AYAHUASCA CHARACTERIZATION, METABOLISM IN HUMANS, AND RELEVANCE TO ENDOGENOUS N,N-DIMETHYLTRYPTAMINES

A Dissertation

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in

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by

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Abstract

Ayahuasca denotes an Amazonian psychotropic plant tea obtained from *Banisteriopsis caapi*, which contains β -carboline alkaloids, chiefly harmine, harmaline and tetrahydroharmine. The tea usually incorporates the leaves of *Psychotria viridis*, which are rich in *N*,*N*-dimethyltryptamine (DMT), a psychoactive 5-HT_{2A} agonist. The β -carbolines reversibly inhibit monoamine-oxidase (MAO), effectively preventing oxidative deamination of the orally inactive DMT and allowing its absorption and access to the central nervous system. Despite increased use of the tea worldwide, easy to perform and validated methods for its characterization do not exist and the metabolism and excretion of DMT and the β -carbolines has not been studied systematically in humans following ayahuasca consumption. Thus, we developed a liquid chromatographyelectrospray ionization-tandem mass spectrometry procedure for the simultaneous quantification of the major alkaloid components of ayahuasca, including several known and potential metabolites. The assay was applied to a variety of ayahuasca samples and modified to be applicable to human blood and urine samples before and after consumption of ayahuasca. The major components present in ayahuasca samples were tetrahydroharmine and harmine, followed by N,N-dimethyltryptamine and harmaline. The major metabolite of DMT was the corresponding *N*-oxide, DMT-N-oxide which was found in both blood plasma and urine, although not detectable in ayahuasca samples. Less than 1% of the administered DMT dose was detected in urine or blood plasma, despite the inhibition of monoamine oxidase afforded by the presence of the harmala alkaloids in ayahuasca. The major harmala alkaloid excreted was tetrahydroharmine. The methods developed would be suitable for the study of ayahuasca in human and ethnobotanical research, as well as in forensic examinations of ayahuasca preparations. The characteristics of the methods suggest that their sensitivity, selectivity and reproducibility are

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adequate for use in further toxicological and clinical research on ayahuasca as well as functioning as an assay to screen biological samples for endogenous hallucinogens. Based on the results of these studies we also present a critical review of 69 published studies reporting the detection in human body fluids of three indole alkaloids that possess differing degrees of psychedelic activity. Suggestions for the future directions of ayahuasca and endogenous psychedelics research are offered.

Chapter 1. Introduction

1.1 Semantics

N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), and bufotenine (5-OH-DMT) may be classified generally as chemical derivatives of the mood and behavioral modulating essential monoamine neurotransmitter 5-hydroxytrptamine (5-HT or serotonin) given that they all share an indole moiety and a basic nitrogen atom connected by an alkyl chain. More specifically these molecules may be classified as indoleamine or indolealkylamine hallucinogens [1.1]. The word 'hallucinogen' has become the most common designation in current scientific literature to describe a variety of substances including psilocybe mushrooms, peyote, iboga, bufo alvarius toad venom, and ayahuasca along with their corresponding psychoactive compounds psilocybin (4-OH-DMT), mescaline, ibogaine, 5-MeO-DMT, and DMT, respectively. In addition there are synthetic hallucinogen molecules such as lysergic acid diethylamide (LSD-25) and a growing number of other structurally related tryptamine and phenethylamine molecules which have been synthesized in laboratories [1.1-.13]. Still more hallucinogenic or potentially hallucinogenic compounds have been identified in nearly 100 species and a wide range of genera and families [1.4].

When consumed and experienced individually or in combination, as in the case of ayahuasca, these hallucinogenic compounds may potentially induce involuntary perceptual phenomena (hallucinations) within a conscious and awake organism while in the absence of any apparent empirically verifiable externally known stimuli. Terrence McKenna once defined a hallucination as being in the presence of that which could previously not be imagined. We may call these substances 'hallucinogens' because the term has been most commonly used in the biomedical literature. The most generic and therefore perhaps more appropriate term could be

psychedelic (mind manifesting; Osmond, [1.5]). Other terms such as: psychotomimetic (psychosis mimicking; [1.6]), entheogenic (generating the divine; [1.7]), or holotropic [1.8] have all generally been used interchangeably with 'hallucinogen' depending on a given paradigm or context (social or recreational, experimental or therapeutic, religious or mystical, spiritual or sacramental). All could possibly be considered misnomers [1.1, 1.9] given that they seem to lack the qualitative descriptors necessary to heuristically express the phenomenal possibilities for such substances to not only potentially influence perception but also cognition, affect, mood, and behavior [1.10, 1.11]. These substances may afford changes in the processing of fundamental information regarding the ontology and epistemology of self, emotions, social interactions, dependencies, language, as well as the overall integration of conscious, unconscious, subconscious, and preconscious information in the brain. This may result in a novel non-ordinary 'psychointegrative' state of consciousness, where limbic system processes may become particularly more integrated with neocortical processes [1.1, 1.12].

Perhaps a more appropriate distinction for these types of compounds would be as 'psychointegrators' [1.9] which attempts to communicate the holistic and psychodynamic nature of the often transpersonal experiences that these compounds may allow for, with potential effects upon sensory, behavioral, emotional, and cognitive processes. These substances, such as psilocybin, may occasion mystical-type experiences mediating the attribution of substantial and sustained personal meaning and spiritual significance [1.13, 1.14]. Perhaps these substances may afford a more integrated ontological connection with the quantum-holographic [1.15] to cosmological scale unified space-time manifold such as could be described by the schwarzschild proton paradigm [1.16].

We may ask the questions: Why do humans produce endogenous psychedelics? Does consciousness represent a product or epiphenomenon of the brain? Or does the brain act as a tuner directing a certain resolution of awareness to a specific frequency of an omnipresent panexperiential consciousness? Could consciousness be a result of an orchestrated objective reduction of quantum coherence in brain microtubules [1.17, 1.18]? While this manuscript does not claim to answer these questions, future studies of exogenous and endogenous hallucinogens could very well lead to clearer empirically testable hypotheses, theories and conclusions. If we choose to see these substances, such as ayahuasca, as molecular tools to explore the conscious, unconscious and preconscious mind, or as useful neuroscientific and psychological instruments to explore novel aspects of the brain and self, we could potentially use them to reveal new resolutions of perceived existence and felt experience much like the microscope has done for biology or the telescope for astronomy.

"We do not see the world as it is, we see the world as we are" -the Talmud

1.2 Historical and social context

Throughout the thousands of years of recorded and, we may assume, unrecorded history and across the entire world, accounts may be found in many religious and mythical texts describing what could be called spontaneous or drug induced hallucinogenic, psychointegrative, mystical, religious, spiritual, or entheogenic types of experiences. This includes the use of 'kykeon' in the Eleusinian mysteries of ancient Greece [1.19], descriptions in the Rig Veda of the use of 'Soma' in India [1.20], Mesoamerica's use of sacred psilocybin mushrooms, Native Americans' use of mescaline-containing Peyote cactus, sacramental use of Iboga among the African Bwiti [1.21], Caribbean use of Anadenanthera peregrine seed-based Cohoba snuff containing bufotenine, 5-MeO-DMT, and DMT [1.22, 1.23], first described by Friar Ramon

Pane in 1496, as well as the ongoing use of the synergistically brewed tea Ayahuasca among the Amazonians which typically combines the DMT containing leaves of Psychotria viridis with MAO inhibiting harmala beta-carbolines (β -carbolines) found in the vines of Banisteriopsis *caapi* [1.10]. The speculative use of ayahuasca has been dated to sometime between A.D. 800 and 1200 based on the gas chromatography-mass spectrometry (GC-MC) detection of harmine alkaloids in ancient Andean hair samples found in northern Chile from an adult male and a one-year-old baby [1.24]. It has even been speculated that perhaps the biblical Moses, while under the MAO inhibiting effects of beta-carbolines found in Peganum harmala (Syrian rue), may have had a 'psychointegrative' experience when in the presence of, as accounted in the Old Testament, the smoke of a burning Acacia bush - which has been found to contain various concentrations of DMT [1.25].

Whether spontaneous or drug-induced, psychointegrative or psychedelic experiences have often played vital roles cross-culturally and across history as primary sources of spiritual inspiration, inquisitions, oppression, as well as religious participation, potentially providing motivation for the institutionalization of many religious sacraments, beliefs, and activities [1.26, 1.27]. The historical, social, and cross-cultural similarities found in many reports concerning various non-ordinary states of consciousness (NOSC) may often represent the probable occurrence of a 'psychointegrative' phenomenon which could be potentially mediated spontaneously by endogenous processes or intentionally by a variety of meditation practices, breathing techniques such as pranayama and holotropic breath work [1.28], physical practices such as yoga or tantra, fasting, hypnosis techniques, electromagnetic or auditory / visual entrainment procedures [1.29], initiation rites or rituals, as well as the therapeutic, recreational, sacramental, or shamanic applications of various exogenously administered hallucinogenic

substances, which could collectively imply an underlying, potentially endogenous, shared neurobiological mechanism of action [1.30]. Thus epistemological claims of supernatural, occult, or magical powers, as well as accounts of contact with spirits, angels, demons, deities, gods, mythical creatures, the dead, or extraterrestrials which may have been initially acquired through psychointegrative or hallucinogenic-related phenomenon may potentially be replaced by empirical scientific paradigms evaluating the significance of such subjective experiences, their etiology and the biochemical mechanism by which they might be produced and reproduced [1.31].

Several N,N-dimethylated indolamines have been characterized as endogenous hallucinogens in humans and other species. These endogenous hallucinogens have been hypothesized to play major roles in diverse phenomena such as sleep and dreaming [1.32], reducing anxiety [1.33], near death experiences, out of body experiences, childbirth [1.15, 1.34], establishment and maintenance of perceptual homeostasis [1.35], mystical or religious experiences [1.13, 1.14, 1.34], psychosis or schizophrenia [136, 1.37], autism, severe depression, mania, post-traumatic stress disorder, dissociative disorders, panic disorders, and even UFO abduction experiences [1.15, 1.34].

The first 25 years of scientific research examining endogenous hallucinogens appears to be primarily motivated by and limited to psychotomimetic or hallucinogenic paradigms [1.38] which propose that the seemingly hallucinatory group of symptoms often associated with the heterogeneous psychiatric diagnosis commonly referred to as 'schizophrenia' by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) may actually be a consequence of endogenous hallucinogen synthesis leading to the "transmethylation hypothesis of schizophrenia" [1.39-1.41]. This hypothesis speculated that dietary tryptophan (TRP) could be

decarboxylated to form tryptamine (TA) which could then undergo two methylations using Sadenosylmethionine (SAMe) as the methyl donor [1.42] to endogenously form the hallucinogenic molecule DMT and thus potentially contribute to the symptoms often associated with schizophrenia. DMT has hence been identified as a naturally occurring potential product of tryptophan metabolism [1.31]. Tryptamine has been identified in the brain [1.43], and the enzyme that methylates tryptamine, indole-N-methyltransferase (INMT), was first identified in the lungs of rabbits and later in the brain, lung, blood, and cerebral spinal fluid of some mammals, including humans [1.43-1.45].

Certain similarities may potentially be associated between hallucinogen phenomenology and endogenous schizophrenia [1.45-1.47]. One study claimed finding a positive correlation between urinary DMT and measures of psychotic symptoms in a group of psychotic patients [1.48], and while other studies have reported identifying DMT in the blood and urine of certain, but not all, patients diagnosed with schizophrenia, DMT was also reported in some normal control participants [1.49-1.50]. Many initial reports identifying DMT levels in the body fluids of patients diagnosed with schizophrenia have not always been replicated successfully [1.51-1.57] given that no statistically significant differences in detectable DMT levels could be found between patients diagnosed with schizophrenia relative to control populations, leading to the conclusion that if DMT was present it was in too low a concentration to be accurately quantitated [1.58].

Unfortunately, clinical research on all hallucinogens was made more difficult during the early 1970's when the Controlled Substance Act was passed [1.59]. Nevertheless, the biological and subjective effects of exogenously administered pharmacological doses of intravenous DMT have since been well characterized in a healthy group of experienced hallucinogen users [1.60-

1.62], and the gene encoding for the human INMT enzyme, which catalyzes the final steps of endogenous hallucinogenic tryptamine formation, has been isolated, cloned, and transfected into mammalian cells [1.63, 1.64]. Northern blot analysis indicated that INMT mRNA transcripts were expressed in many human tissues, appearing to be most highly expressed in the lung although not appearing in brain. In addition to methylation of indoleamines, the endogenous formation of β -carbolines from primary tryptamines such as serotonin (5-HT) and 5methoxytryptamine has also been suggested [1.65]. Interestingly, pinoline (6-methoxy-THBC) which like other β -carbolines may inhibit MAO-A [1.66], has been identified by GC/MS to be an endogenous component of the pineal gland with similar concentrations and circadian release patterns to those of melatonin [1.67], possibly potentiating the effects of endogenous hallucinogens by inhibiting the major route of metabolism and potentially mediating phenomena such as dreaming [1.32]. This endogenous pinoline / DMT interaction could possibly produce an endogenous experience similar to that induced by the consumption of ayahuasca. In this regard, a further investigation into the metabolism and clearance of ayahuasca, which contains MAOinhibiting beta-carbolines and DMT, could lead to a better understanding of endogenous hallucinatory or psychointegrative phenomenon.

1.3 Ayahuasca

The term 'Ayahuasca' denotes a traditional Amazonian psychotropic tea obtained by boiling the stems and bark of the jungle liana *Banisteriopsis caapi* typically in combination with the leaves of *Psychotria viridis* or *Diplopterys cabrerana* [2.1-2.6]. The tea, also known as caapi, yage, mihi, dapa, natema, pinde, hoasca, daime, or vegetal, has a millennia-old cultural history of indigenous religious and medical use where it continues to hold a highly esteemed position in the pharmacopeia of the various South American groups. These cultures still often regularly

consume Ayahuasca to promote healing, commune with ancestors, and induce modified states of consciousness during religious ceremonies and sacred cleansing rituals [2.5, 2.16].

In recent history, the firmly established ancestral use of ayahuasca has been adapted into sacramental use by three growing syncretic religious groups which have established churches across the Americas and Europe. Two of these religious movements, the Santo Daime and União do Vegetal (UDV) claim to have over 10,000 members and have received substantial legal protections to consume ayahuasca in the United States in ceremonial practices if appropriate paper work has been established [2.17]. In addition, an increasing number of foreigners are traveling to the Amazon to participate in ayahuasca retreats and traditional healers are organizing ayahuasca ceremonies around the world. This increased use has implications for public health [2.18].

An increasing interest exists for potential medical applications of ayahuasca [2.6, 2.15], including its antioxidant, antimutagenic and antigenotoxic activity [2.19]. In addition, there are suggestions of its putative psychotherapeutic and rehabilitative effects for conditions such as alcoholism, violence, suicidal behaviors, and severe depression, as well as other disorders [2.20-2.25]. Thus, a detailed examination and understanding of the biochemical parameters affected by ayahuasca would add to our understanding of this Amazonian medicine. Such an undertaking will require scientifically controlled studies conducted as clinical trials. This will require the development of analytical methodologies with the capability to characterize ayahuasca extracts, as well as conduct pharmacokinetic and metabolic studies of its components

Previous research has revealed that *Banisteriopsis caapi*, contains the β -carboline alkaloids harmine, harmaline and tetrahydroharmine [2.7]. The other traditional plant component

of the tea: *Psychotria viridis* or *Diplopterys cabrerana*, contains *N*, *N*-dimethyltryptamine (DMT), a psychedelic 5-HT_{2A/1A/2C} agonist [B.11-B.15]. It has been demonstrated that orally administered DMT does not produce psychoactive activity by itself [2.14]. Therefore, the dominant theory of ayahuasca's oral activity has been that the β -carbolines reversibly inhibit monoamine-oxidase A (MAO-A), effectively preventing oxidative deamination of the usually orally inactive DMT and thus allowing its absorption and access to the central nervous system [1.10]. More detailed information on the issues of ayahuasca composition, metabolism and historical background may be found in the respective introduction of the subsequent chapters.

1.4 Chapter Contents

Given the interests and capabilities of our laboratory, and recognition that clinical research assessing the potential medicinal uses for ayahuasca will require information regarding the composition, pharmacokinetics, metabolism, and clearance of ayahuasca's major components led us to the research presented here. Thus, specific methods for the characterization and quantitation of the major constituents of ayahuasca and their metabolites in blood and urine have been established. In so doing, we hoped to develop methodology that would be useful in the study of ayahuasca in clinical and ethnobotanical research as well as in forensic examinations of ayahuasca preparations. In an effort to characterize and quantify the active chemical components present in ayahuasca we developed a direct injection/liquid chromatography–electrospray ionization-tandem mass spectrometry procedure with the capability to simultaneously quantify 11 compounds potentially found in ayahuasca. The method utilizes a deuterated internal standard for quantitation and affords rapid detection of the alkaloids by a simple dilution assay, requiring no extraction procedures. Chapter 2 presents this manuscript entitled "Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca

by liquid chromatography–electrospray ionization-tandem mass spectrometry" which was published in the Journal of Chromatography A in December 2009 [3.7]. We hypothesized that a simple sample dilution protocol could be used to detect and quantitate the components of ayahuasca using LC/MS technology, greatly simplifying the process of characterization and providing tremendous analytical advantages over previously reported studies and methods. We anticipated that this research would confirm much of the existing data concerning ayahuasca, detecting the major components present in ayahuasca as tetrahydroharmine and harmine, followed by DMT and harmaline. However, the method also had the capability of looking for and detecting other potential components such as harmalol, NMT (N-methyltryptamine), harmol, and DMT-N-oxide (DMT-NO) as described in Chapter 2.

Given that clinical research will require standardization of various ayahuasca preparations, we used the new method described in Appendix A in research in collaboration with Dr. Leanna Standish of Bastyr University (Kenmore, WA) in a study to measure variations in the active constituents of ayahuasca collected from teas brewed and used in ceremonies conducted in Peru, Brazil, Hawaii and mainland United States. Appendix A presents this manuscript entitled "Cross cultural variations in alkaloid content of ayahuasca teas used in spiritual ceremonies" which was submitted to the Journal of Ethnopharmacology in September 2011 and remains under review. We hypothesized that application of the new methodology would allow a more detailed characterization of ayahuasca preparations. Indeed, the research revealed that levels of DMT and harmine remain remarkable similar between different batches of ayahuasca brewed in Peru, Brazil, or Hawaii.

Gaining knowledge of the metabolism of the compounds found in ayahuasca represents another aspect of conducting clinical trials. Despite increased use of the ayahuasca worldwide, the metabolism and excretion of DMT and the β -carbolines in ayahuasca had yet to be studied systematically in humans following ayahuasca administration. Because of an increasing interest in the potential for modern medical applications of ayahuasca, as well as concerns regarding potential for abuse, both toxicological and clinical research will require information regarding the metabolism and clearance of its components. For these reasons, following the successful development of a method to characterize ayahuasca, we applied our approach to the study of the metabolism of ayahuasca by measuring excretory products in human urine following ayahuasca administration. The method for urine analysis, as developed for ayahuasca itself, uses sample dilution and high performance liquid chromatography (HPLC)-electrospray ionization-selected reaction monitoring tandem mass spectrometry, affording a rapid, sensitive and specific characterization and quantitation of the major constituents and metabolites of ayahuasca in human urine. We demonstrate the validity and application of our analytical protocol to urine samples collected in a pilot study by Dr. Jordi Riba's lab (Sant Pau, Barcelona, Spain) from three individuals that were administered ayahuasca in Chapter 3 in our manuscript entitled "Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine" which was published in the Journal of Biomedical Chromatography in September 2010 [5.18]. Again avoiding the complications of having to conduct multiple extracts and analyses which characterized previous literature methods, we hypothesized that the direct analysis-sample dilution protocol and LC/MS/MS analysis of urine would permit detection and quantitation of known metabolites of the constituent compounds and would also permit detection of previously unreported metabolites. We observed that tetrahydroharmine appears excreted as the major harmala alkaloid and that the major metabolite of the hallucinogenic component of ayahuasca, DMT, appears as the corresponding

N-oxide: *DMT-N*-oxide, a new finding in humans. Very little DMT was detected in urine, despite the inhibition of monoamine oxidase afforded by the presence of the harmala alkaloids in ayahuasca

Further toxicological and clinical research on ayahuasca will also require research on the pharmacokinetics and clearance of the major constituents of ayahuasca in human blood. Hence we modified our method for quantification in urine and created a suitable method for quantification in human blood plasma. A combination of two analytical techniques [HPLC with ultraviolet and/or fluorescence detection and gas chromatography with nitrogen-phosphorus detection], have historically been used for the analysis of some of the constituents of ayahuasca in blood following its oral consumption. We report here a single methodology for the direct analysis of 14 of the major alkaloid components of ayahuasca, including several known and potential metabolites of DMT and the harmala alkaloids in blood plasma. We hypothesized that a method using a 96-well plate/protein precipitation/filtration approach for plasma samples, and analysis by HPLC-ion trap-ion trap-mass spectrometry using heated electrospray ionization to reduce matrix effects, would permit the rapid, specific and sensitive analysis of the target components in plasma following ayahuasca administration. Chapter 4 presents the manuscript entitled "Methodology for determining major constituents of ayahuasca and their metabolites in blood" which was published in Journal of Biomedical Chromatography in April 2011 [B.24]. We demonstrate for the first time that DMT-N-oxide also represents a major circulating product of metabolism in the blood following ayahuasca administration. The method also demonstrates adequate sensitivity, specificity and reproducibility to make it useful for future clinical research with ayahuasca.

In further collaboration with Dr. Jordi Riba, we were also able to apply both the urine and blood methodology to further and more fully characterize the metabolism and urinary disposition of DMT and the harmala alkaloids in a group of healthy volunteers following ayahuasca administration. Twenty-four hour urine samples were obtained by Dr. Riba's lab from 10 healthy male volunteers following administration of an oral dose of encapsulated freeze-dried ayahuasca (1.0 mg DMT/kg body weight). These urine samples were assayed using our established analytical method involving HPLC / electrospray ionization /selected reaction monitoring / tandem mass spectrometry. Appendix B presents this manuscript entitled 'Metabolism and disposition of N,N-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca' which was published in the Journal of Drug Testing and Analysis in April 2012. We observed that harmol, harmalol and tetrahydroharmol conjugates were most abundant in urine suggesting that O-demethylation plus conjugation represent an important but probably not the only metabolic route for the harmala alkaloids in humans. We found that less than 1% of the administered DMT dose was excreted unchanged and that most was recovered as indole-3-acetic acid with around 10% being recovered as DMT-N-oxide, suggesting the existence in humans of alternative metabolic routes for DMT other than biotransformation by MAO.

One of the major reasons for undertaking studies of ayahuasca was to gain further insight into the metabolism and clearance of DMT in the presence of an MAO inhibitor, such as the harmalas. Elucidating the presence and purpose of endogenous N, N-dimethyltryptamines such as DMT in humans has been a major focus of our laboratory. The results of our investigation into ayahuasca metabolism suggest that N-oxidation represents a major metabolic route for DMT clearance in humans, particularly if MAO becomes inhibited, such as occurs with ayahuasca administration. However even with no MAOI present, a 6 times higher concentration of DMT- NO compared to DMT has been found in rodents [4.47] suggesting that DMT-NO may represent a major in vivo metabolite of DMT, thus potentially functioning as a more readily detectable marker for endogenous DMT production than DMT itself [4.31, 4.45, 4.47]. The data from our studies represent the first reports of DMT-NO as a metabolite of DMT in human urine and blood following ayahuasca administration. This finding has implications for the further study of DMT in general, particularly its occurrence or absence as a naturally occurring trace amine in humans.

Appendix C assesses what we know about endogenous psychedelics and asks the question "Are DMT and related indole alkaloids truly present in human biochemistry?" Three indole alkaloids that possess differing degrees of psychotropic/psychedelic activity have been reported as endogenous substances in humans; DMT, 5-hydroxy-DMT (bufotenine) and 5methoxy-DMT. We have undertaken a critical review of 69 published studies reporting the detection or detection and quantitation of these compounds in human body fluids. Appendix C presents this invited review entitled "A Critical Review of Reports of Endogenous Psychedelic N, N-Dimethyltryptamines in Humans: 1955-2010" which was published in the Journal of Drug Testing Analysis in February 2012. In reviewing this literature, we addressed the methods applied and the criteria used in the determination of the presence of three N, Ndimethyltryptamines. The review provides a historical perspective of the research conducted from 1955 to 2010, summarizing and critiquing the methods and findings for the individual compounds in blood, urine and/or cerebrospinal fluid. The review also discusses the shortcomings of the existing data in light of more recent findings and how these may be overcome. In particular, we frame the question and address the needs for future research in light of our findings with ayahuasca and how the methods developed may be applied to further pursue

answers to the question "What could be the functional significance of endogenous psychedelics?"

In Chapter 5, the final chapter, a summary of results and general conclusions are presented. We briefly discuss four future collaborative ayahuasca characterization studies for which data have already been collected. These studies are intended to prepare for future clinical trials with ayahuasca. Some potential future studies are presented and suggestions for the future directions of endogenous psychedelics research are offered.

1.5 References

- [1.1] D.E. Nichols. Hallucinogens. *Pharmacology & Therapeutics* **2004**, 101, 131-181.
- [1.2] A. Shulgin, A. Shulgin. PIHKAL: A chemical love story. Transform Press, Berkeley, California **1991**
- [1.3] A. Shulgin, A. Shulgin. TIHKAL: the continuation. Transform Press, Berkeley, California **1997**
- [1.4] R.E. Schultes, A. Hofmann. *The botany and chemistry of hallucinogens*. Charles C. Thomas, Springfield, IL **1980**.
- [1.5] H. Osmond. A review of the clinical effects of psychotomimetic agents. *Ann NY Acad Sci* 1957, 66, 418–434.
- [1.6] A. Hoffer. A program for the treatment of alcoholism: LSD, malvaria and nicotinic acid. In H.A. Abramson (Ed.), The Use of LSD in Psychotherapy and Alcoholism (pp. 343– 406). Indianapolis: Bobbs- Merrill 1967.
- [1.7] C.A. Ruck, J. Bigwood, D. Staples, J. Ott, R.G. Wasson. Entheogens. J Psychedelic Drugs 1979, 11, 145–146.
- [1.8] S. Grof, C. Grof (Eds.). *Spiritual emergency: When personal transformation becomes a crisis.* Los Angeles: Tarcher **1989**.
- [1.9] M. Winkelman. Psychointegration: The Physiological Effects of Entheogens. *Entheos* 2003, 2(1), 51-61

- [1.10] J. Riba. Human Pharmacology of Ayahuasca [Doctoral Thesis]. Universitat Autònoma de Barcelona, 2003. Available at: http://www.tdx.cat/handle/10803/5378 [23 January, 2012]
- [1.11] R. J. Strassman. Human psychopharmacology of N,N-dimethyltryptamine. *Behav. Brain Res.* 1996, 73, 121.
- [1.12] G.C. Lin, R.A. Glennon (eds). Hallucinogens: An Update. U.S. Government Printing Office, Washington, D.C. 1994.
- [1.13] R.R. Griffiths, W.A. Richards, U. McCann, R. Jesse. Psilocybin can occasion mysticaltype experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology (Berl)* 2006, 187, 268-83; discussion 284-292
- [1.14] R.R. Griffiths, W.A. Richards, M. Johnson, U. McCann, R. Jesse. Mystical-type experiences occasioned by psilocybin mediate the attribution of personal meaning and spiritual significance 14 months later. *J Psychopharmacol* **2008**, 22, 621-632
- [1.15] R. Strassman, S. Wojtowicz, L.E. Luna, E. Frecska. Inner Paths to Outer Space. Park Street Press 2008, 162-54
- [1.16] N. Haramein. The Schwarzschild Proton, Proceedings of the 9th International Conference CASYS'09. University of Liege, Belgium AIP CP 1303, ISBN 978-0-7354-0858-6 2010, 95-100
- [1.17] S. Hameroff, R. Penrose. Conscious events as orchestrated space-time selections. *Consciousness Studies* **1996a**, 2(1), 36-53.
- [1.18] S. Hameroff, R. Penrose. Orchestrated Objective Reduction of Quantum Coherence in Brain Microtubules: The "Orch OR" Model for Consciousness. In Toward a Science of Consciousness. Cambridge: MIT Press 1996b
- [1.19] R.G. Wasson, A. Hofmann, C.A.P. Ruck. The Road to Eleusis. Unveiling the Secret of the Mysteries. New York: Harcourt Brace Jovanovich 1978
- [1.20] R.G. Wasson, D.H. Ingalls. The soma of the Rig Veda: what was it? J Am Orient Soc 1971, 91, 169–187
- [1.21] C. Rätsch. The Encyclopedia of Psychoactive Plants, Enthnopharmacology and Its Applications. Rochester, Vermont: Park Street Press 1998/2005
- [1.22] M.S. Fish, N.M. Johnson, E.P. Lawrence, E.C. Horning. Oxidative N-dealkylation. Biochemical and Biophysical Acta 1955, 18, 564-565.
- [1.23] S.H. Wassen. Anthropological survey of the use of South American snuffs. In: D.H. Efron, B. Holmstedt, N.S. Kline(eds.), Ethnopharmacologic search for psychoactive drugs. US Gov Printing Office, Washington DC **1967**, 233-289.

- [1.24] J.P. Ogalde, B.T. Arriaza, E.C. Soto. "Identification of Psychoactive Alkaloids in Ancient Andean Human Hair by Gas Chromatography/Mass Spectrometry," *Journal of Archaeological Science* 2009, 36(2), 467-472.
- [1.25] B. Shanon. Biblical Entheogens: a Speculative Hypothesis. *Time and Mind (Berg)* **2008**, 1(1), 51–74
- [1.26] M. Dobkin de Rios. Visionary vine: hallucinogenic healing in the Peruvian Amazon. Waveland Press, Prospect Heights, Illinois 1984
- [1.27] R.E. Schultes, A. Hofmann. *The botany and chemistry of hallucinogens*. Charles C. Thomas, Springfield, **1980**.
- [1.28] S. Grof, H. Bennett. *The Holotropic Mind: The Three Levels of Human Consciousness* and How They Shape Our Lives. HarperCollins, **1992**
- [1.29] M.A. Persinger. Correlated cerebral events between physically and sensory isolated pairs of subjects exposed to yoked circumcerebral magnetic fields. *Neuroscience Letters* 2010, 486(3), 231–234
- [1.30] J. Mabit, R. Giove, J. Vega. In M. Winkelman and W. Andritzky (Eds.), Yearbook of Cross-Cultural Medicine and Psychotherapy 1996, VMB Press, Berlin 257.
- [1.31] S.A. Barker, J.A. Monti, S.T. Christian. N,N-Dimethyltryptamine: an endogenous hallucinogen. *International Review of Neurobiology* **1981**, 22, 83-110.
- [1.32] J.C. Callaway. A proposed mechanism for the visions of dream sleep. *Med Hypotheses* 1988, 26, 119–24
- [1.33] M.J. Jacob, D.E. Presti. Endogenous psychoactive tryptamines reconsidered: an anxiolytic role for dimethyltryptamine. *Med Hypotheses* 2005, 64, 930–7
- [1.34] R. Strassman. DMT: The Spirit Molecule: A Doctor's Revolutionary Research into the Biology of Near-Death and Mystical Experiences. Park Street Press, Rochester, Vermont, 2001
- [1.35] J.V. Wallach. Endogenous hallucinogens as ligands of the trace amine receptors: a possible role in sensory perception. *Med Hypotheses* **2009** 72(1), 91-4
- [1.36] R. M. Murray, M. C. Oon, R. Rodnight, J. L. Birley, A. Smith. Increased excretion of dimethyltryptamine and certain features of psychosis: a possible association. *Arch. Gen. Psychiat.* **1979**, *36*, 644.
- [1.37] J. Ciprian-Ollivier, M.G. Cetkovich-Bakma . Altered consciousness states and endogenous psychoses: a common molecular pathway? *Schizophr Res* 1997, 28, 257–65
- [1.38] S. Szara. DMT at fifty. *Neuropsychopharmacol. Hungar.* 2007, 9, 201.
- [1.39] H. Osmond and J. Smythies. Schizophrenia: A new approach. *Brit. J. Psychiat.* **1952**, *98*, 309.

- [1.40] G. G. Brune, H. H. Hohl, H. E. Himwich. Urinary excretion of bufotenin-like substance in psychotic patients. *J. Neuropsychiat.***1963**, *4*, 14.
- [1.41] J.C. Gillin, J. Kaplan, R. Stillman, R.J. Wyatt. The psychedelic model of schizophrenia: the case of N,N-dimethyltryptamine. *American Journal of Psychiatry* 1976, 133, 203-208.
- [1.42] J. Axelrod. Enzymatic formation of psychotomimetic metabolites from normally occurring compounds. *Science* **1961**, *134*, 343.
- [1.43] W.R. Martin, J.W. Sloan, J.D. Sapira, D.R. Jasinski. Physiologic, subjective, and behavioral effects of amphetamine, methamphetamine, ephedrine, phenmetrazine, and methylphenidate in man. *Clin Pharmacol Ther* **1971**, 12, 245-258.
- [1.44] R. J. Wyatt, L. R. Mandel, H. S. Ahn, R. W. Walker, W. J. Vanden Heuvel. Gas chromatographic-mass spectrometric isotope dilution determination of N,Ndimethyltryptamine concentrations in normals and psychiatric patients. *Psychopharmacol.* 1973, 31, 265.
- [1.45] L.R. Mandel, R. Prasad, B. Lopez-Ramos, R.W. Walker. The biosynthesis of dimethyltriptamine in vivo. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *16*, 47-58.
- [1.46] L.E. Hollister. Chemical Psychoses. Charles C. Thomas, Springfield, Illinois 1968
- [1.47] R.J. Wyatt, E.H. Cannon, D.M. Stoff, J.C. Gillin. Interactions of hallucinogens at the clinical level. Ann NY Acad Sci 1976, 281, 456-486
- [1.48] R. M. Murray, M. C. Oon, R. Rodnight, J. L. Birley, A. Smith. Increased excretion of dimethyltryptamine and certain features of psychosis: a possible association. *Arch. Gen. Psychiat.* **1979**, *36*, 644.
- [1.49] F. Franzen, H. Gross. Tryptamine, N,N-dimethyltryptamine, N,N-dimethyl-5hydroxytryptamine and 5-methoxytryptamine in human blood and urine. *Nature* **1965**, 206, 1052.
- [1.50] B. Heller, N. Narasimhachari, J. Spaide, L. Haskovec, H. E. Himwich. N-Dimethylated indoleamines in blood of acute schizophrenics. *Experientia* **1970**, *26*, 503.
- [1.51] R. W. Walker, H. S. Ahn, G. Albers-Schonberg, L. R. Mandel, W. J. Vandenheuvel. Gas chromatographic-mass spectrometric isotope dilution assay for N,N-dimethyltryptamine in human plasma. *Biochem. Med.* **1973**, 8, 105.
- [1.52] R. W. Walker, L. R. Mandel, J. E. Kleinman, J. C. Gillin, R. J. Wyatt, W. J. Vandenheuvel. Improved selective ion monitoring mass-spectrometric assay for the determination of N,N-dimethyltryptamine in human blood utilizing capillary column gas chromatography. J. Chromatogr. 1979, 162, 539.

- [1.53] W. T. Carpenter, Jr., E. B. Fink, N. Narasimhachari, H. E. Himwich. A test of the transmethylation hypothesis in acute schizophrenic patients. *Amer. J. Psychiat.* **1975**, *132*, 1067.
- [1.54] B. Angrist, S. Gershon, G. Sathananthan, R. W. Walker, B. Lopez-Ramos, L. R. Mandel, W. J. Vandenheuvel. Dimethyltryptamine levels in blood of schizophrenic patients and control subjects. *Psychopharmacol.* **1976**, *47*, 29.
- [1.55] M. C. Oon, R. M. Murray, R. Rodnight, M. P. Murphy, J. L. Birley. Factors affecting the urinary excretion of endogenously formed dimethyltryptamine in normal human subjects. *Psychopharmacol.* **1977**, *54*, 171.
- [1.56] M. C. Oon and R. Rodnight. A gas chromatographic procedure for determining N, Ndimethyltryptamine and N-monomethyltryptamine in urine using a nitrogen detector. *Biochem. Med.* 1977, 18, 410.
- [1.57] L. Corbett, S. T. Christian, R. D. Morin, F. Benington, J. R. Smythies. Hallucinogenic Nmethylated indolealkylamines in the cerebrospinal fluid of psychiatric control populations. *Brit. J. Psychiat.* **1978**, *132*, 139.
- [1.58] L.M. Hryhorczuk, J.M. Rainey, C. Frohman, E. Novak. A new metabolic pathway for N,N-dimethyltryptamine. *Biological Psychiatry* 1986, 21, 84-93.
- [1.59] R. J. Strassman. Human hallucinogenic drug research in the United States: a present-day case history and review of the process. J. Psycho. Drugs **1991**, 23, 29.
- [1.60] R. J. Strassman, C. R. Qualls, L. M. Berg. Differential tolerance to biological and subjective effects of four closely spaced doses of N,N-dimethyltryptamine in humans. *Biol. Psychiat.* **1996**, *39*, 784.
- [1.61] R.J. Strassman, C.R. Qualls, E.H. Uhlenhuth, R. Kellner. Dose-response study of N,Ndimethyltryptamine in humans. II. Subjective effects and preliminary results. *Archives of General Psychiatry* 1994, 51, 98-108.
- [1.62] R. J. Strassman. Human psychopharmacology of N,N-dimethyltryptamine. *Behav. Brain Res.* 1996, 73, 121.
- [1.63] M. A. Thompson, R. M. Weinshilboum. Rabbit lung indolethylamine Nmethyltransferase. cDNA and gene cloning and characterization. J. Biol. Chem. 1998, 273, 34502.
- [1.64] M. A. Thompson, E. Moon, U. J. Kim, J. Xu, M. J. Siciliano, R. M. Weinshilboum Human indolethylamine N-methyltransferase: cDNA cloning and expression, gene cloning, and chromosomal localization *Genomics* 1999, *61*, 285.

- [1.65] M.M Airaksinen, I. Kari. Beta-carbolines, psychoactive compounds in the mammalian body. Part I: Occurrence, origin and metabolism. *Med Biol* **1981**, 59, 21-34.
- [1.66] R.W. Fuller. Selective inhibition of monoamine oxidase. Adv Biochem Psychopharmacol 1972, 5, 339-354
- [1.67] I. Kari. 6-methoxy-1,2,3,4-tetrahydro-beta-carboline in pineal gland of chicken and cock. *FEBS Lett* **1981**, 127(2), 277-80

Chapter 2. Direct Analysis of Psychoactive Tryptamine and Harmala Alkaloids in the Amazonian Botanical Medicine Ayahuasca by Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry*

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2.1 Introduction

The term ayahuasca comes from the Quechua culture of South America and may be translated into English as "vine of the souls". Ayahuasca is a decoction or tea prepared from the large, woody, jungle vine (liana) Banisteriopsis *caapi* in combination with other psychoactive plants [2.1]. The traditional preparation of ayahuasca involves boiling or soaking the bark and stems of B. *caapi* and the leaves of the Psychotria *viridis*, a member of the coffee family (Rubiaceae) [2.1-2.6].

B. *caapi* contains the beta-carboline harmala alkaloids harmine (1), harmaline (2), and tetrahydroharmine (2.3, Figure 2.1) as the major active components [2.7] as well as lesser amounts of O-demethylated beta-carboline derivatives [harmol (2.4) and harmolol (2.5), Figure 2.1] [2.3, 2.4, 2.8-2.10]. P. *viridis* contains the hallucinogen N, N-dimethyltryptamine [DMT (2.6); Figure 2.1], as well as lesser amounts of N-methyltryptamine [NMT (2.7)] and 2-methyl-1,2,3,4-tetrahydro-beta-carboline [2-MTHBC (2.8), Figure 2.1] [2.3, 2.4, 2.8, 2.11]. The phytochemical composition and pharmacology of ayahuasca have been extensively described [2.6, 2.12-2.14]. Ayahuasca is, however, often brewed with a variety of other plants. With the practice of using different plant combinations, which may contain other hallucinogens such as 5-methoxy- (2.9, Figure 2.1) and/or 5-hydroxy-DMT (2.10, Figure 2.1), the actual composition and, thus, the effects of the brew may vary from region to region as well as with the change of seasons [2.1, 2.15].

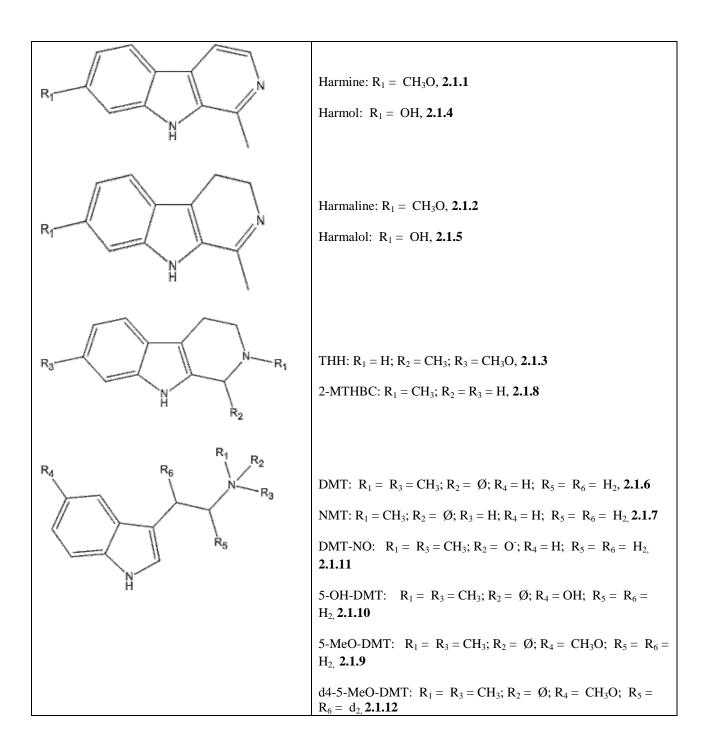


Figure 2.1: Structures of the compounds examined in ayahuasca.

Ayahuasca is also known as caapi, yage, mihi, dapa, natema, pinde, hoasca, daime, or vegetal among various South American groups. Ayahuasca holds a highly esteemed and millennia-old position in these cultures' medical and religious pharmacopeia [2.5, 2.16]. Several syncretic religious movements use ayahuasca as a sacrament in Brazil. These movements, the União do Vegetal (UDV), the Santo Daime, and the Barquiña, claim to have close to 10,000 members, and branches have now spread throughout the industrialized West. The UDV and Santo Daime sects have recently received substantial legal protections to use ayahuasca in their ritual ceremonies in the US [2.17].

Due to these legal developments and the attendant media publicity in the US, a bourgeoning "ayahuasca tourism" trade in South America, an influx of imported ayahuasca into the West for illicit use, and home-brewed preparations utilizing both B. *caapi* and P. *viridis* (or similar plants), ayahuasca use is increasing significantly. Such use has implications for public health [2.18]. However, the possible medical benefits of ayahuasca are also of interest [2.6, 2.15]. For example, the alkaloids in B. *caapi* have been demonstrated to be antioxidant with antimutagenic and antigenotoxic activity [2.19] as well as demonstrating putative psychotherapeutic and rehabilitative effects [2.20, 2.21]. Ayahuasca use also appears to produce a persistent, long term up-regulation in the number of serotonin reuptake transporters in blood platelets, as has been documented in UDV members relative to ayahuasca-naïve age matched controls [2.22]. Conditions such as alcoholism with tendencies toward violence, suicidal behaviors, and severe depression [2.23-2.25] have all been correlated with decreased numbers of serotonin reuptake transporters, suggesting a potential therapeutic biochemical mechanism for ayahuasca treatment in these disorders.

Given the potential medical applications of ayahuasca, along with increasing use and potential for abuse in the US, it is important to be able to characterize its constituents, determining their presence and concentrations. In addition, clinical research will require standardization of various ayahuasca preparations and the generation of information regarding storage stability. A rapid, sensitive, and specific method for characterization of ayahuasca preparations is therefore desirable.

The present manuscript describes a rapid, flexible, specific and sensitive method for the direct qualitative and quantitative analysis of the alkaloid components of ayahuasca. The

protocol uses a small sample size, requires no sample extraction as it is based on sample dilution followed by direct injection, and is capable of analyzing 11 known or potential components of ayahuasca by liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS), quantitating them using a deuterated internal standard.

2.2 Materials and Methods

2.2.1 Reagents and Chemicals

HPLC-grade methanol was purchased from Honeywell Burdick and Jackson (Morristown, New Jersey, USA). HPLC-grade water, high purity formic acid, and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA). N-methyltryptamine (NMT), DMT, 5-5-methoxy-DMT hydroxy-DMT (5-OH-DMT), (5-MeO-DMT), harmine. harmaline hydrochloride dihydrate, harmol hydrochloride dihydrate, and harmalol hydrochloride dihydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydroharmine was purchased from THC Pharm (Frankfurt an Main, Germany). DMT-N-oxide (DMT-NO, 2.11, Figure 2.1) and 2-MTHBC were prepared as previously described [2.25]. Deuterated (d_4) 5-MeO-DMT (alpha, alpha, beta, beta-tetradeutero; 2.12, Figure 2.1) was a gift from Dr. David E. Nichols, Purdue University Department of Medicinal Chemistry and Molecular Pharmacology, West Lafayette, IN, USA.

2.2.2 Botanical Raw Material and Ayahuasca

Ayahuasca samples were prepared by Dr. Leanna Standish at Bastyr University Research Center, Kenmore, WA, USA. The test material was prepared from herbicide- and pesticide-free specimens of *Banisteriopsis caapi* and *Psychotria viridis* maintained in cultivation at 2000 ft elevation in the South Kona District of Hawaii. These materials were vegetative clones of specimens collected in Pucallpa, Peru, in 1982 [2.4]. *B. caapi* vine and leaves of *P. viridis* were harvested in late January 2009. The *B. caapi* vine was collected from a single plant which was in light pink bloom. *P. viridis* leaves were collected from several plants. Samples of the plants were sent to the Missouri Botanical Garden where they were authenticated. The three unique samples used for the analyses were prepared from fresh *P. viridis* leaves 8-12 cm in length and freshly cut *B. caapi* vine 3-5 cm in diameter with five codyleons showing on cross section. Upon arrival at

Bastyr University Botanical Medicine Laboratory the fresh plant materials were refrigerated. At the start of decoction plants materials and water were at room temperature.

Aqueous decoctions of each plant were prepared from freshly harvested material. The vine and leaves were washed in distilled water; the vine macerated, then slowly decocted in 11 liters of double distilled water for 10 hours. Samples derived from two extracts made at the same time which varied in the number of *P. viridis* leaves (300 or 150) while keeping the *B. caapi* vine biomass constant. At the end of the 10 hour decoction the extracts were bottled, frozen at -70°C, then shipped on dry ice to Dr. Barker's laboratory.

All ayahuasca samples were thawed upon receipt, aliquoted and stored at -80°C until analyzed. No sample was assayed that had undergone more than two freeze-thaw cycles.

2.2.3 Preparation of standards and samples for analysis

Stock standard solutions (1 mg/ml) of the 11 selected compounds and the internal standard were prepared individually in methanol in 10 ml amber glass vials with Teflon lined screw-cap closures and stored in a freezer at -20° C. Working standards were prepared in methanol at selected concentrations by serial dilution. The standard mixes were pipetted into 5 ml conical tubes. The methanol was removed by gentle evaporation over dry nitrogen and the residues were dissolved in amounts of 90% H₂O/0.1% formic acid:10% acetonitrile/0.1% formic acid mobile-phase solution corresponding to the sample dilution experiment being conducted. Standard curve data points at 6 selected concentrations (including zero) specific to the expected concentration range for each compound (determined from preliminary analysis) were prepared to determine linearity of response, to collect data for the quantitation of samples, and to determine method performance.

Ayahuasca samples were well mixed and 100 μ l of the sample were transferred to a 5 ml conical glass tube. The samples were then diluted, with mixing, to varying concentrations (10X, 20X, 100X and 200X) using 90% H₂O/0.1% formic acid:10% acetonitrile/0.1% formic acid mobile phase solutions.

Methanol (20 μ ul) containing 1ng/ μ ul internal standard d₄-5-MeO-DMT was added directly into individual autosampler vials and dried completely under nitrogen. Various serial

dilutions of the 11 compound stock standard mixes or Ayahuasca samples (200 ul of diluted sample) were filtered through 0.4 um filters (Nalgene, NalgeNunc, Rochester, NY, USA) and 100 μ l of the filtrate were added to individual autosampler vials containing dried internal standard. The samples were thoroughly mixed by vortexing. The ayahuasca samples and mixed standards were transferred to the HPLC autosampler and 10 μ l were injected for analysis.

2.2.4 LC-ESI-MS/MS analysis

Analyses were conducted using an Agilent 1200 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent G1367A HiP ALS autosampler, an Agilent G1311A Quaternary micropump, and an Agilent G1332A degasser interfaced to a TSQ Quantum Access 1.5 SP1 tandem MS (Thermo Fisher Scientific, Waltham, MA, USA) with electrospray ionization (ESI) operated in the positive ion mode.

Chromatographic separation was achieved on a 1.8 μ m 4.6 x 50 mm (i.d.) Agilent ZORBAX Eclipse Plus C18 rapid resolution HT threaded column with an Alltech Direct-Connect Column 2 μ m pre-filter (Deerfield, IL, USA) using gradient elution. The following gradient system was used as the mobile phase; A (0.1% formic acid in H₂0) and a mobile phase B (0.1% formic acid in acetonitrile) delivered at a constant flow rate of 0.3 ml/min; A:B 90:10 (0 min)- 90:10 (1 min)- 50:50 (5 min)- 50:50 (6 min)- 2:98 (7 min)- 2:98 (9 min) – 90:10 (10 min)- 90:10 (23 min), allowing for re-equilibration. Samples and standards were analyzed using a 20 μ l injection volume.

The MS/MS analysis was performed using selected reaction monitoring (SRM) of the protonated molecular ions for the analytes. The m/z transitions and MS parameters for analytes and internal standards are given in Table 1. The spray voltage was 4000 V, sheath gas (nitrogen) pressure 50 psi, capillary temperature 310° C, and collision pressure was 1.5 psi. Generation of detection data and integration of chromatographic peaks were performed by Xcalibur 2.0.7 Thermo Fisher Scientific (Waltham, MA, USA) LCquan 2.5.6 QF 30115 software.

Identification of the compounds was based on the presence of the molecular ion at the correct retention time, the presence of three transition ions and the correct ratio of these ions to one another (+/- 25% relative).

2.2.5 Calculations

The concentration of compounds in ayahuasca samples was determined from the peak area ratio of the target analyte to that of the internal standard (d₄-5-MeO-DMT; Table 2.2), by reference to calibration curves prepared by spiking water with each of the 11 substances and an equivalent amount of internal standard. If the concentration of the sample was observed to be outside the measured range, the sample was re-analyzed after further dilution with mobile phase and the original concentration calculated by extrapolation. Values determined from repeated analyses of n aliquots of samples or standards were expressed as their arithmetic mean. The percent relative standard deviation (%RSD) was also calculated for standards, as well as the method bias (100 times the observed concentration. Standard deviations were calculated for n determinations of samples as noted. Inter- and intra-assay %RSDs were also determined. The method limits of detection (LOD; concentration response greater than 3-times baseline noise) and limits of quantitation (LOQ: multiplying the LOD by 10 and adding the %RSD) were also determined for each compound using fortified water samples.

2.3 Results

2.3.1 Validation of the Method

Each of the compounds gave high yields of molecular ions $(M+H)^+$ using the mobile phase system and electrospray ionization parameters described (Table 2.1). Product ions were also formed with good efficiency. However, for many of the compounds, the third product ion was less than 10% of the base peak. Nonetheless, adequate sensitivity was achieved to allow inclusion of these ions in the identification criteria.

Analytes	MW	[M + H]+	Product Ion 1	CE 1 (v)	Product Ion 2	CE 2 (v)	Product Ion 3	CE 3 (v)
5-OH-DMT	204	205.2	160.1	16	58.4	14	115.2	32
HARMALOL	200	201.1	160.1	23	184.1	21	185.1	35
NMT	174	175.2	144.1	12	115.2	35	117.2	29
HARMOL	198	199.1	171.1	25	103.2	37	131.1	31
DMT	188	189.2	144.1	17	58.4	14	115.2	39
5-MeO-DMT	218	219.2	174.1	16	130.1	42	58.4	14
2-MTHBC	186	187.2	144.1	12	44.4	37	143.1	30
ТНН	216	217.1	188.1	14	200.1	12	130.1	41
DMT-NO	204	205.2	144.1	15	115.2	40	143.1	32
HARMINE	212	213.1	170.1	29	198.1	22	169.1	40
HARMALINE	214	215.1	172.1	30	200.1	23	171.1	37
d ₄ -5-MeO-DMT (IS)	222	223.2	134.2	43	178.1	15	60.4	15

Table 2.1: Mass spectrometric parameters for analytes and deuterated internal standard (IS) (CE = collision energy in volts, v; MW= molecular weight)

Using the LC-MS/MS multi-component method described, the chromatographic and/or mass separation of the 11 selected compounds was completed within 23 minutes. The first analyte (5-OH-DMT) eluted after 2 min while the last analyte (harmine) eluted at less than 9 min. The remaining time was used for column cleaning and re-equilibration to assure consistent analysis. Data for the retention time of each compound and their consistency, and the analytical imprecision for each analyte are presented in Table 2.2. Representative chromatograms for each standard analyte are shown in Figure 2.2, and a representative ayahuasca sample is shown in Figure 2.3.

Calibration curves for each compound were linear over the range of concentrations examined (5 – 100 ng/mL or 5 – 100 μ g/ml, depending on the compound; Table 2.2). The linear regression equations and coefficients of correlation are presented in Table 2.2 where y and x represent the relationship between the peak area ratio (compound/internal standard) and the

respective corresponding calibration concentrations. The confidence parameters for the method (Bias, %RSD, LOD, LOQ, intra- and inter-assay precision) for determination of the 11 compounds are presented in Table 2.2. Calculations of method bias and %RSDs suggest that the dilution approach and the rather modest sample manipulations do not lead to any significant losses of analytes in the process, with recoveries being near quantitative.

Matrix effects were assessed by analyzing ayahuasca samples at various dilutions (10X, 20X, 100 X and 200X). When diluted less than 20X, some retention time variability (<1%) and ion suppression (<10%) due to apparent matrix effects were noted (data not shown) but were not significant. Observed effects were likely due to overloading the capacity of the column with the major alkaloid components of the ayahuasca. In support of this interpretation, samples could be assayed at 10:1 dilutions without significantly compromising overall analytical performance or results. However, such lower dilution analyses had to be conducted with smaller injection volumes since the concentrations of some components (THH, for example) overwhelmed the column capacity and dramatically affected peak shape. Dilution of the sample up to 200:1 gave highly reproducible retention times (Table 2.2), no indication of ion suppression or enhancement and did not compromise the ability to achieve detection and/or quantitation of the components of ayahuasca. Further dilution could also have been readily applied if necessary. A dilution factor of 200:1 was subsequently used for all analyses.

Other sample components did not produce any evident interference in the analysis although a few peaks possessing the same $(M+H)^+$ ions as the target analytes were evident in the chromatograms. Only 5-MeO-DMT and harmol were affected by background but this did not compromise either detection or confirmation of the presence or absence of either compound (Figure 2.3).

Table 2.2: Performance characteristics of the method. %RSD= percent relative standard deviation; RT= retention time; LOD= limit of detection; LOQ= limit of quantitation; S/N = Signal to noise ratio

	T 1	Mean			Mean RT				Inter-	Intra-
	Target	Observed Conc.		%RSD	(min)	LOD			Assay %RSD	Assay %RSD
Analytes (conc)	Conc.	(n=9)	Bias (%)	(n=9)	(mn) (n=9)	S/N = 3	LOQ	Y and r ²	(n=3)	(n=4)
5-OH-DMT (ppm)	0.5	0.44	-11.74	0.01	2.87	0.0079	0.09	y=2.86+256.30*X	0.00	0.01
J-OII-DWII (ppiii)	1	1.02	1.54	0.01	2.87	0.0079	0.09	r ² =0.9992	0.03	0.01
	2.5	2.52	0.64	0.02	2.87			1 -0.9992	0.02	0.03
	5	5.45	9.00	0.02	2.86				0.02	0.10
	10	9.98	-0.19	0.02	2.86				0.02	0.02
HARMALOL (ppb)	5	4.73	-5.38	0.02	7.21	0.0343	0.43	v=0.10+0.02*X	0.02	0.02
initialities (ppo)	10	10.15	1.53	0.17	7.20	0.0040	0.45	r ² =0.9993	0.72	0.46
	25	26.15	4.60	0.26	7.19			1-0.5555	0.47	0.61
	50	51.72	3.44	0.23	7.20				0.04	0.16
	100	99.89	-0.11	0.27	7.18				0.01	0.87
NMT (ppb)	5	5.17	3.41	0.07	7.42	0.1885	1.95	v=0.08+0.05*X	0.14	0.04
41-2	10	10.84	8.35	0.07	7.42			r ² =0.9988	0.34	0.14
	25	25.50	1.98	0.13	7.42				0.55	0.21
	50	51.61	3.23	0.18	7.43				0.55	0.29
	100	99.66	-0.34	0.13	7.41				0.07	0.16
HARMOL (ppb)	5	4.93	-1.44	0.10	7.43	0.8408	8.51	y=0.05+0.01*X	0.15	0.24
	10	10.80	8.03	0.14	7.41			r ² =0.9991	0.58	0.30
	25	26.27	5.08	0.25	7.42				0.52	0.59
	50	51.37	2.74	0.24	7.42				0.10	0.52
	100	98.84	-1.16	0.28	7.42				0.18	0.77
DMT (ppm)	5	4.64	-7.19	0.08	7.58	0.0064	0.14	y=31.46+27.03*X	0.04	0.13
	10	11.16	11.63	0.06	7.57			r ² =0.9995	0.05	0.19
	25	26.44	5.76	0.20	7.57				0.52	0.41
	50	52.23	4.45	0.20	7.56				0.53	0.42
	100	99.25	-0.75	0.33	7.66				0.19	1.26
5-MeO-DMT (ppb)	5	5.01	0.14	0.10	7.66	0.1451	1.56	y=0.02+0.02*X	0.36	0.25
	10	11.23	12.28	0.09	7.66			r ² =0.9979	0.40	0.07
	25	25.34	1.35	0.29	7.66				0.12	0.53
	50 100	51.81 98.96	3.62 -1.04	0.32	7.66 7.66				0.21 0.17	0.93
2-MTHBC (ppb)	5	4.41	-11.04	0.30	7.88	0.2027	2.12	v=0.21+0.11*X	0.17	0.92
2-MILLEC (ppo)	10	11.12	11.19	0.09	7.89	0.2027	2.12	r ² =0.9995	0.21	0.12
	25	26.40	5.59	0.25	7.91			1-0.5555	0.94	0.48
	50	53.62	7.23	0.42	7.91				0.52	0.63
	100	103.53	3.53	0.49	7.60				0.50	0.80
THH (ppm)	5	4.52	-9.59	0.08	8.02	0.0005	0.08	v=13.23+26.07*X	0.33	0.15
41	10	10.88	8.77	0.18	8.02			r ² =0.9993	0.62	0.51
	25	26.39	5.58	0.20	8.02				0.46	0.28
	50	53.68	7.37	0.31	8.02				0.55	0.52
	100	99.77	-0.23	0.24	8.01				0.18	0.55
DMT-NO (ppb)	5	4.95	-0.95	0.09	8.01	0.0150	0.24	y=0.72+0.59*X	0.24	0.23
	10	11.06	10.64	0.08	8.01			r ² =0.9993	0.20	0.09
	25	25.51	2.04	0.18	8.02				0.28	0.31
	50	53.14	6.29	0.31	8.02				0.90	0.25
	100	99.31	-0.69	0.31	8.03				0.30	0.96
HARMINE (ppm)	5	5.34	6.90	0.06	8.31	0.0005	0.06	y=60.37+63.80*X	0.27	0.10
	10	11.16	11.62	0.08	8.29			r ² =0.9994	0.23	0.18
	25	25.81	3.23	0.11	8.30				0.47	0.17
	50	51.16	2.32	0.20	8.31				0.84	0.15
	100	99.85	-0.15	0.16	8.33	0.0020	0.04	10.40.02.01.07	0.44	0.26
HARMALINE (ppm)	0.5	0.50	0.62	0.01	8.25	0.0028	0.04	y=10.48+27.01*X	0.03	0.02
	1	1.12	12.41	0.01	8.26			r ² =0.9965	0.04	0.02
	2.5 5	2.57 5.04	2.93 0.84	0.02	8.26 8.26				0.07	0.04
	10	5.04 9.98		0.04 0.05	8.26				0.18	0.05
D4 5-MeO-DMT	10	9.90	-0.18	0.05	8.20				0.09	0.10
(ppb)	20				7.65					

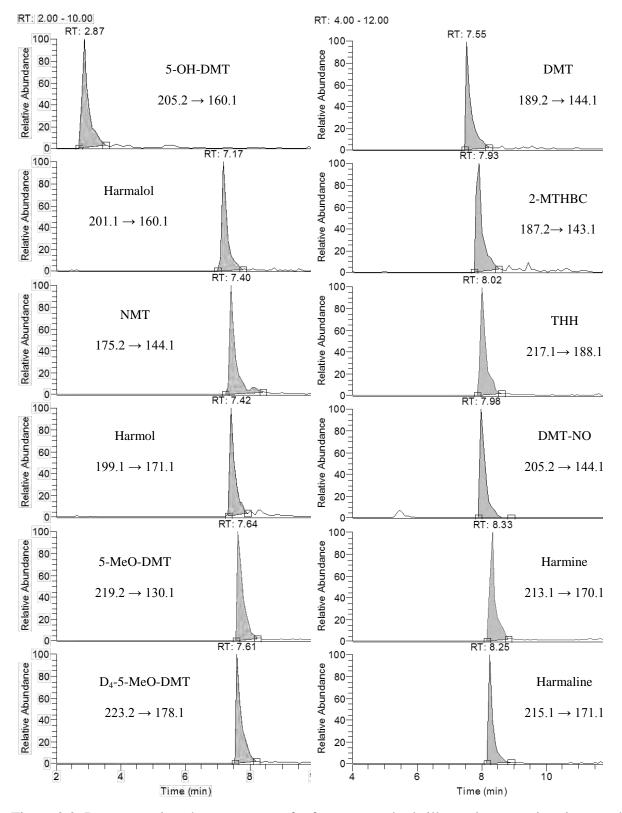


Figure 2.2. Representative chromatogram of reference standards illustrating retention times and the molecular ion and product ion monitored (200 ng/ml of each; RT= retention time in minutes).

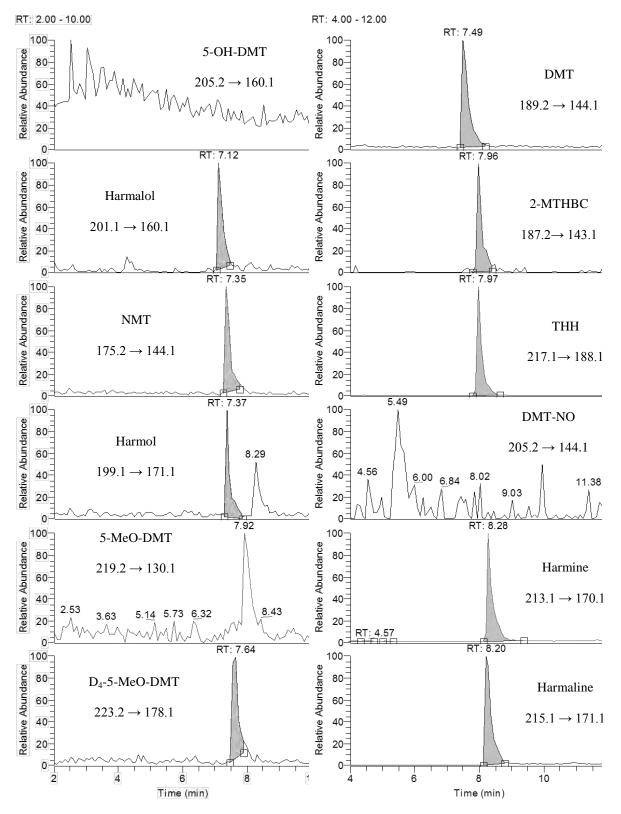


Figure 2.3. Representative chromatogram of an ayahuasca sample (Sample 5 shown at 200:1 dilution; RT= retention time in minutes).

2.3.2 Characterization of ayahuasca preparations

Quantitation of the components of ayahuasca in various preparations (3 unique samples) is presented in Table 2.3. DMT-NO, a major metabolite of DMT in mammalian species, was not detected in the ayahuasca preparations examined (Table 2.3). Detectable concentrations of 5-MeO-DMT, 5-OH-DMT, and 2-MTHBC were observed in some samples (data not shown). The major components observed were THH and harmine, followed by DMT and harmaline. Harmalol, NMT and harmol were also present but in significantly smaller concentrations.

Several of the samples (3 and 5 as single-blind duplicates, 6 as a concentrate of 3 and 2 as a concentrate of 1) were derived from the same batch but underwent further heating and concentration. This process seems to have altered the concentration ratios of some of the components in a non-linear fashion, suggesting that the process of continued heating/boiling or extended storage may lead to analyte decomposition or interconversion.

Solutions of the target compounds in water were stable for the period of time examined (6 months) when stored at -20° C and kept from exposure to light by use of amber vials. Similarly, ayahuasca samples were stable for the same period of time when retained under the same conditions. However, preliminary data (not shown) indicate that the ayahuasca preparations are susceptible to degradation and compound interconversion through prolonged exposure to room temperature conditions and these changes are accelerated by exposure to light (data not shown) and changes in pH that occur as the preparations age. These and related data will be the subject of a forthcoming publication.

Sample # Runs	HARMALOL	NMT	DMT	ANALYTE THH	HARMOL	HARMINE	HARMALINE
II IXIIIS	minilot		DIVII		mmmul		
1	0.0026	0.0052	0.1300	1.2200	0.0026	0.9100	0.0540
(n=6)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
	0.0000	0.0006	0.0337	0.1645	0.0007	0.0948	0.0062
2	0.0260	0.0280	3.1900	11.9000	0.0120	16.1400	1.5500
(n=3)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
	0.0036	0.0011	0.3601	1.4346	0.0024	1.0013	0.1263
3	0.0047	0.0065	0.1796	1.4249	0.0009	1.2199	0.2186
(n=6)	(+/-)		0.1790 (+/-)	(+/-)	(+/-)	(+/-)	
	0.0004	(+/-) 0.0006	0.0206	0.0901	0.0002	0.1835	(+/-) 0.0172
4	0.0004	0.0000	0.0200	0.0901	0.0002	0.1655	0.0172
4 (n=6)	0.0054	0.0053	0.1270	1.8681	0.0011	1.5586	0.2852
(II– 0)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
	0.0004	0.0010	0.0211	0.1529	0.1529	0.1814	0.0227
5	0.0102	0.0094	0.1171	1.6371	0.0197	1.3714	0.3579
(n=3)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
	0.0015	0.0015	0.0572	0.2904	0.0019	0.2977	0.0404
6	0.0310	0.0313	0.9334	6.3706	0.0633	6.6096	1.0857
(n=3)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)	(+/-)
	0.0015	0.0030	0.1289	0.3370	0.0046	0.5712	0.0561

Table 2.3. Mean concentrations (mg/mL +/- standard deviation) plus or minus standard deviations of various alkaloids found in ayahuasca samples quantified using the herein described multi-component LC-MS/MS method.

2.4 Discussion

A range of different alkaloids, other than the 11 examined here, have been reported to be present in ayahuasca; tetrahydro-beta-carboline (THBC), 6- and 5-methoxy-tryptamine, 2-methyl-6-methoxy-THBC, 1, 2-dimethyl-6-methoxy-THBC [2.2, 2.6, 2.8, 2.12-2.14], in addition to the pyrrolidine alkaloids shihunine and dihydroshihunine [2.27]. These and others are apparently minor components and the variation in composition is, in many cases, thought to be due to the addition of other plant species to the brews. For this reason 5-MeO-DMT and 5-OH-DMT have been included in the present assay, as they are also known to be present in some of the plants used in ayahuasca preparations and their presence could potentially contribute significantly to their effect [2.2, 2.28]. The samples were also examined for the presence of DMT-NO which, to our knowledge, represents the first such effort to assay for this compound in

ayahuasca preparations. Although a major DMT metabolite in mammals, it appears to be absent from the preparations of ayahuasca examined here.

B. caapi has been reported to contain between 0.05% dry weight to 1.95% dry weight harmala alkaloids [2.3, 2.4, 2.8] with a mean of 0.51%, consisting of primarily harmine, harmaline, tetrahydroharmine (THH), harmol, and harmalol [11] The results of the present analyses are consistent with these observations, and the quantities of the compounds observed are very similar to those reported by others [2.28].

Preparation of ayahuasca for chemical analysis has often involved liquid-liquid extraction of the target alkaloids [2.3, 2.8, 2.29-2.31]. A solid phase extraction method has also been described [2.32]. These methods subsequently analyze the extracted components by gas chromatography using various detectors, such as flame ionization, nitrogen-phosphorous or mass spectrometry [2.3, 2.8, 2.29-2.32]. Unfortunately, many of the manuscripts provide little or no data concerning recovery efficiencies for the targeted analytes, and the number of analytes in each case is somewhat limited.

However, because the alkaloids are already extracted in an aqueous decoction, several researchers have chosen to use sample dilution and filtration, or centrifugation followed by liquid chromatographic analysis [2.3, 2.7, 2.33] using UV or fluorescence detection [2.3, 2.7] or mass spectrometry [2.33] (LC/atmospheric pressure chemical ionization APCI/MS). Such direct analysis avoids the analytical complications that encompass liquid-liquid extraction protocols, such as pH adjustments, solvent evaporation, the relative selectivity of the extraction, and analyte recovery.

The HPLC-fluorescence method developed by Callaway et al. (1996) [2.7] utilized either a 10:1 [2.7, 2.28] or 100:1 [2.7] mobile phase dilution protocol. The 10:1 dilution protocol gave excellent limits of detection and quantitation. Since the concentrations of the major components are quite high in these extracts this is not a concern, and the method has been applied to many ayahuasca preparations in the last decade.

Kawanishi et al. (1998) [2.33] reported using freeze-dried material (1 mg) dispersed into 40 μ l of ethanol (approximately 40:1 dilution) which was subsequently directly injected after centrifugation. The latter method was not, however, used to quantitate the alkaloids present, and

did not acquire multiple product-ion data (Multiple Reaction Monitoring, MRM). Instead, it was used to conduct targeted $(M+H)^+$ comparisons to reference materials, identifying a number of ayahuasca constituents in this manner.

A sample dilution protocol was chosen for the present study for several reasons. First, by utilizing modern mass spectrometric instrumentation and methods, we are able to detect and quantitate 11 different alkaloids in our ayahuasca preparations at dilutions up to 200:1. At the same time, we attained better overall sensitivity for detection and limits of quantitation than previous methods while eliminating potential matrix effects. Furthermore, the method confirms the identity of the analyte by monitoring the $(M+H)^+$ ions and obtaining up to three product ions whose presence and ratios can be used to assure the absence of interferences that may arise from direct injection. Further an examination of ion-mass contribution (cross-talk) for each the compounds/masses monitored gave no indication that any of them interfered with the detection, confirmation or quantitation of any of the other substances.

The present method has several distinct advantages over the classical LC with UV or fluoresence detection approach. The LC/MS/MS method allows use of a relatively short LC gradient program that, while not always obtaining temporal separation of components, allows for complete separation by mass. The LC program employed by Kawanishi et al. (1998) [2.33] was at least 70 minutes long, precluding its use for routine analysis. A dilution protocol also avoids the problems noted above with liquid-liquid extraction, and makes feasible the analysis of a range of different polarity components in a single sample. In this regard, the method has the flexibility to be expanded to include other compounds of interest by simply adding other components to the analysis after a simple determination of mass spectral properties and retention time data. In most cases the analyst does not have to consider extraction protocol changes or recovery of the new components because no extraction is involved.

The data also suggest that prolonged heating/boiling of the ayahuasca preparations leads to inter-conversion of some of the components. This appears to occur to a significant degree for the changes seen in THH, harmine and harmaline levels between samples undergoing further heating to attain higher concentrations of the components (See Table 2.3). This phenomenon, an apparent acid-driven chemical reduction of harmine-to-harmaline-to-THH, has also been reported by Callaway, et al. [2.28] and constitutes another source of potential variability of results among ayahuasca preparations.

Several solutions to the variability in composition of ayahuasca preparations have been utilized or proposed by researchers that have conducted human studies. One theoretical approach is the use of "pharmahuasca" [2.14], combining pure DMT and harmala alkaloids to produce a pharmaceutical-grade ayahuasca. This approach, however, eliminates from consideration the possible synergistic role of the trace components of ayahuasca brews. A more popular and effective approach has been the use of encapsulated, freeze-dried material obtained from large batches of ayahuasca prepared under an exacting protocol [2.15]. Once prepared, this standardized preparation can be assayed, freeze-dried and stored under dry conditions for further use. For purposes of human studies, this approach also avoids the placebo-vs-drug problem that arises from the taste and odor characteristic of ayahuasca. Another approach has been the use of large, well-characterized batches of ayahuasca which are then frozen at -80° C and thawed for use. Masking the taste remains a problem, however. In either of these cases, analytic methodology for the full characterization of the materials is required.

The assay described here is ideal for such purposes given the demonstrated ability to provide rapid, flexible, accurate and precise analysis over the range of concentrations examined. The use of d_4 -5-MeO-DMT as an internal standard appears to be quite adequate in terms of linearity and quantitation of the range of alkaloid concentrations determined.

The present method may also provide an analytical approach to study metabolism of ayahuasca in blood and urine. Indeed, preliminary data obtained from urine samples collected from individuals who have consumed ayahuasca suggest that this methodology may be directly applied to diluted and filtered urine samples before or after enzyme treatment of the samples to release conjugates (data not shown). Similar results have also been obtained using a protein precipitation/dilution approach for blood samples (data not shown).

2.5 Conclusions

A direct injection/liquid chromatography-electrospray ionization- tandem mass spectrometry procedure has been developed for the simultaneous quantitation of 11 compounds potentially present in the Amazonian botanical medicine and sacrament ayahuasca. The method utilizes a deuterated internal standard, d_4 -5-MeO-DMT, for quantitation. This method affords rapid detection of alkaloids by a simple dilution assay requiring no extraction procedures. In addition, the method affords an extremely high degree of specificity for the compounds in question, as well as low limits of detection and quantitation. Its application to 3 different ayahuasca preparations has been demonstrated. This method should find application in the study of ayahuasca in human and ethnobotanical research, as well as in forensic examinations of ayahuasca preparations.

2.6 References

- [2.1] R.E. Schultes. The identity of the maipighiaceous narcotics of South America. Botanical Museum Leaflets, Harvard University **1957**, 18, 1-56.
- [2.2] R.E. Schultes, A. Hofmann. *The botany and chemistry of hallucinogens*. Charles C. Thomas, Springfield, **1980**.
- [2.3] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. *Journal of Ethnopharmacology* 1984, 10, 195-223.
- [2.4] DJ. McKenna, G.H.N. Towers. Biochemistry and pharmacology of tryptamines and betacarbolines: A minireview. *Journal of Psychoactive Drugs* **1984**, 16, 347-358.
- [2.5] D.J. McKenna, L.E. Luna, G.H.N. Towers, in von Reis S, Schultes RE (Eds.). Ethnobotany: Evolution of a Discipline, Dioscorides Press, Portland, OR, **1995**, p. 349.
- [2.6] D.J. McKenna. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacology and Therapeutics* **2004**, 102, 111-129.
- [2.7] J.C. Callaway, L.P. Raymon, W.L. Hearn, D.J. McKenna, C.S. Grob, G.S. Brito, D.C. Mash. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* **1996**, 20, 492-497.
- [2.8] L. Rivier, J.E. Lindgren. "Ayahuasca," the South American hallucinogenic drink: an ethnobotanical and chemical investigation. *Economic Botany* **1972**, 26, 101-129.
- [2.9] Y. Hashimoto, K. Kawanishi. New organic bases from Amazonian *Banisteriopsis caapi*. *Phytochemistry* **1975**, 14, 1633-1635.
- [2.10] Y. Hashimoto, K. Kawanishi. New alkaloids from *Banisteriopsis caapi. Phytochemistry* **1976**, 15, 1559-1560.
- [2.11] J. Riba. Human Pharmacology of Ayahuasca [Doctoral Thesis]. Universitat Autònoma de Barcelona, **2003**. Available at: http://www.tdx.cat/handle/10803/5378 [23 January, 2012]

- [2.12] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *Journal of Pharmacology and Experimental Therapeutics* **2003**, 306, 73-83.
- [2.13] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O. Andrade, D.C. Mash. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *Journal of Ethnopharmacology* 1999, 65, 243-256.
- [2.14] J. Ott. Pharmahuasca: Human pharmacology of oral DMT plus harmine. Journal of *Psychoactive Drugs* **1999**, 31, 171-177.
- [2.15] J. Riba, M.J. Barbanoj. Bringing ayahuasca to the clinical research laboratory. *Journal of Psychoactive Drugs* **2005**, 37, 219-230.
- [2.16] D.J. McKenna, in: R. Metzner, (Ed.), Ayahuasca: Hallucinogens, Consciousness, and the Spirit of Nature, Thunder's Mouth Press, New York, **1999**, p. 243.
- [2.17] Supreme Court of the United States. 2006. Certiorari To The United States Court Of Appeals For The Tenth Circuit. No. 04-1084. Alberto R. Gonzales, Attorney General et al., Petitioners V. O Centro Espirita Beneficente Uniao Do Vegetal et al. (http://www.erowid.org/chemicals/ayahuasca/ayahuasca_law23.pdf)
- [2.18] R.S. Gable. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction* **2007**, 102, 24-34.
- [2.19] D.J. Moura, M.F. Richter, J.B. Boeira, J.A.P. Henriques, J. Saffi. Antioxidant properties of β -carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis* **2007**, 22, 293-302.
- [2.20] W. Andritzky. Sociopsychotherapeutic functions of ayahuasca healing in Amazonia. *Journal of Psychoactive Drugs* **1989**, 21, 77-89.
- [2.21] J. Mabit, R. Giove, J. Vega, in: M. Winkelman and W. Andritzky (Eds.), Yearbook of Cross-Cultural Medicine and Psychotherapy, VMB Press, Berlin, **1996**, p. 257.
- [2.22] J.C. Callaway, M.M. Airaksinen, D.J. McKenna, G.S. Brito, C.S. Grob. Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology* **1994**, 116, 385-387.
- [2.23] T. Hallikainen, H.M. Saito, J. Lachman, T. Volavka, O.P. Pohjalainen, J. Ryynanen, J. Kauhanenm, E. Syvalahti, J. Hietala, J. Tiihonen. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Molecular Psychiatry* 1999, 4, 385-388.
- [2.24] T. Mantere, E. Tupala, H. Hall, T. Sarkioja, P. Rasanen, K. Bergstorm, J.C. Callaway, J. Tiihonen. Serotonin transporter distribution and density in the cerebral cortex of alcoholic and nonalcoholic comparison subjects: a whole-hemisphere autoradiography study. *American Journal of Psychiatry* 2002, 159, 599-606.

- [2.25] J. Tiihonen, J.T. Kiukka, K.A. Bergstorm, J. Karhu, H. Viinamaki, J. Lehtonen, T. Hallikainen, J. Yang, P. Hakola. Single-photon emission tomography imaging of monoamine transporters in impulsive violent behavior. *European Journal of Nuclear Medicine* 1997, 24, 1253-1260.
- [2.26] S.A. Barker, J.A. Monti, S.T. Christian. Metabolism of the hallucinogen N,Ndimethyltryptamine in rat brain homogenates. *Biochemical Pharmacology* 1980, 29, 1049-1057.
- [2.27] K. Kawanishi, Y. Uhara, Y. Hasimoto. Shinunine and dihydroshinunine from Banisteriopsis caapi. *J Nat Prod* **1982**, 45, 637-639.
- [2.28] J.C. Callaway, G.S. Brito, E.S. Neves. Phytochemical analyses of *Banisteriopsis caapi* and *Psychotria viridis*. *Journal of Psychoactive Drugs* **2005**, 37, 145-150.
- [2.29] R.E. Schultes, The beta-carboline hallucinogens of South America. J Psychoactive Drugs 1982, 14, 205.
- [2.30] R.G. Santos, J. Landeira-Fernandez, R.J. Strassman, V. Motta, A.P.M Cruz. Effects of ayhuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. *Journal of Ethnopharmacology* 2007, 112, 507-513.
- [2.31] C. Gambelunghe, K. Aroni, R. Rossi, L. Moretti, M. Bacci. Identification of N,Ndimethyltryptamine and beta-carbolines in psychotropic ayahuasca beverage. *Biomed. Chromatogr* 2008, 22, 1056.
- [2.32] A.P.S. Pires, C.D.R. De Oliveira, S. Moura, F.A. Dorr, W.A.E. Silva and M. Yonamine. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochemical Analysis* 2009, 20, 149-153.
- [2.33] K. Kawanishi, K. Saiki, H. Tomita, Y. Tachibana, N.R. Farnsworth, M. Bohlke. Chemical components of the Brazilian shamanistic drink "Ayahuasca." Advances in *Mass Spectrometry* 1998, 14, D053560/1.

Chapter 3. Methodology for and the Determination of the Major Constituents and Metabolites of the Amazonian Botanical Medicine Ayahuasca in Human Urine*

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3.1 Introduction

The term 'ayahuasca' denotes a decoction or tea traditionally prepared by South American tribes by boiling or soaking the bark or the stems of the large, woody, jungle vine (liana) *Banisteriopsis caapi* and the leaves of *Psychotria viridis*, a member of the coffee family (Rubiaceae) [3.1-3.6]. The resulting tea is consumed in varying amounts, depending on the potency of the preparation, so as to attain different states of altered consciousness. Because of this and related effects, ayahuasca continues to hold a highly esteemed and millennia-old position in the culture of various South American groups as part of their medical and religious pharmacopeia.

There is now an increasing interest in modern medical applications of ayahuasca, as well as growing concern regarding its potential for abuse. Indeed, several syncretic religious movements originating in South America that use ayahuasca as a sacrament have spread throughout the industrialized West. A bourgeoning "ayahuasca tourism" trade in South America, an influx of ayahuasca into the West for illicit use, and the consumption of home-brewed preparations utilizing both B. *caapi* and P. *viridis* (or similar plants), are seen as having implications for public health [3.7] and for various national and/or international regulatory agencies. The possible medical benefits of ayahuasca [3.6, 3.8] include its antioxidant, antimutagenic and antigenotoxic activity [3.9] as well as putative psychotherapeutic and rehabilitative effects [3.10, 3.11] and a potential to treat conditions such as alcoholism with tendencies toward violence, suicidal behaviors, and severe depression [3.12-3.15]. Thus, an examination of the biochemical parameters of ayahuasca, including its metabolism and clearance, will add to our understanding of this Amazonian medicine.

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The psychotropic effects of ayahuasca are produced by the combination of components extracted by the boiling process described above. B. caapi contains the beta-carboline harmala alkaloids harmine (3.1.1), harmaline (3.1.2), and tetrahydroharmine [THH (3.1.3)] as the major active components as well as lesser amounts of the O-demethylated beta-carboline derivatives harmol (3.1.4) and harmalol (3.1.5) (Figure 3.1) [3.3, 3.4, 3.16-3.19]. P. viridis contains the psychedelic compound N, N-dimethyltryptamine [DMT (3.1.6); Figure 3.1)], as well as lesser amounts of N-methyltryptamine [NMT (3.7)] and 2-methyl-1,2,3,4-tetrahydro-beta-carboline [2-MTHBC (3.1.8), Figure 3.1] [3.5, 3.6, 3.16, 3.20]. Extensive information regarding the phytochemical composition and general pharmacology of traditional ayahuasca has appeared in previous publications [3.6, 3.19-3.23]. Similarly, blood pharmacokinetic data for many of the major constituents of ayahuasca and, subsequently, methods for their analysis in blood either individually or in combination, have been described [3.19, 3.22, 3.24-3.27]. However, no literature exists concerning urinary excretion, clearance and metabolism of the known major constituents of ayahuasca in combination. Only two reports of the analysis of a select few of ayahuasca's known components in urine have been published [3.28, 3.29] and no data concerning the fate of ayahuasca derived DMT.

The present manuscript describes the development of a rapid, specific, and sensitive method for the direct qualitative and quantitative analyses of 12 known or potential major alkaloid components and metabolites found in human urine following oral administration of ayahuasca. Such a method is needed in order to understand the metabolism and clearance of ayahuasca's major constituents, particularly for conducting toxicological, clinical or other basic science research. To demonstrate the utility of the procedures described, the method has been applied to urine samples collected from individuals administered encapsulated, freeze-dried

ayahuasca of a known composition by an oral route [3.8, 3.20, 3.22, 3.30]. The results of the analyses of these urine samples before and after treatment with sulfatase/glucuronidase are also reported.

3.2 Experimental

3.2.1 Solvents and Standards

HPLC-grade methanol was purchased from Honeywell Burdick and Jackson (Morristown, New Jersey, USA). HPLC-grade water, high purity formic acid, and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA). N-methyltryptamine (NMT), DMT, 5hydroxy-DMT (5-OH-DMT), 5-methoxy-DMT (5-MeO-DMT), harmine, harmaline hydrochloride dihydrate, harmol hydrochloride dihydrate, and harmalol hydrochloride dihydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydroharmine was purchased from THC Pharm (Frankfurt am Main, Germany). DMT-NO (3.1.11, Figure 3.1) and 2-MTHBC were prepared as previously described [3.31]. N,N-Dimethylkynuramine (DMK, 3.1.13, Figure 3.1) was a gift from Dr. Laurent Micouin, Laboratoire de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France. Deuterated (d₄) 5-MeO-DMT (alpha, alpha, beta, beta-tetradeutero; 3.1.12, Figure 3.1) was a gift from Dr. David E. Nichols, Purdue University Department of Medicinal Chemistry and Molecular Pharmacology, West Lafayette, IN, USA.

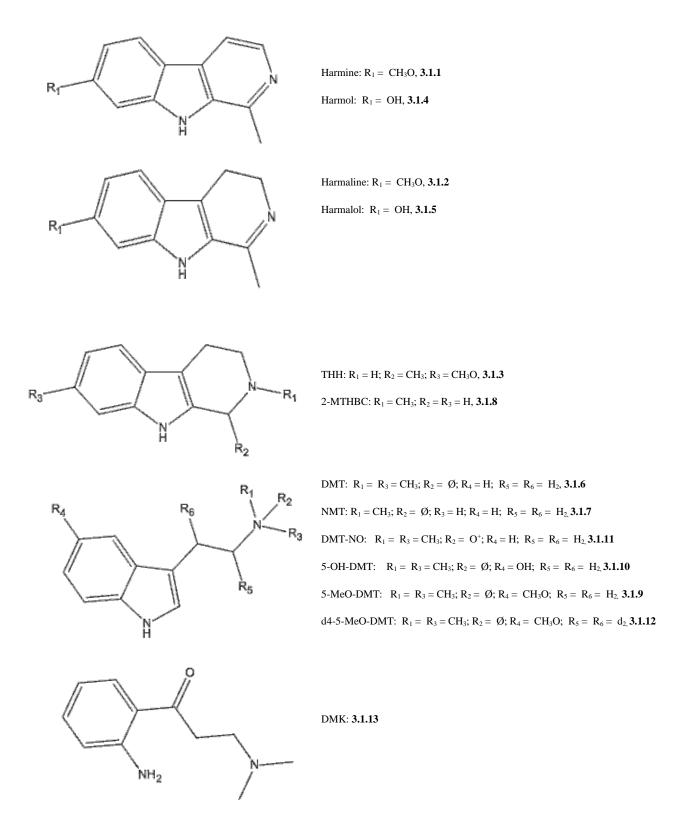


Figure 3.1. Structures of the compounds examined in urine samples.

Stock standard solutions (1 mg/ml) of the 12 selected compounds and the internal standard were prepared individually in methanol in 10 ml amber glass vials with Teflon-lined screw-cap closures and stored in a freezer at -20° C. Working standards were prepared in methanol at selected concentrations by serial dilution and pipetted into 5 ml conical tubes. The methanol was removed by gentle evaporation over dry nitrogen and the residues were dissolved in amounts of 90% H₂O/0.1% formic acid: 10% acetonitrile/0.1% formic acid mobile-phase solution corresponding to the appropriate sample dilution experiment being conducted.

3.2.2 Preparation of Urine Samples

Urine samples were well-mixed and 100 μ l of the sample were transferred to a 5 ml conical glass tube. The samples were then diluted to the appropriate volume using 90% H₂O/0.1% formic acid:10% acetonitrile/0.1% formic acid mobile phase solution. Dilutions of standards in blank urine (pooled, 0 time point/pre-ayahuasca-administration collections) of 5:1, 10:1 and 20:1 were examined. Standard curve data points at 7 or 8 selected concentrations (including zero) specific to the expected concentration range for each compound (determined from preliminary analyses) were prepared in blank urine and used to examine linearity of response, to collect data for the quantification of samples, and to determine method performance. Spiked water samples were similarly prepared and used to examine matrix effects and relative analyte recovery.

Methanol (20 μ l) containing 1ng/ μ l internal standard d₄-5-MeO-DMT was added directly into individual autosampler vials and dried completely under nitrogen. Various serial dilutions of the 12 compound stock standard mixes or urine samples (1 ml) were filtered through 0.4 um filters (Nalgene, NalgeNunc, Rochester, NY, USA) and 200 μ l of the filtrate were added to

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individual autosampler vials containing dried internal standard. The urine samples were thoroughly mixed by vortexing. The urine samples and mixed standards, spiked into urine or water, were transferred to the HPLC autosampler and 10 µl were injected for analysis.

3.2.3 Freeze Dried Ayahuasca Administration and Urine Collection

Ayahuasca was administered orally as an encapsulated lyophilizate obtained from a large, 10 L batch which was freeze-dried, homogenized and analyzed for alkaloid contents by HPLC [3.19]. One gram of this freeze-dried material contained 4.77 mg DMT, 10.45 mg harmine, 0.68 mg harmaline, and 9.04 mg THH. Based on previous ayahuasca dosing studies [3.8, 3.20, 3.22, 3.30], a dose of 0.75 mg DMT/kg body weight was chosen. Freeze-dried ayahuasca was administered in 00 size gelatin capsules containing 0.5, 0.25, or 0.125 g. Each volunteer received their calculated individual dose by consuming the correct combination of these capsules.

Urine samples were obtained following dosing to 3 healthy male volunteers with previous experience in psychedelic drug use. The study was approved by the local ethics committee (Hospital de Sant Pau, Barcelona) and the Spanish Ministry of Health. Signed informed consent was obtained from all participants. Urine was collected for the following time intervals relative to ayahuasca administration: 0 (predose), 0-4h, 4-8h and 8-24h. The collected urine volume across each time interval was noted, the pooled urine was well mixed and 50 ml aliquots were separated and stored at -80 °C until analysis. Samples were shipped and received on dry ice and underwent a single freeze-thaw cycle prior to analysis.

3.2.4 Enzyme Hydrolysis

Enzyme hydrolysis of the urine samples was performed by preparing 1.0 M sodium acetate buffer at pH 5.0 by dissolving 82 g of anhydrous sodium acetate with 967 mL HPLC

water and 33 ml of glacial acetic acid. Glucuronidase/sulfatase buffer was prepared by dissolving 1 bottle (2 million units) of B β -glucuronidase/sulfatase from limpets (*Patella vulgata*) Type L-II (Sigma-Aldrich, St. Louis, MO, USA) in 400 ml of distilled water. Acetate buffer (1200 ml) was then mixed with 400 ml glucuronidase/sulfatase buffer. Glucuronidase/sulfatase /acetate buffer mix (300 μ l) was then added to each tube containing 500 μ l of individual urine samples and incubated for 1 hour in a 65° C shaking water bath, and then allowed to cool to room temperature. Samples were then treated as described above, accounting for the dilution involved in the hydrolysis process.

3.2.5 Determination of Matrix Effects

Matrix effects were examined using three blank urine samples (0-time samples from the three individuals' samples examined here, prior to ayahuasca administration) and water (n = 3). The urines and water were treated as described above (only a 10:1 dilution was examined) and then fortified at five different concentrations of each of the standards (0, 10, 25, 50 and 100 ng/ml). The data were examined to determine the slope of the curves so generated and the slopes were compared, determining the percent relative standard deviation (%RSD) for each compound.

3.2.6 LC-ESI-MS/MS analysis

The LC/MS/MS analyses were conducted as previously described [3.23] using a validated method for the analysis of 12 of these alkaloids in ayahuasca preparations themselves, with modifications, and with the addition of the compound DMK (3.13, Figure 3.1) to the analysis. Thus, analyses were conducted using an Agilent 1200 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent G1367A HiP ALS autosampler, an Agilent G1311A Quaternary micropump, and an Agilent G1332A degasser. An Agilent

G131gA TCC column oven operating at 25°C was interfaced to a TSQ Quantum Access 1.5 SP1 tandem MS (Thermo Fisher Scientific, Waltham, MA, USA) with electrospray ionization (ESI) operated in the positive ion mode.

Chromatographic separation was achieved on a 1.8 μ m 4.6 x 50 mm (i.d.) Agilent ZORBAX Eclipse Plus C18 rapid resolution HT threaded column with an Alltech Direct-Connect Column 2 μ m pre-filter (Deerfield, IL, USA) using gradient elution. The following gradient system was used as the mobile phase: A (0.1% formic acid in H₂O) and a mobile phase B (0.1% formic acid in acetonitrile) delivered at a constant flow rate of 0.3 ml/min throughout the analysis; A:B 90:10 (0 min)- 90:10 (1 min)- 50:50 (5 min)- 50:50 (6 min)- 2:98 (7 min)- 2:98 (9 min) – 90:10 (10 min)- 90:10 (23 min), allowing for re-equilibration. The MS/MS analysis was performed using selected reaction monitoring (SRM) of the protonated molecular ions for the analytes. The spray voltage was 4000 V, sheath gas (nitrogen) pressure 50 psi, capillary temperature 310° C, and collision pressure was 1.5 psi. of high purity argon. Generation of detection data and integration of chromatographic peaks were performed by Xcalibur 2.0.7 Thermo Fisher Scientific (Waltham, MA, USA) LCquan 2.5.6 QF 30115 software.

Identification of the compounds was based on the presence of the molecular ion at the correct retention time, the presence of three transition ions and the correct ratio of these ions to one another (+/- 25% relative).

3.2.7 Determination of Inter- and Intra-assay Variation

A set of three standard curves prepared in blank urine were assayed on the same day and the results compared to generate interassay variability. Three separate curves were generated and analyzed on three separate days to provide data for inter-assay variability. The %RSD for concentrations ranging from 5-200 ng/ml was calculated for each experiment.

3.2.8 Calculations

The concentration of compounds in urine samples was determined from the peak area ratio of the target analyte to that of the internal standard (d₄-5-MeO-DMT; 3.1.12, Figure 3.1), by reference to calibration curves prepared by spiking blank urine with each of the 12 substances and a consistent amount of IS (internal standard). The transition ions used for quantitation are shown in Figure 3.2. If the concentration observed was outside the measured range, the sample was re-analyzed after further dilution with mobile phase and the original concentration calculated by extrapolation. Values determined from repeated analyses of n aliquots of samples or standards were expressed as their arithmetic mean. For the standards the percent relative standard deviation (%RSD) was calculated, as was the method bias, as a function of concentration. Standard deviations were calculated for n determinations of samples as noted. Inter- and intra-assay %RSDs were also determined. Limits of detection (LOD; concentration response greater than 5X baseline noise) and limits of quantification (LOQ: the lowest injected concentration having a %RSD of less than 15%) were also determined for each compound. To examine matrix effects, slopes were calculated for standard curves generated from fortifying 3 different urine samples and 3 water samples. The slope and mean of the slopes and of individual urine samples were compared to each other and that obtained from the mean of spiked water curves and the %RSD was calculated for each compound.

3.3 Results and Discussion

3.3.1 Evaluation of the Method

Several methods have been reported for the analysis of the major components of ayahuasca itself [3.20, 3.23, 3.25, 3.27, 3.32-3.35] and for the analysis of multiple components in blood [3.19, 3.22 3.25, 3.26]. However, no comprehensive method measuring major components and known or potential metabolites, as reported here, has ever been developed or applied to urine samples obtained following ayahuasca administration to humans. This report provides such data for the first time, and would be applicable to future toxicological or clinical research of this Amazonian medicine. The method described provides a simple, flexible, sensitive and selective protocol, as reflected in the performance parameters measured (Table 3.1) and in the chromatograms shown. This approach to conducting the analysis of the components of ayahuasca has previously been applied to ayahuasca itself [3.23] using a 200:1 dilution of the "tea". Such direct analysis following dilution has several advantages, permitting analysis of a range of compounds in a single injection that would otherwise have to be isolated and analyzed by one or more extraction/detection schemes.

Each of the compounds assayed, including DMK, gave high yields of molecular ions $(M+H)^+$ using the mobile phase system and electrospray ionization parameters previously described [3.23]. The LC/ESI/MS/MS characteristics of DMK have not previously been reported, however. DMK gave an excellent yield of the molecular ion $(M+H^+, 193.1)$ and product ions were also formed with good efficiency (58.3, 42.4 and 130.1 at voltages of 13, 63 and 21 V, respectively). Although for many of the compounds the yield of the third product ion

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was less than 10% of the base peak, adequate sensitivity was achieved to allow inclusion of all ions in the identification criteria.

Using the LC method described, all of the compounds examined were not separated temporally. However, all of the compounds were completely separated by mass. A thorough examination of ion contribution (cross-talk) between each of the compounds and the masses monitored indicated no discernible interference, even at the highest concentrations. The LC method employed yielded good peak shape for each analyte, despite the rather diverse chemical characteristics of the compounds examined, and appears to also assist in removing possible matrix interferences. No substances having the same masses as the target analytes were observed, in either the method blanks or zero-time urine samples collected from subjects, which would potentially interfere with the detection of the compounds of interest. Similarly, enzyme treatment did not produce any compounds that interfered with the analysis. These results suggest that the method has a high degree of specificity. A representative chromatogram for analyte standards spiked into urine is shown in Figure 3.2, and a representative chromatogram for a blank urine sample containing only the internal standard is shown in Figure 3.3.

Of the three dilutions examined, 5:1, 10: 1 and 20:1, the 10:1 dilution protocol was chosen. At 5:1 some issues with peak shape (peak broadening and tailing due to overloading) were noted which were eliminated by dilution at 10:1. Dilutions at 20:1 were also quite acceptable but the results were, of course, not as sensitive as the 10:1 dilution. Thus, a 10:1 dilution was subsequently used for all analyses.

Data for the retention time of each compound and their reproducibility, as well as the analytical imprecision (%bias) for each analyte, are presented in Table 3.1. The calibration

curves for each compound were linear over the range of concentrations examined (5-200 ng/ml Table 3.1) and the linear regression equations and coefficients of correlation for each are presented in Table 3.1. The performance parameters for the method (%bias, %RSD, LOD, LOQ, intra- and inter-assay precision) for determination of the 12 compounds are also presented in Table 3.1. Calculations of method bias and %RSDs show that using a 10:1 dilution and performing minimal sample manipulations does not lead to any significant loss of analytes in the process, with recoveries being near quantitative (compared to responses of equal concentrations spiked into water). The limits of detection were in the sub-nanogram range for most of the analytes and were more than adequate for the detection and determination of concentrations of the compounds excreted into the urine following ayahuasca administration. The highly linear response of the analytes over the range of concentrations examined demonstrates the validity of the use of the single deuterated internal standard for all 12 compounds. The modest inter- and intra-assay variations observed reflect a reliable and reproducible method for detection of the relevant compounds found in human urine after ayahuasca administration.

Table 3.1. Method performance parameters. LOD = limit of detection (ng/ml); LOQ = limit of quantitation (ng/ml); RT = retention time (minutes); %RSD = % relative standard deviation.

Molecule	Target Conc (ng/ml) (ppb)	Observed conc mean (n=5) (ng/ml)	% Bias	% RSD (n=5)	RT (min) (n=5)	LOD S/N =5	LOQ	y and r^2 5-200 ng/ml	Inter-assay % RSD (n=3)	Intra-assay % RSD (n=3)
5-OH-DMT					2.87	0.22	5.00	y=0.003+0.003*X		
	5	4.577	-8.451	4.37	2.87			r ² =0.9996	2.02	5.26
	10	10.259	2.591	3.89	2.86				3.18	0.92
	25	25.935	3.740	5.96	2.86				7.04	6.88
	50	51.276	2.552	5.26	2.88				7.02	6.29
	100	100.077	0.077	4.88	2.86				7.61	2.75
	200	199.544	-0.228	0.94	2.87				1.01	1.08
DMK					4.35	0.23	5.00	y = 0.03+0.005*X		
	5	5.257	5.138	3.29	4.07			$r^2 = 0.9982$	2.90	2.10
	10	9.857	-1.430	4.28	4.39				5.17	1.16
	25	27.165	8.661	5.09	4.48				2.18	5.92
	50	49.056	-1.889	1.79	4.37				3.01	1.65
	100	97.018	-2.982	8.13	4.37				9.51	0.02
	200	194.051	-2.975	4.61	4.41				6.89	1.56
HARMALOL					7.19	0.18	5.00	v=0.01+0.001*X		
	5	4.789	-4.228	7.47	7.21			r ² =0.9986	3.24	8.37
	10	11.062	10.623	5.71	7.20				6.22	6.20
	25	25.390	1.562	4.87	7.19				2.24	4.30
	50	50.907	1.814	3.79	7.20				5.02	2.25
	100	103.350	3.350	3.44	7.18				3.47	1.80
	200	200.504	0.252	2.26	7.18				3.14	2.62
NMT					7.42	0.04	5.00	y=0.03+0.006*X		
	5	5.285	5.694	14.43	7.42			r ² =0.9995	7.89	0.33
	10	10.506	5.061	6.41	7.42				7.46	3.64
	25	24.951	-0.197	1.79	7.42				0.88	2.19
	50	48.112	-3.776	3.73	7.43				4.10	3.75
	100	100.717	0.717	2.59	7.41				2.17	2.67
	200	201.668	0.834	1.55	7.41				2.67	0.58
HARMOL					7.42	0.57	5.00	y=0.009+0.0009*X		
	5	6.018	20.356	5.27	7.43			r ² =0.9995	8.73	2.87
	10	11.517	15.173	8.33	7.41				7.97	3.26
	25	25.000	0.001	5.33	7.42				7.93	3.04
	50	49.704	-0.593	5.23	7.42				3.99	5.92
	100	100.560	0.560	1.94	7.42				2.97	0.72
	200	200.878	0.439	1.65	7.42				2.35	1.72
DMT					7.57	0.12	5.00	y=0.01+0.004*X		
	5	5.115	2.293	7.34	7.58			r ² =0.9997	4.45	9.31
	10	10.533	5.325	4.42	7.57				3.74	5.58
	25	25.020	0.078	7.29	7.58				10.32	6.84
	50	49.120	-1.760	2.82	7.58				1.30	3.81
	100	101.869	1.869	4.12	7.56				5.49	1.01
	200	200.098	0.049	1.41	7.56				1.13	1.00

Table 3.1 Continued

5-MeO-DMT					7.66	0.15	5.00	y=0.02+0.004*X		
	5	5.174	3.488	3.71	7.66			r ² =0.9991	4.93	0.65
	10	10.246	2.459	1.89	7.66				0.52	1.96
	25	25.852	3.407	3.88	7.66				6.68	3.99
	50	50.690	1.380	6.56	7.66				7.04	5.06
	100	102.188	2.188	2.35	7.66				4.29	1.75
	200	200.723	0.362	1.57	7.66				2.48	0.22
2MTHBC					7.90	0.14	5.00	v=0.05+0.006*X		
	5	4.769	-4.619	9.93	7.89			r ² =0.9991	1.71	6.43
	10	10.457	4.568	4.26	7.88				3.04	6.06
	25	26.179	4.716	5.93	7.91				11.12	3.75
	50	49.490	-1.021	2.79	7.91				3.85	1.67
	100	99.613	-0.387	4.36	7.91				1.62	4.27
	200	201.753	0.877	2.34	7.89				0.47	2.52
THH					8.02	0.21	5.00	y = 0.019+0.002*X		
	5	5.912	18.241	8.37	8.02			$r^2 = 0.9990$	8.32	5.35
	10	10.901	9.014	2.86	8.02				4.00	2.40
	25	26.680	6.719	2.70	8.02				3.25	2.62
	50	48.127	-3.746	4.31	8.02				5.61	4.37
	100	100.768	0.768	3.25	8.01				2.84	3.59
	200	200.594	0.297	1.49	8.02				1.38	1.63
DMT-NO					8.02	0.07	5.00	y=0.01+0.003*X		
	5	5.307	6.132	6.68	8.01			r ² =0.9995	6.81	3.12
	10	10.523	5.226	4.45	8.01				5.97	3.88
	25	26.897	7.590	8.15	8.02				11.47	6.38
	50	49.640	-0.719	4.49	8.02				5.64	5.11
	100	100.932	0.932	0.96	8.03				0.94	1.18
	200	198.701	-0.649	0.94	8.02				1.12	1.02
HARMINE					8.30	0.18	5.00	y=0.06+0.01*X		
	5	4.917	-1.669	7.23	8.31			r ² =0.9996	1.91	8.74
	10	10.573	5.733	4.31	8.29				2.35	4.31
	25	26.636	6.546	5.25	8.30				0.53	6.06
	50	50.396	0.791	2.94	8.31				3.52	2.86
	100	100.238	0.238	3.14	8.30				3.02	3.01
	200	202.034	1.017	1.34	8.30				1.95	1.16
HARMALINE					8.26	0.07	5.00	y=0.02+0.002*X		
	5	5.412	8.245	9.71	8.25			r ² =0.9992	3.95	9.13
	10	10.788	7.876	6.22	8.26				0.55	2.80
	25	25.357	1.429	4.36	8.26				2.06	4.22
	50	51.153	2.305	1.66	8.26				1.12	1.50
	100	102.195	2.195	1.57	8.26				1.11	1.81
	200	195.361	-2.319	0.49	8.26				0.20	0.60
D4 5-MeO- DMT (IS)	20				7.65					

Table 3.2. Comparison of line slopes over the range of concentrations examined showing relative (urine versus urine) and general [urine versus mobile phase (MP)] matrix effects (%RSD).

	MP Slope (n = 3)	r ²	Urine 1	r²	Urine 2	r²	Urine 3	r²
5-OH-DMT	0.2858	0.9996	0.2950	0.9995	0.3047	0.9997	0.2784	0.9993
DMK	0.0017	0.9998	0.0020	0.9997	0.0023	1.0000	0.0018	0.9997
HARMALOL	0.3606	0.9998	0.4316	0.9997	0.4567	1.0000	0.4390	0.9994
NMT	0.2565	0.9991	0.2922	0.9998	0.2773	0.9998	0.2487	0.9996
HARMOL	0.3692	1.0000	0.3418	0.9999	0.3012	0.9997	0.3785	0.9997
DMT	0.2159	1.0000	0.2056	0.9998	0.2017	0.9997	0.1919	0.9995
5-MeO-DMT	0.3001	0.9996	0.3108	0.9998	0.3080	0.9999	0.2735	0.9999
2MTHBC	0.3397	0.9997	0.3835	0.9996	0.3680	1.0000	0.3611	0.9997
THH	0.2094	0.9999	0.2337	0.9998	0.2432	1.0000	0.2288	0.9962
DMT-NO	0.7197	0.9998	0.6902	0.9998	0.6551	1.0000	0.6753	0.9998
HARMINE	1.0555	0.9999	1.0452	0.9998	1.1257	0.9999	0.9948	1.0000
HARMALINE	0.4169	1.0000	0.3950	1.0000	0.4675	0.9999	0.3937	0.9995

Avg. Urine Slope	Urine %RSD	Urine v. MP %RSD
0.2927	4.54	5.32
0.0020	13.70	14.66
0.4441	2.91	11.33
0.2728	8.10	7.36
0.3405	10.91	10.35
0.1997	3.53	4.88
0.2974	6.98	5.71
0.3709	3.10	5.01
0.2352	3.11	8.85
0.6736	2.62	3.62
1.0553	6.26	5.11
0.4187	10.08	8.96

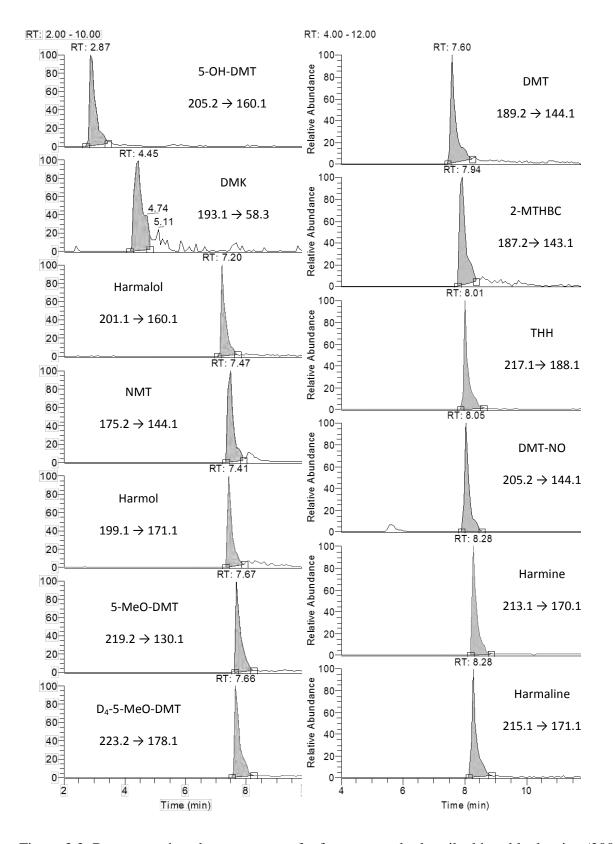


Figure 3.2. Representative chromatogram of reference standards spiked into blank urine (200 ng/ml of each; RT= retention time in minutes).

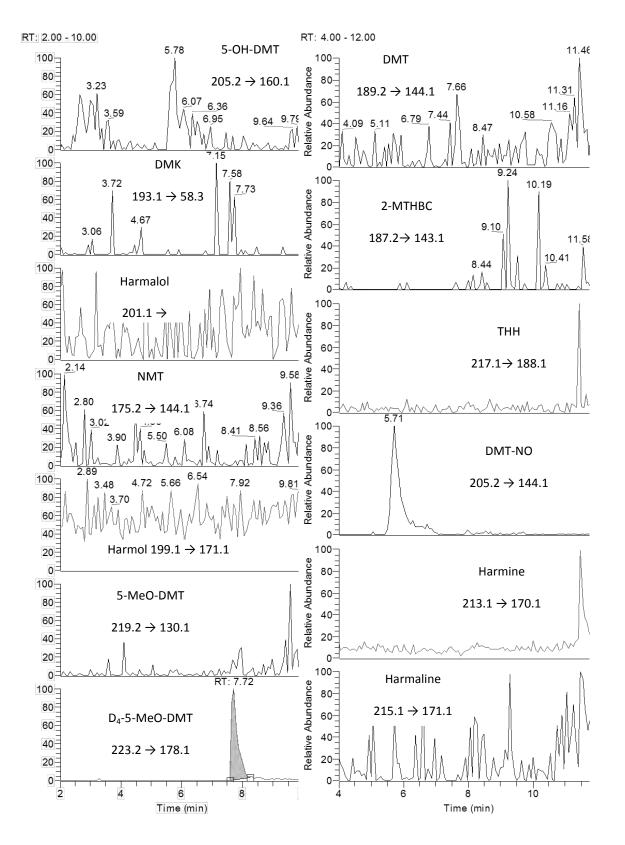


Figure 3.3. Representative chromatogram of a blank urine sample (4RH3) obtained preayahuasca administration (shown at 10:1 dilution; RT= retention time in minutes).

3.3.2 Matrix Effects

One concern involving the use of a direct-injection/sample dilution protocol, especially with one based on LC/ESI/MS/MS analysis, is matrix effects [3.36-3.40]. Thus, following the examples of Matuszewski [3.36, 3.37], we examined both relative and general or absolute matrix effects for the twelve compounds examined by comparing the slopes of the standard curves generated from spiking standards into water (no matrix) over a range of concentrations versus standards similarly spiked into blank urine (n = 3 for each) and by comparing the urines to each other (Table 3.2). Ion suppression or ion enhancement would be expected to cause significant deviation of the slope of the corresponding compound linear regression curve and would be reflected in the %RSD calculated for the mean of n curves. In the present study, no significant deviation (>= 15%) of the slope for any of the compounds was observed (Table 3.2), either between urine samples or for urine versus water, illustrating that relative and absolute matrix effects were generally not of concern [3.38]. Matrix effects are further mitigated in this method by the use of the matrix itself to generate standard curves. DMK was affected to the greatest degree of all the compounds examined. While of interest, this observation should not be an issue since it was determined from the present study that DMK does not appear as a urinary metabolite of DMT following ayahuasca administration and need not be included in future studies.

3.3.3 Major Constituents and Metabolites of Ayahuasca in Urine

The application of this method to actual samples has also been demonstrated and the results obtained offer new data concerning the metabolism and clearance of the major harmala alkaloids of ayahuasca and of DMT, in particular. Because of the limited number of subjects, calculations as to various parameters of clearance and overall metabolism were not deemed

appropriate. A larger study will need to be conducted that includes more subjects and correlation of the data with urine creatinine as well as other values.

The overall chromatography and the detection of the selected alkaloids and metabolites present in pre- and post-enzyme treated urine samples collected after ayahuasca administration, are presented in Figures 4 and 5, respectively. Tabulation of the concentrations of these compounds pre- and post-enzyme treatment of urine as a function of collection interval are shown in Table 3.3. As shown in Table 3.3, prior to enzyme treatment of urine, the overall major metabolite observed was DMT-NO, peaking at 4-8 hour after ayahuasca administration with concentrations of approximately 11 μ g/ml (n = 3 individuals). THH was the major component in the 8-24 hours samples, with concentrations greater than 5 μ g/ml. Free harmalol and harmol were also observed in significant μ g/ml concentrations. Much smaller amounts of unchanged DMT, harmaline and harmine and of 5-OH-DMT, and 2-MTHBC were also detected.

In urine samples which underwent enzyme hydrolysis with glucuronidase/sulfatase, a 40-60 fold increase (Table 3.3) in the amount of harmol in the urine samples was observed, making harmol (or harmol-glucuronide/sulfate) the most abundant product exctreted. Harmalol concentration was increased by a factor of at least 4-fold. Little discernible change in concentration was observed for excreted THH, harmine, harmaline, or DMT as a consequence of enzyme hydrolysis. DMT-NO concentrations were observed to decrease with enzyme treatment of urine but this may be caused by direct chemical effects on the compound produced by heating and hydrolysis [3.31]. Enzyme treatment of urine resulted in release of NMT, which was not detected in unhydrolyzed samples, and of 2-MTHBC. The presence of 5-OH-DMT was also noted in unhydrolyzed urine samples and its concentration was increased following enzyme treatment of urine (Table 3.3).

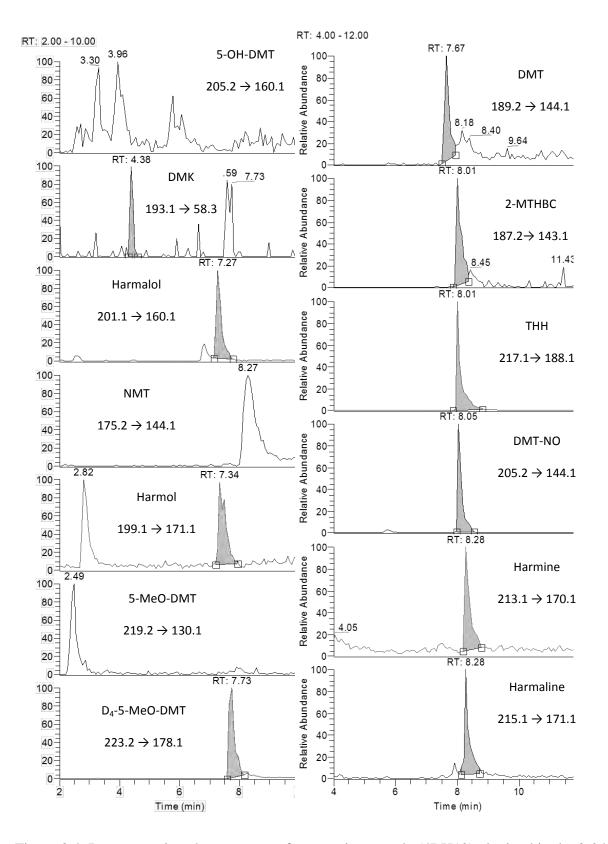


Figure 3.4. Representative chromatogram from a urine sample (4RH12) obtained in the 8-24h interval after ayahuasca administration.

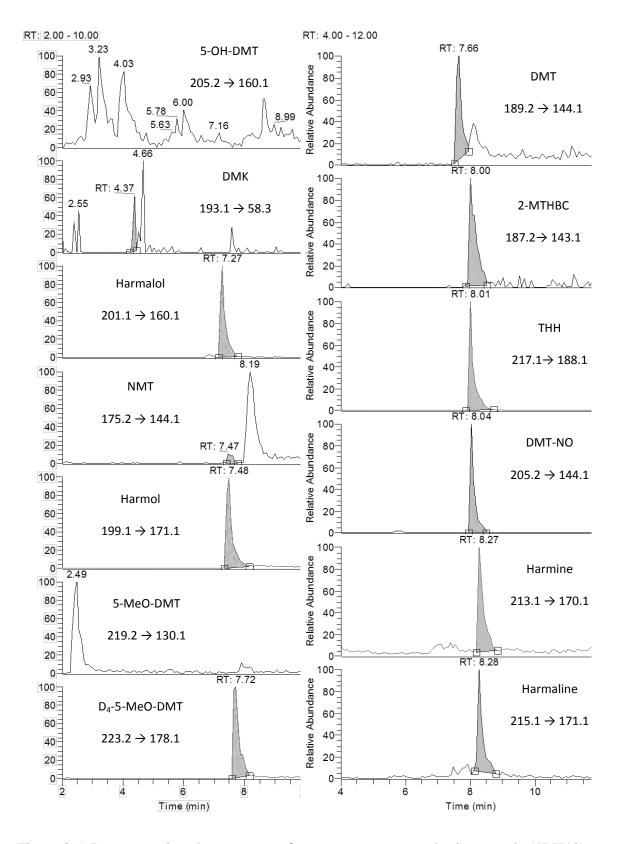


Figure 3.5. Representative chromatogram from an enzyme treated urine sample (4RH12) obtained in the 8-24h interval after ayahuasca administration.

				Concentrati	on of Compou	inds in Urine				
		DMT	DMT-NO	THH HARMINE HARMALINE			HARMALOL	HARMOL	2MTHBC	5-OH-DMT
	0	0	0	0.01	0	0.02	0	0.04	0	0
Collection Interval	0 to 4	0.45	8.66	6.03	0.16	0.51	3.61	2.3	0.02	0.14
(hours)	4 to 8	0.6	11.06	6.27	0.12	0.5	4.04	3.09	0.13	0.16
	8 to 24	0.03	1.27	5.4	0.01	0.32	1.25	0.77	0.01	0
		Co	oncentration	n of Compou	nds in Urine Fo	ollowing Enzym	e Treatment			
		DMT	DMT DMT-NO THH HARMINE HARMALINE HARMALOL HARMOL						2MTHBC	5-OH-DMT
	0	0	0	0	0	0	0	0.17	0	0
Collection Interval	0 to 4	0.5	8.74	7.25	0.21	0.53	14.16	126.18	0.12	0.2
(hours)	4 to 8	0.48	8.67	6.3	0.18	0.36	11.06	115.49	0.11	0.2
	8 to 24	0.02	1.02	4.85	0.06	0.25	5.32	38.93	0	0.02

Table 3.3: Concentration of compounds (μ g/ml) in urine as a function of collection interval (hours) and enzyme (glucuronidase/sulfatase) treatment.

The metabolism of the two major harmala alkaloids in ayahuasca, harmine and harmaline, have previously been examined as individual compounds, in separate studies, to undergo metabolism by O-demethylation to harmol and harmalol, respectively, followed by glucuronidation and/or sulfation [3.41-3.44]. The data from the present study are in agreement with these previous findings. The amounts of harmol and harmalol present in the ayahuasca consumed in the present study would be expected to provide a minor contribution to the total observed in urine, being in the 10's of μ g/ml range versus the 100's of μ g/ml to mg/ml range for harmine and harmaline [3.8, 3.20, 3.22, 3.23, 3.30]. Thus, there appears to be significant metabolism of these latter compounds.

In the current study, higher levels of THH were initially detected compared to harmine and harmaline in urine (Table 3.3). However, following enzyme hydrolysis of the urine samples, harmol and harmalol were found to be at far higher concentrations compared to THH. It is of interest that the hallucinogenic component of ayahuasca, DMT, when consumed alone by the oral route elicits no discernible psychoactivity, due to rapid oxidative deamination and peripheral degradation to indoleacetic acid (IAA) mediated by the enzyme monoamine oxidase A (MAO-A) [3.20, 3.24, 3.25 3.31, 3.45-3.50]. However, the beta-carboline alkaloids, such as harmine and harmaline and, to a lesser extent the THH also found in ayahuasca, are reversible inhibitors of MAO-A [3.3, 3.50]. Thus, co-administration of MAO inhibitors (MAOI) such as harmine or harmaline allows DMT to become orally active [3.3, 3.46] and would be expected to also affect its overall metabolism and excretion profile.

Several studies have examined the pharmacokinetics of parenterally-administered DMT and it is well established that intramuscular (i.m.) or intravenous DMT administered without MAO inhibition is rapidly cleared from blood, [3.51, 3.52] with less than 0.1% of the parent DMT being recoverable in human urine within a 24-hour collection period [3.51, 3.53]. *In vivo* animal studies have also demonstrated a very rapid clearance of DMT from various tissues such as plasma, brain, and liver [3.47, 3.48, 3.54, 3.55].

While oxidative deamination appears to be a major metabolic route, *N*-oxidation has also been identified as an important metabolic pathway of DMT both in vitro [3.31, 3.51, 3.56] and in vivo in rodents and rabbits [3.24, 3.47-3.49]. It is of interest to note that DMT-NO does not appear to be a substrate for MAO [3.49, 3.56]. NMT has also been identified as a minor metabolite of DMT [3.24, 3.31, 3.51, 3.57] as has 2-methyl-THBC (2-MTHBC) and traces of tryptamine and 1,2,3,4-tetrahydro-ß-carboline (THBC) [3.31]. NMT is also a substrate for MAO and is subject to be metabolized to IAA. The finding of NMT in the present study suggests that formation of NMT from DMT in ayahuasca may also be a minor pathway *in vivo* and that it may be conjugated before excretion. One must also consider the possibility that the 2-MTHBC

detected arises from the ayahuasca itself and not necessarily from DMT metabolism. Nonetheless, the latter possibility cannot be excluded.

It has been demonstrated that iproniazid pretreatment (MAO-A inhibition) increases the levels of DMT *in vivo* in rat brain, liver, kidney, blood, as well as DMT-NO in rat liver [3.48], and to increase the urinary excretion of DMT, DMT-NO and NMT in rodents [3.47]. These data suggest that MAO inhibition may shift metabolism to these other routes as part of a possible compensatory metabolic mechanism. Given the MAO-inhibition effects of ayahuasca, similar effects on DMT metabolism would be expected and may explain some of the results.

The present results suggest that *N*-oxidation represents a major metabolic route for DMT clearance in humans, particularly if MAO becomes inhibited, such as occurs with ayahuasca administration, in agreement with the work of Sitaram et al. [3.24]. Sitaram et al. found an approximately 10 times higher concentration of DMT-NO compared to DMT following iproniazid pretreatment in rodents [3.47] and noted a 6 times higher concentration of DMT-NO compared to DMT in control animals when no MAOI was administered. This suggests that DMT-NO may represent a major *in vivo* metabolite of DMT and may, thus, serve as a better marker for endogenous DMT production and metabolism in mammals as well [3.31, 3.45, 3.47].The data from the present study represent the first report of DMT-NO as a metabolite of DMT in the urine of humans following ayahuasca administration or, for that matter, DMT alone. This finding has implications for the further study of DMT in general, particularly its occurrence as a naturally occurring trace amine in man. The necessity for such studies has gained greater impetus with the recent report that DMT may be the endogenous ligand for the sigma-1 receptor [3.58, 3.59].

The origins of the 5-OH-DMT observed are somewhat obscure. It is possible that the ayahuasca used in this study contained small quantities of either 5-OH or 5-MeO-DMT, which were not originally detected. However, 5-OH-DMT may also be endogenous and may, with MAO inhibition afforded by ayahuasca consumption, reach high enough concentrations to become more detectable in urine. Further analyses will be required to answer this question.

DMK has also been reported to be a metabolite of DMT [3.60]. However, this compound has only been reported *in vitro* in whole blood following spiking with DMT. No study has yet to examine or determine the presence of this compound as an *in vivo* metabolite of DMT in any species. The present study suggests that, if formed in blood or in the tissues of humans, it is not consistently excreted as DMK in the urine, even with MAO inhibition, and after treatment with sulfatase/glucuronidase no free DMK is released. However, it remains possible that this compound undergoes metabolism to another, as yet undetermined, compound prior to excretion.

The data also suggest that future studies with clinical administration of ayahuasca would benefit from urine collection beyond 24 hours after administration in order to monitor the complete disappearance of DMT-NO, THH, harmine, and harmol.

3.4 References

- [3.1] R.E. Schultes. The identity of the maipighiaceous narcotics of South America. Botanical Museum Leaflets, Harvard University **1957**, 18, 1-56.
- [3.2] R.E. Schultes, A. Hofmann. The Botany and Chemistry of Hallucinogens. Charles C. Thomas, Springfield, Illinois, **1980**.
- [3.3] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. *Journal of Ethnopharmacology* **1984**, 10, 195-223.

- [3.4] DJ. McKenna, G.H.N. Towers. Biochemistry and pharmacology of tryptamines and betacarbolines: A minireview. *Journal of Psychoactive Drugs* **1984**, 16, 347-358.
- [3.5] D.J. McKenna, L.E. Luna, G.H.N. Towers, in von Reis S, Schultes RE (Eds.). Ethnobotany: Evolution of a Discipline, Dioscorides Press, Portland, OR, **1995**, p. 349.
- [3.6] D.J. McKenna. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacology and Therapeutics* **2004**, 102, 111-129.
- [3.7] R.S. Gable. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction* **2007**, 102, 24-34.
- [3.8] J. Riba, M.J. Barbanoj. Bringing ayahuasca to the clinical research laboratory. *Journal of Psychoactive Drugs* **2005**, 37, 219-230.
- [3.9] D.J. Moura, M.F. Richter, J.B. Boeira, J.A.P. Henriques, J. Saffi. Antioxidant properties of β -carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis* **2007**, 22, 293-302.
- [3.10] W. Andritzky. Sociopsychotherapeutic functions of ayahuasca healing in Amazonia. *Journal of Psychoactive Drugs* **1989**, 21, 77-89.
- [3.11] J. Mabit, R. Giove, J. Vega. in M. Winkelman and W. Andritzky (Eds.), Yearbook of Cross-Cultural Medicine and Psychotherapy **1996**; VMB Press, Berlin 257.
- [3.12] J.C. Callaway, M.M. Airaksinen, D.J. McKenna, G.S. Brito, C.S. Grob. Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology* **1994**, 116, 385-387.
- [3.13] J. Tiihonen, J.T. Kiukka, K.A. Bergstorm, J. Karhu, H. Viinamaki, J. Lehtonen, T. Hallikainen, J. Yang, P. Hakola. Single-photon emission tomography imaging of monoamine transporters in impulsive violent behavior. *European Journal of Nuclear Medicine* 1997, 24, 1253-1260.
- [3.14] T. Hallikainen, H.M. Saito, J. Lachman, T. Volavka, O.P. Pohjalainen, J. Ryynanen, J. Kauhanenm, E. Syvalahti, J. Hietala, J. Tiihonen. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Molecular Psychiatry* 1999, 4, 385-388.
- [3.15] T. Mantere, E. Tupala, H. Hall, T. Sarkioja, P. Rasanen, K. Bergstorm, J.C. Callaway, J. Tiihonen. Serotonin transporter distribution and density in the cerebral cortex of alcoholic and nonalcoholic comparison subjects: a whole-hemisphere autoradiography study. *American Journal of Psychiatry* 2002, 159, 599-606.
- [3.16] L. Rivier, J.E. Lindgren. "Ayahuasca," the South American hallucinogenic drink: an ethnobotanical and chemical investigation. *Economic Botany* **1972**, 26, 101-129.
- [3.17] Y. Hashimoto, K. Kawanishi. New organic bases from Amazonian *Banisteriopsis caapi*. *Phytochemistry* **1975**, 14, 1633-1635.

- [3.18] Y. Hashimoto, K. Kawanishi. New alkaloids from *Banisteriopsis caapi. Phytochemistry* **1976**, 15, 1559-1560.
- [3.19] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O. Andrade, D.C. Mash. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *Journal of Ethnopharmacology* 1999, 65, 243-256.
- [3.20] J. Riba. Human Pharmacology of Ayahuasca. Doctoral Thesis, Universitat Autonoma de Barcelona. **2003**, <u>http://www.tdx.cesca.es/TDX-0701104-165104/</u>
- [3.21] J. Ott. Pharmahuasca: Human pharmacology of oral DMT plus harmine. Journal of *Psychoactive Drugs* **1999**, 31, 171-177.
- [3.22] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *Journal of Pharmacology and Experimental Therapeutics* 2003, 306, 73-83.
- [3.23] E.H. McIlhenny, K.E. Pipkin, L.J. Standish, H.A. Wechkin, R.J. Strassman, S.A. Barker. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Chromatography A* 2009, 1216, 8960-8968.
- [3.24] B.R. Sitaram, W.R. McLeod. Observations on the metabolism of the psychotomimetic indolealkylamines: Implications for future clinical studies. *Biological Psychiatry* **1990**, 28, 841-848.
- [3.25] J.C. Callawa, L.P. Raymon, W.L. Hearn, D.J. McKenna, C.S. Grob, G.S. Brito, D.C. Mash. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* **1996**, 20, 492-497.
- [3.26] M. Yritia, J. Riba, J. Ortuno, A. Ramirez, A. Castillo, Y. Alfaro, R. De La Torre, M.J. Barbano. Determination of N,N-dimethyltryptamine and beta carboline alkaloids in human plasma following oral administration of Ayahuasca. *Journal of Chromatography B* 2002, 779, 271-281.
- [3.27] J.C. Callaway, G.S. Brito, E.S. Neves. Phytochemical analyses of *Banisteriopsis caapi* and *Psychotria viridis*. *Journal of Psychoactive Drugs* **2005**, 37, 145-150.
- [3.28] G. Frison, D. Favretto, F. Zancanaro, G. Fazzin, S.D. Ferrara. A case of beta-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. *Forensic Science International* **2008**, 179, e37-e43.
- [3.29] K. Bjornstad, O. Beck, A. Helander. A multi-component LC-MS/MS method for the detection of ten plant-derived psychoactive substances in urine. *Journal of Chromatography B* **2009**, 877, 1162-1168.
- [3.30] J. Riba, A. Rodríguez-FornellS, G. Urbano, A. Morte, R. Antonijoan, M. Montero, J.C. Callaway, M.J. Barbanoj. Subjective effects and tolerability of the South American

psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology*(Berlin) **2001**, 154, 85-95.

- [3.31] S.A. Barker, J.A. Monti, S.T. Christian. Metabolism of the hallucinogen N,Ndimethyltryptamine in rat brain homogenates. *Biochemical Pharmacology* **1980**, 29, 1049-1057.
- [3.32] K. Kawanishi, K. Saiki, H. Tomita, Y. Tachibana, N.R. Farnsworth, M. Bohlke. Chemical components of the Brazilian shamanistic drink "Ayahuasca." Advances in *Mass Spectrometry* 1998, 14, D053560/1.
- [3.33] R.G. Santo, J. Landeira-Fernandez, R.J. Strassman, V. Motta, A.P.M. Cruz. Effects of ayhuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. *Journal of Ethnopharmacology* **2007**, 112, 507-513.
- [3.34] G. Gambelungh, K. Aroni, R. Rossi, L. Moretti, M. Bacci. Identification of N,Ndimethyltryptamine and beta-carbolines in psychotropic ayahuasca beverage. *Biomedical Chromatography* **2008**, 22, 1056-1059.
- [3.35] A.P.S. Pire, C.D.R. De Oliveir, S. Moura, F.A. Dorr, W.A.E. Silva, M. Yonamine. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochemical Analysis* **2009**, 20, 149-153.
- [3.36] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry* 2003, 75, 3019-3030.
- [3.37] B.K. Matuszewski. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. *Journal of Chromatography B* **2006**, 830, 293-300.
- [3.38] Food and Drug Administration, Guidance for Industry on Bioanalytical Method Validation, Federal Register 23 May **2001**, 66, 28526.
- [3.39] P.J. Taylor. Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometery. *Clinical Biochemistry* **2005**, 38, 328-334.
- [3.40] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, J.R. Mazzeo. Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. Journal of *Chromatography B* **2007**, 852, 22-34.
- [3.41] T.A. Slotki, V. DiStefano, W.Y.W. Au. Blood levels and urinary excretion of harmine and its metabolites in man and rats. *Journal of Pharmacology and Experimental Therapeutics* **1970**, 173, 26-30.
- [3.42] D.J. Tweedie, M.D. Burke. Metabolism of the beta-carbolines, harmine and harmol, by liver microsomes from phenobarbitone- or 3-methylcholanthrene-treated mice. Identification and quantitation of two novel harmine metabolites. *Drug Metabolism and Disposition* **1987**, 15, 74-81.

- [3.43] A. Yu, J.R. Idle, K.W. Krausz, A. Küpfer, F.J. Gonzalez. Contribution of individual cytochrome P450 isozymes to the O-demethylation of the psychotropic beta-carboline alkaloids harmaline and harmine. *Journal of Pharmacology and Experimental Therapeutics* **2003**, 305, 315-322.
- [3.44] A. Yu. Indolealkylamines: biotransformations and potential drug-drug interactions. *American Association of Pharmaceutical Scientists Journal* **2008**, 10, 242-253.
- [3.45] S.A. Barker, J.A. Monti, S.T. Christian. N,N-Dimethyltryptamine: an endogenous hallucinogen. *International Review of Neurobiology* **1981**, 22, 83-110.
- [3.46] O. Suzuki, Y. Katsumata, M. Oya. Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochemical Pharmacology* 1981, 30, 1353-1358.
- [3.47] B.R. Sitaram, L. Lockett, G.L. Blackman, W.R. McLeod. Urinary excretion of 5methoxy-N,N-dimethyltryptamine, N,N-dimethyltryptamine and their N-oxides in the rat. *Biochemical Pharmacology* **1987a**, 36, 2235-237.
- [3.48] B.R. Sitaram, L. Lockett, R. Talomsin, G.L. Blackman, W.R. McLeod. *In vivo* metabolism of 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine in the rat. *Biochemical Pharmacology* **1987b**, 36, 1509-1512.
- [3.49] B.R. Sitaram, R. Talomsin, G.L. Blackman, W.R. McLeod. Study of metabolism of psychotomimetic indolealkylamines by rat tissue extracts using liquid chromatography. *Biochemical Pharmacology* **1987c**, 36, 1503-1508.
- [3.50] H. Kim, S.O. Sablin, R.R. Ramsay. Inhibition of monoamine oxidase A by beta-carboline derivatives. *Archives of Biochemistry and Biophysics* **1997**, 337, 137-142.
- [3.51] J. Kaplan, L.R. Mandel, R. Stillman, R.W. Walker, W.J.A. VandenHeuvel, J.C. Gillin, R.J. Wyatt. Blood and urine levels of N,N-dimethyltryptamine following administration of psychoactive doses to human subjects. *Psychopharmacologia* 1974, 38, 239-245.
- [3.52] J.C. Gillin, J. Kaplan, R. Stillman, R.J. Wyatt. The psychedelic model of schizophrenia: the case of N,N-dimethyltryptamine. *American Journal of Psychiatry* **1976**, 133, 203-208.
- [3.53] R.J. Strassman, C.R. Qualls, E.H. Uhlenhuth, R. Kellner. Dose-response study of N,Ndimethyltryptamine in humans. II. Subjective effects and preliminary results. *Archives of General Psychiatry* 1994, 51, 98-108.
- [3.54] I. Cohen, W.H. Vogel. Determination and physiological disposition of dimethyltryptamine and diethyltryptamine in rat brain, liver and plasma. *Biochemical Pharmacology* **1972**, 21, 1214-1216.
- [3.55] L.R. Mande, R. Prasad, B. Lopez-Ramos, R.W. Walker. The biosynthesis of dimethyltryptamine *in vivo*. *Research Communications in Chemical Pathology and Pharmacology* **1977**, 16, 47-58.

- [3.56] M.S. Fish, N.M. Johnson, E.P. Lawrence, E.C. Horning. Oxidative N-dealkylation. *Biochemical and Biophysical Acta* **1955**, 18, 564-565.
- [3.57] S. Szara, J. Axelrod. Hydroxylation and N-demethylation of N,N-dimethyltryptamine. *Experientia* **1959**, 15, 216-217.
- [3.58] D. Fontanilla, M. Johannessen, A.R. Hajipour, N.V. Cozzi, M.B. Jackson, A.E. Ruoho. The hallucinogen N,N-dimethyltryptamine is an endogenous Sigma-1 receptor regulator. *Science* 2009, 323, 934-937.
- [3.59] T-P. Su, T. Hayashi, D.P. Vaupel. When the endogenous hallucinogenic trace amine N,N-dimethyltryptamine meets the Sigma-1 receptor. *Science Signaling* **2009**, 2, 1-4.
- [3.60] L.M. Hryhorczuk, J.M. Rainey, C. Frohman, E. Novak. A new metabolic pathway for N,N-dimethyltryptamine. *Biological Psychiatry* **1986**, 21, 84-93.

Chapter 4. Methodology for Determining Major Constituents of Ayahuasca and Their Metabolites in Blood*

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4.1 Introduction

Ayahuasca, also known as hoasca, yage, daime, or vegetal in the pharmacopeia of various South American groups, is a psychotropic plant tea that has a long cultural history of indigenous medical and religious use. The major components of ayahuasca are the hallucinogen *N*,*N*dimethyltryptamine (DMT) and members of the harmala alkaloid family [4.1-4.6].

There is an increasing interest in potential medical applications of ayahuasca [4.7-4.8], including its antioxidant, antimutagenic and antigenotoxic activity [4.9]. In addition, there are suggestions of its putative psychotherapeutic and rehabilitative effects for conditions such as alcoholism, violence, suicidal behaviors, and severe depression, as well as other disorders [4.10-4.17]. Thus, a detailed examination and understanding of the biochemical parameters affected by ayahuasca is needed and will no doubt add to our understanding of this Amazonian medicine. Clinical research assessing the potential medicinal uses for ayahuasca will require information regarding the pharmacokinetics, metabolism, and clearance of ayahuasca's major components. Thus, specific methods for the characterization and quantitation of the major constituents of ayahuasca and their metabolites in blood and urine are needed. In this regard, the authors have recently reported such a method for urine [4.18]. The present research provides a protocol for conducting such analyses in blood.

Historically, a combination of two analytical techniques, one based on high performance liquid chromatography (HPLC) with ultraviolet (UV) and/or fluorescence detection [4.19-4.20] and another, using gas chromatography with nitrogen-phosphorus detection (GC-NPD) [4.19-3.20], have been used for the blood analysis of many of the constituents of ayahuasca following oral administration. The major components analyzed in these studies were *N*,*N*dimethyltryptamine (DMT, 4.1..8 in Table 4.1; GC-NPD) and the harmala alkaloids: harmine (1, Table 4.1), harmaline (4.1.3), and tetrahydroharmine (THH, 4.1.5; HPLC-UV or fluoresence) [4.19-4.20], as well as harmol (4.1.2) and harmalol (4.1.4; HPLC-fluoresence; [4.20]). These methods have been applied to blood samples to examine the pharmacokinetics of these compounds [4.11, 4.19-4.21]. Such analyses have also been used to correlate blood levels of the major components with ayahuasca's effects on subjective and cardiovascular variables as well on monoamine metabolite excretion [4.21].

Other than the obvious complexity created by this approach in using two different methods to obtain data from the same sample, samples analyzed by these methods also required separate isolation of the DMT from blood by liquid-liquid extraction [4.19-4.20], as well as preparation of the samples for HPLC analysis by solid phase extraction [4.20] or protein precipitation/dilution [4.19]. However, recent data [4.6] show that a larger number of compounds in ayahuasca, as well as several previously known and potential metabolites of both DMT and the harmala alkaloids [4.18], also need to be monitored in blood in such studies. Given the increasing interest in ayahuasca and the potential for future clinical research, it was determined that a need existed for newer methods of analysis, an expanded list of compounds of interest, and simpler analytical and analyte isolation procedures.

We report here a single methodology for the direct analysis of the known alkaloid components of ayahuasca as well as several known and potential metabolites of DMT and the harmala alkaloids in blood. The method developed is based on a 96-well plate/protein precipitation/filtration of plasma samples and analysis by HPLC-ion trap-ion trap-mass spectrometry using heated electro-spray ionization to reduce matrix effects. In the present work the list of compounds examined in previous studies has been expanded to include indoleacetic acid (IAA, 4.1.15), DMT-*N*-oxide (DMT-NO, 4.1.10), 5-hydroxy- and 5-methoxy-DMT

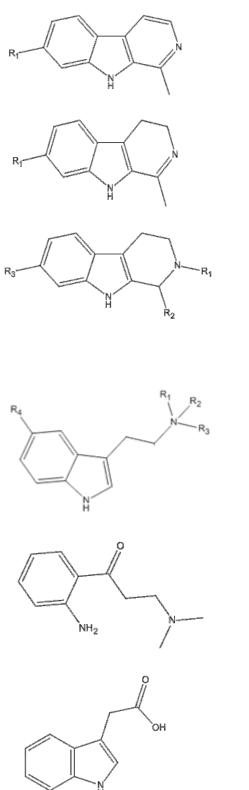
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(OHDMT, 4.1.11, MeODMT, 4.1.12, potential components of ayahuasca itself), *N*,*N*-dimethylkynuramine (DMK, 4.1.14), N-methyltryptamine (NMT, 4.1.9), 2-methyl-tetrahydrobeta-carboline (2-MTHBC, 4.1.6) as well as a recently identified *O*-desmethyl-metabolite of THH, the major harmala component of ayahuasca, 7-hydroxy-THH (THHOH, 4.1.7; [4.22]). As a demonstration of its performance, the method described has also been applied to a small number of blood samples collected from individuals administered ayahuasca. Further, selected blood samples were treated with glucuronidase/sulfatase and analyzed by the same method to determine the presence of glucuronic acid or sulfate conjugates of ayahuasca's major constituents and metabolites.

4.2 Experimental

4.2.1 Standards and reagents

HPLC-grade methanol was purchased from Honeywell Burdick and Jackson (Morristown, New Jersey, USA). HPLC-grade water, high purity formic acid, and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA). *N*-methyltryptamine (NMT), DMT, 5-hydroxy-DMT (5-OH-DMT), 5-methoxy-DMT (5-MeO-DMT), harmine, harmaline hydrochloride dihydrate, harmol hydrochloride dihydrate, harmalol hydrochloride dihydrate, indoleacetic acid (IAA) and *N*,*N*-diethyltryptamine (DET, 4.1.13, Table 4.1, used as an internal standard), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetrahydroharmine was purchased from THC Pharm (Frankfurt am Main, Germany). DMT-NO and 2-MTHBC were prepared as previously described [4.23]. *N*,*N*-Dimethylkynuramine (DMK) was a gift from Dr. Laurent Micouin, Laboratoire de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France. An authentic reference standard of 7-hydroxy-tetrahydroharmine (THHOH) was a gift from THC Pharm (Frankfurt am Main, Germany).

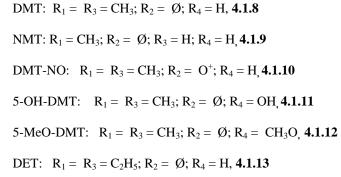


Harmine: $R_1 = CH_3O$, **4.1.1** Harmol: $R_1 = OH$, **4.1.2**

Harmaline: $R_1 = CH_3O$, **4.1.3**

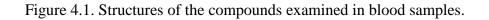
Harmalol:
$$R_1 = OH, 4.1.4$$

THH: $R_1 = H$; $R_2 = CH_3$; $R_3 = CH_3O$, **4.1.5** 2-MTHBC: $R_1 = CH_3$; $R_2 = R_3 = H$, **4.1.6** THHOH: $R_1 = H$; $R_2 = CH_3$; $R_3 = OH$ **4.1.7**



DMK: **4.1.14**

IAA: **4.1.15**



Stock standard solutions (1 mg/ml) of the 14 selected compounds and of the internal standard (DET) were prepared individually in methanol in 10 ml amber glass vials with Teflonlined screw-cap closures and stored in a freezer at -20° C. Working mixed standards were prepared in methanol at selected concentrations (0, 1, 2.5, 5, 10, 25, 50, 100, 250 500 ng/ml) by serial dilution and pipetted into 5 ml conical tubes. The methanol was removed by gentle evaporation over dry nitrogen and the residues were dissolved in amounts of 97% H₂O/0.1% formic acid: 3% acetonitrile/0.1% formic acid mobile-phase (MP) solution corresponding to the appropriate sample concentrations desired.

4.2.2 Freeze Dried Ayahuasca Administration and Plasma Collection

Ayahuasca was prepared as an encapsulated lyophilizate obtained from a previously analyzed, freeze-dried and homogenized 10 L batch. One gram of freeze-dried material contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg THH. A dose of 1.0 mg DMT/kg body weight was chosen based on previous ayahuasca dosing studies [4.8, 4.21, 4.24, 4.25].

Subjects were 3 healthy male volunteers with previous experience with psychedelic drugs. The study was approved by the local ethics committee (Hospital de Sant Pau, Barcelona) and the Spanish Ministry of Health. Signed informed consent was obtained from all participants. After oral administration of 1mg/kg DMT blood was collected in10 ml EDTA tubes and centrifuged at 2000 rpm for 10 min at 4 °C and the resulting plasma immediately frozen at -20 °C. The frozen plasma samples were stored at -80 °C until analysis. The following time points relative to ayahuasca administration were collected for pilot analyses: Basal (pre-dose), 1.5 h, 4.5

h and 10 h. Samples were shipped and received on dry ice and underwent a single freeze-thaw cycle prior to analysis.

4.2.3 Sample preparation

Protein precipitation 96-well plates (Thermo Scientific, Waltham, MA, USA) were used to prepare the samples. Basal plasma was used for negative controls and for spiking to generate standard curves. Standard curve data points at 7 selected concentrations (0, 1, 2.5, 5, 10, 25, 50 ng/ml; IAA was examined at 0, 10, 25, 50 100, 250 and 500 ng/ml) specific to the expected concentration range for each compound (determined from preliminary analyses) were prepared in blank plasma and used to examine linearity of response, to collect data for the quantification of samples, and to determine method performance. Spiked MP which did not go through the protein precipitation process was also prepared and was used to examine matrix effects and to determine relative analyte recovery.

Two sets of 200 µl mixed standards in MeOH were dried completely under nitrogen and brought up with 200 µl of MP or 200 µl of blank plasma. To each well of a 96-well protein precipitation plate were added 580 µl ACN, 20 µl of 1.0 µg/ml DET (20 ng; IS) in MeOH and either 200 µl spiked MP (MP curve), 200 µl MP (Blank with IS), 200 µl spiked plasma (recovery curve), 200 µl blank plasma (matrix effects curve), or 200 µl of ayahuasca administration plasma sample. The plate was shaken for 3 minutes using a microplate genie (Scientific Industries, Bohemia, NY, USA) and then placed in a -40 °C freezer for 30 minutes. The protein precipitation plate was then placed on a Porvair Sciences (Leatherhead, UK) vacuum manifold with an Agilent Technologies (Santa Clara, CA, USA) 96 deep-well receiver plate placed underneath to collect the filtrate. Minimum vacuum was applied for approximately 3 minutes. The filtrate was shaken for 3 minutes and transferred to a new 96-well protein precipitation plate which was again placed in a -40° C freezer for another 30 minutes. The protein precipitation plate was then placed back onto the vacuum manifold with minimum suction for 3 minutes and filtrate was collected in a new Agilent 96 deep well plate which was shaken for 3 minutes and then dried completely using a SPE Dry 96 Dual plate dyer (Argonaut, Mid Glamorgan, UK). All wells were brought up to 200 µl with MP. Thus, there was no relative dilution of the original sample.

4.2.4 Determination of Matrix Effects

Matrix effects were examined using four sets of blank plasma that were processed through the protein precipitation protocol and then fortified with 0, 10, 25, 50, 100, 250, and 500 ng/ml of the 15 analytes, and samples of similarly spiked MP (n = 3) which were not processed through the protocol. The resulting samples were analyzed as described with 20 µl being injected for analysis. The data were examined to determine the slopes of the curves so generated. The slopes were compared (plasma samples to one another and plasma samples to MP samples) in order to determine the percent relative standard deviation (%RSD) of the slopes of extracted/fortified plasma samples with the slopes for the MP spiked samples for each compound.

4.2.5 Enzyme Hydrolysis

A 1.0 M sodium acetate buffer at pH 5.0 was prepared by dissolving 82 g of anhydrous sodium acetate with 967 mL HPLC water and 33 ml of glacial acetic acid. Glucuronidase/ sulfatase buffer was prepared by dissolving 1 bottle (2 million units) of βglucuronidase/sulfatase from limpets (*Patella vulgata*) Type L-II (Sigma-Aldrich, St. Louis, MO, USA) in 400 ml of distilled water. Acetate buffer (1200 ml) was then mixed with 400 ml glucuronidase/sulfatase

buffer. Glucuronidase/sulfatase /acetate buffer mix (120 μ l) was then added to each tube containing (100 μ l) of individual plasma as well as 10 μ l of 1.0 μ g/ml DET in MP. Standard curves were similarly treated and prepared using 90 μ l of spiked blank plasma. The samples, blanks, and standards were vortexed and incubated for 1 hour in a 65° C shaking water bath, then allowed to cool to room temperature. Samples were then treated as described above for protein precipitation. The dilution caused by the hydrolysis process was accounted for by using 680 μ l ACN plus 320 μ l of hydrolysis mixture and bringing the final volume up to only 100 μ l MP (no dilution of original sample).

4.2.6 LC-HESI-MS/MS analysis

The LC/MS/MS analyses were based, in part, on direct methods of analysis we have previously described for ayahuasca itself and for ayahuasca's major constituents [4.16], as well as for their metabolites in urine [4.18], with modifications. In the present study the compounds IAA and THHOH have been added to the analyses (as compared to [4.18]) and the internal standard used in the previous studies was changed from a deuterated 5-methoxy-DMT to DET. Furthermore, the LC mobile phase/separation system and the mass spectrometer were changed as was the sample preparation step.

Thus, analyses were conducted using a Thermo Open Autosampler and a Thermo Accela pumping system interfaced to a Thermo Velos linear ion trap-ion trap system equipped with a heated electrospray ionization (HESI) probe and operated in the positive ion mode. Chromatographic separation was achieved on a 1.8 μ m 4.6 x 50 mm (i.d.) 600 bar Agilent ZORBAX Eclipse Plus C₁₈ rapid resolution HT threaded column with an Alltech Direct-Connect Column 2 μ m pre-filter (Deerfield, IL, USA) using gradient elution. The following gradient

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system was used as the mobile phase: A (0.1% formic acid in H₂0) and a mobile phase B (0.1% formic acid in acetonitrile) delivered at a constant flow rate of 0.3 ml/min throughout the analysis; A:B 97:3 (0 min)- 50:50 (14 min)- 2:98 (16 min)- 2:98 (18 min)- 97:3 (21 min)- 97:3 (25 min), allowing for re-equilibration. The MS/MS analysis was performed using selected reaction monitoring (SRM) of the protonated molecular ions for the analytes and scanning the target ions in seven (7) different segments (Table 4.1). The heated ESI source temperature was 300° C, sheath gas pressure was 25 psi, the auxillary gas pressure was 7 psi, the spray voltage (kV) was 5, the capillary temperature was 275° C and the S-lens RF level was 35%. The collision pressure was 1.5 psi of high purity argon. Data for the molecular ions and energies utilized to generate diagnostic fragment ions are shown in Table 4.1.

Detection data were collected and integration of chromatographic peaks was performed by Xcalibur 2.0.7 Thermo Fisher Scientific (Waltham, MA, USA) LCquan 2.5.6 QF 30115 software.

Identification of the compounds was based on the presence of the molecular ion at the correct retention time (+/- 1%), the presence of at least two transition ions, and the correct ratio of these ions to one another (+/- 25% relative).

Table 4.1: Mass spectrometric parameters for the analytes and internal standard (IS) (CE = collision energy in volts, v; MW= molecular weight; Product ion 1 was used for quantitation in every case). (Segment): Runtime in minutes (1): 5.35 (2):0.50 (3): 0.90 (4): 1.60 (5): 0.90 (6): 1.80 (7): 13.95

Analytes	Scan Segme nt	MW	[M + H]+	Product Ion 1 (Scan Range)	CE 1 (v)	Product Ion 2 (Scan Range)	CE 2 (v)	Product Ion 3 (Scan Range)	CE 3 (v)
5-OH-DMT	1	204	205	160.0 (159-161)	30	134.0 (133-135)	30		
ТННОН	2	202	203	174.0 (173-175)	30	186.0 (185-187)	30	160.0 (159-161)	30
DMK	3	192	193	58.0 (57-59)	30	148.0 (147-149)	30		
HARMALOL	4	200	201	160.0 (159-161)	35	184.0 (183-185)	35	68.0 (67-69)	35
NMT	4	174	175	144.0 (143-145)	25	132 (131-133)	25		
DMT	4	188	189	58.0 (57-59)	38	144.0 (143-145)	38		
5-MeO-DMT	4 & 5	218	219	174.0 (173-175)	25	148.0 (147-149)	25		
2-MTHBC	4 & 5	186	187	144.0 (143-145)	25	158.0 (157-159)	25		
DMT-NO	5&6	204	205	144.0 (143-145)	42	160.0 (159-161)	42		
ТНН	5	216	217	188.0 (187-189)	25	200.0 (199-201)	25	174.0 (173-175)	25
DET (IS)	5 & 6	216	217	86.0 (85-87)	25	144.0 (143-145)	25	74.0 (73-75)	25
HARMOL	4 & 6	198	199	171.0 (170-172)	40	157.0 (156-158)	40	181.0 (180-182)	40
HARMALINE	6	214	215	174.0 (173-175)	35	200.0 (199-201)	35	215.0 (214-216)	35
HARMINE	6	212	213	198.0 (197-199)	35	213.0 (212-215)	35	185.0 (184-186)	35
IAA	7	175	176	130 (129-131)	34	158.0 (157-159)	34		

4.2.7 Calculations

The concentration of compounds in blood samples was determined from the ratio of the peak area of the target analyte to that of the internal standard (DET), by reference to calibration curves prepared by spiking blank plasma samples with each of the 14 substances plus a consistent amount of internal standard. The transition ions used for quantitation are shown in Table 4.1. If the concentration observed for the actual administration samples tested was outside the measured range, the samples were re-analyzed after further dilution with mobile phase and the original concentration calculated by extrapolation. Values determined from repeated analyses of n aliquots of samples or standards were expressed as their arithmetic mean. For the standards the percent relative standard deviation (%RSD) was calculated, as was the method bias, as a function of concentration. Standard deviations were calculated for n determinations of samples as noted. Inter- and intra-assay %RSDs were also determined. Limits of detection (LOD; concentration response greater than 3 times baseline noise) and limits of quantification (LOQ: the lowest concentration having a proven %RSD of less than 15%) were also determined for each compound.

To examine matrix effects, slopes were calculated for standard curves generated from fortifying 4 pre-extracted plasma samples and 4 water samples as described. The slope of individual plasma samples were compared to each other and the %RSD was determined. The mean of these slopes was also compared to the mean of spiked water curves and the %RSD was calculated for each compound.

4.3 Results and Discussion

4.3.1 Method Performance

Each of the 15 compounds gave high yields of molecular ions $(M+H)^+$ using the mobile phase and heated electrospray ionization parameters described. However, for many of the compounds the yield of a third product ion was less than 5% of the base peak and was not sufficient to be included in the identification criteria (Table 4.1). However, the criteria for identification were sufficiently rigorous [correct retention time, molecular ion plus two transitions, and an acceptable ratio of ions (+/- 25% absolute)] to provide high confidence in the data.

Using the LC/HESI/MS/MS multi-component method described, almost all of the 15 compounds examined were separated temporally (Figure 4.2A). All of the compounds were, nonetheless, completely separated by mass. A thorough examination of ion contribution between each of the compounds and the masses monitored indicated that, even at the highest concentrations, no measurable interference ("cross-talk") occurred. The LC method gave excellent peak shapes and consistency of retention time for the 15 compounds despite their rather diverse chemical character. Furthermore, no potentially interfering naturally occurring substances from plasma with the same masses and retention times were observed, in either the method blanks or basal samples collected from subjects. Similarly, enzyme treatment did not produce any new compounds that interfered with the analysis. Although this is a limited examination, it appears that the necessary specificity for the assay can be met, especially when additional parameters of ion fragmentation and ratios are also applied. A representative chromatogram for 50 ng/ml standards spiked in blank plasma is shown in Figure 4.2A, and a

representative chromatogram for a blank plasma sample containing only the internal standard (DET) is shown in Figure 4.2B. A representative chromatogram from the analysis of a volunteer's sample is shown in Figure 4.2C.

Data for the retention time of each compound and their reproducibility are presented in Table 4.2. The calibration curves for each compound were linear over the range of concentrations examined (1-50 ng/ml for all compounds except IAA which was 10-500 ng/ml). The linear regression equations, coefficients of correlation, and the performance parameters for the method (% bias, %RSD, LOD, LOQ, intra- and inter-assay variation) for the 14 compounds using DET as the internal standard are also presented in Table 4.2. Calculations of method bias and %RSDs show that performing the minimal sample manipulations required in conducting protein precipitation/filtration result in acceptable accuracy and precision for quantitation, as well as the appropriateness of DET as the internal standard for all of the compounds tested. The modest inter- and intra-assay variations observed also reflect the method's reliability and reproducibility for analysis of human blood samples after ayahuasca administration. Similarly, the limits of detection observed are more than adequate. Proven limits of quantitation (% bias less than 15%) at 1.0 ng/ml were met for all of the 14 analytes.

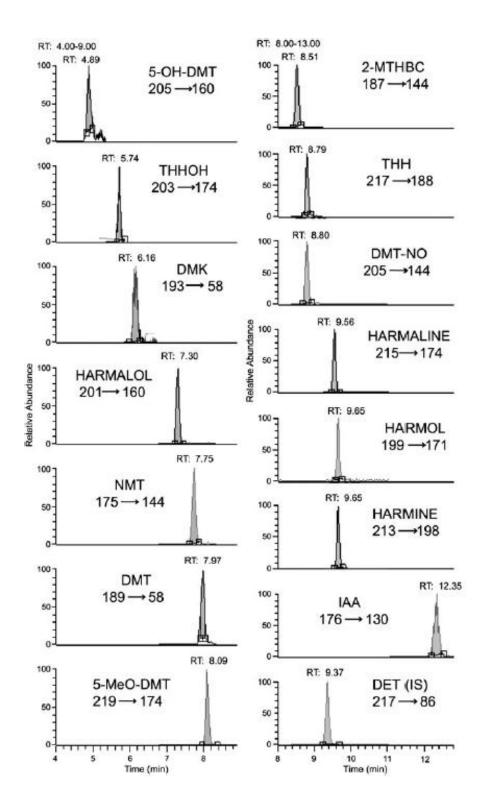


Figure 4.2A. Representative chromatogram of reference standards spiked into blank plasma illustrating retention times and the molecular ion and product ion monitored (50 ng/ml of each; RT= retention time in minutes).

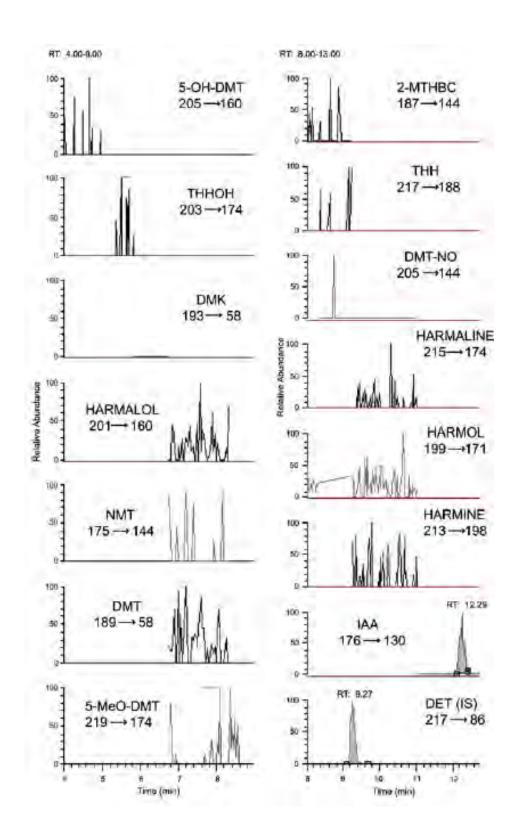


Figure 4.2B. Representative chromatogram of a blank plasma sample (Basal) obtained preayahuasca administration.

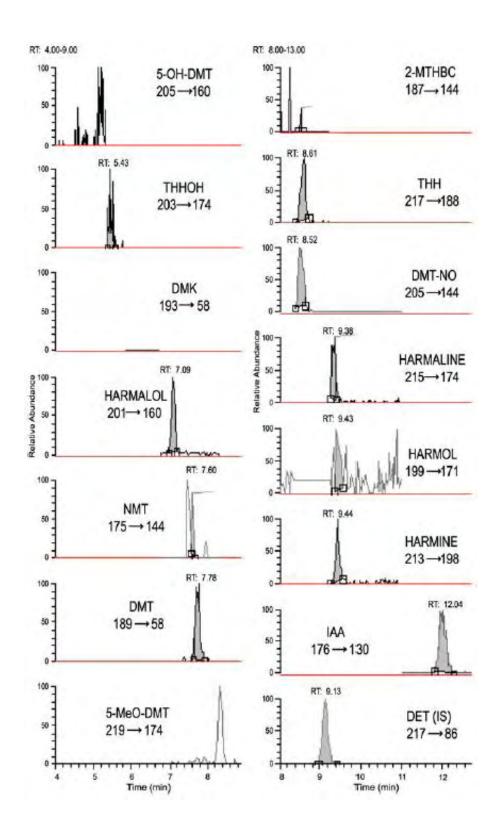


Figure 4.2C. Representative chromatogram from a plasma sample obtained 1.5 hours after ayahuasca administration.

Molecule	Target	Observed	%Bias			LOD (N=7)			Mean R ²	Inter-		Mean %
	concentratio (ng/mL)	n PPT concentratio		(N = 7)		(S/N = 3; ng/mL)	LOQ (%	slope (V = 7)	(N=7)	assay %RSD	assay %RSD	(N=8)
	(ng/mL)	mean (N=7			(4-7)	ng/mg	(% RSD ≤15)				(N=5)	(PPTR/MP)
	(66.6)	(ng/mL)	,							(1-5)	(*100 ± SD
5-OH-	1.00	1.17	14.25	10.34	4.88	0.37	1.00	0.00035	0.9990	12.55	7.24	72.21 ± 10.10
DMT	2.50	2.44	-2.27	12.77	4.90					11.99	11.44	
	5.00	4.75	-5.33	10.70	4.92					8.12	11.02	
	10.00	10.09	0.91	4.11	4.87					3.13	4.04	
	25.00	25.06	0.25	2.66	4.85					2.73	2.87	
тннон	50.00 1.00	49.97 1.13	-0.07 11.68	0.74 15.41	4.90 5.58	0.39	1.00	0.00015	0.9993	0.73	0.74	71.96±10.92
INNON	2.50	2.53	1.23	10.36	5.59	0.59	1.00	0.00015	0.9995	9.90	10.53	71.90 ± 10.92
	5.00	4.49	-11.46	5.71	5.60					6.85	5.71	
	10.00	10.24	2.35	2.26	5.61					2.61	2.08	
	25.00	25.17	0.67	3.15	5.60					3.72	3.43	
	50.00	49.86	-0.27	0.87	5.66					0.99	0.87	
DMK	1.00	1.16	13.67	13.36	6.05	0.41	1.00	0.00004	0.9986	13.38		102.23 ± 14.28
	2.50	2.58	2.93	9.73	6.05					11.52	9.57	
	5.00	4.64	-7.81	11.05	6.06					13.28	9.19	
	10.00	10.12	1.14	5.21	6.07					5.71	5.30	
	25.00 50.00	25.05 49.98	0.19	2.29	6.05 6.11					2,44 0.58	2.47 0.57	
Harmalol	1.00	1.18	15.25	10.49	7.20	0.38	1.00	0.001.99	0.9994	10.49	9.28	66.54 ± 8.25
narmaior	2.50	2.60	3.78	9.54	7.21	0.50	1.00	0.00133	0.3334	7.88	10.24	00.0410.20
	5.00	4.89	-2.19	4.52	7.18					4.87	4.82	
	10.00	10.07	0.72	6.09	7.21					5.41	6.67	
	25.00	24.89	-0.45	1.61	7.20					1.15	1.75	
	50.00	50.07	0.13	0.32	7.26					0.17	0.32	
NMT	1.00	1.01	1.11	7.82	7.65	0.32	1.00	0.00198	0.9993	9.18	7.82	59.40 ± 6.00
	2.50	2.57	2.67	7.65	7.63					3.09	8.10	
	5.00 10.00	4.92 9.87	-1.64 -1.36	10.69	7.62 7.63					12,71 1.57	8.46 2.05	
	25.00	25.06	0.24	5.91	7.62					7.19	6.46	
	50.00	48.71	-2.64	4.47	7.69					4.76	4.47	
DMT	1.00	1.15	12.82	14.59	7.92	0.45	1.00	0.00148	0.9995	13.12	14.85	60.28 ± 7.17
	2.50	2.54	1.50	6.83	7.86					6.16	7.47	
	5.00	4.99	-0.24	7.67	7.84					8.53	8.34	
	10.00	9.85	-1.57	3.89	7.86					4.48	3.97	
	25.00	25.32	1.25	3.62	7.84					4.12	3.96	
	50.00	49.82	-0.36	0.98	7.91					1.01	0.98	
5-MeO- DMT	1.00 2.50	0.97	-3.17	14.22	8.00 7.98	0.09	1.00	0.00710	0.9982	14.09 5.98	13.25 6.97	65.75±8.18
DMI	5.00	4.49	-11.26	12.04	7.96					4.84	13.15	
	10.00	10.63	5.93	3.83	7.99					4.44	2.47	
	25.00	25.08	0.32	1.37	7.98					1.43	1.17	
	50.00	49.84	-0.33	0.23	8.04					0.26	0.23	
2-MTHBC	1.00	0.97	-2.78	14.48	8.44	0.33	1.00	0.00207	0.9986	15.90	20.25	64.21 ± 7.52
	2.50	2.61	4.17	8.09	8.40					5.12	8.85	
	5.00	4.72	-6.02	8.73	8.40					9.29	9.53	
	10.00	10.48	4.57	4.15	8.42					4.60	4.49	
	25.00	24.82	-0.72	2.41	8.42					2.29	2.64	
DMT-NO	50.00 1.00	50.03 1.06	0.05	0.57	8.48 8.68	0.25	1.00	0.00212	0.9990	0.45 12.48	0.57	87.61 ± 9.24
Din NO	2.50	2.55	2.12	5.76	8.66	0.20	1.00	0.00212	0.3330	7.03	6.09	07.01 2 7.24
	5.00	4.79	-4.46	10.35	8.64							
	10.00	10.20	1.99	4.23	8.67					4.60	2.75	
	25.00	24.85	-0.60	2.08	8.65					2.51	2.26	
	50.00	50.07	0.14	0.51	8.73					0.59	0.51	

Table 4.2 Method Perform Parameters

Table 4.2 Continued

Molecule c	Target concentration (ng/mL) (ppb)	Observed PPT concentratio mean (N=7 (ng/mL)	n		(min)	LOD (N =7) (S/N = 3; ng/mL)	Proven LOQ (% RSD ≤15)	Mean slope (N=7)	Mean R ² (N = 7)	assay	assay %RSD	Mean % recovery (N=8) (PPTR/MP) *100±SD
THH	1.00	1.09	7.93	15.41	8.70	0.36	1.00	0.00147	0.9992	15.38	15.57	69.88±5.43
	2.50	2.57	2.69	6.29	8.70					4.65	6.03	
	5.00	4.68	-6.83	4.70	8.68					5.34	5.15	
	10.00	10.06	0.63	4.27	8.69					4.58	4.67	
	25.00	24,71	-1.17	3.44	8.70					3.85	3.72	
	50.00	48.92	-2.21	4.99	8.74					5.61	4.99	
Harmol	1.00	1.08	7.55	10.15	9.51	0.30	1.00	0.00037	0.9992	12.16	9.31	76.31 ± 7.78
	2.50	2.42	-3.31	3.55	9.57					3.94	3.87	
	5.00	4.95	-1.04	9.28	9.54					8.50	9.69	
	10.00	9.93	-0.73	5.99	9.55					6.19	6.56	
	25.00	25.13	0.52	3.32	9.55					2.34	3.63	
	50.00	49.94	-0.12	0.83	9.61					0.49	0.83	
Harmaline	1.00	1.05	4.75	13.70	9.46	0.22	1.00	0.00231	0.9986		8.60	64.89 ± 7.82
	2.50	2.67	6.31	11.85	9.45					11.49	12.98	
	5.00	4.98	-0.39	7.36	9.46					7.99	7.72	
	10.00	10.01	0.08	4.63	9.47					3.29	5.03	
	25.00	24.08	-3.83	6.43	9.47					7.43	4.79	
	50.00	50.28	0.56	1.02	9.51					1.13	1.02	
Harmine	1.00	1.07	6.61	15.91	9.57	0.25	1.00	0.00251	0.9990		15.81	74.75 ± 10.31
	2.50	2.65	5.78	8.77	9.62					10.06	9.56	
	5.00	4.66	-7.30	9.51	9.54					10.42	7.97	
	10.00	10.50	4.78	3.73	9.55					4.01	2.75	
	25.00	24.26	-3.07	3.84	9.56					4.38	2.67	
	50.00	50.18	0.36	0.67	9.60					0.77	0.67	
IAA	10.00	18.30	45.36		12.20			0.00027	0.9896	2.39	4.45	
	25.00	31.20	19.87		12.20					2.33	10.25	
	50.00	42.90			12.21					0.71	1.60	
	100.00	94.30	-6.04		12.19					0.57	0.99	
	250.00	247.80	-0.89		12.19					0.56	0.71	
DET (IS)	500.00	504.10	0.81	0.20	12.26					0.13	0.20	65 00 + 4 40
DET (IS)					9.30							65.08 ± 4.48

LOD, limit of detection; LOQ, limit of quantitation; %RSD, percentage relative standard deviation; RT, retention time in minutes; S/N, signal-to-noise ratio; MP, mobile phase; PPT, protein precipitation; PPTR, PPT recovery; IS, internal standard; SD, standard deviation. 5-OH-DMT, 5-hydroxy-DMT; THHOH, 7-hydroxy-tetrahydroharmine; DMK, *N*,*N*-dimethylkynuramine; NMT, *N*-methyltryptamine; DMT, *N*,*N*-dimethyltryptamine; 5-MeO-DMT, 5-methoxy-DMT; 2-MTHBC, 2-methyl-tetrahydro-beta-carboline; DMT-NO, DMT-*N*oxide; THH, tetrahydroharmine; IAA, indoleacetic acid; DET, *N*,*N*-dimethyltryptamine.

Absolute recovery of the individual analytes was also examined (Table 4.2) and ranged from a mean of 59-102% (n = 8) for these 15 chemically diverse compounds compared to responses of equal concentrations spiked into MP and analyzed directly. Much of the loss of analytes appears to occur during the protein precipitation/filtration steps since the remaining procedures involve little more than pipetting, diluting, or drying without transfers. Because of the natural occurrence of IAA in all plasma samples, an absolute recovery could not be accurately calculated but its response was observed to be linear with concentration (Table 4.2). Correction for endogenous IAA suggests that its recovery is greater than 90%.

The method described has distinct advantages over previous methods used for determination of ayahuasca's major components in blood in that a greater number of relevant compounds can be monitored, only a single analysis is required, and sample manipulation is minimal.

4.3.2 Matrix Effects

Matrix effects were assessed by comparing the linear regression line slopes of standards spiked into MP over a range of concentrations versus standards similarly spiked into 4 blank plasma samples after being processed through the protein precipitation protocol (n = 4 for each) and by comparing the slopes for the compounds spiked into extracted plasmas from individuals to each other [4.26, 4.27]. Table 4.3 shows the %RSD for the slopes of standards spiked into preextracted plasma and water. Comparing the slopes of the 4 plasmas to each other, a %RSD of 15% or less was observed for all compounds. This suggests that little variability will be observed in conducting patient-to-patient plasma analyses due to matrix effects and that standard curves derived from spiking blank plasma will likewise provide appropriate results. Comparing the slopes for the compounds spiked into extracted plasma to the slopes for the same compounds spiked into water, a %RSD of less than 15% was also obtained for all compounds except for DMK. This suggests that some matrix effects occur for this compound but, in terms of attaining accurate quantitation, they may be overcome by fortification into blank plasma. There was little variability or deviation from linearity for standards spiked in water or in different plasma matrices. Reference standards spiked into extracted plasma showed no deviation from a linear

response, and the accuracy of the results was readily reproduced over a broad range of concentrations (Table 4.3). Similarly, shifts in retention times were not evident, regardless of the relative concentrations of the standards (Table 4.1). Thus, the use of a HESI probe, which has been shown to greatly diminish matrix effects [4.26-4.29], as well as the overall methodology, provides an accurate, sensitive and specific analysis, apparently unaffected by co-eluting or other interferences.

Table 4.3. Matrix effects. The slopes of the individual plasma samples, pre-extracted and fortified with analytes, were compared to each other and to fortified mobile phase (MP).

Analyte	MP average slope	MP average R ²	Plasma 1 slope	R ²	Plasma 2 slope	R ²	Plasma 3 slope	R ²	Plasma 4 slope	R ²	Average plasma slope	Plasma %RSD	Plasma vs MP %RSD
	N=3		N = 2		N = 2		N=2		N=2		N=8		
5-OH-DMT	0.00035	0.9965	0.00037	0.9984	0.00030	0.9962	0.00037	0.9985	0.00031	0.9979	0.00034	10.9509	9.5311
THHOH	0.00016	0.9952	0.00022	0.9999	0.00020	0.9994	0.00023	0.9999	0.00019	0.9986	0.00021	9.2847	14.7156
DMK	0.00011	0.9955	0.00006	0.9973	0.00006	0.9984	0.00006	0.9995	0.00008	0.9954	0.00006	14.7295	31.4694
Harmalol	0.00178	0.9971	0.00217	0.9999	0.00193	0.9992	0.00217	0.9999	0.00185	0.9987	0.00203	8.1646	9.1821
NMT	0.00223	0.9990	0.00271	0.9998	0.00228	0.9993	0.00268	0.9994	0.00234	0.9994	0.00250	8.9529	9.3600
DMT	0.00123	0.9990	0.00152	0.9999	0.00134	0.9989	0.00148	1.0000	0.00136	0.9974	0.00143	6.1587	8.3854
5-MeO-DMT	0.00448	0.9987	0.00563	0.9994	0.00451	0.9991	0.00529	0.9995	0.00451	0.9977	0.00499	11.3614	11.0433
2-MTHBC	0.00105	0.9992	0.00106	0.9994	0.00109	0.9999	0.00123	1.0000	0.00109	0.9989	0.00112	6.8423	6.5545
DMT-NO	0.00102	0.9977	0.00145	1.0000	0.00113	0.9981	0.00138	0.9993	0.00112	0.9987	0.00127	13.3219	14.9302
THH	0.00103	0.9984	0.00131	0.9984	0.00108	0.9994	0.00127	0.9997	0.00106	0.9998	0.00118	10.9758	11,4411
Harmaline	0.00285	0.9994	0.00298	0.9994	0.00286	0.9990	0.00327	0.9999	0.00292	0.9998	0.00301	5.9734	5.7292
Harmine	0.00243	0.9990	0.00265	0.9999	0.00252	0.9992	0.00285	0.9995	0.00250	0.9991	0.00263	6.1200	6.4415
Harmol	0.00033	0.9988	0.00037	0.9998	0.00034	0.9982	0.00039	0.9994	0.00035	0.9989	0.00036	6.5323	7.3124
IAA	0.00004	0.9944	0.00004	0.9984	0.00004	0.9945	0.00004	0.9968	0.00004	0.9986	0.00004	2.9991	4.0907

5-OH-DMT, 5-hydroxy-DMT; THHOH, 7-hydroxy-tetrahydroharmine; DMK, N,N-dimethylkynuramine; NMT, N-methylkryptamine; DMT, N,N-dimethylkynuramine; S-MeO-DMT, 5-methoxy-DMT; 2-MTHBC, 2-methyl-tetrahydro-beta-carboline; DMT-NO, DMT-N-oxide; THH, tetrahydroharmine; IAA, indoleacetic acid.

Table 4.4. Mean (n = 6) concentrations (ng/ml) of ayahuasca components and metabolites detected in plasma from three (3) volunteers.

Mean ($V = 6$) concentration or compounds in plasma from three volunteers (V13, V20, V22), ng/mL													
Collection hour	DMT	DMT-NO	THH	THHOH	Harmine	Harmol	Harmaline	Harmalol	2-MTHBC	IAA			
Basal (0)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.27			
1.5	15.09	45.18	37.63	2.50	5.18	5.55	3.96	1.74	0.48	207.80			
4.5	3.12	12.89	55.44	2.94	3.11	3.01	4.53	3.27	ND	197.55			
10	ND	0.25	25.91	0.96	0.54	0.32	2.23	2.96	ND	66.27			

Mean (N=6) concentration of compounds in plasma from three volunteers (V13, V20, V22), ng/mL

DMT, N,N-dimethyltryptamine; DMT-NO, DMT-N-oxide; THH, tetrahydroharmine; THHOH, 7-hydroxy-tetrahydroharmine; 2-MTHBC, 2-methyl-tetrahydro-beta-carboline; IAA, indoleacetic acid.

4.3.3 Analysis of Administration Samples

Representative chromatograms for the detection and quantitation of the ayahuasca alkaloids and metabolites present in plasma samples collected before and after administration are presented in Figures 4.2(B and C), respectively. Tabulation of the concentrations of these compounds in samples from three individuals is shown in Table 4.4. Enzyme treatment of a selected volunteer's samples did not show any significant increase in any of the major alkaloids or their metabolites (data not shown). Considering that conjugates have been observed in urine [4.22], their absence in plasma suggests that they are rapidly excreted.

In the three individuals' samples examined, DMT concentrations were observed to be lower at all time points than the major DMT metabolite recently identified in human urine [4.18], DMT-NO. This metabolite was observed to be present in the plasma at 3-to-4 times the concentration of DMT itself, peaking at 1.5 hrs after ayahuasca administration at approximately 45 ng/ml. The formation of the *N*-oxide appears to occur rapidly after ayahuasca administration. Enzymes responsible for this conversion may be *N*-oxidases in blood as well as liver or kidney CYPs (cytochrome P450 superfamily) [4.23, 4.30-4.34]. Others have reported DMT-NO to be a major metabolite of DMT in rat plasma and urine [4.31-4.34]. The present study is the first report of its presence in human blood following ayahuasca or DMT administration. In addition, inhibition of MAO-A in rats, such as occurs with the harmala alkaloids in ayahuasca [4.35], shifts the normal metabolism of DMT to IAA to the formation of the *N*-oxide *in vitro* and *in vivo* [4.31-4.34]. It may be assumed that a similar mechanism is at work following ayahuasca administration to humans. Nonetheless, rather modest levels of DMT continue to be observed and are consistent with previous studies of ayahuasca pharmacokinetics [4.11, 4.19-4.21].

For the major harmala components, THH was observed to peak in the 4.5 hr samples with concentrations greater than 55 ng/ml. THH is the major harmala component of ayahuasca and it is also the major harmala excretion product in urine in man [4.6, 4.18]. Harmalol and harmol as well as THHOH, harmaline, harmine, and 2-MTHBC were also detected in most samples. 5-OH-DMT, a potential component of some ayahuasca preparations, and NMT, a known demethylation metabolite of DMT, were randomly detected and no 5-MeO-DMT, another potential component of ayahuasca, was observed. The compound DMK, a DMT metabolite reported to be formed in human blood *in vitro* [4.36], was not detected. This was also the case in our examination of urine samples collected from humans administered ayahuasca [4.18], and suggests that, while this compound was identified in *in vitro* studies, apparently it is not a metabolite of DMT *in vivo* in man.

Despite the MAO inhibiting effect of the harmala alkaloids [4.25, 4.35, 4.37], significant increases in the plasma levels of IAA were observed following ayahuasca administration. The reason for this observation is, at present, unclear. However, the data suggest that, to the degree that MAO inhibition and a resultant shift to *N*-oxide formation occur, a significant quantity of the administered DMT may still be converted to IAA, apparently via incomplete inhibition of MAO-A.

4.4 Conclusions

The present method expands the list of compounds capable of being monitored in blood following ayahuasca administration in humans while providing a simplified approach to their analysis. The characteristics of the method suggest that its sensitivity, specificity, and reproducibility are adequate for future clinical research with ayahuasca. The results also show for the first time that the major DMT urinary metabolite, DMT-*N*-oxide, is also a major circulating product in the blood following ayahuasca administration. Thus, the method and data provide the most complete profile of DMT, harmala alkaloids, and their respective metabolite concentrations in the blood following ayahuasca administration to date.

4.5 References

- [4.1] L. Rivier and J.E. Lindgren. "Ayahuasca," the South American hallucinogenic drink: an ethnobotanical and chemical investigation. *Economic Botany* **1972**, 26, 101-129.
- [4.2] J.C. Callaway. Various alkaloid profiles in decoctions of *Banisteriopsis caapi*. Journal of *Psychoactive Drugs* **2005**, 37, 1-5.
- [4.3] J.C. Callaway, G.S. Brito and E.S Neves. Phytochemical analyses of *Banisteriopsis caapi* and *Psychotria viridis*. *Journal of Psychoactive Drugs* **2005**, 37, 145-150.
- [4.4] D.J. McKenna and G.H.N. Towers. Biochemistry and pharmacology of tryptamines and beta-carbolines: A minireview. *Journal of Psychoactive Drugs* **1984**, 16, 347-358.
- [4.5] A.P.S. Pires, C.D.R. De Oliveira, S. Moura, F.A. Dorr, W.A.E. Silva and M. Yonamine. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochemical Analysis* 2009, 20, 149-153.
- [4.6] E.H. McIlhenny, K.E. Pipkin, L.J. Standish, H.A. Wechkin, R.J. Strassman and S.A. Barker. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Chromatography A* 2009, 1216, 8960-8968.
- [4.7] D.J. McKenna. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacology and Therapeutics* **2004**, 102, 111-129.

- [4.8] J. Riba and M.J. Barbanoj. Bringing ayahuasca to the clinical research laboratory. *Journal of Psychoactive Drugs* **2005**, 37, 219-230.
- [4.9] D.J. Moura, M.F Richter, J.B. Boeira, J.A.P. Henriques and J. Saffi. Antioxidant properties of β -carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis* **2007**, 22, 293-302.
- [4.10] W. Andritzky. Sociopsychotherapeutic functions of ayahuasca healing in Amazonia. *Journal of Psychoactive Drugs* **1989**, 21, 77-89.
- [4.11] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O. Andrade, D.C. Mash. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *Journal of Ethnopharmacology* 1999, 65, 243-256.
- [4.12] J. Mabit, R. Giove, J. Vega. In M. Winkelman and W. Andritzky (Eds.), *Yearbook of Cross-Cultural Medicine and Psychotherapy* **1996**, VMB Press, Berlin 257.
- [4.13] J. Tiihonen, J.T. Kiukka, K.A. Bergstorm, J. Karhu, H. Viinamaki, J. Lehtonen, T. Hallikainen, J. Yang, P. Hakola. Single-photon emission tomography imaging of monoamine transporters in impulsive violent behavior. *European Journal of Nuclear Medicine* 1997, 24, 1253-1260.
- [4.14] T. Hallikainen, H.M. Saito, J. Lachman, T. Volavka, O.P. Pohjalainen, J. Ryynanen, J. Kauhanenm, E. Syvalahti, J. Hietala, J. Tiihonen. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Molecular Psychiatry* 1999, 4, 385-388.
- [4.15] T. Mantere, E. Tupala, H. Hall, T. Sarkioja, P. Rasanen, K. Bergstorm, J.C. Callaway, J. Tiihonen. Serotonin transporter distribution and density in the cerebral cortex of alcoholic and nonalcoholic comparison subjects: a whole-hemisphere autoradiography study. *American Journal of Psychiatry* 2002, 159, 599-606.
- [4.16] R.G. Santos, J. Landeira-Fernandez, R.J. Strassman, V. Motta, A.P.M Cruz. Effects of ayhuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. *Journal of Ethnopharmacology* 2007, 112, 507-513.
- [4.17] J.M. Fábregas, D. González, S. Fondevila, M. Cutchet, X. Fernández, P.C. Barbosa, M.Á. Alcázar-Córcoles, M.J. Barbanoj, J. Riba, J.C. Bouso. Assessment of addiction severity among ritual users of ayahuasca. *Drug and Alcohol Dependence* 2010, 111, 257-261.
- [4.18] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomedical Chromatography* 2010, (wileyonlinelibrary.com) DOI 10.1002/bmc.1551.
- [4.19] J.C. Callaway, L.P. Raymon, W.L. Hearn, D.J. McKenna, C.S. Grob, G.S. Brito, D.C. Mash. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* **1996**, 20, 492-497.

- [4.20] M. Yritia, J. Riba, J. Ortuno, A. Ramirez, A. Castillo, Y. Alfaro, R. De La Torre, M.J. Barbanoj. Determination of N,N-dimethyltryptamine and beta carboline alkaloids in human plasma following oral administration of Ayahuasca. *Journal of Chromatography B* 2002, 779, 271-281.
- [4.21] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: Subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *Journal of Pharmacology and Experimental Therapeutics* 2003, 306, 73-83.
- [4.22] J. Riba, E.H. McIlhenny, M. Valle, M. Barbanoj, S.A.Barker. Metabolism and disposition of N,N-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca. *Drug Testing and Analysis* 2012, DOI 10.1002/dta.1344
- [4.23] S.A Barker, J.A. Monti, S.T Christian. Metabolism of the hallucinogen N,N-dimethyltryptamine in rat brain homogenates. *Biochemical Pharmacology* **1980**, 29, 1049-1057.
- [4.24] J. Riba, A. Rodríguez-Fornells, G. Urbano, A. Morte, R. Antonijoan, M. Montero, J.C. Callaway, M.J. Barbanoj. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology*(Berlin) 2001, 154, 85-95.
- [4.25] J. Riba. Human Pharmacology of Ayahuasca. Doctoral Thesis, Universitat Autonoma de Barcelona. 2003, <u>http://www.tdx.cesca.es/TDX-0701104-165104/</u>
- [4.26] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry* 2003, 75, 3019-3030.
- [4.27] B.K. Matuszewski. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. *Journal of Chromatography B* **2006**, 830, 293-300.
- [4.28] P.J. Taylor. Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometery. *Clinical Biochemistry* **2005**, 38, 328-334.
- [4.29] E. Chambers, D.M Wagrowski-Diehl, Z. Lu and J.R. Mazzeo. Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. *Journal of Chromatography B* **2007**, 852, 22-34.
- [4.30] S.A. Barker, J.A. Monti, S.T. Christian. N,N-Dimethyltryptamine: an endogenous hallucinogen. *International Review of Neurobiology* **1981**, 22, 83-110.
- [4.31] B.R. Sitaram, W.R. McLeod. Observations on the metabolism of the psychotomimetic indolealkylamines: Implications for future clinical studies. *Biological Psychiatry* **1990**, 28, 841-848.

- [4.32] B.R. Sitaram, L. Lockett, G.L. Blackman, W.R. McLeod. Urinary excretion of 5methoxy-N,N-dimethyltryptamine, N,N-dimethyltryptamine and their N-oxides in the rat. *Biochemical Pharmacology* **1987a**, 36, 2235-237.
- [4.33] B.R. Sitaram, L. Lockett, R. Talomsin, G.L. Blackman, W.R. McLeod. *In vivo* metabolism of 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine in the rat. *Biochemical Pharmacology* **1987b**, 36, 1509-1512.
- [4.34] B.R. Sitaram, R. Talomsin, G.L. Blackman, W.R McLeod. Study of metabolism of psychotomimetic indolealkylamines by rat tissue extracts using liquid chromatography. *Biochemical Pharmacology* **1987c**, 36, 1503-1508.
- [4.35] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. *Journal of Ethnopharmacology* **1984**, 10, 195-223.
- [4.36] L.M. Hryhorczuk, J.M. Rainey, C. Frohman, E. Novak. A new metabolic pathway for N,N-dimethyltryptamine. *Biological Psychiatry* **1986**, 21, 84-93.
- [4.37] H. Kim, S.O. Sablin, R.R Ramsay. Inhibition of monoamine oxidase A by beta-carboline derivatives. *Archives of Biochemistry and Biophysics* **1997**, 337, 137-142.

Chapter 5. Summary and Conclusions

5.1 Summary and Conclusions

In the research presented in this dissertation we have established, expanded, and modified an analytical method which we applied to study various ayahuasca samples and urine or blood samples before and following administration of ayahuasca to humans. This method has proved useful in the study of ayahuasca in human and ethnobotanical research, and examinations of ayahuasca preparations or human samples. The characteristics of the methods suggest their sensitivity, specificity and reproducibility are adequate for use in further toxicological and clinical research on ayahuasca as well as functioning as a potential assay to screen biological samples for endogenous hallucinogens.

In chapter 2 we presented a manuscript describing the development of a liquid chromatography-electrospray ionization-tandem mass spectrometry procedure capable of the simultaneous quantitation of 11 of the major alkaloid components of ayahuasca, including several known and potential metabolites. This method affords rapid detection of alkaloids by a simple dilution assay requiring no extraction procedures while demonstrating an extremely high degree of specificity for the compounds in question, as well as lower limits of detection and quantitation than reported by previous methods while also eliminating potential matrix effects. The major components present in ayahuasca were identified as THH and harmine, followed by DMT and harmaline with quantities appearing very similar to those reported by others. The samples were also examined for the presence of DMT-NO which, to our knowledge, represented the first such effort to assay for this compound in ayahuasca preparations. Although a major DMT metabolite in mammals, it appears to be absent from the preparations of ayahuasca examined.

After establishing the method we applied the assay to 11 ayahuasca samples collected by Dr. Leanna Standish in either North or South America as presented in the manuscript contained in Appendix A. DMT, harmine, harmol and tetrahydroharmine concentrations among ayahuasca teas made and used in healing ceremonies in South or North America were remarkably similar. Concentrations of two of the three minor beta-carbolines (harmaline and harmalol) were statistically different. North American tea samples were higher in harmaline and harmolol suggesting that the tea formula made and used in North America may include a higher ratio of *B. caapi* vine relative to *P. viridis* leaves or possibly a different strain of *B. caapi*. Seasonal, growth and or methodological practices could also account for some differences.

We were able expand the method to include a number of potential metabolites and modify the preparation protocol in order to make the method suitable to analyze urine samples from participants before and after they had consumed a freeze dried form of ayahuasca thanks to the help of our collaborator Dr. Jordi Riba whose lab collected the samples. This manuscript was presented as Chapter 4: the overall major metabolite observed was DMT-NO, peaking at 4-8 h after ayahuasca administration with concentrations of approximately 11 μ g/ml representing the first report of DMT-NO as a metabolite of DMT in the urine of humans and suggesting that *N*oxidation may represent a major metabolic route for DMT clearance in humans, particularly if MAO becomes inhibited, such as occurs with ayahuasca administration. DMT-NO could also represent a major *in vivo* metabolite of DMT and thus, may serve as a better marker for endogenous DMT production and metabolism in mammals. THH represented the major component in the 8-24 h samples, with concentrations greater than 5 μ g/ml. However, following enzyme hydrolysis harmol and harmalol were found to be at far higher concentrations compared to THH because enzyme hydrolysis with glucuronidase / sulfatase appeared to produce a 40-60

fold increase in the amount of harmol, making harmol the most abundant product excreted in urine. DMK did not appear to be consistently excreted in urine, even with MAO inhibition. However; it remains possible that this compound undergoes metabolism to another, as yet undetermined, compound prior to excretion.

Next we modified and expanded the method to make it appropriate for the analysis of blood plasma samples following freeze-dried ayahuasca administration as presented in Chapter 4. The method expanded the list of compounds capable of being monitored in blood following ayahuasca administration in humans while providing a simplified approach to their analysis. The results also demonstrated for the first time that the major DMT urinary metabolite, DMT-NO, was also a major circulating product in the blood following ayahuasca administration. DMT-NO was observed to be present in the plasma at 3-to-4 times the concentration of DMT itself, peaking at 1.5 h after ayahuasca administration at approximately 45 ng/ml. THH was observed to peak in the 4.5 h samples with concentrations greater than 55 ng/ml. THH thus represented the major harmala component of ayahuasca and also the major harmala excretion product in urine and plasma in man. Despite the MAO inhibiting effect of the harmala alkaloids, significant increases in the plasma levels of IAA were observed following ayahuasca administration. The reason for this observation remains unclear. However, the data suggest that a significant quantity of the administered DMT may still be converted to IAA. The method applied and the resulting data provide the most complete profile of DMT, harmala alkaloids, and their respective metabolite concentrations in the blood following ayahuasca administration to date.

In a recent study we applied our established blood method and collaborated with Rafael Santos and Dr. Jordi Riba's lab on a study entitled 'Autonomic, Neuroendocrine, and Immunological Effects of Ayahuasca: A Comparative Study With D-Amphetamine' which was

published in December 2011 in the Journal of Clinical Psychopharmacology. This study was not included here as it constituted part of the dissertation of one of Dr. Riba's students (Santos). Nonetheless, we found that ayahuasca led to measurable DMT plasma levels and distinct subjective and neurophysiological effects that were absent after amphetamine. Prolactin levels were significantly increased by ayahuasca but not by amphetamine, and cortisol was increased by both, with ayahuasca leading to the higher peak values. Natural killer cells were also increased by both. These findings indicate that ayahuasca has a moderate impact on the nervous system with a modulatory capacity on cell-mediated immunity [B.25]. If present, endogenous DMT and β -carbolines could also potentially produce these effects.

In a follow up to our preliminary urine study where we established the urine method in Appendix B, we presented a manuscript further characterizing the metabolism and disposition of DMT and harmala alkaloids after oral administration of ayahuasca. *O*-demethylation plus conjugation seems to represent an important but probably not the only degradation route for the harmala alkaloids in humans. Harmol and harmalol concentrations were 10-fold and 5-fold the amounts ingested with ayahuasca demonstrating, that the vast majority of harmol and harmalol recovered in urine after ayahuasca ingestion must necessarily be formed through the metabolic breakdown of harmine and harmaline. The recoveries of each harmala alkaloid plus its *O*-demethylated metabolite varied greatly between 9 and 65%.

N-oxidation appears to represents a major degradation pathway of DMT in humans when administered together with β -carbolines in ayahuasca suggesting the existence of an alternative metabolic route to biotransformation by MAO. Less than 1% of the administered DMT dose was recovered as the unmetabolized parent compound. The main DMT metabolite found in the urine was IAA. The second highest concentration metabolite detected was DMT-NO, with recoveries around 10%. DMT and metabolite excretion was maximal during the first third of the 24 h collection period. DMT-NO made up around 20% of the compounds measured. Recovery of DMT plus metabolites reached 68%. These findings support the notion that MAO plays a prominent role in the degradation of DMT. However, MAO-inhibition after ayahuasca appeared to be either incomplete or short-lived, as large amounts of IAA were already detected in the first 4 h collection interval. Partial inhibition of MAO by the harmalas in ayahuasca appeared to be sufficient to allow psychoactive effects. DMT-NO does not seem to function as an intermediate during the formation of IAA by MAO-A, but it does appear to represent the major metabolite of DMT in the absence of or after inhibition of mitochondrial MAO. MAO inhibition could consequently shift metabolism from oxidative deamination to *N*-oxidation as a compensatory metabolic mechanism.

Future investigations could address the metabolism of oral DMT in humans without the presence of β -carbolines, in order to assess the contribution of the different metabolic pathways for its degradation under physiological conditions. This would allow us to estimate the degree of metabolic compensation induced by the harmala alkaloids in ayahuasca. We have already collected and analyzed preliminary data for this study which compared urine samples from participants following orally administered or smoked DMT. Alternatively no studies have yet pretreated humans with MAO inhibitors alone and measured the parent compounds and their corresponding *N*-oxides. The advantage of such a study would be that the *N*-oxide, as opposed to the indoleacetic acid, retains the original structure of the parent molecule, permitting a cumulative association. Therefore, monitoring the *N*-oxide metabolites rather than the parent compounds alone in MAO-inhibited humans could provide a substantial advantage in detecting and quantitating endogenous psychedelic compounds. We suggest that MAO inhibition in

humans could substantially enhance detection and quantitation of endogenous hallucinogenic compounds in the periphery, especially if the *N*-oxide metabolites are monitored. It would appear necessary that in order to sufficiently detect parent compounds sensitivity of assays must be improved to 1.0 pg/ml or less and include more frequent sampling and longer collection times given the possible intermittent presence of these compounds in the periphery, blood and urine.

In this regard, much of our interest in ayahuasca pharmacology and metabolism was based on the need to further understand the possible role and needed methods of analysis for endogenous psychedelics and to provide a more definitive answer to the question, "Are 'hallucinogenic' tryptamines naturally present in human metabolism?" In Appendix C this question was addressed, and we concluded that the quantity of mass spectral evidence in the literature demonstrated that DMT and 5-OH-DMT do indeed appear endogenously and may be sufficiently measured in human body fluids although the tissue sources of these compounds in human remains unknown. The highest levels of INMT mRNA have been reported in adrenal gland and lung, although no large amount of INMT mRNA was detectable in brain [C.96, C.97]. The active transport of DMT across the blood-brain barrier [C.98] suggests that peripheral synthesis may still affect central function. A fluorescent antibody to INMT and confocal microscopy [C.99], have identified INMT in spinal cord, brain, retina, and pineal gland, and suggest the future possibility for mapping and characterizing the regulation of the endogenous psychedelic pathway. Future molecular biological approaches and advances in assay methodology could potentially help characterize the biochemistry and physiology of these compounds in humans. Data regarding psychodynamics, concentrations, circadian variation, metabolism and clearance as assessed by validated analytical methods applied to biological samples represent an accessible approach to more clearly determining potential roles in human

psychophysiology. We propose that future studies assay CSF, blood and urine and monitor for DMT, 5-OH-DMT, 5-MeO-DMT and their corresponding *N*-oxides using validated mass spectrometric methodology. Pretreatment of study subjects with an MAO inhibitor could optimize results.

The search for endogenous psychedelic tryptamines could also turn toward solid organs such as adrenal, brain, lung, pineal, retina, and other tissues in which INMT activity has been demonstrated. Mapping of INMT within certain cell types and locations could reveal its intracellular distribution and possible associations with various receptors. The creation of an INMT knockout mouse and characterization of the phenotype could also greatly aid in understanding the role of this enzyme and endogenous psychedelics.

Why might organisms produce endogenous psychointegrative substances such as DMT and 5-MeO-DMT? The current research conducted leaves this question open to speculation and hypotheses, which abound. Could DMT represent a molecular interface to the experience of consciousness? Indeed, there are many hypotheses that have attempted to link our more mundane experiences of the world to the more extraordinary psychic states through quantum mechanical mechanisms and the possibilities of a multidimensional reality beyond normal perception. These quantum mechanical-to-cosmological hypotheses appear to be untestable at present. However, it may not necessarily remain this way. Perhaps further study of these molecules which have been claimed to afford the capability of perceived transport to "other worlds" might help answer some of these questions and expand our knowledge of the origins of religious and mystical states and other extraordinary states of consciousness. Perhaps these molecules function as dynamic regulators or integrated "tuners" of conscious, unconscious and preconscious perception, sensation, emotions, cognition, intuition, inspiration and intention. Perhaps they afford an awareness of different resolutions of perceived ontological existence.

Appendix A. Cross-cultural variations in psychoactive alkaloid content in ayahuasca teas used in spiritual ceremonies

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BASTYRUNIVERSITY

May 15, 2012

The Graduate School Louisiana State University Baton Rouge, Louisiana

Regarding: Contributions of Ethan H. McIlhenny to the Manuscript entitled "Cross-cultural variations in psychoactive alkaloid content in ayahuasca teas used in spiritual ceremonies"

As first author of the above mentioned manuscript, I have direct knowledge of the contributions made by Mr. McIlhenny in its creation. Mr. McIlhenny developed the analytical method described therein, performed the analysis of the samples collected and quantified the compounds measured. He also contributed ideas regarding the composition of compounds present in ayahuasca and to the overall writing and editing of the manuscript. The results presented in the manuscript are the result of the collaboration between the Department of Comparative Biomedical Sciences at LSU and my own department. I am the first author because of my contribution as principal investigator of the clinical trial in which the biological samples were obtained.

Given the significant contributions made by Mr. McIlhenny to the research project and the manuscript, it is appropriate that the manuscript be included in his dissertation.

Sincerely,

L StandiiL

Leanna Standish, ND, PhD, LAc, FABNO

Clinical Research Professor, Bastyr University Research Institute Clinical Professor, University of Washington, School of Public Health Affiliate Research Professor, UW School of Medicine, Radiology Department

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A.1 Introduction

The purpose of this study was to compare the concentrations of psychoactive alkaloids present in ayahuasca teas used in healing ceremonies conducted in South and North America. Ayahuasca, an ethnobotanical medicine used by indigenous peoples of South America for thousands of years, is primarily derived from the synergistic interactions of two Amazonian plants, *Psychotria viridis* and *Banisteriopsis caapi*. The leaves of *P. v*iridis contain the alkaloid N, N-dimethyltryptamine (DMT). The woody vine of *B. caapi* contains three main active indole alkaloids, beta-carboline alkaloids, harmine, tetrahydroharmine (THH) and harmaline [A.1].

Ayahuasca tea is a unique serotonin agonist with both short and long-term action that, with repeated use, modifies 5-HT receptor sites to increase serotonin availability. DMT contained in ayahuasca tea is a potent serotonin agonist at the 5-HT1A presynaptic and 5-HT2A post-synaptic membrane receptor sites [A.1, A.2] and possibly increases neocortical GABA levels [A.4].

DMT is believed to be primarily responsible for the psychoactive effects of ayahuasca. Orally administered DMT is metabolized by an enzyme in the gut and liver, monoamine oxidase A (MAO-A) before it can cross the blood-brain barrier. Inhibition of MAO by beta-carbolines present in ayahuasca tea protects DMT from oxidative deamination in gut and liver, thus enabling DMT to be active when administered orally [A.2, A.5].

Beta-carbolines are tricyclic indole alkaloids that are structurally related to tryptamines and have been identified in both plant and mammalian tissue [A.6]. The primary mechanism of action of two of the three principal beta carbolines found in ayahuasca, harmine and harmaline, is the reversible inhibition of MAO-A, which is responsible for breaking down serotonin, dopamine and norepinephrine [A.3]. The third beta carboline, tetrahydroharmine weakly inhibits the uptake of serotonin via serotonin transporter binding.

We sought to measure the concentration of nine known active alkaloid constituents present in ayahuasca tea: N,N-dimethyltryptamine (DMT), 5-OH-DMT (bufotenine) and N-methyltryptamine (precursor of DMT) from the *P. viridis* plant and six beta-carbolines (harmine, harmol, tetrahydroharmine, harmalol, harmaline and tetrahydroharmol (THHO)) from the *B. Caapi* vine [A.3, A.7].

A.2 Materials and methods

A.2.1 Materials

Eleven ayahuasca tea samples were collected from two regions, South America (N=6) and North America (N=5). Five ayahuasca ceremony leaders were approached to provide research samples of the tea used in a ceremony that had been conducted within the previous 24 hours. Each of the ayahuasqueros approached agreed and 10 ml of the tea was collected into sterile brown glass vials then immediately frozen at -80 degrees C until batch analysis. Eleven samples were provided to the researchers. Six of the tea samples were brewed by traditional ethnomedicine methods in South America (N=6) and five samples from non-denominational ayahuasca ceremonies brewed and conducted on the Hawaiian Islands or in the mainland U.S. South American samples differed from the North American samples by level of freshness of the tea decoction. South American samples were shipped from indigenous groups in the Amazon basin to the ayahuasquero ceremony leader and thus were not as fresh as those made for ceremonies conducted in Hawaii and on the U.S. mainland.

A.2.2 Methods

The concentration of nine known - constituents of each sample of ayahuasca tea were measured (mg/ml) using high performance liquid chromatography-electrospray ionization-

tandem mass spectroscopy methods developed by our research team and performed at the Louisiana State University in a single-blind manner; see [A.7] for detailed methods. In brief, standard solutions of the 9 compounds analyzed were prepared using a 10 ppm stock standard in MeOH stored in -80°C. A 1.0 ml aliquot of this solution was dried under nitrogen and brought up with 1.0 ml 97/3 (water + 0.1% formic acid: acetonitrile +0.1% formic acid) mobile phase (MP). This 10 ppm stock solution in 97/3 MP was then serially diluted to 5ppm, 2.5ppm, 1ppm, 500ppb, 250ppb, 100ppb, 50ppb, 25ppb, 10ppb, 5ppb, 2.5ppb, and 1 ppb with MP.

Extracts were prepared and analyzed in duplicates. For all the ayahuasca extracts 50 μ l of each were diluted with 950 μ l MP (=20x dilution) in a 96 well Thermo protein precipitation plate (PPT), then shaken for 3 minutes on an orbital shaker. The samples were then transferred to a second PPT using a vacuum manifold, shaken again for 3 minutes and then placed again on a vacuum manifold. Vacuum was applied for 5 minutes with the filtrate being collected in a 96 well deep-well plate from which the samples were injected for analysis.

To 180 µl aliquots of the 9 compound mix-serial dilutions (5ppm-1ppb) were added 20ul of 1 ppm DET internal standard in a separate Thermo PPT plate. Similarly, 100 ul aliquots of the 20X diluted samples were mixed with 100 ul of 1 ppm DET and 800 ul MP in a Thermo PPT plate, shaken for 3 minutes, placed on the vacuum manifold and again suctioned with minimal vacuum for 5 minutes. Standards were treated in the same manner. The filtrate was collected into a new 96 well deep-well plate and 20 ul were injected for analysis.

Analyses were conducted in duplicate and as described in [A.7] with two modifications; the mobile phase conditions for LC separation were slightly altered to afford improved separation of the target compound, as described in [A.8] and the instrument used was a Thermo Velos ion trap [A.8] as opposed to a Thermo TSQ [A.7].

We evaluated whether concentration of the nine alkaloids present in the 11 ayahuasca tea samples was related to the concentration of any of the other eight active constituents measured in this study in two ways, first as a simple correlation then in a regression model. T-tests were used to detect statistically significant difference in alkaloid concentrations between ayahuasca made in South versus North America. Correlations and regression models were used to investigate the relationship among the nine alkaloids. Principle components analysis was used to detect the effect of geographic location (South versus North American) tea samples.

A.3 Results

A.3.1 DMT and 5-OH-DMT concentrations in ayahuasca teas

DMT concentrations vary widely but there were no differences in mean DMT concentration between South and North American ayahuasca tea samples. Nine alkaloid constituents were measured in each of 11 tea samples. See Table A.1 for raw data, mean and standard deviation for the South American samples (N=6) and North American samples (N=5). DMT concentrations ranged from 0.26 - 0.78 mg/ml, a 3 fold difference in concentration across all 11 samples. However, there were no significant differences between the mean DMT concentration between North (0.49 mg/ml ± 0.18) and South (0.45 mg/ml ± 0.71) American teas. Higher levels of 5-OH-DMTwere found in South American samples (p= 0.048) compared to North American samples (Table A.2).

DMT concentration among ayahuasca teas made and used in healing ceremonies in South America or North America are remarkably similar in concentrations of the main psychoactive alkaloids present in ayahuasca tea (DMT, harmine, harmol and tetrahydroharmine).

Concentrations of two of the three minor beta-carbolines (harmaline and harmalol) were

statistically different. North American tea samples were higher in harmaline and harmalol.

Sample #	Source Raw Plant Materials	Location of Ceremony	Ethno Tradition	DMT	Harmine mg/ml	Harmaline mg/ml	THH mg/ml	Harmol mg/ml	Harmalol mg/ml	THHO mg/ml	NMT mg/ml	5-OH- DMT mg/ml
South America												
AY025	Brazil	Brazil	Barquinha Church	0.260	1.302	0.049	0.765	1.084	0.000	0.020	0.008	0.000
AY003A3	Peru	US Mainland	Shipibo	0.646	1.406	0.064	0.656	1.159	0.004	0.000	0.008	0.000
AY009A3	Peru	US Mainland	Shipibo	0.371	2.512	0.223	2.070	1.657	0.030	0.127	0.025	0.005
AY010A3	Peru	US Mainland	Shipibo	0.277	2.028	0.172	1.872	1.467	0.021	0.116	0.021	0.003
AY011A3	Peru	US Mainland	Shipibo	0.413	2.004	0.157	1.475	1.427	0.026	0.077	0.018	0.006
AY024	Peru	US Mainland	Shipibo	0.708	2.423	0.178	1.580	1.598	0.023	0.080	0.017	0.004
N=6												
Mean				0.446	1.946	0.141	1.403	1.399	0.018	0.070	0.016	0.003
Std. Deviation				0.189	0.503	0.069	0.577	0.232	0.013	0.051	0.007	0.002
North America												
AY001B3	Hawaii	Hawaii	Non-denom	0.317	1.706	0.281	1.877	1.293	0.002	0.081	0.022	0.001
AY005B3	Hawaii	Hawaii	Non-denom	0.784	2.343	0.315	1.898	1.717	0.000	0.036	0.023	0.000
AY026	Hawaii	US Mainland	Non-denom	0.393	1.114	0.215	1.441	0.980	0.000	0.045	0.016	0.000
AY027	Hawaii	US Mainland	Non-denom	0.524	2.063	0.348	2.137	1.612	0.002	0.052	0.027	0.000
AY020	Hawaii	Hawaii	Non-denom	0.425	3.916	0.228	2.116	2.743	0.007	0.097	0.031	0.000
N=5												
Mean				0.489	2.229	0.277	1.894	1.669	0.002	0.062	0.024	0.000
Std. Deviation				0.181	1.049	0.057	0.280	0.666	0.003	0.026	0.005	0.001

Table A.1. Concentration of Major Alkaloid Constituents in Ayahuasca Teas Collected in
South and North America

A.3.2 DMT, harmine, THH and harmol concentration

Figure A.1 shows the mean concentration of the four alkaloids found in highest concentration in ayahuasca teas made in South and North America: DMT, harmine, THH and harmol. None of the differences in concentration were statistically significant.

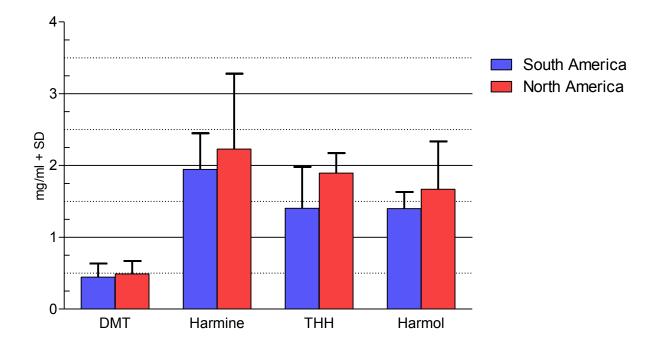


Figure A.1. Mean concentration in mg/ml of DMT, harmine, THH and harmol in ayahuasca teas made for ceremonial use in South American (n=6) compared to North American (n=5). There were no significant differences.

A.3.3 Beta-carboline alkaloid concentrations in ayahuasca teas

The six beta-carbolines from the B. caapi vine were measured in the 11 ayahuasca tea samples and ranked by greatest to least concentration present in each tea sample. In South American teas harmine > harmol > THH > harmaline > THHO > harmalol. In North American teas harmine > THH > harmol > harmaline > THHO > harmalol. Harmine was found in highest concentration in all of the South American samples and three of the five North American samples. However, there were no statistical differences in mean harmine concentration between South (1.95 ± 0.51) and North American (2.23 ± 1.05) tea samples (Table A.2).

Concentrations of the minor beta-carboline alkaloids present in ayahuasca (harmaline, harmalol and THHO) were found in lower concentrations compared to the major beta-carbolines

(harmine, harmol and THH). There was more variability in the minor beta-carbolines among tea samples. For example, harmaline varied widely among all 11 samples, ranging from 0.049 - 0.348 (a 7 fold difference). Harmaline was found in higher concentrations in North American versus South American samples and this difference was statistically significant (p = 0.006). Higher levels of harmalol were found in South America than in North American samples (p=0.029). There were no significant differences in concentrations of the other beta-carbolines, harmine, harmaline, THH, Harmol, or THHO.

 Table A.2. Comparison of active constituents of ayahuasca tea samples from South

 America versus North America using T tests.

	South America	North America	t	Р
DMT	0.4459	0.4888	382	0.711
Harmine	1.9457	2.2286	589	0.571
Harmaline	0.1406	0.2773	-3.551	0.006
THH	1.4031	1.8938	-1.728	0.118
Harmol	1.3988	1.6691	936	0.374
Harmalol	0.0175	0.0021	2.942	0.029
ТННО	0.0699	0.0623	.301	0.770
NMT	0.0163	0.0237	-1.972	0.080
5-OH DMT	0.0028	0.0003	2.511	0.048

A.3.4 Bivariate and multivariate relationships among the 11 ayahuasca alkaloids

Because the DMT present in ayahuasca tea derives only from *the P. viridis* plant admixture, we did not expect, nor detect, any significant correlations of DMT with the concentration of the beta-carbolines measured. Concentrations of 5-OH-DMT or Nmethyltryptamine (precursor of DMT) concentrations did not correlate with DMT (Table A.3).

-		Harmine	Harmaline	THH	Harmol	Harmalol	THHO	NMT	5-OH-DMT
DMT	r	.168	.228	018	.163	108	387	003	123
	р	.621	.501	.958	.632	.753	.239	.993	.719
	Ν	11	11	11	11	11	11	11	11

 Table A.3. Correlations of DMT concentration with the other eight alkaloid constituents

 present in 11 ayahuasca tea samples

Table A.3 shows that there were no significant bivariate relationships between DMT and any of the other eight alkaloids present in ayahuasca, including 5-OH-DMT. This later result is surprising in that DMT and 5-OH-DMT are constituents in *P. viridis* and might be expected to co-vary. However, a regression where DMT is the dependent variable and the other eight alkaloid constituents as predictors turned out to be significant in that, other things being controlled, harmaline and harmalol had both a positive predictive relationship with DMT. Higher DMT concentrations were associated with higher harmaline and harmalol, which are two of the less significant beta carbolines present in ayahuasca tea. DMT levels did not correlate with concentrations of the major beta-carbolines, harmine, harmol or THH. There were also marginal significant negative relationships between NMT and DMT, and 5-OH-DMT with DMT (Table A.4).

			Standardized Coefficients		
	В	Std. Error	Beta	t	Р
(Constant)	.283	.199		1.422	.291
Harmine	239	.386	-1.034	619	.599
Harmaline	8.656	1.868	4.563	4.634	.044
THH	-1.040	.422	-3.013	-2.468	.132
Harmol	1.167	.745	3.117	1.567	.258
Harmalol	31.401	5.947	2.141	5.280	.034
ТННО	4.165	3.036	.935	1.372	.304
NMT	-81.934	24.015	-3.257	-3.412	.076
5-OH-DMT	-94.689	26.626	-1.180	-3.556	.071

Table A.4. Regression coefficients where DMT is the constant dependent variable with each of the concentration of the other eight ayahuasca alkaloids

To better understand the relationships among the nine active constituents of ayahuasca teas collected from 11 ceremonies, we performed a Principal Component Analysis (PCA), which produced a map to determine how closely related were the samples and their relationship with the chemical components (Figure A.2).

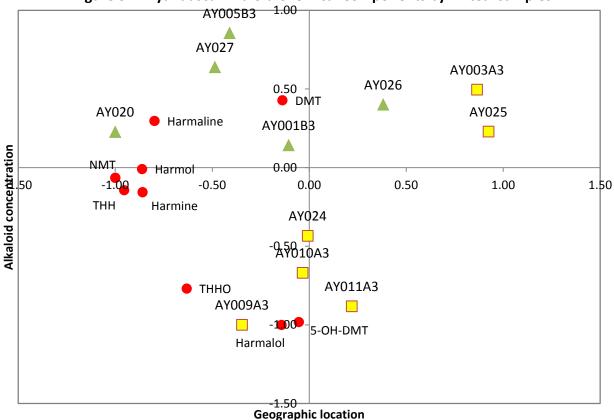


Figure 3.2. Ayahuasca Alkaloid Chemical Components by 11 tea samples

Figure A.2. Principal Components Analysis of 11 ayahuasca tea samples collected in either South or North America. Red represents the nine alkaloids. Green represents the North American samples and Yellow represents the South America samples. North American samples tend to fall on the upper left quadrant, the South American tend to fall at the upper right or lower middle of the map. Component 1 (geographical location of ceremony) and component 2 (psychoactive alkaloid concentrations) describe the 11 samples well. Geographical location (South versus North America) and psychoactive alkaloid concentrations, account for 76.2% of the variability. We compared results from our 11 samples to results published by Callaway et al (1996) [A.9] who measured DMT, harmine, harmaline and THH in an ayahuasca tea used in a União do Vegetal Church ceremony (UDV) in the Brazilian Amazon rain forest near Manaus [A.8]. The UDV ayahuasca tea contained DMT at 0.24 mg/ml, harmine at 1.7 mg/ml, harmaline at 0.2 mg/ml and THH at 1.07 mg/ml. The 11 samples measured in the current study that were collected from 2000-2011 had a higher average concentration of DMT (0.47 ± 0.18) compared to the 1996 data, but had similar concentrations of the beta-carbolines harmine (2.1 + 0.77 mg/ml), harmaline (0.21 + 0.09 mg/ml) and tetrahydroharmine (1.63 + 0.51 mg/ml).

A.4 Discussion

Given the variation between South America and Hawaii in climate, seasonality of harvest and variations in ayahuasca tea formulas used cross-culturally, we expected to observe greater differences in concentration of the major known active constituents. Mean concentrations of DMT, the main psychoactive alkaloid present in ayahuasca tea, did not differ between South and North American tea samples. Concentrations of the major beta-carbolines (harmine, harmol and THH) also did not statistically differ between South and North American samples.

Of the eight beta-carbolines measured in the 11 tea samples, only harmaline and harmalol concentrations significantly differed. Harmaline was nearly two-fold higher in North American tea samples suggesting that relatively more *B. caapi* vine is added to the decoctions made in North America compared to South America. However, this conclusion is complicated by our data showing that harmalol, a low concentration beta-carboline present in *B. caapi*, was found in much higher average concentration (nine-fold) in South American samples. Concentrations of 5-OH-DMT (with no known CNS effects) and harmalol were higher in South American samples.

The difference between South and North American ayahuasca tea samples may be due to variations in season of harvest, strain and maturity of plant used, climate, tea recipe used, and freshness of decoction. North American samples were decocted within 24 hours of the time that the sample was frozen. We do not have information regarding the data of decoction of Brazilian or Peruvian samples.

A.5 Conclusions

DMT concentration among ayahuasca teas made and used in healing ceremonies in South America or North America are remarkably similar in concentrations of the main psychoactive alkaloids present in ayahuasca tea (DMT, harmine, harmol and tetrahydroharmine). Concentrations of two of the three minor beta-carbolines (harmaline and harmalol) were statistically different. North American tea samples were higher in harmaline and harmolol suggesting that the tea formula made and used in North America may include a higher ratio of *B. caapi* vine relative to *P. viridis* leaves or possibly a different strain of *B. caapi*. Seasonal, growth and or methodological practices may also account for some differences.

A.6 Future Ayahuasca Characterization Publications

We have further collaborated with Dr. Standish using the established ayahuasca method on two future ayahuasca characterization publications and two studies preparing ayahuasca for clinical trials. The data for these studies has already been collected and analyzed and the manuscripts are currently in the process of being written. The first examined anatomical distribution of DMT in *P. viridis* and beta-carbolines in *B. caapi* and was conducted by Reardon, McIlhenny, Barker, and Standish. We found no DMT present in *B. caapi* in any anatomical structure. Harmine concentrations were highest in *B. caapi* leaves, with lesser in the glands at the

base of the leaves, still less in petiole, and the least amount was found in the woody vine. Interestingly the traditional brewing of ayahausca only included the vine of *B. caapi* and no parts of the leaf were included. Both the leaf and meristematic nodes in *P. viridis* contain DMT at similar levels.

The second characterization study was a comparison of psychoactive alkaloids in ayahuasca tea formulations that varied in *P. viridis* and *B. caapi* weight ratios conducted by Standish, Reardon, McIlhenny, and Barker. We found that systematic increase of *P. viridis* and *B. caapi* weight ratios from 1:2 to 1:16 resulted in a fairly linear curve of ratio to concentration of harmine while DMT concentrations remained fairly constant.

A.7 Future Publications to Prepare Ayahuasca for Clinical Trials

The first study intended to prepare for potential ayahuasca clinical trials examined chemistry, manufacturing and controls and was conducted by Standish, Martzen, Reardon, McIlhenny, and Barker. We found that ayahuasca alkaloid concentration and therefore dose potency can be controlled by decocting the two plants separately then recombining and that ayahuasca extracts are stable when stored as there appears to be no loss of DMT or harmine after 12 months of refrigeration or freezing at - 80°C. It was further observed that ayahuasca can also be sterilized by lyophilization or autoclave without loss of psychoactive alkaloids and that the experimental ayahuasca extracts examined were free of heavy metals.

The second clinical preparation was an ayahuasca dose consideration for planning a phase I dose escalation safety and tolerability study in healthy adults and was conducted by Standish, Reardon, Martzen, McIlhenny, and Barker. We asked the question: What Phase I doses should be used in the dose escalation? We found that an initial water volume of 12 liters may be boiled

down to at least 833 ml to result in a potent ayahuasca extract that would be similar to what has been used as traditional medicine: 833 ml to provide 16 doses at 50 ml volume per dose. Dosing may start at DMT 0.025, 0.05, 0.10, 0.25. 0.50, and 1.0 mg/ml. Since harmine posed fewer safety concerns we will retain the same concentration of harmine at 1.5 mg/ml. Extracts may be made with *B. caapi* vine 3-5 cm in diameter cut fresh within one week and refrigerated from time of harvest to time of decoction. Extracts may be made with distal *P. viridis* leaves until we know DMT concentration in mature leaves. Extracts can be sterilized using 0.22 micron filtration and placed into 100 ml sterile brown bottles for freezing until administration when the extract will be removed from the freezer and rendered liquid again in a dedicated secure refrigerator.

A.8 References

- [A.1] M. Yritia, J. Riba, J. Ortuno, A. Ramirez, A. Castillo, Y. Alfaro, R. De La Torre, M.J. Barbano. Determination of N,N-dimethyltryptamine and beta carboline alkaloids in human plasma following oral administration of Ayahuasca. *Journal of Chromatography B* 2002, 779, 271-281.
- [A.2] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and beta-carboline constituents of ayahuasca. *Journal of Ethnopharmacology* 1984, 10, 195-223.
- [A.3] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O. Andrade, D.C. Mash. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *Journal of Ethnopharmacology* 1999, 65, 243-256.
- [A.4] W.M. Abi-Saab, M. Bubser, R.H. Roth, A.Y. Deutch. 5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. *Neuropsychopharmacology* 1999, 20, 92-96.
- [A.5] J. Riba, A. Rodríguez-FornellS, G. Urbano, A. Morte, R. Antonijoan, M. Montero, J.C. Callaway, M.J. Barbanoj. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology*(Berlin) 2001, 154, 85-95.
- [A.6] M.M Airaksinen, I. Kari. Beta-carbolines, psychoactive compounds in the mammalian body. Part I: Occurrence, origin and metabolism. *Med Biol* **1981**, 59, 21-34.
- [A.7] E.H. McIlhenny, K.E. Pipkin, L.J. Standish, H.A. Wechkin, R.J. Strassman, S.A. Barker. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian

botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Chromatography A* **2009**, 1216, 8960-8968.

- [A.8] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed Chromatogr.* 2012, 26, 301-313.
- [A.9] J.C. Callawa, L.P. Raymon, W.L. Hearn, D.J. McKenna, C.S. Grob, G.S. Brito, D.C. Mash. Quantitation of N,N-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. *Journal of Analytical Toxicology* **1996**, 20, 492-497.

Appendix B. Metabolism and disposition of *N*,*N*-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca*

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The Graduate School Louisiana State University Baton Rouge, Louisiana

Regarding: Contributions of Ethan H. McIlhenny to the Manuscript entitled "Metabolism and disposition in of N,N-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca" Drug Testing and Analysis doi: 10.1002/dta.1344

As first author of the above mentioned manuscript, I have direct knowledge of the contributions made by Mr. McIlhenny in its creation. Mr. McIlhenny developed the analytical method described therein, performed the analysis of the samples collected and quantified the compounds measured. Mr. McIlhenny also contributed new ideas regarding the metabolism of DMT and harmala alkaloids and to the overall writing and editing of the manuscript. The results presented in the manuscript are the result of the collaboration between the Department of Comparative Biomedical Sciences at LSU and my own department. I am the first author because of my contribution as principal investigator of the clinical trial in which the biological samples were obtained.

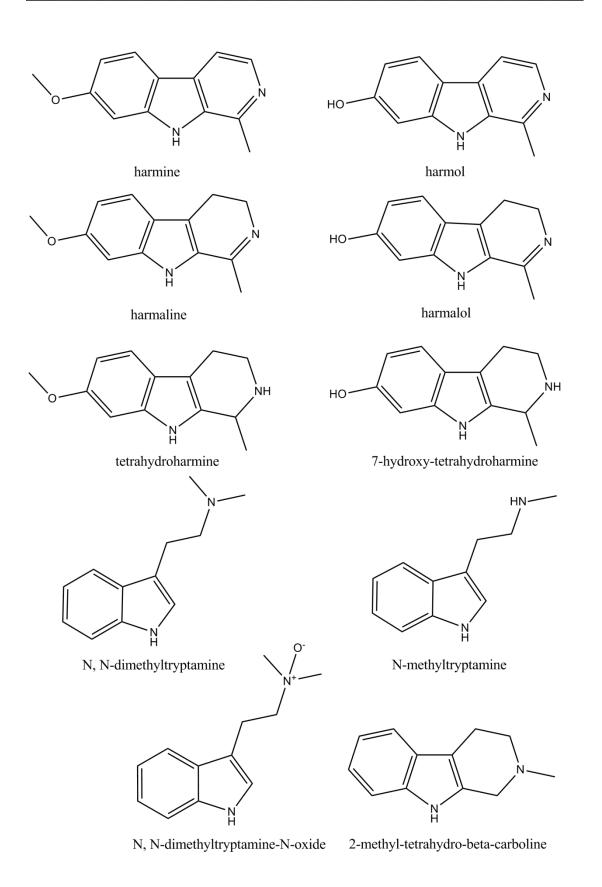
Given the significant contributions made by Mr. McIlhenny to the research project and the manuscript, it is appropriate that the manuscript be included in his dissertation.

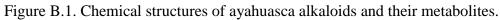
Jordi Riba, PhD

B.1 Introduction

Ayahuasca is a psychotropic plant tea obtained from the stems of the jungle liana *Banisteriopsis caapi* and usually the leaves of *Psychotria viridis* or *Diplopterys cabrerana* [B.1, B.2]. The tea is used by many Amazonian peoples to attain a modified state of consciousness, which is a central element of rites of passage, religious ceremonies and shamanic medicine [B.2]. In recent years, the firmly established ancestral uses of ayahuasca have given way to new forms of consumption. Syncretic religious groups using ayahuasca as a sacrament have appeared and have expanded their activities to the urban areas of South America and also to Europe and North America. An increasing number of foreigners travel to the Amazon to participate in ayahuasca retreats and traditional healers travel to Europe to organize ayahuasca ceremonies. The growing attention ayahuasca is attracting worldwide has raised public health concerns [B.3].

The powerful psychotropic effects of ayahuasca arise from the pharmacological interaction between the β -carboline alkaloids present in *B. caapi* and the tryptamines found in *P. viridis* and *D. cabrerana*. On the one hand, the β -carbolines, mainly harmine, harmaline and tetrahydroharmine, are reversible inhibitors of the enzyme monoamine-oxidase A (MAO-A) [B.4, B.5]. On the other hand, *P. viridis* and *D. cabrerana* contain DMT [B.5], a potent psychedelic [B.6-B.8], which is a priori not active orally [B.7] due to extensive first pass metabolism by MAO-A. Both the β -carbolines, also known as harmala alkaloids, and the DMT present in the plants are extracted into the ayahuasca infusion and ingested by users [B.9]. The blockade of visceral MAO brought about by the β -carbolines is believed to render DMT orally active, allowing its access to systemic circulation and subsequently to the central nervous system [B.10]. There, DMT interacts with serotonergic 5-HT2A, 5-HT1A and 5-HT2C and other receptor sites [B.11-B.15] eliciting psychedelic effects in humans [B.16-B.17].





Early studies involving the administration of pure DMT to humans had already observed that it lacked psychoactive effects after oral administration [B.7]. Following parenteral DMT, Szára failed to find the unmetabolized drug in urine and identified indole-3-acetic acid (IAA), formed by oxidative deamination, as the drug's degradation product [B.6]. Kaplan and coworkers found that following an intramuscular injection, DMT disappeared from plasma very rapidly. They reported that less than 0.1% was recovered in 24 h urine but they did not attempt to identify the putative metabolites [B.18].

The role of MAO in the metabolic breakdown of DMT has been stressed in the literature based in the aforementioned presence of IAA in urine after DMT and in the efficacy of the harmala alkaloids and other MAO-inhibitors to render DMT psychoactive *per os* [B.19]. However, oxidative deamination by MAO may not be the sole metabolic pathway in humans. *In vitro* and animal studies have described *N*-oxidation, *N*-demethylation and cyclization as alternative metabolic routes, [B.20-B.22] as depicted in Figure B.2.

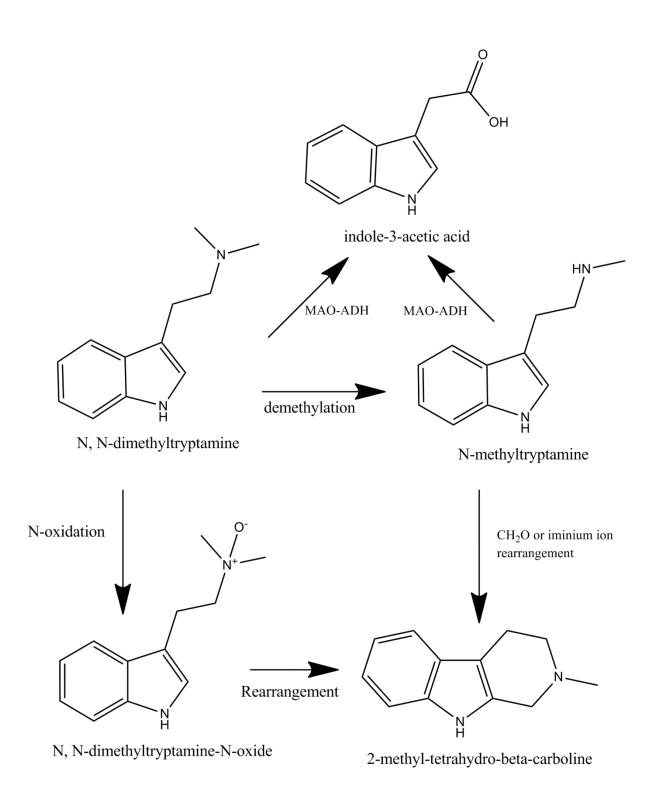


Figure B.2. Metabolic pathways of *N*,*N*-dimethyltryptamine. MAO=monoamine-oxidase; ADH= aldehyde-dehydrogenase.

To date no study has addressed the fate of DMT and the harmala alkaloids when administered in combination in ayahuasca. In a preliminary assessment conducted by our group in the course of analytical method validation, DMT-*N*-oxide (DMT-NO) and harmol and harmalol, the *O*-demethylation products of the harmine and harmaline, respectively, were detected in the urine and blood of three individuals after ayahuasca intake [B.23, B.24]. The present manuscript describes the assessment of the metabolism and urinary disposition of DMT and the harmala alkaloids in a group of healthy volunteers following ayahuasca administration.

B.2 Materials and Methods

B.2.1 Volunteers

Ten young healthy male volunteers were recruited. Participants were experienced psychedelic drug users. The most commonly used substances were psilocybin mushrooms and LSD, followed by ketamine, peyote and mescaline. None of the participants had used ayahuasca before. Volunteer mean age was 29.0 years (range 20-38); mean weight was 67.0 kg (range 60-85); and mean height was 1.77 m (range 1.69-1.96). Volunteers underwent a structured psychiatric interview (DMS-IV) to exclude current or past history of Axis-I disorders and alcohol or other substance dependence. General good health was confirmed by medical history, laboratory tests and ECG.

The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. All volunteers gave their written informed consent to participate.

B.2.2 Drugs

Ayahuasca was administered orally as an encapsulated lyophilizate. The freeze-dried material was obtained from a Brazilian batch of ayahuasca and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline and 11.36 mg tetrahydroharmine (THH) per gram. The lyophilizate was also tested for harmol and harmalol and was found to contain 0.30 mg/g harmol and 0.07 mg/g harmalol. Freeze-dried ayahuasca was administered in doses equivalent to 1.0 mg DMT/kg body weight.

B.2.3 Study Design and Sample Collection

Urine samples were obtained in the course of a clinical trial involving three experimental sessions. In a double-blind crossover balanced design, participants received in each experimental session one of the following treatments: a lactose placebo, 20 mg d-amphetamine, and 1.0 mg DMT/kg body weight ayahuasca. In addition to urine collection, the study involved the measurement of various pharmacodynamic parameters including subjective, neuroendocrine and immunomodulatory data. A detailed description of the methods used and the results concerning these variables have been published elsewhere [B.25]. In the present article we report only the data obtained from the analyses of urine samples collected following ayahuasca administration. The amounts of harmine, harmaline and tetrahydroharmine recovered in urine are reported together with the amounts of their potential O-demethylated metabolites, harmol, harmalol and tetrahydroharmol (7-hydroxy-tetrahydroharmine). Samples were also analyzed for DMT and its potential biotransformation products IAA, DMT-NO, N-methyltryptamine (NMT) and 2-methyltetrahydro-betacarboline (2MTHBC). Additionally, samples collected after placebo administration were also quantified for IAA, which is known to be excreted under normal physiological conditions. In each experimental session, 24h urine was collected, subdivided into

the following time intervals relative to ayahuasca (and placebo) administration: 0-4h, 4-8h, 8-16h and 16-24h. The collected urine volume at each time interval was noted, the pooled urine was well mixed and 50 ml aliquots were separated and stored at -80 °C until analysis. Samples underwent a single freeze-thaw cycle prior to analysis. Samples were analyzed with and without enzyme hydrolysis. Enzyme hydrolysis was achieved using β -glucuronidase/sulfatase from limpets (*Patella vulgata*) Type L-II (Sigma-Aldrich, St. Louis, MO, USA) as described by McIlhenny and coworkers [B.23].

B.2.4 Analytical Method

Urine sample analyses were conducted by the methods of McIlhenny and coworkers, which uses HPLC with electrospray ionization and tandem mass spectrometry [B.23]. Thus, 100 μ l of well mixed urine were diluted to a volume of 1.0 ml (900 μ l of LC mobile phase; 97:3 water with 0.1% formic acid:acetonitrile with 0.1% formic acid) and filtered [B.23]. A volume of 10 μ l was injected for the analysis.

The LC/MS/MS method had been validated for the determination of the following compounds: DMT, IAA, DMT-NO, NMT, 5-hydroxy-DMT, dimethylkynuramine, 2MTHBC, 5-methoxy-DMT, harmine, harmaline, tetrahydroharmine, harmol, harmalol and tetrahydroharmol. Thus, analyses were conducted using an Agilent 1200 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent G1367A HiP ALS autosampler, an Agilent G1311A Quaternary micropump, and an Agilent G1332A degasser. An Agilent G131gA TCC column oven operating at 250 °C was interfaced to a TSQ Quantum Access 1.5 SP1 tandem MS (Thermo Fisher Scientific, Waltham, MA, USA) with electrospray ionization (ESI) operated in the positive ion mode.

Chromatographic separation was achieved on a 1.8 µm 4.6 x 50 mm (i.d.) Agilent ZORBAX Eclipse Plus C18 rapid resolution HT threaded column with an Alltech Direct-Connect Column 2 µm pre-filter (Deerfield, IL, USA) using gradient elution. The MS/MS analysis was performed using selected reaction monitoring (SRM) of the protonated molecular ions for the analytes. The spray voltage was 4000 V, sheath gas (nitrogen) pressure 50 psi, capillary temperature 3100 °C, and collision pressure was 1.5 psi. of high purity argon. Generation of detection data and integration of chromatographic peaks were performed by Xcalibur 2.0.7 Thermo Fisher Scientific (Waltham, MA, USA) LCquan 2.5.6 QF 30115 software.

Identification of the compounds was based on the presence of the molecular ion at the correct retention time, the presence of three transition ions and the correct ratio of these ions to one another (+/- 25% relative). The proven limit of quantitation (LOQ) was 5 ng/ml for all compounds. The limits of detection for the compounds examined were comparable to results previously attained, [B.23] ranging from 0.07 ng/ml for DMT-NO to 0.57 ng/ml for harmol. Tetrahydroharmol was observed to have a LOD of 0.17 ng/ml.

B.2.5 Statistics

Descriptive statistics (mean and standard deviation) were used to report the amounts of the different compounds measured. Percentage recoveries were calculated relative to the amount of parent compound administered. Differences in percentage recoveries between enzymatically-treated and non-treated samples were analyzed using paired-samples *t*-tests. Pearson's correlation coefficient was used to explore potential linear relationships between measures. All comparisons were considered statistically significant for p values <0.05.

Table B.1: Mean (SD) DMT and metabolite amounts excreted in each collection interval.

Amounts are expressed as micrograms (μ g) and micromoles (μ mol). Percent recovered relative to the administered DMT dose. Percent recoveries were compared using paired samples *t*-tests between non-enzyme and enzyme treated samples. * p < 0.05, ** p < 0.01,

·	Time Interval							
Non-Enzyme treated	0-4h (μg)	4-8h (µg)	8-16h (µg)	16-24h (µg)				
DMT	237.1(282.2)	224.7(120.1)	22.0(41.3)	0.4(1.2)				
IAA	8785.8(6358.6)	8476.9(6310.6)	6534.3(5790.6)	1768.3(6398.6)				
DMT-NO	3631.5(2356.4)	2535.6(1697.6)	597.1(440.2)	238.2(216.2)				
2MTHBC	28.5(24.3)	24.5(20.7)	12.4(16.7)	29.8(38.7)				
NMT	9.4(15.0)	4.7(8.4)	0.8(1.9)	3.8(5.1)				
Parent & Metabolites								
Enzyme treated								
DMT	186.2(211.7)	181.5(111.1)	10.4(23.7)	0.0(0.0)				
IAA	8497.6(7423.4)	12226.5(6763.6)	9234.0(7890.5)	1519.3(10101.2)				
DMT-NO	4143.3(2188.5)	2829.1(1438.9)	768.3(640.6)	184.1(163.2)				
2MTHBC	29.4(31.8)	18.9(15.6)	9.2(17.1)	22.9(35.2)				
NMT	13.8(12.1)	9.7(11.0)	2.6(3.3)	4.6(5.6)				
Parent & Metabolites								

	Total					
Non-Enzyme treated	0-24h (µg)	0-24h (µmol)	% Recovered			
DMT	484.1(323.1)	2.6(1.7)	0.8(0.5)			
IAA	25565.3(11068.8)	146.1(63.3)	44.2(19.6)			
DMT-NO	7002.5(3429.1)	34.3(16.8)	10.2(4.8)			
2MTHBC	95.3(79.8)	0.5(0.4)	0.16(0.1)			
NMT	18.7(27.3)	0.1(0.2)	0.03(0.05)			
Parent & Metabolites			55.4(22.5)			
Enzyme treated						
DMT	378.0(242.4)	2.0(1.3)	0.6(0.4)**			
IAA	31477.3(12390.5)	179.9(70.8)	54.9(22.8)			
DMT-NO	7924.8(2560.6)	38.8(12.6)	11.6(3.5)			
2MTHBC	80.4(80.0)	0.4(0.4)	0.13(0.1)			
NMT	30.8(25.0)	0.2(0.1)	0.05(0.04)**			
Parent & Metabolites			67.3(23.7)			

Table B.2: DMT and metabolite amounts measured for each study participant in 24h urine in the absence

 (Non-Enz.) and presence (Enzyme) of enzymatic hydrolysis. Percent values refer to the total amounts

 measured. Mean=average for the 10 study participants. SD=standard deviation

	Micromols in 24h					
Subject	DMT	IAA	DMT-NO	2MTHBC	NMT	Total
Non-Enz.						
1	1.6	262.5	70.8	0.9	0.5	336.3
2	5.3	42.3	49.3	0.6	0.0	97.6
3	0.8	143.3	21.8	0.5	0.2	166.6
4	3.9	154.9	23.7	0.1	0.0	182.6
5	1.6	199.1	31.7	1.3	0.2	233.8
6	5.5	139.1	30.4	1.0	0.2	176.1
7	1.4	145.1	21.1	0.1	0.0	167.8
8	1.4	72.6	24.1	0.2	0.0	98.3
9	2.5	110.5	20.7	0.1	0.0	133.8
10	1.8	191.5	49.6	0.3	0.0	243.2
Mean	2.6	146.1	34.3	0.5	0.1	183.6
SD	1.7	63.3	16.8	0.4	0.2	72.4
Enguna						
Enzyme		102.6	53.3			212.1
1	1.1	193.6	53.3	0.6	0.4	249.1
2	3.6	49.9	55.6	0.8	0.2	110.1
3	0.9	96.6	21.8	0.4	0.3	120.0
4	2.6	239.0	34.0	0.0	0.1	275.7
5	1.4	189.0	25.8	1.1	0.3	217.5
6	4.7	259.8	30.2	1.0	0.2	296.0
7	1.1	183.3	38.6	0.0	0.1	223.1
8	1.0	128.1	39.1	0.1	0.0	168.3
9	2.3	188.0	33.1	0.0	0.0	223.4
10	1.3	271.3	57.1	0.2	0.1	330.1
Mean	2.0	179.9	38.8	0.4	0.2	221.3
SD	1.3	70.8	12.6	0.4	0.1	72.0

			Percentage		
Subject	DMT	IAA	DMT-NO	2MTHBC	NMT
Non-Enz.					
1	0.5	78.1	21.0	0.3	0.1
2	5.5	43.3	50.6	0.6	0.0
3	0.5	86.0	13.1	0.3	0.1
4	2.1	84.8	13.0	0.1	0.0
5	0.7	85.1	13.6	0.6	0.1
6	3.1	79.0	17.2	0.5	0.1
7	0.8	86.5	12.6	0.1	0.0
8	1.4	73.8	24.6	0.2	0.0
9	1.8	82.6	15.5	0.1	0.0
10	0.7	78.7	20.4	0.1	0.0
Mean	1.7	77.8	20.1	0.3	0.0
SD	1.6	12.8	11.4	0.2	0.1
Enzyme					
1	0.4	77.7	21.4	0.3	0.2
2	3.3	45.3	50.5	0.8	0.2
3	0.7	80.5	18.1	0.3	0.3
4	1.0	86.7	12.3	0.0	0.0
5	0.6	86.9	11.8	0.5	0.1
6	1.6	87.8	10.2	0.3	0.1
7	0.5	82.2	17.3	0.0	0.0
8	0.6	76.1	23.2	0.1	0.0
9	1.0	84.2	14.8	0.0	0.0
10	0.4	82.2	17.3	0.1	0.0
Mean	1.0	79.0	19.7	0.2	0.1
SD	0.9	12.4	11.6	0.3	0.1

Table B.2 Continued

Table B.3: Mean (SD) amounts of excreted harmala alkaloids and their metabolites. Amounts are expressed as micrograms (μ g) and micromoles (μ mol). Percent recovered relative to the respective parent comound, i.e, harmine for harmol, harmaline for harmalol and tetrahydroharmine for tetrahydroharmol. Percent recoveries were compared using paired samples *t*-tests between non-enzyme and enzyme treated samples. * p < 0.05, ** p < 0.01, *** p < 0.001.

	Time Interval					
Non-Enzyme treated	0-4h (µg)	4-8h (µg)	8-16h (µg)	16-24h (µg)		
Harmine	9.2(10.9)	11.9(9.2)	13.3(16.9)	5.6(8.0)		
Harmaline	48.9(50.2)	150.4(102.4)	140.0(157.9)	174.6(142.3)		
Tetrahydroharmine	472.4(546.8)	1456.2(965.3)	1921.5(2149.5)	2106.4(1363.0)		
Harmol	224.3(177.7)	191.9(187.6)	86.3(67.1)	98.4(49.5)		
Harmalol	260.2(164.1)	296.8(144.9)	187.2(116.7)	262.7(133.6)		
Tetrahydroharmol	370.6(319.6)	438.6(389.7)	393.0(502.4)	502.2(384.1)		
Enzyme treated						
Harmine	20.6(20.1)	32.1(21.7)	34.8(25.5)	31.8(24.1)		
Harmaline	40.6(41.0)	124.2(76.4)	131.6(139.2)	168.3(105.9)		
Tetrahydroharmine	448.2(516.8)	1348.8(846.0)	1624.9(1749.5)	1952.8(1261.7)		
Harmol	10965.8(5057.1)	7984.9(3505.9)	3543.9(1791.5)	5360.4(2162.6)		
Harmalol	625.6(434.2)	917.6(505.4)	778.2(712.5)	973.9(391.4)		
Tetrahydroharmol	534.8(532.3)	656.8(547.6)	693.3(898.4)	820.6(738.7)		

	-	Total	
Non-Enzyme treated	0-24h (µg)	0-24h (µmol)	% Recovered
Harmine	40.0(35.2)	0.2(0.2)	0.04(0.04)
Harmaline	513.8(399.8)	2.4(1.9)	8.5(6.3)
Tetrahydroharmine	5956.5(4178.2)	27.6(19.3)	6.6(4.1)
Harmol	600.8(324.9)	3.0(1.6)	0.6(0.2)
Harmalol	1006.9(248.2)	5.0(1.2)	17.7(4.3)
Tetrahydroharmol	1704.4(1199.5)	7.3(5.2)	1.7(1.1)
Enzyme treated			
Harmine	119.4(74.6)	0.6(0.4)	0.1(0.1)***
Harmaline	464.7(317.0)	2.2(1.5)	7.7(5.0)
Tetrahydroharmine	5374.7(3742.7)	24.9(17.3)	6.0(3.7)**
Harmol	27855.0(6010.2)	140.7(30.4)	27.8(5.3)***
Harmalol	3295.3(1378.3)	16.5(6.9)	56.9(21.1)***
Tetrahydroharmol	2705.5(2320.0)	11.7(10.0)	2.7(2.3)*

B.3 Results and Discussion

Mean (SD) urine volume collected was 1632 (519) ml after placebo and 1535 (366) ml after ayahuasca. These volumes did not differ statistically [t(9)=0.62]. Mean excreted creatinine was 5537(1368) mg/dl after placebo and 6525(1303) mg/dl after ayahuasca. Despite the larger values after ayahuasca, differences were not statistically significant [t(9)=-2.02, p=0.074].

After placebo administration, concentrations for all measured compounds were below the LOD (limit of detection) except for IAA.

The amounts of DMT and its potential metabolites measured at the different collection intervals are presented in Table B.1. To control for physiological IAA, amounts after placebo have been substracted from amounts after ayahuasca. As shown in the table, less than 1% of the administered DMT dose was recovered as the unmetabolized parent compound. Recovery was significantly less following enzymatic treatment. This may be due to degradation of DMT produced by heating and hydrolysis (1 hr at 65°C) [B.23]. The main DMT metabolite found in the urine was IAA, the oxidative deamination product obtained from the MAO pathway. Recovery was increased after enzymatic treatment, although the difference was not statistically significant. The second highest concentration metabolite detected was DMT-NO, with recoveries around 10%. Another 0.2% was made up by 2MTHBC and NMT. The cyclization product 2MTHBC accounted for 0.13-0.16% of the administered DMT dose. Enzymatic treatment increased the amount of NMT by a factor of 1.5.

DMT and metabolite excretion were maximal during the first third of the 24h collection period. Thus, in the first 8 hours after ingestion, 95-97% of all measured DMT (free/total) was

excreted, 68/66% of all measured IAA, 88/88% of all measured DMT-NO, 57/60% of all measured 2MTHBC and 73/77% of all measured NMT were excreted.

In order to address the relative contribution of oxidative deamination and of MAOindependent metabolism, mainly *N*-oxidation, to the biotransformation of DMT, we performed additional calculations. In addition to calculating the percentage of each metabolite relative to administered DMT dose, we assessed the percentage relative to the overall DMT plus metabolites recovered in urine. This way we controlled for potential individual differences in DMT absorption. Table B.2 shows the amounts of DMT and metabolites found in 24h urine for each participant expressed in micromoles and percentage. As shown therein, IAA was roughly 80% of all substances measured. Individual values were as low as 43% and as high as 88%. DMT-NO made up around 20% of the compounds measured, varying between 10% and 50%.

Potential linear relationships between excreted DMT, DMT-NO and IAA were explored. We had hypothesized that an inverse relationship might exist between the amounts of DMT-NO and IAA excreted. However, no statistically significant correlation was found.

Results for the harmala alkaloids and their metabolites are shown in Table B.3. THH was the most abundant alkaloid in urine followed by harmaline and harmine, both prior to and after enzymatic treatment. Relative to the respective administered dose, the highest recovery rates were found for harmaline and THH. Recovery for harmine was two orders of magnitude lower. Enzymatic treatment caused a threefold increase in the amount of recovered harmine. However, this procedure decreased the amounts of recovered harmaline and THH.

Before enzymatic treatment, the most abundant *O*-demethylated product in urine was tetrahydroharmol, followed by harmalol and harmol. However, the largest recovery, was obtained for harmalol. Following enzymatic hydrolysis with glucuronidase/sulfatase, large increases were seen in the measured amount of these three compounds. A near 50-fold increase in the amount of harmol was observed. Harmalol levels were increased by a factor of 3 and tetrahydroharmol by a factor of 1.5. Thus, the degree of conjugation varied greatly from one metabolite to another. Whereas only 2% of harmol was present in free form, free harmalol was 36% and free tetrahydroharmol was as high as 68%. The combined recoveries of harmine plus harmol, harmaline plus harmalol and THH plus tetrahydroharmol after enzymatic treatment were approximately 28%, 65% and 9%, respectively.

Given that the ayahuasca used in the study contained small amounts of harmol and harmalol, we calculated also the percent recoveries of these two compounds relative to the amounts present in the tea. The obtained values were 1028% for harmol and 516% for harmalol, that is 10-fold and 5-fold the amounts ingested with ayahuasca. These figures show that the vast majority of harmol and harmalol recovered in urine after ayahuasca ingestion must necessarily be formed through the metabolic breakdown of harmine and harmaline. These calculations could not be performed for tetrahydroharmol because the analytical standard for this compound was not available prior to the start of the clinical trial, when the ayahuasca batch was analyzed for alkaloid content. Analyses of other preparations of ayahuasca have indicated, however, that tetrahydroharmol is not present in such extracts (preliminary data, Barker).

Disposition of the harmala alkaloids and their metabolites was more evenly distributed throughout the 24h collection period than that of DMT and its breakdown products. In the first eight hours after dosing, 53/45% of all measured harmine (free/total) was excreted. Lower recoveries were obtained in this initial 8h period for harmaline, 39/35%, and THH, 32/33%.

Consistent with these differences, recovery in the first 8h was 68% for total harmol, 47% for total harmalol and 44% for total tetrahydroharmol.

No statistically significant correlations were found between the amounts of harmala alkaloids and their metabolites in 24h urine and the amounts of DMT and its metabolites recovered.

The amounts of β -carbolines recovered in the global 24h collection period expressed in micromoles were used to compute the following metabolic ratios: harmol/harmine, harmalol/harmaline and tetrahydroharmol/tetrahydroharmine. Statistically significant correlations were found between them: harmol/harmine vs. harmalol/harmaline, r=0.800, p<0.01; harmol/harmine vs. tetrahydroharmol/THH, r=0.803, p<0.01; harmalol/harmaline vs. tetrahydroharmol/THH, r=0.967, p<0.001.

These metabolic ratios were also tested for correlations with excreted DMT, DMT-NO, IAA and the metabolic ratios IAA/DMT and DMT-NO/DMT. However, no statistically significant results were found.

In the present study we assessed the urinary disposition of ayahuasca alkaloids and their metabolites following administration of a lyophilized sample of the tea to humans. In line with previous findings after intramuscular administration [B.18], DMT was found to undergo extensive metabolism, with less than 1% being detected unchanged in urine and increased IAA excretion.

Early investigations in humans had found lack of psychoactivity when pure DMT was administered alone *per os* in doses as high as several hundred milligrams [B.26]. These studies found IAA as a degradation product of DMT in urine [B.6]. A more recent study assessing human plasma concentrations of DMT following its i.v administration found DMT to be measurable in blood at 30 minutes but almost undetectable at 1h [B.27]. Our present findings support the notion that MAO plays a prominent role in the degradation of DMT, as previously noted by other researchers [B.21-B.22, B.28-B.32]. However, MAO-inhibition after ayahuasca appears to be either incomplete or short-lived, as large amounts of IAA were already found in the first four hour collection interval. Partial inhibition of MAO by the harmalas in ayahuasca appears to be sufficient to allow psychoactive effects.

Another interesting finding is that MAO-catalyzed oxidative deamination is not the only metabolic pathway available to DMT when administered together with the β -carbolines in ayahuasca. We found DMT-NO to be a major metabolite accounting for 20% of all tryptamine derivatives measured in urine and 10% of the administered DMT dose. The cyclization product 2MTHBC and the *N*-demethylation derivative NMT were also found. Studies with pure tryptamines and tryptamine derivatives, including DMT, 5-MeO-DMT, and 5-OH-DMT, have found oxidative deamination by MAO-A to be a major metabolic route in brain, liver and kidney *in vitro*, producing the corresponding indoleacetic acid [B.32-B.35]. IAA has been identified in both rodent [B.35] and human urine [B.6] following DMT administration but the amount recovered represented only 2.7% and 8.3%, respectively of the dose administered, with no detectable DMT being observed. In another study, ^[14C]-IAA was identified as the major metabolite representing up to 23% of the radioactivity in the blood of rabbits 60 min after i.v. injection of ^[14C]DMT. However, the majority of radioactivity, and thus other possible metabolites, was not successfully identified [B.36].

In line with our findings after ayahuasca, *in vitro* studies have shown that other pathways besides oxidative deamination also contribute to DMT metabolism. Studies of DMT metabolism in vitro have identified DMT-NO as a major NADH dependent metabolite using mouse liver homogenates [B.20], liver microsomal fractions from rabbits [B.37], as well as rat brain homogenates [B.21]. Two of these studies also identified NMT as a metabolite of DMT [B.21, B.37]. *N*-oxidation has also been identified as an important metabolic pathway of DMT *in vivo* [B.31], while *N*-demethylation seems to function as a minor degradation route [B.31]. NMT could also act as a substrate for MAO and become further metabolized to IAA. Cyclization to form the beta-carboline species 2-MTHBC has also been shown to be an alternative metabolic pathway *in vivo* and *in vitro* [B.21, B.38].

DMT-NO does not seem to function as an intermediate during the formation of IAA by MAO-A but it does appear to represent the major metabolite of DMT in the absence of or after inhibition of mitochondrial MAO [B.20, B.30]. MAO inhibition could consequently shift metabolism from oxidative deamination to *N*-oxidation and the above mentioned alternative routes as a compensatory metabolic mechanism. Sitaram's group demonstrated that iproniazid inhibited the formation of IAA from DMT in liver homogenates although not the formation of DMT-NO [B.32]. Iproniazid was found to increase the levels of DMT *in vivo* in rat brain, liver, kidney and blood as well as DMT-NO in rat liver [B.31], and urinary excretion of unmetabolized DMT, DMT-NO and NMT [B.30]. In the present study we found a 80:20 ratio for IAA:DMT-NO after ayahuasca. A future study could address the metabolism of DMT in humans after oral administration without the harmalas and evaluate whether this ratio is shifted toward lower DMT-NO formation in the absence of MAO inhibition.

The β -carbolines appeared to also undergo extensive metabolism with low urine recoveries. Harmine appeared to be the most metabolically labile of the three, as only 0.1% of the parent compound was recovered unchanged. In agreement with previous research that had shown harmine and harmaline to undergo *O*-demethylation [B.39-B.42], we found large amounts of harmol and harmalol in urine. These findings are in line with results from a clinical study involving oral dosing with ayahuasca and in which these compounds were measured in plasma [B.17]. More recently, harmol and harmalol were found in urine in a three subject sample following ayahuasca intake [B.23]. Enzymatic treatment of the samples in the present study showed that most harmol and harmalol was excreted as sulfate and glucuronide conjugates, as previously found by McIlhenny and coworkers [B.23]. Here we also found for the first time that tetrahydroharmol, the *O*-demethylation product of tetrahydroharmine, is also formed *in vivo* following ayahuasca.

Recoveries of each harmala alkaloid plus its *O*-demethylation product were lower than expected. Whereas harmaline+harmalol in urine accounted for 65% of the total harmaline dose administered with ayahuasca, harmine+harmol only reached 28% of the administered parent comopound; and percentage recovery for THH+tetrahydroharmol was as little as 9%. A potential explanation is that THH undergoes an intense first pass effect and does not reach systemic circulation. Although low plasma concentrations have been observed for harmine after oral administration of ayahuasca [B.17], THH levels in blood have consistently been found to be quite high [B.17, B.43]. A plausible alternative explanation is that the harmalas, and particularly THH, are degraded by other metabolic routes. In this respect, various hydroxylated metabolites have been described following incubation of harmine in mouse liver microsomes [B.40]. Despite this possibility and the large variation in recoveries observed, it is worth noting that the calculated metabolic ratios in the present study showed a high degree of correlation, suggesting a common enzymatic route for the *O*-demethylation of the parent compounds.

B.4 Conclusion

To conclude, the present results show that *N*-oxidation is also a major degradation pathway of DMT in humans when administered together with β -carbolines in ayahuasca. This finding demonstrates the existence of an alternative metabolic route for biotransformation by MAO. Also, that *O*-demethylation plus conjugation is an important but probably not the only degradation route for the harmala alkaloids in humans. Finally, we propose that future investigations address the metabolism of oral DMT in humans in the absence of β -carbolines, in order to assess the contribution of the different metabolic pathways to its degradation under physiological conditions. This would allow us to estimate the degree of metabolic "shift" induced by the harmala alkaloids in ayahuasca.

B.5 References

- [B.1] R.E. Schultes, A. Hofmann. *The botany and chemistry of hallucinogens*. Charles C. Thomas, Springfield, **1980**.
- [B.2] R.E. Schultes, A. Hofmann. *Plants of the gods: origins of hallucinogenic use*. A. van der Marck Editions, New York, **1987**.
- [B.3] K.W. Tupper. The globalization of ayahuasca: harm reduction or benefit maximization. *Int. J. Drug Policy* 2008, 19, 297–303.
- [B.4] N.S. Buckholtz, W.O. Boggan. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: structure-activity relationships and substrate specificity. *Biochem. Pharmacol.* **1977**, *26*, 1991–1996.

- [B.5] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β -carboline constituents of ayahuasca. J. *Ethnopharmacol.* **1984**, *10*, 195–223.
- [B.6] S. Szára S. Dimethyltryptamine: its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia* **1956**, *12*, 441-442.
- [B.7] S. Szára. The comparison of the psychotic effect of tryptamine derivatives with the effects of mescaline and LSD-25 in self-experiments. in *Psychotropic Drugs*, (Eds: S. Garattini, V. Ghetti). Elsevier, Amsterdam, **1957**, pp. 460-467.
- [B.8] R.J. Strassman, C.R. Qualls, E.H. Uhlenhuth, R. Kellner. Dose-response study of N,Ndimethyltryptamine in humans, II. Subjective effects and preliminary results of a new rating scale. Arch. Gen Psychiatry 1994, 51, 98–108.
- [B.9] J. Riba. Human Pharmacology of Ayahuasca [Doctoral Thesis]. Universitat Autònoma de Barcelona, 2003. Available at: http://www.tdx.cat/handle/10803/5378 [23 January, 2012]
- [B.10] J. Riba, S. Romero, E. Grasa, E. Mena, I. Carrió, M.J. Barbanoj. Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. *Psychopharmacology* 2006,186, 93–98.
- [B.11] R.A. Glennon, M. Dukat, B. Grella, S. Hong, L. Costantino, M. Teitler, C. Smith, C. Egan, K. Davis, M.V. Mattson. Binding of β-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors. *Drug Alcohol Depend.* 2000, 60, 121–132.
- [B.12] P.A. Pierce, S.J. Peroutka. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* 1989, 97, 118–122.
- [B.13] D.J. McKenna, D.B. Repke, L. Lo, S.J. Peroutka. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor sybtypes. *Neuropharmacology* 1990, 29, 193–198.
- [B.14] D. Fontanilla, M. Johannessen M, A.R. Hajipour, N.V. Cozzi, M.B. Jackson, A.E. Ruoho. The hallucinogen *N*,*N*-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science* 2009, *116*, 1591-1599.
- [B.15] T.S. Ray. Psychedelics and the human receptorome. Plos One 2010, 5: e9019. doi:10.1371/journal.pone.0009019
- [B.16] J. Riba, A. Rodriguez-Fornells, G. Urbano, A. Morte, R. Antonijoan, M. Montero, J.C. Callaway, M.J. Barbanoj. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology* 2001, 154, 85-95.
- [B.17] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J. Pharmacol. Exp. Ther. 2003, 306, 73–83.

- [B.18] J. Kaplan, L.R. Mandel, R. Stillman, R.W. Walker, W.J.A. VandenHeuvel, J.C. Gillin, R.J. Wyatt . Blood and urine levels of N,N-dimethyltryptamine following administration of psychoactive dosages to human subjects. *Psychopharmacologia* 1974, 38, 239-245.
- [B.19] J. Ott. Pharmacotheon: entheogenic drugs, their plant sources and history. Natural Products Co., Kennewick, Washington, 1993.
- [B.20] M.S. Fish, N.M. Johnson, E.P. Lawrence, E.C. Horning. Oxidative N-dealkylation. Biochem Biophys Acta 1955, 18, 564-565.
- [B.21] S.A. Barker, J.A. Monti, T. Christian. Metabolism of the hallucinogen *N*,*N*-dimethyltryptamine in rat brain homogenates. *Biochem Pharmacol* **1980**, *29*, 1049-1057.
- [B.22] B.R. Sitaram, W.R. McLeod. Observations on the metabolism of the psychotomimetic indolealkylamines: implications for future clinical studies. *Biol Psychiatry* 1990, 28, 841-848.
- [B.23] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomed Chromatogr.* 2011, 25, 970-984.
- [B.24] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed Chromatogr.* 2012, 26, 301-313.
- [B.25] R.G. Dos Santos, M. Valle, J.C. Bouso, J.F. Nomdedéu, J. Rodríguez-Espinosa, E.H. McIlhenny, S.A. Barker, M.J. Barbanoj, J. Riba. Autonomic, neuroendocrine, and immunological effects of ayahuasca: a comparative study with d-amphetamine. J. Clin. Psychopharmacol. 2011, 31, 717-726.
- [B.26] W.J. Turner, S. Merlis. Effect of some indolealkylamines on man. Arch Neurol Psychiatry 1959, 81, 121-129.
- [B.27] R.J. Strassman, C.R. Qualls CR (1994) Dose-response study of N,N-dimethyltryptamine in humans, I. Neuroendocrine, autonomic and cardiovascular effects. *Arch Gen Psychiatry* 1994, 51, 85-97.
- [B.28] S.A. Barker, J.A. Monti, S.T. Christian. N,N-dimethyltryptamine: an endogenous hallucinogen. *Int. Rev. Neurobiol.* 1981, 22, 83-110.
- [B.29] O. Suzuki, Y. Katsumata, M. Oya. Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem Pharmacol* 1981, 30, 1353–1358.
- [B.30] B.R. Sitaram, L. Lockett, G.L. Blackman, W.R. McLeod. Urinary excretion of 5methoxy-*N*,*N*-dimethyltryptamine, *N*,*N*-dimethyltryptamine and their *N*-oxides in the rat. *Biochem. Pharmacol.* 1987, *36*, 2235-2237.

- [B.31] B.R. Sitaram, L. Lockett, R. Talomsin, G.L. Blackman, W.R. McLeod. *In vivo* metabolism of 5-methoxy-*N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine in the rat. *Biochem. Pharmacol.* **1987**, *36*, 1509-1512.
- [B.32] B.R. Sitaram, R. Talomsin, G.L. Blackman, W.R. McLeod. Study of metabolism of psychotomimetic indolealkylamines by rat tissue extracts using liquid chromatography. *Biochem. Pharmacol.* 1987, 36, 1503-1508.
- [B.33] F. Raynaud, P. Pévet. 5-Methoxytryptamine is metabolized by monoamine oxidase A in the pineal gland and plasma of golden hamsters. *Neurosci Lett.* **1991**, *123*, 172-174.
- [B.34] R.W. Fuller, H.D. Snoddy, K.W. Perry. Tissue distribution, metabolism and effects of bufotenine administered to rats. *Neuropharmacol.* 1995, 34, 799-804.
- [B.35] V. Ersparmer. Observations on the fate of indolealkylamines in the organism. J. Physiol., 1955, 127, 118-133.
- [B.36] L.R. Mandel, R. Prasad, B. Lopez-Ramos, R.W. Walker. The biosynthesis of dimethyltriptamine in vivo. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *16*, 47-58.
- [B.37] S. Szára, J. Axelrod. Hydroxylation and N-demethylation of N,N-dimethyltryptamine. *Experientia* 1959, 15, 216-217.
- [B.38] S.A. Barker, J.M. Beaton, S.T. Christian, J.A. Monti, P.E. Morris. In vivo metabolism of α,α,β,β-tetradeutero-N,N,dimethyltryptamine in rodent brain. *Biochem. Pharmacol.* 1984, *33*, 1395-1400.
- [B.39] T.A. Slotkin, V. DiStefano, W.Y.W. Au. Blood levels and urinary excretion of harmine and its metabolites in man an rats. *J. Pharmacol. Exp. Ther.* **1970**, *173*, 26-30.
- [B.40] D.J. Tweedie, M.D. Burke. Metabolism of the beta-carbolines, harmine and harmol, by liver microsomes from phenobarbitone- or 3-methylcholanthrene-treated mice. Identification and quantitation of two novel harmine metabolites. *Drug Metab. Dispos.* 1987, 15, 74–81.
- [B.41] A. Yu, J.R. Idle, K.W. Krausz, A. Küpfer, F.J. Gonzalez. Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic β -carboline alkaloids harmaline and harmine. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 315-322.
- [B.42] A. Yu. Indolealkylamines: biotransformations and potential drug–drug interactions. AAPS J. 2008, 10, 242–253.
- [B.43] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, E.N. Andrade, E.O., Andrade, D.C. Mash. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *J Ethnopharmacol* 1999, 65, 243-256.

Appendix C. A Critical Review of Reports of Endogenous Psychedelic N, N-Dimethyltryptamines in Humans: 1955-2010*

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April 17, 2012

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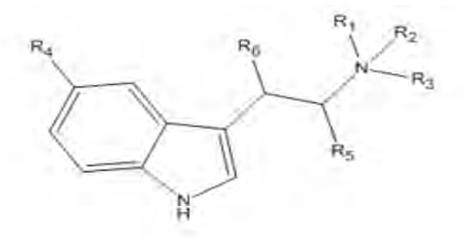
Regarding: Contributions of Ethan H. McIlhenny to the manuscript "A Critical Review of Reports of Endogenous Psychedelic N, N-Dimethyltryptamines in Humans: 1955-2010" by Steven A. Barker, Ethan H. McIlhenny and Rick Strassman

As Mr. McIlhenny's major professor and as first author of the above mentioned manuscript, I have firsthand knowledge of the contributions made by the candidate in its creation. While not the first author, Mr. McIlhenny nonetheless contributed in a significant and collaborative way to the creation of the rather voluminous Table in the manuscript that summarizes 69 different studies on endogenous hallucinogens; no small undertaking. Mr. McIlhenny also contributed to the information and ideas discussed regarding the metabolism of the endogenous hallucinogens, especially in the sections that reference his published work in this area using ayahuasca as a model, and to the overall writing and editing of the manuscript. I chose to be first author because of my own contributions to its writing and the fact that it was an invited review to be published in a special issue of the journal Drug Testing and Analysis.

Given the significant collaborative contributions made by Mr. McIlhenny in this regard, it is appropriate that this manuscript be included in his dissertation.

C.1 Introduction

Three indole alkaloids that possess differing degrees of psychotropic/psychedelic activity have been reported as endogenous substances in humans. These compounds, all metabolites of tryptophan, are N,N-dimethyltryptamine (DMT, C.1.1, Figure C.1), 5-hydroxy-DMT (bufotenine, HDMT, C.1.2) and 5-methoxy-DMT (MDMT, C.1,3). Their presence has been reported in human cerebrospinal fluid (CSF), urine, and/or blood utilizing either paper and/or thin layer chromatography (TLC), direct ultraviolet (UV) or fluorescence (FI) measurements, gas chromatography (GC) using various sensors (nitrogen-phosphorous detector [NPD]; electron capture detector [ECD]; mass spectrometry detector [MSD]), high-performance liquid chromatography (HPLC) using UV and/or FI detection, HPLC-radioimmunoassay, HPLC-electrochemical detection, and liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Tables C.1-C.3, references [C.1-C.69]). Indeed, the review of the 55 year history of the development of methodology for the analysis of these compounds shows how closely it has paralleled the evolution of analytical technology itself, with each researcher seeking more specific and sensitive techniques.



DMT: $R_1 = R_3 = CH_3$; $R_2 = \emptyset$; $R_4 = H$; $R_5 = R_6 = H_2$, **1** HDMT: $R_1 = R_3 = CH_3$; $R_2 = \emptyset$; $R_4 = OH$; $R_5 = R_6 = H_2$, **2** MDMT: $R_1 = R_3 = CH_3$; $R_2 = \emptyset$; $R_4 = CH_3O$; $R_5 = R_6 = H_2$, **3** NMT: $R_1 = CH_3$; $R_2 = \emptyset$; $R_3 = H$; $R_4 = H$; $R_5 = R_6 = H_2$, **4** DMT-NO: $R_1 = R_3 = CH_3$; $R_2 = O^+$; $R_4 = H$; $R_5 = R_6 = H_2$, **5**

Figure C.1. Structures of the compounds discussed.

A renewed interest in these compounds as naturally occurring substances in humans has occurred, in part, due to DMT's recent characterization as an endogenous substrate for the ubiquitous sigma 1 receptor [C.70] and for its possible action at trace amine receptors [C.71]. In both cases, the roles of DMT and the receptors themselves in regulating some aspect(s) of human physiology are poorly understood. Given their known psychedelic effects, there remains an interest in their possible role in naturally occurring altered states of consciousness such as psychosis, dreams, creativity and imagination, religious phenomena and even near-death experiences [C.72]. Although the vast majority of research into the presence of these compounds sought their role in mental illness, no definitive conclusions yet exist. A determination of the role of these compounds in humans awaits further research, much of which awaits the development of adequate analytical methodology.

Interest in DMT has also increased because of the burgeoning use and popularity of the religious sacrament ayahuasca which contains DMT and several harmala alkaloids, which serve to make DMT orally active. Ayahuasca tourism in South America and the establishment of syncretic churches using ayahuasca as a sacrament [C.73, C.74] have stimulated research into the mechanisms of its effects and its possible use as a therapeutic [C.75]. The resumption of human research characterizing DMT's psychopharmacology [C.76-C.84] and the ongoing use of pure DMT for therapeutic and recreational purposes have also focused interest on this and related psychedelics. The dimethylated-tryptamines (DMTs) increasing visibility within medical, non-medical, religious and/or recreational contexts [C.75] reinforce the importance of determining their endogenous role.

This review addresses several fundamental issues regarding these three endogenous psychedelics. For example, are DMT, HDMT and/or MDMT truly present in humans [C.85]? Early criticisms of reports of endogenous psychedelics were directed at the fact that rather non-specific chemical tests were being applied, double-blind analyses were not always being performed and dietary or medication sources were not always adequately ruled out as responsible for the identifications [C.2, C.12]. Further, it was claimed that possible artifacts produced from the extraction solvents and conditions of analysis may have led to misidentification of the DMTs in some early studies [C.20] and, more recently, that the use of halogenated solvents in the analysis may have affected their detection [C.86]. Biological factors that may have affected their rapid metabolism [C.87, C.88]. Finally, there have been concerns that the studies searching for their presence and an association with specific clinical disorders have failed to understand and fully characterize their metabolism or monitor their metabolites [C.88-C.91].

To address these issues we have undertaken a critical review of 69 published studies reporting the detection or detection and quantitation of these compounds in human body fluids. In reviewing this literature, we address the methods applied and the criteria used in the determination of the presence of DMT, MDMT, and HDMT. We begin with the original report of the presence of bufotenin (HDMT) in human urine in 1955 using paper chromatography [C.1] and end with the most recent report concerning the presence of bufotenin (HDMT) in human urine using LC/MS/MS [C.69].

We will be addressing the following questions; How valid were early studies regarding the presence and/or quantities of these compounds in human cerebrospinal fluid (CSF), blood and/or urine? Were the analytical methodologies and the identification criteria adequate? Are they truly there? When present, are they of dietary origin? When and where in the human body are they produced? Can we influence their detection in biological samples by pharmacologically inhibiting their metabolism by monoamine oxidase (MAO). How does turnover rate and metabolism of these substances influence their detectability? Have the precursors and/or metabolites of these compounds been adequately monitored? Is monitoring these compounds in biological samples such as CSF, blood and/or urine the best, or even most practical way to determine their role? What will such data tell us about the function of these compounds? Where does the research on endogenous psychedelics go from here?

C.2 Historical Perspective

The search for endogenous psychedelics soon followed the discovery of the psychedelic effects of mescaline and lysergic acid diethylamide (LSD) in humans. Observations of these effects gave rise to hypotheses that they were related to the symptomology observed in a heterogeneous group of mental disorders, especially psychoses—either mania or schizophrenia

[C.92]. It was proposed that schizophrenics may biochemically produce similar compounds as "schizotoxins" [C.93]. A search for mescaline-like compounds proved unrewarding [C.94], but in studies examining urine samples for serotonin-like compounds, researchers reported in 1955 [C.1] and 1956 [C.2] the presence of 5-hydroxy-N,N-DMT (HDMT, bufotenin) in humans. Subsequently, Axelrod (1961) [C.95] reported the presence of an enzyme capable of N-methylating indole-ethylamines and producing DMTs. Following these reports, attention began to focus in earnest on the possible endogenous formation of the indole-ethylamine psychedelics. During the next fifty years many studies reported finding DMT, HDMT and/or MDMT in human CSF, urine and/or blood. Most of these studies sought differences in levels between controls and psychiatric, especially psychotic, patients. Some studies claimed higher concentrations and significant differences in levels between the groups; some reported not finding the compounds at all in either patients or controls.

It is of interest to note that in its original conception, the schizotoxin hypothesis proposed that the formation of an endogenous psychedelic schizotoxin would be an aberration of metabolism and that "normals" would not form such compounds [C.92]. However, numerous studies subsequently reported finding one or more of these compounds in controls as well as patients. Despite many such efforts, there has yet to be demonstrated a definitive link between the blood and/or urine levels of these compounds and any psychiatric diagnosis [C.85, C.93].

The earliest studies (1950s-1960s) in the search for endogenous psychedelics applied the technology available at the time. These were mainly paper and thin-layer chromatography (TLC) using different reagents as visualization (color development) sprays, as well as comparing Rf values with spotted standards as the criteria for identification. In 1967, thin-layer spots were isolated and derivatized in an attempt to confirm their identification by gas-liquid-

chromatography (GC) using a flame-ionization detector (FID) [C.21]. In this case, Rf values from TLC and relative retention time (Rt) from GC that were consistent with known standards served as the confirmation criteria. Subsequent studies applied this technology utilizing other detectors, such as nitrogen-phosphorous, electron capture and, eventually, mass spectrometry (MS). In many of these studies the sole criterion for identification was retention time compared to a reference standard. However, in the case of the early MS data, the presence of a single major fragment ion [C.38] (m/z 58) or one or two minor ions [C.39], served as additional confirmation. Liquid chromatography with UV and fluorescence detection was also applied, with the collected peaks being confirmed by GC/MS in some cases. As the analytical technology evolved, so too did the methods applied to detect and measure the compounds of interest, with resultant gains in sensitivity, specificity and validity.

The most recent methods have applied LC/MS/MS technologies in combination with more stringent confirmation criteria [C.67-C.69]. These criteria are based on specific protonated molecules, fragment ions and their ratios to one another, and on relative retention times. However, as the criteria have become more exacting and the specificity of the methodology has improved, detection of the endogenous psychedelics appears to have become less frequent and, where detection has occurred, at significantly lower concentrations than originally reported.

Tables C.1-C.3 are a compilation of 69 studies directed toward detecting or detecting and quantitating the three indole psychedelics--DMT, HDMT, and MDMT--in human (patient and/or control) CSF, blood, and/or urine. The entries for each study were taken from copies of the original publications. In some cases, the published studies neglected to address the relevant analytical issues reviewed.

Table C.1; Review of 69 studies regarding endogenous psychedelics showing the year, reference, compounds analyzed, type of sample and method of extraction. Acronyms and abbreviations; IV, intravenous; HNMT, 5-hydroxy-N-methyltryptamine; ext, extraction; vol, volume; w/wo, with or without; evap, evaporate; ppt, precipitate; sat., saturated; TLC, thin-layer chromatography; cent, centrifuge; TFAA, trifluoro-acetic anhydride; SPE, solid phase extraction; LC, liquid chromatography.

Year	Author	Compounds Analyzed	Collection	Extraction Method
1055			24-hour urine 10 ml portions, HCl;	Evap, Acetone, evap, MeOH, evap, AlO3
	Bumpus and Page 1	HNMT, HDMT	turease	column
1956		HNMT, HDMT	24-hour urine; 75-120 ml extracted	Zeo-Karb 226 resin, EtOH/acetone ppt, evap
1961		HDMT	1 L of urine	NaHCO3 sat., butanol, evap, acetone
1961	Fischer et al. ⁴	HDMT	1 L of urine 8 hour urines; IV/oral 14C serotonin	NaOH pH 9, butanol, evap, acetone
1961	Feldstein et al. ⁵	HDMT	(130 µg) 24-36 hour urine; ext vol 500 mg	not described Amberlite CG-120, CG-50; ethanol-acetone
1962	Perry et al.6	HDMT, conjugate	creatinine; w/wo hydrolysis	ppt
1963	Brune et al. ⁷	HDMT; DMT	24 hour urine	pH 10, ethyl ether ext, evap, acetone
1963	Perry ⁸	HDMT; DMT	24 or 48 hour urine; ext vol 500 mg creatinine	Amberlite CG-120, CG-50; ethanol-acetone ppt pH 10, ethyl ether-butanone ext, evap,
1063	Sprince et al.9	DMT, HDMT	24 hour wine	acetone
1905	oprince et al.	DMI, IDMI	24-36 hour urine; ext vol 250-350 mg	Amberlite CG-120, CG-50; ethanol-acetone
1963	Perry and Schroeder ¹⁰	HDMT	creatinine	ppt Extensive multi-step extraction, ppt and
1965	Franzen and Gross ¹¹	DMT, HDMT	blood and urine (24 hour)	clean-up
1965	Siegel ¹² Nishimura and	HDMT	fresh urine, 100 ml fresh urine vol 500-1,000 mg	pH 10, ethyl ether ext, evap, acetone
1965	Gjessing ¹³	HDMT	creatinine	Dowex 50, Amberlite CG 50,
1965	Takesada et al.14	HDMT	24 hour urine	Ext, Dowex 50 column, alumina column
1966	Runge et al.15	HDMT	1 L of urine	pH 8-9, butanol ext, acetone ppt, acetone
1966	Perry et al.16	DMT. HDMT	48 hour urine	Dowex 50W, Amberlite CG-50; HC1 hydrolysis
1,00	r cury et ur		to how there	Lyactyus
1966	Heller ¹⁷	HDMT	1 L of urine	NaHCO3 sat., butanol, evap, acetone
	Fischer and Spatz ¹⁸	HDMT	100 ml fresh urine	NaCO3, ether ext, evap, acetone
		1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 - 1999 -	24 hour urine; vol 600 mg creatinine	
1967	Kakimoto et al.19	DMT, HDMT	analyzed	Ext, Dowex 50 column, alumina column
1967	Tanimukai ²⁰	HNMT, HDMT, NMT, DMT, MDMT	24 hour urine; 1/4th used in assay	Dowex 50 W X2; w/wo HCl hydrolysis

Table C.1 Continued

1967	Tanimukai et al.21	HDMT	24 hour urine;1/3rd used in assay	cation exchange resin; w/wo HCL hydrolysis
1967	Tanimukai et al.22	HDMT	24 hour urine; 1/4th used in assay	Dowex 50 W X2; HCl hydrolysis
1967	Acebal and Spatz ²³	HDMT	100 ml urine	NaCO3, ether ext, evap, acetone column chromatography, sublimation,
1968	Faurbye and Pind24	HDMT	24 hour urine, hydrolyzed at pH1.6	paper/TLC
1969	Sireix and Marini ²⁵	HDMT	100 ml fresh urine	NaCO3, ether ext, evap, acetone pH 10 NaOH, ethyl acetate; diazo-reagent o
1969	Spatz et al.26	HDMT	50 ml fresh urine; 100 ml fresh urine	TLC pH 10 NaOH, ethyl acetate; diazo-reagent
1970	Fischer and Spatz ²⁷	HDMT	50 ml fresh urine; acid hydrolysis	and TLC
1970	Saavedra and Udabe ²⁸	HDMT	50 ml fresh urine; acid hydrolysis	pH 10 NaOH, ethyl acetate; diazo-reagent and TLC
1970	Tanimukai et al.29	HNMT, HDMT, DMT, MDMT	24 hour urine; 1/4th used in assay	Dowex 50 W X2; HCI hydrolysis
1970	Heller et al. ³⁰	DMT, MDMT, HDMT	fasting blood, oxalate tube; acid hydrolyzed	Dowex 50; HCl ext and ethyl acetate at pH 10.2
1971	Narsimhachari et al.31	DMT, MDMT, HDMT	24 hour urine; 75% used in assay	Dowex 50; HCl ext and ethyl acetate at pH 10.2 Dower 50; HCl ext and ethyl acetate at pH
1971	Narasimhachari et al.32	NMT, DMT, MDMT	fasting blood, oxalate tube 50 ml moming urine; w/wo	Dowex 50; HCl ext and ethyl acetate at pH 10.2 Liquid Liquid ext, w/wa glucuronidase
1971	Fischer et al.33	HDMT, glucuronide	50 ml morning urine; w/wo glucuronidase	Liquid-Liquid ext, w/wo glucuronidase treatment
				Dowex 50; HCl ext and ethyl acetate at pH
1972	Himwich et al. ³⁴ Narasimhachari et	HDMT, DMT, MDMT	24 hour urine	10.2 Franzen and Gross; HCl ext ethyl acetate a
1972		HDMT, DMT, MDMT	24 hour urine plasma; DMT stable for 60 days at 6	pH 10.2 HCL ext acid pH with CHCl3, pH 9, ext
1973	Walker et al.36	DMT	degrees C	CHC13, evap HCL ext acid pH with CHC13, pH 9, ext
1973	Wyatt et al. ³⁷ Narasimhachari and	DMT	plasma	CHC13, evap Dowex 50; HC1 ext and ethyl acetate at pH
1973	Himwich ³⁸	DMT, HDMT	24-hour urine	10.2
1974	Lipinski et al. ³⁹	DMT	plasma separated by centrifugation Heparinised plasma or whole blood;	HCL ext acid pH with CHC13, pH 9, ext CHC13, evap HCL ext acid pH with CHC13, pH 9, ext
1974	Bidder et al. ⁴⁰ Narasimhachari et	DMT	24 hr urine	CHCl3, evap Dowex 50; HCl ext and ethyl acetate at pH
1974		HDMT, DMT, MDMT	24 hour urine	10.2
1075	o		244	Dowex 50; HCl ext and ethyl acetate at pH
	Carpenter et al. ⁴² Christian et al. ⁴³	DMT, HDMT	24 hour urine, 90% used in assay	10.2 Dependencies timid timid and CIDCD
	Narasimhachari and Himwich ⁴⁴	DMT, MDMT	Cerebrospinal fluid	Deproteinization, liquid-liquid ext, CH2Cl2 Dowex 50; HCl ext and ethyl acetate at pH
	Angrist et al.45	DMT, HDMT DMT	24 hour urine, 80% used in assay non-fasting blood; heparin; 10 ml assayed	10.2 HCL ext acid pH with CHC13, pH 9, ext CHC13, evap
	Rodnight et al.46	DMT	24-hour urine	Dowex 50; HCl ext and ethyl acetate at pH 10.2
				Dowex 50; HCl ext and ethyl acetate at pH
	Murray and Oon47	DMT	24-hour urine	10.2
	Huszka et al.48	HDMT, DMT, MDMT	24 hour urine; 1/3rd used in assay	Dowex 50 W X2; HC1 hydrolysis HCL ext acid pH with CHC13, pH 11, ext
	Cottrell et al.49	HDMT	24 hour urine	CHC13, evap
1977	Oon et al. ⁵⁰	DMT, NMT	24-hour urine; 50% used; DMT, NMT stable 90 days at -15 C	50% concentrated and extracted with toluene purified by TLC, derivatized with TFAA
		DACT AD CT	24-hour urine; 50% used in assay	50% concentrated and extracted with toluene
1977	Oon and Rodnight ⁵¹	DMT, NMT	2, 10 , 1 , 10	purified by TLC, derivatized with TFAA

Table C.1 Continued

	Corbett et al.53	DMT, MDMT	Cerebrospinal fluid 10 ml whole blood; arterial and	CH2C12 HCL ext acid pH with CHC13, pH 9, ext
	Walker et al.54	DMT	venous	CHC13, evap
	Murray et al.55	DMT, NMT	24-hour urine; 50% used in assay	acidified with HCl 50% concentrated and extracted with toluene purified by TLC, derivatized with TFAA
	Checkley et al. ⁵⁶ Raisanen and Katkkainen ⁵⁷	DMT DMT. HDMT	24 hour wine; 50% used in assay 150 ml morning wine samples	acidified with HCl 50% concentrated and extracted with toluene purified by TLC, derivatized with TFAA pH 11, XAD resin, ethyl acetate elution, evap, TLC
1979	Smythies et al. 58	DMT, MDMT	Cerebrospinal fluid Serial 24 hour urine; longitudinal	Deproteinization, liquid-liquid ext, CH2C12
1980	Checkley et al. 59	DMT	study	acidified with HCl 50% concentrated and extracted with toluene purified by TLC, derivatized with TFAA
1983	Uebelhack et al. ⁶⁰	DMT, MDMT	Cerebrospinal fluid 12 hr specimens (8pm-8am); 200 ml	Deproteinization, liquid-liquid ext, CH2Cl2 ion pair ext CHCl3, LC-silica column
1983	Sitaram et al.61	HDMT	assayed	purification pH11, XAD resin, ethyl acetate elution,
1984	Raisanen et al. ⁶²	HDMT	not stated	evap, TLC pH11, XAD resin, ethyl acetate elution,
1988	Karkkainen et al. ⁶³ Karkkainen and	HDMT	morning urine samples	evap, TLC pH11, XAD resin, ethyl acetate elution,
1992	Raisanen ⁶⁴	HDMT	individual urine samples; w/wo nialamide treatment	evap, TLC
1995	Karkkainen et al. ⁶⁵	HDMT	morning urine samples; 50-100 ml	pH11, XAD resin, ethyl acetate elution, evap, TLC centrifugation, direct injection of 80 µl of
1995	Takeda et al.66	HDMT. HNMT	morning urine samples	urine
1995	Takeoa et al.	DMT, MDMT, HDMT.	morning and afternoon urines; 5 ml	urine centrifuged and ext on Oasis SPE
2001	Forsstrom et al. ⁶⁷	NMT	assayed	cartridge
			urine (5ml), plasma or serum (1ml),	
2005	Karkkainen et al.68	DMT, HDMT	stool:	urine cent and ext on Oasis HLB cartridge;
0.00		10000000000000000000000000000000000000	tissues (0.5-1.5g)	Prep LC for blood
2010	Emanuele et al. ⁶⁹	HDMT	random urine samples	urine cent and ext on Oasis HLB cartridge

Table C.2; Review of 69 studies regarding endogenous psychedelics showing the year, reference, compounds analyzed, detection methods, limits of detection (LOD) and confirmation criteria. Acronyms and abbreviations; HNMT, 5-hydroxy-N-methyltryptamine; TLC, thin-layer chromatography; 2-D, two dimensional; GC-FID, gas chromatography-flame ionization detector; derive, derivative; HFBI, heptafluoro-butyryl-imidazole; IS, internal standard; HPLC, high performance liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; ND, not determined; RT, retention time; UV, ultraviolet; TI, total ion; m/z, mass-to-charge ratio; CI, chemical ionization; IA, immunoassay; MRM, multiple reaction monitoring.

6.000	1.1	Compounds	Sector Sector Sector	and a state	California California
Year	Author	Analyzed	Detection Methods	Limit of Detection	Confirmation Criteria
	Bumpus and Page		paper chromatography (1 system), color		
1955	1	HNMT, HDMT	reaction, bioassay	ND	Rf and color (1 system)
1956	Rodnight ²	HNMT, HDMT	paper chromatography (3 systems), color reaction, bioassay	>5 µg/ 24 hour for HDMT	Rf and color (3 systems)
1961	Fischer et al.3	HDMT	paper chromatography (1 system)	ND	Rf and color (1 system)
1961	Fischer et al.4	HDMT	paper chromatography (1 system) paper chromatography and auto-	ND	Rf and color (1 system) Rf and color (1 system).
1961	Feldstein et al. ⁵	HDMT HDMT	radiographs paper chromatography (2-D), color	ND	radioactive spot
1962	Peny et al.6	conjugate	reaction	ND	Rf and color (2-D)
			paper chromatography (2-D), color		
1963	Brune et al.7	HDMT; DMT	reaction	20 ng/ml	Rf and color (2-D)
1963	Peny ⁸	HDMT; DMT	2-D paper chromatography, color reaction	ND	Rf and color (2-D)
1963	Sprince et al. ⁹ Perry and	DMT, HDMT	2-D paper chromatography, color reaction	ND	Rf and color (2-D)
1963	Schroeder ¹⁰ Franzen and	HDMT	paper chromatography (3 systems)	ND	Rf and color (3 systems)
1965	Gross ¹¹	DMT, HDMT	Fluorescence	2ng/ml	Fluoresence reading
1965	Siegel ¹² Nishimura and	HDMT	TLC (1 system), color reaction	0.1 µg/100 ml	Rf and color (1 system)
1965	Gjessing ¹³	HDMT	TLC (2-D), color reaction	ND	Rf and color (2-D)
1965	Takesada et al.14	HDMT	paper chromatography, color reaction	20 µg/24 hour	Rf and color
1966	Runge et al. ¹⁵	HDMT	paper chromatography, color reaction paper chromatography (2-D), color	ND 2 µg/24 hr for DMT and	Rf and color (2-D)
1966	Perry et al.16	DMT, HDMT	reaction	HDMT	Rf and color (2-D)
1966	Heller ¹⁷	HDMT	paper chromatography (2-D), color reaction	ND	Rf and color (2-D)
1967	Fischer and Spatz ¹⁸	HDMT	paper chromatography (2-D), color reaction	ND	Rf and color (2-D)
1967	Kakimoto et al.19	DMT, HDMT	paper chromatography (3 systems), color reaction	10 µg/24 hour	Rf and color (3 systems)

Table C.2 Continued

1967	Tanimukai ²⁰	HNMT, HDMT, NMT, DMT, MDMT	paper and TLC (2-D); color reaction; GC- FID of HDMT	5 ng/ml HDMT; 1 ng/ml others	Rf and color (2-D paper, TLC)
1967	Taninukai et al. ²¹	HDMT	paper chromatography, TLC (2-D), color reaction; GC-FID	>0.1 µg/24 hour	Rf and color (2-D); GC- RT
1967		HDMT	paper and TLC (2-D); color reaction; GC- FID of HDMT	ND	Rf and color (2-D paper, TLC); GC-RT
1967		HDMT	paper chromatography (2-D), color reaction.	ND	Rf and color (2-D)
1968	Faurbye and Pind ²⁴ Sireix and	HDMT	paper chromatography and TLC, color reaction	>0.7 µg/24 hour	Rf and color (paper and 2-D TLC)
1969	Marini ²⁵	HDMT	UV; paper chromatography, color reaction UV of diazo-deriv; paper chromatography,		Rf and color (2-D)
1969	Spatz et al. ²⁶ Fischer and	HDMT	color reaction	ND	UV; Rf and color
1970	Spatz ²⁷	HDMT	UV; TLC, color reaction	ND	UV; Rf and color
1970	Saavedra and Udabe ²⁸	HDMT	UV; TLC, color reaction	ND	UV: Rf and color
		UNINE UDINE			Pf and color (2 Denner
1970	Taninukai et al. ²⁹	HNMT, HDMT, DMT, MDMT DMT, MDMT,	paper and TLC (2-D); color reaction; GC- FID of HDMT	ND	Rf and color (2-D paper, TLC); GC-RT GC-RT and TLC or
1970	Heller et al. ³⁰ Narsimhachari et	HDMT DMT. MDMT.	GC-FID, TLC, and Spectrofluorometry TLC and GC-FID, verified with	2 ng/ml 5µg/ml per 24hour for	spectrofluorometer TLC and GC-FID,
1971	al. ³¹ Narasimhachari et	HDMT	spectrofluorometer TLC and GC-FID, verified with	DMT	spectrofluorometer TLC and/or GC-FID.
1971	al.32	MDMT HDMT.	spectrofluorometer	2 ng/ml	spectrofluorometer
1971	Fischer et al.33	glucuronide	UV; paper chromatography, color reaction	ND	UV; Rf and color
	Sec. 14	HDMT, DMT,	TLC (3 systems), color reaction; verified		
1972	Himwich et al.34 Narasimhachari et	HDMT. DMT.	with spectrofluorometer paper and TLC (2-D); color reaction; GC-	ND	Rf, color and fluoresence Rf and color (2-D); GC-
1972	al. ³⁵	MDMT	FID GC-MS; 2 ft. SE-30 glass capillary	0.05 μg/24 hour 0.5 ng/ml; m/z 202/204,	RT GC-RT, two ions and
1973	Walker et al.36	DMT	column, DMT-d2 IS, TMS deriv	260/262	ratio
	Wyatt et al. ³⁷ Narasimhachari	DMT	GC-MS; 2 ft. SE-30 glass capillary column, DMT-d2 IS, TMS deriv TLC DACA and OPT spray on cellulose	0.5 - 1.8 ng/ml; m/z 202/204, 260/262 Sng/ml HDMT: 1 ng/ml	GC-RT, two ions and ratio Rf and color (2-D); GC-
	and Himwich ³⁸	DMT, HDMT	and silica; GC/MS, 58 m/z only	5ng/ml HDMT; 1 ng/ml DMT	RT; GC/MS 58 mz TI spectrum match with
			GC-MS; 2 ft. SE-30 glass capillary		DMT standard GC-RT, two ions and
1974	Lipinski et al. ³⁹	DMT	column, DMT-d2 IS, TMS deriv GC-MS; 2 ft. SE-30 glass capillary	0.5ng/ml blood 0.05-2 ng/ml; urine	ratio GC-RT, two ions and
	Bidder et al. ⁴⁰ Narasimhachari et	DMT HDMT, DMT,	column, DMT-d2 IS, TMS deriv TLC DACA and OPT spray on cellulose	0.07-0.2 ng/ml 5ng/ml HDMT; 1 ng/ml	ratio Rf and color (2-D); GC-
1974		MDMT	and silica; GC/MS, 58 m/z only	DMT	RT; GC/MS 58 mz TI spectrum match with DMT, HDMT
1075	0	DAGE TEDAGE	TLC DACA and OPT spray on cellulose	5ng/ml HDMT; 1 ng/ml	Rf and color (2-D); GC-
	Carpenter et al.42	DMT, HDMT	and silica; GC/MS, 58 m/z only	DMT DMT 10 pg/ml; MDMT 5	RT; GC/MS 58 mz
	Christian et al.43 Narasimhachari	DMT, MDMT	GC-ECD; packed column TLC DACA and OPT spray on cellulose	pg/ml 5ng/ml HDMT; 1 ng/ml	RT Rf and color (2-D); GC-
1975	and Himwich ⁴⁴	DMT, HDMT	and silica; GC/MS, 58 m/z only GC-MS; 2 ft. SE-30 glass capillary	DMT	RT; GC/MS 58 mz
1976	Angrist et al.45	DMT	column, DMT-d2 IS, TMS deriv GC-FID,TLC on cellulose; GC/MS 2	0.05 ng/ml	RT, two ions and ratio Rf and color; GC-RT;
1976	Rodnight et al. ⁴⁶ Murray and	DMT	patients and pooled (10) extract GC-FID,TLC on cellulose; GC/MS 2	0.5µg/24hour	matching TI MS Rf and color; GC-RT;
1976		DMT HDMT, DMT,	patients and pooled (10) extract TLC and GC-FID, verified with	20 ng /24hour	GC-MS TLC and GC-FID,
1976	Huszka et al.48	MDMT	spectrofluorometer	4 ng/ml	spectrofluorometer
	Cottrell et al.49	HDMT	HFBI derivatives, GC-ECD	<1 nmol/24 hour	RT
1977	Oon et al. ⁵⁰	DMT, NMT	GC/NPD;GC/MS	20ng/24hour for DMT; 50 ng/ml NMT	RT; CI MS confirmation

Table C.2 Continued

1978	Corbett et al. 53	DMT, MDMT	GC-ECD; HFBI derivative	DMT 10 pg/ml; MDMT 5 pg/ml	RT; MS of selected samples
1979	Walker et al. ⁵⁴	DMT	GC/MS, Selective Ion Monitoring capillary column gas chromatography GC-NPD.TLC on cellulose: GC/MS 2	10pg/ml whole blood 20ng/24hour DMT; 50	GC/MS RT, m/z 58 only RT: MS of selected
1979	Murray et al.55	DMT, NMT	patients and pooled (10) extract	ng/24 hour NMT	samples
1979	Checkley et al. ⁵⁶	DMT	GC with nitrogen-sensitive detector	0.5µg/ml per 24hour	RT
1979	Raisanen and Karkkainen ⁵⁷	DMT, HDMT	TMS derivatives; GC/MS, multiple ion detection	0.1-0.15 ng/ml DMT; 0.25-0.3 ng/ml HDMT	RT, molecular ions or fragments
1979	Smythies et al.58	DMT, MDMT	GC/MS selected ion monitoring; d4-DMT, d4-MDMT IS	70 pg/ml DMT, MDMT	RT, ion fragments, ratios
1980	Checkley et al. 59	DMT	GC with nitrogen-sensitive detector	0.5µg/ml per 24hour	RT
1983	Uebelhack et al. ⁶⁰	DMT, MDMT	GC-FID	ND	RT RT and fluoresence
1983	Sitaram et al. ⁶¹	HDMT	HPLC/fluoresence spectrum TMS derivatives; GC/MS, multiple ion	>0.01 ng/ml per 12 hr 0.1-0.15 ng/ml DMT;	spectrum RT, molecular ions or
1984	Raisanen et al.62	HDMT	detection	0.25-0.3 ng/ml HDMT	fragments
1000	Karkkainen et al. ⁶³	HDMT	TMS derivatives; GC/MS, multiple ion detection		RT, molecular ions or fragments
1700	Karkkainen and	III)WII	TMS derivatives; GC/MS, multiple ion	-	RT, molecular ions or
1992	Raisanen ⁶⁴	HDMT	detection		fragments
1995	Karkkainen et al. ⁶⁵	HDMT	TMS derivatives; GC/MS, multiple ion detection	0.25-0.3 ng/ml HDMT	RT, molecular ions or fragments RT and electrochemical
1995	Takeda et al.66	HDMT, HNMT DMT, MDMT,	3-D-HPLC-electrochemical detection	50 pg/ml 0.35ng/ml HDMT;	response RT. Pseudo molecular
2001	Forsstrom et al. ⁶⁷	HDMT, NMT	HPLC/ESI-MS-MS	-	ion, MRM
2005	Karkkainen et al. ⁶⁸	DMT, HDMT	HPLC/ESI-MS/MS	0.3 ng/ml HDMT; 0.2 ng/ml DMT	RT, Pseudo molecular ion, MRM
2010	Emanuele et al. ⁶⁹	HDMT	HPLC/ESI-MS/MS	ND	RT, Pseudo molecular ion, MRM

Table C.3; Review of 69 studies regarding endogenous psychedelics showing the year, reference, compounds analyzed, the subjects (patients and controls), the results positive or negative out of the total (i.e, 4/12) and the concentrations of the compounds observed. Acronyms and abbreviations; HNMT, 5-hydroxy-N-methyltryptamine; meds, medications; MAOI, monoamine oxidase inhibitor; admin, administration; schizo, schizophrenia; neg, negative; ND, not detected; NA, not applicable; psychiat, psychiatric; sig dif, significant difference;

		Compounds			
Year .	Author	Analyzed	Subjects	Positive/Negative	Concentration
	Bumpus and	HNMT,	and the second	pooled sample; 5-	
1955	Page 1	HDMT HNMT,	4 healthy adults	HNMT, HDMT no HNMT or HDMT	ND
1956	Rodnight ²	HDMT	11 healthy adults	detected 5/5 schizophrenics	ND
1961	Fischer et al. ³	HDMT	5 acute schizophrenics, 4 controls	HDMT, 4 controls neg 14/15 HDMT; 0/10	400 ng/ml
1961	Fischer et al. ⁴	HDMT	15 schizophrenics, 10 controls 15 schizophrenics, 10 controls; no	HDMT	ND
1961	Feldstein et al. ⁵	HDMT	meds for 2 weeks 20 control children: 6 received	no HDMT detected	ND
		HDMT,	MAOI pheniprazine (3) or	1/20 HDMT; 4/6 HDMT	0.3 µg/ 100 mg creatinine; 0.5-2.2
1962	Perry et al.6	conjugate	nialamide (3) 3 on a plant-free diet during admin	following MAOI	μ g/100 mg creatinine with MAOI
			of neomycin to reduce intestinal		
			flora 5 schizophrenics; 3 mentally	9 of 17 urine samples;	
		HDMT:	deficient patients; MAOI	0/3: MAOI increased	20-30 µg/24 hour HDMT; DMT
1963	Brune et al. ⁷	DMT	isocarboxazid plus betaine	schizo symptoms no DMT detected; 2	negative in all samples
		HDMT;	18 juvenile psychotics; Some on	positive for HDMT after	
1963	Perry ⁸	DMT	plant-free diet and MAOI 4 schizophrenics, 2	MAOI	30 ng/100 mg creatinine
		DMT.	psychoneurotics; MAOI	no DMT or HDMT	
1963	Sprince et al.9	HDMT	tranylcypromine, methione or tryptophan	detected	NA
1903	sprince et al.	HDMI	7 control and 2 psychotic children;	1/2 psychotics HDMT;	NA
	Perry and		1 control on plant-free diet; 2	2/2 controls receiving	
	Schroeder ¹⁰	HDMT	controls received MAOI	MAOI	NA
	Franzen and	DMT.	blood 37 controls; urine 46	11/37 blood DMT;37/37	
1965	Gross ¹¹	HDMT	controls	urine DMT 12/37 blood HDMT:	8-55 ng/ml; 42.98 +/- 8.6 μg/24 hour
				46/46 urine HDMT	1-40 ng/ml; 62.8 +/- 7.2 µg/24 hour
1965	Siegel ¹²	HDMT	5 normals, 21 schizophrenics	no HDMT detected	NA
		HDMT	2 periodic catitonia patients; strict	no HDMT detected	NA

Table C.3 Continued

	Gjessing ¹³		dietary control; phenelzine MAC	I	
			7 schizophrenics, 8 controls; no		
1965	Takesada et al.14	HDMT	meds 30 days	no HDMT detected	NA
			22 schizophrenics no meds; 14		
	all shares		schizophrenics on meds, 17		
1966	Runge et al.15	HDMT	controls; no meds 60 days	no HDMT detected	ND
			12 male schizophrenics, 7 male		
	Sec. Sec.	DMT,	controls; MAOI phenelzine	no HDMT or DMT	
1966	Perry et al.16	HDMT	administered;	detected	NA
			no meds for 6 weeks; no plants of	or and the second se	
			cheese in diet		
			11 schizophrenics, 4 controls; 10		
			schizophrenics, 4 controls	HDMT; 10/10 HDM	1.10
1966	Heller ¹⁷	HDMT	received MAOI	0/4 HDMT	ND
	Fischer and		95 schizophrenics w/o treatment		
1967	Spatz ¹⁸	HDMT	43 with treatment; 102 controls	HDMT; 0/102 HDM	T ND
		in the second	8 schizophrenic females; treated		
1775		DMT,	with methionine and	HDMT or DMT	
1967	Kakimoto et al.19		isocarboxazide (MAOI)	detected	NA
		HNMT,			
		HDMT,	4 male chronic schizophrenics;		
		NMT.	MAOI tranylcypromine; special	4/100 samples HDM	
1967	Tanimukai ²⁰	DMT,	diet; no meds 4-6 weeks	3/100 conjugated	ND
				DMT and MDMT	
		1		observed in some	
		MDMT	and a second second	samples	
			6 male schizophrenics, 4 male		
	Tanimukai et		mentally defective patients; spec		n
1967	Tanimukai et al. ²¹	HDMT	mentally defective patients; special diet; no meds 7 weeks	all 10, free in 7/10	1 ug/14 hour
1907	al	HDMI	4 schizophrenics, MAOI	1/4 free HDMT, 3/4	>1 µg/24 hour
	Tanimukai et		tranylcypromine, cysteine admin;	conj; MAO 4/4 free, 3/4	
1967	al.22	HDMT	special diet	conj	HDMT 4-10 µg/24 hour
1001	Acebal and	110,111	10 schizophrenics; 7 controls;	7/10 HDMT, 0/10 after	Indian +10 hg/14 hour
1967		HDMT	patients administered trifluperidol	trifluperidol: 0/7 HDMT	ND
	Faurbye and		1	6/7 schizophrenics, 3/5	schizophrenics 0-3.7 µg/24 hour;
1968	Pind ²⁴	HDMT	7 schizophrenics, 5 controls	controls	controls 0-7.5 µg/24 hour
	Sireix and		20 schizophrenics, 20 non-	19/20 HDMT, 19/20	schizophrenic mean of 155 ng/ml, non-
1969	Marini ²⁵	HDMT	schizophrenics, 20 controls;	HDMT, 18/20	21 ng/ml, controls 29 ng/ml; no dietary

Table C.3 Continued

			special diets	Service Services	effects
		TITO I I		65/65 schizophrenics.	65/65 mean 172 ng/ml; 73/73 mean 30
1969	Spatz et al.20	HDMT	65 schizophrenics, 73 controls	73/73 controls	ng/ml
	Fischer and		67 controls, 11 epilepsy, 9 depression, 8 psychopathic, 86	67/67 normals, 11/11	norm 12-89 ng/ml, epilepsy 26-67 ng/ml, depress 12-42 ng/ml, psycho 2
1970	Spatz ²⁷	HDMT	non-treated schizophrenics	epilepsy, 9/9 depression	
10/10	oparz	1101011	non-actice scinzopincines	8/8 psychopathic,	schizo 17/86 12-96 ng/ml, 69/86 100-
				86/86, 45/45	375 ng/ml, 33/45 10-100 ng/ml, 12/45
			45 treated schizophrenics	schizophrenics	101-212 ng/ml
			4 controls, 25 psychiatric patients,		
	Saavedra and		11 non-treated schizo, 4 treated, 4		norm 17+/- 2.7 ng/ml, psychiat 24 +/-
1970	Udabe ²⁸	HDMT	hysteria	all positive	2.8 ng/ml, schizo untreated 160+/-22.
				1993 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 -	treated 35+/-10 ng/ml, hysteria 69+/- 9
					ng/ml
		HNMT,			
	-	HDMT,	4 schizophrenics, MAOI		
	Tanimukai et	DMT,	tranylcypromine, methionine or	4/4 HDMT, 3/4 MNMT	
19/0	al.29	MDMT	cysteine admin; special diet	3/4 DMT, 2/4 MDMT	HDMT 4-10 µg/24 hour
		DMT,	5 acute schizophrenics, 9 chronic	5/5 DMT, 5/5 MDMT,	
1070	Heller et al.30	MDMT, HDMT	schizophrenics, 2 normals, 1 depressive	2/5 HDMT; 0/12 for others	NA
1970	fieller et al.	DMT.	2 schizophrenics, 6 controls;	2 schiziphrenics	NA
	Narsimhachari et		MAOI tranylcypromine, cysteine	positive; 6 controls	
1971		HDMT	admin	negative	10-40 μg/ml
		NMT.		15/22 DMT and/or	
	Narasimhachari	DMT,	22 acute schizophrenics, 20 non-	MDMT, 2/22 HDMT;	
1971	et al.32	MDMT	schizophrenics	2/20 positive	2 ng/ml
					controls (Free or total) 63+/- 14.3 or
	and the second	HDMT,	4 each, control, acute and chronic	Cherry and	93+/- 21 ng/ml; chronic 91+/- 21.6 or
19/1	Fischer et al.33	glucuronide	scizophrenics	all positive	188+/- 16 ng/ml;
					acute 200+/- 47.5 or 289+/- 78 ng/ml
		HDMT,		6 controls neg for all;	
1072	TT:	DMT, MDMT	Constitution Constanting and all distant	5/6 autistics positive for	
1972	Himwich et al.34	HDMT.	6 autistics, 6 controls; special diets 6 chronic schizophrenics, 7	HDMT 4/6 schizo DMT.	<3-5 µg/24 hour
	Narasimhachari	DMT.	controls; special diets, restricted	HDMT: 7/7 controls	<5 µg/ 24 hour DMT; 3-5 µg/ 24 hour
1972	et al.35	MDMT	meds	negative;	HDMT; 0/6, 0/7 for MDMT
	Walker et al.36	DMT	45 controls	6/45 DMT	1-2 ng/ml
1010	wanter eval.	Dini	45 controls	OF TO DAME	1-2 19 11
	The second	a	11 controls, 29 psychiatric	Longitz Longitz	013501135010
	Wyatt et al.37	DMT	patients; no meds for 30 days	1/11 DMT; 1/29 DMT	1.0 ng/ml; 10.6 ng/ml
1973		DMT,		AND DATE AND IT	HDMT 1-3 µg/24 hour; DMT 1 µg/24
	Narasimhachari	TTDI CT			
	Narasimhachari and Himwich ³⁸	HDMT	6 chronic schizophrenics	3/6 DMT, 6/6 HDMT	hour
		HDMT	7 control	STO DATE, OF TIDATE	nour
1973	and Himwich ³⁸		7 control 6 chronic schizo, 11 acute schizo,		
1973		HDMT	7 control 6 chronic schizo, 11 acute schizo, 11 hepatic coma	2/11 acute schizo DMT	(1) .6, (1) 1.8
1973	and Himwich ³⁸		7 control 6 chronic schizo, 11 acute schizo, 11 hepatic coma 34 with acute psychotic illness, 3	2/11 acute schizo DMT	
1973 1974	and Himwich ³⁸ Lipinski et al. ³⁹	DMT	7 control 6 chronic schizo, 11 acute schizo, 11 hepatic coma 34 with acute psychotic illness, 3 with non psychotic illness, 1	2/11 acute schizo DMT 2/38 blood DMT; 1/44	(1) .6, (1) 1.8
1973 1974	and Himwich ³⁸		7 control 6 chronic schizo, 11 acute schizo, 11 hepatic coma 34 with acute psychotic illness, 3 with non psychotic illness, 1 control	2/11 acute schizo DMT	
1973 1974	and Himwich ³⁸ Lipinski et al. ³⁹	DMT	7 control 6 chronic schizo, 11 acute schizo, 11 hepatic coma 34 with acute psychotic illness, 3 with non psychotic illness, 1	2/11 acute schizo DMT 2/38 blood DMT; 1/44	(1) .6, (1) 1.8

Table C.3 Continued

1975	Carpenter et al.42	DMT, HDMT	26 acute schizophrenics; 10 controls; no meds for 3 weeks	4/26 DMT, 6/26 5- HDMT; 4/10 DMT, 8/10 HDMT	HDMT mean 1.67 µg/24 hr schizo, 1.73 µg/24 hr controls; DMT not quantitated
		DMT,		positive for DMT.	
1975	Christian et al.43 Narasimhachari	MDMT DMT.	1 control cerebrospinal fluid	MDMT 24/47 HDMT, 10/47	ND
1975	and Himwich44	HDMT	47 infantile autism, 46 controls	DMT; 14/46 HDMT	ND
1976	Angrist et al.45	DMT	23 psychiatric patients, 17 controls	13/23 DMT; 7/17 DMT 37/122 DMT; 1/20	0.05-0.79 ng/ml; 0.06-0.22 ng/ml
1976	Rodnight et al.46	DMT	122 psyciatric patients; 20 controls 54 psychiatric patients, 14		>500 ng/24 hour DMT> 500 ng/24 hour, Mean range
	Murray and		controls; 1 patient strict diet, 2		226-1,717 ng/ 24 hour; control 228 ng/
1976	Oon ⁴⁷	DMT HDMT.	patients on neomycin	23/54 DMT; 1/14 DMT	24 hour
		DMT,	7 schizophrenics, special diet;	No HDMT, DMT,	
1976	Huszka et al. ⁴⁸	MDMT	MAOI phenelzine	MDMT detected 15/20 HDMT; 0/2 HDMT; no DMT or	NA
1977	Cottrell et al.49	HDMT	20 psychiatric patients; 2 controls	MDMT detected 19/19 DMT; 19/19	1-120 nmol HDMT/24 hour DMT range 20-2500 ng/24 hour; NMT
1977	Oon et al. ⁵⁰	DMT, NMT	19 normal	NMT No diurnal variation, no dietary source	range 121-3000 ng/24 hour
1977	Oon and	DMT, NMT	69 patients, 24 normal	69/69 DMT; 17/24	DMT range 0.1-4.5 µg/ 24 hr; DMT
	Rodnight ⁵¹		••••	DMT	range 0.1-0.5 µg/ 24 hr
		DMT,			
1978	Riceberg and Van Vunakis ⁵ 2	HDMT, MDMT	6 controls	3/4 DMT, 1/4 MDMT, 3/4HDMT, plasma 4/4 DMT, 2/4 MDMT,	HDMT 0.25-0.38pmol/ml, MDMT 0.09pmol/ml, DMT 0.77-3.69pmol/ml HDMT 0.11-2.64pmol/ml, MDMT 0.7-
				4/4 HDMT, whole blood 2/6 DMT, 2/6 MDMT, 6/6 HDMT, urine 17/57 DMT, 14/57	2.89pmol/ml, DMT 0.27-14pmol/ml HDMT 1.1-10.3nmol/ml, MDMT 1.3- 8.7nmol/ml, DMT 9.1-13.1nmol/ml
1070	0.1.1.1.1.5	DMT,		MDMT; 9/41 DMT,	177
1978	Corbett et al. ⁵³	MDMT	57 psychiatric patients; 41 controls	2/41 MDMT	ND arterial range 24-118 pg/ml; venous
1979	Walker et al.54	DMT	0 schizonbranice	6/9 DMT	range 18-103 pg/ml; no sig dif between
1919	walker et al.	DMI	9 schizophrenics 74 psychiatric patients; 19	74/74 DMT; 19/19	two sources DMT range 0.1-4.5 µg/ 24 hr; DMT
1979	Murray et al. ⁵⁵	DMT, NMT	controls; no meds for 2 weeks	DMT	range 0.1-0.5 µg/ 24 hr
			18 schizophrenics;20 patients with		10/18 >500ng/24 hr; 12/20 > 500 ng/24
1979	Checkley et al.56	DMT	liver disease; 19 controls	all DMT positive	hr; 1/19 >500 ng/24 hr
	Raisanen and	DMT.		all DMT and HDMT	DMT mean 96 ng/g creatinine; HDMT
1979	Karkkainen ⁵⁷	HDMT	26 controls	positive	mean 950 ng/g creatinine
1979	Smythies et al.58	DMT, MDMT	11 patients undergoing lumbar puncture	11/11 DMT; 1/11 MDMT	DMT range from <0.12-100.4 ng/ml;
1980	Checkley et al. 59	DMT	5 schizophrenics	2/5 DMT	
1900	Checkley et al.	DMI	4 manic-depressives	2/5 DMT	~1-3 μg/ml, ~1-2 μg/ml ~0.5-1 μg/ml, ~0.5-3 μg/ml
				14/14 DMT, 12/14	Sum DMT+MDMT; 1,404.3+/-481
	Uebelhack et	DMT.		MDMT; 12/12 DMT,	ng/ml patients; 234.4+/-213.6 ng/ml
1983	al. ⁶⁰	MDMT	14 schizophrenics; 12 controls	10/12 MDMT	controls
1983	Sitaram et al.61	HDMT	8 healthy adults	5/8 HDMT	0.02-7.8 nmol/12 hour
1984	Raisanen et al. ⁶²	HDMT	48 male violent offenders; 23 controls	48/48 HDMT; 23/23 HDMT	range 0.15-103 nmol/g creatinine; 1.23- 14.1 nmol/g creatinine
	Karkkainen et	HDMT	75 psychiatric patients; 51 controls	75/75 HDMT; 51/51	range 0.05-96.3 nmol/g creatinine; 0.29-
	al.63		HDM		Vg creatinine; MAOI 407 reat
			1/1 be	fore; HDMT range 0.0	02-1.785 nmol/ mmol Table

C.3 Continued

1992		HDMT	1 healthy male; with and without MAOI nialamide	1/1 before; HDMT greatly increased after MAOI	range 0.002-1.785 nmol/ nmol creatinine; 0.06-16.6 nmol/nmol creatinine MAOI no diurnal variation observed; bufotenin excretion was intermittent
1005	Karkkainen et al. ⁶⁵	HDMT	112 male violent offenders	112/112 HDMT	A 01 171 1/1 1 1/1
1995	al	HDM1	112 male violent offenders	112/112 HDM1	range 0.01-17.1 nmol/mol creatinine range 2.5-288 ng/ mg creatinine, HNMT
1995	Takeda et al. ⁶⁶	HDMT, HNMT DMT, MDMT,	140 psychiatric and non- psychiatric patients; 200 controls	89/140 HDMT, 46/140 HNMT; 2/200 HDMT	2.0-102 ng/mg; mean 10.9 ng/mg creatinine
	Forsstrom et	HDMT.		7/23 HDMT, 3/23 DMT,	HDMT 0.43-33.57µg/l, DMT 0.16-
2001	al. ⁶⁷	NMT	23 surgical patients; ;	7/23 NMT 14/29 HDMT, 0/29	.28µg/l, NMT 0.1229µg/l HDMT 0.81-24.9µg/l, NMT 0.05-
			29 psychiatric patients	DMT, 13/29 NMT 2/13 HDMT, 2/13 DMT,	0.25µg/1 HDMT 0.48-7.2µg/1, DMT 0.4254µg/1,
			13 internal medicine patients	2/13 NMT	NMT 0.05-0.13µg/1
	Karkkainen et	DMT,		0/137 plasma or serum	
2005	al. ⁶⁸	HDMT	137 hospital patients; 9 control	DMT, HDMT	NA
				9/9 urine controls HDMT; 0/9 DMT 13/13 controls stool samples HDMT, 1/13	<0.05-9.1 ng/ml; NA (Kidney tissue 15 pg/g HDMT and DMT, 14 pg/g DMT in lung)
				DMT	1.0-180 ng/g HDMT; 0.13 ng/g DMT
	Emanuele et		15 autistic spectrum disorder; 15	15/15 HDMT; 15/15	3.3+/- 0.49 ng/ml; 4.39+/-0.43 ng/ml;
2010	al. ⁶⁹	HDMT	schizophrenics; 18 controls	HDMT; 18/18 HDMT	1.53+/-0.30 ng/ml

C.3 Study Review

69 studies were reviewed. Other studies that do exist were either not accessible through current abstract search engines, were sufficiently obscure as not to be abstracted, or were not available in a translated form for inclusion in this analysis. Articles were obtained through SciFinder (Chem Abstracts Selects; <u>https://scifinder.cas.org</u>) and PubMed (<u>http://www.ncbi.nlm.nih.gov/pubmed/</u>) database searches.

C.3.1 HDMT: urine

• 51 studies examined urine samples for HDMT (27 assayed urine for HDMT only). Taking into account the presence of the 5-hydroxyl group on HDMT, 7 studies specifically addressed the issue of the excretion of HDMT as a conjugate by using hydrolysis with HCl or enzyme treatment. From these studies we know that approximately 50% of the total HDMT is excreted as a glucuronide conjugate. The remaining 44 studies did not conduct hydrolysis or enzyme treatment and thus did not determine the total amount of HDMT excreted but rather free HDMT alone.

- 1,912 individuals' urine samples were assayed; 1,249 patients (predominantly diagnosed with schizophrenia) and 663 controls. Among patients 886 were positive for HDMT (71%) and 363 were negative. Among controls 363 were positive for HDMT (55%) and 300 were negative. Thus, 1,249 individuals were positive (65%) and 663 were negative. Most of the urine samples were obtained from 24 hour collections with varying quantities of the total collection being used for analysis. However, many other studies only used morning or random samples, while a few used 8 or 12 hour collections. Varying amounts of urine were used in the assays, based on volume or total mg creatinine. The range of extraction techniques is shown in Table C.1 and the analytical approaches employed are shown in Table C.2. One study examined and failed to find a diurnal variation in urine concentrations of HDMT [C.50], while another reported that HDMT excretion did not vary diurnally but rather was intermittent [C.64]. Several studies examined dietary influences on detection of HDMT but none established a dietary source (Table C.3).
- Concentrations of HDMT were usually reported as μg/24 hours while other studies reported concentrations as μg/g or μg/mg creatinine, nmol or pmol/ml or per 24 hours, and ng/ml or μg/L. Using the most common methods of reporting, these studies demonstrated concentrations ranging from 1 to 62.8 μg/24 hours, and from 0.48 to 218 ng/ml.

C.3.2 HDMT: blood

• Of the 69 studies, 4 examined blood for the presence of HDMT.

- 240 individuals' blood samples were examined; 166 patients and 74 controls. Plasma, serum, and whole blood were used. A single study provided 146 of these total samples [C.67]; it used a limit of detection of 0.3 ng/ml and a 1.0 ml sample of plasma or serum for analyses. For all of the studies combined, 4 patients were positive for HDMT (2.4%) and 162 were negative. Eighteen controls were positive for HDMT (24%) and 56 were negative. Thus, a total of 22 individuals were positive for HDMT (9%) in blood and 218 were negative. One study reported higher concentrations of HDMT were obtained from extraction of whole blood compared to serum [C.52].
- When concentrations were reported (rather than simply present or not present) the concentrations of HDMT in blood ranged from 22 pg/ml (HPLC-radioimmunoassay)
 [C.52] to 40 ng/ml (direct fluorescence assay of extracts) [C.11].

C.3.3 HDMT: cerebrospinal fluid

• None of the 69 studies examined CSF for HDMT.

C.3.4 DMT: urine

- Of the 69 studies, 29 examined urine for DMT.
- There were 861 individuals' urine samples examined: 635 patients and 226 controls. Among patients, 276 were positive for DMT (43%) and 359 were negative. Among controls, 145 were positive (64%) and 81 were negative. Thus, a total of 421 individuals were positive for DMT (49%) in urine and 440 were negative. Most of the urine samples were 24 hour collections and analytical samples varied in volume. However, many also used morning or random samples, while a few used 8 or 12 hour collections. Various amounts of the urine were used in the assays, based on a set volume of urine or that containing a predetermined amount of creatinine. The range of extraction techniques is

shown in Table C.1 and analytical approaches employed are shown in Table C.2. Several studies examined dietary influences on detection of DMT and were uniformly negative (Table C.3). One study reported that DMT and NMT (N-methyltryptamine; C.1.4, Figure C.1) concentrations in urine were stable when stored at -15oC for up to 90 days [C.50].

Concentrations of DMT were usually reported as μg/24 hours while others used μg/g or μg/mg creatinine, nmol/ml or pmol/ml nmol/24 hours, pmol/24 hours, ng/ml or μg/L, etc.
 Concentrations ranged from 0.02 to 42.98 +/- 8.6 (SD) μg/24 hours, and from 0.16 to 19 ng/ml.

C.3.5 DMT: blood

- Of the 69 studies, 11 examined blood for DMT.
- 417 individuals' blood samples were examined for the presence of DMT: 300 patients and 117 controls. Blood samples used were plasma, serum and/or whole blood. Among patients 44 were positive (15%) and 256 were negative. A single study is responsible for 137 of these negative samples [C.68]; the authors--who used a 1.0 ml sample of plasma or serum--reported a limit of detection of 0.2 ng DMT/ml. Among controls 28 were positive (24%) and 89 were negative. Thus, a total of 72 individuals were positive for DMT (17%) in blood and 345 were negative. The range of extraction methods used is shown in Table C.1 and analytical approaches employed are shown in Table C.2. One study demonstrated that higher concentrations of DMT were found by extracting whole blood rather than using plasma [C.52]. One study demonstrated that there was no difference in DMT blood levels between venous and arterial blood [C.54]. One study reported that DMT concentrations were stable in plasma when stored for 60 days at 6oC [36] (Table C.3).

When concentrations were reported (rather than simply present or not present) the concentrations of DMT in blood ranged from 51 pg/ml (HPLC-radioimmunoassay)
 [C.52] to 55 ng/ml (direct fluorescence assay of extracts) [C.11].

C.3.6 DMT; cerebrospinal fluid

- Of the 69 studies, 4 examined CSF for DMT.
- A total of 136 individuals' CSF samples have been examined for the presence of DMT;
 82 patients and 54 controls. Among patients 34 were positive for DMT (41%) and 48 were negative. Among controls 22 were positive (41%) and 32 were negative. Thus, 56 individuals were positive (41%) and 80 were negative.
- Concentrations of DMT in CSF ranged from 0.12 to100 ng/ml (Table 3).

C.3.7 MDMT: urine

- Of the 69 studies, 9 examined urine for the presence of MDMT.
- A total of 113 individuals' urine samples were examined: 94 patients and 19 controls. A single study was responsible for 65 of these samples [C.67]. Combining all studies, two patients were positive for MDMT in urine (2%) and 92 were negative. Two controls were positive (10.5%) and 17 were negative.
- The concentrations of MDMT in urine ranged from 0.3 to 1.3 ng/ml (HPLC-radioimmunoassay) [C.52].

C.3.8 MDMT: blood

- Of the 69 studies, 2 examined blood for the presence of MDMT.
- A total of 39 individuals' blood samples were examined: 36 patients and 3 controls. Among patients, 20 were positive (51%) and 16 were negative. None of the 3 controls was positive for MDMT (Table C.3).

• A single estimate of 2.0 ng/ml was reported by one study (HPLC-radioimmunoassay) [C.52].

C.3.9 MDMT; cerebrospinal fluid

- Of the 69 studies, 4 examined CSF for MDMT.
- A total of 136 individuals' CSF samples were assayed: 83 patients and 53 controls. Among patients 28 were positive (34%) and 55 were negative. Among controls 12 were positive (23%) and 41 were negative. Thus, a total of 40 individuals were positive (29%) and 96 were negative.
- Only one study reported concentrations of MDMT in CSF, in which case the mean combined concentrations of DMT and MDMT were approximately 1,400 ng/ml for patients and 230 ng/ml for controls with quite large standard deviations (GC-FID) [C.60].

The above does not address the analytical methods' sensitivity and specificity, and assumes that all of the data as collected and reported are accurate, either in their detection or non-detection of the target analyte(s) or the concentrations observed. However, this is almost certainly not the case. As can be seen from Table C.2, almost every study conducted between 1955-1972 used paper or thin-layer chromatography for detection, quantitation, and confirmation of one or more of these compounds. Several studies did use multiple chromatographic conditions and detection reagents in attempting to "confirm" their results. It is well-known, however, that paper chromatography is limited in specificity and sensitivity in that spots tend to be diffused and the mobility of the compounds of interest is influenced by the presence of other components and salts. Thin-layer chromatography is somewhat better but is also susceptible to these same factors in addition to many other variables such as humidity. Other studies used 2-D

chromatographic conditions and very sensitive and moderately specific detection reagents. Nevertheless, the criteria for detection relied on Rf values and color reactions relative to standards (Table C.2). There were no data regarding the structure of the detected compounds. Much of the literature acknowledged their limitations and qualified results by referring to the compounds detected as, for example, "bufotenin-like" [C.4, C.7, C.15].

In many studies, large volumes of urine were extracted and concentrated (Table C.1), resulting in a final extract less than optimal for such analysis. For example, in order to precipitate salts and other compounds, acetone was often used in the final steps of sample purification. However, Tanimukai demonstrated that acetone forms adducts with primary amines co-extracted in the process leading to formation of compounds that behaved similarly to bufotenin, for example, on paper or thin layer chromatography [C.20]. Although there do not seem to be any published replications of Tanimukai's findings, they did lead to modification of many of the extraction procedures that were subsequently designed to fractionate tertiary from primary amines (Table C.1).

As can be seen from Table C.1, the extraction methods employed were predominantly classical liquid-liquid extractions with appropriate pH adjustments or the use of ion exchange resins or packings. The earliest studies, and especially those extracting large volumes of urine, often used a combination of methods in sequence in an attempt to obtain an adequately purified and appropriate extract for paper or thin-layer chromatographic analysis. Almost none of these studies reported analyte recoveries, however. The most recent methods have all employed ion exchange solid-phase extraction for the isolation of the target compounds from urine [C.67-C.69].

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In addition to methodological complications, misidentifications of compounds may also have occurred because both paper and thin layer chromatography using color reagents require a somewhat subjective interpretation. For example, Rodnight [C.2] and Siegel et al. [C.12] proposed that the substance detected by Bumpus and Paige [C.1] was tryptamine and not HDMT. Another potential problem, involving co-injection of extracted indole-ethylamines in GC analyses using the solvent methylene chloride, was addressed by Brandt, et al. [C.86]. These authors showed that the compounds of interest react with methylene chloride under such conditions, forming quaternary salts and analytical artifacts.

Some early studies used more than one method for their analyses, increasing the likelihood that their identifications were accurate; for example, combining thin layer chromatography and gas chromatography with packed column technology. However, the resolving power of packed column technology is low and individual "peaks" were often broad humps, sometimes several minutes wide. Subsequent studies using capillary chromatography have consistently demonstrated that some peaks observed using packed columns were often a composite of several compounds. In addition, the flame ionization detector that many studies used also lacked specificity. Although these approaches used two different technologies, the technologies themselves were relatively non-specific and yielded equivocal results.

Some investigators added, or used exclusively, GC with ECD or NP detectors. While these detectors added sensitivity --and in the case of NPD a degree of specificity-- they continued to rely on Rt and detector response as their identification criteria. No structural data were generated. Other research teams used ultraviolet spectrometry and/or spectrofluorometry to detect and quantify the relevant compounds in extracted samples, either directly or after thinlayer or paper chromatography purification. However, the non-specificity of these methods also

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did not provide data regarding structural identity. For example, Siegel [C.12] demonstrated that the fluorescence method used by Franzen and Gross [C.11] did not actually measure a maxima from HDMT but instead the tail of the fluorescence spectrum of another compound. These findings bring into question studies that applied these and similar methods.

Inconsistent findings in previous research suggest that sensitivity was also an issue. Data concerning extraction efficiency and recovery, limits of detection, specificity, reproducibility, storage stability, the use of double blind and replicate analyses, and other variables that are now basic requirements in assay research are lacking either altogether or in part in earlier studies. At best, some early papers point to other references for some of these data. However, we found direct comparisons of methods in either positive or negative studies difficult to conduct.

The first applications of mass spectrometry to the detection and quantitation of putative endogenous psychedelics in man occurred in 1973. Walker et al. [C.36] and Wyatt et al. [C.37] employed an isotope dilution method monitoring two ions to detect and quantitate DMT in blood. Soon thereafter, Narasimhachari and Himwich used gas chromatography/mass spectrometry with single ion monitoring (m/z 58) to detect DMT from urine extracts [C.38]. These latter authors also extracted sufficient material, using TLC for clean-up, to obtain a total ion mass spectrum of the detected substance, and demonstrated its identity with authentic DMT. These data were the first methodologically credible regarding DMT's presence in humans. Subsequent studies by these and other authors applied different MS capabilities for the detection, quantitation, and unequivocal confirmation of DMT and HDMT in humans. In 1974, Narasimhachari et al., providing a matching total ion spectrum of an extracted compound, reported the unequivocal identification of HDMT from human urine [C.41]. In 1976 Rodnight et al. [C.46], using similar methods, published a matching total ion spectrum for DMT in human urine. Other MS techniques matched the retention time and protonated molecule ions (chemical ionization MS) for DMT and HDMT in urine [C.50, C.51]. Additional studies detected, quantified, and confirmed the identity of DMT, NMT, and HDMT in human blood and urine using selected ion monitoring (SIM) of multiple fragment ions (Table C.2). It is important to note that MDMT has yet to be unequivocally detected by any MS-based method in blood or urine. However, there are two reports of its presence in CSF using GC/MS/SIM [C.53, C.58].

Continual improvement in MS technologies has greatly enhanced detection, sensitivity, and specificity of analytic studies searching for these compounds; for example, capillary chromatography for GC, and more advanced LC-mass spectrometers. This being the case, it is encouraging to note that all studies since 1973 using MS methodology have confirmed the presence of one or more of these compounds in human body fluids (Table C.2). The most recent methods utilize LC/MS/MS which afford analyses and confirmation by several additional chemical processes; LC separation and matching of Rt, molecular ion matching, and fragment ion presence and ratio matching. This technique also allows for the detection of these compounds in the pg/ml range while providing unequivocal mass spectrometric confirmation of structural identity.

Thus, while many early studies lacked today's more definitive technology, it is likely that many have been confirmed by later MS-based studies. On the other hand, most early studies that reported rather high concentrations on these compounds were most likely in error.

C.4 Discussion and Conclusions

The answer to the question, "Are the tryptamine psychedelic substances DMT, HDMT and MDMT present in the human body?" is most likely "Yes." We believe that the preponderance of the mass spectral evidence proves, to a scientific certainty, that DMT and HDMT are indeed endogenous and can be measured in human body fluids. The evidence is less compelling for MDMT where the only two MS-based positive studies--in CSF--were performed by the same research group. There is no mass spectral data on detection of MDMT in blood or urine. Thus, further studies are necessary to determine whether MDMT exists in humans. Similarly, there are no data on the possible presence of HDMT in CSF. This too requires examination.

With respect to the paucity of data regarding endogenous MDMT, it should also be noted that HDMT is both a metabolite of and precursor for MDMT. The relationship of these two compounds may help explain why HDMT is so much more frequently detected than MDMT. Future studies will help explicate this relationship.

As to the question, "Were the analytical methodologies and the criteria for compound identification adequate?", the answer is less certain. Undoubtedly, some studies misidentified the target compounds or, at the minimum, greatly overestimated their concentrations.

Are they of dietary origin? Many early studies attempted to determine if diet or gut bacteria were responsible for positive results. Sterilization of the gut with antibiotics or feeding subjects special diets had no effect on these studies' results. In addition, no evidence suggested that medication(s) played a role. More recently, however, Karkkainen et al. [C.68] isolated significant quantities of HDMT from stool samples, and hypothesized that HDMT may be synthesized by cells of the intestinal epithelium or the kidney, but not by gut flora.

When are these compounds produced? The very small numbers of studies that have looked for diurnal, circadian, or ultradian variations in levels of DMT or HDMT in humans have been negative. This may be due, in part, to too infrequent sampling times and inadequate assay methodologies. However, one longitudinal study and one assessing diurnal rhythms of DMT in human urine suggest that measurable concentrations occur only intermittently [C.50]. The same is apparently true for HDMT [C.64]. There are no comparable data available for MDMT. The two DMT studies cited were conducted in urine only and such analyses are probably best conducted in blood. They do stand, however, as examples of one of the possible further complications in understanding the source, role and function of these compounds.

Where in the human body are they synthesized? The tissue source or sources of these compounds in humans remains unknown and, that being the case, we should not assume that monitoring blood, urine, or CSF will answer this question. DMT synthesis has been proposed to occur in adrenal and lung, where high levels of the enzyme responsible for its synthesis--indole-N-methyltransferase (INMT)--have been reported [C.96, C.97]. While these studies did not demonstrate high INMT levels in brain, the active transport of DMT across the blood-brain barrier [C.98] suggests that peripheral synthesis may nevertheless affect central function. In addition, the mapping of INMT sites thus far has been based solely on INMT mRNA studies which only establish where active enzyme translation is occurring. However, recent studies by Cozzi et al.[C.99], using a fluorescent antibody to INMT and confocal microscopy, have identified INMT in spinal cord, brain, retina, and pineal, and suggest the possibility of applying other powerful molecular biology tools and methods for mapping the location and characterizing the regulation of the endogenous psychedelic pathway. Their findings suggest that INMT may be an inducible enzyme. These molecular biological approaches, in combination with advances in assay methodology, may help finally characterize the biochemistry and physiology of these compounds in humans.

The next questions, "Can we influence the detection of endogenous psychedelics in humans by pre-treatment with MAO inhibitors?" "How does the turnover rate and metabolism of these substances influence their detectability?" "Have the precursors and/or metabolites of these compounds been adequately monitored?" require synthesizing several parallel lines of evidence. In humans, only a very small percentage of exogenously administered DMT is excreted in urine as the parent compound [C.88]. This is also true for HDMT [C.100] and MDMT [C.101]. Despite this fact, every cited study monitored, without exception, only the parent compounds themselves in the various biological fluids examined. These compounds all have a very short half-life—a few minutes—and blood levels are undetectable in less than an hour after administration. This rapid metabolism is due to their being excellent substrates for MAO-A. This enzyme's action on the psychedelic tryptamines results in the formation of their corresponding indoleacetic acids, which are indistinguishable from these same acids resulting from other better-known sources, such as tryptamine and serotonin. Several studies attempted to maximize detection of these substances by treating subjects with MAO inhibitors such as tranylcypromine and phenelzine (Table C.3). In most cases, this did result in higher concentrations of the target compounds. Nevertheless, even with significant MAO inhibition, the concentrations of parent compounds remained quite small. This observation has, perhaps, a ready explanation: the other metabolic pathways for DMT, MDMT, and HDMT.

Recognition and understanding of these compounds' pathways for degradation may afford an approach to circumventing the low concentrations of the parent compounds observed even after MAO inhibition. Sitaram et al. [C.89-C.91] have shown that, in MAO inhibited rats, metabolism of these psychoactive tryptamines is shifted away from MAO-A and indoleacetic acid formation to the N-oxidase and the respective N-oxides. However, no studies have yet pretreated humans with MAO inhibitors and measured the parent compounds and their corresponding N-oxides. The advantage of such a study is that the N-oxide, as opposed to the indoleacetic acid, retains the original structure of the parent molecule, permitting a cumulative association. As a proof of concept, we, have measured blood and urine levels of DMT and its N-oxide (C.1.5, Figure C.1) in humans administered a botanical preparation of DMT and MAO-A inhibiting harmala alkaloids—the Amazonian brew *ayahuasca* [C.102, C.103]. Concentrations of the N-oxide of DMT in these subjects were 3-4 times greater in blood, and 20 times greater in urine, than DMT itself. Therefore, monitoring the N-oxide metabolites rather than the parent compounds alone in MAO-inhibited humans may provide a substantial advantage in detecting and quantitating the endogenous psychedelic compounds.

Several of the studies reviewed did examine samples for the corresponding NMT, which is both a precursor for and a metabolite of the three endogenous psychedelics (NMT, HNMT, MNMT). However, in humans administered ayahuasca NMT was only intermittently detected in blood and urine and concentrations were quite low (pg/ml)[C.102, C.103]. This also may be the result of a shift in metabolism of DMT to the N-oxide after MAO inhibition and suggests that monitoring NMT in vivo may not be necessary or possible. Nonetheless, several of the reviewed studies suggested that the corresponding NMT was detected, however (Table C.3). That data must now also be in question.

DMT-N-oxide is neither a substrate for MAO-A nor for N-demethylases. Since similar metabolic pathways exist for HDMT and MDMT, we suggest that MAO inhibition in humans will enhance detection and quantitation of these compounds in the periphery, especially if the N-oxide metabolites are monitored.

Thus, we can respond to the questions, "Is monitoring these compounds in biological samples such as CSF, blood and/or urine the best, or even most practical way to determine their activity?" and "What will such data tell us about the possible normal function of these

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compounds in humans?" Data regarding their peripheral dynamics—concentrations, circadian variation, and metabolism, as assessed by rigorous analytic methods applied to biological samples represent the most accessible approach to beginning to determine their possible role in human psychophysiology and should be pursued.

Our last question, "Where does the research on endogenous psychedelics go from here?" One avenue for future studies concerns the endogenous nature of MDMT. This review has illustrated the convincing evidence that DMT and HDMT are endogenous in humans. However, MDMT has not been reported in human blood or urine but is apparently present in CSF. However, CSF has not been examined for the presence of HDMT. We propose that future studies of CSF, blood (including whole blood where higher concentrations may be observed) and urine monitor all three compounds and their N-oxides using superior, fully validated mass spectrometric methodology. Pretreatment of study subjects with an MAO inhibitor should optimize results and may prove critical to such studies. A technical issue regarding HDMT analysis also must be considered in future studies. Assays for this compound should include an enzyme hydrolysis step to free conjugates that may be formed from both the parent compound and it's N-oxide.

Another area for future research concerns assay sensitivity. We believe it is necessary to improve sensitivity of assays of the parent compounds to 1.0 pg/ml or less. Given the possible intermittent presence of these compounds in the periphery, blood and urine analyses may require more frequent sampling and longer collection times.

The search for endogenous psychedelic tryptamines should also turn towards other human tissues than blood, urine and CSF; that is, solid organs such as adrenal, brain, lung, pineal, retina, and other tissues in which INMT activity has been noted using molecular biology

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tools. The combination of assaying relevant compounds with cell and molecular biology approaches will provide the most detailed possible assessment of the location(s) of synthesis and, ultimately, the role of these compounds in human physiology.

For example, mapping of INMT and its presence within certain cell types and locations, should reveal its intracellular distribution and possible associations with various receptors. The introduction of an INMT knockout mouse to the research effort could greatly assist in understanding the role of this enzyme and, by inference, the endogenous psychedelics. With these tools in hand, the research that can be conducted may finally provide us an answer to the question, "Why do humans produce endogenous psychedelics?" The research thus far is limited but there are many possibilities awaiting further inquiry.

C.5 References

- [C.1] F.M. Bumpus and I.H. Page. Serotonin and its methylated derivatives in human urine. J. Biol. Chem. 1955, 212, 111.
- [C.2] R. Rodnight. Separation and characterization of urinary indoles resembling 5hydroxytryptamine and tryptamine. *Biochem.* **1956**, *64*, 621.
- [C.3] E. Fischer, F.A. Vazquez, T.A. Fernandez, L. Liskowski. Bufotenin in human urine. *The Lancet* **1961**, 890.
- [C.4] E. Fischer, T.A. Fernandez Lagravere, A J. Vazquez, A.O. Di Stefano. A bufotenin-like substance in the urine of schizophrenics. *J. Nerv. Ment. Dis.* **1961**, *133*, 441.
- [C.5] A. Feldstein, H. Hoagland, H. Freeman. Radioactive serotonin in relation to schizophrenia. *Arch. Gen. Psychiat.* **1961**, *5*, 54.
- [C.6] T.L. Perry, K. N.F. Shaw, D. Walker, D. Redlich. Urinary excretion of amines in normal children. *Pediatrics* 1962, 30, 576.
- [C.7] G.G. Brune, H.H. Hohl, H.E. Himwich. Urinary excretion of bufotenin-like substance in psychotic patients. *J. Neuropsychiat.***1963**, *4*, 14.
- [C.8] T.L. Perry. N-Methylmetanephrine: Excretion by juvenile psychotics. *Science* **1963**, *139*, 587.

- [C.9] H. Sprince, C.M. Parker, D. Jameson, F. Alexander. Urinary indoles in schizophrenic and psychoneurotic patients after administration of tranylcypromine (Parnate) and methionine or tryptophan. J. Nerv. Ment. Dis. 1963, 137, 246.
- [C.10] T.L. Perry and W.A. Schroeder. The occurrence of amines in human urine: Determination by combined ion exchange and paper chromatography. J. Chromatog. 1963, 12, 358.
- [C.11] F. Franzen, H. Gross. Tryptamine, N,N-dimethyltryptamine, N,N-dimethyl-5hydroxytryptamine and 5-methoxytryptamine in human blood and urine. *Nature* 1965, 206, 1052.
- [C.12] M. Siegel. A sensitive method for the detection of N,N-dimethylserotonin (bufotenin) in urine; Failure to demonstrate its presence in the urine of schizophrenic and normal subjects. J. Psychiat. Res. 1965, 3, 205.
- [C.13] T. Nishimura and L.R. Gjessing. Failure to detect 3,4-dimethoxyphenylethylamine and bufotenine in the urine from a case of periodic catatonia. *Nature* **1965**, *206*, 963.
- [C.14] M. Takesada, E. Miyamoto, Y. Kakimoto, I. Sano, Z. Kaneko. Phenolic and indole amines in the urine of schizophrenics. *Nature* 1965, 207, 1199.
- [C.15] T.M. Runge, F. Y. Lara, N. Thurman, J.W. Keyes, S.H. Hoerster, Jr. Search for a bufotenin-like substance in the urine of schizophrenics. J. Nerv. Ment. Dis. 1966, 142, 470.
- [C.16] T.L. Perry, S. Hansen, L. MacDougall, C.J. Schwarz. Urinary amines in chronic schizophrenia. *Nature* 1966, 212, 146.
- [C.17] B. Heller. Influence of treatment with an amine oxidase inhibitor on the excretion of bufotenin and the clinical symptoms in chronic schizophrenic patients. *Inter. J. Neuropsychiat.* 1966, 2, 193.
- [C.18] E. Fischer, H. Spatz. Determination of bufotenin in the urine of schizophrenics. *Intern. J. Neuropsychiat.* 1967, 3, 226.
- [C.19] Y. Kakimoto, I. Sano, A. Kanazawa, T. Tsujio, Z. Kaneko. Metabolic effects of methionine in schizophrenic patients pretreated with a monoamine oxidase inhibitor. *Nature* 1967, 216, 1110.
- [C.20] H. Tanimukai. Modifications of paper and thin layer chromatographic methods to increase the sensitivity for detecting N-methylated indolamines in urine. J. Chromatog. 1967, 30, 155.

- [C.21] H. Tanimukai, R. Ginther, J. Spaide, H.E. Himwich. Psychotomimetic indole compound in the urine of schizophrenics and mentally defective patients. *Nature* 1967, 216, 490.
- [C.22] H. Tanimukai, R. Ginther, J. Spaide, J.R. Bueno, H.E. Himwich. Occurrence of bufotenin (5-hydroxy-N,N-dimethyltryptamine) in urine of schizophrenic patients. *Life Sci.* 1967, 6, 1697.
- [C.23] E.M. Acebal, H. Spatz. Effect of trifluperidol (R 2498) on the urinary elimination of bufotenin in schizophrenia. *Intern. J. Neuropsychiat.* 1967, 3, 472.
- [C.24] A. Faurbye and K. Pind. Occurrence of bufotenin in the urine of schizophrenic patients and normal persons. *Nature* 1968, 220, 489.
- [C.25] D.W. Sireix and F.A. Marini. Studies on the elimination of bufotenin in urine. *Behav. Neuropsychiat.* **1969**, *1*, 29.
- [C.26] H. Spatz, D. W. Sireix, F.A. Marini, E. Fischer, A. Bonhour, E.M. Acebal. Laboratory and animal studies on the chemistry of bufotenin. Quantitative determination on bufotenin in human urine. *Behav. Neuropsychiat.* **1969**, *1*, 25.
- [C.27] E. Fischer and H. Spatz. Studies on urinary elimination of bufotenin-like substances in schizophrenia. *Biol. Psychiat.* 1970, 2, 235.
- [C.28] J.M. Saavedra and U. Udabe. Quantitative assay of bufotenine in psychiatric outpatients. *Psychosom.* **1970**, *11*, 90.
- [C.29] H. Tanimukai, R. Ginther, J. Spaide, J.R. Bueno, H.E. Himwich. Detection of psychotomimetic N, N-dimethylated indoleamines in the urine of four schizophrenic patients. *Brit. J. Psychiat.* **1970**, *117*, 421.
- [C.30] B. Heller, N. Narasimhachari, J. Spaide, L. Haskovec, H.E. Himwich. N-Dimethylated indoleamines in blood of acute schizophrenics. *Experientia* 1970, 26, 503.
- [C.31] N. Narasimhachari, B. Heller, J. Spaide, L. Haskovec, M. Fujimori, K. Tabushi, H.E. Himwich. Urinary studies of schizophrenics and controls. *Biol. Psychiat.* 1971, 3, 9.
- [C.32] N. Narasimhachari, B. Heller, J. Spaide, L. Haskovec, H. Meltzer, M. Strahilevitz, H.E. Himwich. N,N-Dimethylated indoleamines in blood. *Biol. Psychiat.* 1971, 3, 21.
- [C.33] E. Fischer, H. Spatz, T. Fledel. Bufotenin like substances in form of glucuronide in schizophrenic and normal urines. *Psychosom.* 1971, 12, 278.
- [C.34] H.E. Himwich, R. L. Jenkins, M. Fujimori, N. Narasimhachari, M. Ebersole. A biochemical study of early infantile autism. J. Autism Childhood Schiz. 1972, 2, 114.

- [C.35] N. Narasimhachari, J. Avalos, M. Fujimori, H.E. Himwich. Studies of drug free schizophrenics and controls. *Biol. Psychiat.* 1972, 5, 311.
- [C.36] R.W. Walker, H.S. Ahn, G. Albers-Schonberg, L.R. Mandel, W.J. Vandenheuvel. Gas chromatographic-mass spectrometric isotope dilution assay for N,N-dimethyltryptamine in human plasma. *Biochem. Med.* 1973, 8, 105.
- [C.37] R.J. Wyatt, L. R. Mandel, H.S. Ahn, R.W. Walker, W.J. Vanden Heuvel. Gas chromatographic-mass spectrometric isotope dilution determination of N,Ndimethyltryptamine concentrations in normals and psychiatric patients. *Psychopharmacol.* 1973, 31, 265.
- [C.38] N. Narasimhachari and H.E. Himwich. Gas chromatographic-mass spectrometric identification of N, N-dimethyltryptamine in urine samples from drug-free chronic schizophrenic patients and its quantitation by the technique of single (selective) ion monitoring. *Biochem. Biophys. Res. Commun.* 1973, 55, 1064.
- [C.39] J.F. Lipinski, L.R. Mandel, H.S. Ahn, W.J. Vanden Heuvel, R.W. Walker. Blood dimethyltryptamine concentrations in psychotic disorders. *Biol. Psychiat.* 1974, 9, 89.
- [C.40] T.G. Bidder, L.R. Mandel, H.S. Ahn, W. J. VandenHeuvel, R.W. Walker. Letter: Blood and urinary dimethyltryptamine in acute psychotic disorders. *Lancet* **1974**, *1*, 165.
- [C.41] N. Narasimhachari, P. Baumann, H.S. Pak, W.T. Carpenter, A.F. Zocchi, L. Hokanson, M. Fujimori, H. E. Himwich. Gas chromatographic-mass spectrometric identification of urinary bufotenin and dimethyltryptamine in drug-free chronic schizophrenic patients. *Biol. Psychiat.* 1974, 8, 293.
- [C.42] W.T. Carpenter, Jr., E.B. Fink, N. Narasimhachari, H.E. Himwich. A test of the transmethylation hypothesis in acute schizophrenic patients. *Amer. J. Psychiat.* 1975, 132, 1067.
- [C.43] S.T. Christian, F. Benington, R.D. Morin, L. Corbett. Gas-liquid chromatographic separation and identification of biologically important indolealkylamines from human cerebrospinal fluid. *Biochem. Med.* 1975, 14, 191.
- [C.44] N. Narasimhachari and H.E. Himwich. Biochemical studies in early infantile autism. Biol. Psychiat. 1975, 10, 425.
- [C.45] B. Angrist, S. Gershon, G. Sathananthan, R.W. Walker, B. Lopez-Ramos, L.R. Mandel, W.J. Vandenheuvel. Dimethyltryptamine levels in blood of schizophrenic patients and control subjects. *Psychopharmacol.* **1976**, *47*, 29.
- [C.46] R. Rodnight, R.M. Murray, M.C. Oon, I.F. Brockington, P. Nicholls, J.L. Birley. Urinary dimethyltryptamine and psychiatric symptomatology and classification. *Psycholog. Med.* 1976, 6, 649.

- [C.47] R.M. Murray, M.C. Oon. The excretion of dimethyltryptamine in psychiatric patients. *Proc. Royal Soc. Med.* **1976**, *69*, 831.
- [C.48] L. Huszka, D.H. Zabek, J.W. Doust. Urinary excretion of N, N-dimethylated tryptamines in chronic schizophrenia. A review of the present status of the hypothesis. *Can. Psychiat. Assoc. J.* 1976, 21, 541.
- [C.49] A.C. Cottrell, M.F. McLeod, W.R. McLeod. A bufotenin-like substance in the urine of schizophrenics. Amer. J. Psychiat. 1977, 134, 322.
- [C.50] M.C. Oon, R.M. Murray, R. Rodnight, M.P. Murphy, J.L. Birley. Factors affecting the urinary excretion of endogenously formed dimethyltryptamine in normal human subjects. *Psychopharmacol.* 1977, 54, 171.
- [C.51] M.C. Oon and R. Rodnight. A gas chromatographic procedure for determining N, Ndimethyltryptamine and N-monomethyltryptamine in urine using a nitrogen detector. *Biochem. Med.* 1977, 18, 410.
- [C.52] L.J. Riceberg and H.V. Vunakis. Determination of N,N-dimethylindolealkylamines in plasma, blood and urine extracts by radioimmunoassay and high pressure liquid chromatography. J. Pharmacol. Exper. Therap. 1978, 206, 158.
- [C.53] L. Corbett, S.T. Christian, R.D. Morin, F. Benington, J.R. Smythies. Hallucinogenic Nmethylated indolealkylamines in the cerebrospinal fluid of psychiatric control populations. *Brit. J. Psychiat.* 1978, 132, 139.
- [C.54] R.W. Walker, L.R. Mandel, J.E. Kleinman, J.C. Gillin, R.J. Wyatt, W.J. Vandenheuvel. Improved selective ion monitoring mass-spectrometric assay for the determination of N,N-dimethyltryptamine in human blood utilizing capillary column gas chromatography. J. Chromatogr. 1979, 162, 539.
- [C.55] R.M. Murray, M.C. Oon, R. Rodnight, J.L. Birley, A. Smith. Increased excretion of dimethyltryptamine and certain features of psychosis: a possible association. Arch. Gen. Psychiat. 1979, 36, 644.
- [C.56] S.A. Checkley, M.C.H. Oon, R. Rodnight, M.P. Murphy, R.S. Williams, J.L.T. Birley. Urinary excretion of dimethyltryptamine in liver disease. Am. J. Psychiat. 1979, 136, 439.
- [C.57] M. Raisanen and J. Karkkainen. Mass fragmentographic quantification of urinary N,Ndimethyltryptamine and bufotenine. J. Chromatogr. 1979, 162, 579.
- [C.58] J.R. Smythies, R.D. Morin, G.B. Brown. Identification of dimethyltryptamine and Omethylbufotenin in human cerebrospinal fluid by combined gas chromatography/mass spectrometry. *Biol. Psychiat.* **1979**, *14*, 549.

- [C.59] S.A. Checkley, R.M. Murray, M.C. Oon, R. Rodnight, J.L. Birley. A longitudinal study of urinary excretion of N,N,-dimethyltryptamine in psychotic patients. *Brit. J. Psychiat.* 1980, 137, 236.
- [C.60] R. Uebelhack, L. Franke, K. Seidel. Methylierte und nichtmethylierte indolamine in zisternalen liquor bei akuten endoenen psychosen. *Biomed. Biochim. Acta* 1983, 42, 1343.
- [C.61] B.R. Sitaram, G.L. Blackman, W.R. McLeod, G.N. Vaughan. The ion-pair extraction, purification, and liquid chromatographic analysis of indolealkylamines in human urine. *Anal. Biochem.* 1983, 128, 11.
- [C.62] M.J. Raisanen, M. Virkkunen, M.O. Huttunen, B. Furman, J. Karkkainen. Increased urinary excretion of bufotenin by violent offenders with paranoid symptoms and family violence. *Lancet* 1984, 2, 700.
- [C.63] J. Karkkainen, M. Raisanen, H. Naukkarinen, J. Spoov, R. Rimon. Urinary excretion of free bufotenin by psychiatric patients. *Biol. Psychiat.* 1988, 24, 441.
- [C.64] J. Karkkainen, M. Raisanen. Nialamide, an MAO inhibitor, increases urinary excretion of endogenously produced bufotenin in man. *Biol. Psychiat.* 1992, 32, 1042.
- [C.65] J. Karkkainen, M. Raisanen, M.O. Huttunen, E. Kallio, H. Naukkarinen, M. Virkkunen. Urinary excretion of bufotenin (N,N-dimethyl-5-hydroxytryptamine) is increased in suspicious violent offenders: a confirmatory study. *Psychiat. Res.* 1995, 58, 145.
- [C.66] N. Takeda, R. Ikeda, K. Ohba, M. Kondo. Bufotenine reconsidered as a diagnostic indicator of psychiatric disorders. *NeuroReport* 1995, 6, 2378.
- [C.67] T. Forsstrom, J. Tuominen, J. Karkkainen. Determination of potentially hallucinogenic N-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. Scand. J. Clin. Lab. Invest. 2001, 61, 547.
- [C.68] J. Karkkainen, T. Forsstrom, J. Tornaeus, K. Wahala, P. Kiuru, A. Honkanen, U.H. Stenman, U. Turpeinen, A. Hesso. Potentially hallucinogenic 5-hydroxytryptamine receptor ligands bufotenine and dimethyltryptamine in blood and tissues. *Scand. J. Clin. Lab. Invest.* 2005, 65, 189.
- [7.69] E. Emanuele, R. Colombo, V. Martinelli, N. Brondino, M. Marini, M. Boso, F. Barale, P. Politi. Elevated urine levels of bufotenine in patients with autistic spectrum disorders and schizophrenia. *Neuroendo. Let.* 2010, *31*, 117.
- [C.70] M.J.D. Fontanilla, A.R. Hajipour, N.V. Cozzi, M.B. Jackson, A.E. Ruoho. The hallucinogen N,N-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator *Science* 2009, 323.

- [C.71] T.H. Tsung-Ping Su and D. Bruce Vaupel. When the endogenous hallucinogenic trace amine N,N-Dimethyltryptamine meets the sigma-1 receptor. *Sci. Signal.* 2009, 2, doi: 10.1126/scisignal.261pe12.
- [C.72] R. Strassman, DMT: The Spirit Molecule: A Doctor's Revolutionary Research into the Biology of Near-Death and Mystical Experiences. Park Street Press, Rochester, Vermont, 2001.
- [C.73] M. Winkelman. Drug tourism or spiritual healing? Ayahuasca seekers in Amazonia. J. Psycho. Drugs 2005, 37, 209.
- [C.74] K.W. Tupper. The globalization of ayahuasca: Harm reduction or benefit maximization? Inter. J. Drug Pol. 2008, 19, 297.
- [C.75] D.J. McKenna. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacol.Therap.* **2004**, *102*, 111.
- [C.76] R.J. Strassman. Human hallucinogenic drug research in the United States: a present-day case history and review of the process. J. Psycho. Drugs **1991**, 23, 29.
- [C.77] R.J. Strassman and C.R. Qualls. Dose-response study of N,N-dimethyltryptamine in humans. I. Neuroendocrine, autonomic, and cardiovascular effects. Arch. Gen. Psychiat. 1994, 51, 85.
- [C.78] R.J. Strassman, C.R. Qualls, E.H. Uhlenhuth, R. Kellner. Dose-response study of N,Ndimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch. Gen. Psychiat. 1994, 51, 98.
- [C.79] R.J. Strassman, C.R. Qualls, L.M. Berg. Differential tolerance to biological and subjective effects of four closely spaced doses of N,N-dimethyltryptamine in humans. *Biol. Psychiat.* 1996, 39, 784.
- [C.80] R.J. Strassman. Human psychopharmacology of N,N-dimethyltryptamine. *Behav. Brain Res.* 1996, 73, 121.
- [C.81] E. Gouzoulis-Mayfrank, K. Heekeren, A. Neukirch, M. Stoll, C. Stock, M. Obradovic, K. A. Kovar. Psychological effects of (S)-ketamine and N,N-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. *Pharmacopsychiat.* 2005, *38*, 301.
- [C.82] J. Daumann, D. Wagner, K. Heekeren, A. Neukirch, C.M. Thiel, E. Gouzoulis-Mayfrank. Neuronal correlates of visual and auditory alertness in the DMT and ketamine model of psychosis. J. Psychopharmacol. 2010, 24, 1515.

- [C.83] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J. Pharmacol. Exper. Therap. 2003, 306, 73.
- [C.84] J. Riba, P. Anderer, F. Jane, B. Saletu, M.J. Barbanoj. Effects of the South American psychoactive beverage ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low-resolution electromagnetic tomography. *Neuropsychobiol.* 2004, 50, 89.
- [C.85] S. Szara. DMT at fifty. Neuropsychopharmacol. Hungar. 2007, 9, 201.
- [C.86] S.D. Brandt, C.P. Martins, S. Freeman, N. Dempster, P.G. Riby, J. Gartz, J.F. Alder. Halogenated solvent interactions with N,N-dimethyltryptamine: formation of quaternary ammonium salts and their artificially induced rearrangements during analysis. *Foren. Sci. Inter.* 2008, 178, 162.
- [C.87] S.A. Barker, J.A. Monti, S.T. Christian. Metabolism of the hallucinogen N,Ndimethyltryptamine in rat brain homogenates. *Biochem. Pharmacol.* **1980**, *29*, 1049.
- [C.88] S.A. Barker, J.A. Monti, S.T. Christian. N, N-dimethyltryptamine: An endogenous hallucinogen. *Inter. Rev. Neurobiol.* 1981, 22, 83.
- [C.89] B.R. Sitaram, L. Lockett, G.L. Blackman, W.R. McLeod. Urinary excretion of 5methoxy-N,N-dimethyltryptamine, N,N-dimethyltryptamine and their N-oxides in the rat. *Biochem. Pharmacol.* 1987, 36, 2235.
- [C.90] B.R. Sitaram, L. Lockett, R. Talomsin, G.L. Blackman, W.R. McLeod. In vivo metabolism of 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine in the rat. *Biochem. Pharmacol.* **1987**, *36*, 1509.
- [C.91] B.R. Sitaram, W.R. McLeod. Observations on the metabolism of the psychotomimetic indolealkylamines: Implications for future clinical studies. *Biol. Psychiat.* 1990, 28, 841.
- [C.92] H. Osmond and J. Smythies. Schizophrenia: A new approach. Brit. J. Psychiat. 1952, 98, 309.
- [C.93] J.C. Gillin, J. Kaplan, R. Stillman, R.J. Wyatt. The psychedelic model of schizophrenia: The case of N,N-dimethyltryptamine. *Amer. J. Psychiat.* 1976, 133, 203.
- [C.94] J.C. Gillin and R.J. Wyatt. Evidence for and against the involvement of N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) in schizophrenia. *Psychopharmacol. Bull.* **1976**, *12*, 12.
- [C.95] J. Axelrod. Enzymatic formation of psychotomimetic metabolites from normally occurring compounds. *Science* **1961**, *134*, 343.

- [C.96] M.A. Thompson, R.M. Weinshilboum. Rabbit lung indolethylamine N-methyltransferase. cDNA and gene cloning and characterization. J. Biol. Chem. 1998, 273, 34502.
- [C.97] M.A. Thompson, E. Moon, U.J. Kim, J. Xu, M.J. Siciliano, R.M. Weinshilboum Human indolethylamine N-methyltransferase: cDNA cloning and expression, gene cloning, and chromosomal localization *Genomics* 1999, 61, 285.
- [C.98] N.V. Cozzi, A. Gopalakrishnan, L.L. Anderson, J.T. Feih, A.T. Shulgin, P.F. Daley, A.E. Ruoho. Dimethyltryptamine and other hallucinogenic tryptamines exhibit substrate behavior at the serotonin uptake transporter and the vesicle monoamine transporter. J Neural Transm. 2009, 116, 1591.
- [C.99] N.V. Cozzi, T.A. Mavlyutov, M.A. Thompson, A.E. Ruoho. Indolethylamine Nmethyltransferase expression in primate nervous tissue. *Soc. Neurosci. Abs.* 2011, 37, 840.19 (2011)
- [C.100]E. Sanders-Bush, J.A. Oates, M.T. Bush. Metabolism of bufotenine-2'-14C in human volunteers. *Life Sci.* **1976**, *19*, 1407.
- [C.101] X.-L. J. Hong-Wu Shen, Winter, Jerrold C., Ai-Ming Yu. Psychedelic 5-methoxy-N,N-dimethyltryptamine: Metabolism, pharmacokinetics, prug interactions, and pharmacological actions. *Cur. Drug Metab.* 2010, 11, 659.
- [C.102] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed. Chromatogr.* 2011, doi: 10.1002/bmc.1657.
- [C.103] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S. A. Barker. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomed. Chromatogr.* 2011, 25, 970.

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11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

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Vita

Ethan Hamilton McIlhenny was born to Henry C. and Bonnie L. McIlhenny in Brevard, North Carolina. He attended Hendersonville High School and graduated in 2002. He attended Skidmore College in Saratoga Springs New York and received his Bachelor of Arts degree in Neuroscience in 2006. Ethan entered a Neuroscience Ph. D program with a teaching assistanceship at Tulane University in New Orleans Louisiana and completed with a Masters of Science degree in 2008. Ethan transferred to a Ph D. program at Louisiana State University in the Department of Comparative Biomedical Sciences at the LSU School of Veterinary Medicine in 2008 under the mentorship of Dr. Steven Barker where he received a 4 year board of regents grant fellowship. Ethan expects to receive his Doctor of Philosophy degree in August of 2012.