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PAPER**CRIMINALISTICS**

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An Efficient Ambient Ionization Mass Spectrometric Approach to Detection and Quantification of the Mescaline Content of Commonly Abused Cacti from the *Echinopsis* Genus^{*,†}

ABSTRACT: Unregulated cacti from the genus *Echinopsis* are used recreationally as mescaline-containing alternatives to the outlawed peyote. *Echinopsis*-derived plant materials appear in a variety of nondescript forms, making rapid assessment of whether they are mescaline-containing materials or simply innocuous plant-derived food products, very challenging. Reported here is a DART-HRMS approach for the rapid detection of mescaline in whole plant material and a validated method for the quantification of mescaline in cactus tissue, using mescaline-*d*₉ as the internal standard. Calibration curves exhibited R^2 values of ≥ 0.995 , and the method exhibited a LLOQ and a linear range of 1 ppm and 1–100 ppm, respectively. Application of the method to commercially available *Echinopsis* spp. yielded results consistent with previous studies performed by GC- and LC-MS, with mescaline levels of $< 2\%$ dry weight in all cases. Therefore, DART-HRMS is a suitable technique for the rapid screening of mescaline and its subsequent quantification within complex plant-derived matrices.

KEYWORDS: forensic science, criminalistics, mescaline, cactaceae, mass spectrometry, direct analysis in real-time

The cactus *Lophophora williamsii* (Lem.) J.M.Coult., otherwise known as peyote, is proposed to have been used by native North Americans for over five thousand years for its psychoactive properties and as a treatment for many different ailments including fever, pain, rheumatism, and wounds (1,2). Mescaline, the phenethylamine-type hallucinogen responsible for peyote's psychoactive effects, is capable of inducing states of altered consciousness and has been used extensively in various ceremonial rituals for millennia (3–5). However, in 1970, the U.S. Drug Enforcement Administration criminalized the possession and use of mescaline and *L. williamsii* specifically, with the introduction of the Controlled Substances Act, where it was listed as a Schedule I substance (6). Consequently, there has been a rise in the use of other mescaline-containing cacti, including those of the *Echinopsis* genus. Some of the most popular, such as *Echinopsis pachanoi* (Britton and Rose) Friedrich and Rowley, *Echinopsis peruviana* (Britton and Rose) Friedrich and Rowley, and *Echinopsis lageniformis* (Forst.) Friedrich and Rowley, are

sought after for their mescaline content, while new clones such as “psycho0” are touted as having high potency (7–10).

The rise in the recreational use of cacti species has resulted in an increasing need for analytical techniques that can be used to readily analyze and quantify their psychoactive components, most notably mescaline, for toxicological studies and other investigative purposes. While typical analytical techniques such as GC- or LC-MS rightfully enjoy widespread use and acceptance for the analysis of drugs of abuse, the complex cactus matrix requires significant sample processing in order for methods using these techniques to be applied for detection and quantification of mescaline. These can include defatting, lyophilization, extraction, pH adjustment, and recrystallization steps, among others, prior to analysis (11–14). For example, one sample preparation method for analysis of mescaline in peyote by LC-MS requires Soxhlet extraction with methanol at 40°C for 8 h, followed by rotary evaporation, resuspension in water, acidification, defatting twice with an organic solvent, alkalization, extraction twice with solvent, subsequent evaporation of the solvent, resuspension in methanol, and syringe filtering before analysis can occur (15). Another method reported the need for four extractions with diethyl ether over 24 h, five extractions with methanol–ammonia for 24 h, rotary evaporation, resuspension in methanol, and syringe filtering (16). In addition to the long run times, some methods have an additional requirement for derivatization (17,18). Thus, there remains a need for the development of alternative methods for the facile and rapid detection and quantification of drugs such as mescaline within plant matrices, with more streamlined sample preparation steps.

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Direct analysis in real time—high-resolution mass spectrometry (DART-HRMS) is one of the newer ambient ionization mass spectrometric methods that has entered the mainstream as a means for the rapid analysis of compounds in complex matrices. It features the use of a DART ion source which enables open-air ionization of analytes. When the source is coupled to a high-resolution mass spectrometer via an atmospheric pressure interface, mass spectra that reveal the presence of the protonated forms of a range of molecules are acquired within a few seconds. The open-air analysis means that samples can be presented in their native state (i.e., gas, liquid, or solid), an attribute which potentially offers numerous advantages. While other ambient techniques may perform similarly, including direct-injection electrospray ionization (DI-ESI) (19), easy ambient sonic-spray ionization (EASI) (20), and paper spray ionization (PSI) (21), DART-HRMS, which is enjoying increasing use as a sample screening tool for forensic laboratories, offers extremely fast analysis times and minimal methods development. The approach has been shown to work well for the rapid direct analysis of very complex matrices, including whole botanical samples such as leaves or seeds, extracts, and other plant products (22–24). Additionally, common sample pretreatment steps that are a requirement of the technique used (such as enhancing the volatility of analytes of interest through sample derivatization) can be circumvented. However, relatively few reports have appeared demonstrating the exploitation of the capabilities of DART-HRMS for the quantification of small molecules in complex matrices (25,26). Furthermore, while it has recently been shown that DART-HRMS can be used to quantify the content of a psychoactive compound (i.e. atropine) in seeds (27), it remains unknown whether the ease of this technique can be readily applied to cactus-type matrices which are composed of chlorophyllaceous parenchymal tissue that is inherently quite different from that of seeds and other woody samples.

Here, we present a validated DART-HRMS-based method for the quantification of mescaline in *Echinopsis* spp. of forensic importance. It is also demonstrated that DART-HRMS can be used as a rapid screening tool to determine the possible presence of mescaline simply by presenting the sample in its native form to the open-air space between the DART ion source and mass spectrometer inlet. The results of the application of this validated method to the determination of the mescaline content of several commercially available *Echinopsis* spp. products are reported.

Methods and Materials

Mescaline and deuterated mescaline (mescaline- d_9) certified reference materials (CRM) were purchased from Cerilliant (Round Rock, TX). *E. pachanoi* cacti were purchased from World Seed Supply (Mastic Beach, NY). *E. peruviana*, *E. lageniformis*, and *E. lageniformis* “psycho0” cacti were purchased from eBay (<https://www.ebay.com/usr/kc121004>). DIP-it[®] tips were purchased from IonSense (Saugus, MA).

Preparation of Calibration Series and Quality Controls

Deuterated mescaline stock solutions at concentrations of 5 and 2.5 ppm were made by diluting the 100 ppm CRM with methanol. A mescaline stock solution was made by diluting the CRM with the 5 ppm mescaline- d_9 solution, affording a solution with 500 ppm mescaline and 2.5 ppm mescaline- d_9 . The 2.5 ppm stock of mescaline- d_9 was then used to make the calibration curve series (CCs) by serial dilution, with concentrations

ranging from 1 to 100 ppm, all containing 2.5 ppm mescaline- d_9 . In a similar fashion, and from a separate stock, quality control samples (QCs) ranging from 1 to 90 ppm were also made, in duplicate. For each CC or QC, 200 μ L was pipetted into a 0.6-mL Eppendorf tube for analysis.

Preparation of Cactus Samples

Samples encountered by crime laboratories can range from whole live cacti, to plant materials that have undergone various levels of processing to yield the drug in powdered or capsule form. To ensure that the devised DART-HRMS mescaline quantification method was relevant to the types of samples abused by users, online forums such as “Erowid” and “Bluelight” were consulted, as both contain a large repository of user-contributed information on optimal *Echinopsis* spp.-based recipes for the creation of mescaline-containing mind-altering substances (7,8). A representative recipe was followed to generate the samples for which the DART-HRMS-based mescaline quantification method was optimized. Briefly, segments of approximately 10 cm by 2 cm of chlorophyllaceous parenchymal tissue per live cactus were removed with a knife and cut into small pieces. These were dehydrated under vacuum for 12 h and then ground to a fine powder using a coffee grinder (Hamilton Beach, Glen Allen, VA). Methanol extracts were created by suspending a given amount (see Table 1) of the dried powder (that was contained within a 20 mL scintillation vial—VWR Scientific, Radnor, PA) in 5 mL methanol and subjecting the mixture to gentle overnight shaking using a rocking table. The suspension was then decanted, and the supernatant was centrifuged at 5000 rpm to pellet any remaining solids which were subsequently discarded. The retained solution, which constituted the extract, was diluted using methanol to a concentration that fell within the limits of the calibration curve, where after the internal standard was added to a concentration of 2.5 ppm mescaline- d_9 , and the total dilution factor recorded. Table 1 lists the dry weights of the various sample sections analyzed (according to species), as well as the end dilution factors.

DART Analysis and Data Processing

DART mass spectra of all samples were acquired in positive-ion mode using a DART-SVP (standard voltage and pressure) ion source (IonSense, Saugus, MA) coupled to a JEOL AccuTOF high-resolution time-of-flight mass spectrometer (JEOL USA, Peabody, MA). The parameters for the DART ion source

TABLE 1—*Cactus species from which extracts for analysis by DART-HRMS were made using cuttings from live plants.*

Cactus Sample	Dry Mass (g)	Total Dilution Factor
<i>Echinopsis pachanoi</i> 1	0.6952	1:20
	0.6042	1:20
<i>Echinopsis pachanoi</i> 2	0.6123	1:20
	0.6499	1:20
<i>Echinopsis peruviana</i>	0.1843	1:2
	0.1556	1:2
<i>Echinopsis lageniformis</i>	0.3596	1:20
	0.3475	1:20
<i>Echinopsis lageniformis</i> “psycho0”	0.2112	1:20
	0.2585	1:20

The resulting dry mass of each cutting, as well as the factor by which the extract was diluted to create the analysis samples, is indicated in each case.

were as follows: grid voltage, 250 V; and gas heater temperature, 350°C. The settings for the mass spectrometer were as follows: ring lens voltage, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; and peaks voltage, 600 V. Spectra were collected over the m/z range 60–800, with an averaged spectrum produced at the user-selected interval of 1 spectrum per s. The helium flow rate for the DART ion source was 2.0 L/sec, and the resolving power of the mass spectrometer was 6000 FWHM. A 12 DIP-it[®] sampler (IonSense) was used to automate the analyses. A constant linear rail speed of 1.0 mm/s was used. For the sampling of calibrators, quality controls, and cactus extracts, a DIP-it[®] tip was deposited into the bottom of a 0.6-mL Eppendorf tube containing 200 μ L of sample. The tip was subsequently removed and allowed to dry before being affixed to the linear rail for analysis. Data calibration and peak integration were done using TSSPro3 software (Shrader Software Solutions, Detroit, MI). Integration of peaks was performed for protonated mescaline at m/z 212.1281 and for protonated mescaline- d_9 at m/z 221.1852. Polyethylene glycol (PEG) 600 was used as the mass calibration standard. Using profile MS data, the peak area ratio (PAR) of mescaline to mescaline- d_9 was used for calibration curves and determination of the concentration of mescaline in the plant samples, in order to account for variations in instrument response.

Method Validation and Mescaline Quantification

For creation of the validated method, the FDA's guidelines for bioanalytical methods were followed. In summary, it is required that three accuracy and precision runs be completed over several days. Each accuracy and precision (A and P) run consisted of the calibrator series (including a solvent blank and an internal standard zero calibrator) with at least 6 nonzero points and the quality control samples from lowest concentration to highest. In this case, the calibrator series ranged from 1 to 100 ppm, and two DIP-it[®] tips were deposited into each solution. Quality control samples were made in duplicate at four concentrations: the lower limit of quantification (LLOQ), and low-(3 \times LLOQ), medium-(30 ppm), and high-(90 ppm) concentrations (designed to span the full range of the calibration curve), and 5 DIP-it[®] tips were deposited into each replicate solution of each QC. It is also stipulated that for an A and P run to be valid, the following conditions should be met: (1) the analyte response at the LLOQ should be 5 \times the zero calibrator; (2) the accuracy should be \pm 15% of the nominal concentration of nonzero calibrators and \pm 20% for the LLOQ; and (3) the nonzero calibrators' coefficients of variation value should be \pm 15%, except for \pm 20% at the LLOQ. These parameters must also be met for each QC solution. For both requirements (2) and (3), 75% or more of the nonzero calibrators should meet the listed criteria.

Results and Discussion

Calibrator and Quality Control Analyses and Method Validation

Prior to the initiation of method development, several aspects of the experimental design were considered. Because DART is an open-air ionization technique, several factors can affect sample signal intensity, including humidity, air flow, and other ambient conditions. As such, an internal standard was used to help mitigate these effects by using the peak area ratio between the analyte and internal standard, as this would remain constant even in the midst of these environmental factors. Additionally,

due to the nature of DART ionization, which relies on the relative proton affinities of analytes relative to that of water, the internal standard used must exhibit an ionization efficiency similar to that of the analyte. This condition was effectively satisfied by using the deuterated form of mescaline (i.e., mescaline- d_9) as the internal standard.

A representative DART mass spectrum from direct analysis of *Echinopsis* spp. tissue is presented in Fig. 1 (Panel A) and that of a methanol extract is shown in Panel B. Both spectra show mescaline as a prominent peak at nominal m/z 212. Similar results were observed for direct analysis of all tissue samples analyzed, indicating that this rapid approach (i.e., \sim 3 sec), using samples in their native form with no pretreatment, can be used as a screening tool and a presumptive test for mescaline in the complex plant matrix (through detection of m/z 212). While observation of this peak does not constitute a proof that mescaline is present, it is strongly suggestive and would alert the analyst of the need for further directed confirmatory analysis. A series of calibration standards ranging from a concentration of 1–100 ppm mescaline, each containing the deuterated mescaline internal standard at a concentration of 2.5 ppm, were analyzed by DART-HRMS. Figure 1 (Panel C) shows a representative DART mass spectrum generated from analysis of a 100 ppm calibration series sample, and Fig. 1 (Panel D) shows a DART mass spectrum of a cactus extract containing the deuterated internal standard. In Panels C and D, nominal m/z values 212 and 221 correspond to mescaline and mescaline- d_9 , respectively, and these peaks were used for constructing the calibration curves and/or evaluating the QCs. Each calibrator sample was analyzed in duplicate, from the lowest to the highest concentration. In addition, QC samples were interspersed with the calibrator samples to ensure reliability for quantification of unknowns. Figure 2 shows a portion of both the total ion current (Panel A) and the extracted ion currents for mescaline and mescaline- d_9 (Panels B and C respectively) from a typical A and P run.

Peak areas were calculated for mescaline and mescaline- d_9 , and the ratio between these was used to evaluate samples. To validate the method, three separate A and P runs were completed over the course of several days, with fresh samples made each time. Figure 3 shows the calibration curves and quality controls plotted for each of the three runs. Each calibration curve had a coefficient of determination (R^2) of 0.995 or better, and the averages, relative errors, and within-run and between-run coefficients of variance for quality controls are listed in Table 2. The mean relative errors and coefficients of variance were all within 15%, except for the LLOQ samples, which were within 20%, per the FDA guidelines. Thus, the method created here is valid for quantification of mescaline in the range of 1–100 ppm.

Quantification of Mescaline in Commercially Available *Echinopsis* Cacti Products Using the Validated Method

The species of *Echinopsis* cacti chosen for analysis of mescaline content were based on reports from online user forums such as Erowid and Bluelight, which serve as current repositories of information on product sourcing and potency, as well as sample preparation approaches (7,8). This led to the selection of several *Echinopsis* cacti that are legal to own and purchase in the United States (unlike peyote), but which proved difficult to obtain from mainstream vendors. As such, in common with recreational users, specimens were purchased from eBay.

For quantification experiments, samples derived from the chlorophyllaceous parenchymal tissue were used (the mescaline

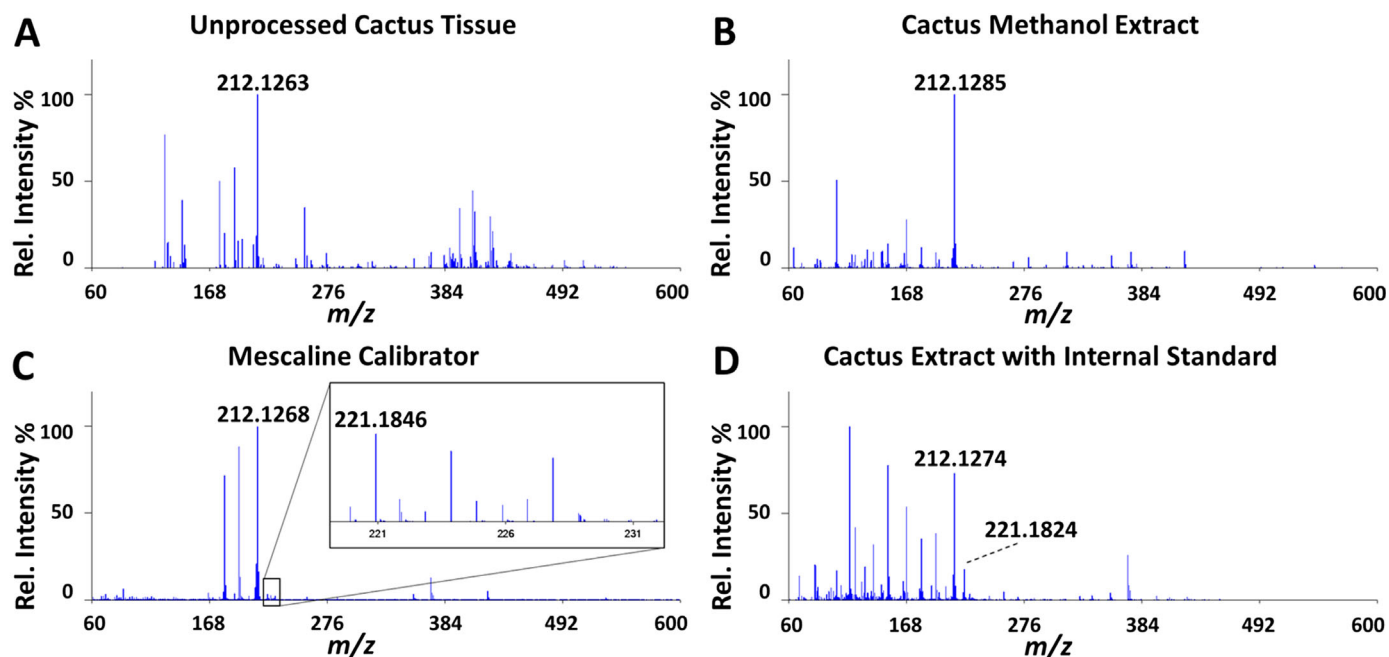


FIG. 1—(A) DART mass spectrum obtained by analyzing an unprocessed cutting from an *Echinopsis pachanoi* cactus. Detection of protonated mescaline at nominal m/z 212 enables DART to be used as a rapid, presumptive screening method; (B) DART mass spectrum of a methanolic extract of *E. pachanoi* cuttings before addition of the deuterated internal standard; (C) DART mass spectrum of a mescaline calibration series sample (100 ppm mescaline). The peak area ratios of mescaline and mescaline- d_9 (nominal m/z 212 and 221 respectively) were used to construct calibration curves; (D) DART spectrum from a processed cactus extract. As in the calibrators, the peak area ratio of mescaline to mescaline- d_9 was used for quantification. [Color figure can be viewed at wileyonlinelibrary.com]

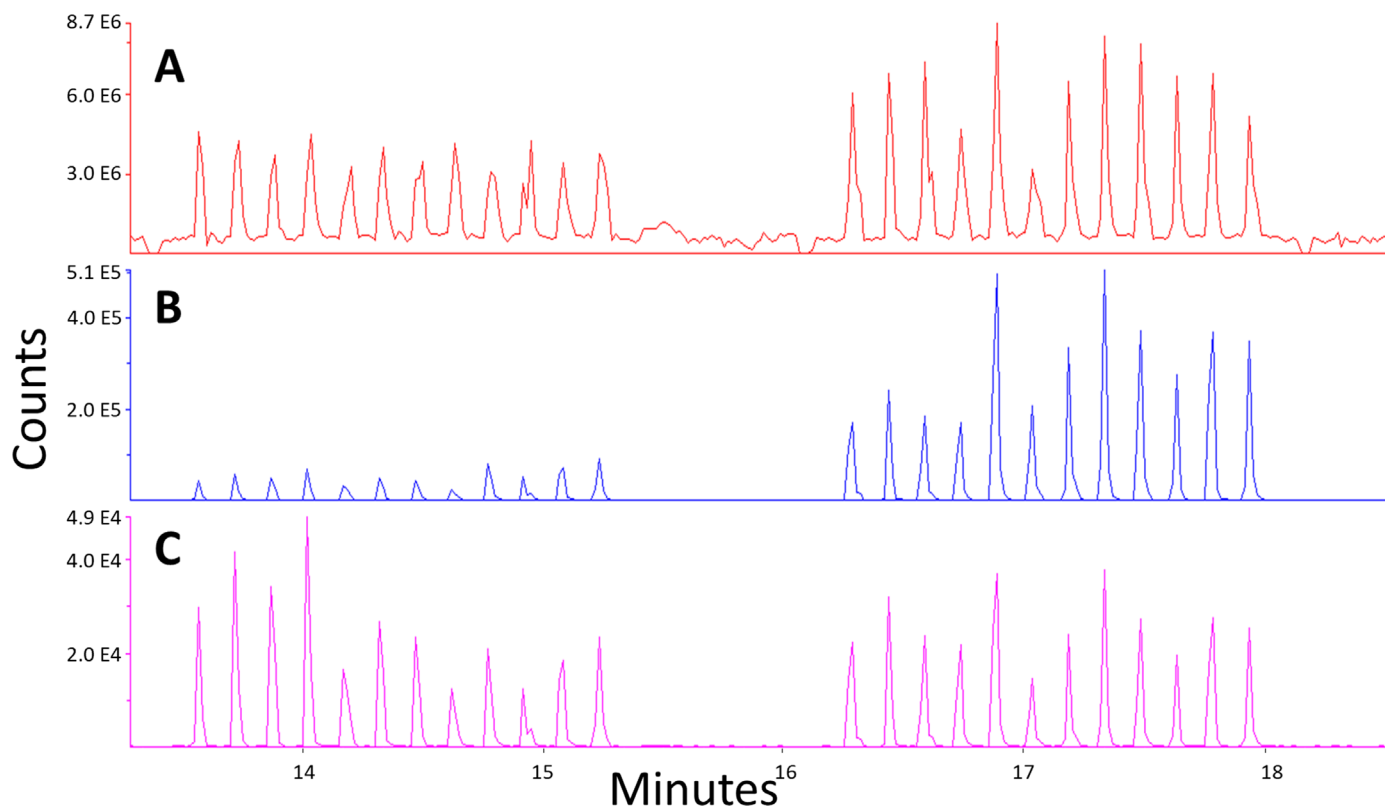


FIG. 2—A portion of the total ion current (TIC) and reconstructed ion currents (RICs) of a typical A and P run. Panel (A) shows the total ion counts, where each peak is the total intensity detected by DART-HRMS for each replicate. Panels (B) and (C) are the extracted ion intensities of mescaline and mescaline- d_9 , respectively. The peak area ratios (PARs) between mescaline and mescaline- d_9 were used to plot the calibration curves. [Color figure can be viewed at wileyonlinelibrary.com]

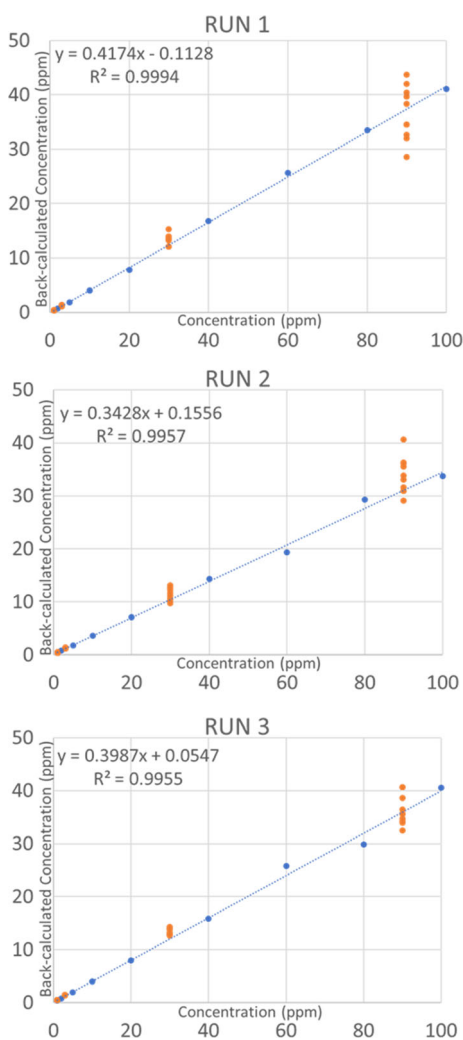


FIG. 3—Plots of the calibration series and quality control samples of the three validated accuracy and precision runs. Blue points represent the calibration series, while the orange dots represent the QCs. Passing QCs fell within 15% relative error, except for the LLOQs, which were within 20%. At least half of the QCs of a given concentration must pass, and two-thirds of all QCs must pass for a valid run. [Color figure can be viewed at wileyonlinelibrary.com]

content of these cacti are mainly localized to the skin and outer portion of the flesh (28,29)). These were dried under vacuum, ground into powder, and extracted with methanol as described previously. This method is similar to homebrew-style extraction protocols developed and reported by users for extraction of the alkaloids from columnar cacti, save for the crystallization and clean-up steps (30). After sample preparation and addition of the internal standard, four DIP-it[®] tips were deposited into each replicate sample, and following DART-HRMS analysis of the sample-coated tips, the mescaline/mescaline-*d*₉ PARs were compared with those of the standard curve. Table 3 lists the observed average concentrations of mescaline determined in each of the samples and the calculated undiluted concentrations and total mescaline content of the dry weight of each cactus sample.

Concentrations of mescaline in *E. pachanoi*, *E. peruviana*, and *E. lageniformis* tissues have been previously reported and were found to be within the range of 0–5% by weight (15,16). In agreement with previous studies, the concentrations determined here fell within this range. Interestingly, the concentration of mescaline in *E. lageniformis* “psycho0” was determined to be

TABLE 2—Concentration and statistics for within-run and between-run calculations of quality control samples for all three validated A and P runs.

Nominal QC Concentration (ppm)	Within-run			Between-run		
	Mean (ppm)	RE%	CV%	Mean (ppm)	RE%	CV%
90						
Run 1	88.07	2.15	12.66	92.36	−2.62	11.04
Run 2	98.14	−9.04	9.62			
Run 3	90.88	−0.97	7.35			
30						
Run 1	32.17	−7.24	7.30	33.07	−10.23	7.59
Run 2	33.65	−12.16	9.51			
Run 3	33.39	−11.30	4.12			
3						
Run 1	3.37	−12.21	5.01	3.34	−11.47	5.62
Run 2	3.24	−8.07	7.33			
Run 3	3.42	−14.15	1.77			
1						
Run 1	1.20	−19.99	7.78	1.04	−3.84	17.19
Run 2	0.83	17.46	12.82			
Run 3	1.09	−9.00	3.66			

All relative errors (RE) and coefficients of variance (CV) were within 15%, except for the LLOQs, which were within 20%.

TABLE 3—Concentration of mescaline in *Echinopsis cacti* extracts determined using the validated DART-HRMS method.

Cactus Sample	Mean Concentration (ppm)	CV (%)	Calculated Stock Concentration (ppm)	Mass % of Dry Weight
<i>Echinopsis pachanoi</i> 1	38.45	4.29	769.0	0.553
<i>Echinopsis pachanoi</i> 2	30.02	4.50	600.4	0.497
<i>Echinopsis pachanoi</i> 2	32.15	4.07	643.0	0.525
<i>Echinopsis peruviana</i>	29.34	4.02	586.8	0.450
<i>Echinopsis lageniformis</i>	0	—	—	—
<i>Echinopsis lageniformis</i>	0	—	—	—
<i>Echinopsis lageniformis</i>	54.06	27.65	1081.2	1.503
<i>Echinopsis lageniformis</i>	57.15	9.42	1143.0	1.645
<i>Echinopsis lageniformis</i>	10.59	26.91	211.8	0.502
<i>Echinopsis lageniformis</i> “psycho0”	9.98	7.12	199.6	0.386

The concentrations listed represent the mean of 12 replicates made from each cactus section. These were used to calculate the stock concentration of the original extract, as well as the mescaline content in the dry mass of chlorophyllaceous parenchymal tissue.

approximately one-third that of the standard variant of *E. lageniformis*, despite its being advertised by some users as being “exceptional” in terms of the “high” experienced from its use.

While the mescaline content of *Echinopsis* cacti has been studied before using conventional methods, the application of DART-HRMS to its determination has several key advantages. For example, reports of GC-MS and LC-MS analysis of such samples often involve extensive sample pretreatment steps (including Soxhlet extraction, defatting, pH manipulations and even derivatization) and the analytical run of a single sample can take the better part of an h (15,18). By comparison, DART-HRMS could be used to analyze a whole, unprocessed plant sample as shown in Fig. 1A) to confirm the presence of mescaline in a matter of seconds, while the entire analytical run of blanks, calibrator solutions, quality control samples, and replicates of unknown plant material can be completed in less than 30 min. The adoption of this approach would greatly streamline the analysis process and allow for a large number of samples to be analyzed within a shorter time period.

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