Supporting information:

Enantioselective sulfoxidation reaction catalyzed by G-quadruplex DNA metalloenzyme

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Materials

All DNA oligodeoxynucleotides (purified by PAGE) and 3-(N-morpholino) propanesulfonic acid (MOPS) were purchased from Sangon (Shanghai, China). The concentration of DNA was determined in ddH_2O by the absorbance value at 260 nm at 20 ^oC. The extinction coefficient of each oligodeoxynucleotide was obtained from IDT website (http://www.idtdna.com/calc/analyzer). All sulfides, phenyl methyl sulfoxide, phenyl methyl sulfone, 1,10-phenanthroline (**L1**), 2,2'-bipyridine (**L3**), 4,4'-bimethyl-2,2'-bipyridine (**L4**) and 4,4'-bimethoxy-2,2'-bipyridine (**L5**) were purchased from J&K (Beijing, China). 5,6-dimethyl-1,10-phenantiroline (**L2**) was purchased from TCI. $Cu(NO_3)_2 \cdot 3H_2O$ (>99%), KCl (>99%) and NaCl (>99%) were purchased from the Shanghai Chemical Reagent Company of the Chinese Medicine Group. Copper complexes were prepared according to the previous procedure (Yinghao Li, Can Li, *et al., Tetrahedron*, 2013, **69**, 6585-6590). All reagents and solvents were used without further purification. Water was distilled and deionized using a Milli-Q A10 water purification system.

Typical oxidation procedure

Sulfides oxidation procedure: DNA (10 μM) was added to a 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (1 mL, 20 mM, pH 7.0) containing KCl (150 mM), heated for 3 min at 98 ^{o}C and annealed slowly over the course of 2 h to room temperature. Then, an aqueous solution of copper complex (50 μM) was added, stirring for 0.5 h at 15 ^{o}C. Then, sulfide dissolved in CH_3CN (10 μL of a 0.5 M solution) was added. The reaction was started with the addition of H_2O_2 (7.5 μL of 3 % aqueous solution). The reaction mixture was stirred for another 5 h at reaction temperature. Then diethyl ether (3mL×3) was added to extract the products and remained substrate. After drying the extracted mixture with anhydrous Na_2SO_4 and removal of the solvent under reduced pressure, the crude products were directly analyzed by a chiral HPLC.

Characterization Experiments

DNA samples (5 μM) dissolved in MOPS buffer (20 mM, pH 7.0) containing KCl (150 mM) or NaCl (150 mM) were heated for 3 min at 98 ^{o}C and slowly annealed over the course of 2 h to room temperature. After that, these aqueous samples were incubated at 4 ^{o}C overnight to ensure the equilibrium between folding and unfolding. CD measurements (220 to 360 nm) were done on a dual beam DSM 1000 CD spectrophotometer (Olis, Bogart, GA) at 20 ^{o}C. The CD spectra presented are the average of five consecutively measured scans. UV-Vis absorption (250 to 360 nm) experiments were carried out on Shimadzu 2450 spectrophotometer (Shimadzu, Japan) at room temperature. UV melting (20~95 ^{o}C, 0.5 ^{o}C/min) experiments were done on Shimadzu 2450 spectrophotometer equipped with a Peltier temperature control accessory. The concentration of Cu**L4** was 25 μM. All samples were dissolved in 20 mM MOPS buffer (pH 7.0). All spectra were recorded using a sealed quartz cell with a path length of 1.0 cm. High-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1100 Series instrument with the eluents of hexane and isopropanol (*i*-PrOH), using a Daicel Chiralcel ODH or ASH column.

Calculation the conversion of 1a~m sulfides

Conversion of **1a** is calculated using the following formula:

Conversion of **1a** (%) = $PA_{2a}/(PA_{2a} + PA_{1a}/f)$ *100,

Where PA_{1a} and PA_{2a} are the HPLC peak areas of **1a** and **2a**, respectively. ***f*** is the correction factor determined to be 0.45 from a fitting curve (Figure **S1**).

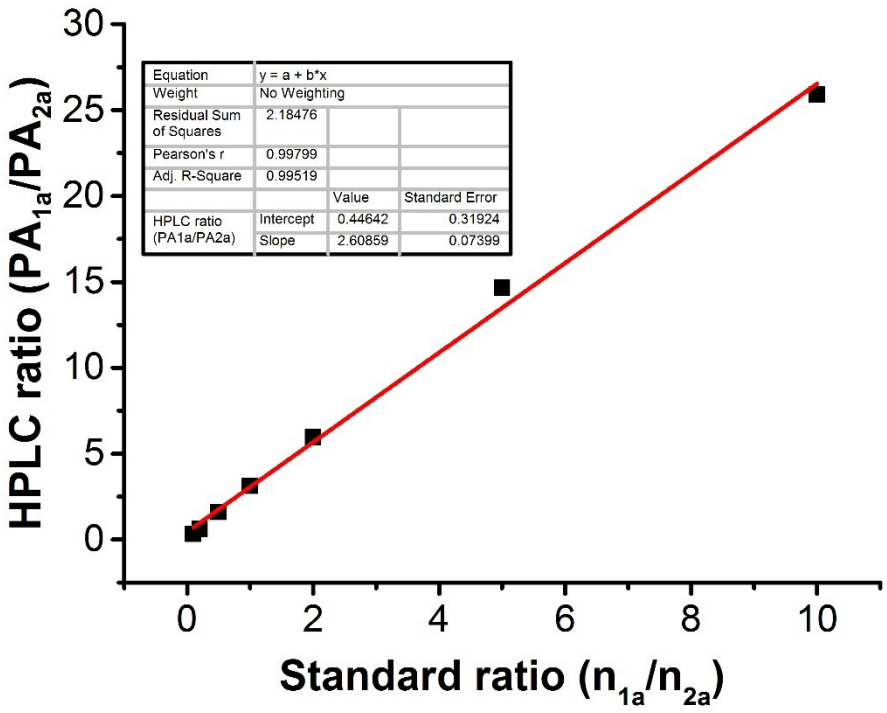


Fig. S1 Determination of the correction cofactor between **1a** and **2a**. The HPLC ratios of peak areas (PA_{2a}/PA_{1a}) were determined with the standard molar ratios (n_{2a}/n_{1a}) of 1/10, 1/5, 1/2, 1, 2, 5, 10.

Table S1 Correction factor and HPLC conditions of substrates (**1a**~**m**) and products (**2a**~**m**).

Entry	1	2	R_1	R_2	f [a]	HPLC Method [b]	Retention Time / min		
							1	2	
1	**1a**	**2a**	phenyl	methyl	0.45	Ox-1	3.9	18.3	25.3
2	**1b**	**2b**	2′-chlorophenyl	methyl	0.39	Ox -2	4.3	26.1	33.7
3	**1c**	**2c**	3′-chlorophenyl	methyl	0.42	Ox -2	4.0	33.9	53.2
4	**1d**	**2d**	3'-tolyl	methyl	0.37	Ox -2	3.8	32.7	41.6
5	**1e**	**2e**	4'-tolyl	methyl	0.50	Ox -1	3.8	18.0	29.2
6	**1f**	**2f**	4′-methoxyphenyl	methyl	1.14	Ox -1	6.2	32.3	51.7
7	**1g**	**2g**	2′-naphthyl	methyl	0.41	Ox -1	4.3	23.3	32.5
8	**1h**	**2h**	phenyl	ethyl	0.24	Ox -1	3.8	14.4	16.3
9	**1i**	**2i**	phenyl	benzyl	0.80	Ox -3	5.8	22.9	28.7
10	**1j**	**2j**	2′-pyridine	methyl	0.53	Ox -1	4.3	12.1	19.8

[a] ***f*** is the correction factor.

[b] HPLC methods:

Ox-1 (Daicel chiralpak-ASH, hexane/*i*-PrOH 70:30, 1.0 mL/min, 250 nm);

Ox-2 (Daicel chiralpak-ASH, hexane/*i*-PrOH 85:15, 1.0 mL/min, 250 nm);

Ox-3 (Daicel chiralcel-ODH, hexane/*i*-PrOH 95:5, 1.0 mL/min, 250 nm).

Fig. S2

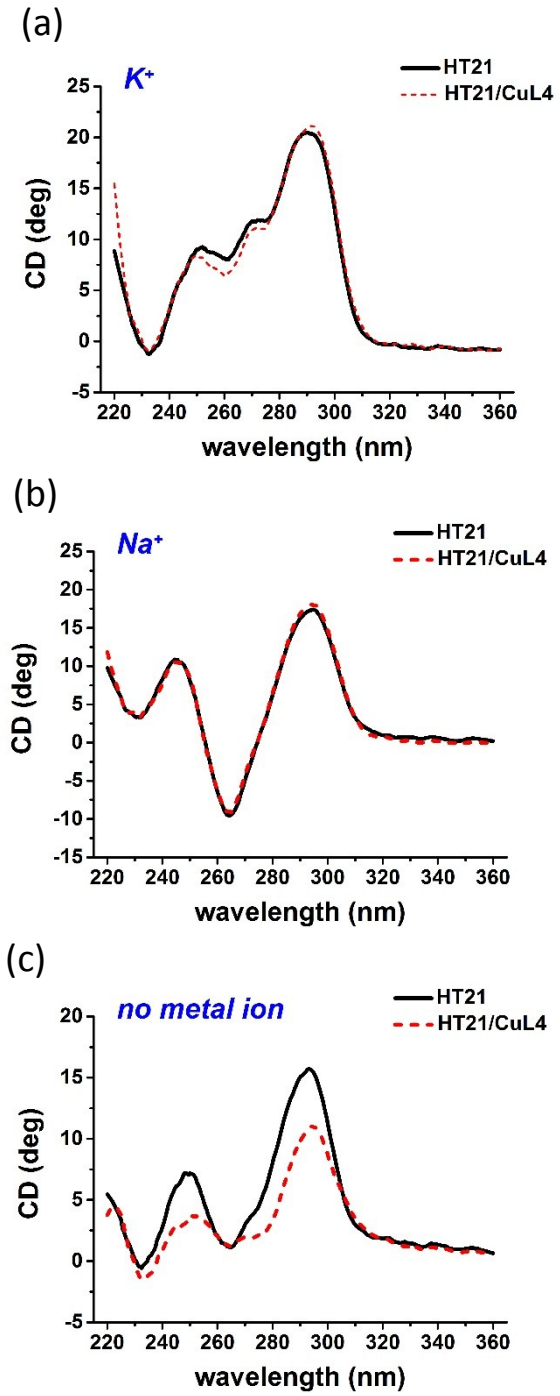


Fig. S2 CD spectra of HT21 alone and HT21•Cu**L4** in the presence of (a) K^+, (b) Na^+ ion and (c) in the absence of metal ion, respectively.

Fig. S3

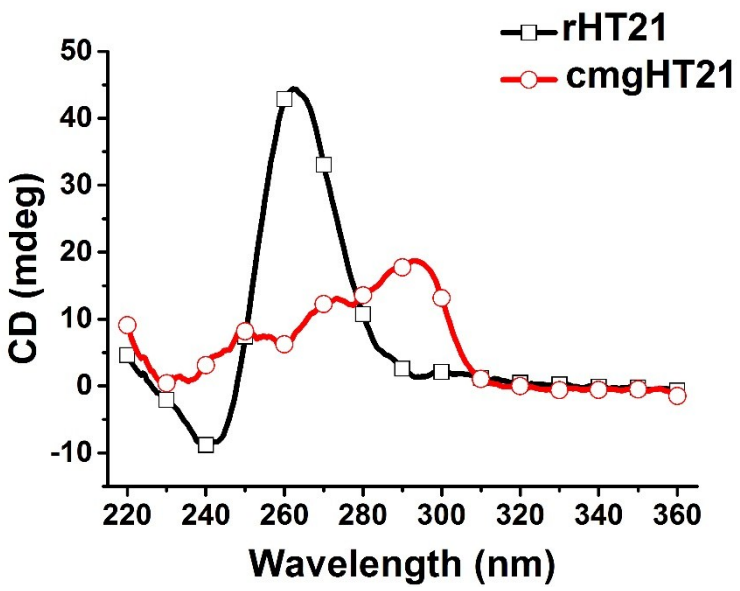


Fig. S3 CD spectra of rHT21 and cgmHT21 in K^+ (150 mM) solution.

Fig. S4

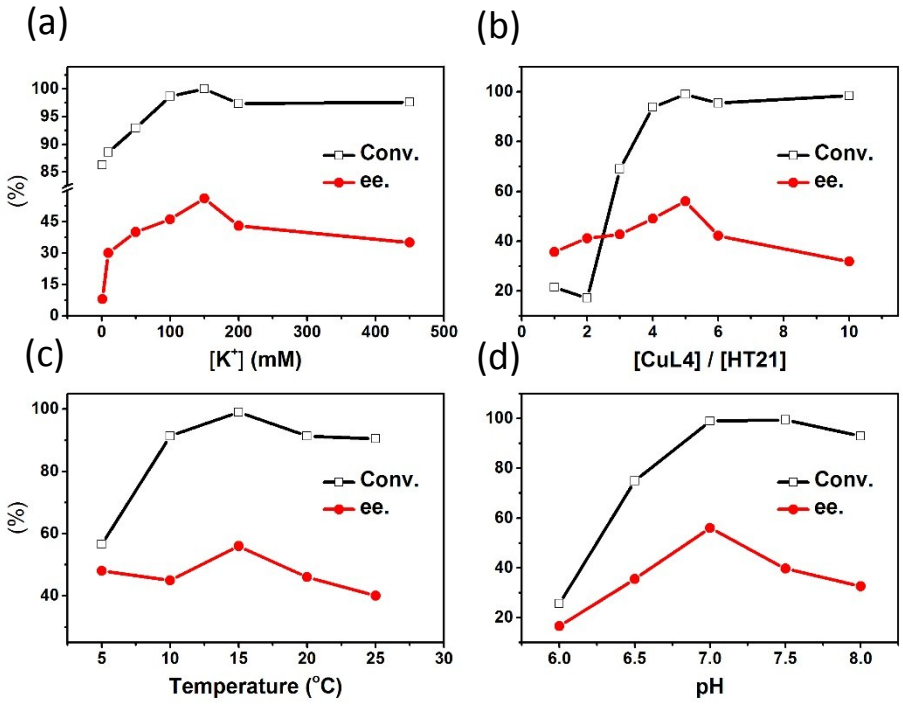


Fig. S4 Optimization of HT21•Cu**L4** catalyzing sulfoxidation reaction conditions on conversion (□) and enantioselectivity (●): (a) Concentration of potassium ion; (b) Molar ratio between Cu**L4** and HT21; (c) Reaction temperature and (d) pH.

Fig. S5

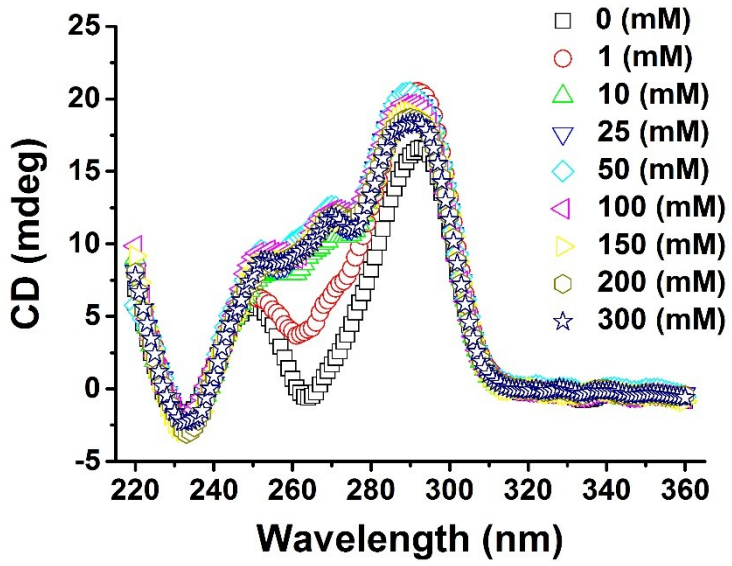


Fig. S5 CD spectra of HT21 in various concentrations of K^+.

Fig. S6

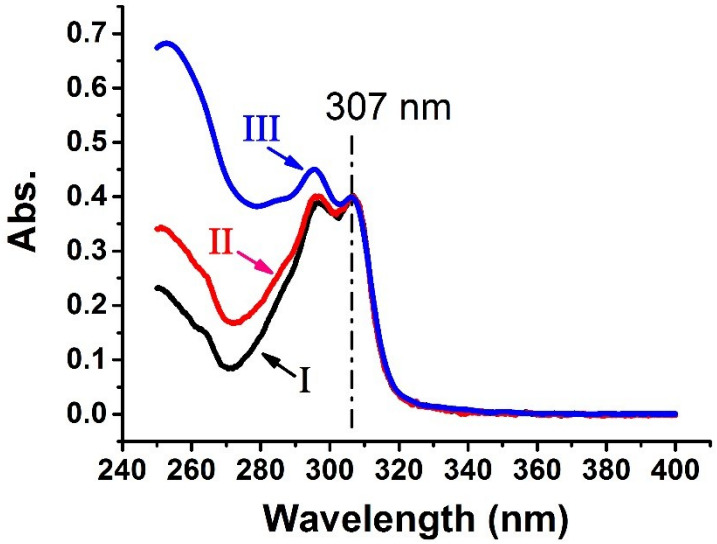


Fig. S6 UV/Vis absorption spectra of (I) Cu**L4** (25 μM) alone; (II) Cu**L4** (25 μM) with HT21 (0.5 μM) and (III) HT21 (2.5 μM) in MOPS buffer (20 mM, pH 7.0).

Fig. S7

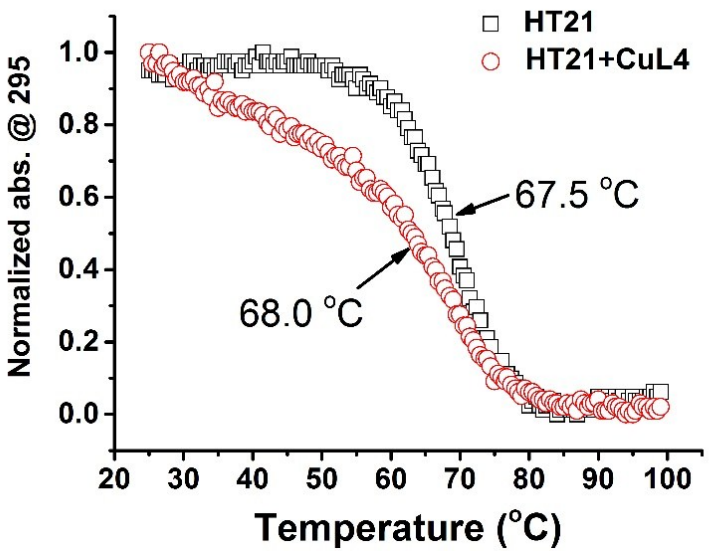


Fig. S7 UV-melting curves of HT21 with and without Cu**L4**.

Oxidative degradation of DNA

In order to figure out whether the degradation of DNA happened or not, CD spectroscopy and electrophoresis experiments were conducted. CD spectra of HT21 G-quadruplex/CuL complexes were obtained after adding 7.5 mM H_2O_2 (Fig. S8). The profile of CD spectra are not significantly changed: the characteristic positive bonds at 295 and 270 nm exist. And the little decrease of intensity at 295 and 270 nm may indicate the slight oxidative degradation of DNA. Thus, we can conclude that slight degradation of DNA happens and structure of G-quadruplex remains. In addition, the DNA integrity was investigated by using SDS-PAGE assay in which HT21/Cu**L4** samples were incubated with or without 7.5 mM H_2O_2 (Fig. S9). The slight degradation of DNA metalloenzyme can be observed by using loading samples with high concentration (20 μL of 200 μM DNA). Considering that the oxidation of thioanisole catalyzed by H21 G-quadruplex/Cu**L4** metalloenzyme can achieve 99 % conversion, this slight degradation of DNA may not influence the catalytic performance significantly.

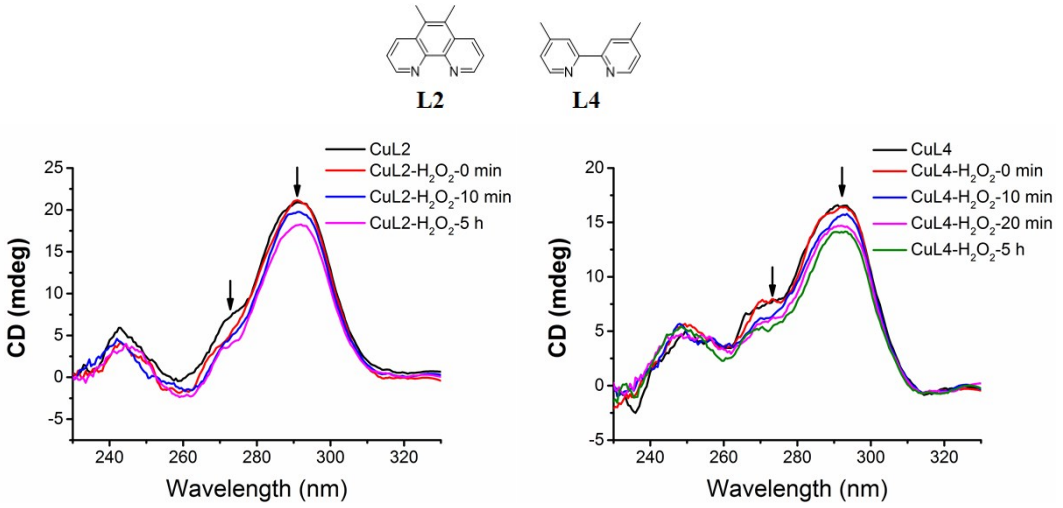


Fig. S8 CD spectra of HT21 G-quadruplex (5 μM)•Cu**L2** (left) or Cu**L4** (right) (25 μM) complexes without (black) and with (red) 7.5 mM H_2O_2. After the H_2O_2 was added, the spectra was recorded at 0, 10, 20 minutes and 5 hours.

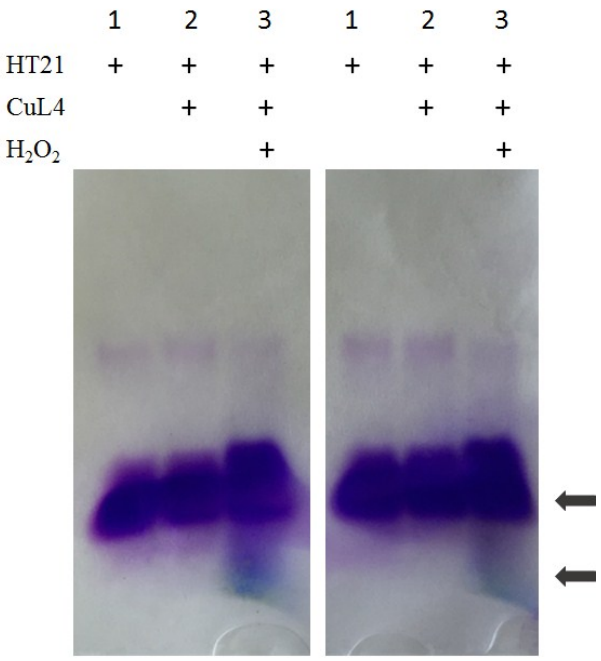
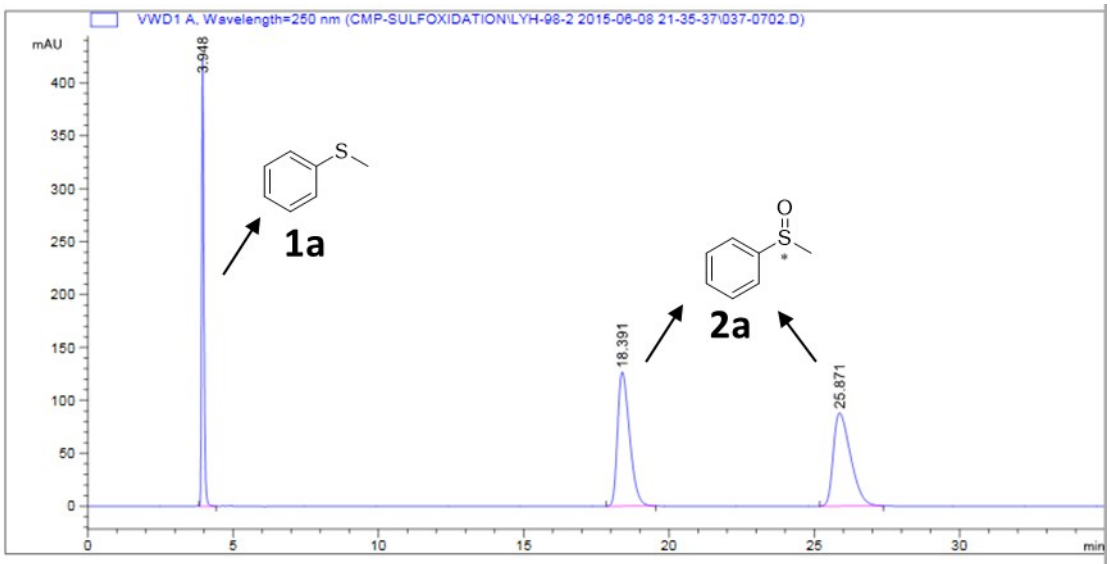


Fig. S9 Electrophoresis of HT21 G-quadruplex•CuL4 with and without treated by 7.5 mM H_2O_2.The experiments were conducted twice.

HPLC trace 1. Racemic **2a**:

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Use Multiplier & Dilution Factor with ISTDs


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   1   3.948 VB    0.0820 2292.92578  423.83615  23.9359
   2  18.391 BB    0.4447 3643.14478  126.69430  38.0309
   3  25.871 BB    0.6341 3643.36157   88.07066  38.0332

Totals :                  9579.43213  638.60111


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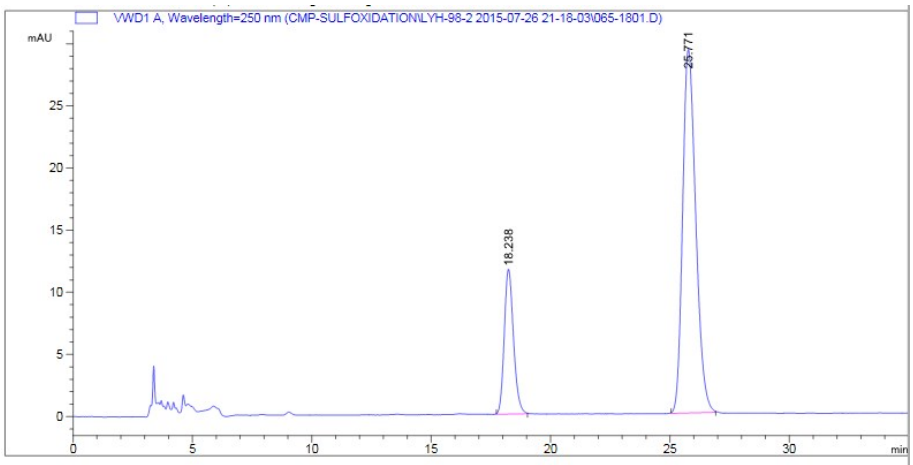
HPLC trace 2. Sulfoxidation of **1a** catalyzed by HT21 G-quadruplex•Cu**L4** catalyst in the presence of K^+.

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Peak RetTime Type  Width     Area      Height     Area
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   1  18.238 BB    0.4152  312.93854   11.65404  21.8742
   2  25.771 BB    0.5944 1117.68750   29.24189  78.1258

Totals :                  1430.62604   40.89593
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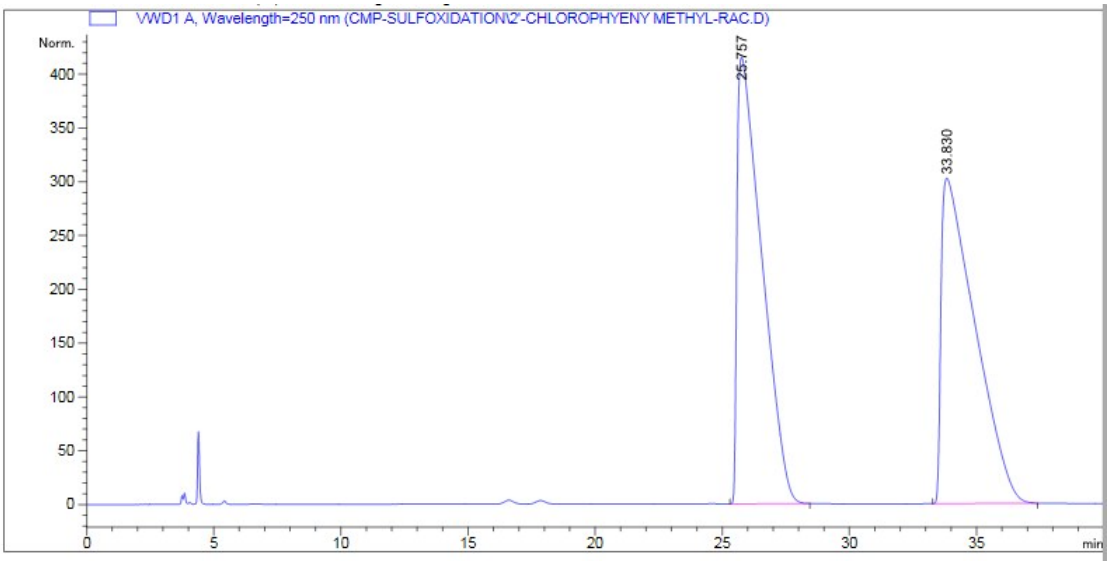
HPLC trace 3. Racemic 2b:

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Peak RetTime Type  Width     Area      Height     Area
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   1  25.757 BB    1.0136 2.79886e4   415.19519  50.0340
   2  33.830 BB    1.2758 2.79506e4   302.55017  49.9660

Totals :                  5.59392e4   717.74536
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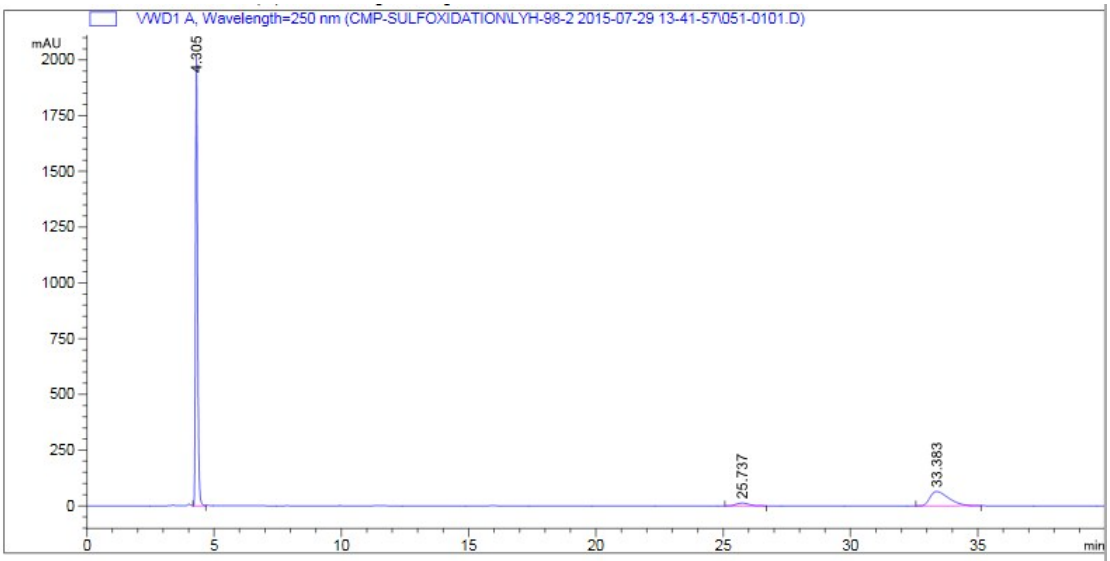
HPLC trace 4. Sulfoxidation of **1b** catalyzed by HT21 G-quadruplex•Cu**L4**.

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Peak RetTime Type  Width     Area      Height     Area
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   1   4.305 VV    0.0869 1.14615e4  2009.93298  75.3871
   2  25.737 BB    0.5478  438.83560   12.41540   2.8864
   3  33.383 BB    0.7910 3303.18237   64.03970  21.7265

Totals :                  1.52035e4  2086.38809
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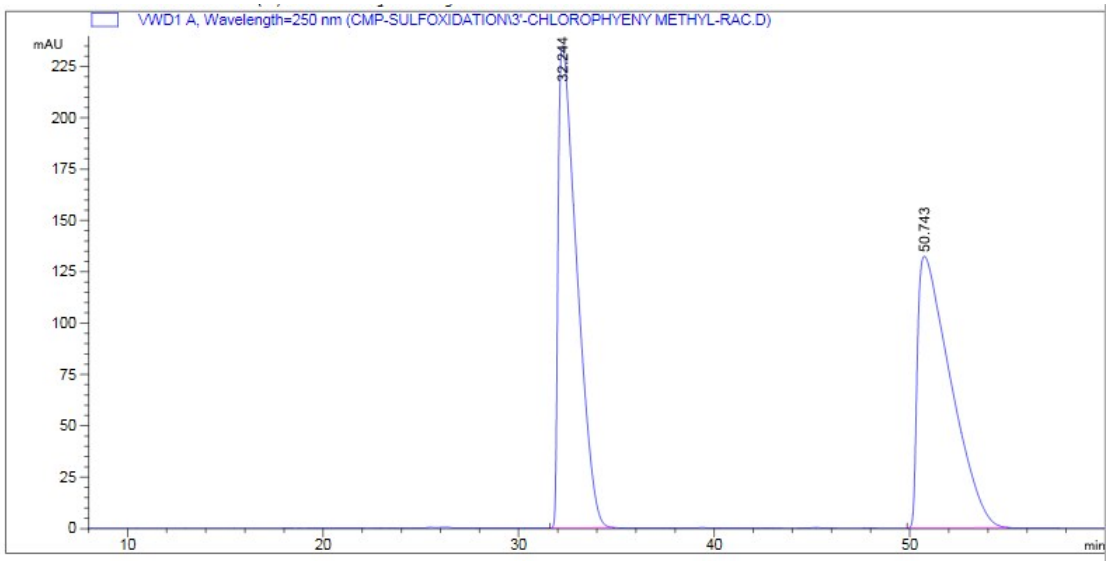
HPLC trace 5. Racemic **2c**:

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Peak RetTime Type  Width     Area      Height     Area
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   1  32.244 BB    0.9627 1.55714e4   234.87790  49.9839
   2  50.743 BB    1.6557 1.55814e4   132.37233  50.0161

Totals :                  3.11527e4   367.25023
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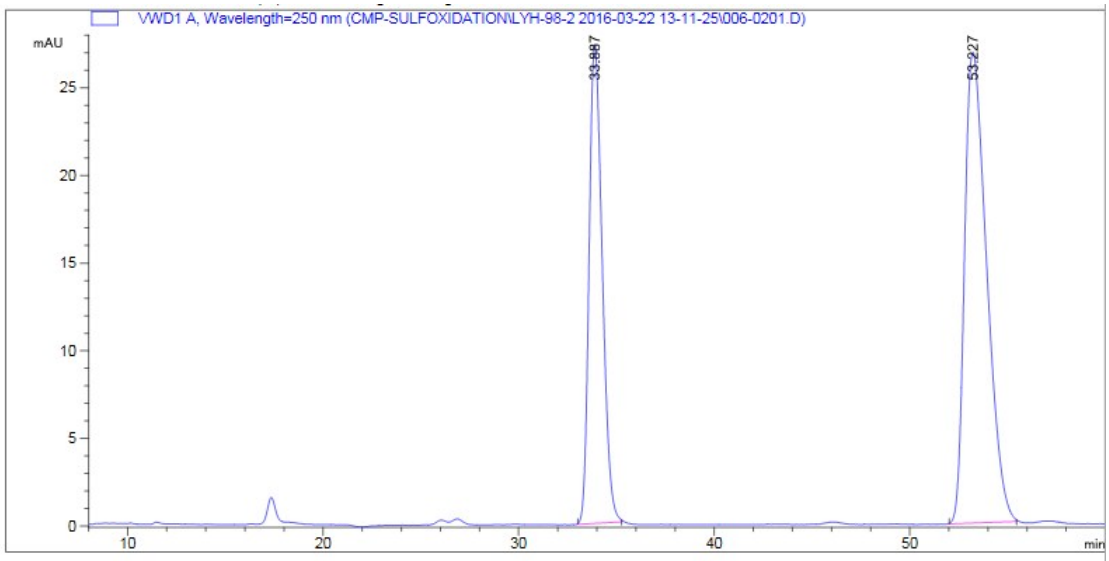
HPLC trace 6. Sulfoxidation of **1c** catalyzed by HT21 G-quadruplex•Cu**L4**.

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   1   4.042 VV    0.0767 8510.94238 1676.26953  71.5953
   2  33.887 BB    0.7157 1273.97180   27.32649  10.7168
   3  53.227 BB    1.1363 2102.66089   26.85168  17.6879

Totals :                  1.18876e4  1730.44770
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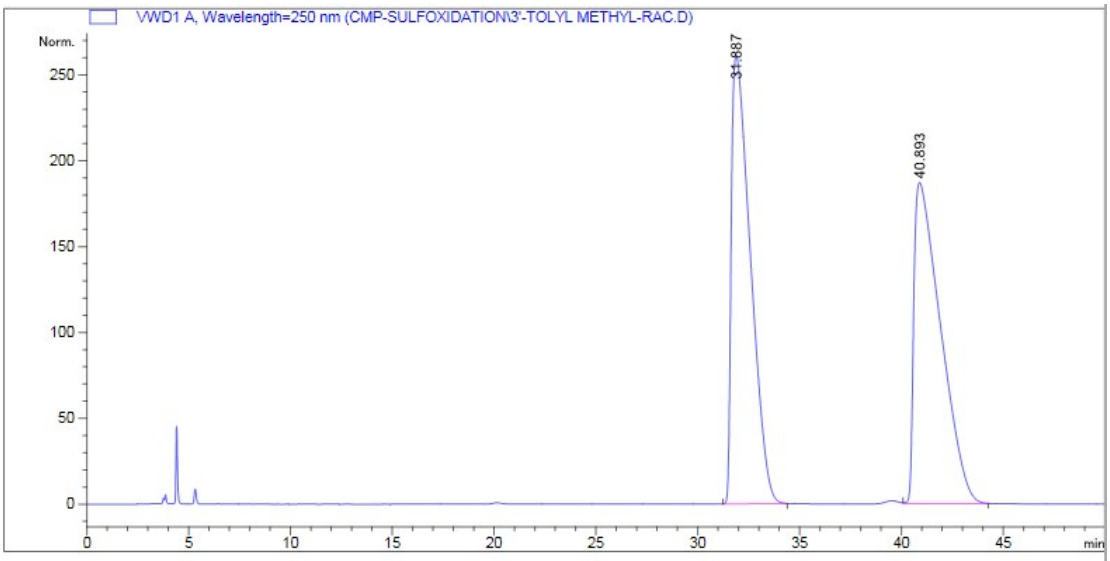
HPLC trace 7. Racemic **2d**:

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   1  31.887 BB    0.9943 1.69971e4   260.95648  49.9238
   2  40.893 VB    1.3207 1.70490e4   187.11017  50.0762

Totals :                  3.40461e4   448.06665
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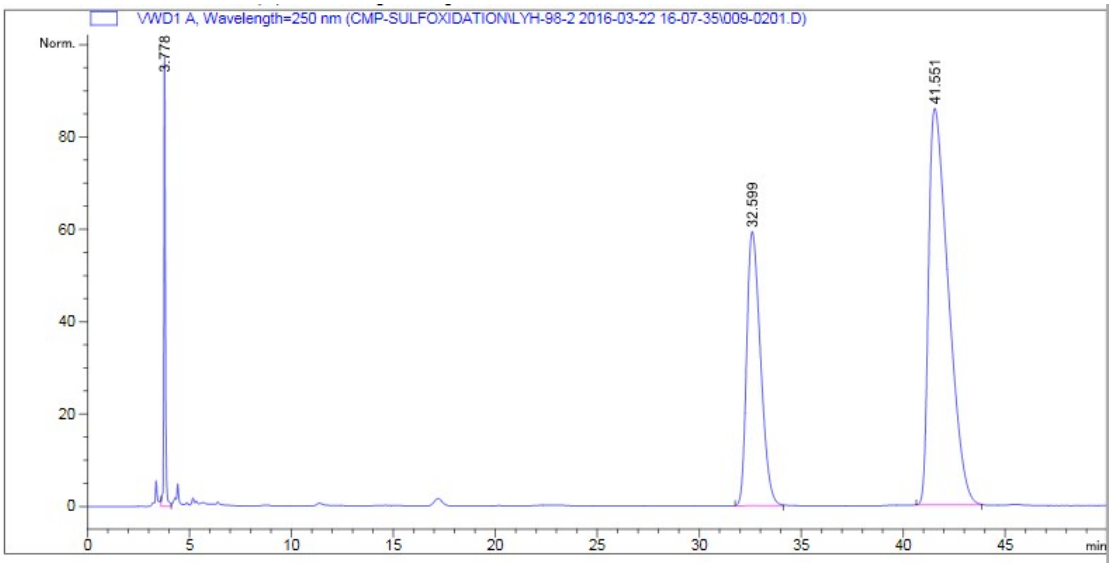
HPLC trace 8. Sulfoxidation of **1d** catalyzed by HT21 G-quadruplex•Cu**L4**.

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----|-------|----|-------|----------|----------|--------|
   1   3.778 VV    0.0771  497.88788   97.46080   5.5000
   2  32.599 BB    0.7328 2787.21997   59.35525  30.7897
   3  41.551 BB    0.9958 5767.32471   85.90492  63.7102

Totals :                  9052.43256  242.72097
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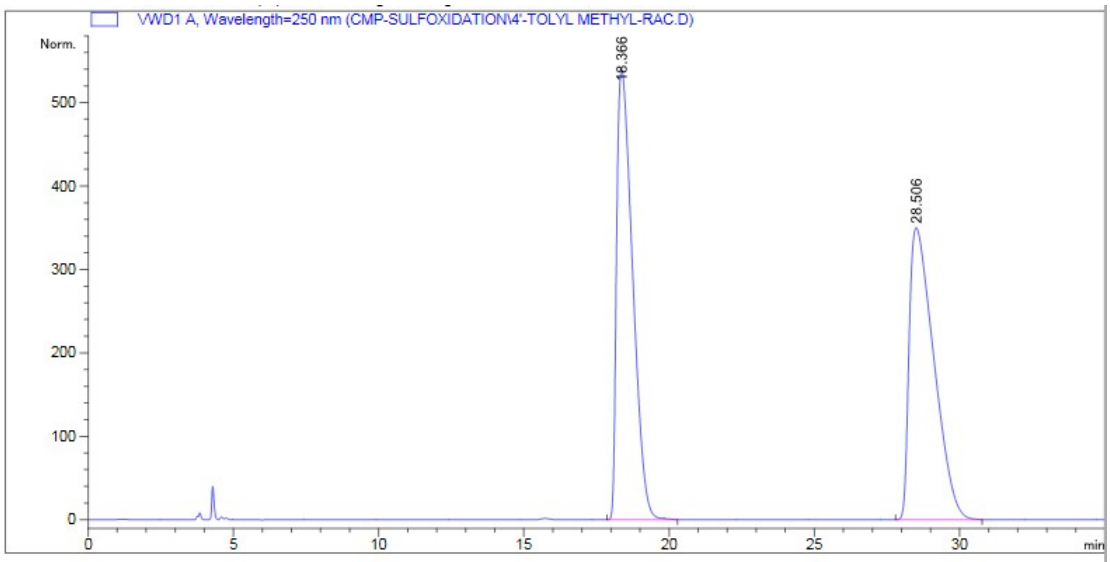
HPLC trace 9. Racemic **2e**:

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----|-------|----|-------|----------|----------|--------|
   1  18.366 BB    0.6050 2.05652e4   539.36499  49.9387
   2  28.506 BB    0.9276 2.06156e4   349.91254  50.0613

Totals :                  4.11808e4   889.27753
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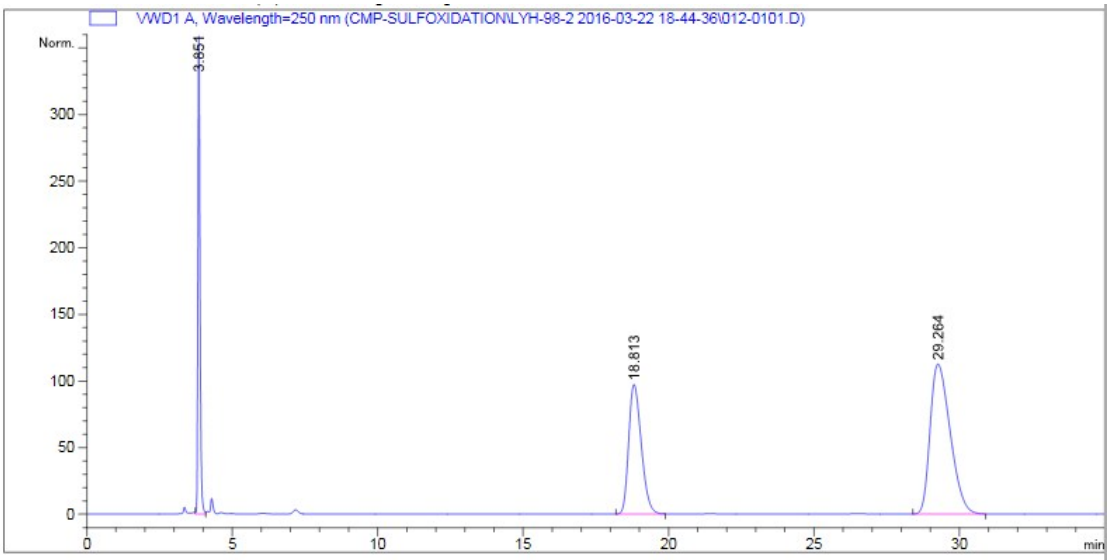
HPLC trace 10. Sulfoxidation of **1e** catalyzed by HT21 G-quadruplex•Cu**L4**.

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Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
   1   3.851 VV    0.0757 1792.47034  359.35315  17.7064
   2  18.813 BB    0.4695 2929.77100   97.23229  28.9410
   3  29.264 BB    0.7519 5401.02197  112.58060  53.3526
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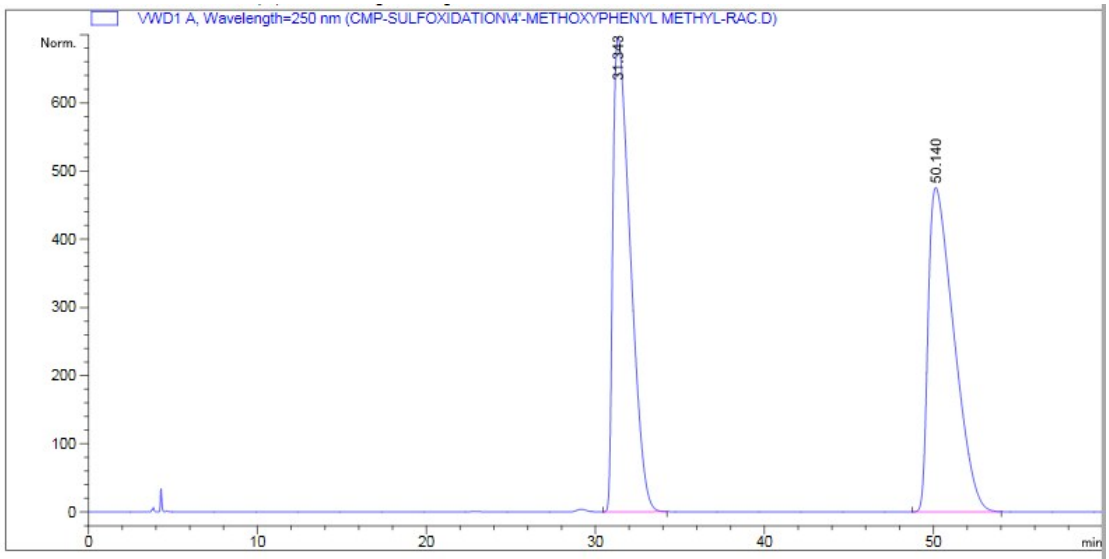
HPLC trace 11. Racemic **2f**:

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Peak RetTime Type  Width     Area      Height     Area
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----|-------|----|-------|----------|----------|--------|
   1  31.343 BB    1.1183 4.97724e4   695.91046  49.8359
   2  50.140 BB    1.6082 5.01003e4   475.91589  50.1641

Totals :                  9.98727e4  1171.82635
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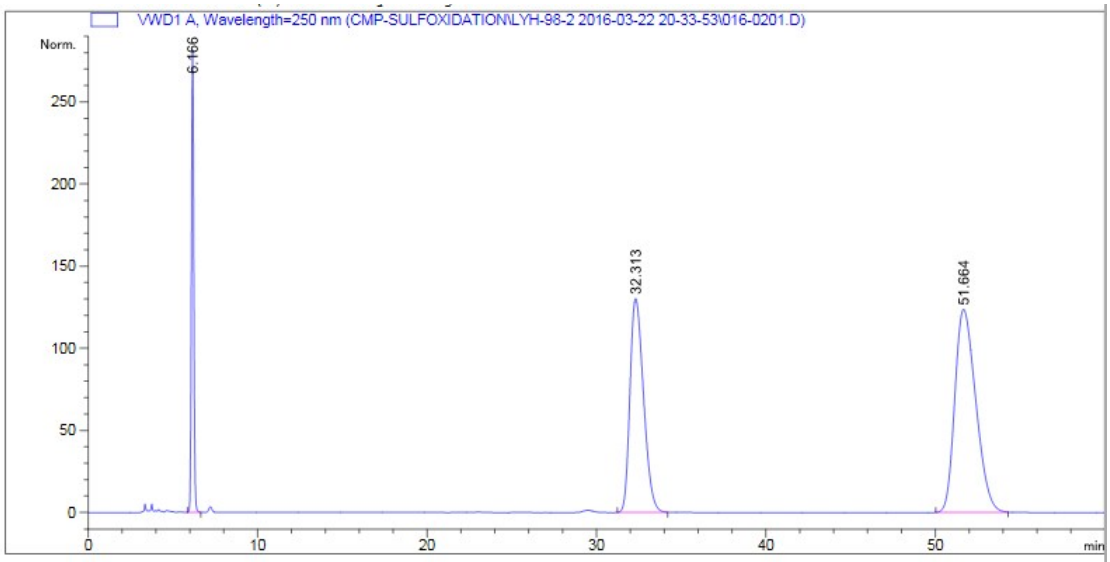
HPLC trace 12. Sulfoxidation of **1f** catalyzed by HT21 G-quadruplex•Cu**L4**.

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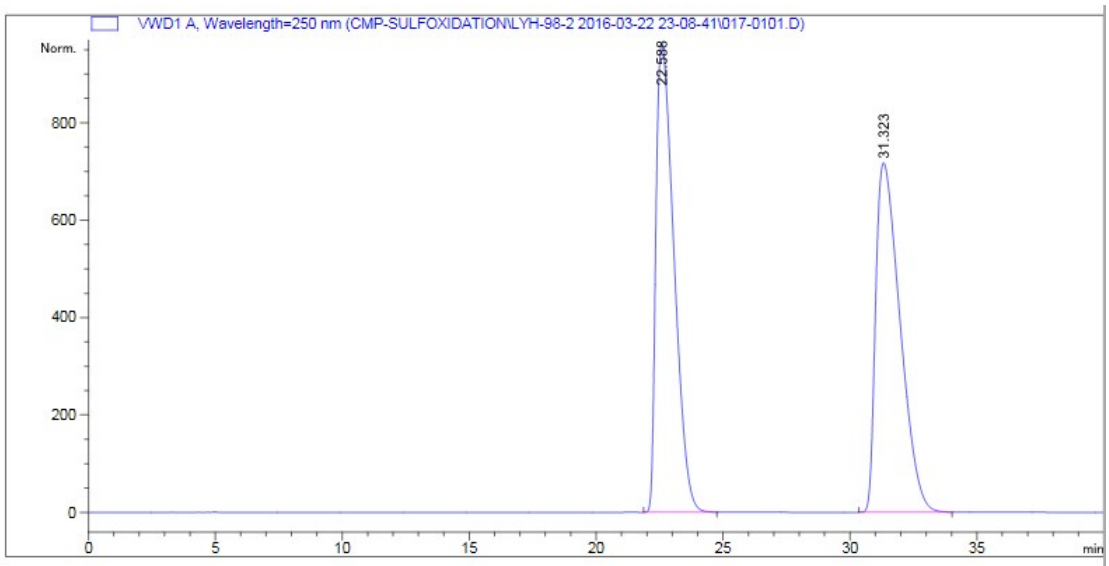
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   6.166 BB    0.1507 2725.87549  281.75613  13.2290
   2  32.313 BB    0.8692 7245.59766  130.19032  35.1638
   3  51.664 BB    1.3338 1.06338e4   123.64821  51.6071

Totals :                  2.06053e4   535.59467
```

HPLC trace 13. Racemic **2g**:




```
=====================================================================
                      Area Percent Report
=====================================================================

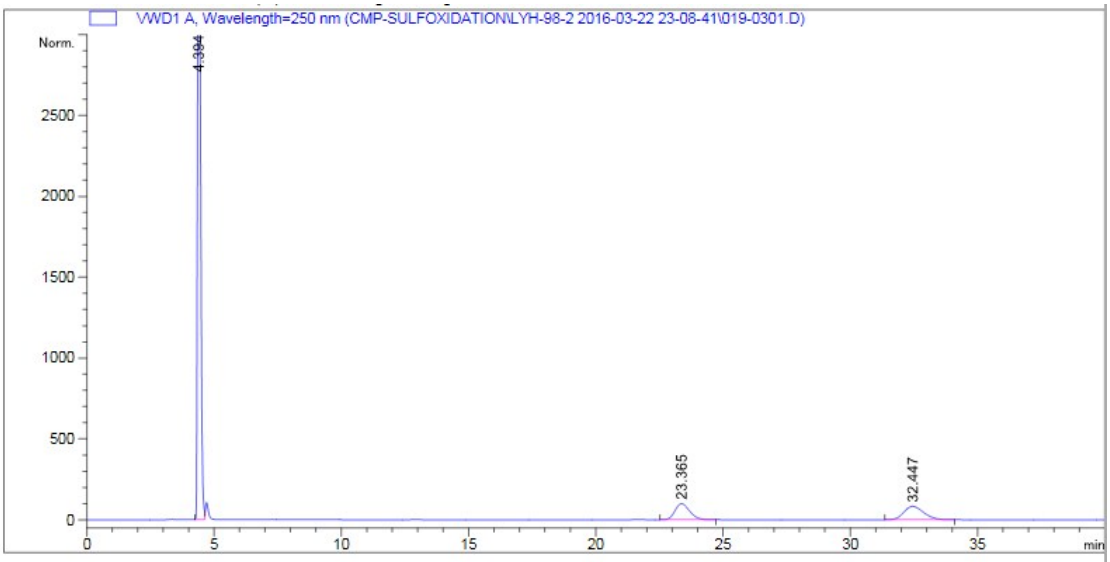
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  22.588 BB    0.7794 4.75284e4   963.36499  49.9035
   2  31.323 BB    1.0363 4.77122e4   717.59125  50.0965

Totals :                  9.52406e4  1680.95624
```

HPLC trace 14. Sulfoxidation of **1g** catalyzed by HT21 G-quadruplex•Cu**L4**.



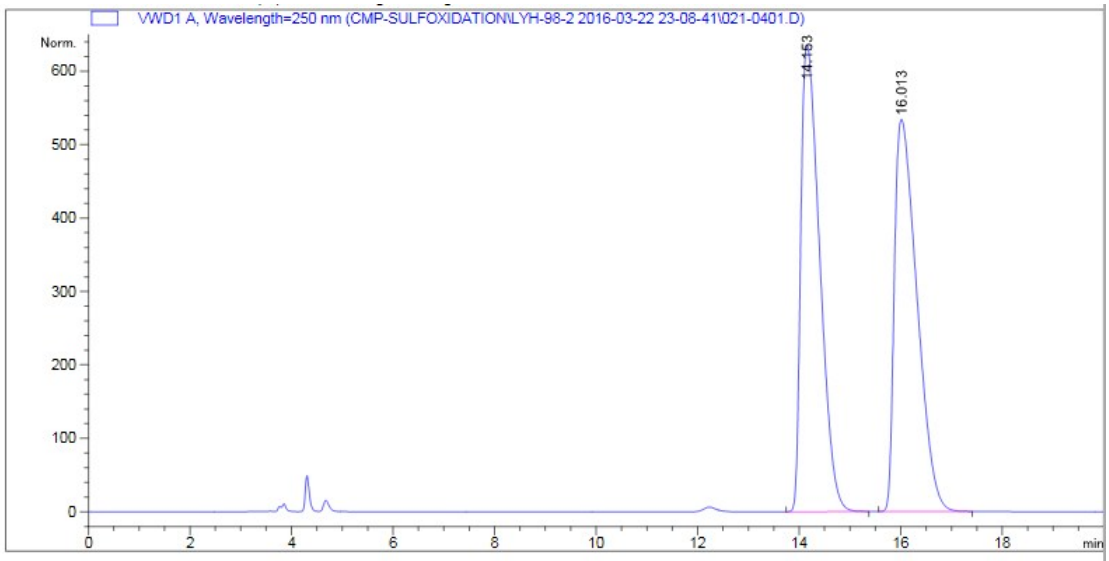

```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   4.394 VV    0.1561 3.07979e4  3197.16626  78.4766
   2  23.365 BB    0.6252 3969.24805   98.95496  10.1141
   3  32.447 BB    0.8467 4477.52441   82.18416  11.4093
```

HPLC trace 15. Racemic **2h**:




```
======================================================================
                      Area Percent Report
======================================================================

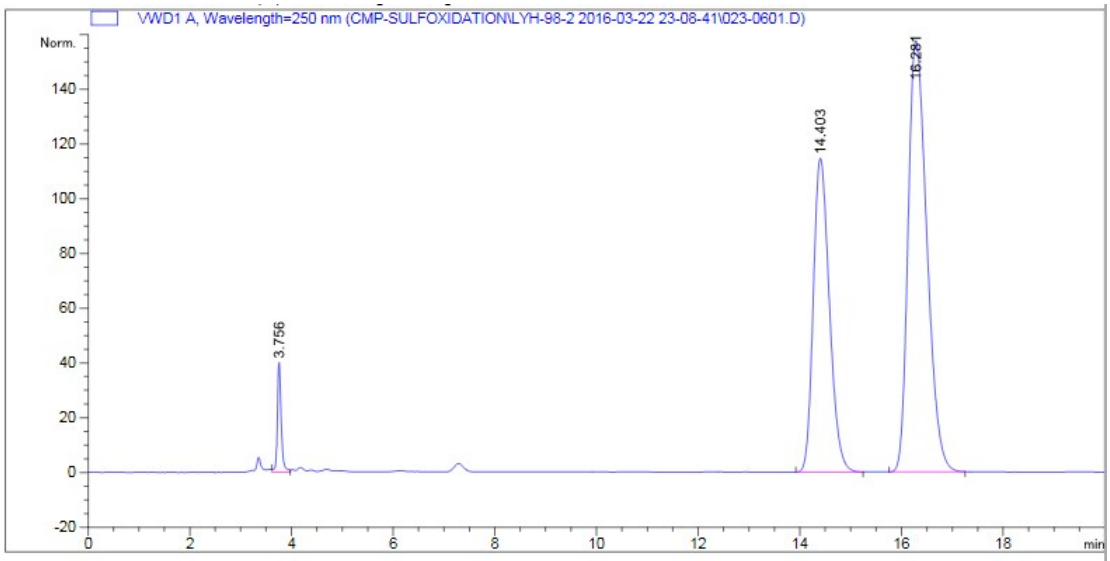
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  14.153 BB    0.4076 1.64171e4   635.64294  49.9721
   2  16.013 BB    0.4827 1.64354e4   534.20465  50.0279

Totals :                  3.28525e4  1169.84760
```

HPLC trace 16. Sulfoxidation of **1h** catalyzed by HT21 G-quadruplex•Cu**L4**.



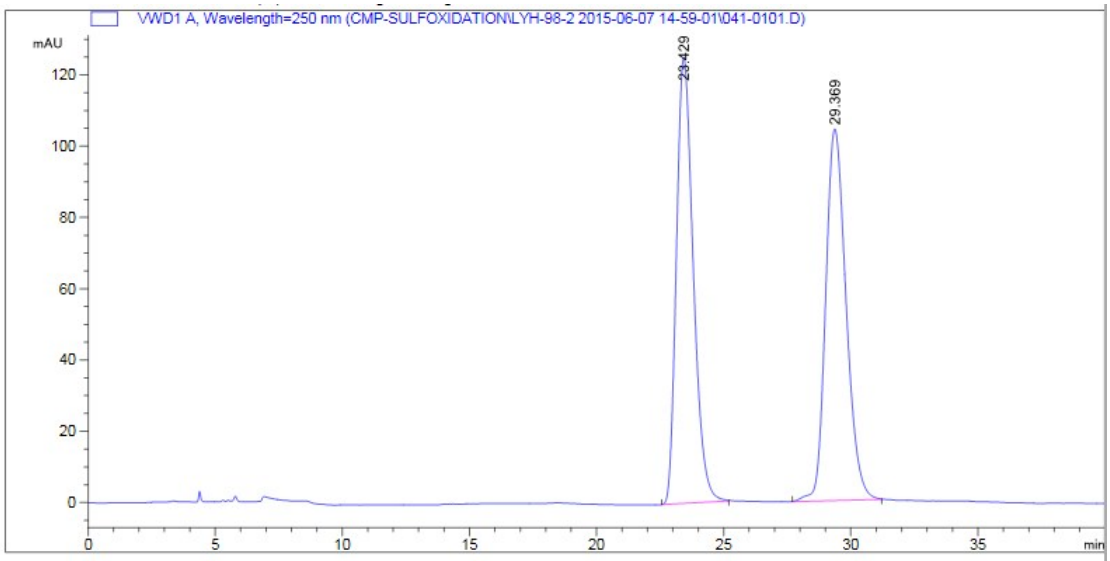

```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s    [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   3.756 VV    0.0790  211.40416   40.09943   3.1176
   2  14.403 BB    0.3427 2525.41333  114.73286  37.2427
   3  16.281 BB    0.3999 4044.14185  157.54330  59.6397
```

HPLC trace 17. Racemic **2i**:




```
=====================================================================
                      Area Percent Report
=====================================================================

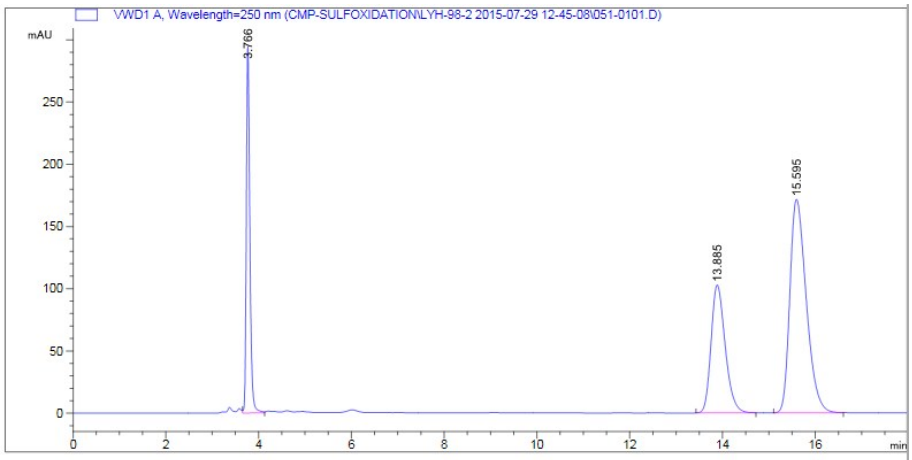
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  23.429 BB    0.7065 5748.58496  125.08639  49.6998
   2  29.369 BB    0.8694 5818.03906  104.27059  50.3002

Totals :                 1.15666e4   229.35698
```

HPLC trace 18. Sulfoxidation of **1i** catalyzed by HT21 G-quadruplex•Cu**L4**.




```
=====================================================================
                        Area Percent Report
=====================================================================

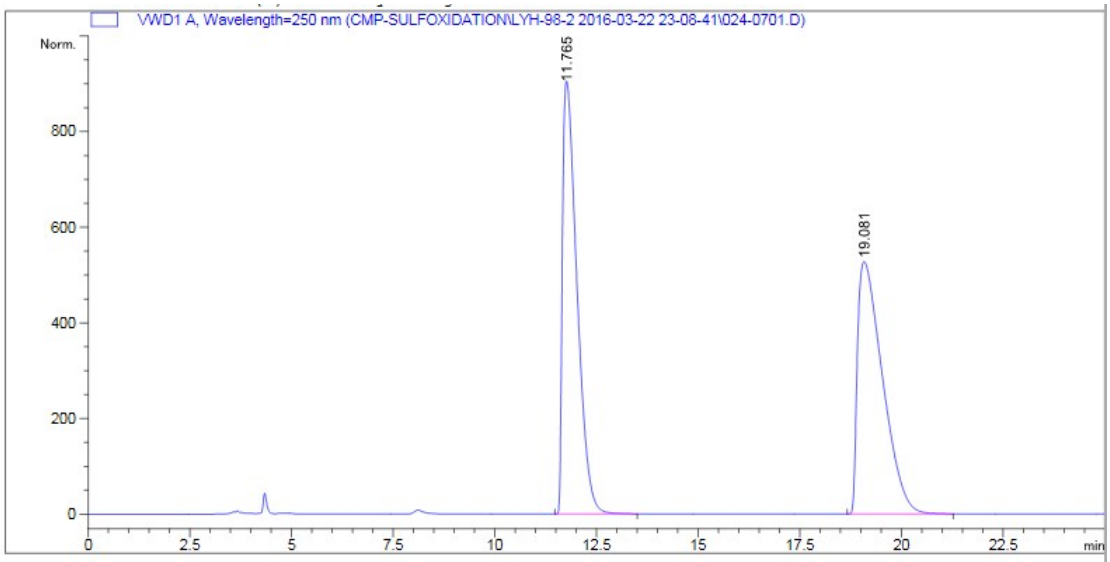
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   3.766 VV    0.0804 1551.92444  294.44034  19.5504
   2  13.885 BB    0.3255 2174.57837  102.77210  27.3944
   3  15.595 BB    0.3795 4211.54736  171.59660  53.0552

Totals :                  7938.05017  568.80904
```

HPLC trace 19. Racemic **2j**:




```
=====================================================================
                      Area Percent Report
=====================================================================

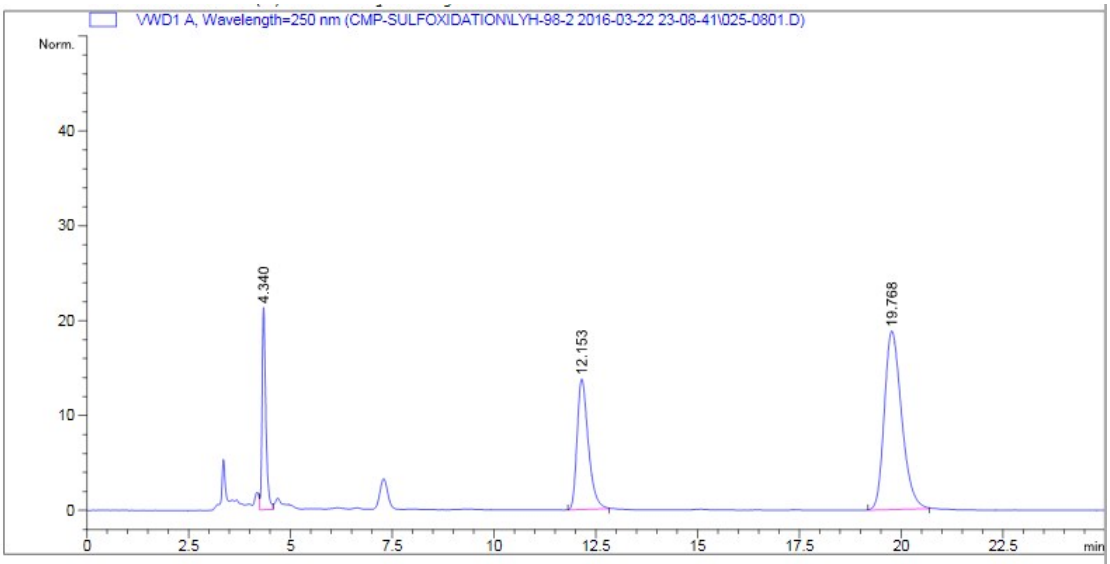
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1  11.765 BB    0.3787 2.23912e4   905.72357  49.7977
   2  19.081 BB    0.6591 2.25730e4   527.80420  50.2023

Totals :                  4.49642e4  1433.52777
```

HPLC trace 20. Sulfoxidation of **1j** catalyzed by HT21 G-quadruplex•Cu**L4**.




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=250 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]       %
----|-------|----|-------|----------|----------|--------|
   1   4.340 VB    0.0933  135.74539   21.30796  14.2147
   2  12.153 BB    0.2920  263.40250   13.74804  27.5825
   3  19.768 BB    0.4525  555.81525   18.80664  58.2028
```