

Certificate of Analysis

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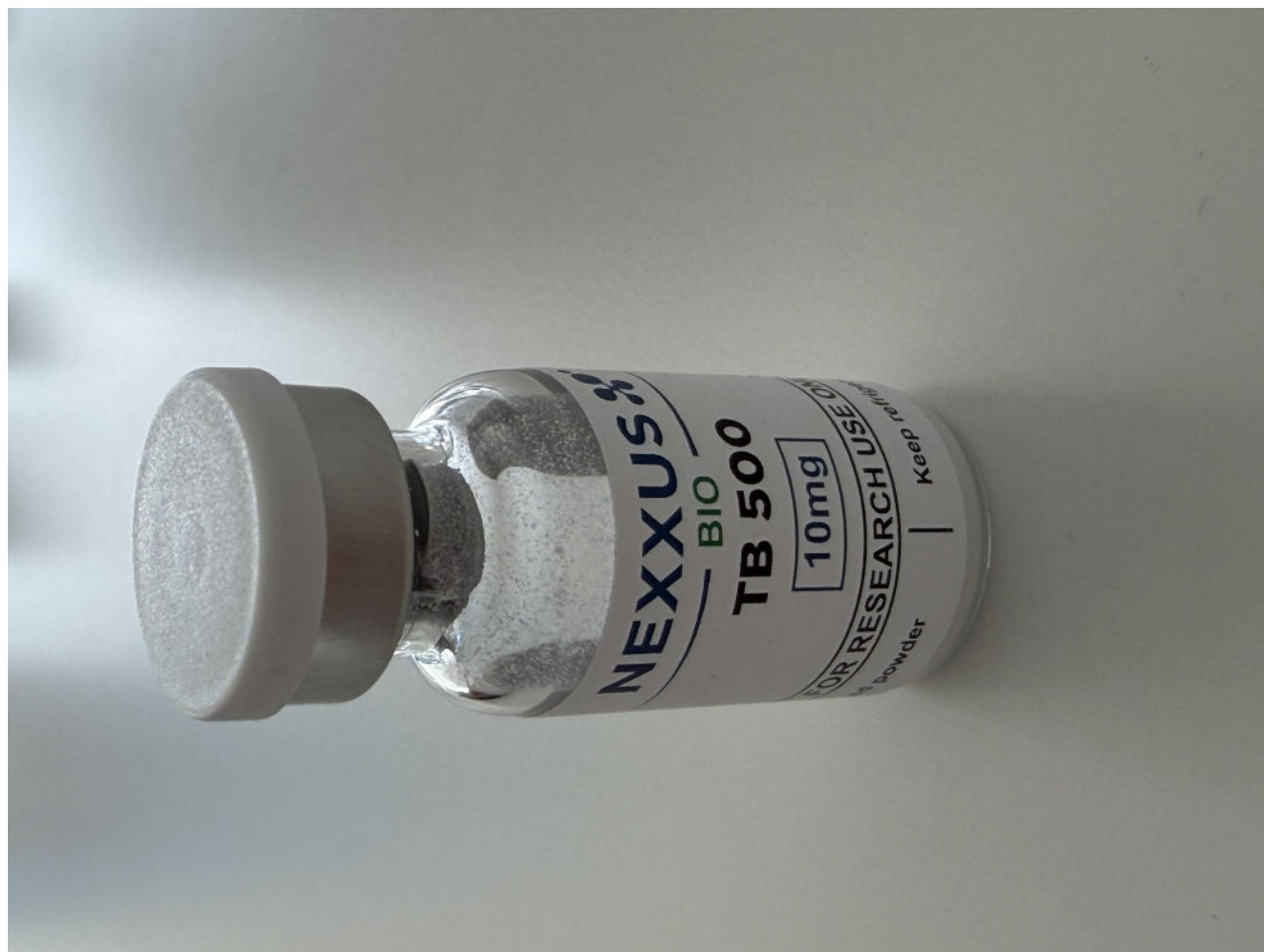
[Verify Results Online](#)

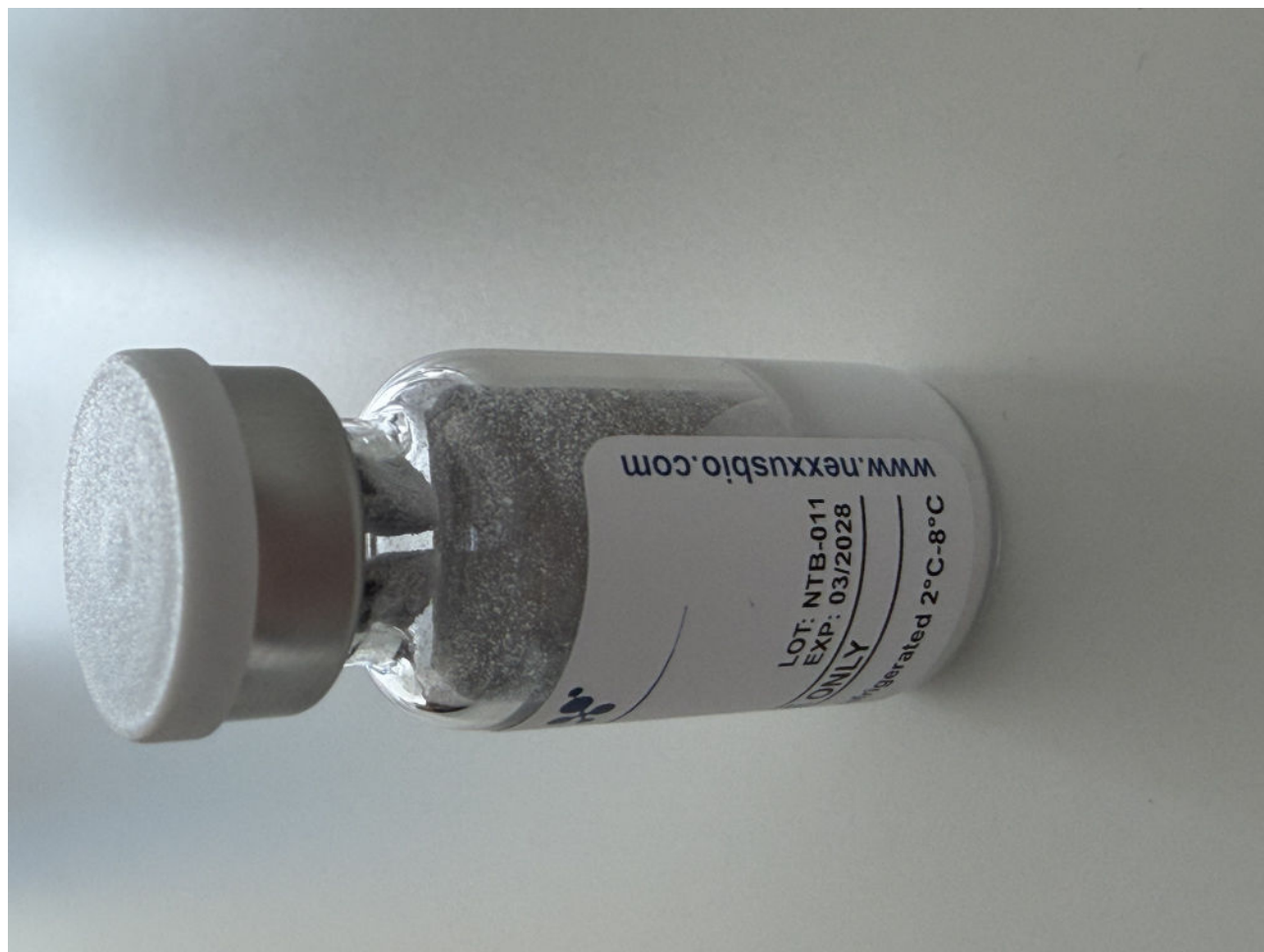
Sample Identification

Sample Name TB500 10 mg
Batch Number NTB-011
Date Published 2026-08-25 15:59

Results for LYO-0623

Peptides	Result	Unit	Uncertainty	Acceptable Range
Thymosin Beta 4 (TB-500) Assay Peptide Screening 0.1% TFA	11.43	mg	[± 0.06]	
Thymosin Beta 4 (TB-500) Purity Peptide Screening 0.1% TFA	98.7	%	[± 0.5]	
Thymosin Beta 4 (TB-500) Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	980		[± 5]	
Thymosin Beta 4 (TB-500) Identification by RT Peptide Screening 0.1% TFA	0.996		[± 0.005]	
Microbiology	Result	Unit	Uncertainty	Acceptable Range
Bacterial Endotoxin Chromgenic USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test	0.005	EU/mg	[± 0]	
Elemental Impurities	Result	Unit	Uncertainty	Acceptable Range
Arsenic Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Cadmium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Quicksilver Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Lead Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Nickel Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Vanadium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
Cobalt Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		
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_008_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Superseded document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	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

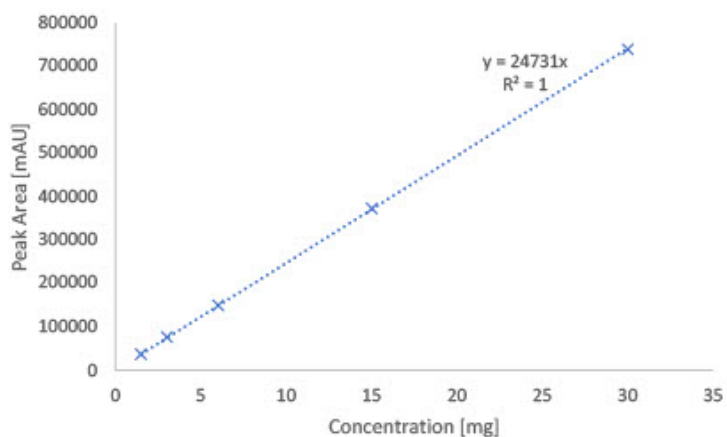
Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	end	

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	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	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 225 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	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.8 mL/min
Program	Gradient elution
Injection volume	2 µL
Column Temperature	60°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection	MS 220-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
1	98	2
13.99	35	65
14	5	95
15	5	95
15.01	98	2
19	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

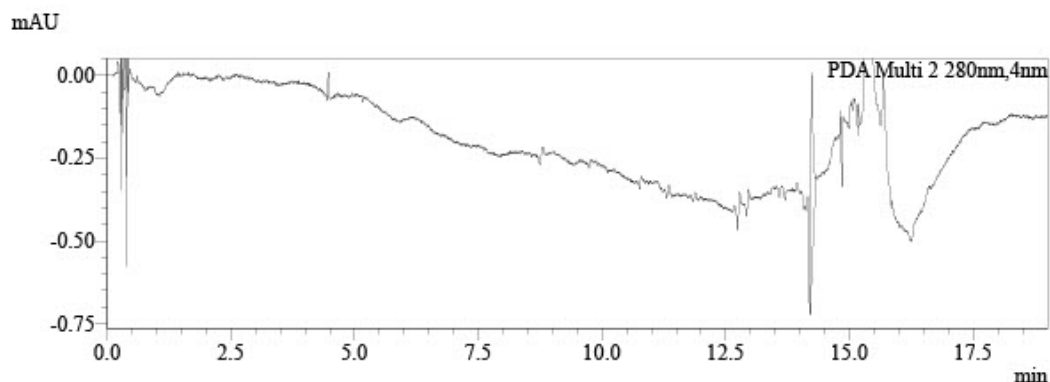
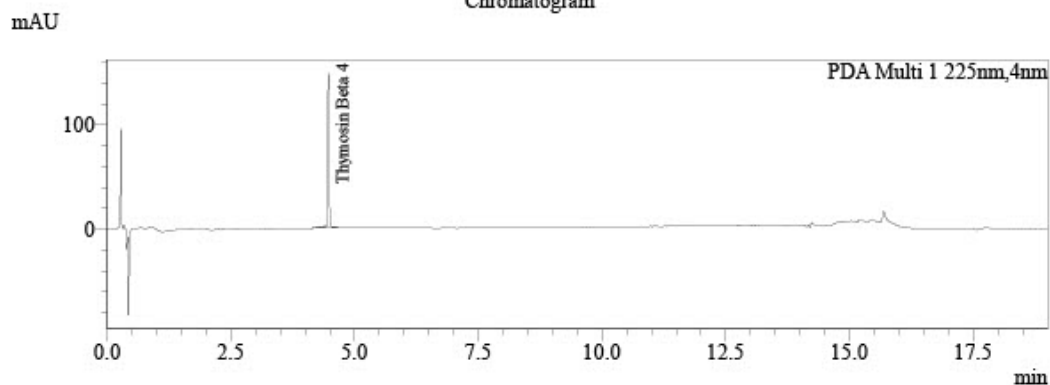


Analysis of quantity of active ingredient by UHPLC with MS detection

Sample Information

Injection Volume : 2
Data File : LYO-0623__007.lcd
Method File : Peptide Screening_DFA_Group C.lcm
Date Acquired : 8/20/2026 6:30:18 PM

Chromatogram



Peak Table

PDA Ch1 225nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		4.165	426	0.148	0.000	
2		4.262	260	0.090	0.000	
3		4.324	522	0.182	0.000	
4		4.396	2007	0.698	0.000	
5	Thymosin Beta 4	4.470	283906	98.705	11.433	mg
6		4.562	389	0.135	0.000	
7		4.603	119	0.041	0.000	
Total			287630	100.000		

Peak Table

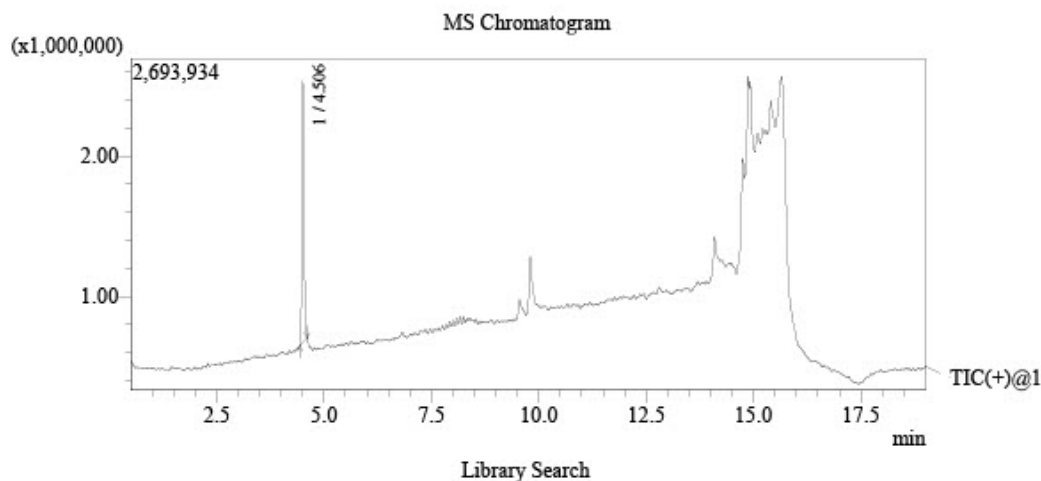
PDA Ch2 280nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		14.240	2019	100.000	0.000	
Total			2019	100.000		

Analysis Report

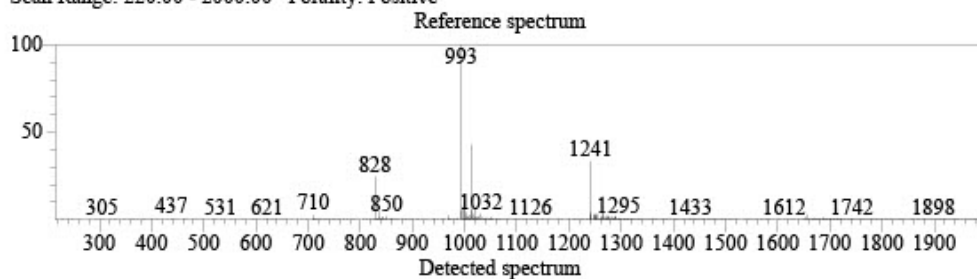


Identification of active ingredient by UHPLC Mass Spectrometry



Base Peak: 993(439990)

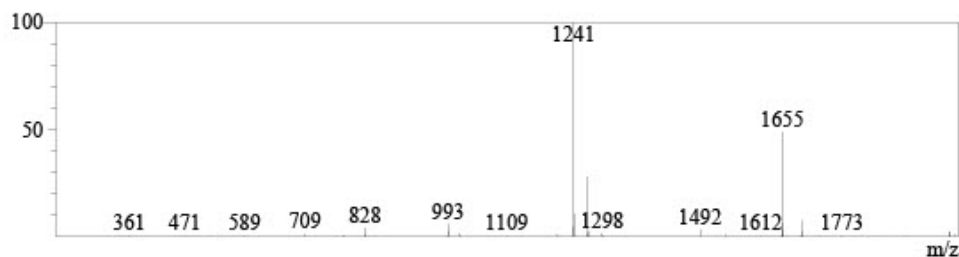
Scan Range: 220.00 - 2000.00 Polarity: Positive



Library: Peptide screening.lib

Formula: C₂₁₂H₃₅₀N₅₆O₇₈S CAS: 77591-33-4 Mol.Weight: 4963(0!)=

Compound Name: Thymosin Beta 4



Endotoxin Determination Report

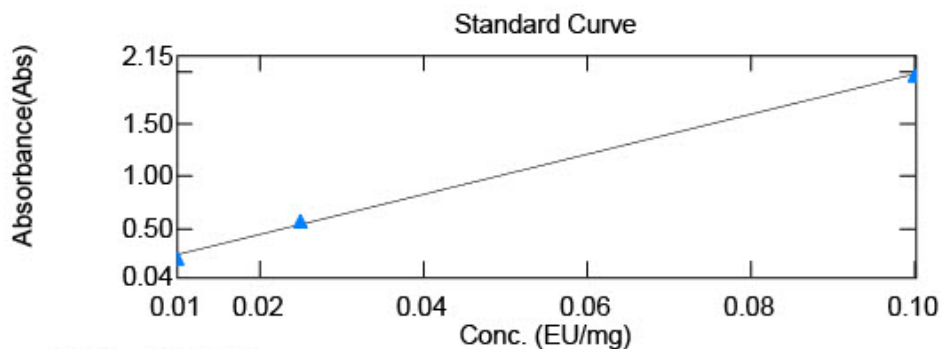


File Information

Filename: C:\UVVis-Data\Data\File_260820_135540.vqud
Date/Time: 08/20/2026 03:18:46 PM

Instrument Information

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



$$y = 19.2242x + 0.0569806$$
$$r^2 = 0.99866$$

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
1	LIQ-0055	0.003	0.1086	1.00
2	LIQ-0056	0.734	1.4685	10.00
3	LYO-0620	5.092	1.0358	100.00
4	LYO-0621	0.032	0.0880	20.00
5	LYO-0622	0.906	0.9279	20.00
6	LYO-0623	0.057	0.1122	20.00
7	LYO0624	2.521	1.2687	40.00
8	LYO-0625	0.073	0.1270	20.00
9	LYO-0626	0.043	0.0981	20.00
10	LYO-0627	0.394	0.4356	20.00
11	LYO-0628	0.051	0.1056	20.00
12	LIQ-0057	0.056	0.1113	20.00
13	LYO-0629	2.182	1.1055	40.00
14	LYO-0630	0.053	0.1084	20.00
15	LYO-0631	1.632	0.8411	40.00
16	LYO-0645	1.560	0.8067	40.00
17	LYO-0646	0.058	0.1130	20.00
18	LYO-0647	0.155	0.2056	20.00
19	LYO0648	2.452	1.2354	40.00
20	LYO-0649	1.360	0.7105	40.00

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
21	LYO-0650	0.043	0.0986	20.00
22	LYO-0651	2.908	1.4546	40.00
23	LYO-0652	0.129	0.1805	20.00
24	LYO-0653	0.156	0.0870	100.00

Responsibles



Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.

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