From ICE3 to Maurice : Qualification of the Maurice system and implementation of the icIEF test method for mAb charge heterogeneity.

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INTRODUCTION

Lonza's new IBEX® comprehensive program combines speed, guarantees and quality while minimizing risk, in the delivery of your CMC path to IND in only 11 months for monoclonal antibodies. One of the biggest challenges encountered is shortening timelines without increasing the risks. To this end, product quality is assessed at different stages of the development process. Thus arises the need of analytical methods with quick turnaround, to provide high quality data to make rapid decisions. Charge variants are used to monitor post-translational modifications. It is a Critical Quality Attribute indicating product purity and stability. Within this framework, we decided to qualify and implement the current imaged capillary isoelectric focusing (icIEF) method based on ICE3 by the Maurice. A total of 18 assays were performed, testing 5 monoclonal antibodies (mAb) to assess 11 parameters. All the qualification parameters passed the acceptance criteria. Accordingly, the icIEF method using the Maurice instrument is thereby considered fit for purpose and can be used for charge variant analysis for process development samples.

MATERIAL & METHOD

Acquisition → Empower 3® (Processing) → Excel® (Analysis)

Current icIEF method included 2 different testing conditions – Wide and Narrow Pharmalyte range - and the option of using Urea as an additive to prevent aggregation and/or precipitation.

The drug substance of 5 commercially available mAb was tested at Lonza's validated platform load (standard load). Every sample was injected in duplicate. All acquisition in Maurice was performed using the Absorbance mode.

DEVELOPMENT PLAN

ASSAY	PARAMETER	ACCEPTANCE CRITERIA
LINEARITY	Linearity	✓ $R^2 \geq 0.98$ for peak area responses (≥ 5 % (w/w)) against load for major isoforms. ✓ Lonza's standard load must be within the defined linear range.
	Working Range	✓ Working range must be at least 80 % to 120 % of Lonza's standard load.
	LOD LOQ	✓ LOD : relative % peak area of lowest isoform detected at standard load is ≤ 2%. ✓ LOQ : relative % peak area of the lowest level isoform is ≤ 5%.
	Accuracy	✓ Recovery of % peak areas for all isoforms are within 100 ± 30 %.
PRECISION	Intermediate precision (inter-assay)	✓ CV % ≤ 15 % for relative % peak area for major isoforms. ✓ CV % ≤ 5 % for the peaks pI for major isoforms .
	Repeatability (intra-assay)	✓ CV % ≤ 5 % for relative % peak area of major isoforms. ✓ CV % ≤ 2 % for the peaks pI of major isoforms .
SPECIFICITY	Assessing stability indicating method	Significant difference if: ✓ Relative % peak area difference ≥ 5.6 % for major isoforms. ✓ pI difference ≥ 0.21 pI units for major isoforms.
EQUIVALENCY	Comparing both instruments	
ROBUSTNESS	Challenging the focusing time	
	Autosampler hold-time for sample stability	

RESULTS

LINEARITY, WORKING RANGE, LOD, LOQ

In a single assay, loads of mAbs ranging from 0.01 to 1 mg/mL were tested (Figure 1). For each molecule, the mean peak area response (µV*sec) was plotted against load mass (mg /ml) and regression analysis was performed (Figure 2). Linear range showed visual proportional increase in mean peak area with load mass.
$R^2 > 0.98$ was observed for all major peaks.
Overall working range = 0.1 mg/ml to 1.0 mg/ml (40% to 400% of the standard load). LOD = 0.8%. LOQ = 2.8%.

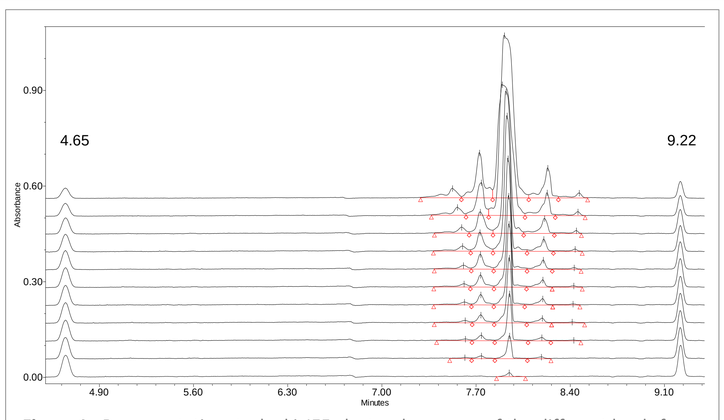

Figure 1 : Representative stacked icIEF electropherogram of the different loads from single mAb tested for linearity.

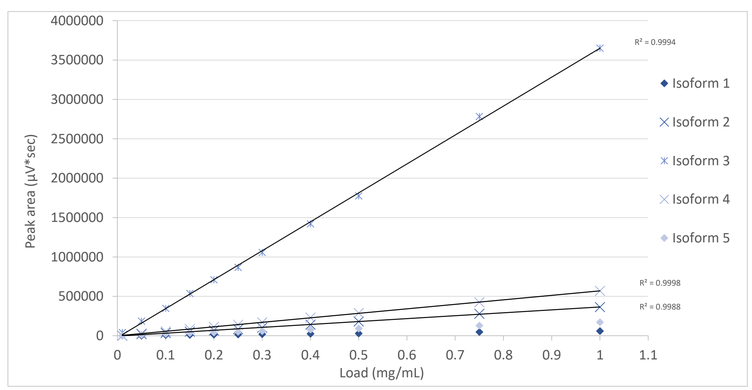

Figure 2 : Graphical representation of the linear regression plot obtained from single mAb (major isoforms 2, 3, 4) .

SPECIFICITY

Assessing the ability of the method to distinguish between stressed and unstressed samples. The profiles of the stressed mAbs were visually distinguishable, showing elevated acidic species (between +6% and +30%) and correspondingly decreased basic species and main peak (between -7% and -30%) when compared to untreated mAbs (Figure 3).

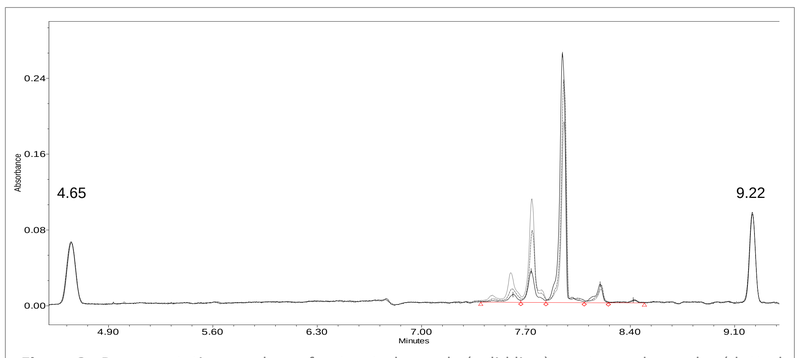

Figure 3 : Representative overlays of untreated sample (solid line) vs. stressed samples (dotted lines, time points 1 and 2) from single mAb for specificity.

EQUIVALENCY

Assessing the ability of the Maurice instrument to be comparable to the standard icIEF method run with iCE3 instrument (Figure 4).
Maximal pI difference for major isoforms = 0.08 pI units.
Maximal relative % peak area for main isoform = 1.58%.

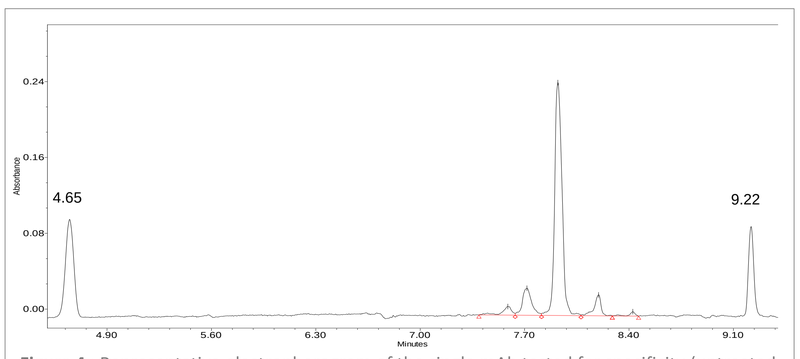

Figure 4 : Representative electropherogram of the single mAb tested for specificity (untreated sample) was also tested in the iCE3 for equivalency.

ACCURACY

Experimental determined peak area (for the different loads) was compared to the theoretical peak area (based on standard load). For all major isoforms, recovery of % peak areas were within 100 ± 30 % (Figure 5).

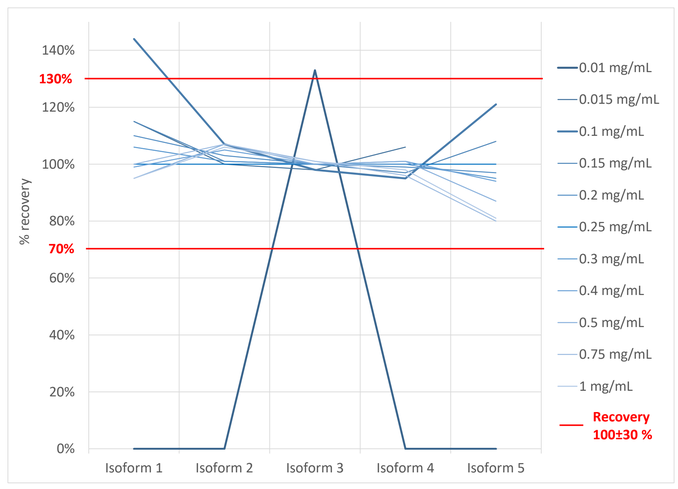

Figure 5 : Graphical representation of the recoveries obtained for accuracy from single mAb (major isoforms 2, 3, 4).

PRECISION

Repeatability : 6 independent preparations were compared (Figure 6, in blue).
For major isoforms, CV % ≤ 2.1 % for relative % peak area and CV % ≤ 0.1% for the peaks pI were observed.
Intermediate precision : The data from at least 3 separate assays, 3 analysts and 2 different cartridges was acquired (Figure 6, in orange).
For major isoforms, CV % ≤ 3.3 % for relative % peak area and CV % ≤ 0.3% for the peaks pI were observed.

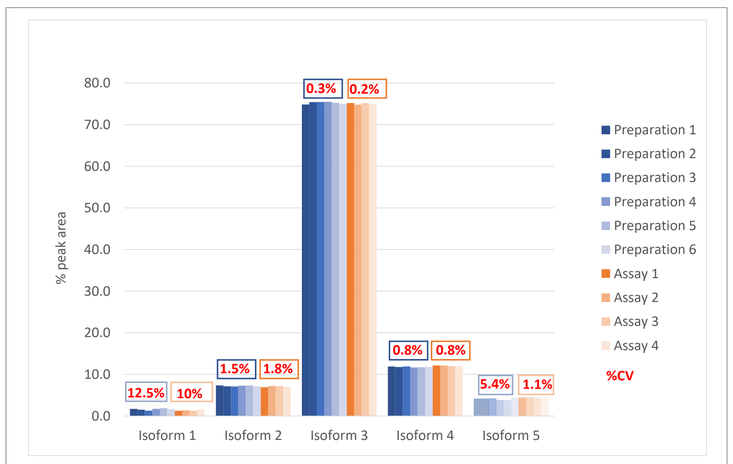

Figure 6 : Representative % peak areas obtained for precision from single mAb (major isoforms 2, 3, 4). Repeatability samples (in blue) with their corresponding %CV. Intermediate precision samples (in orange) with their corresponding %CV.

ROBUSTNESS

Assessing if the Lonza platform method using Maurice was robust under challenging conditions.
Robustness 1 : Challenging the focusing time (standard condition ± 3min).
Robustness 2 : Autosampler hold-time for sample stability in the autosampler (t0, t6h, t12h, t24h)
The method was considered robust for the focusing time tests and for the holding time tests below or equal to 12h.
For sequences beyond 12h, additional SST bracketing is recommended.

CONCLUSION

The icIEF method using the Maurice instrument passed all the acceptance criteria set in the development plan. This method is thereby considered fit for purpose and can be used for charge variant analysis for process development samples. Therefore, Maurice will gradually replace iCE3, allowing fast and high quality analysis and without compromising the timelines. Moving forward, additional development will be performed in Maurice instrument using fluorescence mode (e.g. evaluation of alternate Maurice pI markers and other combinations of Pharmalyte ranges).