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Short communication

Determination of *N*,*N*-dimethyltryptamine in beverages consumed in religious practices by headspace solid-phase microextraction followed by gas chromatography ion trap mass spectrometry

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ABSTRACT

A novel analytical approach combining solid-phase microextraction (SPME)/gas chromatography ion trap mass spectrometry (GC-IT-MS) was developed for the detection and quantification *N*,*N*-dimethyltryptamine (DMT), a powerful psychoactive indole alkaloid present in a variety of South American indigenous beverages, such as ayahuasca and *vinho da jurema*. These particular plant products, often used within a religious context, are increasingly consumed throughout the world following an expansion of religious groups and the availability of plant material over the Internet and high street shops. The method described in the present study included the use of SPME in headspace mode combined GC-IT-MS and included the optimization of the SPME procedure using multivariate techniques. The method was performed with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber in headspace mode (70 min at 60 °C) which resulted in good precision (RSD < 8.6%) and accuracy values (71–109%). Detection and quantification limits obtained for DMT were 0.78 and 9.5 mg L^{-1}, respectively and good linearity (1.56–300 mg L^{-1}, r^2=0.9975) was also observed. In addition, the proposed method showed good robustness and allowed for the minimization of sample manipulation. Five jurema beverage samples were prepared in the laboratory in order to study the impact of temperature, pH and ethanol on the ability to extract DMT into solution. The developed method was then applied to the analysis of twelve real ayahuasca and *vinho da jurema* samples, obtained from Brazilian religious groups, which revealed DMT concentration levels between 0.10 and 1.81 g L^{-1}.

1. Introduction

Ayahuasca is an indigenous brew produced as a decoction using the leaves of chacrona (*Psychotria viridis*) and sections of the stem of the yage vine (*Banisteriopsis caapi*) which originates from the Amazon region. *Vinho da jurema*, commonly referred to as jurema wine probably due to its visual similarity with the ordinary red wine, is also an indigenous brew but prepared with both root and stem barks of the *jurema preta* tree (*Mimosa tenuiflora*) from the arid Northeast of Brazil [1]. Both are used worldwide by various religious groups and in neo-shamanic urban rituals. Brazilian legislation permits the consumption of ayahuasca within a religious context and may also include pregnant women and children provided parental consent is given [2].

Previous studies based on gas chromatography (GC) have been reported for the determination of DMT in ayahuasca matrices which employed liquid–liquid extraction (LLE) [3–5] and solid phase extraction (SPE) procedures [6]. Sample preparation techniques based on LLE can be manually intensive, often involve large amounts of toxic organic solvents and may be time-consuming [7], in addition to the risk of analyte loss. A reliable alternative approach is the use of solid-phase microextraction (SPME). SPME is a solvent-free sample preparation technique that reduces sample preparation requirements and allows both extraction and concentration to be achieved in a single step [8].

There are several SPME applications in chemical analysis, bioanalysis, food and environmental sciences, and a growing number of publications describing pharmaceutical and medical studies [9]. The aim of this study was to evaluate the performance

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of a new solid phase microextraction method for the determination of DMT content in ayahuasca and *vinho da jurema* samples, using multivariate optimization techniques as factorial design and central composite design to identify the critical points involved in the SPME extraction procedure. To the best of the authors' knowledge, this is the first study based on a SPME procedure followed by gas chromatography mass spectrometry (GC–MS) to determine DMT levels present in twelve real ayahuasca and *vinho da jurema* samples, and five jurema beverage samples prepared in the laboratory.

2. Experimental

2.1. Standards, reagents and supplies

Methanol was of HPLC grade (Sigma-Aldrich, Gillingham, Dorset, UK). Analytical grade anhydrous sodium hydroxide was supplied by Merck (Darmstadt, Germany). Chemicals were used as received and without further purification. A manual solid phase microextraction (SPME) holder and fiber, coated with polydimethylsiloxane/divinylbenzene (65 μm, PDMS/DVB), was acquired from Supelco (Bellefonte, PA, USA). Prior to use, the fiber was conditioned in accordance with the manufacturer's recommendations. A magnetic stirrer (Fisher Scientific, Pittsburg, PA, USA) was used to homogenize beverage samples prior to SPME. *N,N*-Dimethyltryptamine (DMT) (95.8% ± 0.6) was isolated from *M. tenuiflora* inner barks, using a previously published method [10]. This standard was used in a previous work to quantify DMT in the barks of *M. tenuiflora*, by matrix solid-phase dispersion followed by GC–MS [11]. A total number of twelve ayahuasca and jurema beverage samples were obtained from Brazilian religious groups.

2.2. Preparation of DMT standard solutions and beverage samples

A working standard of 100 mg L^{-1} DMT was prepared by addition of 50 μL DMT standard stock solution (10 g L^{-1} in methanol) to 5 mL of 0.001 mol L^{-1} NaOH solution (pH 11). Standard solutions were prepared similarly with spiked stock solution, leading to concentration standards between 0.78 to 400 mg L^{-1}. These alkaline standard solutions were stored at 4 °C, and were stable for a period of at least 2 weeks. The real beverage samples were prepared by either 10- or 25-fold dilution of ayahuasca or *vinho da jurema* samples using a 0.001 mol L^{-1} NaOH aqueous solution. The final volume was 5 mL. 50 μL of methanol was added and the pH was corrected to pH 11, using small crystals of sodium hydroxide and a pH meter. These real samples were analyzed immediately after preparation.

2.3. SPME procedure

A 5 mL beverage sample was added to a 9 mL headspace vial and sealed with a cap containing a PTFE-faced silicone septum. The vial was placed into an aluminum block to ensure uniform heating at 60 °C. The sample was stirred (900 rpm) for 10 min to ensure thermal equilibration. The SPME syringe needle penetrated the vial septum, and the PDMS/DVB (65 μm) fiber was then lowered into the headspace located above the sample solution. The extraction time was 70 min followed by removal of the SPME fiber and insertion into the GC injection port for a desorption (and cleaning) period of 5 min. Statistical procedures were performed using Statistica 8.0 (StatSoft, Tulsa, USA).

2.4. GC-ion trap-MS system and operating conditions

GC–MS analysis was performed using a Varian 450-GC gas chromatograph (Walnut Creek, CA, USA) coupled to a Varian 200-MS ion trap (IT) mass spectrometer. The 1177 injector was operated in splitless mode for 60 s and heated at 250 °C. A straight SPME liner (L × o.d. × i.d., 105 × 2.75 × 0.75 mm) was used for sample introduction. The system was operated by the Saturn GC/MS Workstation, version 6.9. Separation was carried out on a Supelco SLB-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) purchased from Supelco (Bellefonte, PA, USA). Helium (purity 99.995%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 50 °C (held for 3.5 min) to 280 °C at 20 °C min^{-1} (held for 10 min). The ion trap mass spectrometer was operated in the electron ionization (EI) mode. Manifold, ion trap, ion source and transfer line temperatures were maintained at 80, 220, 300 and 300 °C, respectively. Helium was also used as damping gas at a flow of 0.8 mL min^{-1}. In the full scan mode the mass range was varied from *m/z* 40 to 400 at 0.6 s $scan^{-1}$. The identification of DMT in beverages (GC retention time and mass spectral comparison) was verified with reference material. For quantification purposes the scan range was restricted to *m/z* 57–59 which reflected the dominating abundance of the *m/z* 58 iminium ion in the mass spectrum of DMT.

3. Results and discussion

3.1. Optimization of the SPME method

Initially, a sample of herbal beverage was used to determine the optimum sample volume (5 mL), desorption time (5 min) and pH value (pH 11), respectively, which were carried out by univariate analyses. The PDMS/DVB fiber was well suited for analyzing volatile and semi-volatile compounds of medium polarity. In order to select the optimal experimental conditions for extraction, a multivariate optimization strategy was employed to assess the influence of the main factors on the SPME procedure. Several tests were carried out in order to select the factors and the domain to be considered in the multivariate experimental approach to maximize the yield of DMT extracted from beverages, and to obtain a good precision for the method. The factors included in the 2^3 factorial design (Table 1) were temperature (*T*), equilibrium time (t_{EQU}) and extraction time (t_{EXT}), respectively. The relationship between each investigated variable and impact of possible cross effects on DMT signal response was determined by using the Pareto graph (Fig. 1). Analysis of the Pareto graph indicated that, within the studied domain, the impact of temperature and extraction time was significant. The temperature was fixed at 60 °C. In order to find the critical factors of the sample preparation method, a response surface technique was employed as a central composite design. Although equilibration time factor did not appear to show any significant influence in the domain previous studied (15–25 min), it was decided to include this in the central composite design with a new domain, ranging between 5 and 15 min. The extraction time was also

Table 1
Factors and levels for 2^3 factorial design.

Factor	Low level (-)	Central point (C)	High level (+)
Temperature (T,°C)	40	50	60
Equilibrium time (t_{EQU}, min)	15	20	25
Extraction time (t_{EXT}, min)	20	35	50

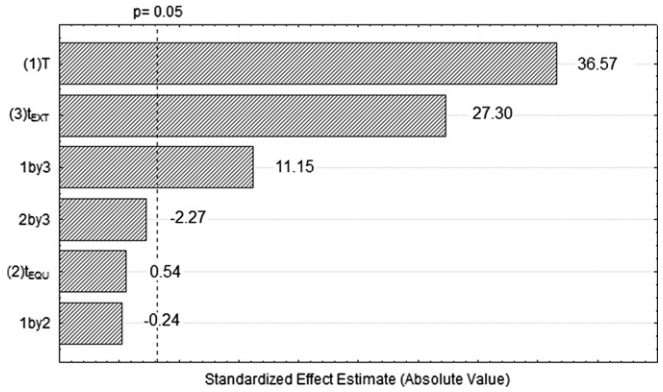

Fig. 1. Pareto chart used to describe the optimization of SPME variables.

Table 2
Factors and levels for central composite design.

Factor	Low level (-)	Central point (C)	High level (+)
Equilibrium time (t_{EQU}, min)	5	10	15
Extraction time (t_{EXT}, min)	20	50	80

evaluated in the central composite design, which considered time intervals varying from 20 to 80 min (Table 2). The response surface model generated by the execution of the central composite design matrix confirmed that the equilibrium time factor had no impact on the peak area of DMT (Fig. 2). For this factor, it was adopted on the central point level, 10 min, long enough to ensure that the fiber reaches thermal equilibrium with the sample medium, at 60 °C, before exposition on the headspace. The extraction time factor, on the other hand, had a major influence on the signal response and the surface (Fig. 2) revealed that chemical equilibrium was reached after 60 min, in the region where maximum DMT peak area values were obtained. In order to verify the prediction a set of extraction time experiments were carried (5–110 min) while maintaining all other factors unchanged, i.e. pH 11, equilibrium time 10 min, desorption time 5 min and temperature 60 °C. The results are presented in Fig. 3. Following this evaluation an extraction time of 70 min was chosen in order to achieve improved method robustness. In the all optimization designs, triplicate analyses were carried out at the central point of the studied domains in order to provide statistically meaningful figures.

3.2. Method validation

3.2.1. Linearity

The DMT calibration curve was prepared using twelve concentration levels between 1.56 and 300 mg L^{-1} using the solution standards described on Section 2, and duplicate analysis per concentration level. The slope and intercept values, together with their standard deviations (see below), were determined using regression analyses which yielded a correlation coefficient for DMT of $r^2=0.9975$.

3.2.2. Accuracy

To determine the accuracy of the developed method, a recovery study was carried out using beverage samples and the standard addition method. In this case, a known amount of isolated DMT was added to the sample at three different concentration levels, i.e. 9.5, 50 and 152 mg L^{-1}, respectively. Each concentration level analyzed in triplicate employing seven samples and the results were expressed as mean recovery and %RSD.

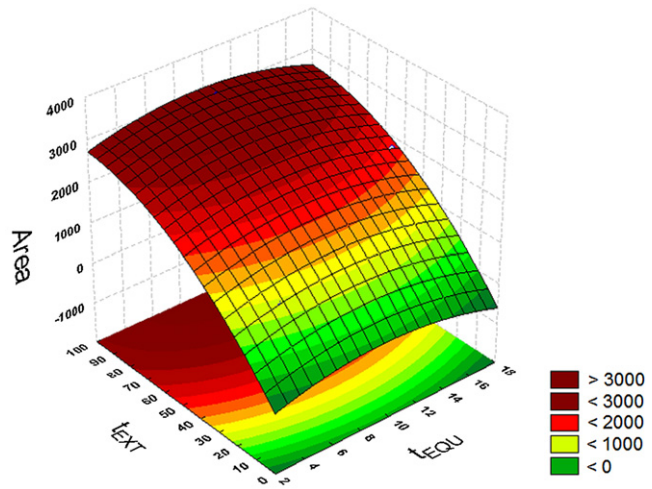

Fig. 2. Surface response model.

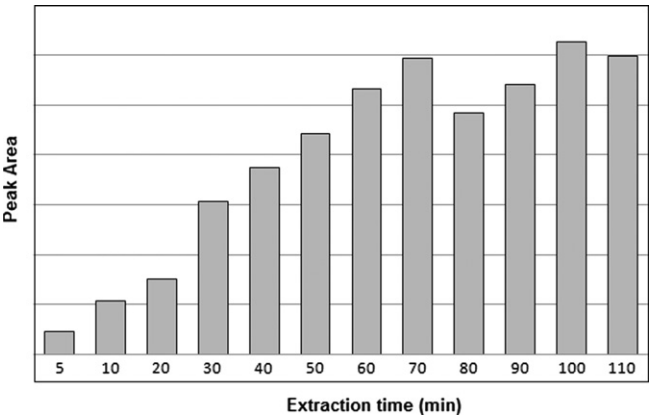

Fig. 3. Effect of extraction time on DMT peak area.

Under standard addition conditions, consistent and high recovery values were obtained and mean absolute recovery values for DMT were found to range between 71 and 109%. This recovery study indicated that the method was suitable for the determination of DMT from herbal preparations.

3.2.3. Precision

The precision of the method was determined by repeatability studies and expressed as relative standard deviation (%RSD). The repeatability (intra-assay precision) was measured by comparing standard deviation values obtained from recovery percentages derived from spiked samples (concentration levels 9.5, 50 and 152 mg L^{-1}) that were run on the same day. Each concentration level was determined seven times followed by RSD calculations. The RSD values for DMT levels were found to be below 8.3% at all spiked levels and considered suitable.

3.2.4. Limits of detection and quantification

The limit of detection (LOD) was calculated considering the standard deviation of the analytical noise (a value seven times the standard deviation of the blank) and the slope of the regression line, and it was equal to 0.78 mg L^{-1}. The limit of quantification (LOQ) was determined as the lowest concentration that provided a response of ten times the average of the baseline noise, and were calculated using seven unfortified samples. The LOQ value for this compound was 9.5 mg L^{-1}.

3.2.5. Robustness

The robustness of this proposed method was estimated by testing the reliability of analysis with respect to small but deliberate variation of optimized method parameters. A three-factor face-centered design consisting of fifteen experiments was employed. The extraction time (69.5–70.5 min), temperature (59–61 °C) and pH of aqueous phase (pH 10.5–11.5) were considered critical factors. Pareto graph plots (Fig. 4) indicated that the obtained response remained unaffected by small changes in these critical method parameters. Statistical analysis revealed that there was good agreement between experimental and predicted values. Furthermore, none of the factors studied in these domains showed significant effect on system efficiency.

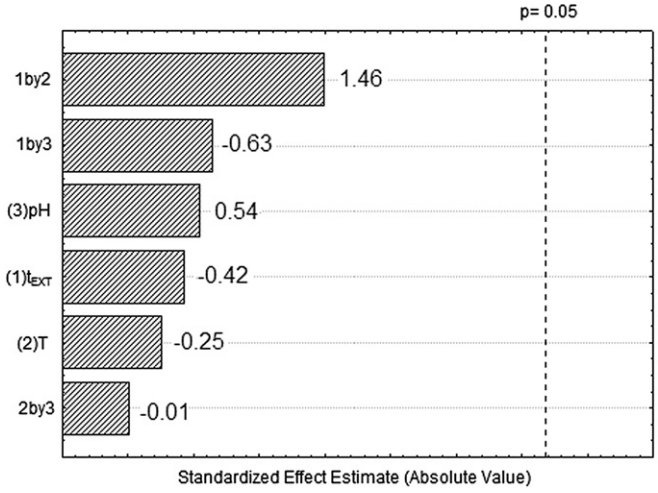

Fig. 4. Evaluation of robustness using the Pareto chart.

Table 3
DMT concentration in jurema beverage samples prepared in the laboratory under different conditions.

	Jurema beverage sample preparation condition	DMT conc. (g L^{-1})
1	Extraction into water at room temperature	1.32
2	Extraction into acidified water (HCl, pH=1) at room temperature	1.57
3	Extraction into water at 100 °C	1.17
4	Extraction into acidified water (HCl, pH=1) at 100 °C	1.79
5	Extraction into water:ethanol (50:50, v/v) at room temperature	1.73

3.2.6. Estimation of DMT content in jurema beverages

The analytical procedure described above was also used to study the influence of several parameters typically involved in the preparation of jurema products from plant material on DMT content. This included an assessment of temperature (room temperature Vs. 100 °C), pH of aqueous phase and the percentage of ethanol in the aqueous extraction medium. Thus, five jurema beverages samples were prepared in the laboratory using 5 g of inner bark of *jurema preta* (*M. tenuiflora*) per 100 mL of sample, employing the same time of extraction. The concentrations of DMT found in the analyzed samples are summarized in Table 3. For example, it was found that simply heating the decoction during preparation did not lead to increased DMT levels, however, concentrations of DMT did increase when an aqueous acid medium (pH 1), or a mix of water and ethanol (50:50, v/v), was employed. All samples were analyzed in triplicate.

Table 4
DMT levels in ayahuasca (A) and *vinho da jurema* (J) preparations obtained from Brazilian religious groups.

Samples	DMT conc. (g L^{-1})
A1	0.44
A2	1.14
A3	0.58
A4	0.57
A5	0.72
A6	0.29
A7	0.17
J1	1.76
J2	1.81
J3	0.73
J4	0.10
J5	0.68

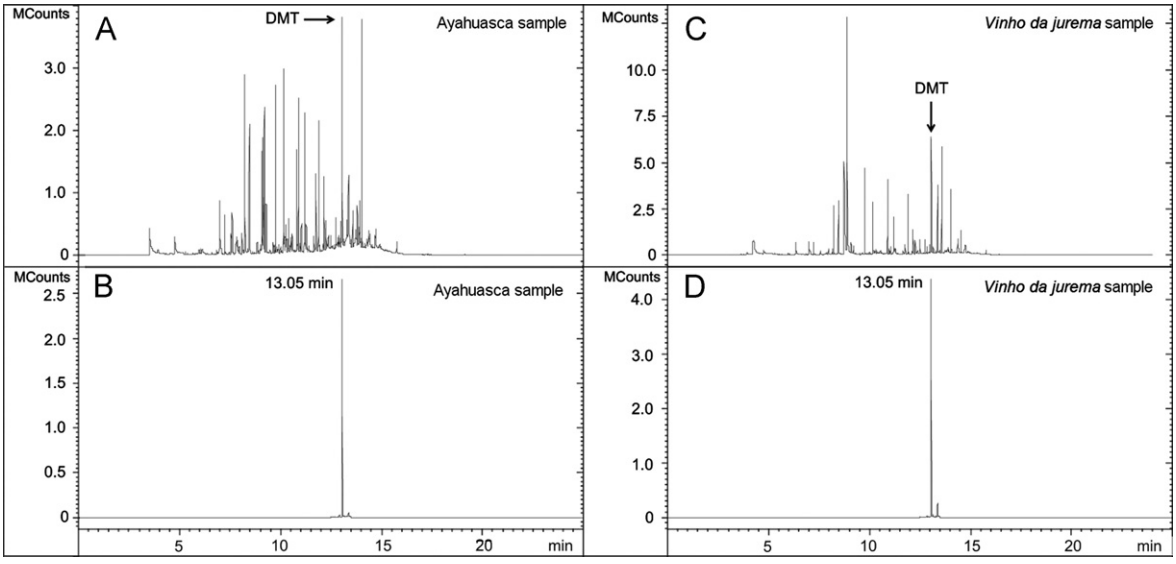

Fig. 5. Representative SPME GC-ion trap-MS traces obtained from real samples of ayahuasca (A and B) and vinho da jurema (C and D). A and C: full scan mode; B and D: quantification of DMT (13.05 min) at a reduced scan range between *m/z* 57–59 which reflected the formation of the *m/z* 58 iminium ion base peak.

3.3. Application of the method

Twelve samples of ayahuasca (A1–A7) and *vinho da jurema* samples (J1–J5) were collected from different Brazilian religious groups and analyzed in triplicate by using the developed SPME/GC-IT-MS method. All samples were diluted by a factor of 10 or 25, depending on the level of DMT present in the beverages. Representative chromatograms are shown in Fig. 5. High levels of DMT were found in both sample types. The DMT concentration in the *vinho da jurema* samples ranged from 0.10 to 1.81 g L^{-1}, whereas ayahuasca products revealed the presence of DMT in the range of 0.17 to 1.14 g L^{-1}, (Table 4), which was consistent with previous reports on liquid samples [1].

4. Conclusions

The present study provided a new method for the determination of DMT in ayahuasca and *vinho da jurema* matrices based on headspace solid-phase microextraction gas chromatography ion trap mass spectrometry. The optimization of SPME-related parameters were carried out by multivariate techniques and provided excellent figures of merit. The fact that it was possible to work with a small sample size and that the extent of sample manipulation was minimized, made the SPME/GC–MS technique particularly useful. There were considerable variations in DMT levels detected in ayahuasca and *vinho da jurema* samples obtained from Brazilian religious groups.

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