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Research Article

A PRECISE RP-HPLC METHOD FOR THE ESTIMATION OF DARIFENACIN IN BULK AND TABLET DOSAGE FORM

E. Manasa*, K. Vanitha Prakash, P. Ravi Pratap and S. Susena

SSJ College of Pharmacy, V.N. Pally, Gandipet, Hyderabad-500 075, Andhra Pradesh, India.

ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the estimation of Darifenacin in bulk and Tablet Dosage form. The proposed method is based on the separation of drugs in reversed-phase mode using Waters HPLC 22695 model,Inertsil ODS column(250 x 4.6 mm, 5µm particle size).The optimum mobile phase consisted of methanol: phosphate buffer in the ratio of 80:20 v/v(Phosphate buffer pH 5.5) was selected as a mobile phase, flow rate of 1.0 ml/min and UV detection was set at 282 nm. The retention times was 3.21.The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 20-150 µg/ml . The percentage RSD for precision and accuracy of the method was found to be less than 2%. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

Keywords: Darifenacin ,RP-HPLC, Validation.

INTRODUCTION

Darifenacin works by blocking the M3 muscarinic acetylcholine receptor, which is primarily responsible for bladder muscle contractions. It thereby decreases the urgency to urinate. It is not known whether this selectivity for the M3 receptor translates into any clinical advantage when treating symptoms of overactive bladder syndrome.

It should not be used in people with urinary retention. Anticholinergic agents, such as Enablex, may also produce constipation and blurred vision. Heat prostration (due to decreased sweating) can occur when anticholinergics such as Enablex are used in a hot environment.

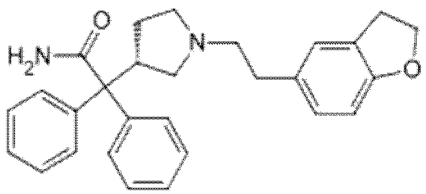


Fig. 1: Darifenacin stracture

EXPERIMENTAL

Chemicals and Reagents

HPLC grade, Methanol and phosphate Buffer (PH-5.5)

Instrumentation and Analytical Conditions

The analysis of drug was carried out on a Waters HPLC 22695 series consisting 4 pump, Auto sampler, equipped with PDA detector, thermostat column connected with waters (alliance) Empower software, Inertsil ODS C18 column (250x4.6mm, 5µm in particle size). Isocratic elution with Methanol: phosphate Buffer (PH-5.5) 85:15(V/V) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Standard and Sample preparation

Darifenacin standard and marketed formulation (equivalent to 10mg was accurately weighed and transferred into a 10ml volumetric flask containing HPLC grade Methanol as the diluent. It was sonicated, dissolved completely and made volume up to the mark with the same solvent. 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with HPLC grade Methanol. The

contents were mixed well and filtered through 0.45µm nylon filter paper to get this stock solution (1mg/ml).

Method validation
The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

Linearity
The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 20-150 µg/ml Linear regression data for the calibration curves are given in Table 1.

Accuracy
The % mean recovery obtained for Darifenacin was 101% .The %RSD is less than 2, results were given in Table 2.

Precision
Results for repeatability expressed as %RSD, results were given in Table 3. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the %RSD values, which indicates that the proposed method was reproducible, results were showed in Table 3.

Detection limit and quantification limit
LOD for Darifenacin was 0.24 µg/ml respectively, while LOQ was 0.72 µg/ml.

Robustness
There was no significant change in the peak areas and retention times of Darifenacin, when the composition of mobile phase ±1ml and flow rate was varied by ±0.2 ml. The results are showed in Table 4.

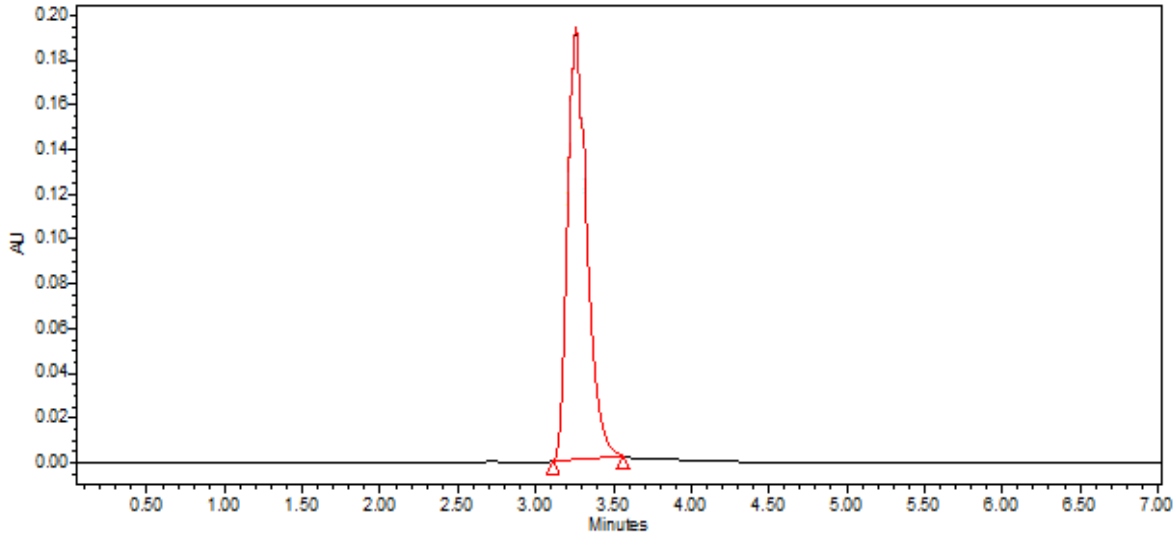
Specificity
No interference from any of the excipients was found at retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram received by the standard solution at the wavelengths applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

System suitability
The acceptance criteria are % RSD of peak areas and retention time less than 2%, theoretical plates numbers (N) at least 4500 per each peak and tailing factors less than 1.5 for Darifenacin ethe results are shown in the Table 5.

Quantification of Liraglutide in parentral dosage form
The proposed method was applied to the estimation of Darifenacin in Tablet dosage form. The results of the assay 99.84±0.24%, of label claim of the tablet. The assay results showed that the method was selective for the estimation of Darifenacin without interference from the excipients used in the tablet form.The results are shown in the Table 6.

DISCUSSION
In order to achieve the estimation of the Darifenacin initial trials was performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wave length and concentrations of the standard solutions were carefully studied. Several solvents were tested in varying proportions. Finally, a mixture of methanol:Phosphate buffer (80:20 v/v) was selected as the optimum mobile phase. The optimized chromatographic conditions were selected based on sensitivity, retention times and peak shape. The method was validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness and specificity as per ICH guidelines. The accuracy data shows that the method is accurate within desired range. The LOD and LOQ values were low which indicates that the method is sensitive. The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and retention times of Darifenacin .

CONCLUSION
A validated RP-HPLC method has been developed for the determination of Darifenacin in tablet form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Darifenacin in pharmaceutical dosage form. The limit of detection for Darifenacin was found to be 0.24 µg/ml and the limit of quantification was found to be 0.72 µg/ml. It proves the sensitivity of method.



Name	Retention Time	Area	% Area	Height	USP Tailing	Symmetry Factor	USP Plate Count
Darifenacin	3.212	3250591	100.00	374481	1.329972	1.339972	9283

Fig. 2: Typical chromatogram of Darifenacin standard

Table 1: Linear regression data for the calibration curve

Concentration of Darifenacin	Mean peak area of Darifenacin
20	1675220
30	2475064
40	3250591
50	4200120
60	5121031
70	6124613
80	7205132

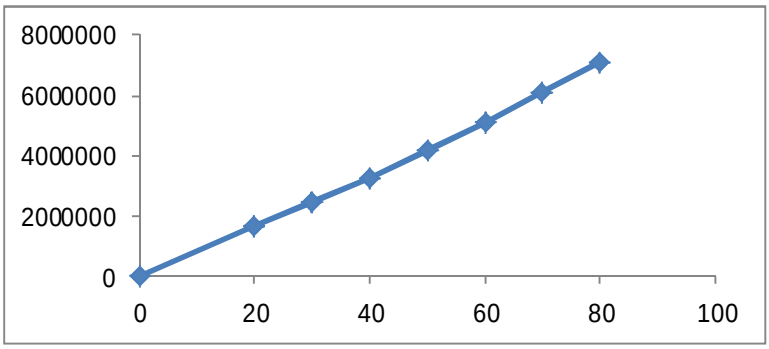


Table 2: Accuracy data for proposed method

Spiked level of drug (%)	Amount of drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery
50	20	20.29	105.0
100	40	40.4	101.0
150	60	60.7	98.0

Table 3: Precision of the proposed HPLC method

Conc. of Darifinacine (40 µg/ml)	Peak area of Darifinacine	
	Intra-day	Inter-day
Injection-1	3218338	3318621
njection-2	3230606	3326451
Injection-3	3250591	3412131
Injection-4	3242511	3332153
Injection-5	3257321	3365143
Average	3239873.4	3350899.8
Standard Deviation	7802.8	14435.5
% RSD	0.2	0.4

Table 4: Results of robustness for proposed method

Factor	Level	Retention time	Asymmetry
A: Flow rate (ml/min)			
0.8	-0.2	3.90	1.34
1.0	0	3.21	1.32
1.2	+0.2	2.89	1.28
%RSD		0.3	0.7
B: % of methanol (ml)			
84	-1	3.70	1.34
85	0	3.26	1.32
86	+1	3.10	1.28
%RSD		0.2	0.7

Table 5: System suitability parameters

Parameters	
Linearity (µg/ml)	20-150
Correlation coefficient	0.998
Theoretical plates	9248
Tailing factor	1.32
LOD (µg/ml)	0.24
LOQ (µg/ml)	0.72

Table 6: Results of sample analysis for proposed method

Brand name	Analyte	Label claim per Tablet(mg)	% Analyte estimated (mean±SD)	%RSD
ENABLEX	Darifenacin	15	99.84±0.24	0.193

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