Poster Reprint

ASMS 2026
MP-288

Automated LC/MS System Readiness Assessment for MAM Using Purpose Designed Peptide Standards

Mahsan Miladi[1], Mike Knierman[1], Melissa Sato[1], Tristan Chutka[1], Andrew McEachran[1]

[1]Agilent Technologies Inc., Santa Clara

Introduction

Multi-Attribute Method (MAM) workflows require consistent LC/MS performance to ensure the instrument is functioning within predefined criteria (e.g., mass accuracy, retention time (RT) stability, and detector response) and is "fit-for-purpose" before valuable samples are injected.[1-3] Traditional system suitability checks often rely on manual reviews of complex protein digests, which introduces subjectivity, analyst variability, and delays. [3-4]

We present an automated system readiness workflow using a purpose-designed peptide standard mixture. This approach offers an objective, scalable framework for confirming and documenting instrument suitability prior to routine MAM analysis in biopharmaceutical development and QC environments.[5]

Experimental

The system readiness assessment utilizes an Agilent 13 peptide standard mixture (1 pmol/µL). While specific peptides within this mixture are grouped into diagnostic pairs to intentionally challenge instrument stressors (e.g., oxidative stress, metal contamination, or in-source fragmentation), the full set of 13 peptides is strategically distributed across the analytical gradient. This broad coverage ensures that general metrics such as mass accuracy, retention time stability, and peak area precision are verified across the entire range where critical quality attributes (CQAs) typically elute. The lyophilized standard was reconstituted in 200 µL of a solvent consisting of water with 1% (v/v) formic acid and 5% (v/v) acetonitrile. This defined mixture eliminates the inherent variability and lengthy preparation times associated with traditional complex protein digests, providing a more consistent and objective measure of system health.

Diagnostic Probe	Peptides Involved	Performance Attribute Assessed
Oxidation-prone peptide pair	DTLMISR/DTLMoxyISR	Column aging or system-related oxidative stress
Isotopically labeled pair	ALPAPIEK/ALPAPIEK*	MS dynamic range
Metal affinity peptide pair	VLQPGYLEVDY/VLQPGYLEVDY+Fe	Column aging or system metal contamination
Metal affinity peptide pair	ALPAPIEK/ALPAPIEK+Fe	Column aging or system metal contamination
Deamidation pair	VVSVLTVLHQDWLNGK/VVSVLTVLHQDWLDGK	Mass accuracy and chromatographic resolution
Sodium adduct pair	SNFR/SNFR + Na	Buffer composition or system salt contamination
Fragile peptide	ALELFR	Insource fragmentation

Experimental

A defined peptide mixture is analyzed by LC/MS and processed using automated system readiness workflow to generate an objective pass/fail readiness assessment that documents LC/MS suitability prior to MAM analysis.

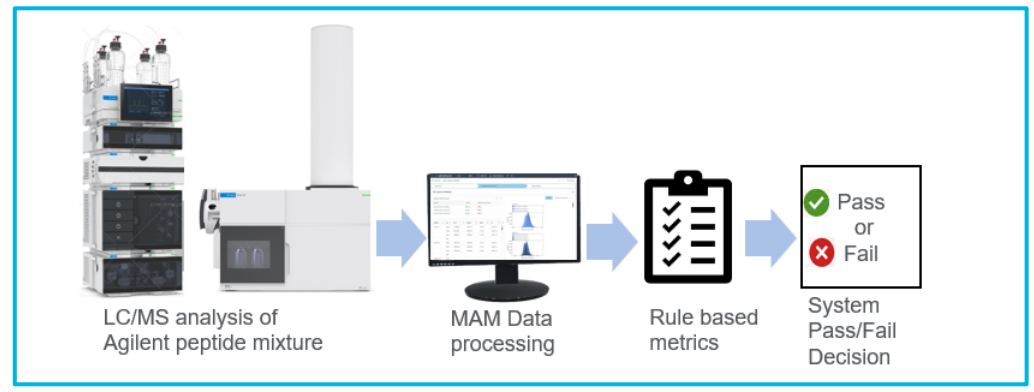


Figure 1. Automated LC/MS system readiness workflow implemented within the MAM software.

LC Conditions	
Column	AdvanceBio Peptide Mapping C18, 2.1 x 150 mm, (p/n 653750-902)
Solvent A	Water with 0.1% FA
Solvent B	ACN with 0.1% FA
Gradient	0 min, 2% B 2 min, 2% B 20 min, 35% B 21 min, 80% B 22 to 14 min, 80%B 23.1 min, 2%B 27 min, 2%B
Flow rate	0.4 mL/min
Column temperature	50 °C
MS Conditions (G6230C)	
Ion Source	Agilent Jet Stream ESI source
Polarity	Positive
MS Scan Range	m/z 200 to 2200
Acquisition Rate	3
Fragmentor	45 V
Skimmer	35 V
Data Storage	Profile
Drying Gas Flow	13 L/min
Nebulizer Pressure	35 psi
Sheath Gas Flow	12 L/min
Sheath Gas Temperature	300 °C
Capillary Voltage	3000 V
Nozzle Voltage	0 V
Gas Temperature	300 °C

Results and Discussion

Targeted Feature Extraction & Grouping

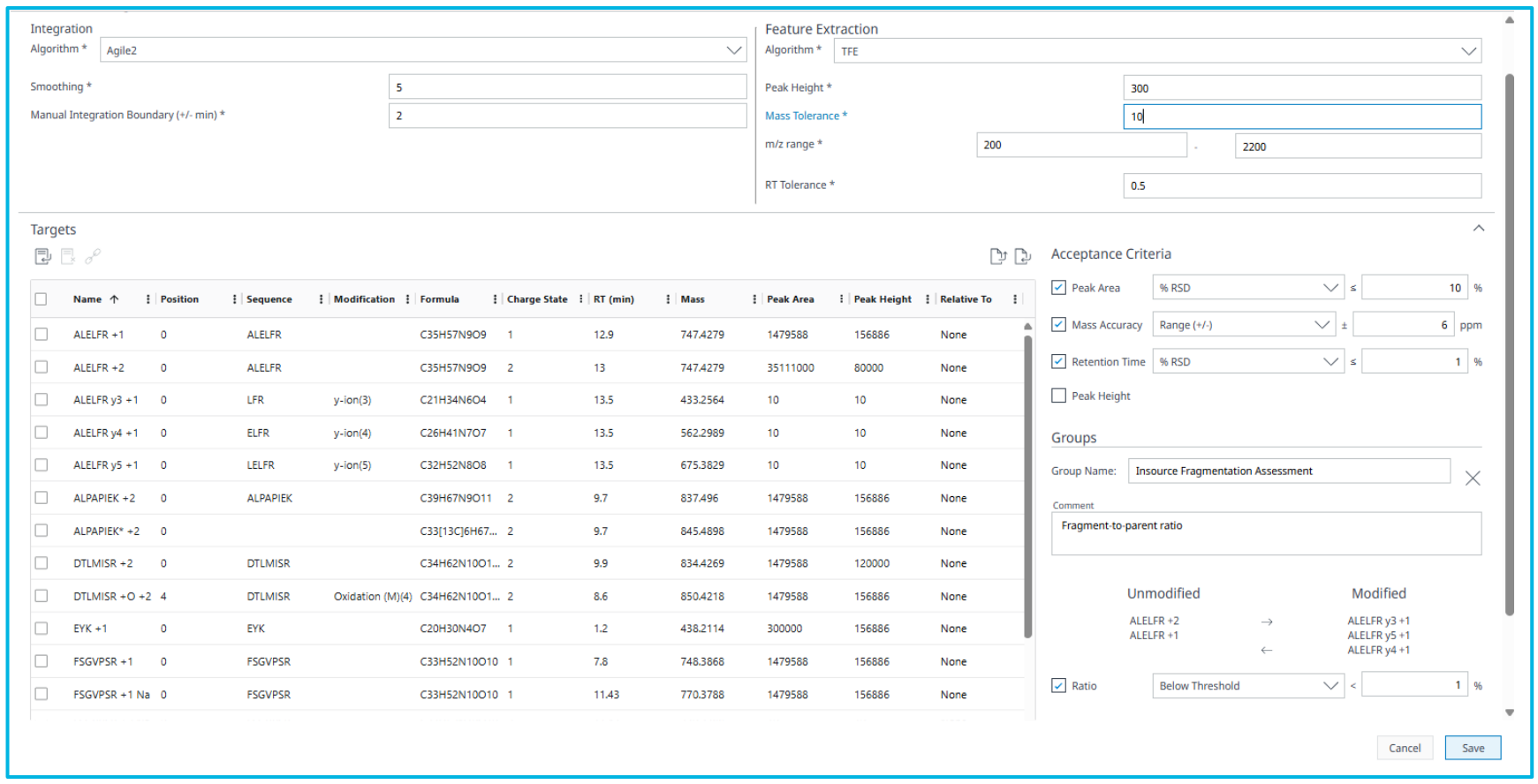
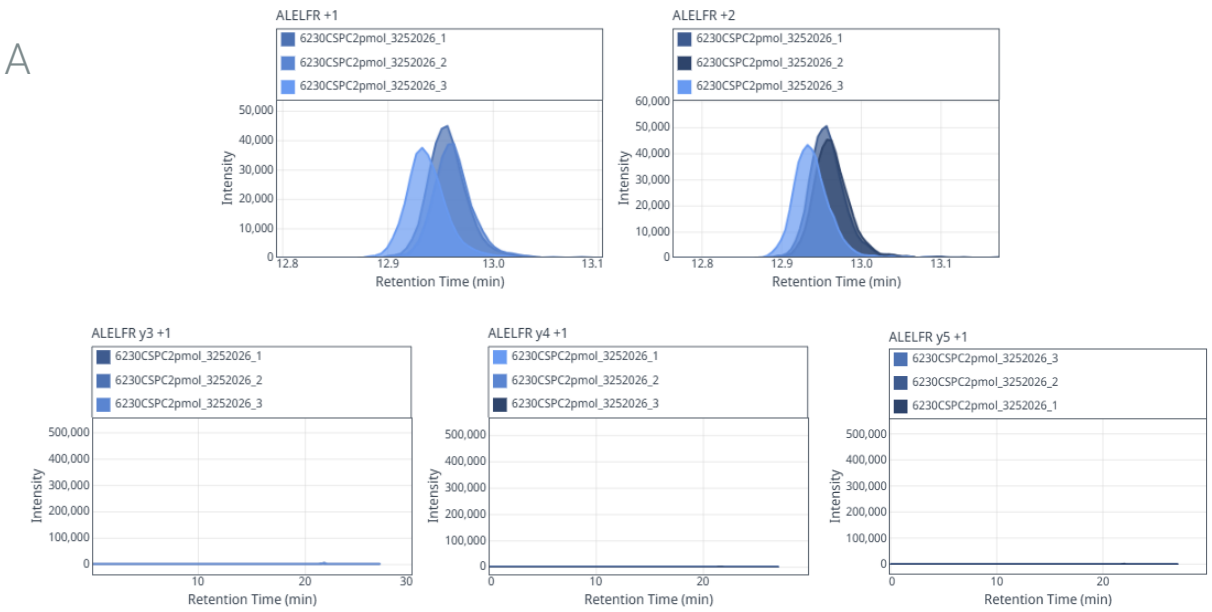


Figure 2. The software automatically identifies and extracts targeted peptides and related molecular species including in source fragments, adducts, and isotopic variants using predefined mass and retention time windows. Peptide level grouping enables automated and reproducible calculation of system readiness metrics such as mass accuracy, retention time stability, and peak area response, eliminating manual data review and ensuring consistent instrument verification across runs.

In Source Fragmentation Assessment Using the Fragile Peptide ALELFR

A



B

Peptide	M...	Group Name	Inj.	Relative ...	Accepta...	Result
ALELFR y3 +1, ALELFR y5 +1, ALELFR y4 +1	y-ion;...	Insource Fragmentation Assessment	6230...	0.00%	<1%	Pass
	y-ion;...	Insource Fragmentation Assessment	6230...	0.00%	<1%	Pass
	y-ion;...	Insource Fragmentation Assessment	6230...	0.00%	<1%	Pass
		Insource Fragmentation Assessment	RSD			Pass

C

Peptide	Inj.	Area	Height	Mass	RT	Result
ALELFR +1	6230...	113892.46	44827.77	-1.06 ppm (±5 ppm)	12.96 min	Pass
	6230...	97233.99	38853.54	-0.32 ppm (±5 ppm)	12.96 min	Pass
	6230...	95995.85	37399.83	-2.78 ppm (±5 ppm)	12.93 min	Pass
	RSD	7.97% (≤10%)			0.10% (≤1%)	Pass
ALELFR +2	6230...	150617.59	50450.92	2.68 ppm (±5 ppm)	12.96 min	Pass
	6230...	137060.43	45252.88	2.92 ppm (±5 ppm)	12.96 min	Pass
	6230...	132237.46	43208.82	1.83 ppm (±5 ppm)	12.93 min	Pass
	RSD	5.56% (≤10%)			0.09% (≤1%)	Pass
ALELFR y3 +1	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	RSD					Pass
ALELFR y4 +1	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	RSD					Pass
ALELFR y5 +1	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	6230...	-	-	-	-	Pass
	RSD					Pass

Figure 3. (A) Extracted ion chromatograms (EICs) of ALELFR parent ions ($[M+H]^+$ and $[M+2H]^{2+}$) and representative y-type fragment ions ($y5^+$, $y4^+$, $y3^+$) acquired at 2 pmol on column. The absence of detectable fragment ion signals indicates minimal in-source fragmentation under the evaluated conditions. (B) Automated evaluation of the fragment-to-parent peak area ratio confirms negligible fragmentation, consistent with stable ion source conditions. (C) Summary of calculated performance metrics, including mass accuracy, retention time stability, and peak area precision, confirming reproducible measurement of the parent peptide.

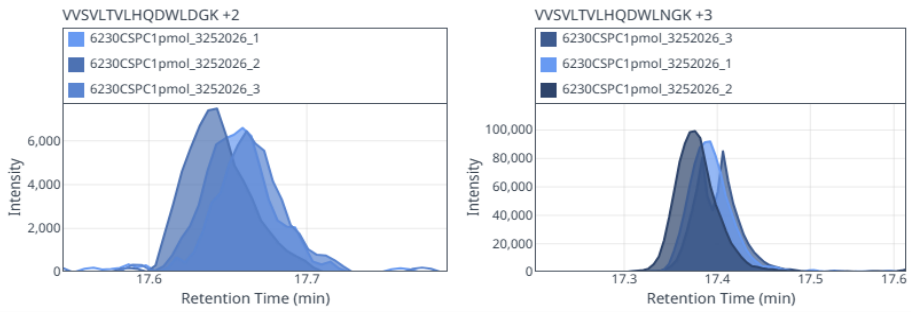
Results and Discussion

Mass Accuracy and Resolution Assessment Using Deamidation Pair Peptide

A

Peptide	Group Name	Inj.	Relative Area	Acce...	Result
VVSVLTVLHQDWLDGK +2	Mass Accuracy and Resolution Assessment	6230CSPC2pmol_...	7.34%	4% - 8%	Pass
	Mass Accuracy and Resolution Assessment	6230CSPC2pmol_...	7.06%	4% - 8%	Pass
	Mass Accuracy and Resolution Assessment	6230CSPC2pmol_...	7.00%	4% - 8%	Pass
	Mass Accuracy and Resolution Assessment	RSD			Pass

B



C

Peptide	Inj.	Area	Height	Mass	RT	Result
VVSVLTVLHQDWLDGK +2	6230...	20079.00	6566.04	-1.07 ppm (±5 ppm)	17.66 min	Pass
	6230...	21060.44	7451.09	0.12 ppm (±5 ppm)	17.64 min	Pass
	6230...	17946.51	6421.08	2.92 ppm (±5 ppm)	17.66 min	Pass
	RSD	6.60% (≤10%)	6.68% (≤10%)		0.05% (≤1%)	Pass
VVSVLTVLHQDWLNGK +3	6230...	282007.24	91466.70	2.51 ppm (±5 ppm)	17.39 min	Pass
	6230...	304858.95	98874.54	3.58 ppm (±5 ppm)	17.38 min	Pass
	6230...	239457.51	84650.32	2.60 ppm (±5 ppm)	17.41 min	Pass
	RSD	9.84% (≤10%)	6.34% (≤10%)		0.07% (≤1%)	Pass

Figure 4. (A) Automated ratio evaluation of VVSVLTVLHQDWLNGK and its deamidated variant VVSVLTVLHQDWLDGK acquired at 1 pmol on column, compared against a predefined acceptance range (4–8%) generates an objective system readiness decision. (B) EICs of the parent and deamidated peptides demonstrate reliable chromatographic resolution and feature extraction. (C) Mass accuracy (ppm) and peak area %RSD confirm stable and reproducible measurements across replicates.

MS Dynamic Range Assessment Using an Isotopically Labeled Peptide Pair

A

Peptide	Group Name	Inj.	Relative...	Accepta...	Result
ALPAPIEK* +2	MS Dynamic Range Assessment	6230...	0.81%	0.2% - 2%	Pass
	MS Dynamic Range Assessment	6230...	0.57%	0.2% - 2%	Pass
	MS Dynamic Range Assessment	6230...	0.73%	0.2% - 2%	Pass
	MS Dynamic Range Assessment	RSD			Pass

B

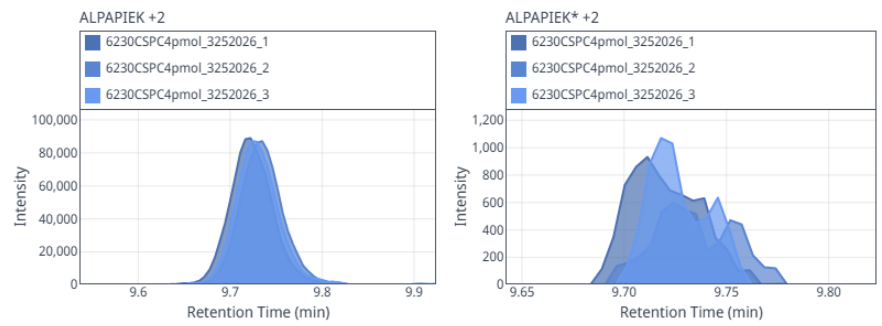


Figure 5. (A) Automated evaluation of the peak area ratio for ALPAPIEK and its isotopically labeled analog ALPAPIEK* (Lys-$^{13}C_6$, $^{15}N_2$) measured at 4 pmol on column and compared against predefined acceptance criteria to confirm adequate MS dynamic range. (B) EICs of the ALPAPIEK and ALPAPIEK*peptides demonstrate consistent signal response and robust feature extraction.

System Related Oxidation Assessment Using DTLMISR Peptide

Peptide	Modif...	Group Name	Relative ...	Result
DTLMISR +O +2, DTLMISR +O +1	Oxidation ...	System-Related Oxidation Assessm...	0.14%	Pass
	Oxidation ...	System-Related Oxidation Assessm...	0.12%	Pass
	Oxidation ...	System-Related Oxidation Assessm...	0.33%	Pass
		System-Related Oxidation Assessm...		Pass

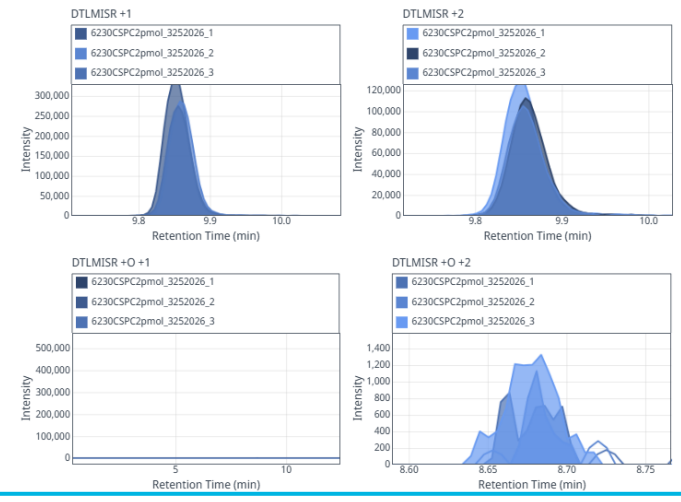


Figure 6. DTLMISR parent and oxidized species (+1 and +2 charge states) acquired at 2pmol on column, were grouped to calculate oxidized to parent ratios. Automated comparison to predefined acceptance criteria enables objective monitoring of system related oxidative stress.

Conclusions

- The automated peptide-based workflow enables objective assessment of LC/MS system readiness for MAM.
- Targeted peptide grouping verifies mass accuracy, RT stability, signal precision, and dynamic range.
- Targeted peptide groups detect common system stressors (e.g., oxidation, in-source fragmentation, ..).
- Successful completion of the SPC workflow provides documented confidence that the LC/MS system is fit for MAM analysis.
- Automated metrics and pass/fail decisions remove manual calculations and improves efficiency and consistency.

References

[1]Nature Protocols, 17,3565-3603.

[2]Rogers, R. S., et al. (2015) mAbs, 7(5), 881-890.

[3] Rogstad, S., et al. (2019) Analytical Chemistry, 91(22), 14170–14177.

[4] Yang et al. (2023), mAbs 15, 2197668

[5] Millán-Martín, S., et al. (2023) Nature Protocols, 18, 1056–1089.

https://www.agilent.com/en/promotions/asms

DE-014827

Published in USA, June 10, 2026

Agilent
Trusted Answers