CHAPTER VII

DEVELOPMENT OF ANALYTICAL METHODS FOR RABEPRAZOLE
7.1 NEW ANALYTICAL METHODS FOR THE DETERMINATION OF RABEPTRAZOLE (RBZ)

The structure of Rabeprazole is given below

Rabeprazole (RBZ) is a synthetic anti ulcer agent. Chemically it is 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl] -1H–benzimidazole. RBZ is not official in any pharmacopoeia. Literature survey reveals the presence of some HPLC and spectrophotometric methods for the estimation of RBZ. But the problem of assay of RBZ in pharmaceutical formulations has been the subject of limited number of investigations.

Very few analytical methods have been reported for the estimation of RBZ using visible spectrophotometry. The analytically important functional groups of RBZ have not been exploited for designing sensitive, accurate and flexible visible spectrophotometric methods for the determination of RBZ in pharmaceutical formulations. A total of three visible spectrophotometric methods and have been established for RBZ in this chapter. The materials and methods used and the principle involved in these methods is also discussed in this chapter. These principles are also extended for the pharmaceutical formulations containing RBZ.
This chapter deals with the details of the materials and instruments used for the present investigations. A summary of different reagents used for different methods are explained below.

7.2.1 INSTRUMENTATION:

All spectral and absorbance measurements were made on Shimadzu UV- 150 – 02 (double beam) and Elico UV- VIS SL- 150 spectrophotometers with 1 cm matched quartz cells. pH measurements were made on Systronics digital pH meter model 335. HPLC methods were developed on Shimadzu- LC-10 ATVP.

7.2.2 MATERIALS:

All the chemicals and reagents used were of analytical or pharmacopoeial grade and all solutions were freshly prepared in double distilled water. A brief account of the materials employed in the present investigations is furnished here.

Method M\textsubscript{37}:

Folin- Ciocalteu reagent (Loba 2 N)

Sodium Carbonate (Loba 10 % w/v in water)

0.1N HCl

Method M\textsubscript{38}:

Ferric Chloride solution (Loba 0.003 M): prepared by dissolving 1.6 gm of FeCl\textsubscript{3} in 100ml of water from this 1 ml was taken and diluted to 250ml with distilled water.

2,2'-Bipyridine (Qualigens 0.01M): prepared by dissolving 198 mg in 100 ml of water.

Ortho Phosphoric Acid (Qualigens 0.02M): prepared by dissolving 0.2 ml of Orthophosphoric Acid in 100 ml of water.

0.2N HCl (Qualigens)
Method M39:

Vanillin (Loba 0.5% w/v)
Methanol (Qualigens)
Conc. sulphuric acid (Qualigens)

7.3 EXPERIMENTAL

Method M39:

Aliquots of solution (0.5-2.5 ml, 200 µg/ml) were transferred to a series of 20 ml graduated tubes. Then 2.5 ml of FC reagent and 9 ml of sodium carbonate solution were added simultaneously and kept aside for 10 min at room temperature. The solution was made up to volume with distilled water. The absorbance was measured at 780 nm against reagent blank. The colored species was stable for 3 hrs. The amount of drug in the sample was computed from Beer’s Plot. All the spectral characteristics are given in Table 7.1

For Pharmaceutical Preparations

Tablets

Four types of commercially available tablets were taken for analysis. Twenty tablets were accurately weighed and powdered separately. An accurately weighed quantity of tablet powder equivalent to 100mg of RBZ was taken and the sample solution was made in the same manner described above and analyzed by the procedure described above. The results are given in Table 7.2.

Chemistry of the colored species:

The color formation by FC reagent with RBZ under alkaline conditions may be explained in the following manner based on the analogy with the reports of the earlier workers, the mixed acids in the FC reagent involve the following chemical species.
RBZ probably effects a reduction of 1,2 or 3 oxygen atoms of tungstate and/or molybdate in FC reagent thereby producing one or more of the possible reduced species, which have a characteristic intense blue color.

**Method M_{38}:**

To a series of 10 ml graduated tubes, aliquots of RBZ ranging from 1 to 5.0 ml (1ml = 200 µg), 1.5 ml of Ferric Chloride (0.003 M) and 2.5 ml of 2,2'-Bipyridine (0.01 M) were added successively to each tube. The tubes were then heated on a boiling water bath for 15 min, cooled to room temperature and 2 ml of O- phosphoric acid (0.02 M) was added to each tube and the total volume was brought to 10 ml with distilled water. The absorbance of the red colored species was measured at 530 nm against reagent blank within 60 min. the amount of RBZ was computed from the calibration graph. All the spectral characteristics are given in Table 7.1

**For Pharmaceutical Preparations**

**Tablets**

The method is the same that is discussed in Method M_{37}. The results are given in Table 7.2

**Chemistry of the colored species:**

The above method is based on the oxidation of RBZ by Fe (III) to produce Fe (II), which subsequently reacts with 2,2'-Bipyridine to form a red colored ferroin complex. O- phosphoric acid is used in this method to combine with residual iron to form Fe (PO₄)₂⁻³ and to prevent photochemical reduction, thus facilitating the determination.
Method M₃₉:

Into a series of 10ml graduated tubes, 0.2-2 ml of methanolic RBZ solution (500 μg/ml) was transferred and the volume was adjusted to 3 ml with methanol. To each of the tubes 2 ml of Vanillin and 2 ml of conc. H₂SO₄ were successively added while cooling under a tap with constant shaking and the volume was made up to the mark with methanol. They were measured within 20 min at 420 nm against a reagent blank prepared under identical conditions. The exact amount of RBZ present was calculated from the calibration graph. All the spectral characteristics are given in Table 7.1.

For Pharmaceutical Preparations

Tablets

The method is the same that is discussed in Method M₃₇. The results are given in Table 7.2.

Chemistry of the colored species:

The method is based on the principle of Schiff’s base formation of RBZ with Vanillin giving a red colored chromogen.
FIG 7.1

ABSORPTION SPECTRA OF RBZ IN F.C. REAGENT $M_{37}$

BEER'S LAW PLOT OF RBZ IN F.C. REAGENT $M_{37}$
FIG 7.2

ABSORPTION SPECTRA OF RBZ IN FERRIC CHLORIDE/2,2'-BIPYRIDINE REAGENT M₃₈

BEER'S LAW PLOT OF RBZ IN FERRIC CHLORIDE/2,2' BIPYRIDINE REAGENT M₃₈
ABSORPTION SPECTRA OF RBZ IN VANILLIN REAGENT M₃⁹

WAVELENGTH (nm)

BEER’S LAW PLOT OF RBZ IN VANILLIN REAGENT M₃⁹

CONCENTRATION (mcg/ml)

ABSORBANCE
FIG 7.1a
RINGBOM PLOT OF RBZ IN F.C.REAGENT M37

% Transmission

0 20 40 60 80 100
0 1 2 3 4
Log Concentration In mcg

FIG 7.2a
RINGBOM PLOT OF RBZ IN FERRIC CHLORIDE/2,2',BIPYRIDINE REAGENT M38

% Transmission

0 20 40 60 80 100
0 1 2 3 4
Log Concentration In mcg
FIG 7.3a

RINGBOM PLOT OF RBZ IN VANILLIN REAGENT M_{39}

% Transmission

Log Concentration In mcg
## Table 7.1

Optical Characteristics, Precision and Accuracy of the Proposed Methods

<table>
<thead>
<tr>
<th>DATA</th>
<th>Proposed Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M_{35}$</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>780</td>
</tr>
<tr>
<td>Beer's law limits (ug/ml)</td>
<td>10-50</td>
</tr>
<tr>
<td>Molar absorptivity (lit.mole$^{-1}$.cm$^{-1}$)</td>
<td>5.685x10$^3$</td>
</tr>
<tr>
<td>Sandell's Sensitivity (ug/cm$^2$/0.01 abs.unit)</td>
<td>0.0606</td>
</tr>
<tr>
<td>Regression Equation ($Y=a+b+c$) slope (b)</td>
<td>1.07x10$^{-3}$</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>5.0x10$^{-3}$</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
</tr>
<tr>
<td>% Relative Standard Deviation</td>
<td>0.29</td>
</tr>
<tr>
<td>% Range of error 0.05 level</td>
<td>±0.32</td>
</tr>
<tr>
<td>0.01 level</td>
<td>±0.51</td>
</tr>
</tbody>
</table>
**TABLE 7.2**  
ASSAY AND RECOVERY OF THE DRUG IN DOSAGE FORMS

<table>
<thead>
<tr>
<th>DATA</th>
<th>LABELLED AMOUNT</th>
<th>Amount found (mg)*</th>
<th>Found by reference method**</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$M_{17}$</td>
<td>$M_{28}$</td>
<td>$M_{39}$</td>
</tr>
<tr>
<td>Tablet 1</td>
<td>20</td>
<td>20±0.30</td>
<td>19.9±0.21</td>
<td>19.8±0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 0.70</td>
<td>t = 0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.92</td>
<td>F = 2.12</td>
<td></td>
</tr>
<tr>
<td>Tablet 2</td>
<td>20</td>
<td>19.9±0.23</td>
<td>20.1±0.20</td>
<td>19.9±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.23</td>
<td>t = 1.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 3.29</td>
<td>F = 2.59</td>
<td></td>
</tr>
<tr>
<td>Tablet 3</td>
<td>40</td>
<td>39.7±0.50</td>
<td>39.8±0.75</td>
<td>40.5±0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 0.63</td>
<td>t = 1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 3.1</td>
<td>F = 2.73</td>
<td></td>
</tr>
<tr>
<td>Tablet 4</td>
<td>40</td>
<td>40.8±0.86</td>
<td>40.2±0.12</td>
<td>39.4±0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 0.70</td>
<td>t = 0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.50</td>
<td>F = 1.94</td>
<td></td>
</tr>
</tbody>
</table>

*Average±standard deviation of six determinations the t-and f - values refer to comparison of the proposed methods with the reference method. Theoretical values at 95% confidence limits.

**U.V method (λmax 292 nm in 0.1 N HCl)

***Recovery of 10 mg added to the pharmaceutical formulations (Average of 3 determinations).
7.4. CONCLUSION

Since Rabeprazole is a relatively new drug and the analytical methods available for its assay are very limited, it is worthwhile to develop some methods for its assay. As part of the present investigations, three methods have been developed for the purpose of assay of RBZ. It can be seen from the results presented above that the proposed methods have reasonable sensitivity. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in them.

The order of sensitivity among the three proposed methods is $M_{37} > M_{38} > M_{39}$. Beer's law limits ($\mu g/ml$) of the proposed methods are better than many of the reported spectrophotometric methods. All the proposed methods are simple sensitive and reliable with good precision and accuracy. These methods can be used for the routine determination of RBZ in bulk samples and in pharmaceutical formulations.