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Distribution of the hallucinogens *N*,*N*-dimethyltryptamine and 5-methoxy-*N*,*N*-dimethyltryptamine in rat brain following intraperitoneal injection: application of a new solid-phase extraction LC–APcI–MS–MS–isotope dilution method

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Abstract

A method for the solid-phase extraction (SPE) and liquid chromatographic–atmospheric pressure chemical ionization–mass spectrometric–mass spectrometric–isotope dilution (LC–APcI–MS–MS–ID) analysis of the indole hallucinogens *N*,*N*-dimethyltryptamine (DMT) and 5-methoxy DMT (or *O*-methyl bufotenin, OMB) from rat brain tissue is reported. Rats were administered DMT or OMB by the intraperitoneal route at a dose of 5 mg/kg and sacrificed 15 min post treatment. Brains were dissected into discrete areas and analyzed by the methods described as a demonstration of the procedure's applicability. The synthesis and use of two new deuterated internal standards for these purposes are also reported.

Keywords: *N*,*N*-Dimethyltryptamine; 5-Methoxy-*N*,*N*-dimethyltryptamine

1. Introduction

N,*N*-Dimethyltryptamine (DMT: 1, Fig. 1) and 5-methoxy-DMT (or *O*-methyl-bufotenin, OMB: 2, Fig. 1) are hallucinogenic indole alkaloids that occur naturally in a variety of plants and preparations used by both ancient and modern South American cultures to perform shamanistic divination rituals (for example, see [1–4]). DMT-use in Western culture flourished during the 1960s and was known as ''the businessman's LSD'' (lysergic acid diethylamide), a reflection of its rapid onset (30 s to 2 min) and short duration of action (30–60 min). OMB-use has been less prevalent in Western culture but is known to be more potent as a hallucinogen than DMT and to have a relatively delayed time to onset (10–15 min) and longer duration of action (1–2 h) [5,6].

It is of interest to note that DMT and OMB, as well as the related compound bufotenin (5-hydroxy-DMT), have been postulated to occur naturally in mammalian brain as neurotransmitters [7–11]. Evidence for their presence in man has been demonstrated in blood, urine and cerebrospinal fluid, particularly in studies designed to elucidate their pos-

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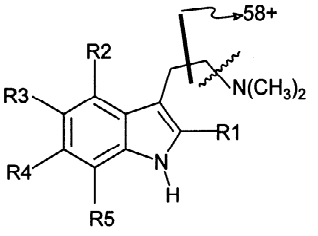

DMT, R1=R2=R3=R4=R5= H: M+1$^+$ = 189.
Product ions = 58$^+$ (100%), 144$^+$ (40%) ~~~

DDMT, R1=R2=R3=R4=R5= D: M+1$^+$ = 194.
Product ions = 58$^+$ (100%), 149$^+$ (40%) ~~~

OMB, R1=R2=R4=R5= H: R3= -OCH3: M+1$^+$ = 219.
Product ions = 58$^+$ (100%), 174$^+$ (30%) ~~~

DOMB, R1=R2=R4=R5= D: R3= -OCH3: M+1$^+$ = 223.
Product ions = 58$^+$ (100%), 178$^+$ (30%) ~~~

Fig. 1. The structure of DMT and OMB and their deuterated analogs. Fragmentation patterns consistent with the ions observed in APcI–MS and APcI–MS–MS (product ions) and their relative percent yields are shown.

sible role in psychiatric disorders, by a variety of analytical methodologies [12–47]. However, in retrospect it is obvious that many of these reports used analytical methodologies that were less than ideal in terms of specificity and analyte identification and newer methodology that takes advantage of more modern technologies for the analysis of these hallucinogens is needed.

The levels of administered DMT and OMB attained in whole rat brain as a function of time and dose have also been reported. Both compounds attain levels in the μg/g range and have rather short half-lives in this tissue [48–51]. However, the areas of the brain to which these compounds are distributed following dosing have not been demonstrated. Such information would be relevant to a determination of their mode of action and for comparative examination in relation to other hallucinogenic and non-hallucinogenic drugs.

We present here a new, highly sensitive, specific and rapid method for the extraction and analysis of both DMT and OMB in biological samples. The procedure provides new deuterated analogs for isotope dilution quantification and possesses a high degree of specificity that is afforded by the use of liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry–mass spectrometry–isotope dilution (LC–APcI–MS–MS–ID) techniques. The method employs an efficient solid-phase extraction procedure for the isolation of these compounds and is applied here to the analysis of administered DMT and OMB from rat brain areas, assaying tissue weights ranging from 0.014 to 0.310 g. The extracts were analyzed on a reversed-phase LC system followed by introduction to a triple quadruple MS operated in the positive ion, APcI mode. Product ions of the precursor molecular ions ($M+1^+$) were monitored in the multiple reaction monitoring mode (MRM). This method provided highly accurate quantification of DMT and OMB in various brain areas following their administration (5 mg/kg) to rats by intraperitoneal (IP) injection.

2. Experimental

2.1. Animal studies

2.1.1. Animals

Male (two) and female (four) Sprague–Dawley rats (Louisiana State University School of Veterinary Medicine Department of Laboratory Animal Medicine colony) weighing between 291 and 372 g, were housed in pairs, exposed to a 12-h light–dark cycle and provided food (commercial rat chow) and water (tap) ad libitum. Animals were divided into two groups (one male and two females each): one received DMT and the other OMB. Additional rats were used in the preliminary method development and extra brain tissues from these untreated animals were used to provide negative (blank) samples for generating standard curves and for quality control analyses.

2.1.2. Drug administration

Saline solutions (85 mg NaCl in 10 ml HPLC grade water adjusted to a pH of approximately 6 using 0.1 *M* HCl: Fisher Chemicals, Fair Lawn, NJ, USA) of DMT and OMB (10 mg: Sigma Chemical Co., St. Louis, MO, USA) were prepared in separate 20-ml scintillation vials. Solutions were stored at 4°C in a capped vial. All solutions were allowed to come to room temperature before use.

Animals were weighed and the dose volume was calculated for an administration of 5 mg/kg (5 mg/kg×animal wt. in kg×1 ml/1 mg=dose in ml). Animals were sedated with CO_2 and were injected by the intraperitoneal route, with the time of injection being noted.

2.1.3. Animal sacrifice and brain collection

Animals were observed and then sacrificed by CO_2 hypoxia at approximately 15 min post injection. The animals were exsanguinated and the brain was removed. Each whole brain was placed in a labeled tissue cassette and wrapped in labeled aluminum foil. The cassette was placed in liquid nitrogen and transferred to a −80°C freezer (Forma Scientific, Marietta, Ohio, USA) for storage prior to analysis. Blank tissues were collected in the same manner from untreated rats.

2.1.4. Brain dissection

Using a scalpel, the brain was dissected on a glass plate resting on a bed of ice. The following areas were collected: cerebral cortex, cerebellum, medulla oblongata, pons, basal ganglia, thalamus, hypothalamus and hippocampus. After each area had been removed, the tissue was placed in a pre-weighed 20-ml glass scintillation vial, capped and stored at −20°C until further processing. An anatomical map of the rat brain [52] was used as a reference to help identify the anatomical boundaries of these brain areas.

Blank tissues were collected similarly but were not divided into brain areas. Rather, the entire brain was minced with a sharp scalpel and pooled for use in generating standard curves and providing tissue sample blanks for quality control.

2.2. Synthesis of 2,4,5,6,7-pentadeutero-N,N-dimethyltryptamine (DDMT) and 2,4,6,7-tetradeutero-5-methoxy-DMT (DOMB)

The deutero analogs were prepared by the method of Wishart, et al. [53] as described for the preparation of deuterated tryptophan, with minor modifications. Thus, 200 mg of either DMT or OMB were added to 10 ml of D_2O (99.99%, Aldrich Chemical Co., St. Louis, MO, USA) in a 25-ml round-bottom flask. The DMT or OMB was dissolved by addition of DCl (deuterium chloride: 20% w/w solution in D_2O, 99.5%; Aldrich Chemical Co., St. Louis, MO, USA) and stirring via a magnetic stirring bar. To the solution was added 0.10 g of reduced Adam's catalyst (Pt°, platinum black: Aldrich Chemical Co., St. Louis, MO, USA). The remaining steps were conducted as described [53].

The product was assayed by LC–APcI–MS (total ion: see conditions below) to ascertain the percent conversion of the proteo to the deutero species. The deuterium exchange procedure described above was repeated a total of three times each to obtain material that was >98% deuterated in the proscribed positions and that had <0.001% contribution to ions monitored for the proteo species.

The oily residues from these syntheses were stored at −20°C and crystallized on standing. Aliquots of this material were used to prepare stock solutions for isotope dilution internal standard spiking of samples.

2.2.1. Sample preparation and extraction

Stock solutions: DMT, DDMT, DOMB and OMB (1 mg) were weighed, placed in separate 15-ml tubes and dissolved in 10 ml MeOH (HPLC grade: Fisher Chemicals, Fair Lawn, NJ, USA: 100 ng/ul). For individual standards, 100 μl of each stock were transferred to new tubes and 9.9 ml MeOH were added (1 ng/ul). A combined working stock for DOMB and DDMT was prepared for pipetting by adding 100 μl of each and bringing to volume with 9.8 ml of MeOH. Stored at −20°C, these solutions were proven to be stable for up to 90 days.

The extraction procedure for brain tissue was a modification of the method reported by Ishii et al. [54] for DMT in blood and urine. Thus, brain areas from administration studies and blended brain tissues for use in preparing standard curves, blanks and controls stored at −80°C, were allowed to thaw in a refrigerator (4°C). Brain areas assayed weighed between 0.014 and 0.310 g. Cortical tissues were blended by mincing and approximately 0.25 g was used for extraction. Similarly, approximately 0.25 g of blank brain tissue was used for each point in the standard curve and for tissue blanks. The method blank received no tissue.

To each sample homogenization tube (15 ml polypropylene conical, calibrated), except as indicated below, 100 μl of combined DDMT and

DOMB working solution (100 ng each) were added as internal standards (I.S.). Internal standards were not added to the method blank (negative control) or tissue blank (negative tissue control). For standard curve tubes 5, 10, 20, 50 or 100 μl of OMB working stock solution and 25, 50, 100, 250 or 500 μl of DMT working stock solution were combined, respectively. These values were adjusted to reflect the study being conducted so as to properly bracket brain tissue levels observed in pilot experiments. The methanol was evaporated under a stream of dry nitrogen (N-Evap, Organomation Associates Inc., South Berlin, MA, USA) in a water bath (30°C).

To each 20-ml scintillation vial containing tissue, 8 ml of HPLC grade water and 1 ml of 1 *M* $NaHCO_3$ were added. The vials were gently swirled and the contents were transferred to the appropriate polypropylene homogenization tube by decantation. The samples were homogenized (Tekmar Tissuemizer, Cincinnati, OH, USA: previously washed with H_2O and MeOH and dried; speed setting of 90 for 30 s) beginning with the method blank and proceeding to the final sample, washing with H_2O and MeOH between each sample and drying the blade–probe. Tubes were centrifuged (Damon IEC, DPR-6000, International Equipment Co., Needham, MA, USA) at 15°C at 5×10^3 rpm for 30 min.

During centrifugation, SPE columns [Sep-Pak cartridges (Waters, Milford, MA, USA) fitted with 12-ml reservoirs with frits (Varian, Palo Alto, CA, USA)] were prepared as described by Ishii, et al. [54] and used in a 24-port solid-phase extraction (SPE) vacuum box (Supelco Inc., Bellefonte, PA, USA) fitted with Teflon needles and luer-lock valves. The columns were rinsed sequentially with 3×10 ml $CHCl_3$ (HPLC grade, Fisher Chemicals, Fair Lawn, NJ, USA), MeOH and then water, being careful at the end not to allow the columns to go dry.

Sample supernatant was transferred by decantation to the appropriate column reservoir. The samples were drawn into the columns by application of vacuum (flow-rate of approximately 2 ml/min). Once the sample was completely into the cartridge 2×10 ml HPLC H_2O were added (10 ml at a time opening the valve and draining). The column was allowed to dry by leaving the valve open after the final addition of water. Vacuum was removed, the box was emptied and pre-labeled 10-ml, screw cap, glass conical tubes were placed in their respective positions. Using a repipetter (Oxford Model 1/10), 4 ml of $CHCl_3$:MeOH (9:1) were added to each column. After all of the elution solvent had been added, the vacuum was briefly applied to begin elution. The flow-rate was approximately 2 ml/min. When all samples were eluted, the box was vented and the tubes removed. Each sample was briefly vortexed and centrifuged (Dynac/Clay Adams, Becton Dickinson, Franklin Falls, NJ, USA) for 5 min at a 60% setting using a desktop centrifuge. A water layer and a $CHCl_3$ layer were evident. The bottom $CHCl_3$ layer was transferred to a clean 10-ml screw-cap conical tube using a disposable Pasteur pipette. Samples were placed in a N_2 evaporator at 30°C and evaporated to dryness. To the residue were added 250 μl of 50:50 isopropanol:acetonitrile (both HPLC grade, Fisher Chemicals, Fair Lawn, NJ, USA). The sample was vortexed and transferred (disposable pipette) to a 1-ml syringe (Becton Dickinson, Franklin Falls, NJ, USA) fitted with a 0.45-micron luer lock filter (Waters, Milford, MA, USA) positioned over a 250-μl, self-aligning LC vial insert and vial (Hewlett-Packard, Palo Alto, CA, USA) and filtered by addition of pressure using a syringe plunger. Samples were capped and submitted for LC–MS–MS [Fisons Quattro II (Micromass Ltd., Manchester, UK): HP 1090 Series 2 LC (Hewlett-Packard, Palo Alto, CA, USA)] analysis. **Note:** Samples may be kept frozen (−20°C) overnight prior to analysis. Data indicate that DMT and OMB are quite stable under these conditions but will not survive storage for >48 h at RT.

2.2.2. LC–APcI–MS–MS analysis

The LC–MS–MS system was prepared for APcI (atmospheric pressure chemical ionization) analysis. The APcI function was tuned for the production of the $M+1^+$ ions of DMT and OMB [direct infusion of a 100 μg/ml solution using an infusion pump (Cole-Parmer 74900 Series, Vernon Hills, IL, USA: flow of 16 μl/min) coupled in-line to the LC (flowing at 0.2 ml/min of initial mobile phase: 50:50 isopropanol:acetonitrile each containing 0.05% v/v formic acid: (95–98%, Aldrich, Milwaukee, WI, USA)] to maximize the yield of these ions, usually involving adjustment of the cone voltage only. From this tune and with the infusion pump still running,

the MS–MS function performance was determined by utilizing a multiple reaction monitoring (MRM) program, examining the yield and relative distribution of product ions (collision energy of 20 V, argon pressure of 3.1×10^{-4} Torr). The system was tuned to maximize product ion yield. This usually involved adjustment of the collision energy and de-tuning of the high- and low-mass resolutions. Analyses were conducted using the tune for these conditions and an electron multiplier setting of 900 eV.

The LC column was a 10 μm particle size, PRP-1 column (150×4.1 mm, 100-angstrom pore size, copolymer of styrene and divinylbenzene: Hamilton, Reno, NV, USA) fitted with an in-line filter (4 mm, 2-μm filter element and holder: Alltech Assoc. Inc., Deerfield, IL, USA). The mobile phase and solvent delivery program for sample runs consisted of: (A) isopropanol–0.05% formic acid, (B) HPLC grade water–0.1% formic acid and (C) acetonitrile–0.05% formic acid. An LC gradient was used: initial condition of 50:50 A:C, 0% B for 3 min, changing to 0% A by 10 min, 60% B and 40% C. The mobile phase returned to the initial conditions by 15 min and was held at these conditions for 4 min prior to the injection of the next sample. The flow-rate was maintained at 0.5 ml/min throughout the analysis. Analyses were conducted following injection of 50 μl of sample from a total sample volume of 250 μl.

Standards for the proteo and deutero compounds were run through the entire program to assess the peak shape and overall response as well as cross-talk. The MRM program monitored the $M+1^+$ ions for the four analytes and the base peak product ion 58^+ m/z, which was common to all four compounds, at a scan rate of one scan every 0.2 s.

2.2.3. Calculations

The standard curves from the fortified tissues were generated by calculating the ratio of the response of the proteo standard to the deutero internal standard and plotting as a function of this ratio (y axis) to increasing concentration (x axis). A least squares fit of the line so obtained was required to possess an r^2 value of no less than 0.995 to be acceptable. Quantification of target compounds in actual samples was obtained by comparing the responses as described to the ratios plotted in the standard curve. Data were expressed as ng compound/g of tissue, correcting for total tissue weight extracted, as well as total ng/brain area±the standard deviation (SD, $n=3$ for each brain area and each compound).

3. Results

The APcI–LC–MS spectra of the proteo and deutero DMT and OMB gave a high yield of $M+1^+$ ions (Fig. 1). Contribution between parent or precursor ion masses for the different compounds (proteo and/or deutero) was undetectable. In this regard, synthesis of the deuterated analogs required three rounds of Adam's catalyst–D_2O treatment to obtain a material that was near quantitatively converted to the respective D_5-DMT and D_4-OMB, with small amounts (<2%) of the respective D_4- or D_3-species being evident.

Product ion generation from these compounds via introduction of argon collision gas (collision induced dissociation: CID) showed a base peak ion of 58^+ m/z for all four compounds as well as another major fragment ion unique to each that is of significant relative percent yield (Fig. 1). These fragment ions, consisting of the indole ring and the alpha- and beta-methylenes of the ethylamine side chain, can be used for monitoring or for identification and verification of DMT or OMB by calculating and comparing ion ratios with standards. The postulated fragmentation pathways consistent with the ions observed are shown in Fig. 1. Multiple reaction monitoring (MRM) of the 58^+ m/z ion for each compound showed that there was imperceptible ''cross-talk'' or mass overlap of the proteo to the deutero species and vice versa at the concentrations examined. Thus, the 58^+ m/z ion was chosen for monitoring and quantifying DMT and OMB and for obtaining the I.S. response for each deuterated analog.

Standard curves derived from fortified brain tissue samples produced highly linear regression lines (r^2) that averaged 0.9995±0.0003 ($n=3$) for DMT in the range of 25 to 500 ng/tube (5–100 ng on-column; 100–2000 ng/g of tissue based on extraction of 0.25 g brain tissue wet weight) and 0.9998±0.0002 ($n=3$) for OMB in the range of 5–100 ng/tube (1–20 ng on column; 20–400 ng/g of tissue). In separate experiments, both compounds were observed to

provide linear curves over three orders of magnitude, ranging from 1 to 1000 ng/tube (0.2–200 ng on column). I.S. concentrations of DDMT and DOMB were held constant (100 ng/tube) in all of the analyses.

Absolute recoveries of DMT and OMB from the SPE procedure were variable but averaged 62±12% for DMT and 68±18% for OMB. Ishii et al. [54] reported recoveries of greater than 86% for urine and greater than 98% for blood using the method from which the present approach was adapted.

Validated limits of quantification (LOQ) were 5 ng on-column for DMT (100 ng/g of tissue as conducted here) and 1 ng on column for OMB (20 ng/g). However, due to the very low cross-talk and the limitation of background noise accomplished using MS–MS procedures, absolute limits of detection (LOD: signal-to-noise ratio of 3:1) of 0.025 ng on column for both DMT and OMB were observed (0.5 ng/g of tissue). The demonstrated limits of quantification reported here were more than adequate for the examination of administered drug in rat brain, bracketing the concentrations observed.

The accuracy and precision of the method for both DMT and OMB were well within acceptable limits. For DMT, accuracy measurements of 96.1±8.8%, 100.1±4.4% and 100.4±1.3% (mean±standard deviation) were observed for the range of concentrations (25-, 50-, 100-, 250- and 500-ng spikes) examined on three analytical occasions, for an overall reproducibility (precision) of 98.9±2.4%. Individual concentration accuracy values ranged from 90 to 109%. Similarly, accuracy values of 98.75±4.2, 99.6±5.2 and 99.5±5.3% were obtained for the range of concentrations (5-, 10-, 20-, 50- and 100-ng spikes) examined on three analytical occasions for OMB, for an overall reproducibility of 99.2±0.58%. Individual concentration accuracy ranged from 91.9 to 107.8%.

Analysis of method and tissue blanks produced no detectable peaks or interferences at the retention time for these compounds. Indeed, the overall ion chromatograms from these analyses were consistently free of peaks. This is an indication that the tissues and the materials employed in the method do not contribute significantly to the apparently unique $M+1^+$ ions monitored and/or, even where they do, they do not produce product ion fragments of 58^+ m/z that would complicate the interpretation of the results.

The LC–MS–MS-compatible mobile phase used here did not resolve DMT and OMB (Fig. 2A–D). This was not considered to be a problem since the compounds are completely resolved in terms of precursor ion mass parameters and no interference is produced from the use of the deuterated I.S. for each. Thus, the specificity of the method is quite high for the tissues examined.

The distribution of IP administered (5 mg/kg dose) DMT and OMB into regions of rat brain 15 min post injection is shown in Table 1. The concentrations are expressed as a function of ng/g of brain area. Values are also given for ng/brain area, since many of the areas were themselves only tens-of-milligrams in weight.

4. Discussion

The present method overcomes a number of difficulties posed by previously reported procedures for the extraction, identification and quantification of DMT and OMB in biological samples. For example, the SPE method applied here greatly speeds the extraction of these compounds from biological samples when compared to classical approaches [48–51]. To our knowledge, there has been no other SPE method described for the isolation of DMT and/or OMB from brain tissues. The method, adapted from one originally intended for plasma and urine [54], is efficient and rapid, permitting processing of up to 24 samples (including spiked tissue controls and QC samples) within a 3-h time span. Classical methods based on homogenization, acid precipitation of proteins, pH changes, countercurrent extraction and derivatization (for GC–MS analyses) often take several days to process a limited number of samples [48–51].

The use of APcI–MS–MS provides an extremely high degree of specificity for the compounds in question and, coupled with isotope dilution, affords a high degree of certainty for the proper assignment of structure and quantification for the compound detected. The only other deuterated internal standards for these purposes were first reported by Shaw et al. [55] and were synthesized to contain four deuterium

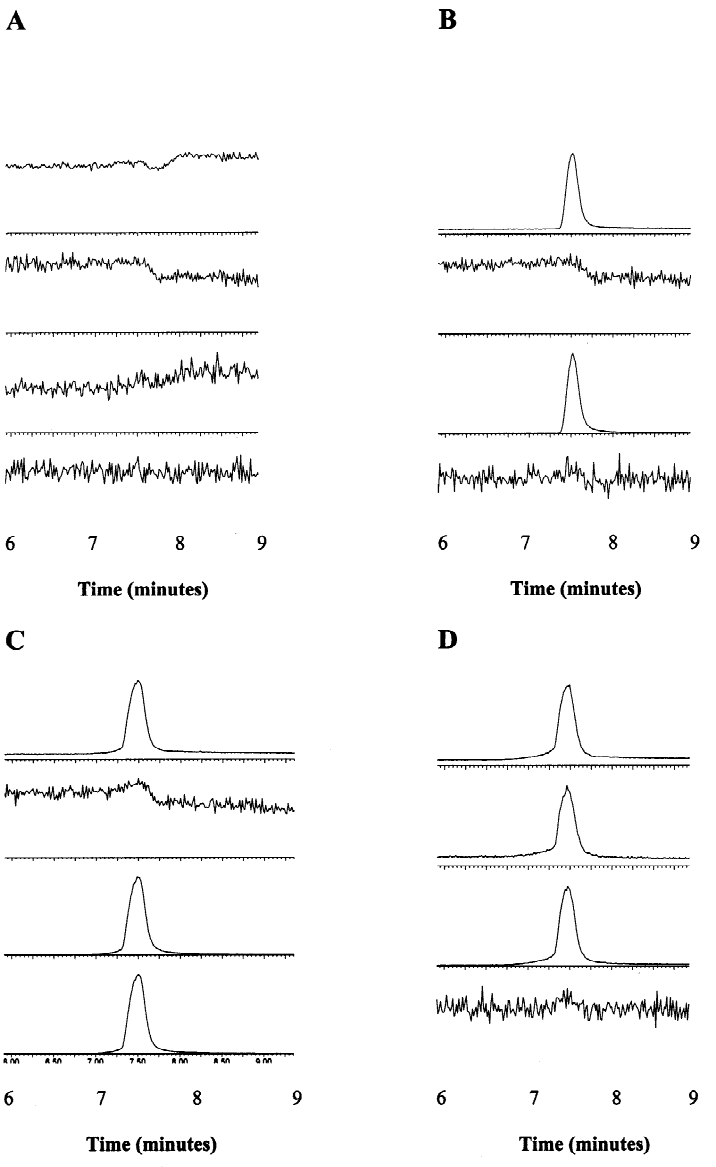

Fig. 2. (A) Representative LC–APcI–MS–MS–isotope dilution (ID) trace of a blank brain tissue sample, monitoring the collision induced dissociation of the precursor $M+1^+$ ions to the product ion 58^+ m/z common to all four compounds. From top to bottom: DOMB, 223→58, OMB, 219→ 58, DDMT, 194→ 58 and DMT→ 189→ 58. (B) Representative LC–APcI–MS–MS–ID trace of a blank brain tissue sample spiked with deuterated internal standards. (C) Representative LC–APcI–MS–MS–ID trace of a brain tissue sample from the thalamus of a rat administered DMT intraperitoneally at a dose of 5 mg/kg and sacrificed 15 min later. (D) Representative LC–APcI–MS–MS–ID trace of a brain tissue sample from the thalamus of a rat administered OMB intraperitoneally at a dose of 5 mg/kg and sacrificed 15 min later.

Table 1
Concentrations (in ng/g) of DMT and OMB in the brain areas examined for each animal as well as the mean and standard deviation (SD) for each brain area collectively[a]

DMT	Concentration (ng/g)						
Animal #	A	B	C	Mean	SD	Mean brain wt±SD	ng/Brain area
Cortex	1592.90	1598.30	1808.00	1666.40	122.66	0.27±0.02	456.59
Hypothalamus	<LOQ	<LOQ	<LOQ	<LOQ		0.11±0.12	<LOQ
Thalamus	936.20	1104.90	1554.70	1198.60	319.72	0.21±0.01	246.91
Hippocampus	498.90	471.10	1100.40	690.13	355.57	0.14±0.02	93.17
Basal ganglia	1182.50	1122.40	1466.30	1257.07	183.68	0.07±0.02	93.02
Medulla	1156.40	1319.80	4171.60	2215.93	1695.63	0.11±0.05	239.32
Pons	546.50	1312.70	1063.60	974.27	390.83	0.14±0.01	137.37
Cerebellum	1861.40	1243.10	1841.80	1648.77	351.45	0.26±0.05	422.08
5-MeO-DMT	Concentration (ng/g)						
Animal #	D	E	F	Mean	SD	Mean brain wt±SD	ng/Brain area
Cortex	211.40	113.30	179.40	168.03	50.03	0.27±0.01	44.53
Hypothalamus	<LOQ	<LOQ	<LOQ	<LOQ		0.04±0.01	<LOQ
Thalamus	231.40	92.60	116.70	146.90	74.16	0.17±0.02	24.83
Hippocampus	342.80	75.80	53.20	157.27	161.07	0.13±0.03	20.76
Basal ganglia	304.90	105.10	109.90	173.30	113.99	0.13±0.02	22.36
Medulla	158.60	231.60	106.20	165.47	62.98	0.12±0.03	19.69
Pons	623.70	55.60	206.20	295.17	294.31	0.11±0.04	32.47
Cerebellum	266.00	121.00	210.30	199.10	73.15	0.27±0.02	54.55

[a] Data for the mean brain area weights±SD are also given as well as the average ng level for each brain area.

atoms on the ethyl-amine side chain of these compounds. Electron impact–positive ion fragmentation of these deuterated compounds, exclusively reported in GC–MS analyses, produced fragment ions of 60^+ m/z and small amounts (<15% of the base peak) of other fragment ions, carrying the remaining two deuterium atoms on the beta-methylene of the ethyl-amine side chain. The new deuterated internal standards reported and utilized here retain a difference of 4–5 amu in both the base peak ion ($M+1^+$) and in the other major fragment ions formed from the indole ring, while yielding 58^+ m/z ion for all four compounds. The MS–MS mode, of course, allows the 58^+ m/z ion common to all four compounds to be completely distinguishable for each.

Efforts to obtain chromatographic resolution of DMT and OMB required the addition of salts and/or other modifiers to the mobile phase. Indeed, resolution was attained using an ammonium acetate–acetonitrile mobile phase (pH 11) on an extended pH range, octadecylsilane, narrow-bore column. However, in this example, the presence of the ammonium acetate suppressed ion yields some 100-fold and made the LOQ of the method unacceptable. This effort was based on a recent study by Kawanishi, et al. [56] which demonstrated the ability to identify and resolve DMT as well as a variety of harmala alkaloids in the shamanistic drink ''ayahuasca'' using APcI–MS and an ammonium acetate-containing mobile phase. Other LC analyses have been devised that resolve these or related alkaloids but the mobile phases required are not readily compatible with LC–MS [38,45,57], at least in the model and configuration of instrument used here.

The present method provides an analytical approach that meets the need to address endogenous hallucinogen detection and measurement with the necessary level of specificity. The proven limits of quantification demonstrated here could be readily lowered with proper validation so as to apply this approach to measurement of endogenous DMT and/or OMB in the femtomole/g of brain tissue range.

Certainly, in hindsight, the older literature that purports to identify and quantify DMT and/or OMB in vivo must be viewed with a degree of caution. This is due to the fact that the levels observed in

these reports were often in the low nanogram/ml or /g range and were determined, in many cases, by methods that were less than ideal in terms of specificity (thin layer chromatography, gas chromatography with electron capture or nitrogen–phosphorous detection: [12–19,23,24,29,32–38,41,43–45]). The reports employing gas chromatographic analysis required derivatization of the indole ring nitrogen (position 1) with trimethylsilyl or fluorinated-hydrocarbon to obtain acceptable peak shape and/or sensitivity, a process that can also complicate the method and results and that is eliminated in the present method.

The most acceptable studies in this regard are, thus, those that employed capillary chromatography, isotope dilution internal standardization mass spectrometry and that found the ratios of the base peak to the confirmatory ions for the proteo species to match with the standards. The present method accomplishes these attributes and adds another dimension of mass resolution afforded by MS–MS analyses. Combined with the SPE method, its application to the purposes of DMT and OMB measurement should be readily accomplished.

In the present study, both DMT and OMB were detected in the hypothalamus of all rats examined. However, the levels were below the proven LOQ for each compound. Extrapolating to ng/g levels below the proven LOQ indicated, however, a concentration range of 2–20 ng/g for DMT and 0.5–5 ng/g for OMB. Although the hypothalamus was the smallest of the brain areas examined, these findings stand in sharp contrast to the values seen in other brain tissues, particularly for DMT. In terms of concentration, the mean values for the cortex and cerebellum, followed by the thalamus, basal ganglia and pons/medulla are the highest for DMT. The levels in the brain at 15 min post injection for OMB appear to be somewhat homogeneous for all brain areas when viewed on a ng/g basis. However, for both DMT and OMB there is significant individual animal variability, as reflected in the SD values for some areas. It is also necessary to recognize that these data represent a ''snapshot'' of the brain levels attained for these compounds, presenting a comparison of data for two different drugs administered 15 min before sacrifice only, as a demonstration of the capabilities of the methods presented. A further complication is the fact that the dissections are gross dissections and have not been determined histologically to include or exclude contributions of neighboring areas.

What is quite clear from these data (Table 1), however, is the rather dramatic differences in brain region levels attained for DMT and OMB following IP injection of 5 mg/kg and sacrifice at 15 min post administration. On average, the concentrations of DMT at 15 min are 7–8 times greater than those observed for OMB and, for specific brain regions, range from 3 to 13 times greater.

Studies of the metabolism of DMT and OMB in brain tissues indicate that it is appropriate to monitor only the parent compounds rather than to pursue metabolites. The major route of metabolism for DMT and OMB has been shown to occur via monoamine oxidase (MAO), yielding an indole acetaldehyde, which is then converted to an indole acetic acid. This fact has been reported by numerous researchers both in vitro and in vivo and confirmed by examination of the ability of MAO inhibitors (MAOI) to prolong the appearance and the effects of DMT in brain tissues. DMT has been shown to be converted to *N*-methyl-tryptamine (NMT), 1,2,3,4-tetrahydro-beta-carboline (THBC), 2-methyl-THBC and DMT-*N*-oxide in brain tissues in vitro and in vivo. Similar results have been reported for OMB both in vitro and in vivo as well and some reports have demonstrated the ability of OMB to be converted to bufotenin via *O*-demethylation. However, these are apparently minor pathways for metabolism of both DMT and OMB in the brain where oxidative deamination overwhelmingly predominates. It has been shown that use of MAOIs does not significantly shift the pattern of metabolism in favor of *N*-oxidation or *N*-demethylation in the brain, however [48–51,59–70], a fact which can be taken advantage of in examining endogenous levels of these compounds in the brain.

The data reported here are consistent with those obtained from the examination of whole brain levels of DMT and OMB in previous studies. These data also reflect the results from a variety of human and animal behavioral studies conducted for DMT and OMB in that DMT has a much shorter time to onset for hallucinations than OMB. Indeed, the transport of DMT into brain has been postulated to occur by an

active transport mechanism that assists in speeding DMT to the brain [51,59,71]. Nonetheless, the significant differences in concentrations is also an interesting observation in light of the fact that OMB is considered to be more potent as a hallucinogen than DMT. These differences may become less apparent at later time points of a brain-depletion curve study. The possibility that these large differences in the brain level can also be explained by delayed adsorption from the intraperitoneal site for OMB or increased metabolism or clearance of OMB is diminished by the fact that the half-lives of DMT and OMB in rat whole brain have been shown to be essentially equivalent ($t_{1/2}$ DMT=5.7 and OMB=6.0 min) [58] following intraperitoneal injection, as conducted here. DMT has been shown to have a longer blood half-life, however ($t_{1/2}$ DMT=15.8 and OMB=6.4 min) [58]. Nonetheless, the data presented here also appear to indicate that the relative distribution of these compounds into selected brain areas also differs and is worthy of further study.

The SPE and LC–MS–MS-isotope dilution method presented here should provide adequate extraction and analytical capabilities for the examination of these compounds in other biological matrices, as well as the ability to conduct further examination of DMT and OMB as naturally occurring compounds in brain tissues.

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