VALIDATED RP-HPLC METHOD FOR ESTIMATION OF RABEPRAZOLE AND ACECLOFENAC FROM HUMAN PLASMA

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ABSTRACT

Objective: Aim of present study was to develop a simple, fast and precise RP-HPLC method for estimation of Rabeprazole and Aceclofenac from human plasma.

Methods: Chromatographic separation of the said drugs was achieved on a HiQ SiL C8 column (250 mm x 4.6 mm i.d, 5 µ) with a mobile phase comprising of 0.05 M Potassium diphosphate: Acetonitrile: Methanol in the ratio 4:4:3 (v/v/v) at a flow rate of 1 ml/min. UV detection was carried at 215 nm. Preparation for spiked plasma samples mobile phase to get desired concentrations. Appropriate dilutions were prepared from these stock solutions using appropriate dilutions were prepared from these stock solutions using.

Result: The described method was found to be linear over a concentration range of 0.5-7.5 µg/ml. Separation was achieved within 8 mins. The recovery was calculated by standard addition method. Average recovery and precision was found to be in the limit given by USFDA guidelines.

Conclusion: Method was validated as per US-FDA guidelines for selectivity, precision, recovery, accuracy and stability and robustness. Thus proposed method was found to be simple, accurate, precise and rapid for the estimation of Rabeprazole and Aceclofenac from human plasma. It can be used for estimation of said drugs in human plasma, pharmacokinetic and interaction studies.

Keywords: RP-HPLC, Aceclofenac, Rabeprazole, Human plasma.

INTRODUCTION

Aceclofenac (Molecular formula: C14H12Cl2NO4; Molecular weight: 354.19), 2-[2-2-(2,6- dichlorophenyl)aminophenyl]acetamido]oxyacetic acid is an effective anti-inflammatory drug. It has been widely used for the treatment of arthritis. It works by blocking the action of cyclooxygenases. Cyclooxygenases are involved in the production of various prostaglandins. It is official in Indian Pharmacopoeia [1].

Rabeprazole (Molecular formula: C16H17N3O2S; Molecular weight: 381.4 g/mol) is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in the treatment of Ulcerative gastrosesophageal reflux disease. It is chemically 2H-[1,4]benzoxazepine-3(4H)-yl]-3-methyl-2-pyridyl|[sulfimyl]-H-benzimidazole. Rabeprazole belongs to a class of anti secretory compounds that suppress gastric acid secretion by inhibiting the gastric H+, K+ ATPase at the secretory surface of the gastric parietal cell. Because this enzyme is regarded as the acid pump within the parietal cell, rabeprazole has been characterised as a gastric proton pump inhibitor. It is official in Indian Pharmacopoeia [2].

A thorough literature survey has indicated several HPLC[3-9], HPTLC[10] and UV[11-13] methods for estimation of Aceclofenac and Rabeprazole as a single drug or in combinations with other drugs in pharmaceutical dosage forms and biological fluids. But there is no single HPLC method for simultaneous estimation of said drugs in human plasma. The present work describes a simple and rapid isocratic RP-HPLC method for simultaneous estimation of Aceclofenac and Rabeprazole in bulk drug and in human plasma. The developed method was validated as per US FDA guidelines [14].

MATERIALS AND METHODS

Reagents and Chemicals

Pure drug samples of Aceclofenac and Rabeprazole were obtained as gift samples from Aristo Pharmaceuticals, Mumbai and Wockhardt Pvt. Ltd, Aurangabad respectively with certificate of analysis. Methanol and Acetonitrile (HPLC grade) were procured from Thomas baker (Chemicals) Pvt. Ltd, Mumbai. Potassium Dihydrogen Phosphate (AR grade) was purchased from SISCO Research Lab. Pvt Ltd, Mumbai. HPLC grade water was used throughout the study. Pooled human plasma was purchased from Sahyadri Hospital blood bank, Pune. Calibrated glassware’s were used for the study.

Instrumentation

Chromatographic separation was performed with JASCO HPLC 2000 Series having PU-2080 HPLC isocratic pump, a JASCO UV- 2075 variable wavelength detector and Rhedynie injector (20µl). Borwin software version 1.5 was used for data analysis.

Preparation of stock solution

10 mg of each drug was taken in a separate 10 ml of volumetric flask and was dissolved in sufficient quantity of mobile phase. Solutions were diluted up to the mark with mobile phase to get stock solutions of strength 1000 µg/ml of each drug. For each validation parameter, appropriate dilutions were prepared from these stock solutions using mobile phase to get desired concentrations.

Preparation for spiked plasma samples

Human Plasma was distributed in small eppendrof tubes and stored at -20˚C until analysis. For each study, plasma was thawed at room temperature and spiked with appropriate pure drug solutions (Drug-Plasma Stock). It was then treated with acetonitrile (4 times...
the actual volume of solution) for protein precipitation and centrifuged at 4°C, at 5000 RPM for 5min. The clear supernatant liquid was evaporated; residue was reconstituted with mobile phase and injected in the stabilized HPLC system. The blank plasma sample was prepared by repeating whole procedure without drug sample.

Validation parameters

System suitability
Solution of standard and spiked plasma sample was analyzed for System suitability parameters namely resolution, tailing factor, number of theoretical plates and capacity factor.

Linearity
Solutions in the range of 0.5-7.5 µg/ml for both the drugs (bulk form and spiked in human plasma) were prepared separately. 20 µL of each solution was injected in six replicates. The chromatograms were recorded and the peak areas were computed. Peak areas were plotted versus concentration to get the correlation. First concentration of linearity range was taken as LLOQ (Lowest limit of quantification).

Recovery and Precision
The absolute recovery of Aceclofenac and Rabeprazole was determined at 3 concentrations (Low, medium and High level) by comparison of the peak areas from standard and that of extracted (spiked plasma) samples in triplicate. The intra-day and inter day precision was determined at three different concentrations (six replicates). The % Coefficient of variation (CV) at each QC level was calculated for both the parameters.

Stability
According to USFDA guidelines stability study of stock solutions, spiked samples and processed samples was performed. All the analysis was performed at low and High concentration levels.

Freeze and thaw stability
Freeze and thaw stability of the spiked samples was determined after performing three freeze thaw cycles of samples stored at -20 °C and comparing them against the freshly spiked samples.

Short term room temperature stability
Short-term room temperature stability of the spiked samples was determined for a period of 4 hours. Samples were stored at room temperature and comparison was made against the freshly spiked samples.

Long term stability
Long-term stability of the spiked plasma solutions was determined for a period of 5 days by storing at 4°C and comparing them against the freshly prepared spiked solutions.

Stock solution stability
Stock solution stability of the drug was determined for a period of 6 hrs stored at room temperature and by comparing them against the freshly prepared stock solution.

Post- preparative stability
It is the stability of the processed sample. Stability of the extracted samples for LQC and HQC levels was determined for a period of 5 hours at room temperature.

Robustness
To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, wavelength and flow rate of the mobile phase were altered and their effect on chromatographic characteristics was evaluated in terms of % RSD.

RESULTS AND DISCUSSION

Pure drug’s chromatogram were run in different mobile phases containing Methanol, Acetonitrile, Water and different Buffers in different ratios, different stationary phases (e.g. C8, C18 ) of different dimensions. Detection was performed at 215 nm where both the drugs show significant absorption. The retention time and tailing factor was evaluated for each drug. Finally 0.05 M Potassium dihydrogen Phosphate, Acetonitrile and Methanol as a mobile phase in the volume ratio 4:4:3 v/v and HiQ SLC8 analytical column was selected which gave a sharp and symmetrical peaks with minimum tailing and appropriate retention time. Fig. 1 indicates a chromatogram of blank plasma while Fig. 2 shows a typical chromatogram of Rabeprazole and Aceclofenac in human plasma.

The chromatogram developed has well resolved peaks of said drugs without any interference of each other as well as plasma components. Results of system suitability parameters are shown in Table 1.

Calibration graph was found to be linear at range 0.5 to 7.5 µg/ml for both the drugs. Correlation coefficient (r²) was found to be 0.990 and 0.997 for aceclofenac and rabeprazole respectively. Details are shown in Table 2. It indicates that excellent correlation exists between peak area and concentration.

Fig. 1: Chromatogram of blank plasma
Fig. 2: A typical chromatogram of Rabeprazole and Aceclofenac in human plasma

Table 1: System suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rabeprazole</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>5.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Resolution</td>
<td>6.56</td>
<td>4.60</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>5351.44</td>
<td>6104.35</td>
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<td>Asymmetry</td>
<td>1.03</td>
<td>1.00</td>
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</table>

Table 2: Results of Linearity

<table>
<thead>
<tr>
<th>Name of drugs</th>
<th>( R^2 )</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceclofenac</td>
<td>0.990</td>
<td>( y = 10222x + 77049 )</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>0.997</td>
<td>( y = 12509x + 14664 )</td>
</tr>
</tbody>
</table>

In Precision Repeatability was performed and % RSD (Relative Standard Deviation) was found to be 1.51 and 1.03 for aceclofenac and rabeprazole respectively. The results obtained for intra-day precision (%RSD) were 3.34 and 1.01. For inter day precision % CV was 1.76 and 1.97 for aceclofenac and rabeprazole respectively. The low values of standard deviation and coefficient of variation at each level indicate high reproducibility of the method. Percent recovery was determined by comparing the standard solution and the spiked drug. The percentage recovery of the aceclofenac and rabeprazole was found to be 100.66% and 100.25% respectively. Stability of the drugs aceclofenac and rabeprazole was checked as short term room temperature, long term, freeze and thaw, stock solution and post preparative stability. % Mean stability for short term room temperature was found to be 91.82 and 88.38 for rabeprazole and aceclofenac respectively. % Mean stability for long term stability, freeze and thaw and stock solution stability was found to be 93.47 and 94.65, 92.65 and 89.23, 96.13 and 88.35 for rabeprazole and aceclofenac respectively. % Mean stability for post preparative stability studies was found to be 101.41 and 89.63 for rabeprazole and aceclofenac respectively. These all results indicate that plasma samples obtained for testing can be stored up to stated stability periods before analysis. Table 3 shows summary of results obtained for all validation parameters.

Table 3: Summary of all validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rabeprazol</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>0.5-7.5</td>
<td>0.5-7.5</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>6.7</td>
<td>5.4</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.990</td>
<td>0.997</td>
</tr>
<tr>
<td>Repeatability</td>
<td>3.34</td>
<td>1.01</td>
</tr>
<tr>
<td>Precision (% CV)</td>
<td>1.76</td>
<td>1.97</td>
</tr>
<tr>
<td>Mean Recovery (%)</td>
<td>100.66</td>
<td>100.25</td>
</tr>
<tr>
<td>Short-term room temperature stability</td>
<td>91.82</td>
<td>88.38</td>
</tr>
<tr>
<td>Long-term stability</td>
<td>93.47</td>
<td>94.65</td>
</tr>
<tr>
<td>Stock solution stability</td>
<td>96.13</td>
<td>88.35</td>
</tr>
<tr>
<td>Post-Preparative stability</td>
<td>101.41</td>
<td>89.63</td>
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<tr>
<td>Freeze-Thaw stability</td>
<td>92.65</td>
<td>89.23</td>
</tr>
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</table>
As all results are within limits of USFDA guidelines, the proposed HPLC method can be successfully applied for measurement of said drugs in human plasma and as bulk drugs.

CONCLUSION

The high performance liquid chromatographic method for the determination of Aceclofenac and Rabeprazole from human plasma was found to be rapid, accurate and precise. Thus, the proposed RPHPLC method can be successfully applied for the estimation of drug levels in human plasma, Pharmacokinetic evaluation and drug interaction studies.

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REFERENCES