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Evaluation of a quantitative analytical method for psilocin and psilocybin using HPLC-DAD

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Resumo

Introduction: The determination of psilocin and psilocybin obtained from hallucinogenic mushrooms, is usually performed by gas chromatography (GC). However, psilocybin is not thermally stable due to high-temperature dephosphorylation under GC conditions [1]. Alternatively, using in liquid chromatography (HPLC) system, a mobile phase containing phosphate buffer and ammonia, a stationary phase column chromatography with silica and octadecyl silica, a reversed-phase separation, and a diode-array detector (DAD), is suggested in the scientific literature [2] as efficient alternatives to GC in the determination of these target compounds. **Objectives:** The aim of the present study was to develop and validated a procedure for the reliable quantification of psilocin and psilocybin obtained from hallucinogenic mushrooms extracts by applying an HPLC-DAD isocratic separation. The linearity, analytical limits, precision, and robustness were determined to validate the method evaluated. **Material and methods:** Separation chromatographic of psilocin and psilocybin was achieved using a Merck Hitachi LaChrom D7000 HPLC, equipped with a diode array detector (DAD) L-7455 and, a quaternary bomb system and an L7200 autosampler and a InfinityLab Poroshell 120 column, 3.0x5 mm, 2.7. A binary mobile phase comprising 10 mmol/L ammonium formate with 0.1 % (v/v) formic acid (MPA) and acetonitrile (MPB) were used. The flow rate was 0.5 mL/min, and the injection volume of the sample was 10 µL. The oven temperature of the chromatography column was maintained at 30 °C and the temperature of the autosampler at 5 °C. **Results:** The proposed method for HPLC-DAD was fully developed and validated. In the obtained chromatogram, the peaks of all the analyzed compounds are well defined with an overall separation time below 8 min. It can also be found that the elution times increase in the order psilocin < psilocybin. **Conclusions:** This analytical method has been successfully used for measuring levels of psilocin and psilocybin samples laboratory and analytical standards and proved to be fast, providing an analysis of high throughput and reducing the cost of analysis. The results of the method tested for linearity, accuracy, precision, and robustness, are framed to consider this method as valid. Project co-financed by the European Union Fund – Portugal 2020.

Keywords: mushrooms; psilocybin; psilocin; HPLC-DAD analysis

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