psychoactives

Supplemental Material

1. Chemical Analysis

1.1. Standards and Reagents

N,N-Dimethyltryptamine (DMT) was purchased from Cerilliant Corporation (Round Rock, Texas, EUA). Harmine (HRM) and harmaline (HRL) were acquired from Sigma-Aldrich (Saint Louis, USA). The internal standard, deuterated dimethyltryptamine (DMT-d6) was synthesized as described previously (Oliveira et al., 2012). Tetrahydroharmine (THH) was synthesized from harmaline (HRL), according to a method previously described (Callaway et al., 1996).

1.2. Sample preparation

Sample preparation consisted of a fully validated dilution procedure using 2 mM ammonium formate buffer with 0.1% formic acid (solution A). The dilution was performed to a final ratio of 1:5000 in three steps (1:10 x 1:10 x 1:50). First, an aliquot of 100 µL was diluted in 900 µL of solution A. This step was repeated once, and then a new aliquot of 100 µL was diluted in 4900 µl of solution A. Finally, 100 uL of this last dilution was combined with 10 uL of the internal standard (DMT-d 6 1 µg/mL). After this procedure, 5 µL of the diluted sample was injected into the UPLC-ESI-MS/MS system. DMT-d 6 was added to all samples as the internal standard.

1.3. UPLC-ESI-MS/MS analysis

Analyses were performed using a Waters UPLC Acquity System coupled to a Quattro Premier tandem MS with electrospray ionization (ESI) operated in the positive ion mode (Waters Corporation, Milford, MA). Chromatographic separation was conducted on a UPLC BEH C18 2.1 mm x 100 mm, ID 1.7 µm Acquity column using the following gradient elution: A (2 mM ammonium formate buffer with 0.1% formic acid) and a mobile phase B (0.1% formic acid in methanol) at a constant flow rate of 0.3 ml/min, 10% B (0 to 0.5 min), 10 - 50% B (0.5 to 7.0 min), 50 - 10% B (7.0 to 7.1 min), and 10% B until 8 minutes. Samples were analyzed using a 5 µL injection volume. The MS/MS analysis was performed using multiple reactions monitoring (MRM), considering three transitions for each analyte. MS settings were established as follows: desolvation gas flow rate, 1100 L/h; cone gas flow rate, 200 L/h; desolvation temperature, 350ºC; source temperature, 100ºC; and capillary voltage, 1000 V. For the retention times, capillary voltage, collision energy, and m/z transitions used for quantification of each analyte, data published in Silveira et al. (2020) were used.