An integrated LC-MS system performance evaluation test for peptide mapping and monitoring

Authors: Hao Yang[1], Melissa Sato[1], Royston Quintyn[2], Catharina Crone[3], Julia Kraegenbring[3] Kai Scheffler[4], Min Du[5],

[1]Thermo Fisher Scientific, San Jose, CA, US

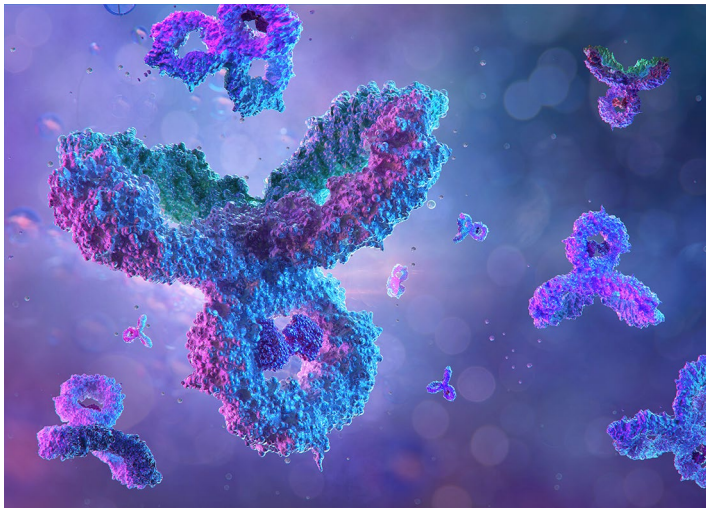
[2]Thermo Fisher Scientific, Somerset, NJ, US

[3]Thermo Fisher Scientific, Bremen, Germany

[4]Thermo Fisher Scientific, Germering, Germany

[5]Thermo Fisher Scientific, Boston, MA, US

Keywords: Liquid chromatography mass spectrometry (LC-MS), liquid chromatography high-resolution accurate-mass mass spectrometry (LC-HRAM-MS), high resolution multi-attribute method (HR MAM), Orbitrap Exploris 240 mass spectrometer, Orbitrap Exploris MX mass detector, system performance evaluation test (SET), bovine serum albumin (BSA) protein digest standard, critical quality attribute, biopharmaceutical characterization and quantitation, Chromeleon CDS, eWorkflow procedure



- Use of a Thermo Scientific™ Chromeleon™ CDS eWorkflow™ procedure comprising the instrument method, processing method, and report template that allows for the assessment of sequence coverage using full MS-only data collected on the Thermo Scientific™ Orbitrap Exploris™ 240 system or Thermo Scientific™ Orbitrap Exploris™ MX system
- Automated data acquisition, processing, and reporting under compliance-ready enterprise deployable software to satisfy the needs of regulated environments

Application benefits

- Simplicity in deployment of a system performance evaluation test based on a pre-digested sample, pre-defined instrument and processing methods, and a report template allowing the user to jump from sample queuing straight to the report that represents the results on a pass/fail basis

Goal

Demonstrate the use of an LC-MS system test for assessing the expected performance level based on a protein digest sample, and defined LC and MS performance-related attributes that are relevant for peptide mapping and monitoring analyses

ThermoFisher SCIENTIFIC

Introduction

An evaluation of the biologics license applications (BLAs) approved by the United States Food and Drug Administration (FDA) between the years 2000 and 2015 revealed that 79 of the 80 electronically submitted monoclonal antibody and protein biotherapeutic BLAs utilized mass spectrometric workflows for protein or impurity characterization.[1] The use of mass spectrometry is not limited to early discovery phase but is employed in all stages of the product life cycle, up to routine quality control.[2]

Of all the mass spectrometric workflows, LC-MS based peptide mapping plays an integral role in the characterization process, as it can indicate product quality by confirming the amino acid sequence and specific attributes at the molecular level on a lot-to-lot basis.[3] 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 issue, the FDA recently designed a workflow to evaluate the LC-MS system suitability for performing peptide mapping.[4] Although they were able to identify several critical performance metrics with proposed acceptance criteria, these metrics focused primarily on the mass spectrometer, and the workflow required the use of expensive synthetic peptides. Herein, we developed an integrated system performance evaluation test (SET) that utilizes a Thermo Scientific™ Pierce™ protein digest standard to monitor relevant metrics of the mass spectrometer as well as of the ultra-high performance liquid chromatography (UHPLC) system based on a comprehensive set of acceptance criteria. Figure 1 shows all the associated items required to run the SET using an eWorkflow, which is an electronic procedure for automatically creating an injection sequence. An eWorkflow contains all the associated methods and reporting template required to setup the injection sequence, acquire and process the data in the Chromeleon Chromatography Data System (CDS) software, and summarize the results in a report with a visual pass/fail representation.

Experimental

Reagents and consumables

- Water (H_2O), UHPLC-MS grade, Thermo Scientific (P/N W8-1)
- Methanol (MeOH), UHPLC-MS grade, Thermo Scientific (P/N A458-1)
- Isopropanol (IPA), Optima™ LC-MS grade, Thermo Scientific (P/N A461-1)
- Acetonitrile (ACN), UHPLC-MS grade, Thermo Scientific (P/N A956-1)
- Formic acid (FA), Pierce™ LC/MS grade, Thermo Scientific (P/N TS-28905)
- Pierce BSA protein digest standard, MS grade, UD294474 (P/N 88341)
- 9 mm Autosampler inserts, Thermo Scientific (P/N 033753A)
- 9 mm Clear glass screw thread vials, Thermo Scientific (P/N C5000-1)

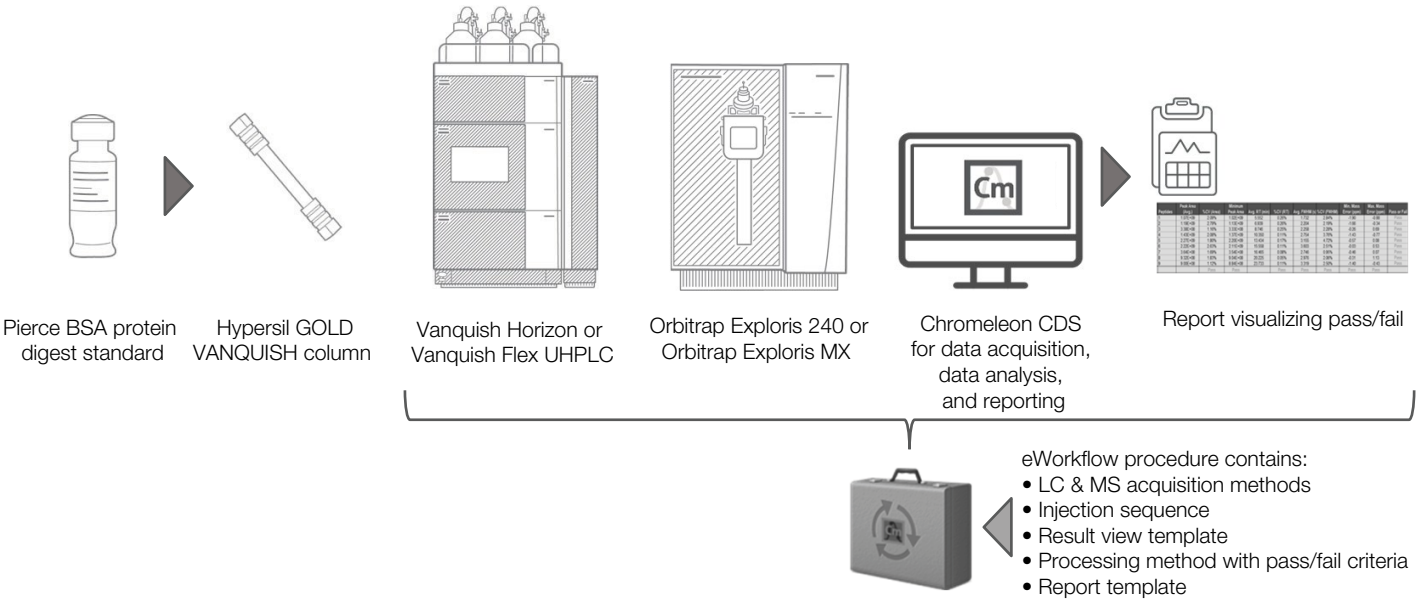


Figure 1. Overview of all components involved in performing the SET

Sample preparation

BSA protein digest sample preparation: 1 pmol/µL working solution of BSA protein digest samples were prepared by adding 1 mL of water to the vial containing 1 nmol of lyophilized BSA protein digest sample. The solution was vortexed for 10 s followed by micro-centrifugation at 500 rpm for 60 s. Ten aliquots were prepared by transferring 100 µL each to 9 mm autosampler inserts. The vials were screw capped and stored at -80 °C until used. Prior to analysis, fresh aliquots were thawed in a Thermo Scientific™ Vanquish™ Flex/Horizon autosampler set at 6 °C for 1 hour, the vials were then vortexed for 10 s to achieve homogeneity before sample injections.

eWorkflow procedure

An eWorkflow procedure consists of all the associated items required from setting up the injection sequence through processing the data to reporting of the results. Here, two eWorkflow templates were created, one for the Orbitrap Exploris 240 system and one for the Orbitrap Exploris MX system. The injection sequence for the Orbitrap Exploris 240 system providing MS^2 capability includes one additional injection using a data-dependent MS^2 ($ddMS^2$) method for assessing the fragmentation efficiency. Figure 2 shows an example of a SET injection sequence for the Orbitrap Exploris 240 system that contains a total of 12 injections. The first five injections are set up to condition a new column. Injections 6 to 11 comprise the injections for the system performance evaluation with injection 6 applying the $ddMS^2$ method and injection 12 finishing the sequence with a blank injection for column cleaning. In the following sections, the instrument methods (both chromatography and mass spectrometry), processing method, view setting, and reporting template are described, all of which are included in the SET eWorkflow template.

Chromatography

For peptide separation, either a Thermo Scientific™ Vanquish™ Flex UHPLC system or a Thermo Scientific™ Vanquish™ Horizon UHPLC system can be used as both exhibit similar performance for the applied gradient detailed in Table 1. The modules present in each UHPLC system are listed in Table 2. For all analyses, 5 µL containing a total of 5 pmol of BSA protein digest samples were injected onto a Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 UHPLC column using the LC gradient and conditions outlined in Table 1.

Table 1. LC and autosampler conditions

Parameter	Value
UHPLC column	Hypersil GOLD VANQUISH C18 UHPLC column, 150 × 2.1 mm, 1.9 µm (P/N 25002-152130-V)
Column temperature	50 °C
Flow rate	0.25 mL/min
Sovent A	H_2O + 0.1% FA
Solvent B	ACN + 0.1% FA
Gradient	*Time (min)* / *% Solvent B*: 0.0 / 2; 0.5 / 2; 1 / 9; 22 / 35; 23 / 90; 26 / 90; 27 / 2; 45 / 2
Injection volume	5 µL
Needle wash solution	10% MeOH with 0.1% FA
Seal rinse solution	10% MeOH (Vanquish Flex UHPLC) 75% IPA with 0.1% FA (Vanquish Horizon UHPLC)
Autosampler temperature	6 °C
Thermostatting mode	Still Air
Needle wash option	Before and after injection
Wash speed and time	34 µL/s for 10 s

Sequence Preview

#	Chromatogram	Name	Type	Position	Volume	Instrument Method	Processing Method
1	None	Blank_pre	Check Standard	R:A1	2.0000	MAM2.0_SET_MS1 Method	
2	None	NewColumnConditioning	Check Standard	R:A2	5.0000	MAM2.0_SET_MS1 Method	
3	None	NewColumnConditioning	Check Standard	R:A2	5.0000	MAM2.0_SET_MS1 Method	
4	None	NewColumnConditioning	Check Standard	R:A2	5.0000	MAM2.0_SET_MS1 Method	
5	None	NewColumnConditioning	Check Standard	R:A2	5.0000	MAM2.0_SET_MS1 Method	
6	None	MAM2.0_SET_BSA_xxx_MSMS	Unknown	R:A2	5.0000	MAM2.0_SET_MS2 Method	MAM2.0_SET_Processing Method_OE240
7	None	MAM2.0_SET_BSA_xxx_MSOnly_1	Unknown	R:A2	5.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
8	None	MAM2.0_SET_BSA_xxx_MSOnly_2	Unknown	R:A2	5.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
9	None	MAM2.0_SET_BSA_xxx_MSOnly_3	Unknown	R:A2	5.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
10	None	MAM2.0_SET_BSA_xxx_MSOnly_4	Unknown	R:A2	5.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
11	None	MAM2.0_SET_BSA_xxx_MSOnly_5	Unknown	R:A2	5.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
12	None	Blank_end	Matrix	R:A1	2.0000	MAM2.0_SET_MS1 Method	MAM2.0_SET_Processing Method_OE240
13	None	Shutdown	Matrix	R:A1	2.0000	MAM2.0_Shutdwon Method	

Figure 2. SET injection sequence included in the eWorkflow template for the Orbitrap Exploris 240 system

Table 2. Vanquish UHPLC system modules and part numbers

Modules	Vanquish Flex (P/N)	Vanquish Horizon (P/N)
System Base F/H	VF-S01-A-02	VF-S01-A-02
Binary Pump F/H	VF-P10-A-01	VH-P10-A-01
Split Sampler FT/HT	VF-A10-A-02	VH-A10-A-02
Column compartment H	VF-C10-A-03	VH-C10-A-03

Mass spectrometry

A full MS-only method covering a mass range of *m/z* 280–1,600 and a resolution setting of 120,000 was applied for both the Orbitrap Exploris 240 system and the Orbitrap Exploris MX system. This method is used for assessing all relevant system performance criteria except for the fragmentation efficiency, which requires a second ddMS^2 method applied only on the Orbitrap Exploris 240 system. Detailed instrument method and source parameters for both systems are summarized in Table 3.

Processing method

A single processing method was built using the MS quantitative template in Chromeleon CDS. As shown in the above injection sequence layout (Figure 2, Processing Method column), only the BSA protein digest sample injections were analyzed using this processing method. The processing method applies the following settings: MS detection algorithm Genesis, manually defined mass tolerance 5 ppm, inhibit integration for TIC channel, Gaussian smoothing 5 points. Composite scoring using isotopic dot product, mass accuracy, and peak apex alignment options were applied with scoring results to pass only if three criteria are met. A representative selection of peptides was chosen to assess sequence coverage based on peptide precursors *m/z* values. A subset of these peptides, which differ in signal intensity, hydrophobicity, and observed charge states ranging from 2 to 5, was used for monitoring.

Table 3. Instrument method parameters for the Orbitrap Exploris 240 and Orbitrap Exploris MX systems (Note: Unless otherwise indicated, default parameters were used)

Orbitrap Exploris 240 and Orbitrap Exploris MX instruments	
MS source parameters	
Spray voltage (V)	3,500
Sheath gas (Arb)	30
Aux gas (Arb)	10
Sweep gas (Arb)	1
Ion transfer tube temperature (°C)	225
Vaporizer temperature (°C)	200
Full scan parameters	
Expected LC peak width (s)	3
Resolution (at *m/z* 200)	120,000
Scan range (*m/z*)	280–1,600
Time range (min)	0–24
Data dependent MS^2 scan parameters (Exploris 240 only)	
MIPS filter: Monoisotopic peak determination	Peptide
Intensity filter: Intensity threshold (counts)	5.0e3
Charge states filter: Include charge state(s)	2–8

View settings

An eWorkflow procedure contains details regarding customizable view settings. For the SET template, we have defined the view settings as displayed in Figure 3.

Reporting template

A report template was built for optimal presentation of the obtained results. For the SET, the critical system performance attributes and acceptance criteria were defined and applied as summarized in Table 4. The monitored attributes are divided into four sections: composite scoring, a general system check, a special system, sequence coverage, and MS^2 check (applies to the Orbitrap Exploris 240 system only). While for the composite scoring and general system check the same set of five peptides is used, the special system check monitors another four peptides, one of which includes a deamidation.

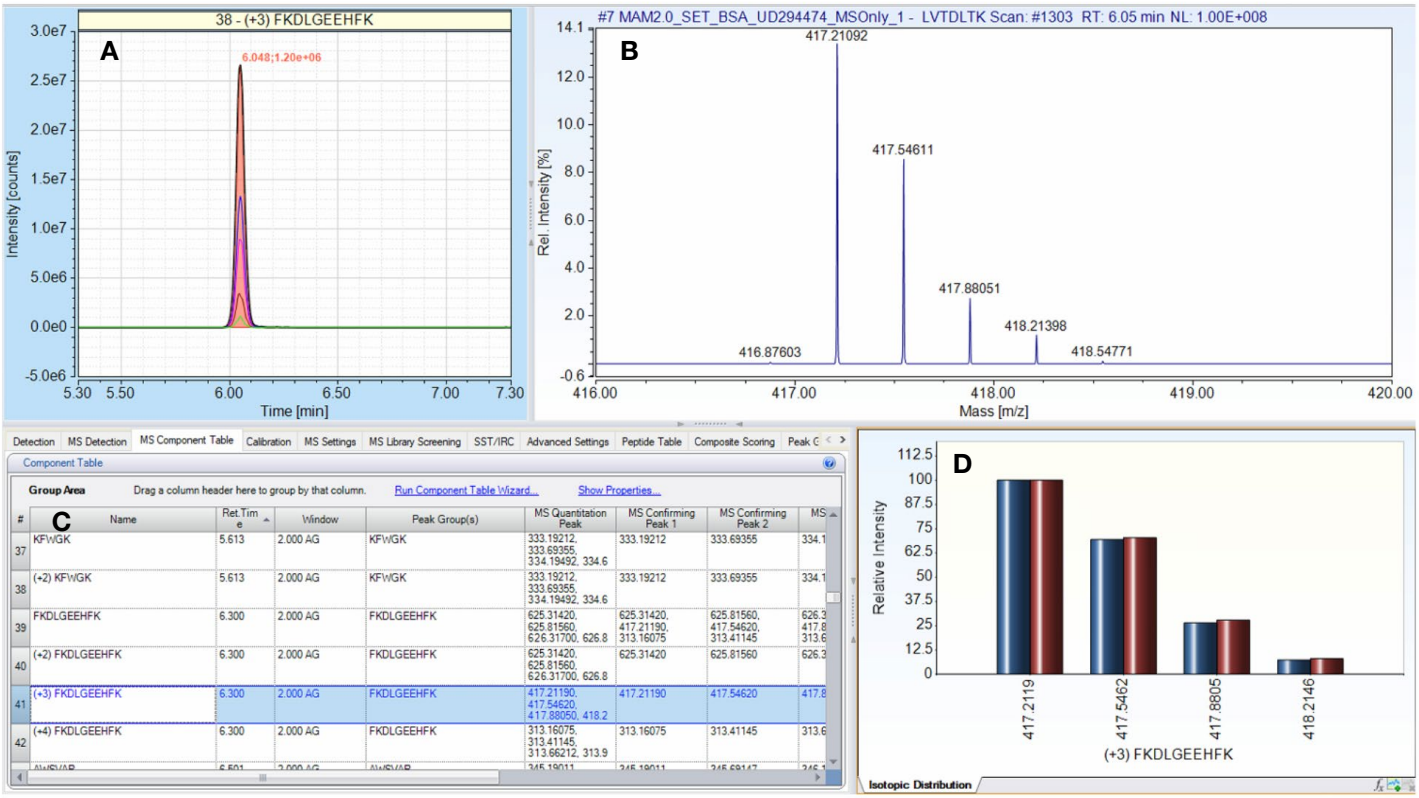


Figure 3. SET eWorkflow procedure view settings, showing an overlay of the XICs of the four most abundant isotopes of the selected peptide FKDLGEEHFK (A, MS component pane), the MS spectrum of the doubly charged precursor (B), the component table (C), and comparison of observed and theoretical isotopic distribution for the selected component (D)

Table 4. Monitored BSA peptides, critical system performance attributes, and 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%
MS² check†	SLHTLFGDELC*K	% y_6	46% ≤ x ≤ 56%
		% y_2	24% ≤ x ≤ 32%
		% y_3	17% ≤ x ≤ 25%

* Carbamidomethylated

† MS² check is only available in the SET for the Orbitrap Exploris 240 mass spectrometer

The SET is defined and reflected as “pass” when all measured critical system performance attributes for all monitored peptides meet the acceptance criteria. An example of obtained results representing the attributes monitored in the general system check section are shown in Figure 4, with a summary of the sequence details and acceptance criteria in the upper section, the peak summary table listing the monitored five peptides and the attributes avg. RT (min), %CV (RT), %CV (Area), min. peak height, max. peak height, %CV peak width, and max. peak width, and the last row reflecting the pass/fail results obtained for every monitored peptide across all five injections. The third section shows a detailed view into the retention time attributes and results obtained across the five individual injections for the five monitored peptide and the “pass/fail” visual result.

Software

Chromeleon CDS version 7.3.1 was used for data acquisition, processing, and reporting.

Results and discussion

The developed eWorkflow procedures as described in the Experimental section were applied on both instruments, and the obtained results relating to monitored system performance attributes will be laid out in the following sections.

Composite scoring test

For composite scoring, three MS criteria were defined:

- Mass accuracy
- Isotopic dot product, which is a Pearson correlation between the isotope distribution of the observed versus theoretical. For the peptide to pass, there must be at least a 90% match.
- Verification of the retention time peak apex alignment for a specific confirming peak across charge states.

For a peptide to pass composite scoring, it must pass all three MS criteria, thus providing the highest confidence in the identified peptide and confirming that the correct peak was selected for integration.

The applied instrument methods do not use internal lock mass but rely on external mass calibration. The maximum mass deviation for confirming ion 1 (most abundant isotope) of all monitored peptides including multiple charge states did not exceed 3 ppm across multiple datasets collected (Figure 5). If we consider four confirming ions, on the least abundant ion which demonstrates the largest mass deviation we can comfortably satisfy a mass deviation below the required 5 ppm, specified in the acceptance criteria (Table 4)..

SET Report Example

ThermoFisher SCIENTIFIC

Sequence Details

Name:	Sequence Name	Criteria		
Instrument:	Instrument Name	RT %CV	< or =	2%
Imported Data:		Peak Area %CV	< or =	10%
First Injection:	First Injection Name	Min Peak Height	> or =	2.40E+07
Processing Method:	Processing Method Name	Max Peak Height	< or =	1.30E+09
MS Acquisition Time [min]	24.01	Max. Peak Width 10% Height (s)	< or =	10
Method Length [min]:	45.00	Peak Width 10% Height %CV	< or =	10%
Total Time [hrs]	8.78			
Data Vault:	Data Vault Name			
No. of Injections:	12			

Peak Summary

BSA Peptides	Avg. RT (min)	%CV (RT)	%CV (Area)	Min. Peak Height (counts/sec)	Max. Peak Height (counts/sec)	%CV (Peak Width 10% Height)	Max. Peak Width 10% Height (s)	Pass or Fail
SHC[Carbamidomethylation]IAEVEK	5.00	0.15%	2.85%	2.88E+08	3.19E+08	1.40%	4.06	Pass
FKDLGEEHFK	7.20	0.11%	1.77%	1.09E+08	1.17E+08	1.15%	5.18	Pass
KVPQVSTPTLVEVSR	11.90	0.08%	1.49%	6.57E+08	6.99E+08	1.59%	6.53	Pass
SLHTLFGDELC[Carbamidomethylation]K	13.74	0.06%	0.77%	7.06E+08	7.79E+08	1.46%	6.29	Pass
HPYFYAPELLYYANK	17.47	0.03%	2.54%	2.14E+08	2.32E+08	1.67%	6.08	Pass

Retention Time

Injection Name	RT min SHC[Carbamidomethylation]IAEVEK	RT min FKDLGEEHFK	RT min KVPQVSTPTLVEVSR	RT min SLHTLFGDELC[Carbamidomethylation]K	RT min HPYFYAPELLYYANK
MAM2.0_SET_BSA_UD279474_MSOnly_1	4.99	7.21	11.91	13.73	17.47
MAM2.0_SET_BSA_UD279474_MSOnly_2	5.00	7.20	11.91	13.73	17.48
MAM2.0_SET_BSA_UD279474_MSOnly_3	5.00	7.21	11.89	13.75	17.47
MAM2.0_SET_BSA_UD279474_MSOnly_4	4.99	7.19	11.90	13.75	17.47
MAM2.0_SET_BSA_UD279474_MSOnly_5	5.00	7.20	11.90	13.74	17.47
Average RT	5.00	7.20	11.90	13.74	17.47
%CV (RT)	0.15%	0.11%	0.08%	0.06%	0.03%
Pass or Fail	Pass	Pass	Pass	Pass	Pass

Figure 4. SET report excerpt representing a section with sequence details and acceptance criteria, the peak summary table for five peptides, and obtained results for the defined critical system performance attributes

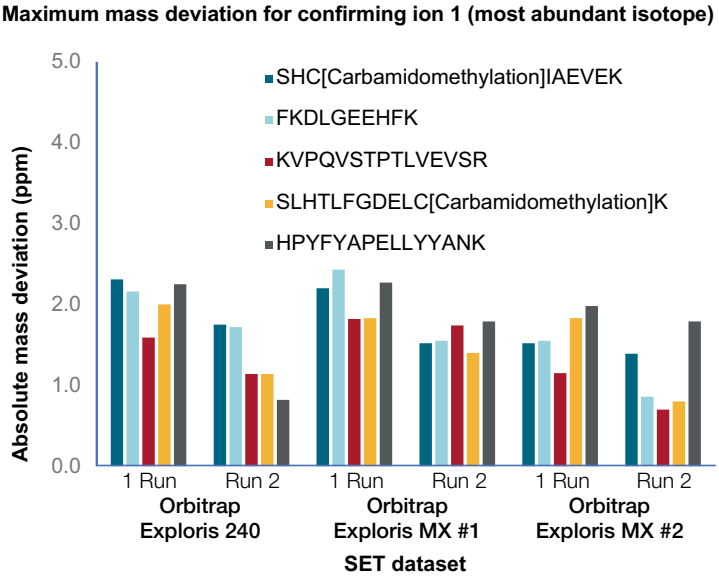


Figure 5. Maximum mass deviation for confirming ion 1 (most abundant isotope) of peptides across multiple datasets obtained from one Orbitrap Exploris 240 and two Orbitrap Exploris MX systems. Each peptide is composed of 2 charge states, +2 and +3, and the mass accuracies of both charge states across all injections are monitored. Only the maximum mass deviation of the monoisotopic ion is reported here.

General system check

As outlined in Table 3, the general system check relates to critical performance attributes for both LC and MS, examining chromatographic precision, injection reproducibility, column efficiency, and MS sensitivity. The general system check monitors high abundant peptides. Overlaying the TIC trace of replicate injections provides insight into reproducibility across all injections. Figure 6 shows five TIC traces that overlap almost completely. A closer look at the test result revealed that the system passed with less than 0.2% retention time, 3% peak area, 5% peak height, and 2% peak width at 10% height variation for all monitored peptides across the entire gradient. This test also captures peak width at 10% height, a critical metric for column screening, and absolute peak height, which can be used to estimate the amount of injected sample. These tests not only provide a pass/fail result, reflecting the required performance level of the instrument, but can also be used to track system performance over time.

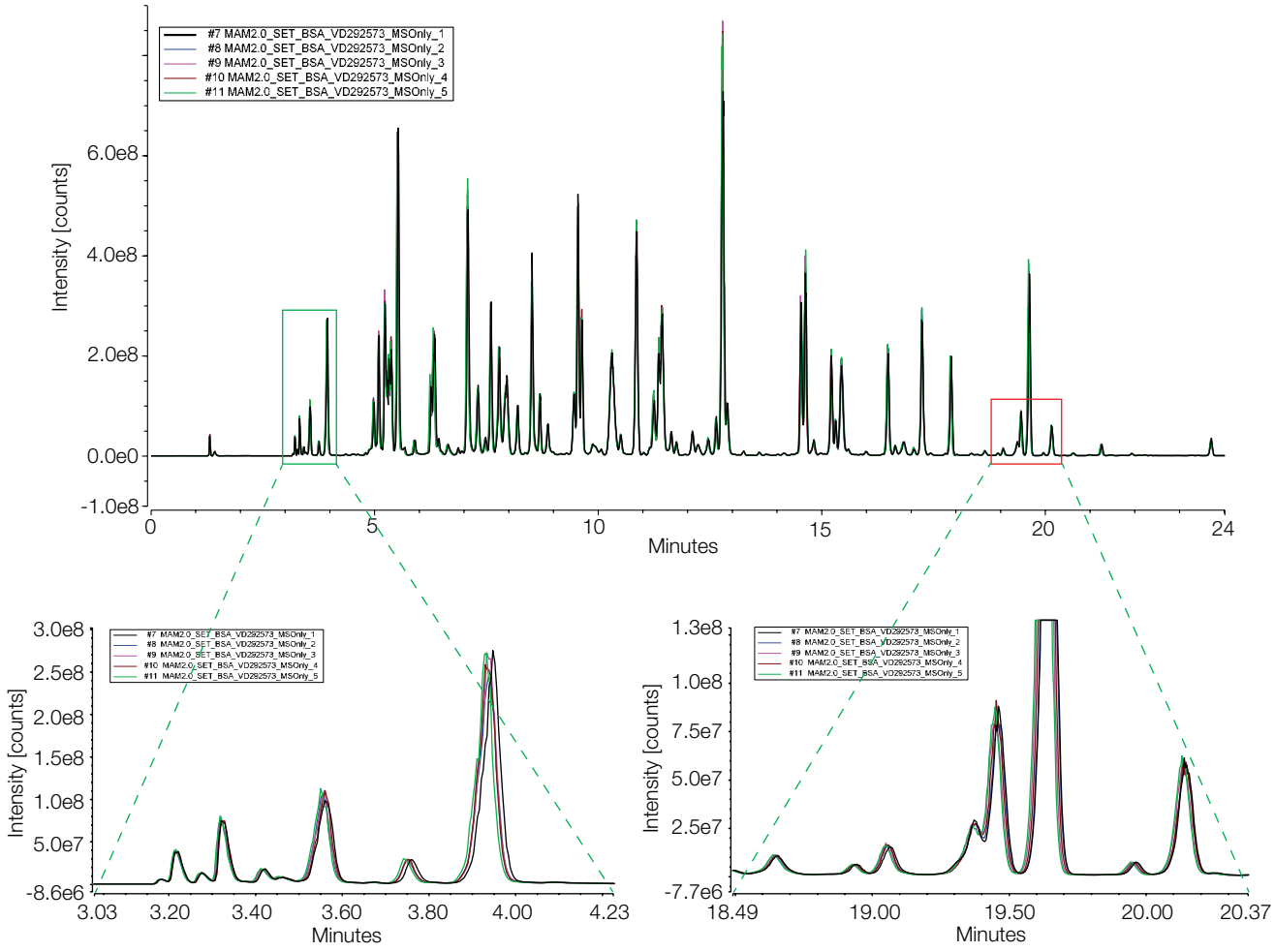


Figure 6. Overlay of 5 TIC traces from the SET injection sequence

Special system check

Unlike the general system check in which the monitored peptides are highly abundant, the special system check tracks the relative quantitation of low abundant, modified peptides. The peptide HLVDEPQNLIK was chosen as it has three deamidated forms, and the sum of all three was used to calculate % deamidation. As shown in Figure 7, percentage deamidation is consistent across multiple datasets collected from the same tested lot across three different systems. The acceptance criteria for % deamidation is set to account for lot-to-lot variations. The special system check also inspects the relative retention time rather than absolute retention time of the selected peptides to account for gradient delays and extra void volume attributed from different column lots and LC configurations.

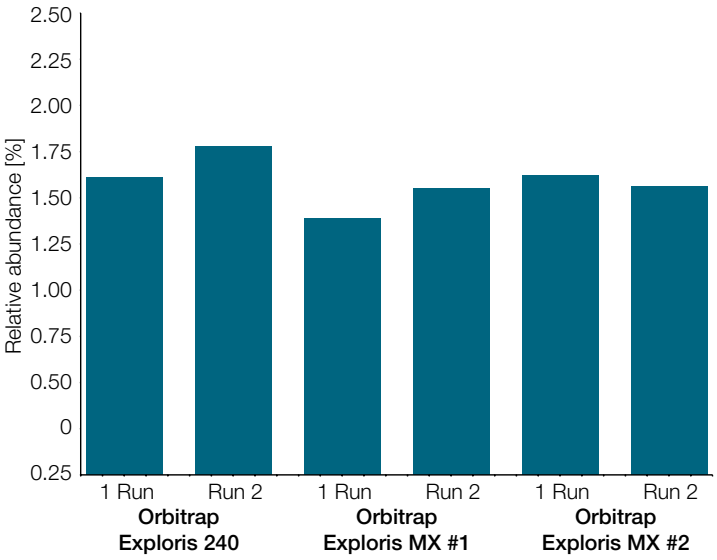


Figure 7. % modification for monitored peptides across two separate SET experiments, each from the Orbitrap Exploris 240 and Orbitrap Exploris MX systems

Sequence coverage

The SET relies on a commercially available, validated BSA protein digested standards, yet improper sample storage or use of expired samples can lead to failure of the SET. Therefore, including sequence coverage as an attribute in the SET supports the assessment of sample quality. Here, we have implemented a step in the report template to calculate the sequence coverage based on *m/z* values of a selected set of 42 tryptic peptides and one peptide containing a missed cleavage site, covering 81.1% of the BSA sequence. This procedure is an alternative approach and does not relate to a traditional database search. The intensities of the three most abundant isotopes of each peptide detected in its most abundant charge state are summed and compared with 0.1% of the base peak. If the summed intensity is higher than the threshold, the number of amino acids in the peptide is counted. If both the miscleaved peptide and the overlapping fully cleaved peptides are found, only the miscleaved peptide is counted. The sum of the counted amino acids is divided by the total of 607 amino acids contained in BSA to yield the % sequence coverage.

Among several different BSA lots tested, the obtained sequence coverages were in all cases above the required 80% to pass the acceptance criteria, and with that, very comparable with the results from Thermo Scientific™ BioPharma Finder™ software using the same full MS only datasets (data not shown).

MS^2 test

The evaluation of the MS^2 fragmentation efficiency is exclusive for the Orbitrap Exploris 240 MS SET and based on the peptide SLHTLFGDELC[Carbamidomethylation]K by applying a fixed collision energy. The relative abundances of the three most abundant fragment ions y_2, y_3, and y_6 were monitored. Acceptance criteria were based on results obtained from large sample datasets and defined as ranges to allow for an expected level of variation.

thermo scientific

Conclusion

- A system performance evaluation test was developed based on a pre-digested BSA sample, a Hypersil GOLD VANQUISH UHPLC column, a Vanquish Horizon or Vanquish 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 is relevant for peptide mapping and monitoring.
- All methods required for data acquisition, analysis, and reporting were optimized and built into eWorkflow procedures.
- These eWorkflow templates can be readily applied to assess the expected performance of the entire LC-MS system, during install and beyond for troubleshooting purposes.
- Chromeleon CDS is a compliance-ready, enterprise-deployable software enabling the application of the SET eWorkflow procedure also in regulated environments.

References

1. Rogstad, S.; Faustino, A.; Ruth, A.; Keire, D; Boyne, M. and Park, J. A Retrospective Evaluation of the Use of Mass Spectrometry in FDA Biologics License Applications, *J. Am. Soc. Mass Spectrom.* **2017,** *28,* 786–794.
2. Rogstad, S.; Yan, H.; Wang, X.; Powers, D.; Brorson, K.; Damdinsuren, B.; Lee, S. Multi-Attribute Method for Quality Control of Therapeutic Proteins, *Anal. Chem.* **2019,** *91,* 14170–14177.
3. Moore, S.; Dougherty, J and Mhatre, R. Using Peptide Maps as Identity and Purity Tests for Lot Release Testing of Recombinant Therapeutic Proteins, *BioPharm International* **2003,***16,* 54–58.
4. Zhou, M.; Gucinski, A.C.; Boyne II, M.T., Performance metrics for evaluating system suitability in liquid chromatography—Mass spectrometry peptide mass mapping of protein therapeutics and monoclonal antibodies, *mAbs* **2015,** *7*(6), 1104–1117.
5. Rochat, B.; Kottelat, E.; McMullen, J. The future key role of LC–high-resolution-MS analyses in clinical laboratories: a focus on quantification. *Bioanalysis* **2012,** *4,* 2939–2958.
6. Olsen, J.V. et al., Parts per Million Mass Accuracy on an Orbitrap Mass Spectrometer via Lock Mass Injection into a C-trap, *MCP* **2011,** *4*(12), 2010–2021.

Find out more at **thermofisher.com/mam**

ThermoFisher SCIENTIFIC