

Certificate of Analysis



Grail Formula B.V.

Grail Formula B.V.
Hingenderstraat 39
6111 AB Sint Joost
Netherlands
info@24peptides.com

Liquilabs s.r.o.

Inovační 122
252 41 Zlatníky-Hodkovice
Czechia
www.liquilabs.cz



[Verify Results Online](#)

Sample Identification

Sample Name	PE-22-28 10 mg
Batch Number	GF-PE2228-B242
Date Published	2026-08-05 18:24

Results for LYO-0404

Peptides	Result	Unit	Uncertainty	Acceptable Range
PE-22-28 Assay Peptide Screening 0.1% TFA	9.77	mg	[± 0.05]	
PE-22-28 Purity Peptide Screening 0.1% TFA	99.7	%	[± 0.5]	
PE-22-28 Identification by Spectrum Peptide Screening 0.1% TFA	100.0		[± 0.5]	
PE-22-28 Identification by RT Peptide Screening 0.1% TFA	0.987		[± 0.005]	
Microbiology	Result	Unit	Uncertainty	Acceptable Range
Total Aerobic Microbial Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	0	CFU/g	[±]	0 - 1000
Total Yeast and Mold Count USP <61>/Eur. Ph. 2.6.12. Plate Count Method	0	CFU/g	[±]	0 - 100
Bacterial Endotoxin Chromogenic USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromogenic Test	0.021	EU/mg	[± 0]	0 - 0.5
Elemental Impurities	Result	Unit	Uncertainty	Acceptable Range
Arsenic Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Cadmium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 0.5
Quicksilver Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Lead Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 1.5
Nickel Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 25
Vanadium Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20	< 0.001	ppm		0 - 25
Cobalt 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) Mass Spectrometry Identity	773	Da	[± 1]	

	Method Specification	
Determination of identity, content and purity of PE-22-28		
<i>Document number</i> PE2228_007_2026	<i>Superseded document</i> -	<i>Number of pages</i> 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	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	280nm

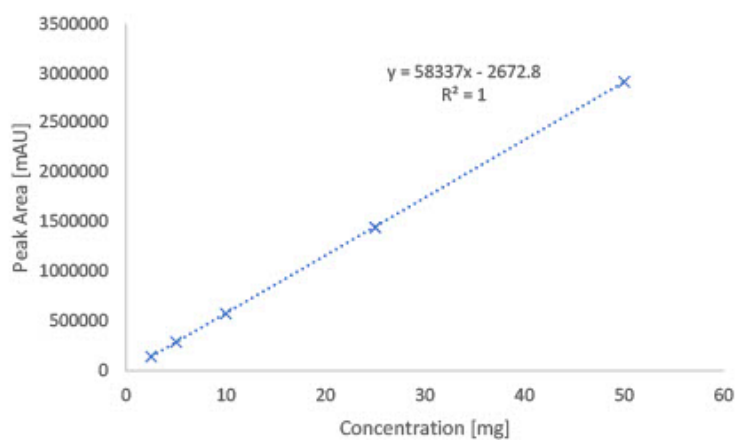
Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	end	

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	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Colum Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Detection wavelength	225nm

Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	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
Column 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	0.05% TFA in Water (HPLC, Gradient Grade)
Eluent B	0.0425% TFA in Acetonitrile (HPLC, Gradient Grade)
Flow rate	0.9 mL/min
Program	Gradient elution
Injection volume	2 µL
Column Temperature	55°C
Column	Waters XSelect CSH C18, 100x2.1mm 2.5µm
Mass spectrometry	Scan: positive 280-2000 Da

Gradient Program		
Time [min]	A [%]	B [%]
1.5	95	5
13	45	55
13.5	1	99
14.5	1	99
14.51	95	5
16	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



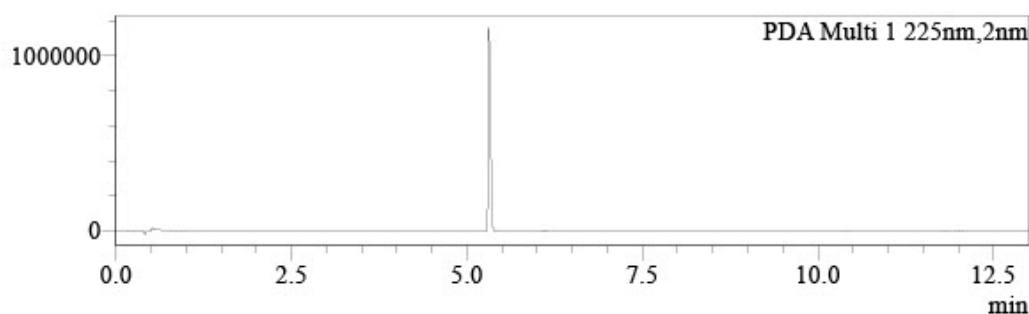
Analysis of quantity and purity of active ingredient by UHPLC with UV detection

Sample Information

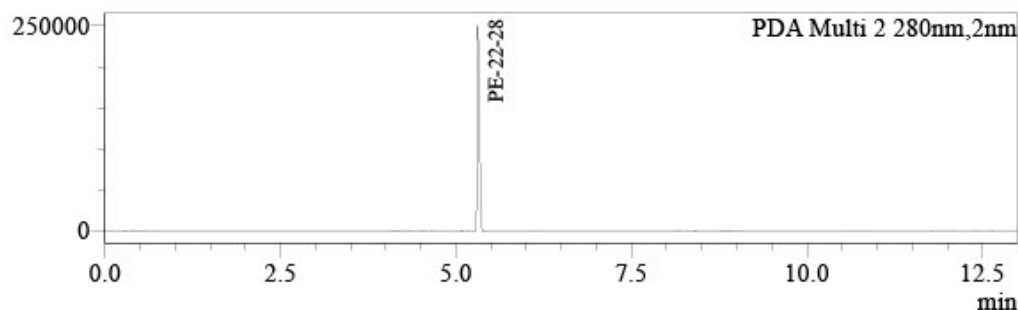
Injection Volume : 2
Data File : LYO-0404__014.lcd
Method File : Peptide screening_V7_Group F.lcm
Date Acquired : 7/23/2026 7:10:25 PM

Chromatogram

uAU



uAU



Peak Table

PDA Ch1 225nm

Name	Ret. Time	Area	Conc.	Unit	Area%
	4.659	428	0.000		0.016
	4.734	143	0.000		0.005
	4.847	110	0.000		0.004
	5.077	248	0.000		0.009
	5.189	672	0.000		0.025
	5.240	346	0.000		0.013
	5.313	2691860	0.000		99.677
	5.565	1045	0.000		0.039
	5.861	287	0.000		0.011
	5.974	276	0.000		0.010
	6.105	4600	0.000		0.170
	6.750	55	0.000		0.002
	6.919	258	0.000		0.010
	7.130	250	0.000		0.009
		2700578			100.000

Peak Table

PDA Ch2 280nm

Name	Ret. Time	Area	Conc.	Unit
PE-22-28	5.313	567267	9.770	mg
		567267		

Analysis Report

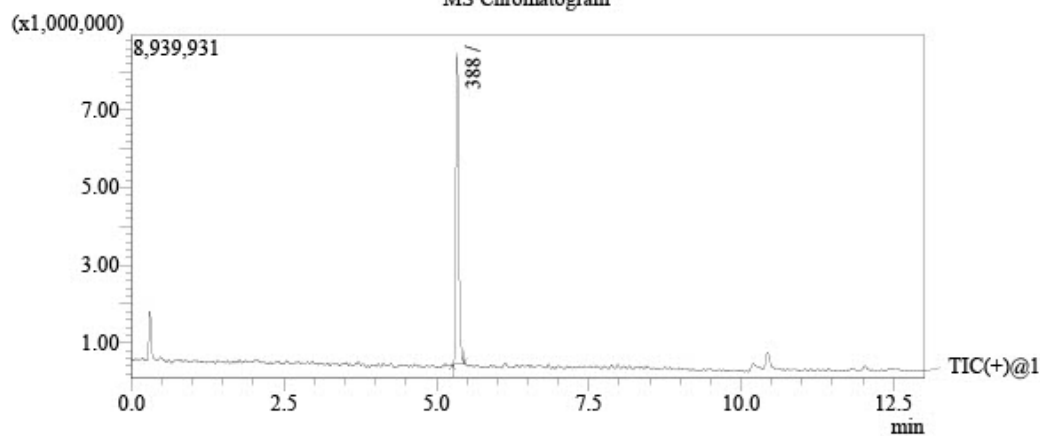


LIQUILABS

Analytical services

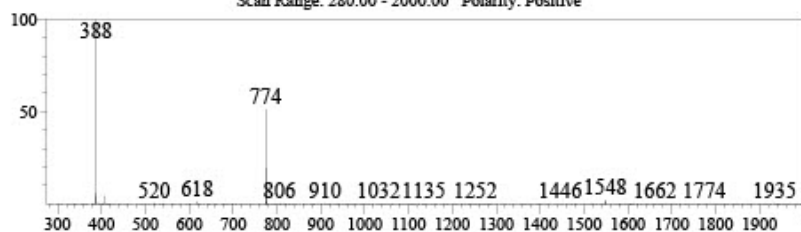
Analysis of identity of active ingredient by UHPLC with mass spectrometric detection

MS Chromatogram

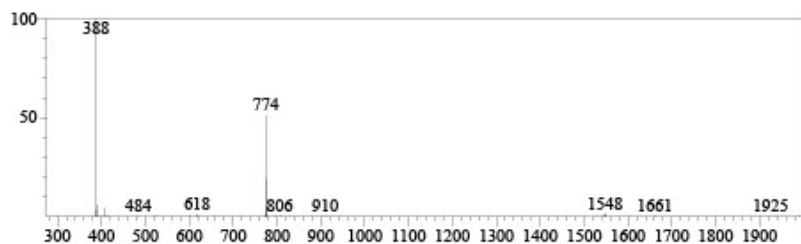


Library Search

Detected compound
Ret. Time: 5.268 Base Peak: 388
Scan Range: 280.00 - 2000.00 Polarity: Positive



Library: Peptide screening lib
Formula: C₃₅H₅₅N₁₁O₉ CAS: 181959-12-5 Mol. Weight: 773 Ret. Index: 0
Compound Name: PE-22-28 (Spadin)



Endotoxin Determination Report

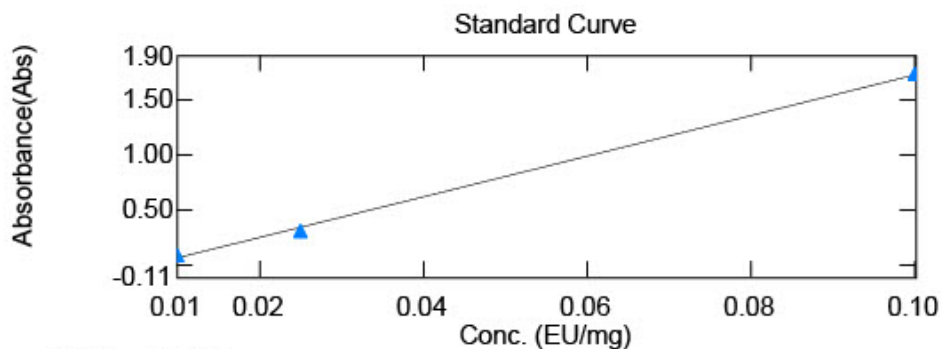


File Information

Filename: C:\UVVis-Data\Data\File_260723_122453.vqud
Date/Time: 07/23/2026 01:13:01 PM

Instrument Information

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



$$y = 18.5422x - 0.127539$$
$$r^2 = 0.99855$$

	Sample Name	Conc	Raw_WL545.0	Dilution Rate
1	LYO-0389	0.756	0.5732	20.00
2	LYO-0390	0.211	0.0677	20.00
3	LYO-0391	0.802	0.6164	20.00
4	LYO-0392	0.275	0.1271	20.00
5	LYO-0393	0.231	0.0863	20.00
6	LYO-0394	0.171	0.0309	20.00
7	LYO-0395	0.285	0.1364	20.00
8	LYO-0396	0.199	0.0569	20.00
9	LYO-0397	0.209	0.0660	20.00
10	LYO-0398	0.159	0.0201	20.00
11	LYO-0399	0.174	0.0337	20.00
12	LYO-0400	0.360	0.2067	20.00
13	LYO-0401	0.203	0.0605	20.00
14	LYO-0404	0.204	0.0613	20.00
15	LYO-0405	1.231	1.0135	20.00
16	LYO-0406	0.160	0.0206	20.00
17	LIQ-0039	0.008	0.0270	1.00

	Method Specification	
Determination of bioburden of lyophilized samples		
Document number MIC_001_2025	Superseded document -	Number of pages 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.

This document shall not be reproduced except in full, without the written approval of Liquilabs s.r.o.