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Short communication

Ultra-high-pressure liquid chromatography tandem mass spectrometry determination of hallucinogenic drugs in hair of psychedelic plants and mushrooms consumers

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ABSTRACT

A procedure based on ultra-high-pressure liquid chromatography tandem mass spectrometry has been developed for the determination of mescaline, N,N-dimethyltryptamine, psilocin, psilocybin, salvinorin A in hair of consumers of psychedelic vegetal material such peyote or trichocereus cacti, psilocybe mushrooms, *Salvia divinorum* or psychedelic beverage ayahuasca. After hair washing with methyl alcohol and diethyl ether and subsequent addition of mescaline-d_9 and 3,4-methylenedioxypropylamphetamine as internal standards, hair samples were treated with 250 μl VMA-T M3 reagent for 1 h at 100 °C. After cooling, 100 μl M3 extract were diluted with 400 μl water and a volume of 10 μl was injected into chromatographic system. Chromatographic separation was achieved at ambient temperature using a reverse-phase column and a linear gradient elution with two solvents: 0.3% formic acid in acetonitrile and 5 mM ammonium formate pH 3. The mass spectrometer was operated in positive ion mode, using multiple reaction monitoring via positive electrospray ionization.

The method was linear from the limit of quantification (0.03–0.05 ng/mg depending on analyte under investigation) to 10 ng/mg hair, with an intra- and inter-assay imprecision and inaccuracy always less than 15% and an analytical recovery between 79.6% and 97.4%, depending on the considered analyte. Using the validated method, mescaline was found in concentration range of 0.08–0.13 ng/mg in hair of peyote smokers, 3.2 ng salvinorin A per mg hair were determined in hair from a *S. divinorum* smoker, 5.6 ng N,N-dimethyltryptamine per mg hair from an ayahuasca user and finally 0.8 ng psilocybin per ng hair of a psilocybe consumer.

1. Introduction

Even though currently "traditional drugs of abuse" (such as opioids, cocaine, cannabinoids and amphetamines) represents the 90% consumers' demand, new psychotropic substances with stimulant or hallucinogenic properties have become increasingly popular among recreational drug users in recent years [1,2]. In particular, an increase in the consumption of vegetable substances with a hallucinogenic effects has been observed [3]. This group includes the well-known "magic" mushrooms of the species *Psilocybe* that contain psilocybin and psilocin; Peyote (*Lophophora williamsii*) and Trichocereus cacti rich in mescaline, *Salvia divinorum* leaves containing salvinorin A, as well as *Mimosa hostilis* roots bark or *Ayahuasca* decoction containing N,N-dimethyltryptamine [3–5].

These plants and mushrooms are obtainable not only in countries of origin [2], but also on internet web sites where, even if illegal in many countries, they can be easily bought and received anonymously avoiding normal law controls [6]. Buyers of psychedelic natural products look for mind-altering effects similar to those of LSD, acute perceptual changes (e.g. hearing colors and seeing sounds), subjective experiences, mystical experience with physiological effects similar to sympathetic arousal state [7].

Whereas analytical procedures for the determination of the above-reported hallucinogenic drugs are available in conventional biological matrices such as blood and urine [8–15], only mescaline has been successfully determined in a non conventional biological matrix such as hair to provide a reliable investigation tool to disclose a long-term abuse or past drug exposure [11].

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In this concern, we developed and validated an ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) method to simultaneously identify and quantify mescaline, N,N-dimethyltryptamine, psilocin, psilocybin and salvinorin A in hair samples. The validated method was checked for its applicability in hair obtained from psychedelic plants and mushrooms consumers.

2. Experimental

2.1. Chemicals and materials

Standard of mescaline (MES), N,N-dimethyltryptamine (DMT), psilocin (PSC), psilocybin (PSB), salvinorin A (SAL) and internal standards mescaline-d_9 (MES-d_9) and 3,4-methylenedioxypropylamphetamine (MDPA) were obtained from Cellirant (Austin, TX, USA). VMA-T M3 (acidic aqueous buffer) reagent was provided by Comedical s.a.s. (Mattarello, Trento, Italy). Ultrapure water and all other reagents for UHPLC–MS/MS analytical grade were obtained from Sigma–Aldrich (Milan, Italy).

2.2. Hair samples

Drug-free human hair samples obtained from 30 healthy individuals were analyzed during method validation to exclude any source of chromatographic interference and mixed to obtain a homogeneous pool of blank hair to be used for calibration standards and quality control (QC) samples.

The entire length of hair shafts was collected from consumers of hallucinogenic plants and mushrooms. It consisted in 6 cm length each, from a female and male individuals smoking peyote cacti, a 2 cm hair shaft from a *S. divinorum* male smoker, a 2.5 cm hair shaft from an Ayahuasca male user and finally 1.5 cm hair shaft from a psilocybe male consumer were obtained by Pediatric Department of Hospital del Mar, Barcelona, Spain within the framework of a survey carried out with young consumers of hallucinogenic plants and mushrooms.

2.3. Calibration standards and quality control samples

Stock standard solutions (1 mg/ml) were prepared in methyl alcohol and stored at −20 °C. From stock solutions, working solutions of 10, 1 and 0.1 μg/ml were prepared and used for the preparation of calibration curves and quality control samples. Working solutions of MES-d_9 and MDPA at a concentration of 0.1 μg/ml were also prepared in methyl alcohol and stored at −20 °C.

Calibration standards containing limit of quantification (LOQ) concentrations, 0.1, 0.5, 1.0, 5.0 and 10.0 ng analytes under investigation per mg hair were prepared daily for each analytical batch by adding suitable amounts of working solutions to 25 mg of pre-checked drug-free hair pool.

Quality Control (QC) samples of 0.06, 4.0 and 8.5 ng analytes under investigation per mg hair were also daily prepared to be included in each analytical batch to check validation parameters (e.g. calibration, inaccuracy, imprecision, analytical recovery, etc.).

2.4. Sample preparation

Aliquots of 25 mg finely cut hair samples were weighed in a glass test tube with hermetic cap. The samples were washed with two aliquots 5 ml methyl alcohol for five min and two aliquots 2.5 ml diethyl ether for other five minutes and then dried. They were then added with 10 μl internal standards (MES d_9 and MDPA 0.1 μg/ml) and treated with 250 μl M3 reagent for 1 h at 100 °C in a thermoblock. Finally, the treated samples were cooled at room temperature and 100 μl M3 extract were diluted with 400 μl water and a volume of 10 μl diluted extract was injected into the chromatographic system.

2.5. Ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC–MS/MS)

The analyses were carried out on an ultra-high pressure liquid chromatography system (Waters Acquity UHPLC, Waters Corporation, Milan, Italy) coupled with a triple quadrupole mass spectrometer (Waters Xevo TQ, Waters Corporation). Chromatographic separation was carried out on a Acquity UHPLC HSS C18 column (2.1 mm × 150 mm, 1.8 μm) using a linear gradient elution with two solvents: 0.3% formic acid in acetonitrile (solvent A) and 5 mM ammonium formate pH 3 (solvent B). Solvent A was maintained at 5% for the first 0.50 min. It was increased to 55% from 0.50 to 10.00 min, then increased to 90% from 10.00 to 10.75 min, held at 90% from 10.75 to 12.85 min, and then decreased back to 5% from 12.85 to 13.00 min and held at 5% from 13.00 to 16.50 min for re-equilibration. The flow rate was kept constant at 0.40 ml/min and the column temperature was set at 50 °C.

The separated analytes were identified and quantified with a triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI). The applied ESI conditions were the following: capillary voltage 3 kV, desolvation temperature 600 °C, source temperature 150 °C, cone gas flow rate 20 l/h, desolvation gas flow rate 1000 l/h and collision gas flow rate 0.12 ml/min. Cone energy voltages, MRM transitions, and collision energy voltages were established for each analyte and the values are listed in Table 1.

2.6. Validation procedures

Validation protocol applied in the present study included linearity, limits of detection (LOD) and quantification (LOQ), imprecision, inaccuracy, selectivity, carryover, matrix effect, recovery and process efficiency, as reported elsewhere [16,17]. Validation

Table 1
Ultra-performance liquid chromatography tandem mass spectrometry parameters for the multiple reaction monitoring (MRM) acquisition mode.

Analytes	Retention time (min)	MRM transitions					
		Quantification			Confirmation		
		m/z	CV (V)	CE (eV)	*m/z*	CV (V)	CE (eV)
Psilocybin	1.48	285.0 > 205.0	16	20	285.0 > 240.0	16	15
Psilocin	2.74	205.2 > 58.2	18	15	205.2 > 160.0	18	10
Mescaline	3.45	212.2 > 180.1	16	20	212.2 > 165.0	16	24
N,N-dimethyltryptamine	3.86	189.1 > 143.9	16	13	189.1 > 58.2	16	13
Salvinorin A	10.28	433.2 > 373.1	16	10	433.2 > 313.1	16	15
Mescaline-d_9	3.39	221.2 > 186.2	16	20	221.2 > 170.4	16	24
3,4-Methylenedioxypropylamphetamine	5.05	222.06 > 163.0	16	15	222.06 > 86.0	16	20