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Development and validation of rapid HPTLC method for determination of Darifenacin Hydrobromide in bulk drug and pharmaceutical dosage form

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ABSTRACT

A simple, selective, precise, sensitive and accurate high performance thin layer chromatographic method of analysis for the determination of darifenacin hydrobromide in both bulk drug and in formulation was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The solvent system consists of n-hexane: ethyl acetate: triethylamine (7:2.5:0.5 v/v/v). This system was found to give compact spots for darifenacin hydrobromide (R_f value of 0.52 ± 0.02). Dentiometric analysis of darifenacin hydrobromide was carried out in the absorbance mode at 210 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.9964 with respect to peak height and peak area, in the concentration range of 500-3000 ng per band. The method was validated for accuracy, precision and recovery.

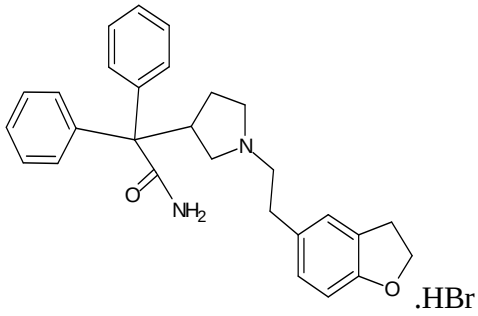
Keywords: Darifenacin hydrobromide, HPTLC, Accuracy, Precision, Recovery.

INTRODUCTION

Darifenacin hydrobromide is a novel muscarinic receptor antagonist developed for the treatment of overactive bladder (OAB). Overactive bladder was characterized by symptoms of increased frequency of micturition, urgency and urge incontinence [1]. It displays up to 59-fold selectivity for M_3 receptors relative to other muscarinic receptor subtypes in vitro [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]. Consequently, these agents

block a range of other muscarinic receptor subtypes that are widely distributed throughout the body, which gives rise to a diverse profile of adverse effects [7].

In this study a simple, selective, precise, sensitive and accurate high performance thin layer chromatographic method for the analysis of darifenacin hydrobromide both as bulk drug and in formulation was developed and validated.



Darifenacin hydrobromide

MATERIALS AND METHODS

Materials

Darifenacin hydrobromide is obtained from Ranbaxy Bangalore. Silica gel 60 F254 TLC plates (20 × 20 cm, layer thickness 0.2mm E-Merck, Darmstadt, Germany) were used as the stationary phase. Tablets were purchased from local pharmacy. Solvents of AR grade were procured from Merck Ltd., Mumbai. Linomat V sample applicator , twin trough developing chamber and TLC scanner III and CATS evaluation software (Version 1.4.2) were used in the studies (Camag, Muttenz, Switzerland).

Preparation of standard solution

A sample of pure darifenacin hydrobromide was accurately weighed (5mg), transferred into a 10ml volumetric flask. It is dissolved in 5ml of methanol and then diluted up to the mark with methanol to give standard stock solution having concentration of 500ng/µl.

Preparation of sample solution

Tablet powder equivalent to 5mg of darifenacin hydrobromide was accurately weighed and transferred into a 10ml volumetric flask. It is dissolved in 5ml of methanol and then diluted up to the mark with methanol. It is then filtered through whatmann filter paper No 41 and 2 µl of filtrate was applied on HPTLC plate.

HPTLC Instrumentation

The samples were spotted in the form of bands of width 3mm with a camag micro liter syringe, on precoated Silica gel aluminium plate 60F-254 (20 cm × 10 cm ; with 0.2mm thickness, E-Merck, Germany) using a Camag Linomat (Switzerland). A constant application rate of 150 nl/s was employed. The slit dimension was kept at 6.00 × 0.45 mm and 20 mm/s scanning speed is used. The mobile system consisted of n-hexane: ethyl acetate: triethylamine (7:2.5:0.5 v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 10 min at room temperature.

The length of chromatogram run was 90 mm subsequent to the development. TLC plates were dried in a current of air with the help of an air drier. Dentiometric scanning was performed on Camag TLC scanner IV in the absorbance mode at 210 nm. The source of radiation utilised was duterium and tungsten lamp.

Calibration Curve

A stock solution of darifenacin hydrobromide (500ng/µl) was prepared in methanol. Different volumes of stock solution 1, 2, 3, 4, 5 and 6 µl were spotted in duplicate on TLC plate to obtain concentrations at 300-500 µg of darifenacin hydrobromide respectively. The data of peak height / area were treated by linear least square regression method.

Accuracy

The accuracy of the analysis was evaluated by recovery studies at three different concentration levels of 50, 100 and 150 ng. The results indicated that, the method enables accurate estimation of the drugs in the pharmaceutical formulations**.**

Precision

Repeatability of the sample application and measurement of peak area were carried out using six replicates of same spot (1000 ng/spot). The intra and inter day variation for the determination of darifenacin hydrobromide was carried out at 1000 ng/band.

Recovery

The analyzed samples were spiked with extra 50, 100 and 150 ng of the standard darifenacin hydrobromide and the mixture was reanalyzed by the proposed method. The experiment was conducted in triplicates. This was done to check for the recovery of the drug at different levels in the formulation.

Analysis of the darifenacin hydrobromide in prepared formulation

The tablets were powdered and the powder equivalent to 5mg of darifenacin hydrobromide were weighed and transferred into a 10ml volumetric flask and dissolved in 5ml of methanol and then was diluted up to mark with methanol. It was then filtered through whattman filter paper No 41 and 2ml of the filtrate was applied on TLC plate followed by development and scanning. The analysis was repeated in triplicates. The possibility of excepients interfering in the analysis was studied.

RESULTS AND DISCUSSION

Development and Optimisation of mobile phase

TLC procedure was optimized with a view to develop a stability indicating assay method. Initially chloroform: methanol in varying ratios were tried. The mobile phase chloroform: methanol (9.5: 0.5 v/v) gave good resolution with R_f value of 0.52 for darifenacin but typical peak nature was missing. Finally the mobile phase constituting of n-Hexane: ethyl acetate: triethylamine (7:2.5:0.5 v/v/v) gave sharp and well defined peaks at R_f value of 0.52 as shown in **Figure-1.** Well defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. The characteristic parameters for the proposed HPTLC method are given in **Table-1**.

Table 1: Characteristic Parameters for the Proposed HPTLC method

Parameters	HPTLC
Calibration range(ng/band)	500-3000
Detection wavelength	210nm
Mobile Phase (Toluene : methanol))	n-Hexane :ethyl acetate:triethyl amine (7:2.5:0.5 v/v/v)
R_F value	0.52
Regression equation (Y*)	Y=1.2544 X +20.96
Slope (m)	1.2544
Intercept (c)	20.96
Correlation coefficient (r^2)	0.9964

Table-2: Linearity of Darifenacin hydrobromide tablet

Sl No	Concentration (ng/band)	R_f	Area
1	500	0.53	624.8
2	1000	0.52	1275.4
3	1500	0.52	1848.3
4	2000	0.52	2657.6
5	2500	0.52	3176.1
6	3000	0.53	3714.0

Table-3: Accuracy of Darifenacin hydrobromide

% Level	% Recovery	% Average Recovery
50	97.4	98.946
100	99.34	
150	100.1	

Table-4: Precision of Darifenacin hydrobromide

Concentration (ng/Spot)	Intraday Precision (% RSD, n=3)	Interday Precision (% RSD, n=3)
1000	0.79	1.05
2000	1.34	1.67
3000	1.12	1.83

Table-5: Recovery studies of Darifenacin hydrobromide

Sample	Label Claim (mg)	Initial Amount (ng/ml)	Amount Added (ng)	Amount Recovered	Recovery± SD (%)	% RSD
Darilong 7.5	7.5	500	50	487.1	97.42 ± 0.496	0.509
		500	100	496.7	99.34 ± 1.491	1.500
		500	150	500.6	100.12 ± 0.944	0.944

Table-6: Analysis of Darifenacin hydrobromide in tablet Formulation

Sl No	Label claim	% Assay	S.D
1	7.5 mg/tablet	98.30	0.291

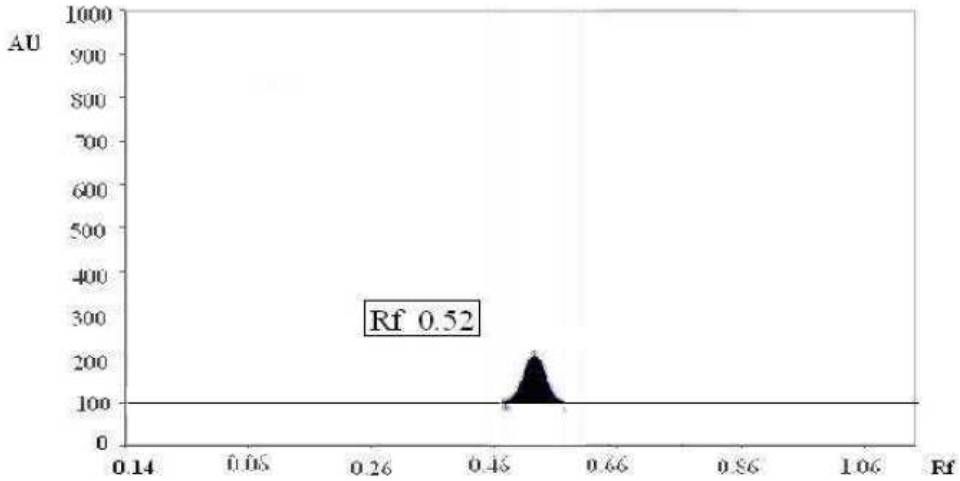


Figure-1: A Typical densitogram of Darifenacin hydrobromide

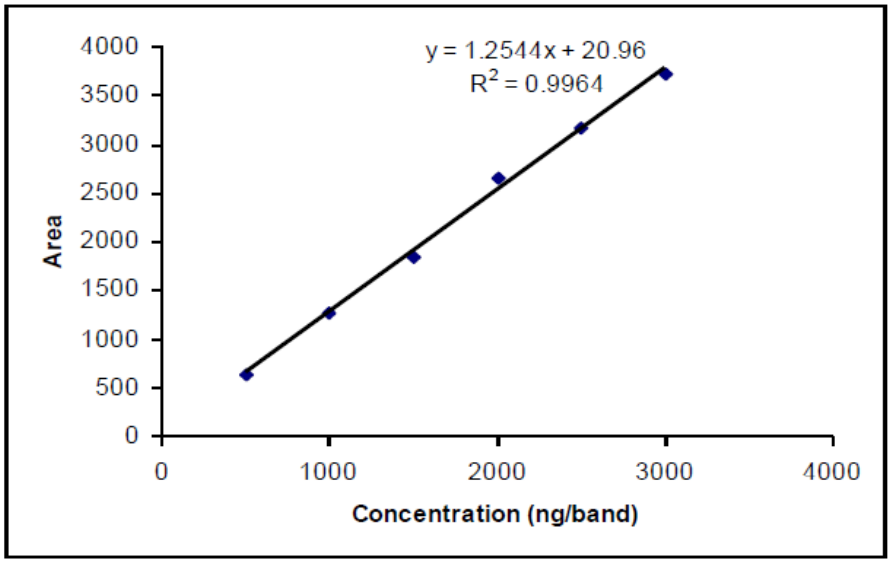


Figure-2: Linearity graph of Darifenacin hydrobromide by HPTLC

Calibration curves

The linearity regression data for the calibration curves as shown in the **Figure-2.** Results showed a good linear relationship over the concentration range of 500-3000 ng of darifenacin hydrobromide/spot with respect to peak area. No significant difference was observed in the slops of standard curves (**Table-2).**

Validation of the method

Accuracy

The values of % recovery and % mean recovery are shown in **Table-3,** with % recovery ranging from 97.4% to 100.1% and % mean recover of 98.946% indicate satisfactory accuracy of the proposed method.

Precision
The repeatability of the sample application and measurement of peak area were expressed in terms of % RSD and results are depicted in **Table-4,** which revealed intra and inter day variation of darifenacin hydrobromide at three different concentration level of 1000, 2000 and 3000 ng per spot. The percentage relative standard deviations ranged from 0.79-1.34 and 1.05-1.83 for intraday and inter day precisions respectively.

Recovery
The proposed method when used for the extraction and subsequent estimation of darifenacin hydrobromide for Pharmaceutical dosage forms after spiking with 50, 100 and 150 ng of additional drug afforded recovery of 97.42 % to 100.12% as listed in **Table-5.**

Analysis of the Darifenacin hydrobromide in prepared formulation
A single spot at R_f 0.52 was observed in the chromatogram of darifenacin hydrobromide samples extracted in the tablets. There was no interference from the excepients commonly present in the tablets. The darifenacin content was found to be 98.30 % with a S.D of 0.291. It may therefore be inferred that degradation of darifenacin hydrobromide had not occurred in the formulations that were analyzed by this method. The low S.D values indicated the stability of this method for routine analysis of darifenacin hydrobromide in Pharmaceutical dosage forms (**Table-6).**

CONCLUSION

The validated HPTLC method proposed is particularly appropriate for the routine analysis of darifenacin hydrobromide in tablet dosage form. This method has the advantages of simplicity, precision, accuracy and sensitivity of darifenacin hydrobromide compared with other reported methods and can be employed for its assay in dosage form.

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