

Magic truffles or Philosopher's stones: a legal way to sell psilocybin?

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"Magic mushrooms" is the most common name given to hallucinogenic fungi containing the psychoactive alkaloids psilocybin and psilocin. In recent years, fungus' sclerotia, commonly called "magic truffles" have become a form of supply of psychoactive Psilocybe alkaloids since Psilocybe sclerotia are not specifically included in the laws banning the sale, the purchase and the use of such substances and mushrooms containing them.

A liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed for the rapid determination of psilocybin and psilocin in Psilocybe sclerotia. Following a simple step extraction with methanol, the alkaloids were separated on a reversed-phase column using a gradient of 0.1% formic acid – acetonitrile as a mobile phase at a flow rate of 0.2 mL/min. Separated analytes were detected by electrospray ionization tandem mass spectrometry in the positive ion mode using multiple reaction monitoring. The developed method was linear over the calibration range for all two substances under investigation, with a $r^2 > 0.99$. The detection and quantification limits were 0.3 µg and 1 µg per 100 mg truffles, for both psilocin and psilocybin and the intra- and inter-day coefficients of variation were always better than 15%.

Using this method, the presence of only psilocybin was demonstrated in examined Psilocybe sclerotia. The content of psilocybin was found to vary over a concentration range of 59.3 to 167.8 µg per 100 mg of fresh sclerotia. Copyright © 2012 John Wiley & Sons, Ltd.

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Introduction

"Magic mushrooms" is the most common name given to hallucinogenic fungi containing the psychoactive alkaloids psilocybin and psilocin. There are approximately 190 species mushrooms containing these two alkaloids and most of them fall in the genus *Psilocybe*.^[1] Most known varieties are e.g.: *Psilocybe cubensis*, *Psilocybe semilanceata* and *Psilocybe Mexicana*, being this last variety the one from where psilocin and psilocybin were firstly isolated and identified in 1958 by Albert Hofmann.^[2]

Psilocybin and psilocin are two psychedelic alkaloids with mind-altering effects similar to those of LSD and mescaline.^[3] Once in the body, psilocybin is rapidly dephosphorylated in the gut to psilocin, a substance structurally similar to serotonin (5-HT), which acts as a 5HT_{2A}, 5HT_{2C} and 5HT_{1A} agonist.^[3] Psilocybin produces a range of acute perceptual changes (e.g. hearing colours and seeing sounds), subjective experiences, and labile moods including anxiety. It is reported to increase measures of mystical experience (e.g. psychedelic effects) and its physiological effects are similar to a sympathetic arousal state: tachycardia, increased body temperature, headache, sweating and chills, nausea and dilated pupils.^[4–7]

Psilocybin and psilocin are contained in the different *Psilocybe* fungi at a total concentration of approximately 1–2%.^[8,9] Since the average dose of psilocybin that induces hallucinogenic effects is 4–10 mg, the minimum amount of mushrooms needed to get the desired psychedelic and recreational effects is about 1 g dried or 10 g fresh mushrooms.^[8,9]

Psilocin and psilocybin are scheduled drugs since 1971 in United States,^[10] and mushrooms containing them are prohibited by extension. In the 2000s decade, in response

to increases in prevalence and availability, all European countries banned possession or sale of psychedelic mushrooms, with the Netherlands being the last country to enact such laws in 2008.^[11]

However, some varieties of *Psilocybe* fungi, specifically *Psilocybe tampanensis*, *Psilocybe mexicana* and *Psilocybe atlantis* (a close relative of *Psilocybe mexicana*) formed yellowish to brownish sclerotia in established conditions.^[12,13] Sclerotia are hard pieces of mycelium containing food reserves which the mushroom grows throughout the substrate and are wrongly defined as "truffles", and commonly called "magic truffles" or Philosopher's Stones.^[8]

In recent years, "magic truffles" have become a new form of supply of psychoactive *Psilocybe* alkaloids, since *Psilocybe* sclerotia are not specifically included in the laws banning the sale, the purchase and the use of such substances and mushrooms containing them.

Sclerotia are typically sold in "Smart Shops" but principally through internet web sites, where "magic truffles" are advertised to contain "very high concentrations of psilocybin". To our knowledge, few scientific reports for identification and quantification of

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Psilocybe alkaloids in sclerotia are present in the international literature.^[14–16]

In the past months, the Analysis Department of Italian anti-adulteration and safety bureau (Carabinieri per la tutela della salute- NAS) seized several plastic packs containing 0.7–1.2 cm diameter brownish-blue sclerotia as a part of an investigation coordinated by the Anti-Mafia District Directorate of Catania Public Prosecutor and requested specific analyses of seized products. We here present the results of analyses.

Experimental

Chemicals and reagents

Powder standards (99% purity as reported in the manufacturer certificates) of psilocybin, psilocin and 4-hydroxy N,N-

diisopropyltryptamine (used as internal standard) were obtained from LGC Standards (Milano, Italy). Ultrapure water and all other reagents of HPLC grade were obtained from Sigma-Aldrich (Milano, Italy).

Fourteen different sclerotia (or truffles) (for a total weight of 10.3 g, with each single sclerotia weighing from 0.5 to 2 g; Figure 1) seized by the Italian anti-adulteration and safety bureau police (Carabinieri per la tutela della salute- NAS) were received at the Istituto Superiore di Sanità to be analyzed for the presence of eventual psychoactive compounds. These truffles were sampled by the safety bureau police at the time of seizure from different plastic packs found together with other plastic packs containing unknown tablets and capsules in private houses during a search as a part of an investigation coordinated by the Anti-Mafia District Directorate of Catania Public Prosecutor.



Figure 1. Sclerotia (or truffles) seized by the Italian anti-adulteration and safety bureau police.

Calibration standards and sample preparation and extraction

Standard solutions (1 mg/ml) of the three compounds were prepared in methanol and corresponding working solutions at lower concentrations were prepared by appropriate dilution. Calibration standards (from 1 to 500 µg substance per 100 mg powdered blank commercial truffles, Tartufo Estivo “Tuber Aestivum Vitt. By Alfonso Fortunati Producer” Perugia, Italy) and quality control (QC) standards (1.2 µg, 200 µg and 425 µg per 100 mg powdered blank commercial truffles) were prepared to be used for the calculation of linearity and other principal validation parameters.^[17] In this concern, it has to be said that real recoveries could not be calculated as sclerotia are a solid matrix and spiked blank samples do not give information on real alkaloids recovery from the sclerotia, but only on solvent extraction efficiency.

Calibration standards and hallucinogenic truffles samples (aliquots of 100 mg from the fourteen different sclerotia), added

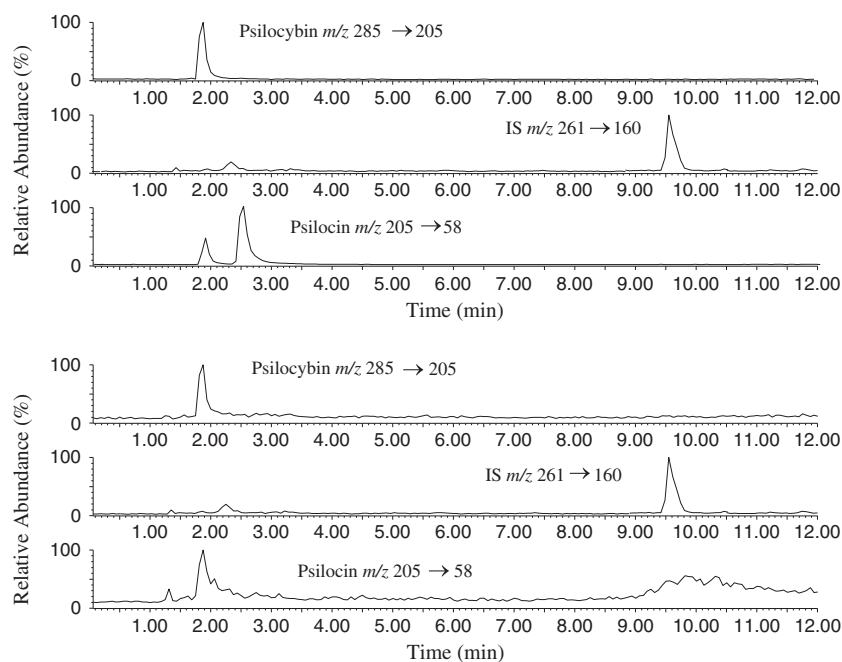


Figure 2. A) LC-MS/MS chromatogram of a blank commercial truffle spiked with 1 µg/mg psilocybin and psilocin and 0.5 µg/mg 4-hydroxy N,N-diisopropyltryptamine (IS); B) LC-MS/MS chromatogram of a real truffle containing 0.59 µg/mg psilocybin.

with 50 µl of 1 mg/ml internal standard were powdered in a ball mill (Restech, Munchen Germany) for five minutes. The powdered samples were extracted overnight with 5 mL methanol using horizontal shaker. After centrifugation, methanol was separated and evaporated to dryness at 40°C under a nitrogen stream. The dry aliquot was re-dissolved in 50 µl of mobile phase to be analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Liquid chromatography- tandem mass spectrometry

The LC-MS/MS analyses were performed using an Alliance HPLC system (Waters, Etten-Leur, The Netherlands) interfaced to a Micromass Quattro micro API triple quadrupole mass spectrometer (Waters) equipped with electrospray ionization (ESI) probe. Chromatographic separation was achieved using a Poroshel 120 SB-C18 column (100 × 2.1 mm; 2.7 µm) (Agilent Technologies, Palo Alto CA, USA). The experiment was carried out at a flow rate of 0.2 mL/min with a mobile phase A (0.1% formic) and B (acetonitrile). The gradient elution started at 5% B, ramped linearly to 20% B in 5 min, and maintained at 20% for 3 min, then directly returned to initial percentage and maintained for 4 min.

MS/MS characterization of the compounds under investigation was achieved using the triple quadrupole using an electrospray ionization (ESI) probe. The analytes dissolved in methanol at a concentration of 10 µg/ml, were infused through an integrated syringe pump into the ESI probe at a rate of 10 µl/min for tuning the mass spectrometer and optimizing the acquisition parameters. The following optimized conditions were used: collision energy at 15 V; capillary voltage at 4.5 kV, cone voltage at 30 V, source temperature at 150°C, and desolvation temperature at 450°C. The cone and desolvation gas flows were set at 60 and 800 L/h, respectively. The collision gas was argon at a collision cell pressure of 0.25 Pa (2.5 × 10⁻³ mbar). The transitions chosen for each compounds were: m/z 205 → 160 and **205 → 58** for psilocin, m/z 285 → 240 and **285 → 205** for psilocybin, **261 → 160** and 261 → 114 for 4-hydroxy N,N-diisopropyltryptamine (internal standard). The transitions in bold were used for compounds quantification.

Results and discussion

A chromatographic run was completed in 12 min, with 1.8 min as retention time for psilocybin, 2.5 min for psilocin and 9.5 min for 4-hydroxy N,N-diisopropyltryptamine (Figure 2). The internal standard run time so distant from those of the two psilocybe alkaloids was due to the fact that methodology was also set up to determine mescaline (retention time: 6.7 min) and dimethyl-tryptamine (retention time: 5.1 min) from other plants preparations. Linear calibration curves were determined by least-squares regression with 1/x² weighting and were based on peak area ratios of the compounds under investigation with the internal standards. They showed correlation coefficients (r²) higher than 0.99 in all cases and intra-assay and inter-assay precision and accuracy always better than 15% were considered adequate for the purposes of the present study. These and other validation parameters are reported in Table 1.

With respect to the matrix effect, the comparison between peak areas of analytes spiked in extracted blank samples versus those for pure diluted standards showed less than 10% analytical signal suppression due to coeluting endogenous substances.

Table 1. Validation parameters data*.

Analyte	Slope ± SD		Intercept ± SD		Correlation coefficient (r ²) ± SD		LOD (ng/mg)		LOQ (ng/mg)	
	Recovery (%)		Standard Deviation		Intra-day Accuracy (Error%)		Inter-day Precision (CV%)		Inter-day Accuracy (Error%)	
Psilocybin	Low QC	98.01	98.12	87.12	High QC	0.998 ± 0.002	3	10	Low QC	10
Psilocin	Low QC	91.03	91.72	85.14	High QC	0.997 ± 0.002	3	10	Low QC	10
Matrix effect (%)										
Process Efficiency (%)										
Psilocybin	Low QC	98.10	92.50	93.29	High QC	0.998 ± 0.002	3	10	Low QC	10
Psilocin	Low QC	91.42	97.01	98.23	High QC	0.997 ± 0.002	3	10	Low QC	10

* Number of replicates: slope, intercept, LOD, LOQ, intra-day precision and accuracy, matrix effect and process efficiency (n = 5); Inter-day precision and accuracy (n = 15).

** Low QC = 1.2 µg/100 mg truffle; Medium QC = 200 µg/100 mg truffle; 425 µg/100 mg truffle.

Only psilocybin could be determined in the analyzed sclerotia and no trace of psilocin could be found, at least at the limit of detection of the assay, calculated at 0.3 µg substance per 100 mg truffles. The psilocybin content in the different aliquots of sclerotia ranged between 59.3 to 167.8 µg (mean and standard deviation: 113.7 ± 54.3 µg) per 100 mg truffles corresponding to 0.06–0.16% alkaloid per fresh sclerotia.

Few comparisons were possible with other data from literature. Similarly to our results, a report published on a web site of the Italian Department Anti-Drug Policies in 2010 provided a **psilocybin** content of 0.07% in examined sclerotia,^[16] while Laussmann T. *et al.* always in 2010 reported a concentration of 119 µg psilocybin per 100 mg of fresh sclerotia from *Psilocybe Tampanensis*.^[15] In early investigations, Gartz found a higher content of psilocybin of 0.68% and psilocin 0.32% in sclerotia of *Psilocybe tampanensis* and *Psilocybe Mexicana*, the two percentages were calculated in dry truffles weight.^[14,18] However, differently from our analyses, all these investigations also found **psilocybin** in analyzed sclerotia.

Nonetheless, it has to be said that both scientific reports and websites which commercialize “magic truffles” claimed that psilocybin and psilocin are light and temperature-sensitive and that degradation of the two alkaloids, particularly psilocin, may occur if mushrooms (and consequently sclerotia) are not stored at low temperature or shipped in vacuum.^[8,15,19,20] Usually bluing of mushrooms corresponds to alkaloids oxidation and consequent degradation.^[8,19]

This was what probably occurred with sclerotia seized the Italian anti-adulteration and safety bureau (Carabinieri per la tutela della salute- NAS). Indeed, at the time of seizure, vacuum packed products (with no information on storage conditions and time period before seizure) were opened for appropriate controls, transported at ambient temperature to the NAS department and stored in the fridge (4–8°C) for some days before they were received at laboratory, where they presented a brownish-blue colour (Figure 1).

Conclusion

At moment, magic truffles or Philosopher's stones are a way to sell psilocybin from Internet websites and “smart shops” or illegal black markets since it has not yet officially established if “sclerotia” can be considered mushrooms or part of them. At moment, only psilocybe alkaloids and mushrooms are included in the Italian and European banning laws on illegal drugs. Thus, formally this occurrence creates a legal vacancy which de facto allows the free selling of sclerotia, as it happens.

The results of our analyses can be an incentive for the policy makers to include *Psilocybe* sclerotia in the list of controlled plants or herbal products that contain psychotropic alkaloids in the corresponding laws banning the sale, the purchase and the use of illegal drugs in European and non European Countries.

Figure 1. “Magic Truffles” or “Philosopher stones” seized by the Italian anti-adulteration and safety bureau police

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