Frontier Analysis, Ltd

TECHNICAL SERVICE RESPONSE NO.: UT016

Subject: Analysis of Samples from a Cow Mutilated June 27, 2001 (Conrad, Montana)

Date: December 10, 2001

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Background/Objective:

A Red Angus cow was found mutilated in Conrad, Montana. There were 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. When the hide was cut away from the neck by law enforcement officers and the rancher, they found an area that was dark green. They did this to determine whether the animal had been injected. Tissue samples were taken underneath the left jaw-bone from area of green colored tissue. Additionally, samples of vitreous fluid and a maggot mass were obtained. The object is to look for any components that should not be normally present in the animal.

Conclusions:

1.) One unusual compound is present in both the green tissue and vitreous fluid of the mutilated cow. This is oxindole. This component is known to possess a sedative property. It is also a decomposition product of tryptophan, another powerful sedative. [1] The other materials detected appear to consist of the expected natural products or decomposition products from the animal.

2.) The dark tissue (mostly muscle tissue) is suggested to be in more advanced state of decay when compared to the pink tissue (primarily fatty tissue) from the mutilated animal. Furthermore, the analysis suggests the mutilated cow is in a greater state of putrefaction than the control heifer.

[1] "The Merck Index, 10th Edition, Published b y Merck & Co., Inc., Rahway, N.J. 1983; Personal communication Colm Kelleher.

Procedure:

Samples: The following samples were submitted.

(Tissue Samples)

•Two pieces of tissue from the same approximate area of green colored tissue underneath the left jaw-bone of the mutilated cow were received 8/22/2001. The sample was received in a plastic vial inside cold packs.

Portions of green tissue and normal pink tissue were excised from two larger 'as received" lumps of tissue. They were allowed to "dry" at ambient temperature in the laboratory to diminish interfering moisture. This took about 3 – 5 hours. Several infrared spectra were obtained from both tissues. Difference spectra were generated between selected pink and dark spectra. An extraction procedure was then performed. This will be designated "Extraction Procedure #1" in this report, because two other extraction procedures were done on the samples. The two sections of dried pink and dark tissues were extracted with progressively more polar solvents: hexane, chloroform, acetone and water. The procedure involved using approximately 3 mls of solvent per wash and three washes per extractant. For each wash the mixture was agitated manually for two minutes. Infrared spectra were also obtained from each extract. Subtraction spectra were also generated between the extract spectra in order to enhance any differences. The extracts were then combined as follows: hexane + chloroform and acetone + water. The solvents were completely removed and sent for GC/MS analysis. Microscope photographs were taken of tissues using a Leica GZ6 stereomicroscope interfaced to a Kodak digital Science MDS 120 camera.

•Two pieces of green tissue from the mutilated cow were additionally submitted on 9/18/2001. Two different extraction procedures were done on these samples. Using "Extraction Procedure #2" the sample was extracted first with chloroform and then by acetone. This was done on samples that had not been dried. Roughly 3 mls of solvent was added and the sample agitated for 2 minutes. This was done three times per solvent type. Solvents were reduced to 0.5 to 2 mls, but not completely removed before GC/MS analysis. "Extraction Procedure #3" involved only a chloroform extraction. 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 reduced to 0.5 to 1 ml and not completely removed, as in the #2 procedure. GC/MS analysis was then performed on all the extracts. Infrared spectra were obtained from selected extracts.

•Muscle tissue from a control heifer which was not mutilated was submitted for reference 11/13/200. It was subjected to "Extraction Procedures #1 and #3". Infrared spectra were obtained from the #1 and #3 extracts. GC/MS analysis was done on the #3 extract.

(Vitreous Fluid)

•The vitreous fluid sample was obtained from the mutilated cow's eye and submitted on 9/18/20001. The "as received" sample was examined by GC/MS analysis. Quantitative values for some components were also obtained by rerunning the fluid using dioxane as an external standard.

•Samples of vitreous fluid from the left and right eye were additionally submitted from a control heifer for reference 11/13/2001. Both were examined by GC/MS using the same conditions as the above vitreous fluid.

(Maggot Mass)

•The sample was submitted on 9/18/2001. The sample was extracted using "Extraction Procedures #2 and #3, i.e. the same as the above green tissue also received 9/18/001. See those descriptions. Additionally, an infrared spectrum was obtained of the chloroform extract. The chloroform was totally removed for the later analysis.

•A very small amount of maggot mass was also submitted from the control heifer for reference on 11/13/2001. It was extracted by using Extraction Procedure #3. There were trace levels of extracted material, which was insufficient for infrared and GC/MS analysis.

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

Results:

The results of the individual tests performed on the tissues follow. These results are summarized in the conclusions section on page one of this report. All tables and figures referenced in this report can be found in an appendix.

TISSUE SAMPLES

Following is a photograph showing the dark green area underneath the left jaw-bone of the mutilated cow. This is the sampling area of the tissues.

Photograph procured from NIDS

Microscope Examination: The "as received" dark and muscle and pink fatty tissues from the mutilated animal, as well as muscle tissues from the control heifer, were observed under the microscope. The cow tissues are darker in color compared to those from the control heifer. Following are the microphotographs.

Mutilated Cow Tissue **Control Heifer Tissues**

GC/MS Analysis: Three different extraction procedures performed on the tissues were done to establish the best conditions for concentrating and detecting any foreign materials by GC/MS. In other words, some method development was required.

"Extraction procedure #1" involved using a progressively polar solvent sequence on the dried tissues from the mutilated cow. The hexane, chloroform, acetone, water extracts were combined, i.e. hexane + chloroform and acetone + water, from the pink and dark tissues before they were subjected for GC/MS analysis. GC/MS was done in order to detect components that may have been masked by the strongly absorbing esters and acids detected in the infrared analysis. (See the following infrared section.) Numerous individual molecules were identified. However, they seemed to be natural and degradation products from the animal. It was suspected that this analytical approach induced more deterioration of the sample due to the long time of exposure to ambient temperature (many hours) between the extraction and the GC/MS analysis. The four GC chromatograms of the extracts are shown in figures 1, 2, 3 and 4. The MS identifications are displayed in Table I.

"Extraction Procedure #2, done by using chloroform followed by acetone solvents, was performed on the "as received" sample. This reduced the time exposed to ambient temperature. Furthermore, not all the solvent was removed before GC/MS analysis. The GC/MS data show that not much material was extracted by this procedure due to short solvent contact time. For this reason the data were not very informative. The components that were in amounts to be detectable by GC/MS are natural and decomposition products (previously detected). Figures 5 and 6 display the GC chromatograms from the chloroform and the acetone extracts. Table II presents the MS identifications of each peak.

"Extraction Procedure #3" involved a refrigerated chloroform extraction for 8 days. It was successful in removing a large amount of solubles from the tissues of the mutilated cow and the control heifer and minimized as much as possible any further degradation. Both chromatograms display strong peaks. This analysis shows the expected predominance of natural and degradation products. However, when comparing the data from the mutilated animal and the control, an unusual compound is uniquely observed in the extract from the mutilated cow. This is oxindole. This molecular structure, as well as some derivatives of this structure such as tryptophan, 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 chromatograms of the extracts from the tissues of the mutilated cow and the control heifer are shown in figures 7 and 8. The mass spectrum along with a reference of oxindole is shown in figure 9.

Infrared Analysis: Infrared analysis actually was initially done on the tissues from the mutilated cow to detect a possible foreign component responsible for the green color. As most analysis dealing with the identification of unknowns, the approach develops as the analysis is in progress. Infrared is a good test to begin with because many times it is all that's needed to solve a problem. If it doesn't solve the problem, it shows which additional procedure/test should be done. This cursory examination indicated any possible foreign material, if present, was in amounts too low for infrared detection. Therefore, additional analysis should be done to try and concentrate this material and identify it using an alternative, more sensitive technique. This is why the extractions followed by GC/MS analyses, as previously described, were done. Infrared did indicate the dark color was a result of progressed degradation. Following are details of some of the pertinent infrared results.

Spectral data from the dried "as received" tissues from the mutilated animal show the difference in composition between the pink and dark tissues. The spectrum of pink tissue displays a predominance of fatty acid ester materials and moderate amount of protein clearly reflective a fatty tissue. The spectrum of the dried dark tissue is primarily protein type material with some carboxylic acid, and significantly less ester. No foreign components are detected.

Further examination of spectra from "Extraction Procedure #1" tissue extracts from the mutilated cow revealed more information. The extraction procedure, using progressively polar solvent sequence (hexane, chloroform, acetone, water), was done on the dark green tissue and the pink fatty tissue of the mutilated animal to concentrate any possible foreign materials for identification, and to compare the tissues. These extract data suggest the green tissue is more deteriorated. The dark areas contain significant amounts of free carboxylic acids and small amounts of carboxylic acid salts with possibly an ammonium cation. A small amount of sulfate may also be present. The pink tissue contains minimal amounts of carboxylic acids and no carboxylic acid salts. No obvious foreign components were suggested. Though spectra were acquired from the control heifer extracts, there was no useful information that could be related to the presence of a foreign contaminant in the tissue from the mutilated animal. The mutilated cow extracts were additionally examined by GC/MS analysis for a more detailed look at the individual molecules comprising the extracts. (See above section.) Table III includes interpretation of pertinent spectra of the "as received" tissues and the extracts as well as references to the spectral figures (10 through 25. References a long chain fatty acid and a triglyceryl ester are shown in figures 26 and 27.

The fact that carboxylic acid salts are present in the dark material suggests it has a basic pH. This was confirmed by testing the water solubles for pH using ColorpHast™ pH paper. It indicated a pH of close to 8. The pH of the water solubles of the pink material was 7. It was noted that a water extract from the control heifer tissue was on the acid side with a pH of 6.

VITREOUS FLUID

GC/MS Analysis: GC chromatograms of "as received" vitreous fluids from the mutilated cow and control heifer are well endowed with peaks showing the presence of numerous components. As with the tissue analysis, most of these are identified as natural and putrefaction products. Both GC chromatograms are very similar. However, under close inspection there are subtle but very significant and informative differences. There are additional weak GC peaks in the chromatogram of the mutilated animal. Those additional peaks identified by MS as acetic acid, propionic acid, butanoic acid, urea etc., suggests the mutilated cow was in greater state of putrefaction than the control heifer. But there is one

additional component at a GC retention time of 18.22 min. which may not be attributable to a decay product. MS identifies it as oxindole, which was also detected in the tissue. The amount of this material could be roughly estimated from the GC chromatogram by comparing it to a chromatogram from another run containing a known amount of dioxane standard. It was determined that the oxindole content is about 50 to 100 ppm (0.005 to 0.01 wt.%). The chromatograms of the fluids from both animals are shown in figures 28 and 29. The MS spectrum of this material is shown along with an oxindole reference is shown in figure 30. Table IV shows the MS identification of many of the components from the mutilated cow. The GC chromatogram from the control heifer does have peaks close to retention times of oxindole. However, an ion scan for the region shows it is definitely not present in the control heifer. 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 is shown in figure 31. Both of these major peaks would be expected if oxindole is present. They are absent. The ion scan of the left eye fluid is identical.

MAGGOT MASS

GC/MS Analysis: There was an insufficient amount of material retrieved from extraction procedure #3 of the maggot mass of the cow that was amenable to GC analysis, i.e. the chloroform extract of the "as received" sample.[2] There was not enough maggot mass from the control animal for extraction.

Infrared Analysis: Mostly natural products and decomposition products are detected in the infrared spectra of the small amount of material extracted from the maggot mass (#3 extraction) of the mutilated cow. The spectrum of this chloroform extract (figure 32) shows significant amounts of both glycerol ester and carboxylic acids. The acids are indicative of degradation products.

Acknowledgments: I wish to thank and acknowledge the following people for their contributions to this effort: Richard L. Wilson for the GC/MS analysis; Bruce O. Budinger for some of the solvent extractions.

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Phyllis A. Budinger

Distribution:

R. L. Wilson

[2] A method for extraction was found by Colm Kelleher which is used by forensic toxicologists after the chloroform extraction. There was an insufficient amount of maggot mass to do the method.

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 Extraction Procedure #1 of Pink and Dark Tissues (Hexane + Chloroform, Acetone + Water) from the Mutilated Cow

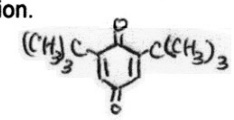
PINK			DARK		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
•Hexane/Chloroform Extracts					
-	-		C_7H_{16} Heptane Hydrocarbon	80	5.562
C_8H_{18} Octane Hydrocarbon	81	6.727	C_8H_{18} Octane Hydrocarbon	38	6.729
Xylene (Dimethyl Benzene)	64	7.602	Xylene (Dimethyl Benzene)	42	7.604
Indene MW 116	64	9.760	-	-	
Nonanal $(CH_3(CH_2)_{7(}C=O)H$	72	10.168	Nonanal $(CH_3(CH_2)_{7(}C=O)H$	90	10.170
Phthalic Anhydride $C_8H_4O_3$	91	12.326	Phthalic Anhydride	43	12.327
$C_{19}H_{40}$ Nonadecane Hydrocarbon	64	13.259	C_{14} to C_{19} Hydrocarbons Tetradecane	97	13.260
-	-		$C_{14}H_{20}O_2$(Dione) MW 220 See Attached Structure	95	14.019
$C_{16}H_{32}O_2$ Hexadecanoic Acid	93	17.632	$C_{16}H_{32}O_2$ Hexadecanoic Acid	95	17.634
$C_{18}H_{34}O_2$ Heptadecane-(8)Carbonic acid-(1) C_{18} Acid	91	19.324	-	-	
$C_{18}H_{34}O_2$ Octadecanoic Acid	64	19.499	$C_{18}H_{34}O_2$ Octadecenoic Acid	91	19.326
			Heptane-(8)-carbonic acid-(1)	90	
$C_{23}H_{48}$Tricosane MW 321	93	20.140	-	-	
C_{20} to C_{23} Eicosane Hydrocarbon	98	22.881	-		
C_{20} to C_{21} Fatty Acid/Ester	66	23.873	Fatty Acid/Ester/Aldehyde Assorted		
			9-Octadecenoic acid-, 9-hexadecenyl ester	10	23.874
			9-Octadecenal $C_{18}H_{34}O$	38	23.874
			Hexadecanedioic acid	14	24.283
$C_{28}H_{58}$ 9-Octyl Eicosane Hydrocarbon	58	24.281	-		
-	-		A Phthalate Ester 1,2-Benzene dicarboxylic acid, dicyclohexyl ester	37	25.216

TABLE I (Continued)
GC/MS Data from Extraction Procedure #1 of Pink and Dark Tissues (Hexane + Chloroform, Acetone + Water) from the Mutilated Cow

PINK			DARK		
Compound	**Match**	**GC Retention Time (min.)**	**Compound**	**Match**	**GC Retention Time (min.)**
MW 330 See Attached Structures	86, 80, 78	25.214	-	-	-
MW 386 See Attached Structure	64	25.564	-	-	-
C_{23} to C_{30} Hydrocarbon			-	-	-
Tricosane $C_{23}H_{48}$	90	26.788			
Heptacosane $C_{27}H_{56}$	86, 90	29.471			
C_{28} Hydrocarbon 9-Octyl-Eicosane $C_{28}H_{58}$	46	32.679	-	-	-
C_{30} Hydrocarbon Dotriacontane $C_{32}H_{66}$	45	36.644	-	-	-
MW 386 $C_{27}H_{46}O$ (Another 386 Compound) Cholest-5-en-3-ol (3.beta)- See Attached Structure	96	49.999	MW 386 Cholest-5-en-3-ol (3.beta)-	-	50.059
•Acetone/Water Extracts					
Dimethyl Benzene (Xylene) + Butyrolactone	53, 64	7.604	Dimethyl Benzene (Xylene) + Butyrolactone		7.602
3-Methylhydantoin MW=114 $C_4H_6N_2O_2$ See Attached Structure	80	11.220	-	-	-
Phenylacetic Acid MW 136	30	11.511	-	-	-
C_{12} to C_{20} Hydrocarbon Undecane	47	13.261	-	-	-

Structures of Selected Compounds

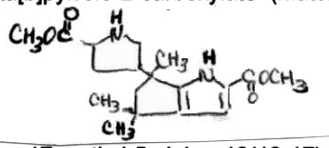
• MW 220 $C_{14}H_{20}O_2$ 2,5-Cyclohexadiene-1,4-dione, 2,6- bis(1,1-dimethylethyl)- This is a positive identification.



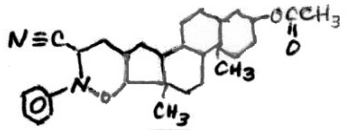
•MW 330 There are three good matches. There's a high probability that it's one of the following structures or a very similar structure.

$C_{23}H_{22}O_2$ 9,10-dihydro-9,10-dimethoxy-9,10-(1',7']-tricyclo(4.1.0.0.(2,7)heptanol anthracene (Not sure of the structure. Match 86)

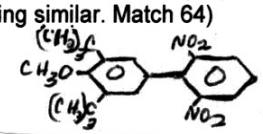
$C_{18}H_{22}N_2O_4$ Methyl-1,4,5,6-tetrahydro-6-[5-(methoxycarbonyl)-1H-pyrrol-3-yl]-4,4,6-trimethylcyclopenta[b]pyrrole-2-carboxylate (Match 80)



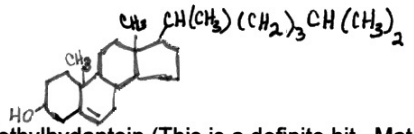
$C_{22}H_{34}O_2$ 3.beta.-Acetoxy-17-methyl-5.alpha.-18(13-17)abeoandrost-13-ene (The literature structure does not correlate with the molecular formula, but here it is. Match 78)



•MW 386 4-Methoxy-2',6'-dinitro-3,5-di-t-butylbiphenyl (The probability is good for this structure or something similar. Match 64)



•MW 386 $C_{27}H_{46}O$ (Another 386 compound) Cholest-5-en-3-ol (3.beta)- (This is a definite hit. Match 96.)



•MW=114 $C_4H_6N_2O_2$ 3-Methylhydantoin (This is a definite hit. Match 80)

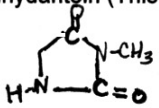


Table II
GC/MS Data from Extraction Procedure #2 Dark (Chloroform, Acetone) from the Mutilated Cow

Green Tissue		
Compound	**Match**	**GC Retention Time (min.)**
•***$CHCl_3$ Extracts***		
2-Ethylhexyl ester of Butanoic Acid	56	9.701
5-Methyl-2,4-Imidazolidinedione	56	14.461
Indole	87	15.778
Hexadecanoic Acid	94	23.779
1,1'-Dodecylidenebis [4-Methyl] Cyclohexane	43	25.703
-	-	-
*4-Methoxy-2''6'-Dinitro-3,5-Di-t-Butylbiphenyl	59	32.083
-	-	-
-	-	-
-	-	-
•*Acetone Extracts*		
Propanoic Acid	95	5.549
2,5-Dimethyl-Furan	81	6.157
C6 Ketone (4-Methyl-3-Penten-2-One)	62	7.524
C6 Ketone (4-Hydroxy-4-Methyl-2-Pentanone)	47	8.183
C5H12N2 (1-Methyl-Piperazine)	56	8.588
Hexadecanoic Acid	97	23.779
Fatty Acid [Heptadecene- (8)- Carbonic Acid- (1)]	81	25.703
Fatty Acid/Ester [Hexadecanoic Acid 2-Hydroxy-1-(Hydroxymethyl)ethyl Ester,	30	27.628
Fatty Acid/Ester [Di-(9-Octadecenoyl)-Glycerol]	41	30.463
M/Z 281 [2-(14-Carboxytetradecyl)-2-Ethyl-4,4-Dimethyl-1,3-Oxazolidine-N-Oxide]	10	30.919
[9,10-Dihydro-9,10-Dimethoxy-9,10-([1',7']-Tricyclo[4.1.0.0(2,7)]Heptano) Anthracene	59	31.729
*M/Z 386, 371 (4-Methoxy-2',6'-Dinitro -3,5-di-t-Butylbiphenol)	45	32.084

*The hit is really not that good. It could be something else, possible siloxane.

Table III

Infrared Analysis of Dried Pink and Dark Tissues and Extraction #1 Fractions from the Mutilated Cow

Spectrum	Infrared Identification	Figures
•Tissues		
"Dried" Pink	Significant amounts of both glycerol esters and protein material.	10
"Dried" Dark	Primarily protein type material; trace glycerol esters and carboxylic acids.	11
"Dried" Control		
•Extracts		
Hexane Pink	Glycerol triesters; possible trace carboxylic acids.	12
Hexane Dark	Significant amount of glycerol triester; moderate amount carboxylic acids; the esters appear to be of a higher molecular weight compared to the extract from the pink. (See difference spectrum Fig. 22.)	13
Chloroform Pink	Glycerol triester; possible trace carboxylic acids.	14
Chloroform Dark	Significant amounts of both glycerol triester and long chain carboxylic acids.	15
Acetone Pink	Predominantly glycerol triester; some carboxylic acid.	16
Acetone Dark	Significant amounts of carboxylic acid and glycerol triester	17
Water Pink	Primarily protein type material and trace glycerol ester.	18
Water Dark	Protein type material; carboxylic acid salts[3] (see difference spectrum Fig. 25)	19
•Insolubles		
Pink	Glycerol triester.	20
Dark	Protein type material; trace ester.	21
•Difference Spectra		
C_6 Ext: Dark vs Pink	Long chain carboxylic acid; higher molecular weight ester than in the pink sample.	22
$CHCl_3$ Ext: Dark vs Pink	Long chain carboxylic acid.	23
$(CH_3)_2C=O$ Ext: Dark vs Pink	Long chain carboxylic acid.	24
H_2O Ext: Dark vs Pink	Carboxlic acid salt with a possible ammonium cation; possible sulfate; carboxlic acid.	25

[3] It was noted that the water solubles from the dark tissue foamed when agitated. This implies detergency which is typical for carboxylic acid salts, i.e. soaps. The water solubles from the pink tissue did not foam.

TABLE IV
GC/MS Data from the Vitreous Fluid of the Mutilated Cow

Vitreous Fluid (As Received)		
Compound	**Match**	**GC Retention Time (min.)**
Acetaldehyde	91	3.380
Trimethylamine	86	3.579
Butane	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	12	8.159
Dimethyl Sulfone	59	9.055
GBL Butyrolctone	83	9.254
Phenol	91	10.698
Urea	86	10.848 & 10.997 & 11.196
C8H16 Hydrocarbon (1-Ethyl-3-Methyl-Cyclopentane)	83	12.142
4-Methyl-Phenol	95	12.341
Amine? (1-Piperazineethanamine)	12	12.441
2-Piperidinone	35	13.735
2-Piperidinone	50	13.835
5-Methylhydantoin	50	14.581
N-Butyl-1-Hexanamine	42	14.731
Amine (N-Ethyl-Cyclopentanamine)	37	15.030
C3H6N4 Amine (4-Methyl-1,2,4-Triazol-3-Amine)	72	15.278
5-Methylhydantoin	83	15.577
Indole	93	15.926
MW=112 (4,5-Dihydro-6-Methyl-3(2H)-Pyridazinone)	32	16.324
2-Methoxy-5-Methyl-2,5-Cyclohenadiene-1,4-Dione	40	16.573
M/Z 42, 98, 111 (1,1'-Methylenebis-Piperidine)	47	16.772 to 16.822
MW= 152 Aromatic Oxygenate (2-Hydroxy-5-Methoxy-Benzaldehyde)	43	17.120
M/Z 100 Nitrogen Compound (2,4-Imidazolidinedione)	64	17.419
Tyramine	72	17.469
MW=152 ?Oxygenate (3-Hydroxy-2-Isobut-1-Enylcyclopent-2-En-1-One	90	17.817
Oxindole	93	18.216
(4-Hydroxy-3-Methoxy-Benzaldehyde)	23	18.365
M/Z 165 (2-Amino-1,7-Dihydro-7-Methyl-6H-Purine-6-One)	38	18.465
MW=166 [3-(1-Amino Ethylidine)-6-Methyl-1H, 3H-2, 4-Pyridinedione]	35	18.614
M/Z 100 (2-Methyl-2-Butenoic Acid)	49	18.813
(1-Nitroso-Pyrrolidine)	45	
Thymin	87	19.211
MW=180 [4-(Acetyloxy)-Benzoic Acid]	49	19.361
(Glutamic Acid)	72	19.709 to 19.759
MW=194 C12H18O2 Lactone Type (Lactone of 5-Acetyl-1,3,3,4,5-Pentamethylbicyclo[2.1.0]Pentan-2-One)	27	19.958
M/Z 120? Phenylalanine Deriv. (L-Phenylalanine-4-Nitroanilide)	50	20.307
M/Z 168 (Imidazo[2,1-a]Isoquinoline)	11	20.954
M/Z 123, 165 Acetanilide Deriv. (3-Methoxyacetanilide)	25	21.153
M/Z 114, 41, 83 Amine? [3-(Hexylamine)-Propanenitrile]	25	21.302
M/Z 116 Glutaminic Acid Deriv. (Glutaminic Acid Dimethyl Ester)	32	21.551
M/Z 154, 70		22.298 & 22.547
M/Z 154, 70		23.493 & 23.642
C18 Fatty Acid (Octadecanoic Acid)	91	23.891
M/Z 186 Indole Deriv. (Fragments for Indole itself +186)		24.538
M/Z 200 Indole Deriv. (Fragments for Indole + 200)		25.285
Phenoxy Components?		25.883 & 26.082 & 26.530 & 28.222
Cholest-5-en-3-ol	89	56.948

File : C:\HPCHEM\4\DATA\BSB\PAB90601.D
Operator : [BSB1]RLW 9/6/01
Acquired : 6 Sep 2001 11:32 using AcqMethod DB1SVO
Instrument : GC/MS #4
Sample Name: Dark C6 + CHCl3 Extract 9/601 in methyl salicylate
Misc Info : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 1

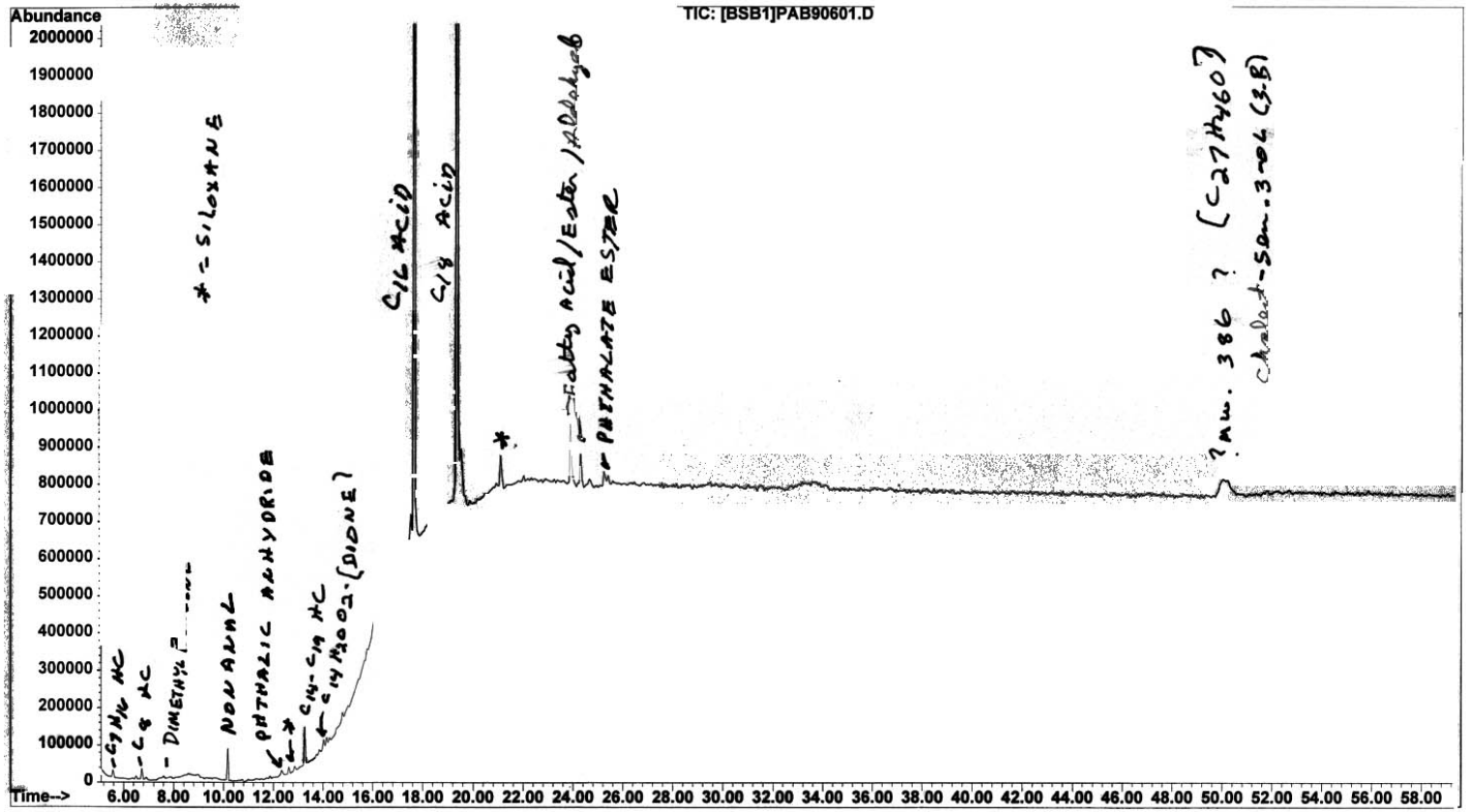


Figure 1. GC chromatogram of the hexane + chloroform extract from the dark tissue of the mutilated cow (extraction #1).

```
File        : C:\HPCHEM\4\DATA\BSB\PAB90603.D
Operator    : [BSB1]RLW 9/6/01
Acquired    :  6 Sep 2001  13:52     using AcqMethod DB1SVO
Instrument :   GC/MS #4
Sample Name: Dark  AC + H20 Extract  9/601   in MeOH
Misc Info   : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 2
```

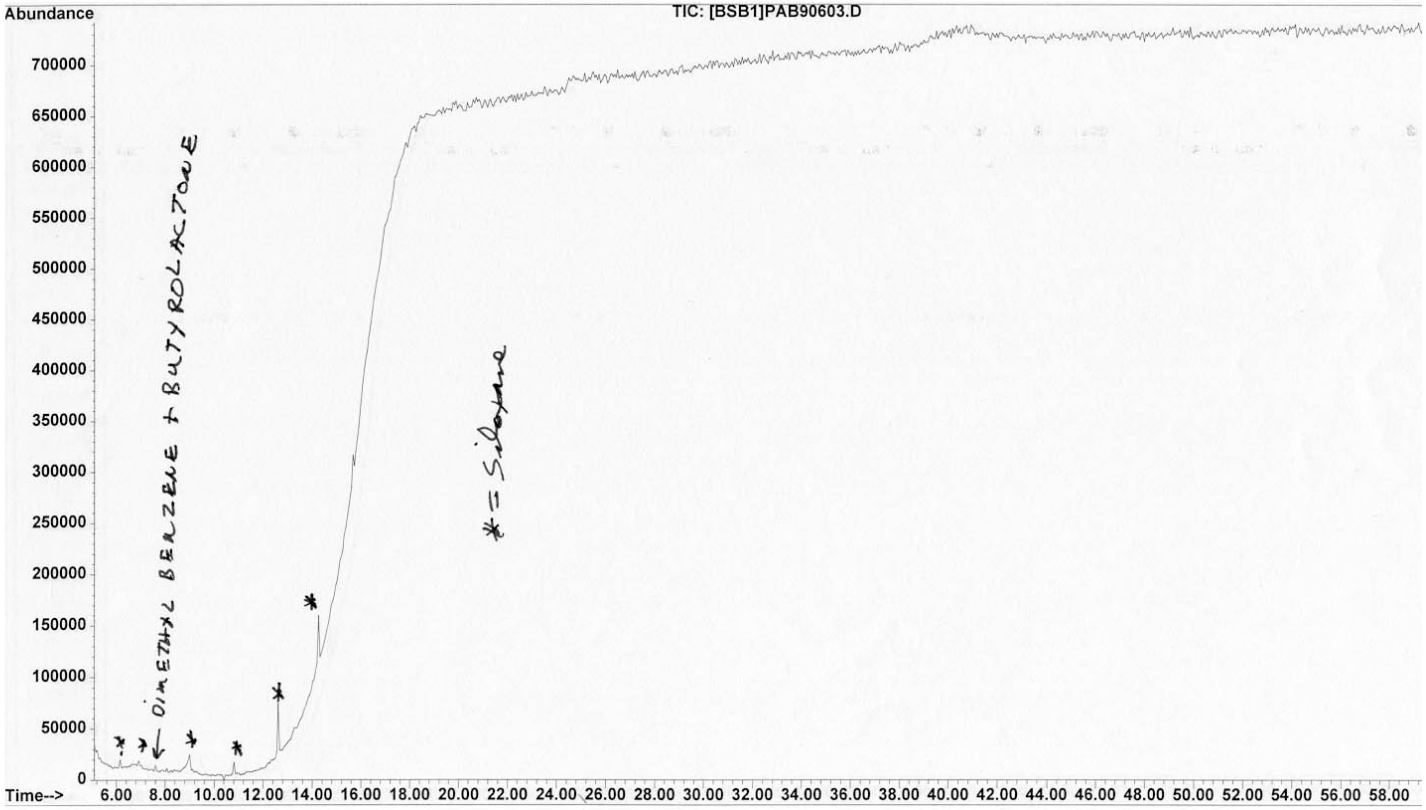


Figure 2. GC chromatogram of the acetone + water extract from the dark tissue of the mutilated cow (extraction #1).

File : C:\HPCHEM\4\DATA\BSB\PAB90600.D
Operator : [BSB1]RLW 9/6/01
Acquired : 6 Sep 2001 10:05 using AcqMethod DB1SVO
Instrument : GC/MS #4
Sample Name: Pink C6 + CHCl3 Extract 9/601 in CH_2Cl_2 solvent
Misc Info : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 1

Col: DB-1 60m x 0.32mm x 1.0 μm Oven: 50°C (0 min) 15→290°C (44 min)

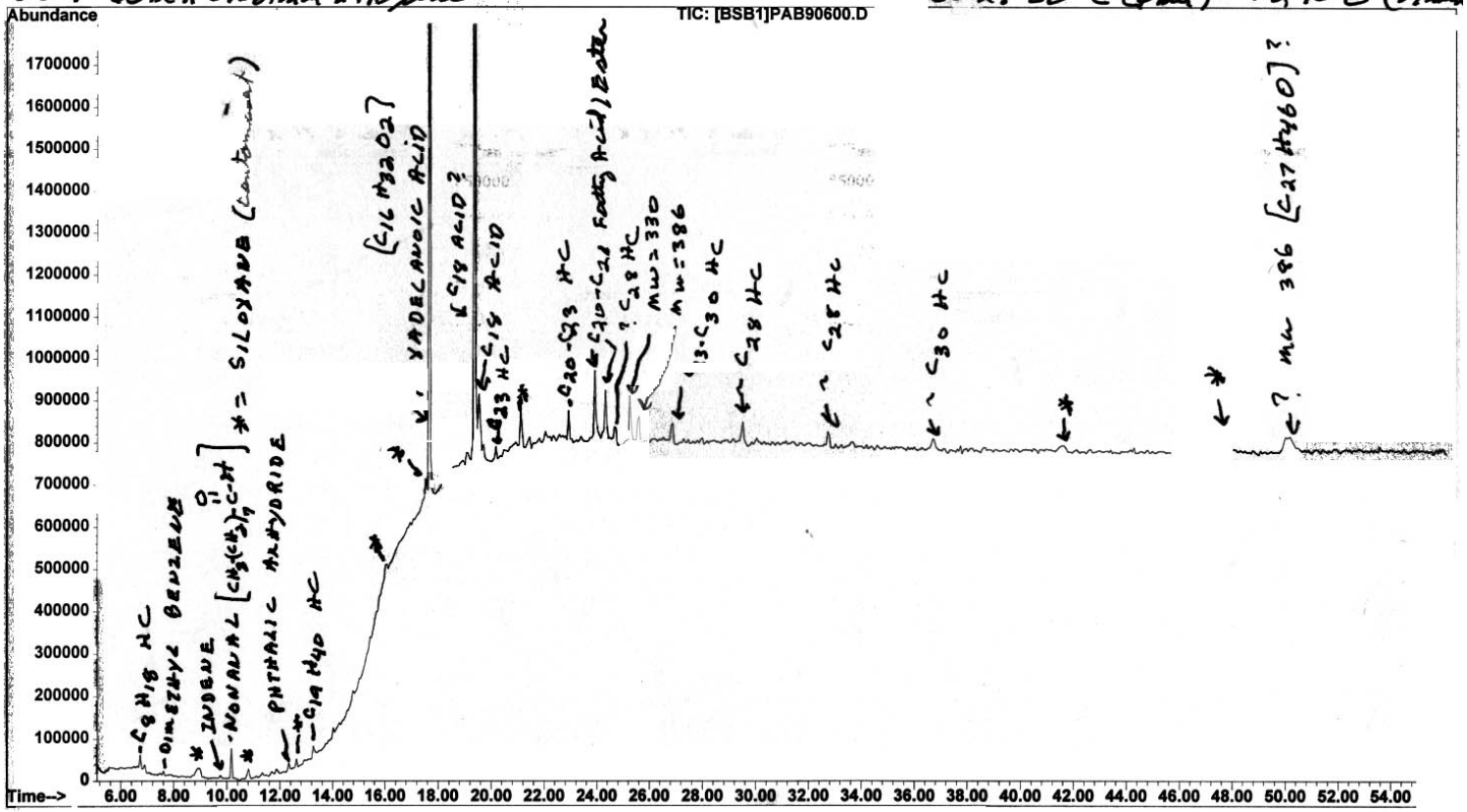


Figure 3. GC chromatogram of the hexane + chloroform extract from the pink tissue of the mutilated cow (extraction #1).

```
File       : C:\HPCHEM\4\DATA\BSB\PAB90602.D
Operator   : [BSB1]RLW 9/6/01
Acquired   :  6 Sep 2001  12:42     using AcqMethod DB1SVO
Instrument :   GC/MS #4
Sample Name: Pink  AC + H20 Extract  9/601   in methanol
Misc Info  : Semivol Org Analysis 1 ul Splitless EM+2
Vial Number: 1
```

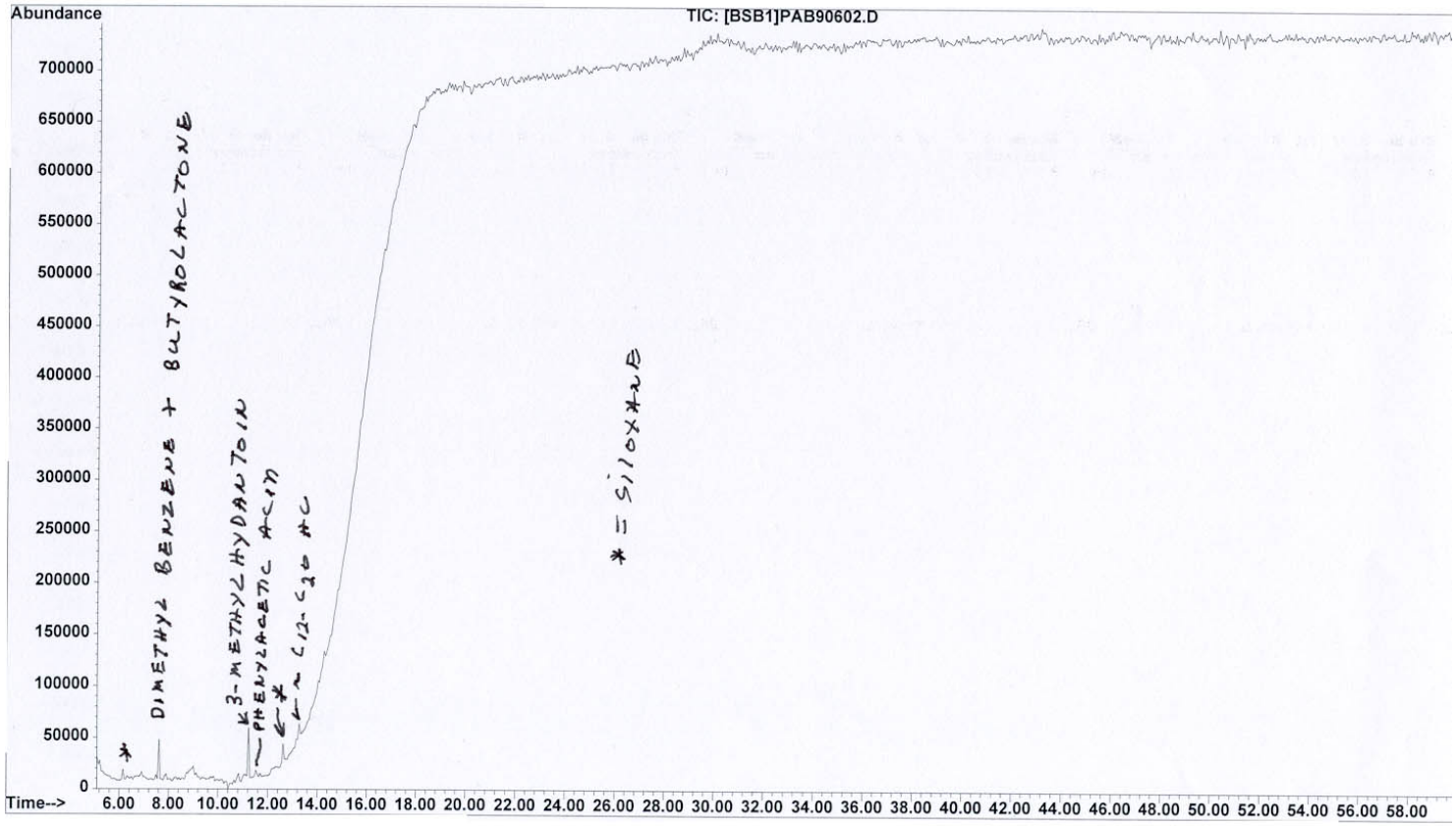


Figure 4. GC chromatogram of the acetone + water extract from the pink tissue of the mutilated cow (extraction #1).

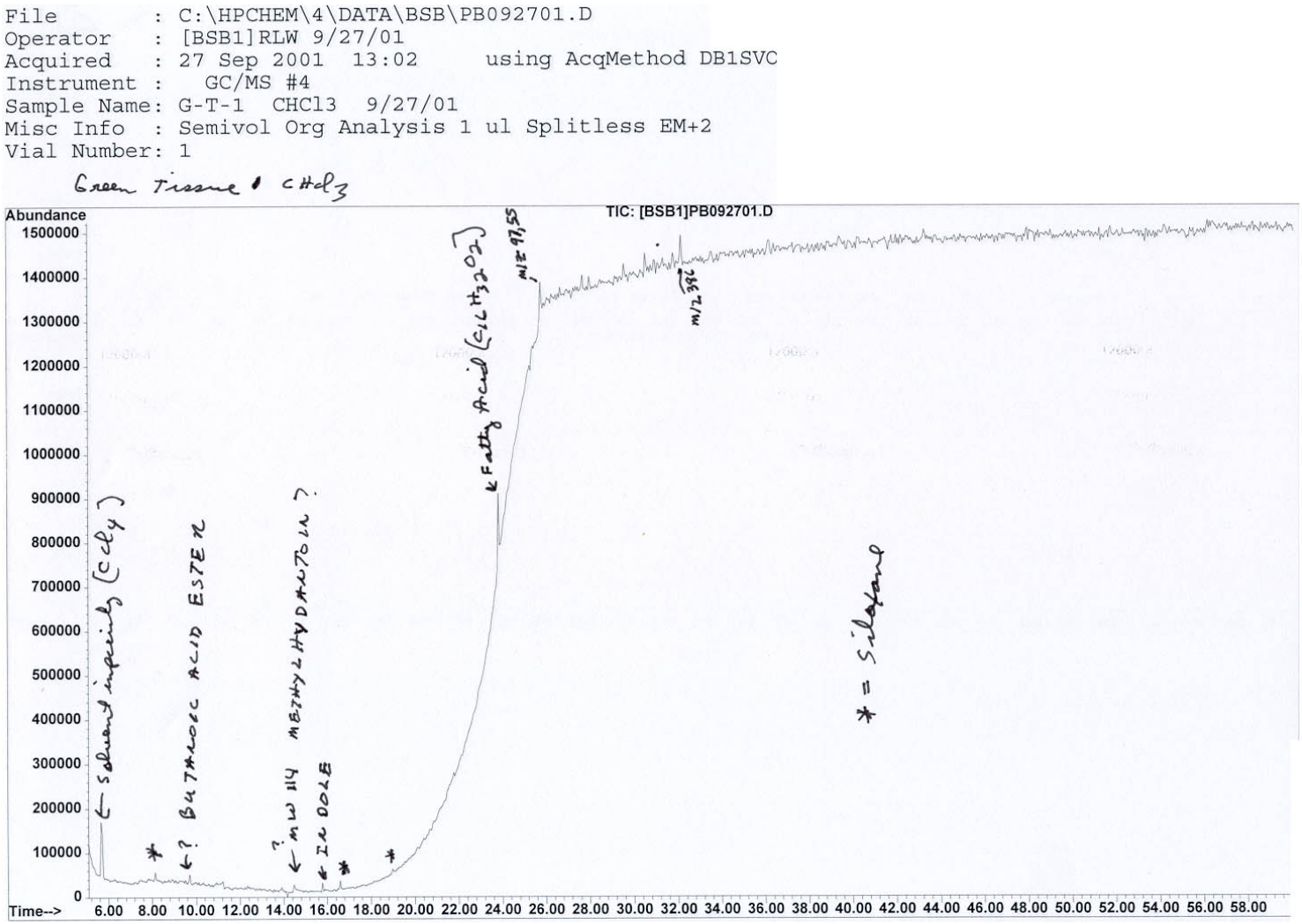


Figure 5. GC chromatogram of the chloroform extract from the mutilated cow tissue (extraction #2).

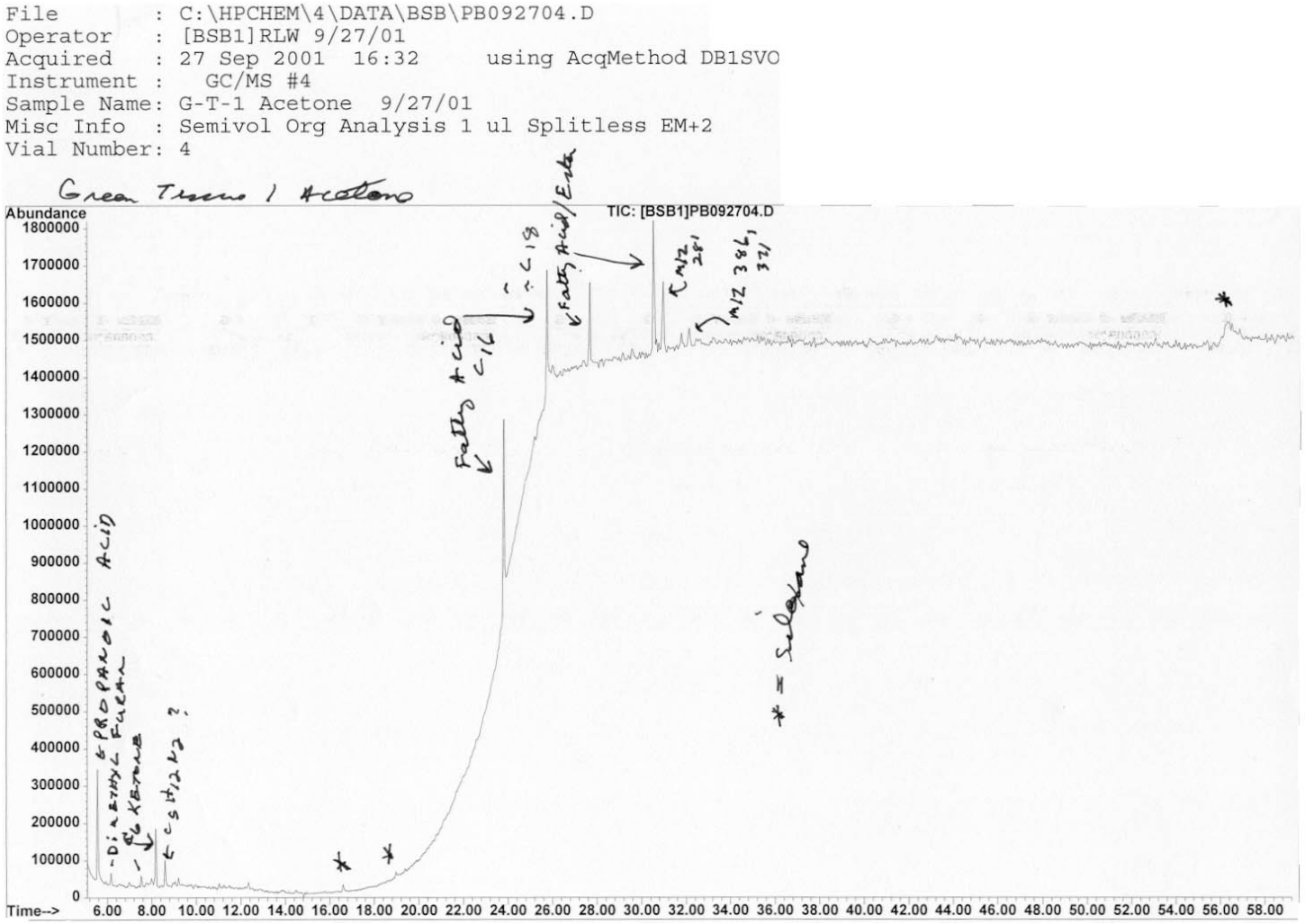


Figure 6. GC chromatogram of the acetone extract from the mutilated cow tissue (extraction #2).

```
File       : C:\HPCHEM\4\DATA\BSB\PAB10162.D
Operator   : [BSB1]RLW 10/16/01
Acquired   : 16 Oct 2001  17:54     using AcqMethod RWSVM
Instrument :   GC/MS #4
Sample Name: Green Tissue  10/16/01
Misc Info  : Semivol Organic Analysis 1ul splitless EM+2
Vial Number: 4
```

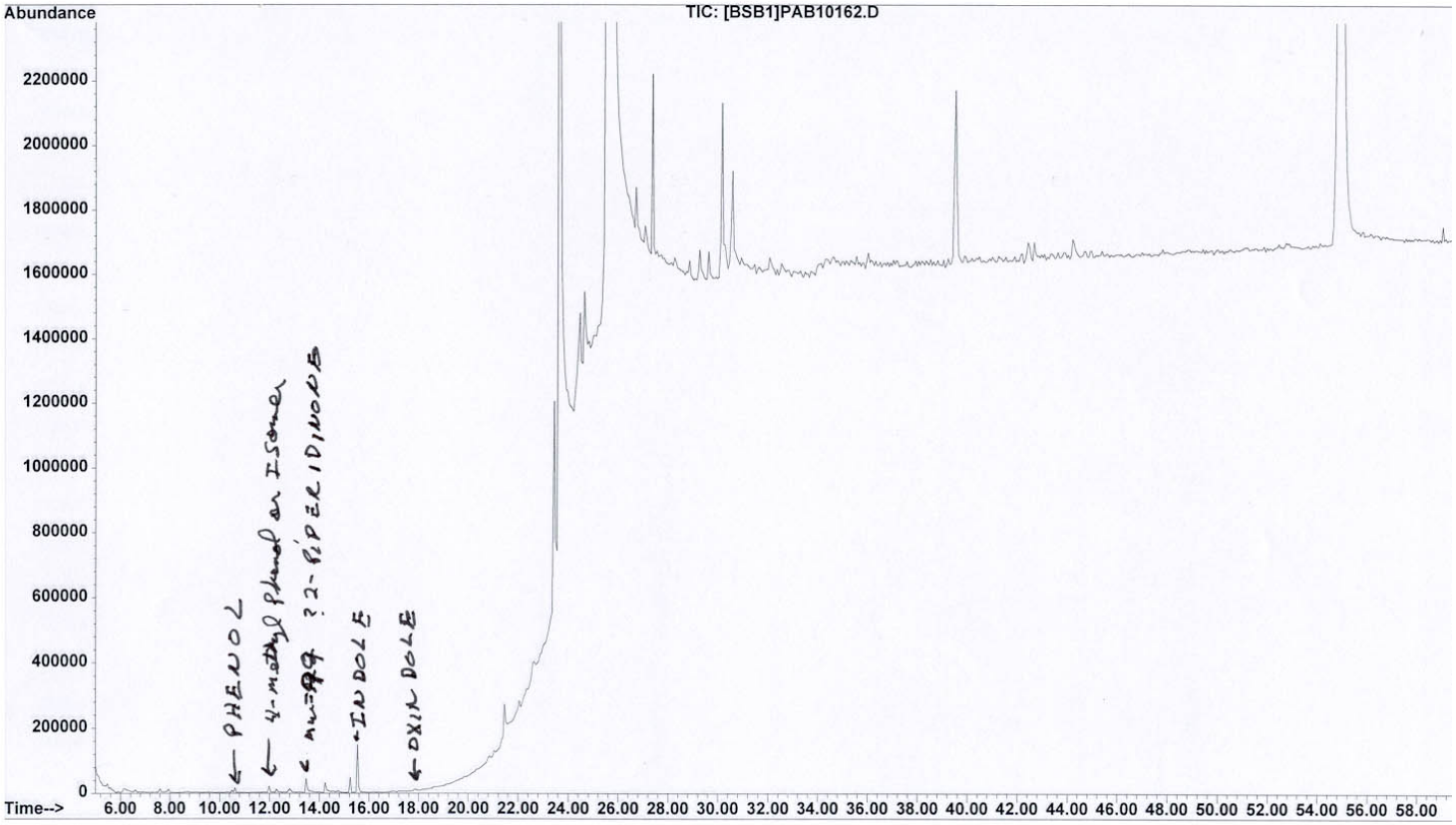


Figure 7. GC chromatogram of the refrigerated chloroform extract from the mutilated cow tissue (extraction #3).

```
File        : C:\HPCHEM\4\DATA\BSB\PAB1204.D
Operator    : [BSB2]RLW 12/4/01
Acquired    :  4 Dec 2001  17:10      using AcqMethod RWSVM
Instrument :   GC/MS #4
Sample Name: Control ( CHCl3 Ext Muscl2), 12/4/01
Misc Info   : Semivol Organic Analysis 1ul Splitless EM+2
Vial Number: 2
```

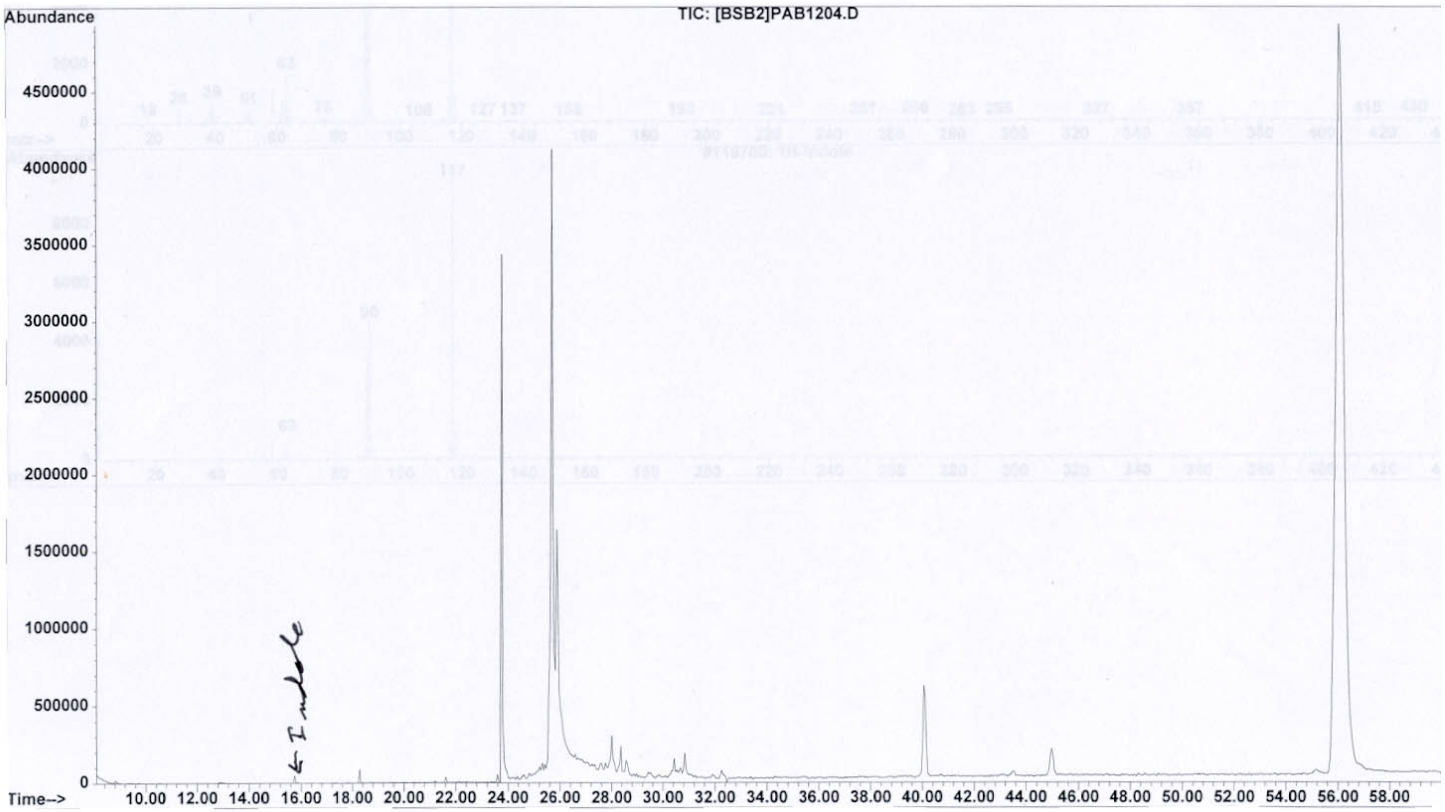


Figure 8. GC chromatogram of the refrigerated chloroform extract from the control heifer tissue (extraction #3).

Library Searched C:\DATABASE\wiley.L
Quality 41
ID 2H-Indol-2-one, 1,3-dihydro-

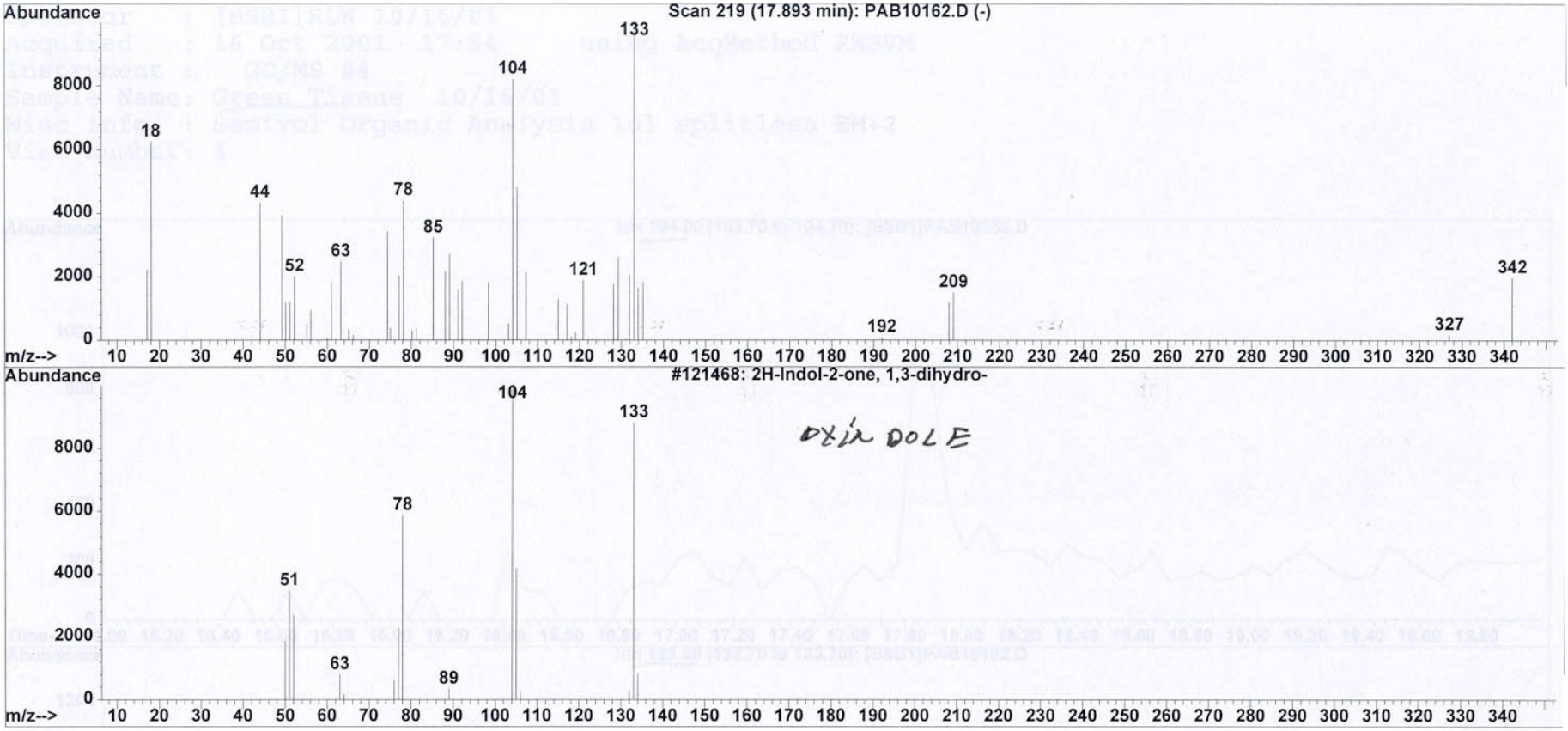
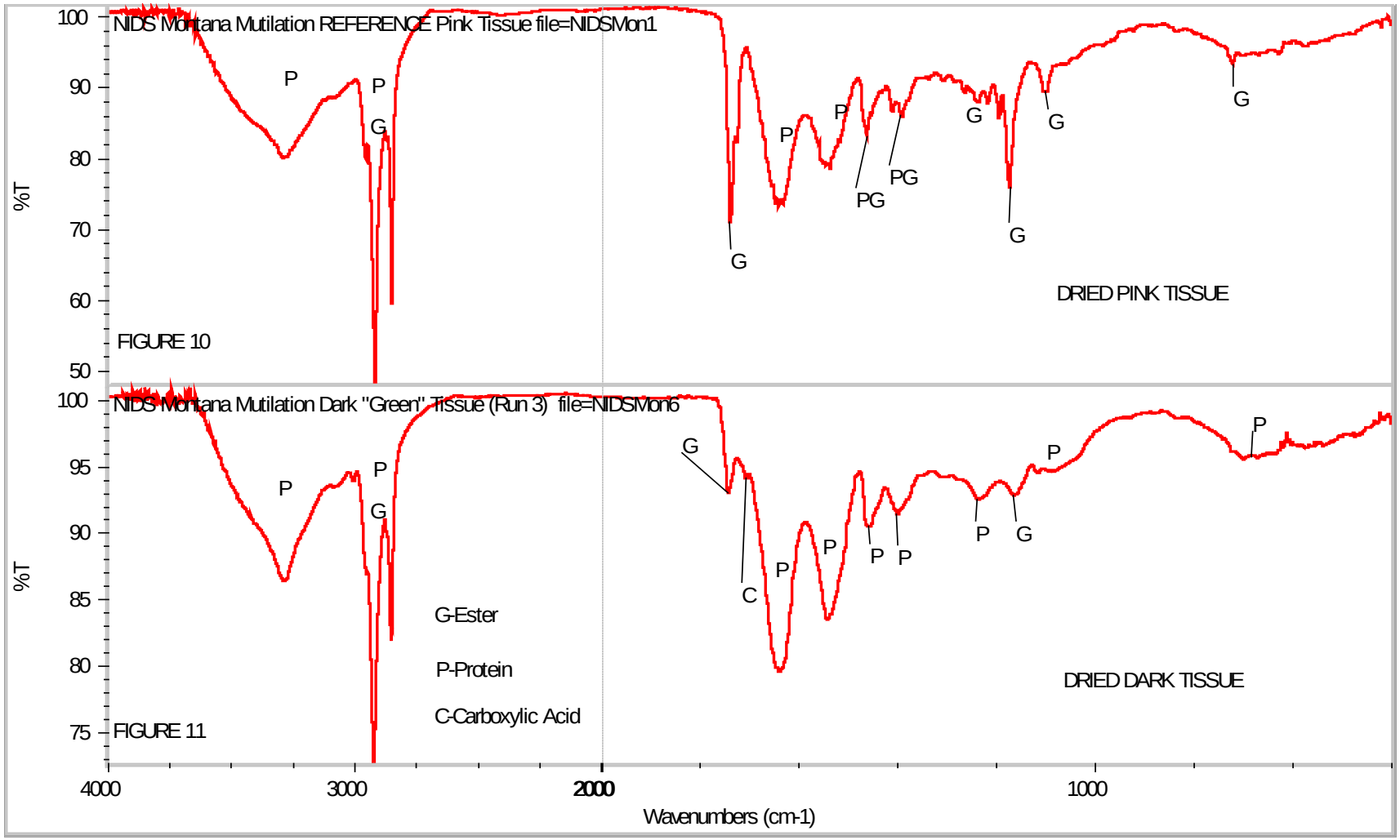
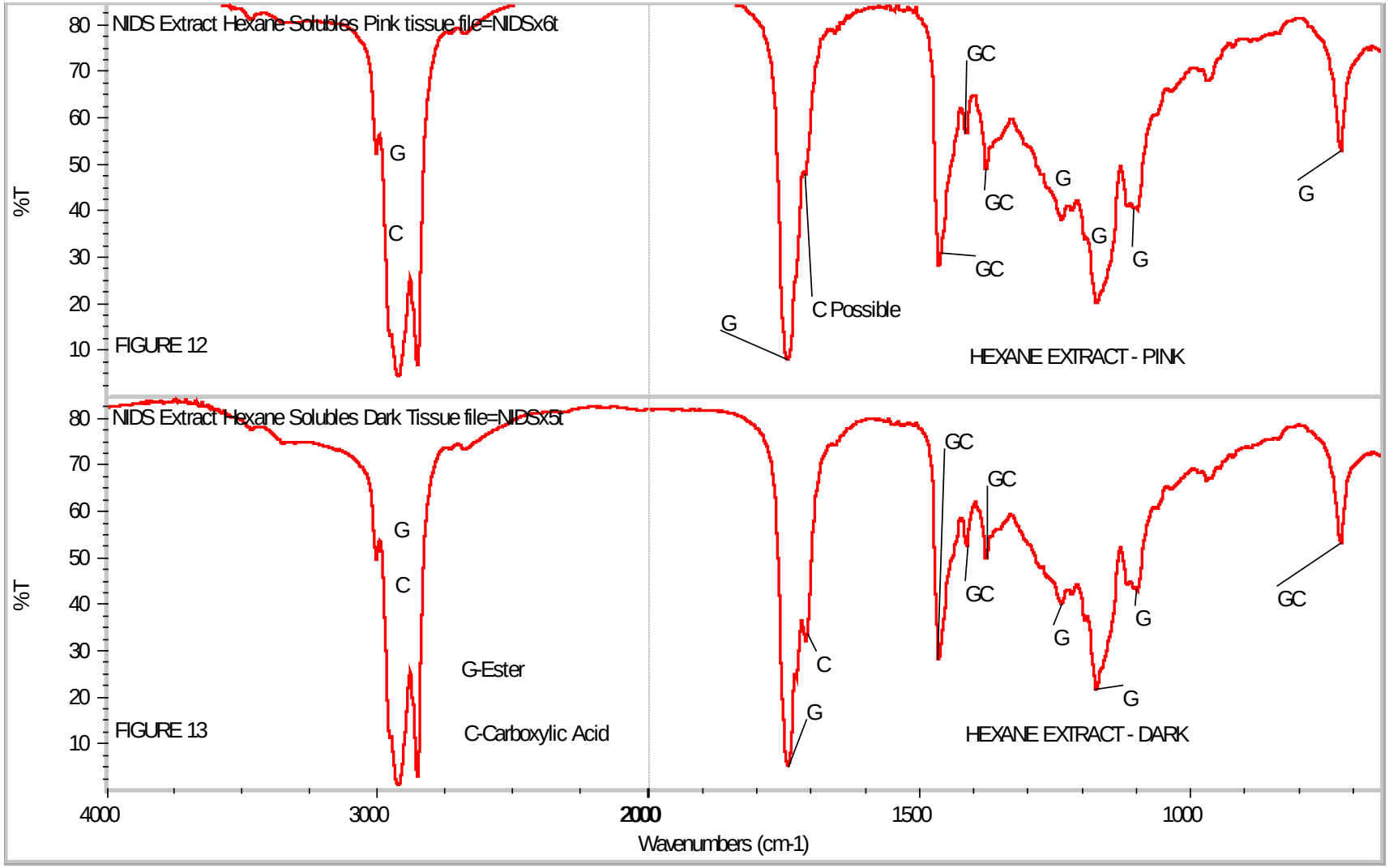


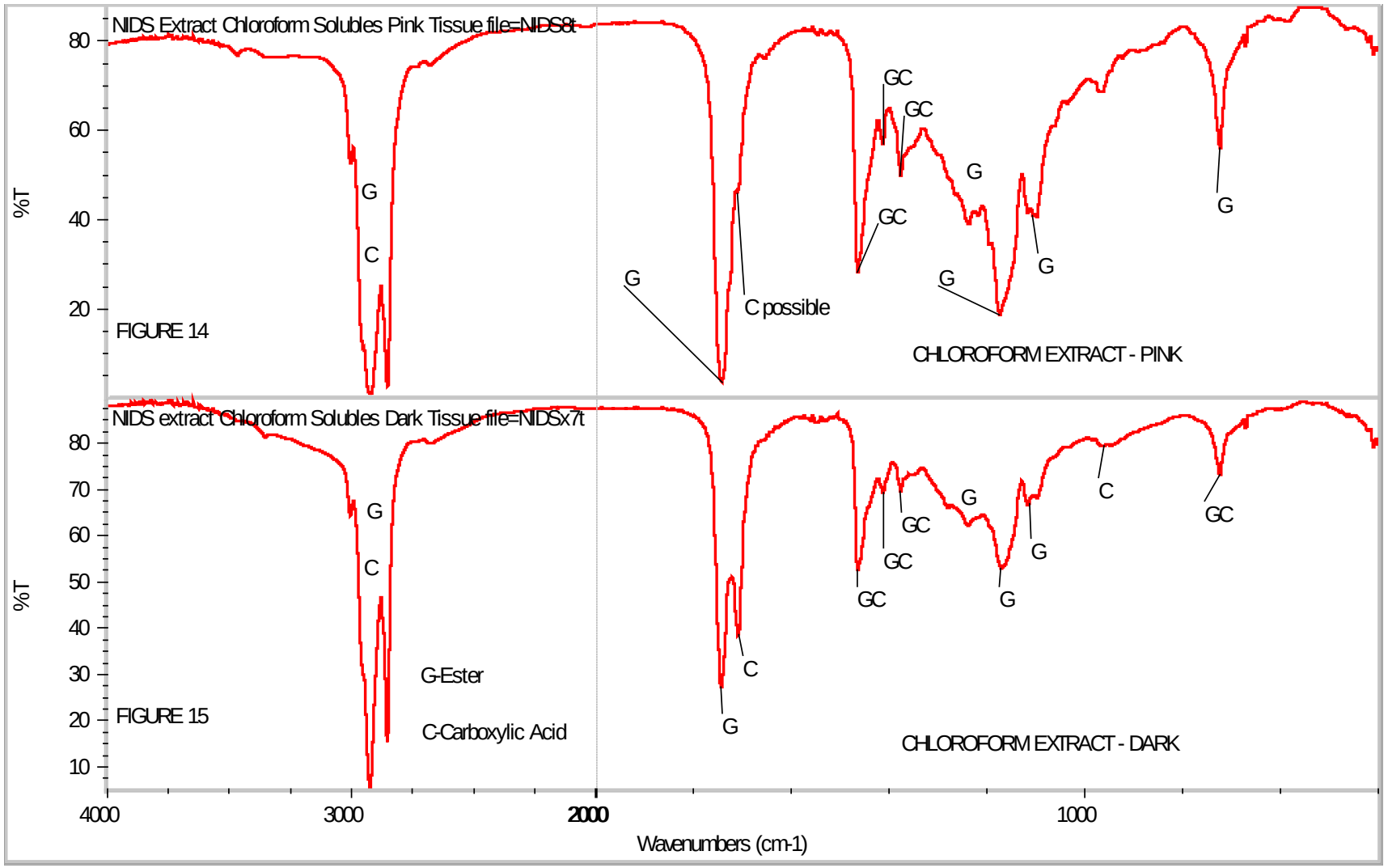
Figure 9. MS spectrum of oxindole from GC peak with retention time 17:54 min. of the chloroform extract from the mutilated cow (extraction #3) and oxindole reference.



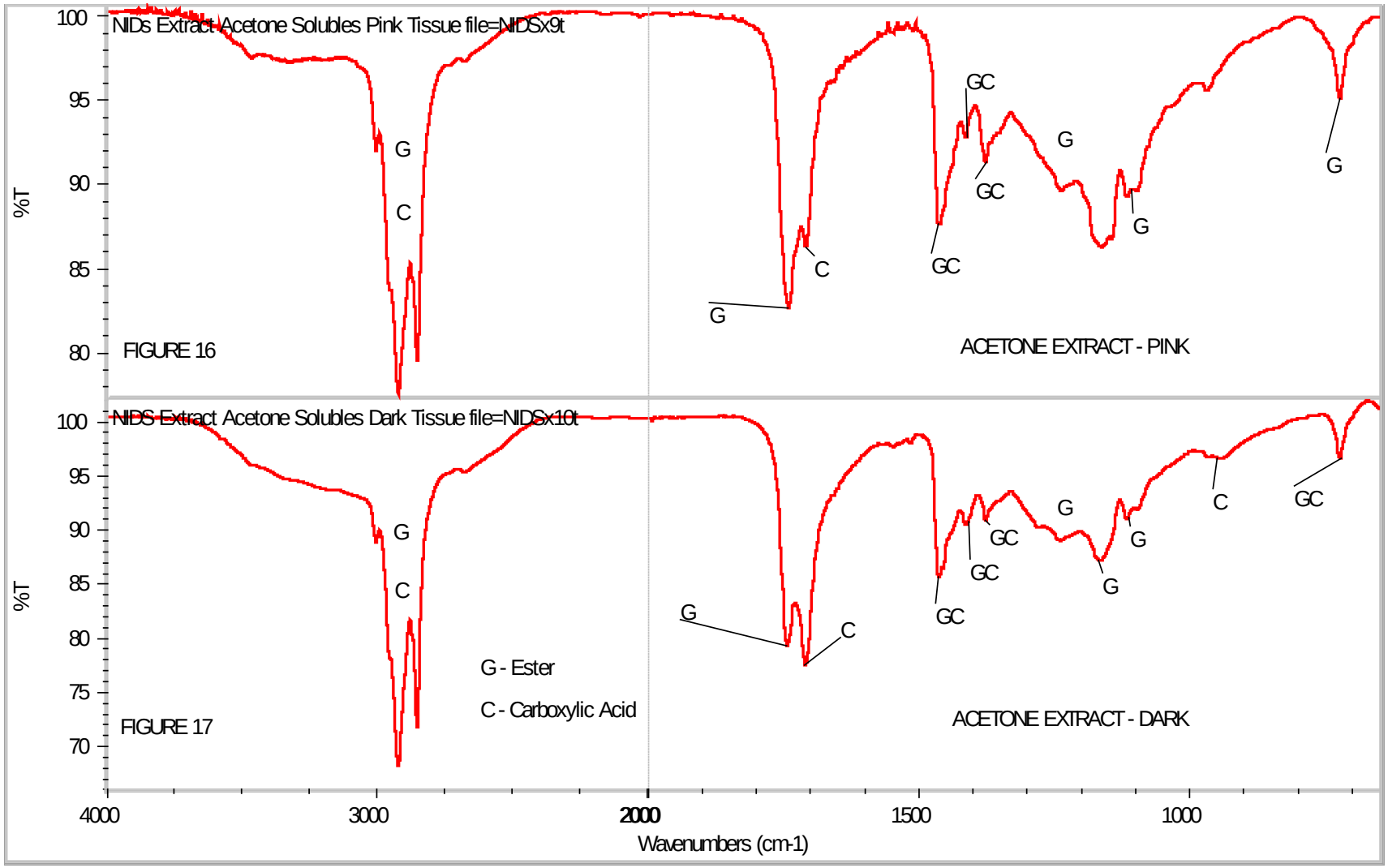
Figures 10, 11: Infrared spectra of dried pink and dark tissues from the mutilated cow.



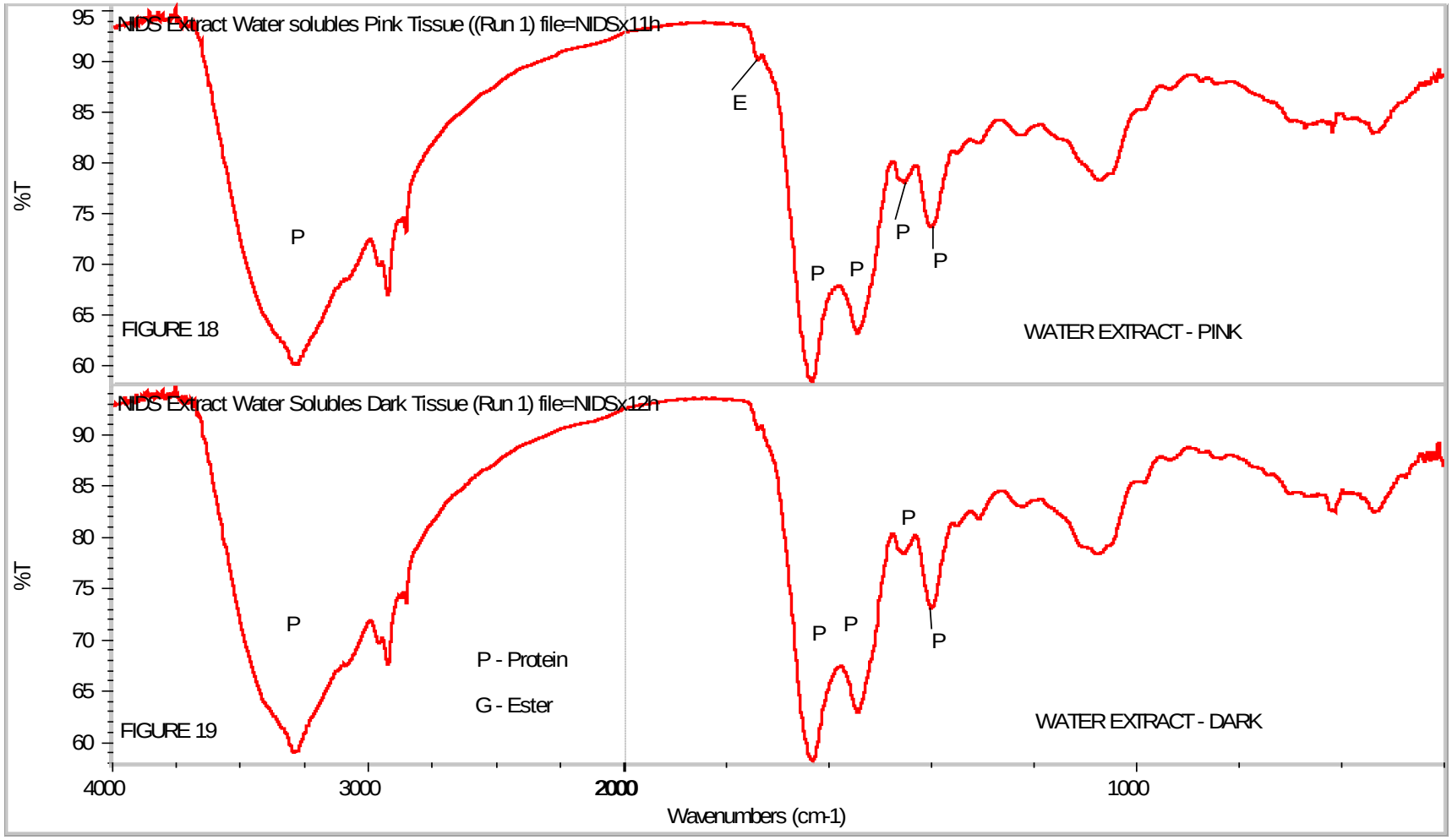
Figures 12,13: Infrared spectra of hexane extracts from the pink and dark tissues from the mutilated cow.



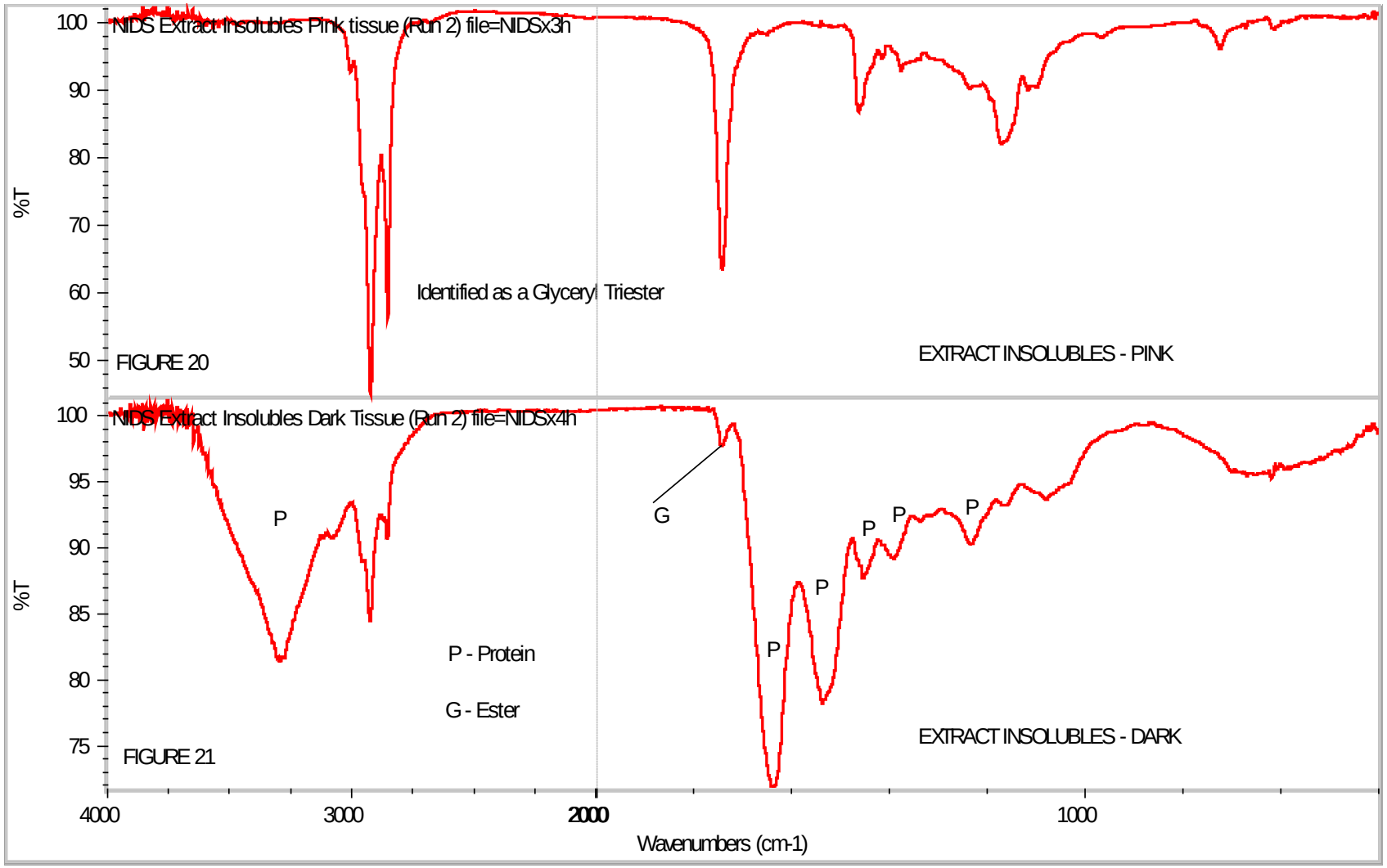
Figures 14,15: Infrared spectra of chloroform extracts from the pink and dark tissues from the mutilated cow.



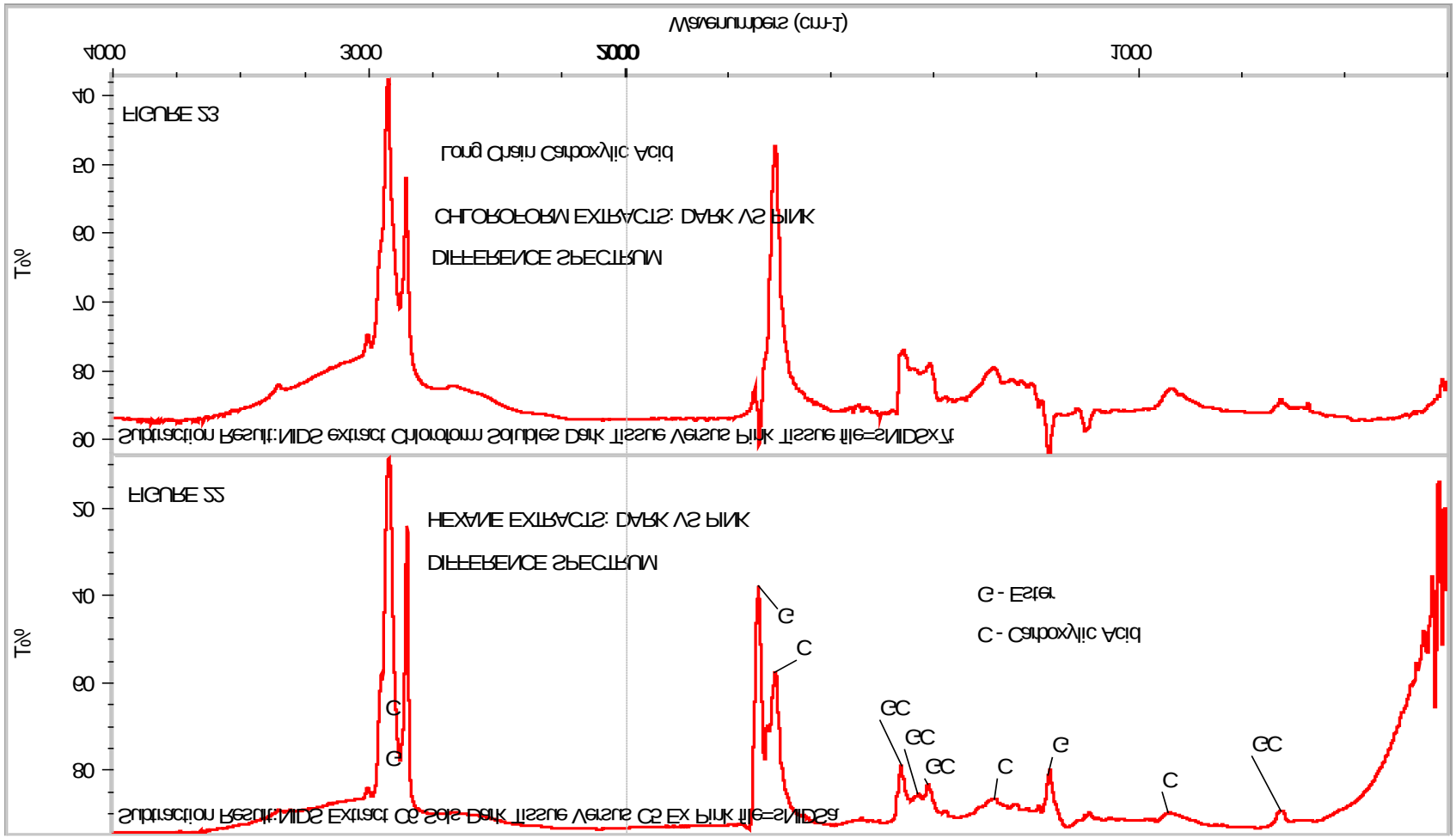
Figures 16, 17: Infrared spectra of acetone extracts from the pink and dark tissues from the mutilated cow.



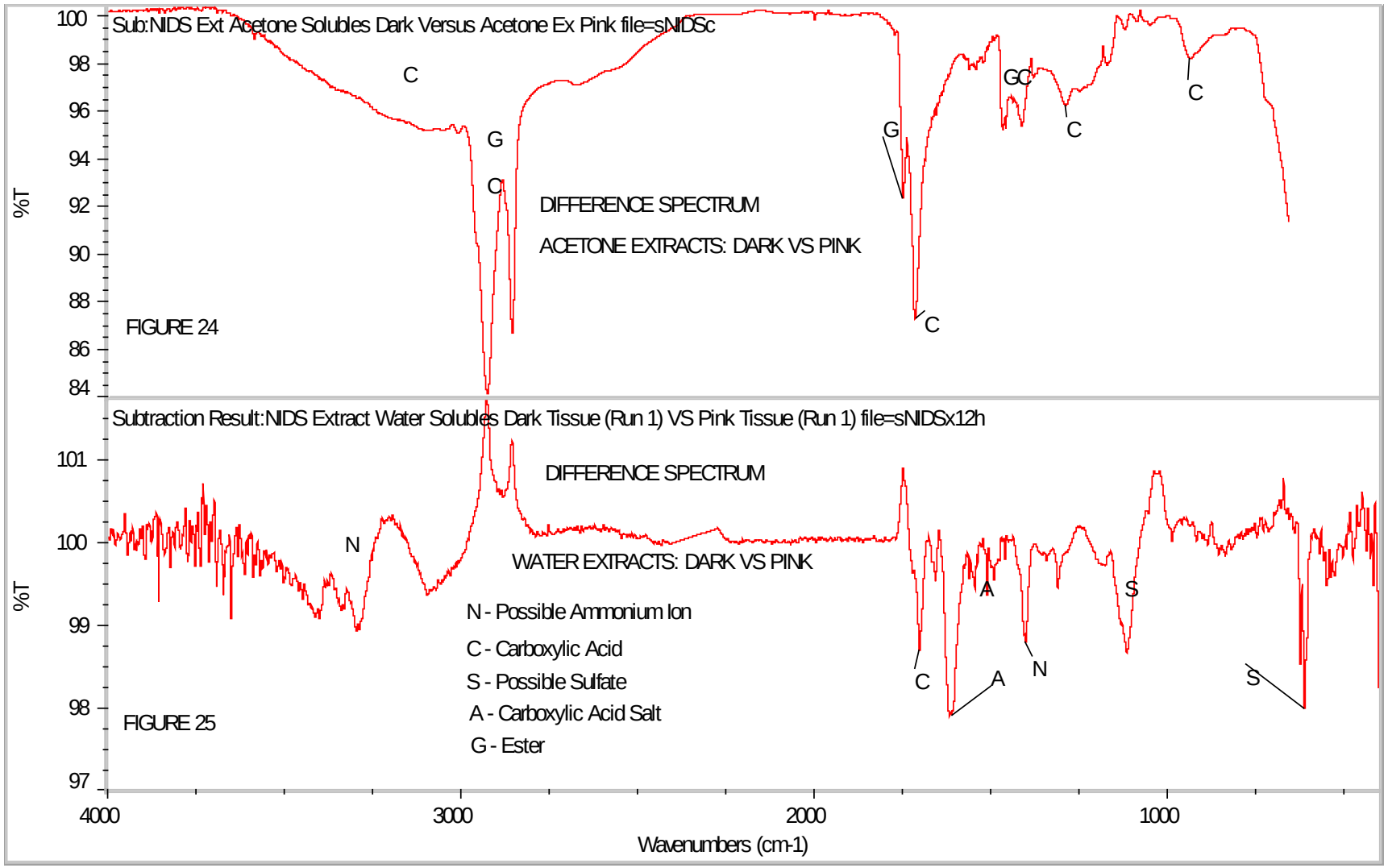
Figures 18, 19: Infrared spectra of water extracts from the pink and dark tissues from the mutilated cow.



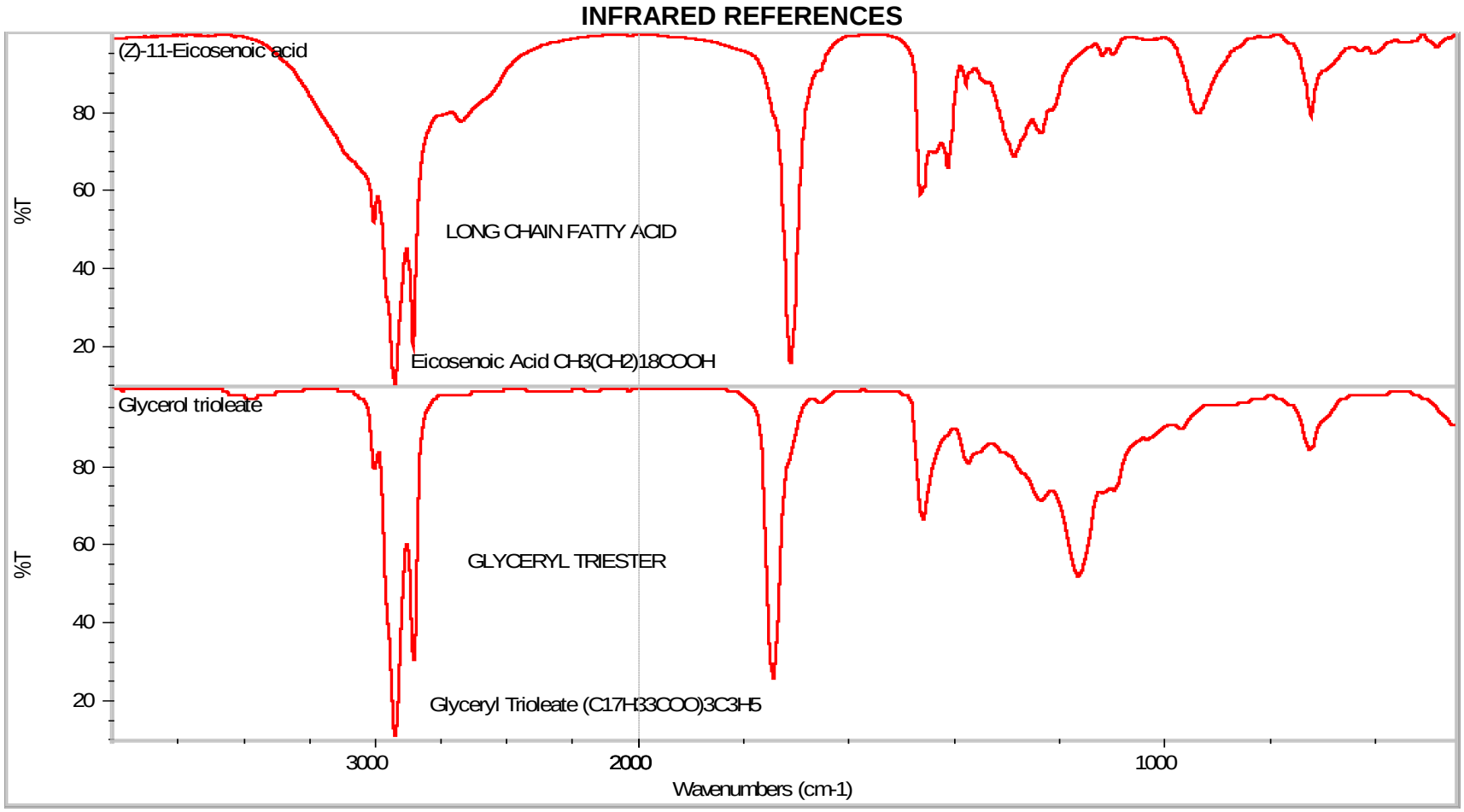
Figures 20, 21: Infrared spectra of water extracts from the pink and dark tissues from the mutilated cow.



Figures 22, 23: Difference spectra of hexane and chloroform exacts from the pink and dark tissues from the mutilated cow.



Figures 24, 25: Difference spectra of acetone and water exacts from the pink and dark tissues from the mutilated cow.



Figures 26, 27: Infrared reference spectra of a long chain fatty acid and a glyceryl triester.

File : C:\HPCHEM\4\DATA\BSB\PAB09201.D
Operator : [BSB2]RLW 9/20/01
Acquired : 20 Sep 2001 14:42 using AcqMethod RWSVM
Instrument : GC/MS #4
Sample Name: Vitreous Fluid (Montana Cow 6/01) 9/20/01
Misc Info : Semivol Organic Analysis 1 ul Splitless
Vial Number: 1

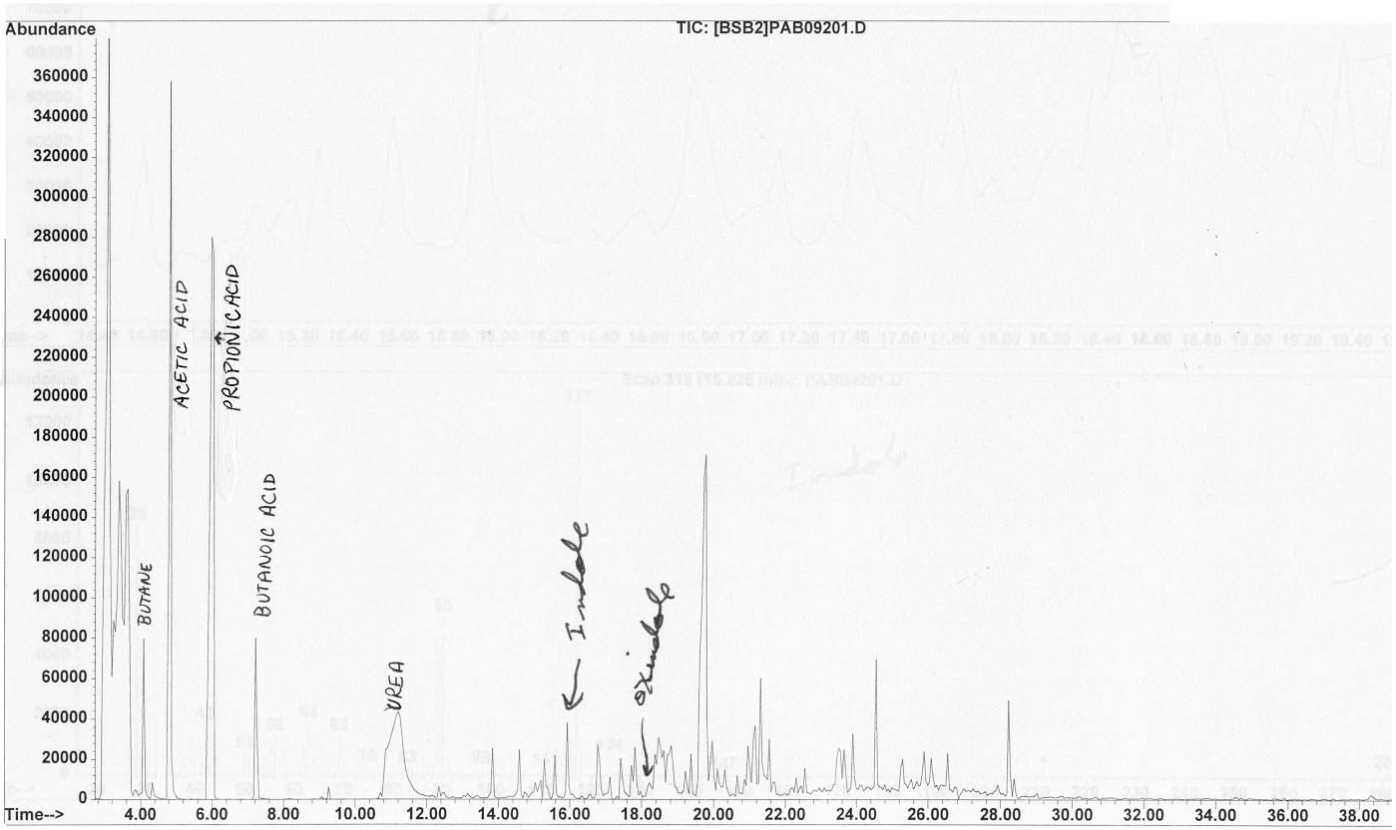


Figure 28. GC Chromatogram of the vitreous fluid from the mutilated cow.

File : C:\HPCHEM\4\DATA\BSB\PAB11130.D
Operator : [BSB3]RLW 11/13/01
Acquired : 13 Nov 2001 13:05 using AcqMethod RWSVM
Instrument : GC/MS #4
Sample Name: Left Eye Fuid 10/18/01 11/13/01
Misc Info : Semivol Organic Analysis 1ul splitless EM+2
Vial Number: 1

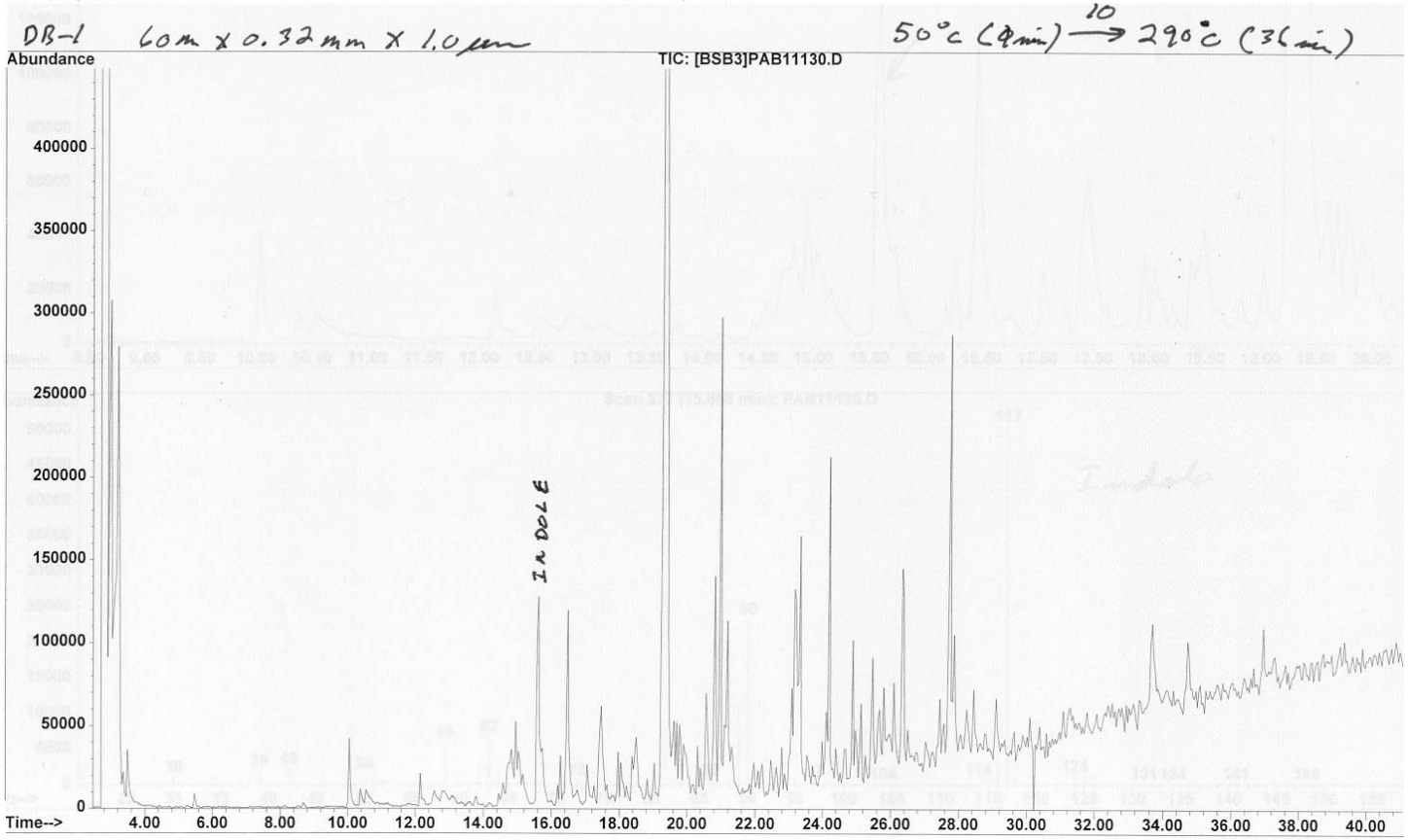


Figure 29. GC Chromatogram of the vitreous fluid from the control heifer.

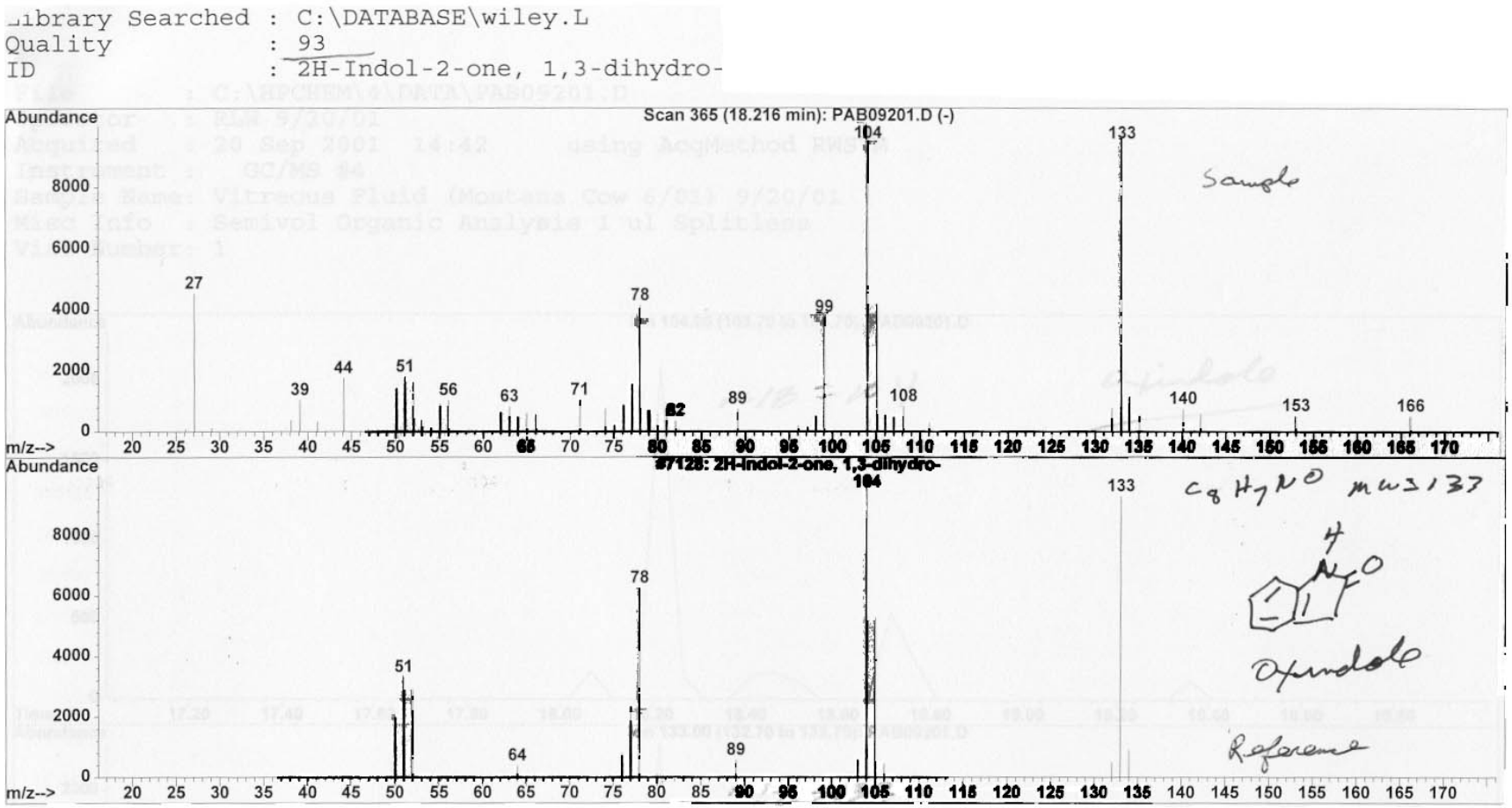


Figure 30. MS spectrum of oxindole from GC peak with retention time 18.22 min. from the vitreous fluid of the mutilated cow.

```
File        : C:\HPCHEM\4\DATA\PAB11131.D
Operator    : RLW 11/13/01
Acquired    : 13 Nov 2001  15:23      using AcqMethod RWSVM
Instrument  :   GC/MS #4
Sample Name: Right Eye Fluid  11/13/01
Misc Info   : Semivol Organic Analysis 1ul splitless EM+2
Vial Number: 3
```

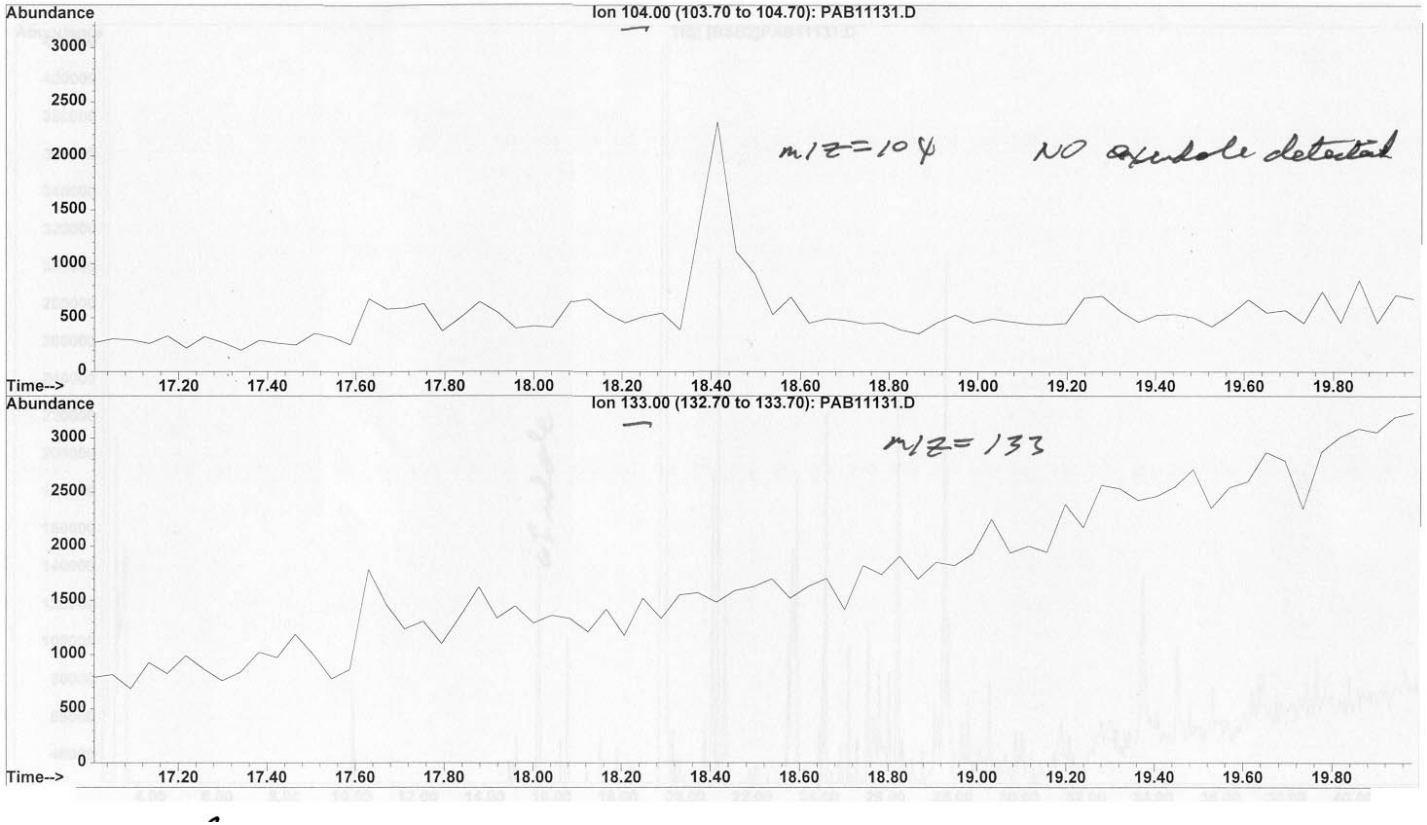


Figure 31. Ion scan for masses of 104 (Top) and 133 (Bottom) of the vitreous fluid from the control heifer.

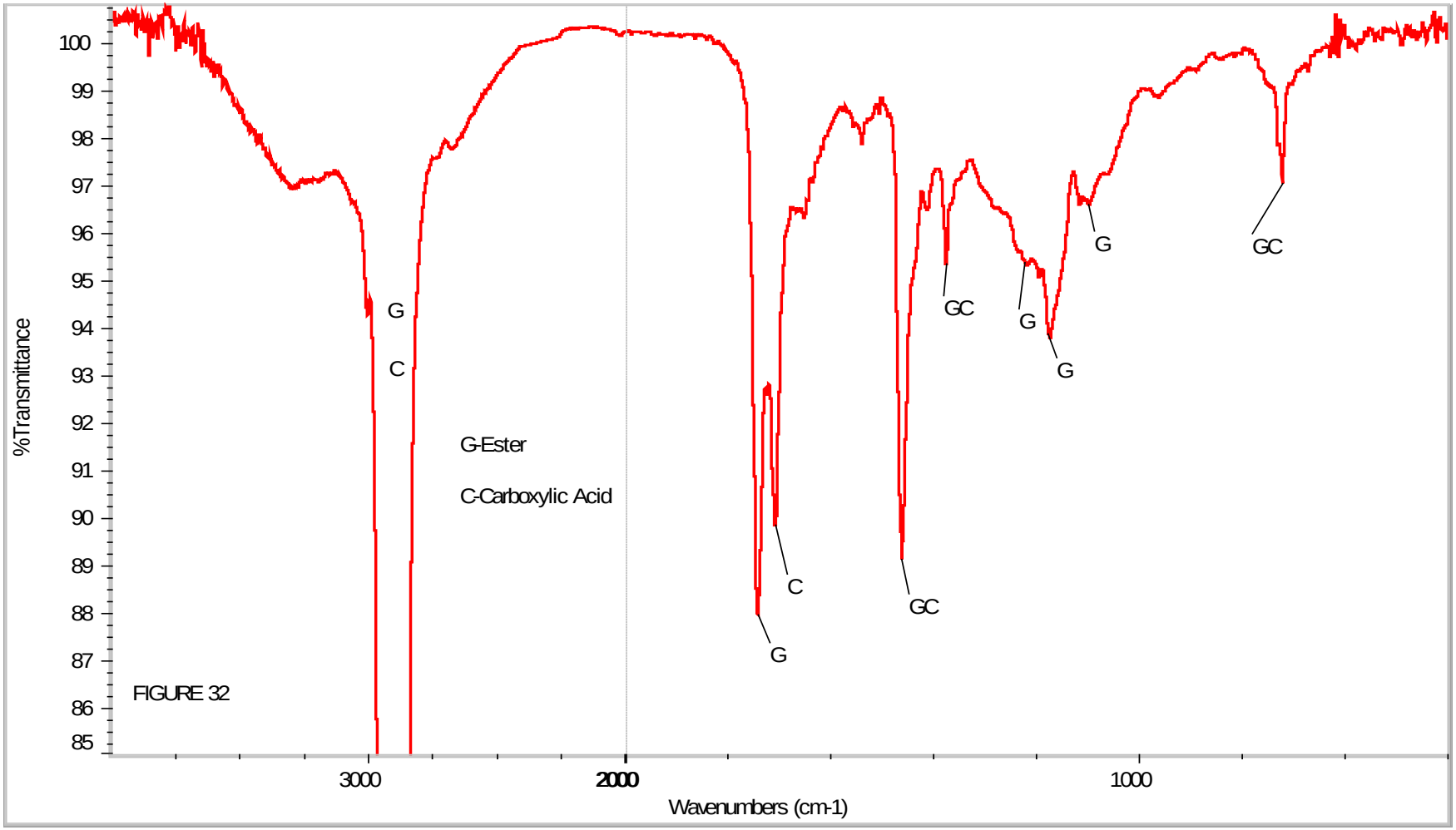


Figure 32. Infrared spectrum of the maggot mass chloroform extract (extraction#3) from the mutilated cow.