Supplementary data for the article:

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Supplementary Material

Synthesis and evaluation of thiophene-based guanylhydrazones (iminoguanidines) efficient against panel of voriconazole-resistant fungal isolates

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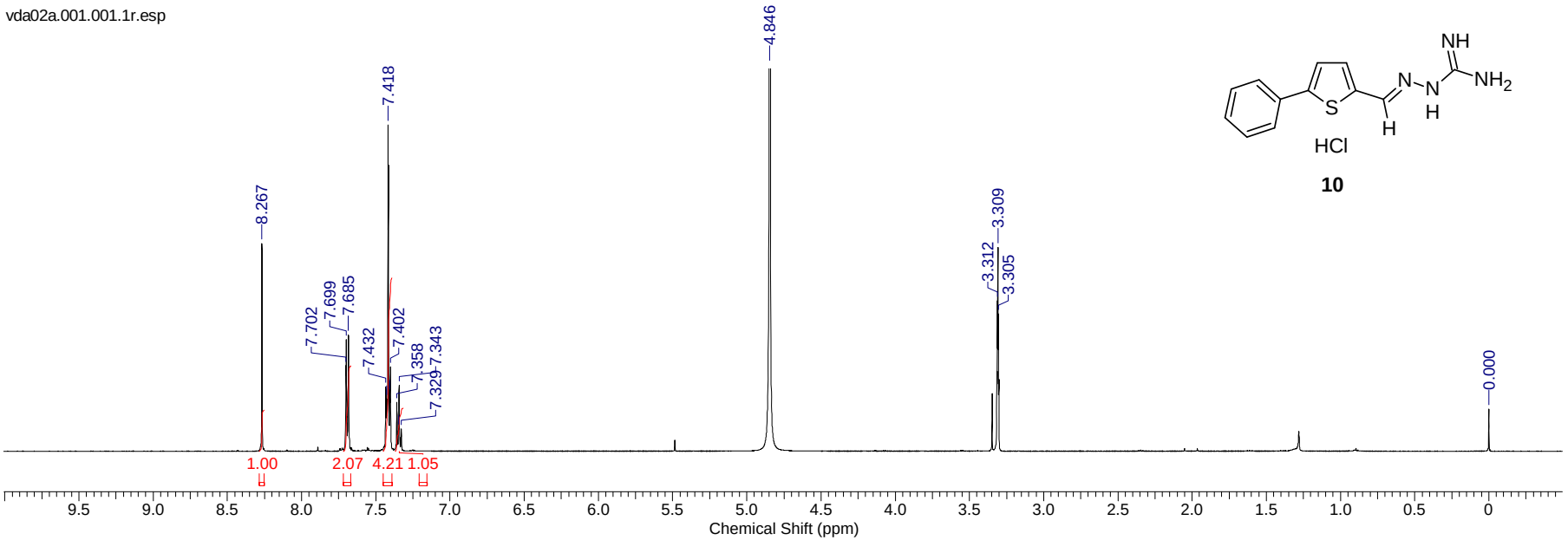
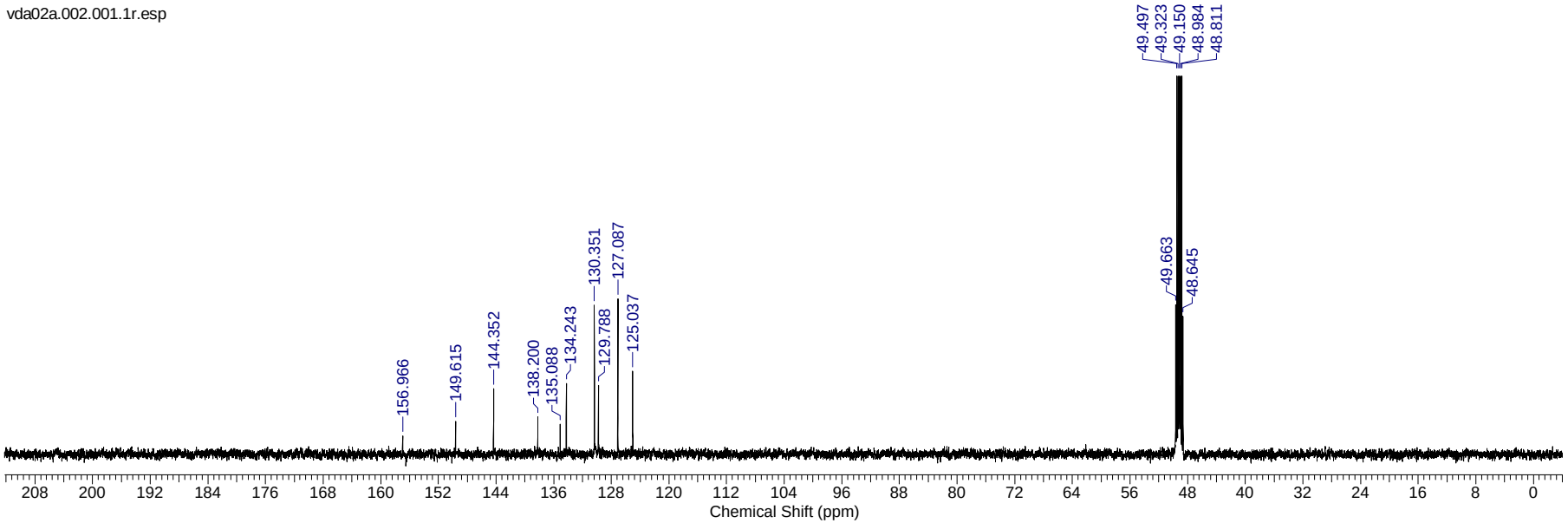
[d] Faculty of Medicine, Department of Chemistry, University of Niš, 18000 Niš, Serbia

Supplementary Material Contents: **Page**

2-[(5-phenylthiophen-2-yl)methylidene]hydrazinecarboximidamide hydrochloride (10)





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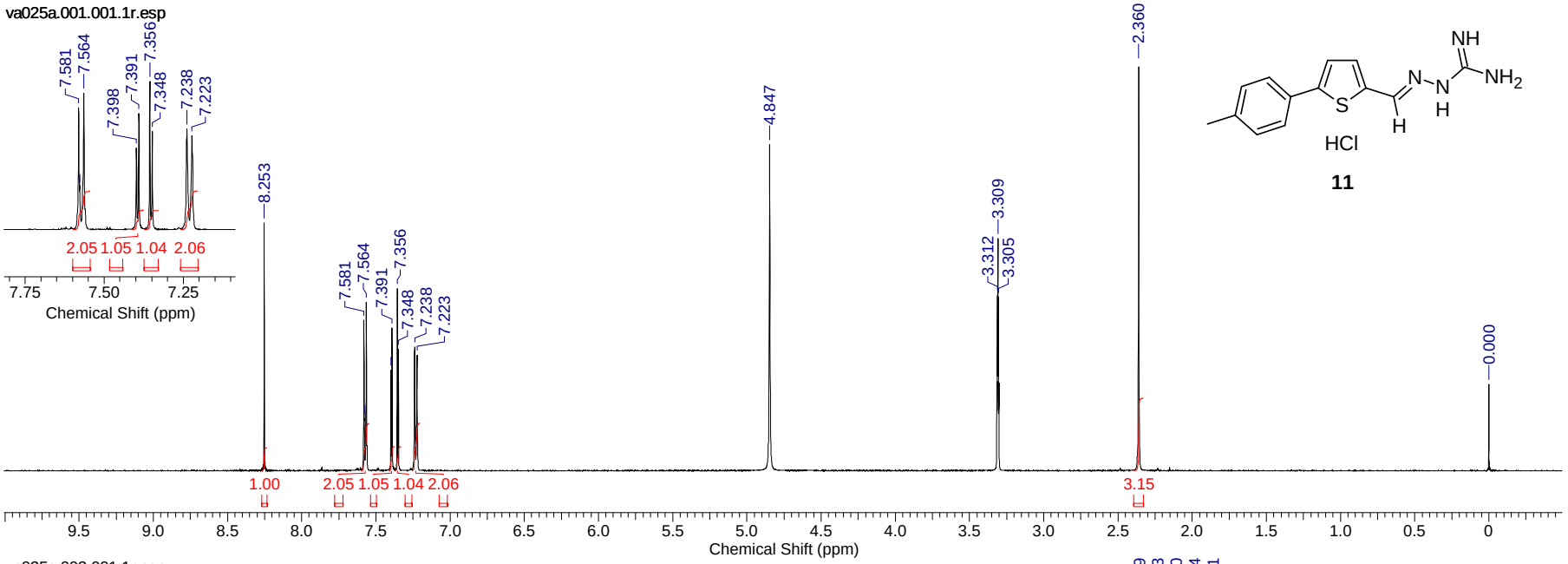
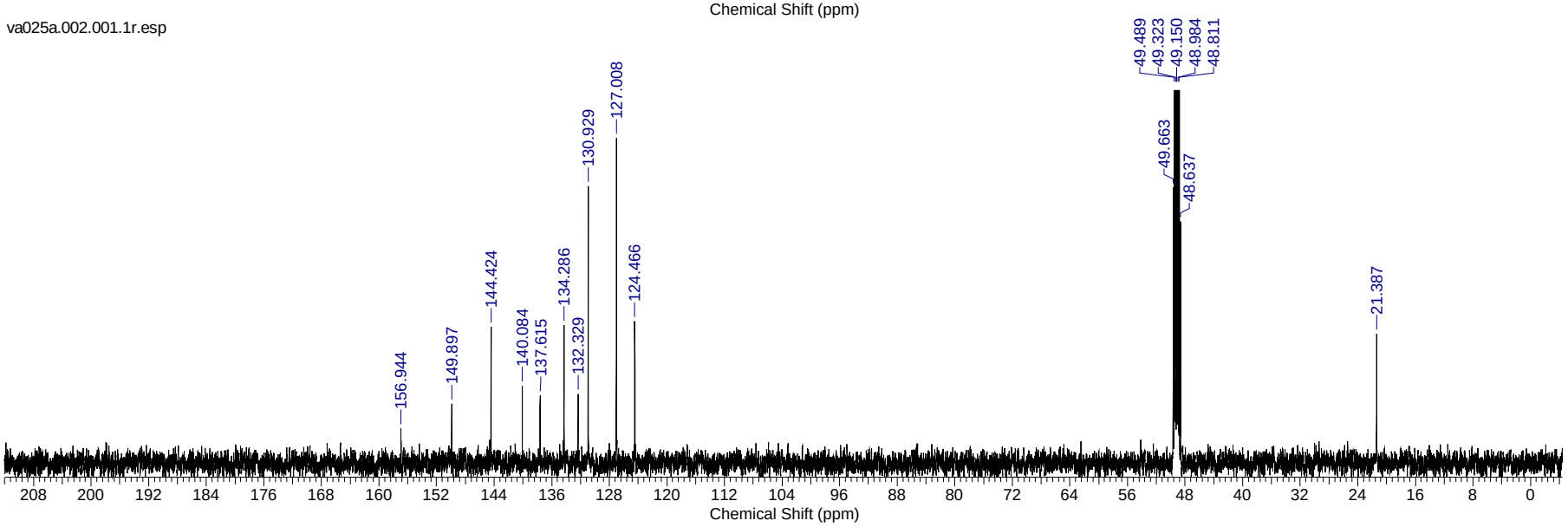
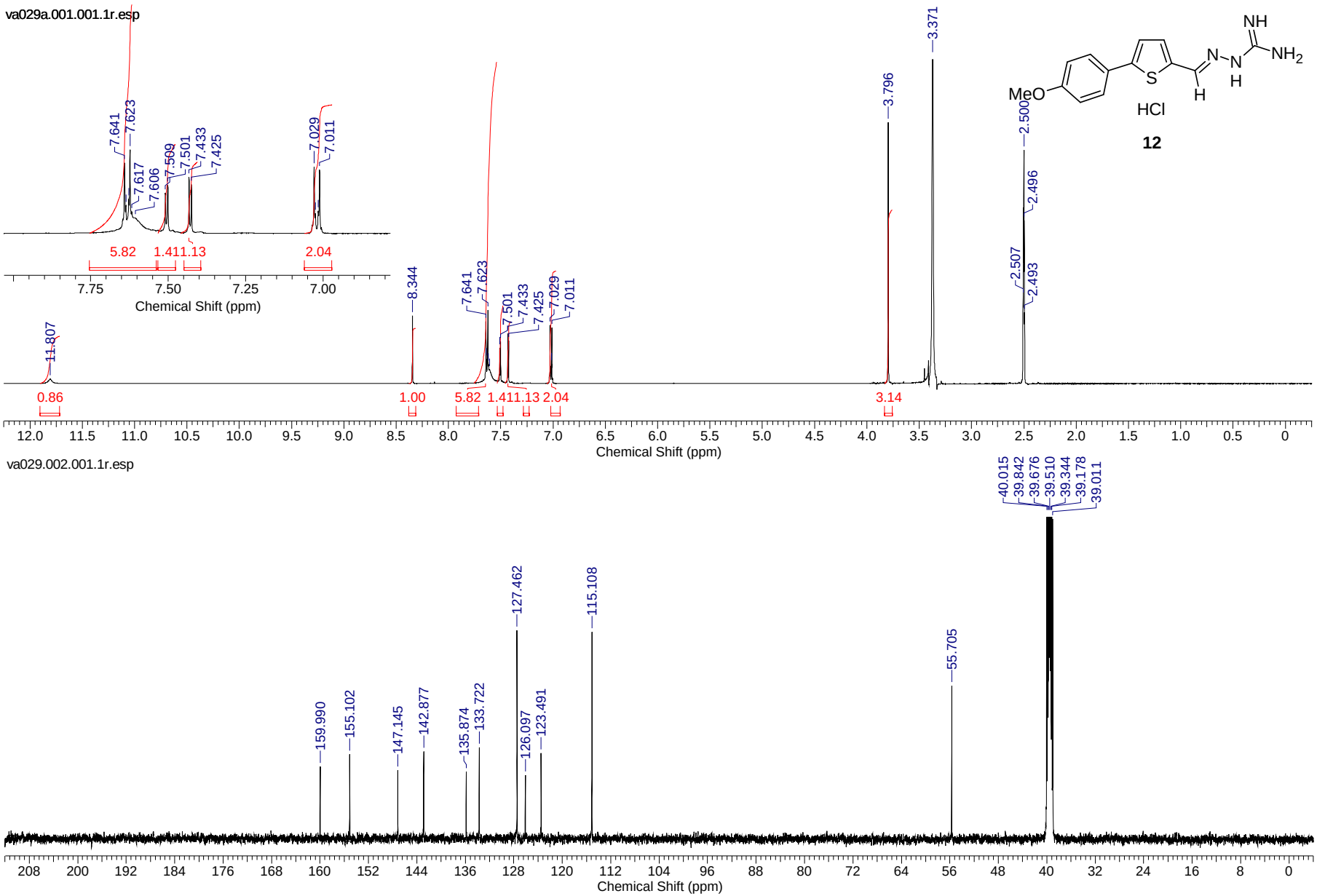




2-{[5-(4-methoxyphenyl)thiophen-2-yl]methylidene}hydrazinecarboximidamide hydrochloride (12)



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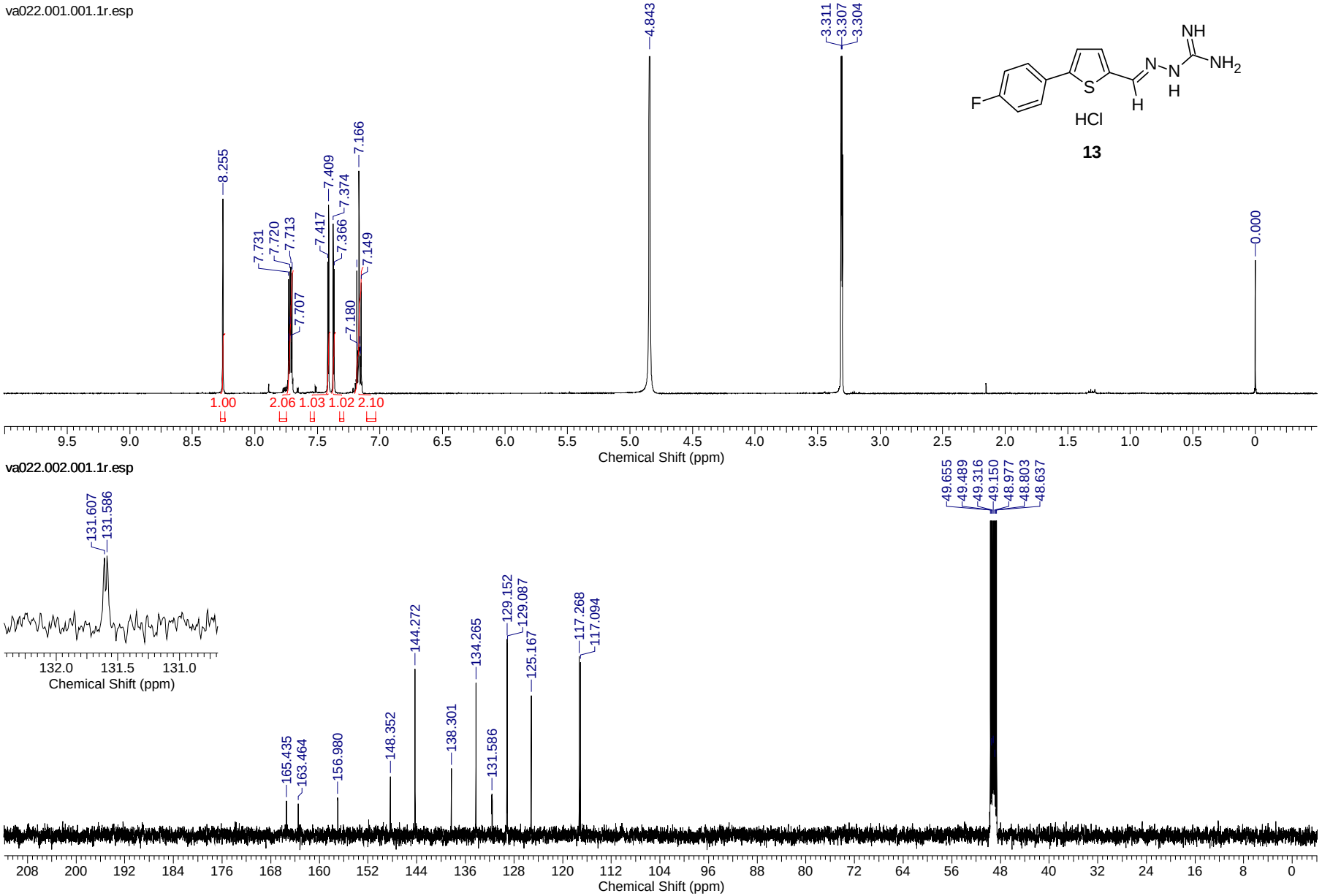

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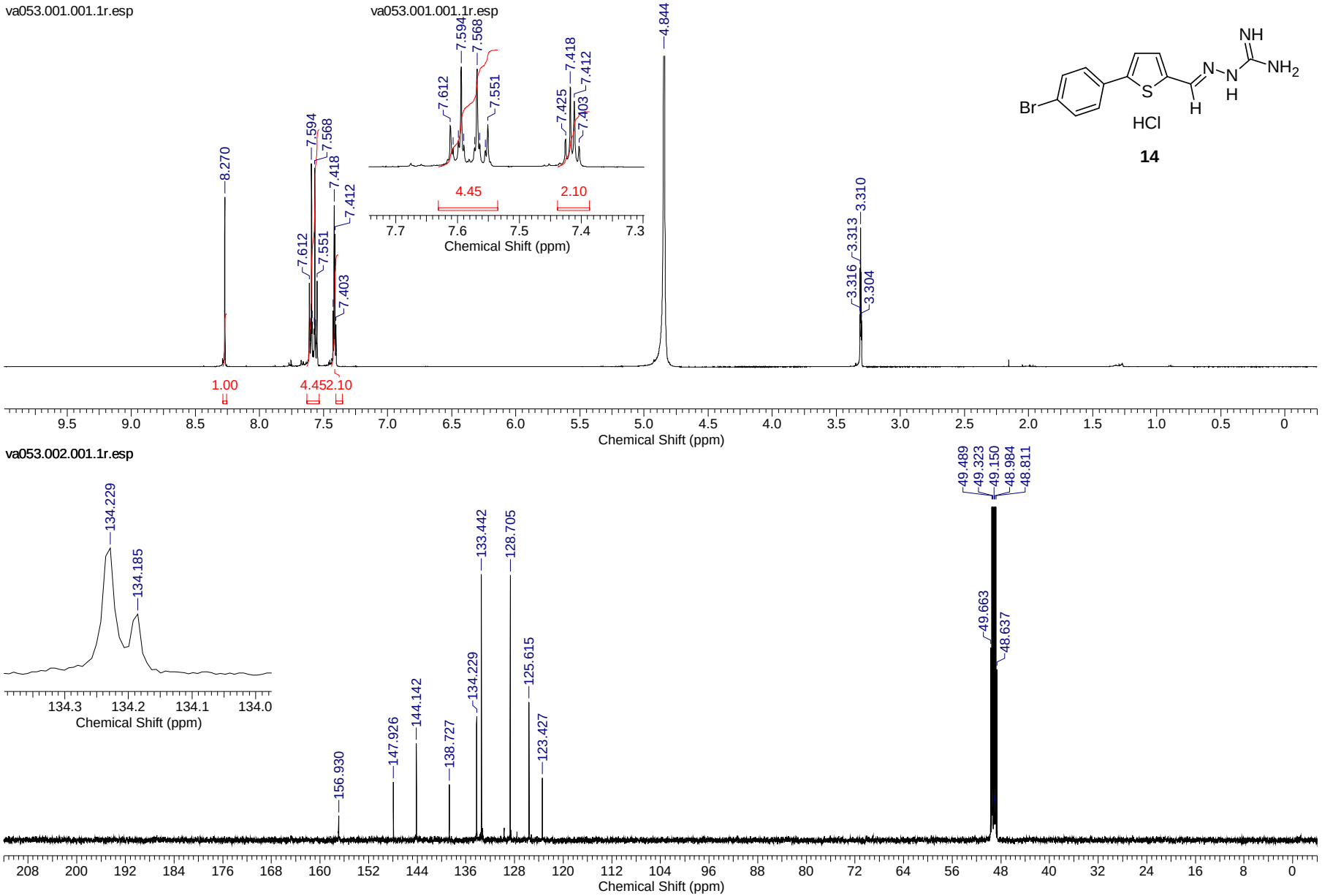

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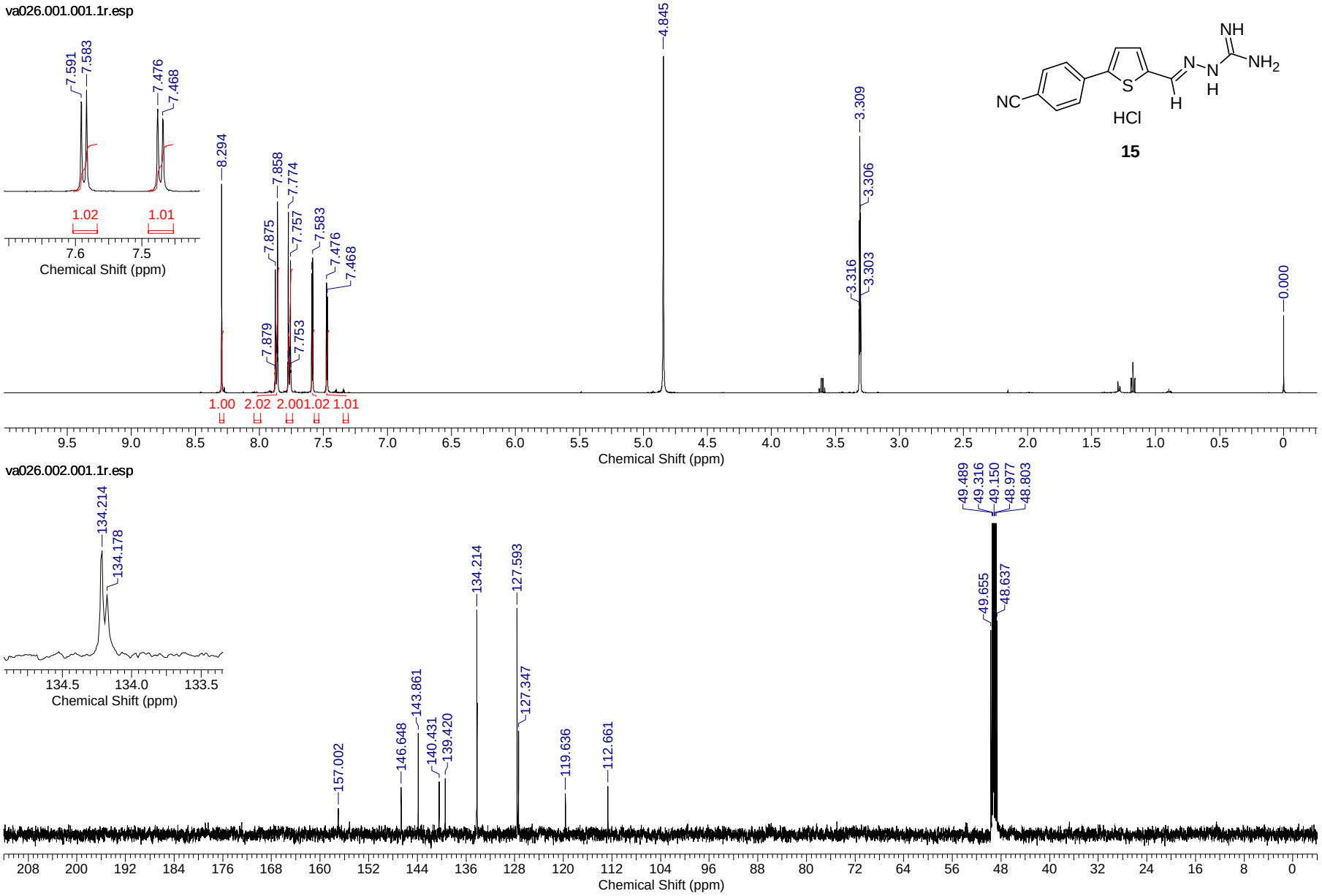
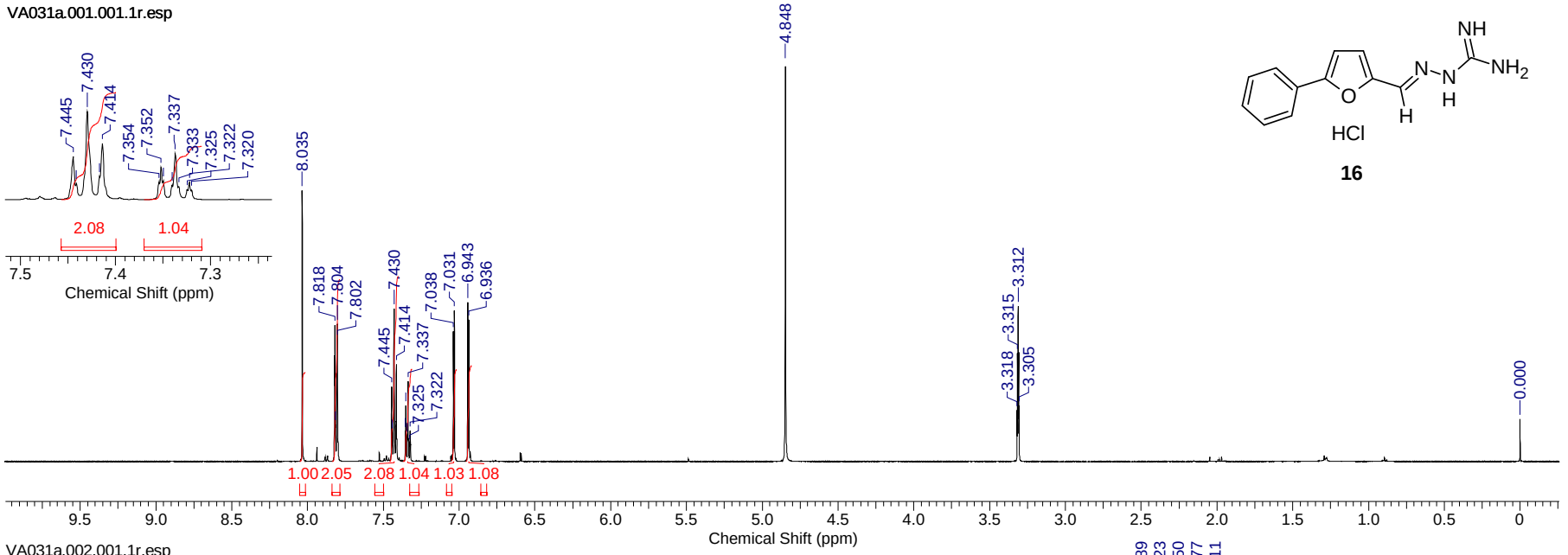
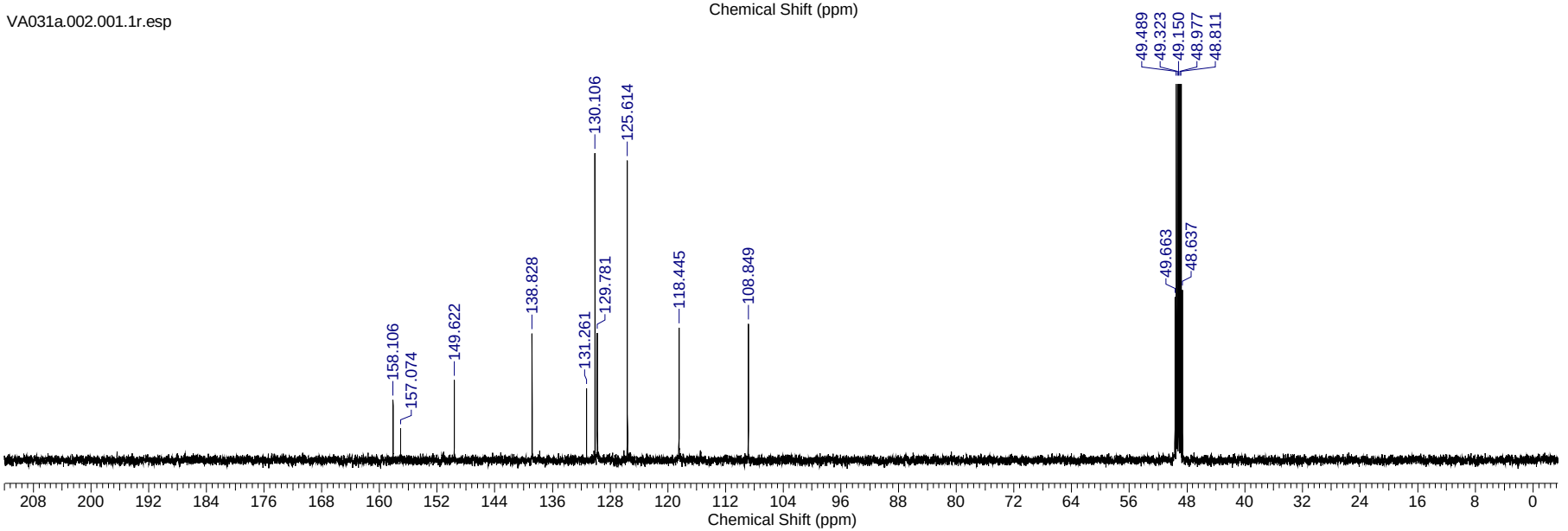
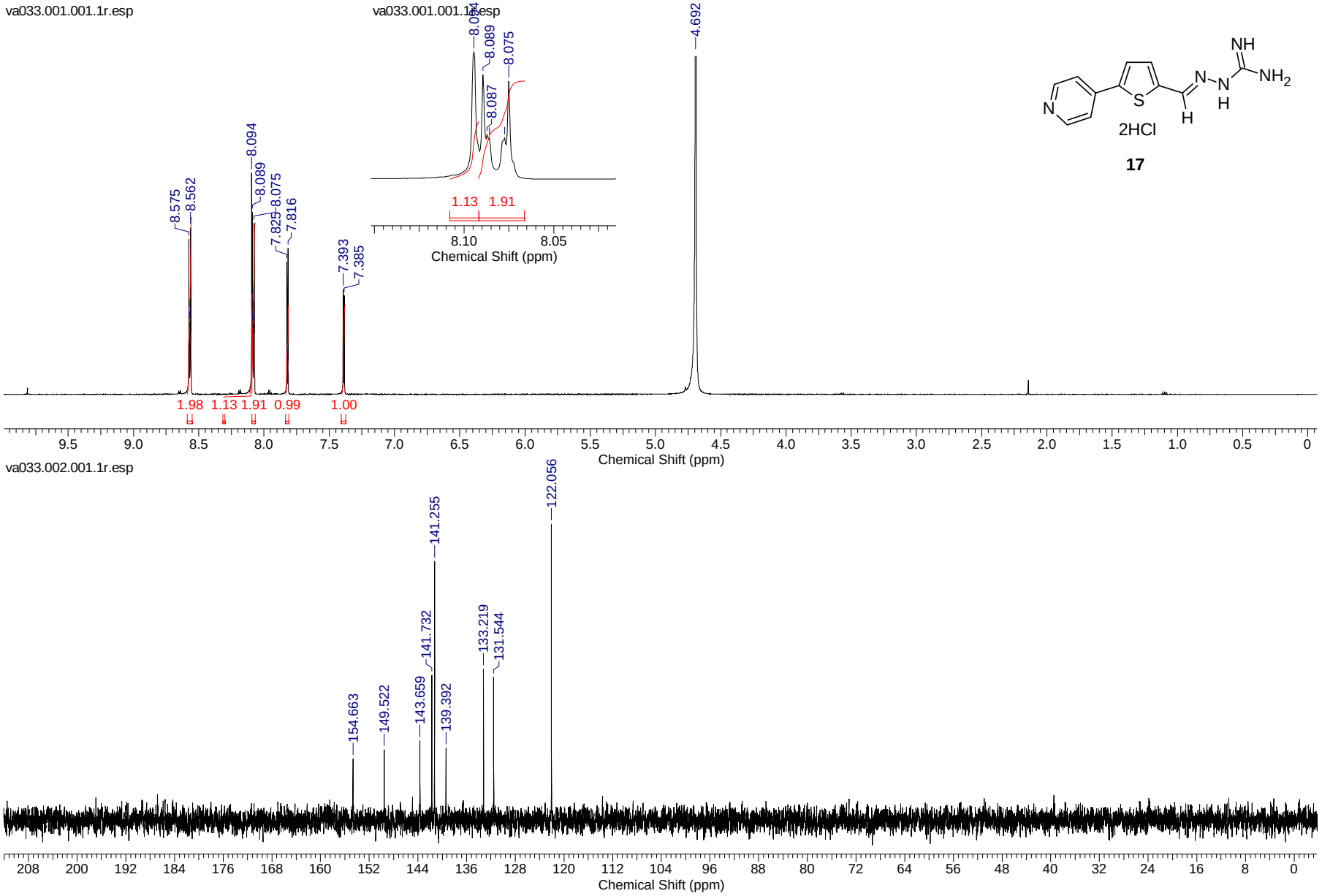


2-[(5-phenylfuran-2-yl)methylidene]hydrazinecarboximidamide hydrochloride (16)



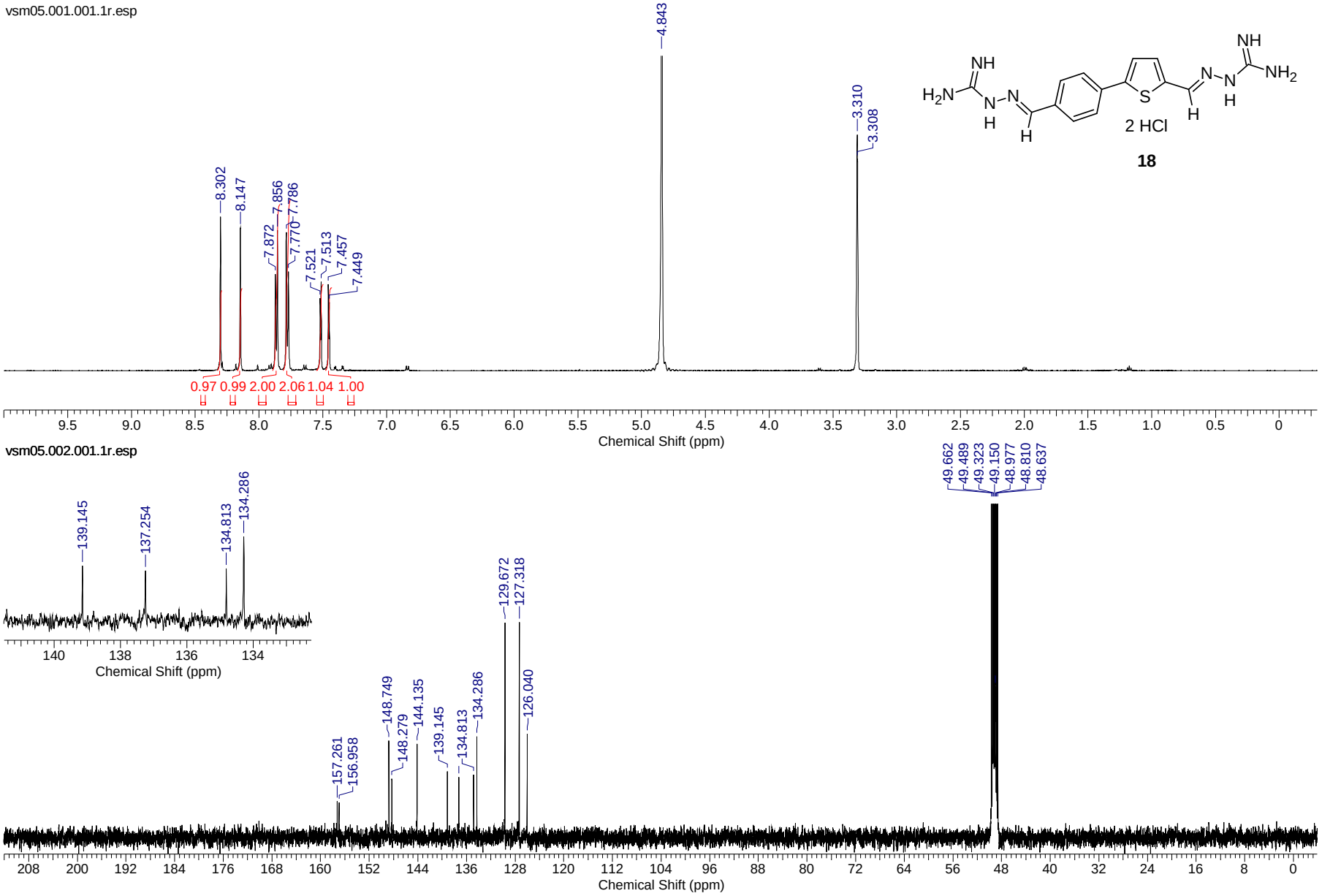


2-{[5-(pyridin-4-yl)thiophen-2-yl]methylidene}hydrazinecarboximidamide dihydrochloride (17)

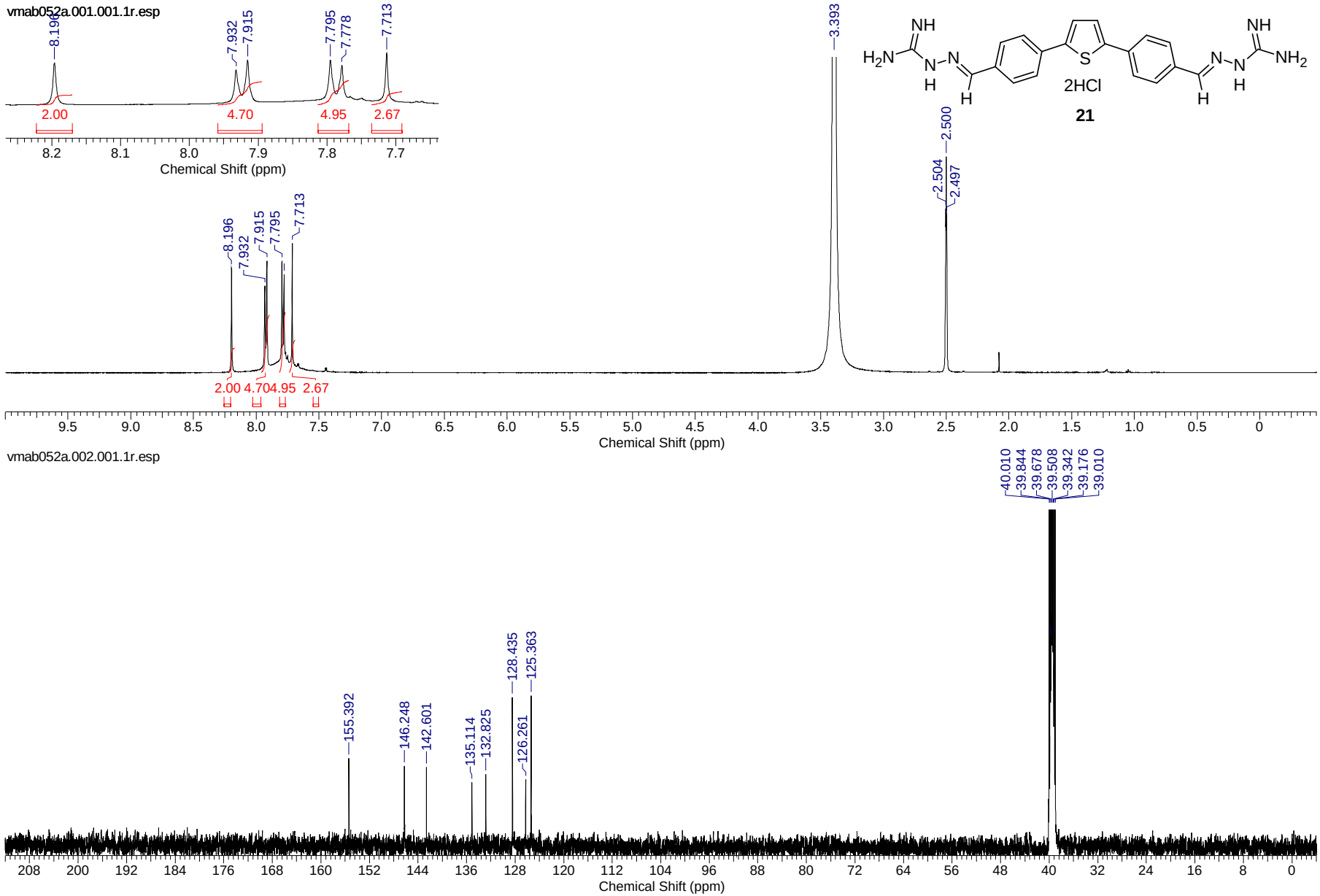


17

2-[(5-{4-[(2-carbamimidoylhydrazinylidene)methyl]phenyl}thiophen-2-yl)methylidene]hydrazinecarboximidamide dihydrochloride (18)



(2,2'-{thiene-2,5-diylbis[benzene-4,1-diylmethylylidene]}dihydrazinecarboximidamide dihydrochloride (21)



HPLC analyses for purity.

Compounds were analyzed for purity (HPLC) using a Agilent 1200 HPLC system equipped with Quat Pump (G1311B), Injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and Detector 1260 DAD VL+ (G1315C). HPLC analysis was performed in two diverse systems:

Method A

Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8µ, S.N. USWKY01594 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were ~ 1mg/mL. Flow rate was 0.5mL/min. Compounds **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A.

Method B

Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8µ, S.N. USWKY01594 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and methanol (B). The analysis were performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were ~ 1mg/mL. Flow rate was 0.5mL/min. Compounds **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A.

Method C

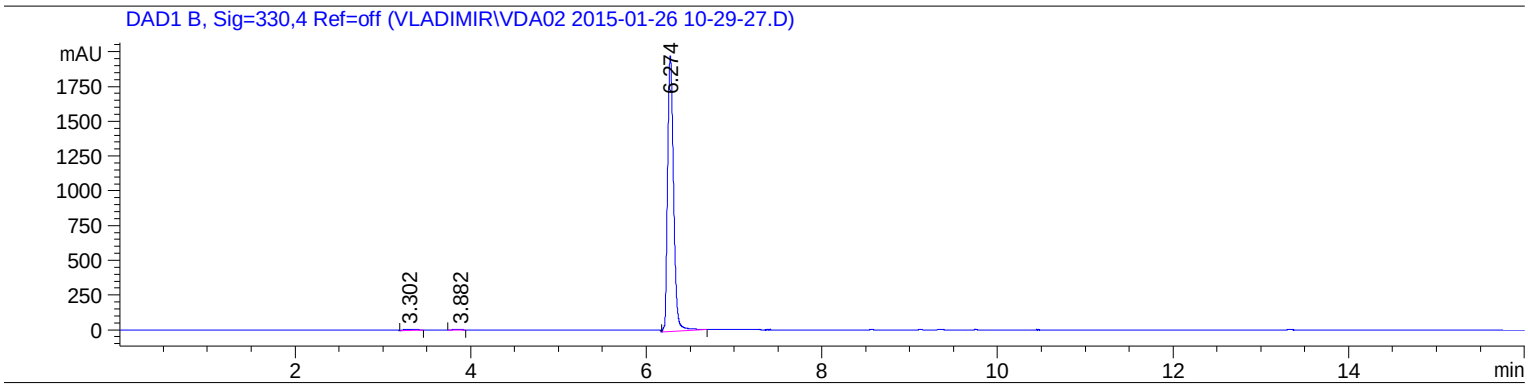
Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8µ, S.N. USWKY01594 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were ~ 1mg/mL.

Flow rate was 0.5mL/min. Compounds **18** and **21** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A.

Method D

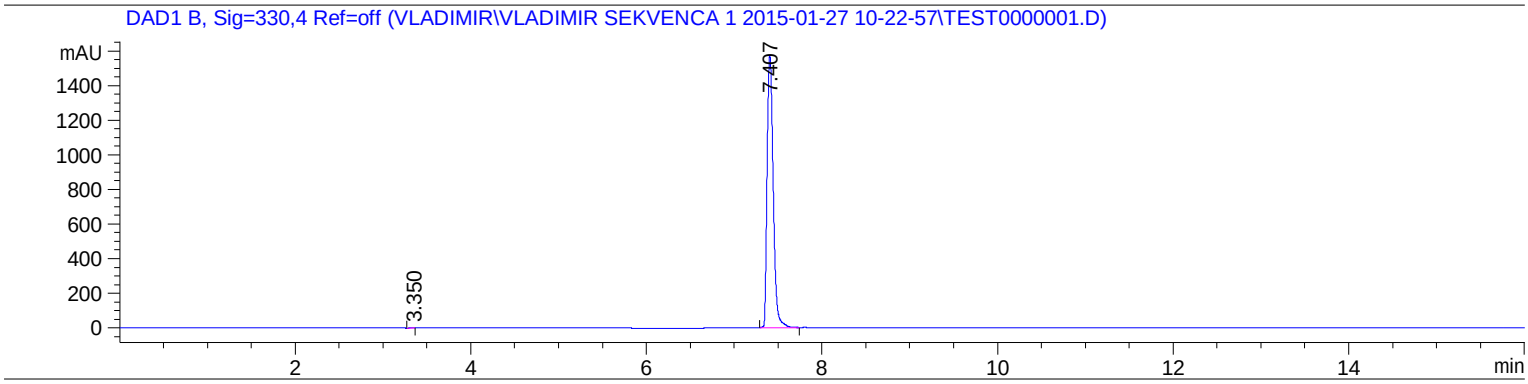
Poroshell 120 EC-C18, 4.6 x 50mm, 2.7µ, S.N. USCFU07797 was used as the stationary phase. Eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). The analysis were performed at the UV max of the compounds to maximize selectivity. Compounds were dissolved in methanol, final concentrations were ~ 1mg/mL. Flow rate was 1 mL/min. Compounds **18** and **21** were eluted using gradient protocol: 0 – 0.5 min 95%A, 0.5 - 3 min 95%A→ 5%A, 3 - 13 min 5%A, 13 – 14 min 5%A→ 95%A, 14 – 16 min 95%A.

Sample Name: 10



Signal 2: DAD1 B, Sig=330,4 Ref=off

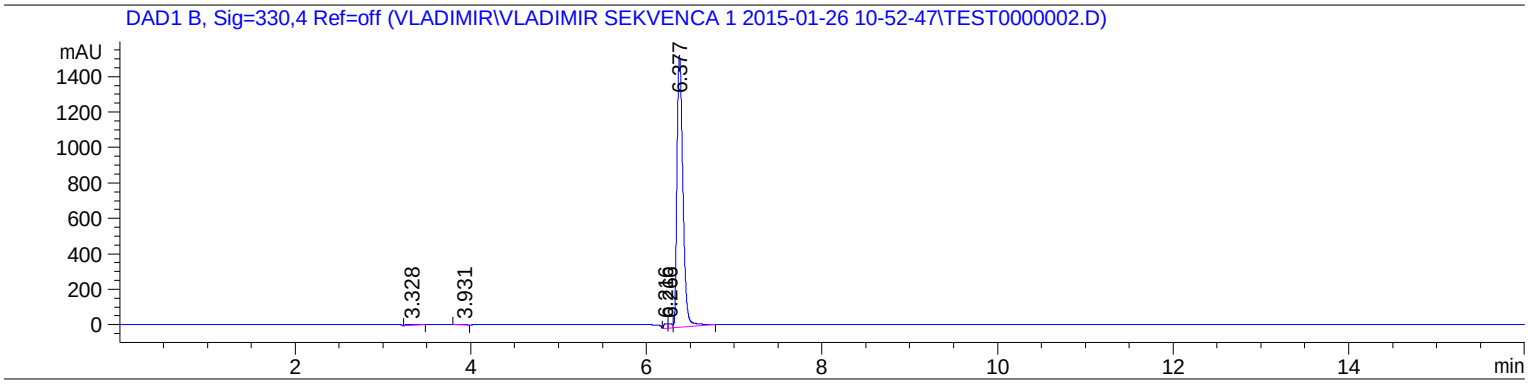
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.302	BB	0.1195	49.38033	4.88304	0.5216
2	3.882	BB	0.0784	18.91366	2.86875	0.1998
3	6.274	BB	0.0748	9398.13281	1981.04675	99.2786
Totals :				9466.42681	1988.79855	



Signal 2: DAD1 B, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.350	BV	0.0557	9.48410	2.07890	0.1247
2	7.407	BV	0.0762	7593.43994	1575.08484	99.8753
Totals :				7602.92404	1577.16374	

Sample Name: 11



Signal 2: DAD1 B, Sig=330,4 Ref=off

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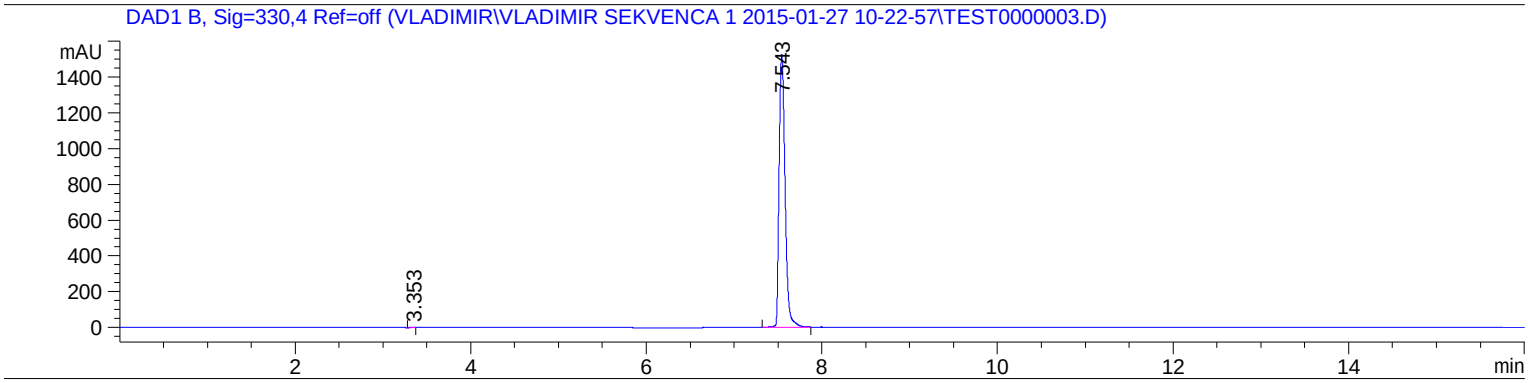
1	3.328	BB	0.1163	44.57111	4.54784	0.5979
2	3.931	BB	0.0739	12.12517	1.96841	0.1626
3	6.216	BV	0.0420	74.38695	26.32635	0.9978
4	6.266	VV	0.0454	74.01833	21.90184	0.9928
5	6.377	VB	0.0741	7250.05371	1536.45154	97.2489
Totals :				7455.15528	1591.19597	



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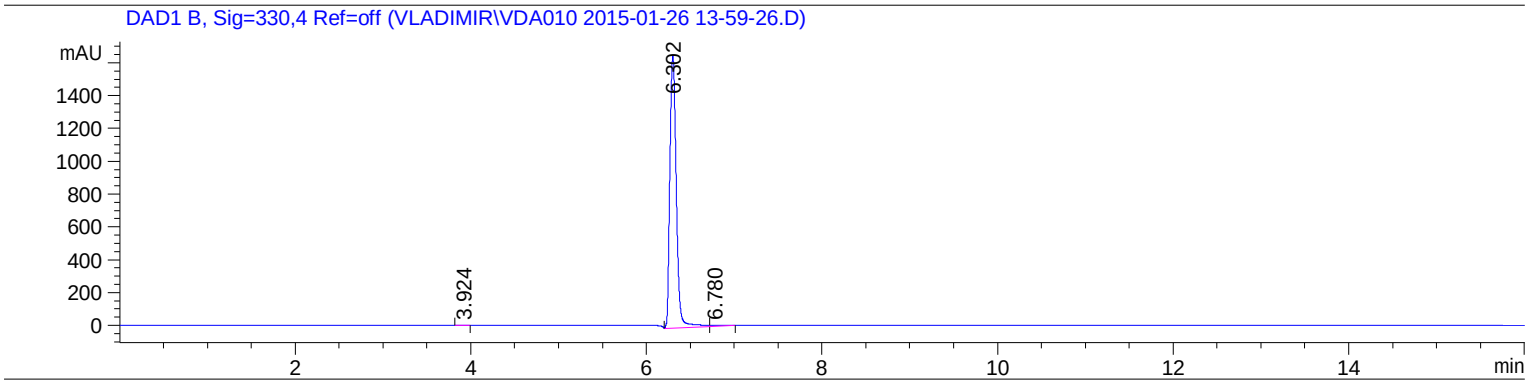
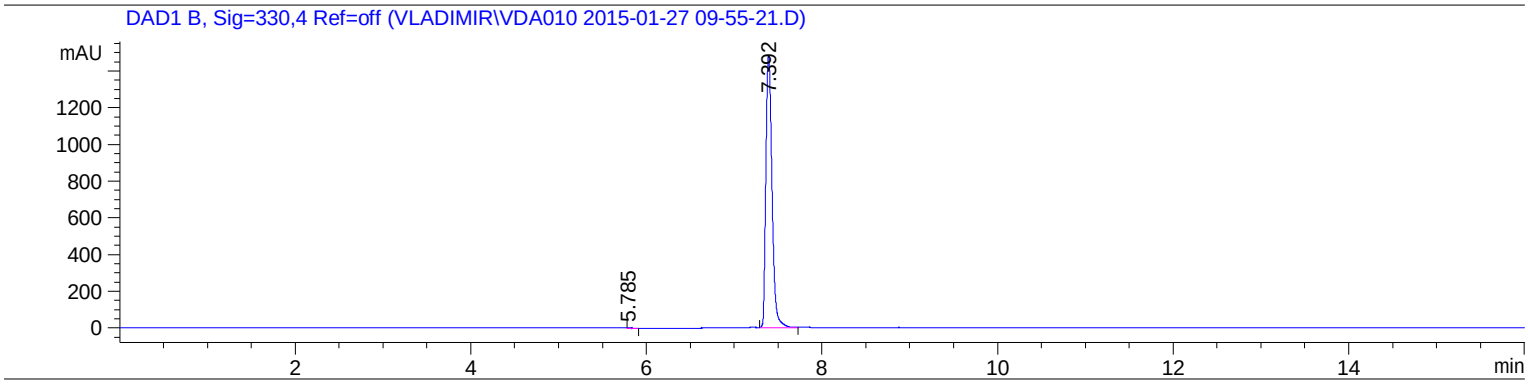
1	3.353	BV	0.0542	9.23323	2.08225	0.1281
2	7.543	VV	0.0746	7199.53369	1525.37170	99.8719
Totals :				7208.76692	1527.45396	

Sample Name: 12



Signal 2: DAD1 B, Sig=330,4 Ref=off

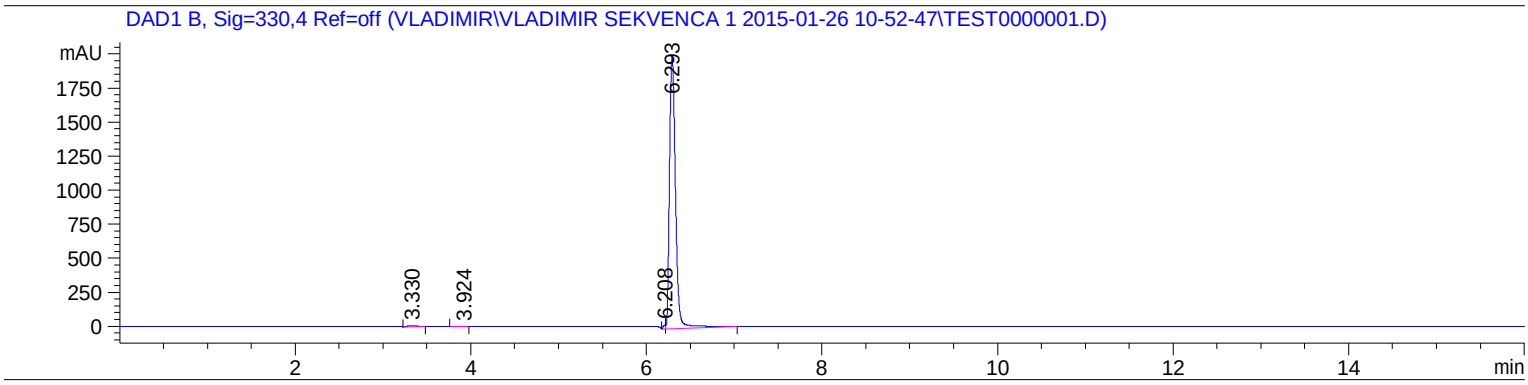
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.924	BB	0.0710	7.27251	1.25710	0.0843
2	6.302	BV	0.0814	8552.31934	1664.55688	99.1009
3	6.780	VB	0.1234	70.31726	6.84459	0.8148
Totals :				8629.90910	1672.65858	



Signal 2: DAD1 B, Sig=330,4 Ref=off

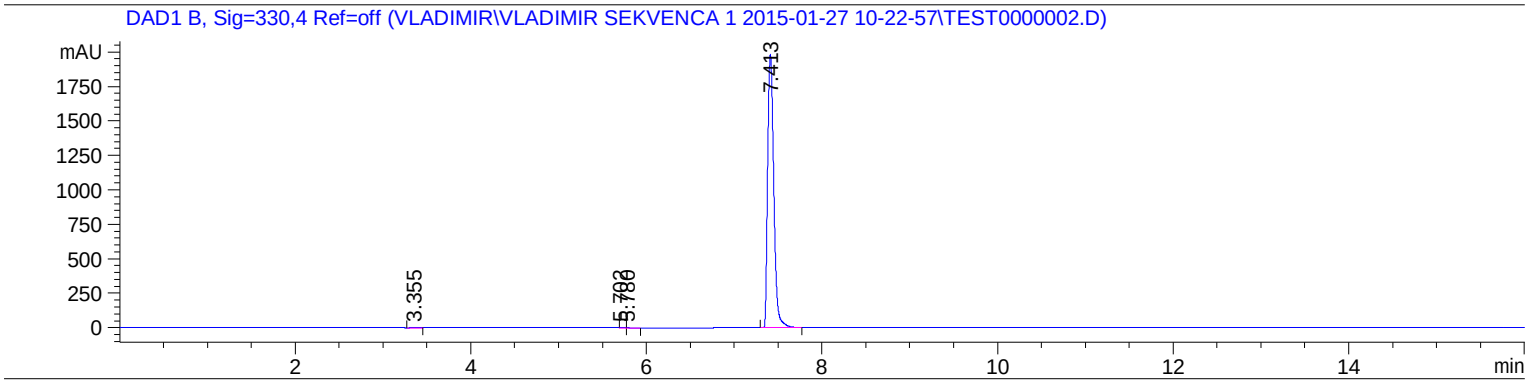
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.785	VB	0.0623	6.58275	1.27393	0.0911
2	7.392	VV	0.0763	7222.63281	1484.15979	99.9089
Totals :				7229.21556	1485.43372	

Sample Name: 13



Signal 2: DAD1 B, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.330	BB	0.1195	45.02869	4.45232	0.4636
2	3.924	BB	0.0840	14.17799	2.05400	0.1460
3	6.208	BV	0.0298	48.47131	26.53604	0.4991
4	6.293	VB	0.0743	9604.24414	2006.67505	98.8913
Totals :				9711.92214	2039.71742	



Signal 2: DAD1 B, Sig=330,4 Ref=off

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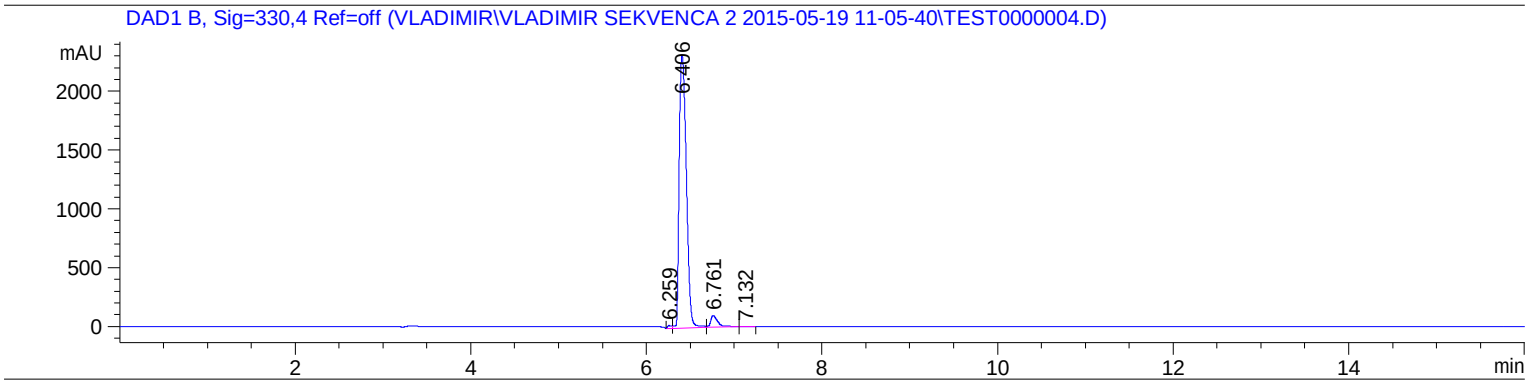
1	3.355	BV	0.0958	20.81204	2.60252	0.2164
2	5.702	VV	0.0629	7.90974	1.51545	0.0823
3	5.780	VB	0.0722	11.06988	1.85420	0.1151
4	7.413	VV	0.0769	9576.01855	1979.12439	99.5862
Totals :				9615.81022	1985.09656	

Sample Name: 14



Signal 2: DAD1 B, Sig=330,4 Ref=off

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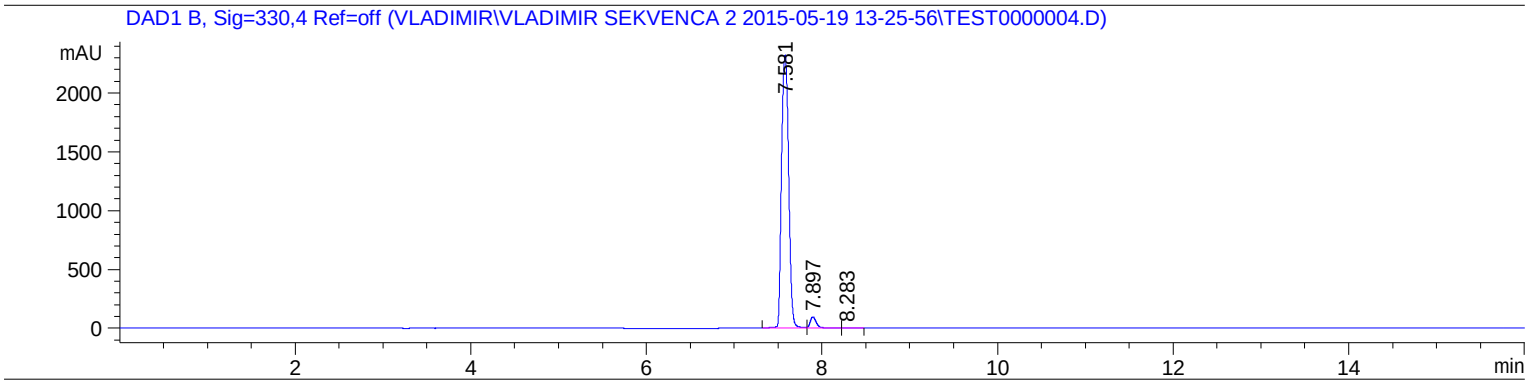
1	6.259	BV	0.0500	85.14626	24.82037	0.6195
2	6.406	VV	0.0923	1.30998e4	2320.94336	95.3077
3	6.761	VB	0.0836	554.59460	98.60591	4.0350
4	7.132	BB	0.0591	5.20267	1.07213	0.0379
Totals :				1.37447e4	2445.44177	



Signal 2: DAD1 B, Sig=330,4 Ref=off

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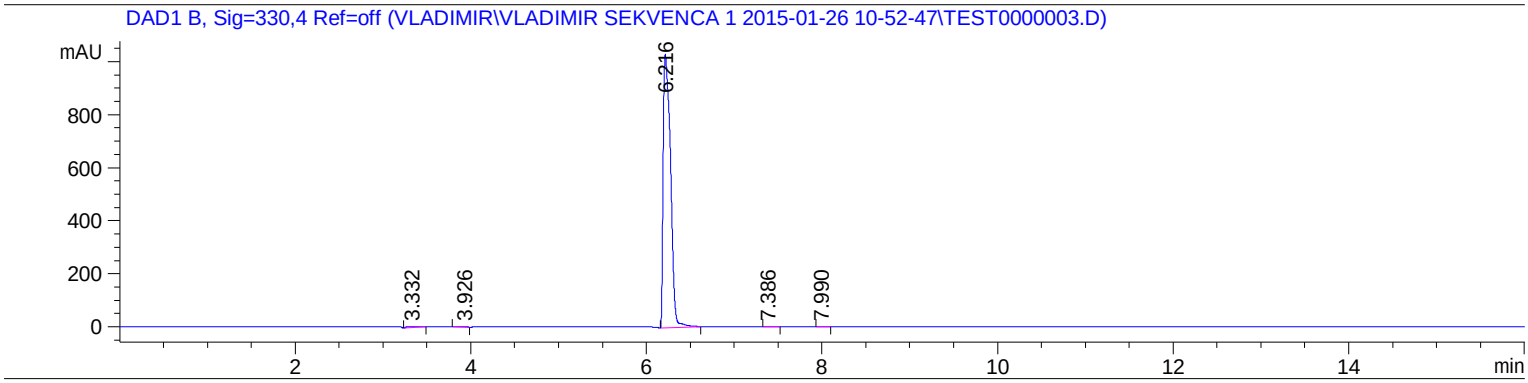
1	7.581	BV	0.0926	1.30506e4	2320.32397	96.5340
2	7.897	VV	0.0763	460.06067	95.27193	3.4030
3	8.283	VB	0.0885	8.50615	1.16775	0.0629
Totals :				1.35191e4	2416.76365	

Sample Name: 15



Signal 2: DAD1 B, Sig=330,4 Ref=off

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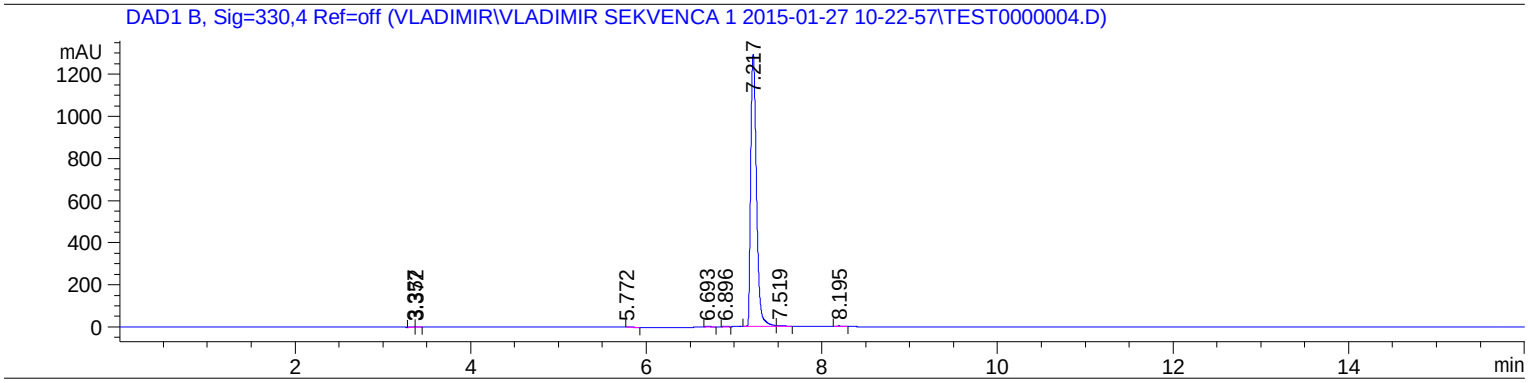
1	3.332	BB	0.1197	44.04861	4.44219	0.7252
2	3.926	BB	0.0800	12.55613	1.86605	0.2067
3	6.216	BB	0.0825	6003.23340	1030.41504	98.8347
4	7.386	BB	0.0546	7.44944	1.82589	0.1226
5	7.990	BB	0.0471	6.72418	1.75800	0.1107
Totals :				6074.01176	1040.30718	



Signal 2: DAD1 B, Sig=330,4 Ref=off

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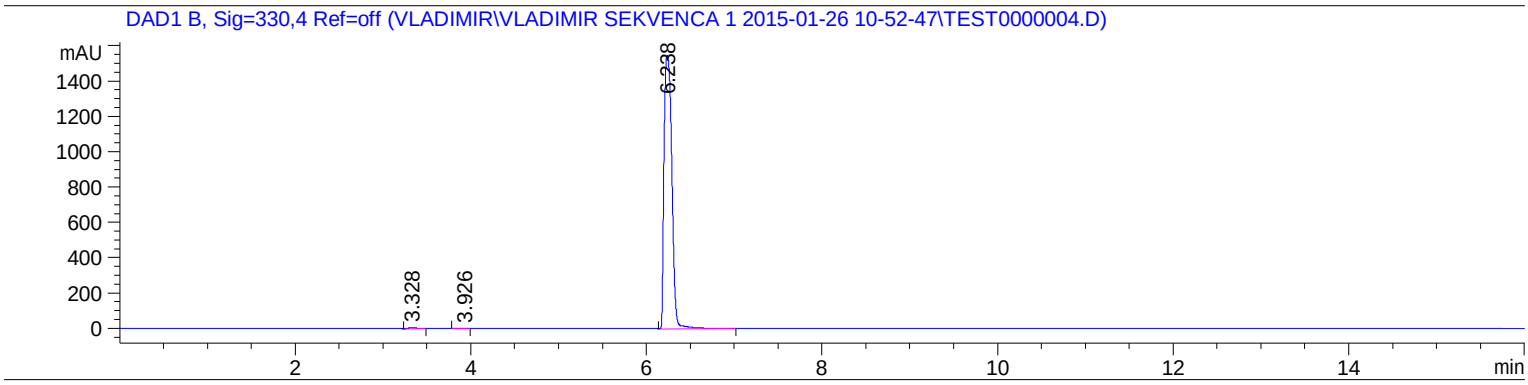
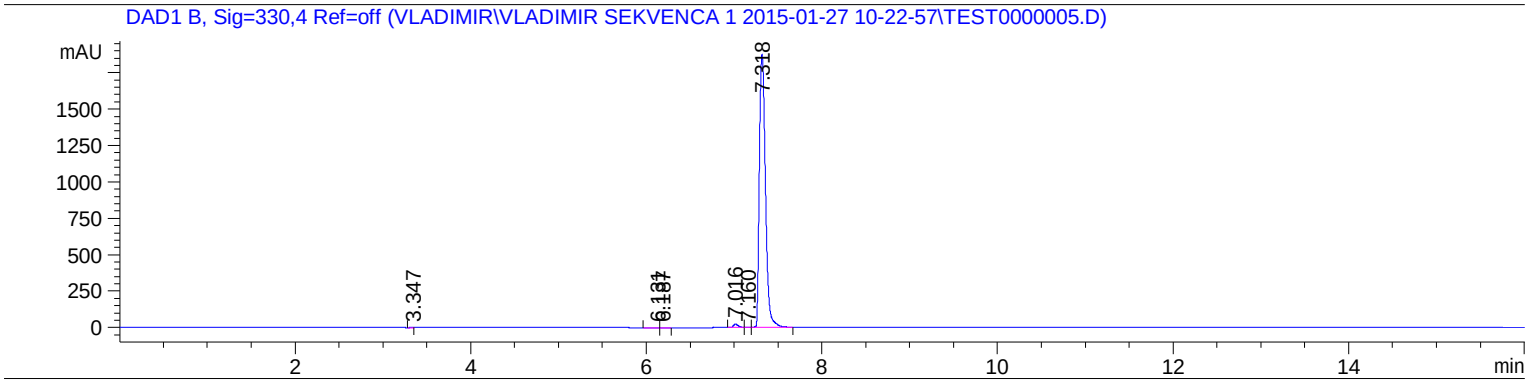
1	3.357	BV	0.0502	8.78893	2.14858	0.1467
2	3.372	VV	0.0405	6.29390	1.93199	0.1051
3	5.772	VB	0.0848	9.48388	1.33696	0.1583
4	6.693	VB	0.0503	16.37825	4.36267	0.2734
5	6.896	BV	0.0444	10.27753	3.16214	0.1716
6	7.217	BV	0.0717	5897.11621	1294.16467	98.4440
7	7.519	VV	0.0722	29.88900	4.96551	0.4990
8	8.195	VB	0.0614	12.09583	3.00990	0.2019
Totals :				5990.32352	1315.08243	

Sample Name: 16



Signal 2: DAD1 B, Sig=330,4 Ref=off

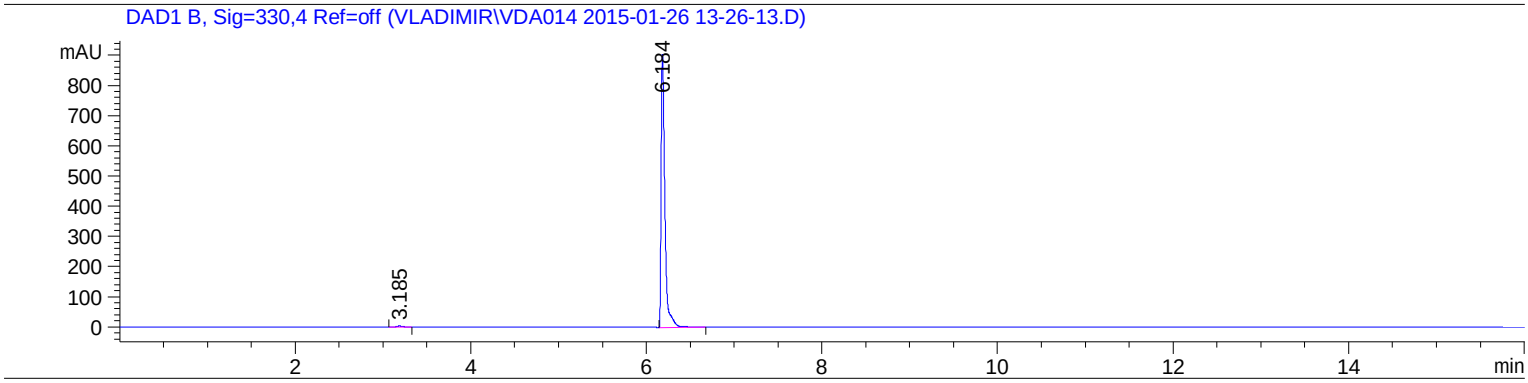
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.328	BB	0.1178	44.44191	4.51776	0.4615
2	3.926	BB	0.0829	13.06229	1.87265	0.1356
3	6.238	BB	0.1017	9572.34180	1546.55591	99.4029
Totals :				9629.84599	1552.94632	



Signal 2: DAD1 B, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.347	BV	0.0403	6.34454	1.93414	0.0693
2	6.131	BV	0.0366	5.95917	2.13038	0.0651
3	6.187	VB	0.0487	8.02346	2.00423	0.0876
4	7.016	BV	0.0552	88.41167	23.62583	0.9657
5	7.160	VV	0.0505	11.44698	2.84109	0.1250
6	7.318	VV	0.0762	9034.82715	1874.22632	98.6872
Totals :				9155.01296	1906.76198	

Sample Name: 17



Signal 2: DAD1 B, Sig=330,4 Ref=off

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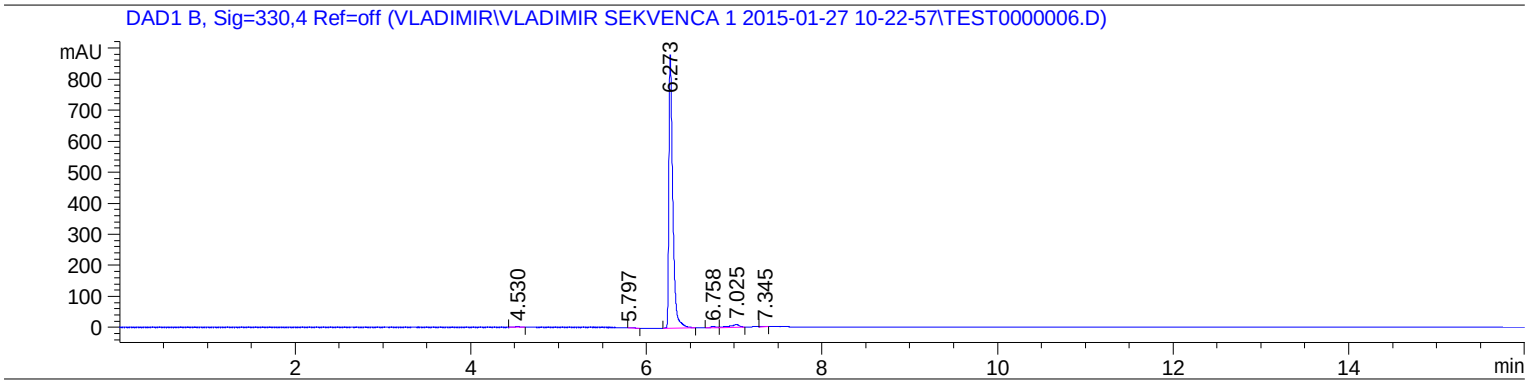
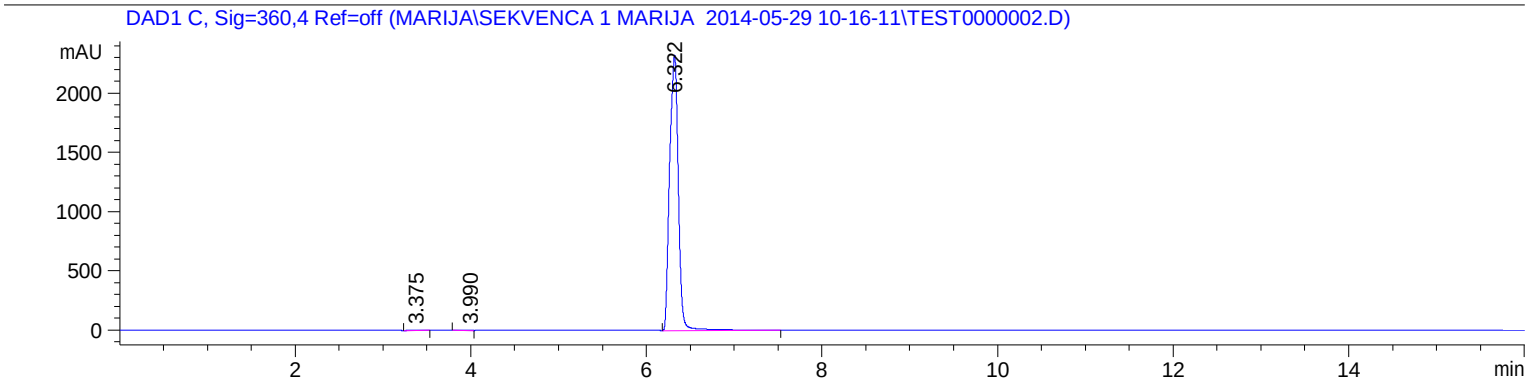
1	3.185	BB	0.0633	13.45634	3.06154	0.4945
2	6.184	BB	0.0449	2707.78149	905.71075	99.5055
Totals :				2721.23783	908.77229	



Signal 2: DAD1 B, Sig=330,4 Ref=off

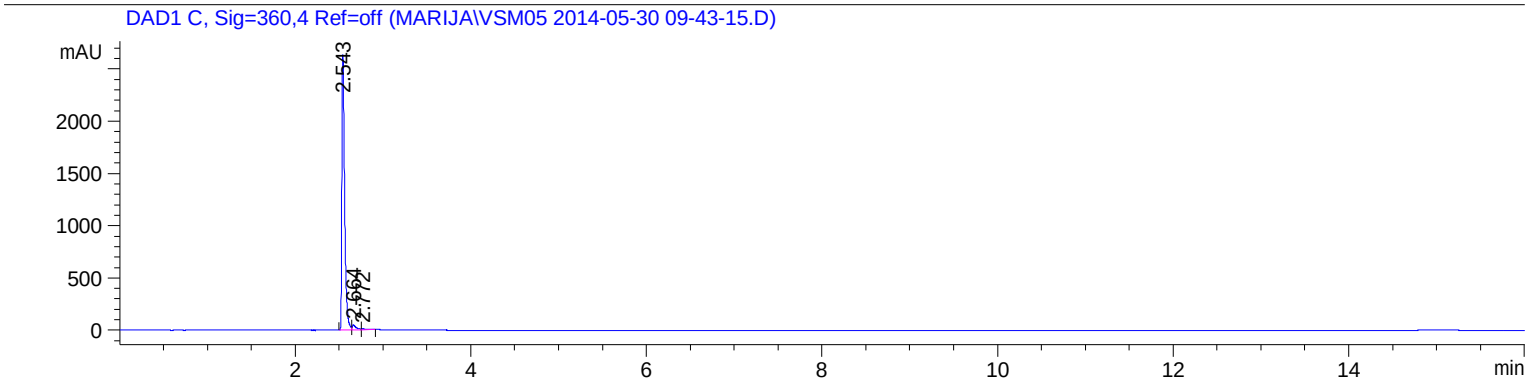
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.530	BV	0.0662	13.22246	2.58311	0.4699
2	5.797	VB	0.0585	7.72265	1.59497	0.2744
3	6.273	BV	0.0454	2711.84814	880.10980	96.3671
4	6.758	BV	0.0531	14.38486	3.63975	0.5112
5	7.025	VV	0.0901	61.49099	8.63360	2.1851
6	7.345	BV	0.0482	5.41134	1.38198	0.1923
Totals :				2814.08044	897.94322	

Sample Name: 18



Signal 3: DAD1 C, Sig=360,4 Ref=off

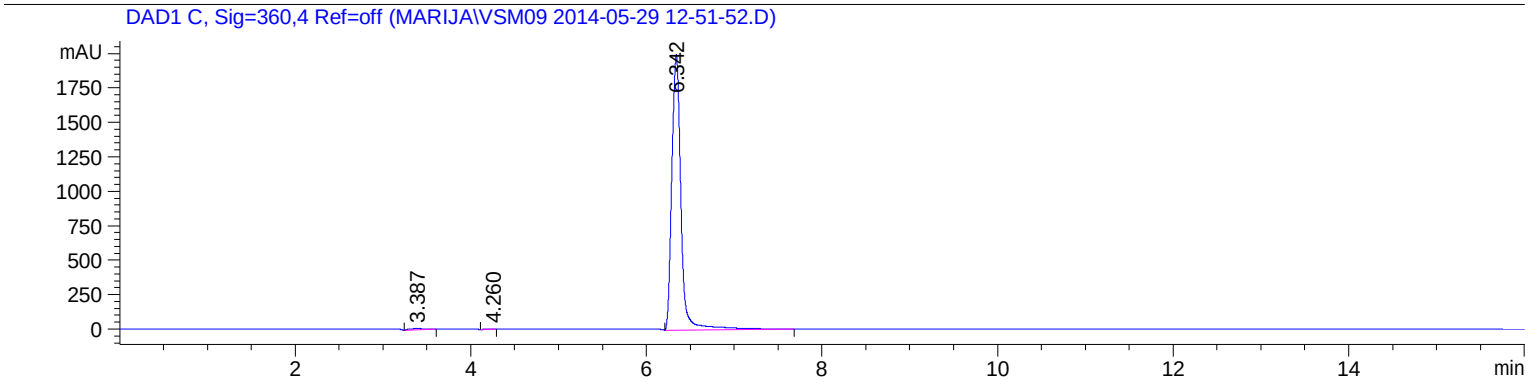
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.375	BB	0.1362	52.79151	4.57361	0.3279
2	3.990	BB	0.1025	24.20163	3.05247	0.1503
3	6.322	BB	0.0984	1.60241e4	2326.64111	99.5218
Totals :				1.61011e4	2334.26719	



Signal 3: DAD1 C, Sig=360,4 Ref=off

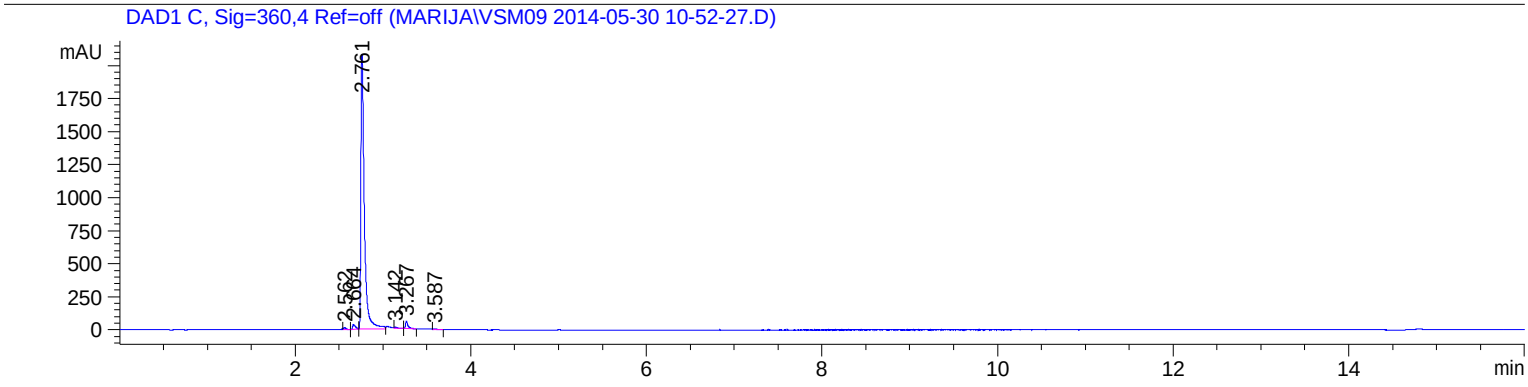
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.543	BV	0.0337	5800.49463	2634.54150	96.8555
2	2.664	VV	0.0439	150.13902	47.42028	2.5070
3	2.772	VB	0.0573	38.17900	8.43770	0.6375
Totals :				5988.81265	2690.39949	

Sample Name: 21



Signal 3: DAD1 C, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.387	BB	0.1649	117.33532	8.48345	0.7937
2	4.260	BB	0.2333	20.86127	1.05235	0.1411
3	6.342	BB	0.1164	1.46447e4	2001.37256	99.0652
Totals :				1.47829e4	2010.90836	



Signal 3: DAD1 C, Sig=360,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.562	VB	0.0296	31.39400	15.55090	0.5281
2	2.664	BB	0.0368	86.51556	37.03198	1.4552
3	2.761	BV	0.0404	5691.38965	2078.74487	95.7303
4	3.142	VB	0.0362	27.87110	10.58320	0.4688
5	3.267	BB	0.0281	102.37231	52.83767	1.7219
6	3.587	VB	0.0340	5.68775	2.45528	0.0957
Totals :				5945.23035	2197.20390	

Table S1 Effect of selected guanylhydrazones in the zebrafish embryo toxicity assay (FET) at 96 hpf

Compound concentration	Dead embryos[a]	Teratogenic embryos[a]	Normal embryos[a]	Growth retardation[b]	Notochord[b]	Eyes[b]	Otoliths[b]	Pericardial edema[b]	Yolk edema[b]	Heart beat[b]	Blood circulation[b]	Unhatched[b]	Head malformation[c]	Skeletal deformities[c]	Tail tip[c]
Voriconazole															
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
10 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
5 µg/mL	80.00	20.00	0.00	100.00	0.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	100.00	100.00	0.00
2.5 µg/mL	36.67	63.33	0.00	100.00	0.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00	100.00	94.74	0.00
11															
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
10 µg/mL	50.00	50.00	0.00	100.00	0.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00	100.00	6.67	0.00
5 µg/mL	20.00	80.00	0.00	0.00	0.00	0.00	62.50	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
2.5 µg/mL	10.00	6.67	56.67	0.00	0.00	0.00	0.00	7.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14															
50 µg/mL	100.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-
25 µg/mL	73.33	26.67	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
10 µg/mL	30.00	23.33	46.67	0.00	0.00	0.00	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 µg/mL	10.00	10.00	80.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.5 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18															
50 µg/mL	3.33	0.00	96.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25 µg/mL	3.33	0.00	96.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.5 µg/mL	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Abrevitation used: (hpf) hours post fertilisation; (-) data not available due to 100% mortality.

[a] Percentage of mortality based on all eggs.

[b] Percentage of teratogenic effect based on all alive embryos at the time of assessment.

[c] Percentage of teratogenic effect based on all alive hatched embryos at the time of assessment.

Table S2 Minimal biofilm eradication concentrations (MBEC) against mature biofilms three *Candida* strains (μg mL^{-1}).

Compd.	Strain		
	C. albicans ATCC10231	*C. krusei* ATCC34135	*C. parapsilosis* CA27
3	>1000	>1000	>1000
10	1000	>1000	>1000
11	125	250	125
12	500	1000	1000
13	>1000	>1000	>1000
14	125	125	125
15	250	>1000	1000
16	500	500	1000
17	1000	>1000	>1000
18	250	500	500
21	250	1000	1000
VOR[a]	250	250	R

[a]Control drug: VOR as a voriconazole.

Table S3 Molecular docking data for selected guanylhydrazones.

Compd.	Docking Score	Glide evdw (kcal mol^{-1})	Glide ecoul (kcal mol^{-1})	Glide Energy (kcal mol^{-1})	Glide Emodel (kcal mol^{-1})	Hbond (kcal mol^{-1})
			C. albicans CYP51			
11	-5.336	-35.244	-2.305	-37.549	-47.964	0.000
15	-5.324	-40.027	-0.187	-40.258	-55.270	-0.663
13	-5.229	-34.873	-2.998	-37.871	-48.577	-1.758
17	-4.953	-34.340	-4.213	-38.553	-57.370	-0.661
10	-2.949	-36.202	-5.277	-41.478	-58.695	-1.330
14	-2.701	-39.549	-2.899	-42.449	-55.012	-1.330
			Human CYP51			
13	-5.617	-33.981	-9.564	-43.545	-63.410	-0.991
10	-5.509	-35.150	-6.522	-41.672	-53.597	-0.907
15	-5.482	-34.258	-14.320	-48.578	-64.878	-1.571
3	-5.007	-24.995	-3.180	-28.175	-34.964	-1.349
11	-4.609	-38.551	-5.779	-44.330	-48.185	-0.766
14	-4.295	-25.987	-2,275	-28.262	-45.407	-1.098

Table S4 Calculated molecular properties of studied compounds used for the assessment of druglikeness ("Rule of five" ≤ 5)

Comp.	miLogP[a]	TPSA[b]	N_{atoms}[c]	MW[d]	N_{ON}[e]	N_{OHNH}[f]	$N_{viol.}$[g]	$N_{rotb.}$[h]	Vol[i]
3	3.54	17.07	13	188.25	1	0	0	2	165.15
10	2.96	74.27	17	244.32	4	4	0	4	215.41
11	3.41	74.27	18	258.35	4	4	0	4	231.97
12	3.02	83.5	19	274.35	5	4	0	5	240.96
13	3.12	74.27	18	262.31	4	4	0	4	220.34
14	3.77	74.27	18	323.22	4	4	0	4	233.3
15	2.71	98.06	19	269.33	5	4	0	4	232.27
16	2.32	87.41	17	228.25	5	4	0	4	206.27
17	1.67	87.16	17	245.31	5	4	0	4	211.26
18	2.17	148.53	23	328.4	8	8	1	7	284.66
21	3.96	148.53	29	404.5	8	8	1	8	356.07
VOR[j]	1.49	76.73	25	349.32	6	1	0	5	285.11

[a]Octanol–water partition coefficient. [b]Topological polar surface area (Å^2). [c]Number of nonhydrogen atoms. [d]Molecular weight. [e]Number of hydrogen-bond acceptors (O and N atoms). [f]Number of hydrogen-bond donors (OH and NH groups). [g]Number of "Rule of five" violations. [h]Number of rotatable bonds. [i]Molecular volume (Å^3). [j]Control drug: VOR as a voriconazole.