

# Certificate of Analysis

**Veyronalabs**  
Veyrona Labs  
Slovakia  
[support@veyronalabs.com](mailto:support@veyronalabs.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

**Sample Name** Thymalin 10 mg  
**Batch Number** THYMALIN-10MG-A  
**Date Published** 2026-10-05 14:45

## Results for LYO-0990

| Peptides                                                                              | Result | Unit | Uncertainty | Acceptable Range |
|---------------------------------------------------------------------------------------|--------|------|-------------|------------------|
| Thymalin Assay<br>Peptide Screening 0.1% TFA                                          | 11.00  | mg   | [± 0.06]    |                  |
| Thymalin Purity<br>Peptide Screening 0.1% TFA                                         | > 99.8 | %    |             |                  |
| Thymalin Identification by<br>Spectrum<br>Peptide Screening 0.1% TFA                  | 992    |      | [± 5]       |                  |
| Thymalin Identification by RT<br>Peptide Screening 0.1% TFA                           | 0.992  |      | [± 0.005]   |                  |
| Mass Spectrometry                                                                     | Result | Unit | Uncertainty | Acceptable Range |
| Molecular Ion Mass Identification<br>(MS Deconvolution)<br>Mass Spectrometry Identity | 858    | Da   | [± 1]       |                  |

|                                                                                   |                                 |                             |  |
|-----------------------------------------------------------------------------------|---------------------------------|-----------------------------|--|
|  | <b>Method Specification</b>     |                             |  |
| <b>Determination of identity, content and purity of Thymalin</b>                  |                                 |                             |  |
| <i>Document number</i><br><b>THYM_008_2026_P</b>                                  | <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Superseded document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                   | 10 mM Ammonium Formate in ACN (HPLC, Gradient Grade)   |
| Eluent B                   | 10 mM Ammonium Formate in Water (HPLC, Gradient Grade) |
| Flow rate                  | 0.6 mL/min                                             |
| Program                    | Gradient elution                                       |
| Injection volume           | 2 µL                                                   |
| Colum Temperature          | 40°C                                                   |
| Column                     | Merck SeQuant ZIC-HILIC 150x2.1mm 3.5µm                |
| Detection wavelength       | 225nm                                                  |

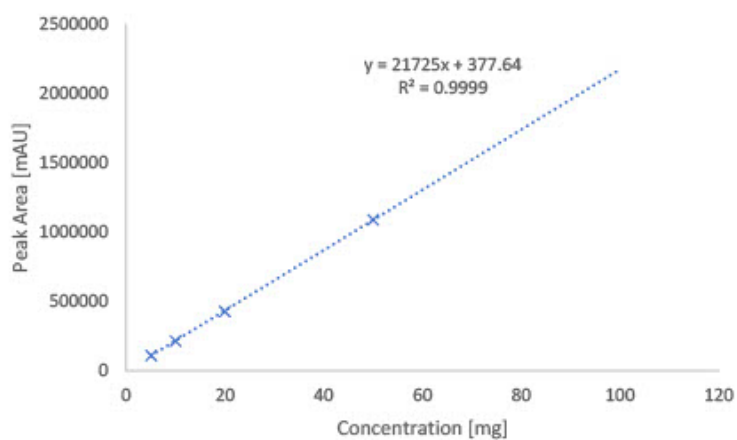
| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 2                | 100   | 0     |
| 18               | 5     | 95    |
| 20               | 5     | 95    |
| 22               | 100   | 0     |
| 30               | end   |       |

### 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 | 4                 |
| Force through Zero           | Disabled          |
| 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                   | 10 mM Ammonium Formate in ACN (HPLC, Gradient Grade)   |
| Eluent B                   | 10 mM Ammonium Formate in Water (HPLC, Gradient Grade) |
| Flow rate                  | 0.6 mL/min                                             |
| Program                    | Gradient elution                                       |
| Injection volume           | 2 µL                                                   |
| Colum Temperature          | 40°C                                                   |
| Column                     | Merck SeQuant ZIC-HILIC 150x2.1mm 3.5µm                |
| Detection wavelength       | 225nm                                                  |

| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 2                | 100   | 0     |
| 18               | 5     | 95    |
| 20               | 5     | 95    |
| 22               | 100   | 0     |
| 30               | 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 225 nm.

### 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                   | 10 mM Ammonium Formate in ACN (HPLC, Gradient Grade)   |
| Eluent B                   | 10 mM Ammonium Formate in Water (HPLC, Gradient Grade) |
| Flow rate                  | 0.6 mL/min                                             |
| Program                    | Gradient elution                                       |
| Injection volume           | 2 µL                                                   |
| Colum Temperature          | 40°C                                                   |
| Column                     | Merck SeQuant ZIC-HILIC 150x2.1mm 3.5µm                |
| Detection w                | MS 155-2000 Da                                         |

| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 2                | 100   | 0     |
| 18               | 5     | 95    |
| 20               | 5     | 95    |
| 22               | 100   | 0     |
| 30               | 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

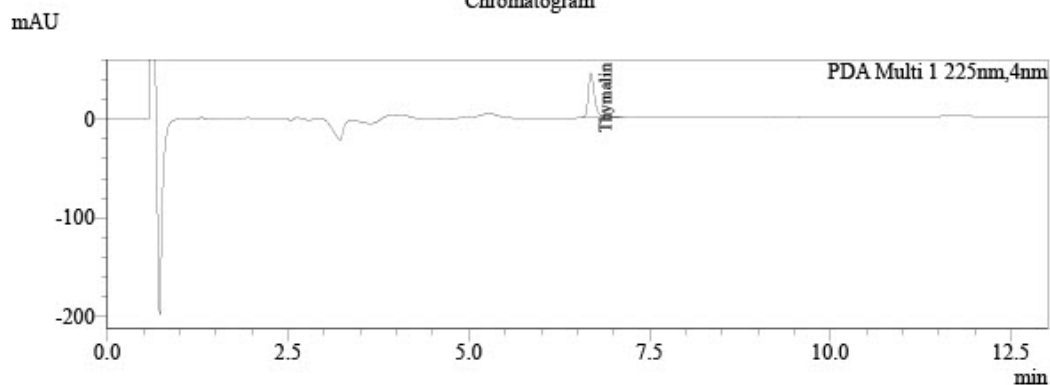


## Analysis of quantity of active ingredient by UHPLC with MS detection

### Sample Information

Injection Volume : 2  
Data File : LYO-0990\_009.lcd  
Method File : Peptides HILIC.lcm  
Date Acquired : 10/2/2026 2:45:28 PM

### Chromatogram



### Peak Table

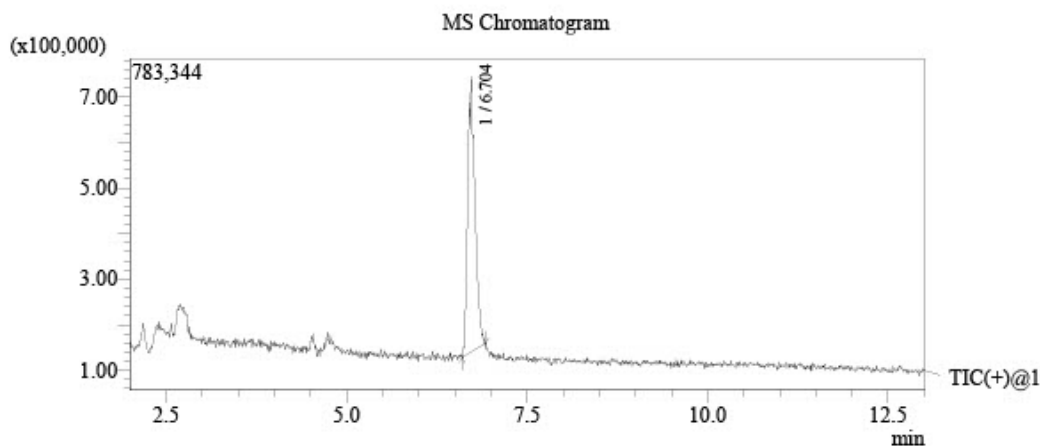
| PDA Ch1 225nm |          |           |        |         |        |      |
|---------------|----------|-----------|--------|---------|--------|------|
| Peak#         | Name     | Ret. Time | Area   | Area%   | Conc.  | Unit |
| 1             | Thymalin | 6.691     | 274121 | 99.942  | 11.002 | mg/L |
| 2             |          | 7.023     | 160    | 0.058   | 0.000  |      |
| Total         |          |           | 274280 | 100.000 |        |      |

### Peak Table

# Analysis Report

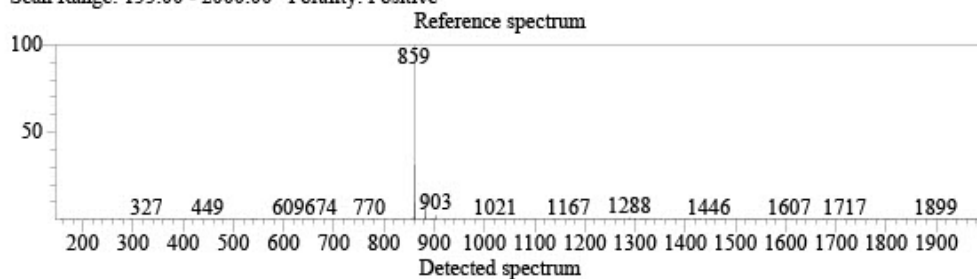


## Identification of active ingredient by UHPLC Mass Spectrometry

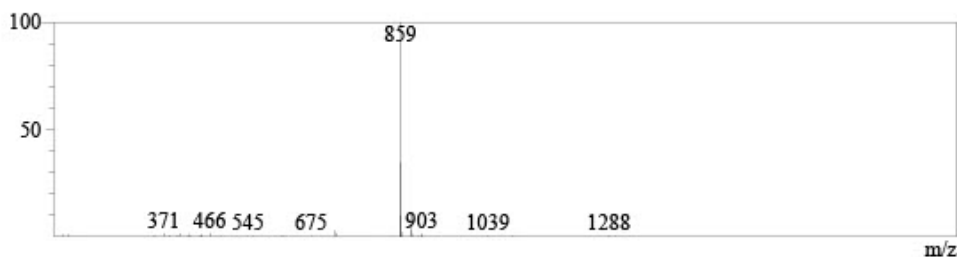


### Library Search

Base Peak: 859(106082)  
Scan Range: 155.00 - 2000.00 Polarity: Positive



Library: Peptide HILIC.lib  
Formula: C<sub>33</sub>H<sub>54</sub>N<sub>12</sub>O<sub>15</sub> CAS: 63958-90-7 Mol.Weight: 858(0!=)  
Compound Name: Thymalin



## Responsibles



**Mr. Ján Galbavý**  
*CEO*

Analysis results relate only to the samples tested.

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