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Development and Validation of RP HPLC Method for Simultaneous Estimation of Ebastine and Montelukast Sodium In Combined Dosage Form

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ABSTRACT

A specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Ebastine (EBA) and Montelukast Sodium (MONTE) in Combined dosage forms. The analysis was carried out using Phenomenex $C_{18,}$ column (250 mm × 4.6 mm id, 5 µm particle size) with Mobile phase consisting of Methanol : Phosphate buffer (65:35 v/v) pH 5.0±0.05 was pumped at a flow rate was 1.0 ml/ min and Quantification was achieved with photodiode array (PDA) detection at 261 nm over the concentration range of 10 - 50 µg/mL for ebastine & Montelukast Sodium with mean recovery of 100.32 ± 0.85 % and 100.15 ± 0.94 % for Ebastine and Montelukast Sodium, respectively. These methods were found to be simple, sensitive, accurate, precise and economical and applicable for the simultaneous determination of Ebastine & Montelukast Sodium in combined dosage form.

Keywords: Ebastine, Montelukast Sodium, RP-HPLC, Recovery.

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INTRODUCTION

Ebastine (EBA) chemically is 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl) butan-1-one. It is a second-generation H1 receptor antagonist that is indicated mainly for allergic rhinitis and chronic idiopathic urticaria [1-3]. The chemical structure of EBA is shown in Figure 1.

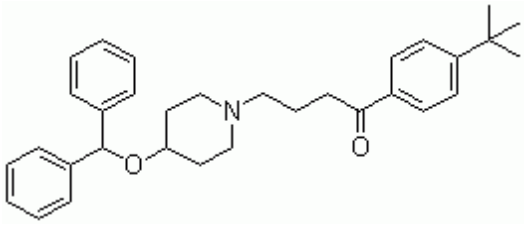

Figure 1 Chemical Structure of Ebastine

Montelukast sodium (MONTE), (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl) vinyl) phenyl)-3-(2-(2-hydroxypropan-2-yl) phenyl) propylthio) methyl) cyclopropyl) aceticacid. It is a leukotriene receptor antagonist (LTRA) used in the treatment of asthma and to relieve of seasonal allergies [4]. It is usually administered orally. Montelukast Sodium blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it. This reduces the broncho constriction results reduce inflammation. The Chemical structure of MONTE is shown in Figure 2.

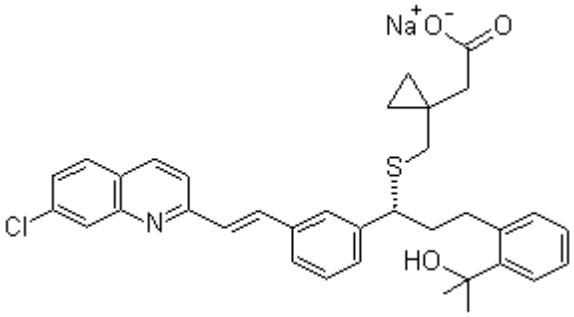

Figure 2 Chemical Structure of Montelukast Sodium

Literature survey reveals few method have been reported in literature for the Spectrophotometric estimation of ebastine single and combined dosage form [5-9],there have been also reported several method for single and combined dosage for estimation of ebastine by RPHPLC[10,11] .Few method have been reported in literature for spectrophotometric methods and RP-HPLC for estimation of Montelukast Sodium.[12-17] Hence attempt to develop RPHPLC and validate method in accordance with ICH guidelines.

MATERIAL AND METHODS

Instrument & Apparatus

A HPLC instrument (LC-2010$C_{HT,}$ Shimadzu, Japan) equipped with a photodiode array detector,

manual injector with 20 µL loop, Phenomenex C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software were used, Digital pH meter,Analytical balance (Sartorius balance).

Reagents & Materials

EBA and MONTE bulk powder was gifted by Pharmaceutical Company Gujarat (India), with 99.96% purity. The commercial fixed dose combination product containing 10 mg EBA and 10 mg MONTE was procured from the local pharmacy. HPLC grade Methanol (Merck Ltd., Mumbai, India), AR grade potassium dihydrogen phosphate (S.D Fine Chemicals Ltd, Mumbai, India)

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature. Mobile Phase comprised of Methanol: Phosphate buffer (65:35 v/v) pH 5.0±0.02, Flow rate: 1.0 mL/min , Injection volume: 20 µL Detection: At 261 nm with PDA detector.

Preparation of EBA and MONTE mixed Standard stock solutions

Accurately weighed EBA (10 mg) and MONTE (10 mg) was transferred to 100 mL amber colored volumetric flask and dissolved and diluted to the mark with methanol to obtain a mixed standard stock solutions having concentration EBA (100 µg/mL) and MONTE (100 µg/mL).

Determination of analytical wavelength

The standard solution of EBA and MONTE were injected under the chromatographic condition described above. Detection was carried out at different wavelength best response was achieved at 261 nm with PDA detector. So both drugs were detected at this analytical wavelength.

METHOD VALIDATION

(i)Calibration curve (Linearity)

Calibration curves were constructed by plotting peak areas Vs concentrations of EBA and MONTE, and the regression equations were calculated. Calibration curves were plotted over a concentration range of 10–50 µg/mL for EBA and MONTE. Accurately measured standard working solutions of EBA and MONTE (1.0, 2.0, 3.0, 4.0 and 5.0 ml) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions described in section above. Each reading was average of three determinations.

(ii)Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of EBA and MONTE by

the standard addition method. Known amounts of standard solutions of EBA and MONTE were added at 50, 100 and 150 % level to prequantified sample solutions of EBA and MONTE (20 µg/mL). The amounts of EBA and MONTE were estimated by using the regression equation of the calibration curve.

(iii)Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) standard solutions of EBA and MONTE (30 µg/mL) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) or coefficient of variation (CV) should not be more than 2 %.

(iv)Intermediate Precision (Reproducibility)

It express within laboratory variation as on different day analysis or equipment within the laboratory as on the proposed method was evaluated within the laboratory .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 EBA and MONTE (30,40 and 50 µg/mL). The results were reported in terms of relative standard deviation (RSD)

(v)Limit of Detection and Limit of Quantification

LOD and LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where S= the standard deviation of the response

σ = Slope of calibration curve.

(vi) Robustness

The robustness of the method was established by introducing small changes in various parameters like mobile phase composition, pH of mobile phase and flow rate. For the same, mobile phases having different compositions, like Change in Mobile phase to Methanol Phosphate Buffer(63: 37,v/v), (67: 33 v/v) were tried and chromatograms were run. The changes made flow rate and pH were ± 0.2 units, respectively. The change in flow rate $\pm$ 0.2. Robustness of the method was evaluated by calculating the % RSD values. Robustness of the method was done at different concentration levels in the range of 10-50 µg/mL for both EBA and MONTE.

Analysis of EBA And MONTE In Combined Dosage Form

The response of the sample solution was measured at 26nm under the chromatographic condition

mentioned above for the quatitation of EBA and MONTE. The amounts of the EBA and MONTE present in the sample solution were calculated by fitting the responses into the regression equation for EBA and MONTE.

RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for EBA and MONTE was obtained with a mobile phase Methanol: Phosphate Buffer (65:35, v/v) pH 5.0 ± 0.02 at a flow rate of 1.0 mL/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 261 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 3). System suitability test parameters for EBA and MONTE for the proposed method are reported in (Table 1)

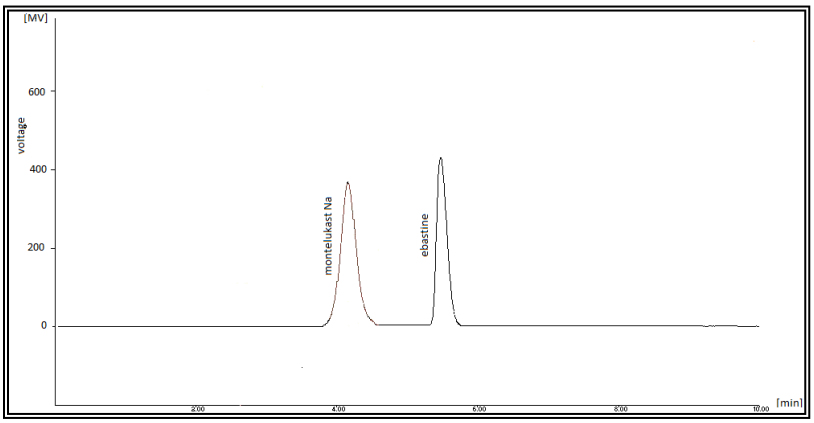

Figure 3 RP HPLC Chromatogram of ebastine & Montelukast sodium

Table-1 System Suitability Parameters of Chromatogram for Ebastine & Montelukast Sodium

Parameters	EBA ± RSD[a](n = 6)	MONTE ± RSD (n = 6)
Retention time (min)	5.51 ± 0.592	4.13 ± 0.390
Tailing factor	1.22 ± 0.738	1.141 ± 0.999
Theoretical plates	5411 ± 1.069	6719 ± 1.296
Resolution	3.18±1.625	

Validation of The Proposed Method[18]

Linearity

Linear correlation was obtained between peak areas versus concentrations of EBA and MONTE in the ranges of 10-50 µg/mL. Regression parameters are mentioned in (Table 2) and the calibration curves of EBA and MONTE at 261 nm, respectively.

Accuracy

The recovery experiment was performed by the standard addition method. The recoveries

obtained were 100.32 ± 0.85 % and 100.15 ± 0.94 % for EBA and MONTE respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in (Table 3)

Table 1 Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (µg/mL)	Mean % Recovery ± SD*
EBA	50 %	20	10	100.00 ± 0.30
	100 %	20	20	100.67 ± 1.21
	150 %	20	30	100.31 ± 1.05
MONTE	50%	20	10	100.20 ± 1.08
	100%	20	20	99.81± 0.97
	150%	20	30	100.62 ± 0.79

* Mean % Recovery ± SD of six observations.

Method Precision (% Repeatability)

The RSD values for EBA and MONTE were found to be 0.678 % and 0.7018 %, respectively The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.(Table 2)

Table 2: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Parameters	EBA	MONTE
Detection wavelength (nm)	261	261
Concentration range (µg/mL)	10-50	10-50
Slope	2891.7	3229.6
Intercept	5439.9	3307.8
Correlation coefficient (r)	0.9961	0.9981
LOD[a] (µg/mL)	0.7498	0.223
LOQ[b] (µg/mL)	2.2721	0.675
% Recovery (Accuracy, n = 5)	100.37± 0.53%	100.43 ± 0.42 %
Repeatability (RSD[c], n = 6), %	0.678 %	0.701%
Precision (RSD), %		
Interday (n = 6)	1.62-1.77 %	1.62-1.85%
Intraday (n = 6)	0.61-0.866 %	0.54-0.783 %

[a] LOD = Limit of detection

[b] LOQ = Limit of quantification

[c] % RSD = Percent relative standard deviation.

Intermediate Precision (Reproducibility)

The low RSD values of Interday (1.62-1.77 % and 1.51-1.75%) and intraday (0.61-0.861 % and 0.54-0.783 %) variations for EBA &MONTE, respectively, reveal that the proposed method is precise.(Table 2)

LOD and LOQ

LOD values for EBA & MONTE were found to be 0.7498 µg/mL and 0.223µg/mL, respectively and LOQ values for EBA and MONTE were found to be 2.271 µg/mL and 0.675 µg/mL, respectively (Table 2) These data show that the proposed method is sensitive for the determination of EBA & MONTE.

Robustness

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %. The low value of % RSD indicates robustness of the proposed method.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine EBA and MONTE in their tablet dosage form. The result obtained for EBA and MONTE was comparable with the corresponding labelled amounts (Table 4)

Table 4 Assay Results for the Combined Dosage Form Using the Proposed Method

Sample	Label Claim (mg/tab)	Amount Found ± SD[a]	% Label Claim ± SD[a](n[b] =6)
EBA	10	10.01 ± 0.066	100.14 ± 0.66
MONTE	10	10.04 ± 0.042	100.43 ± 0.42

[a]SD = Standard deviation.

[b]*n* = Number of determinations.

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 10–50 µg/mL with co-efficient of correlation, (r^2) = 0.9961 and (r^2) = 0.9981 for EBA and MONTE, 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 method can be used for the routine analysis of the EBA and MON in combined dosage form without any interference of the excipients.

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