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Review

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Received: 18 November 2011 Revised: 23 February 2012 Accepted: 23 February 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/dta.1343

Analytical techniques for the determination of tryptamines and β-carbolines in plant matrices and in psychoactive beverages consumed during religious ceremonies and neo-shamanic urban practices

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The consumption of ayahuasca, a hallucinogenic beverage used by indigenous communities in the Amazon, is increasing worldwide due to the expansion of syncretic religions founded in the north of Brazil in the first half of the twentieth century, such as *Santo Daime* and *União do Vegetal*. Another example is the jurema wine, a drink that originated from indigenous cultures of the northeast of Brazil. It is currently used for several religious practices throughout Brazil involving urban neo-shamanic rituals and syncretic Brazilian religions, such as Catimbó and Umbanda. Both plant products contain *N,N*-dimethyltryptamine which requires co-administration of naturally occurring monoamine oxidase inhibitors, for example β-carboline derivatives, in order to induce its psychoactive effects in humans. This review explores the cultural use of tryptamines and β-carbolines and focuses on the analytical techniques that have been recently applied to the determination of these compounds in ayahuasca, its analogues, and the plants used during the preparation of these beverages. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: ayahuasca; jurema wine; plants; tryptamines; β-carbolines; detection; hallucinogens

Introduction

Since the emergence of civilizations, the consumption of psychoactive plants has been used to induce altered states of consciousness. In pre-Columbian societies, the use of these plants was normally associated with mystical-religious rituals and preparation for war. Colonization of the Americas resulted in European explorers coming into contact with a variety of psychoactive plants, including tobacco (*Nicotiana* spp.), maracujá or passion fruit (*Passiflora* spp.), guaraná (*Paulinia cupana*) and yopo (*Anadenanthera peregrina*).[1–4] Four centuries after the global spread of tobacco, consumption of the plant-derived beverage ayahuasca, which originated in indigenous Amazon cultures, is attracting devotees throughout the world as a result of the creation of syncretic religious groups in Brazil during the twentieth century.[5] Two of these religions, Santo Daime and União do Vegetal (UDV), are represented in various countries around the world including Australia, the United States, and Europe. In some countries, a number of legal disputes have been described concerning the legalization of ayahuasca and consumption during religious rituals.[6–9] In addition, 'ayahuasca tourism' is becoming increasingly common in those equatorial South American countries that share areas of the Amazon rainforest.[6,10] Moreover, the Internet also offers a great variety of opportunities to purchase psychoactive plant materials.[11–13] Among the many compounds found in some of these plants, the tryptamine and β-carboline derivatives (Figure 1) represent simple indole alkaloids that are commonly present in the biota.

Ayahuasca is most commonly produced as a decoction using leaves of chacrona (*Psychotria viridis*) and sections of the stem of the yage vine (*Banisteriopsis caapi*). Important key components of the vine are β-carboline derivatives that act as inhibitors of monoamine oxidase (MAO). The leaves of *P. viridis* contain the psychoactive/hallucinogenic *N,N*-dimethyltryptamine (DMT) and

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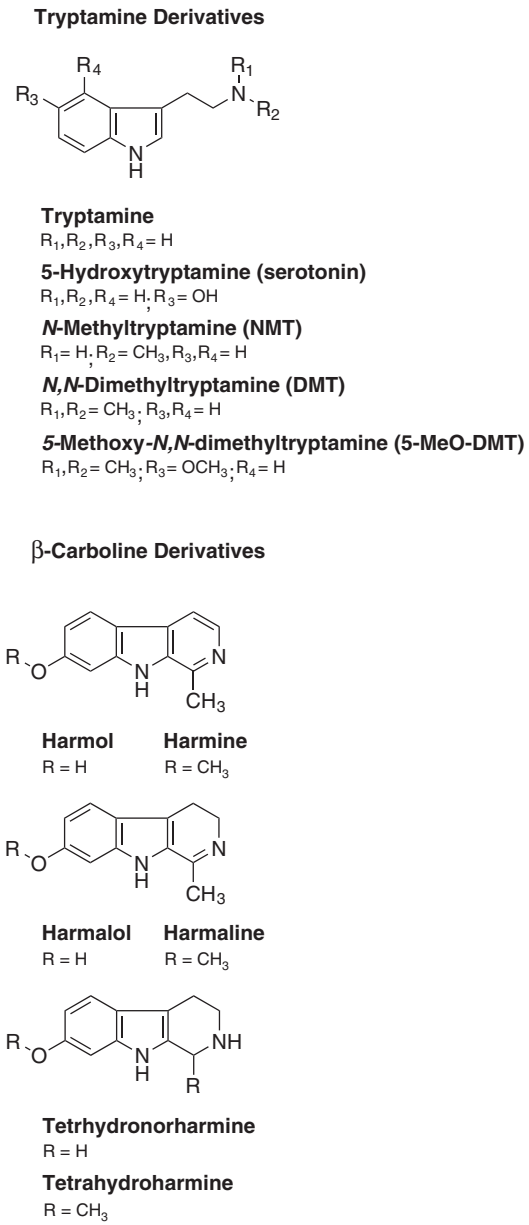

Figure 1. General chemical structure of the tryptamine and β-carboline derivatives.

the combination with reversible MAO inhibitors (MAOIs) renders the DMT orally active.[14–17]

In an analogous fashion, the jurema wine, originally consumed only by pre-colonial indigenous tribes in the northeast of Brazil, has become a part of the liturgy of the Catimbó and Afro-Brazilian religious groups since colonization. The wine is predominantly produced using the root bark of the jurema tree (*Mimosa* spp.), which also contains DMT.[18–21] In large urban centres, it is common to obtain the bark of *M. tenuiflora* (black jurema) from online sources[11,13] and to use the seeds of *Peganum harmala* as a source of MAOIs. *P. harmala* is a Mediterranean shrub that contains a number of β-carbolines also present in *B. caapi*.[22]

Studies involving the chemical characterization of these plants, together with the development of analytical techniques for the measurement of tryptamines and β-carbolines in plant matrices, as well as in ritual beverages, are essential given the current expansion in their use for religious, recreational, and clinical research purposes. The need for an in-depth approach towards analytical characterization becomes obvious in cases of untoward effects or even fatal intoxications which can, for example, arise from ill-informed combinations of plant products with other psychoactive substances.[23–26] At the same time, consideration needs to be given to the promising therapeutic potential that was reported for constituents present in these plant materials.[27–33] In addition, a wide variation of concentration levels of ayahuasca components that differ not only from church to church, but also between different batches of the same church, were also reported.[34] Occasionally, an extremely concentrated form called 'ayahuasca honey' can also be encountered which derives its name from high viscosity similar to honey syrup. Detailed studies on the identity and levels of psychoactive substances found in these preparations and appropriately defined criteria for their determination are required. This might be of particular interest in cases where there is the concomitant use of other additives such as *Cannabis*,[35,36] *P. harmala*, tobacco, and jurema wine, where precautions or quality control might be lacking. The objective of this review is to present some cultural and chemical features of DMT-containing plant products. An account is provided of recent developments in analytical approaches towards the determination of tryptamines, β-carbolines and tetrahydro-β-carbolines detected in tissues of *M. tenuiflora, P. viridis, P. aquatica, B. caapi* and *P. harmala*, as well as in ayahuasca samples.

Psychoactive beverages used for ritual purposes: ayahuasca and jurema wine

Ayahuasca

Ayahuasca (aya = soul, spirit; huasca = vine), a word belonging to the Quechua dialect still spoken in some regions of South America, is a drink that is mostly prepared using a decoction of two plants: the leaves of the DMT-containing chacrona (*Psychotria viridis*) and sections of the stem of the jagube vine (*Banisteriopsis caapi*) that provides three major MAOI components such as harmine, harmaline and tetrahydroharmine (THH) (Figure 1). The chemical composition of ayahuasca can differ between indigenous tribes due to the use of different plant species[1,14,15,37] although the same psychoactive constituents are present in all preparations.[38–41] Ayahuasca is known by various indigenous names, including *yajé*, *natema* and *caapi*, and was first described by Villavicencio in 1858. Seven years earlier, the English explorer Richard Spruce made contact with the Tukanoan Indians, in Rio Uaupés (Brazilian Amazonia), but his findings concerning the use of a liana called *caapi* were not published before 1908 when the plant was identified as *Banisteria caapi*.[37] Clinical research on the physiological and psychological effects of ayahuasca in humans re-emerged in the early 1990s which offered important insights into psychopharmacological, biochemical and pharmacokinetic properties of this hallucinogenic plant mixture. More importantly, these investigations set the stage for a range of clinical studies that followed across several disciplines until the present day.

Brazilian legislation, based on a constitutional right to freedom of religion, permits the consumption of ayahuasca within a religious context, including children and pregnant women which, in this case, requires parental consent.[42,43] Norms concerning the use of ayahuasca in Brazil for religious purposes were published by the

Brazilian National Council on Drug Policies (CONAD) in January 2010[44] which prohibits the marketing of ayahuasca, its therapeutic use, ayahuasca tourism, and its use with illicit drugs. Under this Resolution, consumption is permitted in a religious context and the same document also emphasizes the need for more multidisciplinary areas of research on ayahuasca.[9,44] The government of the State of Acre in Brazil has published a Resolution concerning the authorization of extraction and transport of *Banisteriopsis* spp. vines, as well as the leaves of the *Psychotria viridis* shrub carried out by religious organizations in the State of Acre for the purposes of ayahuasca preparation.[45] It should be noted that while the Brazilian government only legitimized the production and consumption of ayahuasca derived from the *B. caapi* vine[44] the Acre Resolution covers every species of the *Banisteriopsis* genus.

Jurema wine

Species of the Mimosaceae botanical subfamily, locally known in northeast Brazil as jurema (from the Tupi *yurema*, meaning succulent thorn bush), are considered to be amongst the most potent plant sources of DMT. These medium-sized trees are used by various indigenous groups, such as the Kariri-Xocó whose communities are located on the left border of the São Francisco River, the boundary between the Brazilian States of Sergipe and Alagoas. The inner barks of stems and roots are used to prepare a beverage called *vinho da jurema* (jurema wine), or *ajucá* (by the Pancarú Indians) and *cotcha-lhâ*, by the Fulniô Indians.[46] During the *Toré*, a ritual dance designed to demonstrate the power of resistance and express the depth of Brazil's northeastern indigenous culture, the Indians drink *vinho da jurema* including a number of additives such as tobacco and *Passiflora* juice or a tea made from its leaves.[47] This beverage can be produced using several species such as *M. tenuiflora* (black jurema), *M. ophthalmocentra* (red jurema) and *M. verrucosa* (white jurema or sweet jurema according to the Kariri-Xocó Indians) and other plants of the Mimosaceae subfamily.[19,20,47] A mixture of plants is essential to potentiate the psychoactive activity of the DMT, since the *Mimosa* spp. does not appear to contain any appreciable quantities of MAO inhibitors. Although there have not been any studies that reported oral psychoactivity of jurema wine, it may be relevant to observe that indigenous groups and members of Brazilian syncretic religions use large quantities of tobacco.[19] It is known that tobacco smoke contains a number of constituents that possess MAOI activity[48–50] which indicates that orally administered DMT might become psychoactive under these conditions.

The concomitant use of plants belonging to the *Passiflora* species is common in these indigenous communities, while in the syncretic Brazilian groups, besides the smoked tobacco used during the rituals, sugarcane alcohol (cachaça) is also widely used together with other additives during the preparation of the psychoactive beverage.[47,51] A number of studies have identified the presence of MAOI constituents in *Passiflora* species, especially in *P. incarnata*.[52–55] Seeds of *Peganum harmala*, which have been shown to be highly effective in inhibiting monoamine oxidase, have also been described to potentiate the oral psychoactivity of jurema wine.[56,57]

The use of jurema wine has a long history, stretching from its indigenous origins in the *sertão*, i.e. the northeastern region of Brazil, to current days where it is consumed throughout the country by the members of Catimbó-Jurema and followers from other religions. This is a typical example of syncretic evolution of the original indigenous tradition. The jurema use was adopted by the Afro-Brazilian religions which incorporated the Jurema cult in their own traditions when fugitive African slaves were harboured by the northeastern indigenous tribes during their escape to the *quilombos* (communities of escaped slaves). Nowadays, we can see the incorporation of jurema use into neo-shamanic practices and its popularization via the Internet. In contrast to ayahuasca, the additives used in the preparation of jurema wine, by the Indians and members of these religions, remain a closely guarded secret. In Brazil, there is historical documentation describing the indictment and imprisonment of indigenous Indians who consumed the drink.[46,51] Jurema rituals were almost extinguished by the devastating impact of Portuguese Christian colonization; however, since the end of the twentieth century the movement has witnessed a substantial resurgence.[47]

Plants used for Ayahuasca

Psychotria spp

Psychotria spp. belongs to the Rubiaceae family, which also includes coffee. Some species of the genus *Psychotria* are used by Amazonian Indians as additives in the preparation of ayahuasca, namely, *P. viridis, P. carthaginensis*, *P. psychotriaefolia* and *P. poeppigiana*. In the Amazon, *P. viridis* (Figure 2b), is a shrub that reaches a maximum height of 2–3 m[1] and which is popularly known as 'chacrona', 'chacruna', or 'rainha'. Native to the Amazon rainforest, where the plant is becoming increasingly rare, it has become commercially cultivated due to the demand for its leaves, which are used to prepare ayahuasca, although this practice is frowned upon by the Brazilian authorities. In Brazil, the churches tend to be located in the countryside nearby urban centres where there is always a possibility of maintaining their own plantations called *reinados das rainhas* (kingdoms of the Queens). Plantations have also been reported in Hawaii and California.[58] The leaves of *P. viridis* are collected in the early morning or the late afternoon for the production of ayahuasca. The first description of the presence of DMT in these leaves was published in 1970, as was the first report of the presence of the chemical in a member of the Rubiaceae family.[59] The leaves have been reported to contain between 0.10 and 0.61% DMT, together with traces of *N*-methyltryptamine (NMT) and 2-methyl-tetrahydro-β-carboline (MTHC).[58]

Banisteriopsis spp

Some species of the *Banisteriopsis* genus, including *B. argentea*, *B. inebrians, B. caapi* and *B. muricata*, are used to prepare ayahuasca and other similar psychoactive beverages, since they contain the MAOI needed to ensure oral psychoactivity of DMT.[60,61] Nonetheless, *B. caapi* (Figure 2d), is the plant most commonly used for this purpose.[14,37,58] The entire plant contains β-carboline and tetrahydro-β-carboline alkaloids (in concentrations varying from 0.11 to 1.95%), although the stem of the vine is the part normally used. The main alkaloids present are harmine, harmaline, and THH. The levels of harmine, which exerts a reversible MAOI effect, are equivalent to between 40 and 96% of the total alkaloid content of the plant. On the other hand, it has also been reported that these constituents were absent in *B. caapi* samples.[58] Similarly the species *P. viridis*, *B. caapi* is also cultivated in Brazil by some religious groups and plantations have also been reported in Hawaii.[41]

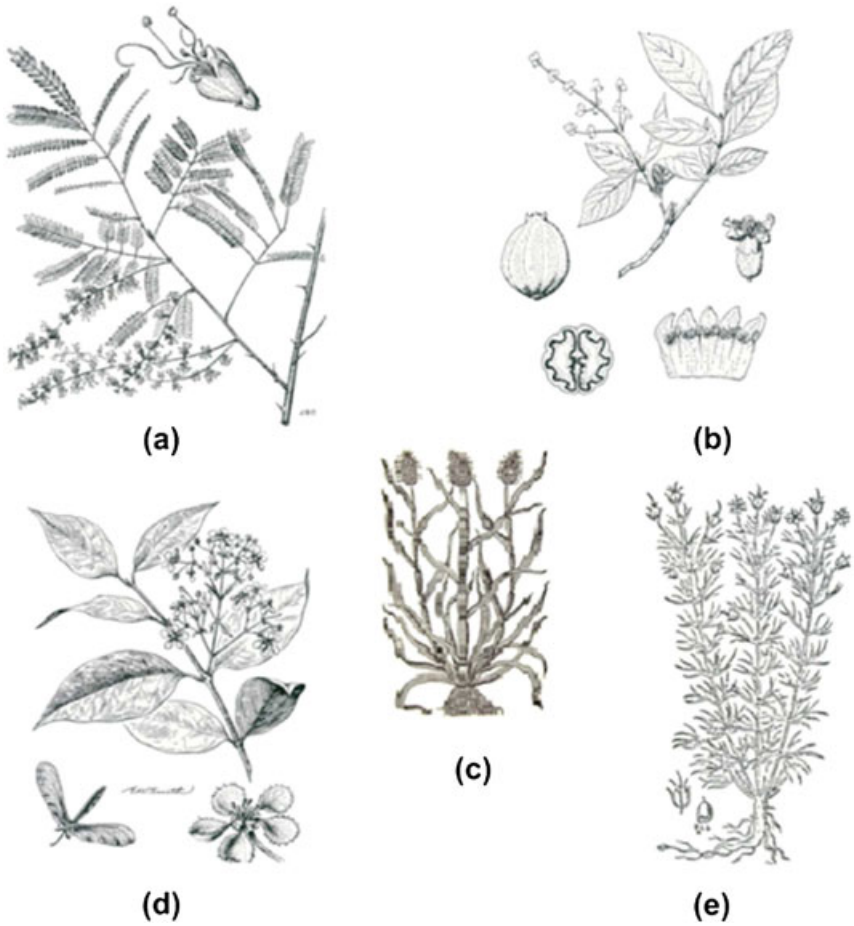

Figure 2. (a) Morphology of *M. tenuiflora* (illustration from J.B. Clark);[37] (b) Morphology of *P. viridis* (illustration from I. Brady);[37] (c) *Phalaris aquatica*, eighteenth-century illustration;[58] (d) Morphology of *B. caapi* (Illustration from E.W. Smith);[37] (e) *Peganum harmala*, seventeenth-century illustration.[58]

Plants used for Jurema wine

Mimosa spp

Several members of the *Mimosa* genus (Leguminosae family) are known as jurema by rural communities in Brazil.[1] Some species such as *M. tenuiflora*, *M. Ophthalmocentra*, *M. verrucosa* and *M. scabrella* may contain considerable amounts of psychoactive tryptamines, especially in their barks.[19,47,58,62,63]

In Brazil, *M. tenuiflora* (Willd.) Poir. [*syn. M. hostilis* (Mart.) Benth.] (Figure 2a), known as 'jurema-preta' (black jurema), is used as the main ingredient in jurema wine since the inner bark of the stem and roots is rich in DMT. Native to low rainfall regions that experience periodical drought, this plant is abundantly found in northeast Brazil, in dry valleys in southern Mexico, in the north of Venezuela and Colombia, as well as in Honduras and El Salvador. In its native habitat it reaches a height of 2.5–5 m and readily colonizes degraded terrain, grows rapidly, and is able to generate new shoots after cutting.[64] In Mexico, where it is known as *tepescohuite*,[21] it appears that there are no reports of its usage as a psychotropic product, although the dried and ground bark is used for wound healing and treatment of skin burns.[65] Jurema wine made from the inner bark of *M. tenuiflora* in addition to *Peganum harmala* seeds which provide the same active principles found in ayahuasca, namely DMT and MAOIs.[56,57] The illicit use of *M. tenuiflora* has become a concern that is currently being addressed by the Brazilian Federal Police.[13]

Plants used for ayahuasca and jurema wine analogues

Phalaris spp

The presence of tryptamines in *Phalaris* species was first described in phytochemical studies for agricultural purposes. *P. arundinacea* (reed canary grass), *P. canariensis* and *P. aquatica* are found worlwide. *P. aquatica* (Figure 2c) is a grass native to the Mediterranean region, and is common in wetlands and that is considered to be toxic to ruminant livestock. Instances of animal poisoning involving *Phalaris* species, sometimes fatal, have been reported in Australia, South Africa, Argentina, Brazil, and the USA.[66–70] Within its genus, *P. aquatica* contains the highest levels of DMT in addition to other tryptamines, such as 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) and NMT.[71] It has also been increasingly used for the preparation of ayahuasca analogues.[56,57]

Peganum harmala L

Syrian rue, or *P. harmala* (Figure 2e), is a shrub native to the dry regions of the Mediterranean, North Africa, the Middle East, India, and Mongolia.[1,22] In North Africa, its seeds are used to the present day as ritual incense. It is an ancient ritual plant, and in folk medicine it is still used for gynecological purposes and as a vermifuge. This plant is increasingly used in North America and

Europe to produce drinks containing DMT and β-carbolines that are analogous to ayahuasca.[22,57] Three to four grams of the seeds is considered to be sufficient to inhibit the action of monoamine oxidase. The alkaloid content of *P. harmala* seeds is around 2–6 %, and consists principally of harmine and harmaline.[58]

Analytical methods

Earlier work

The first descriptions of methods used for the determination of tryptamines and β-carbolines in these plant species and their beverages date from the mid-twentieth century. Most of them were based on liquid-liquid extraction (LLE) and purification was usually performed by column chromatography and crystallization techniques.[46,72–77] Since the late 1960s, the use of high performance liquid chromatography (HPLC) and gas chromatography (GC) coupled with mass spectrometry (MS) became more prominent.[14,59–61]

The Brazilian chemist Oswaldo Gonçalves de Lima was the first scientist to study the chemical composition of the jurema wine, as well as its preparation, and also the first to isolate DMT from the bark of black jurema. This study also provided a detailed account of the ceremony and preparation of the *vinho da jurema* by Indians of the Pancarú tribe (Pernambuco, Brazil). This study also described the isolation of the alkaloid fraction of the root bark of *M. tenuiflora* which led to the identification of 'Nigerina', i.e. 'nigerine' (0.31% dry weight, DW).[46] Years later, this was confirmed to be DMT following the analysis of *M. tenuiflora* bark by Pachter and co-workers. This sample was provided by de Lima and was found to contain up to 0.57% of DMT in the dried plant.[77] Meckes-Lozoya *et al*.[21] identified serotonin and DMT in samples of *M. tenuiflora* root bark using GC-MS and Batista *et al*.[20] isolated the alkaloid fraction of *M. ophthalmocentra* and reported the presence of DMT (1.6%, DW) and NMT (0.0012% DW), respectively.

Regarding ayahuasca, and the plants employed in its preparation, descriptions of analytical quantitative methods date back to the early 1970s. The first description of *P. viridis* analysis was provided in 1969[78] and Rivier and Lindgren carried out a major investigation into the analytical chemistry of ayahuasca and reported the findings in a landmark paper in 1972.[14] The authors reported the results of their work carried out on the upper Rio Purus region near the border between Peru and Brazil, in which they reported the use of ayahuasca by the Sharanahua and Culina Indians. The procedure for chemical analysis of the parts of the plants used in its preparation has also been described. Implementation of liquid-liquid extraction (LLE) was followed by an analysis by GC-MS. The leaf samples of *P. viridis* showed a DMT content (DW) of approximately 0.34%. The same substance was also found at higher concentration levels (0.66% DW) in the leaves of *P. carthaginensis*. The presence of NMT and MTHC was also detected at trace levels. However, one of the leaf samples was reported to contain 85% NMT and 12% MTHC (total alkaloid content 0.11%, DW). Dry matter samples of stems, branches, leaves, and roots of *B. caapi* revealed the presence of β-carbolines ranging from 0.05 to 1.90% with the majority being represented by harmine, followed by THH, harmaline, and harmol, respectively.[14] Also in this work, some samples of ayahuasca were analyzed, stating for each 100 mL of the beverage the presence of 6.6–19 mg for harmine, 1.5–9.8 mg for tetrahydroharmine, 0.3–1.6 mg for harmaline and 5.4–16.0 mg for DMT.

In 1984, and with the use of appropriate standard solutions, samples of ayahuasca from Peru were analyzed qualitatively and quantitatively using two-dimensional thin-layer chromatography (TLC), HPLC, and GC-MS.[15] The majority of alkaloids obtained from five Peruvian ayahuasca samples were quantified by HPLC-UV (260 nm) which led to the detection of DMT (0.6 mg/mL), harmine (4.67 mg/mL), THH (1.60 mg/mL) and harmaline (0.41 mg/mL), respectively. The same samples were also freeze dried and subjected to analysis by HPLC. The reported values were DMT (6.4 mg/g or 0.64%), harmine (23.8 mg/g or 2.38%), THH (11.1 mg/g or 1.11%), and harmaline (5.1 mg/g or 0.51%). Six samples of *B. caapi* were also evaluated quantitatively by HPLC leading to harmine (0.57–6.35 mg/g or 0.057–0.635%), THH (0.25–3.8 mg/g or 0.025–0.38%), harmaline (0.5–3.8 mg/g or 0.05–0.38%), harmol (0.01–1.2 mg/g or 0.001–0.12%) and harmalol (trace– 0.35 mg/g or trace – 0.035%). Analyses conducted by GC-MS also confirmed the presence of DMT (1–1.6 mg/g or 0.1–0.16%) in leaves of *P. viridis*.[15]

Recent analyses

Sample preparation techniques

Most of the methods described for the determination of tryptamines and β-carbolines present in plant matrices and ayahuasca employ sample preparation techniques that require large quantities of toxic organic solvents and that are time-consuming. For herbal samples (Tables 1 and 2), maceration in a suitable solvent, LLE and use of a continuous-flow Soxhlet extraction, are the most commonly used procedures.[22,38,79–83] An effective alternative technique, especially useful when combined with GC, is matrix solid-phase dispersion (MSPD).[84,85] This approach was described first in 1989[86] and was recently employed by Gaujac *et al*. for the quantitative determination of DMT in the bark of *M. tenuiflora*.[87] This procedure offered the advantage of low solvent consumption while providing excellent indices of selectivity, precision, and recovery. Following optimization using a multivariate procedure, recoveries were reported in the range of 81.7–116.2%.[87] Callaway *et al*. reported the quantification of β-carbolines and DMT in *P. viridis* leaves and *B. caapi* stems obtained by sonication of a 100 mg sample for 10 min using a minimal volume of methanol (2 mL). The mixture was allowed to stand for 24 h and then centrifuged before dilution a small aliquot of the supernatant in the mobile phase. Validation data for the proposed technique were not reported.[38]

The extraction of tryptamines was described by Zhou *et al*. who used 0.2 g of dry plant matrix (*P. aquatica*) and macerated the sample in 10 mL of 1% HCl, with periodic agitation.[71] After 3–4 days the mixture was centrifuged and the supernatant passed through a solid-phase extraction (SPE) column. Analytes retained on the column were eluted with 2 mL of an alkaline alcoholic mixture containing NH_4OH. No recovery tests were reported.[71] Wang *et al*. used various parts of *B. caapi*, including leaves, stems, large branches, and bark, and employed an extraction into hot water followed by HPLC analysis although method validation data were not provided.[32] Maceration in methanol and extraction of β-carbolines by LLE with chloroform was reported for the analysis of *P. harmala* seeds[79,82,83] and Pulpati *et al*. offered a methanol extraction of *P. harmala* seeds (1 g) with methanol (3 x 50 mL) under reflux conditions (1 h).[88]

Table 1. Methods for the determination of β-carbolines in *B. caapi* and *P. harmala*

	B. caapi		*P. harmala*			
Reference	Callaway *et al.* [38]	Wang *et al.* [32]	Kartal *et al.* [79]	Hemmateenejad *et al.* [82]	Monsef-Esfahani *et al.* [83]	Pulpati *et al.*[88]	Herraiz *et al.*[22]
Material analyzed	Stem	Leaves, stems, large branches and bark	Seeds	Seeds	Seeds	Seeds	Leaves, sections of stem, flowers, roots, fruits and seeds
Analytes of interest	Harmine, harmaline and THH	THNH, harmol, THH, harmaline, harmine and compounds from other chemical classes	Harmol, harmalol, harmine and harmaline	Harmine, harmane, harmalol and harmaline	Harmol, harmalol, harmine and harmaline	Harmine and harmaline and compounds from other chemical classes	Harmol, harmalol, harmine, harmaline and THH
Sample preparation method	Sonification in methanol and resuspension of residue in mobile phase	Maceration in hot water	Maceration in methanol and extraction by LLE with chloroform	Maceration in methanol and extraction by LLE with chloroform	Maceration in methanol and extraction by LLE with chloroform	Reflux in methanol (1 h)	Maceration in an acid solution of $HClO_4$ and methanol (1:1)
Separation / detection technique	HPLC and detection by fluorescence	HPLC-DAD	HPLC-UV at 330 nm	HPLC-UV at 330 nm	HPLC-UV at 330 nm	HPTLC-UV at 366 nm (densitometer-TLC scanner)	HPLC-DAD
Figures of merit	–	Linear range: 1.0–500.0 μg/mL (for THNH and THH) 0.2–100 μg/mL, (for harmol, harmaline & harmine)	$r^2 > 0.999$; LOD < 10.25 μg/mL; LOQ < 31.0 μg/mL; RSD < 4.609%	Linear range: 1.0–10.0 μg/mL; 94 < R** < 107% RSD < 5%;	Linear range: 0.5–20 μg/mL $r^2 > 0.998$ LOD < 0.1 μg/mL LOQ = 0.5 μg/mL 0.6 < RSD < 10.2%	Linear range: 4–24ng/spot (for harmine) 8–24ng/spot (for harmaline); $r^2 > 0.993$; LOD = 2 ng; LOQ = 4 ng; 97.7 < R < 98.4% Instrumental precision: RSD < 1.53%; Repeatability: RSD < 1.62%;	–
Concentration levels*	Harmine: 0.31 – 8.43 mg/g (0.031 – 0.843%); Harmaline: 0.03 – 0.83 mg/g (0.003 – 0.083%); THH: 0.05 – 2.94 mg/g (0.005 – 0.294%)	Harmine: 10^{-3} – 0.672%; Harmaline: 10^{-4} – 0.058%; THH: 0.004–0.34%; THNH: 0 – 0.014%; Harmol: 4.10^{-4} – 0.019%	Harmol: 1.094%; Harmine: 0.476%; Harmaline: 0.611%;	Harmine: 1.84%; Harmane: 0.18%; Harmaline: 3.90%; Harmalol: 0.25%	(in dried seeds) Harmine: 0.465 g/100 g (0.465%); Harmaline: 0.355 g/100 g (0.355%)	Harmine: 0,44% (w/w); Harmaline: 0.096% (w/w);	(in seeds) Harmalol: 6 mg/g (0.6%); Harmol: 0.03 mg/g (0.003%); Harmaline: 56 mg/g (5.6%); Harmine: 43 mg/g (4.3%); THH: 1.1 mg/g (0.11%)

* Concentration levels based on dry weight (DW) of vegetable matter.
** Recovery (R).

Table 2. Methods for the determination of tryptamines in *M. tenuiflora*, *P. viridis* and *P. aquatica*

	M. tenuiflora		***P. viridis***		***P. aquatica***
Reference	Nicasio *et al.* [81]	Vepsäläinen *et al.*[80]	Gaujac *et al.* [87]	Callaway *et al.* [38]	Zhou *et al.* [71]
Material analyzed	Inner bark, seeds, leaves and flowers. Cultures *in vitro* (plantules and callus)	Root inner bark	Seeds and flowers. Inner barks of stems and roots.	Leaves	Entire plant
Analytes of interest	DMT, tryptamine, serotonin	DMT and yuremamine	DMT	DMT	DMT, 5-MeO- DMT, gramine, hordenine, bufotenine and tryptamine
Sample preparation method	Soxhlet reflux extraction with chloroform and NH_3 solution (27%) (49:1)	Extracts obtained by maceration in methanol and resuspension in mobile phase	Matrix solid-phase dispersion (MSPD)	Maceration in methanol (67%) + acetonitrile (11%) + 0.1 mol/L ammonium acetate (22%)	Maceration in HCl (1%), centrifugation and solid-phase extraction (SPE)
Separation / detection technique	HPLC-UV at 280 nm	HPLC-DAD NMR (^{13}C- and ^{1}H NMR)	GC-MS	HPLC and fluorescence detection	HPTLC and HPLC- MS
Figures of merit	Linear range: 2 – 40 μg/mL	–	Linear range: 0.62 – 20 mg/g; $r^2 = 0.9962$; LOD = 0.12 mg/g; LOQ = 1.25 mg/g; 81.7 < R** < 116.2%; Precision inter-day: RSD < 16.8%; Precision intra-day: RSD < 7.4%	–	Linear range: 120 – 3840 ng/spot; $r^2 > 0.991$; Precision intra-day: RSD < 5%
Concentration levels*	(in barks) DMT: 0.11 – 0.35%; Tryptamine: 0.0022 – 0.0071%; (in flowers) DMT: 0.03%; Tryptamine: 0.0075%; (in leaves) DMT: 0.01 – 0.09%; Serotonin: 0.009%; (in cultures) For all analytes: < 0.08%	–	(in inner barks) DMT: 1.26 – 9.35 mg/g (0.126 – 0.935%) (in seeds and flowers) DMT: Below the LOQ	DMT: 0 – 17.75 mg/g (0 – 1.775%)	DMT: 66.3 – 177 mg/kg (0.00663 – 0.0177%); 5-MeO-DMT: 176 mg/kg (0.0176%)

* Concentration levels based on dry weight (DW) of vegetable matter.
** Recovery (R).

Herraiz *et al.*, on the other hand, described the maceration of 0.2–0.5 g of *P. harmala* components (leaves, sections of stem, flowers, roots, fruits, or seeds) in 20 mL of a 1:1 mixture containing 0.6 mol/L $HClO_4$ and methanol (1:1). Following centrifugation HPLC analysis was employed after dilution of the supernatant.[22]

The procedures required to prepare ayahuasca samples (Table 3) for GC analysis can be more time-consuming than those required for HPLC analyses, largely due to incompatibility of GC capillary columns with water. However, a successful implementation of LLE has been reported using *n*-butyl chloride as the organic solvent.[89] A variation of the theme was offered by Gambelunghe *et al.* who reported a GC-MS analysis of an ayahuasca sample seized in Italy.[40] In this particular case, sodium hydroxide and an internal standard (diphenylhydramine) were added to 5 mL ayahuasca followed by extraction into ethyl ether and centrifugation. Method validation data were not reported.[40] On the other hand, a C_{18} cartridge has also been employed for the determination of ayahuasca alkaloids by GC nitrogen phosphorus detection (NPD) which showed that SPE procedures can be equally applied. Minimal sample manipulation and small amounts of organic solvents were required and recoveries exceeded 68% for measurements in triplicate at concentrations of 0.3, 1.5, and 3.0 mg/mL.[39] McIlhenny *et al.* prepared ayahuasca samples from parts of specimens of *P. viridis* and *B. caapi* collected from cultivations in the district of South Kona, Hawaii (established using clones originating from Peru).[41] The *B. caapi* vines were macerated and boiled slowly, together with *P. viridis* leaves, for 10 h in 11 litres of double-distilled water. Aliquots (100 μL) of each ayahuasca preparation were diluted and analyzed by HPLC tandem mass spectrometry (MS/MS). The samples of ayahuasca derived from two extracts prepared simultaneously, in which the biomass of *B. caapi* was maintained constant and the quantity of *P. viridis* was varied (either 150 or 300 leaves).[41] Moura *et al.* prepared extracts of *P. viridis* for the quantification of DMT by LLE with hexane. An average recovery of 70% was obtained in experiments using three different concentration levels.[90]

Separation and quantification methods

Kartal and colleagues carried out a full validation exercise for the determination of harmol, harmalol, harmine and harmaline in *P. harmala* seeds using HPLC-UV. Several chromatographic parameters were also measured, including capacity factor and resolution. Harmol, harmine and harmaline were determined in samples at concentrations of 1.0, 0.4 and 0.6%, respectively.[79] The method described by Gaujac *et al.* included a full evaluation of figures of merit throughout all stages of the process of GC-MS analysis of the *M. tenuiflora* bark. The levels of DMT varied from 1.26 to 9.35 mg/g in samples of stem and root bark that had been collected in regions characterized by different pluviometric regimes.[87] Vepsäläinen *et al.*, using HPLC-UV and nuclear magnetic resonance (NMR) spectroscopy, discovered the presence of a new phytoindole in the bark of *M. tenuiflora* and it was also observed that heat or pH fluctuations impact on stability of this molecule. The phytoindole was termed yuremamine and further studies would be required in order to determine any potential MAOI activity.[80] Nicasio *et al.* used reversed phase HPLC, with UV detection at 280 nm, to measure tryptophan, tryptamine, serotonin and DMT in the bark, flowers, and leaves of *M. tenuiflora*, as well as in the callus and plantules using micropropagation techniques. The authors conducted two measurements in different times of the year to assess the variation of concentration levels of these analytes between winter and summer seasons.[81]

An HPLC method with a non-polar column and fluorescence detection was reported by Callaway *et al.*,[38] who employed a method that was based on their earlier work,[89] to measure β-carbolines and DMT in parts of *B. caapi* and in the leaves of *P. viridis*, respectively. In dry *B. caapi* material the concentrations obtained were 0.31–8.43 mg/g (harmine), 0.03–0.83 mg/g (harmaline) and 0.05–2.94 mg/g (THH), respectively. In dry leaves of *P. viridis* the maximum concentration of DMT measured was 17.75 mg/g. Diurnal fluctuations were also reported where higher concentrations were detected during daytime (with peaks at 06:00 am and 06:00 pm). Since DMT levels tended to reduce at dusk it was suggested that DMT might be produced in the leaves to aid absorption of solar radiation.[38] A proof-of-principle study using capillary electrophoresis laser-induced fluorescence electrospray ionization mass spectrometry (CE-LIF-ESI-MS) method was presented by Huhn *et al.*[91] The combination of both detection systems was particularly helpful as it allowed for the ability to obtain favorable peak shapes (50 Hz sampling rate) and structural information based on ESI-MS/MS detection. In case of co-elution or incomplete resolution of peaks, quantitative determinations would be possible only with the use of extracted ion electropherograms. Both detection methods could conveniently detect a set of six β-carboline standards around 770 amol levels. A diluted ayahuasca sample revealed the presence of DMT, harmaline, harmine and THH (no quantitation) and an ethanolic extract obtained from *P. viridis* leaves (ultrasonication at 45 °C) showed DMT and an unidentified species with a protonated molecule at *m/z* 189 and product ions at *m/z* 165, 147, 119, 104, and 87, respectively.[91]

Hemmateenejad *et al.* applied multivariate statistical procedures to optimize an HPLC procedure (UV detection at 330 nm) for the determination of harmine, harmane, harmalol and harmaline in *P. harmala* seeds[82] and the chromatographic conditions, including column and mobile phase, were similar to those described earlier by Kartal *et al.*[79] In validation tests the method gave a precision value of 4.6%, excellent linearity ($r^2 > 0.999$) and limits of detection and quantification in the ranges of 3.1–10.3 μg/mL and 9.3–31.0 μg/mL, respectively. In seeds collected from plants in Iran, concentrations of harmine, harmane, harmaline and harmalol were 1.84, 0.16, 3.90, and 0.25%, respectively.[79] Other excellent validation results were obtained by Monsefi-Esfarani *et al.* using an adaptation of the same method with changes in mobile phase pH. Calibration curves were linear ($r^2 > 0.998$) for all analytes in the concentration range of 0.5–20 μg/mL and method RSD values ranged from 0.6–10.2% for all analytes. LODs were less than 0.1 μg/mL and LOQs equal to 0.5 μg/mL.[83] Pulpati *et al.* reported a high-performance thin-layer chromatography (HPTLC) method for the quantification of harmine, harmaline, vasicine and vasicinone from *P. harmala* seeds.[88] These compounds were detected by a densitometric method and the seeds were found to contain 0.44% of harmine and 0.096% of harmaline (both DW) with suitable figures of merit. Herraiz *et al.* measured harmol, harmalol, harmine, harmaline, and THH in extracts prepared using different parts of *P. harmala*. Quantification of the β-carbolines employed reversed phase HPLC with UV-diode array detection (DAD).[22]

Standardized aqueous extracts of *B. caapi* were obtained by Wang *et al.*[32] These were prepared using different parts of the

Table 3. Methods for the determination of tryptamines and β-carbolines in ayahuasca

	Ayahuasca					
Reference	Callaway[34]	Huhn *et al.*[91]	Pires *et al.*[39]	Gambelunghe *et al.*[40]	McIlhenny *et al.* [41]	Moura *et al.*[90]
Material analyzed	Real ayahuasca samples	Real ayahuasca sample	Real ayahuasca samples	Real ayahuasca sample	Extracts prepared in the laboratory from *P. viridis* leaves and the *B. caapi* vine	Extracts prepared from *P. viridis* leaves, and real ayahuasca samples
Analytes of interest	DMT, harmine, harmaline and THH	DMT, norharmane, harmane, harmine, harmaline, harmol and THH	DMT, harmine, harmaline and THH	DMT, harmine and harmaline	DMT, NMT, harmol, harmalol, harmine, harmaline and THH	DMT
Sample preparation technique	For DMT: LLE; For β-carbolines: dilution of extracts in mobile phase	Ayahuasca sample was diluted with a buffer	Solid-phase extraction	Extraction with ethyl ether and centrifugation	Dilution of samples in the mobile phase	LLE
Separation / detection techniques	DMT: GC-NPD; β-carbolines: HPLC-FL	CE -LIF-MS	GC-NPD	GC-MS	HPLC-MS/MS	qNMR
Figures of merit	–	–	Linear range: 0.02 – 4.0 mg/mL; $0.9941 < r^2 < 0.9971$; LOD = 0.01 mg/mL; LLOQ = 0.02 mg/mL; 1.3% < RSD < 9.7%	–	Linear range: 5 – 100 ng/mL and 5 – 100 μg/mL; $r^2 > 0.9965$; LOD < 0.0079 ppm; LOQ < 0.24 ppm	Linear range: 25 – 1000 mg/L; $r^2 = 0.999$; LOD = LLOQ = 12.5 mg/L; R*= 70% RSD < 5.1%;
Concentration levels	DMT: 0 – 14.15 mg/mL; THH: 0.48 – 23.80 mg/mL; Harmaline: 0 – 0.90 mg/mL; Harmine: 0.45 – 22.85 mg/mL	–	DMT: 0.42 – 0.73 mg/mL; Harmine: 0.37 – 0.83 mg/mL; Harmaline: 0.64 – 1.72 mg/mL; THH: 0.21 – 0.67 mg/mL	DMT: 24 mg/100 mL; Harmaline 6 mg/100 mL Harmine: 34 mg/100 mL	DMT: 0.12 – 3.19 mg/mL; NMT: 0.0052 – 0.0313 mg/mL; Harmalol: 0.0026 – 0.0310 mg/mL; Harmol: 0.0009 – 0.0633 mg/mL; Harmine: 0.91 – 16.1 mg/mL; Harmaline: 0.054 – 1.55 mg/mL; THH: 1.22 – 11.90 mg/mL	–

* Recovery (R).

plant, including leaves, stem bark, and entire branches, and were collected from different geographical locations in the Hawaiian Islands of Oahu and Hilo during different seasons. Determinations of tetrahydronorharmine (THNH), harmol, THH, harmaline and harmine were performed using HPLC-DAD. The concentrations measured are listed in Table 1. Validation test results were not reported. Zhou *et al*. developed a method to quantify tryptamines and a β-carboline in 14 *P. aquatica* populations using HPTLC.[71] Visualization of spots included the use of an acidified anisaldehyde reagent spray that produced intense colours which were amenable to quantitation using a flatbed digital scanner. Good linearity was obtained in the concentration range between 120–3840 ng per spot, with a correlation coefficient above 0.991, for hordenine, methyltyramine, gramine, and 5-MeO-DMT. The method provided good specificity for the analytes of interest, as well as adequate repeatability with a variation of less than 5%, on average, for analyses in duplicate. Compound identification was confirmed by atmospheric pressure chemical ionization (APCI) LC-MS.[71]

Of particular note amongst the methods used to quantify tryptamines and β-carbolines in ayahuasca (Table 3) is GC coupled with either an NPD or a mass spectrometer.[34,39,40] NMR has also been used to quantify DMT.[90] Overall, an expansion towards method validation seems indicated in order to examine the reliability of measurements. Callaway[34] presented a compilation of the results obtained for a large number of ayahuasca samples with measurements of DMT, THH, harmine, and harmaline. Decoctions of *B. caapi* were prepared in Brazil by the three main religious groups involved in its use (*Santo Daime*, *União do Vegetal*, and *Barquinha*), and preparations of ayahuasca were also obtained from the Ecuadorian Shuar Indian tribe. An earlier method[89] was used for the detection of β-carbolines with separation by HPLC and fluorescence detection. DMT was determined by GC-NPD and large differences were found in the concentrations of the analytes in the samples, with DMT levels varying between zero and 14.15 mg/mL.

Pires *et al*. appeared to be the first to report a validated method for the simultaneous determination of both DMT and the β-carbolines harmine, harmaline, and THH in real samples of ayahuasca using GC-NPD.[39] For all of the analytes the calibration curves showed excellent linearity in the concentration range 0.02–4.0 mg/mL, with r^2 values varying between 0.9941 and 0.9971. The precision of the method was between 94.0 and 105.4%, and intra-day and inter-day coefficients of variation were lower than 9.7%. LODs and LOQs were provided. In stability tests using spiked water and ayahuasca, losses were less than 10% after 24 h of storage at ambient temperature. The ranges of concentrations measured in eight real ayahuasca samples were 0.42–0.73 mg/mL (DMT), 0.37–0.83 mg/mL (harmine), 0.64–1.72 mg/mL (harmaline) and 0.21–0.67 mg/mL (THH). Despite originating from the same religious group in Araçoiaba da Serra (Brazil), the concentrations in the beverages varied between samples probably due to the use of different quantities and proportions of the plants in each preparation, as well as different alkaloid contents present in the plant specimens.[39]

McIlhenny *et al*. developed an HPLC electrospray ionization (ESI) MS/MS method for the determination tryptamines and β-carbolines present in ayahuasca samples prepared in the laboratory.[41] This comprehensive MS/MS procedure was optimized for the detection of 11 alkaloids and revealed that major constituents of ayahuasca included THH, harmine, DMT, and harmaline, followed by harmalol and NMT. In addition, 5-MeO-DMT, 5-HO-DMT (bufotenin), and MTHC were also detected in some but not all samples. Method validation included determination of precision, method bias, inter- and intra-day precisions, limits of detection and quantification. The analytical curves for the compounds were linear in the concentration ranges employed (5–100 ng/mL and 5–100 μg/mL, depending on the compound), with r^2 above 0.996.[41]

Gambelunghe *et al*. measured concentrations of DMT and harmine of 24.6 mg/100 mL and 34 mg/100 mL, respectively, in a sample of ayahuasca that had been seized in Italy, but did not provide any validation data.[40] An alternative approach was offered by Moura *et al*.[90] who demonstrated that ^{1}H NMR could be successfully employed for the detection of DMT in ayahuasca. The optimized method was applied to water samples spiked with DMT and 2,5-dimethoxybenzalde as the internal standard, and excellent figures of merit were obtained in validation experiments (Table 3). The authors analyzed extracts prepared from the leaves of *P. viridis*, as well as eight samples of ayahuasca but results were not reported.[90] Earlier work,[34] however, indicated that typical levels of DMT in ayahuasca could well exceed the linear range cited by Moura *et al*. (25–1000 mg/L).[90] The main advantages of the ^{1}H NMR technique, compared to chromatography, are that the analysis is fast (~30 s), non-destructive, and that it can provide structural information as well. However, a possible influence of matrix effects was not reported and the method was developed in the absence of any β-carbolines present in fortified aqueous samples. It is well-known that ayahuasca preparations invariably contain material from one of the *Banisteriopsis* spp., which provides the β-carbolines that are vital for the oral psychoactivity of the DMT present in *P. viridis* leaves. Further studies can shed more light on the question as to whether such compounds could interfere with the determination of DMT by NMR.

In summary, while the majority of analytical methods employed in recent years involved the implementation of HPLC-UV procedures, less expensive approaches towards quantitative estimations of at least the major alkaloids found in these psychoactive plant matrices, such as HPTLC, were found to be suitable as well. The key alkaloids, i.e. DMT and the main β-carbolines, are sufficiently volatile to undergo GC-based analysis after extraction into a suitable organic solvent without the need for derivatization. The complementary approach offered by HPLC-based methods is increasingly supported by mass spectrometric applications that offer improved sensitivity and specificity when compared to UV/DAD detection. As described above, the first comprehensive HPLC-MS/MS-based target screening approach of ayahuasca and method validation was reported by McIlhenny *et al*.[41] who also demonstrated that matrix effects, particularly relevant where ESI is employed, were not observed. The need for such a robust, sensitive, and selective mass spectrometric analysis method is especially helpful when considering bioanalytical research following ayahuasca administration studies in humans.[92,93] In addition, it is anticipated that future research on the characterization of these diverse psychoactive brews will benefit from more comprehensive unknown screening methodologies based on full-scan modes in order to identify additional constituents that have not yet been identified. Finally, a more thorough application of sensitive and selective MS/MS-based methodologies is expected to shed more light on the role of psychoactive tryptamines present in mammalian tissues including humans.[94]

Conclusion

The use of psychoactive plant products known to contain bioactive tryptamine and β-carboline derivatives is increasing worldwide which reflects the expansion of syncretic religions derived from South America and straightforward access of plant products that contain these alkaloids. Recent research has been reviewed concerning the implementation of analytical techniques used for the detection of tryptamines and β-carbolines present in plants and psychoactive beverages consumed for religious and recreational purposes. For further studies, an increased focus on method validation procedures is recommended. Given the increasing interest in these plants and the ritual beverages derived from them it is clear that suitable routine analytical techniques will increasingly be required to accurately measure the associated psychoactive compounds in a variety of different matrices. This is especially the case within the clinical and forensic context. An additional avenue for further explorations includes a move towards minimal sample manipulation and low to zero use of environmentally toxic solvents.

Acknowledgements

Helpful comments from Adjunct Professor J.C. Callaway, Ph.D. and Prof. Mark Wainwright, Ph.D. are gratefully appreciated.

References

[1] R.E. Schultes, A. Hofmann, C. Rätsch, *Plants of the Gods: Their Sacred, Healing and Hallucinogenic Powers*, Healing Art Press. Rochester, NY, **2001**.
[2] G. Montenegro. O uso de psicotrópicos na América Pré-Colombiana a partir de uma perspectiva religiosa. *Amerindia* **2006**, *2*, 1.
[3] J.P. Ogalde, B.T. Arriaza, E.C. Soto. Identification of psychoactive alkaloids in ancient Andean human hair by gas chromatography/mass spectrometry. *J. Archaeol. Sci.* **2009**, *36*, 467.
[4] S.T. Martinez, R. Almeida, A.C. Pinto. Alucinógenos naturais: Um voo da Europa medieval ao Brasil. *Quim. Nova* **2009**, *32*, 9.
[5] 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.
[6] M. Dobkin de Rios, R. Rumrrill. *A Hallucinogenic Tea, Laced with Controversy: Ayahuasca in the Amazon and the United States*. Praeger, Westport, CT, **2008**.
[7] R.K. Bullis. The "Vine of the Soul" vs. the controlled substances act: Implications of the hoasca case. *J. Psychoactive Drugs* **2008**, *40*, 193.
[8] K.W. Tupper. The globalization of ayahuasca: harm reduction or benefit maximization? *Int. J. Drug Policy* **2008**, *19*, 297.
[9] B.C. Labate, K. Feeney. Ayahuasca and the process of regulation in Brazil and internationally: implications and challenges. *Int. J. Drug Policy* **2011**, DOI: 10.1016/j.drugpo.2011.06.006
[10] C. Holman. Surfing for a shaman: Analyzing an ayahuasca website. *Ann. Tourism Res.* **2011**, *38*, 90.
[11] F. Schifano, P. Deluca, A. Baldacchino, et al. Drugs on the web; the Psychonaut 2002 EU project. *Prog. Neuro-Psychoph.* **2006**, *30*, 640.
[12] P. Dalgarno. Buying Ayahuasca and other entheogens online: a word of caution. *Addict. Res. Theory* **2008**, *16*, 1.
[13] M.G.R. Leite. *Estudo do uso ilícito de uma planta alucinógena, Mimosa tenuiflora (jurema-preta), da Caatinga nordestina*, Academia Nacional de Polícia. Brasília, **2009**.
[14] L. Rivier, J.E. Lindgren. "Ayahuasca," the South American hallucinogenic drink: An ethnobotanical and chemical investigation. *Econ. Bot.* **1972**, *26*, 101.
[15] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and beta-carboline constituents of ayahuasca. *J. Ethnopharmacol.* **1984**, *10*, 195.
[16] M. Yritia, J. Riba, J. Ortuño, 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. *J. Chromatogr. B* **2002**, *779*, 271.
[17] Z. Mishor, D.J. McKenna, J.C. Callaway. DMT and human consciousness, in *Altering Consciousness, Volume 2: Biological and Psychological Perspectives*, (Eds: E. Cardeña, M. Winkelman). Preager, Westport, CT, **2011**, pp. 85.
[18] C.N. da Mota, Jurema e identidades: um ensaio sobre a diáspora de uma planta poderosa. in *O uso ritual das plantas de poder*, (Eds: B.C. Labate, S.L. Goulart). Mercado das Letras, Campinas, **2005**, pp. 219.
[19] R.S.O. de Souza, U.P. Albuquerque, J.M. Monteiro, L.C. de Amorim. Jurema-Preta (*Mimosa tenuiflora* [Willd.] Poir.): A review of its traditional use, phytochemistry and pharmacology. *Braz. Arch. Biol. Technol.* **2008**, *51*, 931.
[20] L.M. Batista, R.N. Almeida, E.V.L. da Cunha, M.S. da Silva, J.M. Barbosa-Filho. Isolation and identification of putative hallucinogenic constituents from the roots of *Mimosa ophthalmocentra*. *Pharm. Biol.* **1999**, *37*, 50.
[21] M. Meckes-Lozoya, X. Lozoya, R.J. Marles, C. Soucy-Breau, A. Sen, J.T. Arnason. *N,N*-Dimethyltryptamine alkaloid in *Mimosa tenuiflora* bark (tepescohuite). *Arch. Invest. Med.* **1990**, *21*, 175.
[22] T. Herraiz, D. González, C. Ancín-Azpilicueta, V.J. Arán, H. Guillén. beta-Carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO). *Food Chem. Toxicol.* **2010**, *48*, 839.
[23] R.J. Warren. Fatal nicotine intoxication resulting from the ingestion of Ayahuasca. *J. Anal. Toxicol.* **2004**, *28*, 287.
[24] D.E. Brush, S.B. Bird, E.W. Boyer. Monoamine oxidase inhibitor poisoning resulting from Internet misinformation on illicit substances. *J. Toxicol. Clin. Toxicol.* **2004**, *42*, 191.
[25] J. Sklerov, B. Levine, K.A. Moore, T. King, D. Fowler. A fatal intoxication following the ingestion of 5-methoxy-*N,N*-dimethyltryptamine in an ayahuasca preparation. *J. Anal. Toxicol.* **2005**, *29*, 838.
[26] J.C. Callaway, C.S. Grob, D.J. McKenna, D.E. Nichols, A. Shulgin, K.W. Tupper. A demand for clarity regarding a case report on the ingestion of 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) in an ayahuasca preparation. *J. Anal. Toxicol.* **2006**, *30*, 406.
[27] R.J. Strassman. Hallucinogenic drugs in psychiatric research and treatment. Perspectives and prospects. *J. Nerv. Ment. Dis.* **1995**, *183*, 127.
[28] R. Metzner. Hallucinogenic drugs and plants in psychotherapy and shamanism. *J. Psychoactive Drugs* **1998**, *30*, 333.
[29] D.J. McKenna. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. *Pharmacol. Ther.* **2004**, *102*, 111.
[30] J. Schripsema, D. Dagnino, G. Gossman, Alcalóides Indólicos, in *Farmacognosia: da planta ao medicamento*, (Eds: C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petrovick). Editora da UFSC, Florianópolis, **2007**, pp. 819.
[31] N.G.M. Gomes, M.G. Campos, J.M.C. Orfão, C.A.F. Ribeiro. Plants with neurobiological activity as potential targets for drug discovery. *Prog. Neuro-Psychoph.* **2009**, *33*, 1372.
[32] Y.H. Wang, V. Samoylenko, B.L. Tekwani, et al. Composition, standardization and chemical profiling of *Banisteriopsis caapi*, a plant for the treatment of neurodegenerative disorders relevant to Parkinson's disease. *J. Ethnopharmacol.* **2010**, *128*, 662.
[33] R.S. Gable. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. *Addiction* **2007**, *102*, 24.
[34] J.C. Callaway. Various alkaloid profiles in decoctions of *Banisteriopsis caapi*. *J. Psychoactive Drugs* **2005**, *37*, 151.
[35] E.J.B.N. MacRae. Santo Daime and Santa Maria-the licit use of ayahuasca and the ilicit use of cannabis in an Amazonian religion. *Int. J. Drug Policy* **1998**, *9*, 325.
[36] E.J.B.N. MacRae. The religious uses of licit and illicit psychoative substances in a branch of the Santo Daime religion. *Fieldwork in Religion* **2006**, *2*, 393.
[37] R.E. Schultes, A. Hofmann. *The Botany and Chemistry of Hallucinogens*, Charles C. Thomas. Springfield, IL, **1980**.
[38] J.C. Callaway, G.S. Brito, E.S. Neves. Phytochemical analyses of *Banisteriopsis caapi* and *Psychotria viridis*. *J. Psychoactive Drugs* **2005**, *37*, 145.
[39] A.P.S. Pires, C.D.R. de Oliveira, S. Moura, F.A. Dörr, W.A.E. Silva, M. Yonamine. Gas chromatographic analysis of dimethyltryptamine and β-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochem. Anal.* **2009**, *20*, 149.

[40] C. Gambelunghe, K. Aroni, R. Rossi, L. Moretti, M. Bacci. Identification of *N,N*-dimethyltryptamine and beta-carbolines in psychotropic ayahuasca beverage. *Biomed. Chromatogr.* **2008**, *22*, 1056.
[41] 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. *J. Chromatogr. A* **2009**, *1216*, 8960.
[42] Conselho Nacional Antidrogas (CONAD). Resolução n. 05 *[Resolution n. 05]*. Brasília, 4 November, **2004**.
[43] B.C. Labate. Consumption of ayahuasca by children and pregnant women: medical controversies and religious perspectives. *J. Psychoactive Drugs* **2011**, *43*, 27.
[44] Conselho Nacional Antidrogas (CONAD). Resolução n. 01 [Resolution n. 01]. Brasília, 25 January, **2010**.
[45] Conselho Estadual de Meio Ambiente, Ciência e Tecnologia & Conselho Florestal Estadual (CEMACT/CFE). Resolução n. 004 *[Resolution n. 004]*. Acre, 20 December, **2010**.
[46] O.G. de Lima. Observações sobre o 'vinho da jurema' utilizado pelos índios Pancarú de Tacaratú (Pernambuco). *Arq. Inst. Pesqui. Agron. Recife* **1946**, *4*, 45.
[47] C.N. da Mota, *Os filhos de Jurema na floresta dos espíritos: ritual e cura entre dois grupos indígenas do nordeste brasileiro*, Edufal. Maceió, **2007**.
[48] T. Herraiz, C. Chaparro. Human monoamine oxidase is inhibited by tobacco smoke: β-carboline alkaloids act as potent and reversible inhibitors. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 378.
[49] J. van Amsterdam, R. Talhout, W. Vleeming, A. Opperhuizen. Contribution of monoamine oxidase (MAO) inhibition to tobacco and alcohol addiction. *Life Sci.* **2006**, *79*, 1969.
[50] A. Lewis, J.H. Miller, R.A. Lea. Monoamine oxidase and tobacco dependence. *Neurotoxicology* **2007**, *28*, 182.
[51] R.A. Grünewald, Sujeitos da jurema e o resgate da "ciência do índio", in *O uso ritual das plantas de poder*, (Eds: B.C. Labate, S.L. Goulart). Mercado das Letras, Campinas, **2005**, pp. 239.
[52] J. Löhdefink, H. Kasting. Zur Frage des Vorkommens von Hamanalkaloiden in Passiflora-Arten. *Planta Med.* **1974**, *25*, 101.
[53] J.R.F. Allen, B.R. Holmstedt. *The simple β-carbolines. Phytochem.* **1980**, *19*, 1573.
[54] B. Meier. Passionsblume: Portrait einer Arzneipflanze. *Z. Phytother.* **1995**, *16*, 115.
[55] A. Rehwald, O. Sticher, B. Meier. Trace analysis of harman alkaloids in *Passiflora incarnata* by reversed-phase high performance liquid chromatography. *Phytochem. Anal.* **1995**, *6*, 96.
[56] J. Ott. Pharmahuasca: Human pharmacology of oral DMT plus harmine. *J. Psychoactive Drugs* **1999**, *31*, 171.
[57] J. Ott. Farmahuasca, anahuasca e jurema preta: farmacologia humana de DMT oral mais harmina, in *O uso ritual da Ayahuasca*, (Eds: B.C. Labate, W.S. Araújo). Mercado das Letras, Campinas, **2009**, pp. 711.
[58] C. Rätsch. *The Encyclopedia of Psychoactive Plants: Ethnopharmacology and its Applications*, Park Street Press. Rochester, **2005**.
[59] A.H. der Marderosian, K.M. Kensinger, J.M. Chau, F.J. Goldstein. The use of hallucinatory principles of the psychoactive beverage of the Cashinahua tribe (Amazon basin). *Drug Depend.* **1970**, *5*, 7.
[60] S. Agurell, B. Holmsted, J.E. Lindgren. Alkaloid content of *Banisteriopsis Rusbyana*. *Am. J. Pharm. Sci. Support. Public Health* **1968**, *140*, 148.
[61] S. Agurell, B. Holmsted, J.E. Lindgren, R.E. Schultes. Identification of two new beta-carboline alkaloids in South American hallucinogenic plants. *Biochem. Pharmacol.* **1968**, *17*, 2487.
[62] E.H.F. Moraes, M.A. Alvarenga, Z.M.G.S. Ferreira, G. Akisue. As bases nitrogenadas da *Mimosa scabrella* Bentham. *Quim. Nova* **1990**, *13*, 308.
[63] L.M. Batista, R.N. Almeida. Central effects of the constituents of *Mimosa ophthalmocentra Mart. Ex Benth. Acta Farm. Bonaerense* **1997**, *16*, 83.
[64] L.P. Queiroz. *Leguminosas da Caatinga*, Universidade Estadual de Feira de Santana in association with Royal Botanic Gardens, Kew and Associação Plantas do Nordeste, Feira de Santana, **2009**.
[65] E. Rivera-Arce, M.A. Chavez-Soto, A. Herrera-Arellano, S. Arzate, J. Aguero, I.A. Feria-Romero, A. Cruz-Guzman, X. Lozoya. Therapeutic effectiveness of a Mimosa tenuiflora cortex extract in venous leg ulceration treatment. *J. Ethnopharmacol.* **2007**, *109*, 523.
[66] C.A. Bourke, M.J. Carrigan. Mechanisms underlying Phalaris aquatica "sudden death" syndrome in sheep. *Aust. Vet. J.* **1992**, *69*, 165.
[67] N. Anderton, P.A. Cockrum, D.W. Walker, J.A. Edgar. Identification of a toxin suspected of causing sudden death in livestock grazing *Phalaris* pastures, in *Plant Associated Toxins: Agicultural, Phytochemical, and Ecological Aspects*, (Eds: S.M. Colegate, P.R. Dorling). CAB International, Wallingford, UK, **1994**, pp. 269.
[68] A. Gava, R.S. Sousa, M.S. de Deus, C. Pilati, J. Cristani, A. Mori, D.S. Neves. *Phalaris angusta* (Gramineae) como causa de enfermidade neurológica em bovinos no Estado de Santa Catarina. *Pesq. Vet. Bras.* **1999**, *19*, 116.
[69] R.S. Sousa, L.F. Irigoyen. Intoxicação experimental por *Phalaris angusta* (Gramineae) em bovinos. *Pesq. Vet. Bras.* **1999**, *19*, 116.
[70] J.H. Skerritt, S.L. Guihot, S.E. McDonald, R.A. Culvenor. Development of immunoassays for tyramine and tryptamine toxins of *Phalaris aquatica* L. *J. Agric. Food Chem.* **2000**, *48*, 27.
[71] L. Zhou, A.A. Hopkins, D.V. Huhman, L.W. Sumner. Efficient and sensitive method for quantitative analysis of alkaloids in hardinggrass (Phalaris aquatica L.). *J. Agric. Food Chem.* **2006**, *54*, 9287.
[72] E. Perrot, R. Hamet. Le Yagé, plante sensorielle des Indiens de la région amazonienne de l'Equateur et de la Colombie. *Comptes Rend.* **1927**, *184*, 1266.
[73] L. Lewin. Untersuchungen über *Banisteria caapi* Spr. (ein südamerikanisches Rauschmittel) *Arch. Exp. Path. Pharmakol.* **1928**, *129*, 133.
[74] F. Elger. Ueber das Vorkommen von harmin in einer südamerikanischen Liane (Yagé). *Helv. Chim. Acta* **1928**, *11*, 162.
[75] O. Wolfes, K. Rumpf. Über das Vorkommen von Harmin in einer südamerikanischen Liane. *Arch. Pharmakol.* **1928**, *266*, 188.
[76] F.A. Hochstein, A.M. Paradies. Alkaloids from *Banisteria caapi* and *Prestonia amazonicum*. *J. Am. Chem. Soc.* **1957**, *79*, 5735.
[77] I.J. Pachter, D.E. Zacharias, O. Ribeiro. Indole alkaloids of *Acer saccharinum* (the Silver Maple), *Dictyoloma incanescens*, *Piptadenia colubrina*, and *Mimosa hostilis*. *J. Org. Chem.* **1959**, *24*, 1285.
[78] H.V. Pinkley. Etymology of *Psychotria* in view of a new use of the genus. *Rhodora* **1969**, *71*, 535.
[79] M. Kartal, M.L. Altun, S. Kurucu. HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of *Peganum harmala* L. *J. Pharm. Biomed. Anal.* **2003**, *31*, 263.
[80] J.J. Vepsäläinen, S. Auriola, M. Tukiainen, N. Ropponen, J.C. Callaway. Isolation and characterization of yuremamine, a new phytoindole. *Planta Med.* **2005**, *71*, 1053.
[81] M.P. Nicasio, M.L. Villarreal, F. Gillet, L. Bensaddek, M.A. Fliniaux. Variation in the accumulation levels of *N,N*-dimethyltryptamine in micro-propagated trees and in in vitro cultures of *Mimosa tenuiflora*. *Nat. Prod. Res.* **2005**, *19*, 61.
[82] B. Hemmateenejad, A. Abbaspour, H. Maghami, R. Miri, M.R. Panjehshahin. Partial least squares-based multivariate spectral calibration method for simultaneous determination of beta-carboline derivatives in *Peganum harmala* seed extracts. *Anal. Chim. Acta* **2006**, *575*, 290.
[83] H.R. Monsef-Esfahani, M.A. Faramarzi, V. Mortezaee, M. Amini, M.R. Rouini. Determination of harmine and harmaline in *Peganum harmala* seeds by high-performance liquid chromatography. *J. Appl. Sci.* **2008**, *8*, 1761.
[84] S.A. Barker. Matrix solid-phase dispersion. *J. Chromatogr. A* **2000**, *885*, 115.
[85] S.A. Barker. Matrix solid phase dispersion (MSPD). *J. Biochem. Bioph. Meth.* **2007**, *70*, 151.
[86] S.A. Barker, A.R. Long, C.R. Short. Isolation of drug residues from tissues by solid phase dispersion. *J. Chromatogr.* **1989**, *475*, 353.
[87] A. Gaujac, A. Aquino, S. Navickiene, J.B. de Andrade. Determination of *N,N*-dimethyltryptamine in Mimosa tenuiflora inner barks by matrix solid-phase dispersion procedure and GC-MS. *J. Chromatogr. B* **2012**, *881/882*, 107.
[88] H. Pulpati, Y.S. Biradar, M. Raiani. High-performance thin-layer chromatography densitometric method for the quantification of harmine, harmaline, vasicine, and vasicinone in *Peganum harmala*. *J. AOAC Int.* **2008**, *91*, 1179.
[89] 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. *J. Anal. Toxicol.* **1996**, *20*, 492.

[90] S. Moura, F.G. Carvalho, C.D. Rodrigues de Oliveira, E. Pinto, M. Yonamine. qNMR: An applicable method for the determination of dimethyltryptamine in ayahuasca, a psychoactive plant preparation. *Phytochem. Lett.* **2010**, *3*, 79.

[91] C. Huhn, C. Neususs, M. Pelzing, U. Pyell, J. Mannhardt, M. Putz. Capillary electrophoresis-laser induced fluorescence-electrospray ionization-mass spectrometry: a case study. *Electrophoresis* **2005**, *26*, 1389.

[92] 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.

[93] 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.

[94] S.A. Barker, E.H. McIlhenny, R. Strassman. A critical review of reports of endogenous psychedelic *N,N*-dimethyltryptamines in humans: 1955–2010. *Drug Test. Analysis* **2012**. DOI: 10.1002/dta.422