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Detection of psilocin in body fluids

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Abstract

Active compounds of some mushrooms e.g. *Psilocybe cubensis*, *Paneolus subalteatus* or *Stropharia coronilla*, the psychotropic agents psilocybin and psilocin, have hallucinogenic effects. In one case of 'magic mushroom' intake, we had to analyse blood and urine. Psilocin was detected in the urine with REMEDi HS. Most of the psilocin was excreted as the glucuronide. Therefore an enzymatic hydrolysis should be the first step in analysis. Free psilocin was determined at a concentration of 0.23 mg/l while the total amount was 1.76 mg/l urine. The concentration of psilocin in serum was too low for detection with REMEDi HS. We proved a GC–MS-method with d_3-morphine as internal standard and silylation with MSTFA. Similarly to urine, most of the psilocin in serum was found in the conjugated form. The concentration of free psilocin was 0.018 mg/l, that of total psilocin, 0.052 mg/l serum.

Keywords: Psilocin; Psilocybin; Body fluids; Magic mushrooms

1. Introduction

In recent years so called 'magic mushrooms' play a growing role among controlled substances. For instance, the species *Psilocybe cubensis* is offered as a set for cultivation (Fig. 1). At a corresponding time of the year in our region, mushrooms like *Paneolus subalteatus* or *Stropharia coronilla* can be collected [1]. Active components are the psychotropic substances psilocybin and psilocin (Fig. 4). The content of psilocybin is normally far higher than that of psilocin which does not have the protecting phosphorous ester group in its molecule. The mushrooms are consumed because of their hallucinogenic effects. They are illegal drugs with no accepted medical use but a high abuse potential (schedule I controlled substances). A rapid dephosphorylation of psilocybin to psilocin takes place in the organism [2], and it can be assumed that psilocin is similar to morphine glucuronidated to a high degree. Additionally psilocin should have comparable extraction properties as morphine because of the presence of a phenolic and a tertiary amino group. Symptoms after intake of psilocybin containing mushrooms can be very pronounced as the following example demonstrates.

2. Case report

Two young male people drove from Cologne to The Netherlands to buy narcotic drugs. Among others, mushrooms were offered. Each of the men consumed 4 to 5 g of mushrooms. Subsequently they doubled up with laughter. Sometime later the house wall seemed to come up to the persons. After the effects had worn off and the persons felt fit again, they started homeward. During the trip on the

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Fig. 1. In a journal, a set for cultivation of mushrooms was offered.

highway the driver stopped his car because he turned sick. Then policemen came, confiscated the rest of the drugs and forbade further driving of the car. Twenty min later the policemen observed the car again but with insecure driving and they stopped it. The reaction ability of both occupants was retarded, stability in standing was insecure and the eyes were glassy and reddened. Two h later, 5 to 6 h after consuming the mushrooms, the doctor taking blood noted the following observations concerning the driver: insecure and sluggish walk, inarticulate speech, strongly enlarged pupils, delayed pupillary reaction, dazed consciousness, retarded behaviour, depressive mood, illogical thinking, slight problems of naming time and local position.

3. Experimental

3.1. Reagents

Psilocin (100 μg/ml methanol) was obtained from Sigma–Aldrich. d_3-Morphine was synthesised in our laboratory.

β-Glucuronidase from *E. coli* K 12 (Boehringer/Mannheim).

MSTFA (*N*-Methyl-*N*-trimethylsilyltrifluoroacetamide) (MachereyNagel/Düren).

Varian Bond Elut LRC Certify 300 mg per 10-ml columns (Phenomenex/Hösbach) Reagent Kit and other supplies for drug determination with REMEDi HS.

3.2. Instrumentation

REMEDi HS drug profiling system (Bio-Rad).

Hewlett-Packard 5995A gas chromatograph/mass spectrometer connected to a 59970A Workstation. Split ratio, 1:10. Injector temperature, 250°C; detector temperature 200°C, and the oven temperature was programmed from 200°C at 5°C/min to 250°C. An HP Ultra-1 capillary column (12 m×0.2 mm I.D.× 0.33 μm film thickness) was used. Carrier gas: helium 5.0.

3.3. Performance of analysis

3.3.1. REMEDi

One ml of urine was prepared as recommended for drug analysis with REMEDi HS. Cleavage of the glucuronide in a second sample was performed in a water bath at 45°C during 1 h after addition of 10 μl of glucuronidase solution.

3.3.2. GC–MS

A solution of 25 ng of d_3-morphine in 40 μl

methanol was added to 0.5 ml of serum or urine, to a parallel sample, additionally 10 μl of glucuronidase solution for hydrolysis of the glucuronide as described above. The samples were diluted with 5 ml of phosphate buffer pH 8.

Column conditioning: the following solutions were aspirated through Varian Certify LRC 300-mg columns: 2 ml of methanol, 2 ml of phosphate buffer pH 8. Sample addition: the prepared samples were aspirated through the columns at 1 to 2 ml/min until the columns went to dryness.

Column wash: 2 ml water, 2 ml acetate buffer pH 4, 2 ml methanol/water (30% v/v). The columns were then dried under vacuum for 2 min.

Sample elution: the elution was performed with methanol/conc. ammonium hydroxide (98+2 v/v) at slight vacuum, first with 2 ml and then with 1 ml.

Derivatization: The solution was evaporated under nitrogen at 40°C and after addition of 50 μl MSTFA the tube was closed with parafilm and heated for 15 min at 80°C.

The injection volume was 10 μl. The detector was used in the electron impact mode (El) at 70. Mass spectra were recorded in the mass range of m/z 50–550.

Qualitative and quantitative analyses were performed using single-ion monitoring (SIM) mode by comparison of retention times and relative abundance of three ions (psilocin-di-TMS 290, 291, 348) resp. four ions (d_3-morphine-di-TMS 404, 417, 432, 433). The retention indices were calculated to 2099 for psilocin-di-TMS and 2520 for d_3-morphine-di-TMS.

4. Results

The calibration ranges were from 1 to 10 ng psilocin and d_3-morphine as di-TMS-derivatives corresponding to concentrations of 10 to 100 ng/ml serum or urine. There was linearity within these ranges ($R^2>0.99$). Recoveries of psilocin and d_3-morphine after addition of 25 ng/ml to 0.5 ml serum were nearly complete. Recovery of psilocin related to d_3-morphine as internal standard amounted to 98% with a standard deviation of 12% ($n=3$). The limit of detection was about 5 ng/ml serum.

After a conceded intake of *Psilocybe cubensis* blood and urine were available for investigation. Using REMEDi HS drug profiling system psilocin could be detected in urine. By GC–MS analysis in both body fluids psilocin was detectable. In urine a complete mass spectrum was to achieve as Fig. 2 demonstrates. The library search of the system led to psilocybin but it is considered that the phosphorous ester group is cleaved by the derivatization reaction.

Fig. 3 shows ion chromatograms of the serum sample. The characteristic mass peaks 290, 291, and 348 of psilocin-di-TMS are present at a retention time of 4.54 min in expected relative intensities.

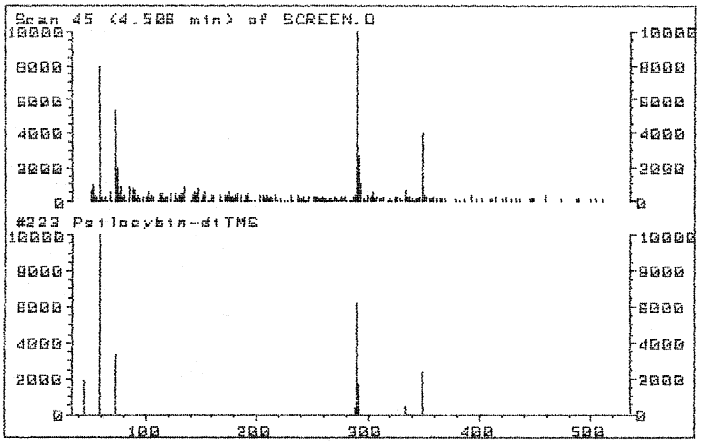

Fig. 2. Comparison of mass spectra of psilocybin-TMS-derivative and of a compound from an urine extract.

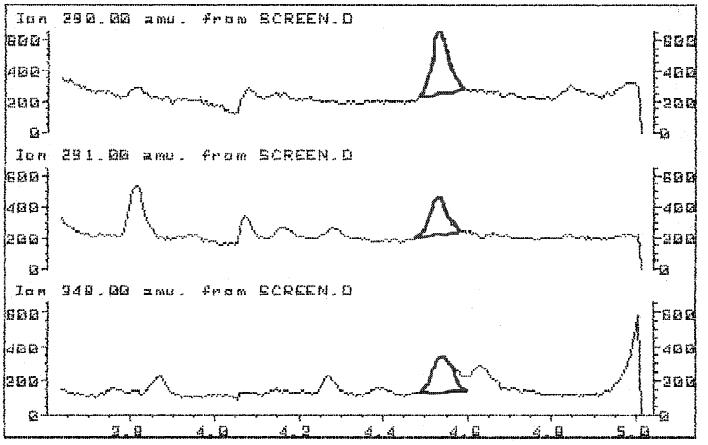

Fig. 3. Ion chromatograms of a serum extract (psilocin-di-TMS derivative marked with an arrow).

The following concentrations were measured (Table 1).

5. Discussion

Keller et al. [3] have recently described a GC–MS-method for the detection of psilocin in mushrooms after derivatization with MSTFA. In our procedure for estimation of morphine and benzoylecgonine, we use the same derivatization technique [4]. Protection of the two polar groups of psilocin, a phenolic and a NH-group, by trimethylsilyl (TMS) may avoid thermal decomposition. The structure of psilocin–di-TMS is confirmed by the occurrence of the molecular peak m/z 348 and the typical fragment m/z 290 formed by loss of m/z 58 in the mass spectrum.

Psilocybin, the main active component of 'magic mushrooms' is transformed into psilocin by rapid dephosphorylation which is indicated by a short period between oral and a very short interval between intravenous administration of psilocybin and maximum plasma levels, 105 resp. 1.9 min [2]. Hasler et al. [2] found peak plasma levels of 8.2 ± 2.8 ng/ml psilocin after oral administration of 0.224 ± 0.02 mg/kg (10–20 mg) psilocybin. Our investigations demonstrate that psilocin can be detected in body fluids after consumption of corresponding mushrooms. Glucuronidation seems to be an important detoxification step (Fig. 4). In the presented case, the free part of psilocin in serum is 35% and in urine only 13%. Similar high parts of bound psilocin in blood, urine and bile of two individuals are referred by Wennig [5].

Table 1
Psilocin concentrations in serum and urine after consumption of 'magic mushrooms'

	Serum	Urine
Psilocin (free) (mg/l)	0.018	0.23
Psilocin (total) (mg/l)	0.052	1.76

The REMEDi HS system is suitable for screening of urine for psilocin. But in spite of an expected obviously higher yield a general enzymatic hydrolysis is not to recommended because by far more interfering substances appear in the chromatogram which can overlap the internal standard 1.

In serum, not even after hydrolysis concentration of psilocin is high enough for a detection with REMEDi HS and therefore GC–MS analysis is the method of choice.

Extraction and derivatization are successful in the same way as with morphine. Psilocin can be detected therefore by the procedure used for opiate and cocaine detection without problems. Morphine or deuterated morphine can serve as internal standards because of similarities with psilocin in the structure and properties in the extraction procedure. For screening of blood or urine with GC–MS an enzymatic hydrolysis is recommended because the

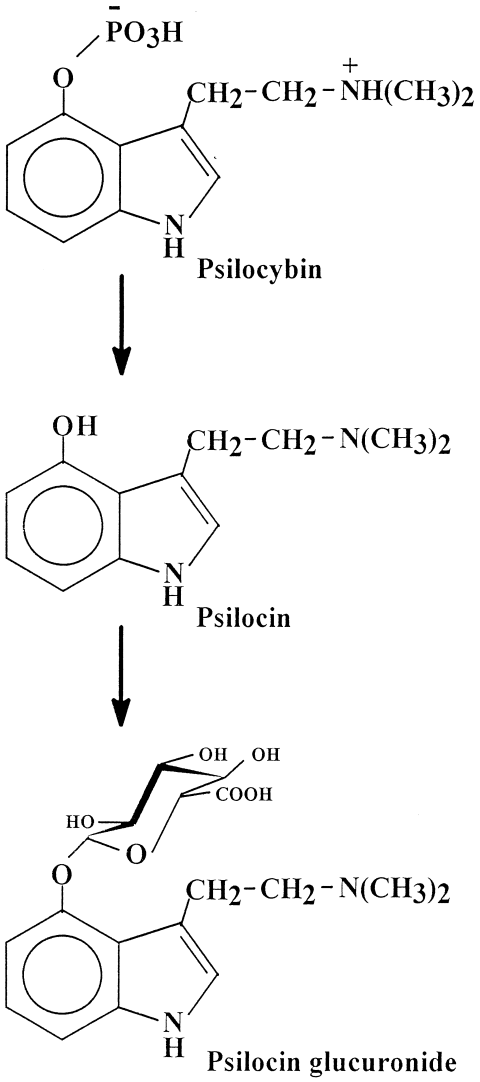

Fig. 4. Structures of psilocybin, psilocin and psilocin glucuronide.

detectable amounts of psilocin increase remarkably by this procedure.

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