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Research Article

A NEW GRADIENT RP- LC METHOD FOR QUANTITATIVE ANALYSIS OF DARIFENACIN HYDROBROMIDE AND ITS RELATED SUBSTANCES IN API

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ABSTRACT

The present research describes a validated, stability- indicating and novel RP-LC method for the quantitative analysis of darifenacin hydrobromide and its four related substances in API (Active Pharmaceutical Ingredient). The chromatographic separation was achieved on a C_{18} stationary phase with a gradient mobile phase prepared from acetonitrile and phosphate buffer pH 3.5. The flow rate of the mobile phase used was 1.0 mL/min and the quantification was carried out by UV detection at 210 nm. The developed RP-LC method was validated according to ICH guidelines. Forced degradation studies were carried out under acidic, alkaline, oxidative, photolytic, thermal and humidity conditions and the drug underwent degradation under oxidative stress condition. In all the above conditions, the main peak of the drug was well separated from its degradation peaks. The percent recovery for individual substances at 25, 50, 100 and 150% of specification concentrations were found to be between 95 to 105% indicating the accuracy of the method. The %RSD for system precision was found to be less than 2.0. The %R.S.D for repeatability and intermediate precision for the process-related impurities in darifenacin hydrobromide were found to be less than 0.8. The correlation coefficient of darifenacin hydrobromide and its four related substances was found to be greater than 0.99. The developed LC method can be used for the quantitative determination of darifenacin hydrobromide and its four related substances in bulk drug.

Keywords: Stability-Indicating, Darifenacin hydrobromide, Forced degradation, ICH guidelines

INTRODUCTION

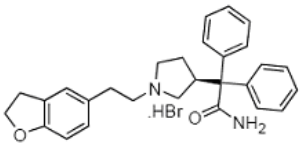
Darifenacin hydrobromide is chemically (S)-2-{1-[2-(2, 3 –dihydrobenzofuran-5-yl) ethyl]-3-pyrrolidinyl}-2, 2-diphenylacetamidehydrobromide, indicated for the treatment of overactive bladder [1]. As a selective antagonist of the M_3 receptor (the major subtype that modulates urinary bladder muscle contraction), darifenacin has a clinically significant effect on bladder function and control [2].

During the manufacturing of drugs, little amount of impurities are always carried in to bulk drug[3] and there is also a possibility for the formation of impurities during formulation and long term storage conditions [4]. Degradation products are the common impurities in the medicines resulting from storage or formulation to different dosage forms or aging [5] It is required to detect the impurities in the drug substance obtained from batches manufactured during the development process, batches from the commercial process, and stress conditions[6]. There are many methods reported in the literature which include HPLC [7, 9], spectroscopic [10-13] and HPTLC methods [14]. Literature survey further revealed the availability of chemo metric evaluation of darifenacin hydro bromide using a stability- indicating HPLC method [15]. However there is no reported stability- indicating LC method by an exhaustive computer- Assisted literature survey which would resolve its four impurities along with its degradation products. In view of the above fact it is felt essential to develop a rapid and reliable HPLC procedure for the separation and quantification of all four process related impurities encountered during the synthesis of darifenacin hydro bromide API. Moreover the main active component along with its degradation and process related impurities were eluted before 14 minutes. The developed method was validated according to ICH guidelines [16]. Here we describe the investigation in detail.

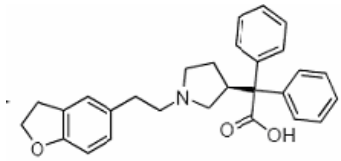
MATERIALS AND METHODS

Chemicals and Reagents

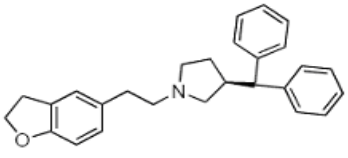
Samples of darifenacin hydrobromide and its related impurities (Fig 1) were procured from Aurabindo Pharmaceuticals (Hyderabad, India). The potential impurities in darifenacin hydrobromide are (S)-2-[1-[2-(2, 3 –dihydrobenzofuran-5-yl) ethyl]-3-pyrrolidinyl]-2,2-diphenylacetic acid (impurity A), 3- benzhydryl-1-[2-(2,3-dihydro-benzofuran-5-yl)-ethyl]-pyrolidine (impurity- B), 2-(1-{2-[3 –(2-sec- Butoxy-ethyl)-4-hydroxy-phenyl]-ethyl}-pyrrolidin-3-yl)-2,2-diphenyl-acetamide(impurity C) and 2-{1-[2-(4- Hydroxy-3-vinyl- phenyl)-ethyl]-pyrolidin-3-yl}-2,2- diphenylacetamide (impurity D). HPLC grade acetonitrile and analytical grade ortho phosphoric acid was obtained from S.D. Fine chemicals Ltd. (Mumbai, India). High purity water was prepared by Millipore Milli-Q plus purification system (Millipore, Bedford, USA).



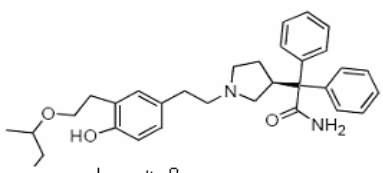
Darifenacin hydrobromide



Impurity A



Impurity B



Impurity C

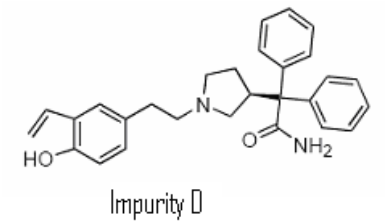


Fig. 1: Structure of darifenacin hydrobromide and its related impurities.

Equipment

The Liquid Chromatographic system consisted of quarternary gradient pump, autosampler, column oven and a PDA detector. The output signal was monitored and integrated using LC solutions chromatography manager software (Prominence HPLC, Shimadzu, Japan).

Chromatographic conditions

The chromatographic column used was X bridge C_{18}, 250 x 4.6 mm, 5 µm particle. Mobile phase consisted of A and B. Mobile phase A was prepared by dissolving 2.72g of potassium dihydrogen orthophosphate and 2mL of triethyl amine in 1000mL of water. The pH of the solution was adjusted to 3.5 ± 0.05 with dilute ortho phosphoric acid. The above solution was filtered through 0.45 µm nylon filter. Mobile phase B used was HPLC grade acetonitrile. The flow rate of the mobile phase was 1.0mL/ min. The gradient program applied is shown in Table 1. The sample injection volume was 20 µL and the quantification was achieved by PDA detector at 210nm.

Diluent

A mixture of pH 3.0 phosphate buffer solutions and methanol in the ratio of 50:50 V/V was used as diluent. The above solution was filtered through a 0.45µ nylon membrane filter prior to use.

2.5. Preparation of test solution. About 25.0mg of darifenacin hydrobromide sample was weighed accurately and transferred into 25mL standard volumetric flask. It was dissolved in sufficient quantity of diluent and made up to the mark with the same diluent.

Preparation of impurity mixture

About 5.0mg of each darifenacin hydrobromide related impurities viz: impurity-A, B, C and D were weighed accurately and transferred to 25mL standard volumetric flask and dissolved in diluent and made up to the mark. 0.5mL of this solution was diluted to 10mL with darifenacin hydrobromide test solution (i.e. 1% of each impurity spiked with respect to darifenacin hydrobromide at the concentration of 1mg/mL and injected in to the system.

Preparation of standard solution of darifenacin hydrobromide

Darifenacin hydrobromide working standard was prepared by accurately weighing 25 mg in sufficient amount of diluent in 25mL standard volumetric flask and sonicated for about 30 min, diluted with same solvent and made upto the mark to give a stock solution containing 1mg/mL of darifenacin hydro bromide.

Method Validation

Forced degradation of sample for specificity study

The specificity of the developed LC method for related substances of darifenacin hydrobromide was evaluated by forced degradation studies of darifenacin hydrobromide sample. Darifenacin hydrobromide was refluxed with aqueous 0.1N hydrochloric acid and 0.1N sodium hydroxide at 60^{0}C for 12 hrs seperately to study the formation of degradation products under acidic and basic conditions. Darifenacin hydrobromide sample was also refluxed with 3% hydrogen peroxide solution at 60^{0}C for half-an hour to study the formation of degradation products under oxidative condition. Degradation products under photolytic and thermal conditions was studied by exposing darifenacin hydrobromide sample to ultraviolet light (254 nm) for 12 hrs while other sample was kept at 70^{0}C temperature for 12 hrs, respectively. Purity of darifenacin hydrobromide peak was evaluated by using PDA detector in case of samples subjected to stress conditions and control sample.

Linearity

Standard solutions at nine different concentration levels ranging from LOQ to 0.15µg/mL (150% of specification limit) were prepared and analyzed in duplicate to demonstrate the linearity for darifenacin hydrobromide and all the four impurities. The calibration curves were plotted for all the impurities using area counts versus corresponding concentrations. The slope, Y-intercept and correlation coefficient were calculated. The response factors were calculated by comparing the slope of the calibration curve for the impurities (A, B, C, D) with that of the darifenacin hydrobromide. The related substances were quantified against area count of darifenacin hydrobromide in standard solution and multiplied with their response factors to obtain the results (%w/w).

Accuracy

Accuracy of the method was demonstrated by using darifenacin hydrobromide drug substance spiked with all related impurities at four different concentration levels in triplicate. The analyses were carried out at 25%, 50%, 100% and 150% of specification limit as per ICH guide lines. The mean recoveries of all the impurities were calculated.

Precision

System precision of the method was evaluated by injecting darifenacin hydrobromide standard solution six times and calculated the percent relative standard deviation (%RSD) for area of darifenacin hydrobromide peak. The precision of the method for the determination of impurities related to darifenacin hydrobromide, was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing the synthetic homogeneous solution containing 0.1 % of each impurity spiked to darifenacin hydrobromide sample for six times. The %RSD for peak area of each impurity was calculated. Intermediate precision of the method was demonstrated by analyzing same sample of darifenacin hydrobromide on three different days (inter day) and intra-day variations of impurities of darifenacin hydrobromide are expressed in terms of %R.S.D values.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection and limit of quantitation for darifenacin hydrobromide impurities A, B, C and D were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity

Precision LOQ level

Precision of the method was also evaluated by injecting standard solutions of known impurities A, B, C and D at about the predicted LOQ levels for six times separately and calculated the percent relative standard deviations (%RSD) for area of each impurity peak.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.1 mL/ min, change in pH of the buffer by ±0.1 unit and change in the column temperature by ±5ºC, keeping the rest of the chromatographic conditions for each alteration study constant.

Stability of the solution

The solution stability of darifenacin hydrobromide in test solution was carried out by analyzing sample solution (prepared by using darifenacin hydrobromide (1mg/mL) spiked with the known related substances at 1.0 %w/w level) at a time interval of 1h for 24hrs by keeping sample solution at room temperature (at 28ºC) and at refrigerated (6ºC) conditions separately. The percent differences between the responses obtained at different time intervals were calculated.

RESULTS AND DISCUSSION

Initial separation experiments

The main aim of the chromatographic method is to separate darifenacin hydrobromide and its four related impurities in the drug substance. Different reverse phase stationary phase were employed during method development and different kind of mobile phase with pH 5 were studied with combination of acetonitrile. The resolution between impurity B and C was critical also the tailing of the peak was observed high. The gradient program was optimized in order to elute all the known impurities and the degradation products. The chromatographic separation of all the impurities was achieved successfully by following the gradient program tabulated in Table 1 using the mobile phase as mentioned under the experimental section. The typical retention times of darifenacin hydrobromide, impurity A, B, C and D are about 13.8, 4.8, 5.8, 6.8 and 8.3 min, respectively. Standard chromatogram of darifenacin hydrobromide (1mg/mL) is shown in Fig.2. A typical chromatogram of darifenacin hydrobromide spiked with 1%w/w of each of the related substances is also shown in (Fig. 3). It is clear that all the compounds were eluted and separated with good peak shapes and resolution.

Table 1: Gradient Program

Time (min)	% A	%B
0.01	75	25
10	60	40
20	45	55
30	45	55
40	75	25
45	75	25

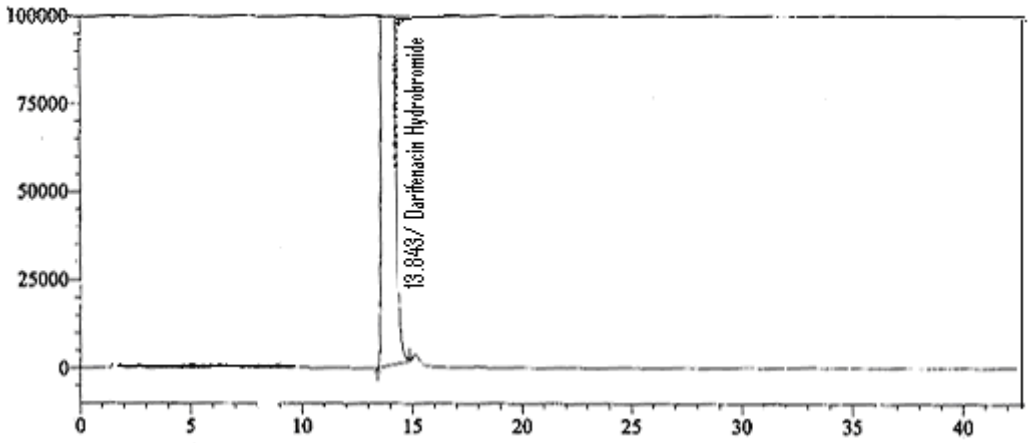


Fig. 2: HPLC Chromatogram showing (1mg/mL) of standard solution of darifenacin hydrobromide

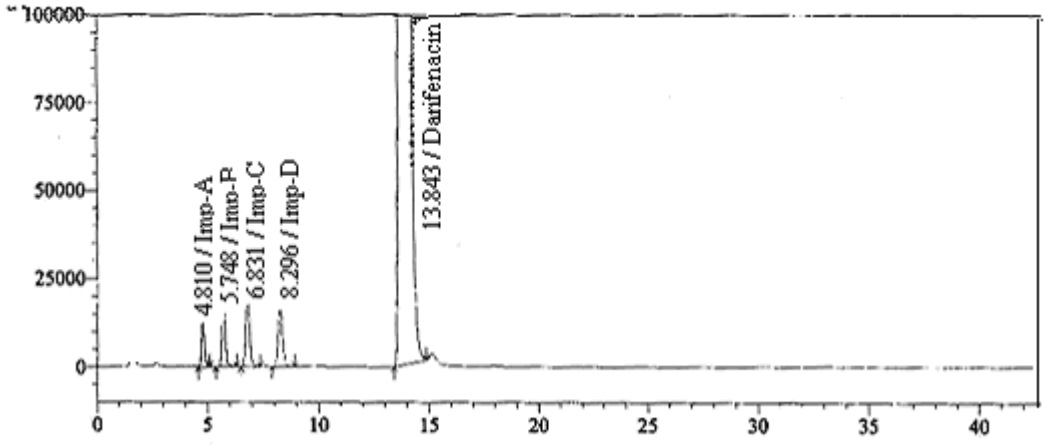


Fig. 3: Typical chromatogram of darifenacin hydrobromide spiked with 1%w/w of each of the related substances.

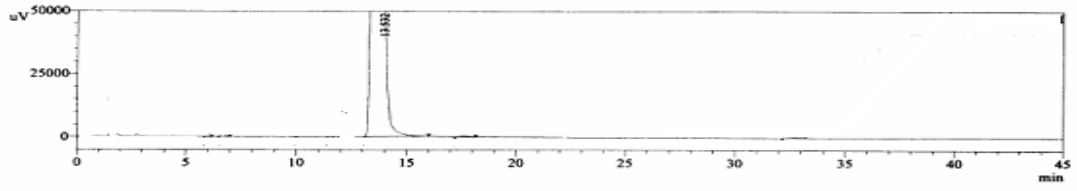
Method Validation

Forced degradation of sample for specificity study

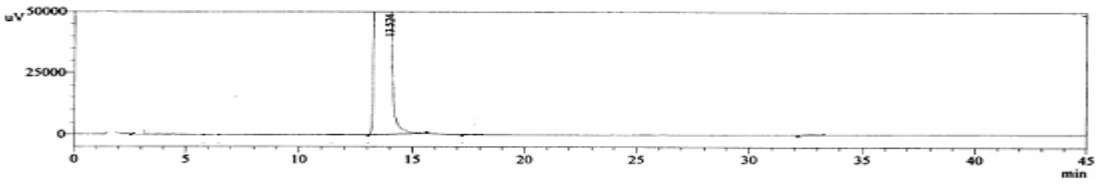
Forced degradation studies were performed to demonstrate the validity of the method. Degradation of darifenacin hydrobromide drug substance was observed under stress condition like oxidative degradation. The degraded peak was found to be impurity A and it was identified by injecting and comparing with the retention time of the standard impurity A. No degradation was observed in case of acidic, alkali, thermal, photolytic and humidity conditions (Table 2 and Fig 4). From the above results, it is clear that the method is specific and able to resolve all the process-related impurities and degradation products.

Table 2: Forced degradation studies of control sample (1mg/mL) solution.

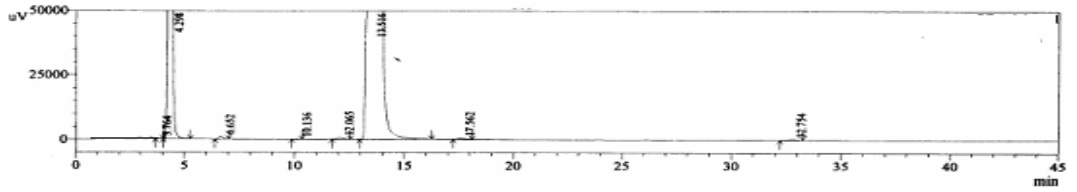
Type of Degradation	No of unknown impurities	Darifenacin hydrobromide peak area %	% Degradation
Control sample	Nil	99.95	_
Acid	Nil	99.95	_
Alkali	Nil	99.96	--
Peroxide	1	89.54	10.35
Thermal	Nil	99.96	_
Photolytic	Nil	99.89	_
Humidity	Nil	99.97	_



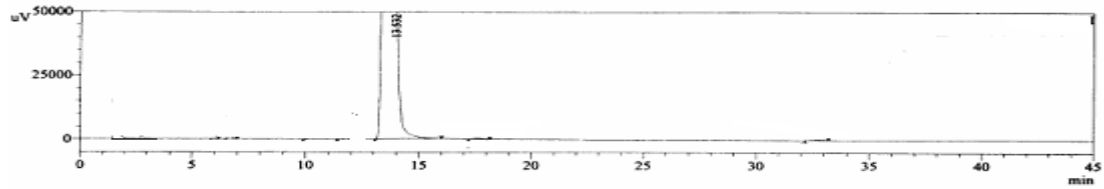
HPLC Chromatogram of acid degradation of darifenacin hydrobromide



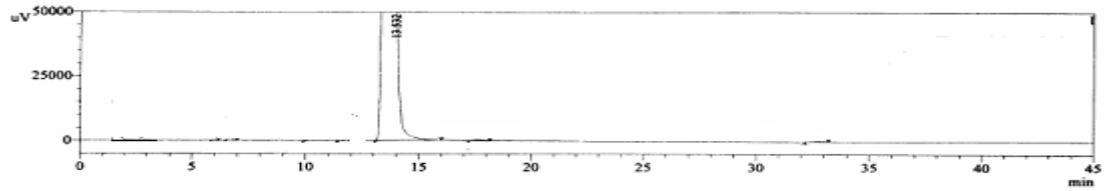
HPLC Chromatogram of alkali degradation of darifenacin hydrobromide



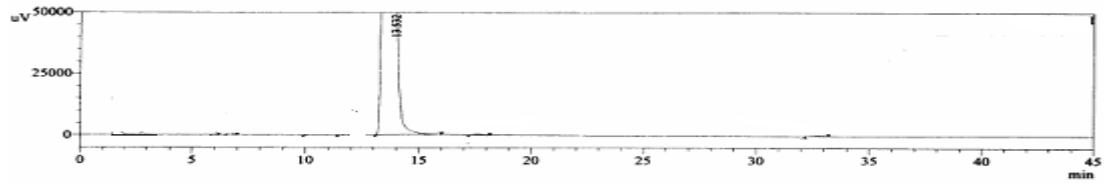
HPLC Chromatogram of H_2O_2 degradation of darifenacin hydrobromide



HPLC Chromatogram of thermal degradation of darifenacin hydrobromide



HPLC Chromatogram of photolytic degradation of darifenacin hydrobromide



HPLC Chromatogram of humidity degradation of darifenacin hydrobromide

Fig. 4: Forced degradation chromatograms of darifenacin hydrobromide under various stressed condition.

Linearity

The linearity regression analysis was demonstrated to check the acceptability of the method for quantitative determination range of LOQ to 150% of the specification limit. The coefficient of correlation was found to be more than 0.9999. The values of slope, intercept, correlation coefficient, LOD and LOQ for each impurity were shown in Table 3. The weight % of the impurity present in darifenacin sample was calculated using its RRF value and peak response. The RRF values of all the impurities are shown in Table 3.

Precision at LOQ level

Precision of the method was also checked at about predicted level of LOQ. The %R.S.Ds for each impurity was shown in Table 4. Precision chromatogram at LOQ level is shown in Fig 5.

Accuracy

The percent recovery for individual substances at 25, 50, 100 and 150% of specification concentrations (Table 5) were found to be between 95 to 105% indicating the accuracy of the method.

Table 3: Summary of linearity, LOD and LOQ

Substance	Slope	Intercept	Correlation coefficient	Relative response factor	LOD (µg/ml)	LOQ (µg/ml)
Impurity –A	18980.7	39.2	0.9999	0.70	0.027	0.097
Impurity –B	10892.8	-56.8	0.9998	0.40	0.045	0.148
Impurity –C	27695.8	-306.5	0.9998	1.1	0.036	0.109
Impurity –D	24554.8	-64.2	0.9998	0.87	0.033	0.107

Table 4: Precision studies for impurities at LOQ level.

Name	LOQ level Mean peak area	
	Mean* peak area	%RSD (n=6)
Darifenacin hydrobromide impurity-A	1960	4.9
Darifenacin hydrobromide impurity-B	1480	3.6
Darifenacin hydrobromide impurity-C	2706	2.7
Darifenacin hydrobromide impurity-D	2715	3.3

* Average of six determinations

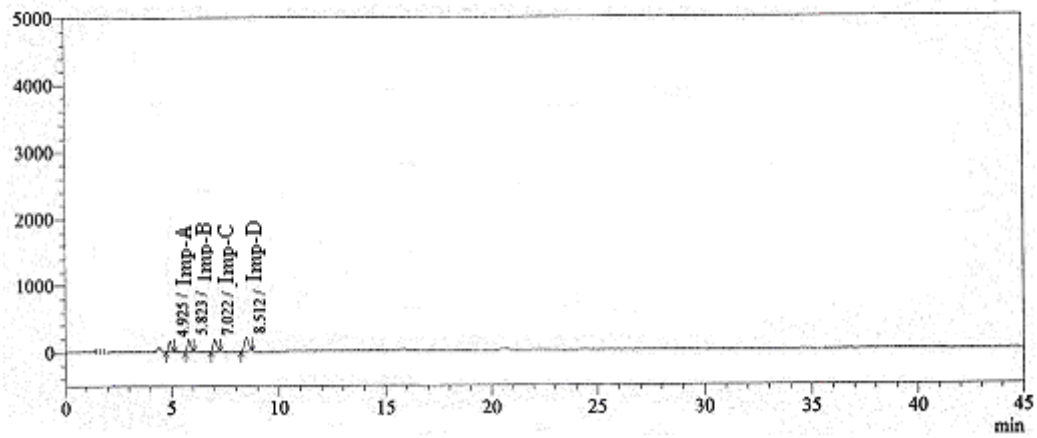


Fig. 5: Precision chromatogram at LOQ level.

Table 5: Parameters of recovery of impurities of Darifenacin hydrobromide

Name	Spike level %	Added 'µg'	Recovered'µg'* ± S.D.	% Recovery* ± S.D
Impurity – A	25	6.24	5.95	95.4
	50	12.49	12.32	98.6
	100	24.97	23.93	95.8
	150	37.46	36.13	96.5
Impurity – B	25	6.37	6.39	100.4
	50	12.75	12.11	95.0
	100	25.50	25.34	99.4
	150	38.25	37.75	98.7
Impurity – C	25	6.49	6.18	95.2
	50	12.99	12.01	92.5
	100	25.97	24.12	92.9
	150	38.96	41.26	105.9
Impurity – D	25	5.98	5.72	95.7
	50	11.97	11.43	95.5
	100	23.94	23.59	98.5
	150	35.90	36.53	108.8

* Average of three determinations

Table 6: Precision studies for Darifenacin hydrobromide and its impurities

Substance	Method precision %RSD(n=6)	Intermediate precision	
		Intra day %RSD(n=6)	Inter day %RSD(n=6)
Darifenacin hydrobromide Impurity –A	0.39	0.55 ±0.08	0.66 ±0.08
Impurity –B	0.68	0.89 ±0.07	0.99 ±0.15
Impurity –C	0.86	0.77 ±0.03	0.96 ±0.08
Impurity –D	0.77	0.67 ±0.05	0.77 ±0.12
	0.88	0.88 ±0.05	0.77 ±0.07

Precision

The %RSD for system precision was found to be less than 2.0. The precision of the method for the determination of impurities related to darifenacin hydrobromide was studied in spiked sample. Repeatability and intermediate precision for the process-related impurities in darifenacin hydrobromide were found to be less than 0.8 %R.S.D. The results are presented under Table 6, which confirm good precision of the method.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD values were found to be 0.028, 0.044, 0.032 and 0.031 µg/mL for impurities A, B, C and D respectively. LOQ values were found to be 0.096, 0.149, 0.108 and 0.105 µg/mL for impurities A, B, C and D respectively (Table 3)

Robustness

The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions. The system suitability results within the acceptable limits and selectivity of individual substances were also not affected when subjected deliberately for varied chromatographic conditions. The result of the study confirms the robustness of the method.

Stability of the solution

The test solution is stable at least for 24hrs at 25 ^{0}C and at 6 ^{0}C. Test samples belonging to two batches were analyzed using proposed chromatographic conditions. The results are incorporated in Table 7.

Table 7: Real sample analysis of Darifenacin hydrobromide

Substance	Sample (%w/w) (Batch-I)	Sample (%w/w) (Batch-II)
Darifenacin hydrobromide	99.85	99.84
Unknown Impurity	0.02	0.02
Impurity –A	N.D*	N.D*
Impurity –B	N.D*	N.D*
Impurity –C	N.D*	N.D*
Impurity –D	0.06	0.06

*ND – Not detectable

CONCLUSION

The validated RP- LC method developed for the quantification of darifenacin hydrobromide and its related substances in bulk drug was reported. The method was found to be selective, sensitive, precise and accurate. The developed RP- LC method showed satisfactory results for all tested method validation parameters in API. The formulated method indicated stability and can be used for assessing the impurities in the drug substance. The developed method can be conveniently used by quality control departments to determine the related substance and assays in regular production samples of drugs and stability samples.

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