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Simultaneous Spectrophotometric and Spectrofluorimetric Assay of Silodosin and Solifenacin in their Co-formulated Binary Mixture

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ABSTRACT

Objectives: New pharmaceutical combinations are routinely developed and marketed to improve treatment of different conditions, increase patient compliance and simplify the medication regimen. However, such case necessities the development of analytical procedures to assay these new mixtures in different matrices. This is the case for Silodosin (SI) & Solifenacin (SO) new combination marketed to treat patients' stent-related symptoms and urological disorders. The simplest and greenest known analytical methods are the spectrophotometric and spectrofluorimetric ones. Thus, these two techniques were chosen to resolve this new binary mixture and assay the drugs in their bulk and dosage form to be routine methods for their analysis. **Methods:** Method I relies on applying third derivative treatment on the two drugs' absorption spectra to measure SI at 280 nm and SO at 222 nm. Method II is direct spectofluorimetric measurement of SI at its λ_{em} of 445 nm and SO at λ_{em} of 276 nm. **Results:** The methods are validated according to "ICH guidelines" to be the first valid reported methods for this new mixture. Linearity was achieved at 6.50-19.20 & 2.50-10.00 µg/mL for SI and SO, respectively, in case of method I and at 0.30-12.80 & 1.00-22.00 µg/mL for SI and SO, respectively, in case of method II. The two proposed methods showed high sensitivity, accuracy and selectivity for each drug. **Conclusion:** The methods in this study were applied successfully to determine SI and SO in their bulk and laboratory prepared tablets with acceptable validation parameters.

Keywords: *Derivative spectrophotometry, Spectrofluorimetry, Silodosin, Solifenacin & Validation*

INTRODUCTION

The known "Double J stent" has been routinely used for decades to resolve ureteral obstructions which are caused by several disorders. Frequency, sexual dysfunction, low work capacity, dysuria, urgency, pain and many other urological symptoms affects quality of life-QOL of patients negatively and collectively called "Stent related symptoms-SRS".[1] Several pharmacological strategies are applied for better compliance with stents using drugs as α-blockers, anticholinergics and their combinations to decrease SRS.[1]

Silodosin (SI), a known α_1 antagonist, is used to manage SRS as it helps relaxing the lower urinary tract smooth muscles. Anticholinergics such as solifenacin (SO) can also improve SRS by decreasing involuntary bladder contraction. Studies show that combination of silodosin with solifenacin improves SRS and QOL to a great extent with less need for analgesics.[1]

Silodosin (SI, **Figure 1a**) is ((-)-1-(3-Hydroxypropyl)-5-[(2R)-2-({2-(2,2,2-tri-fluoroethoxy) phenoxy] ethyl} amino) propyl-2, 3-dihydro-1H-indole-7-carboxamide) and solifenacin, (SO, Fig.1b) is 1-azabicyclo[2.2.2]oct-3-yl (1R)-1-phenyl-3,4-dihydro-1H-isoquinoline-2- carboxylate. SI has been analyzed using HPLC-MS ([2]), electrochemistry [4], spectrofluorimetry(6), spectrophotometry[8], HPTLC[10] and HPLC([12]). Meanwhile, using the same techniques, SO has been also analyzed in the literature with HPLC-MS [3], electrochemistry [5], spectrofluorimetry[7], spectrophotometry[9], HPTLC[11] and HPLC[13].

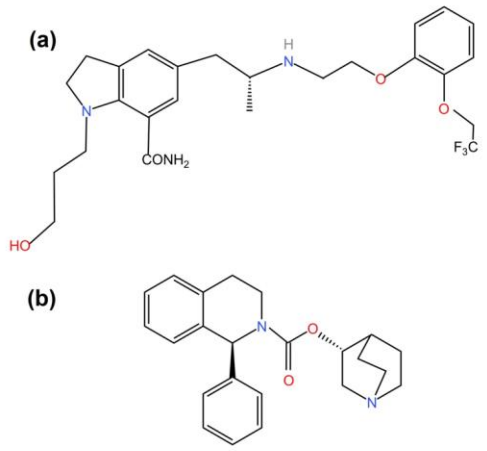

Figure 1. Chemical Structure of Silodosin (SI) (a) and Solifenacin (SO) (b).

Spectrophotometry and spectrofluorimetry are considered the techniques of choice for routine analysis due to their greenness (minimal use of solvents, wastes and energy), simplicity, availability and low cost. However, multi-component analysis with those techniques is difficult due to interferences and strong overlaps. This problem can be overcomed by using derivative treatments which resolve strong spectral overlaps and minimizes interferences.[9, 14]

No reports, till now are present for SI and SO simultaneous analysis in the literature. Thus, this work aimed to introduce simple methods for analysis of these two drugs to be applied for their routine analysis in their combined marketed dosage forms marketed in some countries such as India.

MATERIAL AND METHODS

Experimental

Instrumentation, Reagents and Chemicals

- For spectrophotometric measurements: UV-Vis spectrophotometer - Thermo-Spectronic connected to Harvest computer system was used with 1-cm quartz cells.
- For spectrofluorimetric measurements: Cary Eclipse Spectroflourimeter (Agilent technologies) connected to a Cary Eclipse software with 1 cm quartz cells was used.
- Analytical grade SI was brought from Merck-SA, Dermstadt, Germany and SO was supplied by Medizen Pharmaceuticals, Borg El Arab, Egypt certified to be ≥ 98.00 % and 99.90%, respectively. Analytical grade methanol (SDFCL, India) was used.

Preparation of standards and synthetic mixtures

Stock standard solution, 500.00 µg/mL of each drug was prepared in methanol as a solvent. Prepared stock solutions can be stored at 4°C under light-protected conditions for two weeks. Working standards, for each method, were prepared by further dilution using methanol within the concentration ranges stated in **Table 1**.

To prepare synthetic mixtures of the two drugs in different ratios, aliquots of SO and SI stock solutions were transferred into 10.00 mL volumetric flasks and completed with methanol to volume to achieve mixtures with concentrations stated in **Table 2**.

Preparation of laboratory prepared Pharmaceutical Preparation

SO and SI combined dosage form is unavailable in our commercial markets. Thus, laboratory-prepared tablets containing 8.00 and 5.00 mg of SI and SO, respectively, per tablet were prepared using common tablet fillers from Pharco Pharmaceuticals Co., Egypt. Accurately weighed tablets' powder equivalent to 50.00 and 31.25 mg SI and SO, respectively, was dissolved into approximately 20.00 mL methanol & sonicated ~10 min. After completing to volume (50.00 mL) with methanol, the solution was filtered and 120 µL of the filtrate was transferred into a 10 mL volumetric flask to prepare 12.00 and 7.50 µg/mL of SI and SO sample solution in methanol.

Procedure

For each working standard, sample solution & synthetic mixture, the absorption and emission spectra (λ_{exc} = 217 nm) were recorded. All measurements were corrected against a methanolic blank and computed using Microsoft Excel®.

Table 1. Regression parameters of methods 1 and 2

Method	Method I		Method II	
Parameter	**SI**	**SO**	**SI**	**SO**
Linearity µg/mL	6.50-19.20	2.50-10.00	0.30-12.80	1.00-22.00
Intercept, a	-4.27×10^{-3}	-1.08×10^{-3}	100.61	31.45
Slope, b	5.74×10^{-3}	1.45×10^{-2}	46.99	14.87
LOQ, µg/mL	2.63	0.69	1.64	2.61
LOD, µg/mL	0.87	0.23	0.54	0.86
Correlation coefficient (r)	0.9994	0.9998	0.9991	0.9991
S_a[a]	1.51×10^{-3}	1.00×10^{-3}	7.70	3.87
S_b[b]	1.11×10^{-4}	1.58×10^{-4}	1.17	0.36
$S_{y/x}$[c]	1.12×10^{-3}	9.51×10^{-4}	11.94	6.17
F	2664.77	8360.05	1610.48	1683.58
Significance F	1.60×10^{-5}	2.88×10^{-6}	3.40×10^{-5}	3.19×10^{-5}

[a] S_a: standard deviation of intercept, [b] S_b: standard deviation of slope and [c] $S_{y/x}$: standard deviation of residuals.

Table 2. Evaluation of intra- & inter-day precision & accuracy of methods 1 and 2

Method		Method I				Method II	
(a) Accuracy / Intra-day precision, n=3							
Concentration (µg/mL)		**Mean % Recovery±%RSD ***		**Concentration (µg/mL)**		**Mean % Recovery±%RSD ***	
SO	**SI**	**SO**	**SI**	**SO**	**SI**	**SO**	**SI**
10.00	19.20	100.99 ± 0.80	99.96 ± 0.66	1.00	2.00	100.59 ± 0.56	99.90 ± 1.02
2.50	12.80	100.95 ± 0.76	101.50 ± 1.24	2.50	12.80	101.60 ± 1.50	99.99 ± 0.94
10.00	6.50	99.85 ± 0.88	100.76 ± 0.84	10.00	6.50	100.95 ± 0.92	100.56 ± 0.80
8.00	12.80	100.50 ± 0.97	100.53 ± 0.90	8.00	12.80	99. 50 ± 1.07	101.20 ± 0.69
5.00	6.50	99.93 ± 1.09	101.01 ± 0.55	5.00	6.50	99.10 ± 1.00	100.45 ± 1.11
(b) Accuracy / Inter-day precision, n=3							
Concentration (µg/mL)		**Mean % Recovery±%RSD ***		**Concentration (µg/mL)**		**Mean % Recovery±%RSD ***	
SO	**SI**	**SO**	**SI**	**SO**	**SI**	**SO**	**SI**
10.00	19.20	100.20 ± 0.66	98.50 ± 1.22	1.00	2.00	99.99 ± 1.03	99.10 ± 1.50
2.50	12.80	100.88 ± 1.40	99.84 ± 1.65	2.50	12.80	101.50 ± 1.80	100.64 ± 1.97
10.00	6.50	99.93 ± 0.90	101.99 ± 0.99	10.00	6.50	101.78 ± 1.19	99.06 ± 1.25
8.00	12.80	98.99 ± 1.20	100.95 ± 1.50	8.00	12.80	99.66 ± 1.22	101.77 ± 1.09
5.00	6.50	100.95 ± 1.55	99.20 ± 1.60	5.00	6.50	100.55 ± 0.97	101.25 ± 0.85

Method I: Spectrophotometric Method:

The 3rd derivative (D^3) values of the recorded absorption spectra were computed at 280 nm and 222 nm for SI and SO determination, respectively, using $\Delta\lambda$ of 6 nm.

Method II: Spectrofluorimetric Method:

The fluorescence values were measured at λ_{em} = 276 and 445 nm for SI and SO assay, respectively.

RESULTS AND DISCUSSION

Spectrophotometric & spectrofluorimetric properties of the studied drugs

UV spectra of the two drugs in their dosage form ratio, shown in **Figure 2**, show that they cannot be determined with direct spectrophotometric measurement because of the strong overlap between their absorption spectra. Also, SO is weakly absorbing due to lack of strong conjugation and it absorbs maximally at a very short wavelength of 210 nm. By applying the derivative technique, this strong overlap was resolved with successful determination of SO without any physical separation required.

Meanwhile, upon spectrofluorimetric scanning of SI and SO in methanol, SI had λ_{exc}= 270 nm and λ_{em} = 443 nm while SO showed λ_{exc}= 217 nm and λ_{em}= 279 nm . The chosen λ_{exc} for subsequent measurements was that of SO at 217 nm for both drugs as upon excitation at 270 nm, SO showed zero fluorescence. At the chosen λ_{exc}= 217 nm there was no overlap between the emission spectra of both drugs (**Figure 2**) which enabled their direct measurement without any mathematical transformations which shows the selectivity and superiority of the fluorimetric techniques.

Method I: Spectrophotometric Method

The third derivative amplitudes at 280 nm (zero-crossing of SO) and 222 nm (zero-crossing of SI) as shown in **Figure 3**, were chosen to measure SI and SO, respectively. Influence of $\Delta\lambda$ on the derivative spectra was tested by applying different wavelength intervals finding 6 nm to be optimum for both drugs.

Method II: Spectrofluorimetric Method

Upon selecting the suitable λ_{exc} of 217 nm for SO, SI and their mixture, each drug was determined at its previously mentioned λ_{em} as shown in **Figure 3**.

Solvent effect

To reach the highest absorbance and relative fluorescence readings, the two drugs were measured in different solvents. The highest relative fluorescence intensity for SO (the minor component) was achieved in methanol, while SI had significant decrease in its fluorescence intensity in water. Thus, methanol was the solvent of choice for the spectrofluorimetric measurements to be able to measure SO with high readings while still getting acceptable reading for SI. On the other side, SO showed slightly higher absorbance reading in water than in methanol, but to unify the solvent used in all measurements and to minimize the waste, methanol was the solvent of choice for the spectrophotometric measurements as enough sensitivity was reached using methanol. Basic (0.10N NaOH) & acidic (0.10N HCl) caused decrease in all readings for both drugs.

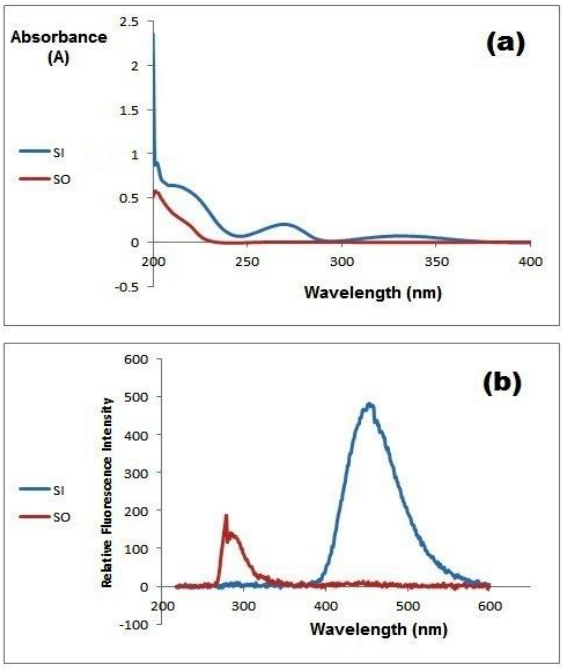

Figure 2. Methanolic absorption (a) and emission curves (b) of Silodosin (SI) and Solifenacin (SO) in concentrations of their dosage form ratios.

Validation

Validation of the two methods was conducted based on the ICH guidelines.[15]

Linearity and range

Linearity was checked by plotting the derivative amplitudes of method I and the fluorescence intensity of method II versus concentrations of the two drugs to obtain the calibration curves. Statistical analysis of the data showed high correlation coefficients (r) values as demonstrated in **Table 1**. Also, all statistical parameters shown in Table 1 confirm the acceptable linearity of the two methods. Lower sensitivity was achieved using the spectrofluorimetry method indicating its superiority.

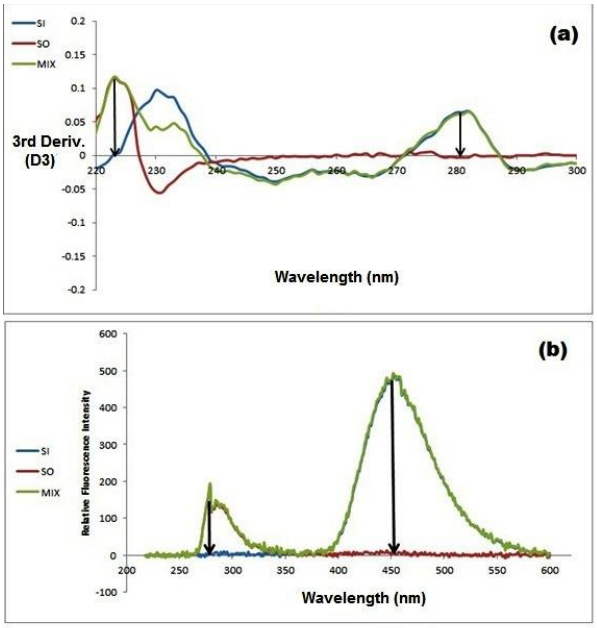

Figure 3. Methanolic Third derivative absorption spectra (a) and zero order emission spectra of of 12.8 µg/mL SI and 8 µg/mL SO.

Limits of detection and quantitation

Both limits of detection (LOD) and quantitation (LOQ) were calculated using the formulae: $3.3S_a/b$ (where S_a is standard deviation of intercept and b is the slope from the regression equations) for the LOD and $10S_a/b$ in case of LOQ. The obtained values are shown in table 1 for both methods.

Accuracy and Precision

Analysis of three replicates of each synthetic mixture of the two drugs was done in the ratios shown in table 2 by both methods. Each mixture was analyzed by the two methods on same day (n = 3) for intraday precision and on five different days (n = 3) for interday precision. Relative standard deviation percent-RSD % & absolute error-E_a values were < 2% indicating good precision and accuracy (**Table 2**).

Selectivity

As previously mentioned, the synthetic mixtures with their different ratios were analyzed with percentage recoveries (98.00-102.00%) indicating high selectivity and lack of interference. In addition, the two methods were conducted to assay laboratory prepared tablets as demonstrated in **Table 3** and no interference from dosage form excipients was observed as the percentage recoveries where acceptable.

Assay of laboratory prepared Pharmaceutical Preparations

The useful application of the proposed methods was tested by determination of the two drugs in their dosage form ratio in mixture with common excipients usually present in tablets, since the tablets dosage form is not available in our commercial markets. Satisfactory recoveries were obtained for both drugs as seen previously in table 3. Results of the proposed methods were not compared with any reported methods since the proposed methods are considered the first to analyze this newly available mixture. The results were within acceptable limits of percentage recoveries and standard deviations and the common tablet excipients used and mentioned earlier did not interfere with the assay.

Table 3. Assay results for SI & SO determination in their laboratory-prepared pharmaceutical preparation by methods 1 & 2.

Test	Method I	Method II
% Found ± % RSD (n=5)	101.89 ± 1.76	100.50 ± 0.99

Solution stability

Standard and sample solutions of SO & SI were stored for 1, 3 and 6 h. at room temperature then analyzed and results were consistent indicating that solutions were stable for at least 6 h.

CONCLUSION

Two basic and simple spectrophotometric & spectrofluorimetric procedures for assay of SI and SO in their newly available combination in some markets have been developed in this work. The two methods were fully validated and established to be ready for routine analysis of this new binary mixture. The developed methods are considered green as they do not involve consumption of large amount of organic solvents, nor use of complicated instruments with high energy consumption nor evolving large amount of waste. Above all, new marketed drug combinations should have a valid method for their routine analysis and these methods are the first reported methods for analysis of this mixture.

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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