ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF BILASTINE AND MONTELUKAST SODIUM BY UV SPECTROPHOTOMETRY

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ABSTRACT
A new, simple, precise, sensitive and specific UV spectrophotometric method was developed for the simultaneous estimation of Bilastine and Montelukast Sodium in bulk and in tablet dosage form. The wavelength maxima (λmax) for Bilastine were 214 nm Montelukast Sodium were found to be 281 nm in methanol. The linearity for this method was found to be in the range of 4-20 μg/ml and 2-10 μg/ml for Bilastine and Montelukast sodium. The correlation coefficient (r) was found to be of 0.9992 and 0.9996 for Bilastine and Montelukast sodium. This sensitive method was capable to recover accurately and precisely from 50%, 100% and 150% level of target concentration. The proposed method may be suitably applied for the analysis of Montelukast Sodium in bulk and in tablet pharmaceutical formulation for routine analysis. The method was validated as per ICH Guidelines.

KEYWORDS: Bilastine, Montelukast sodium, UV-Spectrophotometry, Simultaneous Equation.

INTRODUCTION
Bilastine
Bilastine is a novel new generation antihistamine that is highly selective for the H1 histamine receptor antagonist, has a rapid onset and prolonged duration of action. During allergic response mast cells undergo degranulation which releases histamine and other substances. By
binding to and preventing activation of the H1 receptor, bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.

Figure 1: Chemical structure of bilastine.

Montelukast sodium
Montelukast is a leukotriene receptor antagonist that demonstrates a marked affinity and selectivity to the cysteinyl leukotriene receptor type-1 in preference to many other crucial airway receptors like the prostanoid, cholinergic, or beta-adrenergic receptors. It is 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-[(E)-2-(7-chloroquinolin-2yl)ethenyl]phenyl]-3-[2-(2-hydroxypropan-2yl)phenyl]propyl]sulfanyl methyl] cyclopropyl] acetic acid. It is an official drug in Indian Pharmacopeia and British Pharmacopeia.

Figure 2: Chemical structure of montelukast sodium.
From the literature review it was found that no analytical methods have been reported for the simultaneous estimation of Bilastine and Montelukast Sodium by RP-HPLC and UV spectroscopy.

MATERIALS AND METHODS
All the reagents were used are AR Grade. Bilastine and Montelukast sodium were obtained as a gift sample from SYMED LABS LTD. Hyderabad, India. Methanol AR Grade. Tablet formulation BILAZEST-M™ (Abbott) containing Bilatine 20mg and Montelukast sodium 10mg was procured from local pharmacy.

Equipments
Spectrophotometric determinations were carried out in LABINDIA 3000 double beam UV-Visible spectrophotometer with UVWin software, spectral bandwidth of 1.00nm, wavelength accuracy ±0.1 nm- uv region with 1cm matched paired quartz cells. All weighing was done on electronic balance (Model Shimadzu AUX-220).

Preparation of standard stock solution
20 mg of Bilatine and 10 mg of Montelukast Sodium was accurately weighed and transferred into 25 ml volumetric flask separately, dissolved in minimum quantity of methanol, finally made up to the mark with methanol to get 800 µg/ml of Bilastine and 400 µg/ml of Montelukast Sodium. From the solution 1ml taken in a 10 ml volumetric flask made upto the volume with same (80µg/ml) of Bilastine and (40µg/ml) of Montelukast Sodium.

Selection of wavelength
Bilastine and Montelukast Sodium (10 µg/ml each) were prepared separately by appropriate dilution of standard stock solution with methanol and scanned between 200-400nm. From the overlain spectra of these drugs, Bilastine showed the wavelength maxima (λmax) at 214nm and Montelukast at 281nm. These wavelength were selected for the quantitation.

The stability studies were performed by measuring the absorbance at 80µg/ml and 40µg/ml of Bilastine and Montelukast Sodium at different time intervals. It was found that Bilastine and Montelukast Sodium is stable more than 5 hours at all the selected wavelength. The individual and overlaid spectrum of Bilastine and Montelukast Sodium shown in Fig.
Preparation of calibration graph
The aliquots of stock solution of Bilastine (0.5, 1, 1.5, 2, and 2.5ml) of 80µg/ml and Montelukast Sodium (0.5, 1, 1.5, 2, and 2.5ml) of 40µg/ml were transferred into 10ml volumetric flask and made up to the volume with methanol. The absorbance of different concentration solutions were measured at 214 nm and 281 nm. The calibration curves were plotted using concentration versus absorbance. Both drugs were linear with of 4-20µg/ml for Bilastine and 2-10µg/ml for Montelukast Sodium respectively at their respective wavelengths and thus, it obey’s the Beer’s law.

Quantification of formulation
20 tablets of formulation (BILAZEST-M) containing 20mg of Bilastine and 10mg of Montelukast Sodium were weighed accurately. The average weight of tablets were found and powdered. The tablet powder equivalents to 20mg of Bilastine was weighed and transferred into 25ml volumetric flask and add a minimum quantity of solvent to dissolve the substance by using Ultrasonicator for 15 minutes and made up to the volume with the same (800µg/ml). From the solution 1ml taken in a 10 ml volumetric flask made upto the volume with same (80µg/ml). The content was filtered through the Whatmann filter paper No.41. From the clear solution further dilutions were made by diluting 1 ml to 10ml volumetric flask with methanol to obtain 8µg/ml of Bilastine and 4µg/ml of Montelukast Sodium were determined by using Simultaneous method. The procedure was repeated for 6 times for each percentage.

Method validation
The developed method was validated as per ICH guidelines.

Linearity
A calibration curve was plotted between concentration and absorbance. Bilastine was linear with the concentration range of 4-20 µg/ml at 214 nm and 281 nm, Montelukast sodium was linear with the concentration range of 2-10 µg/ml at 281 nm and 214 nm by obeying Beer’s law.

Accuracy (Recovery studies)
Accuracy of the method was confirmed by recovery studies. To the pre-analyzes formulation, a known quantity of raw materials of Bilastine and Montelukast sodium were added and the procedure was followed as per the analysis of formulation. The amount of each drug
recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

**Precision**

The repeatability of the method was confirmed by the analysis of formulation and repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated.

**LOD and LOQ**

The linearity study was carried out for six times. The LOD and LOQ were calculated by using average value of slope and standard deviation of response (intercept).

**DISCUSSION**

The method developed in this work is a convenient and reliable method for the quantitative determination of Bilastine and Montelukast Sodium in Fixed dose combination. The determination was carried out in the wavelength range of 214 nm for Bilastine and 281 nm for Montelukast sodium.

Different aliquots of Bilastine in methanol were prepared in the concentration range of 4-20 µg/ml. The absorbance of those solution was measured at the 214 nm and 281 nm. The calibration curve was constructed using concentration versus absorbance.

Different aliquots of Montelukast Sodium in methanol were prepared in the concentration range of 2-10 µg/ml. The absorbance of those solution was measured at the 214 nm and 281 nm. The calibration curve was constructed using concentration versus absorbance.

The preparation of calibration curve was repeated for six times for each drug at their selected wavelengths. The Correlation coefficient for the two drugs was found to be above 0.999. This indicates that all the Beer's law in the selected concentration ranges. Hence the concentration was used to be linear.

The tablet formulation BILAZEST-M (containing Bilastine 20mg and Montelukast Sodium 10 mg) was selected for the analysis. The drugs Bilastine and Montelukast Sodium, were in the ratio of (2:1) in the formulation.
The percentage purity of the drugs in the formulation was found to be good concord with the label claim 99.55 ± 0.203511 and 99.78 ± 1.235179 for Bilastine and Montelukast sodium, respectively. The result listed in Table-1.

The precision of the method was confined by the repeated analysis of formulation for six times. The percentage RSD values were found to be 0.204430 and 1.237902 for Bilastine and Montelukast sodium, respectively.

The accuracy of the method was confirmed by the recovery studies. To the pre-analysed formulation, a known quantity of raw material was added and the percentage recovery was calculated. The percentage of raw material added was added 50%, 100%, and 150% Bilastine and Montelukast sodium. The percentage recovery was found to be in the range of 99.66-100.25% and 98.75-99.50% for Bilastine and Montelukast sodium. The percentage RSD value was found to be 0.31823, and 0.39644 for Bilastine and Montelukast sodium.

The low percentage RSD value indicated there was no interference due to excipients in formulation. Hence, the accuracy of the method was conformed. The test results are listed in Table-2.

**CONCLUSION**

Simple, rapid and accurate UV Spectroscopic method was excellent Sensitivity, Reproducibility, Accuracy, Repeatability which is evidenced by low %RSD. The results obtained in recovery studies were indicating that there is no interference from the excipients undo in the formulation. UV Spectroscopic method was found to be economical.

Hence it is suggested that the proposed UV Spectroscopic method can be effectively applied for the routine analysis of Bilastine and Montelukast sodium in tablet formulation and the obtained results will be presented elsewhere.

**ACKNOWLEDGEMENT**

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Table 1: Quantification of formulation BILAZEST-M.

<table>
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<tr>
<th>Drug</th>
<th>Labeled amount (mg/tab)</th>
<th>Amount found (mg/tab)*</th>
<th>Percentage Obtained*</th>
<th>Average (%)</th>
<th>S.D</th>
<th>% R.S.D.</th>
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Table 2: Recovery analysis of formulation BILAZEST-M.

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<th>Amount added* (µg ml⁻¹)</th>
<th>Amount estimated* (µg ml⁻¹)</th>
<th>Amount recovered* (µg ml⁻¹)</th>
<th>% Recovery*</th>
<th>S.D.</th>
<th>% R.S.D.</th>
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Figure 3: Overlaid spectrum of Bilastine and Montelukast sodium in methanol.
REFERENCES

15.