Frontier Analysis, Ltd

TECHNICAL SERVICE RESPONSE NO.: UT023

Subject: Analysis of Samples from a Cow Mutilated in the Fall of 2001 (Logan, Utah)

Date: May 13, 2002

Requested By: Colm Kelleher
NIDS
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Background/Objective:

A cow was found mutilated in northern Utah (near Logan) in the fall of 2001. The object is to look for any components that should not be normally present in the animal. To accomplish this, samples of liver tissue, blood, and vitreous fluid from the mutilated animal were submitted for analysis. Additionally, the analytical results of the vitreous fluid from the mutilated animal are compared to that from a control animal as an aid to achieve this objective.

Conclusions:

1.) Comparison of the analytical results from the vitreous fluids from a control heifer and the mutilated animal reveals differences in phenolic type materials. The vitreous fluid contains higher amounts of as well as additional phenolic types. It is speculated that these could originate from decomposition products of drugs and/or controlled substances. Many of these substances have similar phenolic functionalities as part of their structures. See Table IV.

2.) A trace amount of oxindole (ca. 0.6 pm) was also found in the vitreous fluid of the mutilated animal. The low amount indicates it is a byproduct of natural biological compounds found in the animal such as low levels of tryptophan.[1]

3.) A specific drug structure is not identified by this analysis for the possible following three reasons: 1) it is totally deteriorated, that is, only the byproducts exist; 2) the chemical

[1] Oxindole was found in very high concentration in the vitreous fluid and tissues from a mutilated animal found June 2001 in Montana. Its concentrations were too high (50-100 ppm in the vitreous fluid) to be from a natural origin. This indicated the animal was sedated before death. See T. S. R. Nos. UT016 and UT022.

nature of the drug is not amenable to detection by the analytical procedure employed[2], 3) a foreign material (drug) was not administered to the animal before euthanasia. (This analyst feels the reasons are a combination of the 1 and 2 speculations. The 3 speculation is least likely because of the presence of high concentrations of phenolics.)

Procedure:

Samples: The following samples were submitted in plastic vials surrounded by cold packs. All samples were from the mutilated Utah animal and received 2/27/2002.

- Liver tissue
- Blood
- Vitreous fluid

The liver tissue and blood were extracted with HPLC grade 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 performed on all the extracts to characterize their chemical nature. The vitreous fluid was examined "as received" by GC/MS using the same conditions reported in T.S.R. Nos.: 016 and 022. The GC/MS results of the vitreous fluid were compared to vitreous fluid from a control animal. The control animal had been obtained from a slaughterhouse. It was exposed to environmental conditions expected for mutilated animal carcass. It was laid out for 4 days, and protected from predators and scavengers.

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

Results:

The results of the individual tests performed on the blood, liver tissue and vitreous fluid follow. All tables and figures referenced in this report can be found in an appendix.

LIVER TISSUE

GC/MS Analysis: Significant amounts of liver tissue were methylene chloride extractable. GC/MS analysis shows mostly natural products dominated by fatty acids and esters. No unexpected foreign materials are detected. The GC chromatogram of the extract is shown in figure 1. The MS identifications presented in Table I.

Infrared Analysis: This analysis supports the GC/MS results. The spectrum shows a predominance of fatty acids and some ester (specifically suggested is a glycerol fatty acid ester derivative). Additionally, a minor amount of possibly a phosphorus or sulfonated component is indicated. Some of these materials were not detected by GC/MS because they would not pass through the GC column. The spectrum with pertinent peaks labeled is shown in figure 2.

[2] 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.

BLOOD

GC/MS Analysis: Only a small amount of material was extracted by methylene chloride from the blood. The GC/MS analysis of the extract detects components that appear to be natural. No oxindole or other unusual materials are detected. Figure 3 shows the GC chromatogram. Table II displays the MS identifications.

Infrared Analysis: An infrared spectrum of the methylene chloride extract shows the extract is primarily long chain fatty acid esters which are probably attached to glycerol. Must of this high molecular weight material will not pass through a GC column, and therefore would not be detected by GC/MS analysis. The spectrum is displayed in figure 4.

VITREOUS FLUID

GC/MS Analysis: Comparison of the analytical results of the vitreous fluids from the mutilated animal and the control heifer expectedly show mostly natural products and putrefaction products. Additionally, the data suggest small, but significant, differences in phenolic type materials. The mutilated cow vitreous fluid contains higher amounts as well as additional phenolic types. Phenol in the mutilated cow amounts to 80 ppm, which is significantly higher than the 15 ppm observed in the control fluid. The GC chromatogram is shown in figure 5. The chromatogram of the control fluid can be found in figure 6. The MS identifications of the GC peaks of the vitreous fluids from both animals are presented in Table III. It is speculated that the excess phenolics could originate from decomposition products of drugs and/or controlled substances. Many of these substances have similar phenolic functionalities as part of their structures. The phenolic structures suggested by the MS analysis are singled out and shown along with a few drugs and controlled substances having structural similarities in Table IV.

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® 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 Extraction of Liver Tissue from a Mutilated Cow

Compound	Match	GC Retention Time (min.)
•Propanoic acid, ethyl ester	94	5.794
•C5 Amine		5.993
(1-Butanamine, 3-methyl-)	72	
•Butanoic acid	87	6.690
•Butanoic acid, ethyl ester (<20 ppm)	96	7.237
•Butanoic acid, propyl ester	74	8.930
•Benzeneethanamine (<80 ppm)	72	12.714
•M/Z 56 Nitrogen Compound		13.809
(Aziridine, 1-(2-phenylethyl)-)	64	
•Benzeneacetaldehyde, .alpha.-ethylidene-	96	15.153
•1H-Indole	95	15.402
•5-Methyl-2-phenyl-2-hexenal	94	18.240
•MW= 195		19.186
(9H-Carbazole, 9-ethyl-)	35	
•C13-C15 Fatty Acid		21.277
(Pentadecanoic acid)	90	
•~C16 Fatty Acid		23.417
(Hexadecanoic acid)	99	
•~C18 Fatty Acid		25.259
(9-Octadecenoic acid (Z)-)	80	
•~C18 Fatty Acid		25.508
(Octadecanoic acid)	99	
•M/Z 85 Nitrogen Compound		27.848
Decanamide, H-(2-hydroxyethyl)-	72	
•Cholesterol	99	53.736

Table II

GC/MS Data from Methylene Chloride Extraction* of Blood from a Mutilated Cow

Compound	Match	GC Retention Time (min.)
•Butanoic acid, ethyl ester	80	7.234
•2-Piperidinone (<10 ppm)	86	13.358
•Indole (<2 ppm)	64	15.449
•M/Z 171, 152 (Hydrazinecarbothioamide, 2-cyclohexylidene-)	38	22.120
•Cholesterol	70	53.684

*Very small amounts of components were extracted from blood.

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

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
•Acetaldehyde	39	3.231	•Acetaldehyde	39	3.191
•Methanamine, N,N-dimethyl- (Trimethylamine)	59	3.480	•Methanamine, N,N-dimethyl- (Trimethylamine)	72	3.480
•M/Z 44, 28 Amine		3.829	-	-	-
2-Butanamine, 3-methyl-	42				
•M/Z 43 Urea Derivative		4.077	-	-	-
Urea	9				
•Acetic Acid (~ 53 ppm)	72	4.725	-	-	-
•Propanoic Acid	23	5.820	-	-	-
•M/Z 56 Possible C6 Nitrile or Protein Fragment		7.861	-	-	-
Pentanenitrile, 4-methyl-	37				
•Methane, sulfonylbis- (Dimethyl Sulfone)	78	8.757	-	-	-
-	-	-	•MW=97 C4H3NO3		10.039
			1H-Pyrrole-2.5-dione (Maleimide)	78	
•2(5H)-Furanone, 3-methyl-	81	10.151	-	-	-
•Phenol (~80 ppm)	90	10.350	•Phenol (~15 ppm)	64	10.369
•Urea	72	10.699	-	-	-
•MW=94		10.998	-	-	-
2-Pyridinamine or Derivative	9				
Pyrimidine, 2-methyl-	9				
•2,5-Pyrrolidinedione (Succinimide) (~80 ppm)	83	12.093	•MW=99 C4H4NO2		12.143
			Succinimide (~21 ppm)	80	
•Pentanamide	10	12.392	-	-	-
-	-	-	•M/Z 44, 98 Nitrogen Compound		12.597
-	-	-	•M/Z 112, 56 (MW=112)		13.793
			1,4-Cyclohexanedione	38	
•W=98 Ketone		14.134	-	-	-
2(5H)-Furanone, 5-methyl-	59				
•Benzenepropanenitrile	87	14.532	-	-	-
-	-	-	•M/Z 70		14.742
			L-Proline	35	
•MW=98		14.931			
1,3-Cyclopentanedione	78				
•MW=114		15.130			
Methylthiofuran	37				

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

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
-	-	-	•MW=114		15.154
			Parabanic acid	47	
•1H-Indole (~375 ppm)	97	15.528	•1H-Indole	94	15.608
-	-	-	•M/Z 98		15.732
			Mepivacaine	43	
•MW=112		15.976			
1,4-Cyclohexanedione	43				
			•MW=138		16.474
•MW=138 Aromatic Compound		16.623	-	-	-
3-Methoxy-2-methylphenol	12				
2,5-diamino-p-benzoquinone	25				
Benzene, 1-methyl-2-(methylthio)-	12				
•MW=152		16.673			
Benzaldehyde, 2-hydroxy-5-methoxy-	43				
-	-	-	•MW=152		16.763
			4(3H)-Pyrimidinone, 2-ethyl-3,6-dimethyl-	38	
			2-Methyl-3-(2-thienyl)-2-propenal	64	
•MW=131		16.922	-	-	-
1H-Indole, 3-methyl-	90				
•MW=100		17.022	-	-	-
2,4-Imidazolidinedione (Hydantoin)	45				
-	-	-	•M/Z 100		17.052
			4-Morpholinebutyric acid, .beta.-methyl.alpha.,. alpha.-diphenyl	42	
			4,9-Decadien-2-amine, N-butyl-	42	
•M/Z 69, 56, 152 Very Poor Matches (Fragments suggest material with long chain olefinic hydrocarbons.)		17.420	-	-	-
-	-	-	•M/Z 98 Ketone		17.423
			3-n-Butylcyclohexanone	32	
•MW=152 Aromatic		17.569	-	-	-
Phenol, 5-methoxy-2,3-dimethyl-	22				
•Oxindole (~0.6 ppm)	*	17.82	-	-	-
•MW=166		17.918	-	-	-
2-Methoxy-4-dimethylaminoaniline	43				

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

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
-	-	-	•MW=166		17.959
			Phenol, 3-methoxy-2,4,6-trimethyl-	30	
•MW=166		18.216	-	-	-
2,6-Dimethyl-4-oxa-endo-tricyclo(5.2.1.0**2,6)decane	64				
•2,4-Imidazolidinedione (Hydantoin)	47	18.366			
•MW=166		18.465			
2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydro-	27				
-	-	-	•M/Z 100, 166		18.496
			Hexahydropyrimidin-2-one	40	
•MW=107 Phenol or Pyridine Derivative		18.764	-	-	-
Phenol, 4-(2-phenylethyl)-	53				
Pyridine, 2,5-dimethyl-	53				
•M/Z 138 Aromatic Compound		18.963	-	-	-
2-Methoxy-4-methylphenol	38				
2-Methoxy-1,4-benzenediamine	43				
2,5-Diamino-p-benzoquinone	43				
-	-	-	•M/Z 138, 180		19.032
			Acetamide, N-(2-nitrophenyl)-	38	
			3-Methoxy-2-methylphenol	38	
•L-Glutamic Acid	64	19.361	•M/Z 84 Glutamic Acid or Derivative		19.321
			L-Glutamic Acid	72	
•M/Z 138		20.258	-	-	-
4,5,6-Trimethyl-2-pyrimidone	52				
1,3,7,7-Tetramethyl-2-oxa-bicyclo(4.4.0)dec-5-ene	9				
•M/Z 138		20.506	-	-	-
	-	-	•M/Z 138, 70		20.558
-			Bicyclo [2.2.1]heptane-2-one, 3,3-dimethyl-	53	
			Endo-6-methylbicyclo[2.2.2]octan-2-one	47	
•M/Z 70 Hydrocarbon		20.755			
Nonane, 3-methylene-	43				
Octane, 3-methyl-6-methylene-	43				
•M/Z 41, 114, 70 Nitrogen Compound		20.905			
1H-Imidazole, 4,5-dihydro-2,4-dimethyl-	27				

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

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
-	-	-	•MW=154		20.971
			6,8-Diazabicyclo[3.2.2]nonane-7,9-dione	35	
			2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	14	
			•M/Z 116, 61		21.177
			Hexanoic, 2-methylpropyl ester	12	
•M/Z 116, 61, 99 Glutaminic Acid Derivative		21.254	-	-	-
Glutaminic acid dimethyl ester	35				
•M/Z 152 Benzaldehyde Derivative		21.303	-	-	-
Benzaldehyde, 2-hydroxy-5-methoxy-	64				
•MW=154		21.900	-	-	-
Benzene, 2-chloro-1,3,5-trimehyl-	64				
•M/Z 154 Possible Phenol Derivative		22.149	-	-	-
Phenol, 3,5-dimethoxy-, acetate	53				
•M/Z 130 Indole Derivative (~15 ppm)		22.697	-	-	-
Tryptophane	80				
1H-Indole-3-acetic acid, ethyl ester	80				
•MW=154		23.095	-	-	-
Phenol, 3,5-dimethoxy-	25				
1,2-Cyclopentanedione, 3,3,5,5-tetramethyl-	36				
-	-	-	•MW=154		23.157
			2,4(1H,3H)-Pyrimidinedione, 1,3,6-trimethyl-	38	
•MW=154		23.245			
1,2-Cyclopentanedione, 3,3,5,5-tetramethyl-	42				
-	-	-	•MW=154		23.322
			2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	17	
			Phenol, 3,4-dimethoxy-	27	
•M/Z 186, 117 Indole Derivative Most Probable		24.141	-	-	-
4-Fluroro-2', methyldiphenyl	83				
1H-Indole	43				
-	-	-	•M/Z 186, 117 Indole Derivative Probable		24.188
			1H-Indole	50	
			4-fluoro-2', methylbiphenyl	83	
•M/Z 91 Aromatic		24.340	-	-	-
Benzene, 1,1'-[thiobis(methylene)]bis-	38				
Benzoic acid, 2-hydroxy-, phenylmethyl ester, ion(1-)	14				

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

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
•M/Z 91 Aromatic		24.589	-	-	-
1-(p-Tolyl)-3-methyl-pyrazol-5-one	35				
•M/Z 117, 200 Indole Derivative Most Probable		24.838	-	-	-
1H-Indole	35				
Benzenemethanol, 3-phenoxy-	25				
-	-	-	•M/Z 200, 117 Indole Derivative		24.890
			1H-Indole	43	
•MW=228		25.087	-	-	-
3 (paramethoxyphenyl) 4,5,6,7 tetrahydro indazole	43				
•M/Z 91		25.435			
.Delta.2-1,3,4-oxadiazolin-5-one, 4-phenyl-2-propyl-	50				
-	-	-	•M/Z 91 Aromatic (Phenyl Group)		25.467
			Benzene, 1-nitro-4-(2-phenylethyl)-	35	
			Benzaldehyde, 2-hydroxy-6-methyl-4-(phenol?)	35	
•M/Z 186		26.033	-	-	-
Phenol, 4-phenoxy-	46				
Imidazolo (4,5-B) quinoxaline	43				
•M/Z 70 Amine		26.282	-	-	-
Isomenthylamine	46				
Menthylamine	43				
-	-	-	•M/Z 70		26.334
			Tetramethyl-1,2-cyclopentanedione	50	
•M/Z 70		26.431	-	-	-
L-Alanine, N-methyl-N-(trifluroacetyl)-,butyl ester	32				
•M/Z 186 Aromatic		27.377			
Phenol, 3-phenoxy-	36				
•M/Z 186 Aromatic Phenoxy		27.675			
Phenol, 4-phenoxy-	53				
-	-	-	•M/Z 186 Phenoxy Group		27.736
			Phenol, 3-phenoxy-	59	
•MW=244 Phenylalanine Derivative		27.775			
Phenylalanine-proline diketopiperazine	50				
-	-	-	•Phenylalanine Derivative		27.860
			Phenylalanine-proline diketopiperazine	39	

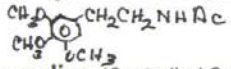
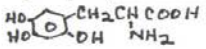
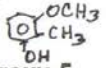
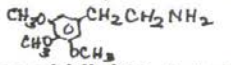
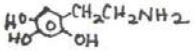
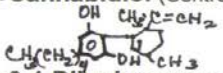
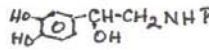
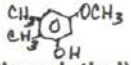
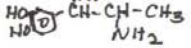
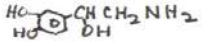
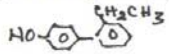
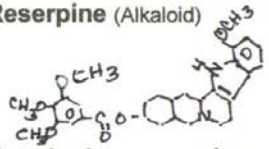
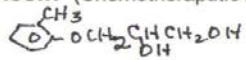
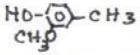
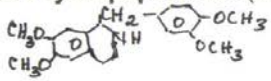
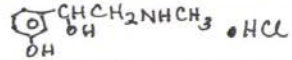
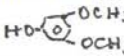
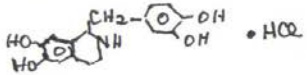
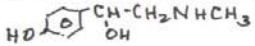
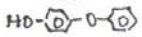
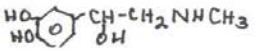
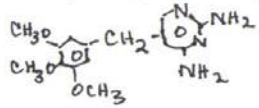
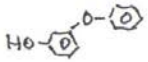
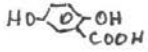
TABLE III (Continued)
GC/MS Data from the Vitreous Fluid of the Mutilated Cow and the Control Heifer

Mutilated Utah Cow			Control Heifer		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
•MW=244 Phenylalanine Derivative Phenylalanine-proline diketopiperazine	38	28.372	-	-	-
•M/Z 226 2-Cinnamylidene-6-methylcyclohexanone	22	29.020	-	-	-
•M/Z 97 Possible Amide 1H-Pyrazole-1-carboxamide, 4,5-dihydro-3,5,5-trimethyl-	40	29.269	-	-	-

*Oxindole was detected in ion chromatogram scans of ions 104 and 133 between GC retention times of 16.00 – 18.40 minutes.

Table IV

Phenolic Material Suggested by GC/MS Analysis of the Mutilated Animal's Vitreous Fluid and Some Drugs/Controlled Substances Containing Phenolic/Phenoxy Functionalities

Suggested Phenolics in Vitreous Fluid	Drugs/Controlled Substances with Phenolic/Phenoxy Functional Groups	
•**Phenol** 	•**N-Acetylmescaline** (Controlled Substance) 	•**6-Hydroxydopa** (Chemotheraputic Agent) 
•**3-Methoxy-2-methylphenol** 	•**Mescaline** (Controlled Substance) 	•**6-Hydroxydopamine** (Chemotheraputic Agent) 
•**2-Hydroxy-5-methoxybenzaldehyde** 	•**Cannabidiol** (Controlled Substance) 	•**Isoproterenol** (Chemotheraputic Agent) 
•**5-Methoxy-2,3-dimethylphenol** 	•**3,4-Dihydroxynorephedrine** (Alkaloid) 	•**Arterenol** (Chemotheraputic Agent) 
•**4-(2-Phenylethyl)-phenol** 	•**Reserpine** (Alkaloid) 	•**Mephenesin** (Chemotheraputic Agent) 
•**2-Methoxy-4-methylphenol** 	•**Tetrahydropapaverine** (Alkaloid) 	•**Phenylephrine•HCl** (Chemotheraputic Agent) 
•**3,5-Dimethoxyphenol** 	•**Tetrahydropapaveroline •HCl** (Alkaloid) 	•**Synephrine** (Chemotheraputic Agent) 
•**4-Phenoxyphenol** 	•**Epinephine** (Chemotheraputic Agent) 	•**Trimethoprim** (Chemotheraputic Agent) 
•**3-Phenoxyphenol** 	•**Gentisic Acid** (Chemotheraputic Agent) 	

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Acquired : 19 Mar 2002 19:50 using AcqMethod RWSVM
Instrument : GC/MS #4
Sample Name: Lv Extract (Utah) 3/19/02
Misc Info : Semivol Organic Analysis 1ul Splitless EM+2
Vial Number: 7

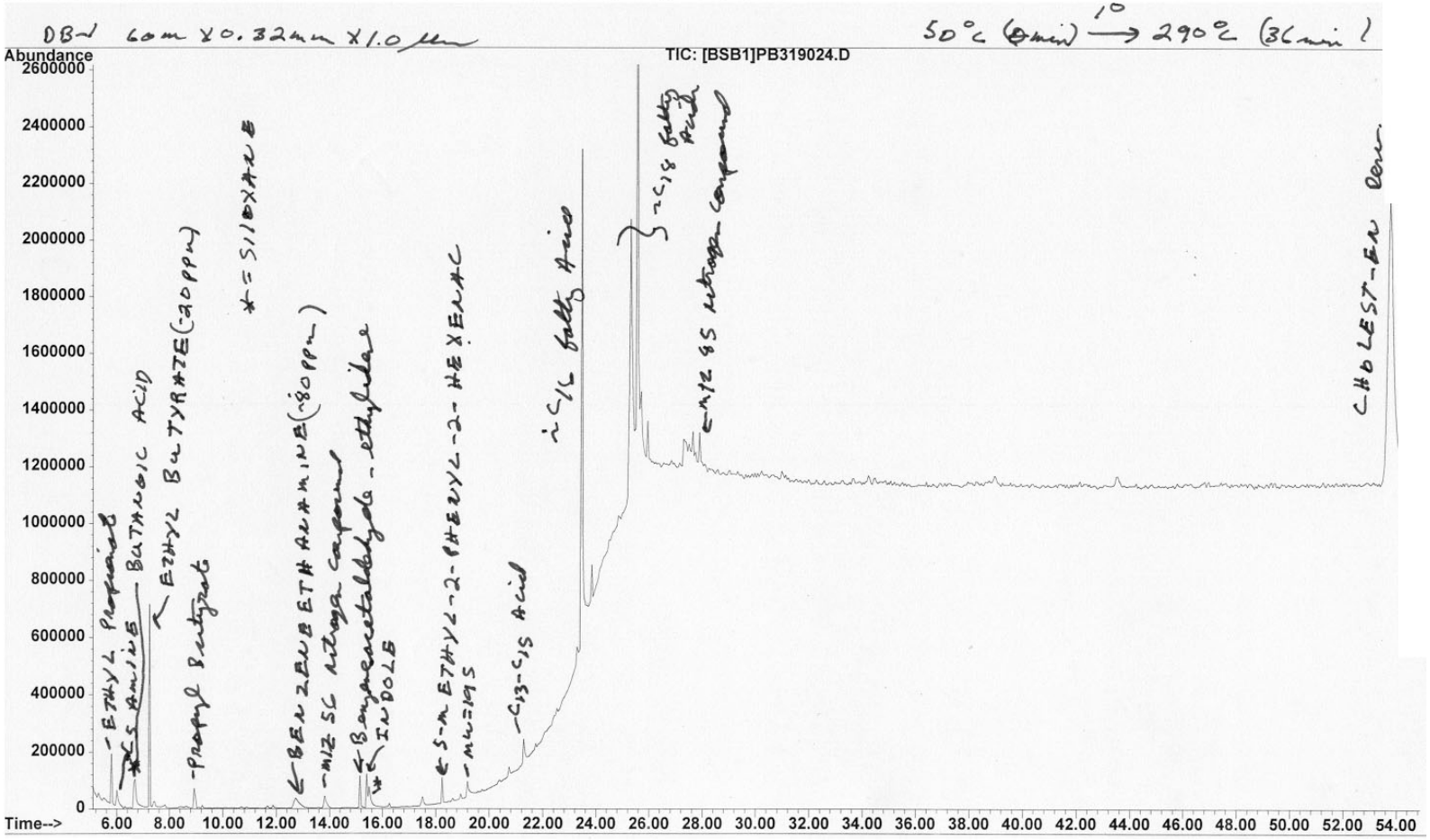


Figure 1. GC chromatogram of the methylene chloride extract from the liver of the mutilated cow.

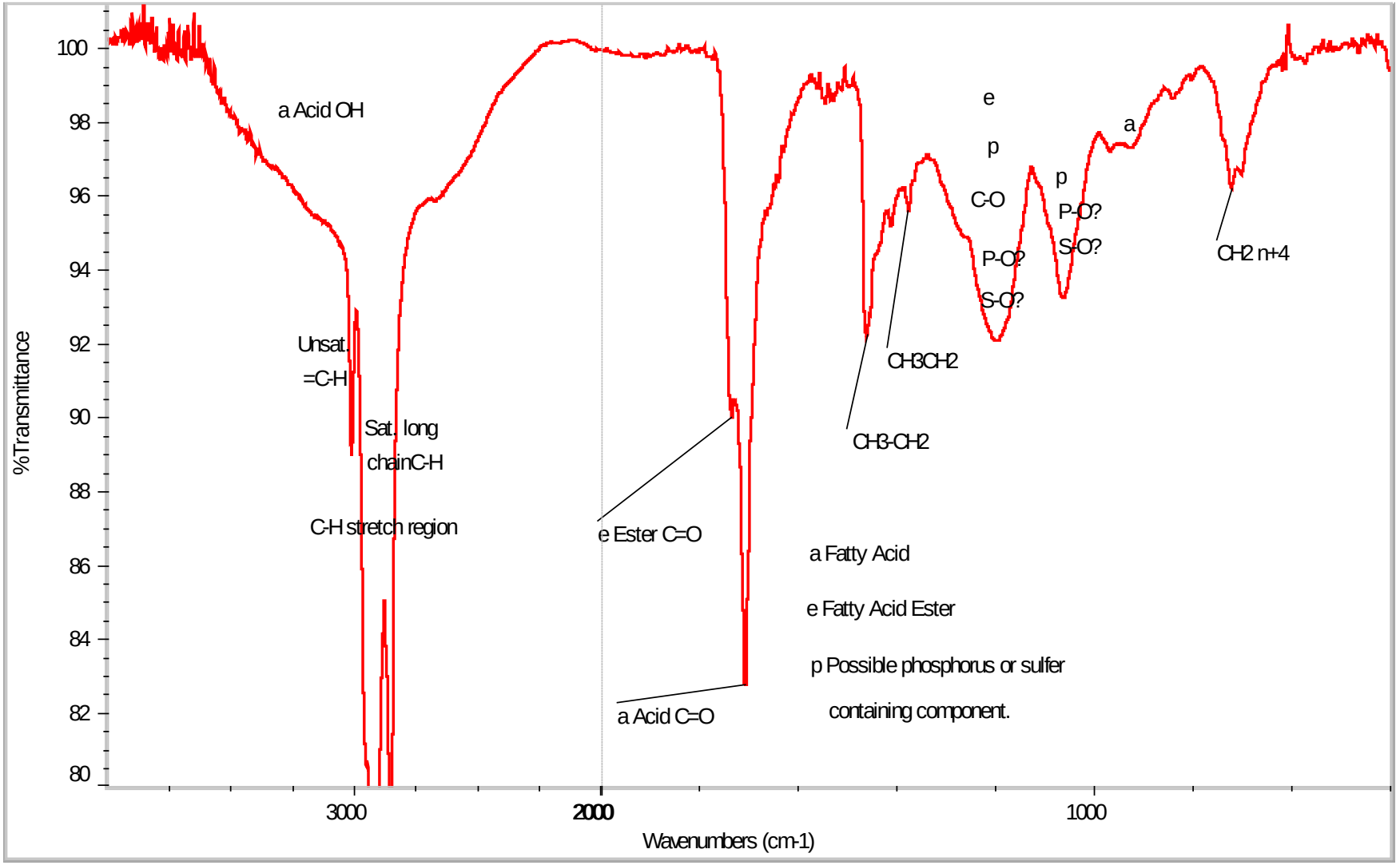


Figure 2. Infrared spectrum of the methylene chloride extract from the liver of the mutilated cow.

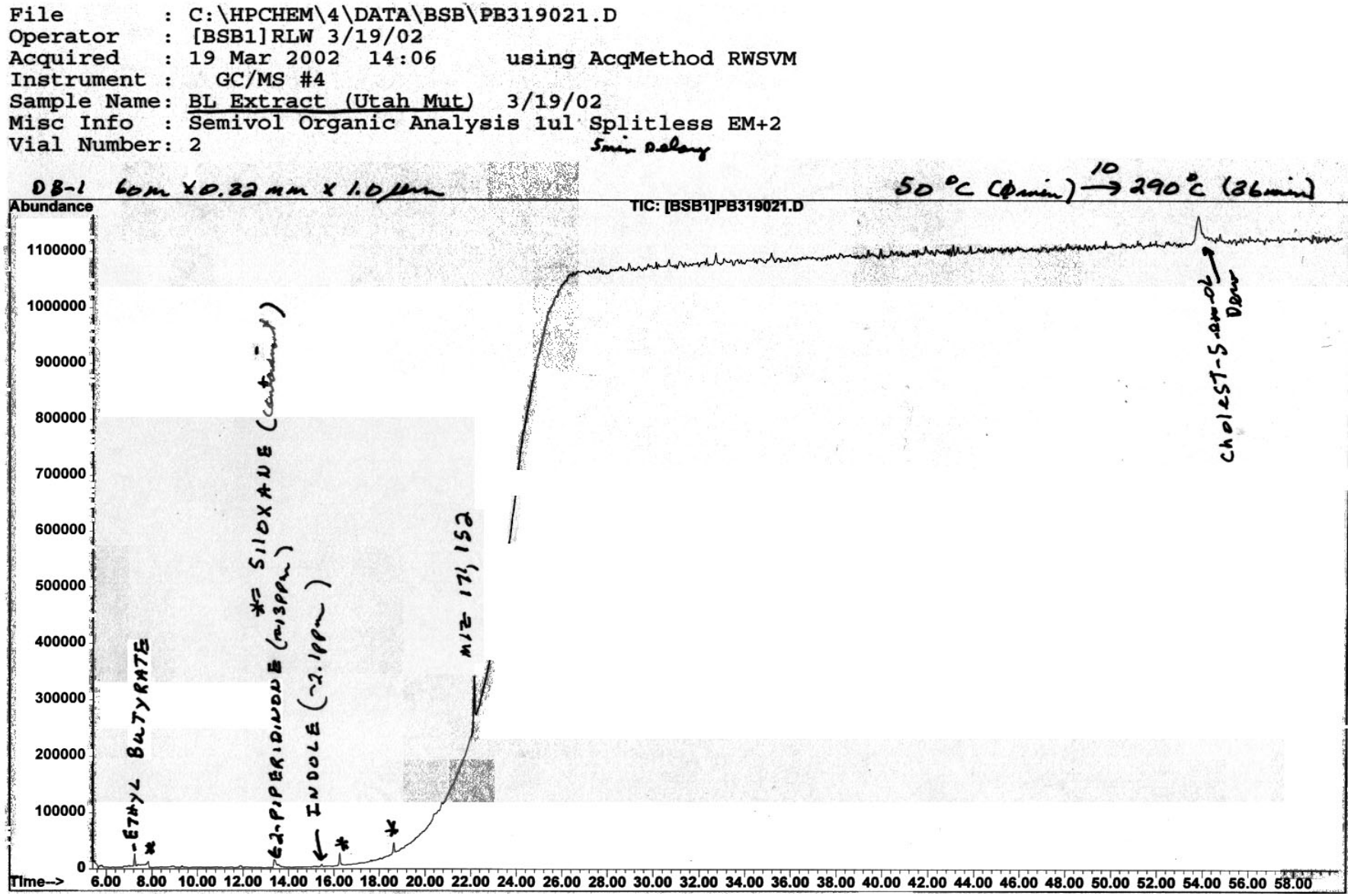


Figure 3. GC chromatogram of the methylene chloride extract from the blood of the mutilated cow.

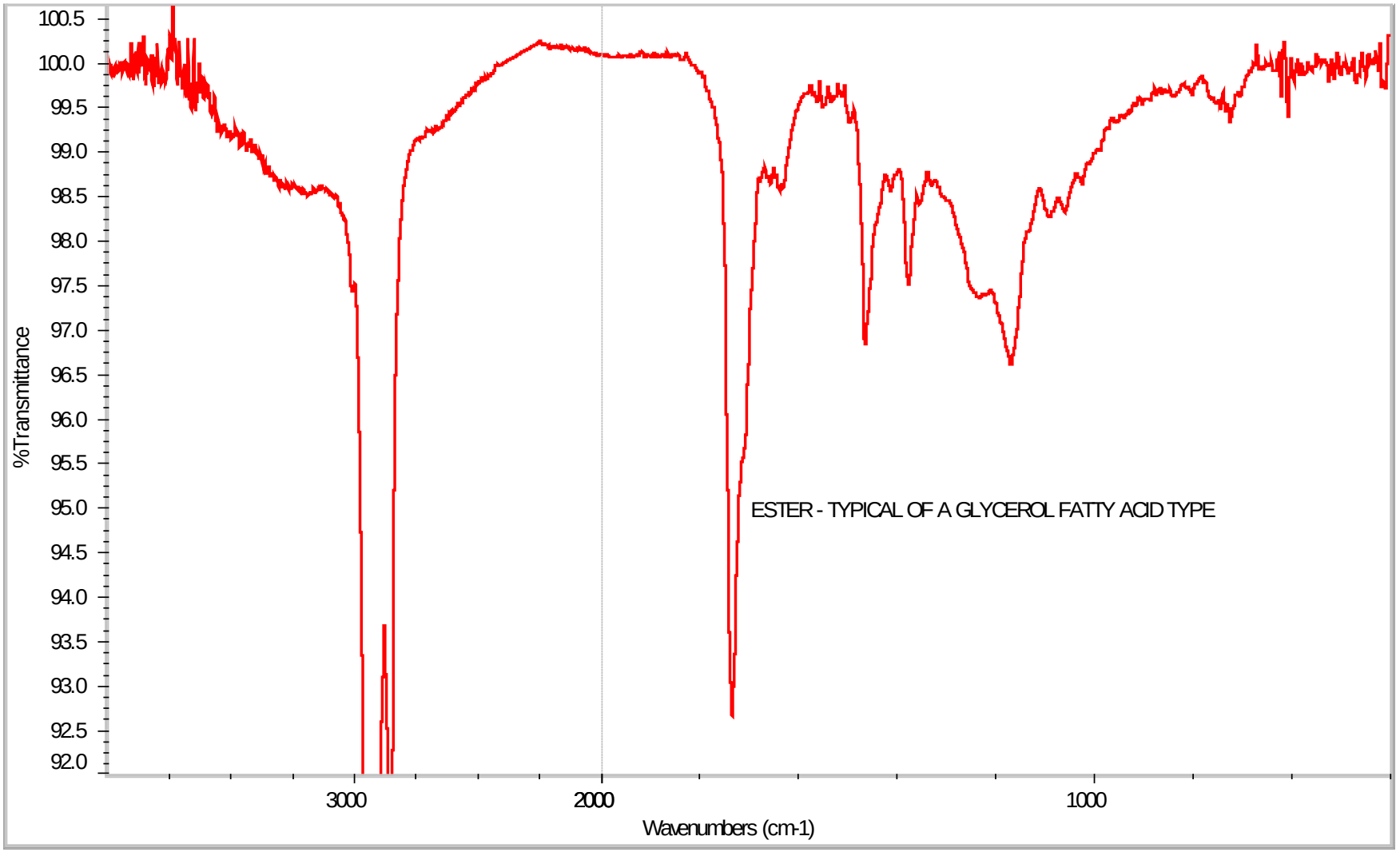


Figure 4. Infrared spectrum of the methylene chloride extract from the blood of the mutilated animal.

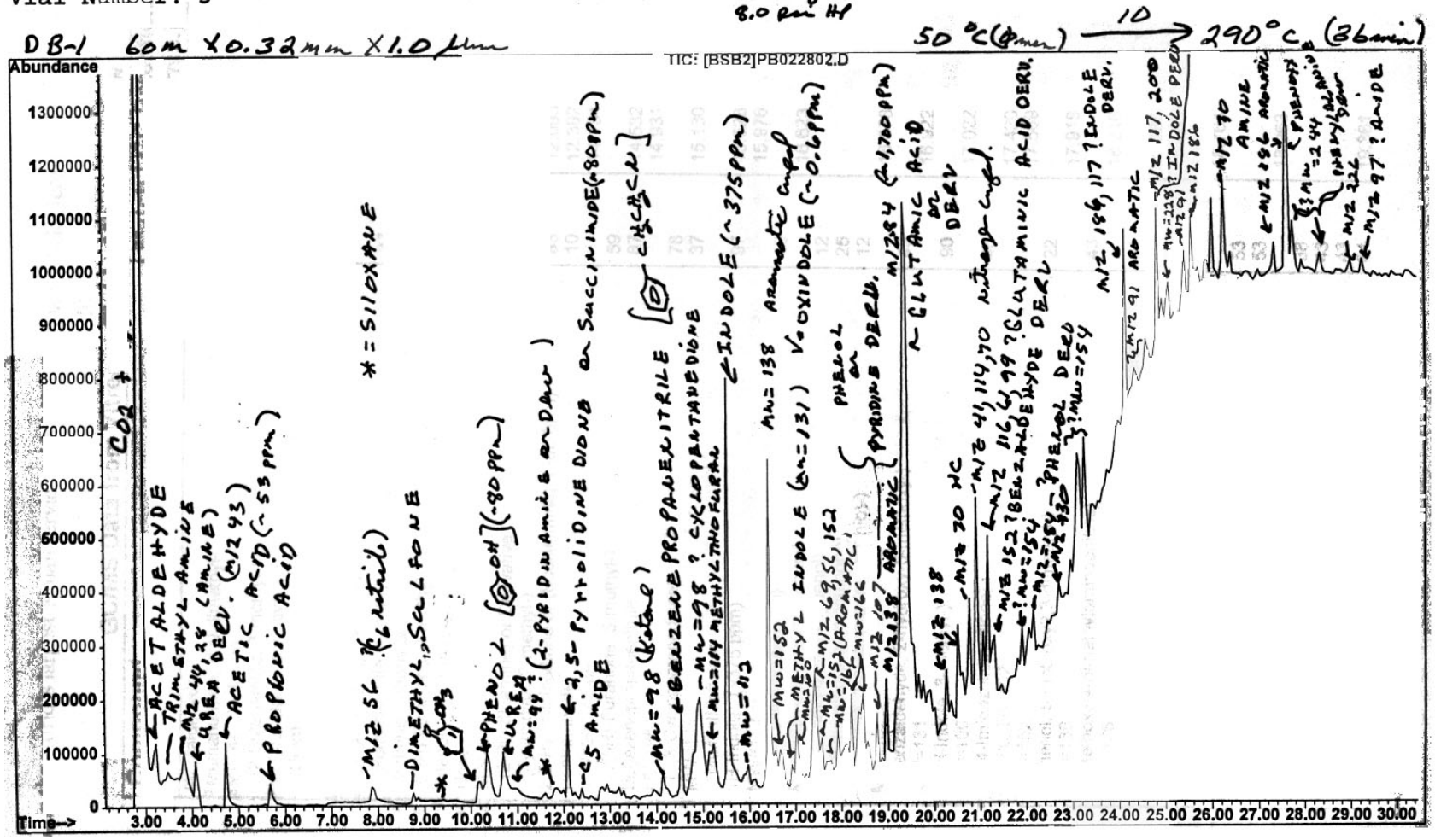
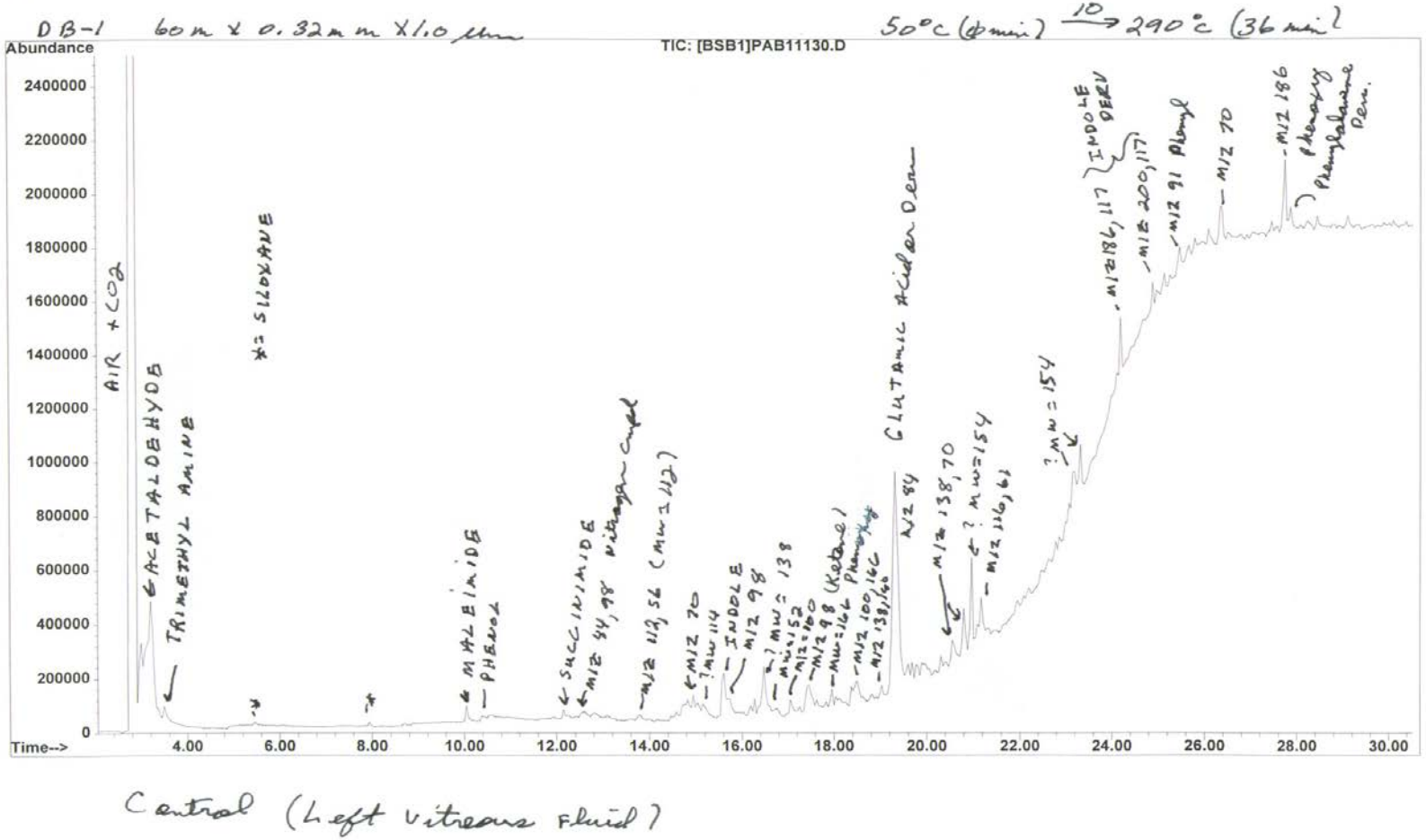


Figure 5. GC chromatogram of the vitreous fluid from the liver of the mutilated cow.



Control (Left vitreous fluid)

Figure 6. GC chromatogram of the vitreous fluid from the control heifer.