

APPENDIX A

File : C:\HPCHEM\1\DATA\TIM\RESEARCH\PHARM\FRAC8A.D
 Operator : TIM
 Acquired : 25 Aug 2000 9:46 using AcqMethod STEROIDS
 Instrument : GC/MS Ins
 Sample Name: FRACTION 8
 Misc Info : Higher concentration
 Vial Number: 1

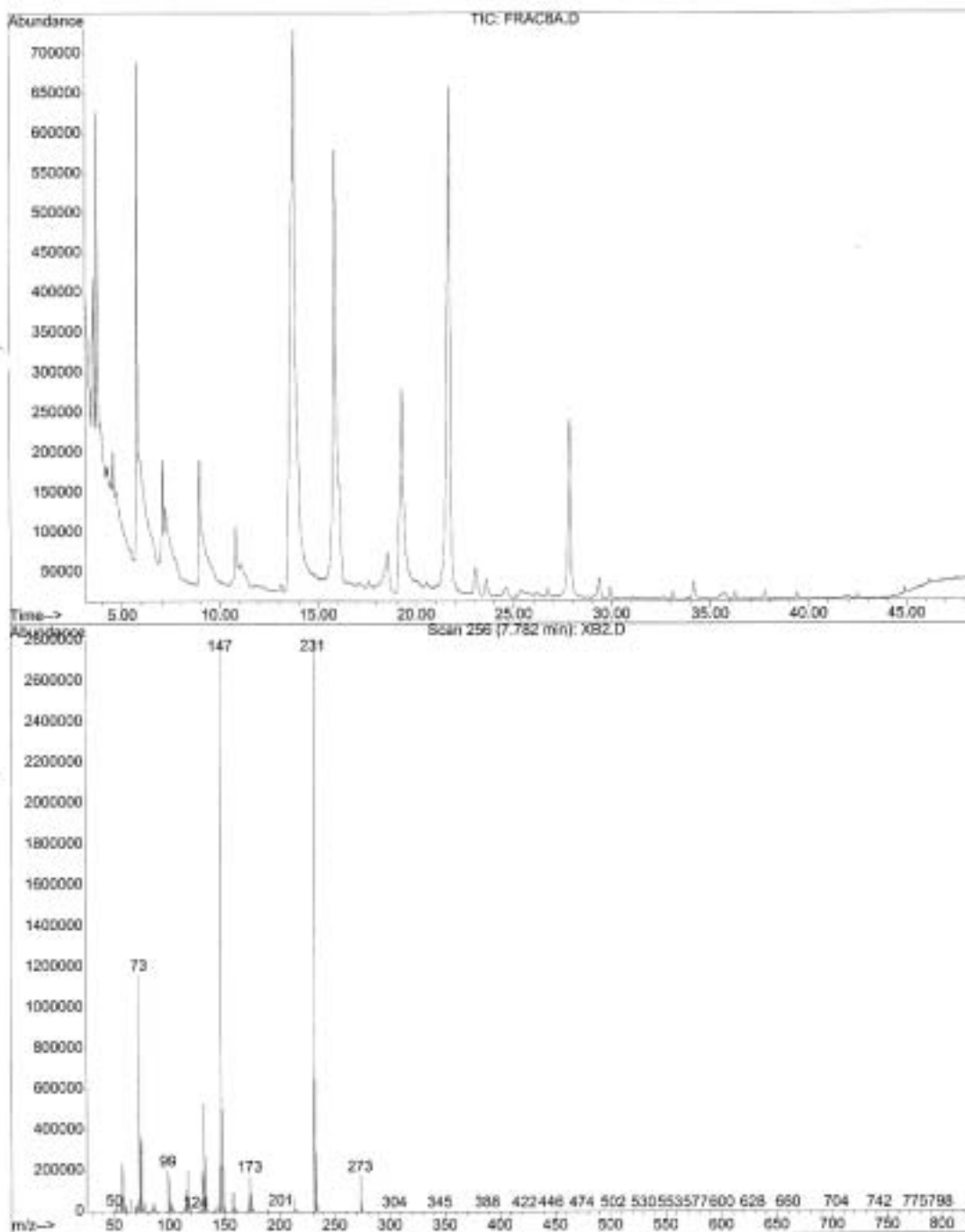


Figure A1: GC/MS spectrum of the whole Fraction 8 precipitate

Library Searched : C:\DATABASE\WILEY275.L
 Quality : 95
 ID : Tetradecanoic acid (CAS) \$\$ Myristic acid \$\$ MYRIST
 INIC ACID \$\$ n-Tetradecanoic acid \$\$ neo-Fat 14 \$\$
 Univol U 316S \$\$ n-Tetradecoic

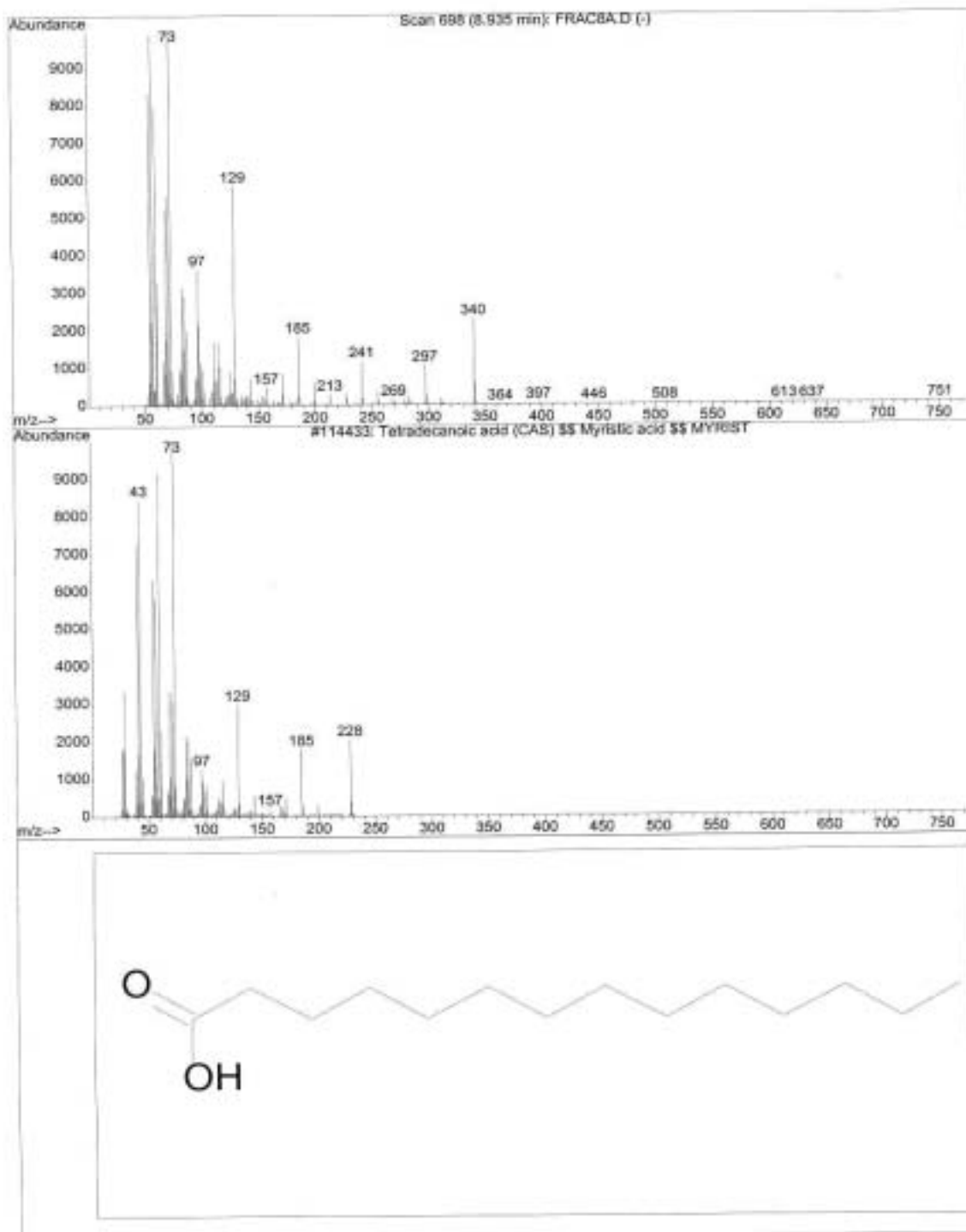


Figure A2: GC/MS data of one possible compound isolated from the Fraction 8 precipitate depicting a 95% probability of tetradecanoic acid

Library Searched : C:\DATABASE\WILEY275.L
 Quality : 64
 ID : Octadecanoic acid (CAS) \$\$ Stearic acid \$\$ n-Octadecanoic acid \$\$ PD 185 \$\$ NAA 173 \$\$ Vanicol \$\$ Kam 3000 \$\$ Kam 1000 \$\$ Kam 2000 \$

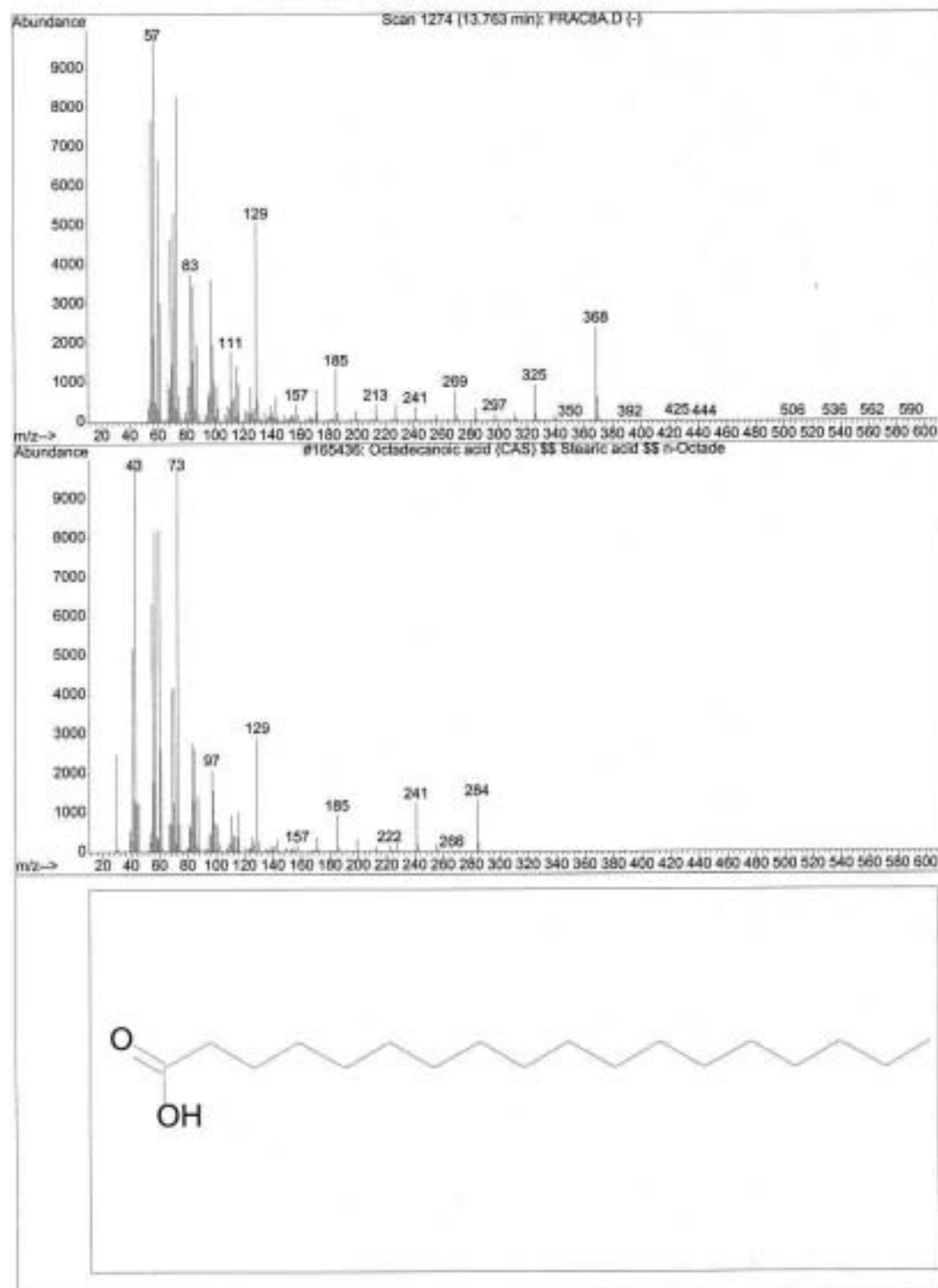


Figure A3: GC/MS data of one possible compound isolated from the Fraction 8 precipitate depicting a 64% probability of octadecanoic acid

Library Searched : C:\DATABASE\WILEY275.L
Quality : 95
ID : Octacosane (CAS) \$\$ n-Octacosane

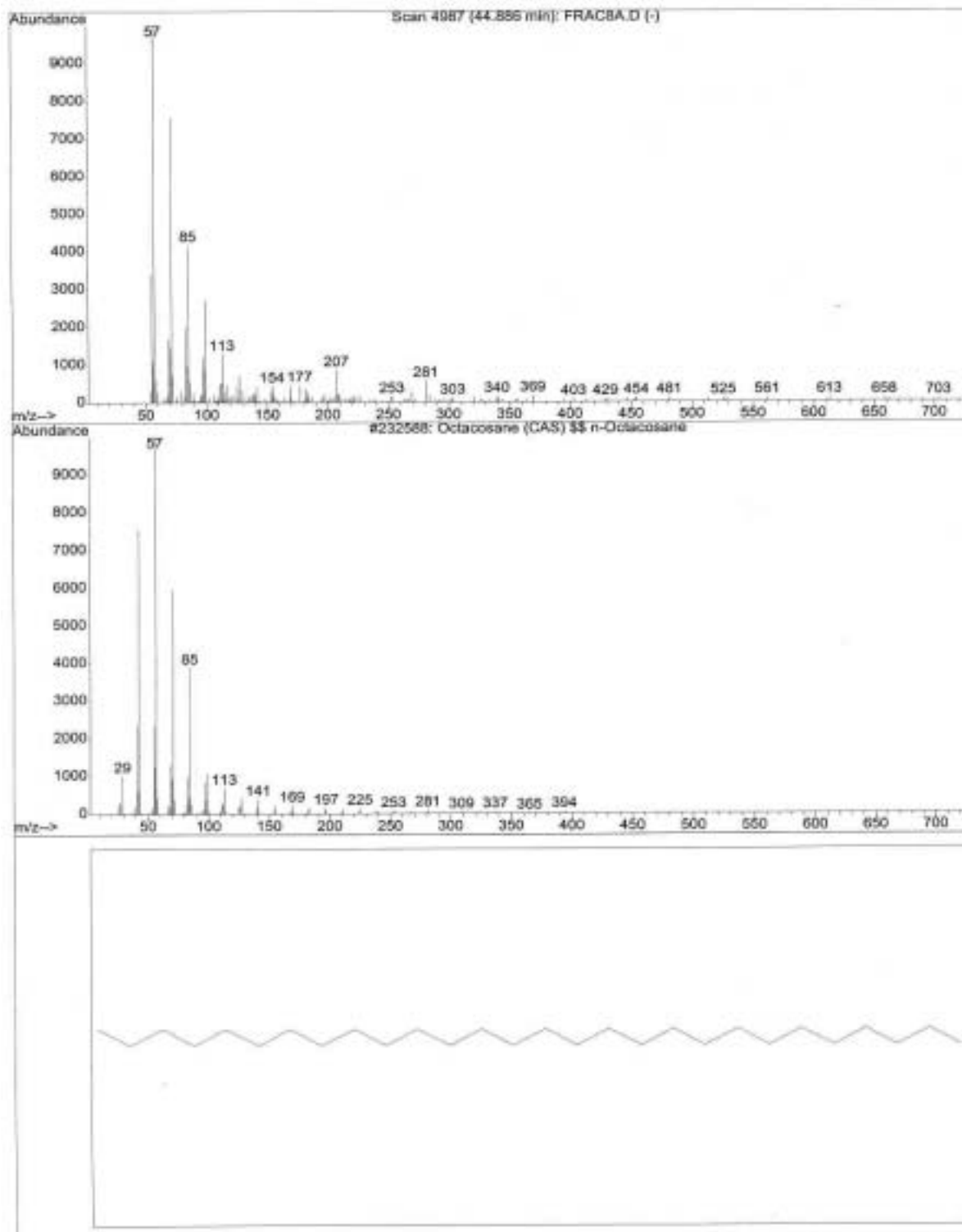


Figure A4: GC/MS data of a possible compound isolated from the Fraction 8 precipitate showing a 95% probability of the alkane octacosane

Library Searched : C:\DATABASE\WILEY275.L
 Quality : 25
 ID : Pregnan-20-one, 3,11-dihydroxy-, (3.beta.,5.beta.,11.beta.)- (CAS) §§ 3.BETA.,11.BETA.-DIHYDROXY-5.BETA.A.-PREGNAN-20-ONE

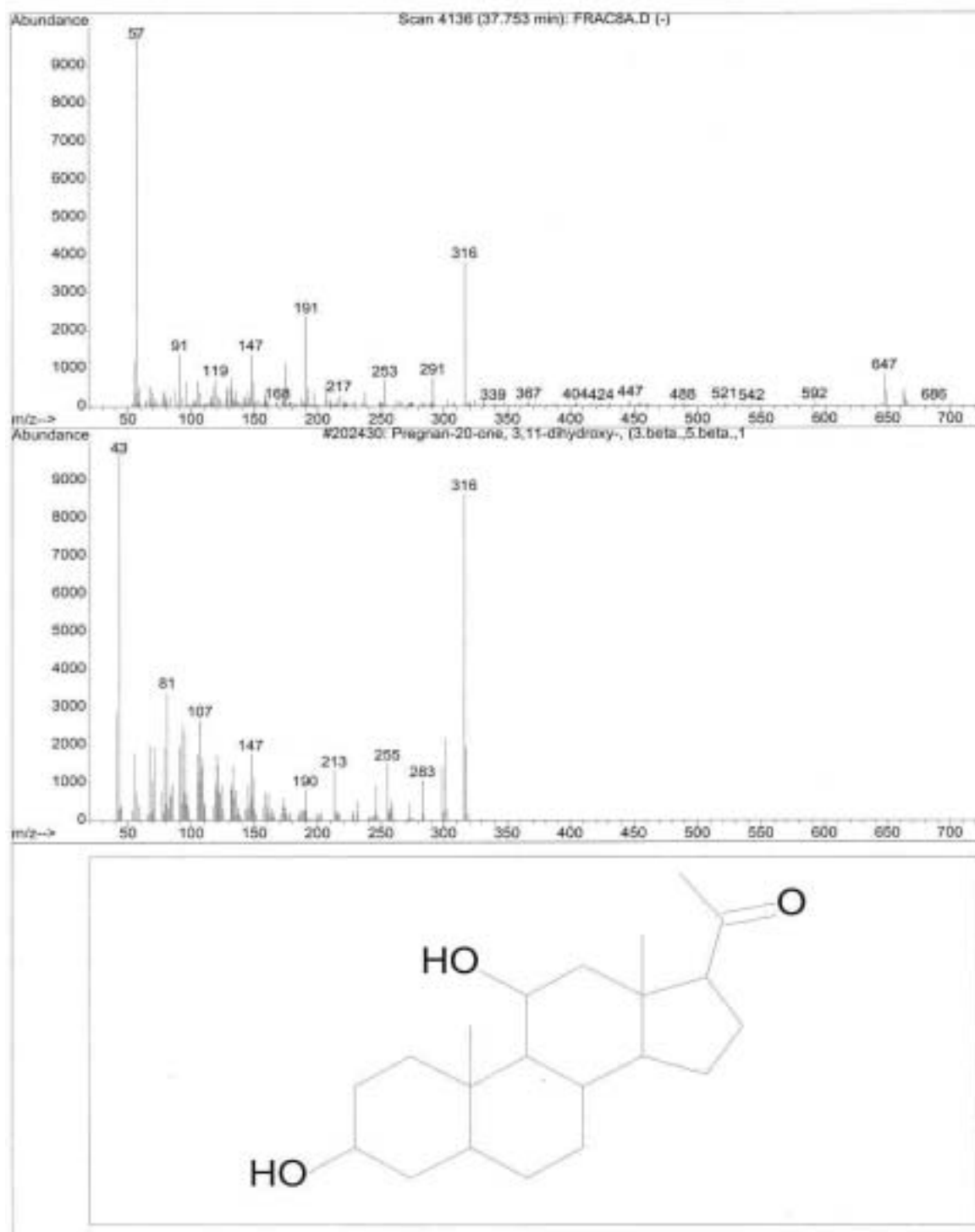


Figure A5: GC/MS data of Fraction 8 precipitate showing a steroid structure (25% probability)

File : C:\HPCHEM\1\DATA\TIM\RESEARCH\PHARM\FRAC8S.D
 Operator : TIM
 Acquired : 25 Aug 2000 13:08 using AcqMethod PHARM1
 Instrument : GC/MS Ins
 Sample Name: FRAC8S - SUPERNATANT OF 8 - ACETONE
 Misc Info :
 Vial Number: 1

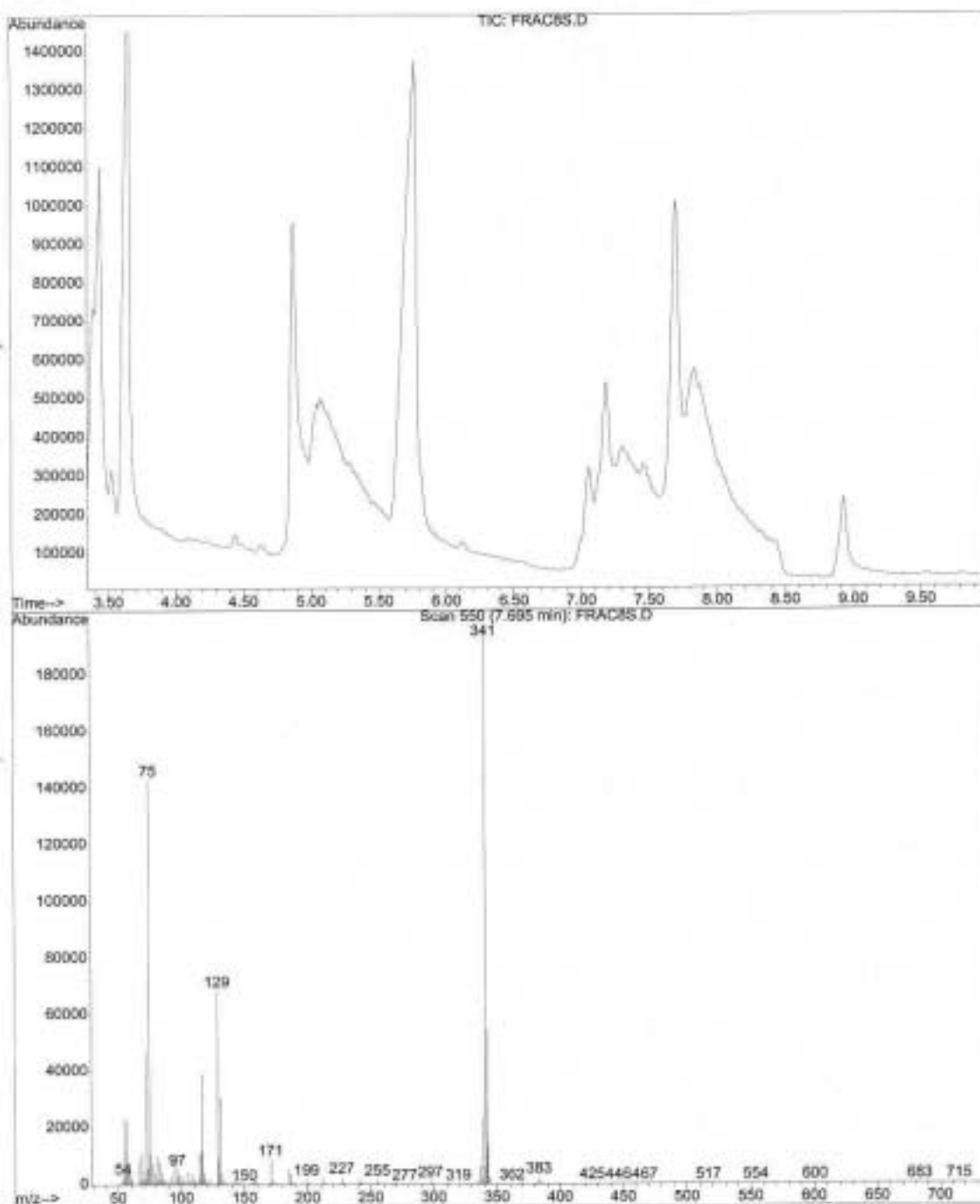


Figure A6: GC/MS data of the Fraction 8 supernatant

Library Searched : C:\DATABASE\PMW_TOX2.L
 Quality : 95
 ID : Stearic acid P1389

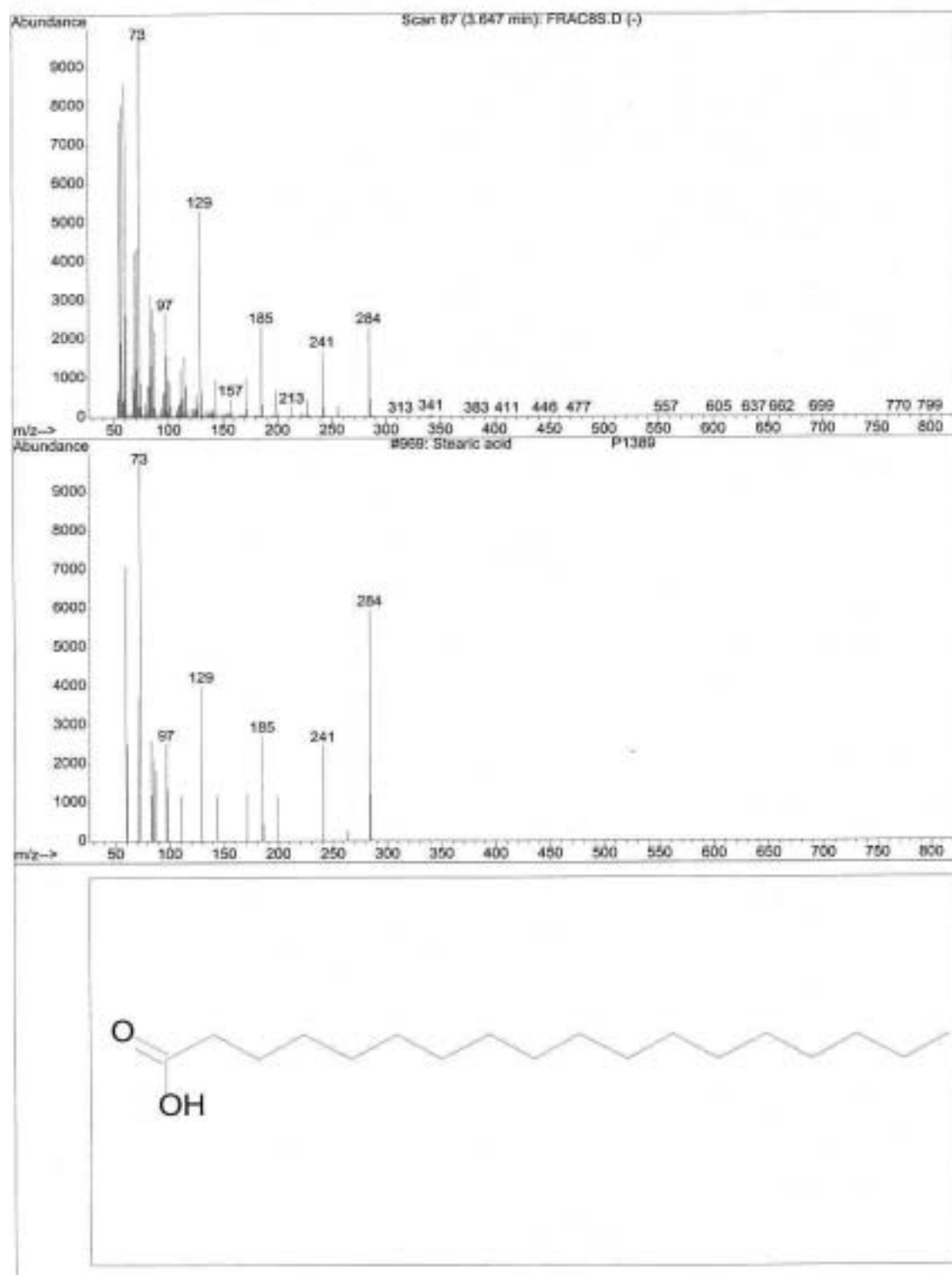


Figure A7: GC/MS data of one possible compound isolated in the supernatant of Fraction 8 depicting a 95% probability of stearic acid

File : C:\HPCHEM\1\DATA\TIM\RESEARCH\PHARM\FRAC8A1.D
Operator :
Acquired : 29 Aug 2000 8:51 using AcqMethod STEROID5
Instrument : GC/MS Ins
Sample Name: frac8a1
Misc Info : Silinized frac8 - TMSI
Vial Number: 1

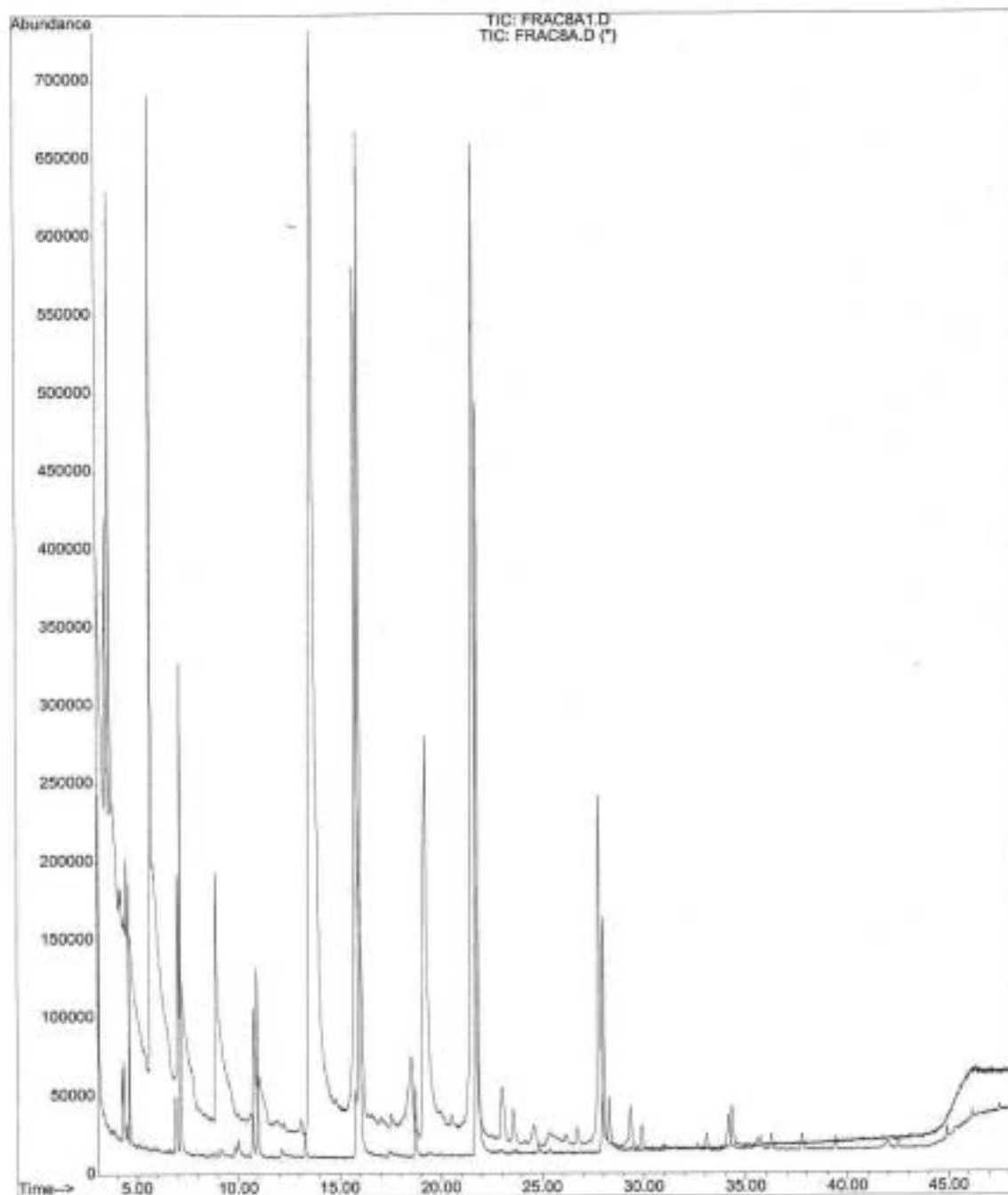


Figure A8: GC/MS showing the precipitate of Fraction 8 superimposed on the silinized precipitate

Library Searched : C:\DATABASE\WILEY275.L
Quality : 53
ID : 2,6-Octadiene, 4-methyl- (CAS)

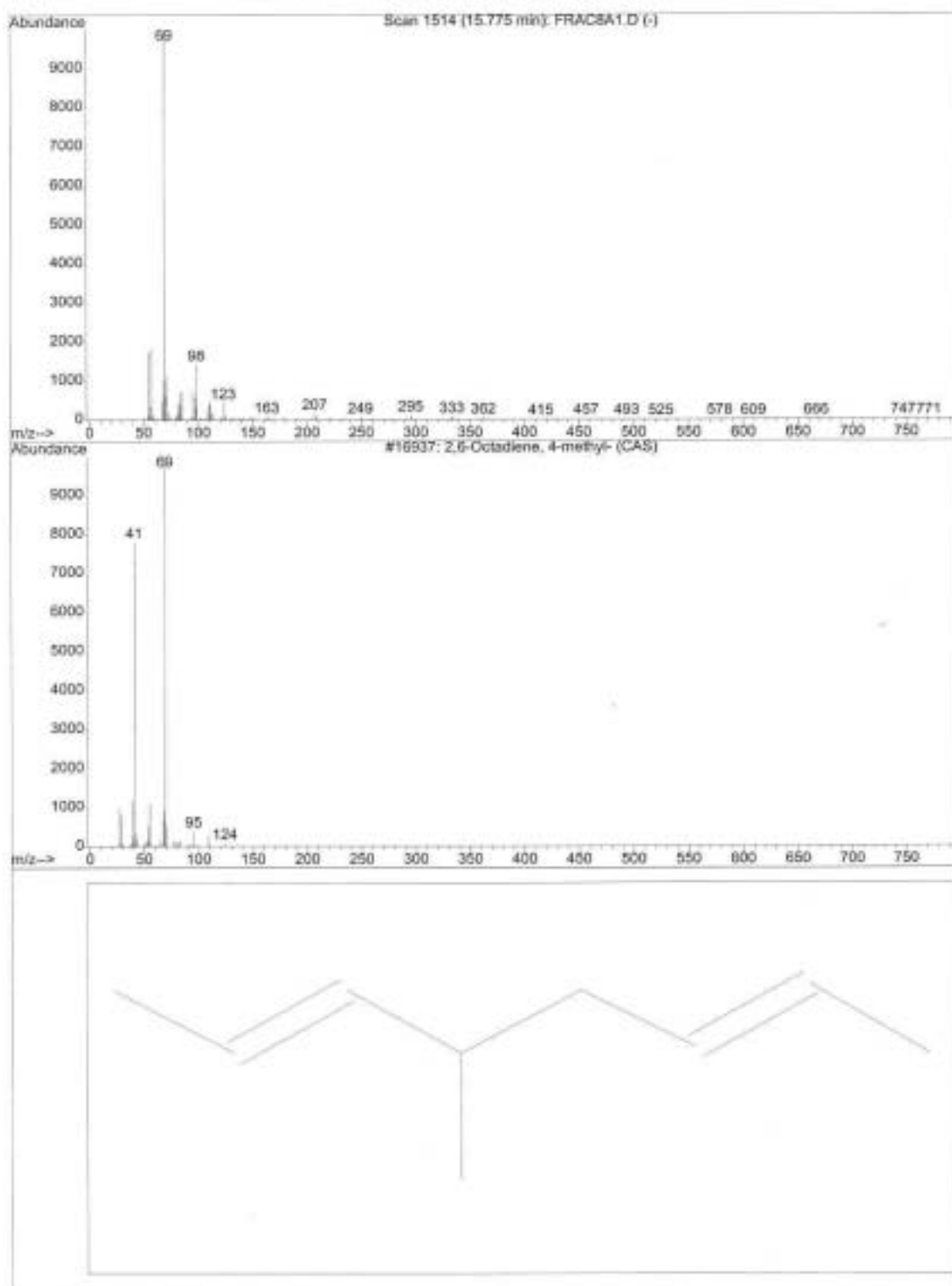


Figure A9: GC/MS data of one possible compound isolated in the silinized precipitate of Fraction 8 depicting a 53% probability of an alkene

Library Searched : C:\DATABASE\WILEY275.L
 Quality : 78
 ID : Eicosanoic acid, trimethylsilyl ester (CAS) \$\$ MONO
 TRIMETHYLSILYL ARACHIDIC ACID \$\$ Arachidic acid, tr
 imethylsilyl ester

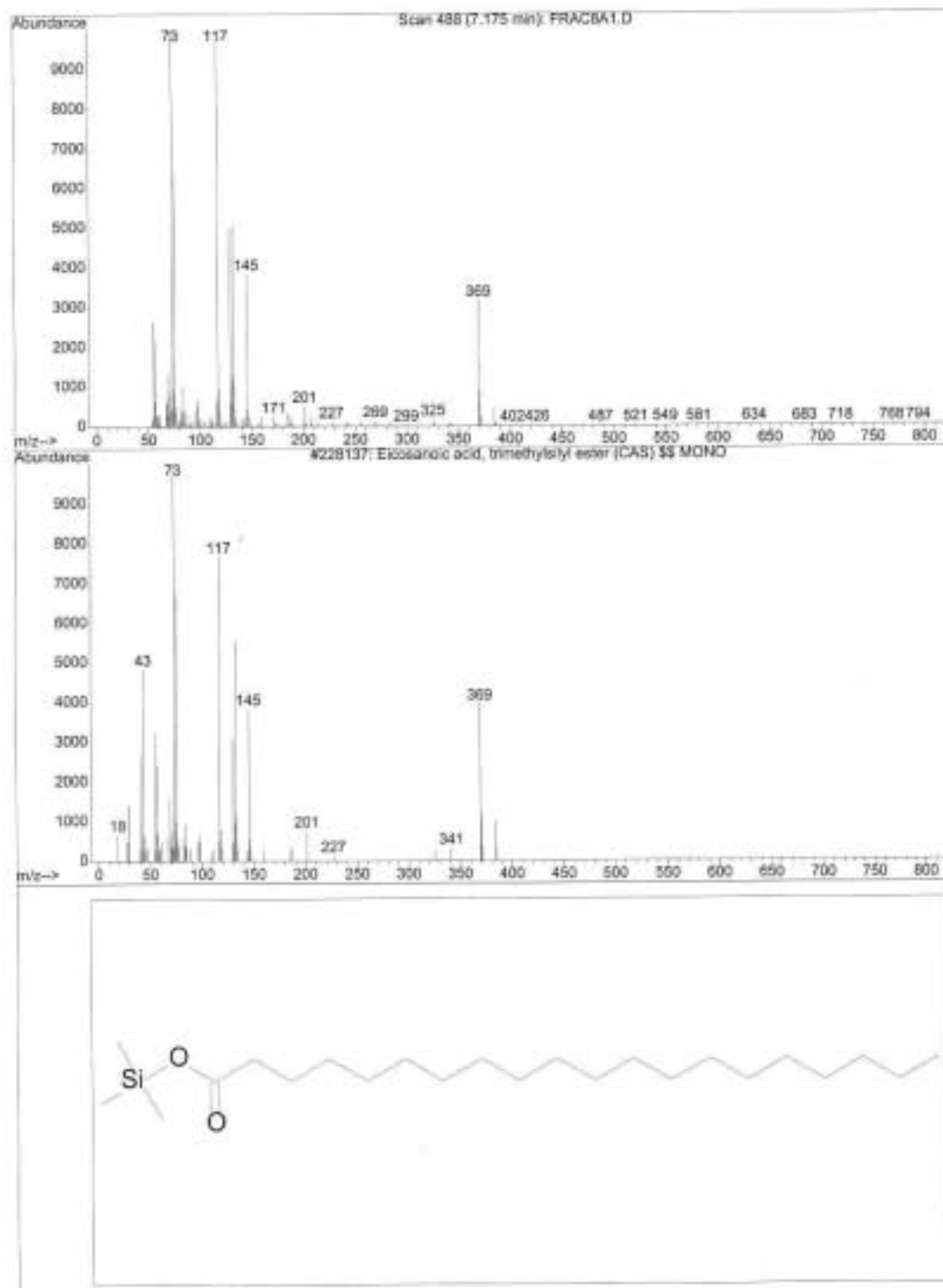


Figure A10: GC/MS data of one possible compound isolated in the silinized precipitate of Fraction 8 depicting a 78% probability of eicosanoic acid

Library Searched : C:\DATABASE\WILEY275.L
 Quality : 95
 ID : TRIMETHYLSILYL ESTER OF TETRACOSANOIC ACID

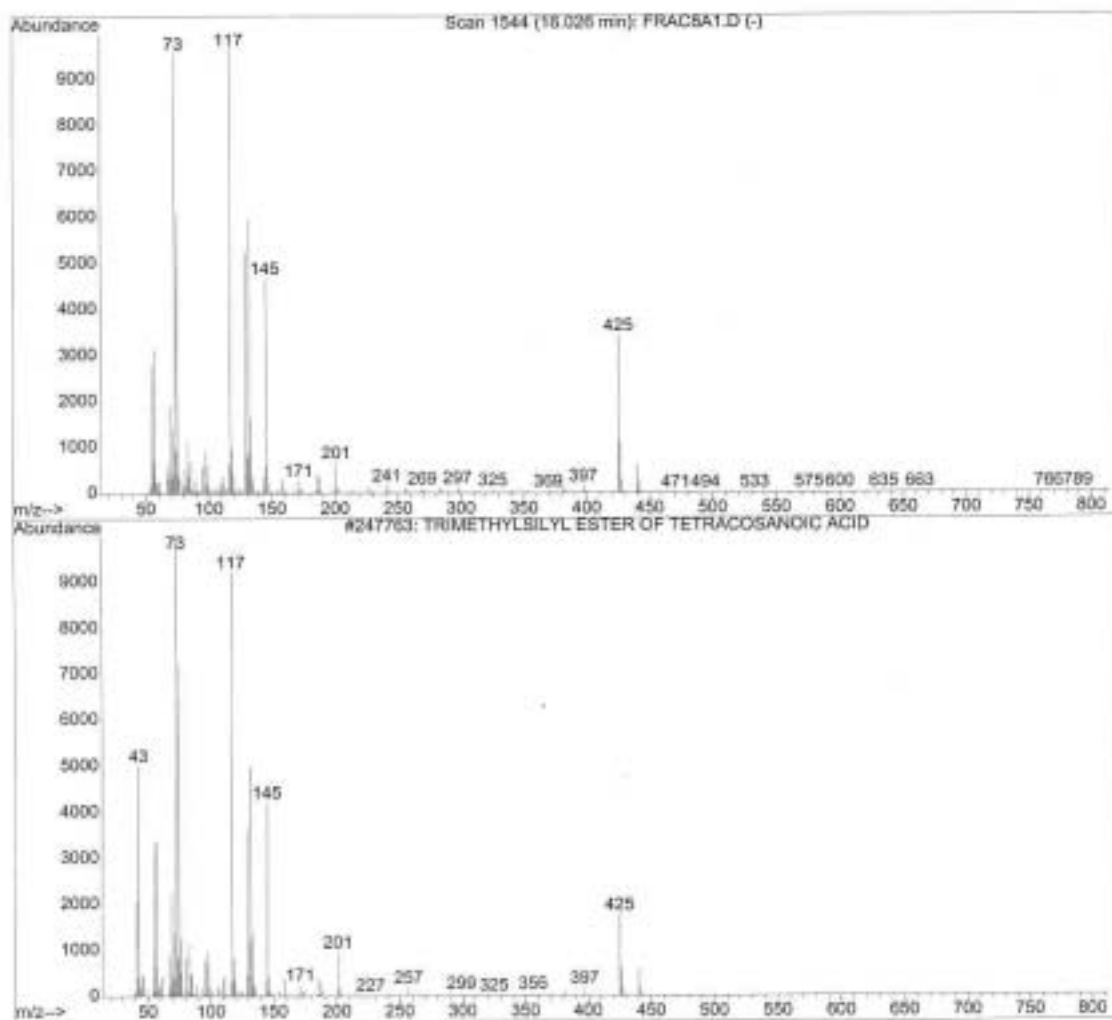
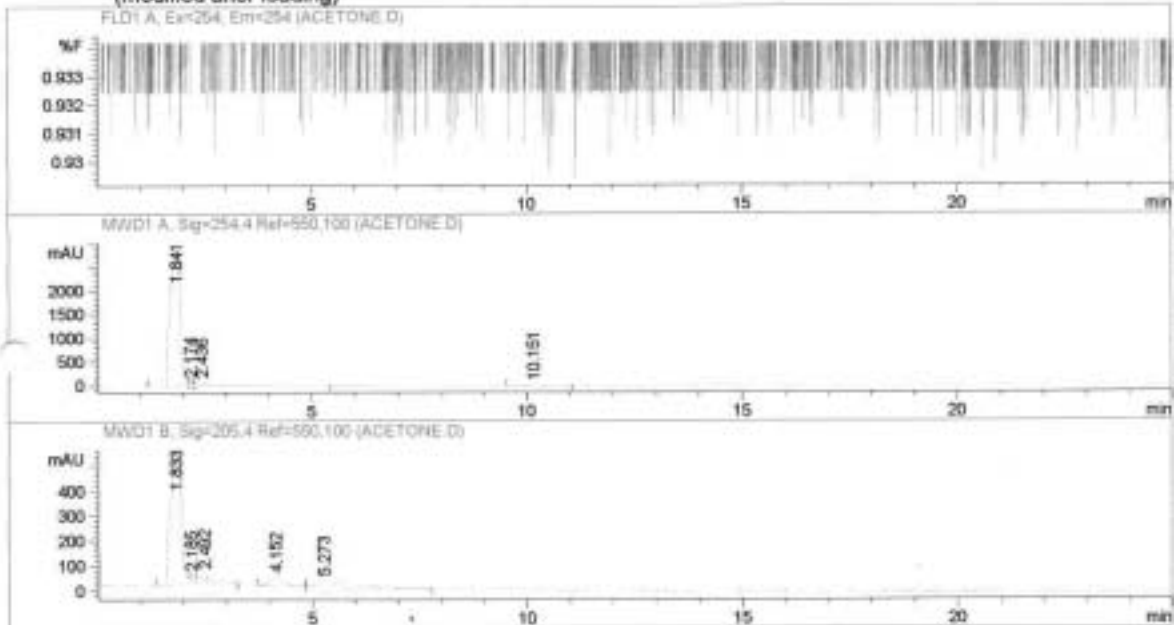


Figure A11: GC/MS data of a possible compound isolated in the silinized precipitate of Fraction 8 depicting a 95% probability of an ester of tetracosanoic acid

APPENDIX B

Injection Date : 2001/02/09 01:58:38 PM
 Sample Name : acetone test Vial : 6
 Acq. Operator : nataly
 Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 01:50:44 PM by nataly
 (modified after loading)



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 Area Percent Report
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Sorted By : Signal
 Multiplier : 1.0000
 Dilution : 1.0000
 Sample Amount : 10.00000 [ng/ul] (not used in calc.)

Signal 1: FLD1 A, Ex=254, Em=254

Signal 2: MWD1 A, Sig=254,4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.841	PV	0.2718	4.71329e4	2867.14868	93.6749
2	2.174	VV	0.1092	438.22711	66.89174	0.8710
3	2.436	VB	0.5411	2680.06567	63.41471	5.3265
4	10.151	BB	0.6168	64.18256	1.32582	0.1276

Totals : 5.03154e4 2998.78096

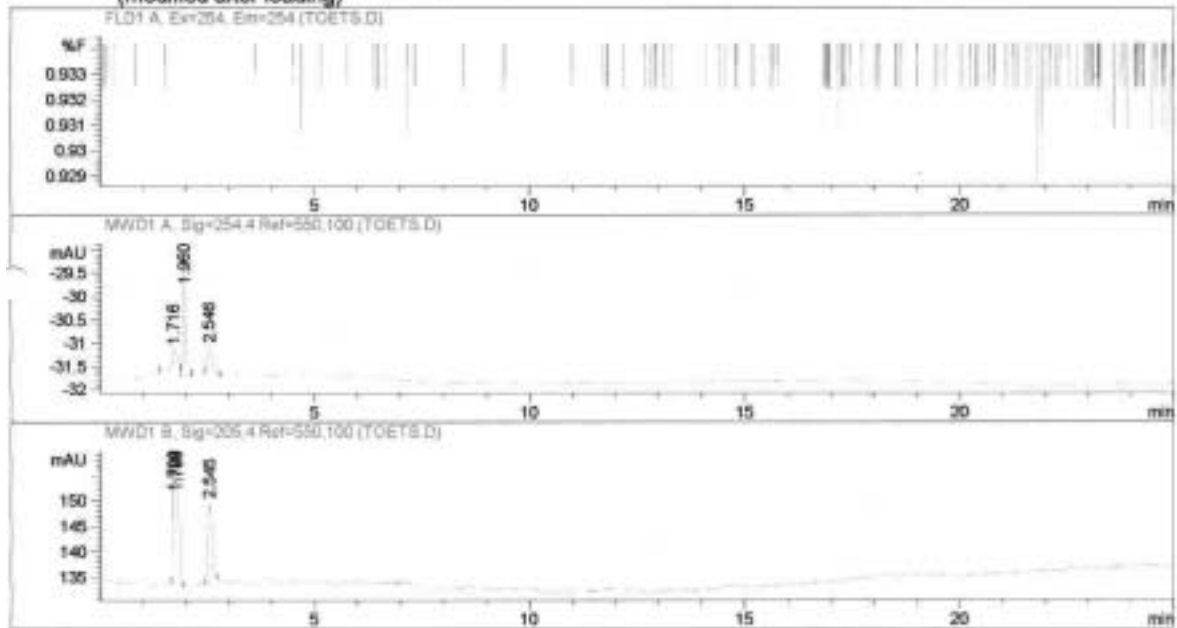
Signal 3: MWD1 B, Sig=205,4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.833	BV	0.2816	8851.36035	512.25281	73.6833
2	2.185	VV	0.1197	285.48730	32.11817	2.3765
3	2.492	VB	0.3300	1064.31470	41.11828	8.8599
4	4.152	PB	0.3401	670.81012	31.03953	5.5842
5	5.273	BP	0.6842	1140.73071	20.27998	9.4960

Totals : 1.20127e4 636.80877

Figure B1: HPLC data of acetone run at a flow rate of 1.5 ml/min

Injection Date : 2001/02/09 11:14:25 AM
 Sample Name : methanol Vial : 4
 Acq. Operator : nataly
 Acq. Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 09:54:40 AM by nataly
 (modified after loading)
 Analysis Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 11:40:30 AM by nataly
 (modified after loading)



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 Dilution : 1.0000
 Sample Amount : 10.00000 [ng/ul] (not used in calc.)

Signal 1: FLD1 A, Ex=254, Em=254

Signal 2: MWD1 A, Sig=254,4 Ref=550,100

Signal 3: MWD1 B, Sig=205,4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.716	BV	0.1831	7.38356	5.34787e-1	34.1738
2	1.960	VB	0.0540	9.58527	2.66080	44.3642
3	2.546	BB	0.1201	4.63705	5.62845e-1	21.4620

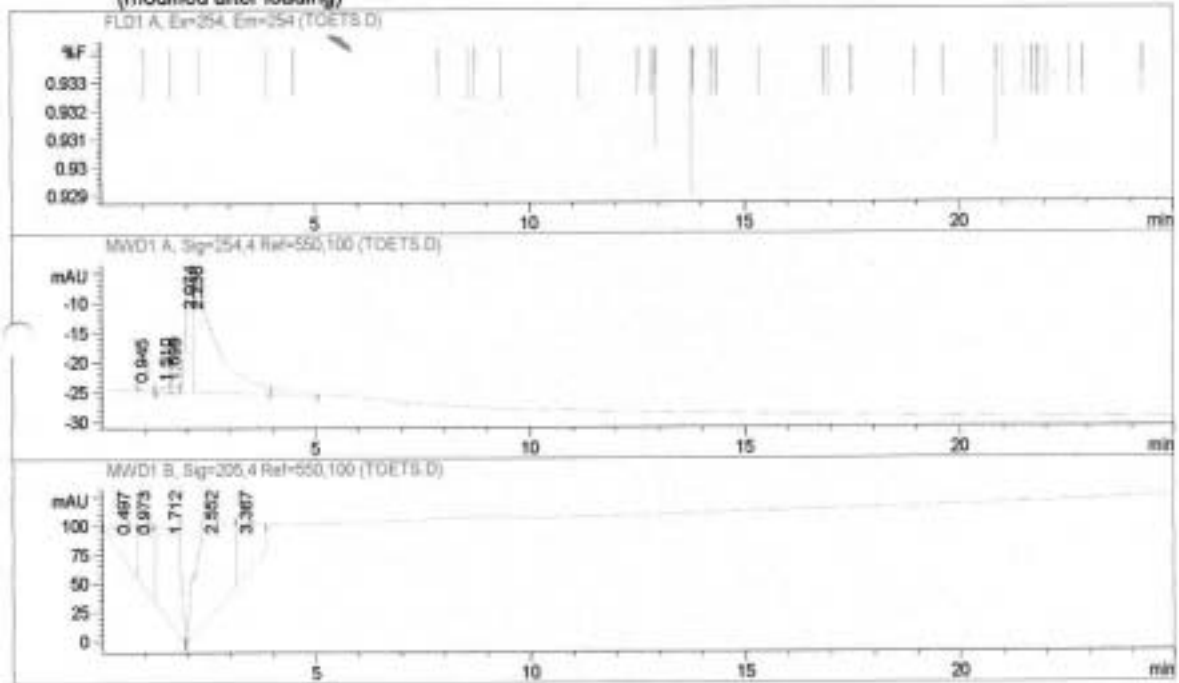
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.709	BV	0.0448	59.86104	21.28381	18.8685
2	1.786	VB	0.0871	148.46460	25.05907	46.7917
3	2.545	PB	0.1056	108.96241	15.57163	34.3418

Totals : 21.60589 3.75843

Totals : 317.28805 61.91450

Figure B2: HPLC data of methanol run at a flow rate of 1.5 ml/min

Injection Date : 2001/02/09 09:56:29 AM
 Sample Name : ~~sampleB~~ Vial : 4
 Acq. Operator : nataly
 Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 09:54:40 AM by nataly
 (modified after loading)



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 Area Percent Report
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Integ. By : Signal
 Multiplier : 1.0000
 Dilution : 1.0000
 Sample Amount : 10.00000 [ng/ul] (not used in calc.)

Signal 1: FLD1 A, Ex=254, Em=254

Signal 2: MWD1 A, Sig=254.4 Ref=550,100

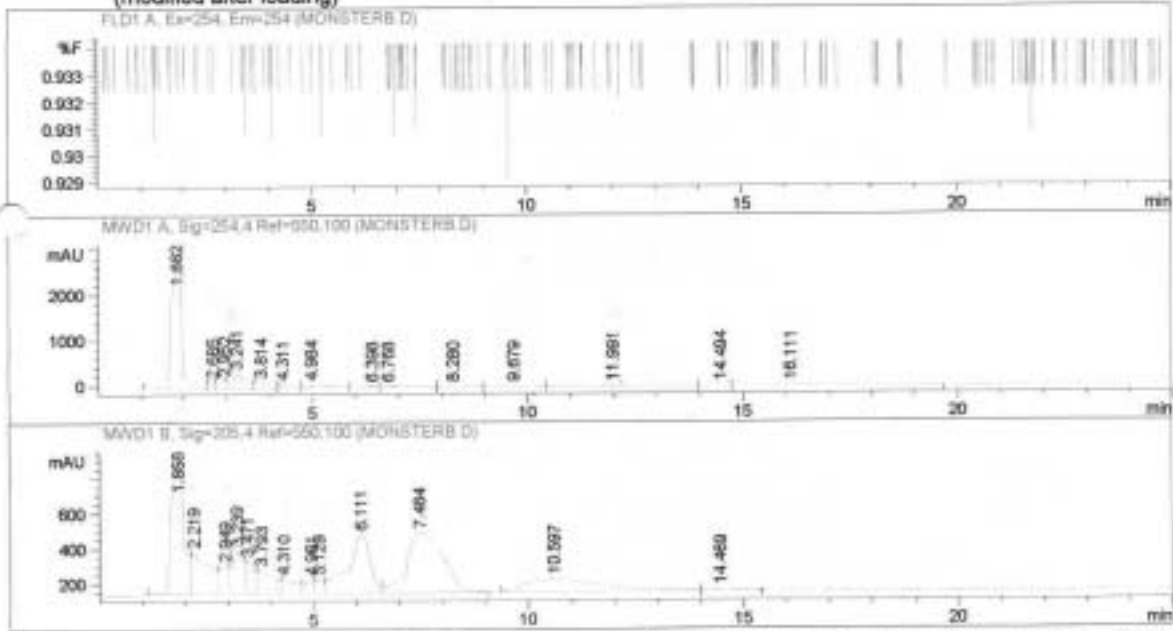
Signal 3: MWD1 B, Sig=205.4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.945	VP	0.1616	7.04754	5.80712e-1	0.7002
2	1.510	VV	0.2169	13.19358	8.70076e-1	1.3108
3	1.699	VV	0.1360	10.36509	1.04252	1.0298
4	2.074	VV	0.1908	243.72751	19.36458	24.2143
5	2.236	VB	0.4780	732.20880	18.48874	72.7449

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.467	BV	0.4618	1086.69458	31.24513	9.3295
2	0.973	VV	0.3413	1333.60535	51.64213	11.4493
3	1.712	VP	0.4289	2984.38452	90.46104	25.6215
4	2.552	VV	0.7035	4560.66357	86.44473	39.1542
5	3.367	VB	0.4553	1682.61987	46.81979	14.4456

Figure B3: HPLC data of purified water run at a flow rate of 1.5 ml/min

Injection Date : 2001/02/09 11:45:48 AM
 Sample Name : sample B Vial : 5
 Acq. Operator : nataly
 Acq. Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 11:40:30 AM by nataly
 (modified after loading)
 Analysis Method : C:\HPCHEM\1\METHODS\NATALY.M
 Last changed : 2001/02/09 12:12:25 PM by nataly
 (modified after loading)



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 Area Percent Report
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 Multiplier : 1.0000
 Dilution : 1.0000
 Sample Amount : 10.00000 [ng/ul] (not used in calc.)

Signal 1: FLD1 A, Ex=254, Em=254

Signal 2: MWD1 A, Sig=254,4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.862	BV	0.1978	4.67141e4	2996.96582	72.3573
2	2.685	VV	0.1394	980.42694	95.77476	1.5186
3	2.952	VV	0.1904	1912.69397	144.49033	2.9626
4	3.241	VV	0.3377	6756.17139	287.98752	10.4649
5	3.814	VV	0.3506	2436.35278	100.59360	3.7738

Hewlett Packard 1050 HPLC 2001/02/09 12:12:52 PM nataly

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
6	4.311	VV	0.3531	1029.00806	38.34810	1.5939
7	4.984	VV	0.5365	1553.67346	37.10403	2.4065
8	6.398	VV	0.4281	414.12177	12.84757	0.6414
9	6.768	VB	0.5113	529.69379	12.89420	0.8205
10	8.280	BV	0.5178	313.77634	8.21145	0.4860
11	9.679	VV	0.7476	293.11731	4.77907	0.4540
12	11.991	VV	1.1302	994.80328	11.26577	1.5409
13	14.494	VV	0.5082	88.54098	2.20624	0.1371
14	16.111	VB	1.5368	543.86914	4.18332	0.8424

Signal 3: MWD1 B, Sig=205,4 Ref=550,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.858	BV	0.2474	1.53135e4	772.00037	19.2821
2	2.219	VV	0.3564	6691.14355	234.81612	8.4252
3	2.949	VV	0.2252	2381.81274	156.79865	2.9991
4	3.239	VV	0.2672	4585.38916	244.09071	5.7737
5	3.471	VV	0.1780	2535.64624	182.59360	3.1928
6	3.793	VV	0.3677	3535.30273	130.65259	4.4515
7	4.310	VV	0.3031	1839.65771	78.00828	2.3164
8	4.961	VV	0.2344	1190.57874	72.95084	1.4991
9	5.129	VV	0.1642	901.63892	77.24799	1.1353
10	6.111	VV	0.5414	1.23268e4	328.80881	15.5213
11	7.484	VB	0.8142	1.95920e4	339.04996	24.6693
12	10.597	BP	1.3691	8399.85547	73.07578	10.5767
13	14.469	BP	0.2698	125.19398	5.75437	0.1576

Figure B4: HPLC data of Fraction B, dissolved in acetone, eluted with 10% water in methanol and run at a flow rate of 1.5 ml/min

APPENDIX C

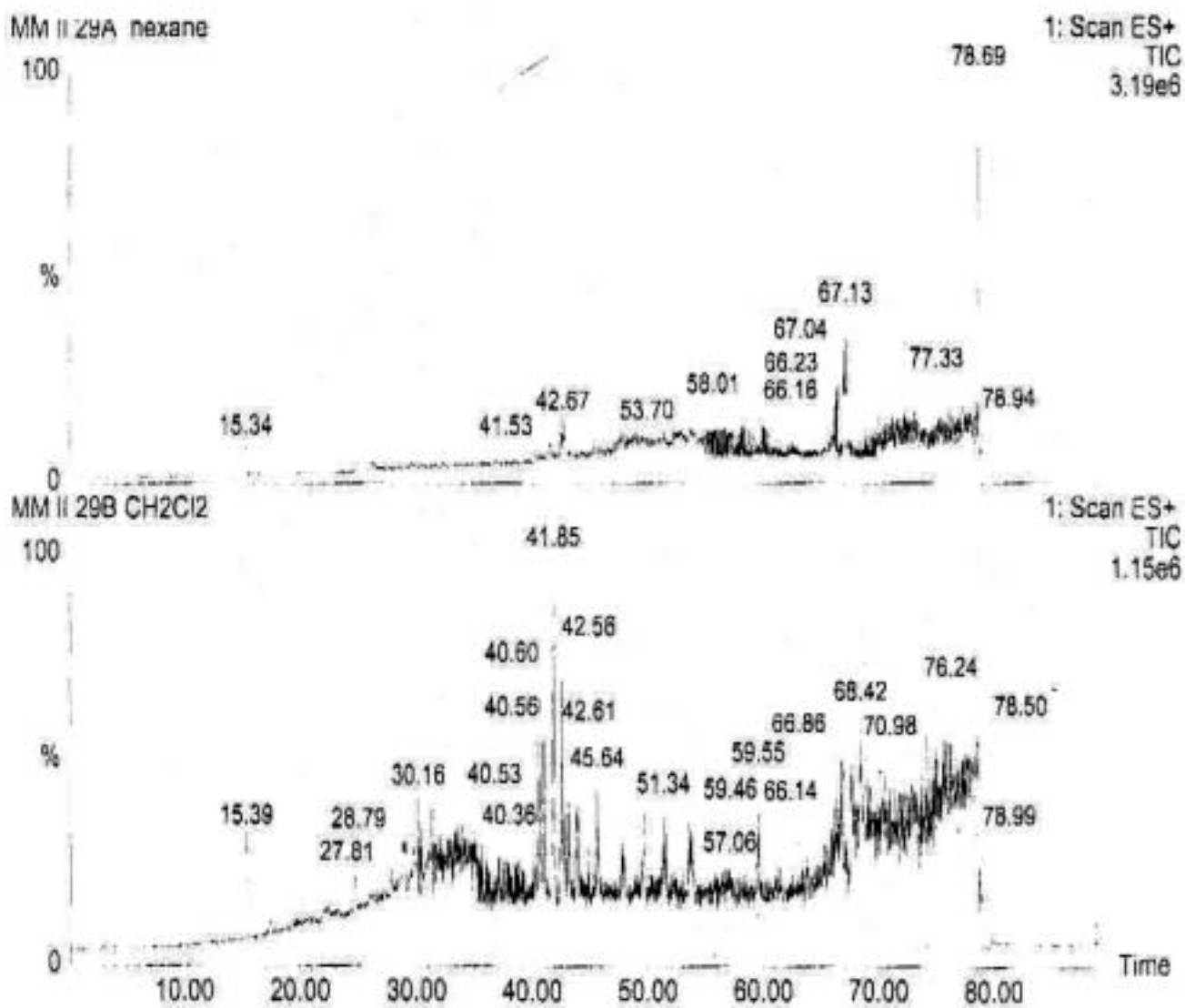


Figure C1: Preparative HPLC of the hexane (top) and chloroform (bottom) fractions following solvent/ solvent extraction

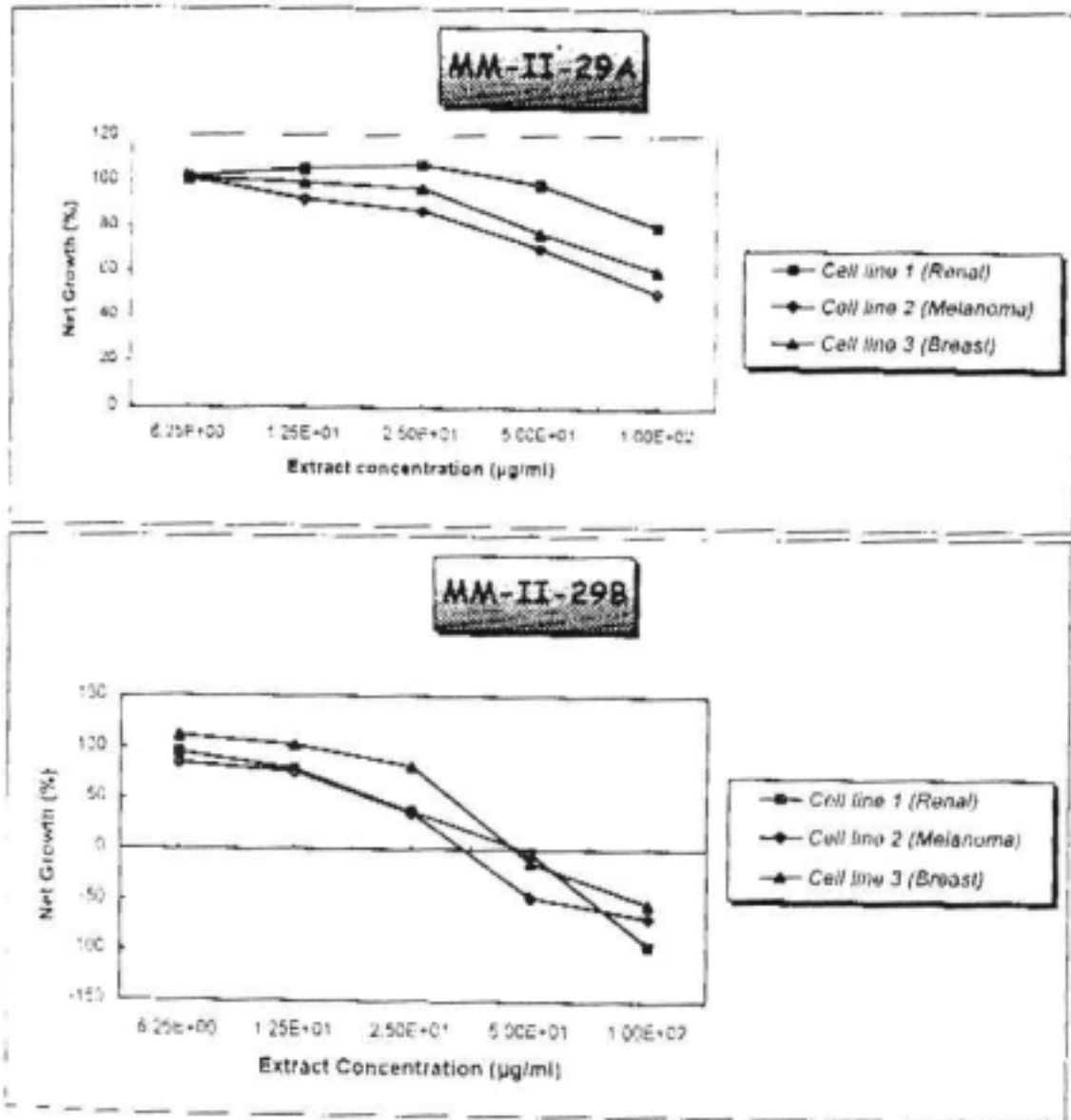


Figure C3: Cytotoxicity of the hexane (top) and chloroform (bottom) fractions on 3 cell lines i.e. renal, melanoma and breast. The chloroform fraction shows inhibition of all three cells lines

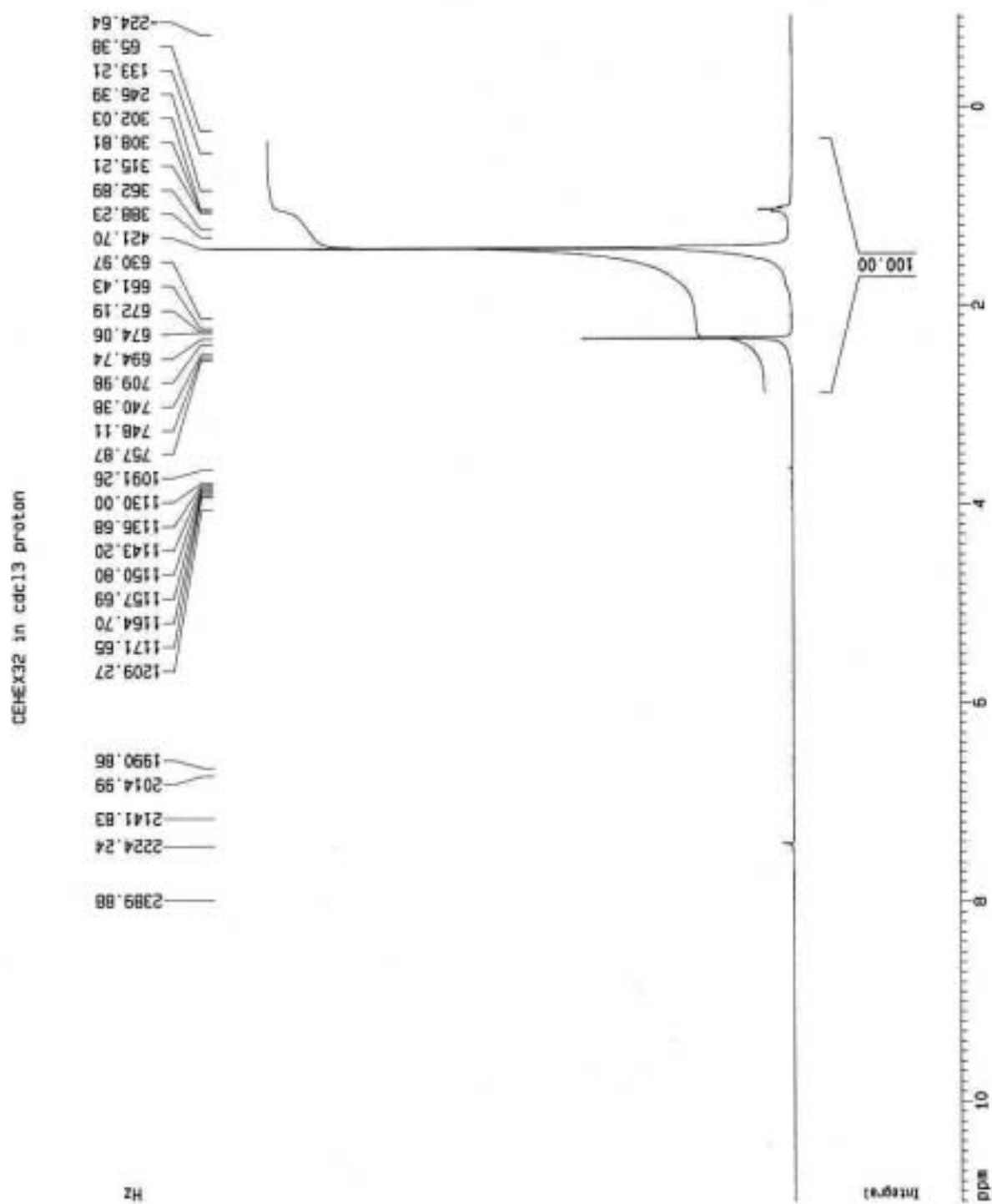


Figure C4: ^1H NMR (300MHz, CDCl_3) of an isolated compound from the hexane fraction

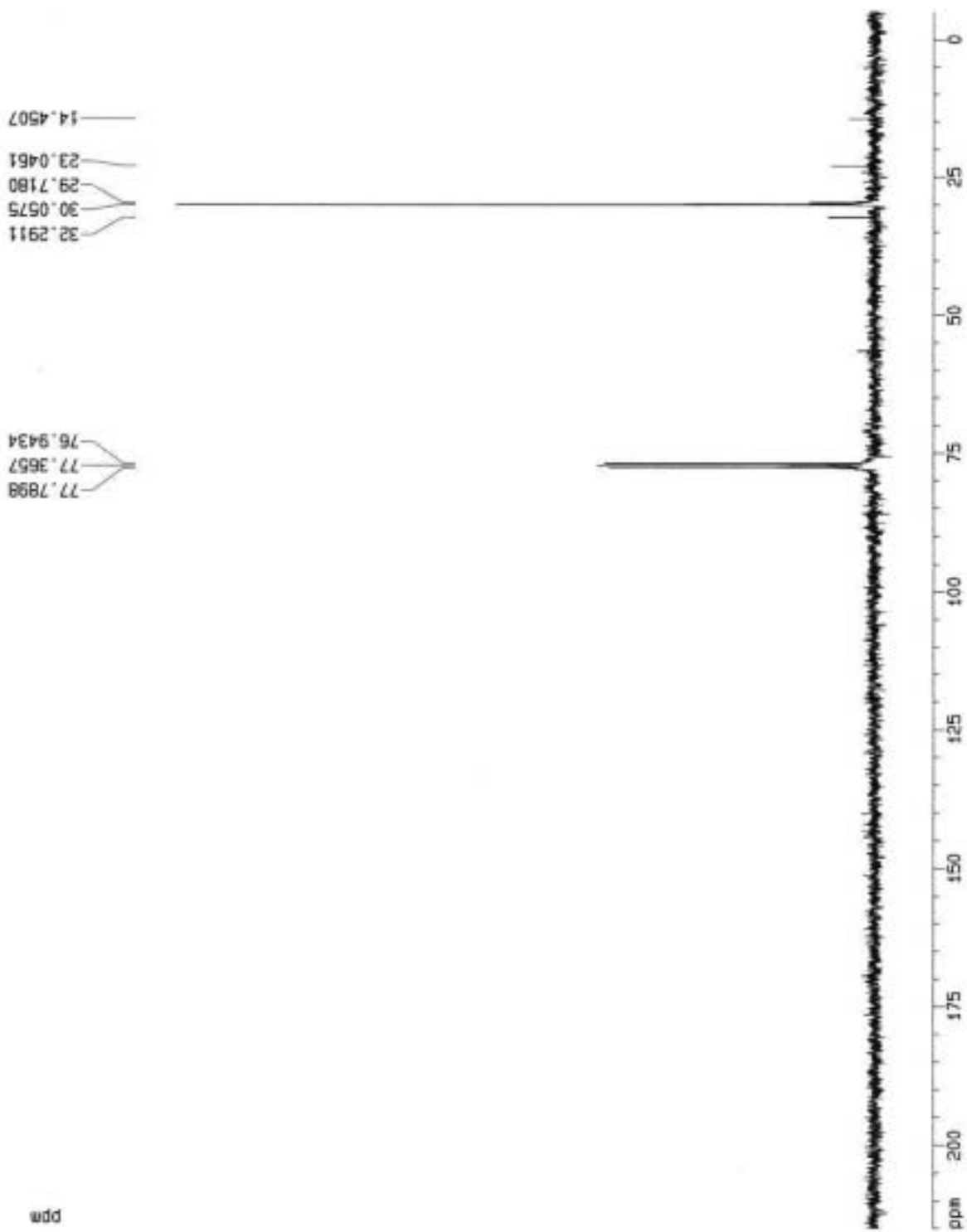


Figure C5: ^{13}C NMR (75MHz, CDCl_3) of an isolated compound from the hexane fraction

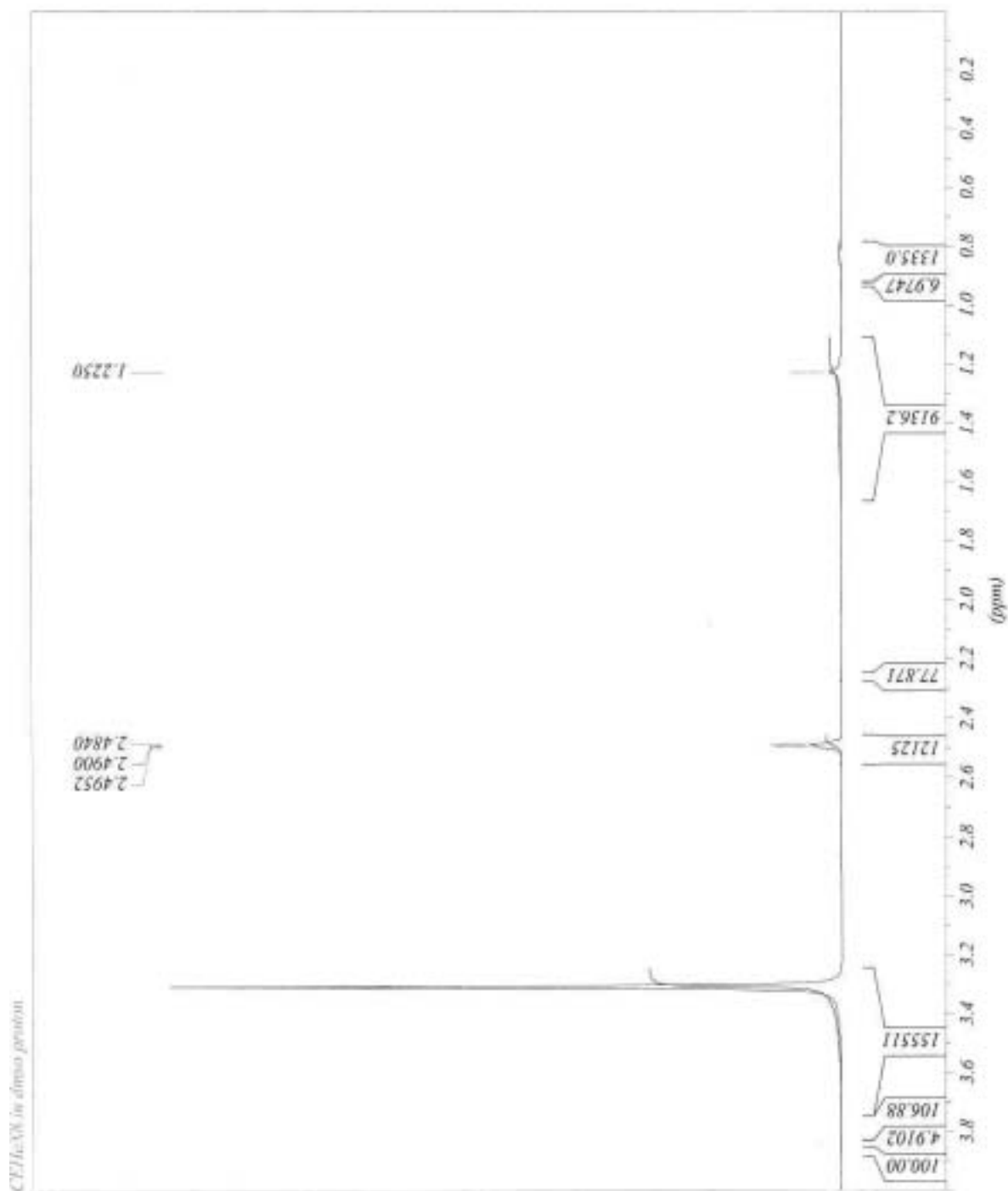


Figure C6: ^1H NMR (300MHz, DMSO- d_6) of an isolated compound from the hexane fraction

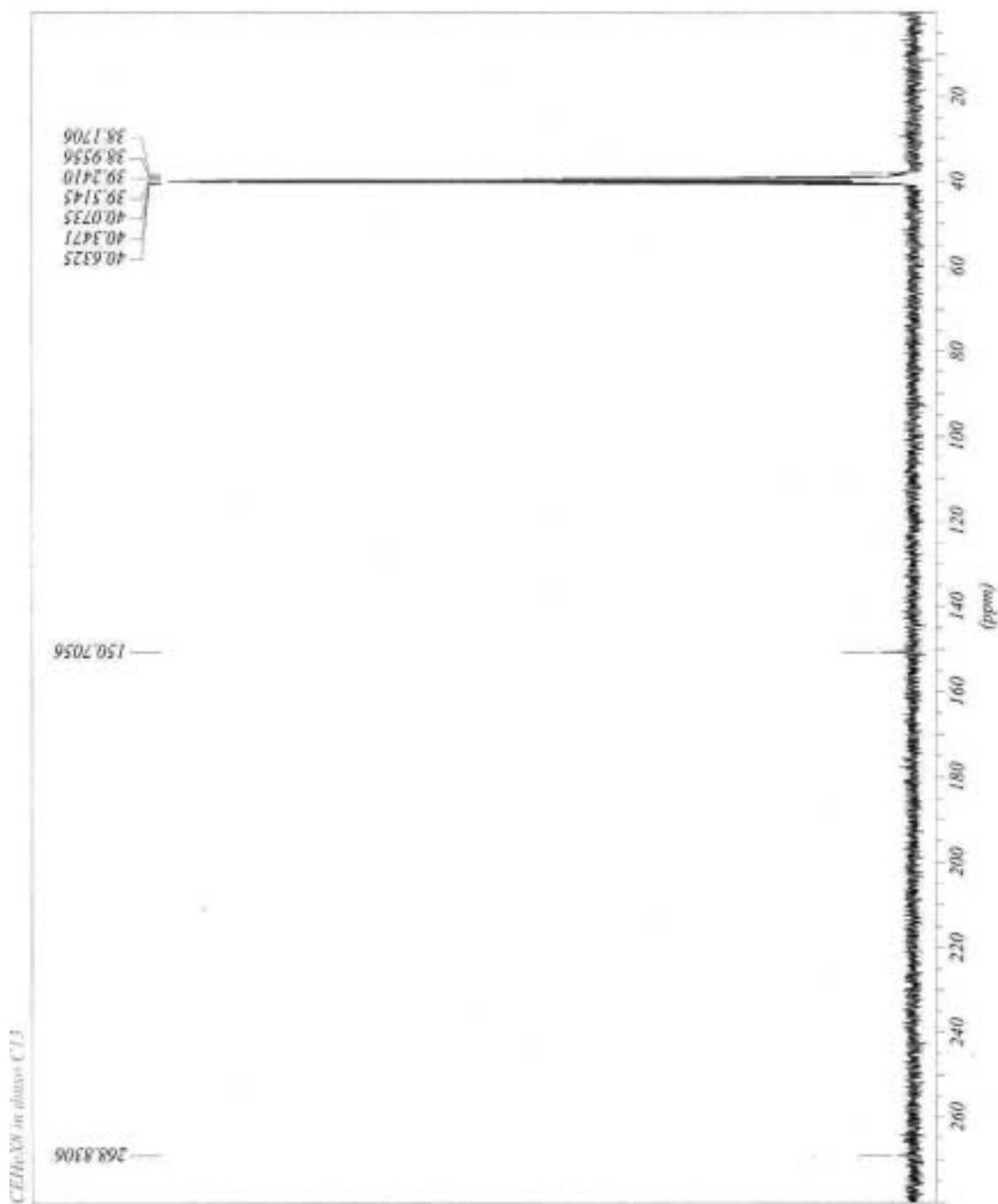


Figure C7: ^{13}C NMR (75MHz, DMSO- d_6) of a isolated compound from the hexane fraction