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High Performance Liquid Chromatographic Quantification of Salvinorin A from Tissues of *Salvia divinorum* Epling & Játiva-M†

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A reversed-phase high performance liquid chromatographic method for the determination of salvinorin A, a psychotropic diterpene isolated from the Mexican sage *Salvia divinorum,* has been developed. Extracts from several plant collections were examined on a C-18 column with UV detection and isocratic elution with acetonitrile: water (45:55). This assay allowed quantification of salvinorin A in extracts of leaves and stems of *S. divinorum* and has also been applied to the screening of related species for the production of salvinorin A. Levels of salvinorin A in leaves range from 0.89 to 3.70 mg/g dry weight. © **1999 John Wiley & Sons, Ltd.**

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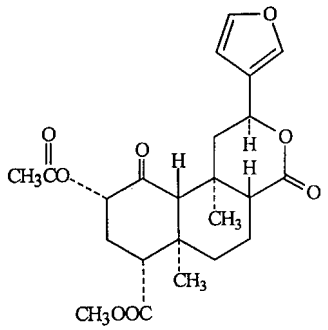
INTRODUCTION

Salvia divinorum Epling et Játiva-M. (Lamiaceae) is native to Oaxaca in Central Mexico. Among the indigenous Mazatec people of Oaxaca, *S. divinorum* has long been used in ceremonial healing rituals as a means of inducing a visionary state that allows the participants to divine the cause of illness or ailment (Wasson, 1962; Valdés *et al.*, 1983). The psychotropic activity of this mint species has been attributed to a neoclerodane diterpene found in the leaves, salvinorin A (**1**) (Ortega *et al.*, 1982; Valdés *et al.*, 1984, 1987; Siebert, 1994). It has been demonstrated that salvinorin A is an extraordinarily potent drug in humans, exhibiting threshold effects at doses in the range of 200–500 μg, making it the most potent natural hallucinogen ever studied (Siebert, 1994). The site of action and mechanism of pharmacological activity of **1** remain unknown. When submitted for major neurotransmitter and second messenger receptor site screening, **1** showed no significant binding to these sites at concentrations of 10^{-5} M (Siebert, 1994). It has been suggested that the extraordinary potency of salvinorin A, and the absence of any known mechanism for its effects, could indicate that a new receptor system is involved in the activity of the compound (McKenna, 1996).



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Salvinorin A has thus far been attributed uniquely to *S. divinorum*; no other natural source for this compound has been identified nor has it been synthetically produced. The current distribution of *S. divinorum* suggests that all existing stands of the plant have been intentionally cultivated by humans; no clearly wild populations of the species have been identified (Reisfeld, 1993). Furthermore, while the plant is readily propagated through asexual reproduction by rooting stem cuttings, it appears that it rarely if ever reproduces from seed in nature. It has been proposed that *S. divinorum* may in fact be a hybrid, resulting in substantially reduced fertility within the species (Reisfeld, 1993). The parentage for such a hybrid

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may remain unknown as no obvious candidates have been proposed as likely progenitors.

Since its initial isolation, no analytical assay has been published in order to quantify precisely the amount of salvinorin A present in samples of *S. divinorum* or any other plant species. The purpose of the current study was to develop a sensitive quantitative assay to determine the amount of **1** present in leaf samples of *S. divinorum*. In addition, the method has been used to screen for this compound in other species.

EXPERIMENTAL

General experimental procedures. Salvinorin A (**1**) used for standard curve development was purified from *S. divinorum* leaf extracts and authenticated by nuclear magnetic resonance spectroscopy and by thin layer chromatographic (TLC) comparison with authentic **1** (Valdés *et al.*, 1994). All solvents used for extraction and chromatography were of high performance liquid chromatographic (HPLC) grade from Fisher Scientific (Fair Lawn, NJ, USA). Water used in HPLC mobile phase mixtures was distilled and subsequently filtered through a 0.22 μm membrane (Millipore Corp., Bedford, MA, USA).

Plant materials. Asexually propagated plants of *S. divinorum* used in establishing the HPLC methods were purchased from Logee's Greenhouses (Danielson, CT, USA) and multiplied by rooting additional cuttings in the S.B. Penick Experimental Greenhouse at the Philadelphia College of Pharmacy and Science. Voucher specimens were deposited in the herbarium of the Philadelphia College of Pharmacy and Science. Additional *S. divinorum* samples used for analysis were collected from established outdoor stands.

Extraction of samples. Plant tissues were lyophilized after harvest and ground to a homogeneous powder in a Wiley mill (no. 20 mesh). Samples (0.500 g) of lyophilized whole leaf were extracted in 125 mL round-bottom flasks by steeping in 25 mL of chloroform for 30 min. The extract was filtered and the filtrate set aside. The extraction flask and filtered solids were rinsed with an additional 15 mL of fresh chloroform. The filtrate from the rinse was then combined with the original filtrate and the resulting solution was evaporated to dryness with a rotary evaporator. The dry solids were redissolved in a mixture of 20.0 mL methanol and 5.0 mL acetone using sonication to assist in dissolving of all solid material.

Chromatography conditions. HPLC was performed using a Milton Roy HPLC system (Riviera, FL, USA), consisting of a Constametric 3000 Series isocratic pump, a Rheodyne injector (Rheodyne L.P., Cotati, CA, USA), and a Spectromonitor 3100 variable wavelength UV-VIS detector. The analogue detector output was acquired by an advanced computer interface (Dionex Corp., Sunnyvale, CA, USA), converted to a digital signal, and then processed by AI-450 Chromatography Automation Software (Dionex). The HPLC column was a Zorbax (MAC-MOD Analytical, Inc., Chadds Ford, PA, USA) 300 SB-C18 column (250 × 4.6 mm i.d.; 5 μm particle diameter; 300 Å average pore size). In order to protect the integrity of the analytical column, all analyses were performed with a coupled C-18 guard column. A mobile phase of acetonitrile:water (45:55) was used for all analyses at a flow-rate of 1.0 mL/min, and **1** was detected by UV absorption at 208 nm.

Composite standard curve. A standard curve for **1** was produced using a solution of 0.051 mg/mL of standard dissolved in the HPLC mobile phase. By varying injection size, six different amounts of salvinorin A (0.255, 0.510, 0.765, 1.02, 1.28 and 1.53 μg) were chromatographed: for each amount analysed, three injections were made.

In order to validate the HPLC method, three separate determinations of the standard curve were performed. The data collected for each amount for all three curves (i.e. nine data points per amount) were averaged and replotted to yield a composite standard curve for salvinorin A.

Quantification of salvinorin A in plant tissue samples. For each sample, 20 μL of the reconstituted methanol: acetone extract was injected into the HPLC and the area of the peak due to **1** was integrated. This peak area was used to calculate the amount of salvinorin A present in the tissue sample by applying the linear equation obtained from the composite standard curve.

RESULTS AND DISCUSSION

Under the isocratic chromatographic conditions, authentic salvinorin A (**1**) eluted in approximately 8.0–8.1 min (Fig. 1A). The analysis of the plant tissue extracts described below allowed rapid elution of polar compo-

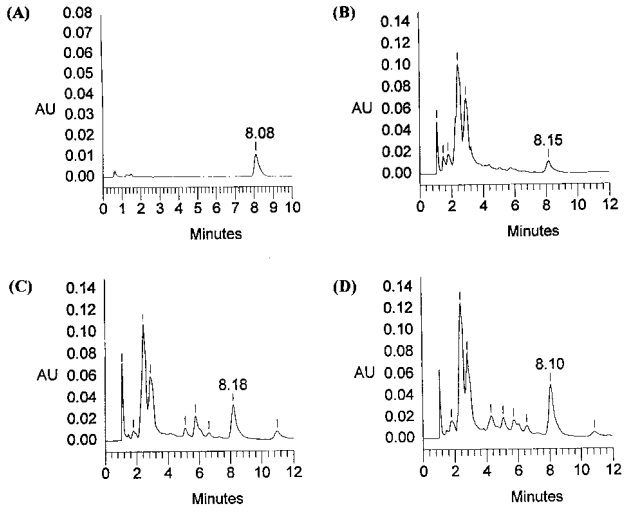

Figure 1. HPLC chromatograms of an authentic sample (0.255 μg) of salvinorin A (**A**), and of representative *Salvia divinorum* tissue extracts obtained from "Palatable" clone (Bret Blosser) (**B**), from Cerro Rabón clone (L. J. Valdés) (**C**), and from a seed grown plant DS03 (D. J. Siebert) (**D**). In each case the retention time of the peak representing salvinorin A is indicated. (For chromatographic protocol see Experimental section).

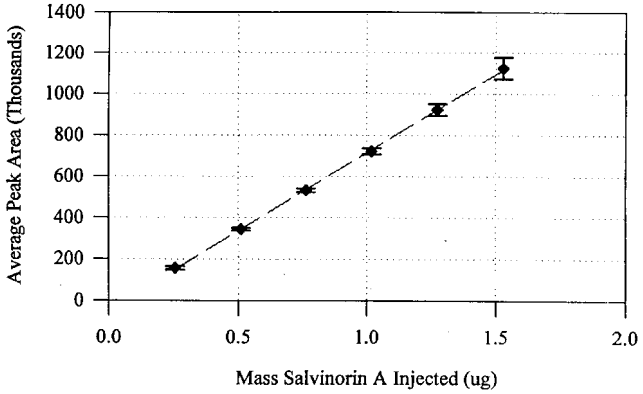

Figure 2. The composite standard calibration for the quantification of salvinorin A by HPLC (the error bars indicate ±1 standard deviation; the linear regression equation for the calibration curve is $y = 759{,}334x - 44{,}127$).

Table 1. Salvinorin A content of extracts of *S. divinorum*

Sample Description	Salvinorin A content (mg/g) dry wt.)
Wasson & Hofmann clones	3.70
	2.83
	2.76
	2.25
	1.94
"Palatable" clones	2.83
	2.32
	0.90
	0.89
Seed-grown plants	3.70
	3.29
	2.79
	2.78
	2.21
Cerro Rabón collection	2.61
Huatla de Jimenez collection	1.89
Clones uncertain	2.74
	2.39
	2.28
	1.91
Wasson & Hofmann stem	<0.63

nents, baseline resolution of the analyte of interest, and a short analysis time (for examples, see Fig. 1).

A composite standard curve for **1** was compiled from three individual determinations of the standard curve (Fig. 2). The correlation coefficients for these three standard curves were 0.9997, 0.9997 and 0.9967. The coefficient of variation among the three curves was 5.9%. The coefficients of variation for each injected amount across all three days ranged from 1.7 to 5.7%.

The peak identified as salvinorin A (**1**) from authentic standard solutions was observed in extracts of leaves and stem from *S. divinorum*. In order to examine the concentration range of **1** found in various populations of *S. divinorum*, 20 different samples of leaves were collected from cultivated plants in private collections as well as endemic populations of the plant in Oaxaca. Representative chromatograms for three different samples of *S. divinorum* are shown in Fig. 1 Estimations were based on the average of two separate injections. The determinations of the leaf content of salvinorin A showed a broad range of values ranging from 0.89 to 3.70 mg/g dry weight (Table 1). Stem material showed considerably lower values of **1** equivalent to approximately 4% of the level found in leaves.

Since the genetic heterogeneity of different populations of *S. divinorum* is unknown, it is not possible to attribute the different levels of **1** in these plants to any specific environmental or genetic conditions. Four separate collections of the plant which may represent distinct clones showed that variability in levels of **1** between clones is as great as the variability between plants of the same clone grown in different locations. These results are in contrast to earlier TLC analyses that suggested that there were no appreciable differences in levels of salvinorin A among plants grown in Michigan, Louisiana, or Mexico (Valdés, 1994). The Wasson and Hofmann clone samples showed a range of 1.94 to 3.70 mg/g of **1** with an average value of 2.69 mg/g (±0.67, $n = 5$). The "palatable" clone showed a range of 0.89 to 2.83 mg/g of **1** with an average of 1.73 mg/g (±0.99, $n = 4$). Two other samples from distinct collections made in Oaxaca contained 2.61 and 1.89 mg/g of salvinorin A.

An early report by Epling and Játiva (1962) showing that *S. divinorum* was most closely allied to *S. concolor* Lamb ex. Benth led us to screen this species for salvinorin A content. Ethnobotanical reports suggesting that *Coleus blumei* was similarly used as a psychotropic by the Mazatec (Wasson, 1962) caused us to screen one sample of this species as well for possible content of **1**. HPLC analysis of the leaf extracts of these two species did not show any salvinorin A present, nor was **1** found in leaf extracts of *S. blepharophylla, S. chiapensis, S. gregii* var. San Isidro, *S. leucantha, S. membranacea* or *S. recurva*.

In summary, application of a novel HPLC methodology to quantify salvinorin A in *S. divinorum* leaves established the typical levels of this compound in a number of samples of the plant. Further screening of additional sage species for **1** may help elucidate the origins and taxonomic position of *S. divinorum* within the genus *Salvia*. Additionally, this analytical assay can be utilized in screening for salvinorin A in other *S. divinorum* plant parts.

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