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ORIGINAL PAPER

Fluorometric Study for the Reaction Between Sertraline and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole: Kinetics, Mechanism and Application for the Determination of Sertraline in Tablets

Ashraf M. Mahmoud · Ibrahim A. Darwish · Nasr Y. Khalil

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Abstract A fluorometric study has been carried out, for the first time, to investigate the reaction of the new generation antidepressant sertraline (SRT) with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl). In an alkaline buffered medium (pH 8.0), a green fluorescent product exhibiting maximum fluorescence intensity at 532 nm after excitation at 470 nm was produced. The factors affecting the reaction were carefully studied and the conditions were optimized. The kinetics of the reaction was investigated, the stoichiometry of the reaction was determined, and the mechanism was postulated. The activation energy of the reaction was determined and found to be 27.34 KJ mole^{-1}. Under the optimum reaction conditions, a linear relationship with good correlation coefficient (r=0.9998, n=6) was found between the fluorescence intensity of the reaction product and SRT concentrations in the range of 0.3–20.0 μg ml^{-1}. The limit of detection and limit of quantitation were 0.07 and 0.21 μg ml^{-1}, respectively. The intra- and inter-assay precisions were satisfactory; the relative standard deviations did not exceed 2.61%. The proposed method was successfully applied to the determination of SRT in its pharmaceutical tablets with good accuracy; the recovery percentages were 96.97–102.23± 1.01–1.62%. The results were compared favorably with those of the reported method.

A. M. Mahmoud
Department of Pharmaceutical Analytical Chemistry,
Faculty of Pharmacy, Assiut University,
Assiut 71256, Egypt

I. A. Darwish (✉) · N. Y. Khalil
Department of Pharmaceutical Chemistry, College of Pharmacy,
King Saud University,
P.O. Box 2457, Riyadh 11451, Saudi Arabia
e-mail: idarwish@ksu.edu.sa

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Introduction

Sertraline (SRT); (1*S*, 4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalen-amine, is a potent new generation antidepressant drug. It exerts its antidepressant activity by selective inhibition of the serotonin reuptake. It is also prescribed in obsessive-compulsive disorder, social phobia, and panic attacks [1, 2]. Sertraline is comparable to tricyclic antidepressants (TCAs) in its clinical efficacy, but it is better tolerated by patients, and remarkably safer than TCAs in the overdose resulting in a better quality of life [2, 3]. For these reasons, SRT was the most prescribed antidepressant on the US retail market [4]. Because of the therapeutic importance and clinical success of SRT, numerous analytical methods have been developed for its quantitative determination in pure, pharmaceutical dosage forms and/or biological fluids. These methods include spectrophotometry [5–12], capillary electrophoresis [13, 14], electrochemical methods [15], gas chromatography [16–18], and HPLC [19–24]. These methods, except the spectrophotometric ones, were adapted to expensive and sophisticated instruments and thus their use in quality control laboratories for routine determination of SRT in its pharmaceutical preparations were limited. Fluorometry is considered one of the most convenient analytical techniques, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories.

No attempt has yet been made for the fluorometric determination SRT. The present study describes, for the first time, a simple, sensitive and economical fluorometric

method for the determination of SRT in its bulk and tablets. The method was based on the reaction of SRT with 7-chloro-4-nitro-2,1,3-benzoxadiazole (NBD-Cl) in slightly alkaline buffered medium to produce a green fluorescent product that was measured fluorometrically at 532 nm after excitation at 470 nm.

Experimental

Apparatus

Spectrofluorimeter, Kontron SFM 25 equipped with a 150-W xenon high-pressure lamp was used for measuring the fluorescence intensity. MLW type thermostatically controlled water bath (Memmert GmbH, Co. Schwa bach, Germany).

Chemicals and materials

Sertraline was obtained from (Pfizer Egypt, S.A.E., Cairo, Egypt). 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) were purchased from Sigma Chemical Co., St. Louis, USA. All other solvents and materials were of analytical grade (Merck, Darmstadt, Germany).

Dosage forms

Lustral (Pfizer Egypt, S.A.E., Cairo, Egypt), moodapex (Apex Pharma, Cairo, Egypt), and sirto (Hi Pharma, Cairo, Egypt) tablets are labeled to contain 50 mg sertraline HCl per tablet.

Preparation of solutions

Stock standard solution

An accurately weighed amount (20 mg) of SRT was quantitatively transferred into a 100-ml calibrated flask, dissolved in 30 ml water, completed to volume with water to produce a working standard solution of 200 μg ml^{-1}. The solution was found to be stable for at least one weak when kept in the refrigerator.

Tablet solution

Twenty tablets of each formulation were weighed and finely powdered. A quantity of the mixed powder equivalent to 20 mg of the active component was transferred into a 100-ml calibrated flask, dissolved in 30 ml water, swirled and sonicated for 5 min, completed to volume with water, shaken well for 10 min, and filtered. The first portion of the filtrate was rejected, and further dilutions were made to obtain the suitable drug concentrations.

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole derivatizing reagent

Accurately weighed amount of NBD-Cl (15 mg) was quantitatively transferred into a 50-ml calibrated flask, dissolved in 5 ml ethanol, completed to volume with ethanol to produce a working solution of 0.03% (w/v). The solution was freshly prepared daily and protected from light during use.

Borate buffer solution

A weighed amount of 1.238 g of boric acid and 1.490 g of potassium chloride were dissolved in 100 ml distilled water. A volume of 8.1 ml of 0.2 M NaOH and 80 ml of ethanol were added and the mixture was diluted to 400 ml with distilled water. The pH of the solution was adjusted to 8.0± 0.1 by a calibrated pH-meter (Microproessor pH meter BT-500, Boeco, Germany).

General analytical procedure

One milliliter of the standard or sample solution (3–200 μg ml^{-1}) was transferred into test tubes, then 1 ml of borate buffer solution (pH 8.0) and 2 ml of NBD-Cl reagent (0.03% w/v in ethanol) were added, respectively. The tubes were capped, mixed well and left to stand in a thermostatically controlled water bath at 70 °C for 35 min. Then the tube was cooled rapidly in ice bath, and a volume of 0.1 ml of concentrated HCl was added. The contents of the tubes were quantitatively transferred into 10.0-ml calibrated flasks and the flasks were diluted to the mark with dimethylformamide. The fluorescence intensity of the resulting solutions was measured at 532 nm after excitation at 470 nm against reagent blanks treated similarly.

Results and discussion

Design and strategy for assay development

Sertraline has no native fluorescence, thus its direct fluorimetric determination of SRT was not possible without derivatization. NBD-Cl is an activated halide derivative that has been used as a fluorogenic reagent for the determination of amino group-containing compounds [24–26]. Our preliminary experiments have showed the reactivity of SRT, via its secondary amino group, with NBD-Cl in an alkaline buffered medium yielding a highly green fluorescent product that exhibited its highest fluorescence intensity at 532 nm after excitation at 470 nm. Figure 1 shows the spectral characteristics of the fluorescent reaction product. The present study was devoted to investigate the kinetics

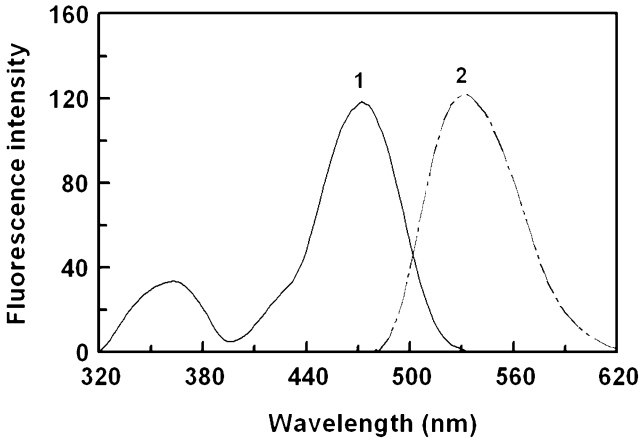

Fig. 1 Excitation (1) and emission (2) spectra of the reaction product of sertraline (6.0 μg ml^{-1}) with NBD-Cl reagent (0.003%, w/v) in a borate buffer (pH 8.0) at 70 °C. The excitation spectra was recorded at λem 532 nm whereas the emission spectrum was recorded at λex 470 nm

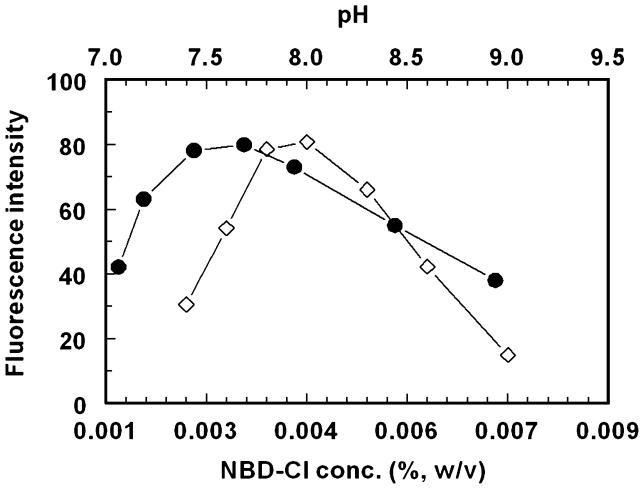

Fig. 2 Effect of NBD-Cl concentration (●) and pH (◊) on the derivatization reaction of SRT (5.0 μg/ml) with NBD-Cl. The measurements were carried out at 532 nm after excitation at 470 nm

and mechanism of the reaction, and its employment in the development of a fluorometric method, for the first time, for the determination of SRT in its pharmaceutical tablets.

Optimization of reaction conditions

The factors affecting the reaction conditions (pH, the concentrations of NBD-Cl reagent, reaction time, and temperature, and the diluting solvent) were studied by altering each variable by turn while keeping the others constant. All measurements of this study were carried out at 532 nm after excitation at 470 nm.

For investigating the effect of pH, the reaction was performed at different pH values (7.2–9.5). The results indicated that the fluorescence intensity was pH dependent (Fig. 2). The optimum pH was found to be 8.0. The reaction was studied as a function of NBD-Cl reagent concentration. The results revealed that the fluorescence intensity was dependent on NBD-Cl concentration (Fig. 2), and the higher fluorescence intensity was attained when the concentration of NBD-Cl in the final solution was 0.003% (w/v); 1 ml of 0.03%, w/v.

The effect of temperature on the reaction time was also studied by performing the reaction at different temperatures (40, 50, 60, 70, 80, and 90 °C) and the results indicated that maximum readings were obtained at 70–80 °C, however the readings at 80 °C were not reproducible. At higher temperatures ≥80 °C, a rapid progressive decrease in the readings occurred. Therefore, further experiments were carried at 70±2 °C. The results obtained from the optimizing of the reaction time indicated that the maximum fluorescence intensity was attained after 35 min. Longer reaction time decreased the fluorescence intensity. This was attributed to the degradation of the reagent at high temperature. This observation was coincident with the results reported by E.S. Akta et al. [27]. In order to select the most appropriate solvent for dilution, different solvents were tested: water, methanol, ethanol, isopropanol, acetonitrile, dimethylformamide, and acetone. Dimethylformamide was found to be an ideal diluting solvent as it afforded maximum sensitivity, and therefore it was selected for further investigations.

Under these conditions, significantly high fluorescence background was also observed. This was attributed to the hydrolysis of NBD-Cl to its corresponding hydroxyl-

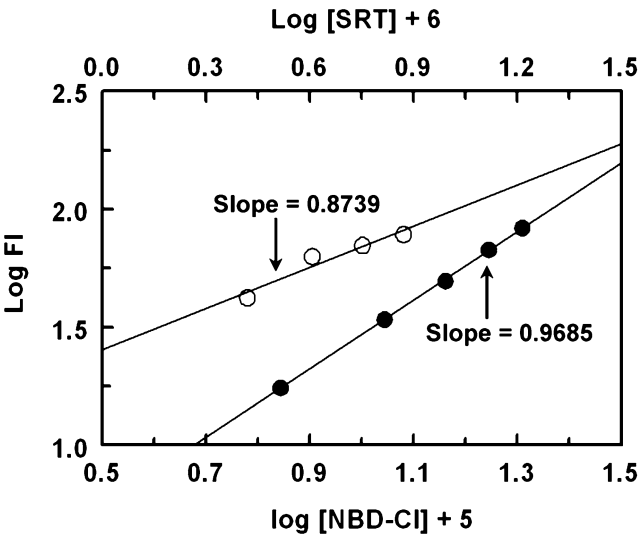

Fig. 3 Limiting logarithmic plots for the molar reactivity of sertraline with NBD-Cl: (○) log [fluorescence intensity] vs. log [NBD-Cl] with [sertraline] kept at 1.63×10^{-5} M; (●) log [fluorescence intensity] vs. log [sertraline] with [NBD-Cl] kept at 1.5×10^{-4} M; FI is the fluorescence intensity. 5 and 6 were added to the values of log [NBD-Cl] and log [SRT] to eliminate the negative sign values

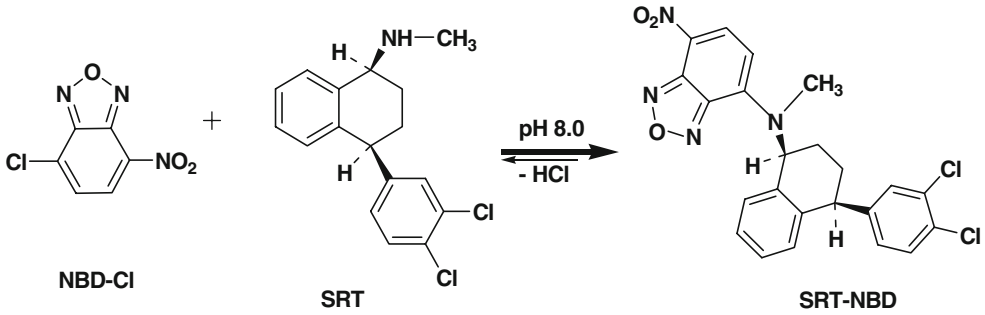

Fig. 4 A scheme for the reaction of sertraline with NBD-Cl reagent

derivative [28]. The fluorescence of NBD-OH was found to be quenched in acid medium [25]. Therefore acidification of the reaction mixture prior to its dilution and measurement steps was necessary to remarkably decrease the background signal. Meanwhile, the reaction product was not affected, thus the sensitivity was ultimately increased. The amount of hydrochloric acid required for acidification was found to be 0.1 ml concentrated hydrochloric acid.

Stoichiometry of the reaction

The stoichiometry of the reaction was studied under the optimum conditions adopting the limiting logarithmic method [29]. The results showed that two straight lines were obtained. The first line was obtained by using increasing concentrations of NBD-Cl with fixed concentration of SRT (1.63×10^{-5} M). The second line was obtained by using increasing concentrations of SRT with fixed concentration of NBD-Cl (1.5×10^{-4} M). The comparable values of the slopes of the two lines (0.8739 and 0.9685, Fig. 3), indicated that the molar ratio of SRT : NBD-Cl may be 1:1 with existence of some sort of equilibrium. Accordingly, the reaction pathway was postulated as described in Fig. 4.

Order and specific rate constant of the reaction

The order of the reaction with respect to NBD-Cl was determined by studying the reaction at different concentrations of NBD-Cl (6×10^{-5} –3.6×10^{-4} M) with fixed concentration of SRT (1.6×10^{-5} M) and the fluorescence intensity-time curves were generated. The initial rate at each concentration was determined from the slope tangent of the curve. Also, the order of the reaction with respect to SRT was determined using different concentrations of SRT (0.55×10^{-6} –1.6×10^{-5} M) with fixed concentration of NBD-Cl (1.5×10^{-4} M). The logarithm of initial rate was plotted versus the logarithm of the molar concentration of either NBD-Cl reagent or SRT. In both cases, straight lines with slopes≈1 and passing through the origins were obtained. This indicated that the order of the reaction with respect to either NBD-Cl or SRT was one. The overall order of the reaction in this case is second order. However, under the optimum conditions, the reaction occurs with relative excess amount of the reagent which can be considered as constant (as sustained release case). Therefore, the reaction was considered as a pseudo-first order and obeyed the following equation [30]:

$$V = \Delta F/\Delta t = K'C^n$$

Where: V is the initial reaction rate, F is the fluorescence intensity, t is the measuring time, K' is the pseudo-first order rate constant, C is the molar concentration of SRT, and n is the order of the reaction. The logarithmic form of the above equation is written as follow:

$$\mathrm{Log}V = \log \Delta F/\Delta t = \log K' + n \log C$$

The order of the reaction was obtained from the slopes (n) of logV (for different SRT concentrations) vs. logC. Figure 5 shows the fluorescence—time curves for the reaction of NBD-Cl (1.5×10^{-4} M) at 70 °C with different concentrations of SRT under the conditions of pseudo-first order kinetics. The results obtained indicated that the value of (n) was 0.8267 (≈ 1) proving that the order of the studied reactions was first order with respect to SRT. The pseudo

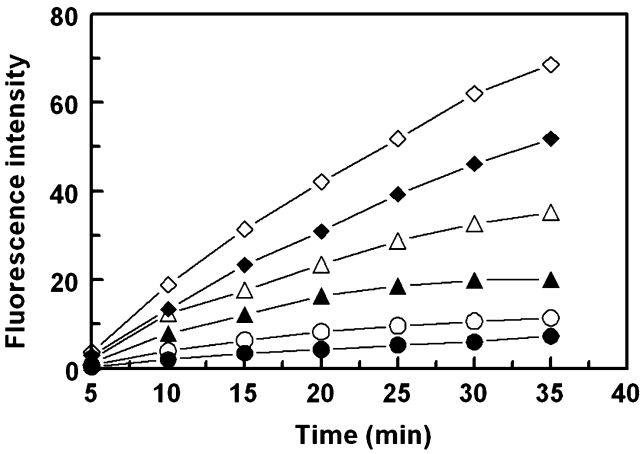

Fig. 5 The fluorescence-time curve for the reaction of sertraline with NBD-Cl (1.5×10^{-4} M) at 70 °C, the concentrations of sertraline were 0.55×10^{-6} (▲), 1.10×10^{-6} (Δ), 2.19×10^{-6} (♦), 4.38×10^{-6} (◊), 6.58×10^{-6} (○), and 8.77×10^{-6} M (●)

first-order rate constants (k) were calculated from the slope of the plot of log F_t against time, where F_t represent the fluorescence intensity of the formed product at time t. The results indicated that the value of the specific rate constant of the reaction was 0.035 min^{-1}.

Because the aim of our work was targeted to the development of a new fluorometric determination of SRT rather than the determination of the order of reaction of SRT with NBD-Cl, we simplified the kinetic assumptions (i.e. we have not determined the kinetics by higher sophisticated methods)

Activation energy of the reaction

The activation energy, the minimum kinetic energy a molecule must possess in order to undergo the reaction, can be determined from Arrhenius equation [31]:

$$V = k = Ae^{-\mathrm{Ea/RT}}$$

Where: (k) is the initial rate of the reaction , (A) is a constant known as frequency factor, (Ea) is the activation energy, (T) is the absolute temperature, and (R) is the gas constant; 1.987 calories $degree^{-1}$ $mole^{-1}$. The logarithmic form of the above equation is written as follow:

$$\mathrm{Log\ k} = \log A - \mathrm{Ea}/2.303\mathrm{RT}$$

The activation energy of the reaction of SRT with NBD-Cl was determined by studying the reaction at different temperatures; 40, 50, 60, 70, and 80 °C using fixed concentrations of SRT and the reagent. The fluorescence-time curves at these temperatures were constructed to determine the initial rates, then plotting 1/T against log k to determine the slope of the line; —Ea / 2.303 R (Fig. 6). The obtained activation energy was 27.34 kJ $mole^{-1}$. This low activation energy indicated the suitability of NBD-Cl as a derivatizing fluorogenic reagent for the determination of SRT under mild conditions.

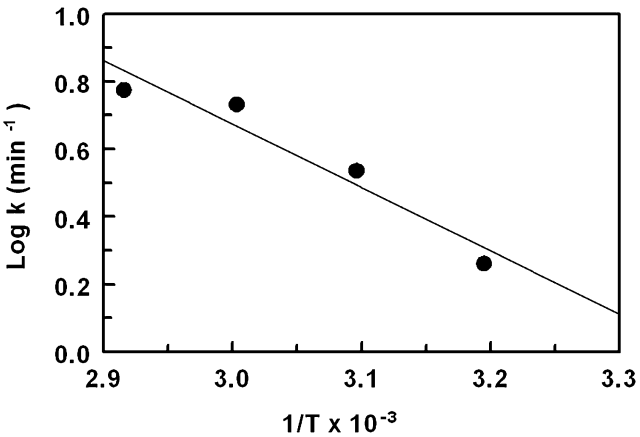

Fig. 6 Arrhenius plot for the activation energy of the reaction of sertraline with NBD-Cl

Table 1 Recovery studies for the proposed fluorometric method for determination of SRT

Added concentration (μg ml^{-1})	Recovery (% ± RSD)[a]	
	Intra-assay	Inter-assay
1.0	97.17±2.05	99.05±2.61
5.0	100.62±1.62	101.59±2.15
10.0	101.81±1.29	102.66±1.59
15.0	99.88±0.65	99.89±1.13
20.0	100.58±0.94	100.71±1.20

[a] values are mean of five and three determinations for intra- and inter-assay, respectively

Validation of the proposed method

Linearity and limit of detection

The fluorescence intensity of the reaction mixtures containing varying amounts of SRT was measured, and the calibration plots of fluorescence intensity versus the concentrations of SRT were established. The regression equation: FI=3.303 (±0.420)+20.316 (±0.418) C, where FI and C are the fluorescence intensity and SRT concentration in μg ml^{-1}. The correlation coefficient was 0.9998. The limits of detection (LOD) and limits of quantitation (LOQ) were determined [32] using the formula: LODorLOQ = kSD_a/b, where κ=3.3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope. The LOD and LOQ were 0.07 and 0.21 μg ml^{-1}, respectively.

Precision and accuracy

Intra-assay precision was studied at five concentration levels (1.0, 5.0, 10.0, 15.0, and 20.0 μg ml^{-1}) of SRT. Five aliquots were prepared from each concentration level and determined. The RSD calculated for the values was ≤2.05% (n=5). The inter-assay precision was carried out using the

Table 2 Determination of SRT in tablets by the proposed and reported methods

Tablets	Recovery (% ± SD)[a]		t-value[c]	F-value[c]
	Proposed method	Reported method[b]		
Lustral	100.74±1.01	100.62±1.54	0.16	2.34
Sirto	96.97±1.62	97.54±1.68	0.47	1.08
Moodapex	102.23±1.27	101.43±1.35	0.97	1.14

[a] Values are mean of five determinations

[b] Reference [6]

[c] The tabulated values at 95% confidence limit are 2.228 and 5.051, respectively

same concentration levels at three consecutive days. The RSD values of the determinations were ≤2.61 (Table 1).

The accuracy of the proposed method was determined by the recovery studies. The recovery values were presented as percentages, calculated by the formula: (calculated concentration / nominal concentration)×100]. The recovery values ranged from 97.17 to 102.66 (± 0.65–2.61%) indicating the accuracy of the method (Table 1).

Robustness and ruggedness

In order to measure the extent of the method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged. The reaction conditions were interchanged within the range of 1–10% of the optimum recommended conditions. The studied parameters were: the pH of the buffer used and the temperature. The results revealed that the method was robust for the small change in the pH of the derivatization reaction, where the results did not significantly change in the range of 7.9–8.1. However, increasing the pH value above 8.3 resulted in dramatic decrease in the fluorescence intensity. With respect to the temperature of the reaction, the results did not significantly change in the range of 70±2 °C. The ruggedness of the method was evaluated by applying the recommended analytical procedures on the same spectrofluorometer (independently on different days) on the analysis of series of SRT samples. The obtained RSD values from three operators were not more than 3%.

Application of the proposed method to analysis of SRT in tablets

It is evident from the above-mentioned results that the proposed method gave satisfactory results with SRT in bulk. Thus its tablets were subjected to the analysis of their contents from the active ingredient by the proposed and the reported [6] methods. The tablets content, as percentage, were 96.97–102.23±1.01–1.62 % (Table 2). These results were compared with those obtained by the reported method by statistical analysis with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence limit proving similar accuracy and precision in the analysis of SRT in its tablets.

Conclusions

The present fluorometric study investigated, for the first time, the reaction of SRT with NBD-Cl, and described a novel simple, sensitive and accurate fluorometric method for the determination of SRT in tablets. The order, specific rate constant and activation energy of the fluorometric reaction were determined. The proposed method was successfully applied to the determination of SRT in its pharmaceutical tablets. The proposed method is easy to perform and yielded highly reliable and accurate analytical results. Thus, it is suitable for the determination of SRT in quality control laboratories.

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