

## Rapid Spectrophotometric Methods for the Determination of Moxifloxacin and Sildenafil in Pharmaceutical Preparations Based on Reaction with 4-Aminoantipyrine

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Received: January 28, 2020

Published: March 09, 2020

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### Abstract

Two simple and rapid spectrophotometric methods by the application of oxidative coupling reactions in aqueous medium for the determination of moxifloxacin hydrochloride (MOXF) and sildenafil citrate (SC) in either pure form or in its pharmaceutical preparations have been carried out. MOXF and SC react with 4-aminoantipyrine in the presence of  $KIO_4$  to yield red colored products exhibit maximum absorption at 530 and 526 nm for MOXF and SC, respectively. Beer's law plot showed linear relationship in the concentration ranges 2.65 - 230.0 and 1.66 - 98.0  $\mu\text{g/mL}$  with detection limit of 0.55 and 0.44  $\mu\text{g/mL}$  for MOXF and SC, respectively. Molar absorptivity for the above two methods were found to be  $4.40 \times 10^3$  and  $8.25 \times 10^3$  L/mol cm, respectively. The developed methods were applied to estimate MOXF and SC in tablets dosage form and the results compare favorably with those of pharmacopeial methods. Both the methods are accurate, precise and highly reproducible, cheap, rapid and suitable applied for routine analysis of MOXF and SC in bulk and formulations.

**Keywords:** 4-Aminoantipyrine; Moxifloxacin Hydrochloride; Sildenafil Citrate; Oxidative Coupling Reactions; Spectrophotometry; Dosage Forms

### Introduction

Moxifloxacin (MOXF), 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-3-quinoline carboxylic acid [1] is an antibiotic prescription medicine for the treatment of certain bacterial infections [2]. British Pharmacopoeia (BP) describes HPLC method for the assay of moxifloxacin hydrochloride in pharmaceutical preparations [3]. Other methods in common use for estimation of moxifloxacin hydrochloride in pharmaceutical and biological samples involved high performance liquid chromatography [4,5], HPTLC [6], spectrophotometry [7-10], atomic absorption spectroscopy [9,10], conductometry [9], kinetics spectrophotometry [10,11], extractive spectrophotometry [12], polarography [13], spectrofluorimetry [14], flow injection with chemiluminescence detection [15].

Sildenafil citrate (SC) is 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl]

sulfonyl]-4-methylpiperazine citrate [1]. It is phosphodiesterase-5 inhibitors used for erectile dysfunction. USP describes HPLC method for the examine sildenafil citrate [16]. Sildenafil citrate was determined by different methods including HPLC in pharmaceutical products [17,18], HPLC in Pharmaceutical products with other drugs [19,20], HPLC in dietary supplements [21], CGC in pharmaceutical formulations and dietary supplements [22,23], HPTLC in commercial lifestyle products [24], electroanalytical methods in pharmaceutical preparations [25-27] and flow injection analysis in tablets using UV-detection [28] and amperometric detection [29]. Spectrophotometric methods, such as spectrophotometry through ion-associate formation reactions [30,31], ion-pair reactions [30,32], charge-transfer complexes [33], extractive spectrophotometry [34] and direct UV-spectrophotometry [35] were also communicated for the analysis of sildenafil citrate in pharmaceuticals.

The topical of our work is to evolve methods based on reaction of moxifloxacin hydrochloride and sildenafil citrate with 4-amino-

antipyrine (4-AP) in the presence of an oxidizing agent (potassium periodate) in aqueous medium, and then spectrophotometric determination of moxifloxacin hydrochloride and sildenafil citrate in raw materials and tablets.

## Materials and Methods

### Instrumentation

Bioteck digital spectrophotometer (England) provided with matched 10 mm quartz cells was used for all spectral measurements of the resulting solutions.

### Chemicals and reagents

Moxifloxacin hydrochloride pure material was obtained from Matrix Laboratories Limited (India). Its purity was evaluated and found to be 99.85% [3]. Sildenafil citrate pure material was obtained from Cipex Specialities Pvt Ltd., Mumbai (India). Its purity was evaluated as 99.83% [16]. Tablets were purchased from Syrian market. All chemicals used were of AR grade and bidistilled water was used to prepare all the solutions. Aqueous solutions of  $1 \times 10^{-2}$  M 4-aminoantipyrine (Ferack) and  $1 \times 10^{-2}$  M  $\text{KIO}_4$  (BDH) were prepared daily. All the solutions were stored in the refrigerator and found to be stable for one week at least.

### Standard drug solutions

Standard stock solutions of MOXF (1.0 mg/mL) and SC (0.5 mg/mL) were prepared in double distilled water. A suitable dilution of the standard solution with bidistilled water was made to prepare working standard solutions.

### Reaction stoichiometry

The stoichiometric ratio of the complexes formed was studied by continuous variation (Job's method) and molar ratio method [36].  $1 \times 10^{-3}$  M of drug and  $1 \times 10^{-2}$  M of 4-AP solutions were utilized. In the molar ratio method a constant volume of  $1 \times 10^{-3}$  M drug solution was treated with varying volume of 4-AP to obtain a ratio for the complex (0:1, 0.1:0.9, 0.2:0.8,....., 1:0), while in Job's method, a series of drug-reagent solutions was prepared to obtain a ratio for the ion pair complex (0:1, 0.1:0.9, 0.2:0.8,....., 1:0) where  $C_{\text{drug}} + C_{4\text{-AP}} = 2.0 \times 10^{-3}$  M. The absorbance of the resulting solutions was measured at  $\lambda_{\text{max}}$  against the reagent blanks treated similarly.

### Formation constant ( $K_f$ ) and the standard free energy ( $\Delta G^\circ$ ) of colored species

The formation constant ( $K_f$ ) of the complexes MOXF-4AP and SC-4AP was evaluated spectrophotometrically by substituting the data of Job's method in the equation (1).

$$K_f = \frac{A/A_m}{[1 - (A/A_{\text{max}})]^{n+2} \cdot C_M(n)^n} \dots\dots\dots(1)$$

Where A is the maximum observed absorbance and  $A_m$  is the absorbance value when all the amount of drug is associated.  $C_M$  is the mole concentration of drug at the maximum absorbance and n is the combination ratio of the ion-pair considered [37].

The equation (2) represents the relation between Gibbs free energy ( $\Delta G^\circ$ ) of the reaction product and the formation constant. This equation was used to calculate  $\Delta G^\circ$  [38]:

$$\Delta G^\circ = -2.303 RT \log K \dots\dots\dots(2)$$

Where  $\Delta G^\circ$  is the free energy change of the complex, R is the gas constant 1.987 cal/mol degree, T is the temperature in Kelvin, K is the association or formation constant of drug-reagent complex (L/mol).

### Validation of the proposed methods

The methods were validated in accordance with the International Conference on Harmonization (ICH) guidelines [39].

### Linearity

To determine MOXF, five separate series of MOXF solutions were prepared and analyzed. Aliquots of 0.085–2.00 mL standard solution of MOXF (2.65 - 230.0  $\mu\text{g/mL}$ , 1.0 mg/mL) were transferred into a series of 10 mL calibrated volumetric flasks. 2.0 mL of 0.01 M 4-AP was added into each flask and kept aside for 4 min. Finally, 2.0 mL of 0.01 M  $\text{KIO}_4$  was added and the volume was made up to the mark with bidistilled water. The content of flasks were mixed and the absorbance of the resulting colored solutions were measured at 530 nm against a similar reagent blank within a period of 10 min.

For the determination of SC, five separate series of solutions of SC were prepared and analyzed. Aliquots of 0.067–1.96 mL standard solution of SC (1.66 - 98.0  $\mu\text{g/mL}$ , 0.5 mg/mL) were transferred into a series of 10 mL calibrated volumetric flasks. 1.4 mL of 0.01 M 4-AP and 0.8 mL of 0.01 M  $\text{KIO}_4$  were added into each flask and kept aside for 2 min. The volume was made up to the mark with bidistilled water. The content of flasks were mixed and the absorbance of the resulting colored solutions were measured at 526 nm against a similar reagent blank within a period of 10 min.

The standard curve of absorbances versus concentration was constructed for the determination of MOXF and SC. The obtained

graph by least-squares method is described by regression equation,  $A = mC + b$  (where  $A$  is the absorbance of a 1 cm layer,  $m$  is the slope,  $b$  is the intercept and  $C$  is the concentration of the cited drug in  $\mu\text{g/mL}$ ) [40].

### Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs were obtained experimentally by calculating the signal to-noise (S/N) ratio (i.e. 3.3 for LOD and 10 for LOQ) using the formula:

$$\text{LOD} = 3.3 \times S_a / b \quad \dots\dots\dots(3)$$

$$\text{LOQ} = 10 \times S_a / b \quad \dots\dots\dots(4)$$

Where  $S_a$  is the standard deviation of the intercept and  $b$  is the slope of the calibration curve [41].

### Precision

Precision of the methods was established as repeatability. Various degrees of drug concentrations were prepared and analyzed as discussed above. The percent relative standard deviation (% RSD) of the anticipated concentrations from the regression equation was taken as precision (Table 1).

### Accuracy

For accuracy assays the same different levels of drug concentrations mentioned above were analyzed by the proposed methods and the percentage relative error and mean percentage recovery were calculated (Table 2).

The accuracy and specificity of the proposed methods were tested by recovery experiments of the formulations and the proposed methods were followed. From the amount of drug found, percentage recovery was calculated (Table 3).

### Procedure for formulations

A total 20 tablets of each MOXF formulation were accurately weighed and pulverized. An amount of the powder equivalent to 50 mg MOXF was transferred to 100 mL measuring flasks, 25 mL of methanol was added and shaken in ultrasonic bath for about 5 min. The solutions were filtered through Whatman filter paper number 40 into separate 50 mL measuring flasks. The methanol was evaporated and the residue of solution was diluted to the volume with bidistilled water to achieve a concentration of 1 mg/mL. the samples were analyzed using proposed method.

20 tablets were weighed, crushed, and mixed in a mortar and pestle. A portion of the powdered tablets equivalent to the weight of one tablet was accurately weighed and transferred into a 100 mL measuring flask and made up to the volume with bidistilled water to achieve a concentration of 0.5 mg/mL. The solution was mixed well and filtered. The solution was further diluted stepwise with bidistilled water to get working solutions and analyzed as described earlier.

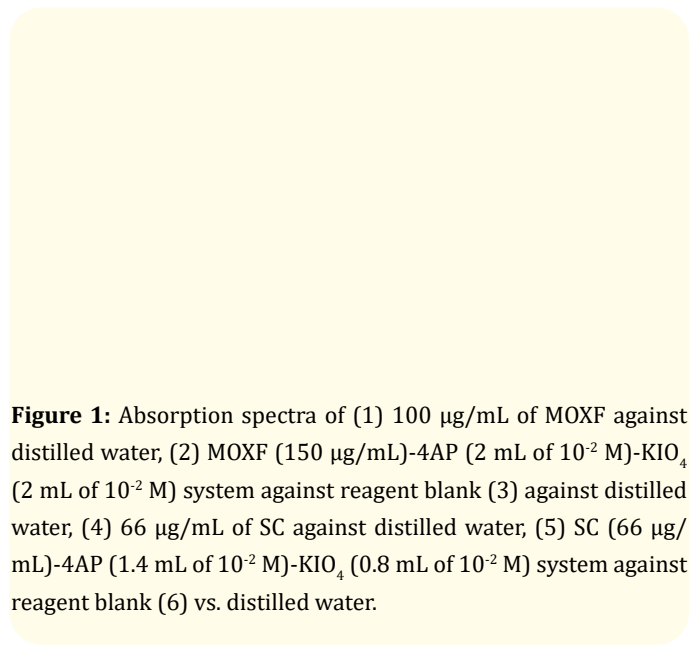
### Robustness

Robustness was tested by evaluating the influence of a small variation of the method variables including the concentration of analytical reagents, reaction time and temperature on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time.

## Results and Discussion

### Absorption spectra

An intense red-coloured oxidizing coupling products with an absorption maximum at 530 and 526 nm are formed when MOXF and SC, respectively, were allowed to react with 4-AP in the presence of  $\text{KIO}_4$  as an oxidant in aqueous medium. Figure 1 shows the spectra of red products formed and of the reagent blanks. The absorbance is directly related to concentration of MOXF and SC and can be used for its spectrophotometric determination.



**Figure 1:** Absorption spectra of (1) 100  $\mu\text{g/mL}$  of MOXF against distilled water, (2) MOXF (150  $\mu\text{g/mL}$ )-4AP (2 mL of  $10^{-2}$  M)- $\text{KIO}_4$  (2 mL of  $10^{-2}$  M) system against reagent blank (3) against distilled water, (4) 66  $\mu\text{g/mL}$  of SC against distilled water, (5) SC (66  $\mu\text{g/mL}$ )-4AP (1.4 mL of  $10^{-2}$  M)- $\text{KIO}_4$  (0.8 mL of  $10^{-2}$  M) system against reagent blank (6) vs. distilled water.

### Optimization of reaction conditions

The influence of the variables on the absorption intensity of the species formed was studied and the reaction conditions are optimized. The optimum values of the variables were maintained throughout the experiment.

### Effect of temperature and order of addition

The species products of the proposed methods were studied at different temperatures. The results indicate that the absorbance values remain constant at laboratory temperature ( $25 \pm 2^\circ\text{C}$ ), whereas, at higher temperatures the absorbance value decrease, indicating the dissociation of the product on prolonged heating. Therefore room temperature is selected.

To obtain maximum absorbance and then optimum results the order of addition of reagents should be as follow: drug, 4-AP and  $\text{KIO}_4$ , otherwise a loss in colour intensity was observed.

### Effect of reaction time

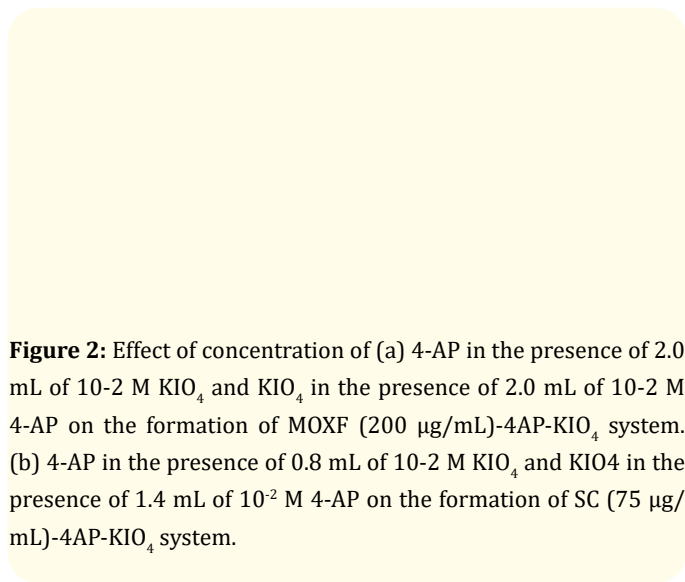
The colour intensity reached its maximum within 5 min after the drug had been reacted with 4-AP in the presence of  $\text{KIO}_4$  and remains stable for 10 min, therefore 5 min development time was selected as optimum in the general procedure.

### Effect of reagent concentration

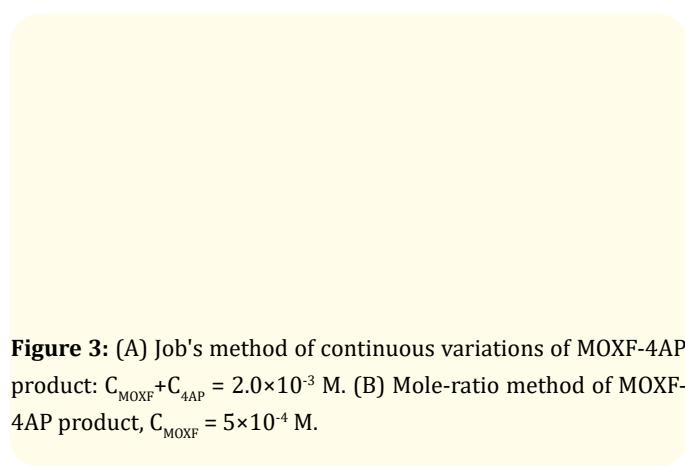
For maximum color development, various concentrations of 4-AP and  $\text{KIO}_4$  solutions were added to fixed amount of the drug solution. 2.0 mL of 0.01 M 4-AP and 2.0 mL of 0.01 M  $\text{KIO}_4$  solutions were found to be optimal for MOXF, while 1.4 mL of 0.01 M 4-AP and 0.8 mL of 0.01 M  $\text{KIO}_4$  solutions were found to be enough for SC, to develop the colour to its full intensity and give a minimum blank value and was considered to be optimum for concentration range of 2.65 - 230.0 and 1.66-98.0  $\mu\text{g}/\text{ml}$  of MOXF and SC, respectively (Figure 2).

### Stoichiometric relationship

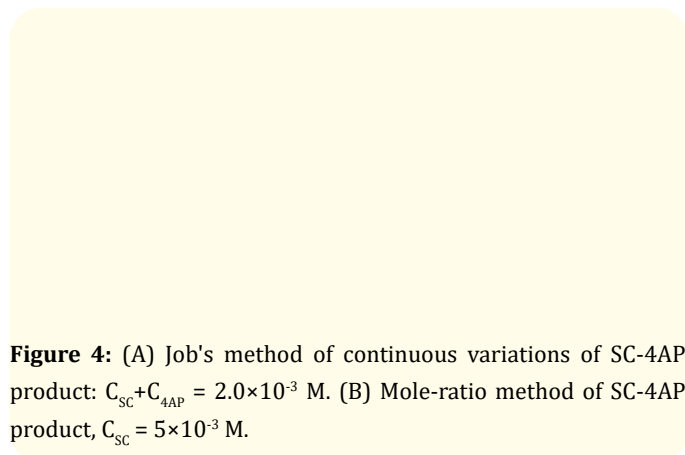
Job's method using equimolar solutions of MOXF and SC and 4-AP was applied to know the composition of MOXF-4AP and SC-4AP colored species. The method revealed 1:2 and 1:1 molar ratio (drug to 4-AP) for MOXF (Figure 3A) and 1:1 for SC (Figure 4A). Also, the mole-ratio method showed the formation of 1:1 and 1:2 for MOXF (Figure 3B) and 1:1 for SC (Figure 4B).



**Figure 2:** Effect of concentration of (a) 4-AP in the presence of 2.0 mL of  $10^{-2}$  M  $\text{KIO}_4$  and  $\text{KIO}_4$  in the presence of 2.0 mL of  $10^{-2}$  M 4-AP on the formation of MOXF (200  $\mu\text{g}/\text{mL}$ )-4AP- $\text{KIO}_4$  system. (b) 4-AP in the presence of 0.8 mL of  $10^{-2}$  M  $\text{KIO}_4$  and  $\text{KIO}_4$  in the presence of 1.4 mL of  $10^{-2}$  M 4-AP on the formation of SC (75  $\mu\text{g}/\text{mL}$ )-4AP- $\text{KIO}_4$  system.

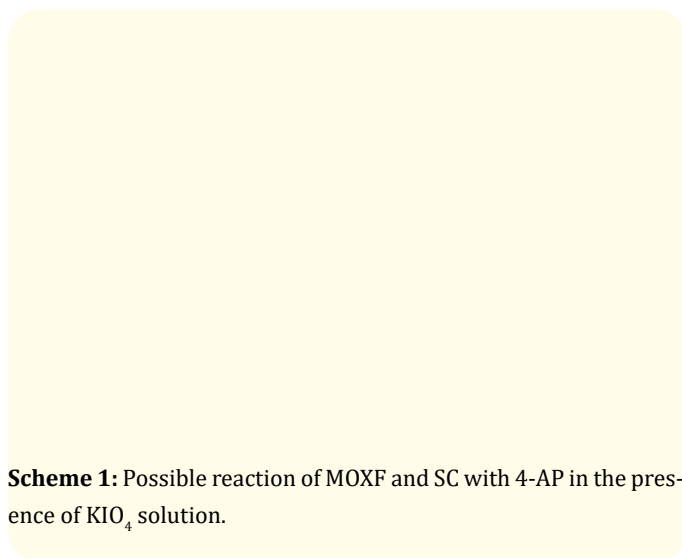


**Figure 3:** (A) Job's method of continuous variations of MOXF-4AP product:  $C_{\text{MOXF}} + C_{4\text{AP}} = 2.0 \times 10^{-3}$  M. (B) Mole-ratio method of MOXF-4AP product,  $C_{\text{MOXF}} = 5 \times 10^{-4}$  M.



**Figure 4:** (A) Job's method of continuous variations of SC-4AP product:  $C_{\text{SC}} + C_{4\text{AP}} = 2.0 \times 10^{-3}$  M. (B) Mole-ratio method of SC-4AP product,  $C_{\text{SC}} = 5 \times 10^{-3}$  M.

4-aminoantipyrine in the presence of potassium periodate, undergoes oxidation with loss of two protons and yields N-substituted quinone imine which in turn is known to spontaneously react with methoxy compounds to yield a red colored antipyrine dye [42]. The electrophilic intermediate couples with MOXF due to the presence of methoxy group by electrophilic attack on the nucleophilic site of the heterocyclic ring to the nitrogen and the resulting intermediate species is spontaneously oxidized with periodate to form the colored oxidative coupling product as shown in Scheme 1.



### The formation constant and the standard free energy

The formation constant ( $K_f$ ) and free energy ( $\Delta G^\circ$ ) values of MOXF-4AP and SC-4AP complexes were calculated using equations (1) and (2) and are tabulated in table 1. The negative values of  $\Delta G^\circ$  indicate the stability of these complexes and their spontaneous formation.

### Validation of the proposed methods

#### Linearity, LOD and LOQ

In quantitative analysis the calibration curve was constructed for both MOXF and SC after analysis of consecutively increased concentrations. Beer's law limits, molar absorptivity and Sandell's sensitivity values were determined for the developed methods and are given in Table 1. Regression analysis of the Beer's law plots reveals a good correlation. Limit of detection (LOD) and limit of quantification (LOQ) decide about the sensitivity of the method. LOD and LOQ for MOXF and SC were calculated according to the equations 3 and 4, respectively. LOD was 0.55 and 0.44  $\mu\text{g/mL}$  and LOQ was 1.65 and 1.33  $\mu\text{g/mL}$  for MOXF and SC, respectively.

Parameters	MOXF	SC
$\lambda_{\text{max}}$ (nm)	530	526
Beer's law range ( $\mu\text{g/mL}$ )	2.65 - 230.0	1.66 - 98.0
Molar absorptivity (L/mol cm)	$4.40 \times 10^3$	$8.25 \times 10^3$
Ringbom optimum photometric range ( $\mu\text{g/mL}$ )	15.0 - 180.0	10.0 - 70.0
Formation constant, $K_f$ (L/mol)	$3.16 \times 10^4$ (1:1)	$1.79 \times 10^8$ (1:1)
	$6.31 \times 10^8$ (1:2)	-
$\Delta G^\circ$ (Kcal/mol)	-6.14, -12.00	-11.25
LOD ( $\mu\text{g/mL}$ )	0.55	0.44
LOQ ( $\mu\text{g/mL}$ )	1.65	1.33
Sandell's sensitivity ( $\mu\text{g/cm}^2$ per 0.001 abs unit)	0.092	0.081
Regression equation <sup>a</sup>	A = 0.0038C+0.0388	A = 0.0077C+0.0439
Correlation coefficient, r	0.9999	0.9999
Mean % recovery $\pm$ SD	100.40 $\pm$ 0.16	100.12 $\pm$ 0.22
<sup>a</sup> With respect to A = mC+b, where A is the absorbance and C is the concentration ( $\mu\text{g/mL}$ ).		

**Table 1:** Optical characteristics of the proposed methods for MOXF and SC.

### Precision

To check the precision and reproducibility of the method, six samples of the same concentration (n=6) of MOXF and SC were prepared and analysed (Table 2). The low %RSD values obtained for MOXF (0.52) and SC (0.11) indicated that the method had high precision and reproducibility.

### Accuracy

Accuracy of the method for the analysis of MOXF and SC in its pure state was checked. The accuracy of the method indicated by the excellent recovery (Table 2).

The % recovery for MOXF and SC were found to be in the range of 100.43-101.04% and 101.27-102.10%, respectively, for the formulations tested (Table 3). The high recovery rate indicated that the method had a good accuracy and specificity, as there was no interference from the excipients present in formulations.

Drug	mg/mL		Relative error (%)	RSD (%)	% Recovery ± SD
	Taken	Found*			
MOXF	2.65	2.67	0.75	1.89	100.75 ± 0.21
	20.00	20.15	0.75	1.52	100.75 ± 0.19
	40.00	39.98	-0.05	1.06	99.95 ± 0.14
	80.00	80.17	0.21	0.92	100.21 ± 0.17
	160.00	161.25	0.78	0.63	100.78 ± 0.13
	200.00	199.73	-0.14	0.52	99.86 ± 0.15
SC	2.00	1.99	-0.50	1.76	99.50 ± 0.24
	5.00	5.01	0.20	0.82	100.20 ± 0.22
	10.00	10.03	0.30	0.64	100.30 ± 0.19
	20.00	20.05	0.25	0.51	100.25 ± 0.21
	40.00	40.11	0.27	0.43	100.27 ± 0.20
	80.00	80.23	0.28	0.11	100.28 ± 0.26

\*Average of six determinations.

**Table 2:** Analysis of MOXF and SC in bulk powder by the proposed method.

### Application to the pharmaceutical dosage forms

The proposed methods were successfully applied to determine MOXF and SC in their commercial tablets. The concentration of the studied drugs was calculated using the corresponding calibration equation shown in Table 1. The commonly used excipients and additives in the preparation of tablets were found not to interfere

in the analysis. The % recoveries of the two drugs in their tablets compared with those of the official methods of BP [3] and USP [16] for the studied drugs, respectively, are given in table 3. The results were treated statistically. Since F-test and t-test showed that there was no significant difference between the proposed methods and the standard methods.

Formulation	% Recovery ± SD <sup>a</sup>		t-value <sup>b</sup>	F-value <sup>b</sup>
	Proposed method	Pharmacopeial method		
Moxicin <sup>c</sup> (400mg/tablet)	100.43 ± 0.23	99.98 ± 0.19	1.68	1.46
Aviloxin <sup>d</sup> (400mg/tablet)	101.04 ± 0.29	99.98 ± 0.18	1.84	2.59
Vaigran <sup>e</sup> (50mg/tablet)	102.08 ± 0.17	101.14 ± 0.13	1.96	1.71
Extra <sup>f</sup> (50mg/tablet)	102.10 ± 0.23	100.53 ± 0.17	1.94	1.83
Vega <sup>g</sup> (50mg/tablet)	101.27 ± 0.24	100.83 ± 0.19	1.59	1.59

a. Five independent analyses. b. Theoretical values for t and F-test at five degree of freedom and 95% confidence limit are t = 2.776 and F = 6.26. c. Supplied by Ibn Al Haytham, d. supplied by Ibn hayan, e. supplied by Barakat Pharmaceutical Industry, f. supplied by adamco and g. supplied by Oubari Pharma, Syria.

**Table 3:** Determination of MOXF and SC in tablets by the proposed and pharmacopeial methods.

### Robustness

The robustness of the methods was studied by using five replicates at MOXF and SC concentration level of 100 and 75 µg/mL, respectively. It was found that small variations in these variables

did not affect the method significantly. This was an indication of the reliability of the proposed method during its routine application for analysis of the investigated drugs (Table 4).

Parameter variation	Recovery % ± SD <sup>a</sup>			
		MOXF		SC
No Change		100.12 ± 0.16		100.06 ± 0.11
Change in 4-AP 0.01 M volume	1.8 mL	96.91 ± 0.18	1.3 mL	98.71 ± 0.12
	2.2 mL	99.48 ± 0.17	1.5 mL	97.41 ± 0.14
Change in KIO <sub>4</sub> 0.01 M volume	1.8 mL	96.94 ± 0.19	0.7 mL	89.43 ± 0.21
	2.2 mL	97.45 ± 0.15	0.9 mL	81.30 ± 0.23
Change in measurement time	4 min	97.85 ± 0.11	4 min	96.09 ± 0.17
	10 min	100.08 ± 0.14	10 min	100.11 ± 0.13
Change in temperature	20°C	99.97 ± 0.13	20°C	100.01 ± 0.15
	30°C	100.09 ± 0.16	30°C	100.05 ± 0.14

<sup>a</sup> Average of five determinations.

**Table 4:** Results of robustness study of MOXF and SC.

### Conclusion

The methods that are proposed in this work for the quantitation of moxifloxacin and sildenafil are simple, direct and sensitive. Moreover, they are less time consuming and do not require elaboration treatment and tedious extraction procedures required in chromatographic and other traditional extractive spectrophotometric methods. These, in addition to satisfactory sensitivity (0.44 µg/mL) and reproducibility compared to the official non-aqueous titrimetric methods, make the methods applicable for routine analysis of the two drugs both in pure form and in tablets.

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