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FARMASÖTİK PREPARATLARDA NORTRİPTİLİN HİDROKLORÜR TAYİNİ İÇİN NBD-Cl İLE REAKSİYONA DAYANAN SPEKTROFLUOROMETRİK YÖNTEM*

SPECTROFLUOROMETRIC DETERMINATION OF NORTRIPTYLINE HYDROCHLORIDE WITH NBD-Cl IN PHARMACEUTICAL DOSAGE FORMS*

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SUMMARY

A spectrofluorometric method was developed, based on the formation of fluorescent derivative with NBD-Cl and nortriptyline in aqueous medium. The optimum reaction conditions were established as follows: the pH was found to be 8.0 and NBD-Cl/amin mole ratio was 15. The reaction completed at 70°C within 30 min. After completion of the reaction, the reaction product was acidified with 0.1 N HCl and diluted with dioxane. The fluorescence intensity was measured at 530 nm using 436 nm excitation filter. A linear relationship existed between relative fluorescence intensity and concentration over the 10-50, 50-250 and 250-1250 ng. ml^{-1} concentration ranges of amine. The proposed method was applied to determination of this substance in tablets. The results were compared with those obtained by the British Pharmacopoeia (BP) method at 95% confidence level in respect of t- and F- tests of significance.

ÖZET

Nortriptilinin NBD-Cl ile sulu ortamda fluoresans gösteren türev oluşturmasına dayanan spektrofluorometrik bir yöntem geliştirildi. Optimum reaksiyon koşulları araştırıldığında reaksiyonun pH 8.0 de NBD-Cl/amin mol oranı 15 olması halinde 70°C de 30 dak. da kantitatif olarak yürüdüğü saptandı. Reaksiyonun tamamlanmasından sonra reaksiyon ürünü 0.1 N HCl ile asidlendirilip, dioksan ile seyreltildi. Fluoresans şiddeti, 436 nm

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eksitasyon filtresi kullanılarak 530 nm de ölçüldü. Bağıl fluoresans şiddeti ile konsantrasyon arasında 10-50, 50-250 ve 250-1250 ng. ml^{-1} konsantrasyon aralıklarında doğrusal bir ilişki olduğu saptandı. Geliştirilen yöntem, bu maddenin tabletlerde miktar tayinine uygulandı. Elde edilen sonuçlar, BP yönteminin sonuçlarıyla % 95 olasılık düzeyinde t- ve F- testi uygulanarak kıyaslandı.

INTRODUCTION

Nortriptyline is a tricyclic antidepressant agent. Several methods have been reported to determine this compound such as spectrophotometry (1, 2), fluorodensitometry (3), GC (4), HPLC (5) and radioimmunoassay (6).

NBD-Cl (7-chloro-4-nitrobenzofurazan) has become widely used as a fluorigenic reagent for primary and secondary aliphatic amines. This reagent has been utilized for the detection of amino acids (7), sulphonamides (8) and for the determination of aliphatic amines (9), amphetamine (10), propoxyphene (11) and baclofen (12).

This report presents a new spectrofluorometric method for the assay of nortriptyline hydrochloride and its dosage forms with NBD-Cl in aqueous solution.

EXPERIMENTAL

Apparatus: All fluorescence measurements were carried out with a Zeiss PMQ II spectrofluorometer equipped with ZFM 4 fluorescence attachment and St 41 mercury lamp. Zeiss 436 nm filter served as the excitation light source. The emission monochromator was set at 530 nm. The slit width was varied between 0.5 - 0.15 nm. The response of the spectrofluorometer was calibrated daily using appropriate concentration of sodium fluoresseine (reference standard) solutions in 0.1 N sodium hydroxide.

Chemicals: Nortriptyline hydrocloride and its tablets were obtained from Mustafa Nevzat İlaç San. A.Ş. Istanbul, Turkey and perphenazine was obtained from Farmos Group LTD, Finland. Other chemicals were purchased from E. Merck A.G., Darmstadt, G.F.R. All solvents used were analytical grade and used without further purification.

Standard solutions: Aqueous solutions of nortriptyline hydrochloride (1-5, 5-25 and 25-125 $\mu g.\ ml^{-1}$) were prepared. These solutions were stable for at least 2 weeks at + 4°C.

Reagent solutions: Solutions of NBD-Cl in methanol (0.005%, 0.025% and 0.13%) were prepared daily.

Buffer solutions were prepared according to the official procedures (2, 13).

Assay procedure: A 0.1 ml of standard drug solution was mixed with 0.1 ml of borate buffer (pH 8.0) in a glass tube with a screw cap. After addition of 0.1 ml of NBD-Cl solution the mixture was heated at 70°C for 30°C min. in a thermostated water bath protected from light. To stop the reaction, the mixture was cooled and acidified with 25 µl of 0.1 HCl. The reaction product was transferred into a 10 ml calibrated flask with 3 ×2 ml of dioxane and diluted to volume with the same solvent.

The fluorescence intensity was measured at a wavelength combination of 536/530 nm against a blank prepared similarly. The reference standard solution was also measured at the same wavelength combination.

Assay procedure for notriptyline hydrochloride tablets: Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to about 20 mg nortriptyline hydrochloride was transferred into a 50 ml calibrated flask, 30 ml of water was added and shaken for 15 min. The volume was adjusted to 50 ml with water, mixed and filtered. The first portion (20 ml) of the filtrate was discarded. A 10 ml of the filtrate was transferred into a 50 ml calibrated flask and diluted to volume with water. Then 0.1 ml of the resulting solution was transferred into a test tube and the assay procedure above was followed. The concentration was calculated from the regression equation of the calibration graph over the 250-1250 ng. ml^{-1} concentration range.

Perphenazine, the other active substituent of the tablets, was examined for its reactivity with NBD-Cl according to the manual procedure. No fluorophore formation was observed.

RESULTS and DISCUSSION

The factors effecting the reaction between nortriptyline and NBD-Cl were investigated. For this purpose, the optimum pH, temperature, reaction time, amount of the reagent, proper solvent, and wavelength were established. These parameters were investi-

gated at the concentration of 1 μg. ml^{-1} of nortriptyline hydrochloride and instrument settings were the followings: excitation filter, 436 nm; emission maxima, 530 nm; sensitivity, 1 × 4 and slit width 0.4 mm.

The fluorescence intensity and the position of the maxima are dependent on the nature of the solvent used. As it can be seen in Table 1; chloroform, dichlormetane and dioxane gave the highest response, we selected dioxane because of its lower volatility.

Table 1. Fluorescence intensities and emission maxima of nortriptyline - NBD derivative in different solvents

Solvent	λem. max. (nm)	Fluorescence intensity (arbitrary units)
Methanol	540	10.0
Methylisobuthylketone	530	25.5
Ethylacetate	530	29.0
Chloroform	525	77.5
Dichlormetane	528	78.0
Dioxane	530	79.0

The results of the pH study shown in Figure 1 indicated that maximum fluorescence was obtained at pH 8.0.

The reaction of nortriptyline with NBD-Cl was processed in thermostated water bath at different temperatures and at various periods. As it is seen from the Figure 2 the reaction was completed at 70°C within 60 min.

To determine the amount of the reagent, varying concentrations of the NBD-Cl were added to a fixed quantity of amine. 15 fold excess of reagent was enough to complete the reaction (See Table 2).

Table 2. Effect of reagent concentration on the reaction of nortriptyline with NBD-Cl

Mole ratio of NBD-Cl/amine	5	10	15	20	25	30	40
Relative fluorescence (arbitrary units)	70.0	83.0	83.5	83.5	82.5	82.5	81.5

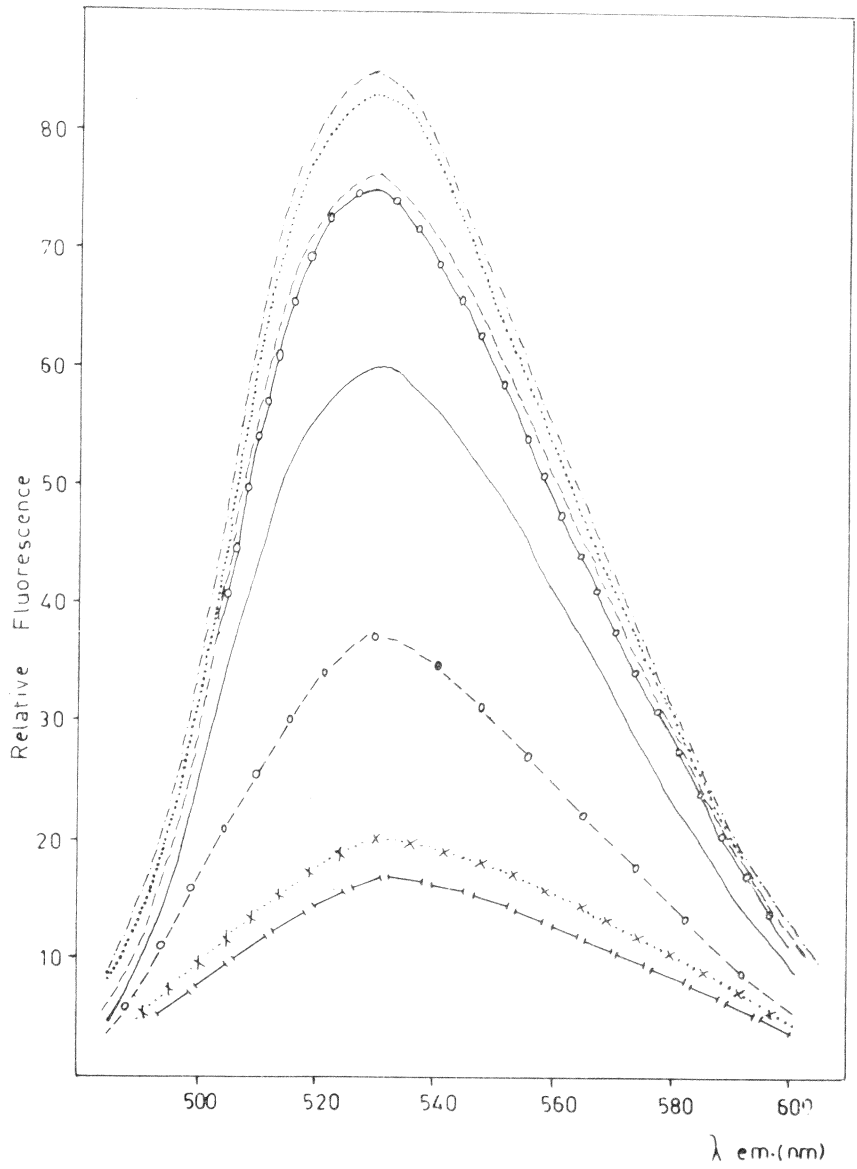

Figure 1. Emission spectra of the Nortriptyline-NBD derivative in dioxane at various pH values —o—o— pH 7.0, ... pH 7.5 and pH 8.5, .—.—.—.— pH 8.0, - - - - pH 9.0, ———— pH 9.5, -o- - -o- pH 10.0, ...x...x pH 11.0, ı—ı ı—ı ı—ı ı—ı pH 12.0).

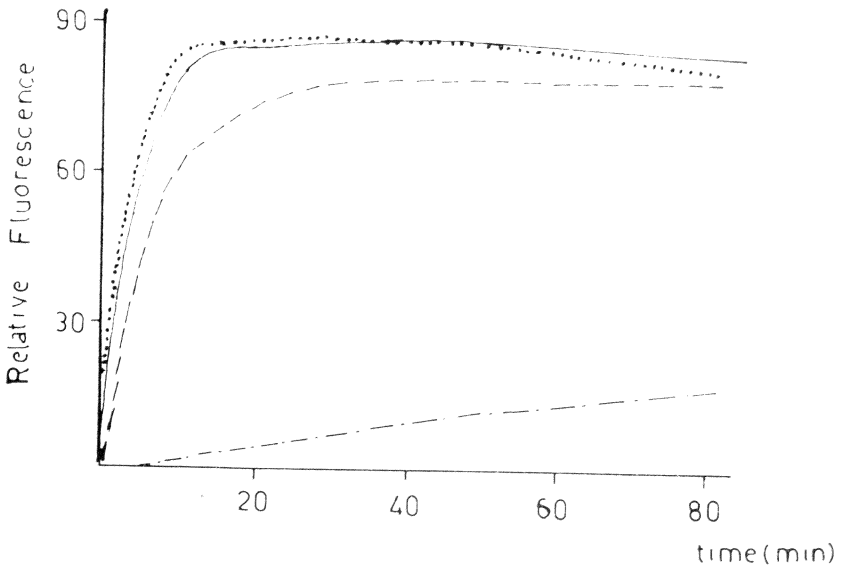

Figure 2. Effect of heating time on fluorescence intensity of fluorophore (— . — . — . room temperature, — — — — — 60°C, ——— 70°C, ... 80°C).

Stability of the derivative in dioxane was investigated by performing repeated readings of the same sample at different times. Fluorescence intensity was found to be stable for at least three days at + 4°C in dark.

Under the experimental conditions, a linear relationship existed between relative fluorescence intensity and concentration over the 10-1250 ng. ml^{-1} concentration range of nortriptyline hydrochloride. The regression equations were $I_F = 3.105\ C + 2.933$ ($r = 0.9994$), $I_F = 0.557\ C + 2.566$ ($r = 0.9997$) and $I_F = 0.123\ C + 1.247$ ($r = 0.9999$) for 10-50, 50-250 and 250-1250 ng. ml^{-1} concentration ranges, respectively.

Results obtained by applying the proposed method to commercially available nortriptyline hydrochloride tablets are presented in Table 3. Comparison of the experimental data with those obtained by the British Pharmacopoeia (BP) (2) shows a good correlation. The results were compared statistically with each other in terms of t- and F- tests of singnificance at 95% confidence level (Table 4).

The advantages of the present method is the sensitivity, the simplicity and the ease of performing the assay.

Table 3. Assay results of commercially available nortriptyline hydrochloride tablets (20 mg nortriptyline hydrochloride/tablet).

assay number	Spectrofluorometric method		Spectrophotometric method	
	mg/tablet	%	mg/tablet	%
1	16.91	84.55	16.81	84.05
2	16.97	84.85	16.81	84.05
3	17.09	85.45	17.02	85.10
4	17.21	86.05	17.02	85.10
5	17.33	86.65	17.23	86.15
6	17.38	86.90	17.45	87.25
mean	17.15	85.74	17.06	85.28

Table 4. Statistical evaluation of the results in Table 3.

Statistical values	Spectrofluorometric method	Spectrophotometric method
number of determinations	6	6
mean	17.15	17.06
standart deviation	0.19	0.25
relative standard deviation	1.11	1.46
confidence limits	16.95-17.35	16.80-17.32
t test of significance	0.72	($p = 0.05$ $t = 2.23$)
F test of significance	1.69	($p = 0.05$ $F = 5.05$)

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