Enhance Productivity in Biomolecular Charge Heterogeneity Analysis from Discovery to Product Release:

Introducing the Maurice icIEF 400 Cartridge

Introduction

As the name suggests, the new **Maurice icIEF 400 cartridge** gives you up to 400 injections! Suitable for up to 40 batches, this extended sample capacity of the cartridge doesn't just save you capital but also helps reduce material waste.

While designed to increase output, the new icIEF 400 cartridge offers the same resolution and data quality as the popular **Maurice cIEF cartridge**, which offers up to 200 injections. To demonstrate comparability between the two cartridges, this study illustrates the charge heterogeneity analysis of three molecules—NISTmAb, and biosimilars of Cetuximab and Rituximab—side-by-side using both cartridges. Data are presented on charge profiles, reproducibility, limit of detection (LOD), and dynamic range. For brevity, the icIEF 400 Cartridge is referred to as IEF 400, and the cIEF Cartridge is IEF 200 throughout this technical note.

Materials & Methods

TABLE // 01

Material	Vendor	Catalog #
NISTmAb	NIST	8671
Cetuximab – Research Grade Biosimilar	R&D Systems	MAB9577
Rituximab – Research Grade Biosimilar		MAB9575
Maurice icIEF 400 Cartridge (IEF 400)	ProteinSimple, a Bio-Techne brand	**PS-MC02-400C**
Maurice cIEF Cartridge (IEF 200)		**PS-MC02-C**
Maurice cIEF Method Development Kit		**PS-MDK01-C**
Iminodiacetic Acid	Millipore Sigma	220000-500G

Table 1 lists all the materials and reagents used in this study, including the Maurice cIEF Method Development Kit which contains all the necessary reagents for analyzing charge heterogeneity.

For icIEF analysis, NISTmAb was prepared at a final concentration of 0.15 mg/mL. Cetuximab and Rituximab were prepared at a final concentration of 0.2 mg/mL. Each of the three samples was prepared in an ampholyte mixture containing Pharmalytes and other solubilizers in varying concentrations, which are listed in Table 2. The samples were loaded onto the instrument and separated for 1 minute at 1500 V, followed by separation for 8 minutes at 3000 V. The same methods were used to analyze samples with both cartridge types. Both absorbance and native fluorescence (NF) detection modes were used. All data generated with the **Maurice system** were analyzed using the **Compass for iCE software**.

TABLE // 02

Concentration of Components in Ampholyte Mixture

Components	NISTmAb*	Cetuximab	Rituximab
1% Methylcellulose (MC)	0.35%	0.35%	0.35%
500 mM Arginine	5 mM	NA	6.26 mM
Pharmalyte 3-10	1%	0.48%	NA
Pharmalyte 5-8	NA	0.88%	1.25%
Pharmalyte 8-10.5	3%	2.64%	3.75%
pI Marker 7.05	0.80%	1%	1%
pI Marker 10.17	0.80%	1%	1%
200 mM Iminodiacetic Acid	NA	NA	6.26 mM
10 M Urea	NA	NA	4 M

Table 2. Concentrations of various components in the ampholyte mixture were used for each sample.

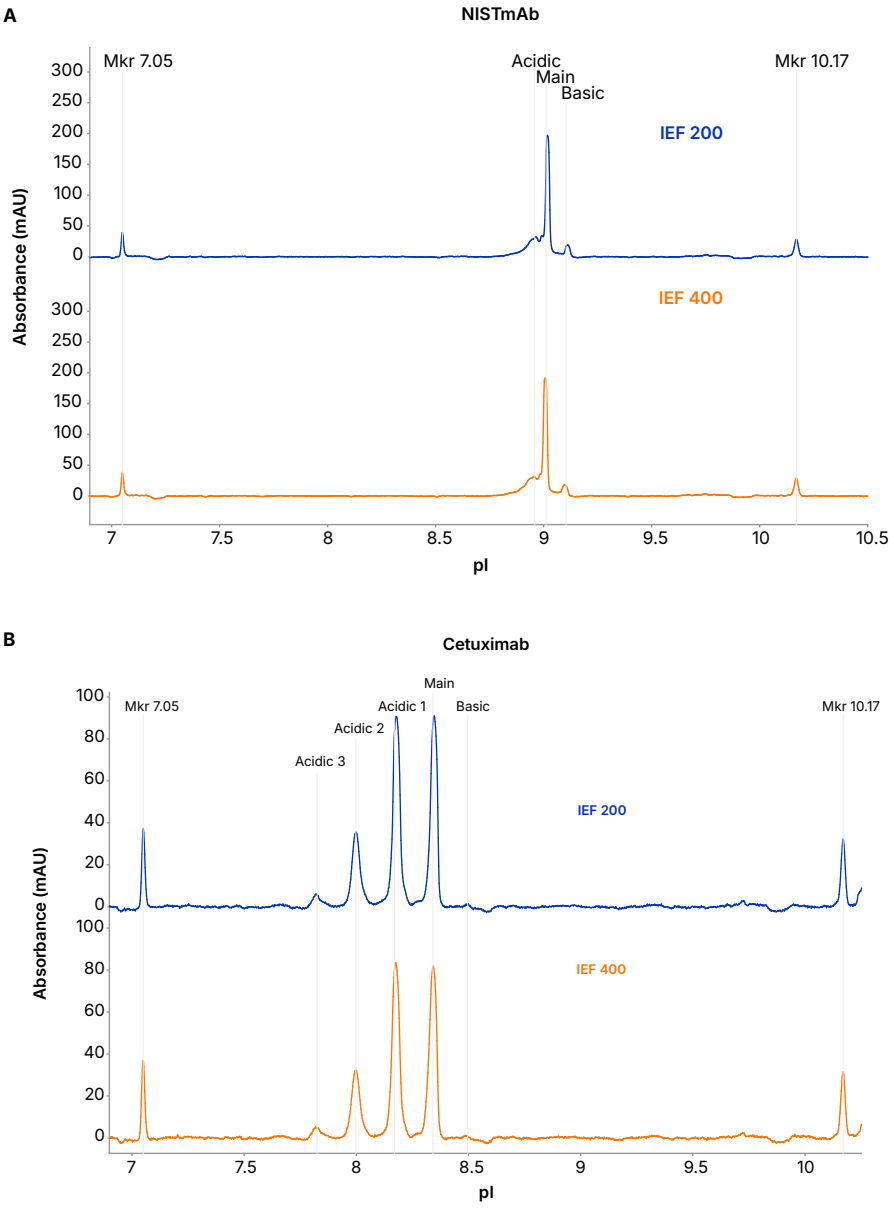
*For LOD and dynamic range studies, the ampholyte mixture for NISTmAb was simplified and contained deionized water, 0.35% MC, Pharmalyte 3-10 (4% v/v), 10 mM arginine, pI Markers 7.05 and 10.17.

Results

Charge Profiles

Figures 1A, 1B, and 1C show the charge profiles of NISTmAb, Cetuximab, and Rituximab respectively, obtained from the IEF 200 and IEF 400 cartridges. Data analyzed with absorbance are shown. For each molecule, the data are highly comparable between the two cartridges, with percent peak area values summarized in Table 3. While not shown here, it should be noted that icIEF data obtained with NF detection were also comparable between both cartridges, for all three samples.

FIGURE // 01



C

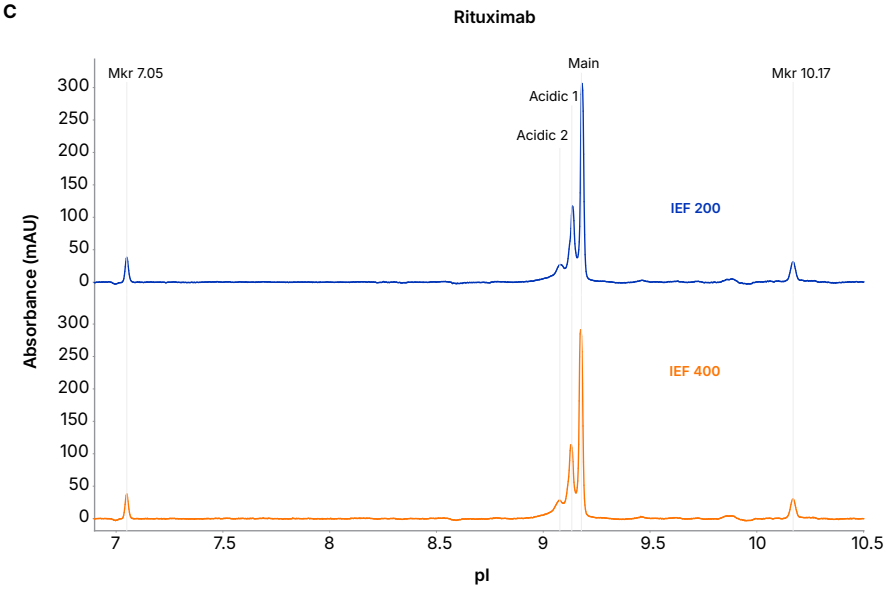


Figure 1. The IEF 200 and IEF 400 cartridges generate comparable charge profiles. Charge heterogeneity data are shown for NISTmAb (1A) Cetuximab (1B) and Rituximab (1C). For each sample, the peaks detected correlate well between both cartridges.

TABLE // 03

Comparison of pI and % Peak Area Values for IEF 200 and IEF 400

	IEF 200				IEF 400			
Peak	**Mean (pI)**	**RSD (%) (pI)**	**Mean (%PA)**	**RSD (%) (%PA)**	**Mean (pI)**	**RSD (%) (pI)**	**Mean (%PA)**	**RSD (%) (%PA)**
NISTmAb								
Acidic	8.963	0.01	29.23	1.77	8.955	0.02	28.95	0.92
Main	9.017	0.00	64.43	0.75	9.007	0.01	63.87	0.54
Basic	9.111	0.01	6.34	1.80	9.101	0.03	7.18	1.91
Cetuximab								
Acidic 3	7.819	0.02	3.01	1.72	7.820	0.06	2.91	2.84
Acidic 2	7.998	0.02	18.25	0.56	7.997	0.04	18.16	0.42
Acidic 1	8.172	0.02	40.49	0.26	8.169	0.03	40.48	0.17
Main	8.341	0.03	37.90	0.48	8.351	0.06	38.12	0.25
Basic	8.499	0.03	0.36	23.64	8.493	0.04	0.34	14.39
Rituximab								
Acidic 2	9.080	0.01	15.38	1.29	9.075	0.02	15.49	1.80
Acidic 1	9.137	0.01	30.57	0.47	9.131	0.02	30.33	1.10
Main	9.181	0.01	54.06	0.26	9.176	0.01	54.18	0.37

Table 3. The pI and percent peak area (%PA) values are comparable between both cartridges for each sample.

Repeatability

Being a critical factor in analytical methods, repeatability ensures that results are consistent and reliable across multiple runs. To assess the repeatability of the new IEF 400 cartridge, 10 consecutive injections of NISTmAb were run, with RSD values reported in Table 4. These data were compared with a repeatability assessment of the IEF 200 cartridge, conducted under similar conditions, resulting in highly comparable data. Together, these data indicate that either cartridge can be reliably used without compromising the precision of the analysis.

FIGURE // 02

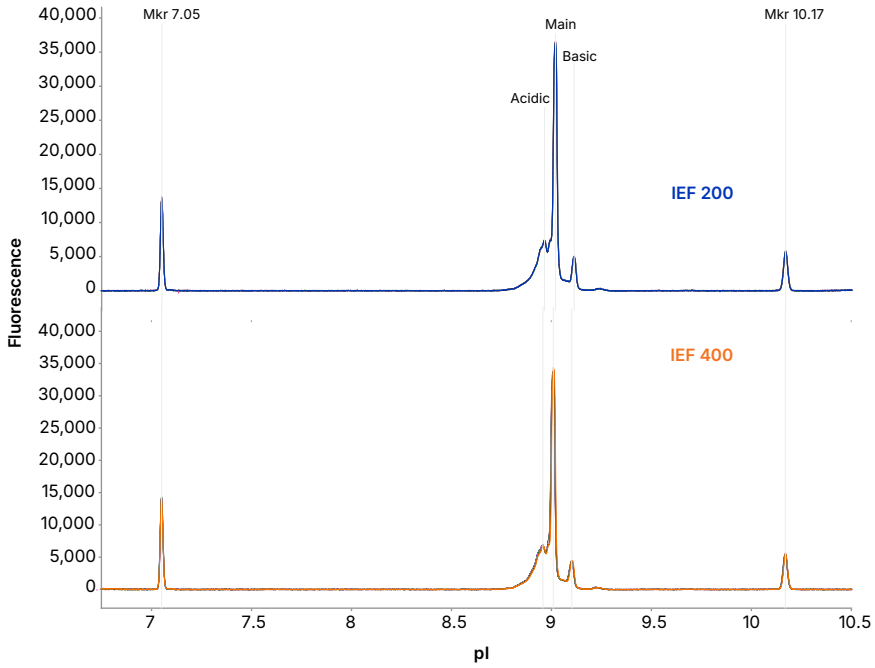


Figure 2. The repeatability of the IEF 400 cartridge is comparable to the IEF 200. Ten consecutive injections of NISTmAb were analyzed with both cartridges. For the IEF 400 cartridge, the overall RSD of the percent area was ≤1.19%, which is close to the IEF 200 cartridge's RSD of ≤1.45%.

TABLE // 04

NISTmAb %Peak Area (n=10)

	IEF 200		IEF 400	
Peak	**Mean (%PA)**	**RSD (%) (%PA)**	**Mean (%PA)**	**RSD (%) (%PA)**
Acidic	29.8	1.23	29.7	1.19
Main	61.2	0.56	60.6	0.60
Basic	9.0	1.45	9.8	1.11

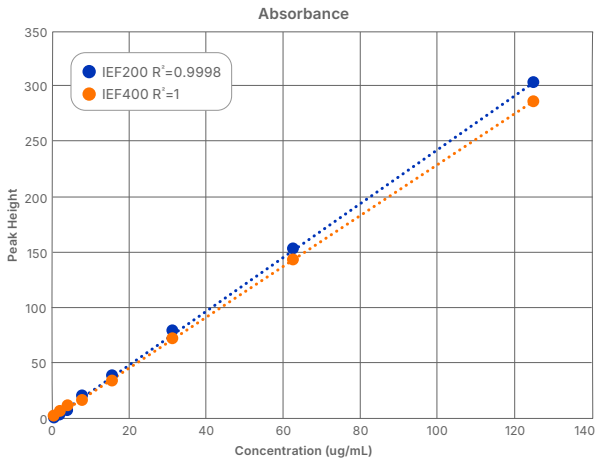
Table 4. The percent peak area (%PA) of NISTmAb, averaged from ten consecutive injections, is comparable between both cartridges.

Limit of Detection

The LOD was measured to ascertain the lowest quantity of sample that can be analyzed with the IEF 400 cartridge. NISTmAb was serially diluted two-fold. With absorbance detection, a range of 125-0.49 µg/mL was analyzed, and with NF, the selected range was 31.25-0.49 µg/mL. Three injections were run at each concentration. The LOD was calculated by dividing three times the standard deviation of the noise for an injection without protein sample by the slope of the linear regression for peak height of the main peak. Plots are shown for both absorbance and native fluorescence (Figures 3A and 3B), and these data are compared with those for the IEF 200 cartridge. Notably, the LOD determined for both cartridges with NF detection, while comparable to each other, were lower than the LOD values determined with absorbance. Such an observation was expected since NF detection with the Maurice system is more sensitive than absorbance.

FIGURE // 03

A



B

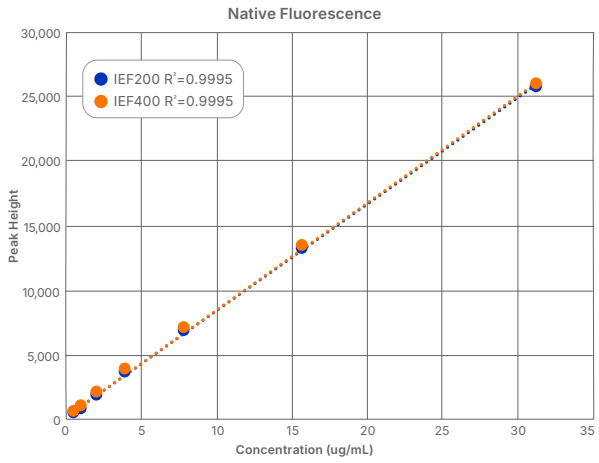


Figure 3. The limit of detection (LOD) comparison between the IEF 200 and IEF 400 cartridges shows highly similar values between both, measured with absorbance (3A) and NF detection modes (3B).

TABLE // 05

Limit of Detection

	IEF 200	IEF 400
Absorbance	0.98 µg/mL	1.03 µg/mL
Native Fluorescence	0.11 µg/mL	0.12 µg/mL

Table 5. LOD values are listed for each cartridge and each detection mode.

Dynamic Range

NISTmAb samples, serially diluted two-fold, ranging from 125-0.49 µg/mL were analyzed with the IEF 400 cartridge, using three injections per concentration. Figures 4 and 5 show charge profiles of NISTmAb at each concentration with absorbance and NF, respectively, including a comparison with charge profiles detected with IEF 200. For NF detection, samples ranging from 31.25-0.49 µg/mL were analyzed. The dynamic range was at least 2 logs based on total peak area (absorbance & native fluorescence), and highly comparable with data from IEF 200. Additionally, plots are shown for both absorbance and NF modes in Figures 6A and 6B.

FIGURE // 04

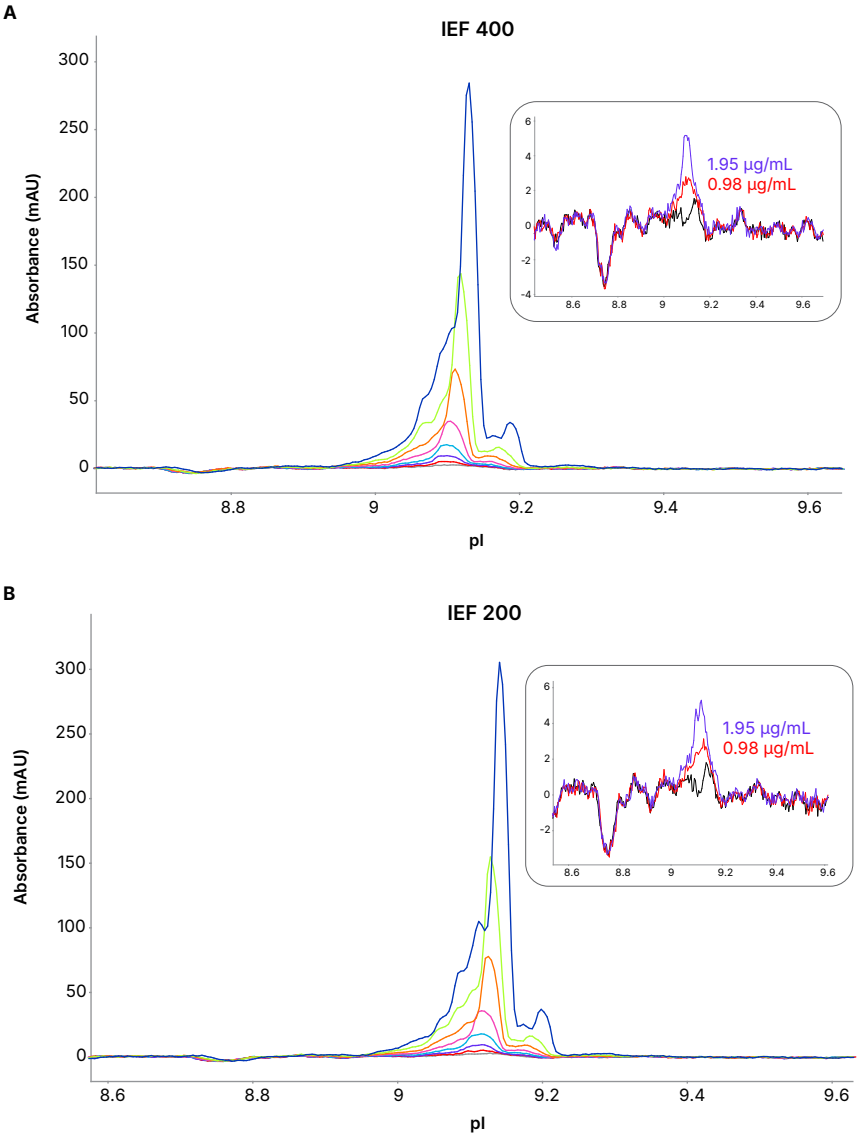


Figure 4. The dynamic range for the IEF 400 cartridge with absorbance detection (4A) is comparable to that of the IEF 200 (4B). Insets in both figures zoom into the lowest detected concentrations for both cartridges, which is at 0.98 µg/mL for both. The concentration of samples analyzed ranged from 125-0.49 µg/mL.

FIGURE // 05

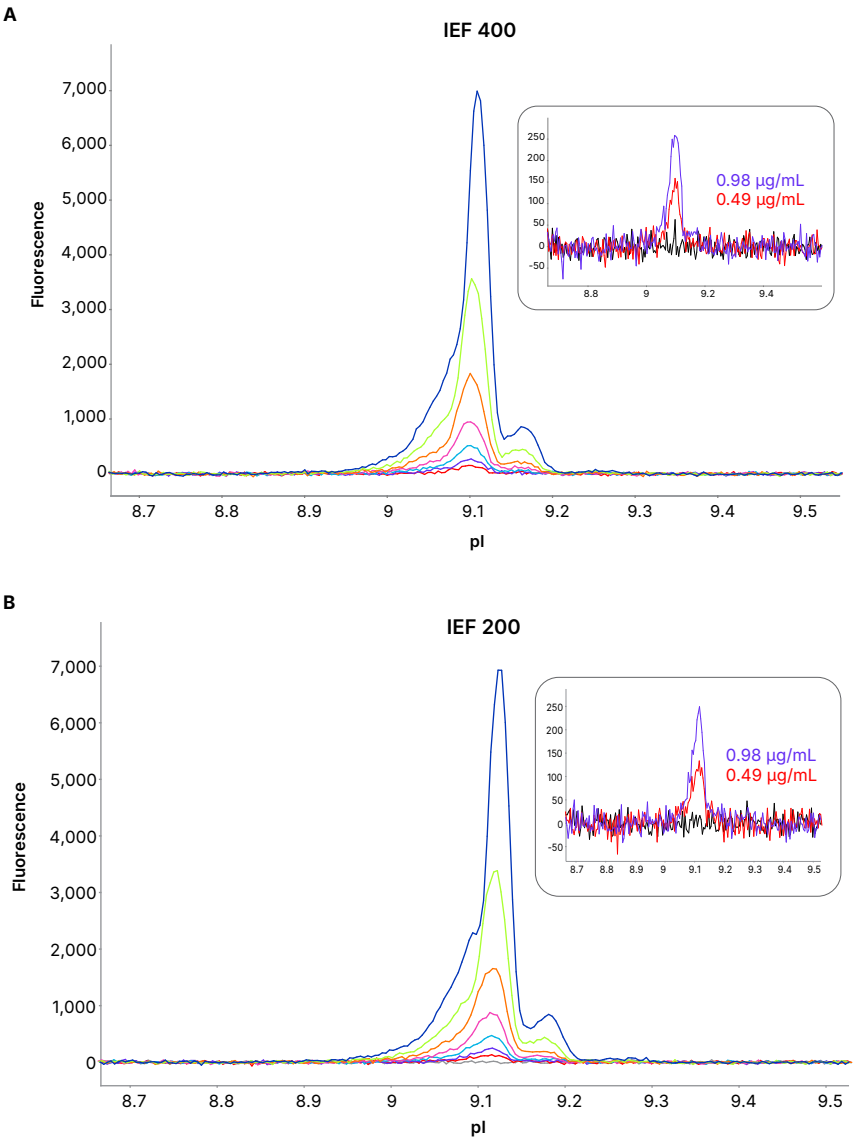
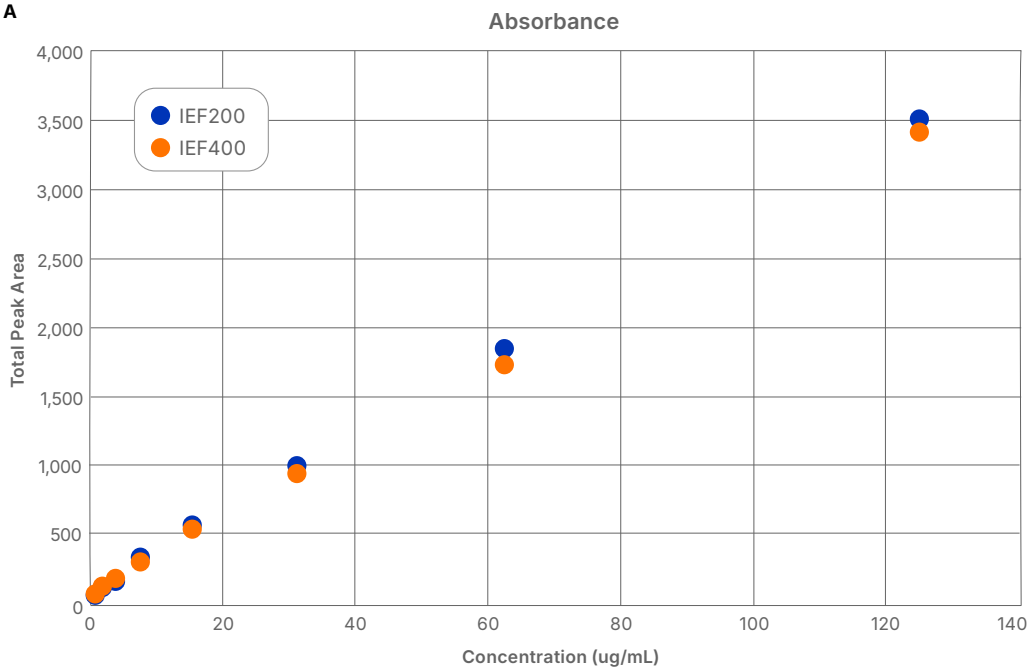


Figure 5. The dynamic range for the IEF 400 cartridge with NF detection (5A) is comparable to that of the IEF 200 (5B). Insets in both figures zoom into the lowest detected concentrations for both cartridges, which is at 0.49 µg/mL. The concentration of samples analyzed ranged from 31.25-0.49 µg/mL.

FIGURE // 06

A



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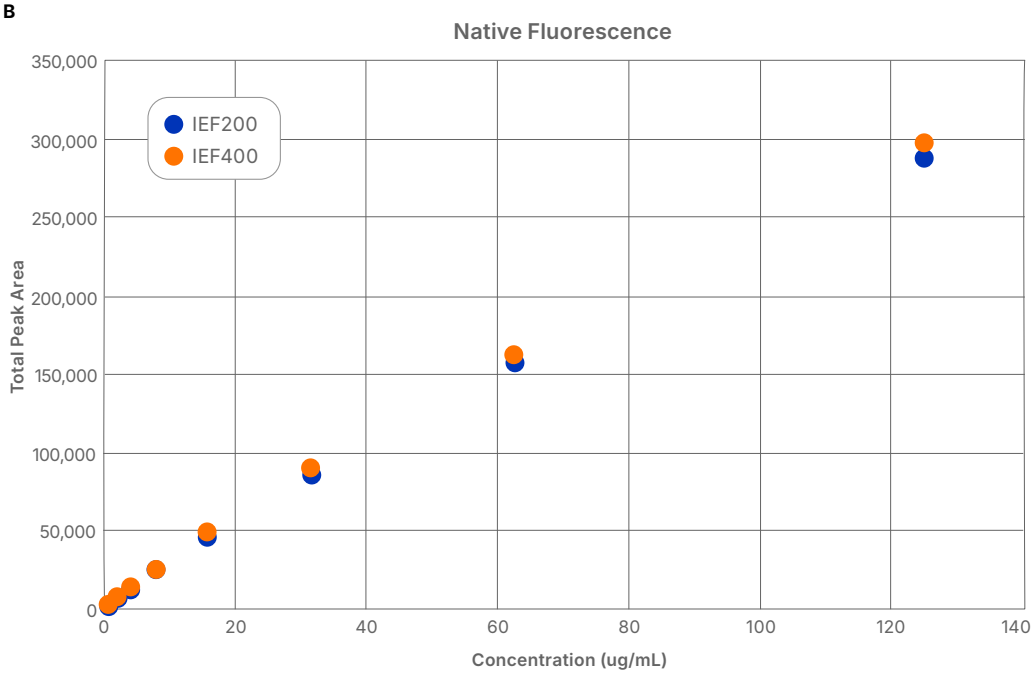

Figure 6. The dynamic range of the IEF 400 cartridge is at least 2 logs based on peak area, as compared with the dynamic range for IEF 200. Data are shown for absorbance (6A) and NF detection modes (6B).

Conclusion

The study presented here successfully demonstrated comparability between the two icIEF cartridges—one designed for up to 200 injections and the other for 400 injections. Through the analysis of standard charge profiles from three distinct molecules, repeatability assessments, LOD, and dynamic range evaluations, the results showed that both cartridges performed with a high degree of similarity. The comparable performance across all tested parameters ensures that either cartridge can be reliably employed for icIEF analysis, providing flexibility in injection capacity without sacrificing precision or sensitivity.

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