

# ANALYTICAL CERTIFICATE

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<b>Sample name</b>	<b>GHK-Cu</b>
<b>Batch No.</b>	<b>2025240</b>
<b>Sample No.</b>	<b>NA</b>
<b>Sequence</b>	<b>Gly-His-Lys (Cu<sup>2+</sup> salt)</b>
<b>Manufacturing date</b>	<b>NA</b>
<b>Submitter of analytical request</b>	<b>Particle s.r.o., Slovakia</b>

## 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 $\mu$  C4-BIO 300 A, 4.6 x 100 mm  
Serial No 221258

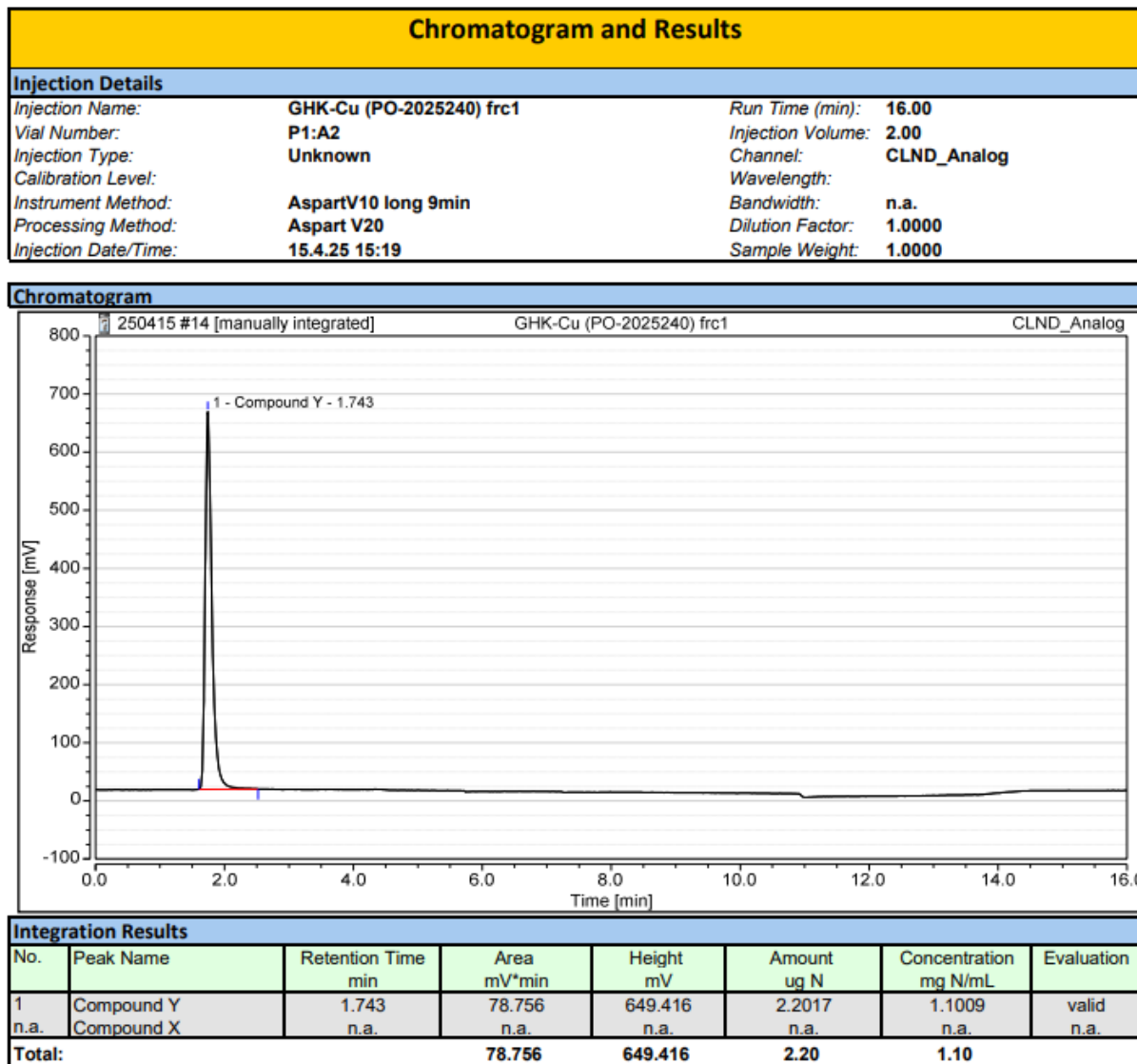
### 1.3 Sample preparation:

The whole amount of GHK-Cu (50 mg) was dissolved in 10 mL of MilliQ water.  
Injection: 2  $\mu$ L

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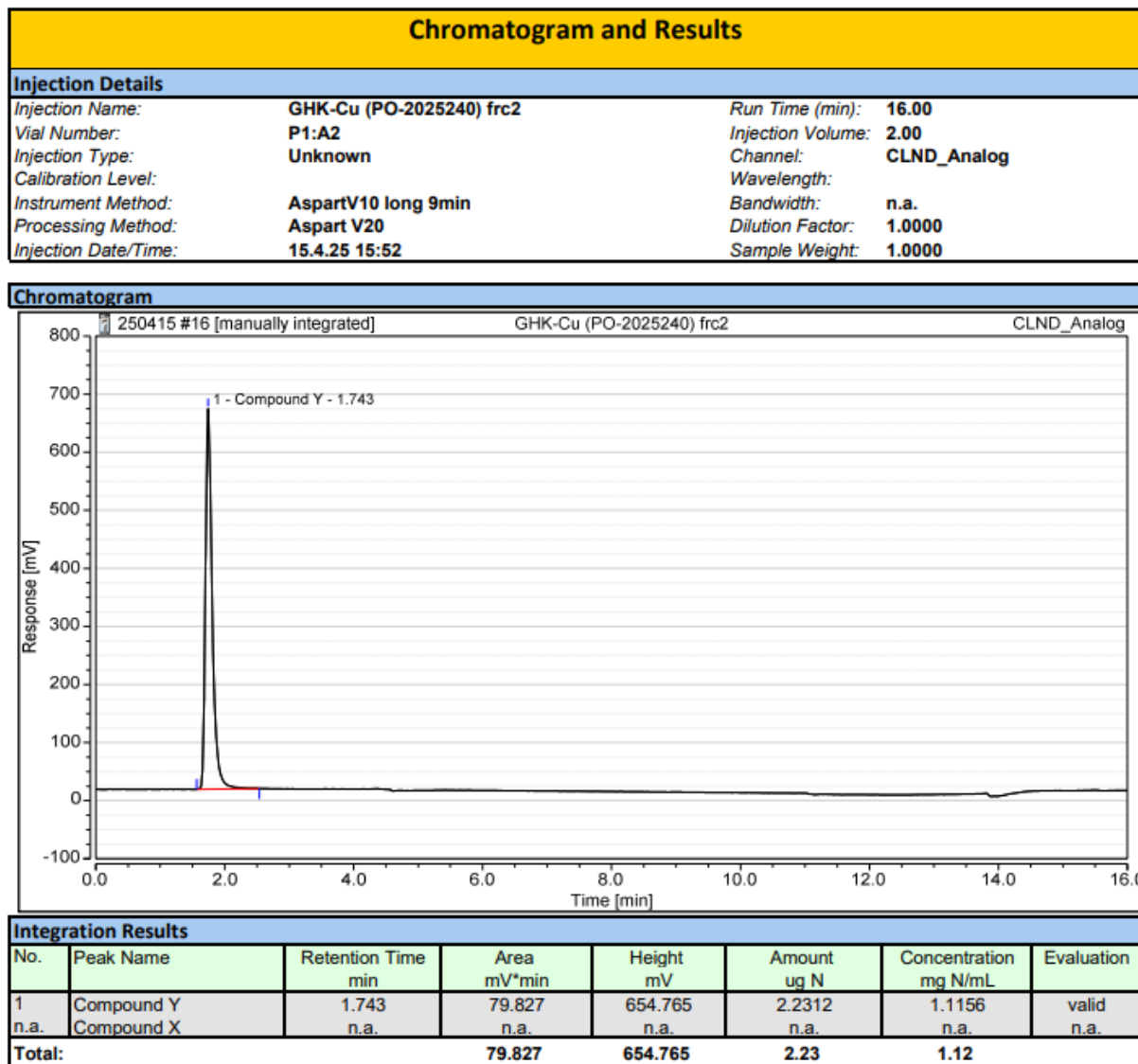
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## 1.4 Chromatograms and calibration curve:



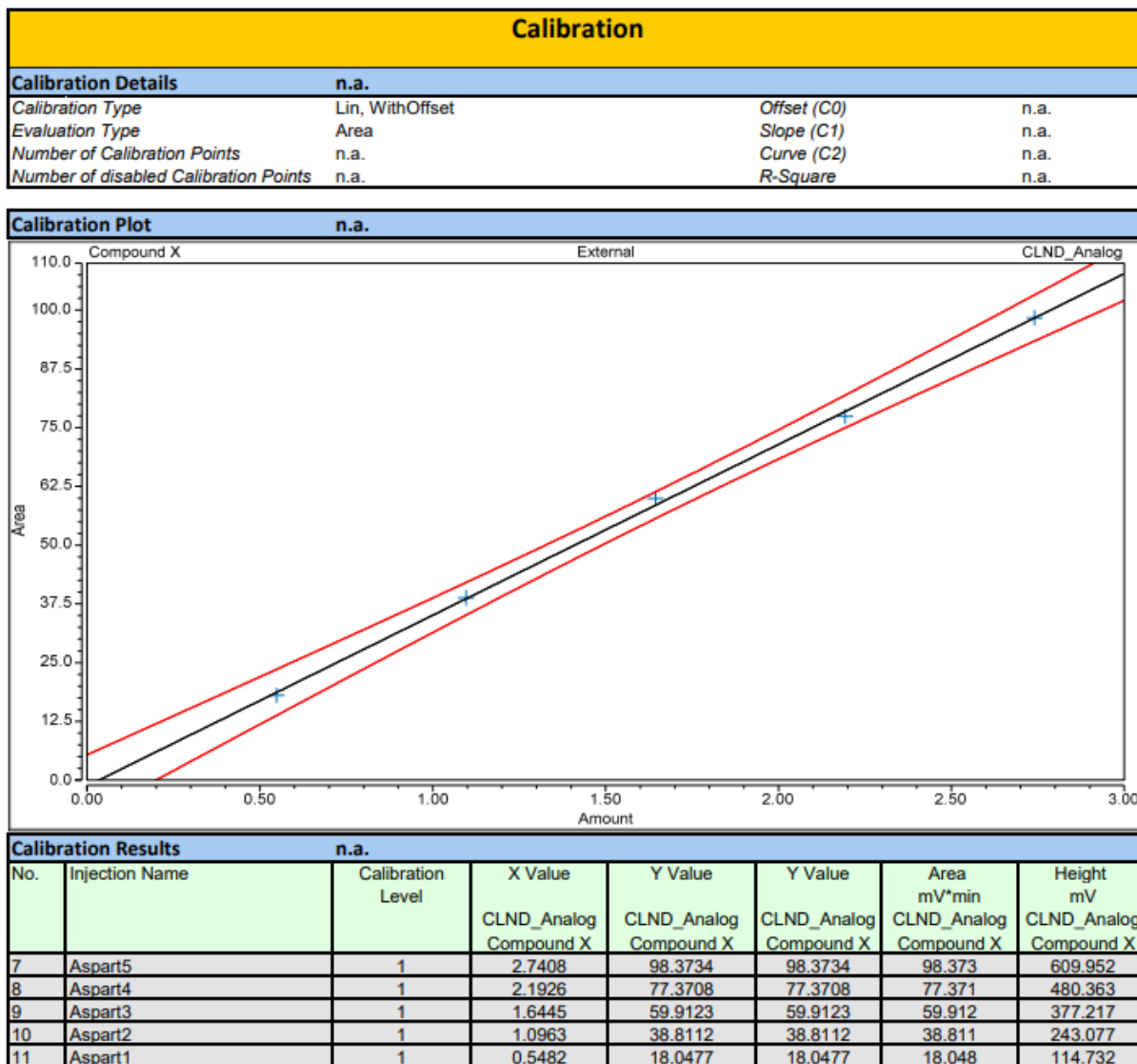
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## 1.4 Results:

NNC: GHK-Cu (PO-2025240)		Salt:	AcOH
MW (calculated) g/mol	N content (calculated) %	N conc. (measured) mg × N/ml	
403,93	15,73	1,1083	
Theoretical Volume ml		Lyophilizate amount mg	
10,00		50,00	
Peptide concentration mg/ml                  nmol/ml		Quantified amount mg                          nmol	
7,05	17442	70,5	174 423
Peptide content assay %			
140,9			

## 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 the label.
GHK-Cu	50	108,2	70,5	65,1	140,9 %

**Content of the peptide was analyzed larger than the stated value on label presumably because larger amount of lyophilizate was put into the vials.**

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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:                0.45 mL/min  
Gradient:                from 0% B to 0% B in 4 min, according to chromatogram results  
Column:                Waters Acquity HSS T3, C-18, 1.8µm, 2.1mm x 150mm  
                              Part No 186003540

#### **2.3 Sample preparation:**

An aliquote of GHK-Cu (2,1 mg) was dissolved in 1 mL of MilliQ water.  
Injection:                1,0 µL

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## 2.4 Chromatogram

blank

### Sample information

#### UPLC1 ZQ

Channel Description ACQUITY TUV ChA 214nm

Vial : 1:A,1 Vol. : 1.00 ul

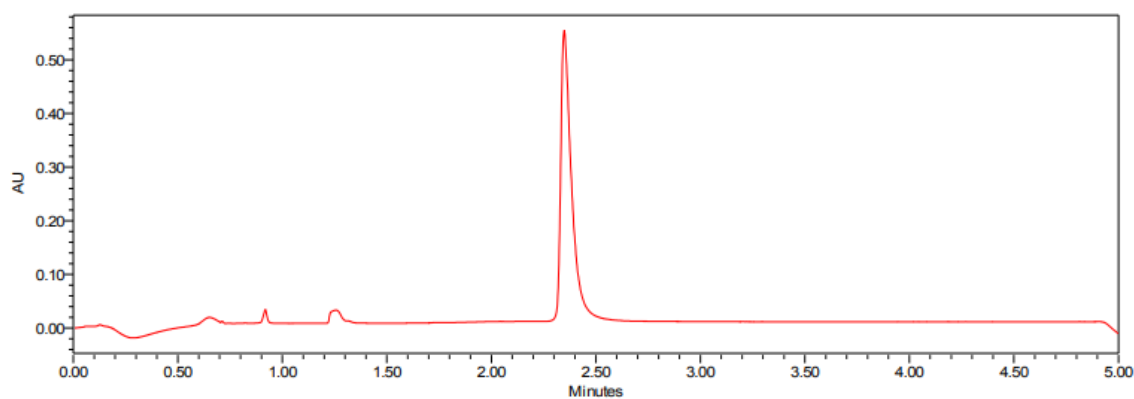
**Sample: blank**

Date Acquired 4/15/2025 11:05:39 AM CEST

Date Processed 4/15/2025 1:35:08 PM CEST

Acq Method Set :

Gr0\_0\_MS\_4min\_0\_45ml\_K1\_me\_s



	RT	Area	Height (μV)	% Area
1				

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient :

0.0 - 4 min 0 % B

4 - 5 min 5 - 95 % B

5 - 5.5 min 95 % B

5.5 - 6 min 95 - 0 % B

6 - 8 min 0 % B

0.45ml/min

Acquity UPLC HSS T3, 1.8μm, 2.1 x 150 mm column

column oven temp. = 40 °C

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**Standard of GHK-Cu acetate (Cayman Chemicals, Batch No 0566241-6)**

## Sample information

### UPLC1 ZQ

Channel Description ACQUITY TUV ChA 214nm

Vial : 1:F,1 Vol. : 0.70 ul

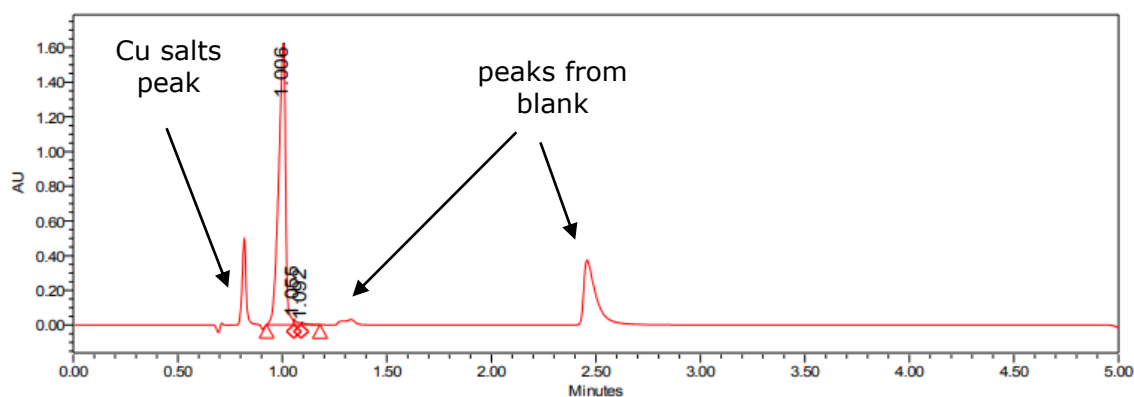
**Sample: GHK-Cu (Cayman)**

Date Acquired 4/15/2025 11:41:49 AM CEST

Date Processed 4/15/2025 1:34:22 PM CEST

Acq Method Set :

Gr0\_0\_MS\_4min\_0\_45ml\_K1\_me\_s



	RT	Area	Height (μV)	% Area
1	1.006	3733679	1628768	98.73
2	1.055	32176	27034	0.85
3	1.092	15974	10439	0.42

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient :

0.0 - 4 min 0 % B

4 - 5 min 5 - 95 % B

5 - 5.5 min 95 % B

5.5 - 6 min 95 - 0 % B

6 - 8 min 0 % B

0.45ml/min

Acquity UPLC HSS T3, 1.8μm, 2.1 x 150 mm column  
column oven temp. = 40 °C



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## Chromatogram of GHK-Cu (PO-2025240)

### Sample information

#### UPLC1\_ZQ

Channel Description ACQUITY TUV ChA 214nm

Vial : 1:F,2 Vol. : 1.30 ul

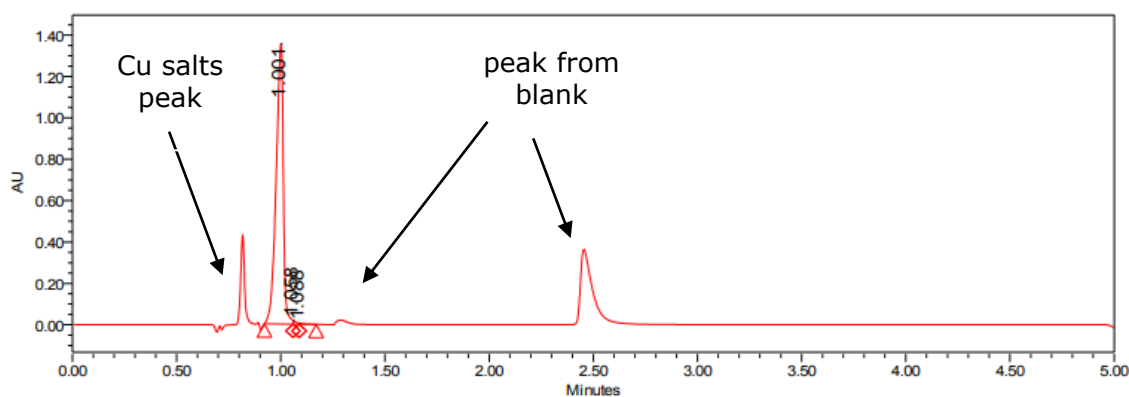
**Sample: GHK-Cu (PO-2025240)**

Date Acquired 4/15/2025 11:52:46 AM CEST

Date Processed 4/15/2025 1:36:19 PM CEST

Acq Method Set :

Gr0\_0\_MS\_4min\_0\_45ml\_K1\_me\_s



	RT	Area	Height (μV)	% Area
1	1.001	3253381	1359353	99.23
2	1.058	17770	18263	0.54
3	1.088	7556	5425	0.23

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient :

0.0 - 4 min 0 % B

4 - 5 min 5 - 95 % B

5 - 5.5 min 95 % B

5.5 - 6 min 95 - 0 % B

6 - 8 min 0 % B

0.45ml/min

Acquity UPLC HSS T3, 1.8μm, 2.1 x 150 mm column  
column oven temp. = 40 °C

## 2.5 Result of purity assessment

After subtraction of blank chromatogram and subtraction of salts peaks from chromatogram of standard the overall purity is 99.23 % and fully corresponds to the standard sample from Cayman Chemicals.

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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 GHK-Cu (1 mg) was dissolved in 1 mL of water.  
Injection:                      1.0 µL

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## 3.5 Mass Spectra of GHK-Cu (PO-2025240)

### Sample information

#### UPLC1 ZQ

Sample: **GHK-Cu(PO-2025240)**

Channel Description ACQUITY TUV ChA 214nm

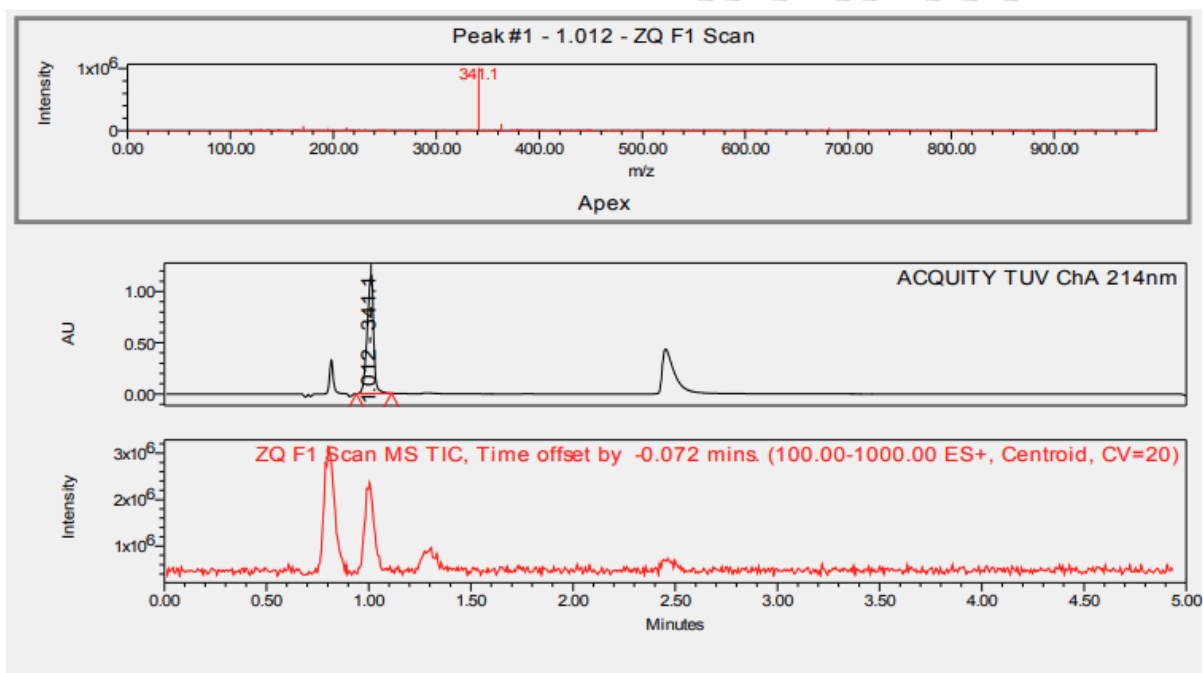
Date Acquired 4/17/2025 11:47:32 AM CEST

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

Date Processed 4/17/2025 11:53:14 AM CEST

Acq Method Set :

GrO\_0\_MS\_4min\_0\_45ml\_K1\_me\_s



**MS Result Table**

	Name	RT	Base Peak (m/z)
1		1.012	341.06

UPLC conditions:

A: 0.05% TFA in water

B: 0.05% TFA in acetonitrile

Gradient :

0.0 - 4.0 min 0 % B

4.0 - 5.0 min 0 - 95 % B

5.0 - 5.5 min 95 % B

5.5 - 6.0 min 95 - 0 % B

6.0 - 16.0 min 0 % B

Theoretical values of m/z:

Peptide MW	$[M+1H]^+$	$[M+2H]^+$	$[M+4H]^+$	$[M+5H]^+$	$[M+6H]^+$	$[M+7H]^+$
<b>340,2</b>	341,2	171,1				
<b>Found</b>	<b>341,1</b>	<b>NA</b>				

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### 4. Endotoxin test:

#### 4.1 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).

#### 4.3 Result:

NEGATIVE (-)

#### CONCLUSION:

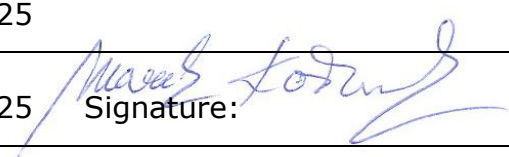
**The sample GHK-Cu (Batch No. 2025240) for peptide content, UV purity at 214 nm, identity and endotoxins.**

**Peptide content is 140,9 % (70,5 mg in 50 mg stated on label)**

**Purity is 99.23 % (UPLC at 214 nm) – fully identical with standard.**

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

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

<b>ANALYSIS COMPLETED:</b>	Date: 15.04.2025
<b>Issued by QC:</b>	Date: 17.04.2025  Signature:

# Analytical report AR-25-KT-013008-04



## 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: 10.04.2025 Date of Testing: 10.04.2025 - 14.04.2025

Issue date: 28.04.2025

**Sample information:** 104-2025-00014056  
# Sample description: GHK-Cu(PO-2025240)  
Material: Food  
Material: POTRAVINY

## Physical and chemical tests

Parameter	Unit	Allowed Value	Measured Value	Uncertainty of Method measurement*	Testing method	E	SL	TT
Arsenic (As)	mg/kg	max, 1,5	0,058	25%	ICP-MS	LS-PP-CH-85	S	TR A
Cadmium (Cd)	mg/kg	max, 0,2	<0,10	-	ICP-MS	LS-PP-CH-85	S	TR A
Lead (Pb)	mg/kg	max, 0,5	<0,30	-	ICP-MS	LS-PP-CH-85	S	TR A
Mercury (Hg)	mg/kg	max, 0,3	0,02	25%	ICP-MS	LS-PP-CH-85	S	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:

RNDr. Hana Benkovičová  
Deputy Head of Laboratory Turčianske Teplice

Worked out by: Zuzana Kubisová

Validity check of document

**Test Certificate approved by**

RNDr. Hana Benkovičová  
Deputy Head of Laboratory Turčianske Teplice

