DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF MONTELUKAST SODIUM AND BAMBUETEROL HYDROCHLORIDE IN TABLETS

S. A. PATEL

Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India.

ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of montelukast sodium and bambuterol hydrochloride in combined tablet dosage form. The method is based on the simultaneous equations for analysis of both the drugs using chloroform as solvent. Montelukast sodium has absorbance maxima at 345 nm and bambuterol hydrochloride has absorbance maxima at 266.5 nm in chloroform. The linearity was obtained in the concentration range of 5-40 μg/ml and 10-80 μg/ml for montelukast sodium and bambuterol hydrochloride, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 99.63 ± 0.47 and 99.57 ± 0.36 for montelukast sodium and bambuterol hydrochloride, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of montelukast sodium and bambuterol hydrochloride in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Bambuterol hydrochloride, Montelukast sodium, Recovery, Simultaneous equations method, Tablet, Validation.

1 Corresponding Author: Phone and Fax - +91 – 2762 - 286082
Email: satishpatel_77@yahoo.com
INTRODUCTION
Montelukast sodium (MTKT) is chemically 1-{[(R)-m-[(E)-2-(7-chloro-2-quinolyl) vinyl]-α-[o-(1-hydroxy-1-methylethyl)phenethyl]benzyl]thio)methyl} cyclopropaneacetate sodium[1], is a leukotriene receptor antagonist, used in the treatment of chronic asthma and allergic rhinitis[2, 3]. It is official in IP. IP[4] describes liquid chromatography method for its estimation. Literature survey reveals voltametric[5], spectrofluorimetric[6], HPLC[7,8,9,10], HPLC and derivative spectroscopic method with loratidine[11], stability indicating HPLC method[12], and LC/MS[13] methods for estimation of MTKT in pharmaceutical dosage forms as well as in biological fluids. Bambuterol hydrochloride (BAM) is chemically (RS)-5-(2-tert-butylamino-1-hydroxyethyl)–m-phenylene bis (dimethylcarbamate) hydrochloride[14], is a long acting bronchodilator for the treatment of asthma[15]. Bambuterol hydrochloride is official in BP. BP[16] describes potentiometric titration method for its estimation. Literature survey reveals HPLC[17,18], solid-state NMR[19] methods for the determination of BAM. The combined dosage forms of MTKT and BAM are available in the market for the prophylaxis and treatment of chronic asthma and chronic bronchitis in pediatrics. Literature survey reveals HPLC[20] and spectrophotometric[21,22] methods for simultaneous determination of MTKT and BAM in tablets. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic spectrophotometric method based on simultaneous equations for simultaneous estimation of montelukast sodium and bambuterol hydrochloride in tablet dosage form.

MATERIALS AND METHODS
Apparatus
A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials
MTKT and BAM bulk powder was kindly gifted by Sun Pharmaceuticals Ltd. Vadodara, Gujarat, India. The commercial fixed dose combination product was procured from the local market. Chloroform AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of standard stock solutions
An accurately weighed quantity of MTKT (10 mg) and BAM (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with chloroform to obtain standard solution having concentration of MTKT (100 µg/ml) and BAM (100 µg/ml).

Methodology
The standard solutions of MTKT (20 µg/ml) and BAM (20 µg/ml) were scanned separately in the UV range of 200-400 nm. Maximum absorbance was obtained at 345 nm and 266.5 nm for MTKT and BAM, respectively. These two wavelengths can be employed for the determination of MTKT and BAM without any interference from the other components in their combined formulations.

Validation of the proposed method
The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[23].

Linearity (Calibration curve)
The calibration curves were plotted over a concentration range of 5-40 µg/ml for MTKT and 10-80 µg/ml for BAM. Accurately measured standard solutions of MTKT (0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml) and BAM (1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with chloroform. The absorbances of the solutions were measured at 345 and 266.5 nm against chloroform as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)
The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for MTKT and BAM (10, 20 µg/ml for MTKT and 20, 40 µg/ml for BAM) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of
standard solutions of MTKT and BAM (10, 20, 30 µg/ml for MTKT and 20, 40 , 60 µg/ml for BAM). The result was reported in terms of relative standard deviation (% RSD).

**Accuracy (recovery study)**
The accuracy of the method was determined by calculating recovery of MTKT and BAM by the standard addition method. Known amounts of standard solutions of MTKT and BAM were added at 50, 100 and 150 % level to prequantified sample solutions of MTKT and BAM (10 µg/ml for each drug). The amounts of MTKT and BAM were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

**Limit of detection and Limit of quantification**
The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines \[23\].

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the response and \( S \) = slope of the calibration curve.

**Analysis of MTKT and BAM in combined tablet dosage form**
Twenty Tablets were weighed and powdered. The powder equivalent to 10 mg of MTKT and 10 mg of BAM was transferred to a 100 ml volumetric flask. Chloroform (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with chloroform. This solution is expected to contain 100 µg/ml of MTKT and 100 µg/ml of BAM. This solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with chloroform to get a final concentration of MTKT (10 µg/ml) and BAM (10 µg/ml). The responses of the sample solution were measured at 345 nm and 266.5 nm for quantitation of MTKT and BAM, respectively. The amounts of the MTKT and BAM present in the sample solution were calculated by fitting the responses into the regression equation for MTKT and BAM in the proposed method.

**RESULTS AND DISCUSSION**
The standard solutions of MTKT and BAM were scanned separately in the UV range, and zero-order spectra for MTKT (Figure 1) and BAM (Figure 2) were recorded. Maximum absorbance was obtained at 345 nm and 266.5 nm for MTKT and BAM, respectively. These two wavelengths can be employed for the determination of MTKT and BAM without any interference from the other drug in their combined formulations.

Linear correlation was obtained between absorbances and concentrations of MTKT and BAM in the concentration ranges of 5-40 µg/ml and 10-80 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values of MTKT were found to be 0.23 and 0.32 % at 345 and 266.5 nm, respectively. The RSD value of BAM was found to be 0.52 % at 266.5 nm. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.38-0.50% and 0.41-0.72% for MTKT at 345 and 266.5 nm, respectively and 0.38-0.64% for BAM at 266.5 nm) and intraday (0.11-0.68% and 0.21-0.53% for MTKT at 345 and 266.5 nm, respectively and 0.21-0.51% for BAM at 266.5 nm) variation for MTKT and BAM, reveal that the proposed method is precise (Table 1). LOD and LOQ values for MTKT were found to be 1.21 and 1.27 µg/ml and 4.0 and 4.2 µg/ml at 266.5 and 345, respectively. LOD and LOQ values for BAM were found to be 2.80 and 9.25 µg/ml at 266.5 nm. These data show that method is sensitive for the determination of MTKT and BAM.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.63 ± 0.47 and 99.57 ± 0.36 for MTKT and BAM, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine MTKT and BAM in their combined dosage form. The results obtained for MTKT and BAM were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared;
hence the proposed method is applicable for the routine simultaneous estimation of MTKT and BAM in pharmaceutical dosage forms.

CONCLUSION
Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 5-40 μg/ml and 10-80 μg/ml for MTKT and BAM, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of MTKT and BAM. The method can be used for the routine analysis of the MTKT and BAM in combined dosage form without any interference of excipients.

ACKNOWLEDGEMENT
The authors are thankful to Sun Pharmaceuticals Ltd. Vadodara, Gujarat, India for providing gift sample of MTKT and BAM for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

REFERENCES
23. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 2005.
TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHOD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MTKT</th>
<th>BAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>345</td>
<td>266.5</td>
</tr>
<tr>
<td>Beer’s law limit (µg /ml)</td>
<td>5-40</td>
<td>5-40</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²/0.001 Absorbance Unit)</td>
<td>0.0243</td>
<td>0.0273</td>
</tr>
<tr>
<td>Regression equation (y = a + bc)</td>
<td>(y = 0.008x - 0.0007)</td>
<td>(y = 0.037x - 0.004)</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.008</td>
<td>0.037</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.0007</td>
<td>-0.004</td>
</tr>
<tr>
<td>Correlation coefficient (r^2)</td>
<td>0.9980</td>
<td>0.9990</td>
</tr>
<tr>
<td>LOD(^a) (µg/ml)</td>
<td>1.21</td>
<td>1.27</td>
</tr>
<tr>
<td>LOQ(^b) (µg /ml)</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Repeatability (% RSD, n = 6)</td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>Precision (% RSD, n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>0.38-0.50</td>
<td>0.41-0.72</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.11-0.68</td>
<td>0.21-0.53</td>
</tr>
<tr>
<td>Accuracy (% recovery, n = 5)</td>
<td>99.63 ± 0.47</td>
<td>99.57 ± 0.36</td>
</tr>
</tbody>
</table>

\(^a\)LOD = Limit of detection. \(^b\)LOQ = Limit of quantification. \(^c\)RSD = Relative standard deviation.
TABLE 2: RECOVERY DATA OF PROPOSED METHOD

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (µg/ml)</th>
<th>Amount added (%)</th>
<th>% Recovery ± S. D. (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>50</td>
<td>99.30 ± 0.42</td>
</tr>
<tr>
<td>MTKT</td>
<td>10</td>
<td>100</td>
<td>100.2 ± 0.90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>150</td>
<td>99.40 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50</td>
<td>98.10 ± 0.12</td>
</tr>
<tr>
<td>BAM</td>
<td>10</td>
<td>100</td>
<td>99.20 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>150</td>
<td>101.4 ± 0.80</td>
</tr>
</tbody>
</table>

S. D. is Standard deviation and n is number of determinations

TABLE 3: ANALYSIS OF MTKT AND BAM BY PROPOSED METHOD

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim ± S. D. (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTKT</td>
<td>BAM</td>
<td>MTKT</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>10</td>
<td>10.04</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>10</td>
<td>9.97</td>
</tr>
</tbody>
</table>

S. D. is Standard deviation and n is number of determinations

FIGURE 1: Zero-order absorption spectra of MTKT in chloroform
FIGURE 2: Zero-order absorption spectra of BAM in chloroform

FIGURE 3: Overlain zero-order absorption spectra of MTKT and BAM in chloroform