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Validated TLC-densitometry method for determination of cetirizine dihydrochloride in tablet dosage form

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ABSTRACT
A rapid, reproducible and accurate TLC method was developed for the determination of Cetirizine Dihydrochloride in tablet. The analytes were dissolved with ethanol 70% and chromatographed on silica Gel GF 254 TLC plate using chloroform : methanol : ethyl acetate in the ratio of 2 : 7 : 3 (v/v) as mobile phase. Quantitative analysis was done through densitometric measurement at wavelength 234 nm. Method was found linear over the concentration range of 400 – 1600 ng/spot with the correlation coefficient of 0.996. Specificity showed calculation of purity and identity more than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) of the method were 75.54 and 226.64 ng/spot. The relative standard deviation of this method was 0.86% whereas the means of the recovery data was 100.54 ± 0.11%. The proposed method has been applied to the determination of Cetirizine Dihydrochloride in commercial tablet formulations and the result were 96.97 ± 0.86 % for brand A and 100.57 ± 1.17 % for brand B. The developed method was successfully used for the assay of Cetirizine Dihydrochloride. This method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

***Key Words*:** Cetirizine Dihydrochloride, validation, TLC densitometry, tablet, chromatography method.

INTRODUCTION

Cetirizine Dihydrochloride (CTZ) (Figure 1) is the dihydrochloride of 2-(4-(4-chlorobenzhydryl)piperazin-1-yl) ethoxyacetic acid, a non-sedating type histamine H1-receptor antagonist is used, mainly, in symptomatic treatment of seasonal rhinitis and conjunctivitis, perennial allergic rhinitis as well as pruritus and urticaria of allergic origin (Reynolds, 1996). It is used to treat several allergy symptoms, including runny nose, sneezing, Itchy or watery eyes and Itchy nose or throat. Analytical method including spectrophotometric (Walily *et al*., 1998) and HPLC (Arayne *et al*., 2008) have been reported for the determination of Cetirizine Dihydrochloride. The disadvantages of those methods are disability to analyze several samples simultaneously in parallel and need much solvents as mobile phase. In this presentation, we report a simple and rapid assay with sufficient sensitivity for the quantitation of CTZ using TLC densitometry method. The objective of this study was to develop, optimize and validate a simple and rapid TLC densitometry method for determination of CTZ in tablets.

EXPERIMENTAL

Material and reagents

Cetirizine Dihydrochloride working standard (Glochem Industries Limited, India), ethanol, methanol, chloroform and ethyl acetate (Merck, Germany). Commercial tablets contain Cetirizine Dihydrochloride were procured from local chemist shop.

Preparation of standard solution & pharmaceutical samples

Standard solution was always freshly prepared by dissolving 50 mg of CTZ in ethanol 70% ad 25 ml. The standard solution of CTZ (2000 ppm) was diluted to get solutions in concentration range of 200-800 ppm. For sample preparation, a total of 20 tablets containing CTZ as the active ingredient were weighed and finely powdered. A portion of the powder equivalent to 5 mg CTZ was weighed accurately, transferred to a 10 ml volumetric flask and suspended in 5 ml ethanol 70%. The flask was placed in ultrasonic bath before completion to volume with the same solvent.

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Chromatographic condition

Planar chromatography was performed by spotting the sampel on precoated TLC silica gel GF 254 (20 x 10 cm) using 2.0 µl glass capillaries. A Camag Twin Through Chamber containing a mixture of chloroform : methanol : ethyl acetate (2 : 7 : 3) (v/v) was saturated. The spots move to a distance of 9 cm. Densitometric scanning was performed on Camag TLC Scanner 3 in the absorbance mode at 234 nm for all measurements. The slit dimension was kept at 6.00 mm x 0.30 mm and a scanning speed of 20 mm/s was employed. Cetirizine Dihydrochloride was detected at Rf 0.49. Quantitative evaluation was performed via peak areas by WinCats software (version 1.4.1.8154).

Method validation

The developed method was validated in accordance with the procedures described by Kristiningrum *et.al.* (2012).

Specificity

The Specificity of this method was determined by analyzing standard and sample. Specificity was showed by purity and identity test that determined by scanning at 200nm – 400 nm. Calculations for identity checks (*r*S.S and *r*S,A where S is spectrum standard and A is spectrum sample and purity checks (*r*S,M and *r*M,E where S = start, M = center; and E = end of spectrum).

Linearity

The evaluation of the calibration curve's linearity was done based on spots of the standard solutions prepared in

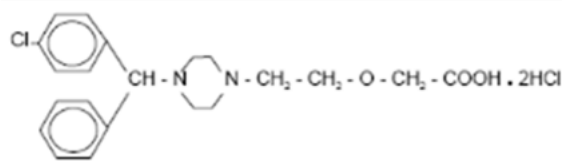

Figure 1. Structure of Cetirizine dihydrochloride.

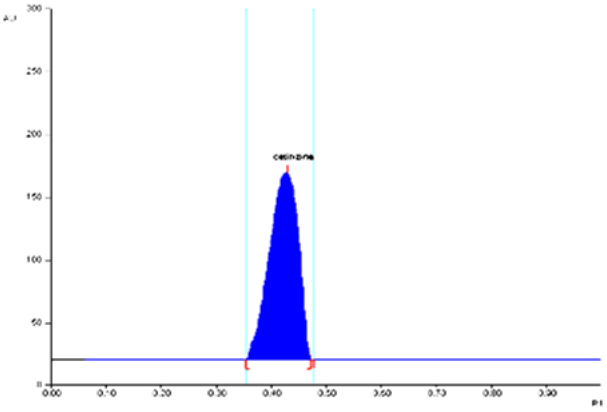

Figure 2: Densitogram of standard Cetirizine Dihydrochloride (Rf: 0.43), mobile phase chloroform : methanol : ethyl acetate (2 : 7 : 3 v/v).

ethanol 70% at the concentrations 200, 400, 450, 300, 533, 600, 700, and 800 ppm. The 2 µl of each of these solutions was spotted on the TLC plate Peak area was record for each concentration and a calibration curve was obtained by plotting peak area vs concentration.

Limit of detection and quantification

Standard solution were prepared at the concentration 80, 120, 160, 280, 320, 360 and 400 ppm. The 2 µl of each of these solutions was spotted on the TLC plate. Peak area was recorded for each concentration. Limit of detection (LOD) and Limit of Quantification (LOQ) were determined using software validation method version 1.13.

Precision

The precision of this method was performed by repeatability and intermediate precision studies. Repeatability studies was performed by analyzing one concentration of the drug for six times on the same day. The intermediate precision was checked by repeating studies on three different days.

Accuracy

The accuracy of this method was evaluated through recovery experiments by adding three different amounts of Cetirizine Dihydrochloride standards i.e. 30, 45 and 60% of the concentration samples. Each concentration were replicated (n=3).

Analysis of marketed formulations

The Samples that is contain of Cetirizine Dihydrochloride (brand A and brand B) were prepared as sample preparation method. Each of samples were replicated (n=3) and spotted on plates. The analysis was done in the same way as described earlier.

Table 1: Optimum condition for analysis of Cetirizine dihydrochloride.

Parameters	Data
Solute	Ethanol 70%
Eluent	Chloroform : methanol : ethyl acetate = 2:7:3 (v/v)
Stationary phase	Silica gel GF 254
Wavelength	234 nm
Concentration optimum	500 ppm

Table 2: Result of precision evaluation of Cetirizine dihydrochloride.

Measurement [a]	RSD value (%) (n=6)[b]
1	0.76 %
2	0.98 %
3	0.85 %
Average RSD	0.86%

[a]Each measurement was perfomed by the same analyst and on a different plate and different days

[b]Evaluted by one analyst on one plate (repeatability)

Table 3: Accuracy result of Cetirizine dihydrochloride commercial tablets.

Analyte	Label claim [%] (mean ± SD)	Added [%]	Recovery
Cetirizine dihydrochloride	100.65 ± 1.164%	30%	100.66 ± 0.10%
		45%	100.51 ± 0.11%
		60%	100.44 ± 0.12%
	Avg. recovery ± SD		100.54 ± 0.11%

Table 4: Validation parameters for standard Cetirizine dihydrochloride.

Parameter	Cetirizine dihydrochloride
Specificity	Purity test ≥0.99 Identity test ≥0.99
Linierity	r= 0.996 Vxo = 3.451%
Sensitivity	LOD=75.54ng/spot
	LOQ=226.64 ng/spot
Precision	Average RSD= 0.86%
Accuracy	Average recovery±SD = 100.54 ± 0.11%

Table 5: Results of analysis of Cetirizine dihydrochloride in pharmaceutical formulation.

No.	Formulation	%Recovery ± SD of Cetirizine dihydrochloride
1.	Brand A	96.97 ± 0.86%
2.	Brand B	100.57±1.17%

RESULTS AND DISCUSSION

Optimum Condition

Table 1 showed the optimum conditions for analysis of Cetirizine Dihydrochloride using TLC densitometry. The mobile phase of chloroform : methanol : ethyl acetate (2 : 7 : 3) (v/v) give the efisien chromatogram. Efficiency of chromatogram was evaluated by the value of *Number of Theoritical Plate* (N), *Height Equivalent to a Theoritical Plate* (H) and *resolution* (Rs). The Rf of analytes are 0.43 (figure 2). Concentration optimum for Cetirizine Dihydrochloride was 500 ppm.

Method validation

From the TLC densitometry, showed that analyte spots in samples were identical with standards. Purity check of the

analyte spots using winCATS software also showed that analyte spots were pure. The values of rS,M and rM,E were >0.999, demonstrating that proposed TLC method is highly specific. Linierity of Cetirizine Dihydrochloride give the equation Y = 300.403 + 2.784X with correlation coefficient (r) 0.996. The LOD and LOQ (Limit Detection and Quantitation) were found to be 75.54 and 226.64 ng/spot. All the values of the repeatability and intermediate precision evaluation were less than 2% (table 2). The three measurement were perfomed within one laboratory by same analyst on different plates and different days. The accuracy of the proposed method were 100.54 ± 0.11 % (table 3). The summary of data validation parameters as listed in table 4.

Analysis of Marketed formulations

The result of the analysis of marketed formulations brand A and brand B showed that there was no interference from the excipients. The result given in table 5.

CONCLUSION

A new TLC method has been developed for the identification and quantification of Cetirizine Dihydrochloride. The method was found to be simple, rapid, specific, sensitive, precise and accurate for estimation and can be conveniently employed for the routine quality control analysis of Cetirizine Dihydrochloride in tablet.

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