Differentiation of ayahuasca samples according to the origin, religious groups, and botanical varieties using multivariate statistical analysis of UHPLC-MS qualitative data

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Liquid chromatography coupled to mass spectrometry analysis

The chromatographic separation was held at Waters Acquity UPLC system (Waters, USA), and at the chromatographic column was an Acquity™ UPLC BEH C18 (50 mm x 2.1 mm, 1.7 µm, Waters Corp.). The mobile phase was water (A) and acetonitrile (ACN) (B), the flow rate was 0.35 mL/min, and the injection volume was 1 µL. HPLC grade acetonitrile was purchased from J.T. Baker (Center Valley, USA). The column temperature was 40 ºC. The optimized gradient program of the mobile phase was set as follows: 0-3 min, 5-90% B; 3-4.5 min, 90% B; 4.5-4.6 min, 90-5% B; and 4.6-7.5 min; 5% B.

Detection was performed using a XEVO-G2XS QTOF (Waters, Manchester, UK). with an electrospray ionization source. The desolvation gas was 600 L/h at 400 ºC, the cone gas was 50 L/h, and the source temperature was 150 ºC. The capillary voltage was – 2.5 kV (negative mode) and + 3.0 kV (positive mode), and the cone voltage was 40 kV. MS data were acquired in the centroid mode from mass spectra range was *m/z* 50-1000 Da, and the scan time was 0.5 seg/scan using the MS^E approach (6 V for low-energy, and a 10- 30 V ramp for high-energy scanning). During MS analysis, a leucine enkephalin (Waters®, molecular mass = 555.62; 200 pg/µL in 1:1 ACN: H_2O) was continuously infused into MS at a flow rate of 30 µL/min, and the ions $[M-H]^-$ = 554.26 e $[M+H]^+$ = 556.27 were used as a lock mass for accurate mass measurement. Data acquisition was controlled by MassLynx V4.2 (Waters®). Calibration was performed before sample analysis via infusion of 0.5 mmol/L sodium formate solution, which was used for calibration procedures. Samples were randomly analyzed, and the quality control (QC) was a sample pool that was injected four times at the beginning of the chromatographic run and then once after every ten experimental samples.

The data matrix exported from the *Progenesis QI 2.0* software (Waters, Milford, MA, USA) contained retention time, *m/z*, and intensity of each feature with sample and groups' names were uploaded on the Generic Format module of *MetaboAnalyst 5.0* (https://www.metaboanalyst.ca/).