Reproducibility study of an imaged capillary isoelectric focusing (icIEF)-UV/MS analysis using intact monoclonal antibody

Featuring an icIEF-UV/MS workflow using the Intabio ZT system from SCIEX

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This technical note highlights the reproducibility of a streamlined icIEF-UV/MS workflow for charge variant separation, UV quantitation, and peak identification of monoclonal antibodies (mAbs) by Intabio ZT system coupled to ZenoTOF 7600 system. The icIEF-UV/MS measurements of NISTmAb were performed across 45 injections with 3 cartridges using the Intabio ZT system, resulting in high inter- and intra-cartridge reproducibility (%CVs <5%).

Recombinant mAbs are widely used in biotherapeutic applications due to their high specificity and efficacy. One challenge associated with the characterization of antibody-based biotherapeutics is sample heterogeneity caused by physiochemical transformations and post-translational modifications (PTMs) that might occur during mAb manufacturing.[1] Monitoring and characterizing the heterogeneity of mAbs is important to assess critical quality attributes (CQA) of biotherapeutics to ensure drug safety and efficacy.

The Intabio ZT system offers direct chip-based integration of icIEF-UV with ZenoTOF 7600 system that can deliver rapid and reproducible separation and characterization of intact mAb charge variants, and assessment of proteoforms.[2,3] The Intabio ZT system coupled with ZenoTOF 7600 system offers a streamlined workflow for reproducible separation, identification and relative quantitation of mAb charge variants in a high-throughput manner.[3, 4]

In this technical note, the icIEF-UV/MS workflow was employed to provide reproducible separation of the main, acidic and basic variants of NISTmAb across 45 injections using 3 different cartridges (Figure 1).

Key features of the icIEF-UV/MS workflow

- Reproducible separation, identification and relative quantitation of mAb charge variants with a seamless, microfluidic chip-based integrated icIEF-UV/MS workflow offered by the Intabio ZT system

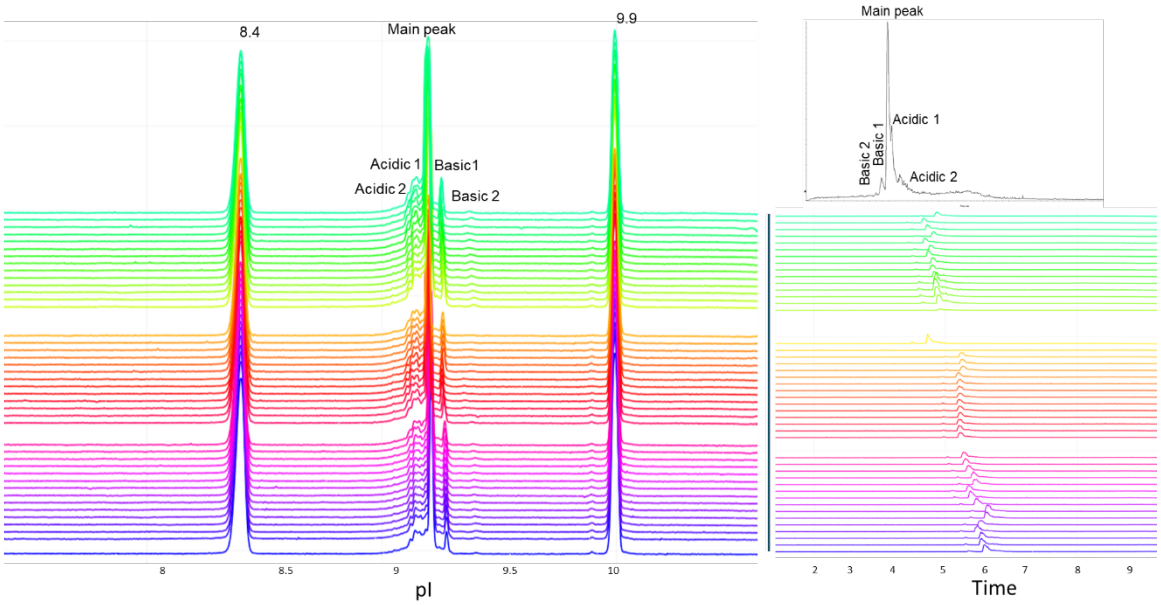

Figure 1. Reproducible separation and detection across 45 runs on 3 cartridges using the Intabio ZT system coupled to the ZenoTOF 7600 system. Comparable NISTmAb charge variant patterns were observed between the icIEF-UV profile (left) and the MS base peak electropherograms (BPEs, right). Consistent separation of main, acidic and basic charge variant peaks was achieved across 45 injections using 3 cartridges.

- The 30-min sample analysis is significantly faster than conventional cIEF and IEX workflows requiring fractionation for the following identification.
- icIEF separation and UV relative quantitation correlate well with standard icIEF techniques
- Streamlined, intuitive data analysis software for rapid reporting and results sharing

Methods

Equipment: Intabio ZT system (SCIEX) and Intabio ZT cartridge (SCIEX, P/N 5088248) were used for the separation of NISTmAb and its charge variants. MS detection was performed on the ZenoTOF 7600 system (SCIEX, P/N 5080337) equipped with OptiFlow interface components (SCIEX, P/N 5084645).

Chemicals and reagents: The Intabio system – Electrolyte and Mobilizer kit (P/N 5088205) was used for anolyte, catholyte and mobilizer. Anolyte and mobilizer were used undiluted. The stock catholyte solution was 1% and diluted to 0.25% for use in the reagent drawer. The stock anolyte is 1% formic acid and catholyte is 1% diethylamine. The mobilizer is composed of 25% acetic acid25% acetonitrile and 50% water.

A 500mM cathodic spacer solution containing free base L-arginine (Arg) (purity ≥ 98.5%, Sigma-Aldrich, P/N A8094-25G) was prepared by dissolving 0.870 mg of Arg powder into 10 mL of Milli-Q water. The electrolytes and cathodic spacer solutions were stored at room temperature. pI markers (CanPeptide) were individually dissolved in Milli-Q water at 5 mg/mL.

Prior to icIEF-UV/MS analysis, NISTmAb was desalted with a Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL (Thermo Fisher Scientific, P/N 89882).

Samples containing 400 µg/mL NISTmAb, 10mM Arg, 1 % Pharmalyte 3 to 10 (Cytiva P/N 17045601), 2.5% Pharmalyte 8 to 10.5 (Cytiva, P/N 17045501) and 6.0 µg/mL peptide pI markers were vortexed and then degassed by centrifugation at 3900 cf.

icIEF-UV/MS analysis: For the reproducibility test, 400 ug/mL NISTmAb solution was mixed with carrier ampholytes, and internal pI markers. The sample was separately analyzed with 3 different Intabio ZT cartridges.

The icIEF separation was achieved using the parameters shown in Table 1. UV absorbance measurements were collected at 1 Hz during the focusing and mobilization steps. The samples were introduced into the ZenoTOF 7600 system with a metered 2 µL/min flow of chemical mobilizer. The TOF MS data were acquired using the parameters shown in Table 2.

Table 1. icIEF separation parameters.

Hold time (s)	Anode voltage (V)	Cathode setting	Mobilization setting	Step
60	*1500*	*0 V*	*0 A*	*Focusing*
60	*3000*	*0 V*	*0 A*	*Focusing*
300	*4500*	*0 V*	*0 A*	*Focusing*
600	*8500*	*0 A*	*5500 V*	*Mobilization*

Data processing: UV profiles and mass spectra from the icIEF-UV/MS analysis of NISTmAb were analyzed using the Biologics Explorer software. Each peak in the icIEF-UV profile was integrated to determine peak area and percent composition. Intact masses were estimated from the raw mass spectrum under each peak of the icIEF-MS profile utilizing a charge deconvolution algorithm with a mass range setting between 145,000 and 150,000 Da.

Table 2. TOF MS parameters.

Parameter	Value
Curtain gas	*15 psi*
Spray voltage	*5500 V*
TOF start mass	*2000 m/z*
TOF stop mass	*6000 m/z*
Accumulation time	*0.5 s*
Source temperature	*100°C*
Declustering potential	*210 V*
Collision energy	*55 V*
Time bins to sum	*150*

Results and discussion

Intra- and inter-cartridge relative abundance reproducibility

The combination of icIEF separation with UV quantitation and MS identification provides a high-resolution separation and comprehensive characterization of intact mAbs and their charge variants, as well as proteoform identification. The charge variants of NISTmAb were monitored across 45 injections using 3 different cartridges to evaluate the reproducibility of the icIEF-UV/MS workflow, Figure 1.

The intra- and inter-cartridge reproducibility of NISTmAb separation and quantitation were assessed based on 3 metrics. First, the relative percent abundances of the main, acidic 1, and basic 1 and basic 2 variants were determined using each of 3

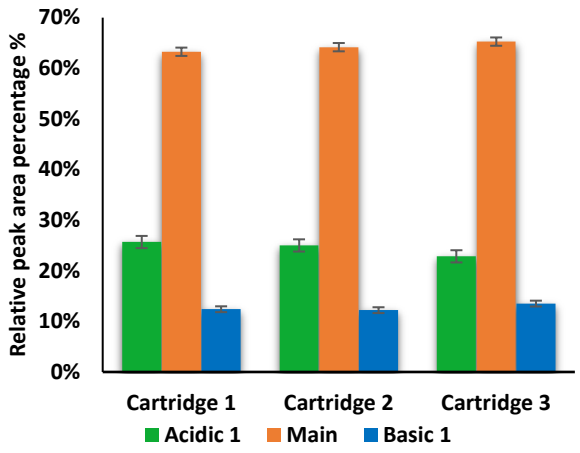

Figure 2. Relative abundances of the main, acidic 1 and basic 1 peaks of NISTmAb based on icIEF-UV/MS analysis of 45 injections on 3 different cartridges. The %CVs for the relative abundance of each charge variant were <5% based on the icIEF-UV profiles.

different cartridges. Additionally, the icIEF-UV and icIEF-MS profiles acquired from the 3 cartridges were correlated and compared. Finally, the pI and peak separation were compared across multiple runs on the same cartridge.

The icIEF-UV/MS analyses of NISTmAb using 3 different cartridges provided consistent pI values and relative abundances of the main, acidic1, basic 1 and basic 2 charge variants (Figure 2 and Table 3). The %CVs of the pI of the main peaks were <0.1% and the %CVs of the relative abundances of all variants based on the icIEF-UV profiles were <5% (Table 3). These results demonstrate the high inter-cartridge reproducibility of the icIEF-UV/MS workflow.

Intra-cartridge pI value and resolution reproducibility

The icIEF-UV profiles were compared to evaluate the reproducibility of charge variant separation and detection across 15 injections on the same cartridge. Figure 3 shows the overlay of icIEF-UV profiles from 15 injections (blank injections are not shown).

Table 3. Separation reproducibility and relative peak area results for multiple runs on 3 cartridges.

	Main peak pI	Basic 1 peak pI	Main peak UV area %	Acidic 1 peak UV area %	Basic 1 peak UV area %	Basic 2 peak UV area %
Cartridge 1	*9.1*	*9.2*	*64.8%*	*26.2%*	*8.9%*	*7.5%*
Cartridge 2	*9.2*	*9.2*	*64.3%*	*28.1%*	*8.5%*	*7.2%*
Cartridge 3	*9.1*	*9.2*	*65.4%*	*26.1%*	*8.4%*	*7.8%*
Average	*9.1*	*9.2*	*64.9%*	*26.8%*	*8.6%*	*7.5%*
Standard deviation	*0.006*	*0.007*	*0.44*	*1.122*	*0.2*	*0.28*
%CV	*0.06*	*0.08*	*0.68*	*4.18*	*3.4*	*3.74*

These data demonstrate the ability of a single cartridge to reliably separate intact NISTmAb and its charge variants. High intra-cartridge separation reproducibility was measured based on the resolution between the main peak and the variant containing 1 C-terminal lysine across 45 injections on 3 different cartridges. This measurement was obtained directly from the software. A resolution of 1.5 is often indicative of baseline separation in icIEF analysis. Here, a separation resolution of 2-2.5 was consistently

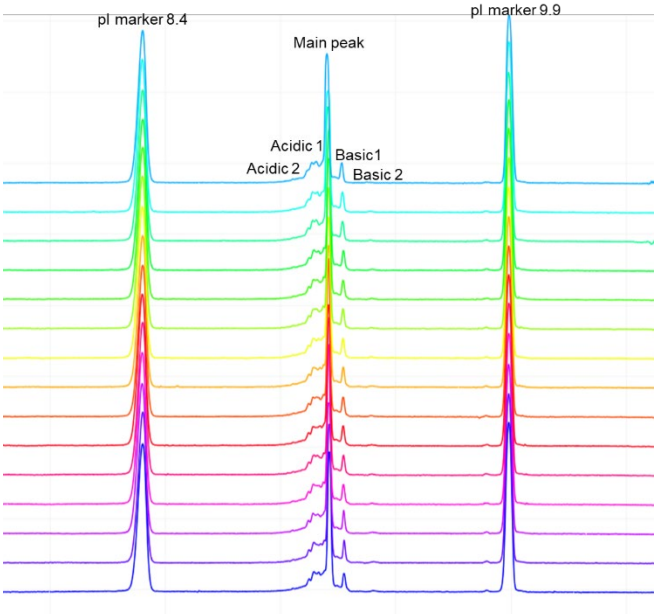

Figure 3. Intra-cartridge reproducibility. The icIEF-UV profiles showing charge variant separation from 15 sequential injections demonstrate the reproducibility of analysis on the Intabio ZT system. Consistent separation of main, acidic 1, basic 1 and basic 2 variants was observed from 15 sequential injections.

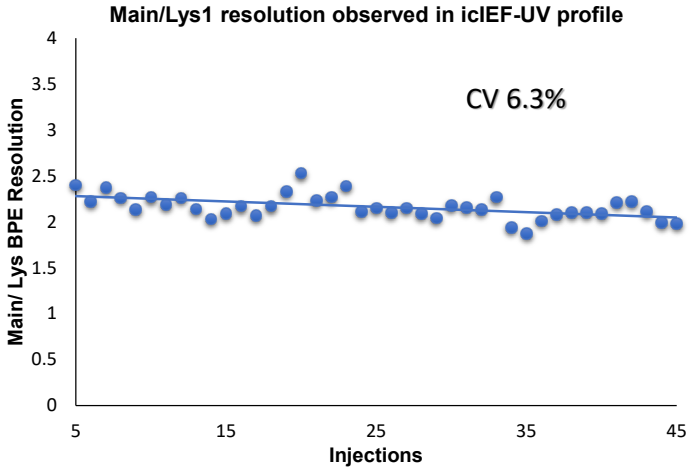

Figure 4. Consistent resolution was observed across 45 injections on 3 different cartridge. The scatter plot shows a 2-2.5 separation resolution in the icIEF-UV profile between the main and basic 1 (1 C-terminal Lys) variant peaks based on 45 consecutive injections. A %CV of 6.3% was observed. These results demonstrate the consistency between technical replicates on the Intabio ZT system.

obtained for the icIEF-UV profiles of these 2 species across all runs (Figure 4). These results demonstrate the separation power and reproducibility of analysis on the Intabio ZT system.

Evaluation of carryover between runs

To assess the carryover between runs for the charge variant analysis, an alternating sequence of blank and highly concentrated NISTmAb (up to 1 mg/mL) samples was injected into the Intabio ZT system. The icIEF-UV profiles of the alternating blank and NISTmAb injections show that carryover was not detected in the blank runs between sample injections (Figure 5). These results demonstrate the absence of carry over in the described analysis on the Intabio ZT system.

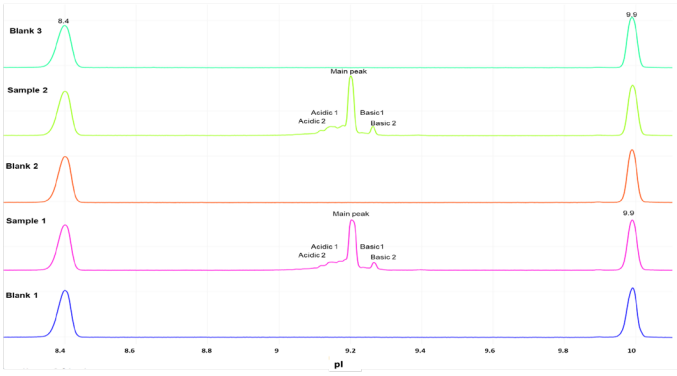

Figure 5. icIEF-UV profile of blank runs followed by injections of highly concentrated NISTmAb (1 mg/mL) sample using the Intabio ZT system. No carryover was detected in the blank injections between sample runs.

In summary, this technical note demonstrates the high inter- and intra-cartridge reproducibility of the Intabio ZT system and the power of this system for separating and characterizing different proteoforms of mAbs.

Conclusions

- The Intabio ZT system is an innovative, chip-based integrated platform that offers high-resolution and high-throughput separation of intact mAbs and their charge variants
- The icIEF-UV/MS analysis demonstrates high reproducibility for both quantitation and resolution across 45 injections using 3 different cartridges
- The Intabio ZT system demonstrates a streamlined workflow to separate charge variants by icIEF and identify them with ZenoTOF 7600 system
- The Intabio ZT system is a robust platform that provides reproducible and high-resolution separation for intact protein analysis
- No carryover was observed for inter- and intra-cartridge injections on the Intabio ZT system
- The Intabio ZT system is a commercially available platform that offers workflow combining icIEF separation, UV quantitiation and MS-based identification.

References

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