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Development and validation of an HPLC-DAD analytical method for psilocybin and psilocin

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Abstract

Background: "Magic mushrooms", also known as hallucinogenic mushrooms, belong to the genus *Psilocybe* and contain tryptamines, especially psilocybin and psilocin, with potential medicinal benefits for mental health [1]. Psilocybin is usually found in higher concentrations than psilocin and both are present in dried mushrooms at a total concentration of approximately 0.5 to 1.5%. *Psilocybe* mushrooms are used for religious, recreational, and therapeutic purposes due to their psychedelic effects [1]. The increasing use of these tryptamines and other psychoactive drugs led to regulatory legislation, specifically Portuguese Decree-Law No. 15/93 of January 22, which aims to control the production, distribution, possession, and use of these substances. High-performance liquid chromatography (HPLC) coupled with UV-Vis, diode array (DAD) [2], or mass spectrometry (MS) [3] are commonly used analytical methods to quantify tryptamines. **Objective:** Development and validation of an HPLC-DAD analytical method for quantification of psilocybin and psilocin in *Psilocybe cubensis* extracts. **Methods:** Mushrooms were pulverised using a porcelain mortar and pestle and extracted with cold methanol by kinetic maceration on a magnetic stirrer plate. 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 analytical determinations. The optimised chromatographic method was established with mobile phase solvents: 10 mM ammonium formate with 0.1% formic acid and acetonitrile; oven temperature: 40 °C; flow rate: 0.8 mL/min; total run time: 12 minutes; injection volume: 1 µL; and UV detection wavelength: 220 nm. **Results:** To optimise the HPLC-DAD analytical method, various parameters were evaluated: mobile phase gradients and solvents, oven temperature, flow rate, run time, post-run time, injection volume and UV detection wavelength. This analytical procedure was validated following the ICH Q2 guideline. Linearity was tested over a range of 9 concentrations (0.50-200 µg/mL for psilocybin and 0.25-100 µg/mL for psilocin), obtaining R^2 values > 0.999. Method precision (%RSD) was ≤ 10%, with an accuracy (%bias) ≤ 15% for both compounds. **Conclusions:** An HPLC-DAD analytical method for the detection and quantification of psilocybin and psilocin was optimised, validated, and effectively applied to *Psilocybe cubensis* extracts.

Keywords: psilocybin; HPLC-DAD; mushrooms.

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