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Monitoring Stability and Impurity Products of GLP-1 Agonists Using a Novel Single Quadrupole LC/MS

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Introduction

Glucagon-like peptide-1 (GLP-1) receptor agonists, such as semaglutide and tirzepatide, are used to treat type 2 diabetes mellitus (T2DM) and obesity by lowering serum glucose and improving metabolic control. GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that enhance insulin secretion post-meal but are rapidly degraded by dipeptidyl peptidase-4. While this response is diminished in T2DM, pharmacological GLP-1 levels can restore insulin release.[1]Tirzepatide and semaglutide presents analytical challenges due to their chemical degradations. The US Food and Drug Administration (FDA) considers the types and amounts of impurities present in a proposed generic drug compared to its reference listed drug (RLD). According to the FDA, a proposed generic synthetic peptide should not contain (i) impurities at levels greater than those found in the RLD nor (ii) any new specified peptide-related impurity that is greater than 0.5% of the drug substance.[2] This study evaluates the stability of tirzepatide and semaglutide using the Agilent InfinityLab Pro iQ Plus LC/MS system under various pH and storage conditions.

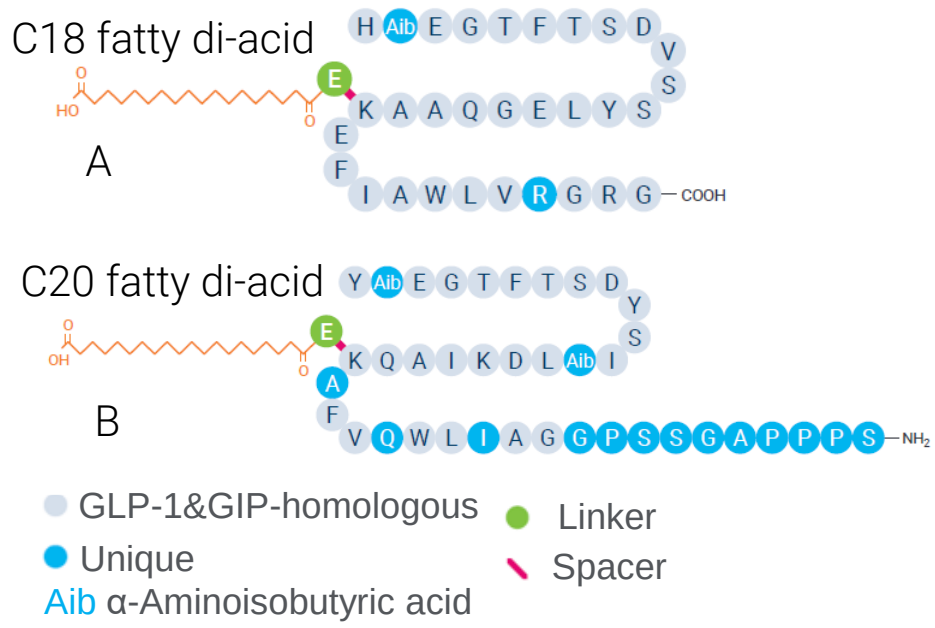

Figure 1. Semaglutide (A) and Tirzepatide (B) amino acid composition.

Experimental

GLP-1 peptide stock was prepared at 2 mg/mL in 15% acetonitrile/water and diluted to 50 ng/µL in buffers at pH 5, 7, and 9. Samples were injected directly without further purification and stored at 5 °C in the autosampler. LC/MS analysis was performed over seven days using the Agilent 1290 Infinity II Bio LC system with the Pro iQ Plus mass detector. Agilent ZORBAX RRHD 300 Å StableBond C18 column was used for separation. Data were processed using Agilent OpenLab CDS v2.8.

Experimental

Pro iQ Plus	
Mass range	m/z 2-3000
Sensitivity	<25 fg
Detection	Routine, trace, and extended mass range

Figure 2. Agilent 1290 Infinity II Bio LC system and Agilent Pro iQ Plus single quadrupole mass spectrometer.

LC Conditions	
Solvent A	Water with 0.1% FA
Solvent B	ACN with 0.1% FA
Gradient	0 min, 20% B 5 min, 48% B 10 min, 58% B 11 min, 60% B 12 to 14 min, 80%B 14.1 min, 20%B 15 min, 20%B
Injection volume	1 µL
Flow rate	0.4 mL/min
Column temperature	60 °C
MS Conditions	
Ion Source	Agilent Jet Stream ESI source
Polarity	Positive
MS Scan Range	m/z 500 to 2500
Scan Time	500 ms
Fragmentor	95 V
Data Storage	Profile
Gas Flow	11 L/min
Nebulizer	30 psi
Sheath Gas Flow	12 L/min
Capillary Voltage	3500 V
Nozzle Voltage	0 V
Gas Temperature	300 °C
Sheath Gas Temperature	250 °C

Table 1. LC/MS conditions for GLP-1 Peptides analysis

Tirzepatide related impurities

The total ion chromatogram (TIC) of tirzepatide after seven days of storage at 5 °C and pH 7 shows peaks corresponding to tirzepatide and its related impurity products. The mass spectrum at 7.89 minutes confirms the elution of tirzepatide, with the presence of +3, +4, and +5 charge states (Figure 3).

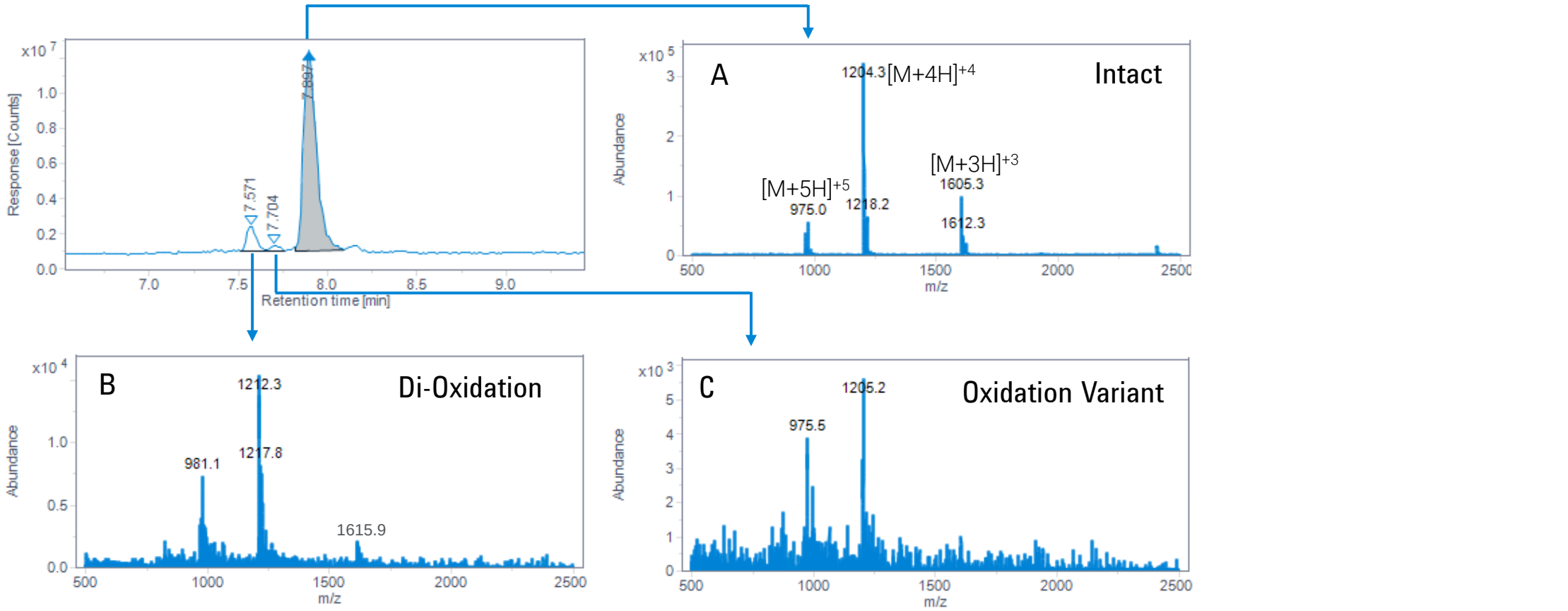

Figure 3. Mass spectra of tirzepatide and its related impurities. Panel A shows the mass spectrum of intact tirzepatide. Panel B displays the impurity eluting at 7.57 minutes, identified as dioxidation (O_2). Panel C shows the impurity eluting at 7.71 minutes, identified as an oxidation variant (O_2–CO).

Semaglutide related impurities

The TIC of semaglutide after seven days of storage at 5 °C and pH 9 shows peaks corresponding to semaglutide and its related impurity products. The mass spectrum at 6.45 minutes confirms the elution of tirzepatide, with the presence of +3, +4, and +5 charge states (Figure 4).

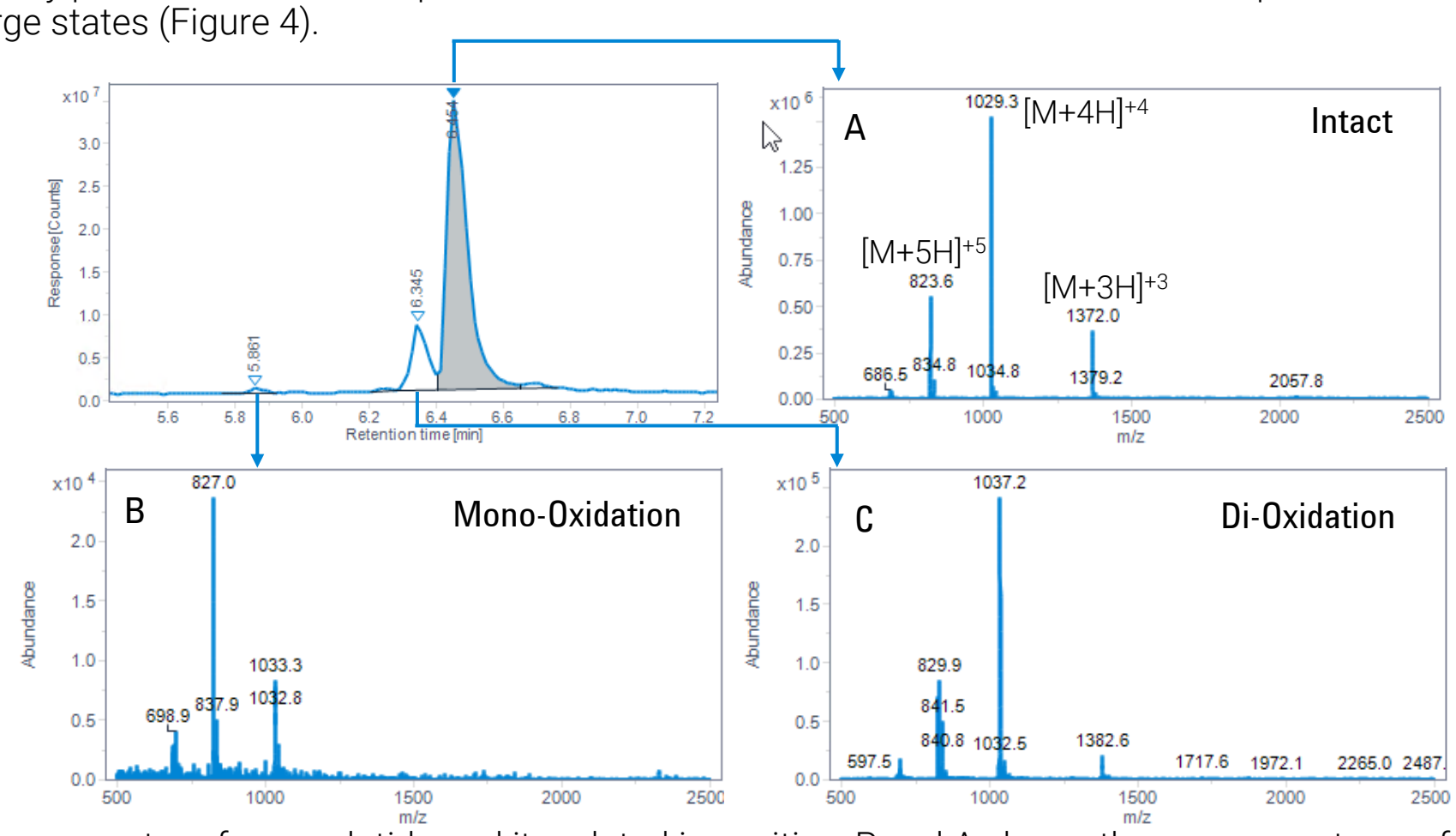

Figure 4. Mass spectra of semaglutide and its related impurities. Panel A shows the mass spectrum of intact semaglutide. Panel B displays the impurity eluting at 5.86 minutes, identified as mono-oxidation (O). Panel C shows the impurity eluting at 6.34 minutes, identified as dioxidation (O_2).

Results and Discussion

Deconvoluted results

The deconvoluted mass spectra show tirzepatide with a measured molecular weight of 4,813.1 Da (theoretical 4,813.5 Da) and semaglutide with 4,113.2 Da (theoretical 4,113.6 Da), both within the instrument's expected mass accuracy (±0.3 Da) .

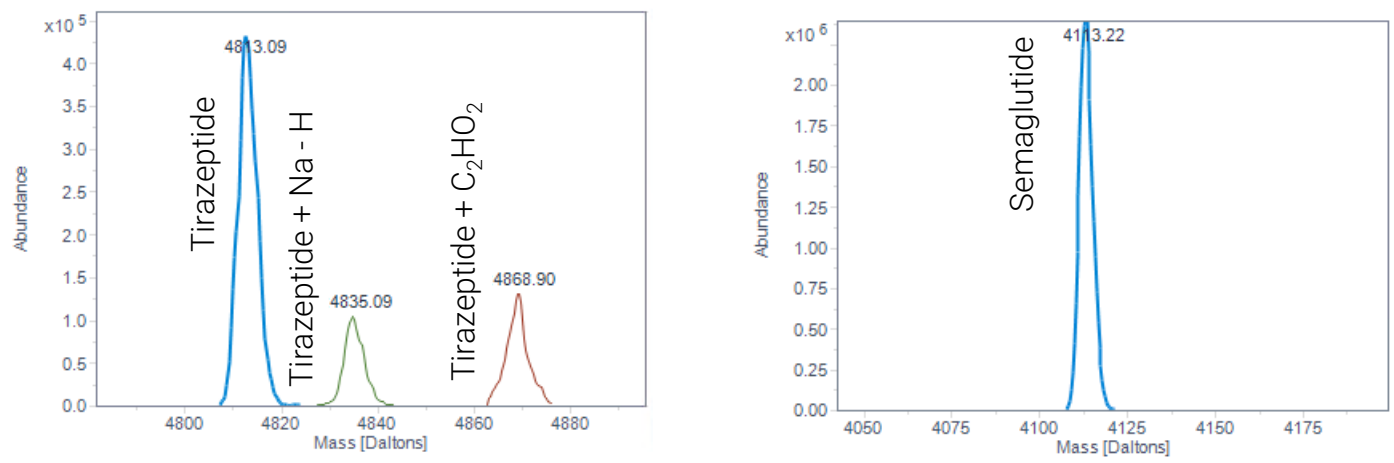

Figure 5. Deconvoluted mass spectra of GLP peptides. Panel A shows tirzepatide with a major peak at 4,813.1 Da and two adduct peaks: (tirzepatide + Na − H) and (tirzepatide + C_2HO_2). Panel B shows semaglutide with a single major peak at 4,113.2 Da.

Temporal monitoring of impurity generation

Proper storage and handling of GLP-1 medications are critical to maintain their stability, potency, and safety. Degradation due to improper conditions can compromise therapeutic efficacy and pose safety risks. Figure 6 illustrates the formation of oxidation products for tirzepatide and semaglutide under different pH conditions over time. The data show that tirzepatide is most prone to oxidation at pH 5, even when stored at 5 °C, while semaglutide shows the greatest instability at pH 9.

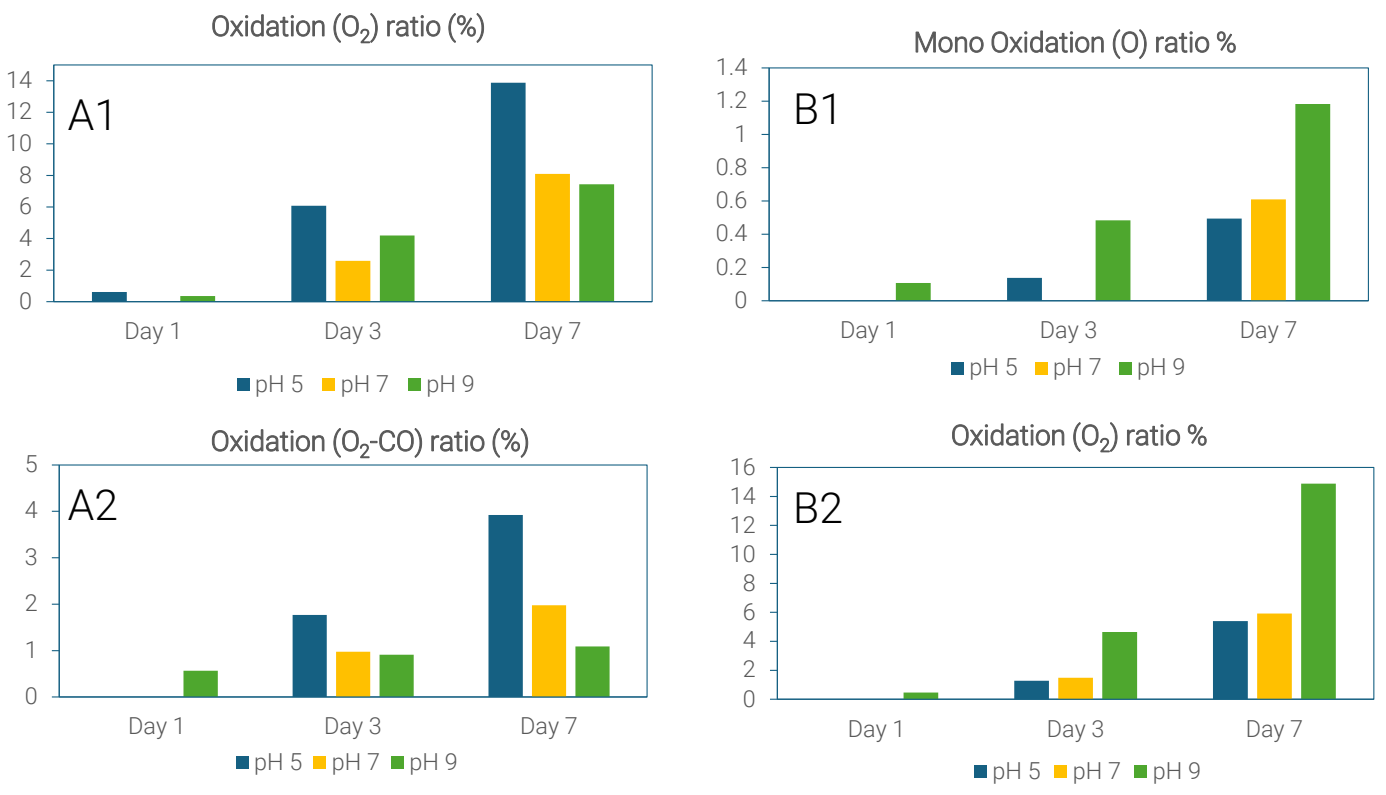

Figure 6. Temporal plots showing the percentage of oxidation products for tirzepatide (plot A1and A2) and semaglutide (plot B1 and B2) under various pH conditions. Tirzepatide displays the highest oxidation at pH 5, while semaglutide is most susceptible to oxidation at pH 9.

Conclusions

- Impurity profiling of tirzepatide and semaglutide under stress with the Agilent Pro iQ Plus mass detector shows the instrument fit-for-purpose in detecting low-level peptide impurities.
- Impurities with relative peak areas below 2% were detected, demonstrating excellent sensitivity.
- Both peptides showed oxidation-related degradation, influenced by pH and storage conditions.
- Proper storage and handling are essential to maintain stability and efficacy.
- This cost-effective, sensitive, and easy-to-use LC/MS system is well-suited for routine QC and QA in pharma/biopharma labs.

References

1. Vilsbøll, T.; Christensen, M.; Junker, A. E.; Knop, F. K.; Gluud, L. L. Effects of Glucagon-Like Peptide-1 Receptor Agonists on Weight Loss: Systematic Review and Meta-Analyses of Randomised Controlled Trials. BMJ 2012, 344, d7771. DOI: 10.1136/bmj.d7771.
2. U.S. Food and Drug Administration, Center for Drug Evaluation Research. ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs for rDNA Origin, Guidance for Industry. U.S. Department of Health and Human Services, May 19, 2021. DOI: 10.1002/psc.3652.

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