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Liquid chromatography–electrospray mass spectrometry determination of ibogaine and noribogaine in human plasma and whole blood Application to a poisoning involving *Tabernanthe iboga* root

Violeta Kontrimavičiūtė[a,b], Hélène Breton[c,d,1], Olivier Mathieu[c,1], Jean-Claude Mathieu-Daudé[c], Françoise M.M. Bressolle[a,*]

[a] *Clinical Pharmacokinetic Laboratory, Faculty of Pharmacy, University Montpellier I, France*
[b] *Department of Analytical and Toxicological Chemistry, Kaunas University of Medicine, Faculty of Pharmacy, Kaunas, Lithuania*
[c] *Medical Pharmacology and Toxicology Unit, Lapeyronie Hospital, France*
[d] *INSERM U 454, Arnaud de Villeneuve Hospital, Montpellier, France*

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Abstract

A liquid chromatography/electrospray ionization mass spectrometry (LC-ESI-MS) method was developed for the first time for the determination of ibogaine and noribogaine in human plasma and whole blood. The method involved solid phase extraction of the compounds and the internal standard (fluorescein) from the two matrices using Oasis®HLB columns. LC separation was performed on a Zorbax eclipse XD8 C8 column (5 μm) with a mobile phase of acetonitrile containing 0.02% (v/v) trimethylamine and 2 mM ammonium formate buffer. MS data were acquired in single ion monitoring mode at *m/z* 311.2, 297.2 and 332.5 for ibogaine, noribogaine and fluorescein, respectively. The drug/internal standard peak area ratios were linked via a quadratic relationship to plasma (0.89–179 μg/l for ibogaine; 1–200 μg/l for noribogaine) and to whole blood concentrations (1.78–358 μg/kg for ibogaine; 2–400 μg/kg for noribogaine). Precision ranged from 4.5 to 13% and accuracy was 89–102%. Dilution of the samples had no influence on the performance of the method. Extraction recoveries were ≥94% in plasma and ≥57% in whole blood. The lower limits of quantitation were 0.89 μg/l for ibogaine and 1 μg/l for noribogaine in plasma, and 1.78 μg/kg for ibogaine and 2 μg/kg for noribogaine in whole blood. In frozen plasma samples, the two drugs were stable for at least 1 year. In blood, ibogaine and noribogaine were stable for 4 h at 4 °C and 20 °C and 2 months at −20 °C. The method was successfully used for the analysis of a poisoning involving *Tabernanthe iboga* root.

Keywords: Ibogaine; Noribogaine; Plasma and whole blood; Quantitation; LC-ESI-MS; Forensic samples

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-5H-6,9-methanopyrido[1′,2′:1,2]azepino[4,5-b]indole) is a naturally occurring indole alkaloid derived from the root bark of the African *Tabernanthe iboga* shrub and used for centuries in West African ceremonies [1]. This drug has previously been reported to have CNS stimulant, anxiogenic and hallucinogenic properties [2–4]. Recent studies, reported the efficacy of this drug in the treatment of drug addiction. Thus, ibogaine attenuated both dependence and withdrawal symptoms to a variety of abuse drugs including morphine, heroin, cocaine, amphetamine, alcohol and nicotine [1,5–18]. The neurochemical mechanisms explaining the antiaddictive properties of ibogaine are not clearly understood. Ibogain 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-5H-6,9-methanopyrido[1′,2′:1,2]azepino[4,5-b]indol-2-ol). Noribogaine is formed by the action of cytochrome P450 enzymes in the liver [19]. As ibogaine, this drug demonstrated to decrease morphine, cocaine and alcohol self-administration

* Corresponding author at: Laboratoire de Pharmacocinétique Clinique, Faculté de Pharmacie Montpellier, 15 Avenue Charles Flahault, B.P. 14491, 34093 Montpellier, Cedex 5, France. Fax: +33 4 6754 8075.
E-mail address: Fbressolle@aol.com (F.M.M. Bressolle).
[1] These authors contributed equally to this work.

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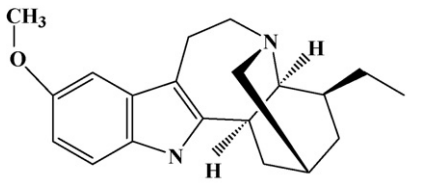
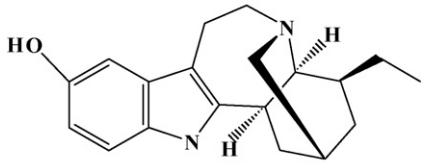


Ibogaine, 12-methoxyibogamine



Noribogaine, 12-hydroxyibogamine

Fig. 1. Chemical structures of ibogaine and noribogaine.

in rat [1,16–18]. However, noribogaine appears less likely to produce the adverse effects associated with ibogaine (i.e., tremors and stress-axis activation), suggesting that the metabolite may be a safer alternative for medication development [18]. Structures of these compounds are presented in Fig. 1.

Several analytical methods have been published to quantify ibogaine in biological matrices [20–29]. Both thin-layer chromatography and gas chromatography have been used. These methods involved liquid–liquid or solid-phase extraction of the biological samples and in the majority of cases, derivatization of the compound was required. Only two methods reported the quantitation of both ibogaine and noribogaine [26,27]. A method to determine opiate agonists including ibogaine by liquid chromatography-atmospheric-pressure chemical-ionization mass spectrometry has been also described [28]. However, the possible interference with noribogaine was not studied by the authors. Recently, a high-performance liquid chromatographic (LC) method with fluorimetric detection was developed in our laboratory for the simultaneous quantitation of ibogaine and noribogaine in human plasma [29]. In this paper, extensive stability testing was undertaken using a wide range of storage conditions, and the method gave good results. However, the use of mass spectrometry coupled with LC in forensic toxicological analysis is more relevant due to the high specificity of mass spectrometry. At the present time, no bio-analytical methods have been described and validated for the determination of ibogaine and noribogaine in the whole blood.

The objective of this study was to develop and validate a robust, reliable, specific and sensitive liquid chromatography-electrospray mass spectrometry (LC-ESI-MS) method for the simultaneous quantitation of ibogaine and noribogaine in human plasma and whole blood that can be used in forensic toxicology. The method was validated according to validation procedures, parameters and acceptance criteria based on USP XXIII and FDA guidelines [30–33]. The method was successfully applied to the analysis of a poisoning case involving the root bark of the *Tabernanthe iboga* shrub.

2. Materials and methods

2.1. Chemicals 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 (molecular weight, 296.4) was kindly supplied by Reform Italia (Endine, Italy). Ibogaine and noribogaine were stored protected from light at normal room temperature (20 °C). HPLC-grade acetonitrile, methanol and acetic acid were obtained from Carlo Erba (Val de Reuil, France). The formate buffer solution (pH 3) consisted of ammonium formate (126 mg/l) in purified water.

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

For method validation, whole blood and human plasma were obtained from pooled blood samples collected from healthy volunteers not undergoing drug therapy (Etablissement Français du sang, Montpellier, France). Coagulation was prevented by adding EDTA-sodium salt. The blood was centrifuged at 2000 × *g* for 10 min to obtain plasma. The drug-free whole blood and plasma were stored at −20 °C and then used during the study in the preparation of standards and quality control (QC) samples.

Stock solutions of ibogaine hydrochloride and the internal standard were prepared by dissolving accurately weighed amounts of the drugs in purified water to give solutions containing 89.5 and 81 mg/l of free form equivalents of each compound, respectively. A stock solution of noribogaine was prepared in methanol at concentration of 100 mg/l. For each compound, two separate stock standard solutions were prepared; one of which was used for the preparation of the calibration curve standards and the other for the preparation of quality control (QC) samples. Stock solutions were stored at 4 °C and protected from light.

2.2. Instrumentation

Liquid chromatography-mass spectrometry (LC-MS) analysis was performed using an Agilent 1100 quadrupole mass spectrometer equipped with an electrospray interface and a data acquisition station (HPChem software, version 08.04) (Agilent Technologies, Les Ulis, France). The mass spectrometer was coupled to a Hewlett Packard LC system equipped with a quaternary pumping unit, a diode-array UV detector and an autosampler with a loading valve fitted with a 100-μl sample loop (Interchim, Montluçon, France) and set at 4 °C.

Optimization of various experimental parameters including the type of stationary phase, eluent composition, nature of the organic modifier, capillary voltage, nebulizer pressure and sampling cone voltage was carried out (data not shown).

Separation of the analytes was performed at room temperature (20 °C) on a Zorbax eclipse XD8 C8 column [150 × 4.6 mm (i.d.)] packed with 5 μm particles (Agilent Technologies, Palo Alto, CA, USA). A C_{18} symmetry column [20 × 3.9 mm (i.d.), 5-μm particles] obtained from Waters (Paris, France) was used

Table 1
Variations in proportions of solvents A and B

Time (min)	Percentage of solvent A	Percentage of solvent B
0	15	85
5	35	65
11.2	50	50
15	80	20
16	80	20
20	15	85

as guard column. A 20-min mobile phase gradient was used. Mobile phase A was 0.02% (v/v) trimethylamine in acetonitrile and mobile phase B consisted of 2 mM formate buffer (pH 3). Table 1 shows the variations in the proportions of solvents A and B. The flow rate started at 1 ml/min then decreased to 0.5 ml/min from 1 to 5 min and remained unchanged for 6.2 min. It increased directly to 1 ml/min in the next 4.8 min and then remained stable. The injection volume was 20 μl.

The mass spectrometer was calibrated in the positive ion mode (ESI+) using a mixture of NaI and CsI (peak width of the mass: 0.6–0.7 amu). The MS system was operated with a capillary voltage of 4.0 kV and a cone voltage of 135 V. The drying gas temperature and flow were maintained at 350 °C and 10 l/min, respectively and the nebulizer pressure was set at 13 psi. Mass spectrometric data were acquired in single ion monitoring (SIM) mode (SIM dwell time: 98 ms).

2.3. Working standards

Working solutions of ibogaine and noribogaine were prepared daily in light-protected vials. They were obtained by diluting the stock solutions with purified water to obtain 12 working standards ranging from 0.0224 to 44.7 mg/l for ibogaine and 0.025 to 50 mg/l for noribogaine. These solutions were used to spike the plasma or whole blood samples prior to extraction. The stock solution of fluorescein was diluted 4-fold (20.25 mg/l) in purified water before use.

A reference standard solution containing ibogaine (22.4 μg/l) and noribogaine (25 μg/l) was prepared daily in a mixture of 1 ml/l TFA in water and acetonitrile (85:15, v/v), and injected before each run to verify the performance of the LC-ESI-MS system. Using diode-array and MS (scan mode) detection, in sequence peaks corresponding to the two analytes were identified. The number of theoretical plates, capacity factors, peak shew and the resolution of the analytes were calculated from the chromatogram obtained by UV detection.

2.4. Preparation of standards and QC samples

Calibration standards were prepared in drug-free human plasma (0.5 ml) or drug-free human whole blood (0.25 g diluted with 0.25 ml of purified water). The calibration set consisted of eight concentrations, prepared by spiking drug-free matrices with 20 μl of the appropriate working solutions. Concentration ranges were 0.89 to 179 ng/ml for ibogaine and 1 to 200 ng/ml for noribogaine in plasma, and 1.78 to 358 ng/g for ibogaine and 2 to 400 ng/g for noribogaine in whole blood. QC samples were prepared in the same way as the calibration standards by mixing drug-free plasma or whole blood with appropriate volumes of working solutions to obtain three different concentrations, low, medium and high (ibogaine: 2.24, 33.6 and 134.2 ng/ml in plasma, and 4.48, 67.2 and 268.4 ng/g in whole blood; noribogaine: 2.5, 37.5 and 150 ng/ml in plasma and 5, 75 and 300 ng/g in whole blood).

Before the sample pretreatment procedure, standards and QC samples previously vortex-mixed (10 s) were incubated at 20 °C protected from light, for 20 min, to allow a steady state with the matrix components.

2.5. Sample preparation

Plasma and whole blood samples were subjected to solid-liquid extraction (SPE) for the removal of proteins and interfering components; fluorescein was used as the internal standard. During the SPE procedure the vacuum apparatus was kept under a plastic black cover in order to protect the products from light. The 1 ml Waters Oasis HLB cartridges were conditioned with 1 ml of methanol followed by 1 ml of distilled water prior to use.

A 0.5 ml aliquot of plasma was mixed with 20 μl of internal standard (fluorescein) solution (20.25 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 column was then rinsed with 2× 1 ml of purified water and dried for 2 min by vacuum aspiration (approximately 27 kPa). The elution was carried out with 2× 1 ml of methanol under a light vacuum (approximately 86 kPa). The eluate fractions were evaporated to dryness under a stream of nitrogen for 50 min at 40 °C. The dried residue was reconstituted in 100 μl of a mixture of 1 ml/l TFA in water and acetonitrile (85:15, v/v). A 20-μl aliquot was injected onto the system.

Twenty microliters of internal standard (20.25 mg/l) were added to 0.5 ml of diluted whole blood samples (0.25 g of whole blood plus 0.25 ml of purified water) and mixed with 0.5 ml of 10% methanol in water. The methanolic solution was added drop-wise while the mixture was vortex-mixed in order to obtain smaller precipitate particles which avoid significant analyte loss. The mixture was centrifuged at 4 °C for 10 min at 17,562 × *g*. Thereafter, the assay procedure was as described above for the plasma samples.

2.6. Data analysis

Analyte-to-internal standard peak area ratios were calculated and calibration curves were constructed from the calibration standard data using a quadratic equation, $Y = aX^2 + bX + c$, in which Y is the peak area ratio and X is the concentration of the analyte. The regression curve was not forced through zero. The resulting a, b and c parameters were used to determine back-calculated concentrations, which were then statistically evaluated [30–33]. The normal distribution of the residuals

(the difference between nominal and back-calculated concentrations) was verified. Moreover, the mean residual value (or mean predictor error) was calculated and compared to zero (Student's *t*-test); the 95% confidence interval was also determined.

2.7. Ion suppression study

The absence of ion suppression attributable to the matrix effects was demonstrated by the method of Matuszewski [34]. For this procedure, ten different batches (from 10 different donors) of both drug-free matrices were treated as described above in duplicate ($n = 20$ per matrix and per concentration studied). The dried extracts obtained from plasma and whole blood were reconstituted with 100 μl of a mixture of 1 ml/l TFA in water and acetonitrile (85:15, v/v), then enriched with 168 and 671 ng/ml for ibogaine and 187.5 and 750 ng/ml for noribogaine, and with 4.05 μg/ml of the internal standard. A reference solution comprising 100 μl of the TFA–acetonitrile–water mixture was also enriched with the three drugs at the same nominal concentrations. The reconstituted extracts and reference solutions were injected onto the analytical column. Peak areas of ibogaine, noribogaine and internal standard obtained from the reconstituted extracts were compared with the corresponding peak areas produced by the reference solutions ($n = 6$ injections). The peak area ratios (reconstituted extracts in the two studied matrices/reference solutions) were as follows: ibogaine, 0.95 (R.S.D., 5%) in plasma, 0.96 (R.S.D., 8%) in blood; noribogaine, 1.05 (R.S.D., 6%) in plasma, 1.05 (R.S.D., 10%) in blood; and internal standard, 1.01 (R.S.D., 4.5%) in plasma, 0.98 (R.S.D., 3.2%) in blood. These findings confirmed that the matrix had no influence on the detection of either ibogaine, noribogaine or the internal standard.

2.8. Validation procedure

The validation criteria considered here were specificity, accuracy, precision, extraction recovery and lower limit of quantitation. During the validation process it was decided to use two different columns. Half of samples were analyzed on the first column and the remainders were analyzed on the second column.

The specificity of the analytical methods was determined by the analysis of ten different independent sources of the same biological matrix obtained from ten different donors. The retention times of endogenous compounds in the matrices were compared with those of the compounds of interest.

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

Between-day precision and accuracy of the assay were assessed by performing replicate analyses of QC samples (ibogaine: 2.24, 33.6 and 134.2 ng/ml in plasma, and 4.48, 67.2 and 268.4 ng/g in whole blood; noribogaine: 2.5, 37.5 and 150 ng/ml in plasma and 5, 75 and 300 ng/g in whole blood) against a calibration curve. The accuracy was evaluated as [mean found concentration/nominal concentration] × 100. Precision was given by the percent relative standard deviation (R.S.D.).

In order to test whether it is possible to apply the described method to samples whose concentrations are higher than the highest calibration point, the following spiked samples were prepared: ibogaine, 447 and 895 ng/ml in plasma and 894 and 1790 ng/g in whole blood; noribogaine, 500 and 1000 ng/ml in plasma and 1000 and 2000 ng/g in whole blood. They were diluted 5- and 10-fold with drug-free human matrices in order to bring concentration within the range of standard curve. Each analysis was performed 10–14 times for each concentration, using calibration curves and QC samples. The found concentrations were reported and compared to the nominal concentrations by calculating accuracy and precision.

Extraction recoveries of ibogaine and noribogaine from plasma and whole blood were measured 3 times at each QC sample concentration by calculating the percentage difference between the peak areas of extracted QC samples and those of authentic (unextracted) standards in the relevant concentration range prepared in a mixture of water containing 1 ml/l TFA and acetonitrile (85:15, v/v). The extraction recovery was also determined for the internal standard.

The lower limit of quantitation (LLOQ) estimated from QC samples was defined as the lowest drug concentration which can be determined with a R.S.D. ≤ 20% and an accuracy between 100 ± 20% on a day-to-day basis [30–33].

2.9. Stability studies

The stability of stock solutions was determined at 4 °C over a span of 1 year.

Short-term (6 h) and long-term (2 months) stability assays in plasma have been previously published [29]. We have shown that at 20 °C with daylight exposure, ibogaine and noribogaine concentrations declined rapidly. Therefore, special attention must be paid during sample handling to avoid photo-degradation of the compounds. In this report, the stability of ibogaine and noribogaine in plasma was determined at −20 and −80 °C over a 1-year period.

Stability assays in whole blood were performed using QC samples at the following concentrations: ibogaine: 4.48, 67.2 and 268.4 ng/g; noribogaine: 5, 75 and 300 ng/g. These QC samples were analyzed, against a calibration curve, immediately following preparation, then after 0.5, 1 and 4 h storage under both routine laboratory conditions (20 °C protected from light) and at 4 °C. The stability of the two drugs in human whole blood stored at −20 °C was also investigated. For this experiment, QC samples spiked with appropriate concentrations of ibogaine and noribogaine were aliquoted into polypropylene tubes and stored at −20 °C. At specific time intervals (0.5, 1 and 2 months), three replicates of each spiked samples were thawed at room temperature and thoroughly vortex-mixed. They were analysed alongside replicates of identical concentrations of freshly prepared samples against a calibration curve. The stability during up to three freeze–thaw cycles was investigated. Run-time stability at room temperature and at 4 °C over 24 h for processed whole blood samples after extraction was also determined for each calibration point.

Each result represents the mean of three separate samples. Compounds were considered stable when losses were <15%.

2.10. Application of the method to a poisoning involving ingestion of root bark of the Tabernanthe iboga shrub

The inquest of a 48-year-old Caucasian male, with a history of drug abuse who appeared to have died by poisoning, suggested that he had ingested powdered root bark of *Tabernanthe iboga* mixed with sweet concentrated milk. A 250 μl aliquot of sub-clavian blood drawn at the death scene was subjected in a preliminary (screening) step to the SPE procedure described above, and analyzed using diode-array and MS (scan mode at two cone voltages: 135 and 180 V) detection in sequence to identify peaks present on the chromatogram and to verify that each observed peak elutes free from potential interferences. In the second step, ibogaine and noribogaine were quantified by LC-MS (SIM mode) using 8 μg of blood sample diluted to 250 μl with drug-free blood matrix.

3. Results

3.1. Mass spectra

Noribogaine, ibogaine and fluorescein were characterized by their protonated species $[M+H]^+$ at *m/z* 297.2 (Fig. 2A), *m/z* 311.1 (Fig. 2B) and *m/z* 333 (Fig. 2C), respectively. Fragment ions were observed at *m/z* 122.2 and 174 for ibogaine, and *m/z* 122.2 and 160 for noribogaine. In accordance with the paper published by Taylor [35], the fragmentation scheme is presented in Fig. 2. Mass spectrometric data were acquired in the SIM mode at *m/z* 311.1, 297.2 and 333 for ibogaine, noribogaine and fluorescein, respectively.

3.2. Retention times and specificity

Fig. 3 shows typical chromatograms obtained from extracts of drug-free human matrices spiked with the three analytes. Ibogaine (retention time $t_r = 10.2 \pm 0.011$ min, $n = 20$), noribogaine ($t_r = 6.74 \pm 0.018$ min, $n = 20$) and fluorescein ($t_r = 14.7 \pm 0.005$ min, $n = 20$) exhibited well separated, narrow and symmetrical peaks under the chromatographic conditions described. The number of theoretical plates (calculated from the ibogaine peak) was approximately 8800. The column was replaced when the number of theoretical plates decreased below 4900 (after 700 analyses).

The specificity of this method was demonstrated by representative chromatograms of blank matrices (Fig. 4), which indicated that each analyte was well resolved from the human matrix endogenous peaks. No interference was found with any tested drugs.

3.3. Drug/response relationship

For all compounds, the quadratic regressions indicated a mean coefficient of determination higher than 0.996. For each compound, mean parameters of the quadratic equation are presented in Table 2. For each point on the calibration curves,

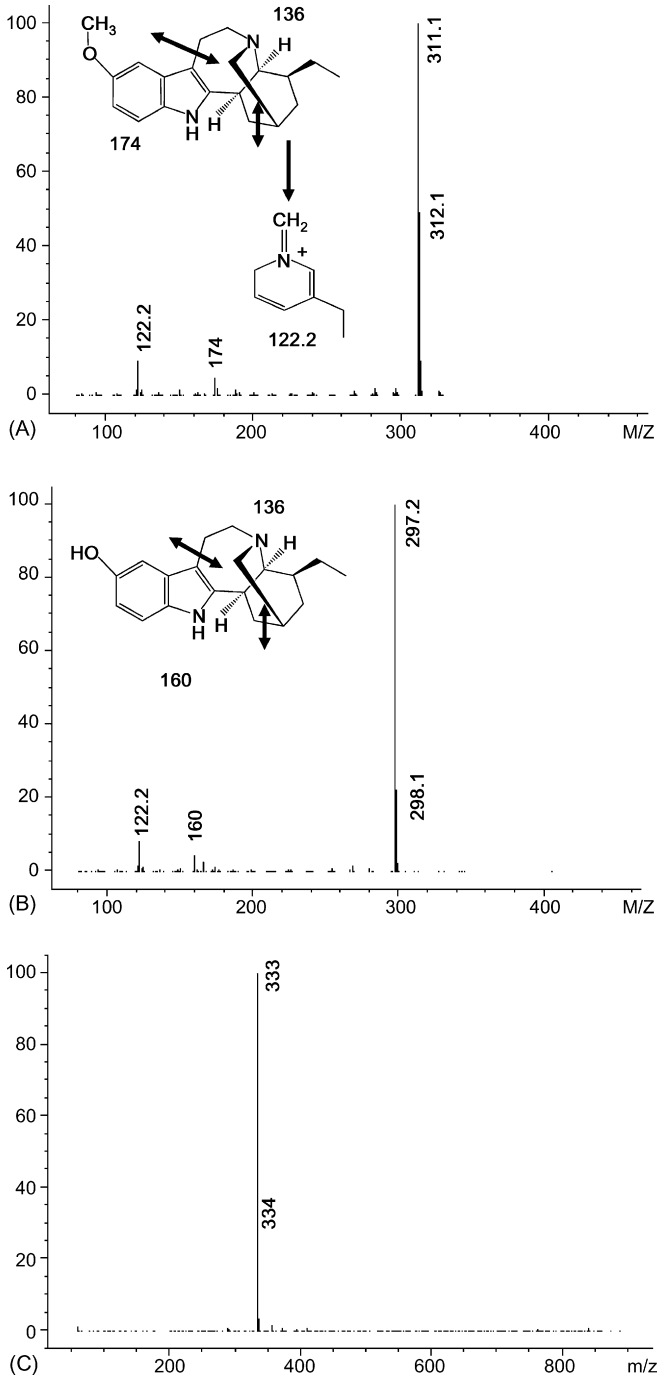


Fig. 2. Mass spectra (scan mode) of (A) ibogaine, (B) noribogaine and (C) fluorescein.

the concentrations were back-calculated from the corresponding quadratic equation parameter, and mean ± S.D. values were calculated. The results are presented in Table 3. For each analyte, the goodness of fit between back-calculated concentrations and nominal concentrations was statistically evaluated (i) by comparing the regression line of back-calculated versus nominal concentrations to the reference line (slope = 1 and intercept = 0); no significant different was observed; (ii) by studying the frequency distribution histogram of the residuals, which were normally distributed and centered around zero, the number of positive and negative values being approximately equal; and (iii) by compar-

ing the bias to zero; a *t*-test showed that this parameter was not statistically different from zero; moreover, the 95% confidence interval included the zero value.

3.4. Extraction efficiency, precision, accuracy and lower limit of quantitation (LLOQ)

Extraction recoveries in plasma as previously published [29], were satisfactory for all substances: 94.2 ± 5.35% for ibogaine, 96.9 ± 3.3% for noribogaine and 95.0 ± 8.7% for the internal standard ($n = 24$). Extraction recoveries in whole blood ($n = 14$) were adequate, 57% (R.S.D., 7.1%) for ibogaine, 62% (R.S.D., 5.4%) for noribogaine and 81% (R.S.D., 3.5%) for the internal standard.

SPE extraction and subsequent LC-ESI-MS analysis displayed excellent inter-assay precision and accuracy for either ibogaine and noribogaine. Table 4 lists the method validation results. At low, medium and high concentrations, precision

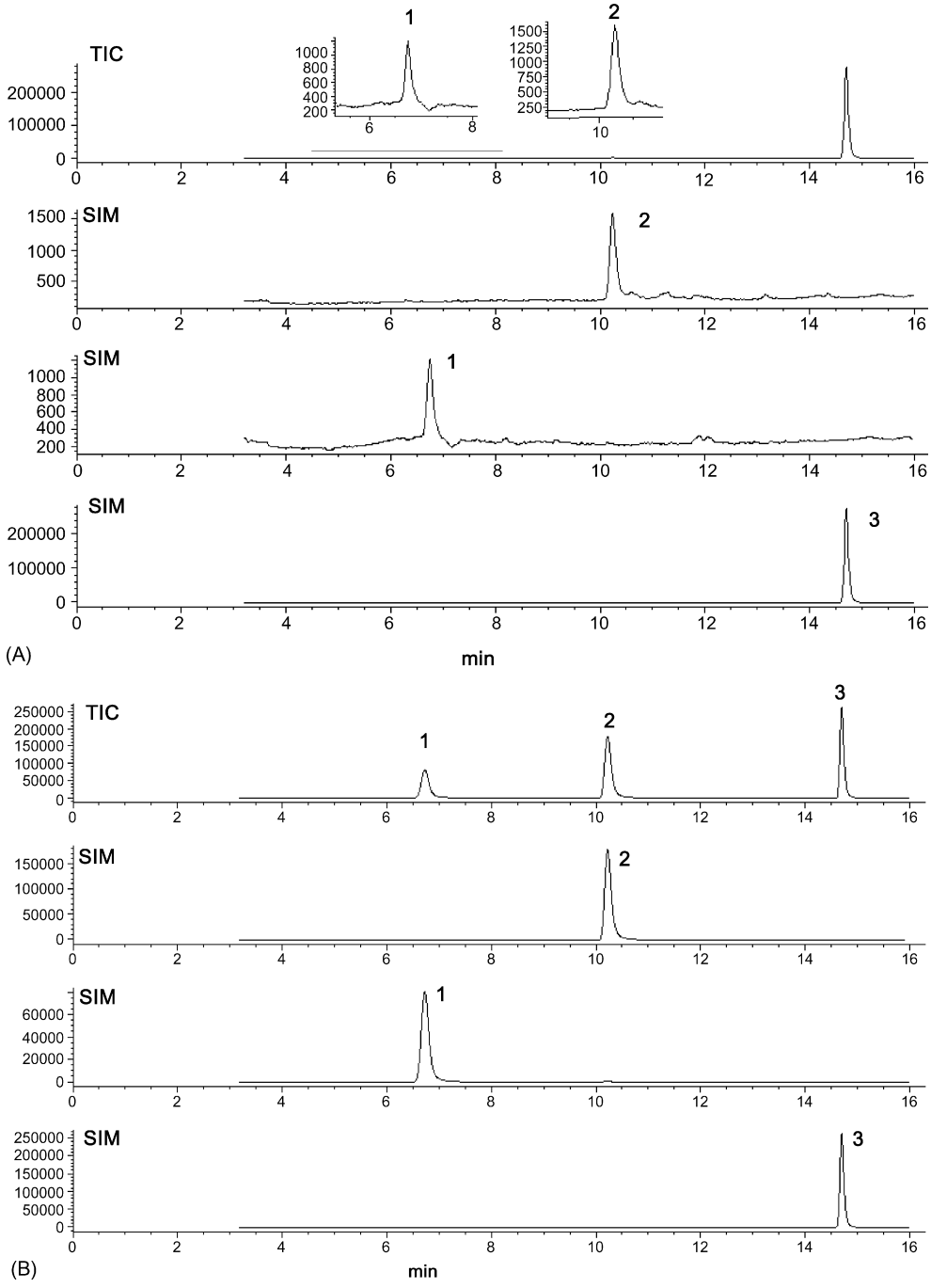


Fig. 3. Typical mass chromatograms of blank human matrices spiked with the two analytes at the following concentrations: (A) 0.89 ng/ml of ibogaine and 1 ng/ml of noribogaine in plasma; (B) 179 ng/ml of ibogaine and 200 ng/ml of noribogaine in plasma; (C) 1.78 ng/g of ibogaine and 2 ng/g of noribogaine in whole blood; and (D) 358 ng/g of ibogaine and 400 ng/g of noribogaine in whole blood. For LC-MS conditions see instrumentation section. Peak 1 = noribogaine; peak 2 = ibogaine; peak 3 = internal standard (TIC, total ionic current; SIM, single ion monitoring).

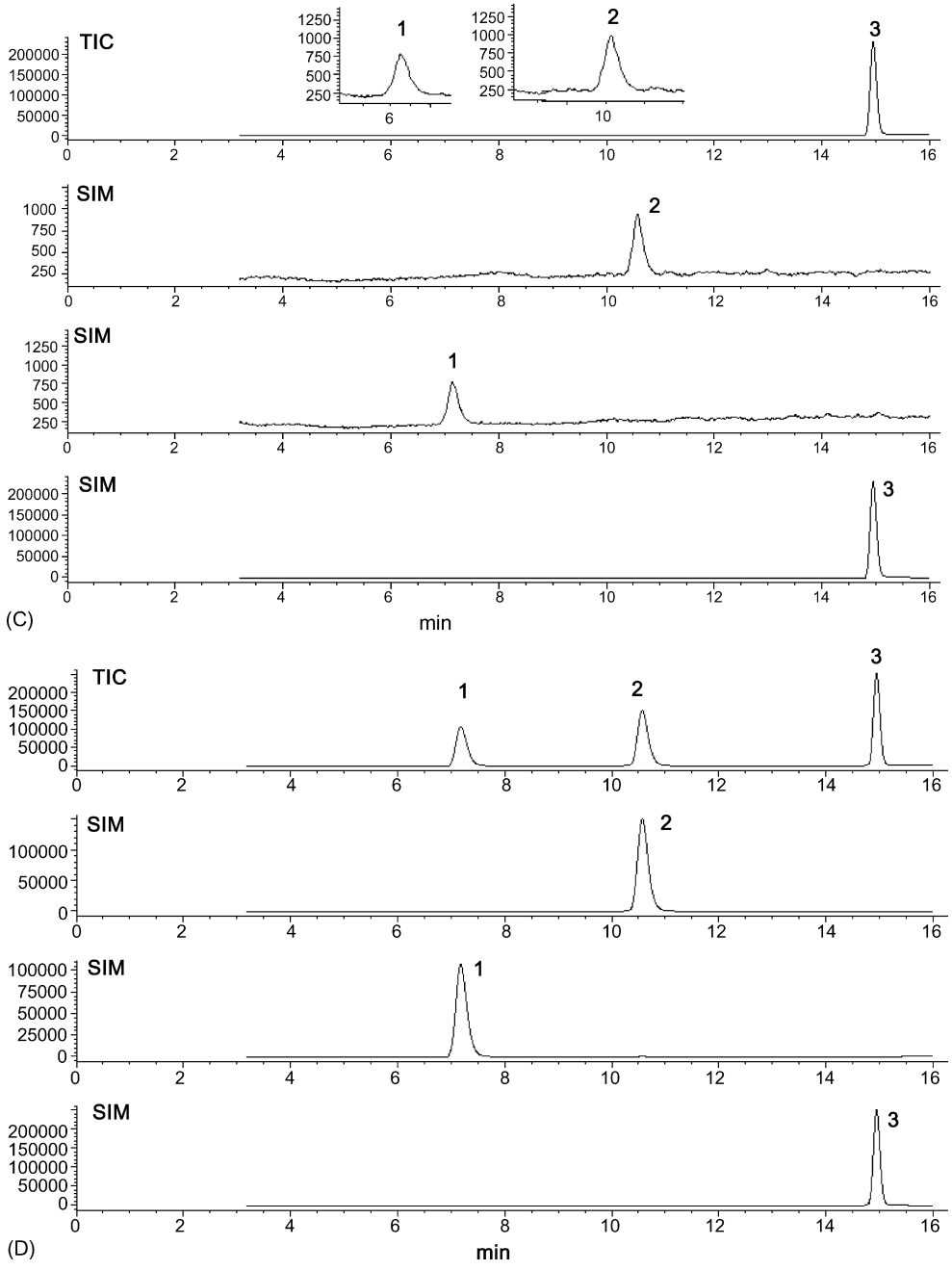


Fig. 3. (*Continued*).

Table 2
Results of calibration curves

	Ibogaine		Noribogaine	
	Mean correlation coefficient of the quadratic regression[a]	*b* (Mean)	Mean correlation coefficient of the quadratic regression[a]	*b* (Mean)
Human plasma ($n = 10$)	0.998	0.0129	0.9997	0.0079
R.S.D. (%)	0.038	5.9	0.033	14
Whole blood ($n = 14$)	0.998	0.0020	0.9999	0.0014
R.S.D. (%)	0.012	7.5	0.017	11

[a] $Y = aX^2 + bX + c$; R.S.D., relative standard deviation.

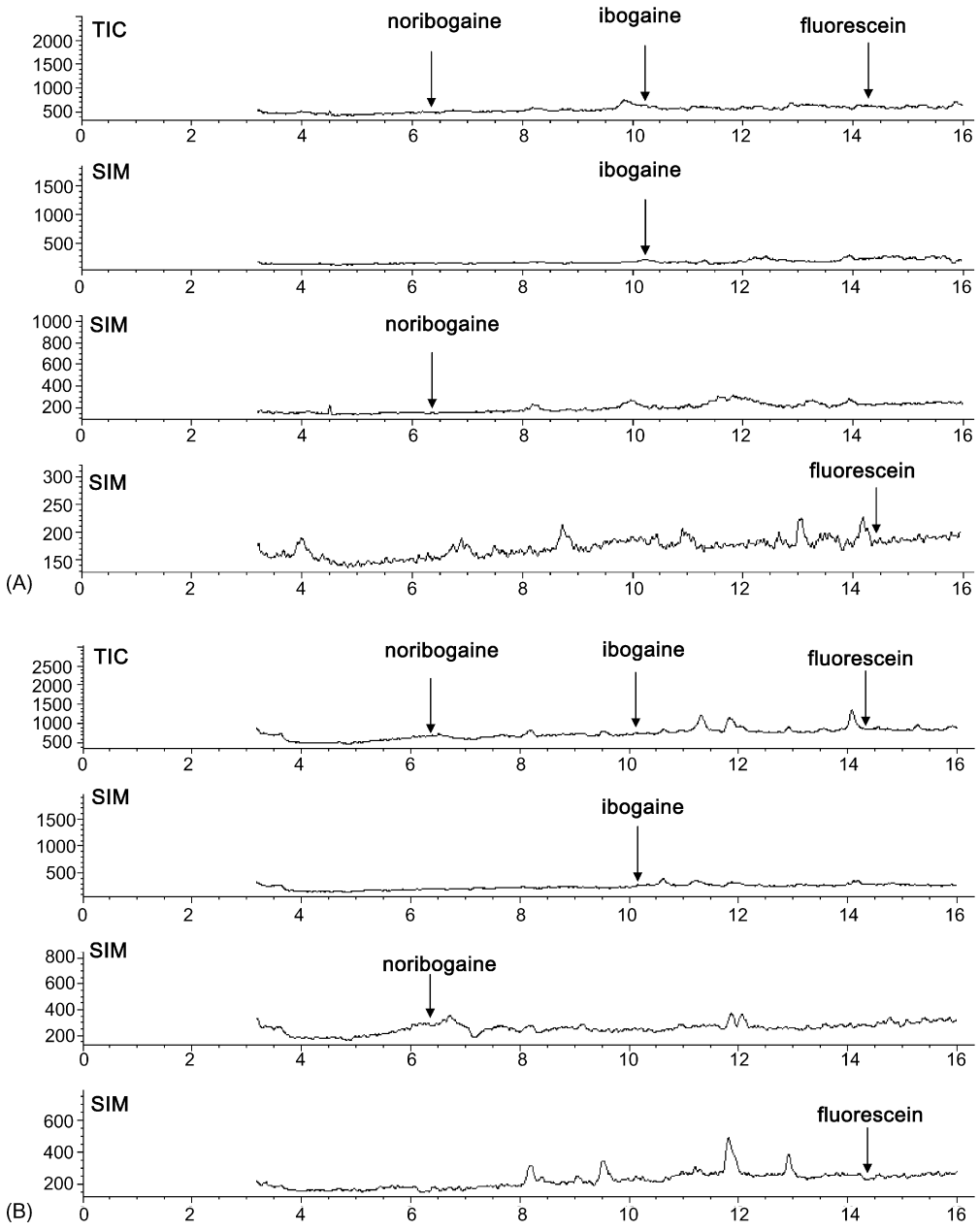


Fig. 4. Mass chromatograms obtained from (A) 0.5 ml of blank human plasma and (B) 0.25 g of blank human whole blood. For LC-MS conditions see instrumentation section (TIC, total ionic current; SIM, single ion monitoring).

(expressed as R.S.D.) varied between 4.5 and 13%. The measured concentrations of QC samples were found to be in good agreement with the actual concentrations; accuracy of measurements ranged from 89 to 102%.

Dilution has no influence on the performance of the method. Following 5- and 10-fold dilutions of plasma and whole blood, QC sample precision ranged from 4.3 to 9.5% and accuracy was 95–110% (Table 4).

Based upon the analysis of low concentration replicate standards in each validation run, the LLOQ values were 0.89 ng/ml for ibogaine and 1 ng/ml for noribogaine in plasma when using a 0.5 ml plasma sample for extraction and a 20 μl injection volume for LC-ESI-MS analysis. In whole blood, the corresponding values were 1.78 ng/g for ibogaine and 2 ng/g for noribogaine when using a 0.25 g whole blood sample for extraction and the same injection volume. At these levels, the precision was <14% R.S.D. and accuracy was 95–110%.

3.5. Stability studies

When stored at 4 °C in the refrigerator for a period of 1 year, stock solutions of ibogaine and noribogaine did not reveal any appreciable degradation. In QC plasma samples frozen at −20 and −80 °C, ibogaine and noribogaine were stable for at least 1 year; there was no statistical difference compared to the reference values. Mean recoveries ranged from 94 to 106%.

Data obtained for the stability assays of ibogaine and noribogaine in whole blood indicate that these two drugs were stable

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

Ibogaine[a]			Noribogaine		
Theoretical concentration (ng/ml or ng/g)	R.S.D. (%)	Recovery (%)	Theoretical concentration (ng/ml or ng/g)	R.S.D. (%)	Recovery (%)
Inter-assay reproducibility in human plasma ($n = 10$)					
0.89	8.1	110	1	9.8	105
4.47	5.7	98	5	3.9	98
8.95	8.0	93	10	5.9	99
17.9	4.9	94	20	6.1	98
44.7	6.1	105	50	5.7	100
67.1	9.1	105	75	7.0	100
89.5	4.5	100	100	4.3	98
179	1.2	99	200	2.2	99
Inter-assay reproducibility in whole blood ($n = 14$)					
1.78	14	107	2	12	95
8.94	8.2	91	10	8.9	93
17.9	5.1	105	20	8.8	105
35.8	6.8	105	40	7.8	109
89.4	4.4	101	100	3.7	100
134.2	2.4	101	150	2.0	99
179	1.7	99	200	5.4	101
358	0.7	99	400	0.9	100

[a] Expressed in free base equivalent.

under the tested conditions. Recovery values were in the range 90–107% at the three concentrations (ibogaine, 4.48, 67.2 and 268.4 ng/g; noribogaine: 5, 75 and 300 ng/g).

3.6. Application of the method in a poisoning case involving the root of Tabernanthe iboga

The screening analysis, using the analytical conditions described above, revealed the presence of six compounds at retention times of 6.0 min (C1), 7.3 min (C2), 9.0 min (C3), 9.6 min (C4), 10.7 min (C5) and 11.3 min (C6) (Fig. 5A, TIC data). Each peak elutes free from interferents and was well separated from the internal standard (fluorecein) peak. By comparison of the protonated molecule and qualifier ions (scan mode) obtained for each peak, at two cone voltages, with those obtained for the reference compounds, peak C2 was noribogaine, peak C5 was ibogaine and peak C6 was ibogamine. They were characterized by the protonated molecules $[M+H]^+$ at *m/z* 297.2, 311.1 and 281.2, respectively. A fragment ion was obtained at *m/z* 122.2 for the three compounds. Additional fragment ions were obtained at *m/z* 174 for ibogaine, *m/z* 160 for noribogaine and *m/z* 144.1 for ibogamine. The other three compounds could be attributed to the following oxidation products: desmethoxyiboluteine (C1, $[M+H]^+$ at *m/z* 313) and iboluteine (C4, $[M+H]^+$ at *m/z* 327), and to ibogaline (C3, $[M+H]^+$ at *m/z* 341) known to be one of the most quantitatively important iboga alkaloids after ibogaine in the root of *Tabernanthe iboga* [36]. Fragment ions characteristic of iboluteine [35,37] were obtained at *m/z* 122.2

Table 4
Accuracy and precision of the LC-ESI-MS assay

Ibogaine[a]			Noribogaine		
Theoretical concentration (ng/ml or ng/g)	Precision (%)	Accuracy (%)	Theoretical concentration (ng/ml or ng/g)	Precision (%)	Accuracy (%)
Human plasma ($n = 10$)					
2.24	11	89	2.5	9.3	100
33.6	9.2	98	37.5	6.0	97
134.2	6.5	100	150	5.4	97
447	5.5	109	500	5.7	100
895	6.6	110	1000	7.8	104
Whole blood ($n = 14$)					
4.48	12	97	5	13	101
67.2	4.5	92	75	7.6	102
268.4	7.4	96	300	7.9	97
894	9.5	100	1000	4.3	95
1790	7.7	96	2000	8.9	97

[a] Expressed in free base equivalent.

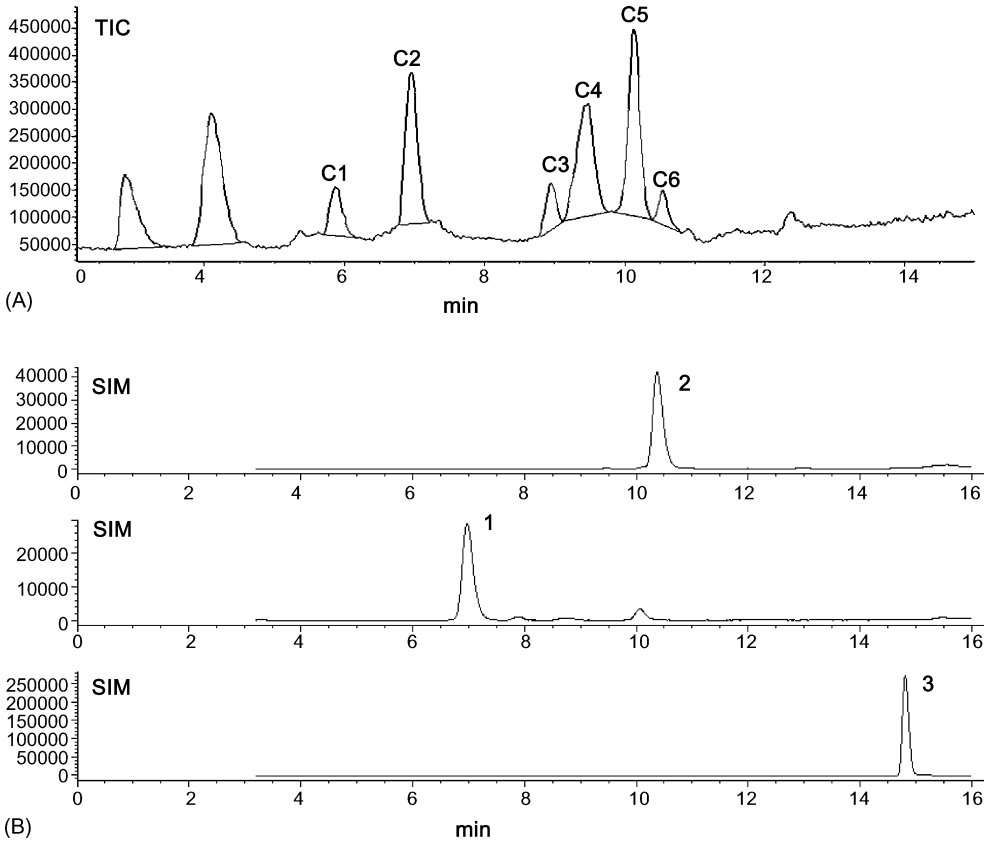


Fig. 5. Chromatogram obtained from sub-clavian blood drawn at the death scene (A) Total ionic current (TIC) from 250 mg of blood (screening analysis): peak C1 could be attributed to desmethoxyiboluteine ($[M+H]^+$ *m/z* 313), peak C2 = noribogaine ($[M+H]^+$ *m/z* 297.2), peak C3 could be attributed to ibogaline ($[M+H]^+$ *m/z* 341), peak C4 could be attributed to iboluteine ($[M+H]^+$ *m/z* 327), peak C5 = ibogaine ($[M+H]^+$ *m/z* 311.1) and peak C6 = ibogamine ($[M+H]^+$ *m/z* 281.2); (B) single ion monitoring (SIM) from 8 μg of blood (Peak 1 = noribogaine; peak 2 = ibogaine; peak 3 = internal standard). For LC-MS conditions see instrumentation section.

and 150.1 for both iboluteine and desmethoxyiboluteine; for ibogaline fragment ions were obtained at *m/z* 122.2 and 204.1. These mass spectra concur with the results published by Clivio et al. [37] and Taylor [35]; but due to the unavailability of reference substances for these compounds, their structures were not confirmed.

Using a 8-μg aliquot of blood sample (accurately weighed), concentrations found (SIM mode, Fig. 5B) were 10.8 ± 0.4 μg/g for ibogaine and 20.8 ± 3.0 μg/g for noribogaine ($n = 3$).

4. Discussion and conclusion

This paper reports for the first time, the development of a fully validated LC-ESI-MS assay for the simultaneous measurement of ibogaine and noribogaine in human plasma and whole blood using fluorescein as internal standard. The method described is selective and no endogenous interfering peaks were visible in blank matrices at the retention time of the analytes. The limits of quantitation were 0.89 μg/l for ibogaine and 1 μg/l for noribogaine in plasma using 0.5 ml plasma samples, and 1.78 μg/kg for ibogaine and 2 μg/kg for noribogaine using 0.25 g of whole blood samples. The SPE procedure can be easily automated either by robotisation or with an automated sample preparation system giving high-quality, high-throughput analyses to support pharmacokinetic and toxicological studies. Among the opiate agonists extensively used by drug abusers worldwide, ibogaine is taken because of its purported hallucinogenic effects. In this report, the described procedure was successfully applied to the analysis of blood samples following a poisoning case involving the root of *Tabernanthe iboga*. Under the same chromatographic conditions, four additional compounds apart from ibogaine and noribogaine were detected, one of which was identified as ibogamine. Among the three non-identified compounds, two could be attributed to the oxidation products, iboluteine and desmethoxyiboluteine, due to the similarity of their mass spectra with the literature data [35,37]. The concentrations of ibogaine and noribogaine found in the blood sample in the *Tabernanthe iboga* poisoning case were 10–20 times greater than those reported by Mash et al. [38] after a single oral dose of 800 mg of ibogaine in humans.

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