Introduction

Hallucinogens are substances which alter thought, perception and mood without affecting memory, producing intellectual impairment or leading to addiction. They also have negligible autonomic side effects. This definition is often considered too limited because besides the two main hallucinogenic classes’ indolylalkylamines and phenylalkylamines other classes like cannabinoids and N-methyl-d-aspartate (NMDA) antagonists, which are not classified as hallucinogens and possess different mechanism of action may produce similar and overlapping effects. Therefore the definition was expanded by Glennon (1996) who added that ‘the hallucinogens are also agents that with an affinity for the 5-HT2A receptors’[1] (Fig. 1).
Fig. 1 Hallucinations arise from agonism at 5-HT2A receptors[1].

These drugs are also known as classical or serotonergic hallucinogens [2]. Phenylalkylamines contain a phenetyl amine group similar to endogenous neurotransmitters dopamine and noradrenaline. Mescaline, 2,5-dimethoxy-4-methylamphetamine (DOM) and 2,5-dimethoxy-4-iodoamphetamine (DOI) are examples of phenylalkylamines. Indolylalkylamines possess an indole nucleus similar structurally to 5-hydroxytryptamine or serotonin. They are divided into two subgroups – simple triptamines like N,N-dimethyltryptamine (DMT), bufotenin and psilocybin and ergolines like LSD [3]. The research into the psychopharmacology of hallucinations is conducted in two directions: first, hallucinogenic agents are investigating to determine their molecular effect on the brain [4, 5]; second, psychosis-associated diseases, which commonly include hallucinations, are successfully treated using specific molecular approaches as well as another diseases [6, 7]. Because of the growing importance of the hallucinogens as drugs of abuse, analytical methods are needed in forensic and clinical toxicology, which allow reliable screening and quantification of these substances in biological matrices [8].

The aim of the current review is to summarize current information related to the analysis, pharmacology and toxicology of some classic hallucinogens with phenylalkylamine and indolylalkylamine structure.
Results and discussion

LSD

Lysergic acid diethylamide or LSD is a substance first synthesized in 1938 by Albert Hofmann, who investigated the compounds of ergot – *Claviceps purpurea* in the Sandoz Laboratories (Fig. 2). The use of the hallucinogenic drug LSD is prohibited. At doses greater than or equal to 25 µg LSD affects the behavior. A dose of 25 µg usually leads to euphoria and facilitates introspection. The optimal dose of LSD which triggers a trip is within the 100-200 µg range. The effects observed following the LSD intake depend on the individual characteristics of the patient and on the specific environment, where the substance is taken. Calm and relaxing surroundings are more likely to trigger the so-called “good trip”, while tense and hostile environment would cause a “bad trip”. There are no documented cases of death after an LSD overdose. Following the administration of LSD the psychiatric complications observed could be panic attacks, depression and “flashbacks” – a recurrence of visions when the substance is absent. They appear most often after a “bad trip”. These complications are not lethal, however, they increase the risk of suicidal reactions [9].

(Table 1) A reexamination of LSD’s potential as a treatment for addictions indicates that optimism, albeit cautious, may be appropriate [10]. Although no teratogenic effects have been registered after its use, the drug alters the expression of genes within mammalian brain cells. Even a single dose of LSD has been demonstrated to alter gene expression patterns [11]. Genes that exhibit differential expression following treatment with LSD include: c-fos, krox-20, NOR-1, arc, IKβ-α, sgk, and Ania3. Increased expression of these genes, which are involved in a wide variety of cellular functions, alters synaptic plasticity, glutamatergic signaling, cytoskeletal architecture, as well as communication between the synapse and nucleus.

LSD is a white crystalline substance, odorless and tasteless with a melting point at 80-85 °C. Tartrate salt melts at 198-200 °C and dissolves in water and ethanol to give clear and odorless solutions. LSD is an unstable compound oxidized easily by air and light to form inactive products. The substance exists in three isomeric forms. The hallucinogenic effect is due to the right rotating isomer. LSD extraction is made with methanol-water 1:1 solution at 20 °C with the use of ultrasound. The substance can be determined colorimetrically with Ehrlich test with para-dimethylaminobenzaldehyde to give a blue coloration [12]. The use of high perfor-

**Fig. 2** Chemical structures of Lysergic acid diethylamide (LSD) and natural analog - Lysergic acid amide (LDA)
mance liquid chromatography (HPLC) with UV detector gives an absorption at 313 nm [13]. Gas chromatography with Mass spectrometry (GC-MS) and Infrared spectroscopy (IR) are also an option for its analysis [14]. The detection and quantification of LSD in biosamples is difficult due to low oral dose (50-250 μg), biotransformation in the body and low concentrations in blood and urine [15]. Its metabolites N-desmethyl-LSD and mainly 2-oxo-3-hydroxy-LSD (OH-LSD) (Fig. 3) have longer biological half-life and are found at a 16-43 times higher concentrations in urine [16, 17].

Besides blood and urine, hair samples also could be tested even at a low dose and after a single administration of the substance[18]. The detection in body fluids is carried out by immunological methods - RIA, CEDIA, EIA, Abusscreen OnLineetc [19]. Radio-immunoassay (RIA) gives a positive result in the content of LSD in urine more than 0.5 ng/ml [20]. The microplate enzymeimmunoassay (EIA) is more sensitive and does not require the use of radioactive materials, making it more accessible when it can work with both urine and blood.

Fig. 3 Metabolites of LSD identified in human urine. The 2-oxo-3-hydroxy-LSD is the major metabolite
samples [21]. The results from these tests and quantification analysis could confirmed by GC or HPLC, combined with MS [22]. A combination of GC and MS is used to determine the presence and concentration of LSD and OH-LSD in urine with a limit of detection 0.5 ng/ml [23]. A gas chromatography – ion trap tandem mass spectrometry method (GC-ion trap-MS-MS) shows that $m/z$ fragments of 2-oxo-3-hydroxy-LSD-bis-TMS are 309 and 499. The determination with GC, however, has its drawbacks, which result in an irreversible adsorption of the substance in the column. LSD is a relatively non-volatile substance and requires derivatization and obtaining of the volatile components trifluoroacetyl (TFA) or trimethylsilyl (TMS)[23]. As GC works at higher temperatures, the results in losses are due to thermolability [24]. That leads to the development of different liquid chromatographical methods. The method of HPLC with fluorescence detector (HPLC-FLD) shows a wavelength of excitation at 315 nm and emission at 420 nm [22]. Bodin et al., used a combination of HPLC and MS to determine the substance in urine with a limit for detection 0.02 ng/ml [25]. Chung et al., validated a method for confirmation and quantification of LSD, iso-LSD, nor-LSD and O-H-LSD in blood and urine. They applied ultra-performance liquid chromatography (UPLC) combined with tandem mass spectrometry (MS-MS). The relative retention time and the ion ratios are used as identification parameters. The limits of detection are 5 pg/ml for LSD and iso-LSD, and 10 pg/ml for nor-LSD and OH-LSD in blood and 10 pg/ml for all substances in urine. The limits of quantitation (LOQ) in blood and urine are 20 pg/ml for LSD and iso-LSD and 50 pg/ml for nor-LSD and OH-LSD [26]. Another method for analysis of LSD, iso-LSD, nor-LSD and OH-LSD from hair was developed by Martin et al. The substances are extracted with HCL and methanol and quantified by liquid chromatography coupled with electrospray tandem mass spectrometry [27].

**Mescaline**

Mescaline (3,4,5-trimethoxyphenethylamine) is a well-known hallucinogen since ancient times (**Fig. 4**). It is a phenethylamine compound which is a natural constituent of the peyote cacti – *Lophophora williamsii, Cactaceae* [28]. Found in the southwestern USA and northern Mexico, the dried tops of this cactus has been used by Native Americans in different religious ceremonies. Mescaline can be extracted from peyote or produced synthetically.

![Mescaline](image)

**Fig. 4 Chemical structures of mescaline and modifications**
Mescaline is used primarily as a recreational drug and is also used to supplement various types of meditation and psychedelic therapy. The use of peyote is illegal and its consumption leads to a hallucinogenic effect similar to those of LSD, but 4000 times weaker although the duration of the trip is longer [29, 30]. Although mescaline does not lead to addiction, it could provoke a lot of adverse reactions like tachycardia, hypertension, nausea, psychosis and paranoia [29]. Some death cases are reported after the consumption of peyote but on extremely rare occasions. In one of them, a 32 year-old Native American having drunk peyote tea died due to Mallory-Weiss esophageal laceration caused by vomiting which mescaline had induced [31]. With continued drug use, the long term effects of mescaline take the form of addiction where a person’s lifestyle revolves around getting and using the drug. Mescaline interacts with the brain’s cognitive and emotion-based centers, oftentimes bringing users to vulnerable and even helpless states of mind. With frequent drug use, the long term effects of mescaline can compromise severely the psychological well-being of a person (Table 1).

Mescaline is a white crystalline substance with melting point of 35-36 °C, boiling at 180 °C. It is moderately soluble in water and soluble in ethanol, chloroform and benzene [32]. The usual dose of mescaline which provokes a hallucinogenic reaction is within the range of 200 to 500 mg [30] (equals to 5-10 peyote buttons) [33]. Mescaline could be detected by a colorimetric test, e.g. Marquis test which is used for alkaloids and amphetamines. The reagent is a mixture of 100 ml of concentrated sulfuric acid and 1 ml of 40 % solution of formaldehyde dripped onto the tested substance. The test is quick and does not require specific equipment. Mescaline gives orange color and its limit of detection is 1 µg [34]. Froehde reagent could also be used to identify mescaline. It consists of molybdic acid or sodium molybdate and concentrated sulfuric acid, mixed, heated and then added dropwise on the substance tested. If mescaline is available, it gives a greenish to brown color [35]. Detection of mescaline could be made with GC combined with MS [36, 37]. The m/z top peak is 30, m/z 2nd highest is 182 and m/z 3rd highest is 166. MS-MS peaks are m/z 180, m/z 195 and m/z 179(35)(25). The quantification of mescaline is carried out by HPLC with UV detector. The UV absorbance maximum for mescaline is 205 nm [34]. Urine could be screened for mescaline using biochip array immunoassay as it is done by Battal et al. They validate GC-MS by an electron impact ionization method for quantification of the substance [38]. LC-MS-MS method was developed by Bjornstad et al., in order to confirm the presence of mescaline in urine samples. The limit of detection in a urine matrix is 3-5 µg/l. Separation is made on a HyPURITY C18 column using methanol gradient in ammonium acetate buffer. The observed transitions in the MS-MS analysis are m/z 212.3 to m/z 180.3 for mescaline and m/z 221.3 to m/z 186.3 for the deuterated internal standard – mescaline-d9 [39]. Peyote tea, hair samples and urine from boy suspected in mescaline abuse were analyzed using GC-MS and GC-MS-MS methods, validated by Gambelunghe et al., [40]. GC-MS analysis detected mescaline in the tea but not in the urine (because it was already excreted) and GC-MS-MS analysis found mescaline in the proximal segments of the hair where the substance accumulated for longer periods.

**DMT**

\( N,N \)-dimethyltryptamine (DMT) is a psychoactive indolealkylamine which was found naturally in the human brain, in some animals including mammals, and also in a broad range of species of the plant kingdom (Fig. 5). DMT is a potent, fast-acting hallucinogen with short duration in humans. It could be administrated via different routes: inhalation 6-20 mg, intravenous injection 0.7–3.1 mg, sublingual or intranasal insufflation 10 mg, and oral administration 30 mg (only with MAO inhibitor like harmaline). \( 5'-\text{Methoxy}-N,N\text{-dimethyltryptamine} \) (5-MeO-DMT) – a designer drug obtained from DMT, produces psychedelic effects which...
DMT is a white crystalline compound. It melts at 46 °C and boils at 60-80 °C. DMT is dissolved easily in diluted acetic and mineral acids. The solubility in water is limited for the base and good for the fumarate salt. It emits toxic fumes of nitrogen oxides when heated [45]. DMT could be determined colorimetrically. Ehrlich’s reagent reacts with tryptamines producing blue to purple color. The limits of detection are 10-50 µg/ml [46]. Gaujac et al., was applied different analytical methods to the structural characterization and purity assessment of DMT. The results show that the infrared spectrum for DMT has peaks at 742, 809, 862, 1008, 1031, 1110, and 1178 cm⁻¹. GC/MS demonstrates a peak at 21.2 min. The ions m/z 130 and m/z 58 are selected for tandem mass spectrometry but a mass spectrum is obtained only from m/z 130. After a direct insertion of the crystals into the mass spectrometer occur the molecular ion peaks at m/z 188, and a base peaks at m/z 58 [45]. These results are similar to the spectrum provided in the NIST Mass Spectral Database: m/z top peak is 58, m/z 2nd highest is 130 and

![Fig. 5 Metabolic pathways for N,N-dimethyltryptamine (DMT) in humans.](image)

start 3-4 min after insufflation, a peak about 35-40 min, and an end around 60-70 min. It distorts time perception and provokes visionary and auditory changes. Those who take the substance often experience visions of mythological alien creatures [41, 42]. The data about DMT toxicity is scarce, except from one arguable report of a death case after drinking 5-MeO-DMT contained in an Ayahuasca preparation [43]. Intravenously administrated DMT is known to increase the systolic and diastolic blood pressure, as well as the heart rate. However, like LSD most adverse reaction is psychotic. DMT is capable of inducing psychological reactions or transient psychotic episodes like the appearance of unpredictable frightening images and thoughts that disappear shortly. Nausea and diarrhea, could also be observed after the administration of the substance [44] (Table 1).
m/z 3rd highest is 188. The UV absorbance maximum for DMT is at 280 nm [47]. Ishii et al., [48] used GC with surface ionization detection (SID) for identification and quantization of DMT in whole blood and urine using gramine as an internal standard. Solid-phase extraction of the samples is carried out with Sep-Pak C18 cartridge before the analysis. The limit of detection of DMT is 0.5 ng/ml. The retention times are 7.9 min for gramine and 9.4 min for DMT. The intensity of the peaks is similar, regardless of the different quantity - 500 ng for gramine and 10 ng for DMT thus the response of SID to DMT is higher [48]. Another method for the analysis of designer triptamines and phenethylamines in blood and urine was developed by Vorce and Sklerov. They used a screening method based on GC/MS with electrospray ionization (ESI), optimizing the separation of the substances. The retention time for DMT is 10.14 min. The different drugs are identified by selected ion monitoring (SIM). The SIM ions for DMT are m/z 58.129 and 180 [49]. There are other analytical methods for identification and quantization of DMT as a component of the Ayahuasca preparation such as: analysis of plasma with GC with a nitrogen-phosphorus detection after liquid-liquid extraction with diphenhydramine as an internal standard [50]; or with n-pentane [51]; analysis of urine with LC-ESI-MS/MS [52].

**Psilocybin and psilocin**

Psilocybin is a hallucinogenic tryptamine with chemical name - 4-phosphoryloxy-\(N,N\)-dimethyltryptamine. It was first isolated by the Swiss chemist Albert Hofman in 1957. The substance is a natural ingredient in *Psilocybe mexicana* distributed mainly in Central America. It is found also in many other mushroom species worldwide all known by the common name – “magic mushrooms”. They are one of the most common hallucinogenic used today. Psilocybin was first synthesized by Hofman in 1958. Pharmacokinetic studies show that after oral administration psilocybin is metabolized to psilocin in the liver (Fig. 6). Both substances produce similar psychedelic effects. The hallucinogenic effect after an oral dose of 8-25 mg of psilocybin exhibits in 70-90 min., lasting about 360 min. It changes perception by enhancing introspection, producing illusions and altering thought and time sense. Being low toxic psilocybin produces no physical damage when used. However, it could lead to many psychiatric complications under uncontrolled conditions in individuals who are emotionally vulnerable [53] (Table 1).

It is a substance forming colorless crystals soluble in water and methanol and insoluble in chloroform and benzene. Under acidic conditions it dephosphorylates into psilocin [54, 55]. The colorimetric determination of psilocin and psilocybin could be made by Marquis test giving a greenish-brown color for psilocin and orange color for psilocybin. Gas chromatography is not useful for the determination of psilocybin due to its instability and dephosphorilation to psilocin when heated.

![Fig. 6 Metabolic pathways for psilocybin in humans](image)
HPLC could be used with retention time for psilocin – 6.08 min. and for psilocybin 7.61 min. [56, 57]. The UV maximum for detection is at 210 nm. Qualification could be performed with ultraviolet spectrophotometry; the absorbance maximums when aqueous acid is used as a solvent are at 266, 283 and 292 nm for psilocin and at 268 for psilocybin. Mass spectrum for psilocin shows highest pick at \( m/z \) 58, 2\(^{nd} \) highest at \( m/z \) 204, 3\(^{rd} \) highest at \( m/z \) 42. The values for psilocybin are \( m/z \) 58 for the highest pick, \( m/z \) 204 for the 2\(^{nd} \) highest pick and \( m/z \) 146 for the 3\(^{rd} \) highest pick[58, 59].

**Bufotenine**

Bufotenine or Bufotenin (Fig. 7), 5-HO-DMT, \( N,N \)-dimethylserotonin, 5-hydroxy-\( N,N \)-dimethylserotonin) is a hallucinogenic substance, secreted in the parotoid glands of Bufo toads, in mushrooms in the genus Amanita and in the seeds of different Anadenathera and Piptadenia species [60]. Its effects are similar to DMT and 5-MeO-DMT. Structurally, bufotenine is an indole hallucinogen that is capable of blocking the action of serotonin, which is the indole amine transmitter of nerve impulses and can be found in normal brain tissue (and in toad poison) [61]. Bufotenine also functions as a powerful constrictor of blood vessels, causing a rise in blood pressure. However Bufotenine is unable to pass blood-brain barrier due to its low lipid solubility, thus its psychoactive effects is controversial [62] (Table 1).

![Chemical structures of Bufotenine](image-url)

**Fig. 7 Chemical structures of Bufotenine**

Color tests used for the determination of bufotenine are Marquis test – green brown and Van Urqs test – violet to dark purple color. Other screening methods are GC and HPLC [63]. GC is performed with a flame ionization detector (FID). The retention time for bufotenine is 3.85 min.[64] The HPLC method uses a UV detector set at 210 nm and the retention time is 2.23 min. Capillary electrophoresis is also an option for screening. Qualitative data could be obtained on a UV spectrophotometer. The absorbance maximum is at 278 nm. Mass spectrum data is \( m/z \) 58 – highest pick and also \( m/z \) 146, 42 and 204[65].
Table 1. Specifics of hallucinogens effect

<table>
<thead>
<tr>
<th>Substance</th>
<th>Common source(s)</th>
<th>Pharmacology</th>
<th>Somatic Effects</th>
<th>Toxicological Data</th>
<th>Lethal</th>
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<tr>
<td>LSD (6-9H)</td>
<td>Found in mushroom such as Agaricus semilanceata</td>
<td>A moderate dose (75–150 μg p.o.): euphoria, enhanced capacity for introspection, hypnagogic experience and dreams, illusions, pseudo-hallucinations, alterations of thinking and time experience; “bad trips”; flashback phenomena; irrational acts leading to suicide or accidental death are extremely rare; psychosis-like symptoms; impaired time perception, distorted perception of the size and shape of objects, movements, color, sounds, touch and the user’s own body image.</td>
<td>Changes of attention and concentration; thinking processes are can be also affected; intellectual functions are impaired; psychomotor functions (coordination and reaction time) are frequently impaired; memory was also affected.</td>
<td>No documented human deaths from an LSD overdose eight individuals had plasma levels of 1000–7000 μg per 100 mL blood plasma and suffered from comatose states, hyperthermia, vomiting, light gastric bleeding, and respiratory problems.</td>
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<tr>
<td>Psilocybin and Psilocin</td>
<td>[70-72]</td>
<td>Hallucinations/illusions, external hallucinations; shadow people, emotion enhancement, language suppression, addiction suppression; auditory, tactile, and visual hallucinations, analysis enhancement, catharsis, cognitive euphoria; conceptual thinking, confusion, creativity enhancement, déjà vu, ego replacement, ego death, existential self-realization; feelings of externatism, of impending doom, of interdependent opposites, of predestination, of self-design, of unity with the environment; increased muscle appreciation, memory suppression, personal bias suppression, rejuvenation, spirituality enhancement; subconscious communication, time distortion, wakefulness; brighter colors, sharper visual definition; increased hearing acuity, synesthesia (melding of the senses: seeing music or hearing colors); difficulty focusing, maintaining attention, concentrating, and thinking; impaired judgment and preoccupation with trivial. Thoughts, experiences, or objects sense of detachment from body and surroundings and loss of boundaries between the two altered perception of space and time, inability to distinguish fantasy from reality melding of past experiences with present</td>
<td>Short-lasting tolerance; shadow people, shadow people; paranoid experiences, derealization, depersonalization; long-lasting unpleasant experiences (bad trips), including frightening hallucinations; psychotic reactions and hallucinogen persisting perception disorder (HPPD); attention disturbance, distractibility, working memory; delusions; associative deficits; spontaneous tactile sensations; a complete psychotic episode of audio or visual hallucinations will occur, but may continue in users with additional mental illness.</td>
<td>Has an extremely low toxicity relative to dose the natural levels of psilocybin within magic mushrooms are much higher than psilocin, Psilocybin - LD₅₀ for rats and mice is 280-285 mg/kg, and for rabbits it is 12.5 mg/kg. Psilocin - LD₅₀ is significantly lower for mice and rats 75 mg/kg and for rabbits 7 mg/kg</td>
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Pharmaco - toxicological aspects and analysis...

PHARMACIA, vol. 64, No. 1/2017 41
**Conclusion**

Most hallucinogens are found as natural ingredients in many plants, mushrooms or animals and have been known to man since ancient times. Nowadays with the discovery of novel synthetic and semi-synthetic substances, they are still used despite of the unpredictable consequences that might occur as a result of their application. Most of the substances exhibit low toxicity but the risks of damaging mental health or causing physical traumas during the hallucinogenic trips should not be underestimated. Moreover, hallucinogenic substances are illegal in most countries worldwide, thus it is important to know different sensitive analytical methods for their determination and quantification in different biological matrices such as blood, plasma, urine and hair.
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