

Supporting Information

Novel 1,4-Dihydropyridines as Specific Binders and Activators of SIRT3 Impair Cell Viability and Clonogenicity, and Downregulate Hypoxia-Induced Targets in Cancer Cells

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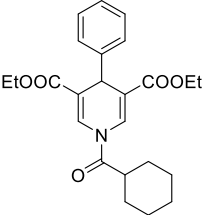
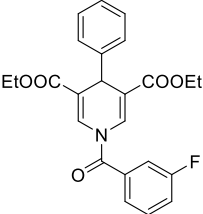
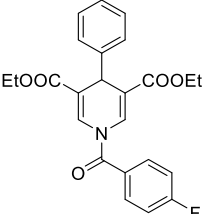
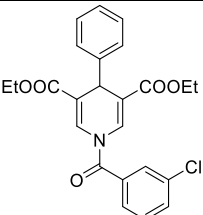
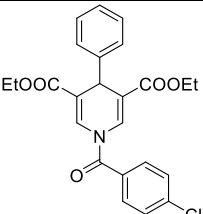
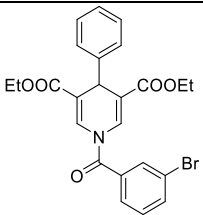
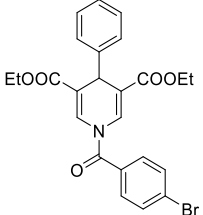
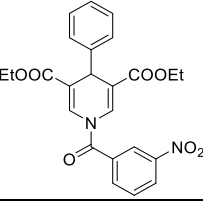
(M. Tafani) E-mail address: marco.tafani@uniroma1.it

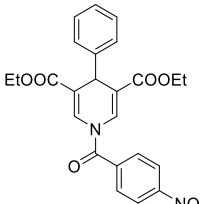
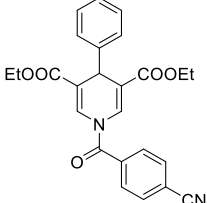
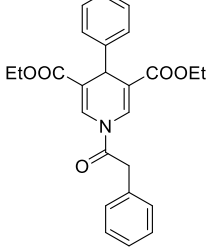
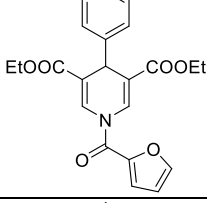
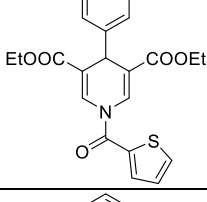
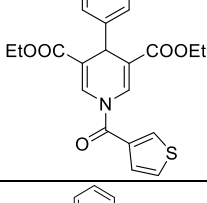
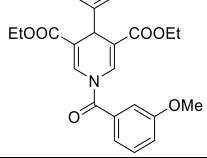
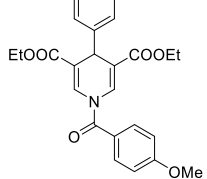
(S. Valente) E-mail address: sergio.valente@uniroma1.it

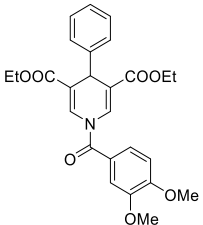
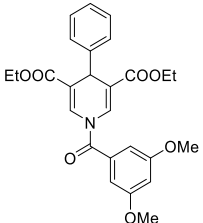
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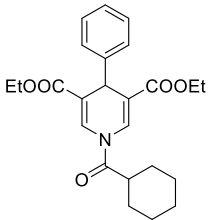
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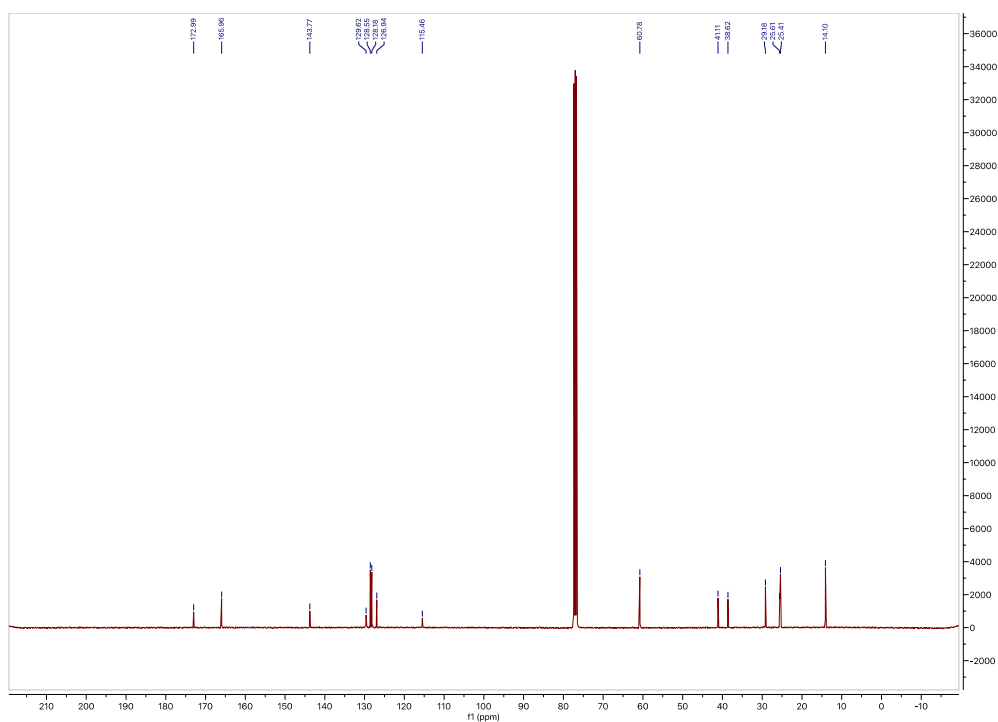
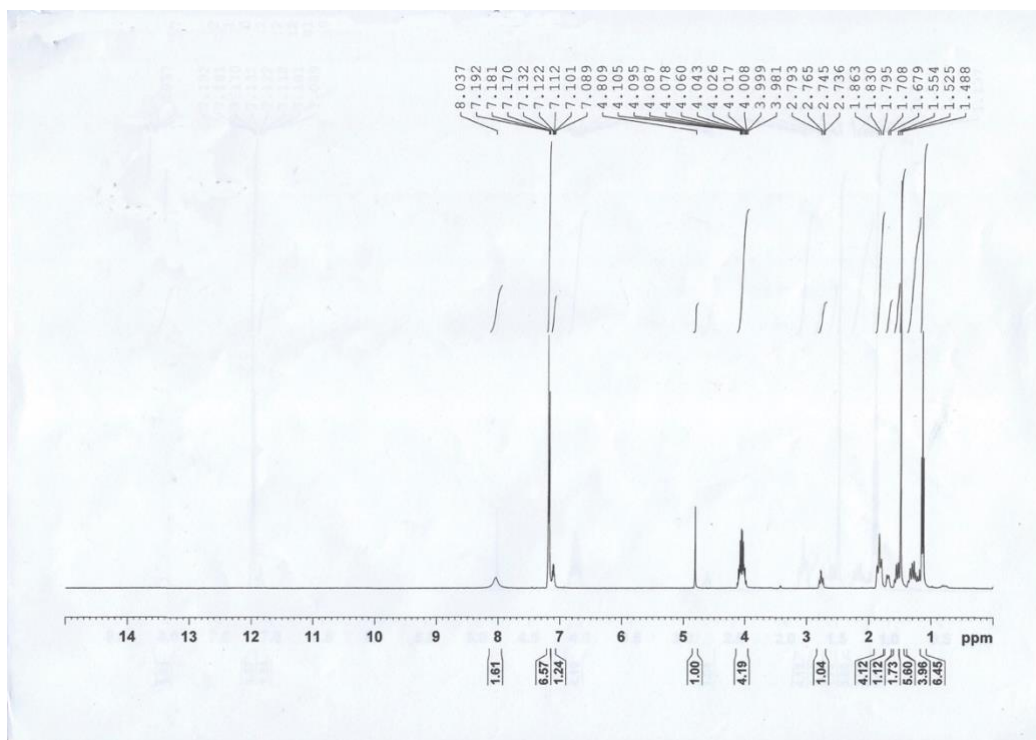
Bayreuth code	Lab Code	Compound	Chemical structure	M.P. (°C)	Yield
UBCS392	MC4199	2a		92-93°C	17.0%
UBCS399	MC4158	2b		124-125°C	85,2 %
UBCS400	MC4168	2c		123-124°C	93,6 %
UBCS401	MC4172	2d		154-155°C	61.8 %
UBCS402	MC4166	2e		122-123°C	65.0%
UBCS403	MC4181	2f		154-155°C	68,7 %
UBCS404	MC4185	2g		140-141°C	71,6 %
UBCS393	MC4165	2h		147-148°C	63,1 %

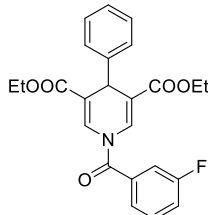
UBCS394	MC4167	2i		109-110°C	65,6 %
UBCS405	MC4177	2j		155-156°C	60,4 %
UBCS409	MC4208	2k		153-154°C	14,1 %
UBCS408	MC4204	2l		125-126°C	12,2 %
UBCS406	MC4188	2m		142-143°C	56,4%
UBCS407	MC4186	2n		114-115°C	51,2%
UBCS395	MC4164	3a		134-135°C	58,2 %
UBCS396	MC4182	3b		131-132°C	17,9%

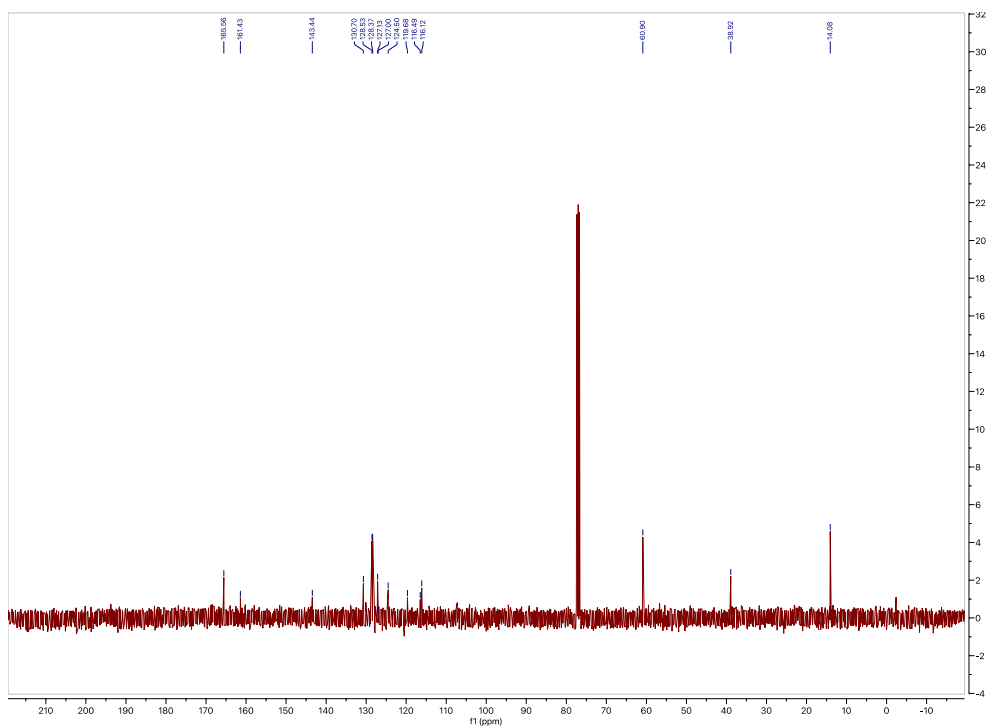
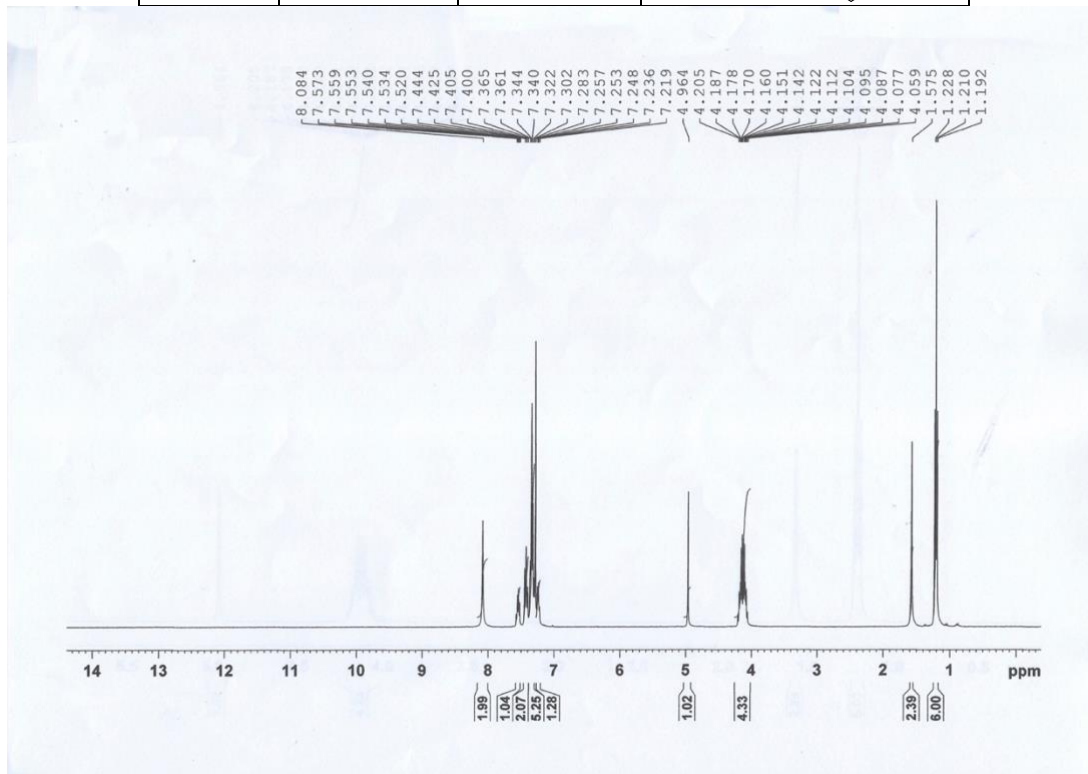
UBCS398	MC4175	3c		174-175°C	27,6 %
UBCS397	MC4176	3d		159-160°C	64, 3%

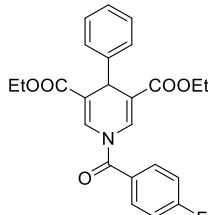
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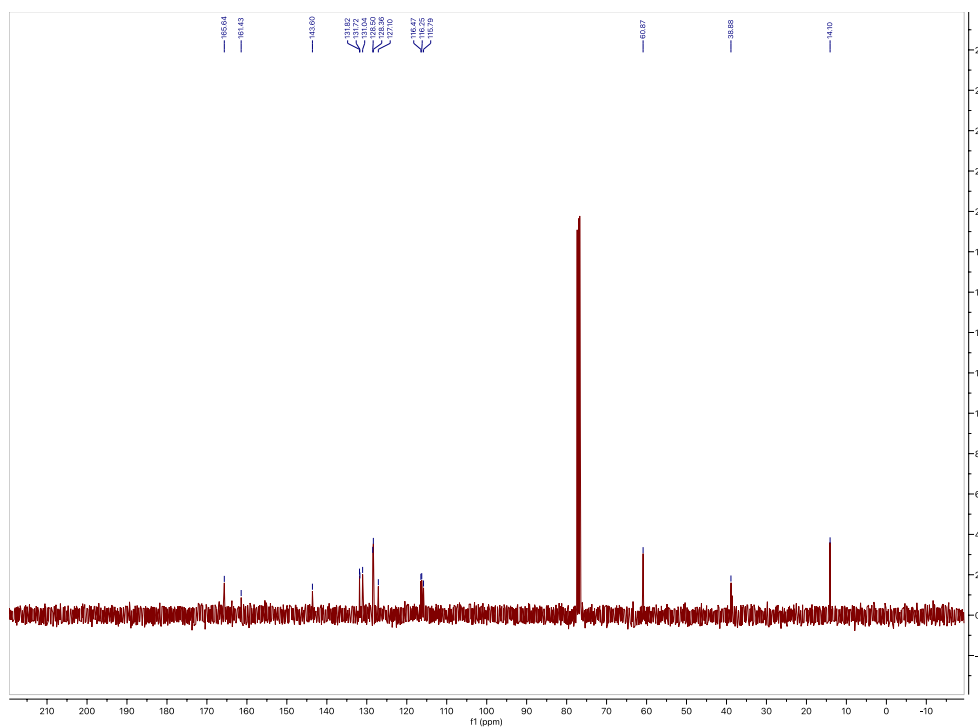
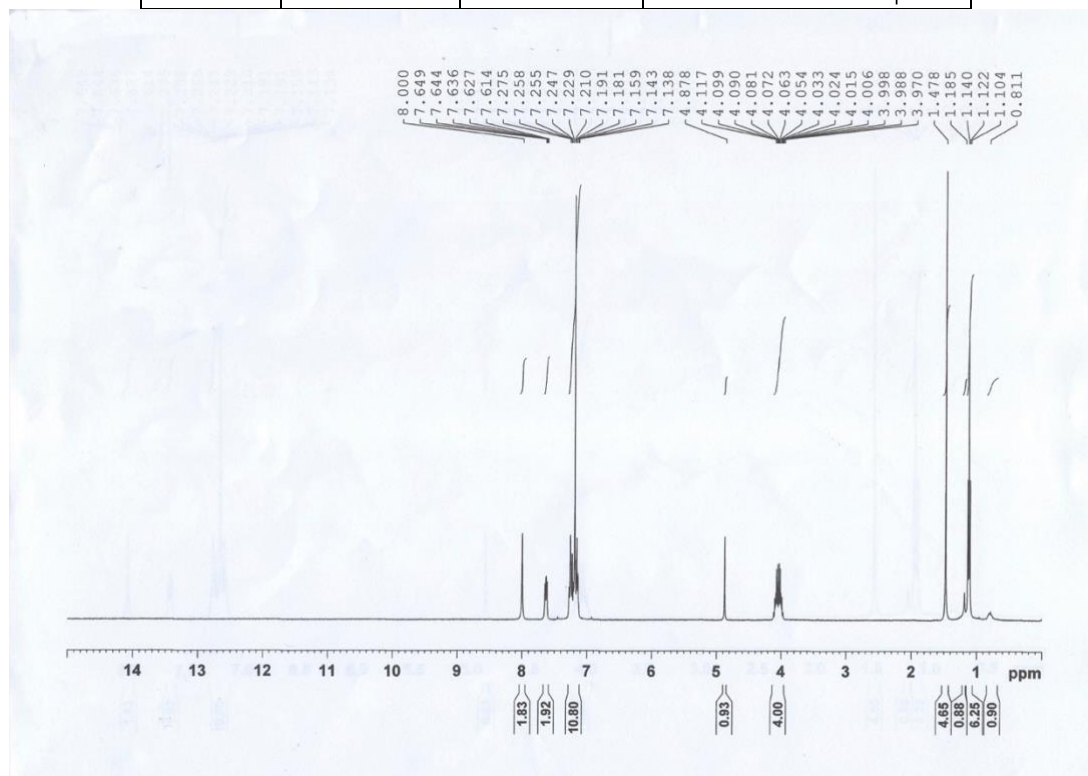
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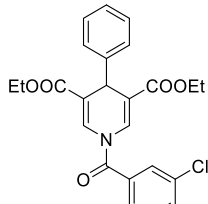


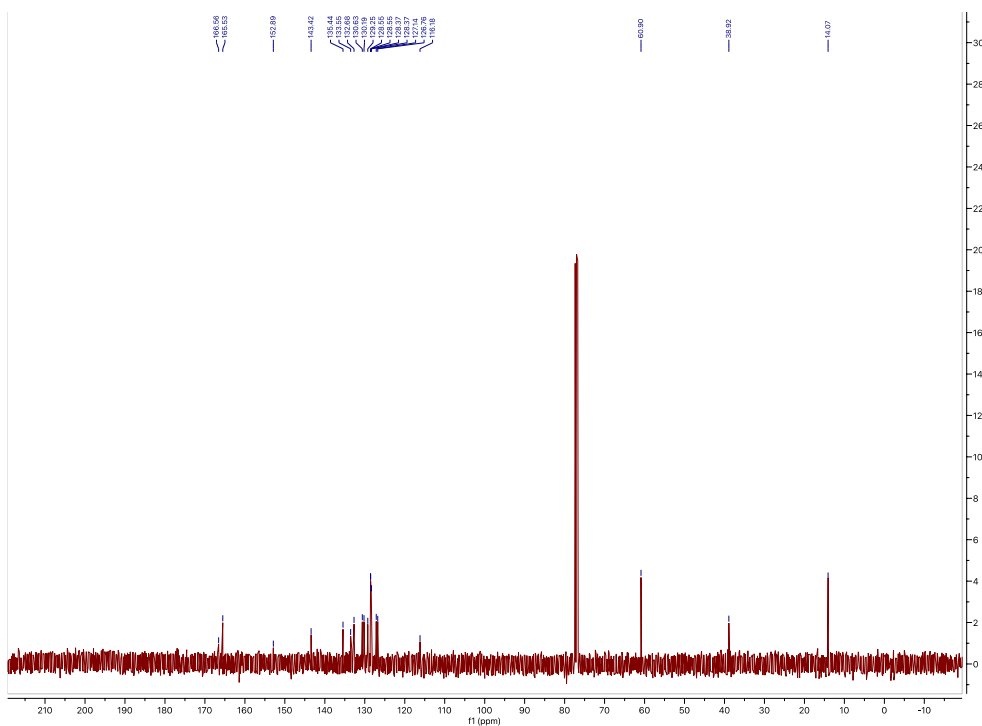
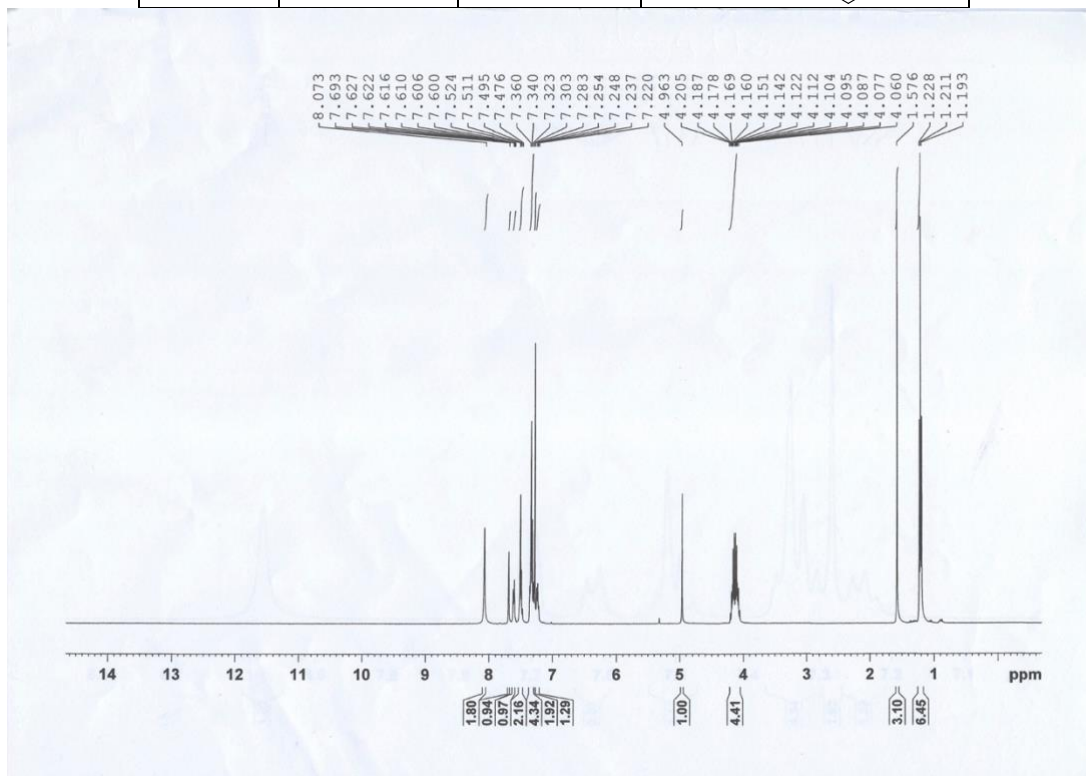
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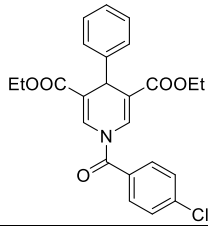


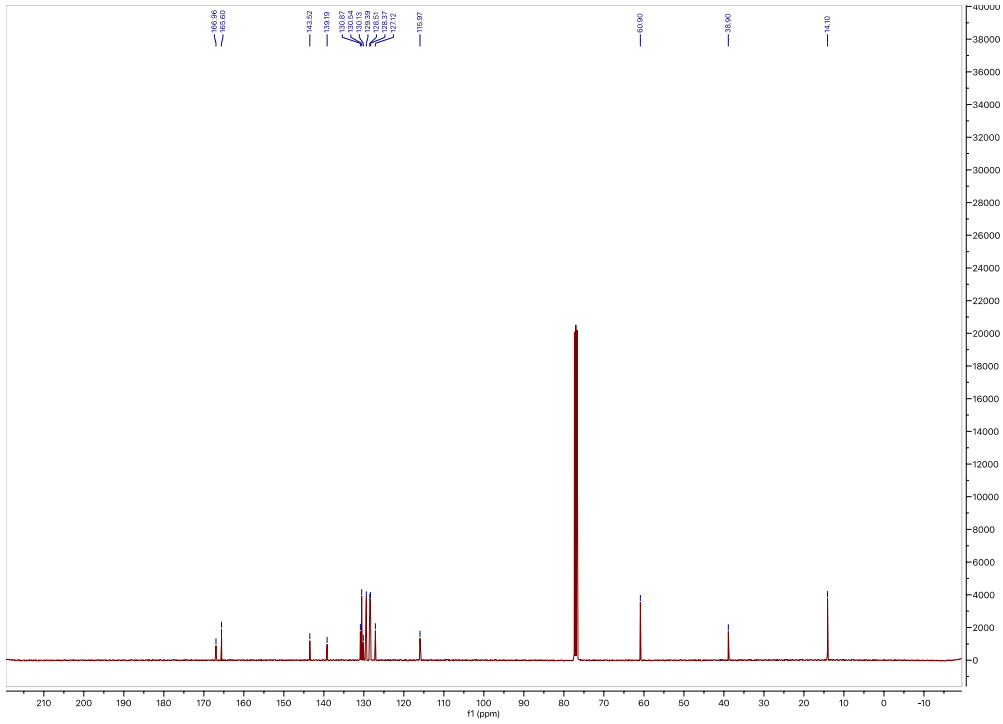
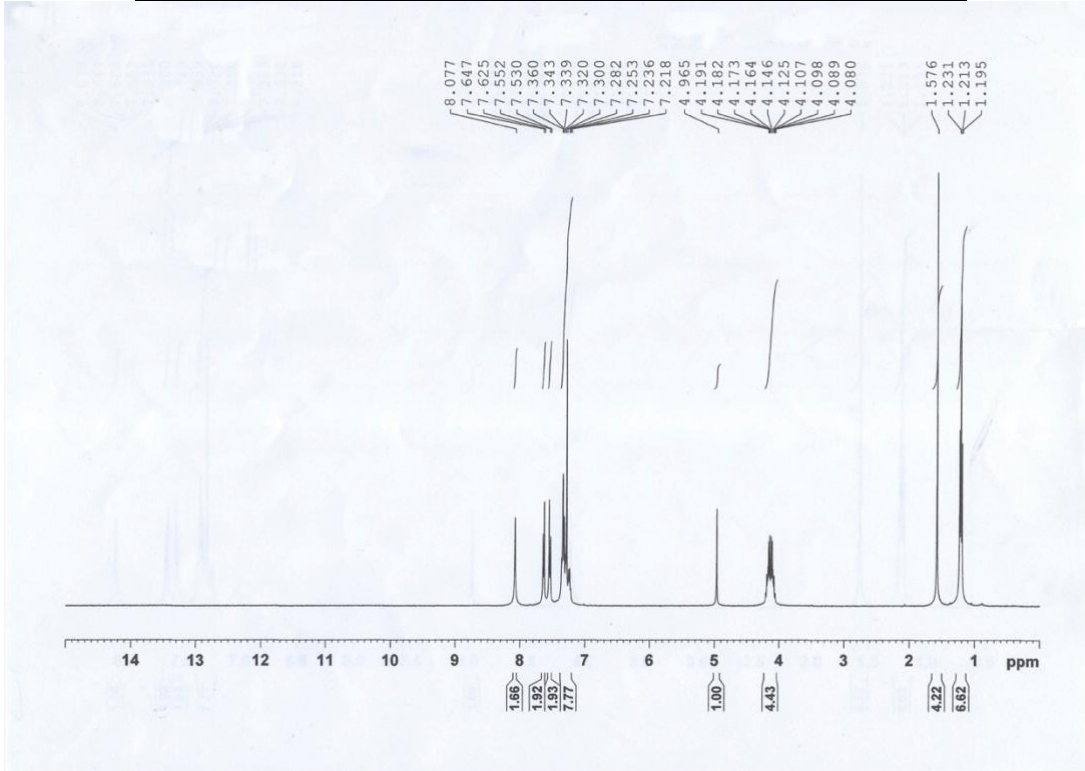
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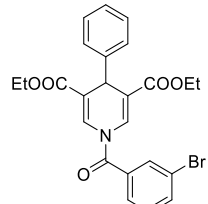


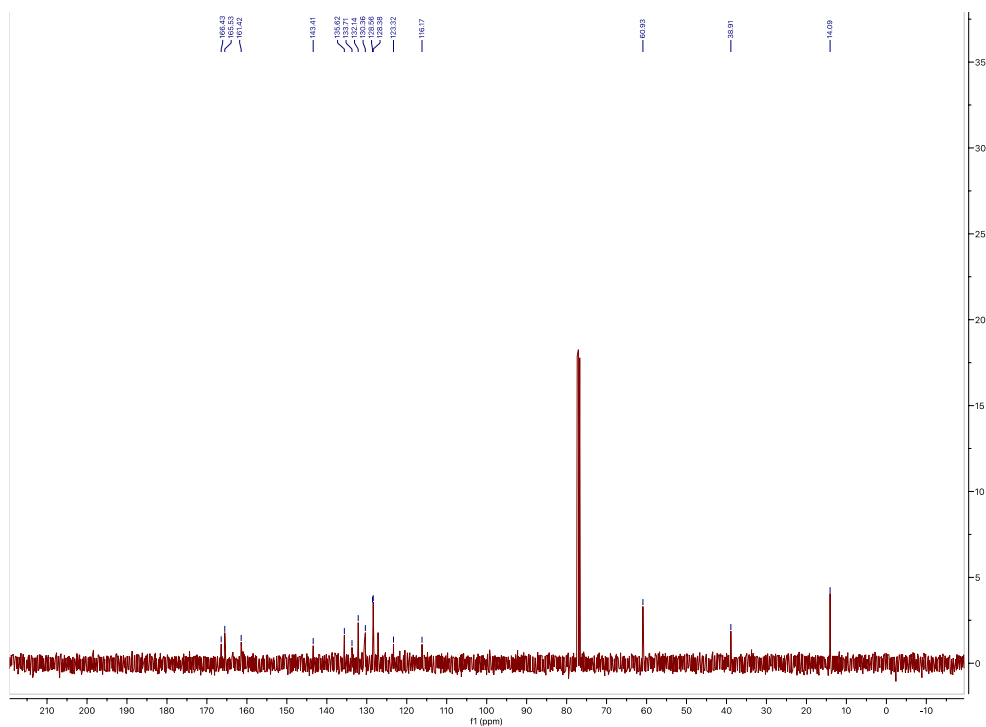
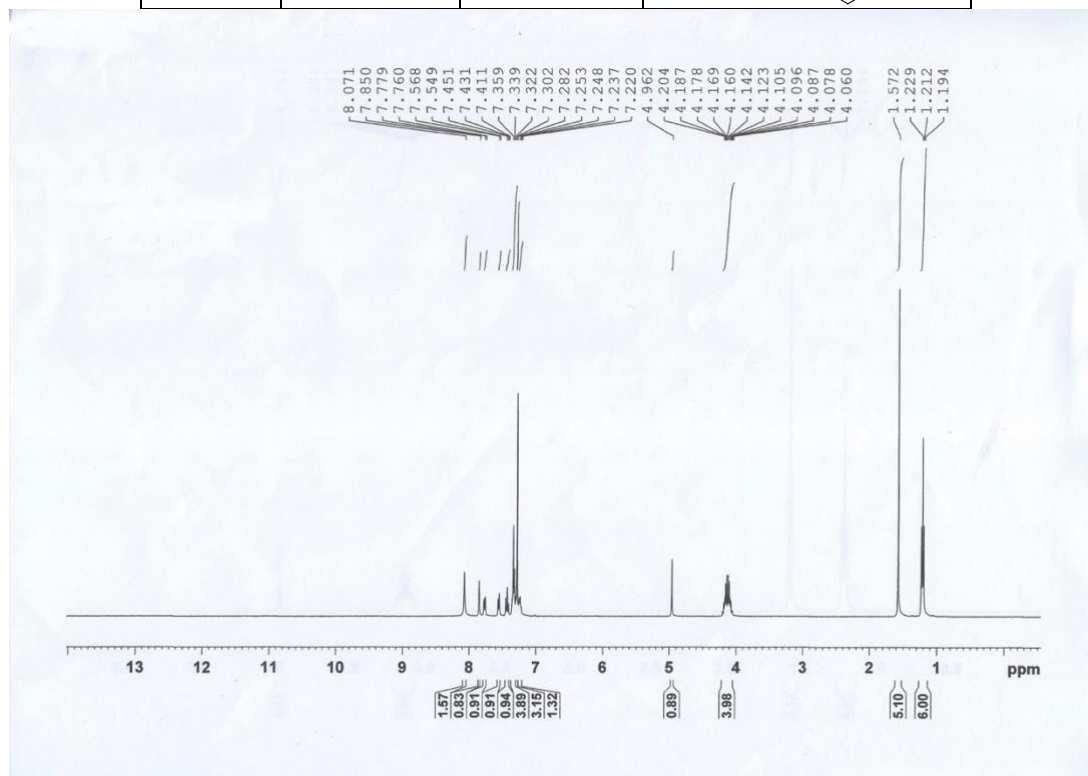
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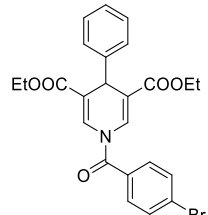


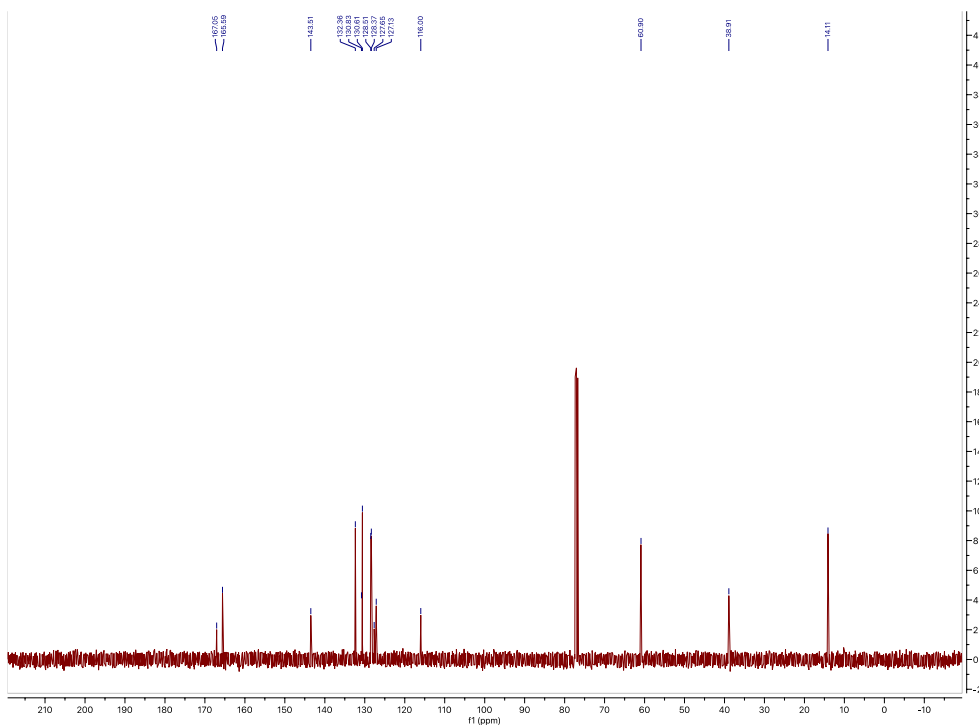
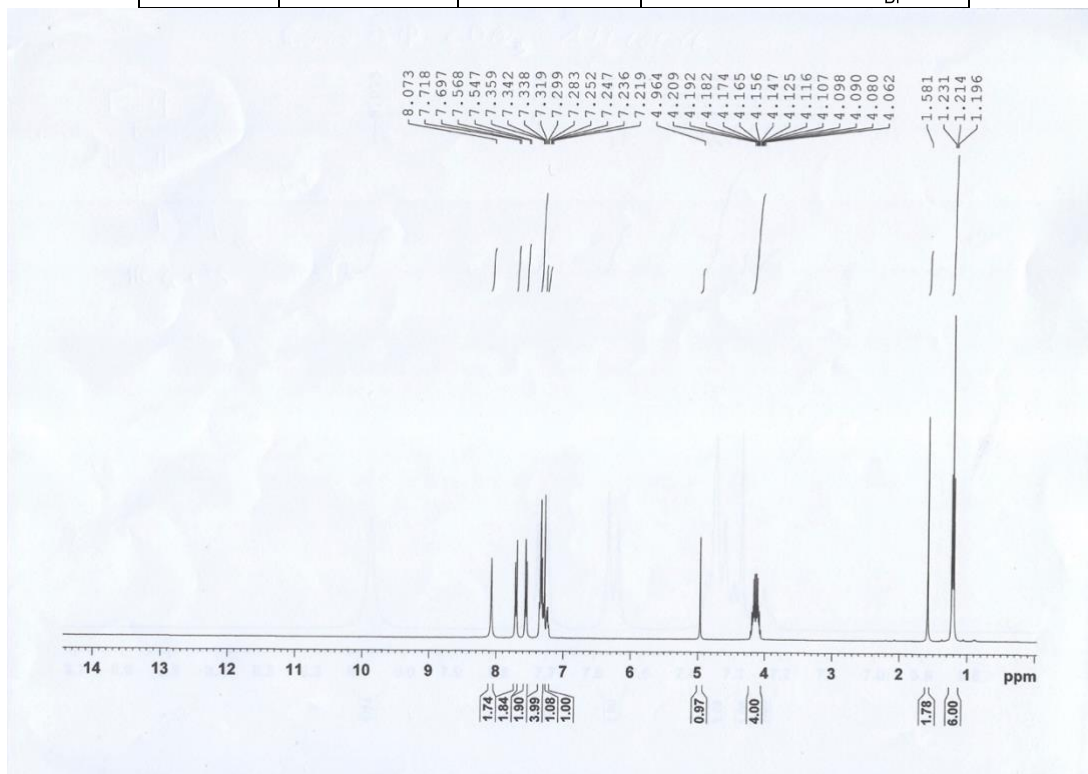
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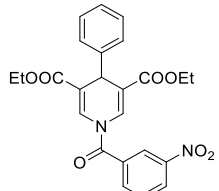


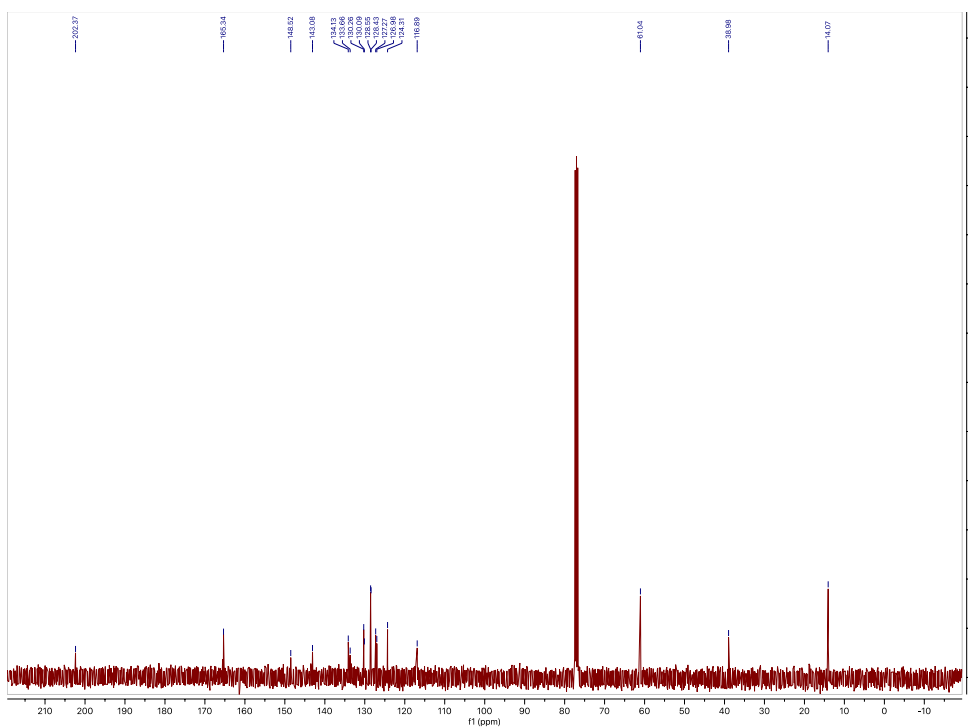
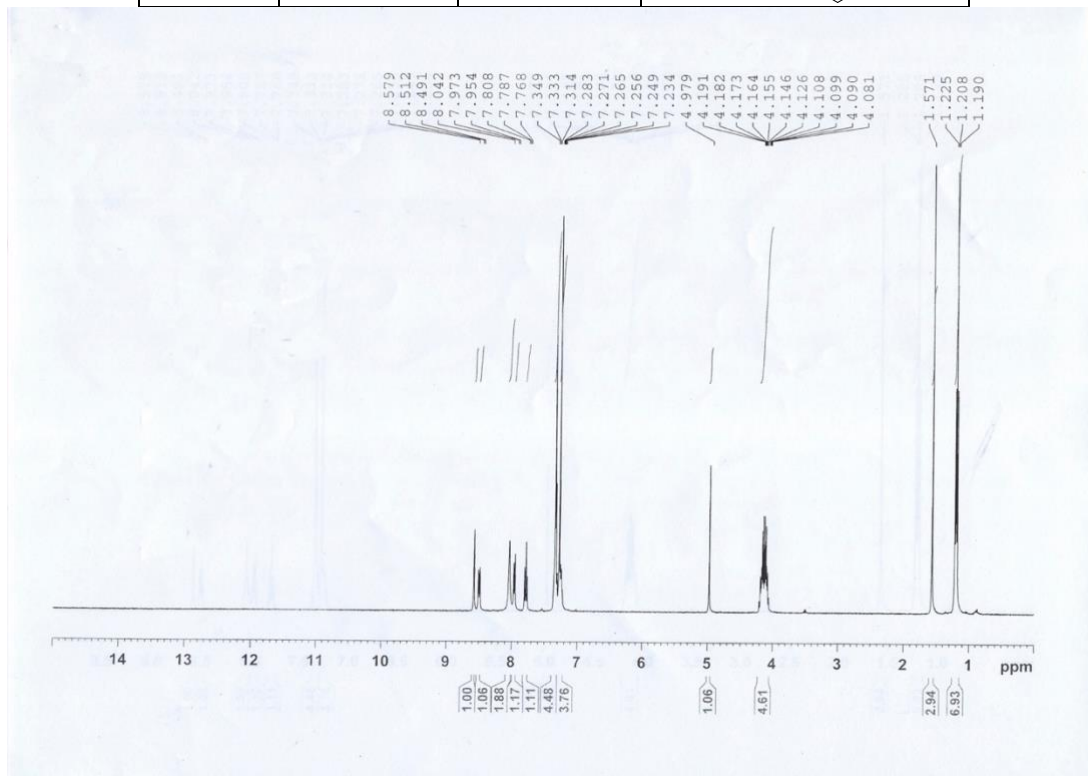
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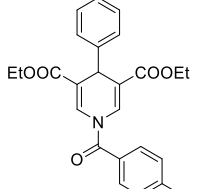


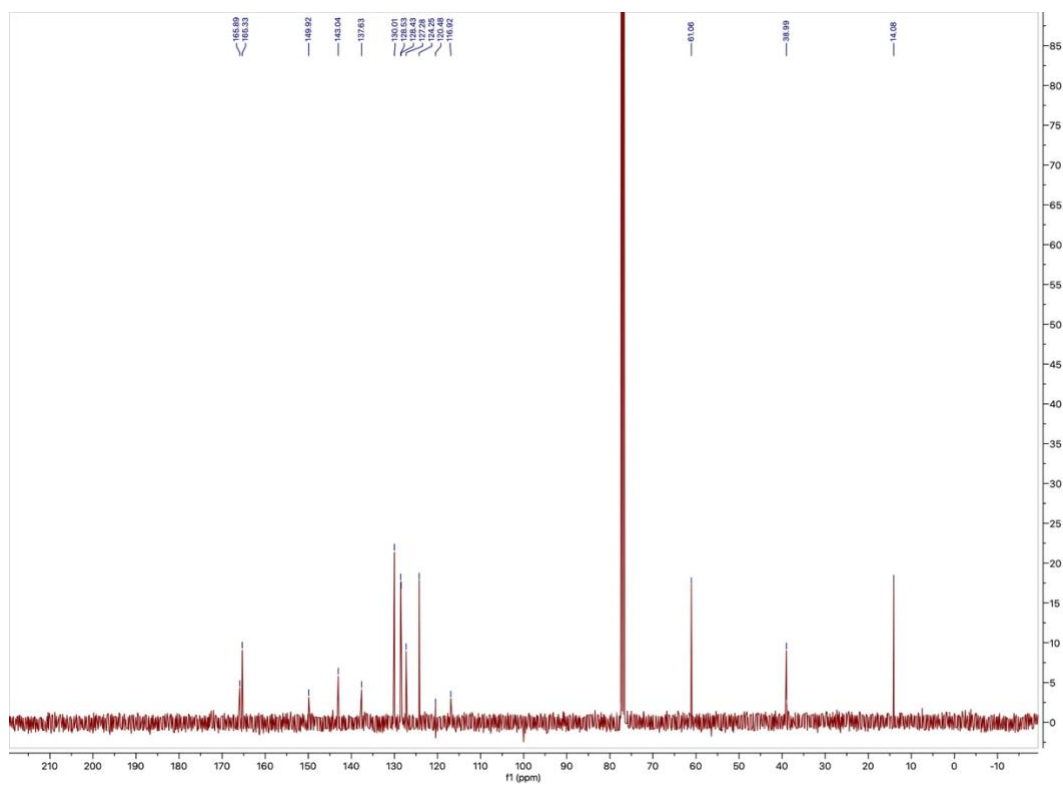
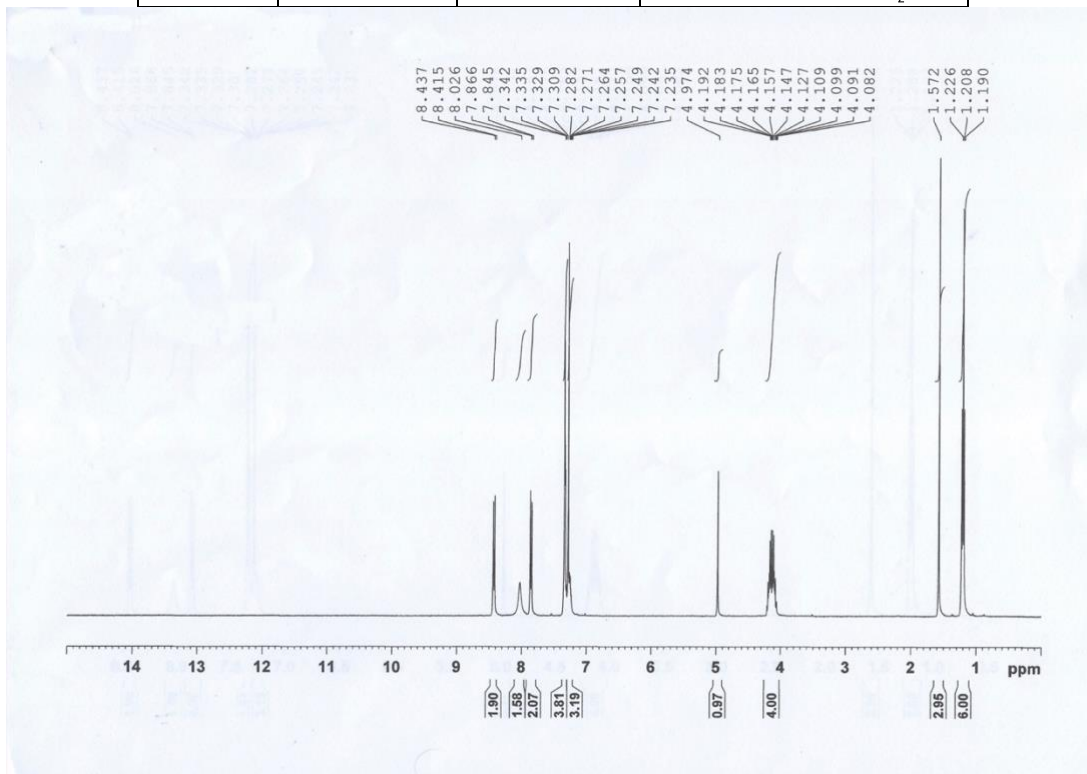
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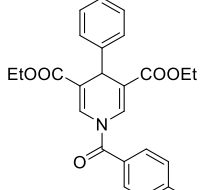


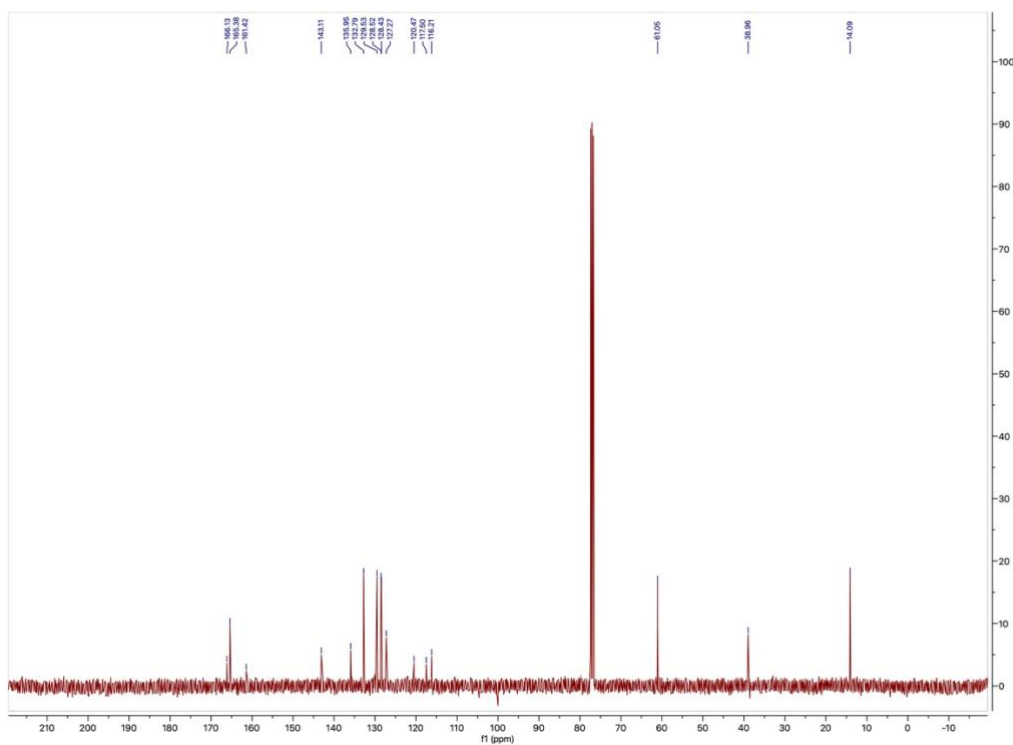
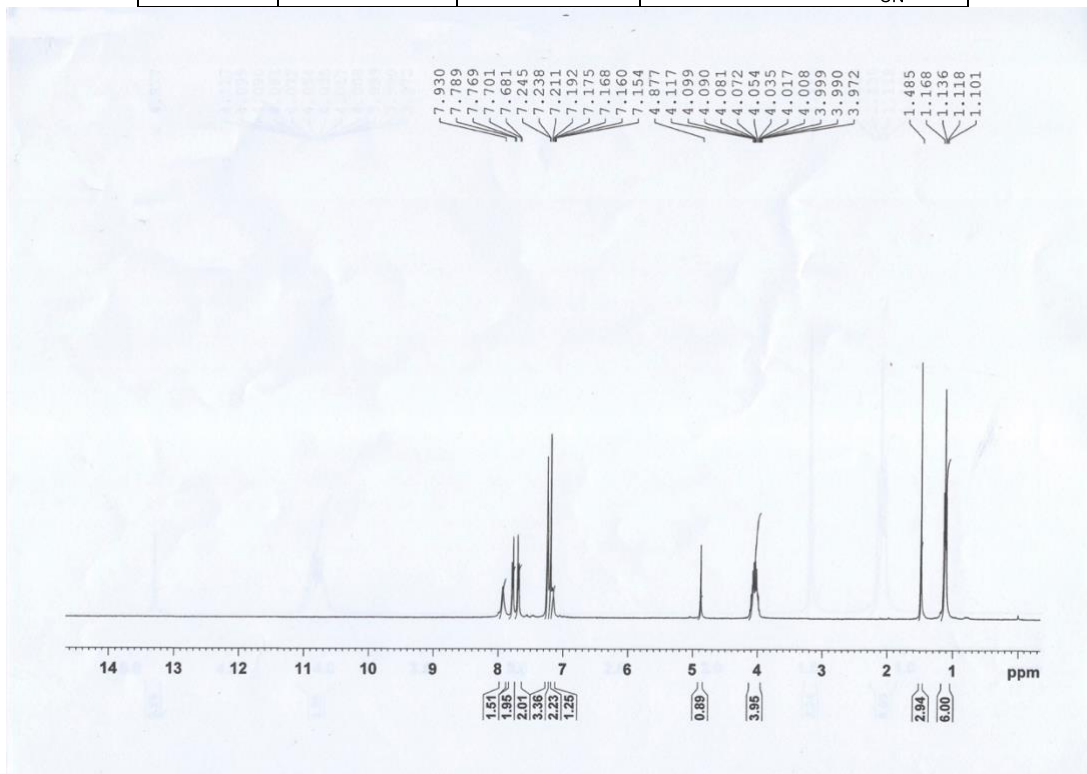
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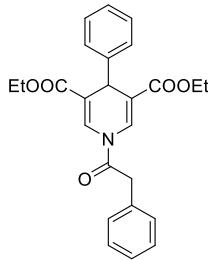


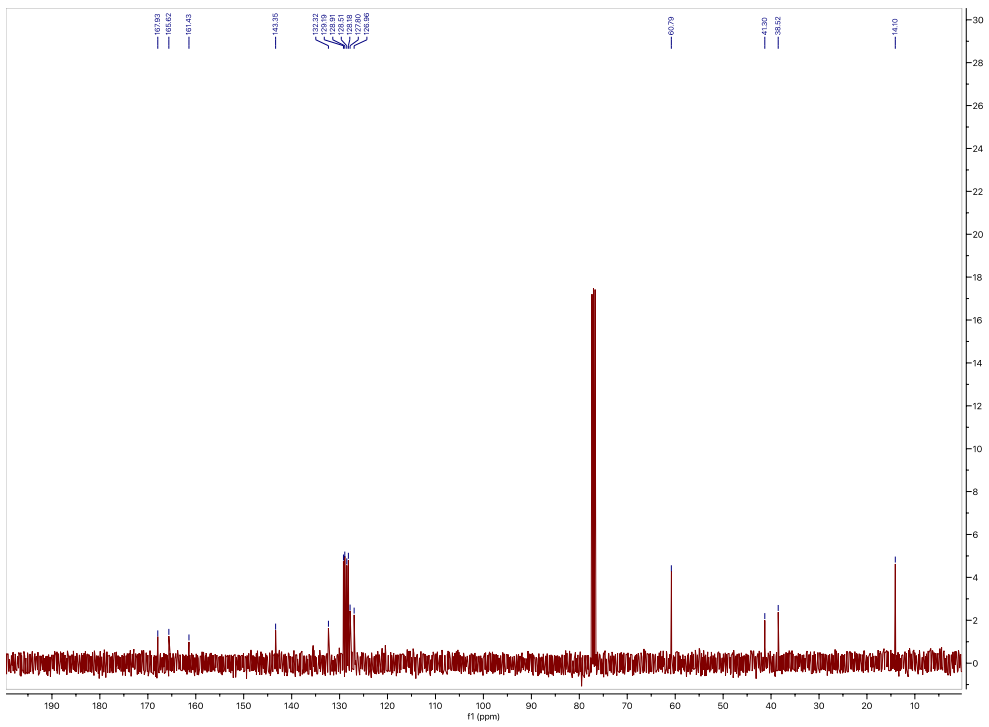
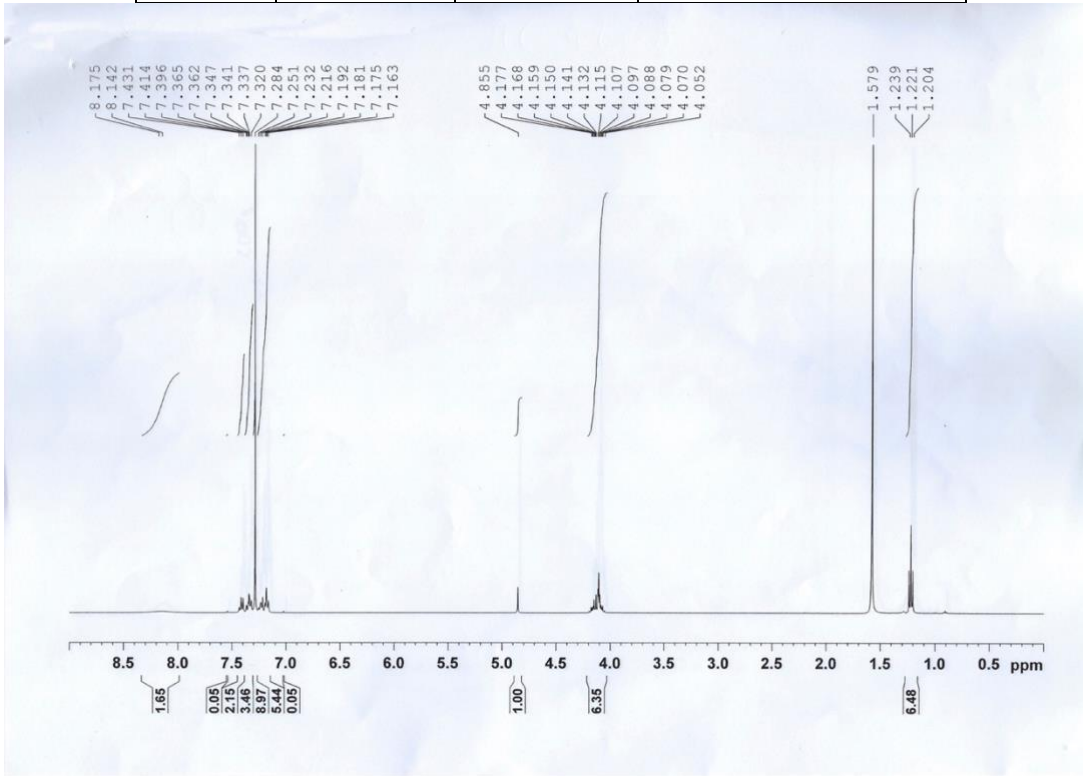
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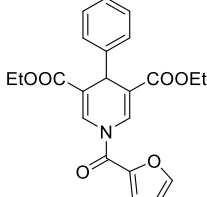


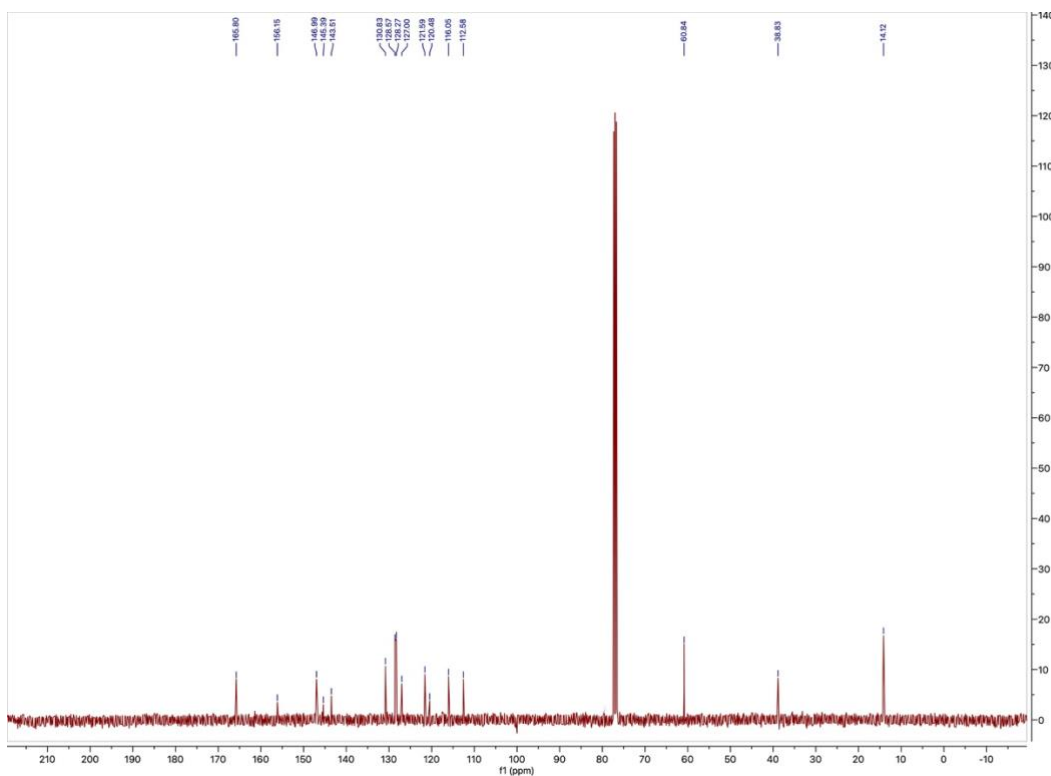
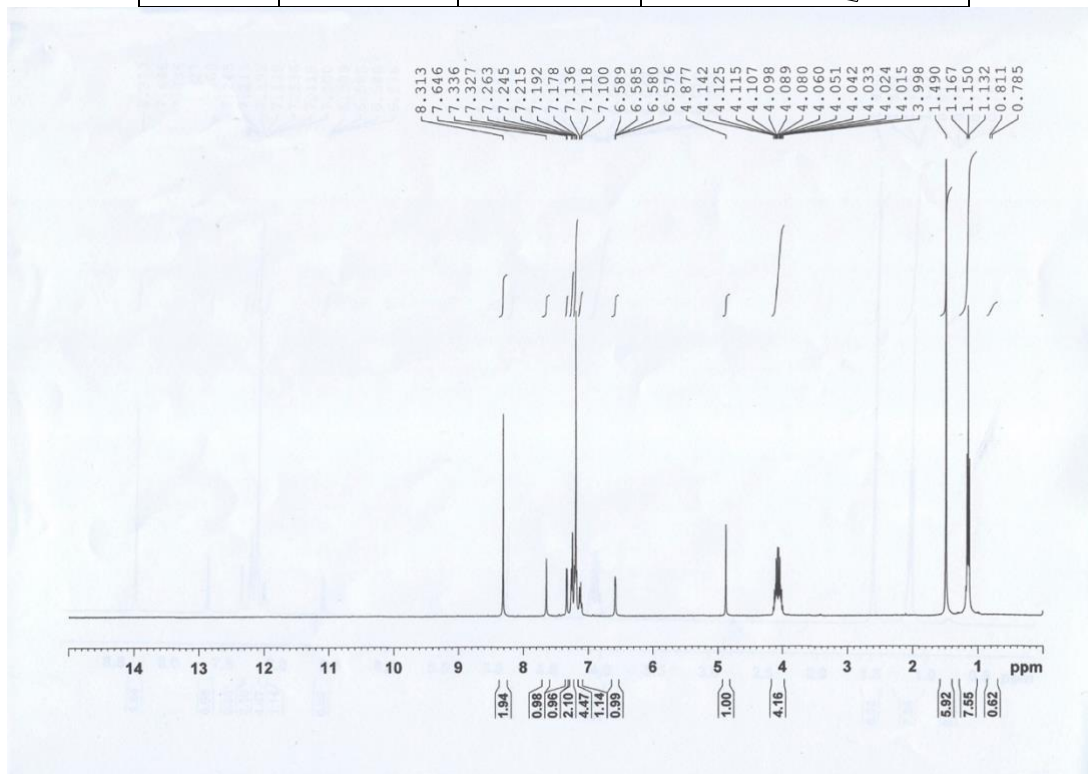
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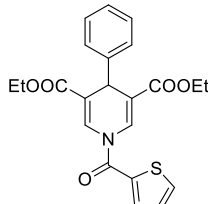


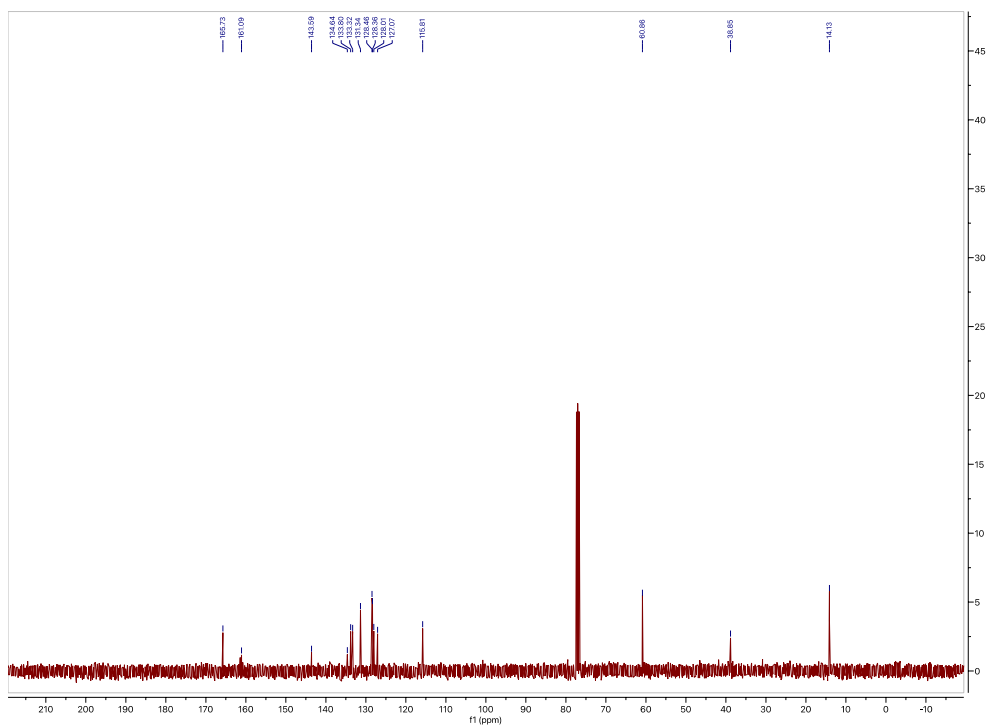
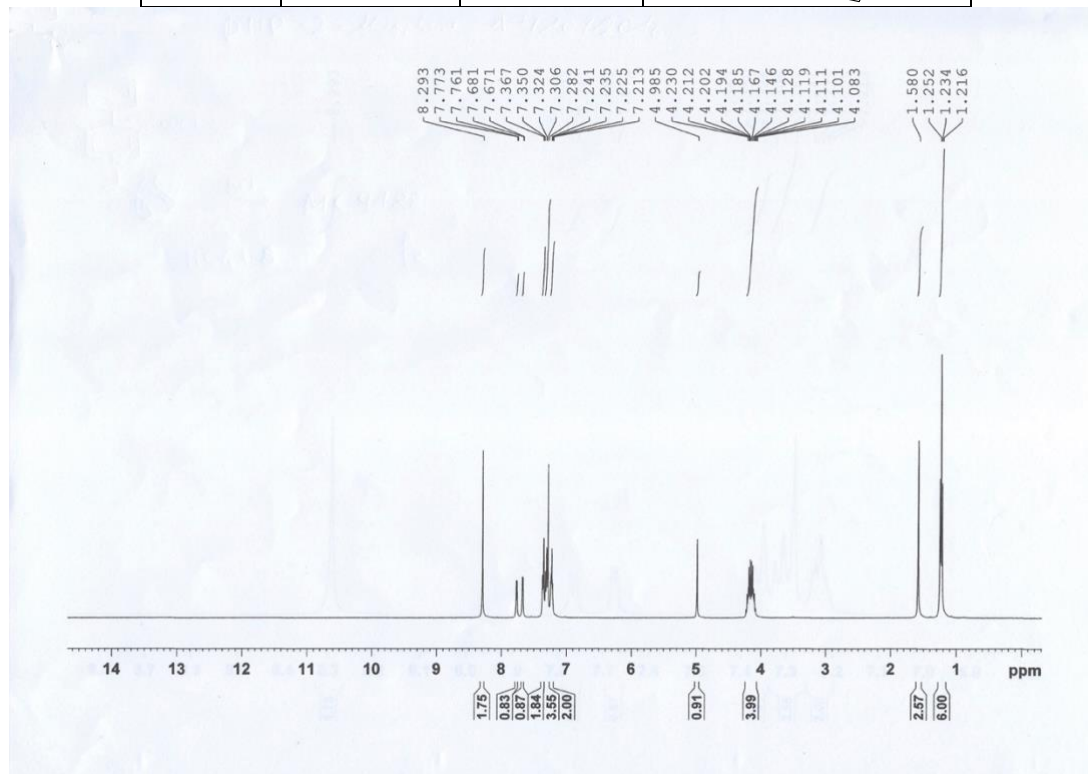
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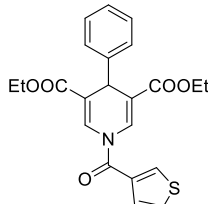


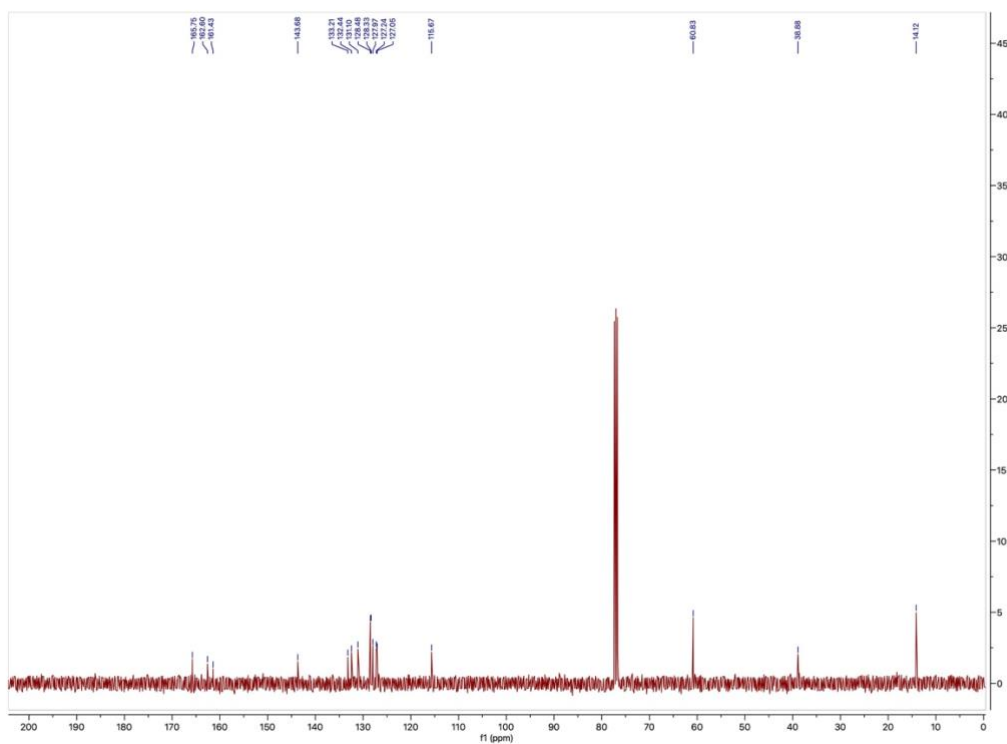
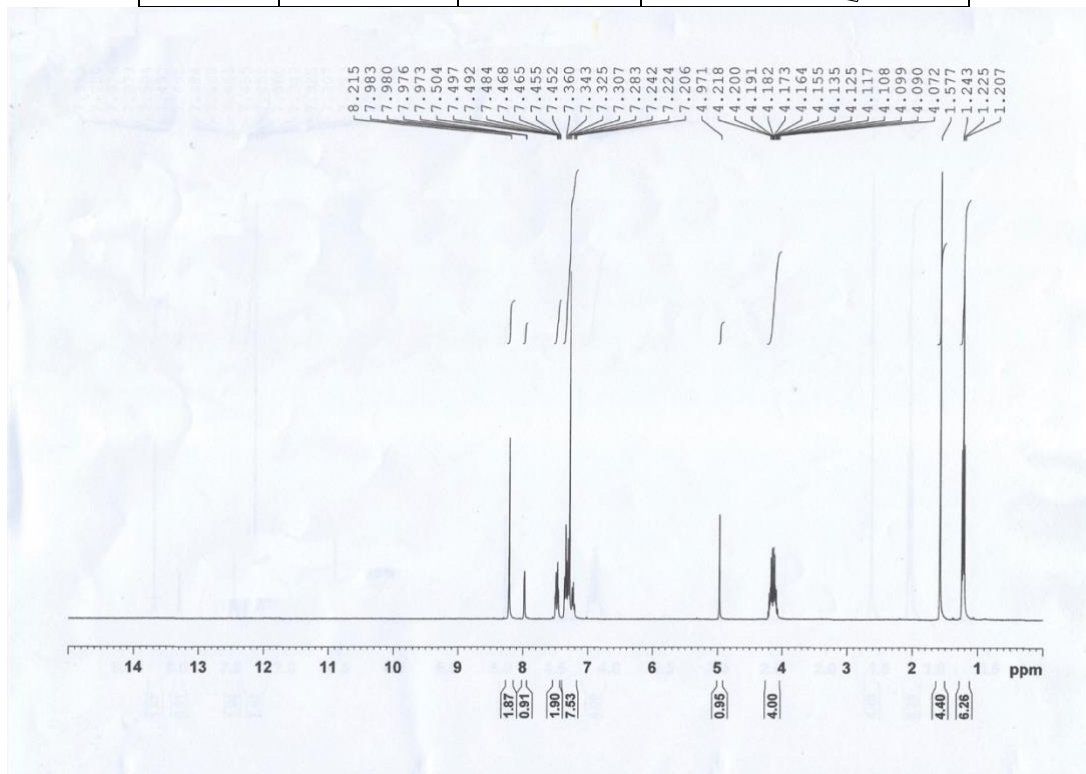
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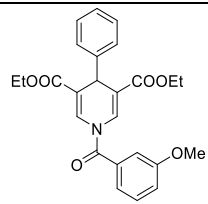


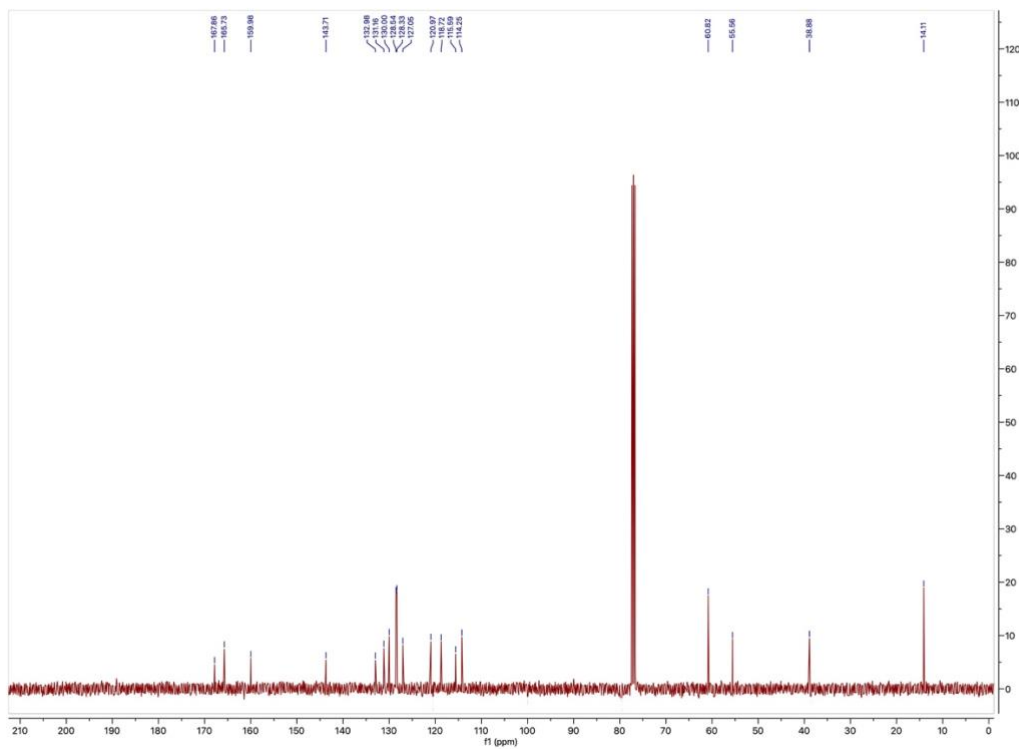
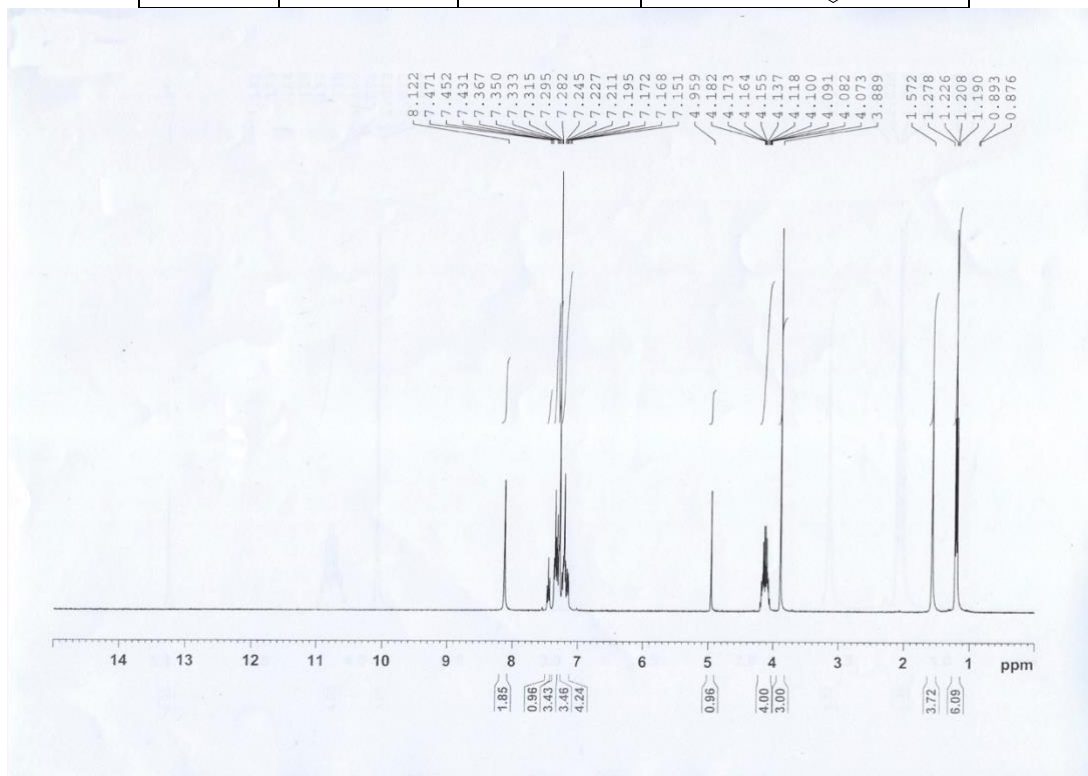
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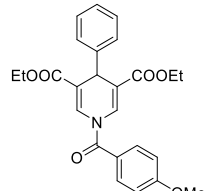


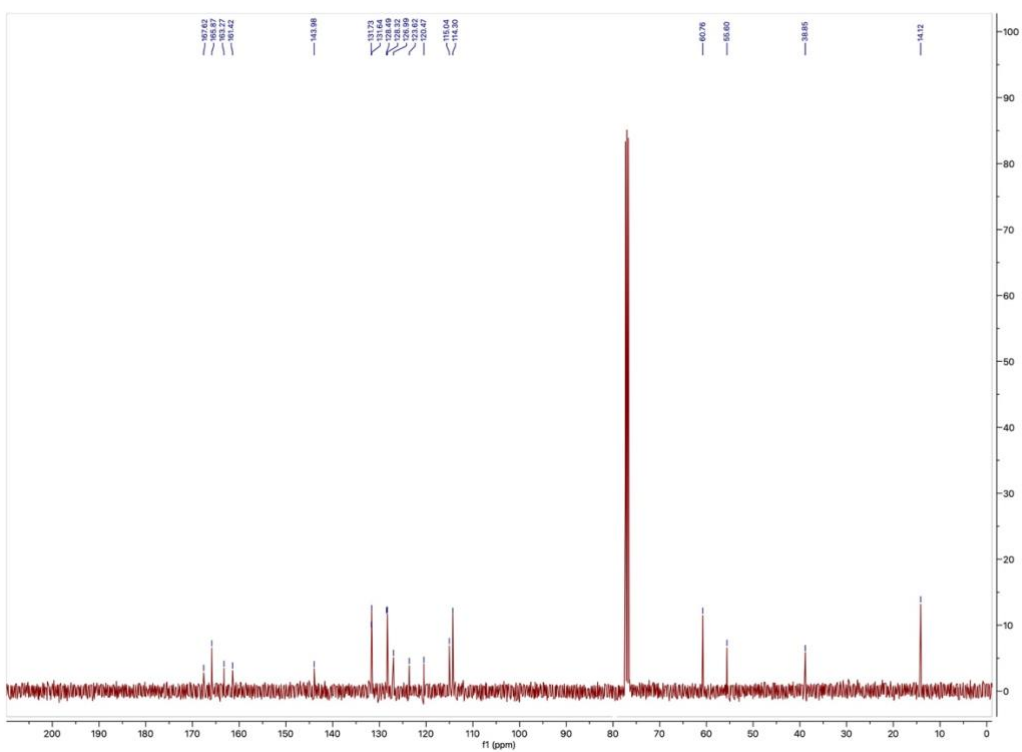
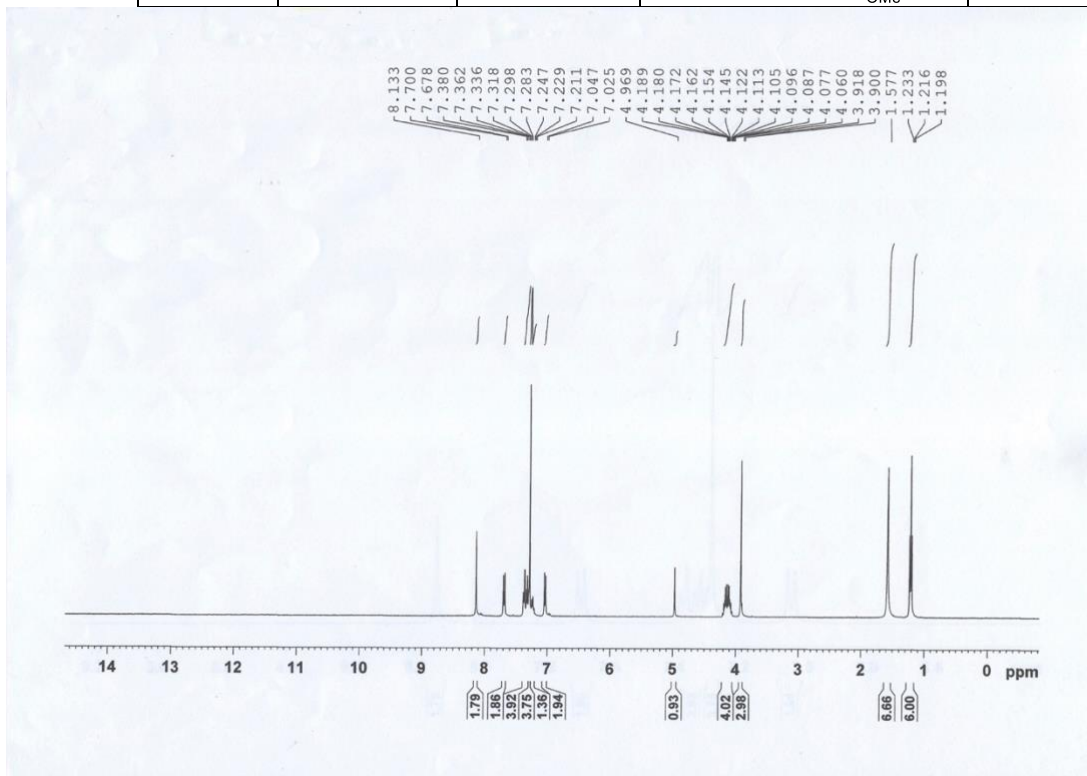
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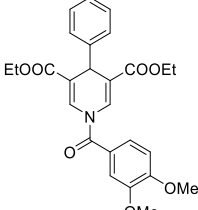


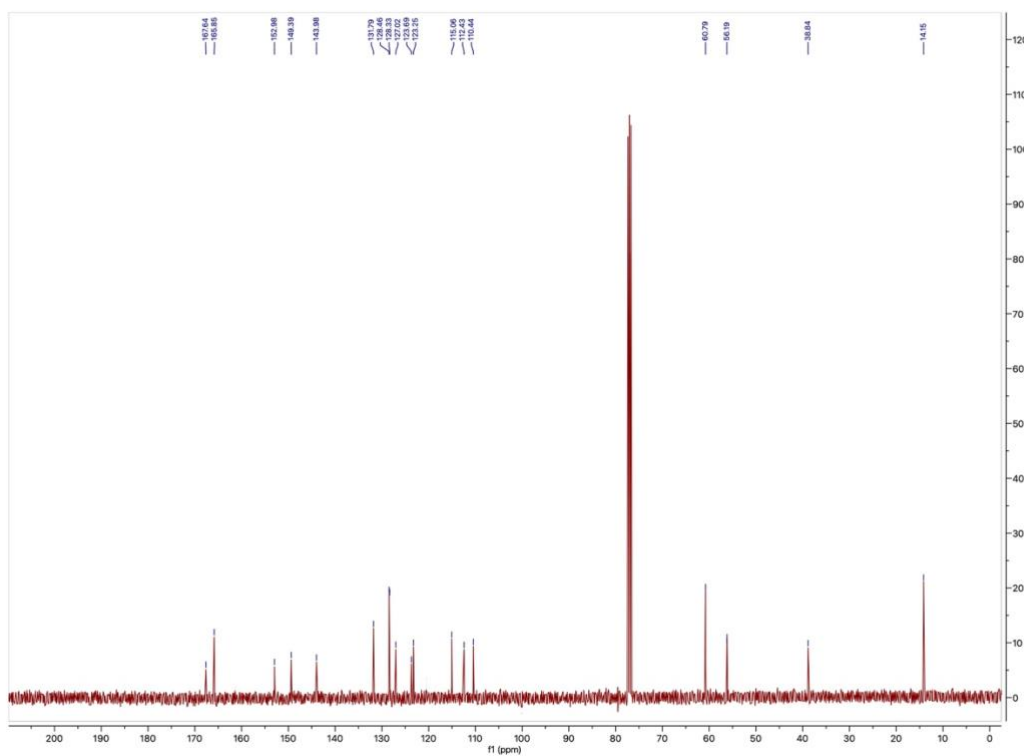
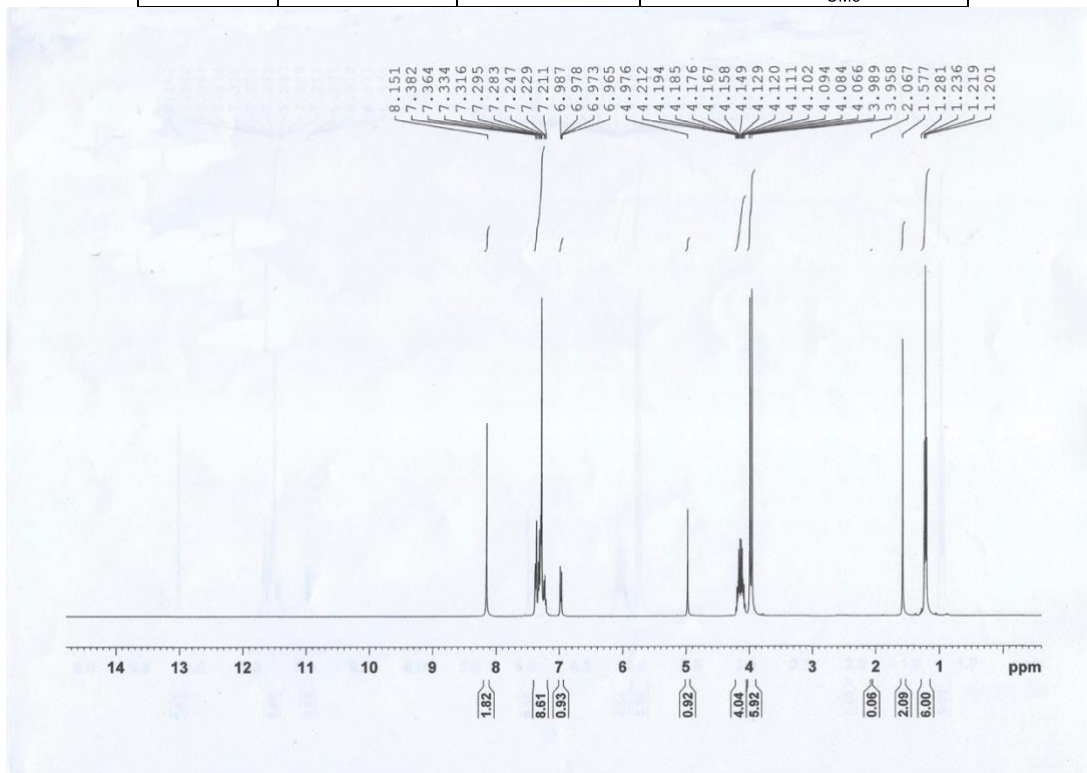
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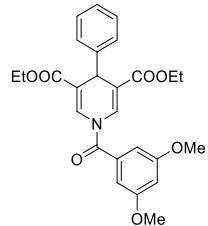


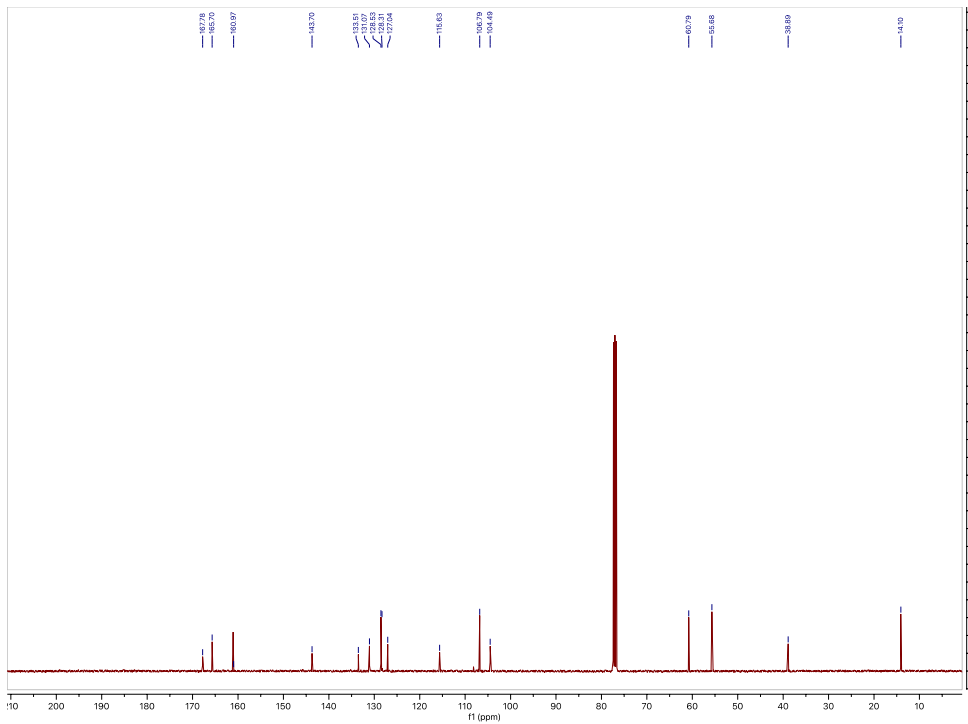
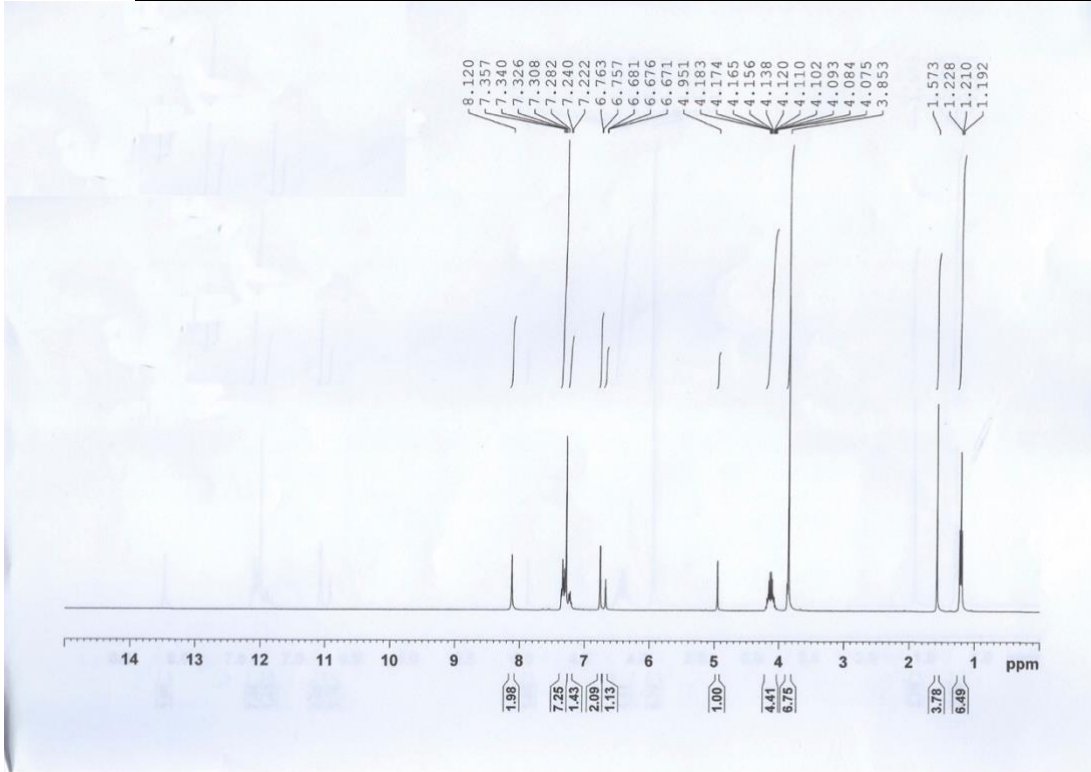
UBCS396	MC4182	3b		132°C	17,9%
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UBCS398	MC4175	3c	
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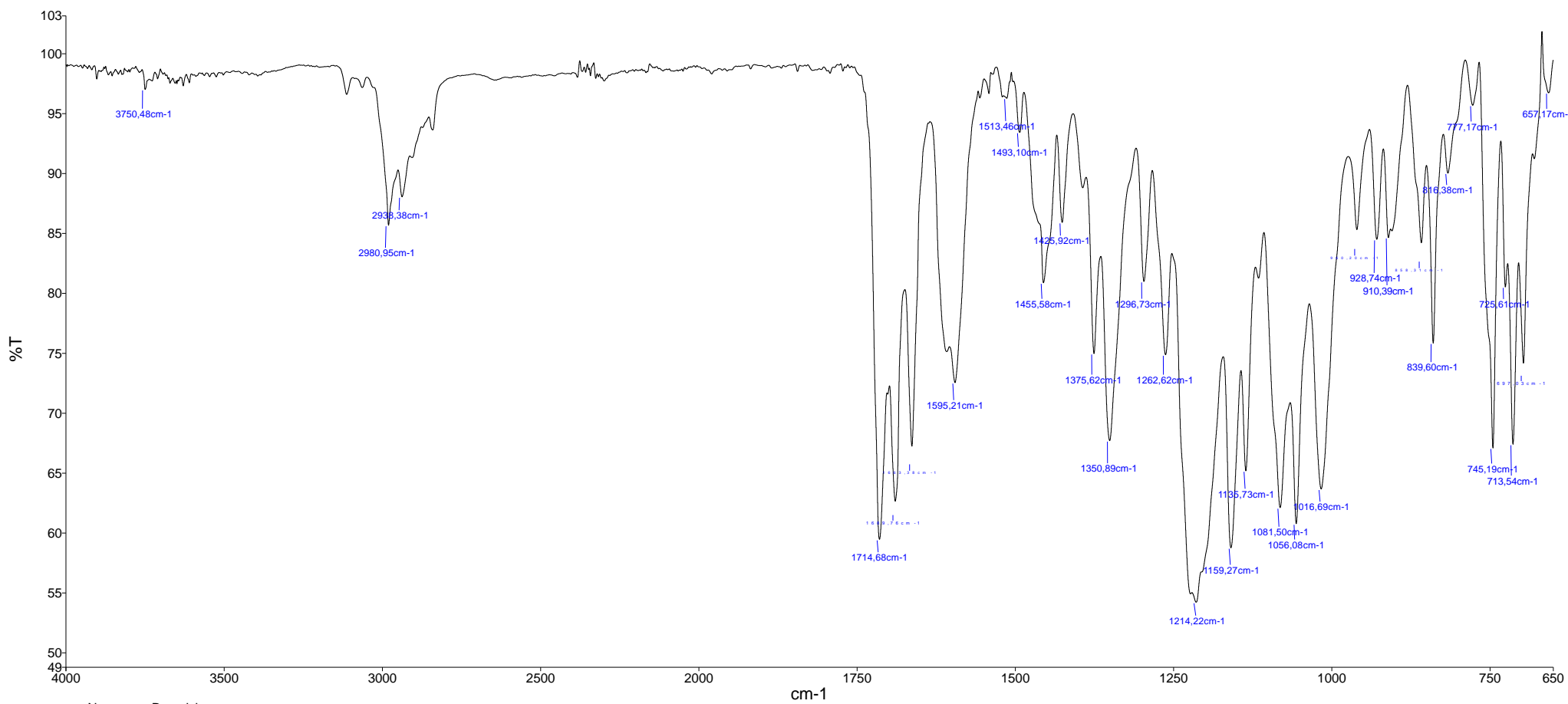


UBCS397	MC4176	3d		159°C	64, 3%
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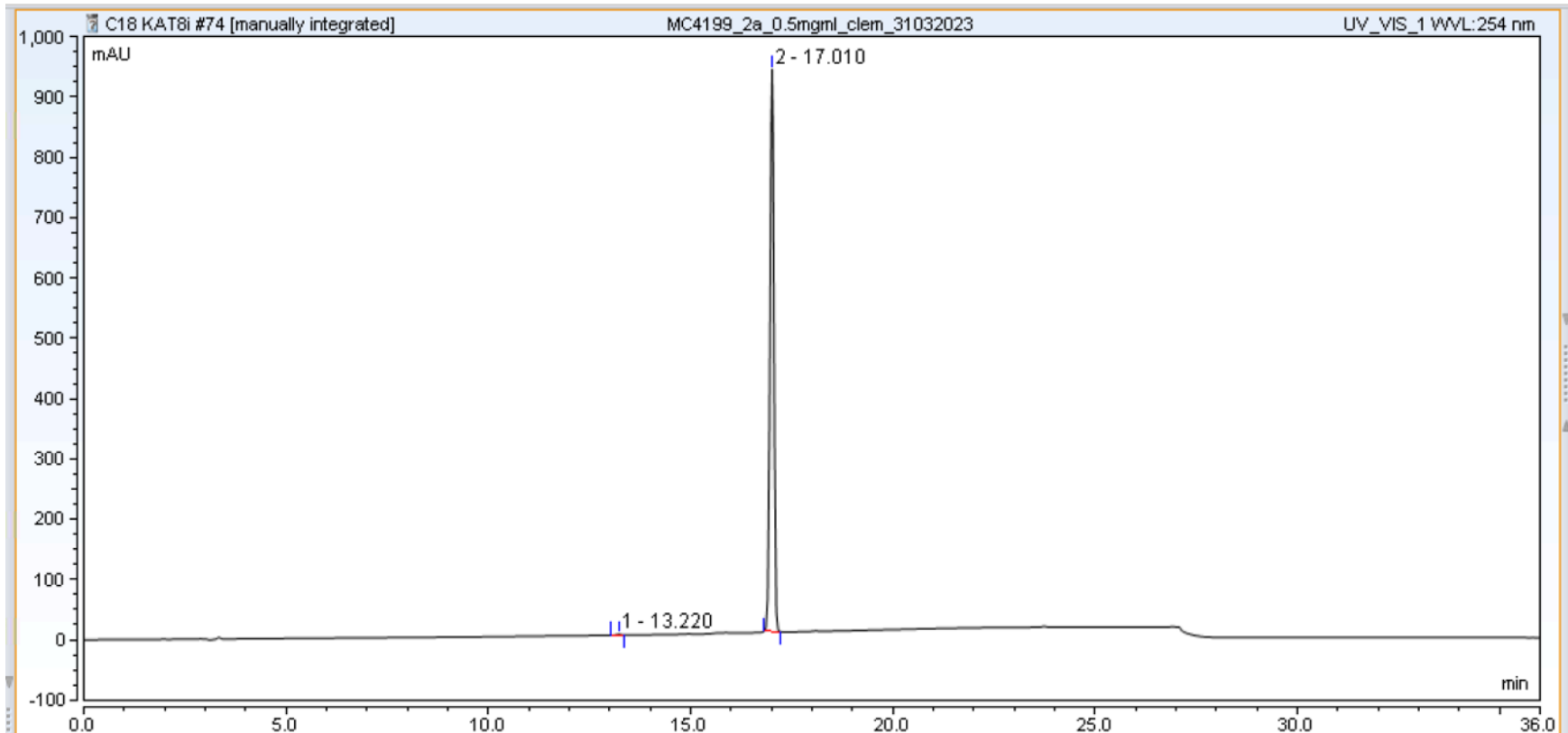


IR spectra and HPLC traces of final compounds 2a-n and 3a-d and high resolution mass spectra for final compounds 3a-d

2a MC4199

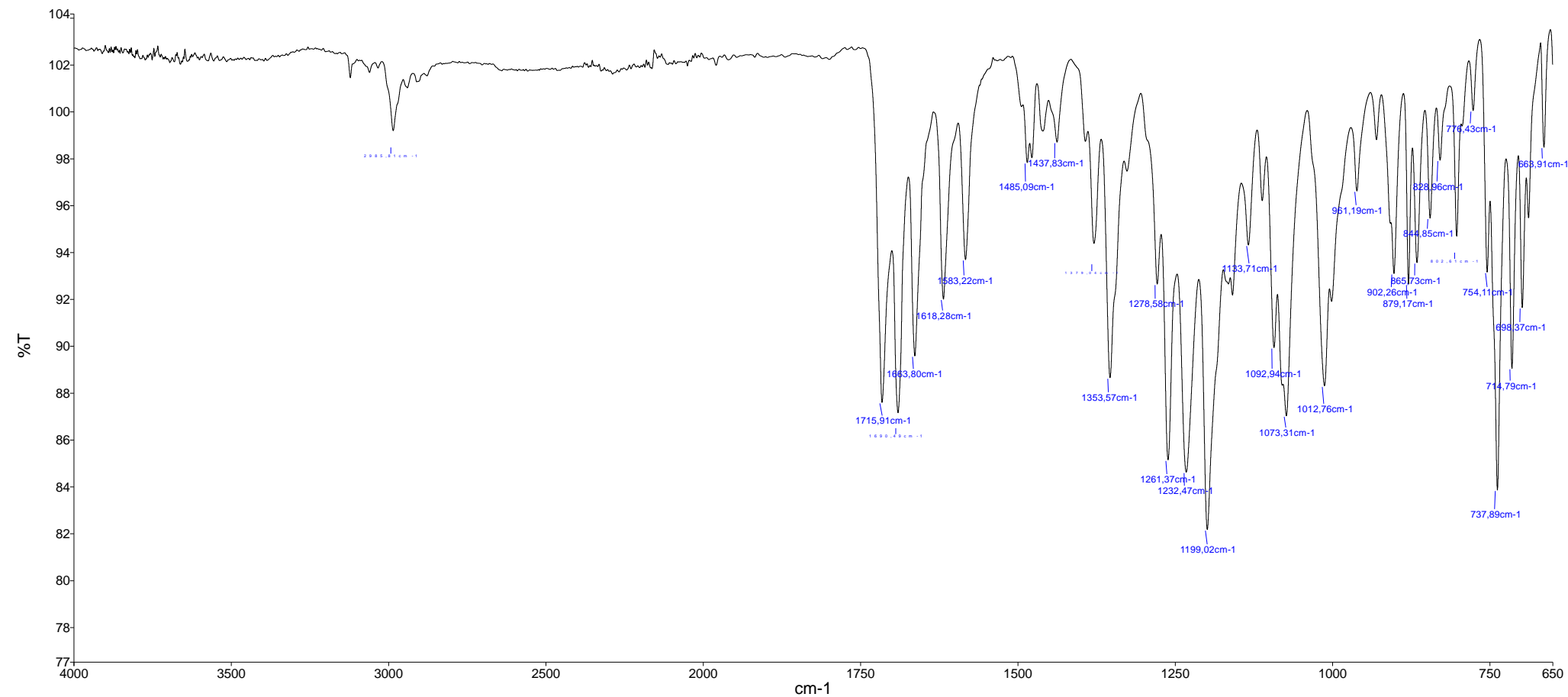


Nome Descrizione
MC4199_1

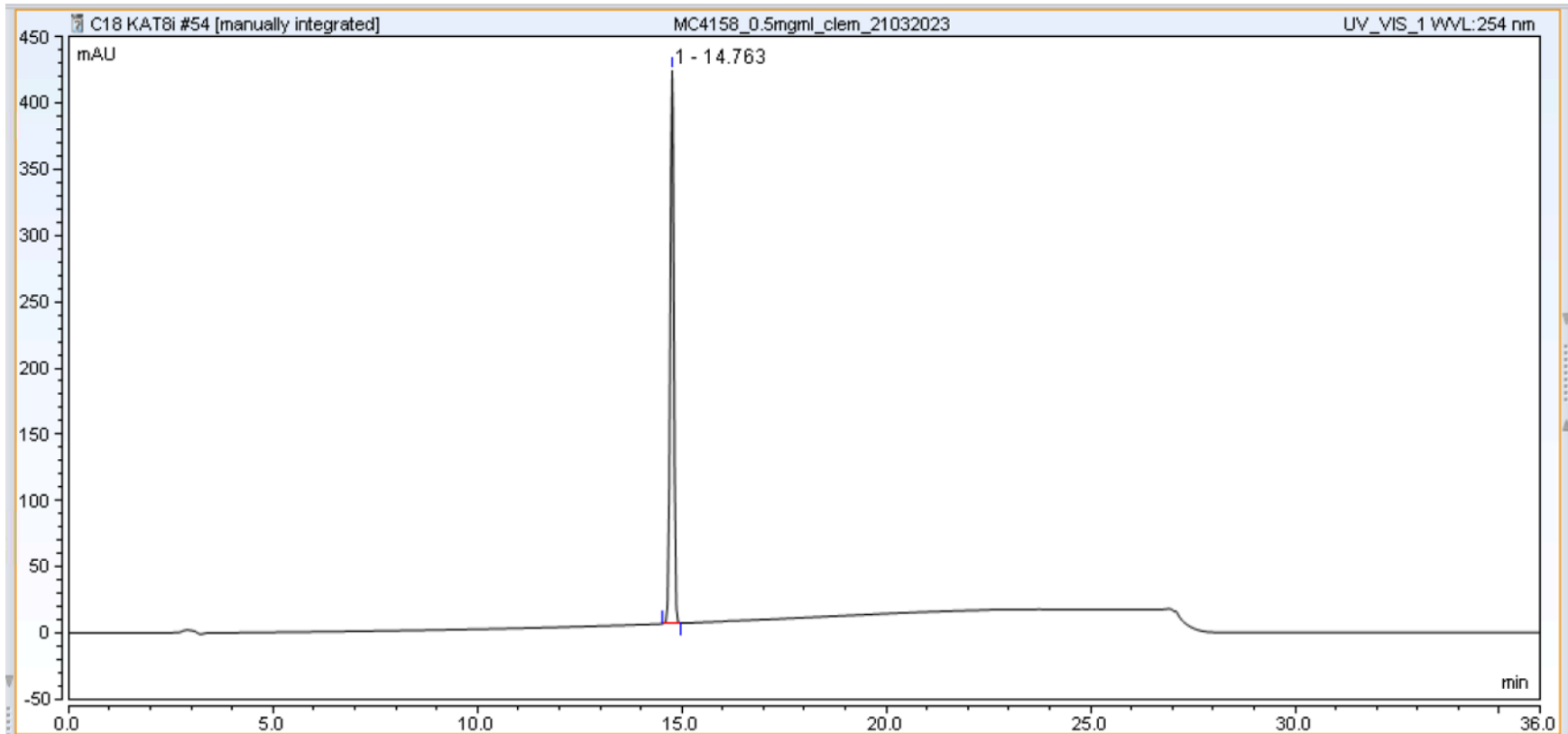


	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret.Time min	Amount	Rel.Area %	Area mAU*min	Height mAU	Type	Width (50% min)	Asym. EP	Resol. EP	Plates EP
4	1	13.220	n.a.	0.18	0.1865	1.70	BMB	0.103	0.91	21.53	91718
5	2	17.010	n.a.	99.82	104.0530	932.86	BMB*	0.105	0.94	n.a.	145478
6	Maximum		0.0000	99.82	104.0530	932.86		0.105	0.94	21.53	145478
7	Minimum		0.0000	0.18	0.1865	1.70		0.103	0.91	21.53	91718
8	Sum		0.0000	100.00	104.2396	934.56					
9											

2b MC4158

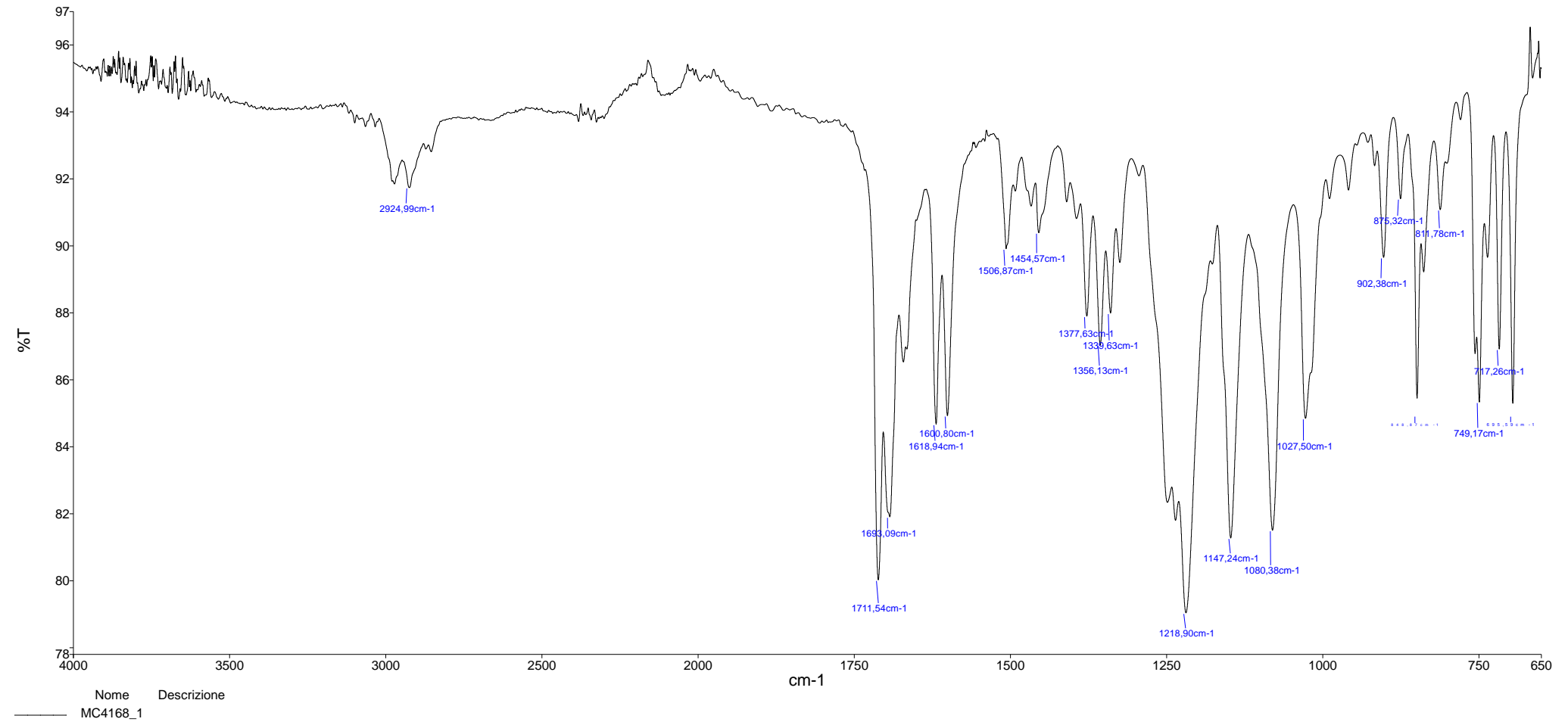


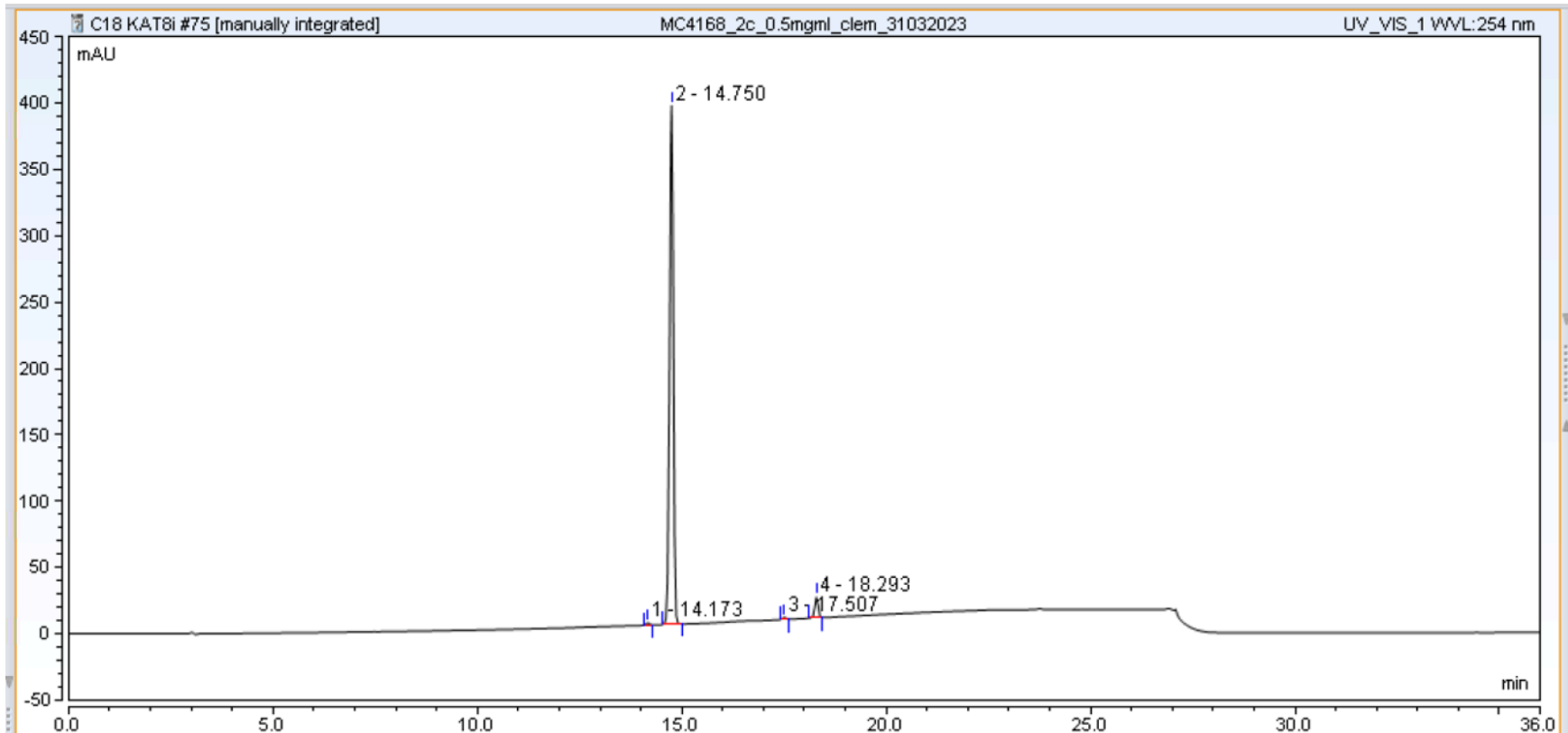
Nome Descrizione
MC4158_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	14.763	n.a.	100.00	42.6207	417.36	BMB*	0.095	0.92	n.a.	133470
6	Maximum		0.0000	100.00	42.6207	417.36		0.095	0.92	0.00	133470
7	Minimum		0.0000	100.00	42.6207	417.36		0.095	0.92	0.00	133470
8	Sum		0.0000	100.00	42.6207	417.36					
9											

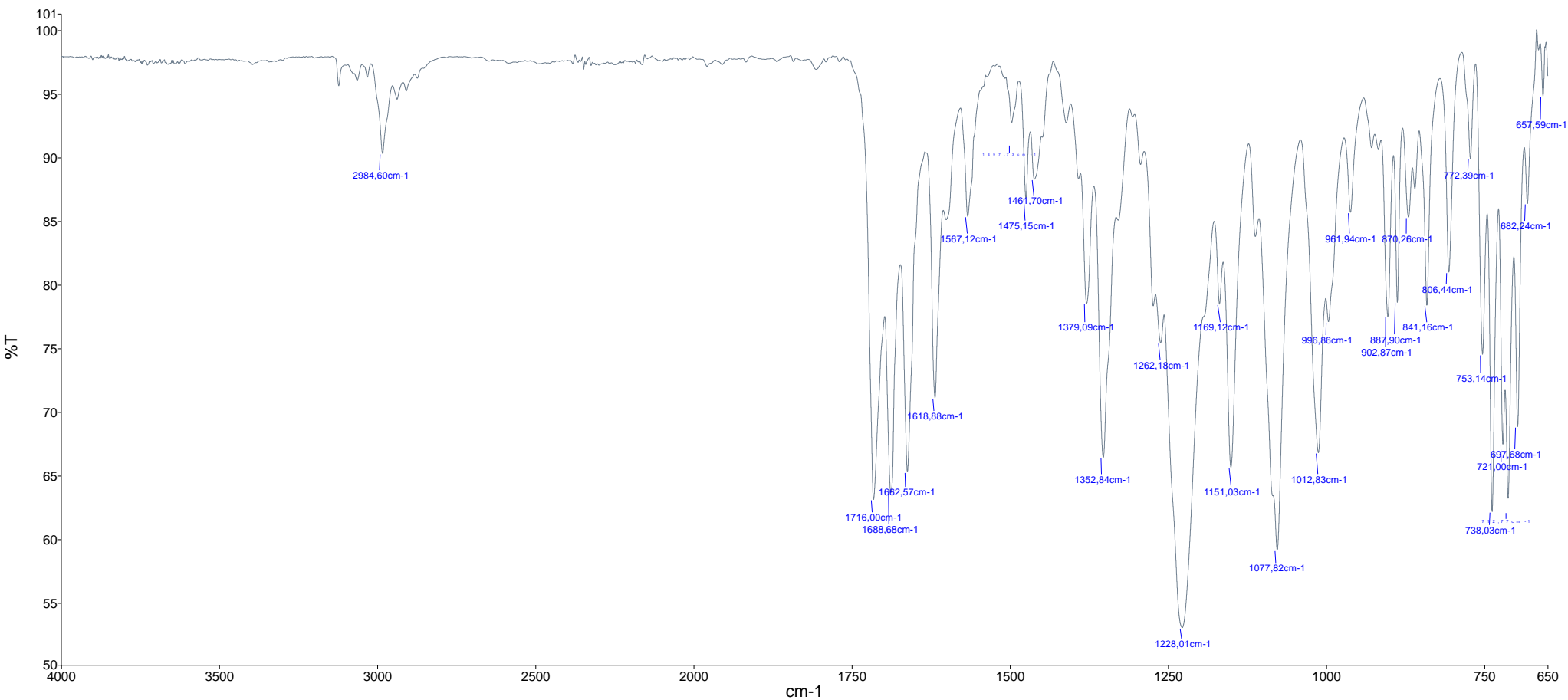
2c MC4168

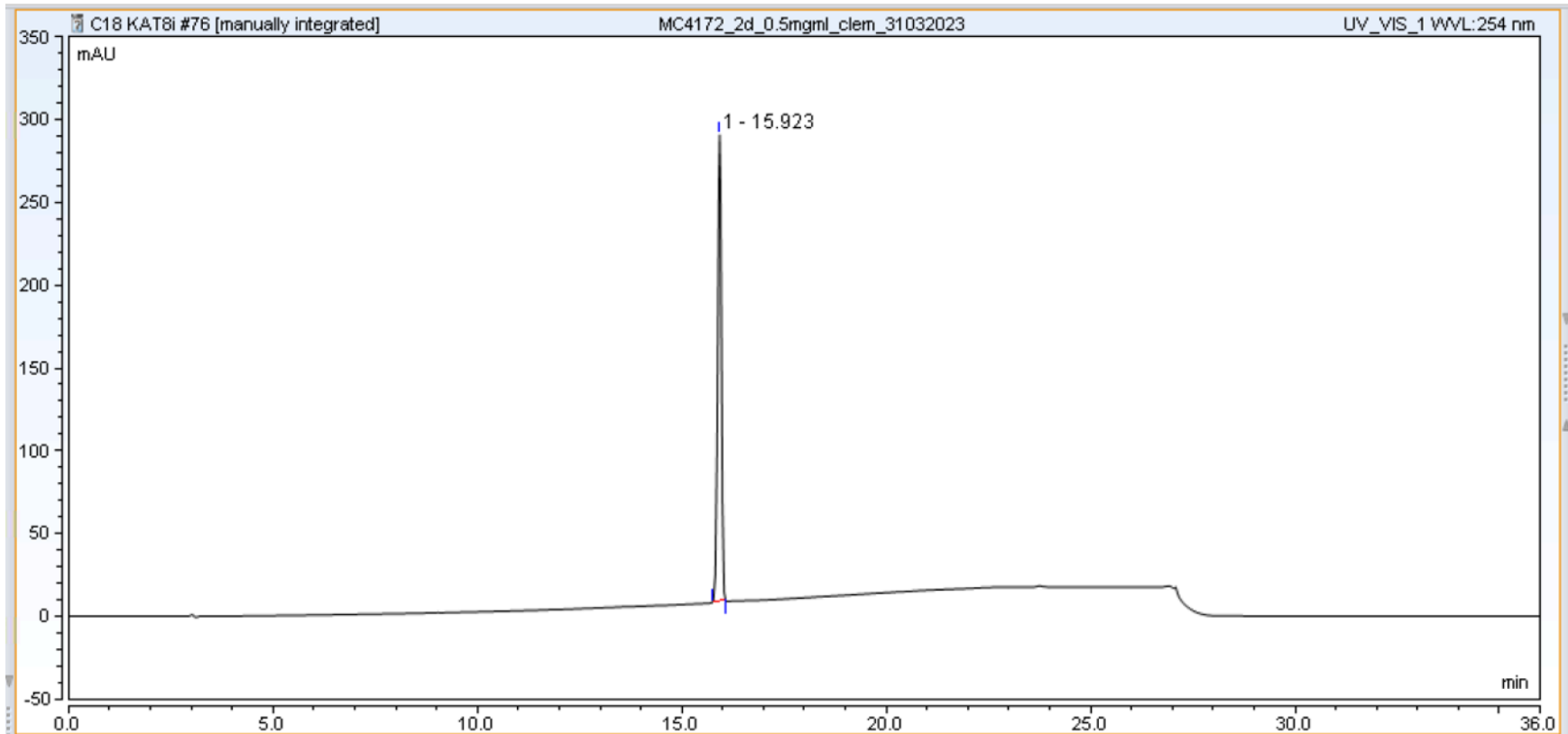




	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	14.173	n.a.	0.43	0.1898	1.98	BMB*	0.094	0.92	3.51	126697
5	2	14.750	n.a.	95.72	41.7944	391.18	BMB*	0.100	0.92	17.07	120452
6	3	17.507	n.a.	0.34	0.1483	1.57	BMB*	0.091	0.94	5.13	207287
7	4	18.293	n.a.	3.51	1.5306	15.88	BMB*	0.090	0.96	n.a.	227374
8	Maximum		0.0000	95.72	41.7944	391.18		0.100	0.96	17.07	227374
9	Minimum		0.0000	0.34	0.1483	1.57		0.090	0.92	3.51	120452
10	Sum		0.0000	100.00	43.6631	410.61					
11											

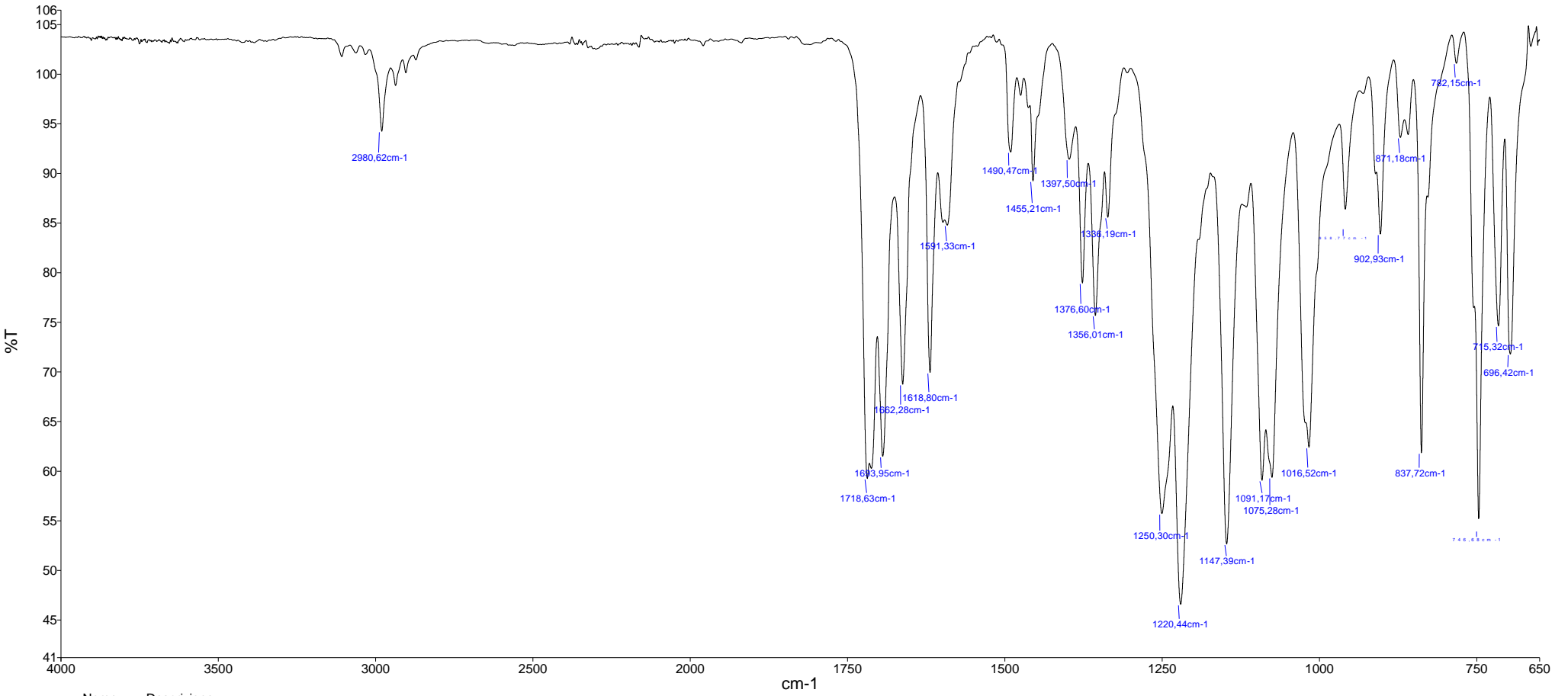
2d MC4172

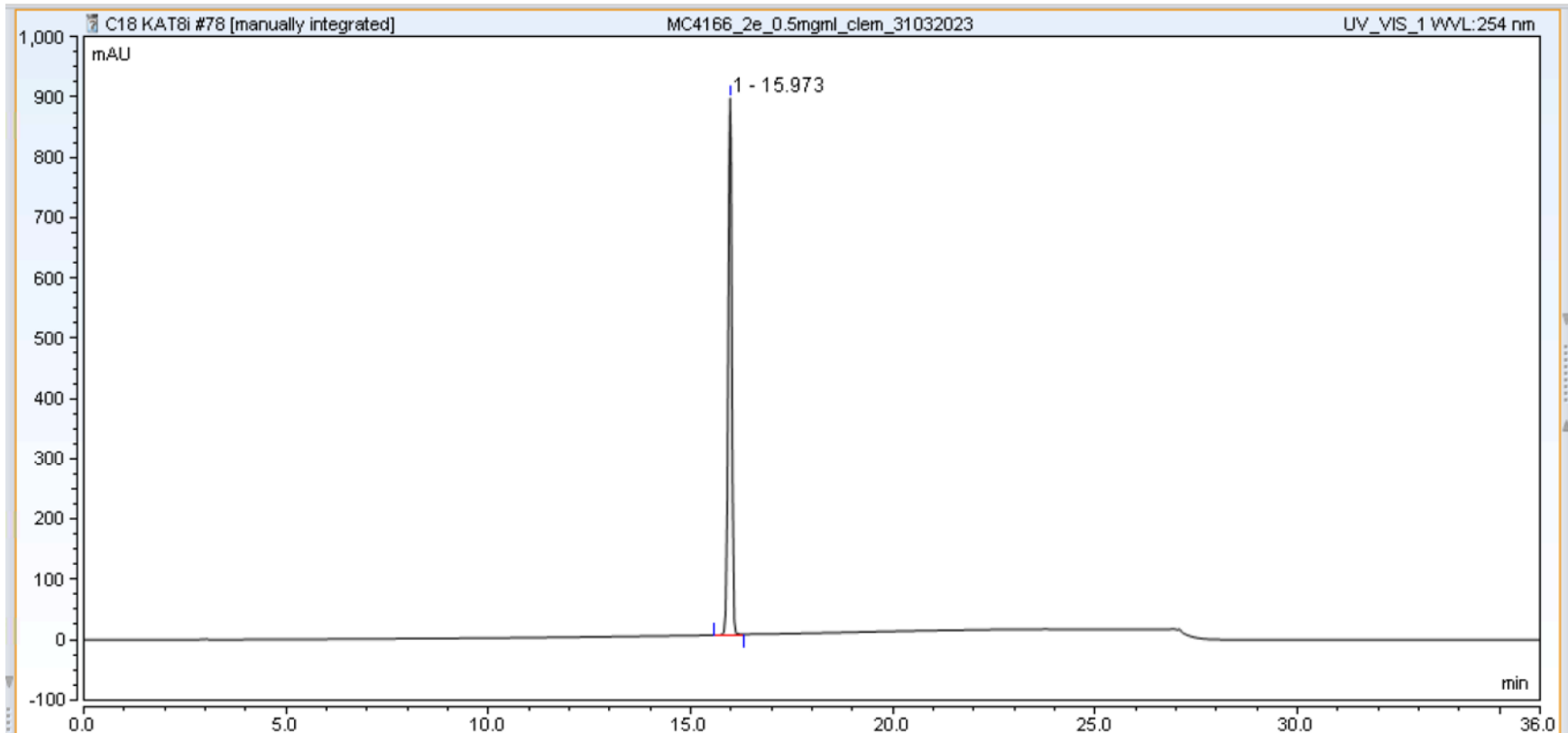




	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	15.923	n.a.	100.00	29.6950	281.30	BMB*	0.099	0.93	n.a.	142353
6	Maximum		0.0000	100.00	29.6950	281.30		0.099	0.93	0.00	142353
7	Minimum		0.0000	100.00	29.6950	281.30		0.099	0.93	0.00	142353
8	Sum		0.0000	100.00	29.6950	281.30					
9											

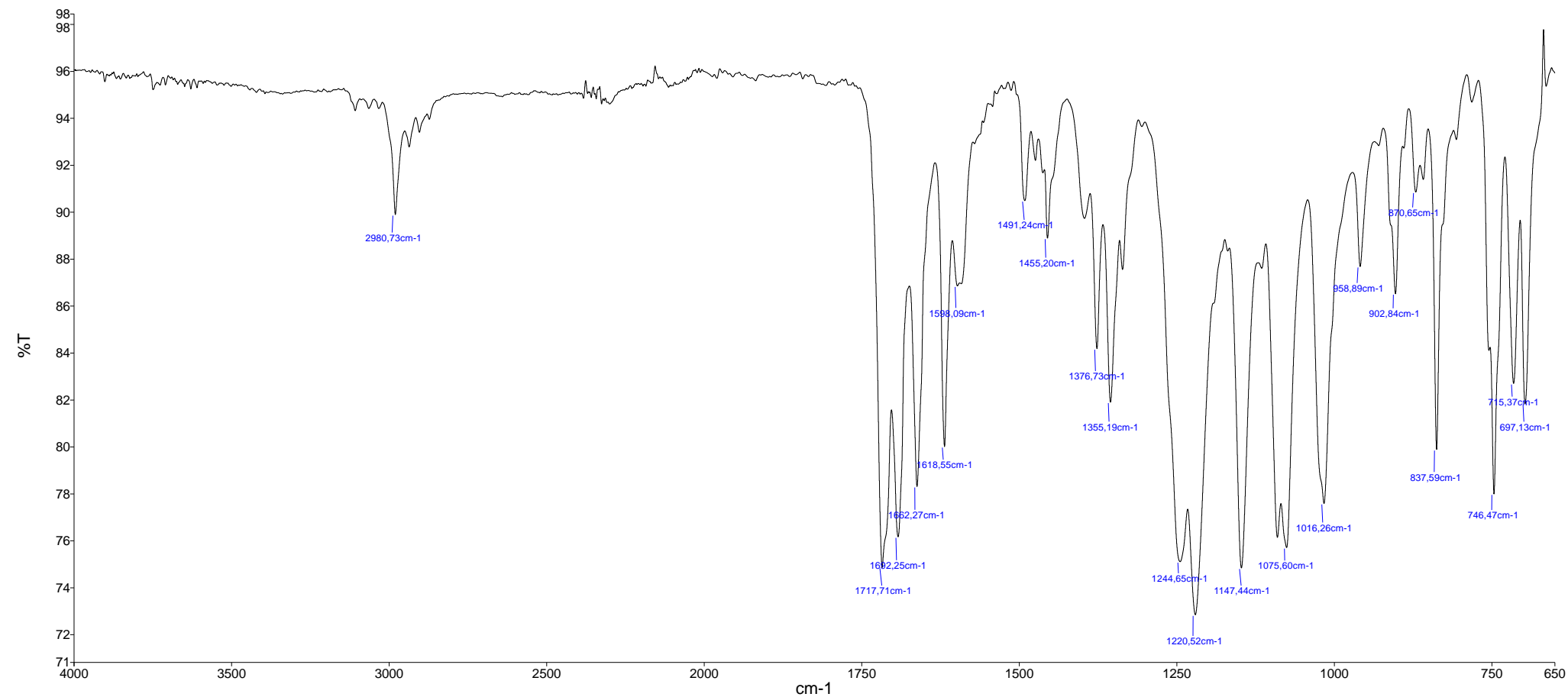
2e MC4166



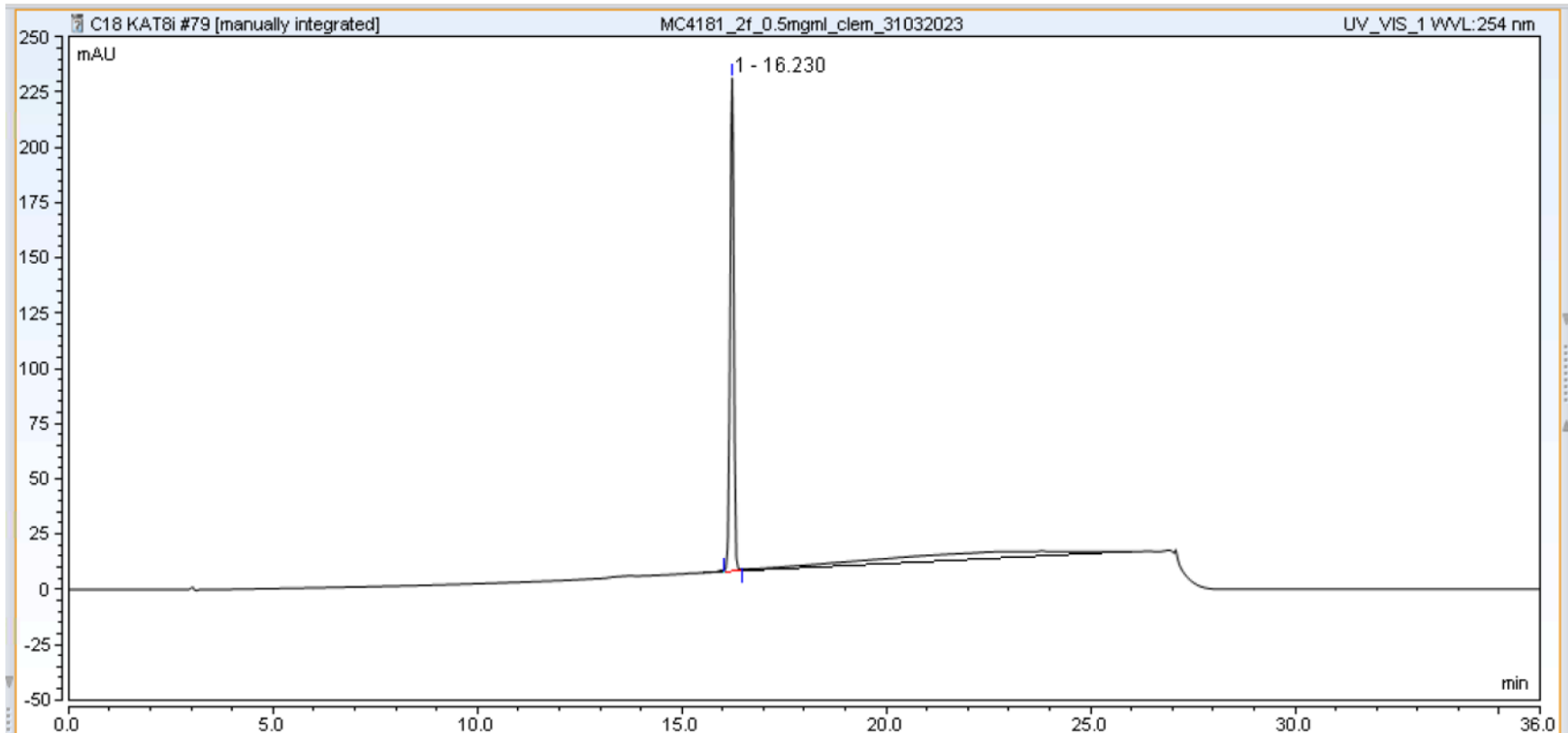


	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	15.973	n.a.	100.00	92.9384	890.43	BM	0.097	0.92	n.a.	150105
6	Maximum		0.0000	100.00	92.9384	890.43		0.097	0.92	0.00	150105
7	Minimum		0.0000	100.00	92.9384	890.43		0.097	0.92	0.00	150105
8	Sum		0.0000	100.00	92.9384	890.43					
9											

2f MC4181

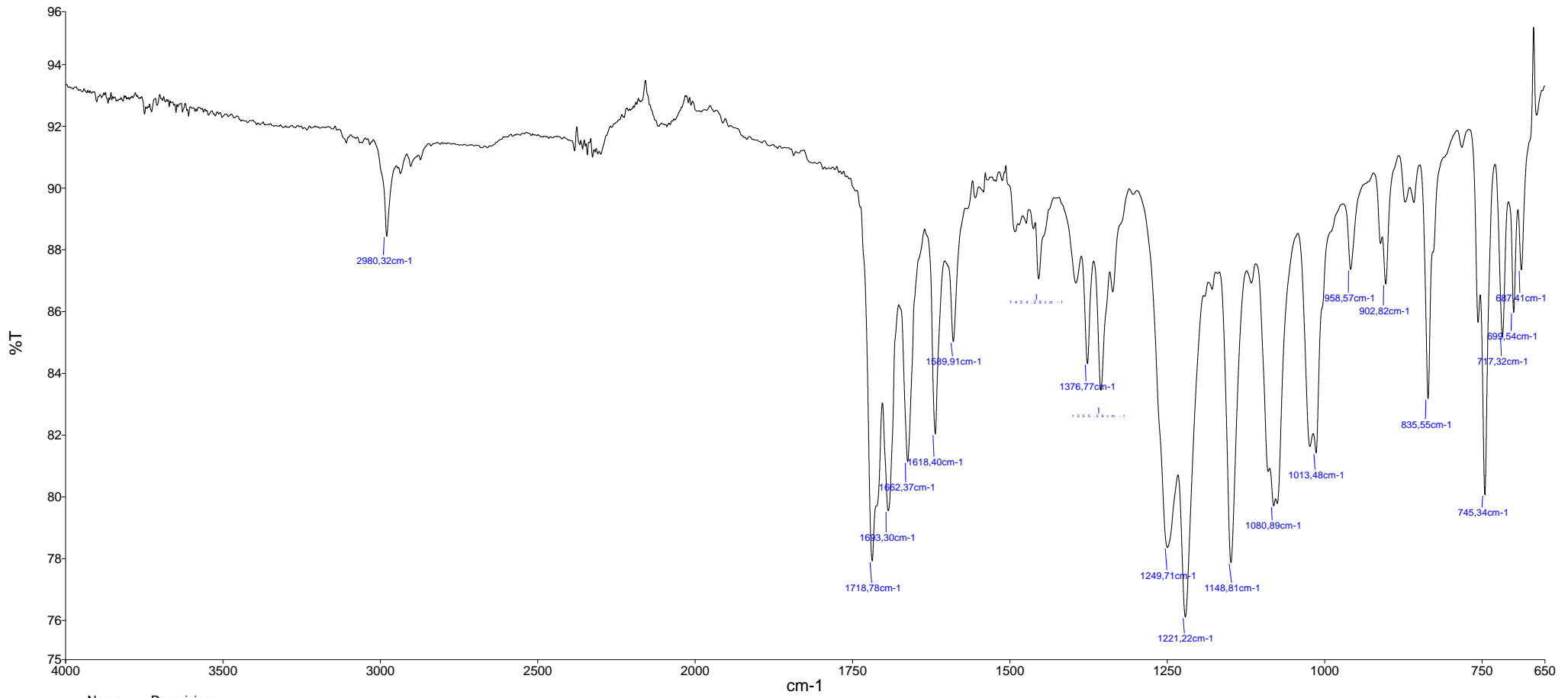


Nome Descrizione
MC4181_1

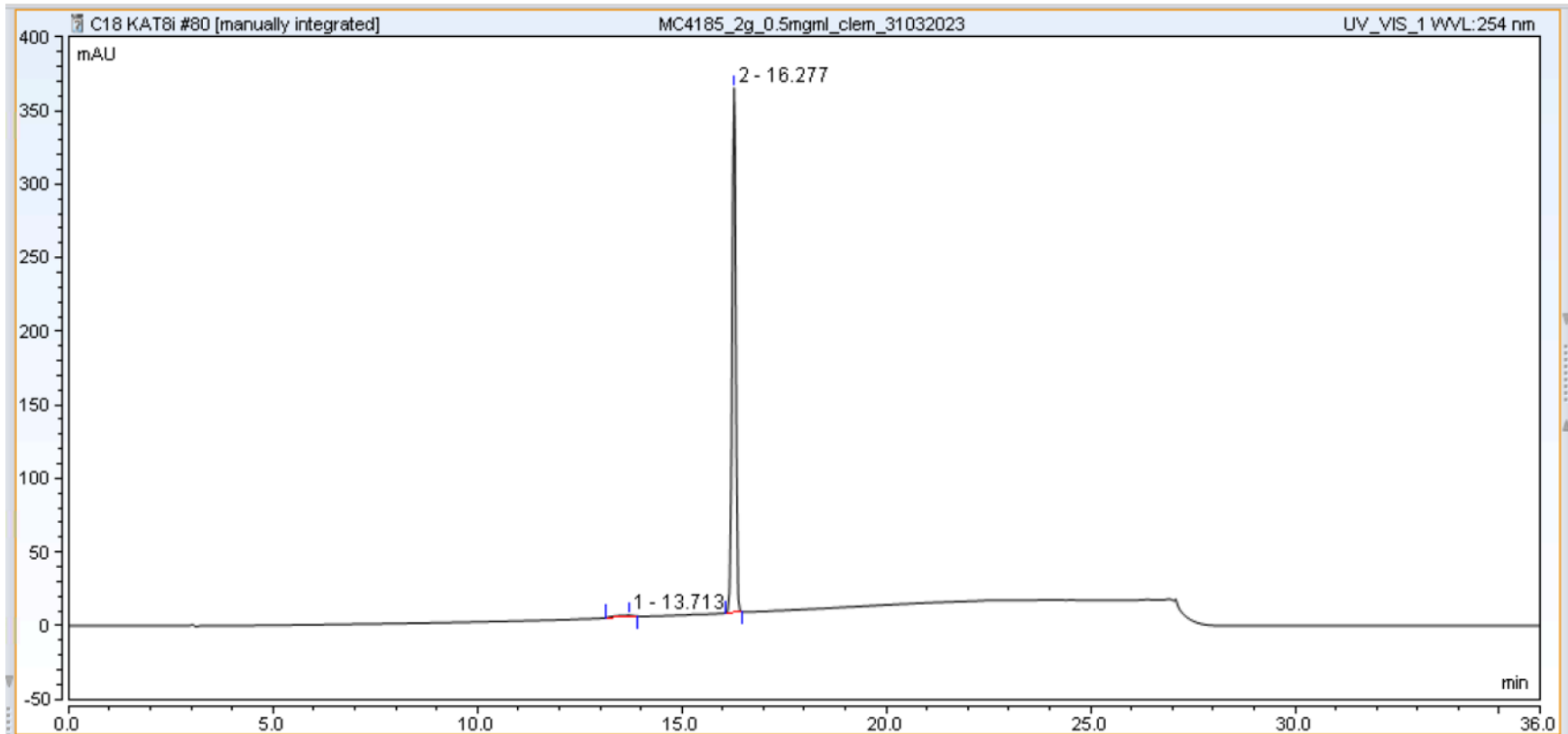


	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	16.230	n.a.	100.00	23.6146	223.14	M	0.098	0.92	n.a.	150911
6	Maximum		0.0000	100.00	23.6146	223.14		0.098	0.92	0.00	150911
7	Minimum		0.0000	100.00	23.6146	223.14		0.098	0.92	0.00	150911
8	Sum		0.0000	100.00	23.6146	223.14					
9											

2g MC4185

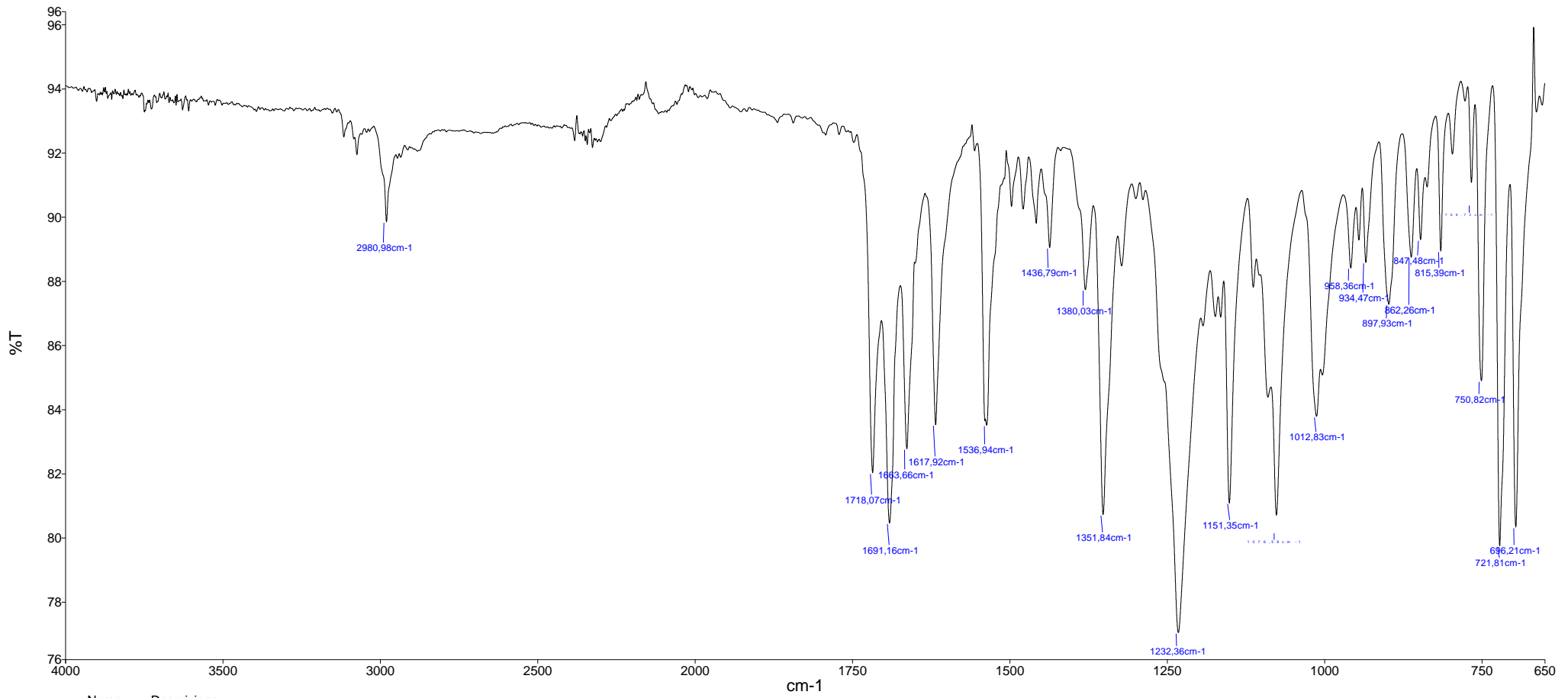


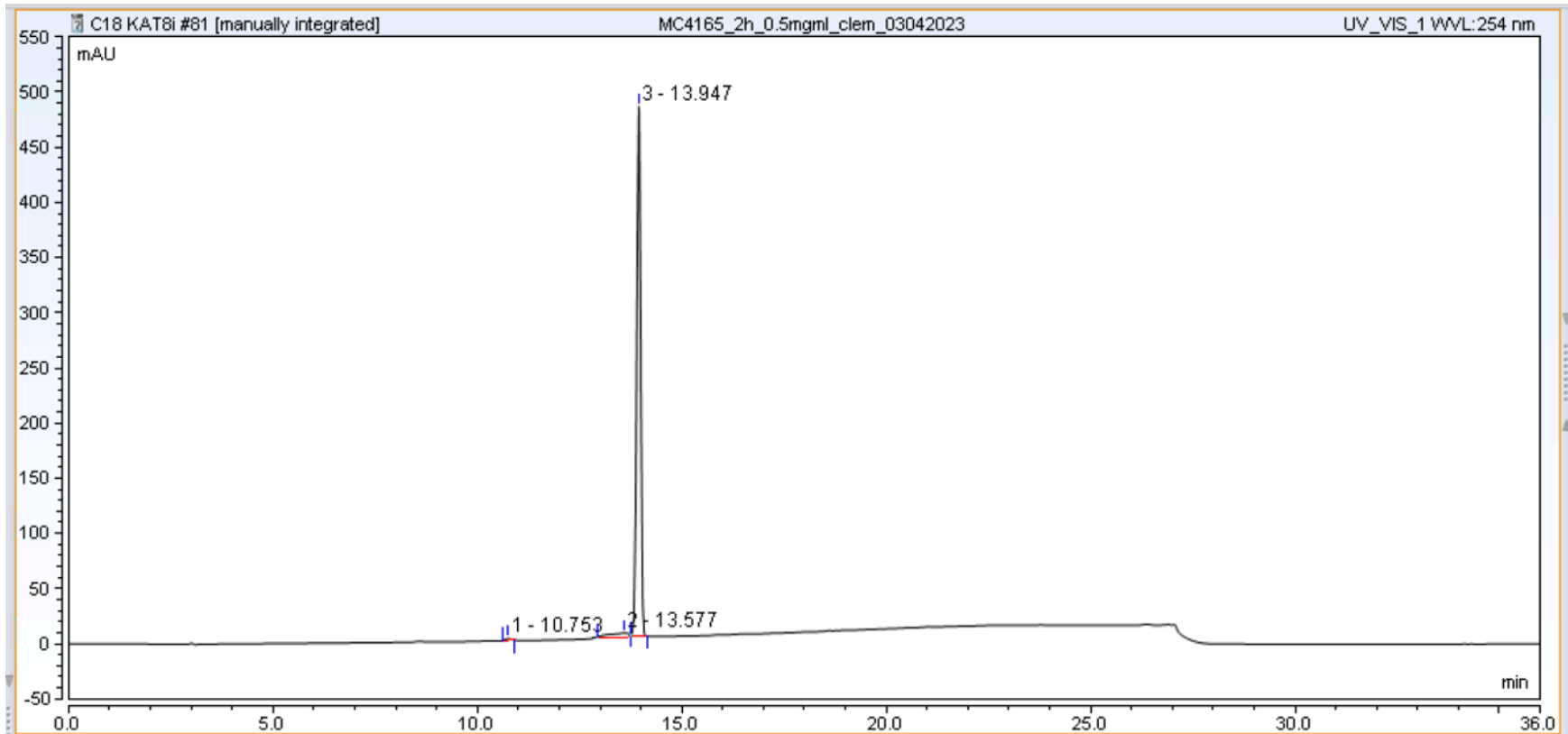
Nome Descrizione
MC4185_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret. Time min	Amount n.a.	Rel. Area %	Area mAU*min	Height mAU	Type	Width (50% min)	Asym. EP	Resol. EP	Plates EP
4	1	13.713	n.a.	1.40	0.5283	0.86	BMB*	0.597	0.67	4.36	2926
5	2	16.277	n.a.	98.60	37.2587	356.38	BMB*	0.098	0.93	n.a.	153624
6	Maximum		0.0000	98.60	37.2587	356.38		0.597	0.93	4.36	153624
7	Minimum		0.0000	1.40	0.5283	0.86		0.098	0.67	4.36	2926
8	Sum		0.0000	100.00	37.7869	357.24					
9											

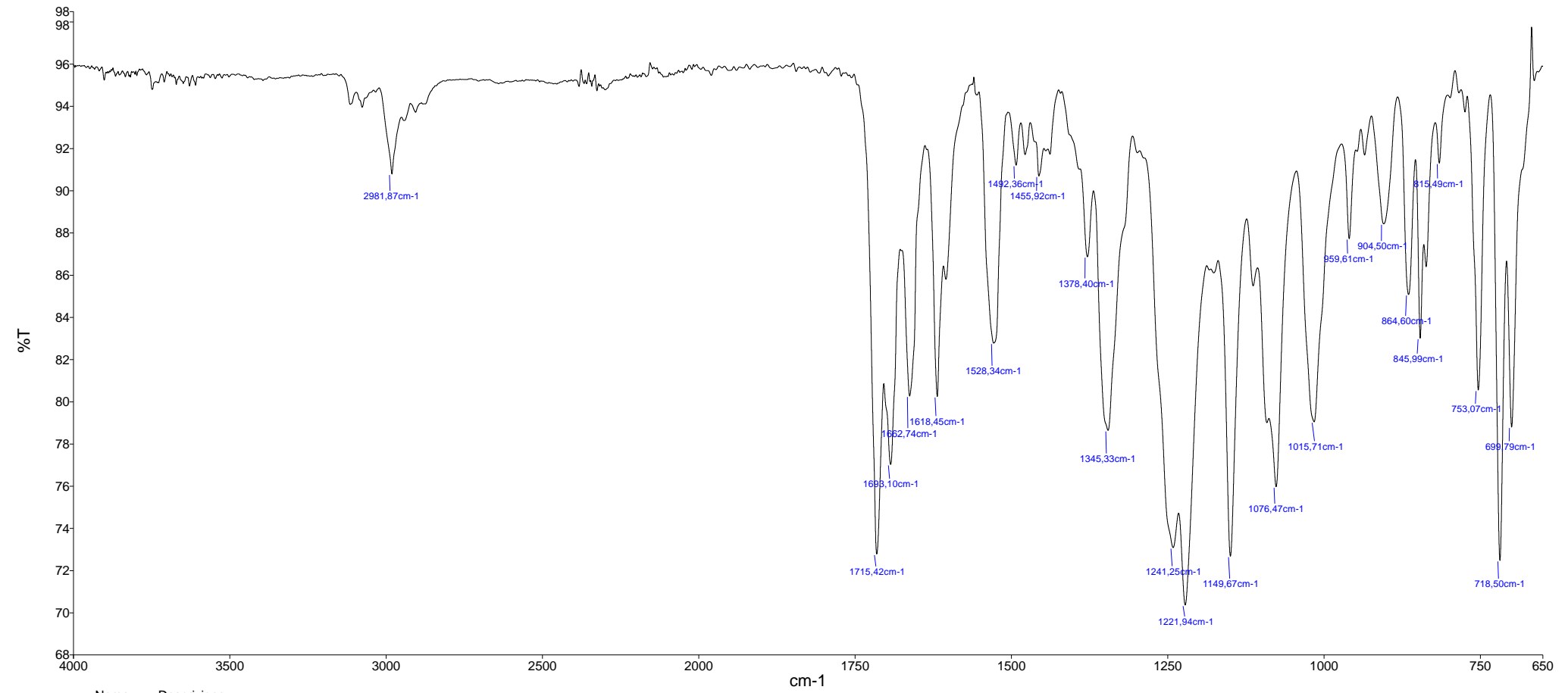
2h MC4165



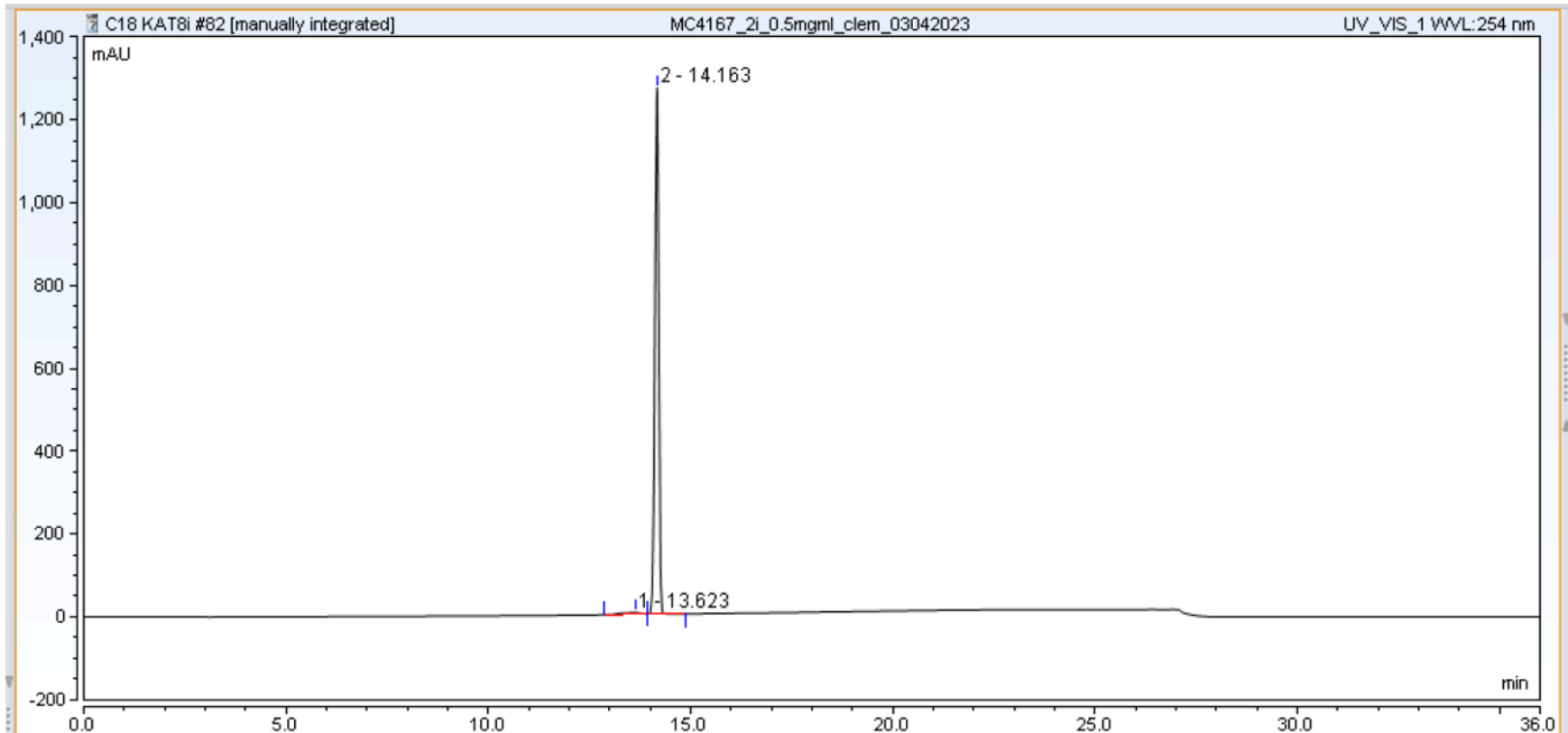


	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret.Time min	Amount n.a.	Rel.Area %	Area mAU*min	Height mAU	Type	Width (50% min)	Asym. EP	Resol. EP	Plates EP
4	1	10.753	n.a.	0.48	0.2688	1.66	BMB*	0.162	1.08	n.a.	24371
5	2	13.577	n.a.	4.29	2.4166	3.79	M *	n.a.	n.a.	n.a.	n.a.
6	3	13.947	n.a.	95.23	53.6503	480.57	MB*	0.105	0.93	n.a.	97269
7	Maximum		0.0000	95.23	53.6503	480.57		0.162	1.08	0.00	97269
8	Minimum		0.0000	0.48	0.2688	1.66		0.105	0.93	0.00	24371
9	Sum		0.0000	100.00	56.3357	486.02					
10											

2i MC4167

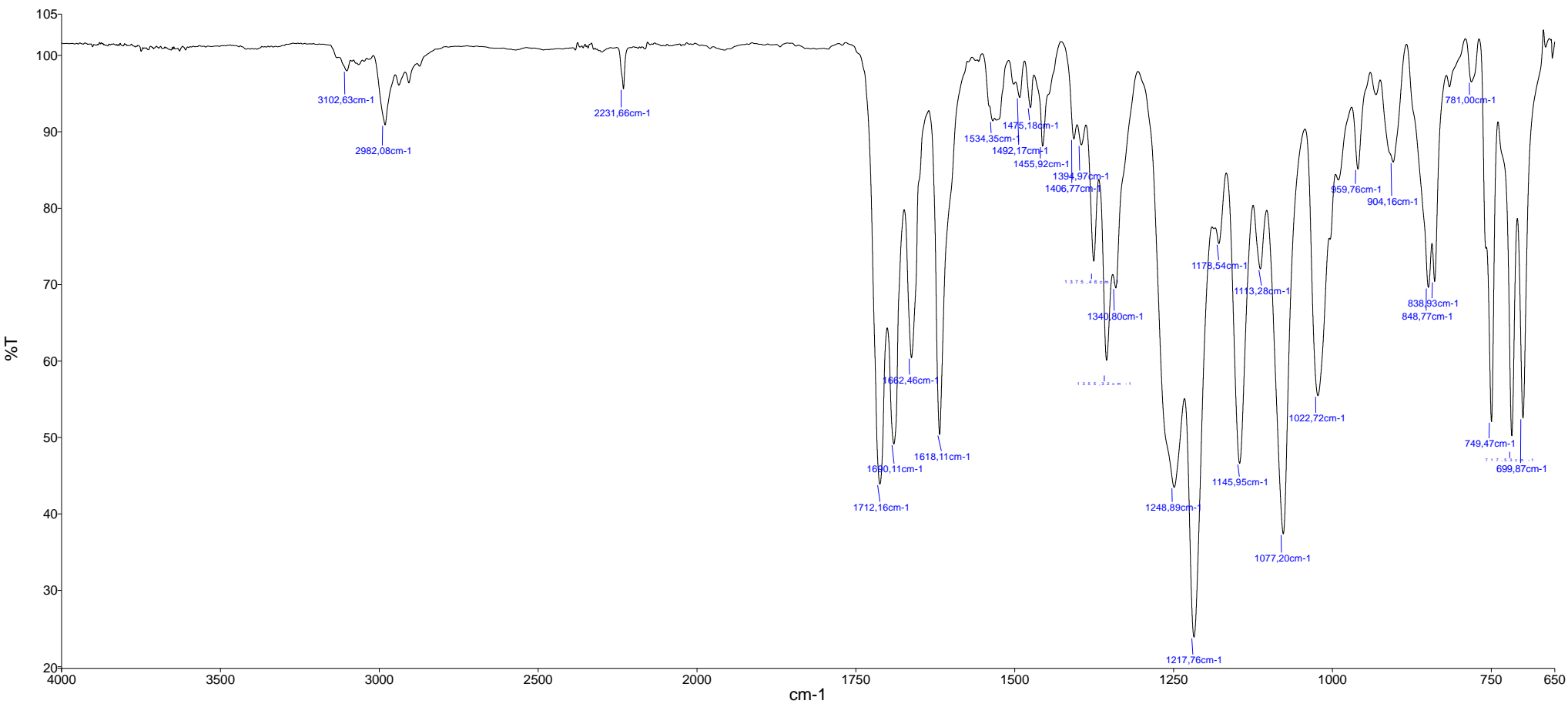


Nome Descrizione
MC4167_1

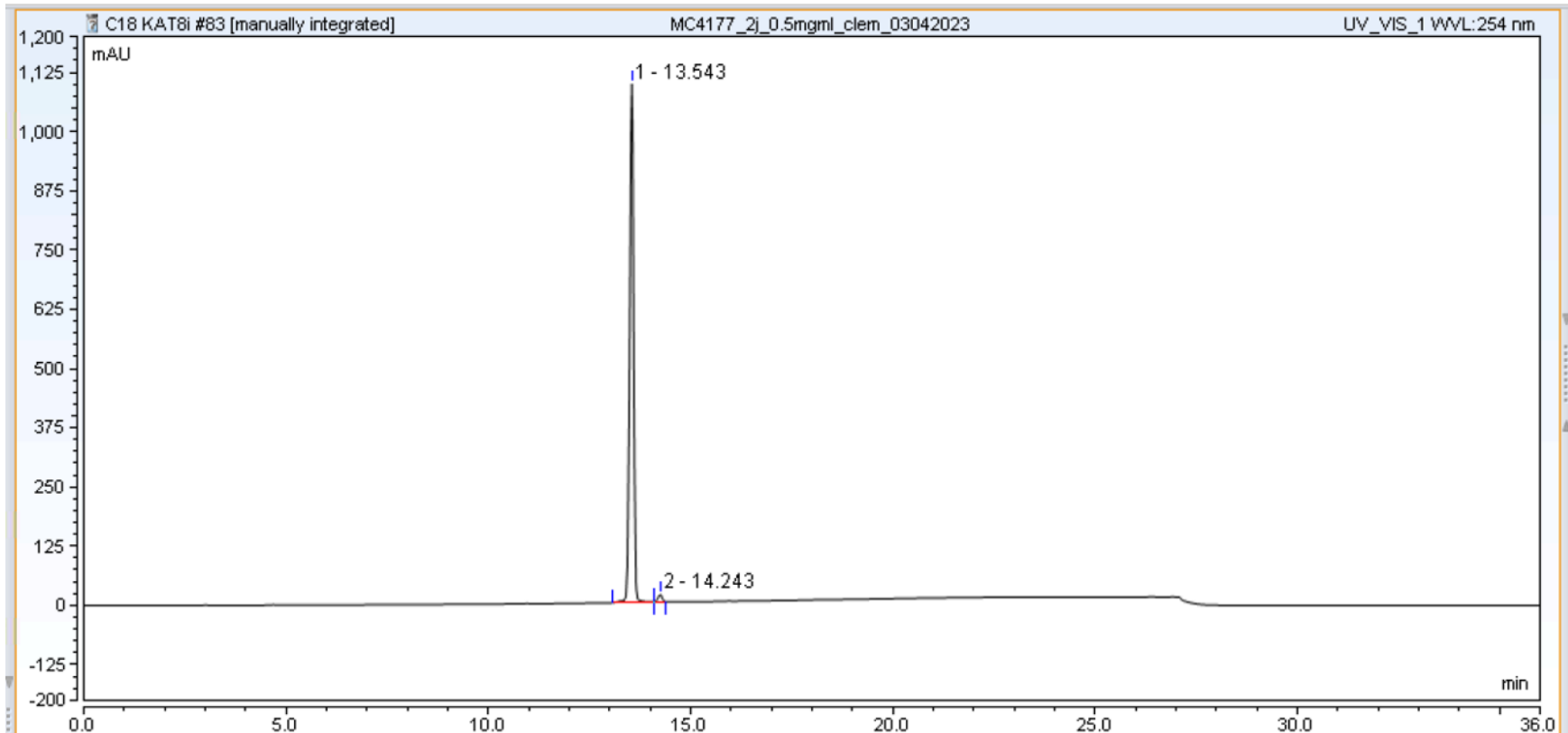


	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	13.623	n.a.	1.90	2.7416	4.18	BM	0.655	n.a.	0.84	2395
5	2	14.163	n.a.	98.10	141.2413	1270.74	MB	0.105	0.93	n.a.	101341
6	Maximum		0.0000	98.10	141.2413	1270.74		0.655	0.93	0.84	101341
7	Minimum		0.0000	1.90	2.7416	4.18		0.105	0.93	0.84	2395
8	Sum		0.0000	100.00	143.9829	1274.92					
9											

2j MC4177

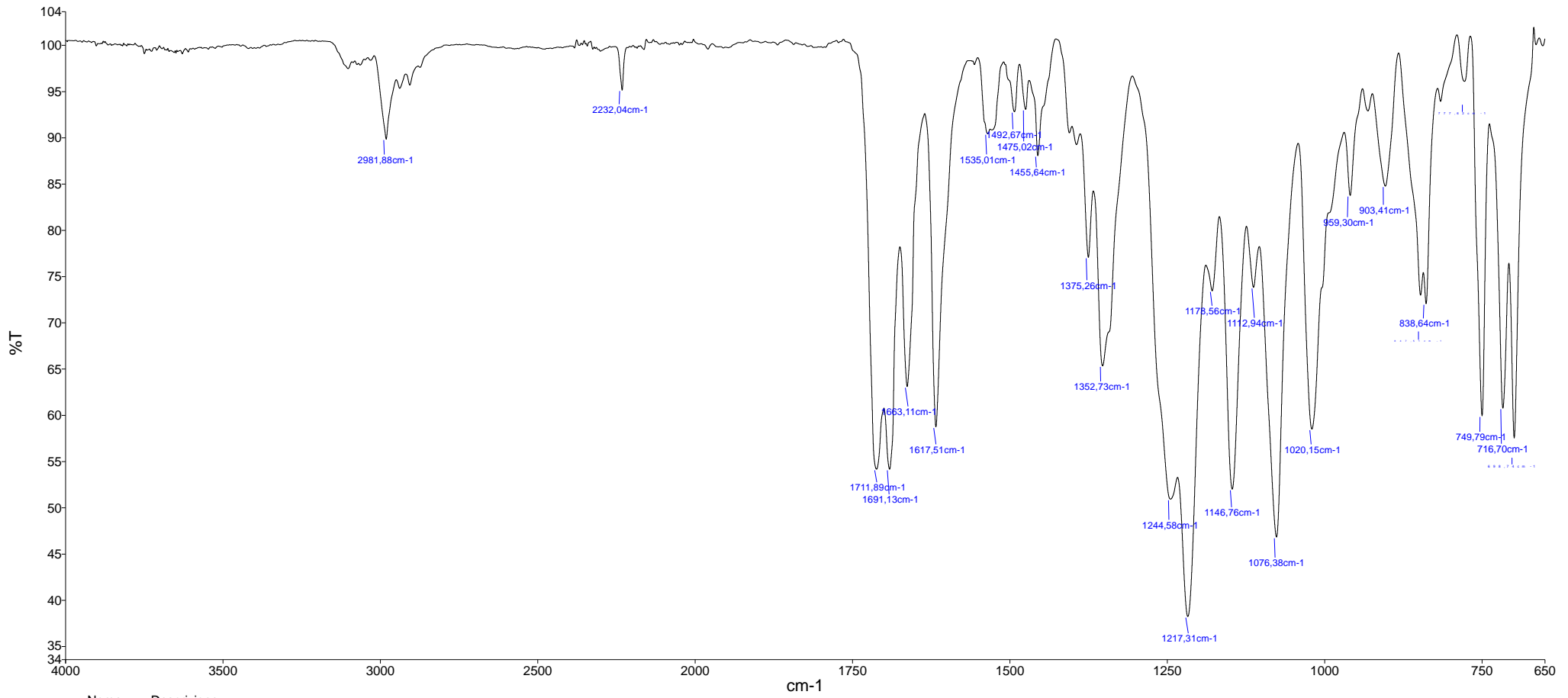


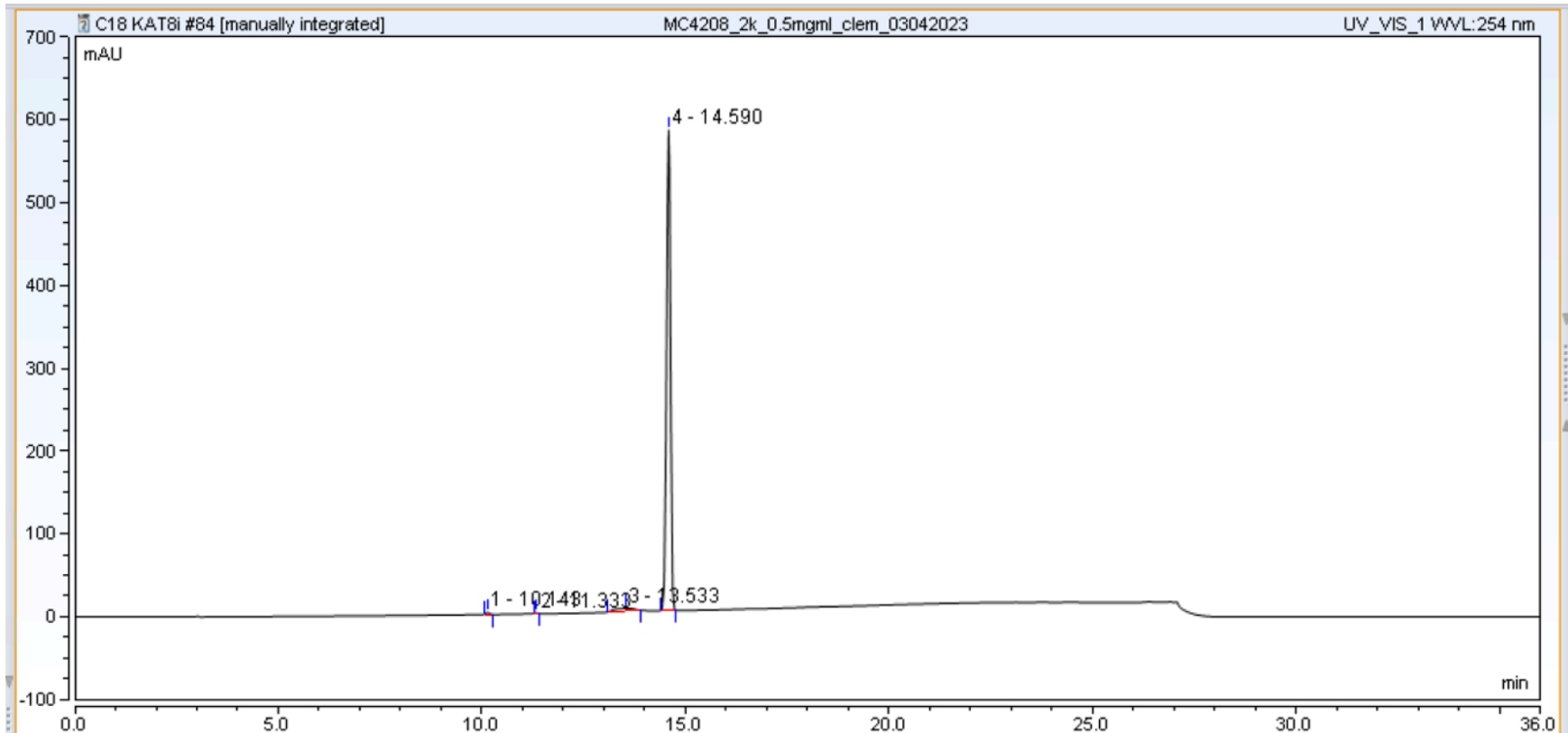
Nome Descrizione
MC4177_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	13.543	n.a.	98.51	124.4290	1094.52	BM *	0.106	0.91	3.85	90784
5	2	14.243	n.a.	1.49	1.8771	16.17	MB*	0.109	0.87	n.a.	95132
6	Maximum		0.0000	98.51	124.4290	1094.52		0.109	0.91	3.85	95132
7	Minimum		0.0000	1.49	1.8771	16.17		0.106	0.87	3.85	90784
8	Sum		0.0000	100.00	126.3062	1110.69					
9											

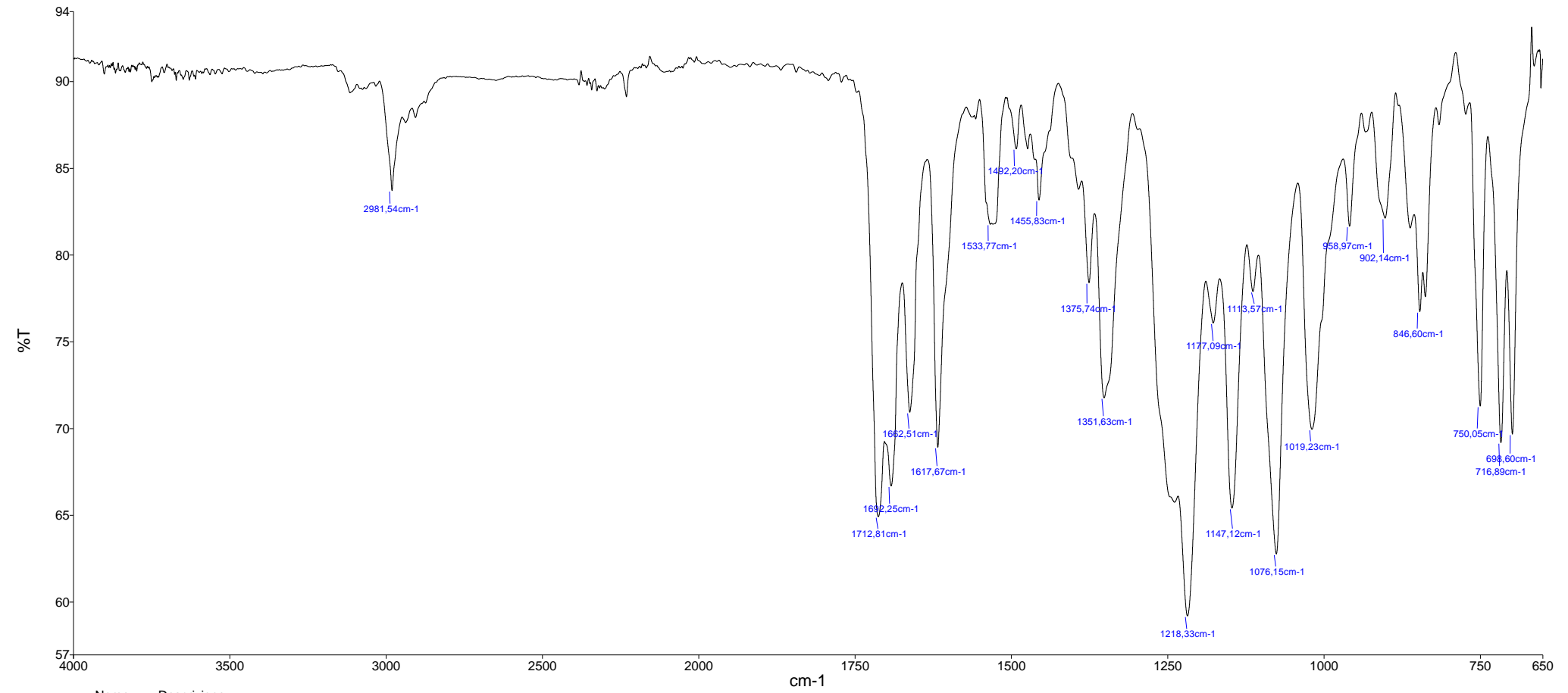
2k MC4208



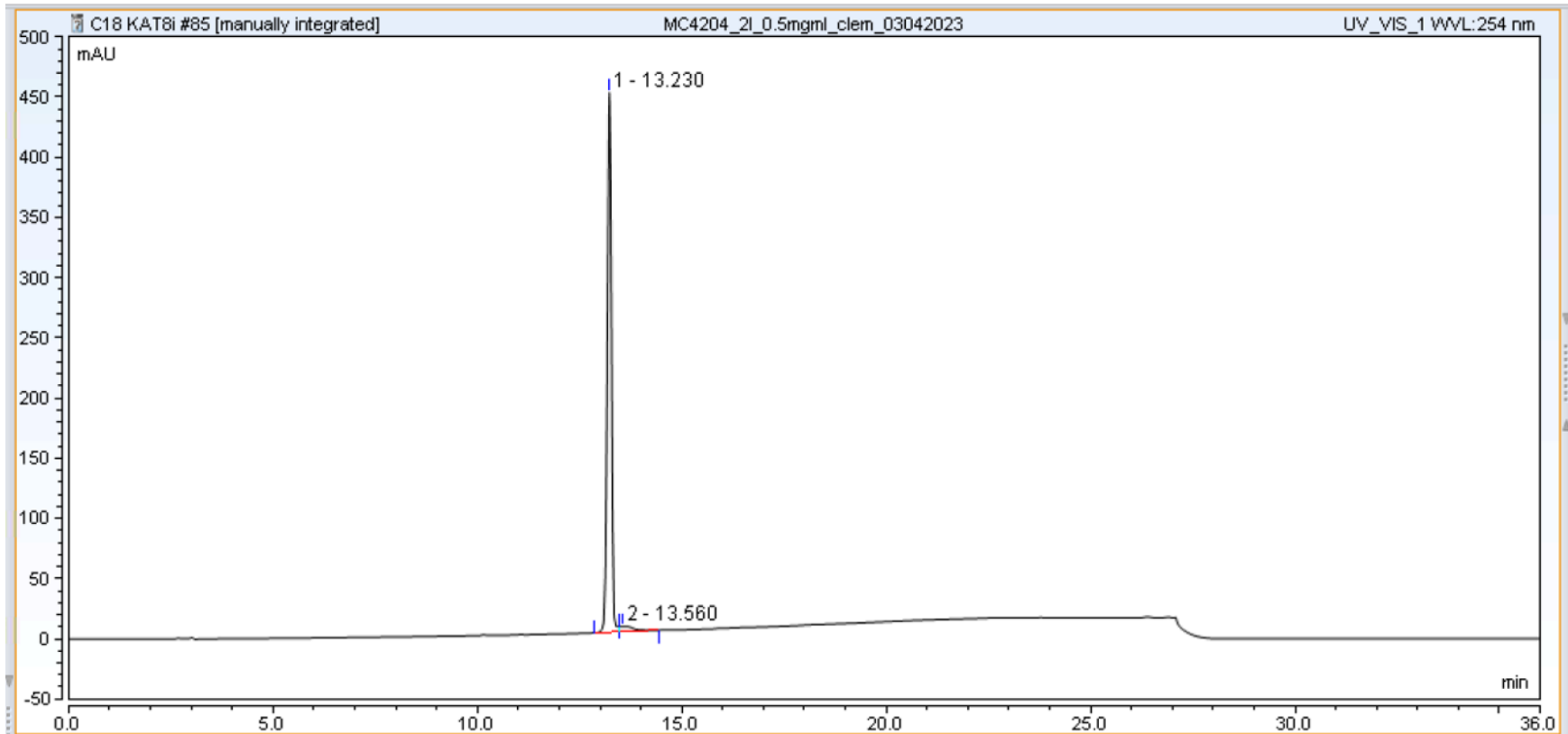


	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	10.143	n.a.	0.37	0.2459	2.42	BMB*	0.100	1.02	7.80	57444
5	2	11.333	n.a.	0.11	0.0772	0.98	BMB*	0.080	1.10	3.98	109900
6	3	13.533	n.a.	2.68	1.8011	3.39	BMB*	0.573	0.91	1.84	3095
7	4	14.590	n.a.	96.84	65.1264	579.71	BMB*	0.106	0.92	n.a.	104457
8	Maximum		0.0000	96.84	65.1264	579.71		0.573	1.10	7.80	109900
9	Minimum		0.0000	0.11	0.0772	0.98		0.080	0.91	1.84	3095
10	Sum		0.0000	100.00	67.2506	586.51					
11											

2I MC4204

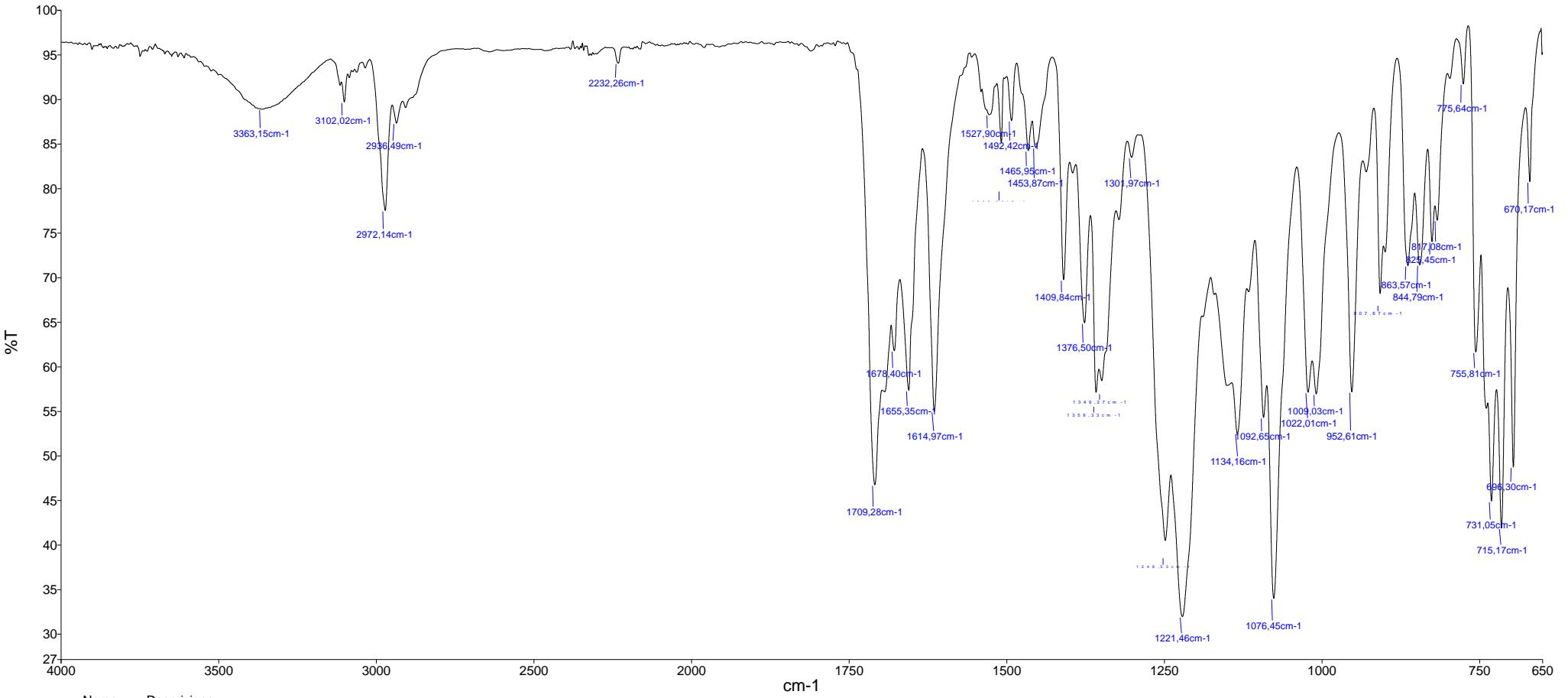


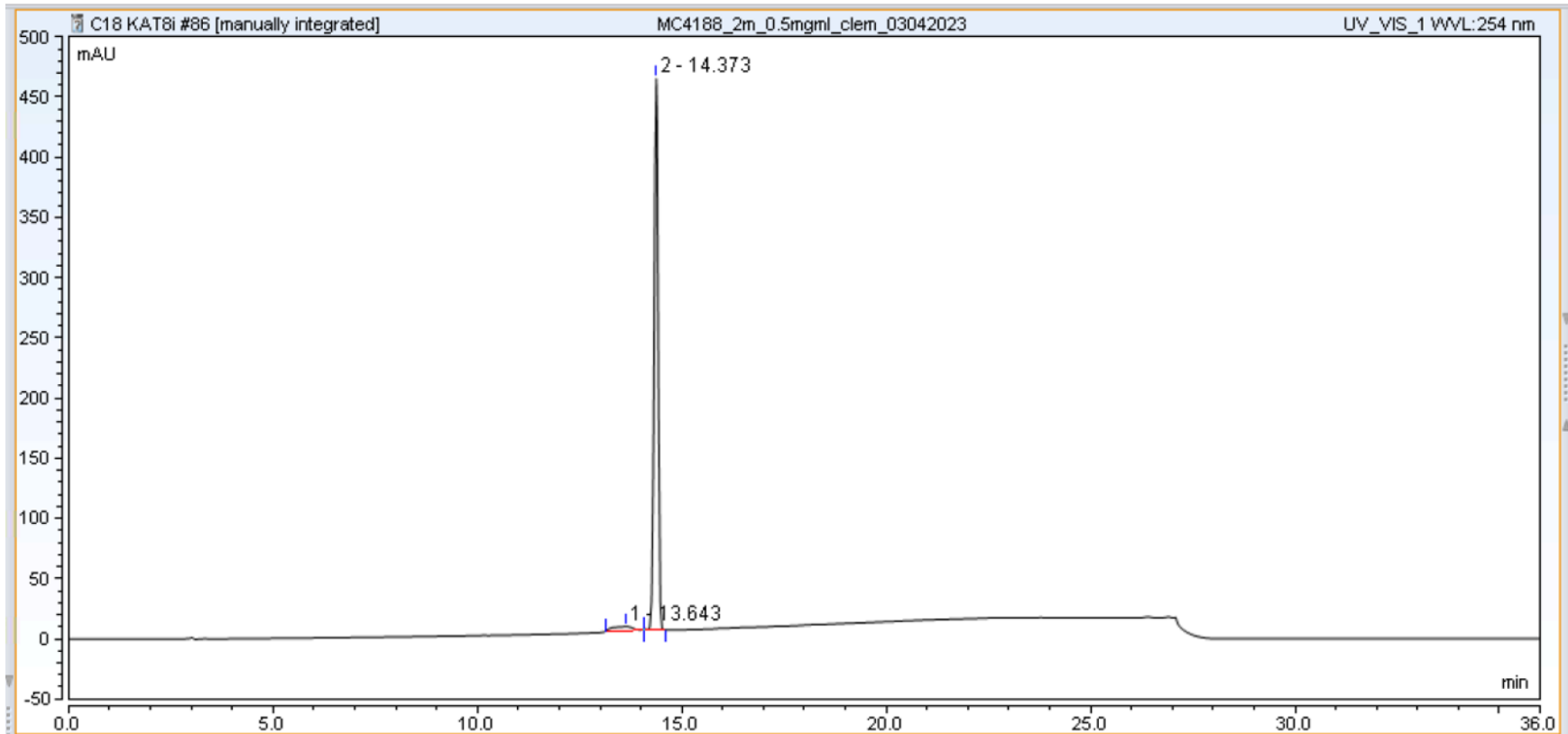
Nome Descrizione
MC4204_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min		%	mAU*min	mAU		min	EP	EP	EP
4	1	13.230	n.a.	96.66	52.0938	448.22	BM	0.108	0.93	n.a.	83258
5	2	13.560	n.a.	3.34	1.8026	4.35	MB	n.a.	n.a.	n.a.	n.a.
6	Maximum		0.0000	96.66	52.0938	448.22		0.108	0.93	0.00	83258
7	Minimum		0.0000	3.34	1.8026	4.35		0.108	0.93	0.00	83258
8	Sum		0.0000	100.00	53.8964	452.57					
9											

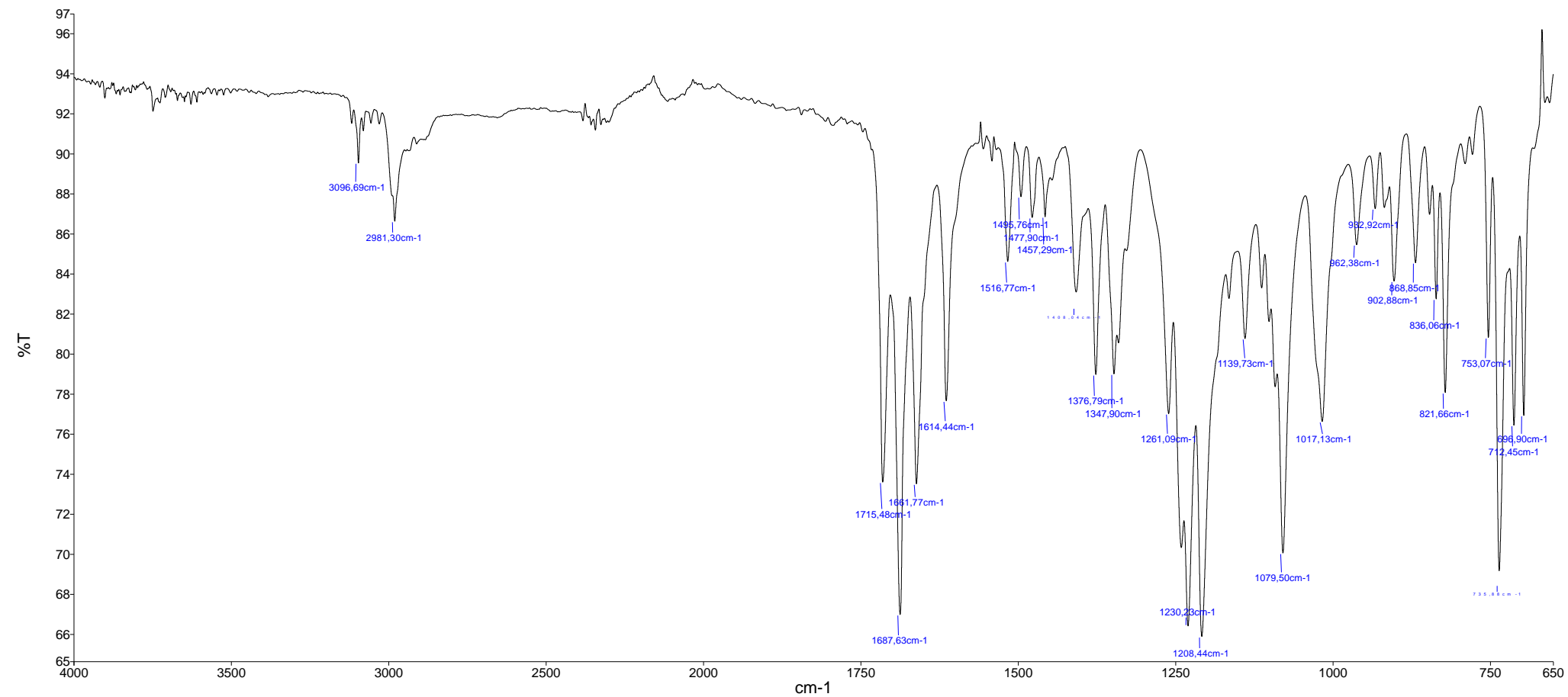
2m MC4188

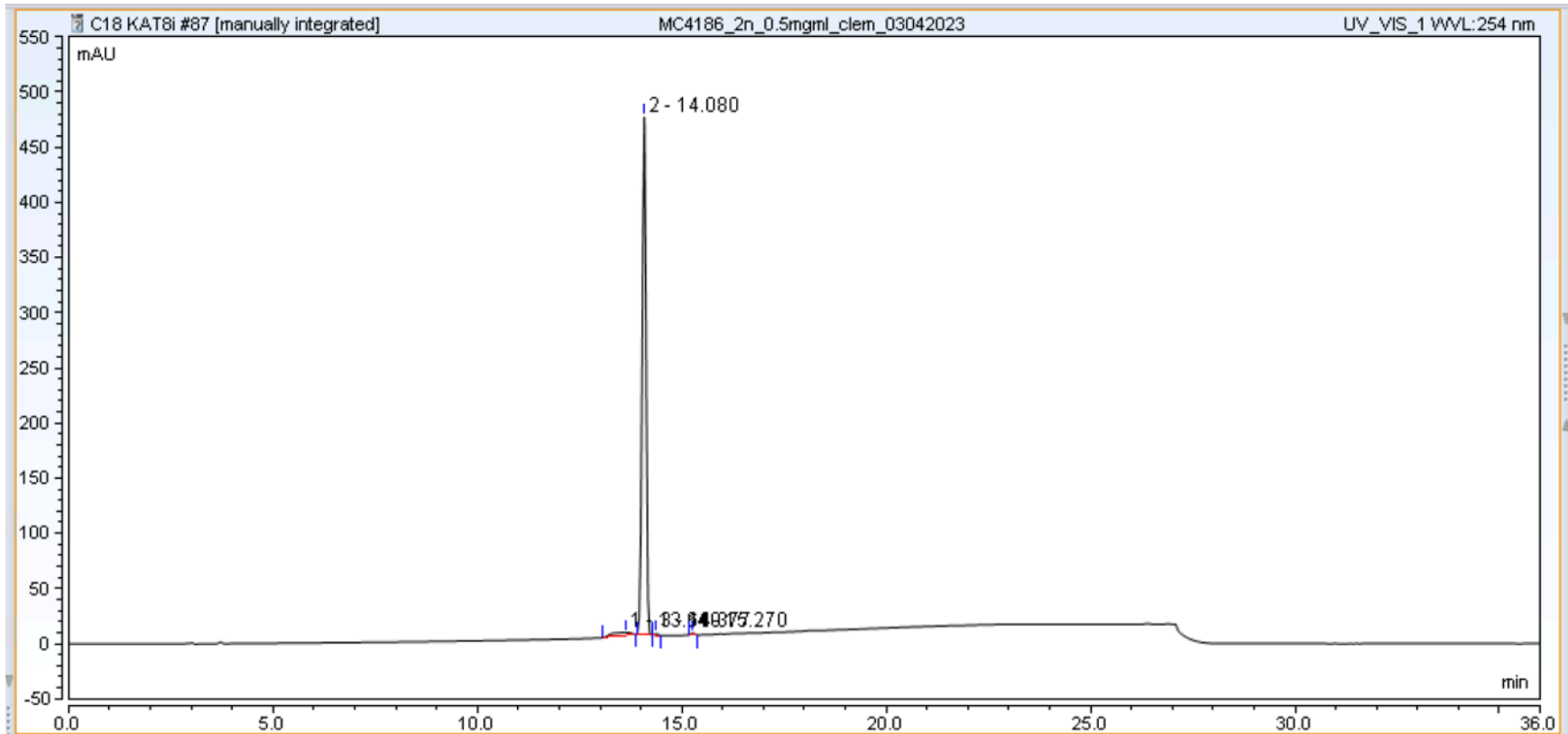




	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret. Time min	Amount	Rel. Area %	Area mAU*min	Height mAU	Type	Width (50% min)	Asym. EP	Resol. EP	Plates EP
4	1	13.643	n.a.	3.96	2.1446	3.42	BM *	0.617	n.a.	1.19	2712
5	2	14.373	n.a.	96.04	52.0605	458.00	MB*	0.107	0.92	n.a.	99766
6	Maximum		0.0000	96.04	52.0605	458.00		0.617	0.92	1.19	99766
7	Minimum		0.0000	3.96	2.1446	3.42		0.107	0.92	1.19	2712
8	Sum		0.0000	100.00	54.2051	461.42					
9											

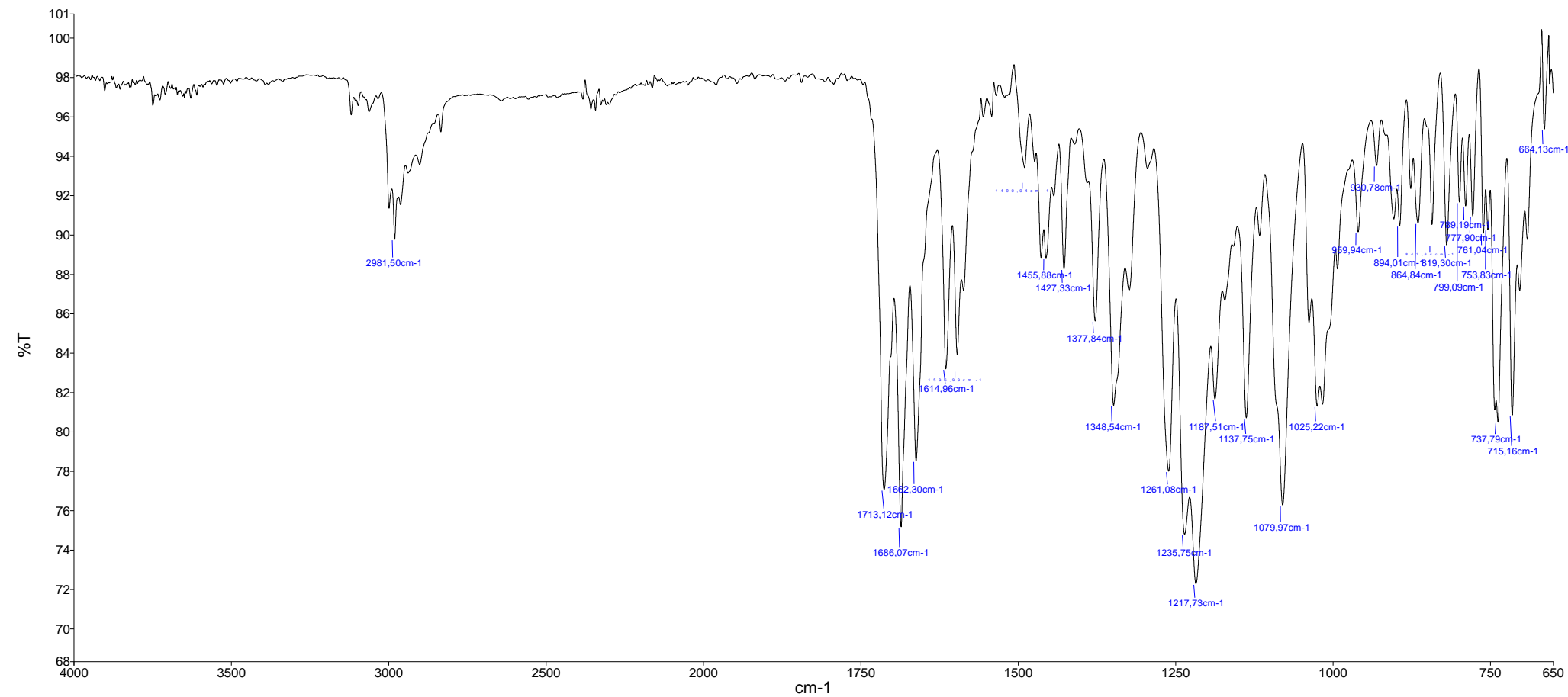
2n MC4186

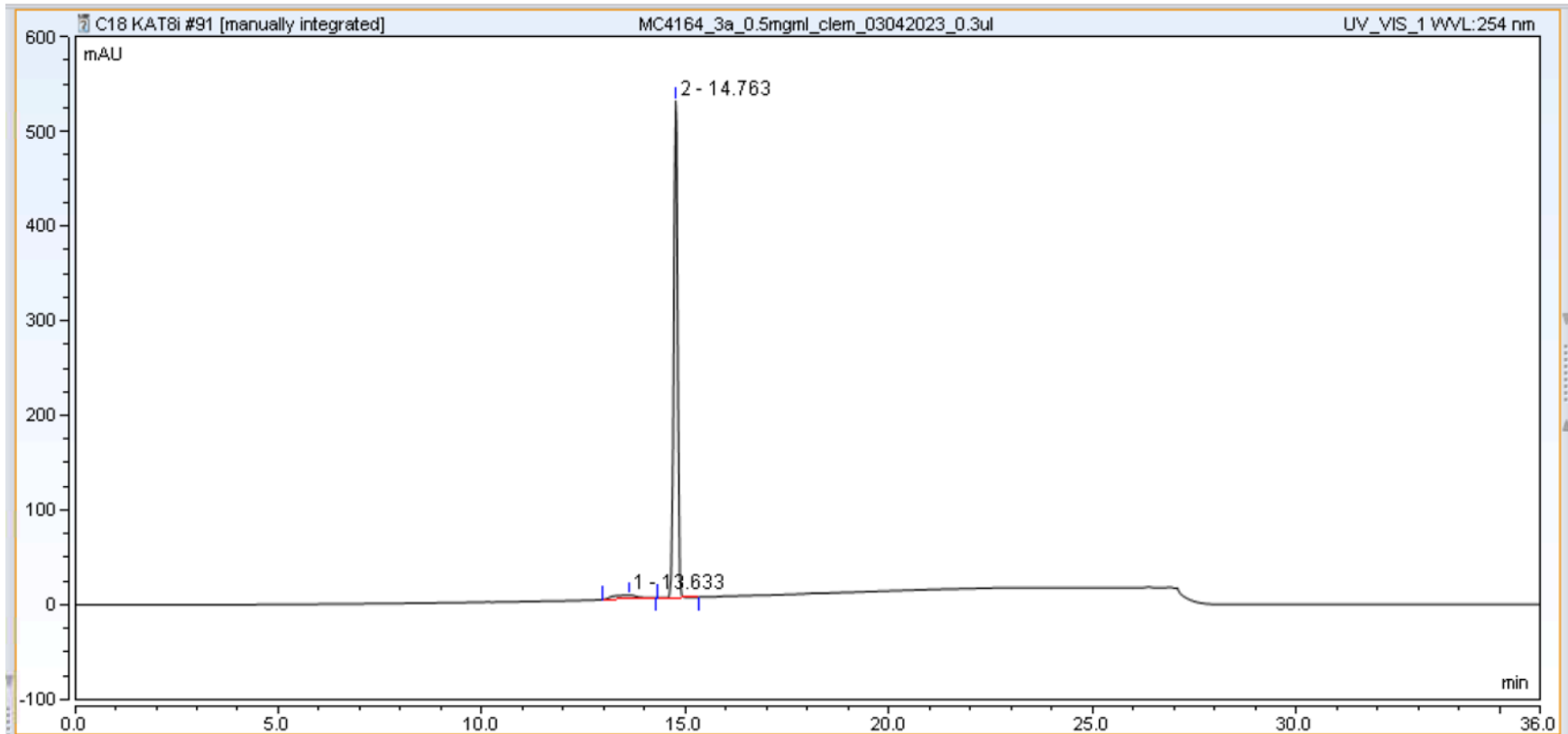




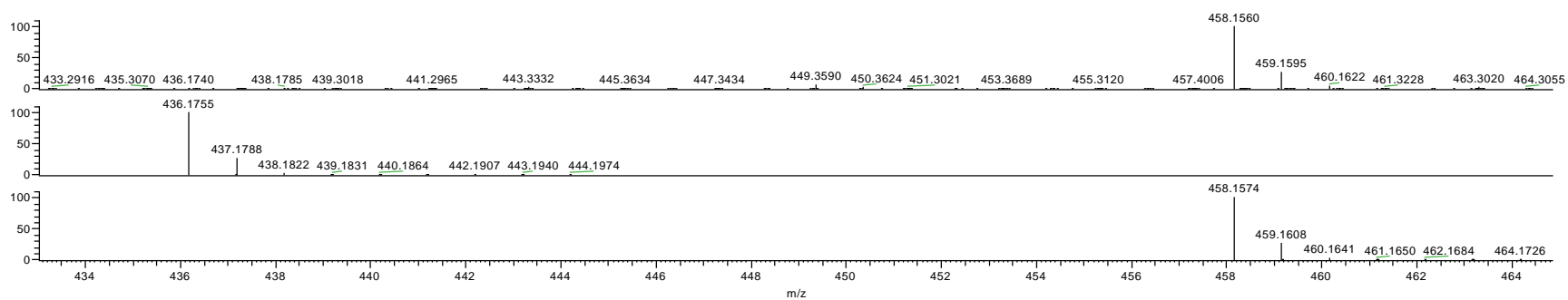
	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret. Time min	Amount n.a.	Rel. Area %	Area mAU*min	Height mAU	Type	Width (50% min	Asym. EP	Resol. EP	Plates EP
2											
4	1	13.640	n.a.	3.02	1.6319	2.56	BMB*	0.597	0.71	0.74	2892
5	2	14.080	n.a.	96.36	52.0346	469.17	BMB*	0.105	0.92	1.74	100569
6	3	14.377	n.a.	0.33	0.1798	1.83	BMB*	0.096	1.00	5.58	123520
7	4	15.270	n.a.	0.29	0.1545	1.66	BMB*	0.093	1.03	n.a.	150522
8	Maximum		0.0000	96.36	52.0346	469.17		0.597	1.03	5.58	150522
9	Minimum		0.0000	0.29	0.1545	1.66		0.093	0.71	0.74	2892
10	Sum		0.0000	100.00	54.0007	475.21					
11											

3a MC4164





	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	13.633	n.a.	4.58	2.7398	3.79	BMB*	0.690	0.92	1.68	2163
5	2	14.763	n.a.	95.42	57.0931	524.87	BMB*	0.102	0.94	n.a.	116027
6	Maximum		0.0000	95.42	57.0931	524.87		0.690	0.94	1.68	116027
7	Minimum		0.0000	4.58	2.7398	3.79		0.102	0.92	1.68	2163
8	Sum		0.0000	100.00	59.8328	528.66					
9											

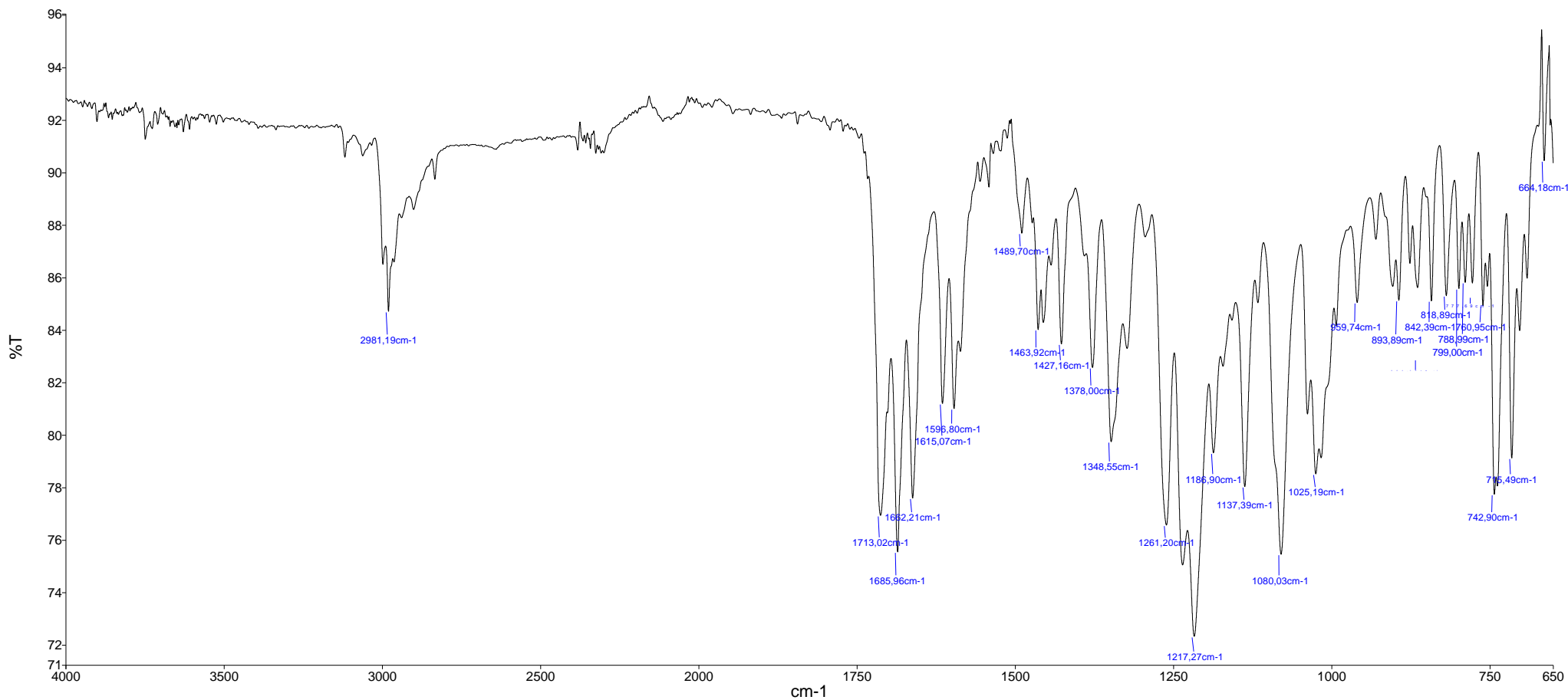


NL:
 2.35E7
 MC4164_20230329120503#986-1000
 RT: 5.36-5.43 AV: 15 T: FTMS + c ESI
 Full ms [106.7000-1600.0000]

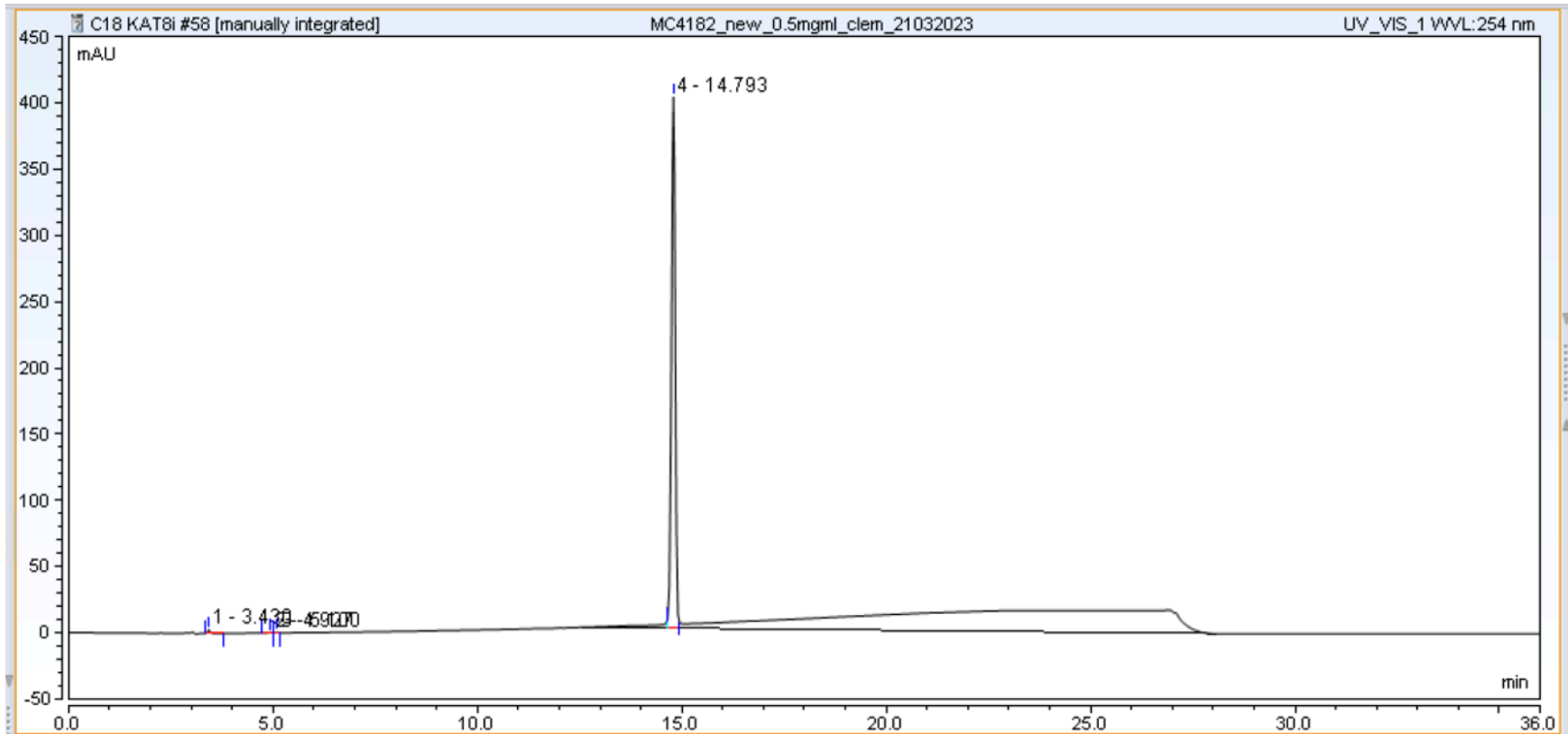
NL:
 7.48E5
 C₂₅H₂₅O₆N₁+H:
 C₂₅H₂₅O₆N₁
 pa Chrg 1

NL:
 7.48E5
 C₂₅H₂₅O₆N₁+Na:
 C₂₅H₂₅O₆N₁Na₁
 pa Chrg 1

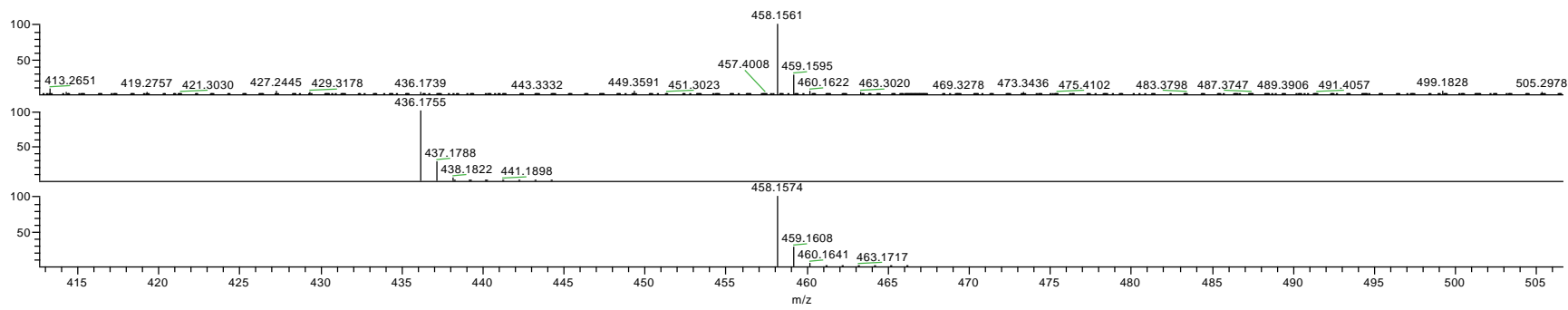
3b MC4182



Nome Descrizione
MC4182_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	µl	%	mAU*min	mAU		min	EP	EP	EP
4	1	3.430	n.a.	0.40	0.1731	3.09	BMB*	0.054	0.99	14.16	22415
5	2	4.927	n.a.	0.12	0.0496	0.65	BM *	0.071	0.90	1.46	26827
6	3	5.100	n.a.	0.10	0.0436	0.61	MB*	0.070	0.94	68.12	29613
7	4	14.793	n.a.	99.38	42.5068	401.33	M *	0.098	0.93	n.a.	125825
8	Maximum		0.0000	99.38	42.5068	401.33		0.098	0.99	68.12	125825
9	Minimum		0.0000	0.10	0.0436	0.61		0.054	0.90	1.46	22415
10	Sum		0.0000	100.00	42.7731	405.68					
11											

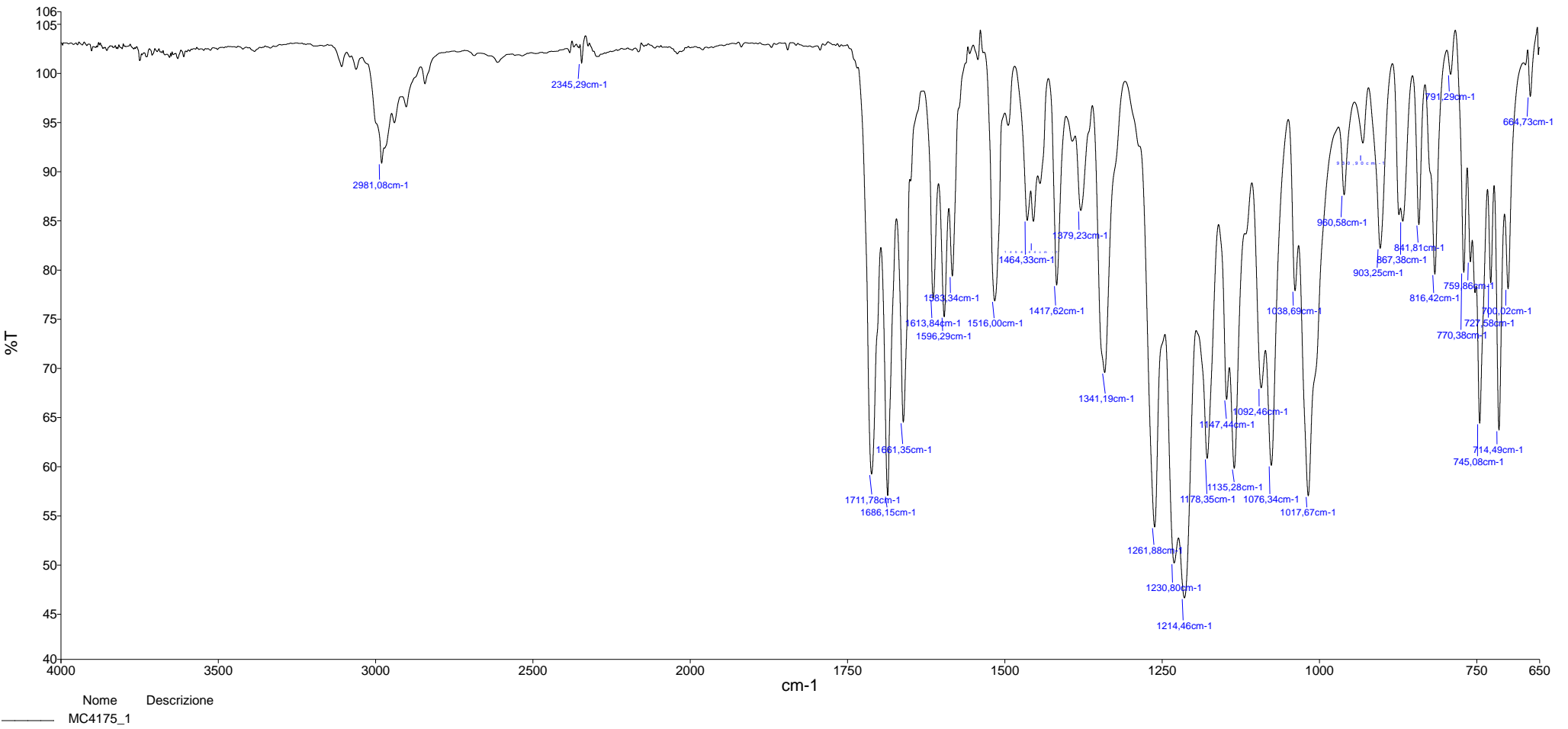


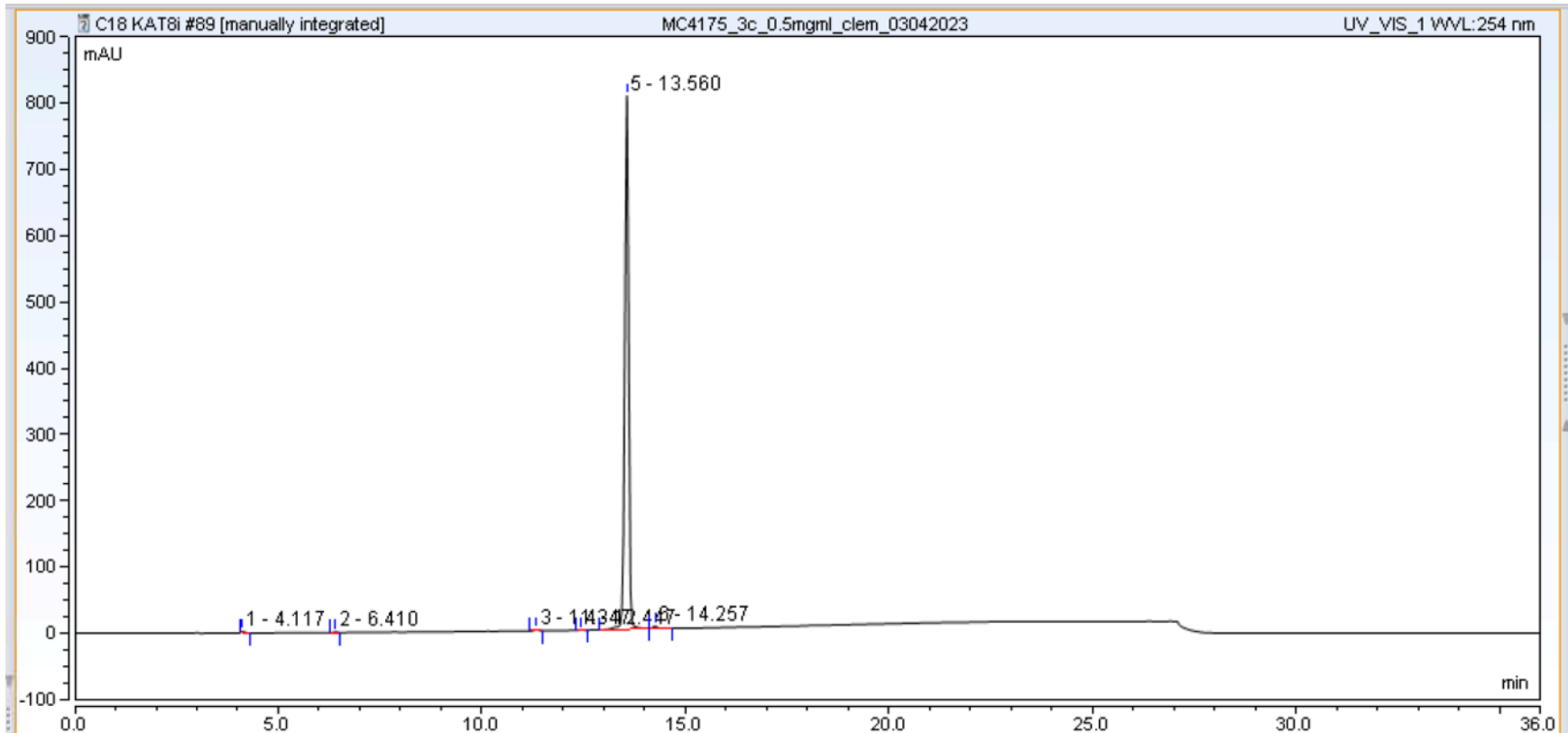
NL:
 4.50E7
 MC8182_20230329115602#978-995
 RT: 5.32-5.41 AV: 18 T: FTMS + c
 ESI Full ms[106.7000-1600.0000]

NL:
 7.48E5
 C₂₅H₂₅O₆N₁+H:
 C₂₅H₂₆O₆N₁
 pa Chrg 1

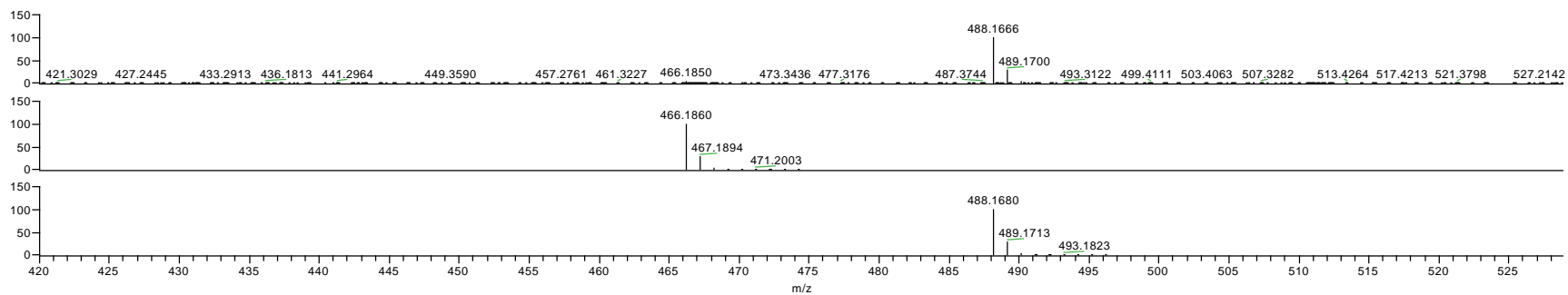
NL:
 7.48E5
 C₂₅H₂₅O₆N₁+Na:
 C₂₅H₂₅O₆N₁Na₁
 pa Chrg 1

3c MC4175





	A	B	C	D	E	F	G	H	I	J	K
1	Peak No.	Ret.Time min	Amount µl	Rel.Area %	Area mAU*min	Height mAU	Type	Width (50% min	Asym. EP	Resol. EP	Plates EP
4	1	4.117	n.a.	0.14	0.1257	1.85	BMB*	0.068	1.63	17.50	20052
5	2	6.410	n.a.	0.11	0.1000	1.09	BMB	0.086	0.96	30.88	30654
6	3	11.347	n.a.	0.24	0.2192	2.00	BMB	0.102	0.89	6.35	67932
7	4	12.447	n.a.	0.07	0.0637	0.60	BMB*	0.102	0.90	6.45	82676
8	5	13.560	n.a.	98.81	89.8827	805.24	BM	0.102	0.93	3.90	98028
9	6	14.257	n.a.	0.63	0.5746	4.39	MB	0.109	n.a.	n.a.	95469
10	Maximum		0.0000	98.81	89.8827	805.24		0.109	1.63	30.88	98028
11	Minimum		0.0000	0.07	0.0637	0.60		0.068	0.89	3.90	20052
12	Sum		0.0000	100.00	90.9661	815.17					
13											

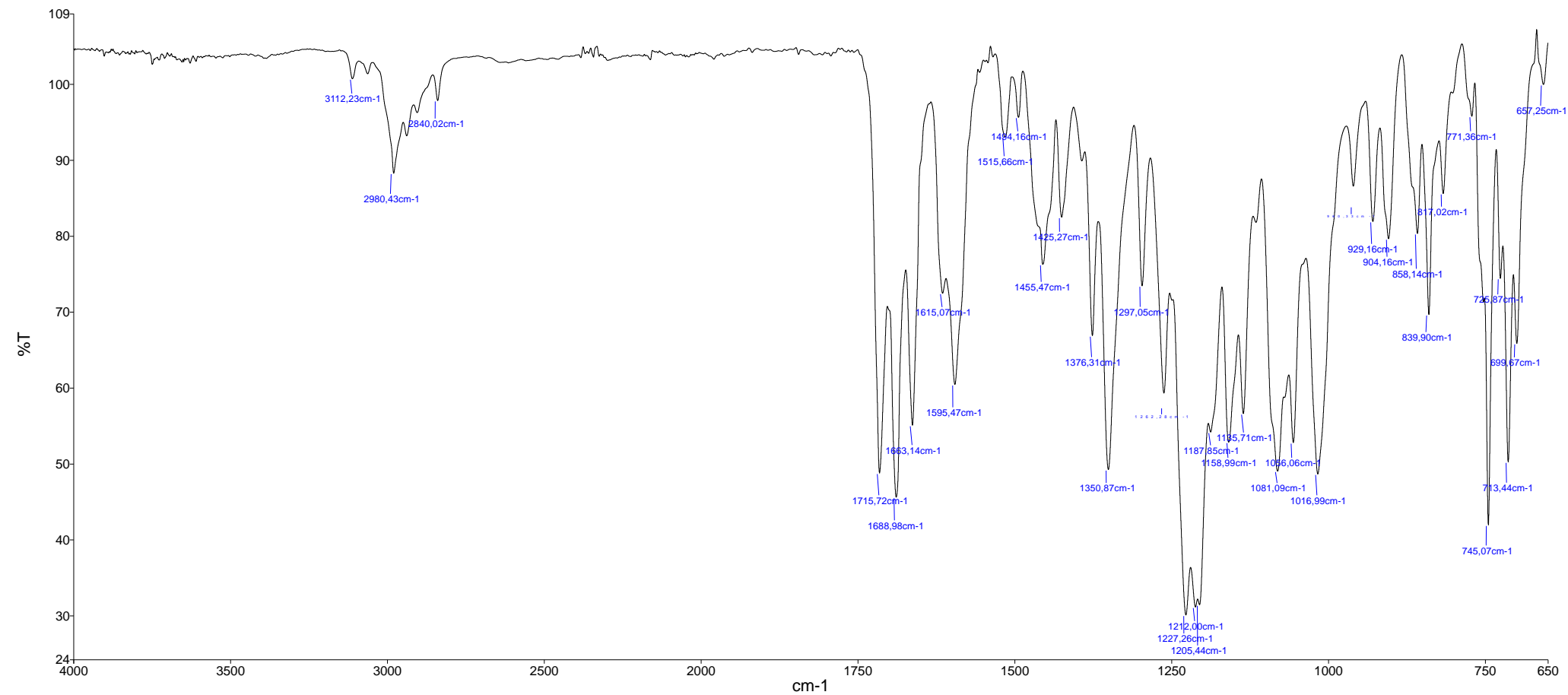


NL:
 5.07E7
 MC4175_20230329122258#956-977
 RT: 5.20-5.31 AV: 22 T: FTMS + c
 ESI Full ms [106.7000-1600.0000]

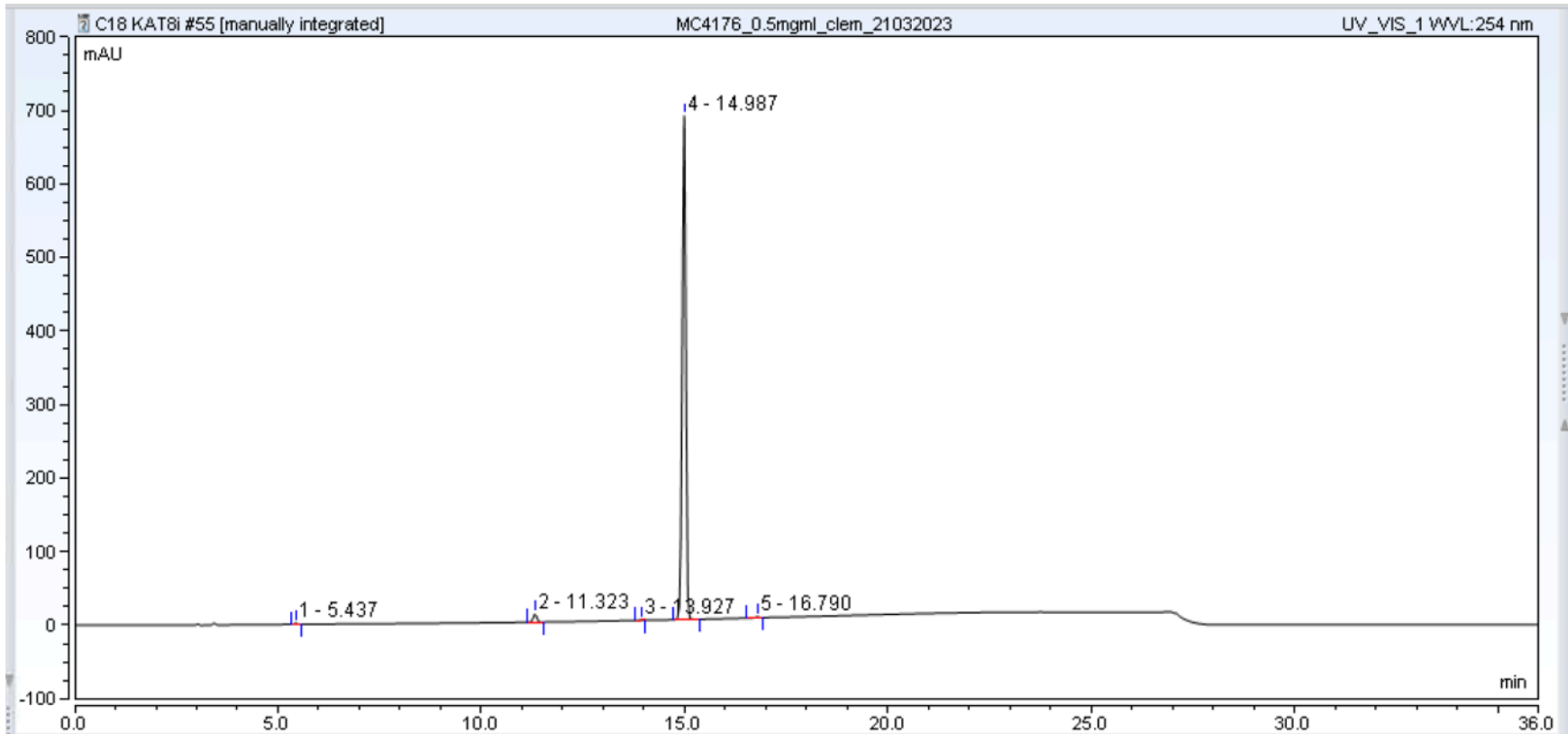
NL:
 7.38E5
 C₂₈H₂₇O₇N₁+H:
 C₂₈H₂₈O₇N₁
 pa Chrg 1

NL:
 7.38E5
 C₂₈H₂₇O₇N₁+Na:
 C₂₈H₂₇O₇N₁Na₁
 pa Chrg 1

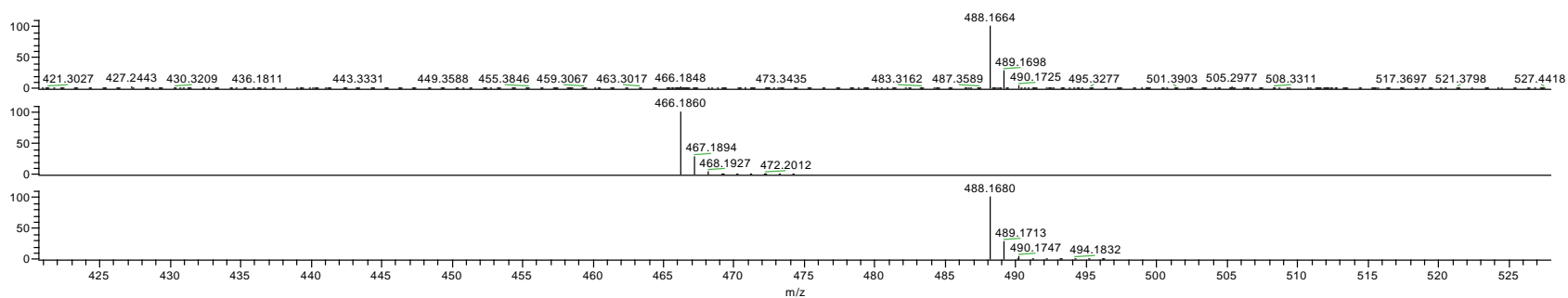
3d MC4176



Nome Descrizione
MC4176_1



	A	B	C	D	E	F	G	H	I	J	K
1	Peak	Ret.Time	Amount	Rel.Area	Area	Height	Type	Width (50%	Asym.	Resol.	Plates
2	No.	min	n.a.	%	mAU*min	mAU		min	EP	EP	EP
4	1	5.437	n.a.	0.17	0.1215	1.48	BMB	0.076	0.96	40.30	28036
5	2	11.323	n.a.	1.62	1.1677	11.38	BMB	0.096	0.94	16.35	77188
6	3	13.927	n.a.	0.08	0.0543	0.57	BMB	0.092	0.93	6.64	126928
7	4	14.987	n.a.	97.91	70.7309	684.85	BMB	0.096	0.94	10.96	134149
8	5	16.790	n.a.	0.23	0.1654	1.53	BMB	0.098	0.88	n.a.	163418
9	Maximum		0.0000	97.91	70.7309	684.85		0.098	0.96	40.30	163418
10	Minimum		0.0000	0.08	0.0543	0.57		0.076	0.88	6.64	28036
11	Sum		0.0000	100.00	72.2398	699.80					
12											



NL:
5.90E7
MC4176_20230329121401#982-997
RT: 5.35-5.43 AV: 16 T: FTMS + c
ESI Full ms [106.7000-1600.0000]

NL:
7.38E5
C₂₆H₂₇O₇N₁+H:
C₂₆H₂₈O₇N₁
pa Chrg 1

NL:
7.38E5
C₂₆H₂₇O₇N₁+Na:
C₂₆H₂₇O₇N₁Na₁
pa Chrg 1

Table S2. Elemental analysis of final compounds 2a-n and 3a-d

LAB	CMP	Elemental analysis							
		Calculated				found			
		C	H	N	O	C	H	N	O
MC4199	2a	70.05	7.10	3.40	19.44	69.92	7.18	3.39	19.48
MC4158	2b	68.08	5.24	3.31	18.89	68.13	5.27	3.26	18.94
MC4168	2c	68.08	5.24	3.31	18.89	68.01	5.19	3.37	18.95
MC4172	2d	65.53	5.04	3.18	18.19	65.45	5.10	3.13	18.22
MC4166	2e	65.53	5.04	3.18	18.19	65.55	4.98	3.20	18.16
MC4181	2f	59.52	4.58	2.89	16.52	59.47	4.64	2.84	16.59
MC4185	2g	59.52	4.58	2.89	16.52	59.49	4.56	2.86	16.48
MC4165	2h	64.00	4.92	6.22	24.86	64.05	4.98	6.17	24.79
MC4167	2i	64.00	4.92	6.22	24.86	63.97	4.88	6.26	24.93
MC4177	2j	69.76	5.15	6.51	18.58	69.71	5.18	6.48	18.65
MC4208	12k	71.58	6.01	3.34	19.07	71.63	6.07	3.29	19.10
MC4204	2l	66.83	5.35	3.54	24.28	66.88	5.33	3.59	24.21
MC4188	2m	64.22	5.14	3.40	19.44	64.17	5.19	3.35	19.50
MC4186	2n	64.22	5.14	3.40	19.44	64.26	5.11	3.42	19.39
MC4175	3a	67.09	5.85	3.01	24.06	67.13	5.89	2.94	24.11
MC4176	3b	67.09	5.85	3.01	24.06	67.02	5.91	3.08	24.12
MC4164	3c	68.95	5.79	3.22	22.04	68.99	5.83	3.19	22.08
MC4182	3d	68.95	5.79	3.22	22.04	69.03	5.72	3.27	21.99

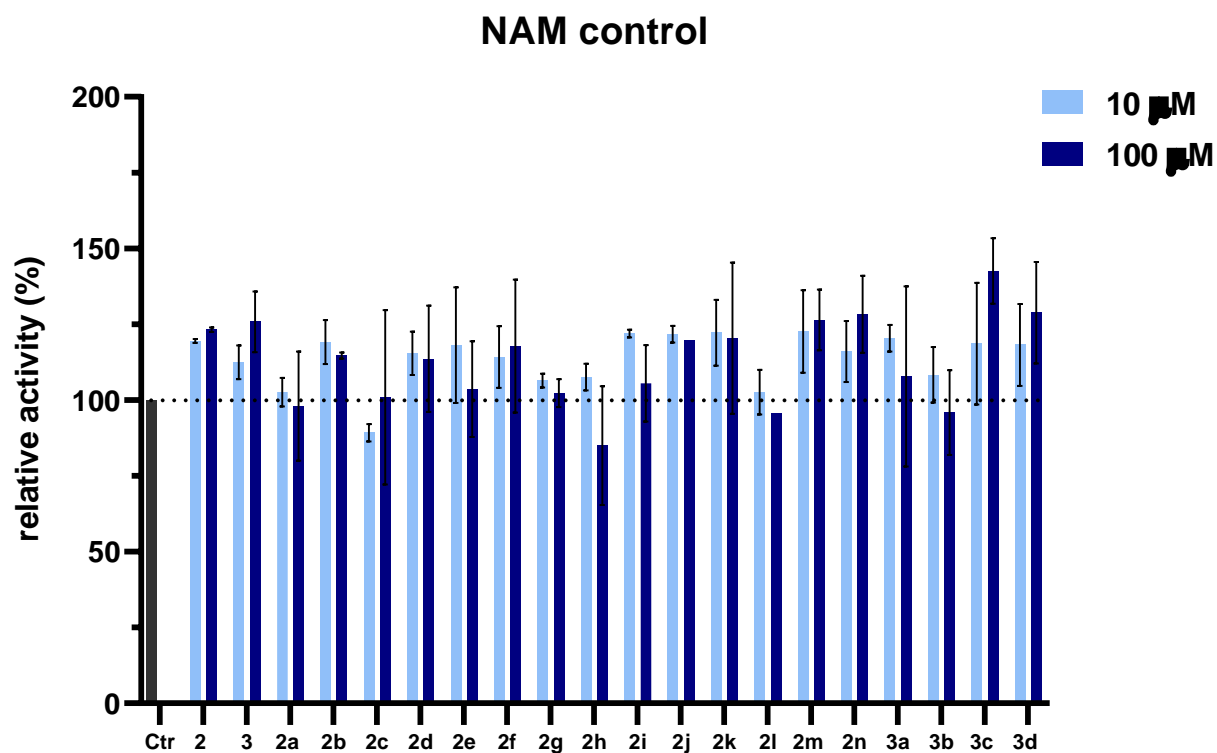


Figure S1. Control experiments for potential compound effects on the coupled enzymes of the assay system. The conversion of 50 μ M NAM, the product of the Sirtuin-catalyzed reaction, by the coupled enzymes was tested in presence of DMSO (reference) and 10 μ M or 100 μ M of each compound.

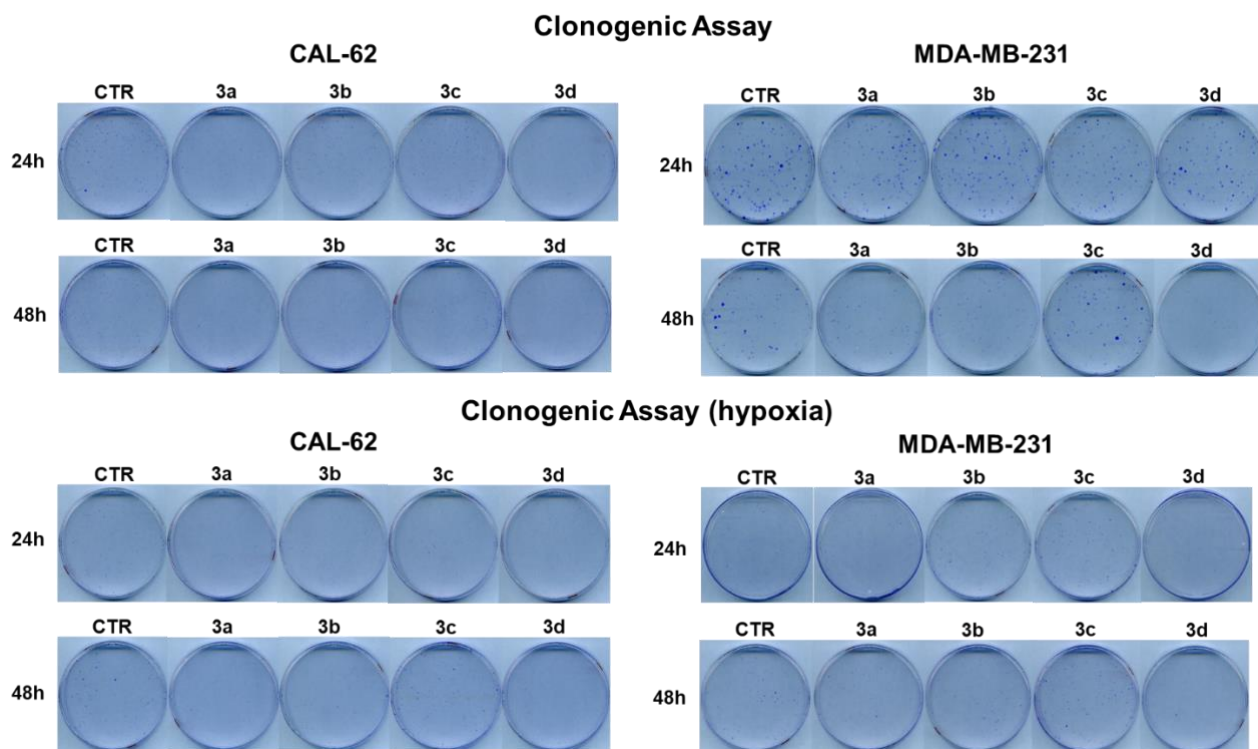
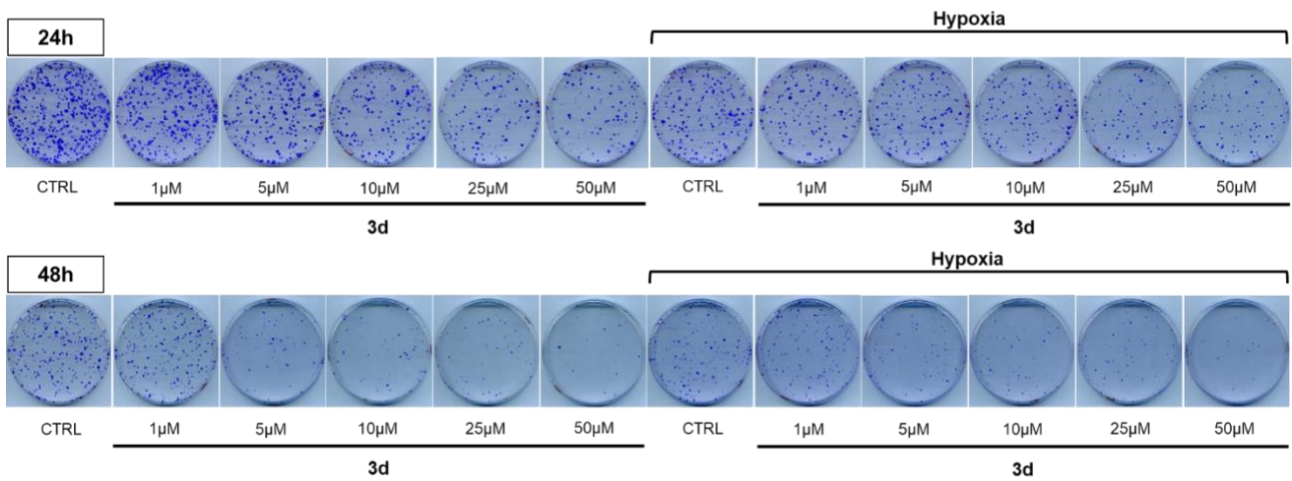


Figure S2. Representative plate images of the colony formation assay for CAL-62 and MDA-MB-231 cells treated with the SIRT3 activators **3a-d** at 50 μ M for 24 and 48 h.

Clonogenic Assay, CAL-62



Clonogenic Assay, MDA-MB-231

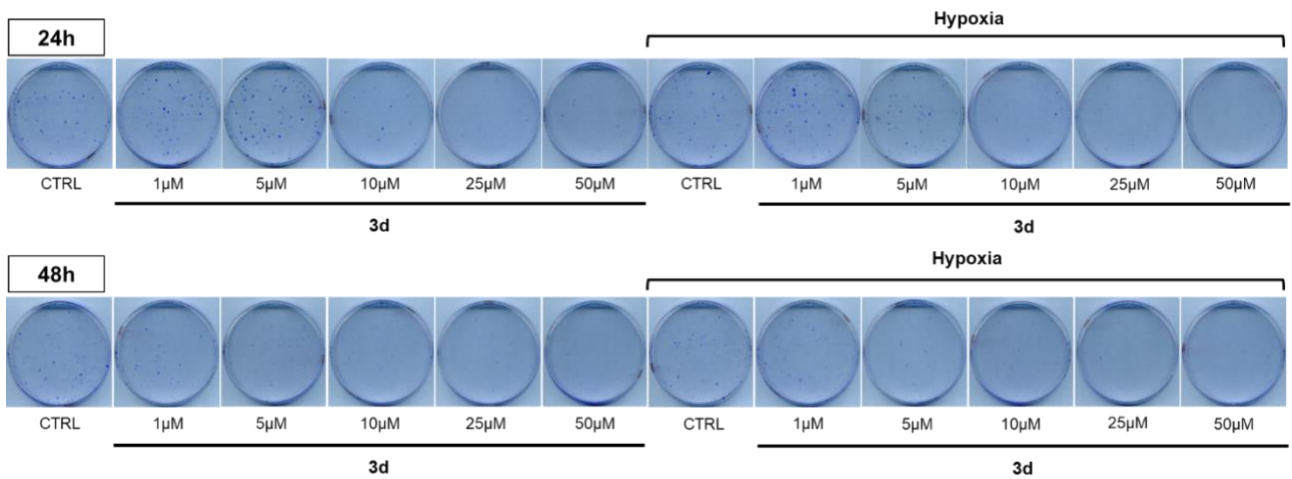


Figure S3. Representative plate images of the colony formation assay for CAL-62 and MDA-MB-231 cells treated with increasing dose (1, 5, 10, 25 and 50 μM) of the SIRT3 activator **3d** for 24 and 48 h.

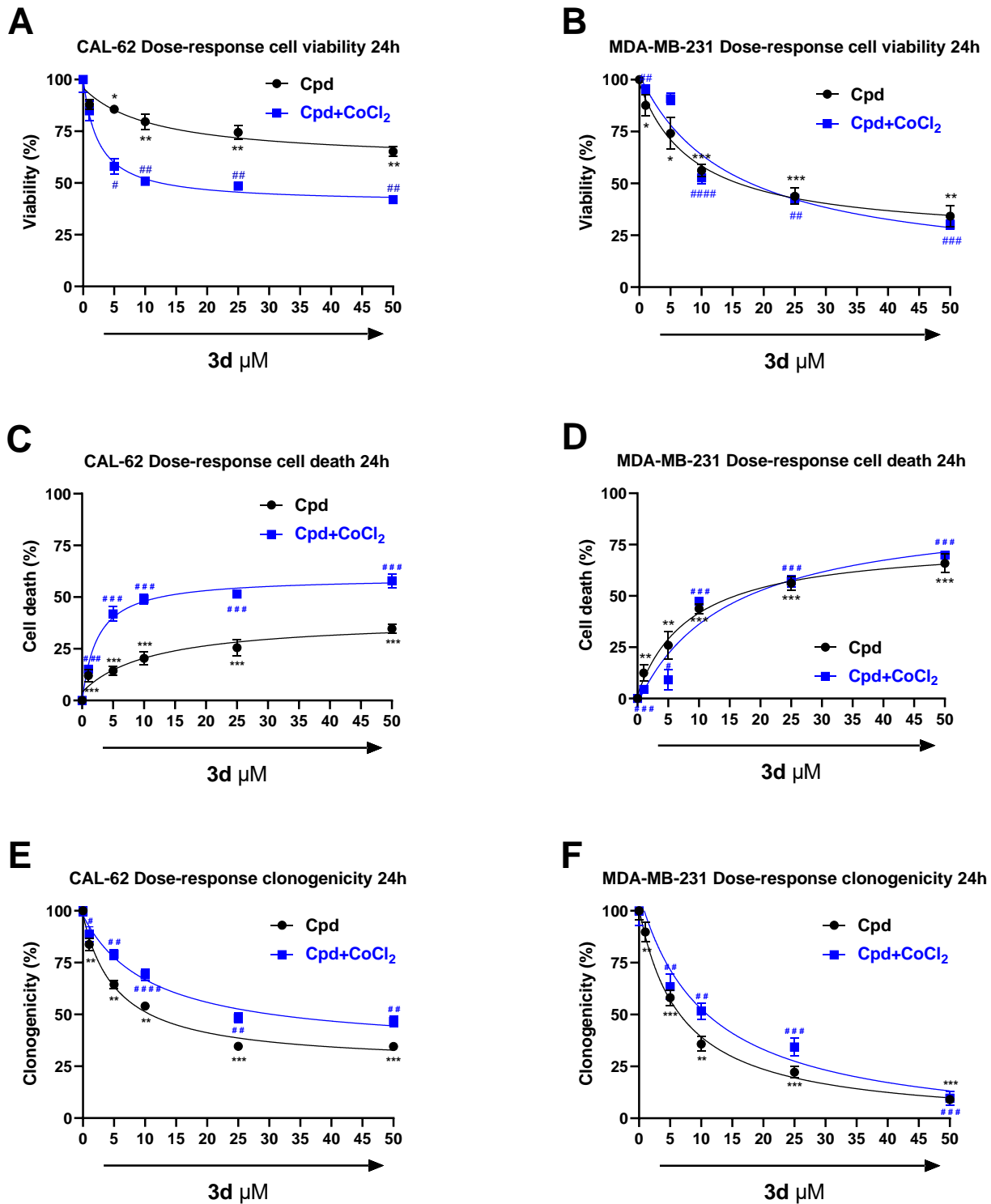


Figure S4. Dose-dependent effects of compound **3d** on cell viability, cell death and clonogenicity in CAL-62 (A, C, E) and MDA-MB-231 (B, D, F) cells upon 24 h of treatment. p-values were obtained using a Student's t-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$); # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$) for three independent experiments.

Table S3. List of Primers used in gene expression analysis.

OLIGO FORWARD	OLIGO REVERSE
L32: GGAGCGACTGCTACGGAAG	L32: GATACTGTCCAAAAGGCTGGAA
SLUG: ATGAGGAATCTGGCTGCTGT	SLUG: CTCTCTCTGTGGGTGTGTGT
ZEB1: CAGGGAGGAGCAGTGAAAGA	ZEB1: CTCTTCAGGTGCCTCAGGAA
MMP2: ATGCCGCCTTTAACTGGAG	MMP2: GGAAAGCCAGGATCCATTTT
SNAIL: CACTATGCCGCGCTCTTTC	SNAIL: GCTGGAAGGTAAACTCTGGATTAGA
MMP9: CTACTGTGCCTTTGAGTCC	MMP9: AGAGAATCGCCAGTACTTCC
COL1A2: TGCAAGAACAGCATTGCATAC	COL1A2: GGCAGGCGAGATGGCTTATTTGTT

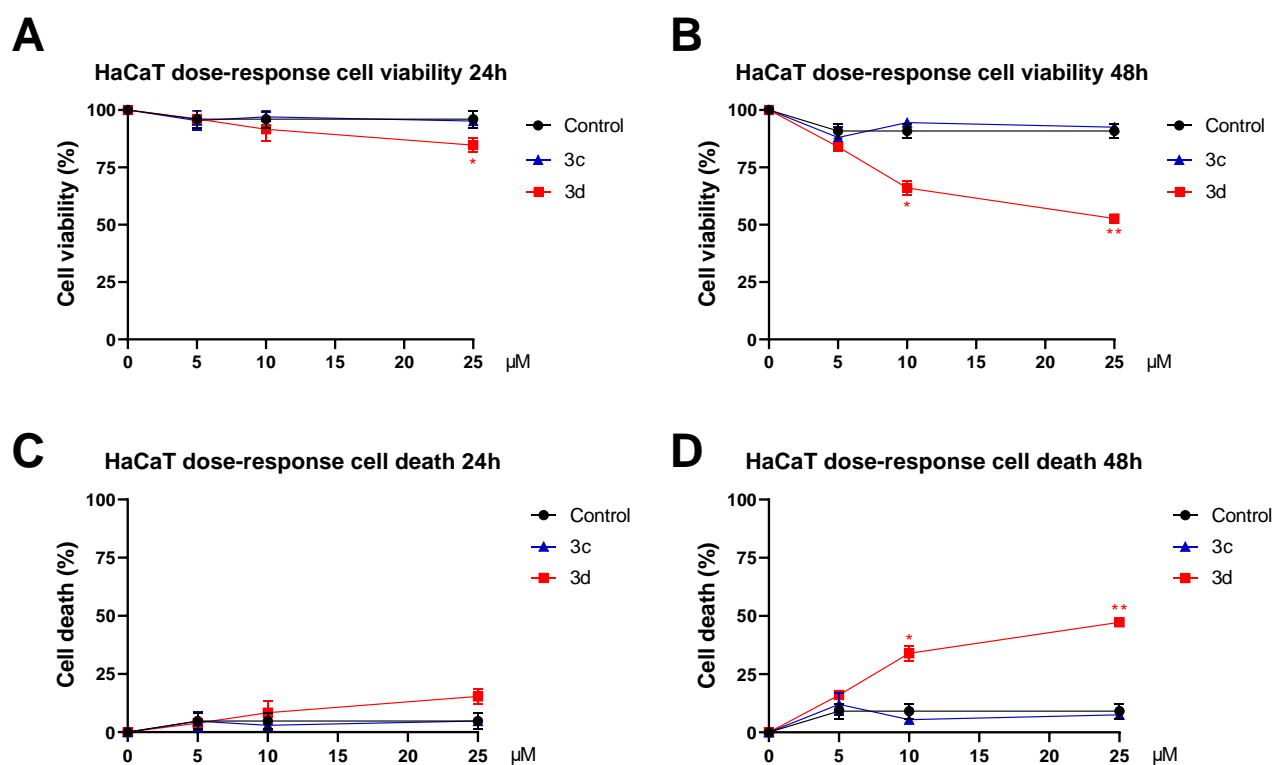


Figure S5. Dose-dependent effects of compounds **3c** and **3d** on cell viability (A, B) and cell death induction (C, D) in non-cancer HaCaT cells upon 24 h (A, C) and 48 h (B, D) of treatment. p-values were obtained using a Student's t-test (*p < 0.05, **p < 0.01) for three independent experiments.

A) Cell viability (%) T24 cpd 3d			
Cpd conc.	CAL-62	MDA-MB-231	HaCaT
5 μ M	85,7	74,1	96,1
10 μ M	79,6	56,2	91,6
25 μ M	74,4	42,8	84,7

B) Cell viability (%) T48 cpd 3d			
Cpd conc.	CAL-62	MDA-MB-231	HaCaT
5 μ M	78,3	51,7	83,9
10 μ M	63,5	36,3	66,1
25 μ M	33,8	21,1	52,7

C) Cell death (%) T24 cpd 3d			
Cpd conc.	CAL-62	MDA-MB-231	HaCaT
5 μ M	14,3	25,9	3,9
10 μ M	20,4	43,8	8,4
25 μ M	25,6	57,2	15,3

D) Cell death (%) T48 cpd 3d			
Cpd conc.	CAL-62	MDA-MB-231	HaCaT
5 μ M	21,7	48,3	16,1
10 μ M	36,6	63,6	33,9
25 μ M	66,1	78,8	47,3

Tables S4 (A-D). Quantification of living (A, B) and death cells (C, D) upon 24 h (A, C) and 48 h (B, D) of treatment relative to three independent experiments. Cell lines are indicated.