İstanbul Ecz., Fak. Mec.
32 (1998)

J. Fac. Pharm. İstanbul
32 (1998)

SPECTROPHOTOMETRIC DETERMINATION OF AMANTADINE HYDROCHLORIDE IN CAPSULES

A.GÜRSOY*, S.ÖZKIRIMLI*, O.ERSOY**

SUMMARY

Amantadine HCl is used as an antiviral and an antiparkinson drug. We described spectrophotometric determination of amantadine HCl by derivatization with sodium 1,2-naphtoquinone-4-sulfonate in aqueous medium. The structure of the derivative was determined by IR, H^1-NMR and mass spectral methods. The derivative was obtained quantitatively at pH 10 and 60 °C in 30 minutes and showed maximum absorption at 451 nm. The calibration curve obeys Beers's Law in the 5-25 µg/ml range. The proposed method was applied to the determination of amantadine HCl in pharmaceutical dosage form (capsule). The results were statistically compared with USP XXIII procedure.

ÖZET

Amantadine HCl antiviral ve antiparkinson etkili bir ilaçtır. Bu çalışmada amantadine HCl in 1,2-naftokinon-4-sulfonik asit sodyum tuzu ile oluşturduğu türev yardımı ile spektrofotometrik miktar tayini geliştirilmiştir. Reaksiyon sulu ortamda, pH 10 da, 60 °C da ve 30 dakikada tamamlanmaktadır. Oluşan türevin maksimum absorpsiyonu 451 nm'de gözlenmiştir. Ölçü eğrisi 5-25 µg/ml arasında doğrusaldır. Geliştirilen yöntem amantadin HCl içeren kapsüllere uygulanmış ve sonuçlar USP XXIII yöntemi ile istatistik olarak kıyaslanmıştır.

Keywords: Amantadine HCl, spectrophotometric determination, 1,2-naphtoquinone-4-sulphonic acid.

* University of Istanbul Faculty of Pharmacy Pharmaceutical Chemistry Dept. 34452 Beyazıt/İSTANBUL
** Refik Saydam Hygiene Centre Istanbul Regional Institute.

INTRODUCTION

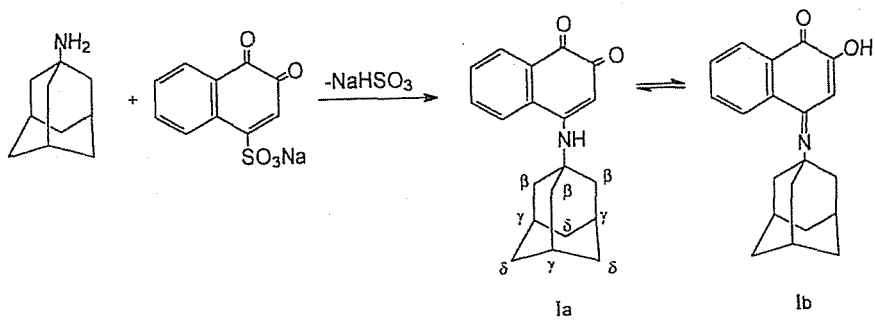
Amantadine HCl, (A) chemically 1-aminotricyclo[3.3.1.1.^{3,7}]decane HCl, is used in medicine due to its antiviral and antiparkinson activity.

Several methods have been published for the assay of the drug including GC (1, 2), HPLC (3, 4), GC-MS (5). There are also some reports on the spectrophotometric determination of amantadine HCl (6-8).

We described spectrophotometric determination of amantadine by derivatization with sodium 1,2-naphtoquinone-4-sulfonate (NQS) in aqueous medium.

RESULTS AND DISCUSSION

The proposed method is based on the reaction of A with NQS which gives a colored stable product (Scheme I).

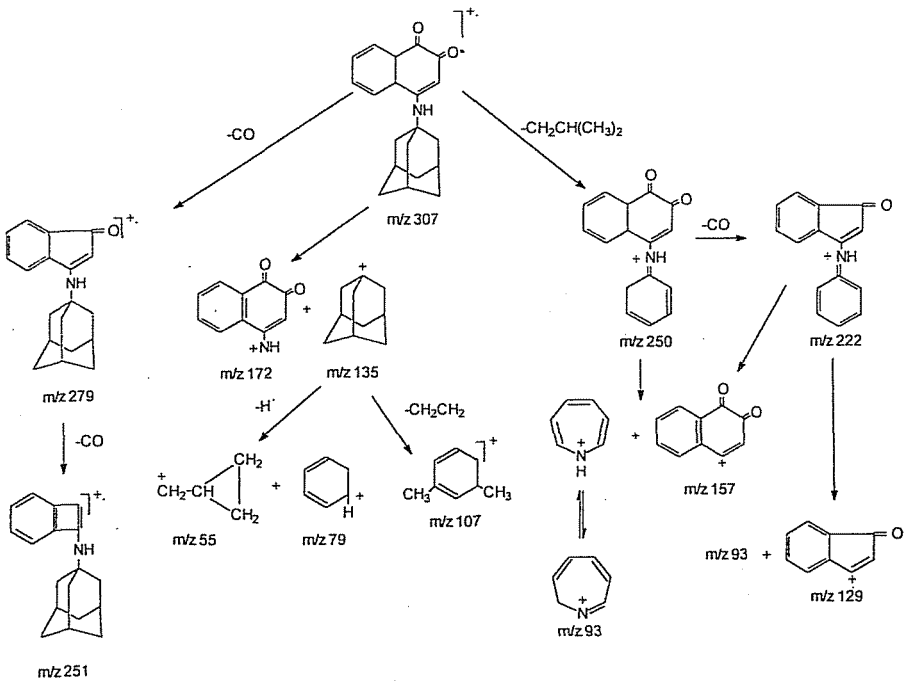


Scheme 1

The compound (A-NQ) may exist in one of the tautomeric forms; 4-alkylamino-1,2-naphtoquinone (I_a) or 2-hydroxy-N-alkyl-1,4-naphtoquinone-imine (I_b).

Two maxima at 276 and 453 nm characterize 1,2-naphtoquinone structure in the electronic spectrum (9). IR spectrum exhibited the NH stretching band at 3370 cm^{-1} which was attributed to the secondary amine. In the NMR spectrum the resonance at 5.52 ppm which is exchangeable with D_2O assigned to the NH proton provided strong confirmation for the 4-[(tricyclo[3.3.1.1^{3,7}]decane-1-yl)amino]-1,2-naphtoquinone (I_a).

The mass fragmentation pattern of Ia was in accordance with those of A and NQS (10) (Scheme 2).



Scheme 2

Making use of the absorption of A-NQ in the visible region a new spectrophotometric quantitation method was developed. Examination of the optimum reaction conditions revealed that the derivative was formed quantitatively at pH 10 and 60° in 30 min. Low stability of A-NQ in aqueous medium necessitated its extraction into an organic matrix: chloroform-butanol (1:1) in which the color intensity of A-NQ was stable for more than 72 hours. The measurements were performed at 451 nm. Stoichiometric relationship between A and NQS was determined as 1:1 by Job's continuous variation experiment (11, 12). Eight fold excess of NQS was sufficient for the quantitative formation of A-NQ derivative.

Under the experimental conditions described a linear relationship existed between absorbance and concentration of A in the range of 5-25 µg/ml. The regression equation was given as follows:

$A = 1.52 \times 10^{-2} (\pm 1.90 \times 10^{-4}) C - 1.40 \times 10^{-3} (\pm 3.18 \times 10^{-3})$

A: Absorbance

C: Amantadine HCl concentration (µg/ml)

The proposed method was applied to the determination of amantadine HCl in a pharmaceutical dosage form (Symmetrel-capsules). The coefficient of variation was 1.13 %.

The results obtained were compared with the USP XXIII method (13) and given in Tables 1 and 2.

Table 1: Results obtained in the analysis of A in capsules (100 mg/capsule)

Spectrophotometric Method %	USP XXIII %
101.20	102.10
99.06	102.26
101.20	102.60
101.20	97.06
99.47	102.60
98.60	98.36
99.47	98.53

Table 2: Statistical evaluation of results

Statistical values	Spectrophotometric method	USP XXIII
X	100.03	100.50
S	1.13	2.41
S^2	1.28	5.76
S_{Rel}	1.13	2.40
Confidence limits	±1.05	±2.23
n=6	P=0.05	

The proposed and the reference methods were compared using student's t test of significance and F test of significance. The t and F values were found to be 0.47 and 4.51 respectively (n=7). The proposed method is simple and sensitive for routine analysis of amantadine HCl, in pharmaceutical dosage forms.

EXPERIMENTAL

Materials

Chemicals: Amantadine HCl (A) (Dinçel Lab), sodium 1,2-naphtoquinone-4-sulfonate (NQS), boric acid, potassium chloride, sodium hydroxide were obtained from Merck. Water was glass distilled.

Instruments: A Shimadzu UV-240 and Spectronic 20 Bausch & Lomb spectrophotometers, Orion pH-meter (model 407-A)

Reagent Solution: 24 mg of NQS was dissolved in water and diluted to 25 ml (the solution was prepared daily and kept in dark).

a) **Preparation of derivative (A-NQ)**

755 mg of amantadine base in 50 ml of ethanol was refluxed with 2.6 g of NQS in 15 ml of water for 30 min at 60°.

The precipitate was filtered, washed with water and crystallized from methanol (yield 63.5 %) Dark red crystalline substance m.p. 269°C UV: EtOH λ_{max} (ε) 236.2 (25657); 276.8 (19819); 453.6 (6026) nm. IR [ν, cm^{-1}, KBr]3370 (N-H str.) 1688, 1684 (C=O str.) 1595 (N-H bending). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.64-1.75 (6H, s, β-CH); 2.16 and 2.17 (6H, 2s, δ-CH); 2.21 (3H, s, γ-CH); 5.52 (1H, s, NH) (disappeared with D_2O); 6.19 (1H, s, C_3-H); 7.42 (1H, dd, C_5-H); 7.58 (1H, ddd C_7-H); 8.19 (1H, dd, C_8-H) Mass (70 eV) m/z 307 (M^+), 279, 250, 222, 172, 135 (base peak), 107, 102, 93, 79, 67.

Elemental analysis: Calcd. for $C_{20}H_{21}NO_2$: C, 78.15; H, 6.89; N, 4.56. Found. C, 78.38; H, 7.23; N, 4.56.

b) **Assay Procedure**

2.52 mg of amantadine HCl was dissolved in 10 ml of water. 20-100 µl aliquots of the stock solution was placed in a 15 ml centrifuge tube. 1 ml of pH 10 borate buffer and 1 ml of NQS solution were added. The tubes were heated on a water bath at 60°C for 15 min. The mixture was vortexed with 7 ml of chloroform-butanol (1:1) for 1 min. The phases were separated by centrifugation (10 min at 1750 rpm). The aqueous phase was dried over anhydrous sodium sulfate and transferred into a 10 ml volumetric flask. The volume was made up to 10 ml with chloroform-butanol (1:1) mixture. The absorbance of the solution was measured at 451 nm against blank.

c) **Sample Preparation**

Commercially available dosage form (capsule) was analyzed as follows: Ingredient of capsules were weighed. An amount of powder equivalent to 300 mg of A was accurately weighed, transferred into a 100 ml volumetric flask, 25 ml of water was added and sonicated in an ultrasonic bath for 30 min., diluted to volume with water and filtered. The first portion (20 ml) of the filtrate was discarded. 50 µl Aliquot of the solution was transferred to a centrifuge tube, and then the procedure described for standard solutions were followed.

REFERENCES

1. Bélanger, P.M., Grech-Bélanger, O., *J.Chromatogr.*, **228**, 327 (1982).
2. Sioufl,A., Pommier,F., *ibid.*, **183**, 33 (1980).
3. Van-der-Horst, F.A.L., Teeuwsen, J., Holthuis, J.J.M., Brinkman,U.A.T., *J. Pharm. Biomed. Anal.*, **8**, 799 (1990).
4. Fujino, H., Goya, S., *Chem.Pharm.Bull.*, **38**, 544 (1990).
5. Narassimhachari, N., Helgeson, E., Parakash, U., *Chromatographia*, **12**, 523 (1979).
6. Tsuboushi, S., Goromaru, T., Iguchi, S., *Japan Analyst.*, **19**, 95 (1970).
7. Sinsheimer, J.E., Hong, D.D., Stewart, J.T., Fink, M.L., Burckhalter, J.H., *J.Pharm.Sci.*, **60**, 141 (1971).
8. Silva, J.A.F., Strojny ,N., *Anal.Chem.*, **47**, 714 (1975).
9. Harmon, R.E., Phipps, L.M., Howell, J.A., Gupta, S.K., *Tetrahedron, 25*, 5807 (1969).
10. Amal, H., Gürsoy, A., Kreutzberger, A., *J.Fac.Pharm. İstanbul*, **12**, 8 (1976).
11. Özkırımlı, S., Sevingil, M., *Acta Pharm.Turc.*, **31**, 77 (1989).
12. Job, P., *Ann.Chim.*, **9**, 113 (1928).
13. The United States Pharmacopeia XXIII, p. 73 United States Pharmacopeial Convention Inc. Rockville (1995).

Accepted 04.09.1998