

Certificate of Analysis

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Sample Identification

Sample Name TIR15 15 mg
Batch Number 20265521
Date Published 2026-07-27 16:19

Results for LYO-0389

Peptides	Result	Unit	Uncertainty	Acceptable Range
Tirzepatide Assay Peptide Screening 0.1% TFA	13.41	mg	[± 0.07]	
Tirzepatide Purity Peptide Screening 0.1% TFA	99.8	%	[± 0.5]	
Tirzepatide Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	995		[± 5]	
Tirzepatide Identification by RT Peptide Screening 0.1% TFA	0.997		[± 0.005]	
Microbiology	Result	Unit	Uncertainty	Acceptable Range
Bacterial Endotoxin Chromgenic USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test	0.056	EU/mg	[± 0.001]	0 - 0.5
Mass Spectrometry	Result	Unit	Uncertainty	Acceptable Range
Molecular Ion Mass Identification (MS Deconvolution) Mass Spectrometry Identity	4813	Da	[± 1]	

	Method Specification	
Determination of identity, content and purity of Tirzepatide		
<i>Document number</i> TIRZ_006_2026	<i>Superseded document</i> -	<i>Number of pages</i> 4

1. Content Assessment

1.1. Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Colum Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

1.2. Chromatographic conditions

Chromatographic conditions	
Eluent A	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	280nm

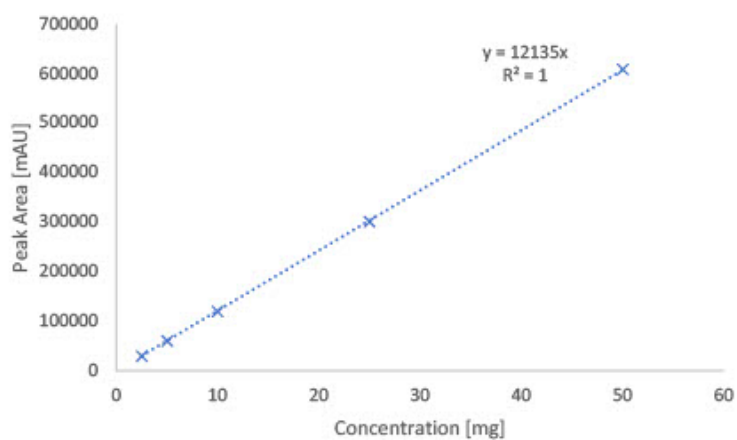
Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	end	

1.3. Sample preparation

Whole amount of container was dissolved in 2mL of water (LCMS Grade). 100 µL of sample was transferred to HPLC vial and diluted by 900 µL water (LCMS Grade) and submitted for analysis.

1.4. Calibration curve

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



2. Purity assessment

2.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Colum Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

2.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	end	

2.3 Purity assesment

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

3. Identity Assessment

3.1 Instrumentation

Module	Name	Serial Number
System Controller	Shimadzu CBM-40 Lite	L221226351398
Degassing Unit	Shimadzu DGU-403	NA
Pump	Shimadzu LC-40B XR	L22146350580
Autosampler	Shimadzu SIL-40C XR	L22216351622
Column Thermostat	Shimadzu CTO-40S	L22236351602
PDA Detector	Shimadzu SPD-M40	L22276352808
SQ MS Detector	Shimadzu LCMS-2050	O12476200760

3.2 Chromatographic conditions

Chromatographic conditions	
Eluent A	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Column Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Mass spectrometry	Scan: positive 280-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	end	

3.3 Molecular Ion Mass evaluation

Molecular ion mass was determined by deconvolution of multiply charged ESI-MS spectra to calculate the average neutral (zero-charge) molecular mass by equation:

$$M(\text{neutral}) = (z_i((mz_i) - H)) - ME$$

Where:

mz_i - Measured mass of charged particle

z_i - charge

H - proton mass (1.0076 Da)

ME – mass error

Analysis Report



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Analytical services

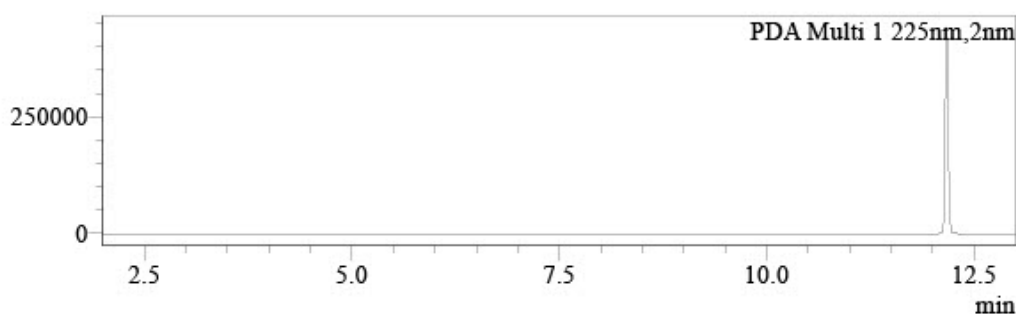
Analysis of quantity and purity of active ingredient by UHPLC with UV detection

Sample Information

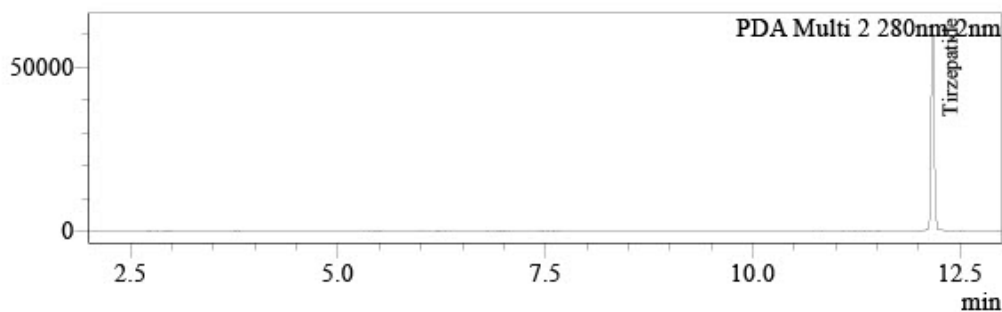
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Data File : LYO-0389__004.lcd
Method File : Peptide screening_V7_Group A.lcm
Date Acquired : 7/23/2026 4:26:40 PM

Chromatogram

uAU



uAU



Peak Table

PDA Ch1 225nm

Name	Ret. Time	Area	Conc.	Unit	Area%
	11.822	444	0.000		0.038
	12.029	676	0.000		0.058
	12.168	1160922	0.000		99.757
	12.284	1713	0.000		0.147
		1163756			100.000

Peak Table

PDA Ch2 280nm

Name	Ret. Time	Area	Conc.	Unit
Tirzepatide	12.168	162089	13.407	mg
		162089		

Analysis Report

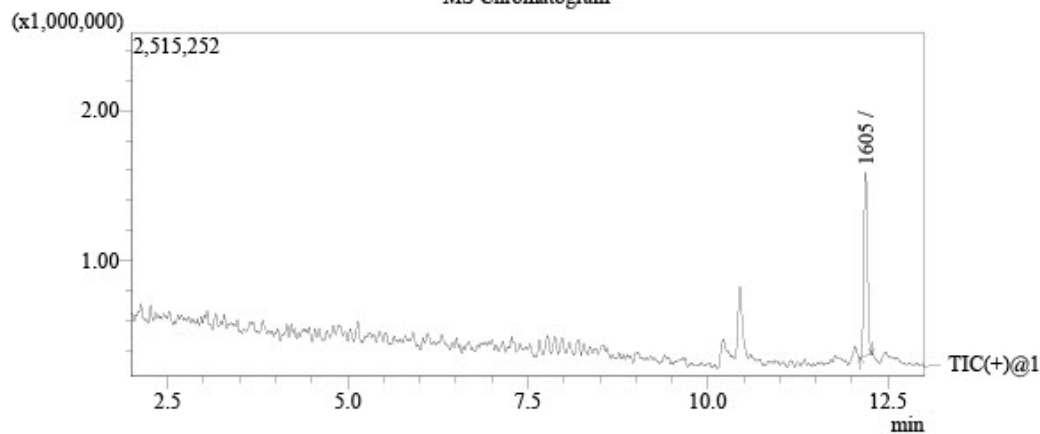


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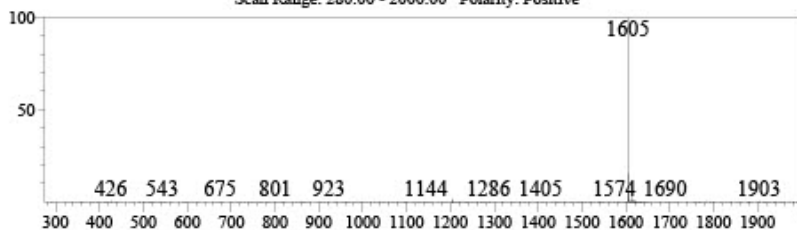
Analysis of identity of active ingredient by UHPLC with mass spectrometric detection

MS Chromatogram

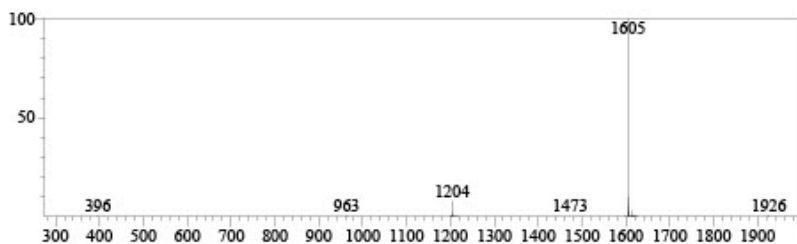


Library Search

Detected compound
Ret.Time: 12.122 Base Peak: 1605
Scan Range: 280.00 - 2000.00 Polarity: Positive



Library: Peptide screening lib
Formula: C225H348N48O68 CAS: 223788-19-2 Mol.Weight: 4813 Ret.Index: 0
Compound Name: Tirzepatide



Endotoxin Determination Report

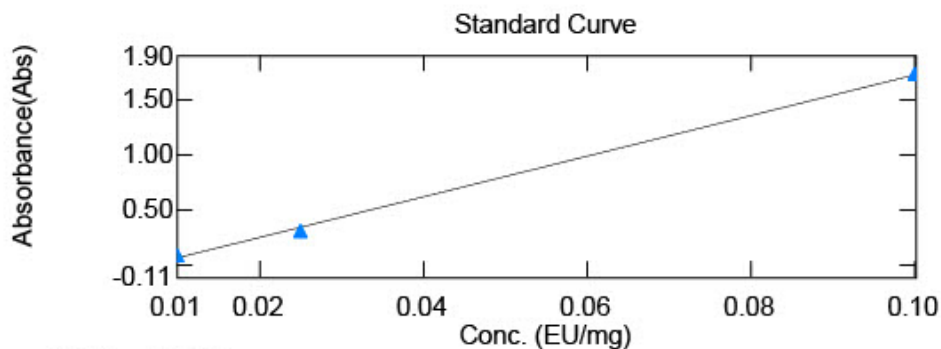


File Information

Filename: C:\UVVis-Data\Data\File_260723_122453.vqud
Date/Time: 07/23/2026 01:13:01 PM

Instrument Information

Instrument Type: UV-1900 Series
Model (S/N): UV-1900i (A12536253123)



$$y = 18.5422x - 0.127539$$
$$r^2 = 0.99855$$

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
1	LYO-0389	0.756	0.5732	20.00
2	LYO-0390	0.211	0.0677	20.00
3	LYO-0391	0.802	0.6164	20.00
4	LYO-0392	0.275	0.1271	20.00
5	LYO-0393	0.231	0.0863	20.00
6	LYO-0394	0.171	0.0309	20.00
7	LYO-0395	0.285	0.1364	20.00
8	LYO-0396	0.199	0.0569	20.00
9	LYO-0397	0.209	0.0660	20.00
10	LYO-0398	0.159	0.0201	20.00
11	LYO-0399	0.174	0.0337	20.00
12	LYO-0400	0.360	0.2067	20.00
13	LYO-0401	0.203	0.0605	20.00
14	LYO-0404	0.204	0.0613	20.00
15	LYO-0405	1.231	1.0135	20.00
16	LYO-0406	0.160	0.0206	20.00
17	LIQ-0039	0.008	0.0270	1.00

Responsibles



Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.

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