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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CARBIMAZOLE API IN BULK AND FORMULATION

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ABSTRACT
The development and validation of analytical method for the determination of active pharmaceutical ingredients (API) in bulk and formulated products are critical to make sure the quality, welfare, and efficacy of pharmaceutical products. The methodical creation and exacting validation of an analytical technique for the measurement of carbimazole is the main emphasis of this study. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) has produced a simple and accurate method for measuring carbimazole. A simple and precise method was developed by Reverse Phase High Performance liquid Chromatography(RP-HPLC) for determination of Carbimazole. The analytical method validation was performed within the ICH guidelines. This innovative approach also complies with regulatory requirements. Carbimazole was separated on Inertsil 8ODS (250x4.6mm, 5µ) column at a UV wavelength of 298nm. Methanol and Acetonitrile was used as a mobile phase for the determination of Carbimazole. After several trials, 80:20v/v Methanol and Acetonitrile were selected with 0.7mL/min flow rate. Following that, tests were conducted to determine whether the validation parameters such as linearity, accuracy, precision within and between days, limit of detection (LOD), and limit of quantification (LOQ) were within acceptable bounds. Carbimazole recovery and assay results fell between 98-102%, suggesting that the suggested approach can be used for carbimazole quality control analysis.

Keywords: Carbimazole, RP-HPLC, ICH guidelines, Methanol, Analytical Method

INTRODUCTION
The chemical name of Carbimazole is Ethyl-3-methyl-2-sulfanylidene-imidazole-1-carboxylate. It's an Anti-hyperthyroidism drug for the treatment of hyperthyroidism. After being absorbed by the body, carbimazole is changed into its active form, methimazole. This form of carbimazole inhibits the thyroid peroxidase enzyme from coupling with the tyrosine residues on thyroglobulin, which lowers the thyroid hormone production of T3 and T4.
There are numerous methods for determination of Carbimazole The approaches reported include HPTLC[1,4] , RP-HPLC[2] , UV spectrophotometric method[3] , HPLC[4]. A kinetic method for determination of Carbimazole in pharmaceutical preparation by oxidation with bichromate in sulfuric acid [5]. The Chemical structure of Carbimazole and drug profile are depicted in figure 1 and table 1 as illustrated.

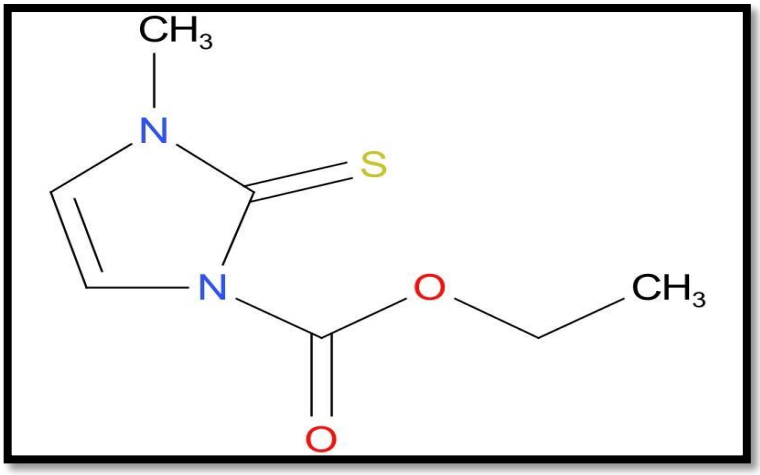

Figure 1: Chemical structure of Carbimazole

Table 1: Drug Profile of Carbimazole

ATTRIBUTE	DESCRIPTION
NAME OF DRUG	CARBIMAZOLE
FUNCTIONAL CATEGORY	Anti-hyperthyroidism
MELTING POINT	122-125^{0}c
MOLECULAR FORMULA	$C_7H_{10}N_2O_2S$
IUPAC	Ethyl3-methyl-2-sulfanylidene-imidazole-1-carboxylate
MOLECULAR MASS	186.3 g/mol
APPEARANCE	White Solid.
SOLUBILITY	Soluble in water, ethanol, chloroform and acetone. Slightly soluble in water.

MATERIALS AND METHODS

Materials

Carbimazole was received as a gift sample from USV pharma private limited. NEO MERCAZOLE tablet was purchased from local pharmacy shop. Analytical grade solvents were all used in the investigation.

Equipment

A JASCO Extrema LC system-4000 RP-HPLC instrument was employed to record chromatograms of the study. The data from the drug analysis were obtained and analysed by ChromNAV software. Electronic analytical balance was used to weigh the materials.

Selection of Diluent

Methanol was selected as diluent as it gives good and well resolved peaks with the mobile phase composition.

Standard solution Preparation

Measure Carbimazole 10mg precisely then put it in the 10ml standard flask consist of methanol resulting in 1000µg ml^{-1} of solution. Further dilutions of this solution were made as per requirement.

Optimisation of Chromatographic conditions

Mobile Phase

Numerous mobile phase trials were conducted in order to identify an ideal composition that yields clear, symmetric, and well-resolved peaks. The combination of Methanol and Acetonitrile was selected as mobile phase in ratio of 80:20 (v/v).Figure 2 depicts the optimized chromatographic conditions for this study.

Setting up of Calibration curve

The standard solution was diluted to yield concentrations between 5 to 25µg ml^{-1}. The calibration curve was constructed by performing the analysis at 298nm.

Method validation [6-8]

Validation of an analytical procedure involves laboratory studies to ensure that its performance is suitable for the proposed use. The parameters validated for the proposed spectrophotometric method include linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness.

Linearity

Different concentrations of Carbimazole standard solutions were utilised in the setting of calibration curve. The curve was obtained at a concentration range of 5-20 µg ml^{-1}. After injecting 10µl of each solution, the chromatogram was obtained at 298 nm. Coefficient of determination was calculated using regression analysis.

Accuracy

Accuracy was determined by mean percent recovery method. The solutions of 80%, 100% and 120% was prepared. The area of each level was used for the calculation of %recovery. The samples were analysed at 298nm.

Precision

Repeatability precision (intra-day and inter-day reproducibility) were taken into consideration when determining the method's precision. Six individual sample of same concentration was prepared. Based on the collected data, the repeatability of the retention duration and peak area was assessed and expressed as mean and %RSD.

LOD & LOQ

LOD and LOQ was calculated from the data obtained from precision of intraday and inter-day. The lowest amount that had a signal-to-noise ratio of at least three (S/N3) was defined as LOD, while the lowest concentration that had a signal-to-noise ratio of at least ten (S/N10) was defined as LOQ.

RESULTS AND DISCUUSION

Method development

Inertsil 8ODS (250x4.6mm, 5µ) was used as a column. The flow rate was set at 0.7mL/min with the injection volume of 10µl. Methanol and Acetonitrile with the ratio of 80:20 v/v was use as a mobile phase. The retention time was found to be 3.9min.

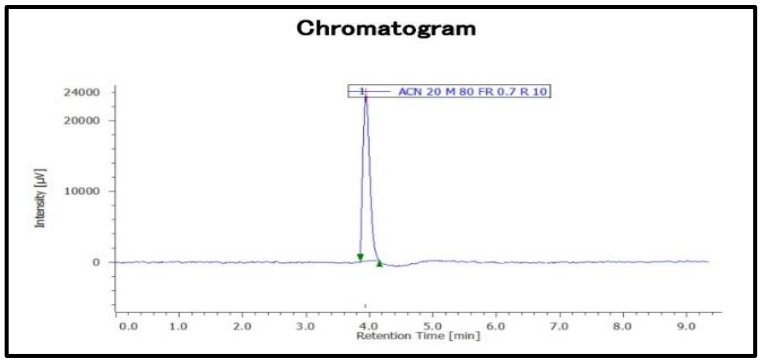

Figure 2. Chromatogram of Carbimazole

Linearity

The linearity was evaluated by constructing a calibration curve using standard solutions with known concentrations. The correlation coefficient (r^2) obtained from the calibration curve is 0.9993, indicating a strong linear relationship between the concentration of the analyte and the measured absorbance. This indicates that the target analyte can be precisely quantified using the HPLC method across a broad concentration range. The linearity of the method is depicted in Table 2 and Figure 3.

Table 2: Concentration of Carbimazole

Concentration	Peak Area
5	178486
10	406683
15	657628
20	887585
25	1155707

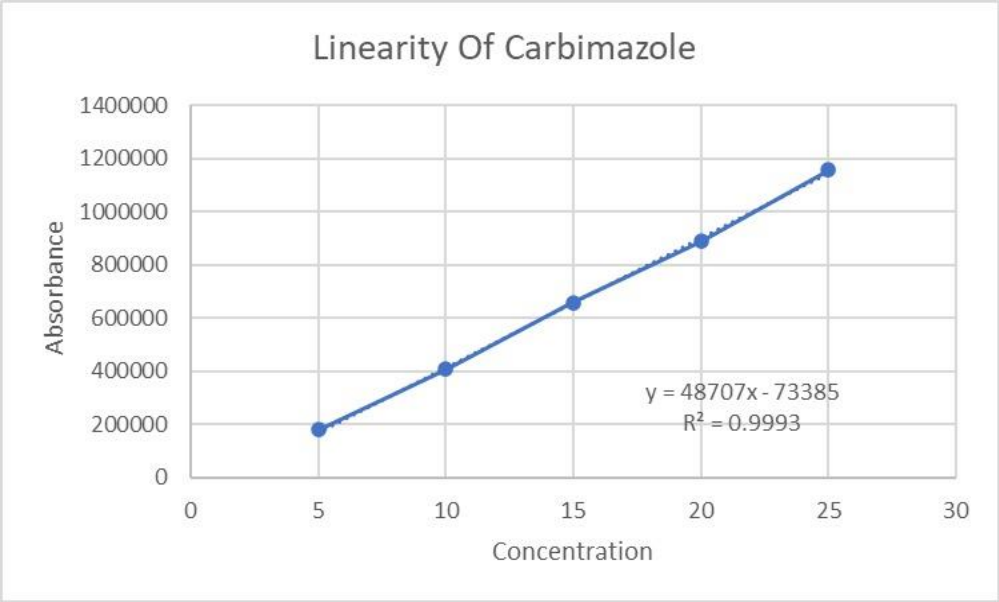

Figure 3: Calibration Curve of Carbimazole

Accuracy

To assess the accuracy of the HPLC method, percentage recovery experiments were determined by adding known amounts of the analyte into different matrix. The accuracy was performed for 80%, 100%, and 120%.The results show that %recovery was found between 99-102%. The outcomes are depicted in Table 3.

Table 3: Accuracy results of Carbimazole

Concentration	Area	Concentration obtained	% Recovery	
80%	321786	8.11	101.4	
80%	319770	8.07	100.89	
80%	315548	7.98	99.8	
100%	412693	9.9	99.7	
100%	435548	10.1	101	
100%	423333	10.19	101.9	
120%	514515	12.07	100.58	
120%	516165	12.10	100.86	
120%	509696	11.97	99.75	

Precision

Method precision was determined in terms of intermediate precision i.e intraday and interday precision. The %RSD for both intraday and interday was found to be less than 2.0% which indicated that the method is precise.

Table 4: Intraday Precision Results(n=6±S.D)

Concentration	Peak Area	Obtained Concentration	S.D	% RSD
20	915853	20.30		
20	946395	20.93		
20	919401	20.38	13233.33	1.43%
20	921480	20.42		
20	913836	20.26		
20	908748	20.16		

Table 5: Interday Precision Results(n=6±S.D)

Concentration	Peak Area	Obtained concentration	S.D	RSD
20	895934	19.90		
20	914561	20.28		
20	896634	19.91	14333.48	1.60%
20	894175	19.86		
20	869637	19.36		
20	893494	19.85		

Detection Limit (LOD)

The LOD for carbimazole were found to be 0.84µg ml^{-1}

Quantification Limit (LOQ)

The LOQ for carbimazole were found to be 2.55µg ml^{-1}

Robustness

The repeatability of same sample were taken. The retention time and peak area were not considerably impacted by small, intentional adjustments to several experimental parameters, such as flow rate (±2%) and wavelength (±2 units).

Table 6: Robustness results of Carbimazole

Sr. No	Changed condition	% RSD
1)	Flow rate(0.5)	1.55%
2)	Flow rate(0.9)	1.78%
3)	Wavelength (296)	1.63%
4)	Wavelength (300)	1.69%

CONCLUSIONS

A quick, easy, and innovative RP-HPLC technique was effectively created to measure carbimazole.. The proposed method was optimized and validated for the various experimental process. The developed RP-HPLC has shown excellent linearity, precision, accuracy and robustness for Carbimazole. The suggested approach might also be appropriate for quality control, drug analysis in pharmaceutical manufacturing, and other analytical uses.

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