International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.4, pp 2003-2010, Oct-Dec 2011

Stability Indicating Normal Phase HPTLC Method for Estimation of Alfuzosin and Solifenacin in Pharmaceutical Dosage Form

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Abstract: This paper presents the development and validation of normal phase HPTLC methods for simultaneous analysis of afluzosin and solifenacin in tablets. Chromatography was performed on silica gel $60F_{254}$ plate as stationary phase and the mobile phase comprised of methanol: ethyl acetate (7:3, v/v). Detection wavelengths selected were 254 nm for Alfuzosin and 220 nm for solifenacin. The Rf values were 0.71 ± 0.03 and 0.32± 0.02 for Alfuzosin and solifenacin, respectively. A TLC scanner set at 254 nm and 220 nm for Alfuzosin and Solifenacin, respectively was used for direct evaluation of the chromatograms in reflectance/absorbance mode. Method was validated according to ICH guidelines. Determination coefficients of calibration curves were found 0.9903 and 0.9981 in the concentration ranges 500-2500 ng/band for Alfuzosin and solifenacin, respectively. Method had an accuracy of 99.30 % for Alfuzosin and 98.91 % for solifenacin. Both the HPTLC methods had the potential to determine these drugs from dosage forms without any interference.
Keywords: Alfuzosin hydrochloride, Solifenacin succinate, HPTLC, Stability Indicating, Validation.

1. INTRODUCTION

The symptoms associated with benign prostatic hyperplasia (BPH) such as urinary frequency, nocturia, weak stream, hesitancy and incomplete emptying are related to two components, anatomical (static) and functional (dynamic) (1). Alfuzosin hydrochloride (ALF) chemically is (R, S)-N-[3-[(4-amino-6, 7-dimethoxy-2-quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride (2). It exhibits selectivity for alpha1-adrenergic receptors in the lower urinary tract. It is used to treat the signs and symptoms of benign enlargement of the prostate, by increasing the flow in urine which is reduced by benign prostatic hypertrophy (3-5).

Solifenacin succinate (SOL), a muscarinic receptor antagonist, chemically is butanedioic acid, compounded with (1S)-(3R)-1-azabicyclo [2.2.2] oct-3-yl-3, 4-dihydro-1-phenyl-2(1H)-isoquinoline carboxylate. Muscarinic receptors play an important role in several major cholinergically mediated functions, including contractions of urinary bladder smooth muscle and stimulation of salivary secretion. It is used in the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency (6, 7).

Literature survey revealed rapid tandem mass spectrometry method for alfuzosin in plasma (8), HPLC (9, 10) methods reported for analysis of alfuzosin alone in pharmaceutical dosage form and in biological samples. However Solifenacin succinate is reported to be estimated in tablets by HPLC (11). There is lack of HPLC equipment in many resource limited countries. In poor countries, where such equipment is available, the high cost of HPLC grade solvents and columns, and the lack of possibility to analyze many samples simultaneously significantly affect timely release of laboratory results for action.

Therefore alternative methods are needed to facilitate and increase the speed of analysis with relatively few costs. Mehta et al. developed HPTLC method for analysis of Alfuzosin in pharmaceutical formulation with mobile phase composed of toluene: methanol: Triethylamine (3: 1: 0.2 v/v/v) (12). In laboratory it is preferred to reduce the use of triethyl amine as it causes irritation to eyes, skin and respiratory system. Whereas the HPTLC method for analysis of solifenacin in pharmaceutical dosage form is not yet reported in the literature. In this study a high performance thin layer chromatography method (with a mobile phase without triethyl amine; with compact and symmetrical bands) for estimation of alfuzosin and solifenacin in tablets, was developed and validated for accuracy, precision, specificity and robustness, as recommended by ICH guidelines (13, 14).

2. MATERIAL and METHOD

2.1 MATERIAL

Pure drugs Alfuzosin and Solifenacin were obtained as gift sample from Ranbaxy Laboratories Ltd. Dewas, India. Methanol and Ethyl acetate was obtained from Qualigens Fine Chemicals Ltd. All were of analytical grade.

HPTLC aluminum plates pre-coated with silica gel 60F254 (10 cm X 10 cm) were from Merck. Densitometry was carried out using Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) fitted with win-CATS software version 4. Samples were applied as band on the HPTLC plates using the spray-on technique of Camag Linomat V under nitrogen gas flow, and developed in a Camag 10 cm X 10 cm twin trough chamber.

2.2 METHOD

2.2.1 Method development:

Standard stock solutions (0.5 mg/ml) of ALF and SOL were prepared in methanol as solvent. Solutions of 2 µl were applied on the HPTLC plates as spot bands of 6 mm using Linomat V. Application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left to saturate with mobile phase vapor for 30 min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7 cm. Then the plates were dried on a hot plate. Room temperature and relative humidity were always maintained at 25^0C ± 2 and 60 % ± 5. Densitometric scanning was done in absorbance mode at 254 nm and 220 nm for ALF and SOL, respectively using a deuterium lamp. The slit dimensions were 5 mm X 0.45 mm and the scanning speed was 20 mm/s and the data resolution at 100 µm/step.

2.2.2 Method validation:

Linearity of detector response

Different volumes of standard stock solutions (1, 2, 3, 4, and 5 µl) were applied separately on HPTLC plate to deliver 500, 1000, 1500, 2000 and 2500 ng/band of ALF and SOL. Each concentration was applied six times on the HPTLC plate. The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

Precision

Precision of the developed method was studied by considering intra-day precision, inter-day precision and variation between analysts.

Accuracy

The pre-analyzed tablet powder was spiked with drug component at 80 %, 100 % and 120 % of the target sample concentration. Extraction and dilutions were performed with methanol and the amounts ALF/SOL applied on the HPTLC plate were 900, 1000 and 1100 ng/band. Solutions were prepared in triplicate and analyzed. Accuracy was determined and expressed as percentage recovery.

Robustness

(Variation in composition of the mobile phase, chamber saturation time, developing distance, band size). The composition of the mobile phase and chamber saturation time were varied in the range of ± 0.1 ml and ±10 %, respectively, of the used optimized conditions. Developing distance and band size were varied ± 1 cm and ± 1 mm of the used optimized condition. The effect of these changes on both the Rf values and peak areas were evaluated by calculating the relative standard deviations (RSD) for each parameter.

Limit of detection and Limit of quantification

To determine the limits of detection and quantification, concentrations in the lower part of the linear range of the calibration plot were used. Stock solution of ALF/SOL (1000 µg/ml) was prepared and different volumes in the range 200-1000 ng/band were applied in triplicate. Amount of ALF/SOL per band was plotted against average response (peak area) and the regression equations were determined. The standard deviation (SD) of responses and average standard deviation (ASD) were calculated. Detection limit was calculated as (3.3 X ASD)/b and quantification limit was calculated as (10 X ASD)/b, where b denotes the slope obtained in the linearity study.

2.2.3. Analysis of Marketed Formulation

The methods were used for quantitation of alfuzosin and solifenacin in tablets. For sample preparation, methanol was used as solvent for extraction and dilution. Twenty tablets of ALF/SOL were

individually weighed and ground into fine powder. Portions of tablet powder equivalent to 10 mg ALF/5 mg SOL was accurately weighed and transferred separately to 10.0 ml volumetric flask. About 6 ml of methanol was added and the mixture was sonicated for 15 min. the mixture was diluted to volume with methanol, mixed well and filtered through whatmann filter paper to obtain sample stock solution.

For the determination of ALF, sample stock solution was used as such. For SOL determination, 5.0 ml of SOL sample stock solution was diluted to 10.0 ml with methanol. Six sample solutions were prepared and analyzed according to the method procedure. Sample and standard solutions were applied on the same plate. The possibility of excipients interference in the analysis was studied.

2.2.4. Forced Degradation Studies

In order to ensure that the analytical method was stability indicating, stress studies were performed.

a) **Acid Degradation Studies**: 1 ml of 0.1N hydrochloric acid was added to 9 ml of drug solution (1000ng/band). This solution was allowed to stand for 24 hrs.
b) **Alkali Degradation Studies**: 1 ml of 0.1N Sodium hydroxide was added to 9 ml of drug solution (1000ng/band). This solution was allowed to stand for 24 hrs.
c) **Oxidation Studies**: 1 ml of 3% hydrogen peroxide was added to 9 ml of drug solution (1000ng/band). This solution was allowed to stand for 24 hrs.

3. RESULTS AND DISCUSSION

3.1 Method development

In an attempt to achieve the desired Rf value range (0.2-0.8) with a compact band, several trials were made by using different solvent systems containing non-polar solvents and relatively polar solvents. Among the different mobile phase combinations tested ethyl acetate and methanol (7:3, v/v) gave compact bands which showed symmetrical peak on chromatogram. The Rf values with their standard deviation were 0.71 ± 0.03 for Alfuzosin (Fig. 1) and 0.32 ± 0.02 for solifenacin (Fig. 2), respectively.

3.2 Method validation

Linearity of detector response

Linearity for both the drugs was tested in the concentration range 500 – 2500 ng/band. The solutions were chromatographed six times, in accordance with the International Conference on Harmonization (14).Separate calibration plots for ALF and SOL were constructed by plotting peak area against the respective concentrations, and the method was evaluated by determination of the correlation coefficient and intercept, calculated in the corresponding statistical study (ANOVA; $P < 0.05$). R^2 values >0.999 and intercepts very close to zero confirmed the good linearity of the method.

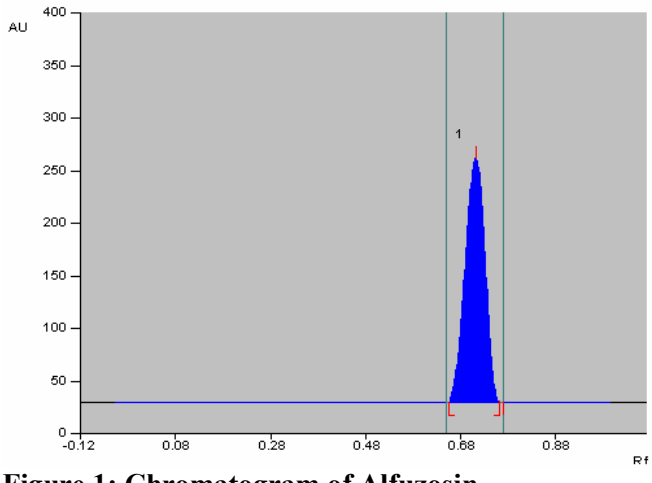

Figure 1: Chromatogram of Alfuzosin

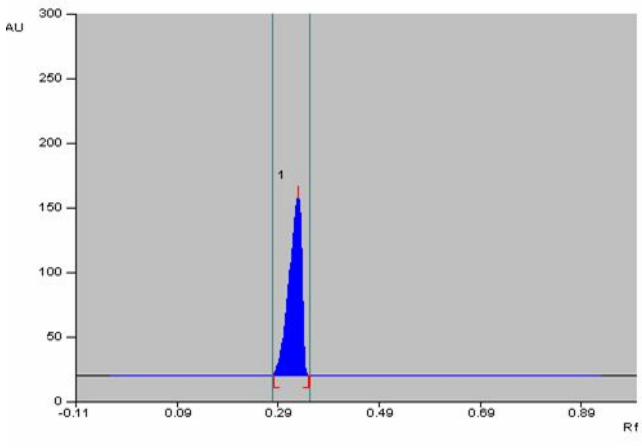

Figure 2: Chromatogram of Solifenacin

Table 1: Liner regression data for ALF

Parameter	ALF	SOL
Wavelength/nm	254 nm	220 nm
Concentration Range/ μg ml^{-1}	500-2500 ng/band	500-2500 ng/band
Determination of coefficient, r^2	0.990	0.998
Slope ±* S.D.	4.28	1.27
Intercept ±* S.D	3716	304.8
Significance level	P<0.005	P<0.005

* Average of 3 determinants (n=3)

Precision

Repeatability and intermediate precision of the developed method were expressed in terms of coefficients of variation (CV) of the peak area. The results showed that intra- and inter-day variation of the results at the concentration 1500 ng/band for ALF/SOL were within the acceptable range. The coefficients of variation for both the inter-day and intraday precision of the method was found to be less than 2% for both drugs (Table 2.a). The dosage forms were also analyzed by three different analysts within the same day and the results revealed that there is good intermediate precision between analysts (Table 2.b) with coefficients of variation less than 2% for Alfuzosin and solifenacin, respectively. In conclusion, the precision values obtained in our method are considered acceptable.

Accuracy: The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The results obtained for ALF at 80 %, 100 % and 120 % concentration levels were 98.82 ± 1.14, 99.82 ± 0.35 and 99.27 ± 0.99. The range of % recovery was 98.03-100.2 %, while the mean recovery for all the concentration levels was 99.30 ± 0.89. For SOL, the % recoveries at the same concentration level were 98.38 ± 0.96, 99.33 ± 0.98 and 99.03 ±0.78. The range of % recovery was 97.4-100 %, while the mean recovery for all the concentration levels was 98.91 ± 0.87 (Table 3). In conclusion, the method was considered to have an acceptable recovery and trueness.

Table 2: a) Statistical evaluation of precision of developed method (n=3)

Drug	Conc.(ng/band)	Intra day				Inter day			
		*%mean	*SD	*%RSD	*S.E	*%Mean	*S.D	*%RSD	*S.E
ALF	1500	100.12	1.15	0.014	0.66	100.20	0.90	0.011	0.54
SOL	1500	99.35	0.98	0.068	0.56	99.91	1.15	0.08	0.66

*Mean of three determinations, S.D: Standard Deviation, R.S.D: Relative Standard Deviation

b) Statistical evaluation of precision of developed method (n=3)

Drug	Conc.(ng/band)	Analyst 1				Analyst 2			
		*%mean	*SD	*%RSD	*S.E	*%Mean	*S.D	*%RSD	*S.E
ALF	1500	100.11	1.88	0.02	1.09	99.76	1.90	0.01	1.10
SOL	1500	99.49	0.89	0.07	1.12	99.81	1.5	0.11	0.91

*Mean of three determinations, S.D: Standard Deviation, R.S.D: Relative Standard Deviation

Table 3: Recovery Study Data

Drug	Level of % recovery	Label claim(mg)	Amount of pure drug added(mg)	%*Mean	S.D.*(±)	R.S.D*
ALF	80	10	3	98.82	1.14	1.15
	100	10	5	99.82	0.35	0.35
	120	10	7	99.27	0.99	0.99
SOL	80	5	3	98.38	0.96	0.97
	100	5	5	99.33	0.98	0.98
	120	5	7	99.03	0.78	0.78

*Mean of three determinations, S.D: Standard Deviation, R.S.D: Relative Standard Deviation

Robustness

The standard deviations of peak areas were calculated for the aforementioned four parameters (variation in composition of the mobile phase, band size, developing distance and chamber saturation time) and coefficients of variation were found to be less than 2% in all cases as shown in Table 4. The low CV values indicate the robustness of the method.

Limit of detection and Limit of quantification

The limit of detection and limit of quantification values, calculated as described above, for alfuzosin were found to be 5.44 ng/band and 16.5 ng/band, respectively. For solifenacin the values were 4.35 ng/band and 13.2 ng/band, respectively.

3.3 Analysis of Marketed Formulation:

Analysis of samples of marketed tablet formulation containing alfuzosin tablet (10 mg) and solifenacin tablet (5 mg) was carried out and the amounts recovered were expressed as percentage label claim. The Rf value for ALF and SOL extracted from tablet sample was found to be 0.71 and 0.32, respectively. A single band was observed in the chromatogram indicated that there is no interference from the tablet excipients. The percentage amounts of Alfuzosin and solifenacin were between 97.87-99.99 % and 98.85-100.2 %, respectively. The results are indicated in Table 5. Low values of % RSD indicated the suitability of this method for routine analysis of alfuzosin and solifenacin in pharmaceutical dosage form.

Table 4: Results of Robustness

Drug	ALF		SOL	
Parameters	*S.D.	%RSD	*S.D.	%RSD
Variation in composition of the mobile phase (±0.1 ml)	0.169	0.170	0.792	0.788
Band size (±1mm)	0.106	0.106	0.558	0.557
Developing distance (±1 cm)	0.357	0.360	0.392	0.395
Chamber saturation time (±3 min)	0.325	0.327	0.447	0.452

*Mean of three determinations, S.D: Standard Deviation, R.S.D: Relative Standard Deviation

Table 5: Results of formulation analysis

Formulation	Label claim (mg)	Amount of drug estimated*±S.D* (mg)	%mean amount Estimated*±S.D*
ALF	10	9.89±0.04	98.99±0.91
SOL	5	4.94±0.09	99.43±0.54

*Mean of three determinations, S.D: Standard Deviation.

3.4 Forced Degradation Studies

HPTLC studies of the samples obtained during the stress testing of ALF and SOL under different conditions. Different degradations peak as shown in figures 3-8. The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error (15). The amount of drug recovered after degradation studies and the Rf of the degradation products are given in table 6. (Table 6)

a) Acid induced degradation:

The drugs were degraded in the acidic condition and shows different degradation products at Rf 0.01, 0.17, 0.89 for ALF and 0.16, 0.20, 0.97 for SOL as shows in the figures 3 and 4.

b) Base induced degradation:

The drugs were degraded in the alkaline condition and shows different degradation products at Rf 0.02, 0.23, 0.37 for ALF and 0.17, 0.63, 0.65, 0.80, 0.86 for SOL as shows in the figures 5 and 6.

c) Hydrogen peroxide-induced degradation:

The drugs were degraded in hydrogen peroxide (3%) at room temperature shows different degradation products at Rf 0.01, 0.06, 0.80 for ALF and 0.13, 0.71, 0.81 for SOL as shows in the figures 7 and 8.

Table 6: Results of forced degradation studies

Stress condition	Drugs	Time hrs	Mass balance (%assay of recovered + %impurities + % degradents)	Rf values of degradation products
Acid hydrolysis (0.1 M HCl)	ALF	24	99.88	0.01, 0.17, 0.89
	SOL	24	100.20	0.02, 0.23, 0.37
Alkali hydrolysis (0.1 N NaOH)	ALF	24	99.96	0.01, 0.06, 0.80
	SOL	24	99.01	0.16, 0.20, 0.97
Oxidation (3%H_2O_2)	ALF	24	100.11	0.17,0.63,0.65,0.80,0.86
	SOL	24	98.99	0.13, 0.71, 0.81

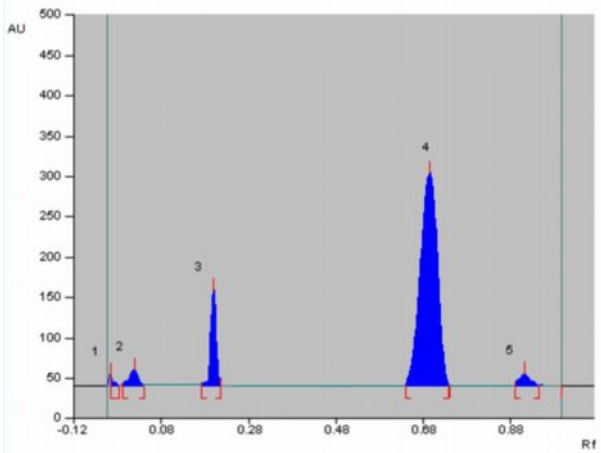

Figure 3: Chromatogram of acid hydrolysis of Alfuzosin

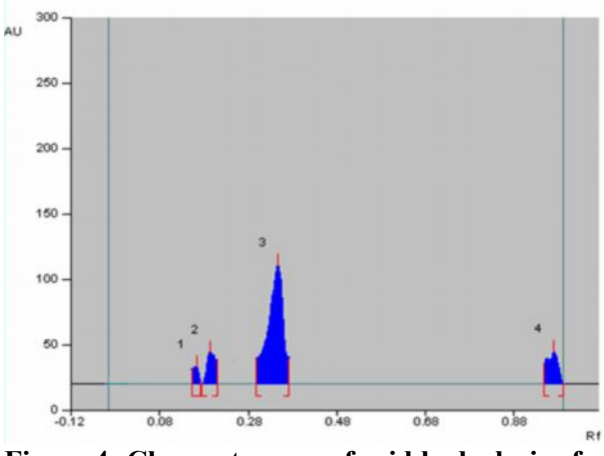

Figure 4: Chromatogram of acid hydrolysis of Solifenacin

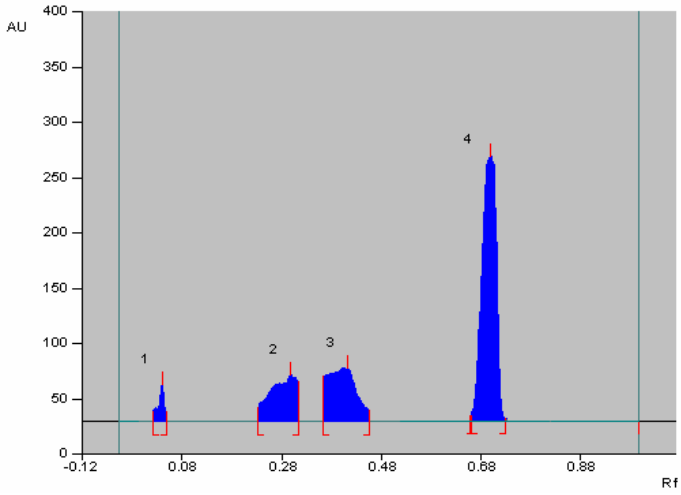

Figure 5: Chromatogram of alkali hydrolysis of Alfuzosin

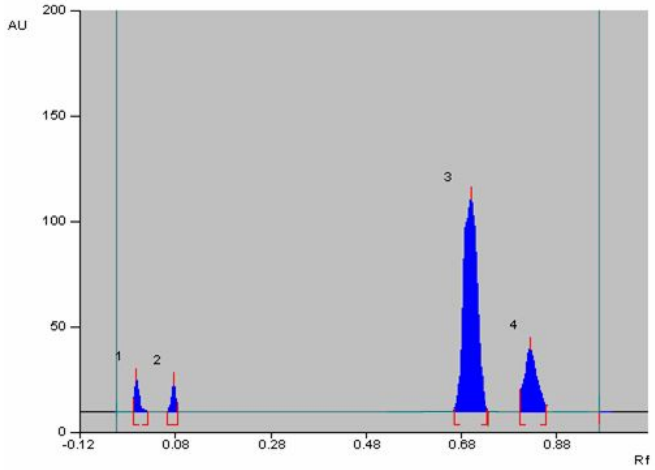

Figure 7: Chromatogram of oxidative degradation of Alfuzosin

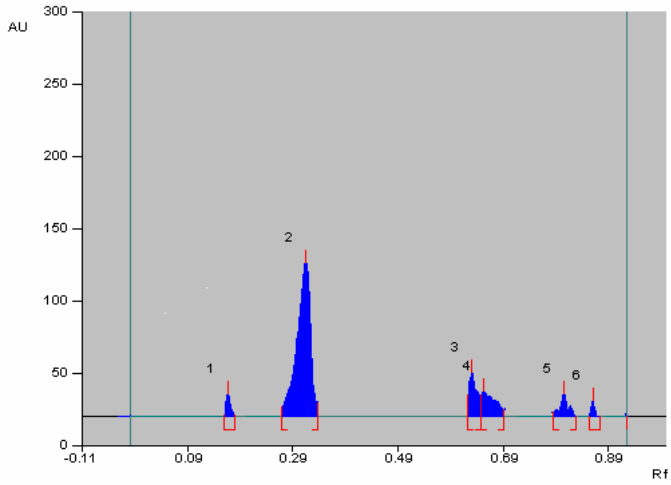

Figure 6: Chromatogram of alkali hydrolysis of Solifenacin

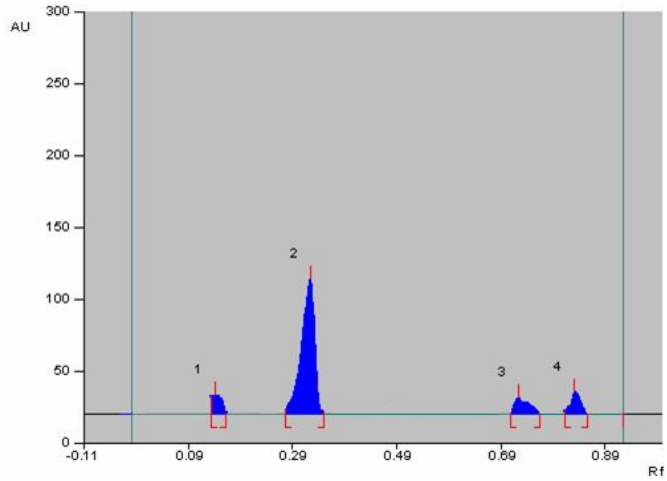

Figure 8: Chromatogram of oxidative degradation of Solifenacin

4. CONCLUSION

The proposed method based on the HPTLC was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy for the proposed method. Hence, it can be concluded that the developed and chromatographic is accurate, precise and selective and can be employed successfully for the estimation of Alfuzosin and Solifenacin in bulk and formulation.

ACKNOWLEDGEMENT

The authors express their gratitude to Dr. A. D. Deshpande, Director, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, MH, India, for providing necessary facilities, and to Ranbaxy Laboratories Ltd. Dewas, India, for the generous gift samples of pure Alfuzosin hydrochloride and Solifenacin succinate.

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