

Pen peptide certificate of analysis (CoA)

Product:	Retatrutide		
Source:	Hybrid synthesis (recombinant using peptide secretion system and chemical synthesis)		
Intended use:	For stability, viability and activity testing only.		
Order number:		Lot:	25AUG21RET
Production:	08/2025	Expiry:	08/2027
Formulation:	0.2 µm-filtered solution in 20mM glycine, 200mM Mannitol, 20mM NaH ₂ PO ₄ , pH 8.4; 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

Retatrutide

Data Provided:

- Initial concentration of Retatrutide: **99,28%**.
- Concentration after 7 days at:
 - **+4°C: 98,63%**.
 - **+42°C: 98,83%**.

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: 315 days.**

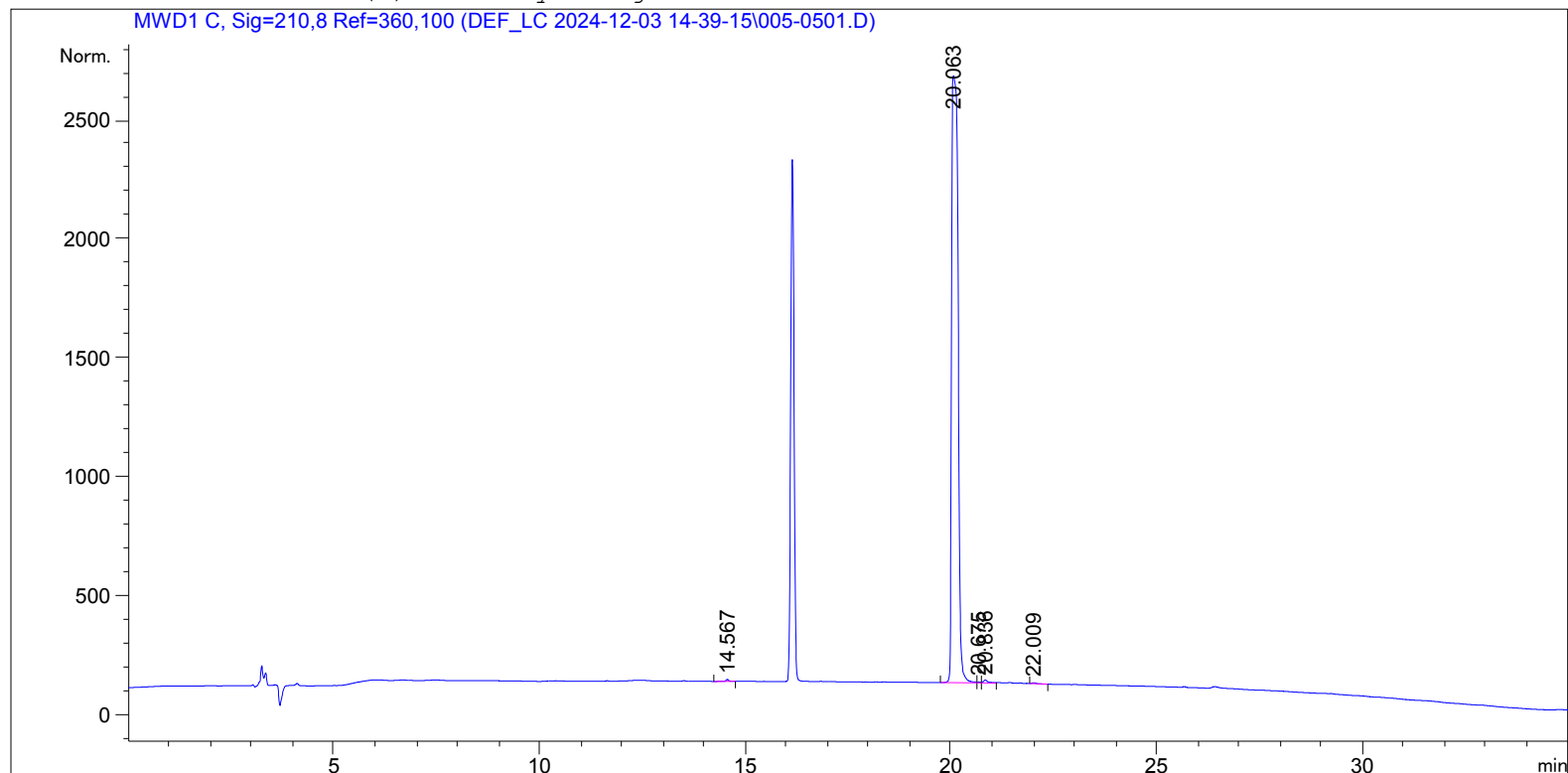
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Acq. Operator	:		Seq. Line	:	5
Acq. Instrument	:	Instrument 1	Location	:	Vial 5
Injection Date	:	12/3/2024 5:27:09 PM	Inj	:	1
			Inj Volume	:	10.0 µl

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	14.567	BB	0.0699	42.10877	8.78942	0.1504
2	20.063	BV	0.1791	2.77929e4	2551.35107	99.2821
3	20.675	VV	0.0804	20.99424	3.80064	0.0750
4	20.836	VV	0.1196	106.71061	12.75552	0.3812
5	22.009	VB	0.1027	31.14668	4.39302	0.1113

Totals : 2.79939e4 2581.08967

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*** End of Report ***

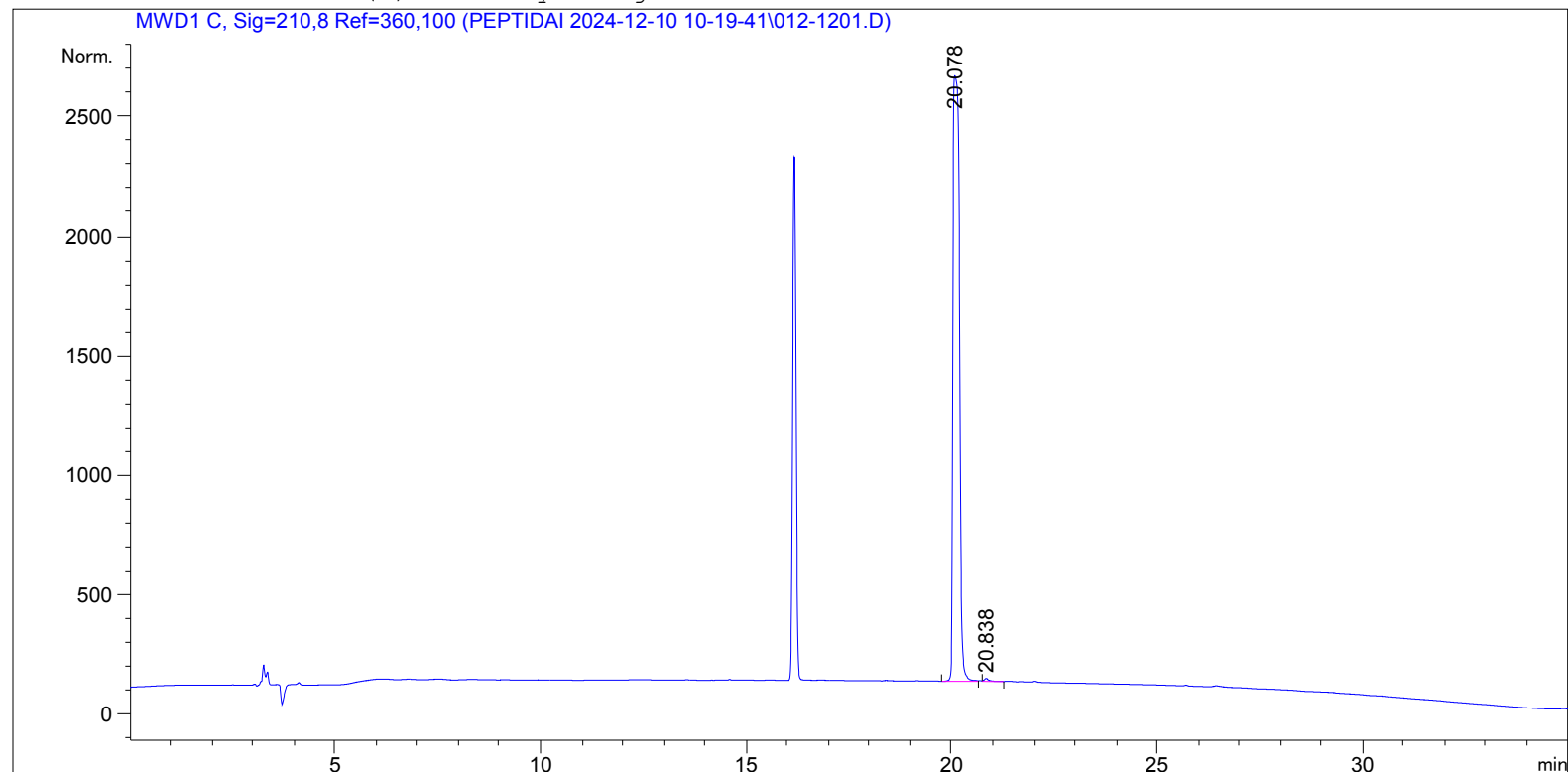
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Acq. Operator	:		Seq. Line	:	12
Acq. Instrument	:	Instrument 1	Location	:	Vial 12
Injection Date	:	12/10/2024 5:57:08 PM	Inj	:	1
			Inj Volume	:	10.0 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\1\DATA\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

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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	20.078	BV	0.1797	2.77428e4	2534.70532	99.6150
2	20.838	VB	0.1221	107.21180	12.49352	0.3850

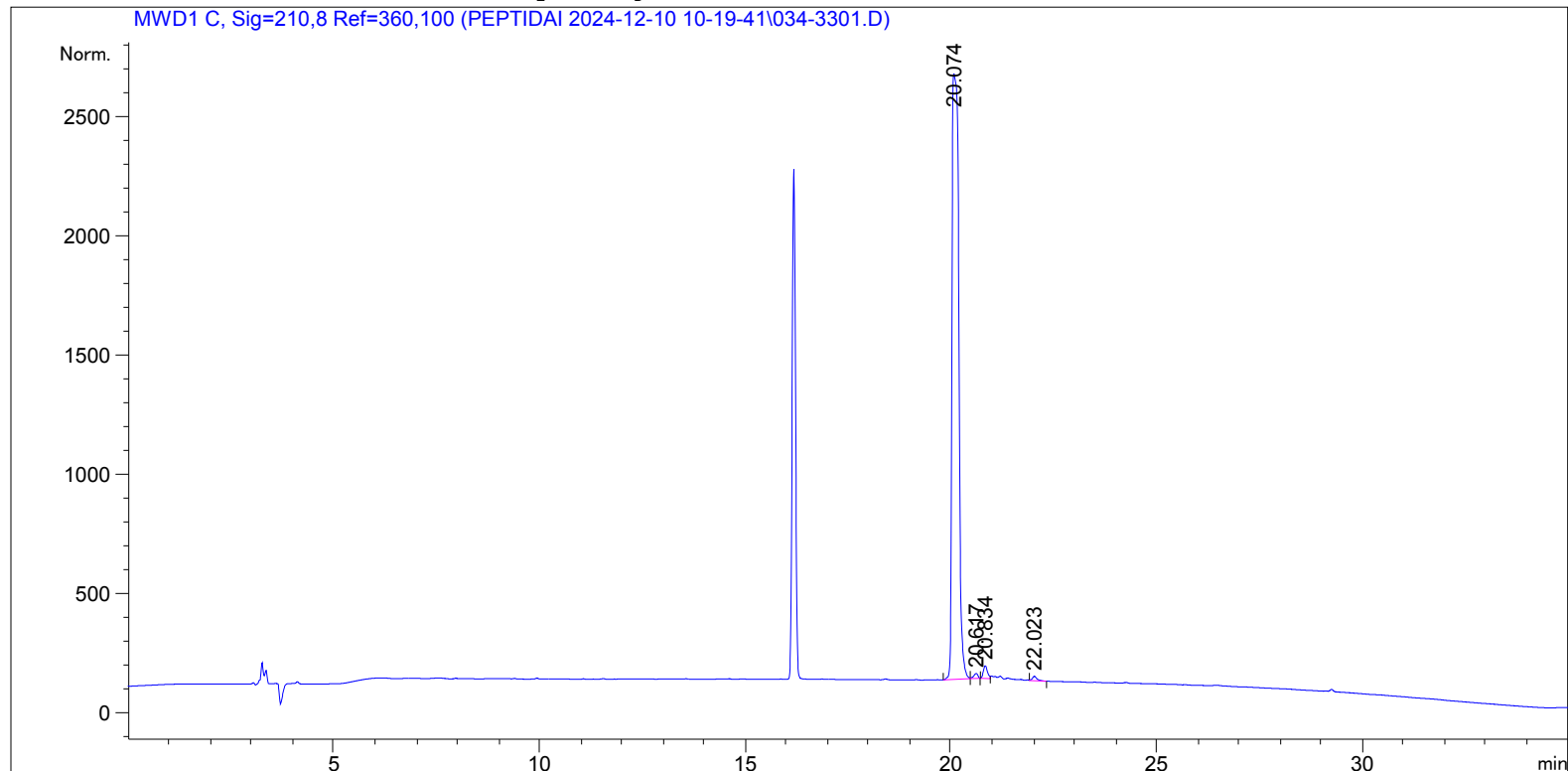
Totals : 2.78500e4 2547.19885

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*** End of Report ***

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Acq. Operator	:		Seq. Line	:	33
Acq. Instrument	:	Instrument 1	Location	:	Vial 34
Injection Date	:	12/11/2024 8:29:45 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 4:40:41 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

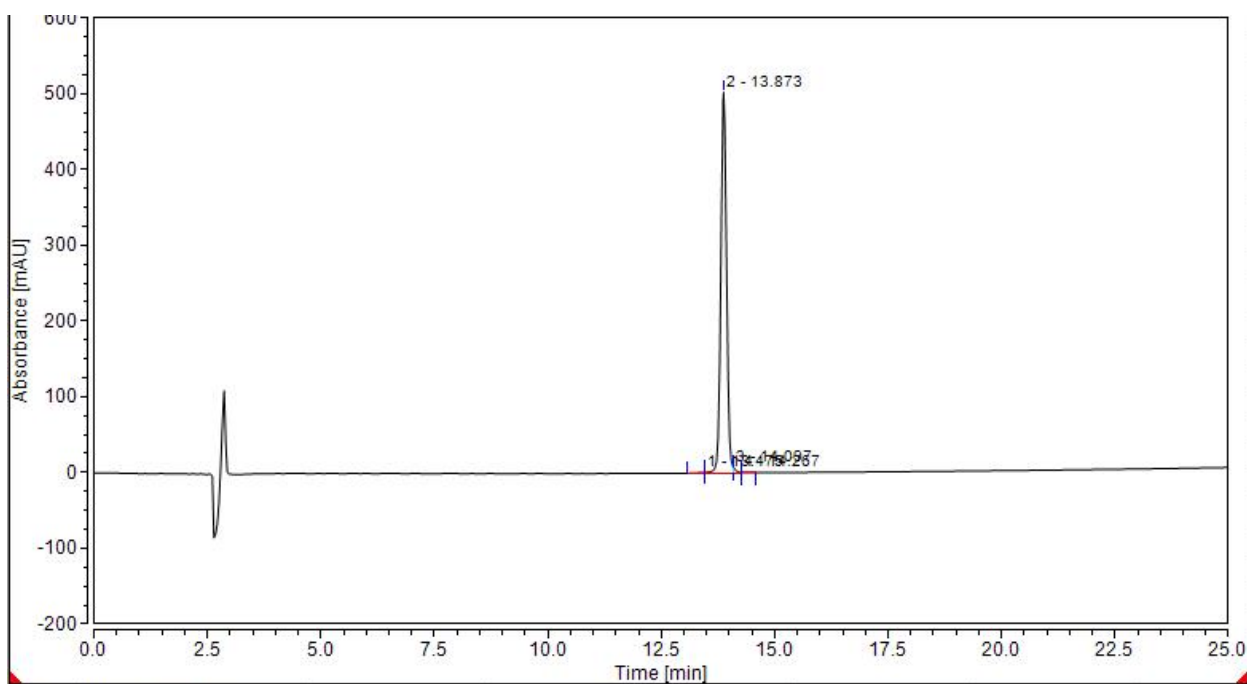
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.074	BV	0.1867	2.88939e4	2539.85767	97.7360
2	20.617	VB	0.1106	148.99915	21.03558	0.5040
3	20.834	BV	0.1103	372.88998	52.85370	1.2613
4	22.023	VB	0.1151	147.40880	18.87267	0.4986

Totals : 2.95632e4 2632.61962

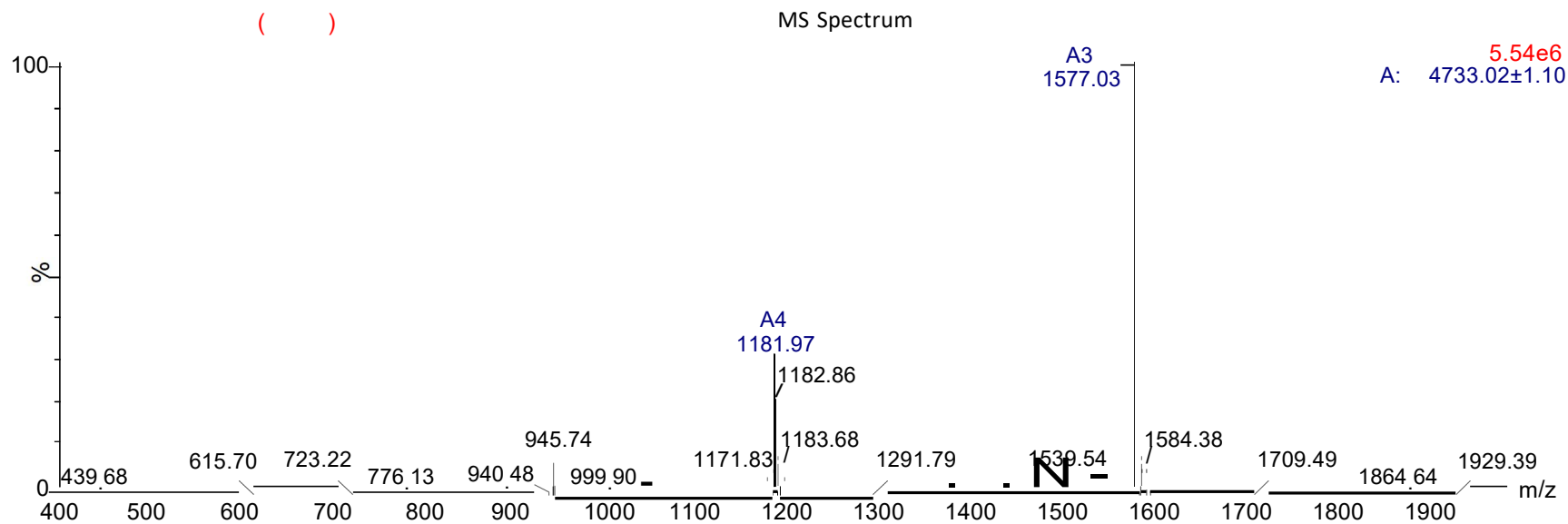
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*** End of Report ***

Sample Information

Product Name :Retatrutide
 Lot. No :
 :Tyr-{Aib}-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Ile- α -Me-Leu}-Leu-Asp-Lys-{diacid-C20-gamma-Glu-(AEEA)-Lys}-Ala-Gln-{Aib}-Ala-Phe-Ile-Glu-Tyr-Leu-Leu-Glu-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH2
 Sequence
 Column :Welch,XB-C18,(4.6mm i.d.,250 mm L)
 Buffer : A: 0.1% TFA in Acetonitrile B: 0.1% TFA in H2O
 Gradient :40-65% in 25 min
 Flow rate :1.0ml/min
 Wavelength :220nm
 Dissolution method :100%H2O



Integration Results							
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1		13.475	0.160	0.850	0.22	0.17	n.a.
2		13.873	72.924	502.311	99.18	98.40	n.a.
3		14.097	0.374	6.814	0.51	1.33	n.a.
4		14.267	0.066	0.480	0.09	0.09	n.a.
Total:			73.524	510.454	100.00	100.00	



Dissolution	:0.1%HCOOH+ACN	Interface	:ESI	Prerod Bias	:+3.5kv
Date	:2025/08/21	Nebulizing Gas Flow	:500L/hr	Detector	:-0.2kv
Injection	20:18:45 :2.5ul	CDL Temp	:400C	T.Flow	:0.35ml/min
Block Temp	:150	CDL Volt	:-45V	B.conc	:50%H2O/50%MEOH

Product Name : Retatrutide

Sequence : Tyr-{Aib}-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Ile-α
-Me-Leu}-Leu-Asp-Lys-{diacid-C20-gamma-Glu(AEEA)-Lys}-Ala-Gln-{Aib}-Ala-Phe-Ile-Glu-Tyr-Leu-Leu-Glu-Gly-Gly-Pro-Ser-Gly-Ala-Pro-Pro-Ser-NH₂

Lot.No : 202508

Theoretical : 4731.33

Observed : 4731.92