

Pen peptide certificate of analysis (CoA)

Product: Melanotan 2
Source: Hybrid synthesis (recombinant using peptide secretion system and chemical synthesis)
Intended use: For stability, viability and activity testing only.
Order number: **Lot:** 25AUG05MEL
Production: 08/2025 **Expiry:** 08/2027
Formulation: 0.2 µm-filtered solution in 20mM glycine, 200mM Mannitol, 20mM NaH₂PO₄, pH 7.4; m-cresol 1 mg/ml, glycerol 2 mg/ml (when liquid)
Protein/peptide concentration per 3 ml cartridge: 30 mg

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

Activity was determined using a Nb2-11 cell proliferation assay.
 Purity was determined by SDS PAGE and coomassie stain (under reducing and non-reducing conditions).
 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 was determined using fluorimetric assay.

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.

Stability report

Melanotan 2

Data Provided:

- Initial concentration of Melanotan 2: **100%**.
- Concentration after 7 days at:
 - **+4°C: 99,93%.**
 - **+42°C: 99,93%.**

Analysis Plan:

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

Summary of Results:

- **Activation Energy (Ea): 10 kJ/mol.**
- **Time to 90% Stability at +4°C: 2030 days**

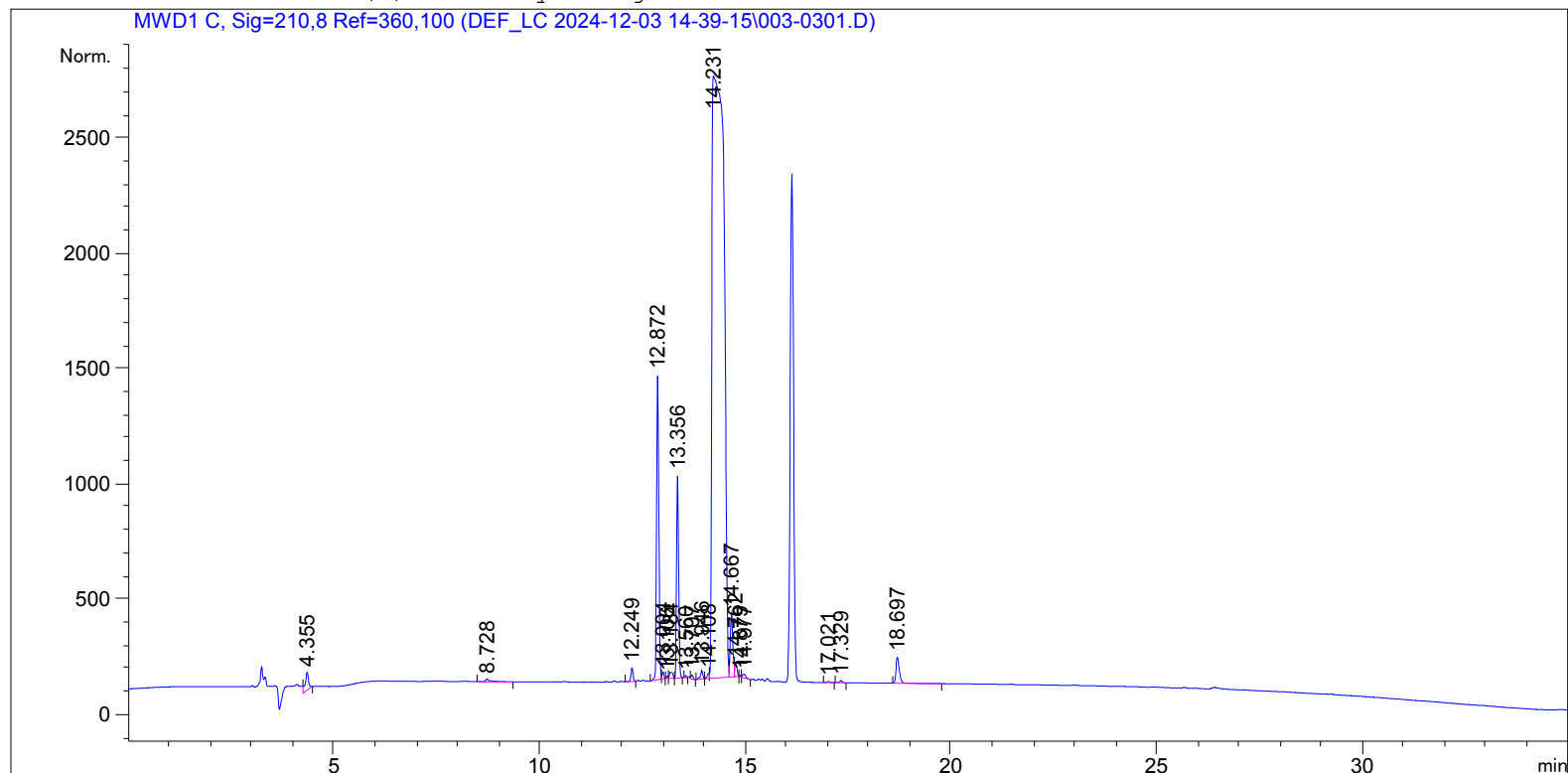
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Acq. Operator	:		Seq. Line	:	3
Acq. Instrument	:	Instrument 1	Location	:	Vial 3
Injection Date	:	12/3/2024 4:04:08 PM	Inj	:	1
			Inj Volume	:	10.0 µl

Different 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	4.355	VB	0.0788	428.75476	77.24622	0.6748
2	8.728	VB	0.1367	119.23534	11.52138	0.1877
3	12.249	BV	0.0557	214.26282	59.93133	0.3372
4	12.872	BV	0.0605	5042.84473	1322.32727	7.9366
5	13.004	VB	0.0485	95.36370	30.51199	0.1501
6	13.106	BV	0.0436	27.53863	10.16362	0.0433
7	13.184	VB	0.0762	151.18065	27.46019	0.2379
8	13.356	BV	0.0556	3132.19092	879.21667	4.9295

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	13.560	BV	0.0495	31.75516	9.88879	0.0500
10	13.707	VB	0.0549	62.70338	17.87820	0.0987
11	13.946	BB	0.0608	150.12251	37.44070	0.2363
12	14.108	BV	0.0548	81.29839	22.15911	0.1279
13	14.231	VV	0.2513	5.17661e4	2611.08984	81.4711
14	14.667	VV	0.0635	1170.60437	275.93045	1.8423
15	14.762	VB	0.0620	235.49153	59.75946	0.3706
16	14.879	BV	0.0385	11.43790	4.67971	0.0180
17	14.977	VB	0.0905	87.43967	15.33252	0.1376
18	17.021	BB	0.0700	22.80446	4.93029	0.0359
19	17.329	BB	0.0657	40.74974	9.56350	0.0641
20	18.697	VV	0.0889	667.35626	113.03426	1.0503

Totals : 6.35392e4 5600.06549

*** End of Report ***

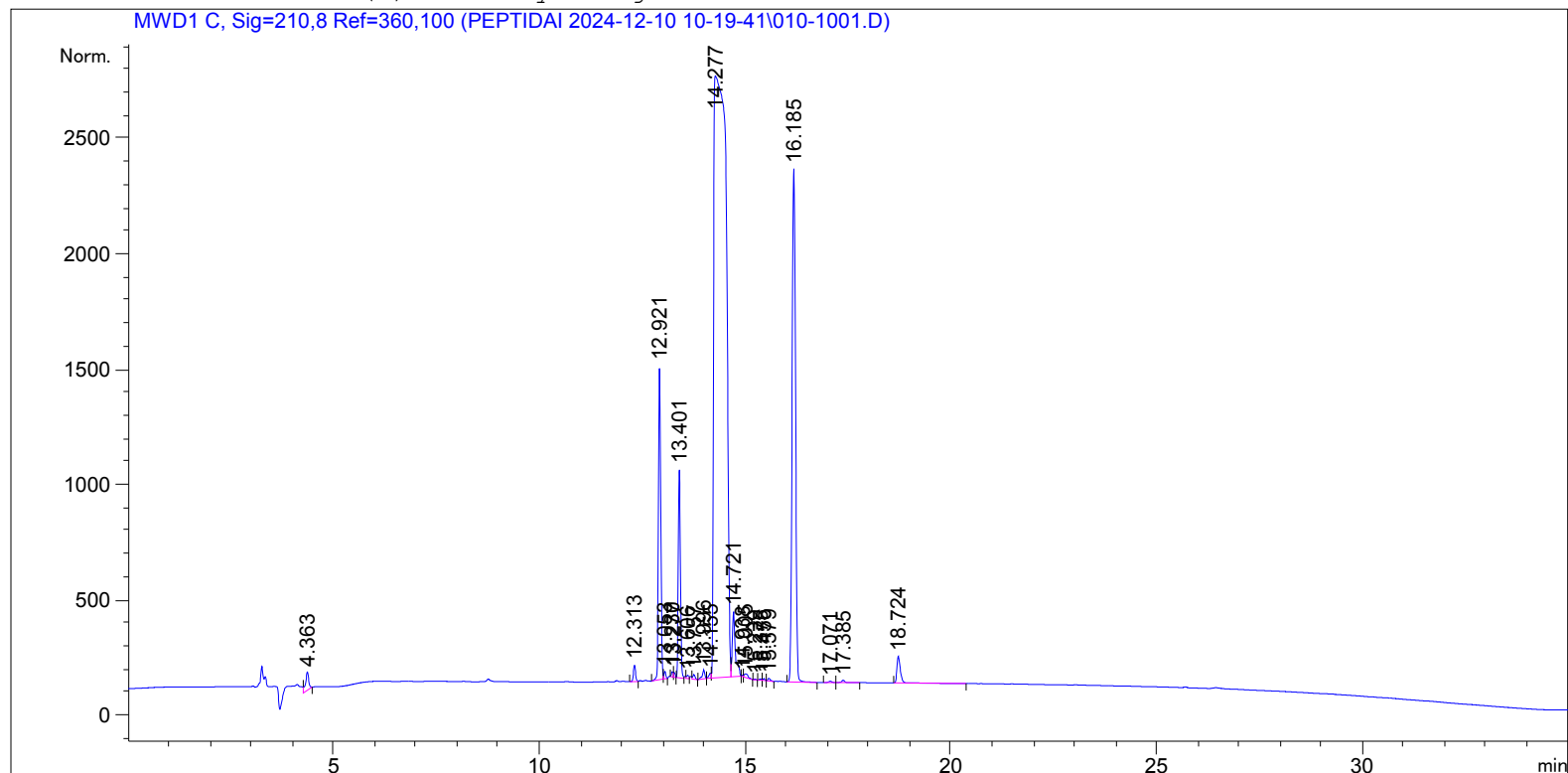
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Acq. Operator	:		Seq. Line	:	10
Acq. Instrument	:	Instrument 1	Location	:	Vial 10
Injection Date	:	12/10/2024 4:33:57 PM	Inj	:	1
			Inj Volume	:	10.0 µl

Different 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 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	4.363	VB	0.0780	416.19717	78.34694	0.5303
2	12.313	BV	0.0556	253.09528	70.97786	0.3225
3	12.921	BV	0.0617	5332.77295	1360.19507	6.7947
4	13.053	VB	0.0488	101.30499	32.12597	0.1291
5	13.230	BV	0.0441	62.10098	21.24787	0.0791
6	13.277	VB	0.0437	55.40018	20.41612	0.0706
7	13.401	BV	0.0587	3313.25659	906.30316	4.2216
8	13.606	VV	0.0581	57.50664	14.55860	0.0733

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	13.757	VB	0.0579	75.45711	20.03699	0.0961
10	13.996	BB	0.0624	160.93283	38.78497	0.2051
11	14.155	BV	0.0551	79.47566	21.49830	0.1013
12	14.277	VV	0.2560	5.32399e4	2609.27515	67.8354
13	14.721	VB	0.0765	1466.48157	282.76068	1.8685
14	14.938	BV	0.0331	10.95662	5.09542	0.0140
15	15.005	VB	0.0919	104.54166	14.90004	0.1332
16	15.239	BB	0.0497	10.59188	3.46175	0.0135
17	15.375	BV	0.0575	22.18423	5.95004	0.0283
18	15.456	VB	0.0545	25.07720	7.22376	0.0320
19	15.579	BB	0.0616	45.50474	11.15349	0.0580
20	16.185	BB	0.0935	1.28752e4	2225.41187	16.4049
21	17.071	VV	0.0770	27.70311	5.29725	0.0353
22	17.385	VV	0.0831	58.86740	10.54869	0.0750
23	18.724	VV	0.0870	689.44879	116.64630	0.8785

Totals : 7.84839e4 7882.21627

*** End of Report ***

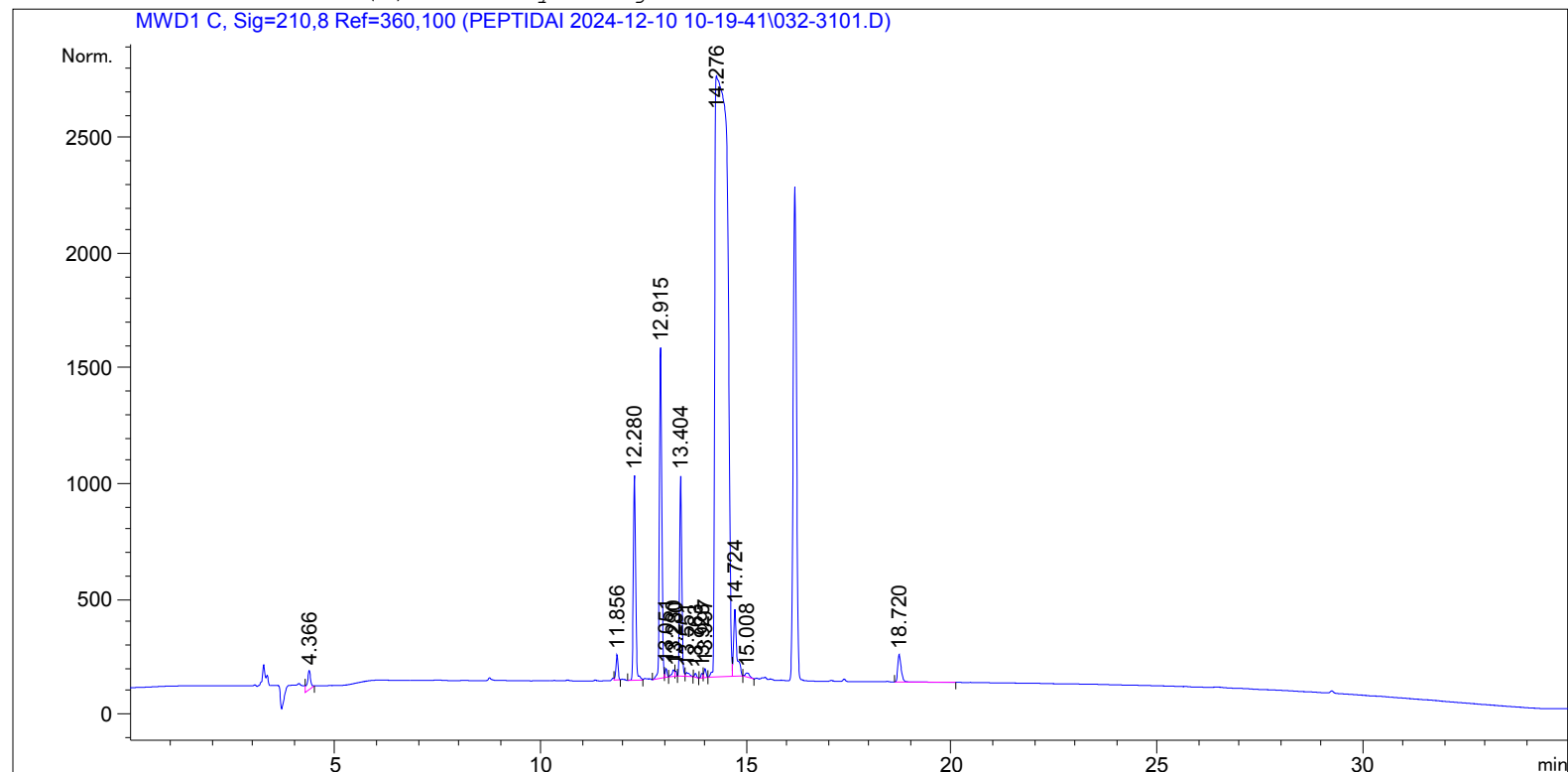
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Acq. Operator	:		Seq. Line	:	31
Acq. Instrument	:	Instrument 1	Location	:	Vial 32
Injection Date	:	12/11/2024 7:06:39 AM	Inj	:	1
			Inj Volume	:	10.0 µl

Different 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 3:15:57 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	4.366	VB	0.0803	456.91794	82.89652	0.6501
2	11.856	VB	0.0554	388.57358	109.57032	0.5529
3	12.280	BV	0.0620	3511.80908	889.73138	4.9969
4	12.915	BV	0.0629	5772.88574	1435.58496	8.2141
5	13.051	VB	0.0516	122.11559	37.84670	0.1738
6	13.230	BV	0.0632	123.85581	28.22560	0.1762
7	13.280	VB	0.0426	56.59282	21.55673	0.0805
8	13.404	BV	0.0589	3206.44092	872.96228	4.5624

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	13.551	VB	0.0848	103.88914	16.20423	0.1478
10	13.763	BB	0.0527	55.15992	16.61440	0.0785
11	13.925	BV	0.0486	60.95539	19.44437	0.0867
12	13.997	VB	0.0555	138.68478	39.01685	0.1973
13	14.276	BV	0.2591	5.39256e4	2609.48389	76.7294
14	14.724	VB	0.0777	1546.17273	292.59558	2.2000
15	15.008	BB	0.1013	128.09209	16.73544	0.1823
16	18.720	BB	0.0867	682.50165	119.38790	0.9711

Totals : 7.02802e4 6607.85714

*** End of Report ***

REPORT

Product Name: Melanotan 2

Instrument No: 0200047

Lot No : 20250805

Column : 4.6*250mm, kromasil C18-5

Solvent A : 0.1% Trifluoroacetic in 100% Acetonitrile

Solvent B : 0.1% Trifluoroacetic in 100% Water

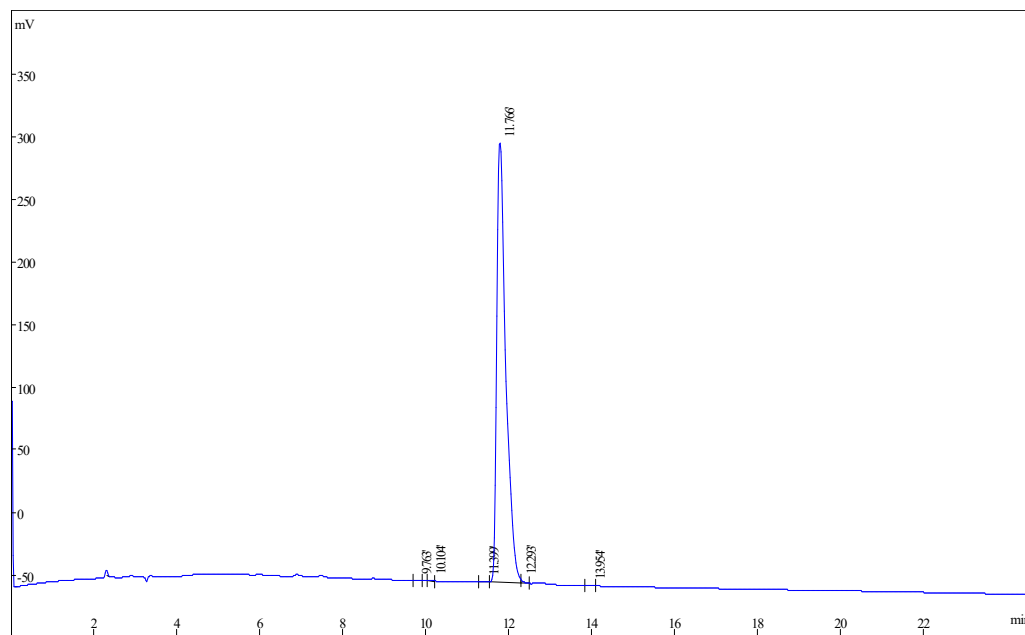
Gradient	:	A	B
0.01min		30%	70%
25min		55%	45%
25.1min		100%	0%
30min		Stop	

Flow rate : 1.0ml/min

Wavelength : 220nm

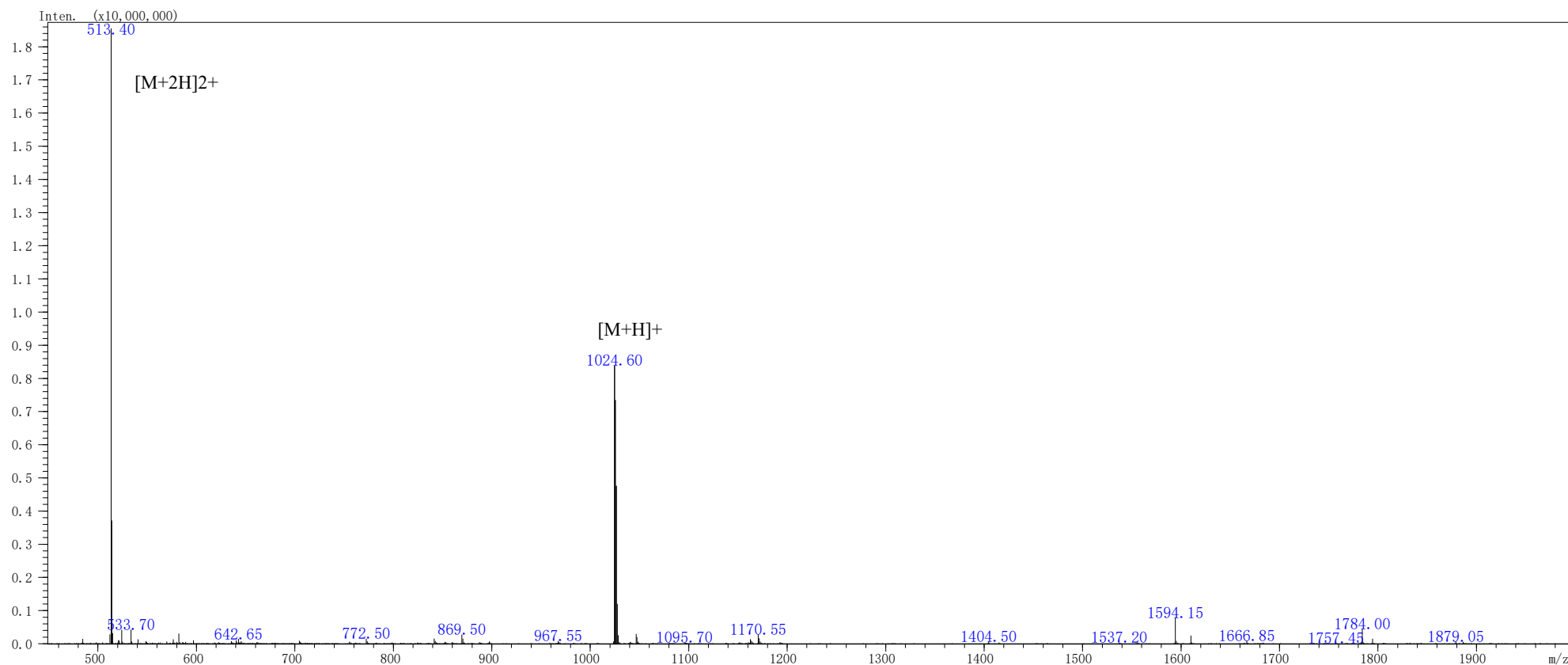
Volume: 5ul

File opened: F:\ 477171-30-55.hw, where



Rank	Time	Conc.	Area	Height
1	9.763	0.0465	2310	302
2	10.104	0.03982	1978	316
3	11.399	0.2248	6199	505
4	11.766	99.27	4945535	350988
5	12.293	0.2347	6690	1937
6	13.954	0.08226	4086	463
Total		100		

MASS SPECTROMETRY REPORT



Sample Description

Analyzed date: 2025/08/05

Analyst: PY

Sample: Melanotan 2

M.W.: 1024.20

Lot. No.: 20240620

Instrument

Probe: ESI

Nebulizer Gas Flow: 1.5L/min

CDL: -20.0v

CDL Temp.: 250 °C

Block Temp.: 400 °C

SHIMADZU LCMS-2020

Probe Bias: +4.5kv

Detector: 1.2kv

T. Flow: 0.2ml/min

B. Conc.: 50%H2O/50%ACN