Urine Mescaline Screening With a Biochip Array Immunoassay and Quantification by Gas Chromatography–Mass Spectrometry

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Abstract: Mescaline, the primary psychoactive chemical in peyote cactus, has been consumed for thousands of years in ancient religious ceremonies. The US military wanted to determine if mescaline intake was a problem for personnel readiness. Twenty thousand seventeen urine specimens negative for cannabinoids, cocaine, opiates, and amphetamines were tested for mescaline with the Randox Drugs of Abuse V (DOA-V) biochip array immunoassay at the manufacturer's recommended cutoff of 6 mcg/L. A sensitive and specific method for mescaline quantification in urine was developed and fully validated. Extracted analytes were derivatized with pentafluoropropionic anhydride and pentafluoropropanol and quantified by gas chromatography–mass spectrometry (GC/MS) with electron impact ionization. Standard curves, using linear least squares regression with $1/x^2$ weighting, were linear from 1 to 250 mcg/L with coefficients of determination >0.994. Intra- and interassay imprecision was <4.4 coefficient of variation (%CV), with accuracies >90.4%. Mean extraction efficiencies were >92.0% across the linear range. This fully validated method was applied for the confirmation of urinary mescaline in 526 presumptive-positive specimens and 198 randomly selected presumptive-negative specimens at the manufacturer's 6 mcg/L cutoff. No specimen confirmed positive at the GC/MS limit of quantification of 1 mcg/L. Results indicated that during this time frame, there was insufficient mescaline drug use in the military to warrant routine screening in the drug testing program. However, mescaline stability, although assessed, could have contributed to lower prevalence. We also present a validated GC/MS method for mescaline quantification in urine for reliable confirmation of suspected mescaline intake.

Key Words: mescaline, peyote, GC/MS, herbal drugs, urine

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INTRODUCTION

Naturally occurring plant-based psychoactive drugs recently gained popularity because of wide distribution over the Internet.[1] In 2012, the United Nations Office on Drugs and Crime (UNODC) evaluated the emergence of new psychoactive substances (NPS) by surveying 240 respondents from 80 countries and territories.[2] Eighty-three percent reported NPS appearance in their drug markets with over 20 substances identified from plant-based origins, including mescaline. Mescaline (3,4,5-trimethoxyphenylethylamine), the primary peyote cactus (*Lophophora williamsii*) hallucinogen, contains button-shaped seeds that are soaked in water to prepare a tea or dried and chewed to produce psychedelic effects.[3] Ancient Toltec and Aztec Indians included peyote in their religious ceremonies and to relieve hunger, fatigue, and treat diseases.[4] The Native American Church continues to preserve peyote rituals, and despite efforts to ban the peyote, federal courts exempted sacramental use of peyote from criminal penalties.[5]

Mescaline, a naturally occurring alkaloid, also can be produced synthetically. It was first isolated in 1897 by Heffter and synthesized in 1919 by Späth.[6,7] Mescaline's chemical structure was the lead compound for hundreds of homologs, analogs, and related derivatives.[8] Peyote and mescaline are listed as Schedule I hallucinogens under the Controlled Substances Act in the United States.[9]

Mescaline is a serotonin receptor agonist, with affinity for 5-HT1A and 5-HT2A/B/C receptors.[10–12] The proportion of mescaline agonism on each receptor subtype is less clear, as is the action on dopaminergic and noradrenergic systems.[11–14] Because of its low lipid solubility and polar molecular characteristics, mescaline does not easily pass the blood–brain barrier, and therefore, higher (180–360 mg) doses are needed to produce psychotropic effects.[13,14] Mescaline is rapidly absorbed, with onset of effects between 30 minutes and 2 hours after ingestion, lasting 10–12 hours. In humans, 87% of an oral dose is excreted within 24 hours and 92% within 48 hours,[15] with a plasma half-life of 6 hours.[10,13,15–19]

Clinical effects of mescaline, similar to the psychedelic action of lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA), include euphoria, hallucinations, depersonalization, and psychoses.[16,20–22] In rodent models, mescaline modulates locomotion, exploration, cognitive function,[19,23–25] aggression, and startle[19,26,27] and motor responses.[28–30] Mescaline is reported to undergo

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O-demethylation, N-acetylation, and amine oxidation[31,32]; however, all metabolites are believed to be inactive.[18]

There are multiple published analytical methods for mescaline quantification in plasma,[33,34] urine,[35] hair,[36] and postmortem tissues,[37] or as part of a sympathomimetic amine screening assay.[38] It is critical for military readiness for their drug testing laboratories to identify new drugs of abuse (synthetic cannabinoids, cathinones) or other compounds entering or re-entering the illicit drug market. The mission of the US military Drug Demand Reduction Program (DDRP) is to deter illicit and prescription drug abuse through education, outreach, and awareness programs to ensure fitness for duty. The primary aims of this research were to screen 20,000 US military urine specimens by the Randox Drugs of Abuse V (DOA-V) mescaline biochip array immunoassay and to quantify urine mescaline by gas chromatography–mass spectrometry (GC/MS).

MATERIALS AND METHODS

Authentic Specimens

Urine specimens were screened and quantified for mescaline by GC/MS under an interagency agreement between the Department of Defense (DoD) Drug Demand Reduction Initiative and the National Institutes on Drug Abuse, National Institutes of Health. We tested 20,017 anonymized DoD urine specimens previously screened negative for amphetamines, cannabinoids, cocaine, opiates, and phencyclidine. Per US military sample collection standard operating procedures, all urine specimens were stored at room temperature (RT) before initial analysis. These specimens were collected from US service members stationed worldwide between July 2011 and June 2012.

Randox DOA-V Biochip Array Technology Screening

The Randox (Crumlin, Co, Antrim, United Kingdom) DOA-V biochip array technology allows simultaneous multiple competitive immunoassays. Drug in the specimen and drug labeled with horseradish peroxidase compete for binding sites on immobilized polyclonal antibodies. Signal reagent is added to the biochip, generating a chemiluminescent signal that is compared with the calibrators' intensities.

The DOA-V biochip has 11 antibodies, 1 cross-reacting 100% with mescaline. A 9-point calibration curve (0, 0.17, 0.33, 0.67, 1.3, 2.7, 5.3, 10.7, and 21.3 mcg/L) and 2 controls (2.3 and 10 mcg/L) prepared in phosphate buffer were included in the kit. Any specimen with a result ≥6 mcg/L, the manufacturer's recommended mescaline cutoff, was presumptive positive. One hundred ninety-eight randomly selected negative and 589 presumptive-positive mescaline specimens were stored at 4–7°C before GC/MS confirmation. Sixty-three presumptive-positive specimens were unable to be confirmed for the presence of mescaline because of insufficient sample volume, leaking container, or unsuitable specimen with evidence of contamination. Seven hundred twenty-four authentic urine specimens were processed with our fully validated GC/MS method.

Chemicals and Reagents

Mescaline and deuterated mescaline (mescaline-d9) internal standard (IS) were purchased from Cerilliant Corporation (Round Rock, TX). Pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH) were obtained from Thermo Scientific (Waltham, MA). Clean Screen ZSDAU020 (10 mL per 200 mg) extraction columns were acquired from United Chemical Technologies (Bristol, PA). Organic solvents were high-performance liquid chromatography grade. Potassium phosphate monobasic, potassium phosphate dibasic, and acetic acid were from JT Baker (Phillipsburg, NJ), and ammonium hydroxide, methylene chloride, 2-propanol (IPA), methanol, ethyl acetate, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ).

Calibrators and Quality Control Samples

Working mescaline standard solutions (100, 1000, and 10,000 mcg/L) were prepared by diluting 1.0 g/L stock solution with methanol. Working urine calibrators (1.0, 5.0, 10, 25, 50, 100, and 250 mcg/L) were prepared daily by fortifying appropriate amounts of working standard into 1.0 mL blank urine from drug-free volunteers. Quality control (QC) solutions were prepared in methanol from different lots of stock solutions than calibrators. Low, medium, and high QC samples across the dynamic range of the assay were prepared daily in drug-free urine at final concentrations of 3.0, 30, and 150 mcg/L. Deuterated stock IS (mescaline-d9) was diluted with methanol to achieve a working IS concentration of 500 mcg/L. Fifty-microliter working IS was added to each sample before extraction, yielding a final IS concentration of 25 mcg/L. All standards, QCs, and IS solutions were stored in amber vials at −20°C.

Extraction and Derivatization Procedure

Drug-free urine (1 mL) was pipetted into 10-mL polypropylene tubes and fortified with appropriate amounts of calibrator or QC solutions and 50 μL working IS. One hundred-microliter methanol was added to authentic specimens to account for the volume of methanolic standard added to calibrators and QC samples. All samples were diluted with 2 mL 0.1 M potassium phosphate buffer (pH 6.0), vortexed, and centrifuged at 4°C for 5 minutes (1800×*g*). Supernatants were loaded onto solid-phase extraction (SPE) columns preconditioned with 3 mL methanol, 3 mL deionized water, and 2 mL 0.1 M potassium phosphate buffer (pH 6.0). After sample application under gravity flow, columns were washed with deionized water (3 mL), 0.1 M acetic acid (3 mL), and methanol (3 mL). Samples were dried under full vacuum for 10 minutes and eluted with 3 mL methylene chloride/2-propanol/ammonium hydroxide (78:20:2) into 10-mL conical glass centrifuge tubes. Fifteen microliters of 1% hydrochloric acid in methanol (vol/vol) was added before evaporation under nitrogen at 40°C. Subsequently, samples were derivatized with 50 μL PFPA and 30 μL PFPOH for 30 minutes at 70°C. Derivatized extracts were cooled to RT, dried under nitrogen at 40°C, reconstituted with 50 μL ethyl acetate, and briefly centrifuged at 1800×*g* for 3 minutes. Extracts were transferred to autosampler vials and quantified by GC/MS.

GC/MS Analysis

All analyses were performed with an Agilent 6890 gas chromatograph interfaced with an Agilent 5973 mass selective detector operating in electron impact ionization mode. Analytes of interest were separated within 9.5 minutes using a DB-35 MS capillary column (30 m × 0.32 mm × 0.25 μm). The initial oven temperature was held at 70°C for 0.5 minutes, followed by ramps of 40°C per minute to 200°C, 10°C per minute to 235°C, and finally 75°C per minute to 310°C and held for 1.25 minutes to clean the column before re-equilibrating to the initial starting temperatures. Injection port temperature was maintained at 250°C, and a 2 μL splitless injection was used for each sample. Helium was the carrier gas at a constant flow of 1.5 mL/min. Interface, ion source, and quadrupole temperatures were 280, 230, and 150°C, respectively. Selective ion monitoring mode was used with dwell times of 20 milliseconds and electron multiplier set to +200 eV relative to the daily autotune. Three ions for mescaline and 3 for mescaline-d9 were monitored (quantification ions are underlined): mescaline, *m/z* 181.1, 194.1, 357.1; and mescaline-d9, *m/z* 190.1, 203.1, 366.1. All ions and ion ratios were used.

Method Validation Procedures

This analytical method was fully validated in accordance with the Scientific Working Group for Toxicology (SWGTOX) standard practices for method validation in forensic toxicology.[39] We evaluated imprecision, cross-reactivity, and interferences and characterized sensitivity, specificity, and diagnostic efficiency.

Sensitivity

Assay sensitivity was evaluated in triplicate by determining limits of detection (LOD) and limits of quantification (LOQ) for mescaline on 3 occasions. The LOD was defined as the lowest analyte concentration with acceptable peak shape, chromatographic resolution, retention time, qualifier ion ratios (±20% of average calibrator ratios), and a signal-to-noise ratio (determined by peak height) of at least 3:1. The LOQ was defined as the lowest calibrator that met LOD criteria and had analyte concentrations within ±20% of target.

Linearity

Daily calibration curves were prepared in each analytical batch using peak abundance ratios of analyte to IS. Linearity was determined by the method of least squares with a $1/x^2$ weighting factor and expressed as the determination coefficient (r^2). Each point on the calibration curve was required to have acceptable chromatography, ion ratios within ±20% of mean calibrator ratios, and when quantitated against the full 7-point curve (1.0, 5.0, 10, 25, 50, 100, and 250 mcg/L) must quantify within 15% of the target concentration, except LOQ within 20%.

Imprecision and Bias

Imprecision and bias were evaluated over the linear range with 3 QC samples at target concentrations of 3.0, 30, and 150 mcg/L for mescaline. Intra-assay data were assessed by comparing analyte concentrations of low and high QC samples (n = 10), and interassay data were evaluated from triplicates of low, medium, and high QC samples from 5 analytical batches (n = 15). Imprecision was expressed as coefficient of variation (%CV) of the measured values. Bias was expressed as a percentage of target concentration.

Specificity

Specificity is defined as the ability of the method to identify and quantify analyte with or without the presence of other endogenous or exogenous constituents. Drug-free urine samples were prepared as described above to check for the absence of potentially interfering endogenous compounds. In addition, blank urine matrix with IS added was evaluated in each analytical batch to verify that there were no interferences from the IS. To assess potential exogenous interferences, the method was challenged with 86 structurally similar or commonly coadministered compounds, metabolites, and over-the-counter medications. The following compounds were individually fortified at 1000 g/L into low QC samples (3 mcg/L): Δ^9-tetrahydrocannabinol, 11-hydroxy-Δ^9-tetrahydrocannabinol, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol, cannabidiol, cannabinol, nicotine, cotinine, norcotinine, hydroxycotinine, ephedrine, pseudoephedrine, methamphetamine, amphetamine, *p*-hydroxymethamphetamine, *p*-hydroxyamphetamine, *p*-methoxymethamphetamine, *p*-methoxyamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, 4-hydroxy-3-methoxymethamphetamine, 4-hydroxy-3-methoxyamphetamine, cocaine, benzoylecgonine, cocaethylene, norcocaethylene, *m*-hydroxycocaine, *p*-hydroxycocaine, *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine, ecgonine ethyl ester, ecgonine methyl ester, anhydroecgonine methyl ester, ecgonine, buprenorphine, norbuprenorphine, morphine, codeine, normorphine, norcodeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, noroxymorphone, noroxycodone, 6-acetylmorphine, 6-acetylcodeine, morphine-3-glucuronide, morphine-6-glucuronide, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP), phencyclidine, ketamine, propoxyphene, diazepam, nordiazepam, lorazepam, oxazepam, alprazolam, nitrazepam, 7-aminonitrazepam, flunitrazepam, 7-aminoflunitrazepam, 7-aminoclonazepam, temazepam, bromazepam, clonazepam, flurazepam, paroxetine, fluoxetine, norfluoxetine, imipramine, clomipramine, clonidine, pentazocine, acetaminophen, ibuprofen, acetylsalicylic acid, caffeine, dextromethorphan, phentermine, chlorpheniramine, brompheniramine, and diphenhydramine. All compounds were purchased from Cerilliant Corporation.

Extraction Efficiency

Recovery or extraction efficiency was determined for each analyte at 2 concentrations (3 and 30 mcg/L) by adding IS to a set of spiked samples (n = 10) after SPE but before evaporation (set 1) and to a second set (n = 10) before extraction (set 2). All samples were derivatized and analyzed by GC/MS. Extraction efficiency (%) was calculated by dividing

TABLE 1. Mescaline in Urine by GC/MS: LOD, LOQ, Slope, Intercept, and Linear Range (n = 5) for Calibration Results

Compound	IS	LOD, mcg/L	LOQ, mcg/L	Mean Slope (±SD)	Mean Intercept (±SD)	Linear Range, mcg/L
Mescaline	Mescaline-d9	0.5	1.0	0.0407 (±0.002)	−0.0006 (±0.007)	1–250

mean concentrations of set 2 by mean concentrations of set 1 and multiplied by 100.

Carryover

Carryover in the chromatographic system was evaluated in triplicate by injecting extracted negative specimens (blank urine containing IS) immediately after specimens containing 2000 mcg/L mescaline.

Dilution Integrity

Dilution integrity was assessed by diluting the high QC samples (150 mcg/L), 1:2, 1:5 and 1:10 vol/vol, with blank urine. Each dilution was evaluated in triplicate. A multiplication factor was applied to diluted samples, and assayed concentrations were required to be within ±20% of target, with acceptable peak shape, retention time, and qualifier ion ratios within ±20% of averaged calibrator ratios.

Stability

Mescaline stability at low and high QC concentrations (3.0 and 150 mcg/L) was evaluated under several conditions. Concentrations of fortified control samples (n = 3) were evaluated after storage at RT for 16 hours, 72 hours at 4°C, and after 3 freeze/thaw cycles at −20°C. After these storage conditions, IS was added and samples extracted as described above.

Stability of derivatized extracts was also evaluated after storage at RT for 48 hours. Samples were re-injected and quantified against the initial calibration curve with acceptable concentrations within 20% of initial injections.

RESULTS

It was necessary to develop and validate a mescaline GC/MS assay to confirm the DOA-V presumptive-positive and negative mescaline urine screening results.

Method Validation

Linearity was determined with a 7-point calibration curve in each analytical batch (n = 5). Linear regression analysis using $1/x^2$ weighting yielded coefficients of determination (r^2) ≥0.994 and linearity from 1 to 250 mcg/L (Table 1). Sensitivity was evaluated by extracting decreasing mescaline concentrations in fortified blank urine. The LOD, evaluated in 3 assays with urine specimens (n = 3) from 3 different sources, was 0.5 mcg/L (Table 1). The 1.0 mcg/L calibrator (LOQ) was within 2.1% of target and the remaining calibrators within 11.5%. Interassay analytical bias and imprecision, determined with triplicates of low, medium, and high QC samples over 5 batches (n = 15) (Table 2), were 96.2%–98.0% and 3.6%–4.4%, respectively. Intra-assay bias was >90.4% and intra-assay imprecision 2.2%. Additionally, all potential sources of method uncertainty were considered, and we report an expanded uncertainty of 13% with a 95% confidence level.

Assay specificity, evaluated with blank urine from 10 drug-free volunteers, verified the absence of endogenous interferences. There were no interfering peaks when the method was challenged with 86 exogenous interferents at 1000 mcg/L. All QC samples quantified within ±15.5%. Extraction efficiency was evaluated at 3.0 and 150 mcg/L with urine from 10 drug-free volunteers. Mean peak areas of extracted low and high controls were compared with mean peak areas of controls that were fortified after SPE. Mean mescaline extraction efficiencies were 93.5% and 92.0% for the low and high QC concentrations, respectively.

There was no evidence of carryover in each assay for a negative sample injected immediately after the highest calibrator (250 mcg/L). In addition, no detectable carryover was observed after the injection of samples (n = 3) fortified at 2000 mcg/L. Dilution integrity was investigated to account for specimens exceeding the upper limit of linearity. High QC samples (150 mcg/L) were diluted with drug-free urine (1:2, 1:5, and 1:10 vol/vol). Triplicates of each dilution were evaluated with mean measured concentrations between 92.3% and 98.8% of target. Mescaline stability was evaluated for 16 hours at RT, 72 hours at 4°C, and after 3 freeze–thaw cycles at −20°C by comparing mean analyte concentrations with mean concentrations of freshly prepared QC samples (n = 3) at 3.0 and 150 mcg/L (Table 3). Mean percent differences were within 6.4%. Autosampler stability of derivatized extracts was assessed after 72 hours at RT. Derivatized QC were within 4.1% of target.

Authentic Urine Specimens

All 20,017 urine specimens were shipped and stored at RT (10–233 days) before DOA-V analysis. All presumptive-positive

TABLE 2. Intra- and Interassay Imprecision (%CV) and Analytical Bias (%Target Concentration)

		Imprecision (%CV)		Bias (%Target Concentration)	
	Target Concentration, mcg/L	Intra-Assay (n = 10)	Interassay (n = 15)	Intra-Assay (n = 10)	Interassay (n = 15)
Mescaline	3	0.6	4.4	90.4	98.0
	30	—	3.6	—	96.2
	150	2.2	3.7	99.6	98.5

Data for mescaline in urine were determined by GC–MS.

TABLE 3. Mean Stability Data (%CV) of Mescaline in Urine (n = 3)

Compound	Target Concentration, mcg/L	%Target Concentration (%CV)		
		RT, 16 h	4°C, 16 h	Freeze/ Thaw, ×3
Mescaline	3	88.1 (1.7)	91.3 (2.5)	85.5 (1.0)
	150	95.5 (1.6)	100.0 (2.0)	98.4 (1.0)

(n = 526) and 198 randomly selected presumptive-negative specimens were stored at 4°C for up to 2.4 years before confirmation with our newly validated GC/MS assay. Representative chromatography showing extracted ions for blank urine (Figs. 1A, B) and urine quantified at the LOQ (Figs. 1C, D) is presented. Almost all specimens (n = 721) quantified below the 1 mcg/L LOQ. We were unable to confirm the presence of mescaline in 3 presumptive-positive urine specimens because of the presence of interfering peaks initially and on re-extraction. Because of the lack of any positive urine specimen, we requested positive specimens from Randox and multiple other reference laboratories. Unfortunately, no authentic positive urine specimens were available from any source.

DISCUSSION

An unexpectedly high number of DoD urine specimens screened positive for mescaline (n = 589). Presumptive-positive (526) and presumptive-negative (198) specimens were reanalyzed with the newly developed GC–MS method for confirmation of mescaline. The assay's low sample volume is advantageous, as many clinical and forensic toxicology laboratories are challenged with limited sample size because of polydrug use requiring multiple confirmations. Despite the low sample volume, a 1 mcg/L LOQ was attained. Acceptable sensitivity was achieved without expensive tandem mass spectrometry.[35] The extraction procedure could easily be modified for other matrices, or additional compounds may be incorporated into a full panel screen using PFPA/PFPOH derivatives. A common weakness of immunoassay screening techniques is the lack of specificity, as cross-reactivity of substances other the analyte of interest may produce false-positive (FP) results.

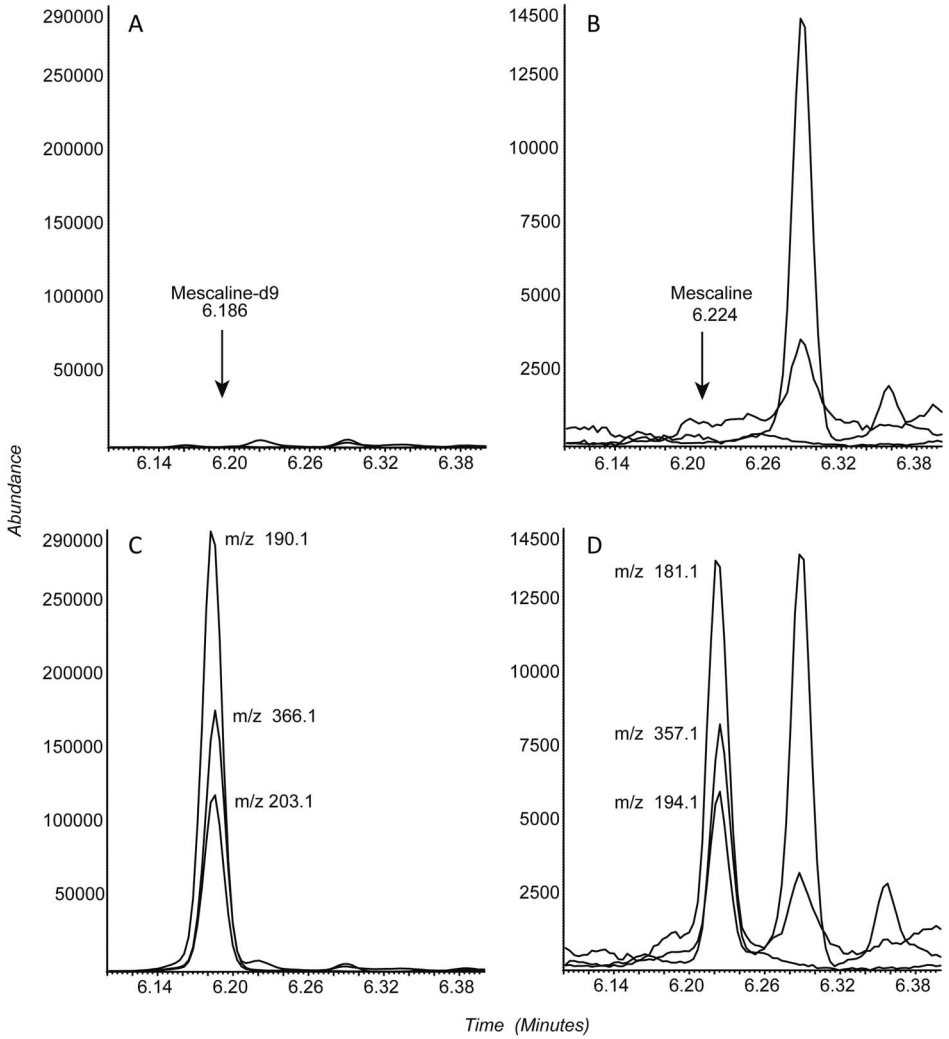

FIGURE 1. Extracted ion chromatograms for blank urine showing IS (A) and mescaline ions (B) and when fortified at the LOQ with mescaline-d9 at 25.0 mcg/L (C) and mescaline at 1.0 mcg/L (D).

Our confirmation assay identified 198 true-negative results, 523 FP results, and 3 specimens that we were unable to confirm. The high FP results could be due to cross-reactivity of synthetic mescaline derivatives or other NPS. It is also possible that the specimens contained low mescaline concentrations at the time of screening that degraded before confirmation. However, we documented acceptable stability at 4°C for up to 1 month, and specimens were stored for varying periods at RT before screening. In fact, 231 of the 589 presumptive-positive specimens (39.2%) were within 20% of the recommended cutoff concentration. It is also possible that the sensitivity of the assay may have been overestimated, as calibration curves (0.0–21.3 mcg/L) were prepared in phosphate buffer, according to manufacturer's recommendations and not matrix matched. Another possible explanation is that the cutoff concentration was too low. We found many FP screening results in the synthetic cannabinoid DOA-V assay at the manufacturer's suggested cutoffs, but when the optimal assay cutoff was determined by liquid chromatography–tandem mass spectrometry results, specificity and efficiency improved greatly.[40] For the synthetic cathinones, we also found many unconfirmed positive biochip array screening results compared with liquid chromatography–tandem mass spectrometry confirmation.[41] However, we could not rule out instability of synthetic cathinones as a source of the FP screening results. Furthermore, when we evaluated linearity of the calibration curve, there was little separation for all calibrators at 6 mcg/L and below. We monitored duplicate blank urine specimens from 10 drug-free volunteers (n = 20). The mean concentration of blank urine (0.31 mcg/L) was similar to the third mescaline calibrator (0.33 mcg/L). Biochip assay LOD was defined as mean observed concentration of these drug-free urine samples + 3 SDs and empirically calculated as 2.0 mcg/L, a concentration between the fifth (1.3 mcg/L) and sixth (2.7 mcg/L) of 9 mescaline calibrators.

A limitation of this study is the long-term RT stability of mescaline in urine, as DoD specimens are transported and stored before analysis at RT. The length of time specimens stored before screening would not affect our confirmation rate but could influence prevalence. However, these randomly collected specimens were from US service members' who routinely undergo urine testing with significant penalties for positive results. Low confirmation rates for synthetic cannabinoids (1.4%),[40] cathinones (0.2%),[41] and piperazines (0.4%)[42] also were observed in the same population. RT storage can produce bacterial growth, alter urinary pH, and affect stability.[43] Bjornstad et al[35] compared short-term storage of fortified urines kept refrigerated or at RT for 7 days. Observed differences were $<7.0\%$ CV. We also documented short-term stability of fortified urine at low and high QC concentrations for 4 weeks at RT, 4°C, and −20°C. All results were within 13.4% of target, with mean percent differences $\leq 4.4\%$ when compared with freshly prepared controls.

Extracted ion chromatograms from the 3 specimens that we were unable to confirm showed no mescaline target ions, yet interfering peaks for the mescaline-d9 target and qualifying ions precluded a definitive determination. It is possible that these samples were unsuitable for analysis because of bacterial growth or possibly contained an interfering compound. However, extensive endogenous and exogenous specificity studies were conducted and showed no interferences.

We present DOA-V and GC/MS results for mescaline from over 20,000 authentic urine specimens collected from US service members stationed worldwide between July 2011 and June 2012. Results indicate that during this time frame, there was insufficient mescaline drug use in the military to warrant routine screening in the drug testing program. However, mescaline stability, although assessed, could have contributed to lower prevalence. We also present a validated GC/MS method for mescaline quantification in urine for reliable confirmation of suspected mescaline intake.

REFERENCES

1. Halpern JH, Pope HG Jr. Hallucinogens on the Internet: a vast new source of underground drug information. *Am J Psychiatry.* 2001;158:481–483.
2. The challenge of new psychoactive substances-United Nations Office on Drugs and Crime (UNODC). Available at: http://www.undoc.org/documents/scientific/NPS_2013_SMART.pdf. Accessed June 8, 2015.
3. Brands B, Sproule B, Marshman J, Psilocybin. In: *Drugs & Drug Abuse* (3rd edition). Toronto, Ontario, Canada: Addiction Research Foundation; DEA, Drugs and Chemicals of Concern: Psilocybin. 1998;462–468.
4. Carson-DeWitt R. *Encyclopedia of Drugs, Alcohol & Addictive Behavior*. 2nd ed. New York, NY: Macmillan Reference USA; 2001.
5. Department of Human Resources of Oregon, Employment Division. Available at: https://supreme.justia.com/cases/federal/us/494/872/#annotation. Accessed June 8, 2015.
6. Manske RHF, Holmes HL. *The Alkaloids*. Vol III, pp 324–328. New York, NY: Academic Press; 1953.
7. Appendino G, Minassi A, Taglialatela-Scafati O. Recreational drug discovery: natural products as lead structures for the synthesis of smart drugs. *Nat Prod Rep.* 2014;31:880–904.
8. Trachsel D. Fluorine in psychedelic phenethylamines. *Drug Test Anal.* 2012;4:577–590.
9. US Drug Enforcement Administration. Available at: http://www.justice.gov.dea/drug_data_sheets/Peyote_Mescaline.pdf. Accessed June 8, 2015.
10. Nichols DE. Hallucinogens. *Pharmacol Ther.* 2004;101:131–181.
11. Monte AP, Waldman SR, Marona-Lewicka D, et al. Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. *J Med Chem.* 1997;40:2997–3008.
12. Harms A, Ulmer E, Kovar KA. Synthesis and 5-HT2A radioligand receptor binding assays of DOMCl and DOMOM, two novel 5-HT2A receptor ligands. *Arch Pharm (Weinheim).* 2003;336:155–158.
13. Spinella M. *The Psychopharmacology of Herbal Medicine: Plant Drugs that Alter Mind, Brain, and Behavior*. Cambridge, MA: MIT Press; 2001.
14. Laing RR, Beyerstein BL. *Hallucinogens: A Forensic Drug Handbook.* Waltham, MA: Academic Press; 2003.
15. Charalampous KD, Walker KE, Kinross-Wright J. Metabolic fate of mescaline in man. *Psychopharmacologia.* 1966;9:48–63.
16. Hermle L, Funfgeld M, Oepen G, et al. Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. *Biol Psychiatry.* 1992;32:976–991.
17. Charalampous KD, Kinross-Wright J. Hallucinogens. Tools for research or therapeutic agents. *Tex Med.* 1963;59:848–852.
18. Baselt RC. Disposition of toxic drugs and chemicals in man. 9th ed. Seal Beach, CA: Biomedical Publications; 2011.
19. Palenicek T, Balikova M, Bubenikova-Valesova V, et al. Mescaline effects on rat behavior and its time profile in serum and brain tissue after a single subcutaneous dose. *Psychopharmacology (Berl).* 2008;196:51–62.
20. Gouzoulis-Mayfrank E, Heekeren K, Thelen B, et al. Effects of the hallucinogen psilocybin on habituation and prepulse inhibition of the startle reflex in humans. *Behav Pharmacol.* 1998;9:561–566.
21. Schwarz BE, Sem-Jacobsen CW, Petersen MC. Effects of mescaline, LSD-25, and adrenochrome on depth electrograms in man. *AMA Arch Neurol Psychiatry.* 1956;75:579–587.

22. Wolbach AB Jr, Miner EJ, Isbell H. Comparison of psilocin with psilocybin, mescaline and LSD-25. *Psychopharmacologia*. 1962;3:219–223.
23. Geyer MA, Light RK, Rose GJ, et al. A characteristic effect of hallucinogens on investigatory responding in rats. *Psychopharmacology (Berl).* 1979;65:35–40.
24. Koupilova M, Herink J, Krs O. Influencing of spatial memory in rats by DSP-4 and mescaline. *Acta Med (Hradec Kralove).* 1999;42:69–72.
25. Sykes EA. Mescaline-induced motor impairment in rats, assessed by two different methods. *Life Sci.* 1986;39:1051–1058.
26. Sbordone RJ, Wingard JA, Gorelick DA, et al. Severe aggression in rats induced by mescaline but not other hallucinogens. *Psychopharmacology (Berl).* 1979;66:275–280.
27. Sbordone RJ, Carder B. Mescaline and shock induced aggression in rats. *Pharmacol Biochem Behav.* 1974;2:777–782.
28. Canal CE, Olaghere da Silva UB, Gresch PJ, et al. The serotonin 2C receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. *Psychopharmacology (Berl).* 2010;209:163–174.
29. Silva MT, Calil HM. Screening hallucinogenic drugs: systematic study of three behavioral tests. *Psychopharmacologia*. 1975;42:163–171.
30. Yamamoto T, Ohno M, Yatsugi S, et al. Nootropic candidates inhibit head-twitches induced by mescaline in mice. *Jpn J Pharmacol.* 1992;59:419–421.
31. Musacchio JM, Goldstein M. The metabolism of mescaline-14-C in rats. *Biochem Pharmacol.* 1967;16:963–970.
32. Scheline RR. *Mammalian Metabolism of Plant Xenobiotics*. London; New York, NY: Academic Press; 1978.
33. Beyer J, Peters FT, Kraemer T, et al. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. *J Mass Spectrom.* 2007;42:150–160.
34. Habrdova V, Peters FT, Theobald DS, et al. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. *J Mass Spectrom.* 2005;40:785–795.
35. Bjornstad K, Helander A, Beck O. Development and clinical application of an LC-MS-MS method for mescaline in urine. *J Anal Toxicol.* 2008;32:227–231.
36. Kim JY, Jung KS, Kim MK, et al. Simultaneous determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom.* 2007;21:1705–1720.
37. Henry JL, Epley J, Rohrig TP. The analysis and distribution of mescaline in postmortem tissues. *J Anal Toxicol.* 2003;27:381–382.
38. Valentine JL, Middleton R. GC-MS identification of sympathomimetic amine drugs in urine: rapid methodology applicable for emergency clinical toxicology. *J Anal Toxicol.* 2000;24:211–222.
39. Scientific Working Group for Forensic T. Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *J Anal Toxicol.* 2013;37:452–474.
40. Castaneto MS, Desrosiers NA, Ellefsen K, et al. Method validation of the biochip array technology for synthetic cannabinoids detection in urine. *Bioanalysis*. 2014;6:2919–2930.
41. Ellefsen KN, Anizan S, Castaneto MS, et al. Validation of the only commercially available immunoassay for synthetic cathinones in urine: Randox Drugs of Abuse V Biochip Array Technology. *Drug Test Anal.* 2014;6:728–738.
42. Castaneto MS, Barnes AJ, Concheiro M, et al. Trazodone and mCPP-positive random workplace urine tests identified with a piperazines biochip immunoassay. *Anal Bioanal Chem.* 2015;407:4639–4648.
43. Cook JD, Strauss KA, Caplan YH, et al. Urine pH: the effects of time and temperature after collection. *J Anal Toxicol.* 2007;31:486–496.