SUPPORTING INFORMATION

Assessing the effect of aromatic residues placement on α-helical peptide structure and nanofibril formation of 21-mer peptides

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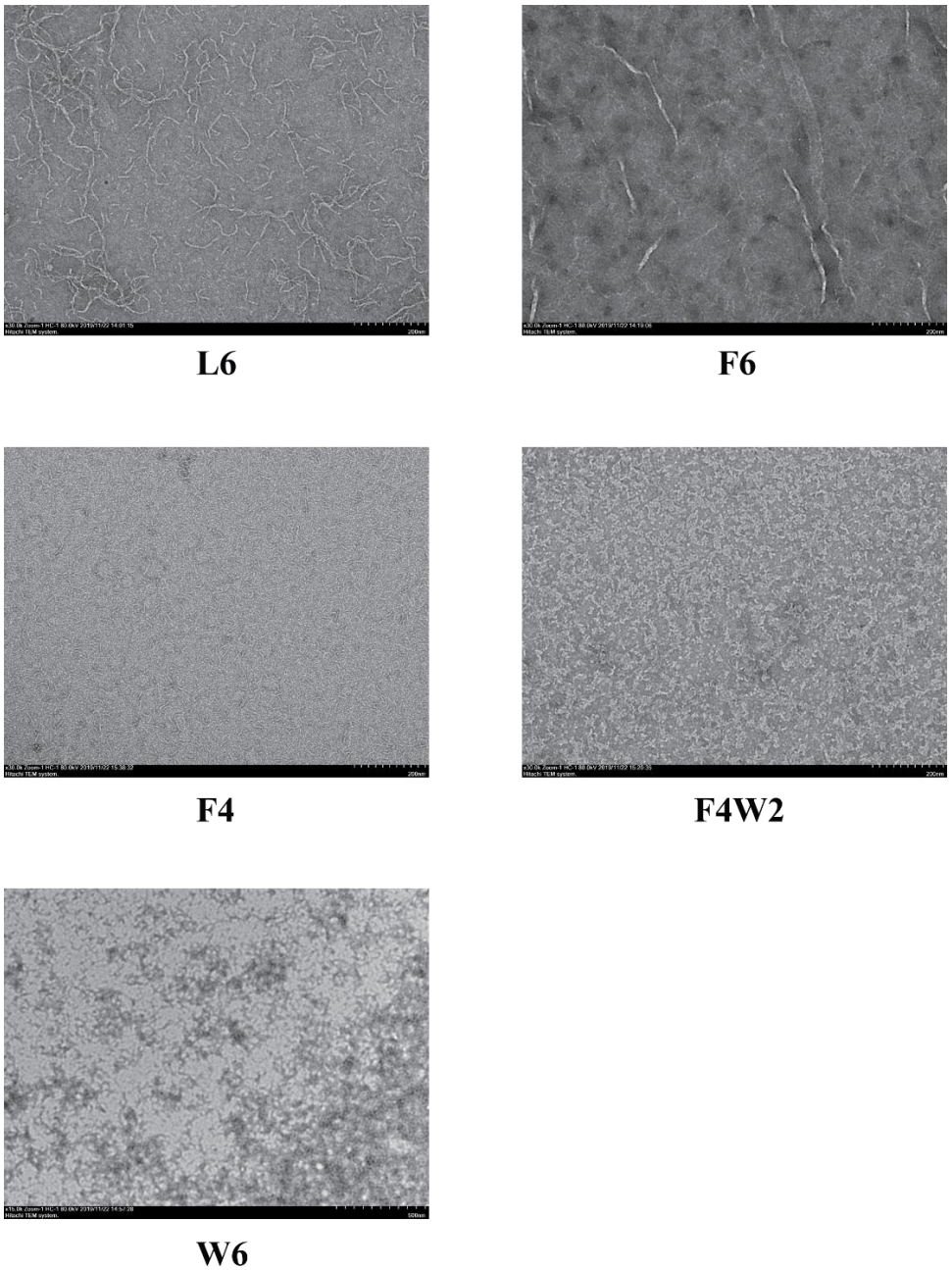


Figure S1. TEM Micrographs of L6, F6, F4, F4W2, and W6 prepared at 1 mM concentration.*

* The TEM samples were prepared and imaged as follows: 10 mM peptides solutions were prepared by dissolving lyophilised peptides in MilliQ water and diluting to 2 mM. Then, the 2 mM peptide solutions were mixed with required sodium bicarbonate (and left at room conditions for 2 hours to reach pH 7.4) to final concentration of 1 mM. TEM samples were prepared upon completion of 2-hour titration period by deposition of 4µL of titrated peptide on 200-mesh carbon-coated copper grid and left for 90s. The excess liquid was wicked away and 4µL of aqueous 1% uranyl acetate statin solution was added and briefly mixed on the grid. After 90 s, the excess was removed, and grids were air-dried. The TEM images were collected using Hitachi-H7700 transmission electron microscope.

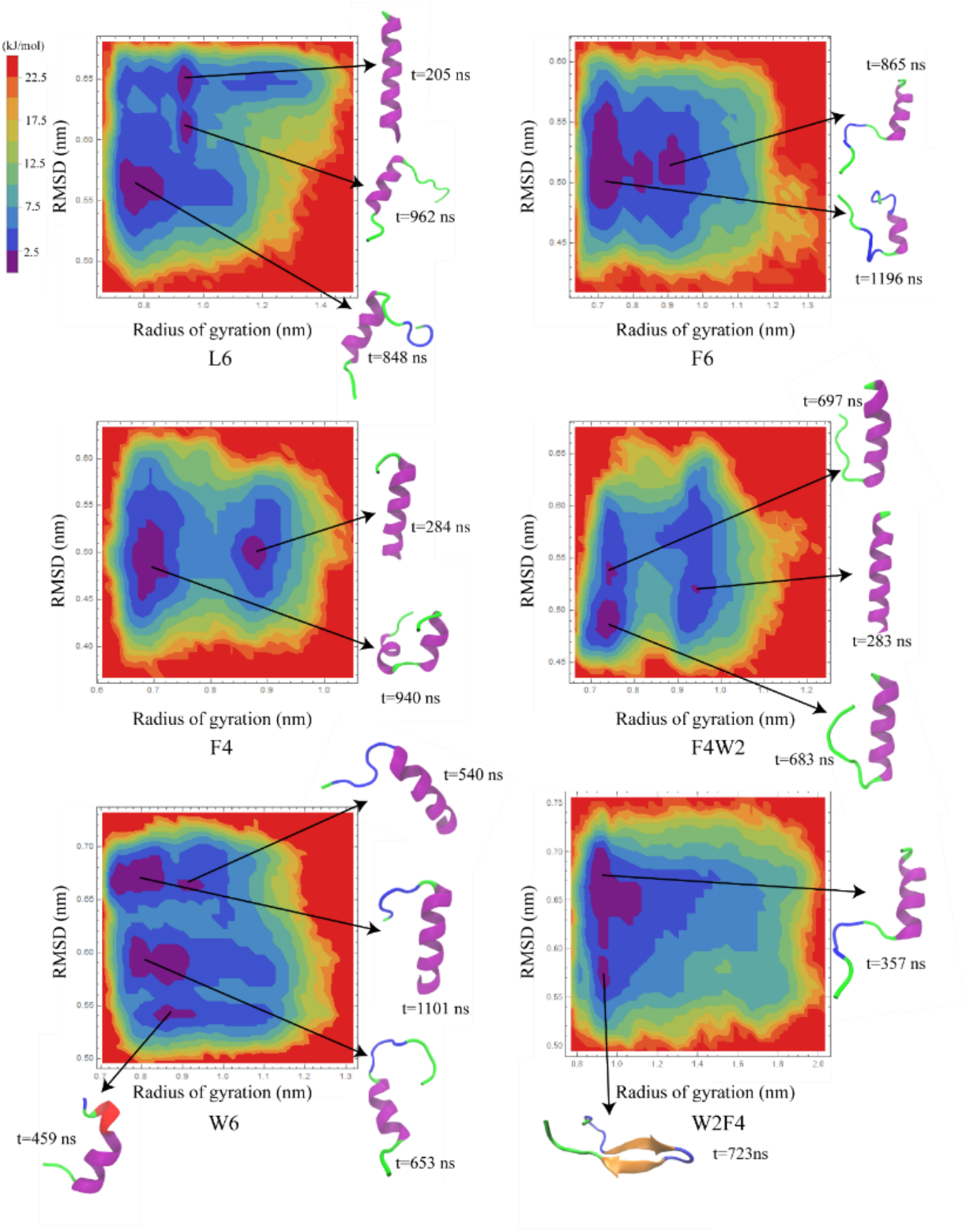


Figure S2. Free energy landscape for full atomistic models of L6, F6, F4, F4W2, W6, and W2F4 measured up to 1.2 μs (first 200 ns of simulation omitted). Radius of gyration of backbone and RMSD of backbone versus average structure of each peptide over the simulation period used as X and Y axis. Representative models at local minimum energies are represented with their occurrence of simulation time. Purple, green, blue, and red, and orange colours indicate parts of the representative models that are α-helix, random coil, turn, and 3-10-helix, and β-sheet secondary structure. Bar legend for all free energy landscapes are the same shown at top left of the figure with kJ/mol units.

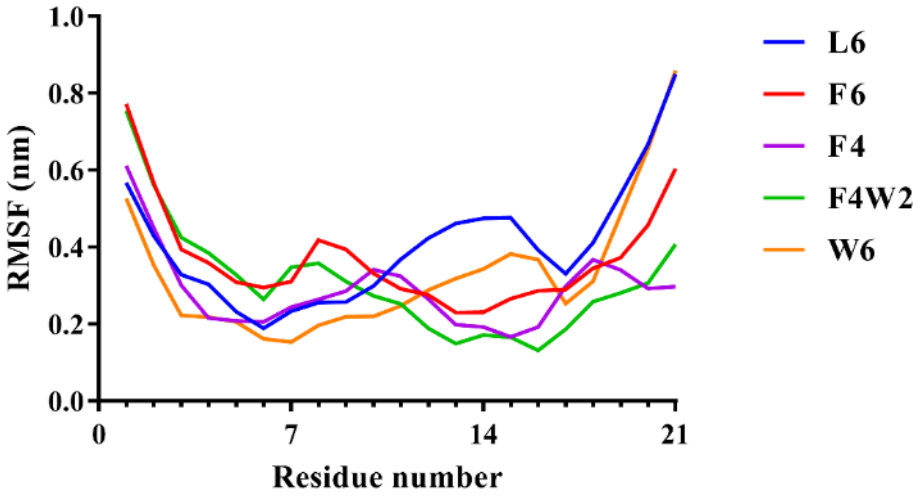


Figure S3. RMSF results for MD simulation of all-atom models up to 1.2 μs (first 200 ns of simulation omitted) of each residue for L6, F6, F4, F4W2, and W6 peptides coloured blue, red, purple, green, and orange, respectively.

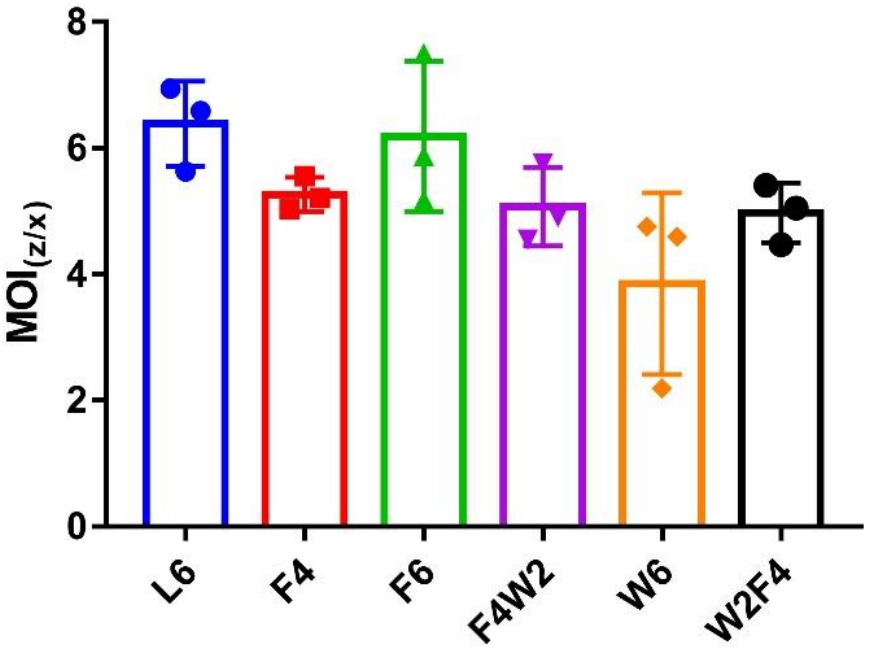


Figure S4. $MOI_{(z/x)}$ of three repeated MD simulations of peptide CG models from different initial peptide distribution in the simulation box.

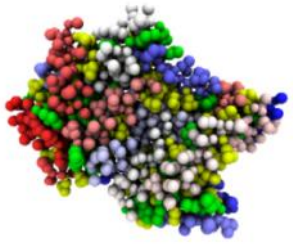


Figure S5. Snapshot of largest CG cluster for W2F4 at 25 μs with Phe and Trp residues coloured yellow and green, respectively and different peptide chains presented in different colours.

Table S1. Moment of Inertia (MOI) along the principles axis of CG models (at 25 μs) calculated for all designed peptides along with solvent accessible surface area (SASA) of them calculated at initial (after energy minimisation) and end of simulation (at 25 μs). Results are average of three repeated MD simulations of peptide CG models from different initial peptide distribution in the simulation box.

Peptide	Run	MOI (10^2 amu Å^2)			MOI (z/x)
		X	Y	Z	
	1	538	2910	3033	5.63
	2	506	3292	3332	6.59
	3	471	3081	3265	6.94
	1	636	3378	3535	5.56
	2	605	3007	3151	5.21
	3	656	3115	3304	5.03
	1	656	3273	3400	5.18
	2	12534	86237	93942	7.50
	3	659	3773	3878	5.88
	1	653	3700	3768	5.76
	2	780	3279	3544	4.55
	3	766	3688	3757	4.91
	1	4172	6981	9156	2.19
	2	859	3802	3953	4.60
	3	837	3725	3987	4.76
	1	808	3523	3614	4.47
	2	728	3547	3681	5.05
	3	733	3910	3966	5.41

Table S2. Secondary structure prediction of L6, F4, F6, F4W2, and W6 peptides at 1 mM, 100 µM, 10 µM, and 1 µM concentrations using BeStSel method.[1,2] Each class of secondary structure is defined by BeStSel. Total α-helical structure is combination of Helix 1 and Helix 2. Total antiparallel is combination of Anti 1, Anti 2, and Anti 3 while total β-sheet is combination of parallel and anti-parallel.

Peptide	Concentration (mM)	Secondary Structure Prediction (%)										
		Helix1	Helix2	Anti1	Anti2	Anti3	Antiparallel (Total)	Parallel	Beta Sheet (Total)	Turn	α-Helical (Total)	Others
	1	69.0	19.7	0.0	0.0	0.0	0.0	0.0	0.0	6.5	88.7	4.7
	0.1	58.1	18.5	0.0	0.0	0.0	0.0	0.0	0.0	7.8	76.6	15.6
	0.01	54.9	18.9	0.0	0.0	0.0	0.0	0.0	0.0	9.6	73.8	16.6
	0.001	38.2	15.1	0.0	0.0	2.5	2.5	3.9	6.4	8.6	53.3	31.7
	1	51.2	24.6	0.0	2.0	0.0	2.0	0.0	2.0	8.7	75.8	13.6
	0.1	28.1	12.3	0.8	7.9	0.0	8.8	0.0	8.8	12.6	40.4	38.3
	0.01	20.1	12.2	2.4	8.0	0.0	10.4	0.0	10.4	12.7	32.2	44.7
	0.001	9.1	4.7	5.4	14.7	11.2	31.3	0.0	31.3	13.4	13.9	41.4
	1	57.6	28.6	5.2	0.0	0.0	5.2	7.5	12.7	1.0	86.3	0.0
	0.1	40.8	26.4	0.0	0.0	0.0	0.0	0.0	0.0	7.9	67.2	24.9
	0.01	15.8	16.0	3.1	1.5	3.0	7.6	0.0	7.6	13.4	31.8	47.3
	0.001	17.8	13.4	4.2	5.4	5.6	15.2	0.0	15.2	13.4	31.3	40.1
	1	46.9	15.7	0.0	6.7	0.0	6.7	0.0	6.7	9.7	62.6	21.0
	0.1	13.0	9.5	2.9	10.6	5.8	19.4	0.0	19.4	13.3	22.5	44.7
	0.01	2.8	4.3	0.0	15.3	14.7	30.0	0.0	30.0	16.3	7.1	46.6
	0.001	1.9	2.4	3.8	13.4	14.2	31.4	0.0	31.4	16.7	4.4	47.6
	1	15.9	8.3	0.0	19.9	11.8	31.7	0.0	31.7	6.8	24.2	37.2
	0.1	25.6	4.7	4.7	14.1	3.0	17.1	0.0	17.1	12.8	30.4	39.7
	0.01	15.1	3.6	0.0	19.9	10.6	30.6	0.0	30.6	11.3	18.7	39.5
	0.001	0.0	1.7	2.9	20.7	17.7	41.4	0.0	41.4	11.2	1.7	45.7

Table S3. Fibre diameter calculation for L6, F4, F6, F4W2, and W6 peptides based on AFM images. It should be noted these measurements are from the images in Figure 1 of the manuscript. These are representative images only.

Fibre diameter	*L6 (pm)*	*F4 (pm)*	*F6 (pm)*	*F4W2 (pm)*	*W6 (pm)*
	1555	2977	1013	3861	4216
	1487	3400	847	5526	3866
	1040	2253	1317	4471	5161
	1678	2504	848	4900	6141
	938	2415	940	5289	6973
	1885	3326	1200	4910	6504
	1828	2439	1324	5834	4130
	1331	2034	1038	7109	5882
	1552	1759	1003	6405	5030
	1047	3020	1182	6786	6774
Average:	**1434.1**	**2612.7**	**1071.2**	**5479.1**	**5467.7**
Standard Deviation:	335.2	547	176.3	1040.4	1148.8

References:

1 A. Micsonai, F. Wien, L. Kernya, Y. H. Lee, Y. Goto, M. Refregiers, et al., Proc Natl Acad Sci U S A, 2015, **112**, E3095-103.
2 A. Micsonai, F. Wien, E. Bulyaki, J. Kun, E. Moussong, Y. H. Lee, et al., Nucleic Acids Res, 2018, **46**, W315-W22.

Appendix: High performance liquid chromatography (HPLC) and mass spectroscopy (MS) data for each peptide

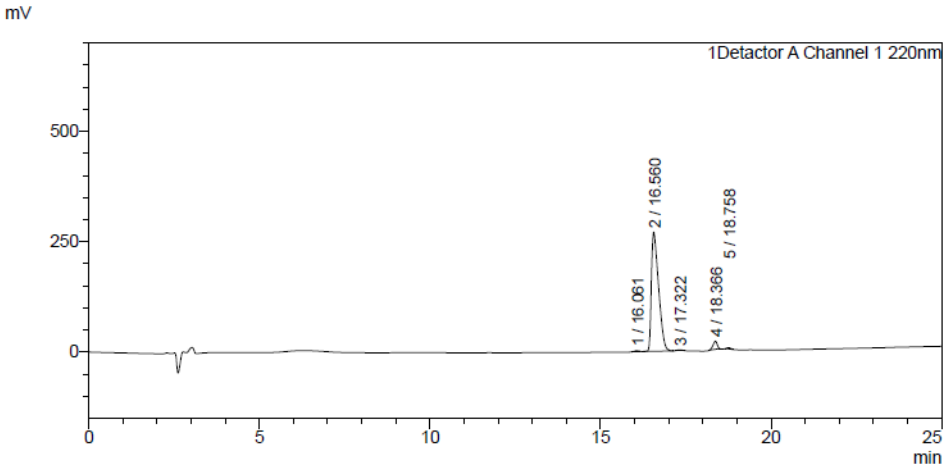
HPLC data for **L6**:

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	Pump B Conc.	35
25.00	Pumps	Pump B Conc.	95
31.00	Pumps	Pump B Conc.	95
31.01	Pumps	Pump B Conc.	35
40.00	Pumps	Pump B Conc.	35
40.01	Controller	Stop	

<<Column Performance>>
<Detactor A>
Column : AlltimaTM C18 4.6 x 250 mm

<Chromatogram>

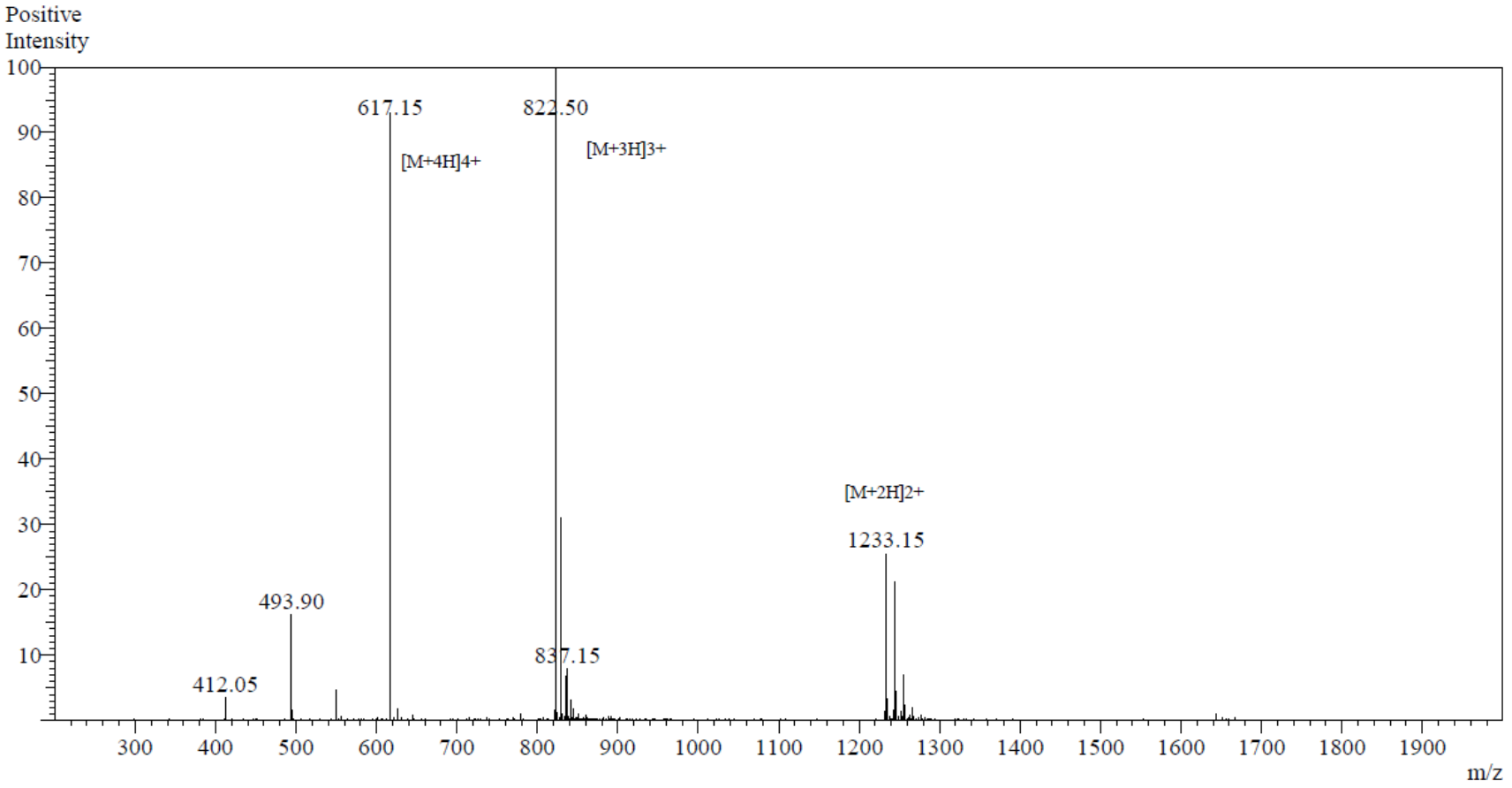


<Peak Table>

Detactor A Channel 1 220nm

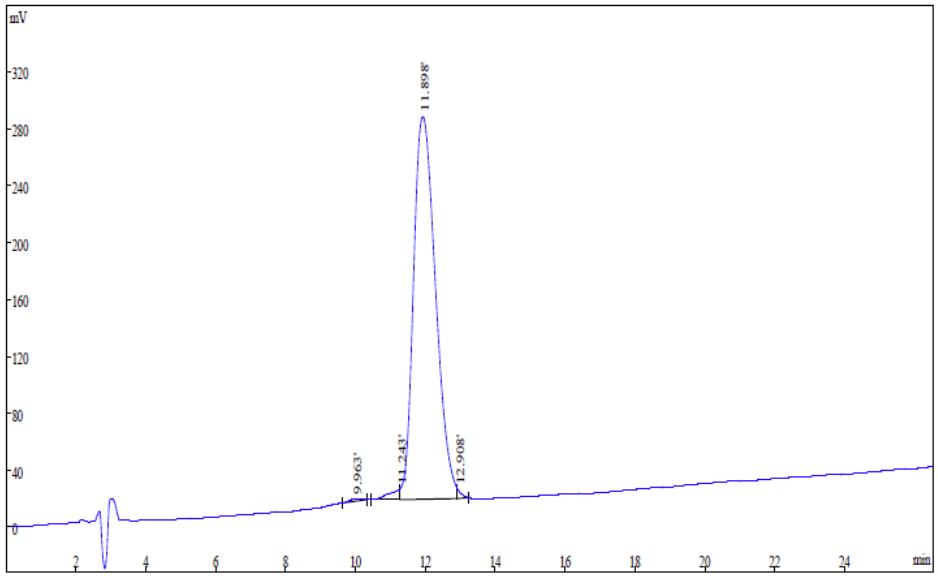
Peak#	Ret. Time	Area	Height	Area%
1	16.061	14921	1926	0.359
2	16.560	3951208	269901	95.032
3	17.322	13923	1660	0.335
4	18.366	156392	18448	3.761
5	18.758	21309	3041	0.513
Total		4157753	294976	100.000

MS data for **L6**:



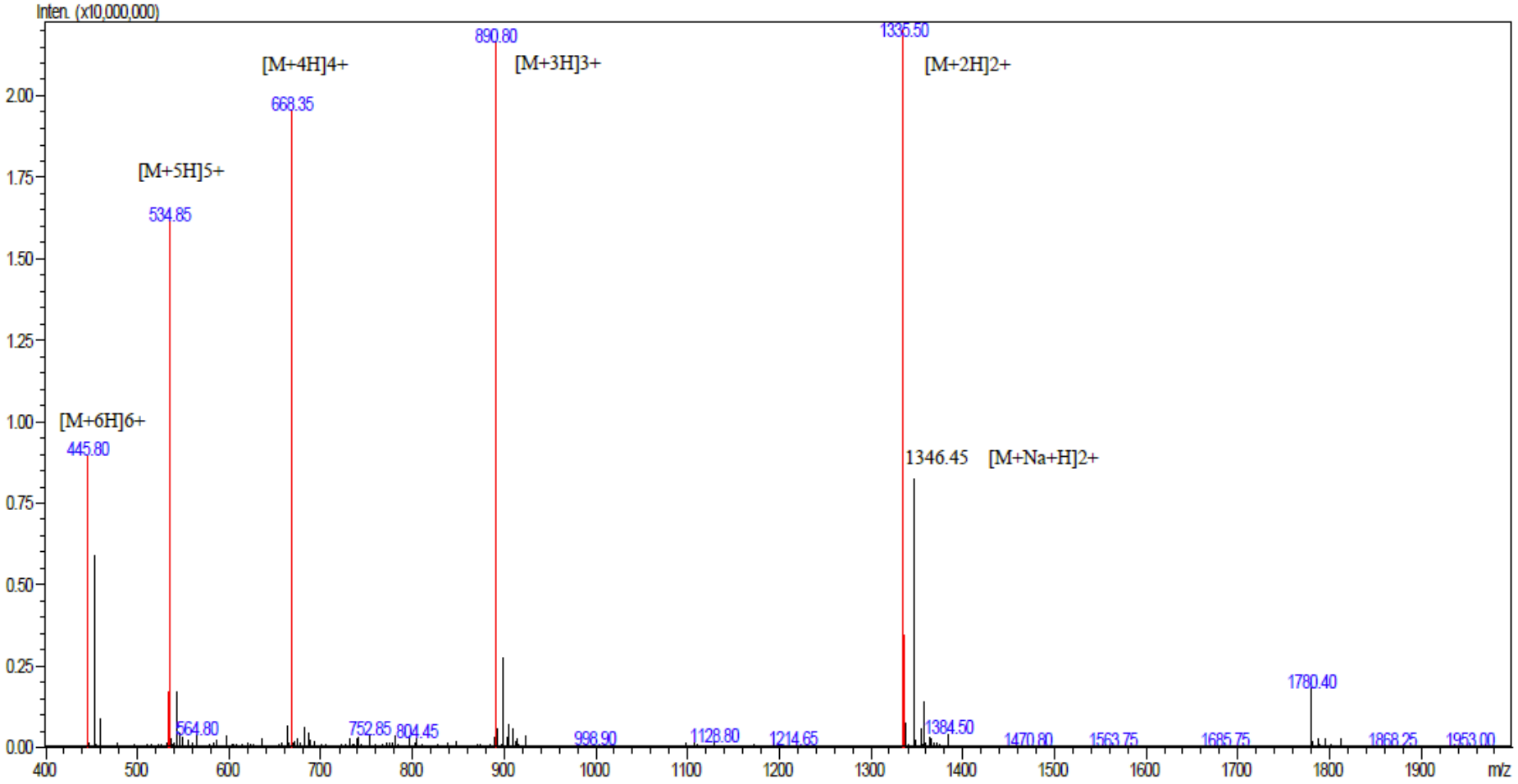
HPLC data for **F6**:

Column	4.6mmx250mm, Agela		
Solvent A	0.1% trifluoroacetic in 100% acetonitrile		
Solvent B	0.1% trifluoroacetic in 100% water		
Gradient		A	B
	0.01min	46%	54%
	25min	71%	29%
	25.1min	100%	0%
	30min	STOP	
Flow Rate	1.0ml/min		
Wavelength	214nm		
Volume	10ul		



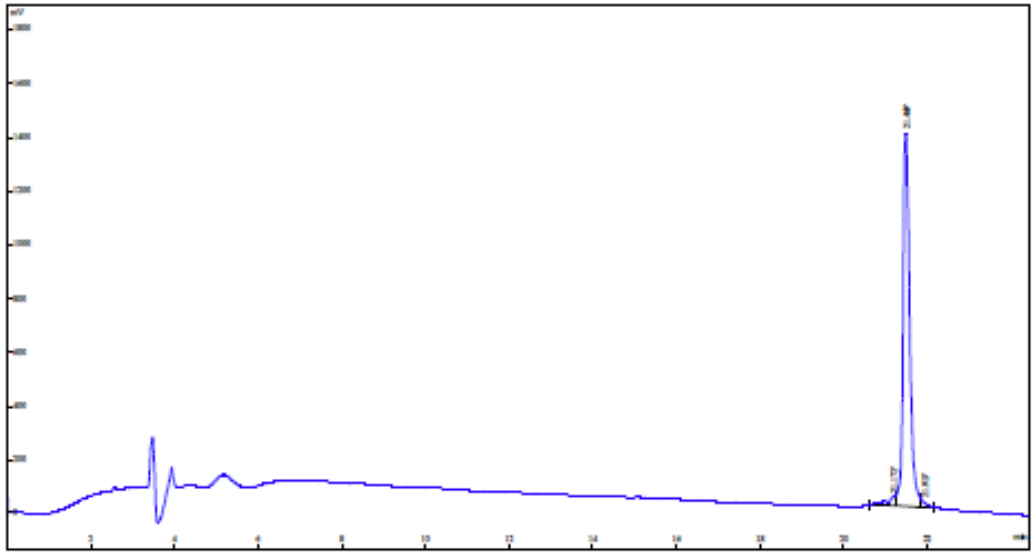
Rank	Time	Conc	Area	Height
1	9.963	0.2918	34099	1515
2	11.243	1.267	148124	6815
3	11.898	98.12	11467832	268351
4	12.908	0.322	37638	5423
				100

MS data for **F6**:



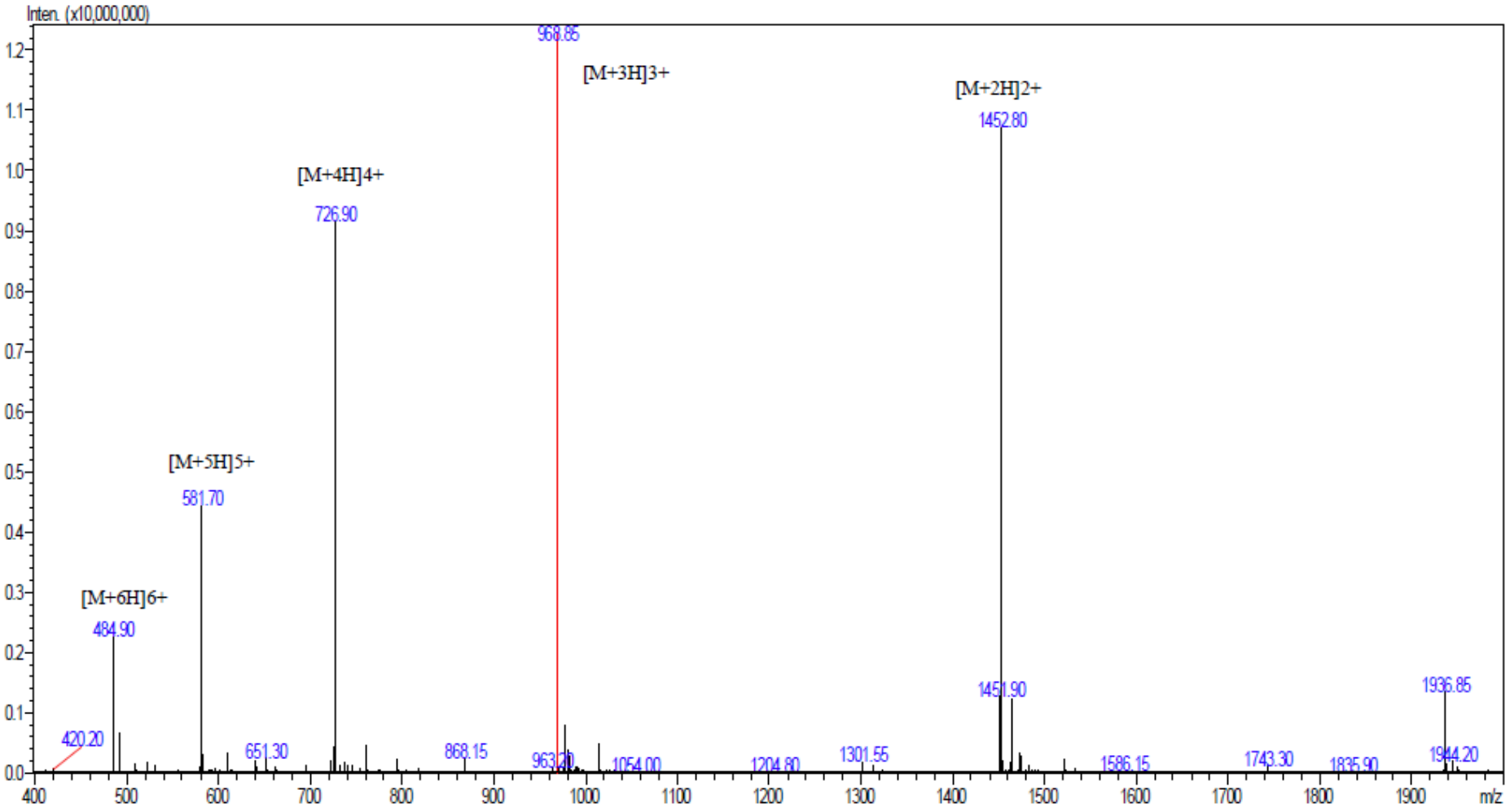
HPLC data for **W6**:

Column	4.6mmx250mm, Sinochrom ODS-Bp 5um		
Solvent A	0.1% trifluoroacetic in 100% acetonitrile		
Solvent B	0.1% trifluoroacetic in 100% water		
Gradient		A	B
	0.01min	10%	90%
	25min	67%	33%
	25.1min	100%	0%
	30min	STOP	
Flow Rate	1.0ml/min		
Wavelength	214nm		
Volume	10ul		



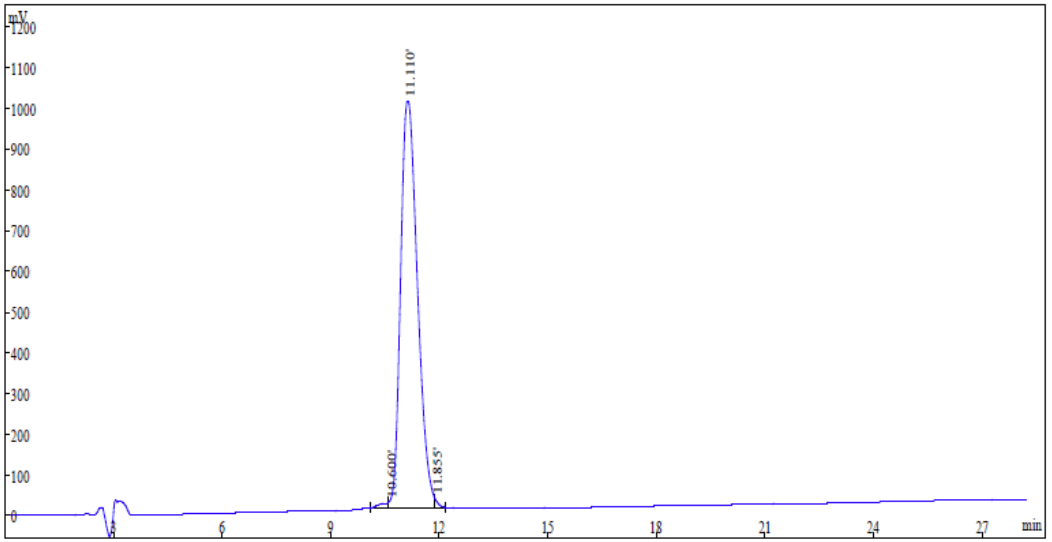
Rank	Time	Conc.	Area	Height
1	21.172	3.439	558536	36006
2	21.469	95.31	15477533	1399945
3	21.915	1.253	203493	15717
Total		100	16239562	1451668

MS data for **W6**:



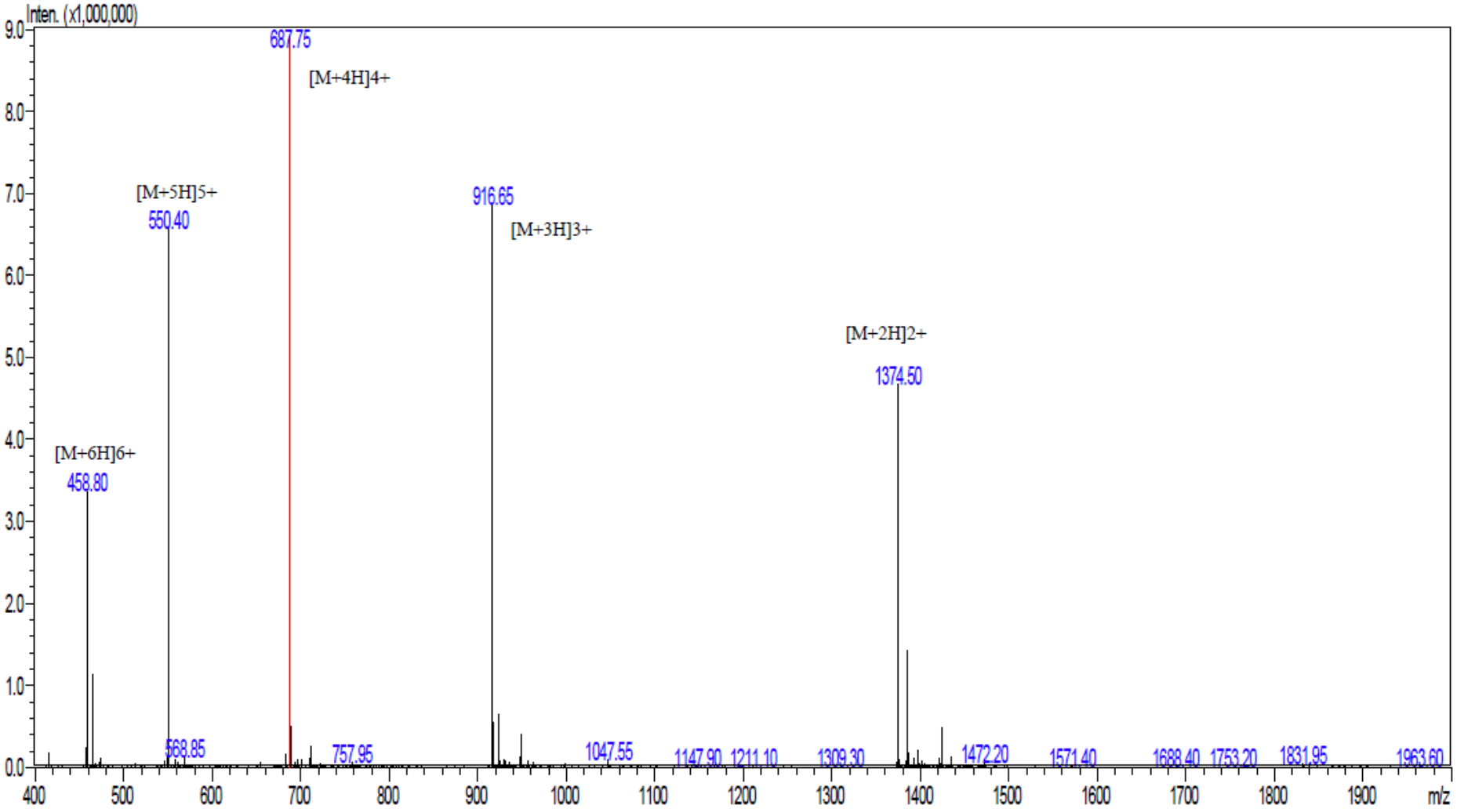
HPLC data for **F4W2:**

Column	4.6mmx250mm, Agela		
Solvent A	0.1% trifluoroacetic in 100% acetonitrile		
Solvent B	0.1% trifluoroacetic in 100% water		
Gradient		A	B
	0.01min	43%	57%
	25min	68%	32%
	25.1min	100%	0%
	30min	STOP	
Flow Rate	1.0ml/min		
Wavelength	214nm		
Volume	10ul		



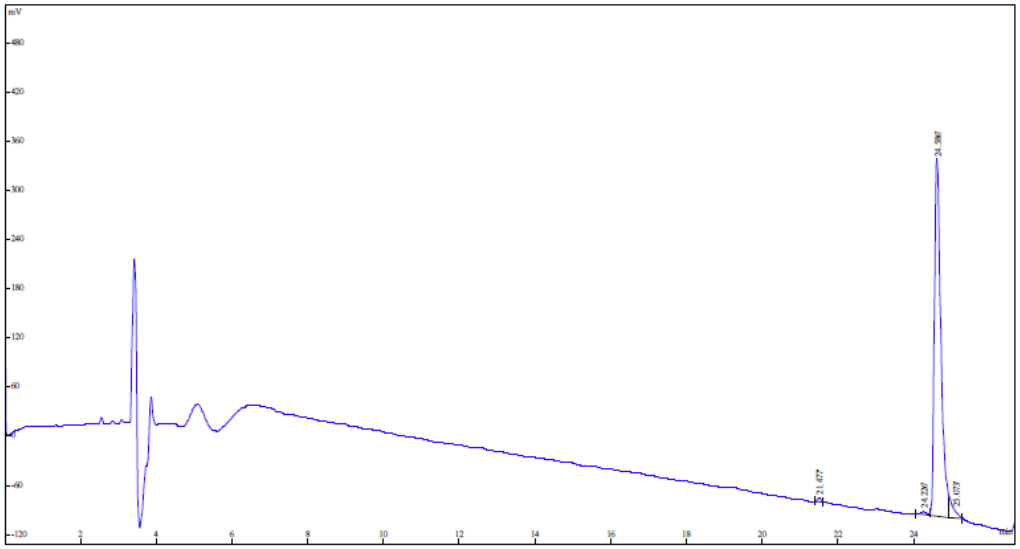
Rank	Time	Height	Area	Conc.
1	10.600	13726	221739	0.7066
2	11.110	997598	31041146	98.92
3	11.855	21009	117844	0.3755
				100

MS data for **F4W2**:



HPLC data for **F4:**

Column	4.6mmx250mm, Sinochrom ODS-Bp 5um		
Solvent A	0.1% trifluoroacetic in 100% acetonitrile		
Solvent B	0.1% trifluoroacetic in 100% water		
Gradient		A	B
	0.01min	10%	90%
	25min	67%	33%
	25.1min	100%	0%
	30min	STOP	
Flow Rate	1.0ml/min		
Wavelength	214nm		
Volume	10ul		



Rank	Time	Conc.	Area	Height
1	21.477	0.5514	30150	5089
2	24.226	0.5848	31976	2887
3	24.586	95.82	5239025	439502
4	25.073	3.048	166660	8422
Total		100	5467811	455900

MS data for **F4**: