Development and Validation of RP-HPLC Method for the Analysis of Carbimazole In Bulk and Marketed Formulation

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ABSTRACT
A simple and reproducible method was developed for carbimazole by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Carbimazole was separated on C18 column [4.6x250mm, particle size 5μm] at the UV detection of 291nm. Methanol and OPA (0.1%) was used as a mobile phase with various ratios and flow rates, eventually 80:20 v/v Methanol and OPA (0.1%) was being set with the flow rate of 0.7mL/min. The statistical validation parameters such as linearity, accuracy, precision, inter-day and intra-day variation were checked, further the limit of detection and limit of quantification of carbimazole concentrations were found to be within the limits. Recovery and assay studies of carbimazole were within 99 to 102% indicating that the proposed method can be adoptable for quality control analysis of carbimazole.

Keywords: Carbimazole, HPLC, Methanol, Ortho phosphoric acid (OPA).

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INTRODUCTION

Carbimazole, ethyl 3-methyl-2-sulfanylidene-imidazole-1-carboxylate, is an antihyper-thyroid is 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

Several analytical methods have been reported for the analysis of carbimazole such as an indirect bromometric,² potentiometric,³ voltammetric,⁴ chromatographic,⁵ spectro-photometric,⁶ polarographic,⁷ and fluorometric titration.⁸ 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).⁹

MATERIALS AND METHOD

Quantitative HPLC was performed on a high performance liquid chromatography-Younglin HPLC system connected with PDA Detector 2998 and Empower 2 Software. The drug analysis data were acquired and processed using Empower 2 software running under Windows XP on a Pentium PC and Thermohypersil BDS C₁₈ column of dimension [4.6x250mm, particle size 5μm]. In addition an analytical balance (DENVER 0.1mg sensitivity), digital pH meter (Equiptronics pH 5-10), a sonicator (Unichrome associates UCA 701) were used in this study.

Standards and chemicals used:

Pharmaceutical grade Carbimazole was kindly supplied as a gift sample by Macleods Pharmaceutical, Goa. Methanol and OPA were of HPLC grade and Purchased from Jinendra Scientifics, Jalgaon. Water HPLC grade was obtained from a Milli-QRO water purification system. Carbimazole tablets available in the market as Neo-mercazole tablet of Abbott Healthcare, India. in composition of carbimazole (5 mg).
Preparation of mobile phase:
The combination of mobile phase is Methanol: OPA (0.01%) in the ratio of 80:20 v/v and filtered through 0.45μ membrane filter and degassed by sonication.

Preparation of standard stock solution:
Standard stock solution
Weigh accurately 10 mg of carbimazole and taken in a 10 ml standard flask. The volume was made up to 50 ml with the mobile phase and sonicated for 5 min. It consists of 1000 μg/ml of carbamizole and filtered through 0.45 μm membrane filter. Then the dilutions are made.

Quantification of Carbimazole
Twenty tablets were finely powdered and an accurately weighed sample of powdered tablets equivalent to carbimazole (5 mg) were transferred to a 10 ml volumetric flask and dissolved in Mobile Phase. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drug. The contents were made up to the mark with Mobile Phase and filtered through a 0.45μ membrane filter. From the filtrate, dilution was made in a 10 ml volumetric flask to get 5μg/ml of carbimazole. The peak area measurements were done by injecting the sample for three times and the amount of carbimazole was calculated.

Chromatographic conditions
For chromatographic analysis: Thermo Hypersil BDS C\textsubscript{18} column [4.6x250mm, particle size 5μm] was used. The solvent system was a mixture of Methanol: OPA (80:20 v/v). It was filtered under vacuum from 0.45 membrane filter and degassed in ultrasonic bath for 15 min before passing through the instrument. The flow rate was 0.7ml/min. UV detection was carried out at 291 nm.

METHOD VALIDATION
As per the International Conference on Harmonization (ICH) guidelines, the method validation parameters such as specificity, linearity, precision, accuracy, limit of detection/quantization and robustness were optimized.

Linearity
To establish linearity, the stock solutions were prepared (1000 μg/ml) of carbimazole using mobile phase as the solvent, again from the stock solution further dilutions were made to yield solutions in the concentration range of 05-30 μg/ml of carbimazole. 20 μl of each solution was injected and records the chromatogram at 291 nm.
The chromatogram optimized given in (Figure-1) and their system suitability parameters were given in (Table -1), the calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation coefficient was found to be above 0.999.

**Table 1: Linearity**

| Sr. NO. | Parameters          | Values  |
|---------|---------------------|---------|
| 1       | Retention Time      | 4.65    |
| 2       | Area %              | 100.00  |
| 3       | Theoretical Plate   | 3567.3  |
| 4       | Tailing Factor      | 1.9286  |

**Precision:**

Precision study of sample Carbimazole was carried out by estimating corresponding responses 6 times on the same day for the 100% target concentration. The percent relative standard deviation (%RSD) is calculated which is within the acceptable criteria of not more than 2.0.

**Repeatability:**

Repeatability is the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

**Accuracy:**

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments, by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

**Robustness:**

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.6 to 0.8 ml/min, wavelength and volume of the mobile phase.

**LOD and LOQ:**

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.302 $\bar{\sigma}$/S and 1.009$\bar{\sigma}$/S, respectively as per ICH guidelines, where $\bar{\sigma}$ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The results of validation parameters and System suitability parameters are discussed as follows.

**RESULTS AND DISCUSSION:**

**Linearity:**
Table 2: Concentration and average area of Carbimazole

| Sr No | Conc | Avg.area |
|-------|------|----------|
| 1     | 5    | 460.89   |
| 2     | 10   | 927.79   |
| 3     | 15   | 1351.37  |
| 4     | 20   | 1772.08  |
| 5     | 30   | 2671.09  |

Figure 1: Linearity graph of Carbimazole

We described more in detail tabulated format as follows

Table 3: Linearity of carbimazole

| Sr No | Conc | Area-I   | Area-II  | Mean  | SD  | % RSD |
|-------|------|----------|----------|-------|-----|-------|
| 1     | 5    | 458.561  | 463.223  | 460.89| 3.30| 0.72  |
| 2     | 10   | 925.389  | 930.184  | 927.79| 3.39| 0.37  |
| 3     | 15   | 1350.845 | 1351.885 | 1351.37| 0.74| 0.05  |
| 2     | 20   | 1762.456 | 1781.695 | 1772.08| 13.60| 0.77  |
| 3     | 30   | 2654.628 | 2687.552 | 2671.09| 23.28| 0.87  |

Table 4: Precision of Carbimazole

| Sr No | Parameters | % estimated | SD  | RSD |
|-------|------------|-------------|-----|-----|
| 1     | Intra-day  | 99.74       | 2.62| 0.56|
|       |            | 101.5       | 1.87| 0.2 |
|       |            | 100.27      | 2.71| 0.2 |
| 2     | Inter-day  | 99.74       | 0.71| 0.15|
|       |            | 99.39       | 6.68| 0.72|
|       |            | 99.73       | 5.59| 0.42|

Precision:
The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. To study precision, three replicate standard solutions of Carbimazole (200 µgm/ml) was prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be...
which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table 4.

**Repeatability**

Repeatability was ascertained by getting the sample analyzed by different analyst and carrying out analysis for number of times. The results are shown in table no.

### Table 5: Repeatability of Carbimazole

| Sr No. | Conc | Peak Area | Amt Found | % Amt Found |
|--------|------|-----------|-----------|-------------|
| 1      | 5    | 461.666   | 4.25      | 100.86      |
| 2      | 5    | 460.212   | 4.35      | 101.19      |
| 3      | 5    | 461.874   | 4.29      | 100.96      |
| Mean   |      | 4.30      |           | 101.00      |
| SD     |      | 0.05      |           | 0.17        |
| % RSD  |      | 1.17      |           | 0.17        |

### Accuracy:

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, results are shown in Table no.

### Table 6: Accuracy of Carbimazole

| Mg/Band | Amt added | Area | Amt found | Amt Rec | % rec |
|---------|-----------|------|-----------|---------|-------|
| 80%     | 5         | 5    | Mean 8.91 | 3.91    | 97.67 |
|         | 5         | 4    | SD 0.03   | 0.03    | 0.76  |
|         | 5         | 4    | %RSD 0.34 | 0.78    | 0.78  |
| 100%    | 5         | 5    | Mean 10.40| 5.40    | 102.10|
|         | 5         | 5    | SD 0.18   | 0.18    | 0.91  |
|         | 5         | 5    | %RSD 1.69 | 3.26    | 0.89  |
| 120%    | 5         | 6    | Mean 11.63| 5.63    | 98.44 |
|         | 5         | 6    | SD 0.06   | 0.06    | 0.25  |
|         | 5         | 6    | %RSD 0.52 | 1.07    | 0.25  |

### Ruggedness:

The ruggedness was calculated by using two different analysts. An appropriate concentration 30 μg/ml of carbimazole was analyzed and concentration was determined.

### Table 7: Ruggedness of Carbimazole

| Sr No. | Area | II    | III    | Mean   | Amt Found | % Amt Found | SD   | RSD  |
|--------|------|-------|--------|--------|-----------|-------------|------|------|
| Analyst-I | 1347.85 | 1355.48 | 1347.22 | 1350.18 | 15.01     | 100.07      | 4.60 | 0.34 |
| Analyst-II | 1341.58 | 1345.88 | 1344.56 | 1344.01 | 14.94     | 99.60       | 2.20 | 0.16 |
Robustness:
The robustness is evaluated by the analysis of Atorvastatin and Fenofibrate under different experimental conditions such as making small changes in flow rate, λ max and mobile phase concentration.

| Parameters | Carcimazole |
|------------|-------------|
| Flow rate (ml/min) | 0.6 | 0.8 |
| % RSD | 0.23 | 0.23 |
| Wavelength | 290 | 292 |
| % RSD | 0.18 | 0.28 |
| Mobile phase | 79+21 | 81+19 |
| % RSD | 1.29 | 0.83 |

LOD and LOQ:

| Parameters | Measured Value |
|------------|----------------|
| LOD | 0.3027 |
| LOQ | 1.0091 |

Assay for formulation of Carbimazole:
The validated method was applied for the determination of Carbimazole in commercially available Neo-mercazole tablets. The results of the assay (n = 6) undertaken yielded 99.13% (%RSD = 0.37%) of label claim carbimazole. The mean retention time of carbimazole was 4.65 min. The results of the assay indicate that the method is selective for the analysis of carbimazole without interference from the excipients used to formulate and produce these tablets.

Table 10: Analysis of Formulation

| Drugs | Labelled amount | Amount | Amount found for assay (µg/mL) (mg) | % |
|-------|----------------|--------|------------------------------------|---|
| Carbimazole | 5 | 4.992+0.371 | 99.90 |
CONCLUSION:
A simple isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed for determination of carbimazole in bulk and pharmaceutical dosage form. Validation of the method was performed for precision, accuracy, linearity, ruggedness, robustness and sensitivity to conform to the ICH guidelines for validation of an analytical method. This method may be suitable for analysis in Quality control units of Pharmaceutical industries.

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