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# Detection of Psilocybin Mushroom Analogs in Chocolate: Incorporating Current Events into the Undergraduate Teaching Laboratory

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**Abstract:** In this experiment, tryptamine is used as a psilocin analog and is dispersed onto a Fisher brand cellulose laboratory sponge to simulate dehydrated mushrooms. The resulting “mushroom” material is ground, molded into chocolate, and presented to student groups for real-world and applied analyses. Students isolate the tryptamine from the chocolate using their knowledge of drug chemistry, solubility, pH, extractions, etc. Qualitative analysis is conducted by comparison to standards (Thin Layer Chromatography or Gas Chromatography) and quantitative analysis is conducted by Gas Chromatography using the method of internal standards.

## Introduction

Psilocin is a hallucinogenic compound and a drug of abuse found in psilocybin mushrooms. The mushrooms are consumed for their hallucinogenic properties and can be eaten raw, dehydrated, or made into a soup. However, law enforcement agencies commonly find the dehydrated mushrooms mixed into chocolate bars to mask their bitter taste [1–3]. In this form, the illicit product is sold as magic chocolate or magic mushroom chocolate. Chocolate has long been a problematic matrix for analysis and is commonly used as an example for sample preparation considerations in popular quantitative analysis textbooks [4]. The analyte is suspended in a colloidal matrix of emulsifiers, lipids, cocoa, plant sterols, and other interferents, including organic molecules that will co-extract with psilocin, mainly theobromine and caffeine [5–6].

Methods currently used in forensic drug analysis are unavailable to undergraduate students due to the illegal status of forensically relevant compounds. For example, psilocin is a Schedule I controlled substance and is unlikely to be found in undergraduate laboratories, even those possessing a DEA license. Several articles have been published which investigate the use of laboratory chemicals and over-the-counter medications as forensic drug analogs in the undergraduate teaching lab [7–10]. For example, Hasan et al. have used 2-chloroacetophenone as a mescaline analog and indole as a substitute for LSD [7].

This experiment expands upon recent work by Huskins, et al. to determine the suitability of tryptamine as an analog for psilocin [11]. Tryptamine is used because of its structural similarity to psilocin, because it is affordable and readily available (~\$30/10g Sigma-Aldrich 98% CAS 61-54-1), and because it is not controlled by the DEA. Additionally, designer or synthetic tryptamine derivatives are becoming forensically relevant. The DEA reports 40 referrals of suspected designer tryptamines in a six month period [12]. 4-Aminoacetophenone was chosen as an internal standard due to its basic substituent group and aromaticity. Structures for psilocin, tryptamine (analog) and 4-aminoacetophenone (internal standard) are shown in Figure 1. During a solid-liquid acid extraction

process psilocin, tryptamine, and 4-aminoacetophenone would all be converted to their respective conjugate acids through protonation of the amine side chain. When positively charged, they can be separated from the colloidal chocolate matrix. Caffeine and theobromine from the chocolate are co-extracted during this process. A liquid-liquid extraction then converts the compounds of interest back to neutral organic molecules that are removed upon washing with an organic solvent.

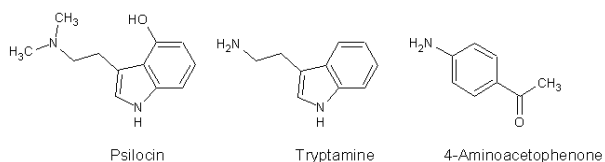
## Situational Context

Students are asked to assume the role of drug chemists in a state forensics laboratory. A State Patrol Officer has confiscated 5 kilograms of suspected psilocybin mushrooms and what appears to be a batch of homemade chocolate confections during a traffic stop. Students are asked to determine if the suspected mushrooms are positive for psilocin and if the chocolate contains the mushroom material.

## Experimental

Mushroom plant matter was substituted with a ground, dried Fisher brand cellulose laboratory sponge (Fisher Scientific Cat. 14-417) to eliminate inferences due to plant sterols and served as the substitute for dehydrated mushrooms. The sponge material was spiked with 1.00 mL of a 12 mg/mL stock solution of tryptamine in ethanol, close to the natural concentration of psilocin in mushrooms [13–14]. After the ethanol was allowed to evaporate, the mushroom substitute was then dispersed into molten dark chocolate ( $\geq 60\%$  cocoa) to simulate illicit preparations. The spiked chocolate was allowed to cool and then broken in to pieces for analysis. Upon acidifying the tryptamine in 0.1M HCl, neutral organic interferents were removed using petroleum ether and dichloromethane. The pH was adjusted to 10 using 0.1M  $\text{NH}_4\text{OH}$  and the tryptamine was extracted with a dichloromethane and ethanol mixture and identified by GC/FID, or subjected to TLC as a screening method. Caffeine (Sigma-Aldrich 99% CAS 58-08-2), theobromine (Fluka 98% CAS 83-67-0), tryptamine (Sigma-Aldrich 98% CAS 61-54-1), and 4-aminoacetophenone (Aldrich 99% CAS 99-92-3) standards were used for comparison.

**Psilocybin Mushroom Substitute.** According to TiHKAL (Tryptamines I Have Known And Loved by Alexander Shulgin), a therapeutic dosage of psilocin is 10-20 mg [14]. This experiment uses



**Figure 1.** Structural comparison of Psilocin, Tryptamine (analog), and 4-Aminoacetophenone (Internal Standard).

36 mg of tryptamine analog in a full serving (based on a 14 g serving) to produce a greater response for student analysis. To simulate psychedelic mushrooms for undergraduate student analysis, the lab sponge was cut into 3 cm squares and hydrated with 15 mL of DI water per gram of sponge. The hydrated squares were then shredded in a coffee grinder and placed in a 90 °C oven for one hour. 50.00 mL of a stock solution containing 12 mg/mL tryptamine and 12 mg/mL of 4-aminoacetophenone internal standard was prepared in ethanol. Using a 1.00 mL pipette, 0.33 g samples of dried, shredded sponge were spiked with the prepared stock solution. The ethanol was allowed to evaporate from the sponge until the surface appeared dry (~10 min). The spiked samples were added to 4.6 g of molten dark chocolate ( $\geq 60\%$  cocoa) and stirred in as the chocolate cooled. When complete, the sample had the appearance of a commercial candy bar.

**Tryptamine Extraction.** A frozen spiked chocolate sample was ground to a powder in a mortar. 5.0 mL of 0.1M HCl was added to the powder and ground until a paste formed. Another 10.0 mL 0.1M HCl was added to produce a slurry. The slurry was equally divided into two 15 mL conical bottom plastic centrifuge tubes and placed in a VWR clinical-50 centrifuge for five minutes at 2000 rpm. After 5 minutes in a centrifuge, the aqueous layer was removed with a transfer pipette and filtered into a separatory funnel. 2.5 mL of 0.1M HCl was then added to each tube and the remaining organic material was re-suspended and centrifuged for 5 more minutes. The aqueous layer was again removed and filtered into the separatory funnel. The aqueous solution was washed three times with 4.0 mL of petroleum ether then dichloromethane. The washed aqueous solution was adjusted to pH 10 with 5% ammonium hydroxide. The resulting neutral organic compounds were extracted with 4.0 mL of 9:1 dichloromethane/ethanol, dried with  $\text{MgSO}_4$  and filtered. The extract was then subjected to TLC and/or GC/FID.

**Qualitative Analysis.** As a screening method, the extracts were compared with standard solutions of tryptamine, caffeine, 4-aminoacetophenone, and theobromine on a silica gel thin layer chromatography plate (Selecto Scientific Cat. 33328). 4-aminoacetophenone was not visible on the plate. The order of elution (in a solvent of 13% ethanol, 85% ethyl acetate, and 2% ammonium hydroxide) was caffeine first, then theobromine, followed by tryptamine. The solvent selected was used in other procedures to compare psilocin with bufotenin, a similar hallucinogen [15]. An ultraviolet lamp was used to visualize the compounds on the plate.

**Quantitative Analysis.** Quantitative analysis of tryptamine serves to validate the method performance by determining the percent recovery and allows students to determine the concentration of tryptamine in an unknown sample. Using 4-aminoacetophenone as an internal standard, the response factor (F) is established by Equation 1 [4].

$$\frac{A_X}{[X]} = F \frac{A_S}{[S]} \quad (1)$$

Known concentrations of the analyte (X) and the internal standard (S) are analyzed in the same solution using GC/FID. The measured instrumental response, integrated peak areas

( $A_X$  and  $A_S$ ) of each, are divided by the known concentrations to determine the response factor (F). Once defined, the response factor can be applied to find the concentration of an unknown sample. Or, when students are provided with known starting concentrations, the chocolate samples are used to determine a percent recovery. A Shimadzu GC-17A with a SS420x Flame Ionization Detector and a Rtx<sup>®</sup>-5 column (Restek 15m, 0.25mm I.D., 0.25 $\mu\text{m}$  Cat. 10220) was employed for this experiment. The injection port and detector were set at 250 °C. Helium was used as the carrier gas at a flow rate of 3.0 mL/min. 1.00  $\mu\text{L}$  injections were made in splitless mode using a Shimadzu AOC-20i autosampler (Shimadzu Cat. 220-90932-02). The column was initially set at 60 °C and held for 2 min. An acquisition delay was set at 3 min. The temperature was then raised by 35 °C/min to a final temperature of 275 °C and held for 8.14 min.

## Results and Discussion

Standard solutions of tryptamine, caffeine, 4-aminoacetophenone and theobromine were prepared to identify their individual retention times by GC/FID (Table 1). The retention time for tryptamine and 4-aminoacetophenone are predicted to be less than the co-extracted interferents using a Rtx<sup>®</sup>-5 GC column.

Figure 2 represents the GC/FID analysis of a sponge extraction spiked with the analyte tryptamine and 4-aminoacetophenone as an internal standard. The areas and concentrations of sponge extractions were used to define the response factor  $F = 0.84 \pm 0.05$ .

Figure 3 shows a representative GC/FID analysis of a chocolate extraction. Using the response factor and Equation 1, students can calculate the concentration tryptamine in the chocolate extraction. The determined concentration was 11 mg/mL, or 93% recovery of tryptamine.

## Conclusion

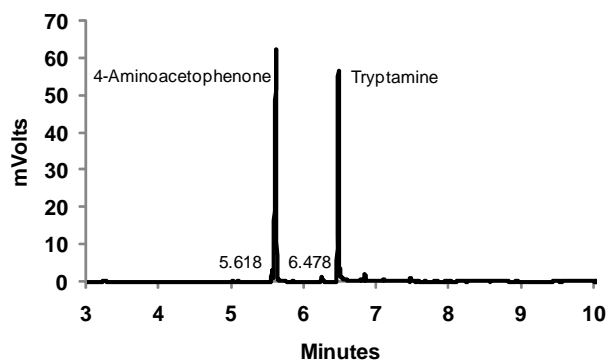
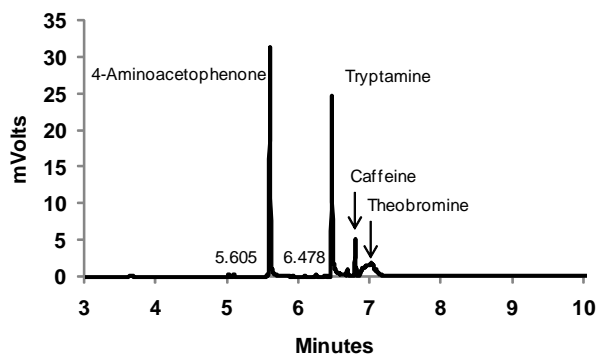
TLC and GC/FID demonstrates that tryptamine can be isolated from chocolate with a high percent recovery using procedures optimized for psilocin extraction [11]. The use of 36 mg of tryptamine per serving allows the student to perform presumptive screening and quantitative analysis on the hallucinogenic analog. After successful completion of this lab the students will have insight to illicit sample preparation and current methods of extraction and detection including: solid-liquid extraction, liquid-liquid extraction, thin layer and gas chromatography as well as drug chemistry and solutions chemistry including acid-base equilibria and solubility.

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**Supporting Materials.** Detailed procedures and example discussion questions are available as supporting materials (<http://dx.doi.org/10.1333/s00897092230a>).

**Table 1.** Retention times of the internal standard, analyte, and interferent standards. All concentrations were 1.0 mg/mL in 9:1 dichloromethane:ethanol

4-Aminoacetophenone	Tryptamine	Caffeine	Theobromine
5.62 ± 0.02 min	6.48 ± 0.01 min	6.70 ± 0.01 min	7.95 ± 0.03 min

**Figure 2.** Representative GC/FID chromatogram of an extracted, spiked sponge sample.**Figure 3.** Representative GC/FID chromatogram of extracted dark chocolate with a spiked sponge.

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