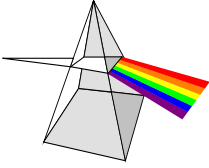




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## **Frontier Analysis, Ltd**

### **TECHNICAL SERVICE RESPONSE NO.: UT029**

**Subject:** Analysis of the Vitreous Fluid from Cow (#8) Mutilated April 7, 2003  
(Valparaiso, Nebraska)

**Date:** October 31, 2003

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

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

**Analyzed By:** Richard Wilson

### **Background/Objective:**

A cow (#8) was found mutilated on the morning of April 7, 2003. "...knife marks were found near the animal's teat which was intact but partially removed. No blackening was noted around the animal's mouth or tongue, but a bite shaped wound was apparent in the roof of the animal's mouth as well as on the tongue, consistent with, according to the veterinarian, the animal biting down hard. The animal was found dead in eight inches of mud, about 70 feet from the roadway. The owner and local law enforcement noticed both fresh vehicle tracks in the snow as well as three separate sets of footprints in the snow."<sup>1</sup> Vitreous fluid was removed from the animal's eye and submitted for GC/MS analysis to determine if any foreign materials are present. Identification of foreign material could indicate whether the animal had been drugged before euthanasia.

### **Conclusions:**

- 1.) Analytical results do not confirm the presence of an unusual substance. However, the possibility of a brominated material is not ruled out. Expensive, additional analysis would be necessary to confirm its presence<sup>2</sup>. Additionally, tryptophan derivatives are present, which would

<sup>1</sup> Background description copied from the NIDS website.

<sup>2</sup> Possible additional tests may include GC/ICP or GC/XRF, but there may not be enough sample available. High resolution MS is also a possibility.

- be expected to be found naturally in an animal. However, forms of it are also known for their sedative properties. It may be speculated that a derivative might be used as a ruse to avoid drug detection. And finally, it is possible the animal was not drugged.
- 2.) No oxindole is detected. This is a foreign substance which had been found in another mutilated cow. (See Frontier Analysis T.S.R. Nos.: UT016 and UT022.) Derivatives of oxindole are known for sedative properties.
  - 3.) Other materials detected in the vitreous fluid are attributed to natural and possible decomposition products.

### **Procedure:**

The vitreous fluid sample from Cow #8 was received by Richard Wilson on 8/27/03. It was enclosed in a cooled 50 ml Falcon culture container. GC/MS data were acquired from the 'as received' sample. These data were compared to the vitreous fluid from a control heifer. Samples from the control animal were submitted for reference on 11/13/2001. These were intended to be used as a benchmark for cattle mutilation events. (See Frontier Analysis TSR# UT022 for background on the control animal and the testing of its vitreous fluid.) The detailed information regarding the instrumental data acquisition conditions for both samples are the same, and can be found in the appendix. GC/MS data were additionally acquired from a reference sample of bromotoluene using the same acquisition conditions as for the vitreous fluid. This was done to confirm, or rule out, the possible identification of a peak with a retention time of 23.65 from the cow #8 fluid.

Two extracted ion chromatogram runs were also done. One scan was made for ions of M/Z 77 and M/Z 130 between 35.00 and 40 minutes for tryptophan and its derivatives. Another scan was for ions of M/Z 77 and M/Z 130 between 16.60 to 18.60 minutes. These are ions characteristic of oxindole. It had been found in another mutilation case. (See Frontier Analysis T.S.R. No.: UT016.)

### **Results:**

The GC chromatogram of the vitreous fluid from cow #8 shows an abundance of components. The majority were identified or postulated by MS spectral analysis. Table I lists the MS identifications of the control heifer GC peaks along with those of the eye fluid from the mutilated animal to conveniently compare the data. The GC chromatograms are displayed in figures 1 and 2.

The data suggest that of most GC peaks from the cow #8 fluid are natural and putrefaction products existing in the animal after euthanasia. However, one peak at a retention time of 23.65 minutes was suspect, because it was tentatively identified as bromomethylbenzene (bromotoluene). The concentration is probably less than 25 ppm.

Further confirmation of the 23.65 peak was necessary. If bromotoluene is present, it would indicate foreign material, for example, halothane a commonly used anesthetic was administered to the animal<sup>3</sup>. For this reason a sample of bromotoluene was purchased, and GC/MS data were collected using identical conditions as the #8 cow fluid. The retention time of bromotoluene was found to be 12.9 minutes which is quite different than that in the #8 cow fluid. However, the mass spectrum is quite similar. A match of both retention time and mass spectrum is necessary for a confirmation of this structure. The 23.65 peak mass spectrum of the #8 cow has a M/Z of 91 showing the material is definitely a toluene derivative. While not specifically bromotoluene, the 23.65 peak could still contain a brominated structure. This would have to be confirmed by another technique. The chromatogram of the bromotoluene reference is shown in figure 3, and the mass spectrum displayed in figure 4. An expanded GC chromatogram from a retention time range of 22 – 25 minutes of the cow #8 fluid is shown in figure 5. The mass spectrum of the 23.65 peak is shown in figure 6.

An extracted ion chromatogram for tryptophan derivatives shows they are present. Two masses, 77 and 130, typical of this type of structure would be present at identical retention times. The scans for these masses ranged between 35 - 40 minutes. Both masses were found at the same retention times. See figure 7 for the ion chromatogram.

An extracted ion chromatogram for oxindole shows it is not present. Figure 8 is the ion chromatogram which shows the results of scans for oxindole expected masses of 104 and 133 between GC retention times of 16.40 to 18.60 min. These ions were not found at identical retention times. Oxindole is an unusual material which had been detected in another animal mutilation event. (See Frontier Analysis T.S.R. Nos: UT016 and UT022.)

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Phyllis A. Budinger

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<sup>3</sup> Com Kelleher, October 1, 2003 email.

**APPENDIX**

### GC/MS Data Acquisitions Conditions

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 the Vitreous Fluid of the Mutilated Cow and the Control Heifer**

Valparaiso Cow #8			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Phosphine? (MW=34 H <sub>3</sub> P)	7	3.353	-	-	-
•Acetaldehyde	80	3.518	•Acetaldehyde	39	3.191
•Methanethiol (MW=48 CH <sub>4</sub> S)	72	3.725	-	-	-
-	-	-	•Methanamine, N,N-dimethyl- (Trimethylamine)	72	3.480
•Urea? (MW=60 CH <sub>4</sub> N <sub>2</sub> O)	9	4.137	-	-	-
•Acetic Acid	72	4.880	-	-	-
•Formamide (MW=45 CH <sub>3</sub> NO)	78	8.799	-	-	-
•Lactic Acid (MW=90 C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> )	78	8.924	-	-	-
•1,2,3-Propanetriol	72	10.449	-	-	-
•1H-Pyrrole-2,5-dione (Maleimide) (MW=97 C <sub>4</sub> H <sub>3</sub> NO <sub>3</sub> )	86	10.572	•1H-Pyrrole-2,5-dione (Maleimide) MW=97 C <sub>4</sub> H <sub>3</sub> NO <sub>3</sub>	78	10.039
-	-	-	•Phenol (~15 ppm)	64	10.369
•2-Propanamine	9	11.150	-	-	-
•2,5-Pyrrolidinedione (Succinimide) (MW=99 C <sub>4</sub> H <sub>4</sub> NO <sub>2</sub> )	78	12.717	•2,5-Pyrrolidinedione (Succinimide) (MW=99 C <sub>4</sub> H <sub>4</sub> NO <sub>2</sub> )	80	12.143
•2-Amino-4-methyloxazole	47	13.089	-	-	-
-	-	-	•Nitrogen Compound (M/Z 44, 98)	-	12.597
•2-Amino-4,5-dimethyloxazole (MW=112 C <sub>5</sub> H <sub>8</sub> N <sub>2</sub> O) or Pyrimidinedione	50	13.419	-	-	-
•Pyrimidinedione Isomer (MW=112 C <sub>4</sub> H <sub>4</sub> N <sub>2</sub> O <sub>2</sub> )	16	14.326	-	-	-
-	12	-	•1,4-Cyclohexanedione (M/Z 112, 56 MW=112)	38	13.793
-	-	-	•M/Z 70 L-Proline	35	14.742
•1,3-Cyclopentanedione or deriv. (MW=98)	86	15.523	-	-	-

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

Valparaiso Cow #8			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Imidazolidinedione, 5-methyl- (MW=114 C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> O <sub>3</sub> )	17	15.729	•Parabanic acid (MW=114)	47	15.154
•1H-Indole (MW=117 C <sub>8</sub> H <sub>7</sub> N)	94	16.183	•1H-Indole	94	15.608
-	-	-	•Mepivacaine (M/Z 98)	43	15.732
•1,4-Cyclohexanedione	53	16.595	-	-	-
•MW=138	-	17.049	-	-	-
•3-Pyridinecarbothioamide? (MW=138)	11	17.090	-	-	-
-	-	-	•MW=138	-	16.474
-	-	-	•4(3H)-Pyrimidinone, 2-ethyl-3,6-dimethyl-2-Methyl-3-(2-thienyl)-2-propenal (MW=152)	38 64	16.763
1-Pentanamine, N-pentyl-	9	17.626	-	-	-
-	-	-	•M/Z 100	-	17.052
-	-	-	4-Morpholinebutyric acid, .beta.-methyl.alpha.,.alpha.-diphenyl	42	-
-	-	-	4,9-Decadien-2-amine, N-butyl-	42	-
•3-Hydroxy-2-isobut-1-enylcyclopent-2-en-1-one (MW=152 C <sub>9</sub> H <sub>12</sub> O <sub>2</sub> )	72	18.080	•M/Z 98 Ketone	-	17.423
-	-	-	3-n-Butylcyclohexanone	32	-
•Nitrogenated Compound (M/Z 85)	-	18.699	•Phenol, 3-methoxy-2,4,6-trimethyl- (MW=166)	30	17.959
•Nitrogenated Compound (M/Z 100)	-	18.988	-	-	-
•1-Methyl-5-amino-D2-1,2,4-triazole	50	19.070	-	-	-
-	-	-	•Hexahydropyrimidin-2-one (M/Z 100, 166)	40	18.496



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

Valparaiso Cow #8			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•3-Methoxy-2-methylphenol	25	19.606	-	-	-
-	-	-	• Acetamide, N-(2-nitrophenyl)- (M/Z 138, 180)	38	19.032
•L-Glutamic Acid (MW=147 M/Z=84 C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub> )	72	19.978	3-Methoxy-2-methylphenol	38	-
3-(2-Cyanophenyl)-2,3-dimethyl-1-butene	38	20.184	•M/Z 84 Glutamic Acid or Derivative L-Glutamic Acid	72	19.321
•M/Z=114	-	20.431	-	-	-
•2-Cyano-3-hydroxy-4,5,5-trimethyl-1-pyrroline 1-oxide (MW=168 C <sub>8</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> )	37	21.174	-	-	-
-	-	-	• Bicyclo [2.2.1]heptane-2-one, 3,3-dimethyl- (M/Z 138, 70)	53	20.558
•Filicinic acid	10	21.587	Endo-6-methylbicyclo[2.2.2]octan-2-one	47	-
-	-	-	-	-	-
•M/Z 116	-	21.793	•MW=154	-	20.971
-	-	-	6,8-Diazabicyclo[3.2.2]nonane-7,9-dione	35	-
•Benzene, (phenoxyethyl)- (M/Z 91 aromatic)	9	21.917	2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl-	14	-
•Benzene, (chloromethyl)- (M/Z 91 aromatic)	9	22.700	-	-	-
•Benzene, (bromomethyl)- (M/Z 91 aromatic)	52	23.649	•M/Z 116, 61	-	21.177
			Hexanoic, 2-methylpropyl ester	12	-
			-	-	-

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

Valparaiso Cow #8			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Benzene, 1-methoxy-3-(methylthio)- (M/Z 91 aromatic)	47	23.773	-	-	-
•2-Hydroxy-3,5,5-trimethyl-2-cyclohexenone (MW=154 C <sub>9</sub> H <sub>14</sub> O <sub>2</sub> )	59	23.814	-	-	-
-	-	-	•MW=154 2,4(1H,3H)-Pyrimidinedione, 1,3,6-trimethyl-	38	23.157
•Phenol, 3,5-dimethoxy (MW=154)	35	23.938	-	-	-
-	-	-	•MW=154 2,4(1H,3H)-Pyrimidinedione, 1,3,5-trimethyl- Phenol, 3,4-dimethoxy-	17 27	23.322
•Indole Derivative (M/Z 186, 117) Ketone, 3,3-dimethyl-3H-indol-2-yl methyl 1H-Indole	59 52	24.804	•M/Z 186, 117 Indole Derivative Probable 1H-Indole 4-fluoro-2', methylbiphenyl	50 83	24.188
•1H-Imidazole-2-methanol, 1-(phenylmethyl)- (M/Z 91 aromatic)	9	25.299	-	-	-
•Indole Derivative (MW=200 M/Z=117)	-	25.547	•M/Z 200, 117 Indole Derivative 1H-Indole	43	24.890
•M/Z=91 aromatic	-	25.670	-	-	-
-	-	-	•M/Z 91 Aromatic (Phenyl Group) Benzene, 1-nitro-4-(2-phenylethyl)- Benzaldehyde, 2-hydroxy-6-methyl-4-(phenol?)	35 35	25.467
•2,6-Piperazinedione, 4-(phenylmethyl)- (MW=204 C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> )	46	26.207	-	-	-
•MW=228 M/Z=186, 117	-	26.867	-	-	-

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

Valparaiso Cow #8			Control Heifer		
Compound	Match	GC Retention Time (min.)	Compound	Match	GC Retention Time (min.)
•Tetramethyl-1,2-cyclopentanedione (M/Z=70)	43	27.115	•Tetramethyl-1,2-cyclopentanedione (M/Z 70)	50	26.334
•Imizazolo(4,5-B)quinoxaline	53	28.311	-	-	-
-	-	-	•M/Z 186 Phenoxy Group Phenol, 3-phenoxy-	59	27.736
•Indole Deriv.. (M/Z=186, 117) 5H-Pyrido[2,3-b]indole, 6,7,8,9-tetrahydro-4-methyl-	38	28.599			
•Phenylalanine Derivative (MW=244) Phenylalanine-proline diketopiperazine	56	28.806	•Phenylalanine Derivative Phenylalanine-proline diketopiperazine	39	27.860
•Phenylalanine Derivative (MW=244) Phenylalanine-proline diketopiperazine	37	29.424	-	-	-
•1,2,3-Oxazaborinane, 2-butyl (M/Z=99)	16	32.477	-	-	-
•Acenaphthylene, 1,2-dihydro- ? (M/Z=154, 107)	32	35.158	-	-	-
• Phenol, 4-undecyl-? (M/Z=154)	10	36.313			
•D-Tryptophan or Deriv. (MW=204 C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> )	59	38.749			
•8-Amino-5-methylbenzo[b]carbazole-6,11(5H)-dione (MW=276)	45	39.120			

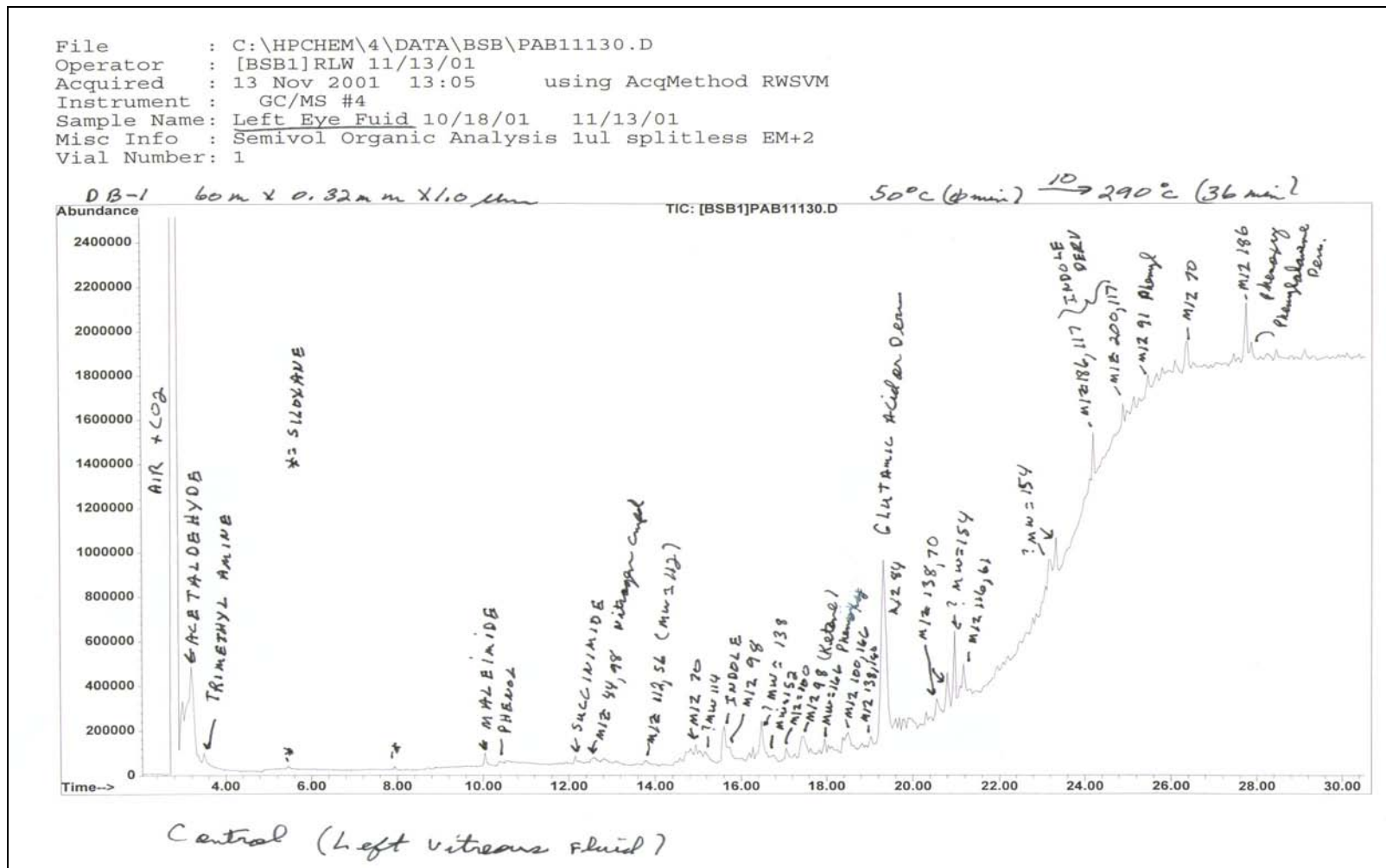


Figure 1. GC chromatogram of the vitreous fluid from the control heifer.

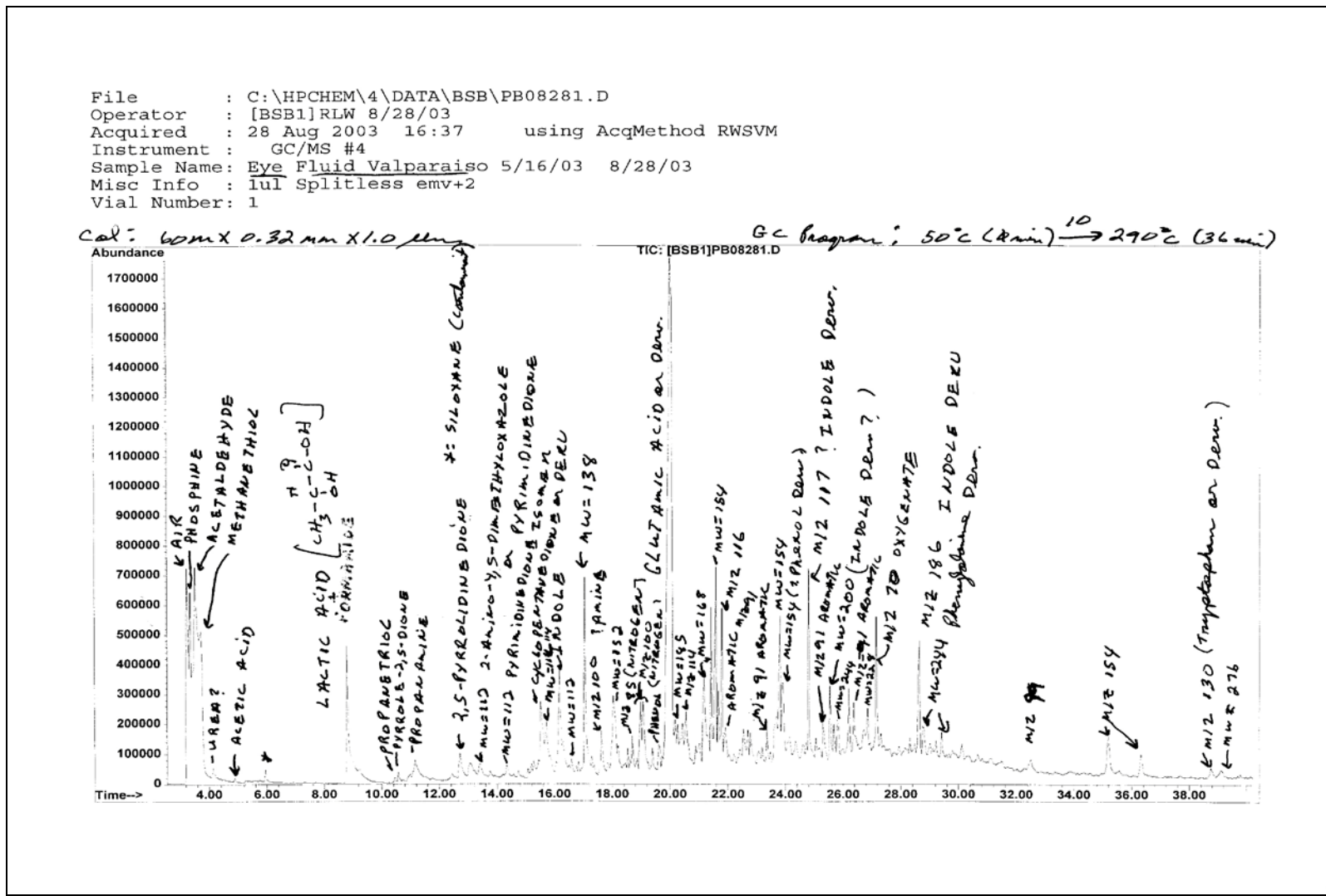


Figure 2. GC chromatogram of the vitreous fluid from the cow #8, Valparaiso, Nebraska.

Abundance

**TIC: Benzyl Bromide Std  
[BSB2]PBSTD1.D**

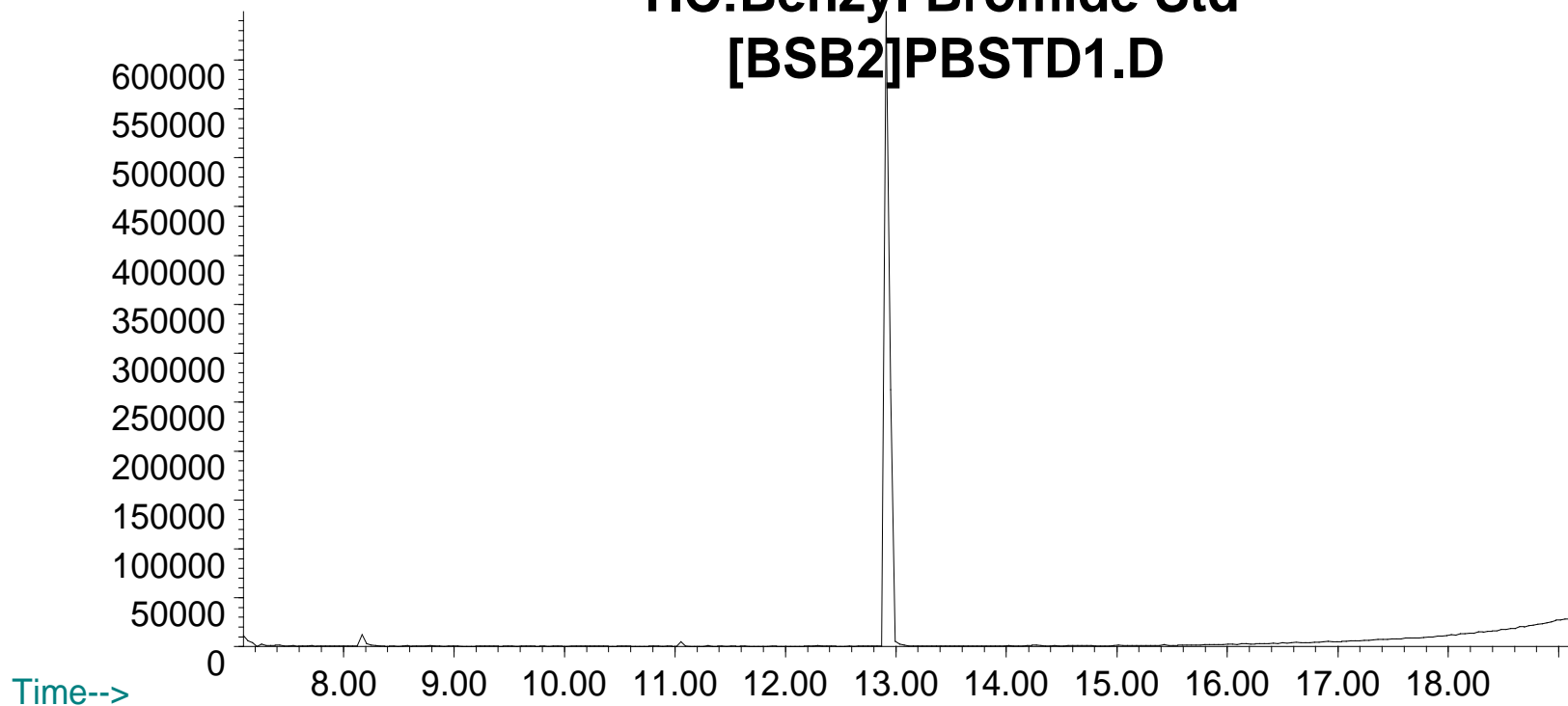


Figure 3. GC chromatogram of bromotoluene.

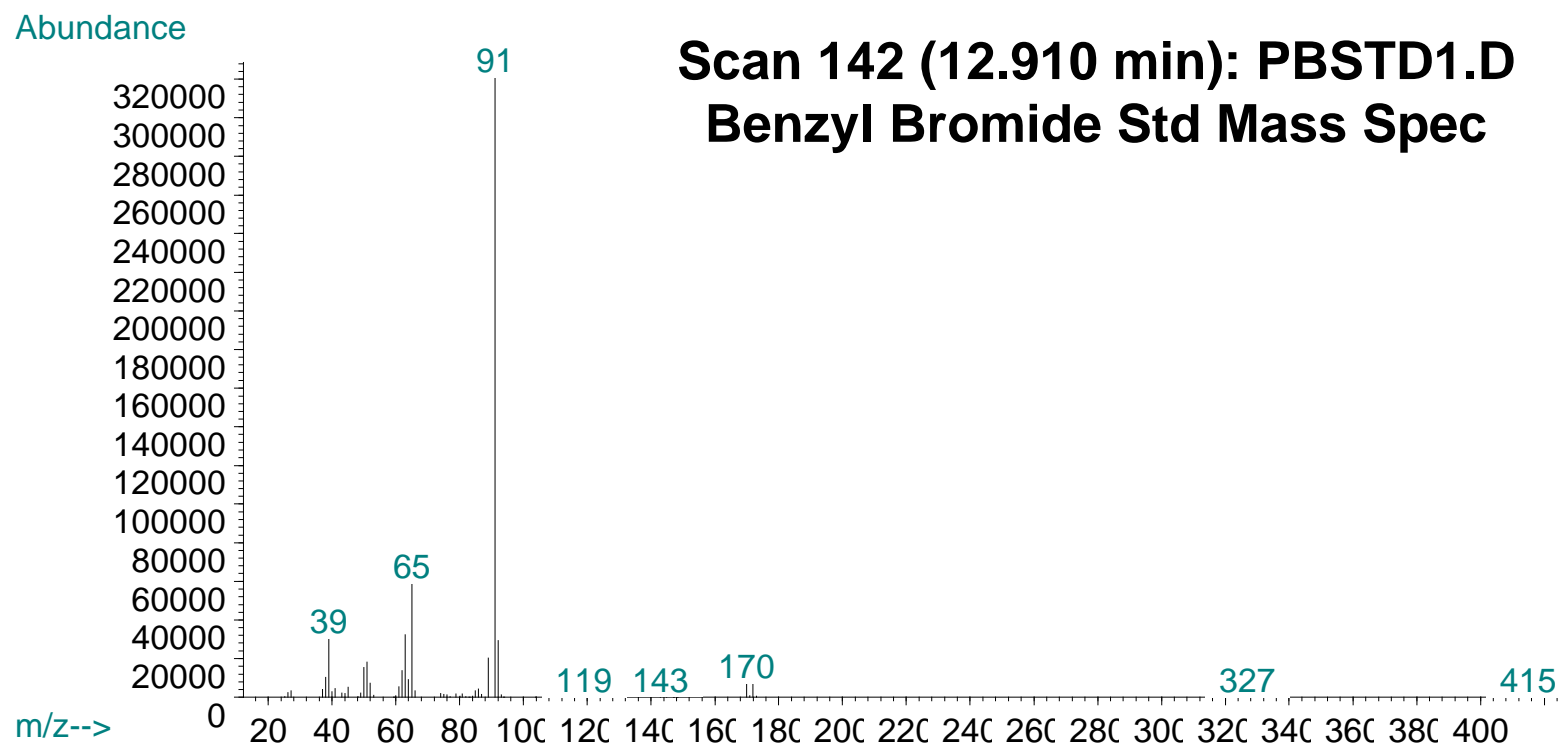


Figure 4. Mass spectrum of bromotoluene.

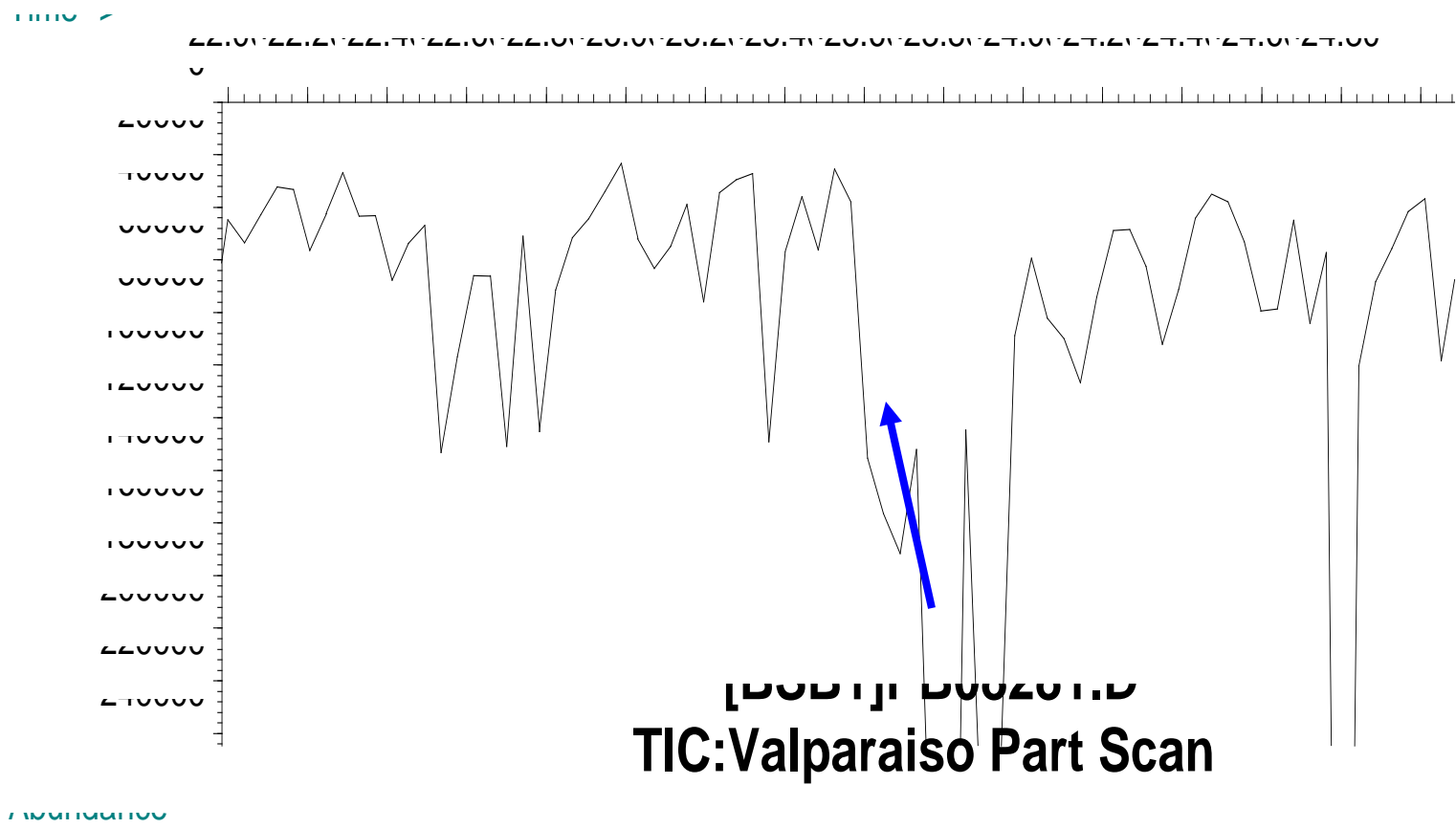


Figure 5. Expanded GC chromatogram of vitreous fluid from #8 cow showing the elusive peak at 23.65 retention time.



Abundance

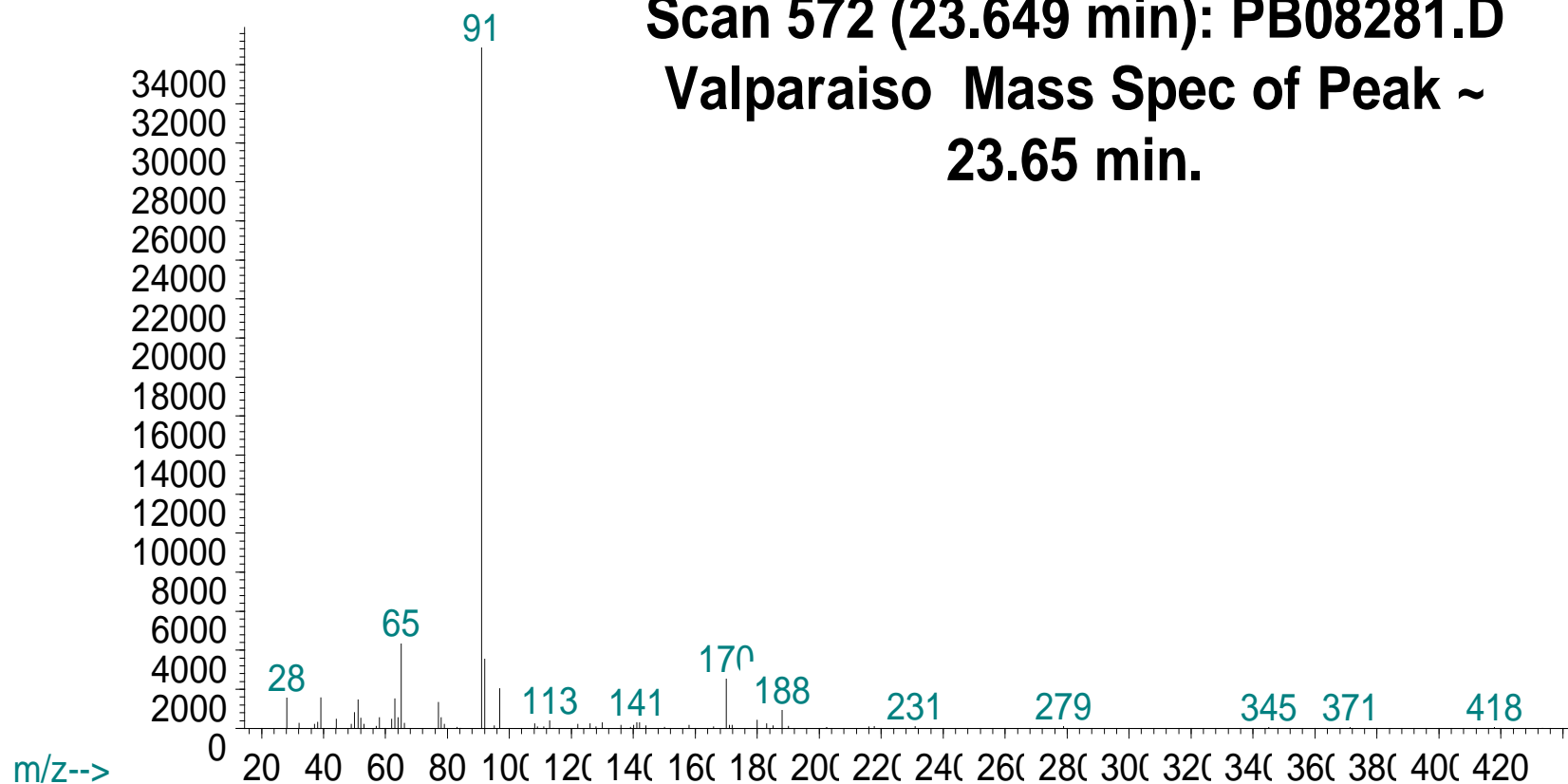


Figure 6. The mass spectrum of peak at 23.65 retention time .

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Acquired : 28 Aug 2003 15:25 using AcqMethod RWSVM  
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Sample Name: Eye Fluid Valparaiso 5/16/03 8/28/03  
Misc Info : 1ul Splitless emv+2  
Vial Number: 1

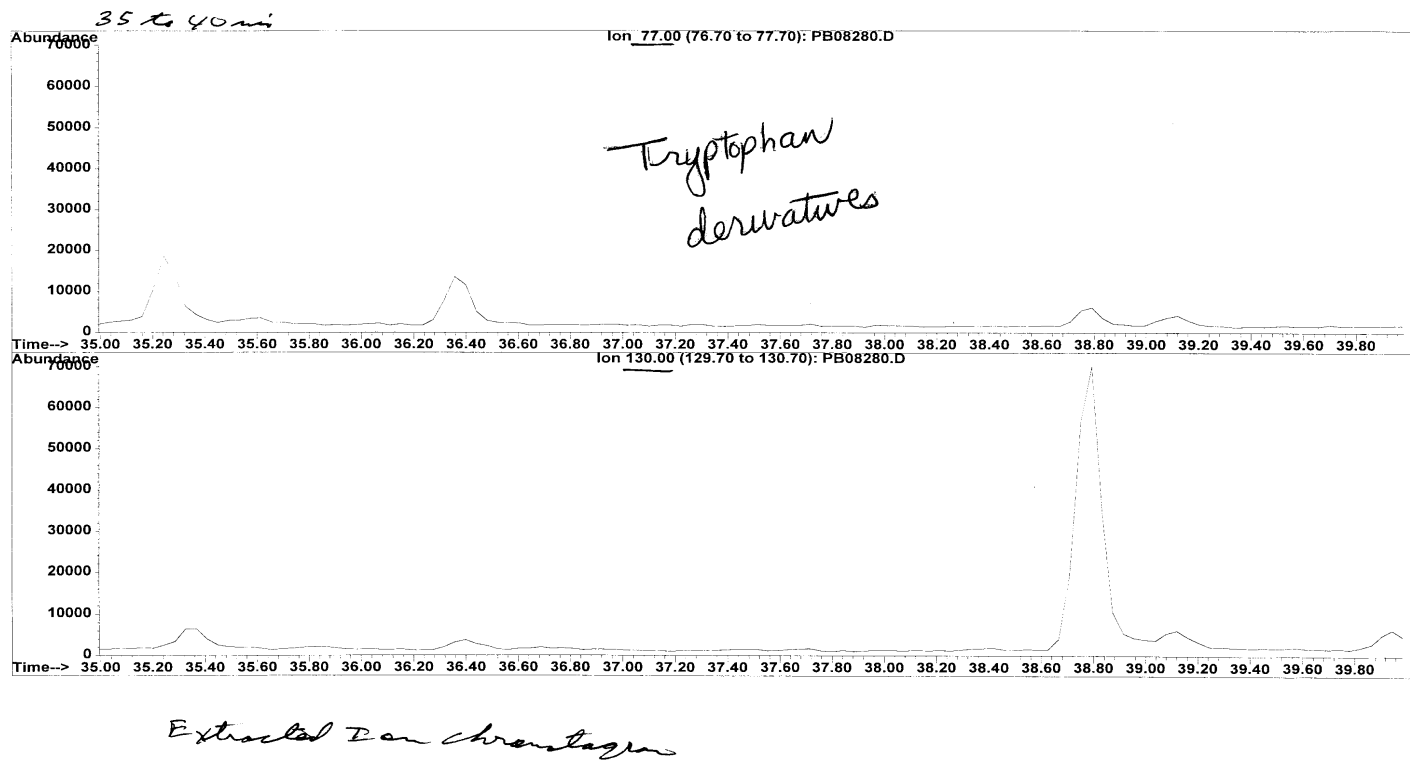


Figure 7. Ion chromatogram scan for tryptophan peaks 77 and 130 between retention times of 35 and 40 minutes.

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Misc Info : 1ul Splitless emv+2  
Vial Number: 1

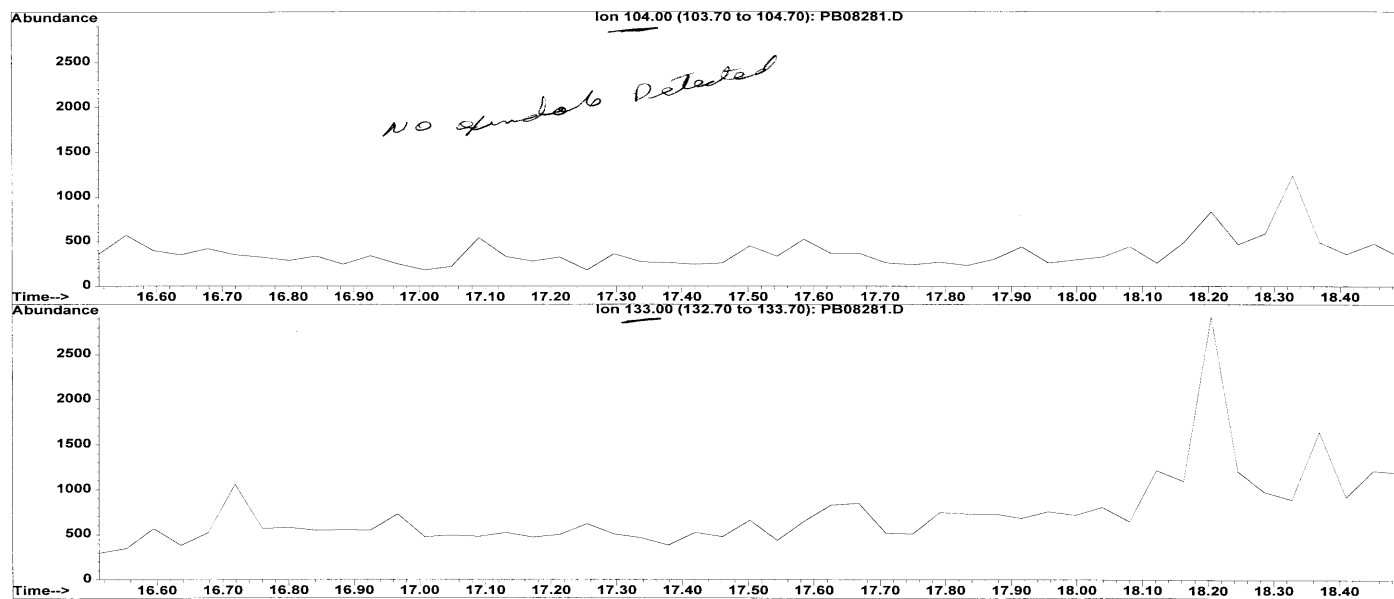


Figure 8. Ion chromatogram scan for oxindole peaks 104 and 133 between retention times of 16.40 and 18.60 minutes.