Salvinorin A: Forensic and Toxicological Aspects

Evgeny Volgram, Leonid Khodasevitch, Tatiana Khodasevitch, Astrida Stirna

Riga Centre of Psychiatry and Addiction Medicine, Latvia

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Introduction. An active ingredient of Mexican mint (*Salvia divinorum*, Epling & Jativa) salvinorin A is one of the most potent naturally occurring psychoactive substances known. It is active at doses as low as 200 µcg and is selective kappa opioid receptor agonist. Leaves of *Salvia divinorum* can be smoked, chewed, brewed and ingested as tea. When converted to liquid extract, it can be also vaporized and inhaled. Immediately after the ingestion, abusers experience vivid hallucinations, travelling through time and space. Since 2009, *Salvia divinorum* and salvinorin A have been included in the List of controlled drugs in Latvia. Their use and abuse is illegal and punishable. Toxicological analysis is complicated due to low concentrations and short elimination half-life.

The aim, materials and methods. The aim of our study was to elaborate simple method of identification of salvinorin A in urine by means of solid-phase extraction and GC/MS. Salvinorin A (standard) was from Ipomed, organic solvents were analytical grade. Solid-phase extraction columns ChemElut 1020 (Varian, Harbor city, CA, USA) were used as part of the research process. The GC/MS was performed using Agilent 6890 N gas chromatograph with 5975 mass spectrometer (splitless mode) and helium used as a carrier gas (1.0 ml/min). The oven temperature was held for 2 min. at 80°C following the injection and programmed at 280°C at the rate 30°C/min. The temperature was held at 280°C for 10 min. The detector was operated in electron impact mode (EI) at 70-eV. Preliminary toxicological screening was performed by means Biochip Array Technology (Randox, UK), using Evidence analyzer (sensitivity 0.06 ng/ml, precision 5.3%). Confirmation of screening results was performed by GC/MS and thin-layer chromatography.

10 ml of urine sample, 1 ml of phosphate buffer (pH 6.86) were applied to a ChemElut 1020 column. After 5 min. salvinorin A was eluted with 2 x 15 ml chloroform-isopropanol (9 : 1). The eluent was evaporated to dryness in N_2 atmosphere, dissolved in 100 µL ethyl acetate and 1 µL was injected into a gas chromatograph. Thin-layer chromatography of eluate was performed on plates Silica gel F_{254} (Merck) in a solvent system n-hexane-ethyl acetate (3 : 1). Salvinorin A was visualized by spraying the plates with vanillin reagent (1.0 g vanillin, 0.3 ml of conc.sulfuric acid, 50 ml 96% ethanol) and slowly heated at 110 °C. The salvinorin A reacts with this chromogenic reagent to produce pinkish-purple spot on the plates.

Results. Preliminary toxicological screening results were confirmed by the mentioned methods. Salvinorin A (Rf 0.42) was identified on TLC plates.

The retention time (GC/MS) of salvinorin A was 10.702 min.

The 70-eV mass spectrum of salvinorin A (m/z): 44, 77, 94, 107, 121, 147, 166, 192, 234, 252, 273, 294, 318, 343, 359, 393, 404, 432 (M+).

Conclusions.

1. Biochip Array Technology (Randox) is an effective tool for preliminary toxicological screening of salvinorin A.
2. Salvinorin A can be identified by means gas chromatography-mass spectrometry and thin-layer chromatography.
3. *Salvia divinorum* and salvinorin A are of great toxicological and forensic significance.