ISSN: 2278-778X
Research Article
www.ijbio.com

A RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SOLIFENACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Saroj Kumar Raul[1]*, BVV Ravi kumar[2], Ajaya Kumar Patnaik[3]
[1]M.R. College of Pharmacy, Phool-Baugh, Vizianagaram, A.P, India
[2]Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India
[3]Departments of Chemistry, Ravenshaw University, Cuttack, Orissa, India

***Corresponding Author:** Mr. Saroj Kumar Raul, Department of Pharmaceutical Chemistry, Maharajah's College of Pharmacy, Vizianagaram-2, A.P, India.

Received for publication: October 11, 2012; **accepted**: October 28, 2012.

Abstract: A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Solifenacin in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.0 mL min^{-1} was employed on XTerra C_{18} (150 × 4.6 mm, packed with 5 µm) column at ambient temperature. The mobile phase consisted of Acetonitrile: phosphate buffer 50:50 (v/v) and the detection wavelengths were at 210 nm. Linearity was observed in concentration range of 20-70 µg/mL. The retention time for Solifenacin was 2.4 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Solifenacin in pharmaceutical dosage forms.

Keywords: Dosage forms, Estimation, Method development, Solifenacin, RP-HPLC, Validation.

INTRODUCTION

The drug Solifenacin (Fig.1) generally available as Solifenacin succinate is a competitive muscarinic receptor antagonist[1]. Muscarinic receptors play an important role in several major cholinergically mediated functions, including contractions of urinary bladder smooth muscle and stimulation of salivary secretion[2]. It has higher selectivity for the urinary bladder than for the salivary gland and used for the treatment of overactive bladder[3]. Chemically it is described as 1-azabicyclo [2.2.2] oct-8-yl (1S)-1-phenyl-3, 4-dihydro-1*H*-isoquinoline-2-carboxylate.

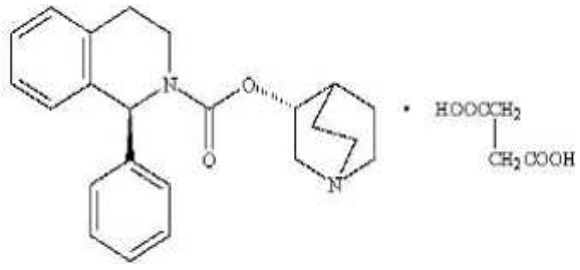

Figure.1: Chemical structure of Solifenacin succinate

Literature survey reveals that few spectrophotometric methods[4-5], HPLC methods[6-8]. HPTLC methods[9-10] has been reported for the estimation of Solifenacin in alone and in combined tablet dosage form. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Solifenacin in pharmaceutical dosage form as per ICH guidelines[11].

MATERIALS AND METHOD

Instrumental and analytical conditions: The HPLC analysis was carried out on Waters HPLC system (2695 module) equipped with 2487 dual lambda detector with auto Sampler and running on Waters Empower software. The column used is XTerraC_{18} (150 × 4.6 mm, packed with 5 µm) and detection was performed at 210 nm. The injection volume of sample was 20 µL and the run time was 5 minutes. An isocratic mobile phase containing acetonitrile and 0.02 M phosphate buffer at 50: 50 (v/v) at the pH 2.5 was carried with the flow rate at 1.0mL min^{-1}. The mobile phase was filtered through 0.4µm membrane filter and degassed before use.

Reagents and chemicals: Solifenacin working standard was kindly gifted by pharma train, Hyderabad. Tablets were purchased from local pharmacy manufactured by Ranbaxy Laboratories Ltd (Soliten). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrilel was obtained from Merck (India) limited. All other chemicals used were AR grade. The optimum chromatographic conditions were summarized in table.8.

Preparation of mobile phase: Dissolved 2.7218 g of Potassium Di hydrogen orthophosphate in 1000 mL of water and mixed, pH adjusted to 2.5 using ortho phosphoric acid, sonicated to degas the buffer. Transferred 500 volumes of acetonitrile and 500 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45 µm

filter under vacuum. The same mobile phase was used as diluent.

Preparation of Standard Solution: Accurately weighed about 10 mg of Solifenacin and transferred into a 10mL volumetric flask and 7 mL of diluent was added and sonicate to dissolve it completely and the volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. Then 0.4 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with mobile phase and filter through 0.45µm filters, which gives a solution of strength 40 µg/mL.

Preparation of sample solution: Weigh 20 Solifenacin tablets and calculates the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Solifenacin into a 50 ml volumetric flask. Add about 25ml of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Method Validation:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity: From the standard stock solution, the various dilutions of Solifenacin in the concentration of 20, 30, 40, 50, 60 and 70 µg/mL were prepared. The solutions were injected using 20µL injection volumes in to the chromatographic system at the flow rate of 1.0 $mLmin^{-1}$ and the effluents were monitored at 210 nm, chromatograms were recorded. Calibration curve of Solifenacin was obtained by plotting the peak area ratio versus the applied concentrations of Solifenacin, given in table.1. The linear correlation coefficient was found to be 0.999, shown in figure2.

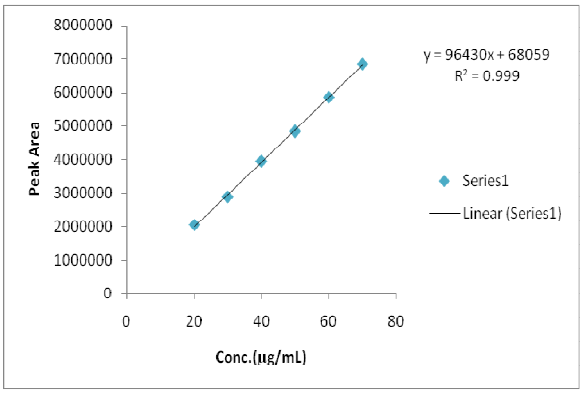

Figure.2: Linearity curve of Solifenacin

Table.1: Linearity of Solifenacin

Concentration (µg/mL)	Average area
20	2060589
30	2886321
40	3958090
50	4834019
60	5845266
70	6860125

Precision: Repeatability of the method was checked by injecting replicate injections of 40 µg/mL of the solution for six times on the same day as intraday precision study of Solifenacin and the % RSD was found to be 0.07, given in table.2.

Table.2: Precision of Solifenacin

Injections	Area
1	3989869
2	3987777
3	3991053
4	3988291
5	3996042
6	3987655
Mean	3990115
SD	3191.066
% RSD	0.07

Accuracy: Solifenacin reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50, 100 and 150 percent). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table.3.

Table.3: Accuracy of Solifenacin

% Conc.	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.96	99.2 %	99.6%
100%	10.0	10.1	101 %	
150%	15.0	14.8	98.6 %	

Specificity: Spectral purities of Solifenacin chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness: To determine the robustness of the method, two parameters (flow rate, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no

significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table.4.

Table.4: Robustness of Solifenacin

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate as per method 1.0mL/min	0.8 mL/min	3898251	2.412	3918.6	0.1
	As it is	3981362	2.414	5993.2	0.15
	1.2ml/min	3889375	2.411	8898.6	0.22
Mobile phase composition Acetonitrile I:Buffer (50:50)	Acetonitrile: Buffer (48:52)	3912525	2.414	10121.8	0.26
	As it is	3916822	2.408	7287.3	0.19
	Acetonitrile: Buffer (52:48)	3878316	2.415	9985.5	0.25

Ruggedness: Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst which is shown in table.5.

Table.5: Ruggedness of Solifenacin

Injections	Area
1	4031865
2	4031027
3	4030086
4	3998892
5	4033713
6	4024564
Mean	4025025
SD	13168.53
% RSD	0.32

Detection and quantitation limits: According to the determined signal-to-noise ratio, Solifenacin presented limits of detection of 0.1 µg/mL and limits of quantitation of 0.4µg/mL, where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the method is the quantitation of Solifenacin so that the values obtained should be considered as the limit of method sensitivity.

System Suitability Parameter: System suitability tests were carried out on freshly prepared standard stock solutions of Solifenacin and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table.6.

Table.6: System Suitability of Solifenacin

Concentration	Injection	Area	R_t
40 µg/mL	Inj-1	3997551	2.415
	Inj-2	3987495	2.414
	Inj-3	3998047	2.414
	Inj-4	3989693	2.408
	Inj-5	4002921	2.413
	Inj-6	3996885	2.406
	Mean	3995432	2.411667
	SD	5752.509	0.003724
Statistical Analysis	% RSD	0.14	0.15
	Tailing Factor	1.3	
	Plate Count	2855.5	

Assay of Solifenacin tablet: Three different batches of Soliten were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Solifenacin was transferred to a 50 ml volumetric flask followed by the addition of 25 ml of mobile phase. The solution was sonicated for 3 minutes and volume adjusted with the mobile phase then filtered through 0.45µm membrane filter. Further dilutions were made to get the final concentration equivalent to 40 µg/mL of Solifenacin. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in table.7.

Table.7: Contents of Solifenacin in tablets (n=6)

Sample tablet	Batch	Labeled amount(mg)	Amount found ± SD	%Amount found
Soliten (10mg)	1	10	9.99±0.14	99.9
	2	10	9.96±0.05	99.6
	3	10	10.06±0.11	100.6

All the analyzed batches presented Solifenacin were very close to the labeled amount. The Solifenacin content in the tablets samples varied from 99.6 to 100.6%.

S.D=Standard Deviation

Table.8: Developed Chromatographic Conditions

Parameters	Method
Stationary phase (column)	XTerra C_{18} (150 × 4.6 mm, packed with 5 µm)
Mobile Phase	50:50 (Acetonitrile : Phosphate Buffer)
pH	2.5 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	5.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	210
Drugs RT (min)	2.4

RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Solifenacin was preferably analyzed by reverse phase chromatography and accordingly C_{18} column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the acetonitrile to phosphate buffer was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of acetonitrile: phosphate buffer at the ratio of 50:50 (v/v).The retention time of Solifenacin was found to be 2.4 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table.6. Developed chromatographic method was applied for the determination of Solifenacin in tablet formulation, given in table.8. A typical chromatogram showing the separation of Solifenacin is shown in figure 3.

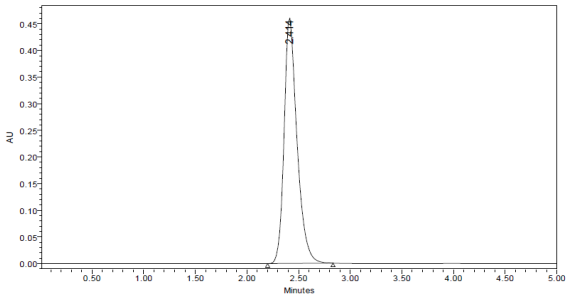

Figure.3: Standard Chromatogram of Solifenacin

CONCLUSIONS

A validated RP-HPLC method has been developed for the determination of Solifenacin in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Solifenacin in pharmaceutical dosage form.

ACKNOWLEDGEMENTS

The authors are thankful to Pharmatrain, Kukatapally, Hyderabad for providing gift sample of Solifenacin and for providing necessary facilities to carry out the research work.

REFERENCES

1. Ohtake A, Saitoh C, Yuyama H, Ukai M, Okutsu H, Noguchi Y, Hatanaka T, Suzuki M, Sato S, Sasamata M, Miyata K, Pharmacological characterization of a new anti-muscarinic agent, Solifenacin succinate, in comparison with other anti-muscarinic agents, Biological and Pharmaceutical Bulletin, 2007, 30 (1), 54–58.
2. Cardozo L, Lisec M, Millard R, Randomized, double blind placebo controlled trial of the once daily anti muscarinic agent Solifenacin succinate in patients with overactive bladder. Journal of Urology, 2004, 172 (5), 1919–1924.
3. Basra R, Kelleher C, A review of Solifenacin in the treatment of urinary incontinence, Journal of Therapeutics and Clinical Risk Management, 2008, 4(1), 117–128.
4. Singh L, Nanda S, Spectrophotometric estimation of Solifenacin succinate in tablet formulations, Pharmaceutical Methods, 2011, 2(1), 21-24.
5. Seetharaman R, Lakshmi KS, Development and validation of first order derivative spectrophotometric method for estimation of Solifenacin succinate in pharmaceutical formulation, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011, 2 (3), 1052-1057.
6. Desai D, Patel G, Shukla N, Rajput S, Development and validation of stability-indicating HPLC method for Solifenacin succinate: isolation and identification of major base degradation product, Acta Chromatographica, 2012, 24 (3), 399-418.
7. Desai N, Hussen SS, Vasanthraju SG, Karthik A, Udupa N, Development and validation of stability indicating HPLC method for determination of Solifenacin in bulk formulations, International Journal of Pharmacy and Pharmaceutical Sciences, 2011,3 (1), 70-74.
8. Nageswara rao K, Ganapaty S, Lakshmana rao A, Development and validation of RP-HPLC method for estimation of solifenacin in bulk and tablet dosage form, Pharmanest, 2012, 3 (5), 366-374.
9. Desai D, Mehta G, Ruikar D, Jain R, Rajput S, Development and Validation of Stability-Indicating HPTLC Method of Solifenacin Succinate, Asian Journal of Pharmaceutical and Biological Research, 2011, 1 (3), 310-316.
10. Wankhede SB, Somani K, Chitlange SS, Stability indicating normal phase HPTLC method for estimation of Alfuzosin and Solifenacin in pharmaceutical dosage Form, International Journal of Chem Tech Research, 2011, 3(4), 2003-2010.
11. ICH Harmonized Tripartite Guideline, Q2 (R1), Validation of Analytical Procedures, Text and Methodology, International Conference on Harmonization, Geneva, 2005, 1-13.

Source of support: Nil
Conflict of interest: None Declared