

GLP-1 Analogs: Accelerating Method Development and Manufacturing with LC-UV/MS

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INTRODUCTION

Glucagon-like-peptide-1 receptor agonist (GLP-1 RA)

- Metabolic regulator for type 2 diabetes and weight management
- GLP-1 analogs: modified to increase resistance to enzyme degradation and reduce clearance rate
- Diverse modification: amino acid sequence change, conjugation with fatty acid/protein fragment
- Modification increases complexity of analytical target profile
- Production: chemical synthesis, recombinant DNA technology

Key Benefits

- Orthogonal mass information enables detection of impurities that may be overlooked using traditional UV-based detection
- ACQUITY QDa II Mass Detector enhances capabilities in raw material screening, process control, lot release, and stability monitoring
- Integrated LC-UV/MS workflow helps manufacturers meet regulatory requirements while maintaining operational productivity

METHODS

LC: ACQUITY Arc™ Premier System

- Samples: exenatide, tirzepatide in formulation of corresponding drug products
- Injection volume: 10 µL
- Sample temp.: 10 °C
- Column: XSelect™ Premier Peptide CSH™ C18 Column, 130Å, 2.5 µm, 4.6 x 100 mm (p/n 186009908)
- Column temp.: 60 °C
- PDA, λ = 210 - 400 nm

Mobile phase
A: H₂O, 0.1% formic acid v/v (MS-grade)
B: MeCN, 0.1% formic acid v/v (MS-grade)

MS: ACQUITY QDa II Mass Detector

- Scan range: 250-15000 m/z @ 5Hz
- ESI mode: positive
- Capillary voltage: 1.5 kV
- Cone voltage: 15 V
- Probe temp.: 600 °C
- Software: Empower™ 3.8.1



ACQUITY™ QDa™ II Mass Detector

- Deployable
- Scalable
- Lower operating cost
- Tech transferable

RESULTS

Orthogonal screening for impurity detection

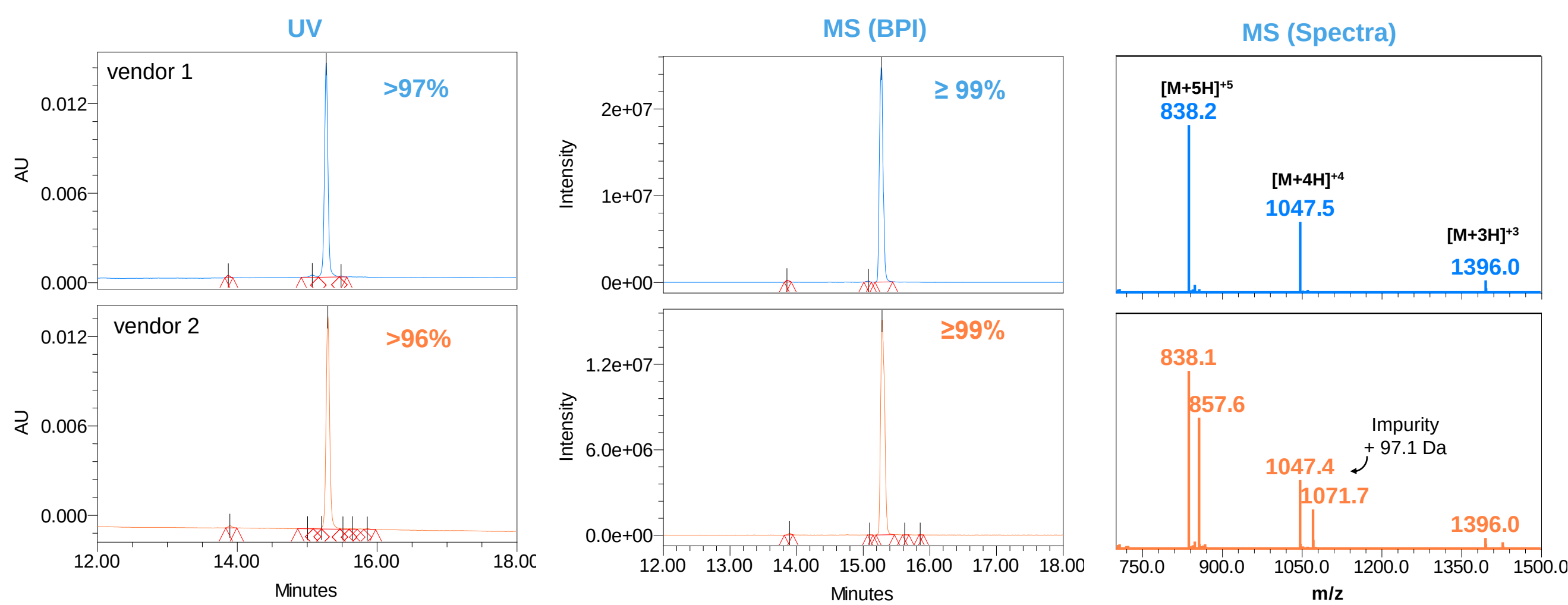
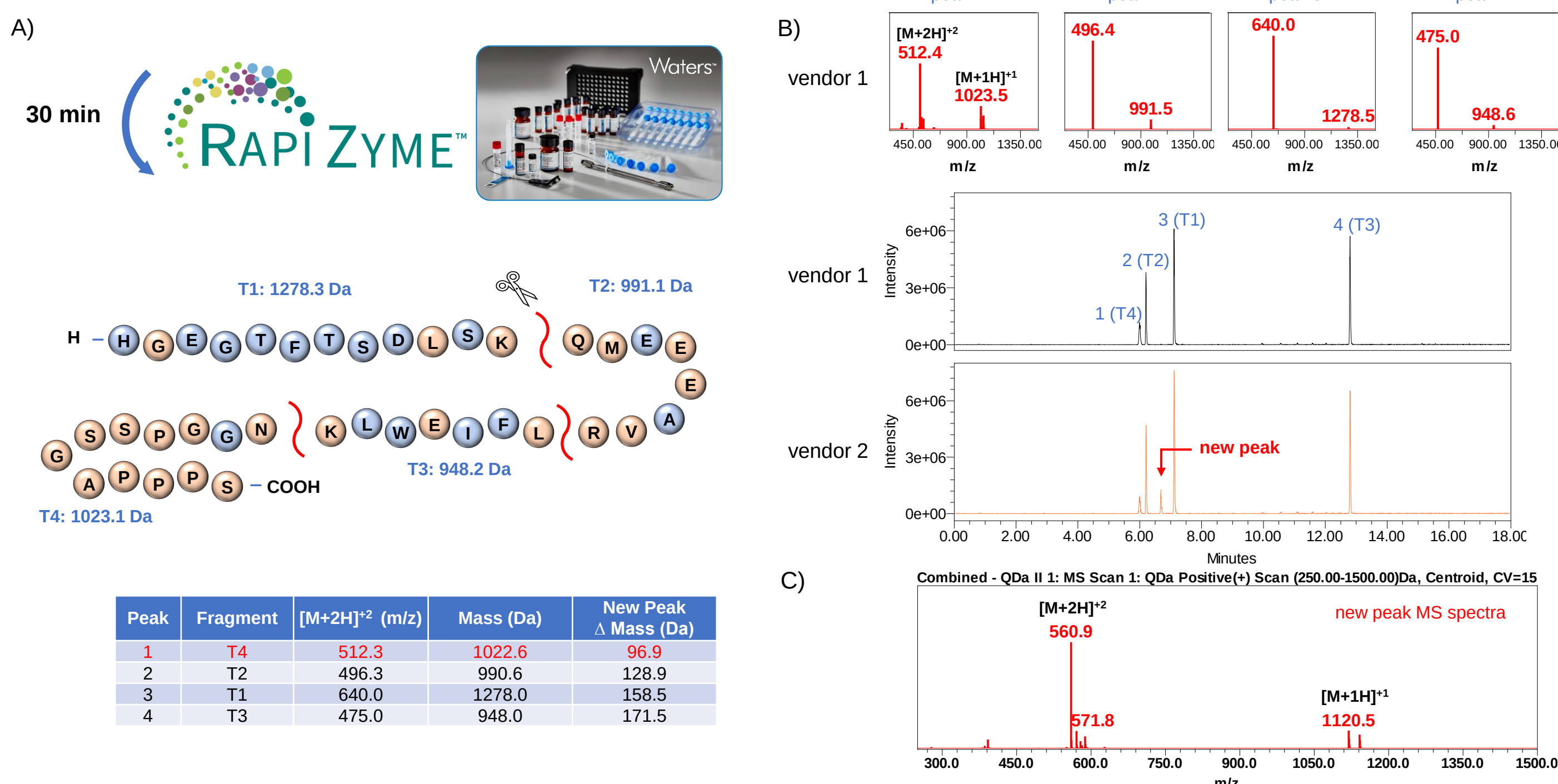


Figure 1 Exenatide was purchased from two different vendors and prepared at the same concentration. UV data (A) showed purity at above 96%. MS base peak intensity (BPI) chromatogram (B) also suggest high purity; however, vendor 2 response is half the intensity of vendor 1. Mass spectra (C) reveals the presence of impurity (mass difference +97.1 Da) in vendor 2 sample. Orthogonal mass detection identifies impurities that would be overlooked using UV-only workflows.

ACQUITY QDa II Mass Detector supports out of specification investigations



Peak	Fragment	[M+2H] ²⁺ (m/z)	Mass (Da)	New Peak Δ Mass (Da)
1	T4	512.3	1022.6	96.9
2	T2	496.3	990.6	128.9
3	T1	640.0	1278.0	158.5
4	T3	475.0	948.0	171.5

Figure 2 Exenatide samples were digested using Waters RapiZyme™ Trypsin 30-minute digestion protocol (A) and produced four fragments (B). Vendor 2 has an additional peak with a neutral mass of 1119.5 Da (C), which has a 96.9 Da mass difference compared to the four expected fragment. This close agreement with the observed intact mass difference, 97.1 Da, putatively suggests the observed impurity is related to a proline insertion in fragment T4. This insertion was confirmed by sequence analysis enabled by Waters Xevo™ QTof G3 Mass Spectrometer configured with UNIFI™ Scientific Information System.

Orthogonal mass data provides insights on API degradants

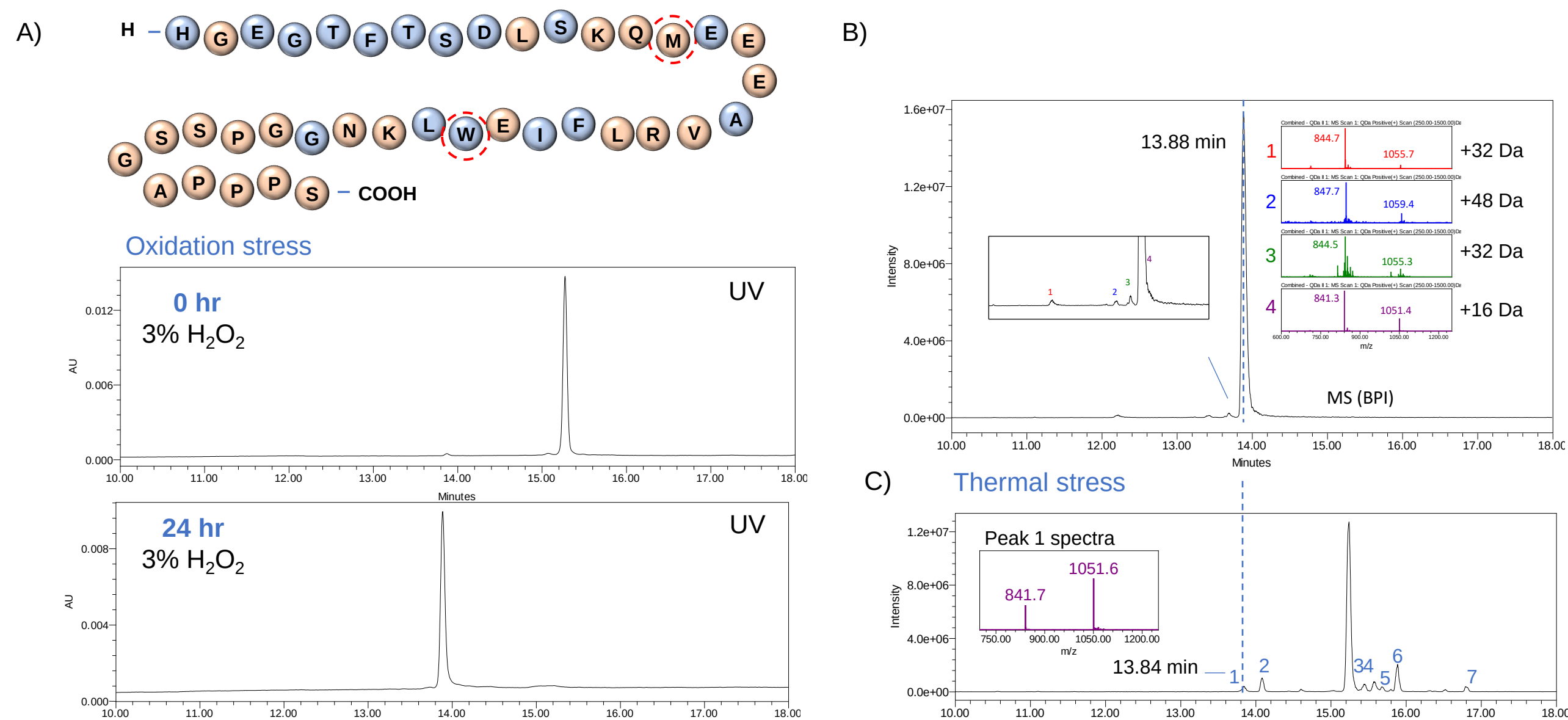


Figure 3 Exenatide contains a methionine and tryptophan sensitive to oxidation(A). After exposed to chemical oxidation for 24 hours, the full-length product was oxidized and shifted to the left. Spectral data (B) shows 4 main oxidation events with the 4th peak being the oxidized species. Following the dashed line, this corresponds to peak 1 in the thermally stressed sample (C), indicating that the sample can undergo a degree of oxidation when exposed to elevated temperature.

RESULTS

Mass spectra provide peptide fragment masses to identify oxidation site

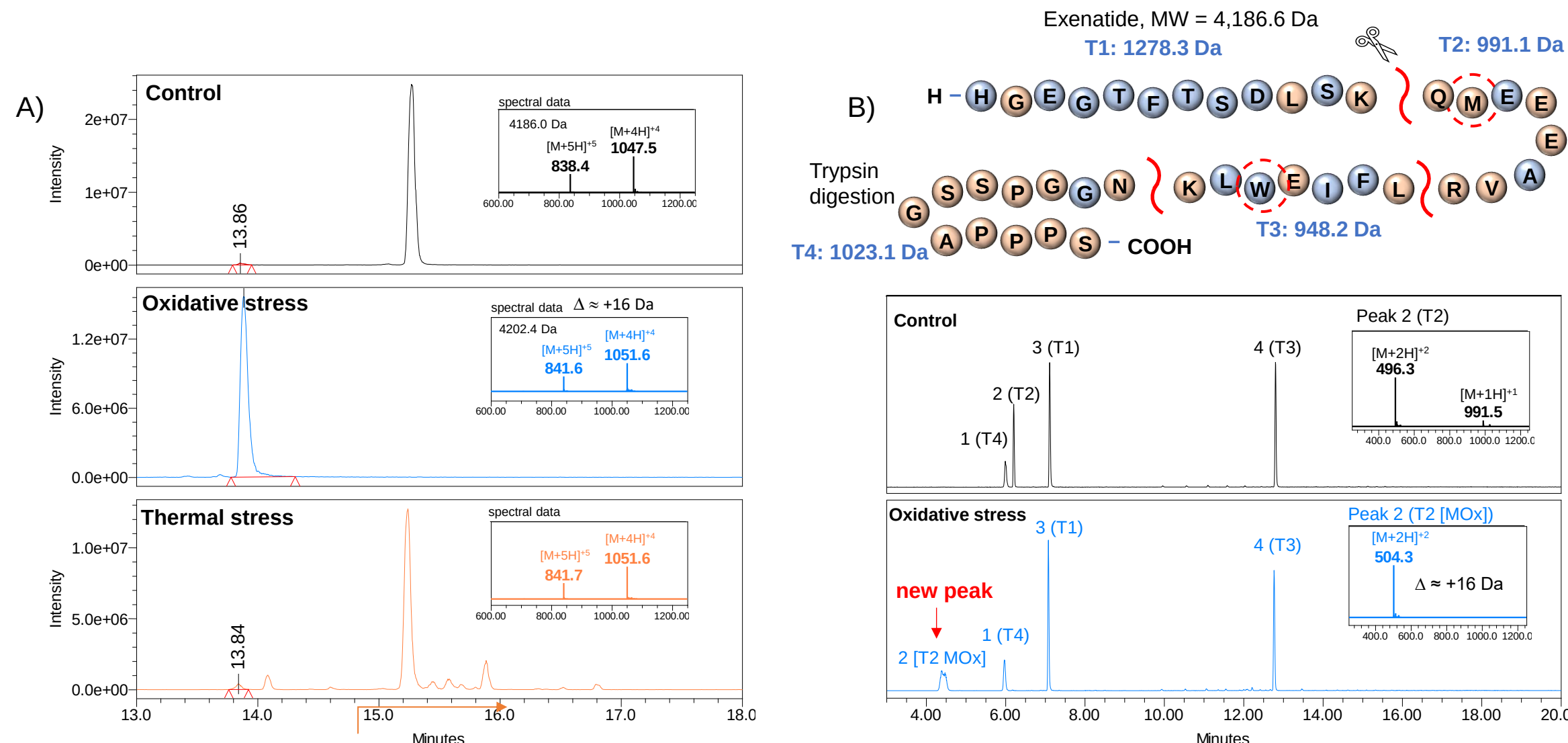


Figure 4 Oxidation is observed in both chemical oxidation and thermal degradation conditions (A). Bottom-up analyses like peptide mapping can help localize modifications. Trypsin digested oxidized exenatide contain four fragments, while fragment 2 in the control sample was replaced by a new peak (B). Mass of the new peak matches fragment 2 at an oxidized state, indicating that the main oxidation species is likely related to methionine.

ACQUITY QDa II Mass Detector identifies stability indicating impurities

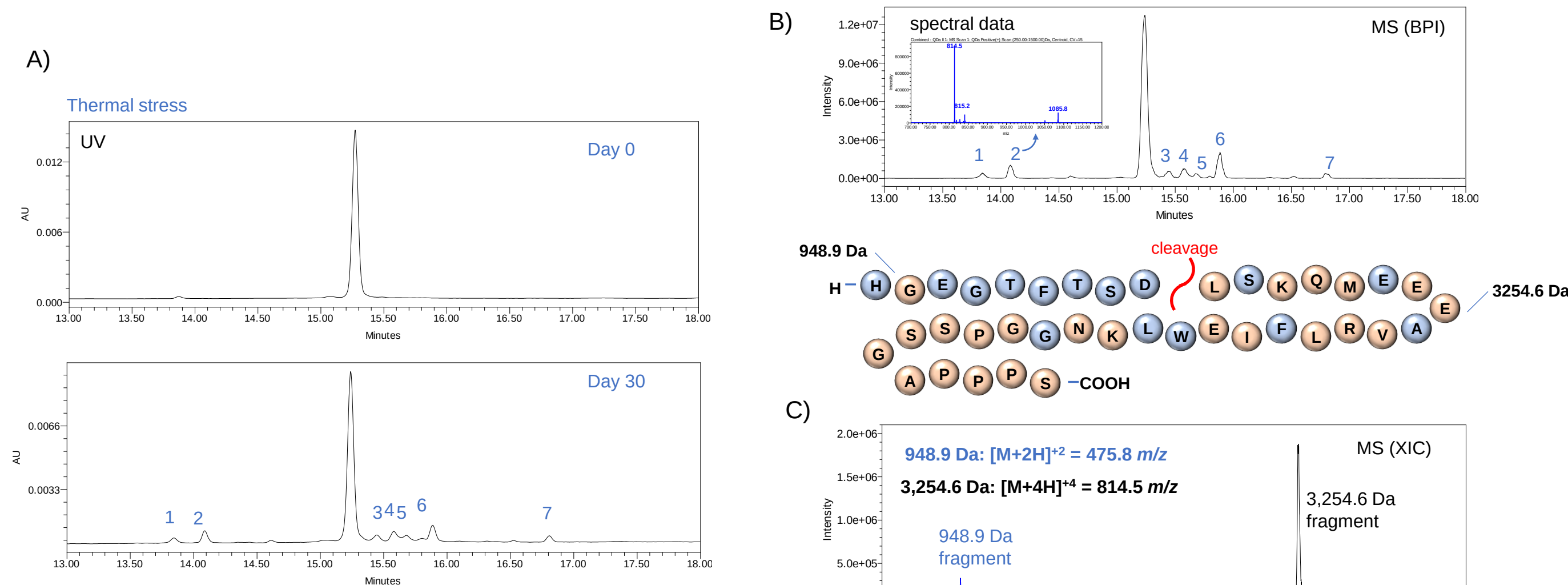


Figure 5 Exenatide generated several new impurities due to thermal stress (A). Peak 2 has a mass of 3,254.6 Da (B), which corresponds to a non-enzymatic cleavage between the aspartic acid and leucine residue. The other fragment from this cleavage was identified by extracted ion chromatogram (C).

Mass detector offers increased sensitivity for degradants

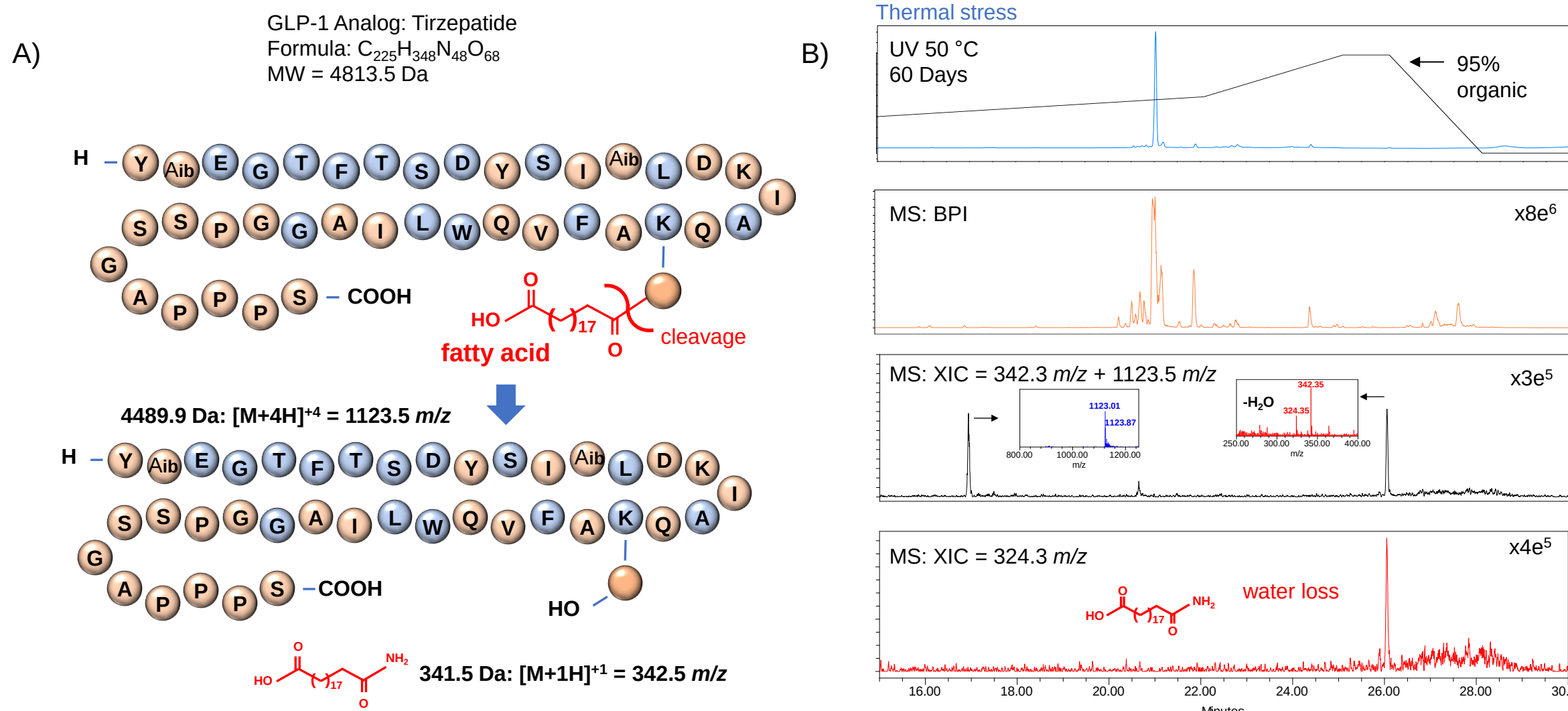


Figure 6 Tirzepatide is thermally stressed, and the base peak intensity (BPI) chromatogram showed impurities not observed in the UV chromatogram (B). A cleavage at the linker species (A) was detected as shown in the two peaks from extracted ion chromatograms for the fatty acid (342.5 m/z) and the remaining GLP-1 analog and linker (1123.5 m/z) species. The fatty acid fragment eluted at 95% high organic condition, and this peak might be dismissed as a reconditioning artifact if MS data were not available.

Compliant-ready scalable CDS provides easy data interpretation and integrated reporting

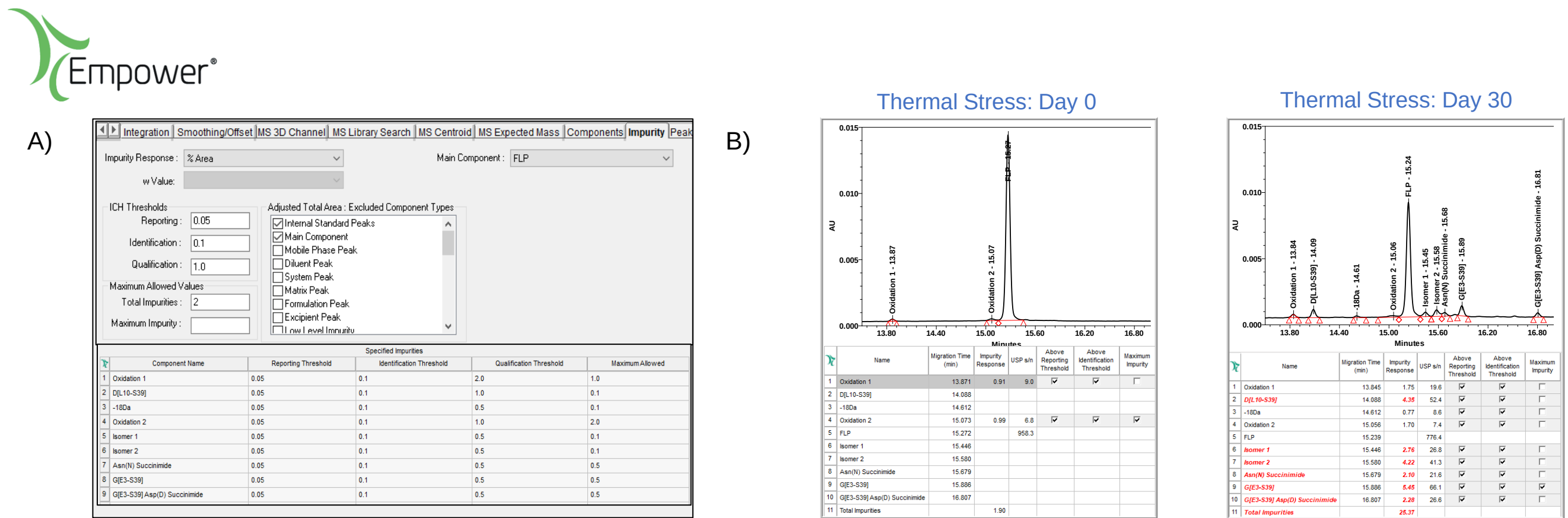


Figure 7 Empower™ Chromatograph Data System offers integrated workflows to address customers needs. For example, the impurity function (A) allows to define impurity thresholds to monitor total or individual impurities. The impurity level is monitored and flagged if exceeded (B), and this allows users to quickly review data to ensure drug substance/product are meeting quality specifications as per regulatory expectations.

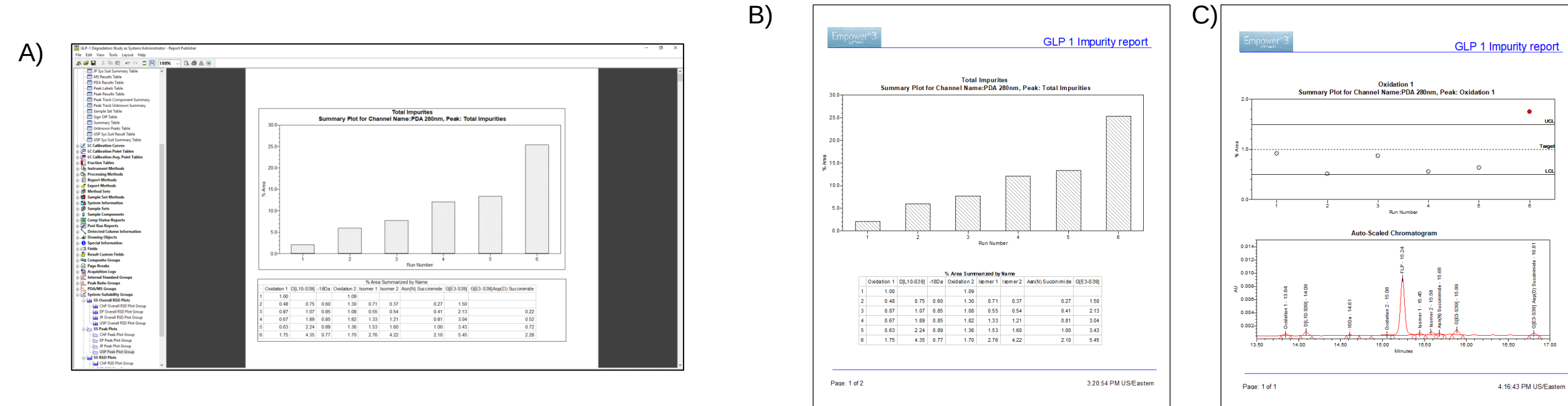


Figure 8 Results can be quickly compiled and formatted for communication or supporting regulatory documentation using the integrated reporting functionality (A) of Empower 3 Software. The total impurity was monitored and reported over a 30-day thermal stress study. The bar plot (B) shows that the total impurities increased rapidly after 7 days (data point Run 2). The control plot (C) shows that the main oxidation peak was consistent in Area % relative to the remaining impurities up to 15 days (data point Run 5) indicating some sample stability towards initial oxidation under thermal stress.

CONCLUSIONS

- ACQUITY QDa II Mass Detector can be easily integrated into existing LC-UV platforms and lower adoption barriers associated with MS and increase analytical capabilities
- Access to mass data provides deeper understanding of drug product behaviors enabling more insightful decision-making during the development and manufacturing process
- In-line acquisition of complementary orthogonal mass data increases confidence in results and product knowledge for improved product safety and regulatory compliance while maintaining productivity

REFERENCES

- D. Han et al. "Accelerating Method Development and Manufacturing of GLP-1 Analogs with LC-UV/MS" Application Note 720008800, May 2025, Waters Corporation
- D. Han et al. "Application of LC-UV/MS Workflows to Increase Efficiency in Impurity Profiling of GLP-1 Analogs" Application Note 720008812, May 2025, Waters Corporation