Research Article

Determination of *N*,*N*-dimethyltryptamine and beta-carbolines in plants used to prepare ayahuasca beverages by means of solid-phase extraction and gas-chromatography–mass spectrometry

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Abstract

The goal of the present work was the development of a method to determine beta-carbolines, i.e., tetrahydroharmine, harmine, and harmaline, together with *N*,*N*-dimethyltryptamine present in five different commercial tea extracts [*Banisteriopsis caapi*, *Psychotria viridis*, *Peganum harmala*, *Mimosa tenuiflora* and *DC AB* (commercial name)] which are used to prepare the ritualistic Ayahuasca tea beverages, that exerts psychoactive effects on the brain, due to interactions between these compounds. The analytes were extracted and preconcentrated by an optimized solid-phase extraction procedure and analysis was performed by gas chromatography coupled to mass spectrometry (GC–MS). Also, the optimization of the solid-phase extraction procedure was accomplished by evaluating different elution solvents and different proportions. Linearity was established from 0.2 to 20 µg/mL for all compounds, except for *N*,*N*-dimethyltryptamine (0.04 to 5 µg/mL), with determination coefficients above 0.99 for all analytes. The limits of quantification achieved for *N*,*N*-dimethyltryptamine and beta carbolines were 0.04 and 0.2 µg/mL, respectively. Recoveries ranged from 44 to 79%. Lastly, intra- and inter-day precision and accuracy values were considered acceptable, in agreement with the chosen guidelines. This is the first method that determined both beta-carbolines and *N*,*N*-dimethyltryptamine present in commercially available tea extracts using GC–MS.

Keywords Ayahuasca · Beta-carbolines · GC–MS · *N*,*N*-Dimethyltryptamine · Solid-phase extraction

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1 Introduction

Ayahuasca has been consumed for centuries amongst Amazonian indigenous groups, both Brazilian and Peruvian [1]. However, its popularity has increased all over the world in religious ceremonies, commonly due to the belief of purgatory and liberator effects associated to consumption. In addition, a faith links the drinking of Ayahuasca tea on ceremonies conducted by shamans and/or healers with the ability to communicate with divine spirits [2, 3].

Traditionally, ayahuasca tea is a concoction of *Banisteriopsis caapi* vine with *Psychotria viridis* leaves [4]. The former contains beta-carboline alkaloids, i.e. harmine (HMN), tetrahydroharmine (THH) and harmaline (HML) [5]. Such chemical compounds have the ability to inhibit monoamine oxidase (MAO) activity, being important on the peripheral and central degradation of neurotransmitters and structurally related compounds, such as dopamine, serotonin and norepinephrine [6, 7]. This inhibition induces the hallucinogenic and sedative effects of *N,N*-dimethyltryptamine (DMT) on the central nervous system (CNS). It has been previously reported that the administration of DMT alone has a short-lasting effect, whether its administration route is parenteral, oral or even by intramuscular injection [8]. In fact, the half-life of this compound is approximately 5–15 min [9, 10], and its degradation by MAO-A in the liver and gut occurs rapidly, preventing its access to the bloodstream. Therefore, oral intake of DMT alone will be inactive in the CNS. Hence, when traditional ayahuasca beverage is consumed, DMT will access the bloodstream and exert its effects on CNS, due to the presence of the beta-carbolines. When this comixture is administered, the effects of DMT can last up to hours [8]. As already mentioned, *B. caapi* and *P. viridis* are constituents of the traditional preparations of this beverage, however, there are other plants that can be used as substitutes, once they contain the same compounds. For instance, *Peganum harmala* can be used in the place of *B. caapi*, whereas *Mimosa tenuiflora* can be used as *P. viridis* substitute, among others [3, 11].

There is still little available literature regarding the quantification of such compounds in ayahuasca tea extracts or plants that contain these compounds, particularly in commercially available mixtures. Many studies are simply based on inquiries, clinical trials or analysis of biological samples. Thus, it is of extreme importance to know, using a validated method, the quantity of the compounds present in each tea extract, allowing to evaluate the amount of compounds ingested.

The present work describes the development and validation of a sensitive and specific method for the determination of both DMT and β-carbolines present in five different commercially available plants, namely *P. viridis*, *B. caapi*, *P. harmala*, *M. tenuiflora* and *DC AB*, by GC–MS, using SPE as an extraction technique.

2 Materials and methods

2.1 Reagents and standards

The analytical standards of HMN, HML and THH and DMT were kindly provided by Nal von Minden, GmbH (Regensburg, Germany). The internal standard (IS) promazine (PRZ) was acquired from Sigma Aldrich (Lisbon, Portugal Methanol, dichloromethane, 2-propanol, hexane were obtained from Fisher Scientific (Enzymatic; Santo Antão do Tojal, Portugal) and ammonium hydroxide from T.J. Baker (Deventer, Holland). These chemicals were all analytical or chromatographic grade. Hydrochloric acid (37% v/v) from Enzymatic (Santo Antão do Tojal, Portugal). Deionized water (DI) was obtained from a Milli-Q system (Millipore Billerica, MA, USA). Oasis® MCX Solid Phase extraction cartridges (3 cm^3/60 mg) were obtained from Waters (Milford, MA, USA).

Working solutions of THH, HMN and HML were prepared by dilution of the stock solutions (1 mg/mL) with methanol to the final concentration of 100 µg/mL and 10 µg/mL. Concerning DMT, the concentration of the stock solution was 100 µg/mL, and then serial dilutions were prepared, to the final concentrations of 10 µg/mL and 1 µg/mL (working solutions).

The concentration of IS was 1 µg/mL. All solutions were stored protected from light at 4 °C.

2.2 Gas chromatographic and mass spectrometric conditions

Chromatography was operated on a gas chromatographer model HP7890B equipped with a model a mass spectrometer model 5977A from Agilent Technologies. Data was acquired in the *selected ion monitoring* (SIM) mode using the ChemStation from Agilent Technologies. The separation of the analytes was achieved using a capillary column (30 m × 0.25 mm; 0.25 µm I.D.) packed with 5% de phenylmethylsiloxane (HP-5MS) supplied by Agilent Technologies. Carrier gas was helium set at a constant flow rate of 0.8 mL/min. The injection volume was 2 µL in splitless mode. The temperatures of the injector and detector were set at 250 °C and 280 °C, respectively. Ion source temperature was set at 230 °C and quadrupole at 150 °C. The oven temperature started at 90 °C for 3 min increasing 15 °C/min up to 300 °C and held for 8 min. The total separation run took 25 min. The mass

spectrometer was operated at 70 eV with a filament of 300 µA in the positive electron ionization mode. Three ions for each analyte and one for the IS were chosen taking into account the selectivity and abundance, in order to maximize the signal-to-noise ratio in matrix extracts. Table 1 summarizes retention times and in the specific conditions at which they were detected.

2.3 Sample preparation

In this study, samples were prepared as a liquid extract based on a recipe kindly provided by Dr. Nicolás Fernández (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires Argentina). The plants used were *B. caapi*, *P. viridis*, *M. tenuiflora*; *P. harmala* and *DC AB* (commercial name) and were acquired online from the *Shayana Shop* (https://shayanashop.com, Amsterdam, Netherlands). All plants were weighed as follows: 0.215 g; 0.212 g; 0.213 g; 0.210 g and 0.208 g. Firstly, 250 mL of ultra-pure water was added to 5 different shot flasks and brought up to boil (100 °C). The plants were then added to the correspondent flask were the boiling process was maintained during 6 h. Nonetheless, as water was evaporating and to avoid total water loss, 2 h after the initial time, 150 mL of the same water was added to each flask. The same happened after 4 h of the initial time, and 50 mL of ultra-pure water was added, and the temperature reduced to 60 °C until the process finish. The final volume obtained for each tea was approximately 200 mL, from this 50 mL of each sample was transferred into falcon tubes so that the samples could suffer a process of freeze-drying to posterior cell exposure and testing. For extraction procedure and compounds determination, 2 mL of each were stored in amber glass containers.

Table 1 Retention times and selected ions for the identification of the β-carbolines and DMT

Analyte	Retention time (min)	Quantifying ion (m/z)	Qualifying ions (m/z)	Dwell time (µs)
DMT	12.27	58	130 188	50
THH	14.50	201	172 216	50
HML	14.69	213	198 214	50
HMN	15.29	169	197 212	50
PRZ[a]	15.84	284	–	50

DMT: *N*,*N*-dimethyltryptamine; THH: tetrahydroharmine; HML: harmaline; HMN: harmine; PRZ: promazine

[a]Internal standard

2.4 Solid phase extraction procedure

For this study, the extraction was performed with solid phase extraction (SPE) using Oasis® MCX3cc 60 mg. SPE procedure conditions were previously optimized and so the final conditions were as described herein. Before the extraction procedure, 250 µL of each tea sample was diluted with 4 mL of phosphate buffer (pH = 5) and spiked with 25 µL of IS. SPE cartridges were conditioned with 2 mL of methanol and 2 mL of DI water. After the sample passed through the cartridge, a washing step was performed in the following order: 2 mL of DI water, 2 mL of hydrochloric acid (0.1 M) and 2 mL hexane. Following this step, the columns were dried under full vacuum for 10 min. Subsequently, the analytes of interest were eluted with 2 mL of a mixture of dichloromethane:isopropanol:ammonium hydroxide (80:20:2 v/v). The resulting extracts were evaporated to dryness under a steam of nitrogen. The remaining residues were dissolved in 50 µL of methanol and vortex mixed and, 2 µL was injected into the GC–MS system in SIM mode.

2.5 Validation procedure

The described method was fully validated in a 5-day validation protocol according to the Food and Drug Administration (FDA) and the Scientific Working Group of Forensic Toxicology SGWTOX guidelines [12, 13]. This study included parameters such as selectivity, linearity, intermediate precision and accuracy, intra and inter-day precision and accuracy, absolute recovery, limits and calibration curves.

3 Results and discussion

3.1 Optimization of the extraction procedure

In order to optimize the extraction procedure, a total of four different techniques were tested. These techniques were chosen based on the available literature for similar compounds, since specific data for the target analytes is scarce [14]. The elution step was the studied parameter, using different solvents and different concentration levels. For this assay, the samples were spiked with compounds at the lowest calibration concentration and the IS was added after extraction. Four techniques were evaluated (n = 3), and are summarized in table S1 (supplementary material).

When comparing all tested elution solvents and different proportions (Fig. 1), better results were observed when using T1. Regarding T2, which uses methanol: isopropanol: ammonium hydroxide [40:60:0.25], this revealed low relative peak areas for DMT and THH, and great relative

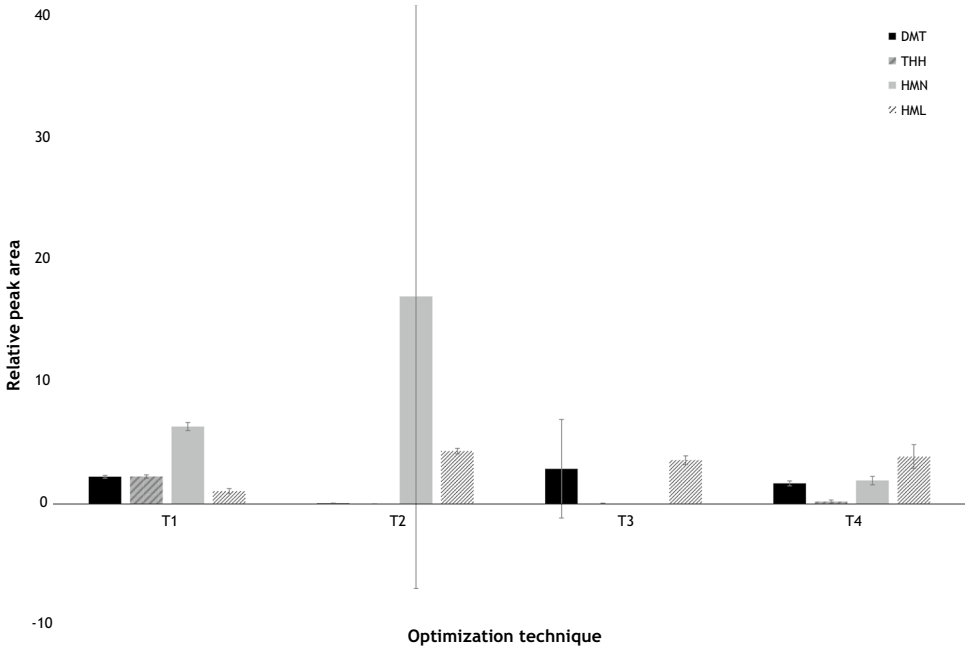

Fig. 1 Evaluation of different solvents and proportions in the elution process of SPE (n = 3)

peak areas for HMN and HML, although a large standard deviation was observed for HMN. Despite obtaining good relative peak area values for DMT and HML, on T3 very poor results were achieved for the remaining compounds. Finally, T4 did show good relative peak area values for DMT, HMN and HML, except for THH. For this reason, overall T1 was the one that showed greater recoveries for most target compounds, as well as better coefficients of variation (CV), and was therefore selected.

3.2 Method validation

3.2.1 Selectivity

Selectivity is the ability of an analytical method to detect the target compounds while evaluating the presence of potential interferences [13]. This parameter was studied in order to verify if other drugs of abuse (cocaine, opiates, cannabinoids and amphetamines) interfered at the retention times and selected ions of the target analytes.

The described method was considered selective given that no interferences were observed at the retention time and respective monitored ions. The criteria for the identification of the analytes were established according to the World Anti-Doping Agency recommendations [15].

3.2.2 Calibration curves and limits

To evaluate the method's linearity, calibration curves were prepared ranging from 0.2 to 20 µg/mL for all compounds, except for DMT (0.04–5 µg/mL). Seven calibrators (six in the case of DMT), were prepared by spiking DI water with the working solutions. The results were expressed by plotting the peak area ratio between each analyte and the IS with the correspondent concentration.

In order to respect the chosen guidelines criteria, the accuracy of the calibrators had to present a relative error (BIAS) within ± 15% of the nominal concentrations, whereas a value within ± 20% was accepted for the lowest limit of quantification (LLOQ) and a coefficient of variation (CV) equal or lower than 20%. Moreover, a determination coefficient (R^2) value equal or above 0.99 for linearity is acceptable (mean values). For all other calibrators, CV's accuracy within ± 15% interval was accepted [13]. Results are summarized in Table 2. The LLOQ is defined as the lowest concentration of analyte that could be measured and quantified with adequate precision and accuracy i.e. CV ≤ 20% and a BIAS within ± 20% (mean values) [13]. The LLOQs obtained were 0.2 µg/mL for all compounds, except for DMT where a LLOQ of 0.04 µg/mL was achieved (Fig. 2). These results were considered satisfactory, when comparing to other studies. Gaujac et al. [3], who have used GC–IT–MS with a SLB-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness and obtained a LLOQ for DMT of 9.5 µg/mL, which was higher than the value presented in this study, however, the authors have only determined DMT. On a different study by Pires et al. [16], the reported LLOQ for DMT, HMN, HML and THH were 20 µg/mL, when using 0.5 mL (double of the volume used herein). Although a similar extraction technique was performed (SPE-C_{18}), a NPD detector was used, which is less sensitive than MS. The remaining publications on quantification of these compounds are applied to biological specimens, making it impossible to compare

Table 2 Linearity data (n = 5)

Analyte	Weighting factor	Linear range (µg/mL)	Linearity[a]		R^{2a}	LLOQ (µg/mL)
			m	b		
DMT	1/x	0.04–5	60.9308 ± 1.9890	− 2.7790 ± 0.0443	0.9967 ± 0.0017	0.04
THH	1/x	0.2–20	38.4787 ± 54.9862	− 10.6541 ± 15.8236	0.9972 ± 0.0007	0.2
HML	1/x	0.2–20	4.0246 ± 0.6677	− 0.7049 ± 0.0009	0.9960 ± 0.0015	0.2
HMN	1/x	0.2–20	44.8453 ± 21.8932	− 3.236 ± 5.1266	0.9971 ± 0.0007	0.2

m: slope; b: intercept

DMT: *N*,*N*-dimethyltryptamine; THH: tetrahydroharmine; HML: harmaline; HMN: harmine

[a]Mean values ± standard deviation

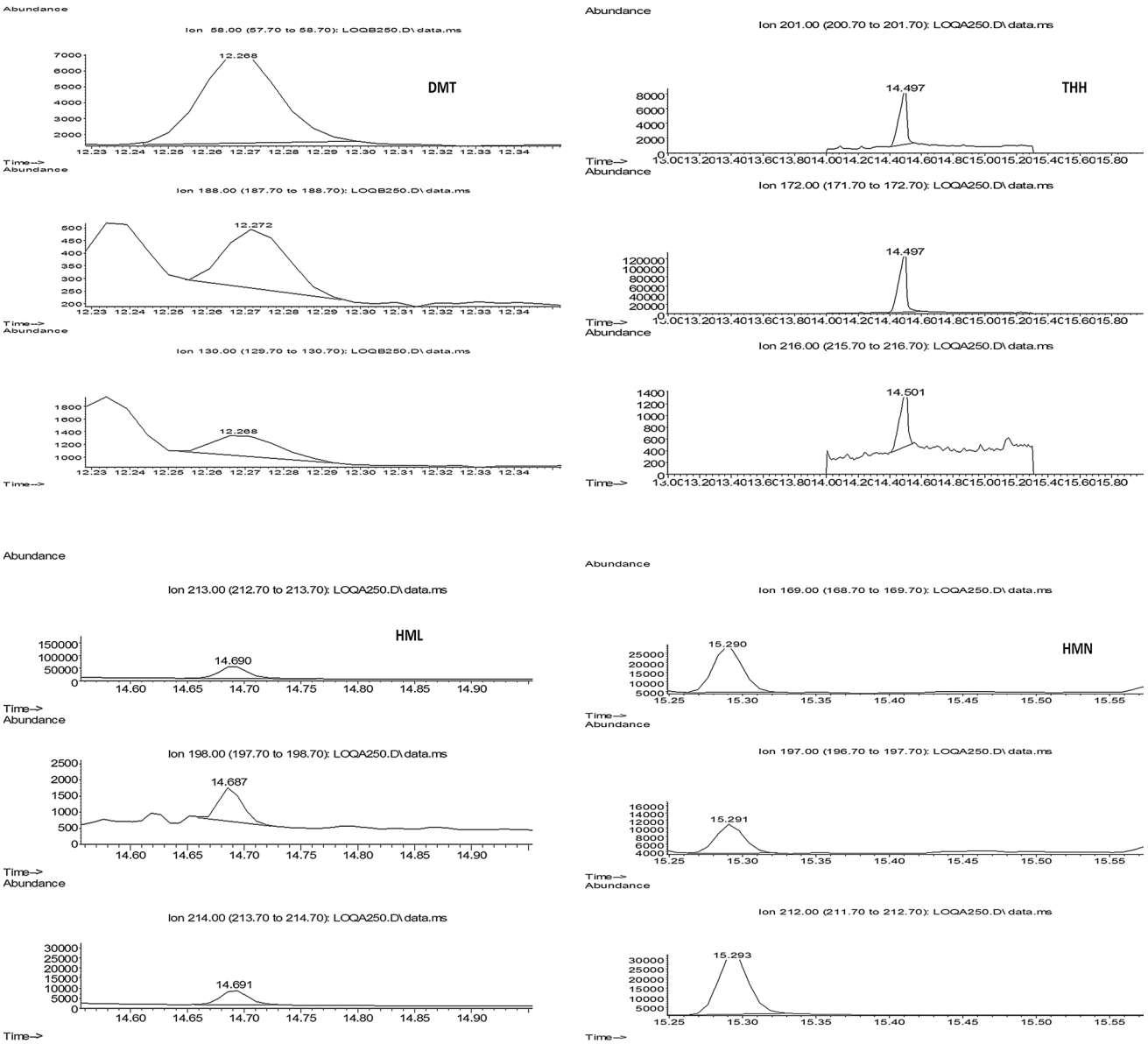

Fig. 2 Chromatogram of the studied compounds at LLOQ concentration

our results [17–19]. In a recent study by Souza et al. [20] linearity ranged from 1.0 to 60 µg/mL, in which the LLOQs were almost three times greater than those obtained in this study, despite having performed a more robust chromatography technique (LC–MS–MS) with direct injection of the sample into the equipment. Table 3 describes the comparison of results between different methodologies described in the literature and the method proposed in this article.

The limit of detection (LOD) is usually considered as the lowest concentration of the analyte that could be distinguished from the background noise [13]. The LOD was not systematically evaluated, hence it was considered the same as the LLOQ.

3.2.3 Intra- inter-day and intermediate precision and accuracy

Quality control (QC) samples were prepared to guarantee the liability of the developed method. Three different levels of concentrations were used for each analyte (0.8, 8 and 16 µg/mL), except for DMT (0.06, 0.8 and 4 µg/mL), samples were performed in triplicates (n = 3) and evaluated along the 5-day validation protocol. To validate the method, the same criteria for CV and RE were applied. Once again, CV values were found to be lower than 15% and a BIAS within ± 15% (mean values).

Intermediate precision and accuracy were calculated by analyzing the QC samples (3 different concentrations levels within the linearity reach). The obtained CVs were lower than 15%, and a BIAS of ± 9.6% (Table 4).

Intra-day precision and accuracy were evaluated by analyzing on the same day (n = 6) spiked samples with DMT, HMN, HML and THH at three different concentrations. The CVs were lower than 11% at the evaluated concentrations and a mean BIAS value within ± 13% (Table 4).

Finally, to determine inter-day precision and accuracy, seven concentration levels for all compounds (6 for DMT) were evaluated within a 5-day period. It was possible to observe that CVs were typically lower than 14%, except for LLOQs, for which a value of 17.21% was obtained for HMN (Table 4).

3.2.4 Extraction recovery

The recovery studies were performed by spiking samples at the three concentrations for the B-CA 0.4; 8 and 16 µg/mL, except for DMT (0.1; 0.8 and 4 µg/mL) each one in triplicate. These samples were divided into two groups. In the first one, all analytes were spiked before SPE procedure, in the second group analytes were added to the eluates after SPE procedure. In all cases, IS was only added after extraction. The second group was used as neat standard. Therefore, absolute recovery (%) can be determined by comparing the peak ratio between the compounds and IS peak areas at each concentration by comparing the two sets of samples. Absolute recoveries ranged from 37 to 97% for all analytes and are shown in Table 5.

On a study by Gaujac and coworkers, the determination of only DMT on ayahuasca teas obtained good results for absolute recovery of DMT, values ranging between 71 and 109%, in our case values were a little lower, ranging from 47 to 63%. This study was only performed for DMT, hence no comparison can be done respecting B-CAs [3].

To the best of our knowledge, there is only one more study available where both DMT and B-CAs were quantified. In this research, Pires et al. [16] were able to obtain good recovery values: for DMT the recovery intervals ranged from 78 to 89%; for HMN from 70 to 87%; for HML from 68 to 95% and finally for THH it ranged from 84 to 99%.

3.3 Method applicability

After validation, the herein described method was evaluated in terms of applicability on authentic tea samples. An aliquot of each one of the extract samples was analyzed by GC–MS in full scan mode, in order to confirm the identity of the extract's chemical compounds.

After evaluating the content of DMT, THH, HMN and HML, these were quantified with the validated method and the results are summarized on Table 6.

Peganum harmala tea has the highest concentrations of B-CA, followed by *B. caapi*. In contrast, *DC AB* tea is the one presenting the lowest concentrations of these compounds.

It is possible to confirm that DMT is present in two tea varieties, i.e., *P. viridis* and *M. tenuiflora*, being the major component. On the other hand, in the remaining tea varieties, only B-CA are present. However, the major compound is different in each tea. While in *DC AB*, THH reveals as the major compound, in *B. caapi* the compound present at higher concentrations is HMN. On the other hand, HML is the predominant compound in *P. harmala* tea. The latter is in accordance with the available literature. Pires et al. [16], that have previously quantified ayahuasca preparations, have also verified that HML was the most abundant substance.

The HML concentrations ranged from 0.20 (*P. viridis*) to 26.95 µg/mL (*P. harmala*). Also, HMN concentrations showed a wide range between teas, being of 0.20 (*DC AB*) to 24.27 µg/mL (*P. harmala*). The remaining compounds did not present such a wide range of concentrations as the latter. When compared to the concentrations present in preparations of both Peruvian and Brazilian Ayahuasca

Table 3 Comparison between different methods described in the literature

Sample	Detected compounds	LOD (μg/mL)				LOQ (μg/mL)				Recovery (%)	Extraction technique	Analytical technique	Reference
		DMT	THH	HMN	HML	DMT	THH	HMN	HML				
Plasma (1 mL)	DMT, THH, HMN, HML	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	0.2×10^{-3}	0.4×10^{-3}	0.3×10^{-3}	0.4×10^{-3}	89–107	SPE: Classic Sep-Pack® C_{18} cartidges	LC–MS/MS	[17][a]
Hair (25 mg)	DMT	0.01 (ng/mg)	–	–	–	0.03 (ng/mg)	–	–	–	80–85	Hydrolysis with 250 μL of VMA-T M3 reagent (acidic aqueous buffer) for 1 h at 100 °C	UHPLC–MS/MS	[19][a]
Plasma (1 mL)	DMT, THH, HMN, HML, harmol and harmalol	0.5×10^{-3}	0.3×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	1.6×10^{-3}	1.0×10^{-3}	0.5×10^{-3}	0.3×10^{-3}	74–102	LLE: *n*-pentane (DMT); SPE: bond-Elut C_{18} (100 mg) cartridges	GC-NPD (DMT) and HPLC-FLD (THH, HMN, HML, harmol and harmalol)	[18][a]
Blood (0.2 mL)	DMT, 5-hydroxy-DMT, 5-methoxy-DMT, DMT-*N*-oxide, *N*-dimethyl-kynuramine, *N*-methyl-tryptamine, 2-methyl-tetrahydro-beta-carboline, THH, 7-hydroxy-tetrahydro-harmine, HMN, HML and indoleacetic acid	0.45×10^{-3}	0.36×10^{-3}	0.25×10^{-3}	0.22×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	60–75	Protein precipitation 96-well plates	LC/MS/MS	(only LOD)[a] [21]

Table 3 (continued)

Sample	Detected compounds	LOD (µg/mL)				LOQ (µg/mL)				Recovery (%)	Extraction technique	Analytical technique	Reference
		DMT	THH	HMN	HML	DMT	THH	HMN	HML				
Ayahuasca beverages (5 mL)	DMT	0.78	–	–	–	9.5	–	–	–	71–109	SPME: polydimethylsiloxane/divinylbenzene (65 mm, PDMS/DVB); headspace mode	GC–IT–MS	[3]
Ayahuasca beverages (0.5 mL)	DMT, THH, HMN, HML	10	10	10	10	20	20	20	20	68–99	SPE: classic Sep-Pack® C_{18} cartidges	GC-NPD	[16][a]
Ayahuasca beverages (1 mL)	DMT, THH, HMN, HML	n.a	n.a	n.a	n.a	1.5	3.5	6.0	1.0	n.a	Direct injection	LC–MS/MS	[20]
Ayahuasca beverages (5 mL)	DMT, HMN, HML	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	LLE: diethyl ether	GC–MS	[22][+]
Ayahuasca beverages (0.2 mL) seized powder (10 mg)	DMT, THH, HMN, HML	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	n.a.	Addition of 0.8 mL of methanol (beverage) 8 mL of methanol (power)	HPLC–DAD	[23][a]
Ayahuasca beverages (0.25 mL)	DMT, THH, HMN, HML	0.04	0.2	0.2	0.2	0.04	0.2	0.2	0.2	47–98	SPE: Oasis® MCX3cc (60 mg) cartidges	GC–MS	This study

DMT: *N*,*N*-dimethiltryptamine; DVB: divinylbenzene; GC–IT–MS: gas chromatography coupled to ion trap mass spectrometry; GC-NPD: gas chromatography coupled to nitrogen phosphorous detector; HML: harmaline; HMN: harmine; HPLC–DAD: high-performance liquid chromatography coupled to diode array detector; HPLC-FLD: high-performance liquid chromatography coupled to fluorescence detector; LC–MS/MS: liquid chromatography coupled to tandem mass spectrometry; LLE: liquid–liquid extraction; LOD: limit of detection; LOQ: limit of quantification; PDMS: polydimethylsiloxane; SPE: solid-phase extraction; SPME: solid-phase microextraction; THH: tetrahydroharmine; UHPLC-MS/MS: ultra-high-pressure liquid chromatography tandem mass spectrometry

[a]The LOD and LOQ were calculated mathematically

[+]Only identification

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Table 4 Inter-day (n = 5), intraday (n = 5) and intermediate (n = 15) precision and accuracy

Analyte	Nominal concentration (µg/mL)	Interday			Intraday			Intermediate		
		Measured concentration[a] (µg/mL)	CV (%)	BIAS (%)	Measured concentration[a] (µg/mL)	CV (%)	BIAS (%)	Measured concentration[a] (µg/mL)	CV (%)	BIAS (%)
DMT	0.04	0.04 ± 0.001	1.89	12.50	0.04 ± 0.004	9.04	3.20			
	0.06							0.054 ± 0.001	2.06	− 9.6
	0.1	0.01 ± 0.003	3.13	− 4.00						
	0.5				0.46 ± 0.054	11.69	− 7.26			
	0.8							0.77 ± 0.100	13.13	− 4.3
	1	0.95 ± 0.065	6.81	− 4.62						
	3	2.92 ± 0.286	9.78	− 2.57						
	4				4.00 ± 0.346	8.64	1.34	3.97 ± 0.174	4.39	− 0.9
	5	5.08 ± 0.191	3.76	1.59						
THH	0.2	0.22 ± 0.002	0.70	9.50						
	0.4	0.44 ± 0.030	6.79	9.93	0.37 ± 0.019	5.07	− 7.35			
	0.8							0.79 ± 0.889	11.29	− 1.59
	1	0.87 ± 0.015	1.72	− 13.10	0.89 ± 0.021	2.32	− 11.49			
	2.5	2.28 ± 0.184	8.09	− 8.84						
	5	4.81 ± 0.259	5.38	− 3.78						
	8							8.14 ± 0.559	6.87	1.78
	10	10.05 ± 0.382	3.80	0.48						
	16							18.34 ± 9.752	15.22	14.65
	20	20.43 ± 0.206	1.01	2.13	22.63 ± 0.301	1.33	13.14			
HMN	0.2	0.20 ± 0.034	17.17	− 1.00						
	0.4	0.40 ± 0.031	7.67	1.01	0.44 ± 0.018	4.06	10.07			
	0.8							0.78 ± 0.076	9.74	− 2.6
	1	1.03 ± 0.088	8.55	2.93	1.03 ± 0.096	9.31	3.17			
	2.5	2.47 ± 0.225	9.13	− 1.39						
	5	4.77 ± 0.513	10.75	− 4.56						
	8							7.66 ± 0.733	9.57	− 4.3
	10	10.19 ± 0.589	5.78	1.94						
	16							16.28 ± 1.407	8.64	1.7
	20	19.98 ± 0.306	1.53	− 0.09	20.29 ± 1.301	6.41	1.44			
HML	0.2	0.22 ± 0.001	0.47	9.00						
	0.4	0.38 ± 0.001	0.27	− 5.75	0.36 ± 0.019	5.21	− 10.30			
	0.8							0.74 ± 0.040	5.45	− 7.69
	1	0.92 ± 0.101	10.98	− 8.00	0.92 ± 0.046	4.97	− 8.11			
	2.5	2.46 ± 0.349	14.20	− 1.72						
	5	4.49 ± 0.071	1.58	− 10.24						
	8							8.37 ± 0.661	7.89	4.67
	10	10.56 ± 0.363	3.44	5.65						
	16							16.69 ± 0.915	5.48	4.30
	20	20.05 ± 0.051	0.25	0.23	20.31 ± 2.108	10.38	1.54			

CV: coefficient of variation; BIAS: relative error [(measured concentration − nominal concentration/nominal concentration) × 100)]

[a]Mean values ± standard deviation

[16], the concentrations are much smaller, sometimes more than an order of magnitude.

The available literature on the quantification of DMT, THH, HMN and HML in ayahuasca preparations or teas remains scarce. In 2009, the quantification of the same compounds in ayahuasca preparations yielded HML as the major compound, however, the authors obtained the preparations already made and the varieties of the plants

Table 5 Recovery (n = 3)

Analyte	Concentration (μg/mL)	Recovery[a] (%)
DMT	0.1	63.05 ± 4.47
	0.8	63.26 ± 10.10
	4	57.23 ± 5.63
THH	0.4	47.24 ± 2.31
	8	57.03 ± 1.64
	16	58.25 ± 7.87
HML	0.4	37.17 ± 5.30
	8	46.55 ± 5.42
	16	48.76 ± 7.31
HMN	0.4	71.61 ± 3.93
	8	97.74 ± 12.70
	16	80.87 ± 6.24

[a]Mean values ± standard deviation; DMT: *N*,*N*-dimethyltryptamine; THH: tetrahydroharmine; HML: harmaline; HMN: harmine

Table 6 Concentrations of *N*,*N*-dimethyltryptamine; tetrahydroharmine; harmaline; and harmine found in *P. viridis*, *B. caapi*, *P. harmala*, *M. tenuiflora* and *DC AB* (n = 2)

	Concentration (μg/mL)[a]			
	DMT	THH	HML	HMN
P. viridis	2.06 ± 0.12	–	0.2 ± 0.21	–
B. caapi	–	1.12 ± 0.01	0.99 ± 0.21	12.81 ± 0.46
P. harmala	–	7.46 ± 0.01	26.95 ± 0.21	24.27 ± 0.46
M. tenuiflora	1.70 ± 0.12	–	–	0.74 ± 0.46
DC AB	–	1.11 ± 0.01	0.30 ± 0.21	0.20 ± 0.46

[a]Mean values ± standard deviation; DMT: *N*,*N*-dimethyltryptamine; THH: tetrahydroharmine; HML: harmaline; HMN: harmine

were not discriminated. On the other hand, the compound detected in a lower amount was THH. However, it is not possible to make direct comparisons, since these four compounds were quantified on the same preparation, which does not happen in the present work, where none of the teas presented simultaneously the four compounds. In 2013, Gaujac et al. [3] focused their work to the determination of DMT in ayahuasca tea. Its concentrations were shown to vary between 0.17 and 1.14 mg/mL, which are much higher than those obtained in the present work. Nevertheless, these concentrations, as well as those presented by Pires et al. [16] changed in function of the volume of tea and the amount of leaves used to prepare the beverages of ayahuasca. These authors did not describe the volume and the weight used, making the comparison impossible.

In a recent study the authors have used LC–MS/MS as an analytical technique to measure these compounds in tea extract [20]. The authors were able to quantify bigger quantities of all compounds, for example, DMT concentrations ranged from 62 to 340 μg/mL, whereas for THH the maximal concentration obtained was 3308 μg/mL. Comparing to this study, concentrations are much higher, however, the authors do not mention the exact quantity of tea extract present in each sample, that is, the amount of *P. viridis* leaves or the stalks of *B. caapi*, once the samples were provided by different centers of União do Vegetal (UDV). In addition, the authors defend that the process of ayahuasca preparation can lead to variances on the final compounds' concentrations in function of the boiling time, also with evaporation the concentration can be modified and by continuously increasing the plant content to the concoction while it is brewing. The same can be taken into consideration in the preparation of the samples in the present work, which could be responsible for some of the discrepancy on the values obtained. It is also interesting to highlight that in the previously mentioned works the samples were obtained from communities recognized has knowing how to prepare such teas (such as the UDV) and not from commercially available mixtures.

4 Conclusion

An analytical method using SPE and GC–MS was developed and fully validated to detect and quantify the major compounds present in commercial plants used to prepare ayahuasca beverages, DMT and the beta-carbolines THH, HMN and HML. A linearity range of 0.2–20 μg/mL was obtained for all compounds, except for DMT (0.04–5 μg/mL). A LLOQ of 0.2 μg/mL was achieved for the beta-carbolines, except for DMT (0.04 μg/mL), using a low volume of sample (250 μL). Moreover, the method's selectivity and precision and accuracy were considered adequate.

This is the first GC–MS method using SPE that was applied to the determination and quantification of these analytes simultaneously. SPE provides low matrix effects and no interferences from other used drugs were observed. In addition, its ease in use and operation, allowing for better laboratorial results using an analytical instrument accessible to most laboratories.

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Compliance with ethical standards

Conflict of interest All the authors have no potential conflicts of interest.

Ethical approval This article does not contain any studies with animals or human participants performed by any of the authors.

Informed consent Not applicable.

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