CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF DARIFENACIN HYDROBROMIDE

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Abstract:

A simple, rapid, precise, specific and accurate RP-HPLC method has been developed for the determination of Darifenacin, a drug is of Urinary antispasmodic in bulk and pharmaceutical dosage form. Chromatography was performed on a Hibar® (250 x 4 mm, 5µm) LiChrosphere® 100 RP-18 in an isocratic mode with mobile phase Acetonitrile: 10mM Tetra Butyl Ammonium Hydrogen Sulphate (70:30%v/v) was used. The flow rate was 1.0ml/ min and effluent was monitored at 285 nm. The retention time was 3.108 min & linearity range was found to be 5 - 300 µg/ml and the %RSD of the method was found to be less than 2%. The proposed method was statistically validated. The excipients present in the formulation do not interfere with the assay procedure.

Key Words: *Darifenacin hydro bromide, Validation, RP-HPLC.*

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QR code

Please cite this article in press as Divya and Padmalaha, ***Development and Validation of High Performance Liquid Chromatography Method for the Determination of Darifenacin Hydrobromide,*** *Indo Am. J. Pharm. Sci, 2015;2(11).*

INTRODUCTION

Darifenacin hydrobromide is the Urinary antispasmodic category. Used for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency and frequency. Darifenacin selectively antagonizes the muscarinic M3 receptor. M3 receptors are involved in contraction of human bladder and gastrointestinal smooth muscle, saliva production, and iris sphincter function. Chemically it is 2-[(3S)-1-[2-(2,3-dihydro-1-benzofuran-5yl)ethyl]pyrrolidin-3-yl]-2,2-diphenylacetamide hydrobromide [1-3].

Literature survey revealed that some analytical methods like LC-MS and HPTLC have been reported for the estimation of Darifenacin hydrobromide. The present work reports simple, sensitive, accurate precise and economical method for determination of Darifenacin hydrobromide by HPLC method in the pure form and its tablet formulation. The method was validated by parameters such as linearity, precision, accuracy, LOD&LOQ, robustness, stability and system suitability as per ICH guidelines and USP requirements.Suitable statistical tests were performed on validation data [4,5]. The structure of Darifenacin hydrobromide is given in figure 1.

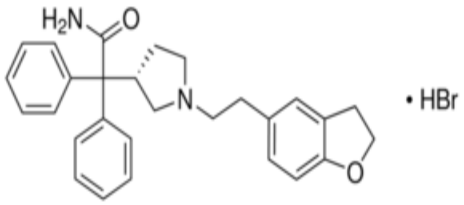

Fig.1.Structure of Darifenacin Hydrobromide

MATERIALS AND METHODS

Instruments and Equipments: SHIMADZU with class-10Vp Software HPLC system with Isocratic mode with UV-Visible Detector (SPD-IOA), PUMP (LC-IOAT) and (LC-IOAT Vp) were used. Hibar® (250 x 4 mm, 5µm) LiChrosphere® 100 RP-18 column was used.

Chemicals/Reagents and Solvents:

Darifenacin hydrobromide pure drug sample was provided by Ranbaxy Pharmaceuticals, Huyderabad.HPLC grade water, Methanol, Acetonitrile was used as solvents. Hydrochloric acid, Sodium hydroxide, Tetra butyl ammonium hydrogen sulphate was of analytical grade. Fixed dose tablet (Brand name: Darilong) containing 15mg of Darifenacin was procured from local pharmacy, Hyderabad, India.

Preparation of Tetra Butyl Ammonium Hydrogen Sulphate:

10mM TBAHS was prepared by dissolving 1.6976 gm of TBAHS salt in 500ml Triple Distilled Water. The prepared solution was sonicated for 30 minutes and was filtered through a 0.45µ membrane filter.

Preparation of Mobile Phase:

Acetonitrile and 10mM Tetra Butyl Ammonium Hydrogen Sulphate (TBAHS) were properly mixed in the ratio of 70:30 & sonicated for 30 min

Preparation of Stock Solution:

Stock solution of Darifenacin hydrobromide (1mg/ml) was prepared by dissolving 10 mg of pure drug in 10 ml of acetonitrile. The solution was sonicated for about 20 minutes.

Method Validation:

Linearity: The linear fit of the system was illustrated graphically. The linearity range was found to be 5 - 300 µg/ml. Least square regression analysis was carried out for the slope, intercept and correlation coefficient.

Precision: The precision of each method was ascertained separately from the peak area obtained by actual determination of three fixed concentrations of drug in three replicates. The percent relative standard deviations were calculated for intra-day & inter-day for Darifenacin hydrobromide.

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples of Darifenacin hydro bromide within the linearity range and adding to the pre-analyzed formulation concentration 50 µg/ml. From that percentage recovery values were calculated.

LOD and LOQ:

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The parameters LOD and LOQ for this method were found to be 0.329 and 0.997 respectively.

System Suitability Parameters:

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. (Or) The USP (2000) defines parameters

that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates, Resolution, Tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Darifenacin hydrobromide in pharmaceutical formulations was validated or not.

Robustness:

The percent recovery of Darifenacin hydrobromide was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for Darifenacin hydrobromide was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

RESULTS AND DISCUSSION:

Method Development and Optimization

The contents of the mobile phase were filtered before use through 0.45µm filter paper, and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The chromatographic separation was achieved using a mobile phase consisting of acetonitrile: TBHS (70:30 %v/v) using a flow rate 1ml/min. The eluent was monitored using UV detection at a wavelength of 285nm.The column was maintained an ambient temperature (25^0C) and an injection volume of 20µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, average peak areas of drug were recorded. A graph was plotted by taking concentration of the drug on X-axis and peak areas on Y-axis. The linearity range was found to be in between 5-300µg/ml.

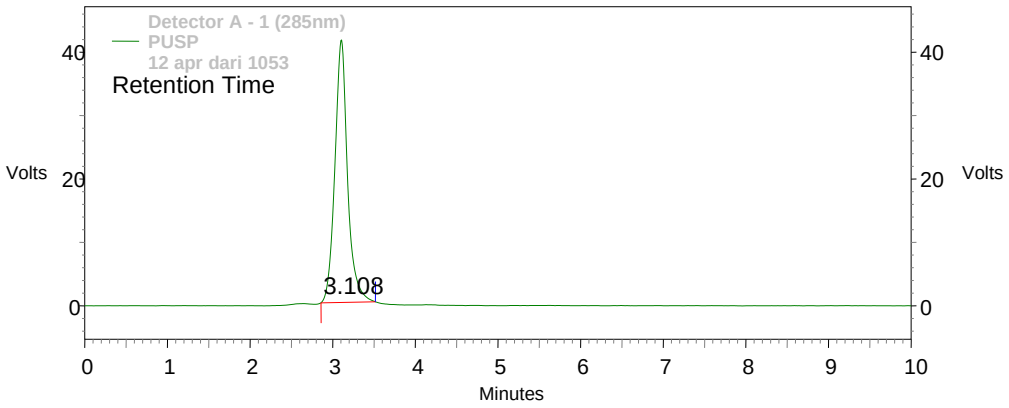

Fig 2: A Typical Chromatogram of Darifenacin hydrobromide (100 µg/ml) in Pure Drug.

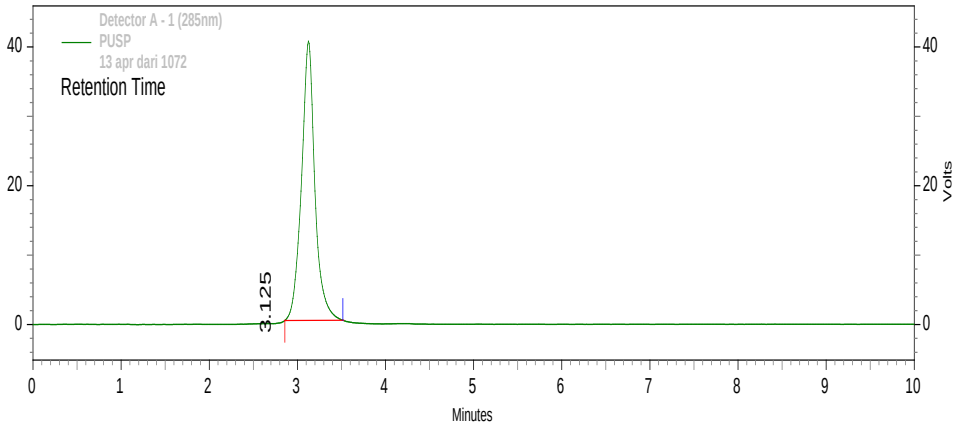

Fig 3: A Typical Chromatogram of Darifenacin hydrobromide (100 µg/ml) in Formulation

Method Validation:

System Suitability:

This test was carried out to find the reproducibility of the system for the analysis. The total results of the system suitability studies summarized in Table 1

Table1: System Suitability Parameters

Parameter	Obtained Values
Theoretical plates (N)	2135
Resolution	2.21
Asymmetry	1.15

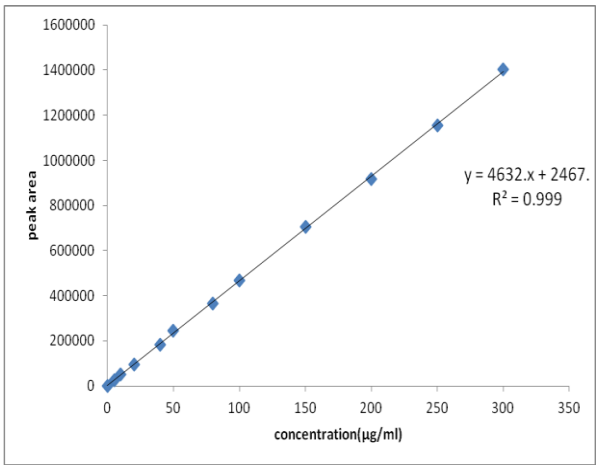

Fig 4: Calibration Curve of Darifenacin hydrobromide

Linearity

The linearity studies were determined at different concentration ranging from 0 to 300µg/ml for Darifenacin hydrobromide. The regression coefficient was 0.999 showing good linearity. This shows the linearity of calibration curve.

Accuracy

The accuracy studies were determined at 3 different concentrations like 80%, 100%, 120% the %recovery was calculated for Darifenacin hydrobromide.

Table 2: Accuracy Observation Of Darifenacin hydrobromide

Sample ID	Concentration (in µg/ml)		% Recovery of pure drug	Statistical analysis
	Pure drug	Formulation		
80 %	8	10	99.92	Mean= 99.96 S.D=0.0862 %RSD=0.0862
100 %	10	10	100.26	Mean=100.79 S.D= 0.6683 %RSD=0.6631
120 %	12	10	101.67	Mean=100.82 S.D=0.8818 %RSD= 0.0862

Precision

System Precision

System precision was determined by injecting sample solution of concentration of Darifenacin hydrobromide for nine times. The chromatograms were recorded for Darifenacin hydribromide and results are mentioned in Table.3. %RSD of Darifencin hydrobromide was 0.3. From the system precision and id precision reports it was found that %RSD values for retention times and peak areas of Darifenacin hydrobromide were found to be less than 2%.

Table3: Intra-day Precision:

Day	Concentration (in µg/ml)	Peak area	Statistical analysis	Retention time	Statistical Analysis
1	10	51167	Mean=50703	3.096	Mean=3.103
2	10	50139	SD=521.2447	3.089	SD=0.0190
3	10	50803	%RSD=1.028	3.125	%RSD=0.6123
1	20	95432	Mean=96306	3.092	Mean=3.102
2	20	96379	SD=840.35	3.103	SD=0.0095
3	20	97108	%RSD=0.872	3.111	%RSD=0.3062
1	50	245399	Mean=245719	3.100	Mean=3.108
2	50	246767	SD=929.45	3.125	SD=0.0147
3	50	244993	%RSD=0.378	3.099	%RSD=0.4729

Table4:Inter-day Precision

Day	Concentration (in µg/ml)	Peak area	Statistical analysis	Retention time	Statistical Analysis
1	10	51167	Mean=50703	3.096	Mean=3.103
2	10	50139	SD= 521.2447	3.089	SD=0.0190
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1	20	95432	Mean=96306	3.092	Mean=3.102
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3	20	97108	%RSD=0.872	3.111	%RSD=0.3062
1	50	245399	Mean=245719	3.100	Mean=3.108
2	50	246767	SD=929.45	3.125	SD=0.0147
3	50	244993	%RSD=0.378	3.099	%RSD=0.4729

Robustness

In the robustness, the changes in the flow rate and mobile phase composition were made to evaluate impact on the method and retention times were significantly changed.

Table 5: Observation of Flow Rate

Parameter	Conditions	Peak area	Retention time
Mobile phase Composition	65:35	458599	3.001
	70:30	**469065**	**3.108**
	75:25	470096	3.236
Flow rate (ml/min)	0.8	460025	3.496
	1	**469065**	**3.108**
	1.2	459583	2.896
UV detection (nm)	283	453649	3.114
	285	**469065**	**3.108**
	287	463252	3.321

Limit of Detection and Limit of Quantification:

The LOD for this method was found to be 0.329 and LOQ for this method was found to be 0.997.

CONCLUSION:

The proposed RP-HPLC method evaluates that, Darifenacin hydrobromide obeys linearity within the concentration range of 5-300 µg/ml in mobile phase Acetonitrile: TBHS (70:30) at 285 nm detection wavelength with 1ml/min flow rate. The retention time of drug was 3.108 minutes. From the results shown in precision tables, it was found that %RSD is less than 2%; which indicates that the proposed method has good reproducibility. From the results shown in accuracy Table, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 99.89-101.56 which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulation were not interfering the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method. From the robustness study done by changing the mobile phase ratio (±5ml), flow rate (±0.2ml) & UV detection (±2nm), it was concluded that the developed method was robust in the experimental condition.

ACKNOWLEDGEMENT

The authors are thankful to Gyana Jyothi College Of Pharmacy, Hyderabad for their valuable guidance, innovate advice, technical and moral support given to me throughout the entire course to carry out this project work.

REFERENCES

1.Barry Kaye, William J. Herron, Paul V. Macrae, Sylvia Robinson, David A. Stopher, Richard F. Venn, and William Wild Rapid; Solid Phase Extraction Technique for the High-Through put Assay of Darifenacin in Human Plasma. *Anal. Chem.*, 1996; 68 (9):1658–1660.

2. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, Vol.2, CBS Publishers and Distributors.

3. ICH Harmonised Tripartite Guideline, Validation Of Analytical Procedures: Text And Methodology, Q2(R1) , Current Step 4 version, Parent Guideline dated 27 October 1994, (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005)

4.L. Stevens, C.R. Chapple, P. Tophill, R.C. Williams, J. Urol., 2004, 171(4), 143 [A535]. [6] L. Stevens, R.C. Williams, C.R. Chapple, J. Urol., 2004, 171(4), 143 [A527]. [7] ICH Harmonized triplicate guidelines on validation of analytical procedures: Text and Methodology Q2 (R1), 2005.

5.MurthyM.Vishnu, Raghavacharyulu.S.V, Krishnaiah Ch., KatkamSrinivas ,K.Mukkanti N.Ramesh Kumar ; A Stereo selective, Stability Indicating Validated LC-Assay Method For The Separation And Quantification Of Darifenacin And Its Enantiomer. Analytical Research and Development, Integrated product development operations,2009; 8(1): 26-30.

6.Praveen P Say, Shaiba,M, Swetha A, Jyothi A Aruna , Nishat Amreen; Spectrophotometric methods for the determination of darifenacin. Research Journal of Pharmaceutical, Biological and Chemical Sciences,2010; 1(4): 35.

7.Radhakrishnanand P., and V. Himabindu ; A Validated LC Method for Determination of the Enantiomeric Purity of Darifenacin in Bulk Drug and Extended Release Tablets. Chromatographia Volume 68, Numbers 11-12, 1059-1062, DOI: 10.1365/s10337.