Technical Note

Identification of Bufotenine in Yopo Seeds via GC/IRD

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ABSTRACT: The analysis of seeds from yopo (*Anadenanthera peregrina*) by GC/IRD and GC/MS is presented. The GC/IRD technique is easily able to discriminate between bufotenine (present in yopo seeds) and its positional isomer psilocin.

KEYWORDS: Yopo, *Anadenanthera Peregrina*, Bufonenine, Psilocin, Tryptamines, GC/IRD, GC/MS, Forensic Chemistry

Introduction

Yopo (*Anadenanthera peregrina*) is a tree that is native to the open plains of South America (1,2). Its leaves, bark, and seeds (sometime called "beans") reportedly contain bufotenine (5-hydroxydimethyltryptamine), dimethyltryptamine (DMT), and 5-methoxydimethyltryptamine (5-MeO-DMT) (1-6). The seeds are ground with a mortar and pestle into a snuff-like powder that is used by indigenous peoples in various religious rituals. Because the various tryptamines that are present in yopo are hallucinogenic, the seeds are also subject to abuse, and so are irregularly encountered in forensic laboratories (3).

Recently, this laboratory (NCIS - RFL - San Diego) received a zip-lock plastic bag that contained approximately 20 suspected yopo seeds (see Photo 1, next page). The exhibit had been confiscated from a U.S. Navy member in Japan (no further details).

Analysis of any substrate containing bufotenine by GC/MS is complicated by the similarity of its mass spectrum with that of its positional isomer psilocin (4-hydroxydimethyltryptamine). Bufotenine and psilocin are both controlled under Schedule I of the U.S. Controlled Substances Act, but bufotenine-containing substrates are

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[1] The NCIS San Diego Laboratory ceased operations in early 2006.

Photo 1

submitted far less commonly to forensic laboratories than psilocin-containing substrates. Analysis and discrimination of the isomers is usually accomplished using a combination of GC and GC/MS, with confirmation (if needed) by additional GC/MS analysis of their respective N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives (7,8). However, GC/IRD is both simpler and gives distinct and easily distinguished spectra (8). Herein, we report the analysis of yopo seeds using a combination of GC/IRD and GC/MS, and compare and contrast the respective spectra for bufotenine and psilocin.

Experimental

Standard Preparation: Bufotenine monooxalate and psilocin standards (Sigma, St. Louis, MO) were provided by the DEA Southwest Laboratory. For GC/MS and GC/IRD analyses, a small amount (not weighed) of these standards were placed in glass vials and dissolved in a few drops of methanol.

Sample Preparation: Using a scalpel blade, the thin hard dark brown outer coating was removed from one of the seeds. The inside, uniform, light brownish-yellow material was placed in a mortar, covered with saturated sodium bicarbonate, and macerated with a pestle. After sitting for several minutes, the resulting solution was transferred to a separatory funnel and extracted with a small amount of chloroform. The extract was filtered through a cotton plug in a disposable Pasteur pipette. After concentrating via evaporation, the extract was analyzed by GC/MS at the NCIS - RFL - San Diego, and also by both GC and GC/IRD at the DEA Southwest Laboratory.

Gas Chromatography: An Agilent Technology 6890N GC equipped with a flame ionization detector was used. The GC was fitted with a 10 m x 0.10 mm i.d. capillary column coated with 0.34 µm 5 % phenylmethyl siloxane (J&W DB-5). The GC was operated in a split mode of approximately 50:1. The injector port and detector temperatures were maintained at 280 ^{O}C. The oven temperature program was as follows: Initial temperature, 100 ^{O}C for 1 minute, ramped up to 280 ^{O}C at 25 ^{O}C per minute, with a final hold of 1.5 minutes. Hydrogen was used at an average velocity of 99 cm/second.

Gas Chromatography/Mass Spectrometry: An Agilent Technology 6890 GC interfaced to an Agilent Technology 5972A Mass Selective Detector was used. The GC was fitted with a 30 m x 0.25 mm i.d. capillary column with 0.25 µm 5 % polyphenylmethyl siloxane (J & W DB-5MS). The GC was operated in a split mode of 50:1. Helium was used as a carrier gas at a column flow rate of 28 cm/second. The injection port temperature was maintained at 250 ^{O}C. The oven temperature program was as follows: Initial temperature, 70 ^{O}C for 2

minutes, ramped up to 300 ^{O}C at 20 ^{O}C per minute, with a final hold of 15 minutes. The MSD transfer line was maintained at 280 ^{O}C. The MSD was operated at 70 eV.

Gas Chromatography/Infrared Spectroscopy: A Varian/Digilab GC/IRD was used. The GC was fitted with a 25 m x 0.32 mm i.d. capillary column with 0.52 µm 5 % phenylmethyl siloxane (HP-5). The GC was operated in a splitless mode, with a purge delay time of 0.50 minute. Helium was used as the carrier gas at a column flow rate of 36 cm/second. The injector port temperature was maintained at 275 ^{O}C. The oven temperature program was as follows: Initial temperature, 70 ^{O}C for 1 minute; ramped up to 300 ^{O}C at 25 ^{O}C per minute, with a final hold of 3 minutes. The flow cell and transfer line temperatures were maintained at 250 ^{O}C.

Results and Discussion

The workup procedure gave a chloroform extract that was surprisingly clean, and that contained a significant amount of bufotenine based on the intensities of the GC, GC/MS, and GC/IRD signals. Clearly, even less than one seed would have provided sufficient sample for analysis and identification. The psilocin eluted prior to the bufotenine on all three instrument systems, and the elution time for the yopo seed extract matched the bufotenine standard (the seed extract's greater concentration caused some peak broadening). Figures 1 through 6 show, respectively, the GC, GC/MS, and GC/IRD instrumental results for the seed extract, the psilocin standard, and the bufotenine standard.

Although others have reported that the seeds (or beans) contain DMT and 5-MeO-DMT in addition to bufotenine (1-6), in fact only bufotenine was found in the seeds in this case. A portion of these seeds were sent to James S. Miller, Ph.D., Curator and Director at the William L. Brown Center for Plant Genetic Resources, Missouri Botanical Garden [P.O. Box 299, St. Louis, MO 63166-0299]; Dr. Miller confirmed that the seeds were from *Anadenanthera peregrina*, "Yopo."

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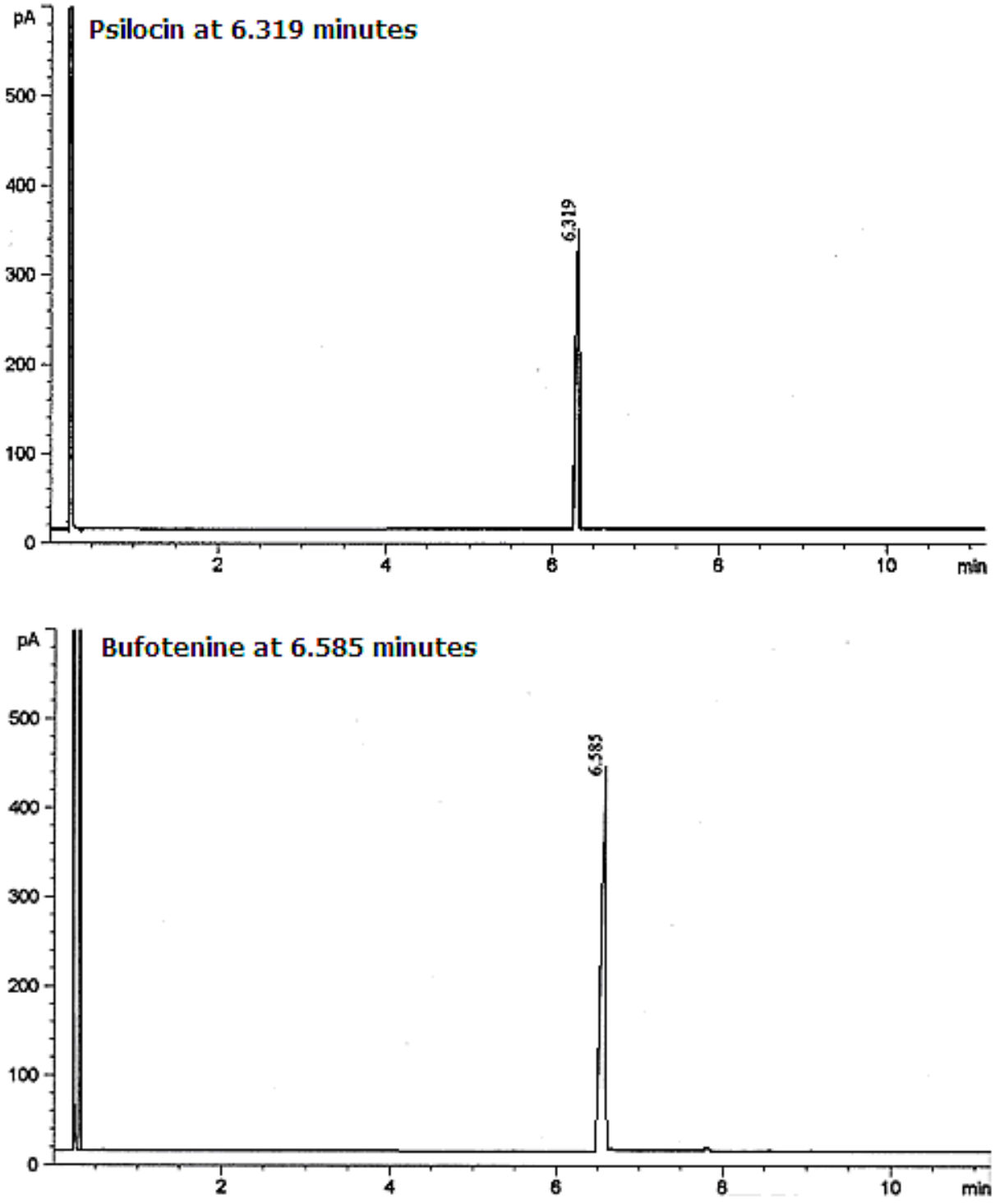

Figure 1. Chromatographs of Psilocin and Bufotenine.

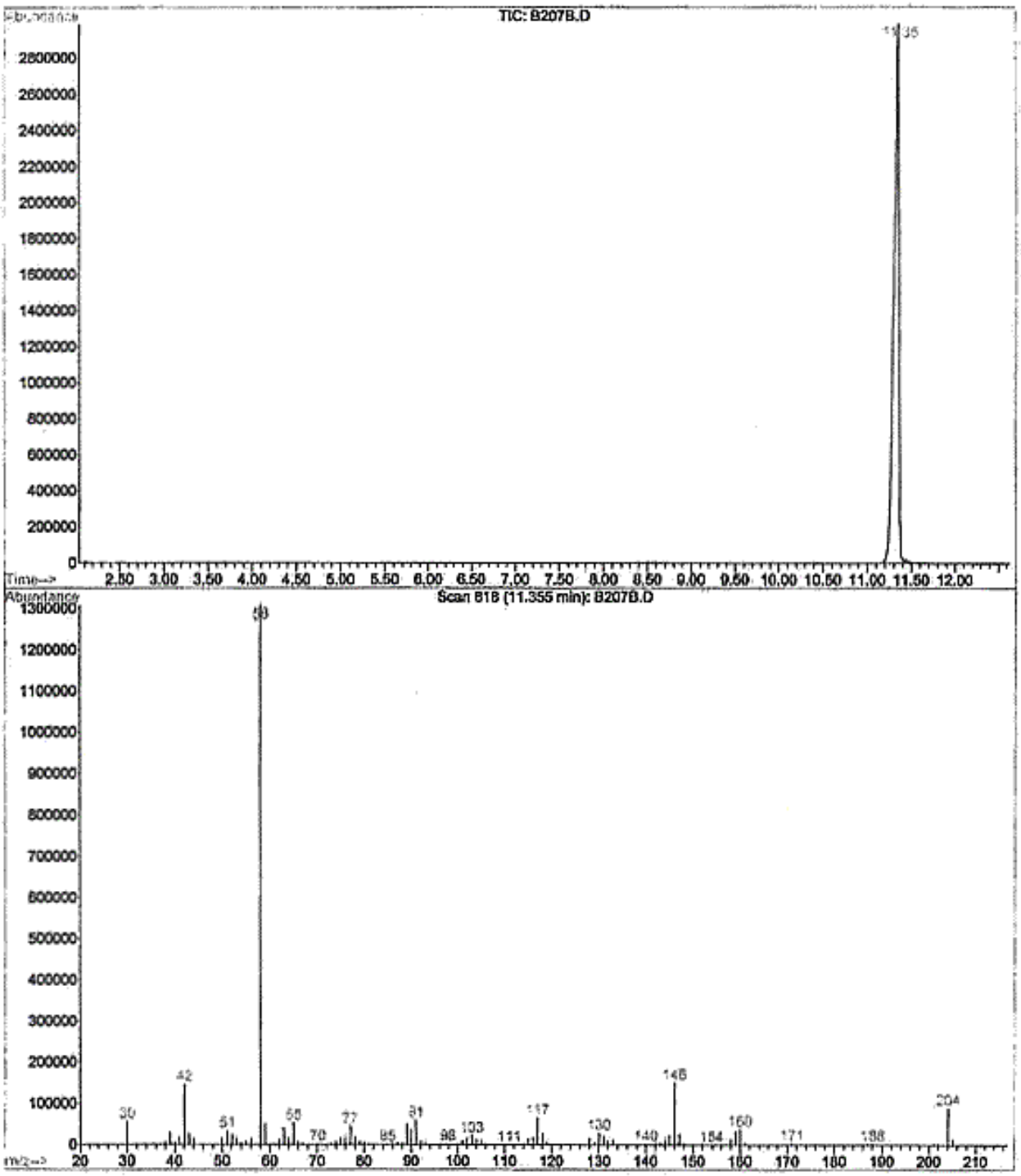

Figure 2. TIC and Mass Spectrum of Yopo Seed Extract.

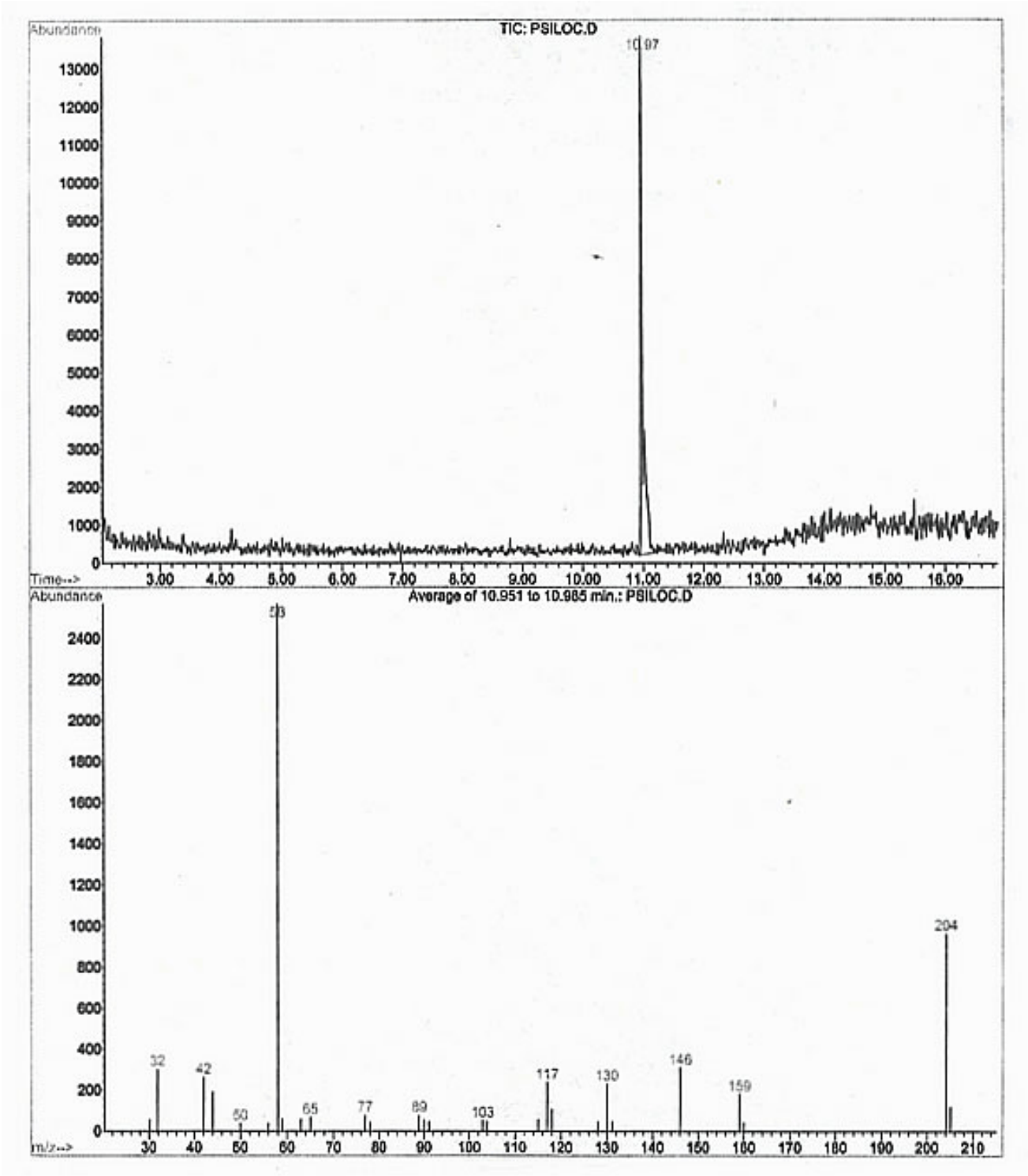

Figure 3. TIC and Mass Spectrum of Psilocin Standard.

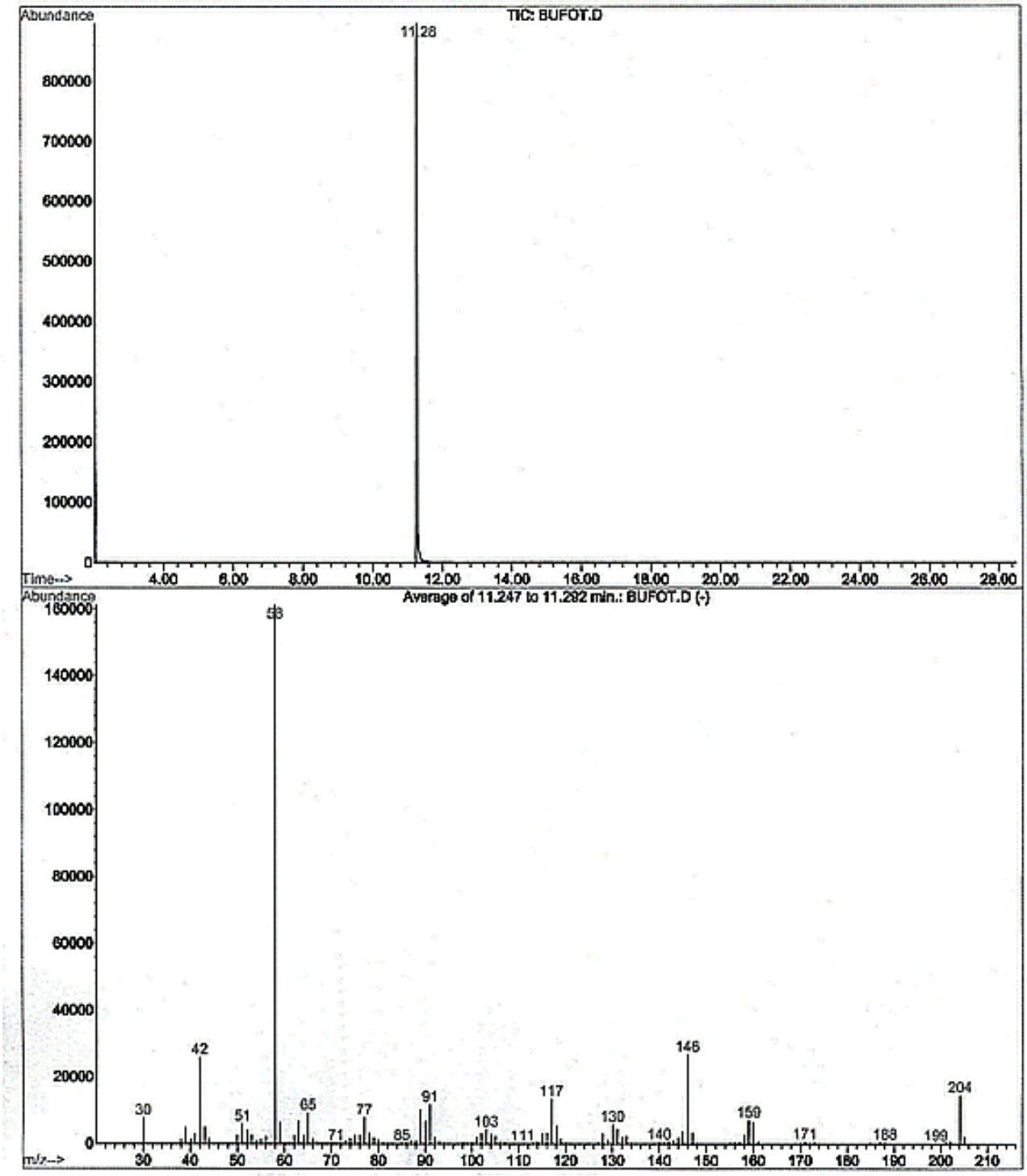

Figure 4. TIC and Mass Spectrum of Bufotenine Standard.

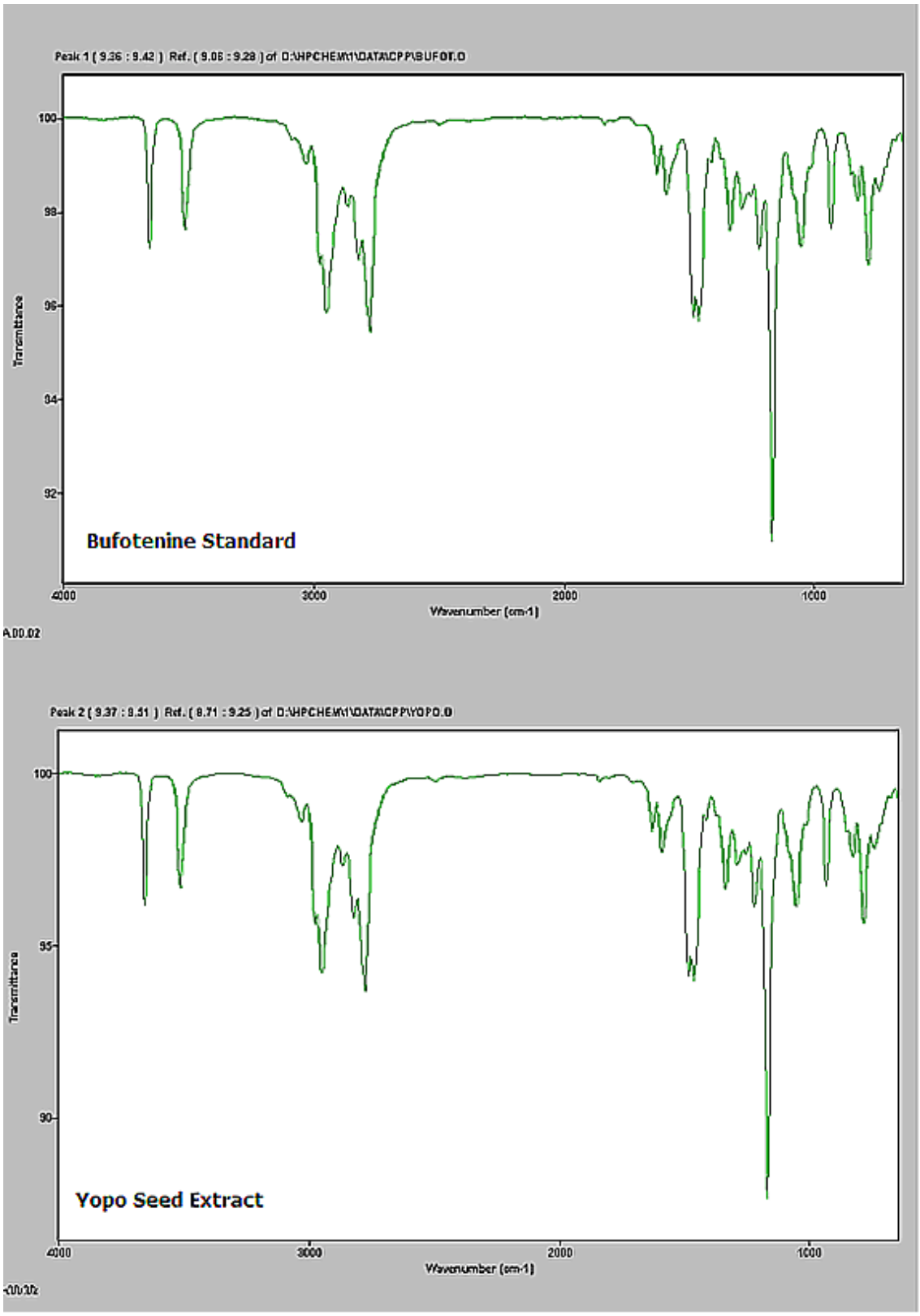

Figure 5. GC/IRD Spectra of Bufotenine Standard and Yopo Seed Extract.

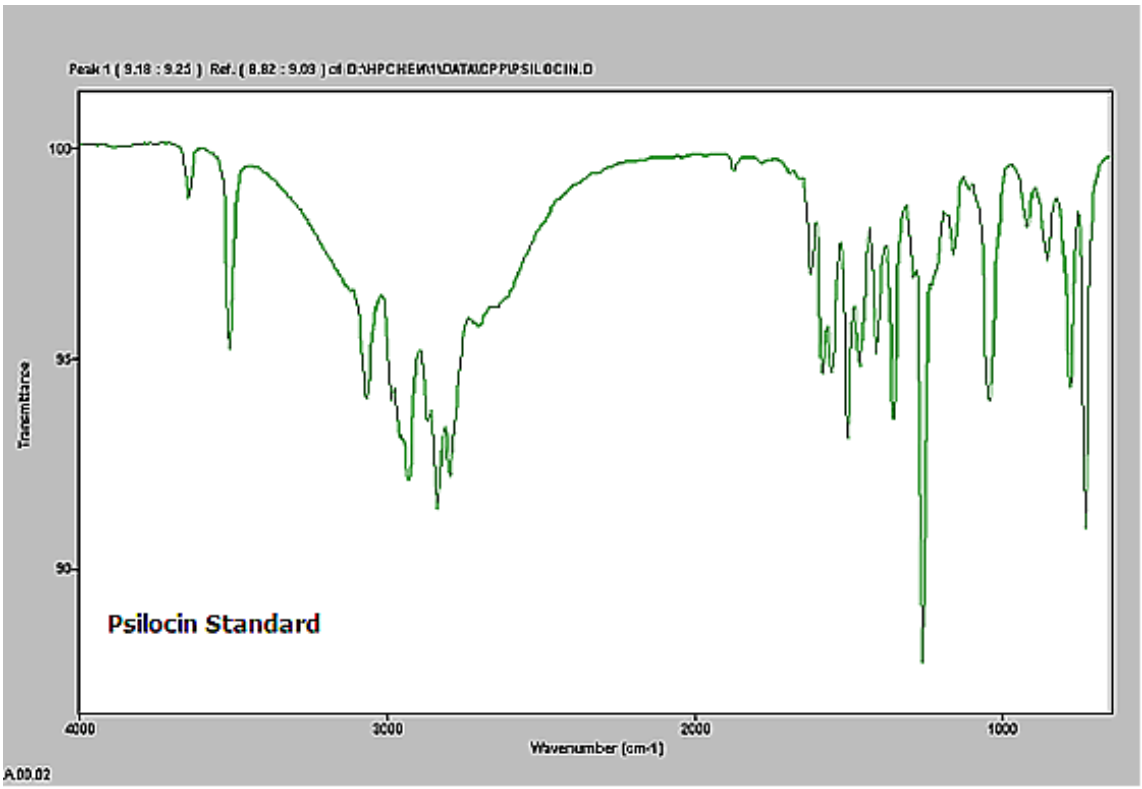

Figure 6. GC/IRD Spectra of Psilocin Standard.

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