Determination of Psilocin and Psilocybin in Magic Mushrooms Using iHILIC®-Fusion and MS

Tibor Veress[1], Norbert Rácz[2], Júlia Nagy[1], and Wen Jiang[3], [1]Department of Drug Investigation, Hungarian Institute for Forensic Sciences, Budapest, Hungary, [2]Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Hungary, [3]HILICON AB

Hallucinogenic mushrooms, known as *magic mushrooms*, contain psychoactive compounds such as psilocin and psilocybin (Figure 1). This hallucinogenic effect means they are constantly offered on the black market. Therefore, the reliable quantification of these compounds is a particularly important task for forensic analysis because their results have a significant impact on the judgement passed by the courts.

Although there are many analysis methods available in forensic laboratories and in the scientific literature, the majority of them are based on reversed-phase liquid chromatography (LC) separation (1–6). Due to the highly hydrophilic nature of psilocybin and psilocin, reversed-phase LC is not able to provide sufficient retention for them. Moreover, it is crucial to develop new methods and techniques that can improve the analysis detectability, selectivity, and productivity. To fulfill these goals, the application of hydrophilic interaction liquid chromatography (HILIC) and mass spectrometry (MS) is investigated.

In this study, we aimed to use a charge modulated iHILIC®-Fusion HILIC column for the analysis of extracts from hallucinogenic mushrooms and evaluate its potential for forensic application.

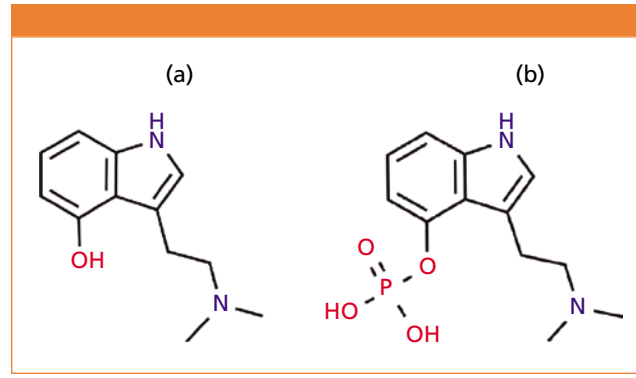

Figure 1: Chemical structures of (a) psilocin and (b) psilocybin.

Experimental

LC–MS System: Agilent 1100 LC system and Bruker Esquire 6000 ion trap mass spectrometer, operated in positive ionization mode (ESI+). Chromatographic data were acquired and evaluated with ChemStation Rev. A. 10.02.

Column: 150 × 4.6 mm, 3.5-µm 100 Å iHILIC®-Fusion (P/N 110.154.0310, HILICON AB, Sweden)

Mobile Phase: 80:20 (*v/v*) acetonitrile–ammonium format (10 mM, pH 3.5)

Flow Rate: 0.5 mL/min

Column Temperature: 12 °C

Sample Preparation: Quasi-counter current extraction with methanol at 60 °C in a Shimadzu 10/A HPLC system. A 50-mg measure of air-dried and homogenized hallucinogenic mushroom was filled in the extractor chamber (an empty 250 × 4.6 mm HPLC column). The standard solutions were 5 µg/mL and 500 µg/mL for psilocin and psilocybin, respectively. Methanol was used as the solvent.

Injection Volume: 1 µL

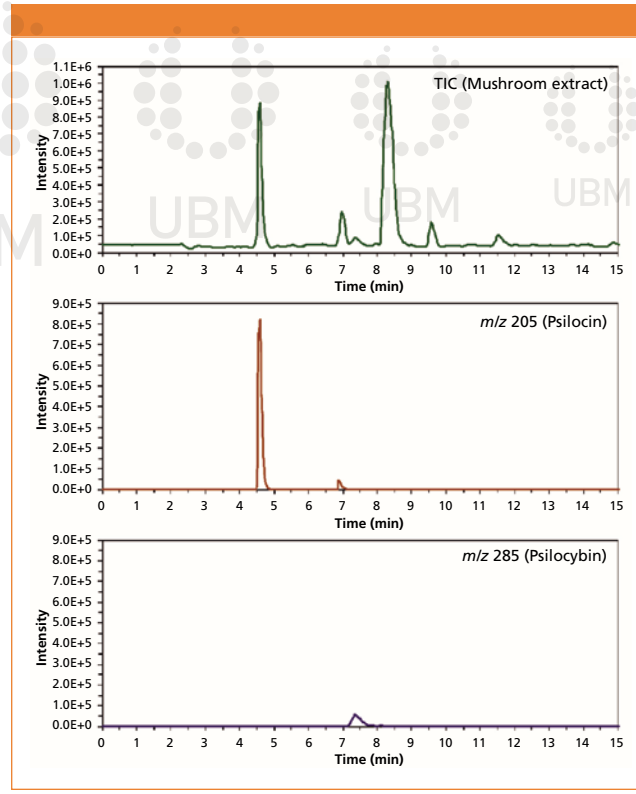

Figure 2: Total ion chromatogram (*m/z* 40–400) of the methanolic mushroom extract and extracted ion chromatograms of *m/z* 205 (psilocin) and *m/z* 285 (psilocybin).

Results and Discussion

In our previous study (2), the methanolic mushroom extract was first separated under the conditions within a designed experimental space with a total of 18 model establishment points and two approval points, considering the mobile phase composition, pH, and temperature. The factors that affect the separation selectivity and resolution on three iHILIC® columns were studied using DryLab® and STATISTICA®. It was found that iHILIC®-Fusion provides best separation regarding separation selectivity and efficiency. Figure 2 illustrates the separation of mushroom extract and also

the extract ion chromatograms at *m/z* 205 (psilocin) and *m/z* 285 (psilocybin), respectively. It is clear that iHILIC®-Fusion was able to separate psilocin and psilocybin from each other and also from the major matrix compounds within 15 min. An unique feature is that psilocybin elutes with a retention factor two times greater than that of psilocin. In addition, the sample preparation consists of few steps to minimize error sources and assure reliable results.

In the second step of this work, we separated the methanolic solution of psilocin and psilocybin standards to confirm the detection of these two alkaloids in the mushroom extract. As shown in Figure 3, both psilocin and psilocybin have identical retention times to the standards compared to those peaks from the mushroom extracts. Therefore, the developed method is selective for the two target compounds and can be used for the quantification as described in our early work (1).

Conclusion

This work illustrates how to use an iHILIC®-Fusion column and MS detection to separate and identify psilocin and psilocybin in hallucinogenic mushrooms or "magic mushroom" extracts. This developed HILIC–MS method can be utilized in forensic and clinical applications.

References

(1) J. Nagy and T. Veress, *J. Forensic Res.* **7**, 356 (2016), DOI: 10.4172/2157-7145.1000356.

(2) N. Rácz, J. Nagy, W. Jiang, and T. Veress, *J. Chromatogr. Sci.* **57**, 230–237 (2019).

(3) M.W. Beug and J. Bigwood, *Journal of Chromatography* **207**, 379–385 (1981).

(4) N. Anastos, S.W. Lewis, N.W. Barnett, and D.N. Sims, *J. Forensic Sci.* **51**, 45–51 (2006).

(5) R. Kysilka and M. Wurst, *Planta Med.* **56**, 327–328 (1990).

(6) V. Gambaro, G. Roda, G.L. Visconti, S. Arnoldi, and E. Casagni, *J. Anal. Bioanal. Tech.* **6**, 277 (2015).

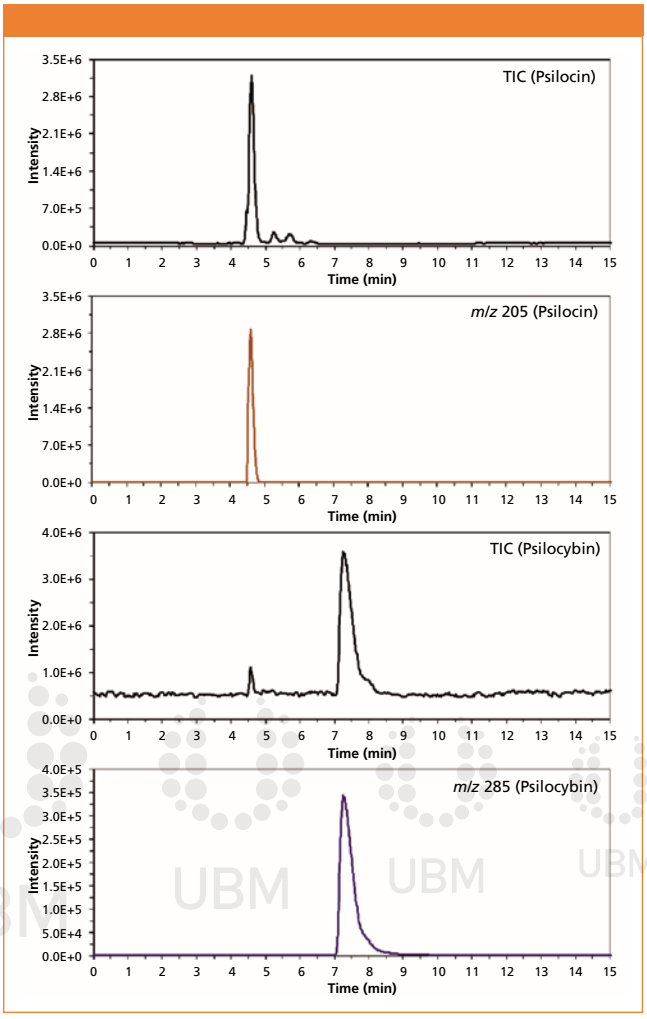

Figure 3: Total ion chromatogram (*m/z* 40–400) of psilocin and psilocybin standards and extracted ion chromatograms of *m/z* 205 (psilocin) and *m/z* 285 (psilocybin).

HILICON AB
Tvistevägen 48, SE-90736, Umeå, Sweden
Tel.: +46 (90) 193469
E-mail: info@hilicon.com
Website: www.hilicon.com