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ISSN: 0975-7538  
Research Article

## A new spectrophotometric method for the determination of Solifenacin in pure form and in pharmaceutical formulations

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### ABSTRACT

Simple, sensitive and rapid extractive spectrophotometric method was developed for the determination of solifenacin in bulk and pharmaceutical dosage form. This method was based on the formation of yellow ion-pair complex between the basic nitrogen of the solifenacin and alizarin red in acid solution. The formed complex was extracted with chloroform and the absorbance was measured at 430 nm. The system obeyed Beer's law in the ranges 2.5–22.5  $\mu\text{g mL}^{-1}$ . The developed method was validated as per ICH guidelines. The effect of optimum conditions via strength of the acid solution on the ion pair formation, reagent concentration, time and temperature, and solvent was studied. The low relative standard deviation (%RSD) values indicate good precision and high recovery values. This method has been successfully applied for the assay of solifenacin in pure form and in pharmaceutical formulations.

**Keywords:** Solifenacin succinate; Alizarin red; Extractive spectrophotometric method; Ion-pair complex

### INTRODUCTION

Solifenacin is an orally administered urinary antispasmodic anticholinergic drug. The Solifenacin is (1S)-quinuclidin-3-yl-1-phenyl-3,4-dihydroisoquinoline-2(H)-carboxylate (Budavari ., 1996). Structure of solifenacin is shown in Fig.1. Solifenacin is an example of anticholinergic or parasympatholytic drug. It is white or pale yellowish white crystalline powder. It is freely soluble in water, methanol, dimethyl sulphoxide and glacial acetic acid. Acetylcholine is an example of cholinergic drug. It is bind with muscarinic receptor particularly  $M_3$  receptor to produce contraction of urinary bladder smooth muscle to cause micturition, urgency and incontinence episodes. Solifenacin is a competitive muscarinic acetylcholine receptor antagonist. Solifenacin preventing the binding of acetylcholine to these receptors particularly  $M_3$  receptor and reduces the contraction smooth muscle in the urinary bladder, allowing the bladder to retain larger volumes of urine and reducing the number of micturition, urgency and incontinence episodes. Solifenacin should not be taken by people with a history of previous hypersensitivity to it, gastric retention, urinary retention and severe liver disease (Cardozo L *et al.*, 2004; Garely AD., 2006).

Simple, rapid and highly sensitive spectrophotometric method is described, for the first time, for the deter-

mination Solifenacin with alizarin red. The aim of present study is to develop and validation of extractive spectrophotometric method (Barary MH *et al.*, 1991; Abdelmageed OH *et al.*, 1993; Botello JC *et al.*, 1995; Sastry CSP *et al.*, 1995) for the determination of solifenacin with alizarin red through ion-pair complex formation. Spectrophotometric method of analysis is used in the analysis of drugs in pharmaceutical dosage forms owing to its good sensitivity and cost effectiveness (Basavaiah K *et al.*, 1999; Madihalli S *et al.*, 2012; Lokesh Singh *et al.*, 2011).

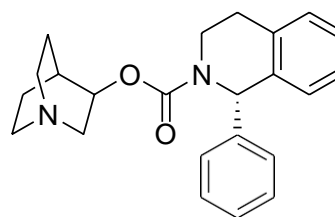


Figure 1: Structure of Solifenacin

### MATERIALS AND METHODS

#### Materials

Solifenacin and its tablets (Soliten® 5 mg) were kindly supplied from Ranboxy, Mohali, Punjab, India. Alizarin red was purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India. Analytical grade chloroform was used for extraction. Solvents and other chemicals were of analytical grade.

#### Apparatus

Analytical UV–Vis spectrophotometer 2060 Plus with 1 cm glass cells was used.

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Received on: 12-03-2013  
Revised on: 04-04-2013  
Accepted on: 07-04-2013

## Solutions

Stock solution of Solifenacin 1.0 mg/mL was prepared in ethanol. Working standard solutions of solifenacin 100 µg/mL was prepared by appropriate dilution of the stock solution with ethanol. Stored in a refrigerator it was stable for 1 month.

Stock solution of alizarin red (0.05%w/v) were prepared by dissolving 0.050 gm of alizarin red in 100 mL distilled water. The acid dye reagent was stable for several weeks.

Standard solution of hydrochloric acid (0.1N) was prepared by dissolving 0.85 ml of hydrochloric acid in distilled water and completed to 100 mL with distilled water. Freshly prepared solutions were always employed.

## General analytical procedure for the analysis of bulk drug

Aliquots of 0.25-2.25 mL of the working drug solutions (100µg/mL) were accurately transferred in to series of 10 mL volumetric flasks and to each flask 1.0 mL of 0.1N HCl solution was added, followed by 1.0 mL of 0.05% w/v alizarin red solution. The content was mixed and the reaction mixture was kept aside for 10 min at room temperature. After 10 min the volume of the each flask was made up to mark with distilled water. The formed ion-pair complex was extracted twice with 5.0 mL chloroform after shaking for 2.0 min and chloroform layer was collected and dried through anhydrous Na<sub>2</sub>SO<sub>4</sub>. The absorbance of the yellow colored complex was measured at 430nm against reagent blank.

## General analytical procedure for the analysis of tablets

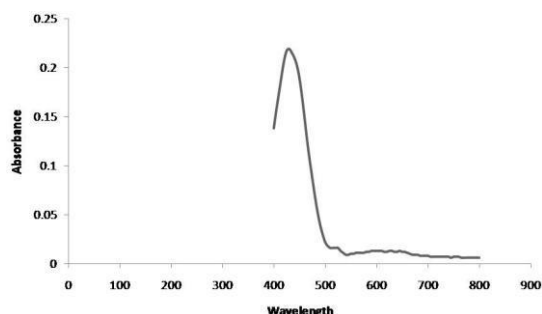
The average tablet weight was calculated from the contents of 20 tablets that had been finely powdered and weighed. A portion of this powder, equivalent to 50 mg of solifenacin, was accurately weighed. The samples were shaken with 25mL of ethanol. The mixtures were then introduced into an ultrasonic bath for 25 min and diluted with ethanol in a 50 mL volumetric flask and filtered. From this filtrate 10 ml was taken into 100 mL volumetric flask and volume of flask was made upto the mark with distilled water (100µg mL<sup>-1</sup>). An appropriate volume of 100 µg mL<sup>-1</sup> sample solution was added and mixed. This solution was analyzed as in the general analytical procedure for the bulk drug. The amount of solifenacin per tablet was calculated using the calibration curve method. The proposed method was applied to the determination of solifenacin in tablets (ICH guidelines., 1996; Shabir GA., 2003).

## RESULTS AND DISCUSSION

### Absorption Spectra

The reaction of solifenacin with alizarin red in an acidic medium to form a yellow ion pair complex was investi-

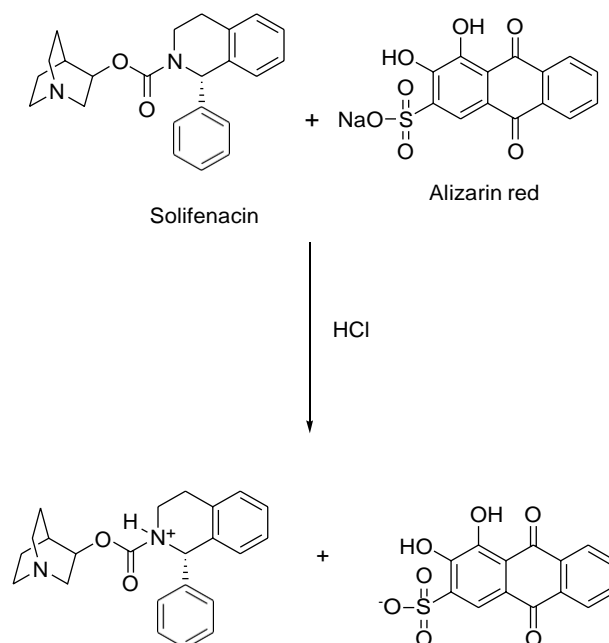
gated and this complex was extracted into chloroform. The absorption spectrum of the formed ion-pair complex was recorded at 400 to 800 nm against the reagent blank and exhibited a maximum absorption at 430 nm. The absorption spectrum of solifenacin was shown in Figure 2. The optimum reaction conditions for determination of the ion-pair complex between solifenacin and alizarin red were established.



**Figure 2: Absorption spectra of ion-associate complexes of solifenacin-alizarin red (10µg/ml) against blank**

### Reaction mechanism

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphophthalein group present mainly in anionic form in acidic condition ( $\geq 3$ ). So, when the solifenacin was treated with an acid dye in acid medium, a yellow ion-pair complex was formed which was extracted with chloroform. The reaction pathway for the formation of ion-pair complex between solifenacin and alizarin red was shown in scheme 1.



**Scheme 1: The reaction pathway for ion-pair complex formation of solifenacin-alizarin red**

The reaction conditions of the method were carefully studied to achieve complete reaction, highest sensitivity and maximum absorbance. Reaction conditions for

the formation of the ion-pair complex were optimized by studying preliminary experiments involving strength of acid medium, type of organic solvent, shaking time and volume of the dye for the extraction of ion-pair complex.

#### Effect of time and temperature

The optimum reaction time was investigated from 0.5 to 4.0 min by following the color development at ambient temperature ( $37\pm 2^\circ\text{C}$ ). Complete color intensity was attained after 2.0 min of mixing for complex are shown in Figure 3. The absorbance remains stable for at least 24 hrs.

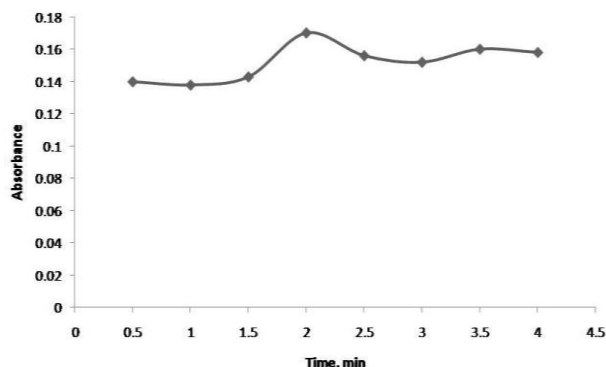


Figure 3: Effect of shaking time on the ion-pair complex

#### Effects of strength of acid medium on the ion-pair formation

The effect of strength of acid medium was studied by extracting the colored complex in the presence of various strength of hydrochloric acid solution such as 0.05, 0.1, 0.2, 0.3, 0.4, 0.5N. The maximum color intensity and highest absorbance value were observed in 0.1N hydrochloric acid solution. The result was shown in Figure 4. Further, 1.0 mL of 0.1N hydrochloric acid solution gave maximum absorbance and reproducible results.

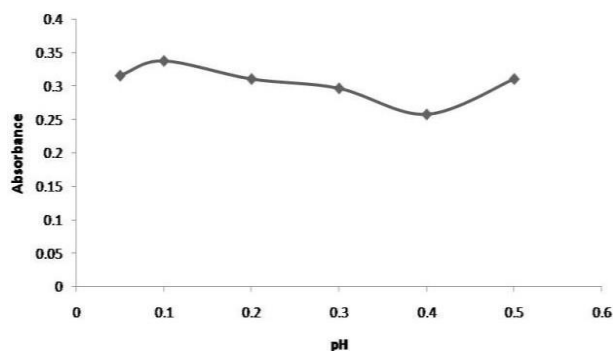


Figure 4: Effect of various strength of acid solution on the absorbance of solifenacin ( $20\mu\text{g/mL}$ )

#### Effect of dye Concentration

The effect of the concentration of the dye on the intensity of the color developed at the selected wavelength and constant drug concentration ( $22.5\mu\text{g mL}^{-1}$ ) was tested using different volumes of alizarin red (0.5 -

2.5 mL). It was observed that 1.0 mL of 0.05% (w/v) dye was necessary for maximum color development of the ion-pair complex. Above this volume, the absorbance remained constant. The result was shown in Figure 5.

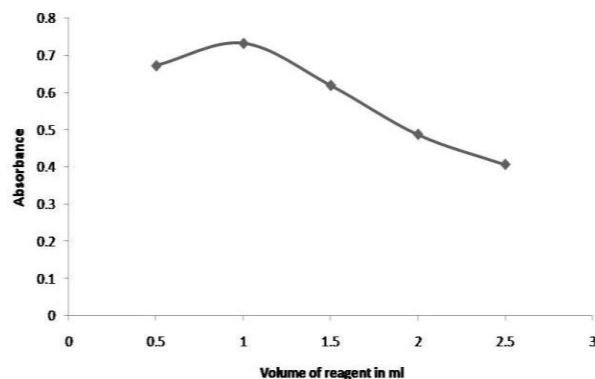


Figure 5: Effect of reagent concentration on the reaction of ( $22.5\mu\text{g mL}^{-1}$ ) solifenacin with alizarin red

#### Effect solvents

The effect of several organic solvents viz., carbon tetrachloride, chloroform, ethyl acetate, xylene, dichloromethane, chlorobenzene, diethyl ether, toluene and butyl acetate were tried for effective extraction of the colored species from aqueous phase. Chloroform was found to be the most suitable solvent for extraction of yellow colored ion-pair complex for alizarin red, yielding maximum absorbance and color intensity and considerably lower extraction ability for the reagent blank.

#### Linearity and range

At a described experimental conditions for the determination of solifenacin, standard calibrations curve were constructed between the concentrations and absorbances.  $\lambda_{\text{max}}$ , Beer's law range, Sandell's sensitivity, molar absorptivity, correlation coefficient, regression equation, limit quantification and limit of detection were determined for the developed method are given in Table 1. Limit quantification and limit of detection are recorded as per IUPAC definitions. A linear relationship was found between the absorbance's and the drug concentrations in the range  $2.5\text{--}22.5\mu\text{g mL}^{-1}$  in the final measured volume of 10 ml. Regression equation ( $A = mC + b$ ) where  $A$  is the absorbance of 1 cm layer,  $m$  is the slope,  $b$  is the intercept and  $C$  is the concentration of the measured solution in  $\mu\text{g mL}^{-1}$ , were calculated and recorded in Table 1. The high molar absorptivities of the resulting colored complexes indicate the high sensitivity of the methods. Calibration curve for proposed method is shown in Figure 6.

#### Validation of the method

Samples of pure solifenacin were prepared and tested at three levels of drug using the proposed procedures. The complete set of validation assays was performed for drug, determined by the proposed methods. The results obtained for pure drugs are given in Table 2. The precision and accuracy of the method were tested

**Table 1: Statistical data of the regression equations for Determination of solifenacin by alizarin red**

S. No	Parameters	Alizarin red
1	$\lambda_{\max}$	430
2	Linearity range ( $\mu\text{g/mL}$ )	2.5 – 22.5
3	Molar absorptivity (L/mol/Xcm)	$1.76 \times 10^4$
4	Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ per 0.001 absorbance unit)	1.890
5	Regression equation (y)	$0.009 + 0.017x$
6	Intercept (b)	0.00893
7	Slope (a)	0.01679
8	Correlation coefficient (r)	0.999
9	SD	0.2106
10	LOD ( $\mu\text{g/mL}$ )	0.041
11	LOQ ( $\mu\text{g/mL}$ )	0.125

LOD, limit of detection; LOQ, limit of quantification

**Table 2: Analysis of solifenacin in bulk powder by alizarin method (n=6)**

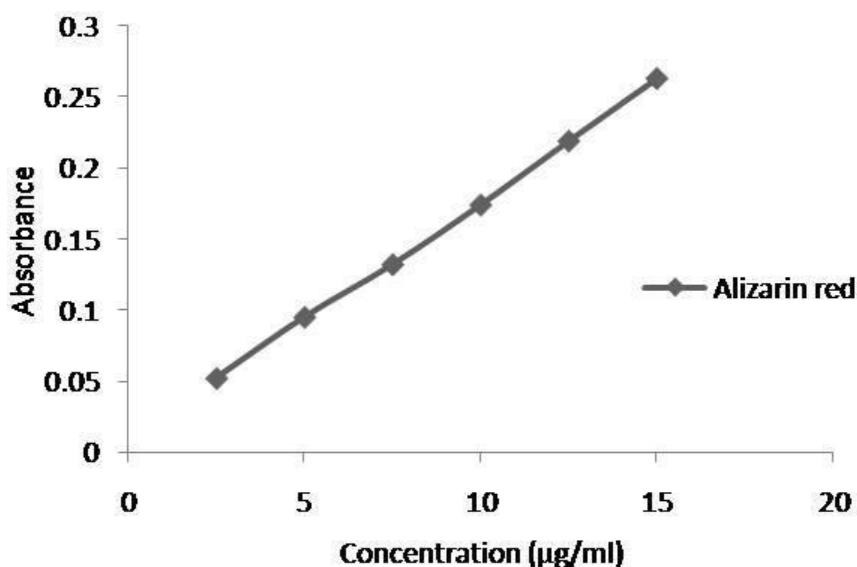
Method	$\mu\text{g/mL}$		S.D	Recovery (%)	Precision <sup>a</sup> R.S.D (%)	Accuracy ER%	Confidence limits <sup>b</sup> (90%)
	Taken	Found					
Alizarin red	2.5	2.501	0.0222	100.04	0.89	0.40	2.483-2.519
	7.5	7.51	0.0260	100.1	0.35	0.13	7.489-7.531
	12.5	12.51	0.0294	100.08	0.24	0.08	12.486-12.534

n, number of determination, R.S.D.%, percentage relative standard deviation; Er %, percentage relative error.

<sup>a</sup>Mean of six determination; <sup>b</sup>Confidence limit at 90% confidence level and five degrees of freedom.

**Table 3: Evaluation of accuracy and precision of solifenacin tablets by standard addition method (n= 6)**

Method	$\mu\text{g/mL}$			S.D	Recovery (%)	Precision <sup>a</sup> R.S.D (%)	Accuracy ER%	Confidence limits <sup>b</sup> (90%)
	Taken	Added	Found					
Alizarin red	2.5	2.5	5.02	0.0544	100.40	1.08	0.40	4.975-5.065
		5.0	7.503	0.0265	100.04	0.35	0.04	7.481-7.525
		7.5	10.02	0.0618	100.20	0.62	0.20	9.969-10.071

**Figure 6: Calibration curve of ion-associate complex of solifenacin-alizarin red against blank**

by analyzing six replicates of the drug. The standard deviation, relative standard deviation, recovery and 90% confidence limits of different amounts tested were determined from the calibration curve, as recorded in Table 2. The accuracy of the method was indicated by the excellent recovery (100.04–100.1%).

#### Tablets analysis

The proposed method were applied to the determination of solifenacin in commercial tablets. The accuracy of the proposed method is evaluated by applying standard addition technique, in which variable amounts of the drug were added to the previously analyzed por-

tion of pharmaceutical preparations and the results are tabulated in Table 3. Six replicates determinations were made. Satisfactory results were obtained for drug and were in a good agreement with the label claims are shown in Table 3. The results were reproducible with low R.S.D. values. The average percent recoveries obtained were quantitative (100.04–100.4%), indicating good accuracy of the methods. The results of analysis of the commercial tablets and the recovery study of drug suggested that there is no interference from any excipients (such as starch, lactose, titanium dioxide, and magnesium stearate), which are present in tablets.

## CONCLUSION

The proposed method (Alizarin red) can be used for determination of solifenacin in tablets. The method is rapid, simple and have great sensitivity and accuracy. Proposed method make use of simple reagents, which an ordinary analytical laboratory can afford. Methods are sufficiently sensitive to permit determination even down to  $2.5 \mu\text{g mL}^{-1}$ . The proposed methods are suitable for routine determination of solifenacin in its formulations. The commonly used additives such as lactose, starch, titanium dioxide and magnesium stearate do not interfere with the assay procedures.

## ACKNOWLEDGEMENT

We are very much grateful to Management, Ratnam Institute of Pharmacy, Andhra Pradesh for providing necessary facilities to conduct the research work.

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