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

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Erin McAllister, Duanduan Han, Samantha Ippoliti, Robert E. Birdsall, Karen Nyholm

Waters Corporation, Milford, MA

CONTACT INFORMATION: Erin McAllister erin_mcallister@waters.com

PURPOSE

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 increases complexity of analysis: amino acid sequence change, conjugation with fatty acid
- 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

Software: Empower™ Chromatography Database System 3.8.1

LC: ACQUITY Arc™ Premier System

- Samples: exenatide and tirzepatide in drug product formulation
- 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
- MPA: H_2O, 0.1% formic acid v/v (MS-grade)
- MPB: MeCN, 0.1% formic acid v/v (MS-grade)

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.96	99	1	Initial
2	0.96	99	1	6
22	0.96	45	55	6
25	0.96	5	95	6
26	0.96	5	95	6
28	0.96	99	1	6
40	0.96	99	1	6

MS: ACQUITY QDa II Mass Detector

- Scan range: 250-1500 m/z @ 5Hz
- ESI mode: positive
- Capillary voltage: 1.5 kV
- Cone voltage: 15 V
- Probe temp.: 600 ˚C

RESULTS

Orthogonal screening for impurity detection

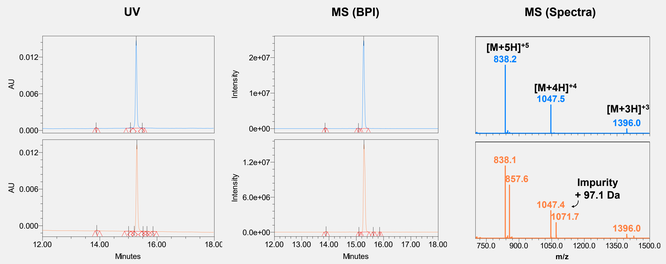
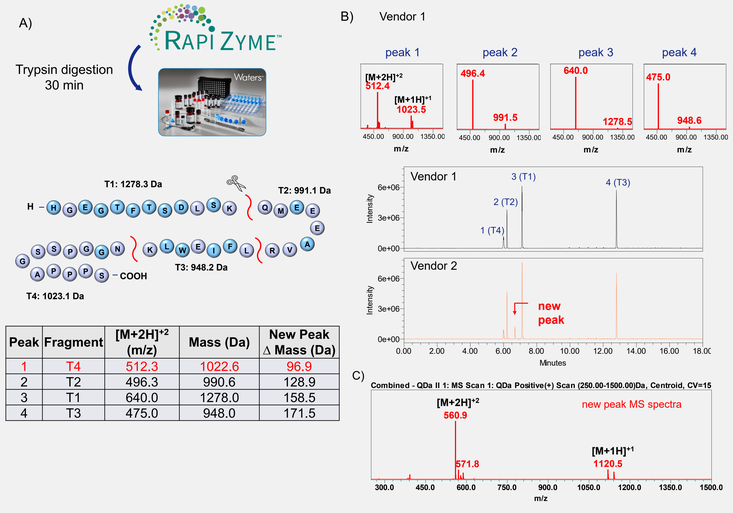


Figure 1 Exenatide was purchased from two different vendors and prepared at the same concentration. UV data (A) showed purity at above 96%. MS base peak intensity (BPI) chromatogram (B) also suggest high purity; however, vendor 2 response is half the intensity of vendor 1. Mass spectra (C) reveals the presence of an impurity (mass difference +97.1 Da) in vendor 2 sample. Orthogonal mass detection identifies impurities that may 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 fragments. 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™ Application through waters_connect™ Informatics Platform.

Orthogonal mass data provides insights on API degradants

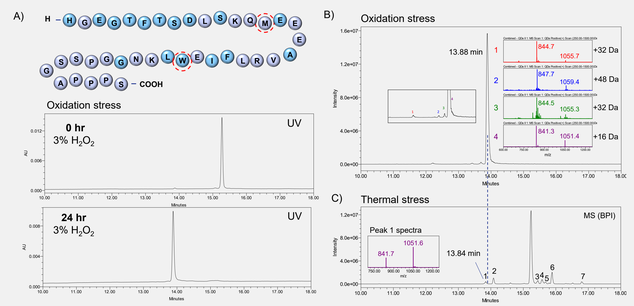


Figure 3 Exenatide contains a methionine and a tryptophan sensitive to oxidation(A). After exposed to chemical oxidation for 24 hours, the full-length product was oxidized and shifted to the left. Spectral data (B) shows 4 main oxidation events with the 4th peak being the oxidized species. Following 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.

Mass spectra provide peptide fragment masses to identify oxidation sites

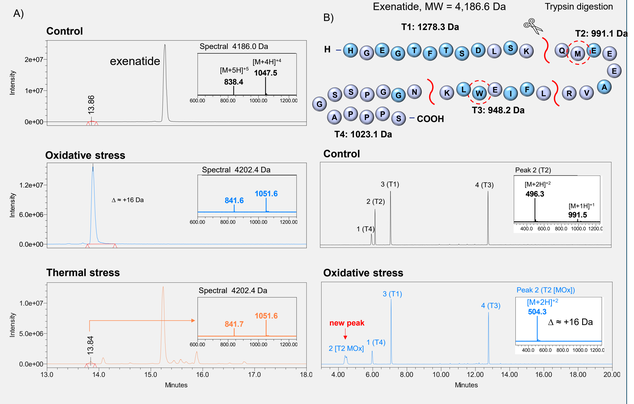


Figure 4 Oxidation is observed in both chemical oxidation and thermal degradation conditions (A). Bottom-up analyses like peptide mapping can help localize modifications. Trypsin digested oxidized exenatide contain four fragments, while fragment 2 in the control sample was replaced by a new peak (B).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

RESULTS

Leveraging MS sensitivity to detect degradants

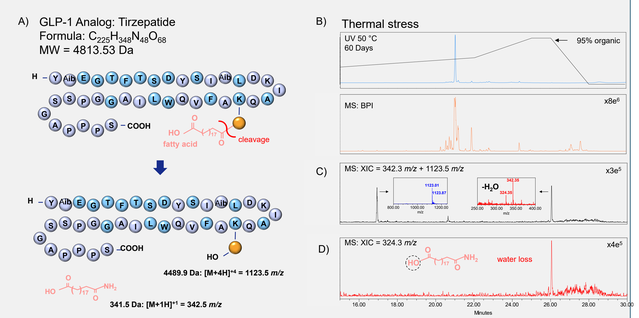


Figure 5 (A) GLP-1 analog tirzepatide contains a fatty acid conjugated via a linker. (B) For a thermal stressed sample, base peak intensity (BPI) chromatogram showed several impurities not observed in the UV chromatogram. (C) We detected masses associated with the cleavage of the fatty acid as shown in extracted ion chromatograms (XIC): the fatty acid (342.5 m/z) and the remaining GLP-1 analog and linker (1123.5 m/z) species. (D) XIC for the fatty acid ion after a water loss (324.3 m/z) and late elution at 95% organic condition agree with fatty acid characteristics. This peak would have been ignored as a reconditioning artifact in UV chromatogram.

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

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