BUFOTENINE

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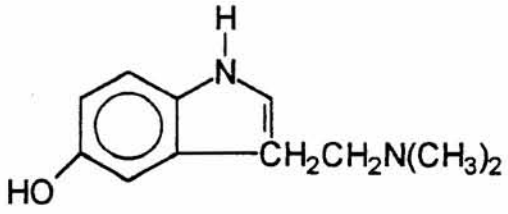
Background:

Bufotenine (5-hydroxy-N,N-dimethyltryptamine) is an isomer of psilocin and is a controlled substance under Federal and New York State laws.



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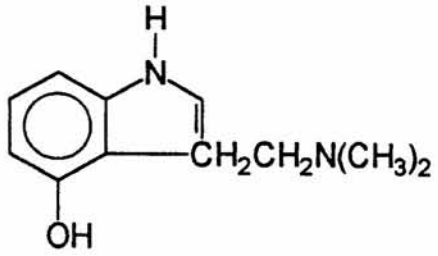

PSILOCIN

In May 1992, this laboratory received case samples alleged to be "Hashish." The samples were small, candy like, irregular cubes, resinous in texture, and reddish brown in color. They were packaged in small clear/red zip lock plastic bags, and weighed about 0.6-1.0 g each. Since November 1992, sixteen more cases were received. They have been seized at different locations throughout the five boroughs of New York City.

Gas chromatography/mass spectrometry (GC/MS) and thin-layer chromatography (TLC) were used to determine the presence of bufotenine. GC gave multiple peaks, two of which were identified as linoleic acid and cholesterol. Initially it was thought that the samples contained psilocin/psilocybin (*Microgram*, August 1984) because the mass spectra were similar, but the sample retention time was 0.3 minutes longer. Further investigation confirmed the presence of bufotenine. Bufotenine has not been identified before in case samples in the New York City Police Laboratory.

Dr. Richard E. Schultes, in a personal communication, indicated that he has not heard of any such cases in the United States. (Dr. Schultes is a Professor of Biology; Director, Botanical Museum of Harvard University [Emeritus], and is the author of many books and papers on hallucinogens of plant origin.)

This is a preliminary report that has been prepared to inform other forensic communities of the analysis of these cases.

Reagents/Equipment:

Bufotenine: Bufotenine monooxalate monohydrate
Sigma Chemical Co., St. Louis, MO USA
Psilocin: Sigma Chemical Co., St. Louis, MO USA
TLC plates: Merck - TLC Plates - Silica Gel 60
Other reagents: ACS Grade
GC/MS: Hewlett-Packard (HP) Model 5890/5970
Data system: HP RTE-A
Injector: 250°C
Oven: 100°C- 270°C @ 23°C/min
GC run time: 15.15 min
Column: HP-1 methyl silicone gum, 12 m x 0.2 mm ID,
0.33 μm film thickness
Transfer line: 280°C
Scan: 4-400 amu
Source: approximately 200°C
Electron ionization mode: 70 eV

Procedure:

Approximately 0.1 g of the sample was ground to a fine powder and soaked in 0.5 mL of methanol for 10 minutes, shaken for a few minutes, centrifuged, and the clear liquid injected into the GC/MS. (Derivatization was easily achieved in a test tube by adding double the volume of acetic anhydride to a portion of the concentrated methanolic extract of the sample, or to the standards. The test tube was then warmed near the injection port [about 50°C] for 20 minutes.) The sample extract was streaked on a TLC plate along with a bufotenine reference spot. The plate was developed almost to the end using solvent system TA [1]. The case sample streak was covered with a glass plate and the bufotenine standard spot was visualized using Van Urk's reagent. The portion of the sample streak corresponding to the bufotenine spot was marked on the TLC plate and that area was scraped off using a spatula. The scrapings were finely powdered and transferred to a test tube. A minimum amount of methanol was added to the test tube to immerse the scrapings, the tube was shaken for a few minutes, and was then centrifuged. The clear liquid was injected into the GC/MS, which gave a single GC peak.

Results and Discussion:

The mass spectra of bufotenine and psilocin/psilocybin could not be distinguished, but their retention times were different. Retention times showed slight variations with concentration (approximately 0.1 min). The relative intensities of the minor peaks in the mass spectra, e.g. 146 and 204, also varied. Either monoacetylated or diacetylated derivatives, or both, were obtained when bufotenine, psilocin, or the sample were derivatized using acetic anhydride. The retention time and the

mass spectra of a peak in the sample matched that of bufotenine. More of the other components were extracted into methanol if soaked for longer periods of time, or with other solvents, such as petroleum ether.

TLC:

System: TA (Clarke) [1]
Methanol:Ammonia, 100:1.5
Spray: Van Urk's reagent (1 g p-dimethylaminobenzaldehyde in 100 mL ethanol plus 10 mL hydrochloric acid)

R_f values:

Bufotenine	0.35
Psilocin	0.39
Psilocybin	0.05

Color Test:

A. Van Urk: A purple color was obtained on the concentrated methanol extract, and also on the powdered sample directly.

B. Weber Test: Step 1 - Three drops of freshly prepared 0.1% solution of diazo blue B)o-dianisidine tetrazotized) in distilled water was added to the concentrated methanolic extract of the case sample. The solution turned red.

Step 2 - Two drops of concentrated hydrochloric acid was added to the red solution. The color turned blue. [2]

GC Retention Times:

	Bufotenine	Psilocin	Case Sample
	6.05	5.75	6.05
Monoacetyl derivative	6.50	6.35	6.50
Diacetyl derivative	7.10	6.90	7.10

Natural sources of bufotenine are: 1. mushrooms (*Amanita mappa, A. citrina,* and *A. porphyrina* [synonym "*mappine*" is derived from *A. mappa*]); 2. secretions of the parotid gland of toads (*Bufo vulgaris, B. viridis, B. marinus,* etc [the name bufotenine is derived from "Bufo"]); 3. plant material, mostly seeds from the genus *Anadenanthera* (formerly *Piptadenia*).

The possibility of this material coming from mushrooms was ruled out after consultation with Dr. Roy E. Hallings, Curator of Mycology, The New York Botanical Gardens. The possibility of the toads being the source is very remote. The most probable source is the seeds of the genus *Anadenanthera*. There is a long history of the use of this material by native Indians of South America and the West Indies, mostly as snuff. Sometimes it was smoked and it was also used as an enema. A detailed review article on this subject is under preparation.

Anadenanthera seeds may also contain some other tryptamine derivatives such as N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO DMT). GC/MS of the samples showed only one tryptamine alkaloid, i.e. bufotenine. TLC, however, showed at least three other intense spots when sprayed with Van Urk's reagent. This matter is still under investigation. To rule out the possibility of bufotenine being an artifact of GC/MS analysis, the preparative TLC was also used.

Summary:

Bufotenine can be identified in case samples by color tests, TLC, and GC/MS. Additional confirmation can be obtained from the retention times and mass spectra of the mono and diacetylated derivatives of bufotenine.

Acknowledgments:

I am especially grateful to Tony Veneziano, Associate Chemist II for his guidance and advice. I am also grateful to Dr. Edward Stanley, Director; Mary Bianchi, Principal Chemist; and Lt. Eddie Huggins for their permission and encouragement to pursue this work.

References:

[1] *Clarke's Isolation and Identification of Drugs*, 2nd Edition, The Pharmaceutical Press, 1986.

[2] Garrette, A.S.; Siemens, S.R.; and Gaskill, J.H.; "The Weber Test for the Presence of Psilocin in Mushrooms," *North Eastern Association of Forensic Scientists Newsletter*, Vol. XVIII, No. 1, 1993.

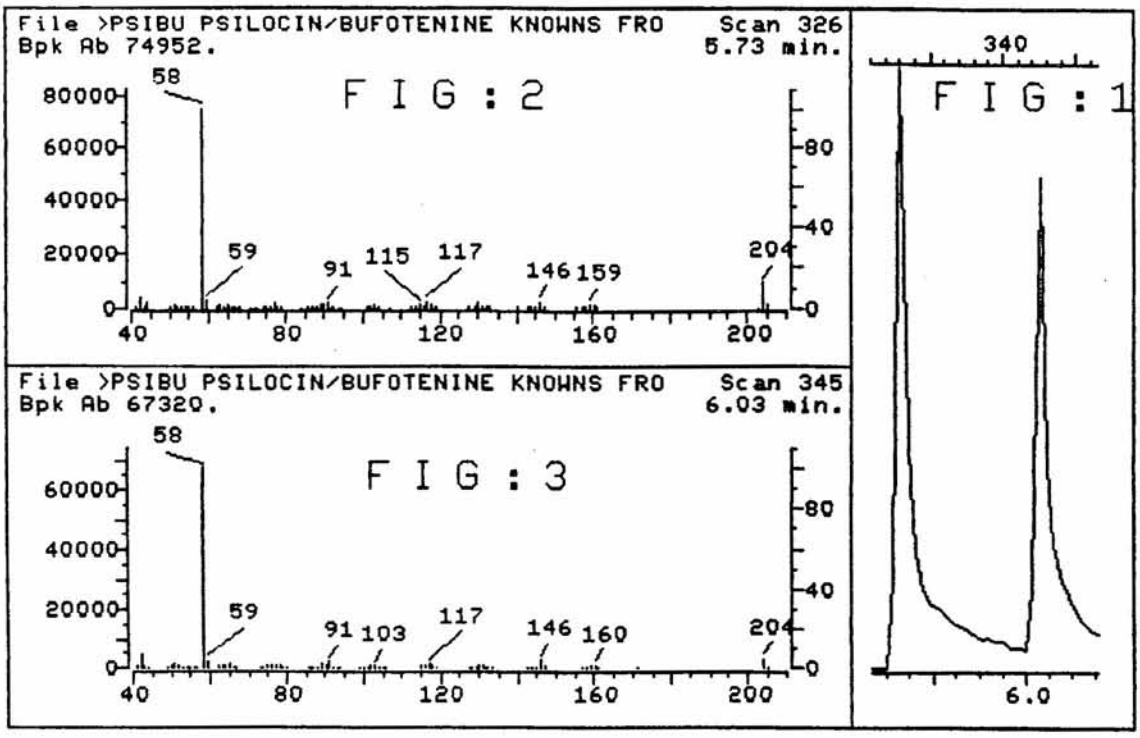

Figure 1: GC separation of psilocin and bufotenine standards
Figure 2: MS of psilocin standard
Figure 3: MS of bufotenine standard

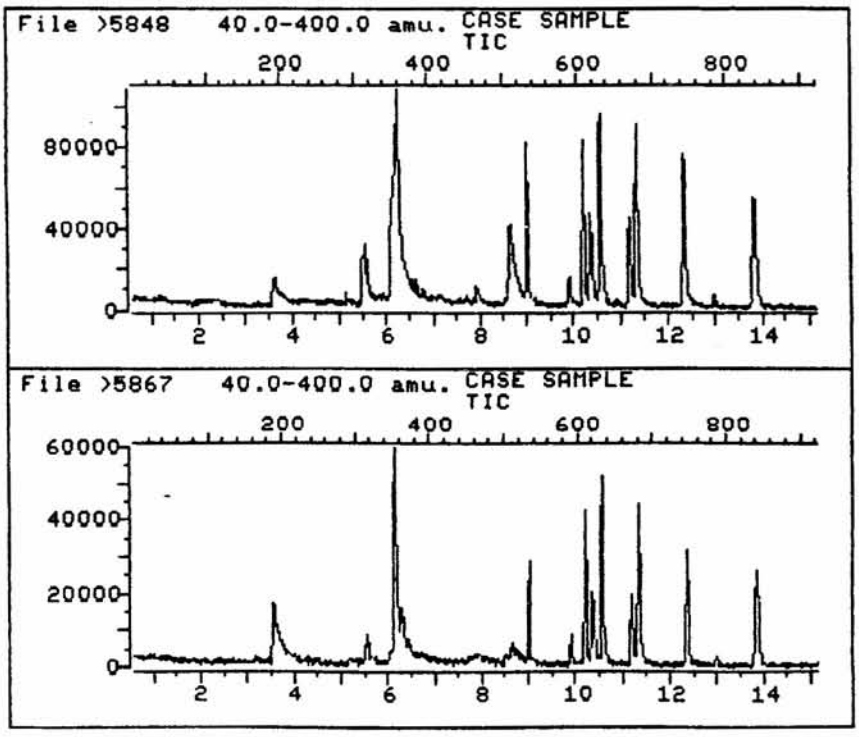

Figure 4: GC-TIC profile of two case samples

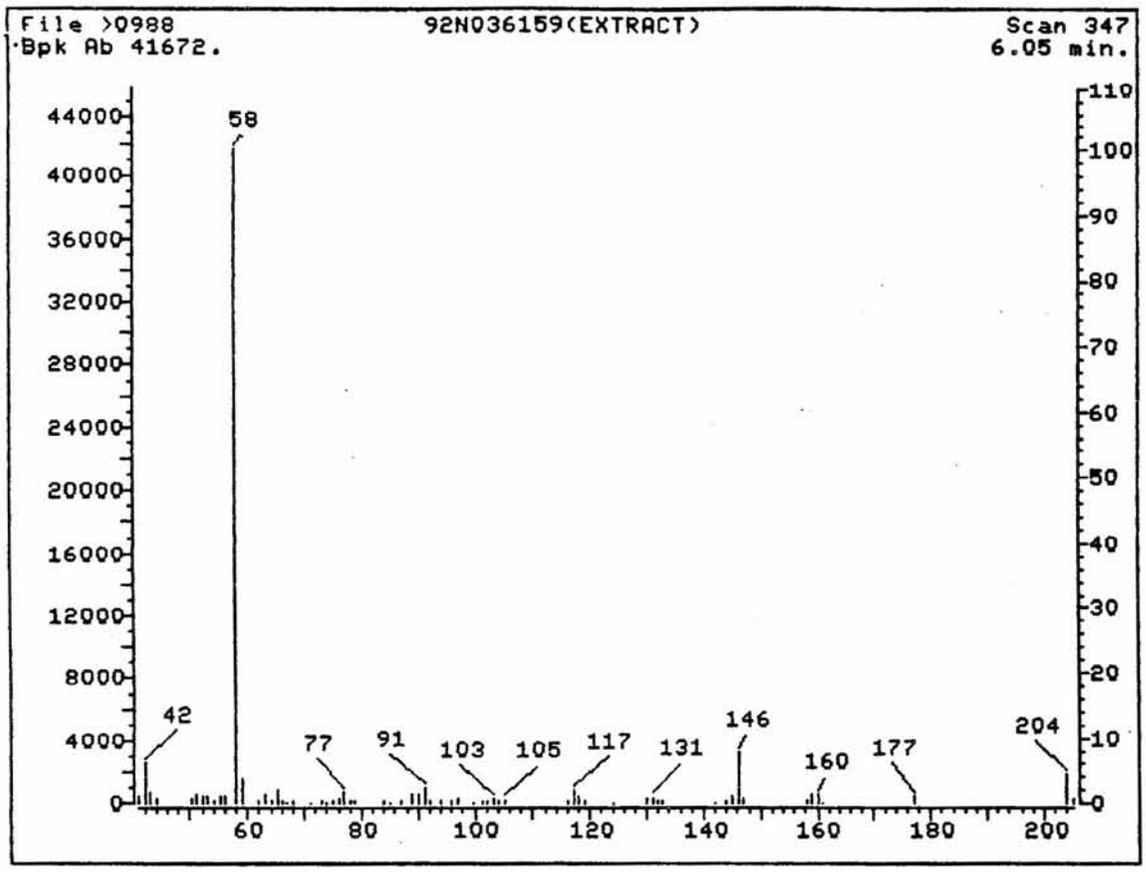

Figure 5: MS of bufotenine in case sample

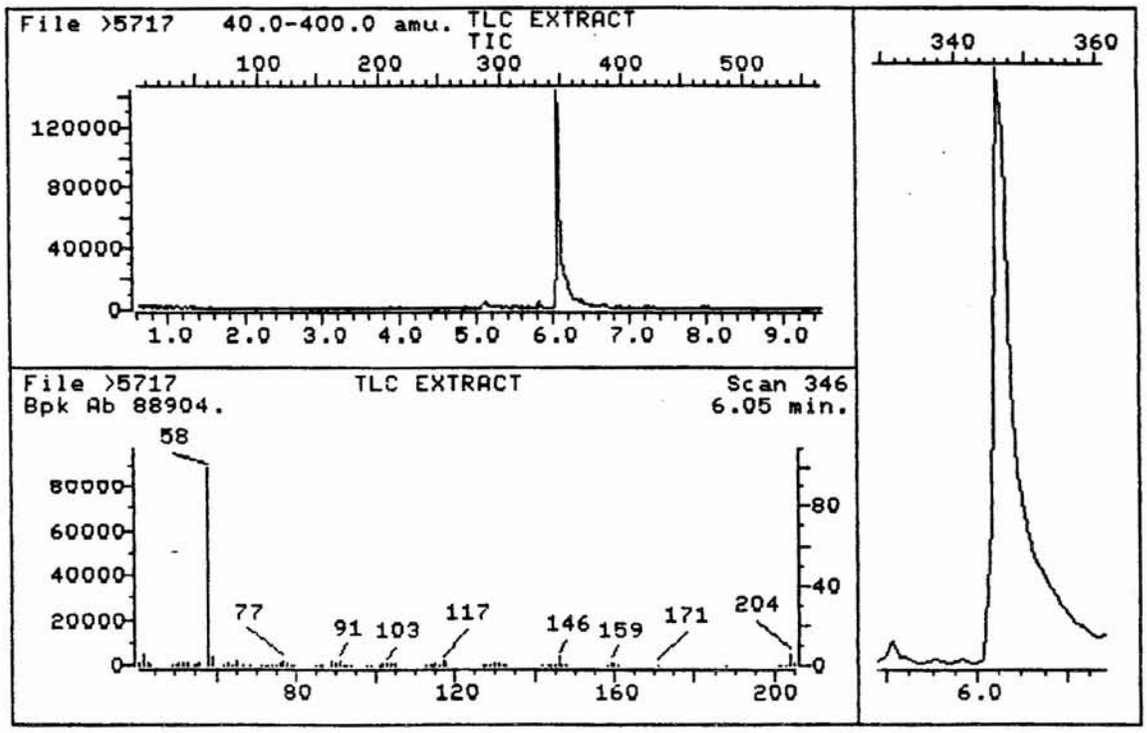

Figure 6: GC/MS of TLC extract of band corresponding to bufotenine standard.

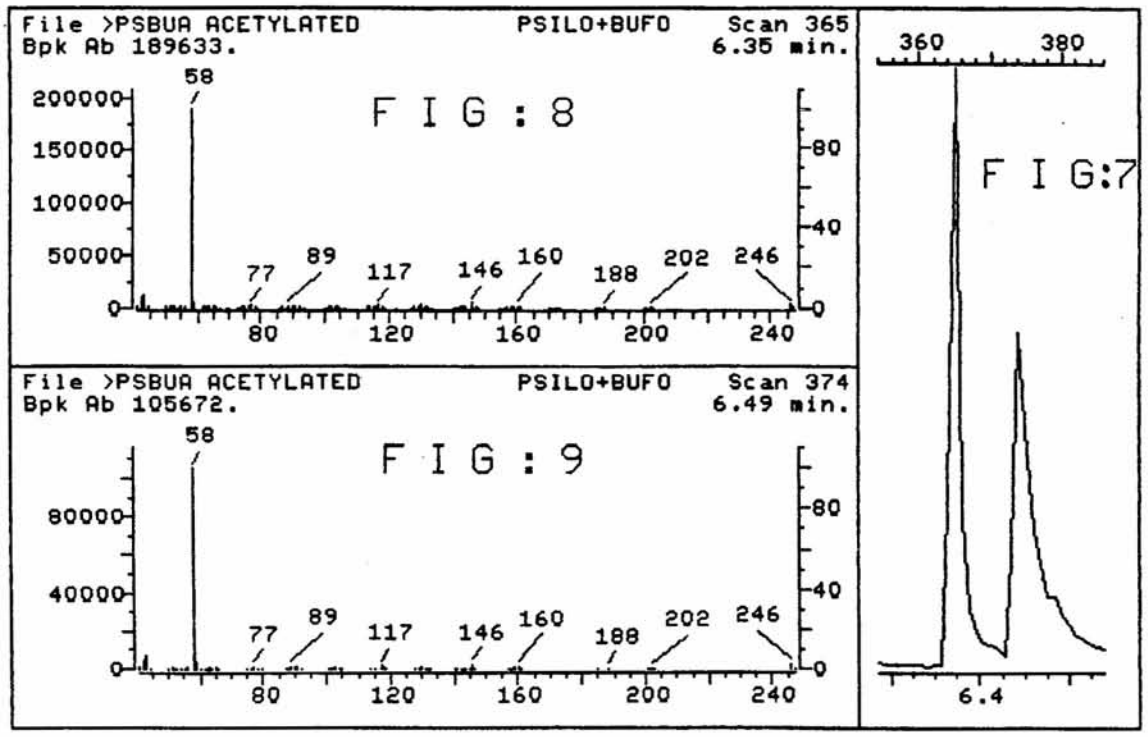

Figure 7: GC separation of monoacetylated psilocin and bufotenine standard.
Figure 8: MS of monoacetylated psilocin standard
Figure 9: MS of monoacetylated bufotenine standard

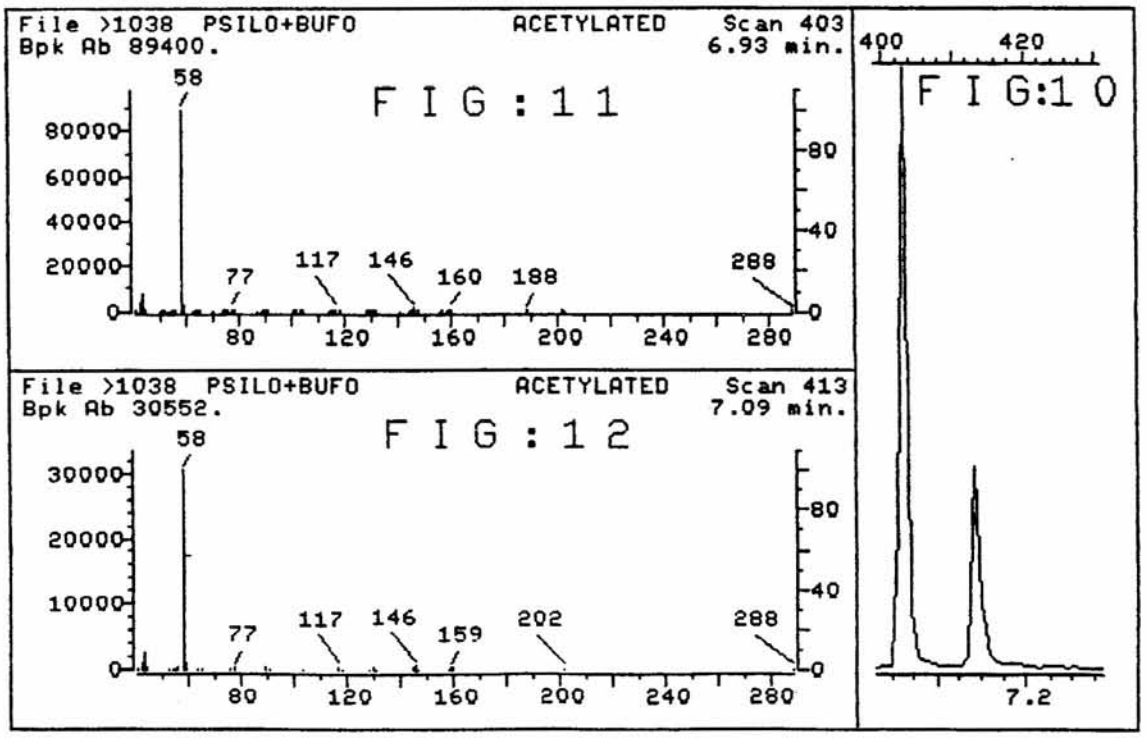

Figure 10: GC separation of diacetylated psilocin and bufotenine
Figure 11: MS of diacetylated psilocin standard
Figure 12: MS of diacetylated bufotenine standard

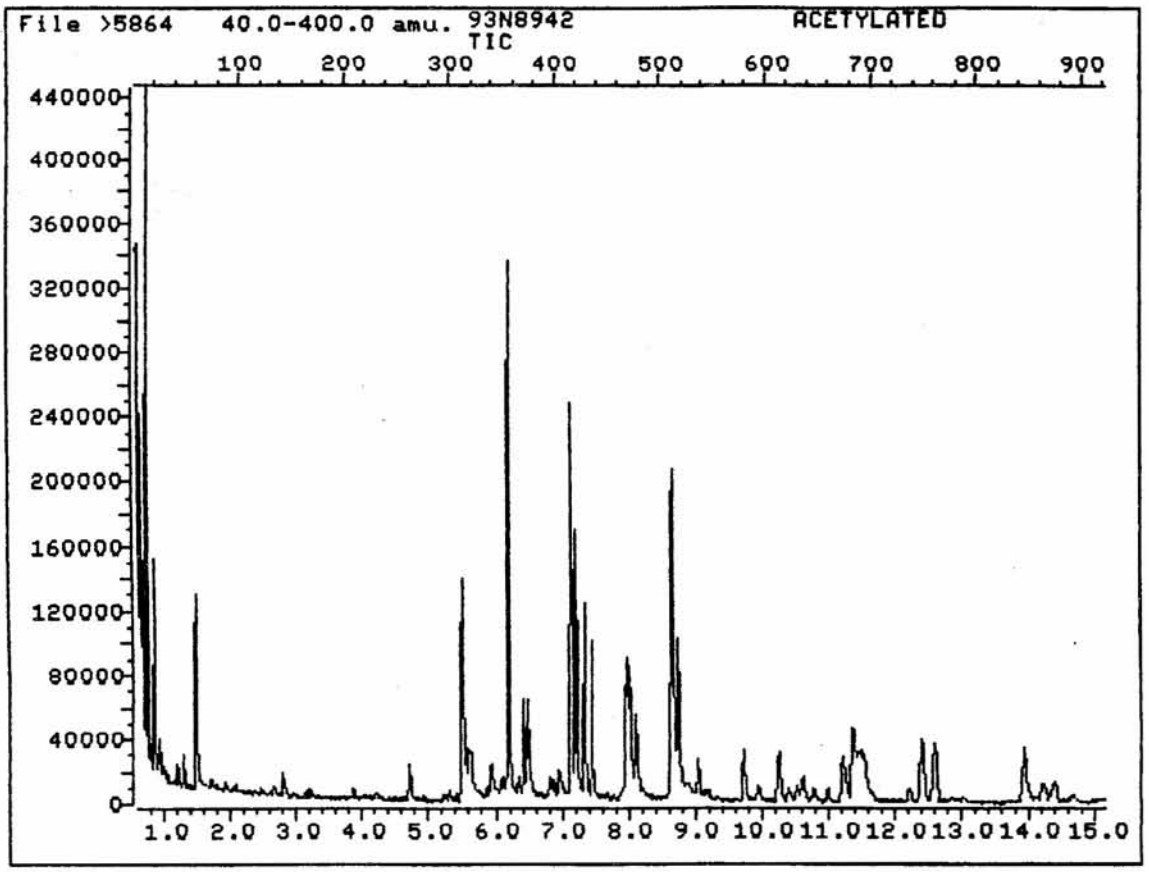

Figure 13: GC-TIC profile of acetylated case sample

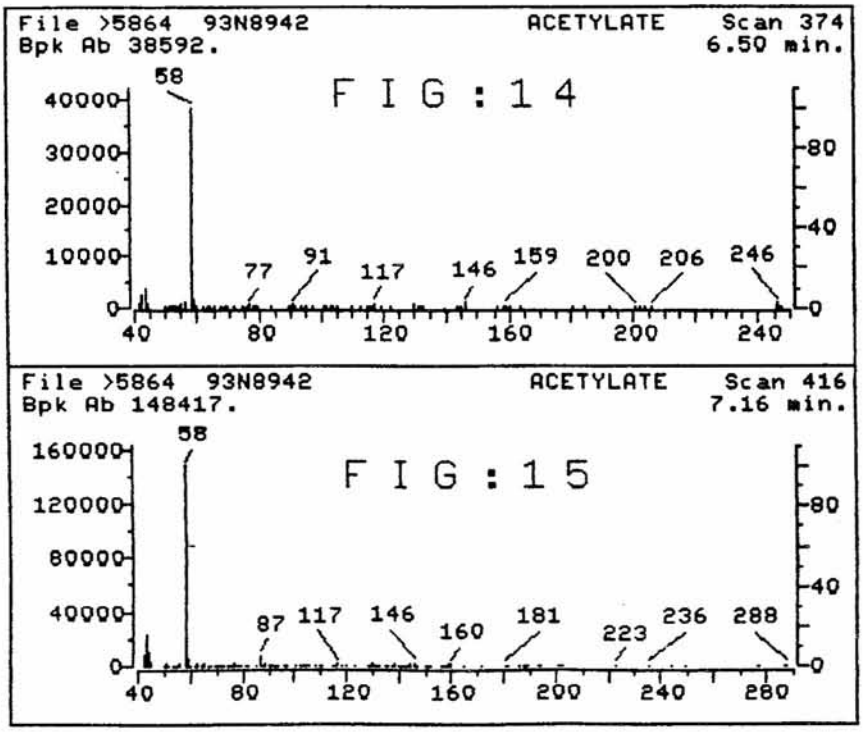

Figure 14: MS of monoacetylated case sample
Figure 15: MS of diacetylated case sample