Interlaboratory comparison of microfluidic chip-based integrated imaged capillary isoelectric focusing (icIEF)-UV/MS characterizing the NISTmAb

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OVERVIEW

This work demonstrates a microfluidic chip-based icIEF-UV/MS technology deployed in 14 different settings (varying systems, locations, and users) to characterize a standard monoclonal antibody. Samples, solvents, systems, and software were provided with integrated workflows to guide operations at various biopharma and analytical development sites. These workflows allowed users newer to icIEF or MS to effectively generate high-quality integrated datasets, enabling the characterization of critical quality attributes and post-translational modifications.

INTRODUCTION

Characterizing the heterogeneity of manufactured monoclonal antibodies is an integral part of the biotherapeutic development and manufacturing processes. Rapid and robust strategies to separate and analyze these compounds expedite and streamline pharmaceutical workflows. Additionally, continuously monitored, holistic data processing and analysis can ensure confidence in instrumentation and protocols.

MATERIALS AND METHODS

Different Intabio ZT systems coupled with different ZenoTOF 7600 systems were used for analysis at each site. Anolyte, catholyte and mobilizer solutions were provided for operation. Each operator used local Milli-Q water for autosampler/wash solutions and catholyte dilution.

Sample preparation:
At each site, samples of the NISTmAb standard were desalted via spin column according to manufacturer protocol, with aliquots analyzed using a provided commercially available formulation containing ampholytes, spacers, and pI 8.4 and 9.99 markers. Cartridges and the system were prepared following the in-software guided procedures. After signal-based optimization of electrospray position and MS conditions, batches of samples were set up for integrated analysis using preset system methods for autonomous priming, icIEF-UV separation, and mobilization to electrospray ionization for MS analysis.

Figure 1. Screencaps showing Helpers in the Intabio ZT software

Integrated software "Helpers" limited differences between individual operators. The instrument-to-instrument and site-to-site variation observed was driven by optimization choices, user-to-user differences in pipetting for sample and cartridge preparation, and possibly variations in storage of the samples of NISTmAb. These procedures were addressed with guidance integrated in the software.

icIEF-UV/MS analysis conditions:
The default method for NISTmAb, preinstalled in the Intabio software, was used to separate the samples in 3 steps of 1500 V, 3000 V, and 4500 V for 60 s, 60 s, and 300 s, respectively, prior to mobilization and electrospray at 5500 V with a 3000 V differential between the anode and mobilizer electrode. Mobilizer flow was automatically set to 3 µlpm with nebulizer gas at 80 psi. A suggested MS data acquisition method was provided within the software, for 10 minute acquisitions of the mobilized compounds.

Data processing was performed in the Intabio Data Analysis, SCIEX OS, and Biologics Explorer software using the icIEF-UV/MS analysis workflow with targeted mass searching.

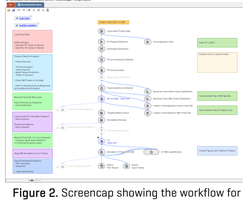

Figure 2. Screencap showing the workflow for integrated icIEF-UV/MS data analysis in Biologics Explorer

RESULTS

The NISTmAb standard sample was used for system evaluation across multiple laboratories and performed with different instruments guided by the Intabio software, shown in Figure 1. This software includes an optimized platform method for focusing and mobilization conditions, in addition to suggested optimized parameters for gas-assisted electrospray ionization and detection. Initial data processing was aided by the ability to automatically assess critical to quality [CTQ] system and sample metrics, per Figure 3, at right as well as Table 2.

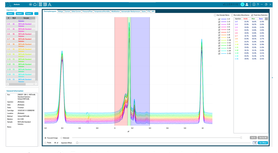

Figure 3. Rapid charge variant electropherogram statistics calculator in the Intabio ZT software

The selected focused UV traces were automatically evaluated, and co-registered with the acquired and calibrated MS files. A Time Resolved Deconvoluted icIEF-UV/MS workflow in the Biologics Explorer software (Figure 2) was used to facilitate visualization and characterization, highlighted in Figures 4 and 6. A 3D rendering of this type of data is also shown in Figure 7 to highlight intensity differences. Libraries generated for known modifications of the NISTmAb were used for targeted mass search based annotation to semi-automatically characterize the MS data, as each peak consists of spectra like those shown in Figure 5, clarifying peaks used for calculating percentages in Table 1.

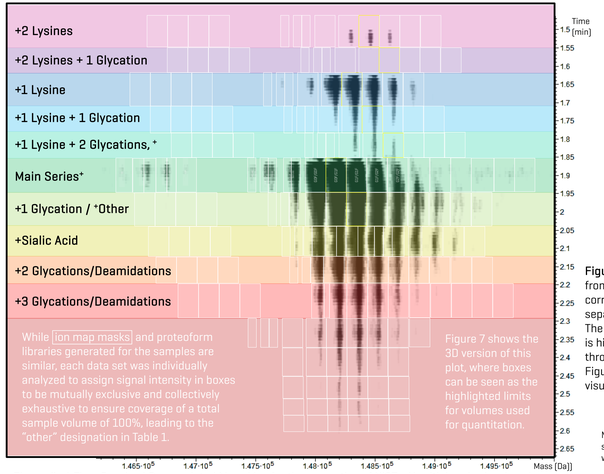

Figure 4. A Time Resolved Deconvoluted Mass Map for the separation of NISTmAb with bands showing charge variants from basic [top] to acidic [bottom] with labeled PTMs on the main glycosylation series shown in boxes.

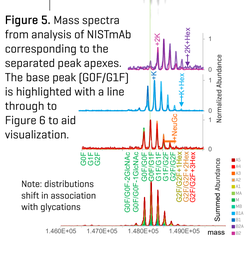

Figure 5. Mass spectra from analysis of NISTmAb corresponding to the separated peak apexes. The base peak [G0F/G1F] is highlighted with a line through to Figure 6 to aid visualization.

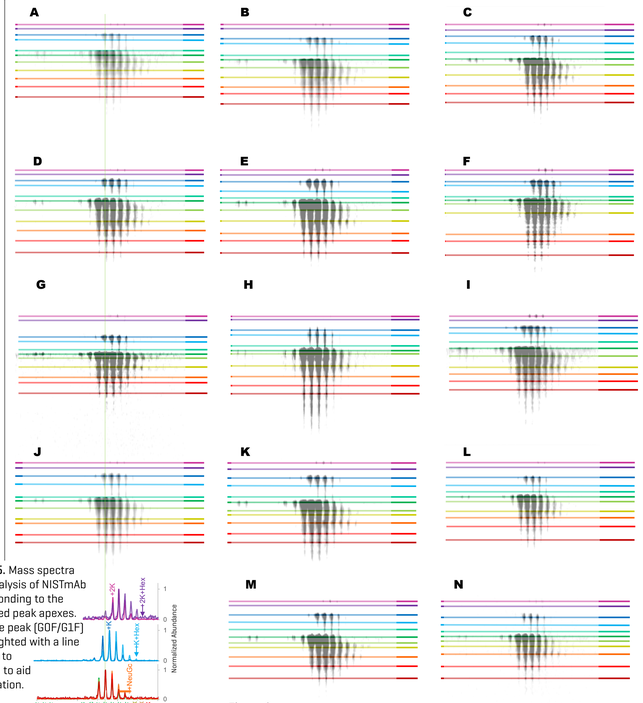

Figure 6.
[A-N] Representative top-down images of the time resolved deconvolution of the icIEF-UV/MS analyses of the NISTmAb at 14 sites, with each showing 1.8 minutes (the equivalent of 1 pI unit) and 4 kDa ranges of the separated data. Peak apexes for modifications from basic to acidic charge variants (with modifications denoted in spectra at left) are highlighted under each map.

Mods	AVG
+ 2K	0.7
+ 2K+Hex	0.2
+ 1K	5.5
+ 1K+Hex	14
+ 1K+2Hex	0.5
Main	53.1
+ 1 Hex	20.1
+ Sialic Acid	6.1
+ 2 Hex*	4.3
+ 3 Hex*	1.7
Other	6.4

Table 1. Average identified modification percentages based on each sample's intensity volume for the icIEF-UV/MS data analyses. "Other" includes volumes not otherwise categorized.

Near isobar assignments were parsed based on charge variant detection time, included in library.

Mods	AVG
UV BASIC	11.6
UV MAIN	59.0
UV ACIDIC	29.4

Table 2. Average percentages from Acidic, Main, and Basic peaks in the icIEF-UV profiles

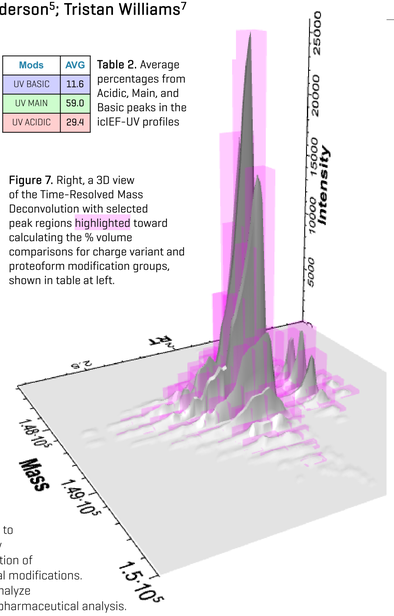

Figure 7. Right, a 3D view of the Time-Resolved Mass Deconvolution with selected peak regions highlighted toward calculating the % volume comparisons for charge variant and proteoform modification groups, shown in table at left.

CONCLUSIONS

This work demonstrates a microfluidic chip-based icIEF-UV/MS technology deployed in 14 different settings (different locations, systems, and users) and used to characterize a standard monoclonal antibody. Samples, solvents, systems, and software were available with integrated workflows to guide operations at various biopharma and analytical development sites.

A new usability approach to facilitate ease of adoption called "Helpers" enabled each site to operate the Intabio ZT system. These guided procedures allowed users newer to icIEF or MS to effectively generate high-quality integrated datasets, enabling the characterization of critical quality attributes and post-translational modifications. Rapid and robust strategies to separate and analyze biotherapeutics may expedite and streamline pharmaceutical analysis.

REFERENCES

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