APPENDICES

APPENDIX 1

Calculation for sample yield

Example,

$$\text{Yield of hexane soluble fraction (\%)} = \frac{\text{Weight of hexane soluble fraction obtained}}{\text{Weight of aqueous ethanol extract used}} \times 100$$

$$= \frac{3.85\ (g)}{52.29\ (g)} \times 100$$

$$= 7.36\ \%$$

APPENDIX 2

Statistical analysis for percentage of neurite bearing cells of aqueous ethanol extract by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	32.451	5	6.4902	4.43	0.0491
Within groups	8.79665	6	1.46611		
Total	41.2476	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 4.43, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	19.91	XX
positive control (NGF)	2	23.01	X
10	2	22.90	X
25	2	22.53	XX
50	2	20.87	XXX
100	2	18.63	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-3.09500*	2.96280
negative control - 10µg/ml	-2.99000*	2.96280
negative control - 25µg/ml	-2.62000	2.96280
negative control - 50µ/ml	-0.96000	2.96280
negative control - 100µg/ml	1.28000	2.96280
positive control - 10µg/ml	0.10500	2.96280
positive control - 25µg/ml	0.47500	2.96280
positive control - 50µg/ml	2.13500	2.96280
positive control - 100µg/ml	4.37500*	2.96280
10µg/ml - 25µg/ml	0.37000	2.96280
10µg/ml - 50µg/ml	2.03000	2.96280
10µg/ml - 100µg/ml	4.27000*	2.96280
25µg/ml - 50µg/ml	1.66000	2.96280
25µg/ml - 100µg/ml	3.90000*	2.96280
50µg/ml - 100µg/ml	2.24000	2.96280

* Denotes a statistically significant difference

3 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 3

Statistical analysis for percentage of neurite bearing cells of hexane fraction by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	360.73	5	72.146	24.17	0.0007
Within groups	17.9126	6	2.98543		
Total	378.642	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 24.17, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	24.58	X
positive control (NGF)	2	28.86	XX
10	2	25.40	XX
25	2	32.29	XX
50	2	34.09	X
100	2	40.59	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.28500*	4.22788
negative control - 10µg/ml	-0.81500*	4.22788
negative control - 25µg/ml	-7.71000*	4.22788
negative control - 50µ/ml	-.9.51000*	4.22788
negative control - 100µg/ml	-16.01000*	4.22788
positive control - 10µg/ml	3.47000	4.22788
positive control - 25µg/ml	-3.42500	4.22788
positive control - 50µg/ml	-5.22500*	4.22788
positive control - 100µg/ml	-11.72500*	4.22788
10µg/ml - 25µg/ml	-6.89500*	4.22788
10µg/ml - 50µg/ml	-8.69500*	4.22788
10µg/ml - 100µg/ml	-15.19500*	4.22788
25µg/ml - 50µg/ml	-1.80000	4.22788
25µg/ml - 100µg/ml	-8.30000*	4.22788
50µg/ml - 100µg/ml	-6.50000*	4.22788

* Denotes a statistically significant difference

5 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 4

Statistical analysis for percentage of neurite bearing cells of ethyl acetate fraction by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	229.567	5	45.9134	26.94	0.0005
Within groups	10.2246	6	1.70411		
Total	239.791	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 26.94, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	20.49	X
positive control (NGF)	2	24.14	X
10	2	23.74	X
25	2	26.51	XX
50	2	27.75	X
100	2	34.53	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-3.65000*	3.19424
negative control - 10µg/ml	-3.25000*	3.19424
negative control - 25µg/ml	-6.01500*	3.19424
negative control - 50µ/ml	-7.26000*	3.19424
negative control - 100µg/ml	-14.04000*	3.19424
positive control - 10µg/ml	0.40000	3.19424
positive control - 25µg/ml	-2.36500	3.19424
positive control - 50µg/ml	-3.61000*	3.19424
positive control - 100µg/ml	-10.39000*	3.19424
10µg/ml - 25µg/ml	-2.76500	3.19424
10µg/ml - 50µg/ml	-4.01000*	3.19424
10µg/ml - 100µg/ml	-10.79000*	3.19424
25µg/ml - 50µg/ml	-1.24500	3.19424
25µg/ml - 100µg/ml	-8.02500*	3.19424
50µg/ml - 100µg/ml	-6.78000*	3.19424

* Denotes a statistically significant difference

4 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 5

Statistical analysis for percentage of neurite bearing cells of water fraction by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	52.6377	5	10.5275	4.52	0.0469
Within groups	13.9704	6	2.3284		
Total	66.6081	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 4.52, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (μg/ml)	replicate	Mean	Homogenous groups
negative control	2	19.02	X
positive control (NGF)	2	24.17	X
10	2	21.86	XX
25	2	24.03	X
50	2	23.46	X
100	2	25.56	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-5.14500*	3.73378
negative control - 10μg/ml	-2.83500	3.73378
negative control - 25μg/ml	-5.00500*	3.73378
negative control - 50μ/ml	-4.43500*	3.73378
negative control - 100μg/ml	-6.54000*	3.73378
positive control - 10μg/ml	2.31000	3.73378
positive control - 25μg/ml	0.14000	3.73378
positive control - 50μg/ml	0.71000	3.73378
positive control - 100μg/ml	-1.39500	3.73378
10μg/ml - 25μg/ml	-2.17000	3.73378
10μg/ml - 50μg/ml	-1.60000	3.73378
10μg/ml - 100μg/ml	-3.70500	3.73378
25μg/ml - 50μg/ml	0.57000	3.73378
25μg/ml - 100μg/ml	-1.53500	3.73378
50μg/ml - 100μg/ml	-2.10500	3.73378

* Denotes a statistically significant difference

2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 6

Statistical analysis for percentage of neurite bearing cells of fraction E1 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	2098.18	5	419.636	103.68	0.0000
Within groups	24.2844	6	4.04739		
Total	2122.47	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 103.68, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	21.51	X
positive control (NGF)	2	26.77	X
10	2	25.09	XX
25	2	35.75	X
50	2	51.27	X
100	2	56.04	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-5.26500*	4.92274
negative control - 10µg/ml	-3.58000	4.92274
negative control - 25µg/ml	-14.24500*	4.92274
negative control - 50µ/ml	-29.76000*	4.92274
negative control - 100µg/ml	-34.53500*	4.92274
positive control - 10µg/ml	1.68500	4.92274
positive control - 25µg/ml	-8.98000*	4.92274
positive control - 50µg/ml	-24.49500*	4.92274
positive control - 100µg/ml	-29.27000*	4.92274
10µg/ml - 25µg/ml	-10.66500*	4.92274
10µg/ml - 50µg/ml	-26.18000*	4.92274
10µg/ml - 100µg/ml	-30.95500*	4.92274
25µg/ml - 50µg/ml	-15.51500*	4.92274
25µg/ml - 100µg/ml	-20.29000*	4.92274
50µg/ml - 100µg/ml	-4.775	4.92274

* Denotes a statistically significant difference

4 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 7

Statistical analysis for percentage of neurite bearing cells of fraction E2 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	1231.14	5	246.228	84.77	0.0000
Within groups	17.4287	6	2.90479		
Total	1248.57	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 84.77, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	20.77	X
positive control (NGF)	2	25.22	X
10	2	28.12	X
25	2	33.46	X
50	2	38.49	X
100	2	51.74	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.44500*	4.17039
negative control - 10µg/ml	-7.34500*	4.17039
negative control - 25µg/ml	-12.68500*	4.17039
negative control - 50µ/ml	-17.72000*	4.17039
negative control - 100µg/ml	-30.97000*	4.17039
positive control - 10µg/ml	-2.90000	4.17039
positive control - 25µg/ml	-8.24000*	4.17039
positive control - 50µg/ml	-13.27500*	4.17039
positive control - 100µg/ml	-26.52500*	4.17039
10µg/ml - 25µg/ml	-5.34000*	4.17039
10µg/ml - 50µg/ml	-10.37500*	4.17039
10µg/ml - 100µg/ml	-23.62500*	4.17039
25µg/ml - 50µg/ml	-5.03500*	4.17039
25µg/ml - 100µg/ml	-18.28500*	4.17039
50µg/ml - 100µg/ml	-13.25000*	4.17039

* Denotes a statistically significant difference

5 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 8

Statistical analysis for percentage of neurite bearing cells of fraction E3 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	231.749	4	57.9372	58.90	0.0002
Within groups	4.9184	5	0.98368		
Total	236.667	9			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 58.90, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	18.67	X
positive control (NGF)	2	22.72	X
10	2	24.45	X
25	2	29.48	X
50	2	32.18	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.04500*	2.54953
negative control - 10µg/ml	-5.77500*	2.54953
negative control - 25µg/ml	-10.80500*	2.54953
negative control - 50µ/ml	-13.50500*	2.54953
positive control - 10µg/ml	-1.73000	2.54953
positive control - 25µg/ml	-6.76000*	2.54953
positive control - 50µg/ml	-9.46000*	2.54953
10µg/ml - 25µg/ml	-5.03000*	2.54953
10µg/ml - 50µg/ml	-7.73000*	2.54953
25µg/ml - 50µg/ml	-2.70000	2.54953

* Denotes a statistically significant difference

4 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 9

Statistical analysis for percentage of neurite bearing cells of fraction E4 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	161.884	4	40.4609	34.09	0.0008
Within groups	5.93435	5	1.18687		
Total	167.778	9			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 34.09, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	18.01	X
positive control (NGF)	2	21.46	X
10	2	17.89	X
25	2	26.42	X
50	2	27.35	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-3.44500*	2.80049
negative control - 10µg/ml	0.12000	2.80049
negative control - 25µg/ml	-8.40500*	2.80049
negative control - 50µ/ml	-9.33500*	2.80049
positive control - 10µg/ml	3.56500*	2.80049
positive control - 25µg/ml	-4.96000*	2.80049
positive control - 50µg/ml	-5.89000*	2.80049
10µg/ml - 25µg/ml	-8.52500*	2.80049
10µg/ml - 50µg/ml	-9.45500*	2.80049
25µg/ml - 50µg/ml	-0.93000	2.80049

* Denotes a statistically significant difference

3 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 10

Statistical analysis for percentage of neurite bearing cells of fraction E5 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	231.366	4	57.8414	143.31	0.0000
Within groups	2.0181	5	0.40362		
Total	233.384	9			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 143.31, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	20.71	X
positive control (NGF)	2	25.48	X
10	2	22.26	X
25	2	28.92	X
50	2	34.08	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.76500*	1.63312
negative control - 10µg/ml	1.54500	1.63312
negative control - 25µg/ml	-8.21000*	1.63312
negative control - 50µ/ml	-13.37000*	1.63312
positive control - 10µg/ml	3.22000*	1.63312
positive control - 25µg/ml	-3.44500*	1.63312
positive control - 50µg/ml	-8.60500*	1.63312
10µg/ml - 25µg/ml	-6.66500*	1.63312
10µg/ml - 50µg/ml	-11.82500*	1.63312
25µg/ml - 50µg/ml	-5.16000*	1.63312

* Denotes a statistically significant difference

4 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 11

Statistical analysis for percentage of neurite bearing cells of fraction E6 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	19.0941	5	3.81882	4.40	0.0497
Within groups	5.2059	6	0.86765		
Total	24.3	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 4.401, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (μg/ml)	replicate	Mean	Homogenous groups
negative control	2	18.74	X
positive control (NGF)	2	22.72	X
10	2	19.88	X
25	2	19.67	X
50	2	19.90	X
100	2	19.33	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-3.97500*	2.27925
negative control - 10μg/ml	-1.13500	2.27925
negative control - 25μg/ml	-0.92500	2.27925
negative control - 50μ/ml	-1.15000	2.27925
negative control - 100μg/ml	-0.58500	2.27925
positive control - 10μg/ml	2.84000*	2.27925
positive control - 25μg/ml	3.05000*	2.27925
positive control - 50μg/ml	2.82500*	2.27925
positive control - 100μg/ml	3.39000*	2.27925
10μg/ml - 25μg/ml	0.21000	2.27925
10μg/ml - 50μg/ml	-0.01500	2.27925
10μg/ml - 100μg/ml	0.55000	2.27925
25μg/ml - 50μg/ml	-0.22500	2.27925
25μg/ml - 100μg/ml	0.34000	2.27925
50μg/ml - 100μg/ml	0.56500	2.27925

* Denotes a statistically significant difference

2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 12

Statistical analysis for percentage of neurite bearing cells of fraction E7 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	51.0189	5	10.2038	7.27	0.0158
Within groups	8.42455	6	1.40409		
Total	59.4435	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 7.27, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups
negative control	2	18.18	X
positive control (NGF)	2	22.21	X
10	2	16.07	X
25	2	16.97	X
50	2	16.64	X
100	2	16.84	X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.03000*	2.89946
negative control - 10µg/ml	2.11000	2.89946
negative control - 25µg/ml	1.20500	2.89946
negative control - 50µ/ml	1.53500	2.89946
negative control - 100µg/ml	1.33500	2.89946
positive control - 10µg/ml	6.14000*	2.89946
positive control - 25µg/ml	5.23500*	2.89946
positive control - 50µg/ml	5.56500*	2.89946
positive control - 100µg/ml	5.36500*	2.89946
10µg/ml - 25µg/ml	-0.90500	2.89946
10µg/ml - 50µg/ml	-0.57500	2.89946
10µg/ml - 100µg/ml	-0.77500	2.89946
25µg/ml - 50µg/ml	0.33000	2.89946
25µg/ml - 100µg/ml	0.13000	2.89946
50µg/ml - 100µg/ml	-0.20000	2.89946

* Denotes a statistically significant difference

2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 13

Statistical analysis for percentage of neurite bearing cells of subfraction sub4b_4 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	1341.1	5	268.62	158.17	0.0000
Within groups	10.19	6	1.69833		
Total	1353.29	11			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 158.17, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (µg/ml)	replicate	Mean	Homogenous groups				
negative control	2	17.01	X				
positive control (NGF)	2	20.65		X			
10	2	20.46		X			
25	2	24.70			X		
50	2	29.56				X	
100	2	48.84					X

Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-3.63500*	3.18883
negative control - 10µg/ml	-3.44500*	3.18883
negative control - 25µg/ml	-7.68500*	3.18883
negative control - 50µ/ml	-12.55000*	3.18883
negative control - 100µg/ml	-31.82500*	3.18883
positive control - 10µg/ml	0.19000	3.18883
positive control - 25µg/ml	-4.05000*	3.18883
positive control - 50µg/ml	-8.91500*	3.18883
positive control - 100µg/ml	-28.19000*	3.18883
10µg/ml - 25µg/ml	-4.24000*	3.18883
10µg/ml - 50µg/ml	-9.10500*	3.18883
10µg/ml - 100µg/ml	-28.38000*	3.18883
25µg/ml - 50µg/ml	-4.86500*	3.18883
25µg/ml - 100µg/ml	-24.14000*	3.18883
50µg/ml - 100µg/ml	-19.27500*	3.18883

* Denotes a statistically significant difference

5 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 14

Statistical analysis for percentage of neurite bearing cells of subfraction sub4b_6 by using one way ANOVA.

ANOVA table

Source of variation	Sum of squares	Df	Mean square	F-ratio	P-value
Between groups	48.5128	4	12.1282	5.36	0.0471
Within groups	11.3161	5	2.26321		
Total	59.8289	9			

The ANOVA table decomposes the variance into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 5.36, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean of neurite bearing cells from one level of concentration to another at the 95.0% confidence level. To determine which means are significantly different from which others, Multiple Range Tests were carried out.

Multiple range tests

Treatment concentration (μg/ml)	replicate	Mean	Homogenous groups
negative control	2	17.24	X
positive control (NGF)	2	21.57	XX
10	2	19.84	XXX
25	2	23.41	X
50	2	18.40	XX

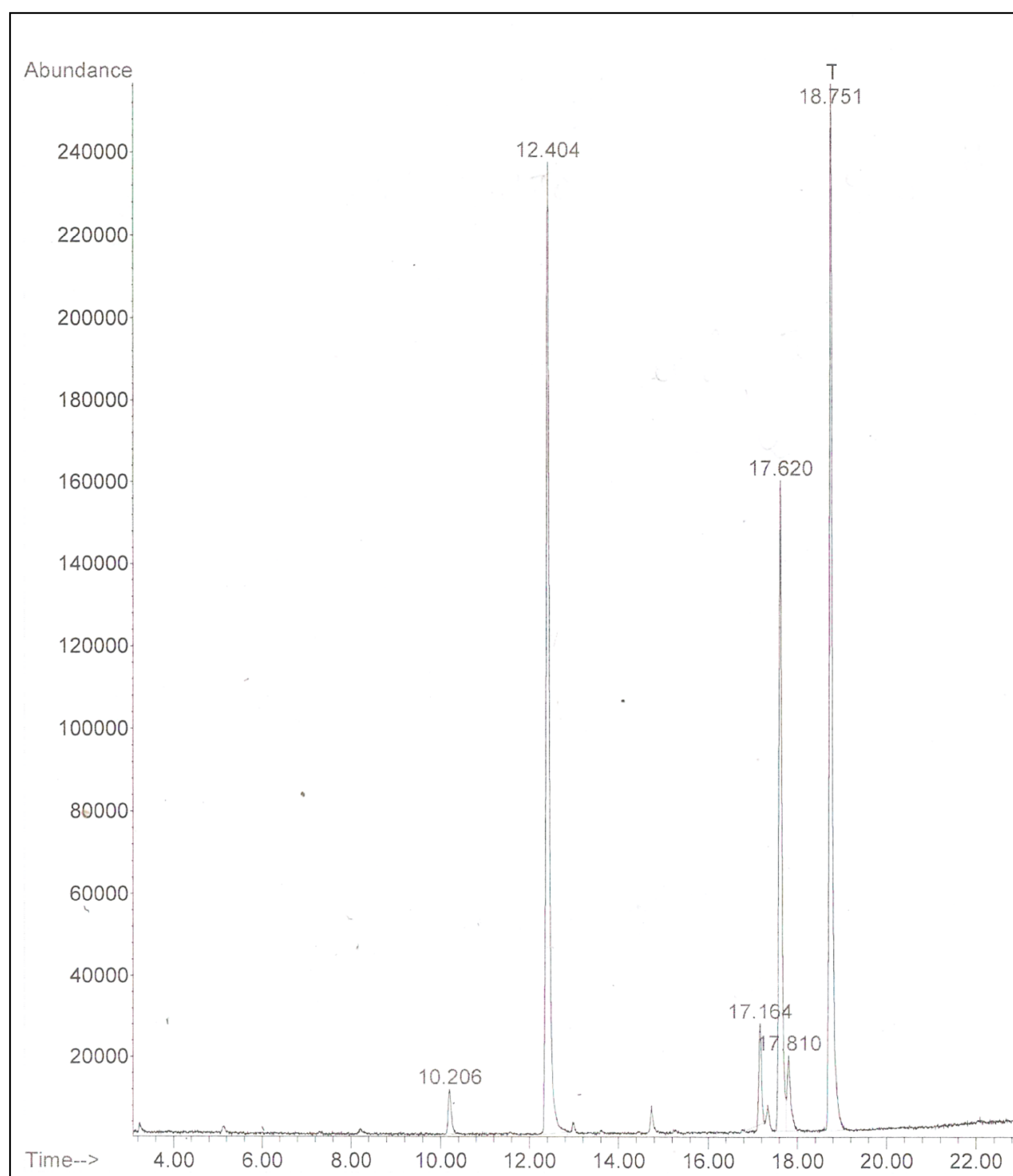
Contrast (concentrations)	Difference	+/- Limits
negative control – positive control	-4.33000*	3.86719
negative control - 10μg/ml	-2.59500	3.86719
negative control - 25μg/ml	-6.17000*	3.86719
negative control - 50μ/ml	-1.16000	3.86719
positive control - 10μg/ml	1.73500	3.86719
positive control - 25μg/ml	-1.84000	3.86719
positive control - 50μg/ml	3.17000	3.86719
10μg/ml - 25μg/ml	-3.57500*	3.86719
10μg/ml - 50μg/ml	1.43500	3.86719
25μg/ml - 50μg/ml	5.01000*	3.86719

* Denotes a statistically significant difference

3 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

APPENDIX 15

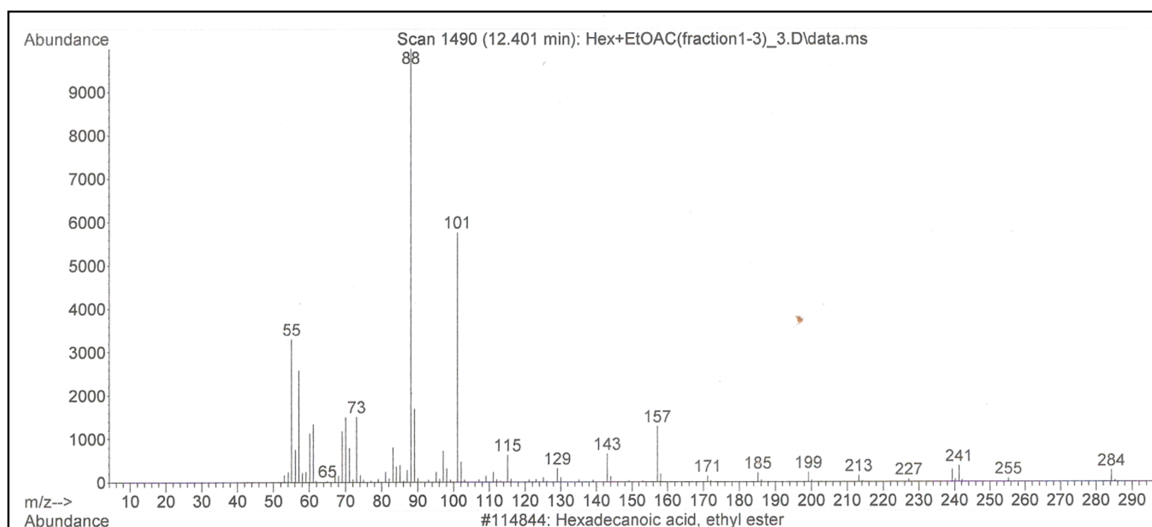
The total ion chromatogram (TIC) of fraction E1 of *Hericium erinaceus*



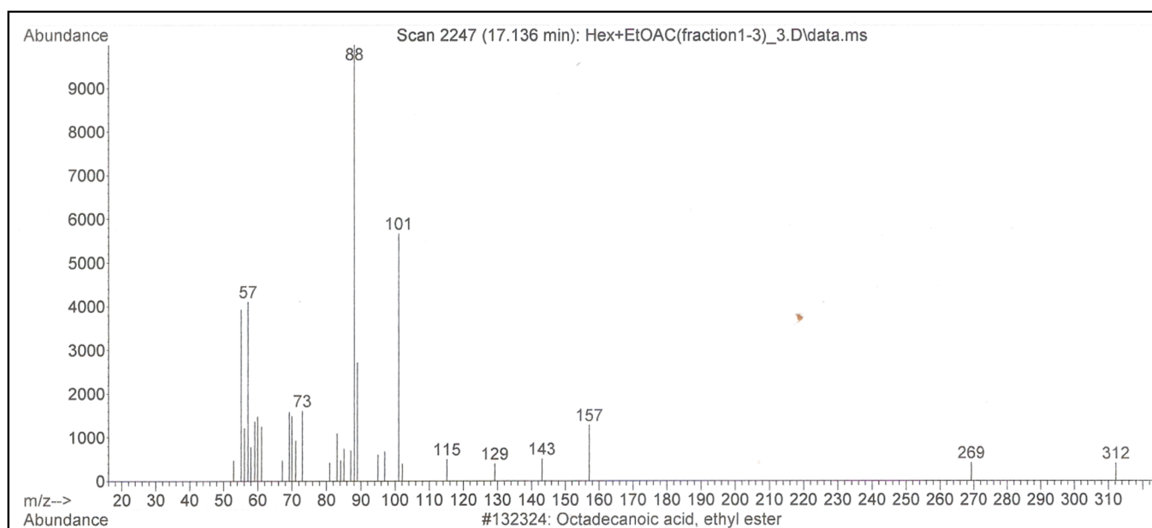
APPENDIX 16

Mass spectrum of fraction E1 of *Hericium erinaceus*

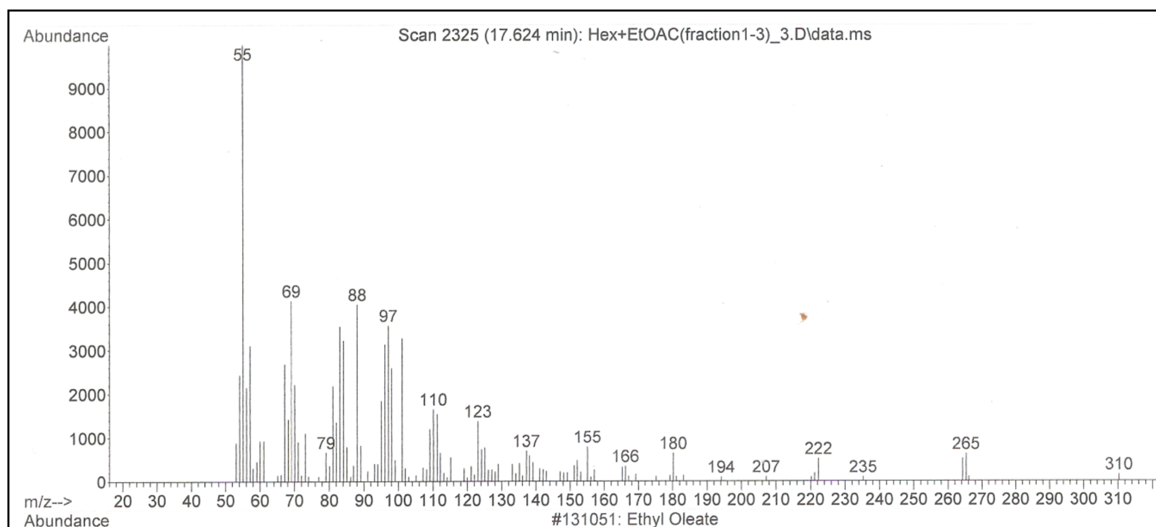
Mass spectrum of ethyl palmitate



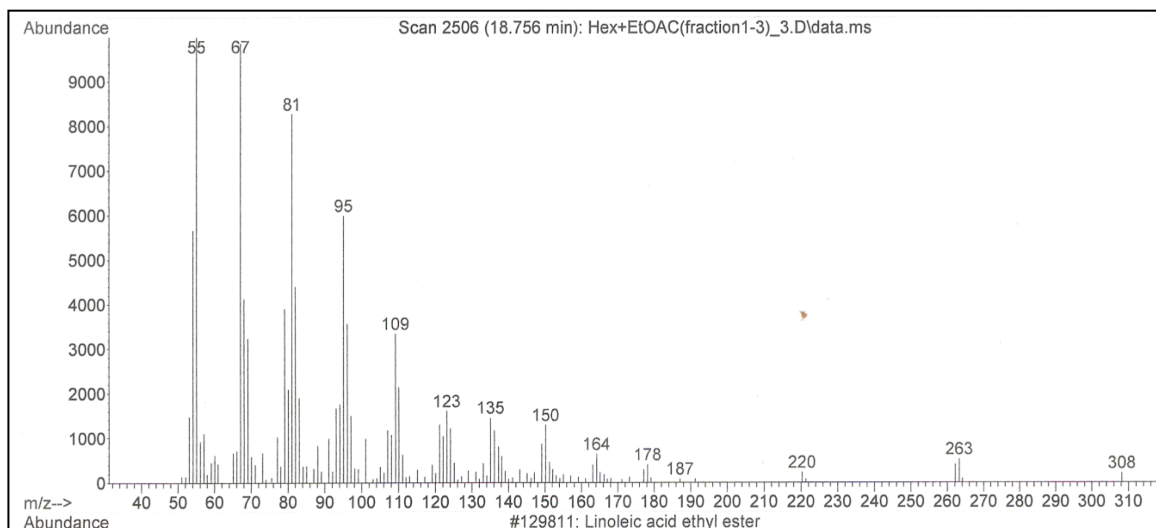
Mass spectrum of ethyl stearate



Mass spectrum of ethyl oleate

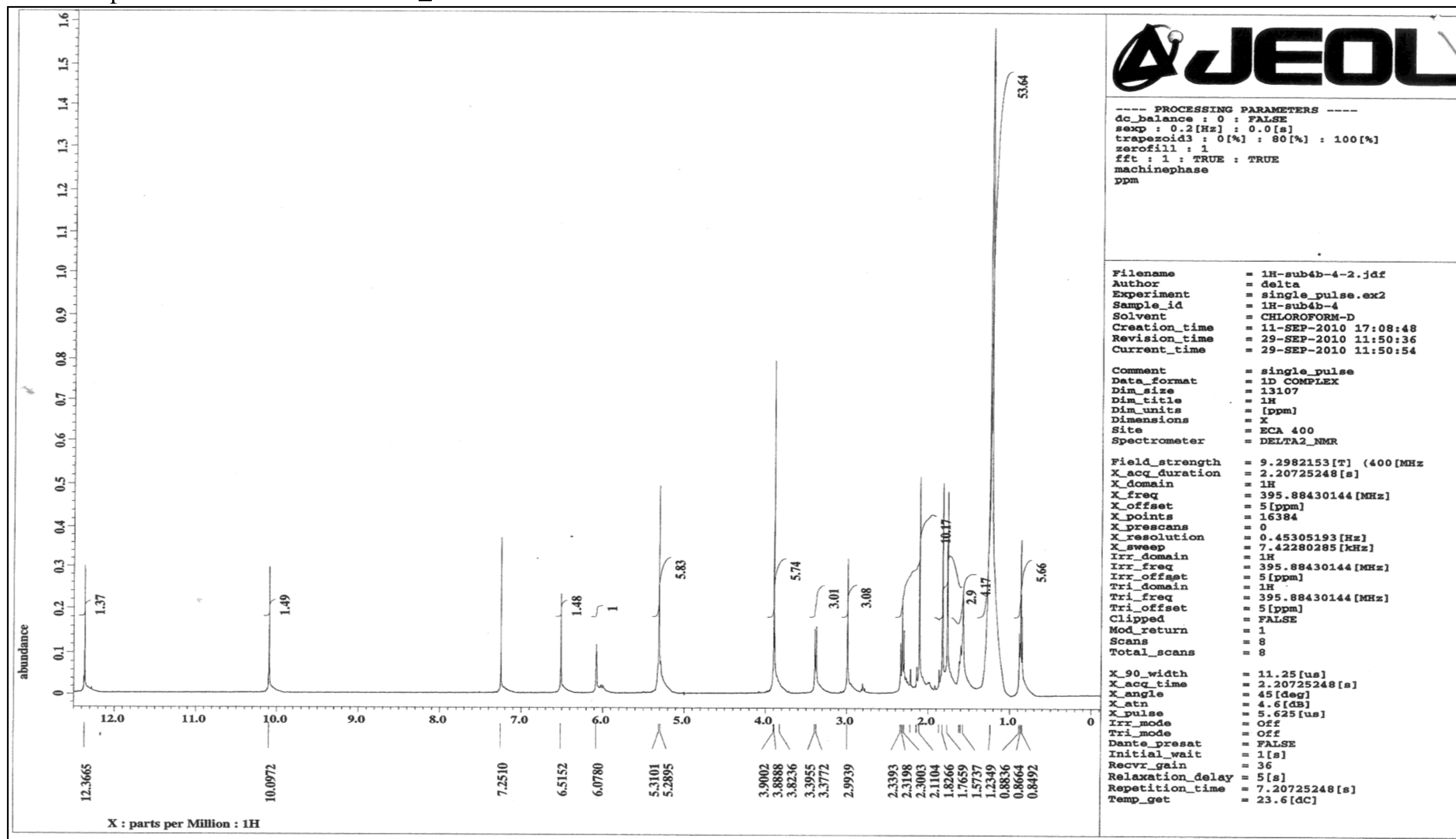


Mass spectrum of ethyl linoleate



APPENDIX 17

^{1}H-NMR spectrum of subfraction sub4b_4



---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename	= 1H-sub4b-4-2.jdf
Author	= delta
Experiment	= single_pulse.ex2
Sample_id	= 1H-sub4b-4
Solvent	= CHLOROFORM-D
Creation_time	= 11-SEP-2010 17:08:48
Revision_time	= 29-SEP-2010 11:52:34
Current_time	= 29-SEP-2010 11:52:40
Comment	= single_pulse
Data_format	= 1D COMPLEX
Dim_size	= 13107
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 2.20725248[s]
X_domain	= 1H
X_freq	= 395.88430144[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.45305193[Hz]
X_sweep	= 7.42280285[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Tri_domain	= 1H
Tri_freq	= 395.88430144[MHz]
Tri_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 8
Total_scans	= 8
X_90_width	= 11.25[us]
X_acq_time	= 2.20725248[s]
X_angle	= 45[deg]
X_atn	= 4.6[dB]
X_pulse	= 5.625[us]
Irr_mode	= Off
Tri_mode	= Off
Dante_presat	= FALSE
Initial_wait	= 1[s]
Recvr_gain	= 36
Relaxation_delay	= 5[s]
Repetition_time	= 7.20725248[s]
Temp_get	= 23.6[dC]

abundance

0.5
0.4
0.3
0.2
0.1
0

5.83
1.37
1.49
1.48
1

12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0

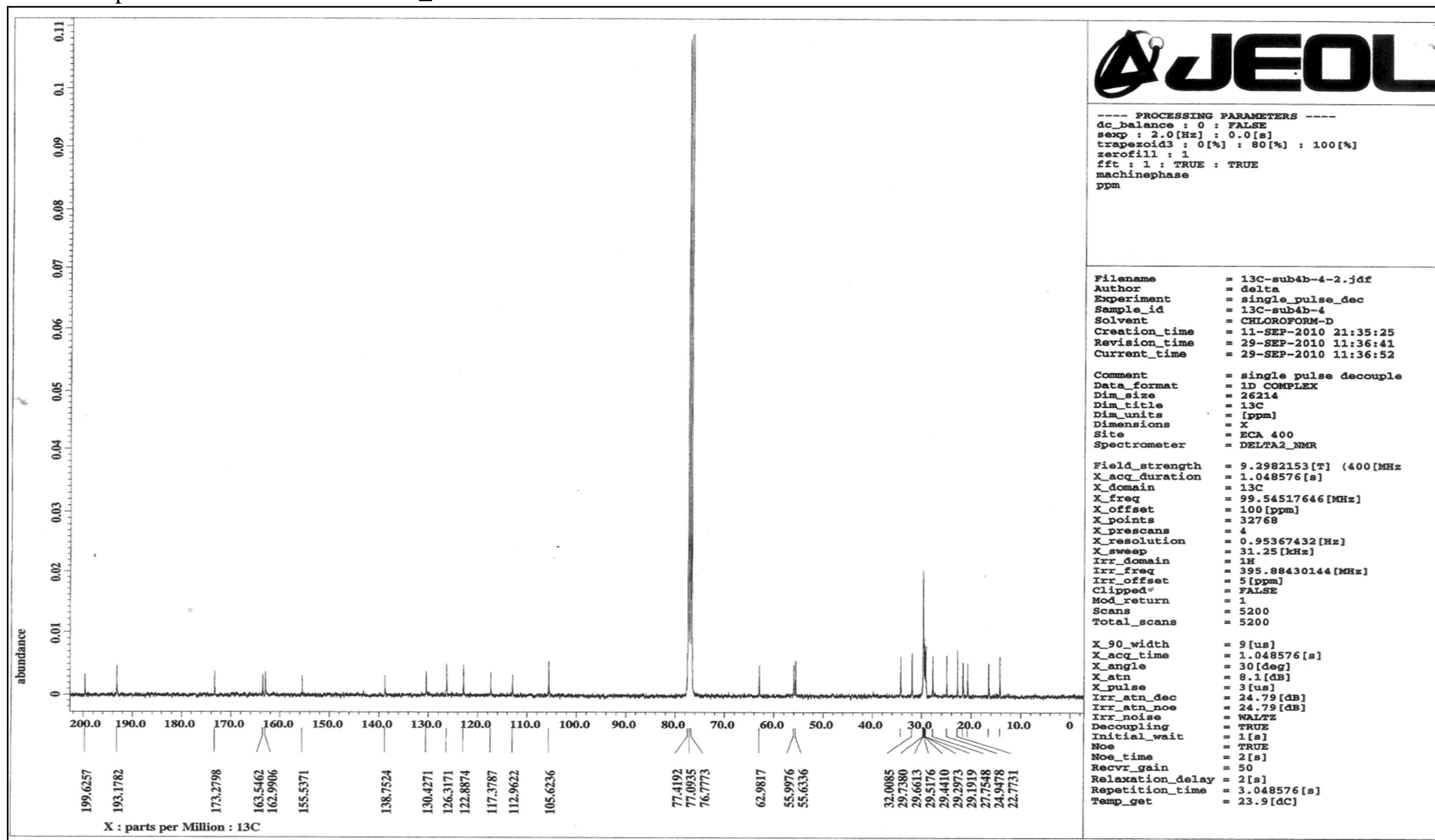
12.3665
10.0972
7.2510
6.5152
6.0780
5.3101
5.2895

X : parts per Million : 1H

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename            = 1H-sub4b-4-2.jdf
Author              = delta
Experiment          = single_pulse.ex2
Sample_id           = 1H-sub4b-4
Solvent             = CHLOROFORM-D
Creation_time       = 11-SEP-2010 17:08:48
Revision_time       = 29-SEP-2010 11:51:16
Current_time        = 29-SEP-2010 11:51:29

Comment             = single_pulse
Data_format         = 1D COMPLEX
Dim_size            = 13107
Dim_title           = 1H
Dim_units           = [ppm]
Dimensions          = X
Site                = ECA 400
Spectrometer        = DELTA2_NMR

Field_strength      = 9.2982153[T] (400[MHz
X_acq_duration      = 2.20725248[s]
X_domain            = 1H
X_freq              = 395.88430144[MHz]
X_offset            = 5[ppm]
X_points            = 16384
X_prescans          = 0
X_resolution        = 0.45305193[Hz]
X_sweep             = 7.42280285[kHz]
Irr_domain          = 1H
Irr_freq            = 395.88430144[MHz]
Irr_offset          = 5[ppm]
Tri_domain          = 1H
Tri_freq            = 395.88430144[MHz]
Tri_offset          = 5[ppm]
Clipped             = FALSE
Mod_return          = 1
Scans               = 8
Total_scans         = 8

X_90_width          = 11.25[us]
X_acq_time          = 2.20725248[s]
X_angle             = 45[deg]
X_atn               = 4.6[dB]
X_pulse             = 5.625[us]
Irr_mode            = Off
Tri_mode            = Off
Dante_presat        = FALSE
Initial_wait        = 1[s]
Recvr_gain          = 36
Relaxation_delay    = 5[s]
Repetition_time     = 7.20725248[s]
Temp_get            = 23.6[dC]
```

APPENDIX 18

^{13}C NMR spectrum of subfraction sub4b_4



(thousandths)

30.0

20.0

10.0

0

60.0 50.0 40.0 30.0 20.0

62.9817 55.9976 55.6336 34.3078 32.0085 29.7380 29.6613 29.5176 29.4410 29.2973 29.1919 27.7548 24.9478 22.7731 21.6809 20.7420 16.4788 14.1987

X : parts per Million : 13C

---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename	= 13C-sub4b-4-2.jdf
Author	= delta
Experiment	= single_pulse_dec
Sample_id	= 13C-sub4b-4
Solvent	= CHLOROFORM-D
Creation_time	= 11-SEP-2010 21:35:25
Revision_time	= 29-SEP-2010 11:36:41
Current_time	= 29-SEP-2010 11:37:16
Comment	= single pulse decouple
Data_format	= 1D COMPLEX
Dim_size	= 26214
Dim_title	= 13C
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 1.048576[s]
X_domain	= 13C
X_freq	= 99.54517646[MHz]
X_offset	= 100[ppm]
X_points	= 32768
X_prescans	= 4
X_resolution	= 0.95367432[Hz]
X_sweep	= 31.25[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 5200
Total_scans	= 5200
X_90_width	= 9[us]
X_acq_time	= 1.048576[s]
X_angle	= 30[deg]
X_atn	= 8.1[dB]
X_pulse	= 3[us]
Irr_atn_dec	= 24.79[dB]
Irr_atn_noe	= 24.79[dB]
Irr_noise	= WALTZ
Decoupling	= TRUE
Initial_wait	= 1[s]
Noe	= TRUE
Noe_time	= 2[s]
Recvr_gain	= 50
Relaxation_delay	= 2[s]
Repetition_time	= 3.048576[s]
Temp_get	= 23.9[dC]

---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename	= 13C-sub4b-4-2.jdf
Author	= delta
Experiment	= single_pulse_dec
Sample_id	= 13C-sub4b-4
Solvent	= CHLOROFORM-D
Creation_time	= 11-SEP-2010 21:35:25
Revision_time	= 29-SEP-2010 11:36:41
Current_time	= 29-SEP-2010 11:37:04
Comment	= single pulse decouple
Data_format	= 1D COMPLEX
Dim_size	= 26214
Dim_title	= 13C
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 1.048576[s]
X_domain	= 13C
X_freq	= 99.54517646[MHz]
X_offset	= 100[ppm]
X_points	= 32768
X_prescans	= 4
X_resolution	= 0.95367432[Hz]
X_sweep	= 31.25[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 5200
Total_scans	= 5200
X_90_width	= 9[us]
X_acq_time	= 1.048576[s]
X_angle	= 30[deg]
X_atn	= 8.1[dB]
X_pulse	= 3[us]
Irr_atn_dec	= 24.79[dB]
Irr_atn_noe	= 24.79[dB]
Irr_noise	= WALTZ
Decoupling	= TRUE
Initial_wait	= 1[s]
Noe	= TRUE
Noe_time	= 2[s]
Recvr_gain	= 50
Relaxation_delay	= 2[s]
Repetition_time	= 3.048576[s]
Temp_get	= 23.9[dC]

(thousandths)

0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0, 16.0, 17.0

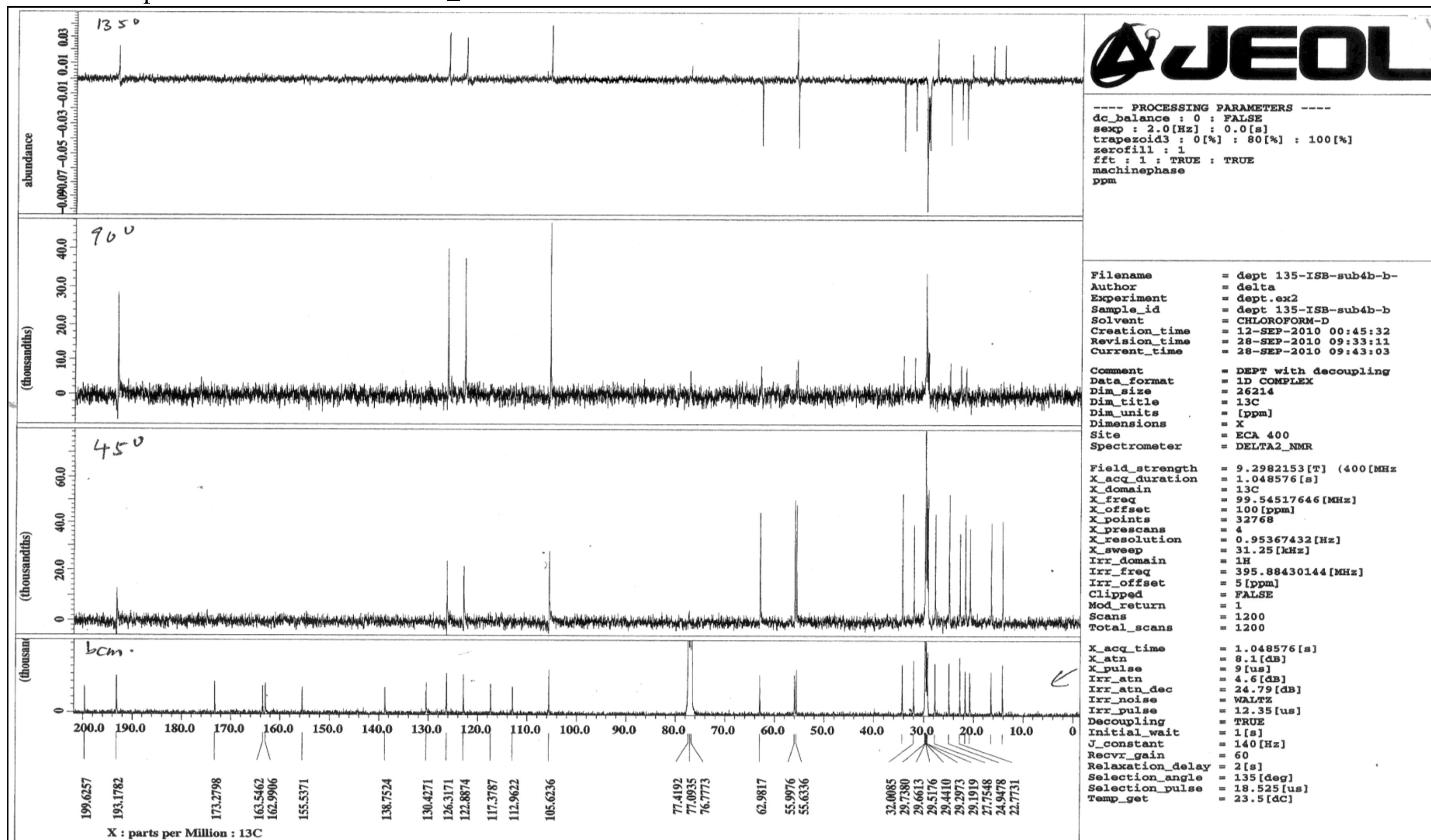
200.0, 190.0, 180.0, 170.0, 160.0, 150.0, 140.0, 130.0, 120.0, 110.0

199.6257, 193.1782, 173.2798, 163.5462, 162.9906, 155.5371, 138.7524, 130.4271, 126.3171, 122.8874, 117.3787, 112.9622, 105.6236

X : parts per Million : 13C

APPENDIX 19

DEPT NMR spectrum of subfraction sub4b_4



abundance

-0.09 -0.07 -0.05 -0.03 -0.01 0.01 0.03

(thousandths)

0 10.0 20.0 30.0 40.0

(thousandths)

0 20.0 40.0 60.0

(thousand

0 2.0 4.0

200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0

199.6257 193.1782 173.2798 163.5462 162.9906 155.5371 138.7524 130.4271 126.3171 122.8874 117.3787 112.9622 105.6236

X : parts per Million : 13C

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename           = dept 135-ISB-sub4b-b-
Author             = delta
Experiment         = dept.ex2
Sample_id          = dept 135-ISB-sub4b-b
Solvent            = CHLOROFORM-D
Creation_time      = 12-SEP-2010 00:45:32
Revision_time      = 28-SEP-2010 09:33:11
Current_time       = 28-SEP-2010 09:41:53

Comment            = DEPT with decoupling
Data_format        = 1D COMPLEX
Dim_size           = 26214
Dim_title          = 13C
Dim_units          = [ppm]
Dimensions         = X
Site               = ECA 400
Spectrometer       = DELTA2_NMR

Field_strength     = 9.2982153[T] (400[MHz
X_acq_duration     = 1.048576[s]
X_domain           = 13C
X_freq             = 99.54517646[MHz]
X_offset           = 100[ppm]
X_points           = 32768
X_prescans         = 4
X_resolution       = 0.95367432[Hz]
X_sweep            = 31.25[kHz]
Irr_domain         = 1H
Irr_freq           = 395.88430144[MHz]
Irr_offset         = 5[ppm]
Clipped            = FALSE
Mod_return         = 1
Scans              = 1200
Total_scans        = 1200

X_acq_time         = 1.048576[s]
X_atn              = 8.1[dB]
X_pulse            = 9[us]
Irr_atn            = 4.6[dB]
Irr_atn_dec        = 24.79[dB]
Irr_noise          = WALTZ
Irr_pulse          = 12.35[us]
Decoupling         = TRUE
Initial_wait       = 1[s]
J_constant         = 140[Hz]
Recvr_gain         = 60
Relaxation_delay   = 2[s]
Selection_angle    = 135[deg]
Selection_pulse    = 18.525[us]
Temp_get           = 23.5[dC]
```

---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Parameter	Value
Filename	= dept 135-ISB-sub4b-b-
Author	= delta
Experiment	= dept.ex2
Sample_id	= dept 135-ISB-sub4b-b
Solvent	= CHLOROFORM-D
Creation_time	= 12-SEP-2010 00:45:32
Revision_time	= 28-SEP-2010 09:33:11
Current_time	= 28-SEP-2010 09:42:33
Comment	= DEPT with decoupling
Data_format	= 1D COMPLEX
Dim_size	= 26214
Dim_title	= 13C
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 1.048576[s]
X_domain	= 13C
X_freq	= 99.54517646[MHz]
X_offset	= 100[ppm]
X_points	= 32768
X_prescans	= 4
X_resolution	= 0.95367432[Hz]
X_sweep	= 31.25[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 1200
Total_scans	= 1200
X_acq_time	= 1.048576[s]
X_atn	= 8.1[dB]
X_pulse	= 9[us]
Irr_atn	= 4.6[dB]
Irr_atn_dec	= 24.79[dB]
Irr_noise	= WALTZ
Irr_pulse	= 12.35[us]
Decoupling	= TRUE
Initial_wait	= 1[s]
J_constant	= 140[Hz]
Recvr_gain	= 60
Relaxation_delay	= 2[s]
Selection_angle	= 135[deg]
Selection_pulse	= 18.525[us]
Temp_get	= 23.5[dC]

abundance: 0.03, 0.01, −0.01, −0.03, −0.05, −0.07, −0.09

(thousandths): 0, 10.0, 20.0, 30.0, 40.0

(thousandths): 0, 20.0, 40.0, 60.0

(thousandths): 0, 10.0

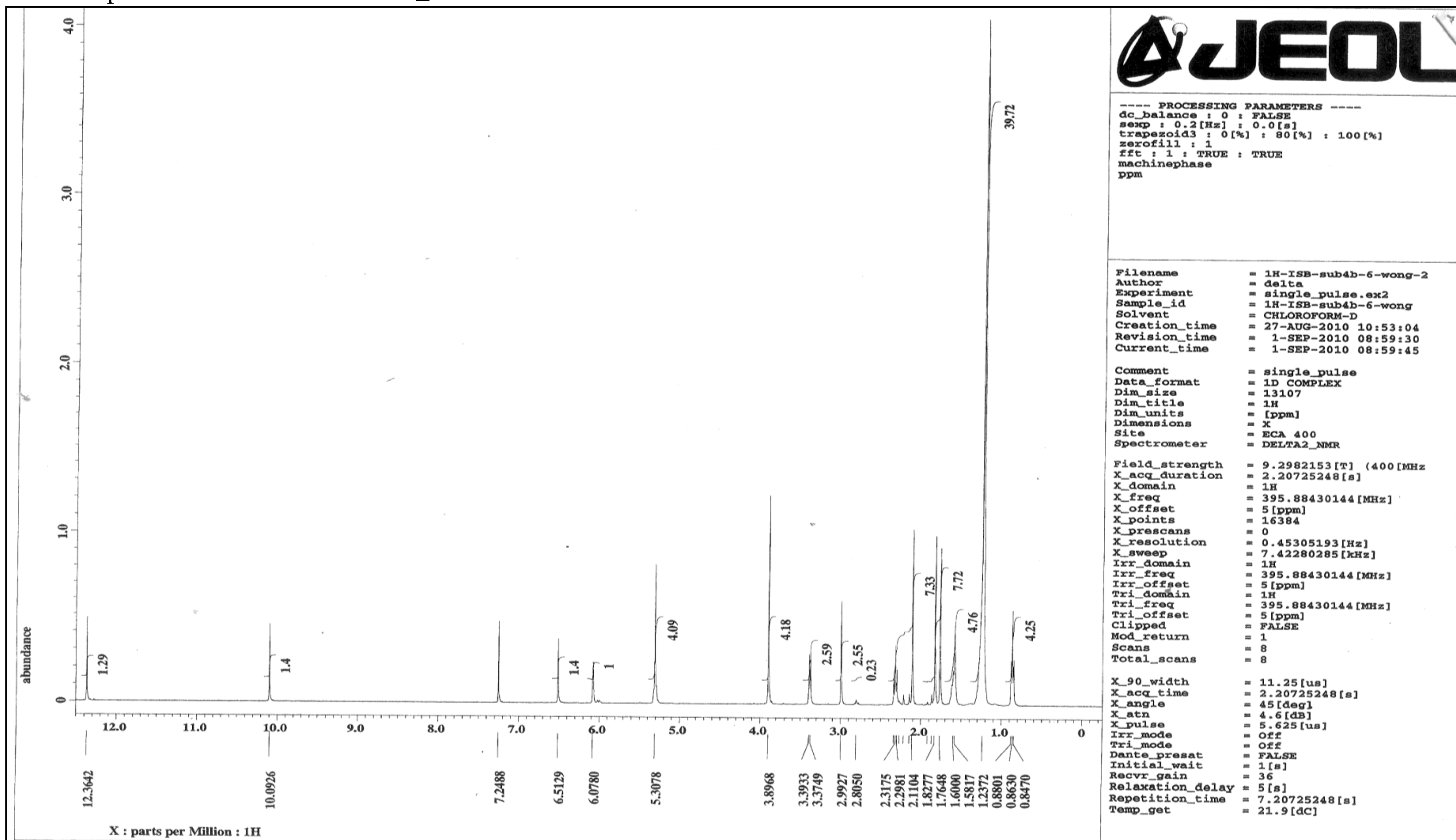
80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

77.4192, 77.0935, 76.7773, 62.9817, 55.9976, 55.6336, 34.3078, 32.0085, 29.7380, 29.6613, 29.5176, 29.4410, 29.2973, 29.1919, 27.7548, 24.9478, 22.7731, 21.6809, 20.7420, 16.4788, 14.1987

X : parts per Million : 13C

APPENDIX 20

^{1}H-NMR spectrum of subfraction sub4b_6



---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Parameter	Value
Filename	= 1H-ISB-sub4b-6-wong-2
Author	= delta
Experiment	= single_pulse.ex2
Sample_id	= 1H-ISB-sub4b-6-wong
Solvent	= CHLOROFORM-D
Creation_time	= 27-AUG-2010 10:53:04
Revision_time	= 1-SEP-2010 08:59:30
Current_time	= 1-SEP-2010 09:00:58
Comment	= single_pulse
Data_format	= 1D COMPLEX
Dim_size	= 13107
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 2.20725248[s]
X_domain	= 1H
X_freq	= 395.88430144[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.45305193[Hz]
X_sweep	= 7.42280285[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Tri_domain	= 1H
Tri_freq	= 395.88430144[MHz]
Tri_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 8
Total_scans	= 8
X_90_width	= 11.25[us]
X_acq_time	= 2.20725248[s]
X_angle	= 45[deg]
X_atn	= 4.6[dB]
X_pulse	= 5.625[us]
Irr_mode	= Off
Tri_mode	= Off
Dante_presat	= FALSE
Initial_wait	= 1[s]
Recvr_gain	= 36
Relaxation_delay	= 5[s]
Repetition_time	= 7.20725248[s]
Temp_get	= 21.9[dC]

abundance

0.7 0.6 0.5 0.4 0.3 0.2 0.1 0

1.29

1.4

12.512.4 12.3 12.2 12.1 12.0 11.9 11.8 11.7 11.6 11.5 11.4 11.3 11.2 11.1 11.0 10.9 10.8 10.7 10.6 10.5 10.4 10.3 10.2 10.1 10.0 9.9 9.8

12.3642

10.0926

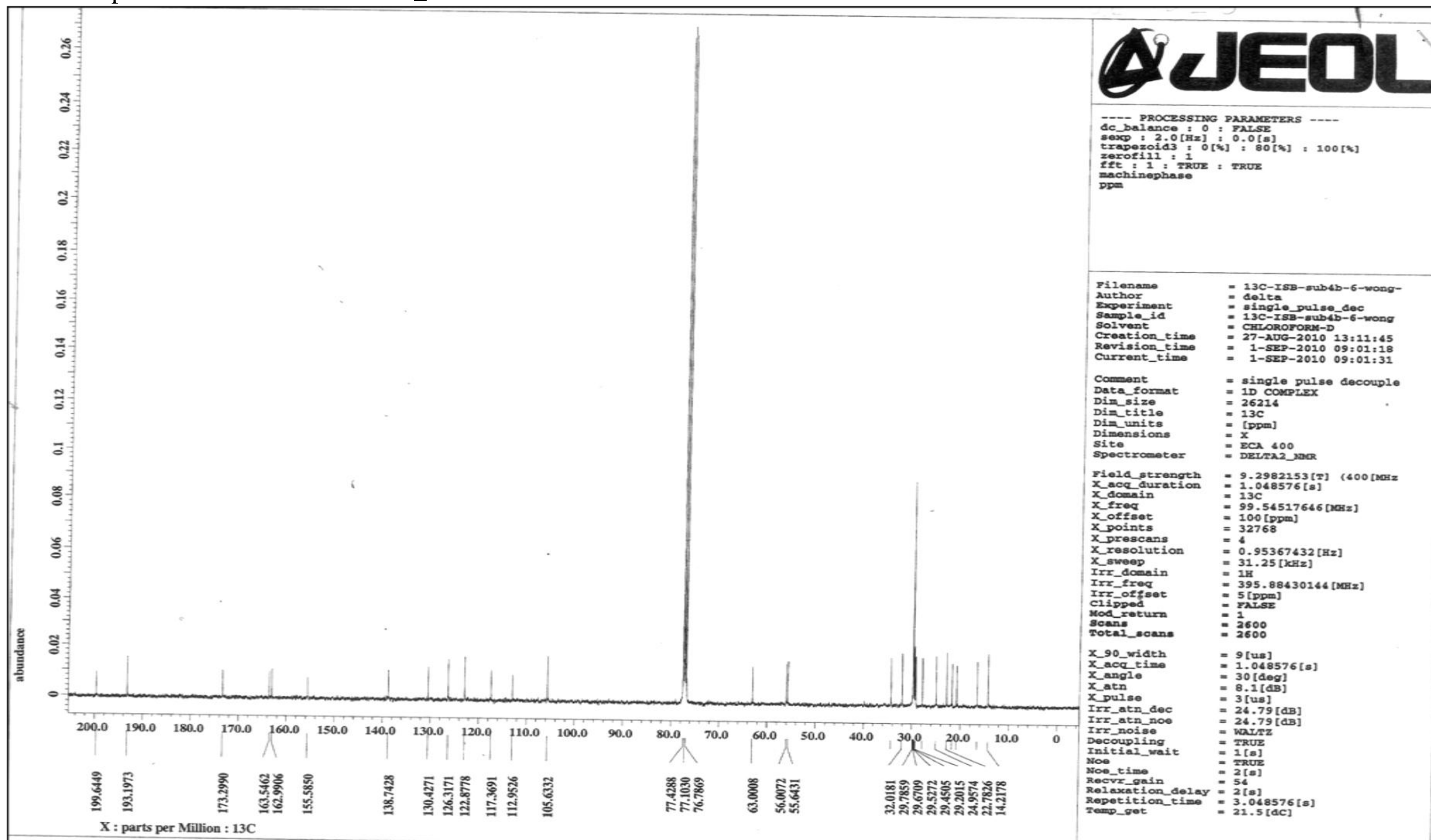
X : parts per Million : 1H

---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename	= 1H-ISB-sub4b-6-wong-2
Author	= delta
Experiment	= single_pulse.ex2
Sample_id	= 1H-ISB-sub4b-6-wong
Solvent	= CHLOROFORM-D
Creation_time	= 27-AUG-2010 10:53:04
Revision_time	= 1-SEP-2010 08:59:30
Current_time	= 1-SEP-2010 09:00:16
Comment	= single_pulse
Data_format	= 1D COMPLEX
Dim_size	= 13107
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 2.20725248[s]
X_domain	= 1H
X_freq	= 395.88430144[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.45305193[Hz]
X_sweep	= 7.42280285[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Tri_domain	= 1H
Tri_freq	= 395.88430144[MHz]
Tri_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 8
Total_scans	= 8
X_90_width	= 11.25[us]
X_acq_time	= 2.20725248[s]
X_angle	= 45[deg]
X_atn	= 4.6[dB]
X_pulse	= 5.625[us]
Irr_mode	= Off
Tri_mode	= Off
Dante_presat	= FALSE
Initial_wait	= 1[s]
Recvr_gain	= 36
Relaxation_delay	= 5[s]
Repetition_time	= 7.20725248[s]
Temp_get	= 21.9[dC]

APPENDIX 21

^{13}C NMR spectrum of subfraction sub4b_6



---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename = 13C-ISB-sub4b-6-wong-
Author = delta
Experiment = single_pulse_dec
Sample_id = 13C-ISB-sub4b-6-wong
Solvent = CHLOROFORM-D
Creation_time = 27-AUG-2010 13:11:45
Revision_time = 1-SEP-2010 09:01:18
Current_time = 1-SEP-2010 09:01:56

Comment = single pulse decouple
Data_format = 1D COMPLEX
Dim_size = 26214
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Site = ECA 400
Spectrometer = DELTA2_NMR

Field_strength = 9.2982153[T] (400[MHz
X_acq_duration = 1.048576[s]
X_domain = 13C
X_freq = 99.54517646[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 0.95367432[Hz]
X_sweep = 31.25[kHz]
Irr_domain = 1H
Irr_freq = 395.88430144[MHz]
Irr_offset = 5[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 2600
Total_scans = 2600

X_90_width = 9[us]
X_acq_time = 1.048576[s]
X_angle = 30[deg]
X_atn = 8.1[dB]
X_pulse = 3[us]
Irr_atn_dec = 24.79[dB]
Irr_atn_noe = 24.79[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Recvr_gain = 54
Relaxation_delay = 2[s]
Repetition_time = 3.048576[s]
Temp_get = 21.5[dC]

(thousandths)
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0

60.0
50.0
40.0
30.0
20.0

63.0008
56.0072
55.6431
34.3078
32.0181
29.7859
29.6709
29.5272
29.4505
29.3068
29.2015
27.7740
24.9574
22.7826
21.6809
20.7612
16.4884
14.2178

X : parts per Million : 13C

---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Filename	= 13C-ISB-sub4b-6-wong-
Author	= delta
Experiment	= single_pulse_dec
Sample_id	= 13C-ISB-sub4b-6-wong
Solvent	= CHLOROFORM-D
Creation_time	= 27-AUG-2010 13:11:45
Revision_time	= 1-SEP-2010 09:01:18
Current_time	= 1-SEP-2010 09:01:43
Comment	= single pulse decouple
Data_format	= 1D COMPLEX
Dim_size	= 26214
Dim_title	= 13C
Dim_units	= [ppm]
Dimensions	= X
Site	= ECA 400
Spectrometer	= DELTA2_NMR
Field_strength	= 9.2982153[T] (400[MHz
X_acq_duration	= 1.048576[s]
X_domain	= 13C
X_freq	= 99.54517646[MHz]
X_offset	= 100[ppm]
X_points	= 32768
X_prescans	= 4
X_resolution	= 0.95367432[Hz]
X_sweep	= 31.25[kHz]
Irr_domain	= 1H
Irr_freq	= 395.88430144[MHz]
Irr_offset	= 5[ppm]
Clipped	= FALSE
Mod_return	= 1
Scans	= 2600
Total_scans	= 2600
X_90_width	= 9[us]
X_acq_time	= 1.048576[s]
X_angle	= 30[deg]
X_atn	= 8.1[dB]
X_pulse	= 3[us]
Irr_atn_dec	= 24.79[dB]
Irr_atn_noe	= 24.79[dB]
Irr_noise	= WALTZ
Decoupling	= TRUE
Initial_wait	= 1[s]
Noe	= TRUE
Noe_time	= 2[s]
Recvr_gain	= 54
Relaxation_delay	= 2[s]
Repetition_time	= 3.048576[s]
Temp_get	= 21.5[dC]

(thousandths)

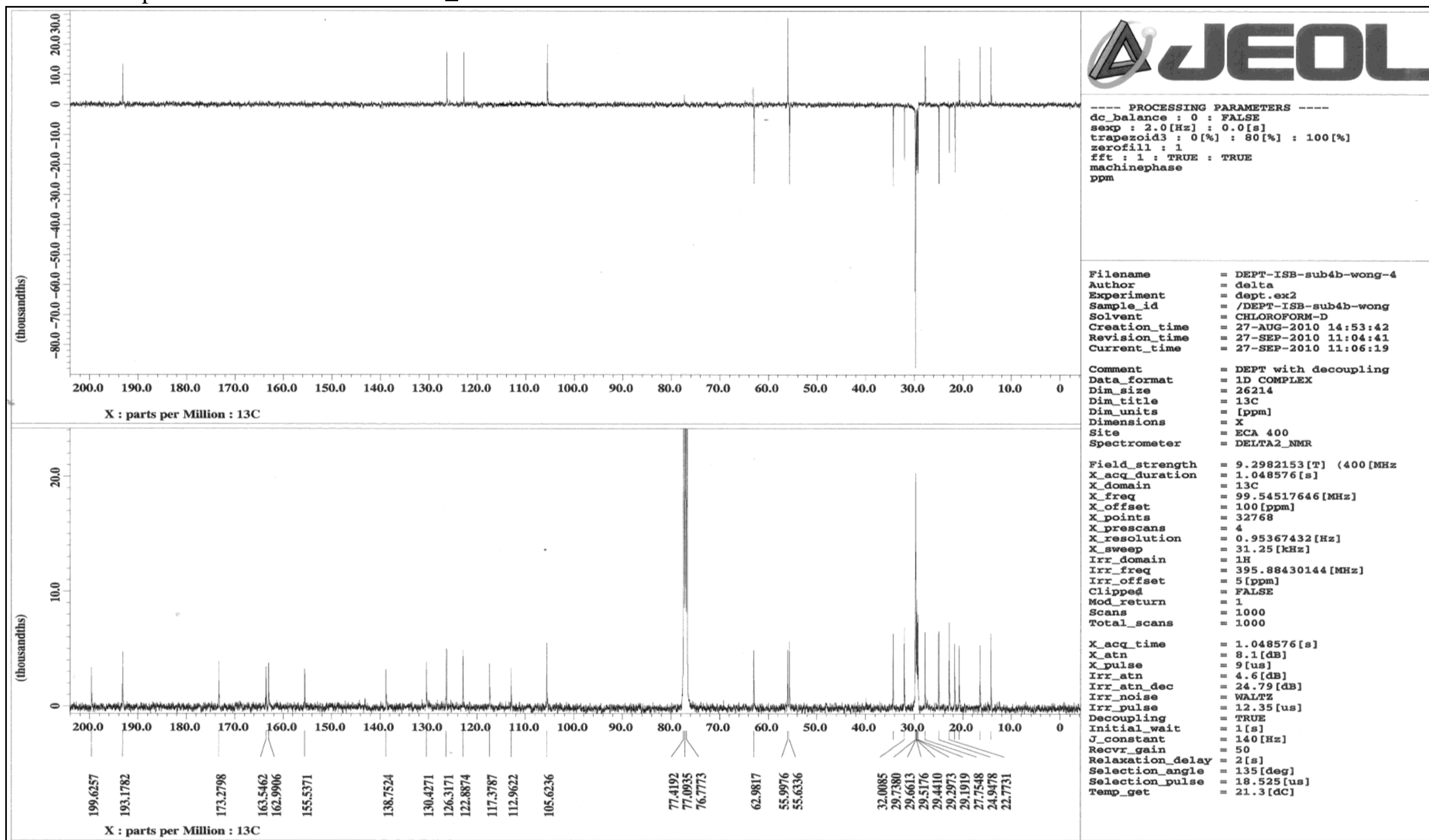
50.0 40.0 30.0 20.0 10.0 0

200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0

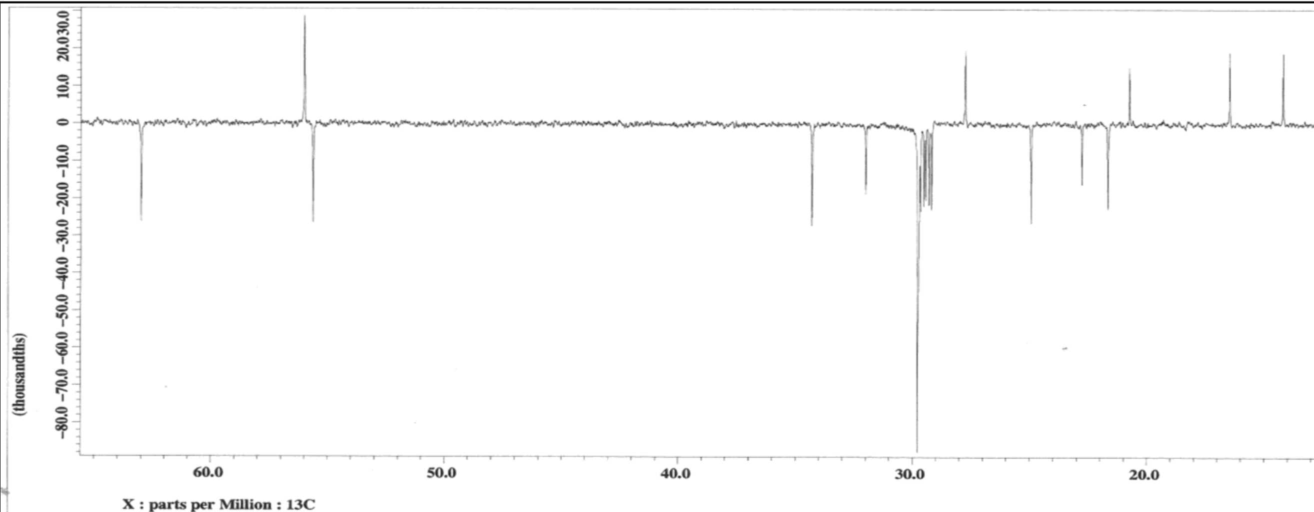
199.6449 193.1973 173.2990 163.5462 162.9906 155.5850 138.7428 130.4271 126.3171 122.8778 117.3691 112.9526 105.6332

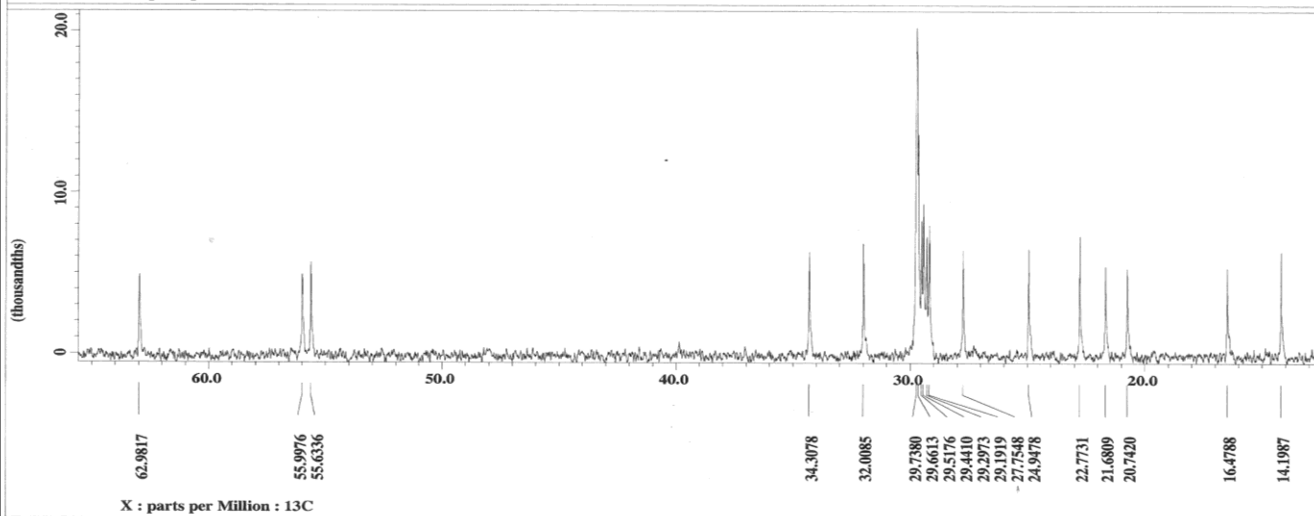
X : parts per Million : 13C

APPENDIX 22
DEPT NMR spectrum of subfraction sub4b_6

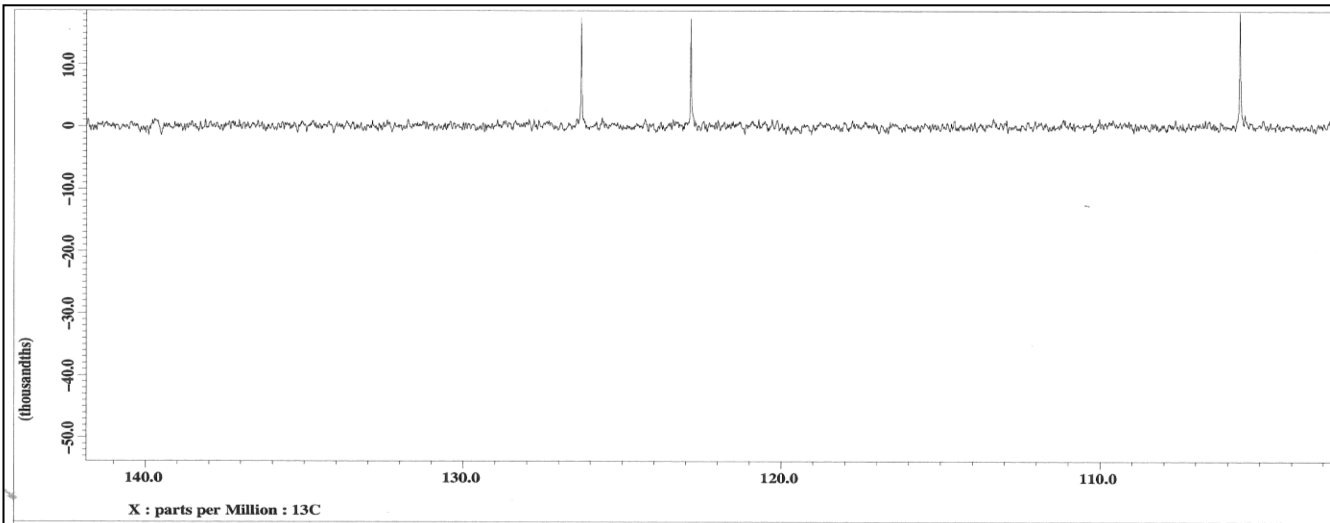


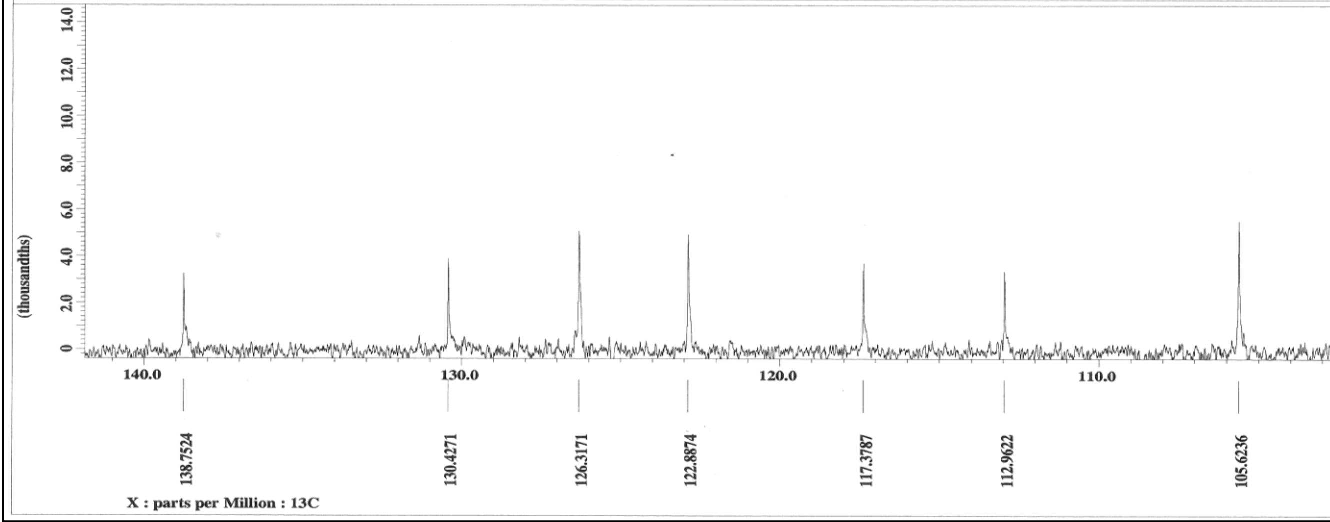
```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm
```






```
Filename           = DEPT-ISB-sub4b-wong-4
Author             = delta
Experiment         = dept.ex2
Sample_id          = /DEPT-ISB-sub4b-wong
Solvent            = CHLOROFORM-D
Creation_time      = 27-AUG-2010 14:53:42
Revision_time      = 27-SEP-2010 11:04:41
Current_time       = 27-SEP-2010 11:08:07

Comment            = DEPT with decoupling
Data_format        = 1D COMPLEX
Dim_size           = 26214
Dim_title          = 13C
Dim_units          = [ppm]
Dimensions         = X
Site               = ECA 400
Spectrometer       = DELTA2_NMR

Field_strength     = 9.2982153[T] (400[MHz
X_acq_duration     = 1.048576[s]
X_domain           = 13C
X_freq             = 99.54517646[MHz]
X_offset           = 100[ppm]
X_points           = 32768
X_prescans         = 4
X_resolution       = 0.95367432[Hz]
X_sweep            = 31.25[kHz]
Irr_domain         = 1H
Irr_freq           = 395.88430144[MHz]
Irr_offset         = 5[ppm]
Clipped            = FALSE
Mod_return         = 1
Scans              = 1000
Total_scans        = 1000

X_acq_time         = 1.048576[s]
X_atn              = 8.1[dB]
X_pulse            = 9[us]
Irr_atn            = 4.6[dB]
Irr_atn_dec        = 24.79[dB]
Irr_noise          = WALTZ
Irr_pulse          = 12.35[us]
Decoupling         = TRUE
Initial_wait       = 1[s]
J_constant         = 140[Hz]
Recvr_gain         = 50
Relaxation_delay   = 2[s]
Selection_angle    = 135[deg]
Selection_pulse    = 18.525[us]
Temp_get           = 21.3[dC]
```

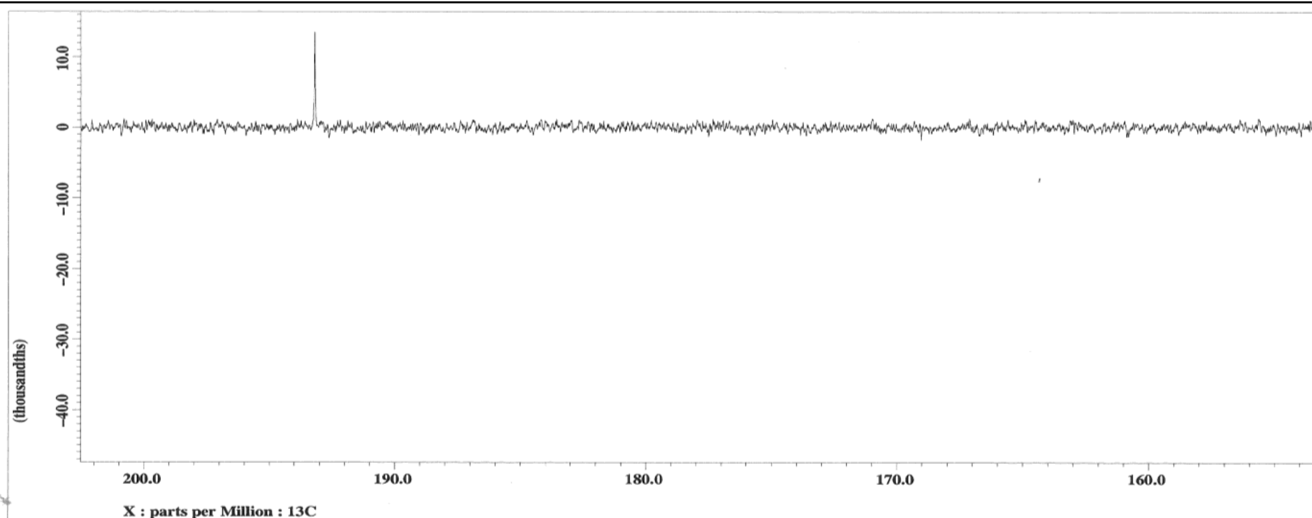




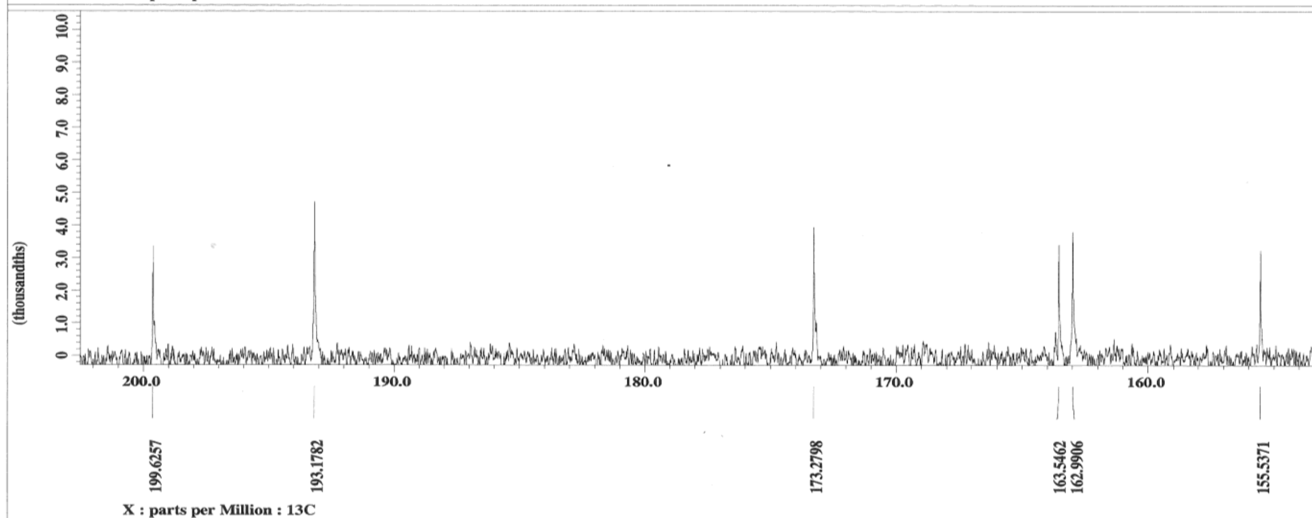
```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm
```

```
Filename          = DEPT-ISB-sub4b-wong-4
Author            = delta
Experiment        = dept.ex2
Sample_id         = /DEPT-ISB-sub4b-wong
Solvent           = CHLOROFORM-D
Creation_time     = 27-AUG-2010 14:53:42
Revision_time     = 27-SEP-2010 11:04:41
Current_time      = 27-SEP-2010 11:06:53

Comment           = DEPT with decoupling
Data_format       = 1D COMPLEX
Dim_size          = 26214
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = ECA 400
Spectrometer      = DELTA2_NMR

Field_strength    = 9.2982153[T] (400[MHz
X_acq_duration    = 1.048576[s]
X_domain          = 13C
X_freq            = 99.54517646[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.95367432[Hz]
X_sweep           = 31.25[kHz]
Irr_domain        = 1H
Irr_freq          = 395.88430144[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 1000
Total_scans       = 1000

X_acq_time        = 1.048576[s]
X_atn             = 8.1[dB]
X_pulse           = 9[us]
Irr_atn           = 4.6[dB]
Irr_atn_dec       = 24.79[dB]
Irr_noise         = WALTZ
Irr_pulse         = 12.35[us]
Decoupling        = TRUE
Initial_wait      = 1[s]
J_constant        = 140[Hz]
Recvr_gain        = 50
Relaxation_delay  = 2[s]
Selection_angle   = 135[deg]
Selection_pulse   = 18.525[us]
Temp_get          = 21.3[dC]
```

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm
```

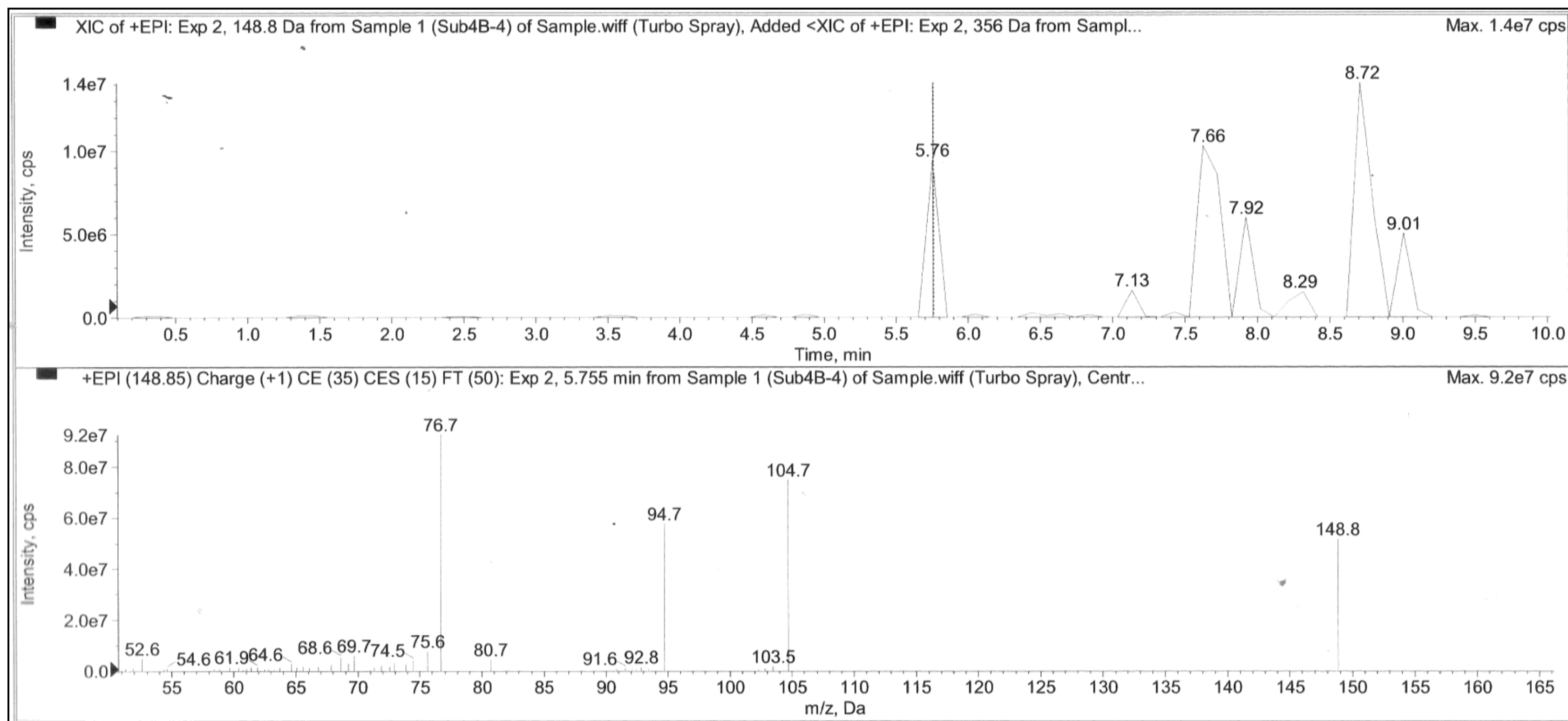



```
Filename          = DEPT-ISB-sub4b-wong-4
Author            = delta
Experiment        = dept.ex2
Sample_id         = /DEPT-ISB-sub4b-wong
Solvent           = CHLOROFORM-D
Creation_time     = 27-AUG-2010 14:53:42
Revision_time     = 27-SEP-2010 11:04:41
Current_time      = 27-SEP-2010 11:06:33

Comment           = DEPT with decoupling
Data_format       = 1D COMPLEX
Dim_size          = 26214
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = ECA 400
Spectrometer      = DELTA2_NMR

Field_strength    = 9.2982153[T] (400[MHz
X_acq_duration    = 1.048576[s]
X_domain          = 13C
X_freq            = 99.54517646[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.95367432[Hz]
X_sweep           = 31.25[kHz]
Irr_domain        = 1H
Irr_freq          = 395.88430144[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 1000
Total_scans       = 1000

X_acq_time        = 1.048576[s]
X_atn             = 8.1[dB]
X_pulse           = 9[us]
Irr_atn           = 4.6[dB]
Irr_atn_dec       = 24.79[dB]
Irr_noise         = WALTZ
Irr_pulse         = 12.35[us]
Decoupling        = TRUE
Initial_wait      = 1[s]
J_constant        = 140[Hz]
Recvr_gain        = 50
Relaxation_delay  = 2[s]
Selection_angle   = 135[deg]
Selection_pulse   = 18.525[us]
Temp_get          = 21.3[dC]
```



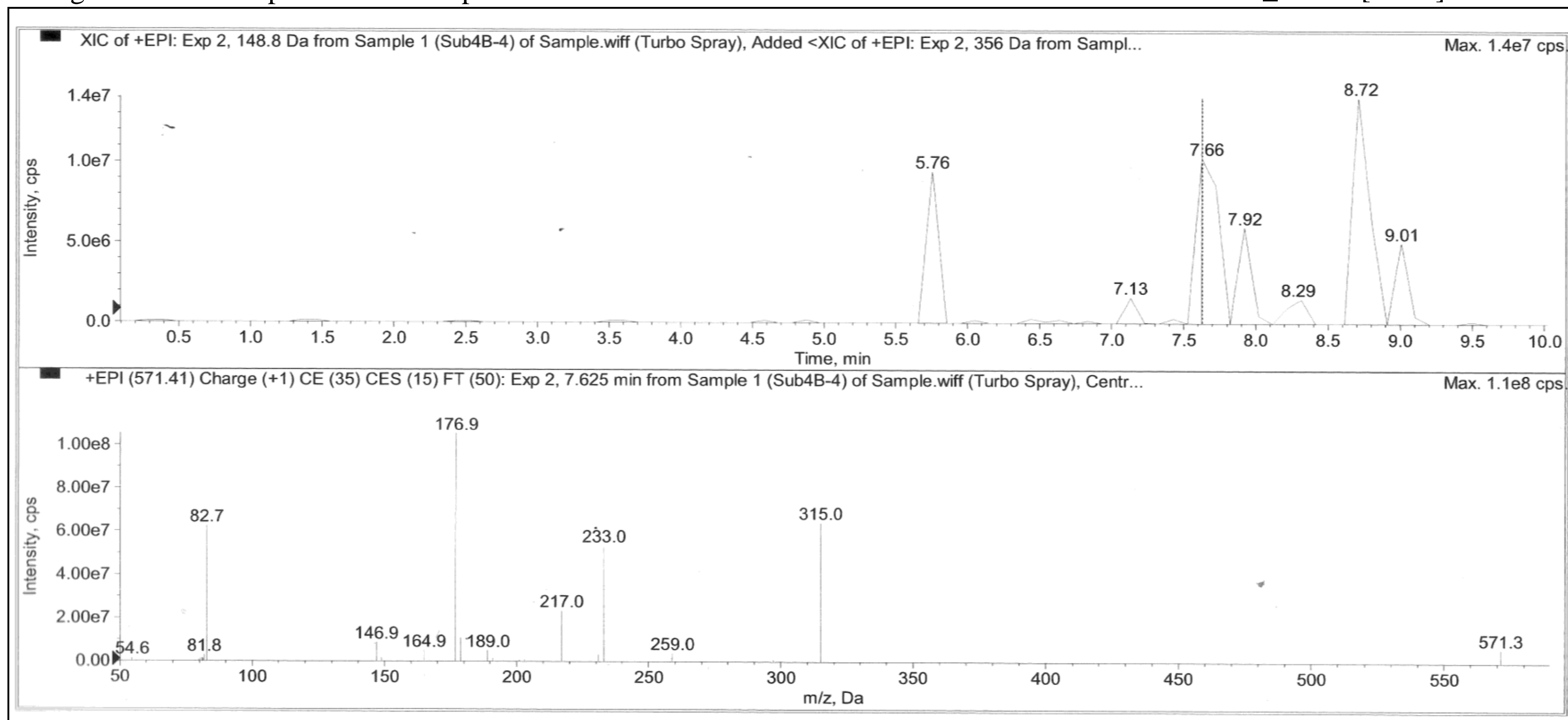
APPENDIX 23

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 5.76 min in subfraction sub4b_4 with $[M+H]^+$ of 148.8



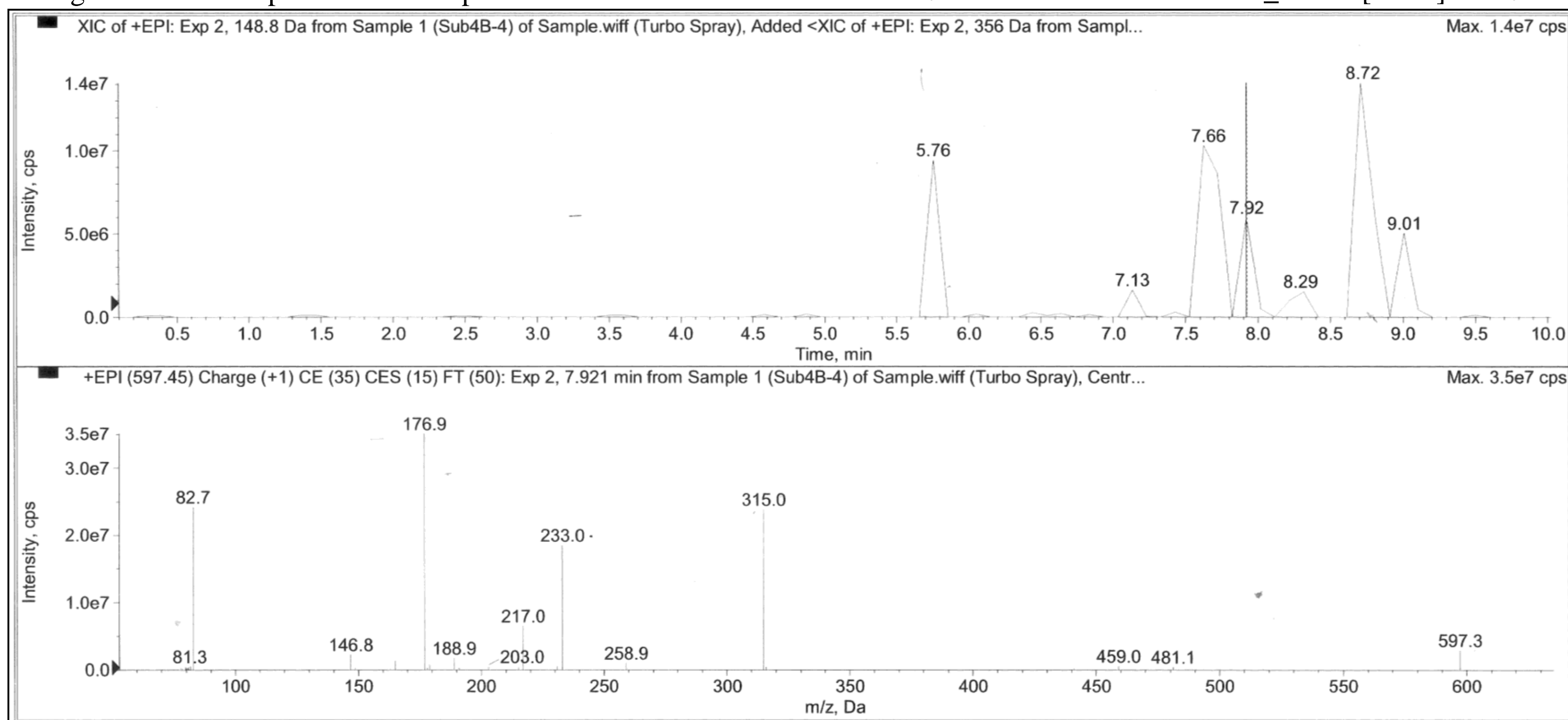
APPENDIX 24

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 7.66 min in subfraction sub4b_4 with $[M+H]^+$ of 571.3



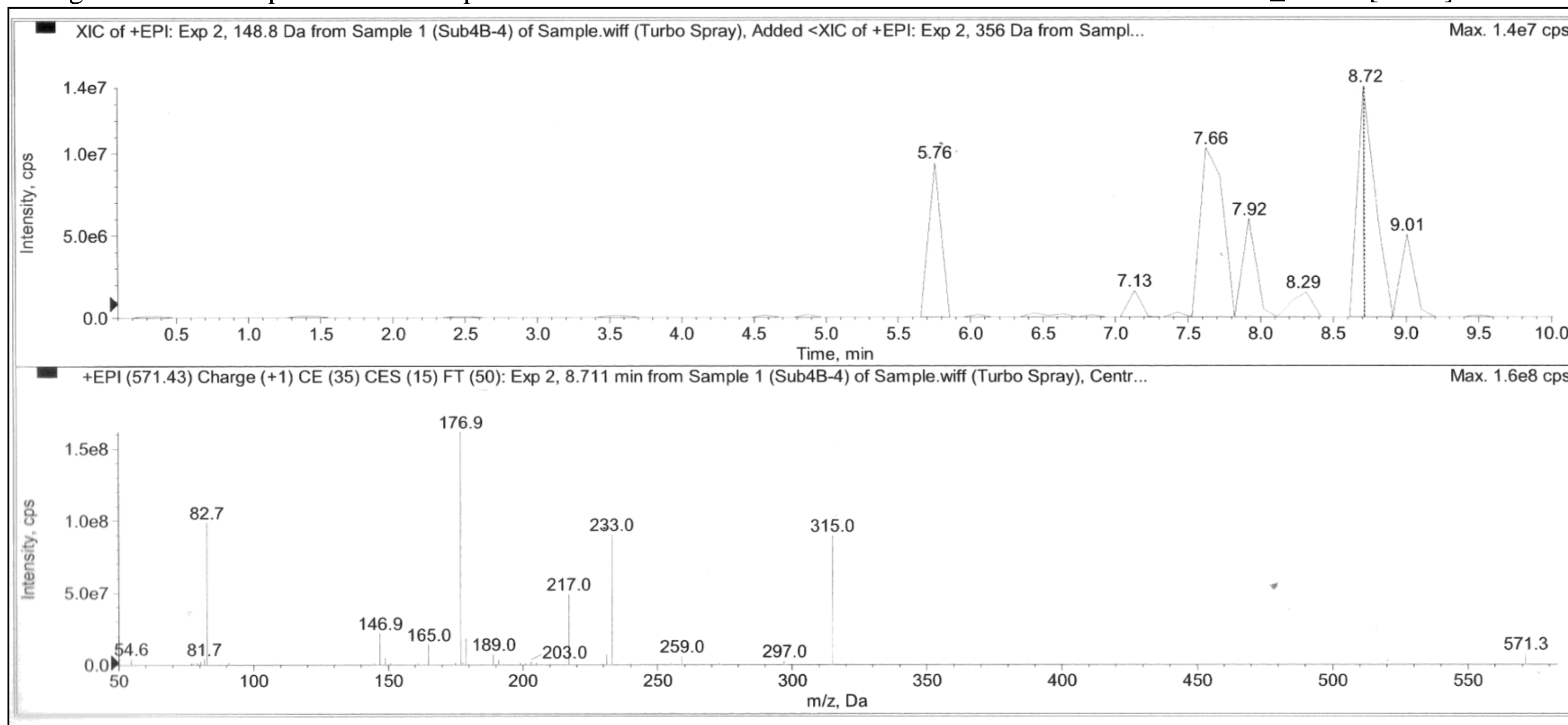
APPENDIX 25

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 7.92 min in subfraction sub4b_4 with $[M+H]^+$ of 597.3



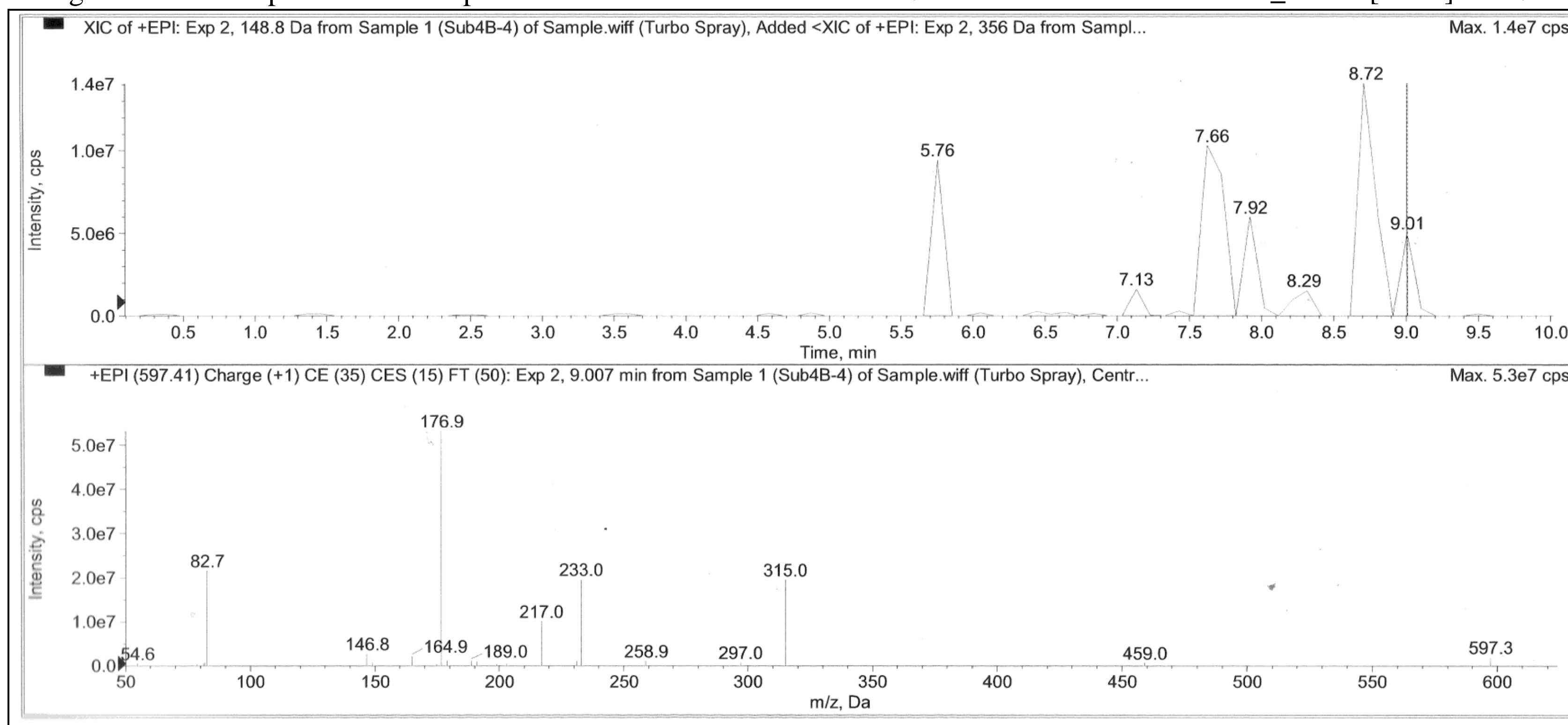
APPENDIX 26

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 8.72 min in subfraction sub4b_4 with $[M+H]^+$ of 571.3



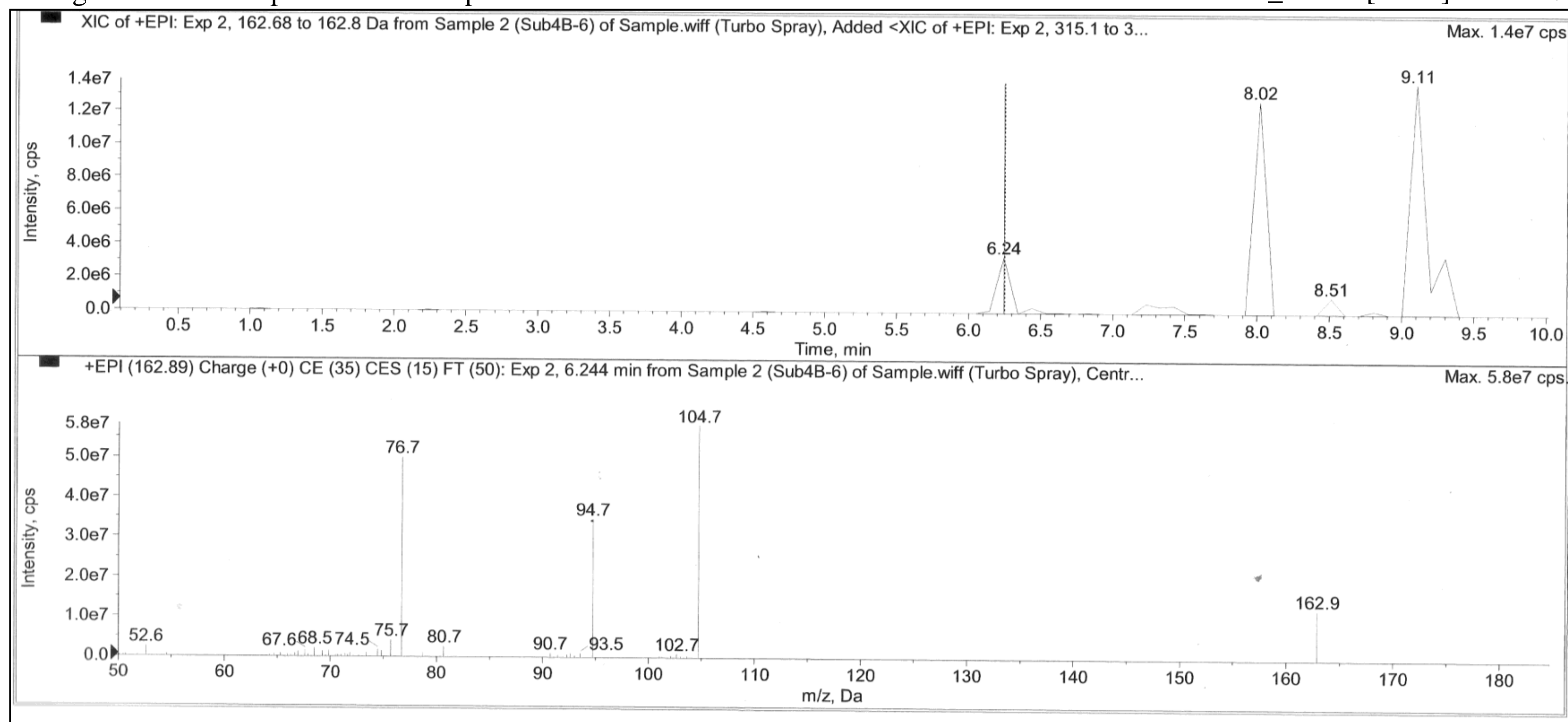
APPENDIX 27

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 9.01 min in subfraction sub4b_4 with $[M+H]^+$ of 597.3



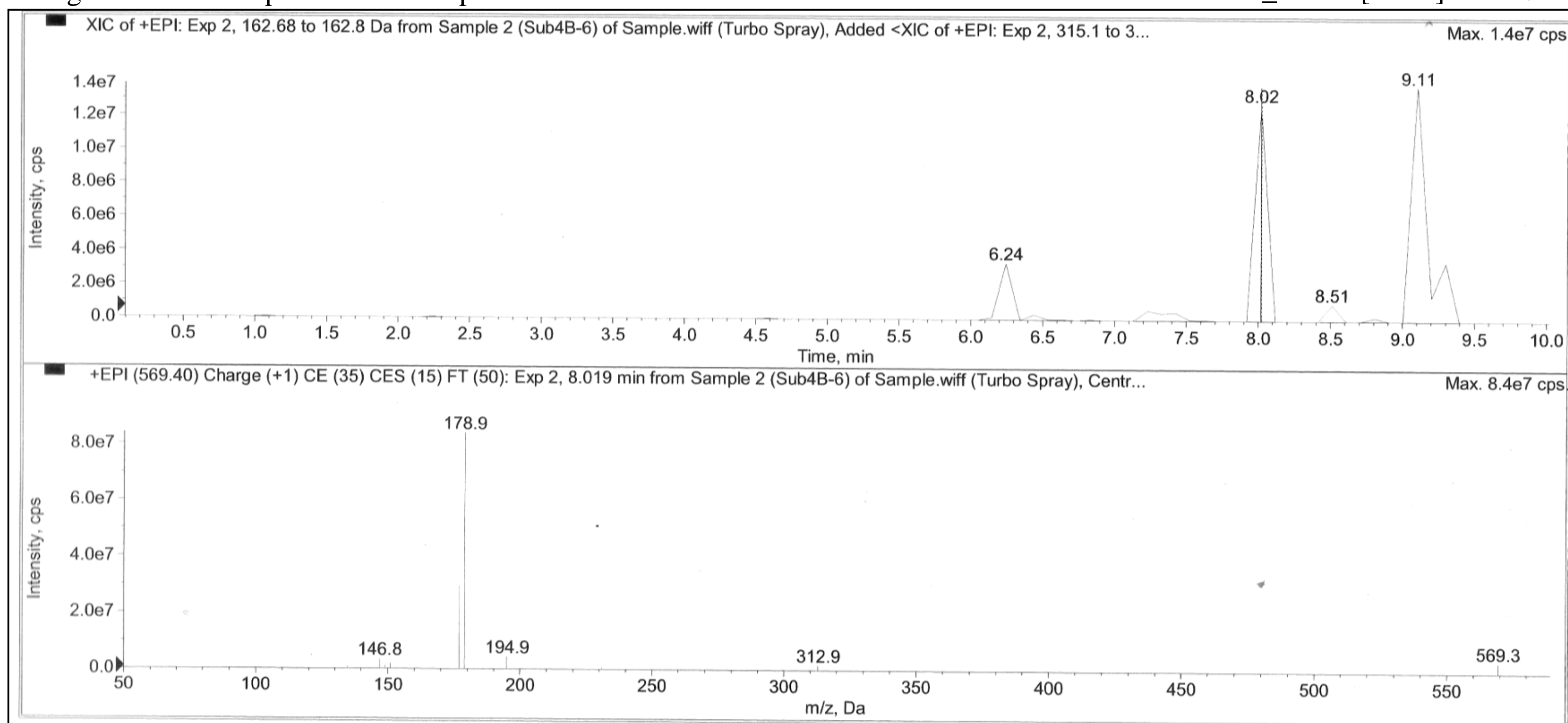
APPENDIX 28

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 6.24 min in subfraction sub4b_6 with $[M+H]^+$ of 162.9



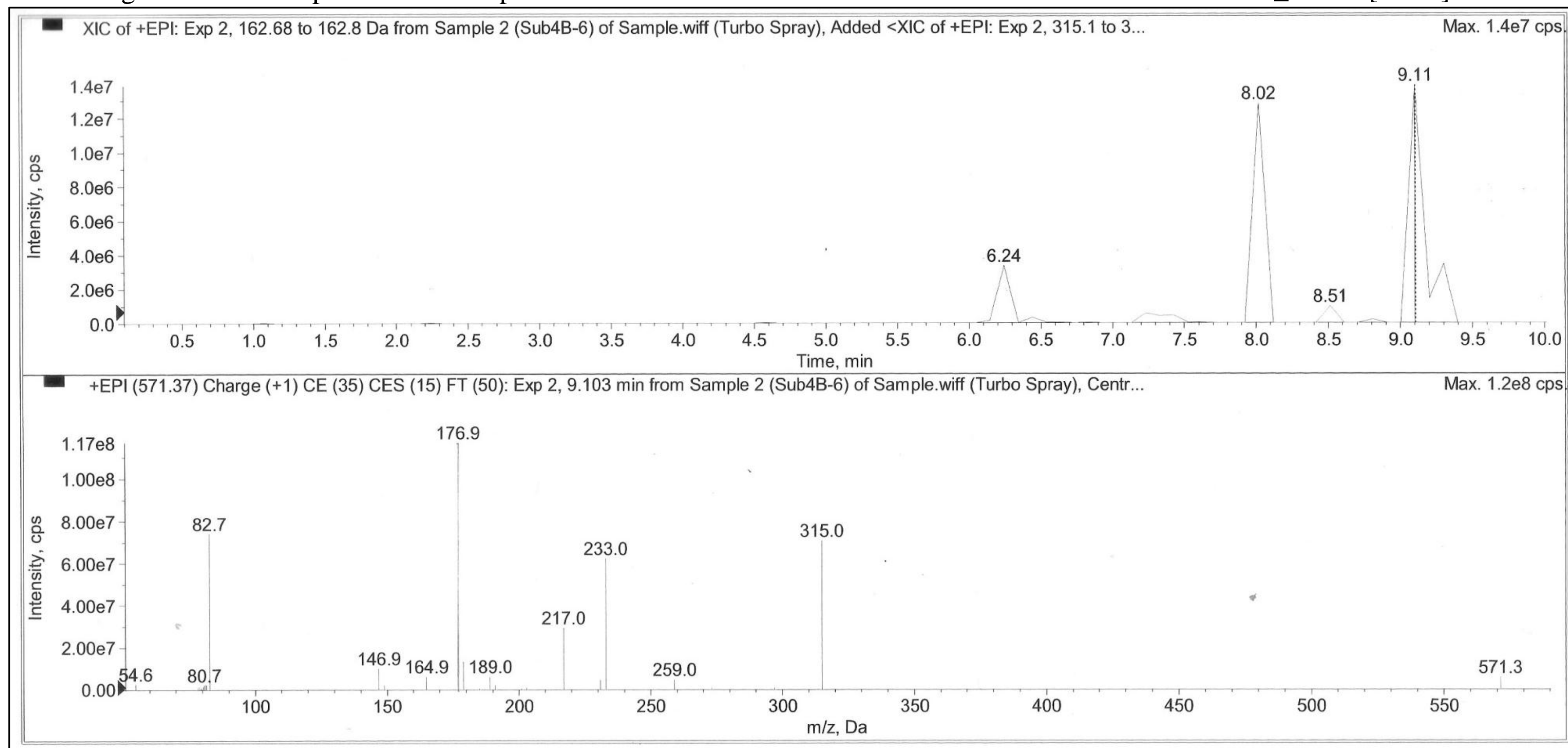
APPENDIX 29

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 8.02 min in subfraction sub4b_6 with $[M+H]^+$ of 569.3



APPENDIX 30

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 9.11 min in subfraction sub4b_6 with $[M+H]^+$ of 571.3



APPENDIX 31

Chromatogram and mass spectrum data of peak in LC/MS/MS at retention time 9.31 min in subfraction sub4b_6 with $[M+H]^+$ of 597.3