Application note | 003920

Pharma

Confident and sensitive identification of semaglutide degradation products and impurities using a UHPLC-HRAM MS platform

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Keywords

GLP-1, semaglutide, degradation products identification, HRAM MS, Orbitrap Exploris 240 mass spectrometer, Vanquish Flex UHPLC, Chromeleon CDS, BioPharma Finder software, Ardia Platform,

Application benefits

- The Thermo Scientific™ Hypersil GOLD™ peptide column enables the effective separation of semaglutide and its degradation products using formic acid-containing eluents.
- Confident and sensitive identification of semaglutide degradation products down to a 0.005% level is achieved using high-resolution accurate mass (HRAM) mass spectrometry (MS).
- High-quality HRAM ddMS^2 data ensures unambiguous identification of co-eluting impurities, including isobaric impurities at trace level.
- Thermo Scientific™ BioPharma Finder™ software connects to the Thermo Scientific™ Ardia™ Platform, enhancing data processing efficiency and delivering comprehensive and clear insights into the drug characterization.

Goal

Demonstrate the capability of the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer coupled with the Thermo Scientific™ Vanquish™ Flex UHPLC system and BioPharma Finder software to provide confident and sensitive identification for semaglutide degradation products and related impurities.

Introduction

Therapeutic peptides have gained substantial interest in recent years and are increasingly being developed as therapeutic agents for various diseases. They are composed of well-ordered amino acids, typically with molecular weights between 500 and 5,000 Da. Currently, over 100 peptide medications are approved globally for treating various diseases.[1] The best-selling and notable synthetic peptide drug is semaglutide, a 32-amino acid glucagon-like peptide-1 (GLP-1) receptor agonist used to treat type 2 diabetes and long-term weight management. Following its approval by the U.S. Food and Drug Administration (FDA) in 2017, semaglutide-containing formulations became the third best-selling drug globally in 2023.[2] In addition, the expiration of patents for several GLP-1 receptor agonists is pushing numerous pharmaceutical companies to develop a generic equivalent.

Chemical synthesis, particularly solid-phase peptide synthesis technology, is a widely utilized technique for producing therapeutic peptides, in addition to recombinant DNA-derived or natural source-based methods. Each production method faces significant challenges related to impurities, especially peptide-related impurities. These impurities can arise from deletions, insertions, substitutions, isomerization of amino acids, or modification of amino acid side chains, leading to variations in the amino acid sequence and its modifications. Any difference in the amino acid sequence can potentially interfere with the peptide's biological activity, affecting its efficacy and safety. According to guidelines published by the FDA for the Abbreviated New Drug Application of highly purified synthetic peptides, peptide-related impurities must be rigorously identified and controlled.[3] High performance liquid chromatography (HPLC) coupled with HRAM MS has emerged as a powerful tool for analyzing peptide-related impurities. HRAM MS enables the identification and structure elucidation of impurities based on mass detection of the intact species and their characteristic fragments generated upon gas-phase fragmentation. The high resolution and accurate mass detection enable the distinction of co-eluting impurities. Additionally, isobaric impurities can be easily distinguished and identified based on the fragment ion spectrum.

In this application note, to achieve the comprehensive coverage of potential impurities that may arise during production, transportation, and storage, the semaglutide sample was subjected to thermal and oxidative stress conditions to generate potential impurities. Following the degradation study, the Vanquish Flex UHPLC system coupled with an Orbitrap Exploris 240 mass spectrometer was used for the semaglutide degradation products analysis. Figure 1 illustrates the overview of the experimental study design. The Hypersil GOLD Peptide column was used on a Vanquish Flex UHPLC system to achieve an effective separation of the semaglutide full-length product (FLP) with its degradation products. The Orbitrap Exploris 240 mass spectrometer offers high sensitivity, facilitating the identification of all degradation products, even those with very low abundance. BioPharma Finder software, powered by the Ardia Platform, provided flexible and comprehensive visualization and reporting tools, enhancing the review and interpretation of semaglutide degradation results.

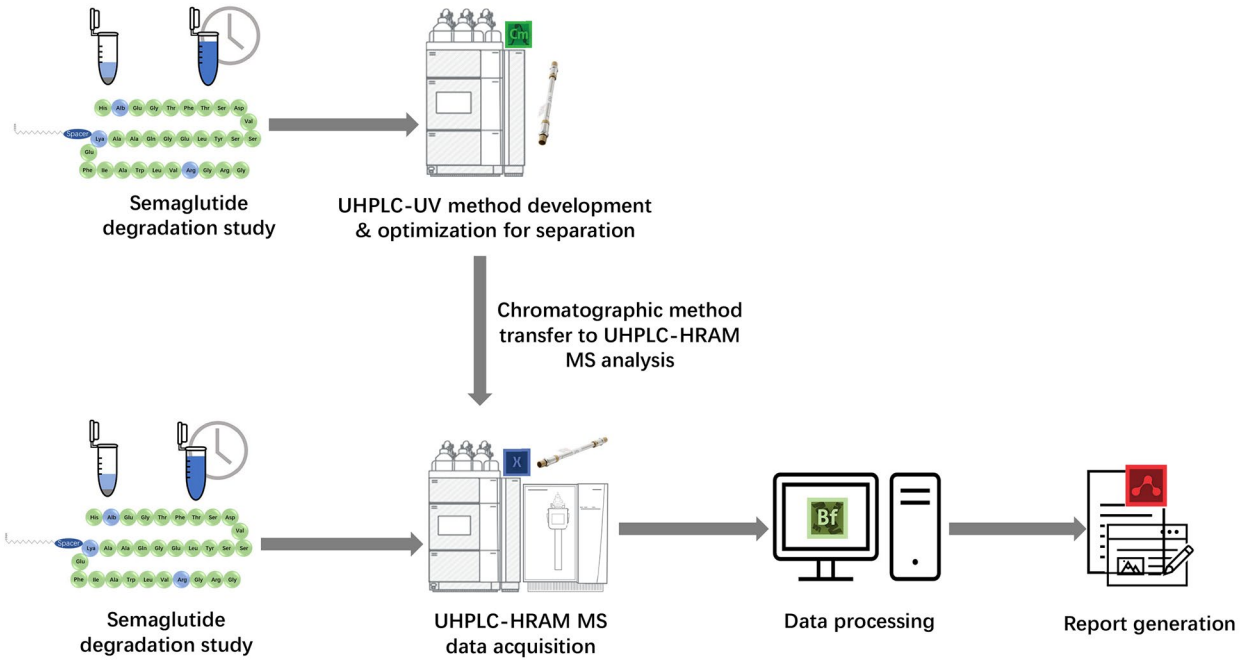


Figure 1. Overview of the experimental study design for semaglutide degradation products identification

Experimental

Chemicals

- Deionized water with 18.2 MΩ·cm resistivity or higher, purified by a Thermo Scientific™ Barnstead™ GenPure™ Pro Water Purification System (P/N 50131948)
- Fisher Chemical™ Acetonitrile, Optima™ LC/MS grade (P/N 5A955-4)
- Fisher Chemical™ Formic acid, Optima™ LC/MS grade (P/N 5A117-50)
- Merck EMSURE™ Hydrogen peroxide 30% (P/N 51.07210)

Sample handling

- Thermo Scientific™ SureSTART™ 2 mL glass vials (amber) (P/N 6ASV9-2P)
- Thermo Scientific™ SureSTART™ 9 mm vial caps with septum (P/N 6ASC9ST1)
- Thermo Scientific™ Finnpipette™ F3 Variable Volume Single Channel Pipettes (P/N 4640030, P/N 4640040, P/N 4640050, P/N 4640060)
- Eppendorf ThermoMixer™ F 1.5 (P/N 5384000071)

Sample preparation

The semaglutide active pharmaceutical ingredient (API) with a purity higher than 99.0% was purchased from Shandong Mopai Biotechnology Co., Ltd.

Semaglutide sample solution: 5.0 mg of semaglutide sample was weighed and transferred into an amber glass vial and then dissolved with 5 mL diluent (20% acetonitrile in water, v/v) to achieve a 1.0 mg/mL semaglutide sample solution.

Forced degradation study samples: The semaglutide sample solution (1.0 mg/mL) was heated at 60 °C for 8, 24, 48, and 72 hours in the thermal stress study. The samples were then allowed to cool to room temperature before being subjected to UHPLC-UV or UHPLC-HRAM-MS analysis. For the oxidative stress study, hydrogen peroxide solution was added to the semaglutide sample solution (1.0 mg/mL) to achieve a final concentration of 0.01%. The solution was incubated at room temperature in the dark for 2, 4, 6, and 14 hours, and then immediately subjected to UHPLC-UV or UHPLC-HRAM MS analysis.

Instrumentation

Liquid chromatography

- Vanquish Flex UHPLC system consisting of:
  - Vanquish System Base (P/N VF-S01-A)
  - Vanquish Binary Pump F (P/N VF-P10-A)
  - Vanquish Split Sampler FT (P/N VF-A10-A)
  - Vanquish Column Compartment H (P/N VH-C10-A)
  - Vanquish Variable Wavelength Detector F (P/N VF-D40-A)
  - Vanquish Standard Bio Flow cell, 11 µL, 10 mm (P/N 6077.0200)

Chromatographic conditions

Table 1. Chromatographic conditions

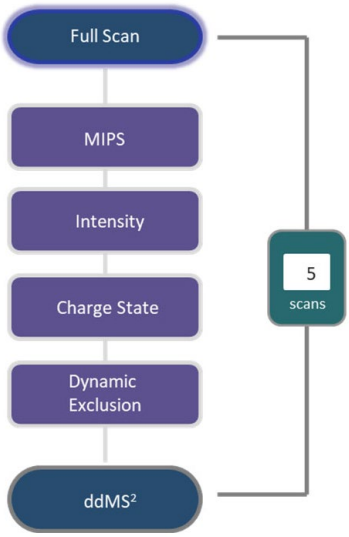
Parameter	Value
Column	Hypersil GOLD Peptide column, 150 × 2.1 mm, 1.9 µm (P/N 26002-152130)
Mobile phase	A: 0.1% formic acid (FA) in water; B: 0.1% FA in acetonitrile
Gradient	Time (min) A (%) B (%) 0 98 2 3 98 2 4 85 15 44 45 55 50 5 95 55 5 95 55.5 98 2 65 98 2
Flow rate	0.4 mL/min
Column temperature	50 °C
Autosampler temperature	4 °C
Autosampler wash solvent	30% MeOH with 0.1% FA in water
Wash mode	Before
Injection volume	5 µL
Detector	280 nm, Data collection rate: 0.2 Hz

Mass spectrometry

- Orbitrap Exploris 240 mass spectrometer system (P/N BRE725535)

Table 2. Instrument and scan settings for the mass spectrometer

Parameter	Value
Ion source properties	
Ion source type	H-ESI
Spray voltage	Static
Positive ion (V)	3,800
Gas mode	Static
Sheath gas (Arb)	35
Aux gas (Arb)	15
Sweep gas (Arb)	0
Ion transfer tube temp (°C)	250
Vaporizer temp (°C)	250
Full scan properties	
Orbitrap resolution @ *m/z* 200	120,000
Scan range (*m/z*)	200–2,500
RF lens (%)	60
AGC target	Custom
Normalized AGC target (%)	300
Maximum injection time mode	Custom
Maximum injection time (ms)	100
Data type	Profile
Polarity	Positive
Dynamic exclusion	8 s



Data-dependent MS² scan properties	
Number of dependent scans	5
Isolation window (*m/z*)	1.2
Collision energy type	Normalized
HCD collision energies (%)	30
Orbitrap resolution	30,000
Scan range mode	Define First Mass
First mass (*m/z*)	110
AGC target	Auto
Maximum injection time mode	Custom
Maximum injection time (ms)	250

Table 3. Parameter settings for the processing method in BioPharma Finder software

Parameter	Value
Peptide sequence	HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG
Static modification	+14.015 Da @ A2 (instead of 2-aminoisobutyric acid)
Variable modifications	Deamidation (Q), Oxidation (MW), Side Chain (+715.426 Da @ K20), Isomerization (D), Double oxidation (MWC)
Peptide identification	
Search by Full MS only	No
Use MS/MS	Use All MS/MS
Maximum peptide mass	11,000
Mass accuracy (ppm)	14
Minimum confidence	0.80
Maximum number of modifications for a peptide	4
Advanced search	
Glycosylation	None
Search for amino acid substitutions	None
Disulfide search	
Perform disulfide bond search	No
Select protease	
Enzyme	Nonspecific
Specificity	High

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3.2 was used for data acquisition and processing during UHPLC method development and optimization.

Thermo Scientific™ Xcalibur™ and BioPharma Finder 5.3 software were used for UHPLC-HRAM-MS data acquisition and data processing, respectively. BioPharma Finder software connected to the Ardia Platform was used for customized data reporting and visualization of results.

Results and discussion

Forced degradation study and UHPLC method development and optimization

To identify potential impurities in semaglutide during production, storage, and transportation, semaglutide sample solutions were periodically tested under thermal and oxidative conditions to achieve a degradation ratio of approximately 5% to 10%. The semaglutide sample solution was used as a control, and the semaglutide FLP peak area was used to calculate the degradation ratio. Ultimately, samples exposed to 60 °C for 72 hours and samples exposed to 0.01% H_2O_2 for 14 hours were selected for further study, with degradation ratios of 4.65% and 8.94%, respectively.

The conventional method for crude peptide analysis usually employs the reversed-phase LC with gradient elution, using aqueous and acetonitrile as mobile phases. Ion pair reagents, such as trifluoroacetic acid (TFA), are commonly added to the mobile phase to increase the hydrophobicity and retention of peptides.[4] However, TFA can cause ion suppression for MS analysis. Formic acid (FA) is a preferred acid for MS analysis and is more green, but it is less effective at suppressing nonspecific interactions and creates a less hydrophobic ion pair with the peptide. The Hypersil GOLD Peptide column provides excellent separation and peak shape for peptide analysis, even using FA-containing mobile phases. In this study, the gradient, injection volume, and column temperature were optimized to separate the degradation products and semaglutide by using the Hypersil GOLD Peptide column with 0.1% FA in acetonitrile and water as the mobile phase. The optimized UHPLC conditions are listed in Table 1. Figure 2 illustrates the final chromatograms for control and stressed samples, demonstrating effective separation even with the mass spectrometry-friendly eluent FA in the mobile phase. The main degradation product peaks in stressed samples are labeled in the chromatogram; a total of five and eleven degradation products were separated and detected under thermal stress conditions and oxidative stress conditions, respectively.

Identification of degradation products in stressed samples

After the chromatographic method optimization, the method was transferred to a UHPLC-HRAM MS system for the identification of degradation products. The instrument and scan settings for the MS are listed in Table 2. The amino acid sequence and the lipid side chain structure of semaglutide are illustrated in Figure 3. In BioPharma Finder software, the non-proteinogenic amino acid 2-aminoisobutyric acid (the second amino acid) is represented as alanine with a fixed modification of +14.015 Da. Additionally, the lipid chain modification on lysine is set as a variable modification of +715.426 Da. Thus, the semaglutide sequence in BioPharma Finder software is HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG, with two modifications on A2 and K20 as listed in Table 3.

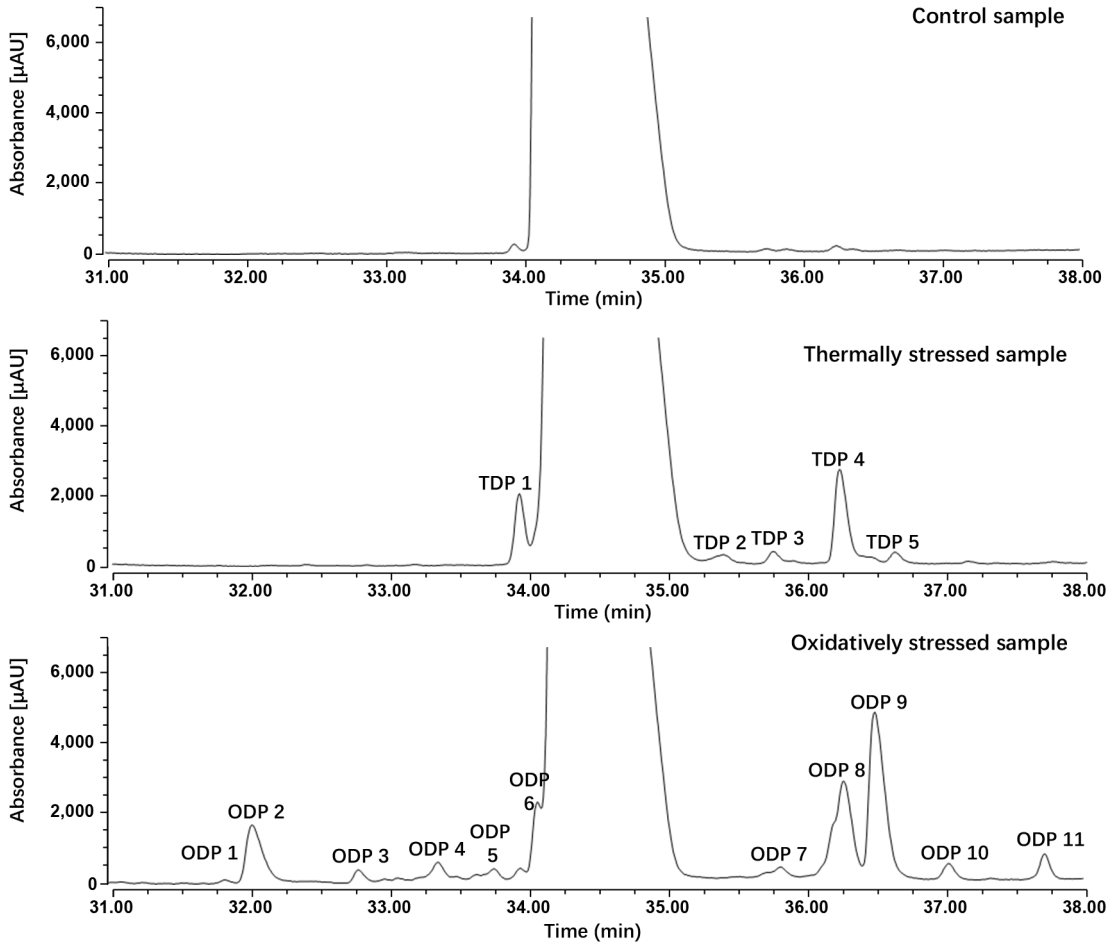


Figure 2. UV chromatograms at 280 nm of the semaglutide control sample, thermal stressed sample, and oxidative stressed sample. TDP stands for thermal degradation product of semaglutide FLP, and ODP stands for oxidative degradation product of semaglutide FLP. The detailed identification results for these degradation products can be found in Figure 5 and Table 4.

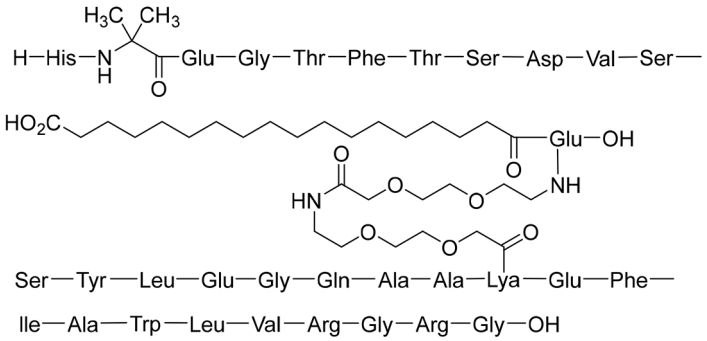


Figure 3. Amino acid sequence and lipid side chain structure of semaglutide

The peptide mapping analysis workflow within BioPharma Finder software was then used for MS data processing, which provides a streamlined process for peptides identification, including their chemical and post-translational modifications, as well as truncated forms. The parameters of the processing method utilized in BioPharma Finder software are detailed in Table 3.

After the data processing, the sequence of the peptide, the modifications such as oxidation, deletion, and other user-defined modifications, and its corresponding modification site can be automatically identified and assigned to peaks by BioPharma Finder software, based on the full MS and ddMS^2 data.

Figure 4 demonstrates the confident identification of semaglutide FLP and one of its oxidative degradation products, ODP 2, which elutes at 32.1 minutes (Figure 2). ODP 2 is identified as semaglutide FLP with a single oxidative modification at the W25 site. The experimentally determined monoisotopic mass of ODP 2 is 4127.1284 Da, reflecting a 15.9907 Da increase relative to the monoisotopic mass of semaglutide FLP, which is 4111.1377 Da. This mass increase is consistent with a single oxidation modification, further supported by the MS^2 data.

The MS^2 fragment spectrum in Figure 4 reveals a +15.995 Da increase in the molecular weight for the y_7^{2+} and y_8^{2+} fragment ions of ODP 2 compared to semaglutide FLP, while the y_6^{2+} fragment ion remains unchanged.

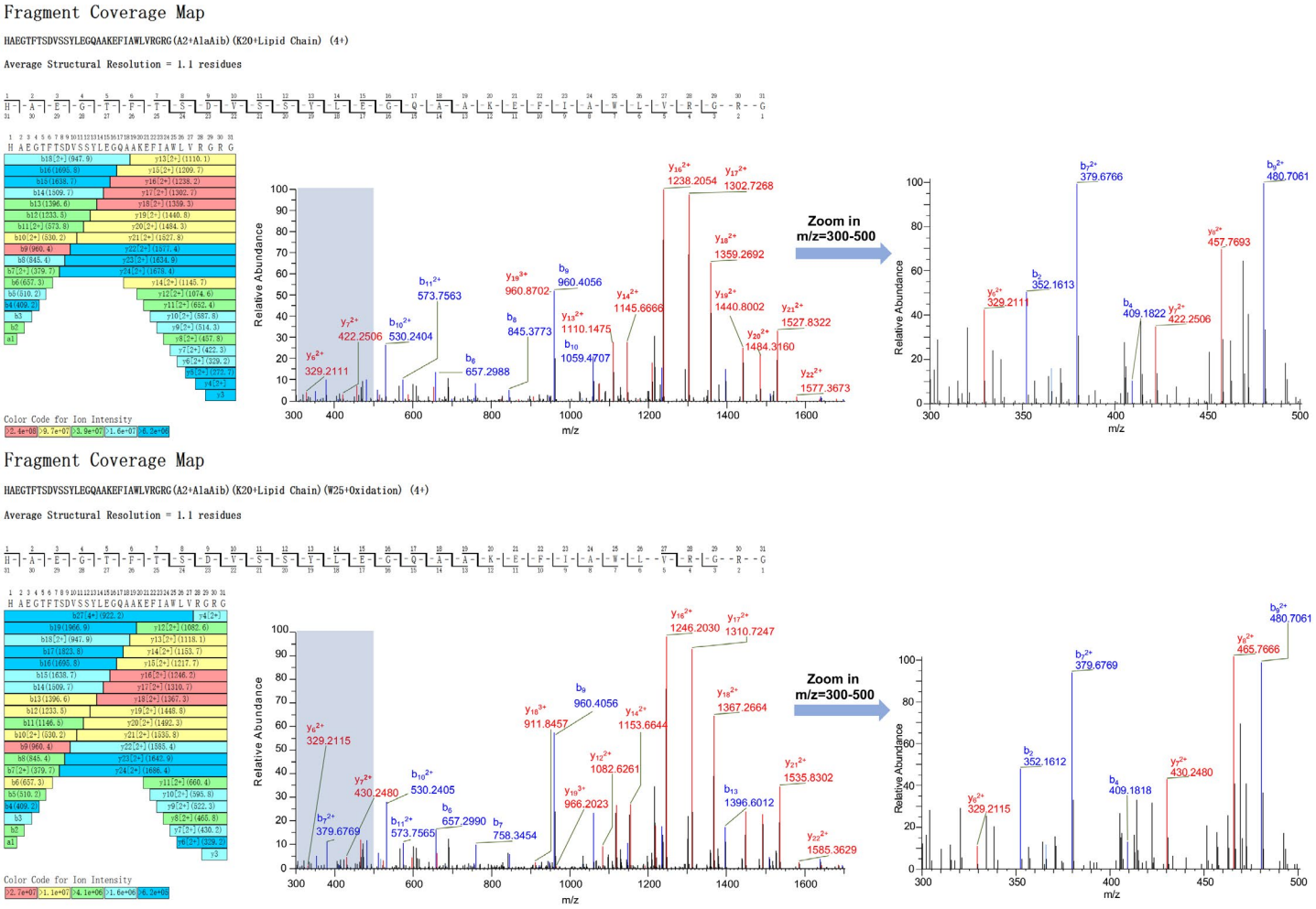


Figure 4. MS^2 spectrum and fragment coverage map of semaglutide (above) and the oxidative degradation product ODP 2 (below) with single oxidation on W25 eluted at 32.1 min. The MS^2 spectrum was zoomed from *m/z* = 300–500 to show the *m/z* changes of the fragments after the single oxidation of semaglutide FLP.

This data provides conclusive evidence of the oxidation site at W25. According to the Biopharma Finder software processed results, both peptides were identified with high confidence, showing a mass deviation of less than 6 ppm, a confidence score of 100, and an Average Structure Resolution (ASR) of 1.1.

Using this approach, efficient identification and relative quantitation of degradation products in the stressed samples were achieved. Figure 5 presents the total ion chromatogram (TIC) of the control sample and each stressed sample. The degradation product peaks were annotated with the most abundant peptides and modifications that were identified under these chromatographic peaks. The identification results indicate that under the thermal stressed conditions, the bonds between amino acids are prone to break, resulting in truncated semaglutide FLP. In the oxidation stress sample, single and double oxidation products on tryptophan (W25) dominate the degradation products. The distinct retention times of several single oxidation products/double oxidation products can be attributed to different locations of the hydroxyl group on the aromatic ring of tryptophan. The proposed mechanism for tryptophan single and double oxidation is illustrated in Figure 6. The different locations of the hydroxyl group, causing a distinct retention time shift, were also reported in the literature.[5,6]

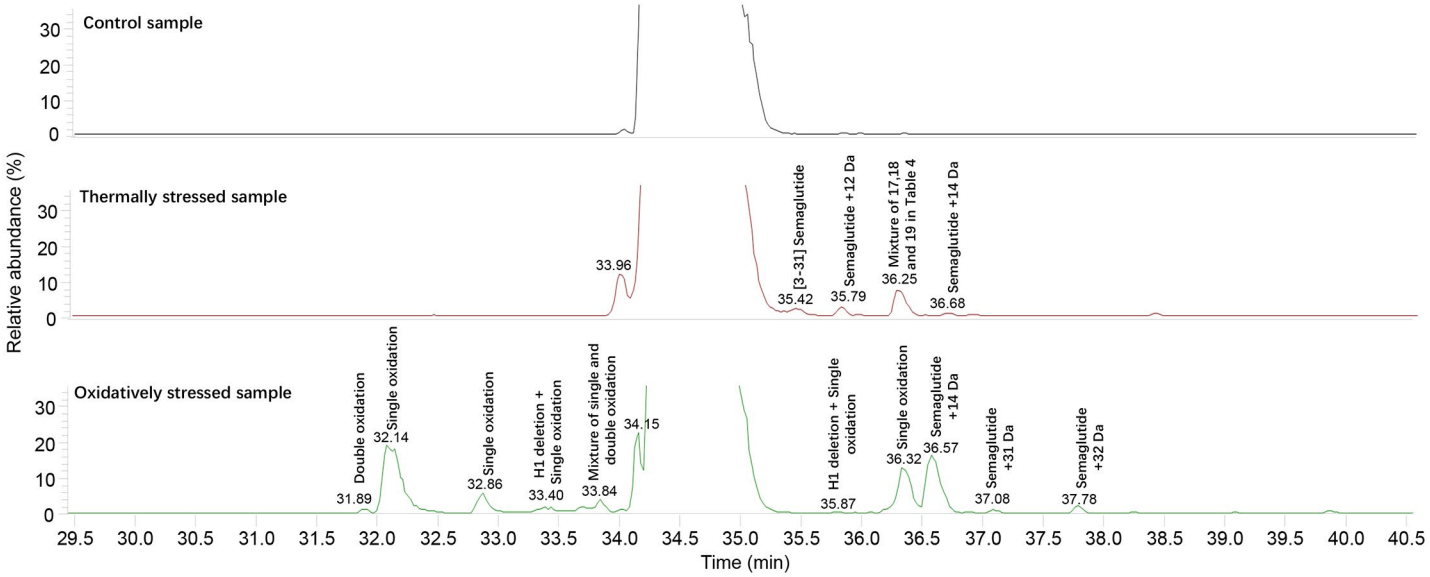


Figure 5. TIC of the semaglutide control sample, thermal stressed sample, and oxidative stressed sample. The degradation product peaks were annotated with the most abundant peptides and modifications identified under these chromatographic peaks.

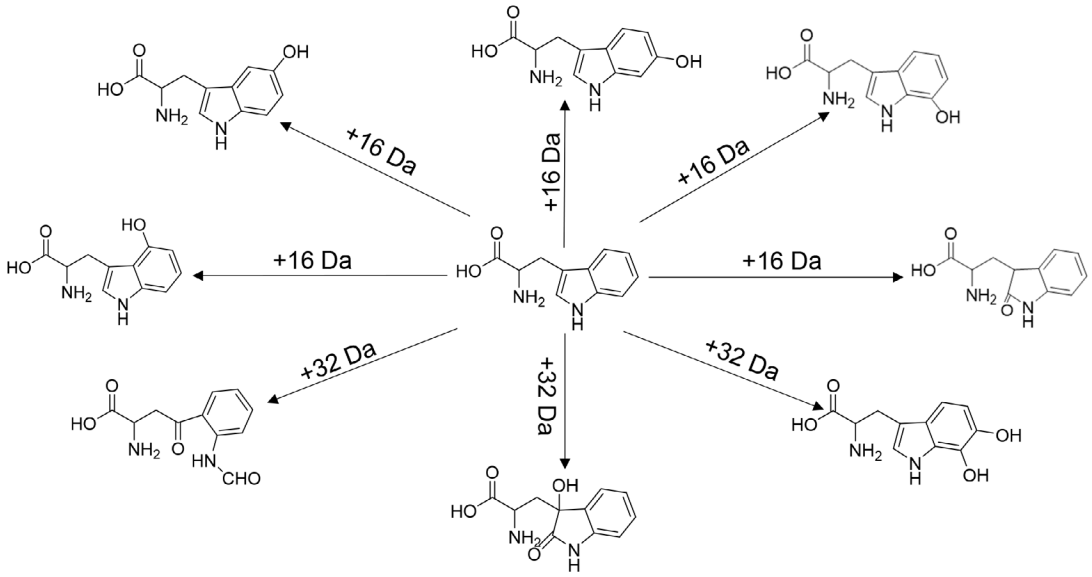


Figure 6. The proposed mechanism for the single and double oxidation of tryptophan

Identification of trace-level, co-eluted isobaric degradation products and impurities using high-quality HRAM ddMS^2 data

It is challenging to separate all the impurities from the semaglutide in LC separation, especially when the impurity has a similar structure and is present at a low level. Benefiting from the high sensitivity of HRAM MS, many peaks that were not visible on the UV chromatogram due to their low abundance or that co-eluted with semaglutide API were detected and identified by the HRAM MS. Table 4 lists all the identified degradation products (generated by stressed samples) and impurities (existing in the control sample) with levels exceeding 0.005% relative abundance of semaglutide FLP, as calculated by the extracted ion chromatogram (XIC), generated using the reporting features in Biopharma Finder software powered by the Ardia Platform.

The identified modifications, including oxidation, amino acid deletion, isomerization, and truncation of semaglutide, reflect potential impurities related to the semaglutide production, transportation, and storage. These modifications can be used for routine monitoring in the laboratory. It is worth noting that one impurity that co-eluted with the semaglutide FLP at 34.24 min was detected at a 0.43% level (above the 0.1% reporting threshold) in the control sample and was confidently identified as semaglutide with T5 deletion (#12 in Table 4). Based on the report generated by the Ardia Platform, the distribution and statistical results of the degradation products and impurities can be generated as a visualized graph, as shown in Figure 7. This provides comprehensive and clear insights into the semaglutide degradation study.

Table 4. UHPLC-HRAM MS-based identification and relative quantitation results of semaglutide and its degradation products. This table was generated using the reporting features in Biopharma Finder software powered by the Ardia Platform. It summarizes all the identified degradation products from thermal and oxidative stressed samples with an MS peak area above 0.005%. All the listed results were identified with mass deviation less than 6 ppm and a confidence score of 100 in BioPharma Finder software.

No	Peptide sequence	Product	Modification	Mod. site	RT (min)	Experimental monoisotopic mass (Da)	Theoretical monoisotopic mass (Da)	ΔMass (ppm)	Level in oxidative stressed sample (%)	Level in thermally stressed sample (%)	Level in control sample (%)
1	HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG	Semaglutide			34.42	4111.1377	4111.1154	5.43	91.2986	97.2746	99.3766
2	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Double oxidation	W25	31.22	4143.1060	4143.1052	0.18	0.0528	0.0002	0.0002
3	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Double oxidation	W25	31.89	4143.0996	4143.1052	-1.35	0.1692	0.0003	0.0001
4	HAEGFTSDVSSYLEGQAAKEFIA**W**LVRGRG	T5 deletion	Single oxidation	W24	31.94	4026.0610	4026.0626	-0.21	0.0213	0.0002	0.0000
5	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Single oxidation	W25	32.13	4127.1284	4127.1103	4.39	6.3980	0.0691	0.0095
6	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Single oxidation	W25	32.89	4127.1167	4127.1103	1.55	0.8745	0.0321	0.0021
7	AEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG	H1 deletion	Single oxidation	W24	33.40	3990.0513	3990.0514	-0.03	0.0117	0.0015	0.0001
8	HEGTFTSDVSSYLEGQAAKEFIAWLVRGRG	A2 deletion			34.16	4026.0527	4026.0632	-2.59	0.0154	0.0220	0.0219
9	HAEGFTSDVSSYLEGQAAKEFIA**W**LVRGRG	T5 deletion	Single oxidation	W24	34.17	4026.0610	4026.0626	-2.46	0.0076	0.0001	0.0000
10	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Single oxidation	W25	34.19	4127.0996	4127.1103	-2.59	0.4760	0.0116	0.0062
11	HAEGTFTSDVSSYLEGQAAKEFIAWLVRGRG		Endo	G4	34.20	4168.1274	4168.1363	-2.13	0.0422	0.0229	0.0289
12	HAEGFTSDVSSYLEGQAAKEFIAWLVRGRG	T5 deletion			34.24	4010.0698	4010.0677	0.53	0.3938	0.3538	0.4275
13	SDVSSYLEGQAAKEFIAWLVRGRG	[8-31] Semaglutide			35.04	3353.7781	3353.7764	0.49	0.0352	0.1699	0.0354
14	EGTFTSDVSSYLEGQAAKEFIAWLVRGRG	[3-31] Semaglutide	Isomerization	D7	35.42	3889.0044	3889.0043	0.04	0.0005	0.0120	0.0005
15	FTSDVSSYLEGQAAKEFIAWLVRGRG	[6-31] Semaglutide			35.85	3601.8872	3601.8925	-1.48	0.0005	0.0216	0.0004
16	AEGTFTSDVSSYLEGQAAKEFIAWLVRGRG	H1 deletion	Single oxidation	W24	35.87	3990.0559	3990.0514	1.13	0.0199	0.0028	0.0002
17	EGFTSDVSSYLEGQAAKEFIAWLVRGRG	[3-31] Semaglutide, T5 deletion			36.25	3787.9565	3787.9566	-0.01	0.0058	0.0114	0.0051
18	AEGTFTSDVSSYLEGQAAKEFIAWLVRGRG	H1 deletion			36.27	3974.0476	3974.0565	-2.23	0.0118	0.0401	0.0015
19	EGTFTSDVSSYLEGQAAKEFIAWLVRGRG	[3-31] Semaglutide			36.28	3888.9985	3889.0043	-1.47	0.0941	1.8558	0.0783
20	HAEGTFTSDVSSYLEGQAAKEFIA**W**LVRGRG		Single oxidation		36.33	4127.1313	4127.1103	5.09	0.4613	0.0002	0.0000
21	DVSSYLEGQAAKEFIAWLVRGRG	[9-31] Semaglutide			36.44	3266.7407	3266.7444	-1.13	0.0008	0.0360	0.0006
22	HAEGTFTSDVSSYLEGQAAKEFIAWLVR	[1-28] Semaglutide			38.20	3840.9756	3840.9713	1.10	0.0003	0.0183	0.0003
23	HAEGTFTSDVSSYLEGQAAKEFIAWLVRG	[1-29] Semaglutide			38.67	3897.9961	3897.9928	0.84	0.0010	0.0267	0.0008

Figure 8 shows how the high resolution, sensitivity, and mass accuracy of the Orbitrap HRAM MS platform empower the user to confidently identify co-eluting components under the main semaglutide FLP peak, even for isobaric species at trace levels. For the impurity co-eluting at 34.16 min under the main peak of semaglutide FLP, A2 deletion of semaglutide present at 0.022% level was confidently identified with high quality Full MS (<3 ppm mass deviation) and MS^2 data (ASR 1.1, Confidence Score: 100) from the control sample (data not shown) and thermal stressed sample. The theoretical monoisotopic mass of this A2 deletion peptide is 4026.0632 Da. In the oxidative stressed sample, four XIC peaks were observed and extracted by using *m/z* 1008.0245 (4+) ± 10 ppm. The degradation product eluted at 31.94 min was confidently identified with high-quality Full MS (<3 ppm mass deviation) and MS^2 data (ASR 1.1, Confident Score: 100) as T5 deletion of semaglutide with a single oxidation occurring at the W24 site. The theoretical monoisotopic mass of this T5 deletion + W24 oxidation peptide is 4026.0626 Da. For the products co-eluted at 34.17 min under the main peak in the oxidative sample, the MS^2 spectrum contained the characteristic ions matching to both the A2 deletion (b/y ions annotated in blue) and the T5 deletion+W24 oxidation (b/y ions annotated in purple) peptides, confirming the co-elution of these two isobaric peptide impurities in the oxidative stressed sample. The total relative abundance of these two co-eluted isobaric peptides in the oxidative stress sample is 0.022%. The fraction abundance of each isobaric peptide was calculated by the top 3 characteristic MS^2 ions. With this approach, the relative abundance of A2 deletion impurity was determined as 0.015% vs. T5 deletion + W24 oxidation as 0.007%.

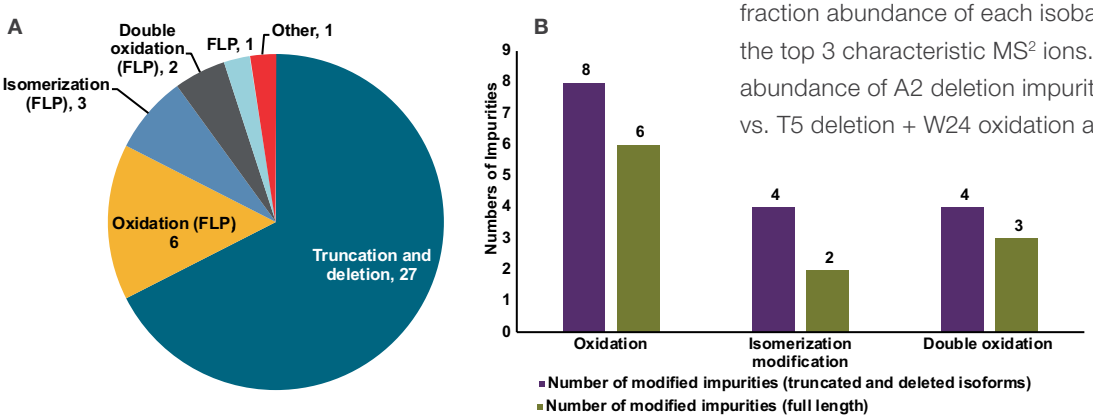


Figure 7. The distribution of identified degradation products and impurities. (A) The types of degradation products and impurities. (B) The distribution of single oxidation/double oxidation/isomerization in semaglutide FLP and truncated/deleted isoforms. DPs represents the degradation products.

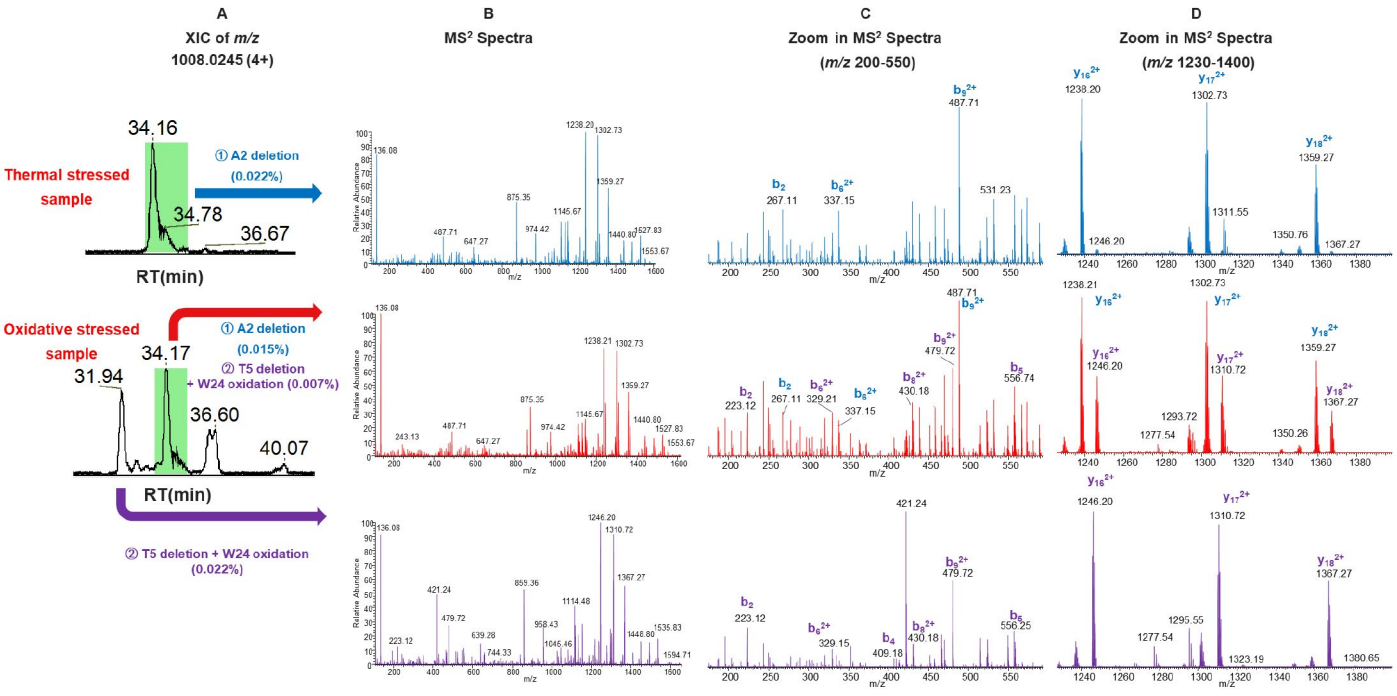


Figure 8. Identification of low-abundance co-eluted isobaric degradation products at 34.17 min in the oxidative stressed sample. (A) XIC of *m/z* 1008.0245 (4+) with 10 ppm isolation window from thermal stressed sample (top) and oxidative stressed sample (bottom). (B) High-quality MS^2 spectra of peptide eluted at 34.16 min in thermal stressed sample (top), peptide eluted at 34.17 min (middle), and 31.94 min (bottom). (C) Zoom in MS^2 spectra (*m/z* 200–550). (D) Zoom in MS^2 spectra (*m/z* 1230–1400). Characteristic b/y ions from the A2 deletion impurity are annotated with blue. Characteristic b/y ions from T5 deletion + W24 oxidation degradation product are annotated with purple.

Conclusion

In this study, we demonstrated a highly sensitive UHPLC-HRAM MS method for confident identification of the degradation products in thermal and oxidative stressed semaglutide samples using a Vanquish Flex UHPLC system, Hypersil GOLD Peptide column, and an Orbitrap Exploris 240 mass spectrometer combined with BioPharma Finder software. The high-quality HRAM MS data from the Orbitrap Exploris 240 mass spectrometer and the subsequent data processing workflow in BioPharma Finder software ensure confident and efficient identification.

- The Hypersil GOLD Peptide column provides excellent separation and peak shape for the semaglutide peptide and its degradation products using FA-containing mobile phases.
- The high sensitivity, high resolution, and high mass accuracy of the Orbitrap Exploris 240 mass spectrometer enabled confident and sensitive peptide identification, especially for accurately identifying low-abundant, co-eluting impurities in an excessive amount of semaglutide API.
- The full scan and ddMS^2 spectra, integrated with the peptide coverage map in BioPharma Finder software, provide intuitive insight into elucidating synthetic peptide structures.
- BioPharma Finder software connects to the Ardia Platform, offering an efficient and streamlined approach to peptide identification and generating a comprehensive report of the identification results.

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