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Investigating Psychedelic Tryptamines: Extraction and Quantification from *Psilocybe* Mushrooms

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Abstract

Background: *Psilocybe cubensis* mushrooms contain the tryptamines psilocybin and psilocin, which are recognized for their therapeutic potential in mental health [1]. Additionally, due to their psychedelic effects, these mushrooms are also used for religious and recreational purposes. The increasing use of these tryptamines and other psychoactive substances has led to regulatory measures, particularly Portuguese Decree-Law No. 15/93 of January 22, which aims to regulate the production, distribution, possession, and consumption of these substances. Psilocybin is generally more prevalent, with concentrations in dried mushrooms ranging from 0.5% to 1.5% [2]. **Objective:** The goal was to develop an efficient method for extracting and quantifying psilocin and psilocybin, as well as a fast and simple technique for identifying tryptamines. **Methods:** The mushrooms were finely pulverized using a cold porcelain mortar and pestle and extracted twice through kinetic maceration on a magnetic stirrer plate, with cold methanol containing 10% water serving as the extraction solvent (0.1 mL/mg of mushroom). An Agilent 1260 Infinity II HPLC-DAD system with a Poroshell 120 EC-C18 3.0 x 150 mm, 2.7 µm column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column was used for psilocin and psilocybin quantification [3]. **Results:** In order to optimize the extraction method, various parameters were taken into account, including solvent, extraction time, the number of extractions, agitation rate, temperature, and the solvent-to-dry material ratio. The concentrations of psilocybin and psilocin in the mushroom were found to be 1.98% and 0.10%, respectively. A Thin Layer Chromatography (TLC) method was developed for the rapid identification of psilocybin and psilocin, utilizing Ehrlich's reagent as the detection solution. **Conclusions:** An optimized extraction protocol was successfully established to maximize the recovery of target compounds. Additionally, a fast TLC identification method was developed for application in forensic sciences.

Keywords: psilocybin; psilocin; HPLC-DAD; mushrooms

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