

# 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>Adria Peptides d.o.o.</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

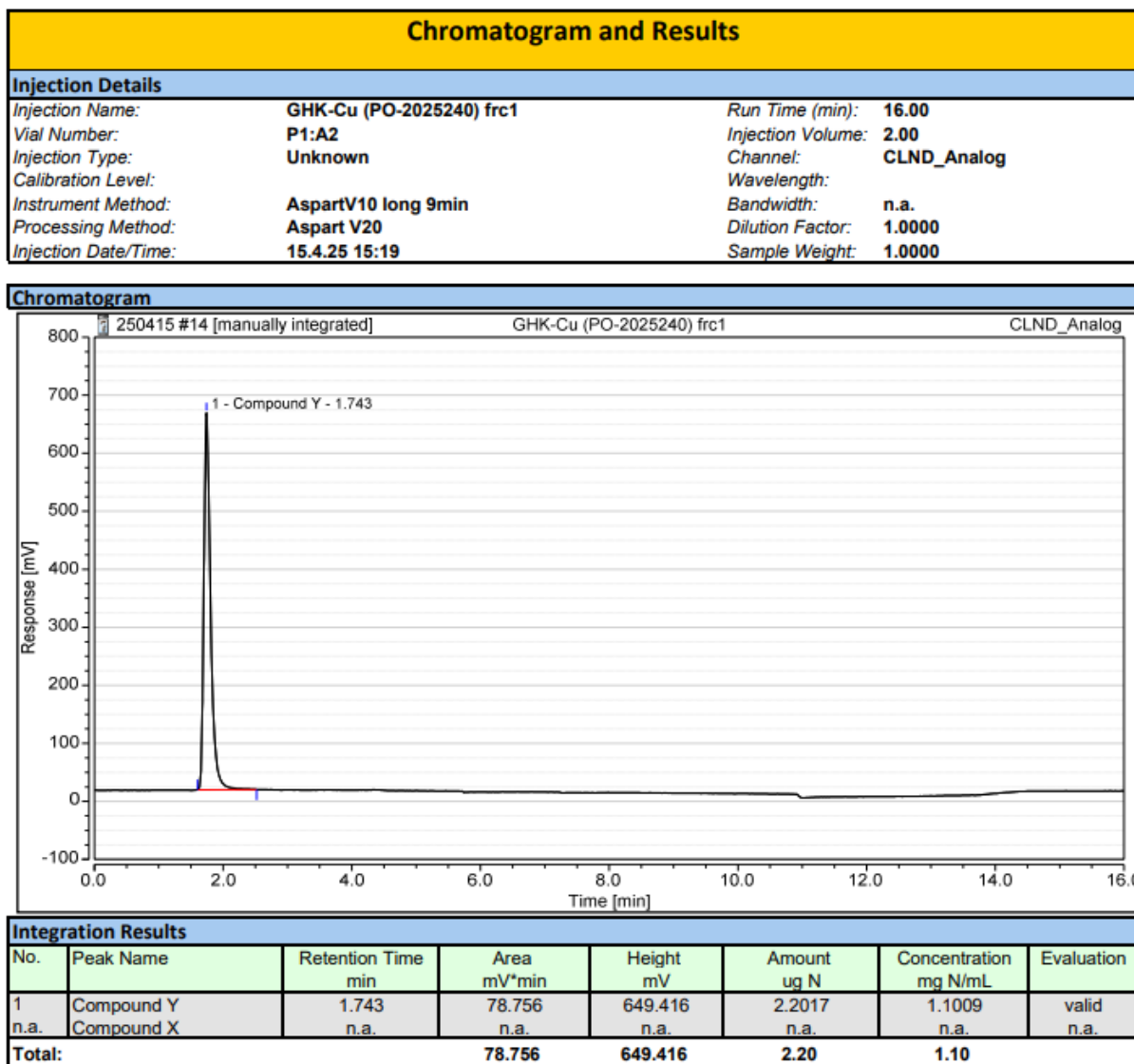
### 1.3 Sample preparation:

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

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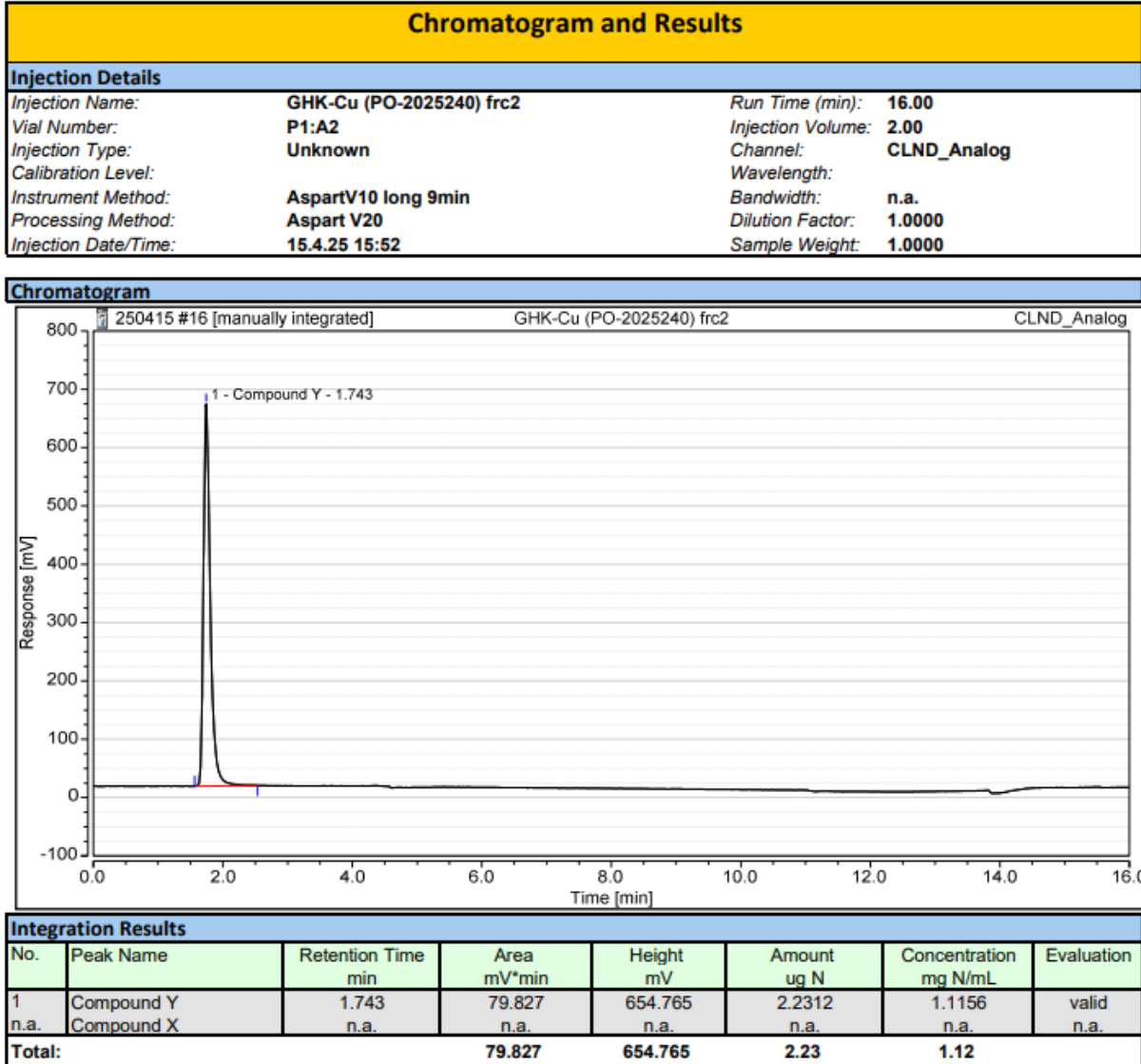
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## 1.4 Chromatograms and calibration curve:



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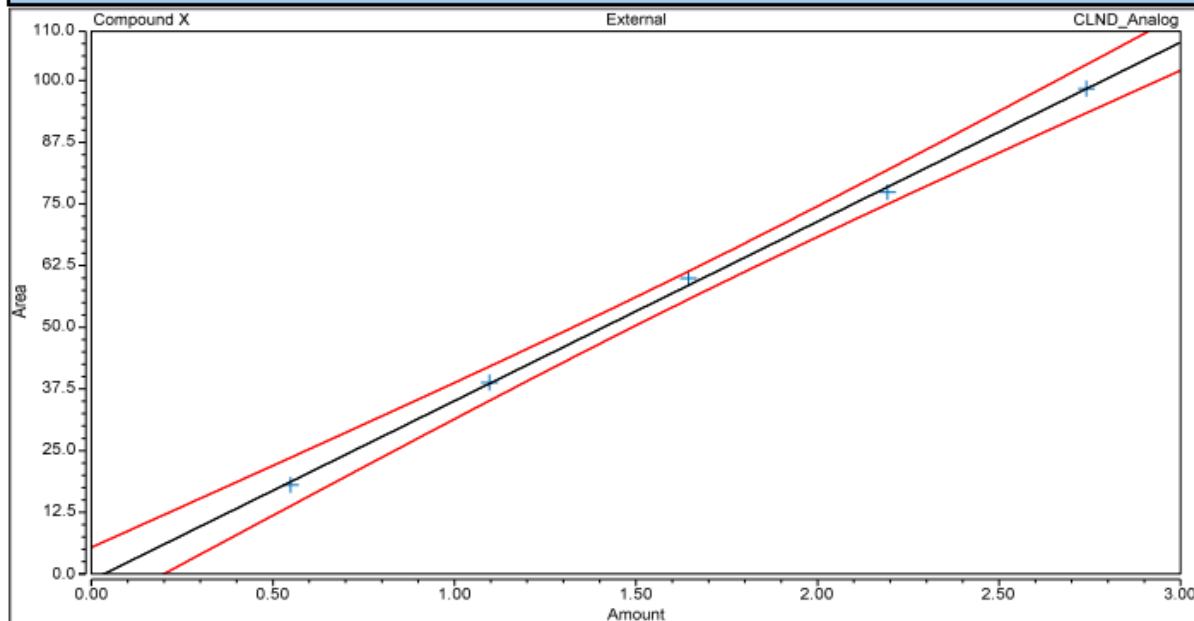
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## Calibration

Calibration Details		n.a.	
Calibration Type	Lin, WithOffset	Offset (C0)	n.a.
Evaluation Type	Area	Slope (C1)	n.a.
Number of Calibration Points	n.a.	Curve (C2)	n.a.
Number of disabled Calibration Points	n.a.	R-Square	n.a.

### Calibration Plot



### Calibration Results

Calibration Results		n.a.					
No.	Injection Name	Calibration Level	X Value	Y Value	Y Value	Area	Height
			CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X	CLND_Analog Compound X
7	Aspart5	1	2.7408	98.3734	98.3734	98.373	609.952
8	Aspart4	1	2.1926	77.3708	77.3708	77.371	480.363
9	Aspart3	1	1.6445	59.9123	59.9123	59.912	377.217
10	Aspart2	1	1.0963	38.8112	38.8112	38.811	243.077
11	Aspart1	1	0.5482	18.0477	18.0477	18.048	114.732

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### 1.4 Results:

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

### 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	<b>70,5</b>	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

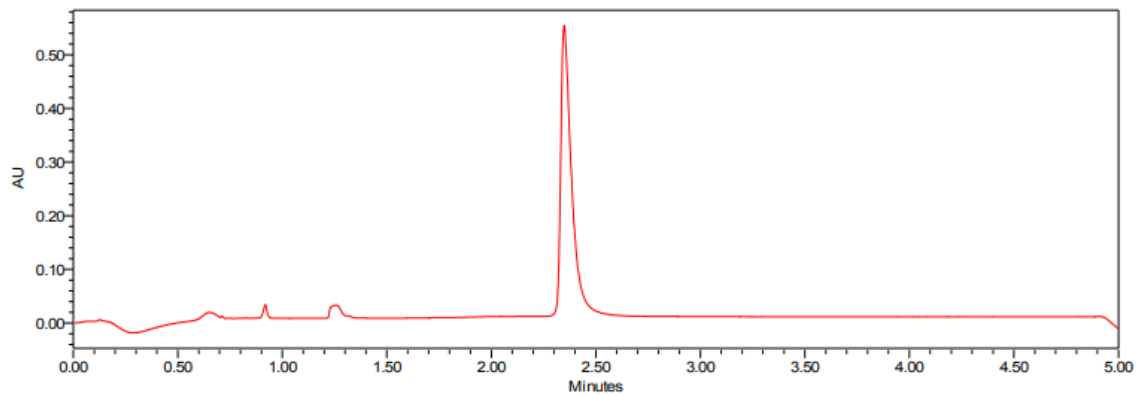
**Sample: blank**

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 acetoni trile

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, 1.8um, 2.1 x 150 mm column

column own 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

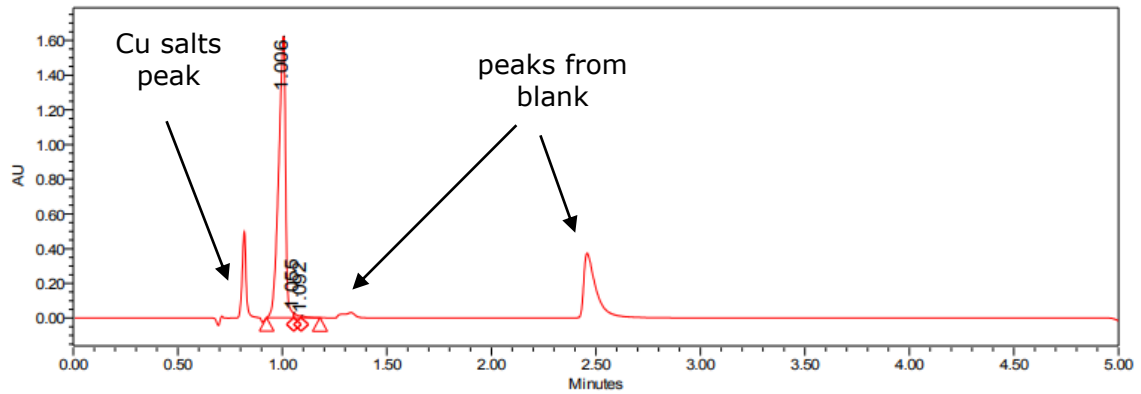
**Sample: GHK-Cu (Cayman)**

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

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

Acq Method Set :

GrO\_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 acetoni trile

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 own 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

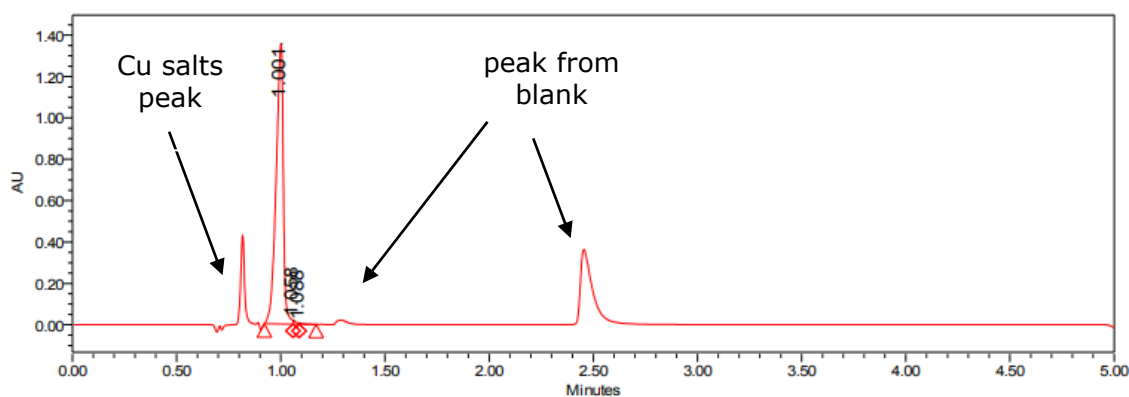
Sample: **GHK-Cu (PO-2025240)**

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 own 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

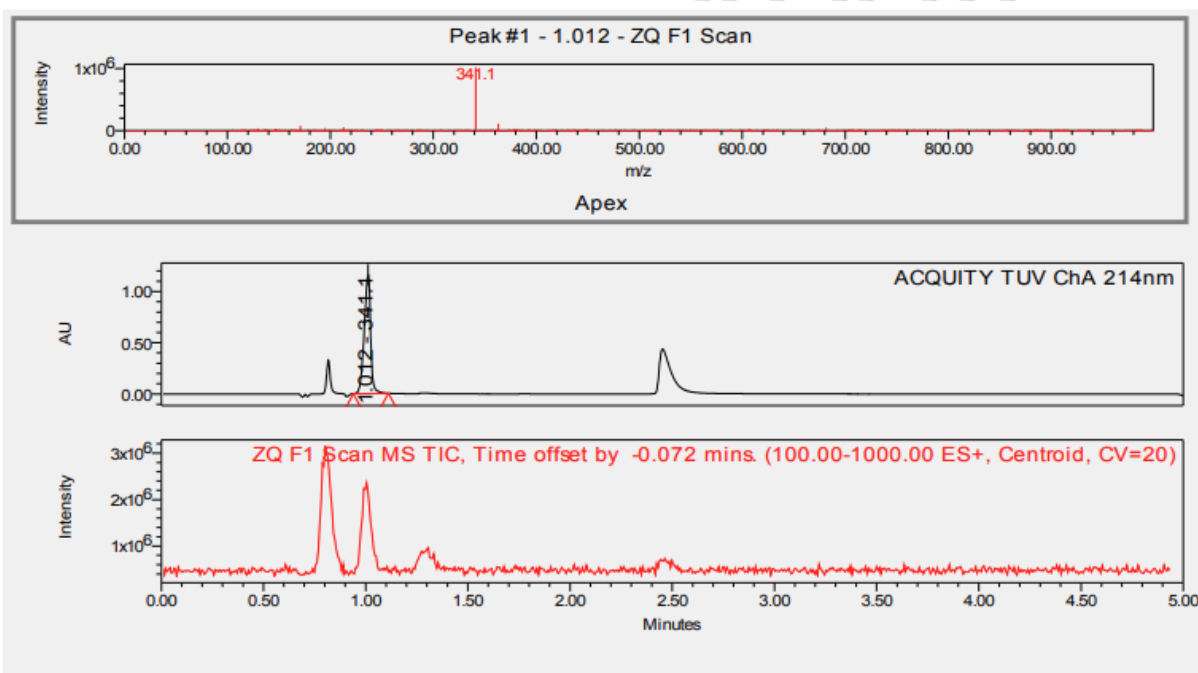
Date Acquired 4/17/2025 11:47:32 AM CEST

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

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

Acq Method Set :

Gr0\_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:

<b>Peptide MW</b>	$[M+1H]^+$	$[M+2H]^2+$	$[M+4H]^4+$	$[M+5H]^5+$	$[M+6H]^6+$	$[M+7H]^7+$
<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  $\mu$ L 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).

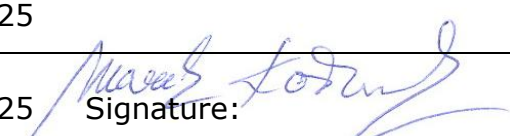
#### 4.3 Result:

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: 



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

