



Short communication

Determination of *N,N*-dimethyltryptamine in *Mimosa tenuiflora* inner barks by matrix solid-phase dispersion procedure and GC–MSAlain Gaujac^{a,c}, Adriano Aquino^d, Sandro Navickiene^d, Jailson Bittencourt de Andrade^{a,b,*}^a Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, s.210-214, 40170-115 Salvador-Ba, Brazil^b Instituto Nacional de Ciência e Tecnologia, Centro Interdisciplinar de Energia e Ambiente, Campus Universitário de Ondina, 40170-115 Salvador-Ba, Brazil^c Instituto Federal de Educação, Ciência e Tecnologia de Sergipe, Br 101, Km 96, 49100-000 São Cristóvão-Se, Brazil^d Departamento de Química, Universidade Federal de Sergipe, Av. Marechal Rondon, s/n, 49100-000 São Cristóvão-Se, Brazil

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ABSTRACT

N,N-dimethyltryptamine (DMT) is a potent hallucinogen found in beverages consumed in religion rituals and neo-shamanic practices over the world. Two of these religions, Santo Daime and União do Vegetal (UDV), are represented in countries including Australia, the United States and several European nations. In some of this countries there have been legal disputes concerning the legalization of ayahuasca consumption during religious rituals, a beverage rich in DMT. In Brazil, even children and pregnant women are legally authorized to consume ayahuasca in a religious context. A simple and low-cost method based on matrix solid-phase dispersion (MSPD) and gas chromatography with mass spectrometric detection (GC–MS) has been optimized for the determination of *N,N*-dimethyltryptamine in *Mimosa tenuiflora* inner bark. The experimental variables that affect the MSPD method, such as the amounts of solid-phase and herbal sample, solvent nature, eluate volume and NaOH concentration were optimized using an experimental design. The method showed good linearity ($r=0.9962$) and repeatability (RSD < 7.4%) for DMT compound, with detection limit of 0.12 mg/g. The proposed method was used to analyze 24 samples obtained locally. The results showed that concentrations of the target compound in *M. tenuiflora* barks, ranged from 1.26 to 9.35 mg/g for these samples.

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1. Introduction

N,N-Dimethyltryptamine is a psychedelic agent widely present in plants [1]. DMT is contained in ayahuasca and in jurema wine, indigenous beverages made with some plants species and consumed in syncretic religions and neo-shamanic rituals. Nowadays, the use of ayahuasca has become increasingly popular in South America, especially in Brazil, North America and Europe [2,3]. Some species of the Mimosoideae botanical subfamily are considered to be amongst the most potent plant sources of *N,N*-dimethyltryptamine. *Mimosa tenuiflora* or “jurema-preta” (black jurema) is a small tree whose barks are used as the main ingredient in jurema wine [3,4].

The extraction procedure is a critical step in the determination of drugs, pollutants and naturally occurring substances in herbal samples. In general, the determination of these compounds including drugs in plant matrices is accomplished using chromatographic

techniques and involves preliminary steps including sampling, extraction and clean-up [5]. Matrix solid-phase dispersion (MSPD) is a method that provides a good alternative to traditional extraction techniques for chromatographic analysis. MSPD can be carried out simultaneously with sample homogenization, extraction and clean-up and requires only a small sample size and small amounts of solvent [6]. It avoids the drawbacks generally associated with liquid–liquid extraction, such as the use of large volumes of solvent, the occurrence of troublesome emulsions, and slow speed [7–9]. Thus, MSPD is an analytical technique used for extraction of analytes from semi-solid and viscous samples.

The principle of the MSPD technique is based on the use of the same bonded-phase solid supports as used in solid-phase extraction (SPE), which are also used as grinding materials for disruption of the sample matrix. During this procedure, the bonded-phase support acts as an abrasive, and the sample disperses over the surface of the support. The classical methods used for sample disruption, such as mincing, shredding, grinding, pulverizing and pressuring are avoided in this procedure, and the MSPD technique has many applications to the processing of samples of biological origin (animal tissues, plant materials, fats, etc.) [10–12]. The sample is placed in a mortar, together with a bonded phase material, and the mixture is then crushed with a pestle. Recently, some tryptamines

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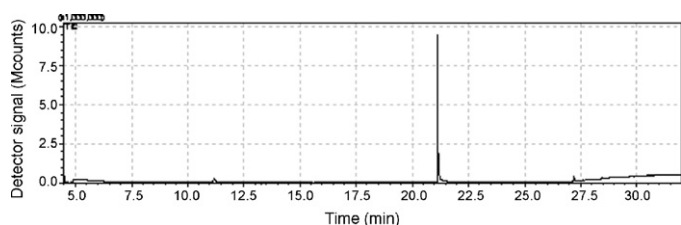


Fig. 1. GC–MS (SCAN mode) chromatogram of DMT isolated from *M. tenuiflora*.

have been studied by GC–MS, HPLC and LC–MS in different matrices [1,2,13–26]. To the best of our knowledge no publication has documented the use of MSPD procedure followed by GC–MS to determine DMT in *M. tenuiflora* barks.

The present work reports a simple method for determination of *N,N*-dimethyltryptamine (DMT) in tissues of *M. tenuiflora* by matrix solid-phase dispersion and gas chromatography–mass spectrometry.

2. Experimental

2.1. Chemicals, reagents and supplies

GC grade solvent *n*-hexane was purchased from Tedia (Fairfield, OH, USA). Analytical grade anhydrous sodium sulfate and Florisil® (80–100 mesh) were supplied from Mallinckrodt Baker (Paris, KY, USA). Analytical grade sodium hydroxide was obtained from Vetec (Duque de Caxias, Brazil).

2.2. Collection and preparation of plant material

Stem bark from *M. tenuiflora* was collected fresh from local habitats, being in two humid coastal counties in municipalities of Aracaju and São Cristóvão (Location A) and three semi-arid regions, municipalities of Simão Dias, Pinhão and Canindé de São Francisco (Location B), all located in the State of Sergipe, Northeast region of Brazil, between August and September 2010. Voucher specimens of these samples have been deposited in the herbarium of the Federal University of Sergipe (ASE18817). Bark samples were powdered by a cutting mill, sieved and dried at 38 °C to a constant mass before use.

2.3. Isolation of *N,N*-dimethyltryptamine from *M. tenuiflora*

Due to the difficult in acquiring a commercial standard of DMT, a method was developed to isolate this compound from the *M. tenuiflora* inner barks. The purity of the *N,N*-dimethyltryptamine obtained was equal to 95% with regard to UV analysis. An aliquot of the isolated compound was analyzed by GC–MS in full scan mode (SCAN) and showed a prominent peak at 21.2 min, Fig. 1. To confirm the identity of the compound, a ¹H and ¹³C NMR spectra of the isolated chemical were generated and are in agreement with others previously described for the DMT in the literature [2,21,26,27].

2.4. Preparation of standard solutions

Standard solutions of DMT were prepared by dissolving 100 mg of DMT in 10 mL acetonitrile to yield concentration of 10.0 mg/mL. Calibration solutions were prepared in a blank stem bark of the *Anadenanthera colubrina* (Vell.) Betran, which presents a chromatographic profile very similar to *M. tenuiflora*, Fig. 2, by adding respective spiking solution to blank extract, to produce final concentrations varying of 0.62–20.0 mg/g. Besides, these two species belong to the same botanical subfamily Mimosoideae, showed

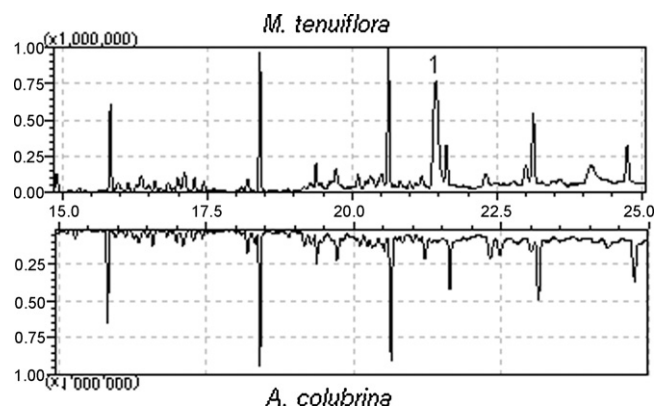


Fig. 2. Comparison chromatograms of *M. tenuiflora* and *A. colubrina* real samples analysis by MSPD/GC–MS (SCAN mode). The numbered peak is *N,N*-dimethyltryptamine.

inner barks with physical properties in good agreement and, according to previous studies, alkaloids were not detected in the inner barks of *A. colubrina* [28]. These standard solutions were stored at 4 °C in the dark, and were stable for a period of at least 1 month.

2.5. MSPD procedure

An aliquot of herbal sample (0.5 g) was placed into a mortar (*ca.* 50 mL), and 0.5 g of Florisil and 0.5 mL of NaOH 0.1 mol/L were added. The sample was then gently blended into the sorbent material with a pestle, until a homogeneous mixture was obtained (*ca.* 3 min). The homogenized mixture was introduced into a 100 mm × 20 mm id polypropylene column, filled with 0.1 g of glass wool at the base and 0.5 g of anhydrous Na₂SO₄. The elution was performed under vacuum with 30 mL of *n*-hexane. The eluent was collected into a round bottom flask and concentrated using a rotary vacuum evaporator (40 °C), and finally purged with a gentle stream of nitrogen to a volume of 1 mL. An aliquot of 1 μL was analyzed by GC–MS.

2.6. GC–MS system and operating conditions

A Shimadzu (Kyoto, Japan) system consisting of a QP-2010 Plus mass spectrometer coupled to a GC 2010 gas chromatograph, with a Shimadzu AOC 20i autosampler and a split/splitless injector, was used for the identification and quantification of DMT. A fused-silica RTX-5MS column (5% phenyl–95% polydimethylsiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness), supplied by Restek (Bellefonte, PA, USA), was employed, with helium (purity 99.995%) as carrier gas at a flow rate of 1.2 mL/min. The GC oven temperature was programmed from 60 °C (3.0 min), then at the rate of 8 °C/min to 200 °C and directly to 280 °C (4 min) at 10 °C/min. The solvent delay was 5 min. The injector port was maintained at 250 °C, and 1 μL sample volumes were injected in splitless mode (50 s). The data were acquired and processed on a personal computer, using Shimadzu GC Solution software. The total analysis time was 27 min, and the equilibration time was 2 min. The eluent from the GC column was transferred, via an interface line heated to 280 °C, into the 70 eV electron ionization source, also maintained at 280 °C. The analysis was performed in the selected ion monitoring (SIM) mode. The ions monitored were *m/z* 58 and 188.

2.7. Recovery studies

A 0.5 g homogenized *A. colubrina* sample was spiked prior to the determination procedure by addition of a DMT standard solution to

Table 1
Factors and levels in the optimization of MSPD procedure.

Factors	Low level	High level
(1) Eluent (E)	Petroleum ether	n-Hexane
(2) Support (S)	Aluminium oxide	Florisil®
(3) Eluent volume (V_E) (mL)	10	30
(4) Support mass (m_S) (g)	0.5	2.0
(5) Sample mass (m_{SA}) (g)	0.5	2.0
(6) NaOH concentration (C_{OH^-}) (mol/L)	0.01	0.1

give 1.25, 2.0, 8.0 and 16.0 mg/g ($n=8$ replicates). Spiking samples were left to stand for around 24 h to allow DMT absorption onto the sample.

3. Results and discussion

3.1. Optimization of the MSPD procedure

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 MSPD procedure. Tests were then carried out in order to select the factors and the domain to be considered in the multivariate experimental approach, Table 1. The factors included in the factorial design were eluent (E) support (S), eluent volume (V_E), support mass (m_S), sample mass (m_{SA}) and NaOH concentration (C_{OH^-}). Statistical procedures were performed using Statistica 8.0 (StarSoft, Tulsa, USA). Analysis of the Pareto graph, Fig. 3, for the DMT compound demonstrated that all factors within the studied domain were significant, with the exception of the eluent, support mass and sample mass, whose values were insignificant in this domain studied. In this case, 0.5 g of support mass and 0.5 g of sample mass were selected, once an additional amount of these factors did not improve the recovery. Based on the preliminary assays, Florisil® and aluminium oxide were selected to compare their performance using petroleum ether and n-hexane as eluting solvents. Although the same results in terms of recovery were obtained, which were around 70% for the two adsorbents, Florisil® and n-hexane were chosen for producing the cleanest chromatographic profiles with lower baselines than the other solid-phase and solvent. The eluent volume was the most significant factor, which considered volumes varying from 10 to 30 mL, with best results (chromatographic peak area of DMT) in the highest level. Besides, NaOH concentration giving the highest responses with Florisil® based on the MSPD column, in the higher level of concentration, 0.1 mol/L.

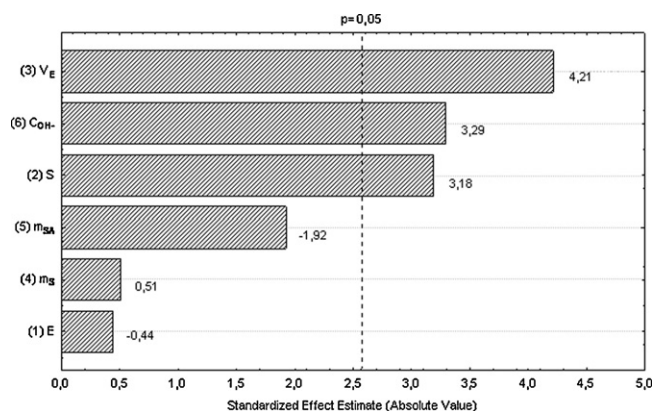


Fig. 3. Experimental design Pareto graph.

3.2. Method validation

3.2.1. Linearity

The linearity of a method is a measure of the range within which detector response is directly proportional to the concentration of the analyte in standard solutions or samples. The linearity for the compound was determined using blank herbal samples fortified at concentration levels ranging from 0.62 to 20.0 mg/g. The slope and intercept values, together with their standard deviations, were determined using regression analyses. Linear regression coefficient for the DMT was equal to 0.9962.

3.2.2. Recovery

DMT compound was extracted from *A. colubrina* by MSPD procedure. Herbal samples were fortified at 1.25, 2.0, 8.0 and 16.0 mg/g before extraction by adding 0.5 mL of the appropriate working standard solution. Eight replicates spiked at each fortification level were assayed. The recovery obtained for DMT ranged from 81.7 to 116.2%.

3.2.3. Repeatability

The precision of the method was determined by repeatability and intermediate precision studies, and expressed by the relative standard deviation (RSD). The repeatability (intra-assay precision) was measured by comparing standard deviation of the recovery percentages of spiked herb samples at five concentration levels (1.25, 3.75, 5.0, 10.0 and 15.0 mg/g) run the same day. The samples were injected 8 times with manual injection and the relative standard deviation values obtained for the retention times were lower than 7.4%. The intermediate precision (as between-day precision) was determined by analyzing spiked herb samples at two concentration levels (8.0 and 16.0 mg/g), besides a real sample, for three alternate days by two different analysts. Replicated ($n=8$ for each concentration level) samples were all run and the RSD value was calculated for the DMT compound. The method was found precise (RSD < 16.8%) for DMT compound studied at all spiking levels. The different origins of the samples did not influence the instrumental response, and routine clean-up of the insert and/or ion source box was sufficient to maintain system performance.

3.2.4. Accuracy

The accuracy tests were performed comparing the results obtained using the traditional acid–base method that employs liquid–liquid for alkaloid extraction, with the data obtained using the proposed method, MSPD/GC–MS. The concentrations of DMT in the samples analyzed by the two methods were in good agreement. For a commercial sample, 8.10 mg/g (traditional LLE acid–base method) and 8.24 mg/g (MSPD/GC–MS method), and for the sample 1042R, 7.02 mg/g and 7.20 mg/g, respectively.

3.2.5. Detection and quantification limits

The limit of detection (LOD) of the method was 0.12 mg/g and was calculated considering the standard deviation of the analytical noise (a value of seven times the standard deviation of the blank) and the slope of the regression line. The limit of quantification (LOQ) was determined as the lowest concentration giving a response of ten times the average of the baseline noise, calculated using seven unfortified samples. The LOQ value for this compound was 1.25 mg/g [29].

3.3. Application of the method

A total of 24 *M. tenuiflora* inner bark samples were collected from different localities and analyzed by developing MSPD combined with GC–MS. The DMT concentration in the samples ranged from 1.26 to 9.35 mg/g (Table 2). High levels of DMT were mostly

Table 2
Concentration of DMT found in herb samples from humid coastal region (location A) and semi-arid region (location B).

Locations	Samples ^a	DMT concentrations in <i>M. tenuiflora</i> inner barks (mg/g)		
		Stem bark (S)	Root bark (R)	
Location A Aracaju-Se lat 11.071150°S lon 37.146190°W	0945S	3.19	6.86	
	1005R			
	1022S	1.35	7.20	
	1042R			
	1015S	6.41	5.93	
	1047R			
	0743S	3.47	6.67	
	0800R			
	0810S	2.04	5.97	
	0836R			
São Cristóvão-Se lat 10.905978°S lon 37.188713°W	0955S	2.16	5.29	
	1002R			
	0850S	2.15	1.26	
	0915R			
	0930S	4.00	6.87	
	0940R			
	Location B Pinhão-Se lat 10.588435°S lon 37.735462°W	1341S	7.55	–
		1408S	7.26	
		1138S	4.88	–
		1220S	9.35	
1232S		5.54		
1246S		4.42		
1130S		1.54	2.12	
1136R				
(specimen centenary)				
Canindé de São Francisco-Se lat 9.627350°S lon 37.807383°W				
Simão Dias-Se lat 10.717147°S lon 37.793817°W				

^a Samples of *M. tenuiflora* inner barks collected at different days between August and September 2010. The first four digits of sample codes refer to the time of the sample collection.

found in root tissue in comparison to the stem bark of *M. tenuiflora*. Stem barks obtained samples from semi-arid regions were richer in DMT than the coastal herbal samples. *M. tenuiflora* flowers and seeds samples were also analyzed by this method, but its DMT levels were below the limit of quantification. The results obtained were in agreement with a recent work involving Soxhlet extraction technique and HPLC separation with UV detection [30].

4. Conclusion

The proposed MSPD procedure followed by GC/MS (SIM) can be applied to determine DMT in tissues of *M. tenuiflora*. The method uses a Florisil® based on the MSPD column and *n*-hexane as elution solvent. The results demonstrate that the accuracy, precision

and selectivity of the proposed method are acceptable for the determination of DMT. In addition, the method requires a small sample size and offers considerable savings in terms of solvent consumption, cost of materials, sample manipulation and analysis time.

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