A Novel Extraction Procedure for Psilocybin and Psilocin Determination in Mushroom Samples

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Received: October 3, 1989

Since 1958, when psilocybin was discovered as an active principle of hallucinogenic mushrooms of the genus *Psilocybe* (1), many papers have been published about the determination of psychotropic indole derivatives. High-performance liquid chromatography is now almost exclusively used for the quantitative determination of these substances (2, 3, 4, 5, 6, 7).

A key step in the analytical procedure is the extraction of the compounds from the biological material. However, meagre attention has been paid to the extraction procedure. Hofmann et al. (2) found that psilocybin and psilocin are well soluble in methanol, and have subsequently used solely methanol for extraction. Other authors then used this solvent with or without modifications (4, 6, 7, 9, 10, 11, 12, 13).

Psilocybin and psilocin were found in concentrations up to 1.0 % and 0–0.16 %, respectively, in fruit bodies of *Psilocybe bohemica* Sebek extracted with methanol.

In this work we optimized the extraction conditions for psilocybin and psilocin from fruit bodies of *Psilocybe* mushrooms and studied the influence of extraction agent composition, its quantity and extraction time on the yield of these compounds. High-performance liquid chromatographic method was used for quantification of psilocybin and psilocin.

Materials and Methods

Extraction

The dried fruit bodies of mushroom *Psilocybe bohemica* were cut and then perfectly homogenized in a glass mortar. The extraction was performed in 20 ml vials at a constant sample weight of 10 mg. It was carried out on a reciprocal shaker with a 15 mm amplitude and a frequency of 1.2 Hz. The crude extract was filtered through 1 mm PTFE filter before injection into the HPLC apparatus.

HPLC analysis

Chromatographic analysis was performed on LC-3B liquid chromatograph (Perkin-Elmer, USA). Column (250 × 4 mm I.D.) was packed with Silasorb SPH C18 7.5 μm (Lachema, Czechoslovakia). Mobile phase consists of methanol, water and acetic acid 10 : 90 : 1 (for psilocybin), or 35 : 65 : 1 (for psilocin). A spectrophotometric and electrochemical detection was used for quantification of both substances (14).

Results and Discussion

The composition of the extraction agent has a fundamental influence on the yield of extraction. For testing we used aqueous solutions of methanol and ethanol, in which the tested substances are soluble (2). The dependence of psilocybin and psilocin yield on the alcohol concentration (pure or in presence of potassium nitrate) is illustrated in Fig. 1. It follows from this that an optimal extraction agent for psilocybin is quite unsuitable for psilocin.

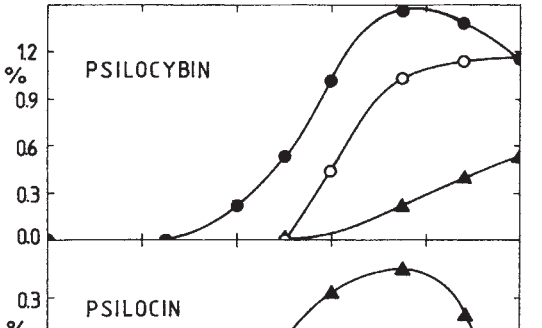

Fig. 1 Dependence of the determined amount of psilocybin and psilocin on the composition of the extracting agent (● methanol/water/saturated KNO_3; ○ methanol/water; ▲ ethanol/water).

We assume that the satisfactory values with methanol obtained by spiking (9) can be attributed to the extracellular supply of the spiked substances whereas in real samples they are intracellular.

A statistically significant dependence of the extraction yield on the amount of solvent was not found in range of 0.05–0.9 ml/mg for both compounds.

We also tested the influence of extraction time (0–10 h) on the yield of both substances. The time dependence may be well described by the equation (1).

$$C = a.(1-e^{b.t}) \qquad (1)$$

in both cases. C is the determined concentration in time of t, a and b are constants (the limit of a is the actual concentration of substance in the sample, b is the rate constant of the extraction). The calculated values for psilocybin and psilocin were a = 1.159, b = −41.429 and a = 0.190, b = −2.580, respectively. The extraction of psilocin thus appears to be much slower than that of psilocybin.

On the basis of these results, we devised an improved procedure for extraction of psilocybin and psilocin from samples of mushroom fruit bodies.

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Psilocybin: 10 mg of finely powdered sample is extracted for 10 minutes in 0.50 ml of 75 % methanol (saturated with potassium nitrate). The extract is then centrifuged, filtered through a membrane filter, and analyzed by HPLC.

Psilocin: An analogous procedure is used for extraction, the difference being in the composition of the extraction agent (75 % water/ethanol) and time (160 min).

The results obtained with the new extraction procedure were compared with the conventional extraction in methanol [e.g. (11)]; the results are summarized in Table 1. The mean yields are significantly different even on a high confidence level. Only 76 % psilocybin and about 8 % psilocin were found with the original extraction procedure in comparison with the new procedure.

Table 1 Comparison of the results of conventional and optimized extraction procedure in the determination of psilocybin and psilocin in *Psilocybe bohemica* (Poricko, middle Bohemia, 1983).

		method		
		conventional	optimized	T[b]
psilocybin	average	0.932	1.223	5.76
	S. D.[a]	0.149	0.129	
psilocin	average	0.041	0.448	29.75
	S. D.	0.019	0.055	

[a] standard deviation for 15 parallel measurements
[b] T criterion for significance test (15), critical value is 2.765 (alpha = 0.010)

The results pose a question if the absence or the very low concentrations of psilocin determined in the presence of substantially higher values of psilocybin may not result from an incorrect analytical procedure. According to our results, the contents of psilocybin and psilocin are comparable in *Psilocybe bohemica*, in contrast to current literature data.

Acknowledgements

We thank Mrs. Marta Semerdzieva, Department of Experimental Mycology of our institute, for providing us with the biological material.

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