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Determination of ibogaine in plasma by gas chromatography–chemical ionization mass spectrometry

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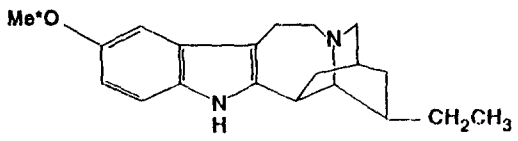
Abstract

Ibogaine is a naturally occurring indole alkaloid that is currently being considered as a treatment medication for drug dependence. Although there have been a variety of investigations regarding the mechanisms of action and pharmacology of ibogaine, relatively little has been reported regarding quantitative methods. Because of the paucity of analytical methodologies, studies involving the pharmacokinetics and metabolism of ibogaine have also been limited. A method is described for the determination of ibogaine levels in plasma by gas chromatography–methane chemical ionization mass spectrometry. [$^{13}C^{2}H_3$]Ibogaine was synthesized and used as an internal standard to control for recovery during sample preparation. The assay requires one ml of plasma and is shown to be a selective and sensitive means of ibogaine quantitation.

Keywords: Ibogaine; Alkaloids

1. Introduction

Ibogaine (Fig. 1), a naturally occurring indole alkaloid found in the root bark of the African shrub *Tabernanthe iboga*, has been used as a stimulant by hunters of the West African forests to ward off the effects of hunger and exhaustion while searching for food. In larger doses, ibogaine was used for its hallucinogenic properties during tribal ceremonies [1]. During the 1960's, ibogaine's hallucinogenic attributes led to its abuse. Surprisingly, abuse of ibogaine was anecdotally noted to decrease the abusers' craving for drugs. Ibogaine has since been patented for its therapeutic effects in substance abusers. Unfortunately, the pharmacological properties of ibogaine are still relatively unknown, despite a concerted effort by many researchers to unequivocally define the mechanism(s) of action of ibogaine and to assess its therapeutic potential.



*For non-labeled ibogaine Me = CH_3
For internal standard Me = $^{13}CD_3$

Fig. 1. Structure of ibogaine.

Laboratory studies have suggested that

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ibogaine may diminish the effects of certain drugs of abuse. Several papers have reported the effects of ibogaine upon locomotor activity in drug-dependent rats and mice. In 1992, Sershen et al. [2] noted that a single dose of ibogaine could attenuate cocaine-induced locomotor stimulation for extended periods of time. However, amphetamine-induced locomotor activity was apparently unaffected by ibogaine treatment [2]. In addition to ibogaine's effects on cocaine-induced locomotor activity, dose levels of 40 mg/kg i.p. have been shown to produce significant decreases in cocaine intake in rats trained to self-administer cocaine [3]. Morphine-dependent rats also showed similar behavior.

Despite the apparent efficacy of ibogaine in attenuating the reinforcing properties of a wide variety of abused substances, some individuals expressed concern regarding any therapy utilizing an extremely potent hallucinogenic compound. More recently, another area of concern with ibogaine treatment has been noted. O'Hearn and Molliver, using microtubule associated protein 2 (MAP2) and calbindin immunoreactivity staining of cerebellar sections, demonstrated that ibogaine administration in rats induces cerebellar degeneration [4]. Although ibogaine produced neuronal injury in the cerebellum of rats, the effects of ibogaine in the central nervous system of humans are unknown. Due to the possibility of neurotoxicity, research in humans has been limited. Researchers have also been required to use substantially lower doses of the drug with fewer subjects, all of which must have taken ibogaine previously.

In laboratory rats [5] and mice [6], the pharmacokinetic half-life of ibogaine has been estimated at approximately 1 h. Thus, the elimination of ibogaine is reasonably rapid in animals. This observation has led to speculation regarding the existence of an active metabolite or a persistent mechanism of action which could thereby explain the apparently long-lasting effects of ibogaine in human drug abusers. Long-term effects of ibogaine on brain dopamine metabolism have been reported in rats [7,8], and reversal of the cerebellar neurotoxicity due to ibogaine has not been reported. Therefore, long-term alterations in brain function or neurological damage may be involved in some of the persistent pharmacological properties exhibited by ibogaine in substance abusers. However, the pharmacokinetics and the metabolic fate of ibogaine have yet to be fully characterized in laboratory animals, and much remains to be known regarding the pharmacokinetics of ibogaine in man.

One factor that has prevented a thorough evaluation of ibogaine pharmacokinetics and metabolism has been the lack of a specific and sensitive assay for ibogaine. Previous techniques include extraction and quantitative analysis using spectrophotometry [8], thin-layer chromatography [9–11], or gas chromatography [10,11]. The sensitivity of the chromatographic assays ranged from approximately 0.1 to 10.0 mg using TLC to 5 ng for gas chromatography assays (e.g. GC-FID). In order to study the pharmacokinetics of ibogaine, these assays required high doses of ibogaine to be administered, or relatively large volumes of biological fluids–tissues be collected and extracted. To utilize the TLC method [10], 10–100 ml of urine was required from a 70-kg individual given 6 capsules of Iperton (ca. 240 mg of *Tabernanthe iboga* extract). At this dosage, ibogaine was not detected using the TLC or the GC method. In individuals given 5 mg of ibogaine-HCl, extraction of 50–100 ml of urine was required. Ibogaine was detected in urine collected within the first 3 h post ibogaine administration. After 4 h, ibogaine was not detected. The utilization of radiolabeled ibogaine could afford a quantitative assay of excellent sensitivity, and radiolabeling of ibogaine has been described [13,14]. However, the use of radioisotopes in humans is problematic.

Due to the recent requirement for the use of low doses of ibogaine in experimental human subjects (as directed by the Food and Drug Administration), there remains a need for an extremely sensitive (<1.0 ng/ml) and selective means of detection for ibogaine in biological samples. The desired method of quantification needs to be capable of detecting low levels of ibogaine in small volumes of biological fluids, particularly plasma or blood, where repeated

sampling of large volumes of fluid is not possible. Through the use of a positive chemical ionization GC–MS method, we have developed a method for the detection of ibogaine in 1-ml samples of plasma that allows quantitation of ibogaine at levels of 0.5 ng/ml or greater (Scheme 1). This technique, utilizing solid-phase extraction and a stable isotope internal standard, possesses the selectivity and sensitivity appropriate for use in pharmacokinetic, pharmacodynamic and bioavailability studies of ibogaine.

2. Experimental

2.1. Chemicals and reagents

Ibogaine was obtained through the National Institute on Drug Abuse from Sigma (St. Louis, MO, USA). All solvents were Burdick and Jackson HPLC grade. Solid-phase extraction columns were Varian C_{18} Bond-Elut containing 200 mg of sorbent in a 3-ml liquid reservoir (Chromtech, Apple Valley, MN, USA). A 12-sample manifold (Supelco, Bellefonte, PA, USA) was used for processing the C_{18} columns under negative pressure (aspiration). Boron tribromide (BBr_3) and [$^{13}C^2H_3I$]iodomethane (99 atom % ^{13}C, 99.5 atom % ^2H) were obtained from Sigma.

2.2. Preparation of internal standard

For the preparation of [$^{13}C^2H_3$]ibogaine, 53 mg of ibogaine-free base was dissolved in 2 ml of methylene chloride (CH_2Cl_2). The solution was then capped and stirred under nitrogen. Boron tribromide (BBr_3) was added in 10-fold molar excess. Upon addition, the reaction immediately turned cream-colored and a dark precipitate formed. The solution was stirred an additional 30 min; thereupon the reaction was quenched slowly with 2 ml of deionized distilled water (dH_2O). Sodium bicarbonate was added to the solution to a pH of approximately 9–10. The CH_2Cl_2 was removed in vacuo leaving the sodium bicarbonate slurry. Addition of 3×3 ml washes of CH_2Cl_2 extracted the desmethyl ibogaine. Analysis by GC–MS showed the predominate component to be the desmethyl ibogaine, with a small amount of unreacted ibogaine also detected. Purification of the desmethyl ibogaine was performed by flash column chromatography (silica gel) with 40 ml chloroform ($CHCl_3$) followed by 40 ml of chloroform–methanol (MeOH)–triethylamine (TEA) (95:5:1, v/v). The fractions containing the desmethyl ibogaine were combined and concentrated in vacuo yielding 26.2 mg of desmethyl ibogaine.

In a dry round-bottom flask, 17.5 mg of a 60% dispersion of sodium hydride (NaH) in mineral oil was washed with 2×30 ml of hexane under a constant stream of nitrogen. The NaH was resuspended in 35 ml of dry tetrahydrofuran (THF) and stirred vigorously to create a suspension. A 33.5-ml aliquot was removed leaving 1.5 ml of THF containing approximately 0.744 mg of NaH. After stirring for 10 min, 5.5 mg of desmethyl ibogaine was dissolved in 8 ml of THF and added to the stirring NaH solution. The vial was rinsed with a second 8-ml wash of THF, which was then added to the mixture. The mixture was stirred for 2 h to generate the phenoxide. Finally, a fresh ampule of [$^{13}C^2H_3I$]iodomethane was opened and 1.3 μl added to the flask. The reaction was allowed to stir for 4 h. GC–MS analysis of the reaction product displayed the isotopically labeled product. Isolation and purification of this material yielded approximately 5.0 mg of [$^{13}C^2H_3$]ibogaine.

2.3. Analytical instrumentation

A Hewlett-Packard (Kennett Square, PA, USA) GC–MS system, consisting of a 5890 Series II gas chromatograph, a 7673A autosampler, a 5989A MS Engine, and a Chemstation (HP-UX) for system control, was used in this study. The chromatographic system for this analysis was a J&W Scientific (Folsom, CA, USA) DB-1 capillary column (30 m $\times$ 0.25 mm I.D., film thickness 0.25 μm) with helium at a flow-rate of approximately 1.5 ml/min serving as the carrier gas. The injection port temperature was held at 250°C. The initial oven temperature,

125°C (0.5 min), was increased at 40°C/min to 250°C (1 min), and then 10°C/min to 300°C (3 min). The temperature of the transfer line to the mass spectrometer was 280°C, and the mass spectrometer source and analyzer temperature were maintained at 200°C and 100°C, respectively. A splitless injection of 3 μl was made with the purge value turning on at 0.75 min. With this methodology, ibogaine eluted at approximately 10 min.

The selected-ion monitoring (SIM) methodology monitored the intense [M + 1] (protonated) and [M − 15] ions of the labelled and non-labelled ibogaine generated by methane chemical ionization. The dwell time for *m/z* 315 and *m/z* 311 was 150 ms, whereas for the two remaining ions, the dwell time was programmed at 25 ms.

2.4. Analytical methods

Plasma (1 ml) was mixed with 20 μl of an ibogaine–MeOH solution (varying concentrations) and 100 ng of internal standard. The plasma samples were then transferred to conditioned C_{18} solid-phase extraction columns. The columns were washed with 2 ml 0.40 *M* K_2HPO_4 (pH 10), 2 ml of dH_2O at pH 10 and 3 ml of 20% MeOH–dH_2O (pH 10) and then dried. Ibogaine was eluted from the column with 2.0 ml of a 0.1% HCl–MeOH solution. After sample concentration to dryness, 3 × 40 μl washes of MeOH were used to transfer the sample to a low-volume sample vial. The sample was again evaporated to dryness and then reconstituted in MeOH to a final volume of 20.0 μl. A 3-μl aliquot was analyzed by GC–MS. For a schematic representation of the method see Fig. 2 .

2.5. GC–MS calibration

All standards used for generating calibration curves or quality-control samples were formulated in MeOH and aliquoted into 1 ml of plasma. These calibration standards were generated from two independently weighed samples, and covered a range extending from 0.1 ng/ml to 33 000 ng/ml plasma. Area ratios were plotted against the actual concentration and then fit by linear regression analysis using a weighting factor of 1 over the concentration squared ($[\text{analyte}]^{-2}$) to control for the large dynamic range (high concentrations having an exaggerated effect on the fit). The calibration curve quality-control samples were extracted and analyzed under the same conditions to determine the reproducibility of the analytical procedure.

2.6. Quality control: intra-assay and inter-assay variability

Inter-assay variability was determined with quality-control standards (containing approximately 3.0, 30.0 and 300 ng of ibogaine/ml plasma) that were generated from a third, independently weighed sample. The quality-control samples were prepared fresh, as needed, in 1.0-ml plasma aliquots. Samples at each concentration were processed and analyzed in triplicate immediately following the generation of the calibration curve. Inter-assay variability results were then calculated and are shown in Table 1 (set 1). On three additional occasions, inter-assay variability was determined from newly prepared samples (Table 1, sets 2, 3 and 4). Intra-assay variability was then calculated from these four determinations and the results shown in Table 2. During the analysis of the control samples, plasma blanks were also extracted and analyzed.

3. Results and discussion

3.1. Synthesis of a stable isotope labeled internal standard

The ability of GC–MS to discriminate between isotopically labeled analytes enabled the use of a stable isotope internal standard to closely control for sample recovery and quantitation. Attempts to control for sample recovery using voacangine (an indole alkaloid similar in structure to ibogaine) as an internal standard were unsatisfactory; recovery of the analyte and internal standard were inconsistent (data not shown). Thus, we chose to label ibogaine in the O-methoxy position of the aromatic ring system. The syn-

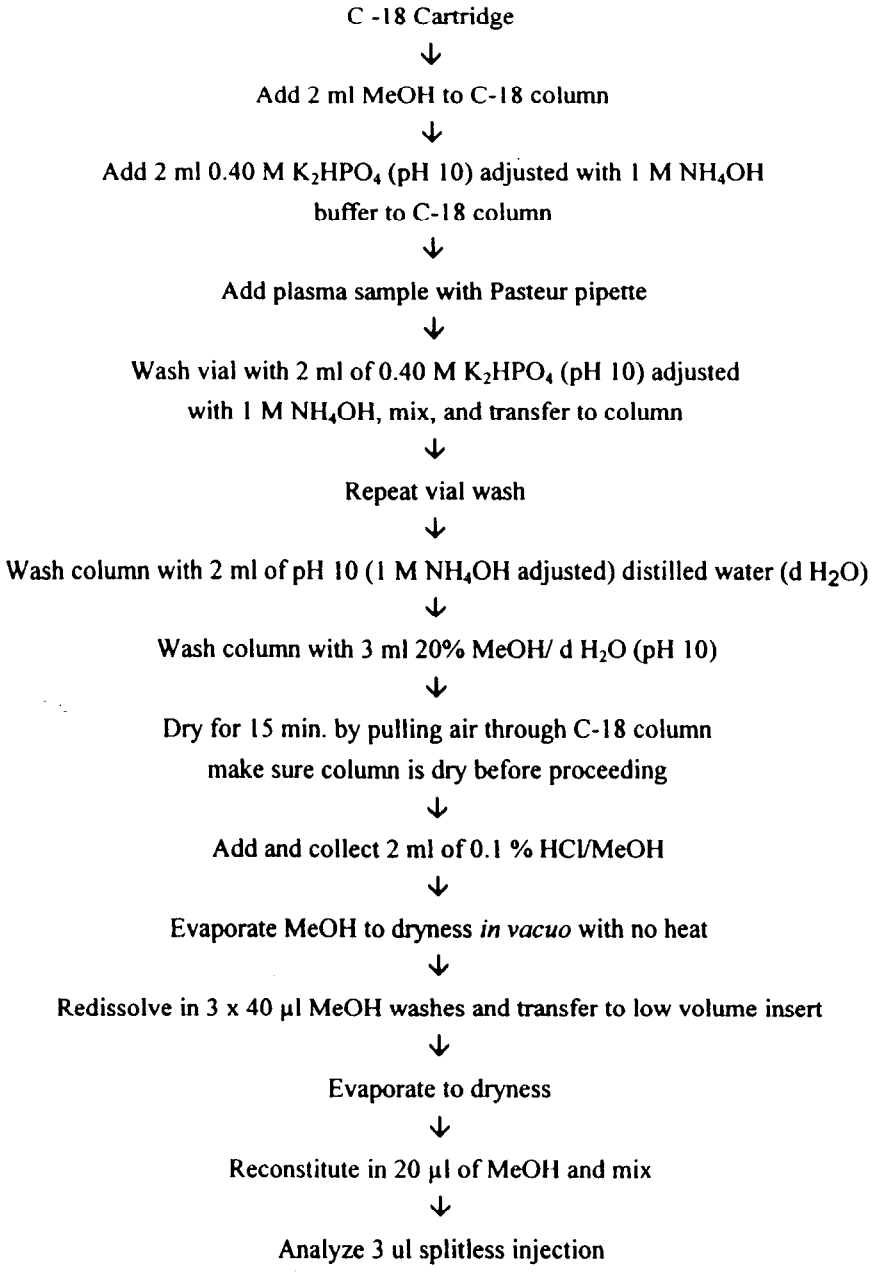

Fig. 2. Flow chart depicting procedure developed for C_{18} solid-phase extraction of ibogaine from 1 ml of plasma.

thetic procedure was relatively straightforward and yielded an ibogaine analog that was four mass units heavier than ibogaine, with little [$^{12}C^1H_3$]ibogaine contamination.

3.2. Chemical ionization mass spectrometry

The electron-impact (EI) and chemical ionization (CI) spectra obtained for ibogaine and

Table 1
Inter-assay variability for ibogaine ($n = 3$)

Added (ng/ml) (mean)	Measured (ng/ml) (mean)	Measured S.D. (ng/ml)	R.S.D. (%)	Difference[a] (%)
Set 1				
292.1	306.2	9.2	3.0	4.8
32.7	30.3	0.4	1.2	−7.34
3.4	4.6	1.0	21.4	37.1
Set 2				
288.8	328.4	14.7	4.5	13.7
31.1	31.8	0.4	1.2	2.4
3.4	5.6	0.4	7.9	62.7
Set 3				
306.1	330.9	6.9	2.1	8.1
32.8	30.8	1.1	3.7	−6.0
3.4	3.7	0.2	5.8	9.0
Set 4				
304.3	335.7	10.9	2.1	10.3
31.2	32.1	0.4	1.1	3.1
3.4	2.9	0.2	5.5	−15.4

[a] % Difference defined as [(measured mean − actual mean)/actual mean].

[$^{13}C^2H_3$]ibogaine are shown in Fig. 3 and Fig. 4. Fig. 3A shows the mass spectrum obtained for ibogaine using EI ionization at 70 eV. An intense molecular ion at m/z 310 is apparent. In comparison, the EI spectra of [$^{13}C^2H_3$]ibogaine (Fig. 3B) resulted in a molecular ion at m/z 314. Methane CI of ibogaine produced an intense [M + 1] ion at m/z 311 and [M + 29] adduct ion at m/z 339 with ibogaine (Fig. 4A), whereas CI of [$^{13}C^2H_3$]ibogaine produced an intense [M + 1] ion at m/z 315 and [M + 29] adduct ion at m/z 343 (Fig. 4B). The degree of isotopic purity in the internal standard is apparent in both the electron-impact ionization spectra and the chemical ionization mass spectra. From the EI data, the internal standard was found to be ≥ 99.75% $^{13}C^2H_3$-labeled ibogaine.

The ion-current profiles shown in Fig. 5 are for a plasma sample containing ibogaine at a concentration of 10 ng/ml. The top panel shows the selected-ion current profile recorded for m/z 315 (the [M + 1] for [$^{13}C^2H_3$]ibogaine). The peak-

Table 2
Intra-assay variability for ibogaine (n = 12)

Added (ng/ml) (mean)	Measured (ng/ml) (mean)	Measured S.D. (ng/ml)	R.S.D. (%)	Difference[a] (%)
297.8	325.3	13.1	4.0	10.3
31.9	31.3	0.4	2.8	2.1
3.4	4.2	1.1	26.2	23.2

[a] % Difference defined as [(measured mean − actual mean)/actual mean].

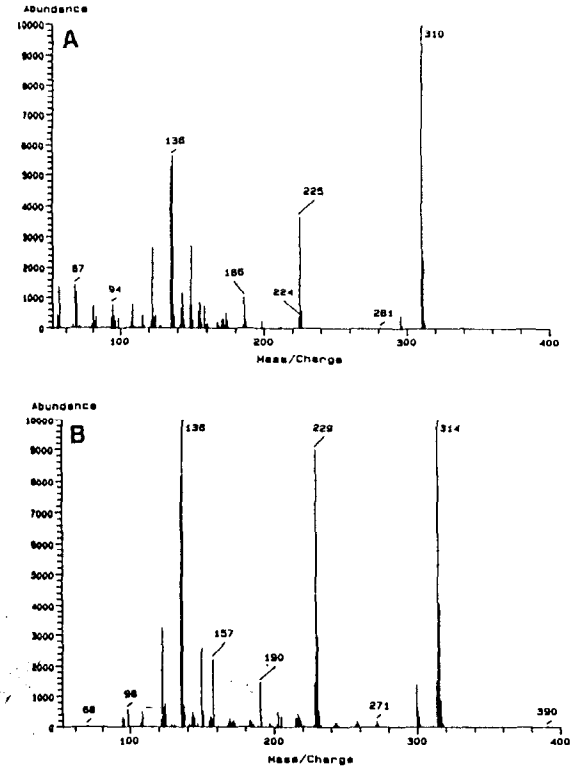

Fig. 3. Mass spectra recorded for: (A) EI spectra of ibogaine; (B) EI spectra of [$^{13}C^{2}H_{3}$]ibogaine.

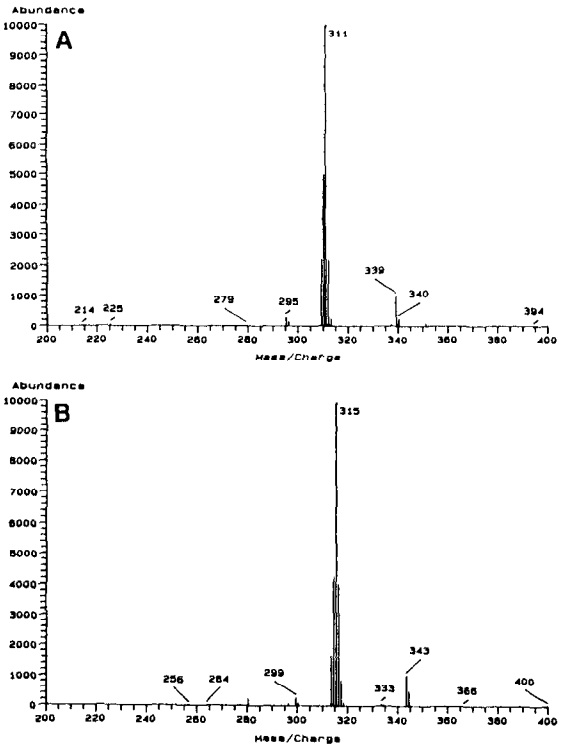

Fig. 4. Methane chemical ionization spectra: (A) methane chemical ionization spectra of ibogaine; (B) methane chemical ionization spectra of [$^{13}C^{2}H_{3}$]ibogaine.

area ratio of this peak to the peak recorded at *m/z* 311 (the [M + 1] for ibogaine) was used to quantitate the concentration of ibogaine. The lower two panels show the selected-ion current profiles for *m/z* 299 and 295, the confirmation ions for [$^{13}C^{2}H_{3}$]ibogaine and ibogaine, respectively.

As noted by Lerner and Katsiaficas [12], derivatizition of ibogaine to the trimethylsilyl derivative is problematic. We found that derivatization of ibogaine using bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was incomplete, resulting in a mixture of derivatized and unconverted material. Somewhat surprisingly, the chromatographic properties of non-derivatized ibogaine are such that a peak of excellent shape and symmetry could be obtained when chromatographed on a DB-1 capillary column. Therefore, our GC–MS analysis was performed on the non-derivatized analyte and internal standard.

3.3. GC–MS calibration

The fifteen calibration standards covered a wide dynamic range from 0.1 to 33 210 ng ibogaine/ml plasma. With such a wide range, the highest concentrations had an exaggerated effect on a simple least-squares linear regression. By using a linear regression with a weighting factor of 1 over the concentration squared ([analyte]$^{-2}$), the line obtained from the linear regression intersected the lower concentrations to a greater extent than with no weighting. The concentrations that could be obtained in certain tissues or fluids with high doses of ibogaine are

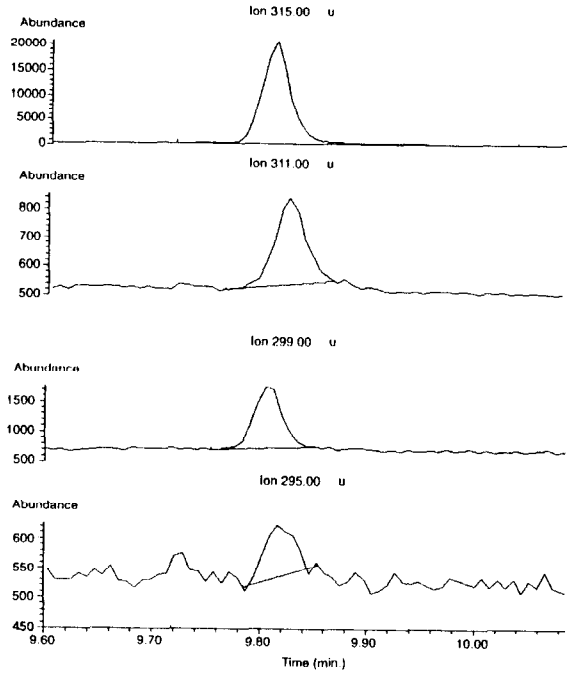

Fig. 5. Selected-ion current profiles for [M + 1] ion of [$^{13}C^2H_3$]ibogaine (top), [M + 1] ion of ibogaine (middle top), [M − 15] ion of [$^{13}C^2H_3$]ibogaine (middle bottom), and [M − 15] ion of ibogaine (bottom) at a concentration of 10 ng ibogaine/ml plasma.

unknown. Therefore, the dynamic range was extended to relatively high concentrations.

3.4. Quality control: inter-assay and intra-assay variability

Results of the initial quality-control samples are shown in Table 1 (set 1). Blank injections were made, but no interfering peaks or carry over from previous injections were found. The greatest error in quantitation occurred at the lowest level. In the first control set, the 3 ng/ml level was overestimated by 37%. In comparison, the mid and high control levels were underestimated by 7.3% and overestimated by 4.8%, respectively. At the two higher control concentrations (30 and 300 ng/ml), the coefficient of variation was 1.2% and 3.0%, respectively, whereas the variation in the low control level was 21.4%. Approximately a week later, the second and third control sets were run. The data obtained during these runs were similar to the first control set with the greatest error in estimation at the 3 ng/ml level. The second set overestimated the 3 ng/ml level by 63% and the third set overestimated by 9%. A final control set was done two weeks later. Again, the lower (3.0 ng/ml) concentration exhibited the least-accurate quantitation, it being underestimated by 15.4%. The variance for 3, 30, and 300 ng/ml levels were 5.5%, 1.1%, and 3.3%, respectively. These four determinations of inter-assay variability were then used to calculate intra-assay variability (Table 2). The 3 ng/ml plasma control had the highest coefficient of variation with 26.2%. The 30 ng/ml and 300 ng/ml had 2.8% and 4.0% coefficients of variation, respectively.

Throughout these control experiments, the coefficients of variation were relatively low for all levels, indicating that the assay possesses a reasonable degree of consistency, particularly at the higher levels of quantitation. However, the 3 ng/ml sample was overestimated by 63% in the second quality-control set, which exceeds the 50% limit imposed by our method validation acceptance criteria. The reason for this overestimate in unknown, but the other determinations at this concentration showed acceptable precision and reproducibility. The increased variability at the lowest level clearly indicates the importance of running control samples with experimental samples. When a control sample is found to be poorly estimated by the method, the batch run should be aborted until recalibration and elimination of the source of the error occurs.

3.5. Conclusion

Alternative techniques possessing the selectivity and sensitivity of our GC–MS methodology have not been reported. The method, along with the synthesis, is relatively straightforward allowing for rapid sample processing. By utilizing small sample volumes, repeated sampling from one subject is also a possibility. One area that needs to be addressed is the effects of various metabolites on the assay. However, the assay appears to be sensitive enough to detect the low

levels of ibogaine in biological fluids that would be expected in clinical trials and pharmacokinetic–pharmacodynamic studies. Therefore, it could serve as a useful method for future researchers.

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