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Research Article

HPTLC and RP-HPTLC Method Development and Validation for the Estimation of Carbimazole in Bulk and Marketed Formulation

Pritam S Jain*, Champalal T Pawara and Sanjay J Surana

R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, India

***Corresponding Author:** Pritam S Jain, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, India.

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Abstract

Carbimazole, ethyl 3-methyl-2-sulfanylidene-imidazole-1-carboxylate, is a antihyperthyroidism drug. HPTLC and RP-HPTLC method has been developed and validated for the determination of carbimazole in bulk drug and marketed formulation. Chromatographic separation was performed on Pre-coated aluminum plates with 250 µm layer of Silica gel 60 F_{254} and Silica gel 60 RP-18 TLC $F_{254}S$ using Toluene: Methanol: formic acid (3.5:1.5% v/v) and water: methanol in the ratio of (2.2:2.8% v/v) and Scanning was carried out densitometrically at 298 nm. Linear relationships were obtained between response and amount of drug in the range 400–2400 ng per band with high correlation coefficients (r^2 =0.995 and r^2 =0.998) for HPTLC and RP-HPTLC method of carbimazole respectively. The accuracy of the method in terms of % recovery was found to be from 98-101 ± 1.04 % and 99-100 ± 0.47 % and the limit of detection and quantification were 20.83, 63.12 and 27.30, 82.74, respectively. This method under statistical analysis proved a selective, repeatable and accurate analysis of the drug. This method can be used for quantitative analysis of Carbimazole in the bulk drug and in tablet formulation.

Keywords: Carbimazole (CBZ); HPTLC: RP-HPTLC Densitometry; Validation

Introduction

Carbimazole, ethyl 3-methyl-2-sulfanylidene-imidazole-1-carboxylate (Figure 1), is an antihyper- thyroidism drug. It is a prodrug and after absorption it gets converted to active form, methimazole. Methimazole acts by preventing the thyroid peroxidase enzyme and reducing the production of the thyroid hormones T3 and T4 (thyroxine).

Figure 1: Chemical Structure of Carbimazole.

This antithyroid drug has also been used to increase growth and weight in animals for human consumption, with harmful consequences for human health [1]. In the literature survey analytical methods have been reported for the estimation of Carbimazole which include Bromometric [2], Potentiometric and Coulometric [3], Polaragraphy and Voltametry [4], Spectrophotometric [5-7], RP-HPLC [8], and stability-indicating RP-HPLC [1]. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive RP-HPTLC method for the estimation of carbimazole in bulk drug and tablet. The method was further optimized and validated in accordance with guidelines suggested by International Conference on Harmonization (ICH).

Experimental

Chemical and reagents

Carbimazole (Pure) were obtained as a gift sample from MacLeod's Pharmaceutical Ltd., Daman. All chemicals and reagents were used of HPLC grade and were purchased from Merck Chemicals, India.

HPTLC instrumentation

The High Performance Thin Layer Chromatography (HPTLC) system (Camag Switzerland) having Camag Linomat 5 applicator with Camag TLC Scanner 3, Camag twin trough developing chambers (20×10 and 10×10 cm), Camag UV Cabinet with dual wavelength UV lamps (254 and 365 nm), Camag Hamilton micro syringe (100 µL), Pre-coated aluminum plates with 250 µm layer of Silica gel 60 F_{254} and Silica gel 60 RP-18 TLC $F_{254}S$ and Electronic analytical balance Shimadzu AUX-120 was used for all the weighing.

Chromatographic Conditions

Chromatographic separation was performed on 10×10 and 20×10 cm pre-coated aluminum plates with 250 µm layer of silica gel 60 F_{254} and Silica gel 60 RP-18 TLC $F_{254}S$ (E. Merck, Darmstadt, Germany) for HPTLC and RP-HPTLC respectively. The plates were prewashed with methanol and activated at 100° C for 10 minutes prior to the application. Sample was spotted in the form of 6 mm width with the help of Camag Hamilton micro syringe (100 µL) on TLC plate from the bottom edge using Linomat 5 applicator. The TLC plate was developed in twin trough developing chamber using Toluene: Methanol (3.5:1.5%v/v) and Water: Methanol (2.2:2.8% v/v) for HPTLC and RP-HPTLC, respectively as mobile phase at room temperature (25°C ± 2) with the 15 minutes of chamber saturation up to the 80 mm development distance. Densitometry scanning was performed at 298 nm on a Camag TLC scanner 3 and was operated by win CATS software version 1.3.0.

Preparation of standard stock solution

An accurately weighed 10 mg of Carbimazole (CBZ) was transferred into a 10 mL volumetric flask, dissolved in methanol and volume made up to the mark with the same solvent to achieve 1000 ng/µL.

Method validation

Linearity

Linearity was performed using working standard stock solution of CBZ in the range of 400-2400 ng/spot by applying 0.4-2.4 µL, for both method HPTLC and RP-HPTLC respectively.

Precision

Precision of the method was estimated as intra-day and inter-day changes by analyzing 800, 1200, 1600 ng/spot in triplicate on the same day for three times for intra-day precision and triplicate for three consecutive days for the inter-day precision for both method HPTLC and RP-HPTLC, respectively.

Limit of detection (LOD) and Limit of quantification (LOQ)

Detection limit and Quantification limit was calculated by the method based on the SD of the response and the slope of the calibration curve. Sensitivity of the proposed method was estimated in terms of limit of detection LOD=3.3×σ/S and LOQ=10×σ/S, where, σ is the standard deviation and S is the slope.

Specificity

The specificity of the method was determined by analyzing standard drug and sample. The spot of the CBZ in the sample was confirmed by comparing the Rf value and the spectra with respect to standard drug. The peak purity was assessed at three different level i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Ruggedness

Ruggedness of the proposed method was studied by spotting 1200 ng/spot of CBZ for both methods HPTLC and RP-HPTLC, respectively with the help of two different analyst keeping same experimental and environmental conditions.

Accuracy

Accuracy was determined in terms of % recovery. Recovery study of CBZ was carried out by over spotting of the known amount of the standard CBZ in the sample at 80, 100 and 120% level. The total concentration of the drug was determined. At each level three determinations were performed.

Robustness

Robustness of the method was performed by spotting 1200 ng/spot of CBZ for both methods HPTLC and RP-HPTLC, respectively on TLC plate by making small deliberate changes in the chromatographic conditions to examine the effects on the results by performing the parameters include mobile phase composition, development distance, activation of plate and duration of chamber saturation.

Application of proposed method to tablet formulation

To determine the amount of Carbimazole in tablets (label claim: 5mg/tablet), twenty tablets were accurately weighed and finely powdered. An amount equivalent to 10 mg from tablets powder were transferred into 10 mL volumetric flask and extracted with methanol by shaking mechanically for 15 min and volume was made up to the mark and filtered using 0.41 µm filter (Millifilter, Milford, MA). From the above solution 1200 ng/spot were applied and analyze by development and scanning as section 2.3 for HPTLC and RP-HPTLC, respectively.

Results and Discussion

Development of optimum mobile phase

Different ratio of toluene: methanol and acetonitrile: water: glacial acetic acid was tried as a mobile phase but different errors was observed such as tailing of spot, less persistence and spreading of spots. In order to overcome the problems, toluene: methanol: formic acid (3.5:1.5% v/v) and methanol: water (2.2:2.8%v/v) was tried and results observed in good resolution, sharp and symmetrical peak with Rf 0.49 (Figure 6) and 0.55 (Figure 7) for HPTLC and RP-HPTLC, respectively. It was observed after prewashing of TLC

with methanol (followed by drying and activation) and pre-saturation of chamber with mobile phases for 20 min.

Calibration curve

The linear regression data for the calibration curve of NAP by HPTLC and RP-HPTLC was determined in the concentration range of 400-2400 ng/spot both methods with the Linear regression equation Y=6.6836x+837 (r^2=0.995) and Y=8.6732x+327 (r^2=0.998) correlation coefficient. The calibration curve shown in Figure 2, 3 and the 3-D linearity chromatogram is shown in Figure 4 and 5, respectively.

Figure 2: Linearity Curve of Carbimazole by HPTLC.

Figure 3: Linearity Curve of Carbimazole by RP-HPTLC.

Figure 4: 3-D NP-HPTLC Linearity Chromatogram of Carbimazole.

Validation of the method

Precision

The precision of the proposed method was estimated in terms of % relative standard deviation (%RSD). The results depicted revealed high precision of the method is presented in table 1.

LOD and LOQ

Sensitivity of the developed method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ) for FDP. The LOD and LOQ of HPTLC and RP-HPTLC were found to be 20.83, 63.12 and 27.30, 82.74 respectively. This indicates the adequate sensitivity of the method.

Figure 5: 3-D RP-HPTLC Linearity Chromatogram of Carbimazole.

Conc. (ng/spot)		Intra-day*				Inter-day*			
	% Amount found ± SD		%RSD		% Amount found ± SD		%RSD		
HPTLC	RP-HPTLC	HPTLC	RP-HPTLC	HPTLC	RP-HPTLC	HPTLC	RP-HPTLC	HPTLC	RP-HPTLC
800	800	100.4851 ± 1.4680	99.9061 ± 0.9925	1.4467	0.9866	99.2785 ± 0.8715	99.0873 ± 1.4336	0.8702	1.4247
1200	1200	100.2461 ± 1.2799	100.8206 ± 0.7721	1.2863	0.7722	100.8206 ± 0.9075	100.6286 ± 1.2790	0.9095	1.2758
1600	1600	99.2342 ± 1.2757	100.2463 ± 0.6077	1.2669	0.6104	99.7670 ± 0.8481	99.6356. ± 1.1868	0.8565	1.1750

Table 1: Precision. *mean of three estimates

Recovery Studies

The proposed method when used for extraction and subsequent estimation of CBZ from the pharmaceutical dosage form after over spotting with 80,100 and 120% of additional drug, mean recovery is within acceptable limit. The % recovery listed in table 2.

Specificity

The peak purity of CBZ was assessed in HPTLC and RP-HPTLC by comparing the spectra at three levels, i.e. peak start (S), peak apex (M) and peak end (E) position of the spot and the result obtained as r^2(S, M) =0.997, r^2(M,E)=0.998 and r^2(S,M)=0.997, r^2(M,E)=0.998, respectively. Good correlation was obtained between standard and sample spectra of CBZ (Figure 8, 9).

Label Claim (mg/tablet)	Amount of standard drug added (%)	Drug Recovered*		% RSD	
		HPTLC	RP-HPTLC	HPTLC	RP-HPTLC
10	80	98.95	99.92	1.7276	0.0814
	100	98.46	98.79	0.9009	0.7745
	120	100.23	99.75	0.5111	0.5673

Table 2: Recovery study. * mean of three estimations at each level

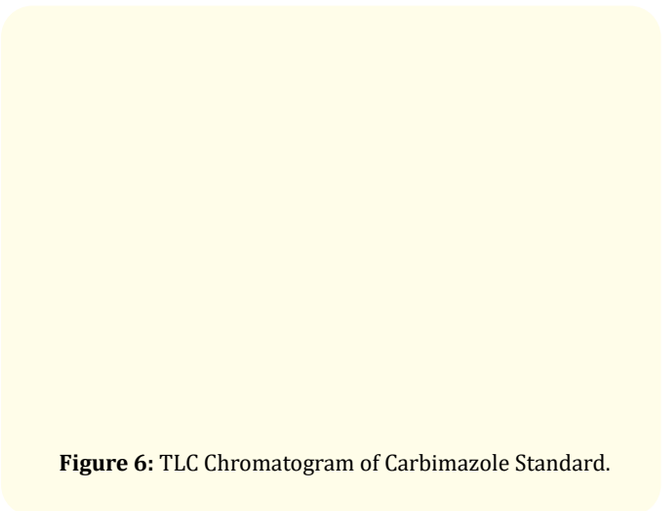

Figure 6: TLC Chromatogram of Carbimazole Standard.

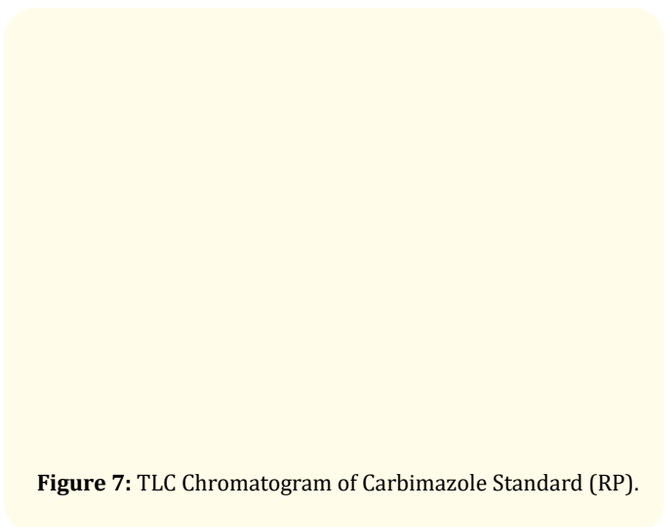

Figure 7: TLC Chromatogram of Carbimazole Standard (RP).

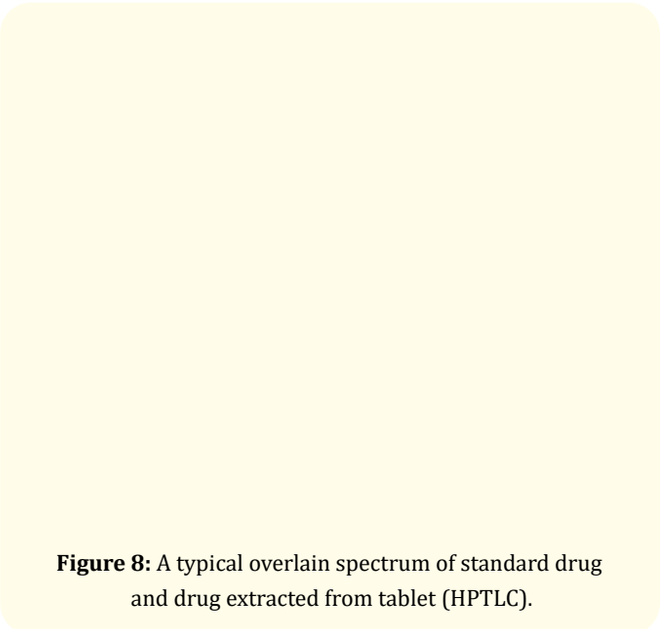

Figure 8: A typical overlain spectrum of standard drug and drug extracted from tablet (HPTLC).

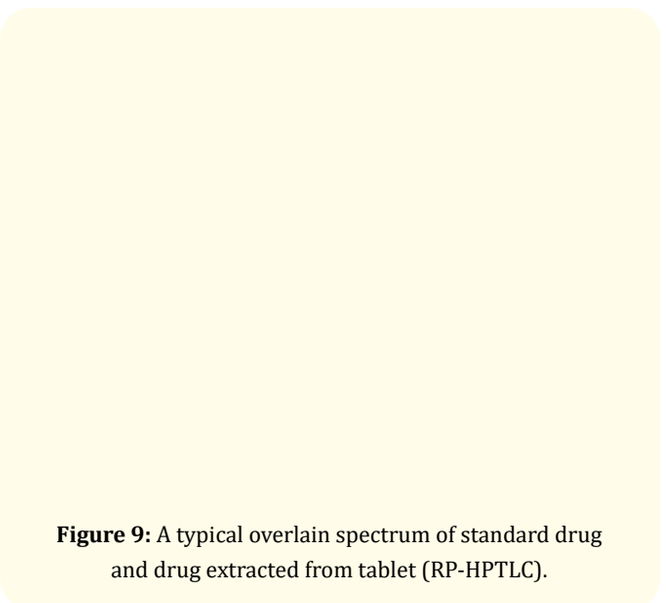

Figure 9: A typical overlain spectrum of standard drug and drug extracted from tablet (RP-HPTLC).

Robustness of the method

Robustness of the method was studied by calculated the standard deviation of peak area for each parameter and % relative standard deviation was found to be less than 2%. Values of %RSD are indicated in table 3.

Parameters		±SD of Peak area*		%RSD	
		HPTLC	RP-HPTLC	HPTLC	RP-HPTLC
Mobile Phase composition	-0.5 mL	94.01	98.15	1.90	1.54
	+0.5 mL	62.05	94.84	1.31	1.77
Activation of Plate	-2 min	79.02	112.97	1.89	1.67
	+2 min	92.56	108.33	1.97	1.88
Duration of saturation	-5 min	78.86	79.61	1.41	1.70
	+5 min	71.59	69.35	1.68	1.28
Development distance	-10 mm	47.01	65.71	0.86	1.17
	+10 mm	38.51	88.60	0.84	1.63

Table 3: Robustness

*mean of six estimations at each level.

Ruggedness of the method

Ruggedness of the method was performed by applying 1200 ng for both methods HPTLC and RP-HPTLC, respectively by two different analyst keeping same experimental and environmental conditions. The results summarized in table 4.

Parameters		HPTLC	RP-HPTLC
Linearity range (ng/spot)		400-2400	400-2400
Correlation coefficient (r^2)		0.995	0.998
Limit of detection LOD (ng/spot)		11.5195	29.9022
Limit of quantification LOQ (ng/spot)		34.9076	90.6127
% Recovery (n=6)		99.21	99.48
Ruggedness (%RSD)	Analyst-I	0.9120	0.4318
	Analyst-II	0.3926	0.2914
Robustness		Robust	Robust
Specificity		Specific	Specific

Table 4: Summery of validation parameter.

Application of marketed formulation

A single spot at Rf 0.40 and 0.53 was observed for HPTLC and RP-HPTLC in the chromatogram of CBZ. There was no interference from the excipients commonly present in the tablet. The % drug content and % RSD were calculated. The low % RSD value indicated the suitability of this method for the routine analysis of Carbimazole in pharmaceutical dosage forms.

Conclusion

HPTLC and RP-HPTLC method has been developed for the identification and quantification of Carbimazole in bulk and pharmaceutical dosage form. The method for the estimation of Carbimazole was simple, accurate, precise, specific and selective. The methods were found to be linear in the concentration range of 400-2400 ng/spot, both methods respectively. The method was validated as per ICH guidelines.

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