LC-MS/MS System Suitability Evaluation with Automated Data Processing for Protein Analysis in a Regulated Environment

Wencheng Ge, Bhavin Patel, Thermo Fisher Scientific, 3747 N Meridian Rd, Rockford, IL, USA, 61101

ABSTRACT

Purpose: Develop an automatic data analysis workflow using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software for LC-MS based proteomics system performance evaluation to ensure compliance in a regulated environment.

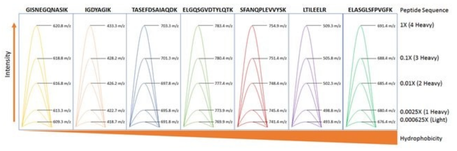
Methods: A quality control standard for the LC-MS system suitability analysis was created using 200ng/µL of HeLa protein digest spiked with different amounts of 7x5 mix standard. This standard was run using Thermo Scientific™ UltiMate™ 3000 RSLCnano System coupled to Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap or Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometers on different days. The standard peptides were separated using the Thermo Scientific™ Acclaim™ PepMap™ 100 C18 TRAP column and Thermo Scientific™ EASY-Spray™ ES800A/ES803A columns. A Chromeleon data analysis and reporting workflow was developed to automatically analyze, quantitate and report results for routine system suitability checks. Attributes such as retention time, linearity of peptide isotopologues and LLOQ were evaluated in both Skyline and Chromeleon CDS software.

Results: We demonstrate that Chromeleon CDS software which enables compliance with GxP and 21 CFR part 11 can be applied for automated data analysis of a LC-MS system suitability standard and is an effective tool to assess system performance in a regulated lab.

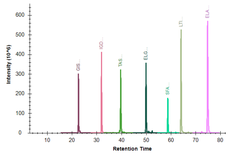
INTRODUCTION

In LC-MS/MS bioanalytical method development and process validation, system suitability assessment plays a vital role to ensure the accuracy and precision of data generated on the system before downstream sample analyses. Although protein digest standards provide a biological relevant matrix of sufficient complexity to qualitatively assess the instrument performance for bottom-up proteomics study, they lack the ability to quantitatively evaluate the sensitivity and dynamic range of LC-MS/MS system. Here, we evaluated the Thermo Scientific™ Pierce™ HeLa Protein Digest Standard combined with the Pierce™ LC-MS/MS System Suitability Standard (7x5 Mix) and developed an automatic data analysis workflow in Chromeleon CDS software for system suitability assessment in regulated environment.

Retention times and dilution series of 7 x 5 Mix



7x5 Mix (TIC)



MATERIALS AND METHODS

Sample Preparation

Pierce HeLa Protein Digest Standard (PN#88328) was reconstituted in 5% ACN with 0.1% formic acid to 200 ng/µl. The HeLa digest was spiked three different amounts (200 fmol, 400 fmol, 600 fmol for highest concentration peptide on column) of Pierce LC-MS/MS System Suitability Standard 7x5 Mix (PN# A40010) using 5%DMSO/1% formic acid to QS to the same volume to generate three different QC standards.

LC-MS analysis

All QC standard samples were analyzed using an EASY-Spray C18 15 cm column with the Acclaim™ PepMap™ C18 trap column. Thermo Scientific™ Dionex™ Ultimate™ 3000RSLCnano system with a gradient run using water with 0.1% formic acid as mobile phase A, and ACN with 0.1% formic acid as mobile phase B. We tested both long and short gradient in the LC method. Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer was operated in data-dependent mode and subsequent parallel reaction monitoring (PRM) mode with the inclusion list targeting a selection of HeLa peptides and all 7x5 peptides. The Orbitrap Fusion Tribrid mass spectrometer was run using the Orbitrap for both MS1 and MS^2 spectra acquisition using the parameters below.

PRM method on QEHF MS	
Resolution	30,000
AGC target	2e5
Maximum IT	115 ms
Isolation window	2.0 m/z

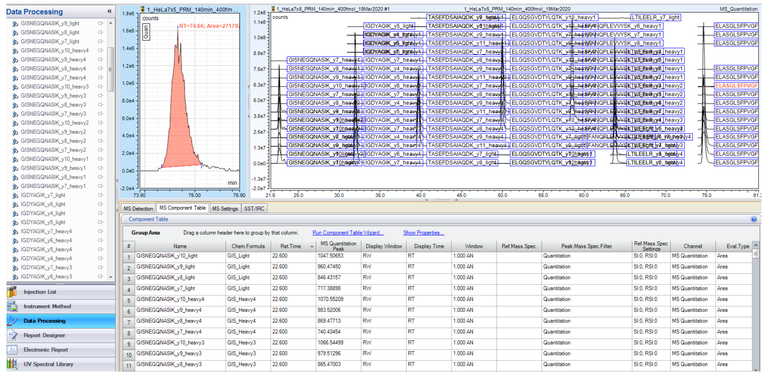
Full MS and Targeted MS2 on Fusion MS			
MS OT		tMS^2 OT HCD	
Orbitrap resolution	60,000	Orbitrap resolution	15,000
Scan range (m/z)	350 – 1500	Scan range (m/z)	200 – 2000
RF Lens (%)	60	Isolation window	1.6 m/z
AGC target	4e5	AGC target	5e4
Maximum IT	50 ms	Maximum IT	22 ms

Data Analysis

Data was analyzed using both Skyline (University of Washington) and Chromeleon 7.2 software.

RESULTS

Figure 1. Chromeleon processing method which extracts MS^2 data of 7x5 peptides automatically.



Adjusting peptide retention time during data processing may be necessary correct RT shifts for proper peak integration.

Figure 2. Calibration curves of the HeLa/7x5 mix generated in Chromeleon report demonstrating a system dynamic range.

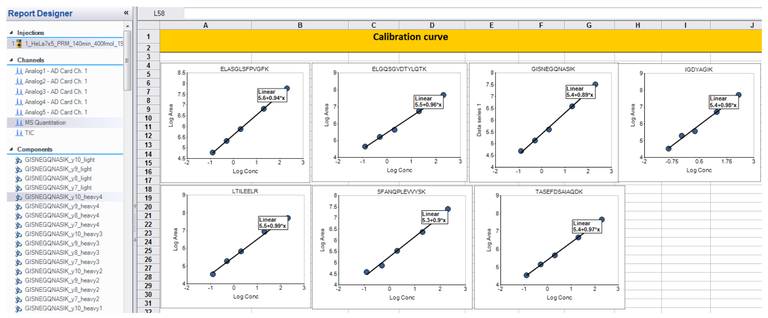
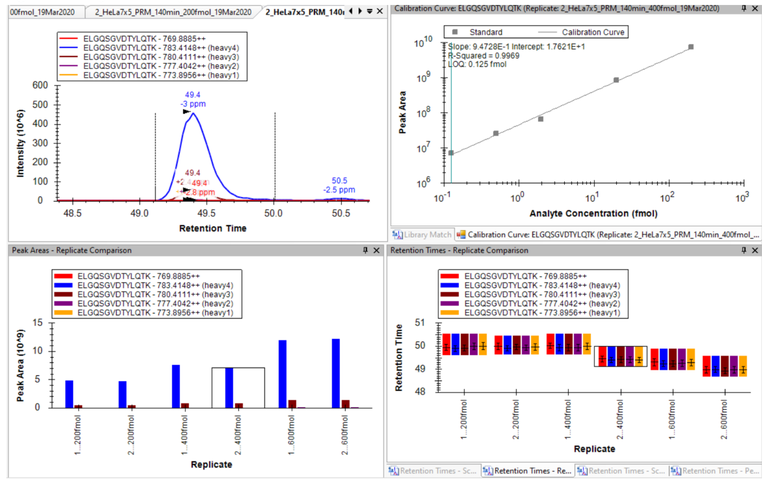
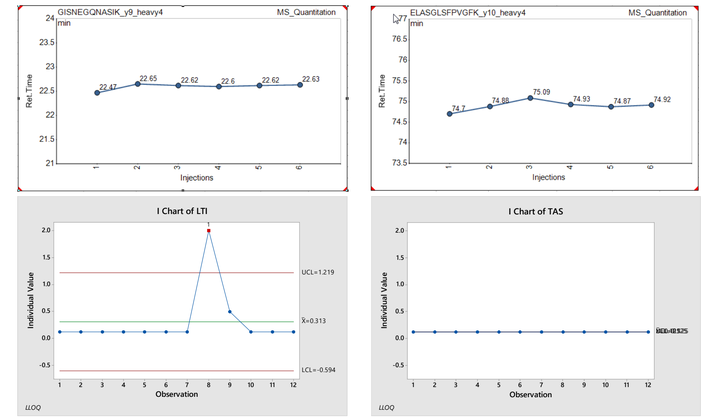


Figure 3. Skyline analysis of HeLa 7x5 Mix showing RT, linearity and LLOQ.



Skyline software was used to assess the peptide RT, dynamic range and linearity of 7x5 Mix. We monitored the response of different sample loading amounts on column, and the result showed increased peak area as desired.

Figure 4. Control chart to track the 7x5 peptide RT and LLOQ over multiple injections.



Control chart showed stable retention time and good LLOQ response over different QC sample injections (Example peptide results were displayed).

Chromeleon report template enables automatic data reporting

7x5 Mix in a HeLa digest proteome matrix to assess LC-MS system performance

The Chromeleon report template automatically generates dynamic range and linearity result for the 7x5 Mix in a HeLa digest matrix QC sample. Note that the user can set their own LLOQ %Bias requirement to determine the LLOQ concentration. Due to retention time (RT) shifts from different sample injections, adjusting the RT while processing the data is required for correct peak integration.

Table 1. Chromeleon report template automatically calculated the LLOQ and linearity from the calibration curve of 7x5 peptides (one peptide example displayed below).

Peptide	Isotope label	Conc. (fmol)	Area	Log Conc.	Log Area	%Bias	R^2
ELASGLSFP VGFK	Light	0.125	60094	-0.9031	4.7788	3.9%	1.000
	Heavy 1	0.5	210000	-0.3010	5.3222	-1.3%	
	Heavy 2	2	752068	0.3010	5.8763	-3.8%	
	Heavy 3	20	6660506	1.3010	6.8235	-1.4%	
	Heavy 4	200	59897387	2.3010	7.7774	2.7%	

CONCLUSIONS

- A processing method and report template to automatically assess the LC-MS/MS system suitability for proteomics analyses were developed in Chromeleon CDS software.
- HeLa 7x5 QC standard results generated from Chromeleon CDS software were comparable to those obtained from the Skyline software to assess the LC-MS method, dynamic range, linearity and LLOQ.
- HeLa 7x5 QC standard can be used in LC-MS method optimization to assess system sensitivity and performance overtime and before subsequent analysis of proteomics samples.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures or protocols.

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