

Supporting Information

Xenopus GLP-1 derived and PEGylated GLP-1 receptor agonists: long-acting hypoglycemic and insulinotropic activities with potential therapeutic utilities

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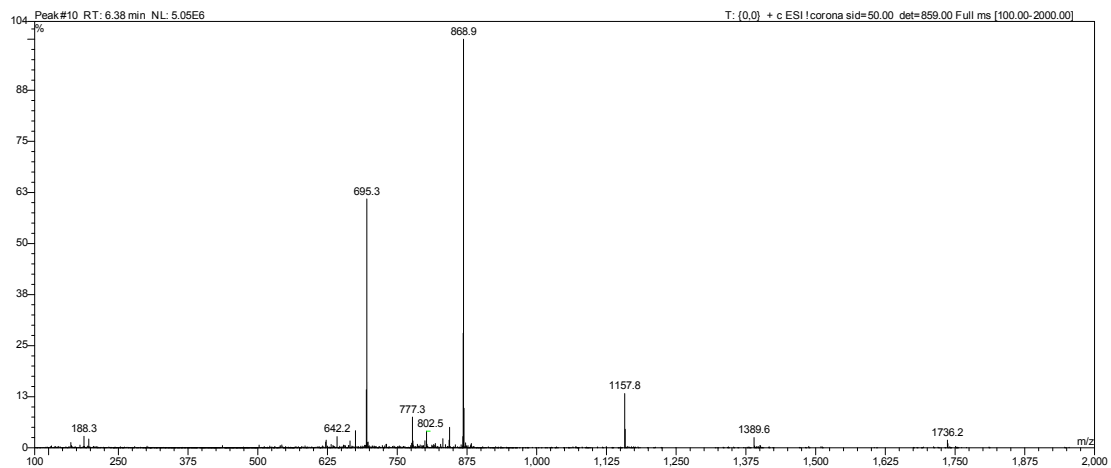
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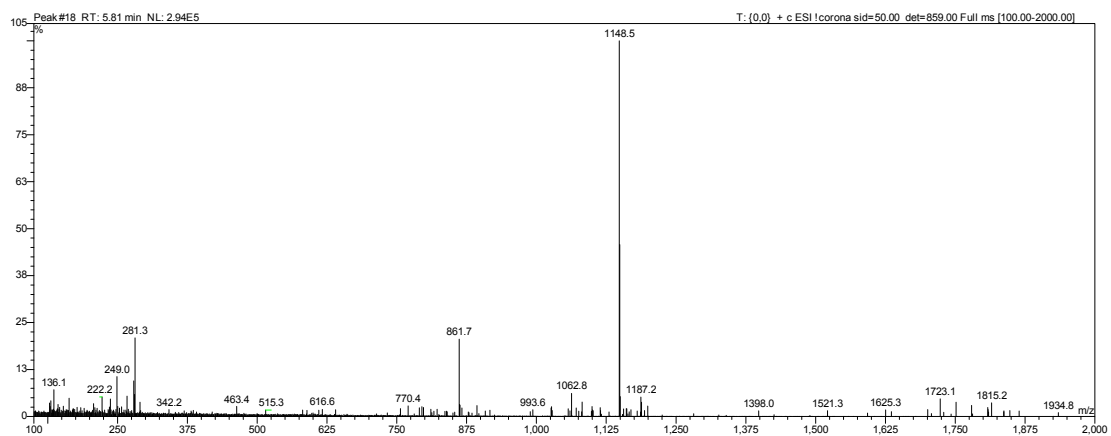
Characterization of peptides 1–20

1



(m/z)calad	(m/z)found
[m+4H] ⁴⁺ 868.5	[m+4H] ⁴⁺ 868.9
[M+5H] ⁵⁺ 695.0	[M+5H] ⁵⁺ 695.3

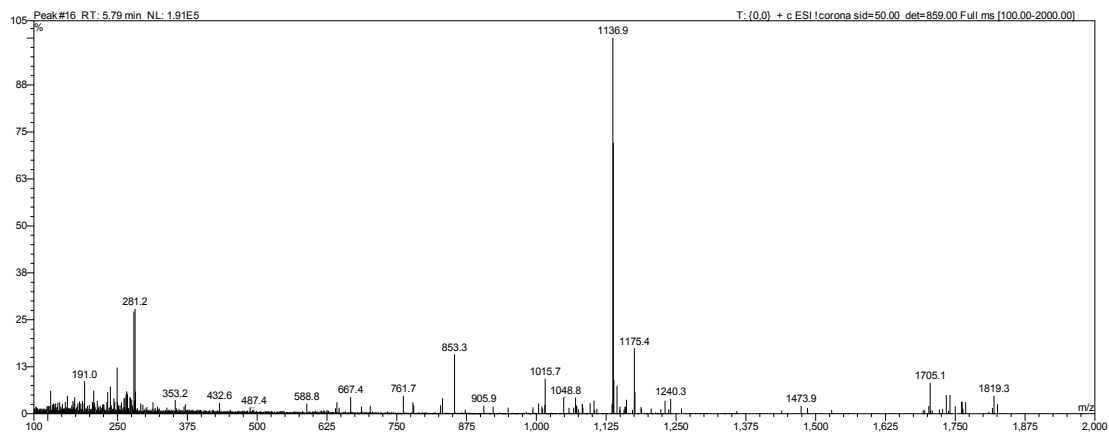
2



(m/z)calad	(m/z)found
[m+3H] ³⁺ 1148.3	[m+3H] ³⁺ 1148.5
[m+4H] ⁴⁺ 861.4	[m+4H] ⁴⁺ 861.7

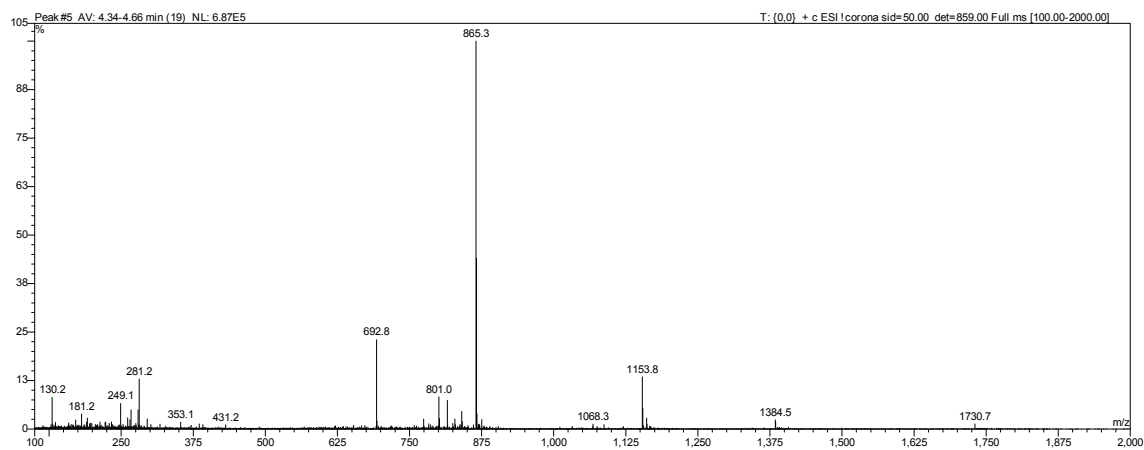
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3



(m/z)calad	(m/z)found
[m+3H] ⁵⁺ 1136.9	[m+3H] ³⁺ 1136.9
[m+4H] ⁴⁺ 852.9	[m+4H] ⁴⁺ 853.3

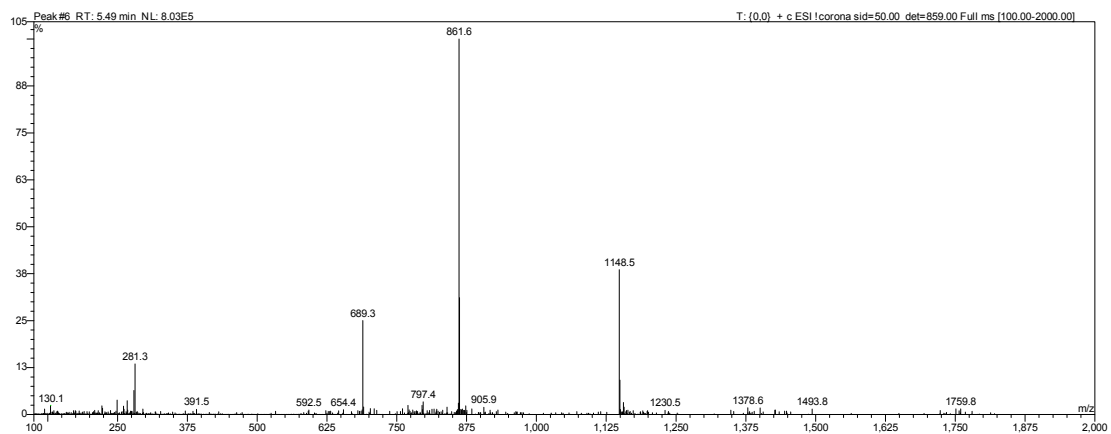
4



(m/z)calad	(m/z)found
[m+4H] ⁴⁺ 865.4	[m+4H] ⁴⁺ 865.3
[m+5H] ⁵⁺ 692.5	[m+5H] ⁵⁺ 692.8

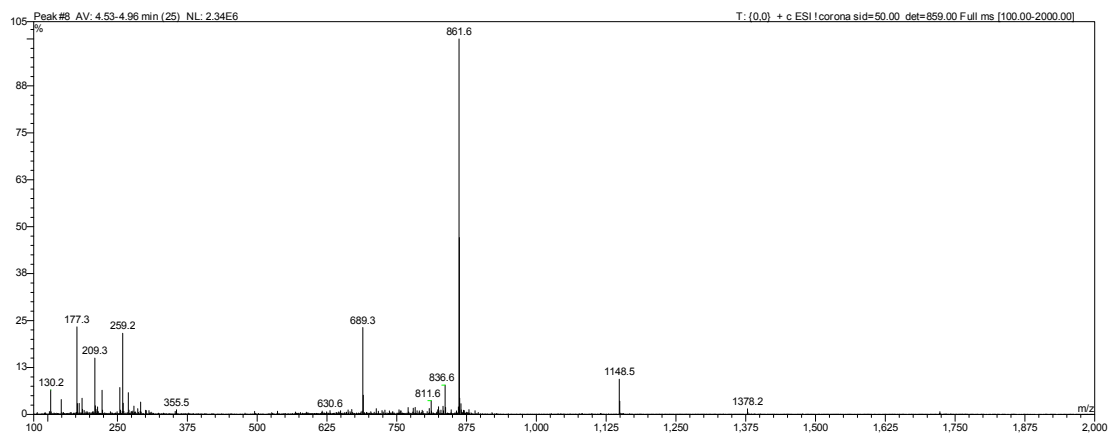
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5



(m/z)calad	(m/z)found
[m+4H] ⁴⁺ 861.5	[m+4H] ⁴⁺ 861.6
[m+5H] ⁵⁺ 689.4	[m+5H] ⁵⁺ 689.3

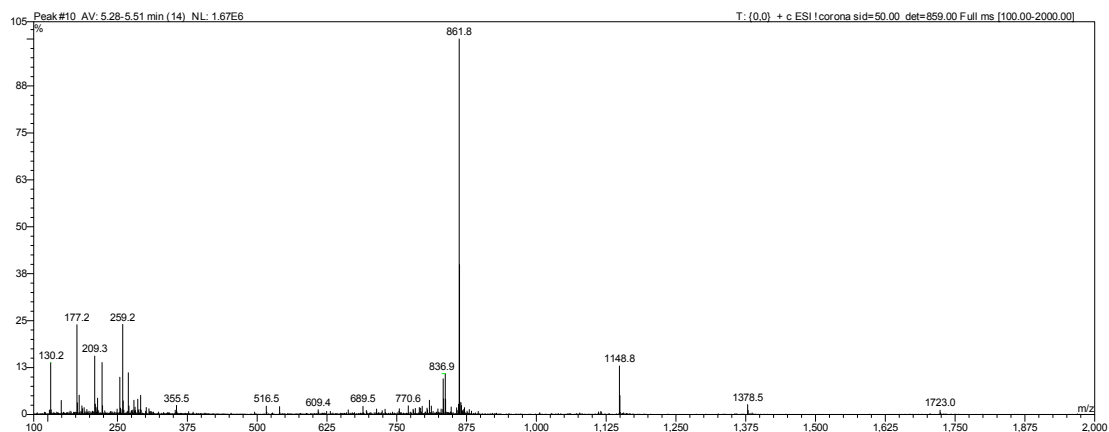
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(m/z)calad	(m/z)found
[m+4H] ⁴⁺ 861.5	[m+4H] ⁴⁺ 861.6
[m+5H] ⁵⁺ 689.4	[m+5H] ⁵⁺ 689.3

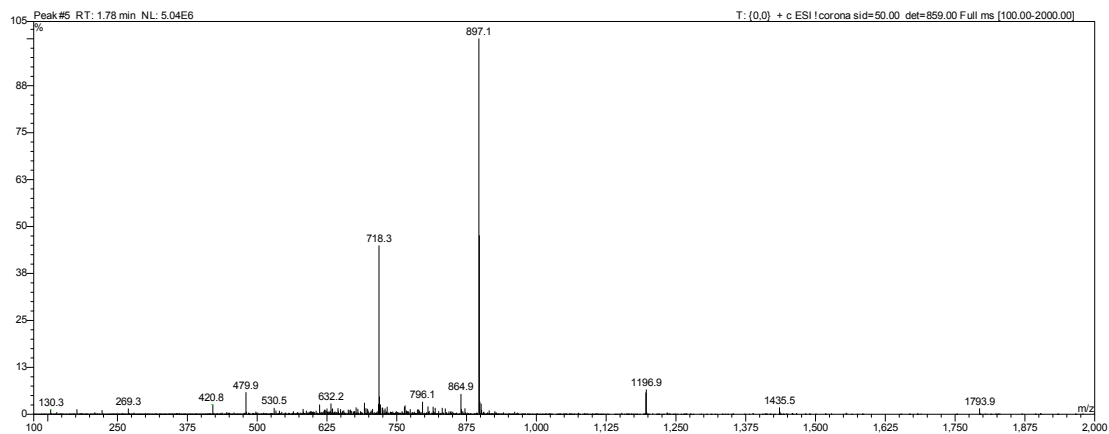
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7



(m/z)calad	(m/z)found
[m+3H] ³⁺ 1148.6	[m+3H] ³⁺ 1148.8
[m+4H] ⁴⁺ 861.7	[m+4H] ⁴⁺ 861.8

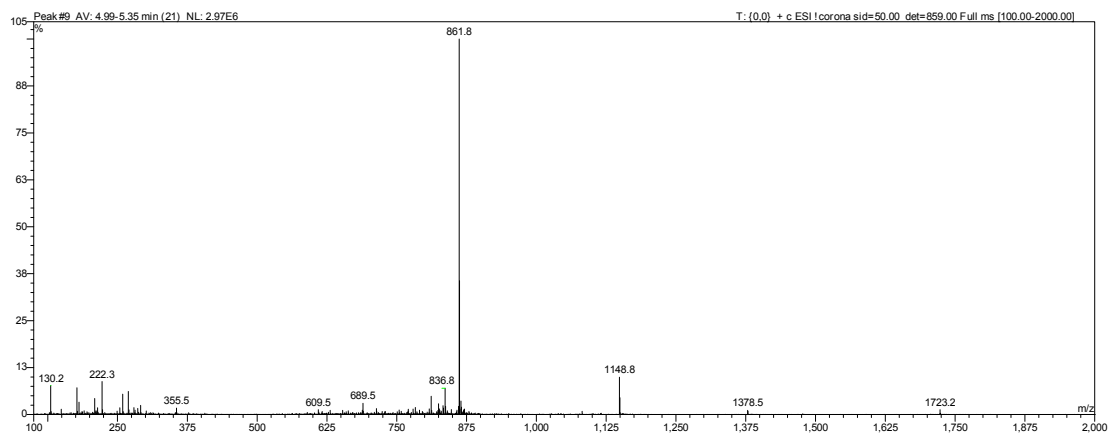
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(m/z)calad	(m/z)found
[m+4H] ⁴⁺ 897.3	[m+4H] ⁴⁺ 897.1
[m+5H] ⁵⁺ 718.0	[m+5H] ⁵⁺ 718.3

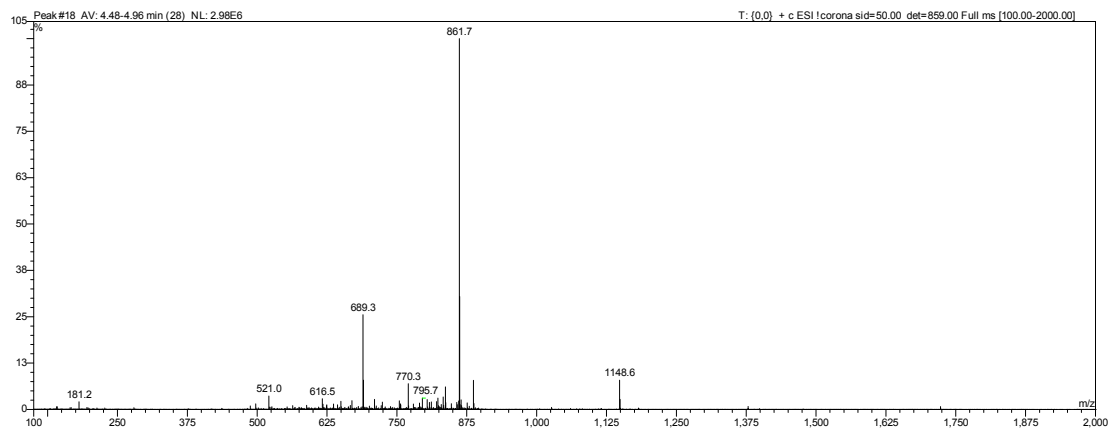
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10



(m/z)calad	(m/z)found
$[m+3H]^{3+} 1148.6$	$[m+3H]^{3+} 1148.8$
$[m+4H]^{4+} 861.7$	$[m+4H]^{4+} 861.8$

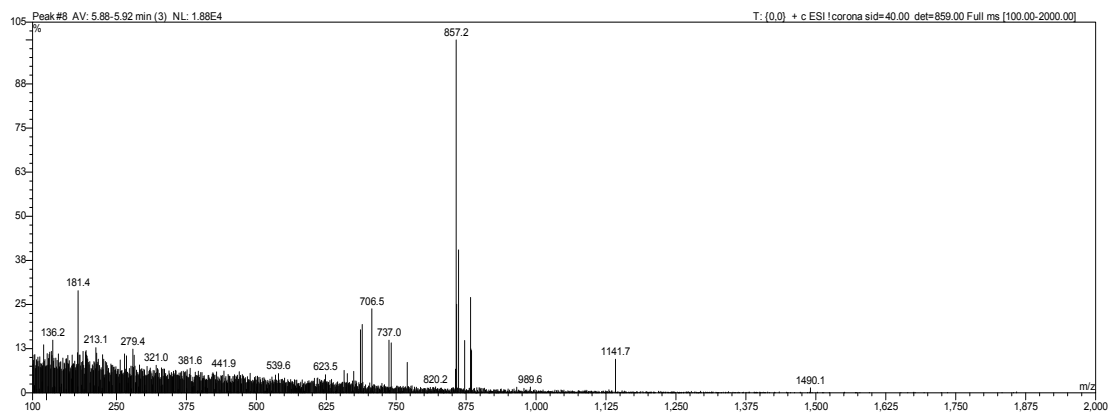
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(m/z)calad	(m/z)found
$[m+4H]^{4+} 861.5$	$[m+4H]^{4+} 861.7$
$[m+5H]^{5+} 689.4$	$[m+5H]^{5+} 689.3$

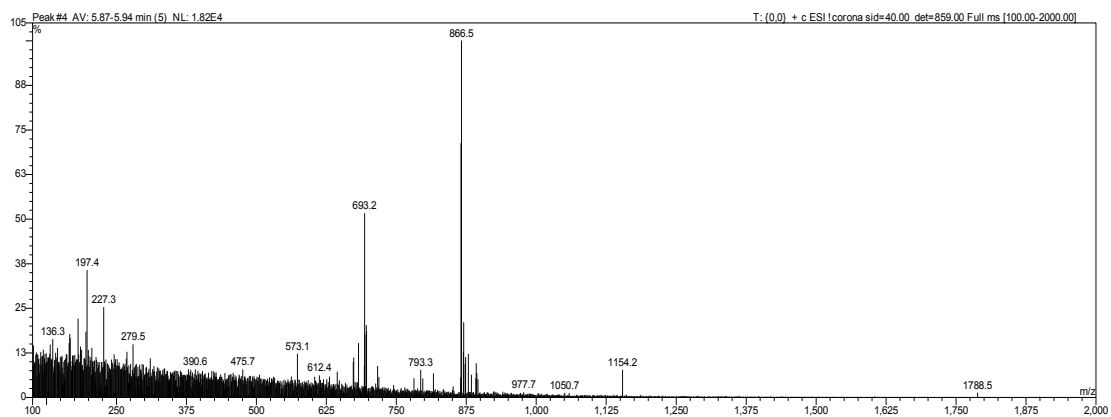
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12



(m/z)calad	(m/z)found
$[m+3H]^{3+}$ 1142.3	$[m+3H]^{3+}$ 1141.7
$[m+4H]^{4+}$ 857.0	$[m+4H]^{4+}$ 857.2

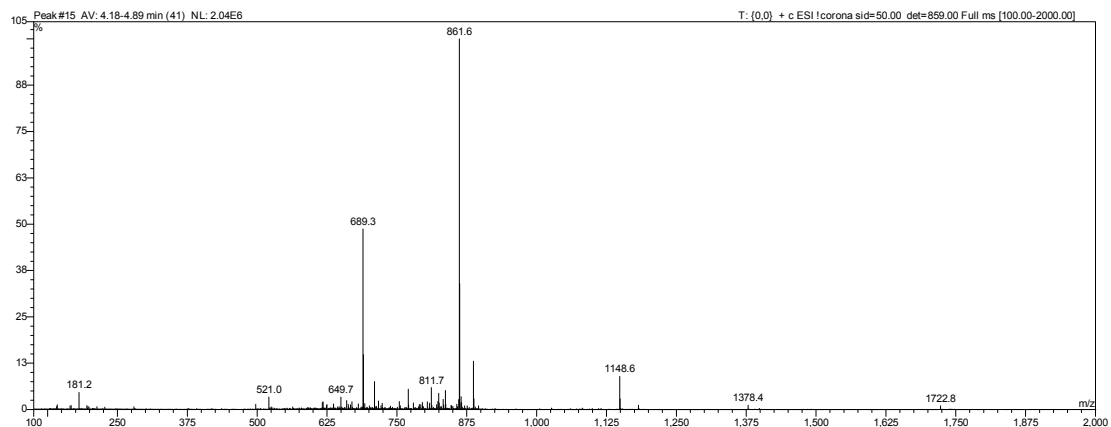
13



(m/z)calad	(m/z)found
$[m+4H]^{4+}$ 865.5	$[m+4H]^{4+}$ 866.5
$[m+5H]^{5+}$ 692.6	$[m+5H]^{5+}$ 693.2

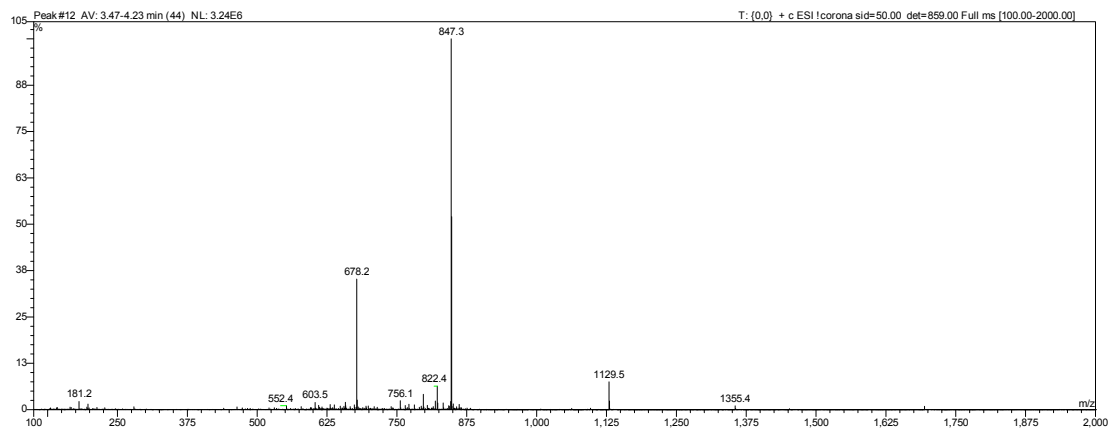
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14



(m/z)calad	(m/z)found
$[m+4H]^{4+} 861.5$	$[m+4H]^{4+} 861.6$
$[m+5H]^{5+} 689.4$	$[m+5H]^{5+} 689.3$

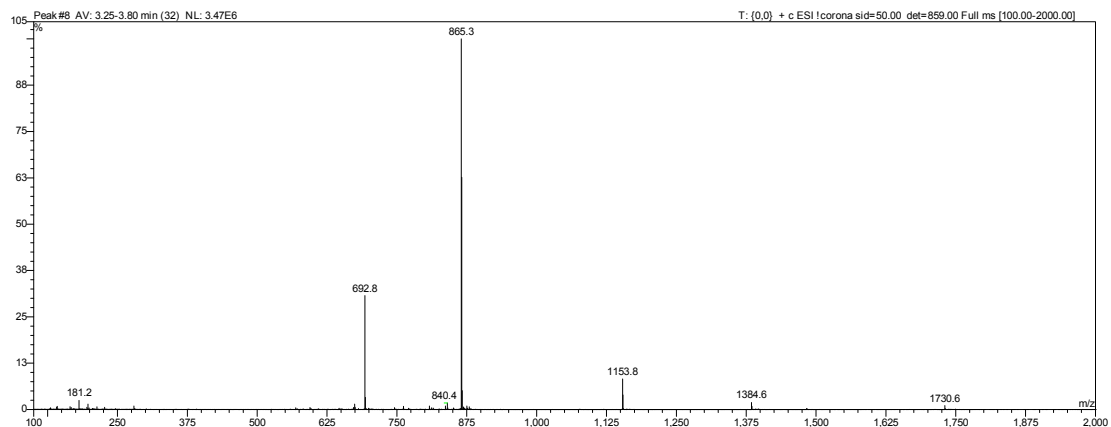
15



(m/z)calad	(m/z)found
$[m+4H]^{4+} 847.2$	$[m+4H]^{4+} 847.3$
$[m+5H]^{5+} 678.0$	$[m+5H]^{5+} 678.2$

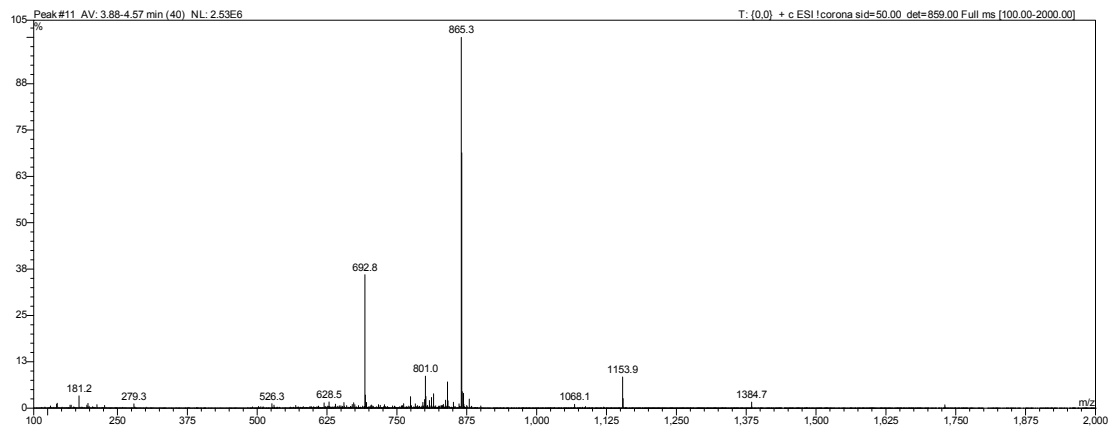
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16



(m/z)calad	(m/z)found
$[m+4H]^{4+}$ 865.5	$[m+4H]^{4+}$ 865.3
$[m+5H]^{5+}$ 692.6	$[m+5H]^{5+}$ 692.8

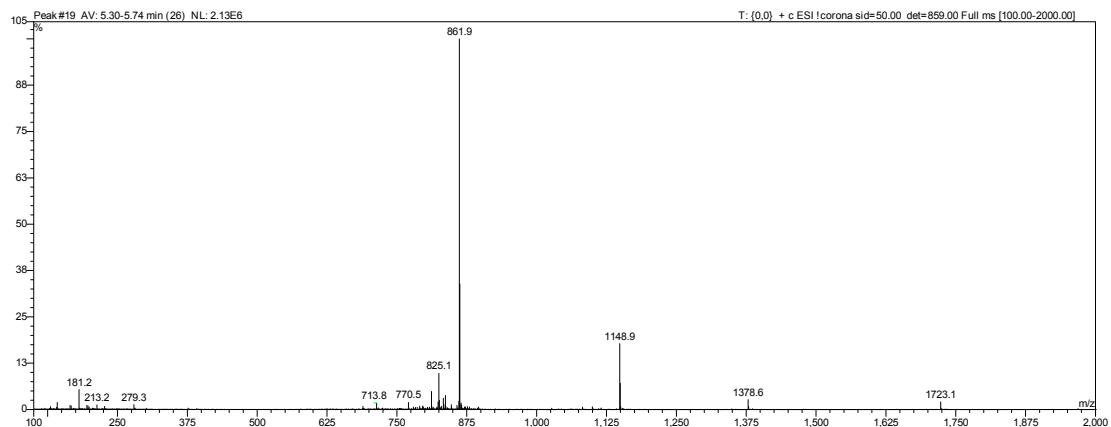
17



(m/z)calad	(m/z)found
$[m+4H]^{4+}$ 865.5	$[m+4H]^{4+}$ 865.3
$[m+5H]^{5+}$ 692.6	$[m+5H]^{5+}$ 692.8

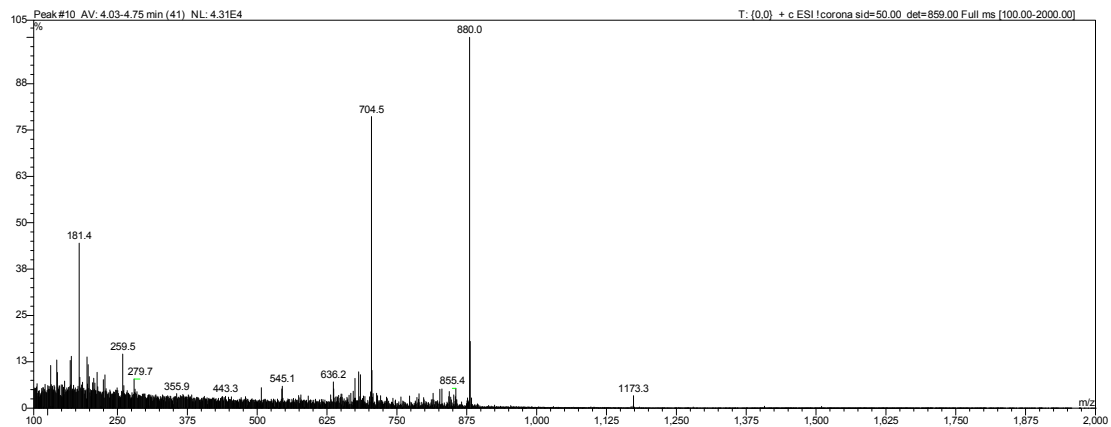
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18



(m/z)calad	(m/z)found
$[m+3H]^{3+}$ 1148.6	$[m+3H]^{3+}$ 1148.9
$[m+4H]^{4+}$ 861.7	$[m+4H]^{4+}$ 861.9

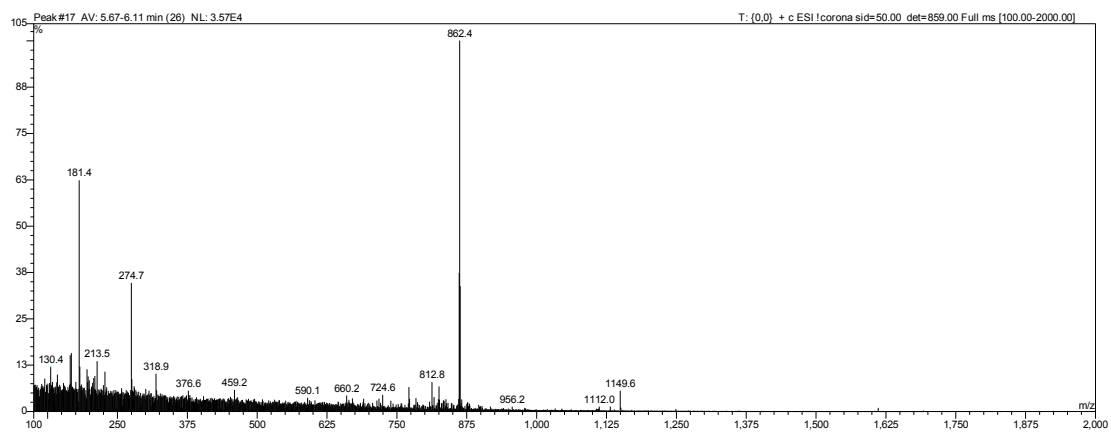
19



(m/z)calad	(m/z)found
$[m+4H]^{4+}$ 879.5	$[m+4H]^{4+}$ 880.0
$[m+5H]^{5+}$ 703.8	$[m+5H]^{5+}$ 704.5

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20

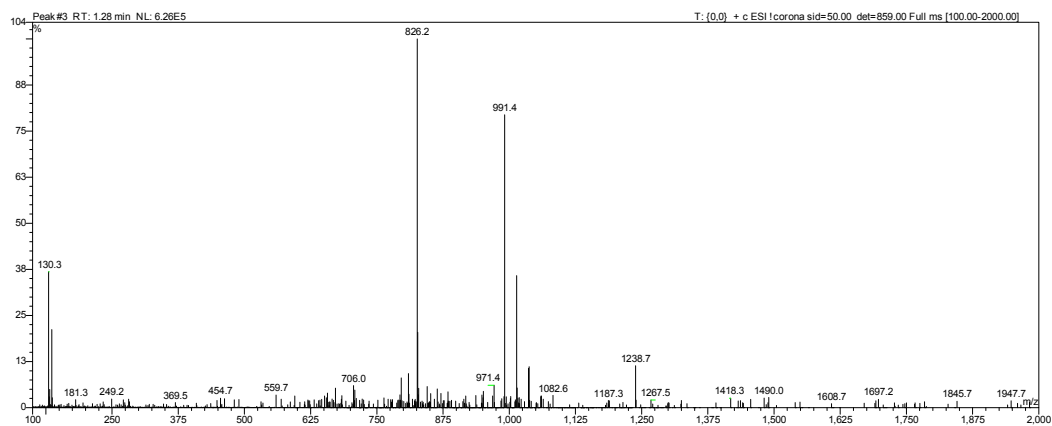


(m/z)calad	(m/z)found
$[m+3H]^{3+}$ 1148.6	$[m+3H]^{3+}$ 1149.6
$[m+4H]^{4+}$ 861.7	$[m+4H]^{4+}$ 862.4

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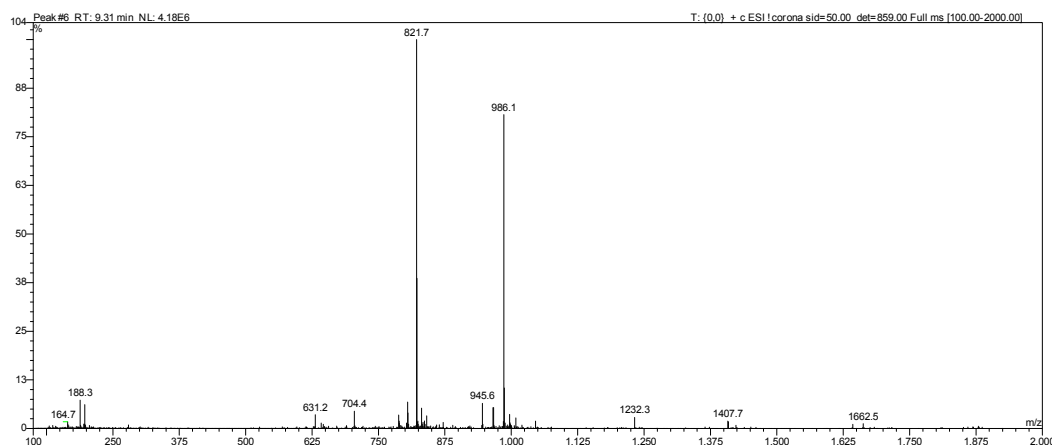
Characterization of xGLP-1B-Sen and peptides 21–24

xGLP-1B-Sen



(m/z)calad	(m/z)found
[m+5H] ⁵⁺ 990.9	[m+5H] ⁵⁺ 991.4
[m+6H] ⁶⁺ 826.0	[m+6H] ⁶⁺ 826.2

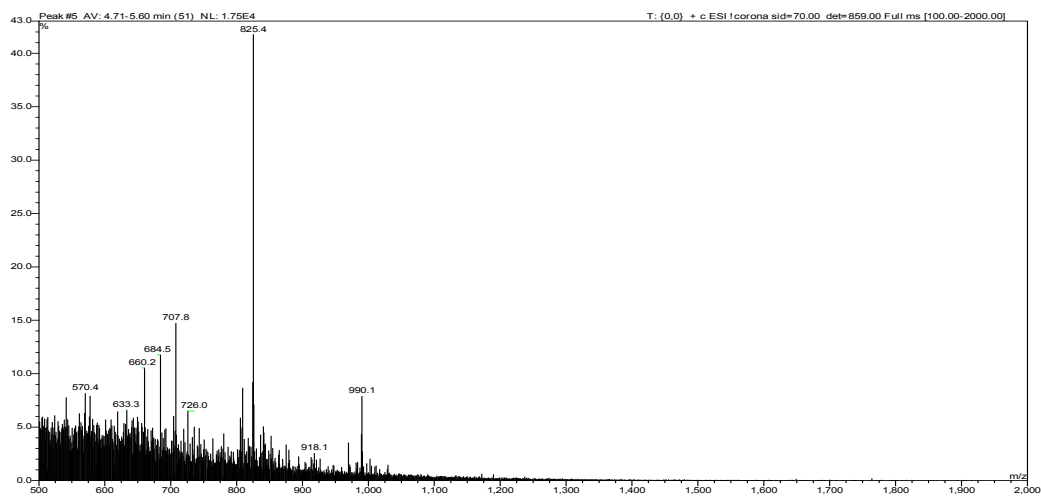
21



(m/z)calad	(m/z)found
[M+5H] ⁵⁺ 985.7	[M+5H] ⁵⁺ 986.1
[M+6H] ⁶⁺ 821.6	[M+6H] ⁶⁺ 821.7

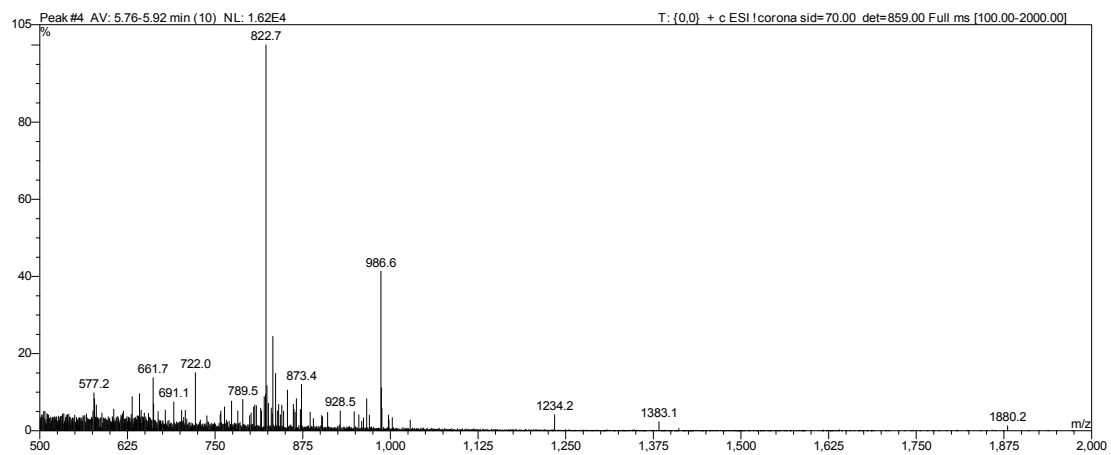
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22



(m/z)calad	(m/z)found
[M+5H] ⁵⁺ 988.9	[M+5H] ⁵⁺ 990.1
[M+6H] ⁶⁺ 824.3	[M+6H] ⁶⁺ 825.4

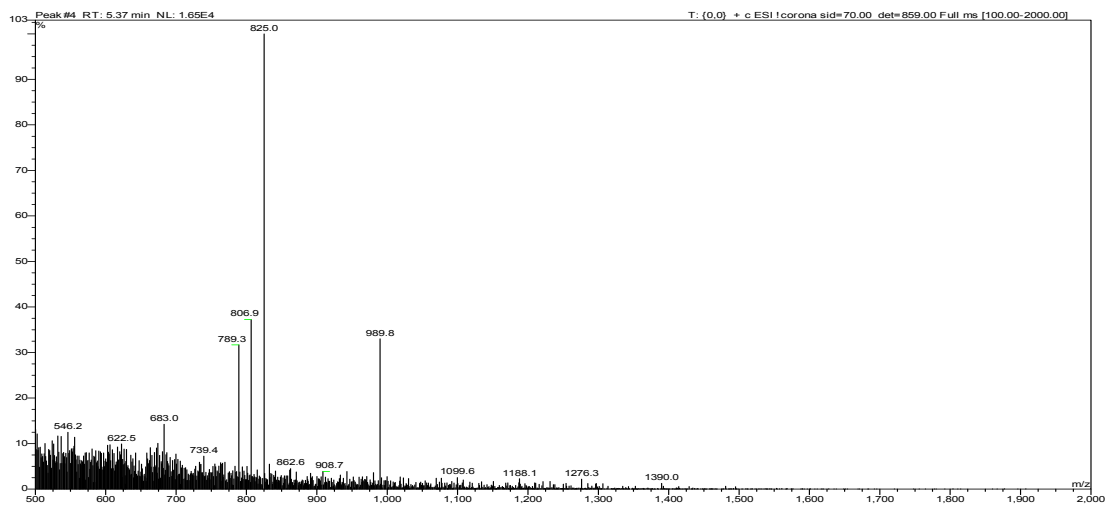
23



(m/z)calad	(m/z)found
[M+5H] ⁵⁺ 985.9	[M+5H] ⁵⁺ 986.6
[M+6H] ⁶⁺ 821.8	[M+6H] ⁶⁺ 822.7

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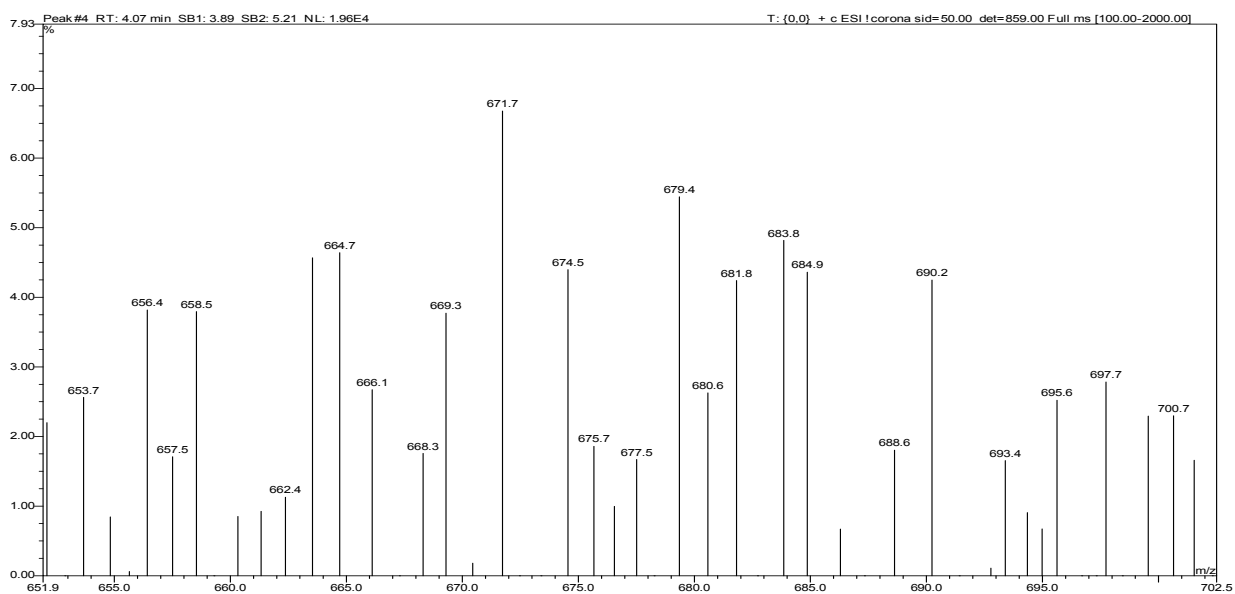
24



(m/z)calad	(m/z)found
$[M+5H]^{5+}$ 988.9	$[M+5H]^{5+}$ 989.8
$[M+6H]^{6+}$ 824.3	$[M+6H]^{6+}$ 825.0

Characterization of PEGylated conjugates 25–36

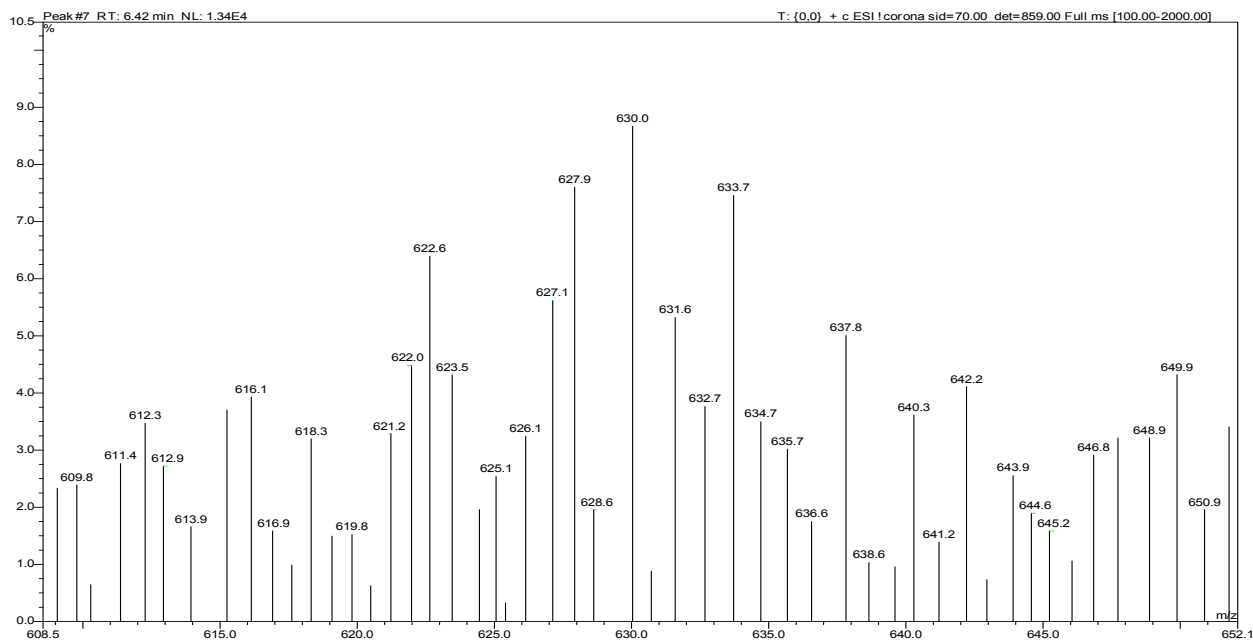
25



	(m/z)calad		(m/z)found	
18	$[m+9H]^{9+}$ 661.2	$[M+8H+Na]^{9+}$ 663.6	$[m+9H]^{9+}$ 661.2	$[M+8H+Na]^{9+}$ 663.6
19	$[m+9H]^{9+}$ 666.1	$[M+8H+Na]^{9+}$ 668.5	$[m+9H]^{9+}$ 666.1	$[M+8H+Na]^{9+}$ 668.3
20	$[m+9H]^{9+}$ 671.0	$[M+8H+Na]^{9+}$ 673.4	$[m+9H]^{9+}$ 671.7	$[M+8H+Na]^{9+}$ 674.5
21	$[m+9H]^{9+}$ 675.9	$[M+8H+Na]^{9+}$ 678.3	$[m+9H]^{9+}$ 676.5	$[M+8H+Na]^{9+}$ 679.4
22	$[m+9H]^{9+}$ 680.8	$[M+8H+Na]^{9+}$ 683.2	$[m+9H]^{9+}$ 680.6	$[M+8H+Na]^{9+}$ 683.8

Supporting Information

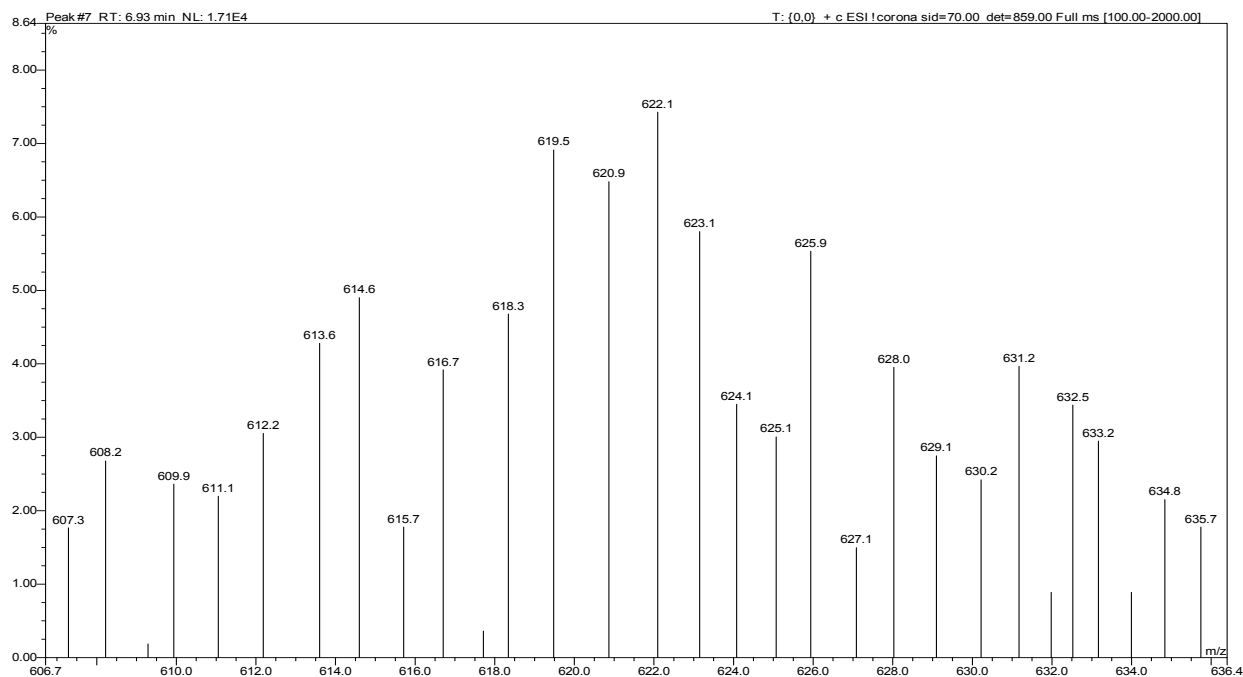
26



	(m/z)calad		(m/z)found	
38	$[m+11H]^{11+}$ 621.2	$[M+10H+Na]^{11+}$ 623.2	$[m+11H]^{11+}$ 621.2	$[M+10H+Na]^{11+}$ 623.5
39	$[m+11H]^{11+}$ 625.2	$[M+10H+Na]^{11+}$ 627.2	$[m+11H]^{11+}$ 625.3	$[M+10H+Na]^{11+}$ 627.1
40	$[m+11H]^{11+}$ 629.2	$[M+10H+Na]^{11+}$ 631.2	$[m+11H]^{11+}$ 630.0	$[M+10H+Na]^{11+}$ 631.6
41	$[m+11H]^{11+}$ 633.2	$[M+10H+Na]^{11+}$ 635.2	$[m+11H]^{11+}$ 633.7	$[M+10H+Na]^{11+}$ 635.7
42	$[m+11H]^{11+}$ 637.2	$[M+10H+Na]^{11+}$ 639.2	$[m+11H]^{11+}$ 637.8	$[M+10H+Na]^{11+}$ 639.6

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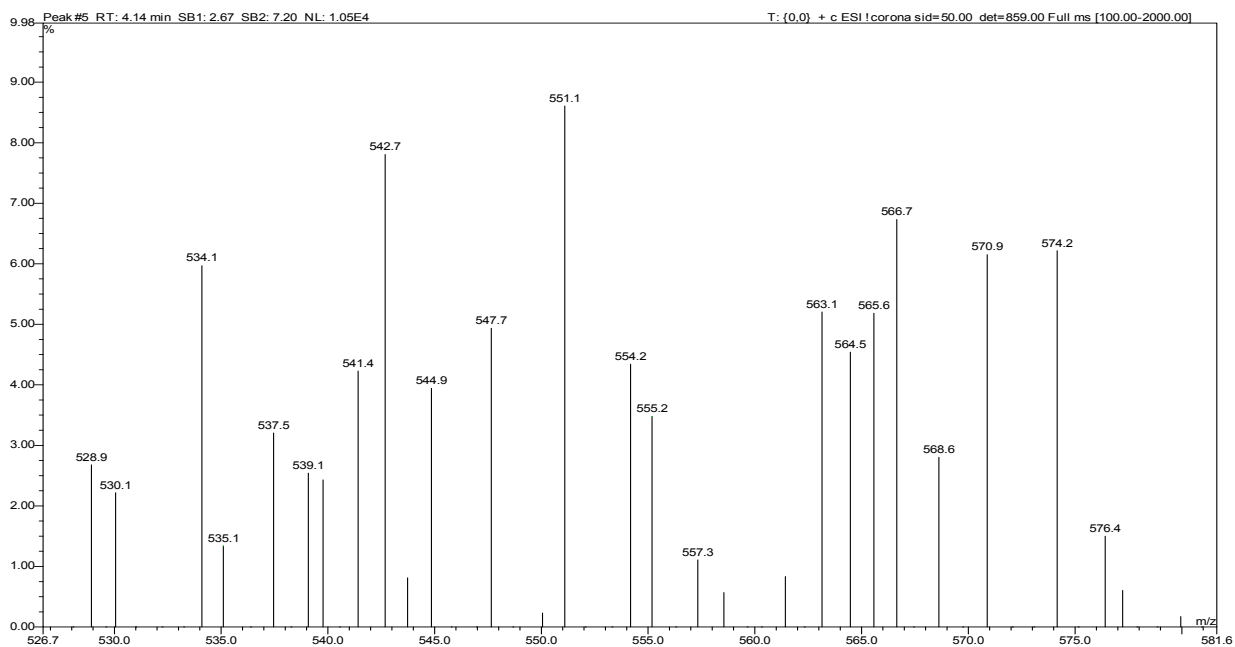
27



	(m/z)calad		(m/z)found	
106	$[m+16H]^{16+}$ 614.4	$[M+15H+Na]^{16+}$ 615.7	$[m+16H]^{16+}$ 614.6	$[M+15H+Na]^{16+}$ 615.7
107	$[m+16H]^{16+}$ 617.1	$[M+15H+Na]^{16+}$ 618.5	$[m+16H]^{16+}$ 617.7	$[M+15H+Na]^{16+}$ 618.3
108	$[m+16H]^{16+}$ 619.9	$[M+15H+Na]^{16+}$ 621.2	$[m+16H]^{16+}$ 619.5	$[M+15H+Na]^{16+}$ 622.1
109	$[m+16H]^{16+}$ 622.6	$[M+15H+Na]^{16+}$ 624.0	$[m+16H]^{16+}$ 623.1	$[M+15H+Na]^{16+}$ 624.1
110	$[m+16H]^{16+}$ 625.4	$[M+15H+Na]^{16+}$ 626.7	$[m+16H]^{16+}$ 625.9	$[M+15H+Na]^{16+}$ 627.1

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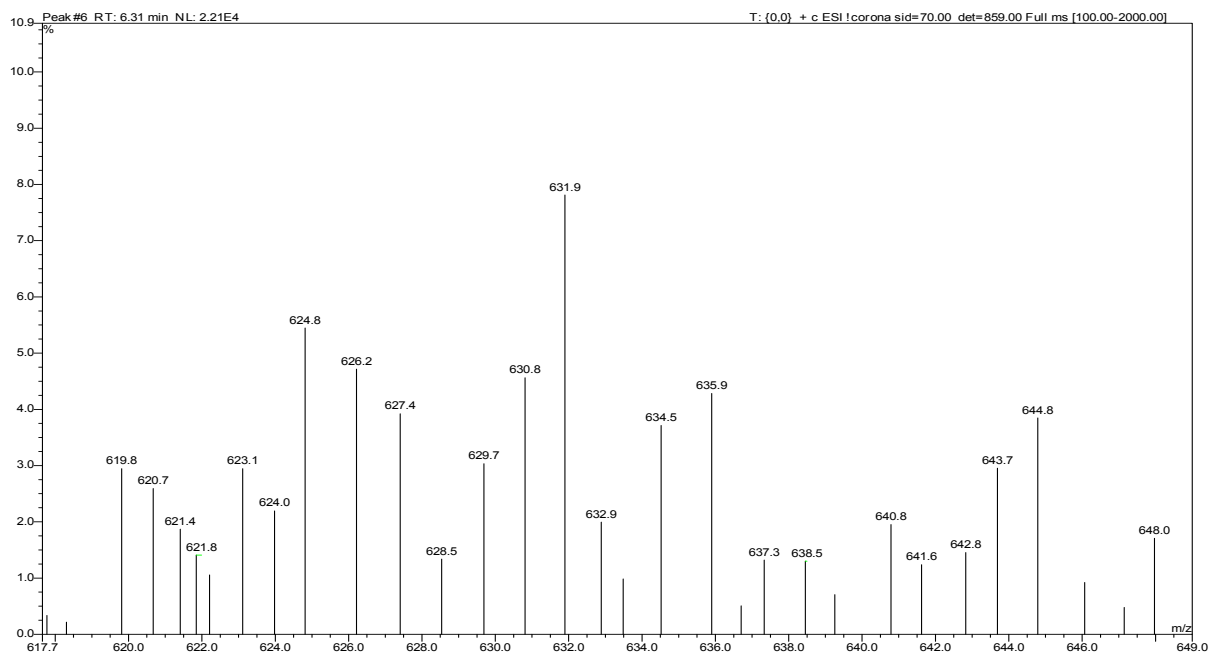
28



	(m/z)calad		(m/z)found	
18	$[m+11H]^{11+}$ 542.6	$[M+10H+Na]^{11+}$ 544.6	$[m+11H]^{11+}$ 542.7	$[M+10H+Na]^{11+}$ 544.9
19	$[m+11H]^{11+}$ 546.6	$[M+10H+Na]^{11+}$ 548.6	$[m+11H]^{11+}$ 547.7	$[M+10H+Na]^{11+}$ not found
20	$[m+11H]^{11+}$ 550.6	$[M+10H+Na]^{11+}$ 552.6	$[m+11H]^{11+}$ 551.1	$[M+10H+Na]^{11+}$ not found
21	$[m+11H]^{11+}$ 554.6	$[M+10H+Na]^{11+}$ 556.6	$[m+11H]^{11+}$ 555.2	$[M+10H+Na]^{11+}$ 557.3
22	$[m+11H]^{11+}$ 558.6	$[M+10H+Na]^{11+}$ 560.6	$[m+11H]^{11+}$ 558.5	$[M+10H+Na]^{11+}$ 561.4

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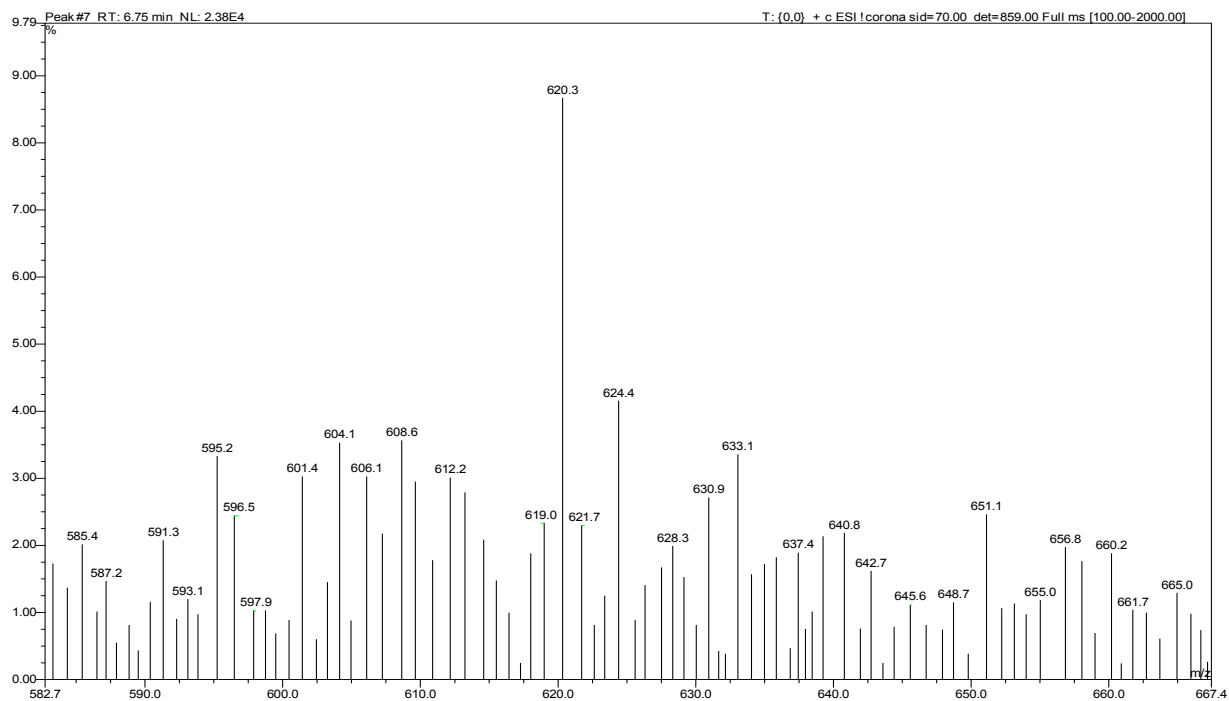
29



	(m/z)calad		(m/z)found	
38	$[m+11H]^{11+}$ 622.6	$[M+10H+Na]^{11+}$ 624.6	$[m+11H]^{11+}$ 623.1	$[M+10H+Na]^{11+}$ 624.8
39	$[m+11H]^{11+}$ 626.6	$[M+10H+Na]^{11+}$ 628.6	$[m+11H]^{11+}$ 626.2	$[M+10H+Na]^{11+}$ 628.5
40	$[m+11H]^{11+}$ 630.6	$[M+10H+Na]^{11+}$ 632.6	$[m+11H]^{11+}$ 630.8	$[M+10H+Na]^{11+}$ 632.9
41	$[m+11H]^{11+}$ 634.6	$[M+10H+Na]^{11+}$ 636.6	$[m+11H]^{11+}$ 634.5	$[M+10H+Na]^{11+}$ 636.7
42	$[m+11H]^{11+}$ 638.6	$[M+10H+Na]^{11+}$ 640.6	$[m+11H]^{11+}$ 638.5	$[M+10H+Na]^{11+}$ 640.8

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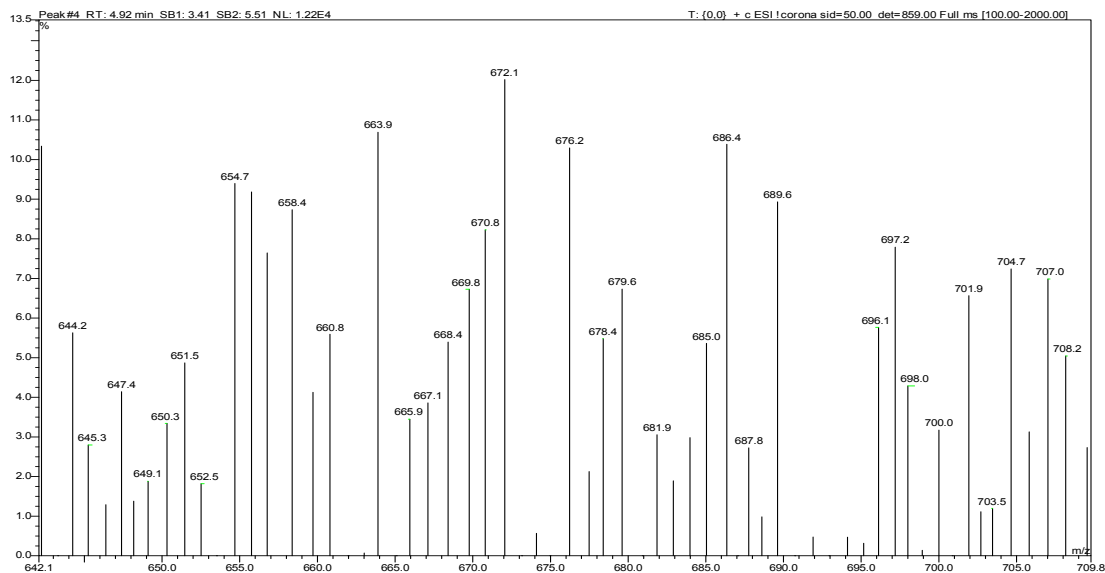
30



	(m/z)calad		(m/z)found	
106	[m+16H] ¹⁶⁺ 615.4	[M+15H+Na] ¹⁶⁺ 616.7	[m+16H] ¹⁶⁺ 615.5	[M+15H+Na] ¹⁶⁺ 616.6
107	[m+16H] ¹⁶⁺ 618.1	[M+15H+Na] ¹⁶⁺ 619.5	[m+16H] ¹⁶⁺ 618.0	[M+15H+Na] ¹⁶⁺ 620.3
108	[m+16H] ¹⁶⁺ 620.9	[M+15H+Na] ¹⁶⁺ 622.2	[m+16H] ¹⁶⁺ 621.7	[M+15H+Na] ¹⁶⁺ 622.8
109	[m+16H] ¹⁶⁺ 623.6	[M+15H+Na] ¹⁶⁺ 625.0	[m+16H] ¹⁶⁺ 624.4	[M+15H+Na] ¹⁶⁺ 625.2
110	[m+16H] ¹⁶⁺ 626.4	[M+15H+Na] ¹⁶⁺ 627.7	[m+16H] ¹⁶⁺ 626.4	[M+15H+Na] ¹⁶⁺ 627.5

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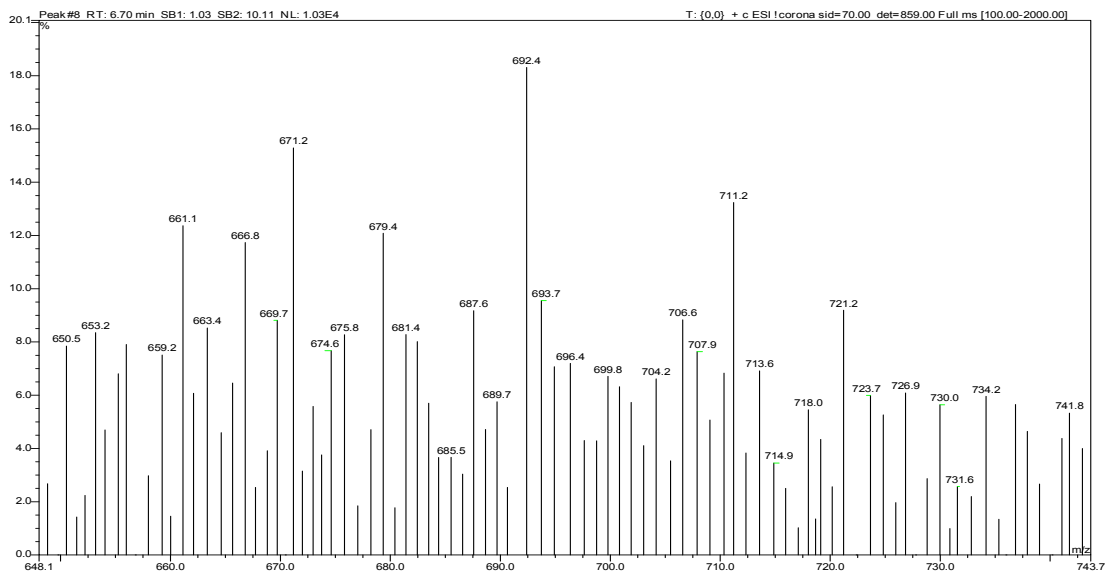
31



	(m/z)calad		(m/z)found	
18	$[m+9H]^{9+}$ 661.3	$[M+8H+Na]^{9+}$ 663.7	$[m+9H]^{9+}$ 660.8	$[M+8H+Na]^{9+}$ 663.9
19	$[m+9H]^{9+}$ 666.2	$[M+8H+Na]^{9+}$ 668.6	$[m+9H]^{9+}$ 667.1	$[M+8H+Na]^{9+}$ 668.4
20	$[m+9H]^{9+}$ 671.1	$[M+8H+Na]^{9+}$ 673.5	$[m+9H]^{9+}$ 672.1	$[M+8H+Na]^{9+}$ 674.1
21	$[m+9H]^{9+}$ 676.0	$[M+8H+Na]^{9+}$ 678.4	$[m+9H]^{9+}$ 676.2	$[M+8H+Na]^{9+}$ 678.4
22	$[m+9H]^{9+}$ 680.9	$[M+8H+Na]^{9+}$ 683.3	$[m+9H]^{9+}$ 681.9	$[M+8H+Na]^{9+}$ 684.0

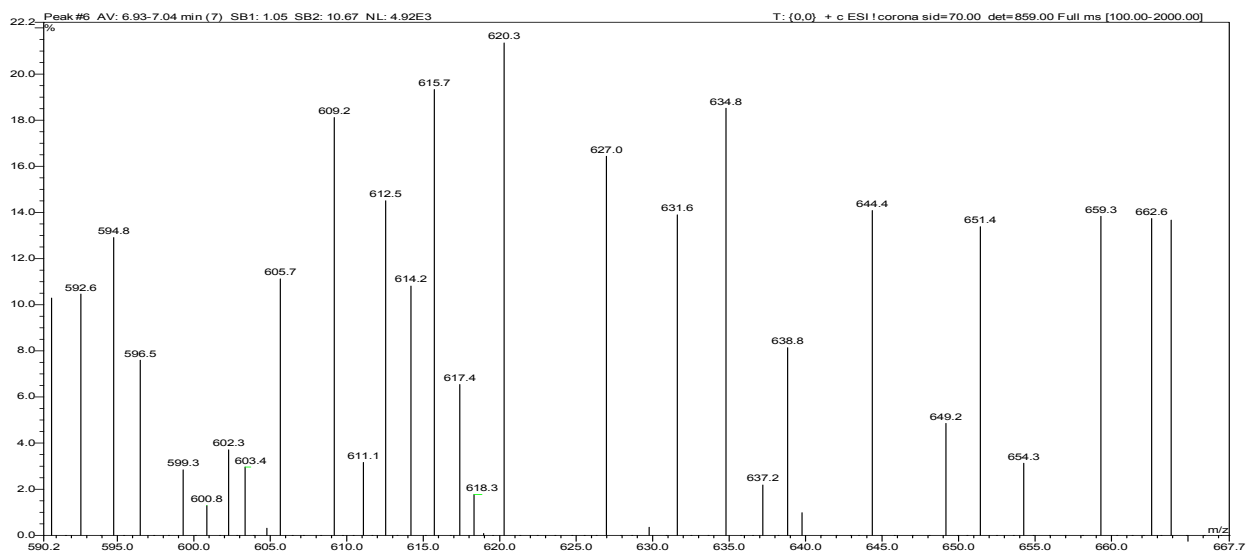
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	(m/z)calad		(m/z)found	
38	$[m+10H]^{10+}$ 683.3	$[M+9H+Na]^{10+}$ 685.5	$[m+10H]^{10+}$ 683.9	$[M+9H+Na]^{10+}$ 685.5
39	$[m+10H]^{10+}$ 687.7	$[M+9H+Na]^{10+}$ 689.9	$[m+10H]^{10+}$ 687.6	$[M+9H+Na]^{10+}$ 689.7
40	$[m+10H]^{10+}$ 692.1	$[M+9H+Na]^{10+}$ 694.3	$[m+10H]^{10+}$ 692.4	$[M+9H+Na]^{10+}$ 694.9
41	$[m+10H]^{10+}$ 696.5	$[M+9H+Na]^{10+}$ 698.7	$[m+10H]^{10+}$ 696.4	$[M+9H+Na]^{10+}$ 698.8
42	$[m+10H]^{10+}$ 700.9	$[M+9H+Na]^{10+}$ 703.1	$[m+10H]^{10+}$ 700.6	$[M+9H+Na]^{10+}$ 703.2

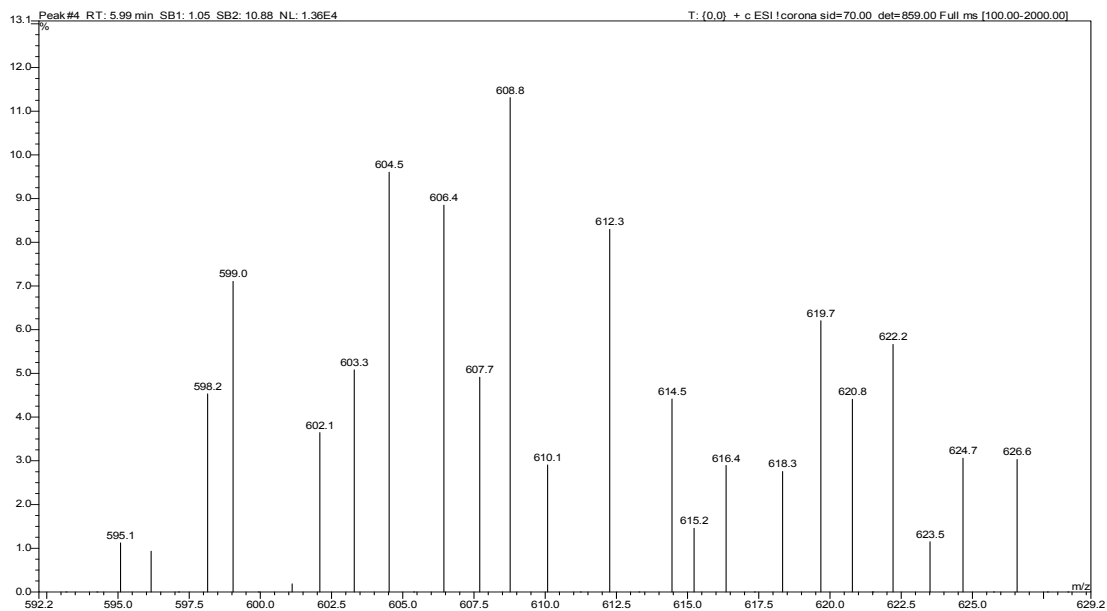
33



	(m/z)calad		(m/z)found	
106	$[m+16H]^{16+}$ 614.4	$[M+15H+Na]^{16+}$ 615.8	$[m+16H]^{16+}$ 614.2	$[M+15H+Na]^{16+}$ 615.7
107	$[m+16H]^{16+}$ 617.2	$[M+15H+Na]^{16+}$ 618.5	$[m+16H]^{16+}$ 617.4	$[M+15H+Na]^{16+}$ 618.3
108	$[m+16H]^{16+}$ 619.9	$[M+15H+Na]^{16+}$ 621.3	$[m+16H]^{16+}$ 620.3	$[M+15H+Na]^{16+}$ not found
109	$[m+16H]^{16+}$ 622.7	$[M+15H+Na]^{16+}$ 624.0	$[m+16H]^{16+}$ not found	$[M+15H+Na]^{16+}$ not found
110	$[m+16H]^{16+}$ 625.4	$[M+15H+Na]^{16+}$ 626.8	$[m+16H]^{16+}$ not found	$[M+15H+Na]^{16+}$ 627.0

Supporting Information

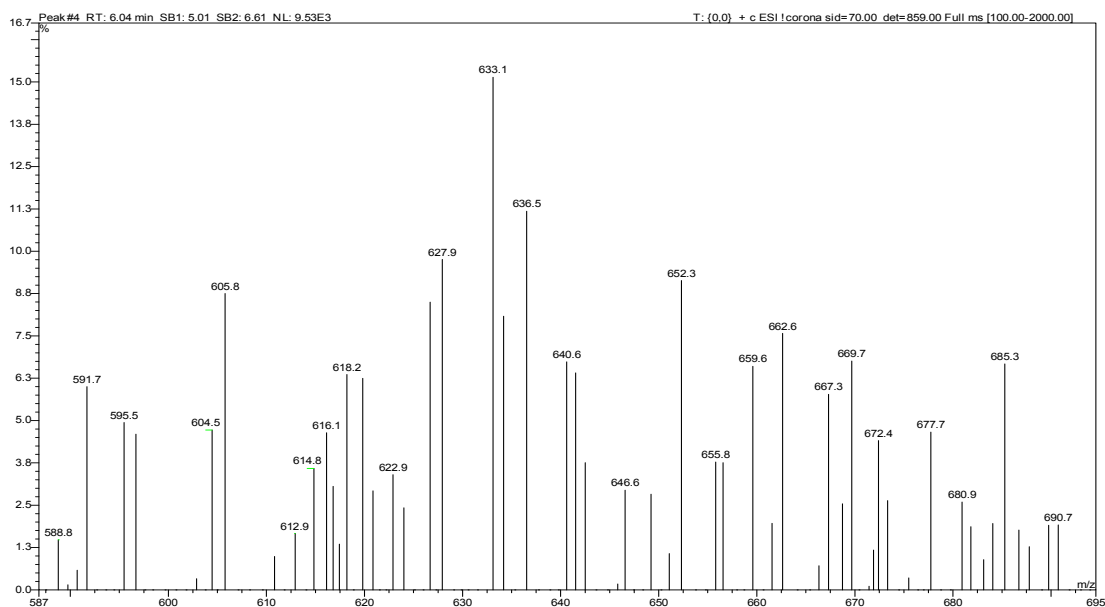
34



	(m/z)calad		(m/z)found	
18	$[m+10H]^{10+}$ 596.8	$[M+9H+Na]^{10+}$ 599.0	$[m+10H]^{10+}$ 596.3	$[M+9H+Na]^{9+}$ 599.0
19	$[m+10H]^{10+}$ 601.2	$[M+9H+Na]^{10+}$ 603.4	$[m+10H]^{10+}$ 601.1	$[M+9H+Na]^{9+}$ 603.3
20	$[m+10H]^{10+}$ 605.6	$[M+9H+Na]^{10+}$ 607.8	$[m+10H]^{10+}$ 606.4	$[M+9H+Na]^{9+}$ 608.8
21	$[m+10H]^{10+}$ 610.0	$[M+9H+Na]^{10+}$ 612.2	$[m+10H]^{10+}$ 610.1	$[M+9H+Na]^{9+}$ 612.3
22	$[m+10H]^{10+}$ 614.4	$[M+9H+Na]^{10+}$ 616.6	$[m+10H]^{10+}$ 614.5	$[M+9H+Na]^{9+}$ 616.4

Supporting Information

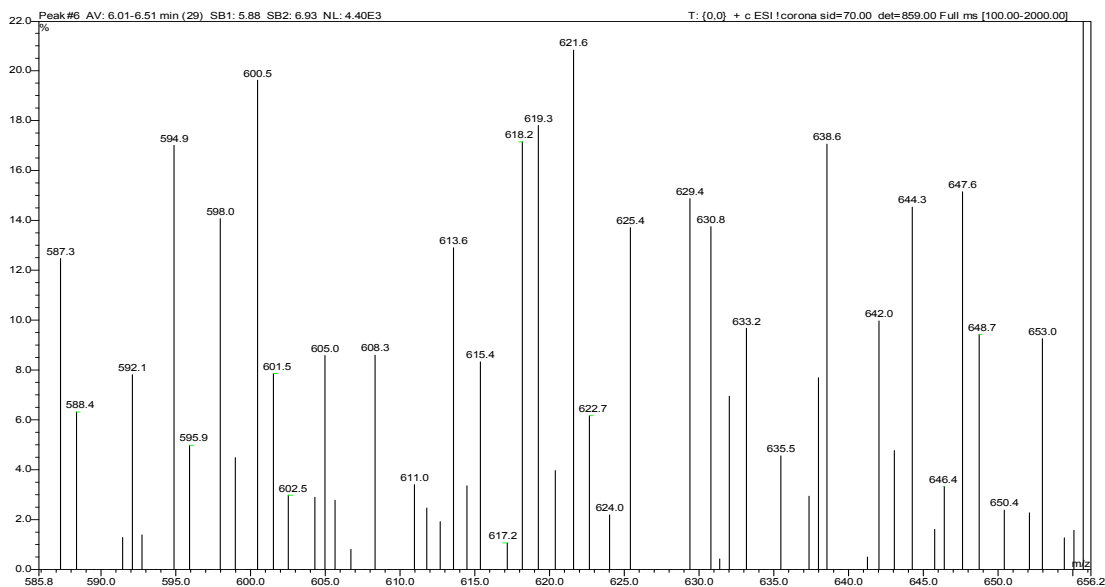
35



	(m/z)calad		(m/z)found	
38	$[m+11H]^{11+}$ 622.6	$[M+10H+Na]^{11+}$ 624.6	$[m+11H]^{11+}$ 622.9	$[M+10H+Na]^{11+}$ 624.3
39	$[m+11H]^{11+}$ 626.6	$[M+10H+Na]^{11+}$ 628.6	$[m+11H]^{11+}$ 626.9	$[M+10H+Na]^{11+}$ not found
40	$[m+11H]^{11+}$ 630.6	$[M+10H+Na]^{11+}$ 632.6	$[m+11H]^{11+}$ not found	$[M+10H+Na]^{11+}$ 633.1
41	$[m+11H]^{11+}$ 634.6	$[M+10H+Na]^{11+}$ 636.6	$[m+11H]^{11+}$ 634.5	$[M+10H+Na]^{11+}$ 636.5
42	$[m+11H]^{11+}$ 638.6	$[M+10H+Na]^{11+}$ 640.6	$[m+11H]^{11+}$ not found	$[M+10H+Na]^{11+}$ 640.6

Supporting Information

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	(m/z)calad		(m/z)found	
106	$[m+16H]^{16+}$ 615.4	$[M+15H+Na]^{16+}$ 616.7	$[m+16H]^{16+}$ 615.4	$[M+15H+Na]^{16+}$ 617.2
107	$[m+16H]^{16+}$ 618.1	$[M+15H+Na]^{16+}$ 619.5	$[m+16H]^{16+}$ 618.2	$[M+15H+Na]^{16+}$ 619.3
108	$[m+16H]^{16+}$ 620.9	$[M+15H+Na]^{16+}$ 622.2	$[m+16H]^{16+}$ 621.6	$[M+15H+Na]^{16+}$ 622.7
109	$[m+16H]^{16+}$ 623.6	$[M+15H+Na]^{16+}$ 625.0	$[m+16H]^{16+}$ 624.0	$[M+15H+Na]^{16+}$ 625.4
110	$[m+16H]^{16+}$ 626.4	$[M+15H+Na]^{16+}$ 627.7	$[m+16H]^{16+}$ not found	$[M+15H+Na]^{16+}$ not found

Supporting Information

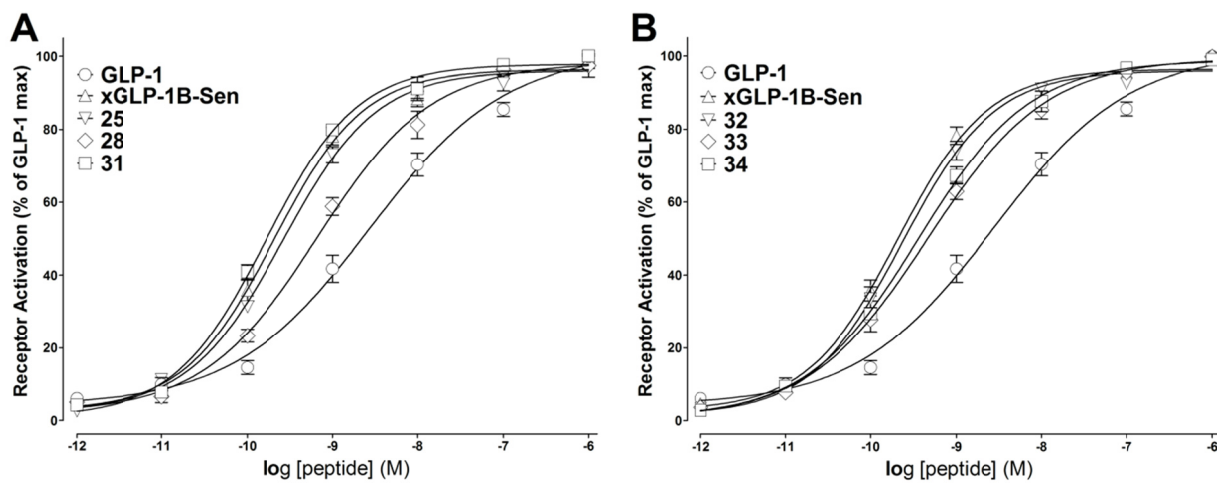


Figure S1. The representative concentration response curves of hGLP-1 and PEGylated conjugates. The data for the test peptides were normalized and plotted as the percentage of the response stimulated by saturating GLP-1 (1000 nM).

Supporting Information

Table S1. Time table of in vivo IPGTT and OGTT tests

Entry	Test	Mice		Peptides			Glucose		Blood collection
		Type	n	Fasted time (h)	Dose (nmol/kg)	Treatment time (min)	Dose (g/kg)	Load time (h)	
1	acute IPGTT	KM mice	6	12	25	-10	2	0	-10, 0, 15, 30, 60, 120 min
2	IPGTT	<i>db/db</i> mice	6	18	25	-30	1	0	-30, 0, 15, 30, 60, 120 min
3	pre-OGTT	<i>db/db</i> mice	6	18		-120	1.5	0	-120, 0, 30, 60, 90, 120 min
4	pre-OGTT	<i>db/db</i> mice	6	18	25 (33 and lixisenatide)	-360	1.5	0	-360, 0, 30, 60, 90, 120 min
5	multiple-OGTT	<i>db/db</i> mice	6	18	or 100 (33)	-30	1.5	0, 6, 12	-0.5, 0, 0.25, 0.5, 1, 2, 3, 6, 6.25, 6.5, 7, 8, 9, 12, 12.25, 12.5, 13, 14, 15, 18 h
6	IPGTT after chronic treatment	<i>db/db</i> mice	6	18	/	/	1	0	0, 15, 30, 60, 120 min