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Development of a validated spectrofluorimetric method for assay of sotalol hydrochloride in tablets and human plasma: application for stability-indicating studies

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Abstract: The native fluorescence of sotalol hydrochloride (SOT) was used as a basis for establishing a new method of analysis for SOT in tablets and spiked human plasma. The fluorescence of SOT in water was measured at 310 nm when excited at 235 nm. The detection limit (LOD) was 0.37 ng/mL and the quantification limit (LOQ) was 1.08 ng/mL. The proposed method offers high sensitivity which permits determination of SOT, even if present in a very small amount, in human plasma. The obtained results were successfully compared to that of a reference pharmacopeial method and statistical analysis proved a good agreement between the results of both methods. Further investigation of the SOT stability upon exposure to various stress conditions, such as acidic, alkaline, oxidative and photolytic degradation conditions was also performed. The kinetics of acidic, alkaline and oxidative degradation of the drug showed a pseudo first order degradation reaction. A proposal of the degradation pathway was suggested and confirmed by developing a thin layer chromatographic method used for separation of SOT and its acidic and alkaline degradation products.

Keywords: Sotalol HCl; Spectrofluorimetry, Stability-study; Tablets; Human plasma; Method validation.

1 Introduction

Sotalol hydrochloride (SOT) is chemically designated as N-[4-[1-hydroxy-2-(propan-2-ylamino)ethyl]phenyl]methane-sulfonamide (Figure 1) [1] and is considered a non-cardio selective beta- blocker. Because of its pro-arrhythmic effects, it is usually used for severe or life-threatening arrhythmias [2].

The official method for the determination of SOT in the United States pharmacopeia [3] is based on HPLC while the official assay method in the British pharmacopeia [4] is based on non-aqueous titration with perchloric acid using potentiometric detection of the end point. A good guide to the work reported up to 1992 on the analysis of SOT is presented as a comprehensive monograph in the series of Analytical Profiles of Drug Substances [5]. Later, different analytical methods were developed for the determination of SOT alone or in combination with other drugs. A spectrofluorometric method for the determination of SOT based on fluorescence quenching technique was reported by Zhang et al. [6]. This method is based on quenching the fluorescence of the cucurbit[7]uril and palmatine complex by SOT with a linearity range of 0.10-3.60 µg/mL. Different spectrophotometric methods were reported for the determination of SOT based on the formation of charge transfer complex with 4-chloro-7-nitrobenzofurazan (NBD-Cl) reagent [7], derivative spectrophotometry [8,9] and chemometric-assisted spectrophotometry [10]. The reported chromatographic methods for SOT determination include HPLC with UV detection [10-13], fluorescence detection [14-20] and MS detection [21-24]. Stability of SOT in oral suspension was studied during its storage at different temperatures using HPLC in order to investigate its shelf-life stability [25,26]. Capillary electromigration techniques were also utilized for the determination of SOT [27,28]. In addition, Ensafi et al. [29] and Heli et al. [30] reported two different electrochemical methods for the analysis of SOT. To the best of our knowledge, no stability-

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Figure 1: Structural formula of SOT.

indicating direct spectrofluorimetric method has been published for the determination of SOT.

The aim of the present study is to develop and validate a stability-indicating sensitive and simple spectrofluorimetric method for the determination of SOT under different stress conditions. The study is extended to the evaluation of SOT in pharmaceutical preparations and spiked human plasma.

2 Experimental

2.1 Apparatus

The spectrofluorimetric measurements were performed using Shimadzu spectrofluorophotometer (RF-1501) with a xenon lamp and excitation and emission slits set at 5 mm. Also, a 1-cm quartz cell was used. The photo stability study was carried out using a Camag UV-Lamp (S/N 29000), dual wavelength (254/336), 2 X 8W. The oxygen of the solvents was eliminated by sonication. For the analysis of spiked human plasma, a nylon membrane syringe filter (Wicom GmbH, Germany) was used with a pore size 0.45 μm . A Jenway pH-meter (model 3505) was used for pH measurements.

2.2 Materials and Methods

2.2.1 Chemicals and reagents

Sotalol hydrochloride was generously provided by AMOUN (Cairo, Egypt) with purity of 99.7% according to the official USP method [3] and was used as received in this study. Betacor[®] tablet containing 80 mg of SOT (a product of AMOUN) was purchased from a local pharmacy in Egypt. All the used solvents are of HPLC grade except for acetone and acetic acid that are of analytical reagent grade. Acetonitrile and n-propanol were products of Sigma-Aldrich, Germany while methanol was obtained from Tedia (USA). Tween 80, Hydroxy propyl β -cyclodextrin

(HP- β -CD), acetone, ethanol, acetic acid (96%), sodium acetate trihydrate, boric acid, sodium hydroxide, hydrogen peroxide (30%) and sulfuric acid, were all obtained from El-Nasr Pharmaceutical Chemical Co. (Adwic, Egypt). Sodium dodecyl sulphate (SDS, 95%) was obtained from Winlab (UK). SDS, CD and Tween-80 were prepared as 0.5% w/v aqueous solutions. 0.2 M Acetate buffer was prepared for covering the pH range of (3.6–5.0) while 0.2 M borate buffer for the pH range of (7.5 – 9.5). Plasma samples were obtained from the Blood Bank of Mansoura University Hospital, Mansoura, Egypt. They were stored at -20°C and were allowed to thaw gradually at room temperature. The analysis of spiked human plasma was performed after approval of the ethical committee of the Faculty of Pharmacy, Mansoura University on 11/6/2016. The double distilled water was obtained using A4000D Aquatron water still, Staffordshire, UK. The conductivity of random samples of the used water was measured and it ranged from 2.4 to 3.0 μS in most cases.

2.2.2 Preparation of Standard solutions

Stock solution of SOT (200.0 $\mu\text{g/mL}$) was prepared by dissolving 20 mg of the pure powder of the drug in 100 mL distilled water using volumetric flask. Several dilutions with distilled water were made to obtain a working standard solution. The stock solution was stable upon storage in the refrigerator for at least one month without alteration.

3 General Procedures

3.1 Construction of calibration graph

Aliquots from the standard solution covering the working concentration range (40–280 ng/mL) were transferred into a set of 10 mL volumetric flasks, then completed to the volume of the flask with distilled water. The prepared solution was then well mixed by sonication. The fluorescence intensities were measured at 310 nm when excited at 235 nm. The relative fluorescence intensities (RFI) obtained were plotted versus the corresponding concentrations of the drug and then the regression equation was derived.

3.2 Analysis of SOT in raw material

Accurate volumes of the working standard solution covering the linearity range of (40 - 280 ng/mL) were

transferred into a set of 10 mL volumetric flasks. The emission intensity of prepared samples were measured and the content of SOT was reported as percentage in the raw material.

3.3 Analysis of SOT in tablets

Twenty tablets of the Betacor® 80 mg were weighed and grinded to fine powder. A precisely weighed amount of the mixed contents of the powdered tablets equivalent to 10 mg SOT was transferred into a 100 mL volumetric flask followed by the addition of about 70 mL of distilled water. The flask contents were then mixed by sonication for thirty minutes. The flask was then completed to the full volume with the distilled water. Filtration of the flask contents was performed to remove the insoluble additives. The filtrate was additionally diluted with distilled water to get the required working standard solution. Procedure mentioned in the “**Construction of calibration graph**” subheading was followed. The nominal contents were calculated through the corresponding regression equation.

3.4 Analysis of SOT in spiked human plasma

In 5.0 mL screw-capped tubes for centrifugation, 1 mL of human plasma was spiked with different volumes of SOT working standard solution and well mixed. Then, 3 mL of methanol was added as a precipitating agent [31] followed by the addition of distilled water to reach the full volume. After mixing by the vortex for 30 s, the screw capped tubes were centrifuged at 3,500 rpm for thirty minutes. The produced clear supernatants were then filtered with a membrane syringe filter (0.45 µm pore size) into a 20 mL beaker. Then, 1 mL of each filtrate was transferred to a 10 mL volumetric flask and completed with distilled water to obtain the final concentration covering linearity range of 40.0-200 ng/mL. The fluorescence intensities were measured at 340 nm when excited at 235 nm. A blank experiment was carried out simultaneously using double-distilled water in spiking the plasma instead of SOT.

3.5 Stability studies for SOT

3.5.1 Application of acidic and alkaline degradation

1 mL of SOT working standard solution (65 µg/mL) was transferred into a set of 25.0 mL volumetric flasks. To each flask, 5 mL of either 2 M NaOH or 2 M HCl were added

separately. The solutions were kept at room temperature for different time intervals ranging from 0.0 to 60 minutes. The contents of each flask were neutralized to pH 7.0 with either 2 M HCl or 2 M NaOH. Degraded samples were diluted as appropriate with distilled water to obtain a measurable fluorescence signal within the concentration range of the calibration curve. Finally, the steps described in “**procedure of calibration graph**” subheading were performed to obtain concentration of SOT in the presence of its degradation products using the corresponding regression equation.

3.5.2 Application of oxidative degradation

1 mL of SOT working standard solution (65 µg/mL) was transferred into a set of 25 mL volumetric flasks, and to each flask, a 5 mL of 0.5% H₂O₂ solution was added. The solutions were kept at room temperature for different time intervals ranging from 0.0 to 60.0 minutes. At the specified time, degraded samples were diluted as appropriate with distilled water to obtain a measurable fluorescence signal within the concentration range of the calibration curve. Finally, the steps described in “**Procedure for calibration graph**” subheading were performed to obtain concentration of SOT in the presence of its degradation products. The drug concentration was then calculated as previously mentioned.

3.5.3 Application of photolytic degradation

1 mL of SOT working standard solution (65 µg/mL) was transferred into a set of 25.0 mL volumetric flasks and completed with distilled water to obtain 2.60 µg/mL. The prepared flasks were exposed to UV-lamp at its two wavelengths for different time intervals ranging from 0.5 to 3.0 hours. At a specified time, a 260 ng/mL solution was prepared from the exposed flasks. Under daylight, 1 mL of SOT working standard solution (65 µg/mL) was transferred into a set of 25.0 mL volumetric flasks and completed with distilled water to obtain 2.60 µg/mL. The prepared flasks were left in daylight for different times ranging from 0.5 to 8.0 hours. At a specified time, a 260 ng/mL solution was prepared from the exposed flasks. The drug concentration was then calculated as previously mentioned.

Ethical approval: The conducted research is not related to either human or animal use.

4 Results and discussion

Based on the previously reported HPLC-fluorescence method [15], the fluorescence intensity was examined at 310 nm after excitation at 235 nm. As a consequence, the present work aims at discovering a new technique for the analysis of SOT in tablets and human plasma in addition to studying the stability of SOT under various stress conditions, according to the ICH guidelines. SOT has a strong native fluorescence at 235/310 nm (Figure 2). Various experimental parameters affecting the fluorescence intensities of SOT were carefully studied and optimized.

4.1 Selection of optimum experimental conditions

4.1.1 pH effect

Various kinds of buffers covering the whole pH range were utilized to investigate the pH effect on the fluorescence intensity of SOT. Among the buffers utilized are 0.2 M acetate buffer which covers the pH range from 3.6 to 5 and 0.2 M borate buffer which covers the pH range from 7.5 to 9.5. For the studied drug, no buffer was used throughout the study, since water alone resulted in the highest fluorescence intensity, as shown in Figure 3.

4.1.2 Diluting solvent effect

Various diluting solvents were investigated to show their influence on the RFI of SOT. The solvents included in this investigation were water, methanol, ethanol, n-propanol, iso-propanol, acetonitrile, and acetone. Also, the effects of 0.1 M HCl, 0.1 M H_2SO_4 and 0.1 M NaOH were studied. Methanol usage resulted in a decrease in the RFI of SOT, while ethanol, n-propanol, acetonitrile and iso-propanol resulted in a very high blank reading. On the other hand, the fluorescence of SOT was greatly quenched by dilution with acetone. Also, all 0.1 M HCl, 0.1 M H_2SO_4 and 0.1 M NaOH resulted in a decrease in the RFI of SOT. So, the distilled water was the optimum solvent, as it had the lowest blank reading, and consequently the highest RFI with reproducible results, as shown in Figure 4.

4.1.3 Surfactant effect

Various types of surfactants, including anionic, nonionic and macromolecule surfactants, such as sodium dodecyl

Figure 2: Fluorescence spectra of 200.0 ng/mL of SOT in water. (A, A''): Excitation and emission spectra of SOT, (B, B''): Excitation and emission spectra of water.

Figure 3: Effect of pH (0.2 M acetate buffer, 0.2 M Borate buffer, 0.1 M H_2SO_4 , 0.1 M NaOH and 0.1 M HCl) on the RFI of SOT (180.0 ng/mL) and $n=5$.

sulphate (SDS), tween 80 and β -cyclodextrin were used to investigate the effect of surfactant on RFI of SOT. All these surfactant did not have any positive effect on the fluorescence intensity of SOT. So, the study was completed without surfactant, as shown in Figure 5.

4.1.4 Time

Time factor effect was investigated and the results showed that the fluorescence intensity stayed unchanged for at least 4 hours.

Figure 4: Effect of diluting solvents on the RFI of SOT (180.0 ng/mL) and $n=5$.

Figure 5: Effect of surfactants on the RFI of SOT (180.0 ng/mL) and $n=5$.

4.2 Method validation

4.2.1 Linearity and range

Calibration curve was constructed in the range of 40 to 280 ng/mL. The relative fluorescence intensity for each concentration was recorded in triplicates then plotted against the corresponding concentration to obtain the calibration graph.

Statistical analysis [32] of the data is summarized in Table 1. The data presented in the table verified the linearity of the calibration graph as homoscedasticity test shows F test value = 2.75 and the tabulated F value = 3.18 at significance level of 95% and degrees of freedom

Table 1: Analytical performance data for the determination of SOT by the proposed method.

Parameter	Value
Linearity range (ng/mL)	40 – 280
Intercept (a)	95.148
Slope (b)	3.113
Correlation coefficient (r)	0.9998
S.D. of residuals ($S_{y/x}$)	2.163
S.D. of intercept (S_a)	0.337
S.D. of slope (S_b)	0.009
S.D.	1.042
% RSD ^a	1.044
% Error ^b	0.330
LOD ^c (ng/mL)	0.358
LOQ ^d (ng/mL)	1.084
Homoscedasticity ^e	Calculated F 2.75 Tabluted F 3.18

^a Percentage relative standard deviation.

^b Percentage relative error.

^c Detection limit.

^d Quantification limit.

^e Significance level 95% and degrees of freedom of 9 for both numerator and denominator [32].

of 9 for both numerator and denominator which ensures the homoscedasticity of the variance and the validity of the proposed method in the studied range. Compared to the previously reported quenching spectrofluorometric method [6], the developed method offers much higher sensitivity with fewer steps and lower cost.

4.2.2 Detection and quantification limit

Detection limit (LOD) and quantification limit (LOQ) were calculated according to the ICH Q2 (R1) recommendation [33] and were found to be 0.37 and 1.08 ng/mL, respectively. They were calculated from the standard deviation of the response and the slope of the calibration curve in accordance to the following equations:

$$\text{LOQ} = 10 S_a / b \quad \text{LOD} = 3.3 S_a / b$$

where, S_a is standard deviation of the intercept of the calibration curve and, b is the slope of the calibration curve. The results are shown in Table 1.

Table 2: Assay results for the determination of SOT in pure form by the proposed method and the reference USP method.

Compound	Proposed method			Reference USP method [3]		
	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (µg/mL)	Amount found (µg/mL)	% Found
SOT	40	39.882	99.71	100	100.007	100.01
	60	60.262	100.44	150	149.988	99.99
	100	98.534	98.53	200	200.005	99.20
	160	161.858	101.16			
	180	178.414	99.12			
	200	201.400	100.70			
	220	220.801	100.36			
	240	241.829	100.76			
	260	258.319	99.35			
	280	278.808	99.57			
Mean			99.79			99.70
± S.D.			1.04			0.49
<i>t</i> -test			0.46 (2.2)*			
<i>F</i> -test			3.3 (19.3)*			

N.B. Each result was the average of three separate determinations.

* The figures between parentheses were the tabulated *t* and *F* values at $p=0.05$ and degrees of freedom for *t*-test 11 and for *F*-test 9 for numerator and 2 for denominator, respectively [32].

4.2.3 Accuracy and precision

Statistical analysis (using Student's *t*-test and variance ratio *F*-test at $p=0.05$ and degrees of freedom for *t*-test 11 and for *F*-test 9 for numerator and 2 for denominator, respectively [32]) of the results obtained by the designed method compared to the official USP reference one [3] revealed no differences between them regarding accuracy and precision, as presented in Table 2.

Precision was calculated in terms of intra-day repeatability and inter-day reproducibility. The intra-day repeatability was evaluated in terms of %RSD by the analysis of three different concentrations of the pure drug for each in triplicates on a single day. The inter-day reproducibility was checked on 3 different days by repeating the analysis of the same concentrations levels as for intra-day repeatability. The intra-day repeatability and inter-day reproducibility results are summarized in Table 3.

4.2.4 Specificity

It was indicated that the excipients of the tablet preparation as microcrystalline cellulose, magnesium stearate and lactose did not affect the results of the designed method as shown in table 4.

5 Application

5.1 Assay of pharmaceutical products

One of the purposes of the current study was to determine SOT in tablet dosage form. Results were compared to the results obtained from the reference method using Student's *t*-test and *F*-test at $p=0.05$ and degrees of freedom for *t*-test 4 and for *F*-test 2 for numerator and 2 for denominator, respectively. Table 4 shows that there was no significant difference between the results of the designed method and the official one.

5.2 Application of the designed method to the determination of the SOT in spiked human plasma

The maximum plasma concentration of SOT was 1.1–1.4 mg/L after daily oral doses of 400 mg for 5 days. SOT was

Table 3: Precision data for the determination of SOT by the proposed method.

Parameter		SOT (ng/mL)		
		80	160	240
Intra-day	% Found	99.09	99.25	100.09
		100.60	100.40	101.40
		100.90	99.37	99.69
	Mean	100.19	99.67	100.39
	S.D.	0.97	0.63	0.89
	% RSD	0.96	0.63	0.89
	% Error*	0.55	0.36	0.51
Inter-day	% Found	100.15	99.35	99.69
		98.57	100.61	100.09
		101.90	102.24	100.89
	Mean	100.21	100.73	100.22
	S.D.	1.66	1.44	0.61
	% RSD	1.66	1.43	0.61
	% Error*	0.96	0.83	0.35

N. B. Each result was the average of three separate determinations.

* % error is calculated considering the average as exact value.

absorbed completely from the gastrointestinal tract (GIT) after oral administration with bioavailability of about 90% [1].

The analysis of SOT in spiked human plasma was performed through a simple extraction procedure [31]. According to the above described experimental conditions, a linear relationship was established by plotting the RFI against the drug concentration in ng/mL. Assay results for the determination of SOT in spiked human plasma samples using proposed method is shown in Table 5.

The proposed method shows satisfactory results for the determination SOT in spiked human plasma over a concentration range of 40 to 200 ng/mL. Figure 6 shows fluorescence spectra for blank and spiked human plasma sample.

5.3 Application of stability studies and degradation kinetics

5.3.1 Degradation in acidic and alkaline medium

As a sulfonamide, SOT was found to be easily degraded by acidic and alkaline hydrolysis. The degradation in 0.2 M-2.0 M HCl or NaOH at room temperature and at 100°C was studied. At room temperature, the degradation was very slow in 0.2 M-1.0 M HCl and NaOH. However, about 30% of the drug degraded in 2 M HCl and NaOH after thirty minutes, as revealed by the decrease in RFI of the SOT. So, 2 M HCl or NaOH was found to be the suitable concentration to be used for degradation studies. The degradation of SOT in acidic and alkaline medium at room temperature was found to be time dependent, as it was noticed that RFI

Table 4: Assay results for the determination of SOT in its tablet dosage form by the proposed method and the reference USP method.

Parameter	Proposed method			Reference USP method [3]		
	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (µg/mL)	Amount found (µg/mL)	% Found
Betacor tablets (80 mg)	80	79.568	99.46	100	100.083	100.08
	160	160.960	100.60	150	149.848	99.90
	240	239.496	99.79	200	200.073	100.44
Mean			99.95			100.14
± S.D.			0.587			0.274
t-test			0.51(2.78)*			
F-test			4.55 (19)*			

N.B. Each result was the average of three separate determinations.

*The figures between parentheses were the tabulated t and F values at p=0.05 and degrees of freedom for t- test 4 and for F- test 2 for numerator and 2 for denominator, respectively [32].

Table 5: Assay results for the determination of SOT in spiked human plasma samples using the proposed method.

Parameter	Concentration taken, (ng/mL)	Concentration found (ng/mL)	% Found
SOT	40	40.361	100.90
	100	101.203	101.20
	160	158.262	98.91
	180	177.69	98.72
	200	202.853	101.43
S.D.			1.25
% RSD ^a			1.24
% Error ^b			0.57
Mean % Found			100.34

^a Percentage relative standard deviation.^b Percentage relative error.

of SOT decreased as time intervals increased in 2 M HCl or NaOH (Figure 7).

Plotting the logarithm of concentrations of SOT against time produced straight lines which indicates pseudo first order reaction (Figure 8 A and B). The apparent pseudo first order rate constants and half-life times for both alkaline and acidic degradation were calculated (Table 6). The acidic and basic treatments of SOT were expected to cause cleavage of its sulfonamide bond [11]. The Proposal of the degradation pathways of SOT were demonstrated in Scheme1.

Thin layer chromatography (TLC) of SOT and its degradation products (after its acidic and alkaline degradation) was developed using aluminum cards of silica gel and a developing TLC eluent system consisting of methanol:chloroform:25% ammonium hydroxide (6:4:0.05). Visualization of the separated spots was carried out under UV lamp at 254 nm. R_f of the acidic and alkaline degradation product was 0.85 while for intact drug was 0.62. The proposal of the degradation pathway is indicated in Scheme 1.

5.3.2 Oxidative degradation

Obvious degradation of SOT was observed with oxidative conditions, as treating the drug with high concentrations of H_2O_2 (30%) solution at room temperature resulted in immediate degradation of SOT, while treating it with lower concentrations of H_2O_2 resulted in very slow degradation.

Figure 6: Emission spectra of SOT in spiked human plasma where (a) plasma blank and (b, c, d, e, f and g) SOT in spiked human plasma in a concentration of 40, 100, 120, 160, 180 and 200 ng/mL. **Figure 7:** Effect of different exposure time with: (A) 2 M NaOH and (B) 2 M HCl on the RFI of SOT (260.0 ng/mL) at room temperature (n=5).

As shown in Figure 9, the RFI of SOT decreased with increasing exposing time to 0.5% H_2O_2 . Plotting the logarithm of concentrations of SOT against time produced straight lines which indicate pseudo first order reaction for the oxidative degradation of SOT (Figure 8C). Moreover, the first order rate constants and half-life times for oxidative degradation were calculated (Table 6).

Upon using 0.5% H_2O_2 solution, about 30% degradation of SOT was observed after 30 minutes, as revealed by the decrease in the RFI of SOT. The pathway of the oxidative degradation of SOT was assumed to proceed by oxidation of hydroxyl group into carbonyl group as

Scheme 1: The proposed pathway of alkaline, acidic and oxidative degradation of SOT.

Table 6: Results of the degradation study of SOT (260ng/mL) under different stress conditions on the kinetic parameters of SOT at room temperature

Degradation condition	Reaction rate constant (K, min ⁻¹)	Half life time (t _{1/2} , min)
Alkaline degradation (2 M NaOH at room temp.)	0.012178	56.9
Acidic degradation (2 M HCl at room temp.)	0.007116	97.4
Oxidative degradation (0.5% H ₂ O ₂ at room temp.)	0.010081	68.7

illustrated in Scheme 1. This assumption can be confirmed by the work published by Abdelmonem *et al.* [11], De Souza *et al.* [34] and Liu and Wang [35]. This proposal can explain the observed decrease in the RFI of SOT upon oxidative degradation due to the electron withdrawing effect of the formed carbonyl group.

5.3.3 Photolytic degradation

The stability of SOT under the effect of UV-light was studied by exposing the SOT to the UV-light. No degradation of SOT upon exposure to the UV-light at 366 nm was observed, even after 48 hr. On the other hand, by exposing SOT solutions to UV-light at 254 nm, very limited degradation of SOT was observed (about 10%) after 24 h of exposure.

Figure 8: Semilogarithmic plot of SOT (260.0 ng/mL) versus different exposure times with: (A) 2 M NaOH, (B) 2 M HCl and (C) 0.5% H₂O₂ at room temperature (n=5).

Figure 9: Effect of different exposure time with 0.5% H₂O₂ on the RFI of SOT (260.0 ng/mL) at room temperature (n=5).

6 Conclusion

This study reported a green, sensitive, rapid and time-saving spectrofluorimetric method for the determination of SOT. The suggested method could be used for the determination of SOT in pharmaceutical preparations and biological fluids. Besides, the importance of the suggested method is extended to be used as a stability-indicating assay method. It does not need the complicated handling related to chromatographic techniques. Furthermore, the developed method offers better sensitivity and less experimental steps than the previously reported quenching spectrofluorimetric method. Also, there is no need for organic solvent usage, which makes the

method of lower toxicity and cost. On the basis of its high sensitivity, greenness and simplicity, the designed method could be convenient for routine quality control in the pharmaceutical industry.

Conflict of interest: Authors declare no conflict of interest.

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