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ATENOLOLUN İYON ÇİFTİ EKSTRAKSİYONU TEKNİĞİ İLE SPEKTROFOTOMETRİK MİKTAR TAYİNİ*

SPECTROPHOTOMETRIC DETERMINATION OF ATENOLOL* BY ION–PAIR EXTRACTION TECHNIQUE

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SUMMARY

A simple method is presented for the determination of atenolol in tablets. The method is based on the formation of an ion–pair between atenolol and bromothymol blue (BTB) in pH 5.8 aqueous solution. The ion–pair was extracted into chloroform and the aosorbance of the chloroform layer was measured at 414 nm against a blank solution. A linear relationship existed between absorbance and atenolol concentration over the 2.5 – 15.0 $\mu g.ml^{-1}$ range. The molar ratio of atenolol–BTB was determined as 1:1. The stability constant of the ion–pair was calculated (log K = 6.39). Using the present method commercially available atenolol tablets were assayed. The results were statistically compared with those obtained by UV–spectrophotometric method by t– and F– tests at 95 % confidence level. Calculated t and F values were both lower than the tabulated values.

ÖZET

Tabletlerde atenolol tayini için basit bir yöntem geliştirildi. Yöntem atenolol ile bromtimol mavisi (BTM) arasında pH = 5.8 olan sulu çözeltide bir iyon çifti oluşmasına dayanmaktadır. İyon çifti kloroforma ekstre edildi ve kloroform tabakasının absorbansı 414 nm'de boş çözeltiye karşı ölçüldü. Atenolol konsantrasyonu ile absorbans arasındaki ilişki 2,5 – 15,0 $\mu g.ml^{-1}$ konsantrasyon aralığında doğrusaldır. İyon çiftinin atenolol – BTM mol oranı 1:1, kararlılık sabiti ise log K = 6,39 olarak saptandı. Piyasada bulunan tabletlerde atenolol önerilen yönem ile tayin edildi. Sonuçlar UV – spektrofotometrik yöntemle elde edilen sonuçlarla t ve F testleri yardımıyla % 95 güvenirlik düzeyinde istatiksel olarak kıyaslandı. Hesaplanan t ve F değerleri tablo değerlerinden küçük bulundu.

INTRODUCTION

Atenolol, 2–(4–(2–hydroxy–3–isopropylaminopropoxy)phenyl) acetamide is a cardioselective beta–adrenoreceptor blocking agent.

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Potentiometric (1), spectrophotometric (2), spectrofluorimetric (3), high performance liquid chromatographic (4, 5) and nuclear magnetic resonance spectroscopic (6) methods have been published for the determination of atenolol in pharmaceutical dosage forms.

Spectrophotometric determination of amines as ion–pairs with anionic dyes has been widely used in pharmaceutical analytical chemistry (7–11). This paper presents an accurate, simple and sensitive spectrophotometric method for the quantitative determination of atenolol in tablet formulations. The method involves the formation of atenolol–bromothymol blue (BTB) ion–pair in pH = 5.8 aqueous solution, extraction with chloroform and subsequent measurement of the absorbance of the chloroform layer.

EXPERIMENTAL

Apparatus

UV–Visible spectrophotometer (Philips PO 8740) with 1 cm. glass cells (Hellma, 100–OS), Vortex Mixer (Nüve), Centrifuge (Janetzki T 30), pH–meter (Metrohm E 510 Herisau) with pH electrode (Metrohm A G CH–9101 Herisau) were used.

Materials

Pharmaceutical grade atenolol (Doğu) was used as received. Other chemicals were all of analytical reagent grade (Merck, Darmstadt, FRG). Bidistilled water was used.

Reagent Solution

BTB solution ($2x10^{-3}$ M) was prepared by dissolving 0,1248 g in 2 ml of 0.1 N NaOH and diluting to 100 ml with water. For the preparation of $2x10^{-4}$ M reagent solutions in various buffer solutions (pH = 4 – 9), 10 ml of $2x10^{-3}$ M solution was adjusted to the studied pH with 0.2 M NaOH or HCl and diluted to 100 ml with the appropriate buffer solution. $5x10^{-4}$ M reagent solution in pH = 5.8 buffer solution was prepared similarly for the construction of Job's curve. The reagent solutions were stable for one month in refrigerator.

Buffer Solutions

The buffer solutins of various pH's were prepared by mixing of the appropriate volumes of 0.2 M potassium hydrogen phtalate – 0.2 M HCl (pH = 4.0), 0.2 M $C_6H_4COOHCOOK$–0.2 M NaOH (pH = 4.2 – 5.8), 0.2

M KH_2PO_4 – 0.2 M NaOH (pH = 5.8 – 8.0) and 0.2 M H_3BO_3 – 0.2 M NaOH (pH = 9.0) solutions (12).

Atenolol Solutions

Standard atenolol solution (100 μg.ml^{-1} in water) and 5x10^{-4} M atenolol solution (in water) were used for the construction of the calibration curve and Job's curve respectively.

Sample Solution

Twenty tablets were weighed and finely powdered. An acurately weighed portion of the powder equivalent to 10–15 mg of atenolol was transferred into a 100 ml calibrated flask. 50 ml of water was added and the mixture was shaken for 10 min. The mixture was diluted to volume with water, mixed and filtered through a dry filter paper. The first 20 ml portion of the filtrate was discarded, and appropriate volume of the filtrate was diluted with water to provide the necessary working concentration (15–90 μg.ml^{-1}).

Assay Procedure

A 1 ml aliquot of the sample solution was pipetted into a 15 ml glass stoppered centrifuge tube, and 3 ml of BTB solution (2x10^{-4} M in pH = 5.8 buffer solution) and 6 ml of chloroform were added. The mixture was vortexed for 1 min and centrifuged for 3 min. The organic layer was separated and its absorbance was measured at 414 nm against a blank solution. Atenolol concentration was calculated from the regression equation of the calibration curve prepared using 1 ml aqueous solution containing 0.15–0.90 ml aliquots of standard atenolol solution.

RESULTS AND DISCUSSION

The spectrophotometric determination of atenolol was based on the formation of its yellow coloured ion–pair with bromothymol blue. The ion–pair was extracted with chloroform and the absorbance of the yellow coloured chloroform layer was measured.

The optimum conditions of the ion–pair formation were investigated. The effect of the pH of the aqueous solution was studied in the pH interval of 4 to 9. When the aqueous solution was buffered at pH 5.8, the maximum absorbance value was observed. Among the various solvents tested for the extraction of the ion–pair such as chloroform, dichloromethane, benzene, toluene and carbontetrachloride, chloroform was found to be the most suitable solvent. To determine the amount of the reagent, the determinations were repeated using increasing concentrations of BTB. It was found that 3 ml of $2x10^{-4}$M BTB solution in pH 5.8 buffer solution is sufficient for 15 $\mu g.ml^{-1}$ final atenolol concentration.

A linear relationship was obtained between absorbance (A) and atenolol concentration (C) in the range of 2.5–15.0 $\mu g.ml^{-1}$. The regression equation of the calibration curve was

$$A = 2.98x10^{-2}\ C - 1.0x10^{-3}\ (r = 0.9994)$$

Reproducibility of the method was calculated for 10 $\mu g.ml^{-1}$ atenolol concentration. The relative standard deviation was 1.4 % (n = 5)

The molar ratio of the ion–pair was determined using Job's Continuous Variation (13) and Molar Ratio (14) methods. The Job's Curve (Fig. 1) prepared at the total concentration of $1x10^{-4}$M, indicated a ratio of 1:1. This value was confirmed by the Molar Ratio Curve constructed for 15 $\mu g.ml^{-1}$ ($5,632x10^{-5}$M) atenolol concentration (Fig. 2).

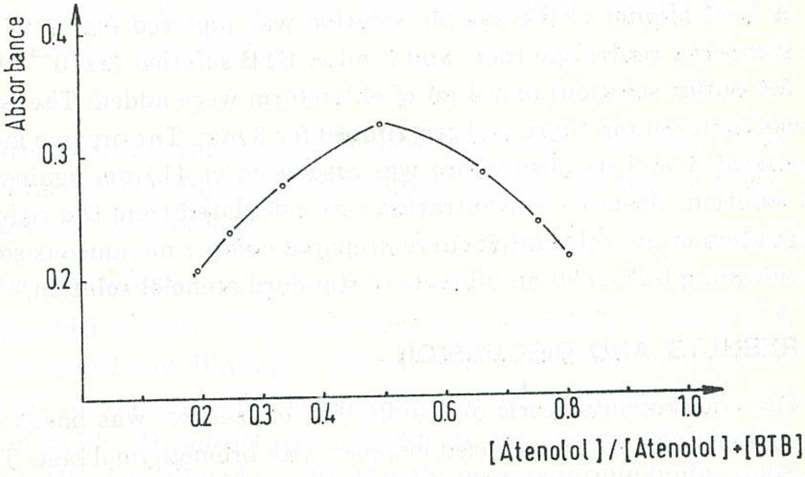

Fig. – 1 : Job's Curve for atenolol–BTB ion–pair in chloroform (Total concentration of atenolol and BTB is $1x10^{-4}$M)

The stability constant of the ion–pair was calculated by Job's Curve as log K = 6.15. This value was confirmed with the value of stability constant calculated as log K = 6.39 using the Molar Ratio Curve (Fig.2) and the following equation.

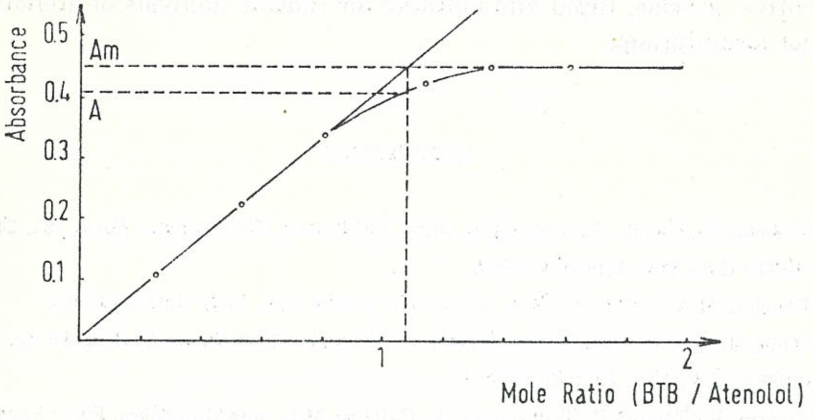

Fig. – 2 : Molar Ratio Curve for atenolol–BTB ion–pair (atenolol concentration = 15 mcg.ml^{-1} , $A_m = 0.446$, $A = 0.408$)

$$K = \frac{A / A_m}{(1 - A / A_m)^2 . C}$$

Where C is the molar concentration of atenolol and A and A_m are the extrapolated absorbance values in the molar ratio curve.

The developed spectrophotometric method was applied to commercially available atenolol tablets satisfactorily. For comparison, the tablets were also assayed with UV – spectrophotometric method since a pharmacopoeial method is not available for the determination of atenolol and its dosage forms. Table 1 shows the results obtained by the proposed and UV – spectrophotometric methods and statistical comparison of these methods in terms of t– and F– tests of significance at 95 % confidence level. As seen from the table, there was no significant difference between the two methods.

Table – 1 : Assay of atenolol tablets (100 mg)

Statistical value	Proposed method	UV–spectrophotometric method (λ_{max} = 226 nm in methanol)
$\overline{X}$	99.3	98.8
Recovery (%)	99.3	98.8
SD	2.4	1.0
SD %	2.4	1.0
n	5	5

t test of significance* t = 0.38
F test of signifcance** F = 5.43

* t = 2.31 (p = 0.05)
** F = 6.39 (p = 0.05)

In conclusion, the proposed spectrophotometric method is simple, sensitive, precise, rapid and suitable for routine analysis of atenolol in tablet formulations.

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