

Certificate of Analysis

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Sample Identification

Sample Name Melanotan 1 10 mg
Batch Number
Date Published 2026-10-05 14:45

Results for LYO-0989

Peptides	Result	Unit	Uncertainty	Acceptable Range
Melanotan I Assay Peptide Screening 0.1% TFA	14.86	mg	[± 0.07]	(RT)
Melanotan I Purity Peptide Screening 0.1% TFA	99.2	%	[± 0.5]	(RT)
Melanotan I Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	976		[± 5]	
Melanotan I Identification by RT Peptide Screening 0.1% TFA	0.995		[± 0.005]	
Mass Spectrometry	Result	Unit	Uncertainty	Acceptable Range
Molecular Ion Mass Identification (MS Deconvolution) Mass Spectrometry Identity	1646	Da	[± 1]	

	Method Specification	
Determination of identity, content and purity of Melanotan 1		
<i>Document number</i> MT1_010_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Superseded document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	0.1% DFA in Water (HPLC, Gradient Grade)
Eluent B	0.1% DFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Colum Temperature	60°C
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection wavelength	280nm

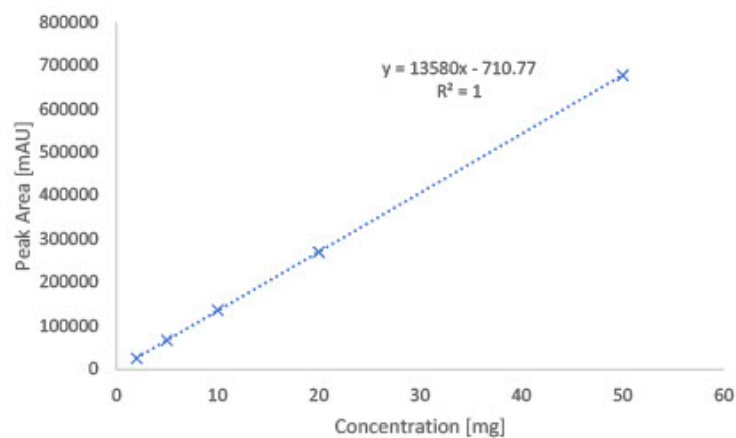
Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	end	

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	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Colum Temperature	60°C
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	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	0.4 mL/min
Program	Gradient elution
Injection volume	1 µL
Column Temperature	60°C
Column	Waters ACQUITY CSH C18, 100x2.1mm 1.7µm
Detection	MS 220-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	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

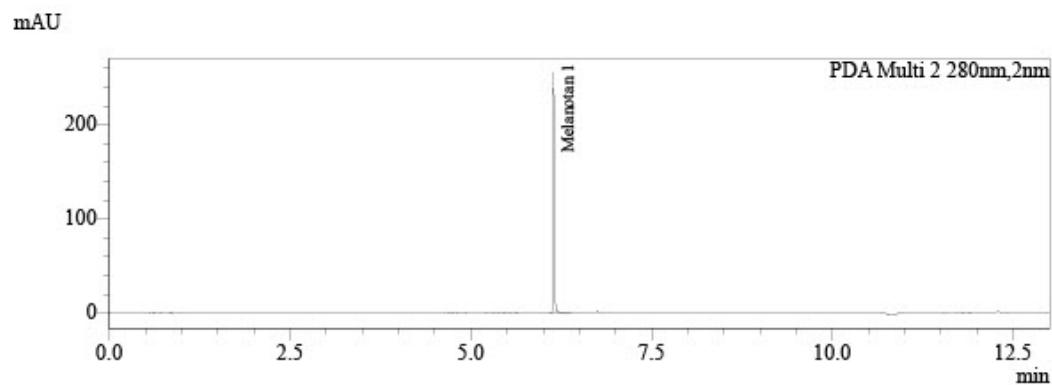
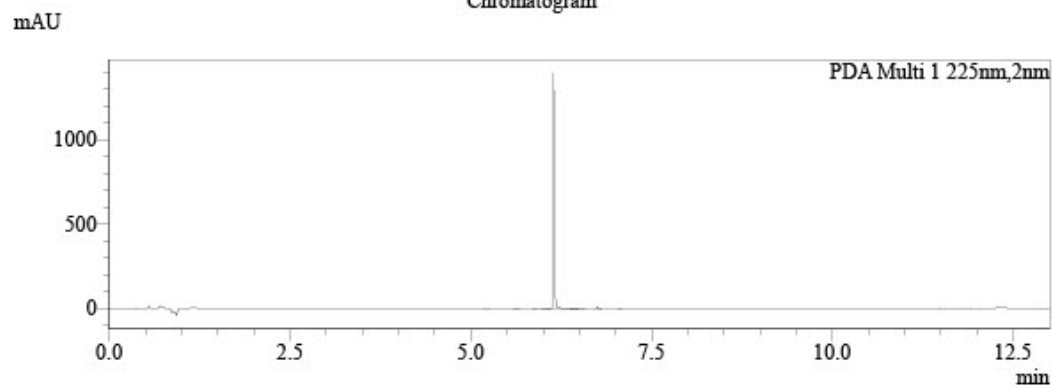


Analysis of quantity of active ingredient by UHPLC with MS detection

Sample Information

Injection Volume : 1
Data File : LYO-0989__002.lcd
Method File : Peptide Screening_DFA_V2_D.lcm
Date Acquired : 10/3/2026 10:52:56 AM

Chromatogram



Peak Table

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		5.629	1749	0.101	0.000	
2		5.875	1053	0.061	0.000	
3		5.992	150	0.009	0.000	
4		6.041	148	0.009	0.000	
5		6.075	221	0.013	0.000	
6		6.147	1722605	99.183	0.000	
7		6.389	124	0.007	0.000	
8		6.432	33	0.002	0.000	
9		6.475	25	0.001	0.000	
10		6.551	510	0.029	0.000	
11		6.750	10172	0.586	0.000	
Total			1736789	100.000		

Peak Table

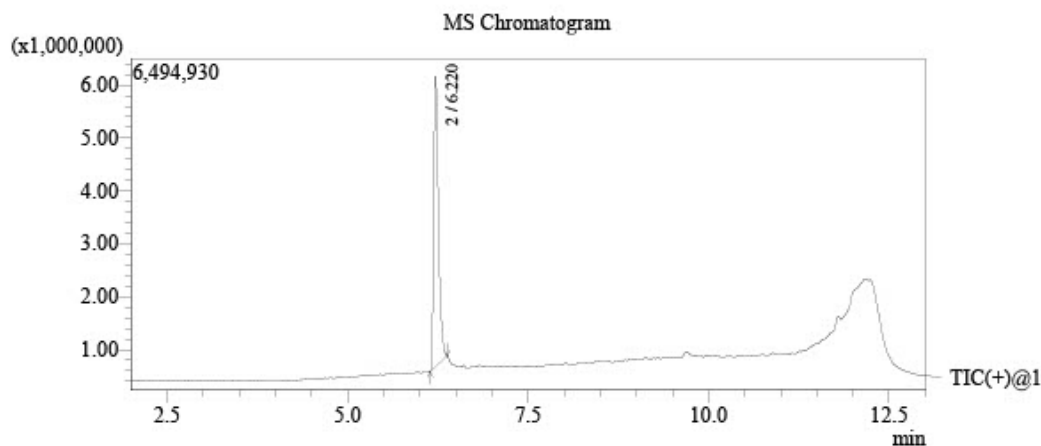
PDA Ch2 280nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1	Melanotan 1	6.147	305020	100.000	14.859	mg
Total			305020	100.000		

Analysis Report

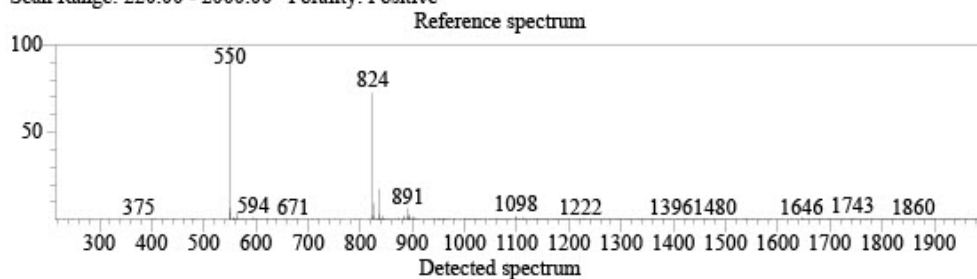


Identification of active ingredient by UHPLC Mass Spectrometry

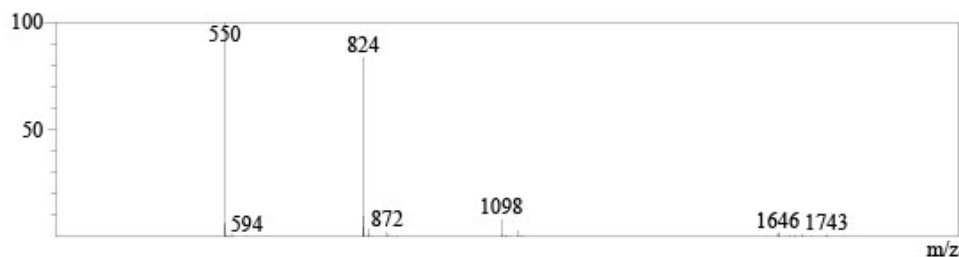


Library Search

Base Peak: 550(1933977)
Scan Range: 220.00 - 2000.00 Polarity: Positive



Library: Peptide Screening DFA.lib
Formula: $C_{78}H_{111}N_{21}O_{19}$ CAS: 75921-69-6 Mol.Weight: 1646(0!=)
Compound Name: Melanotan 1 (Afamelanotide)



Responsibles



Mr. Ján Galbavý
CEO

Analysis results relate only to the samples tested.

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