GLP's: What's the Skinny? A Scientific Perspective

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President and CEO

ARL Bio Pharma

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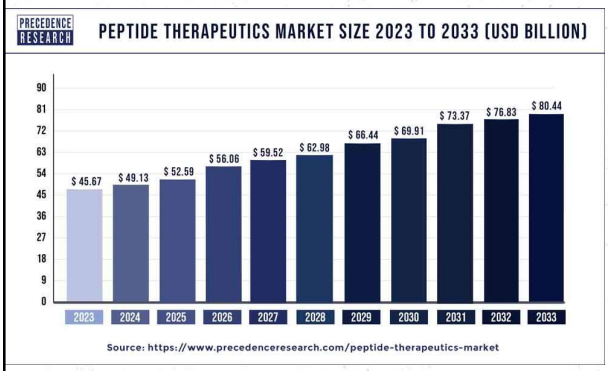
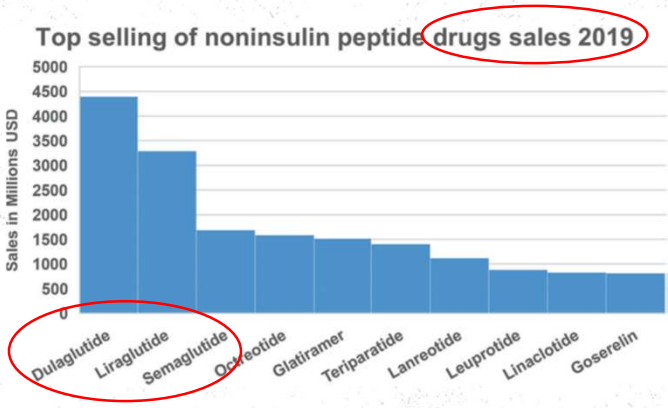
Overview

- Peptides – a simple scientific summary
- Testing of peptides
- Evaluation of peptide's COAs
- Summary and Questions

2

Market and Peptide Drugs





GLP-1

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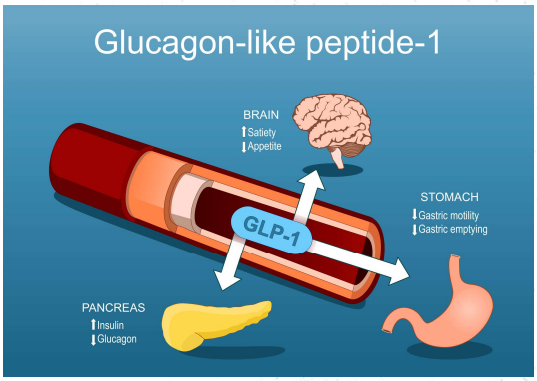
Glucagon-Like Peptide-1 (GLP-1) Peptides

- GLP-1 peptides exhibit significant therapeutic potential and are emerging as promising treatments for various diseases, notably diabetes, obesity, and related conditions.
- Exenatide (Byetta, Bydureon), a **synthetically modified peptide** was the **first GLP-1** receptor agonist **approved by the FDA in 2005**.
- Other approved GLP-1 agonists include: liraglutide (Victoza); dulaglutide (Trulicity); semaglutide (Ozempic, Rybelsus, and Wegovy); lixisenatide (Adlyxin); albiglutide (Tanzeum, Eperzan) and dual **GIP**/GLP-1 agonists tirzepatide (Zepbound and Mounjaro)

Gastric Inhibitory Polypeptide (GIP)



Signal Transduction and Targeted Therapy (2024) 9:234
Endocrinol Diab Metab. 2024;7:e462

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Peptide Definition

- Peptides are short chains of two or more amino acids covalently linked by amide bonds. USP <1503>
- FDA distinguishes proteins from peptides based on size and considers any polymer composed of **less than 40 amino acids to be a peptide**.
- FDA state that a **protein** is any alpha amino acid polymer, with a specific defined sequence that is **greater than 40 amino acids** in size.

https://www.nature.com/scitable/topicpage/protein-structure-14122136/
Acc Chem Res. 2006 Dec;39(12):909-17

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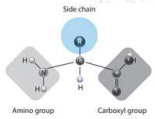
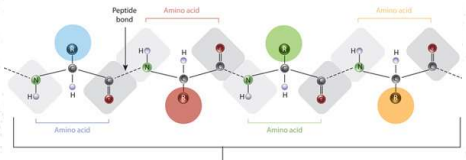
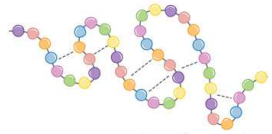
Amino Acids ⟹ Peptides ⟹ Proteins







Amino acids
There are 22 genetically encoded (proteinogenic) amino acids

Peptides
Shorter chains of amino acids, usually between 2 and 40 amino acids.

Proteins
Longer chains amino acids, usually more than 40 amino acids. Proteins processes a range of three-dimensional structures

https://www.nature.com/scitable/topicpage/protein-structure-14122136/
Acc Chem Res. 2006 Dec;39(12):909-17

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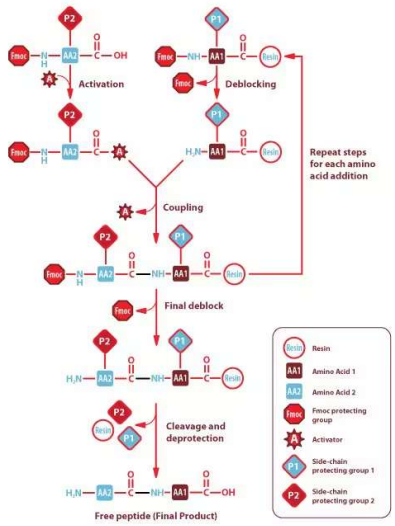
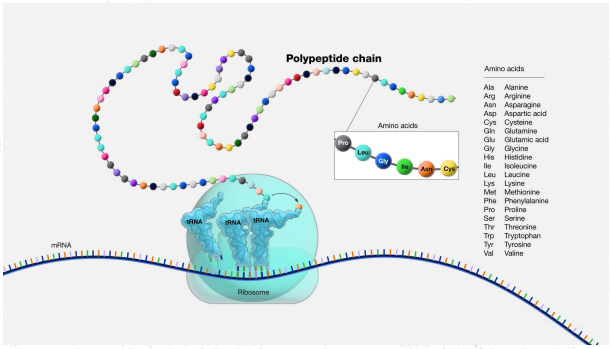
Chemical and Recombinant Peptide Production

Synthetic peptide production



Recombinant DNA peptide production



https://www.genome.gov/genetics-glossary/Peptide
https://www.sigmaaldrich.com/

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Example of Compounded GLP-1 Peptide Drug Products

- Semaglutide injection, tablet, suspension, sublingual and film
- Semaglutide/Methylcobalamin
- Semaglutide/Cyanocobalamin
- Semaglutide/Vitamin B12
- Semaglutide/Vitamin B6
- Semaglutide/Levocarnitine
- Semaglutide/Arginine
- Semaglutide/Carnitine
- Semaglutide/Glycine
- Semaglutide/NAD
- Liraglutide
- Liraglutide/Arginine
- Tirzepatide injection and tablet
- Tirzepatide/Cyanocobalamin
- Tirzepatide/Levocarnitine
- Tirzepatide/Niacinamide
- Tirzepatide/Pyridoxine
- Tirzepatide/Carnitine
- Tirzepatide/P5P
- Tirzepatide/Methylcobalamin
- Tirzepatide/Vitamin B12

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Active Pharmaceutical Ingredient (API) Considerations for Compounding GLP-1 Peptide Drug Products

- USP <797> requirements for Components
  - Must comply with the criteria in the USP–NF monograph, if one exists
  - Must have a **certificate of analysis (COA)** that includes the specifications and that test results for the component show that the API meets expected quality
  - Must be manufactured by an **FDA-registered facility**
- FDA observations related to compounded GLP-1 peptide drug components
  - Conduct at least one test to verify the identity of each component of a drug product
  - Validate supplier's test results at appropriate intervals
  - Salt forms (including semaglutide sodium and semaglutide acetate) should not be used to compound semaglutide

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Example Common Tests for Characterization and Quality Control of the Peptide Drug Substance

Test	Method	Comments
Characteristic		
Appearancea	Visual inspection	—
Identification		
High-performance liquid chromatography (HPLC) coelution with reference standarda	⟨621⟩	The method used for the identification of the active pharmaceutical ingredient (API) may be the same as the one used for the detection of related substances or for determination of the assay based on comparison with a reference standard
Mass spectrometry (MS)	⟨736⟩	Monoisotopic mass
Amino acid analysis (AAA)	⟨1052⟩ and ⟨507⟩	Hydrolysis and derivatization protocols should be specified
Tandem mass spectrometry (MS–MS) sequencing	⟨736⟩	May be complicated for longer sequences
Peptide mapping by chemical or enzymatic cleavage methods	⟨1055⟩	Used for longer sequences (e.g., >20 amino acids); equivalent to MS–MS
....	....	....
Assay		
Assay by HPLC	⟨621⟩	Method is based on a comparison with a reference standard and may be the same method used to measure related substances and for identification
Peptide Content		
Peptide content by AAA	⟨1052⟩ and ⟨507⟩	Hydrolysis protocols must be validated; only well-recovered amino acids should be included in the calculation of mean peptide content
....	....	....
Impurities		
Peptide-related substancesa	⟨621⟩, LC–MS	Method specific for drug substances; must be validated for both process-related impurities and degradation products; limits for total and individual impurities should be specified, LC–MS is a commonly used method for characterization
Residual solventsa	⟨467⟩	If justified, may be limited to solvent used in the final steps of the manufacturing process
Elemental impuritiesa	⟨232⟩,⟨233⟩, and ⟨1065⟩	Required if metal catalysts are used in the manufacturing process; elemental impurities may be required based on the risk assessment
Residual trifluoroacetic acida	⟨503.1⟩	Required if TFA is used during the manufacturing process
....	....	....
Specific Tests		
Counter-ion contenta	For acetate ⟨503⟩, for others ⟨1065⟩	Titration with silver nitrate may be used to determine chloride
Water contenta	⟨921⟩	⟨921⟩, Method I, Method Ic (Coulometric Titration)v preferred
Bacterial endotoxinsa	⟨85⟩	Required for the drug substances used in the manufacture of parenteral drug products
....	....	....

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USP ⟨1503⟩ Quality Attributes of Synthetic Peptide Drug Substances (Raw Material)

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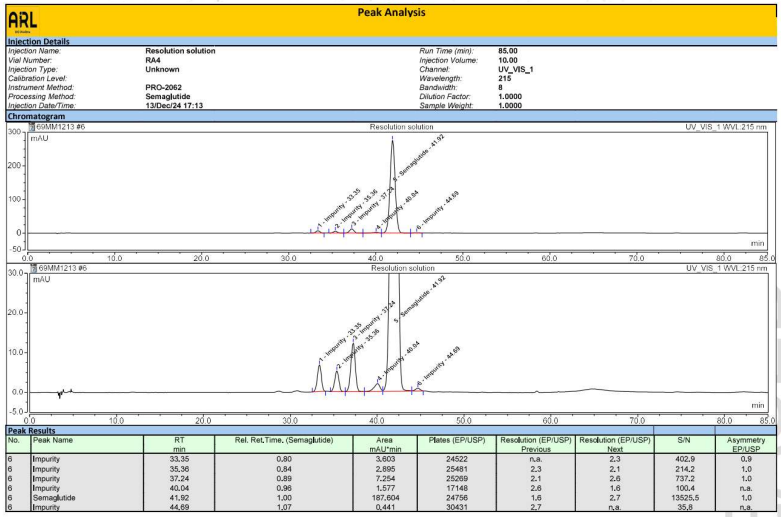
Example of GLP-1 API Retesting Methods for Compounder

Semaglutide Impurity Method Example



Peak Analysis

Injection Details

Injection Name:	Resolution solution	Run Time (min):	85.00
Vial Number:	R44	Injection Volume:	10.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Calibration Level:		Wavelength:	215
Instrument Method:	PRO-2062	Bandwidth:	8
Processing Method:	Semaglutide	Dilution Factor:	1.0000
Injection Date/Time:	13/Dec/24 17:13	Sample Weight:	1.0000

Chromatogram

69MM1213 #6 — Resolution solution — UV_VIS_1 WVL:215 nm

mAU

1 - Impurity - 33.35
2 - Impurity - 35.36
3 - Impurity - 37.24
4 - Impurity - 40.04
5 - Semaglutide - 41.92
6 - Impurity - 44.69

min

Peak Results

No.	Peak Name	RT min	Rel. Ret.Time, (Semaglutide)	Area mAU*min	Plates (EP/USP)	Resolution (EP/USP) Previous	Resolution (EP/USP) Next	S/N	Asymmetry EP/USP
6	Impurity	33,35	0,80	3,603	24522	n.a.	2,3	402,9	0,9
6	Impurity	35,36	0,84	2,895	25481	2,3	2,1	214,2	1,0
6	Impurity	37,24	0,89	7,254	25269	2,1	2,6	737,2	1,0
6	Impurity	40,04	0,96	1,577	17148	2,6	1,6	100,4	n.a.
6	Semaglutide	41,92	1,00	187,604	24756	1,6	2,7	13525,5	1,0
6	Impurity	44,69	1,07	0,441	30431	2,7	n.a.	35,8	n.a.

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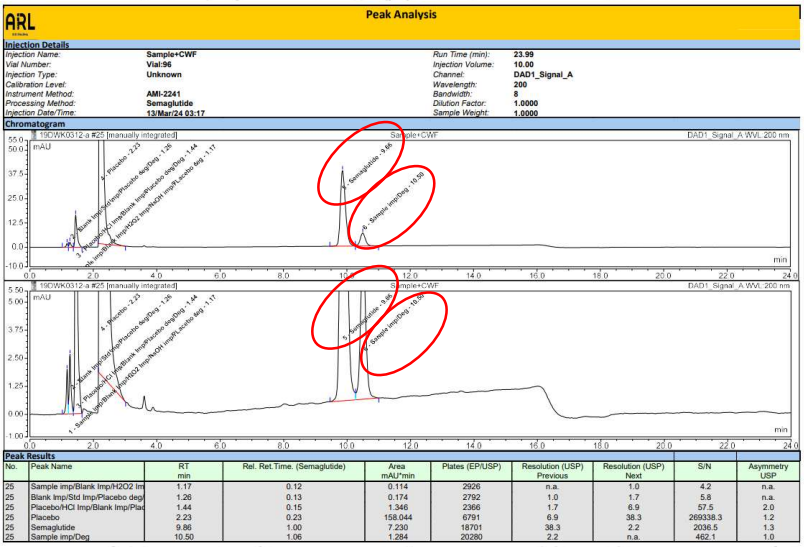
Example of Stability Indicating HPLC Method Development for Semaglutide



Peak Analysis

Injection Details

Injection Name:	Sample+CWF	Run Time (min):	23.99
Vial Number:	Vial:96	Injection Volume:	10.00
Injection Type:	Unknown	Channel:	DAD1_Signal_A
Calibration Level:		Wavelength:	200
Instrument Method:	AMI-2241	Bandwidth:	8
Processing Method:	Semaglutide	Dilution Factor:	1.0000
Injection Date/Time:	13/Mar/24 03:17	Sample Weight:	1.0000

Chromatogram

19CWK0312-a #25 [manually integrated] — Sample+CWF — DAD1_Signal_A WVL:200 nm

mAU

4 - Placebo - 2.23
5 - Semaglutide - 9.86
6 - Sample imp/Deg - 10.50

min

Peak Results

No.	Peak Name	RT min	Rel. Ret.Time. (Semaglutide)	Area mAU*min	Plates (EP/USP)	Resolution (USP) Previous	Resolution (USP) Next	S/N	Asymmetry USP
25	Sample imp/Blank Imp/H2O2 Im	1.17	0.12	0.114	2926	n.a.	1.0	4.2	n.a.
25	Blank Imp/Std Imp/Placebo deg	1.26	0.13	0.174	2792	1.0	1.7	5.8	n.a.
25	Placebo/HCl Imp/Blank Imp/Plac	1.44	0.15	1.346	2366	1.7	6.9	57.5	2.0
25	Placebo	2.23	0.23	158.044	6791	6.9	38.3	269338.3	1.2
25	Semaglutide	9.86	1.00	7.230	18701	38.3	2.2	2036.5	1.3
25	Sample imp/Deg	10.50	1.06	1.284	20280	2.2	n.a.	462.1	1.0

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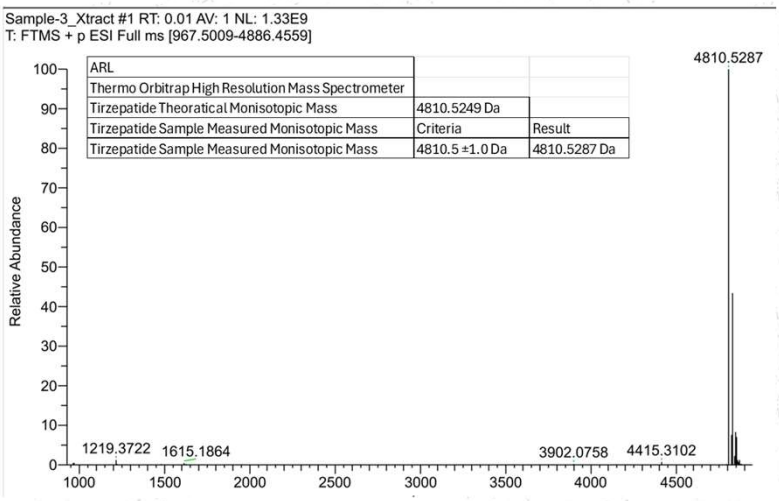
Example of GLP-1 API Retesting Methods for Compounder

Tirzepatide Molecular Weight Method Example



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Example 1 – Semaglutide CoA API

Test Items		Specifications	Results	Method
Appearance		White to off-white powder	White to off-white powder (Conforms)	BPT-QC-SOP-2098 V03
Identification	Molecular Weight (MS)	4113.58±1.0 Da	4114.00 Da	BPT-QC-SOP-2098 V03
	Retention Time (HPLC)	The retention time of the major peak of the sample solution corresponds to that of the standard solution.	Conforms	BPT-QC-SOP-2098 V03
Assay	Purity (HPLC)	≥98.0%	99.8%	BPT-QC-SOP-2098 V03
	Related Substances (HPLC)	Total Impurities(%)≤2.0% Largest Single Impurity(%)≤1.0%	0.2% 0.1%	BPT-QC-SOP-2098 V03
	Peptide Content (HPLC)	≥85.0%	94.1%	BPT-QC-SOP-2098 V03
Specific Tests	Water Content (Karl Fischer)	≤8.0%	4.0%	BPT-QC-SOP-2098 V03; USP<921>
	Residual Solvent (GC; HPLC)	Acetonitrile≤0.041% Trifluoroacetic≤0.500% Acetic Acid≤0.100%	<0.004% <0.05% 0.065%	BPT-QC-SOP-2098 V03
	Bacterial Endotoxins (Chromogenic Technique)	<10 EU/mg	<1 EU/mg	BPT-QC-SOP-2098 V03; USP<85>

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Example 2 – Semaglutide CoA API

Test items		Specifications	Results
Appearance		White to almost white powder or loose lump	Almost white powder
Solubility		Freely soluble in water	Complies
Molecular weight identification		Molecular weight should be 4113.6±1.0Da	4113.8Da
HPLC identification		Examine the chromatograms obtained in the assay. The retention time of main peak obtained in sample solution should be in accordance with retention time of main peak obtained in standard solution.	Complies
pH		7.0~9.0（C=5mg/ml）	7.7
Clarity and color of solution		Colorless and clear liquid (C=10mg/ml)	Complies
Water content		≤10.0%	4.8%
Amino acid analysis	Asp	0.9-1.1	1.0
	Ser	2.1-3.9	2.6
	Glu	4.5-5.5	5.0
	Gly	3.6-4.4	4.0
	His	0.9-1.1	1.1
	Arg	1.8-2.2	2.1
	Thr	1.8-2.2	2.1
	Ala	2.7-3.3	3.0
	Aib	0.7-1.3	1.1
	Tyr	0.9-1.1	0.9
	Val	1.8-2.2	2.1
	Lys	0.9-1.1	1.0
	Ile	0.9-1.1	1.0
	Leu	1.8-2.2	2.0
	Phe	1.8-2.2	2.0
	Trp	Present	Present

Test items		Specifications	Results
substances Ⅰ	G06-IM01	≤0.2%	0.08%
	G06-IM59	≤0.2%	0.13%
	G06-IM28	≤0.2%	0.13%
	Any other individual impurity	≤0.1%	0.10%
	Total impurities	≤2.0%	0.78%
Related substances Ⅱ	G06-IM03	≤0.2%	0.05%
Residual solvents	Acetonitrile	≤410ppm	N.D
	Triethylamine	≤5000ppm	N.D
	N,N'-Diisopropylcarbodiimide	≤100ppm	N.D
Polymer		≤0.5%	0.09%
Bacterial endotoxins		<10EU/mg	<10EU/mg
Microbial limit	Total aerobic microbial count	$\leq 10^2$cfu/g	45cfu/g
	Total yeast and mold count	$\leq 10^2$cfu/g	<10cfu/g
	Escherichia coli	Absent	Present
Peptide content		≥80.0%	94.7%
Assay		95.0%~105.0% (on anhydrous and salt-free substance basis)	101.7%
Sodium ion		1.0%~3.0%	2.1%
Carbonate		≤0.50%	0.12%
Acetic acid		≤0.5%	0.06%
Trifluoroacetic acid		≤0.25%	N.D
Related substances	G06-IM60	≤0.2%	0.11%

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Example 3 – Semaglutide CoA API

Test Items	Acceptance Criteria	Test Results
Appearance	White or off-white loose powder, hygroscopic or very hygroscopic	White loose powder, hygroscopic
Solubility	Freely soluble in water, insoluble in acetonitrile	Freely soluble in water, insoluble in acetonitrile
Identification	The monoisotopic mass should be 4111.12 ±0.5Da	4111.16
	The retention time of major peak in the chromatogram obtained from sample solution should correspond to that obtained from reference solution, as obtained in the Assay (By HPLC)	The retention time of major peak in the chromatogram obtained from sample solution should correspond to that obtained from reference solution, as obtained in the Assay.
Specific rotation	-6.0° to-16.0°，calculated on anhydrous acid radical, ammonium ion and sodium ion-free basis	-11.4°
Water	NMT 10.0%	5.0%
pH	6.0~9.0(1 mg/mL in water)	7.9
Clarity and color of solution	The solution should be clear and colorless. If it is turbid, its opalescence should be not more pronounced than that of reference suspension No. 1; if it is colored, it should be not more intensely colored than that of reference solution No. 1	Clear and colorless
Anion	Chloride ion: NMT 0.5%	0.028%
	Trifluoroacetic acid: NMT 0.5%	Not Detected
	Phosphate ion: NMT 0.5%	Not Detected
	Sulfate ion: NMT 0.5%	Not Detected
Cation	Sodium ion: NMT 4.0%	2.2%
	Ammonium ion: NMT 0.5%	Not Detected

Test Items	Acceptance Criteria	Test Results
Bacterial endotoxins	Less than 2EU/mg	Less than 2EU/mg
Microbiological examination	TAMC: NMT 100cfu/g	Less than 10cfu/g
	TYMC: NMT 50cfu/g	Less than 10cfu/g
	Escherichia coli: absent	Absent
Peptide content	NLT 80.0%	90.9%
Assay	95.0%~105.0% (calculated on anhydrous, acid radical, Ammonium ion and sodium ion-free basis)	99.9%
Related substances(I)	SPC054-Z18: NMT 0.50%	0.02%
	SPC054-Z20: NMT 0.30%	0.06%
	SPC054-Z21: NMT 0.50%	Not Detected
	SPC054-Z26 & SPC054-Z76: NMT 0.20%	0.02%
	SPC054-Z60 & SPC054-Z28: NMT 0.50%	0.01%
	SPC054-Z57: NMT 0.20%	Not Detected
	Any other impurity:NMT 0.10%	0.09%
	Total impurities: NMT 1.0%	0.22%
Related substances(II)	SPC054-Z19: NMT 0.30%	0.03%
	SPC054-Z23: NMT 0.20%	0.02%
	SPC054-Z69: NMT 0.20%	Not Detected
	Total impurities of related substances I and related substances II: NMT 1.5%	0.26%
Residual solvents	Dichloromethane: NMT 600ppm	Below LOQ (200ppm)
	Pyridine: NMT 200ppm	Below LOQ (100ppm)
	N,N-dimethylformamide: NMT 880ppm	Below LOQ (440ppm)
	Triisopropylsilane: NMT 1000ppm	Below LOQ (25ppm)
	1,2-Ethanedithiol: NMT 1000ppm	Below LOQ (220ppm)
	N,N-diisopropylethylamine:NMT 1000ppm	Below LOQ (120ppm)

Test Items	Acceptance Criteria	Test Results
Amino acid ratio	Leu: 1.6~2.4	2.1
	Val: 1.6~2.4	2.1
	Arg: 1.6~2.4	1.9
	Thr: 1.6~2.4	1.9
	Phe: 1.6~2.4	2.0
	AEEA: 1.6~2.4	2.1
	Ser: 2.4~3.6	2.8
	Ala: 2.4~3.6	3.1
	Gly: 3.2~4.8	3.9
	Glu: 4.0~6.0	5.1
	Asp: 0.8~1.2	1.0
	His: 0.8~1.2	1.0
	Tyr: 0.8~1.2	1.0
	Lys: 0.8~1.2	1.0
	Ile: 0.8~1.2	0.9
	Aib: 0.8~1.2	0.9

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Common Question Regarding Peptide API CoA

Assay purity vs. Peptide content

- Different manufacturers recommend one over another. But both are purity, just from different test methods.
- After proper calculation, they should be close.
  - *Purity based on Assay =*

  99.8% X (1-4%-0.065%)= 95.7%

  in comparison to peptide content 94.1%
- If not specified on CoA, Compounders should get clarification from the manufacturer whether the **assay purity is on anhydrous and salt-free basis**
- The water content could change during storage. If happens, compounder can retest water content or peptide content then update purity factor for compounding.

Semaglutide CoA Example 1

Test Items		Specifications	Results	Method
Appearance		White to off-white powder	White to off-white powder (Conforms)	BPT-QC-SOP-2098 V03
Identification	Molecular Weight (MS)	4113.58±1.0 Da	4114.00 Da	BPT-QC-SOP-2098 V03
	Retention Time (HPLC)	The retention time of the major peak of the sample solution corresponds to that of the standard solution.	Conforms	BPT-QC-SOP-2098 V03
Assay	Purity (HPLC)	≥98.0%	99.8%	BPT-QC-SOP-2098 V03
	Related Substances (HPLC)	Total Impurities(%)≤2.0% Largest Single Impurity(%)≤1.0%	0.2% 0.1%	BPT-QC-SOP-2098 V03
	Peptide Content (HPLC)	≥85.0%	94.1%	BPT-QC-SOP-2098 V03
Specific Tests	Water Content (Karl Fischer)	≤8.0%	4.0%	BPT-QC-SOP-2098 V03; USP<921>
	Residual Solvent (GC; HPLC)	Acetonitrile≤0.041% Trifluoroacetic≤0.500% Acetic Acid≤0.100%	<0.004% <0.05% 0.065%	BPT-QC-SOP-2098 V03
	Bacterial Endotoxins (Chromogenic Technique)	<10 EU/mg	<1 EU/mg	BPT-QC-SOP-2098 V03; USP<85>

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Example of Compounder Retesting Semaglutide API

ARL BIO PHARMA

Certificate of Analysis

CLIENT : [redacted]
DESCRIPTION : Semaglutide
LOT # : [redacted]
ARL # :
FORMULATION ID :
DATE RECEIVED : 10/25/2024
STORAGE : -25°C to -10°C

Test	Method	Specifications	Results	Date Tested
Endotoxin	USP <85>	< 2 EU / mg	<0.2 EU / mg	10/30/2024
Total Aerobic Microbial Count	USP <61>	NMT 1000 CFU/g	<10 CFU/g	11/01/2024
Total Yeast and Mold Count	USP <61>	NMT 100 CFU/g	<10 CFU/g	11/01/2024
Test for Escherichia coli	USP <62>	Conforms to USP Specifications	Conforms	11/06/2024
Hygroscopicity	AMIT-2657	Report Value	Very Hygroscopic	12/06/2024
Anion (Chloride ion)	IC	NMT 0.5%	Not Detected	12/13/2024
Anion (Phosphate ion)	IC	NMT 0.5%	Not Detected	12/13/2024
Anion (Sulfate Ion)	IC	NMT 0.5%	Not Detected	12/13/2024
Anion (Trifluoroacetic Acid)	IC	NMT 0.5%	Not Detected	12/13/2024
Cation (Ammonium Ion)	IC	NMT 0.5%	0.01%	11/14/2024
Cation (Sodium Ion)	IC	NMT 4.0%	2.1%	11/14/2024
Residual Solvent - Piperidine	IC	NMT 100ppm	Not Detected	11/15/2024
Oligomer	HPLC	NMT 1.0%	NMT 1.0%	12/03/2024
Related Substances I: Total Impurities Z18 Z20 Z21 Z57 Sum of Z76 and Z26 Sum of Z60 and Z28 Unspecified Impurity 1 Unspecified Impurity 2 Unspecified Impurity 3 Unspecified Impurity 4	HPLC	 NMT 1.0% NMT 0.50% NMT 0.30% NMT 0.50% NMT 0.20% NMT 0.20% NMT 0.50% NMT 0.10% NMT 0.10% NMT 0.10% NMT 0.10%	 0.326% 0.053% 0.088% 0.053% Not Detected 0.048% 0.032% 0.013% 0.019% 0.021% 0.022%	12/05/2024
Related Substances II: Z19 Z23 Z69 Sum of Related Substance 1 and Related Substance 2 Impurity	HPLC	 NMT 0.30% NMT 0.20% NMT 0.20% NMT 1.5%	 Not Detected 0.028% Not Detected 0.548%	12/12/2024
Assay - Semaglutide	HPLC	95.0% - 105.0%	100.2%	01/18/2024

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Release Inspections and Testing for Compounded Sterile Injection Products

- USP <797> describes the minimum standards to be followed for the preparation of compounded sterile preparations (CSPs) for human and animal drugs.
- Release testing procedures must be included in the facility's quality assurance (QA) and quality assurance (QC) program.
  - Visual Inspection
  - Sterility Testing
  - Bacterial Endotoxins Testing
- No USP official monograph for peptide drug product yet

https://online.uspnf.com/uspnf/document/1_GUID-A4CAAA8B-6F02-4AB8-8628-09E102CBD703_8_en-US?source=Search%20Results&highlight=797

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Batch Release Quality Control Testing Example for Compounded GLP-1 Peptide Drug Products

ARL BIO PHARMA

Certificate of Analysis

CLIENT : [redacted]
DESCRIPTION : Semaglutide 1mg/mL Sterile Injection
LOT # : [redacted]
ARL # : [redacted]
FORMULATION ID : [redacted]

DATE RECEIVED : 12/05/2024
STORAGE : 2°C to 8°C

Test	Method	Specifications	Results	Date Tested
Rapid Sterility	ATP Bioluminescence / MBI-199	Sterile	Sterile	12/05/2024
Endotoxin	USP <85>	NMT 300 EU / mL	<20 EU / mL	12/10/2024
Particulate Matter: ≥10µm ≥25µm	USP <788> Method I	 ≤6000 particles/cont ≤600 particles/cont	 517 particles/cont 7 particles/cont	12/09/2024
Appearance	AMI-738	Colorless liquid without visible particulates	Conforms	12/09/2024
pH	USP <791>	7.0 - 9.0	7.6	12/09/2024
Assay - Semaglutide	HPLC / AMI-2241	90.0% - 110.0%	99.3% (0.9929mg / 1mL)	12/06/2024

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Summary and Questions?

Summary of Peptides, definition, structure, production (synthetic and rDNA).

GLP-1 therapeutic and market potential.

Testing

Raw Material (API) and Drug Product

Certificate of Analysis Evaluation

Raw Material (API) and Drug Product

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Contact Information

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Back up slides

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Establishing Beyond-Use Dates (BUD) for Compounded Sterile Injection Products

Table 3. Stability Tests Required for CSPs

Test	⟨797⟩ Requirements
Antimicrobial Effectiveness Test ⟨51⟩	Required: All aqueous multidose CSPs
Container–Closure Integrity ⟨1207⟩	Required: All multidose CSPs
Assay (potency or strength)	Required: Only Category 3 CSPs
Particulate Testing ⟨788⟩/⟨789⟩	Required: Only Category 3 Injectable and Intraocular CSPs
Appearance (e.g., color, clarity, and visible particulates)	Recommended, but not specifically required in the chapter
Sterility Tests ⟨71⟩	Recommended, but not specifically required in the chapter
Bacterial Endotoxins Test ⟨85⟩	Recommended, but not specifically required in the chapter
pH ⟨791⟩	Recommended, but not specifically required in the chapter
Impurities [related substances (e.g., degradants)]	Recommended, but not specifically required in the chapter
Preservative content	Recommended, but not specifically required in the chapter

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https://online.uspnf.com/uspnf/document/2_GUID-3B136D49-D7C0-49CF-B3CD-75BAD5B41BA9_10101_en-US?source=Search%20Results&highlight=797

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Establishing Beyond-Use Dates (BUD) for Compounded Sterile Injection Products

- USP <797> defines BUD as the date, or hour and date, after which a CSP must not be used. The BUD is determined from the date and time that preparation of the CSP is initiated.
- USP <797> provides guidance BUD for different Category CSPs.
- ⟨795⟩ and ⟨797⟩ establish minimum standards for stability studies and formulation testing for CNSPs and compounded sterile preparations (CSPs).
- USP <1225> requires analytical methods for stability study "*must be appropriately validated to ensure that quantitation of the API is reproducible, and no interference occurs from excipients, degradants, or impurities. Forced degradation studies, also referred to as specificity, allow for the unequivocal assessment of the analyte in the presence of components which may be expected to be present (e.g., impurities, degradants, matrix components, etc.)*".

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https://online.uspnf.com/uspnf/document/1_GUID-A4CAAA8B-6F02-4AB8-8628-09E102CBD703_8_en-US?source=Search%20Results&highlight=797

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Example of Stability Study and Beyond-Use Dates (BUD) Determination for Compounded GLP-1 Peptide Drug Products

Stability Data for Semaglutide in Ambient Conditions [redacted]

Controlled Ambient Conditions (25°C ± 2°C and 60% RH ± 5%)

Attribute	Specification (Description)	Initial (To D)	T30 D	T60 D	T90 D	T120 D	T150 D	T180 D
Appearance	Colorless liquid without visible particulates	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
pH	Report Value	7.8	7.8	7.9	8.0	8.1	8.1	8.1
Endotoxin	[redacted]	<20 EU/mL	<20 EU/mL	<20 EU/mL	<20 EU/mL	<20 EU/mL	<21 EU/mL	<20 EU/mL
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
Antimicrobial Effectiveness	Conforms to USP Specifications	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Particulate Matter - Method I	≥ 10 µm; ≤ 6000 Particles/Container	65	104	129	110	112	134	134
	≥ 25 µm; ≤ 600 Particles/Container	1	4	1	5	2	3	3
Container Closure	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Benzyl Alcohol Assay	% of Label [redacted]	105.1%	93.2%	97.9%	97.3%	98.6%	100.0%	99.1%
Semaglutide Assay	% of Label [redacted]	99.8%	96.7%	97.8%	96.7%	92.5%	88.4%	86.9%

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Overview

- Introduction to Peptides and Glucagon-Like Peptide-1 (GLP-1) Therapeutic Peptides
- Batch Release and Quality Control of Compounded GLP-1 Peptide Drug Products
- Active Pharmaceutical Ingredient (API) Considerations for Compounding GLP-1 Peptide Drug Products
- Stability Studies and Beyond-Use Date (BUD) Determination for Compounded GLP-1 Peptide Drug Products
- Summary

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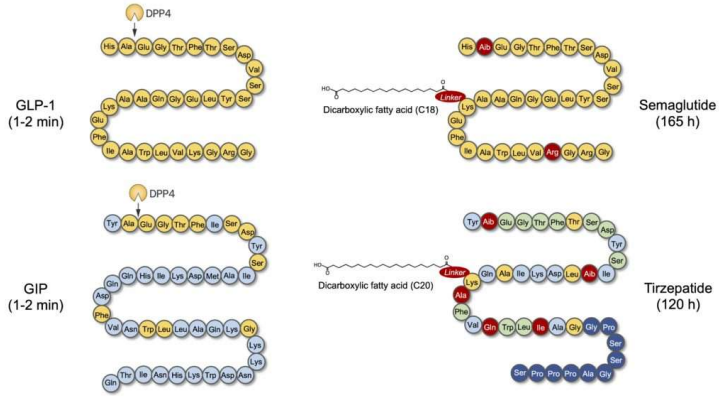
Structure of Semaglutide and Tirzepatide



GLP-1 is a 31 amino acid hormone. Gastric Inhibitory Polypeptide (GIP) is a 42 amino acid hormone. Both hormones have a very short half-life (the time corresponding to a loss of half of their physiological activity), of only 1-2 minutes, in contrast to the long half life of their structurally modified agonists.

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https://observatoireprevention.org/en/2023/03/02/ozempic-semaglutide-and-mounjaro-tirzepatide-a-major-breakthrough-for-the-treatment-of-obesity/

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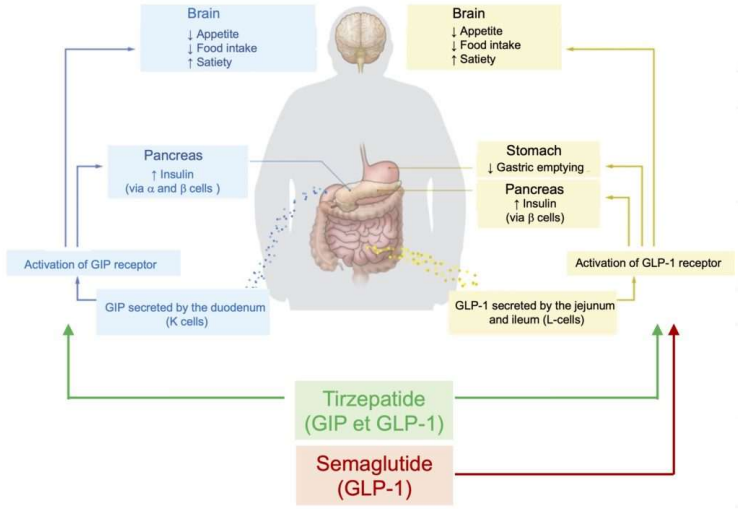
Mechanism Of Action of Semaglutide and Tirzepatide



Mechanisms of action of GIP, GLP-1 and their agonists. Adapted from Bass et al. J Clin Invest. 2023 Feb 1;133(3):e167952. doi: 10.1172/JCI167952. PMID: 36719381
https://observatoireprevention.org/en/2023/03/02/ozempic-semaglutide-and-mounjaro-tirzepatide-a-major-breakthrough-for-the-treatment-of-obesity/

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Slide Heading

- Explore the latest scientific insights into GLP-1 peptides in this session, covering essential topics such as:
  - Peptide composition,
  - Rigorous testing protocols, and
  - The evaluation of Certificates of Analysis (COAs) to ensure quality.
- Join us for an in-depth look at these transformative compounds and their role in modern therapeutic practices.

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Active Pharmaceutical Ingredient (API) Considerations for Compounding GLP-1 Peptide Drug Products

- 503A may only use bulk drug substances in compounding drug products that:
  - Comply with an applicable United States Pharmacopeia (USP) or National Formulary (NF) monograph if one exists;
  - **Are components of FDA-approved drug products if an applicable USP or NF monograph does not exist; or**
  - Appear on FDA's list of bulk drug substances that can be used in compounding (the 503A bulks list) if such a monograph does not exist and the substance is not a component of an FDA-approved drug product
- 503B may only use bulk drug substances in compounding that:
  - **Are used to compound drug products that appear on FDA's drug shortages list at the time of compounding, distribution, and dispensing; or**
  - Appear on FDA's list of bulk drug substances for which there is a clinical need (the 503B bulks list).

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https://www.fda.gov/drugs/human-drug-compounding/bulk-drug-substances-used-compounding

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Therapeutic peptides: current applications and future directions

SYNTHESIS AND MODIFICATION OF THERAPEUTIC PEPTIDES

1. Peptide Synthesis:

- Chemical synthesis (e.g., SPPS technology, Fmoc/Boc strategies)
- Recombinant technology for long or complex peptides

2. Peptide Modification:

- Backbone and side-chain modification for stability and activity
- Secondary structure stabilization (e.g., cyclization, α-helices, β-sheets)

3. Advanced Techniques:

- Genetic code expansion to incorporate non-canonical amino acids
- PEGylation for improved pharmacokinetics
- Covalent peptide/protein drugs for enhanced efficacy

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Wang L et al. Therapeutic peptides: current applications and future directions. Signal Transduct Target Ther. 2022 Feb 14;7(1):48. PMID: 35165272.

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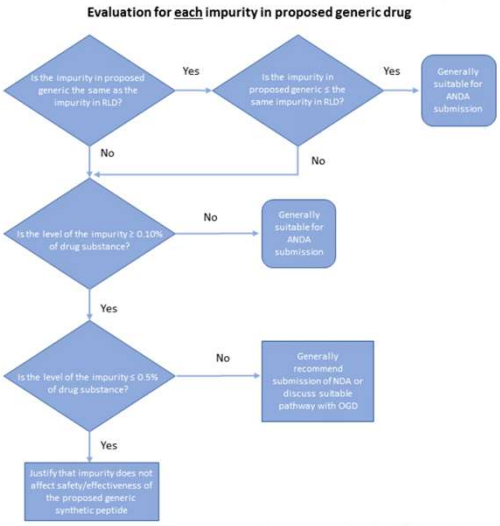
Scientific Considerations for ANDAs for Proposed Generic Synthetic Peptides

A. Active Ingredient Sameness

- Primary sequence and physicochemical properties
- Secondary structure
- Oligomer/aggregation states
- Biological activities

B. Impurities

- Peptide-related impurities
- Host cell-related impurities
- Other (non-peptide-related) impurities



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ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin, FDA-2017-D-5767, May 19, 2021

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Example of Stability Indicating HPLC Method Development for Semaglutide

Stress Condition	Injection #	RT (min)	Area of API Peak (mAU*min)	Average Area (mAU*min)	% Of Control	Additional Peaks not found in Standard, Control, Placebo or Blanks (RRT)*	Stress Condition and Time
Control	1	9.87	8.639	8.550	N/A	1.07	NA
	2	9.87	8.460		N/A	-	
HCl	1	9.84	8.447	8.435	99%	0.13,0.14	1N HCl overnight
	2	9.84	8.423			0.13,0.14	
H2O2	1	9.88	7.971	8.010	94%	0.14,0.81,0.86	30% H2O2 -1 hr
	2	9.88	8.049			0.14,0.82,0.86	
CWF	1	9.88	7.258	7.244	85%	0.13,1.06	4 hours
	2	9.86	7.230			0.13,1.06	
HH	1	9.85	7.930	7.949	93%	0.82,0.86	1 hour
	2	9.82	7.967			0.82,0.86	
NaOH	1	9.88	7.486	7.486	88%	0.81,0.86	1 N NaOH 30 min
	2	9.88	7.486			0.82,0.86	
UV	1	9.82	7.497	7.500	88%	-	10 min
	2	9.83	7.502			-	

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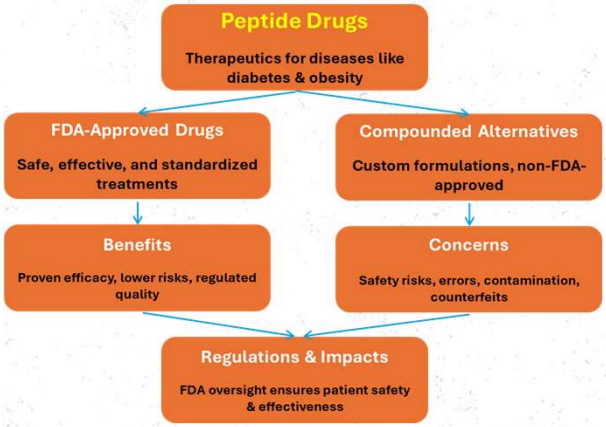
Criticizes of Compounded Peptide Drugs

1. FDA-Approved Drugs:
- Represent standardized treatments that ensure safety, efficacy, and quality.
- Highlighted benefits include proven outcomes, regulated quality, and reduced risks.

2. Compounded Alternatives:
- Custom formulations created for specific needs but not FDA-approved.
- Key concerns include safety risks, dosing errors, contamination, and counterfeiting.



Neumiller JJ et al. Compounded GLP-1 and Dual GIP/GLP-1 Receptor Agonists: A Statement from the American Diabetes Association. Diabetes Care. 2025 Feb 1;48(2):177-181. doi: 10.2337/dci24-0091. PMID: 39620926.

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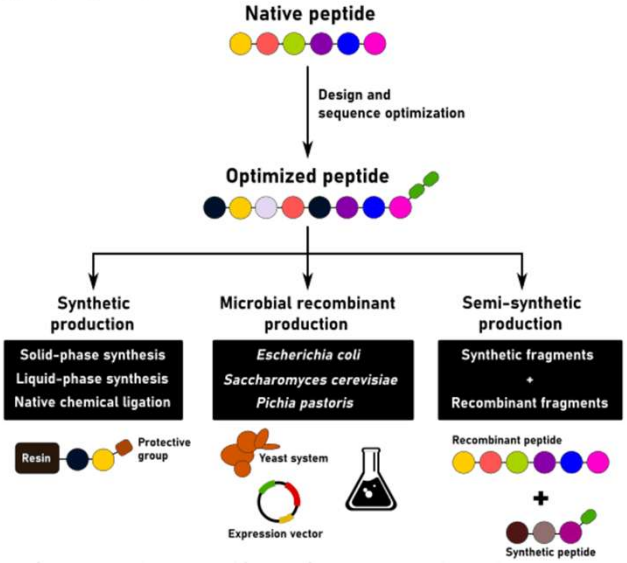
Chemical and Recombinant Peptide Production



https://www.proteogenix.science/scientific-corner/peptide-synthesis/challenges-in-chemical-and-recombinant-peptide-production-processes/

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