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Quantitation of ibogaine and 12-hydroxyibogamine in human plasma by liquid chromatography with fluorimetric detection

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Abstract

A high-performance liquid chromatographic (HPLC) method with fluorimetric detection was developed for the simultaneous determination of ibogaine and noribogaine in human plasma using fluorescein as internal standard. This method involved a solid phase extraction of the compounds from plasma using *N*-vinylpyrrolidone-divinybenzene copolymer cartridges. Separation of the three analytes was performed on a reversed-phase Supelcosil C18 analytical column (75 mm × 4.6 mm i.d., 3 μm particle size). The excitation wavelength was set at 230 nm for the first 15.8 min and then at 440 nm for the following 14.2 min; the emission wavelength was set at 336 nm for the first 15.8 min and then at 514 nm for the following 14.2 min. Obtained from the method validation, inter-assay precision was 6.0–12.5% and accuracy was 95.4–104%. The extraction efficiencies of the assay were higher than 94% and were constant across the calibration range. The lower limits of quantitation were 0.89 ng/ml for ibogaine and 1 ng/ml for noribogaine; at these levels, precision was ≤17% and accuracy was 95–105%. In this paper, extensive stability testing was undertaken using a wide range of storage conditions. Special attention must be paid to sample handling to avoid light degradation of the compounds.

Keywords: Ibogaine; 12-Hydroxyibogaine; Plasma; High-performance liquid chromatography; Fluorimetric detection; Validation

1. Introduction

Ibogaine (12-methoxyibogamine, (6*R*,6a*S*,7*S*,9*R*)-7-ethyl-2-methoxy-6,6a,7,8,9,10,12,13-octahydro-5*H*-6,9-methanopyrido[1′,2′:1,2]azepino[4,5-*b*]indole) is an indole alkaloid derived from the bark of the root of the African shrub *Tabernanthe iboga*, used as a medicinal and ceremonial agent in West Central Africa [1]. Psychoactive properties of ibogaine have been known for decades [2,3]. More recently, based on experimental data from animals and anecdotal reports from addict self-help groups, it has been found that this drug has an efficacy in interrupting addiction to 'narcotics' (morphine and heroin), cocaine, amphetamine, alcohol and nicotine [1,4–17]. Indeed, ibogaine interacts with multiple neurotransmitter systems known to modulate drug addiction [15]. Despite such promising findings, the mechanism responsible for the anti-addictive properties of ibogaine remain unknown. Ibogaine undergoes desmethylation to its principal metabolite, noribogaine, or 12-hydroxyibogamine ((6*R*,6a*S*,7*S*,9*R*)-7-ethyl-6,6a,7,8,9,10,12,13-octahydro-5*H*-6, 9-methanopyrido[1′, 2′:1,2]azepino[4,5-*b*]indol-2-ol). Very few studies have systematically evaluated the neurobiological effects of noribogaine in vivo. As ibogaine, this drug demonstrated to decrease morphine, cocaine and alcohol self-administration in rat [1,15–17]. However, noribogaine appears less likely to produce the adverse effects associated with ibogaine

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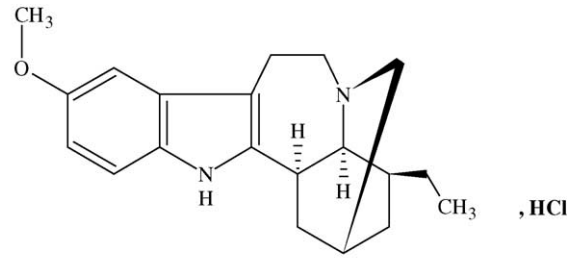
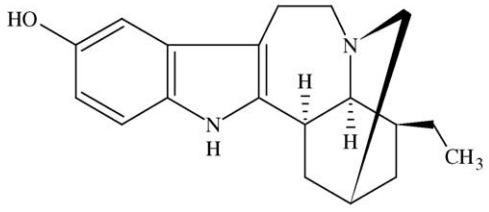


Ibogaine hydrochloride



Noribogaine

Fig. 1. Structural formulae of ibogaine and noribogaine.

(i.e., tremors and stress-axis activation), suggesting that the metabolite may be a safer alternative for medication development [17]. Structures of these compounds are presented in Fig. 1.

To date, ibogaine was determined in complex mixtures of *T. iboga* and in biological matrices (brain homogenate, urine and plasma) by spectrophotometry [18], thin-layer chromatography [19] or gas chromatography (GC) with flame ionization [19–21], nitrogen-specific [21] or mass-spectrometric (electron impact or chemical ionization) [22–25] detection. These methods involved liquid–liquid or solid-phase extraction of the biological samples. Only two of them reported the quantitation of ibogaine and its 12-hydroxy metabolite [24,25]. Most of these methods involved a derivatization procedure. A method for determining opiate agonists including ibogaine by liquid chromatography-atmospheric-pressure chemical-ionization mass spectrometry after solid-phase extraction without any derivatization procedure has been also described [26]. This method provided good results, but involved expensive equipment and is not easily available for routine drug monitoring. Moreover, only ibogaine was quantified and the possible interference with its metabolite was not studied by the authors. Most of these published methods either did not report assay validation or reported assay validation which were incomplete.

This paper describes for the first time a specific, reliable, and sensitive high-performance liquid chromatography (HPLC) method with fluorimetric detection to quantify, simultaneously, ibogaine and 12-hydroxyibogamine (Fig. 1) in human plasma. This method has an enhanced precision due to the use of an internal standard (fluorescein). It was validated according to validation procedures, parameters and acceptance criteria based on USP XXIII guidelines and FDA guidance [27–30]. Moreover, extensive stability testing was undertaken using a wide range of storage conditions.

2. Experimental

2.1. Materials and reagents

Ibogaine hydrochloride (molecular weight, 346.9), fluorescein sodium salt (internal standard) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Noribogaine base was synthesized through the desmethylation of ibogaine using a mixture of hydrobromic acid in water (49%) and acetic acid (80:20, v/v) [31]. Ibogaine and noribogaine were stored protected from light at routine room temperature (20 °C). HPLC-grade acetonitrile, methanol and acetic acid were purchased from Carlo Erba (Val de Reuil, France).

Purified water was generated by a Milli-Q reagent water system (Millipore corporation, Bedford, MA). Oasis HLB cartridges (30 mg of sorbent, average particle diameter 30 μm) were supplied by Waters (Saint Quentin, France).

For the validation of the method, blood samples from healthy volunteers (Etablissement Français du sang, Montpellier, France) were collected in heparinized tubes and plasma was obtained by centrifugation at $1000 \times g$ for 10 min. Pooled drug-free plasma samples were aliquoted, frozen at −20 °C, and then used during the study in the preparation of standards and quality control (QC) samples. Each batch of pooled drug-free plasma was obtained from 10 volunteers.

Drug stock standard solutions were prepared by dissolving accurately weighed quantities of ibogaine hydrochloride or internal standard with purified water to give solutions containing 89.5 and 81 mg/l of free form equivalents of each compound, respectively. The noribogaine stock solutions was prepared in methanol at concentrations of 100 mg/l. Stock solutions were stored at 4 °C. For each compound, two separate stock standard solutions were prepared: one which was used for the preparation of the calibration curve standards and the second which was used for the preparation of quality control (QC) samples.

Stock solutions of ibogaine and noribogaine were diluted daily in purified water to obtain 12 working standards ranging from 0.0224 to 44.7 mg/l and 0.025 to 50 mg/l, respectively. They were used to spike the plasma samples prior to extraction. The stock solution of fluorescein was diluted two-fold (40.5 mg/l) in purified water before use.

Stock and working solutions of ibogaine and noribogaine were prepared in flasks protected from light.

A reference standard solution (22.4 μg/l of ibogaine plus 25 μg/l of noribogaine plus 8.1 μg/l of internal standard) was prepared daily in the mobile phase to check the resolution of the chromatographic system.

2.2. Instrumentation

The HPLC system consisted of the following components: a model Spectra System P1000XR quaternary gradient pump from Thermo Finnigan (San Jose, CA, USA) with a Rheodyne loading valve (model 7010) fitted with a 20 μl sample loop, an oven (Iglo-cil, Cluzeau Info Labo, Sainte Foy la Grande, France) and a stainless-steel column (75 mm × 4.6 mm i.d., Supelco, Bellefonte, PA, USA) packed with Supelcosil C18 (3 μm). The column effluent was monitored with a Spectra System FL 3000 fluorescence detector (Thermo Finnigan). The HPLC system was interfaced with an IBM compatible-computer data station and controlled through Thermo product PC 1000 software, which allowed post-data analysis whilst allowing further on-line acquisition of data.

2.3. Chromatographic conditions

The mobile phase consisted of a mixture of acetonitrile (solvent A) and 1 ml/l TFA in water (solvent B, pH, 1.8) at a flow rate of 0.7 ml/min. Table 1 shows the variations in proportions of solvents A and B. The solvents A and B were filtered through a 0.2 μm cellulose nitrate filter (Sartorius, Goettingen, Germany) prior to use then degassed using a SCM1000 vacuum membrane degasser (Thermo Finnigan) during use. Chromatography was achieved at 30 °C. The injected volume was 20 μl. The excitation wavelength was set at 230 nm for the first 15.8 min and then at 440 nm for the following 14.2 min; the emission wavelength was set at 336 nm for the first 15.8 min and then at 514 nm for the following 14.2 min.

2.4. Preparation of standards and controls

Standards and QC samples were prepared in 5 ml polypropylene tubes protected from light.

Human plasma standards were prepared by aliquoting appropriate volumes of drug working solutions (20 μl) into 0.5 ml of blank plasma (drug-free) to produce a concentration series ranging from 0.89 to 179 ng/ml of ibogaine and 1 to 200 ng/ml of noribogaine. For each calibration curve, eight concentrations were used.

Quality control (QC) samples were prepared as described above by aliquoting appropriate volume of working standard solutions into blank plasma to yield concentrations of 2.24, 33.6 and 134.2 ng/ml of ibogaine, and 2.5, 37.5 and 150 ng/ml of noribogaine.

Before the solid-phase extraction (SPE) procedure, standards and QC samples previously vortex-mixed (10 s) were incubated at 20 °C for 5 min, to allow a steady-state with the matrix components.

Table 1
Variations in proportions of solvents A and B

Time	Solvent A (%)	Solvent B (%)
0	17	83
7	17	83
20	60	40
23	100	0
25	100	0
27	17	83

2.5. Sample preparation

Plasma samples were subjected to a solid–liquid extraction for the removal of proteins and interfering components. During the SPE procedure a protection of the products from light was required. The SPE Oasis HLB cartridges were pre-washed with 1 ml of methanol followed by 1 ml of purified water. A 0.5 ml aliquot of plasma was mixed with 20 μl of internal standard solution (40.5 mg/l) and 0.5 ml of water containing 100 ml/l acetic acid. The mixture was vortex-mixed for 10 s and centrifuged (4 °C) for 10 min at 1500 × *g*. The supernatant was then loaded onto the conditioned extraction column under a light vacuum (approximately 86 kPa) using a Vac Elut 20® (Varian, Les Ulis, France). The extraction column was washed with 2 × 1 ml of purified water and was then dried for 2 min by vacuum aspiration (approximately 27 kPa). The analytes were eluted from the column with 2 × 1 ml of methanol under a light vacuum (approximately 86 kPa). The eluate was dried at 40 °C under a stream of nitrogen for 50 min. The dried residue was reconstituted in 100 μl of the mobile phase. An aliquot of 20 μl was injected onto the HPLC system via the Rheodyne loop.

2.6. Data analysis

Standard calibration curves were obtained from unweighted least-squares linear regression analysis of the data. The slope and intercept of the calibration graphs were determined through linear regression of the drug-to-internal standard peak-area ratio versus drug concentration plot (formula: $y = a + bx$; where x = concentration and y = peak area). Individual peak-area ratios were then interpolared on the calibration graphs to determine values of concentration found (back-calculated concentration) as compared to concentration added.

The quality of fit was evaluated by comparing back-calculated concentrations to the nominal ones. The "Lack of Fit" test was used to confirm the linearity of the method. Moreover, the back-calculated concentrations (C_{TEST}) were compared to the theoretical concentrations (C_{REF}), and the bias (or mean predictor error) was computed as follows:

$$\text{bias} = \frac{1}{n}\sum_{i=1}^{i=n}[C_{TEST(i)} - C_{REF(i)}]$$

where i is the number of concentrations.

The 95% confidence interval for bias was also computed.

2.7. Validation

2.7.1. Specificity

The specificity of the method was investigated by analyzing 10 different batches of pooled blank human plasma samples from healthy volunteers to determine whether endogenous constituents coeluted with the different analytes. The retention times of endogenous compounds in the matrix were compared with that of the compounds of interest.

Plasma samples ($n = 10$) from patients receiving other drugs were analyzed for interference. The following drugs were checked: morphine, codeine, methadone, cocaine, buprenorphine and tramadol.

2.7.2. Precision and accuracy

Within-day and between-day precision and accuracy of the assay were assessed by performing replicate analyses of QC samples in plasma against a calibration curve. The procedure was repeated on different days on the same spiked standards to determine between-day repeatability. Intra-day repeatability was determined by treating spiked samples in replicate the same day.

The accuracy was evaluated as [mean found concentration/theoretical concentration] × 100. Precision was given by the percent relative standard deviation (R.S.D.).

2.7.3. Extraction efficiency

Absolute recoveries of ibogaine and noribogaine were measured three times at all concentrations of calibration standards, based on the comparison of the areas under the peaks of the extracted samples with those of unextracted reference standard solutions containing the corresponding concentrations prepared in the mobile phase. The extraction recovery was also computed for the internal standard. In all cases, the means and standard deviations (S.D.) were calculated.

2.7.4. Determination of the lower limit of quantitation (LLOQ)

The LLOQ estimated on QC samples was defined as the lowest drug concentration which can be determined with a R.S.D. $\leq 20\%$ and an accuracy of $100 \pm 20\%$ on a day-to-day basis [27–30].

2.8. Stability assays

Extensive stability testing was undertaken using a wide range of storage conditions.

The stability of stock solutions of ibogaine in purified water and noribogaine in methanol was determined at 4 °C. Appropriately diluted stock solutions were injected in triplicate into the HPLC system immediately after preparation (reference value) and at periodic intervals after storage at 4 °C over a period of 6 months. The stability of the two compounds in the working solution containing ibogaine at concentration of 22.4 ng/ml and noribogaine at concentration of 25 ng/ml was assessed after storage at 4 °C in a refrigerator for 72 h and at ordinary laboratory conditions (20 °C, with and without daylight exposures) for 4 h. At selected time intervals, a 20 μl aliquot was injected into the analytical column. The areas under the peaks of the stored samples were compared with those obtained for the two compounds immediately after preparation of the solution (reference values). Compounds were considered stable when losses were less than 2%.

The stability of ibogaine and noribogaine in plasma and at various steps of the analysis was explored to assess the substance integrity throughout the procedure, starting from sampling to processing in the laboratory. For stability studies carried out in plasma, QC samples representing the low, middle and high concentrations (2.24, 33.6 and 134.2 ng/ml for ibogaine, and 2.5, 37.5 and 150 ng/ml for noribogaine) were used. QC samples were analyzed immediately after preparation (reference values) and after storage. Each determination was performed in triplicate. Concentrations of each analyte were determined against a calibration curve. The stability assays were carried out as follows:

(i) The short-term stability of ibogaine and noribogaine was assessed at 1, 2, 4 and 6 h after bench-top storage at both ordinary laboratory temperature (20 °C and daylight protection) and in a refrigerator at 4 °C.
(ii) The protocol was repeated after 15, 30 and 60 days storage in a freezer at −20 and −80 °C. Prior to their analyses, samples were brought to room temperature and vortex-mixed well.
(iii) The freeze–thaw stability was also determined. Spiked QC samples were analyzed on a daily basis after repeated freezing–thawing cycles at −20 °C on three consecutive days.

The stability of ibogaine and noribogaine in the reconstituted extracts was inspected over a period of 72 h at 4 °C in a refrigerator.

The potential influence of the evaporating step was also tested.

Stability was defined as <15% loss of initial drug concentration.

3. Results

3.1. HPLC characteristics

Under the chromatographic conditions used, the number of theoretical plates was approximately 38,673 (calculated from the internal standard peak). The column was replaced when the number of theoretical plates had decreased below 15,000. Representative chromatograms of drug-free human plasma and of plasma spiked with ibogaine and noribogaine are shown in Fig. 2. There was clear resolution of the compounds of interest which had retention times of 6.5 ± 0.18 min for noribogaine, 14.8 ± 0.14 min for ibogaine and 16.5 ± 0.06 min for the internal standard ($n = 13$). The mean k'-values were 4.22, 10.9 and 12.3, respectively.

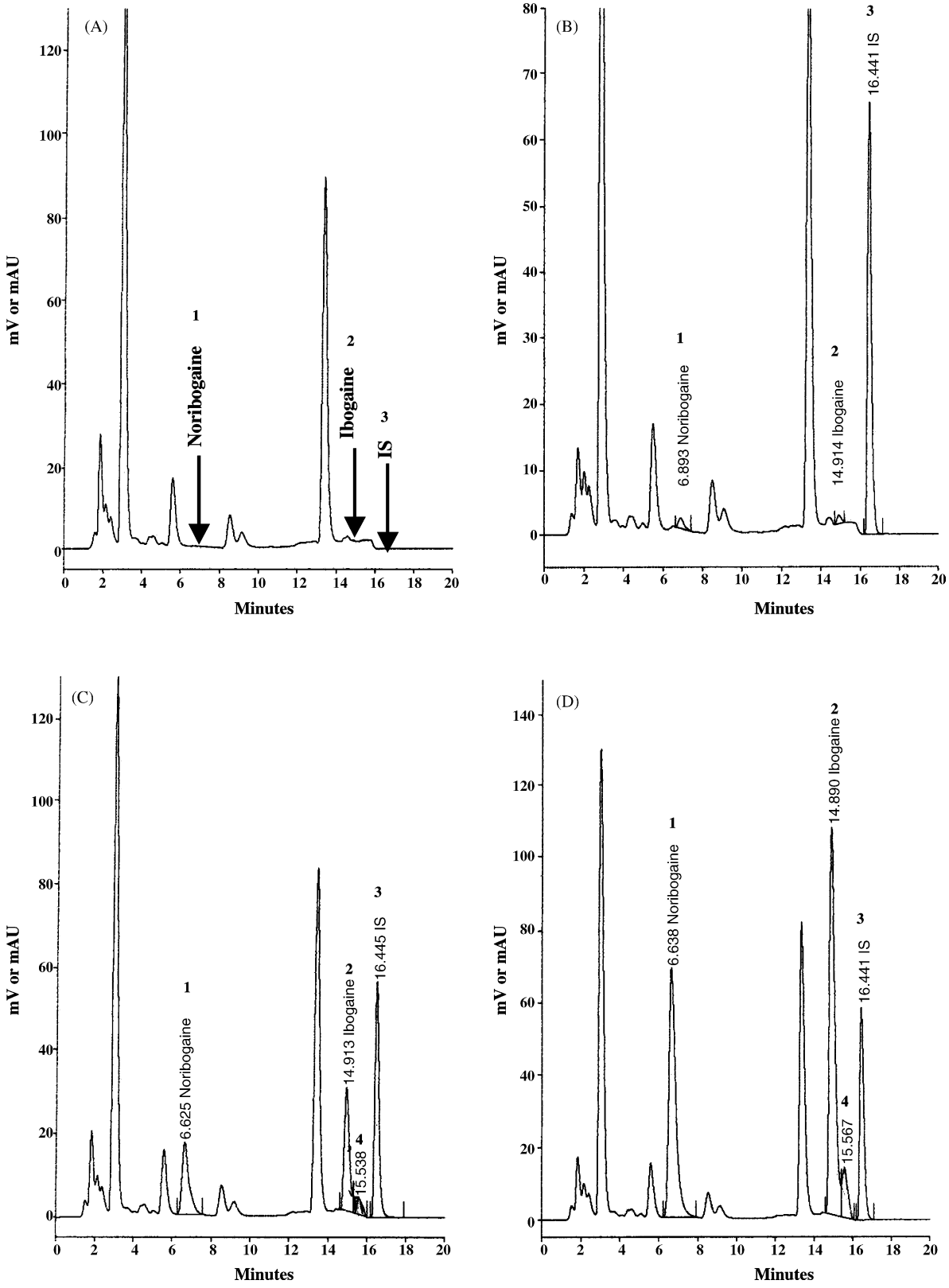


Fig. 2. Chromatograms of blank plasma (A); of blank plasma spiked with 0.89 ng/ml of ibogaine and 1 ng/ml of noribogaine (B), 44.7 ng/ml of ibogaine and 50 ng/ml of noribogaine (C), and 179 ng/ml of ibogaine and 200 ng/ml of noribogaine (D). Peak 1 is noribogaine, peak 2 is ibogaine, peak 3 is the internal standard and peak 4 is an additional product present in ibogaine hydrochloride. For chromatographic conditions see text.

3.2. Linearity

The regression analysis between peak area ratios of ibogaine and noribogaine over the internal standard and plasma concentrations revealed that the method is linear. The correlation coefficients (r) for calibration curves were equal to or better than 0.997. Intra-assay reproducibility was determined for calibration curves prepared the same day in replicate ($n = 7$) using the same stock solutions. Inter-assay reproducibility was determined for calibration curves prepared on different days (1 calibration curve per day over 12 successive days). The mean regression equations are presented in Table 2. For each point of calibration standards, the concentrations were back-calculated from the equation of the linear regression curves and the percent R.S.D. values were computed. Intra- and inter-day variability's at concentrations of calibration standards are presented in Table 3. The linearity of this method was statistically confirmed. The "Lack of Fit" test showed no significant deviation from linearity. Moreover, the residuals (difference between nominal and back-calculated concentrations) were normally distributed and centred around zero. The bias values on residuals, −0.044 ng/ml for ibogaine and 0.18 ng/ml for noribogaine, were not statistically different from zero and the 95% confidence intervals (−0.54/0.46, −0.21/0.56, respectively) included the zero value.

3.3. Specificity

Specificity of this method has been demonstrated by the representative chromatogram in drug-free human plasma (Fig. 2A), which indicated that each analyte was well resolved from the human plasma endogenous material peaks. There were no interferences at the retention times of the compounds of interest.

A number of common drugs of abuse were examined for possible interference with the HPLC method. None of the compounds coeluted with ibogaine, noribogaine and fluorescein.

3.4. Precision, accuracy and recovery

The SPE and subsequent HPLC analysis displayed excellent intra- and inter-assay precision and accuracy values. Table 4 lists the method validation results; the precision (R.S.D.) derived from mean data in the day-to-day (inter-assay, $n = 12$) study varied between 6.0 and 12.5% and the accuracy was 95.4–104%.

The mean extraction efficiencies of ibogaine and noribogaine from human plasma were 94.2 ± 5.35% and 96.9 ± 3.3% ($n = 24$), respectively and were independent of concentration over the range of concentrations studied, an observation which contributes to the good precision and accuracy of the method.

The mean recovery of internal standard averaged 95.0 ± 8.66% ($n = 24$).

Table 2
Assay linearity for ibogaine and noribogaine

	Ibogaine			Noribogaine		
	Mean correlation coefficient of the linear regression analysis[a]	*b*, Mean slope	*a*, Intercept ± S.D.	Mean correlation coefficient of the linear regression analysis[a]	b, Slope	*a*, Intercept ± S.D.
Intra-day reproducibility ($n = 7$)	0.998, R.S.D. = 0.13%	0.016, R.S.D. = 6.3%	0.005 ± 0.032	0.998, R.S.D. = 0.13%	0.010, R.S.D. = 5.0%	0.0035 ± 0.021
Inter-day reproducibility ($n = 12$)	0.998, R.S.D. = 0.20%	0.013, R.S.D. = 11.6%	0.016 ± 0.038	0.998, R.S.D. = 0.12%	0.010, R.S.D. = 11.3%	0.010 ± 0.0257

[a] Linear unweighted regression, formula: $y = a + bx$; R.S.D.: relative standard deviation.

Table 3
Back-calculated concentrations from calibration curves performed in human plasma

Ibogaine[a]			Noribogaine		
Theoretical concentration (ng/ml)	R.S.D. (%)	Recovery (%)	Theoretical concentration (ng/ml)	R.S.D. (%)	Recovery (%)
Intra-assay reproducibility ($n = 7$)					
0.89	11.9	96.5	1	15.2	102
4.47	6.4	102	5	6.5	102
8.95	4.2	94.6	10	4.2	94.6
17.9	2.4	97.9	20	2.0	98.1
44.7	0.68	100	50	0.81	100
67.1	3.1	98.8	75	2.7	98.0
89.5	7.2	99.4	100	7.2	99.5
179	0.96	100	200	1.1	100
Inter-assay reproducibility ($n = 12$)					
0.89	16.1	103	1	13.2	96.3
4.47	5.9	104	5	7.9	99.0
8.95	7.7	96.4	10	9.6	93.9
17.9	10.1	95.7	20	5.2	97.4
44.7	7.1	106	50	2.2	101
67.1	7.5	104	75	4.8	100
89.5	5.8	102	100	3.0	99.7
179	1.6	98.9	200	0.80	99.5

[a] Expressed in free base equivalent.

3.5. Lower limit of quantitation

The LLOQs were 0.89 ng/ml for ibogaine and 1 ng/ml for noribogaine and were chosen as the lowest concentrations of the standard calibration curves. At these levels, precision was ≤17% and accuracy was 95–105%.

3.6. Stability studies

When stored at 4 °C for a period of 6 months, stock solutions of ibogaine and noribogaine did not reveal any appreciable degradation. In aqueous solution containing ibogaine and noribogaine at concentrations of 22.4 and 25 ng/ml, respectively, the two compounds were stable for 4 h at 20 °C (without daylight exposure) and for 72 h at 4 °C in a refrigerator. At 20 °C with daylight exposure, ibogaine and noribogaine showed a monoexponential decline in drug concentrations; the corresponding half-lives were 81.5 min for ibogaine and 11 min for noribogaine (Fig. 3). After 80 min of storage, concentrations were 11.5 ng/ml for ibogaine and <1 ng/ml for noribogaine. Under the chromatographic conditions described above, none degradation products were detected.

After bench-top storage at room temperature for 6 h (with daylight protection), ibogaine (whatever the concentration studied) and noribogaine (37.5 and 150 ng/ml) were stable in plasma for 6 h. At each time studied, no statistical difference appeared by comparison with the reference values. At a concentration of 2.5 ng/ml, the noribogaine was stable for 2 h; after 4 and 6 h of storage, mean percent recoveries were 76.8% (R.S.D., 8.8%) and 71.6% (R.S.D., 5.5%), respectively. At +4 °C, ibogaine and noribogaine were stable in plasma for 6 h.

Frozen at −20 and −80 °C ibogaine and noribogaine were stable for at least 3 months, compared to the reference values, no statistical difference appeared. Mean recoveries ranged from 93 to 108%.

Table 4
Accuracy and precision of the HPLC assay

Ibogaine[a]			Noribogaine		
Theoretical concentration (ng/ml)	Precision (%)	Accuracy (%)	Theoretical concentration (ng/ml)	Precision (%)	Accuracy (%)
Within-day precision and accuracy ($n = 7$)					
2.24	5.5	115	2.5	14.8	104
33.6	4.4	101	37.5	5.2	102
134.2	4.4	98.1	150	4.0	98.4
Between-day precision and accuracy ($n = 12$)					
2.24	12.5	98.7	2.5	10.1	104
33.6	8.7	101	37.5	8.5	95.4
134.2	9.9	99.2	150	6.0	99.7

[a] Expressed in free base equivalent.

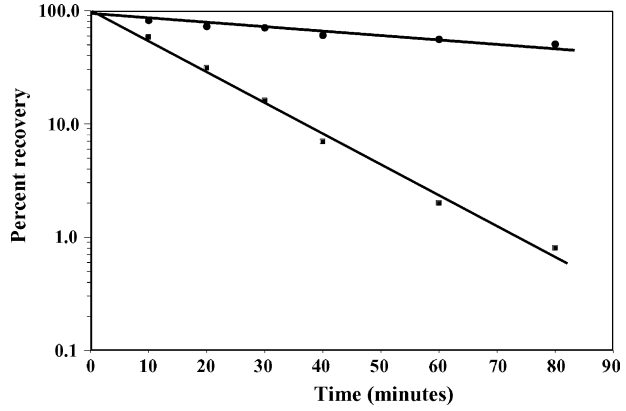


Fig. 3. Stability of ibogaine (22.4 ng/ml, ●) and noribogaine (25 ng/ml, ■) in aqueous solution at 20 °C with daylight exposure.

For ibogaine and noribogaine, at least three freeze–thaw cycles can be tolerated without losses higher than 10%. After the third freezing–thawing process, mean percent recoveries ranged from 94.3 to 101.7% for ibogaine and from 96.1 to 105.8% for noribogaine.

All analytes were stable during the evaporation process. Run-time stability at 4 °C of reconstituted extracts originating from plasma (i.e., in the mobile phase) was determined for each concentration of calibration standard in replicate ($n = 5$). After 72 h, no significant losses occurred.

4. Discussion and conclusion

In this paper we describe an HPLC method to quantify simultaneously ibogaine and noribogaine in human plasma. The use of an internal standard allows the control of minor variations in the recovery during the pre-treatment step. The validation of this analytical method in plasma indicated excellent reproducibility. Assay performance was assessed both on the basis of the statistical characteristics of individual calibration lines and from the results of QC samples. The LLOQ values of 0.89 ng/ml for ibogaine and 1 ng/ml for noribogaine were lower than most published methods; nevertheless, for ibogaine, the LLOQ was similar than that obtained with GC-chemical ionization mass spectrometry by Ley et al. using 1 ml of plasma [23]. Distinct advantages of the present method include the simplicity and rapidity of sample preparation and chromatography, good resolution from endogenous compounds and co-administered drugs, accurate assay of large numbers of samples manually, good sensitivity and the requirement of only common instruments. In this paper, stability tests under various conditions have been performed. Special attention must be paid to sample handling to avoid light degradation of the compounds. The SPE procedure, with one step each for the sample loading, clean-up, and elution, can be easily automated either with robotisation or an automated sample preparation system. The present method validation results indicate that the performance characteristics of the method fulfilled the requirements for a sufficiently accurate and precise assay method to carry out pharmacokinetic studies.

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