Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Montelukast Sodium and Ebastine in Tablet Dosage Form

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A rapid and sensitive RP-HPLC method with UV detection (244 nm) for routine analysis of montelukast sodium and ebastine in a pharmaceutical formulation (Ebast-M) was developed. Chromatography was performed with mobile phase containing a mixture of methanol:acetonitrile:ammonium acetate (80:10:10, % v/v/v), pH of mobile phase was adjusted 5.5 using glacial acetic acid and flow rate was 1.2 ml/min. The method was validated for linearity, accuracy, robustness and intermediate precision. The linearity was established over the concentration range of 0.01–0.06 mg/ml for both drugs. The correlation coefficients (r^2) for ebastine and montelukast were 0.9989 and 0.9955, respectively. Statistical analysis of the data showed that the method was precise, accurate, reproducible and selective for the analysis of ebastine and montelukast drugs. The method was successfully employed for the determination of ebastine and montelukast in commercially available tablet dosage form.

Key words: Ebastine, method validation, montelukast, reversed-phase high-performance liquid chromatography, tablets

Montelukast Sodium (MNK) chemically, (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl) vinyl) phenyl) 3-(2-(2-hydroxypropan-2yl) phenyl) propylthio) methyl) cyclopropyl) acetic acid[1,2] is a cysteinyl leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies[3-5]. Literature survey reveals that assay of MNK in bulk and tablet dosage form is official in Indian Pharmacopoeia 2010[6]. Ebastine (EBA), chemically, 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl) butan-1-one is a nonsedating H_1 antihistamine. Assay of ebastine in bulk form is official in British Pharmacopoeia[7]. Literature survey reveals that analytical methods, including UV spectrophotometer, and HPLC methods, are available for the determination of montelukast in pharmaceutical dosage forms. The analytical methods reported for estimation of MNK and EBA alone and with other drug combination are UV spectrophotometry[8-11], HPLC[12-14], HPLC/PDA[15], LC-MS[16] and HPTLC[17-19] methods. The combination of MNK and EBA has recently been introduced into the market. However, so far, no method was reported for the simultaneous estimation of MNK and EBA, in combination. The proposed method is rapid, simple, accurate and reproducible, and can be successfully employed in the routine analysis of both these drugs simultaneously, in tablet dosage form. The proposed method is optimised and validated as per the ICH guidelines[20]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously using RP-HPLC method. This study attempts to describe a rapid and sensitive HPLC method with UV detection, useful for routine quality control of MNK and EBA in pharmaceutical formulation. The structure of MNK and EBA was depicted in fig. 1.

A pure drug MNK was obtained as gift sample from Alembic Pharmaceuticals, Vadodara and EBA was procured as gift sample from Kivi Labs Pvt, Vadodara. Methanol AR was used as solvent. Calibrated glassware was used throughout the work. The marketed formulation studied was Ebast-M tablets manufactured by Micro Labs Pvt Ltd. Each tablet contains 10 mg MNK and 10 mg EBA.

The HPLC instrument was a Shimadzu chromatographic system equipped with an injection

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valve (Rheodyne 033381); Shimadzu LC 10AT UV dual absorbance detector (SPD 10A) was used. A reversed-phase C18 column (Phenomenex-Gemini 150×4.6 mm, 5 µm) was used. Peak area integration was performed using Sprinchrome software. A mixture of methanol:acetonitrile:ammonium acetate (80:10:10, % v/v/v) pH adjusted 5.5 using glacial acetic acid was used as a mobile phase with 1.2 ml/min flow rate.

Accurately weighed quantity of MNK (100 mg) and EBA (100 mg) were transferred to two separate 100 ml volumetric flasks, dissolved in little amount of methanol and diluted to the mark with methanol and was denoted as stock solutions (1000 µg/ml of MNK and 1000 µg/ml of EBA). From each of this solution, 5 ml was diluted to 50 ml with methanol to give 100 µg/ml of MNK solution and 100 µg/ml of EBA solution.

Ten tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to about 10 mg of MNK and 10 mg of EBA was transferred to 100 ml volumetric flask and dissolved in 50 ml of mobile phase. The volume was made up to the mark using mobile phase as solvent. The resulting solution was filtered through vacuum filter and 2 ml of this filtrate was appropriately diluted to get concentration of 20 µg/ml of MNK and 20 µg/ml of EBA.

UV spectrum of both the standard solutions was taken separately. After overlay, it was found that at 244 nm both drugs showed same absorbance (isobestic point); hence 244 nm was selected as analytical wavelength for both drugs. The standard solution of MNK and EBA were scanned under the chromatographic condition described above.

The calibration curve was plotted with six concentrations of 10-60 µg/ml for MNK and EBA. The curve was repeated thrice for each dilution. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Before injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. Peak area was recorded for all the solutions. The correlation graph was constructed by plotting the peak area obtained at the optimum wavelength of detection *versus* the injected amounts.

The mobile phase was a mixture of methanol:acetonitrile:ammonium acetate (80:10:10% v/v/v), pH of mobile phase was adjusted 5.5 using glacial acetic acid and flow rate was 1.2 ml/min. The UV detector wavelength was set at 244 nm (fig. 2) and the temperature was set at 26±2°C.

The applied chromatographic conditions permitted a good separation of MNK and EBA, (fig. 3), no drug decomposition was observed during the analysis. Results obtained in the study of the solution (both reference and sample solution) where it can be

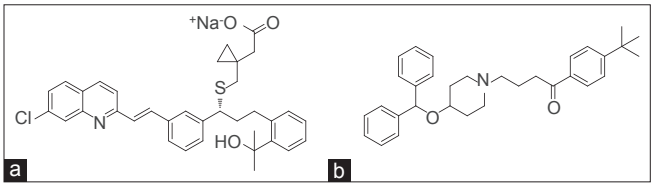

Fig. 1: Chemical structures of the analytes.
(a) Montelukast sodium, (b) ebastine.

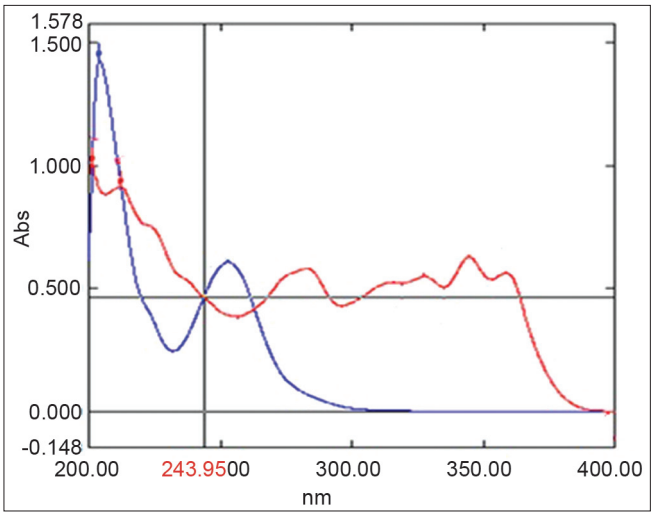

Fig. 2: Overlay UV Spectrum of MNKT and EBA.
Overlay UV Spectrum of MNKT and EBA showing selection of wavelength detection, i.e. isobestic point at 244 nm.

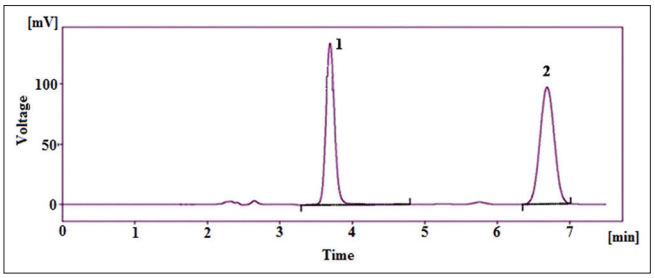

Fig. 3: HPLC chromatogram.
Chromatogram of montelukast sodium (1, 40 µg/ml) and ebastine (2, 40 µg/ml) using methanol:acetonitrile:ammonium acetate (80:10:10, v/v/v) as mobile phase.

noticed that solutions were stable for 48 h, as during this time the results does not decrease below the minimum percentage (96%). The chromatographic separation, as explained above was carried out with HPLC to evaluate the chromatographic parameters, column efficiency (N), tailing factor and resolution between two consecutive peaks (Table 1). Resolution observed for MNK and EBA was 5.940. Number of plates observed for MNK and EBA were 4659.66 and 4312, respectively. Tailing factors observed for MNK and EBA were 0.995 and 1.119, respectively. The data for accuracy for MNK and EBA are presented in Table 2. The recovery range for MNK and EBA were found to be 98.97-99.83% and 98.75-99.63%, respectively. The calibration curve for MNK and EBA was found to be linear over the range of 10-60 µg/ml for both drugs. The %RSD for repeatability was found to be 1.758 and 1.403 for MNK and EBA, respectively. The range for intraday precision %RSD was found to be 1.772-1.86% for MNKT and 1.096-1.944% for EBA. The range for intraday precision %RSD was found to be 0.563-1.248% for MNK and 0.347-1.396% for EBA. The %RSD for pH Change (±0.2) were found to be 1.028-1.284 and 1.046-1.169 for MNK and EBA, respectively, and for mobile phase ratio change were found to be 1.207-1.462 and 1.138-1.308 for MNK and EBA, respectively. The limit of detection for MNK and EBA was found to be 0.819 µg/ml and 0.667 µg/ml, whereas the limit of quantification for MNK and EBA was found to be 2.484 µg/ml and 2.02 µg/ml, respectively. All validation parameters were summarised in Table 3. The proposed high-performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy and specificity and proved to be convenient and effective for the quality control of MNK and EBA in pharmaceutical dosage forms. The measured signal was shown to be precise, accurate and linear over the concentration range tested with a correlation coefficient better. Method validation has been demonstrated by various tests for linearity, accuracy, precision and ruggedness. In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analysed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of MNK and EBA remained almost unchanged (% RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

TABLE 1: SYSTEM SUITABILITY PARAMETERS OF MNK AND EBA

System suitability parameters	Drugs	Observed values	IP' 2007 specification
Resolution (R_s)	Between MNK and EBA	5.94	>1.5
Number of theoretical plates (N)	MNK	4966	Not less than 2000
	EBA	4464	
Tailing factor (T_f)	MNK	1.034	Not greater than 2.0
	EBA	1.152	

MNK=Montelukast sodium, EBA=Ebastine

TABLE 2: ACCURACY DATA OF MNK AND EBA

Amt of sample (µg/ml)		Amt. of drug added (µg/ml)		Amt. recovered mean±SD (*n*=3) (µg/ml)		% recovery *n*=3	
MNK	EBA	MNK	EBA	MNK	EBA	MNK	EBA
10	10	-	-	9.915±0.065	9.963±0.62	99.15	99.63
10	10	20	20	19.91±0.28	19.75±1.55	99.55	98.75
10	10	30	30	29.95±0.26	29.79±0.32	99.83	99.3
10	10	40	40	39.59±0.25	39.69±0.42	98.97	99.22

MNK=Montelukast sodium, EBA=Ebastine, SD=Standard deviation

TABLE 3: SUMMARY OF VALIDATION PARAMETERS

Parameters	MNK	EBA
Linearity range	10-60 µg/ml	10-60 µg/ml
Correlation coefficient	0.9989	0.9955
Precision (% RSD)		
Repeatability	1.758	1.403
Intraday (*n*=3)	1.772-1.86	1.096-1.944
Interday (*n*=3)	0.563-1.248	0.347-1.396
Mean % recovery	98.87-99.83	98.75-99.3
Robustness		
pH Change (±0.2)	1.028-1.284	1.046-1.169
Mobile phase ratio change	1.207-1.462	1.138-1.308
Limit of detection	0.819 µg/ml	0.667 µg/ml
Limit of quantification	2.484 µg/ml	2.02 µg/ml

MNK=Montelukast sodium, EBA=Ebastine, RSD=Relative standard deviation

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