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DETERMINATION OF MESCALINE IN HALLUCINOGENIC *CACTACEAE* BY ION-INTERACTION HPLC.

Key words: mescaline, ion-interaction HPLC, peyote, hallucinogenic *cactaceae*.

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ABSTRACT

An ion-interaction HPLC method is developed for the determination of mescaline in some *Cactaceae* species, namely *Gymnocactus beguinii, Echinocactus polycephalus, Coriphanta radians, Coriphanta scolymoides, Coriphanta palmeri, Lophophora williamsii and Trichocereus pachanoi* .

Mescaline is characterized by hallucinogenic properties and can be prescribed as a psychodrug.

The method makes use of a C18-reversed-phase as the stationary phase and of a 5.0 mM aqueous solution of octylamine o-phosphate as the mobile phase, with spectrophotometric detection at 230 nm.

The method is sensitive (detection limit of 35 μg/L) and matrix interference-free.

The pretreatment of the sample is performed by grinding the fresh cactus and extracting the jelly pulp obtained either with a methanolic-ammonial solution or an aqueous solution buffered at pH 4.0 (phosphate buffer).

The average amounts of mescaline found in *Lophophora williamsii* and in *Trichocereus pachanoi* were respectively 2.55 mg and 3.10 mg/g of fresh cactus.

INTRODUCTION

Psychodrugs are usefully employed in the therapy of different diseases like depression, bulimia, schizophrenia, anxiety[1]. The biggest problem is a correct therapeutic dosology which at the same time is efficaceous and guaranted free from undesirable side-effects.

Recent therapeutic trends suggest the use of naturally-occurring psychotropic species, as those present in some biological matrices.

The hallucinogenic properties of *Lophophora williamsii*, more generally known as cactus peyote or "mescal buttons", were already known in Mexico in the years B.C.[2]. Peyote (or pellote, challote, devil's root) was believed to give strength in fighting, to avoid fear, hunger and thirst and to protect from every kind of danger. *Lophophora williamsii* grows wild in South America in dry areas, cliffs and rocky slopes but it can be grown practically anywhere. The major psychotropic principle seems to be mescaline (3,4,5-trimethoxy-β-phenetylamine) whose structure (see fig.1) is similar to "ecstasy" drugs produced by synthesis[3].

While large doses of mescaline (20-60 mg/Kg) produce in humans vasodilatation, drop in blood pressure, bradycardia, visual and auditory hallucination, disturbance of personality..., studies performed on cats, rabbits, mice and frogs showed that doses around 4mg/kg can enhance the effect of serotonine in the absence of simultaneous undesired side-effects. The habitual use of peyote on humans does not seem to produce habituation or accrued dependence[2].

For the quantitation of mescaline in biological matrices, the literature reports a thin-layer separation[4], a gas chromatographic[5] and two reversed-phase HPLC methods[3,6,7], which both make use of hydroorganic mobile phases with added ion-pair reagents.

Literature information together with the presence in the molecular structure of mescaline of a protonisable nitrogen atom, suggested the development of an ion-

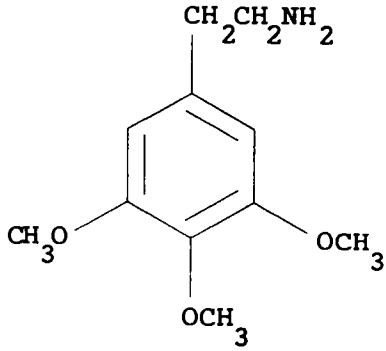

Figure 1. Molecular structure of mescaline.

interaction HPLC method which makes use of a mobile phase of an aqueous solution of octylamine-o-phosphate. The method is matrix interference-free and allows the achievement of a detection limit of 35 μg/L (spectrophotometric detection at 230 nm). It was employed for the identification and determination of mescaline in some *Cactaceae* species grown in Italy.

EXPERIMENTAL

Apparatus

The analyses were carried out with a Merck-Hitachi (Tokyo, Japan) Lichrograph Chromatograph Model L-600, interfaced with a UV-visible detector model L-4200 .

A Metrohm 654 pH-meter equipped with a combined glass-calomel electrode was employed for the pH measurements and a Hitachi (Tokyo, Japan) mod.150-20 spectrophotometer for the absorbance measurements.

Reagents

Ultrapure water from Millipore Milli-Q (Millipore Corporation, Bedford, MA, USA) was used for the preparation of all the solutions.

Octylamine was Fluka (Buchs, Switzerland) analytical grade chemical; mescaline was purchased from Sigma Chemicals (U.K) following authorization obtained from the Italian Ministry of Health to import it for research purposes.

All the other reagents were C.Erba (Milano, Italy) analytical grade reagents.

Chromatographic Conditions

A 5 μm ODS-2 Spherisorb Phase Separations column fully-endcapped 250.0 x 4.6 mm with a carbon load of 12% (0.5 mmol/g), together with a 15.0 x 4.6 mm LiChrospher RP-18 5 μm guard pre-column was used. The mobile phase was an aqueous 5.00 mM solution of octylamine brought to a pH value of 6.4±0.2 by the addition of o-phosphoric acid . The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained; a minimum of 1 h was necessary. Inter-day repeatibility was always within 2%; intra-day reproducibility for different mobile phase preparation was within 6%. After use, the stationary phase was washed by flowing water (1.0 mL/min for 15 min), a 50/50 (V/V) water/methanol mixture (1.0 mL/min for 15 min), and methanol (1.0 mL/min for 5 min).

These conditions have been already employed in previous methods devoted to the separation of anions and amines[8,9].

Standard Solution and Sample Preparation.

Standard solution: 500 mg/L mescaline standard solution was prepared in Millipore ultrapure water and diluted as required.

Extraction procedure: amounts of fresh cactus around 5-10 g were ground. 1.0 g of the jelly pulp obtained was then extracted with 50.0 mL of reagent. The use of two reagents was compared: a) a mixture 99:1 of methanol and 25% NH_4OH and b) ultrapure water phosphate buffered at pH 4.0. The extracts were sonicated for 15 min and then centrifuged at 3600 g for 30 min. The liquid phase was separated, filtered (0.20 μm), diluted as required and injected into the HPLC system. The

chromatographic analysis of successive extracts of the solid residue did not show detectable amounts of mescaline, indicating that the extraction can be considered to be significantly complete in the first step. Before HPLC analysis, the extracts of *Lophophora williamsii* and *Trichocereus pachanoi* were diluted 1/50 L.C. with ultrapure water, while for the other *Cactaceae* investigated, due to the their very much lower mescaline content, dilutions of 1/2 and 1/5 L.C. were performed: the analysis result was therefore more affected by matrix interference and reproducibility was lower.

RESULTS

Standard Separation

Figure 2A shows the separation of a 0.75 mg/L standard solution of mescaline under the following optimized conditions: C18-reversed-phase as the stationary phase; 5.00 mM aqueous solution of octylamine (brought to pH 6.4 with o-phosphoric acid) as the mobile phase; spectrophotometric detection at 230 nm [$\epsilon_{mescaline} = (7.66 \pm 0.10) \times 10^3$ L mol^{-1} cm^{-1}].

The calibration plot constructed for mescaline standard solutions at concentrations ranging between 0.1 and 1.5 mg/L could be fitted with a straight line with coefficient of determination $r^2 > 0.99$. The detection limit evaluated from sensitivity data and for a signal to noise ratio =3 was 35 μg/L.

Sample Extraction

Preliminary experiments were performed for *Echinocactus polycephalus* cactus which naturally does not contain detectable amounts of mescaline. 0.20 mg, 1.0 mg and 2.0 mg of mescaline were respectively added to 1.0 g of the fresh cactus jelly pulp obtained after grinding and percentage recovery yields evaluated. Two extraction procedures were compared, respectively performed a) on fresh cactus without any previous defatting step and b) on liophilized cactus previously defatted

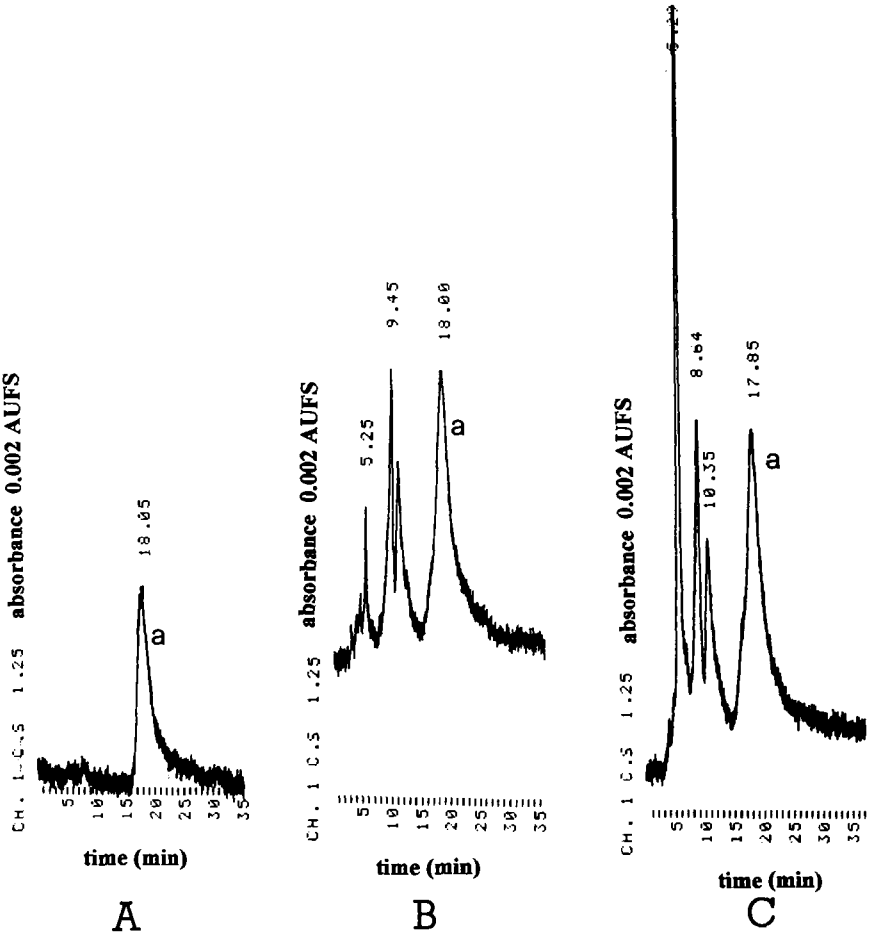

Figure 2. Chromatograms recorded for:
A) standard mescaline solution 0.75 mg/L.
B) *Lophophora williamsii* cactus: aqueous phosphate buffer extract diluted 1/50 V/V.
C) *Lophophora williamsii* cactus: methanolic (99%) + 25% ammonium hydroxide (1%) extract diluted 1:50 V/V.
Conditions:
Stationary phase: 5μm ODS-2 Spherisorb Phase Separation column fully-encapped.
Mobile phase: aqueous 5.00 mM solution of octylamine pH 6.4 for o-phosphoric acid.
Spectrophotometric detection: 230 nm.
Flow rate: 1.0 mL/min. 100 μL injected.

with ethyl-ether as described in literature[6,7]. As the extracting reagents the use of methanolic ammonia solution and of an aqueous buffered solution was compared as described in the experimental section. The extraction process carried out without the previous defatting step gave percent yields greater than 90% and that for defatted liophilized specimens was around 78%. The extracting power of the two reagents was comparable within 10%, being generally higher for the methanolic solution.

***Cactaceae* Analysis**

Different cactus specimens were analyzed, namely: *Gymnocactus beguinii, Echinocactus polycephalus, Coriphanta radians, Coriphanta scolymoides, Coriphanta palmeri, Lophophora williamsii, Trichocereus pachanoi* .

Fig. 2 shows, as an example, the results obtained in the analysis of *Lophophora williamsi* under the optimized chromatographic conditions and after extraction with aqueous phosphate buffer (fig. 2B) and with methanolic ammonium hydroxide (fig. 2C). In both cases the peak of mescaline is free from interference from the other components of the matrix.

Also the sensitivity is comparable for the two extracts. In the two chromatograms a difference can be observed concerning the peak at 5.29 min which was identified as belonging to serotonine, which shows a greater distribution coefficient in the aqueous extract.

Similar results were obtained for *Trichocereus pachanoi,* as shown in figure 3 A (aqueous extract) and 3 B (methanolic extract).

For both aqueous and methanolic extracts, the quantitative analysis was performed by standard addition method and average data were considered.

Fig.4 shows, as an example, the standard addition plot (peak height in relative units, as given by the integrator, vs. concentration) for the methanolic extract (diluted 1/50 V/V) of a *Trichocereus pachanoi* specimen. In order to evaluate an average content, three specimens of *Trichocereus pachanoi* and two of

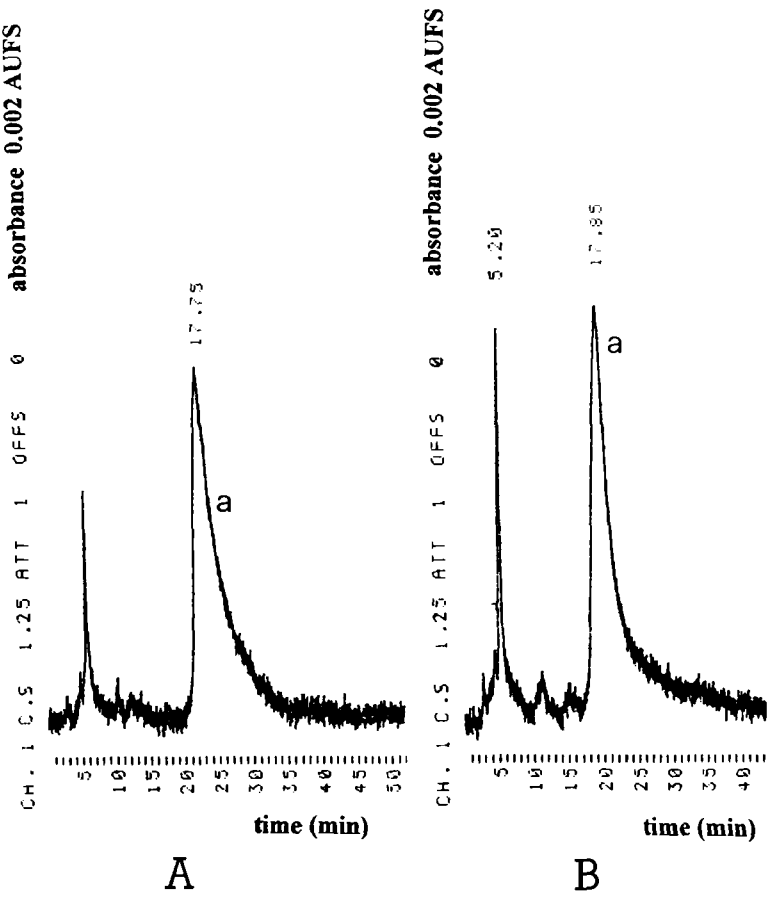

Figure 3.
Chromatograms recorded for:
A) *Trichocereus pachanoi* cactus: aqueous phosphate buffer extract diluted 1:50 V/V.
B) *Trichocereus pachanoi* cactus: methanolic (99%) + 25% ammonium hydroxide (1%) extract diluted 1/50 V/V.
Conditions: as in figure 2.

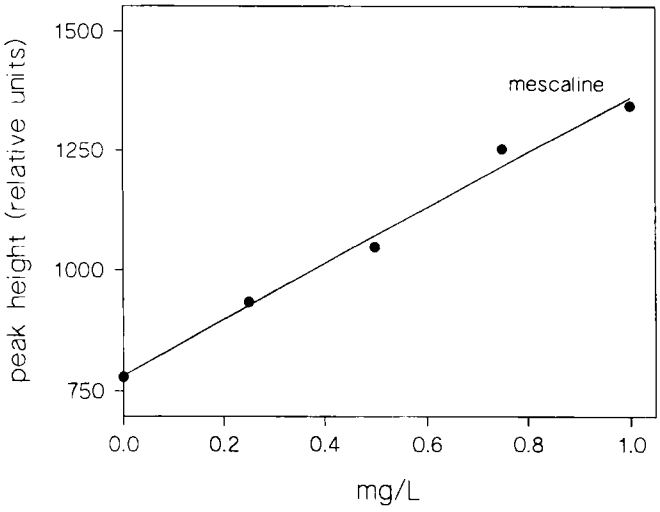

Figure 4.
Typical standard addition plot (peak height vs standard concentrations) for mescaline determination in a methanolic extract of a *Trichocereus pachanoi* specimen.

Lophophora williamsii were analyzed. For the other *Cactaceae* which contain negligible amounts of mescaline, the analysis was performed for only one specimen.

The amounts found in *Gymnocactus beguinii* and *Coryphanta scolymoides* can be evaluated as between 4 and 12 μg/g of fresh cactus. Quantities lower than detection limit are present in *Echinocactus polycephalus*. Relevant amounts of mescaline were found as expected for *Lophophora williamsii* and *Trichocereus pachanoi*. The data are given as the average calculated from the results obtained for the specimens investigated and for fresh cactus and respectively are: in *Lophophora williamsii* 2.55 mg/g of mescaline and for *Trichocereus pachanoi* 3.10 mg/g.

CONCLUSION

Literature data[3] report a mescaline content in *Lophophora williamsii* ranging

between 0.68 and 1.01% for dry weight and reaching 6% for mescal buttons from Mexico[2]. The data found for *Trichocereus pachanoi* are around 2%[3] .
The average mescaline concentrations we found (with respect to dry weight) are around 1.75% in *Lophophora williamsii* and 2.06% for *Trichocereus pachanoi*. The lower average we found for *Lophophora williamsii* with respect to Mexico species can be very likely explained when considering the low age of the specimens we studied, together with the habitat in which they were grown (in Italy, in pot), so different in weather and soil from the native one.

With respect to the new method proposed here, it shows a good sensitivity and an exceptional selectivity towards the other matrix components. Furthermore it does not require complex pretreatment procedures. In particular the extraction in aqueous medium can be of particular interest for a possible use of native mescaline as a psychodrug.

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REFERENCES

1. R. Brenneisen, S. Geisshusler, *Pharm. Acta Helv.*, **60**, 290 (1985).
2. G.J. Kapadia, M.B.E. Fayez, *J. Pharm. Sci.*, **59**, 1699 (1970).
3. H.J. Helmlin, R. Brenneisen, *J. Chromatogr.*, **593**, 87 (1992).
4. J. Lundstrom, S. Agurell, *J. Chromatogr.*, **30**, 271 (1967).
5. J. Lundstrom, S. Agurell, *J. Chromatogr.*, **36,** 105 (1968).

6. H.J. Helmlin, R. Brenneisen, *J. Chromatogr.*, **623**, 381 (1992).
7. M.J. Knox, D. Clark, S.O. Link, *J. Chromatogr.*, **265**, 357 (1983).
8. E. Marengo, M.C. Gennaro, C. Abrigo, *Anal.Chemistry*, **64**, 1885 (1992).
9. M.C. Gennaro, D. Giacosa, *J. Liquid Chromatogr.*, **19**, 149 (1996).

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