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Der Pharmacia Sinica, 2011, 2 (2): 169-176

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

UV spectrophotometric determination of Darifenacin Hydrobromide in bulk and pharmaceutical dosage forms

NK Sathish[1*], Pradeep V. Khandelwal[2], IA Chethan[1] and Ashwinee Kumar Shrestha[3]

[1]*Dept. of Pharmaceutical Chemistry, SAC College of Pharmacy, BG Nagara, Karnataka*
[2]*Dept. of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Bharathi Nagara, Karnataka*
[3]*Department of Studies in Chemistry, University of Mysore, Mysore, Karnataka, India*

ABSTRACT

A simple and sensitive ultra-violet spectrophotometric method was developed for the estimation of darifenacin hydrobromide in bulk and pharmaceutical dosage forms. This method is based on darifenacin hydrobromide, showing absorbance at 286 nm in methanol. This method obeys Beer's law in the concentration range of 10 to100 µg ml^{-1}. The proposed method is precise, accurate and reproducible and can be extended for the analysis of darifenacin hydrobromide in bulk and tablet formulations. The % recovery is greater than 98 %, hence showed that the method was free from the interference of excepients. The mean % recovery ranges from 99.186 ± 0.9627 % to 100.914 ± 0.9648 %. Intraday precision had a % RSD of 0.004939 and for that of intermediate precision the % RSD is found to be 0.636. The value of standard deviation and % R.S.D were found to be less than 2 which shows that the method is highly precise. The % RSD for analysis is found to be around 99.69 ± 0.909. The solutions were found to be stable for the various concentrations ranging from 10-100% at 30 and 60 minutes.

Keywords: Zero-Order, Recovery, Robustness, Analysis, Stability.

INTRODUCTION

Darifenacin hydrobromide (s)-2-{1-[2-(2, 3-dihydrobenzofuran-5-yl) ethyl 3-pyrolidine}-2diphenyl acetamide hydrobromide (**Fig. 1)**, is a novel muscarinic receptor antagonist developed for the treatment of overactive bladder (OAB). Some studies have assessed the benefit of adding behavioral modification to darifenacin treatment for overactive bladder (OAB). Darifenacin treatment provides a degree of normalization of micturition variables [1]. The molecule has a chiral center and the s-enantiomer is selected for the analytical method development [2]. Existing

antimuscarinic agents are not highly selective for the muscarinic M_3 receptor, which are primarily responsible for mediating human bladder contraction [3-6]. The results of analysis were validated using International Conference on Harmonization and USP guidelines [7]. No reports were found in the literature for the quantitative estimation of darifenacin hydrobromide by Ultra-violet. In the present work, a simple and sensitive ultra-violet spectrophotometric method has been developed for the quantitative estimation of darifenacin hydrobromide in bulk drug and pharmaceutical dosage forms.

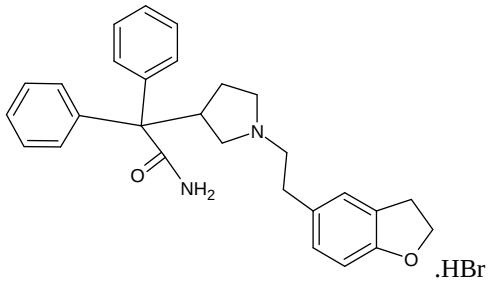

Fig 1: Darifenacin hydrobromide

MATERIALS AND METHODS

Shimadzu UV 1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UVPC software was used for all the spectrophotometric measurements. The spectral bandwidth was 1 nm and the wavelength scanning speed was 2800 nm min^{-1}. The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 200-750 nm.

Reagents and pharmaceutical preparations

Darifenacin hydrobromide was kindly supplied by Ranbaxy Ltd. (Okhla Industrial area, New Delhi, India). The drug was used without further purification. All the solvents used in spectrophotometric analysis were of spectroscopic grade. The commercially available pharmaceutical preparations of Darilong-15 mg (Silvasa-Vapi, Gujarat, India), contained 15 mg of darifenacin hydrobromide which was determined through analysis.

Preparation of standard solution

A stock solution of 1 mg ml^{-1} darifenacin hydrobromide (Stock A) was prepared using methanol. From the above stock solution, a solution of 0.1 mg ml^{-1} darifenacin hydrobromide was prepared by transferring 5 ml of the solution into a 50 ml volumetric flask and the volume was made up with methanol.

Sample preparation (Darilong 15 mg)

A powder weight of 10 tablets was accurately weighed and an amount equivalent to 100 mg was taken and dissolved in 60 ml of methanol and was shaked well for 2 mins. About 10 ml of methanol was further added and sonicate for 10 mins. The mixture was filtered through whattman filter paper No. 40 and transferred to a 100 ml volumetric flask. The residue was washed thrice with 10 ml of methanol and the combined filtrate was made up to the mark with methanol. The sample solution thus prepared was diluted with methanol to get different concentrations of darifenacin hydrobromide which ranged from 10 to 100 µg mL^{-1}.

Calibration
A calibration set of 10 samples was prepared in methanol and graph is obtained as shown in (**Fig 2)**. UV spectra were recorded in the wavelength range 200-700 nm versus solvent blank and the absorbance was recorded with interval of 1 nm (**Fig 3)**. The overlaying zero order spectra were recorded and shown in **(Fig 4).** Absorbance measured at 286 nm (λ max) was used for the preparation of calibration curve.

VALIDATION OF METHOD

Linearity and range
The linearity was tested by using standard calibration curve in the given concentration range. The calibration curves were later evaluated by using correlation co-efficient and intercept values.

Accuracy as Recovery
It was tested (% recovery and % RSD of individual measurements) by analyzing samples in the concentration range of 10 % to 100 %. Fresh samples were prepared for each determination and the assay values were calculated. Recovery was calculated from slope and Y-intercept of the calibration curve obtained in the linearity study. The accuracy was determined from mean relative error for a set of replicate analysis (*i.e.* the difference between measured and nominal concentrations for spiked samples). The absorbance of solution was measured at 286 nm against reagent blank. The percentage recovery data are recorded in, with the help of standard curve. The total amount of darifenacin hydrobromide and percentage recovery of sample darifenacin hydrobromide solution was calculated.

Robustness
The Robustness of the method was determined by using methanol from three different manufacturers for the preparation of stock solution of standard drugs. The average values of % RSD responses for determination of darifenacin hydrobromide are calculated.

Method reproducibility
Method reproducibility was demonstrated by intermediate precision. The intermediate precision was carried out for 10, 20, 40, 60 and 80% of concentration levels for the compound and the average % RSD values for determination of darifenacin hydrobromide were calculated.

Analysis in commercial formulations
Applicability of the method was tested by analyzing the commercially available formulations of darifenacin hydrobromide. The value of % recovery from the formulation was checked for the applicability of the method in determination of darifenacin hydrobromide in the formulations.

Stability
The standard stock solution was diluted with methanol to obtain a solution of concentration 0.1µg ml^{-1}. The absorbance was measured at 286 nm against reagent blank at different time intervals. The solution was found to be unstable after one hours of preparation of sample. So for each study freshly prepared solution shall be used. The prepared solution should be kept in freezer.

RESULTS AND DISCUSSION

Method development

The estimation of darifenacin hydrobromide by UV spectroscopy method was carried out. The standard and sample solutions were prepared and the UV spectra were recorded which is presented in **Fig 1-3.** The absorbance of standard and sample solution was calculated. Accurate results were obtained by utilizing the proposed method for the quantitation of darifenacin hydrobromide. The developed methods were validated for parameters like linearity, precision, accuracy and recovery. For UV spectrophotometric method, linearity was obtained in concentration range of 10-100 µg mL^{-1} for darifenacin with regression equation, intercept and slope, the data for which are presented in the **Table No. 1-7**.

Table No.1: Zero-Order observations

Sl.No	Conc. of Darifenacin hydrobromide in (µg mL^{-1})	Absorbance at 286nm
1.	10	0.072
2.	20	0.120
3	30	0.163
4.	40	0.213
5.	50	0.260
6.	60	0.320
7.	70	0.351
8.	80	0.403
9.	90	0.446
10.	100	0.449

Table No. 2: Calibration Parameters (at zero order)

Sl.No	Parameter	Results
01	Absorption Maxima (nm)	286
02	Beer's Law limits(g/ml)	10-100
03	Molar extinction coefficient (mole-1 cm-1)	0.52
04	Sandell's Sensitivity (µg/cm 0.001absorbance units)	0.192
05	Regression equation (y)*	0.004x+0.024
06	Slope	0.004
07	Intercept	0.024
08	Coefficient of variance	0.999
09	Limit of detection µg mL-1	19.8
10	Limit of quantification µg mL-1	60.0

**y = a + bx; when x is the concentration in µg/ml and y is absorbance unit.*
*** Three replicate sample*

Table No.3: Recovery study from formulation

Sl.No	Concentration taken in (µg mL^{-1})	% Standard addition	% Recovery of Darifenacin hydrobromide
1	10	60	100.1 ± 0.9539
2	10	80	99.42 ± 0.9814
3	10	100	99.552 ±0.9757
4	10	120	99.186 ±0.9627
5	10	140	100.914±0.9648

The % recovery was greater than 98 % and hence showed that the method was free from the interference of excepients used in the formulation. The value of standard deviation and % R.S.D. were found to be less than 2 which showed high precision of the method. The result of analysis shows that the amounts of drug were in good agreement with the label claim of the formulation.

Table No. 4: Robustness (Intra-day precision) for determination of Darifenacin hydrobromide

Sl.No	Conc. of Darifenacin Hydrobromide (µg mL^{-1})	Amplitudes at 286 nm			* Mean ± SD of amplitudes	% RSD
		1	2	3		
1	10	0.072	0.070	0.071	0.071 ± 0.000816	0.0115
2	20	0.120	0.119	0.121	0.120 ± 0.000816	0.00680
3	30	0.163	0.163	0.164	0.1633 ± 0.000471	0.002886
4	60	0.320	0.321	0.320	0.32033 ± 0.00471	0.001472
5	80	0.403	0.401	0.402	0.402 ± 0.000816	0.002031
Average of % RSD						0.004939

Table No. 5: Method Reproducibility (Intermediate precision) for determination of Darifenacin hydrobromide

Sl.No	Conc. of Darifenacin Hydrobromide (µg mL^{-1})	Amplitudes at 286 nm			* Mean ± SD of amplitudes	% RSD
		1	2	3		
1	10	0.069	0.068	0.071	0.0693 ± 0.0012	1.80
2	20	0.119	0.118	0.120	0.119 ± 0.0008	0.69
3	40	0.211	0.212	0.212	0.2117 ± 0.0005	0.220
4	60	0.318	0.320	0.319	0.319 ± 0.0008	0.26
5	80	0.390	0.392	0.391	0.3910 ± 0.0008	0.21
Average of % RSD						0.636

**Average of three determinations.*

Table No.6: Analysis of tablet formulation

Tablet	Label claimed (mg)	Conc. Found (mg)	%Recovery ± SD
Darilong	7.5	99.98	99.69 ± 0.909

** Values correspond to the parameters calculated after accounting for Darifenacin, that is, values without standard addition.*

Table No.7: Stability of the solution

Sl.No.	Concentration (%)	Initial Abs	After 30 Minutes	After 60 Minutes
1	10	0.072	0.115	0.225
2	20	0.120	0.234	0.334
3	30	0.163	0.321	0.445
4	40	0.213	0.415	0.634
5	50	0.260	0.510	0.712
6	60	0.320	0.611	0.914
7	70	0.351	0.654	0.889
8	80	0.403	0.779	1.211
9	90	0.446	0.885	1.201
10	100	0.499	0.876	1.119

Linearity and range

The spectrophotometric method showed good linearity for darifenacin hydrobromide in the range of 10-100 µg/ml with regression equation, intercept and slope of 0.999, 0.004 and 0.024, respectively **Fig 5 (Table No.1 and Table No. 2)**.

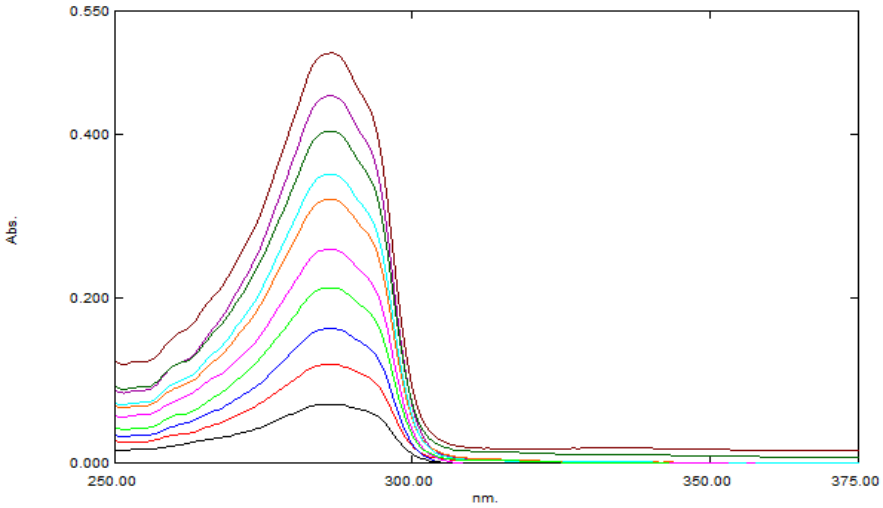

Fig 2: Spectrum of Darifenacin HBr by UV absorption

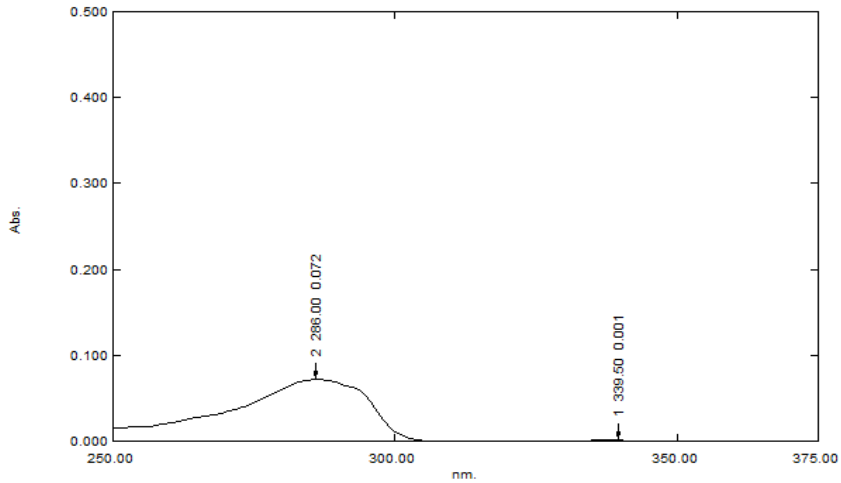

Fig 3: UV absorption spectrum of Darifenacin hydrobromide (286 nm).

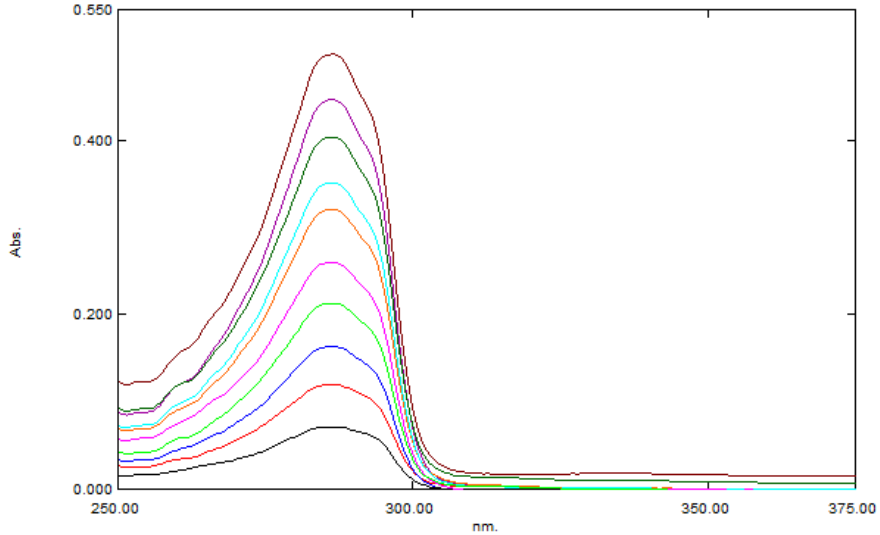

Fig 4: Overlay Graph of UV Spectrum (10% -100 % Concentration)

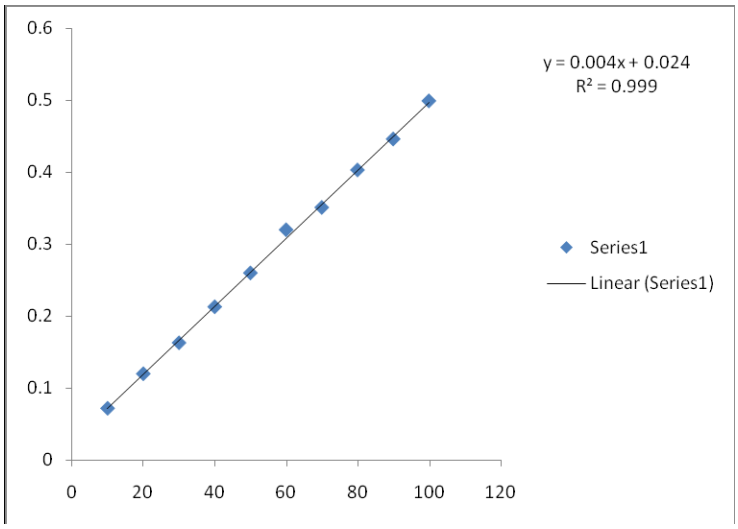

Fig 5: Zero-Order linearity plot of Darifenacin hydrobromide

Accuracy as Recovery

The values of drug recovered ranges from 99.186 ±0.9627 to 100.914±0.9648 when upon addition of standard ranging from 60 to 140% **(Table No.3),** which indicate satisfactory accuracy of the proposed method.

Robustness

The robustness of the method showed that there were no marked changes in chromatographic parameter, which demonstrated that the method develop was robust. The average value of % RSD responses for determination of darifenacin hydrobromide is 0.004939 which is less than 2.0 % indicating the robustness of the method (**Table No. 4)**.

Method reproducibility

The method showed that there were no marked changes in chromatographic parameter, upon repeated trials with different concentrations which demonstrated that the method develop was reproducibility. The average value of % RSD responses for determination of darifenacin hydrobromide is 0.636.The values were found to be in the acceptance range (**Table No. 5)**.

Analysis in commercial formulations

The result obtained **(Table No.6)** showed that percentage recovered was high and SD value were low, which confirm the method is suitable for routine determination of darifenacin hydrobromide in the pharmaceutical preparation. The value of % recovery from the formulation was found to be 99.69 ± 0.909.

Stability

The stability of darifenacin hydrobromide in solution was checked by determining the percentage deviation of the amount present in the solution after 60 min at room temperature and compared with the amount present at 30 min. The result showed no significant variation, the percentage

deviation was less than 2% of the initial amount. This is indication of good stability of each component at the mixture over a period of 1 hours (**Table No. 7)**.

CONCLUSION

The ultra-violet spectrophotometric method proposed, is particularly appropriate for the routine analysis of darifenacin hydrobromide in tablet dosage forms. This method has the advantages of simplicity, precision, accuracy, sensitivity and quantification of darifenacin hydrobromide in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT
The authors are thankful to Manager, Medrich Pharmaceuticals, Bangalore for providing facilities to do this work.

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