LC-PAD Determination of Mescaline in Cactus ''Peyote'' (*Lophophora williamsii*)

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Abstract

A reversed-phase column liquid chromatographic method for the separation and quantification of mescaline present in ''peyote'' has been developed using a Symmetry C_{18} column and isocratic profile. The method can be utilised for the quantitative determination of other alkaloids. This method is economical in terms of the time taken and the amount of solvent used for each analysis. The validity of the method with respect to analysis was confirmed by comparing the UV spectra of peak with the reference compound (mescaline) using a photodiode array detector. The assay method described is simple, rapid and accurate, and may form part of future drug authentication protocols.

Keywords

Column liquid chromatography
HPLC-DAD
Validation
Mescaline and peyote
Lophophora williamsii

Introduction

Lophophora williamsii (Lem. Ex Salm-Dyck) Coult (*Cactacae*) better known as peyote, is a small, spineless cactus whose native region extends along the southwestern United States [1], it is a controlled substance, illegal in all 50 states [2]. Both peyote and mescaline are listed in the CSA as Schedule I hallucinogens. Mescaline is listed as a Schedule III controlled substance under the Canadian Controlled Drugs and Substances Act, but peyote is specifically exempt.

Peyote contains a large spectrum of alkaloids, the principal of which is mescaline (Fig. 1). Although chemically unrelated to lysergic acid diethyl amide (LSD), the hallucinogenic effects of mescaline are similar to that of LSD, although they are longer lasting [3]. Typical hallucinogenic doses range from 200 to 500 mg (equivalent to roughly 5 g of dried peyote) of the mescaline [4].

In the literature a number of methods for the analysis of peyote alkaloids have been described: TLC [5–9]; GC with different detectors [3, 10–13]; Ion-interaction LC [14] and LC-EI-MS [13], but there are no references about LC-PAD methods for their detection and quantification.

The aim of the present study was to analyse mescaline using a newly and rapid developed LC-photodiode array detection (PAD) method that offered clear advantages as compared with the previously described methods. Further, the developed method was validated on according to ICH guidelines [15]. This method can be employed for the quantification of mescaline in cactus peyote.

Experimental

Plant Material and Reagents

D. Jose Antonio de Arístegui supplied the peyote sample analysed. Dr. R. Y. Cavero authenticated the voucher speci-

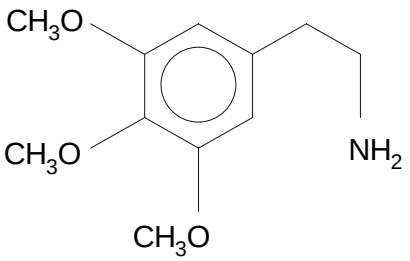

Fig. 1. Structure of mescaline

men deposited in the PAMP Herbarium (No. 20068) of the Faculty of Sciences (University of Navarra). Acetonitrile and phosphoric acid were of LC grade (Merck, Darmstadt, Germany). Distilled water was deionised, double distilled using a quartz distillation unit and filtered through a 0.45 μm filter before use. Mescaline (**1**) was purchased from Sigma (St Louis, MA, USA).

Preparation of Standard Solutions

Standard solutions were prepared dissolving mescaline (5 mg mL^{-1}) in methanol, since the drug is freely soluble in this solvent. Further dilutions were prepared by transferring 0.5–2.5 mL aliquots of stock solution to 5 mL volumetric flasks.

Preparation of Sample Solutions

The plant material was shade dried and powdered coarsely before extraction. In order to eliminate possible interfering with lipids the peyote sample (1 g) was pre-extracted with diethylether (four times × 5 mL; 24 h shaking) at room temperature. After drying the extraction thimble, the sample was extracted with methanol-concentrated ammonia solution (99:1) (five times × 5 mL; 24 h shaking) at room temperature. This solution was concentrated at reduced temperature (40 °C) on a rotary evaporator (Büchi, Labortechnik AC, Flawil, Switzerland) and redissolved in methanol yielding a volume of 10.0 mL. Two millilitres of this solution were filtered through an LC filter and placed in an autosampler vial of 2 mL.

Apparatus and Chromatographic Conditions

Analysis was performed using a liquid chromatograph with Waters (Milford, MA, USA) pumps (Waters 515) equipped with online degasser, a Waters PCM (pump control module), a Waters 2996 photodiode array detector and Waters Empower software. Separation was carried out using a Nova-Pak C-18 (150 × 3.9 mm, i.d., 4 μm).

Elution was carried out in an isocratic solvent system at a temperature of 25 °C, a flow rate of 1 mL min^{-1} and a run time of 8 min. The mobile phase consisted of a mixture of water (pH = 2): acetonitrile (90:10 *w*/*w*). The injection volume was 20 μL, the detection wavelength was 268 nm, whilst PDA scans were measured using a wave step of 2 nm.

Method Validation

The precision of peak area responses for six replicate injections of a standard solution of mescaline was determined. Repeatability of the method was affirmed by multiple measurements ($n = 6$) of mescaline under the same analytical and laboratory conditions and was expressed as %RSD. Variability of the method was studied by analysing aliquots of standard solutions of mescaline on the same day and on different days (inter-day precision) and the results were expressed as %RSD.

Accuracy of the method was tested by performing the recovery studies at three levels. To 1 g of powered sample of *L. williamsii* known amounts of mescaline (50, 100 and 150 mg) were added, extracted and estimated as described above. The percentage recovery as well as average percentage recovery was calculated.

Linearity of the method was verified by analysing in triplicate, three solutions of mescaline in the range of 5–200 μg mL^{-1}. Chromatographic signals were fitted to linear graphs using least square regression.

Results and Discussion

We report an LC method for quantification of the mescaline content in cactus ''peyote''. Under the conditions used the most successful solvent system consisted of a mixture of water (pH = 2) and acetonitrile (90:10 *w*/*w*) which gave the best resolution of mescaline in the presence of other compounds in the extract (Fig. 2). Peak identity of mescaline in the sample extracts was confirmed by overlaying its UV absorption spectrum with that of the standard mescaline. Purity of mescaline in the sample extract was confirmed by comparing the absorption spectra at start middle and end position of the peak.

The method was validated in terms of precision, repeatability, accuracy and linearity. The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). The intra-day coefficient of variation was 0.4–0.7% for mescaline, and the inter-day coefficient was 0.6–0.8%. Results indicate good repeatability and low inter-day variability (RSD maximum 1.0%). Good linearity was observed over the concentration range of 5–200 μg mL^{-1}, with a correlation coefficient of 0.9992 and the linear regression equation $y = 10675x + 65642$. The percentage recovery at three different levels was found to be 99.9 ± 0.898% and was considered highly satisfactory. The limits of detection (LOD) and the limits of quantification (LLOQ) were 0.28 and 1.40 μg of mescaline, respectively.

Application of the LC Method

Five percent of the world's total population over the age of 15 are using illicit drugs, and several hundred thousands of drug intoxications are reported each year in the western world alone. Forensic scientists and law enforcement agencies require screening methods used in forensic intoxication cases to be highly sensitive and fast. Considering the short runtime, this validated method offers a

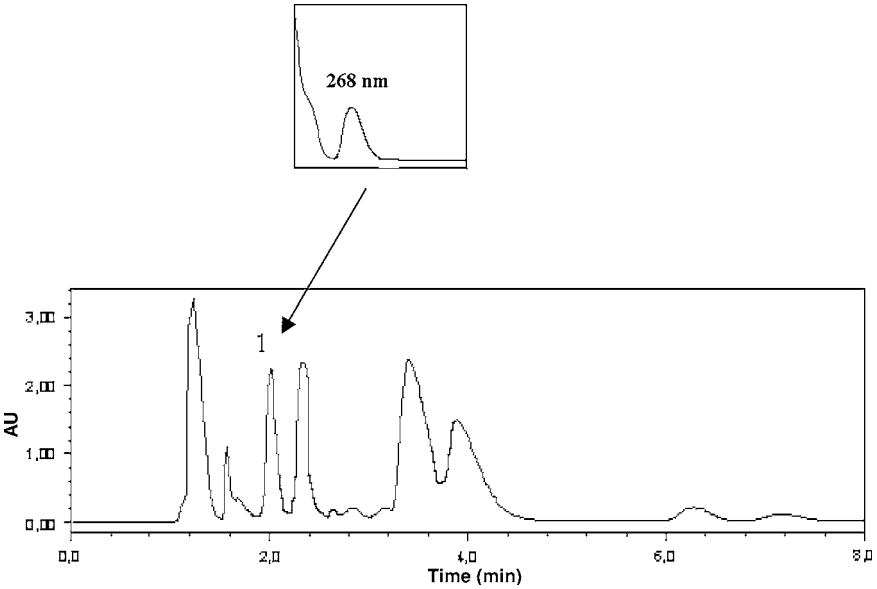

Fig. 2. LC profile of the peyote extract (**1** = mescaline)

clear improvement over previously published methods for the rapid qualitative and quantitative analysis of mescaline in toxically determinations.

Conclusion

In conclusion, this report is the first using LC-PAD for a direct quantification of the mescaline content in peyote cactus. The isocratic LC method described is rapid, and provides a good baseline separation of mescaline at 2.07 min retention time. Also the good recovery, linearity, precision and accuracy, as well as the excellent LOD and LLOQ values, make this method suitable as a standard analytical procedure.

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