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## Note

# Determination of tryptamine derivatives in hallucinogenic mushrooms using high-performance liquid chromatography with photodiode array detection

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In Switzerland and other European countries there has been a marked increase in the abuse of wild hallucinogenic mushrooms, such as *Psilocybe* species, during the last few years. Most of these mushrooms are controlled drugs, *e.g.*, the widespread and often confiscated *Psilocybe semilanceata* (Fr.) Quél. (Strophariaceae). It contains the tryptamine derivatives psilocybin, the main psychotropic compound, psilocin and baeocystin[1,2].

High-performance liquid chromatography (HPLC) is the preferred method for analysis of *Psilocybe* constituents[3–12]. Isocratic normal phase, ion-exchange and reversed-phase systems with buffered mobile phase and ion-pair reagents are used together with UV, fluorescence or electrochemical detection. Most of these methods have disadvantages, *e.g.*, time-consuming extraction procedures, poor resolution of the indole alkaloids and/or interferences with the matrix, lacking determination of minor alkaloids, low sensitivity, inactivation of column packing, etc. It was the aim of the present work to develop a selective, specific and accurate HPLC quantitation method for Psilocybe alkaloids using a simple one-step extraction, a gradient reversed-phase system and computer-controlled high-speed spectrophotometry (photodiode array detection, PAD). The efficiency of PAD in the field of analytical toxicology[13], forensic chemistry and phytochemistry of psychotropic drugs (poppy, khat)[14,15] has been demonstrated previously.

## EXPERIMENTAL

### *Instrumentation*

The HPLC system consisted of an Altex 420 controller/programmer (Kontron, Zurich, Switzerland), two Altex 110A pumps, a pulse-dampener, a Rheodyne 71-25 injection valve with a 20-$\mu$l loop, a HP 1040A high-speed spectrophotometric detector system (Hewlett-Packard, Waldbronn, F.R.G.) and an HP 3390A integrator. The gradient separation was performed on a 250 mm × 4.6 mm I.D. column, packed with Spherisorb 5-$\mu$m ODS-1 using a slurry technique[16]. Solvent A was water containing 0.3 *M* ammonium acetate and buffered to pH 8 with concentrated ammonia, solvent B was methanol containing 0.3 *M* ammonium acetate. The solvents were passed through a 0.45-$\mu$m nylon membrane filter (Supelco, Gland, Switzerland) and

degassed during use with a constant flow of helium. The gradient profile was as follows: 0–2 min, 0% B in A (isocratic); 2–14 min, 0–95% B in A (linear gradient). The flow-rate was 2.0 ml/min. All measurements were carried out at ambient temperature. After 20 experiments the column was washed with water and methanol.

*Chemicals and reagents*

All chemicals were of HPLC or analytical grade, obtained from Fluka (Buchs, Switzerland) and Merck (Darmstadt, F.R.G.). Psilocybin (PY; 4-phosphoryloxy-N,N-dimethyltryptamine), psilocin (PI; 4-hydroxy-N,N-dimethyltryptamine) and 4-hydroxytryptamine (HT) were supplied by Sandoz (Basel, Switzerland), bufotenin hydrogen oxalate (BT; 5-hydroxy-N,N-dimethyltryptamine hydrogen oxalate), serotonin hydrogen oxalate (ST; 5-hydroxytryptamine) and tryptamine (TR) by Fluka, N,N-dimethyltryptamine (DMT) by Laboratoires Plan (Geneva, Switzerland). Baeocystin (BC; 4-phosphoryloxy-N-methyltryptamine) was isolated from *Psilocybe semilanceata* and synthesized[17].

*Mushroom samples*

*Psilocybe semilanceata* (Fr.) Quél. (Strophariaceae), *Panaeolina foenisecii* (Pers. ex Fr.) Mol. (Coprinaceae) and *Amanita citrina* (Schff.) S.F. Gray (Amanitaceae) were collected fresh at several places in Switzerland or confiscated from the illicit drug market. *Psilocybe cubensis* (Earle) Singer (= *Stropharia cubensis* Earle) was cultivated saprophytically on agar and grain media[18].

*Method*

Mushroom samples were lyophilized and stored at −20°C until used for analysis. The dried fruit-bodies (carpophores) were pulverized by squeezing through a 250-μm sieve with a pestle. An accurately weighed amount of a powdered sample, about 20 mg, was extracted with 2.0 ml of methanol, containing 80 μg/ml of BT as internal standard (I.S.), in an ultrasonic bath for 15 min. The filtered extract was then concentrated to about 400 μl under a stream of nitrogen, filtered (if necessary) and aliquots of 10 μl were injected into the HPLC system. Quantitation was done by measuring the peak areas of BC, PY, PI and I.S. at 269 nm (150 m.a.u.f.s.).

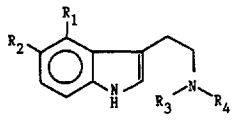
## RESULTS AND DISCUSSION

With methanol (solvent: sample = 100:1, v/w) and ultrasonication (15 min), the Psilocybe-tryptamine derivatives BC, PY and PI as well as the I.S. (Table I) are rapidly and almost quantitatively extracted from the finely powdered mushroom matrix. BT, used as the I.S., is a naturally occurring tryptamine derivative found within the fungi, so far exclusively in *Amanita* species[19]. The efficiency of the simple one-step extraction was tested by spiking "negative" mushroom material (*Panaeolina foenisecii*) with standards. The recovery of Psilocybe-tryptamine derivatives is shown in Table II. The methanolic extracts are stable over several months when stored at −20°C.

An efficient separation with symmetrical peak shapes for all the tryptamine derivatives identified in methanolic extracts of *Psilocybe* species (Figs. 1 and 2) was realized on 5-μm spherical $C_{18}$ packing material using a water–methanol gradient

TABLE I

STRUCTURES, RETENTION TIMES AND CONTENTS OF PSILOCYBE-TRYPTAMINE DERIVATIVES



| *Compound* | *Peak No.* | $t_R$ *(min)* | *Content (%)* | | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|---|---|---|
| | | | *P. semilanceata* | *P. cubensis* | | | | |
| Baeocystin (BC) | 1 | 3.8 | 0.85 | 0.11 | $H_2PO_4$ | H | H | $CH_3$ |
| Psilocybin (PY) | 2 | 6.3 | 1.76 | 1.07 | $H_2PO_4$ | H | $CH_3$ | $CH_3$ |
| Bufotenin (BT = I.S.) | 3 | 10.9 | | | H | OH | $CH_3$ | $CH_3$ |
| Psilocin (PI) | 4 | 11.7 | 0.23 | 0.18 | OH | H | $CH_3$ | $CH_3$ |

and ammonium acetate as buffer. Ammonium acetate is chemically stable, highly soluble in water and methanol, an excellent masking agent for residual silanol groups and able to accelerate the proton equilibrium in the chromatographic process[20]. As for the retention mechanism on reversed-phase columns, hydrophobic interactions between the solutes and non-polar stationary phase, ion pairing and ion exchange have been discussed[20]. The chromatographic system described represents a significant improvement for RP-HPLC separation of most zwitterionic phosphorylated and non-phosphorylated tryptamine derivatives within a wide range of polarity (Fig. 3).

Peak detection and identification was done using a computer-aided high-speed spectrophotometer (photodiode array detector, PAD). As demonstrated in Figs. 1 and 2, the Psilocybe-tryptamine derivatives are identified by their retention times and on-line UV spectra over the range of 240–350 nm in comparison with data for pure standards (Fig. 3). BC and PY have the same characteristic absorption maxima at 269 (detection wavelength), 282 (sh) and 292 (sh) nm, PI shows maxima at 269, 284 (sh) and 293 (sh) nm. An indication of the peak homogeneity is given by the coincident up-slope, apex (maximum) and down-slope spectra. To our knowledge this is the first time that BC, the monomethyl analogue of PY, has been found in *P. cubensis*.

TABLE II

RECOVERY, PRECISION AND DETECTION LIMITS OF THE METHOD

| *Compound* | *Recovery (%, n = 5)* | *Precision (mean, %; C.V., %; n = 5)* | *Detection limit (abs., ng; rel, %*)* |
|---|---|---|---|
| Baeocystin | 98 ± 2 | 0.51; 3.7 | 15; 0.003 |
| Psilocybin | 98 ± 2 | 1.34; 2.9 | 10; 0.002 |
| Psilocin | 98 ± 2 | 0.15; 2.5 | 8; 0.002 |

* 20-mg sample, extract concentrated to 400 μl, 10 μl injected.

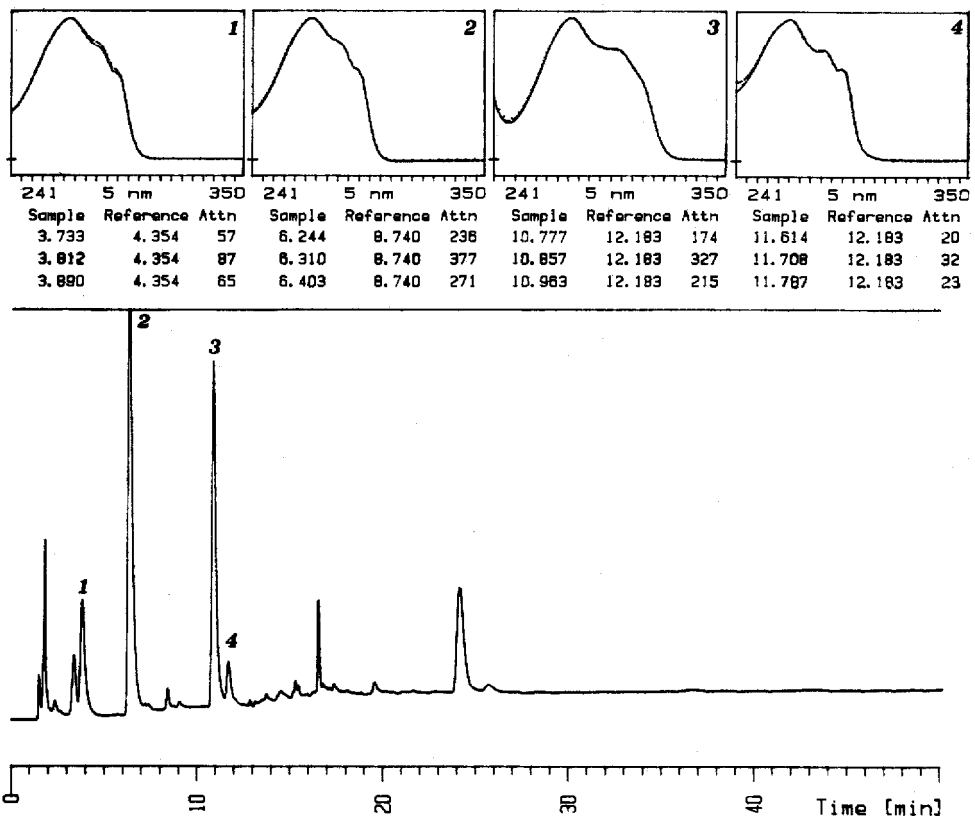



Fig. 1. Chromatogram and on-line UV spectra (up-slope, apex, down-slope) of *Psilocybe semilanceata*, extract. Chromatographic conditions: as described in Experimental. Peaks: see Table I and Fig. 3.

Until now, it was not known whether this phosphorylated indoleamine, which was first isolated from *P. baeocystis*[21,22], produces psychotropic effects like the dimethyl analogue PY. The unidentified peaks eluted between 15 and 26 min are the subject of further investigations. UV spectra of these unknown compounds also indicate indoleamine character.

The sensitivity of PAD allows the detection of even traces of tryptamine derivatives in mushroom samples of 10–20 mg dry weight. At 5 m.a.u.f.s. and a signal-to-noise ratio of 3:1, the minimum detectable amount of PY is 10 ng, corresponding to 0.002% PY when injecting 10 $\mu$l of a concentrated extract of a 20-mg mushroom sample.

The calibration graphs were obtained by measuring standard solutions in the concentration range of 20–200 $\mu$g/ml BC, PY and PI with an addition of 80 $\mu$g/ml I.S., and by use of linear regression analysis. A linear relationship ($r \geqslant 0.999$) was

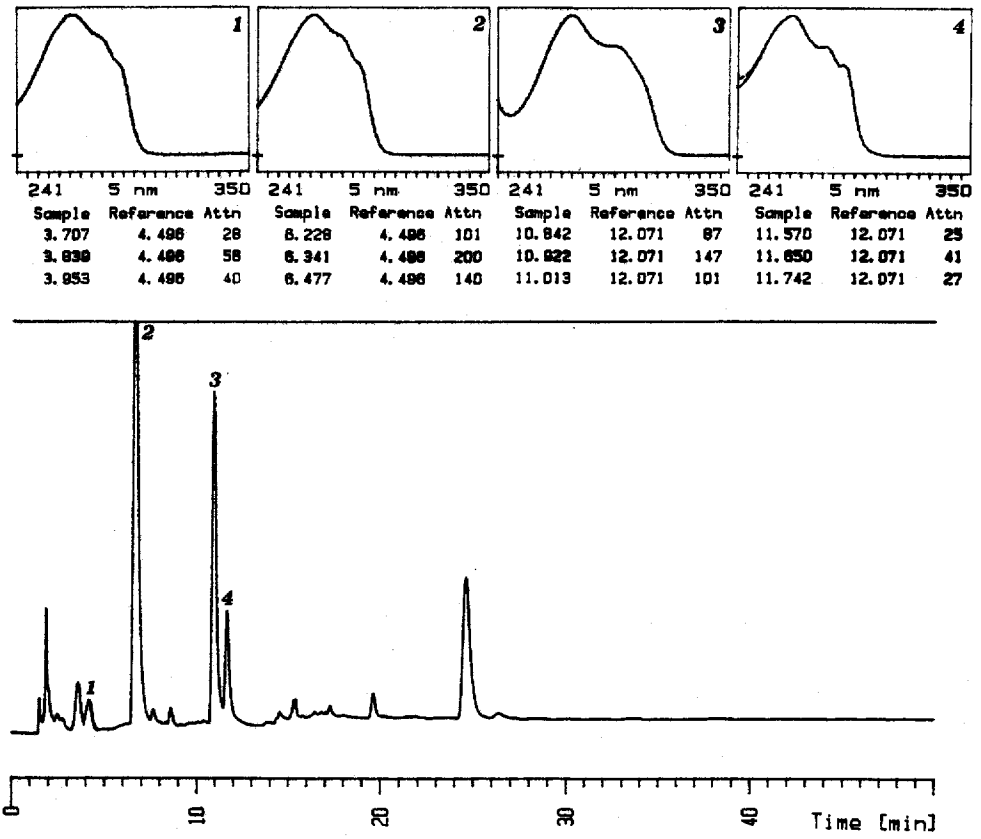



Fig. 2. Chromatogram and on-line UV spectra of *Psilocybe cubensis* extract. Chromatographic conditions: as described in Experimental. Peaks: see Table I and Fig. 3.

found at 269 nm between the peak-area ratio of BC, PY and PI *versus* I.S. and the concentrations of BC, PY and PI. The intra-assay precision of the method was determined by analysing five replicates of a *P. semilanceata* sample. The precision and detection limits of all the Psilocybe-tryptamine derivatives are summarized in Table II.

The method described was used for an extensive phytochemical screening of wild and cultivated mushrooms as well as for chemotaxonomic studies within the genus *Psilocybe*[23]. Its psychotropic potency was derived from qualitative and quantitative HPLC profiles. The contents of tryptamine derivatives in two representative hallucinogenic mushroom samples are given in Table I. The assay is also applicable to the quantitation of mushroom samples containing non-phosphorylated tryptamine derivatives. So 0.2% ST was found in *Panaeolina foenisecii* and 1.8% BT in *Amanita citrina*. In the latter case ST instead of BT has to be used as the I.S.

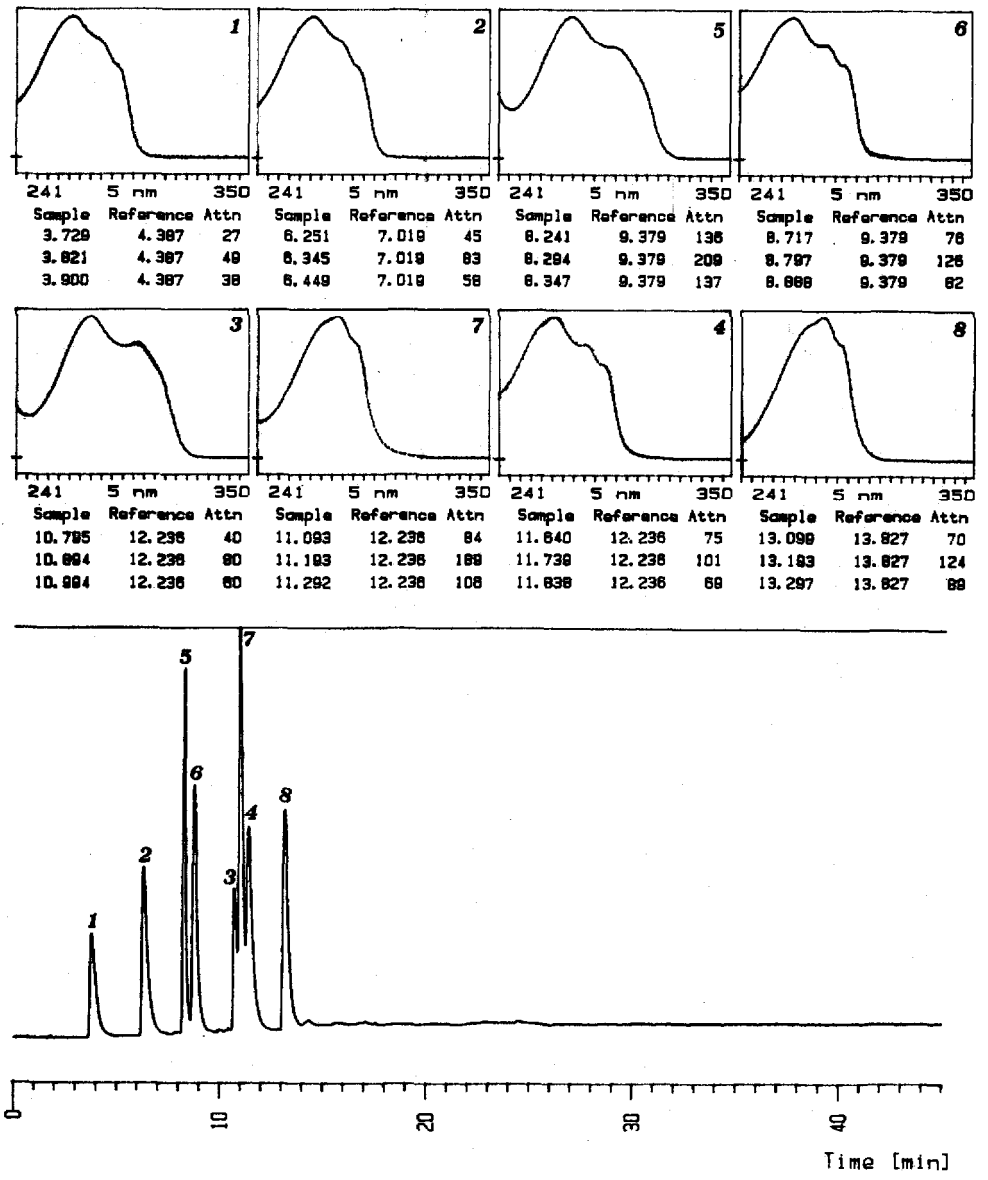



Fig. 3. Chromatogram and on-line UV spectra of tryptamine derivatives. Chromatographic conditions: as described in Experimental. Peaks: 1 = baeocystin; 2 = psilocybin; 3 = bufotenin; 4 = psilocin; 5 = serotonin; 6 = 4-hydroxytryptamine; 7 = tryptamine; 8 = dimethyltryptamine.

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