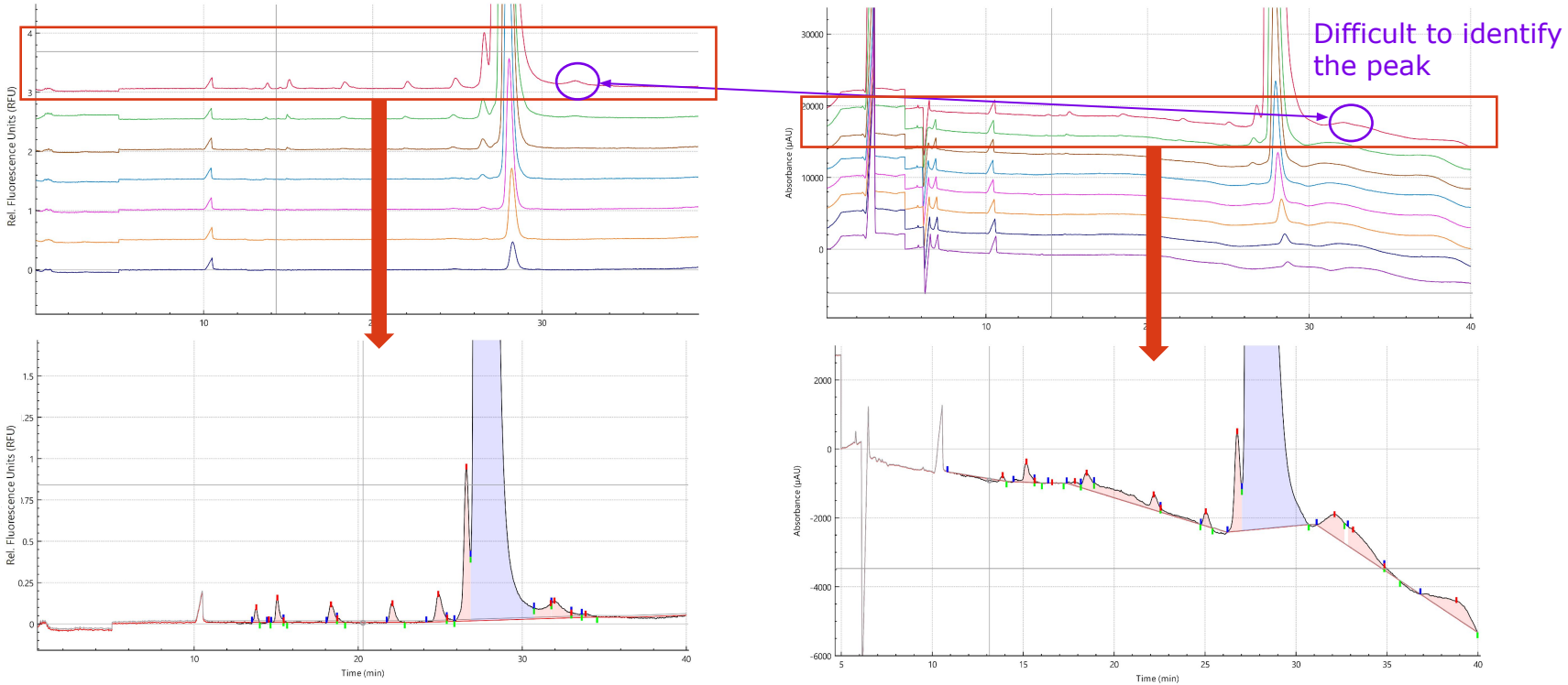




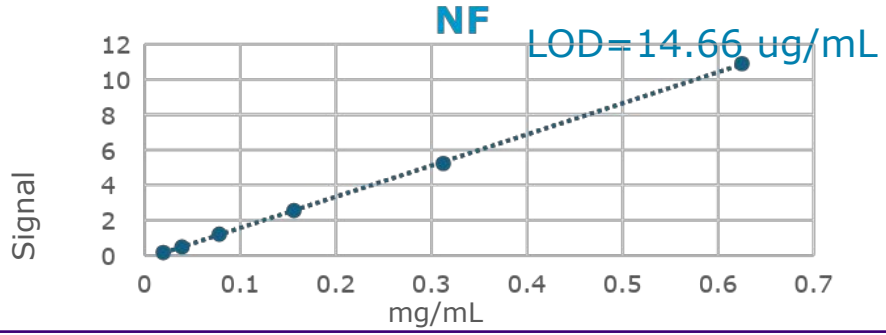
Overlay of NF and UV e-grams for eight NIST IgG dilutions (Baseline zoom-in): NF's straight baseline simplifies analysis

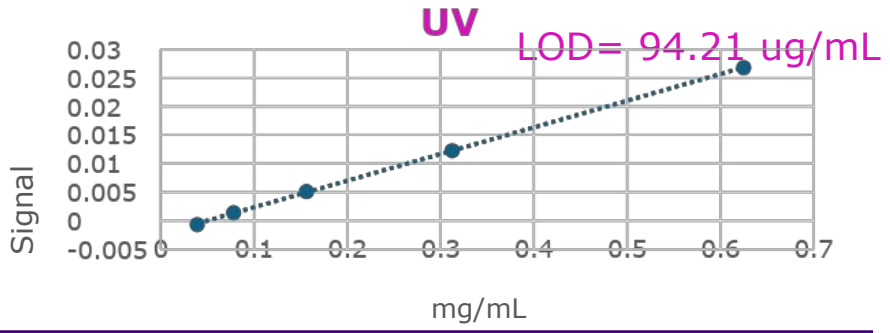
NF Detection

UV Detection

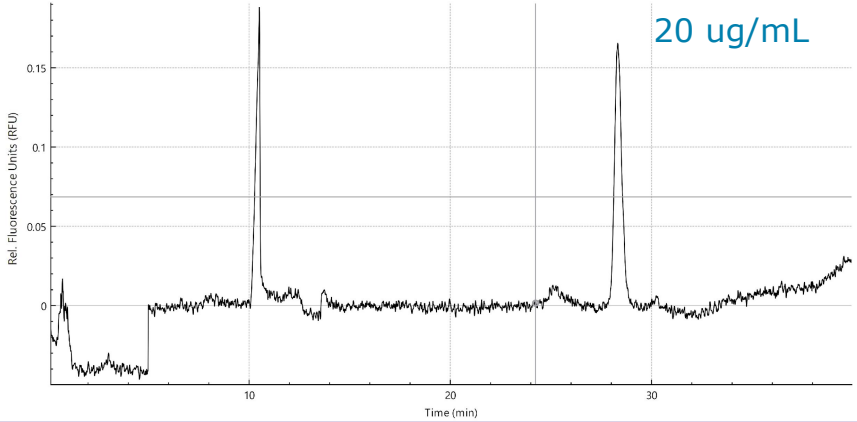


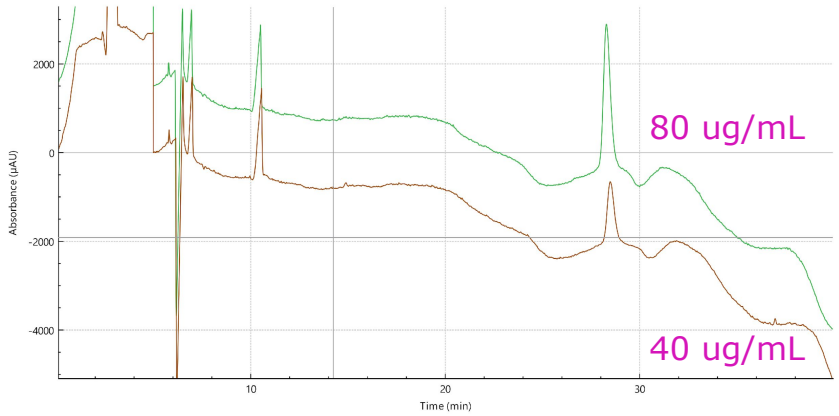
The NF detection of NIST IgG shows 7× higher sensitivity than UV detection





E-gram example





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Conclusion

Protein purity analysis remains a cornerstone of biochemical research, where precision and efficiency are critical. This study highlights the application of multi-capillary electrophoresis integrated with label-free native fluorescence (NF) detection for high-throughput protein analysis (Running 8 capillaries at the same time).

The approach offers several key advantages:

- **Linear dynamic range**
- **Improved Sensitivity (up to 10X)**
- **High-throughput**
- **Stable, Flat baseline for better peak integration**
- **Streamlined Method Transfer and Bridging Strategy (PA 800 plus and BioPhase 8800)**

By eliminating the need for labeling, NF detection preserves the native state of proteins, improving quantitation accuracy and reducing variability. This not only minimizes human error but also enhances assay Precision or Intermediate precision. —especially important for complex protein samples

Acknowledgements

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