

## SUPPLEMENTARY INFORMATION

# Combination of Amphiphilic Cyclic Peptide [R<sub>4</sub>W<sub>4</sub>] and Levofloxacin Against Multidrug-Resistant Bacteria

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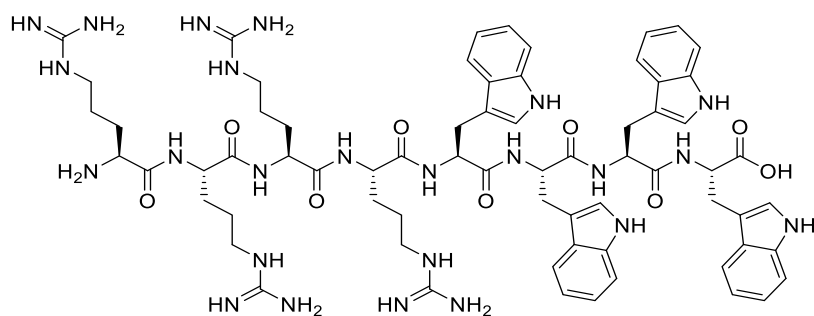
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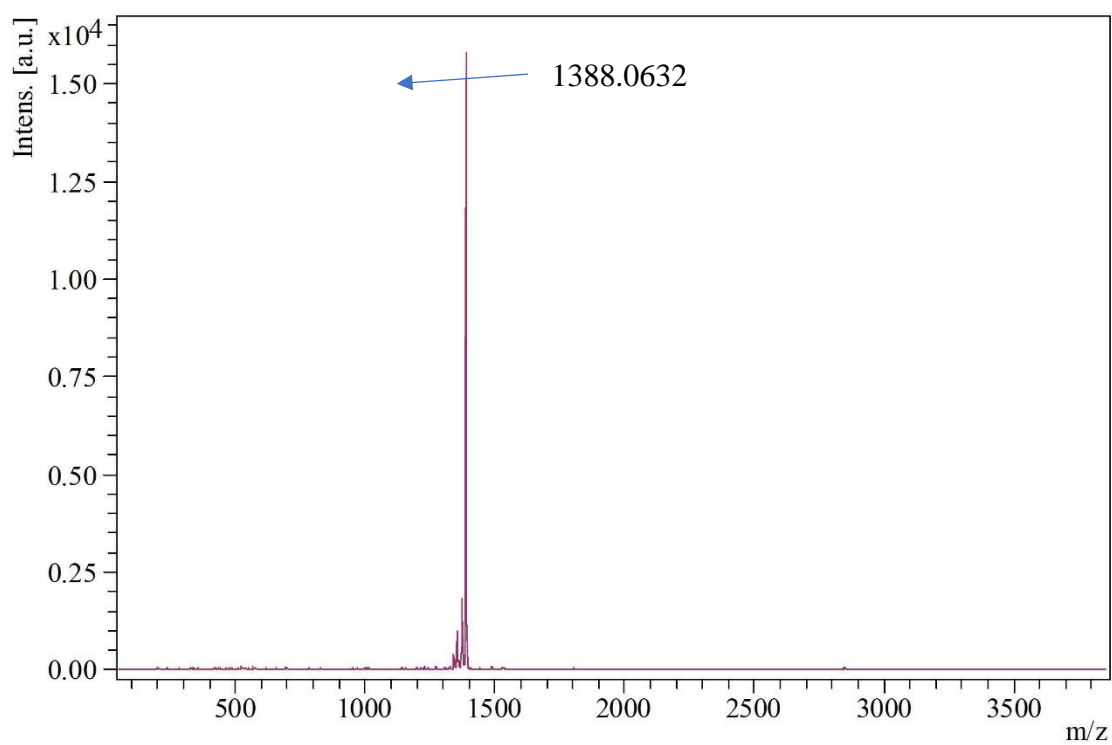
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# 1. MALDI-TOF mass spectrum of linear peptide (R<sub>4</sub>W<sub>4</sub>)

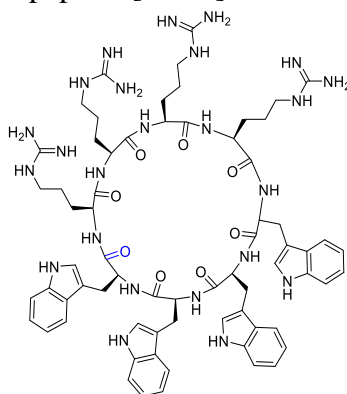


Chemical Formula: C<sub>68</sub>H<sub>90</sub>N<sub>24</sub>O<sub>9</sub>  
Exact Mass: 1386.7323

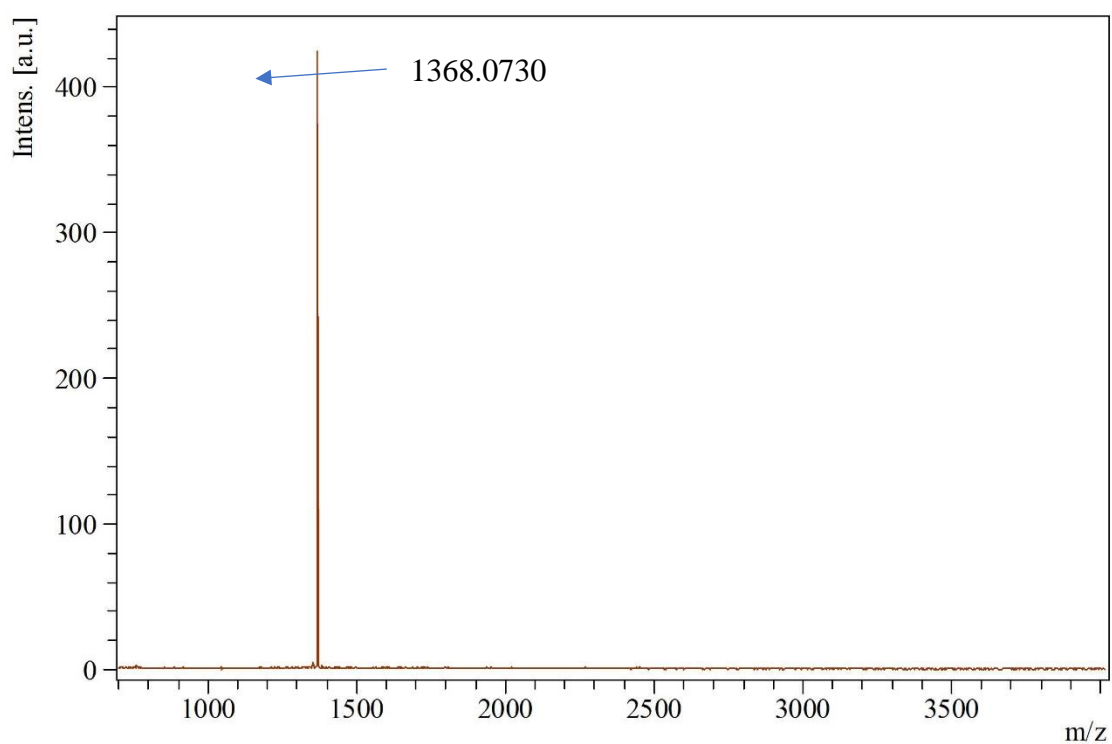


m/z	S/N	Quality Fac.	Res.	Intens.	Area
1340.9943	20	1924	520	207	1363
1354.6184	55	2084	886	733	259
1359.8750	13	2545	762	149	74
1372.0041	13	96.0	538	165	98
1374.4433	95	3748	919	1278	4478
1384.6683	6	4.88	1201	87.1	237
1388.0632	884	235389	957	11904	40730

## 2. MALDI-TOF mass spectrum of cyclic peptide [R<sub>4</sub>W<sub>4</sub>]

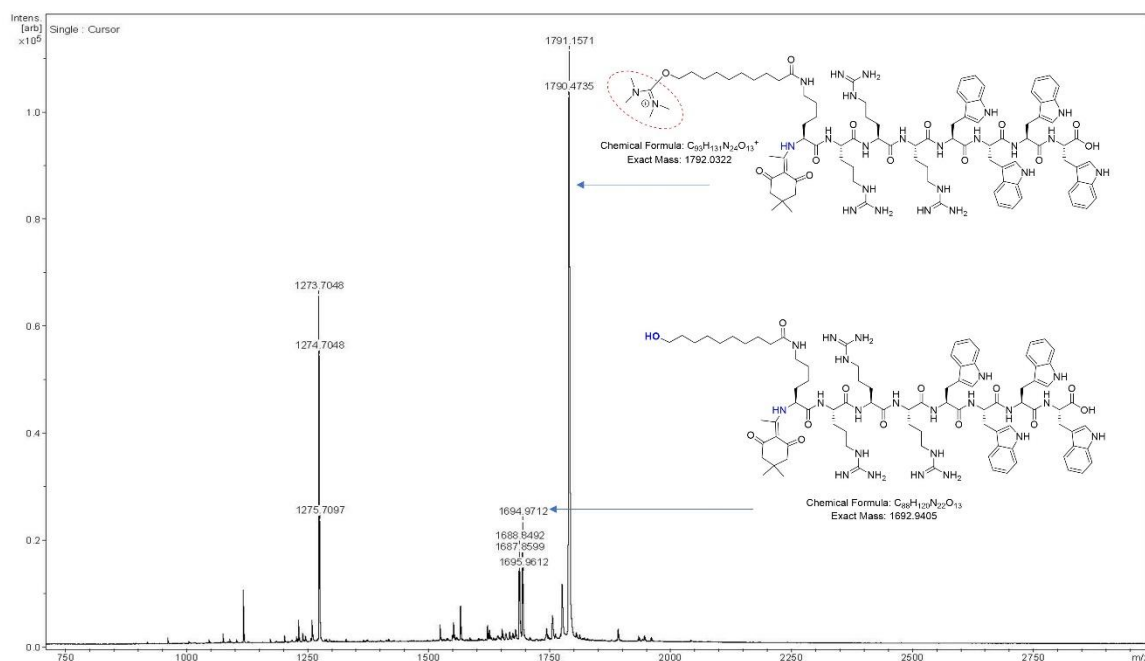


Chemical Formula: C<sub>68</sub>H<sub>88</sub>N<sub>24</sub>O<sub>8</sub>  
Exact Mass: 1368.7217

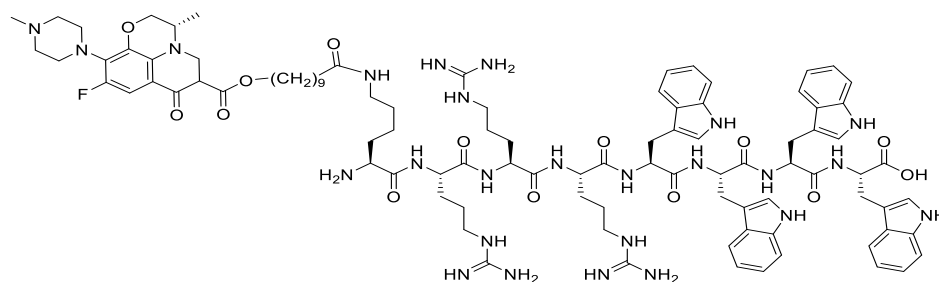


m/z	S/N	Quality Fac.	Res.	Intens.	Area
1368.0730	36	3468	4712	424	288

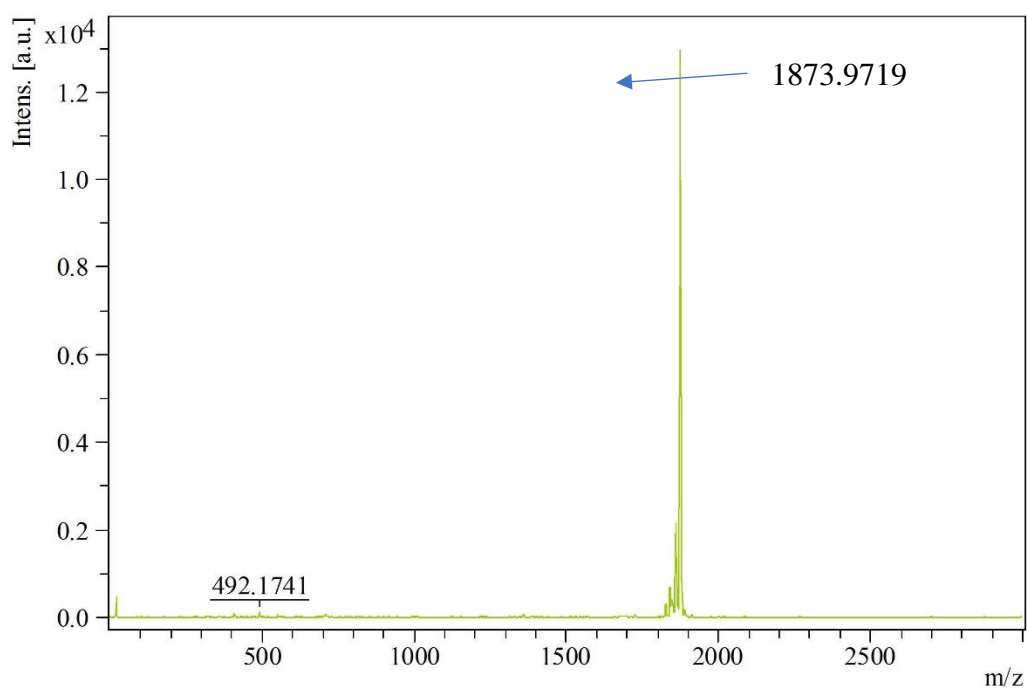
3. MALDI-TOF mass spectrum of the test cleavage of peptidyl resin to monitor the reaction mixture after 2 h for the coupling of 10-hydroxydecanoic acid (10-HDA) with the linear peptide (Dde-K(NH<sub>2</sub>)-(R(Pbf))<sub>3</sub>(W(Boc))<sub>4</sub>) on chlorotrityl resin using HBTU and DIPEA in the DMF.



#### 4. MALDI-TOF mass spectrum of linear peptide Levo conjugate (Levo-C<sub>10</sub>-KR<sub>3</sub>W<sub>4</sub>)

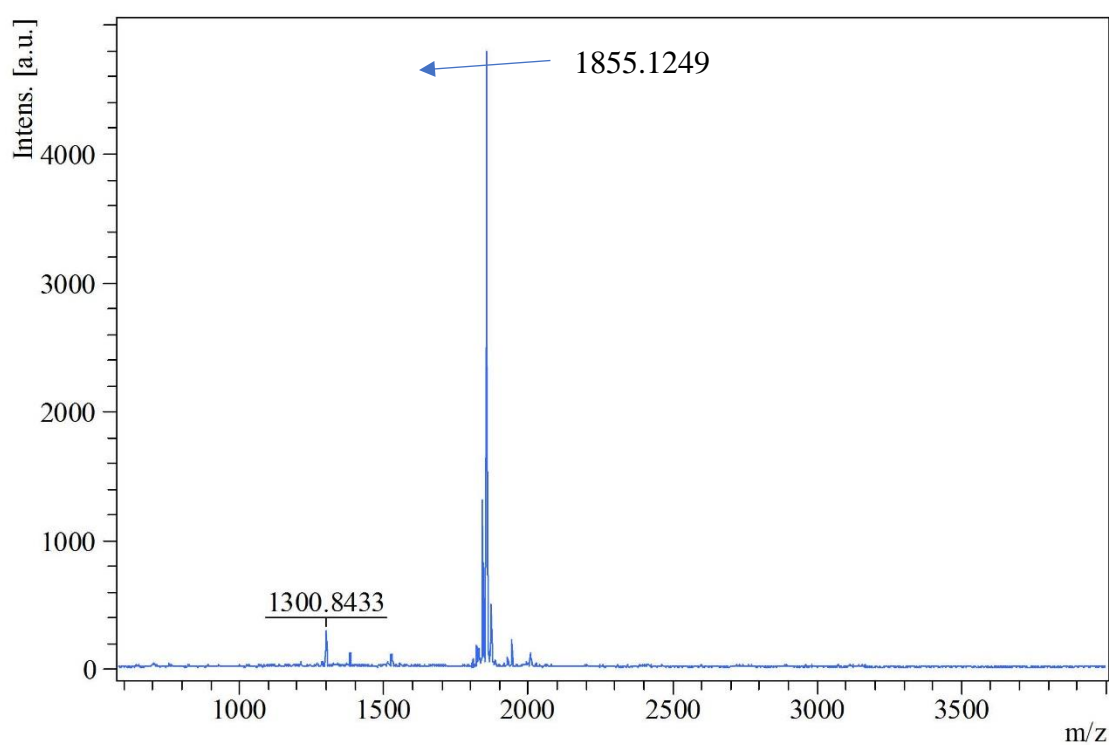
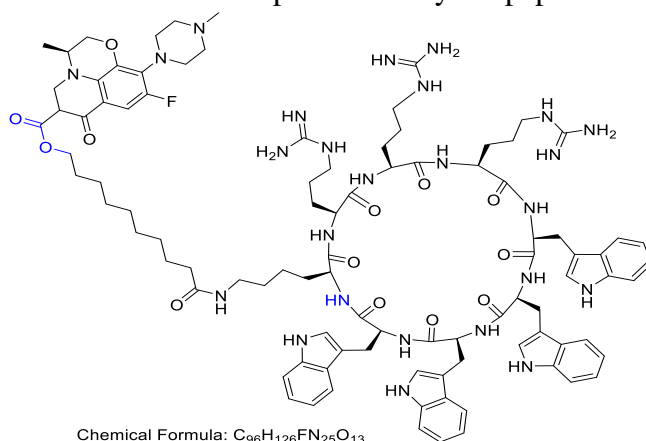


Chemical Formula: C<sub>96</sub>H<sub>128</sub>FN<sub>25</sub>O<sub>14</sub>  
Exact Mass: 1874.0057



m/z	S/N	Quality	Fac.	Res.	Intens.	Area
21.9660	10	158	594	124	5.64	
22.0464	6	147	854	81.4	2.25	
22.1575	17	700	735	207	6.72	
22.2192	19	236	672	237	8.37	
22.2936	36	497	705	458	15.4	
22.3681	40	464	696	501	17.1	
22.4466	22	264	728	278	9.17	
22.5222	7	89.6	851	83.3	2.35	
22.5953	9	159	581	117	5.46	
22.7311	13	166	655	162	7.03	
22.8040	7	137	883	92.4	3.02	
22.8809	6	207	778	78.9	2.52	
23.0239	9	505	582	115	4.84	
492.1741	8	1520	1498	108	55.4	
1826.6876	15	17117	675	132	1152	
1840.1408	41	9663	842	364	2600	
1845.8078	21	4278	613	166	1748	
1859.8388	117	15364	747	998	8321	
1868.1896	9	151	492	88.5	1057	
1873.9719	723	2.174316e6	1017	7393	42699	

## 5. MALDI-TOF mass spectrum of cyclic peptide Levo conjugate [Levo-C<sub>10</sub>-KR<sub>3</sub>W<sub>4</sub>]



m/z	S/N	Quality Fac.	Res.	Intens.	Area
1300.8433	16	58348	2503	292	390
1821.3029	8	3412	1982	130	422
1841.0730	63	168137	2404	1052	2808
1855.1249	236	225664	2436	3910	10399
1868.0990	8	454	2405	129	372
1871.1541	23	3250	2597	437	984
1942.1810	11	32376	3289	191	387
2007.1052	6	4492	2919	93.8	236

**6. Analytical HPLC analysis.** The purity of linear ( $R_4W_4$ ) and cyclic [ $R_4W_4$ ] peptides and their respective Levo conjugates were determined using a reverse-phase analytical HPLC (Shimadzu; LC-20ADXR). Method 1 was used to determine the purity of the linear ( $R_4W_4$ ) and cyclic [ $R_4W_4$ ] peptides. Method 2 was used to determine the purity of Levo conjugates of linear and cyclic peptides.

#### **Method 1:**

**Column:** Phenomenex (Luna), 4  $\mu$ m, C18, 150  $\times$  4.6 mm

**Flow rate:** 0.5 ml/min

**Mobile Phase:** Buffer A-Water containing 0.1% TFA

Buffer B-Acetonitrile containing 0.1% TFA

#### **Gradient**

Time (min)	Buffer B (%)
0.01	5
2	5
5	10
25	70
27	95
32	95
32.01	5
38	5

#### **Method 2**

**Column:** Phenomenex (Luna), 4  $\mu$ m, C18, 150  $\times$  4.6 mm

**Flow rate:** 0.5 ml/min

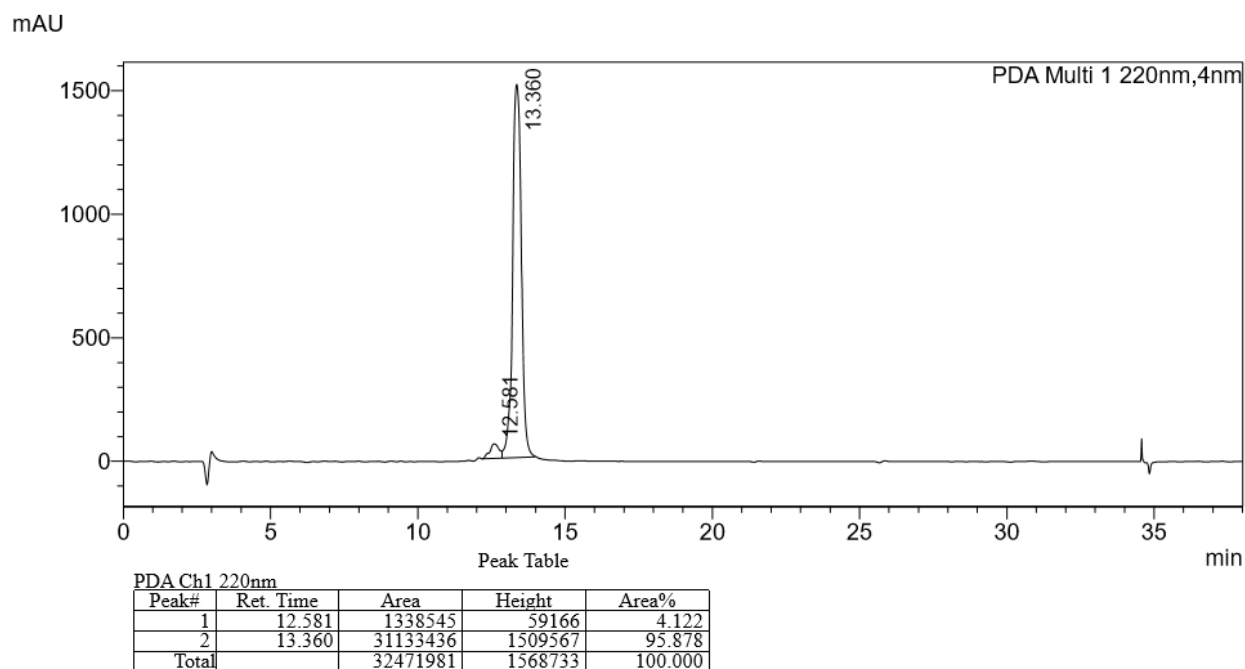
**Mobile Phase:** Buffer A- Water containing 0.1% TFA

Buffer B- Methanol containing 0.1% TFA

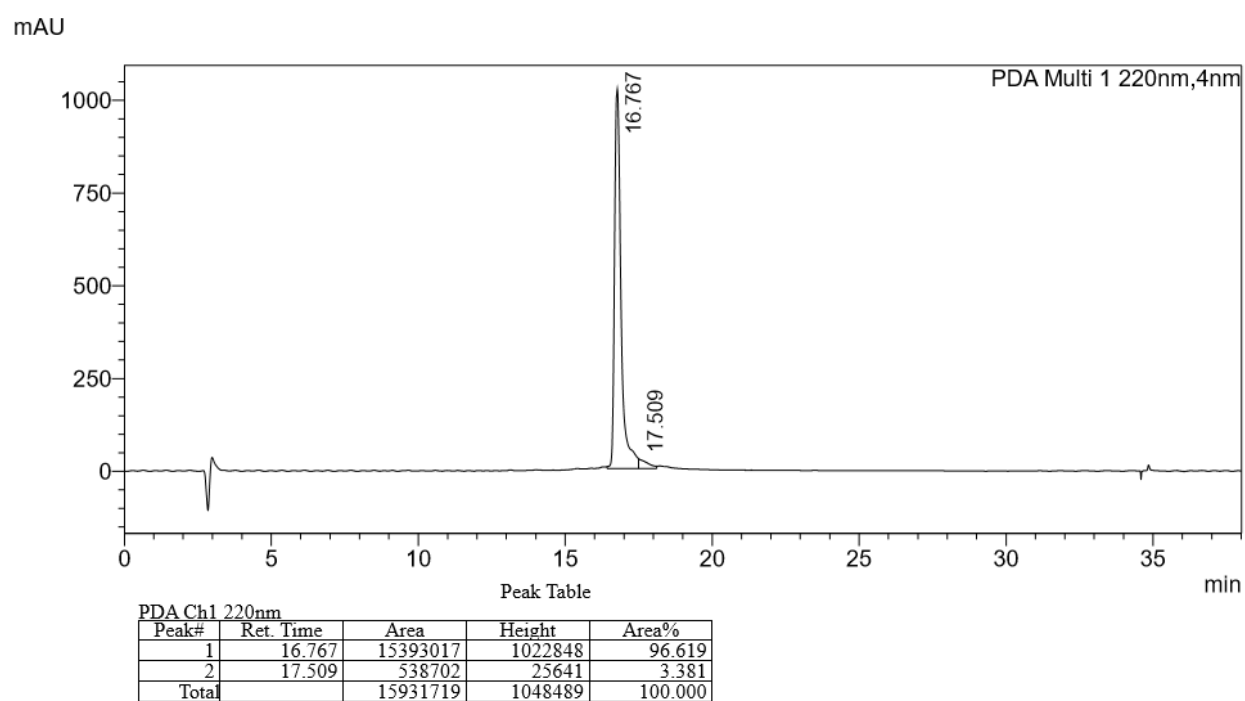
Time (min)	Buffer B (%)
0.01	5
2	5
10	20
25	65
27	95
32	95
32.01	5
38	5

**6A. HPLC Chromatograms of**

linear peptide ( $R_4W_4$ )



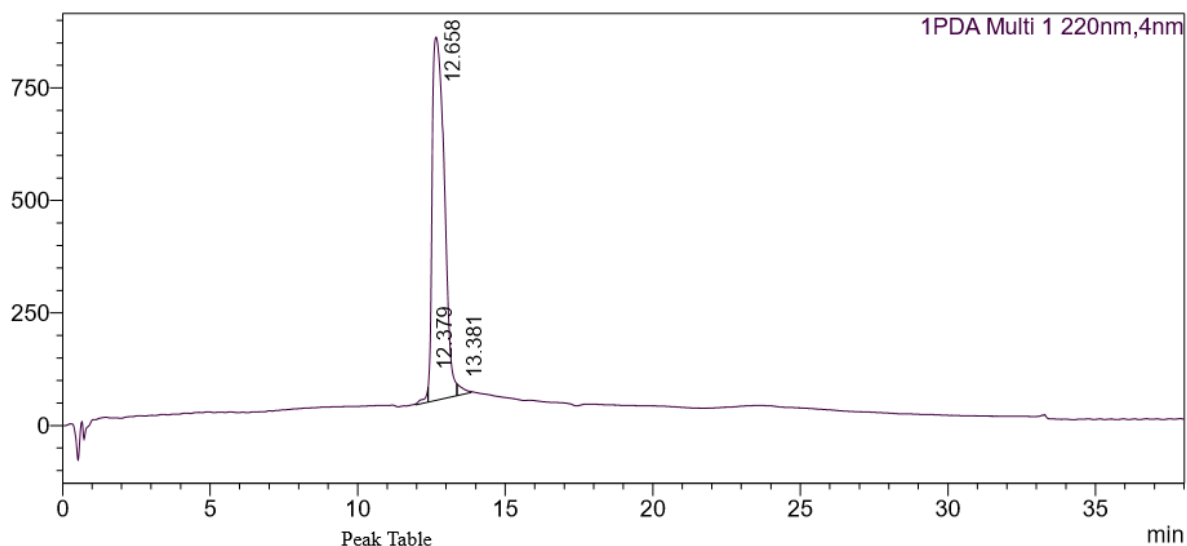
## 6B. HPLC Chromatograms of cyclic peptide [R<sub>4</sub>W<sub>4</sub>]



## 6C. HPLC Chromatograms of linear peptide levofloxacin conjugate (Levo-C<sub>10</sub>-KR<sub>3</sub>W<sub>4</sub>)



mAU



#### 6D. HPLC Chromatograms of cyclic peptide Levo conjugate [Levo-C<sub>10</sub>-KR<sub>3</sub>W<sub>4</sub>]

mAU

