SPECTROFLUORIMETRIC METHOD FOR DETERMINATION OF SOLIFENACIN SUCCINATE IN BULK AND IN TABLET FORMULATION**

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A sensitive, reproducible, and cost-effective spectrofluorimetric method was developed for the quantification of solifenacin succinate in bulk and its tablet formulation. The method was validated in accordance with the International Conference on Harmonization guidelines with respect to linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. Excellent linearity was noted in the concentration range of 15.0–95.0 μg/mL with a correlation coefficient (r^2) of 0.9961. The limits of detection and quantitation for the proposed method were found to be 1.19 and 3.62 μg/mL, respectively. Excellent recovery of the drug was obtained from its marketed tablet formulation with the proposed method.

Keywords: *spectrofluorimetric method, limit of detection, limit of quantification, solifenacin succinate.*

СПЕКТРОФЛУОРИМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ СУКЦИНАТА СОЛИФЕНАЦИНА В НЕФАСОВАННОЙ И ТАБЛЕТИРОВАННОЙ ФОРМАХ

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Разработан чувствительный, воспроизводимый и экономичный спектрофлуориметрический метод для количественного определения сукцината солифенацина в нефасованной и таблетированной формах. Метод проверен в соответствии с рекомендациями Международной конференции по гармонизации на линейность, точность, предел обнаружения, предел количественного определения и надежность. В области концентраций 15.0—95.0 мкг/мл отмечена отличная линейность с коэффициентом корреляции (r^2) 0.9961. Пределы обнаружения и количественного определения 1.19 и 3.62 мкг/мл. С помощью предложенного метода показана хорошая извлекаемость лекарственного средства из выпускаемой таблетированной формы.

Ключевые слова: *спектрофлуориметрический метод, предел обнаружения, предел количественного определения, сукцинат солифенацина.*

Introduction. Solifenacin succinate, [(3*R*)-1-azabicyclo[2.2.2]octan-3-yl] (1*S*)-1-phenyl-3,4-dihydro-1*H*-isoquinoline-2-carboxylate is a selective muscarinic (M_3) receptor antagonist approved by the US FDA, and is widely used for the treatment of neurogenic overactivity of the detrusor muscle with symptoms of urinary incontinence, and urinary frequency [1, 2]. The development of bladder-selective M_3-antagonists like solifenacin has considerably reduced the possibility of antimuscarinic adverse effects including dry mouth, constipation, and blurred vision [3]. A literature survey retrieved several reports on analytical method development for solifenacin in which a variety of analytical approaches have been utilized. The majority of the documented studies are based on chromatographic methods. These include estimation of solifenacin and its related impurities by RP HPLC [4–7], HPTLC [8], UPLC [9], and Raman spectroscopy [10]. Quantitative

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determination of the drug has been reported in bulk and in its pharmaceutical dosage forms (oral tablet formulations) by UV spectrophotometry [11–14]. The drug has also been assayed by derivative UV spectrophotometric methods in bulk [15] and in combination dosage forms [16]. Stability-indicating chromatographic methods have been reported for the drug [17–19], and its stress-degradation products have been identified by LC-MS [20]. Further, clinical determination of the drug in human plasma has been carried out by LC-tandem MS [21, 22] and HPLC-UV [23] methods. The metabolite profiling of the drug in rat plasma has also been reported by HPLC [24] and LC-tandem mass spectrometric [25] methods. A literature review retrieved only one report on discriminative derivative synchronous emission spectroscopy for the estimation of solifenacin. Spectrofluorimetric methods can offer several advantages over spectrophotometric methods including higher sensitivity (nearly 10^3 folds, comparable with HPLC methods) and selectivity (due to the exclusive excitation/emission wavelength combination utilized for a fluorophoric molecule). In comparison with chromatographic methods, spectrofluorimetric methods are more cost-effective and convenient. Hence, the present study was envisaged to develop and validate a simple and sensitive spectrofluorimetric method for the determination of solifenacin in bulk and in its marketed tablet formulation (Soliten™, Sun Pharma Laboratories). The developed method was validated with respect to various parameters outlined in the International Conference on Harmonization (ICH) guideline Q2 (R1) [26].

Experimental. Solifenacin succinate (batch number – MDC160222) was kindly gifted by Cipla Limited, Mumbai (India). A tablet formulation containing solifenacin succinate (label claim 5 mg) (Soliten™), manufactured by Sun Pharma Laboratories Ltd., East Sikkim (batch number EMX2217), was purchased from the local market. Absolute ethanol, sodium hydroxide, hydrochloric acid, and hydrogen peroxide (30%) were procured commercially from Merck India Pvt. Ltd., Mumbai. Deionized water was prepared using Milli-Q plus purification system, Millipore (Bradford, USA).

All the glassware, including volumetric flasks, pipettes, measuring cylinders, beakers, and test tubes were of class A grade purchased from Borosil. Absorption and emission spectra were recorded using a Hitachi spectrofluorimeter F 2500 with a scanning speed of 300 nm/min, 10-mm matched quartz cells, and a resolution of 2.5 nm.

Standard stock solution for spectrofluorimetry (1000.0 μg/mL) was prepared daily by dissolving 10.0 mg of solifenacin succinate in 10 mL of the solvent (methanol). This was diluted 1 in 10 to obtain a stock solution (100 μg/mL). The working standard solutions, ranging from 15.0 to 95.0 μg/mL of solifenacin were prepared by serial dilutions of the stock solution with ethanol, and the test tubes were kept stoppered to avoid evaporation of the solvent.

The excitation and emission spectra for the working standard solutions of solifenacin succinate (ranging from 15.0 to 95.0 μg/mL) were recorded over the wavelength range of 200–400 and 400–800 nm, respectively. The maximum absorbance of the drug was observed at 228 nm (λ_{max}), which was selected as an excitation wavelength. The emission wavelength for measurement of fluorescence intensity was selected as 285 nm. The optimized method was validated with respect to various parameters outlined in the ICH guideline Q2 (R1).

Ten tablets of solifenacin succinate (Soliten™ tablets; label amount is 5 mg per tablet, Sun Pharma Laboratories; average weight of tablets is 0.75 g) were weighed and crushed. Powder weight equivalent to 10 mg of the drug was sonicated with 10.0-mL methanol to prepare the stock solution (1 mg/mL). The stock solution was further diluted with methanol to obtain a concentration of 25 μg/mL and these dilutions were analyzed four times for the drug content.

Results and discussion. A sensitive spectrofluorimetric method of analysis for solifenacin succinate has been developed and validated. A systematic analysis of the fluorescence characteristics of the drug revealed that solifenacin possesses fair native fluorescence in methanol. The pK_α value of solifenacin is 8.88. Hence, the drug solubility was found to be higher in solutions of acidic pH, but insoluble in a basic medium. In preliminary studies, drug solutions were prepared in various acidic buffers and corresponding fluorescence intensities were measured. Fluorescent intensities for the drug solution (10 μg/mL) in ammonium acetate buffer (pH 3.0, 3.5), were found to be very low. Low-intensity fluorescence (around 10 FU) was also noted in phosphate buffer (pH 2.0). Fluorescence intensity in 0.1 N HCl ranged from 7 to 36 FU. Further, spectrofluorimetric analysis was also carried out in various solvents, such as water, ethanol, and methanol. Considering the UV absorption and solubility characteristics of the drug/solvent, ethanol was selected as a solvent, returning good fluorescence intensities at the selected low drug concentrations. The fluorescence intensity of the drug solutions/stress degraded solutions was determined, taking 228 and 285 nm as the excitation and emission wavelengths, respectively, against the solvent blank. Figure 1 shows the excitation and emission spectra of solifenacin succinate in absolute ethanol.

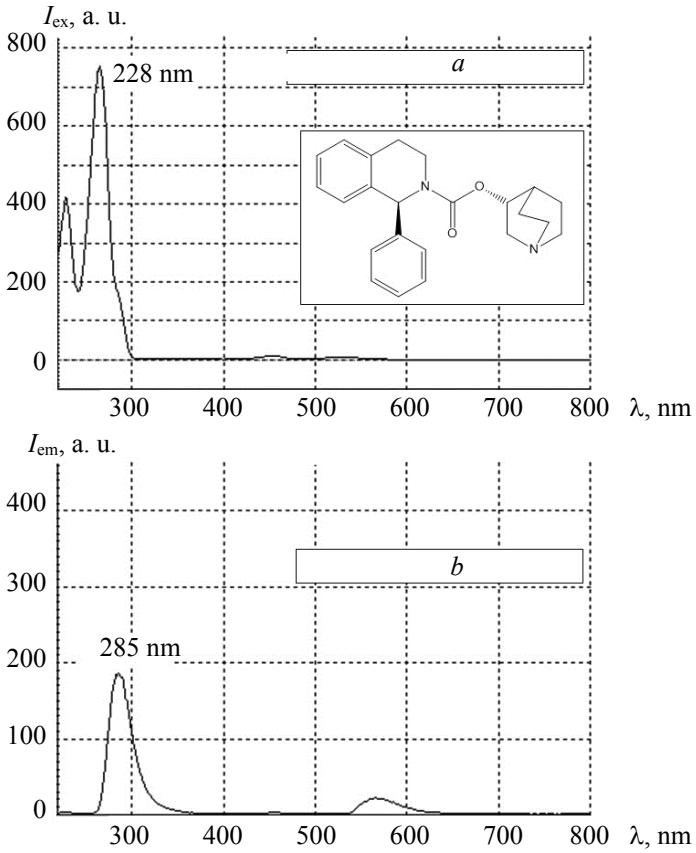

Fig. 1. Excitation (a) and emission (b) scans of solifenacin succinate in ethanol.

The developed method was validated with respect to linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The various validation parameters are summarized in Table 1.

A strictly linear relation was obtained between the fluorescence intensity and concentration of solifenacin in the concentration range of 15.0–95.0 μg/mL. The corresponding calibration curve was described by the equation $y = 0.48x + 10.664$ ($n = 3$, $r^2 = 0.9963$) (Fig. 2).

TABLE 1. Validation Parameters of Solifenacin Succinate for the Proposed Method

Accuracy	Concentration (μg/mL) ± S.D., %RSD			
	Concentration of drug taken, μg/mL	Concentration of standard added, μg/mL	Calculated	Recovery, %
	10.0	5.0 (50%)	14.51±0.33; 1.87%	96.74
	10.0	10.0 (100%)	19.62±0.14; 0.72%	98.11
	10.0	15.0 (150%)	24.53±0.19; 0.89%	98.13
Precision	Calculated concentration (μg/mL) ± S.D.; %RSD			
	Conc. taken, μg/mL	Intra-day (n = 6)	Inter-day (n =3)	
	15.0	13.21±0.083; 0.49%	13.36±0.050; 0.30%	
	25.0	26.49±0.026; 0.11%	26.39±0.057; 0.25%	
	45.0	46.95±0.143; 0.43%	47.5±0.064; 0.19%	
Linearity	Range, μg/mL	Slope	Intercept	Coefficient of correlation r^2
	15.0–95.0	0.48	10.664	0.9963
LOD	1.19 μg/mL			
LOQ	3.62 μg/mL			

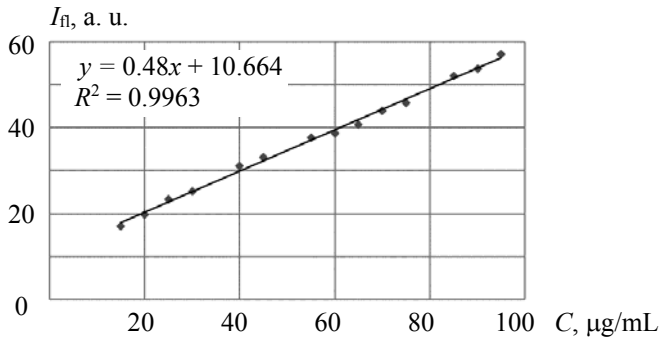

Fig. 2. Calibration plot for solifenacin succinate with the proposed spectrofluorimetric method.

Different concentration levels of the drug for analysis were prepared from independent stock solutions. Assessment of the accuracy of the developed methods was done by spiking the excess drug (50, 100, and 150%) to preanalyzed drug solution samples (10 μg/mL). Accuracy was determined as a mean percent recovery of the spiked drug concentration (Table 1).

Intra-day precision of the developed method was assessed by analyzing varying concentrations of solifenacin succinate in six independent replicates on the same day. Inter-day precision was ascertained from similar determinations carried out on three consecutive days. The method was found to be sufficiently precise and the %RSD for the intra-day and inter-day precision was not found to exceed 0.49 and 0.30%, respectively. The results for the calculated intra-day and inter-day precision of the proposed method of analysis are given in Table 1 and no significant variation in the calculated drug concentration was observed on any day. These results showed that the method was sufficiently precise for determining the drug concentrations.

The LOD and LOQ values were calculated using the formulas 3.3σ/s and 10σ/s, respectively, where σ is the standard deviation of the response (calculated from the standard deviation of intercept) and s is the slope of the calibration curve. The slopes and intercepts of calibration plots for three sets of fluorescence intensities taken from linearity studies were used for the calculation of the LOD and LOQ values, which were found to be 1.19 and 3.62 μg/mL, respectively. Further, solutions of the drug having concentrations corresponding to the LOD and LOQ values were prepared and analyzed six times ($n = 6$).

No significant change in fluorescence intensity was observed when carrying out deliberate changes in the method variables, including the excitation wavelength, emission wavelength, and the analyst performing the study. Hence, the method was found to be robust with %RSD in all cases being less than 2.6%. The results from the robustness studies are shown in Table 2.

TABLE 2. Robustness of the Proposed Method

Parameter	Change	Fluorescence intensity			Mean	SD	%RSD
Optimized conditions	NA	20.5	19.98	19.72	20.06	0.39	1.97
λ_{ex} = 228 nm	235	17.87	17.89	17.07	17.60	0.47	2.68
λ_{em} = 285 nm	300	18.98	18.71	19.07	18.92	0.18	0.99
Analyst I	Analyst II	20.25	19.78	20.01	20.01	0.23	2.51

TABLE 3. Recovery Studies Form Drug Formulation

Conc. taken, μg/mL	Fluorescence intensity	Recovery of drug, μg/mL	Drug content per tablet, mg
25.0	22.11	23.84 (95.36%)	4.76
	22.35	24.34 (97.36%)	4.86
	22.03	24.24 (96.96%)	4.84
Mean	22.16	24.14 (96.56%)	4.82
SD	0.17	0.26	0.05
%RSD	0.75	1.09	1.09

The data from recovery studies of the drug in the marketed oral tablet formulation of solifenacin (Soliten^{TM}) are shown in Table 3. Recoveries ranging from 95 to 97% were obtained with the marketed formulation with low %RSD values (1.09%).

Conclusions. A simple, rapid, reliable, and sensitive spectrofluorimetric method has been proposed for the determination of solifenacin succinate in bulk and in its oral tablet formulation. This is the first-ever report on the spectrofluorimetric analysis of this drug. The developed method was validated for various parameters including sensitivity, reproducibility, precision, accuracy, robustness, limits of detection, and quantification. Good recoveries of the drug from bulk samples and the drug formulation suggest that the method is well suited for routine drug analysis without any interference from the formulation excipients.

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