Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2012, 3 (3):388-393

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Estimation of Carbimazole in Presence of its Degradants Using RP-HPLC

Avinash V. Deosarkar*, Shruti D. Deshpande and Sanjay G. Walode

Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmaceutical Sciences, Lonavala, Pune, Maharashtra, India

---

ABSTRACT

RP-HPLC method has been developed and validated for the determination of carbimazole in bulk drug and marketed formulation. The developed method was found to be specific, reproducible, and stability indicating. Separation was carried out by using Inertsil, ODS-3V C18 (250 X 4.6 mm) 5µm column. Mobile phase comprising of water: acetonitrile (75:25 v/v) was used to achieve good resolution of the analyte and its impurities. The detector linearity was established in concentrations ranging from 2-10 µg mL^{-1}. The method was tested at different levels of specificity and accuracy as per requirements given in ICH guidelines. For stability study the drug was exposed to the stress conditions such as acid, base, oxidation, neutral and sunlight as per the recommendations of ICH guidelines. The method was proved to be robust with respect to changes in flow rate and temperature. The proposed method is found to be sensitive, precise, rapid, reproducible, and offers good column life.

Key words: Carbimazole, HPLC, ICH, Stability indicating, Validation.

INTRODUCTION

Carbimazole, ethyl 3-methyl-2-sulfanylidene-imidazole-1-carboxylate (Fig.1), is an antihyper- thyroidism drug. It is a pro-drug 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) [1].

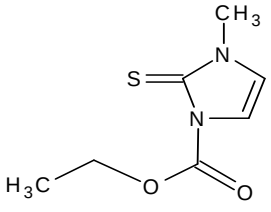

Fig.1 Chemical structure of carbimazole

Several analytical methods have been reported for the analysis of carbimazole such as an indirect bromometric [2], potentiometric [3], voltammetric [4], chromatographic [5-7], spectrophotometric [8, 9], polarographic [10], and fluorometric titration [11]. Literature survey revealed that no stability indicating RP-HPLC method is reported for determination of carbimazole in bulk drug and tablet dosage form. The main objective of the proposed work was to develop a simple, accurate, precise and sensitive RP-HPLC 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) [12].

MATERIALS AND METHODS

Authenticate carbimazole sample was a kind gift from Gens Pharma International Pvt. Ltd., Pune, India. HPLC grade water and acetonitrile (Merck Ltd, Mumbai, India) was used as solvent. All the aqueous reagents were prepared using carbon dioxide free distilled water.

Apparatus:
The HPLC system, Jasco PU-2080 Plus, with manual Rheodyne injector facility operates at 20 µL capacity per injection was used. The column used was Inertsil, C18 (250 X 4.6 mm) 5µm and the detector consisted of UV/VIS (Jasco UV 2075-Plus) operated at 298 nm. The data were acquired and processed using Borwin software version 1.5

Chromatographic Conditions:
Optimizations of chromatographic condition were carried out using water: acetonitrile (75:25 v/v) as mobile phase. Prior to deliver into the system, mobile phase was filtered through 0.45 µm filter and sonicate for 10 min. The samples were introduced by injector with a 20 µL sample loop. The analysis was carried out under isocratic conditions using flow rate 1.5 mL min^{-1} at 18^0C and chromatograms were recorded at 298 nm.

Preparation of standard stock solution:
Weighed accurately 10 mg of carbimazole and transferred to 10 ml volumetric flask, add 5 mL of mobile phase and sonicate for 10 min and volume was made up to mark with mobile phase (1000µg mL^{-1}).

Preparation of standard solution:
From the standard stock solution 0.1mL solution was pipetted out in 10 mL volumetric flask and volume was made up to the mark with mobile phase to get a final concentration 10 µg mL^{-1}.

Analysis of tablet formulation:
Twenty tablets of carbimazole were weighed, triturated, mixed thoroughly and average weight of tablet was calculated. Accurately weighed quantity of tablet powder equivalent to 10 mg of carbimazole (label claim) was transferred to 10 mL volumetric flask, added 5 mL of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter, diluted to volume with mobile phase. 0.1 mL of resultant solution further diluted to 10 mL and injected to HPLC system (Table 1).

Table 1: Assay data of marketed formulation.

Sr. No.	Formulation	Taken amount	Amount estimated	% estimated	% RSD
1	Thyrocab	10 mg	9.9576	99.5767	1.8813
2		10 mg	9.8204	98.2048	
3		10 mg	10.0534	100.5345	
	Mean		9.9438	99.4386	

RSD is Relative standard deviation

System Suitability:
System suitability parameters were evaluated from retention times, asymmetry, capacity factor and theoretical plates of standard chromatograms (Table 2).

VALIDATION:
Limit of detection (LOD) and limit of quantification (LOQ):
The signal-to-noise ratio (*S/N*) method was adopted for the determination of limit of detection and limit of quantification. The limit of detection was estimated as three times the *S/N* ratio and the limit of quantification was estimated as ten times the *S/N* ratio (Table 2).

Specificity:
Specificity is the ability of a method to discriminate between the analyte of interest and other components that may present in the sample. The specificity of the method was evaluated to ensure separation of carbimazole.

Linearity:
Different standard solutions were prepared by diluting standard stock solution with mobile phase in the concentration range 2-10 µg mL^{-1}. Diluted samples were injected and chromatograms (Fig 2) were taken under standard chromatographic conditions. The peak area was plotted against corresponding concentrations to obtain the calibration graph (Fig.3).

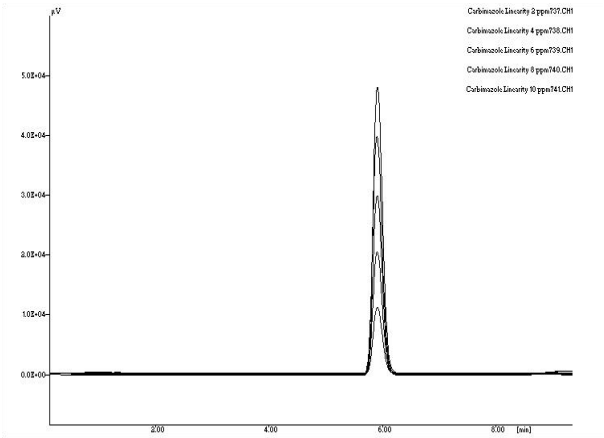

Fig.2. Chromatogram of linear response of carbimazole

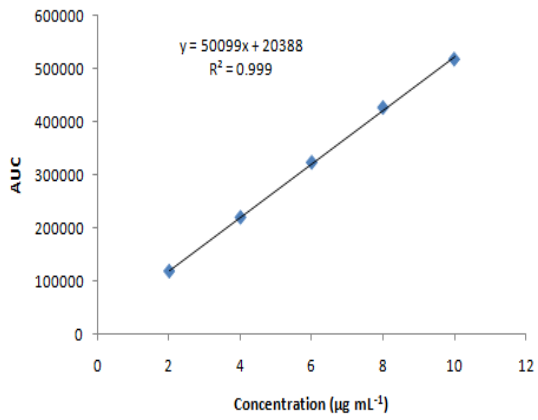

Fig.3 Calibration plot of carbimazole

Table 2: Statistical parameters of Carbimazole

Parameters	Values
Limit of detection (µg mL^{-1})	0.4
Limit of quantification (µg mL^{-1})	1.4
Linearity (µg mL^{-1})	2-10
Regression equation (Y=mx+c)	y = 50099x + 20388
Correlation coefficient (r^2)	0.999
Retention time	5.958
Asymmetry	1.154
Capacity	708.0
Theoretical Plates	8025

Precision:

Precision of analytical method was expressed in relative standard deviation (RSD) of a series of measurements. The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses (i.e. three concentrations / three replicates each) of the sample solution on the same day and on three different days respectively.

Table 3: Precision data of carbimazole

Parameters	% estimated	S.D.	% RSD
Intra-day*	100.13	1.4002	1.3983
	101.11	1.7970	1.7771
	100.63	1.0786	1.0718
Inter-day*	100.80	0.7485	0.7346
	100.76	1.8782	1.8580
	100.83	0.9048	0.8973

** indicates mean of three replicates, SD is standard deviation.*

Recovery:

To check the accuracy of the proposed method, recovery studies were carried out by standard addition method. A known amount of standard carbimazole corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

Table 4: Recovery study data

Level of standard addition (%)	Amount of tablet powder (mg)	Amount of pure drug added (mg)	Amount of pure drug recovered (mg)	% Recovery	% RSD
80	10	8	8.13	101.73	0.9630
100	10	10	10.08	100.87	1.1842
120	10	12	12.14	101.22	1.8362

Robustness:
Robustness is a measure of the performance of a method when small and deliberate changes are made to the conditions of method. Robustness studies were performed by making slight variations in flow rate and temperature changes one at a time.

Table 5: Robustness data for carbimazole

Parameters	% Recovery	S.D.	% RSD
Change in Temperature (18 ± 2^0c)	100.99	1.1258	1.1147
Change in flow rate (1.5 ± 0.2 mL min^{-1})	100.67	0.5137	0.5102

FORCED DEGRADATION:
Acid and base induced degradation product:
To 10 mL of standard stock solution, 10 ml of 0.1 N HCl and 10 mL of 0.01N NaOH were added separately. These mixtures were reflux separately for 45 min for acid and 10 min for base at 50ºC. The forced degradation study in acidic and basic media was performed in the dark in order to leave out the possible degradative effect of light. 0.1 ml of each resultant solution was diluted to 10 mL with the mobile and resultant solution injected into the system.

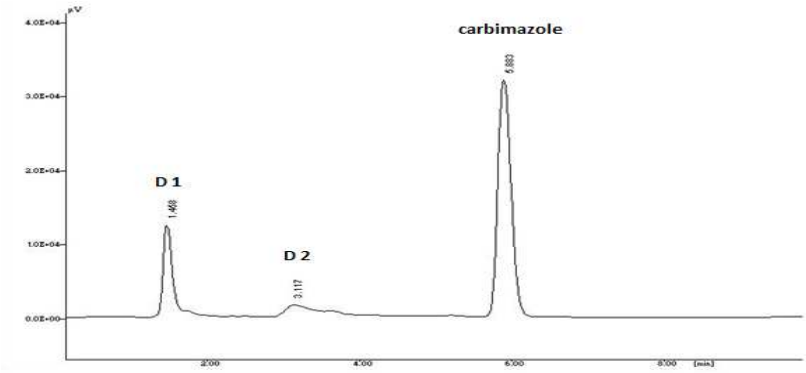

Fig.4 Chromatogram of acid [0.1N HCl (reflux for 45 min at 50°C)] treated sample
Peak D1, degradant [Rt = 1.458]; Peak D2, degradant [Rt = 3.117]; Peak 2, carbimazole [Rt = 5.883]

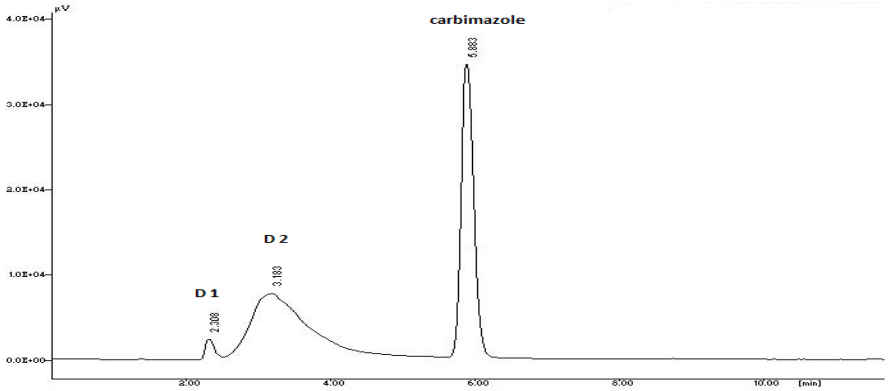

Fig.5 Chromatogram of base [0.01N NaOH (reflux for 10 min at 50°C)] treated sample
Peak D1, degradant [Rt = 2.308]; Peak D2, degradant [Rt = 3.183]; Peak 2, carbimazole [Rt = 5.883]

Hydrogen peroxide induced degradation product:
To 10 mL of standard stock solution, 10 mL of hydrogen peroxide (H_2O_2) (3 % v/v) was added. This solution was heated in boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and reflux for 20 min at 50°C. 0.1 ml of resultant solution was diluted to 10 mL with the mobile phase and resultant solution injected into the system.

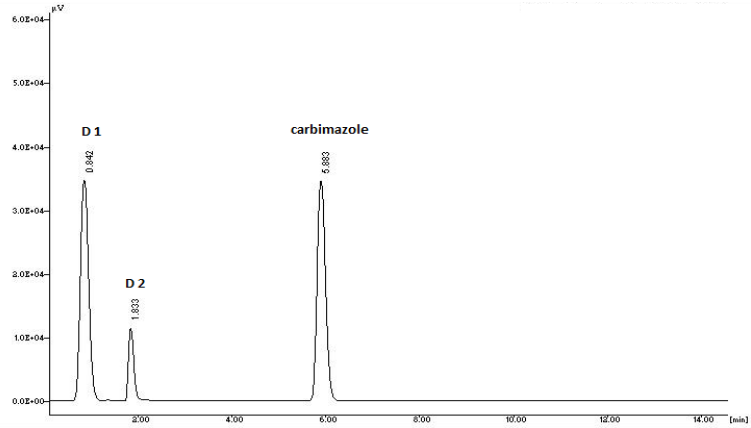

Fig.6 Chromatogram of Hydrogen peroxide [3% H_2O_2 (reflux for 20 min at 50°C)] treated sample
Peak D1, degradant [Rt = 0.842]; Peak D2, degradant [Rt = 1.833]; Peak 2, carbimazole [Rt = 5.883]

Neutral hydrolysis:
Ten millilitres of standard stock solution was mixed with 10 mL water and reflux for 60 min at 60ºC. 0.1 ml solution this solution was diluted to 10 mL with the mobile and resultant solution injected into the system.

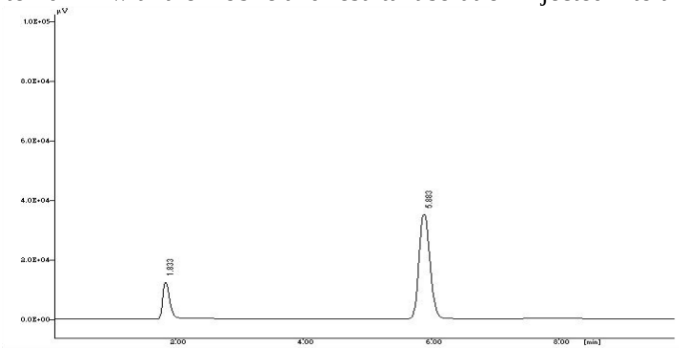

Fig.7 Chromatogram of neutral hydrolysis (reflux for 60 min at 60°C)]
Peak D1, degradant [Rt = 1.833]; Peak 2, carbimazole [Rt = 5.883]

Photolytic induced degradation product:
Ten millilitres of standard stock solution was exposed to direct sunlight for 30 min on a wooden plank and kept on terrace. 0.01 ml of resultant exposed solution was transferred to 10 mL volumetric flask, diluted with the mobile phase and solution was injected into the system.

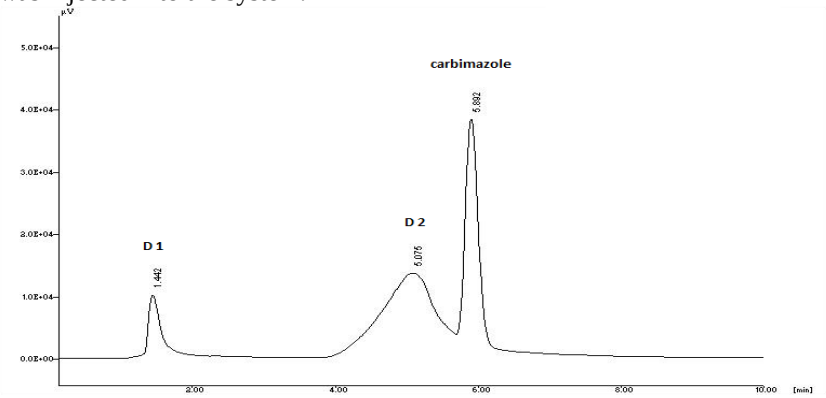

Fig.8 Chromatogram of sunlight exposed (for 30 min) sample
Peak D1, degradant [Rt = 1.442]; Peak D2, degradant [Rt = 5.075]; Peak 2, carbimazole [Rt = 5.892]

RESULTS AND DISCUSSION

The parameters were focused for improvisation of retention time, separation of degradation products and column life. The Inertsil C18 column provided good peak shapes and no peak splitting was observed. Carbimazole showed linear responses in concentrations level ranging from 2-10µgmL^{-1} with correlation co-efficient 0.999 (Table 2).

Table 6: Summary of force degradation data

Sample stress condition	Stress condition	Carbimazole (R.T.)	Degradants (R.T.)	% Area decreased	Fig. No.
Acid degradation	0.1 N HCl reflux for 45 min.	5.883	1.458, 3.117	30.78	4
Alkaline degradation	0.01 N NaOH reflux for 10 min.	5.883	2.308, 3.183	26.92	5
Oxidative degradation	6% H_2O_2 reflux for 20 min.	5.883	0.842, 1.833	26.55	6
Neutral hydrolysis	Purified water reflux for 1 hr.	5.882	1.833	25.37	7
Photolytic degradation	Kept in sunlight for 30 min.	5.892	1.442, 5.075	28.84	8

The measurement at three different concentration levels showed low value of % R.S.D. (<2) and low value of S. E. (<2) for intra- and inter-day variation, which suggested an excellent precision of the method.

The recovery of drug was determined by spiking drug at three different levels and was found to be between 100.87-101.73. The method was found to be robust with respect to flow rate and change in temperature without any changes in system suitability parameters.

Forced degradation of drug was carried out as per the ICH guidelines (ICH Q2B) by subjecting carbimazole to various stress conditions. The percent area decreased at the level of 25-31 % and additional peaks at retention time different to that of well separated peak of carbimazole indicated that carbimazole undergoes degradation in acidic, basic, oxidative, neutral and photolytic conditions. Summary of force degradation data are given in Table – 6.

CONCLUSION

The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. As the method able to separate the parent drug from degradation products it can be employed as a stability indicating method for carbimazole.

REFERENCES

[1] G. Mukherji, N. Agrawal, *Int. J. of Pharm.*, **1992**, 86,153-158.
[2] M.G. El-Bardicy, Y.S. El-Saharty, M.S. Tawakkol, *Talanta*, **1993**, *40*, 577.
[3] W. Ciesielski, A. Krenc, *Anal. Lett.*, **2000,** *33*, 1545-1554.
[4] K. Sarna, Z. Fijalek, *Anal. Lett.*, **1997**, *42*, 425.
[5] H.F. De Brabander, P. Batjones, J. Van Hoof, *J. Planar Chromatogr.Mod. TLC*, **1992,** *5*, 124.
[6] Chen Bo Liang Fanghui Niu, Yanqiu Dong Lidan Changchun., *China Foreign Med.Treat.*, **2009**, *35*, 74.
[7] C. Sanchez-Pedreno, M.I. Alberto, Garcia M.S., V. Rolenas, *Anal. Chim. Acta.* **1995,** *308*, 45.
[8] Y.S. El-Saharty, M. Abdel-Kawy, M.G. El-Bardicy, *Spectrosc. Lett.*, **2001**, *34*, 325-334.
[9] M.G. El-Bardicy, Y.S. El-Saharty, and M.S. Tawakkol, *Spectrosc. Lett.*, **1991**, *24*, 1079-1095.
[10] Z. Fijalek, P. Zuman, *Anal.Lett.*, **1990**, *23*, 1213.
[11] M.M. Bedair, M.A. Korany, M.A. El-Sayed, O.T. Fahmy, *Spectrosc. Lett.*, **1990,** *23*, 161.
[12] 12. Validation of Analytical Procedure: Text and Methodology Q2 (R1), ICH Harmonized Tripartite Guideline **2005**, 1-13.