Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

DEVELOPMENT AND VALIDATION OF RP- HPLC METHOD FOR THE DETERMINATION OF DARIFENACIN HYDROBROMIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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Received 06 January, 2017; Revised 19 May, 2017

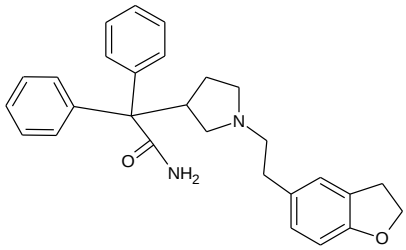
ABSTRACT
The main objective of present study is to develop and validate a new, simple, precise and accurate RP-HPLC method for the determination of Darifenacin Hydrobromide (DFH) in bulk and pharmaceutical dosage forms. The separation and quantification of the drug was achieved on a RP C18 column (250×4.6mm, 5µm) using a mobile phase of acetonitrile: buffer (50:50), pH 3.0 ± 0.2 at a flow rate of 1 mL/min with detection of analyte at 287 nm. The separation was achieved with in 4.0 ± 0.3 min. The method showed good linearity in the range of 10-100 µg/mL. The intra and inter day RSD ranged from 0.20-0.58%. The recovery (mean ± SD) of low, medium and high concentrations were 98.50 ± 0.20, 100.27 ± 0.15 and 100.90 ± 0.09 respectively. The limit of detection and limit of quantification were 0.31 and 0.61 µg/mL, respectively. It can be concluded that the present method could be superior over the methods which were reported earlier.

Keywords: Darifenacin hydrobromide, RP-HPLC, Validation, Recovery.

INTRODUCTION
Darifenacin Hydrobromide is chemically (s)-2-{1-[2-(2, 3-dihydrobenzofuran-5-yl) ethyl 3-pyrolidine}-2-diphenyl acetamide Hydrobromide, which is an anti-muscarinic agent used in the treatment of overactive bladder. It relaxes the destructs muscle in the wall of bladder by preventing acetylcholine from acting on the muscarinic receptor. The molecule has a chiral center and the s-enantiomer is selected for the analytical method development [1].

Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.



Darifenacin Hydrobromide

Few analytical methods have been reported in the literature for the determination of Darifenacin Hydrobromide. S. Thomas et al. reported a selective stability-indicating HPLC method for DRF in the presence of process related impurities and stress degradation products and characterization of degradation products by LC-MS studies [2]. Despite of the separation of several related substances and DRF, the method has limitations of using ion-pairing mobile phase additive, very high run time, and poor peak shape of DRF. M. V. Murthy et al. reported a stability-indicating UPLC method [3]. A stability-indicating LC method with chemometric evaluation of DRF was also reported [4]. A chiral stability-indicating assay method for the determination of DRF and its enantiomer and a chiral LC Method for enantiomeric purity determination of DRF in bulk drugs and extended release tablets were also reported [5]. K. Srinivas et al. reported an impurity profile method for batch analysis of DRF [6]. In addition, B. Kaye et al. reported a solid phase extraction of DRF in human plasma [7]. To the best of our knowledge, very few rapid stability-indicating HPLC methods for the quantitative determination of DRF in drug substance in presence of more susceptible process related impurities in bulk drugs was reported. A method based on the ability to selectively retain Darifenacin from aqueous acetonitrile as a mobile phase in HPLC was done while working on synthesis and properties of molecular imprints of Darifenacin [8].

To the best of our knowledge, only few HPLC methods were reported for the estimation of Darifenacin Hydrobromide in pharmaceutical formulation.

The present work describes a newer, simple, precise and accurate RP-HPLC method for the estimation of Darifenacin hydrobromide in commercial dosage form. The results of analysis were validated using International Conference on Harmonization and USP guidelines [9].

MATERIAL AND METHOD

Experiment

Reagents:

Acetonitrile (HPLC grade) milli-Q water, methanol, potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate were procured from MERCK Ltd India. A reference standard of Darifenacin Hydrobromide was procured from Ranbaxy Ltd India.

Kathmandu University
Journal of Science, Engineering and Technology

Instrumentation:
The HPLC system consisting of isocratic pump (LC-10ATvp pump), mixer (SUS vp Assay (new), Rheodine injector, UV-Vis dual wave length detector (SPD10A-vp) and Hamilton syringe was used. The separation was accomplished on a RP C-18 (250 X 4.6 mm, 5 µm) column. The mobile phase consists of acetonitrile: buffer (50:50 v/v), and the pH is adjusted to 3.0 ± 0.2 using mobile phase phosphate buffer at a flow rate 1.0 ml/m. The mobile phase was filtered through 0.4 µm Millipore nylon membrane filter and degassed by sonicator before use. The analyte was detected at 287 nm (figure 1). The retention time was 4.0 ± 0.3 m.

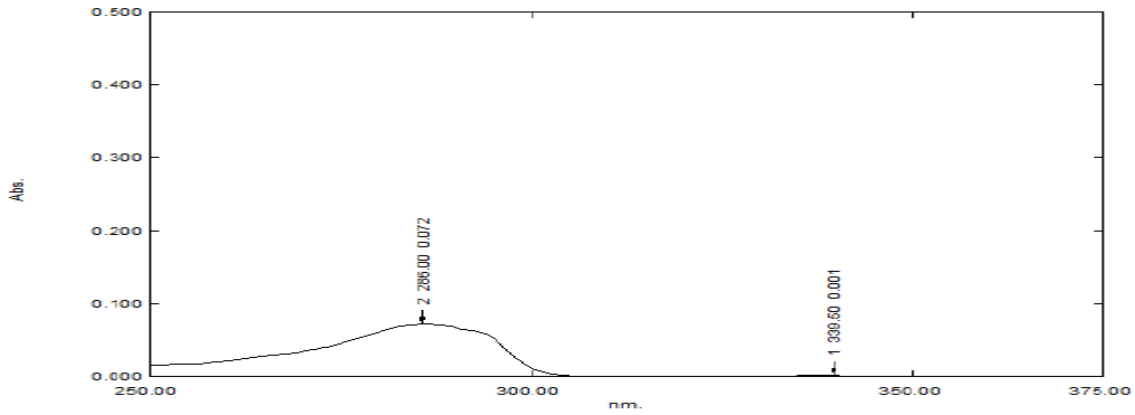


Figure 1. UV Spectrum of Darifenacin hydrobromide

Method Development
Preparation of standard stock solution:
Standard stock solution of Darifenacin Hydrobromide was prepared by dissolving 10 mg of drug in 10 ml of mobile phase.

Preparation of sample solution:
Tablet powder equivalent to 10 mg of drug was weighed, transferred into 10 ml methanol.

Optimization:
For the optimization of the chromatographic conditions, the effect of variables such as mobile phase, pH, flow rate and solvent ratio were studied. Following mobile phase were tried for this purpose; actonitrile: buffer (80:20), acetonitrile: buffer (50:50), methanol: water (70:30), methanol: water (50:50), methanol: water: acetonitrile (35:30:35). The conditions that gave the best resolution and symmetry were selected. Same solvent was used during the extraction of the drug from the pharmaceutical dosage forms.

Calibration curve:
The study was carried out for the drug at six different concentration levels. Aliquots of standard Darifenacin hydrobromide working solutions were taken in volumetric flask and diluted with mobile phase to get the final concentration of Darifenacin hydrobromide in the range 50-100 µg/ml. All stocks

Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

and working solutions were sonicated for 5 minute and filtered through nylon membrane filter (0.45 µ). Triplicate 20 µl injectors were made for each concentration and chromatograph was obtained at ambient temperature (28 ^{o}C).

Method Validation

Linearity:

The linearity was determined by mathematical treatment of the test result obtained by analysis of sample in triplicate with analyte concentration across the claimed range. Area was plotted graphically as a function of analyte concentration, a 20 µl of each solution was injected in triplicate and the chromatogram was obtained. The peak areas obtained at different concentrations are shown in Table 1. A linear relationship was obtained for Darifenacin hydrobromide in the range of 50%-100% µg/mL^{-1} as shown in the Figure 2.

Table 1. Linearity study of HPLC method for Darifenacin hydrobromide

Concentrations (%)	Area 1	Area 2	Average	Regression Coefficient R^2
50	836.460	824.748	830.604	0.999
60	955.821	949.082	952.452	
70	1092.246	1071.436	1081.841	
80	1199.110	1212.946	1206.028	
90	1320.628	1326.207	1323.418	
100	1446.553	1444.647	1445.590	

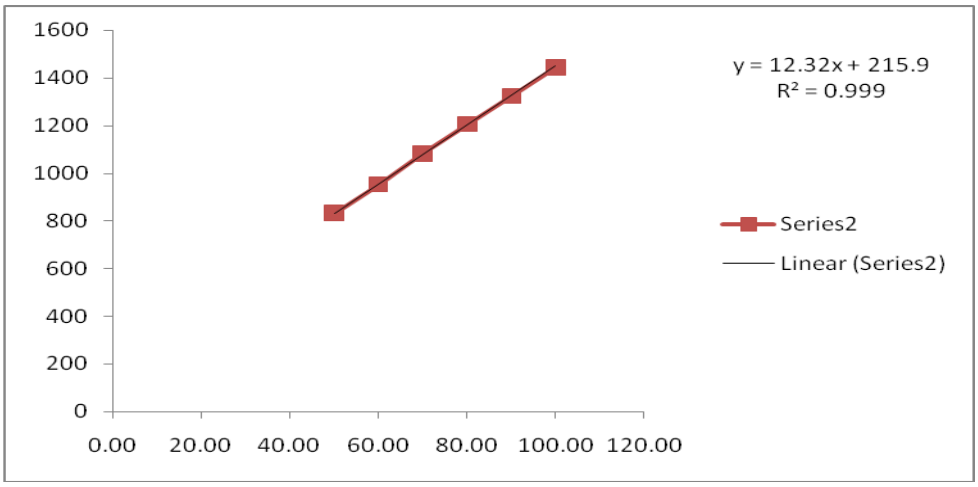


Figure 2. Calibration curve of Darifenacin hydrobromide

Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

Accuracy:
The study was performed by making four different standard concentrations 10%, 30%, 40% and 50% of known amounts of studied drugs. Finally, the final volume was made up with solvent (mobile phase) and mixed well. The resulting mixtures were analyzed by the proposed HPLC method at 287 nm. The excellent mean recoveries and standard deviation (Table 2) indicated good accuracy of the propose methods.

Table 2. Recovery studies of Darifenacin hydrobromide

Sample	Label Claim (mg)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery ±SD %	% RSD
Darilong 7.5	7.5	10	0	9.85	98.50 ± 0.20	0.203
		10	20	29.79	99.30 ± 0.41	0.412
		10	30	40.11	100.27±0.15	0.149
		10	40	49.15	98.30 ± 0.39	0.396
Darilong 15.0	15.0	10	0	9.91	99.10 ± 0.50	0.504
		10	20	29.42	98.06 ± 0.75	0.764
		10	30	39.55	98.87 ± 0.68	0.687
		10	40	50.45	100.9 ± 0.09	0.584

Precision:
The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. Precision for 100 % concentration was determined by injecting six times of same concentration of solution and calculated the % Relative Standard Deviation (% RSD) for area Table 3.

Table 3. System precision of Darifenacin hydrobromide

Sl No	Concentration	Retention Time	Area
1	100	4.095	1217.65
2	100	4.096	1227.61
3	100	4.087	1225.13
4	100	4.093	1226.28
5	100	4.097	1218.60
6	100	4.097	1230.30
Avg	-	4.09	1224.20
S.D	-	0.003	4.62
%RSD	-	0.001	0.004

Kathmandu University
Journal of Science, Engineering and Technology

Robustness:
The robustness of the proposed HPLC method was assessed for peak resolution and symmetric factor. The parameters investigated are apparent to pH of the mobile phase (± 0.5), mobile phase organic content (± 5%) and temperature (± 2%). The results are shown in Table 4. The method showed that there were no marked changes in the chromatographic parameters, demonstrating the robustness of the method.

Table 4. Robustness studies of Darifenacin hydrobromide

Condition	Modification	Mean area	%RSD
Mobile phase composition (Solvent :Buffer) (v/v)	45:55	1732.812	0.020
	50:50	1680.425	
	55:45	1652.862	
Mobile phase pH	2.5	1555.277	0.038
	3.0	1638.652	
	3.5	1708.325	
Column temperature (Degree Celsius)	25	1662.454	0.025
	27	1619.679	
	29	1564.667	

Ruggedness:
The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay of Darifenacin hydrobromide was performed in different condition and by different analyst. As the results in Table 5 are within the acceptance limit, the proposed method is found to be rugged.

Table 5. Ruggedness studies of Darifenacin hydrobromide

Sample	Analyst 1	Analyst 2	Analyst 1	Analyst 2
	Retention time	Retention time	Area	Area
Derifenacin hydrobromide	4.087	4.070	1251.834	1249.393
Average	4.079		1250.610	
S.D	0.0085		1.220	
%RSD	0.002		0.001	

Limit of detector (LOD) and Limit of quantification (LOQ):
The limit of detection (LOD) and limit of quantitation (LOQ) are calculated according to ICH recommendations where the approach based on the signal-to-noise ratio. Chromatogram signals obtained

Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

with known low concentrations of analytes was compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 is considered for calculating LOD and LOQ respectively.

RESULTS AND DISCUSSION

Method development

The proposed HPLC procedure was optimized with a view to develop a suitable analytical method. Mobile phase tried for the propose were acetonitrile:buffer (80:20 and 50:50), methanol:water: acetonitrile (35:30:35). Internal standard was not used as there was no extraction or separation step involved. The chromatogram obtained with acetonitrile: buffer (50:50) solvent system was found to have very good symmetry and sharp peaks were obtained. Therefore, the mixture of acetonitrile: buffer (50:50), pH 3.01 was chosen as mobile phase. The drug was stable for a period of 48 h at laboratory temperature and under refrigerator temperature.

Method validation

Linearity:

The linearity range of Darifenacin hydrobromide solutions was obtained as 10-100 µg/mL.
The linear regression equation was y=12.27x-25.98 with regression coefficient of 0.999.

Accuracy and Recovery:

The values of drug recovered, mean recovery and % RSD are shown in table**,** which indicate satisfactory accuracy of the proposed method.

Precision:

The result (% RSD) of the precision found to be 0.001 % and 0.004% for the retention time and area respectively. It suggested the good precision of the proposed methods.

Robustness:

The robustness of the method showed that there were no marked changes in chromatographic parameter, which demonstrated that the method develop was robust.

Ruggedness:

Ruggedness of the method was determined by analysis of aliquots from homogeneous sample by two analysts, using similar operational and environmental conditions, the % RSD reported was found to be less than 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were calculated based on the signal-to-noise ratio. LOD and LOQ were 0.34 and 0.68 respectively.

Application of method to assay Darifenacin hydrobromide in tablet:

The method was used for the determination of Darifenacin hydrobromide in tablet formulation. The result obtained (Table 6) showed that percentages recovered were high and RSD value were low, which

Kathmandu University
Journal of Science, Engineering and Technology

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

confirm the method is suitable for routine determination of Darifenacin hydrobromide in the pharmaceutical preparation. Figure 3 shows a typical chromatogram obtained from analysis of a tablet formulation.

Table 6. Analysis of Darifenacin hydrobromide dosage form

Assay	%RSD
99.11	0.245

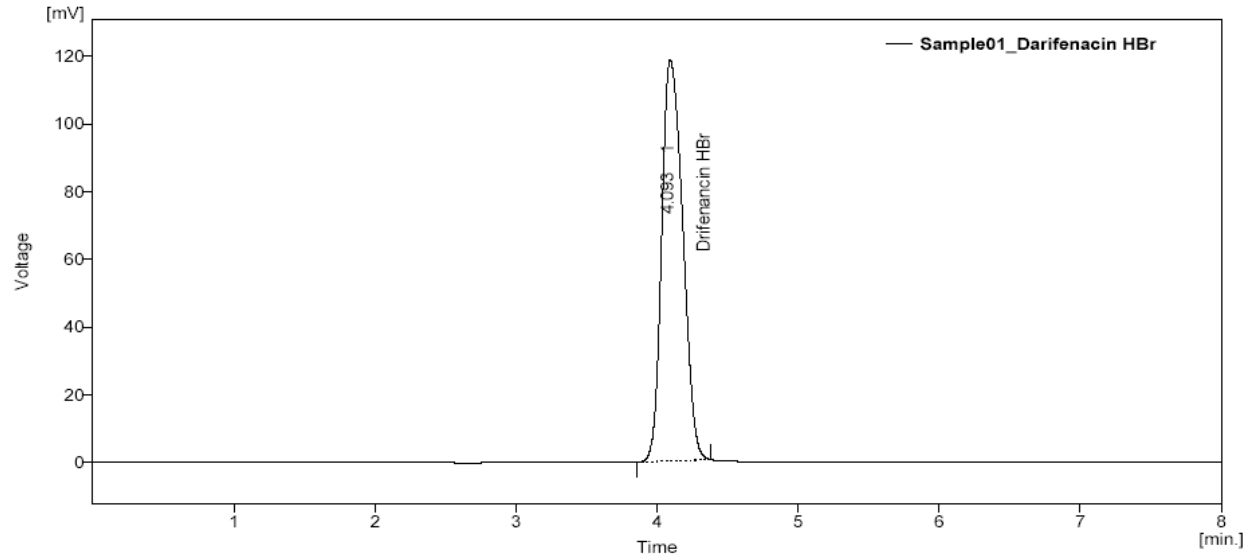


Figure 3. Chromatogram of Darifenacin hydrobromide

Stability:

The stability of Darifenacin Hydrobromide in solution was checked by determining the percentage deviation of the amount present in the solution after 24 h at room temperature and compared with the amount present at zero time. The result showed no significant variation, the percentage deviation was less than 2% of the initial amount. This is an indication of good stability of each component at the mixture over a period of 18 h.

CONCLUSIONS

The proposed HPLC method is particularly appropriate for the routine analysis of Darifenacin Hydrobromide in tablet dosage form. This method has the advantages of simplicity, precision, accuracy, sensitivity and quantification of Darifenacin Hydrobromide compared with other reported methods and can be employed for its assay in dosage form with a single injection. The selectivity of the chosen chromatographic systems was ascertained. Excepients showed no peaks in the range of the retention times corresponding to the analyte.

Acharya *et. al.*, Vol. 13, No. I, October 2017, pp 36-44.

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

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