

Note

**Determination of hallucinogenic components of *Psilocybe* mushrooms using high-performance liquid chromatography**

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The interest in the hallucinogenic effects of mushrooms of the *Psilocybe* and related genera was first aroused through the reports of Wasson<sup>1</sup> on his expeditions to Mexico in the early 1950's. Specimens of *P. mexicana* (Heim) were analyzed by Hofmann<sup>2</sup>, who reported the presence of the two hallucinogenic components psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine) and psilocin (4-hydroxy-N,N-dimethyltryptamine).

The two commonly found hallucinogenic mushrooms in Australia are *P. cubensis* (Earle) Sing<sup>3</sup> and *P. subaeruginosa* (Cleland)<sup>4</sup>. Concern about their abuse in Australia was first shown in the early 1950's<sup>5</sup>, and there is now a noticeable increase in the use of hallucinogenic mushrooms during their natural growing season. In order to establish regulatory control over the use of the hallucinogenic mushrooms, it is essential to develop appropriate assay procedures to determine the amount of active hallucinogens present.

Several techniques have been employed to analyze for psilocybin and psilocin in solvent extracts of the mushrooms. These include column chromatography<sup>6,7</sup>, thin-layer chromatography<sup>8,9</sup>, gas chromatography (GC)<sup>10</sup>, ultraviolet spectroscopy<sup>8</sup> and GC-mass spectrometry<sup>10</sup>. The results obtained from these analyses reveal a wide range of psilocybin and psilocin contents, depending on the species investigated.

These techniques are rather lengthy, and, in GC, it is necessary to derivatize the compounds before analysis. It is important for forensic applications that an accurate and rapid method for the determination of psilocybin and psilocin be available. Accordingly, we have developed a high-performance liquid chromatographic (HPLC) procedure based on ion-exchange chromatography that provides excellent quantitation for psilocin and psilocybin following a simple extraction involving homogeniza-

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tion of the dried mushrooms in methanol. A reversed-phase HPLC method has been recently described, but no quantitation was reported<sup>11</sup>.

## EXPERIMENTAL

### *Apparatus*

The liquid chromatography system incorporated a Perkin-Elmer 1220 pump and column oven, a Perkin-Elmer LC 55 spectrophotometer and a Perkin-Elmer Model 3000 fluorescence spectrometer. Separation was achieved on an ion-exchange column (25 cm  $\times$  4.6 mm) of Whatman Partisil SCX-10. The analytical column was protected by a pre-column (3 cm  $\times$  2.8 mm) packed with 30- $\mu$ m pellicular beads<sup>12</sup>. The mobile phase was methanol-water (20:80) containing 0.2% of ammonium phosphate and 0.1% of KCl (pH 4.5) at a flow-rate of 1 ml/min. The temperature of the column oven was 50°C.

### *Reagents*

The methanol used in this work was redistilled analytical-reagent grade. Psilocin and psilocybin were supplied by Sandoz (Basle, Switzerland) and dimethyl-tryptamine was a product of Sigma (St. Louis, MO, U.S.A.).

### *Extraction procedure*

A sample of dried ground mushrooms (100 mg) was sieved on a Greenburn No. 22 sieve (0.0275 opening/in. BSS) and homogenized for 2 min in 3 ml of methanol using a Polytron-Kinematica homogenizer; the methanol contained N,N-dimethyl-tryptamine (1 mg/ml) as the internal standard. These conditions were considered to be optimum for extraction of the active components, since no further psilocin or psilocybin was extracted by increasing either the volume of methanol or the time of homogenization. This contrast markedly with the methods used by previous workers<sup>6-10</sup>, where extraction times were usually several hours.

The extract was centrifuged at 1600 g for 5 min, and 10  $\mu$ l were injected into the HPLC system.

## RESULTS AND DISCUSSION

### *Fluorescence spectra*

Fluorescence spectroscopy was investigated as a method of selective detection and quantitation of compounds in the HPLC eluent. Excitation and emission spectra of psilocin and psilocybin dissolved in the mobile phase were recorded. Psilocybin was found to fluoresce strongly at 335 nm, with excitation at 267 nm, whereas psilocin displayed weak fluorescence at 312 nm, with excitation at 260 nm.

### *Chromatography*

Representative chromatograms are shown in Fig. 1. The upper traces show the response of the UV detector at 267 nm, corresponding to the maximum in the absorption curve for both compounds. The lower traces are the chromatograms obtained from the fluorescence detector with excitation and emission wavelengths optimized for psilocybin fluorescence. There is a significant improvement in the chromatographic

result. The psilocybin peak was confirmed by halting the flow of mobile phase during the elution of the peak and scanning the emission and excitation spectra. The results are shown in Fig. 2, and the spectra can be seen to correlate very well with those of the pure substance.

Straight-line calibration graphs were obtained for both psilocybin and psilocin over a range of 0–10  $\mu\text{g/ml}$  by using UV detection at 267 nm, and were constructed from a plot of the ratios of peak heights of the analyte (psilocin or psilocybin) relative to the internal standard (N,N-dimethyltryptamine; 10 ng per injection). The lines corresponded to the equations  $y = 0.027 + 1.635x$  and  $y = 0.034 + 1.605x$ , with correlation coefficients of 0.99982 and 0.99938 for psilocybin and psilocin, respectively. Excellent linearity was also obtained in the psilocybin fluorescence response. The standard curve over the range 0–6 ng fitted the equation  $y = 0.035 + 1.187x$ , with a correlation coefficient of 0.99983. For five concentrations in the range 0–10  $\mu\text{g/ml}$  of

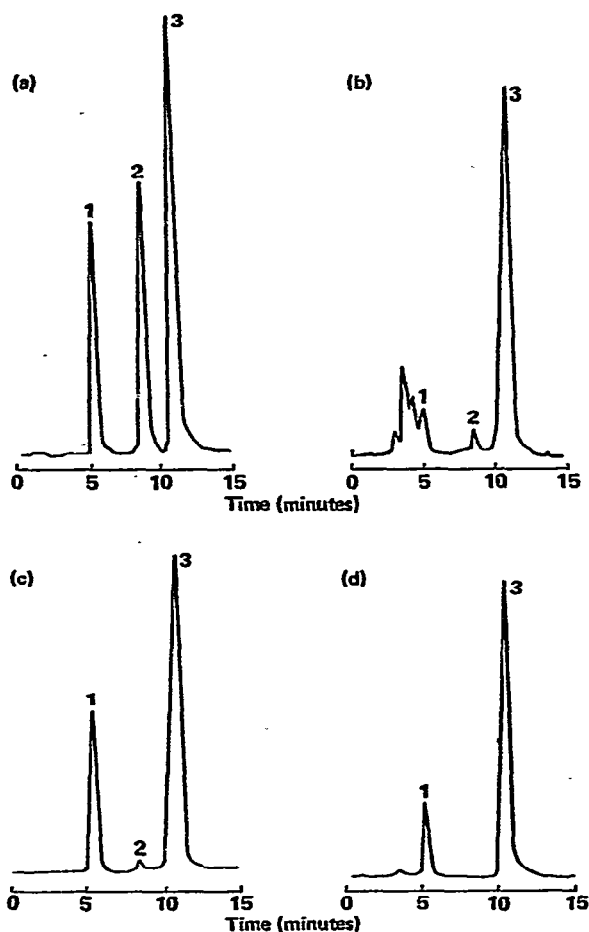


Fig. 1. Liquid chromatograms of psilocybin (1), psilocin (2) and dimethyltryptamine (3). Upper chromatograms: detection by UV absorption at 267 nm of standard mixture (a) and mushroom extract (b). Lower chromatograms: detection by fluorescence at 335 nm (excitation at 267 nm) of standard mixture (c) and mushroom extract (d).

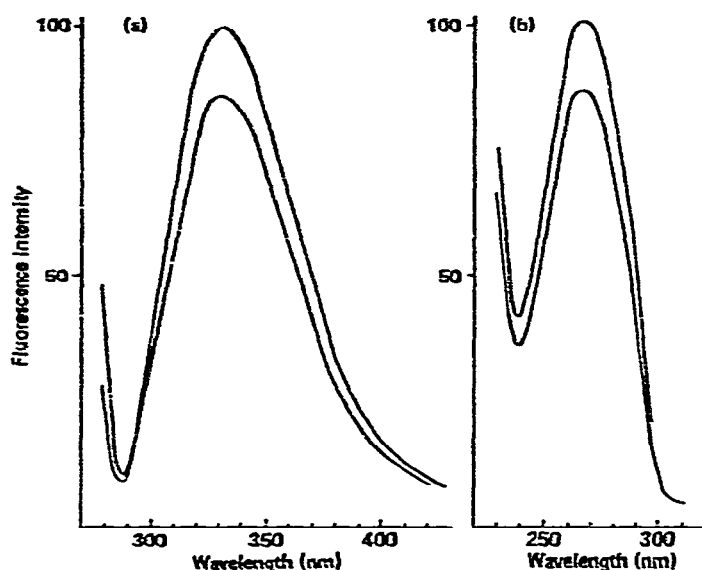


Fig. 2. Fluorescence spectra obtained by halting the flow of mobile phase during elution of psilocybin. (a) Emission spectra with excitation at 267 nm; (b) excitation spectra (emission at 335 nm). The upper traces are from psilocybin standard and the lower traces from mushroom extracts.

psilocybin and psilocin and four determinations per concentration, the average coefficient of variation was 3.9 and 2.8 %, respectively.

The minimum detectable quantities (signal-to-noise ratio  $> 2$ ) for psilocybin and psilocin are 250 pg and 30 ng with fluorescence detection at the optimum wavelengths for each component, and 7 ng and 150 ng by using ultraviolet absorption at 267 nm.

#### *Mushroom extracts*

The analytical procedure described above has been used in a preliminary study of the psilocybin content of a number of mushrooms. Eight different mushroom specimens were extracted and analyzed; the results are given in Table I. There is excel-

TABLE I

PSILOCYBIN CONTENT OF DRIED MUSHROOM SAMPLES DETERMINED USING UV AND FLUORESCENCE MONITORING OF THE HPLC ELUATE

| Sample No. | Psilocybin content (% w/w) |                       |
|------------|----------------------------|-----------------------|
|            | UV detector                | Fluorescence detector |
| 1          | 0.06                       | 0.06                  |
| 2          | —                          | 0.01                  |
| 3          | 0.03                       | 0.03                  |
| 4          | 0.08                       | 0.08                  |
| 5          | 0.21                       | 0.20                  |
| 6          | 0.08                       | 0.07                  |
| 7          | 0.11                       | 0.11                  |
| 8          | 0.06                       | 0.06                  |

lent agreement between the results obtained by both ultraviolet absorption and fluorescence detection. All of the extracts investigated contained trace amounts of psilocin and other (at present unidentified) components. The results of a detailed study of the chemical constituents of the *P. subaeruginosa* mushrooms will be presented in a later publication.

## CONCLUSION

A method has been developed for the extraction and quantitation of the hallucinogenic components psilocin and psilocybin in mushrooms. The use of a fluorescence spectrometer as the HPLC detector improves both the sensitivity and specificity of the assay, particularly for psilocybin. Application of the assay to the analysis of a number of samples of *P. subaeruginosa* yields a range of psilocybin contents from 0.01 to 0.2%. The high specificity and good sensitivity of the assay makes it suitable for forensic work.

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