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RESEARCH ARTICLE

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Comparative study of sample preparation procedures to determine the main compounds in ayahuasca beverages by QuEChERS and high-performance liquid chromatography analysis

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Abstract

Introduction: Ayahuasca is a psychoactive drink originally consumed by indigenous people of the Amazon. The lack of regulation of this drink leads to uncontrolled consumption, and it is often consumed in religious contexts.

Objective: The aim of this work is to compare three miniaturised extraction techniques for extracting the main ayahuasca compounds from beverages.

Methodology: Three sample pretreatment techniques were evaluated (dispersive liquid–liquid microextraction [DLLME], microextraction by packed sorbent [MEPS] and QuEChERS [Quick, Easy, Cheap, Effective, Rugged and Safe]) for the simultaneous extraction of *N*,*N*-dimethyltryptamine (DMT), tetrahydroharmine (THH), harmine, harmaline, harmol and harmalol from ayahuasca beverage samples. Then, the most promising technique (QuEChERS) was chosen to pre-concentrate the analytes, subsequently detected by high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD).

Results: The procedure was optimised, with the final conditions being 500 μL of extractor solvent, 85 mg of primary secondary amine (PSA) and 4 s of vortexing. The analytical method was validated, showing to be linear between 0.16 and 10 μg/mL for β-carbolines and between 0.016 and 1 μg/mL for DMT, with coefficients of determination (R^2) between 0.9968 and 0.9993. The limit of detection (LOD) and lower limit of quantification (LLOQ) were 0.16 μg/mL for all compounds, except for DMT (0.016 μg/mL) and extraction efficiencies varied between 60.2% and 88.0%.

Conclusion: The analytical methodology proved to be accurate and precise, with good linearity, LODs and LLOQs. This method has been fully validated and successfully applied to ayahuasca beverage samples.

KEYWORDS
ayahuasca, DLLME, MEPS, QuEChERS

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1 | INTRODUCTION

Ayahuasca is a decoction prepared from leaves of *Psychotria viridis* Ruiz & Pav. and *Banisteriopsis caapi* (Spruce ex Griseb.) C. V. Morton, being a thick and brown oily liquid.[1,2] The active ingredient of this mixture is the alkaloid *N*,*N*-dimethyltryptamine (DMT), a hallucinogenic compound that acts on serotonergic receptors (5-HT1A, 5-HT2A, and 5-HT2C).[2,3] The actions of DMT are made possible through the actions of the β-carbolines harmaline, harmine, and tetrahydroharmine (THH), derived from *B. caapi*,[2,4] since these components inhibit monoamine oxidase-A (MAO-A), an enzyme that degrades DMT in the digestive tract. The synergy between DMT and β-carbolines results in hallucinogenic and visionary effects associated with this decoction.[2,5,6] The effects of ayahuasca can last for about 4 h, starting 30 to 60 min after ingestion and reaching maximum intensity between 60 and 120 min after.[4] This psychoactive beverage was originally used by indigenous people as a sacred drink for healing purposes (spiritual, mental, and social).[1,4] Later, the consumption of ayahuasca began to be used ritualistically as a religious sacrament and to facilitate self-knowledge, giving rise to the religions of the Barquinha, União do Vegetal and Santo Daime.[1,2] Currently, its consumption occurs all over the world (Europe, United States and Oceania), being known by the most varied names: *yage*, *caapi*, *natem*, *mihi*, *dapa*, *daime* and *hoasca*, among others.[1,4]

The effects of taking ayahuasca may include physical symptoms as vomiting, diarrhoea and nausea.[2] However, this drink leads to changes in auditory, visual and somatosensory perception, causing an altered state of consciousness with transpersonal experiences, visions, autobiographical memories and introspective effects.[2,3] The expansion of consumption of this psychoactive drink worldwide, as well as the report of its therapeutic potential, has aroused much interest.[7] Thus, the reported effects have led to a constant increase in demand for ayahuasca for psychotherapeutic, self-realisation and spiritual enlightenment purposes.[7] Nevertheless, the consumption of *B. caapi* or *Psychotria viridis* is not regulated, and it is inclusively legal practice for religious purposes in Brazil and the United States.[2,8] The lack of regulation leads to uncontrolled consumption, occurring in a religious context from 1 year to a lifetime, with a monthly consumption of two or more times.[2] Therefore, it is important to control the safety regarding the regular consumption of this drink.[2]

Among the compounds present in ayahuasca, indole alkaloids are the most investigated, not only for their structural diversity, but also for their potential beneficial effects.[1] Several analytical methodologies have been developed with the aim of the detection and quantification of DMT and β-carboline alkaloids in samples of plasma,[9–11] urine,[12] hair[13] and blood,[14] or in ayahuasca preparations.[14–20] However, sample pretreatment methodologies are not always applied, and dilution and direct injection into the chromatographic equipment[12,14,17,18] is usually the practice, especially for the analysis of ayahuasca preparations. Additionally, the use of miniaturised techniques is also reduced in this type of samples, and, as far as we know, methodologies for detecting ayahuasca constituents in preparations of the drink have not yet been developed, such as, by the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach, microextraction by packed sorbent (MEPS) or dispersive liquid–liquid microextraction (DLLME). Adequate sample preparation is important for chromatographic analysis, having an important role on analyte isolation.[21]

MEPS functions as a miniaturised version of solid-phase extraction (SPE).[21] Unlike SPE, where the sorbent material is contained in a separate column, MEPS employs the syringe itself for sorbent placement, and can be reused several times. Moreover, MEPS significantly reduces solvent volume and allows for online coupling, enabling automation.[22–25] As for DLLME, it involves introducing an organic solvent with an extracting function and a dispersing solvent into an aqueous sample.[26] Following vigorous agitation, fine droplets form and disperse within the sample. Subsequent centrifugation allows the collected drop in the tube to be injected into the chromatographic equipment.[26] The formation of droplets allows for a large contact area, making extraction immediate.[27,28] Moreover, DLLME is a low-cost, ecological technique that uses very simple and common instrumentation.[28] Concerning QuEChERS, two phases are involved: an initial partitioning and an extract cleanup phase using dispersive solid-phase extraction (d-SPE).[29] This methodology presents several advantages, since small volumes of sample and solvent are required, being a fast, simple and direct technique.[29] Additionally, QuEChERS generally presents a higher recovery rate, with better analytical performance, and a great versatility in the application to the most varied analytes and samples.[29]

In this study, three miniaturised extraction techniques (DLLME, MEPS, and QuEChERS) were employed on four plant mixtures utilised in the preparation of ayahuasca decoctions, four individual plants, and a commercial mixture. Among these techniques, QuEChERS demonstrated superior efficacy in analyte extraction following a preliminary study and was therefore chosen for subsequent optimisation and validation for the quantification of harmine, DMT, harmaline, harmol, harmalol, and THH in ayahuasca decoctions. This technique is usually not considered a miniaturised approach; however, in the present study, it suits this classification, due to the low amounts of solvents that were used (microlitres).

2 | EXPERIMENTAL

2.1 | Reagents and standards

The analytical standards of DMT, harmine, THH, harmaline, harmol and harmalol were provided by Nal von Minden, GmbH (Regensburg, Germany). Methanol (HPLC grade), isopropanol, chloroform, acetic acid, and acetonitrile were obtained from Fischer Scientific (Loughborough, UK). Formic acid and internal standard (IS) 3,4-dihydroxybenzylamine (DHBA) were purchased from Sigma-Aldrich (Sintra, Portugal) and ammonium hydroxide from Enzymatic (Santo Antão do Tojal, Portugal). Primary secondary amine (PSA), magnesium sulphate ($MgSO_4$), and sodium acetate (CH_3COONa)

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were purchased from Laborspirit (Sintra, Portugal). Deionised water was obtained from a Milli-Q System (Millipore, Billerica, MA, USA).

Working solutions of harmol, harmalol, THH, harmine and harmaline at concentrations of 100 and 10 μg/mL were prepared from a stock solution at 1 mg/mL, by dilution with methanol. In the same way, DMT solutions were prepared at concentrations of 10 and 1 μg/mL from a 100 μg/mL solution. The IS was also prepared in methanol at a concentration of 100 μg/mL. All solutions were protected from light and kept at 4°C.

2.2 | Sample preparation

Plant samples were purchased from the Shayana Shop (https://www.shayanashop.com, Amsterdam, The Netherlands) (accessed 25 May 2019). The decoctions were prepared following a recipe provided by an ayahuasca consumer. The individual was admitted to the emergency room due to symptoms of poisoning resulting from this preparation. Accordingly, 0.210 g of each plant sample (*Psychotria viridis*, *B. caapi*, *Mimosa hostilis* Benth, *Peganum harmala* L., and a commercial mixture) were weighed and crushed with water droplets.

Subsequently, the crushed contents were combined in a Schott flask along with 250 mL of ultrapure water and heated for 4 h at 100°C. Additionally, four decoctions comprising mixtures of two plants were prepared: *Psychotria viridis* and *Peganum harmala*; *M. hostilis* and *Peganum harmala*; *Psychotria viridis* and *B. caapi*; *M. hostilis* and *B. caapi*. Upon completion of the heating period, the decoctions underwent refreshing, filtration, freezing at −80°C, and subsequent lyophilisation.

2.3 | Sample pretreatment

2.3.1 | MEPS

The procedure was performed according to the study by Malaca et al.,[30] with some modifications. The MEPS C_{18} sorbent was initially conditioned with 250 μL of methanol (one cycle) followed by 250 μL of water (one cycle). Subsequently, the sample, comprising 100 μL (including 50 μL of IS at 100 μL/mL), was loaded (10 cycles). A washing step was performed using 150 μL of water (one cycle) and 150 μL of a 5% methanol solution (one cycle). Next, the elution of the sample occurred with 100 μL of a solution containing acetonitrile with 2% ammonium hydroxide (four cycles). The resulting extract was evaporated to dryness using a stream of nitrogen, reconstituted in 150 μL methanol, filtered through a 0.2 μm filter, and then injected into the high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) system. To reconstitute the sorbent, 100 μL of two solutions was used (1% formic acid in isopropanol–water [10:90] and ammonium hydroxide in acetonitrile–methanol [1:1]) (four cycles).

2.3.2 | DLLME

The extraction procedure was performed by modifying the technique of Fernández et al.[31] The sample (1 mL) was mixed with 1 mL of acetonitrile, 125 μL of chloroform and 50 μL of IS at 100 μL/mL. Then, the mixture was submerged in a ultrasonic bath for 3 min, and then centrifuged for 5 min at 4000 rpm. Finally, the drop deposited at the bottom of the tube was aspirated and evaporated to dryness under a gentle nitrogen stream. The extract was reconstituted in 150 μL methanol, filtered (0.2 μm filter) and injected into the HPLC-DAD system.

2.3.3 | QuEChERS

The QuEChERS procedure was as follows: 50 μL of IS (100 μL/mL) was added to the sample (1 mL), along with $MgSO_4$ (0.4 g), CH_3COONa (0.1 g), and a solution of 1% acetic acid in acetonitrile (1.5 mL). The mixture was vortexed for 10 s and then centrifuged for 2 min at 4400 rpm. The resulting supernatant was collected, followed by the addition of 150 mg of $MgSO_4$ and 25 mg of PSA. After another 10 s of vortexing, the solution underwent centrifugation at 4400 rpm for 3 min. The supernatant was once again collected, subjected to evaporation under a nitrogen stream until dry, reconstituted in 150 μL of methanol, filtered through a 0.2 μm filter, and subsequently injected into the HPLC-DAD system.

2.4 | Instrumental and chromatographic conditions

The compounds present in the ayahuasca decoctions were quantified using a HPLC system with a DAD (Agilent Technologies Soquímica, Lisbon, Portugal). Samples were kept in the sampler at 4°C and injected onto a YMC-Triart PFP analytical column (5 μm, 4.6 inner diameter [i.d.] × 150 mm) coupled to a Guard-c holder (4 mm × 10 mm) and a Triart PFP (5 μm, 3 mm × 10 mm) pre-column, all from YMC Europe GMBH (Solitica, Lisbon, Portugal), maintained at 25°C. The mobile phase was composed of 0.1% formic acid in methanol in line A and 0.1% formic acid in water in line B, at a flow rate of 1 mL/min. Next, 50 μL was injected, and the elution was performed in gradient mode: 5% A (0–2 min), 50% A (2–50 min) and again, 5% A (50–60 min). Harmol and harmine were detected at 246 nm, harmalol and harmaline at 360 nm, THH and DMT at 278 nm.

2.5 | Validation procedure

In order to validate the described method, guiding principles of the ANSI/ASB Standard 036 were followed,[32] and the studied parameters included selectivity; linearity and limits; intermediate, intra-day and inter-day precision and accuracy, and extraction efficiency. The linearity ($n = 5$) was determined between 0.016 and 1 μg/mL for DMT and 0.16 and 10 μg/mL for the remaining analytes. The calibration curves

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were obtained by plotting the ratio of the peak area between each analyte and the peak area of the IS against the analyte concentration. Accuracy results within 15% (20% at the lower limit of quantification [LLOQ]), with a coefficient of determination (R^2) equal to or higher than 0.99, and coefficients of variation (CVs) not exceeding 15% (20% at the LLOQ) were accepted. The LLOQ was defined as the minimum concentration that could be measured precisely and accurately, that is, presenting a relative error (RE) of less than 20% of the nominal concentration and CV of less than 20%. In order to define the limit of detection (LOD), three replicates of enriched samples were analysed. The LOD was defined as the lowest concentrations where it was possible to visualise a distinct peak, clearly discernible from the blank and with a signal-to-noise ratio of at least 3. To evaluate the intra-day precision and accuracy, on the same day, blank samples spiked with the target analytes (minimum of three different concentration levels) were analysed in triplicate. Within the same period, inter-day precision and accuracy were evaluated at seven concentrations. Intermediate precision and accuracy were calculated with three quality control (QC) samples in triplicate at the concentrations 0.16, 0.63 and 5 μg/mL (0.016, 0.063 and 0.5 μg/mL for DMT) over the 3-day protocol. Two sets of samples in triplicate were prepared at the concentrations 0.16, 0.63 and 5 μg/mL, in order to analyse extraction efficiency. Set 1 represented peaks obtained by analysis of samples spiked before extraction, while Set 2 consisted of peaks obtained by spikes after extraction (representing 100% efficiency), with IS being added to both sets after extraction. The efficiency results were calculated by the ratio between the relative peak areas of sample Set 1 and sample Set 2.

3 | RESULTS AND DISCUSSION

3.1 | Extraction procedure selection

Initially, the best sample pretreatment method was chosen. Thus, the three extraction methods (QuEChERS, DLLME and MEPS) were tested on a sample of ayahuasca beverage, in order to verify which method provided the clearest variations for analysis. Analysing Figure 1, it is possible to verify that the QuEChERS technique clearly showed superior areas for harmine, harmaline, THH and DMT, whereas for harmol and harmalol, this was not so evident. As a multianalyte method was deemed necessary for practicality, QuEChERS was chosen to proceed to the following stages of the work.

DLLME has been used to quantify DMT and β-carboline alkaloids in human plasma in a paper by de Silveira et al.,[33] however none of the described sample treatment approaches was applied to ayahuasca beverage samples.

3.2 | Optimisation of the QuEChERS extraction

In an initial phase of the optimisation process, the Design of Experiments (DOE) statistical tool MINITAB® was used. This tool allows multivariate analysis of parameters that can significantly influence the extraction procedure. Multifactor analysis is crucial in an optimisation process, since its use allows the best analyte recoveries to be obtained, while maintaining the number of experiments at a

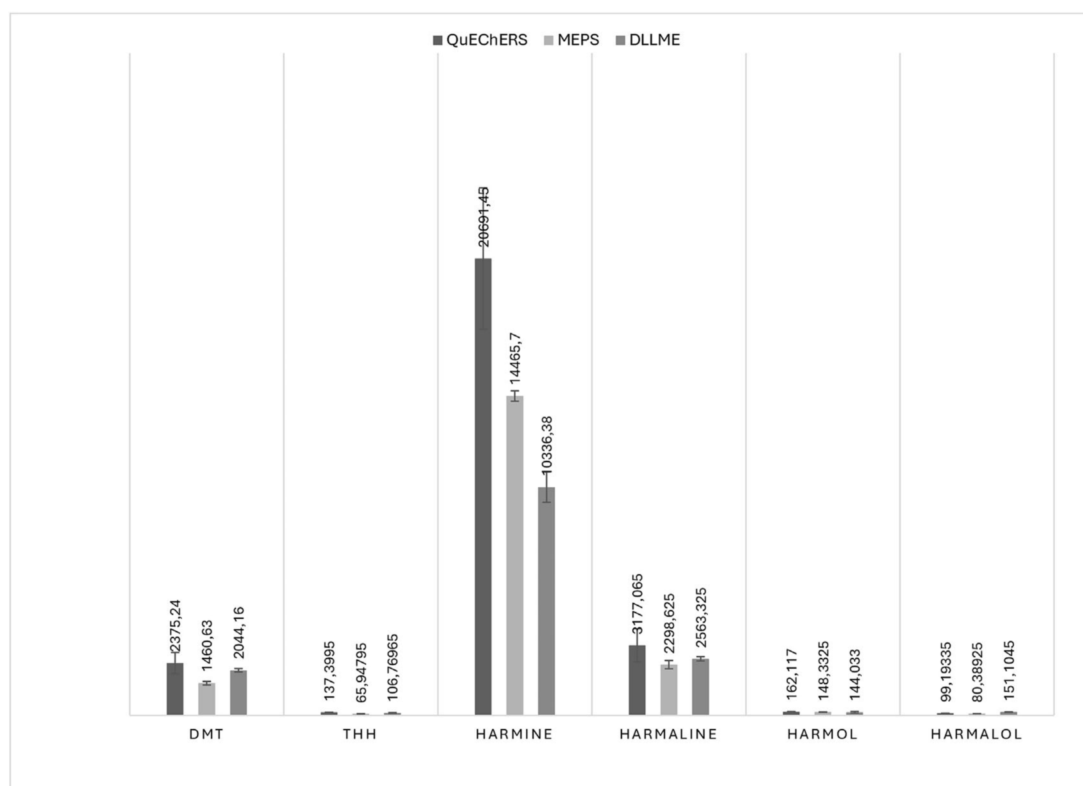

FIGURE 1 Analyte peak areas obtained for each extraction procedure ($n = 3$).

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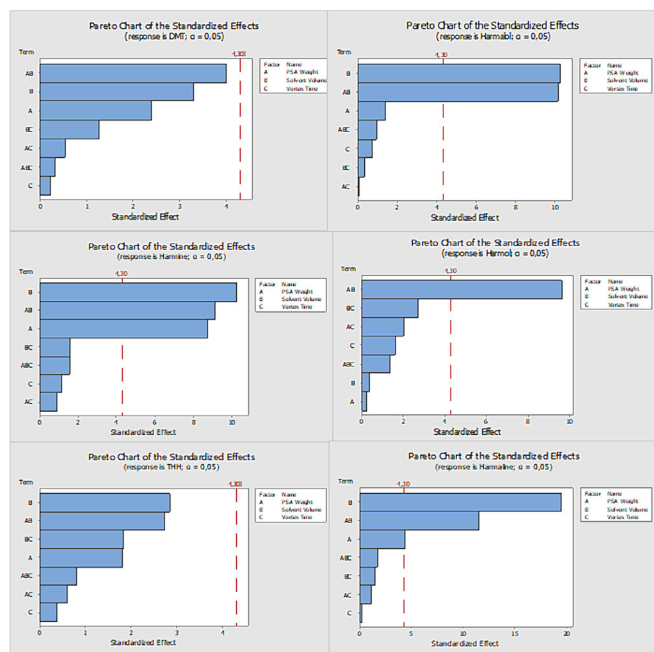
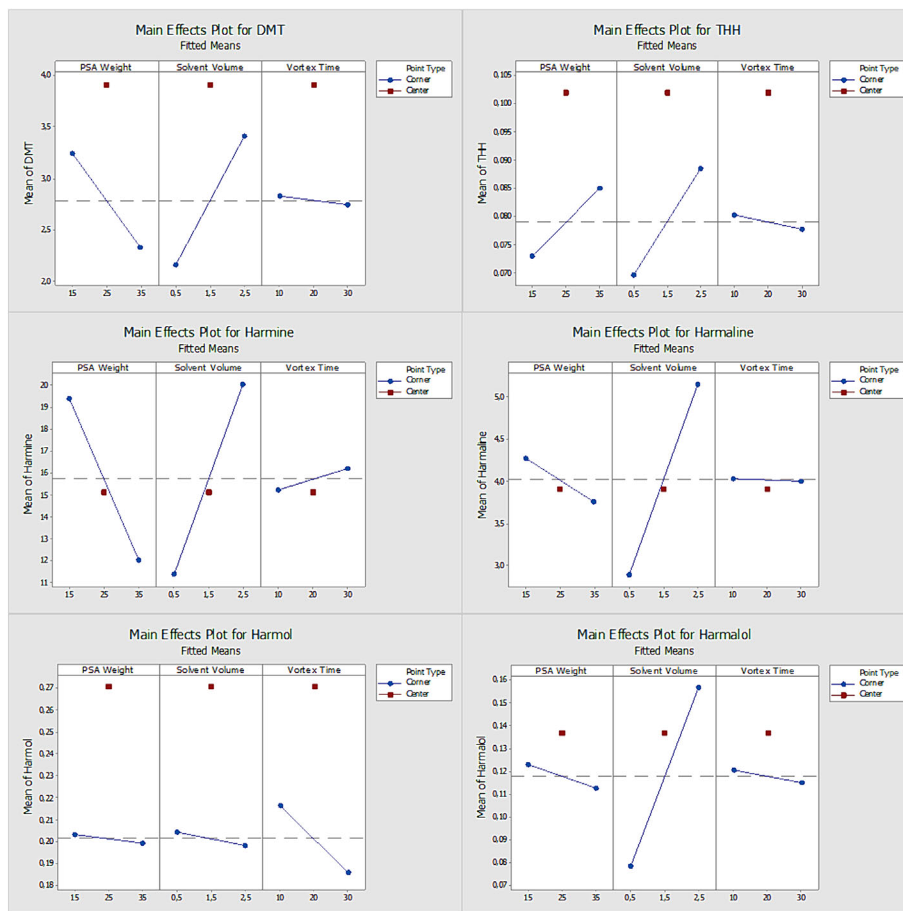


FIGURE 2 Pareto diagrams of the compounds under study. [Colour figure can be viewed at wileyonlinelibrary.com]

minimum.[21] Thus, a two-level, three-component (2^3) factorial design was performed to analyse the relevant variables to the recovery of analytes and their main effects. The independent variables consisted of extractor solvent volume, amount of PSA and vortex time. A total of 11 runs were generated from this factorial design, covering all potential combinations of factor values. The independent variables were systematically tested at high and low levels: the values chosen for the extractor solvent volume were 0.5 and 2.5 mL, for the amount of PSA were 15 and 35 mg, and for the vortex time 10 and 30 s. Each experiment was randomly conducted with a central point in triplicate, aiming to minimise the impact of noise factors and systematic errors. The obtained results are shown in Figures 2 and 3, which represent the main effects and Pareto charts generated for each target analyte through the DOE analysis.

Looking at these Pareto charts, one can see that while DMT and THH were not affected significantly by any of the chosen parameters, harmine and harmaline were affected by the volume of solvent and amount of PSA, and by an interaction of these two factors. Regarding harmol, only one interaction of the solvent volume and amount of PSA was statistically significant, while for harmalol the same interaction and solvent volume also significantly influenced the recovery.

FIGURE 3 Plots of main effects of the factors tested on each parameter of each analyte. [Colour figure can be viewed at wileyonlinelibrary.com]



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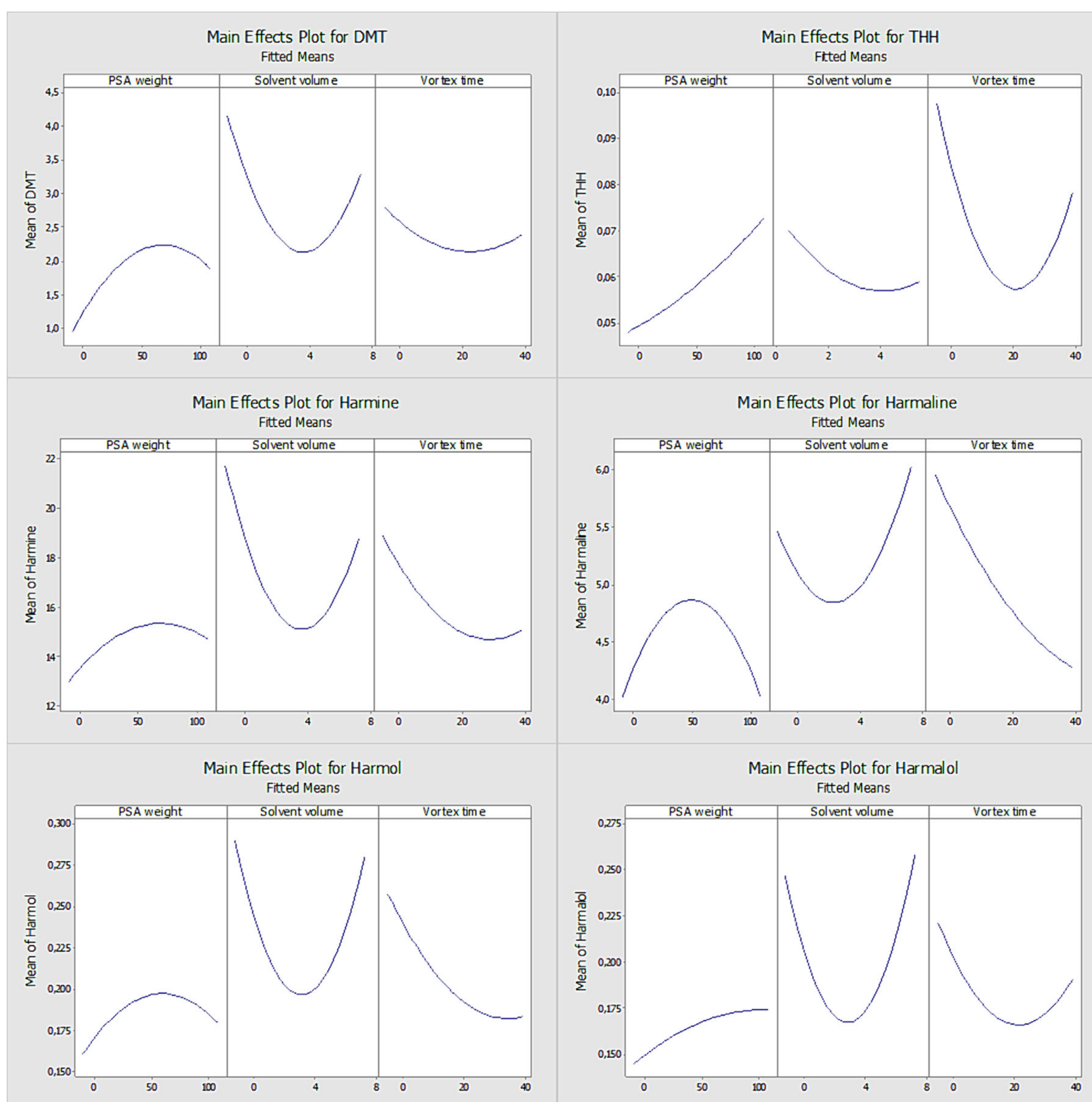

FIGURE 4 Results from RSM response optimiser for analytes. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Linearity data ($n = 5$).

Compound	Linear range (μg/mL)	Linearity (mean ± SD) Slope (m)	Intercept (b)	R^2 (mean ± SD)	LLOQ and LOD (μg/mL)
DMT	0.016–1	0.2651 ± 0.0065	0.0035 ± 0.0005	0.9993 ± 0.0004	0.016
Harmalol	0.16–10	0.1743 ± 0.0159	0.0001 ± 0.0031	0.9982 ± 0.0025	0.16
Harmol	0.16–10	1.0293 ± 0.1758	−0.0261 ± 0.0206	0.9978 ± 0.0035	0.16
THH	0.16–10	0. 0680 ± 0.0067	−0.0062 ± 0.0036	0.9985 ± 0.0016	0.16
Harmaline	0.16–10	0.3941 ± 0.0575	−0.0010 ± 0.0223	0.9968 ± 0.0034	0.16
Harmine	0.16–10	2.2773 ± 0.1989	−0.0515 ± 0.0806	0.9978 ± 0.0025	0.16

Note: DMT, *N*,*N*-dimethyltryptamine; LLOQ, lower limit of quantification; LOD, limit of detection; R^2, coefficient of determination; SD, standard deviation; THH, tetrahydroharmine.

Figure 3 shows the graphs of main effects where the effect of each factor studied for each studied analyte can be observed. Except for THH, a greater recovery was obtained using a lower amount of PSA. Regarding the volume of solvent, a greater amount of analyte is obtained with a greater volume of solvent, except for harmol whose recovery is favoured with a smaller volume of extractor solvent.

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Finally, a shorter vortex time favoured the recovery of all analytes, except for harmine. Despite these results, it must be considered that none of these factors significantly influenced the response obtained for DMT and THH. Likewise, the vortex time factor also did not significantly influence the behaviour of the analytes. However, harmaline, harmol, harmine and harmalol were significantly influenced by the volume of extractor solvent, by the amount of PSA, or even by an interaction between these two variables. Thus, since four of the analytes were significantly influenced by at least one of the variables tested, it was deemed necessary to further optimise the method.

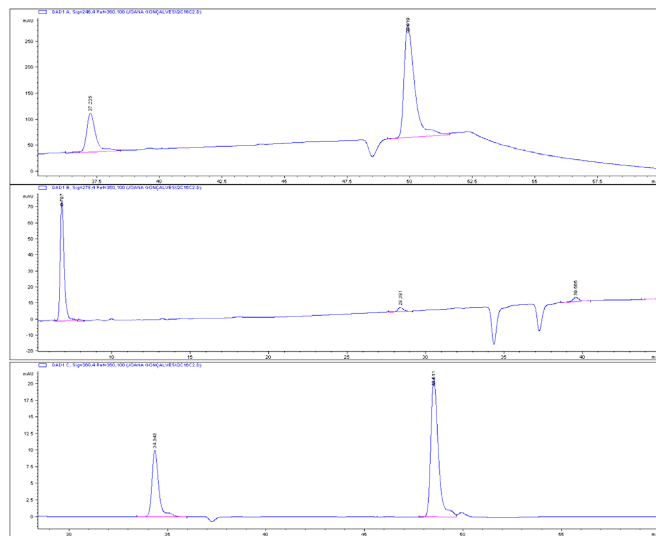

FIGURE 5 Chromatograms of compounds at the LLOQ. 246 nm: harmol (retention time 37.2 min) and harmine (retention time 49.9 min); 278 nm: IS (retention time 6.8 min), DMT (retention time 28.4 min) and THH (retention time 39.6 min) and 360 nm: harmalol (retention time 31.3) and harmaline (retention time 48.5 min). [Colour figure can be viewed at wileyonlinelibrary.com]

With DOE optimisation it was not possible to draw an adequate conclusion, and therefore an experimental response surface methodology (RSM) was applied to all analytes. A new matrix was constructed with the same variables. However, the number of experiments increased, and the testing range was wider (volume of solvent varying between 0.5 and 7.2 mL, amount of PSA between 5 and 108.8 mg and vortex time between 4 and 39 s). The response to the main effects (Figure 4) exhibited that the analytes showed a better response for the minimum vortex time (4 s), although this variable was not significant. In addition, the recovery of the analytes was maximum for the minimum volume of extractor solvent (500 μL) for all analytes, except for harmaline and harmalol. Finally, the amount of analyte was maximum when using 85 mg of PSA for DMT, harmine and harmol. Conversely, a better response was obtained with an amount of PSA between 85 and 100 mg for THH and harmalol. Regarding harmaline, the response was more favourable with only 50 mg of PSA.

The following sample pretreatment procedure was established: 85 mg of PSA, 500 μL of extracting solvent, and vortex for 4 s.

TABLE 2 Intra-day precision and accuracy.

		Intra-day		
Compound	Concentration (μg/mL)	Measured concentration (mean ± SD) (μg/mL)	CV (%)	RE (%)
DMT	0.016	0.01 ± 0.00	2.44	−11.94
	0.063	0.07 ± 0.00	2.04	13.22
	0.5	0.53 ± 0.02	3.80	5.43
Harmalol	0.16	0.16 ± 0.02	9.48	−0.52
	0.63	0.69 ± 0.04	5.62	9.25
	5	5.13 ± 0.50	9.72	2.62
Harmol	0.16	0.16 ± 0.01	5.33	1.65
	0.63	0.64 ± 0.04	5.97	0.85
	5	4.97 ± 0.12	2.51	−0.56
THH	0.16	0.14 ± 0.01	4.47	−10.51
	0.63	0.59 ± 0.06	9.93	−6.82
	5	4.74 ± 0.12	2.54	−5.22
Harmaline	0.16	0.16 ± 0.00	3.01	3.09
	0.63	0.61 ± 0.06	10.14	−3.41
	5	4.98 ± 0.15	3.09	−0.46
Harmine	0.16	0.17 ± 0.00	2.68	8.97
	0.63	0.71 ± 0.02	2.59	12.40
	5	5.15 ± 0.28	5.39	2.94

Note: CV, coefficient of variation; DMT, *N*,*N*-dimethyltryptamine; RE, relative error; SD, standard deviation; THH, tetrahydroharmine.

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TABLE 3 Inter-day precision and accuracy.

		Inter-day		
Compound	**Concentration (μg/mL)**	**Measured concentration (mean ± SD) (μg/mL)**	**CV (%)**	**RE (%)**
DMT	0.016	0.02 ± 0.00	15.18	−4.51
	0.031	0.03 ± 0.00	8.62	1.71
	0.063	0.07 ± 0.00	4.74	9.53
	0.125	0.12 ± 0.01	6.61	−1.90
	0.25	0.25 ± 0.00	0.80	−1.34
	0.5	0.50 ± 0.02	4.48	−0.25
	1	1.00 ± 0.01	1.02	0.13
Harmalol	0.16	0.16 ± 0.02	15.43	2.85
	0.31	0.32 ± 0.01	2.63	2.88
	0.63	0.63 ± 0.04	6.49	0.52
	1.25	1.30 ± 0.14	10.93	3.73
	2.5	2.46 ± 0.12	4.68	−1.51
	5	4.82 ± 0.32	6.70	−3.60
	10	10.07 ± 0.13	1.25	0.66
Harmol	0.16	0.15 ± 0.02	10.16	−4.00
	0.31	0.29 ± 0.02	7.50	−6.33
	0.63	0.60 ± 0.04	7.10	−4.36
	1.25	1.22 ± 0.11	9.11	−2.23
	2.5	2.44 ± 0.10	4.08	−2.52
	5	4.78 ± 0.41	8.62	−4.38
	10	10.04 ± 0.12	1.20	0.40
THH	0.16	0.17 ± 0.01	7.05	10.87
	0.31	0.33 ± 0.03	8.26	5.83
	0.63	0.66 ± 0.03	5.27	5.49
	1.25	1.17 ± 0.05	4.57	−6.53
	2.5	2.38 ± 0.15	6.19	−4.80
	5	5.20 ± 0.22	4.30	3.92
	10	9.94 ± 0.08	0.80	−0.56
Harmaline	0.16	0.18 ± 0.00	1.39	14.23
	0.31	0.31 ± 0.02	5.95	0.24
	0.63	0.66 ± 0.04	5.80	5.84
	1.25	1.27 ± 0.14	10.76	1.78
	2.5	2.47 ± 0.13	5.08	−1.29
	5	4.67 ± 0.38	8.05	−6.52
	10	10.12 ± 0.14	1.41	1.22
Harmine	0.16	0.18 ± 0.01	5.44	16.29
	0.31	0.34 ± 0.01	2.58	12.10
	0.63	0.70 ± 0.03	4.71	10.11
	1.25	1.28 ± 0.14	11.11	7.48
	2.5	2.45 ± 0.17	6.87	0.34
	5	4.77 ± 0.27	5.66	−3.25
	10	10.12 ± 0.12	1.16	−1.69

Note: CV, coefficient of variation; DMT, *N*,*N*-dimethyltryptamine; RE, relative error; SD, standard deviation; THH, tetrahydroharmine.

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TABLE 4 Intermediate precision and accuracy.

		Intermediate		
Compound	Concentration (μg/mL)	Measured concentration (mean ± SD) (μg/mL)	CV (%)	RE (%)
DMT	0.016	0.02 ± 0.00	10.84	3.36
	0.063	0.07 ± 0.00	1.03	14.06
	0.5	0.53 ± 0.02	3.68	6.46
Harmalol	0.16	0.17 ± 0.01	6.06	8.96
	0.63	0.72 ± 0.02	2.39	13.79
	5	5.49 ± 0.21	3.83	9.78
Harmol	0.16	0.18 ± 0.01	3.10	12.62
	0.63	0.70 ± 0.04	5.11	11.70
	5	5.51 ± 0.38	6.92	10.14
THH	0.16	0.17 ± 0.02	10.29	4.89
	0.63	0.68 ± 0.04	5.45	8.45
	5	5.28 ± 0.44	8.43	5.53
Harmaline	0.16	0.18 ± 0.01	3.40	9.45
	0.63	0.69 ± 0.04	5.31	10.05
	5	5.38 ± 0.49	9.07	7.59
Harmine	0.16	0.17 ± 0.02	13.36	0.5
	0.63	0.71 ± 0.01	2.07	12.80
	5	5.65 ± 0.10	1.70	12.97

Note: CV, coefficient of variation; DMT, *N*,*N*-dimethyltryptamine; RE, relative error; SD, standard deviation; THH, tetrahydroharmine.

3.3 | Method validation parameters

3.3.1 | Linearity and calibration model

The analytical method proved to be linear between 0.16 and 10 μg/mL for all β-carbolines, while for DMT it was linear from 0.016 to 1 μg/mL, with the R^2 values ranging from 0.9968 to 0.9993. The accuracy of the calibrators used (mean relative error [BIAS] between spiked and measured values) remained within the range of ±15% (±20% at the LLOQ) for all concentrations and CV below 15% were obtained, indicating an acceptable precision. The data are shown in Table 1.

3.3.2 | Limits of detection and quantification

Except for DMT, where the value was 0.016 μg/mL, the LLOQ and LOD obtained was 0.16 μg/mL for all compounds (Table 1). As a result, the LLOQs are satisfactory compared to other published works where the same analytes were analysed. Thus, comparable or better results were obtained for DMT,[15,34–39] THH,[15,35,37] harmine,[15,35,37] harmaline[15,35,37] and harmalol.[37] However, Eller et al.[38] reported lower LLOQs for harmine, harmaline and THH, but using however a much more sensitive equipment (ultrahigh-performance liquid chromatography–tandem mass spectrometry [UHPLC–MS/MS]). Other works that used LC–MS/MS, reported LLOQs of the same order of magnitude or higher than those of the present study.[17,40]

Analytical methods for the detection of DMT and β-carbolines in biological samples were also described, namely, for plasma,[9–11,33,41] hair,[13] blood,[14] sweat[42] and urine.[43] In these works, LOD and LLOQ values are presented in the order of ng/mL or ng/mg, but once again the equipment used is much more sensitive and almost always associated with mass spectrometry.[11,13,14,33,41–44]

This technique proves to be very advantageous when comparing the limits obtained and volume of solvent (microlitres), amount of reagents and reduced preparation time. This methodology is the first to quickly detect and quantify DMT and β-carboline alkaloids in ayahuasca decoctions, using QuEChERS and liquid chromatography, presenting adequate LLOQs, perfectly comparable to those published elsewhere (Figure 5).

3.3.3 | Intra-day, inter-day, and intermediate precision and accuracy

The evaluation of intra-day accuracy and precision was performed through the analysis of three calibrators of known concentrations, within the linear range ($n = 7$). The results obtained are shown in Table 2, where CVs for all studied concentrations were less than 15%, with a mean relative error of ±13.2%.

Inter-day precision and accuracy were measured for 3 days for a total of seven calibrators. All concentrations had CVs of less than 15%, with a mean relative error of ±16.3%. The results obtained are shown in Table 3.

Finally, for the evaluation of the intermediate precision and accuracy, three QCs of known concentrations were used ($n = 3$). Solutions at 0.16, 0.63 and 5 μg/mL (0.016, 0.063 and 0.5 μg/mL for DMT) were analysed during 3 days, with the CVs obtained being less than 13.4% with an accuracy of ±14.1% (Table 4).

Extraction efficiency

The extraction efficiency of the technique was measured using two sets of three samples, prepared by adding analytes at concentrations of 0.16, 0.63 and 5 μg/mL (0.016, 0.06 and 0.5 μg/mL for DMT) to water. One of the sets was fortified before extraction and the other set was fortified after extraction, corresponding to 100% recovery. The extraction efficiency was calculated through the proportion between the relative peak areas of each sample of the first set to those of the second set. Extraction efficiencies are shown in Table 5.

As far as we know, this is the only analytical methodology that uses the QuEChERS technique in ayahuasca samples. In other methods where the quantification of analytes in ayahuasca samples was performed either did not pretreat the sample[17,34,35,38,40] or have used approaches such as SPE,[15 37] solid-phase microextraction (SPME)[36] and matrix solid phase dispersion (MSPD).[39] The use of SPE as extraction technique showed higher recoveries for DMT,[15,37] THH,[15,37] harmine[15,37] and harmaline.[15,37] However, the present study demonstrated a better extraction efficiency for harmalol

TABLE 5 Extraction efficiency (%) of the target analytes ($n = 3$).

Compound	Concentration (μg/mL)	Efficiency (mean ± SD) (%)
DMT	0.016	70.1 ± 6.4
	0.032	88.0 ± 12.8
	0.5	78.5 ± 5.0
Harmalol	0.16	68.5 ± 7.6
	0.32	73.5 ± 7.5
	5	77.6 ± 3.5
Harmol	0.16	75.8 ± 7.1
	0.32	81.4 ± 10.3
	5	63.9 ± 3.4
THH	0.16	64.8 ± 3.8
	0.32	84.4 ± 5.9
	5	77.3 ± 3.5
Harmaline	0.16	63.8 ± 9.8
	0.32	79.3 ± 7.00
	5	67.4 ± 7.5
Harmine	0.16	60.2 ± 9.2
	0.32	79.1 ± 1.9
	5	64.5 ± 5.6

Note: DMT, *N*,*N*-dimethyltryptamine; SD, standard deviation; THH, tetrahydroharmine.

TABLE 6 Concentrations of compounds found in authentic samples.

Sample	Compound	Concentration (μg/mL)
Psychotria viridis	DMT	31.13 ± 4.78
Banisteriopsis caapi	Harmalol	0.02 ± 0.00
	Harmol	0.08 ± 0.00
	THH	1.22 ± 0.11
	Harmaline	0.11 ± 0.01
	Harmine	2.14 ± 0.33
Peganum harmala	Harmalol	1.20 ± 0.11
	Harmol	0.30 ± 0.03
	THH	1.98 ± 0.17
	Harmaline	17.30 ± 2.00
	Harmine	13.13 ± 1.66
Mimosa hostilis	DMT	15.00 ± 1.78
Commercial mixture	DMT	3.33 ± 0.05
	Harmalol	ND
	Harmol	ND
	THH	ND
	Harmaline	0.03 ± 0.00
	Harmine	0.16 ± 0.02
Psychotria viridis + *Banisteriopsis caapi*	DMT	3.43 ± 0.08
	Harmalol	0.06 ± 0.01
	Harmol	0.07 ± 0.00
	THH	2.87 ± 0.32
	Harmaline	1.45 ± 0.28
	Harmine	2.46 ± 0.05
Psychotria viridis + *Peganum harmala*	DMT	3.81 ± 0.61
	Harmalol	0.21 ± 0.03
	Harmol	0.09 ± 0.00
	THH	1.08 ± 0.08
	Harmaline	3.44 ± 0.66
	Harmine	3.91 ± 0.43
Mimosa hostilis + *Banisteriopsis caapi*	DMT	15.43 ± 0.48
	Harmalol	0.19 ± 0.00
	Harmol	0.41 ± 0.02
	THH	10.66 ± 0.32
	Harmaline	0.44 ± 0.01
	Harmine	7.26 ± 0.19
Mimosa hostilis + *Peganum harmala*	DMT	5.32 ± 0.19
	Harmalol	0.05 ± 0.03
	Harmol	0.40 ± 0.06
	THH	1.43 ± 0.01
	Harmaline	7.03 ± 0.24
	Harmine	5.78 ± 0.23

Note: DMT, *N*,*N*-dimethyltryptamine; THH, tetrahydroharmine.

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(68.9%–75.8%) compared to SPE, where these values varied between 45% and 58.4%.[37] Gaujac et al.[36] obtained better recoveries for DMT (71%–109%) with the application of SPME; however, it is important to mention that in that study only DMT was quantified. The same research group used MSPD as a technique for extracting DMT, again showing better recoveries for only one compound.[39]

It should be noted that despite presenting lower recoveries, the QuEChERS technique is more environmentally friendly compared to SPE, where larger volumes of organic solvents are used, and more economical, since it is not necessary to purchase single-use cartridges.[29] In contrast, although the SPME technique is a miniaturised technique, and uses smaller volumes of solvents, it requires the use of fibres, which, although reusable, are quite expensive.[29] The QuEChERS technique is a quick, simple, easy and economical approach, which does not require the acquisition of specific material, making it very advantageous.[29] However, we must bear in mind that we are dealing with a sample prepared with plant products, which may present variability in compounds between samples of different origins. Additionally, it should be noted that extraction efficiency can also be influenced by parameters such as sample viscosity or analyte solubility, which determines the success of the sample filtration step.

3.4 | Method applicability

The developed methodology was successfully applied to four decoctions of plants used in the ayahuasca preparation, one decoction of a commercial mixture and four decoctions of two mixtures of plants. The results are presented in Table 6.

As far as we know, this is the first study where three sample pretreatment techniques were compared and where QuEChERS were used for extracting ayahuasca constituents from beverages. This analytical approach can be considered advantageous for analytical laboratories, since it is easy, low cost and uses a reduced quantity of organic solvents.

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DATA AVAILABILITY STATEMENT

Data are available in this article.

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