International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 843-848
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Stability Indicating Chromatographic Method for Estimation of Methimazole

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Received: 14 Mar 2019 / Accepted: 16 Apr 2019 / Published online: 1 Jul 2019
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Abstract
A stability indicating high performance thin layer chromatography (HPTLC) method was developed and validated for determination of anti-thyroid drug, Methimazole. HPTLC separation was carried out on Merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using mobile phase as Chloroform: Acetone. Methimazole gave sharp peak at Rf 0.44 ±0.03 at 252nm. Calibration curve was linear in range 200-600ng/band for methimazole. Stress degradation study was carried out according to ICH guidelines Q1A (R2) and the method was validated as per ICH guideline.

Keywords
Methimazole, HPTLC, stability, validation.

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INTRODUCTION
Methimazole is used to treat hyperthyroidism, a condition that occurs when the thyroid gland produces too much thyroid hormone. It directly interferes with thyroid synthesis by preventing iodine and peroxidase from combining with thyroglobulin to form thyroxine (T4) and triiodothyronine (T3). This action decreases thyroid hormone production[https://en.wikipedia.org/wiki/Thiamazole]. Methimazole is chemically 3-methyl-1-imidazole-2-thione. It is a white, crystalline substance that is freely soluble in water. It is metabolite of carbimazole[https://www.drugbank.ca/drugs/DB00763]. Several analytical methods have been reported for the analysis of carbimazole and methimazole such as HPLC method for methimazole in human plasma, urine and fish homogenates [1-6], HPLC and HPTLC method for carbimazole [7 & 8], stability indicating method for carbimazole by HPTLC [9]. Literature survey revealed that no stability indicating HPTLC method is reported for determination of methimazole in bulk drug and tablet dosage form. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive HPTLC method for the estimation of methimazole in bulk drug and tablet. The method was further optimized and validated in accordance with guidelines suggested by ICH guidelines (International Council for Harmonization). Structure of methimazole is given as Fig 1.

MATERIAL AND METHODS
All chemicals and reagents that is Methanol, Chloroform, Acetone, Hydrochloric acid(HCL), Hydrogen peroxide solution 6% w/v (H_2O_2), Sodium hydroxide (NaOH) were purchased from LOBA CHEMIE PVT. LTD., Mumbai.
CHROMATOGRAPHIC CONDITION

Optimisation of chromatographic condition was carried out on aluminum plates precoated with silica gel $60F_{254}$ in (10 cm × 10 cm with 250µm layer thickness). Sample was applied on the plate as a band of 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag Switzerland). The mobile phase was composed of Chloroform: Acetone 7:3v/v. Mobile phase was saturated in Camag (10cm × 10cm) twin trough glass chamber for 15 min. saturation condition and run to distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS (version 1.4.3.6336).

SELECTION OF ANALYTICAL WAVELENGTH

Standard stock solution of 1,000ug/ml was prepared by using methanol. Further dilution was carried out to make solution of 10µg/ml and was scanned over 200 to 400nm in UV-Spectrophotometer. Wavelength 252nm showed maximum absorbance hence it was selected as analytical wavelength. UV spectrum is given as Fig.2

PREAPARATION OF STANDARD STOCK SOLUTION

Standard stock solution is prepared by dissolving 10 mg of methimazole in 10 ml methanol to 1000µg/ml. Further dilutions were made in methanol to make 50µg/ml and applied on TLC plate. Densitogram of standard methimazole is given as Fig 3

STRESS DEGRADATION STUDIES [10, 11]

The strength of reagent, time of exposure and temperature were optimized to obtain 10-30% degradation.

The optimized conditions are as follows

Acid Hydrolysis

1 ml of stock solution (1,000µg/ml) was mixed with 1 ml of 0.2N HCL and volume was made up to 10 ml with methanol. Solution was kept for 30 minutes at room temperature and applied at TLC plate.

Base Hydrolysis

5 ml of stock solution was mixed with 5 ml of 0.2 N NaOH and volume was made up to 50 ml with methanol. Solution was refluxed for 45 minutes at 60°C and applied at TLC plate.

Neutral Hydrolysis

5 ml of stock solution was mixed with 5 ml of Distilled water and volume was made up to 50 ml with methanol. Solution was refluxed for 60 minutes at 60°C and applied at TLC plate.

Oxidation Hydrolysis

1 ml of stock solution was mixed with 1 ml of 0.6% H_2O_2 w/v and volume was made up to 10 ml with methanol. Solution was kept for 15 minutes at room temperature and applied on TLC plate.

Degradation under Dry Heat

Effect of dry heat was studied by keeping drug in oven at 80ºC for 48hours. Sample was weighed 10 mg and dissolved in methanol to get solution of 1000µg/ml of methimazole.

Photo-degradation studies

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hrs/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hours. Sample was weighed, dissolved in methanol to make 1000µg/ml of methimazole.

Table 1: Summary of forced degradation studies

Stress degradation conditions at 252nm	Methimazole		Peak purity at 252nm		Rf of degradation product
	% Recovery	% Degradation	R (s, m)	R (m, e)	
Acid hydrolysis (0.2N, 30mins)	84.99	15.01	0.9980	0.9965	NA
Base hydrolysis (0.2N, Refluxed for 45 mins)	73.88	26.11	0.9961	0.9945	NA
Neutral hydrolysis (Refluxed for 60 mins)	81.77	18.23	0.9912	0.9929	NA
Oxidative hydrolysis (0.6% H_2O_2 w/v, kept for 15mins)	52.59	47.40	0.9961	0.9974	0.11
Dry heat (80ºC, for 48 hrs)	83.19	16.80	0.99654	0.9918	NA
Photo stability UV, 200-watt hrs/square meter	95.20	4.80	0.9988	0.9942	NA
Florescence, 1.2 million Lux. Hours	95.25	4.75	0.9972	0.9945	NA

VALIDATION OF ANALYTICAL METHOD [12]

The method was validated for various parameters according with ICH Q2 (R1) guidelines

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.9900, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

Linear relation between amount spotted vs peak area was obtained in the range of 200-600ng/band of methimazole. The equation was found to be y=19.05x-2837 for methimazole and coefficient of correlation (r^2) was found to be 0.998

Range

Methimazole = 200-600ng/band

Assay

Assay was performed on marketed formulation. Assay was determined by extrapolation of amount spotted vs peak area from linearity equation which was found to be 98.40 % for methimazole.

Accuracy

To check accuracy of the method, recovery studies were carried out by using marketed formulation to which standard was added at three different levels 80, 100, 120%. The drug concentrations were calculated from respective linearity equation. The result of the recovery studies indicated that the method is accurate for estimation of drug in marketed formulation of tablet. The result obtained is shown in table 2.

Table 2: Result of Accuracy

Level %	Recovery%
80	99.31
100	99.65
120	99.34

Precision

The precision of method was demonstrated by intra-day and inter-day variation studies. The result obtained for intra-day and inter-day variation as shown in table 3 & 4

Table 3: Intra-day precision

Sr. No.	Amount	Area	Mean	SD	%RSD
1	400ng/band	4879.4			
2	400ng/band	5047.7			
3	400ng/band	4960.7	4906.13	82.74	1.68
4	400ng/band	4874.5			
5	400ng/band	4834.8			
6	400ng/band	4839.7			

Table 4: Inter-day precision

Sr. No.	Amount	Area	Mean	SD	%RSD
1	400ng/band	4761.2			
2	400ng/band	4925.7			
3	400ng/band	4819.2	4805.25	92.88	1.93
4	400ng/band	4697.1			
5	400ng/band	4728.6			
6	400ng/band	4899.7			

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated as 2.63ng/band and 7.98 ng/band, respectively.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time were altered, Time was also changed from spotting to development and development to scanning, composition of mobile phases and the effects on the peak area was noted.

Table 5: Summary of validation parameter

Sr no	Parameter	Result
1	Linearity	y=19.05x-2837 r^2= 0.998
2	Range	200 to 600 ng/band
	Precision	%RSD
3	Intraday	1.68%
	Interday	1.93%
4	LOD	2.63 ng/band
5	LOQ	7.98 ng/band
6	Assay	98.40 %
		99.31
7	Accuracy	99.65
		99.34
8	Specificity	specific
9	Robustness	robust

RESULTS

The optimized stress degradation condition led to degradation within range of 10-30%. As shown in table except oxidative hydrolysis. Oxidative hydrolysis study showed 47.70% degradation. R_f of standard methimazole is 0.44 ± 0.03. Spectrum of oxidative hydrolysis indicated that at R_f 0.11 there can be well resolved peak of degradation product. 3D densitogram and spectrum of oxidative hydrolysis study is given as fig 4 & 5.

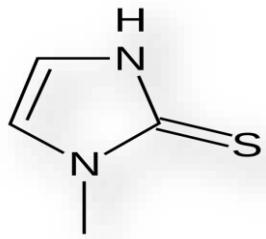

Fig 1. Structure of Methimazole

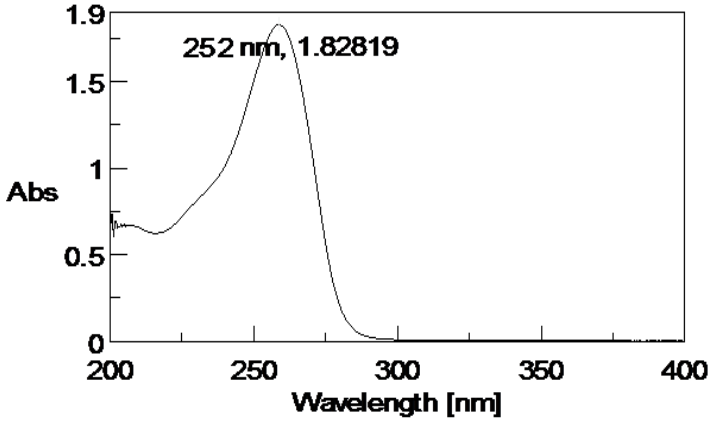

Fig 2. UV spectrum of methimazole solution (10µg/ml)

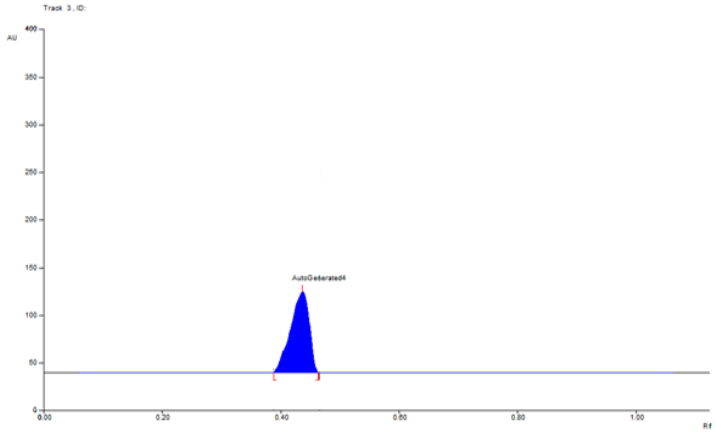

Fig3. Densitogram of standard solution of methimazole 300 ng/band (Rf 0.44 ± 0.03)

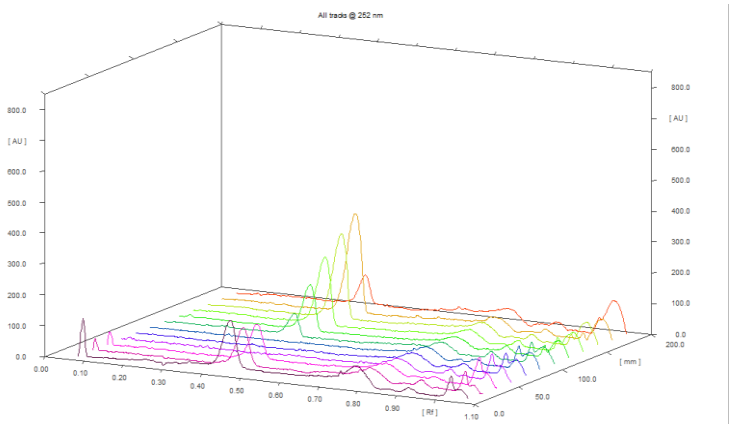

Fig4. 3D Densitogram of oxidative hydrolysis

In above densitogram track first was H_2O_2 treated methimazole of 4000 ng/band, track 2 & 3 was H_2O_2 treated methimazole of 400 ng/band, track 4 to 6 were blank, 7 to 12 (200 to 600 ng/band) standard linearity.

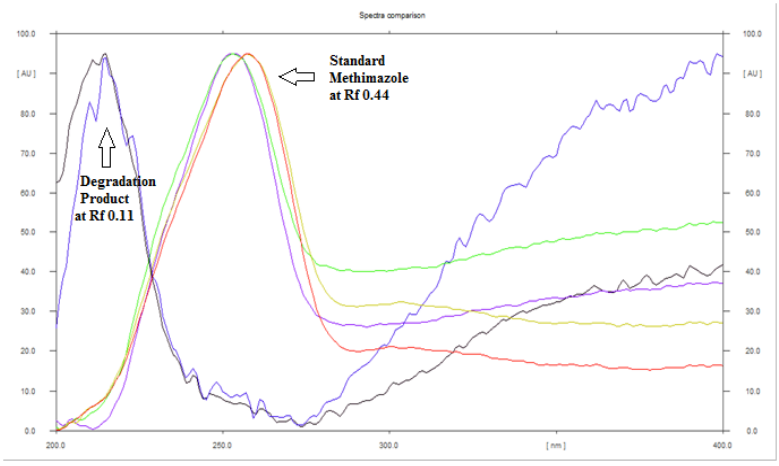

Fig.5. Spectrum of degradation product in oxidative hydrolysis study and standard methimazole

DISCUSSION

One research article of stability indicating method of carbimazole by HPLC was found in literature survey. Research work presented in current paper was compared with the literature search, results mentioned in this paper did not match. The objective of work was to develop stability indicating HPTLC method. We have observed that methimazole is

more sensitive to oxidative hydrolysis with well resolved degradation product. The developed method may be used for estimation and monitoring stability of methimazole.

CONCLUSION

A simple and rapid method was developed and validated. And the degradation product was observed in oxidative hydrolytic condition. The method has been successfully validated according to ICH Q2R1 Guidelines.

ACKNOWLEDGEMENT

Authors are thankful to the principal and the management of AISSMS College of Pharmacy, Pune for providing the necessary facilities for research work.

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