





In solid-phase peptide synthesis, amino acids are sequentially added to a growing chain anchored to a solid resin. The synthesis begins with the attachment of the first amino acid to the resin, followed by repetitive cycles of deprotection and coupling steps. In each cycle, the protective group (usually Fmoc or Boc) is removed from the amino acid, exposing its reactive amine group. Then, the next amino acid, with its reactive group protected, is coupled to the growing chain. This cycle repeats until the desired peptide sequence is completed. Once the synthesis is done, the peptide is cleaved from the resin and further purified. This is the route chosen by Ely Lilly for manufacturing Tirzepatide.

### The Regulatory Pathway for Generic Peptides

On 24th September 2024 we celebrated the 40 years of the Hatch-Waxman Act (officially known as the Drug Price Competition and Patent Term Restoration Act of 1984). This law transformed the generic drug industry by creating a balance between innovation in pharmaceuticals and the availability of affordable generic medicines. By simplifying the process for generic drug approvals, the Hatch-Waxman Act led to a surge in the availability of generic drugs. Today, generics make up the vast majority of prescriptions in the U.S. and significantly reduce healthcare costs. The Act allowed small molecule generics to enter the market more easily by proving "bioequivalence" to the innovator drug, meaning the generic works in the body the same way as the original branded drug. However, for peptides "generic" approval is more complicated.

The US FDA considers any alpha amino acid polymer composed of 40 or fewer amino acids to be a peptide approvable as a New drug application (NDA), regardless of their commercial production method (synthetic or recombinant DNA origin). Proteins are classified as biologics and are licensed as biological products (BLA) under section 351 of the Public Health Service (PHS) Act). Both Semaglutide and Tirzepatide are approved by the US FDA as NDAs.

All generic peptides undergo extensive characterisation with respect to their primary and secondary structures, oligomer and aggregation states, and biological activity, as these should

be the same as in the reference listed drug (RLD). For generic peptide drugs, the primary structure and the position of disulfide bond are critical determinants of the peptide drug substance sameness as compared to the RLD. Most generics have formulations that are qualitatively and quantitatively the same as the RLD. New excipients could lead to altered stability profile including formation of dimers and higher order aggregates, which may impact safety (including immunogenicity) and efficacy of the peptide drug product. Peptides are characterised predominantly by random coil and some degree of secondary structures (e.g., alpha-helix, and/or beta-sheet). Structural ordering may have significant implications for the biological activity of some peptides. Therefore, the elucidation of the secondary structure is often considered to be part of the peptide structure characterisation. Nonetheless, given that many of these peptides exhibit significant conformational flexibility, their higher order structure is dictated by thermodynamics, as opposed to a kinetic folding phenomenon. Therefore, for generic peptides that have the same amino acid sequence and are formulated qualitatively and quantitatively the same as the RLD, it can be generally inferred that they will have the same higher order structure as the RLD.<sup>6</sup>

The USFDA Guidance for Industry "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin" dated May 2021 set out the expectations for generic synthetic peptide drug products of a previously approved peptide drug product of recombinant deoxyribonucleic acid (rDNA). In the case of Semaglutide, Product Specific Guidance has been drafted for the oral<sup>7</sup> and injectable generic Semaglutide drug products, with the possibility for a waiver of *in vivo* bioequivalence study requirements in the later.<sup>8,9</sup> However, the main challenge in ensuring the quality and equivalence between the generic and the brand peptide drug products is with impurities that may be inadvertently introduced or increase during the production process and that may affect a proposed generic drug's performance and safety profile.<sup>10</sup> Peptide-related impurities can be especially difficult to detect, analyse, and control because they usually have similar sequences to the drug itself.

In addition to the analytical methods that characterise and compare the peptide-related impurity profiles and the generic and RLD peptide primary and secondary structures, additional comparative studies (e.g., *in vitro*, *in vivo* animal, clinical PK/PD equivalence) and clinical studies may be required to independently establish the safety or effectiveness of a proposed synthetic peptide. For example, an *in vitro* bioassay providing information on peptide structure-activity relationship is considered an essential aspect of efforts undertaken for characterisation of higher order structure and biological activity for complex peptides. Assessment of biological activity (which can be done by animal-based or cell-based assays, biochemical assays, or immune responses assays) is particularly important in cases where the synthetic peptide is used as a drug substance for a generic drug, if an additional confirmatory test is needed for demonstrating sameness between the proposed generic and the RLD products. FDA's Draft Guidance on Tirzepatide<sup>11</sup> requires comparative analysis of secondary structure, oligomer/aggregation states and biological activities. Where clinical data needs to be provided, the submission of an application under section 505(b) of the FD&C Act would be necessary. Outside the

## ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin Guidance for Industry

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)

May 2021  
Generics



US, generic peptides may need to be approved as a biosimilar, where products must demonstrate high similarity to the reference peptide in manufacturing quality, biologic activity, clinical safety and efficacy, and in the rate of immune reactions and specific clinical studies are required to demonstrate this equivalence. The Guideline on the Development and Manufacture of Synthetic Peptides<sup>12</sup> from the European Medicines Agency (EMA), requires generic applicants to fully quantify all differences in peptides produced by chemical synthesis and peptides produced by recombinant technology and demonstrate that both products are comparable. A broad panel of analytical methods is required for the side-by-side comparability studies between the EU sourced recombinant reference product and the synthetic version.

and more sustainable. Using various testing methods reported by the originator, Aurisco's scientists conducted a quality comparison study of several batches of Aurisco's Semaglutide API, along with the Injection and Tablet RLDs, confirming that Aurisco API shows consistently higher quality in all parameters and is suitable for a generic version of the originator product.

### Materials and Results

The present quality comparison study used seven batches of Injection Formulations and four batches of Oral Tablets from the originator for both USA and China markets and three commercial batches of Aurisco API (see Table 1).

Source	Strength	Sample number	Origin	Batch Number	Expiry Date
Reference Injection	Wegovy®: 2.4mg/0.75ml	I1	USA	NZF7T58	2025.08.31
	Wegovy®: 1.7mg/0.75ml	I2	USA	PZFAE95	2026.02.28
	Ozempic®: 8mg/3ml	I3	USA	PARO638	2027.04.30
	Ozempic®: 2mg/3ml	I4	USA	NZF7M97	2025.06.30
	Ozempic®: 1.34mg/ml	I5	China	202211BEH1	2025.10
	Ozempic®: 1.34mg/ml	I6	China	202211BEM1	2025.10
	Ozempic®: 1.34mg/ml	I7	China	202211BEV1	2025.10
Reference Tablets	RYBELSUS®: 3mg	T1	USA	PA42091	2025.09.30
	RYBELSUS®: 7mg	T2	USA	PA42079	2025.12.31
	RYBELSUS®: 7mg	T3	USA	PA42164	2025.12.31
	RYBELSUS®: 14mg	T4	USA	PA42103	2026.02.28
Aurisco API	API (Lyophilised)	S1	China	SEMA-240901	-
	API (Spray-dried)	S2	China	SEMC-240901	-
	API (Lyophilised)	S3	China	SEM-24071003	-

Table 1: Basic information of Reference Formulations and Aurisco's APIs

### Impact of Manufacturing Process on the Properties and Quality of Semaglutide

On October 2024, a study by a group of scientists from Novo Nordisk was published,<sup>13</sup> comparing a representative selection of commercially available Semaglutide APIs made by solid-phase synthesis against their Reference Listed Drugs (RLDs). Compared with originator, synthetic Semaglutide DSs and DPs had new impurities and impurity patterns, including high molecular weight proteins, trace metals, anions, counterions, and residual solvents. This study concluded that changing the manufacturing process of Semaglutide from recombinant to solid-phase synthesis can result in several changes to the DSs and DPs. Moreover, the authors recommended that the impact of these changes on efficacy and safety outcomes should be investigated by clinical studies. One can read between the lines that these companies, who felt tempted to use the fully synthetic API and leverage the guidance to avoid the clinical work, instead of a fast track to an ANDA, will receive a thick Complete Response Letter (CRL).

### Recombinant Generics

Both Aurisco Pharmaceutical and Novo Nordisk use recombinant technology and semi-synthetic methods to manufacture Semaglutide API, making Aurisco's product quality similar to that of the RLD, but more cost-effective

### Related Substances I by HPLC

Four batches of different strengths of Reference Injection and four batches of Reference Tablets were analysed by HPLC and impurity profiles were compared with Aurisco's API (see method on Appendix 1.1). The results on Table 2 showed that all Reference Formulations had a higher number and total amount of impurities compared to Aurisco's APIs, especially the Reference Tablets (Sample T1), where hydrophilic impurities, hydrophobic 1 impurities, hydrophobic 2 impurities, and total impurities were significantly higher than in Aurisco's APIs (Figure 1).

### Related Substances II by HPLC

Based on an optimised HPLC method for impurity testing (see Appendix 1.2), where all impurities greater than 0.10% were qualitatively studied and included in the quality standards, we analysed seven batches of different strengths of Reference Injection Formulations, four batches of different strengths of Reference Tablets, and two batches of Aurisco's Semaglutide API. The results on Table 3 revealed that in both Aurisco's batches of API, impurities such as SEM-K, SEM-P, SEM-Y and SEM3 were significantly lower than those in the Reference Injection and Tablet Formulations. For other unknown single impurities, Aurisco's API maintained levels below 0.10%. (Figure 2).

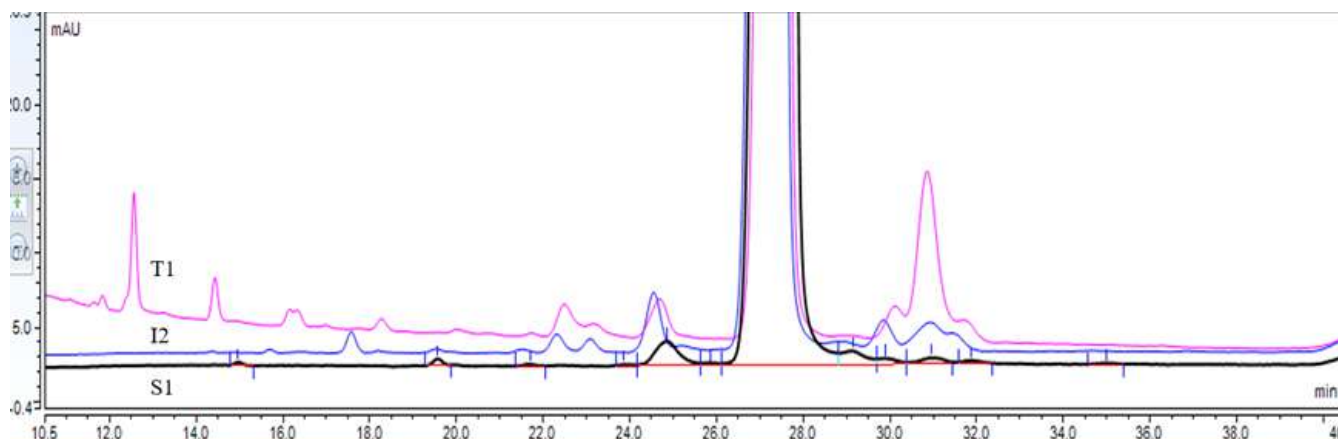


Figure 1: Comparison of Related substance II in Aurisco's API (black) vs Reference Formulations (blue and pink).

Source	Sample number	Hydrophilic Impurities (%)	Hydrophobic 1 Impurities (%)	Hydrophobic 2 Impurities (%)	Total Impurities (%)
Reference Injection	I1	1.07	1.13	0.15	2.34
	I2	1.25	1.30	0.17	2.73
	I3	0.94	1.34	0.30	2.60
	I4	0.90	1.30	0.17	2.36
Reference Tablets	T1	2.92	4.43	0.46	7.82
	T2	1.98	2.83	0.39	5.22
	T3	2.45	2.23	0.29	4.95
	T4	1.79	2.55	0.24	4.63
Aurisco API	S1	0.30	0.32	N/A	0.62
	S2	0.33	0.37	N/A	0.70

**Note:** *Hydrophilic impurities:* The sum of all impurities before the main peak; *Hydrophobic 1 impurities:* The sum of all impurities in the first section of isocratic elution after the main peak; *Hydrophobic 2 impurities:* The sum of all impurities in the second section of gradient elution and second section of isocratic elution after the main peak.

Table 2: Related substances I results for Aurisco API vs Reference Formulations

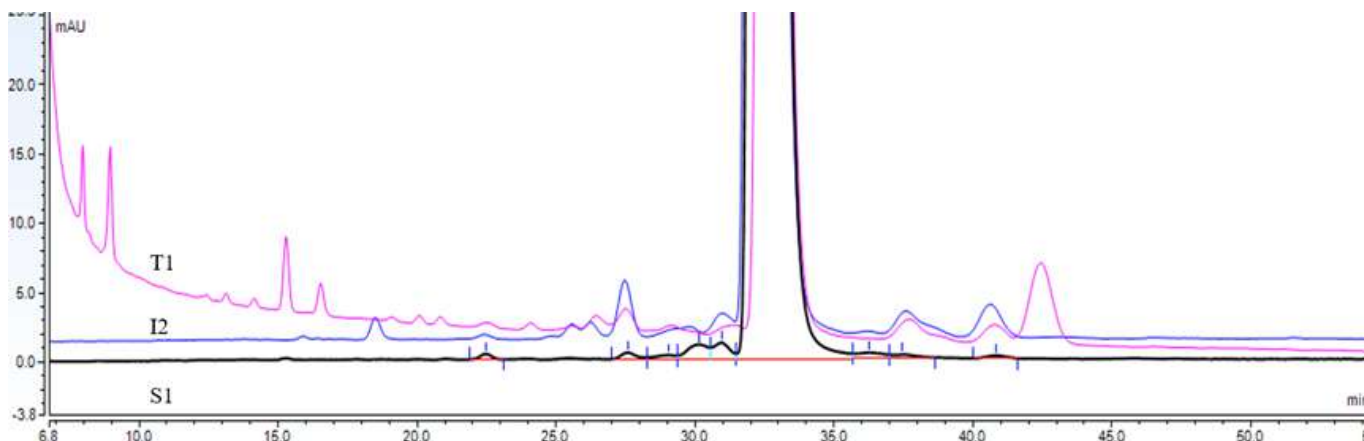


Figure 2: Comparison of Related substance II in Aurisco's API (black) vs Reference Formulations (blue and pink).



Source	Sample number	SEM-K (%)	SEM-N (%)	SEM-P (%)	SEM-A (%)	SEM-Y+SEM-Z (%)	SEM3 (%)	Maximum Unknown Impurity (%)	Total Impurities (%)
		≤0.5%	≤0.4%	≤0.3%	≤0.2%	≤0.5%	≤0.5%	≤0.10%	≤1.5%
Reference Injection	I1	0.42	0.16	0.32	0.10	0.50	0.42	0.15	2.73
	I2	0.58	0.13	0.35	0.11	0.59	0.51	0.27	3.12
	I3	0.46	0.08	0.26	0.08	0.77	0.40	0.20	2.80
	I4	0.42	0.10	0.26	0.09	0.59	0.51	0.28	2.96
	I5	0.41	0.31	0.26	0.15	0.47	0.63	0.14	3.11
	I6	0.45	0.37	0.28	0.17	0.50	0.72	0.18	3.72
	I7	0.43	0.33	0.24	0.16	0.45	0.70	0.19	3.54
Reference Tablets	T1	0.57	0.05	0.34	0.02	0.76	0.58	2.78	9.08
	T2	0.59	0.07	0.32	0.06	0.91	0.67	0.96	6.47
	T3	0.60	0.07	0.32	0.05	0.84	0.68	0.65	5.28
	T4	0.61	0.06	0.36	0.07	0.86	0.70	0.81	5.36
Aurisco API	S1	0.09	0.24	0.19	0.12	0.07	0.05	0.07	0.90
	S2	0.09	0.25	0.19	0.11	0.08	0.04	0.08	0.92

Note: Impurities SEM-K = D-Ser8, SEM-N = β-Asp9, SEM-P = D-Glu15, SEM-A = D-His1, SEM-Y= His1 Formaldehyde adduct, SEM-Z= Ser8 Acetylation, SEM3 = Des-His1Aib2; Threshold: as specified in Aurisco's API quality standards

Table 3: Related substance II Testing Results for Aurisco API vs Reference Formulations

### High Molecular Weight Proteins by SEC

Using the same size exclusion chromatography method as described by Novo Nordisk's researchers<sup>13</sup> (see Appendix 1.3), we tested seven batches of different strengths of Reference Injection Formulations, four batches of Reference Tablets, and two batches of Aurisco's API for High Molecular Weight Protein (HMWP) content. The results on Table 4 showed that both Aurisco's API batches contained particularly low levels of high molecular weight proteins, which were much lower than those in the Reference Formulations (Figure 3).

### Impurities by LC-MS

For qualitative impurity analysis, we followed the LC-MS method reported by the originator<sup>13</sup> (see Appendix 1.5). The results presented on Table 5 showed that no new impurities were detected in Aurisco's API (Figure 4). Among the 33 impurities detected in the Reference Formulations, none were detected

Source	Sample number	HMWP (%)
Reference Injection	I1	0.20
	I2	0.16
	I3	0.23
	I4	0.83
	I5	0.52
	I6	0.65
	I7	0.68
Reference Tablets	T1	0.04
	T2	0.06
	T3	0.05
	T4	0.09
Aurisco API	S1	0.02
	S2	0.02

Table 4: HMWP Content in Aurisco API vs Reference Formulations.

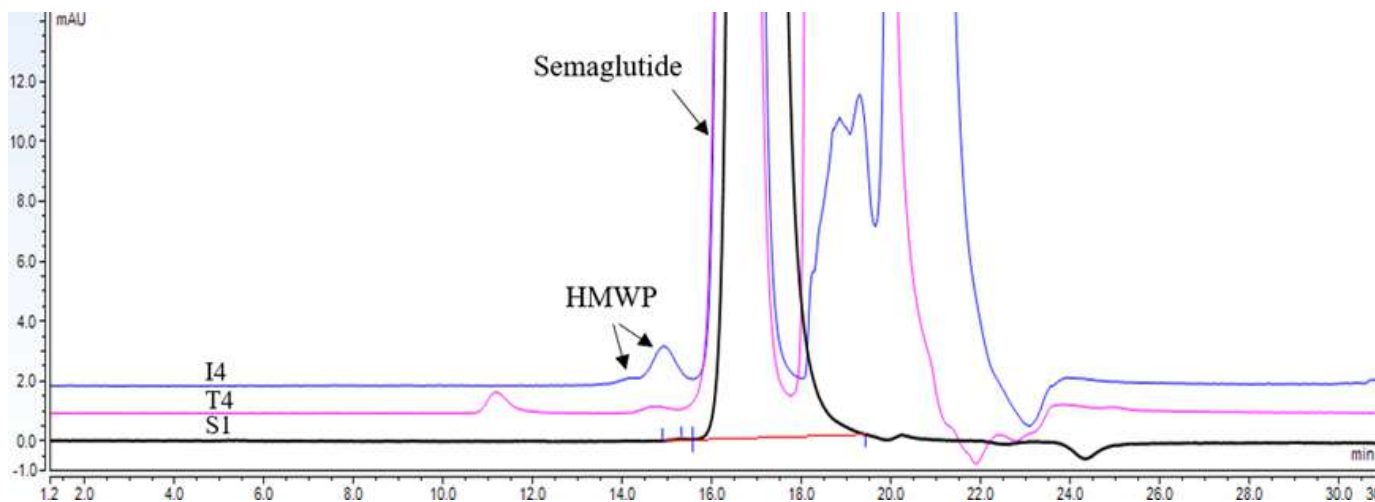


Figure 3: Comparison of High Molecular Weight Protein content in Aurisco's API (black) vs Reference Formulations (blue and pink).



Impurity	Reference Injection	Reference Tablets	Aurisco API
Unknown 1			
Semaglutide backbone			
Unknown 2			
Unknown 3			
Unknown 4			
Semaglutide ox form 1			
Unknown adduct 1			
Unknown adduct 2			
Semaglutide isomer form 1			
Des 1-13 amino acid Semaglutide form			
Unknown 5			
Unknown adduct 3			
Semaglutide di-ox form			
Endo Ala Semaglutide form			
Sidechain Add-on Unit (C6H11NO3)			
Semaglutide di-ox form			
Semaglutide isomer form 2			
Formaldehyde adduct			
Acetaldehyde adduct 1			
Acetylation modification 1			
Unknown 6			
Des Gly Semaglutide form			
Acetylation modification 2			
Acetaldehyde adduct 2			
Des His-Aib Semaglutide			
Des Gly Semaglutide form			
Acetylation modification 3			
Des His-Aib-Glu-Gly Semaglutide			
Des His-Aib-Glu Semaglutide			
Formylation modification 1			
Methylation modification			
Unknown 7			
Des H2O Semaglutide form			
Des Gly-Arg-Gly Semaglutide			
Unknown 8			
Unknown 9			
Acetaldehyde adduct 2			
Formylation modification 2			
Semaglutide dimer form			

**Note:** Light blue box indicates impurities identified in the reference drug; Dark blue box indicates impurities identified in Aurisco's API. Impurities are listed in the order they were extracted.

Table 5: LC-MS Impurity Testing Results for Aurisco API vs Reference Formulations

in Aurisco API. For example, the dimer impurity present in the Reference Injection Formulations, was not detected in Aurisco's API, which is consistent with the findings of size exclusion chromatography.

### Other Non-Peptide Impurities

At Aurisco's QC labs, we used inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) to quantitatively test the metal elements, the results displayed on Table 6 showed that all metal elements in Aurisco's API were equivalent to that of the RLD Formulations. Additionally, based on the "ICH Q3D Element Impurities Guidelines," we tested for potential class 1, class 2A, and class 3 element impurities in Aurisco's API, finding all results well below the acceptable level of 30%. The ion chromatography

Classification	Impurity	Originator (µg/g)	Aurisco (µg/g)
Residual solvents	Trifluoroacetic acid(µg/g)	≤20	≤20
	Acetic acid(µg/g)	≤20	≤20
	Methyl tert-butyl ether (µg/g)	No report	≤30
	Tetrahydrofuran(µg/g)		≤15
	1,2-Dimethoxyethane(µg/g)		≤20
	Acetonitrile		6
	Dichloromethane		4
	Metal elements	B_conc (µg/g)	0.49ppm
K_conc (µg/g)		≤9.7	≤7.0
Fe_conc (µg/g)		0.49ppm	≤0.01
Mg_conc (µg/g)		≤0.24	≤0.33
Ca_conc (µg/g)		≤9.7	≤5.5
Mn_conc (µg/g)		≤0.24	≤0.02
Zn_conc (µg/g)		≤0.24	≤0.08
Li_conc (µg/g)		≤0.24	≤0.01
Cr_conc (µg/g)		≤0.24	≤0.05
Ni_conc (µg/g)		≤0.24	≤0.20
Cu_conc (µg/g)		≤0.24	≤0.17
Cd_conc (µg/g)		No report	≤0.1
Pb_conc (µg/g)			≤0.1
As_conc (µg/g)			≤0.01
Hg_conc (µg/g)			≤0.007
Co_conc (µg/g)			≤0.01
V_conc (µg/g)			≤0.01
Sb_conc (µg/g)			≤0.21
Mo_conc (µg/g)		≤0.05	
Cation	Na_conc (%w/w)	2.1	1.6~2.4
	Chloride (µg/g)	190	≤14
Anion	Phosphate (µg/g)	630	≤78
	Nitrate (µg/g)	≤16	≤16

**Note:** The data for the originator is sourced from the reference.<sup>13</sup>

Table 6: Summary of Other Non-Peptide Impurities

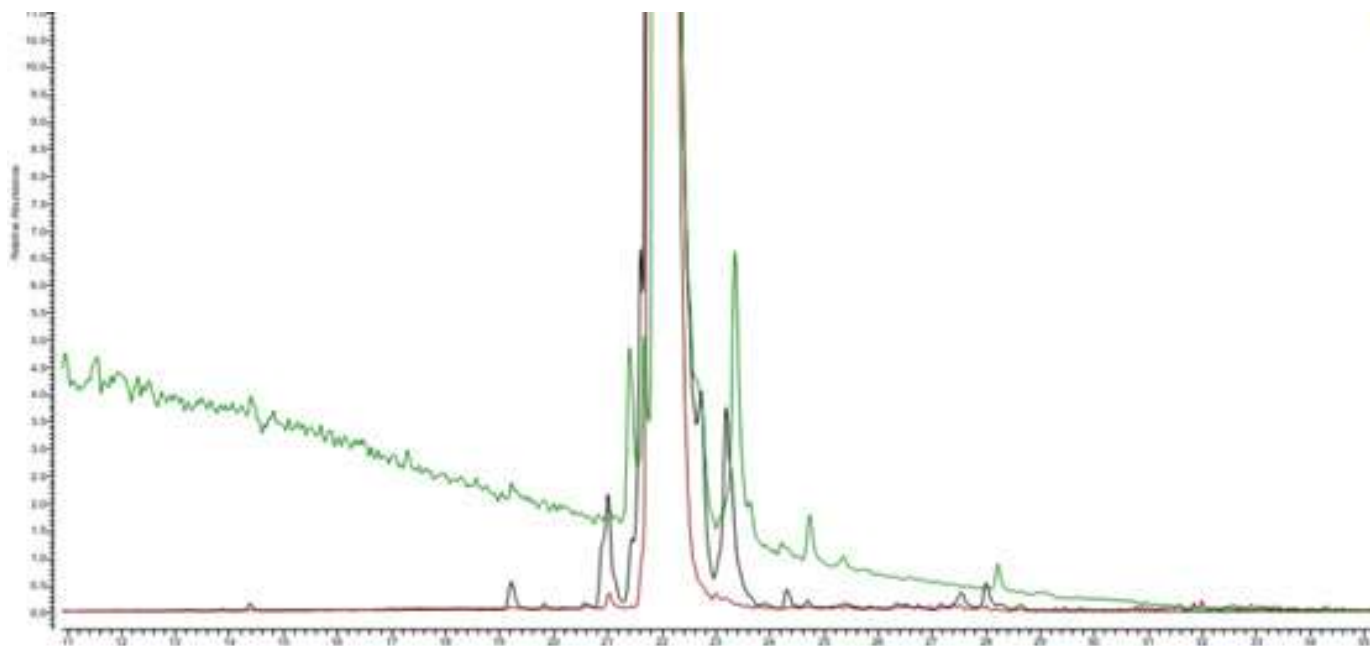


Figure 4: Comparison of LC-MS Impurities in Aurisco's API (brown) vs Reference Injection (black) and Tablet Formulations (green).

(IC) results showed that sodium ion and nitrate ions were similar as the originator, and chloride, phosphate were far lower than the originator levels. High-performance liquid chromatography (HPLC) and gas chromatography (GC) were used for quantitative determination of residual solvents, acetic acid and trifluoroacetic acid were consistent with that of the originator Formulations. The detection values of other residual solvents were extremely low and complied with the "ICH Q3C Residual Solvents Guidelines".

### A Higher Order Structural Assessment

The correct higher order structure of a protein is critical for the biological function. It is therefore highly relevant to ensure structural protein properties remain intact. In this

study, lyophilised API and spray-dried API were tested by 2D NMR spectroscopy (Appendix 1.6), and all of these displayed identical higher order structure when compared with originator injection Semaglutide.

### Photostability Assessment

We exposed the Reference Injection (I1) to light at 25°C and 4500 lux (90  $\mu\text{W}/\text{cm}^2$ ) for 12 days, the results showed that SEM3, SEM-Y and unspecified impurity increased significantly. In comparison, Aurisco's API, exposed to the same conditions for 15 days, showed no significant increase in any single impurity except for SEM-Y. This indicates better stability for Aurisco's API powder compared to the Reference Injection Formulation.

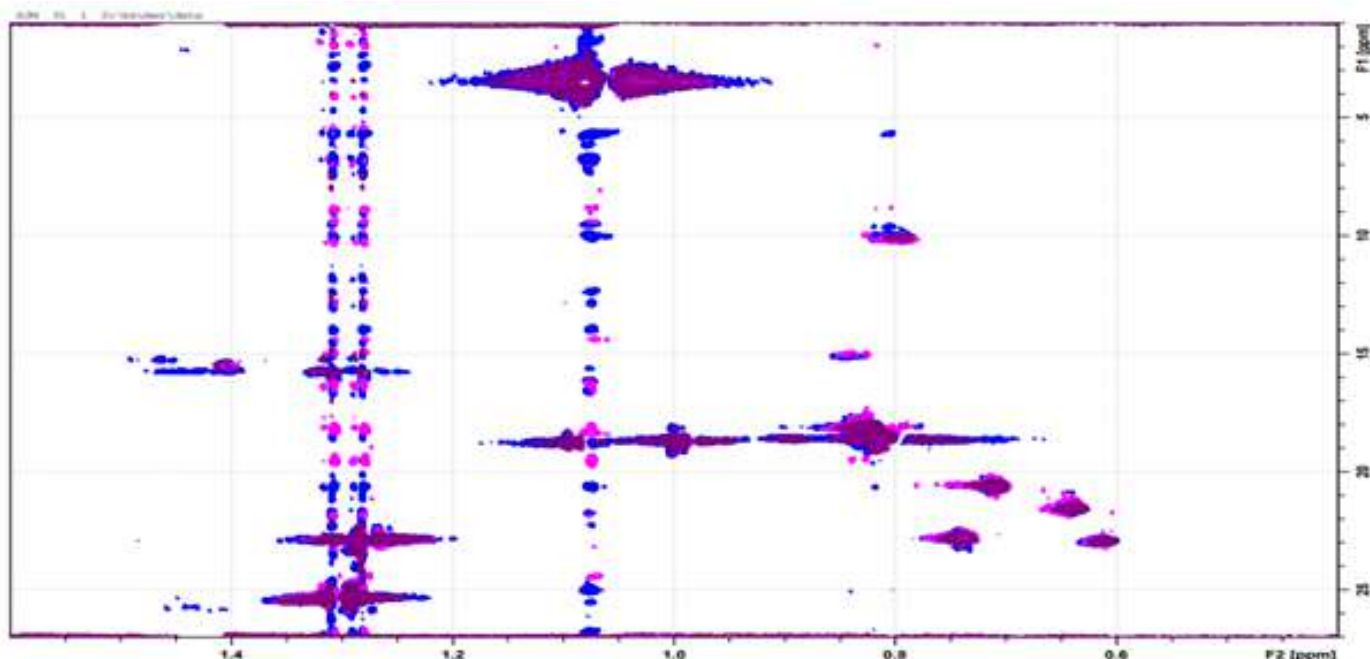


Figure 5: Overlay of HMQC 1H-13C NMR spectra for originator (I6/purple) and Aurisco's API (S1/blue, S2/pink)



Test items	Impurity	Reference Injections (I1)		Aurisco's API (S3)	
		Day 0	12 Days 25°C 4500lux	Day 0	15 Days 25°C 4500lux
Related substances	SEM-K (%)	0.42	0.45	0.06	0.04
	SEM-N (%)	0.16	0.15	0.18	0.20
	SEM-P (%)	0.32	0.28	0.15	0.15
	SEM-A (%)	0.10	0.12	0.12	0.15
	SEM3 (%)	0.42	0.98	0.05	0.05
	SEM-Y+ SEM-Z (%)	0.50	0.80	0.08	0.40
	Unspecified Impurity (RRT0.79) (%)	0.12	0.79	0.02	0.03
	Total Impurities (%)	2.73	5.34	0.75	1.23

**Note:** Impurities SEM-K = D-Ser8, SEM-N = β-Asp9, SEM-P = D-Glu15, SEM-A = D-His1, SEM-Y= His1, SEM-Z= Ser8 Acetylation, Formaldehyde adduct, SEM3 = Des-His1Aib2.

Table 7: Light Stability Testing Results for Aurisco API vs Reference Formulation

## Conclusions

Following the same approach used by Novo Nordisk to compare synthetic versions of Semaglutide against the originator's Reference Listed Drug, and using the same or improved testing methods, we demonstrated that Aurisco's recombinant Semaglutide API outperforms the Reference Listed Drug from Novo Nordisk in terms of impurities, high molecular weight proteins, trace metals, anions, cations, residual solvents, and stability. In addition, Aurisco's API displayed identical higher order structure with originator.

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- <https://www.fda.gov/drugs/regulatory-science-action/impact-story-developing-tools-evaluate-complex-drug-products-peptides>
- [https://www.accessdata.fda.gov/drugsatfda\\_docs/psg/PSG\\_215866.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/psg/PSG_215866.pdf)
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- Hach, M., Englund, D.K., Mysling, S. et al. Impact of Manufacturing Process and Compounding on Properties and Quality of Follow-On GLP-1 Polypeptide Drugs. *Pharm Res* 41, 1991–2014 (2024). <https://doi.org/10.1007/s11095-024-03771-6>

## APPENDIX 1: TESTING METHODS

### Related Substances I

Method based on the test for impurities by HPLC, according to USP General Chapter <621>. A Thermo Fisher Ultimate 3000 liquid chromatography system was used for the analysis, with C18-phenylsilica gel as the stationary phase (Phenomenex Kinetex, C18 4.6×150mm, 2.6μm). Mobile phase A consisted of a 0.08 mol/L ammonium dihydrogen phosphate buffer solution – 10% acetonitrile (prepared by dissolving 9.2g ammonium dihydrogen phosphate in 900mL water, adjusting the pH to 3.6 with concentrated phosphoric acid, adding 100mL acetonitrile, and then filtering). Mobile phase B consisted of acetonitrile-isopropanol-water (3:1:1, v/v/v). The gradient elution conditions are listed in Table 1-1. The column temperature was set to 30°C, and the flow rate was 0.7mL/min. The detection wavelength was 210nm, with a sample injection volume of 10μL. The sample tray temperature was maintained at 5°C.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	54	46
7	47	53
37	47	53
49	10	90
52	10	90
53	54	46
60	54	46

Table 1-1: Gradient Elution Parameters for Related substance I.

In the chromatogram recorded from the sample solution, the hydrophilic impurities were determined as the total of all peaks eluted before the Semaglutide main peak, hydrophobic impurity 1 as the total of all peaks eluted during the first isocratic elution after the Semaglutide main peak, and hydrophobic impurity 2 as the total of all peaks eluted during the gradient elution and second isocratic elution following the Semaglutide main peak.



## Related Substances II

The test for related substances II was also based on HPLC, following the USP General Chapter <621>. The stationary phase was C18-phenylsilica gel (Phenomenex Kinetex, C18 4.6×150mm,2.6µm), and the mobile phase A consisted of 0.08 mol/L ammonium dihydrogen phosphate buffer – 10% acetonitrile (prepared by dissolving 9.2g ammonium dihydrogen phosphate in 900mL water, adjusting the pH to 3.0 with concentrated phosphoric acid, adding 100mL acetonitrile, and then filtering). Mobile phase B consisted of acetonitrile-isopropanol-water (4:1:1, v/v/v). The gradient elution conditions are listed in Table 1-2. The column temperature was set to 30°C, with a flow rate of 0.7mL/min, and the detection wavelength was 210nm. The sample injection volume was 10µL, and the sample tray temperature was maintained at 5°C. In the chromatogram, after subtracting the chromatogram of the blank solution, the impurities were calculated by normalising the peak area.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	53	47
60	53	47
65	10	90
70	10	90
72	53	47
80	53	47

Table 1-2: Gradient Elution Parameters for Related substance II

## High Molecular Weight Proteins

This method is based on molecular size exclusion chromatography (SEC), according to the USP General Chapter <621>. The stationary phase was hydrophilic gel (Waters Insulin HMWP 7.8×300mm, 10µm), and the mobile phase was a phosphate buffer solution with 0.5mol/L sodium chloride (prepared by dissolving 146.1g sodium chloride and 6.9g monosodium

phosphate in 2000mL water, adding 1.7mL concentrated phosphoric acid, then adding 2500mL isopropanol and mixing thoroughly. The final volume was adjusted to 5000mL with water). The flow rate was set to 0.5mL/min, and the column temperature was 50°C. The detection wavelength was set to 280nm. The sample injection volume was 10µL, and the retention time of Semaglutide was used for analysis. All peaks with retention times greater than that of Semaglutide were excluded in the calculation.

## Ion Chromatography – Anion and Cation Measurement

Ion chromatography was conducted using the USP General Chapter <621> method for anion and cation analysis. A Dionex™ IonPac™ CS12A chromatography column from Thermo Scientific™ was used for cation analysis, with 20mM methanesulfonic acid solution as the mobile phase. The isocratic elution was performed for 10 minutes at a flow rate of 1.0mL/min. The sample injection volume was 25µL; the conductivity cell temperature and the column temperature was 35°C; the detector sampling frequency was 5 Hz; and the suppressor current was 60 mA for cation detection.

For anion measurement, a Thermo Fisher Dionex IonPac AS11-HC chromatography column was used, with 30mM sodium hydroxide solution as the mobile phase. The isocratic elution lasted for 15 minutes at a flow rate of 1.0mL/min. The sample injection volume was 10µL, and the conductivity cell temperature was 30°C. The detector's collection frequency was 5Hz, and the suppressor current was 100mA for anion detection.

## Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analysis was conducted using the Thermo Fisher Vanquish ultra-high-performance liquid chromatography (UHPLC) system coupled with the Thermo Fisher Q Exactive™ HF-X mass spectrometer. The stationary phase was C18-phenylsilica gel (Waters Acquity HSS C18, 1.8µm, 2.1×100mm), and mobile phase A consisted of 0.06% trifluoroacetic acid in water, while mobile phase B was 0.055% trifluoroacetic acid in acetonitrile. The gradient elution conditions are outlined in Table 1-3. The column temperature was set to 60°C, with a flow rate of





Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	75	25
35	40	60
37	10	90
49	10	90
49.1	75	25
60	75	25

Table 1-3: LC-MS Gradient Elution Parameters

0.4mL/min and a detection wavelength of 215nm. The sample injection volume was 3µL, and the sample tray temperature was maintained at 5°C.

### Nuclear Magnetic Resonance (NMR)

A higher order structural assessment was made using 2D NMR. 1 mL of the originator injection sample was filtered through an Amicon filtration (3 kDa cutoff). Added water to a total volume of 5 mL, mixed thoroughly, and then centrifuged at 4°C and 7500xg for 30-40 minutes. The ultrafiltration process was repeated twice, the sample was diluted with 600µL deuterium oxide and transferred to a 5 mm NMR sample tube. For API samples without excipients, a simpler approach was used for sample preparation, in which they were diluted in deuterium oxide and transferred to a 5 mm NMR sample tube.

The NMR spectroscopic data were recorded on a Bruker Avance 600 MHz spectrometer equipped with a 5 mm TCI cryoprobe (Bruker, Billerica, MA, United States). Based on signal intensity, the following parameters were used: NS = 32-148, F1 TD = 128, F2 TD = 2048. According to the signal distribution, the spectral width was adjusted as follows: F2: spectral width 1.2 ppm, O1P = 1 ppm; F1 = 26 ppm, O1P = 14 ppm.



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### Dr. Li Jinliang

Dr. Li Jinliang is the Executive Director of Aurisco Pharmaceutical. With a PhD in Chemistry from Nankai University and Post-doc at Tianjin University, he has focused his research on green chemistry, chiral chemistry and oligonucleotides. Dr. Li has broad and diverse experience in both academic and industrial settings. He was Distinguished Professor at Shanghai Institute of Technology, Chairman of China's National R&D and Engineering Center of Anti-HIV Drugs and General Manager at Shanghai Desano Pharma before joining Aurisco. He launched Aurisco Biotech's R&D Center and the generic and biosimilar portfolio expansion through the development of generic peptides such as Semaglutide and Tirzepatide and generic oligonucleotides such as Nusinersen and Inclisiran. He and his teams have more than 50 approved patents.