Toxichem Krimtech 2013;80(Special Issue):262

A convenient method for *Salvia divinorum* analysis and attempt to explain different effects based on the origin

Victoria Spencer[1], Esra Ergin[1], Heike Wollersen[1], Nico Erdmann[2]

[1]Universitätsklinikum Gießen, Institut für Rechtsmedizin, Frankfurter Straße 58, D-35392 Gießen, Germany
[2]RWTH Aachen, D–52062 Aachen

Abstract

Aim: *Salvia divinorum* is not commonly consumed in Europe due to its ability to cause threatening visions, whereas in other countries, like Iran, it is ranked as the second drug of choice among the youth. The reason for the rare abuses in Europe might be the frequently reported bad trips resulting in hysteric outbreaks or hysterical crying. In the Middle East these kinds of reports are very seldom which might be due to a different composition of the sold leaves. Our aim was to define a fast detection method of Salvinorin A, the active ingredient of the *Salvia divinorum* leaves, in blood and urine samples as well as Salvia leaves and to determine the difference in composition of sold Salvia leaves which could explain the putative less frightening effects.

Methods: Blood and urine samples from a Salvia consumer as well as the Salvia leaves obtained in Iran were analyzed via GC/MS and LC/MS-MS.

Results: In the blood sample Salvinorin B was determined as well as a trace of Salvinorin A. The urine samples contained Salvinorin B, which is also known to be the hydrolysis product of Salvinorin A. The extracted leaves contained Salvinorin A-F of which Salvinorin A is the only active compound. Next to the expected ingredients we also detected Cannabigerol, CBD (Cannabidiol), CBN (Cannabinol) and THC (Tetrahydrocannabinol) in the extracted leaves.

Conclusion: A LC/MS-MS method was developed for the detection of Salvinorin A including a simple liquid/liquid extraction preparation step to treat blood or urine samples as well as a method to analyze *Salvia divinorum* leaves by GC/MS. Herein special regards should be laid on the possibility that hemp compounds may be admixed into the salvia leaves, especially when the origin of the *Salvia divinorum* product is the Middle East.

1. Introduction

Salvia divinorum is a hallucinogenic plant naturally occurring in Oaxaca, Mexico and recently parts of California [1]. Known as Mazatec deviner's sage it was used by the Mazatec Indians for healing and religious rituals [2]. It is a member of the mint family Lamiaceae of which mint is the most popular member. The sage *Salvia divinorum* is the only sage with hallucinogenic properties [3]. The plant is characterized by an hallow, square stem and lilac blossoms. The usual height differs between one to two meters. The leaves, which are usually chewed or smoked, are easily obtainable through the internet, although the commercial leaves are usually increased in its potency by extraction.

Fig. 1. Salvia divinorum leaves as sold in Iran.

The only active ingredient of *Salvia divinorum*, Salvinorin A, possesses a high, selective affinity to κ-opioid receptors. It is the most potent natural occurring hallucinogen known [4]. Biological effects of the other naturally occurring Salvinorins (B-F) are not known although Salvinorin B is the deacetylated metabolite of Salvinorin A after a carboxylesterase catalyzed hydrolysis. The rate of hydrolysis was studied in rat plasma at 37°C and 25°C by Tsujikawa *et al.* (Figure 2) [5].

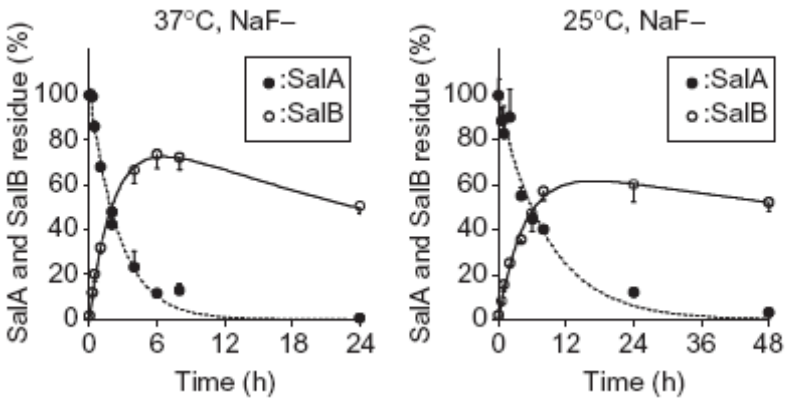


Fig. 2. Hydrolysis of Salvinorin A in rat plasma (Source: Tsujikawa et al. 2009).

There are several reports in the literature of *Salvia divinorum* abuses as recreational drug. [6] The effects of Salvia abuse is described to be psychoactive and spiritual including increased visualizations, vivid colors and shapes, distortions of bodies and objects, dysphory, visions of two-dimensional surfaces, separation of body and spirit ("out-of-body-experiences"), coordination problems, dizziness and speech problems [1,6]. Trips usually last between 20 min to one hour. On the one hand videos in the internet reveal that the amounts of bad trips resulting in hysteric outbreaks or hysterical crying seem to be extraordinary high.

On the other hand the effects of *Salvia divinorum* abuse in the Middle East, namely Iran, are described to be relaxing, comforting and soothing. The trips are only disturbed by short outbursts of uncontrollable laughter and last only a few minutes.

These differences seem to affect the popularity of *Salvia divinorum* abuse as well. Whereas in Europe cases of abuse are not common, in Iran *Salvia divinorum* emerged as the second most popular drug among young people. The good accessibility of *Salvia divi*norum leaves via the internet opens up the possibility that cases of Salvia abuse will increase in the Western countries too as the leaves consumed in Iran could spread quickly. For this reason we aimed to determine the difference of Salvia leaves consumed in Europe and accessed in Iran and to develop a convenient and fast method to analyze blood, urine and leaf samples.

2. Material and Methods

2.1. Materials

Ten mL of urine, 3 blood samples of a *Salvia divinorum* consumer in Iran as well as a methanolic extract of the leaves were accessed. The samples were taken approximately 12 hours after the consumption. A video documentation was made of the consumption, showing the inhalation and the effect of the abuse.

Salvinorin A and Salvinorin B purchased from LGC Standards were used as reference substances. Warfarin was used as internal standard.

2.2. Sample Preparation

The urine sample was incubated with β-glucuronidase for one hour at 55°C followed by a pH adjustment to pH 6 by the addition of phosphate buffer. After extraction with 1-chlorobutane, the sample was analyzed by LC-MS/MS. The blood sample was treated with phosphate-buffer to adjust the pH to 6 followed by the extraction with 1-chlorobutane and analysis via LC-MS/MS. The methanolic extract of the leaves was directly analyzed by GC-MS.

2.3. LC-MS/MS and GC-MS Parameters

For LC-MS/MS analysis the AB Sciex API 4000 QTrap was used employing a PFP column. With a flow rate of 750 µL/min the following gradient was used employing 0.2 % ammonium formate, 0.2 % formic acid as solvent A and ACN as solvent B: 10 % B increase to 90 % B at 13.3 %/min, 90 % B for 1.5 min, decrease to 10 % B at 160 %/min and 10 % B for 1 min.

For GC-MS analysis an Agilent 6850 / 5975C was used. As gas Helium was used. The samples were injected at an injectiortemperature of 250°C and the detectortemperature was set to 280 °C. The following temperature settings were given during the run: Initialtemperature started with 80 °C and rose up to 280 °C with a rate of 20 °C per minute. As column a HP 5 MS column was used with the length of 12m and I.D of 0.2 mm.

2.4. Calibration and Recovery

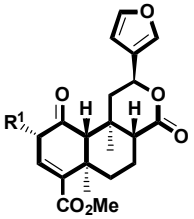
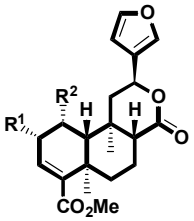
Salvinorin A and B were calibrated in the range 2-30 ng/mL in urine and blood using warfarin as interal standard. The correlation factor of Salvinorin A in urine was 0.9994 in blood 0.9987 for Salvinorin B in urine it was 0.9997 in blood 0.9995. The recovery of Salvinorin A in urine was 107.1 % in serum it was 111.8 %. The recovery for Salvinorin B in urine was 93.8 % in blood it was 93.1 %.

3. Results and Discussion

The analysis of the blood sample lead to the detection of traces of Salvinorin A, 2.2 ng/mL Salvinorin B and traces of THC-COOH. The low amounts of Salvinorin A and B in the blood sample could be explained by the rather long gap between consumption and the time when the samples were taken, although there are no data available which could indicate the range of Salvinorin A concentrations in blood samples. The urine sample contained Salvinorin B which can be formed by the hydrolysis of Salvinorin A as outlined above as well as THC-COOH. The Salvia leaves contained Salvinorin A-F as well as the cannabinoids cannabigerole, THC, CBN and CBD.

The detection of THC-COOH in the blood and urine sample is in line with the detection of Cannabigerole, THC, CBN and CBD in the leaves that was analyzed, proofing that the *Salvia divinorum* samples sold in Iran already have hemp compounds admixed. The solemn detection of THC-COOH might be due to the low concentration of cannabinoids admixed to the Salvia leaves as well as the time gap before gaining the sample. The presence of cannabinoids within the Iranian Salvia samples could lead to a simultaneous antagonisation of the CB1 and CB2 receptors as well activation of the κ-opioid receptor which would explain the different effects of Savia divinorum misconduct in Iran compared to reports from Europe and northern America.

Tab. 1. Results of the LC-MS experiments.

Name	Structure	R^1	R^2
Salvinorin A		–OAc	/
Salvinorin B		–OH	/
Salvinorin C		–OAc	–OAc
Salvinorin D		–OAc	–OH
Salvinorin E		–OH	–OAc
Salvinorin F		–OH	–OH

4. Conclusions

In summary we have presented a fast and convenient method to analyze blood and urine samples to detect Salvinorin A and B. Using this method, we analyzed a blood and urine sample of a *Salvia divinorum* consumer from Iran where we could detect traces of Salvinorin A and 2.2 ng/mL of Salvinorin B in the blood sample as well as traces of Salvinorin B in the urine sample. Furthermore we could show that *Salvia divinorum* leaves consumed in Iran have cannabinoids admixed in the sold samples explaining the milder and shorter effect of *Salvia divinorum* abuse in Iran compared to Europe and northern America. These results in hand, the convenient accessibility of *Salvia divinorum* via the internet as well as the comparably low prices place a great risk that *Salvia divinorum* abuse could rapidly increase within the next years.

5. References

[1] Lozama A, Cunningham CW, Caspers MJ, Douglas JT, Dersch CM, Rothman RB, Prisinzano TE. Opioid receptor probes derived from cycloaddition of the hallucinogen natural product Salvinorin Am J Nat Prod 2011;74:718–726.

[2] Siebert DJ. *Salvia divinorum* and Salvinorin A: new pharmacological findings. J Enthnopharmacol 1994;43:53-56.

[3] Valdés III LJ, Butler WM, Hatfield GM, Paul AG, Koreeda M. Divinorin A, a psychotropic terpenoid and divinorin B from the hallucinogenic Mexican mint *Salvia divinorum*. Org Chem 1983;49:4716-4720.

[4] Sheffler DJ, Roth BL. Salvinorin A: the "magic mint" hallucinogen finds a molecular target in the kappa opioid receptor. Trends Pharmacol Sci 2003;24:107-109.

[5] Tsujikawa K, Kuwayama K, Miyaguchi H, Kanamori T, Iwata YT, Inoue H. In vitro stability and metabolism of Salvinorin A in rat plasma. Xenobiotica 2009;39:391–398.

[6] Giroud C, Felber F, Augsburger M, Horisberger B, Rivier L, Mangin P. *Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland. Forensic Sci Int 2000;112:143-150.