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METOPROLOL TARTARATIN NBD-KLORÜR İLE TÜREVLENDİRİLMESİNDEN SONRA FLUORODANSİTOMETRİK MİKTAR TAYİNİ

FLUORODENSITOMETRIC DETERMINATION OF METOPROLOL TARTRATE AFTER DERIVATIZATION WITH NBD-CHLORIDE

S. SUNGUR*, T. GÜRKAN** and F. İ. MORGİL***

SUMMARY

A fluorodensitometric method was developed for the determination of metoprolol tartrate in pharmaceutucal preparations. The method was based on the reaction of metoprolol tartrate with NBD-Cl at 80° in 30 min, in 0.1 M $NaHCO_3$-MIBK two phase system and the measurement of the fluorescence intensity on silica gel plate after TLC separation.

Fluorescence measurements were performed at 540 nm, using the 366 nm mercury line for the excitation. The method was applied to commercially available tablets and the results were statistically compared with those obtained by hplc method of USP XX, using t- and F- tests, t and F values were found to be 0.20 and 0.82, respectively and both lower than the table values for 95% confidence level (t=2.23 and F=5.05).

ÖZET

Metoprolol tartarat'ın farmasötik preparatlarda miktar tayini için fluorodansitometrik bir yöntem gelitirildi. Yöntem, metoprolol tartarat'ın NBD-Cl ile 0.1 M $NaHCO_3$-MIBK iki fazlı sisteminde 80° de 30 dak. reaksiyonu ve ITK da ayırmadan sonra silika jel tabakada fluoresans şiddetinin ölçülmesine dayanmaktadır.

Fluoresans ölçmeleri 540 nm de, uyarma için 366 nm de cıva çizgisi kullanılarak yapıldı. Yöntem piyasada bulunan tabletlere uygulandı ve sonuçlar USP XX de öngörülen HPLC yöntemi ile elde edilen sonuçlarla t- ve F- testleri kullanılarak istatistik olarak kıyaslandı. t ve F değerleri sırası ile 0.28 ve 0.82 olarak bulundu, bu değerlerin ikisi de % 95 güven seviyesi için verilen tablo değerlerinden küçüktür (t=2.23 ve F=5.05).

* Yıldız Üniversitesi Fen-Edebiyat Fakültesi, Kimya Bölümü, İSTANBUL.
** İstanbul Üniversitesi Eczacılık Fakültesi, Analitik Kimya Anabilim Dalı, İST.
*** Hacettepe Üniversitesi Eğitim Fakültesi, Kimya Bölümü, ANKARA.

INTRODUCTION

Metoprolol, a cardioselective β-adrenergic blocking agent is used in the treatment of hypertension and angina.

Gas chromatographic (1-4), high - performance liquid chromatographic (5-12) and high-performance thin - layer chromatographic (13) methods have been published for the determination of metroprolol in both biological fluids and pharmaceutical preparations. Nonaqueous titrimetric and hplc methods are proposed for the assay of metoprolol tartrate and its determination in tablets respectively, in USP XX (14).

This report describes a fluorodensitometric method for the determination of metoprolol tartrate in pharmaceutical preparations. The method involves derivatization with NBD-Cl (7-chloro-4-nitrobenzofurazan), thinlayer chromatographic separation and insitu measurements of the fluorescence intensity of the spots.

EXPERIMENTAL

Materials and methods:

Chemicals: Metoprolol tartrate and its tablets were kindly supplied by Ciba-Geigy Corp., Istanbul. Aqueous solutions of 4×10^{-4} and 1.2×10^{-3} M metoprolol tatrate were prepared as the stock solutions. NBD-Cl was supplied from E. Merck, Darmstadt, FRG, and 1 % solution in methylisobutylketone (MIBK) was used as the derivatizing reagent. 0.5 mm thinlayer plates (20 × 20 cm) coated with silicagel G 60 and activated at 105° for two hours were used for the TLC seperations.

Apparatus: Zeiss PMQ II spectrophotometer equipped with ZMF 4 fluorescence attachment and Camag Z scanner were used for the insitu fluorescence measurements. A St 41 mercury lamp with 366 nm filter served as the excitation light source. The peaks were recorded using Servegor - Zeiss recorder. Bransonic 221, USA, ultrasonic bath was used to dissolve the tablets.

Assay Procedure for Metoprolol Tartrate: To a 100 µl of aqueous solution of metoprolol tartrate (8×10^{-5} – 1.2×10^{-3} M) in 5 ml glass stoppered centrifuge tube 100 µl of 0.2 M $NaHCO_3$ solution and 200 µl of 1 % NBD-Cl solution in MIBK were added using

100-µl Hamilton syringe. The mixture was vortexed for 10 sec and then kept at 80° for 30 min, after cooling to room temperature, centrifuged for 5 min. An aliquot (5 µl) of the upper organic layer was carefully removed with a 25 µl Hamilton syringe and applied to a TLC plate. The plate was developed for 15 cm using chloroform-methanol (30:1) mixture as the solvent system. Fluorescence intensity of NBD-derivatized ping spots ($R_f = 0.40$) were insitu measured by scanning the air dried plates at 540 nm using 366 nm excitation filter. The peaks were coppied on a uniform transparent paper, then cut and weighed.

Calibration curve was constructed by plotting the peak weights in mg against corresponding metoprolol tartrate amounts in nmole per spot. Regression equation of the calibration curve was calculated by the method of least-squares.

Assay Procedure for Metoprolol Tartrate in Tablets: Twenty tablets were weighed and powdered. An accurately weighed portion of the powder, equivalent to about 10 mg of metoprolol tartrate as transferred to a 50 ml volumetric flask and 25 ml of methanol was added. The mixture was heated at 50° for 10 min, sonicate for 20 min, cooled to room temperature, diluted with methanol to the volume, mixed and filtered through a 0.47 µm millipore filter. An aliquot (100 µl) of the filtrate was evaporated under a stream of nitrogen to dryness. The residue was dissolved in 100 µl of distilled water, derivatized and insitu measured on the same plate together with standart metoprolol tartrate solutions as described under «Assay Procedure for Motoprolol Tartrate». The amount of metoprolol tartrate in tablets was calculated by means of the regression equation obtained by the peak weights of the spots of the standard solutions on the same plate.

RESULTS AND DISCUSSION

The reaction between metoprolol and NBD-Cl was carried out in 0.1 M $NaHCO_3$ – MIBK two phase system. The time course of the reaction was followed at two different temperatures (80° and 60°) by measuring the variation of fluorescence intensities of NBD-derivatized spots (Fig. 1). As it is seen from Fig. 1 derivatization completed in 30 min at 80°.

1 % NBD-Cl solution in MIBK was found to be the optimum reagent concentration when the maximum metoprolol concentration was taken for the reaction.

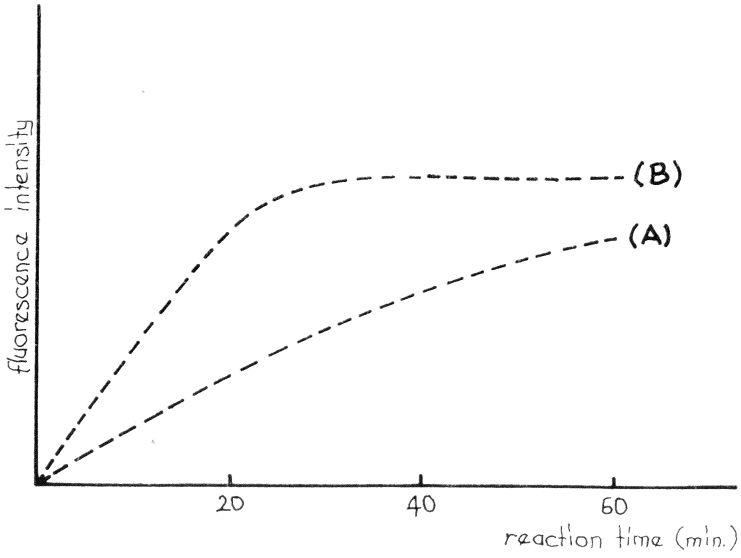

Fig. 1 — Variation of fluorescence intensity at 60° (A) and 80° (B) with time.

The highest fluorescence intensity was yielded with the 366 nm mercury line and it was used for the excitation. Emission wavelenght was 540 nm.

The effect of light on fluorescence intensity of the NBD-derivatized spots was studied by expozing the spots with UV light for 15 min and no change was observed in the fluorescence intensity. It was also remained constant for 48 hr when the plates kept in the dark.

A linear relationship between fuorescence intensity and concentration was obtained over the range of 0.3-3.0 nmole metoprolol per spot. Linear calibration curves for two concentration range are illustrated in Fig. 2.

Detection and determination limits were found to be 0.02 and 0.20 nmole per spot, respectively.

Application of the proposed method for the determination of metoprolol tartrate in tablets gave satisfactory results. For the

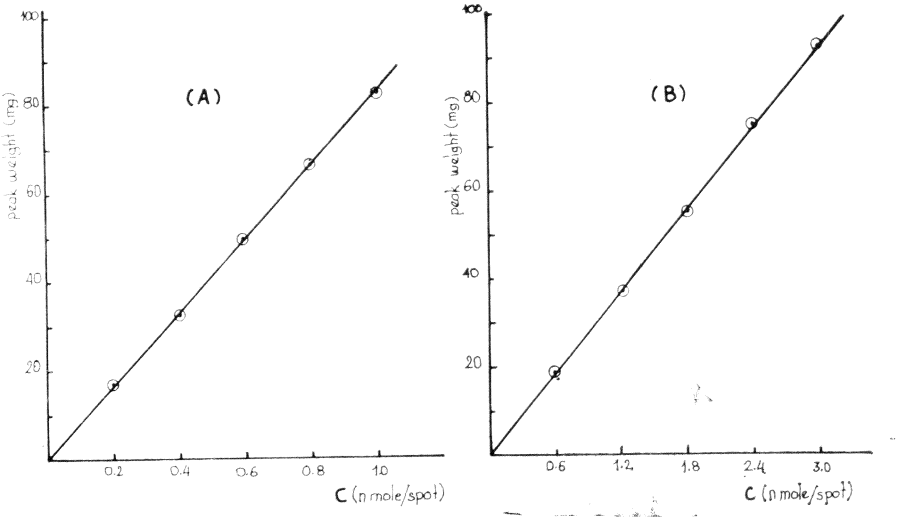

Fig. 2 — Linear calibration curves. (A) $I_F = 80.25 \times C + 1.51$ ($r = 0.999$), (B) $I_F = 30.91 \times C + 0.28$ ($r = 0.999$)

comparison between the present method with a HPLC based pharmacopeia method (USP XX), 100 mg metoprolol tartrate tablets were analysed for both methods. Results obtained by the present method were not significantly different than those obtained by USP XX. The statistical evaluations were shown in Table 1.

Table 1. Determination of Mettoprolol Tartrate in Tablets. (Each tablet contains 100 mg metoprolol tartrate)

Statistical values	Fluorodensitometric Method	USP XX Method
x	99.41	99.17
% Recovery	99.41	99.17
s	1.56	1.41
CV	1.57	1.42
n	6	6
t test of significance	$t = 0.28$ ($p = 0.05$ $t = 2.23$)	
F test of variances	$F = 0.82$ ($p = 0.05$ $F = 5.05$)	

The results obtained in this study indicated that fluorodensitometric analysis of metoprolol with NBD-Cl is accurate and precise for routine pharmaceutical analysis. The coefficient of variation (CV) is found to be 1.57 %.

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