Supplementary Material

Structural and molecular insight into piperazine and piperidine derivatives as histamine H_3 and sigma-1 receptor antagonists with promising antinociceptive properties

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[+] These authors contributed equally to this work.

Contents

1. Purity confirmation data

Mass spectra (LC/MS) were performed on Waters TQ Detector mass spectrometer. High-resolution mass spectra (HRMS) were performed on UltrafleXtreme maldi-tof/tof mass spectrometer. HPLC analyses were performed on Waters Alliance 2695 Separations Module with Waters 2998 Photodiode Array Detector (Chromolith® SpeedROD RP-18 endcapped 50-4.6 HPLC column was used)[1–4]. Elemental analyses (C, H, N) were performed on an Elemental AnalyserVarioEl III (Hanau, Germany) and agreed with theoretical values within ± 0.4%[1,2].

Cpd	LC/MS		HRMS		RP-HPLC			References
	Purity [%]	Retention time [min]	ESI *m/z* $[M+H]^+$	MALDI *m/z* $[M+H]^+$	Purity [%]	Retention time [min]	*k*	
1	100	3.23	326	326.217	99.21	0.923	1.64	3
2	100	4.02	355	-	99.30	1.082	2.09	1
3	100	1.87	341	-	100	0.723	1.07	2
4	100	3.04	366	366.203	100	0.756	1.16	4
5	100	3.28	365	365.209	100	0.788	1.25	4
6	100	3.89	374	374.224	99.64	1.111	2.17	3
7	100	3.67	399	399.234	99.40	0.971	1.77	4
8	97.83	3.18	402	402.212	95.42	0.971	1.77	3
9	97.28	4.07	436	436.183	95.56	1.040	1.97	4
10	95.61	3.61	420	420.195	95.76	0.928	1.65	4
11	100	5.24	296	296.209	100	1.280	2.66	-
12	100	4.47	324	324.201	100	1.140	2.26	-
13	100	4.18	369	-	100	1.168	2.34	1
14	98.46	4.66	383	-	98.06	1.252	2.58	1
15	100	2.26	355	-	-	-	-	2
16	93.73	2.90	382	382.253	92.19	0.942	1.69	3
17	100	3.43	396	396.266	100	1.029	1.94	3
18	100	5.26	396	396.303	100	1.334	2.81	3
19	94.79	3.69	410	410.284	94.47	1.124	2.21	3
20	100	4.03	424	424.296	100	1.202	2.43	3

Cpd 2: Anal. Calcd for $C_{22}H_{31}N_3O$ x $C_2H_2O_4$: C, 64.99, N, 9.47, H, 7.50%. Found: C, 64.96, N, 9.52, H, 7.48.

Cpd 13: Anal. Calcd for $C_{23}H_{33}N_3O$ x $C_2H_2O_4$: C, 65.62, N, 9.18, H, 7.71%. Found: C, 65.60, N, 9.19, H, 7.72.

Cpd 14: Anal. Calcd for $C_{24}H_{35}N_3O$ x $C_2H_2O_4$: C, 66.22, N, 8.91, H, 7.91%. Found: C, 66.19, N, 8.89, H, 7.91.

Cpd 15: Anal. Calcd for $C_{21}H_{27}N_3O_2$ x $C_2H_2O_4$: C, 62.29, N, 9.47, H, 6.59%. Found: C, 62.32, N, 9.49, H, 6.63.

2. Chemistry

Compounds **11** and **12** were obtained by *N*-alkylation of piperidine with proper bromides by the method described by Kuder *et al.*[5] Both compounds have been previously described in the literature[6,7].

*(Biphenyl-4-yloxy)propyl)piperidine hydrogen oxalate (**11**)*
4-(3-Bromopropoxy)-1,1'-biphenyl (CAS113795-28-1) (1.46 g; 5 mmol), piperidine (0.21 g; 2.5 mmol) was refluxed for 24 h in the mixture of ethanol-water (5:1) with the powdered potassium carbonate (0.52 g; 3.8 mmol) and catalytic amount of potassium iodide. After purification oily product was converted into hydrogen oxalate. White solid, yield (28%), m.p. 213-215 ºC, $C_{22}H_{27}NO_5$ (MW=385.46). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.54 - 7.59 (m, 4H), 7.36 - 7.42 (m, 2H), 7.27 (tt, *J*=7.30, 1.15 Hz, 1H), 6.96 - 7.01 (m, 2H), 4.05 (t, *J*=6.01 Hz, 2H), 3.02 - 3.16 (m, 4H), 2.46 - 2.47 (m, 2H), 2.04 - 2.14 (m, 2H), 1.68 (br. s., 4H), 1.49 (br. s., 2H). ^{13}C NMR (DMSO-d_6): 164.85, 158.43, 140.27, 133.34, 129.42, 128.32, 126.71, 115.48, 65.62, 54.09, 52.82, 24.22, 23.36, 22.10. LC-MS: purity 100% t_R= 5.24, (ESI) *m/z* $[M+H]^+$ 296.20.

*Phenyl (4-(3-(piperidin-1-yl)propoxy)phenyl)phenyl)methanone hydrogen oxalate (**12**)*
4-(3-Bromopropoxy)phenyl)(phenyl)methanone (CAS108357-63-7) (0.998 g; 3.85 mmol), piperidine (0.328g; 3.85 mmol) was refluxed for 24 h in the mixture of ethanol-water (5:1) with the powdered potassium carbonate (0.69 g; 5 mmol) and catalytic amount of potassium iodide. After purification oily product was converted into hydrogen oxalate. White solid, yield (45%), m.p. 129-132 ºC, $C_{23}H_{27}NO_6$ (MW=413.47). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.69 - 7.73 (m, 2H), 7.60 - 7.66 (m, 3H), 7.49 - 7.54 (m, 2H), 7.03 - 7.08 (m, 2H), 4.11 (t, *J*=6.16 Hz, 2H), 2.95 - 3.19 (m, 6H), 2.08 - 2.17 (m, 2H), 1.65 - 1.74 (m, 4H), 1.49 (br. s., 2H). ^{13}C NMR (DMSO-d_6): 194.97, 165.21, 162.52, 138.25, 132.73, 129.79, 129.01, 114.90, 66.01, 53.85, 52.67, 23.94, 23.18, 22.06. LC-MS: purity 100% t_R= 4.47, (ESI) *m/z* $[M+H]^+$ 324.18.

None of the final structures has the Pan-assay interference compounds properties[8].

3. Calibration of parameters for the Smina scoring function

Table S1. Well-known σ_1 and σ_2 receptor ligands used to test the new calibrated Smina scoring function, and their experimental and calculated binding constant values.

Compound	Exp. σ_1 K_i (nM)	Calcd K_i σ_1 (nM)	Exp. σ_2 K_i (nM)	Calcd K_i σ_2 (nM)
Ifenprodil	1.02	2.93	18.8	26.62
PRE-084	2.2	3.68	13091	14691.11
Haloperidol	2.6	3.24	77	89.57
Pentazocine	4.3	6.30	1465	1285.65
BD1063	13.7	16.70	204	231.24
S1RA	17	24.00	9300	9795.90
DTG	124	102.65	18	22.23
F-ISO	330	357.87	6.95	8.35
RHM-4	2150	2071.76	0.26	1.61
Pitolisant	0.5	1.09	6.5	8.14

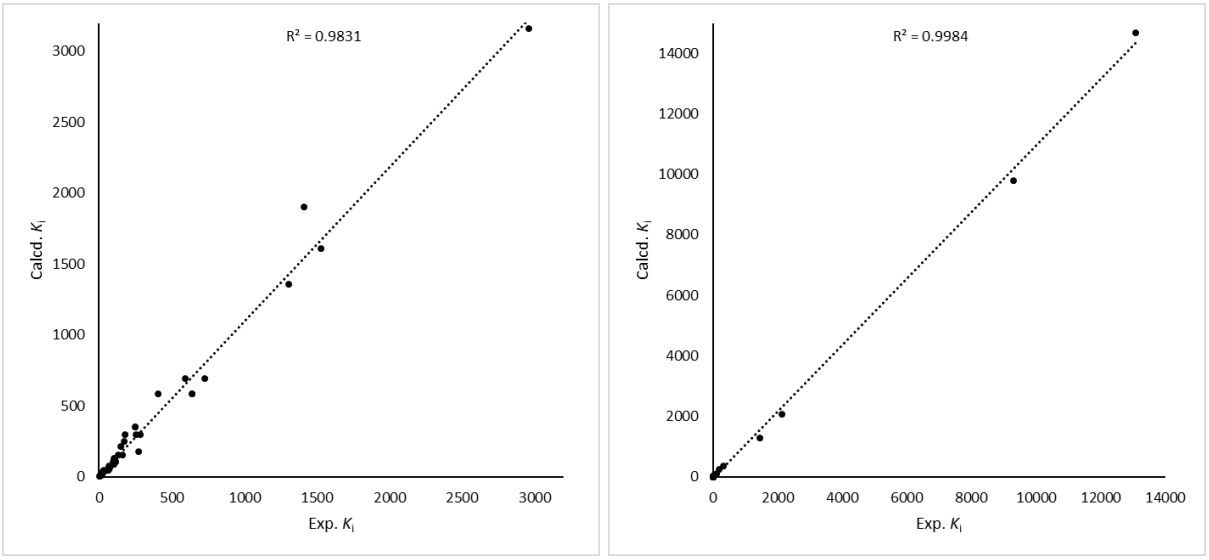

Figure S1. 2D plot of the linear regression fit between experimental and calculated σ_1 and σ_2 binding constants values for (left) compounds **1**–**14**, and **16**–**20** (the training set), reported in **Table 1**, and (right) those of the chosen well-known ligands (the test set), reported in Table **S1**. All values have been calculated by the calibrated Smina scoring function employing the optimized parameters reported in **Table 2**.

4. Molecular modeling – 3D docking poses of compounds 4, 5, and 11 in the binding site of σ_1R, σ_2R, and H_3R

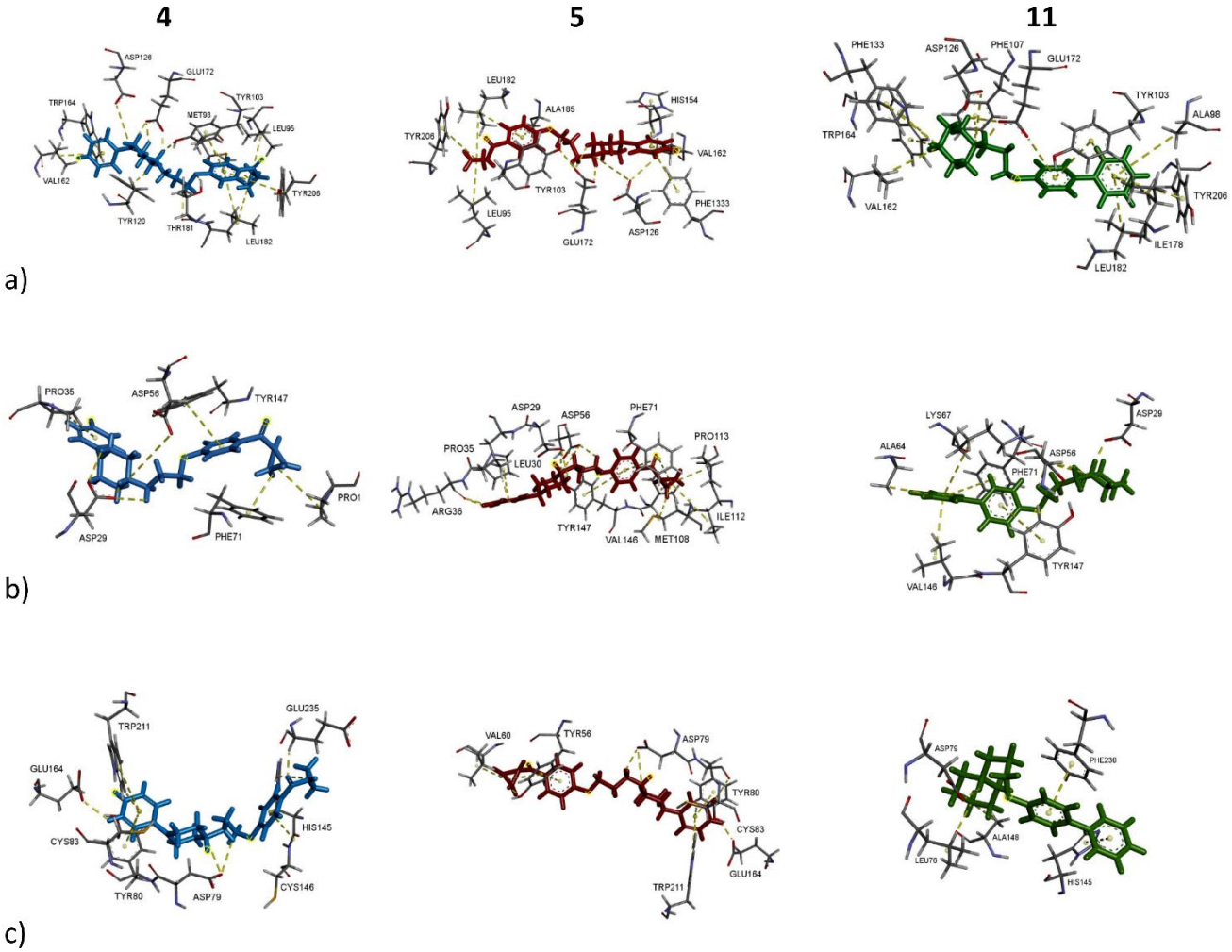

Figure S2. 3D docking poses of compounds **4**, **5**, and **11** in the binding site of a) σ_1R, b) σ_2R, c) H_3R.

5. Outcome of correlational studies between frequency of interaction of ligands with particular amino acid residues of σRs

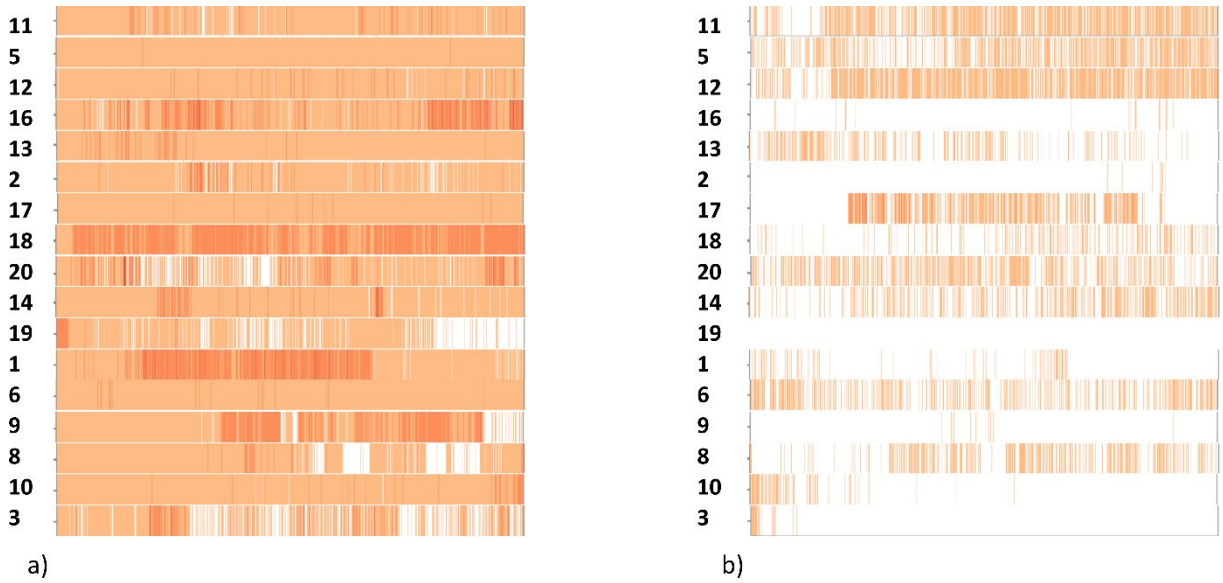

Figure S3. Ligand-protein interaction diagrams obtained during MD simulations for the highest correlated residues of σ_1R: a) E172, b) Y206.

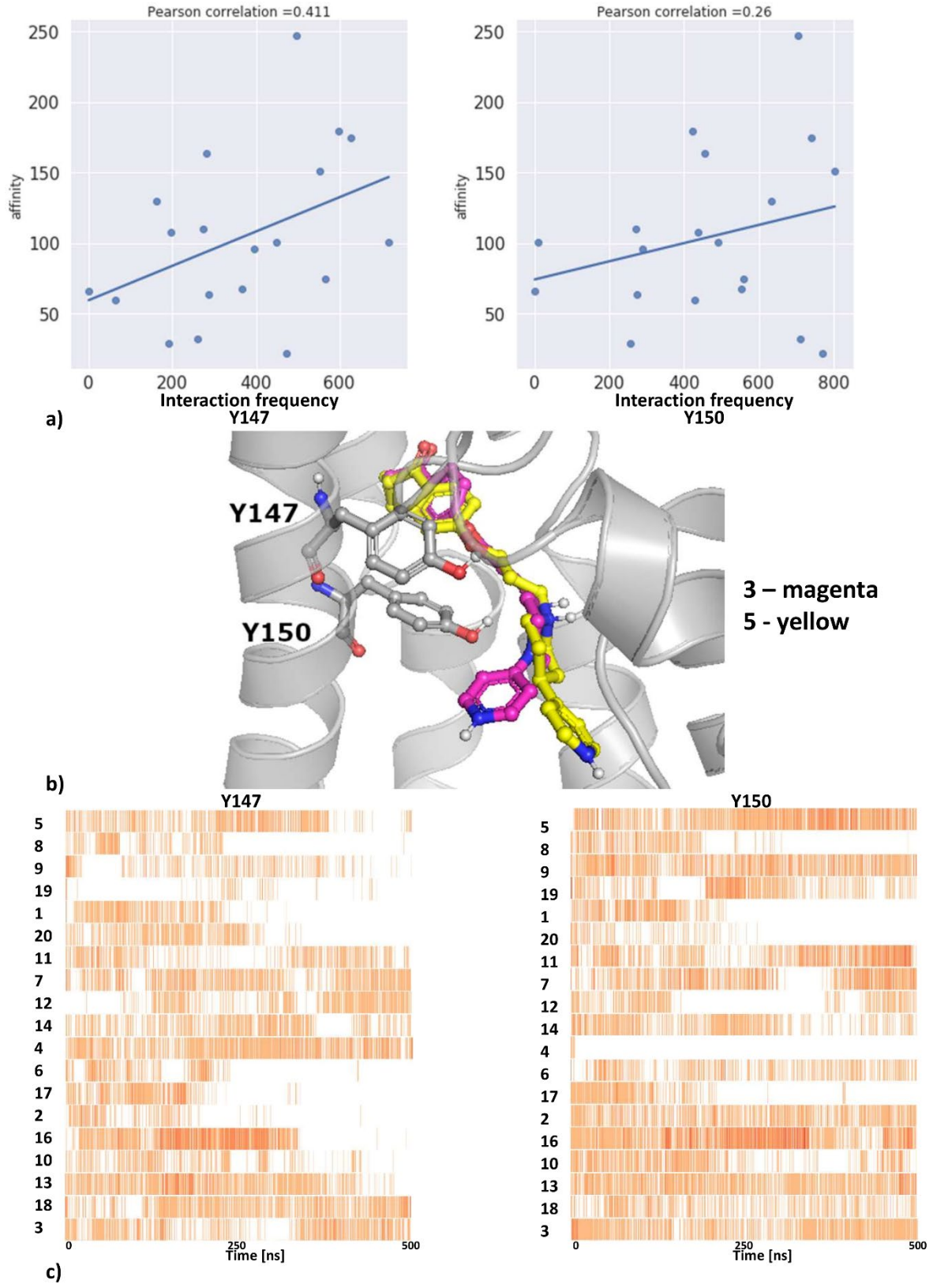

Figure S4. Outcome of correlational studies between frequency of interaction of ligands with particular amino acid residues of σ_2R, a) Pearson correlation coefficients for the highest correlated residues, b) visualization of the highest correlated residues with examples of docked compounds, c) ligand-protein interaction diagrams obtained during md simulations for the highest correlated residues.

6. References

1. Szczepańska K, Karcz T, Mogilski S, Siwek A, Kuder KJ, Latacz G, Kubacka M, Hagenow S, Lubelska A, Olejarz A, Kotańska M, Sadek B, Stark H, Kieć-Kononowicz K. Synthesis and biological activity of novel *tert*-butyl and *tert*-pentylphenoxyalkyl piperazine derivatives as histamine H_3R ligands. *Eur J Med Chem*. 2018; 152: 223-234.

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7. ^{1}H- and ^{13}C-NMR spectra of compounds 11 and 12. HPLC traces of all the final compounds

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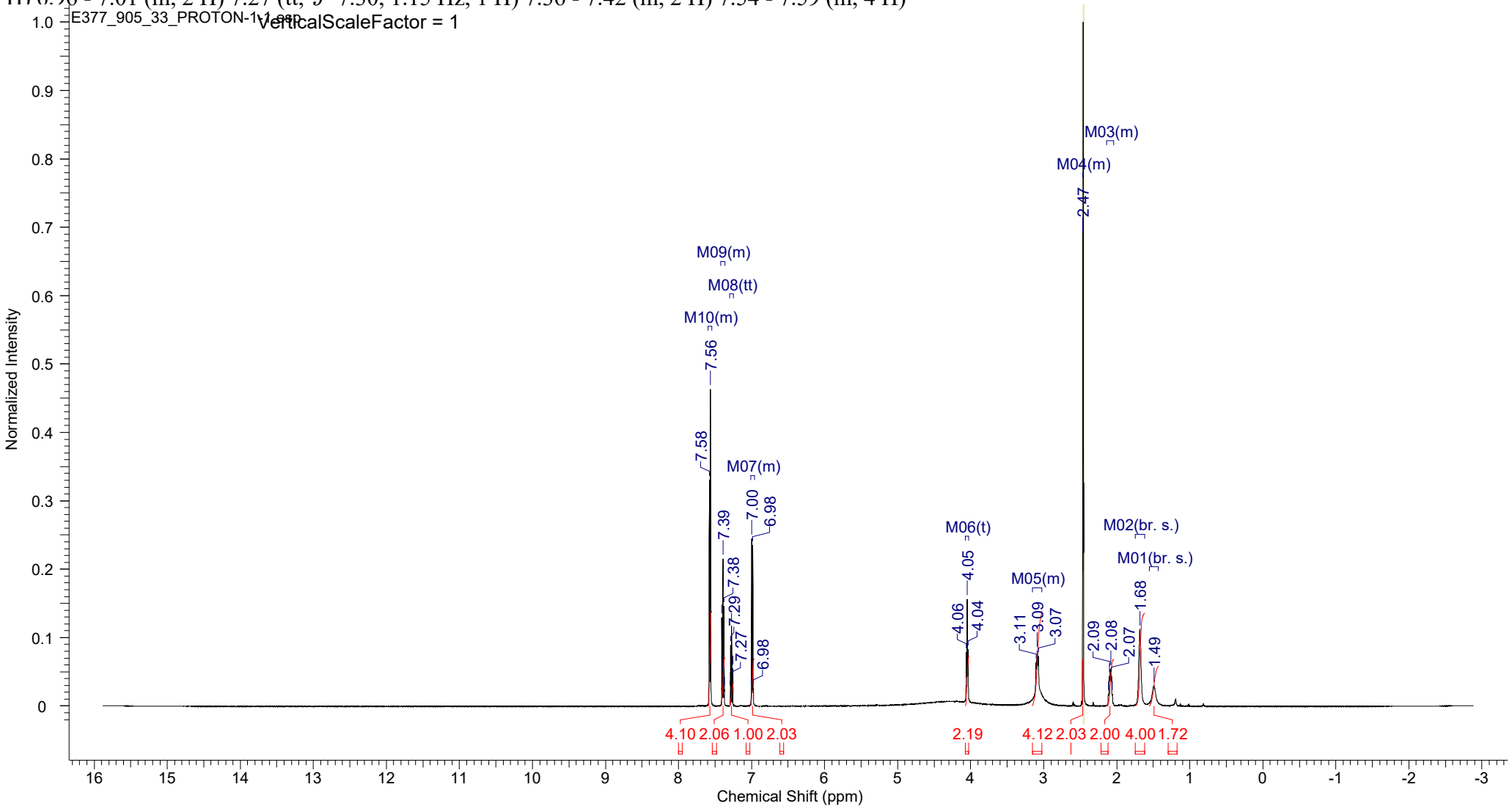

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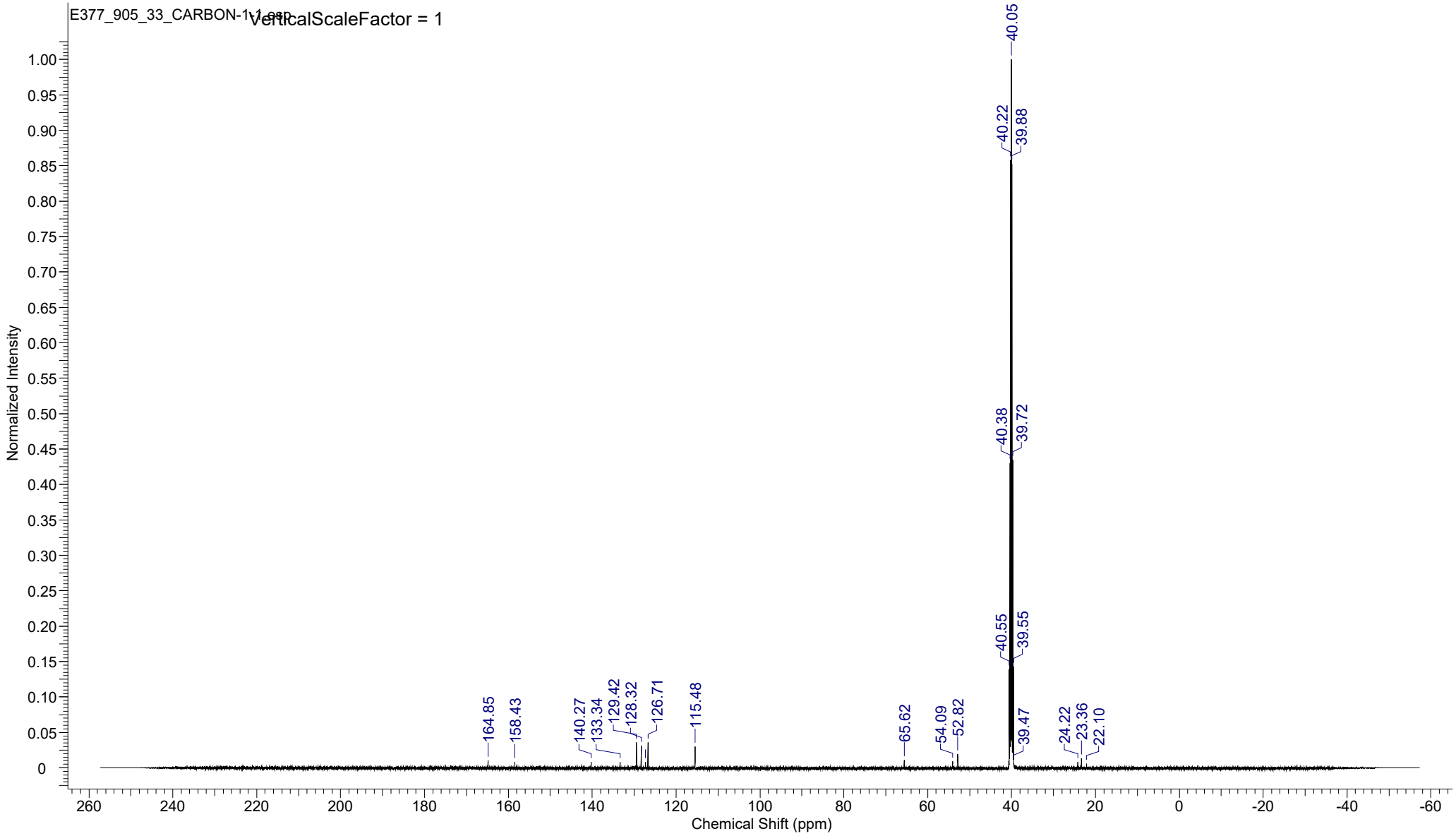

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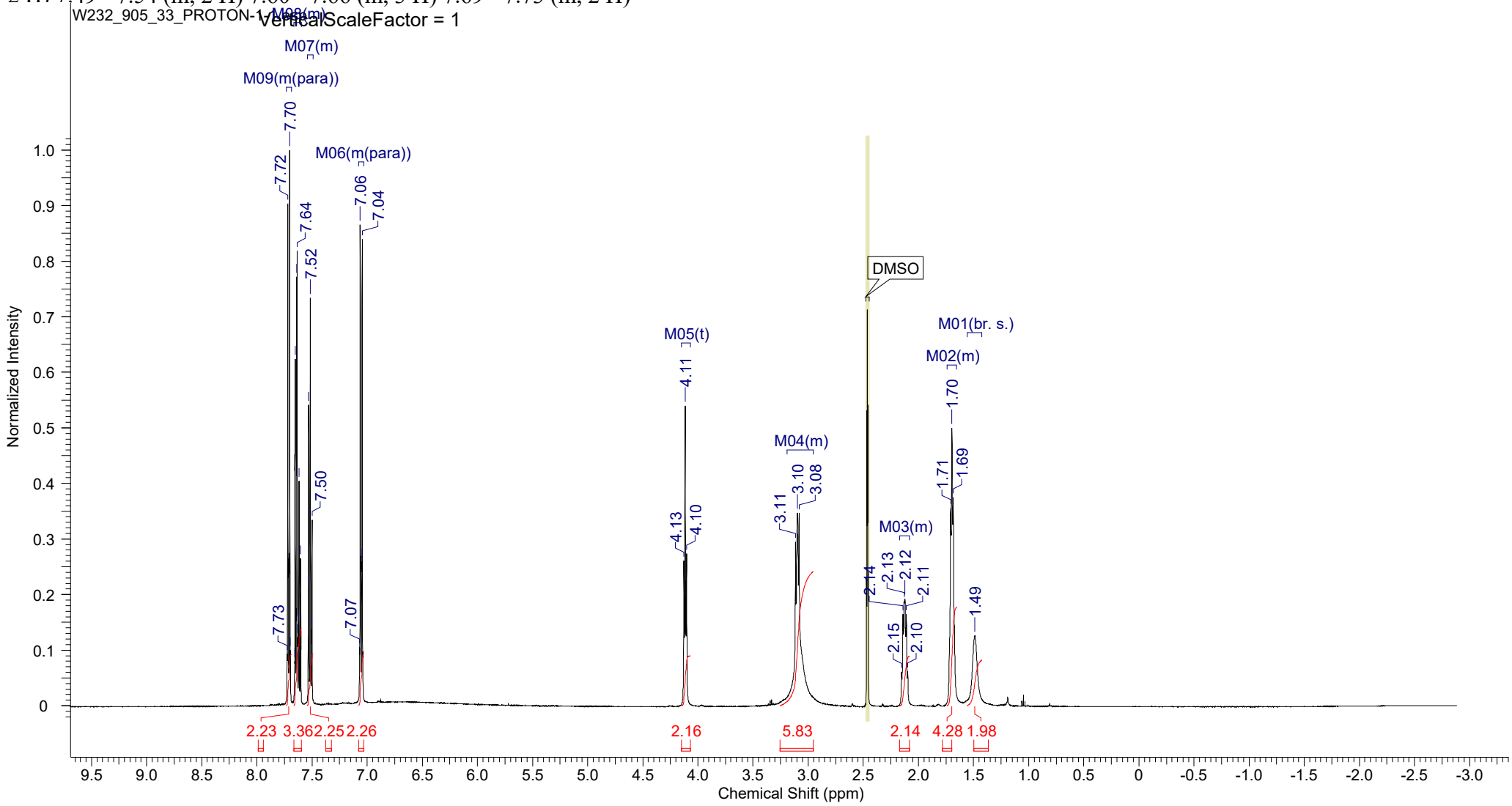
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.49 (br. s., 2 H) 1.65 - 1.74 (m, 4 H) 2.08 - 2.17 (m, 2 H) 2.95 - 3.19 (m, 6 H) 4.11 (t, *J*=6.16 Hz, 2 H) 7.03 - 7.08 (m, 2 H) 7.49 - 7.54 (m, 2 H) 7.60 - 7.66 (m, 3 H) 7.69 - 7.73 (m, 2 H)



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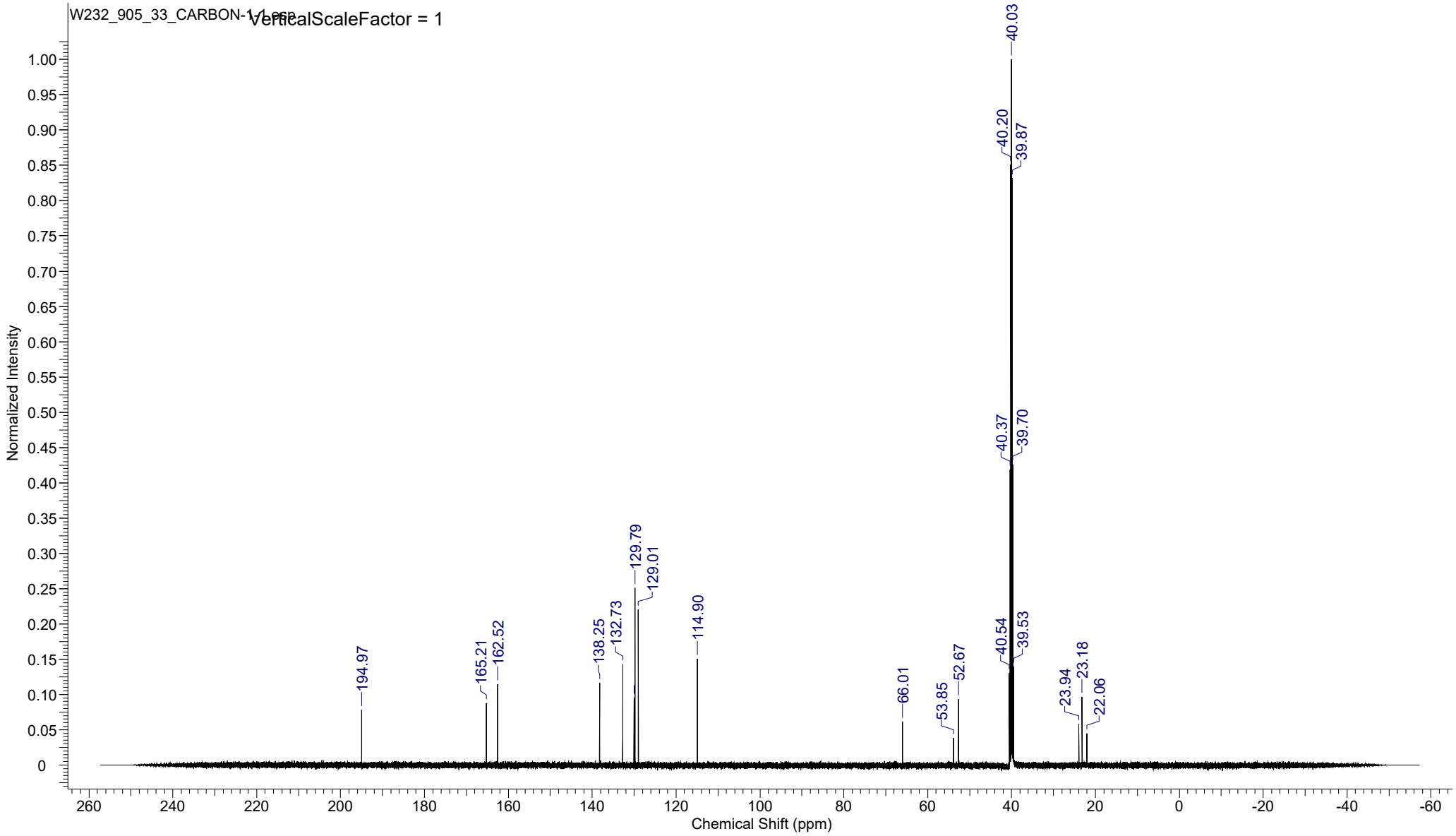

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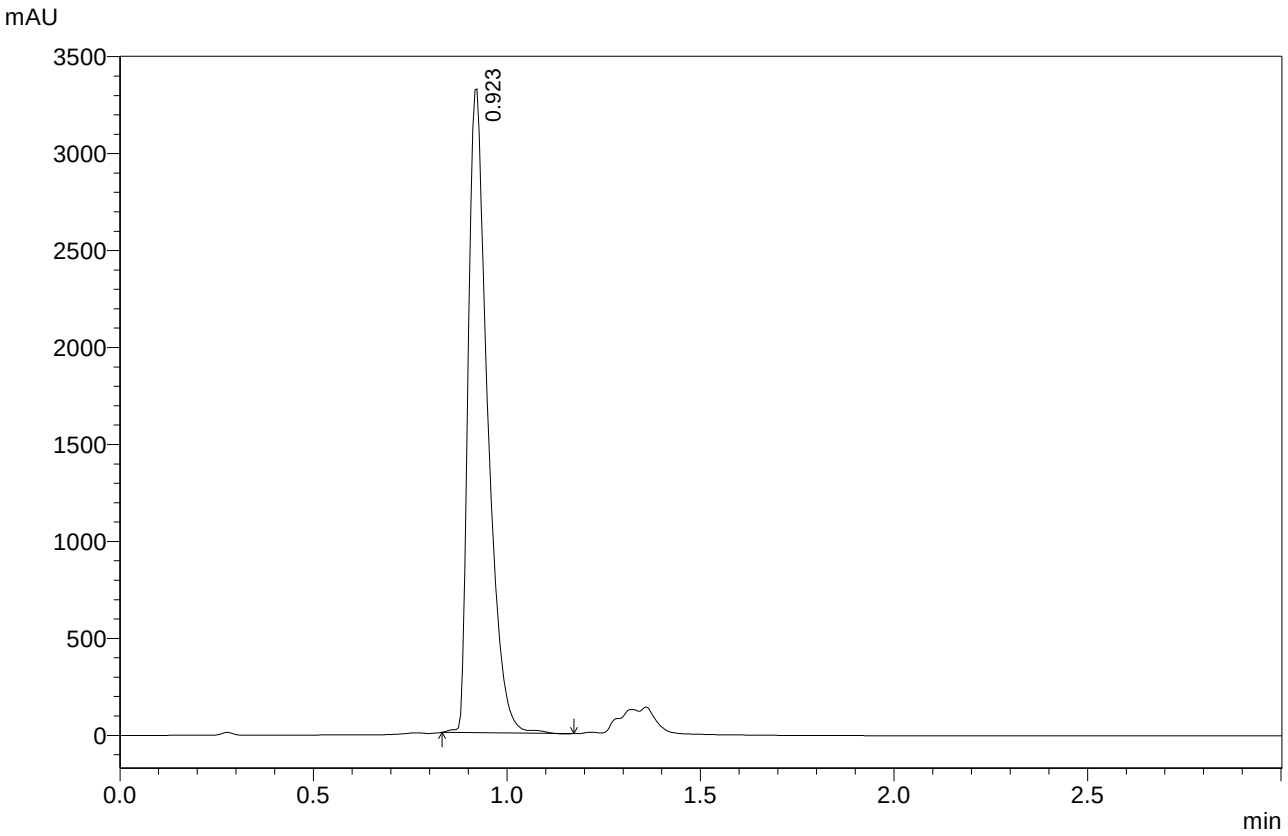
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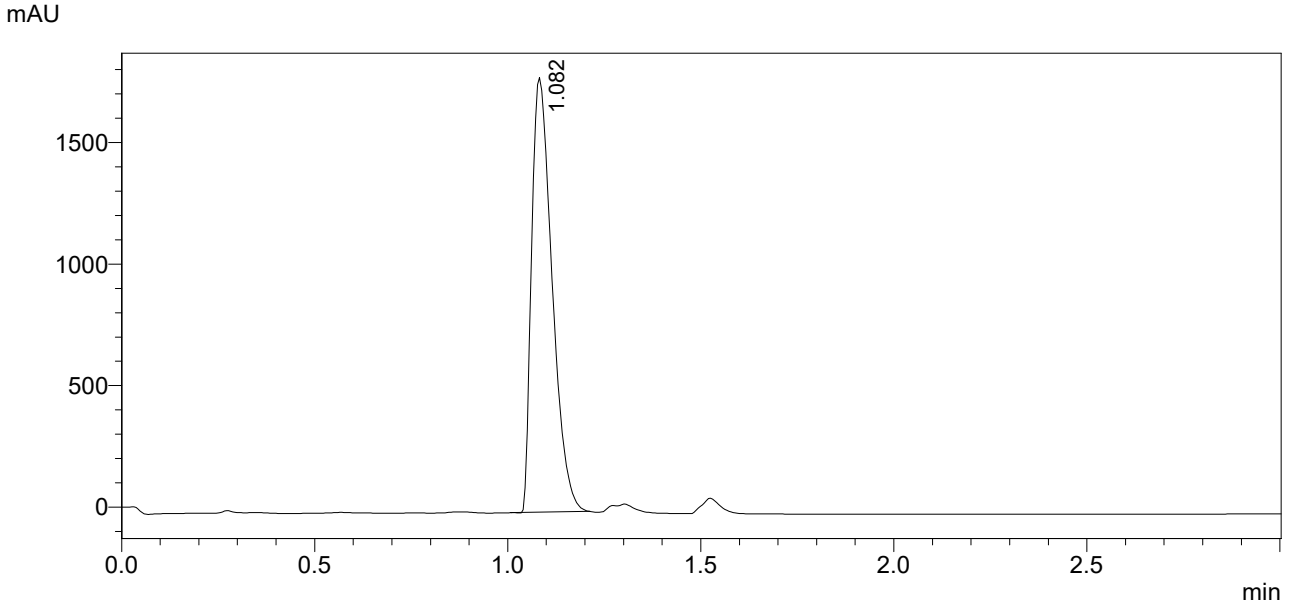

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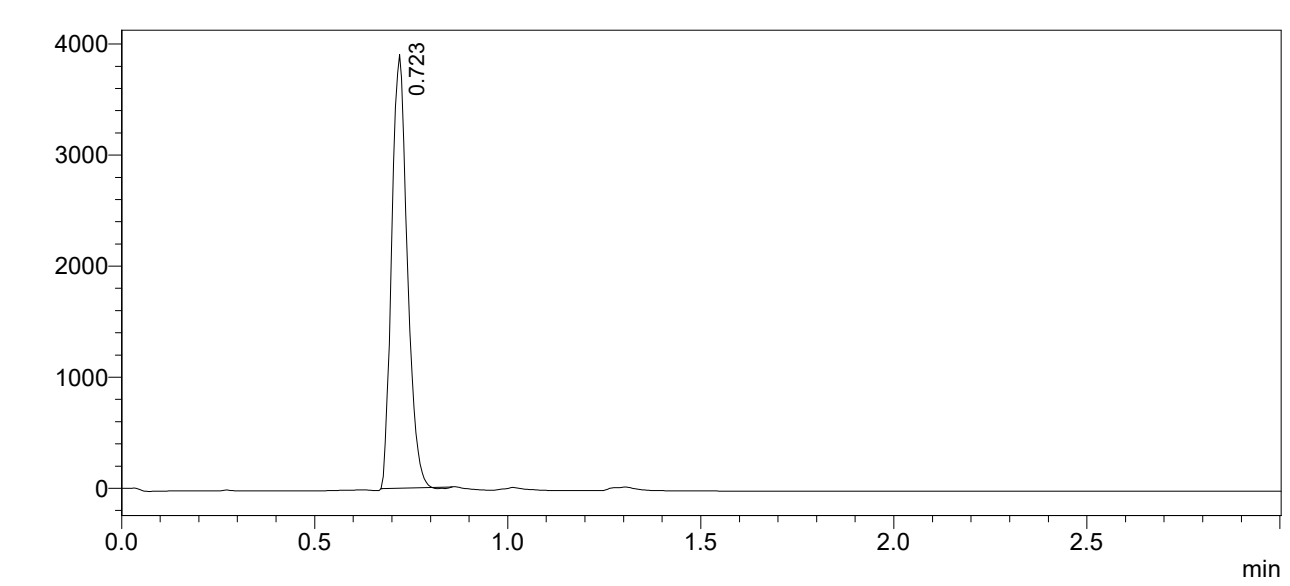

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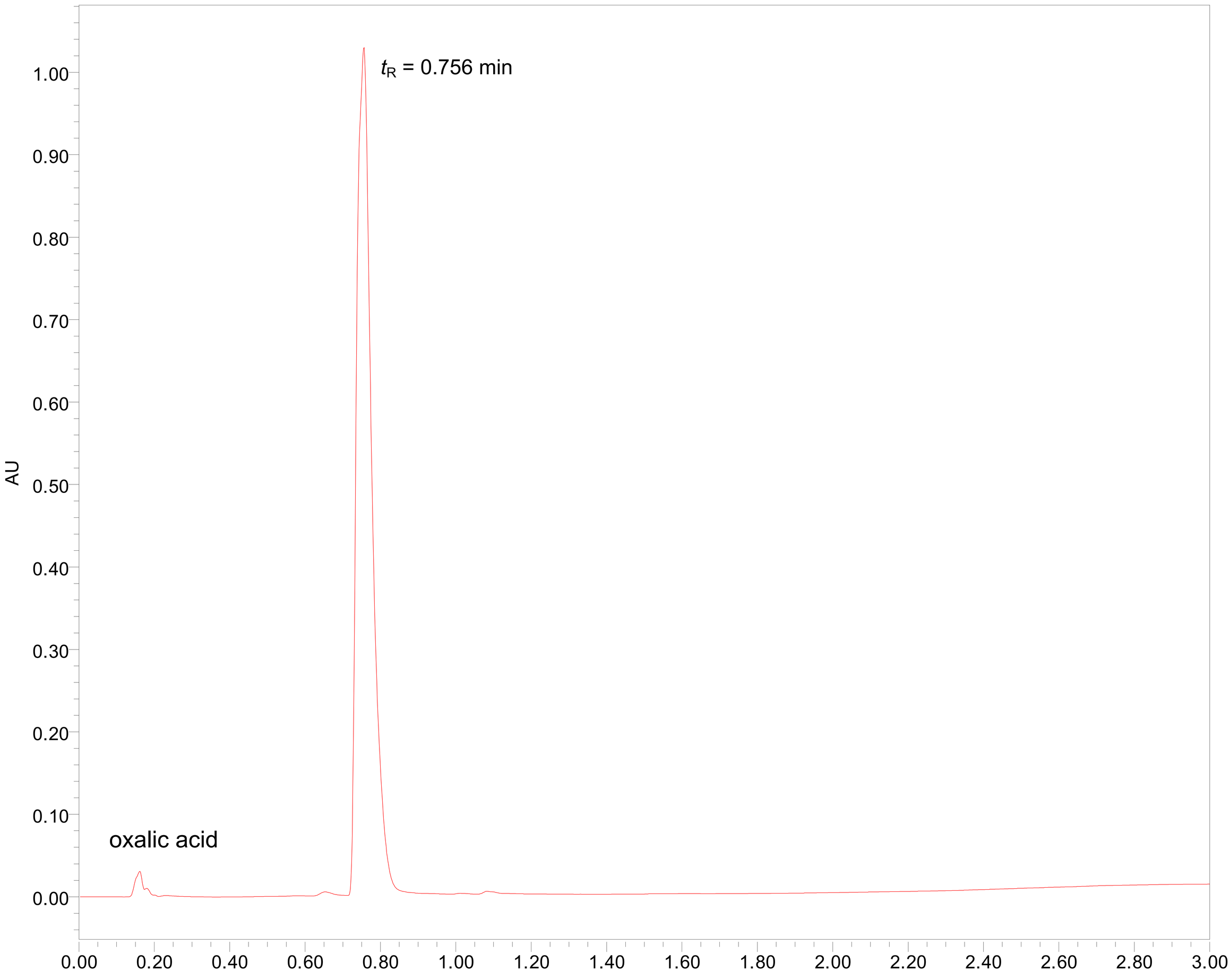
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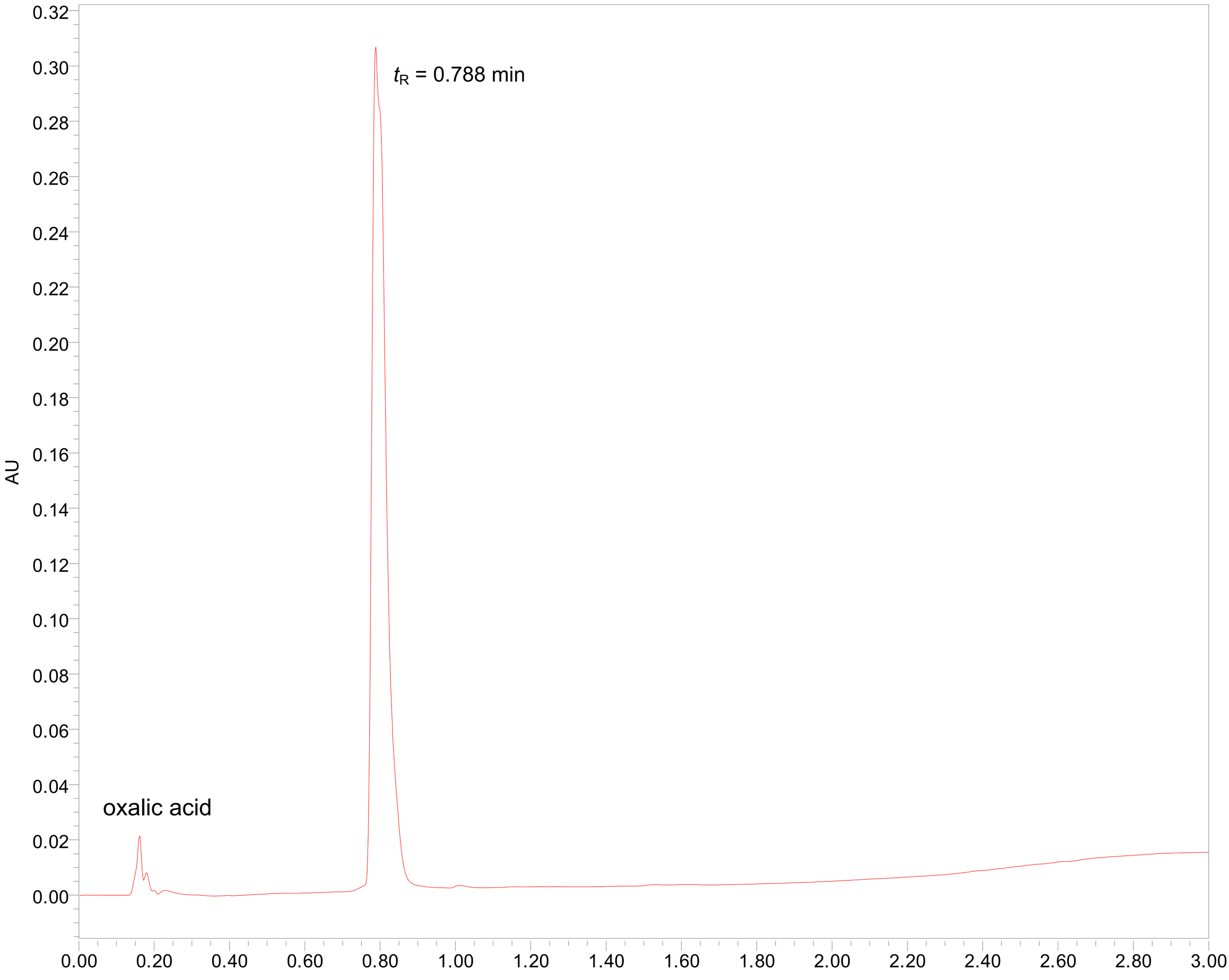
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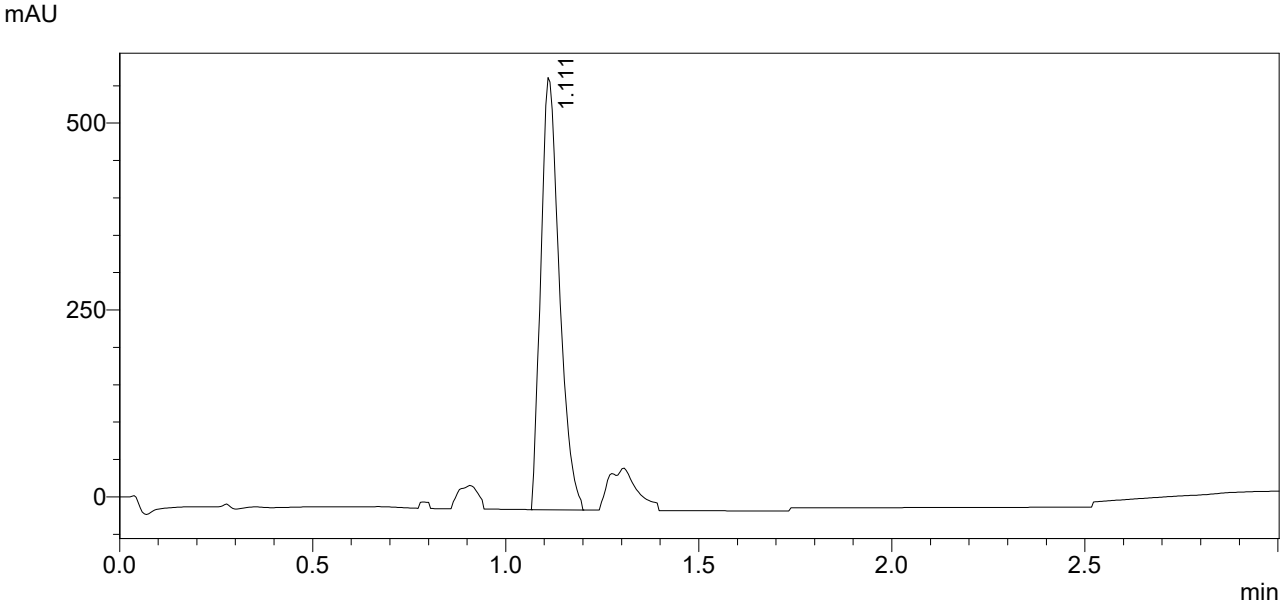

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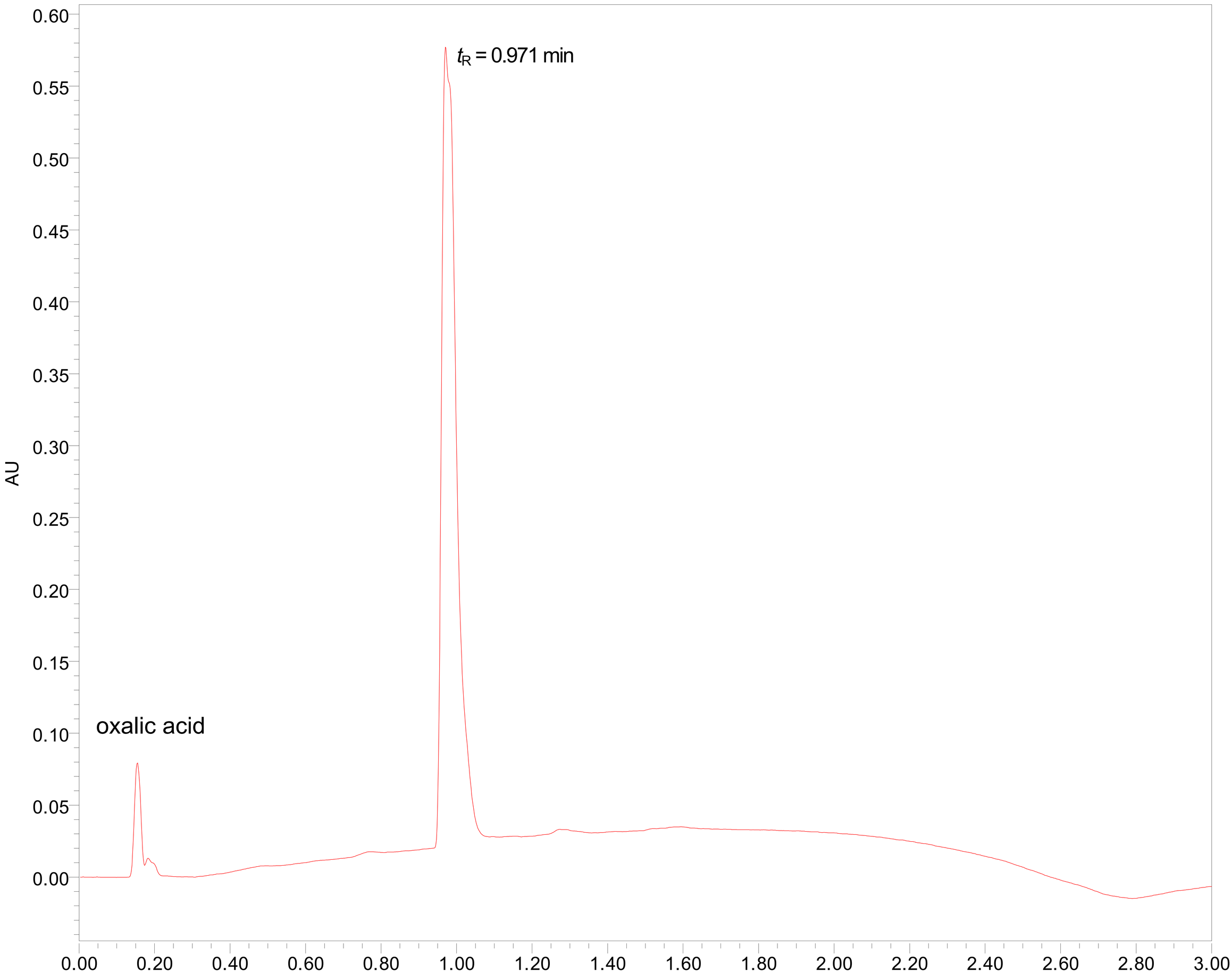
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SHIMADZU LabSolutions

Cpd8

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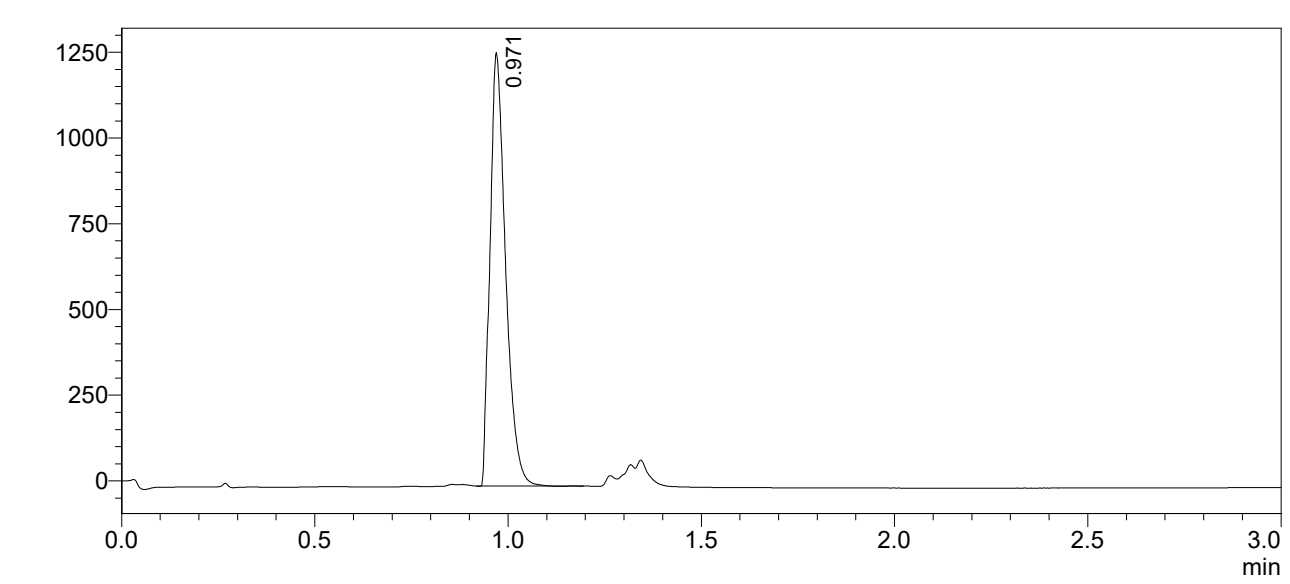
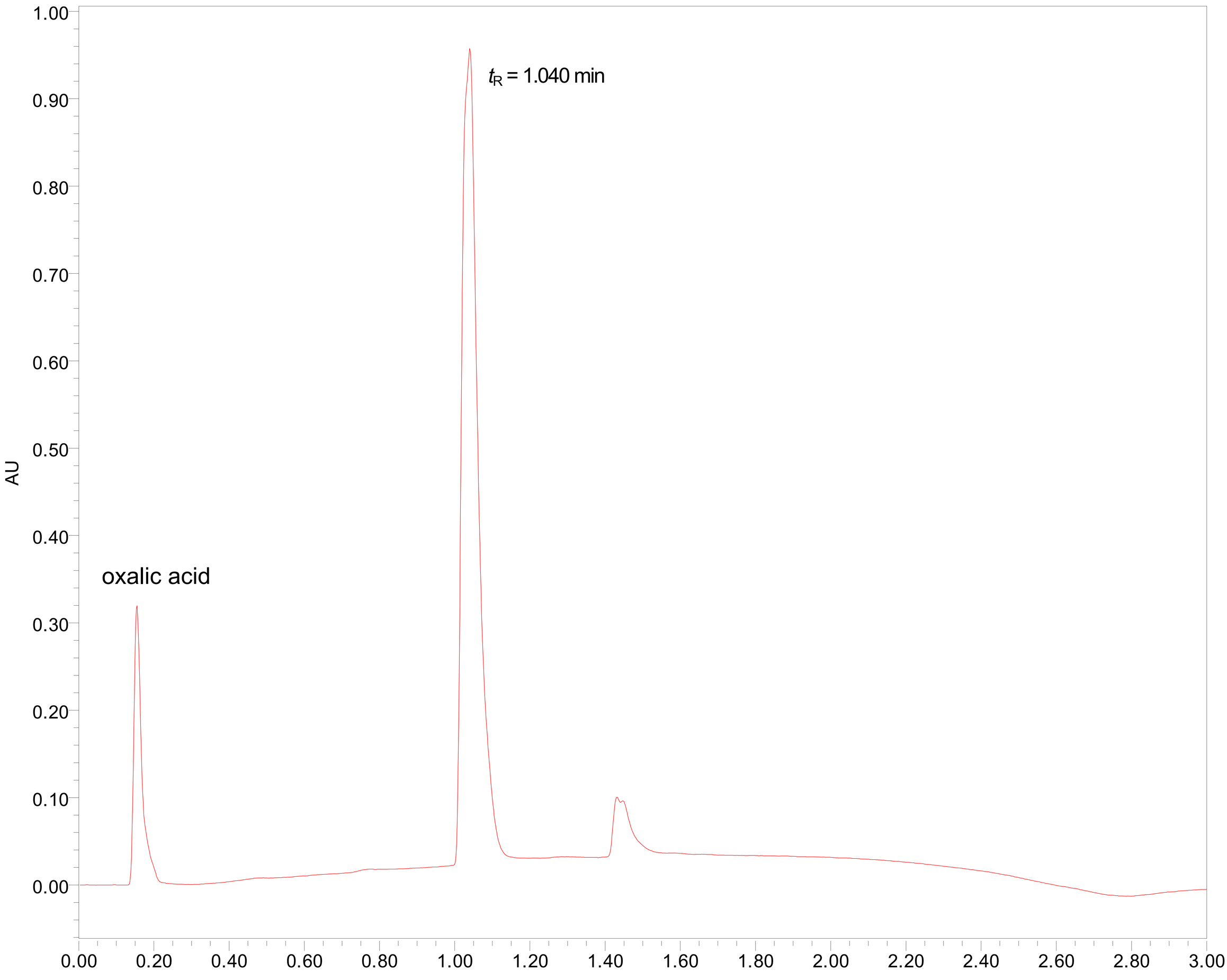
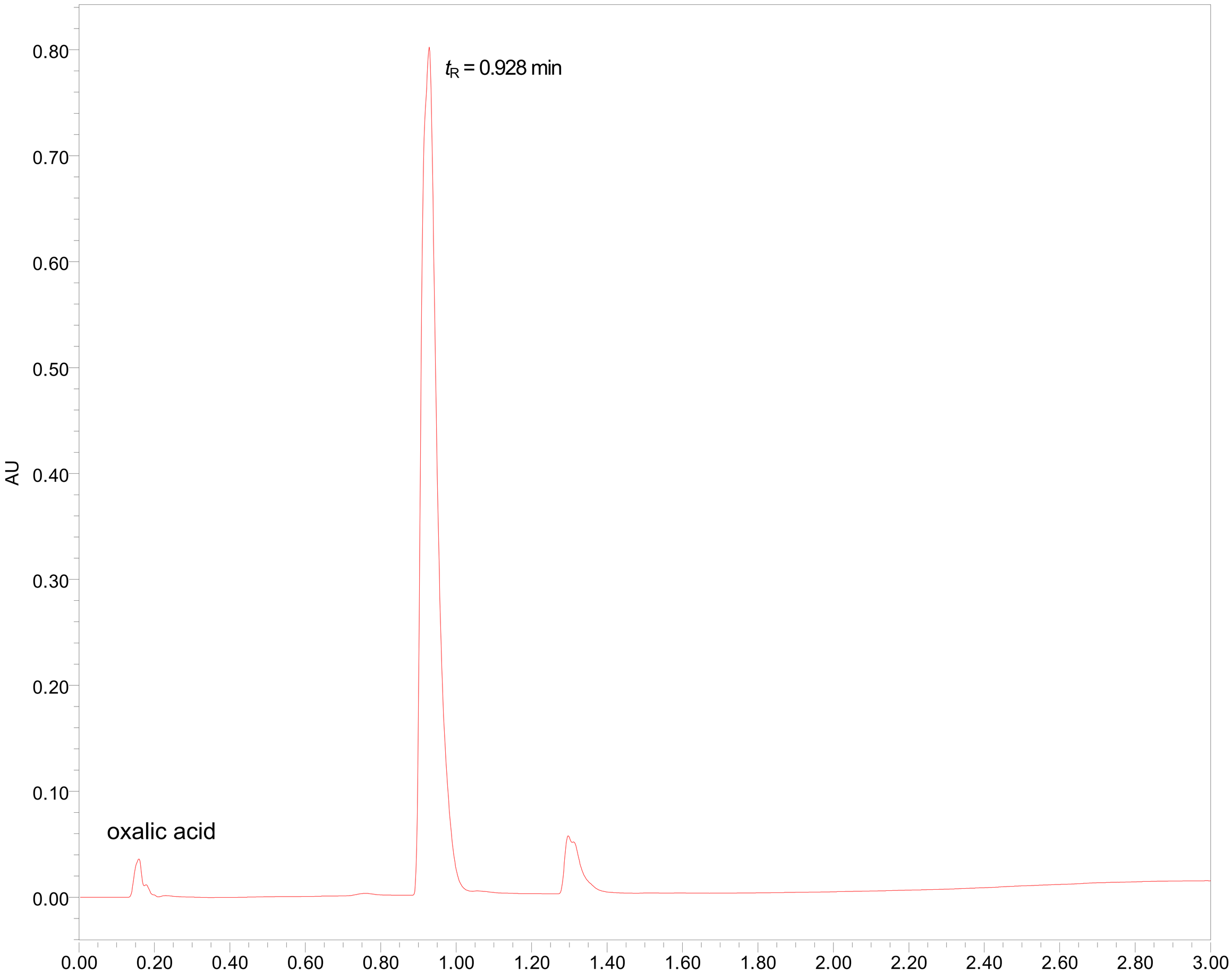


Cpd9


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
AU
t_R = 1.040 min
oxalic acid
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40
2.60
2.80
3.00

Cpd10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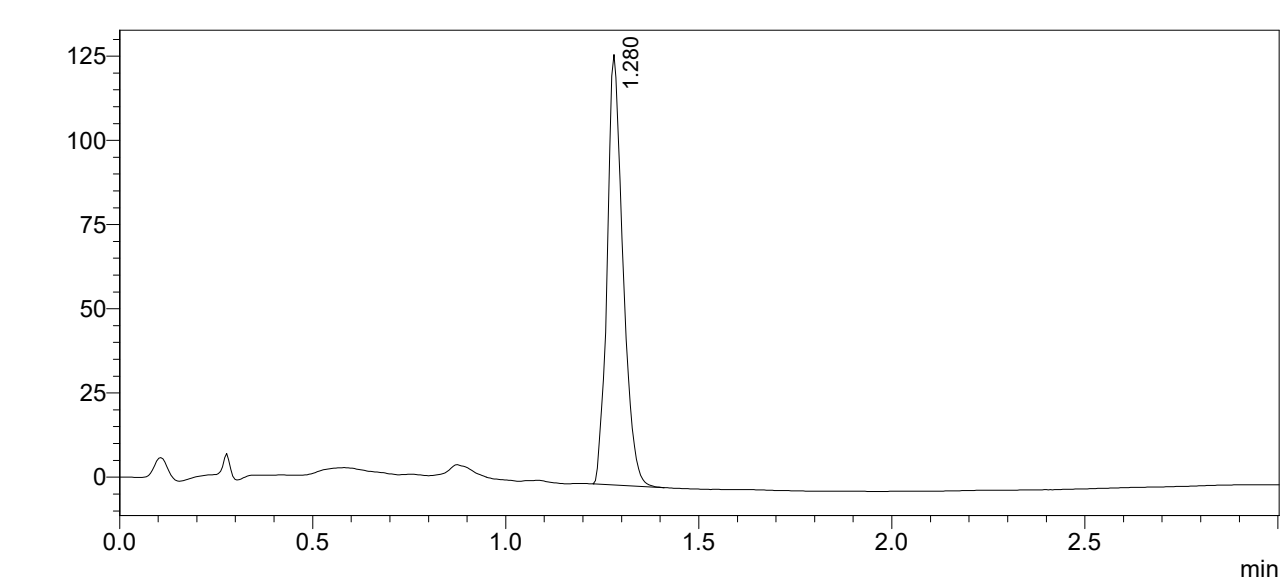
oxalic acid
AU
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40
2.60
2.80
3.00

SHIMADZU LabSolutions

Cpd11

<Chromatogram>



SHIMADZU LabSolutions

Cpd12

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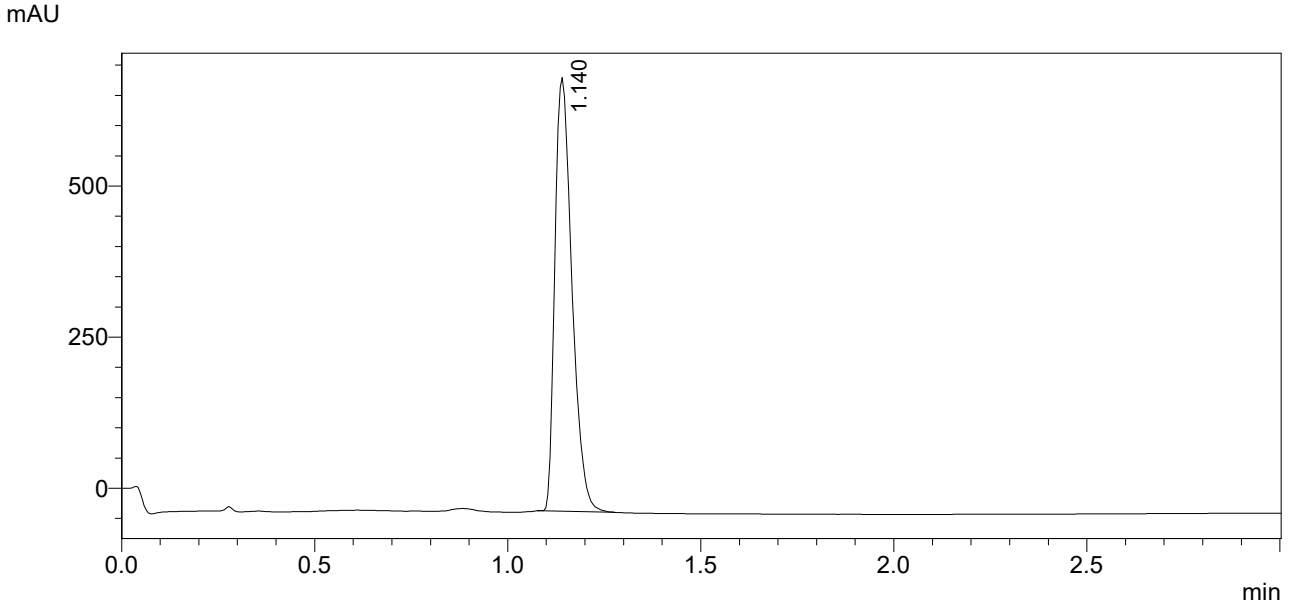
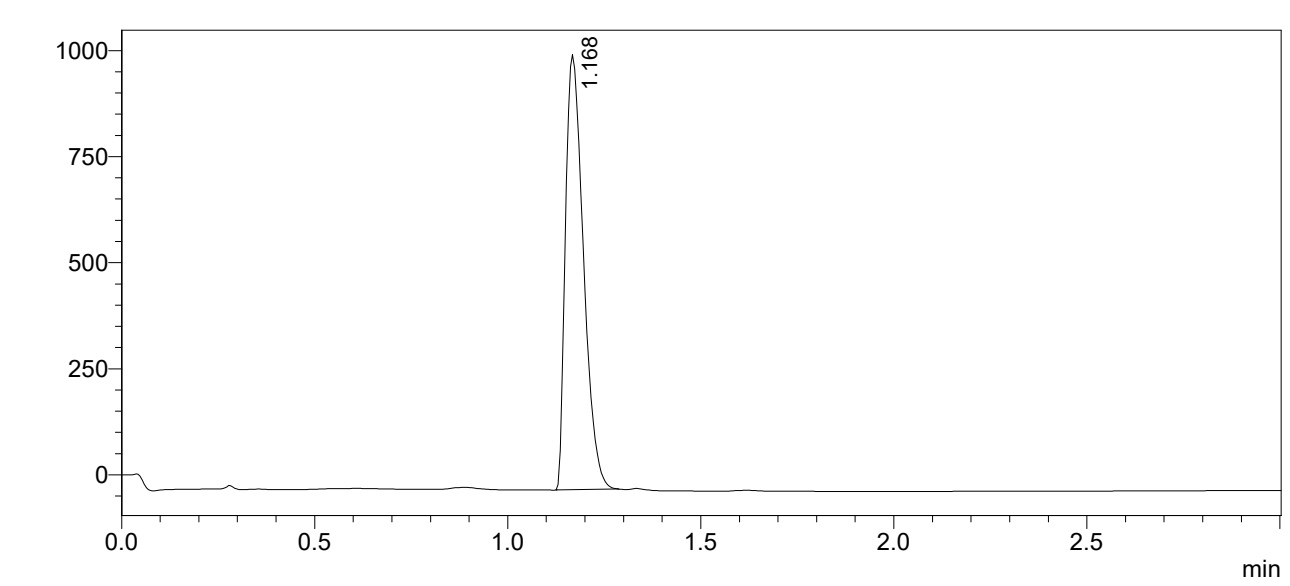


SHIMADZU LabSolutions

Cpd13

<Chromatogram>



SHIMADZU LabSolutions

Cpd14

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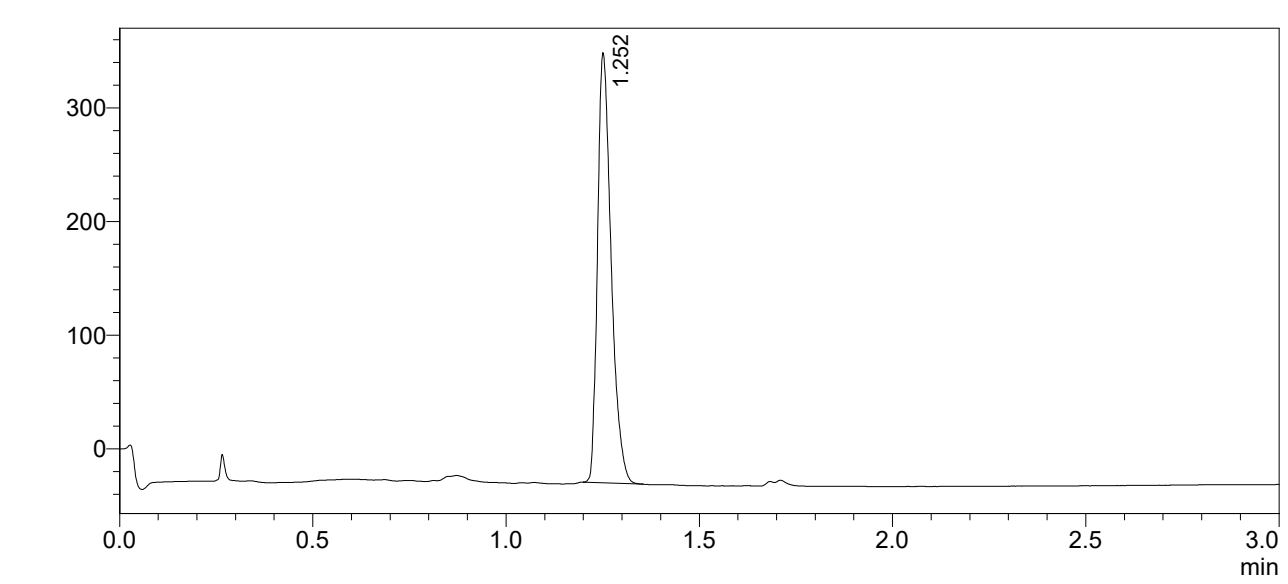

SHIMADZU LabSolutions

Cpd16

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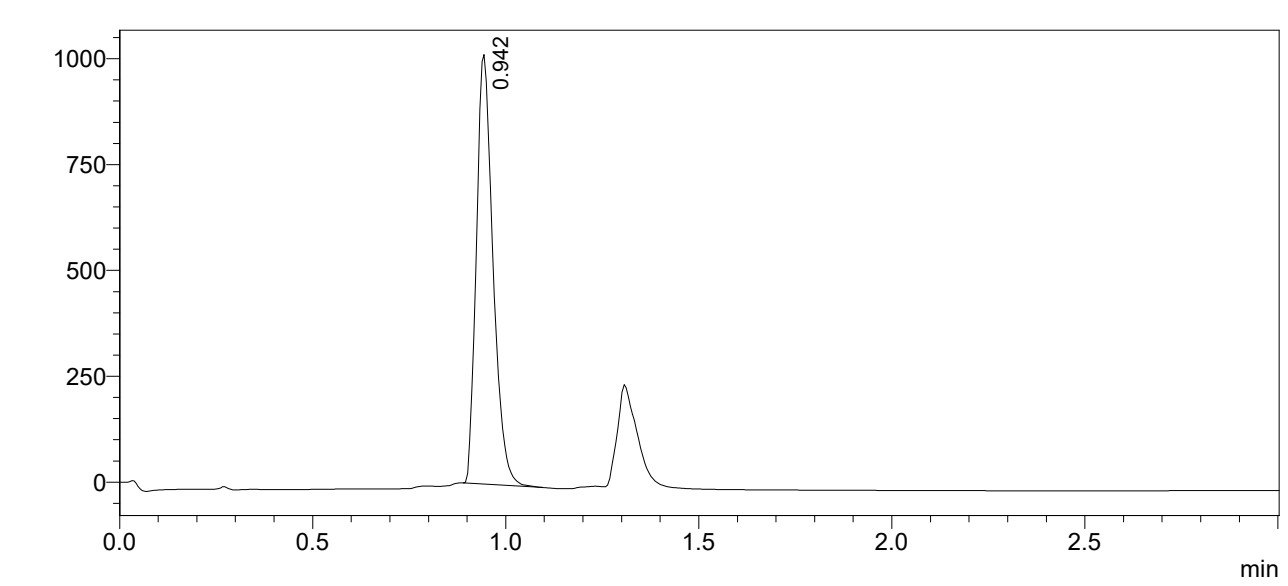

SHIMADZU LabSolutions

Cpd17

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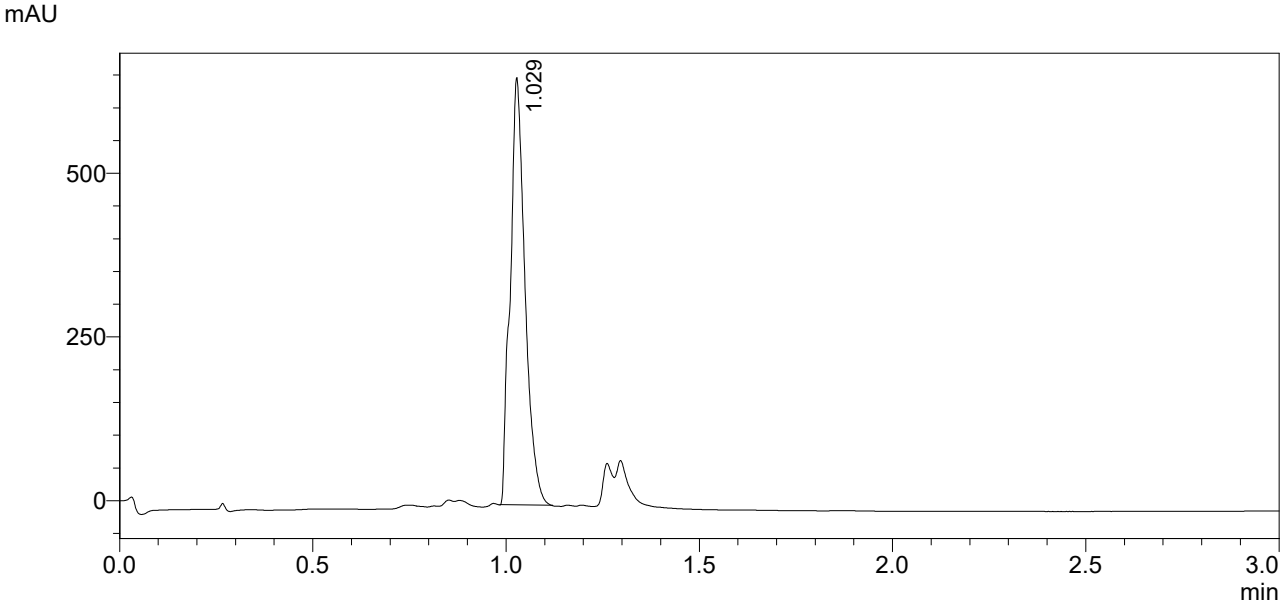

SHIMADZU LabSolutions

Cpd18

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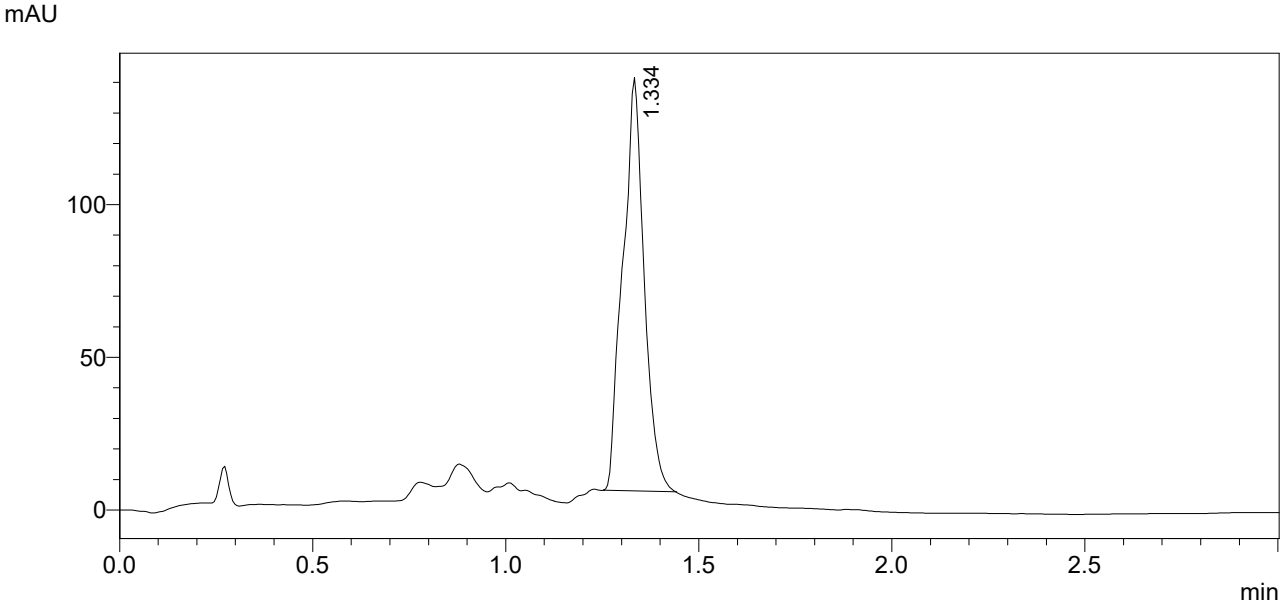

SHIMADZU LabSolutions

Cpd19

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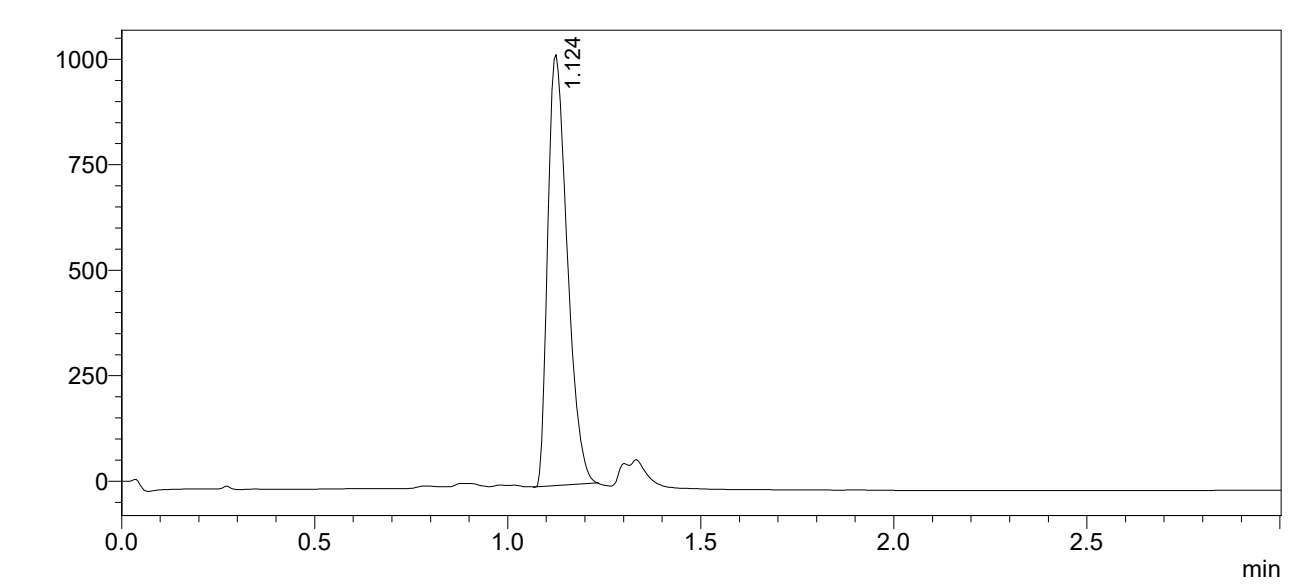

SHIMADZU LabSolutions

Cpd20

<Chromatogram>