**Simultaneous estimation of Montelukast and Bambuterol in tablet dosage forms by RP-HPLC**

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**ABSTRACT**

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Montelukast & Bambuterol in tablet dosage forms. An Inertsil ODS-3CV C18, 250x4.6 mm, column with 5 µm particle size and the mobile phase consisting of 0.1% Trifluoro Acetic acid: Methanol in the ratio of 10:90 v/v was used. The flow rate was 0.8 ml/min and the effluents were monitored at 220 nm. The retention times were 2.373 min for Bambuterol and 6.899 min for Montelukast. The detector response was linear in the concentration of 17.5-420 mcg/ml. The respective linear regression equation being Y = 26705.167x + 67644.9694 for Bambuterol and Y = 75702.306x + 114141.09 for Montelukast. The limit of detection (LOD) is 0.175 mcg and 0.35 mcg for Bambuterol and Montelukast respectively. The limit of quantification (LOQ) is 0.525 mcg for Bambuterol and 1.05 mcg for Montelukast. The percentage assay of Montelukast & Bambuterol was 99.48 % and 99.78 % respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Montelukast & Bambuterol in bulk drug and in its pharmaceutical dosage forms.

**Key words:** Montelukast & Bambuterol, RP-HPLC, estimation, and tablets.
determination of Montelukast & Bambuterol in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Montelukast & Bambuterol in bulk drug samples and in pharmaceutical dosage form.

**EXPERIMENTAL**

**Materials and Methods**

Montelukast & Bambuterol were obtained as a gift samples from MSN Pharmachem Pvt.Ltd, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available tablets (Montair Plus, Cipla) were procured from local market.

**Instrument**

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. An Inertsil-ODS-3V C18 column (250 x 4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

**HPLC Conditions**

The contents of the mobile phase were 0.1% Trifluoro Acetic acid: Methanol in the ratio of 1:1.

**Table 1: Linear Regression Data for Calibration curves**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Montelukast</th>
<th>Bambuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.range (µg/ml)</td>
<td>17.5-420</td>
<td>17.5-420</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>75702.306</td>
<td>26705.167</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>111411.0925</td>
<td>67644.9694</td>
</tr>
<tr>
<td>Correlation coeff.</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.93</td>
<td>0.35</td>
</tr>
<tr>
<td>Standard error of estimate</td>
<td>118206.6442</td>
<td>48619.7117</td>
</tr>
</tbody>
</table>

**Table 2: Assay & Recovery of Montelukast & Bambuterol in Tablet dosage form**

<table>
<thead>
<tr>
<th>Tablet formulation</th>
<th>Amount claim (mg/tablet)</th>
<th>Amount claim (mg/tablet)</th>
<th>Amount Obtained (mg)* by proposed method</th>
<th>** % Recovery by the by Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montelukast</td>
<td>Bambuterol</td>
<td>Montelukast</td>
<td>Bambuterol</td>
<td>Montelukast</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>9.84</td>
<td>99.5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
<td>9.88</td>
<td>99.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10</td>
<td>9.80</td>
<td>99.1</td>
</tr>
</tbody>
</table>

*Average of three determinations. ** After spiking the sample.

Fig 1: Typical chromatogram of montelukast & bambuterol by RP-HPLC

Fig. 2: Calibration curves of the Montelukast & Bambuterol by RP-HPLC
10:90 v/v. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 ml/min. The run time was set at 12.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 220 nm.

**Preparation of standard stock solution**

A standard stock solution of the drug was prepared by dissolving 193.5 mg of Bambuterol hydrochloride (equivalent to 175 mg of Bambuterol) and 182 mg of Montelukast sodium (equivalent to 175 mg of montelukast) in 100 ml volumetric flask containing 30 ml of methanol as diluent, sonicated for about 15 min and then made up to 100 ml with methanol to get standard stock solution of 1.75 mg/ml each of Montelukast and Bambuterol.

**Working standard solution**

10 ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with methanol as diluent to get a concentration of each 350 µg/ml of Montelukast and Bambuterol.

**Preparation of sample solution**

Twenty tablets (Montair Plus, Cipla) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing 175 mg of Montelukast and 175 mg of Bambuterol active ingredients, was mixed with 30 ml of methanol as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to 100 ml to obtain a stock solution each of 1.75 mg/ml of Montelukast and Bambuterol. 10 ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with methanol as diluent to get a concentration of each 350 µg/ml of Montelukast and Bambuterol.

**Linearity**

Aliquots of standard Montelukast & Bambuterol stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Montelukast & Bambuterol are in the range of 17.5-420 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 220 nm and a Calibration graphs were obtained by plotting peak area versus concentration of Montelukast & Bambuterol (Fig 2). The plot of peak areas of each sample against respective concentration of Montelukast & Bambuterol was found to be linear in the range of 17.5-420 µg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in Table I. The respective linear regression equation being $Y = 26705.167x + 67644.9694$ for Bambuterol and $Y = 75702.306x + 114141.09$ for Montelukast. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

**Assay**

20 µl of sample solution was injected into the injector of liquid chromatograph. The retention times were found to be 2.373 min for Bambuterol and 6.899 for Montelukast. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

**Recovery studies**

Accuracy was determined by recovery studies of Montelukast & Bambuterol; known amount of standard was added to the preanalysed

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**Table 3: Validation summary**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Montelukast</th>
<th>Bambuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>System Suitability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical Plates(N)</td>
<td>2400.38</td>
<td>2007.45</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.14</td>
<td>1.15</td>
</tr>
<tr>
<td>Retention time(min)</td>
<td>6.899</td>
<td>2.373</td>
</tr>
<tr>
<td>Resolution</td>
<td>17.52</td>
<td>17.36</td>
</tr>
<tr>
<td>% Peak Area</td>
<td>99.98</td>
<td>99.82</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.35</td>
<td>0.175</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.05</td>
<td>0.525</td>
</tr>
</tbody>
</table>
sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

**RESULTS AND DISCUSSION**

The system suitability tests were carried out on freshly prepared standard stock solutions of Montelukast & Bambuterol. Parameters that were studied to evaluate the suitability of the system are given in Table 3.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The limit of detection (LOD) is 0.175 mcg and 0.35 mcg for Bambuterol and Montelukast respectively. The limit of quantification (LOQ) is 0.525 mcg for Bambuterol and 1.05 mcg for Montelukast.

From the typical chromatogram of Montelukast & Bambuterol as shown in Fig 1, it was found that the retention times were 2.373 min. for Bambuterol and 6.899 min. for Montelukast. A mixture of 0.1% Trifluoro Acetic acid: Methanol in the ratio of 10:90 v/v was found to be the most suitable as mobile phase to obtain the peaks well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship \( r=0.9999 \) was observed between the concentration range of 17.5-420 µg/ml. Low values of standard deviation are indicative of the high precision of the method. The assay of Montelukast & Bambuterol tablets was found to be 98.48\% and 98.78\% respectively. From the recovery studies it was found that about 99.14\% of Montelukast & 100.2\% of Bambuterol was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Montelukast & Bambuterol within a short analysis time.

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**REFERENCES**
