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Journal of Chromatography B, 779 (2002) 271–281

JOURNAL OF
CHROMATOGRAPHY B

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Determination of *N,N*-dimethyltryptamine and β -carboline alkaloids in human plasma following oral administration of *Ayahuasca*

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Received 12 March 2002; received in revised form 28 May 2002; accepted 30 May 2002

Abstract

Ayahuasca is a South American psychotropic beverage prepared from plants native to the Amazon River Basin. It combines the hallucinogenic agent and 5-HT_{2A/2C} agonist *N,N*-dimethyltryptamine (DMT) with β -carboline alkaloids showing monoamine oxidase-inhibiting properties. In the present paper, an analytical methodology for the plasma quantification of the four main alkaloids present in *ayahuasca* plus two major metabolites is described. DMT was extracted by liquid–liquid extraction with *n*-pentane and quantified by gas chromatography with nitrogen–phosphorus detection. Recovery was 74%, and precision and accuracy were better than 9.9%. The limit of quantification (LOQ) was 1.6 ng/ml. Harmine, harmaline, and tetrahydroharmine (THH), the three main β -carbolines present in *ayahuasca*, and harmol and harmalol (*O*-demethylation metabolites of harmine and harmaline, respectively) were measured in plasma by means of high-performance liquid chromatography (HPLC) with fluorescence detection. Sample preparation was accomplished by solid-phase extraction, which facilitated the automation of the process. All five β -carbolines were measured using a single detector by switching wavelengths. Separation of harmol and harmalol required only slight changes in the chromatographic conditions. Method validation demonstrated good recoveries, above 87%, and accuracy and precision better than 13.4%. The LOQ was 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. Good linearity was observed in the concentration ranges evaluated for DMT (2.5–50 ng/ml) and the β -carbolines (0.3–100 ng/ml). The gas chromatography and HPLC methods described allowed adequate characterization of the pharmacokinetics of the four main alkaloids present in *ayahuasca*, and also of two major β -carboline metabolites not previously described in the literature.

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Keywords: *Ayahuasca*; *N,N*-Dimethyltryptamine; β -Carboline alkaloids

1. Introduction

Ayahuasca is a psychotropic plant tea which has traditionally played a central role in the magico-religious practices and folk medicine of indigenous

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peoples native to the Amazon and Orinoco river basins [1,2]. In recent years, *ayahuasca* has become increasingly popular in Europe and North America, where many individuals interested in shamanic practices reportedly use it as a means to facilitate self-knowledge and introspection or as a sacramental drug in the context of syncretic religions [3]. As the use of this tea has spread outside its original geographic area reaching Europe and North America, health issues regarding the safety of its use have been raised, and clinical data on its general pharmacology are warranted.

The psychotropic properties of *ayahuasca* are attributed mainly to the fact that it contains measurable amounts of the hallucinogenic indole DMT, a serotonergic 5-HT_{2A/2C} agonist [4], such as LSD or psilocybin. DMT is known to elicit intense perceptual, cognitive and affective modifications when parenterally administered [5], but has been shown to be devoid of psychoactivity after oral ingestion [6], probably due to first-pass enzymatic degradation by monoamine oxidase (MAO) [6,7]. Interestingly, *ayahuasca* also contains levels of β -carboline alkaloids with MAO-inhibiting properties, which could explain the oral psychoactivity of the tea. The usual

elaboration process of *ayahuasca* involves the combination in a single beverage of two different plants, one of which contributes the orally labile DMT, and the other, the MAO inhibitors. Thus, the pounded stems of *Banisteriopsis caapi* (Malpighiaceae) are infused together with the leaves of *Psychotria viridis* (Rubiaceae). While *B. caapi* contributes varying amounts of five different MAO-inhibiting β -carbolines, i.e. harmine, THH, harmaline and trace amounts of harmol and harmalol [8,9], *P. viridis* is the source of the DMT [8,10]. The chemical structures of DMT, harmine, harmaline, harmol and harmalol are shown in Fig. 1. In vitro, harmine and harmaline, and to a lesser extent THH, potentially inhibit MAO [9], an effect which could prevent in vivo the oxidative deamination of the DMT present in the tea, allowing its access to systemic circulation and the central nervous system after oral ingestion.

Our research group has initiated a project directed at studying the pharmacology of *ayahuasca* in humans. It includes the assessment of the subjective, cardiovascular and neurophysiological effect profile of the beverage in healthy volunteers. In a clinical research setting, *ayahuasca* has demonstrated a

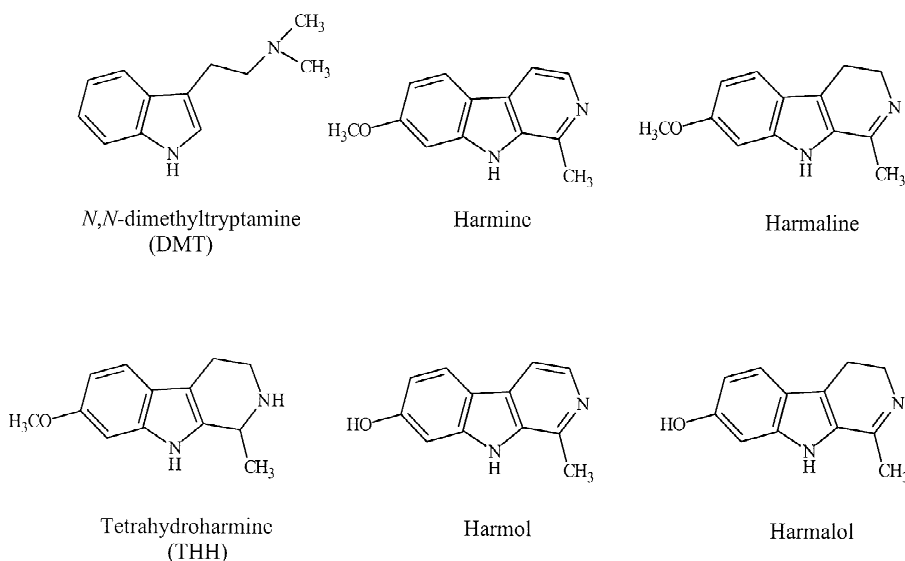


Fig. 1. Chemical structure of alkaloids from *P. viridis* (DMT) and *B. caapi* (harmine, harmaline and THH) typically found in *ayahuasca* brews. Harmol and harmalol are found in trace amounts in *B. caapi* and *ayahuasca* but appear in significant concentrations in human plasma samples following oral dosing with *ayahuasca*. These compounds are presumably formed in vivo by *O*-demethylation of harmine and harmaline, respectively.

combined stimulatory and hallucinogenic effect profile, as measured by subjective effect self-assessment instruments [11], and dose-dependent changes in spontaneous brain electrical activity, which parallel the time course of subjective effects and support the role of 5-HT_{2A/2C} and D₂ agonism in mediating the effects of *ayahuasca* [12]. In the present paper, we describe an analytical methodology which was developed to characterize the pharmacokinetics of *ayahuasca* alkaloids in humans following oral administration of the tea.

2. Experimental

2.1. DMT

2.1.1. Chemicals and reagents

DMT was generously provided by the United Nations International Drug Control Programme, Technical Services Branch, Laboratory Operations. Diphenhydramine and blank plasma were supplied by Uriach Laboratories (Barcelona, Spain) and the blood bank of Hospital del Mar (Barcelona, Spain), respectively. Methanol, *n*-pentane, potassium hydroxide and sodium chloride were reagent-grade and purchased from Merck (Darmstadt, Germany).

2.1.2. Instrumentation

A gas chromatograph equipped with a nitrogen-phosphorus detection system (HP5890 series II, Hewlett-Packard, Palo Alto, CA, USA) was used. Samples were injected in splitless mode (30 s of purge off time) into 5% phenyl-methylsilicone capillary column, 12 m×0.2 mm I.D. and 0.33- μ m film thickness (Ultra 2, Hewlett-Packard). Helium was used as carrier gas at a flow-rate of 0.7 ml/min (measured at 180 °C) and as make up gas at a flow-rate of 47 ml/min. Air and hydrogen detector flow were set at 80 and 5.5 ml/min, respectively. A temperature program for plasma DMT separation starting at 70 °C, was maintained for 1 min, and programmed to 120 °C at 30 °C/min, then to 280 °C at 20 °C/min; it was maintained for 1 min. Total run-time was 12 min. Injector and detector temperature were set at 280 °C.

2.1.3. Working standards

Working solutions of 1 μ g/ml of diphenhydra-

mine (internal standard) and DMT were prepared from a stock solution of 100 μ g/ml by dilution with methanol. All solutions were checked for purity on a routine basis. Standard solutions were stored at –20 °C until analysis.

2.1.4. Preparation of calibration curve and quality control samples

A calibration curve was prepared for each analytical batch. Appropriate volumes of working solutions were added to test-tubes containing 1.0 ml of drug-free plasma and vortexed vigorously. Final concentrations were 2.5, 5.0, 10, 25 and 50 ng/ml. Control plasma samples containing DMT were prepared at three different concentrations, low control 2.5 ng/ml, medium control 25 ng/ml and high control 50 ng/ml. They were kept frozen at –20 °C in 1.0-ml aliquots. Control samples were included in each batch in duplicate.

2.1.5. Sample preparation

Test and control samples were allowed to thaw at room temperature. Aliquots of 1 ml of plasma were pipetted into 15-ml screw-capped tubes and processed together with a calibration curve after addition of 25 ng of internal standard. Samples were treated by adding 0.5 ml of 5 M potassium hydroxide and 1.0 ml of sodium chloride saturated solution. They were then extracted with 5 ml of *n*-pentane for 20 min. The organic phase was separated and evaporated to dryness under a nitrogen stream at 25 °C. The residue was redissolved in 50 μ l methanol. Finally, 1.0 μ l was injected into the chromatographic system.

2.2. β -Carbolines

2.2.1. Chemicals and reagents

Harmine, harmaline, harmol, harmalol, propranolol and yohimbine were purchased from Sigma (St Louis, MO, USA). THH was synthesized and generously provided by Dr James C. Callaway, University of Kuopio, Finland. Blank plasma was supplied by the blood bank of Hospital de Sant Pau (Barcelona, Spain). Acetonitrile, methanol, triethylamine, ammonium acetate and sodium hydroxide were HPLC grade and purchased from Scharlab (Barcelona, Spain). Ultra pure water was obtained using a Milli-

Q purification system (Millipore, Molsheim, France). Boric acid was purchased from Panreac (Barcelona, Spain), glacial acetic acid from Riedel-de-Haën (Seelze, Germany), ammonia from Merck (Darmstadt, Germany), and 0.9% v/v saline solution from B. Braun Medical (Barcelona, Spain). Bond-Elut C₁₈ 100-mg solid-phase extraction cartridges and Vac-Elut SPE24 vacuum system were from Varian (Harbor City, CA, USA).

2.2.2. Instrumentation

Analysis by HPLC was carried out using an autosampler 717, pumps 515 and 510, a variable wavelength fluorescence 474 detector and Millenium³² acquisition and processing software, all from Waters (Milford, MA, USA). The analytical column was a Kromasil 100 C₁₈, 5 μ m, 150 \times 4 mm I.D., and the guard column was a C₁₈ 5 μ m, 10 \times 4 mm I.D., both purchased from Teknokroma (Barcelona, Spain).

The mobile phase for harmine, harmaline and THH consisted of solvent A which was a mixture (63:37 v/v) of ammonium acetate buffer 50 mM pH 8.0 and acetonitrile–methanol (20:30 v/v); and solvent B which was a mixture of acetonitrile–methanol (20:30 v/v). Gradient elution was initiated with 100% solvent A at a flow-rate of 0.8 ml/min for 6.5 min, then changed within 2 min to the following proportions: 68.3% solvent A and 31.7% solvent B and a flow-rate of 1.2 ml/min. These conditions were maintained for 7.5 min, to return to initial conditions thereafter. The fluorescence detector was operated at the following excitation/emission wavelengths: $\lambda=260$ nm/ $\lambda=370$ nm to measure THH, harmine and yohimbine (internal standard), and $\lambda=340$ nm/ $\lambda=495$ nm to measure harmaline.

The mobile phase for harmol and harmalol consisted of solvent A which was a mixture (73:27 v/v) of ammonium acetate buffer 50 mM pH 6.3 and acetonitrile–methanol (20:30 v/v); and solvent B which was a mixture of acetonitrile–methanol (20:30 v/v). Gradient elution was initiated with 100% solvent A at a flow-rate of 0.8 ml/min for 4.5 min, then changed within 1.5 min to the following proportions: 72% solvent A and 28% solvent B and a flow-rate of 1 ml/min. These conditions were maintained for 9 min, to return to initial values thereafter. The fluorescence detector was operated at excitation/

emission wavelengths of $\lambda=340$ nm/ $\lambda=495$ nm to measure harmol and harmalol, and $\lambda=260$ nm/ $\lambda=370$ nm to measure propranolol (internal standard).

2.2.3. Working standards

Working solutions of 100, 10, 1 and 0.1 μ g/ml of harmine and THH were prepared from stock solutions of 1 mg/ml of each alkaloid in methanol. The same working solutions plus 0.01 μ g/ml were prepared for harmaline from a stock solution of 1 mg/ml in methanol. A stock solution of 1 mg/ml yohimbine in methanol was prepared. A 10 μ g/ml solution in methanol was obtained by dilution and from this, a 100 ng/ml yohimbine in saline solution was prepared, which was later added to the samples.

Working solutions of 10, 1 and 0.1 μ g/ml of harmol and harmalol were prepared from stock solutions of 1 mg/ml of each alkaloid in methanol. A stock solution of 10 mg/ml of propranolol in methanol was prepared. A working solution of 1 mg/ml was obtained by dilution of the latter, and this was used to obtain a 50 ng/ml propranolol solution in saline, which was later added to the samples. All standard solutions were stored at +4 °C during sample analysis.

2.2.4. Preparation of calibration curves and quality control samples

Calibration curves were constructed for each of the five β -carbolines (three original alkaloids plus two metabolites). Blank plasma was spiked with working solutions of each of the five compounds. Final concentrations were 0.5, 1, 2, 5, 10, 25 and 50 ng/ml for harmine; 0.3, 0.5, 1, 2, 5, 10 and 25 ng/ml for harmaline; 1, 1.5, 3, 10, 25, 50 and 100 ng/ml for THH; 0.3, 0.5, 1, 2, 5, 10, 25, and 50 ng/ml for harmol; and 0.3, 0.5, 1, 2, 5, 10, 25 and 50 ng/ml for harmalol. Analyte concentrations were calculated by comparison with calibration curves. THH/yohimbine, harmine/yohimbine, harmaline/yohimbine, harmol/propranolol and harmalol/propranolol area ratios were used. Quality control samples were prepared at the following concentrations: 2, 5 and 25 ng/ml for harmine; 1, 2 and 10 ng/ml for harmaline; 3, 10 and 50 ng/ml for THH; 2, 10 and 25 ng/ml for harmol and harmalol. Spiked plasma samples at the following concentrations: 2 and 10 ng/ml for harmine; 1 and 5 ng/ml for

harmaline; 3 and 25 ng/ml for THH; 2 and 25 ng/ml for harmol and harmalol were prepared in the same way for the stability study.

2.2.5. Sample preparation

Calibration, quality control, stability control and test samples were allowed to thaw at room temperature. Six hundred microliter aliquots of an internal standard solution were added to 600 μ l of the samples. This was followed by gentle mixing and centrifugation for 10 min prior to solid-phase extraction. The cartridges were attached to the vacuum station and activated with 2 ml methanol and conditioned with 1 ml Milli-Q water and 2 ml borate buffer (50 mM) at pH 9.0. Next, 1 ml of a mixture

containing 0.5 ml of plasma and 0.5 ml saline solution with 100 ng/ml of yohimbine (for harmine, harmaline and THH) or 50 ng/ml of propranolol (for harmol and harmalol), were transferred into the cartridges. After aspiration of the entire volume through the cartridges, they were washed with 1 ml Milli-Q water, followed by 1 ml acetonitrile–water (10:90 v/v). The cartridges were then dried under full vacuum and eluted with 0.5 ml of a 0.1% triethylamine solution in methanol. The eluate was allowed to dry for 1.5 h, approximately. The solid residue was redissolved in 100 μ l mobile phase, vortexed for 10 s and placed in disposable microvials which were put in the autosampler. Sixty microliters were injected into the chromatographic system

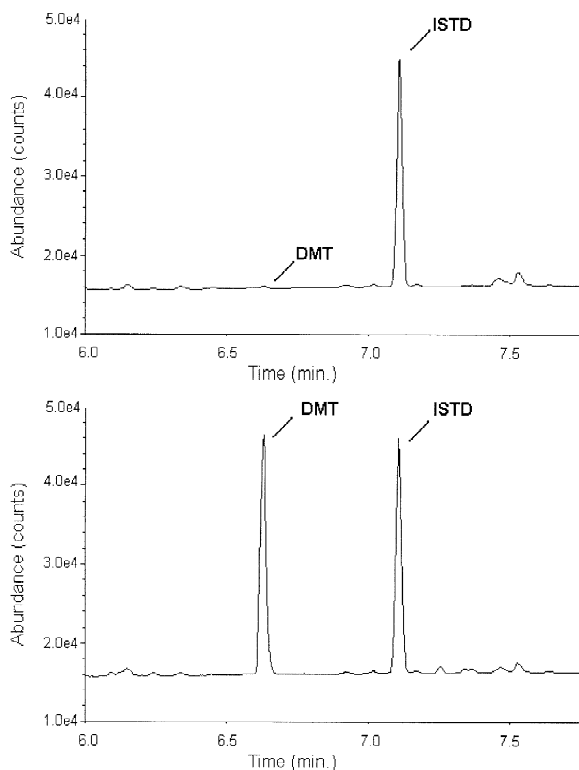


Fig. 2. Chromatogram obtained with gas chromatography with nitrogen–phosphorus detection of a plasma sample from a volunteer administered a 1 mg DMT/kg dose of *ayahuasca*. (A) Baseline plasma sample obtained just before drug administration. Expected retention time for DMT, and peak corresponding to the internal standard (ISTD). (B) Plasma obtained at 2.5 h after drug administration. The calculated concentration for DMT was 10.97 μ g/l.

3. Results

3.1. DMT

Fig. 2 shows a representative plasma chromatogram obtained after method application. No interfering peaks were observed at the elution time of analyte and the internal standard. The DMT retention time was 6.61 min.

3.1.1. Recovery

Extraction efficiencies for DMT and diphenhydramine were calculated by comparing the areas of the chromatographic peaks of equal concentrations of drug extracted and non-extracted. The experiment was carried out with concentrations of analytes (in duplicate) identical to those used for calibration. Recoveries for DMT and the internal standard in the concentration range studied were 74 ± 8.2 and $81 \pm 7.4\%$, respectively.

3.1.2. Linearity

Five concentrations ranging from 2.5 to 50 ng/ml were used to assess the linearity of the method. Results obtained from regression analysis of the theoretic concentrations versus ratio are shown in Table 1.

3.1.3. Precision, accuracy and robustness

Intra-assay precision and accuracy were determined by testing five replicates of blank plasma

Table 1

Linearity parameters of plasma determinations of DMT (gas chromatography) and β -carbolines (HPLC) obtained in the routine phase

	<i>n</i>	Intercept <i>x</i> ±SD	Slope <i>x</i> ±SD	Determination coef. <i>r</i> ² <i>x</i> ±SD
DMT	36	-0.0416±0.0484	0.0754±0.0152	0.9946±0.0032
Harmine	10	0.0051±0.0040	0.0189±0.0016	0.9929±0.0028
Harmaline	10	0.0036±0.0050	0.1115±0.0150	0.9916±0.0020
THH	10	0.0077±0.0069	0.0094±0.0013	0.9948±0.0018
Harmol	10	-0.0006±0.0011	0.0284±0.0021	0.9981±0.0005
Harmalol	10	-0.0005±0.0027	0.0526±0.0042	0.9964±0.0015

n, no. of days.

spiked with 5, 20, and 40 ng/ml (low, medium and high control samples). Each control sample was analyzed in 36 consecutive analytical batches over a 3-month period. Analytical batches were carried out by different scientists, thus emphasizing the method's robustness. Precision was expressed as the %CV of the calculated concentration. Accuracy was expressed as the relative error (R.E.) of the calculated concentration. Results obtained are shown in Table 2. The intra-assay precision was lower than 7.5% for the three concentrations tested.

3.1.4. Estimation of limits of detection and quantification

Five replicates of the low concentration value of the calibration curve of DMT (2.5 ng/ml) were processed for their calculation. An estimate of the limits of detection and quantification was calculated as three and 10 times the standard deviation of the estimated concentration, respectively. The detection and quantification limits obtained following this method were 0.5 and 1.6 ng/ml, respectively.

Table 2

Precision and accuracy of plasma determinations of DMT (gas chromatography) and β -carbolines (HPLC)

	Intra-day		Inter-day	
	Precision (CV, %)	Accuracy (R.E., %)	Precision (CV, %)	Accuracy (R.E., %)
DMT	≤7.5	≤8.7	≤9.9	≤-7.4
Harmine	≤6.2	≤10.1	≤7.8	≤11.2
Harmaline	≤6.9	≤10.4	≤13.4	≤12.5
THH	≤10.9	≤9.5	≤9.8	≤8.8
Harmol	≤6.2	≤13.2	≤7.6	≤11.3
Harmalol	≤6.3	≤5.4	≤8.2	≤7.6

3.2. β -Carbolines

Fig. 3 shows typical sample plasma chromatograms of the β -carbolines. The mean retention times in minutes found in the validation study were 5.42 for THH, 6.95 for harmaline, 11.77 for harmine, and 13.00 for yohimbine (internal standard). Retention times for the *O*-demethylated metabolites were 3.87 for harmalol, 6.02 for harmol, and 14.07 for propranolol (internal standard). Variability in retention times for all compounds tested was less than 5% (CV).

3.2.1. Recovery

Peak areas of the β -carbolines and internal standards were measured after injection of the same amounts of the respective alkaloids and internal standard in mobile phase. These were compared with those obtained with blank plasma spiked with known amounts of the three β -carbolines. Recoveries, expressed in %, near the LOQ were 102.1±6.8 for 2 ng/ml harmine, 91.5±1.6 for 1 ng/ml harmaline, 87.4±2.9 for 3 ng/ml THH, and 90.6±3.7 for yohimbine. Recoveries for the *O*-demethylated metabolites were 89.8±4.4 for 0.5 ng/ml harmol and 91.9±6.3 for 0.5 ng/ml harmalol and 91.2±5.7 for propranolol.

3.2.2. Linearity

Linearity was evaluated for concentrations ranging from 0.5 to 50 ng/ml for harmine, from 0.3 to 25 ng/ml for harmaline, from 1 to 100 ng/ml for THH, and from 0.3 to 50 ng/ml for harmol and harmalol. The response ratio of the peak alkaloid area/internal standard area was fitted versus effective concentration by means of least-squares linear regression. The inverse of the square concentrations

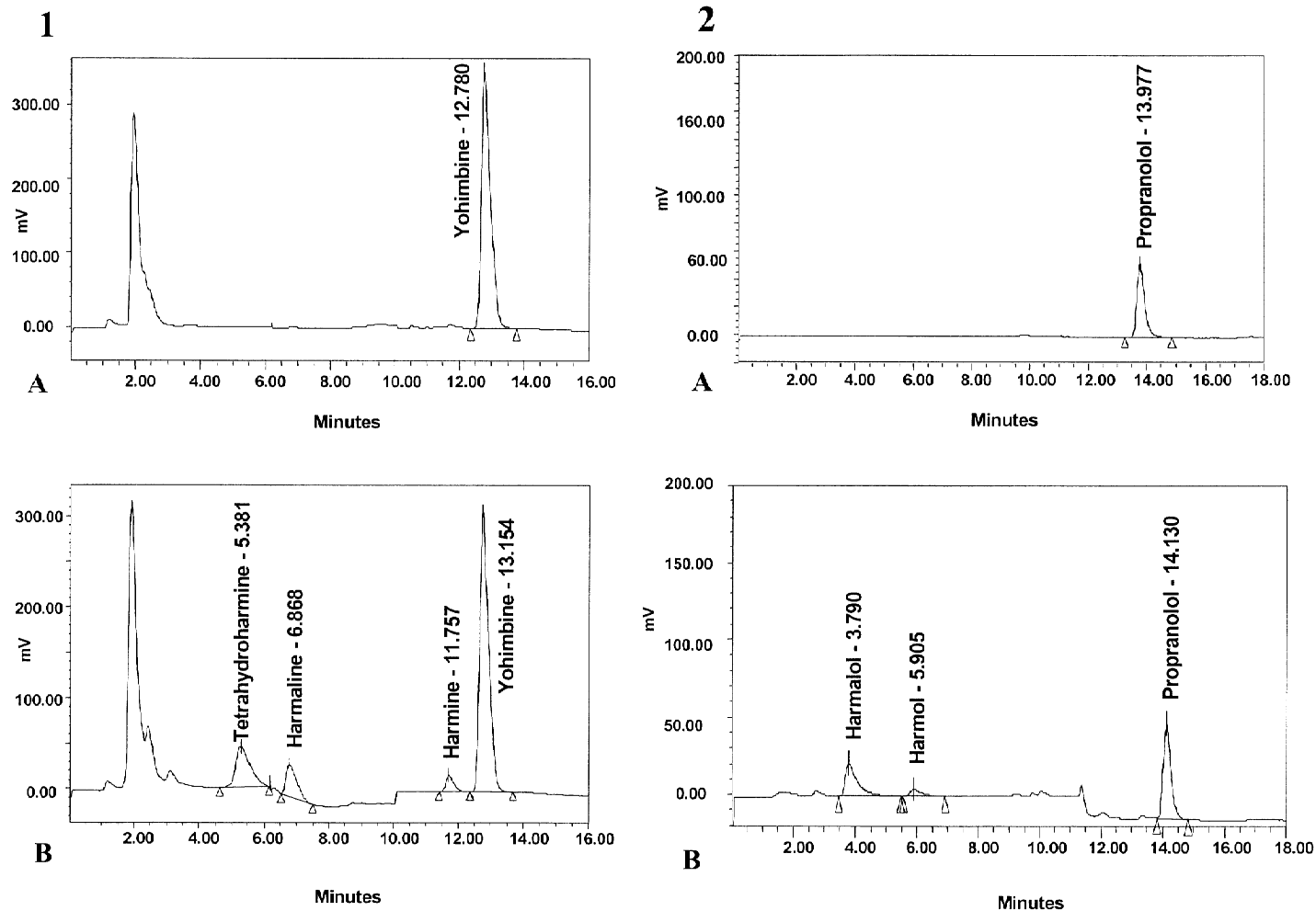


Fig. 3. Representative chromatograms of solid-phase extracted plasma samples obtained with HPLC. (1) Method for the quantification of harmine, harmaline and THH: (a) typical chromatogram of a blank plasma sample with internal standard (yohimbine), free of interfering peaks; (b) typical plasma sample from a volunteer after oral dosing with *ayahuasca*. (2) Method for the quantification of harmol and harmalol, the *O*-demethylated metabolites of harmine and harmaline: (a) typical chromatogram of a blank plasma sample with internal standard (propranolol), free of interfering peaks; (b) typical plasma sample from a volunteer after oral dosing with *ayahuasca*.

($1/C^2$) was used as a weighting factor. Regression analysis yielded the following results in the validation phase ($n=4$ days): $y=0.1620x+0.0018$ ($r^2=0.9912$) for harmine, $y=0.1071x+0.0019$ ($r^2=0.9915$) for harmaline, $y=0.0091x-0.0019$ ($r^2=0.9943$) for THH, $y=0.0265x-0.0017$ ($r^2=0.9962$) for harmol, and $y=0.0519x-0.0011$ ($r^2=0.9962$) for harmalol. Results in the routine phase are shown in Table 1.

3.2.3. Precision, accuracy and robustness

Intra-assay and inter-assay precision was determined by testing six replicates (during method validation) and 10 replicates (during routine analysis) of blank plasma samples spiked with the control samples of the respective alkaloids. Precision (expressed as %CV) and accuracy (expressed as R.E.) results obtained in the method validation phase are shown in Table 2.

In the routine phase, precision values (%CV) between assays for quality controls were below 12.4 for harmine, 11.1 for harmaline, 9.1 for THH, 3.5 for harmol and 5.9 for harmalol; and accuracy values (%R.E.) between 10 assays were below 6.9 for harmine, 9.0 for harmaline, 5.0 for THH, 10.4 for harmol and 5.4 for harmalol.

3.2.4. Estimation of limits of detection and quantification

The LOQ was established as the lowest concentration values in the calibration curve with acceptable precision and accuracy, i.e. 0.5 ng/ml for harmine, 0.3 ng/ml for harmaline, 1.0 ng/ml for THH, and 0.3 ng/ml for harmol and harmalol. The limit of detection was 0.1 ng/ml for harmine, harmaline, harmol and harmalol, and 0.3 ng/ml for THH.

3.2.5. Stability study

Freeze and thaw stability was assessed with two analyte concentrations for each alkaloid (one high and one low) in spiked plasma samples stored at -80°C . The samples were subjected to three freeze and thaw cycles in three consecutive days. Concentrations determined in each cycle were within the method's accuracy ($<10\%$) except for harmaline, which showed a 16% variation in the third cycle. To test the stability of samples in the autosampler, three

sets of spiked samples were analyzed at $t=0$, $t=24$ h, $t=48$ h with a fresh calibration curve. Concentrations determined at each time tested were within the method's accuracy ($<10\%$). Additional stability studies were carried out, i.e. stability in solution kept in the refrigerator and stability in dried residue. All compounds were stable except for harmaline and harmalol. Harmaline showed a 30% concentration decrease and harmalol a 21% concentration decrease at 24 h in dried residue. For this reason, dried residues were redissolved immediately in mobile phase during routine analysis. The stability study showed that sample degradation was low, being inferior to 10% for all the β -carbolines evaluated with the exception of the two cases mentioned. No significant variations were observed in the quality controls during the entire routine process, as indicated in Section 3.2.3.

3.3. Pharmacokinetic results

Blood samples from 18 healthy volunteers with previous experience with hallucinogen drug use were obtained at 0, 30, 60, 90, 120, 150 min, and 3, 4, 6, 8 and 24 h following oral administration of two doses of encapsulated freeze-dried *ayahuasca* in a double-blind placebo-controlled clinical trial. The study was approved by the local ethics committee and the Spanish Ministry of Health. Signed informed consent was obtained from all participants. The freeze-dried material administered in the study was obtained from a 9.6 l batch of *ayahuasca* and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg THH per g. The alkaloid concentrations in the original tea were 0.53 mg/ml DMT, 0.90 mg/ml harmine, 0.06 mg/ml harmaline and 0.72 mg/ml THH. The two doses administered to the volunteers were equivalent to 0.6 and 0.85 mg DMT/kg body weight, and were chosen based on tolerability data obtained in a previous study [11]. The blood samples were collected in tubes containing EDTA, centrifuged, and the plasma frozen at -20°C and stored at -80°C until analysis.

Fig. 4 shows mean concentration versus time curves for DMT, harmine, harmaline, THH, harmol and harmalol obtained after analysis of plasma samples from four male volunteers who received two

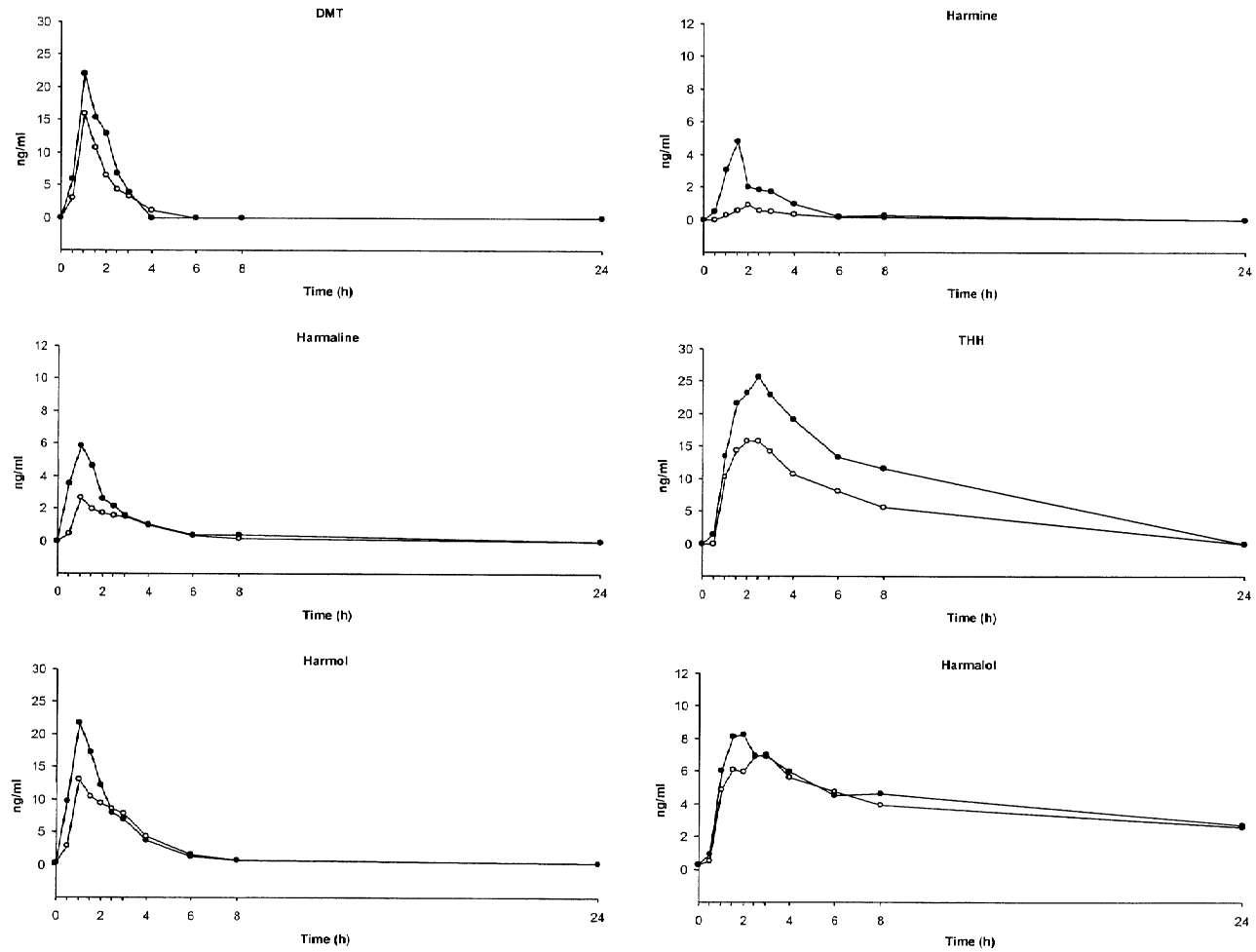


Fig. 4. Plasma concentration–time curves ($n=4$) for the four main alkaloids present in *ayahuasca* (DMT, harmine, harmaline, and THH) and the *O*-demethylated metabolites of harmine (harmol) and harmaline (harmalol); ○, low 0.6 mg DMT/kg dose of *ayahuasca*; ●, high 0.85 mg DMT/kg dose of *ayahuasca*.

oral doses of *ayahuasca* corresponding to 0.6 and 0.85 mg DMT/kg body weight.

4. Discussion and conclusions

There is a high variability in the bioavailability of *ayahuasca* alkaloids in humans, as observed in the present study. Some of the clues to these variations have already been provided in the Introduction. From an analytical perspective, for a given dose, one should be prepared to cover differences of peak plasma concentrations of one order of magnitude. The present work has proven sufficiently specific, sensitive and robust to perform this task. The GC and HPLC methods described in the present paper allowed for the adequate characterization of the pharmacokinetics of the six compounds evaluated after oral ingestion of *ayahuasca*.

Regarding the DMT quantification method, the use as salting-out effect of sodium chloride to saturate the aqueous phase and *n*-pentane as organic solvent, instead of *n*-butyl chloride as described in a previously reported method [13], provides an adequate recovery and very clean extracts. There is an overall improvement of three times the LOQ (from 5 to 1.6 ng/ml) which facilitates the pharmacokinetic study, taking into account the variability in the absorption of DMT when orally administered. The method described for the determination of the β -carbolines also introduces several improvements from previously described procedures [13]. Sample preparation is thus facilitated by solid-phase extraction, and quantification of the three alkaloids plus two metabolites, harmol and harmalol, is accomplished using a single fluorescence detector, the procedure requiring only slight modifications in the chromatographic conditions, and the use of two internal standards. The LOQ is also lower than previously reported values. Results demonstrate the present HPLC methodology is a rapid, simple and sensitive procedure for the determination of β -carboline compounds at low concentrations in human plasma, enabling the automation of the process.

In conclusion, a previously reported method was modified [13] in order to facilitate analyte extraction, improve sensitivity and allow the quantification of two metabolites not previously studied. DMT was

determined by gas chromatography with selective nitrogen–phosphorus detection following liquid–liquid extraction. The three main β -carbolines present in *ayahuasca*, i.e. harmine, harmaline and THH, were determined by means of HPLC with fluorescence detection following solid-phase extraction. Additionally, harmol and harmalol, two alkaloids present in trace amounts in *ayahuasca* but showing significant levels in plasma following ingestion of the tea, were also determined. Harmol and harmalol had not been assessed previously in plasma following oral dosing with *ayahuasca*, and are presumably formed *in vivo* by the *O*-demethylation of harmine and harmaline, respectively. The quantification of these two metabolites was accomplished with slight changes in the chromatographic conditions necessary to determine the parent compounds.

Acknowledgements

We are grateful to Dr James C. Callaway of the Department of Pharmaceutical Chemistry of the University of Kuopio, Finland, for supplying the THH standard, and to the United Nations International Drug Control Programme, Technical Services Branch, Laboratory Operations for generously providing the DMT standard.

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