

# Certificate of Analysis



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**Grail Formula B.V.**

Grail Formula B.V.  
Hingenderstraat 39  
6111 AB Sint Joost  
Netherlands  
[info@24peptides.com](mailto:info@24peptides.com)

**Liquilabs s.r.o.**

Inovační 122  
252 41 Zlatníky-Hodkovice  
Czechia  
[www.liquilabs.cz](http://www.liquilabs.cz)



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**Sample Identification**

|                       |                  |
|-----------------------|------------------|
| <b>Sample Name</b>    | AICAR 50 mg      |
| <b>Batch Number</b>   | GF-AICAR50-B242  |
| <b>Date Published</b> | 2026-07-17 15:24 |

## Results for LYO-0254

| Peptides                                                                                         | Result  | Unit  | Uncertainty | Acceptable Range |
|--------------------------------------------------------------------------------------------------|---------|-------|-------------|------------------|
| AICAR Assay<br>Polar Compound Screening 10mM ammonium formate buffer                             | 50.3    | mg    | [± 0.3]     |                  |
| AICAR Purity<br>Polar Compound Screening 10mM ammonium formate buffer                            | > 99.8  | %     |             |                  |
| AICAR Identification by Spectrum (FTIR)<br>Polar Compound Screening 10mM ammonium formate buffer | 998     |       | [± 5]       |                  |
| AICAR Identification by RT<br>Polar Compound Screening 10mM ammonium formate buffer              | 0.999   |       | [± 0.005]   |                  |
| Microbiology                                                                                     | Result  | Unit  | Uncertainty | Acceptable Range |
| Total Aerobic Microbial Count<br>USP <61>/Eur. Ph. 2.6.12. Plate Count Method                    | 0       | CFU/g | [± ]        | 0 - 1000         |
| Total Yeast and Mold Count<br>USP <61>/Eur. Ph. 2.6.12. Plate Count Method                       | 0       | CFU/g | [± ]        | 0 - 100          |
| Bacterial Endotoxin Chromgenic<br>USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test  | < 0.001 | EU/mg |             | 0 - 0.5          |
| Elemental Impurities                                                                             | Result  | Unit  | Uncertainty | Acceptable Range |
| Arsenic<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                     | < 0.001 | ppm   |             | 0 - 1.5          |
| Cadmium<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                     | < 0.001 | ppm   |             | 0 - 0.5          |
| Quicksilver<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                 | < 0.001 | ppm   |             | 0 - 1.5          |
| Lead<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                        | < 0.001 | ppm   |             | 0 - 1.5          |
| Nickel<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                      | < 0.001 | ppm   |             | 0 - 25           |
| Vanadium<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                    | < 0.001 | ppm   |             | 0 - 25           |
| Cobalt<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                      | < 0.001 | ppm   |             | 0 - 25           |
| Mass Spectrometry                                                                                | Result  | Unit  | Uncertainty | Acceptable Range |
| Molecular Ion Mass Identification (MS Deconvolution)<br>Mass Spectrometry Identity               | 258     | Da    | [± 1]       |                  |

|                                                                                   |                                 |                             |
|-----------------------------------------------------------------------------------|---------------------------------|-----------------------------|
|  | <b>Method Specification</b>     |                             |
| <b>Determination of identity, content and purity of AICAR</b>                     |                                 |                             |
| <i>Document number</i><br>AICAR_006_2026                                          | <i>Superseded document</i><br>- | <i>Number of pages</i><br>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% TFA in Water (HPLC, Gradient Grade)           |
| Eluent B                   | 0.1% TFA in Acetonitrile (HPLC, Gradient Grade)    |
| Flow rate                  | 0.4 mL/min                                         |
| Program                    | Gradient elution                                   |
| Injection volume           | 2 µL                                               |
| Colum Temperature          | 60°C                                               |
| Column                     | Phenomenex Biozen Peptide Polar C18, 150x2.1mm 3µm |
| Detection wavelength       | 225nm                                              |

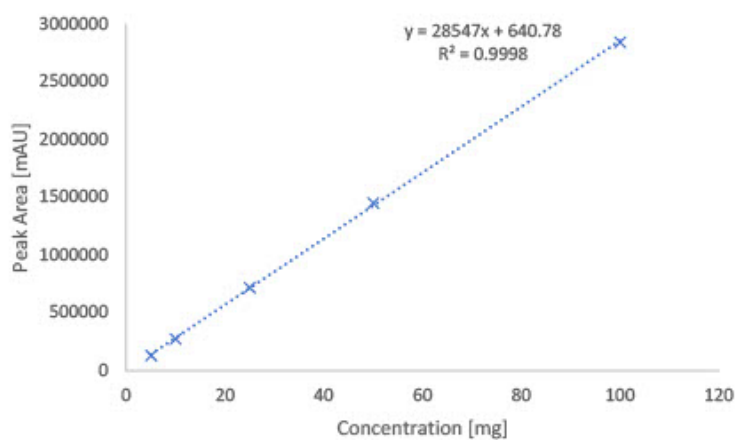
| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 3                | 100   | 0     |
| 18               | 45    | 55    |
| 20               | 1     | 99    |
| 21               | 1     | 99    |
| 21.01            | 100   | 0     |
| 26               | 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% TFA in Water (HPLC, Gradient Grade)           |
| Eluent B                   | 0.1% TFA in Acetonitrile (HPLC, Gradient Grade)    |
| Flow rate                  | 0.4 mL/min                                         |
| Program                    | Gradient elution                                   |
| Injection volume           | 2 µL                                               |
| Colum Temperature          | 60°C                                               |
| Column                     | Phenomenex Biozen Peptide Polar C18, 150x2.1mm 3µm |
| Detection wavelength       | 225nm                                              |

| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 3                | 100   | 0     |
| 18               | 45    | 55    |
| 20               | 1     | 99    |
| 21               | 1     | 99    |
| 21.01            | 100   | 0     |
| 26               | 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 225nm.

### 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  |
| Colum 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% TFA in Water (HPLC, Gradient Grade)           |
| Eluent B                   | 0.1% TFA in Acetonitrile (HPLC, Gradient Grade)    |
| Flow rate                  | 0.4 mL/min                                         |
| Program                    | Gradient elution                                   |
| Injection volume           | 2 µL                                               |
| Colum Temperature          | 60°C                                               |
| Column                     | Phenomenex Biozen Peptide Polar C18, 150x2.1mm 3µm |
| Mass spectrometry          | Scan: positive 280-2000 Da                         |

| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 3                | 100   | 0     |
| 18               | 45    | 55    |
| 20               | 1     | 99    |
| 21               | 1     | 99    |
| 21.01            | 100   | 0     |
| 26               | 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



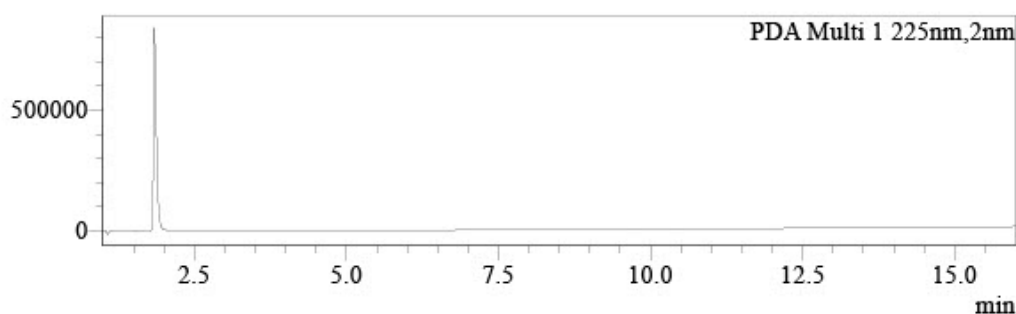
## Analysis of quantity and purity of active ingredient by UHPLC with UV detection

### Sample Information

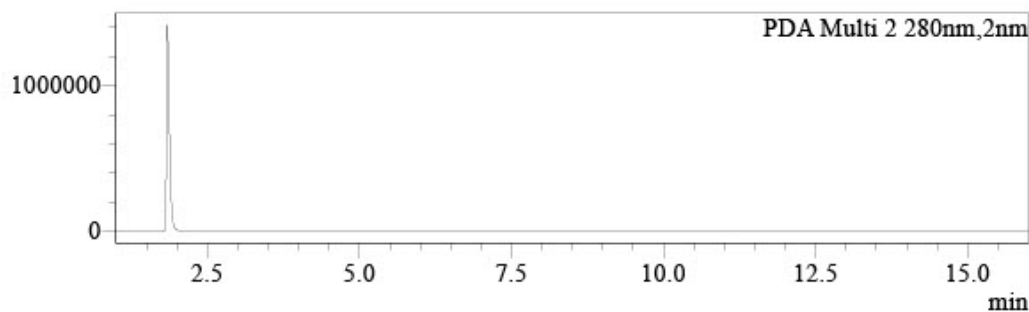
Injection Volume : 2  
Data File : LYO-0254\_\_004.lcd  
Method File : Peptide screening polar.lcm  
Date Acquired : 7/6/2026 8:52:54 PM

### Chromatogram

uAU



uAU



### Peak Table

PDA Ch1 225nm

| Name | Ret. Time | Area    | Conc. | Unit | Area%   |
|------|-----------|---------|-------|------|---------|
|      | 1.843     | 2870621 | 0.000 |      | 100.000 |
|      |           | 2870621 |       |      | 100.000 |

### Peak Table

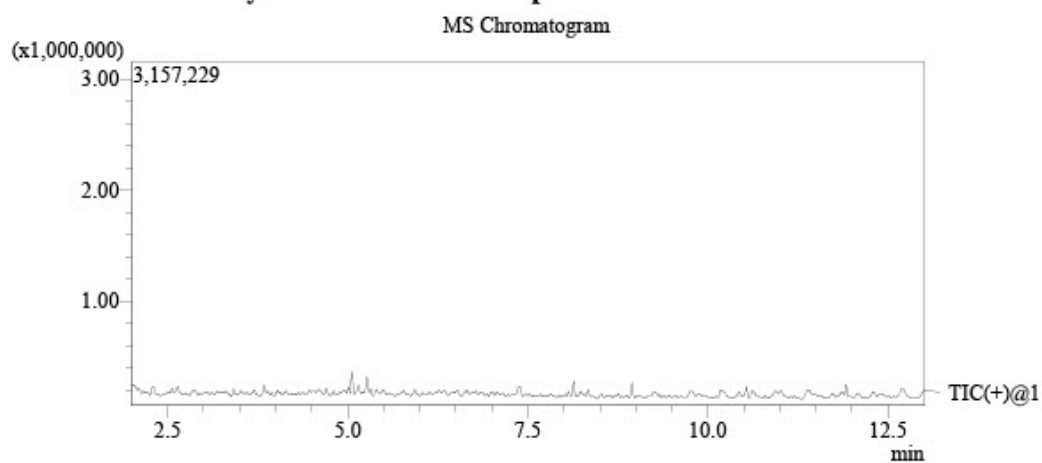
PDA Ch2 280nm

| Name | Ret. Time | Area    | Conc. | Unit |
|------|-----------|---------|-------|------|
|      | 1.843     | 4893624 | 0.000 |      |
|      |           | 4893624 |       |      |

# Analysis Report



## Analysis of identity of active ingredient by UHPLC with mass spectrometric detection





# Endotoxin Determination Report

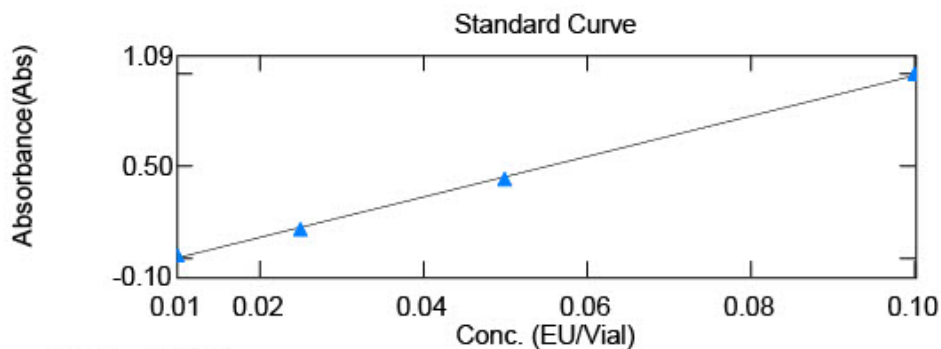


## File Information

Filename: C:\UVVis-Data\Data  
Date/Time: \File\_260618\_2.vqud 06/18/2026  
09:14:52 PM

## Instrument Information

Instrument Type: UV-1900 Series  
Model (S/N): UV-1900i (A12536253123)



$$y = 10.9261x - 0.105573$$

$$r^2 = 0.99912$$

|   | Sample Name | Conc   | Raw_WL545.0 | Result |
|---|-------------|--------|-------------|--------|
| 1 | LYO-0254    | -0.218 | -0.1330     | -0.133 |
| 2 | LYO-0255    | -0.241 | -0.0263     | -0.046 |
| 3 | LYO-0256    | -0.418 | -0.0123     | -0.052 |
|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |
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|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |
|   |             |        |             |        |

|                                                                                                                           |                             |                      |
|---------------------------------------------------------------------------------------------------------------------------|-----------------------------|----------------------|
|  <b>LIQUILABS</b><br>Analytical services | <b>Method Specification</b> |                      |
| Determination of bioburden of lyophilized samples                                                                         |                             |                      |
| Document number<br>MIC_001_2025                                                                                           | Superseded document<br>-    | Number of pages<br>2 |

## 1. Instrumentation and chemicals

### 1.1. Instruments used

- Sterile Syringe 2mL Luer
- Sterile needles
- Ready made PCA Plate ROTI Aquatest
- Ready made Sab4 Plate ROTI Aquatest

### 1.2. Chemicals

Sterile physiological solution (0.9% NaCl)

## 2. Sample preparation and inoculation

### 2.1 Sample preparation

1. Fresh sterile needle and syringe was used for measuring exactly 2 mL of sterile physiological solution.
2. Needle was changed and by new needle rubber top of peptide container was penetrated and 2 mL of sterile physiological solution was dispensed.
3. Content of container was completely dissolved and left for 5 minutes to settle potentially created bubbles.
4. This procedure is repeated for two vials.

### 2.2 Total Aerobic microbial count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with PCA agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with PCA agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 37°C for 120h.

### 2.3 Total Yeast and Mold count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with Sab4 agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with Sab4 agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 25°C for 72h.

## 3. Evaluation of results

After incubation time, colonies are counted as cfu (colonies forming units) and result per 1g of sample is determined as:

$$CFU_{avg} = \frac{\sum CFU_n}{n}$$

$CFU_{avg}$  = average CFU counted form  $n$  inoculations

$CFU_n$  = CFU counted per inoculation

$n$  = number of inoculations

$$CFU \text{ per gram} = \frac{CFU_{avg}}{m_s} * DF$$

$CFU_{avg}$  = Average CFU counted from  $n$  inoculations

$m_s$  = mass of sample (mg)

$DF$  = Dilution factor

If negative control sample is evaluated as positive, process have to be repeated due to possible contamination in the process of inoculation or incubation.

## Responsibles



**Mr. Ján Galbavý**  
*CEO*

Analysis results relate only to the samples tested.

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