Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Montelukast Sodium and Bambuterol Hydrochloride in Bulk and Tablet Dosage Formulation.

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ABSTRACT
Montelukast is leukotrine receptor blocker and bambuterol is β₂ receptor agonist, and a combination of these drugs is used in the management of asthma. A simple, precise, rapid and selective simultaneous equation by corrective absorbance method has been developed and validated for the simultaneous determination of montelukast and bambuterol in bulk and marketed tablet formulation. The method involves solving the simultaneous equation by calculating the concentration of one analyte in the sample and the concentration of the second absorbing component of interest which was then calculated from the corrected absorbance (total absorbance minus absorbance of the interfering substances) in the usual way. Methanol was used as a solvent, the wavelength maxima of montelukast and bambuterol was found to be 283 nm and 265 nm, respectively. The absorptivity of montelukast at 283 and 265 nm was calculated and found to be 383.15 and 285.67 g⁻¹L cm⁻¹ respectively, for bambuterol at 265 nm it was 10.68 g⁻¹L cm⁻¹, while at 283 nm, bambuterol has very negligible absorptivity. An equation was derived to calculate the concentration of montelukast and bambuterol in combined tablet formulation simultaneously. Montelukast and bambuterol were found to be linear in the concentration range of 10-80 µg/mL and 40 – 240 µg/mL, respectively. The method was found sensitive, accurate and precise for simultaneous estimation of montelukast and bambuterol in combined tablet dosage forms.

Keywords: Montelukast, Bambuterol, Spectrophotometry, Absorptivity.

INTRODUCTION
Montelukast is leukotrine receptor blocker, administered orally as tablet in the dose of 5-10 mg per day. Chemically it is represented as (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl) -3-(2-(1-hydroxy-1-methylethyl) phenyl) propyl)thio) methyl) cyclopropaneacetic acid, monosodium salt with molecular formula C₁₅H₁₅ClINaO₃S (Fig. 1). It is not an official drug in any pharmacopoeia.
Bambuterol is a \( \beta_2 \) receptor agonist, administered orally as tablet in the dose of 5-10 mg per day. Chemically it is represented as \([3(\text{dimethylcarbamoyloxy})-5-[1\text{-hydroxy-2-(tert-butylamino) ethyl}]\text{phenyl}] \text{N, N-dimethylcarbamate} \) with the molecular formula \( \text{C}_{18}\text{H}_{29}\text{N}_3\text{O}_5 \). It is an official drug in British pharmacopoeia.

The review of literature revealed that several methods are available for the determination of montelukast and bambuterol individually or in combination with other drugs. But no method mentioned in the literature explained the simultaneous estimation of montelukast and bambuterol in bulk, dosage forms and in biological fluids. The present study describes a simple, rapid, accurate, reproducible and economical method for simultaneous estimation of montelukast and bambuterol using corrective absorbance method.

**EXPERIMENTAL**

**Instruments and Materials**

Pure Montelukast sodium and bambuterol hydrochloride were obtained as gift samples from Sun Pharmaceuticals Pvt. Ltd. India. The spectrophotometer used was Shimadzu UV-Visible spectrophotometer 1601 model with spectral bandwidth of 3 nm and wavelength accuracy (with automatic wavelength correction) of 0.5 nm. Analytical grade methanol was purchased from Qualigens Fine Chemicals, Mumbai, India. All the apparatus and instruments were calibrated and validated as per calibration and validation protocol specified before starting the experimental work.

**Selection and standardization of the solvent**

Drugs should have adequate absorbance in the same solvent for the simultaneous determination. In water, bambuterol absorbs below 230 nm, possibility of water as solvent was ruled-out. In methanol, bambuterol absorbs below 275 nm and montelukast has a very good absorbptivity in entire UV range, hence methanol was selected as a possible solvent for analysis.

**Determination of wavelength maxima and molar absorptivity**

Standard solution consisted of 50 \( \mu \text{g} \text{mL}^{-1} \) of montelukast and bambuterol was prepared separately in 10 mL volumetric flasks. Standard solutions of both drugs were scanned in the UV range 220 to 400 nm, using methanol as blank (Fig 2). The wavelength maxima of montelukast and bambuterol were found to be 283 nm and 265 nm, respectively. The absorbances of both drugs were recorded at 265 and 283 nm; and molar absorptivity (\( \epsilon \)) for drugs was calculated from the formula:

\[
\epsilon = \frac{A}{C} \text{Eqn.1},
\]

Where, \( A \): absorbance, \( C \): concentration of analyte in \( \mu \text{g} \text{mL}^{-1} \).

The absorptivity of montelukast at 283 nm and 265 nm was found to be 383.15 and 285.67, respectively. The absorptivity of bambuterol at 265 nm was found to be 10.68; while at 283 nm it was negligible.
Development of Simultaneous Equation

The equation for simultaneous estimation of montelukast and bambuterol in combination was developed by 'Corrective Absorbance Method'.

\[
C_{\text{MON}} = \frac{A_1}{\varepsilon_{x1}} \quad \text{Eqn. 2},
\]

\[
C_{\text{BAM}} = \frac{A_2 - (C_{\text{MON}} \times \varepsilon_{x2})}{\varepsilon_{y2}} \quad \text{Eqn. 3}.
\]

Where, 
- \( A_1 \) and \( A_2 \): absorbance of the sample at \( \lambda_1 \) and \( \lambda_2 \), respectively,
- \( \varepsilon_{x1} \) and \( \varepsilon_{x2} \): absorptivity of montelukast at \( \lambda_1 \) and \( \lambda_2 \), respectively,
- \( \varepsilon_{y2} \): absorptivity of bambuterol at \( \lambda_2 \),
- \( C_{\text{MON}} \) and \( C_{\text{BAM}} \): concentrations of montelukast and bambuterol in g/100mL

\( \lambda_1: 283 \text{ nm} \)
\( \lambda_2: 265 \text{ nm} \)

The equations 2 and 3 were modified, for the purpose of calculating the concentration of montelukast and bambuterol in the sample, to be as the following:

\[
C_{\text{MON}} = \frac{A_1}{383.15} \quad \text{Eqn. 4},
\]

\[
C_{\text{BAM}} = \frac{A_2 - (C_{\text{MON}} \times 285.67)}{10.68} \quad \text{Eqn. 5}.
\]

Equation (4) and (5) are the modified forms of the equation for the simultaneous estimation of montelukast and bambuterol in combination.

Preparation of standard mixture solution

Accurately weighed 10 mg of each of the standard drugs montelukast and bambuterol was transferred into clean, dry 10 mL volumetric flask and dissolved into a sufficient volume of methanol. The volume was made up to 10 mL with methanol to get the concentration of 1000 \( \mu \text{g/mL} \). Aliquots of this solution were transferred into separate 10 mL volumetric flasks and the volume was then adjusted with methanol to get the concentrations in the range between 1-100 \( \mu \text{g/mL} \).

Validation of Analytical method

Validation is the process of establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

1. Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOD and LOQ were calculated statistically from formula:

\[
\text{LOD} = 3.3 \left( \frac{SD}{S} \right), \quad \text{LOQ} = 10 \left( \frac{SD}{S} \right)
\]

Where, 
- \( SD \): Standard Deviation of y- intercepts of regression lines,
- \( S \): Slope of the calibration curve.

The LOD and LOQ of montelukast and bambuterol by statistical and visualization methods were mentioned in Table (1).

2. Linearity

The linearity of montelukast and bambuterol were found to be 1 – 80 and 40 – 240 \( \mu \text{g/mL} \), respectively.
The values of the percentage curve fittings were in the limits of acceptance criteria (Table 1).

3. Precision

Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision is usually expressed as the standard deviation or relative standard deviation. In the present study, developed method was validated for method, system, inter-day and intra-day precision.

As the values of %RSD of all precision study were within the acceptable limits (less than 2%), both the method as well as the system provides good precision and reproducibility (Table 1).

4. Sensitivity

Absorbance of standard solutions of montelukast and bambuterol was taken at 283 and 265 nm. Sandell’s sensitivity (II) for drugs was calculated from the following formula, at both wavelengths (1):

\[
II \text{ (\(\mu g/cm^3\) AU)} = \frac{\text{Conc. of drug (}\mu g/100 mL^{-1})}{\text{Absorbance}} \times 0.001
\]

The Sandell’s sensitivity for montelukast at 283 and 265 nm was found to be 0.00026 and 0.00035 \(\mu g/cm^3\) 3AU\(^{-1}\), respectively; and for bambuterol at 265 nm it reached 0.009441 \(\mu g/cm^3\) 3AU\(^{-1}\).

5. Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the preanalyzed sample. Results of the recovery study were found to be within the acceptance criteria 100±10 %, indicating a good degree of sensitivity of the method towards detection of analytes in sample. (Table 1).

Table 1. Validation Parameters

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Montelukast</th>
<th>Bambuterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LOD</td>
<td>(\mu g/mL^{-1})</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>LOQ</td>
<td>(\mu g/mL^{-1})</td>
<td>7.66</td>
</tr>
<tr>
<td>2</td>
<td>Linearity</td>
<td>(\mu g/mL^{-1})</td>
<td>10 - 80</td>
</tr>
<tr>
<td></td>
<td>Regression eq&quot;</td>
<td>0.0389x - 0.0129</td>
<td>0.001x + 0.004</td>
</tr>
<tr>
<td></td>
<td>R(^2)</td>
<td>0.996</td>
<td>0.999</td>
</tr>
<tr>
<td>3</td>
<td>Precision</td>
<td>Method (SD)</td>
<td>0.0488</td>
</tr>
<tr>
<td></td>
<td>%RSD</td>
<td>0.101</td>
<td>1.7046</td>
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<tr>
<td></td>
<td>System (SD)</td>
<td>(\mu g/mL^{-1})</td>
<td>0.0018171</td>
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<tr>
<td></td>
<td>%RSD</td>
<td>0.101</td>
<td>0.0816</td>
</tr>
<tr>
<td></td>
<td>Inter-day (SD)</td>
<td>0.0727</td>
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<tr>
<td></td>
<td>%RSD</td>
<td>0.15</td>
<td>1.933</td>
</tr>
<tr>
<td></td>
<td>Intra-day (SD)</td>
<td>0.1112</td>
<td>0.96</td>
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<tr>
<td></td>
<td>%RSD</td>
<td>0.2301</td>
<td>1.9952</td>
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<tr>
<td>4</td>
<td>Sensitivity</td>
<td>265 nm</td>
<td>0.00035</td>
</tr>
<tr>
<td></td>
<td>(\mu g/cm^3) AU</td>
<td>283 nm</td>
<td>0.00026</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy</td>
<td>80%</td>
<td>98.3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>92.34 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120%</td>
<td>98.7 %</td>
</tr>
</tbody>
</table>
Procedure for the analysis of tablet formulation

Sample: Montelukast sodium and Bambuterol hydrochloride Tablet

Brand name: Montek Plus (10mg of Montelukast + 10 mg of Bambuterol).

Manufacturer: SUN Pharmaceutical Ind. Ltd.

Preparation of sample stock solution

193 mg of tablet powder equivalent to 10 mg each of montelukast and bambuterol was weighed. The powder was dissolved in a sufficient volume of methanol and filtered through a Whatmann filter paper. Filtrate was made up to 100 mL with methanol, 5 mL of filtrate was transferred to 10 mL volumetric flasks, and then the volume was made up with methanol. Absorbance of this solution at 283 nm and 265 nm was recorded as A1 and A2. Using equation 4 and 5, concentration of montelukast and bambuterol was calculated in tablets.

The amount of montelukast and bambuterol in marketed tablet formulation conformed to the label claim (montelukast sodium 10 mg and bambuterol hydrochloride 10 mg) (Table 2).

RESULTS AND DISCUSSION

The combination of montelukast and bambuterol tablets is administered for the management of asthma. In the present study, a new, reliable, reproducible, simple UV method for the simultaneous determination of montelukast and bambuterol in combined dosage forms was developed and evaluated. The method was developed by corrective absorbance, using methanol as solvent. The wavelength maxima for montelukast and bambuterol were 283 nm and 265 nm, respectively. The absorptivity of montelukast at 283 and 265 nm was 383.15 and 285.67, respectively. The absorptivity of bambuterol at 265 nm was 10.68. An equation was developed to calculate concentration of montelukast and bambuterol in combined tablet formulation simultaneously. The methods were validated based on ICH; analytical method validation guidelines. The values of R² for linearity, %RSD for precision and recovery study for accuracy were within the limits of acceptance criteria of ICH; and this indicates repeatability, reproducibility and accuracy of the method.

The proposed method for simultaneous estimation of montelukast and bambuterol was found to be simple, accurate, precise, sensitive, economical and rapid. The method can be employed to perform the routine analysis conducted in quality control laboratories.

<table>
<thead>
<tr>
<th>Stock vol.</th>
<th>A₁*</th>
<th>A₂*</th>
<th>Conc. (µg/mL)</th>
<th>% Label Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Montelukast</td>
<td>Bambuterol</td>
</tr>
<tr>
<td>5 mL</td>
<td>1.784</td>
<td>1.38</td>
<td>46.56</td>
<td>46.70</td>
</tr>
</tbody>
</table>

*Average of three determinations

CONCLUSION

The UV method has been developed for the simultaneous estimation of montelukast sodium and bambuterol hydrochloride in bulk and tablet formulation. The methods were validated based on ICH analytical method validation guidelines. The values of R² for linearity, %RSD for precision, and recovery study for accuracy were within the limits of acceptance criteria of ICH; and this indicates repeatability reproducibility, and accuracy of the method. The proposed method for simultaneous estimation of montelukast and bambuterol was found to be simple, accurate, precise, sensitive, economical and rapid. The method can be employed when performing the routine analysis in quality control laboratories.
REFERENCES


(2) http://www.catmanchem.com


للتنقيح الفسيولوجي فوق الأشعاء باستعمال الطيفي التحليل طريقة وتقدير المحاذير في الهيدروكلايريد بامبيوتورل وصدوع اكسيجيزة.  

1.1

نيراللباحثة جامعة الصيدلة، اليمن الهندلية بانغالور، ن، ملخص

لدالة الإيدوخيرات بامبيوتورل لامعة الدواء، الكمية المولدة للتنقيح للمنديلية في المادة المعادلة المشتركة كما

.2

الحالة الغازية الرائحة في العادة تستخدم الدواء هذه في الثانوية، التي تترافق حسب التحليل لوعود الأدلة و لدى

.283

كما أن النمو الوارد عندما تكون 283 و 265 ملم لحمي و 383.51 و 265، 283، 10.86، 285.76 أ. الثمانية 10. اكتوبر

الأوكتوغار الاكسيراتكية إلى كت توريكي лечения لا أنسي و 10.86 لم يتم

1.2 (240-40) أ (80-10) التجربة. ل.6

2008/6/16

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