

## ***Frontier Analysis, Ltd***

### **TECHNICAL SERVICE RESPONSE NO.: UT022**

**Subject:** Addendum to Technical Service Response No.: UT016 Regarding the Analysis of Samples from a Cow Mutilated June 27, 2001 (Conrad, Montana)

**Date:** May 12, 2002

**Requested By:** Colm Kelleher  
NIDS  
Las Vegas, NV

**Reported By:** P. A. Budinger  
Analytical Scientist

### **Background/Objective:**

A mutilated Red Angus cow was found in Montana with no obvious tracks from vehicles, people, or predators around the animal. The mutilations consisted of very clean excisions of the left eye and eyelid, rectum, genitalia, and tongue. A previous Technical Service Response No.: 016 (December 10, 2001) documented the analyses of a green colored tissue from underneath the left jaw bone, vitreous fluid, and a maggot mass. A cursory examination of vitreous fluid from a control heifer was also done. Additional brain tissues samples were submitted for analysis from the Montana cow, as well as the control heifer, on February 27, 2002. The object is to look for any components that should not be normally present in the mutilated animal. An in-depth evaluation of the MS data previously obtained in November from vitreous fluid of the control heifer was completed as an aid to accomplish this objective.

### **Conclusions:**

- 1.) The brain of the Montana animal contains a low level (< 1 ppm) of oxindole. Oxindole was also reported (T.S.R. No.: 016) at much higher levels (50-100 ppm) in the vitreous fluid from the cow. This component is known to possess a sedative property. No oxindole was detected in the brain of the control heifer. The other materials detected appear to consist of the expected natural products or putrefaction products from the animal.
- 2.) Oxindole may also be a byproduct of another drug in which the "parent" molecule may not be identified by this analysis for the possible following two reasons: 1) it is

totally deteriorated; 2) the chemical nature of the drug is not amenable to detection by the analytical procedure employed<sup>1</sup>.

3.) Components such as urea and heterocyclic components are also present, which are not noted in the control heifer. Most of these are probably a result of putrefaction, which would indicate that the mutilated cow is in a more advanced state of decay. However, one may speculate that barbiturate type drugs would have degradation products that would also contribute to these putrefaction products. Barbiturates appear to be unstable in aqueous solutions<sup>2</sup> and therefore, one could surmise that they would also not exist in original form in the animal.

### **Procedure:**

**Samples:** The following samples were submitted.

**(Brain Tissue Samples)** Two samples were received in plastic vials surrounded by cold packs.

- A brain tissue sampling from the mutilated cow received 2/27/2002. On receipt the tissue appeared to be in almost a liquid consistency.

- A brain tissue from a control heifer, which was not mutilated, was submitted for reference. It was also received on 2/27/2002. The control animal, obtained from a slaughterhouse, was exposed to environmental conditions expected for mutilated animal carcass. It was laid out for 4 days, and protected from predators and scavengers.

The above samples were extracted with methylene chloride. Solvent was added to the "as received" sample, and it was allowed to soak for 8 days in the refrigerator. The sample was subjected to ultrasonic agitation for approximately one hour a day. The solvent was not completely removed and reduced to 2 mls. Both GC/MS and infrared analyses were then done to characterize the extracts.

### **(Vitreous Fluid)**

- Vitreous fluids from the left and right eye were additionally submitted from a control heifer for reference on 11/13/2001. (See background on control animal above.) Both had been examined "as received" by GC/MS. A more in-depth examination/interpretation was completed on this previously acquired data and is reported in this report. It is to be used as a "benchmark" to possible future samples.

The detailed information regarding the instrumental data acquisition conditions can be found in the appendix.

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<sup>1</sup> The chemical structure may have not been amenable to GC (gas chromatography) separation because of high molecular weight and boiling point, or possibly it was in salt form such as a hydrochloride salt, sulfate etc. Also, it is possible that the drug may not have been soluble in methylene chloride used in extracting the liver tissue and the blood.

<sup>2</sup> Merck Index, Published by Merck & Co., Inc., Rahway, NJ, 10<sup>th</sup> Edition, 1983.

## **Results:**

The results of the individual tests done on the brain tissues and control vitreous fluids follow. These results are summarized in the conclusions section of this report. All tables and figures referenced in this report can be found in an appendix.

### **BRAIN TISSUE**

**GC/MS Analysis:** The methylene chloride extraction removed a large amount of soluble material from the brain tissue of the mutilated cow and the control heifer. Expectedly, this analysis shows natural and degradation products predominate. Cholesterol and its derivatives are most abundant. However, a very small amount of unusual compound is uniquely observed in the extract from the mutilated cow when comparing the data from the mutilated animal and the control. This is oxindole. This molecular structure, as well as some derivatives of this structure, is known to possess a sedative property. It has a GC retention time of 17.89 minutes and is positively identified in the mass spectrum. The characteristic masses of oxindole are all present (51, 63, 78, 89, 104 and 133). Masses 104 and 133 are the strongest. The GC chromatograms of the extracts from the tissues of the mutilated cow and the control heifer are shown in figures 1 and 2. The mass spectrum along with a reference of oxindole is shown in figure 3. There are other extraneous peaks in the spectrum, which are probably due to interfering noise. These peaks appear intense because of the very low concentration of the oxindole (<1 ppm). Table I displays the MS identifications of the GC peaks for the tissue extracts from both the mutilated and control animal.

### **Infrared Analysis:**

Infrared analysis of the methylene extracts from the Montana and control animal are identical. The spectra can be found in figure 4. Detected are major amounts of ester and cholesterol components. A smaller amount of protein matter is also indicated. The ester is a glycerol derivative, which compares to references of L- $\alpha$ -phosphatidylinositol, 4,5-diphosphate sodium salt and L- $\alpha$ -phosphatidylinositol, 4-monophosphate sodium salt from bovine brain.<sup>3</sup> This material, as well as the protein, would not be detected by the above GC/MS analysis, because it “probably does not pass through a GC column”. References of cholesterol, L- $\alpha$ -phosphatidylinositol, 4,5-diphosphate sodium salt and L- $\alpha$ -phosphatidylinositol, 4-monophosphate sodium salt are shown for comparison in figures 5 and 6.

### **VITREOUS FLUID (Mutilated Montana Cow Versus Control Heifer)**

**GC/MS Analysis:** GC chromatograms of “as received” vitreous fluids from the right and left eyes of the control heifer expectedly match each other. The graphs show a plethora of components. The MS identifications are a good reference to natural and

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<sup>3</sup> Roger J. Keller, “The Sigma Library of FT-IR Spectra”, Edition I, Volume 1, Sigma Chemical company, Inc., 1986, References 1542A, 1542B.

putrefaction products existing in the animal four days after euthanasia. As noted in T.S.R. No.: UT016, no oxindole was found in the control eye fluids. The ion chromatogram scans for masses of 104 and 133 from GC retention times 17:00 to 20:00 min. of the vitreous fluid from the right eye of the control animal would be expected at an identical retention times if oxindole is present. This is not observed. Oxindole was uniquely identified at 50-100 ppm in the vitreous fluid from the mutilated cow. Table II lists the MS identifications of the control heifer GC peaks along with those of the eye fluid from the mutilated animal, which are reported in T.S.R. No.: 016 to conveniently compare the data. The GC chromatograms are displayed in figures 7 and 8.

**Acknowledgments:** I wish to thank and acknowledge Richard L. Wilson for the GC/MS analysis

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Distribution:

R. L. Wilson

## APPENDIX

## Instrumental Data Acquisitions Conditions

**Infrared:** Both transmittance and reflectance infrared spectra were obtained from the samples using a Nicolet Avatar 360 spectrometer. Transmittance spectra were obtained from smears on KBr crystals. Reflectance spectra were acquired using the Harrick SplitPea<sup>®</sup> sampling accessory.

**GC/MS:** A Hewlett-Packard GC/MS (DOS-MSD/ChemStation) employing a 6890 gas chromatography, 5973 Mass selective detector and capillary injection system was used for analysis. Chromatographic separation was accomplished by using a 60m x 0.32mm i.d., 1.0 mm film thickness DB-1 capillary column from J&W Scientific (sn 0433924; Cat # 123-1063). The following GC/MS conditions were used:

Instrument:	GC/MS-4
Injector Temp:	Inj. 300°C
GC Oven Program:	50°C (0.0 min.) to 290°C @ 10.0°C/min. (36.0 min.)
Injection Volume:	1.0 µl, splitless
Run Time:	60.6 min.
MS Run Type:	Scan
Mass Range:	25-600 Da; Scan threshold: 100
Scan Start Time:	0 min.
Sampling:	No.=5
Multiplier Volt.:	Emv offset=200; resulting volt.=1490
Method File:	RWSVM.M
Tune File:	ATUNE.U

**TABLE I**  
**GC/MS Data from Methylene Chloride Extractions of Brain Samples from the Mutilated Cow and from a Control Animal**

Brain Extract of Montana Mutilated Cow			Brain Extract of Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Butanoic Acid	80	6.686	-	-	-
•Possible C6 Nitrile or Protein Fragment (4-Methyl-3-pentenitrile)	37	7.533	•M/Z Possible C6 Nitrile or Protein Fragment (4-Methyl-3-pentenitrile)	38	7.533
•Benzeneacetaldehyde, .alpha.-ethylene- (<5ppm)	91	15.150	-	-	-
•Octanoic Acid	50	16.345	-	-	-
•M/Z 104, 133 Oxindole (<1 ppm)	*	17.75	-	-	-
•MW=256 Amino Derivative (2-Amino-3,9-Dimethyl-5-dimethylamino-3H-1,3,4,6-tetrazacyclopent(E)azulene)	78	18.933	•MW=256 Amino Derivative (2-Amino-3,9-Dimethyl-5-dimethylamino-3H-1,3,4,6-tetrazacyclopent(E)azulene)	78	933
-	-	-	•MW=195 Nitrogen Compound (H-Carbazole, 9-ethyl-)	35	19.182
•MW=195 (Methyl 3-ethylamino-5-Hydroxybenzoate)	43	19.232	-	-	-
•C16-C18 Aldehyde (Tetradecanal)	96	22.070	•C16-C18 Aldehyde (Hexadecanal)	94	22.070
-	-	-	•Benzene, dodecyl-	91	22.966
•Fatty Acid (<215 ppm) (Hexadecanoic acid)	99	23.414	•C16 Acid (Hexadecanoic acid)	93	23.414
•~C18 Aldehyde (9-Octadecenal)	95	23.962	•~C18 Aldehyde (9-Octadecenal)	90	23.961
•~C18 Aldehyde (17-Octadecenal)	93	24.161	•~C18 Aldehyde (17-Octadecenal)	91	24.161
•~C18 Fatty Acid (Heptadecene-(8)-carbonic acid-(1))	97	25.306	•~C18 Fatty Acid (9-Octadecenoic acid (Z)-)	43	25.306
•~C18 Fatty Acid (Octadecanoic acid)	99	25.505	•~C18 Fatty Acid (Octadecanoic acid)	99	25.505
-	-	-	•Cholesterol Derivative [(22S,25S)-22,26-Epiminocholest-5-ene-3beta, 16 alpha-diol 16- acetate) (Muldamine)]	27	27.496
•Nitrogen Compound (Piperidine, 1,1'-methylene bis-)	18	27.546	-	-	-
•Nitrogen Compound (Decanamide, N-(2-hydroxy ethyl)-)	56	27.845	•M/Z 85 Amide (Decanamide, N-(2-hydroxy ethyl)-)	56	27.845
-	-	-	•M/Z 97 Thiophene Derivative (Thiophene, 2-decyl-)	23	30.185
•M/Z 131 (Naphthalene, 1,2,3,4-tetra hydro-1-methyl-)	36	33.570	•M/Z 131 Naphthalene Deriv. (Naphthalene, 1,2,3,4-tetra hydro-1-methyl-)	47	33.620
-	-	-	•M/Z 97 Thiophene Derivative (Thiophene, 2-butyl-)	25	34.167
•Cholesterol Derivative (Cholest-5-en-3-ol (3.beta.)-)	64	38.748	•Cholesterol Derivative (Cholest-5-en-3-ol (3.beta.)-)	12	38.748
•Cholesterol Derivative (Cholesta-3,5-diene)	78	42.083	•Cholesterol Derivative (Cholesta-7,14-diene)	42	42.083
•Cholesterol Derivative (Cholesta-3,5-diene)	99	43.477	•Cholesterol Derivative (Cholesta-3,5-diene)	99	43.477
•Cholesterol	99	53.733	•Cholesterol	99	53.783

\*Oxindole was detected in ion chromatogram scans of ions 104 and 133 between GC retention times of 6.00 - 18.40 minutes.

**TABLE II**  
**GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Mutilated Montana Cow			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Acetaldehyde	91	3.380	•Acetaldehyde	39	3.191
•Trimethylamine	86	3.589	•Methanamine, N,N-dimethyl- (Trimethylamine)	72	3.480
•Butane C <sub>4</sub> H <sub>10</sub>	4	4.077	-	-	-
•1-Propanol	72	4.326	-	-	-
•Acetic Acid	91	4.824	-	-	-
•Methyl Butanal	45	5.421	-	-	-
•Propionic Acid	93	5.969	-	-	-
•Butanoic Acid	90	7.263	-	-	-
•C6 Acid		8.159	-	-	-
Hexanoic Acid	12				
•Dimethyl Sulfone	59	9.055	-	-	-
•Butyrolctone (GBL)	83	9.254	-	-	-
-	-	-	•MW=97 C <sub>4</sub> H <sub>3</sub> NO <sub>3</sub>		10.039
			1H-Pyrrole-2.5-dione (Maleimide)	78	
•Phenol	91	10.698	•Phenol (~15 ppm)	64	10.369
•Urea	86	10.848 & 10.997 & 11.196	-	-	-
		12.142	-	-	-
•C <sub>8</sub> H <sub>16</sub> Hydrocarbon			•MW=99 C <sub>4</sub> H <sub>4</sub> NO <sub>2</sub>		12.143
1-Ethyl-3-methyl-cyclopentane	83	-	Succinimide (~21 ppm)	80	
-	-	-	-	-	-
•4-Methyl-phenol	95	12.341	-	-	-
•Amine?		12.441	-	-	-
1-Piperazineethanamine	12	-	•M/Z 44, 98 Nitrogen Compound		12.597
-	-	-	2-Pentanamine, 4-methyl-	37	
•MW=99		13.735	-	-	-
2-Piperidinone	35	-	•M/Z 112, 56 (MW=112)		13.793
-	-	-	1,4-Cyclohexanedione	38	
•MW=99		13.835	-	-	-
2-Piperidinone	50		-	-	-
•MW=114		14.581			
5-Methylhydantoin	50				
•N-Butyl-1-hexanamine	42	14.731			



**TABLE II (Continued)**  
**GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Mutilated Montana Cow			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
-	-	-	•M/Z 70 L-Proline	35	14.742
•Amine N-Ethyl-cyclopentanamine	37	15.030	-	-	-
-	-	-	•MW=114 Parabanic acid	47	15.154
•MW=98 C <sub>3</sub> H <sub>6</sub> N <sub>4</sub> Amine 4-Methyl-1,2,4-triazol-3-amine	72	15.278	-	-	-
•MW=114 C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub> 5-Methylhydantoin	83	15.577	-	-	-
•1H-Indole	93	15.926	•1H-Indole	94	15.608
-	-	-	•M/Z 98 Mepivacaine	43	15.732
•MW=112 4,5-Dihydro-6-methyl-3(2H)-pyridazinone)	32	16.324	-	-	-
•2-Methoxy-5-methyl-2,5-cyclohexadiene-1,4-dione	40	16.573	•MW=138	-	16.474
-	-	-	•MW=152 4(3H)-Pyrimidinone, 2-ethyl-3,6-dimethyl- 2-Methyl-3-(2-thienyl)-2-propenal	38 64	16.763
•M/Z 42, 98, 111 1,1'-Methylenebis-piperidine	47	16.772 to 16.822	-	-	-
-	-	-	•M/Z 100 4-Morpholinebutyric acid, .beta.-methyl- .alpha.,.alpha.-diphenyl 4,9-Decadien-2-amine, N-butyl-	42 42	17.052
•MW= 152 Aromatic Oxygenate 2-Hydroxy-5-methoxy-benzaldehyde)	43	17.120	-	-	-
•M/Z 100 Nitrogen Compound 2,4-Imidazolidinedione	64	17.419	-	-	-
-	-	-	•M/Z 98 Ketone 3-n-Butylcyclohexanone	32	17.423
•Tyramine	72	17.469	-	-	-
•MW=152 ?Oxygenate 3-Hydroxy-2-isobut-1-enylcyclopent-2-en-1-one	90	17.817	-	-	-

**TABLE II (Continued)**  
**GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Mutilated Montana Cow			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
-	-	-	•MW=166 Phenol, 3-methoxy-2,4,6-trimethyl-	30	17.959
•Oxindole (50-100 ppm)	93	18.216	-	-	-
•4-Hydroxy-3-methoxy-benzaldehyde	23	18.365	•M/Z 100, 166 Hexahydropyrimidin-2-one	40	18.496
•M/Z 165 2-Amino-1,7-dihydro-7-methyl-6H-purine-6-one	38	18.465	-	-	-
•MW=166 3-(1-Amino ethylidene)-6-methyl-1H, 3H-2, 4- pyridinedione	35	18.614	-	-	-
•M/Z 100 2-Methyl-2-butenic Acid	49	18.813	-	-	-
1-Nitroso-pyrrolidine	45	-	•M/Z 138, 180 Acetamide, N-(2-nitrophenyl)- 3-Methoxy-2-methylphenol	38 38	19.032
-	-	-	-	-	-
•Thymin	87	19.211	•M/Z 84 Glutamic Acid or Derivative L-Glutamic Acid	72	19.321
•MW=180 4-(Acetyloxy)-benzoic Acid	49	19.361	-	-	-
•Glutamic Acid	72	19.709 to 19.759	-	-	-
•MW=194 C <sub>12</sub> H <sub>18</sub> O <sub>2</sub> Lactone Type 5-Acetyl-1,3,3,4,5-pentamethylbicyclo[2.1.0] pentan-2-one	27	19.958	-	-	-
•M/Z 120 Phenylalanine Derivative L-Phenylalanine-4-nitroanilide	50	20.307	•M/Z 138, 70 Bicyclo [2.2.1]heptane-2-one, 3,3-dimethyl- Endo-6-methylbicyclo[2.2.2]octan-2-one	53 47	20.558
-	-	-	•MW=154 6,8-Diazabicyclo[3.2.2]nonane-7,9-dione 2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	35 14	20.971
•M/Z 168 Imidazo[2,1-a]isoquinoline	11	20.954	-	-	-
-	-	-			
•M/Z 123, 165 Acetanilide Derivative 3-Methoxyacetanilide	25	21.153			

**TABLE II (Continued)**  
**GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Mutilated Montana Cow			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
-	-	-	•M/Z 116, 61 Hexanoic, 2-methylpropyl ester	12	21.177
•M/Z 114, 41, 83 Amine? 3-(Hexylamine)-propanenitrile	25	21.302	-	-	-
•M/Z 116 Glutaminic Acid Derivative Glutaminic acid dimethyl ester	32	21.551	-	-	-
•M/Z 154, 70 Benzene, 2-chloro-1,3,5-trimethyl-	45	22.298	-	-	-
•M/Z 154, 70 2-Thiatricyclo[3.3.1.1.(3,7)]decane	43	22.547	-	-	-
Phenol, 3,5-dimethoxy-	40	-	-	-	-
-	-	-	•MW=154 2,4(1H,3H)-Pyrimidinedione, 1,3,6-trimethyl-	38	23.157
-	-	-	•MW=154 2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	17	23.322
•M/Z 154, 70	-	23.493	Phenol, 3,4-dimethoxy-	27	-
•M/Z 154, 70	-	23.642	-	-	-
•C18 Fatty Acid Octadecanoic Acid	91	23.891	-	-	-
-	-	-	•M/Z 186, 117 Indole Derivative Probable 1H-Indole	50	24.188
•M/Z 186 Indole Derivative Fragments for Indole itself +186	49	24.538	4-fluoro-2', methylbiphenyl	83	-
-	-	-	•M/Z 200, 117 Indole Derivative 1H-Indole	43	24.890
•M/Z 200 Indole Derivative Fragments for Indole + 200	-	25.285	-	-	-
-	-	-	•M/Z 91 Aromatic (Phenyl Group) Benzene, 1-nitro-4-(2-phenylethyl)-	35	25.467
•M/Z 91 Phenyl Component Nonylbenzene	25	25.883	Benzaldehyde, 2-hydroxy-6-methyl-4-(phenol?)	35	-
			-	-	-

**TABLE II (Continued)**  
**GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Mutilated Montana Cow			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Phenyl Component Methyl(4.alpha.)-2.alpha.,3.beta.-dihydroxy-5,5-dimethyl-11-oxatricyclo(7.2.1.0)dodecane-1.alpha.-carboxylate	27	26.082	-	-	-
-	-	-	•M/Z 70 Tetramethyl-1,2-cyclopentanedione	50	26.334
•M/Z 186 Phenoxy Component Phenol, 3-phenoxy-(1,1'-Biphenyl) 2,5-diol	43	26.530	-	-	-
-	38	-	•M/Z 186 Phenoxy Group Phenol, 3-phenoxy-	59	27.736
-	-	-	•Phenylalanine derivative Phenylalanine-proline diketopiperazine	39	27.860
•M/Z 186 Phenoxy Component Similar to 26.53. Probably oligomer of some large aromatic compound	-	28.222	-	-	-
•Cholest-5-en-3-ol	89	56.948			

5 min Delay

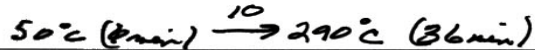


Figure 1. GC chromatogram of the methylene chloride extract from the brain tissue of the mutilated cow.

File : C:\HPCHEM\4\DATA\BSB\PB319022.D  
 Operator : [BSB1]RLW 3/19/02  
 Acquired : 19 Mar 2002 15:14 using AcqMethod RWSVM  
 Instrument : GC/MS #4  
 Sample Name: Br Extract (Control) 3/19/02  
 Misc Info : Semivol Organic Analysis 1ul Splitless EM+2  
 Vial Number: 3

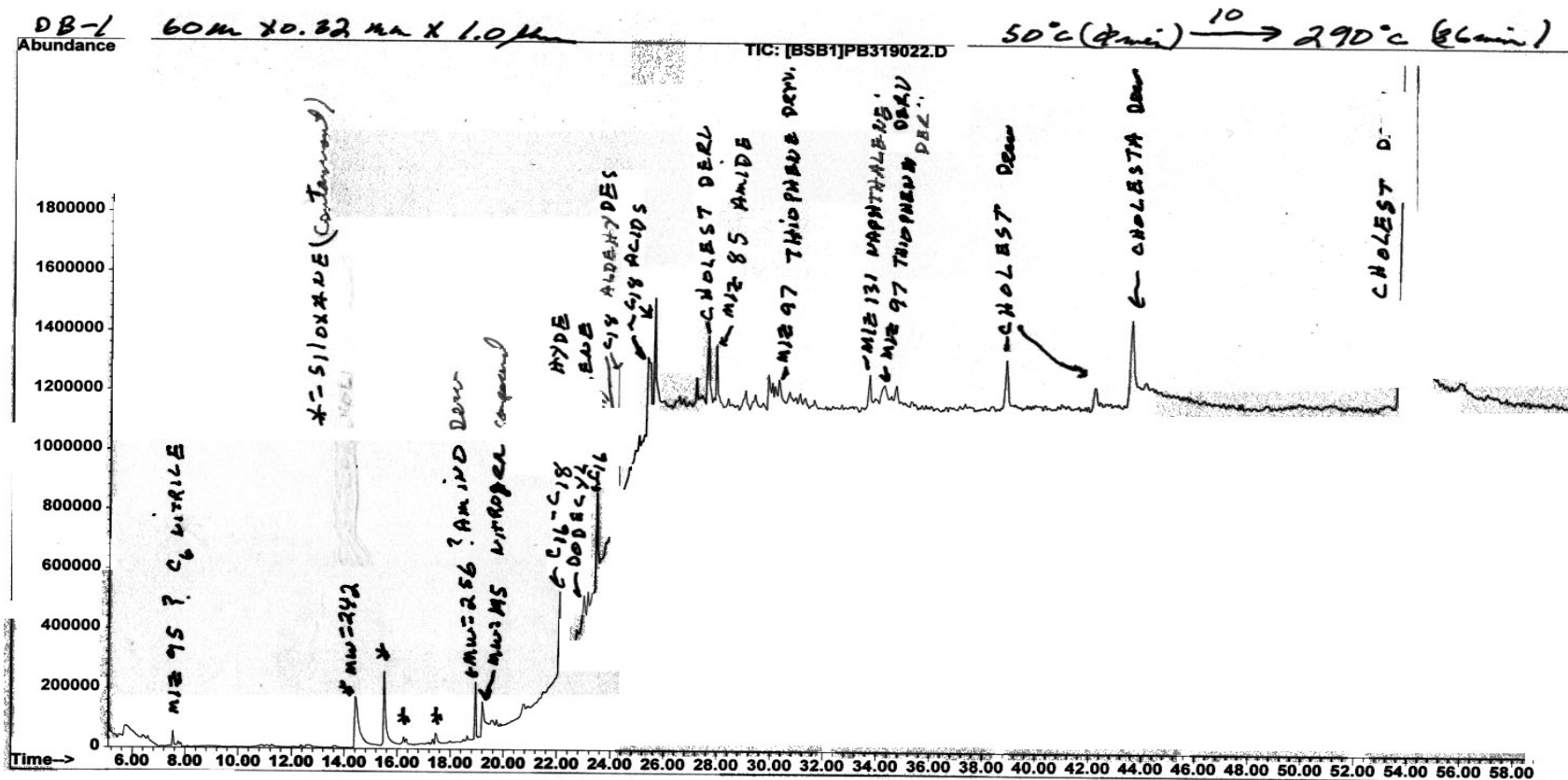


Figure 2. GC Chromatogram of the methylene chloride extract from the brain tissue of the control heifer.

File : C:\HPCHEM\4\DATA\PB319023.D  
 Operator : RLW 3/19/02  
 Acquired : 19 Mar 2002 17:32 using AcqMethod RWSVM  
 Instrument : GC/MS #4  
 Sample Name: Br Extract (Montana) 3/19/02  
 Misc Info : Semivol Organic Analysis 1ul Splitless EM+2  
 Vial Number: 5

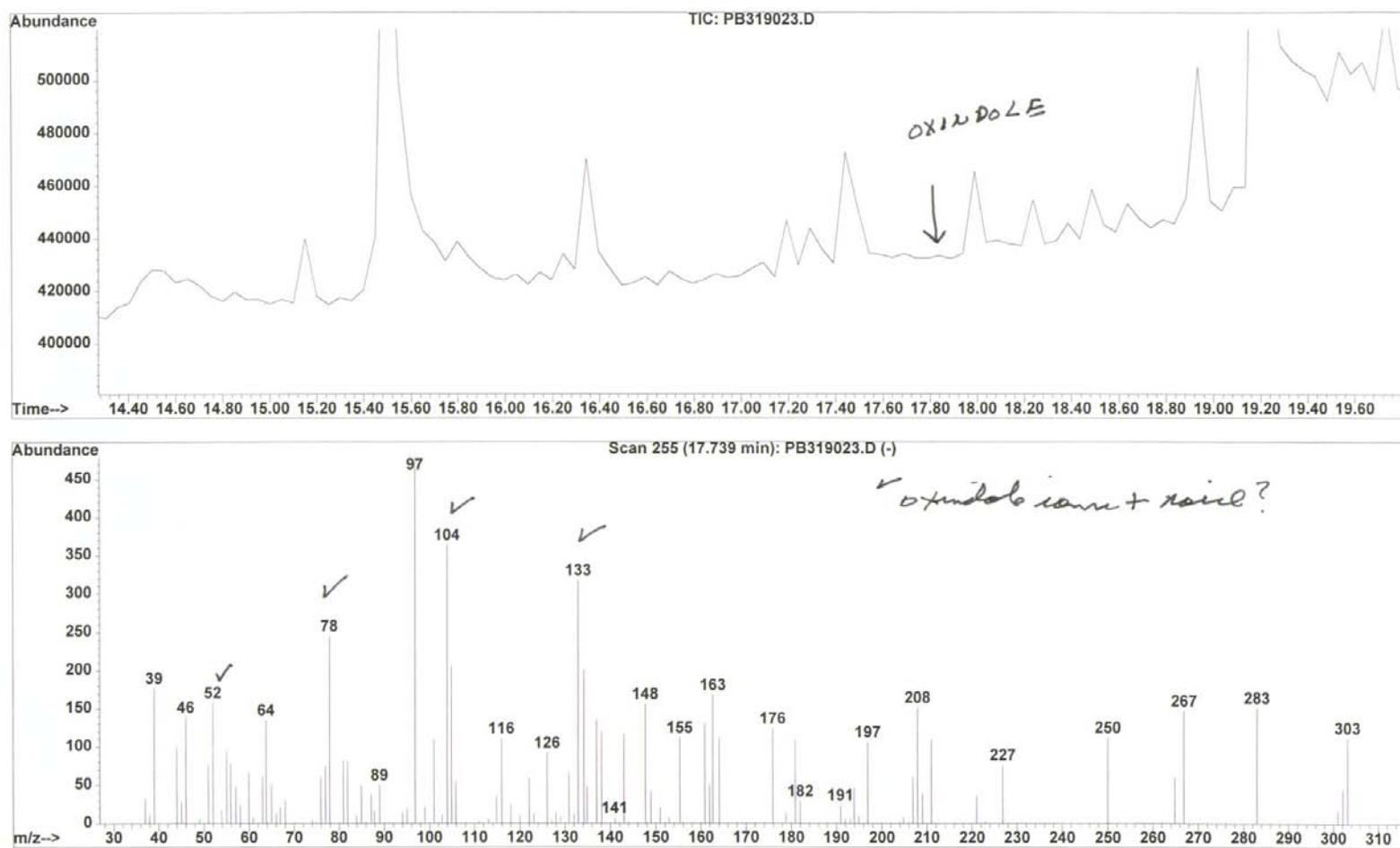


Figure 3. Expanded GC chromatogram and MS spectrum of oxindole from the brain tissue of the mutilated cow.

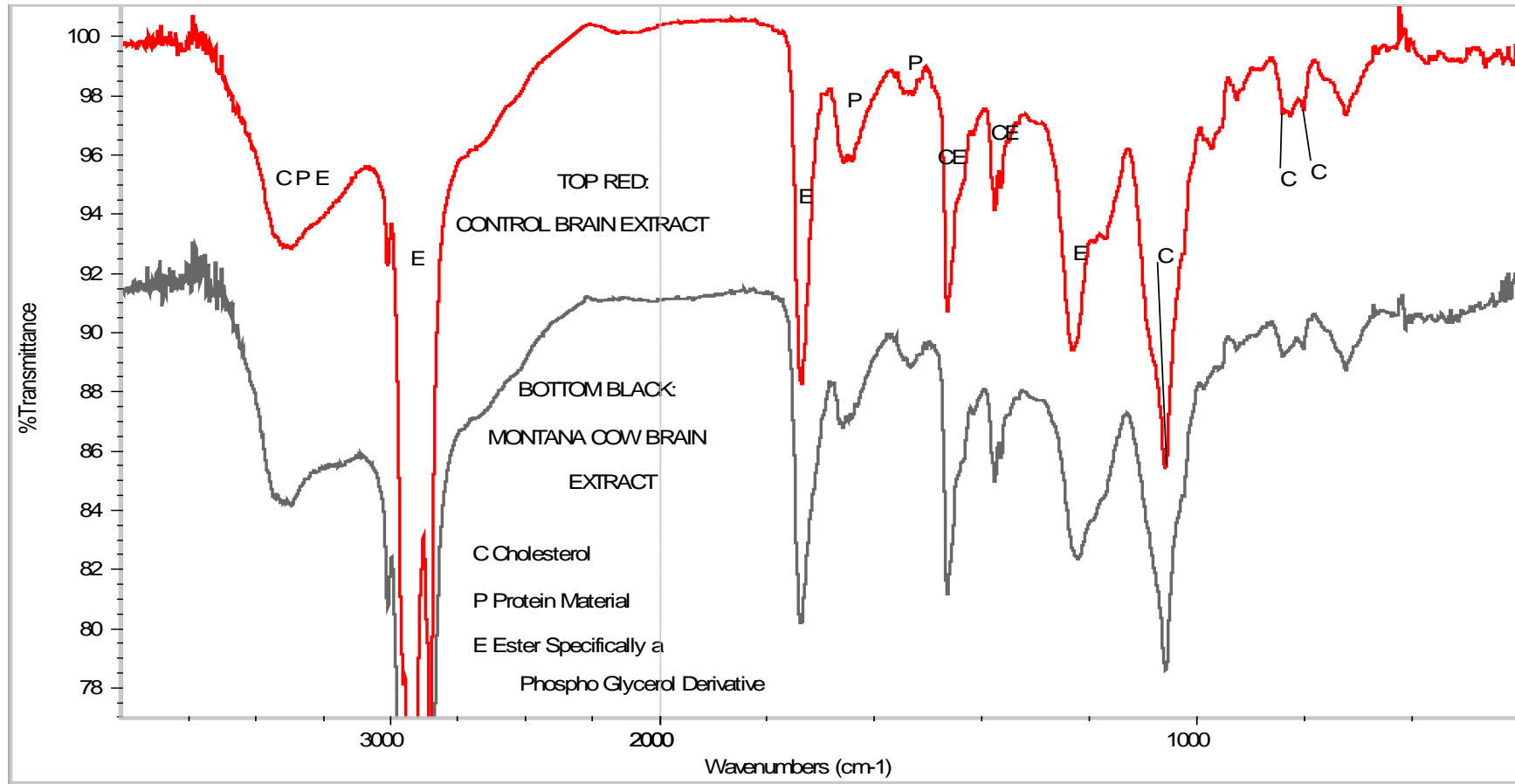


Figure 4. Infrared spectra of the methylene chloride extracts from the brains of the mutilated animal (top, red) and the control heifer (bottom, black).



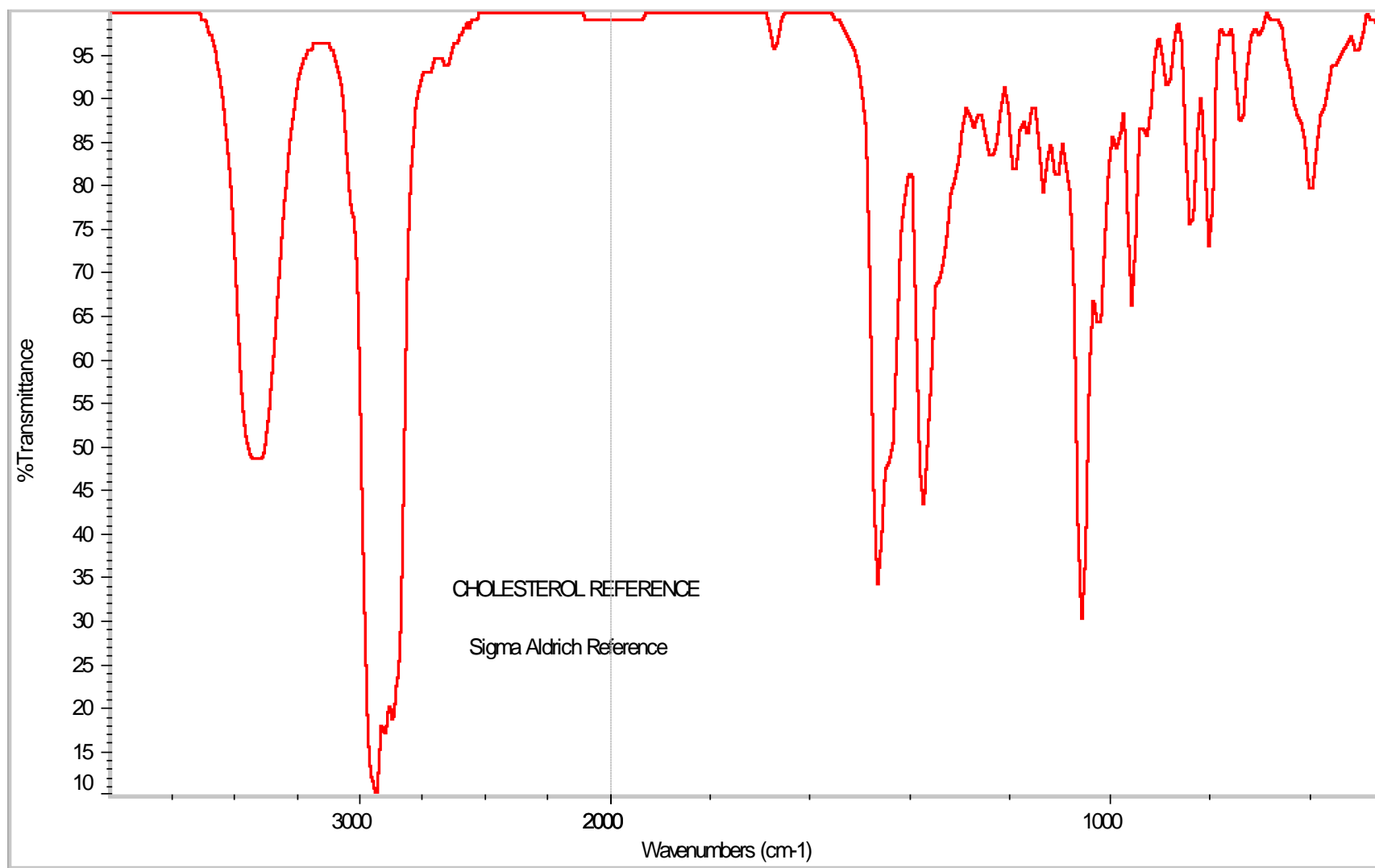


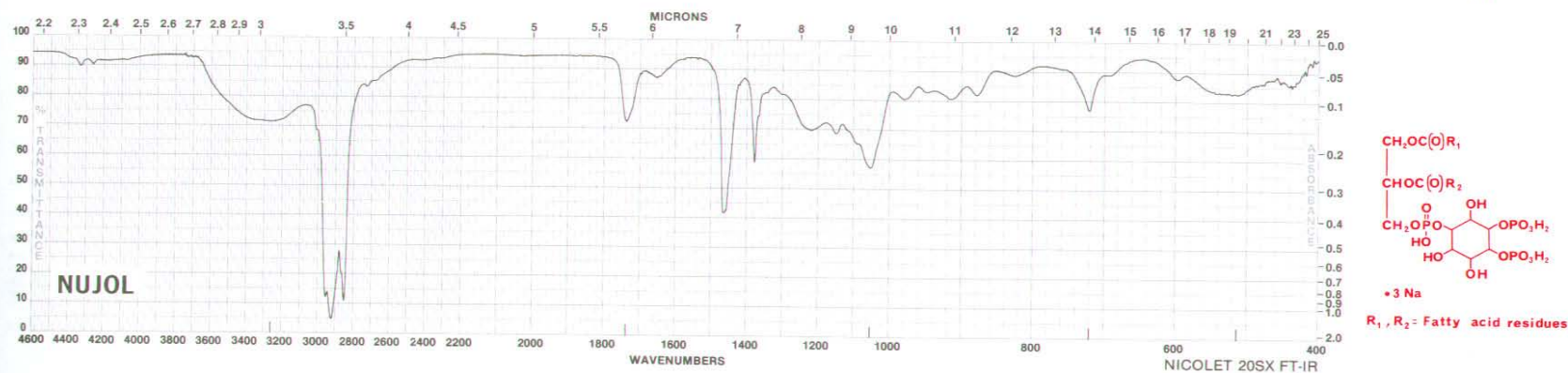
Figure 5. Infrared reference spectrum of cholesterol (Sigma-Aldrich).

## 1542

**L- $\alpha$ -PHOSPHATIDYLINOSITOL 4,5-DIPHOSPHATE**  
Sodium Salt: Approx. 98% From Bovine Brain

3262.1	721.6
1737.6	514.5
1053.5	

**A**



**L- $\alpha$ -PHOSPHATIDYLINOSITOL 4-MONOPHOSPHATE**  
Sodium Salt: Approx. 98% From Bovine Brain

4328.5	1221.3	876.1
3247.6	1047.7	721.8
1738.6	973.2	507.3

B

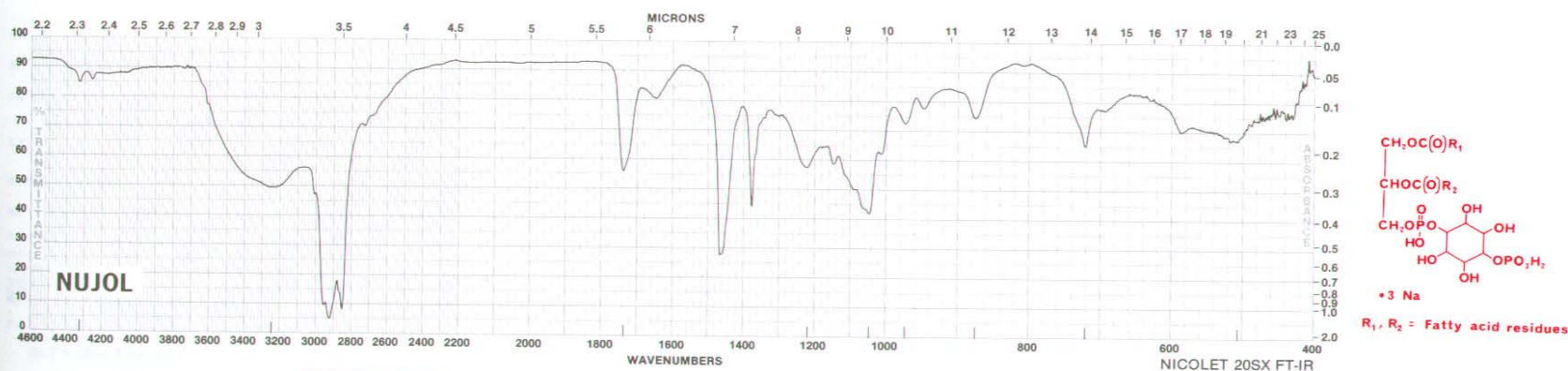
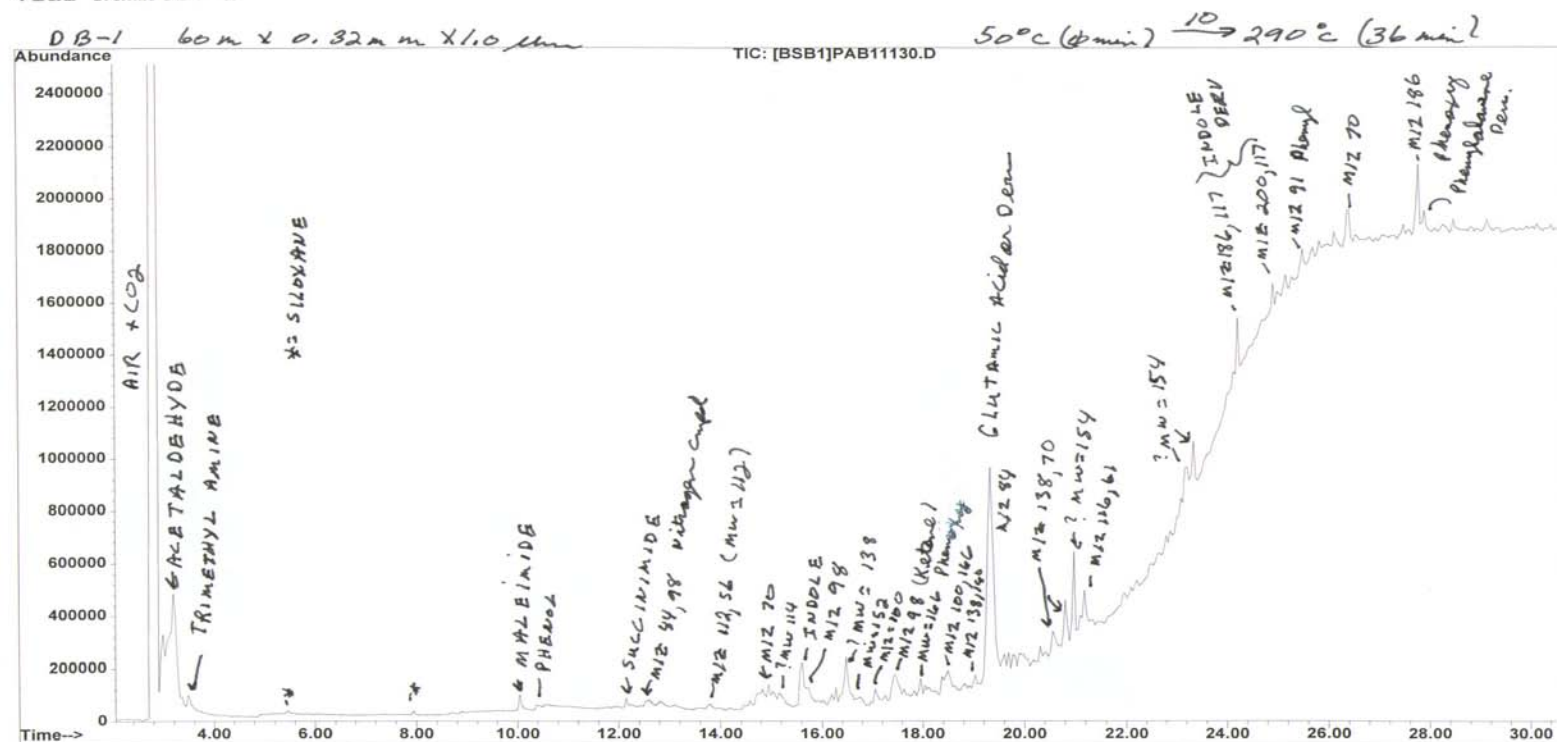


Figure 6. Infrared reference spectra of L- $\alpha$ -phosphatidylinositol, 4,5-diphosphate sodium salt and L- $\alpha$ -phosphatidylinositol, 4-monophosphate sodium salt (Sigma Aldrich).

File : C:\HPCHEM\4\DATA\BSB\PAB11130.D  
 Operator : [BSB1]RLW 11/13/01  
 Acquired : 13 Nov 2001 13:05 using AcqMethod RWSVM  
 Instrument : GC/MS #4  
 Sample Name: Left Eye Fluid 10/18/01 11/13/01  
 Misc Info : Semivol Organic Analysis 1ul splitless EM+2  
 Vial Number: 1



Control (Left vitreous fluid)

Figure 7. GC chromatogram of the vitreous fluid from the control heifer.

Col: DB-1 60m x 0.32 mm x 1.0  $\mu$ m GC Program: 50°C (4 min)  $\xrightarrow{10}$  290°C (36 min)

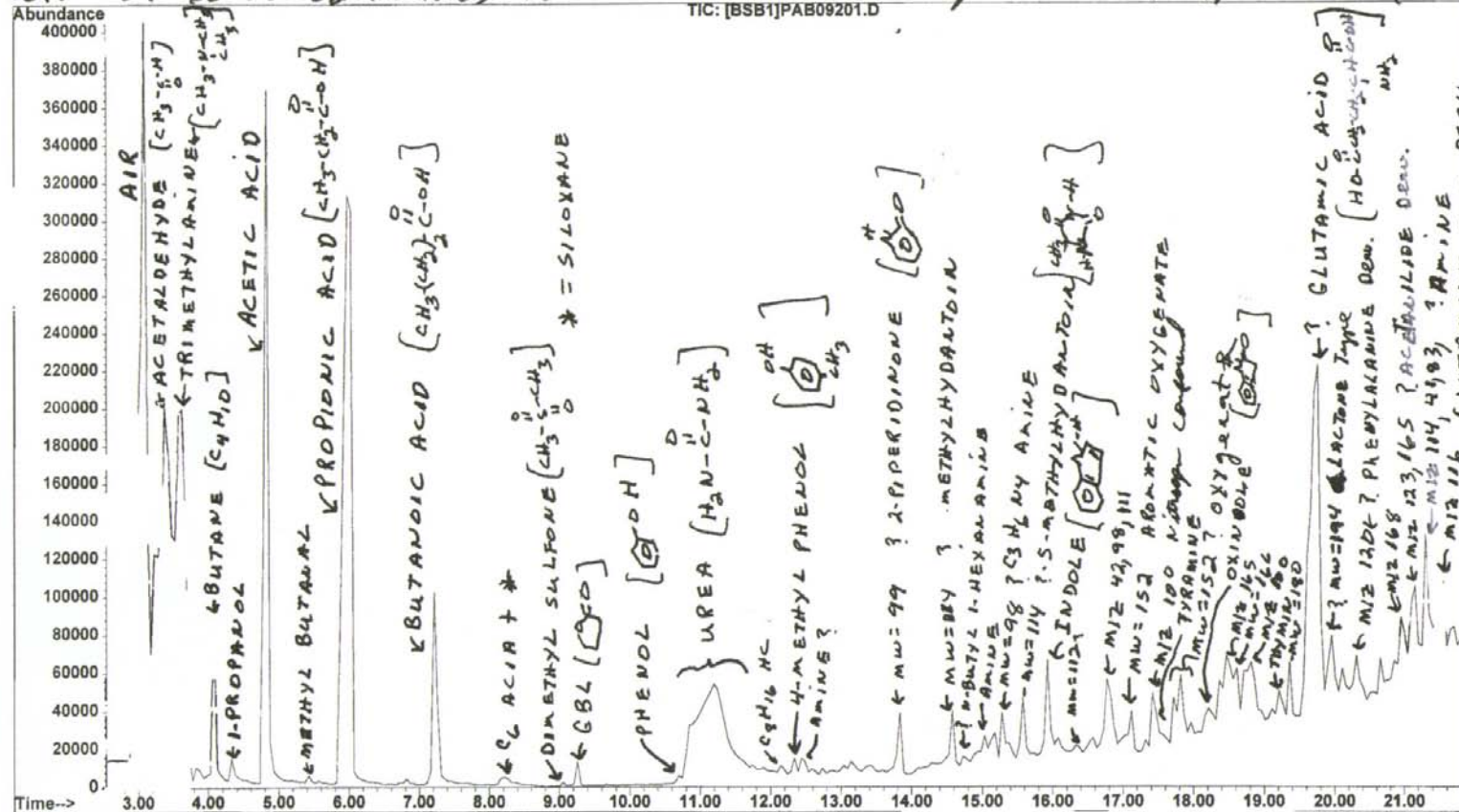


Figure 8. GC chromatogram of the vitreous fluid from the mutilated cow.