

ANALYTICAL CERTIFICATE

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Sample name	EPITHALON
Batch No.	2024200
Sample No.	01
Sequence	Ala-Glu-Asp-Gly
Manufacturing date	NA
Submitter of analytical request	Particle s.r.o., Slovakia

1. Peptide content by HPLC/CAD:

1.1 HPLC Instrument:

Pump: Thermo Scientific Vanquish, Dual Pump F VF-P32-A
Sampler: Thermo Scientific Vanquish, Split Sampler FT VF-A10-A
Detectors: Thermo Scientific Vanquish, Diode Array Detector FG VF-D11-A
Thermo Scientific Vanquish, Charged Aerosol Detector H VH-D20-A

1.2 HPLC conditions:

Eluents: A – 0,1% TFA MilliQ water
B – 0,1% TFA MeCN
Flow rate: 0,45 mL/min
Gradient:

Pump Right – delivers the flow to column for gradient separation

Time	B (%)
0	2
4	95
4.5	95
5	2

Pump Left – creates the reverse gradient and dilutes the flow to CAD detector to make the eluent composition constant

Time	B (%)
0	98
4	5
4.5	5
5	98

Column: ACQUITY UPLC® BEH C18 1.7 µm, 2.1 x 50 mm

1.3 Sample preparation:

The whole amount of EPITHALON (5 mg) was dissolved in 1 mL of H₂O.
Injection: 0,4 µL

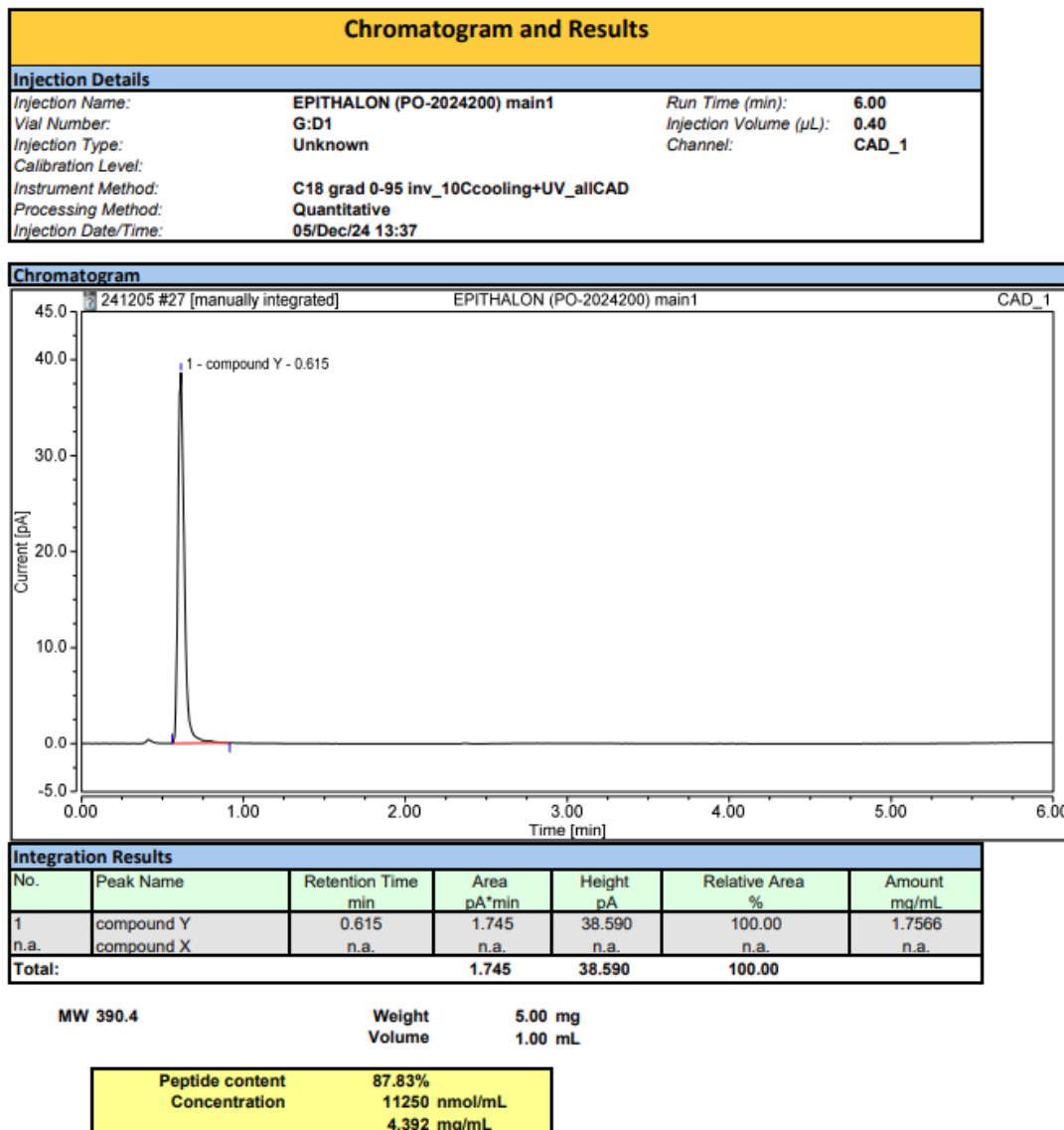
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1.4 Chromatograms and calibration curve:

Instrument: V01_LC Sequence: 241205

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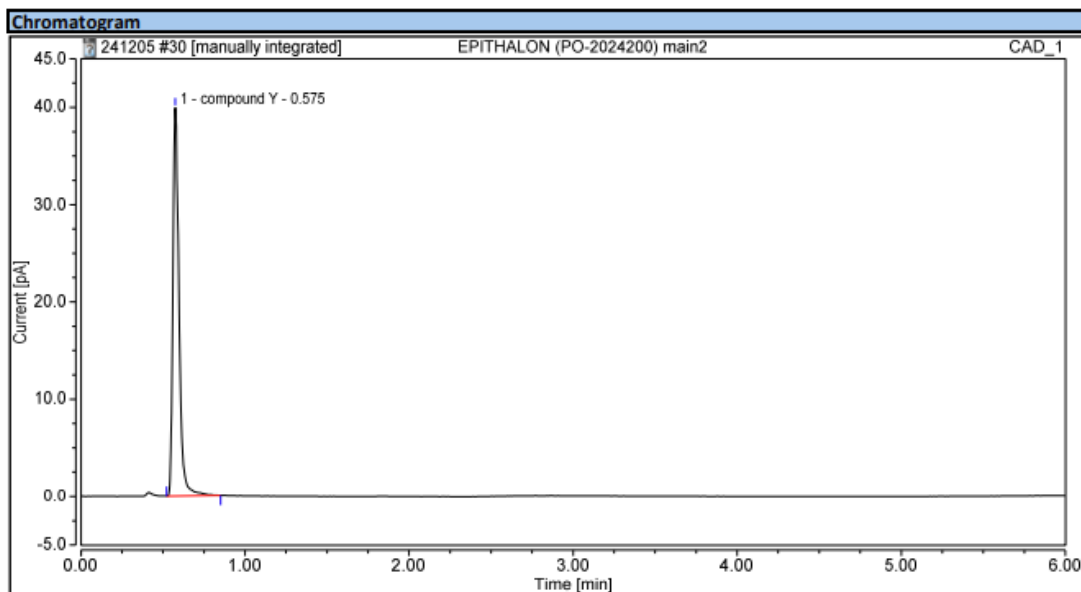
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Instrument: V01_LC Sequence: 241205

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Chromatogram and Results			
Injection Details			
Injection Name:	EPITHALON (PO-2024200) main2	Run Time (min):	6.00
Vial Number:	G:D2	Injection Volume (µL):	0.40
Injection Type:	Unknown	Channel:	CAD_1
Calibration Level:			
Instrument Method:	C18 grad 0-95 inv_10Ccooling+UV_allCAD		
Processing Method:	Quantitative		
Injection Date/Time:	05/Dec/24 14:22		



Integration Results						
No.	Peak Name	Retention Time min	Area pA*min	Height pA	Relative Area %	Amount mg/mL
1	compound Y	0.575	1.728	39.917	100.00	1.7419
n.a.	compound X	n.a.	n.a.	n.a.	n.a.	n.a.
Total:			1.728	39.917	100.00	

MW 390.4 Weight 5.00 mg
Volume 1.00 mL

Peptide content	87.10%
Concentration	11156 nmol/mL
	4.355 mg/mL

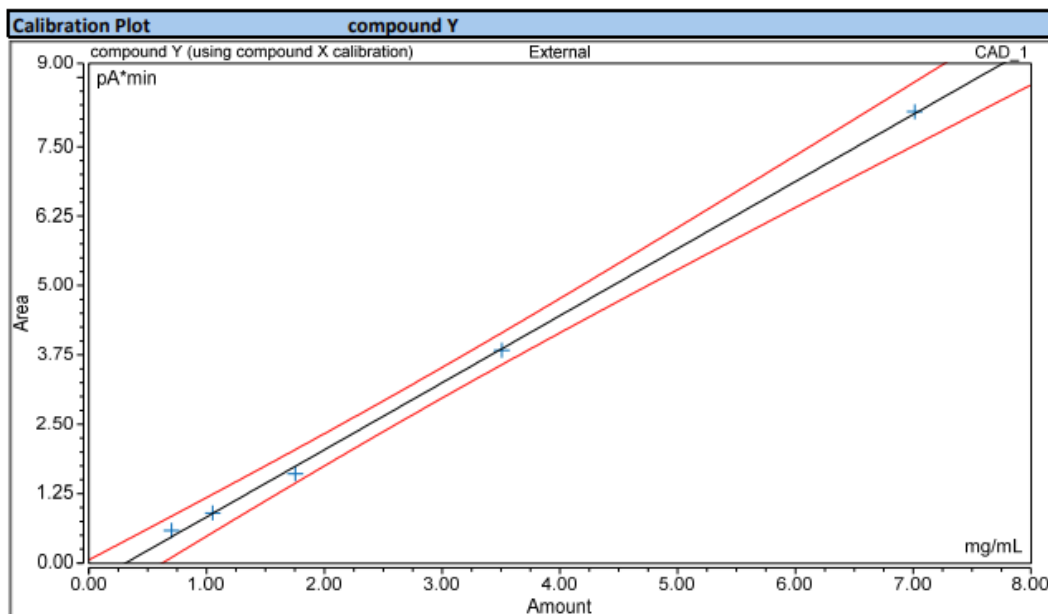
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Instrument: V01_LC Sequence: 241205

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Calibration				
Calibration Details		compound Y		
Calibration Type	Use compound X	Offset (C0)	-0.3769	
Evaluation Type	Area	Slope (C1)	1.2082	
Number of Calibration Points	5	Curve (C2)	0.0000	
Number of disabled Calibration Points	0	R-Square	0.9992	



Calibration Results				
compound Y				
No.	Injection Name	Volume μ L	Area pA*min CAD_1 compound Y	Amount mg/mL CAD_1 compound Y
	std aspart 2.12.	0.20	n.a.	n.a.
	std aspart	0.30	n.a.	n.a.
	std aspart	0.50	n.a.	n.a.
	std aspart	1.00	n.a.	n.a.
	std aspart	2.00	n.a.	n.a.

$$Y = 1.2082 X - 0.3769$$

1.4 Results:

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.
EPITHALON	5	NA	4,38	NA	87.5 %

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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 0% B to 20% B in 16 min, according to chromatogram results
Column: Waters Acquity BEH, C-18, 1.7µm, 2.1mm x 150mm
 Part No 186002353

2.3 Sample preparation:

An aliquote of EPITHALON (0.5 mg) was dissolved in 1 mL of H₂O.
Injection: 10 µL

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2.4 Chromatogram of EPITHALON (PO-2024200)

Sample information

UPLC2

Channel Description ACQUITY TUV ChA 214nm

Vial : 1:A,3 Vol. : 10.00 ul

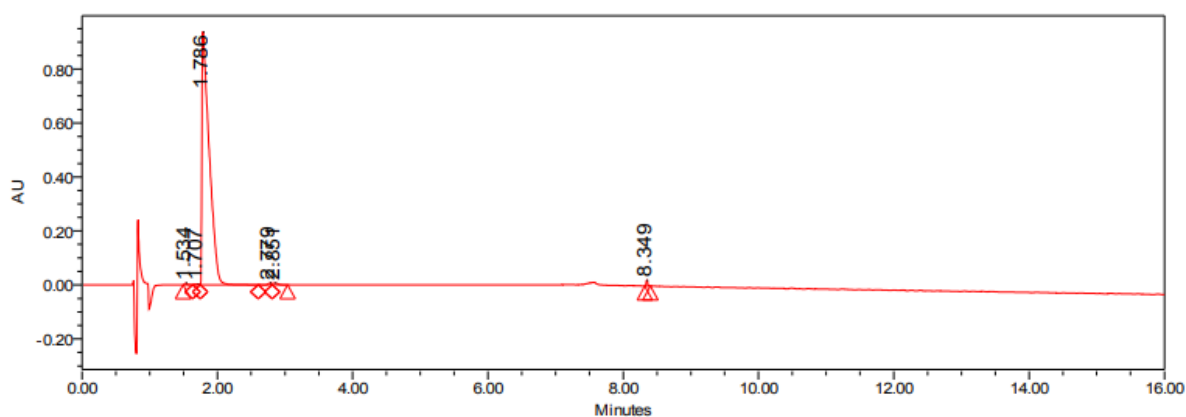
Sample: Epithalon (PO-2024200)

Date Acquired 12/6/2024 8:56:05 AM CET

Date Processed 12/11/2024 11:02:31 AM CET

Acq Method Set :

Gr_0_20_16min_40C_0_4_K1_met_s



	RT	Area	Height (μV)	% Area
1	1.534	16242	6268	0.23
2	1.707	12192	2938	0.17
3	1.786	7042073	937746	98.35
4	2.779	23601	6239	0.33
5	2.851	34495	6475	0.48
6	8.349	31567	21461	0.44

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient : 0-0/5min, 5-16min0--> 20% B, , 0.4ml/min

Acquity UPLC BEH130, 1.7μm, 2.1 x 150 mm column

column own temp. = 40 °C

2.5 Result of purity assessment

The overall purity is 98.35 % at 214 nm.

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3. Endotoxin test:

3.1 Description:

Test tubes: Gel Clot Lyophilized Amebocyte Lysate Single Test in Vial
Manufacturer: Xiamen Bioendo Technology Co., Ltd.
Lot: 24061152
Content: 0.2ml endotoxin-specific Amebocyte Lysate which includes beta-glucan inhibitor in the formulation
Sensitivity of test: 0.5 EU/mL
Sample sensitivity level: 5 EU/mg

4.2 Sample preparation and test:

A sample peptide is dissolved in endotoxin-free water to form a concentration of 0.5 mg/mL. 200 µL of this solution is then transferred to the Amebocyte Lysate Single Test tube and incubated at 37 °C for 60 min. Immediately after incubation the test tube is slowly turned upside down.

A solid gel clot which doesn't come down immediately indicates **positive** result (meaning that endotoxins are above the current sensitivity level).

An absence of solid gel clot so the solution freely flows down from the bottom of test tube indicates **negative** result (meaning that endotoxin are below the current sensitivity level).

3.3 Result:

NEGATIVE (-)


CONCLUSION:

The sample EPITHALON (Batch No. 2024200) was analyzed for peptide content, UV purity at 214 nm and endotoxins.

Peptide content is 87.5 % (4.38 mg in 5 mg)

Purity is 98.35 % (UPLC at 214 nm).

Endotoxin test (sensitivity level 5 EU/mg) - NEGATIVE.

ANALYSIS COMPLETED:	Date: 11.12.2024
Issued by QC:	Date: 17.12.2024  Signature:

Analytical report AR-24-KT-043631-02



Testing laboratory:

Eurofins Environment Testing Slovakia s.r.o.
 Robotnícka 820/36, 039 01 Turčianske Teplice
 IČO: 53 248 376
 Place of work:
Accredited testing laboratory Turčianske Teplice
 Robotnícka 820/36, 039 01 Turčianske Teplice
 tel: 043/490 1562
 RegistrationEnviroSK@etcee.eurofins.com, www.eurofins.sk

Customer:

PARTICLE s.r.o.
 Kolonáda 4490/18
 984 01 Lučenec
 SLOVAKIA

Date of Sample Receipt: 22.11.2024 Date of Testing: 22.11.2024 - 26.11.2024

Issue date: 03.12.2024

Information about Sampling:

Sampler: customer

Sample information: 104-2024-00048097

Sample description: Epithalon (PO-2024200)

Material: Peptidy

Physical and chemical tests

Parameter	Unit	Allowed Value	Measured Value	Uncertainty of Method measurement*	Testing method	E	SL	TT
Arsenic (As)	mg/kg	-	<1,5	-	ICP-MS	LS-PP-CH-85	-	TR A
Cadmium (Cd)	mg/kg	-	<0,2	-	ICP-MS	LS-PP-CH-85	-	TR A
Lead (Pb)	mg/kg	-	<0,5	-	ICP-MS	LS-PP-CH-85	-	TR A
Mercury (Hg)	mg/kg	-	<0,3	-	ICP-MS	LS-PP-CH-85	-	TR A

Notes:

E - evaluation
 S - satisfied
 NS - not satisfied
 (A) - accredited sampling
 (SA) - accredited sampling executed under the subcontract
 ŠPP - Standard operation procedure
 ND - not detected by given method
 LOQ, LQ – limit of quantification
 CFU - Colony forming unit
 NM - necessary quantity
 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
 * - measurement uncertainty – sampling and analysis – determined by extension coefficient k=2 (with probability of 95%). If sample is taken by the customer uncertainty of sampling is not available.
 - uncertainty given in % reflects the uncertainty from the result of measurement.
 ** - Acceptable to consumers and no abnormal change
 SL - analysis laboratory: NZ-Nové Zámky, TR-Turčianske Teplice, RK-Ružomberok, TV-Trebišov
 TT - type of test
 A - accredited test executed at the own test laboratory
 N - non accredited test executed at the own test laboratory
 SA - accredited test executed under the subcontract
 SN - unaccredited test executed under the subcontract
 (TM) - testing outside the laboratory at the customer

Disclaimer:

Laboratory is a disclaimer when the information is supplied by the customer (#) and can affect the validity of 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 Analytical report and marked as non accredited test shall not be a subject of accreditation. The result given in this Analytical report and marked as sub- delivery is the result of a Subcontractors gauging made under the terms and conditions of a contract concluded with him. This Analytical report shall not be reproduced except in full colour version, without written approval of the laboratory. SNAS is a Signatory to the Multilateral Agreement MRA ILAC.

Responsible for correctness:

Michaela Ruttkayová
Specialist worker

Worked out by: Andrea Podušelová

Validity check of document

**Test Certificate approved by**Michaela Ruttkayová
Specialist worker