

Certificate of Analysis

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Sample Identification

Sample Name Melanotan I 10 mg
Batch Number M1-082604
Date Published 2026-10-11 08:42

Results for LYO-1050

Peptides	Result	Unit	Uncertainty	Acceptable Range
Melanotan I Assay Peptide Screening 0.1% TFA	13.16	mg	[± 0.07]	
Melanotan I Purity Peptide Screening 0.1% TFA	> 99.8	%		
Melanotan I Identification by Spectrum (FTIR) Peptide Screening 0.1% TFA	982		[± 5]	
Melanotan I 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	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Superseded document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

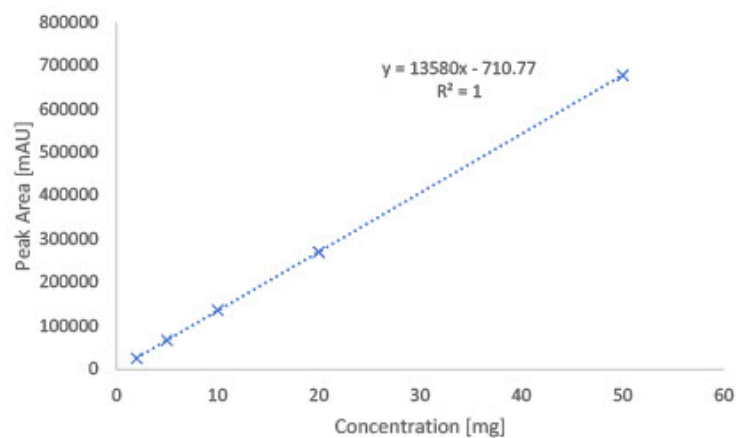
Gradient Program		
Time [min]	A [%]	B [%]
2	98	2
10	35	65
10.01	5	95
11	5	95
11.01	98	2
15	end	

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

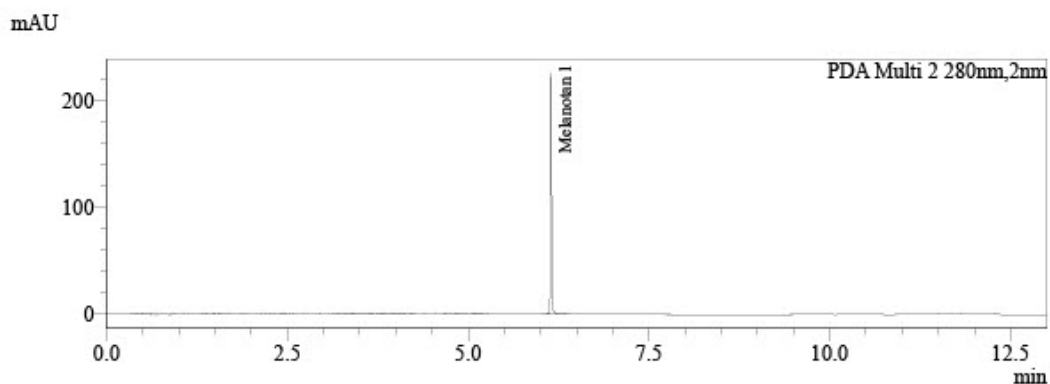
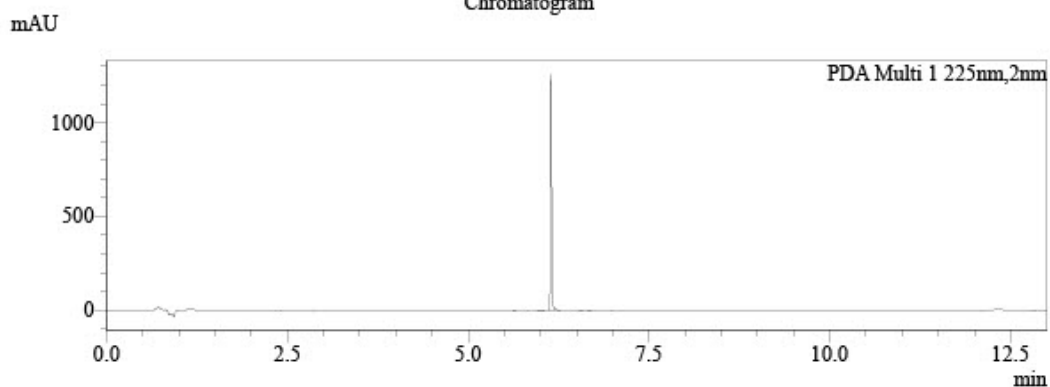


Analysis of quantity of active ingredient by UHPLC with MS detection

Sample Information

Injection Volume : 1
Data File : LYO-1050_013.lcd
Method File : Peptide Screening_DFA_V2_D.lcm
Date Acquired : 10/5/2026 5:37:44 PM

Chromatogram



Peak Table

PDA Ch1 225nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1		5.635	387	0.025	0.000	
2		5.981	270	0.018	0.000	
3		6.037	1193	0.078	0.000	
4		6.145	1531253	99.856	0.000	
5		6.552	153	0.010	0.000	
6		6.676	198	0.013	0.000	
Total			1533455	100.000		

Peak Table

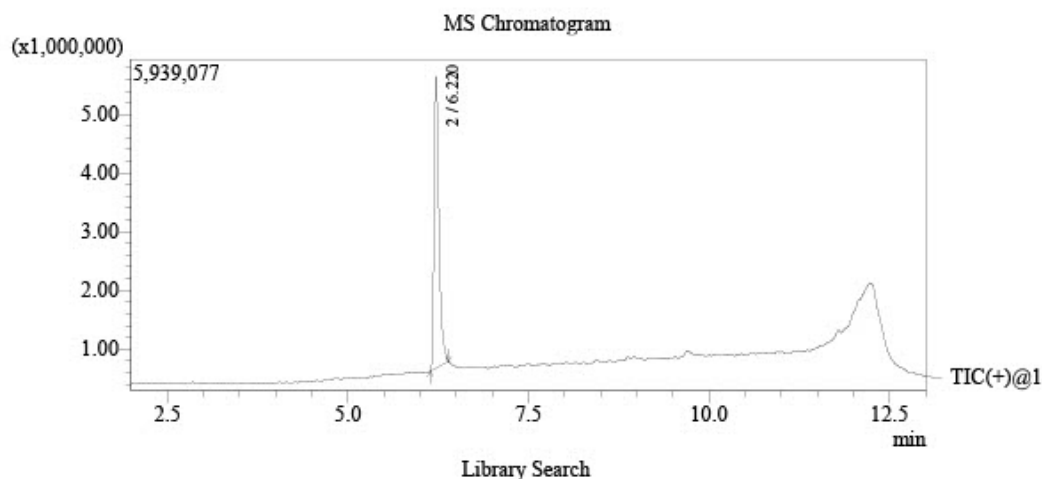
PDA Ch2 280nm

Peak#	Name	Ret. Time	Area	Area%	Conc.	Unit
1	Melanotan 1	6.145	270655	100.000	13.165	mg
Total			270655	100.000		

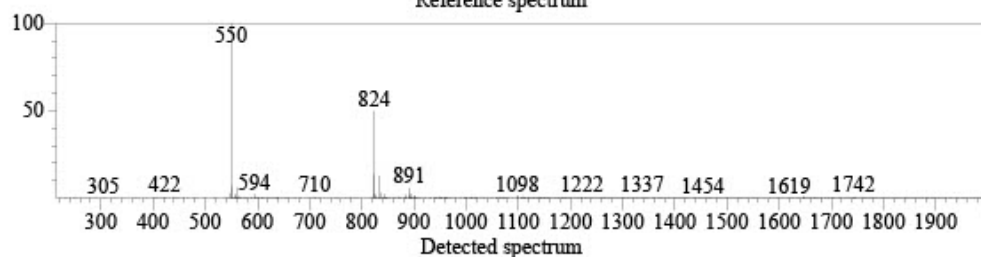
Analysis Report



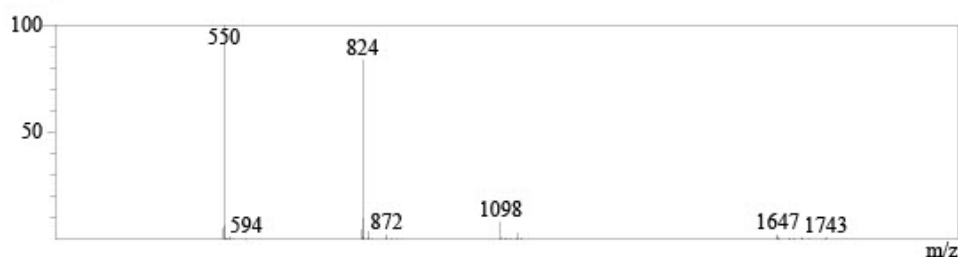
Identification of active ingredient by UHPLC Mass Spectrometry



Base Peak: 550(1836174)
Scan Range: 220.00 - 2000.00 Polarity: Positive
Reference spectrum



Library: Peptide Screening DFA.lib
Formula: C₇₈H₁₁₁N₂₁O₁₉ 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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