Development and statistical validation of spectrophotometry method for estimation of Montelukast in bulk and tablet dosage form

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ABSTRACT

Three simple, precise and economical UV methods have been developed for the estimation of Montelukast in bulk and pharmaceutical formulations. Montelukast has the absorbance maxima at 359nm (Method A), and in the first order derivative spectra, showed zero crossing at 359nm, with a sharp peak at 340.5nm when n=1 (Method B). Method C applied was Area Under Curve (AUC). For analysis of Montelukast the wavelength range selected was 350-370 nm. Drug followed the Beer’s Lamberts range of 5-40 µg/ml for the Method A, B C. Results of analysis were validated statistically and by recovery studies and were found to be satisfactory.

Key words: Montelukast, UV spectophotometry, Derivative Spectroscopy, Area Under Curve

INTRODUCTION

Montelukast is a leukotriene receptor antagonist used as an alternative to anti – inflammatory medications in the management and chronic treatment of asthma and exercise-induced bronchospasm (EIB). Chemically Montelukast is 2-[1-[(1R)-1-[(3-[2-(7-chloroquinolin-2-yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2yl)phenyl]propyl]sulfanyl methyl] cyclopropyl] acetic acid. It is listed in Martindale-The complete drug reference and in Merck Index 12th edition. Literature survey reveals plane HPLC and HPLC in plasma method exists for estimation of Montelukast. No single UV method for Montelukast is reported till date using derivative spectroscopy and AUC method. Hence an attempt has been made to develop new UV method for its estimation in bulk and pharmaceutical formulations with good accuracy, simplicity, precision and economy.

MATERIALS AND METHODS:

Pure sample of Montelukast was obtained from Glenmark Pharmaceutical Limited, Mumbai as a gift sample. A Shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1 cm matches quartz cell. Tablet of 5mg and 10mg were procured from local pharmacy.

Preparation of standard solution:

The pure drug accurately about 5mg was weighed and dissolved 100 ml methanol to give the standard stock solution of concentration 50 µg/ml. Aliquots of standard stock solution were pipette out and suitably diluted with distilled water to get the final concentration of standard solutions.

Zero order spectroscopic method:

The solutions were scanned in the range from 400-200 nm (method A), and the peaks were observed at 280nm and 359 nm. The wavelength selected for the analysis of the drug was 359nm (figure-1). The drug followed the Beer’s- Lamberts law in the range of 5-35 µg/ml using the calibration curve the concentration of the sample solution can be determined.

First order derivative spectroscopic method:

The first order derivative spectra at n=1 (method B), showed a sharp peak at 340.5 (figure 2).the absorbance difference at n=1 (dA/d?) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 5-40 µg/ml and scanned in the first order derivative spectra. The calibration curve of dA/d? against concentration of the drug showed linearity.

Area Under Curve Method (AUC):

The AUC (Area Under Curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 350-370 nm. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (50µg/ml) of the drug were prepared and scanned in the spectrum mode from the wavelength range 400-200 nm (figure 3) and the calibration curve was plotted. All the three method were checked by analyzing the samples with known concentration. As the result obtained were satisfactory, the method was applied for the pharmaceutical formulations.

Analysis of the Tablet formulation:

For the estimation of Montelukast in tablet formulation by three methods, 10 tablets of brand were weighed and triturate to fine powder. Tablet powder equivalent 5mg of Montelukast was weighed
Figure 2: First order derivative spectrum of Montelukast with n=1

Figure 3: Wavelength range selected for AUC method of Montelukast

Table 1: Optical characteristics and other Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>?max (nm)/wavelength range (nm)</td>
<td>359</td>
<td>340.5</td>
<td>294-310</td>
</tr>
<tr>
<td>Beer’s-Lambert’s range (µg/ml)</td>
<td>5-35</td>
<td>5-35</td>
<td>5-40</td>
</tr>
<tr>
<td>Coefficient of Correlation</td>
<td>0.9996</td>
<td>0.9989</td>
<td>0.9987</td>
</tr>
<tr>
<td>Regression Equation Y = mx + c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.Slope(m)</td>
<td>0.033839</td>
<td>0.03431</td>
<td>0.3169</td>
</tr>
<tr>
<td>b.Intercept(c)</td>
<td>0.004429</td>
<td>0.00124</td>
<td>0.1034</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.1</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.3</td>
<td>0.15</td>
<td>0.36</td>
</tr>
</tbody>
</table>

A is zero order derivative spectrum method with n = 0. B is first order derivative method with n = 1. C is AUC method.

Table 2: Result of Analysis of Montelukast in Tablet and Recovery studies

<table>
<thead>
<tr>
<th>Method</th>
<th>Tablet</th>
<th>Label Claim</th>
<th>%estimated</th>
<th>%recovery</th>
<th>S.D.</th>
<th>%COV</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>T1</td>
<td>5</td>
<td>99.84</td>
<td>99.13</td>
<td>0.1834</td>
<td>0.1836</td>
<td>0.1058</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>10</td>
<td>99.69</td>
<td>99.72</td>
<td>0.5852</td>
<td>0.5870</td>
<td>0.3378</td>
</tr>
<tr>
<td>B</td>
<td>T1</td>
<td>5</td>
<td>100.62</td>
<td>100.83</td>
<td>1.1867</td>
<td>1.1793</td>
<td>0.6851</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>10</td>
<td>100.68</td>
<td>98.50</td>
<td>0.7504</td>
<td>0.7453</td>
<td>0.4332</td>
</tr>
<tr>
<td>C</td>
<td>T1</td>
<td>5</td>
<td>99.18</td>
<td>100.14</td>
<td>0.4104</td>
<td>0.0412</td>
<td>0.2369</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>10</td>
<td>99.14</td>
<td>100.89</td>
<td>0.4640</td>
<td>0.4680</td>
<td>0.2879</td>
</tr>
</tbody>
</table>

Where,
A is zero order derivative spectrum method with n = 0.
B is first order derivative method with n = 1.
C is AUC method.
T1 is Montaire 5
T2 is Romilast 10

Table 3: One Way ANOVA (Tukey -Kramer multiple comparison test).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean Difference</th>
<th>q value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A Vs Method B</td>
<td>-0.2455</td>
<td>0.3395</td>
<td>ns P &gt; 0.05</td>
</tr>
<tr>
<td>Method A Vs Method C</td>
<td>-1.095</td>
<td>1.514</td>
<td>ns P &gt; 0.05</td>
</tr>
<tr>
<td>Method B Vs Method C</td>
<td>-0.8495</td>
<td>1.175</td>
<td>ns P &gt; 0.05</td>
</tr>
</tbody>
</table>
and the dissolved and further diluted with quantity sufficient with methanol. It was kept for ultrasonication for 30 min; this was filtered through Whatman filter paper no. 41 to get the stock solution of 50 µg/ml. Various dilutions of the tablet solution were prepared and analyzed for six times and the concentration was calculated by using the calibration curve for three methods.

Validation Of the method:

All these methods were validated according to ICH guidelines by carrying out analysis of six replicate sample of tablet (tablet 2). Recovery studies were carried out at three different levels i.e. 50%, 100%, and 150% by adding the pure drug to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated (table 2).

The ANOVA test i.e. Tukey-Kramer Multiple comparison test was applied to determine whether there is no significant difference between the results of analysis by three different analysis methods (table 3).

RESULT AND DISCUSSION:

All the methods A, B, and C for the estimation of Montelukast in tablet dosage were found to be simple, accurate and reproducible. Beer-lambert’s law was obeyed in the concentration range of 5-35 µg/ml in all these methods. The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The %RSD value is less than 2 indicative of accuracy of the method. The developed method was statistically compared using one way ANOVA. The P value was found to be 0.0953 and was greater than 0.05. Hence the results of the ANOVA indicate no significant difference between three methods. Hence these methods can be useful in routine analysis of Montelukast in bulk drug and formulations.

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REFERENCES:


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