

## Pen peptide certificate of analysis (CoA)

<b>Product:</b>	<b>Thymalin</b>		
<b>Source:</b>	Hybrid synthesis (recombinant using peptide secretion system and chemical synthesis)		
<b>Intended use:</b>	For stability, viability and activity testing only.		
<b>Order number:</b>		<b>Lot:</b>	25AUG25THY
<b>Production:</b>	08/2025	<b>Expiry:</b>	08/2027
<b>Formulation:</b>	0.2 µm-filtered solution in 20mM glycine, 200mM Mannitol, 20mM NaH <sub>2</sub> PO <sub>4</sub> , pH 7.4; m-cresol 1 mg/ml, glycerol 2 mg/ml (when liquid)		
<b>Protein/peptide concentration per 3 ml cartridge:</b>	30 mg		

<b>Release Testing:</b>	<b>Specification</b>	<b>Lot Result</b>
<b>Purity:</b>	≥ 97%	> 98%
<b>Identity:</b>	Complies	Complies
<b>Sterility:</b>	Sterile	Complies
<b>Endotoxin level:</b>	< 10 EU/mg	< 0.20 EU/mg
<b>Host-cell DNA</b>	≤ 200 ng/mg	Complies (1.1 ng/mg)

Activity was determined using in vitro test.

Purity was determined by HPLC.

Identity was confirmed by end-of-production DNA sequencing and N-terminal protein sequencing.

Sterility test of vial product was performed according to Eur.Pharm. (Inoculation method).

Endotoxin was determined using the gel clot assay according to Eur.Pharm.

Host-cell DNA/RNA was determined using fluorimetric assay (if applicable).

### **Handling Instructions:**

**General usage:** Open cap, clean the rubber stopper with disinfectant napkin or other cleaning disinfection method / material. Puncture rubber stopper with sterile needle by screwing needle on. Remove the plastic protective cover. Set the index to physician prescribed position, remove the pink plastic cover and let out the air from the cartridge by several button presses into the air. Put on the plastic protective cover back.

**Using liquid product:** Liquid products are ready to use according to physician recommendations.

**Storage and stability:** Store material at +2 - +8°C. **Do not freeze!**

### **Quality Statement:**

This product is manufactured, tested and realized in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> “ancillary materials for cell, gene, and tissue-engineered product” has been considered in the design of this product.

## Thymalin

### Data Provided:

- Initial concentration of Thymalin: **99%**.
- Concentration after 7 days at:
  - **+4°C: 99%**.
  - **+42°C: 94,41%**.

### Analysis Plan:

- Use the Arrhenius equation to calculate the degradation rate constants.
- Determine the activation energy ( $E_a$ ) based on the temperature and degradation rates.
- Predict time to 90% stability at **+4°C**.

### Summary of Results:

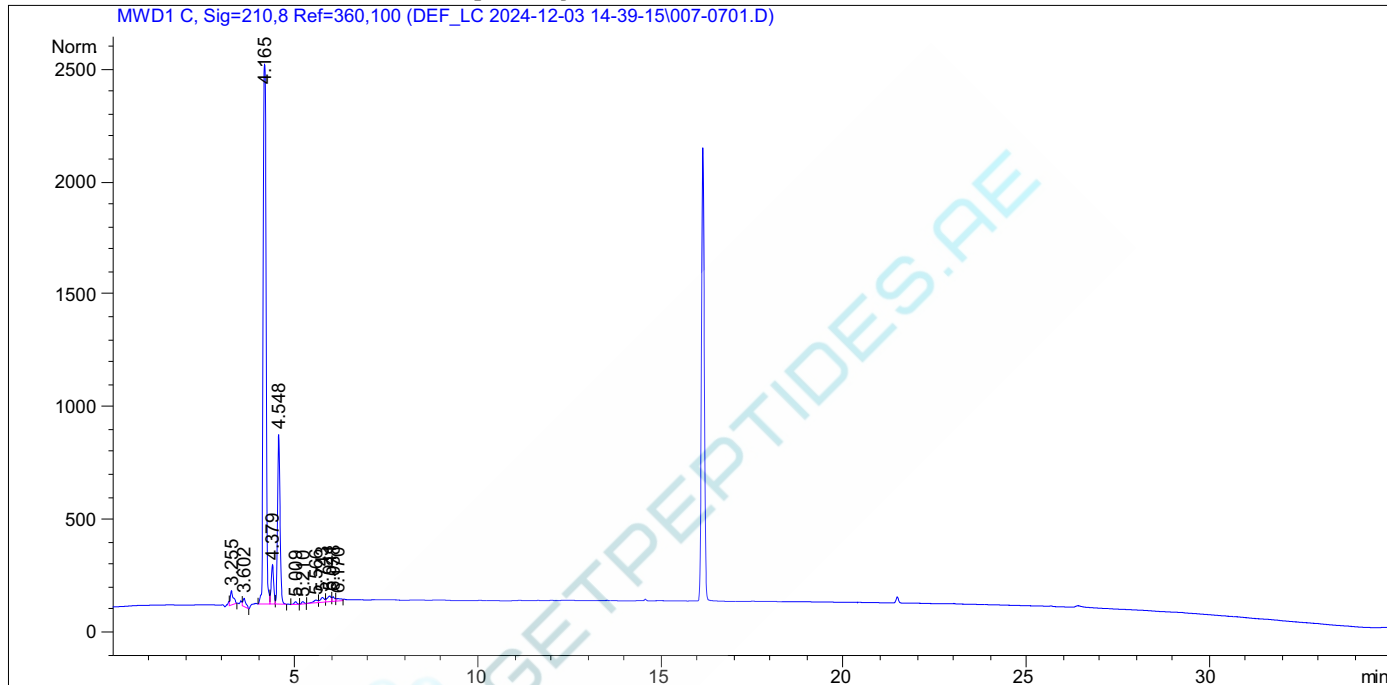
- **Activation Energy ( $E_a$ ): 50 kJ/mol.**
- **Time to 90% Stability at +4°C: 315, but please note, at high temperatures hydrolysis occurs, need more distant data for better evaluation.**



Sample Name: Thymulin 10mg/ml 24-12-03

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=====
Acq. Operator   :                               Seq. Line :    7
Acq. Instrument : Instrument 1                 Location  : Vial 7
Injection Date  : 12/3/2024 6:50:21 PM       Inj       :    1
                                                Inj Volume: 10.0 µl
Differen t Inj Volume from Sequence !      Actual Inj Volume : 5.0 µl
Acq. Method    : C:\CHEM32\1\DATA\DEF_LC 2024-12-03 14-39-15\C18 RP-HPLC ACN 5-95 30 MIN.M
Last changed   : 7/31/2024 10:51:05 AM
Analysis Method : C:\CHEM32\1\METHODS\C414\C4 GLP1 30-70 ACN.M
Last changed   : 12/19/2024 1:44:58 PM
                (modified after loading)
Additional Info : Peak(s) manually integrated
    
```



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 Area Percent Report  
 =====

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Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: MWD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.255	VB	0.0774	355.33032	63.40388	1.8255
2	3.602	VB	0.0781	201.91655	36.75046	1.0374
3	4.165	BV	0.0928	1.37180e4	2396.16602	70.4765
4	4.379	VV	0.0708	822.09454	174.89894	4.2235
5	4.548	VB	0.0736	3593.49365	754.59967	18.4617
6	5.009	BB	0.0743	57.79366	11.97069	0.2969
7	5.210	BB	0.0755	48.66410	10.23061	0.2500
8	5.566	BV	0.1226	104.42091	12.10757	0.5365

Data File C:\CHEM32\1\DATA\DEF\_LC 2024-12-03 14-39-15\007-0701.D  
Sample Name: Thymulin 10mg/ml 24-12-03

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	5.743	VV	0.1095	164.87503	21.50191	0.8471
10	5.941	VV	0.1053	188.61226	25.20093	0.9690
11	6.038	VV	0.0693	101.25887	20.63133	0.5202
12	6.170	VV	0.1433	108.16540	10.23607	0.5557

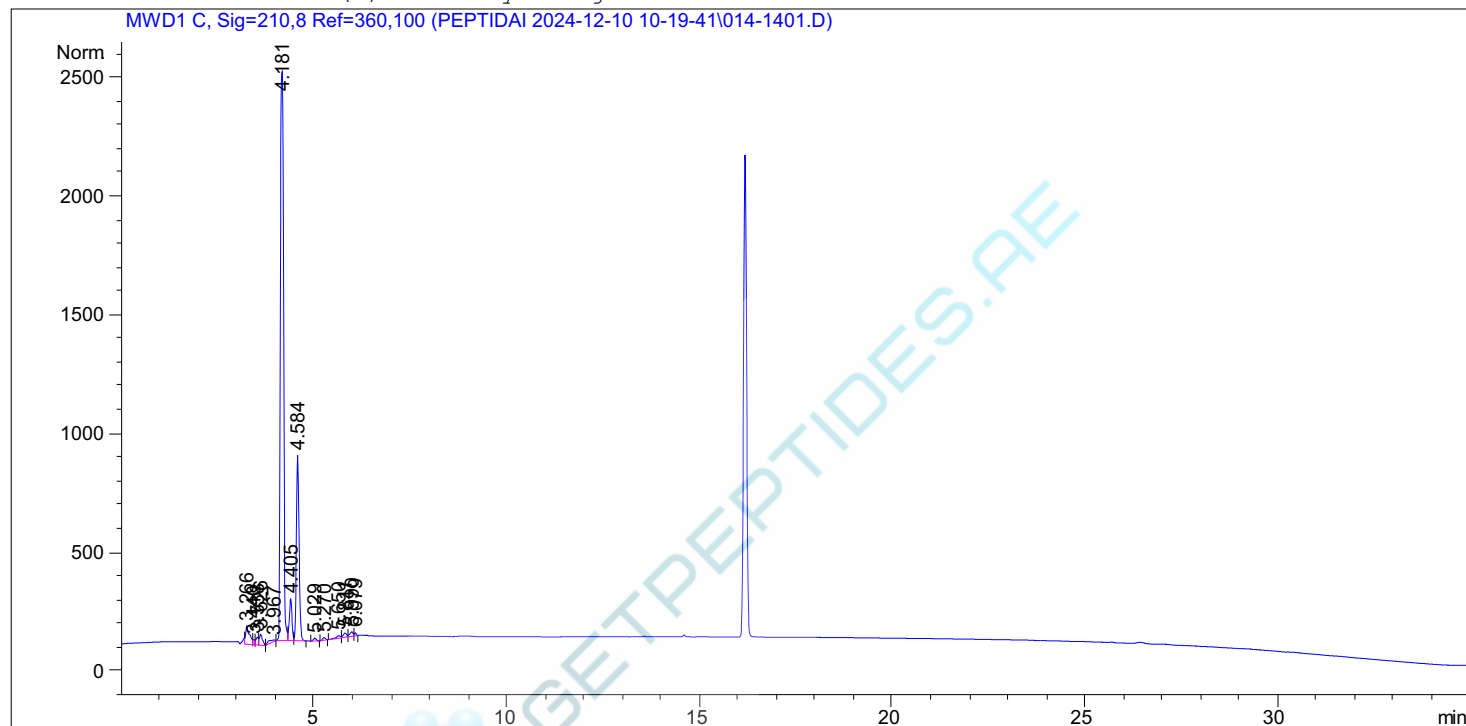
Totals : 1.94646e4 3537.69808

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\*\*\* End of Report \*\*\*



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=====
Acq. Operator   :                               Seq. Line :   14
Acq. Instrument : Instrument 1                   Location  : Vial 14
Injection Date  : 12/10/2024 7:20:14 PM        Inj       :    1
                                                    Inj Volume: 10.0 µl
Differen t Inj Volume from Sequence !      Actual Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\1\DATA\PEPTIDAI 2024-12-10 10-19-41\C18 RP-HPLC ACN 5-95 30 MIN.M
Last changed    : 7/31/2024 10:51:05 AM
Analysis Method : C:\CHEM32\1\METHODS\C414\C4 GLP1 30-70 ACN.M
Last changed    : 12/19/2024 3:04:50 PM
                  (modified after loading)
Additional Info : Peak(s) manually integrated
  
```



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 Area Percent Report  
 =====

```

Sorted By      : Signal
Multiplier     : 1.0000
Dilution      : 1.0000
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: MWD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.266	VV	0.0966	539.12750	76.13059	2.6458
2	3.440	VV	0.0529	79.78048	21.70198	0.3915
3	3.538	VV	0.0689	152.89638	32.51460	0.7503
4	3.626	VB	0.0796	257.40732	45.75732	1.2632
5	3.967	BB	0.2350	128.39185	6.83980	0.6301
6	4.181	BV	0.0965	1.40636e4	2396.70044	69.0178
7	4.405	VV	0.0736	843.96521	176.95781	4.1418
8	4.584	VB	0.0775	3852.76929	781.38062	18.9077

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	5.029	BB	0.0814	60.74250	11.54725	0.2981
10	5.270	BB	0.0778	55.55721	11.21611	0.2726
11	5.650	BV	0.1093	75.80458	10.12927	0.3720
12	5.811	VV	0.0884	108.91453	18.04395	0.5345
13	5.990	VV	0.0868	115.68411	19.05713	0.5677
14	6.079	VB	0.0547	42.13436	12.06728	0.2068

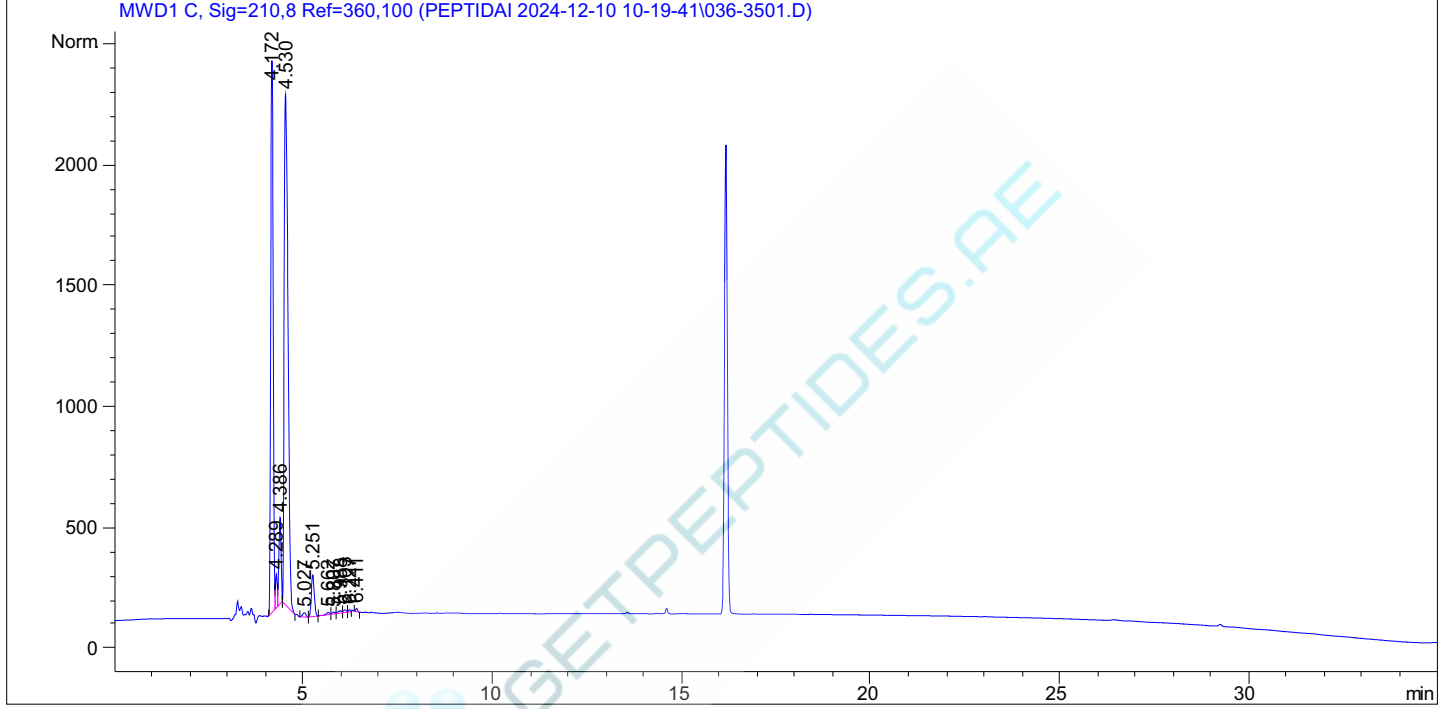
Totals : 2.03768e4 3620.04414

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\*\*\* End of Report \*\*\*



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=====
Acq. Operator   :                               Seq. Line :   35
Acq. Instrument : Instrument 1                  Location  : Vial 36
Injection Date  : 12/11/2024 9:52:49 AM       Inj       :    1
                                                Inj Volume: 10.0 µl
Differen t Inj Volume from Sequence !      Actual Inj Volume : 5.0 µl
Acq. Method    : C:\CHEM32\1\DATA\PEPTIDAI 2024-12-10 10-19-41\C18 RP-HPLC ACN 5-95 30 MIN.M
Last changed   : 7/31/2024 10:51:05 AM
Analysis Method : C:\CHEM32\1\METHODS\C414\C4 GLP1 30-70 ACN.M
Last changed   : 12/19/2024 4:40:41 PM
                (modified after loading)
Additional Info : Peak(s) manually integrated
  
```



Area Percent Report

```

Sorted By      : Signal
Multiplier    : 1.0000
Dilution      : 1.0000
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: MWD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.172	BV	0.0750	1.03720e4	2285.26440	36.5711
2	4.289	VV	0.0510	469.52701	140.34045	1.6555
3	4.386	VB	0.0628	1437.17542	358.43262	5.0674
4	4.530	BB	0.1152	1.47146e4	2113.01563	51.8831
5	5.027	BB	0.0911	109.23606	18.43061	0.3852
6	5.251	BB	0.0847	924.99878	172.15991	3.2615
7	5.662	BV	0.1042	64.24613	9.12251	0.2265
8	5.807	VV	0.0863	47.53238	8.11913	0.1676

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	5.988	VV	0.1177	70.42686	9.58895	0.2483
10	6.103	VV	0.0832	61.15189	11.65080	0.2156
11	6.227	VV	0.0667	39.46252	8.73551	0.1391
12	6.411	BB	0.0568	50.76287	13.83284	0.1790

Totals : 2.83611e4 5148.69337

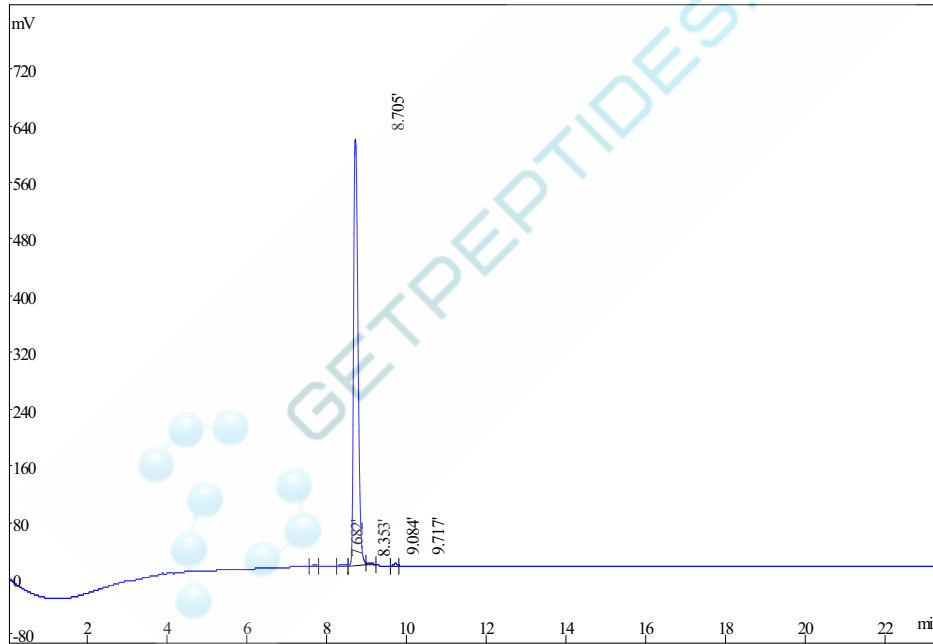
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\*\*\* End of Report \*\*\*



Structure : Thymalin (Pyr-AN-8)  
 Lot NO :250825  
 Number :0200049  
 Column :250\*4.6mm,ChromCore 120-C18-5um  
 Solvent A:0.1%TFA in 100%water  
 Solvent B:0.1%TFA in 100%acetonitrile  
 Gradient :
 

	A	B
0.1min	99%	1%
25.0min	74%	26%
25.1min	0%	100%
30.0min	stop	

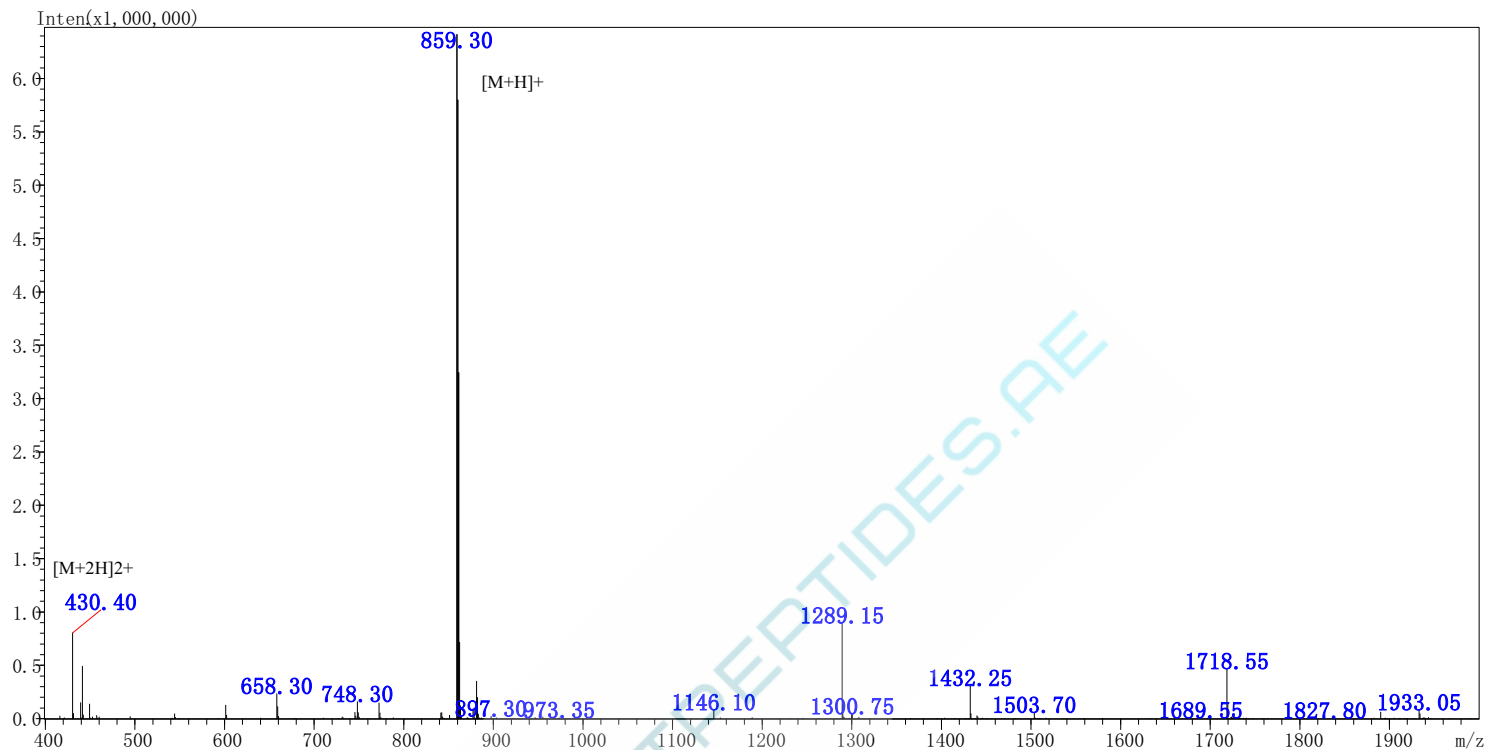
  
 Flow rate:1.0ml/min  
 Wavelength(nm):220  
 Volume :10ul



Rank	Time	Conc.	Area	Height
1	7.682	0.1732	7426	1052
2	8.353	0.1945	8340	934
3	8.705	98.48	4223067	602587
4	9.084	0.7749	33224	3497
5	9.717	0.3727	15980	2819
Total		100	4288037	610889

MS Spectrum

Positive



Acquired by :Shen  
Data Acquired : 2025/08/25  
Injection Volume : 1  
Sample Name : Thymalin  
Mw : 858.85  
Lot No. : 250825

Probe :ESI  
Nebulizer Gas Flow :1.5L/min  
CDL : -20.0v  
CDL Temp °C :250  
Block Temp °C :400

Probe bias :+4.5kv  
Detector :1.2kv  
T.Flow :0.2ml/min  
B.conc :50%H2O/50%ACN