Development and validation of HPTLC method for estimation of Darifenacin Hydro bromide in Pharmaceutical formulation

Rohitkumar S. Singh *, Chintalben Y. Mahida, Praful P. Dedhiya
* Department of Quality Assurance, Maliba Pharmacy College, Bardoli - Mahuva road, Tarsadi, Dist.,Surat,Gujarat, India –394350

ABSTRACT

A specific, accurate, precise and robust HPTLC method was developed and validated for estimation of darifenacin hydrobromide in pharmaceutical formulation. In this method, standard and sample solutions of darifenacin hydrobromide were applied on pre-coated silica gel 60F254 TLC plate and developed using Toluene: Methanol: Acetone: Triethylamine [7: 1.5: 1.5: 0.2, % v/v/v/v] as mobile phase. A Camag HPTLC system comprising of Camag Linomat V applicator, Camag twin trough chamber and Camag TLC IV scanner was used for the analysis. The spots were scanned at 286 nm. The linearity range was achieved in range of 50 to 250 ng/spot. The method was validated as per ICH Q2(R1) guidelines and applied for assay of darifenacin hydrobromide in its marketed formulations.

KEYWORDS:
Darifenacin hydrobromide, HPTLC, ICH Q2(R1),Validation.

1. INTRODUCTION

Darifenacin Hydrobromide, chemically known as (s)-2-[1-[2,3dihydrobenzofuran-5-yl]-2,2-diphenylacetamide (Figure 1), is a selective muscarinic M3 receptor antagonist which is intended for symptomatic treatment of urge incontinence or increased urinary frequency and urgency as may occur in patients with overactive bladder syndrome[1-2]. In literature various analytical methods like spectrophotometric [3-6], HPLC [7-10], UPLC [11] and LC-MS [12-14] methods have been reported for the determination of Darifenacin Hydrobromide in the formulations. The review of literature promoted us to develop an accurate and precise HPTLC method for estimation of Darifenacin Hydrobromide in its dosage forms.

Figure 1: Structure of Darifenacin

Correspondence to:
Rohitkumar S. Singh,
Maliba Pharmacy College, Bardoli
Mahuva road, Tarsadi, Dist.
Surat, Gujarat, India – 394350.
E-Mail: singhrohit1255@live.com

2. MATERIAL AND METHODS

Instrumentation

The HPTLC system (Camag Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttenz, Switzerland), twin-trough developing chamber (10 x 10cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 μl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing.

Chemicals and reagents

Drug was supplied as a gift sample by Alembic Pharmaceuticals Pvt. Ltd., India. All chemicals and reagents used were of LR grade and purchased from s.d. Fine-Chem Limited, Mumbai, India. Formulations were procured from local pharmacy.

Preparation of standard solutions

Darifenacin Hydrobromide 10 mg was dissolved in 10 ml of methanol as stock solution [1000μg/ml]. From the stock solution, 100 μg/ml and 10 μg/ml working standard solutions were prepared by successive dilution with the same solvent.

Chromatographic conditions

The TLC plates were pre washed with methanol and activated by keeping at 120 ºC for about 20 min. The samples were spotted in the form of bands using 100μl
Camag Linomat syringe on the pre-coated silica gel 60F254 plate (10x10) cm. The mobile phase Toluene: Methanol: Acetone: Triethylamine [7:1.5:1.5:0.2, %v/v/v/v] was used and the plate saturation time was 30 min, migration distance was allowed up to 75mm, linear ascending development was carried out in [10x10] cm twin trough glass chamber. Subsequent to the development, plates were dried in oven. The developed plate was scanned at 286 nm using Camag TLC scanner.

Validation of method [15]

Specificity
The specificity of the method was ascertained by analysing standard drug and sample. The band for Darifenacin Hydrobromide in individual samples was confirmed by comparing the Rf and UV spectra of the band with spectra obtained from standard. The peak purity of Darifenacin Hydrobromide was assessed by comparing spectra acquired at three different position of the band, i.e. peak start(s), peak apex(m) and peak end(e).

Linearity
From working standard solution 5, 10, 15, 20, 25μl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. Linearity of proposed method was evaluated by repeating same procedure for five times. Calibration curve for Darifenacin Hydrobromide was obtained by plotting graph of mean peak area of five determination v/s respective concentration of drugs. The correlation coefficient and regression line equation was calculated.

Precision
From working standard solution, 15μl was spotted seven times on a same TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. The peak area of seven spots was measured and % RSD of peak area was calculated.

Repeatability of sample application
From working standard solution, 15μl was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. The spots were scanned for seven times without changing plate position and %RSD for measurement of peak area was calculated.

Intraday and Interday Precision
From working standard solution, 100, 150 and 200 ng were spotted on TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions. Same procedure was carried out three times on same day for intraday precision and on three different days for interdays precision and %RSD of Peak Area was calculated.

Accuracy
The accuracy was determined by standard addition method. The proposed method was applied for estimation of Darifenacin Hydrobromide in their dosage forms. The recovery experiment was carried out in triplicate by spiking previously analysed sample (20 mg) with different concentration of standard drug at 80%, 100% and 120%. The percentage recovery of Darifenacin Hydrobromide was calculated at each level.

LOD and LOQ
LOD and LOQ of the method were calculated using the following equations.

\[
LOD = 3.3 \frac{N}{S} \quad \text{and} \quad LOQ = 10 \frac{N}{S}
\]

Where, \(N\) = Standard deviation of intercepts of five calibration curve
\(S\) = Mean slope of five calibration curves.

Analysis of marketed formulation
Twenty tablets were weighed accurately, finely powdered and mixed. Powder equivalent to 20mg of Darifenacin Hydrobromide was transferred to 100ml volumetric flask and filled about 80% with Methanol. The solution was sonicated for 15 minute and diluted to mark with methanol. The solution was filtered through whatman filter paper. Further, 1ml aliquot from the filtrate was diluted to 10ml with methanol. Aliquot 5ml from above solution was diluted to 10ml with methanol; 15μl from resulting solution was spotted on TLC plate. Amount of Darifenacin Hydrobromide from marketed formulation was calculated using calibration curve data.

3. RESULTS AND DISCUSSION
During the stage of method development, different mobile phases were tried and the mobile phase comprising of Toluene: Methanol: Acetone: Triethylamine [7:1.5:1.5:0.2 v/v/v/v] was confirmed

The Rf value was found to be 0.59±0.02 (Figure 5). Linearity of the drug was determined by the calibration curve and the linearity based on the peak area was in the range of 50 to 250ng/spot (Table 1 and Figure 2).
The regression coefficient value for Darifenacin Hydrobromide is 0.996 (Figure 3). Repeatability of sample application (Figure 4) and repeatability of measurement in terms of RSD were found to be 1.18 and 0.82 respectively. Intraday and interday precision in terms of RSD were found to be 0.93-1.06 % and 1.12-1.16 %. The mean % recovery was found in the range of 98.93-101.15%. The Limit of Detection and Limit of quantitation were found to be 6.97 and 21.14 respectively. Percentage assay of marketed formulations were found to be 98.3 % (Figure 6). The method passes all the validation parameter limits and proves to be specific, sensitive and precise.
CONCLUSION

The developed HPTLC method is precise, specific and accurate; the statistical analysis proved that the method is repeatable and selective for estimation of Darifenacin Hydrobromide in bulk drugs and its pharmaceutical dosage forms without any interference from the excipients. Hence the proposed method can be used for routine assay of Darifenacin Hydrobromide using HPTLC.

CONFLICTS OF INTEREST

The authors do not have any conflict of interest.

REFERENCE


