TECHNICAL COMMUNICATION

Determination of Tryptamines and β-Carbolines in Ayahuasca Beverage Consumed During Brazilian Religious Ceremonies

MÔNICA CARDOSO SANTOS and SANDRO NAVICKIENE[1]
Universidade Federal de Sergipe, Departamento de Química, Av Marechal Rondon, s/n, 49100-000, São Cristóvão-Se, Brazil
ALAIN GAUJAC
Instituto Federal de Educação, Ciência e Tecnologia de Sergipe, Br 101, Km 96, 49100-000, São Cristóvão-Se, Brazil

Ayahuasca is a potent hallucinogenic beverage prepared from *Banisteriopsis caapi* in combination with other psychoactive plants. *N,N*-dimethyltryptamine, tryptamine, harmine, harmaline, harmalol, and tetrahydroharmine were quantified in ayahuasca samples using a simple and low-cost method based on SPE and LC with UV diode-array detection. The experimental variables that affect the SPE method, such as type of solid phase and nature of solvent, were optimized. The method showed good linearity (r > 0.9902) and repeatability (RSD < 0.8%) for alkaloid compounds, with an LOD of 0.12 mg/L. The proposed method was used to analyze 20 samples from an ayahuasca cooking process from a religious group located in the municipality of Fortaleza, state of Ceará, Brazil. The results showed that concentrations of the target compounds ranged from 0.3 to 36.7 g/L for these samples.

Ayahuasca is a beverage that is mostly prepared using a decoction of two plants: the leaves of chacrona (*Psychotria viridis*), which contain the psychoactive/hallucinogenic *N,N*-dimethyltryptamine (DMT); and sections of the stem of the jagube vine (*Banisteriopsis caapi*), which provide three major monoamine oxidase (MAO) inhibitors (i.e., harmine, harmaline, and tetrahydroharmine). The combination of the leaves of *P. viridis* (containing DMT) and the reversible MAO inhibitors renders the DMT orally active. Brazilian legislation, based on a constitutional right to freedom of religion, permits the consumption of ayahuasca within a religious context (1, 2). Norms concerning the use of ayahuasca in Brazil for religious purposes were published by the Brazilian National Council on Drug Policies in January 2010; they prohibit the marketing of ayahuasca, its therapeutic use, ayahuasca tourism, and its use with illicit drugs (3). Under this resolution, consumption is permitted in a religious context. The same document also emphasized the need for more multidisciplinary areas of research of ayahuasca. Studies involving the chemical characterization of these plants, together with the development of analytical techniques for the measurement of tryptamines and β-carbolines in plant matrixes and in ritual beverages are essential, given the current expansion in their use for religious, recreational, and clinical research purposes. The need for an in-depth approach toward analytical characterization becomes obvious in cases of untoward effects or even fatal intoxications that can, e.g., arise from ill-informed combinations of plant products with other psychoactive substances (4).

The present work reports a simple method for the determination of major alkaloid components, namely DMT, tryptamine, harmine, harmaline, harmalol, and tetrahydroharmine, in ayahuasca beverage samples collected from a religious group located in the municipality of Fortaleza, state of Ceará, Brazil, using SPE on silica cartridges and LC with UV diode-array detection (DAD).

Materials and Methods

Chemicals, Reagents, and Supplies

Tryptamine, harmalol, harmine, and harmaline were purchased from Sigma-Aldrich (St. Louis, MO). DMT was prepared as previously described (2). Tetrahydroharmine was prepared according to the method described by Begum (5). The HPLC grade solvents acetonitrile and methanol were purchased from Tedia (Fairfield, OH). Research grade Florisil (80–100 mesh) was supplied from Sigma-Aldrich (Büchs, Switzerland), and silica-gel 60 (70–230 mesh) was from Merck (Darmstadt, Germany). C_{18}-bonded silica (50 μm) SPE cartridges (6 mL, 500 mg) were obtained from Phenomenex (Torrance, CA).

Collection and Preparation of Plant Material

Ayahuasca samples were prepared from specimens of *B. caapi* stems and *P. viridis* leaves by members of a religious group located in the municipality of Fortaleza, state of Ceará, Brazil. At the start of the cooking process, plant materials and water were at room temperature. Aqueous decoctions of each plant were prepared from freshly harvested material. The cooking process for obtaining ayahuasca tea takes 20 h. At each cooking hour a sample is collected and stored. This is the first step in the cooking process, which produces a total of 16 samples (1-16 samples, Figure 1). The 16 samples that have been stored are subdivided into five sets (1-3, 4-6, 7-9, 10-13, and 14-16 samples), from which a sample is taken from each of the five sets. These five samples are combined and are subjected to a new cooking process which takes 4 h. This is the second step of the cooking process that produces another four samples, which are also obtained at each hour of this second step of the cooking process (17-20 samples, Figure 1). These four samples are called apuro or ayahuasca tea. A total of 20 aqueous dark brown liquid samples were separately bottled and shipped to the Department of Chemistry of the Federal University of Sergipe. Before analysis, ayahuasca samples were taken out of the refrigerator and maintained for 30 min to reach room temperature.

Received October 17, 2016. Accepted by SG November 21, 2016.
[1]Corresponding author's e-mail: sandnavi@ufs.br
DOI: 10.5740/jaoacint.16-0337

Samples 1–16 were analyzed directly by the SPE method, whereas samples 17–20 were initially diluted (1:100) due to their viscous characteristics that could clog the filter of the SPE cartridges.

Structural Characterization of Tetrahydroharmine

UV-Vis molecular absorption spectrometry.—A Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) was used for measurements (in triplicate, at 326 nm) of five harmine standard solutions at concentrations in the range of 0.01–0.1 mmol/L to generate an analytical curve (6). The percentage of tetrahydroharmine was determined using a solution concentration of 0.1 mmol/L. The measurements were performed at a wavelength of 296 nm (7).

NMR spectroscopy.—Proton NMR (^{1}H NMR) spectra were recorded at 400 MHz, using a $CDCl_3$ solution. Chemical shifts were referenced to the residual solvent peak or to tetramethylsilane as an external reference. The data were reported in terms of the chemical shift (δ, in parts per million), multiplicity, coupling constant (*J*, in hertz), and integrated intensity. ^{13}C NMR spectra were recorded at 100 MHz (using a $CDCl_3$ solution). The chemical shifts were referred to the $CDCl_3$ solvent peak. The multiplicity of a particular signal was indicated as *s* (singlet), *d* (doublet), *qdd* (quartet of doublet of doublets), *dd* (doublet of doublets), *ddd* (doublet of doublet of doublets) and *dddd* (doublet of doublet of doublet of doublets). The ^{1}H NMR and ^{13}C NMR spectra were measured using a Bruker Spectrospin Avance DPX-400 spectrometer (Fällanden, Switzerland).

GC-MS.—GC/MS analyses were performed with a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) coupled to a Shimadzu TQ8040 mass spectrometer. Samples were injected into a split/splitless injector using an autosampler. A Supelco SBL-5ms capillary column (30 m × 0.25 mm id, 0.25 µm film thickness) was used. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The computer that controlled the system also contained an EI-MS library. The mass spectrometer was calibrated with perfluorotributylamine. Helium (99.999%), at a flow rate of 1.2 mL/min, was used as the carrier gas. The injector temperature was 250°C. The oven temperature program was as follows: initial temperature of 80°C for 1 min, followed by a ramp to 280°C at 10°C/min and a hold at 280°C for 5 min. The mass spectrometer was operated in SCAN mode, and the temperatures of the transfer line and ionization source were set at 280 and 250°C, respectively. The total run time was 26 min.

Preparation of Standard Solutions

Individual stock standard solutions of the indole alkaloids (harmine, harmaline, tetrahydroharmine, harmalol, tryptamine, and DMT) were prepared by dissolving the reference standard in methanol to obtain a concentration of 2 mg/mL. The stock solutions were stored at −18°C. Mixed working standard solutions were prepared at various concentrations by diluting the stock solutions in methanol as required. This solution was prepared monthly to obtain the analytical curves (0.001, 0.005, 0.01, 0.025, 0.05, 0.1, 0.15, 0.02, 0.025 and 0.30 mg/mL) and the optimum experimental conditions.

SPE Procedure

A silica-based cartridge was preconditioned with 4 mL methanol, followed by 4 mL aqueous hydrochloric acid (1 mol/L). The cartridge was placed on top of a vacuum block. An analytical aliquot of 20 mL ayahuasca sample (pH 8, with 0.01 mol/L NaOH solution) was transferred to the cartridge at a flow rate of around 0.5 mL/min, and the solid phase was allowed to dry for 5 min. The alkaloids were eluted with 3.0 mL acidified methanol (pH 3). The eluent was collected into a round-bottom flask and concentrated using a gentle stream of nitrogen to a volume of 1 mL. A 20 µL aliquot was analyzed by HPLC–UV/DAD.

Instrumentation and Operating Conditions

The analysis of the alkaloid components of ayahuasca in the SPE extracts was carried out using an HPLC system (Shimadzu) equipped with a binary solvent pump (LC-20AT), a DGU-20A3 degasser, a Sil-20A autosampler with injection volume set at 20 µL, and an SPD-M20A UV/DAD. Data acquisition and processing were performed with LCsolution software (LabSolutions Series WorkStation v. 2.0). The chromatographic separation was performed on a reversed-phase Zorbax 5 µ Eclipse Plus C_8 analytical column (150 × 4.6 mm id, 5 µm particle size), protected by a guard cartridge (4.6 × 12.5 mm, 5 µm), both obtained from Agilent Technologies (Palo Alto, CA). The elution was carried out with a binary gradient composed of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B), delivered at ambient temperature with a flow rate of 1.5 mL/min. The initial mobile phase composition was 5% B held constant for 2 min, followed by a linear gradient to 50% B at 30 min, and then a return to the initial conditions in 10 min. Spectral data for all peaks were collected in the range of 190–800 nm. The identification of compounds present in the ayahuasca samples was achieved by comparing the characteristics of the DAD spectra and the retention times with those of the standard compounds. The injection volume was 20 µL.

Results and Discussion

Chromatographic Conditions

The best conditions for analysis of the investigated alkaloids were provided by gradient elution using 0.1% formic acid in methanol–0.1% formic acid in water at an initial proportion of 5 + 95 (v/v) maintained for 2 min, and then ramped to 50 + 50 (v/v) at 30 min. Alkaloid components are compounds with high molar absorptivity in the UV-Vis region. They display their highest UV absorption maxima at 278 nm for DMT and tryptamine, at 246 nm for harmine, at 320 nm for tetrahydroharmine, and at 372 for harmalol and harmaline. For this reason, HPLC with DAD could be one of the methods used in their determination.

Characterization of Tetrahydroharmine

Melting point.—The melting point of tetrahydroharmine recrystallized was 199.3 ± 0.2°C (n = 3), which was compatible with the literature value (199°C; 5).

GC-MS analysis.—An aliquot of the synthesized compound was analyzed by GC-MS in full scan mode and showed a prominent peak at 18.600 min. To confirm the identity of the compound, the spectrum of the peak was compared with the spectra available in the Wiley EI-MS library. There was 89% similarity between the measured spectra and the library spectra, and with a molecular ion peak at *m/z* 216 and a base peak at *m/z* 201. These and other peaks in the tetrahydroharmine

spectrum were similar to the spectrum provided in the Wiley Registry of Mass Spectral Data.

NMR spectroscopy.—The chemical shift values of the ^{1}H [400 MHz, 303 K, $CDCl_3$ + MeOD-d_4 (δ in ppm and *J* in Hz)] were 4.15 (1H; *qdd* 6.7, 2.1, and 1.8; H-1); 1.45 (3H; *d* 6.7; 1-CH_3); 3.01 (1H; *ddd* 12.9, 8.9, and 5.2; H-3a); 3.33 (1H; *ddd* 12.9, 5.3, and 5.2; H-3b); 2.73 (1H; *dddd* 15.5, 5.2, 3.7, and 1.8; H-4a); 2.76 (1H; *dddd* 15.5, 8.9, 5.3, and 2.1; H-4b); 7.34 (1H; *d* 8.6; H-5); 6.74 (1H; *dd* 8.6 and 2.2; H-6); 3.84 (3H; *s*; 7-OCH_3), and 6.86 (1H; *d* 2.2; H-8). The chemical shift values of the ^{13}C (100 MHz, 303 K, $CDCl_3$ + MeOD-d_4) δ (ppm) were 156.1 (C-7), 136.7 (C-8), 135.5 (C-10), 121.9 (C-12), 118.5 (C-5), 108.6 (C-6), 107.4 (C-11), 95.4 (C-8), 56.4 (7-OCH_3), 48.4 (C-1), 42.5 (C-3), 22.3 (C-4), and 20.1 (1-CH_3). These values are in agreement with literature data (8).

Optimization of the SPE Procedure

To select the optimal experimental conditions for extraction, an optimization strategy was used to assess the influence of the main factors on the SPE procedure. Silica and methanol were chosen over the other solid phase and solvent, respectively, for producing the cleanest chromatographic profiles with lower baselines. Indole alkaloids are basic compounds, and the pH of the solution was expected to have effect on their recovery. Therefore, before the elution step, the adsorbent was washed with 4 mL methanol and 4 mL aqueous HCl (1 mol/L). The washing of the adsorbent with this solvent composition was found to decrease ion suppression and, thus, improve the detectability of the compounds. The compounds were dissolved in aqueous solution at a concentration of 100 µg/mL, the pH was adjusted to 8, and the sorbent was eluted with 4 mL acidified methanol. Average recoveries of the analytes of 71.2–82.5% were achieved with acidified methanol. Therefore, acidified methanol was chosen as the eluent (9).

Method Validation

Linearity.—Calibration curves were linear over the specified range (0.001–3.0 mg/L). The linear regression equations and coefficients of correlation were as follows: harmine ($y = 7303x + 58996$, r = 0.9905); harmaline ($y = 42766x + 298629$, r = 0.9904); tetrahydroharmine ($y = 41180x + 462149$, r = 0.9902); harmalol ($y = 19228x + 71563$, r = 0.9929); tryptamine ($y = 15839x + 96013$, r = 0.9943); and DMT ($y = 9753x - 169.9$, r = 0.9919), where *y* and *x* = the peak area and the corresponding calibration concentration, respectively.

Recovery.—Aqueous samples were fortified at 1, 5, 10, 50, 100, and 250 µg/mL. Five replicates spiked at each fortification level were assayed. The recovery obtained for alkaloids ranged from 71.7 to 107.4%, and the obtained RSDs ranged from 1.1 to 9.8%, with the exception of harmalol, for which the recovery ranged from 45.0 to 58.4%, with RSDs ranging from 2.3 to 5.5% for 1 and 5 µg/mL.

Repeatability.—The repeatability (intra-assay precision) was measured by comparing the SD of the recovery percentages of spiked aqueous samples at one concentration level (100 µg/mL) run the same day. The samples were injected five times using an autoinjector, and the RSD values obtained for the retention times were lower than 0.8%. The intermediate precision (between-day precision) was determined by analyzing spiked aqueous samples at one concentration level (100 µg/mL) for 3 alternate days. Replicate ($n = 5$) samples were run, and the RSD values were calculated for the alkaloid compounds. The method was found to be precise (RSD < 0.3%) for all compounds studied.

LOD and LOQ.—LOD and LOQ were calculated at an S/N of 3 and 10, respectively. LODs ranged from 6.8 to 18.8 µg/mL (7.5, 18.8, 13.8, 11.6, 6.8, and 17.5 µg/mL for tryptamine, DMT, harmalol, harmine, harmaline, and tetrahydroharmine, respectively). The LOQ values ranged from 20.6 to 57.1 µg/mL (10).

Application of the Method

Twenty samples were collected from an ayahuasca preparation process from a religious group of the municipality of Fortaleza, Brazil, and analyzed by the developed SPE method combined with HPLC–UV/DAD. The harmine, harmaline, tetrahydroharmine, harmalol, and DMT concentrations in the samples ranged from 0.3 to 36.7 g/L (Figure 1). The increase observed for fractions 17–20 can be explained by the addition of all fractions (1–16) to form the apuro (ayahuasca tea) fractions, which contain significant concentrations of DMT and β-carbolines. It is suggested that the absence of tryptamine alkaloid is related to the complete bioconversion to DMT in plants.

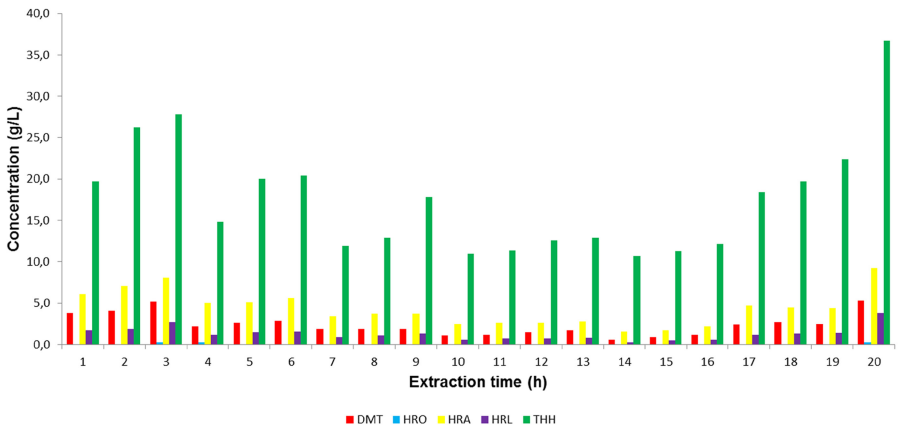

Figure 1. Concentration of indole alkaloids found in ayahuasca samples as a function of collection interval (extraction time) during the cooking process by members of a religious group located in the municipality of Fortaleza, state of Ceará, Brazil. HRO, harmalol; HRA, harmine; HRL, harmaline; and THH, tetrahydroharmine.

Conclusions

The results demonstrate that the accuracy, precision, and selectivity of the proposed method are acceptable for the determination of these alkaloids in ayahuasca samples from an ayahuasca cooking preparation process from a religious group of the municipality of Fortaleza, Brazil.

Acknowledgments

We wish to thank Andersson Barison for providing the laboratory infrastructure for NMR analysis, and Maria de Fátima Costa Santos for her assistance with the NMR spectra. We are also grateful to Mark Ian Collins for providing the ayahuasca samples.

References

(1) Gaujac, A., Dempster, N., Navickiene, S., Brandt, S.D., & de Andrade, J.B. (2013) *Talanta* **106**, 394–398. doi:10.1016/j.talanta.2013.01.017

(2) Gaujac, A., Navickiene, S., Collins, M.I., Brandt, S.D., & de Andrade, J.B. (2012) *Drug Test. Anal*. **4**, 636–648. doi:10.1002/dta.1343

(3) Conselho Nacional de Políticas sobre Drogas (2010) Resolução n°1 de 25 de janeiro de 2010, Brasília, 26 January, 2010

(4) Winkelman, M. (2014) *Curr. Drug Abuse Rev*. **7**, 101–116

(5) Begum, S. (1984) *Studies in the Harmine Series of Alkaloids and Pharmacologically Significant Derivatives of Yohimbine*. PhD thesis, H. E. J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

(6) Balón, M., Hidalgo, J., Guardado, P., Muñoz, M.A., & Carmona, C. (1993) *J. Chem. Soc., Perkin Trans. 2* **1993**, 91–97. doi:10.1039/P29930000091

(7) Balón, M., Hidalgo, J., Guardado, P., Muñoz, M.A., & Carmona, C. (1993) *J. Chem. Soc., Perkin Trans. 2* **1993**, 99–104. doi:10.1039/P29930000099

(8) Wu, J., Talwar, D., Johnston, S., Yan, M., & Xiao, J. (2013) *Angew. Chem*. **52**, 6983–6987. doi:10.1002/anie.201300292

(9) González-Ruiz, V., Olives, A.I., & Martín, M.A. (2011) *Anal. Bioanal. Chem*. **400**, 395–401. doi:10.1007/s00216-010-4562-2

(10) Bliesner, D.M. (2006) *Validating Chromatographic Methods: A Practical Guide*, John Wiley and Sons, Inc. doi:10.1002/0470042206