Technical Note

A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions

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ABSTRACT: A simple, convenient, and rapid method for the identification of psilocyn in hallucinogenic mushroom/chocolate concoctions is presented. A 10% solution of acetic acid is used to extract psilocyn from the mushrooms. The acidic solution is then basified with solid sodium bicarbonate, then extracted with chloroform. The resulting extract is then back-washed to remove theobromine and caffeine from the chocolate, then concentrated and analyzed by TLC and GC/MS. The method takes about 30 minutes for mushroom/chocolate concoctions. A more simplified version of the method can be used for mushrooms, and takes about 15 minutes.

KEYWORDS: Forensic Science, Psilocyn, Extraction, Psilocybe Mushrooms, Mushroom/Chocolate Concoctions.

Introduction

Psilocyn and psilocybin, and the mushrooms containing these substances, are Schedule I substances under both Federal and Illinois state law. Psilocyn and psilocybin are hallucinogens, which act on the central nervous system to produce changes in perception, mood, and thinking ability. The effects produced by psilocyn and psilocybin are similar to those produced by LSD and mescaline (1-2). Since the mushrooms that produce these hallucinogens are easily cultivated, and spores, growing kits, and information are readily available through the Internet, increasing numbers of mushrooms and mushroom containing preparations (especially mushroom/chocolate concoctions, *vide infra*) are being encountered in forensic laboratories.

The life cycle of mushrooms has four stages, namely spores, the mycelium, pinhead or the primordial, and the mature fruit. The spores are actually the seeds of the fungi. Mushrooms cannot be classified as plants because they lack a root system, and do not have leaves, flowers, or the main constituent of plants, chlorophyll. Plants get their food through roots and leaves through photosynthesis, while mushrooms get their food or nutrients from the surrounding environment. The four species of mushrooms that contain psilocyn and psilocybin are *strophariaceae*, *bolbitiaceae*, *coprinaceae*, and *cortinariaceae* (3-5).

Psilocyn, psilocybin, and various other alkaloids are found naturally in all four above listed species of mushrooms. The mature fungi are sold in the underground market in both whole and powdered forms. More recently, various mushroom-containing concoctions have become popular, especially grated or powdered mushrooms in chocolate (6). A number of such cases have been received at this laboratory over the past year.

The most common analytical techniques reported in the literature for analysis of hallucinogenic mushrooms are all based on methanol extraction. In the most common procedure, the mushrooms are simply soaked in methanol overnight, and the resulting extracts condensed to near dryness and then analyzed using TLC and GC/MS (7-8). A more rapid technique involves placing the mushrooms in a closed vial with methanol, heating for a half an hour, then heating to dryness; the resulting residue is taken into 0.1 N sodium hydroxide, then extracted with

butyl chloride. The butyl chloride extract is then back-extracted with 0.1 N sulfuric acid, and the UV spectrum recorded in acidic and basic media. The basic solution is further extracted with butyl chloride, and the extract evaporated to dryness; the resulting powder is then analyzed by IR (9). In another, longer method, the mushrooms are dried at 40 ˚C in an oven for 16 hours, ground, and then soaked in methanol for 24 hours. The volume is reduced and then analyzed by HPLC (10). In a more rapid method using a buffer extraction, ground mushrooms are triturated in a rotary mixer with 10% ammonium nitrate in methanol for 30 minutes, then two methanol extractions are performed, and the combined methanol extracts analyzed by HPLC (11). Quantitative determination of psilocybin and psilocyn is accomplished by stirring freeze dried mushrooms in methanol for 12 hours, followed by analysis by HPLC and TLC (12). In a more refined method, the mushrooms are extracted with methanol, and the co-extracted sugars then precipitated with acetone; the resulting solution is concentrated prior to analysis by GC/MS (13). The aqueous extraction of psilocyn was achieved by using dilute acetic acid, adjusting the pH with glacial acetic acid, and heating the contents for one hour. The pH of the solution was then raised by the addition of ammonium hydroxide, and psilocyn extracted with diethyl ether. The latter method was also applicable to pure mushrooms but was more time consuming (14).

As noted above, mushroom/chocolate concoctions have become popular. The isolation and identification of psilocyn and psilocybin from mushrooms is somewhat problematic when the mushrooms have been grated or powdered and mixed with chocolate, because chocolate is a complex matrix containing a wide variety of components, many of which are soluble in methanol. Thus, the standard methanolic extraction techniques detailed above are almost inapplicable to mushroom/chocolate concoctions. In one recently described method, the concoction is soaked in dilute sulfuric acid and then washed with chloroform or methylene chloride. The aqueous layer is then basified with sodium hydroxide to pH 10, then extracted with chloroform (15-16). However, a clean peak of psilocyn was not obtainable even after multiple washings. Moreover, psilocyn is unstable at higher pH values (17). A short review on the methods of extraction for psilocyn can be read elsewhere (18).

In general, methanolic extraction procedures are very time consuming. Most procedures either involve an "overnight" extraction or heating. In addition, methanolic extractions of psilocybe mushrooms usually co-extract other indolic compounds (and other methanol soluble components), some of which can mask the psilocyn and psilocybin peaks in GC or GC/MS analyses. And as noted above, methanolic extraction is poorly suited for mushroom/chocolate concoctions. Herein, we present a new method for the extraction of psilocyn from such concoctions. The extraction takes about fifteen minutes for pure mushrooms, and about half an hour for mushroom/chocolate concoctions. In addition, large number of samples can be analyzed in a relatively short period of time.

Materials and Methods

Reagents: (1) A 10% acetic acid by volume (Analytical Reagent); (2) Chloroform (A.R.); (3) Sodium bicarbonate (A.R.); (4) Deionized water; and (5) Ehrlichs reagent.

Equipment: GC/MS (HP 6890/5973), centrifuge, pestle and mortar.

Procedure for Pure Mushrooms:

1. About 0.2 to 0.5 gram of mushrooms are transferred into a mortar.
2. The mushrooms are covered with 10 % acetic acid, and ground with the help of a pestle.
3. Another 5 mL of deionized water are added and the mixture is ground into a fine slurry.
4. The slurry is then transferred into a test tube and centrifuged for about 3 minutes.
5. The supernatant is transferred into a small beaker
6. The supernatant is neutralized by adding small amounts of sodium bicarbonate (neutralization is judged to be complete when the foamy effervescence stops). A little excess bicarbonate is then added.
7. The resulting solution is transferred into a test tube and extracted with an equal amount of chloroform.

8. The biphasic solution is centrifuged, and the chloroform layer collected in a shell vial.
9. The chloroform extract is concentrated under air, transferred to a micro vial, and analyzed on the GC/MS.

Total extraction takes around 15 minutes. The results are shown in Figure 1.

Procedure for Mushroom/Chocolate Concoctions:

1. 1.0 to 2.0 gram(s) of sample is transferred into a mortar and ground with a pestle.
2. The resulting powder is covered with 10 % acetic acid, and the sample is further ground with a pestle.
3. An additional 5 to 7 mL deionized water is added, and the mixture is ground for about 2 minutes, creating a thin slurry.
4. This slurry is divided into two equal portions, and each is transferred into a test tube.
5. An equal amount of chloroform is added to each tube, and the tubes are centrifuged for 3 minutes.
6. The aqueous layer is pipetted into a beaker from both of the test tubes.
7. 2 or 3 drops of this solution are placed in a test tube, and treated with the Ehrlich's reagent; a deep purple color is indicative of presence of indolic compounds.
8. The aqueous solution in the beaker is neutralized by slowly adding sodium bicarbonate until the effervescence stops.
9. A little excess bicarbonate is added, and the pH is checked with pH paper to make sure it lies between 8 - 8.5.
10. The resulting solution is then transferred into two test tubes, and each extracted with an equal amount of chloroform.
11. The tubes are centrifuged for about 5 minutes.
12. The chloroform layers are collected into two new test tubes.
13. An excess of 2% sodium bicarbonate solution is added to each test tube.
14. After vigorous shaking, the test tubes are centrifuged for 5 minutes.
15. The chloroform layers are combined in a small beaker.
16. The chloroform extract is concentrated under air, transferred to a micro vial, and analyzed on the GC/MS.

The results are shown in Figure 2.

Results and Discussion

The presented acetic acid facilitated extraction of psilocyn from mushrooms is more rapid and convenient versus traditional methanolic extraction procedures, which require long time frames or potentially destructive heating. In addition, the use of sodium bicarbonate as a neutralization agent keeps the pH below 8.5, thereby avoiding base-facilitated destruction of psilocyn. The Total Ion Chromatogram (TIC) of the mushroom only sample shows a clean psilocyn peak (Figure1). Analysis by TLC also shows only psilocyn. No psilocybin was detected - this is perhaps due to the activity of the phosphatase enzymes present in the mushrooms, which can dephosphorylate psilocybin to psilocyn in aqueous medium (19).

Analysis of mushroom/chocolate concoctions requires additional cleanup steps. Analysis of the chloroform extract at Step 12 (that is, before the extract was washed with sodium bicarbonate) showed three peaks in the TIC (Figure 2). The small peak at 4.223 minutes is due to caffeine, the broad peak at 4.50 minutes is due to theobromine, and the sharp peak at 4.837 minutes is due to psilocyn. Caffeine and theobromine (both purine alkaloids) result from the chocolate; theobromine is the main alkaloid in chocolate (2.8 - 3.5 % in cocoa), and caffeine is another major alkaloid (0.1 - 0.4 %) (20). After washing the chloroform extract (at Step 12) with 2 % sodium bicarbonate solution, the amounts of theobromine and caffeine are very low (see Figure 3), resulting in a nearly clean TIC showing only psilocyn. However, the mass spectrum of psilocyn depicted in Figure 3 showed an extraneous ion 109, probably resulting from trace theobromine. When the chloroform extract was washed with

water only, the peak due to theobromine nearly disappears, and there is no extraneous 109 fragment, but the peak due to caffeine is still present (see Figure 4).

Quantitation was not performed in this study; however, the procedure allows facile identification of psilocyn in mushroom/chocolate concoctions.

The mass spectra acquired in this study for caffeine, theobromine, and psilocyn are presented in Figures 5 -7.

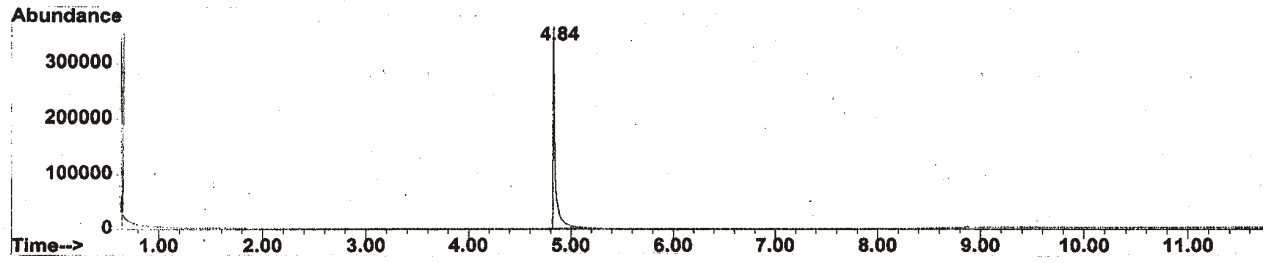

Figure 1. Total Ion Chromatogram of Mushrooms (Only) using Acetic Acid and Sodium Bicarbonate (4.84 Minutes = Psilocyn).

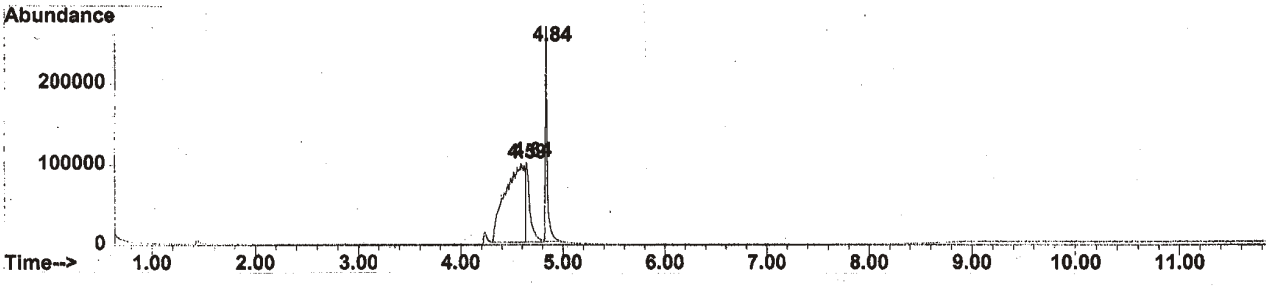

Figure 2. Total Ion Chromatogram of Mushroom/Chocolate Concoctions using Acetic Acid and Sodium Bicarbonate (4.22 Minutes = Caffeine; 4.50 Minutes = Theobromine; and 4.84 Minutes = Psilocyn).

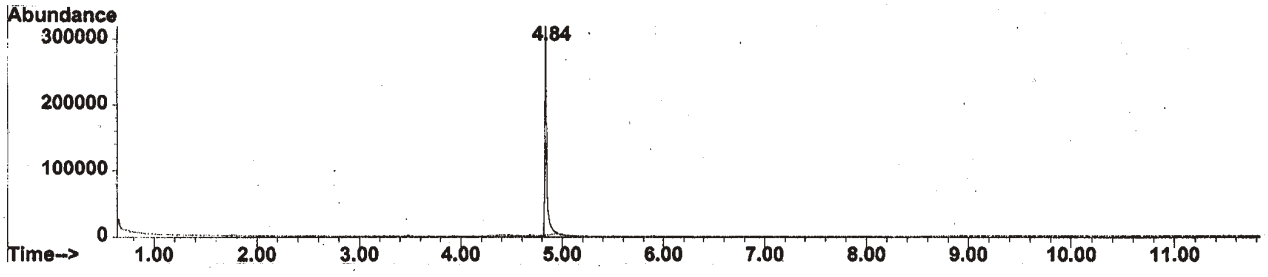

Figure 3. Total Ion Chromatogram of Mushroom/Chocolate Concoctions when the Chloroform Extract was Washed with 2 Percent Aqueous Sodium Bicarbonate.

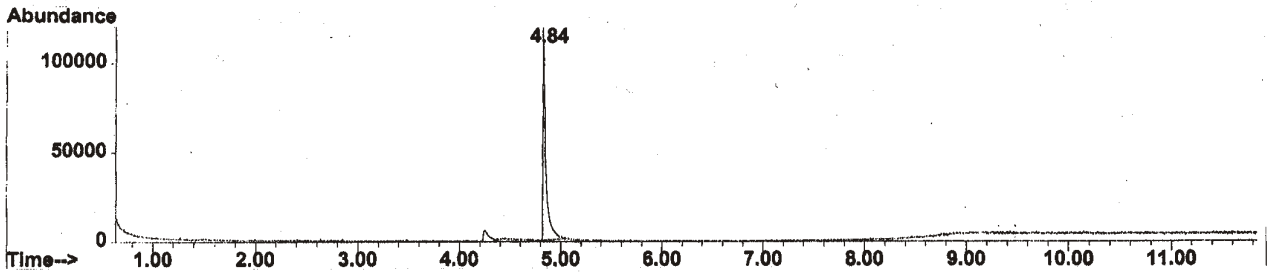

Figure 4. Total Ion Chromatogram of Mushroom/Chocolate Concoctions when the Chloroform Extract was Washed with Water.

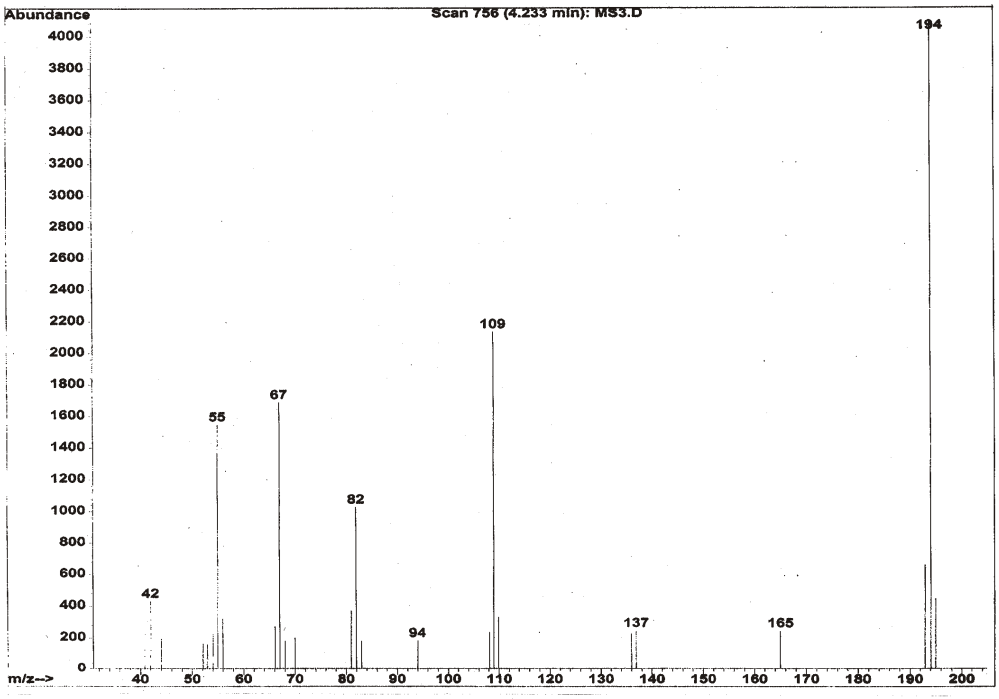

Figure 5. Mass Spectrum of Caffeine.

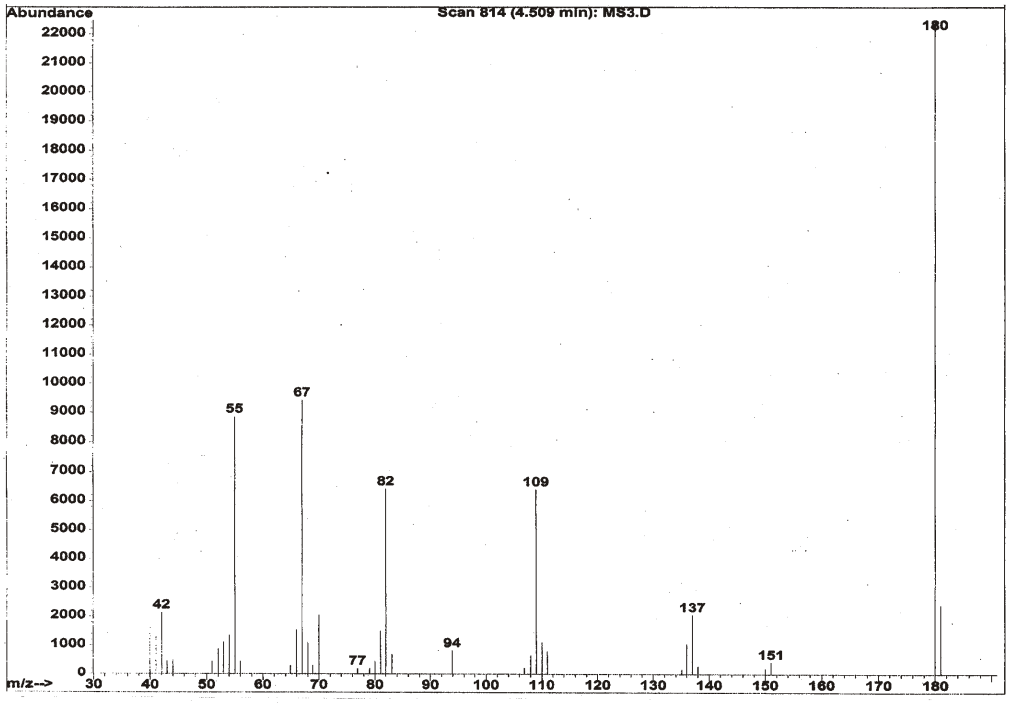

Figure 6. Mass Spectrum of Theobromine

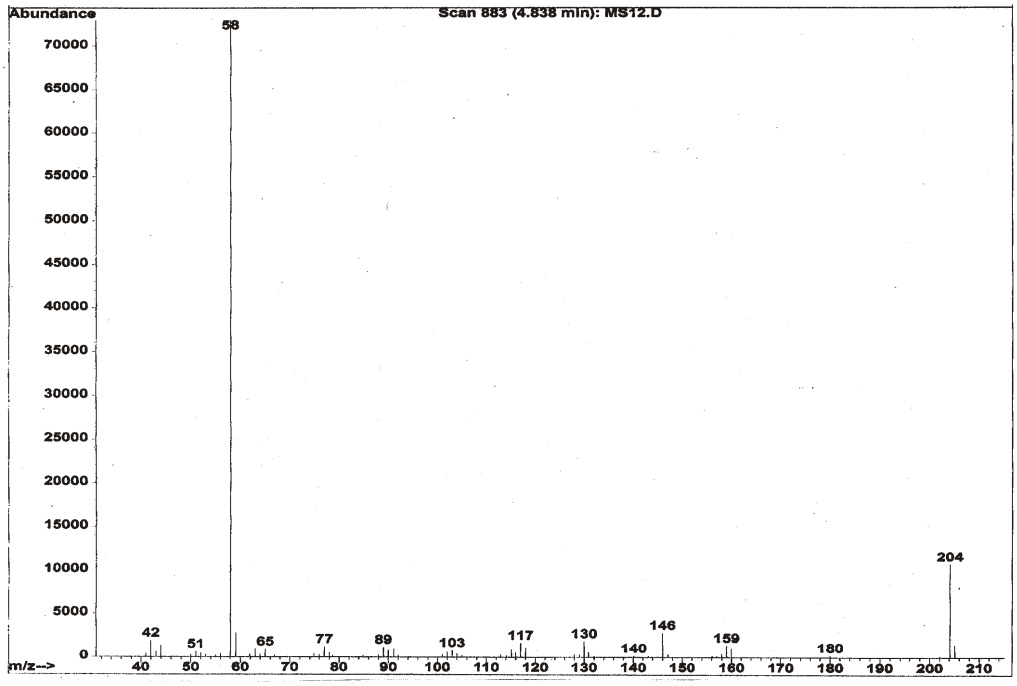

Figure 7. Mass Spectrum of Psilocyn.

Acknowledgements

The authors thank Forensic Scientists Joseph Gillono and Paula Bosco Szum, this laboratory, for their technical assistance.

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