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Article

Novel qNMR Method to Quantify Psilocybin and Psilocin in Psychedelic Mushrooms

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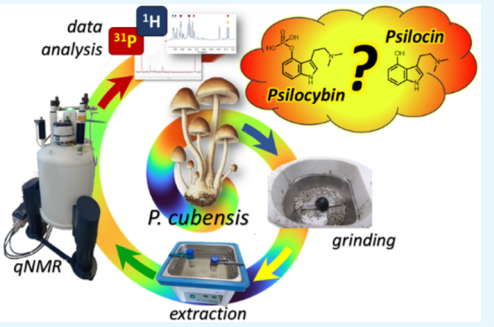
ABSTRACT: Psychedelic mushrooms of the *Psilocybe* genus contain the psychoactive tryptamines psilocybin and psilocin, compounds currently under clinical investigation for the treatment of depression and other psychiatric conditions. However, the accurate quantification of these alkaloids in fungal matrices remains analytically challenging. Here, we report an optimized extraction protocol and a robust, nondestructive quantification method based on quantitative nuclear magnetic resonance (qNMR) spectroscopy. Using a combination of ^{1}H- and ^{31}P NMR, we achieved simultaneous detection and quantification of psilocin and psilocybin in dried *Psilocybe cubensis* samples with high accuracy and reproducibility. Our method revealed significant variability in tryptamine content and psilocybin-to-psilocin ratios among user-provided and laboratory-grown samples, underscoring the potential influence of storage conditions on alkaloid stability. Compared with conventional chromatographic approaches, qNMR offers a rapid and calibration-free alternative for the routine analysis of psychedelic fungi. This approach may facilitate quality control in emerging clinical and regulatory contexts of psychedelic mushrooms.



INTRODUCTION

Psychedelic mushrooms, primarily belonging to the *Psilocybe* genus, have been extensively utilized throughout history for medicinal, religious, and spiritual purposes.[1,2] In the 1960s, they were widely known as "magic mushrooms" due to the intense psychedelic effects induced by their consumption, attributed later to the presence of the psychoactive alkaloids psilocybin (PSB, **1**) and psilocin (PS, **5**) (Figure 1).[3,4] Years later in 1970, psilocybin was included as a Schedule 1 drug, adversely impacting scientific research of their medicinal properties.[5] Since the 1990s, there has been renewed interest in exploring the medicinal potential of psychedelic substances for treating psychiatric disorders. Recent clinical studies indicate the safety and efficacy of PSB-assisted psychotherapy in producing immediate and enduring antidepressant effects in patients with major depressive disorder, results that appear to surpass the efficacy of conventional antidepressants.[6,7] PSB has also shown promise as a therapeutic intervention for various conditions, including substance use disorder,[8] and demoralization and depression in patients with serious illnesses.[9]

PSB-producing mushrooms are distributed worldwide and grow in various substrates. The biosynthesis of PSB and PS stems from a complex evolutionary trajectory shaped by gene-clustering events, convergent evolution, and horizontal gene transfer (HGT) driven by interactions among cohabiting species, alongside selective ecological pressures such as predation by mycophagous animals.[10,11] It has been determined that PSB is a pro-drug for PS, which is the actual active compound that targets the animal central nervous system and, given its close structural relationship with serotonin, intervenes in various physiological functions.[12] The removal of the phosphate group can be undertaken by several phosphatases present in the mushrooms,[13] and in the digestive system and liver of animals.[14,15] PS interacts with most serotonin receptors, and it is well established that its psychedelic effects are due to activation as a partial agonist of the 5HT2A receptor, which could also mediate some of its therapeutic effects.[12]

Although the main psychoactive alkaloids found in "magic mushrooms" species are PSB and PS, recent studies have shown that the universe of secondary metabolites present is far from being fully known and characterized. Structural variants of PSB have recently been described in several species of psychedelic mushrooms (Figure 1), such as baeocystin (4-OP-NMT, **2**), norbaeocystin (4-OP-T, **3**), aeruginascin (4-OP-TMT, **4**), and norpsilocin (4-OH-NMT, **6**).[16−19] In addition, the presence of several β-carbolines, such as harmine, harmane,

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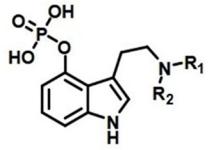

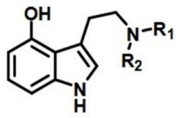

1, $R_1 = R_2$ = Me, **Psilocybin**
2, R_1 = H; R_2 = Me, **Baeocystin**
3, $R_1 = R_2$ = H, **Norbaeocystin**
4, N^+Me_3, **Aeruginascin**

5, $R_1 = R_2$ = Me, **Psilocin**
6, R_1 = H; R_2 = Me, **Norpsilocin**

Figure 1. Tryptamines are described for *Psilocybe* and related psychedelic mushrooms. Reprinted with permission from Anaclara Cabrera Varela.

norharmane, perlolyrine, and cordycinin C/D, has been found in *Psilocybe*.[20] A recent genomic analysis revealed the presence of genes encoding the biosynthesis of various families of secondary metabolites in some *Psilocybe* species, demonstrating unexplored metabolic diversity from the perspective of chemical analysis. Genes involved in the synthesis of terpenes, nonribosomal peptide synthetases (NRPSs), nonreducing polyketide synthases (NR-PKS), and halogenases were detected. Additionally, two new products were reported as metabolites of *Panaeolus cyanescens* and *Psilocybe mexicana*: lumichrome (an isoalloxazine) and Verpacamide A, a diketopiperazine with strong Chitinase activity.[21]

Regarding chemical analysis of psychedelic mushrooms, previous work (see Table S1 in the Supporting Information) has used numerous taxa to extract, detect, and quantify PSB and PS from samples of dried mushrooms, primarily from the *Psilocybe* genus. Regarding the extraction protocol, methanol has consistently been the solvent of choice, but with highly variable concentrations, ranging from 1 to 200 mg of dried mushroom per mL of solvent. Other experimental parameters, like the number of extraction cycles and conditions (e.g., mechanical agitation, ultrasound), show high variability. In terms of chemical and analytical quantification methods, previous studies analyzing psilocybin-producing mushrooms have relied primarily on chromatographic methods such as HPLC and LC-MS, with no reports utilizing NMR spectroscopy. In this regard, a recent study from our group successfully applied NMR-based quantification of tryptamines in ayahuasca,[22] demonstrating its capacity for simultaneous detection and quantification of multiple components in a single spectrum. This method is noninvasive, nondestructive, and rapid, with a linear response spanning 6 orders of magnitude (μM to M), making it highly advantageous for complex natural product matrices. In this regard, a recent report describes the metabolomic profile of edible mushrooms and quantifies several metabolites using qNMR.[23] In this manuscript, we developed and validated an optimized method for exhaustive extraction of PSB and PS from dried mushrooms and their detection and quantification by ^{1}H and ^{31}P NMR spectroscopy.

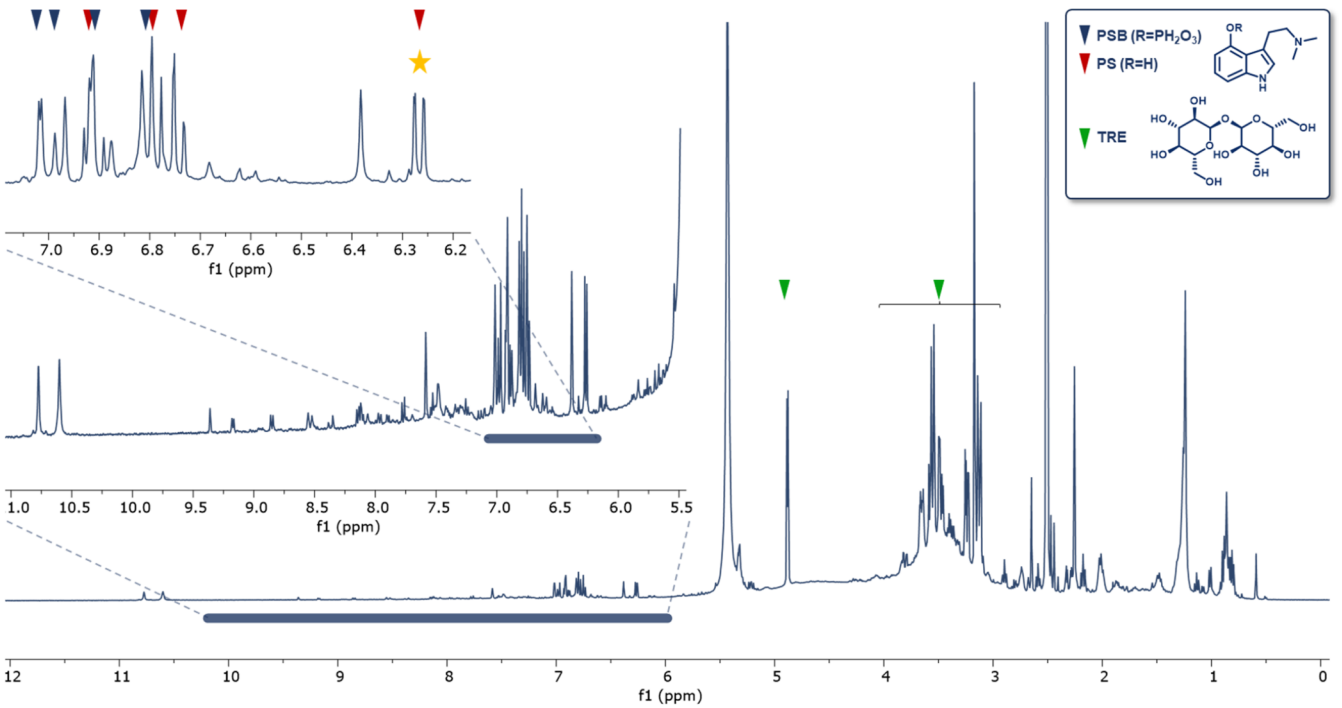

Figure 2. ^{1}H NMR (DMSO-d_6, 400 MHz) spectra of a typical methanolic extract of *P. cubensis* mushroom with signals from PS (red triangles), PSB (blue triangles), and trehalose (green triangles) identified. The signal used for PS quantification is marked with a yellow star.

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RESULTS AND DISCUSSION

Biological Samples and Identification. For this study, six samples of psychedelic mushrooms were analyzed, five of those were donated by users in Uruguay between March 2023 and February 2025 (labeled according to the name stated by the donor as "Golden Teacher", "Thai Pink Buffalo", "Cambodia", "B+" and "Libelula") and the remaining one was isolated from cow dung in a field in the Rocha department (Uruguay, 2022) and grown in the laboratory (labeled as "RA" by the collector). Samples were identified as belonging to the *Psilocybe cubensis* species, as described in the Methods section.

Optimization of Alkaloid Extraction. The optimization of alkaloid extraction from *P. cubensis* focused on maximizing the recovery of PSB and PS while minimizing sample degradation and interference from matrix components, such as sugars. The selected method, involving four consecutive methanolic extractions with ultrasound assistance (Figure S1A), demonstrated high efficiency by recovering 95% of the total PSB and PS content (from now on, total tryptamine content, TTC) by the fourth extraction (Figure S1B).

This optimized protocol was informed by a comprehensive comparison with previously published extraction methodologies (Table S1). Historically, a variety of solvents and extraction approaches have been used, ranging from single macerations with methanol at room temperature to Soxhlet extractions and, in some cases, acidified solvent systems. For example, early protocols employed thin-layer chromatography (TLC) analysis with single methanol extractions at concentrations as low as 2.5−10 mg of dry mushroom per mL of solvent, with no validation of alkaloid recovery.[24,25] More recent studies utilized more refined HPLC and UHPLC-MS/MS approaches with similar methanolic extraction protocols but limited the number of extraction cycles, often stopping after one or two extractions, potentially leaving a significant portion of the alkaloid content in the residual fungal matrix.[26,27]

Our study addressed this limitation through exhaustive sequential extractions, confirming that alkaloid recovery increased with each successive cycle until plateauing at the fourth extraction (Figure S1). This iterative approach contrasts with several previously reported methods that, while simpler, likely underestimate PSB and PS concentrations. Notably, methods using dynamic maceration,[28,29] or overnight extractions,[30] introduce a higher risk of degradation of the alkaloids, as evidenced by the formation of blueish psilocin oligomers as reported in the literature and observed during this work.[31]

One drawback of using methanol is the high trehalose content present in all of the methanol-based extracts. Trehalose, a sugar abundant in fungal matrices,[23] is the major component of the extract (Figures 2 and S2A), and its signals, especially the hydroxyl groups between 4 and 5 ppm, complicated NMR analysis of the much weaker alkaloid signals due to baseline distortions (Figure S2B). An acetone precipitation method was assayed to selectively reduce sugar interference while preserving alkaloid integrity (data not shown). However, to streamline sample preparation, minimize analyte loss, and reduce processing time, the final optimized method employed methanolic extraction without trehalose removal, as a robust baseline correction procedure was developed to obtain precise integrations, *vide infra* (Figure S2B). To the best of our knowledge, this is the first report that identifies trehalose as the main component of the methanolic extracts. A probable reason for this could be the difference in detection methods: previous studies relied on various LC-MS techniques, whereas the present work employed NMR spectroscopy. The difference between these methods resides in that the identification of analytes in the former is based on a targeted approach (where the researcher looks for evidence of specific compounds), while an NMR spectrum shows all components at once. For example, we found trehalose in the LC-MS ion trace as ammonium adducts (Figure S3), something that might easily be missed if one is not specifically looking for it.

Qualitative Analysis of Alkaloid Extracts. Qualitative analysis of the alkaloid content in the *P. cubensis* RA extract was performed using a combination of ^{1}H NMR, ^{31}P NMR, and LC-MS techniques. Methanolic extracts were redissolved in DMSO-d_6 after solvent evaporation. As mentioned before, the proton NMR spectra (Figure 2) revealed major signals in the aliphatic region, especially in the region between 3 and 5 ppm, suggesting the presence of sugars, especially trehalose, as was later confirmed. Most important for this work is the examination of the aromatic region where PS and PSB characteristic signals can be clearly distinguished and represent the major aromatic components, as shown in Figure 2. There are, nevertheless, other minor aromatic components that did not correspond to other reported tryptamines (Figure S4) or β-carbolines (Figure S5), as was evidenced by comparison with authentic samples of these compounds.

The chromatographic analysis with LC-MS further validated these findings, detecting both major alkaloids, which were corroborated through a comparison with authentic standards (Figure S6). In the same manner, no minor tryptamines or β-carbolines were found in the methanolic extract. As mentioned before, trehalose was also identified as the ammonium adduct in the extracted ion chromatogram, corroborating its presence by comparing the mass spectrum with that of the corresponding standard (Figure S3). The qualitative profiles for all samples were consistent across multiple extraction replicates, indicating the robustness of the optimized extraction protocol.

Quantitative NMR Determination of Alkaloids. Quantification of PS and PSB in *P. cubensis* extracts was performed by using quantitative NMR (qNMR), combining both ^{1}H NMR and ^{31}P NMR techniques. Psilocin was successfully quantified by using ^{1}H NMR, taking advantage of its isolated and well-resolved signal at 6.27 ppm, corresponding to the H-5 proton of the indole ring (Figure 2). As mentioned before, the methanolic extract was redissolved in DMSO-d_6 after solvent evaporation; solvents with exchangeable deuterium, i.e., MeOD and D_2O, were purposely avoided since 4-OH indoles can participate in hydrogen exchange mediated by the hydroxyl group. Since psilocin quantification depends on integration of the H5 signal, its exchange with deuterium would invalidate the method. On the other hand, PSB could not be quantified using ^{1}H NMR due to the absence of an isolated peak in the proton spectrum. Instead, ^{31}P NMR was employed to quantify PSB by targeting the phosphate group's signal. Comparing the phosphate resonance of an authentic PSB standard with the corresponding signal in the ^{31}P NMR spectrum of an extract, both externally referenced to 85% phosphoric acid, reveals a pronounced matrix effect, evidenced by a chemical-shift deviation of up to 0.4 ppm. We believe this is due to slight differences in the protonation states of the phosphate group.

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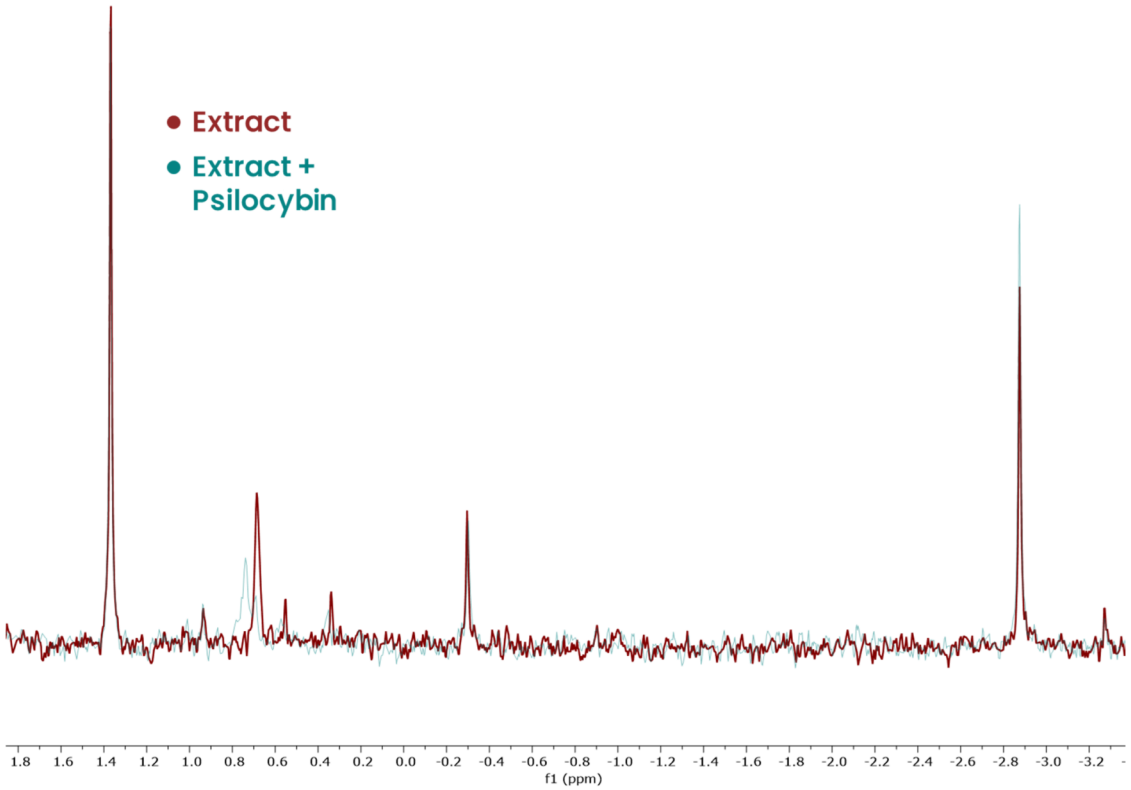

Figure 3. ^{31}P NMR (DMSO-d_6, 161.976 MHz) spectra comparison between a typical *P. cubensis* methanolic extract (maroon line) and the same extract with the addition of pure PSB (cyan line). An increase in the signal at −2.875 confirms its assignment to the PSB phosphate group. This signal was used for quantification purposes (yellow star).

Since the samples are dissolved in DMSO-d_6 and have different amounts of remnant water, it is difficult to assess the pH of the sample. Hence, to confirm the pH dependence of the ^{31}P chemical shift, we have added small amounts of TFA (10 uL total) to the NMR tube for two different extracts. Signal attributed to PSB shows an upfield shift of about 1.97 ppm, confirming the pH dependence of the ^{31}P chemical shift (Figure S7). Furthermore, to unequivocally assign the PSB phosphate group signal, we first looked for a long-range correlation with indole protons through ^{1}H−^{31}P Heteronuclear Multiple Bond Correlation spectroscopy (HMBC). We can observe a cross-peak between the ^{31}P signal at −2.875 ppm and H5 and H7 of PSB, which links the phosphate group to the indole ring (Figures S8 and S9). Also, we performed an additional spiking experiment in which pure PSB was added to the same NMR tube that was used previously to record the ^{31}P NMR spectra of the extract. In this experiment, we look for an increase in the PSB phosphate signal without the appearance of new signals. As shown in Figure 3, we can see a clear increase in the signal at −2.875 ppm, unambiguously assigning this signal to the PSB phosphate group (see also Figure S10).

The use of ^{31}P NMR offered several advantages over ^{1}H NMR for PSB quantification: a clean spectral window, minimal signal overlap, and a flat baseline free from interfering signals. These characteristics allowed for straightforward and reliable integration of the PSB signal.

In contrast, ^{1}H NMR spectra exhibited broad and overlapping peaks between 3 and 5 ppm, where the hydroxyl-rich structure of trehalose gives rise to broad, intense resonances that compromise integration accuracy. Since accurate NMR quantification requires a flat baseline, this interference posed a challenge. To address this issue, a first-order polynomial baseline correction was applied locally to the integration region of interest, effectively improving the psilocin quantification reliability (Figure S2B).

Quantitative results for all samples are summarized in Table 1, expressed as a percentage of the initial mass of dry material.

Table 1. Amounts of PS and PSB Found *in P. cubensis* Samples by ^{1}H and ^{31}P Quantitative NMR

sample	name	% PS	% PSB	% TTC
1	Cambodian	0.160 ± 0.016	0.033 ± 0.003	0.193
2	RA	0.016 ± 0.005	0.440 ± 0.019	0.456
3	Libelula	0.098 ± 0.001	0.056 ± 0.001	0.154
4	Golden Teacher	0.050 ± 0.002	0.250 ± 0.011	0.30
5	B+	0.360 ± 0.001	ND	0.36
6	Thai Pink Buffalo	0.13 ± 0.006	0.14 ± 0.007	0.27

Since it is well-known that PSB can be hydrolyzed to PS under acidic or humid conditions due to the activation of phosphatases present in fungal tissue,[13] we calculated the TTC as the sum of PS and PSB. This provides a more comprehensive metric for the total bioactive alkaloid load in each sample.

Results revealed significant variation in both the TTC and the relative proportions of psilocybin (PSB) and psilocin (PS) in the six *P. cubensis* samples. TTC values ranged from 0.154 to 0.456% of dry biomass, aligning with the mid-to-lower end of values reported in the literature, where TTCs as high as 1.5% of dry weight have been observed under optimal cultivation and storage conditions.[27,32]

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Table 2. Comparison of qNMR and LC-MS Quantification Results for Four Mushroom Samples

sample	% PS NMR	% PS LC-MS	% PSB NMR	% PSB LC-MS
P. cyanescensS1	0.31 ± 0.03	0.36 ± 0.03	0.282 ± 0.016	0.271 ± 0.007
P. cyanescensS2	0.110 ± 0.007	0.131 ± 0.005	0.044 ± 0.007	0.012 ± 0.003
P. cubensis **RA**	0.016 ± 0.005	0.018 ± 0.004	0.44 ± 0.02	0.52 ± 0.10
P. cubensis **Cambodian**	0.160 ± 0.016	0.190 ± 0.001	0.033 ± 0.003	0.044 ± 0.002

At the higher end, RA and B+ samples showed TTC values of 0.456 and 0.362%, respectively. Interestingly, the relative proportions of PS and PSB diverged between these two. RA contained almost exclusively PSB, whereas B+ consisted predominantly of PS. This contrast likely reflects differences in origin and storage: B+ was obtained from users and possibly exposed to environmental moisture, promoting PSB hydrolysis, while RA was cultivated and dried under controlled laboratory conditions that preserved the phosphorylated form. In contrast, samples like Cambodian and Libélula were at the lower end of the TTC spectrum, exhibiting low concentrations of both PS and PSB. This coincides with anecdotal and qualitatively reports on the Cambodian strain being of moderate potency.[32] We found no published information about the "Libelula" strain, suggesting it is a locally coined name. As others have noted, even well-known strain labels are often misapplied and chemically inconsistent, underscoring the need for rigorous characterization of locally named clones.[33]

Finally, Golden Teacher and Thai Pink Buffalo occupy an intermediate position in terms of TTC, with values of 0.30 and 0.27% of dry biomass, respectively. In the case of Golden Teacher, a strain known for its substantial psychotropic effects,[32] the majority of tryptamines were present as psilocybin (0.25%), with only a minor fraction as psilocin (0.05%). On the other hand, Thai Pink Buffalo exhibited a more balanced distribution, with approximately equal contributions from psilocybin (0.14%) and psilocin (0.13%). Together, these results highlight how even samples with comparable TTC values can differ markedly in their psilocybin/psilocin ratio, underscoring the importance of controlling storage conditions to preserve the chemical integrity of these alkaloids, as other authors have also noted.[27,32]

Validation of the qNMR Method for Alkaloid Determination. The qNMR method was validated by determining for each analyte linearity, accuracy, and precision; limit of detection (LOD) and limit of quantification (LOQ); and recovery and extraction efficiency.

Linearity and Precision. External calibration standards were used to construct concentration curves for both alkaloids to confirm the linearity. Benzoic acid (5.26 mg/mL) was used as an external standard for psilocin (^{1}H NMR), while dimethyl methylphosphonate (86 mM) served as the standard for psilocybin (^{31}P NMR). Both calibration curves exhibited excellent linearity throughout the range of concentrations measured, confirming the accuracy of the method (Figures S11 and S12).

The precision of the method was assessed through five independent extractions of *P. cubensis*, each analyzed in triplicate by ^{1}H and ^{31}P NMR (Table S2). The relative standard deviation (RSD) values were 11.3% for psilocin and 16.1% for psilocybin, indicating acceptable reproducibility for a method that includes extraction from a complex natural matrix.

Limit of Detection (LOD) and Limit of Quantification (LOQ). To determine the LOD and LOQ, serial dilutions of *P. cubensis* extracts were analyzed by ^{1}H NMR for psilocin and ^{31}P NMR for psilocybin, and each measurement was performed in triplicate. The LOD was defined as the lowest concentration presenting an RSD $\leq$ 20%, while the LOQ was set as the lowest concentration with an RSD $\leq$ 10%. The LOD and LOQ values obtained were 0.11 and 0.21 mM for psilocin (Figure S13 and Table S3), and 0.06 and 0.16 mM for psilocybin (Figure S14 and Tables S4 and S5).

Recovery Studies and Extraction Efficiency. To evaluate the efficiency of the methanolic extraction, recovery studies were performed using *P. cubensis* samples spiked with commercial psilocin and psilocybin standards, which were obtained as acetonitrile/water solutions. Initial trial results showed a complete disappearance of the psilocybin peak and a corresponding increase in psilocin concentration, suggesting complete enzymatic dephosphorylation of psilocybin by phosphatases present in the fungal matrix. To investigate whether the solvent of the standards was responsible for activating these enzymes, additional tests were conducted using acetonitrile and acetonitrile/water mixtures, which confirmed that the presence of water induced psilocybin hydrolysis (Figures S15−S17 and the text therein).

To circumvent this issue, a final recovery experiment was performed using PSB and PS standards dissolved in pure methanol, avoiding the presence of water to ensure minimal enzymatic activity. Under these conditions, recovery percentages of 92% for psilocin and 91% for psilocybin were achieved, confirming the efficiency of the extraction protocol and the reliability of the qNMR quantification.

Comparison with LC-MS Analysis. The accuracy of the qNMR method was further validated through a comparative analysis with LC-MS quantification using the same fungal extracts. The results summarized in Table 2 showed a strong correlation between the two methods. Notably, ^{31}P NMR provided an especially robust and interference-free approach for psilocybin quantification, highlighting its potential as a rapid and solvent-efficient alternative to chromatographic methods.

CONCLUSIONS

This study presents an optimized extraction protocol that ensures reliable and exhaustive recovery of psilocybin and psilocin from mushroom biomass. The method improves upon the efficiency of previously reported approaches while preserving the alkaloid stability and reducing sample preparation complexity for both qualitative and quantitative analyses.

The developed qNMR method, employing ^{1}H NMR for psilocin and ^{31}P NMR for psilocybin, demonstrated high accuracy, reproducibility, and robustness in the quantification of these psychoactive alkaloids. The use of ^{31}P NMR for psilocybin quantification significantly simplified the analysis, overcoming the limitations associated with matrix interferences in ^{1}H NMR spectra.

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The method was successfully applied to a panel of *P. cubensis* samples obtained from different sources, revealing significant variability in alkaloid content across specimens. These differences may reflect genetic diversity, growth conditions, or postharvest handling, underscoring the importance of robust analytical tools for consistent product characterization.

Together, the combined extraction and qNMR approach offers a rapid, nondestructive, and reagent-free alternative to chromatographic techniques. This workflow is particularly well-suited for the routine analysis of psychoactive fungi, supporting efforts in strain profiling, the standardization of therapeutic materials, and regulatory quality control.

METHODS

Reagents and Chemicals. Chemicals and reagents, including authentic samples of psilocin and psilocybin, were purchased from Sigma-Aldrich and used as received or were synthesized as described below. All solvents were distilled prior to use.

Biological Samples, Cultivation, and Identification. For this study, six samples of psychedelic mushrooms were analyzed. Five of these were donated by users in Uruguay between March 2023 and February 2025. These specimens were provided dehydrated in aluminum jars and were stored at room temperature and in the dark. The remaining sample was obtained through the isolation and cultivation of a native *P. cubensis* strain collected from cow dung during a sampling conducted in the Rocha Department, Uruguay, in 2022. In this case, tissue samples were taken from fresh basidiomata and cultivated on Petri dishes containing Potato Dextrose Agar (PDA). Then, pieces of pure culture were grown (4 weeks at 25 °C) on sterilized, hydrated oat grains (120 °C, 1 h). These cultures were used as inoculum at a 10% ratio in sterilized, hydrated bovine dung bags. The bags were incubated in the dark at 25 °C for 4 weeks. When the substrate was fully colonized, the bags were exposed to light, 80% humidity, and airflow to stimulate the formation of primordia. The mushrooms were subsequently harvested, dried, and stored at 20 °C in the dark and under airflow for future assays.

For the identification of the mushrooms, macroscopic and microscopic observations of basidiomes were conducted using the methodology described by Guzman (1995), Singer and Smith (1958), and Wright and Albertó (2002).[34−36] Macroscopic elements were analyzed, including basidiome dimensions, shape, color, margin characteristics, context, gills, and stipe coverage and base. Microscopic features, including basidiospores and cystidia, were examined by using an optical microscope, and small sections of basidiomes were hydrated with 5% KOH and stained with Congo Red (1%).

NMR Experiments. The qualitative and quantitative analyses of alkaloid extracts were recorded at 25 °C on a Bruker Neo 400 equipped with a BBO *z*-gradient probe operating at ^{1}H, ^{13}C, and ^{31}P frequencies of 400.13, 100.62, and 161.98 MHz, respectively.

qNMR analyses were carried out using the PULse length-based CONcentration (PULCON) method. Concentration measurements with PULCON are based on the reciprocity principle, which states that the sensitivity of a measurement and the radio frequency magnetic field generated in the coil are correlated. Since the rf field is inversely proportional to the pulse lengths of a 90 or 360° pulse, the concentration of an unknown sample can be determined as long as the concentration of a standard is precisely known and the 90° pulse of all of the samples has been calibrated correctly. To this end, a 20 mM benzoic acid in DMSO-d_6 sample was used for calibration, and spectra were recorded with 16 scans, a relaxation delay of 40 to 60s, and a 90° pulse length of 13 μs. All extract samples were redissolved in DMSO-d6 after solvent evaporation.

LC-MS/MS Analysis. For the LC-MS analysis of psilocybin and psilocin, an HPLC-DAD-ESI-MS/MS system was used, specifically a Shimadzu LCMS-8040, equipped with an LC-20AD HPLC pump, a solvent degassing module DGU-20A5R, a DAD SPD-M20A detector, a CTO-20A oven, a SIL-20A injector, and a FCV-32AH high-pressure switching valve with a split connection to the ESI-MS. The software used to process data was LabSolutions LC-MS. For chromatographic separation, the column used was a 100 × 4.6 mm Phenomenex Kinetex C18 Nucleosil with a particle size of 5 μm, a flow rate of 1.25 mL/min, and a temperature of 40 °C. UV−vis spectra were recorded in the range of 220−360 nm with detection at nm. Gradient system—Mobile phase: (A) 0.1% formic acid +2 mM ammonium formate and (B) 0.1% formic acid +2 mM ammonium formate in methanol. The gradient program was as follows: t0′, 0% B, t4′, 50% B, t7′, 90% B, t11′, 90% B, and t11.01′, 0% B. The mass spectrometer was programmed to carry out a full scan over m/z 100−600 (MS1) in positive ion detection mode. The heat-block and desolvation line (DL) temperatures were set to 400 and 250 °C, respectively. The nebulizer gas flow rate, drying gas flow rate, CID gas pressure, and ion spray voltage were 3.0, 15.0, 230, and 4.5 kV, respectively.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c07092.

Mushroom identification procedure, extraction protocols, and validation of the qNMR method (PDF)

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