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Psychometric assessment of the Hallucinogen Rating Scale[☆]

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Abstract

Reliability and convergent-discriminant validity of a Spanish version of the Hallucinogen Rating Scale (HRS) were assessed in two differentiated populations of hallucinogen users involving the retrospective assessment of drug effects. In Study 1 (immediate assessment), 75 European users of the South American hallucinogenic drink *ayahuasca* answered the HRS 4 h after drug intake in their habitual setting. In Study 2 (delayed assessment), 56 adult polydrug users answered the HRS and a short form of the Addiction Research Center Inventory (ARCI) recalling the effects they experienced when they last took a hallucinogen, in order to test the convergent-discriminant validity of HRS with the scales of the standard questionnaire used in most studies involving psychoactive drugs. The HRS scales showed increases after both the immediate and delayed retrospective assessment of drug effects. Reliability data indicated that four of the six scales show an acceptable level of internal consistency. Significant but limited correlations were found between the Perception and Somaesthesia scales and the ARCI LSD scale, pointing out the questionnaire's construct validity. Thus, the HRS was sensitive to hallucinogenic drug effects other than those elicited by intravenous *N,N*-dimethyltryptamine (DMT), for which it was originally designed, and showed reasonable reliability and convergent validity. Results suggest its usefulness in the evaluation of subjective effects elicited by psychoactive drugs with hallucinogenic properties, and constitute a preliminary approach to the effects of *ayahuasca* in European subjects. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Experimental studies involving hallucinogenic drug administration to human subjects have awakened renewed interest since the research of Hermle et al. (1992) and Vollenweider et al. (1997a,b,c) with model psychoses in Europe and Strassman's studies involving the administration of *N,N*-dimethyltryptamine (DMT) to healthy volunteers in the US (Strassman et al., 1994, 1996; Strassman, 1996). Before the resumption of clinical studies, these intriguing drugs had received little attention in psychiatry and human experimental psy-

chopharmacology in the last two decades, despite their popularity as recreational drugs. Hallucinogen consumption, especially of LSD, rather than decreasing, has remained stable or even increased throughout the years and has recently won new impetus in the form of synthetic amphetamine derivatives such as MDMA (Pope et al., 1990; Schuster et al., 1998). Additionally, in Europe, a new pattern of use is emerging involving the so-called 'natural drugs' or 'shamanic inebriants', such as *peyote* (a mescaline-containing cactus) or *ayahuasca* (a DMT containing-drink) rediscovered by religious cults or 'new age' groups. Regarding *ayahuasca*, several groups ingesting this South American hallucinogenic drink have settled in recent years in several European countries, particularly in Holland, Italy, Germany and Spain. This hallucinogenic beverage is obtained from infusing various plants native to the Amazon Basin, habitually *Banisteriopsis caapi* and

[☆] The full text of Appendix A is available at the journal website at <http://www.elsevier.com/locate/drugalcdep> under 'Supplementary Materials'.

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Psychotria viridis (Rivier and Lindgren, 1972). The drink contains both the potent short-acting hallucinogen DMT, the same used parenterally in Strassman's study, and several alkaloids known generically as beta-carbolines, which render DMT orally active by preventing its peripheral metabolism due to their monoamine oxidase inhibiting properties (McKenna et al., 1984; Callaway et al., 1999). This preparation is becoming increasingly popular in the countries mentioned above probably because it is a 'plant' drug (i.e. not the product of chemical synthesis) and because both the tea and the several religious groups which consume it in a ritual setting enjoy legal protection in Brazil. All these aspects have made it possible for a growing number of people to come into contact with *ayahuasca* outside South America.

The maintained prevalence of use of the classical hallucinogens, and the emergence of new patterns of use, including plant drugs and the widespread consumption of the so-called 'entactogenic' amphetamine derivatives, with mixed psychostimulant and hallucinogenic properties, have prompted new research efforts directed to clarify the complex psychological effects elicited by these agents. In this context, new psychometric research tools have been designed to quantify their effects on the central nervous system. By measuring the mood-, perception- and cognition-altering effects that are characteristic of a given drug, these instruments can help characterize their activity profiles over time and establish dose-response data. Although neuroendocrine and neurophysiological measures have also been tested for this purpose, the psychometric approach is still widely accepted, as such biological variables are usually less sensitive and specific. To date, no reliable unique biological measures are available to study dose-response relationships after the administration of hallucinogens to human subjects.

In the present study one intended both to approach the new phenomenon of *ayahuasca* use in Spain and further explore the sensitivity and psychometric properties of a recently developed instrument used to evaluate the perceptual, somatic and psychological effects of hallucinogenic drugs: the Hallucinogen Rating Scale (HRS). This self-report questionnaire was first designed to quantify the subjective effects experienced after the administration of DMT, and thus facilitate neuropharmacological research with these drugs in human subjects (Strassman, 1994, 1995). The items included in the questionnaire were originally generated by analyzing the verbal reports obtained after interviewing a number of experienced American DMT users. The resulting instrument was subsequently modified during the course of a controlled dose-response study conducted with DMT in 12 volunteers (Strassman et al., 1994). Its utility in human ketamine research (Bowdle et al., 1998) has also been demonstrated, and a recent study incor-

porated the HRS in the evaluation of the subjective effects elicited by psilocybin, MDE and methamphetamine in a group of healthy volunteers (Gouzoulis-Mayfrank et al., 1999). The items are grouped in six empirically-derived scales which, in the original dose-response study conducted by Strassman et al., and in subsequent DMT (Strassman, 1996; Strassman et al., 1996) and ketamine (Bowdle et al., 1998) studies, provided a better resolution of effects among doses than other biological variables used. The aim of the present study was thus to test whether the HRS was sensitive to the immediate and delayed assessment of hallucinogenic drug effects, and to explore its reliability and convergent validity, in order to establish its suitability for human psychopharmacological research with hallucinogens other than intravenous DMT.

2. Methods

A Spanish version of the questionnaire (Appendix A) was evaluated by means of two independent studies involving different approaches. Both study protocols had been previously reviewed by the Research Institute's review board. Approval by the hospital's Ethics Committee was deemed unnecessary, since both studies were non-experimental designs, that is the independent variable (drug ingestion) was not manipulated by the researchers. These non-experimental designs are more specifically known as *ex post facto* designs (DePoy and Gitlin, 1993).

In Study 1 the questionnaire was administered to a group of local users (Barcelona area, Spain) of *ayahuasca* immediately after one of their drug sessions (immediate retrospective assessment). In Study 2, one wished to compare the HRS results with those from the Addiction Research Center Inventory (ARCI), by means of a delayed retrospective assessment. The ARCI is the standard questionnaire used in studies involving drugs of abuse and includes a scale theoretically sensitive to the effects of hallucinogens (Haertzen et al., 1963; Haertzen, 1966, 1974). The HRS and a short version of the ARCI already validated into Spanish were administered to a group of polydrug users with experience in the consumption of hallucinogens. This time, volunteers were requested to answer both questionnaires recalling the effects they had experienced the last time they were under the influence of a hallucinogen. In both studies, reliability was assessed for each scale using Cronbach's alpha coefficient, and in the second study a convergent/discriminant analysis between the HRS and the ARCI scales was performed. This methodology would allow one first to cross-evaluate the HRS in two different samples and determine whether or not the instrument was sensitive to hallucinogenic drug effects in two different conditions (im-

mediate vs. delayed retrospective assessment of drug effects) and secondly, establish the HRS' convergent/discriminant validity with the most frequently used instrument in studies involving psychoactive drugs. The delayed retrospective assessment approach had already been used by Lamas et al. (1994) in their validation procedure of the Spanish version of the ARCI used in the present study.

2.1. Questionnaires

The HRS (version 3.06) was adapted into Spanish using the back-translation method, a judgmental method for investigating the conceptual equivalence (i.e. meaning symmetry) of the original and translated versions of a scale, necessary for valid cross-cultural comparisons (Berry, 1980). The HRS was translated independently by two Spanish researchers working in the field of psychopharmacology and with a good knowledge of the English language. These two Spanish versions were then contrasted and a final Spanish version was adopted. This was translated back into English by two independently working translators. The differences between retranslations were discussed and a final retranslated version was agreed upon. Finally, the original source and the back-translated items were compared for non-equivalence of meaning, and any discrepancies were noted. The translation-retranslation process was repeated several times until no semantic differences were noticed between the two questionnaire forms (Brislin, 1980).

The version of the American scale used in the present translation and evaluation is shorter than that used originally by Strassman et al. in their initial DMT dose-response study. The questionnaire has undergone several modifications and it now includes 71 items distributed in six scales: *Somaesthesia*, reflecting somatic effects including interoceptive, visceral and tactile effects; *Affect*, sensitive to emotional and affective responses; *Volition*, indicating the subject's capacity to willfully interact with his/her 'self' and/or the environment; *Cognition*, describing alterations in thought processes or content; *Perception*, measuring visual, auditory, gustatory and olfactory experiences; and finally *Intensity*, which reflects the strength of the overall experience (Strassman et al., 1994). The 71 items, their Spanish translation and their scale location are shown in Appendix A. All items are scored 0–4 (0 = not at all, 1 = slightly, 2 = moderately, 3 = quite a bit, 4 = extremely). Scorings on items 19, 62, 63, 64, 65, 66, 67 and 68 are inverted, since lower values are expected with increasing doses. The scores for the different scales are obtained by summing the scores obtained for the scale's individual items, divided by the number of items included in a given scale. A detailed account of item selection and scale

development can be found in Strassman et al. (1994).

The short version of the ARCI used in the present study was an already existing Spanish version of Martin et al.'s widely used 49-item short form (Martin et al., 1971). It was translated and evaluated by Lamas and colleagues and has shown adequate reliability and discriminant validity (Lamas et al., 1994). It contains five scales or groups: MBG, morphine-benzedrine group, measuring euphoria; PCAG, pentobarbital-chlorpromazine-alcohol group, measuring sedation; LSD, lysergic acid diethylamide scale, which measures dysphoria and psychotomimetic effects; BG, benzedrine group, a stimulant-sensitive scale; and the A scale, amphetamine, sensitive to the effects of *d*-amphetamine. Alpha coefficients for these scales range from 0.49 to 0.87.

2.2. Study 1

2.2.1. Subjects

Prior to the study, several groups taking *ayahuasca* for recreational purposes in the Barcelona area (Spain) were contacted. In this preliminary evaluation of the HRS' sensitivity to the subjective effects elicited by a hallucinogen other than intravenous DMT, emphasis was placed on obtaining as large a sample of users as possible, which inevitably militated against its homogeneity. It was also not feasible to obtain a psychological or psychiatric profile of the participants. Nevertheless, sex, age and number of previous ingestions were recorded. The nature and goals of the present study were explained to them, and a total of 75 adult *ayahuasca* users gave their written informed consent to participate. After pooling the questionnaires, four subjects were excluded because of unanswered items. The final study sample consisted of 71 subjects (38 men and 33 women) with a mean \pm S.D. age of 36.59 ± 7.89 years (range: 18–50). Thirty-three subjects had ingested *ayahuasca* tea between 1 and 5 times (46.5%); 11 had between 6 and 10 times (15.5%) and 27 had taken it more than 10 times (38%).

2.2.2. Study procedure

The subjects were requested to complete the questionnaire on a single occasion 4 h after *ayahuasca* ingestion, once the psychoactive effects had resolved. Instructions were given by one of the researchers or by a trained collaborator in the habitual setting where the subjects ingested the tea. They were asked to answer according to the effects experienced on that particular occasion and to answer each item according to the maximum intensity with which each effect was felt, thus avoiding the possibility of doubt in case the same effect had been felt with different intensities on different occasions.

2.3. Study 2

2.3.1. Subjects

Fifty-six polydrug users (48 men and eight women), with a mean (\pm S.D.) age of 26 ± 6.72 years and previous experience with hallucinogens, were recruited by word of mouth, in collaboration with the methadone maintenance program of the Hospital de Sant Pau, Barcelona. Eligibility criterion was hallucinogen use during the last 5 years. Of the 56 subjects included, four had used a hallucinogen the preceding week (7.1%); 16 during the preceding month (28.6%); another 16 during the preceding year (28.6%) and 20 during the last 5 years (35.7%). Regarding present use of other illicit drugs, 50 of the 56 subjects admitted they were presently taking the following: cannabis ($n = 47$), cocaine ($n = 27$), amphetamine ($n = 21$), MDMA ($n = 15$), opiates ($n = 10$) or others ($n = 10$). Prior to participation, the subjects signed a written informed consent.

2.3.2. Study procedure

In this second study, a delayed retrospective assessment (also termed 'simulation' by some authors, see Lamas et al., 1994) approach was used. That is, subjects were requested to complete the HRS and the ARCI according to their recollection of the effects they had experienced when they last took a hallucinogen. Again, they were instructed to indicate the maximum intensity with which a particular effect had been felt (HRS) and to answer 'true' or 'false' (ARCI) depending on whether a particular statement applied to their last experience with a hallucinogen. The order of administration of the two questionnaires was balanced to avoid an effect of order in the responses.

2.4. Data analysis

Two criteria were considered in the analysis of the Spanish version of the HRS scales: (1) reliability, measured using Cronbach's alpha coefficient (studies 1 and 2); and (2) convergence-discriminance between the HRS and the ARCI scales (study 2). This validity criterion was assessed by studying correlations between the HRS and ARCI scales. The occurrence of a significant correlation was hypothesized between at least the Somaesthesia scale included in the HRS and the ARCI LSD scale, together with an absence of significant correlations between the six HRS scales and the other four ARCI scales, as the ARCI LSD scale is known to reflect basically the somatic-dysphoric effects elicited by hallucinogens. Reliability and intercorrelations of the ARCI scales are also presented in Section 3.

Additionally, in order to cross-evaluate the pattern of responses in both samples, where different methodological approaches had been used, the following analysis was performed: First, Pearson's product-moment corre-

lations among the six HRS scales were computed in both samples. Subsequently, a Principal Component Analysis was performed on the correlation matrix thus obtained.

The purpose of this analysis was merely to compare results obtained in the two different samples. Scale development has been reported previously (Strassman et al., 1994) and one desired to maintain the questionnaire's original structure, so no first order Principal Component Analysis was performed on the individual items. Moreover, the small sample size precluded such an approach.

Once the product-moment correlations were obtained, the decision about the appropriate number of factors was made considering those factors with an eigenvalue greater than 1. Oblique rotation of the principal components extracted was preferred to orthogonal rotation because of the intensity of the correlations obtained between some scales. In order to test the null hypothesis stating that the factor structure was similar in the two samples studied, both factor solutions were compared. The similarity of both oblique factorial solutions obtained in the two samples was evaluated using the following indices: (a) the factorial congruency index (Harman, 1976); (b) the discrepancy or distance index, which is the square root of the average squared difference between the corresponding elements of the respective columns of factor loadings (Mulaik, 1972); (c) the non-parametric salience index, s (Cattell and Baggaley 1960); and (d) Pearson's r between Fisher's Z -transformed factor loadings. Different indices were used because of controversy among authors over the most appropriate index to be employed. However, Cattell (1978) indicated that these four indices tend to yield similar results. Good agreement between factors in the different samples would yield a congruency index near 1 (acceptable values are those above 0.90), a discrepancy index near zero, and salience and Fisher's Z -transformed index values near 1.

3. Results

3.1. Mean scores and reliability of the HRS scales

Both the subjective effects elicited by *ayahuasca* (immediate assessment, Study 1) and the recollection of previous hallucinogen-induced subjective effects (delayed assessment, Study 2) produced increases in the six HRS scales. Mean scores, standard deviations and reliability estimates (Cronbach's alpha) obtained for the six HRS scales in studies 1 and 2 are shown in Table 1. In Study 1, alpha coefficients for Perception, Cognition, Somaesthesia and Affect were adequate and acceptable (range between 0.81 and 0.88), reflecting a good degree of internal consistency. However, alpha coefficients for

the Intensity and Volition scales failed to reach a reasonable value. The Intensity scale showed the lowest internal consistency of the six scales. In Study 2, alpha coefficient values for the HRS scales replicated those found in Study 1, showing similar figures, especially for Perception and Cognition and to a lower extent for Somaesthesia and Affect. Although the Intensity scale reached a higher alpha value in this sample than in the previous sample, it was still low for an acceptable internal consistency.

3.2. Intercorrelations and principal component analysis: cross-evaluation of the two samples

Pearson's product-moment correlations between HRS scales are also shown in Table 1.

The highest correlations were found between Perception and Somaesthesia (Study 1) and between Affect and Cognition (Studies 1 and 2). Volition was the only scale inversely related to the rest, showing the lowest correlations in the matrix. This pattern of correlations between Volition and the other scales suggested that

Volition was measuring a different construct or mapping a different content compared to the other HRS scales, which were all positively related in the correlation matrix.

The pattern of correlations obtained in the two samples showed a high degree of similarity. To further test this behavior a principal component analysis of intercorrelations between the HRS scales was performed. This analysis rendered in Study 1 two factors explaining approximately 75% of the variance in the correlation matrix (first factor: 57.5%; second factor, 16.8%). After an oblique rotation of both factors, a clear pattern appeared, showing a negative association between the two components (see Table 2), the correlation between both being $r = -0.19$. The first component included Somaesthesia, Perception, Cognition, Affect and Intensity; and the second component included only the Volition scale. The same analysis was performed with the factor matrix obtained in Study 2. As shown in Table 2, the factorial matrix was practically equal in both samples. Taken together, the two factors that emerged explained approximately 68% of the variance in the

Table 1
Intercorrelations (Pearson's r) among Hallucinogen Rating Scale (HRS) scales in the two samples studied, plus means \pm S.D. and reliability coefficients associated with each HRS scale in the two samples^a

	Aff.	Cog.	Int.	Per.	Som.	Mean \pm S.D.	Alpha
Aff.	1					1.82 \pm 0.58	0.81
Cog.	0.69**	1				1.92 \pm 0.77	0.87
Int.	0.39**	0.55**	1			2.56 \pm 0.58	0.33
Per.	0.53**	0.65**	0.48**	1		1.87 \pm 0.70	0.88
Som.	0.63**	0.66**	0.52**	0.74**	1	1.45 \pm 0.64	0.82
Vol.	-0.30*	-0.23*	-0.24*	-0.06	-0.13	1.42 \pm 0.44	0.51
Aff.	1					1.75 \pm 0.47	0.72
Cog.	0.70**	1				1.85 \pm 0.72	0.86
Int.	0.34**	0.45**	1			2.49 \pm 0.62	0.50
Per.	0.62**	0.66*	0.49**	1		1.72 \pm 0.73	0.91
Som.	0.49**	0.40**	0.27**	0.47**	1	1.76 \pm 0.63	0.71
Vol.	0.12	-0.09	-0.09	0.05	0.02	1.58 \pm 0.48	0.54

^a Upper and lower panels show data for study 1 ($N = 71$) and study 2 ($N = 56$), respectively. Aff. = affect, Cog. = cognition, Int. = intensity, Per. = perception, Som. = somaesthesia.

* $P < 0.05$;

** $P < 0.01$ (two tailed).

Table 2
Oblique rotated factor pattern matrix for the Hallucinogen Rating Scale (HRS) scales in the two samples studied

	Study 1 ($N = 71$)		Study 2 ($N = 56$)		
	1st Factor	2nd Factor	1st Factor	2nd Factor	
Somaesthesia	0.9074	0.1236	Somaesthesia	0.6690	0.1160
Perception	0.8949	0.2351	Perception	0.8589	0.0411
Cognition	0.8563	-0.0836	Cognition	0.8484	-0.1251
Affect	0.7458	-0.2183	Affect	0.8444	0.1985
Intensity	0.6684	-0.1536	Intensity	0.6277	-0.3084
Volition	-0.0344	0.9639	Volition	0.0480	0.9543

Table 3
Means, S.D., reliability coefficients and intercorrelations (Pearson's r) among Addiction Research Center Inventory (ARCI) scales^a

	A	BG	LSD	MBG	PCAG
BG	0.66**				
LSD	0.05	-0.14			
MBG	0.62**	0.38**	-0.11		
PCAG	-0.19	-0.35**	0.29*	-0.14	
Mean	6.39	3.75	3.02	9.84	-0.80
S.D.	1.87	2.12	2.42	3.54	1.81
No. items	11	13	14	16	15
Alpha	0.42	0.48	0.57	0.70	0.30

^a $N = 56$; PCAG, pentobarbital-chlorpromazine-alcohol group; LSD, lysergic acid diethylamide scale; BG, benzedrine group; A, amphetamine; MBG, morphine-benzedrine group.

* $P < 0.05$;

** $P < 0.01$ (two tailed).

second study (first factor: 50.2%; second factor: 17.9%), and, again, they showed a slight negative association ($r = -0.3$).

The similarity evaluation of the two oblique factorial solutions yielded the following indices for the first and second component, respectively: congruency index = 0.99 and 0.99; discrepancy index = 0.08 and 0.08; salience index = 1 and 1; and Fisher's Z-transform index = 0.97 and 0.99.

3.3. Convergent and discriminant validity of the HRS scales

The ARCI scales were used to assess convergent and discriminant validity of the HRS in Study 2. Mean scores, standard deviations, alpha coefficients and intercorrelations between the ARCI scales are shown in Table 3. As can be seen, the delayed assessment of hallucinogen effects produced increases in the MBG and A scales, and to a lower extent in the BG and LSD scales, in relation to the number of items. Scale A strongly correlated with both the BG and MBG scales. This correlation between the A and BG scales replicates that ($r = 0.70$) reported by Lamas et al. (1994) in their

validation study. In addition, the BG scale was positively related to the MBG scale although with less intensity. As a group, these three scales could be considered to reflect stimulating effects. All three correlated negatively with the PCAG scale. In addition, the LSD scale was positively correlated with PCAG and tended to be inversely related with the three stimulant-sensitive scales, although in no case did correlations reach a significant value.

Correlations between the HRS (including a global score for the HRS obtained by summing the individual scores in the six scales), and ARCI scales are shown in Table 4. As hypothesized, a convergent correlation was obtained between the LSD scale and the HRS scales. The highest significant correlations were found with the Somaesthesia and Perception scales. The global HRS score was also significantly correlated with the LSD scale ($r = 0.32$, $P < 0.05$). In contrast, the stimulant-sensitive scales, A and BG did not correlate significantly with the different HRS or the global score, whereas MBG showed a significant correlation with the Intensity scale ($r = 0.32$, $P < 0.05$), but correlated negatively with the global HRS score. An exception to this behavior was the two significant negative correlations found between Volition and the A and BG scales. These correlations were consistent with the results mentioned above, and further support the hypothesis that the Volition scale was measuring an independent dimension not reflected by the other five scales.

A non-predicted correlation was obtained between PCAG and the HRS scales, mainly Cognition and Somaesthesia, also observed with the global HRS score ($r = 0.39$, $P < 0.01$). It should be noted, however, that PCAG and LSD share a number of items, and as the ARCI correlations already showed these two scales are partially related. In order to remove the influence of the LSD scale on PCAG scores, the partial correlation between the global HRS score and PCAG was computed ($r = 0.29$, $P < 0.05$). Although this correlation was lower than the previous one, it was still significant and indicated a positive relation between PCAG and some HRS scales. This unexpected correlation should

Table 4
Correlations (Pearson's r) among Hallucinogen Rating Scale (HRS) and Addiction Research Center Inventory (ARCI) scales in Study 2^a

	Affect	Cognition	Intensity	Perception	Somaesth.	Volition	Global
A	-0.03	-0.02	-0.06	-0.05	0.13	-0.27*	-0.06
BG	-0.16	-0.06	-0.10	-0.09	-0.10	-0.27*	-0.18
LSD	0.14	0.23	0.23	0.28*	0.33*	0.03	0.32*
MBG	0.04	-0.01	0.32*	-0.01	0.10	-0.19	-0.07
PCAG	0.36	0.29*	0.21	0.19	0.38*	0.15	0.39**

^a $N = 56$; Global, global HRS score; PCAG, pentobarbital-chlorpromazine-alcohol group; LSD, lysergic acid diethylamide scale; BG, benzedrine group; A, amphetamine; MBG, morphine-benzedrine group.

* $P < 0.05$;

** $P < 0.01$ (two tailed).

be interpreted cautiously, given the low alpha value obtained for PCAG in the present study, and considering the fact that Haertzen (1974) had already described a high correlation between the PCAG and LSD scales.

4. Discussion

The purpose of the present study was to test the sensitivity of the HRS to a hallucinogenic drug other than intravenous DMT and to evaluate the reliability and validity of the questionnaire in two different populations of hallucinogen users, implying the immediate and delayed retrospective assessment of hallucinogenic drug effects. Results showed increases in the six scales in both samples and acceptable reliability values in four of the six HRS scales. Significant correlations were also found between the Perception and Somaesthesia scales with the ARCI LSD scale.

One of the most interesting aspects of the data presented above is the notable similarity of the psychometric indices obtained in the two studies undertaken in the evaluation process.

Despite the limitations associated with the heterogeneous nature of the studied samples, similar alpha values were obtained in five of the six HRS scales using quite different approaches, one involving the immediately preceding consumption of a hallucinogen, and the other the recollection of drug effects more distant in time. Alpha values obtained for Perception, Cognition, Somaesthesia and Affect indicated a good internal consistency for these four scales in both studies. Only two scales, Volition and Intensity, yielded poorer levels, indicating a non-uniform covariation of items or, as was very likely the case for the Intensity scale, too low a number of items. The number of items included in this scale is indeed lower than in the other five (only four items), and as alpha values depend on the number of items (McDonald, 1998), this could explain the poor internal consistency found for this scale. To the authors' knowledge, no reliability analyses have been published regarding the original American questionnaire or any of the existing translations of the HRS. One is consequently unable to compare the alpha values with those from other studies, but they objectively reflect a good degree of internal consistency in at least four of the six scales.

The equivalence of the two different assessment approaches used in the present study, that is immediate versus delayed, was further confirmed by the principal component analyses performed. Similarity indices showed a high degree of convergence between the two factorial solutions extracted. Consequently, it can be inferred that the same underlying factors accounted for the relationships among variables in both cases. Furthermore, mean values obtained for the HRS scales in

studies 1 and 2 were similar and higher than those reported by Grob et al. (1996) in a study in which a moderate-low dose of *ayahuasca* was administered, and fall between the scores obtained with dosage levels of 0.2–0.4 mg/kg IV DMT, in the study of Strassman et al. (1994), reflecting a full hallucinogenic effect. These data would support the value of delayed retrospective assessment of drug effects as a questionnaire-validation procedure, as previously suggested by Haertzen (1974) and Lamas et al. (1994) regarding the ARCI.

Before performing the convergent-discriminant analysis between the HRS and the ARCI, the internal consistency of the ARCI scales was studied. For some of them, Cronbach's alpha values tended to be lower in the sample than those reported in the original adaptation of the instrument (Lamas et al., 1994). Alpha coefficients were similar for MBG, LSD and A scales, but PCAG obtained a considerably lower value in the sample than in the original study. The BG scale also yielded a lower value in the present analysis. Showing a pattern described in previous studies, A, MBG and BG correlated strongly with each other. In this study, they showed negative correlations with PCAG and LSD, and these two scales showed a significant positive correlation.

Consistent with the hypothesis, the highest correlations between questionnaires were found between several HRS scales and the LSD scale included in the ARCI. LSD correlated significantly with Perception, Somaesthesia and the global HRS score. This pattern of convergent correlations is also an index of the construct validity of these scales (Cronbach and Meehl, 1955). The results obtained indicate that the ARCI LSD and the HRS (in a global sense) are measuring the same construct, though probably covering different aspects, given the moderate values of the correlations obtained. Regarding the already-mentioned correlation between PCAG and several HRS scales, no explanation was found other than the variance shared between PCAG and LSD scale (8%). Haertzen (1974) reported a 0.44 correlation between the two scales. Although high scores were obtained for the stimulant-sensitive ARCI scales (MBG, A, BG), no correlations were found between these and the HRS scales except between MBG and Intensity, a correlation that was not present when the global HRS score was considered. This can be interpreted as an ability of the Intensity scale to capture the euphoria and the stimulating aspects of hallucinogen-induced phenomena. This ability is not observed when the global HRS score is considered, presumably indicating a greater sensitivity of the HRS to hallucinogenic than stimulant effects. Although the second highest, the correlation between the global HRS score and the LSD scale was modest, explaining only 10% of the variance. This suggests that the dysphoric somatic effects measured by the LSD scale (Haertzen, 1974) are

part of the effects elicited by hallucinogens only to some extent, but are by no means central to the experience, as has long been argued.

Finally, a brief comment on the specificity of the HRS must be made. In Study 2, data on non-hallucinogen effects were not collected, so conclusions on this aspect of the questionnaire can not be drawn. Future studies should address this issue by asking drug abusers to score the HRS for their recollection of a wider variety of drug experiences.

To summarize, the similar results obtained in the two different settings indicate that the HRS was effectively sensitive to hallucinogenic drug effects in the samples, other than intravenous DMT, and also demonstrate the value of delayed retrospective assessment of drug effects in validation procedures. Four out of six scales showed an acceptable degree of internal consistency and therefore reasonable reliability. The HRS Intensity scale showed a positive correlation with an ARCI stimulant-sensitive scale (MBG). This pattern was not seen for the other five scales, probably indicating the questionnaire's greater sensitivity to hallucinogenic than stimulant effects. Finally, the HRS showed a significant but limited correlation with the LSD scale of the ARCI, which met the authors' expectations. In view of the results obtained in the present study, one believes the HRS will prove a valuable instrument in the assessment of subjective effects in those research trials involving the administration of drugs with hallucinogen-like properties. Nevertheless, future dose-response studies using both the HRS and the ARCI will help clarify the ability of the first to reflect and measure additional aspects of hallucinogen-induced phenomena, other than the somatic-dysphoric symptoms measured by the ARCI.

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*Determination of N,N-dimethyltryptamine and β -carboline alkaloids
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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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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

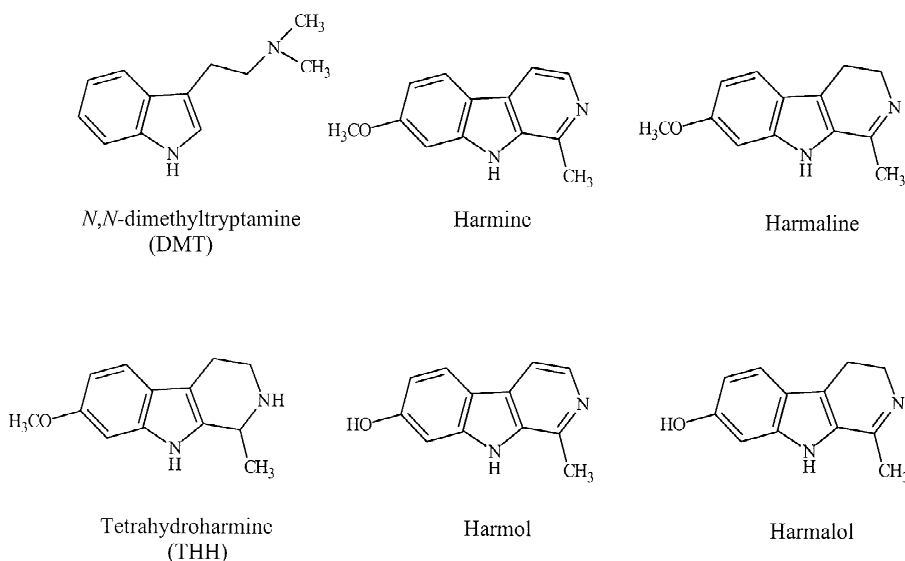


Fig. 1. Chemical structure of alkaloids from *P. viridis* (DMT) and *B. caapi* (harmine, harmaline and THH) typically found in *ayahuasca* brews. Harmol and harmalol are found in trace amounts in *B. caapi* and *ayahuasca* but appear in significant concentrations in human plasma samples following oral 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 µm, 150×4 mm I.D., and the guard column was a C₁₈ 5 µm, 10×4 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 µg/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 µg/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 µg/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 µg/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

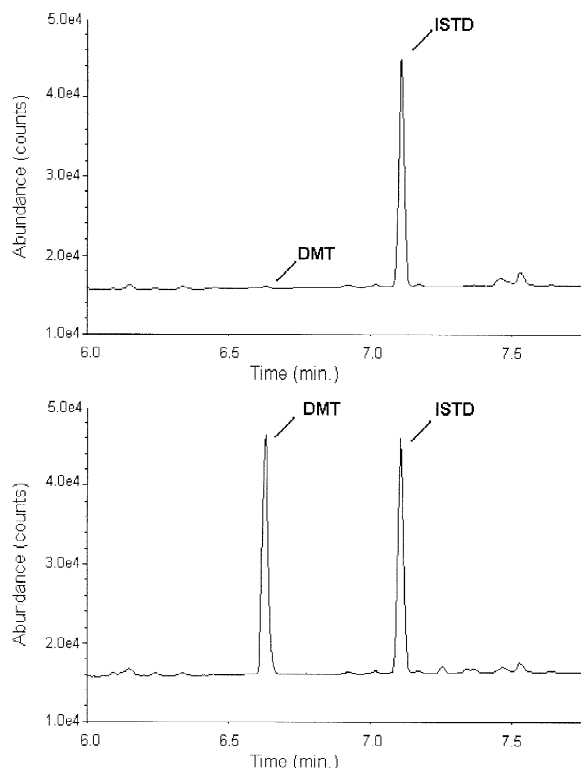


Fig. 2. Chromatogram obtained with gas chromatography with nitrogen–phosphorus detection of a plasma sample from a volunteer administered a 1 mg DMT/kg dose of *ayahuasca*. (A) Baseline plasma sample obtained just before drug administration. Expected retention time for DMT, and peak corresponding to 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

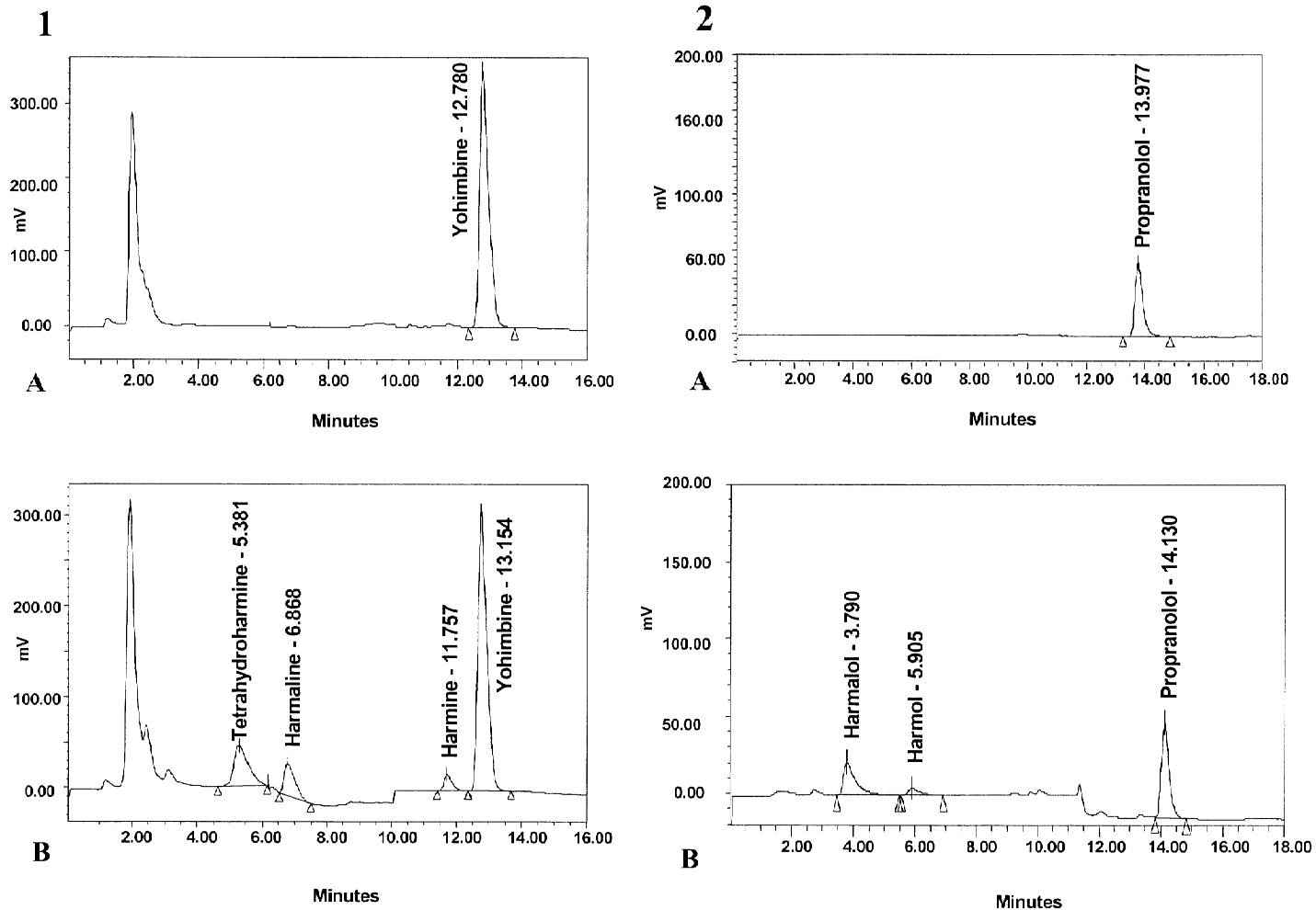


Fig. 3. Representative chromatograms of solid-phase extracted plasma samples obtained with HPLC. (1) Method for the quantification of harmine, harmaline and THH: (a) typical chromatogram of a blank plasma sample with internal standard (yohimbine), free of interfering peaks; (b) typical plasma sample 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

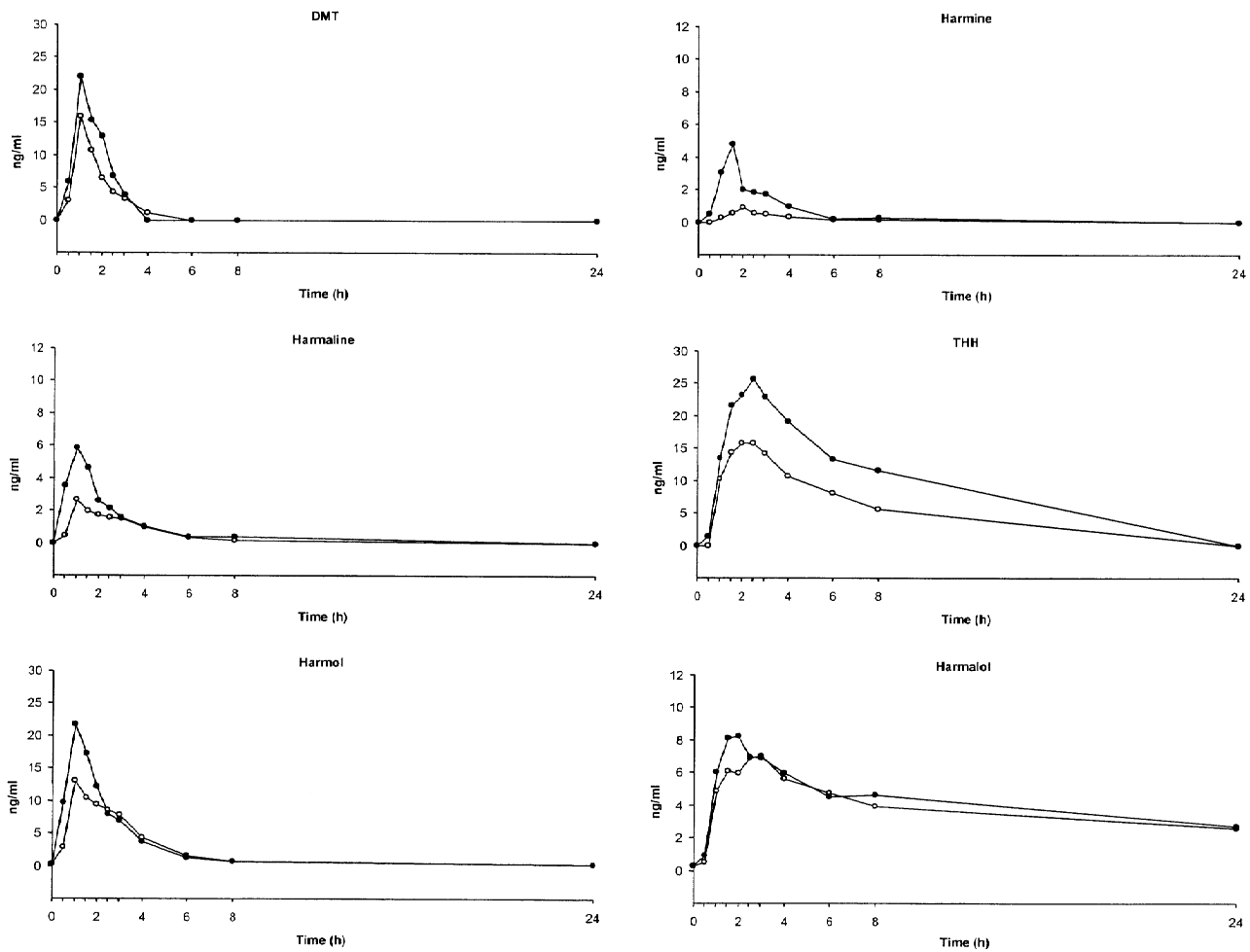


Fig. 4. Plasma concentration–time curves ($n=4$) for the four main alkaloids present in *ayahuasca* (DMT, harmine, harmaline, and THH) and the *O*-demethylated metabolites of harmine (harmol) and harmaline (harmalol); ○, low 0.6 mg DMT/kg dose of *ayahuasca*; ●, high 0.85 mg DMT/kg dose of *ayahuasca*.

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.

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