Demonstrate Method Robustness and Seamless Method Transfer, a Step Towards Enabling High Resolution Accurate Mass Multi-Attribute Method for Biotherapeutic QC

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ABSTRACT

LC-MS based peptide mapping plays an integral role in the biotherapeutic characterization process, as it can simultaneously identify, quantitate, and monitor product quality attributes (PQAs) and impurities during process development and lot releases. Therefore, it is imperative to define a set of operating parameters and performance metrics, under which the LC-MS systems are tested against a pre-defined acceptance criteria, such that the test result can determine whether the systems are deemed fit for their intended purpose. To address this gap, we have developed an integrated system performance evaluation test (SET) that utilizes a pre-digest standard to monitor relevant metrics of LC-MS system based on a comprehensive set of acceptance criteria. This test was conducted on multiple LC-MS setups using an Chromeleon eWorkflow which contains all the associated items from setting up the injection sequence through, processing the data to reporting of the results. eWorkflows enables seamless transfer of all methods and reports, and ensures consistent data acquisition, processing reporting.

INTRODUCTION

Previously, we have described a HRAM MAM workflow for monitoring PQAs of NISTmAb and other monoclonal antibodies as well as demonstrated the capability of detecting changes in PQAs and new impurities resulting from changes in sample preparation processes, storage, and bioprocessing conditions.[1] Although this type of workflow has gained popularity and recognition in the biopharmaceutical industry for early developmental stage of the biologic pipeline, demonstrating method transferability, robustness, ease-of-use, and validation are paramount before it is ready for transfer to a quality control environment. Herein, we demonstrate the use of SET and pre-digested bovine serum albumin (BSA) sample for assessing the performance of LC-MS setups. Once the SET passes, using eWorkflows, these setups are used to evaluate PQAs of a NISTmAb digest and quantitative results are compared between platforms.

MATERIALS AND METHODS

Sample Preparation

Thermo Scientific™ Pierce™ MS grade BSA protein digest (P/N 88341) was used for SET. 1 pmol/µL working solution of BSA protein digest sample was prepared by adding 1 mL of water to the vial containing 1 nmol of lyophilized BSA protein digest sample. Digested NISTmAb humanized IgG1κ monoclonal antibody (NIST, RM 8671, Lot 14HB-D-001) were used for cross platform comparison. Samples were digested using the previously reported method.[1]

LC Methods

2 LC methods were created (See Table 1 and 2)

Table 1: LC methods for SET

UHPLC system	Thermo Scientific™ Vanquish™ UHPLC
UHPLC column	Hypersil GOLD™ Vanquish™ C18 UHPLC column, 150x2.1 mm, 1.9 µm
Column temp.	50°C
Flow Rate	0.25 mL/min
Solvent A	Water + 0.1% formic acid
Solvent B	Acetonitrile + 0.1% formic acid
Gradient	Time (min) / %B
	0.0 / 2
	0.5 / 2
	1 / 9
	22 / 35
	23 / 90
	26 / 90
	27 / 2
	45 / 2
Injection Volume	5 µL

Table 2: LC methods for NISTmAb digest

UHPLC system	Thermo Scientific™ Vanquish™ UHPLC
UHPLC column	Hypersil GOLD™ Vanquish™ C18 UHPLC column, 150x2.1 mm, 1.9 µm
Column temp.	25°C
Flow Rate	0.25 mL/min
Solvent A	Water + 0.1% formic acid
Solvent B	Acetonitrile + 0.1% formic acid
Gradient	Time (min) / %B
	0.0 / 1
	5 / 1
	6 / 10
	70 / 35
	72 / 90
	77 / 90
	79 / 1
	81 / 1
	83.5 / 10
	91.5 / 45
	93 / 90
	99 / 90
	101 / 1
	115 / 1
Injection Volume	2 µL

Mass Spectrometry

For the SET, a full MS only method covering a mass range of m/z 280-1600 and a resolution setting of 120,000 was applied for both the Thermo Scientific™ Orbitrap Exploris™ 240 system and the Thermo Scientific™ Orbitrap Exploris™ MX systems. A 2nd data dependent MS^2 method was applied to Orbitrap Exploris 240 system only for fragmentation efficiency evaluation. Both methods use the same source parameters. Only the first 24 minutes of data were acquired for SET. The same full MS method was used for NISTmAb digest analysis, and only first 70 minutes of data were collected.

Data Analysis

Thermo Scientific™ Chromeleon™ 7.3.1 Chromatography Data System (CDS) software was used for data acquisition, processing, and reporting.

RESULTS

SET for evaluation of LC-MS system performance

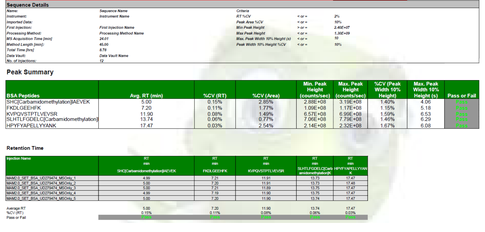
The SET evaluates LC-MS system performance by measuring critical system performance attributes relating to the LC and the MS systems across 5 replicate injections of the BSA protein digest standard. Table 3 outlines the critical system performance attributes and their predetermined acceptance criteria for each test. The SET only passes when all measured critical system performance attributes for all monitored peptides pass, and a good example for general system check is shown in Figure 1

Table 3. Monitored BSA peptides, critical system performance attributes, and associated acceptance criteria

	BSA peptides	Attributes	Acceptance criteria
Composite scoring	SHC*IAEVEK FKDLGEEHFK KVPQVSTPTLVEVSR SLHTLFGDELC*K HPYFYAPELLYYANK	Mass Accuracy	Absolute ≤ 5 ppm
		Isotopic Dot Product	≥ 0.9
		Peak Apex Alignment	≤ 0.5
General System Check	SHC*IAEVEK FKDLGEEHFK KVPQVSTPTLVEVSR SLHTLFGDELC*K HPYFYAPELLYYANK	Retention Time % RSD	≤ 2%
		Peak Area % RSD	≤ 10%
		Peak Height (Min, Max)	Min ≥ 2.4E7 counts Max ≤ 1.3E9 counts
		Peak Width at 10% Height	≤ 10 seconds
		Peak Width at 10% Height %RSD	≤ 10%
Special System Check	HLVDEPQNLIK HLVDEPQ[Deamidation]NLIK SEIAHR GLVLIAFSQYLQQC*PFDEHVK	% Deamidation %CV	≤ 10%
		Δ Retention time (SEIAHR and GLVLIAFSQYLQQC*PFDEHVK)	16.5 ≤ x ≤ 18.5 minutes
		Δ Retention time (HLVDEPQNLIK and HLVDEPQ[Deamidation]NLIK)	0.1 ≤ x ≤ 0.2 minutes
Sequence coverage	BSA peptides that passes 0.1% base peak intensity threshold	% sequence coverage	≥ 80%
MS^2 check[1]	SLHTLFGDELC*K	% y6	46% ≤ x ≤ 56%
		% y2	24% ≤ x ≤ 32%
		% y3	17% ≤ x ≤ 25%

1. MS^2 check is only available in SET for Orbitrap Exploris 240 mass spectrometer
* Carbamidomethylated

Figure 1. Snapshot of the peptides associated to the general system check section (see Table 3) report with acceptance criteria, a peak summary table with monitored system performance attributes for the selected peptides reflecting the pass/fail status, and an example of detailed results showing Retention Time results across the 5 individual injections.



eWorkflow enables seamless method transfer between multiple systems

An eWorkflow was created using pre-defined instrument method(s), processing method, view setting, and report template in a well-defined injection sequence layout. It enables seamless method transfer between multiple systems across multiple sites, a useful feature for routine analysis of quality control samples to ensure consistency from batch-to-batch.

Reliable quantitation of PQAs often depends on the ability of the MS to hold mass accuracy throughout the duration of the experiments without lock mass. As shown in Figure 2, both Orbitrap Exploris 240 and MX systems can maintain less than 3 ppm maximum mass deviation for the top 3 confirming ions. The higher mass deviation (less than 5 ppm) of the 4th confirming ion is due to significantly lower signal intensity.

Figure 2: Maximum mass deviation (not RMS) for each confirming ion (1-4) of selected BSA peptides across multiple datasets obtained from Orbitrap Exploris 240 and Orbitrap Exploris MX systems

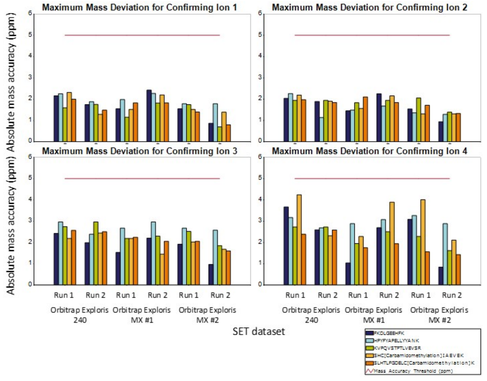
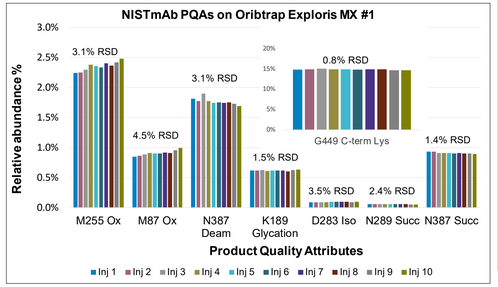


Figure 3: Evaluation of selected NISTmAb PQAs on Orbitrap Exploris MX. Plot a) shows the %M255 and %M87 oxidation, %N287 deamidation, %K189 glycation, % D283 isomerization, %N289 and N387 succinimide profile; and plot b) shows %N-glycosylation profile for NISTmAb digest across 10 replicate injections.

a) %M255 and %M87 oxidation, %N287 deamidation, %K189 glycation, % D283 isomerization, %N289 and N387 succinimide evaluation on Orbitrap Exploris MX (n=10)



b) N-glycosylation evaluation across 10 replicate injections on the Orbitrap Exploris MX system

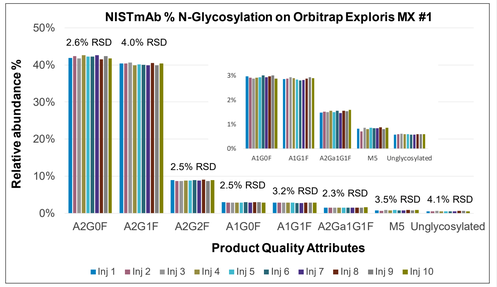
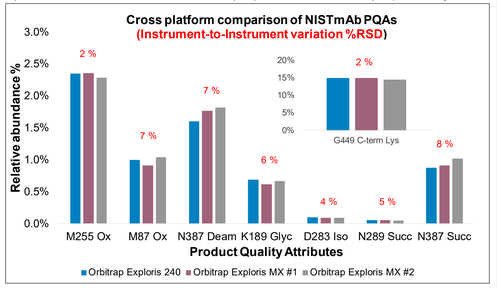
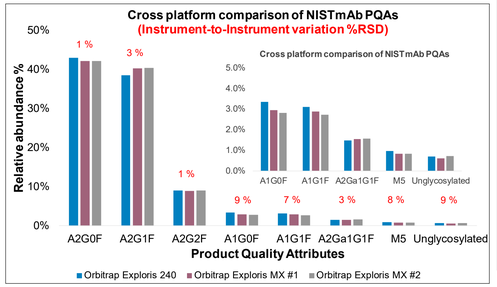


Figure 4: Cross platform comparison of NISTmAb PQAs analysis. Plot a) shows selected PQAs, and b) shows N-glycosylation evaluation across 3 instruments

a) NISTmAb PQAs evaluation across one Orbitrap Exploris 240 and two Orbitrap Exploris MX systems



b) N-glycosylation evaluation across one Orbitrap Exploris 240 and two Orbitrap Exploris MX systems



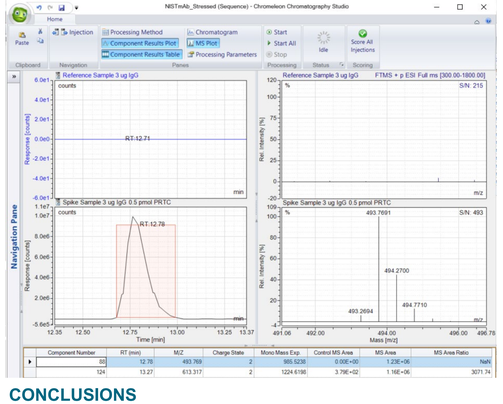
Excellent reproducibility and low instrument-to-instrument variation

Using eWorkflows, we have evaluated quantitative performance of both Orbitrap Exploris 240 and MX systems for analysis of PQAs of NISTmAb digest. Not only can we achieve excellent reproducibility with replicate injections within individual system, with measured %CV of all monitored PQAs well below 5% as shown in Figure 3 (Only MX system data shown here), we can achieve comparable quantitative PQA results across platforms, with minimal variations for the monitored PQAs as shown in Figure 4.

New peak detection for non-targeted screening of impurities, and monitoring changes to PQAs

The new peak detection feature in Chromeleon 7.3.1 uses a newly implemented algorithm allowing for unlimited frames for new component detection, and enables in-depth analysis of tested samples regardless of gradient length and sample complexity. An example of one of the 15 detected PRTC peptides in a spiked NISTmAb sample in comparison with a reference sample is shown in Figure 5.

Figure 5: Detection of one of the PRTC peptides in spiked NISTmAb sample using the new peak detection; other than the 15 PRTC peptides, none were detected as new impurities when comparing the reference NISTmAb against PRTC spiked sample injection



CONCLUSIONS

- A system performance evaluation test was developed based on a pre-digested BSA sample, a Hypersil Gold VANQUISH UHPLC column, a Vanquish Horizon or Flex UHPLC, and the Orbitrap Exploris 240 and Orbitrap Exploris MX systems, only requiring Chromeleon software for all steps from samples to result
- A comprehensive set of acceptance criteria related to LC-MS system performance was developed that are relevant for peptide mapping and monitoring
- All methods required for data acquisition and analysis, and reporting were optimized and built into eWorkflows, and these can be readily applied to assess the expected performance of the entire LC-MS system both during install and beyond for troubleshooting purposes
- Both Orbitrap Exploris 240 and Orbitrap Exploris MX systems demonstrated high repeatability across 10 replicate injections for the analysis of NISTmAb PQAs
- Excellent reproducibility and low instrument-to-instrument variations were shown for the quantitation of NISTmAb PQAs across platforms

REFERENCES

1. Thermo Fisher Scientific, "A high-resolution accurate mass multi-attribute method for critical quality attribute monitoring and new peak detection", Application Note 72916

TRADEMARKS/LICENSING

PO66106 EN0921S