

Certificate Of Analysis



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Sample Identification

Sample Name Retatrutide 10 mg **Batch Number** 2025264 **Date Published** 2025-08-01 08:42

Results for Lyo-0060

Analysis of Peptide Identity, Content and Purity	Result	Unit	Uncertainty	Reporting Limit
Retatrutide Assay Peptide Screening	9.68	mg	[± 0.05]	
Retatrutide Identification by RT Peptide Screening	1.00		[± 0]	
Retatrutide Identification by Spectrum Peptide Screening	994		[± 20]	
Retatrutide Purity Peptide Screening	> 99.8	%		

Bioburden	Result	Unit	Uncertainty	Reporting Limit
Total Aerobic Microbial Count USP <61> Plate Count Method	Not detected	CFU/g		>= 1000 △
Total Yeast and Mold Count USP <61> Plate Count Method	Not detected	CFU/g		>= 100 △

Endotoxin Analysis	Result	Unit	Uncertainty	Reporting Limit
Bacterial Endotoxin USP<85> Bacterial Endotoxin Chromogenic Test	< 0.001	EU/mg		> 0.5 △

Heavy Metals	Result	Unit	Uncertainty	Reporting Limit
Arsenic Elemental Impurities Screening	Not detected	ppm		>= 1.5 △
Cadmium Elemental Impurities Screening	Not detected	ppm		>= 0.5 △
Cobalt Elemental Impurities Screening	Not detected	ppm		>= 25 △

Heavy Metals	Result	Unit	Uncertainty	Reporting Limit	
Lead Elemental Impurities Screening	Not detected	ppm		>= 1.5	△
Nickel Elemental Impurities Screening	Not detected	ppm		>= 25	△
Quicksilver Elemental Impurities Screening	Not detected	ppm		>= 1.5	△
Vanadium Elemental Impurities Screening	Not detected	ppm		>= 25	△

Attachments for Lyo-0060

	Method Specification		
Determination of identity, content and purity of Retatrutide			
Document number <i>RETA_001_2025</i>	Sponsoring document <i>-</i>	Number of pages <i>3</i>	

1. Content Assessment

1.1. Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu SCL-10ADvp	C21014112659
Degassing Unit	Shimadzu DGU-14A	NA
LPGE valve	Shimadzu FCV-10Avp	NA
Pump	Shimadzu LC-10ADvp	C20964130094
Autosampler	Shimadzu SIL-10ADvp	C21054109114
Column Thermostat	Shimadzu CTO-10ACvp	C21033770144
Detector	Shimadzu SPD-10ADvp	C20994233588

1.2. Chromatographic conditions

Chromatographic conditions	
Eluent A	1% TFA in Water (HPLC, Gradient Grade)
Eluent B	1% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	1mL/min
Program	Gradient elution
Injection volume	0.5 µL
Column Temperature	35°C
Column	Aventor ACE UltraCore C18, 150x3,0 mm, 3,5µm
Detection wavelength	280nm

Gradient Program		
Time (min)	A [%]	B [%]
2	99	1
10	70	30
14	70	30
32	5	95
35	5	95
35.01	99	1
40	end	

1

Attachment for Lyo-0060

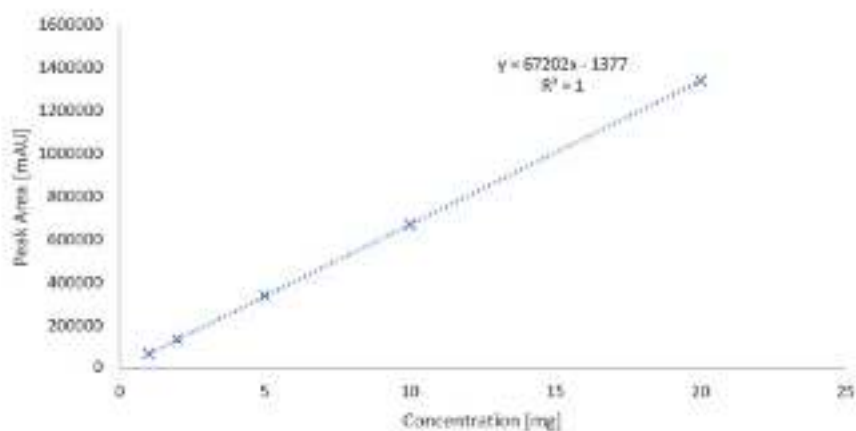
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1.3. Sample preparation

Whole amount of container was dissolved in 2mL of water (HPLC, Gradient Grade). Aliquote part of 1 mL was dispensed into HPLC vial for analysis.

1.4. Calibration curve

Calibration curve detail	
Quantitative method	External Standard
Calibration Type	Linear
Number of calibration points	5
Force through Zero	Disabled
Weighting Method	None



2. Purity assessment

2.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu SCL-10ADvp	C21014112658
Degassing Unit	Shimadzu DGU-14A	NA
LPGE valve	Shimadzu FCV-10Avp	NA
Pump	Shimadzu LC-10ADvp	C20964130094
Autosampler	Shimadzu SIL-10ADvp	C21054109114
Column Thermostat	Shimadzu CTO-10ACvp	C21033770144
Detector	Shimadzu SPD-10ADvp	C20994233588

2.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	1% TFA in Water (HPLC, Gradient Grade)
Eluent B	1% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	1mL/min
Program	Gradient elution
Injection volume	0.5 µL
Column Temperature	35°C
Column	Avantor ACE UltraCore C18, 150x3.0 mm, 3.5µm
Detection wavelength	214nm

Gradient Program		
Time (min)	A (%)	B (%)
2	99	1
10	70	30
14	70	30
32	5	95
35	5	95
35.01	99	1
40	end	

1.5. Sample preparation

Whole amount of container was dissolved in 2mL of water (HPLC, Gradient Grade). Aliquote part of 1 mL was dispensed into HPLC vial for analysis.

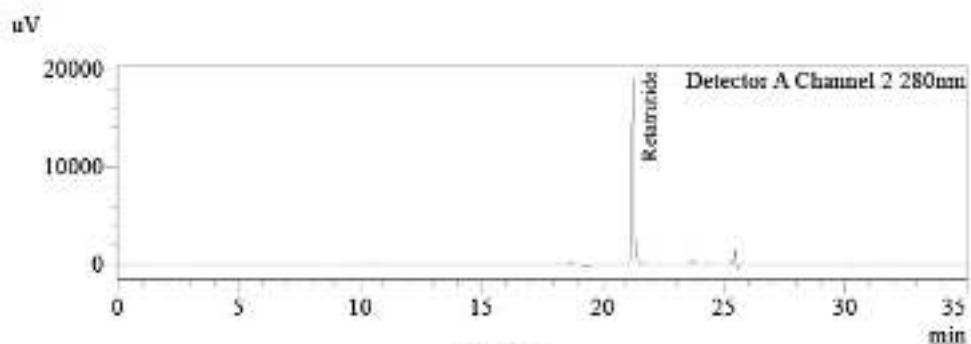
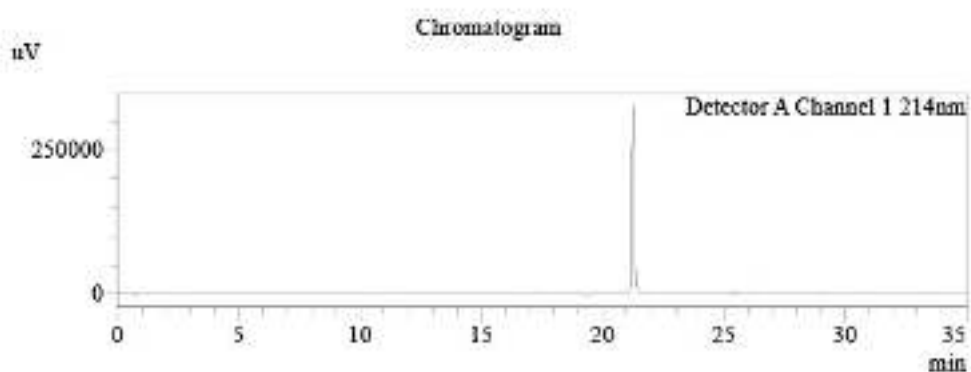
1.6. Purity assesment

Purity of compound assesed by area normalization method, comparing area of each peak to sum of area of all peaks detected at wavelength of 214 nm.

Analysis Report



Sample Information
 Injection Volume : 0.5
 Data File : LYO-0060-P01_002_019.lcd
 Method File : Peptide screening_V3.lcm
 Date Acquired : 25.07.2025 4:22:18



Peak Table

Peak#	Name	Ret. Time	Conc.	Unit	Area%
1		21.247	0.000		100.000
Total					100.000

Peak Table

Peak#	Name	Ret. Time	Conc.	Unit
1	Retatrunda	21.247	9.680	ng
Total				

Attachment for Lyo-0060
 Filename: LYO-0060-P01.jpg

 LIQUILABS laboratory services	Method Specification	
Determination of bioburden of lyophilized samples		
<i>Method number</i> LYO_060_26.25	<i>Specimen description</i> -	<i>Number of pages</i> 3

1. Instrumentation and chemicals

1.1. Instruments used

- Sterile Syringe 2ml Luer
- Sterile needles
- Ready-made PCA Plate Count Agar
- Ready-made 360 Plate Count Agar

1.2. Chemicals

Sterile physiological solution (0.9% NaCl)

2. Sample preparation and inoculation

2.1. Sample preparation

1. Pre-heated sterile needle/syringe was used for measuring exactly 2 ml of sterile physiological solution.
2. Needle was changed and by new sterile rubber tip of petri dish container was penetrated and 2 ml of sterile physiological solution was dispensed.
3. Content of container was completely dissolved and left for 5 minutes to settle potentially created bubbles.
4. This procedure is repeated for two vials.

2.2. Total Aerobic microbial count inoculation and cultivation

1. By sterile needle 1 ml of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 ml of solution was poured into the ready to use sterile petri dish filled with PCA agar and petri dish was closed.
4. Process was repeated for two petri dishes.
5. With sterile needle, 1 ml of sterile physiological solution was filled into the sterile needle and was inoculated into one sterile petri dish filled with PCA agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 37°C for 120h.

1

2.3 Total Yeast and Mold count: inoculation and cultivation

1. By sterile needle 1 ml of solution was filled into the sterile syringe.
2. Needle was placed about 1 cm from the flame for 10 seconds in sterilize.
3. Consequently 1 ml of solution was poured into the ready to use sterile petri dish filled with Sabi agar and petri dish was closed.
4. This was repeated for two petri dishes.
5. By the sterile needle 1 ml of sterile physiological saline was filled into the sterile needle and was inoculated onto one sterile petri dish filled with Sabi agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 25°C, for 72h.

3. Evaluation of results

After incubation time colonies are counted as cfu. Colonies forming units and result per 1g of sample is determined as:

$$CFU_{avg} = \frac{\sum CFU_k}{n}$$

CFU_{avg} = average CFU counted from n inoculations

CFU_k = CFU counted per inoculation

n = number of inoculations

$$CFU \text{ per gram} = \frac{CFU_{avg}}{m_s} \cdot DF$$

CFU_{avg} = Average CFU counted from n inoculations

m_s = mass of sample (mg)

DF = Dilution factor

*Negative control sample is evaluated as positive, process have to be repeated due to possible contamination in the process of inoculation or cultivation.

 LIQUILABS analog to reality	Method Specification		
Determination of bacterial endotoxin content of lyophilized samples			
Document number ENDOTOX_001_2025	Typical document ENDOTOX_001_2025	Number of pages 2	

1. Chromogenic LAL Assay Determination of Bacterial Endotoxin content of sample

1.1. Instrumentation

- Pipette set 1-1000 µL
- Thermostatically controlled water bath
- UV-VIS spectrometer (Shimadzu UV-1601)
- GlauScript ToxinSensor Chromogenic LAL Endotoxin Assay kit

1.2. Chemicals

- LAL Reagent water (endotoxin free)
- Limulus Amebocyte Lysate
- LAL Substrate
- Color Stabilizer #1
- Color Stabilizer #2
- Color Stabilizer #3
- 35% HCl (p.a.)

1.3. Sample preparation

1. Sample container was weighed prior to dissolution and measured weight was marked.
2. Sample was completely dissolved in its container by 2 mL of LAL Reagent water.
3. 100 µL of the sample was aliquoted for analysis.
4. After analysis container was emptied and dried.
5. Dry mass of container was measured and exact weight of dissolved content was determined as:

$$m_{dc} = m_{sample} - m_{container}$$

1.4. Toxin sensor Chromogenic LAL Endotoxin Assay kit preparation

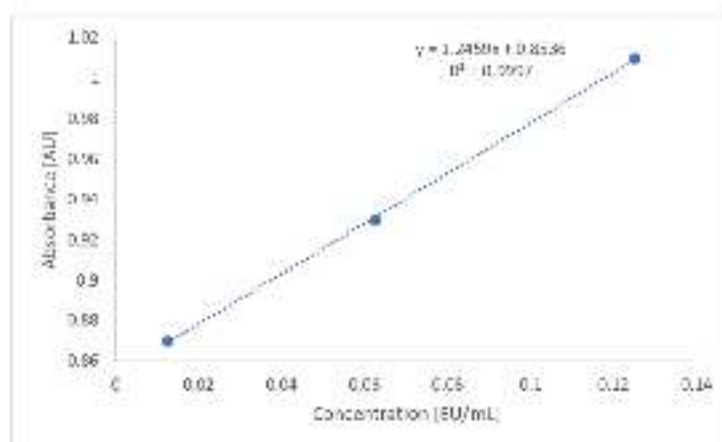
Procedures regarding preparation of reaction solutions possible to find in:

https://www.genscript.com/site/document/5293_20080606231827.PDF

1.5. Measurement procedure

	Standards	Samples	Blank
Standards (mL)	0.1	-	-
Samples (mL)	-	0.1	-
LAL Reagent Water (mL)	-	-	0.1
LAL Solution (mL)	0.1	0.1	0.1
Mix well and incubate at 37°C for 27 min			
Substrate solution (mL)	0.1	0.1	0.1
Mix well and incubate at 37°C for 8 min			
Color Stabilizer #1 solution	0.0	0.0	0.0
Color Stabilizer #2 solution	0.0	0.0	0.0
Color Stabilizer #3 solution	0.0	0.0	0.0
Mix well and read the absorbance at 545nm			

1.6. Calibration curve



1.7. Calculation of endotoxin content

Endotoxin content of the sample was calculated from the calibration curve as:

$$\text{Endotoxin [EU/mg]} = \frac{\left(\frac{ABS_{\text{sample}}}{S_{\text{slope}}} \right) \cdot 20}{m_{\text{sample}}}$$

ABS_{sample} = Measured absorbance of sample

S_{slope} = Slope of calibration curve

m_{sample} = real measured mass of sample

20 = dilution factor of measured sample

2

Attachment for Lyo-0060

Filename: Endotoxin_072025_1_page-0002.jpg

Responsibles



Mr. Ján Galbavý
Founder/Manager

Analysis results relate only to the samples tested.

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