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IDENTIFICATION AND QUANTIFICATION OF THE INDOLE ALKALOID IBOGAINE IN BIOLOGICAL SAMPLES BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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Abstract—A sensitive and highly selective analytical chemical method for measuring the indole alkaloid ibogaine in biological samples has been developed. The method utilizes organic extraction, derivatization with trifluoroacetic anhydride, and detection by combined gas chromatography–mass spectrometry. The deuterated analog of ibogaine, O-[Cd_3]-ibogaine, was synthesized and used as an internal standard for the method. Standard curves, constructed from variable amounts of ibogaine (50–400 ng) and a fixed amount of internal standard (250 ng) were linear. The method has an approximate detection limit of at least 20 ng/mL of tissue extract (180 ng/g tissue), with a coefficient of variation of 8 to 12.5%. Chemical stability studies with the method found that aqueous ibogaine solutions (1–10 mg/mL) could be stored at 10° for up to 7 months with no more than 10% loss. The method was also used to measure brain ibogaine levels in rats 1 and 19 hr after a single dose of drug (40 mg/kg, i.p.); the results suggest a rapid disappearance of the drug after i.p. dosing. The method will help reveal the pharmacokinetic properties of this putative anti-addictive agent in animals and humans.

Key words: ibogaine; drug abuse; quantitative analysis; analytical chemistry; brain; gas chromatography–mass spectrometry

The indole alkaloid ibogaine (Fig. 1) is currently being investigated as a potential anti-addictive substance (U.S. patents 4,499,096 and 4,587,243; H. Lotsof). Despite the possibility that this compound may be capable of reducing or abolishing the self-administration of opiates [1] and cocaine [2], almost no information is available on the absorption, distribution, metabolism or excretion of this agent (see also Refs. 3 and 4 for a discussion of possible mechanisms of action). In fact, it is by no means clear that the reported biological actions of ibogaine are attributable to the unmetabolized parent compound. The lack of availability of a sensitive and selective chemical method for measuring ibogaine in biological specimens is one factor responsible for this dearth of information.

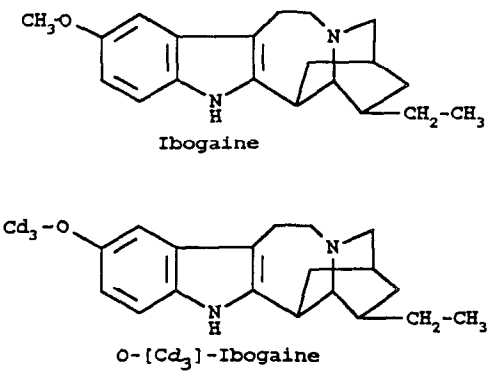

Fig. 1. Chemical structures of ibogaine and the deuterated internal standard O-[Cd_3]-ibogaine.

Dhahir [5] described a quantitative spectrophotometric method for extracting and measuring ibogaine in samples from drug-treated rats, and used the method to estimate tissue and fluid levels of ibogaine after various treatment regimens. Unfortunately, this method depends only on the absorbance of a single wavelength of UV light, and thus would not be expected to discriminate between ibogaine and its metabolites, several of which would probably contain the indole chromophore. Dhahir [5] and others [6] also used thin-layer chromatography to study ibogaine metabolism and elimination, although this method is tedious and only semi-quantitative at best. Cartos and Giarusso [7] used GC to identify ibogaine in urine from drug-treated animals. However, this method failed to identify urinary ibogaine in drug-treated humans, due to a

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lack of sensitivity. Presently, we describe a sensitive and highly selective method which utilizes GC–MS for quantifying very small amounts of ibogaine in biological samples. We have also used the method to conduct preliminary studies of ibogaine levels in brain after acute dosing in rats.

MATERIALS AND METHODS

Synthesis of internal standard. A deuterium-labeled analog of ibogaine (O-[Cd_3]-ibogaine, Fig. 1) was synthesized from ibogaine in a two-step procedure. A solution of 1.9 mL of boron tribromide in 20 mL of anhydrous methylene chloride was added dropwise over a period of 15 min to a stirred suspension of ibogaine hydrochloride (500 mg) in 100 mL of methylene chloride kept at room temperature under anhydrous conditions. After stirring for 8 hr, the reaction mixture was poured into a cold solution of saturated sodium bicarbonate, and extracted with 2 portions of methylene chloride. The combined extracts were washed with water and evaporated to dryness, leaving 265 mg of crude desmethylibogaine (noribogaine [8]). Isolation of the purified compound by silica gel chromatography (ethyl acetate:hexane, 1:1) yielded 160 mg of product. Ibogaine was not detectable in the sample by NMR or mass spectral analysis. In the second step, a solution of 50 mg of noribogaine in 5 mL of tetrahydrofuran was mixed with aqueous potassium hydroxide (5 mL, 1.6%), followed by the addition of hexadeuterodimethylsulfate (95 mg), and the mixture was stirred at room temperature for 5 hr. The solution was poured into water and extracted twice with ethyl acetate. The extracts were washed with water and evaporated to dryness, leaving 40 mg of crude product. After chromatographic purification as above, O-[Cd_3]-ibogaine (13 mg) was obtained. The product gave a molecular ion of 314.25 (calculated 314.42) by chemical ionization mass spectrometry. NMR analysis of the product showed a spectrum identical with that of ibogaine, except for the absence of the methoxy (CH_3O) group.

Ibogaine standards, stock solutions and glassware. Ibogaine hydrochloride obtained from the Sigma Chemical Co. (St. Louis, MO) was indistinguishable by TLC from a sample of the salt obtained from NIDA (also see [9]). R_f values were 0.64 for both samples on silica gel G (LK6D Linear-K plates, Whatman, Hillsboro, OR) with chloroform: methanol (60:35) as the solvent. Stock solutions of ibogaine hydrochloride (Sigma) and O-[Cd_3]-ibogaine were prepared in degassed methanol (1.12 mg salt/mL, equivalent to 1.0 and 0.2 mg base/mL, respectively) and stored at −10° in polypropylene vials. Except for the doses of ibogaine administered to animals (which are specified as amounts of hydrochloride salt), all units of ibogaine are given as the free base. Derivatization reactions (described below) were performed in 1.0 mL Reactivials© (Pierce, Rockford, IL) previously silanized with 10% dimethyldichlorosilane in toluene (see Pierce Catalog and Handbook, Rockford, IL).

Assay of ibogaine by GC–MS. Ibogaine was assayed by organic extraction, derivatization with TFAA*, and detection by GC–MS. Aliquots (1 mL) of supernatant fractions from biological homogenates in 0.4 N perchloric acid were pipetted into 12 mL polyethylene conical tubes (Krackeler Scientific, Albany, NY). Samples then received 250 ng of internal standard, and were made alkaline by the addition of 0.2 mL of 10 N KOH. Each tube was mixed by vortex, received 5 mL of *n*-hexane, followed by 15 min of rapid mechanical shaking, and centrifugation (1200 *g* for 10 min). The hexane (upper) layer was transferred to a tube containing 0.5 mL of 0.01 N HCl, and the extraction and centrifugation steps were repeated. The upper organic layer was removed by aspiration and discarded. The lower aqueous fraction was then transferred to a Reactivial© and evaporated to dryness at room temperature by vacuum centrifugation (200 mTorr). Residues were derivatized in 50 μL of TFAA for 30 min at 60°, and then cooled for 10 min on ice. TFAA was then evaporated under a gentle nitrogen stream at room temperature. The residues were resuspended in 50 μL of toluene, vortexed and analyzed by GC–MS. All samples were automatically injected (1 μL, Hewlett Packard [HP] model 7673B autosampler) into an HP model 5890A gas chromatograph in splitless mode. Gas chromatography was performed on a DB-5ms capillary column (30 m, 0.25 mm i.d., 0.1 μm film thickness, J&W Scientific, Folsom, CA) heated from 90 to 250° at 70°/min after a 1.7-min purge delay (210° inlet, 275° transfer line). Electron impact mass spectra were obtained with an HP 5970A mass selective detector (−70 eV) with computerized data analysis. Chemical ionization mass spectrometry was not available as an interface to the gas chromatograph. Quantitative analysis was performed by the multiple ion monitoring of *m/e* 406 (TFA ibogaine) and *m/e* 409 (TFA O-[Cd_3]-ibogaine) at 9.9 min. A second ion for each derivative was also monitored to confirm identification in all cases (*m/e* 391 and 394 for TFA ibogaine and the internal standard, respectively). Ibogaine was identified successfully in unknown samples when the suspected peak was within 0.05% of the expected retention time and the 391/406 ion ratio was 0.88 ± 0.06 (mean ± 95% tolerance limits). A peak area threshold setting of 10 was used to automatically identify and integrate peaks in the ion traces of each sample.

Animal experiments. Female Sprague–Dawley rats (250–300 g, Charles River, Kingston, NY), were housed individually under normal laboratory conditions with food and water *ad lib*. All animal procedures were approved by the Institutional Animal Care and Use Committee of Albany Medical College. Ibogaine hydrochloride was weighed the day of the experiment, and dissolved in distilled water at 10 mg/mL by sonication and gentle heating in warm water. The drug did not dissolve in isotonic saline. Animals received ibogaine (40 mg/kg, 4 mL/kg, i.p.) with no visible discomfort and were killed 1 or 19 hr later by decapitation. Brains were removed immediately and homogenized in 5 vol. of ice cold

* Abbreviations: HFBA, heptafluorobutyric anhydride; *m/e*, mass/charge ratio; TFA, trifluoroacetyl; and TFAA, trifluoroacetic anhydride.

0.4 N perchloric acid, and the homogenate was centrifuged (20,000 *g* for 20 min). Standard curves were constructed by the addition of varying amounts of ibogaine (50–400 ng) and internal standard (250 ng) to brain homogenates from untreated animals. Identical curves were obtained by the addition of ibogaine and internal standard to perchloric acid solutions containing no tissue.

RESULTS

When ibogaine was reacted with TFAA as described, a single product was consistently detected by GC–MS with a retention time of 9.9 min (Fig. 2A). An analogous product was found after derivatization of O-[Cd_3]-ibogaine (Fig. 2B); the retention time of this compound was routinely 0.05 min earlier than that of the ibogaine derivative. However, GC separation of these compounds was not necessary, since they were specifically detected by different ion scans.

The mass spectra of the TFA derivatives of ibogaine and the internal standard (Fig. 2) support the identities of the peaks at 9.9 min. The structures and mass spectral assignments are given in Table 1. The spectra confirm the molecular weights of TFA ibogaine (*m/e* 406) and TFA O-[Cd_3]-ibogaine (*m/e* 409) and show the expected, similar fragmentation patterns with an offset of +3 amu (due to the trideuterated substituent) for fragments larger than

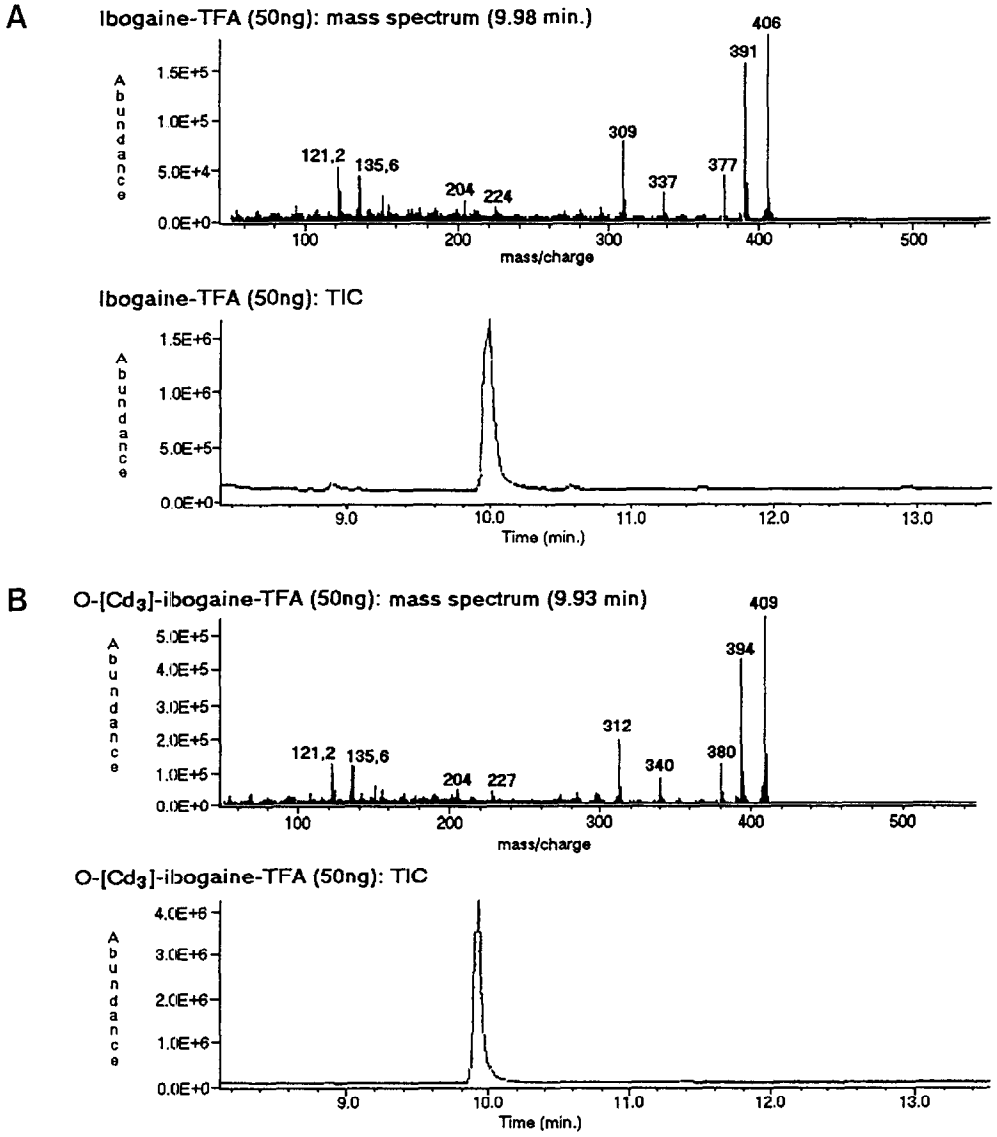
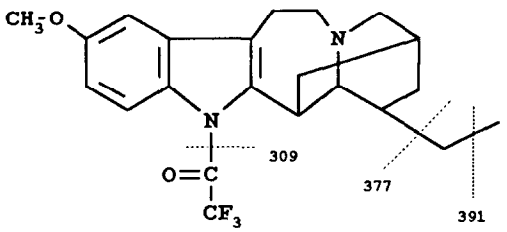
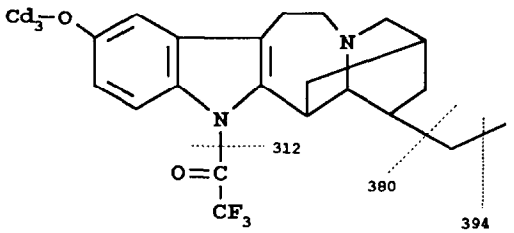
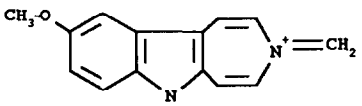
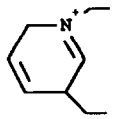
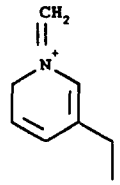


Fig. 2. Gas chromatography and mass spectra of the TFA derivatives of ibogaine (A, top) and O-[Cd_3]-ibogaine (B, bottom). Each compound (50 ng) was added to a Reactivial©, directly derivatized with TFAA and analyzed by GC–MS as described. The lower half of each panel shows the total ion chromatogram of each compound, which plots the total ion abundance (arbitrary units, E = exponential, ordinate) vs time after injection (min, abscissa). The upper half of each panel shows the mass spectrum measured at the times indicated.

Table 1. Proposed mass spectral fragmentation of the TFA derivatives of ibogaine and O-[Cd_3]-ibogaine





	Ibogaine-TFA	O-[Cd_3]-ibogaine-TFA
	m/e	*m/e*
M^+	406	409
(M^+)-97 TFA	309	312
Loss of —CH_3	391	394
Loss of —C_2H_5	377	380
	224	227
	136	135,136
	121,122	121,122

m/e 204. In both spectra, the molecular ions were the base peaks, and thus were chosen for quantitative analysis (see below). The M^+-15 ions (*m/e* 391 and 394, corresponding to the loss of the terminal methyl group) were the second most abundant ions, and were chosen as confirming ions in the quantitative analysis. The mass spectra also support the presence of the TFA group at the nitrogen of the indole nucleus of both compounds. The *m/e* 309 and *m/e* 312 fragments correspond to the loss of the TFA moiety and were present as approximately 40% of the base peak. Other spectral assignments in Table 1 are based on those of Biemann *et al.* [10].

Figure 3 shows the detection of ibogaine and its internal standard from the analysis of brain homogenates by the multiple ion monitoring technique. As shown, ibogaine was detected specifically in the scans of ions 406 and 391, whereas the internal standard was detected with scans of ions 409 and 394. Brain homogenates from naive subjects showed no interfering peaks in any of the ion scans.

A standard curve for the ibogaine assay is shown in Fig. 4. The curve was linear from 50 to 400 ng of ibogaine per mL of brain homogenate. At the threshold setting used, the 50 ng point routinely yielded integrated peak areas of 100,000–200,000 U. Under these conditions, a blank brain or plasma homogenate containing only O-[Cd_3]-ibogaine yielded no identifiable ibogaine peak (see also Fig. 3). Since the smallest peak identified by the integration software at these settings yields a peak area of about 20,000 U, the 50 ng ibogaine standard is detected at 5–10 times this value. Some assays included a 20 ng standard, which was near the lower limit of detection for the method. Repeated determinations of the ratio (406/409) for ibogaine standards yielded coefficients of variation (i.e. standard deviation/mean) of 12.5, 10.4, and 8.7% for the 100, 200, and 400 ng standards, respectively (N = 9–10).

A number of experiments were performed to investigate the chemical stability of ibogaine. Ibogaine hydrochloride readily dissolved in deionized water or methanol at 1 mg/mL; these solutions showed no more than a 10% loss upon storage at 10° for up to 7 months. Thus, for routine assay, it is recommended that ibogaine standards be prepared daily from an aliquot of stock solution (1 mg/mL) in methanol. Stock solutions can be stored at −10° for up to 6 months and thawed at 10° for dilution. When 10 mg of the ibogaine salt was added to 1 mL of deionized water, it did not dissolve completely. After intermittent sonication and vortex mixing of this suspension over an 18-min period, the particulate

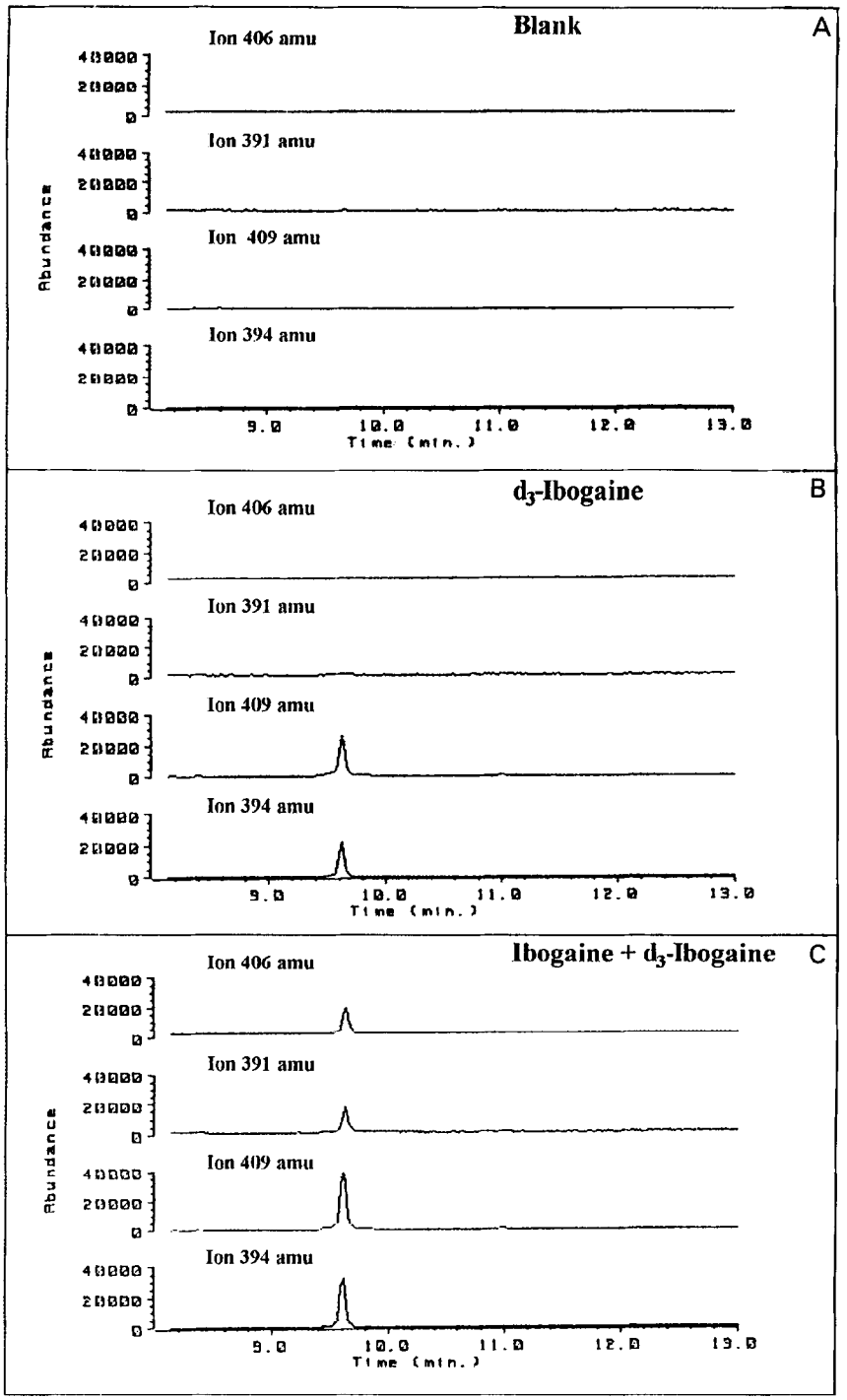


Fig. 3. Determination of ibogaine and its internal standard by multiple ion monitoring. Brain homogenates from untreated rats were prepared as described and received either: (A) nothing (top), (B) the internal standard O-[Cd_3]-ibogaine (250 ng, middle) or (C) internal standard along with ibogaine (250 and 100 ng, respectively, bottom). Homogenates were assayed as described and analyzed by multiple ion monitoring. Amounts injected into the GC–MS are 1/50 of the amounts derivatized, since 1 μL volumes were injected from a total of 50 μL. The abundance (ordinate, arbitrary units) of the four ions scanned are plotted vs the time after sample injection into the GC–MS (abscissa, min).

was allowed to settle, and the aqueous fraction was analyzed by GC–MS. Results showed that the dissolved fraction contained 98–100% of the ibogaine prepared, justifying the use of this solution. Storage of this solution (10 mg/mL) at 10° for up to 7 months also resulted in a loss of no more than 10%. No stability studies of ibogaine in brain homogenates have been performed; thus, samples were stored at

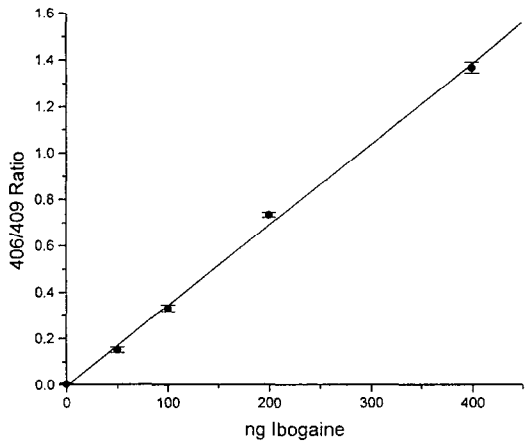


Fig. 4. Standard curve for the determination of ibogaine. Ibogaine standards (ng, abscissa) were mixed with 250 ng of internal standard and added to supernatant fractions (1 mL) from homogenates of drug-free rat brain, and assayed as described. Ions 406 (TFA ibogaine) and 409 (TFA O-[Cd_3]-ibogaine) were quantified by multiple ion monitoring, and the ratio (406/409) is plotted for each sample (ordinate). Shown are means ± SEM for triplicate determinations.

−10° and always assayed within 5 days. Aqueous solutions of more than 10 mg/mL could not be reliably prepared, even with sonication and heating.

The thermal stability of aqueous ibogaine solutions (10 mg/mL) was investigated by assaying ibogaine levels after exposure to boiling water for up to 1 hr. A time-dependent loss of up to 25% of the compound was observed (not shown).

The method was successfully applied to measure ibogaine in brain homogenates from drug-treated rats 1 and 19 hr after dosing. One hour after treatment, brain levels of drug were 3.26 ± 0.62 μg/g (mean ± SEM, N = 9). Levels were much lower 19 hr after administration. The compound was detected in only 9 out of 13 brain homogenates, with a mean of 0.19 ± 0.05 μg/g (N = 9).

DISCUSSION

The present method appears to reproducibly extract, derivatize and detect ibogaine in biological samples. The hexane extraction step from alkaline pH (first implemented by Dhahir [5]) offers maximum purification of ibogaine from more polar basic substances. Derivatization of ibogaine is clearly needed for analytical gas chromatography, since underivatized ibogaine gave an unacceptably broad peak shape and poor sensitivity (our unpublished results, similar to other findings [7]). The results also found that TFAA functions as an acceptable derivatizing agent. Thus, under the described conditions (60°, 30 min), ibogaine and the internal standard reacted with TFAA alone to yield single products. These derivatization conditions were also the mildest ones found to produce optimal yield of product. Perfluorinated anhydrides have been used previously as derivatizing agents for measuring other indoles, notably serotonin [11]. In addition, the mass spectra of the TFA derivatives of ibogaine and deuterated ibogaine verify the proposed structures of the derivatives. The results support and extend the studies of Biemann *et al.* [10], which described the mass spectral properties of ibogaine and several derivatives.

The successful derivatization of ibogaine reported presently was developed after an extensive investigation of numerous derivatizing reagents, derivatizing conditions, and detection parameters (unpublished results). For example, heptafluorobutyryl imidazole, a reagent reported to derivatize a variety of indoles, gave inconsistent results. Use of the derivatizing agent HFBA produced up to four different ibogaine derivatives, depending on the derivatization and GC–MS conditions, but no conditions were found that resulted in only a single ibogaine product. Although TFAA performed adequately when used by itself (see above), the addition of catalysts such as pyridine also resulted in multiple ibogaine adducts with either HFBA or TFAA as the derivatizing agent. With TFAA, results also varied considerably when derivatization time and temperature were varied from the optimal conditions (60°, 30 min). GC conditions (i.e. temperatures of inlets, oven, and transfer line) have also been optimized. For example, reduction of the inlet temperature by as little as 20° caused a dramatic drop in derivative peak area. Similarly, the use of toluene as the injection solvent produced results superior to those found with several other solvents. Samples should be analyzed by GC–MS as soon as possible after preparation of the TFA derivatives, since peak sizes of the products markedly decreased within 24 hr of derivatization.

The present method also offers reliable quantitative results. The deuterated derivative O-[Cd_3]-ibogaine functions as an ideal internal standard, since it formed an analogous TFA derivative to that of ibogaine. In addition, the gas chromatographic and mass spectral properties of the derivative closely resemble those of TFA ibogaine. The linearity of the ibogaine standard curve (Fig. 4) further supports this conclusion. Standard curves constructed presently are appropriate for measuring a wide range of tissue levels of ibogaine. For other applications, it is possible to construct standard curves with smaller amounts of ibogaine and internal standard (e.g. 10–100 ng, with 50 ng of internal standard). Such a standard curve might improve the precision with which smaller amounts of drug could be measured. Preliminary results also suggest that the present method yields quantitative analyses of ibogaine in plasma and in other tissues besides brain, such as liver, kidney and fat.

Because quantitative GC–MS methods depend on the detection of specific combinations of ion fragments in defined proportions at exact gas chromatographic retention times, these methods are extraordinarily selective for the substances being measured. Thus, it is unlikely that ibogaine metabolites could be mistakenly detected as ibogaine in the present method. In contrast, spectrophotometric methods [5] may not have distinguished ibogaine from its unknown metabolites. Preliminary ibogaine levels in brain reported here

may be consistent with this difference. Thus, rat brain levels of ibogaine 1 hr after administration of 40 mg/kg (3.26 μg/g) were approximately 17% of those found by Dhahir in rat brain [5] 1 hr after a slightly larger dose (50 mg/kg).

Previous work has established that a single dose of ibogaine induces profound behavioral changes in rats for at least 19 hr after administration [12]. The present results show that ibogaine levels in brain are very low after this treatment interval, and suggest that either: (1) ibogaine is active at very low concentrations [4], (2) an active metabolite of ibogaine may be responsible for its prolonged duration of action, or (3) the initial exposure to ibogaine leads to irreversible behavioral changes.

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