A Novel Stability Indicating Liquid Chromatographic Assay for the Estimation of Rimonabant Hydrochloride in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A stability indicating HPLC assay method has been developed and validated for the estimation of rimonabant hydrochloride in bulk and pharmaceutical dosage forms. A RP-HPLC isocratic separation was achieved on C18 column (250X4.6 mm i.d., 5 µm) utilizing a mobile phase comprising of methanol and water in the ratio of 90:10 (v/v) and the eluents from the column were monitored using a variable wavelength detector at 280 nm. The stress testing of rimonabant hydrochloride was carried out under acidic, alkaline hydrolysis, oxidation and thermal degradation (dry heat) conditions and rimonabant hydrochloride was well resolved from its degradation products with good resolution. The proposed method has permitted the quantification of rimonabant hydrochloride in the linearity range of 0.2-10 µg/ml and the flow rate was maintained at 1 ml/min. The column was maintained at ambient temperature and the total run time was 10 min. The retention times of rimonabant hydrochloride and saquinavir mesylate were found to be 5.760 min and 4.657 min, respectively and the limit of detection and limit of quantification were found to be 0.0113 µg/ml and 0.034 µg/ml, respectively. The percentage recovery was found to be in the range of 100 to 100.22 and the % RSD of intra-day and inter-day precision was found to be 0.572 and 0.549, respectively. The percentage amount of marketed brand of rimonabant hydrochloride was found to be 100.4. The method was found to be suitable for routine quality control analysis of rimonabant hydrochloride in bulk drug and formulation as well as for stability-indicating studies. The method was validated as per ICH (International Conference on Harmonization) guidelines.

KEYWORDS: Rimonabant hydrochloride, Isocratic, RP-HPLC, Stability-indicating assay, Forced degradation, Validation, ICH guidelines.
INTRODUCTION

The endocannabinoid system controls food intake via both central and peripheral mechanisms, and it may also stimulate lipogenesis and fat accumulation (1). Discovery of the cannabinoid receptors has led to the development of rimonabant, a cannabinoid-1 (CB₁) antagonist (2). Rimonabant hydrochloride (RMT) (Acomplia), specific inhibitor of the endocannabinoid system (3) and it is the first of a new class of selective cannabinoid receptor-1 blockers. It is a peripherally acting endocannabinoid (CB₁) antagonist (4) that offers novel therapeutic approach to appetite control, weight reduction, smoking cessation and it was an investigational agent for the management of cardiovascular risk factors (5). It reduces the over activity of the endo cannabinoid system, improving lipid and glucose metabolism and regulating food intake and energy balance (6). Cannabinoid drugs exert their effects primarily through activation of cannabinoid CB₁ and CB₂ receptors. Both CB₁ and CB₂ receptors have been implicated in a number of cardiovascular processes, including vasodilation, cardiac protection, modulation of the baroreceptor reflex in the control of systolic blood pressure, and inhibition of endothelial inflammation and the progress of atherosclerosis in a murine model (7). These effects are mainly mediated through central and peripheral nervous system CB₁ receptors, vascular CB₁ receptors and immune cell CB₂ receptors. Recent data on potential anti-obesity drugs currently undergoing phase III trials, such as RMT and topiramate, produce greater and more prolonged weight loss (8). It is an anti-obesity drug and an aid to smoking cessation treatment of alcohol dependency cannabinoid CB₁ antagonist (9). Chemically, it is 5-(4-Chloro phenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide mono hydrochloride (figure 1), with empirical formula of C₂₂H₂₁Cl₂N₄O.HCl and the molecular weight of 500.29. Literature review reveals that few analytical methods were evoked for the estimation of RMT in various matrices such as human plasma and hair (11-14), in rat and monkey plasma (15, 16), in fat cells (17), bioanalytical application with RMT (18), estimation of RMT with orlistat (19) and with four major species in slimy foods (20), estimation synthetic drugs in herbal slimming formula (21), pharmacokinetic studies of RMT in rats (22), stability indicating assay of RMT by HPLC (23, 24), HPTLC (25) and HPLC estimation (26), spectrophotometric estimation (27) of RMT were reported.

The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date (28). Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life (29, 30).
In the absence of official RMT monograph in the pharmacopoeias, including the European Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia, development of such a method may prove all the more useful. In this report a simple, rapid, accurate and economic RP-HPLC stability indicating assay for the estimation of RMT in bulk and pharmaceutical dosage forms other than the methods existing in the literature are presented as per ICH (31) guidelines.

**MATERIALS AND METHODS**

Pure standard of RMT (purity 99.89%) was obtained as gift sample from Dr. Reddy’s Laboratories Pvt. Ltd, Hyderabad, India. HPLC grade water and methanol (Qualigens), Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (S.D fine chemicals, Mumbai, India), Hot air oven (Sky lab Instruments & Engineering Pvt. Ltd), Electronic analytical balance (DONA), Micro pipette (In labs, 10-100 µl) and Ribafit tablets (20 mg, Torrent pharmaceuticals) were employed in the study. Working environment was maintained in between 18-22°C. The chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC studies.

**INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS**

The HPLC system consisted of an Agilent LC 1200 HPLC pump, solvent degasser, a variable wavelength detector with deuterium lamp, a rheodyne injector and EZ Chrom Elite software. Isocratic elution of mobile phase comprising of methanol and water in the ratio of 90:10 (v/v) with flow rate of 1 ml/min was performed on C\textsubscript{18} column (250 x 4.6 mm i.d, 5 µm). The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 µl. Prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluents were monitored at 280 nm and data were acquired, stored and analyzed with software EZ Chrom Elite.

**PREPARATION OF SAMPLE AND STANDARD SOLUTIONS**

**Standard preparation**

A standard stock solution of 1mg/ml of RMT was prepared in a volumetric flask. Working solutions were prepared by diluting the stock solution with mobile phase to contain 0.2-10 µg/ml of concentration.

**Sample preparation**

Twenty tablets were weighed to obtain the average tablet weight and were powdered by trituration. A sample of the powder claimed to contain 20 mg of the active ingredient was mixed with known amount of methanol and the mixture was allowed to stand for 30 min with intermittent sonication to ensure complete solubility of the drug; then it was filtered through a 0.45 µm membrane filter, followed by adding methanol to obtain a stock solution of 0.1 mg/ml (100 µg/ml). An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up to a sufficient volume with the mobile phase to give an expected concentration of 10 µg/ml. All determinations were conducted in triplicate.

**FORCED DEGRADATION OF ACTIVE PHARMACEUTICAL INGREDIENT AND TABLETS OF RMT**

In order to establish whether the analytical method and the assay indicated stability, the
tablets and pure active pharmaceutical ingredient of RMT were stressed under various conditions. As RMT was freely soluble and stable in methanol, so methanol was used as solvent in all forced degradation studies. All the solutions prepared for use in forced degradation studies were prepared by dissolving API or drug product in a small volume of methanol and later diluted with aqueous hydrochloric acid, aqueous sodium hydroxide and aqueous hydrogen peroxide. Post degradation the solutions were diluted with mobile phase. To study the effect of thermal stress, Ribafit tablets and API powder were exposed to dry heat in an oven at 70°C for 48 h. In order to evaluate the stability of RMT, the drug at a concentration of 100 µg/ml was prepared and subjected to forced degradation for the detection of RMT and its degraded forms. Post degradation these solutions were diluted with mobile phase to obtain the starting concentration of 10µg/ml.

**Hydrolysis (acid and alkali)**

Initially for hydrolytic degradation, RMT was dissolved in known amount of methanol and diluted with 0.1N HCl or 0.1N NaOH to obtain a concentration of 100 µg/ml. After completion of the degradation process, the solutions were neutralized and dilution with mobile phase to achieve a concentration of 10 µg/ml. The solutions for hydrolysis were prepared in methanol and 0.1 N HCl and 0.1N NaOH (60:40 v/v). The solutions prepared in acid and alkali was injected to the chromatographic system at time 0 (immediately after preparing the solution) and after reflux at 60°C about 12 h. The respective chromatograms were recorded for the study of extent of degradation.

**Peroxide degradation**

The solutions for peroxide degradation were prepared in methanol and 3% hydrogen peroxide (60:40 v/v). The solution prepared was diluted and injected into chromatographic system at time 0 (immediately after preparing the solution) and after reflux at 60°C about 8 h. The respective chromatograms were recorded for the study of extent of degradation.

**Thermal degradation**

The bulk and marketed formulation were subjected to heat treatment for the assessment of thermal degradation and subsequently exposed to dry heat in an oven at 70°C for approximately two days (48 h). The tablets and bulk drug were removed from the oven and the tablets were powdered. The amount which was equivalent to 20 mg of active ingredient and bulk drug were prepared for analysis by the proposed method. The respective chromatograms were recorded for the study of extent of degradation.

**RESULTS AND DISCUSSION**

**OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS (METHOD DEVELOPMENT)**

A simple stability-indicating RP-HPLC method has been developed for determination of RMT in the presence of its degradation products. The method was optimized to provide a good separation of the components (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor < 2) in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules. The chromatographic separation was
achieved using an RP C\textsubscript{18} column because it was suitable to resolve the degradation products from RMT with adequate resolution and gave symmetrical peak shapes. RMT was easily soluble in methanol and it is a well known solvent for various pharmaceutical compounds. Our experiments and data reported in the literature showed that both the methanol and acetonitrile could be used as organic modifiers in the mobile phase. But the tests using methanol was done with water (HPLC grade) as mobile phase eluted the RMT with a significant shorter retention time of 5.760 min. We selected methanol and water in the ratio of 90:10 (v/v) as a mobile phase. The method has many advantages, e.g., simplicity, isocratic conditions, and absence of buffers in the mobile phase that could damage the chromatographic column and equipment. Under these conditions, the retention time of RMT was 5.760 min, with a good peak (peak symmetry), and the chromatographic analytical time was less than 10 min.

Method Validation

The method was validated as per the ICH guidelines for different validation parameters. The method was validated for its specificity, linearity, accuracy, precision, selectivity, Limit of detection (LOD) and Limit of quantification (LOQ).

Specificity

The results from the stress studies indicated that the method was highly specific to RMT. The degradation products were completely distinguishable from the parent compound. Peak purity data (based on forced degradation data) of RMT, every degradation sample showed that the peaks were homogeneous and there were no co-eluting peaks indicating that the method was stability indicating and specific.

Linearity

The standard plot for the RMT was constructed by plotting the ratio of the peak area of RMT to the peak area of the internal standard (Y) against concentration (X). It was found to be linear with a correlation coefficient (r\textsuperscript{2}) of 0.9996, the corresponding linear regression equation being y= 3.5451x+1.3975. In the linear range of 0.2-10µg/ml, the coefficients of variation (CV) based on the peak area ratios for six replicate injections, were found to be between 0.03 to 0.53.

Statistical Data of Calibration Curves

The regression characteristics, such as standard deviation of slope (S\textsubscript{b}, 0.214), the % relative standard deviation (%RSD) of the slope (0.634), the standard deviation of intercept (S\textsubscript{a}, 9.07X \texttimes 10\textsuperscript{-3}), regression equation (3.5451x+1.3975) and correlation coefficient (r\textsuperscript{2}, 0.9996) were calculated.

Precision

The precision of the method was determined by repeatability and intermediate precision studies. Repeatability was evaluated by performing six determinations (n=6) at the same concentration, during the same day, under the same experimental conditions. Intermediate precision was evaluated by comparing the assays on 3 different days using different analysts. The result revealed the precision with %RSD for intra-day and inter-day of 0.572, 0.549, respectively. The results are shown in table 1.
**Table 1:** Intra-day and inter-day precision

<table>
<thead>
<tr>
<th>RMBH</th>
<th>% Recovery</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.4</td>
<td>100.2</td>
<td>100.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100.5</td>
<td>100.9</td>
<td>101.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100.6</td>
<td>99.9</td>
<td>100.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>99.9</td>
<td>99.7</td>
<td>100.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100.2</td>
<td>99.5</td>
<td>101.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>99.1</td>
<td>100.4</td>
<td>99.67</td>
<td></td>
</tr>
<tr>
<td>Intra-day&lt;sup&gt;b&lt;/sup&gt; (n=6)</td>
<td>100.1±0.556</td>
<td>100.1±0.509</td>
<td>100.51±0.572</td>
<td></td>
</tr>
<tr>
<td>Inter-day&lt;sup&gt;b&lt;/sup&gt; (n=18)</td>
<td>100.24±0.549</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Different analysts, <sup>b</sup> Mean ± %RSD

**ACCURACY**

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre analyzed sample and contents were re-analyzed by the proposed method. Accuracy was evaluated at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of RMT standard to a sample of known concentration and the % recovery of RMT with %RSD was calculated. The mean % recoveries are in between 100 to 100.22 and are shown in table 2. There was a high % recovery (100.22) of RMT indicating that the proposed method for the determination of RMT in tablet dosage forms was highly accurate.

**Table 2:** Accuracy of the method

<table>
<thead>
<tr>
<th>RBT</th>
<th>80% level</th>
<th>100% level</th>
<th>120% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100.4</td>
<td>100.5</td>
<td>100.03</td>
</tr>
<tr>
<td>2</td>
<td>99.97</td>
<td>100.2</td>
<td>99.52</td>
</tr>
<tr>
<td>3</td>
<td>100.3</td>
<td>99.71</td>
<td>100.2</td>
</tr>
<tr>
<td>Mean (n=3)</td>
<td>100.22</td>
<td>100.13</td>
<td>100.00</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.22</td>
<td>0.39</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**SYSTEM SUITABILITY**

To determine reproducibility of the method, a suitable test was employed to establish parameters such as tailing factor, theoretical plates, resolution, asymmetry factor, and asymmetry (10%), limit of detection and limit of quantification. The values are shown in table 3.
Table 3: System Suitability Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>5.760</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>7584</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.833</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>0.2-10</td>
</tr>
<tr>
<td>Limit of detection (µg/ml)</td>
<td>0.0113</td>
</tr>
<tr>
<td>Limit of quantification(µg/ml)</td>
<td>0.0345</td>
</tr>
<tr>
<td>Resolution</td>
<td>4.448</td>
</tr>
<tr>
<td>Asymmetry factor</td>
<td>1.198</td>
</tr>
<tr>
<td>Asymmetry (10%)</td>
<td>1.1835</td>
</tr>
</tbody>
</table>

**Selectivity**

The results of stress testing studies indicated a high degree of selectivity of this method for RMT. The degradation of RMT was found to be similar for both the tablets and API powder.

**LOD and LOQ**

Limits of Detection (LOD) and Quantification (LOQ), were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/S and LOQ =10σ/S as defined by ICH.

**Stress Degradation Studies**

To determine whether the method was stability indicating, RMT tablets and active pharmaceutical ingredient (API) powder were stressed under different conditions to promote degradation. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 requires the development and validation of stability indicating potency assays. The degradation products were well separated from parent compound with greater resolution. The drug demonstrated degradation under all conditions but there was almost no degradation in dry heat. RMT was degraded in both acidic and alkali conditions when it was exposed to 0.1N HCl and 0.1N NaOH. In acidic degradation of RMT at 0 h (immediately after preparing the solution) 5 degradation products with retention times of (3.013, 3.173, 3.560, 3.620, and 4.073min) were observed, and RMT eluted with the retention time of 5.817 min with percentage of degradation 9.94. After reflux at 60°C for about 12h RMT demonstrated 3 degradation products with retention times of (3.000, 3.460 and 3.727min) and RMT eluted with the retention time of 5.830 min with percentage of degradation of 15.25. In alkali degradation of RMT at 0 h (immediately after preparing the solution) demonstrated 3 degradation products with the retention times of (3.017, 3.190 and 3.560 min) and RMT eluted at the retention time of 5.810 min with percent of degradation 14.29. After reflux at 60°C for about 12h, RMT demonstrated seven degradation products with retention times of (1.150, 1.313, 1.530, 1.667, 3.003, 3.167 and 3.613min) and RMT eluted with the retention time of 5.820 min with percentage of degradation of 53.52. In hydrogen peroxide environment RMT, demonstrated three degradation products at 0 h with percentage degradation of 7.43 and after reflux at 60°C for about 8 h the percentage of degradation was 21.79. There was considerable degradation observed in dry heat for about 48 h, at
70°C in the oven, with the API and tablets demonstrating percent degradation of 3.08 and 3.46, respectively. The percentage degradation and the summary of forced degradation data are shown in the table 4 and 5 while the chromatograms of degraded RMT in different stress conditions are shown in figures 2 (a-h).

**Table 4:** % of Degradation

<table>
<thead>
<tr>
<th>Degradation mechanism</th>
<th>Degradation condition</th>
<th>Assay (mg/tab)</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un degraded</td>
<td>-</td>
<td>20.08</td>
<td>-</td>
</tr>
<tr>
<td>Acid hydrolysis</td>
<td>0.1 N HCl, at 0 h, 12 h</td>
<td>18.08, 17.01</td>
<td>9.94%, 15.25%</td>
</tr>
<tr>
<td>Alkali hydrolysis</td>
<td>0.1 N NaOH, at 0 h, 12 h</td>
<td>17.21, 9.33</td>
<td>14.29%, 53.52%</td>
</tr>
<tr>
<td>H₂O₂ oxidation</td>
<td>3% H₂O₂ at 0 h, 8 h</td>
<td>18.58, 15.70</td>
<td>7.43%, 21.79%</td>
</tr>
<tr>
<td>Thermal degradation of API</td>
<td>Dry heat, 48 h in oven</td>
<td>19.46</td>
<td>3.08%</td>
</tr>
<tr>
<td>Thermal degradation of tablet</td>
<td>Dry heat 48 h in oven</td>
<td>19.38</td>
<td>3.46%</td>
</tr>
</tbody>
</table>

**Table 5:** Forced Degradation of RMT

<table>
<thead>
<tr>
<th>S.No</th>
<th>Exposure condition</th>
<th>Degradation Products (Retention times)</th>
<th>Drug remained</th>
<th>% recovery</th>
<th>Figure no</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1N HCl at 0 h&amp; after reflux at 60°C about 12h</td>
<td>3 (3.00, 3.460, 3.727)</td>
<td>90.06 at 0 h</td>
<td>90.06</td>
<td>2(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>84.75 at 12 h</td>
<td>84.75</td>
<td>2(b)</td>
</tr>
<tr>
<td>2</td>
<td>0.1 N NaOH at 0 h&amp; reflux at 60°C about 12h</td>
<td>7 (1.150, 1.313, 1.530, 1.667, 3.003, 3.167, 3.613)</td>
<td>85.71 at 0 h</td>
<td>85.71</td>
<td>2(c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46.48 at 12 h</td>
<td>46.48</td>
<td>2(d)</td>
</tr>
<tr>
<td>3</td>
<td>H₂O₂ (3% v/v), at 0 h &amp; after reflux at 60°C about 8h</td>
<td>2 (2.833, 3.293)</td>
<td>92.57 at 0 h</td>
<td>92.57</td>
<td>2(e)</td>
</tr>
<tr>
<td></td>
<td>Thermal treatment of API at 70°C about 48h in an oven</td>
<td>3 (2.737, 3.037, 3.167)</td>
<td>96.92 at 48 h</td>
<td>96.92</td>
<td>2(g)</td>
</tr>
<tr>
<td>5</td>
<td>Thermal treatment of tablet at 70°C about 48h in an oven</td>
<td>3 (2.760, 3.247, 6.503)</td>
<td>96.53 at 48 h</td>
<td>96.53</td>
<td>2(h)</td>
</tr>
</tbody>
</table>
**Figure 2(a):** A chromatogram of RMT degraded in 0.1 N HCl at 0 h

![Figure 2(a)](image)

**Figure 2(b):** A chromatogram of RMT degraded in 0.1 N HCl after reflux at 60°C about 12 h

![Figure 2(b)](image)

**Figure 2(c):** A chromatogram of RMT degraded in 0.1 N NaOH at 0 h

![Figure 2(c)](image)
Figure 2 (d): A chromatogram of RMT degraded in 0.1 N NaOH after reflux at 60 °C about 12 h

![Chromatogram of RMT degraded in 0.1 N NaOH after reflux at 60 °C about 12 h](image)

Figure 2 (e): A chromatogram of RMT degraded in 3% hydrogen peroxide at 0 h

![Chromatogram of RMT degraded in 3% hydrogen peroxide at 0 h](image)

Figure 2 (f): A chromatogram of RMT degraded in 3% hydrogen peroxide at 60 °C about 8h

![Chromatogram of RMT degraded in 3% hydrogen peroxide at 60 °C about 8h](image)
**Figure 2 (g):** A chromatogram of API of RMT degraded in an oven at 70°C about 48 h

![Chromatogram of API of RMT degraded in an oven at 70°C about 48 h](image)

**Figure 2 (h):** A chromatogram of tablet of RMT degraded in an oven at 70°C about 48 h

![Chromatogram of tablet of RMT degraded in an oven at 70°C about 48 h](image)
**Solution Stability**

To demonstrate the stability of standard solutions and tablet sample solutions during analysis, they were analyzed over a period of 48 h at room temperature. The results demonstrated that for both solutions the retention time and peak area of RMT remained almost unchanged with no significant degradation observed during this period, suggesting that both solutions were stable for at least 48 h, which was sufficient for the whole analytical process.

**Assay of the Method**

The assay of commercial tablets was established with present chromatographic conditions developed and found to be more accurate and reliable. The average drug content was found to be 100.4% of the labeled claim. No interference peaks were found in the chromatogram, indicating the estimation of drug free from interference of excipients. The results are shown in *table 4*. The chromatograms of bulk and marketed formulations along with internal standards are shown in *figures 3 and 4*.

**Table 6: Assay of the Method**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Labeled claim (mg)</th>
<th>Mean amount found*±S.D</th>
<th>% purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIBAFIT</td>
<td>20</td>
<td>20.08±0.22</td>
<td>100.4</td>
</tr>
</tbody>
</table>

*Mean of three values
**CONCLUSION**

A validated stability indicating HPLC method has been developed for the determination of RMT in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective, specific and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the tablet dosage forms. The method is suitable for use for the routine analysis of RMT in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and or GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals. This approach has demonstrated its strength in terms of sensitivity, rapidity, cost effectiveness when compared with other methods cited in the literature.
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