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Rapid Detection and Quantification of Hallucinogenic Salvinorin A in Commercial *Salvia divinorum* Products by DART-HRMS

Megan I. Chambers, Justine E. Giffen-Lemieux, and Rabi A. Musah*

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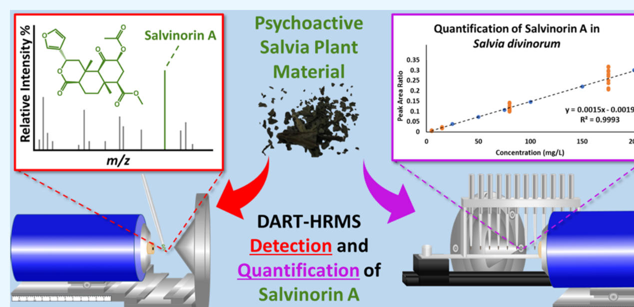
ABSTRACT: In recent years, national laboratories have identified several plant-derived materials as concerns to public health because of their psychoactive effects, potential for abuse, and the lack of federal regulation of their use. One of these is *Salvia divinorum* (aka Salvia), which has received focused attention due to its increasing recreational use and the ease by which it can be acquired. Traditional chromatographic approaches for the detection of the major psychoactive component of Salvia (i.e., salvinorin A) typically require time-consuming sample pretreatment prior to identifying the presence of salvinorin A in plant material unknowns. In this study, direct analysis in real time−high-resolution mass spectrometry (DART-HRMS) was used to rapidly screen for Salvia plant material. This approach facilitated the analysis of bulk material in its native form, thereby bypassing sample pretreatment steps. In addition, a validated DART-HRMS method was developed for the quantification of salvinorin A in commercial Salvia products (e.g., raw plant materials, enhanced leaf extracts). In this regard, cholesterol was found to be a suitable internal standard. The average salvinorin A content in raw Salvia leaves was determined to be 1.54 mg/g, while the salvinorin A quantified in enhanced Salvia leaf extracts was between 13.0 and 53.2 mg/g.



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■ INTRODUCTION

In 2013, the United Nations Office on Drugs and Crime (UNODC) described several emerging new psychoactive substances (NPSs) identified by national laboratories between 2009 and mid-2012.[1] Eight percent of these NPSs, which is equivalent to 20 substances, were plant-based materials now classified as plants of abuse.[1] Their increased recreational use continues to raise public health concerns because of their dangerous health risks and their potential to result in dependence. Further exacerbating the issue is the difficulty of distinguishing these products from innocuous nonpsychoactive plant materials such as culinary items and medicinal herbs. Currently, only two of the plants on the list of 20 are federally classified as Schedule I Controlled Substances (and therefore considered illegal in the United States): (1) *Catha edulis*, commonly known as Khat; and (2) *Lophophora williamsii*, referred to as peyote or peyote cactus.[1] However, the unscheduled status of the other 18 species does not make them any less concerning. *Salvia divinorum*, commonly referred to simply as "Salvia", serves as a case in point. It is a mint-family plant indigenous to Mexico, and is known for its powerful hallucinogenic properties.[2] Although several states in the United States have instituted various types of restrictions on Salvia use and possession (e.g., age restrictions, bans on certain product types, etc.), the lack of regulation at the federal level contributes to its ongoing use and abuse.[3]

Over the millennia, Salvia has been employed by various populations for medicinal, ceremonial, and religious purposes.[2] However, the recreational use of this plant has grown at an exponential pace primarily due in part to the ease of purchasing Salvia products online.[4] Salvia is typically sold as fresh or dried leaves, liquid tinctures, or plant extracts with strengthened potency. Enhanced leaf products are made by increasing the potency of raw Salvia leaves with a Salvia alcohol extract.[5] These are created by subjecting a large amount of raw Salvia leaves (e.g., 10 g) to solvent extraction and then applying the extract to a smaller amount of Salvia leaves (e.g., 1 g). The intense hallucinogenic effects of Salvia are experienced through: (1) smoking dried leaves or other enhanced products; (2) ingesting tinctures; and (3) chewing the leaves.

Because of the differences in *S. divinorum* cultivation conditions, as well as product enhancements that are designed to increase potency, the levels of the psychoactive salvinorin A (also referred to as divinorin A) to which users are exposed vary widely between products. Since the concentrations of this

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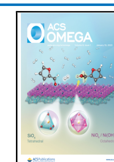

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Figure 1. Commercial Salvia products purchased from online vendors.

compound in the end product are not standardized, the psychoactive effects experienced by users can vary depending on the form of the material (e.g., tincture, dried or fresh leaves, etc.), and from batch to batch even for the same product. The experience that users have, which spans the extremes of peace and tranquility on the one hand, to paranoia and anxiety on the other, in addition to hallucinations and dyskinesia, is known to be dependent upon the salvinorin A levels ingested or inhaled, and the duration of use.[6] However, for the products available for purchase, active ingredient levels are not provided, which makes it impossible for users to gauge their level of exposure, or to make informed decisions about how much to use. Although there have been no reported instances of Salvia overdoses, several deaths have been linked to its use.[7,8] Because of the potential medical applications and/or toxicity of salvinorin A, and interest in this compound in forensic and toxicology contexts, a number of studies of its biological effects have been conducted.[4,6,9−11]

While *S. divinorum* contains several diterpenes, salvinorin A is the only one known to possess psychoactive properties.[12] This molecule is unique in that: (1) it is reported to be one of the most potent natural hallucinogens;[13−15] (2) it is the only known hallucinogen that is not an alkaloid;[14,16] and (3) it was the first non-nitrogenous diterpenoid with psychoactive properties discovered.[17] Heightened concerns over the potential of *S. divinorum* to result in dependence have resulted in attempts to restrict its use. However, the drafting of legislation to accomplish this hinges on the ability to not only accurately identify *S. divinorum*-derived material, but also to readily quantify the salvinorin A contained therein. Because *S. divinorum* cannot always be differentiated from other *Salvia* spp. through visual examination, forensic laboratories currently use the presence of salvinorin A to identify this plant material because it is only known to exist in *S. divinorum*. Studies have applied multivariate statistical procedures (e.g., principal component analysis (PCA)) to gas chromatography−mass spectrometry (GC-MS)-derived data obtained from *Salvia* spp. extracts, to distinguish *S. divinorum* plant materials.[18,19] There have also been investigations that have sought to develop approaches for the quantification of salvinorin A.[20−23] These methods have included GC-MS, liquid chromatography−mass spectrometry (LC-MS), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). While the developed procedures were functional for the analysis of the particular material that was analyzed in each case, it remains challenging to apply them to the full range of forms in which *S. divinorum* products appear. This impacts the extent to which such methods can be readily adopted for the analysis of each type of *S. divinorum* material that emerges on the market. Thus, it is highly desirable to have a more universal analysis approach that can better accommodate the wide range of sample types, and which, at the same time, enables incoming samples to be rapidly triaged and identified as being *S. divinorum* or not, so that informed decisions can be made about the necessity of subjecting the sample(s) to the more time-consuming salvinorin A quantification experiments.

The ambient ionization technique termed direct analysis in real time−high-resolution mass spectrometry (DART-HRMS)[24] has been demonstrated to readily enable qualitative and quantitative analyses of psychoactive molecules in complex plant matrices.[25−34] Samples can be screened in their native form for molecules of interest (i.e., no pretreatment required) by simply introducing them to the open-air gap between the DART ion source and the mass spectrometer inlet. DART-HRMS analyses are typically completed under either soft ionization conditions, which produce primarily protonated/deprotonated precursor molecules (depending on the ionization mode in which the DART ion source is operated) with minimal fragmentation, or collision-induced dissociation (CID) conditions, which result in varying levels of fragmentation.[24,35] More recently, DART-HRMS has shown utility in enabling the quantification of small molecules, with minimal sample preparation, using a semi-automated analysis

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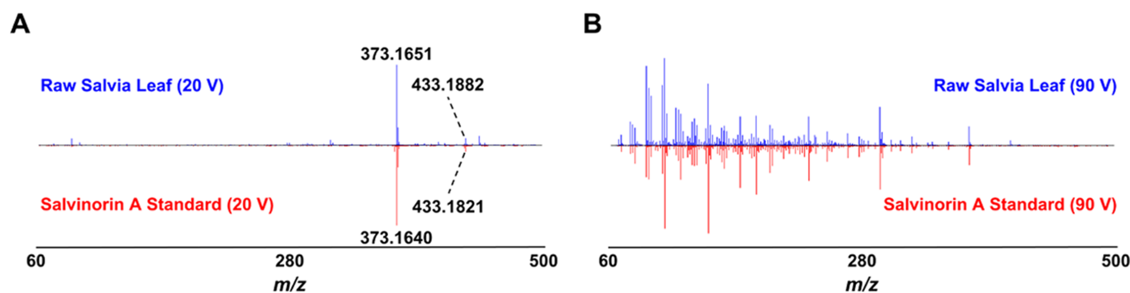

Figure 2. Head-to-tail renderings of the DART mass spectra of raw Salvia leaf and an authentic salvinorin A standard analyzed under soft ionization conditions at 20 V (A) and under CID conditions at 90 V (B).

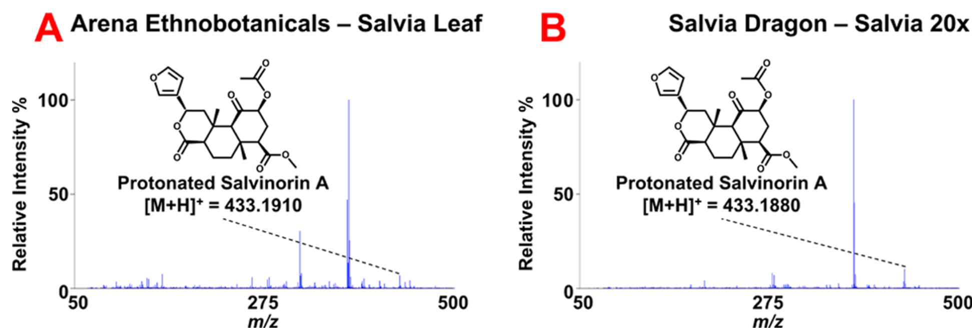

Figure 3. Representative DART mass spectra of two commercial Salvia products: raw Salvia leaf (A) and an enhanced Salvia leaf extract (B).

approach.[25,27,30,31,33,34] Each of these advantages can be leveraged for effective analyses of Salvia.

Our laboratory previously demonstrated two successful investigations of Salvia-genus plant material by DART-HRMS: (1) species-level identification of culinary *Salvia*-genus plants using chemometric processing of DART-HRMS chemical profiles;[36] and (2) identification of *S. divinorum* headspace volatiles collected through the use of solid-phase microextraction (SPME).[37] The study presented here accomplishes the following: (1) rapid detection of salvinorin A in *S. divinorum* plant material and enhanced leaf products; (2) validation of a method for the quantification of salvinorin A, according to the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation: Guidelines for Industry;[38] and (3) application of the aforementioned protocol to eight commercial Salvia products (featured in Figure 1) acquired from online vendors. While salvinorin A is a unique chemical biomarker for *S. divinorum*, and there are other salvinorin derivatives present in Salvia,[39] the method does not aim to distinguish between salvinorin A isomers that are present in minor amounts in Salvia plant material. Therefore, although the salvinorin A concentrations reported here represent the upper limit of salvinorin A that could be present in the samples, the reported method will ultimately allow law enforcement and other laboratories to rapidly identify an unknown plant material as *S. divinorum* or *S. divinorum*-derived based on mass spectral data, and provide information regarding the amount of the psychoactive constituent a Salvia product contains.

RESULTS AND DISCUSSION

Rapid Screening of *S. divinorum*-Related Molecules in Salvia Products. This study commenced with confirmation that DART-HRMS was a suitable method for the detection of the psychoactive compound salvinorin A and rapid screening of Salvia plant materials. DART mass spectra [20 V (Panel A) and 90 V (Panel B)] of raw Salvia leaves (top) and an authentic salvinorin A standard (bottom) were compared in head-to-tail plot renderings such as those shown in Figure 2. Under soft ionization conditions (Panel A), a peak consistent with the protonated $[M + H]^+$ mass of salvinorin A ($[C_{23}H_{28}O_8+H]^+$ calc. 433.1857) is detected in both spectra. Also detected under these conditions is a peak at nominal m/z 373, which is consistent with the fragment of salvinorin A that is formed following the loss of an acetate group ($C_{21}H_{25}O_6$ calc. 373.1651), and which appears as the base peak in both mass spectra. Under CID conditions (Panel B), extensive fragmentation of the molecule(s) present in the samples was observed. Importantly, the salvinorin A fragments are well represented in the top *S. divinorum* spectrum, thereby confirming the presence of this biomarker. Similar analyses and comparisons were performed for an enhanced Salvia product (i.e., Salvia 20× leaf extract) to confirm the ability of DART-HRMS to screen for salvinorin A in different types of Salvia materials. Figure 3 illustrates the detection of salvinorin A in raw Salvia leaf (Panel A) and Salvia 20× (Panel B) products.

Once it was demonstrated that DART-HRMS could detect salvinorin A in representative Salvia product types (i.e., raw plant materials and enhanced leaf extracts), the remaining six Salvia products were screened to confirm the presence of salvinorin A in each product and to ensure that all products were in fact derived from *S. divinorum*. The DART mass spectra for each of the eight Salvia products obtained for this study are displayed in Figure 4. A peak consistent with the protonated $[M + H]^+$ mass of salvinorin A (within 5 mmu) at nominal m/z 433 was detected in each product.

There are several molecules, in addition to salvinorin A, that are characteristic of *Salvia* spp., including salvinorins B–J[39] and divinatorins A–C.[40] Protonated masses consistent (within 10 mmu) with several of these molecules were detected throughout the DART-HRMS analysis of the acquired Salvia

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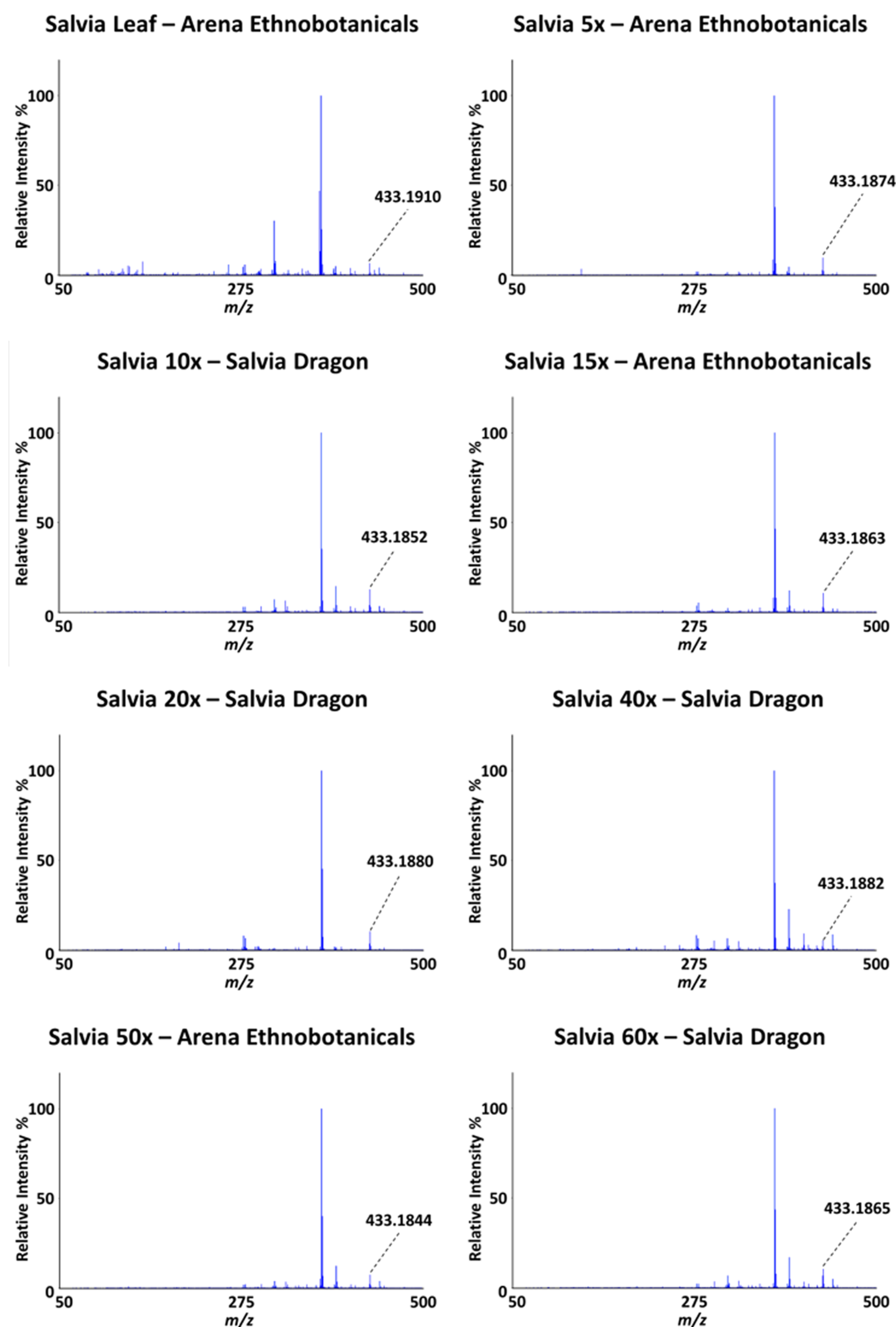

Figure 4. DART-HR mass spectra for eight Salvia products, all of which contain a peak consistent with the protonated mass of salvinorin A.

products. These molecules are listed in Table S1 and further confirm the identity of the Salvia plant materials as derived from *S. divinorum*.

Determining the Feasibility of Cholesterol as an Internal Standard for Quantification Studies. Deuterated internal standards have been used in previously reported DART-HRMS quantification investigations.[27,30,31,34] However, since no deuterium-labeled salvinorin A was commercially available, cholesterol was investigated as an alternative, given the similarity of some elements of its scaffold to that of salvinorin A. Because some plants are known to contain cholesterol, its presence in the various *S. divinorum* products was assessed. Although DART-HRMS soft ionization conditions often detect protonated precursors, there are occasions where adducts (e.g., $+NH_4^+$) or dehydrated (e.g., $-H_2O$) versions of the molecule occur. This is influenced by the unique chemical structures of various molecules and the mechanisms by which they interact with the metastable helium (among other constituents present under ambient conditions). No peaks consistent with the protonated $[M + H]^+$ ($[C_{27}H_{46}O + H]^+$ calc. 387.3627) or the protonated-dehydrated $[M - H_2O + H]^+$ ($[C_{27}H_{46}O - H_2O + H]^+$ calc. 369.3516) masses of cholesterol were observed in any Salvia products. This finding supported its suitability as an internal standard in these

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quantification studies. Furthermore, based on the analyte response (i.e., relative abundance and ion counts) observed by DART-HRMS analysis of cholesterol, the ion (i.e., *m*/*z* value) consistent with $[M - H_2O + H]^+$ was used to generate peak area ratios throughout the quantification experiments.

Validation of DART-HRMS Protocol for the Quantification of Salvinorin A. Method validation for the DART-HRMS quantitative investigations of Salvia products was conducted in accordance with the U.S. FDA Bioanalytical Method Validation: Guidelines for Industry.[38] Following these recommendations, a standard curve was developed with seven nonzero calibrators covering the quantification range. Accompanying the curve were two additional calibrators: (1) a blank calibrator, defined as containing no analyte or internal standard; and (2) a zero calibrator classified as a blank calibrator with internal standard at the concentration used throughout the quantification experiments. To demonstrate the reproducibility and consistency of the developed method, the curve was prepared and analyzed by DART-HRMS on three separate days. The requirements that the blank and zero calibrators not produce any peaks that may interfere with the analyte of interest or that might interfere with quantification efforts were met. Thus, the analyte response of the lowest nonzero calibrator (5 mg/L) was greater than 5 times the response produced by the zero calibrator. This demonstrates method sensitivity, in addition to enabling the determination that the 5 mg/L calibrator coincided with the lower limit of quantification (LLOQ).

The guidelines indicate that two sets of quality control (QC) standards at four concentration levels must span the quantification range, including at the LLOQ, and that these should accompany the curve during each DART-HRMS acquisition. These standards were each analyzed in triplicate at the following levels: 175 mg/L (high), 80 mg/L (medium), 15 mg/L (low), and 5 mg/L (LLOQ). According to the FDA guidelines, for a run to be considered validated, the nonzero calibrators must be within ±15% of their nominal (theoretical) concentration. The one exception is that the LLOQ calibrator can be within ±20% of the nominal concentration. Regarding QCs, ≥50% of the replicates at each level must be within ±15% of their nominal concentrations and ≥67% of the total QC replicates must be within ±15% of the nominal concentrations.

For each of the 3 days that the curve was analyzed, and for all subsequent plant material analyses, all of the recommended validation guidelines were met. In addition, it is worth noting that each of the three validated curves had an R^2 value of ≥0.9977. The three curves used to validate the method are shown in Figure 5.

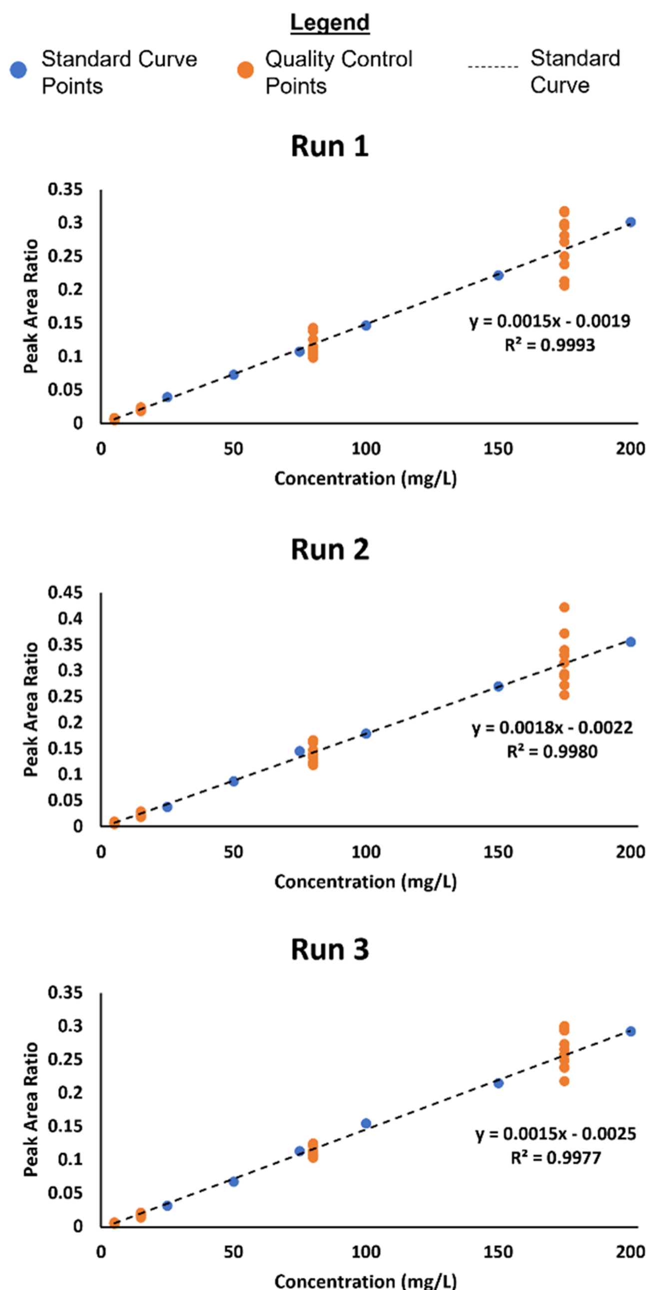

Figure 5. Validated standard curves for salvinorin A quantification developed from DART-HRMS data.

Quantification of Salvinorin A in Commercial Salvia Products. Qualitative and quantitative investigations of salvinorin A in Salvia products and plant materials have been conducted using several common approaches, including GC-MS, LC-MS, TLC, and HPLC. Additional studies have applied other techniques for the analysis of Salvia plant materials including liquid chromatography/electrospray ionization multistage ion trap mass spectrometry ($LC/ESI-IT-MS^n$)[41] and two-dimensional gas chromatography−time-of-flight mass spectrometry (GCxGC-TofMS).[42] Products analyzed in these studies ranged from raw plant materials to leaves with strengthened potency, with samples acquired through various means (e.g., smartshops, online vendors, or grown on-site).[41,42] However, the chromatography-based methods employed typically require time-consuming sample preparation, lengthy run times, and extensive method development.

The concentrations of salvinorin A in raw Salvia leaves reported in previous studies range from 0.89 to 7.8 mg/g.[20−22,41,43] Salvia stems have also been investigated and were found to contain less than 0.63 mg/g of salvinorin A.[21] Significant effort has been dedicated to quantifying salvinorin A in enhanced Salvia leaf products. Concentrations or concentration ranges published to date and organized according to the potency listed on the product labels are as follows: 4.10 mg/g (2×), 1.64 mg/g (3×), 0.13−18.2 mg/g (5×), 6.60 mg/g (7×), 0.56−35.8 mg/g (10×), 15.50 mg/g (14×), 20.9−33.18 mg/g (15×), 0.46−44.5 mg/g (20×), 38.90 mg/g (25×), 28.91−155.6 mg/g (40×), 521.2 mg/g (60×), 36.32 mg/g (70×), and 54.80 mg/g (100×).[20−23,43,44]

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The study presented here features raw Salvia leaves and multiple enhanced leaf products of various potency levels. Salvia extracts were spiked with internal standard (cholesterol) and analyzed by DART-HRMS alongside calibrators and two sets of QC standards. The peak area ratios developed between the salvinorin A $[M + H]^+$ peak ($[C_{23}H_{28}O_8+H]^+$ calc. 433.1857) and the cholesterol ($[C_{27}H_{46}O - H_2O + H]^+$ calc. 369.3516) were used to determine the concentration of salvinorin A in the plant extracts, and by extension, the original amount in the enhanced plant materials and raw leaf. To consider an extract concentration accurate, the peak area ratio between the analyte and internal standard peaks must fall within the linear range of quantification established by the calibration curve; this was the case for all eight of the experimentally acquired peak area ratios. The observed salvinorin A concentrations are listed in Table 1. The product with the lowest salvinorin A content (1.54 mg/g) was the raw leaves from Arena Ethnobotanicals, which was expected considering that all seven of the other products were purchased as enhanced materials. The enhanced leaf products yielded concentrations ranging from 13.0 to 53.2 mg/g. These concentrations represent the average results obtained from three separate runs of the calibration curve and QCs (prepared fresh each day).

Table 1. Salvinorin A Concentrations Measured in Commercial Salvia Products and Plant Materials

commercial Salvia product	vendor	average salvinorin A concentration (mg/g)
Salvia Leaves	Arena Ethnobotanicals	1.54
Salvia 5×	Arena Ethnobotanicals	13.0
Salvia 10×	Salvia Dragon	32.4
Salvia 15×	Arena Ethnobotanicals	20.9
Salvia 20×	Salvia Dragon	16.7
Salvia 40×	Salvia Dragon	24.6
Salvia 50×	Arena Ethnobotanicals	53.2
Salvia 60×	Salvia Dragon	29.3

These salvinorin A results, which are shown in bar chart form in Figure 6, are fairly similar to those appearing in the reports published to date.[20−23,43,44] In particular, the salvinorin A concentration in the raw Salvia leaves and Salvia 5×, 10×, 15×, and 20× products all fall within the ranges reported in the literature. Nevertheless, there were some deviations between the experimentally determined and literature values of salvinorin A, for the Salvia 40× and Salvia 60× products, in that they fell below previously reported values or ranges. To the authors' knowledge, this is the first report of the analysis of a commercial Salvia product with a labeled potency of "50×", and therefore there are no literature-reported values to which to compare the results obtained for analysis of this potency.

Although the Salvia 10× product fell within the range of reported literature concentrations of other 10× products, this material had a higher salvinorin A concentration than the Salvia 20×, Salvia 40×, and Salvia 60× products that were purchased from the same online vendor. This observation may be a consequence of the use of *S. divinorum* leaves with a very high natural concentration of salvinorin A for the preparation of the Salvia 10× extract, while the Salvia 20×, 40×, and 60× extracts may have been derived from leaves with lower natural concentrations of salvinorin A. In addition to the fact that they were purchased from different vendors, this explanation may also account for the differences between the Salvia 50× and

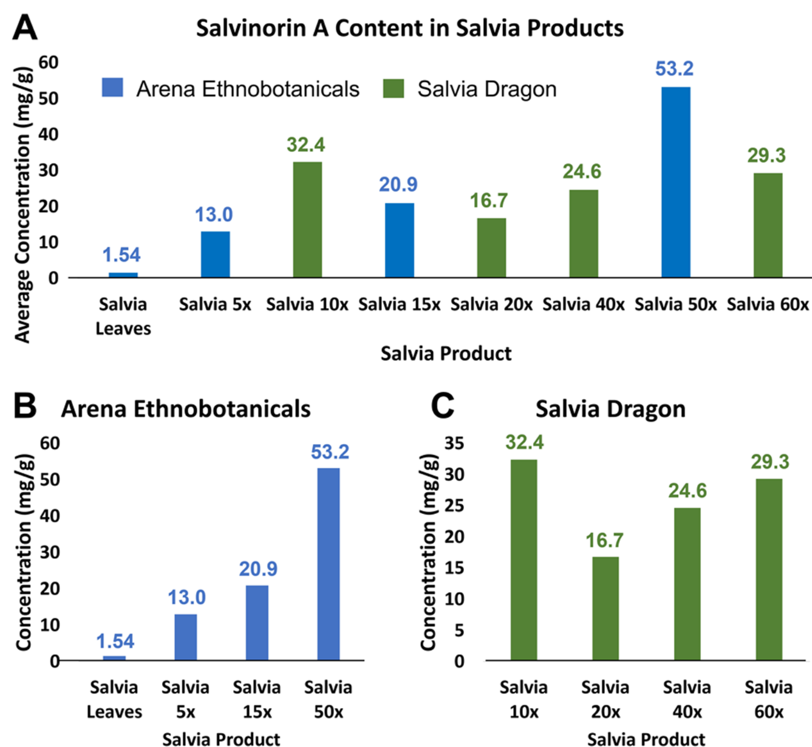

Figure 6. Salvinorin A concentrations measured in commercial Salvia products illustrated in bar chart form, demonstrating the trends between products of different strengthened potency.

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60× products, where the Salvia 60× had a significantly lower salvinorin A concentration than the Salvia 50×.

It is also notable that while *S. divinorum* is the only known Salvia species to contain salvinorin A, there are other known salvinorin derivatives.[39] These include salvinorin B (also referred to as divinorin B), for which an authentic chemical standard is available, and salvinorins D and E which share the same molecular formula as salvinorin A ($[C_{23}H_{28}O_8+H]^+$ calc. 433.1857). Therefore, the salvinorin A concentrations reported here represent the upper limit of salvinorin A that could be present in the various samples. Based on previous studies,[41] in comparison to salvinorin A, the contribution of these isomers (i.e., salvinorins D and E) is minor. Although their exact contributions to the signal observed at m/z 433 could not be confirmed due to the lack of available chemical standards for most salvinorin and divinatorin derivatives, it can be inferred that the majority of the total signal produced at m/z 433 is contributed by the salvinorin A content in the plant material that was analyzed here. While this supposition could not be confirmed in this study, it should be noted that the method presented here is nevertheless useful because it provides rapid assessment of whether confirmatory testing (e.g., GC-MS) is required to determine the extent of any contributions from salvinorins B–J.

■ CONCLUSIONS

The increasing popularity of Salvia, the ease of purchasing and obtaining its related products, and its association with several deaths, all support the UNODC's decision to include Salvia as one of the 20 plants of concern. Therefore, a method to facilitate laboratory identification of otherwise indistinguishable plant materials as *S. divinorum* or *S. divinorum*-derived, as well as the ability to quantify the psychoactive constituent, is highly desirable. The validated DART-HRMS method presented here meets the demand by providing a means by which to rapidly screen for Salvia plant material and quantify salvinorin A. In comparison to other published methods, it proved to be simple, fast, and effective, following a straightforward extraction procedure. The use of a non-deuterated internal standard offers an added benefit. These results further support the use of DART-HRMS protocols for the quantification of psychoactive small molecules in plant species of concern. In addition, the developed method reveals the ability to create additional validated DART-HRMS protocols to aid investigations across multiple fields (e.g., environmental science, forensics, food chemistry, toxicology, etc.).

■ MATERIALS AND METHODS

Salvia Products. All Salvia products were purchased from online vendors. Salvia Leaf, Salvia 5×, Salvia 15×, and Salvia 50× products were purchased from Arena Ethnobotanicals (Del Mar, CA). Salvia 10×, Salvia 20×, Salvia 40×, and Salvia 60× products were purchased from Salvia Dragon (Los Angeles, CA).

Chemical Standards. Salvinorin A was purchased from Cayman Chemical (Ann Arbor, MI). Cholesterol was purchased from Fisher Scientific (Pittsburgh, PA). Methanol was acquired from Pharmco (Brookfield, CT). Nitrogen and ultra high purity helium gases were obtained from AirGas (Albany, NY).

Preparation of Stock Solutions and Calibrators. To quantify the salvinorin A in Salvia products, standard curves were developed using salvinorin A calibrators. Cholesterol was used as an internal standard. Stock solutions of salvinorin A and cholesterol (500 mg/L each) were made by dissolving 10.0 mg in 20 mL of methanol. Seven salvinorin A calibrators ranging from 10 to 400 mg/L with final volumes of 250 μL were prepared by serial dilution. After diluting the 500 mg/L cholesterol stock solution to 50 mg/L, this internal standard was added in 250 μL aliquots to each calibrator, bringing the final volume to 500 μL for each (diluting the original concentrations by half). The final standard curve range was 5 to 200 mg/L, with an internal standard concentration of 25 mg/L. A blank calibrator of 500 μL of methanol and zero calibrator composed of 250 μL of methanol and 250 μL of 50 mg/L cholesterol accompanied the seven calibrators for validation purposes (see below). All calibrators and controls were analyzed by DART-HRMS in triplicate.

Preparation of Quality Control Standards. In accordance with the U.S. FDA Bioanalytical Method Validation: Guidelines for Industry,[38] two QC stock solutions of salvinorin A were prepared independent of the salvinorin A stock solution used to make the curve calibrators. These were created by dissolving 10.0 mg of salvinorin A in 20 mL of methanol and were labeled QC 1 and QC 2 to represent the two sets of QC standards. To follow the validation guidelines, QC standards at four levels (i.e., high, medium, low, and the LLOQ) must accompany the curve in each DART-HRMS acquisition. The concentrations selected for this validation, prepared in the same manner as the curve calibrators, were as follows: 175 mg/L (high), 80 mg/L (medium), 15 mg/L (low), and 5 mg/L (LLOQ). Furthermore, in accordance with the guidelines, the QC standards from each set were freshly prepared each day from their respective stock solutions on the three days the curve was run. All QC standards were analyzed in replicates of five.

Sample Preparation and Data Acquisition for Salvia Plant Materials. All Salvia plant materials were analyzed in replicates of three, meaning that in a single DART-HRMS acquisition, three replicates of the analyzed product were introduced to the DART gas stream independently. Sampling was performed by either holding the material with tweezers (for whole dried leaves) or dipping the closed end of a glass melting point capillary tube into the plant material (for enhanced Salvia leaf products), and presenting it to the open-air gap between the mass spectrometer inlet and DART ion source for approximately 5 s.

Once the DART-HRMS quantification method had been validated, the eight Salvia products were prepared for quantification experiments. Approximately 1 g of the whole dried Salvia leaf was ground using a mortar and pestle to increase the surface area exposed to the solvent during the extraction. The crushed leaves were suspended in 10 mL of methanol in a 20 mL scintillation vial. Approximately 200 mg of the remaining seven enhanced Salvia leaf products was suspended in 5 mL of methanol in a 20 mL scintillation vial. The eight product samples were sonicated for 20 min and placed on a 3D Rotator Mixer (Benchmark Scientific, Inc., Edison, NJ) overnight. The solution was decanted into a 10 mL volumetric flask and diluted accordingly. A 2 mL aliquot was removed and placed into a 2 mL plastic Eppendorf tube (Fisher Scientific, Pittsburgh, PA). All plant samples were centrifuged at 1000 g for 5 min. A 250 μL aliquot of the

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supernatant was added to a new 2 mL plastic tube and diluted to 500 μL with 250 μL of the 50 mg/L cholesterol internal standard solution. To confirm that the salvinorin A was exhaustively extracted from the plant material, the remaining plant samples were subjected to a second round of extraction and the supernatant was analyzed by DART-HRMS. The first round of extraction was considered sufficient because no peak consistent with the protonated mass of salvinorin A was detected in the solutions collected from the second round of extractions.

DART-HRMS Mass Spectral Acquisition and Data Processing. DART-HRMS-derived data for all chemical standards and Salvia products were obtained using a DART-SVP ion source (IonSense, Saugus, MA) coupled to a JEOL AccuTOF high-resolution time-of-flight (TOF) mass spectrometer (JEOL USA, Peabody, MA) in positive-ion mode. The following parameters were used to perform DART-HRMS soft ionization experiments: ring lens voltage, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; peak voltage, 600 V; detector voltage, 2000 V; and resolving power, 6000 FWHM (full width at half-maximum). When experiments were conducted under collision-induced dissociation (CID) conditions, the orifice 1 voltage (which controls the extent to which fragmentation of the molecules occurs) was adjusted to 90 V to increase the extent to which the molecule(s) present fragment. All mass spectra were collected over the range m/z 60−1000 at a rate of 1 spectrum/s. The DART ion source was operated using the following parameters: helium flow rate, 2 L/min; grid voltage, 250 V; and heater temperature, 350 °C. Poly(ethylene glycol) (PEG 600) (Sigma-Aldrich, St. Louis, MO) was used as the mass calibrant for all DART-HRMS acquisitions. Calibration of mass spectral data, spectral averaging, background subtraction, peak centroiding, and peak integration were performed using TSSPro 3.0 software (Shrader Software Solutions, Grosse Point, MI). Analysis of mass spectral data was conducted using the Mass Mountaineer software suite (RBC Software, Portsmouth, NH).

DART-HRMS Semiautomated Quantification of Salvinorin A in Salvia Products. DART-HRMS allows for semiautomated quantification through the use of a 12 DIP-it Holder (IonSense, Saugus, MA) device that is mounted on a linear rail system. The calibrators, standards, and extracts were deposited onto DIP-it Tips (IonSense, Saugus, MA) (referred to as tips) using a dipping method. The tips were inserted into the 2 mL plastic tubes containing the various calibrators and extracts until the tip touched the bottom of the tube. The tips were then removed from the 2 mL plastic tubes and affixed to the holder. A visual assessment was conducted to ensure consistency in the height of the tips. The holder was moved across the DART-HRMS linear rail system at a speed of 1 mm/s. This optimal speed introduced each of the tips to the DART ion stream independently and avoided carryover and cross-contamination from one replicate to the next.

The same calibrators created to validate the standard curve were used to quantify the amount of salvinorin A in the Salvia products. As was performed in the method validation, QC standards were prepared fresh on each of the three days that the curve was run. The calibrators were analyzed in replicates of three, while the QC standards and Salvia extracts were analyzed in replicates of five.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06106.

Mass measurements and relative intensities of salvinorins and divinatorins in Salvia products (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Rabi A. Musah − *Department of Chemistry, University at Albany State University of New York, Albany, New York 12222, United States;* orcid.org/0000-0002-3135-4130; Phone: 518-437-3740; Email: rmusah@albany.edu

Authors

Megan I. Chambers − *Department of Chemistry, University at Albany State University of New York, Albany, New York 12222, United States*

Justine E. Giffen-Lemieux − *Department of Chemistry, University at Albany State University of New York, Albany, New York 12222, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c06106

Notes

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