

# Cyclic gallium-68 labeled peptides for specific detection of human angiotensin-converting enzyme 2

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San Francisco CA 94110, USA

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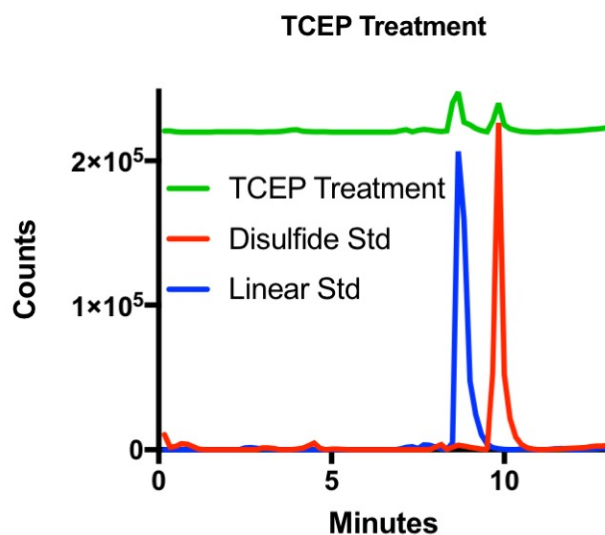
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## A. Determination of ACE2 IC<sub>50</sub>'s:

PEPTIDE	IC <sub>50</sub>
DX600	118.2 nM
6-TCEP	90.93 nM
6+TCEP	412.5 nM

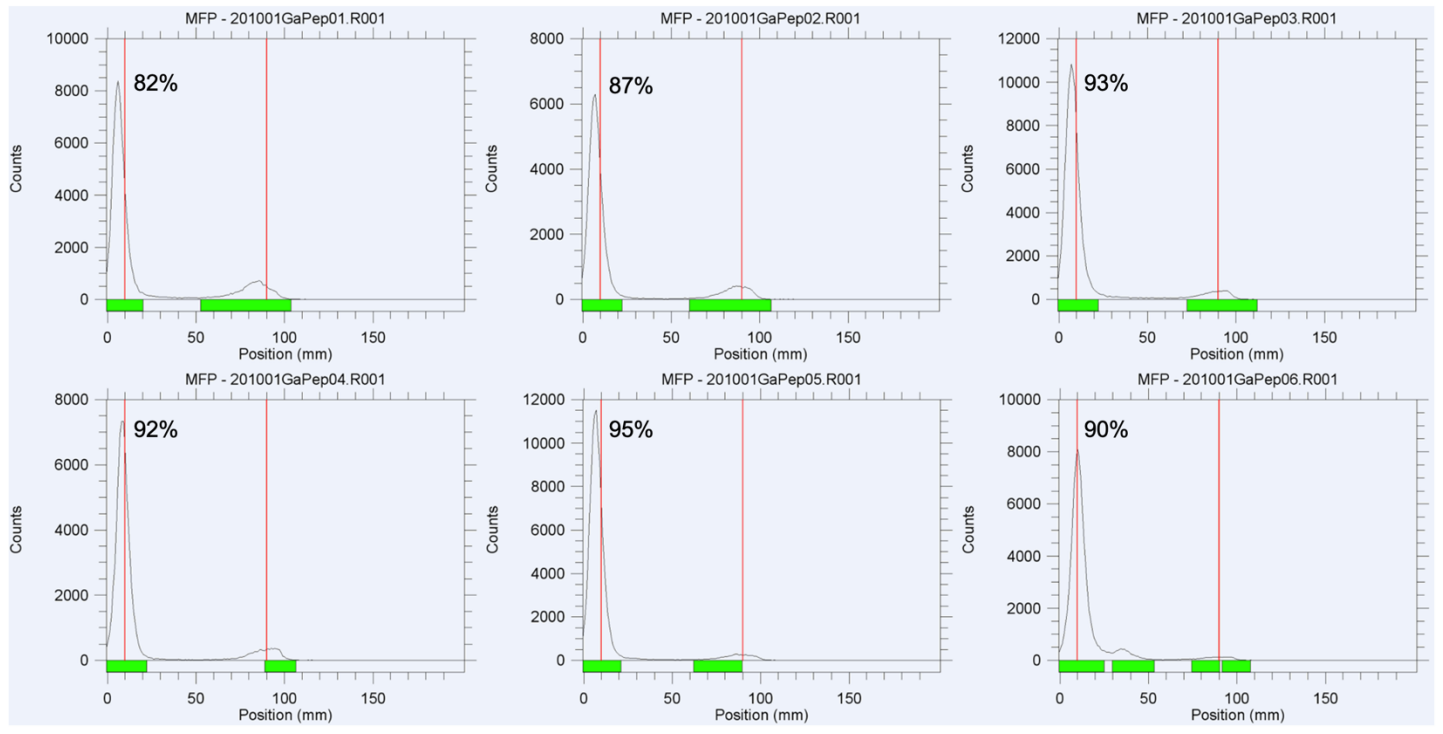
**Supp. Fig. 1:** ACE2 IC<sub>50</sub>'s for NOTA-PEP6 when TCEP is used for disulfide bridge reduction (conversion of cyclic to linear peptide). An approximately 5-fold higher calculated IC<sub>50</sub> was observed in the presence of TCEP.



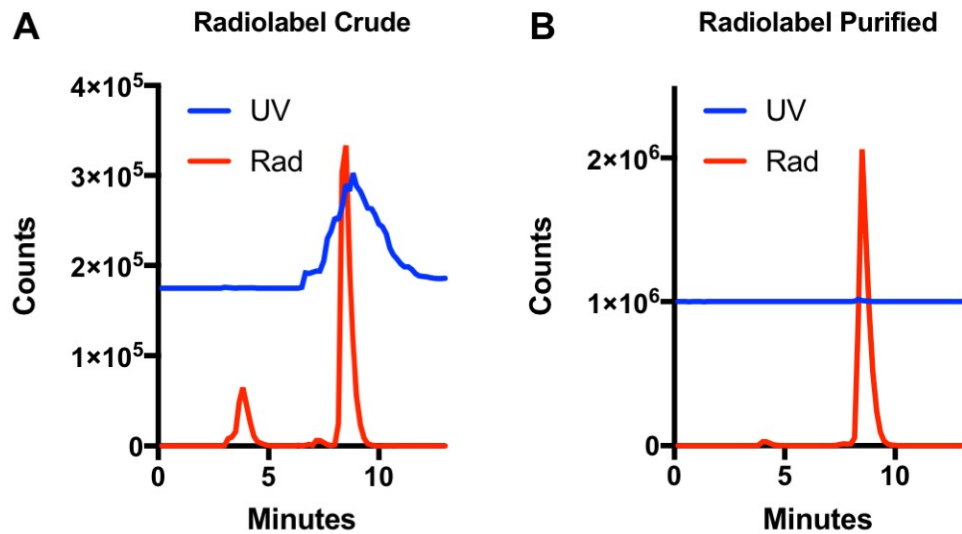
**Supp. Fig. 2:** HPLC (C18; isocratic 70% MeCN in H<sub>2</sub>O) confirmation of reduction of the disulfide bridge in NOTA-PEP6; HPLC confirmed conversion of NOTA-PEP6 to NOTA-PEP5 in the presence of TCEP. A 2 µg/µL solution of NOTA-PEP in PBS was treated with a 0.5M solution of TCEP (100X concentration) to a final concentration of 5mM. The reaction was heated for 1 hour at 37°C and 10µL aliquot was injected on the HPLC. The TCEP treatment converted 60% of NOTA-PEP6 to NOTA-PEP5.

## B. Radiochemistry:

80 µg of NOTA-PEP (2 µg/µL concentration) was diluted into 160 µL of sodium acetate buffer (pH = 5.5) and added to <sup>68</sup>GaCl<sub>3</sub> solution (370-740 MBq in 4 mL 0.05 M HCl; eluted from a generator). The mixture was tested for pH and additional sodium acetate buffer (pH = 5.5) was added to modulate the pH to between 3.5-4. The mixture was heated to 90°C for 15 minutes, diluted with 50 mM ammonium acetate solution and loaded onto a preconditioned C18 Sep-Pak. The cartridge was washed with 50 mM ammonium acetate solution and the labelled peptide was eluted from the cartridge in 200 µL fractions with 70:30 ethanol/50 mM ammonium acetate solution. The most concentrated fraction was diluted 10-fold with 0.9% sodium chloride solution and passed through a sterile filter for use. <sup>68</sup>Ga-NOTA-PEP4 was obtained 63% yield (decay corrected, n = 8) in greater than 99% purity (n = 8), with molar activity greater than or equal to 15.6 GBq/µmol.

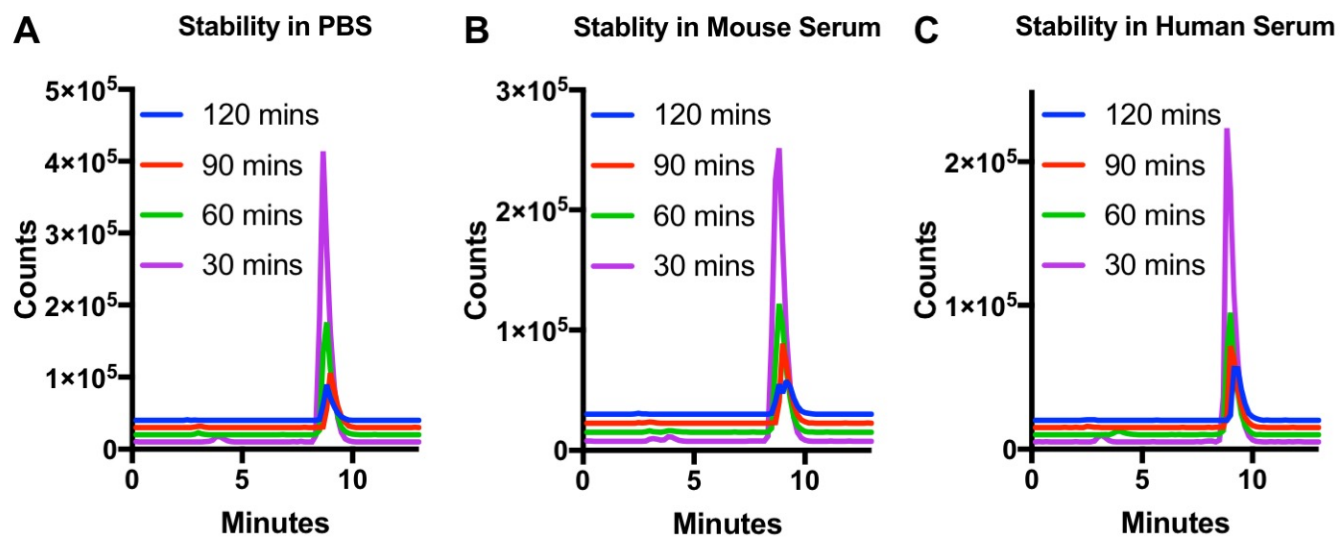


**Supp. Fig. 3:** Radiosyntheses of all six  $^{68}\text{Ga}$ -NOTA-PEP peptides (crude RadioTLC). The chelated  $^{68}\text{Ga}$ -NOTA-PEP was > 80% of radioactive signals observed in the crude product, in all cases.

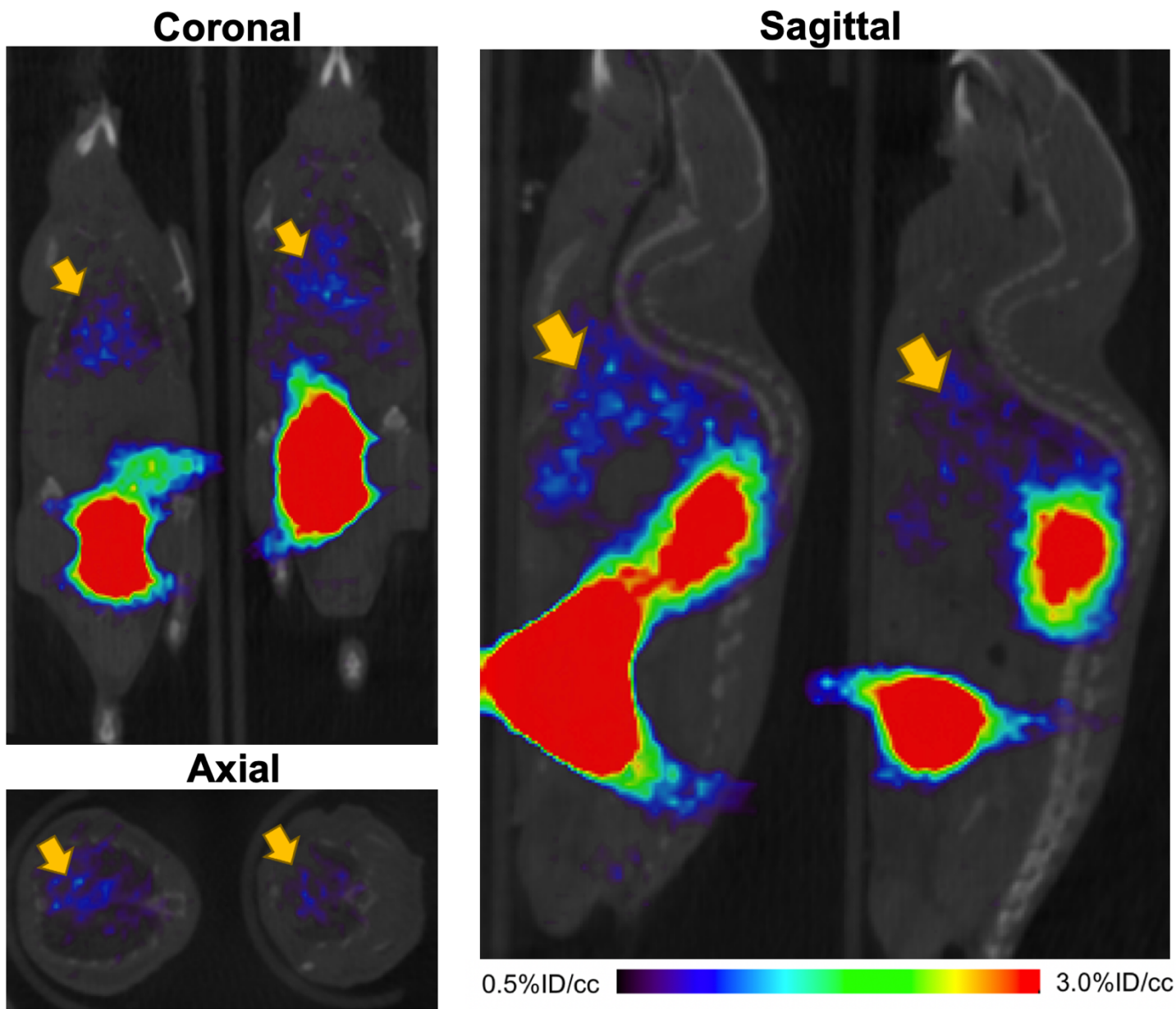


**Supp. Fig. 4.** A) HPLC (C18; gradient of 5-95% MeCN in  $\text{H}_2\text{O}$ ) of crude  $^{68}\text{Ga}$ -NOTA-PEP4 after incubation of NOTA-PEP4 with  $^{68}\text{Ga}$ - $\text{GaCl}_3$ . B) HPLC (C18; gradient of 5-95% MeCN in  $\text{H}_2\text{O}$ ) of purified  $^{68}\text{Ga}$ -NOTA-PEP4 after purification on C18 sep-pak. Analysis of the purified trace shows 99% purity of the labeled peptide which was used subsequently for animal studies.

**C. Supplemental Figures:**

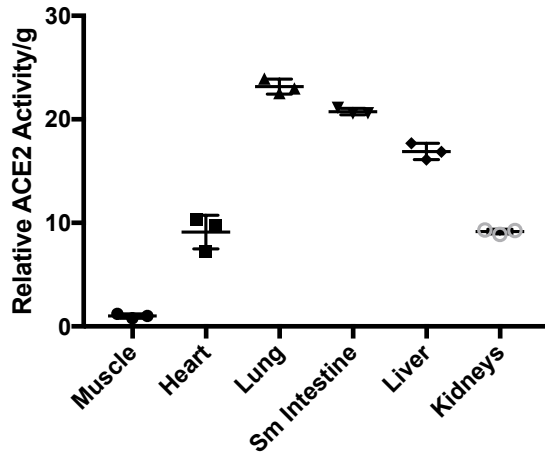


**Supp. Fig. 5.** HPLC C18; gradient of 5-95% MeCN in H<sub>2</sub>O) analysis of <sup>68</sup>Ga-NOTA-PEP4 incubated with PBS, mouse serum, and human serum. 37MBq (1mCi) of <sup>68</sup>Ga-NOTA-PEP4 was added to 1mL of each solution and incubated for 2 hours at 37°C. HPLC were performed at 30 min intervals. <sup>68</sup>Ga-NOTA-PEP4 was > 95% intact in each solution for the 2 hour duration.



**Supp. Fig. 6:** Additional images of  $^{68}\text{Ga}$ -NOTA-PEP4 in hACE2 transgenic mice, obtained via single time-point acquisition.

### Ex Vivo ACE2 Activity of Tissue Isolates



**Supp. Fig. 7:** Confirmation of ACE2 activity in tissue isolates, as correlation to reported *in vivo* data (Anaspec, Fremont CA).

<b>YOUR ORDER</b>	<b>SON</b>	<b>DATE</b>
B001890494	1500055456 	15-Jun-20

<b>CUSTOMER</b>	<b>ADDRESS / INSTITUTION</b>
University of California San Francisco (UCSF)	UNITED STATES San Francisco

<b>PEPTIDE NAME</b>	<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>
75386-1: (linear peptide)	2056275	2071245	Custom	28

**SEQUENCE (N-Term → C-Term)**

**NOTA - GGG DYS HCS PLR YYP WWK CTY PDP EGG G - NH<sub>2</sub>**

PHYSICOCHEMICAL PROPERTIES		REGULAR AA PROPERTIES					
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA	D,S,H,R,K,T,E	9
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA	G,Y,C,P,L,W	1
Isoelectric Point *	6.7	Basic AA	H,R,K	3			

(\* Theoretical values)

**QC DATA**

Attribute	Test method	Acceptance criteria	Result
Appearance	Visual	Report result	White Powder
% Peak Area by HPLC	HPLC	≥ 90 %	96 %
Identity	MS	3433.9 ± 0.2 %	3435.1

**DELIVERABLE**

<b>Format</b>	Dried	<b>Alliquoting</b>	Lot# 2056275 10 mg
		Number of Aliquots	1 (linear peptide)
		Qty by Aliquot (mg)	10 mg

For Laboratory Use Only

**DELIVERY CONDITION**

Room temperature

**STORAGE CONDITION**

-20 °C, dry

**COMMENTS**

**PEPTIDE RECONSTITUTION AND STORAGE**

Please read the entire section before proceeding with the solubilization of your custom peptide.

Peptides are shipped at ambient temperature as a lyophilized powder. Upon receipt store them at -20°C. Allow the vial to equilibrate to room temperature prior to opening.

Peptide solubility is highly dependent on the sequence. Peptides that are more hydrophobic (high propensity of A, F, G, V, L, I, M, W, P) in nature, will require an organic solvent in order to dissolve. Peptides that are acidic in nature (high propensity of D, E in the peptide sequence) require a basic aqueous buffer to dissolve, while peptides that are basic in nature (high propensity of K, H, and R) require an acidic aqueous buffer to dissolve.

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**C-terminus:** OH means free acid (-COOH), NH<sub>2</sub> means amide [-CONH<sub>2</sub>]

Modifications on the side chain of amino acids are depicted in the parenthesis after the corresponding amino acid. For example; phosphorylated serine = S(PO<sub>3</sub>H<sub>2</sub>) or epsilon-N-acetylated lysine = K(Ac)

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E-mail: [info@eurogentec.com](mailto:info@eurogentec.com) Web: [www.eurogentec.com](http://www.eurogentec.com)

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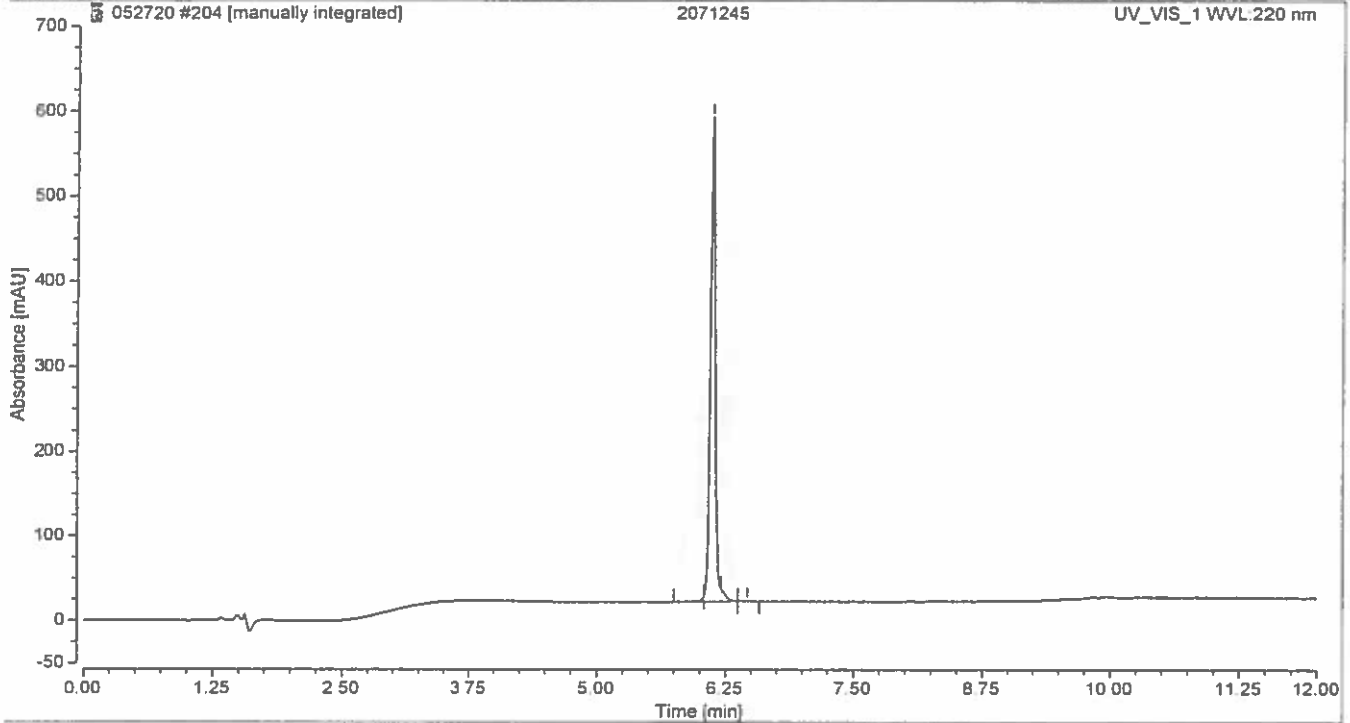
E-mail: [service@eurogentec.com](mailto:service@eurogentec.com) Web: [www.anaspec.com](http://www.anaspec.com)

### Chromatogram and Results

**Injection Details**

Injection Name:	2071245	Run Time (min):	12.00
Vial Number:	GA1	Injection Volume:	1.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Column:	C18,100X4.6mm,H17-231434	Wavelength:	220
Instrument Method:	5-60%B-7minsExtended,Column(6-1)-0.7ml-30C	Bandwidth:	4
Processing Method:	test	Instrument No.	QC-HPLC-10
Injection Date/Time:	15/Jun/20 11:31	Sample Weight:	

**Chromatogram**



**Integration Results**

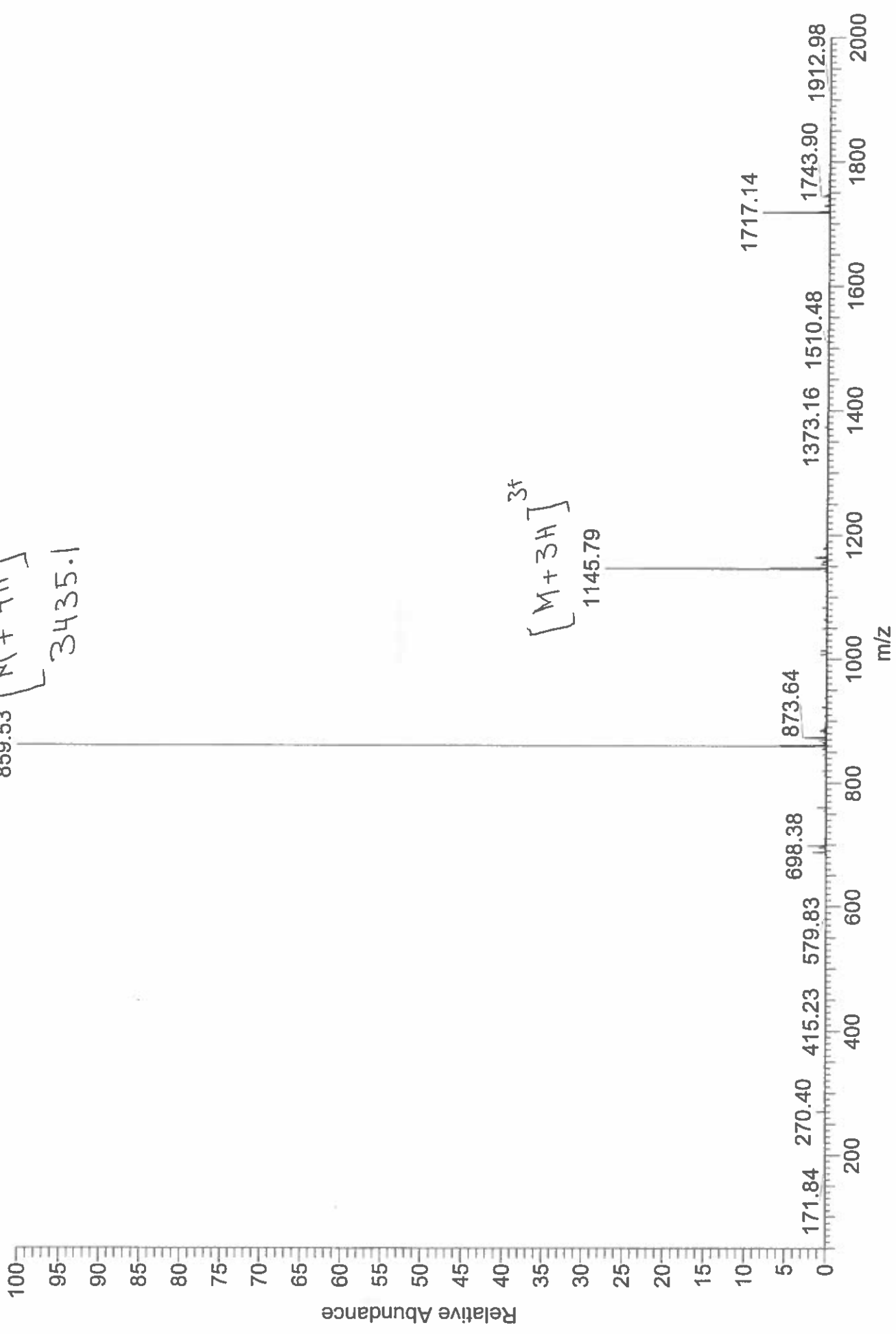
No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	6.040	0.204	6.008	0.64
2	6.120	30.586	571.320	96.41
3	6.207	0.807	15.001	2.55
4	6.463	0.128	1.142	0.40
<b>Total:</b>		<b>31.724</b>	<b>593.470</b>	<b>100.00</b>



06/12/20

2071245 #35-120 RT: 0.22-0.77 AV: 29 NL: 4.36E5

F: ITMS + c ESI E Full ms [50.00-2000.00]



<b>YOUR ORDER</b>		<b>SON</b>		<b>DATE</b>	
B001890494		1500055456		16-Jun-20	
<b>CUSTOMER</b>			<b>ADDRESS / INSTITUTION</b>		
University of California San Francisco (UCSF)			UNITED STATES San Francisco		
<b>PEPTIDE NAME</b>		<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>
75386-2: (disulfide bridge)		2056276	2071246	Custom	28
<b>SEQUENCE (N-Term → C-Term)</b>					
NOTA - GGG DYS HC(S-)S PLR YYP WWK C(S-)TY PDP EGG G - NH <sub>2</sub> , with disulfide bridge					
<b>PHYSICOCHEMICAL PROPERTIES</b>			<b>REGULAR AA PROPERTIES</b>		
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA
Isoelectric Point *	6.7	Basic AA	H,R,K	3	
(* Theoretical values)					
<b>QC DATA</b>					
<b>Attribute</b>	<b>Test method</b>	<b>Acceptance criteria</b>		<b>Result</b>	
Appearance	Visual	Report result		White Powder	
% Peak Area by HPLC	HPLC	≥ 95 %		95 %	
Identity	MS	3431.9 ± 0.2 %		3432.6	
<b>DELIVERABLE</b>					
<b>Format</b>	Dried	<b>Aliquoting</b>		<div style="border: 1px solid black; padding: 5px; text-align: center;">           Corr 2056276 10 mg            (disulfide bridge)  <i>For Laboratory Use Only</i>  <b>ANASPEC</b> </div>	
		Number of Aliquots 1			
		Qty by Aliquot (mg) 10 mg			
<b>DELIVERY CONDITION</b>			<b>STORAGE CONDITION</b>		
Room temperature			-20 °C, dry		
<b>COMMENTS</b>					

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Modifications on the side chain of amino acids are depicted in the parenthesis after the corresponding amino acid. For example; phosphorylated serine = S(PO<sub>3</sub>H<sub>2</sub>) or epsilon-N-acetylated lysine = K(Ac)

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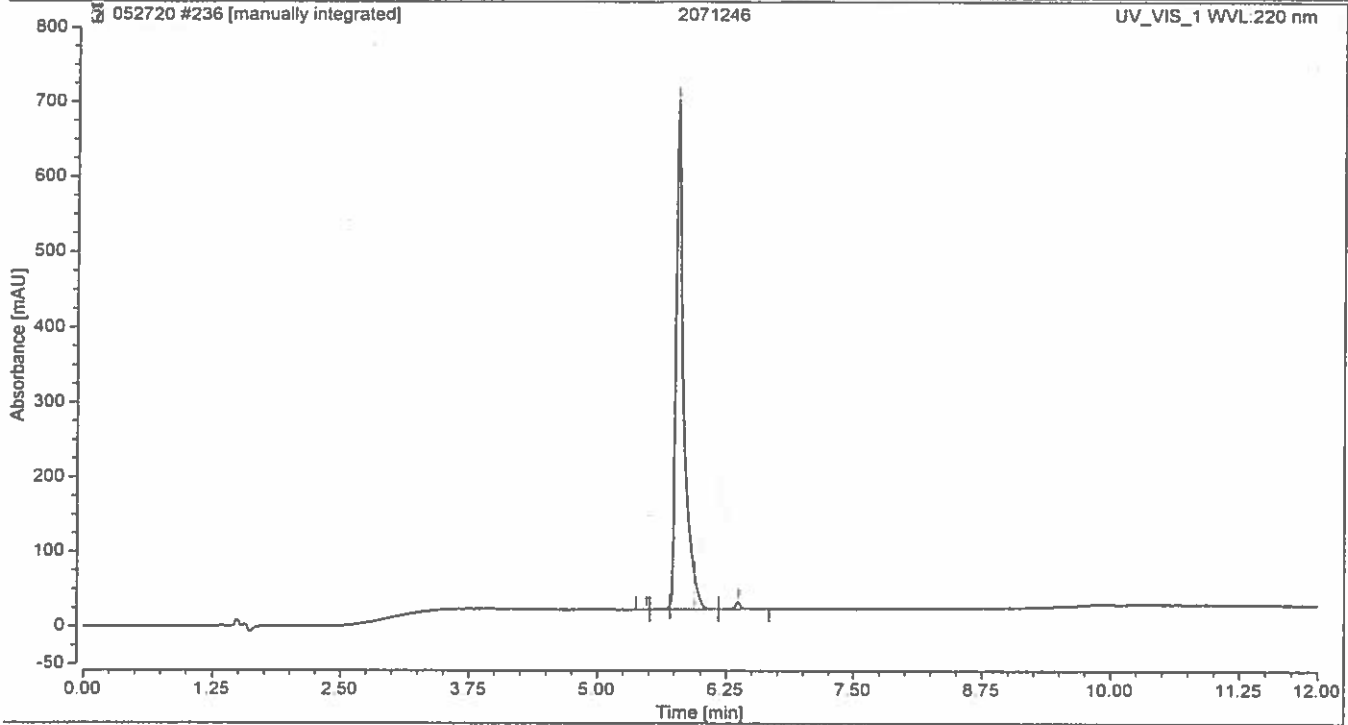
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### Chromatogram and Results

**Injection Details**

Injection Name:	2071246	Run Time (min):	12.00
Vial Number:	GC2	Injection Volume:	1.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Column:	C18,100X4.6mm,H17-231434	Wavelength:	220
Instrument Method:	5-60%B-7minsExtended,Column(6-1)-0.7ml-30C	Bandwidth:	4
Processing Method:	test	Instrument No.:	QC-HPLC-10
Injection Date/Time:	16/Jun/20 13:47	Sample Weight:	

**Chromatogram**



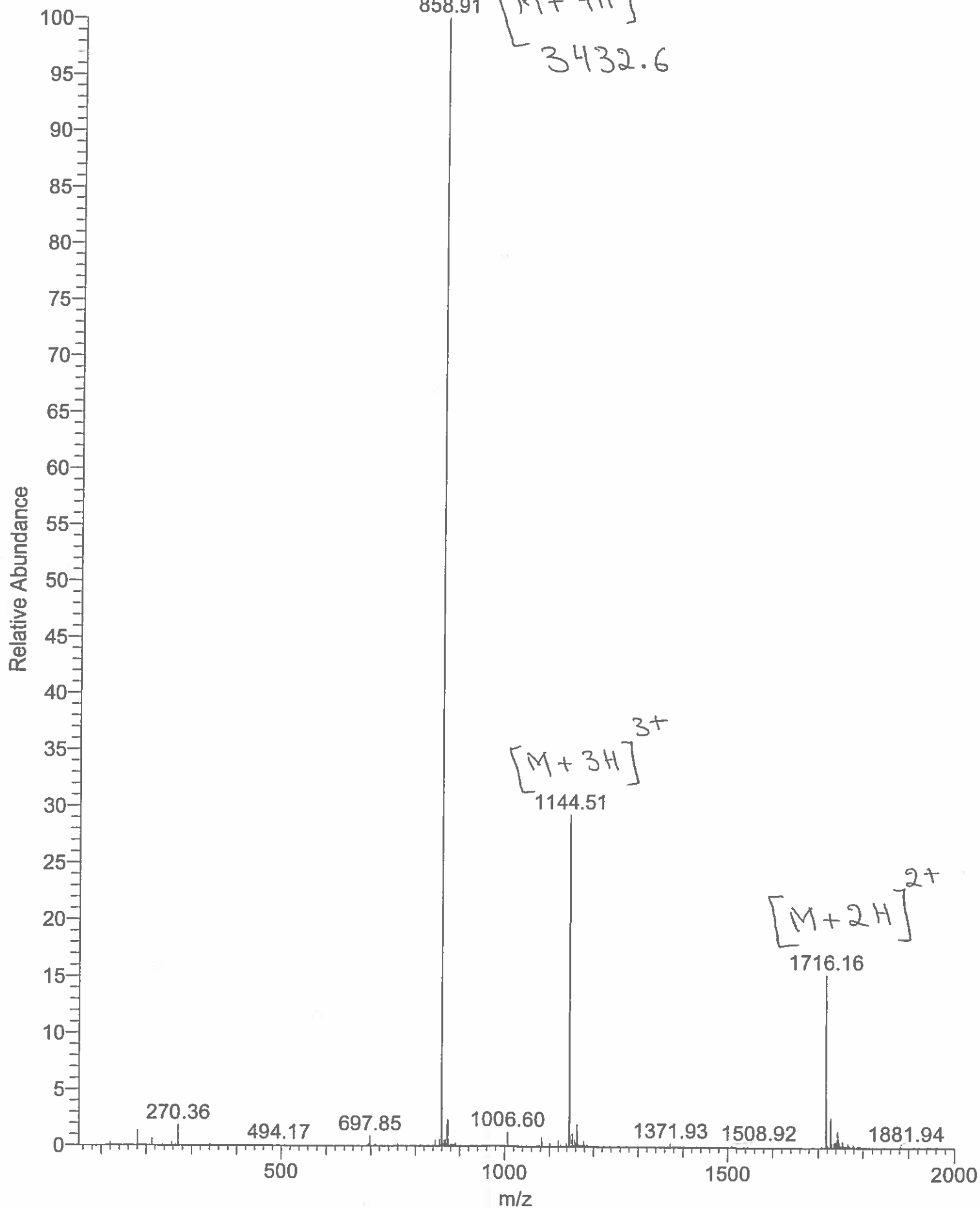
**Integration Results**


No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	5.477	0.003	0.104	0.01
2	5.707	0.096	3.887	0.17
3	5.773	55.557	679.939	95.33
4	5.937	2.010	47.591	3.45
5	6.367	0.611	10.131	1.05
<b>Total:</b>		<b>58.277</b>	<b>741.651</b>	<b>100.00</b>

06/16/20

2071246 #35-114 RT: 0.22-0.73 AV: 27 NL: 2.03E5

F: ITMS + c ESI E Full ms [50.00-2000.00]



<b>YOUR ORDER</b>	<b>SON</b>		<b>DATE</b>
B001890494	1500055456		10-Jun-20

<b>CUSTOMER</b>	<b>ADDRESS / INSTITUTION</b>
University of California San Francisco (UCSF)	UNITED STATES San Francisco

<b>PEPTIDE NAME</b>	<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>
75386-3: (linear peptide)	2056277	2071247	Custom	26

**SEQUENCE (N-Term → C-Term)**

**NOTA** -Ahx- DY SHC SPL RYY PWW KCT YPD PEG GG - NH<sub>2</sub>  
 Ahx=6-aminohexanoic acid linker

PHYSICOCHEMICAL PROPERTIES		REGULAR AA PROPERTIES					
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA	D,S,H,R,K,T,E	9
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA	Y,C,P,L,W,G,-Ahx-	1
Isoelectric Point *	6.7	Basic AA	H,R,K,-Ahx-	4			

(\* Theoretical values)

**QC DATA**

Attribute	Test method	Acceptance criteria	Result
Appearance	Visual	Report result	White Powder
% Peak Area by HPLC	HPLC	≥ 90 %	96 %
Identity	MS	3375.9 ± 0.2 %	3376.8

**DELIVERABLE**

<b>Format</b>	Dried	<b>Aliquoting</b>
		Number of Aliquots 1
		Qty by Aliquot (mg) 10 mg

**DELIVERY CONDITION**

Room temperature

**COMMENTS**

Lot# 2056277 10 mg

(linear peptide)

For Laboratory Use Only

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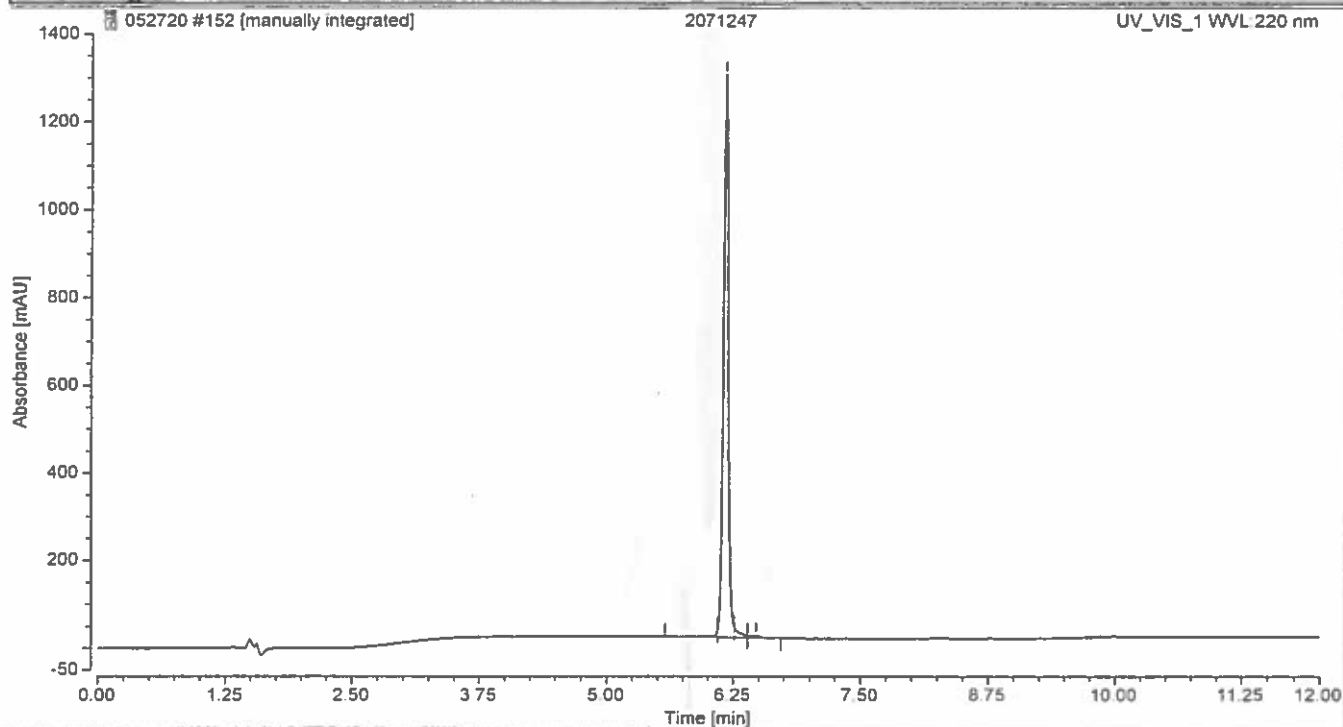
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## Chromatogram and Results

### Injection Details

Injection Name:	2071247	Run Time (min):	12.00
Vial Number:	BA1	Injection Volume:	1.50
Injection Type:	Unknown	Channel:	UV_VIS_1
Column:	C18, 100X4.6mm, H17-231434	Wavelength:	220
Instrument Method:	5-60%B-7minsExtended, Column(6-1)-0.7ml-30C	Bandwidth:	4
Processing Method:	test	Instrument No.:	QC-HPLC-10
Injection Date/Time:	10/Jun/20 13:56	Sample Weight:	

### Chromatogram



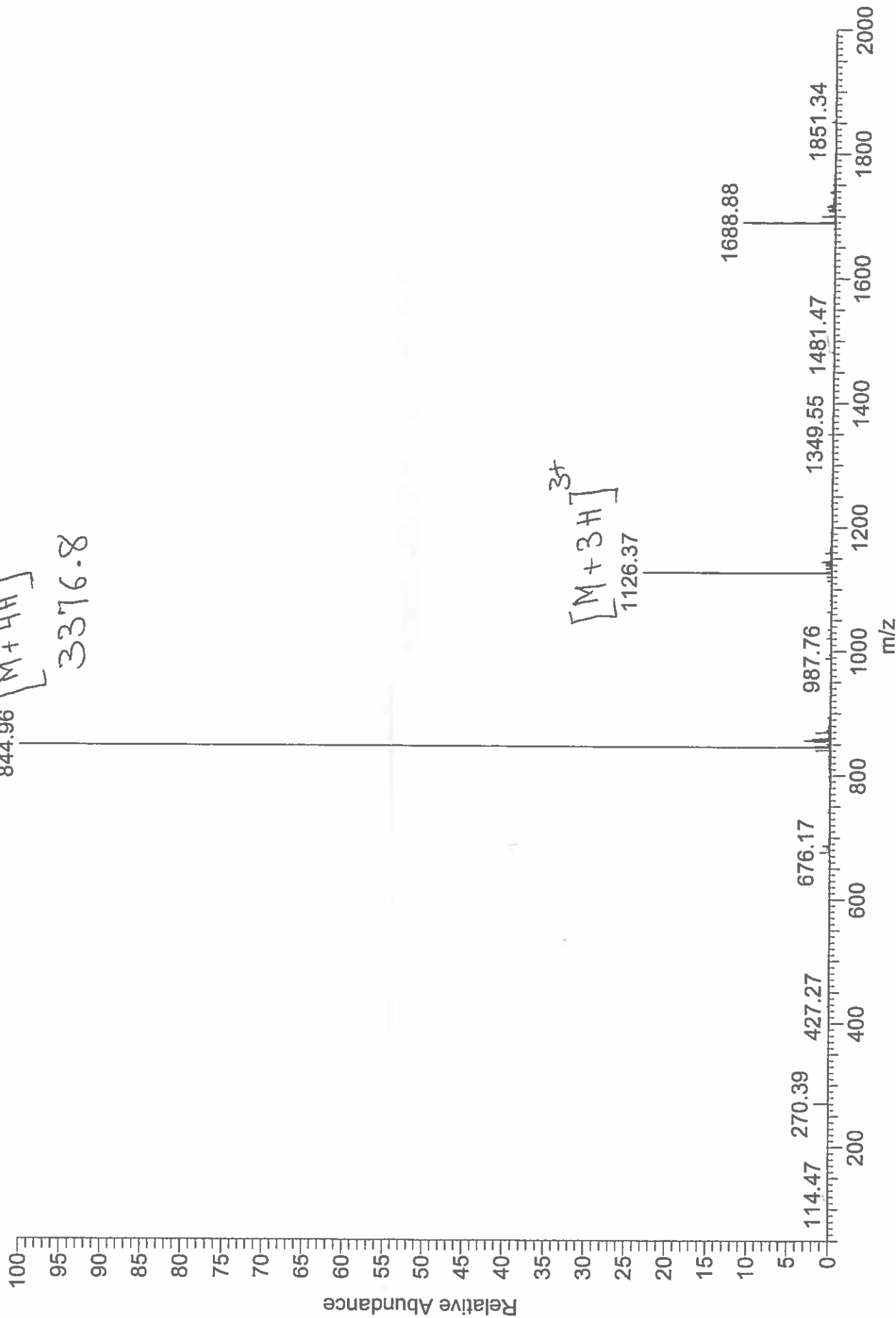
### Integration Results

No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	6.097	0.652	16.476	0.91
2	6.170	69.280	1283.037	96.41
3	6.260	1.376	21.965	1.92
4	6.473	0.555	5.212	0.77
<b>Total:</b>		<b>71.863</b>	<b>1326.691</b>	<b>100.00</b>

06/10/20 *[Signature]*

2071247 #35-109 RT: 0.22-0.69 AV: 25 NL: 2.70E5

F: ITMS + c ESI E Full ms [50.00-2000.00]



<b>YOUR ORDER</b>		<b>SON</b>		<b>DATE</b>	
B001890494		1500055456		10-Jun-20	
<b>CUSTOMER</b>			<b>ADDRESS / INSTITUTION</b>		
University of California San Francisco (UCSF)			UNITED STATES San Francisco		
<b>PEPTIDE NAME</b>		<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>
75386-4: (disulfide bridge)		2056278	2071248	Custom	26
<b>SEQUENCE (N-Term → C-Term)</b>					
NOTA- Ahx- DY SHC(S)- SPL RYY PWW KC(S)-T YPD PEG GG - NH <sub>2</sub> , with disulfide bridge					
Ahx=6-aminohexanoic acid linker					
<b>PHYSICOCHEMICAL PROPERTIES</b>			<b>REGULAR AA PROPERTIES</b>		
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA
Isoelectric Point *	6.7	Basic AA	H,R,K,-Ahx-	4	
(* Theoretical values)					

<b>QC DATA</b>			
<b>Attribute</b>	<b>Test method</b>	<b>Acceptance criteria</b>	<b>Result</b>
Appearance	Visual	Report result	White Powder
% Peak Area by HPLC	HPLC	≥ 95 %	95 %
Identity	MS	3373.8 ± 0.2 %	3374.8
<b>DELIVERABLE</b>			
<b>Format</b>	Dried	<b>Aliquoting</b>	
		Number of Aliquots	1
		Qty by Aliquot (mg)	10 mg
<b>DELIVERY CONDITION</b>		<b>STORAGE CONDITION</b>	
Room temperature		-20 °C, dry	
<b>COMMENTS</b>			

Lot# 2056278 10 mg  
 (Disulfide bridge)  
 For Laboratory Use Only  
**ANASPEC**

**PEPTIDE RECONSTITUTION AND STORAGE**

Please read the entire section before proceeding with the solubilization of your custom peptide.

Peptides are shipped at ambient temperature as a lyophilized powder. Upon receipt store them at -20°C. Allow the vial to equilibrate to room temperature prior to opening.

Peptide solubility is highly dependent on the sequence. Peptides that are more hydrophobic (high propensity of A, F, G, V, L, I, M, W, P) in nature, will require an organic solvent in order to dissolve. Peptides that are acidic in nature (high propensity of D, E in the peptide sequence) require a basic aqueous buffer to dissolve, while peptides that are basic in nature (high propensity of K, H, and R) require an acidic aqueous buffer to dissolve.

To reconstitute a hydrophobic peptide, add 100 µL DMSO and sonicate until a homogenous solution forms. Next, add your buffer of choice to form a 1 mg/mL solution (a higher concentration of peptide will require a greater amount of DMSO). To reconstitute basic or acidic peptides, add 1 mL of the appropriate buffer to the peptide and sonicate to ensure a homogenous solution forms.

Reconstituted peptides can be stored frozen at -20°C for short period of time, but it is advisable to prepare multiple aliquots to avoid multiple freeze thaw cycles. We recommend that all aliquoted solutions be lyophilized if the peptide is going to be stored for extended periods of time at -20 °C.

Additionally, please note that peptides with a high propensity of basic residues (R, K, H) in their sequence may undergo a physical change from solid powder to an oil (via moisture absorption). This physical change does not affect the purity or functionality of the peptide.

Nomenclature used for the sequence termini:  
**N-terminus:** H means free amine (NH<sub>2</sub>), Ac mean acetyl [CH<sub>3</sub>C(O)-NH-], Pyr means pyroglutamic acid  
**C-terminus:** OH means free acid (-COOH), NH<sub>2</sub> means amide [-CONH<sub>2</sub>]

Modifications on the side chain of amino acids are depicted in the parenthesis after the corresponding amino acid. For example; phosphorylated serine = S(PO<sub>3</sub>H<sub>2</sub>) or epsilon-N-acetylated lysine = K(Ac)

**TECHNICAL SUPPORT**

If you have any questions feel free to call our Technical Support Centre

<p><b>EUROPE</b></p> <p>☎ 00 800 666 00 123 (European toll free number),                  ✉ <a href="mailto:info@eurogentec.com">info@eurogentec.com</a>                  Kaneka Eurogentec S.A. Liège Science Park                  Rue Bois Saint-Jean 5 - 4102 SERAING BELGIUM                  Tel: +32(0)4 372 74 00 - Fax: +32(0)4 264 07 88                  E-mail: <a href="mailto:info@eurogentec.com">info@eurogentec.com</a> Web: <a href="http://www.eurogentec.com">www.eurogentec.com</a>                  RPM Liège T.V.A. (BE)-0427.348.346 - ING Belgique Bank - IBAN: BE86 3400 2118 6050 BIC: BBRUBEBB</p>	<p><b>NORTH AMERICA</b></p> <p>☎ +1 800 452-5530 (American toll free number),                  ✉ <a href="mailto:service@anaspec.com">service@anaspec.com</a>                  AnaSpec, Inc. 34801 Campus Drive                  Fremont, CA 94555 - USA                  Tel.: +1 (510) 791 9560 - Fax: +1 510 (791) 9572                  E-mail: <a href="mailto:service@eurogentec.com">service@eurogentec.com</a> Web: <a href="http://www.anaspec.com">www.anaspec.com</a></p>
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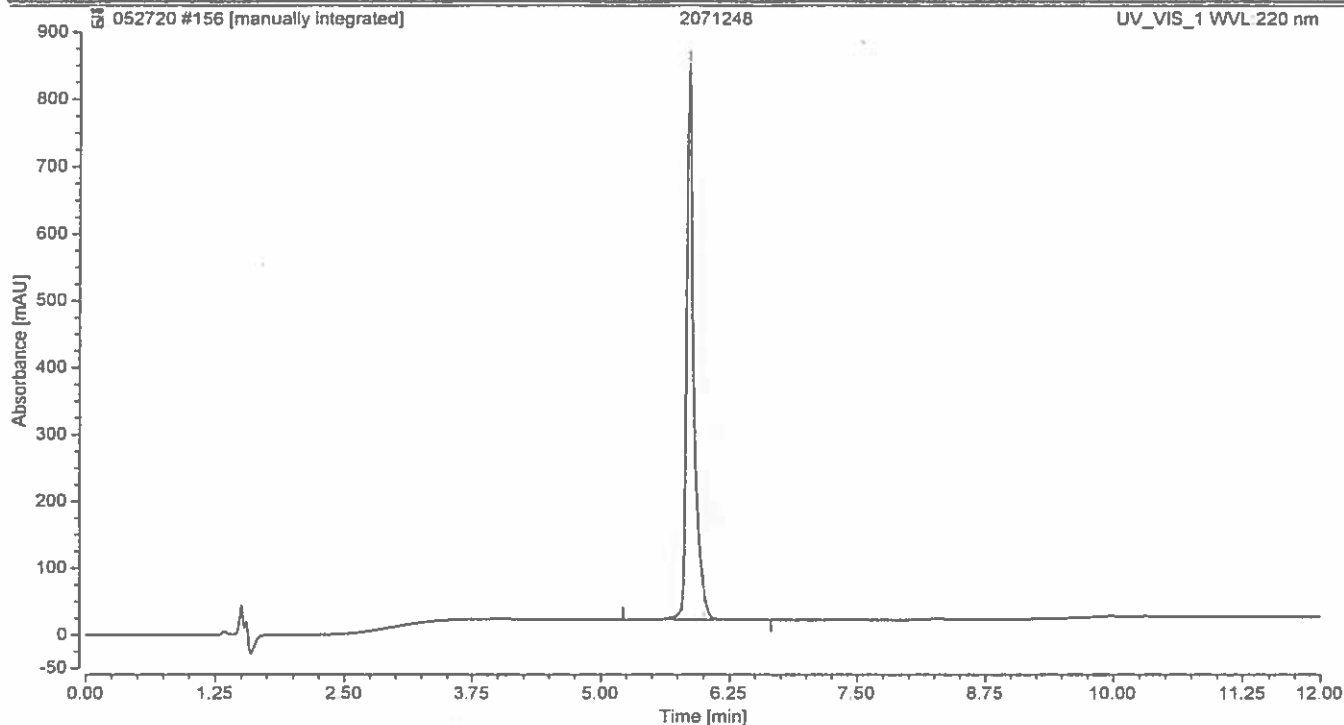


### Chromatogram and Results

#### Injection Details

Injection Name:	2071248	Run Time (min):	12.00
Vial Number:	BA2	Injection Volume:	2.50
Injection Type:	Unknown	Channel:	UV_VIS_1
Column:	C18,100X4.6mm,H17-231434	Wavelength:	220
Instrument Method:	5-60%B-7minsExtended,Column(6-1)-0.7ml-30C	Bandwidth:	4
Processing Method:	test	Instrument No.	QC-HPLC-10
Injection Date/Time:	10/Jun/20 15:31	Sample Weight:	

#### Chromatogram



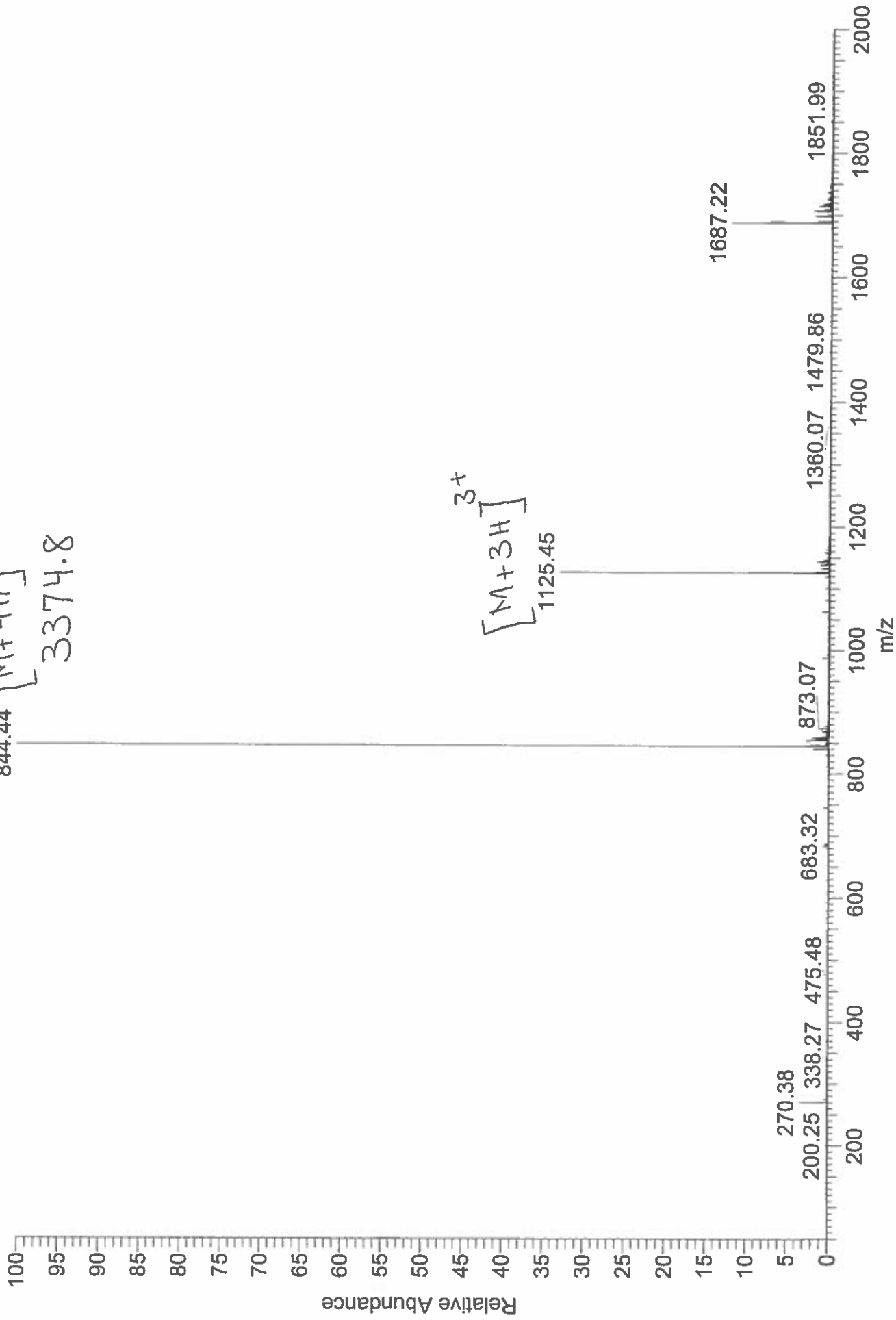
#### Integration Results

No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	5.803	1.364	49.670	2.05
2	5.853	63.551	829.478	95.29
3	5.997	1.779	47.305	2.67
<b>Total:</b>		<b>66.694</b>	<b>926.453</b>	<b>100.00</b>

06/10/2010

2071248 #37-107 RT: 0.24-0.69 AV: 24 NL: 1.54E5

F: ITMS + c ESI E Full ms [50.00-2000.00]



<b>YOUR ORDER</b>		<b>SON</b>		<b>DATE</b>	
B001890494		1500055456		19-Jun-20	
<b>CUSTOMER</b>			<b>ADDRESS / INSTITUTION</b>		
University of California San Francisco (UCSF)			UNITED STATES San Francisco		
<b>PEPTIDE NAME</b>	<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>	
75386-5: (linear peptide)	2056279	2071249	Custom	26	
<b>SEQUENCE (N-Term → C-Term)</b>					
NOTA -X- DY SHC SPL RYY PWW KCT YPD PEG GG - NH <sub>2</sub>					
X=AEEEA					
<b>PHYSICOCHEMICAL PROPERTIES</b>			<b>REGULAR AA PROPERTIES</b>		
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA
Isoelectric Point *	6.7	Basic AA	H,R,K	3	
(* Theoretical values)					

<b>QC DATA</b>			
<b>Attribute</b>	<b>Test method</b>	<b>Acceptance criteria</b>	<b>Result</b>
Appearance	Visual	Report result	White Powder
% Peak Area by HPLC	HPLC	≥ 90 %	95 %
Identity	MS	3451.9 ± 0.2 %	3452.9
<b>DELIVERABLE</b>			
<b>Format</b>	Dried	<b>Aliquoting</b>	
		Number of Aliquots	1
		Qty by Aliquot (mg)	10 mg
<b>DELIVERY CONDITION</b>		<b>STORAGE CONDITION</b>	
Room temperature		-20 °C, dry	
<b>COMMENTS</b>			
			Lot# 2056279 10 mg (linear peptide) For Laboratory Use Only <b>ANASPEC</b>

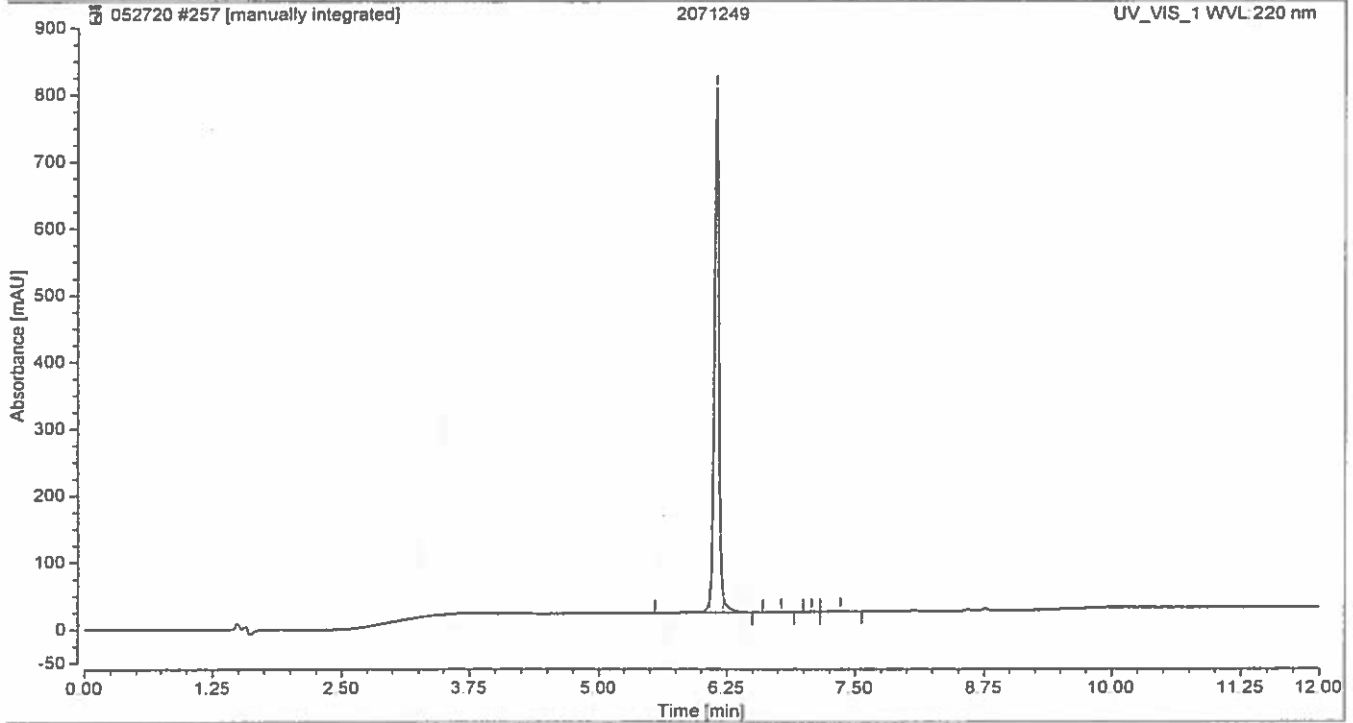
<b>PEPTIDE RECONSTITUTION AND STORAGE</b>	
Please read the entire section before proceeding with the solubilization of your custom peptide.	
Peptides are shipped at ambient temperature as a lyophilized powder. Upon receipt store them at -20°C. Allow the vial to equilibrate to room temperature prior to opening.	
Peptide solubility is highly dependent on the sequence. Peptides that are more hydrophobic (high propensity of A, F, G, V, L, I, M, W, P) in nature, will require an organic solvent in order to dissolve. Peptides that are acidic in nature (high propensity of D, E in the peptide sequence) require a basic aqueous buffer to dissolve, while peptides that are basic in nature (high propensity of K, H, and R) require an acidic aqueous buffer to dissolve.	
To reconstitute a hydrophobic peptide, add 100 µL DMSO and sonicate until a homogenous solution forms. Next, add your buffer of choice to form a 1 mg/mL solution (a higher concentration of peptide will require a greater amount of DMSO). To reconstitute basic or acidic peptides, add 1 mL of the appropriate buffer to the peptide and sonicate to ensure a homogenous solution forms.	
Reconstituted peptides can be stored frozen at -20°C for short period of time, but it is advisable to prepare multiple aliquots to avoid multiple freeze thaw cycles. We recommend that all aliquoted solutions be lyophilized if the peptide is going to be stored for extended periods of time at -20 °C.	
Additionally, please note that peptides with a high propensity of basic residues (R, K, H) in their sequence may undergo a physical change from solid powder to an oil (via moisture absorption). This physical change does not affect the purity or functionality of the peptide.	
Nomenclature used for the sequence termini:	
<b>N-terminus:</b> H means free amine (NH <sub>2</sub> ), Ac mean acetyl [CH <sub>3</sub> C(O)-NH-], Pyr means pyroglutamic acid	
<b>C-terminus:</b> OH means free acid (-COOH), NH <sub>2</sub> means amide [-CONH <sub>2</sub> ]	
Modifications on the side chain of amino acids are depicted in the parenthesis after the corresponding amino acid. For example; phosphorylated serine = S(PO <sub>3</sub> H <sub>2</sub> ) or epsilon-N-acetylated lysine = K(Ac)	
<b>TECHNICAL SUPPORT</b>	
If you have any questions feel free to call our Technical Support Centre	
<b>EUROPE</b>	<b>NORTH AMERICA</b>
☎ 00 800 666 00 123 (European toll free number),	☎ +1 800 452-5530 (American toll free number),
✉ <a href="mailto:info@eurogentec.com">info@eurogentec.com</a>	✉ <a href="mailto:service@anaspec.com">service@anaspec.com</a>
Kaneka Eurogentec S.A. Liège Science Park Rue Bois Saint-Jean 5 - 4102 SERAING BELGIUM Tel: +32(0)4 372 74 00 - Fax: +32(0)4 264 07 88 E-mail: <a href="mailto:info@eurogentec.com">info@eurogentec.com</a> Web: <a href="http://www.eurogentec.com">www.eurogentec.com</a>	AnaSpec, Inc. 34801 Campus Drive Fremont, CA 94555 - USA Tel: +1 (510) 791 9560 - Fax: +1 510 (791) 9572 E-mail: <a href="mailto:service@eurogentec.com">service@eurogentec.com</a> Web: <a href="http://www.anaspec.com">www.anaspec.com</a>
RPM Liège T.V.A.-(BE)-0427.348.346 - ING Belgique Bank - IBAN: BE86 3400 2118 6050 BIC: BBRUBEBB	

### Chromatogram and Results

**Injection Details**

Injection Name:	2071249	Run Time (min):	12.00
Vial Number:	GA3	Injection Volume:	1.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Column:	C18,100X4.6mm,H17-231434	Wavelength:	220
Instrument Method:	5-60%B-7minsExtended,Column(6-1)-0.7ml-30C	Bandwidth:	4
Processing Method:	test	Instrument No.	QC-HPLC-10
Injection Date/Time:	18/Jun/20 16:03	Sample Weight:	

**Chromatogram**

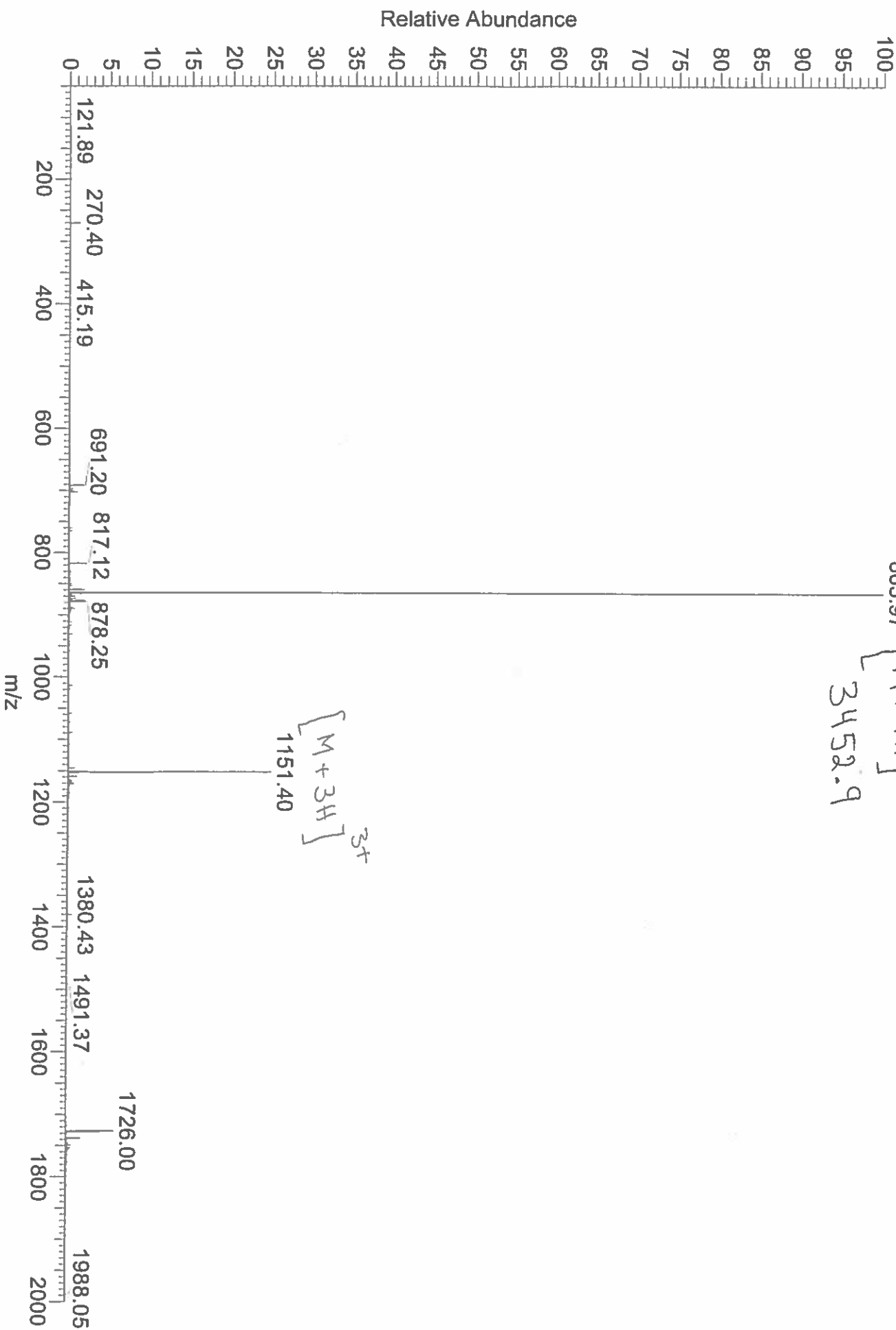


**Integration Results**

No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	6.083	0.670	19.269	1.55
2	6.147	41.100	784.336	95.32
3	6.217	1.101	25.057	2.55
4	6.783	0.024	0.553	0.06
5	7.073	0.036	0.654	0.08
6	7.360	0.186	1.409	0.43
<b>Total:</b>		<b>43.117</b>	<b>831.279</b>	<b>100.00</b>

06/19/20 *RE*

2071249 #36-108 RT: 0.24-0.69 AV: 24 NL: 3.93E5  
F: ITMS + c ESI E Full ms [50.00-2000.00]



<b>YOUR ORDER</b>		<b>SON</b>		<b>DATE</b>	
B001890494		1500055456		19-Jun-20	
<b>CUSTOMER</b>			<b>ADDRESS / INSTITUTION</b>		
University of California San Francisco (UCSF)			UNITED STATES San Francisco		
<b>PEPTIDE NAME</b>		<b>LOT#</b>	<b>INTERIM</b>	<b>SCALE</b>	<b># AMINO</b>
75386-6: (disulfide bridge)		2056280	2071250	Custom	26
<b>SEQUENCE (N-Term → C-Term)</b>					
NOTA -X- DY SHC(S)- SPL RYY PWW KC(S)-T YPD PEG GG - NH <sub>2</sub> , with disulfide bridge					
X=AEEDA					
<b>PHYSICOCHEMICAL PROPERTIES</b>			<b>REGULAR AA PROPERTIES</b>		
1A 280 [mg/ml] *	0.2	Charged AA	D,H,R,K,E	6	Polar AA
Charged at pH 7 *	0.0	Acid AA	D,E	3	Hydrophobic AA
Isoelectric Point *	6.7	Basic AA	H,R,K	3	
(* Theoretical values)					

<b>QC DATA</b>			
<b>Attribute</b>	<b>Test method</b>	<b>Acceptance criteria</b>	<b>Result</b>
Appearance	Visual	Report result	White Powder
% Peak Area by HPLC	HPLC	≥ 95 %	95 %
Identity	MS	3449.8 ± 0.2 %	3451.0
<b>DELIVERABLE</b>			
<b>Format</b>	Dried	<b>Aliquoting</b>	
		Number of Aliquots	1
		Qty by Aliquot (mg)	10 mg
<b>DELIVERY CONDITION</b>		<b>STORAGE CONDITION</b>	
Room temperature		-20 °C, dry	
<b>COMMENTS</b>			

Lot# 2056280 10 mg  
 (disulfide bride)  
 For Laboratory Use Only  
**ANASPEC**

**PEPTIDE RECONSTITUTION AND STORAGE**

Please read the entire section before proceeding with the solubilization of your custom peptide.

Peptides are shipped at ambient temperature as a lyophilized powder. Upon receipt store them at -20°C. Allow the vial to equilibrate to room temperature prior to opening.

Peptide solubility is highly dependent on the sequence. Peptides that are more hydrophobic (high propensity of A, F, G, V, L, I, M, W, P) in nature, will require an organic solvent in order to dissolve. Peptides that are acidic in nature (high propensity of D, E in the peptide sequence) require a basic aqueous buffer to dissolve, while peptides that are basic in nature (high propensity of K, H, and R) require an acidic aqueous buffer to dissolve.

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Additionally, please note that peptides with a high propensity of basic residues (R, K, H) in their sequence may undergo a physical change from solid powder to an oil (via moisture absorption). This physical change does not affect the purity or functionality of the peptide.

Nomenclature used for the sequence termini:  
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**C-terminus:** OH means free acid (-COOH), NH<sub>2</sub> means amide [-CONH<sub>2</sub>]

Modifications on the side chain of amino acids are depicted in the parenthesis after the corresponding amino acid. For example; phosphorylated serine = S(PO<sub>3</sub>H<sub>2</sub>) or epsilon-N-acetylated lysine = K(Ac)

**TECHNICAL SUPPORT**

If you have any questions feel free to call our Technical Support Centre

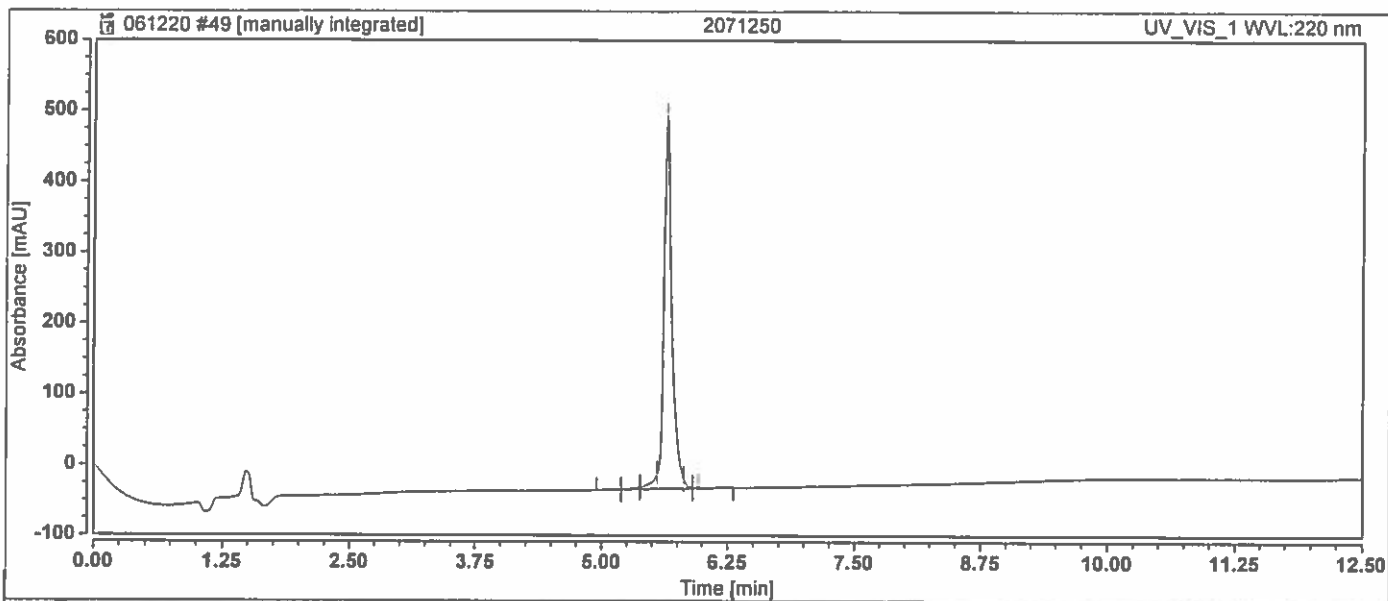
<b>EUROPE</b> ☎ 00 800 666 00 123 (European toll free number), ✉ <a href="mailto:info@eurogentec.com">info@eurogentec.com</a> Kaneka Eurogentec S.A. Liège Science Park Rue Bois Saint-Jean 5 - 4102 SERAING BELGIUM Tel.: +32(0)4 372 74 00 - Fax: +32(0)4 264 07 88 E-mail: <a href="mailto:info@eurogentec.com">info@eurogentec.com</a> Web: <a href="http://www.eurogentec.com">www.eurogentec.com</a> RPM Liège T.V.A.-(BE)-0427.348.346 - ING Belgique Bank - IBAN: BE86 3400 2118 6050 BIC: BBRUBEBB	<b>NORTH AMERICA</b> ☎ +1 800 452-5530 (American toll free number), ✉ <a href="mailto:service@anaspec.com">service@anaspec.com</a> AnaSpec, Inc. 34801 Campus Drive Fremont, CA 94555 - USA Tel.: +1 (510) 791 9560 - Fax: +1 510 (791) 9572 E-mail: <a href="mailto:service@eurogentec.com">service@eurogentec.com</a> Web: <a href="http://www.anaspec.com">www.anaspec.com</a>
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### Chromatogram and Results

#### Injection Details

Injection Name:	2071250	Run Time (min):	12.50
Vial Number:	BA2	Injection Volume:	1.000
Column:	C18, 100 x 4.6mm, H18-074009	Channel:	UV_VIS_1
Instrument Method:	5-60%-7mins-Bextended-0.7ml-30C	Wavelength:	220.0
Processing Method:	test	Bandwidth:	4
Injection Date/Time	18/Jun/20 16:47	Instrument No.	QC-HPLC-9

#### Chromatogram



No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %
1	5.197	0.014	0.000	0.03
2	5.380	0.288	3.131	0.58
3	5.553	1.483	21.673	2.97
4	5.633	47.498	526.989	95.25
5	5.813	0.411	13.648	0.82
6	5.960	0.171	2.507	0.34
<b>Total:</b>		<b>49.865</b>	<b>567.948</b>	<b>100.000</b>

06/19/20 ~~PR~~

2071250 #35-106 RT: 0.22-0.67 AV: 24 NL: 3.28E5

F: ITMS + c ESI E Full ms [50.00-2000.00]

4+  
[M+4H]  
3451.0

3+  
[M+3H]  
1150.73

