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ORIGINAL PAPER

Spectrofluorimetric Determination of Paroxetine HCl in Pharmaceuticals via Derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)

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Abstract A sensitive and simple spectrofluorimetric method has been developed and validated for the determination of the antidepressant paroxetine HCl (PXT) in its dosage forms. The method was based on coupling reaction of PXT with 4-chloro-7-nitrobenzo-2- oxa-1,3-diazole (NBD-Cl) in an alkaline medium (pH 8) to form a highly fluorescent derivative that was measured at 530 nm after excitation at 460 nm. The factors affecting the formation and stability of the reaction product were carefully studied and optimized. The fluorescence–concentration plot is rectilinear over the range 0.2-6 μg/mL with LOD of 0.08 μg/mL and LOQ of 0.24 μg/mL respectively. The method was applied to the analysis of commercial tablets and the results were in good agreement with those obtained using the reference method. The mean percentage recoveries for paxetin and xandol tablets were 101.27±1.79 and 101.33±1.19 respectively. A proposal of the reaction pathway was postulated.

Keywords Paroxetine HCl · 4-chloro-7-nitobenzo -2-oxa-1,3-diazole (NBD-Cl) · Spectrofluorimetry · Dosage forms

Introduction

Paroxetine (PXT) is known as (3S,4R)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl) piperidine hydrochloride (Fig. 1) [1]. It is a phenylpiperidine derivative and it is considered as selective serotinine reuptake inhibitor (SSRI) with actions similar to tricyclic antidepressants. It is useful in the treatment of depression, as generalized anxiety, obsessive-compulsive, panic disorder and social anxiety disorder [2]. Several methods have been reported concerning the analysis of PXT including: HPLC [3–7], voltammetry [8, 9], Gas Chromatography, GC [10–12], capillary electrophoresis [13] and spectrophotometry [14–17].

4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) has been used as a derivatizing reagent for the spectrofluorimetric determination of many amino compounds of pharmaceutical interest [18–21]. The reaction of NBD-Cl with PXT has been investigated spectrophotometrically [14] only. Therefore, there is a need for more sensitive method for the determination of PXT in its dosage forms such as its reaction with NBD-Cl. The fluorimetric reaction product was measured at 530 nm after excitation at 460 nm.

Experimental

Apparatus

The spectrofluorimetric measurements were made using Perkin Elmer LS 45 Luminescence Spectrometer equipped with 150 Watt Xenon arc lamp and quartz cell (1 cm).

Materials and reagents

All reagents and solvents were of Analytical Reagent Grade.

– Paroxetine HCl (PXT) was kindly provided by SmithKline Beecham Pharmaceuticals, Bentford, England. It's purity was found to be 99.8 % according to

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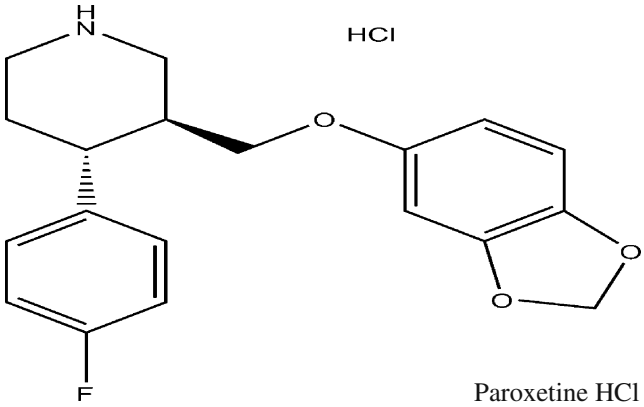


Fig. 1 Structural formula of Paroxetine HCl

the reference method [17]. Tablets were obtained from commercial sources in the local Pharmacy.

- 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was purchased from Sigma (Louis USA). A stock solution containing 0.2% *w/v* of the reagent was freshly prepared in methanol. Borate buffer (0.2 M, pH 8.0) was prepared by mixing appropriate volumes of 0.2 M boric acid and 0.2 M NaOH and adjusting the pH using a pH meter. The buffer solution was kept in refrigerator and left to reach the room temperature before use.
- Methanol and concentrated hydrochloric acid (32%) were purchased from Sigma (Louis, USA).

Standard solution

A stock solution was prepared by dissolving 20.0 mg of PXT in 100 mL of methanol and was further diluted with the same solvent as appropriate. The standard solutions were stable for seven days when kept in the refrigerator.

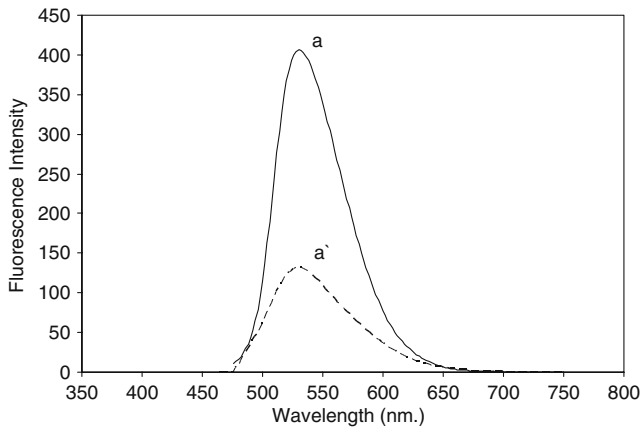


Fig. 2 emission spectra of the reaction product of: (*a*) PXT (5.0 μg/mL) with 0.2% *w/v* NBD-Cl at pH 8.0 (*a`*) blank with 0.2% *w/v* NBD-Cl at pH 8.0

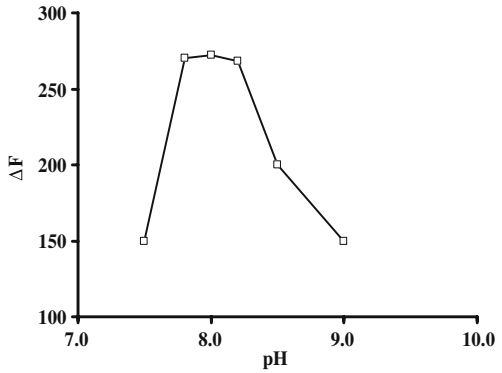


Fig. 3 Effect of pH on the fluorescence intensity of reaction product of PXT (5.0 μg/mL) with NBD-Cl

General procedure

Construction of the calibration curve

Aliquots of PXT standard solution was transfered into a series of 10 mL volumetric flasks. 1 mL of borate buffer (pH 8.0± 0.2) was added, followed by 1 mL±0.2 mL of 0.2% NBD and mixed well. The solution was heated at 55 °C±5 °C for 10± 5 min in a thermostatically-controlled water bath , then cooled to room temperature. 0.2 mL of HCl was added and completed to the volume with methanol and mixed well. The fluorescence intensity of the resulting solution was measured at 530 nm after excitation at 460 nm. The corrected fluorescence intensity was plotted *vs* the final concentration of the drug (μg/mL) to get the calibration curve. Alternatively, the corresponding regression equation was derived.

Application

Procedure for commercial tablets

Ten tablets were weighed and pulverized. A weighed quantity of the powder equivalent to 100.0 mg of PXT

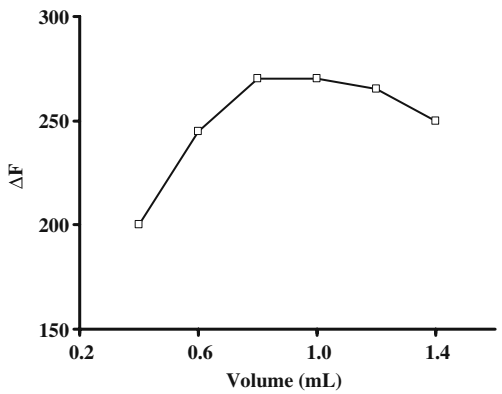


Fig. 4 Effect of volume of borate buffer on the fluorescence intensity of reaction product of PXT (5.0 μg/mL) with NBD-Cl

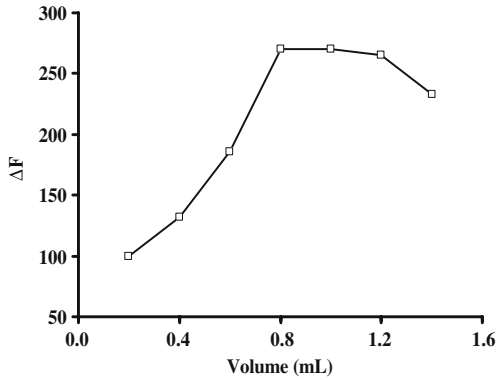


Fig. 5 Effect of volume of 0.2% *w/v* NBD-Cl on the fluorescence intensity of reaction product of PXT (5.0 μg/mL)

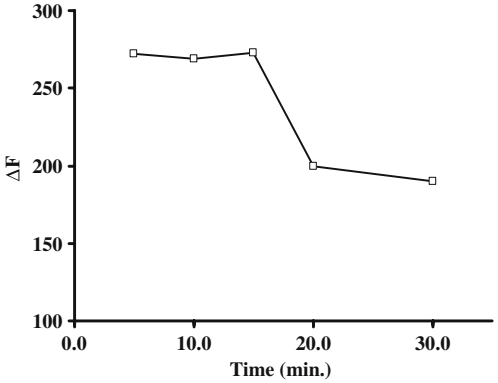


Fig. 7 Effect of heating time (min) on the fluorescence intensity of reaction product of PXT (5.0 μg/mL) with NBD-Cl

was transferred into a small conical flask and extracted with 3×30 mL of methanol on three successive times each with 30 mL. The solution was filtered into a 100 ml volumetric flask. The conical flask was washed with few mls of methanol. The washings was passed into the same volumetric flask and completed to the volume with the same solvent. Aliquots covering the working concentration range was tranfered into 10 mL volumetric flasks. The procedures described under "General Procedure" was performed. The nominal content of the tablets was determined either from the calibration curve or using the corresponding regression equation.

Results and discussion

In the present study, PXT was found to react with NBD-Cl in presence of borate buffer (pH 8.0) producing a yellow color resulting in a strong fluorescence at 530 nm after excitation at 460 nm (Fig. 2).

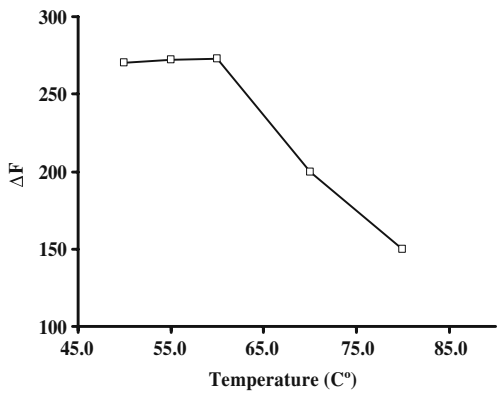


Fig. 6 Effect of heating temperature (°C) on the fluorescence intensity of reaction product of PXT (5.0 μg/mL) with NBD-Cl

Optimization of experimental parameters

The spectrofluorimetric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized. Such factors were changed individually while others were kept constant. The factors include; pH, type of buffer, temperature, time of heating and effect of solvent.

Effect of pH

The influence of pH on the fluorescence intensity of the reaction product was evaluated. Maximum fluorescence intensity was obtained at pH 7.8 and remained constant up to 8.2 after which the fluorescence intensity of the reaction product began to decrease gradually until pH 9.0. Therefore, pH of 8±0.2 was chosen as the optimum pH (Fig. 3). Other buffers having the same pH value such as phosphate and hexamine (pH8.0) were tried and compared with 0.2 M borate buffer. Borate buffer was found to be superior to other buffers having the same pH value since the net fluorescence intensity was highest in case of borate buffer. This is probably, because the rate of hydrolysis of NBD-Cl to NBD-OH was much slower. This result is in agreement with that of Miyano et al. [22].

Effect of volume of borate buffer

It was found that increasing the volume of buffer produces a gradual increase in the fluorescence intensity of the reaction product up to 0.8 mL and it remained constant up to 1.2 mL. Therefore, the optimum buffer volume used during the study was 1 mL for PXT (Fig. 4).

Effect of NBD-Cl concentration

The effect of NBD-Cl concentration was studied using different volumes of 0.2% *w/v* solution of the reagent. It

Table 1 Performance data of the proposed method

Parameter	Spectrofluorimetric Method	Ref. method [17]
-concentration range (μg/ mL)	0.2–6	
-LOD (μg/mL)	0.08	
-LOQ (μg/ mL)	0.24	
-Correlation coefficient (r)	0.9999	
-Slope	46.6	
-Intercept	38.7	
-$S_{y/x}$	1.58	
-S_a	1.1	
-S_b	0.3	
-% Error	0.32	
-%RSD	0.86	
-No.of Experiments	7	5
-Mean found (%)	99.86	99.94
± SD	0.86	1.11
-Student's t-value	0.18 (2.23)	
-Variance ratio F-test	1.24 (4.53)	
-Applications	Tablet preparations	

N.B.

-$S_{y/x}$ = standard deviation of the residuals

-S_a = standard deviation of the intercept of regression line

-S_b = standard deviation of the slope of regression line

-% Error = RSD% / √ n

- Figures between parentheses are the tabulated t and F values respectively, at p=0.05 [24]

was found that, the reaction of NBD-Cl with PXT started upon using 0.2 mL of the reagent. Increasing the volume of the reagent, produces a proportional increase in the fluorescence intensity of the reaction product up to 0.8 mL and remained constant up to 1.2 mL, after which further increase produces a gradual decrease in the fluorescence intensity. Therefore, 1 mL of 0.2% of NBD-Cl solution was chosen as the optimal volume of the reagent (Fig. 5).

Different temperature settings were used with constant heating time. Increasing the temperature of the water bath was found to produce a proportional increase in the

Table 2 Validation of the proposed method for the determination of paroxetine HCl in pure form

Sample concentration	% recovery (repeatability)	% recovery intermediate precision
2 μg/mL		
	98.5	100
	101.5	99.2
	99.5	97.7
X`	99.83	98.97
± SD	1.53	1.17
%RSD	1.53	1.17
% Error	0.58	0.45
4 μg/mL	101.1	101.7
	101.7	101.7
	101.1	100.6
X`	101.3	101.33
± SD	0.35	0.64
%RSD	0.35	0.64
% Error	0.13	0.24
6 μg/mL	99.4	100.3
	100.9	100.9
	101.3	100.0
X`	100.53	100.4
± SD	0.99	0.46
%RSD	0.99	0.46
% Error	0.38	0.17

Table 3 Validation of the proposed method for the determination of paroxetine HCl in tablet preparations

Sample concentration	% recovery (repeatability)	% recovery intermediate precision
PXT		
Paxetin® 4 μg/mL	99.2	102.45
	102.3	100.9
	102.3	101.20
X`	101.27	101.52
± SD	1.79	0.82
%RSD	1.79	0.82
% Error	0.67	0.31
Xandol® 4 μg/ mL	101.7	101.7
	102.3	101.2
	100.0	99.6
X`	101.33	100.83
± SD	1.19	1.1
%RSD	1.19	1.1
% Error	0.44	0.41

fluorescence intensity of the reaction product up to 50 °C and remained constant until 60 °C after which further increase in the temperature produces a gradual decrease in the fluorescence intensity, so the optimum temperature for the study was 55 °C±5 °C (Fig. 6).

Effect of heating time

The time of heating is an essential part of the experiment. Different time intervals were tested to ascertain the time after which the solution attains its highest fluorescence intensity. It was found that after 5 min, the reaction product reaches the highest fluorescence (Fig. 7). It was observed that heating time for 10 min is adequate and the fluorescence intensity of the reaction product is stable for about 40 min. at room temperature.

The fluorescence intensity of the hydrolysis product of NBD-Cl, namely,4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH) is quenched by decreasing the pH of the reaction medium to less than 1. Therefore, acidification of the reaction mixture prior to measurement of the fluorescence intensity remarkably decreased the background fluorescence due to the formation of NBD-OH without affecting the drug reagent adduct, hence the sensitivity was increased.

Effect of the solvent

Dilution with different solvents such as methanol, water, chloroform, dimethylsulfoxide (DMS) and dimethylformamide (DMF) was studied. It was found that, water causes precipitation, chloroform causes phase separation, DMF results in high blank reading and DMSO results in lower

Table 4 Application of the proposed spectrofluorimetric method to the determination of paroxetine HCl in commercial tablets

preparation	Spectrofluorimetric method		Ref. method [17]	
	Amt.Taken (μg/ml)	% found	Amt.Taken (μg/ml)	% found
1-Paxetin® tablets-	2.0	98.35	2.0	100.54
(20.0 mg PXT HCl/Tablet)	4.0	102.0	5.0	101.55
B.No:1228002	6.0	100.7	8.0	100.34
X̄± SD		100.35±1.85	100.81±0.65	
Student's t test		0.40		
Variance ratio F test		8.14		
2-Xandol® tablets	2.0	99.8	2.0	100.05
(20.0 mg PXT HCL/tablet)	4.0	101.34	5.0	100.98
B.No:8235001	6.0	100.55	8.0 100.79	
X̄± SD		100.56±0.77	100.61±0.49	
Student's t test		0.40		
Variance ratio F test		2.46		

N.B.: 1- Product of PHARAONIA pharmaceuticals

2- Product of EUROPIAN EGYPTIAN PHARM. IND

- The tabulated values of t and F are (2.78) and (19.00) respectively, at $p=0.05$ [24]

- Each result is the average of three separate determinations.

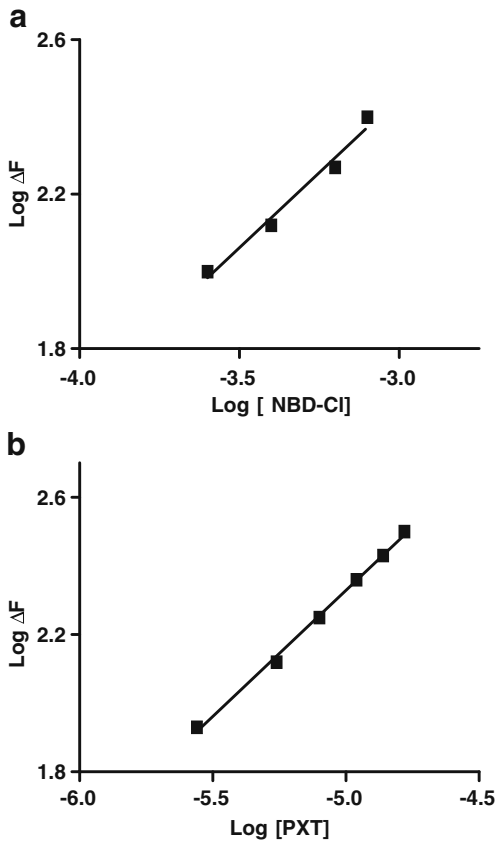


Fig. 8 Stoichiometry of the reaction between PXT and NBD-Cl (0.2% *w/v*) adopting limiting logarithmic method. **a** Log [NBD-Cl] *vs* log ΔF. **b** Log [drug] *vs* log ΔF

fluorescence intensity than methanol. Therefore, the highest fluorescence intensity value was achieved upon diluting with methanol.

Analytical parameters for paroxetine

Validation of the proposed methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to ICH Q2B recommendations [23].

Linearity

The fluorescence-concentration plot was found to be linear over the range of 0.2-6 μg/mL with minimum detection limit (LOD) of 0.08 μg/mL. Linear regression analysis of the data gave the following equation:

$$F = 38.71 + 46.61\ C \quad (r = 0.9999)$$

Where F, is the fluorwescence intensity, C is the concentration of the drug (μg/mL)

The limits of quantification (LOQ) and the minimum detection limit (LOD) were calculated according to ICH Q2B [23] using the following equations:

$$LOQ = 10S_a/b \quad LOD = 3.3S_a/b$$

Where S_a = The standard deviation of the intercept of regression line b = Slope of the calibration curve.

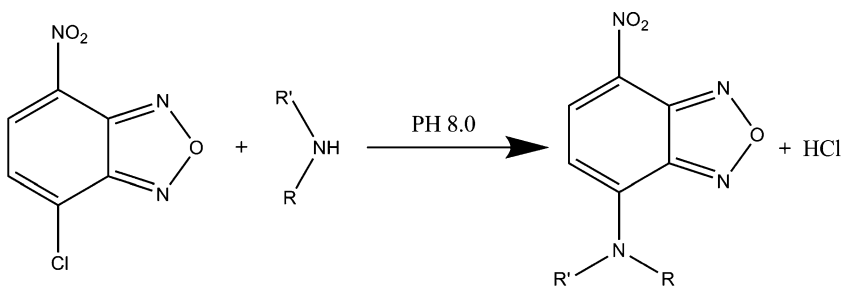
The results are shown in Table 1.

LOQ was found to be 0.24 μg/mL and LOD was found to be 0.08 μg/mL

The proposed method was evaluated by studying the accuracy as percent relative error (% Er) (Table 1) and precision as percent relative standard deviation (% RSD) and the results are shown in Table 1. The small values of % Er and % RSD indicates high accuracy and high precision of the proposed method.

Accuracy

To test the validity of the proposed method it was applied to the determination of pure sample of PXT over the working concentration range. The results obtained were in good agreement with those obtained using reference method [17]. Using Student's t-test and variance ratio F-test [24], revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively . The reference method is based on the reaction of PXT with 1,2-Naphthoquinone-4-sulphonate as a chromogenic reagent to form an orange colored product peaking at 488 nm in Clark and Lubs buffer solution of pH 9. The reference method obey Beer's law over the concentration



Scheme 1 Proposed reaction pathway between NBD-Cl and Paroxetine HCl

range of 1–8 μg/mL which is less sensitive than the proposed method.

The validity of the method was evaluated by statistical analysis of the regression lines regarding the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) . The results are given in Table 1. The small values of the figures point out to the low scattering of the points around calibration graph and the precision of the method.

Precision

Repeatability

The repeatability was performed by applying the proposed method for the determination of three concentrations of PXT in pure form on three successive times, and the results are listed in Tables 2 and 3.

Intermediate precision

It was performed through repeated analysis of PXT in pure form, using the concentrations shown in Tables 2 and 3 for a period of 3 successive days. The results are summarized in Tables 2 and 3.

Robustness of the method

The robustness of the method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor changes in the experimental parameters such as, change in pH 8±0.2, change in the volume of NBD-Cl (0.2 % *w/v*), 1.0±0.2 mL , the change in reaction time 10±5 min. These minor changes that may take place during the experimental operation didn't affect the fluorescence intensity of the reaction product.

Pharmaceutical applications

The proposed methods were then applied to the determination of PXT in its tablets. The methods were tested for linearity, selectivity and accuracy and precision according to ICH Q2B recommendations.

Selectivity

The selectivity of the method was investigated by observing any interference encountered from the common tablet excepients, such as talc, lactose, starch, avisil, gelatine, and magnesium stearate. These excepients did not interfere with the proposed method. As revealed by a placebo blank experiment using tablet additives but omitting PXT.

Effect of co-administered drugs

The selectivity of the method was also investigated by observing any interference encountered from the co-administered drugs such as tricyclic antidepressants. It was found that there is no interference from these drugs such as: amitriptyline, clomipramine and imipramine.

Precision

Repeatability

The repeatability was performed by applying the proposed method for the determination of three concentrations of PXT in tablet preparations on three successive times, and the results are listed in Tables 2 and 3.

Intermediate precision

It was performed through repeated analysis of PXT in tablet preparations, using the concentrations shown in Tables 2 and 3 for a period of 3 successive days. The results are summarized in Tables 2 and 3.

Accuracy

The results of the proposed method was statistically compared with those obtained using the reference method [17]. Statistical analysis [24], of the results, using Student's t-test and variance ratio F-test revealed no significant difference between the performance of the proposed and reference method regarding the accuracy and precision, respectively (Table 4).

Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [25]. The fluorescence of the reaction product was alternatively measured in the presence of excess of NBD-Cl and PXT. A plot of log Δ F versus log [NBD-Cl] and log [PXT] gave straight lines, the values of the slopes are 0.78 and 0.73 respectively (Fig. 8). Hence, it is concluded that, the molar reactivity of the reaction is 0.78 / 0.73 ,i.e. the reaction proceeds in the ratio of 1 : 1. By analogy to previous study [26], it is confirmed that one molecule of the drug reacts with one molecule of NBD-Cl. It is known that the appearance of a red-brown color is due to the intermediacy of Meisenheimer complex [27–29]. The yellow color proves the conversion of the Meisenheimer complex into the reaction product which could be analyzed spectrofluorimetrically, at 530 nm. PXT in alkaline medium reacts with NBD-Cl through its secondary amino group to give the following

final reaction product. A schematic proposal of the reaction pathway is given in Scheme 1.

Conclusion

New simple and sensitive spectrofluorimetric method for the determination of PXT has been successfully developed and validated. The method involved simple derivatization of PXT with NBD-Cl reagent, and subsequent measuring the fluorescence intensity of the fluorescent reaction product. The proposed method is specific, accurate, reproducible, and highly sensitive to be applied for the analysis of PXT in its tablets. Furthermore, the analysis is relied on a simple apparatus, thus the proposed method is suitable for routine analysis of PXT in quality control and clinical laboratories.

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