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TECHNICAL NOTE

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Analyzing *Salvia Divinorum* and its Active Ingredient Salvinorin A Utilizing Thin Layer Chromatography and Gas Chromatography/Mass Spectrometry

ABSTRACT: In recent years, *Salvia divinorum* has become a major focus by state legislatures throughout the United States looking to prohibit the sale of the psychoactive plant. After researching testing procedures presented in the literature and those employed by crime laboratories throughout the country, it was decided that thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS) were the methods to use to analyze plant material for salvinorin A. With TLC, salvinorin A was detected from extracted plant material and was easily distinguishable from 13 other *Salvia* species as well as *Cannabis sativa L.* (marijuana). When using GC/MS, salvinorin A was best extracted from plant material with chloroform at ambient temperature when using a nonpolar solvent and acetone at ambient temperature when using a polar solvent. By utilizing these techniques, criminalists are now able to confirm the presence of salvinorin A in a submitted plant material suspected to be *Salvia divinorum.*

KEYWORDS: forensic science, *Salvia divinorum*, *Salvia*, salvinorin A, salvinorins, marijuana

Salvia divinorum, a powerful psychoactive herb, is a member of the Lamiaceae (mint) family. The psychoactive principal chemical compound has been identified as the neoclerodane diterpene salvinorin A, which is an extremely potent and highly selective kappa-opioid receptor agonist (1). *Salvia divinorum* also contains the structurally related compounds of salvinorin B, salvinorin C, salvinorin D, salvinorin E, salvinorin F, and salvinorin G, which occur in the plant at lower concentrations than salvinorin A (2). The chemical structure of salvinorin A and the compounds of salvinorin B, salvinorin C, and salvinorin D can be viewed in Fig. 1. While salvinorin A has been shown to be a kappa-opioid receptor, receptor assays have demonstrated that salvinorin B, salvinorin D, and salvinorin E are inactive (2). While no results have been published on the effects of salvinorin C and salvinorin F, scientists agree that salvinorin A is the primary psychoactive chemical compound found in *Salvia divinorum* (3). In a previous study, quantitative analysis of 20 dry leaf samples showed salvinorin A contents ranged from 0.089% to 0.37%, with most samples being close to the total average of 0.245% (4). The salvinorins are thus far only known to occur in *Salvia divinorum* and no other natural source has been identified (2).

Native to certain areas of the Sierra Mazateca region of Oaxaca, Mexico, the plant has become increasingly well known and more widely available due in large part to internet businesses selling live plants, dried leaves, and "extracts" ("5×," "10×," and "20×"), which are plant material purportedly enriched with salvinorin A extracted from *Salvia divinorum*. Due to the powerful psychoactive chemical ingredients found in *Salvia divinorum*, Federal and state legislatures have been considering prohibition of the sale of the herb and making it a Schedule I Controlled Substance. Presently, Delaware, Kansas, Illinois, Louisiana, Maine, Missouri, North Dakota, Oklahoma, Tennessee, and Virginia have all passed laws controlling either *Salvia divinorum* or salvinorin A (5). Currently, California Assembly Bill 259 (6) would make the sale or distribution of *Salvia divinorum* or salvinorin A to any person under 18 years of age a misdemeanor. Because of this legislation and the current legal status of the plant throughout the remainder of the country, the San Bernardino County Sheriff's Department's Scientific Investigations Division has taken steps to insure proper testing procedures are available to analyze *Salvia divinorum* and its active chemical ingredient salvinorin A.

To make sure that proper measures are available to analyze salvinorin A, inquiries were made in regards to procedures employed by other crime laboratories. Gas chromatography/mass spectrometry (GC/MS) was the preferred method by all laboratories for analyzing salvinorin A, but the method for extracting the active chemical from *Salvia divinorum* differed for each laboratory. These extraction techniques included using boiling chloroform for 10 min; methanol, chloroform, or acetone extraction at ambient temperature; or basic extraction. By testing each method, it was determined which *Salvia divinorum* extraction technique is best suited in analyzing salvinorin A.

While GC/MS was the most used method to confirm salvinorin A, the application of a screening test would allow for a simple way to examine for *Salvia divinorum*. When testing for marijuana, criminalists utilize microscopy to identify unique trichome characteristics as well as a Duquenois–Levine color test to identify the presence of cannabinoids. While the glandular trichomes of *Salvia divinorum* were observable under the microscope, they could not be differentiated from some of the other *Salvia* species

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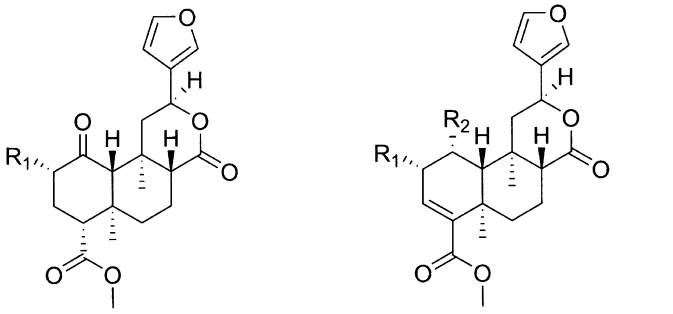

FIG. 1—*Chemical structure of the salvinorins.*

analyzed. Even though *Salvia divinorum* could not be distinguished from other *Salvia* species using microscopy or a color test, it could be differentiated from marijuana utilizing these techniques. In drug analysis, thin layer chromatography (TLC) can be used to identify the presence of a substance, based on the solvent system employed and the developing agent. While this method was not used by any of the crime laboratories to screen for salvinorin A, it is a technique commonly utilized in the identification of methamphetamine, heroin, and cocaine. If a technique could be developed to identify salvinorin A by TLC, it would allow for a much simpler process to identify the active component and provide a complement to GC/MS.

The studies described herein were performed due to the interest in developing procedures to identify the presence of salvinorin A. The two techniques that were utilized for this study were TLC and GC/MS. Raw dried leaves of *Salvia divinorum*, commercial *Salvia divinorum* extracts ("5×," "10×," and "20×"), *Cannabis sativa L.*, and 13 other species of *Salvia* were examined by TLC to determine if salvinorin A could be distinguished from the chemical components in the other plant species. While GC/MS is the preferred method used by surveyed crime laboratories to identify salvinorin A, there are a wide variety of extraction techniques utilized. Several of these extraction procedures were studied to determine which technique is best suited in analyzing salvinorin A.

Materials and Methods

Plant Material and Salvinorin Standards

The raw dried leaves of *Salvia divinorum* and commercial *Salvia divinorum* extracts ("5×," "10×," and "20×") were purchased in June 2007 from HerbalFire (http://www.herbalfire.com). From the company's website, HerbalFire guarantees 25 mg of salvinorin A per gram of leaf in the "10×" and 12.5 mg of salvinorin A per gram of leaf in the "5×." The *Cannabis sativa L.* (marijuana) and the 13 species of *Salvia* were provided by the controlled substances unit of the San Bernardino County Sheriff's Department's Scientific Investigations Division. The 13 species of *Salvia* used were *Salvia clevelandii, Salvia spathacea, Salvia dorrii, Salvia aurea, Salvia officinalis, Salvia greggii, Salvia purpurea, Salvia sclarea, Salvia leucantha, Salvia microphylla, Salvia columbariea, Salvia apiana,* and *Salvia hybrid.* The salvinorin A (CAS# 83729-01-5) standard and the Δ^9-tetrahydrocannabinol (Δ^9-THC) (CAS# 1972-08-3) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). The salvinorin B, C, D, E, and G standards were provided by Dr. Thomas Munro of the McLean Hospital in Belmont, MA.

TLC

The TLC procedure was adapted from the *Annals of Botany* (2). Approximately 0.15 mL (three drops) of a mixture of reagent grade methanol:chloroform (1:1 by volume), a solution currently utilized by the Lab in drug extractions, was added to 0.1 g of raw dried leaves of *Salvia divinorum*, commercial *Salvia divinorum* extracts ("5×," "10×," and "20×"), *Cannabis sativa L.*, and samples of 13 species in the genus *Salvia.* Approximately 4 μL of the resulting solutions of each compound were applied to Whatman 250 μm silica gel plates (catalog no. 4865-820). The plates were developed in reagent grade ethyl acetate:hexane (1:1 by volume). Terpenes were visualized by spraying the plates with vanillin reagent, and then slowly heating on a hot plate. The vanillin reagent was prepared by mixing 50 mL of reagent grade ethanol, 0.3 mL of reagent grade sulfuric acid, and 1 g of vanillin (≥98.5% HPLC Grade). The salvinorins react with this chromogenic reagent to produce pinkish-purple spots on the plates. A standard of salvinorin A was also applied to each TLC plate.

Extraction Techniques Employed by Surveyed Crime Laboratories

The method for extracting the active chemical from *Salvia divinorum* differed for each laboratory contacted. These extraction techniques include: a basic extraction utilizing 0.1 *N* sodium hydroxide then partitioned to methylene chloride (Correspondence with Sean Brooks of the Missouri State Highway Patrol Forensic Laboratory); applying methanol to the *Salvia divinorum* and vortexing the material to extract the salvinorin A (Correspondence with Glenn Everett of the Tennessee Bureau of Investigations Central Laboratory in Nashville); adding boiling chloroform to the plant material for 10 min (Correspondence with Mistie Burris of the Oklahoma State Bureau of Investigation); applying ether at an ambient temperature to the plant material (Correspondence with Ron Porche of the Louisiana State Police Crime Lab); and finally soaking the *Salvia divinorum* in acetone for 1 min at ambient temperature to extract the salvinorin A (Correspondence with Pamela Smith of the Drug Enforcement Administration's Special Testing and Research Laboratory).

GC/MS

A Hewlett-Packard (HP) 5890 Series II Plus Gas Chromatograph was used in combination with a HP MSD Series 5972 Mass Spectrometer and a HP 6890 Series Injector (Palo Alto, CA). The chromatographic separations were run on a J&W Scientific HP-5 (5% phenyl-methyl-siloxane) capillary column (15 m × 0.25 mm ID, 0.25 μm film thickness). The injection volume was 1 μL (splitless injection) with an injection temperature at 280°C, using helium as the carrier gas. The column temperature was programmed from 60°C (initial time: 2 min) to 300°C (rate: 30°C/min) and then held at 300°C for 5 min. Mass spectra were collected in scan mode in the range of m/z 40–450, with the electron energy set to 70 eV.

Results

TLC

Raw dried leaves of *Salvia divinorum*, commercial *Salvia divinorum* extracts ("5×," "10×," and "20×"), *Cannabis sativa L.*, and 13 species in the genus *Salvia* were analyzed using the process of TLC. Terpenes were visualized by spraying the plates with vanillin reagent and then applying heat to the plates. Salvinorin A, the

primary psychoactive constituent, reacted with this reagent to produce a pinkish-purple spot on the plates. No similar spots were detected with an R_f value equal to the salvinorin A in any other sample examined, except *Salvia divinorum*.

Examination of the developed TLC plates revealed that salvinorin A was extracted from the *Salvia divinorum*, using the chloroform:methanol mixture, at a concentration that could be consistently detected. The highest concentration of salvinorin A was extracted in the first minute the plant material was exposed to the solvent. This extract showed no visible chlorophylls or other pigments, which was not the case when the plant material was extracted after a few minutes of exposure to the solvent. TLC was deemed to be a very sensitive method for detecting salvinorins and through preliminary experimentation it was determined that salvinorin A can readily be detected in solution at concentrations as low as one part per 50,000 (0.002%) (2). All of the commercial *Salvia divinorum* extracts ("5×," "10×," and "20×") analyzed showed a pinkish-purple spot equivalent to the salvinorin A standard. When evaluating marijuana and 13 other *Salvia* species, none showed a spot comparable to that of salvinorin A with a similar color and R_f value. Also, salvinorin A could be differentiated from a number of other standards such as salvinorin B, salvinorin C, a mixture of salvinorins D, E, and G (D being the major, and E and G being the minor components), and Δ^9-THC by its R_f value, as shown in Table 1. While salvinorin A could not be distinguished from the other salvinorin standards by color, it could be differentiated from Δ^9-THC, which gave a blue-colored spot on the TLC plate. In all cases where the plant material was exposed to the solvent in the first minute, the salvinorins were consistently extracted and gave reproducible results on the TLC plate. With salvinorin A clearly distinguishable on the plates, this TLC procedure has proven to be a useful technique in the preliminary identification of the presence of *Salvia divinorum*.

GC/MS

GC/MS was the preferred method of analysis by all laboratories surveyed for analyzing salvinorin A. All of the extraction techniques utilized by the five consulted crime laboratories were performed on 0.5 g of dried *Salvia divinorum* leaves and their chromatograms were studied to determine which procedure is more suited in analyzing salvinorin A.

The tested extraction techniques, which included using boiling chloroform for 10 min, methanol and chloroform extractions at ambient temperature, and a basic extraction are all methods employed by crime laboratories to analyze for salvinorin A. While all of these methods were proven to be valid, certain extraction techniques were shown to be better suited in analyzing salvinorin A based on lack of additional plant material interference.

The highest concentration of salvinorin A was extracted in the first minute the plant material was exposed to the different solvents. This extract showed no visible chlorophylls or other pigments, which was not the case when the plant material was extracted after a few minutes of exposure to the solvent.

TABLE 1—*Thin layer chromatography analysis.*

Compound	R_f	Color of Spot
Salvinorin A	35	Pinkish-purple
Salvinorin B	20	Pinkish-purple
Salvinorin C	45	Pinkish-purple
Salvinorin D	24	Pinkish-purple
Δ^9-Tetrahydrocannabinol	78	Blue

The methanol procedure was able to extract salvinorin A from the dried *Salvia divinorum* leaves, but a large amount of residual plant material was observed throughout the chromatogram. The methanol was believed to have penetrated the epidermis of the leaf resulting in the release of chlorophylls and other compounds from the cellular tissues. Also, compared with other extraction techniques utilized, the abundance peak of salvinorin A was much smaller.

The basic extraction procedure was also able to extract salvinorin A from the dried leaves and resulted in very little residual material being observed throughout the chromatogram. Similar to the methanol results, the abundance peak of salvinorin A was smaller than the other extraction techniques.

Chloroform is a common solvent because it is relatively unreactive, miscible with most organic liquids, and conveniently volatile. It is also an effective solvent for alkaloids in their base form and thus plant material is commonly extracted with chloroform for pharmaceutical processing. Acetone, another common solvent, is miscible with water and because of its medium polarity, it dissolves a wide range of compounds. The use of chloroform as a nonpolar solvent and acetone as a polar solvent were deemed the methods to use when extracting salvinorin A from the dried leaves of *Salvia divinorum*. Both extraction techniques resulted in very little residual material being observed in GC/MS and the abundance of salvinorin A was much larger than the other previously mentioned methods. It should also be noted that these two solvents are readily available and the extraction technique was reasonably simple. No differences were observed in the chromatograms of the two chloroform temperature procedures at 1 min exposure, one being at ambient temperature and the contacted forensic lab which utilizes boiling chloroform. But when the plant material was exposed to the boiling chloroform for 10 min, chlorophylls and other compounds from the cellular tissues were visible throughout the chromatogram. Therefore, by using chloroform and acetone at an ambient temperature, salvinorin A can be easily extracted to determine the presence of *Salvia divinorum*. All of the commercial *Salvia divinorum* extracts ("5×," "10×," and "20×") analyzed also showed a significant peak equivalent to the salvinorin A standard.

The reconstructed total ion chromatograms of *Salvia divinorum* extracted by ambient chloroform and ambient acetone are shown in Figs. 2 and 3. Both chromatograms show the apparent locations of salvinorin A, salvinorin B, salvinorin C, and salvinorin D based on their mass spectra. The mass spectrum of salvinorin A obtained by the chloroform (ambient) extraction and the mass spectrum of salvinorin A obtained by the acetone (ambient) extraction were

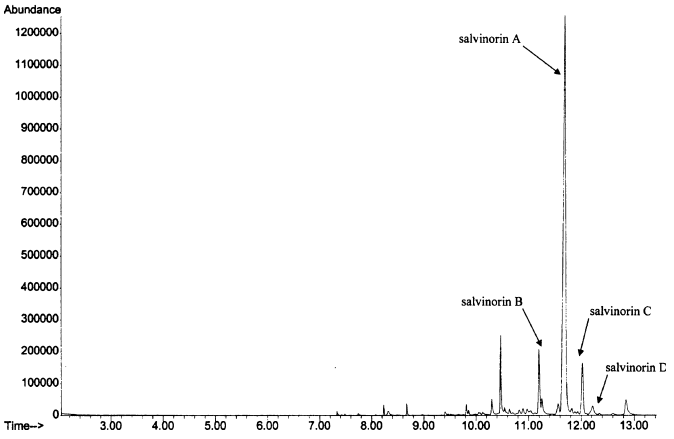

FIG. 2—*Reconstructed total ion chromatogram of* Salvia divinorum *extracted by ambient temperature chloroform.*

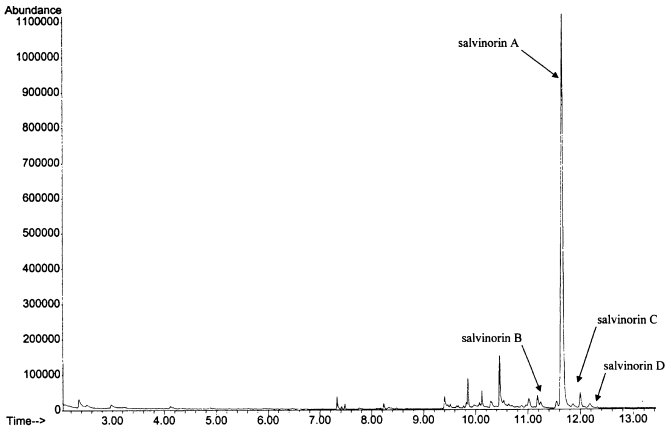

FIG. 3—*Reconstructed total ion chromatogram of* Salvia divinorum *extracted by ambient temperature acetone.*

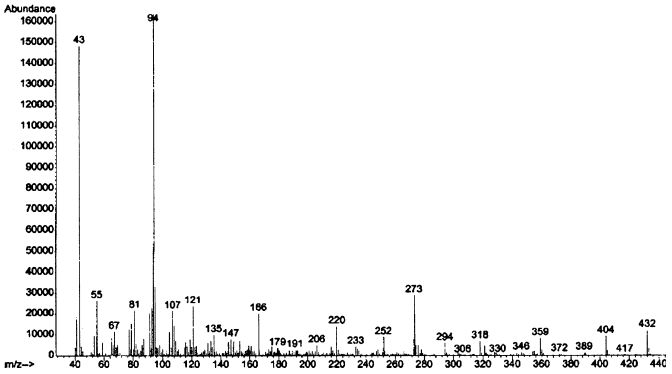

FIG. 4—*Mass spectrum of salvinorin A standard.*

compared with the mass spectrum of the salvinorin A standard in Fig. 4. The mass spectra from each extraction was positively comparable to that of the salvinorin A standard.

While the California Assembly Bill 259 does not regulate any other salvinorins present in *Salvia divinorum* other than salvinorin A, it was determined that their mass spectra should be examined to determine if they are distinguishable from salvinorin A. The mass spectra of salvinorin B, salvinorin C, and salvinorin D in Figs. 5, 6, and 7 were compared with the salvinorin A spectrum and the prominent ions were contrasted in Table 2. It was determined that all of the salvinorins could be distinguished from each other.

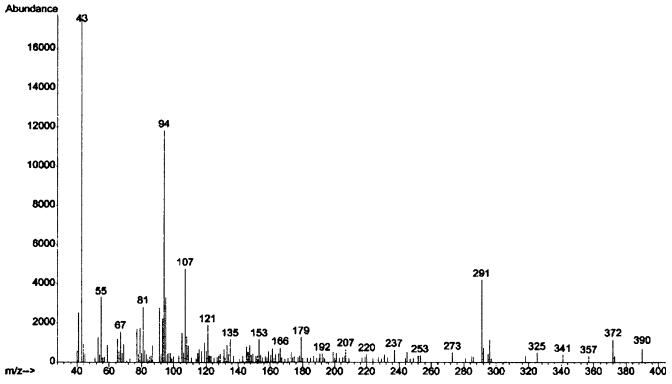

FIG. 5—*Mass spectrum of salvinorin B standard.*

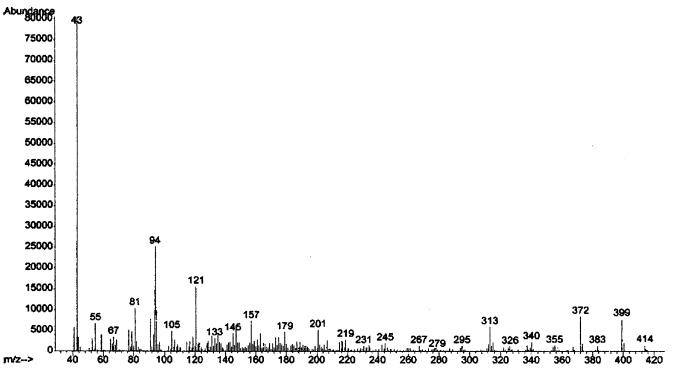

FIG. 6—*Mass spectrum of salvinorin C standard.*

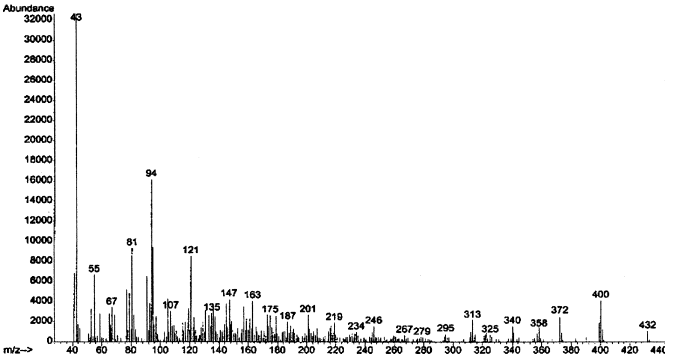

FIG. 7—*Mass spectrum of salvinorin D standard.*

TABLE 2—*Prominent ions by mass spectrometry.*

Compound	Base Peak (m/z)	Other Prominent Ions (m/z)						MW
Salvinorin A	94	43	273	55	121	81	107	432
Salvinorin B	43	94	107	291	55	81	121	390
Salvinorin C	43	94	121	81	372	399	81	474
Salvinorin D	43	94	81	121	55	400	163	432

Discussion

From this study, TLC and GC/MS were determined to be acceptable methods to use when analyzing for salvinorin A. With TLC, salvinorin A was detected in raw dried leaves of *Salvia divinorum* and commercial *Salvia divinorum* extracts (“5×,” “10×,” and “20×”); and was clearly differentiable from 13 other *Salvia* species, *Cannabis sativa L.* (marijuana), as well as salvinorins B, C, D, E, and G. When using GC/MS, salvinorin A was best extracted from plant material with chloroform at an ambient temperature when using a nonpolar solvent and acetone at an ambient temperature when using a polar solvent. By utilizing these two techniques, criminalists are now able to confirm the presence of salvinorin A in a submitted plant material suspected to be *Salvia divinorum.*

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Nashville, Mistie Burris of the Oklahoma State Bureau of Investigation, Ron Porche of the Louisiana State Police Crime Lab, Pamela Smith of the Drug Enforcement Administration's Special Testing and Research Laboratory, Liqun Wong of the DEA's Drug and Chemical Evaluation Section, Terry Dal Cason of the DEA's North Central Laboratory, and Harry Skinner of the DEA's Southwest Laboratory for their critical input into the extraction techniques utilized by their laboratories. We would also like to thank Kenneth Goddard of the National Fish & Wildlife Forensics Laboratory in Ashland, Oregon for supplying the 13 *Salvia* species which he collected while employed at the San Bernardino County Sheriff's Department and especially Dr. Thomas Munro of the McLean Hospital in Belmont, MA, for most generously providing the salvinorin B, C, D, E, and G standards.

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