

Certificate of Analysis

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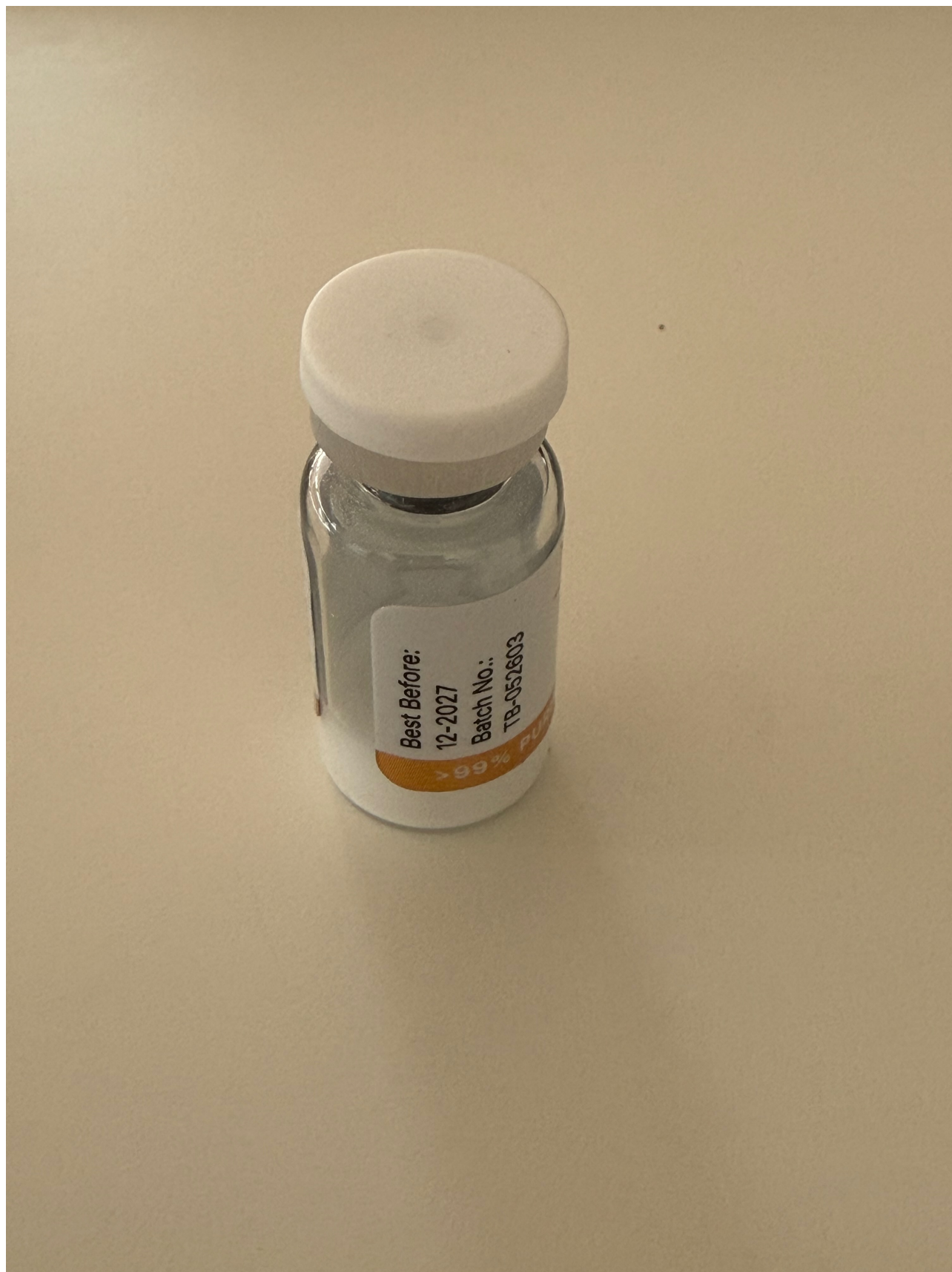
Sample Identification

Sample Name TB-500 10 mg
Batch Number TB-052603
Date Published 2026-06-23 12:49

Results for LYO-0270

Peptides	Result	Unit	Uncertainty	Acceptable Range
Thymosin Beta 4 (TB-500) Assay Peptide Screening 0.1% TFA	12.20	mg	[± 0.06]	
Thymosin Beta 4 (TB-500) Purity Peptide Screening 0.1% TFA	99.3	%	[± 0.5]	
Thymosin Beta 4 (TB-500) Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	0.989		[± 0.005]	
Thymosin Beta 4 (TB-500) Identification by RT Peptide Screening 0.1% TFA	0.997		[± 0.005]	
Mass Spectrometry	Result	Unit	Uncertainty	Acceptable Range
Molecular Ion Mass Identification (MS Deconvolution) Mass Spectrometry Identity	4963	Da	[± 1]	





	Method Specification	
Determination of identity, content and purity of Thymosin Beta 4		
<i>Document number</i> TB4_006_2026	<i>Superseded document</i> -	<i>Number of pages</i> 4

1. Content Assesment

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	225nm

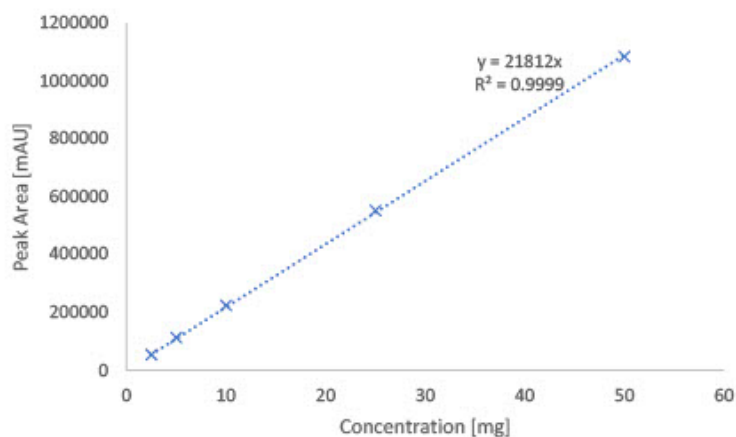
Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	end	

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	97.5	2.5
13	45	55
13.5	1	99
14.5	1	99
14.51	97.5	2.5
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	97.5	2.5
13	45	55
13.5	1	99
14.5	1	99
14.51	97.5	2.5
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



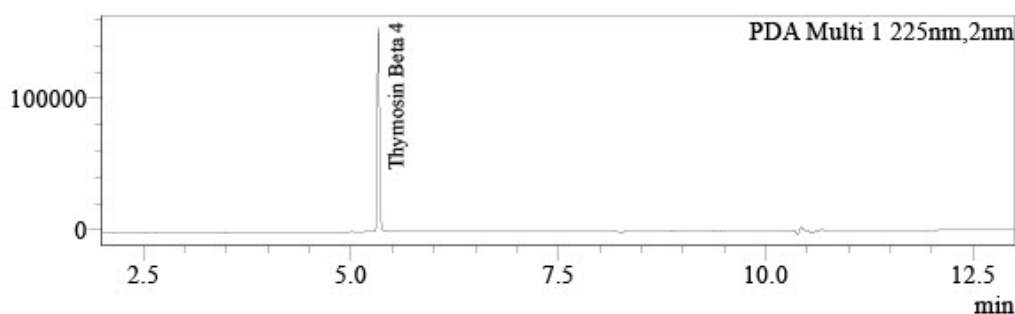
Analysis of quantity and purity of active ingredient by UHPLC with UV detection

Sample Information

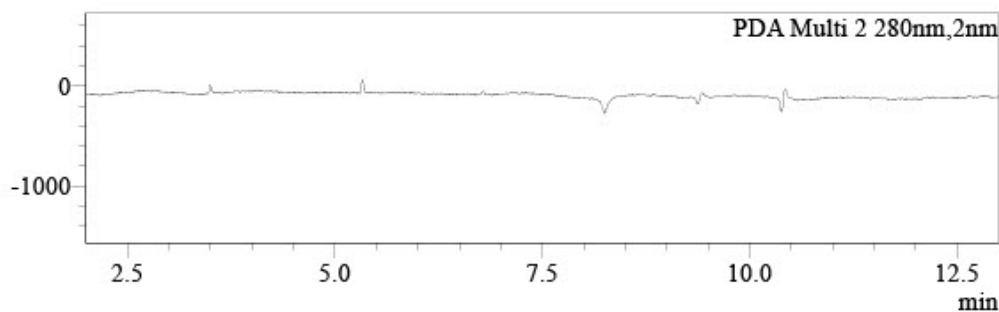
Injection Volume : 2
Data File : LYO-0270__035.lcd
Method File : Peptide screening_V7_Group C.lcm
Date Acquired : 6/19/2026 10:28:24 AM

Chromatogram

uAU



uAU



Peak Table

PDA Ch1 225nm

Name	Ret. Time	Area	Conc.	Unit	Area%
	3.497	675	0.000		0.234
	5.009	319	0.000		0.111
Thymosin Beta 4	5.330	286379	12.195	mg	99.310
	5.704	433	0.000		0.150
	6.788	562	0.000		0.195
		288368			100.000

Peak Table

PDA Ch2 280nm

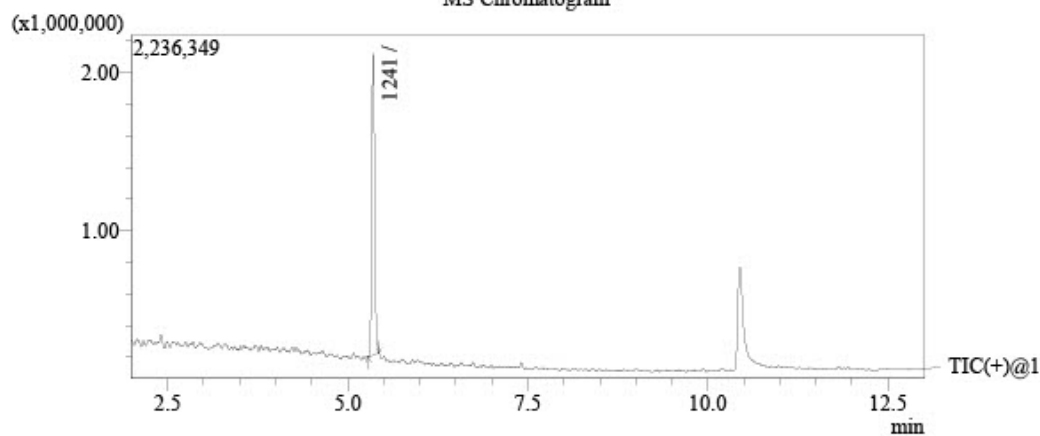
Name	Ret. Time	Area	Conc.	Unit

Analysis Report



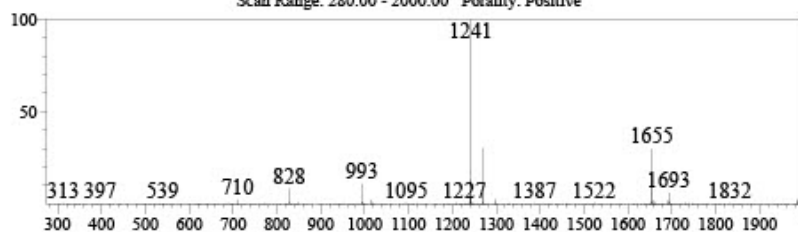
Analysis of identity of active ingredient by UHPLC with mass spectrometric detection

MS Chromatogram



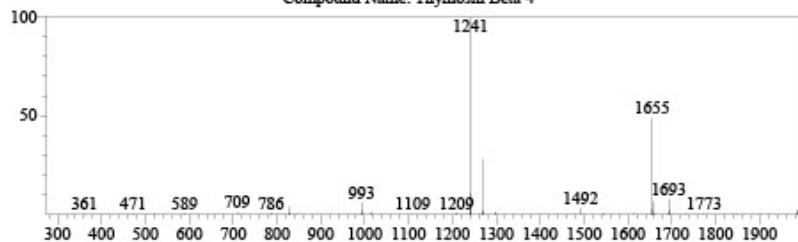
Library Search

Detected compound
Ret.Time: 5.291 Base Peak: 1241
Scan Range: 280.00 - 2000.00 Polarity: Positive



Reference

Library: Peptide screening lib
Formula: C₂₁₂H₃₅₀N₅₆O₇₈S CAS: 77591-33-4 Mol.Weight: 4963 Ret.Index: 0
Compound Name: Thymosin Beta 4



Responsibles



Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.

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