

ANALYTICAL CERTIFICATE

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Sample name	Melanotan 2
Batch No.	2024196
Sample No.	01
Specification	NA
Manufacturing date	NA
Submitter of analytical request	Particle s.r.o., Slovakia

1. Peptide content by HPLC/CLND:

1.1 HPLC Instrument:

Pump: Agilent 1200 Series, Quat Pump G1311A
Sampler: Agilent 1260 Series, Hip ALS G1367E
Degasser: Agilent 1200 Series, Degasser G1379B
Detectors: Agilent 1200 Series, VWD G1314B
Nitrogen detector Antek 8060

1.2 HPLC conditions:

Eluents: A – MilliQ water
B – isopropanol
D – 1% TFA in MilliQ water
Flow rate: 1 mL/min
Gradient:

Time	A (%)	B (%)	D (%)
0	90	0	10
1	90	0	10
9	10	80	10
10	10	80	10
11	90	0	10
15	90	0	10

Column: ARION 5 μ C4-BIO 300 A, 4.6 x 100 mm
Serial No 221258

1.3 Sample preparation:

The whole amount of Melanotan 2 (10 mg) was dissolved in 2 mL of water.
Injection: 2 μ L

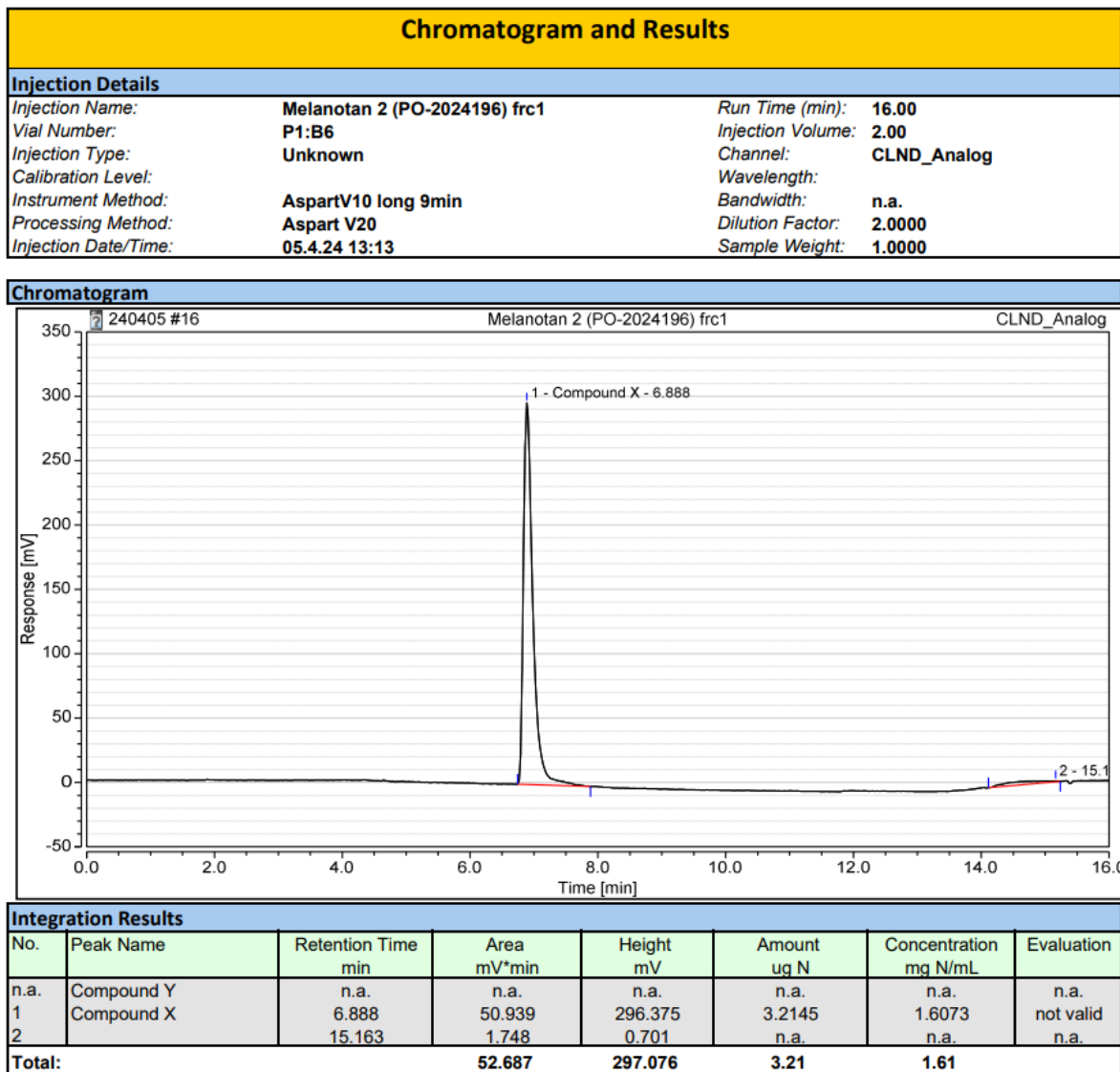
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1.4 Chromatograms and calibration curve:

Instrument:CLND-2 Sequence:240405

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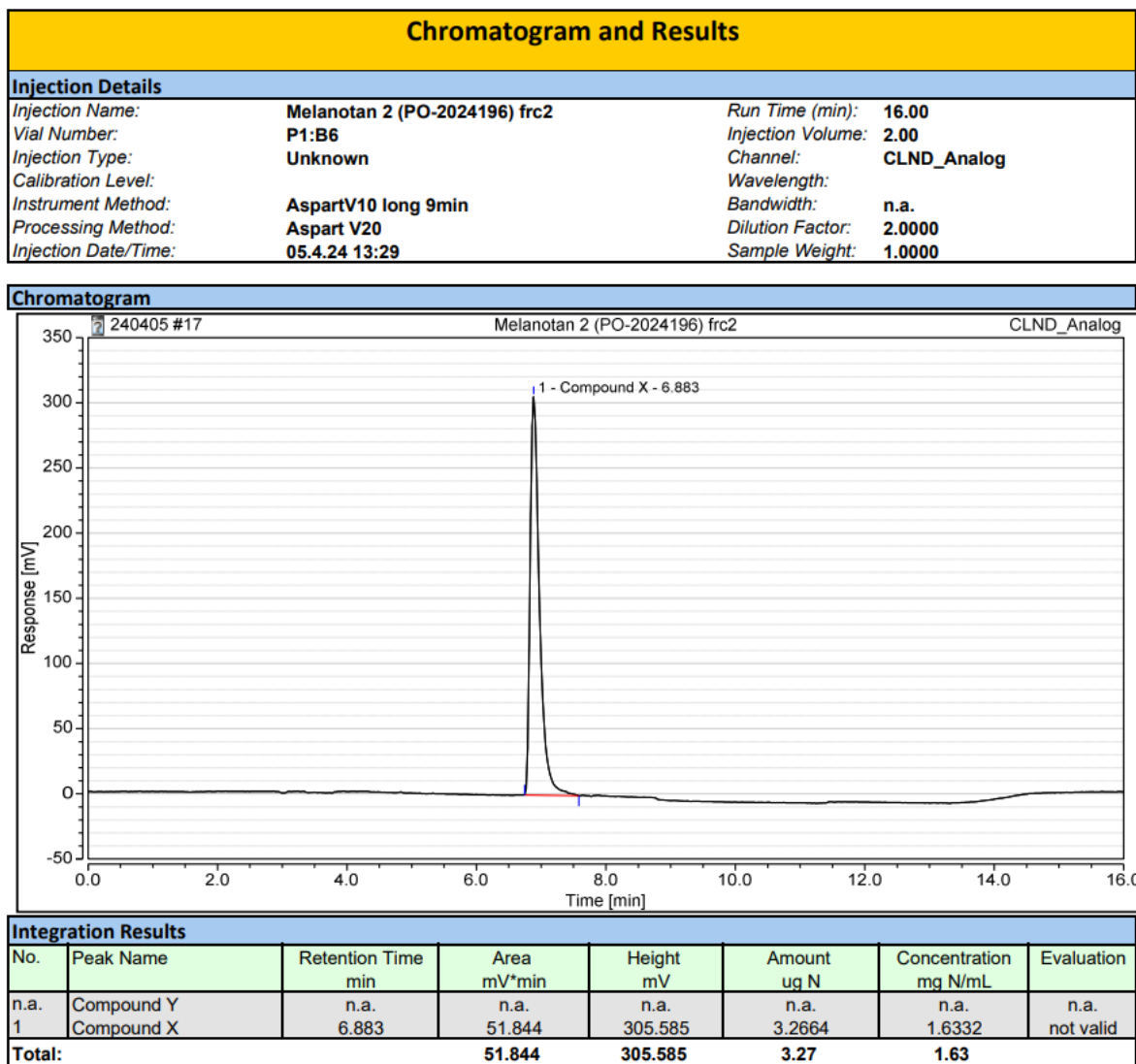


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Instrument:CLND-2 Sequence:240405

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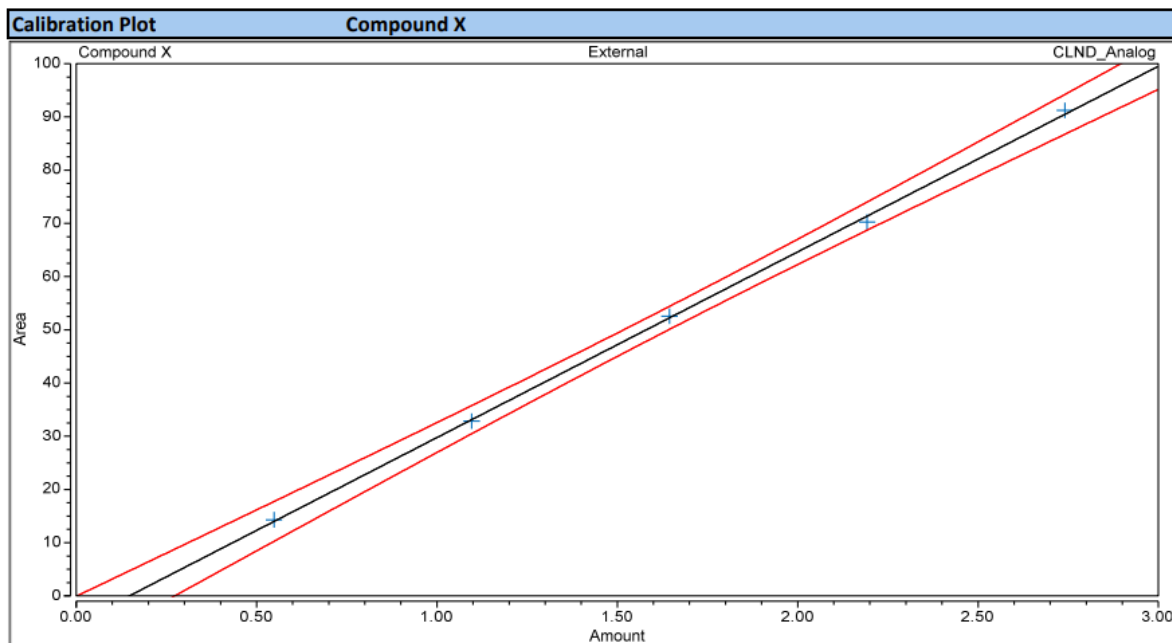
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Instrument:CLND-2 Sequence:240405

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Calibration			
Calibration Details		Compound X	
Calibration Type	Lin, WithOffset	Offset (C0)	-5.1320
Evaluation Type	Area	Slope (C1)	34.8862
Number of Calibration Points	5	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9994



Calibration Results		Compound X					
No.	Injection Name	Calibration Level	X Value	Y Value	Y Value	Area mV*min	Height mV
			CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X
2	Aspart5	1	2.7408	91.2238	91.2238	91.224	532.856
3	Aspart4	1	2.1926	70.2476	70.2476	70.248	415.230
4	Aspart3	1	1.6445	52.5511	52.5511	52.551	310.070
5	Aspart2	1	1.0963	32.8563	32.8563	32.856	190.430
6	Aspart1	1	0.5482	14.3049	14.3049	14.305	84.132

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1.4 Results:

NNC: Melanotan 2 (PO-20241		Salt:	0
MW (calculated) g/mol	N content (calculated) %	N conc. (measured) mg × N/ml	
1024,2	20,51	1,6203	
Theoretical Volume ml		Lyophilizate amount mg	
1,00		10,00	
Peptide concentration mg/ml nmol/ml		Quantified amount mg nmol	
7,90	7713	7,9	7 713
Peptide content assay %			
79,0			

Summary table:

Peptide	Aliquoting (mg)	Total weight of sample (mg)	Content of the peptide by CLND (mg)	Content of the peptide in the sample (%)	Content of the peptide against the amount on label.
Melanotan 2	10	NA	7,9	NA	79.0%

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2. Purity assessment by UPLC:

2.1 HPLC Instrument:

LC-System Waters Acquity UPLC
Detectors: UV or DAD at 214 nm

2.2 HPLC conditions:

Eluents: A – MilliQ water + 0.05% TFA
 B – acetonitrile + 0.05% TFA
Flow rate: 0.40 mL/min
Gradient: from 5% B to 60% B in 4 min, according to chromatogram results
Column: Waters Acquity BEH, C-18, 1.7µm, 2.1mm x 50mm
 Part No 186002353

2.3 Sample preparation:

The whole amount of Melanotan 2 (10 mg) was dissolved in 2 mL of water.
Injection: 0,1 µL

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2.4 Chromatogram of Melanotan 2 (PO-2024196)

Sample information

UPLC5

Channel Description PDA Ch1 214nm@4.8nm

Vial : 1:B,1 Vol. : 0.10 ul

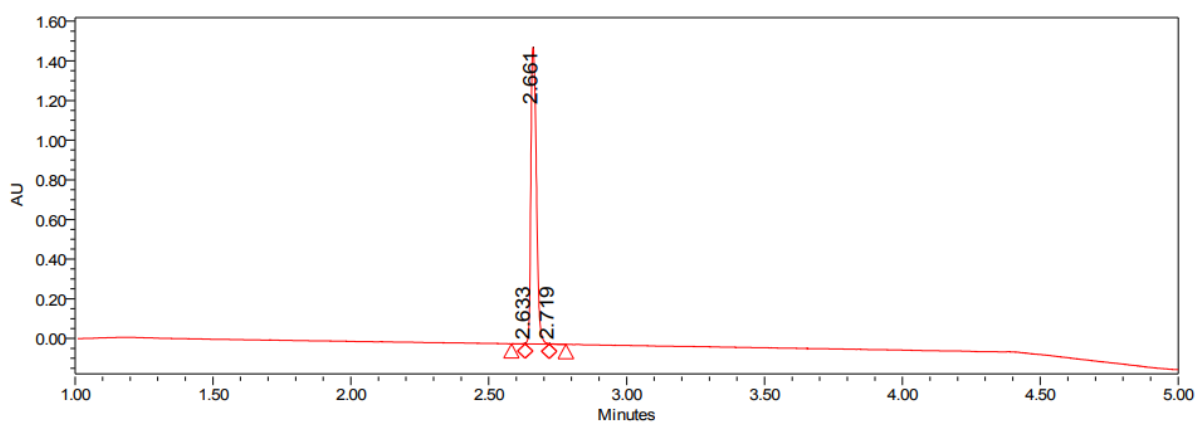
Sample: Melatonan 2 (PO-2024196)

Date Acquired 4/5/2024 3:13:59 PM CEST

Date Processed 4/9/2024 3:30:02 PM CEST

Acq Method Set :

Gr5_60_4mi_40C_0_45ml_K2_met_s



	RT	Area	Height (μV)	% Area
1	2.661	2061905	1496856	99.75
2	2.719	3509	3525	0.17

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient :

0.0 - 0.5min 5 - 5 % B

0.5 - 4 min 5 - 60 % B

4.0 - 4.5 min 60 - 100 % B

4.5 - 5.0min 100 % B

5.0 - 5.5min 100 - 5 % B

6min 5 % B

0.45ml/min

Acquity UPLC BEHC18, 1.7μm, 2.1 x 50 mm column

column oven temp. = 40 °C

2.5 Result of purity assessment

The overall purity is 99.75 % at 214 nm.

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3. Peptide identity by UPLC/MS:

3.1 HPLC Instrument:

LC-System Waters Acquity UPLC
Detectors: UV or DAD at 214 nm

3.2 HPLC conditions:

Eluents: A – MilliQ water + 0.05% TFA
 B – acetonitrile + 0.05% TFA
Flow rate: 0.40 mL/min
Gradient: from 5% B to 60% B in 4 min, according to chromatogram results
Column: Waters Acquity BEH, C-18, 1.7µm, 2.1mm x 50mm
 Part No 186002353

3.3 MS Detector:

Detector Waters (Micromass) ZQ 2000
Ionisation method: ES+
Scanning range: 200 – 2000 amu
Capillary voltage: 3.0 kV
Cone Voltage: 20 V
Scantime: 0.9 s
Interscan delay: 0.1 s
Detection method: quadrupole

3.4 Sample preparation:

An aliquote of Melanotan 2 (1 mg) was dissolved in 1 mL of water.
Injection: 1.0 µL

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3.5 Mass Spectra of Melanotan 2 (PO 2024196)

UPLC1 ZQ

Channel Description ACQUITY TUV ChA 214nm

Vial : 1:F,2 Vol. : 1.00 ul

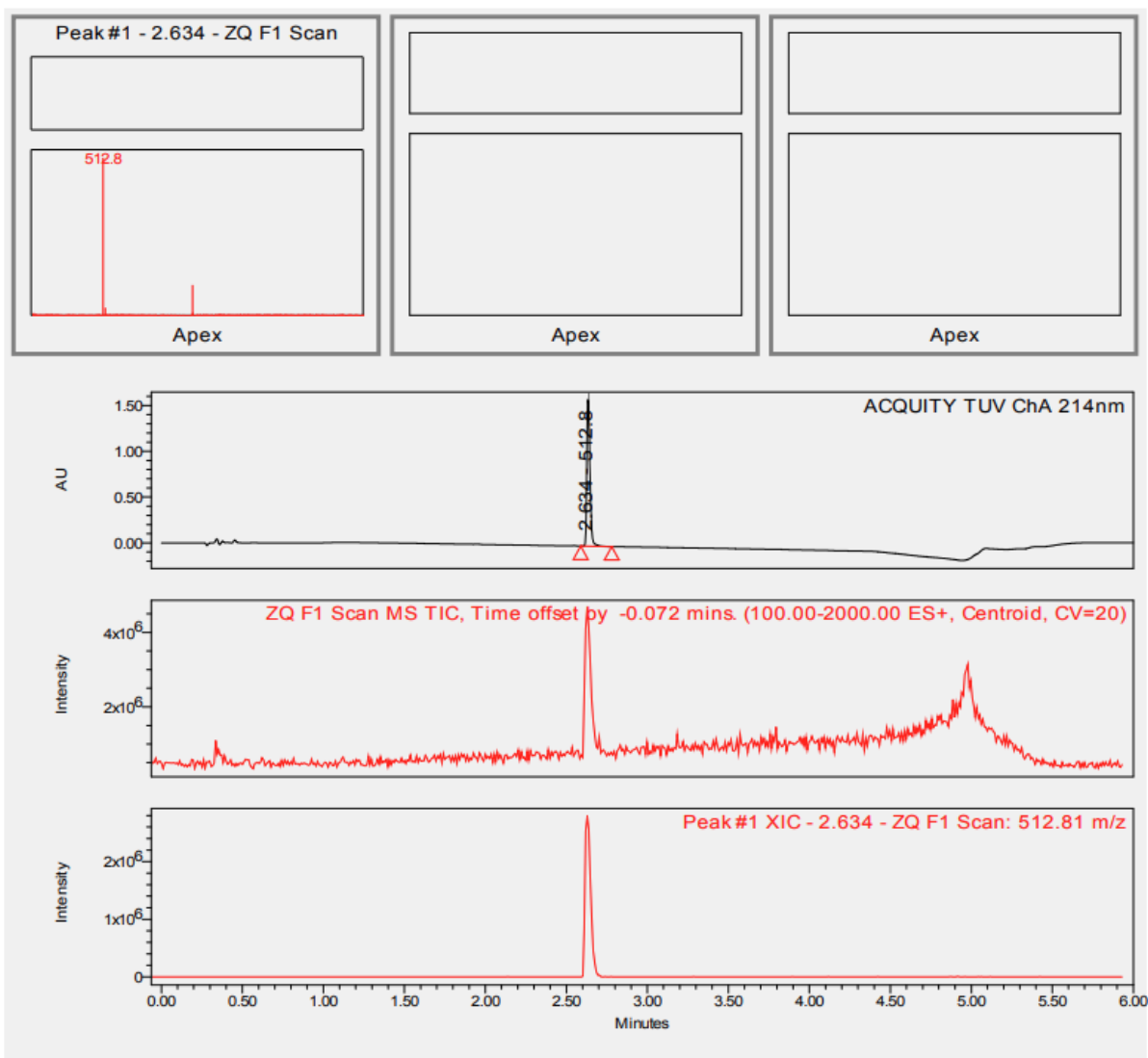
Sample: Melanotan 2 (PO-2024196)

Date Acquired 1/21/2025 9:33:13 AM CET

Date Processed 1/22/2025 2:50:45 PM CET

Acq Method Set :

Gr5_60_MS_4min_0_45ml_K2_me_s



Theoretical values of m/z:

Peptide m/z	[M+2H] ²⁺	[M+3H] ³⁺	[M+4H] ⁴⁺	[M+5H] ⁵⁺	[M+6H] ⁶⁺	[M+7H] ⁷⁺
1023,5	512,8	342,2				
Found	512,8	NA				

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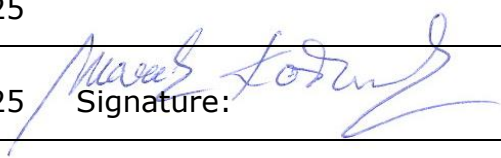
CONCLUSION:

The sample Melanotan 2 (Batch No. 2024196) was analyzed for peptide content, UV purity and identity.

Peptide content is 79.0 % (7,9 mg in 10 mg)

Purity is 99.75 % (UPLC at 214 nm).

MS identity complies with theoretical calculation of m/z values.

ANALYSIS COMPLETED:	Date: 21.01.2025
Issued by QC:	Date: 23.01.2025 Signature: 

Test Certificate No.: 3305/2024/1
Replaces Test Certificate 3305/2024

Testing laboratory Eurofins BioPharma Product Testing Slovakia s.r.o. Radlinského 9, 811 07 Bratislava IČO: 31 329 209 Place of work: Testing laboratory Bratislava Kollárovo nám. 9, 811 07 Bratislava Tel.: 0911 810 533 cspharmask@bpt.eurofinseu.com, www.eurofins.sk	Customer PARTICLE s.r.o. Kolonada 4490/18 984 01 Lučenec
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Product information No.: 3305
Sample description: Melanotan 2 (PO-2024196)
Gross weight (volume): 10 mg glass vials

Information about Sampling:
Sampler: customer

Sample reception date: 25.03.2024 **Date of Testing:** 25.03.2024 - 04.04.2024 **Certificate issued on:** 05.04.2024

Microbiological tests

Parameter	Unit	Allowed Value	Measured Value	Uncertainty*	Testing method	E	SL	TT
Total Aerobic Microbial Count	CFU /5mg	-	0	-	ŠPP MB.M.140.PN	-	PN	A
Total Combined Yeasts/Moulds Count	CFU /5mg	-	0	-	ŠPP MB.M.140.PN	-	PN	A
Bacterial endotoxins	IU / mg	-	<0,5	-	ŠPP MB.M.146.PN	-	PN	A

Notes:

E - evaluation	TT - type of test
S - satisfied	(A) - accredited sampling
NS - not satisfied	A - accredited test executed at the own test laboratory
ŠPP, LS-PP-CH - Standard operation procedure	N - non accredited test executed at the own test laboratory
ND - not detected by given method	SA - accredited test executed under the subcontract
CFU - Colony forming unit	SN - unaccredited test executed under the subcontract
NM - necessary quantity	TM - testing outside the laboratory at the customer
m - the highest allowed value at the case of one sample	
M, c - "M" highest allowed value for the number "c" at the case of 5 sample's evaluation	
* - uncertainty determined by extension coefficient k=2 (with probability of 95%) does not include the uncertainty of sampling.	
- uncertainty given in units of analysed parameter reflects the uncertainty to the result of measurement.	
- uncertainty given in % reflects the uncertainty from the result of measurement.	
SL - analysing laboratory: BA-Bratislava, PN-Piešťany	

Disclaimer: The laboratory is not responsible for the information provided by the customer, which can affect the validity of the results.
If the sample has been provided by the customer, the results refer to the sample as it was received.
Gauges and measuring equipment used for testing were calibrated or attested in accordance with the valid metrological instructions.
The above mentioned test results refer to the tested sample only!
The result given in this Test Certificate and marked as non accredited test shall not be a subject of accreditation.
The result given in this Test Certificate and marked as sub- delivery is the result of a Subcontractor's gauging made under the terms and conditions of a contract concluded eith him.
It's not possible reproduce or incorporate the test certificate into promotional materials without laboratory written authorization!

Test results have been electronically validated by: Ing. Zuzana Šperková, PhD.

Worked out by: Ing. Zuzana Šperková, PhD.
Document No.: 2438/2024