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Development and validation of simultaneous equation method for estimation of ebastine and montelukast sodium in combined tablet dosage form

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ABSTRACT

Simultaneous Equation Method for simultaneous estimation of Ebastine and Montelukast sodium in combined tablet dosage form has been developed. The UV spectrophotometric method is the Simultaneous Equation method, which involves the formation of absorbance equation at 253.00 nm maximum absorption of Ebastine and at 344.00 nm the maximum absorption of Montelukast Sodium. The linearity ranges for Ebastine and Montelukast Sodium were 5-45 µg/ml and 5-45 µg/ml respectively. The accuracy of the method was assessed by recovery studies was found to be and 100.43 ±0.1893 and 100.22 ± 0.3215 for Simultaneous Equation method for Ebastine and Montelukast Sodium respectively. Developed method is simple, accurate and rapid; require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Key words: Ebastine, Montelukast Sodium, Simultaneous Equation Method, UV Spectrophotometric method.

INTRODUCTION

Ebastine (EBS) 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. The chemical structure of EBS is shown in Figure 1.

Montelukast sodium (MNLT), (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid. It is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Montelukast is a $CysLT_1$ antagonist; it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor $CysLT_1$ in the lungs and bronchial tubes by binding to it. This reduces the bronchoconstriction otherwise caused by the leukotriene and results in less inflammation. The chemical structure of MNLT is shown in Figure 2.

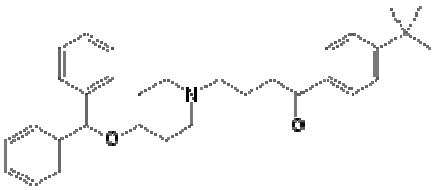


Figure 1

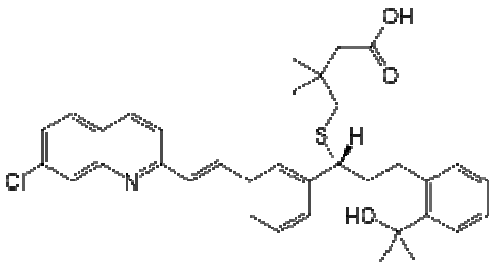


Figure 2

Literature survey reveals that no method for simultaneous estimation of EBS and MNLT in tablet dosage form has been reported. However, simple UV methods [1-3] and RP-HPLC [4] for EBS and simple UV methods [5-8] and RP-HPLC [9-11] for MNLT have been noted. Hence attempt has been made to develop and validate in accordance with ICH guidelines, a simple, precise and accurate spectrophotometric method for Simultaneous estimation of EBS and MNLT in combined tablet.

MATERIALS AND METHODS

Ebastine was supplied by Vashudha Pharma Chem. Limited (Hyderabad, Andhra Pradesh) and Montelukast Sodium was supplied by Shree Pharma International (Vadodara) as a gift sample. Methanol AR grade was purchased from Merck Lab. and Qualigens Fine Chemicals Pvt. Ltd., India. Tablets of Ebast-M were purchased from local market; each tablet was labelled to contain 10 mg of EBS and 10 mg of MNLT.

INSTRUMENTATION:

UV-Visible spectrophotometer (Shimadzu 1800 with UV Probe 2.21 software) and a pair of 1 cm matched quartz cells were used. Shimadzu AUX 220 weighing balance.

SELECTION OF SOLVENT:

Ebastine and Montelukast Sodium are soluble in methanol. So, Methanol is selected as solvent & used for preparation of stock solution & working standards.

SELECTION OF SUITABLE WAVELENGTHS FOR ANALYSIS:

Solutions containing appropriate concentration of EBS and MNLT in methanol were scanned using UV spectrophotometer in "Spectrum mode" in the range of 400 - 200 nm and their spectra were overlaid. From overlaid spectra of both the drugs analytical wavelengths for detection were selected.

Figure 3- Spectrum of Ebastine (45 µg/ml)

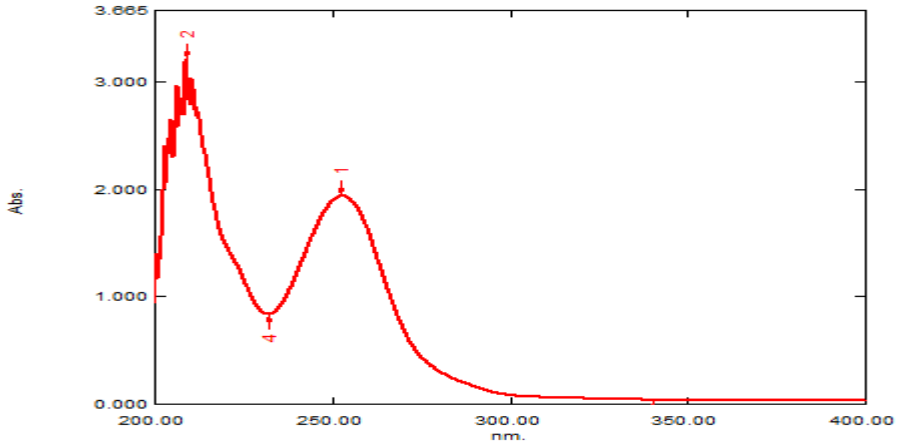


Figure 4- Spectrum of Montelukast Sodium (45 µg/ml)

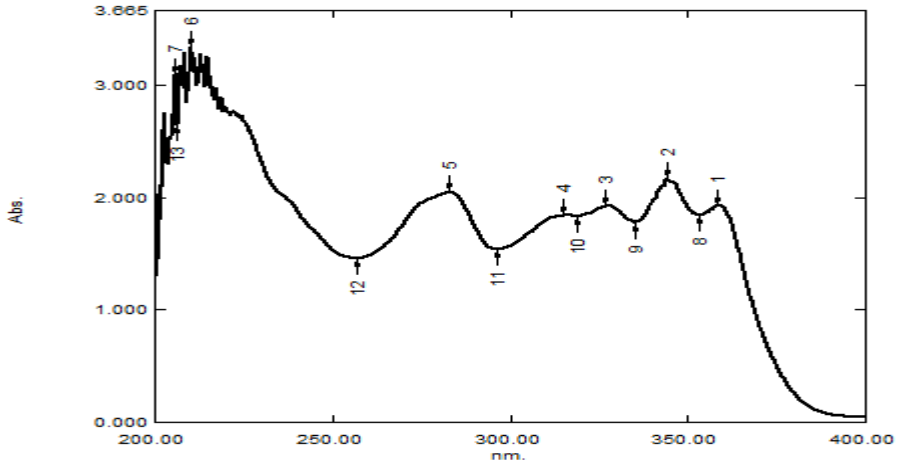
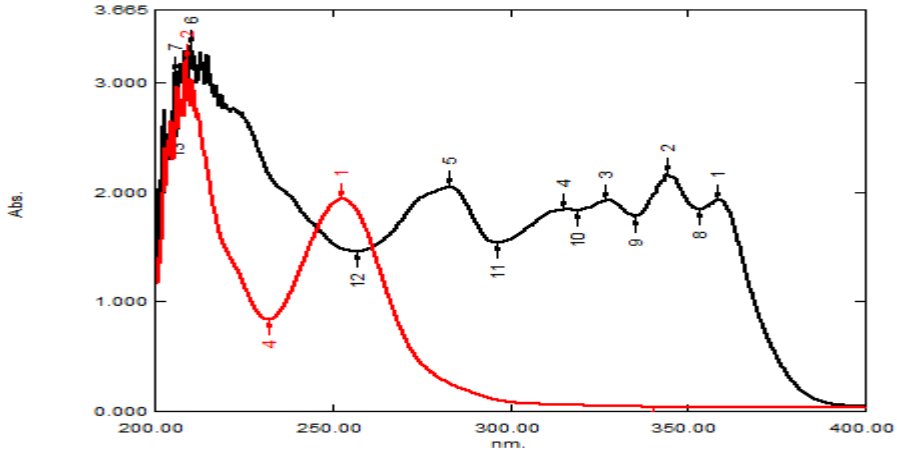


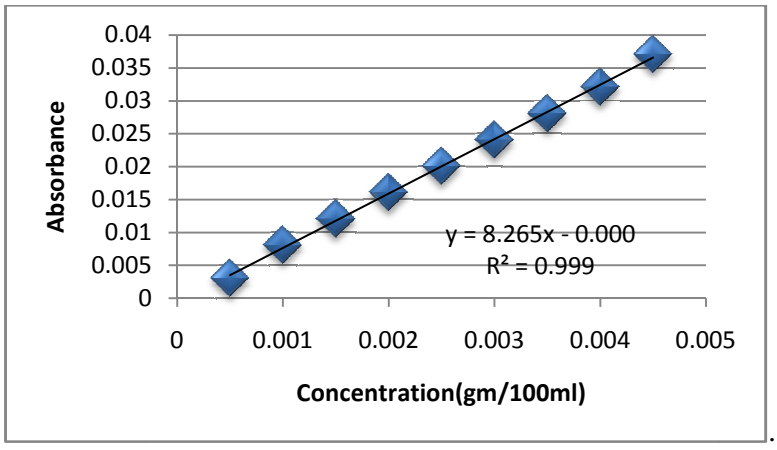
Figure 5- Overlaid spectra of Ebastine and Montelukast Sodium in methanol (45 µg/ml of EBS and 45 µg/ml of MNLT)



SPECTROSCOPIC CONDITIONS:
Following spectroscopic conditions were optimized for analysis:
Solvent: Methanol
Measuring Mode: Spectrum
Scanning Range: 400 - 200 nm
Absorbance Range: 0.0 - 4.0 Abs Unit
Scanning Speed: Medium
Detection Wavelengths: 344.00 nm (λ_{max} of MNLT) and 253.00 nm (λ_{max} of EBS)

PREPARATION OF STANDARD SOLUTIONS:
PREPARATION OF EBS STOCK - WORKING STANDARD SOLUTION:
Accurately weighed 25 mg of EBS was transferred to 25 ml volumetric flask, dissolved and diluted up to mark with methanol to obtain final concentration of 1000 µg/ml EBS. Solution was further diluted with methanol to obtain working standard solutions of 100 µg/ml of EBS.

Figure 6- Calibration curve of Ebastine at 344.00 nm by Simultaneous Equation Method



PREPARATION OF MNLT STOCK - WORKING STANDARD SOLUTION:
Accurately weighed 25 mg of MNLT was transferred to 25 ml volumetric flask, dissolved and diluted up to mark with methanol to obtain final concentration of 1000 µg/ml MNLT, which was used as working standard solution. Solution was further diluted with methanol to obtain working standard solutions of 100 µg/ml of MNLT.

PREPARATION OF SOLUTION OF EBS AND MNLT FOR CALIBRATION CURVE:
Six replicate series of standard solutions were prepared by dilution of the working standard solutions with methanol to reach concentration range of 5 - 45 µg/ml for EBS and 5- 45 µg/ml for MNLT.

PREPARATION OF CALIBRATION CURVES:

Absorbance of prepared standard solutions having concentration 5, 10, 15, 20, 25, 30 , 35, 40 and 45 µg/ml for EBS and 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg/ml for MNLT were measured at 344.00 nm and 253.00 nm. Standard calibration curves of Absorbance against Concentration were plotted. Absorptivity coefficients were determined using calibration curves at both the wavelengths.

Figure 7- Calibration curve of Ebastine at 253.00 nm by Simultaneous Equation Method

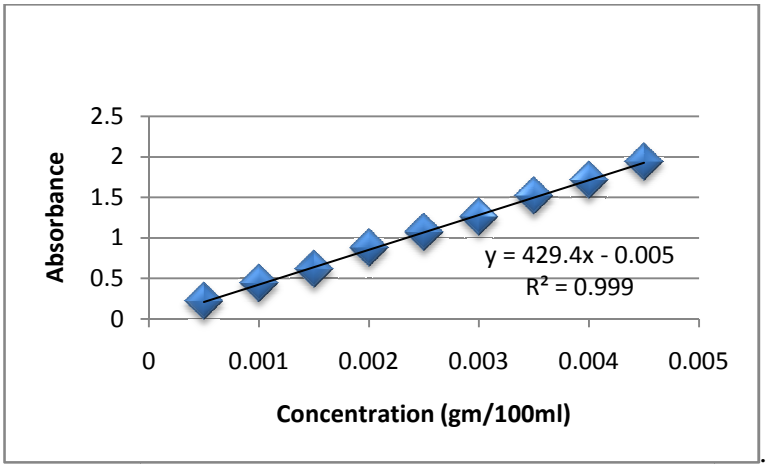


Figure 8- Calibration curve of Montelukast Sodium at 253.00 nm by Simultaneous Equation Method

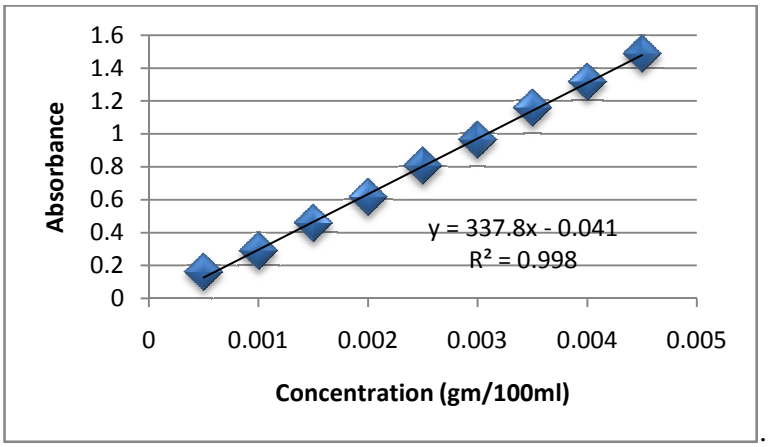
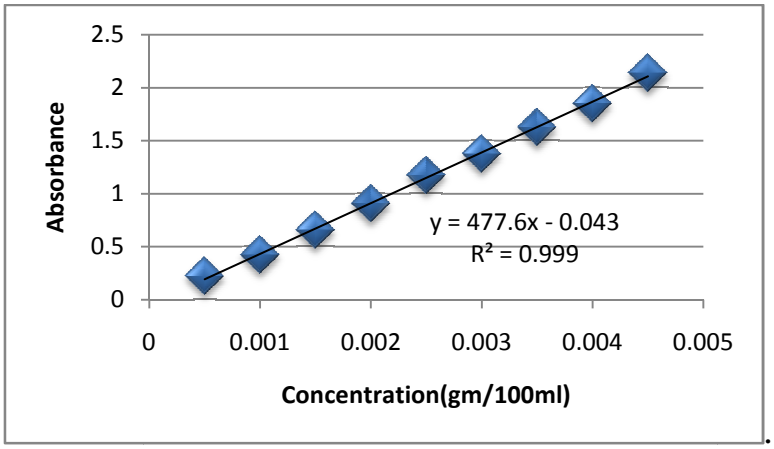


Figure 9- Calibration curve of Montelukast Sodium at 344.00 nm by Simultaneous Equation Method



SIMULTANEOUS METHOD FOR ANALYSIS OF PHARMACEUTICAL FORMULATION: [12-15]

Twenty tablets were weighed accurately and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 25 mg of EBS and 25 mg of MNLT were weighed and

transferred to 25 ml volumetric flask. To this flask, 15 ml methanol was added and the flask was sonicated for 5 min. The volume was adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. Filtrate contained mixture of 1000 µg/ml EBS and 1000 µg/ml MNLT. The filtrate solution was suitably diluted with methanol to get a final concentration of 10 µg/ml of EBS and 10 µg/ml of MNLT. The absorbance of prepared sample solution i.e. A1 and A2 were recorded at 253.00 nm and 344.00 nm respectively. Relative concentration of two drugs in the sample was calculated using equation (1) and (2). The analysis procedure was repeated six times with tablet formulation.

The concentration of Cx and Cy can be obtained as

$$C\,x = \frac{A2\,aY1 - A1\,aY2}{ax2\,aY1 - ax1\,aY2} \qquad (01)$$

$$C\,y = \frac{A1ax2 - A2\,ax1}{ax2\,aY1 - ax1\,aY2} \qquad (02)$$

Where, λ1 = 253.00 nm (λmax of EBS); λ2 = 344.00 nm (λmax of MNLT),
A1 = absorbance of mixture at 253.00 nm,
A2 = absorbance of mixture at 344.00 nm,
aX1 = absorptivity of EBS at 253.00 nm,
aY1 = absorptivity of MNLT at 253.00 nm,
aX2 = absorptivity of EBS at 344.00 nm,
aY2 = absorptivity of MNLT at 344.00 nm.

Figure 10- Spectrum of Standard mixture solution (20 µg/ml of EBS and 20 µg/ml of MNLT)

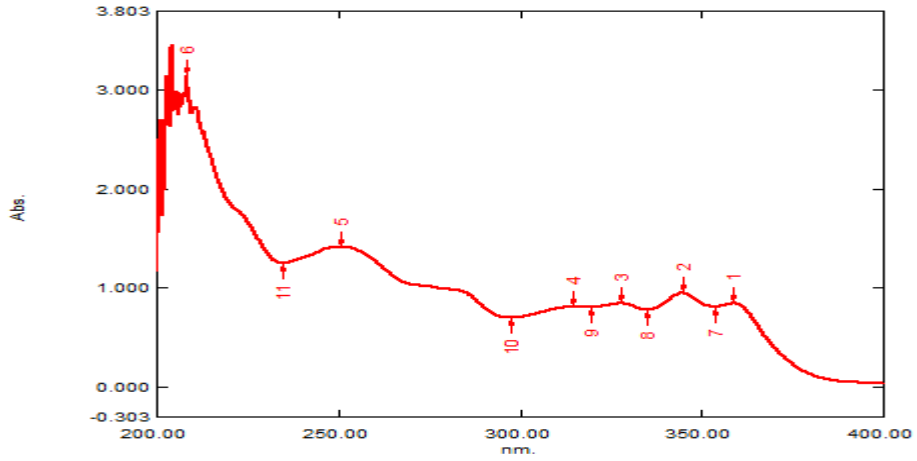
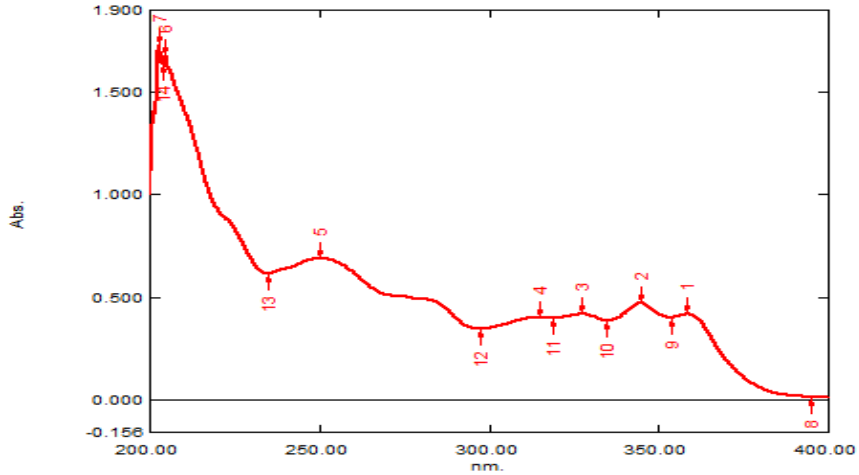


Figure 11- Spectrum of Tablet Formulation by Simultaneous Equation method (10 µg/ml of EBS and 10 µg/ml of MNLT)



VALIDATION OF METHOD: [16]
Validation of developed method was carried out according to ICH guideline for Validation of Analytical Procedures Q2 (R1).

LINEARITY:
Solutions having concentration 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg/ml for EBS and concentration 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg/ml for MNLT were prepared from working standard solution. Prepared solutions were analyzed as per the proposed method. Six replicate analyses were carried out. The mean absorbance with its standard deviation and % relative standard deviation were calculated for both the drugs. Mean absorbance against concentration were plotted to obtain the calibration curves. Regression equations, co-relation coefficients were computed from calibration curves.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):
LOD and LOQ were calculated from the data obtained from the linearity studies. For each of the six replicate determinations, slope and y-intercept of the linearity plot was determined. Average of slope (S) and standard deviation of the y intercept (σ) was computed. From these values, the parameters LOD and LOQ were determined using following equations (On the basis of response and slope of the regression equation):

LOD = 3.3 σ\S
LOQ = 10 σ\S
Where; σ = Standard deviation of Response,
S = Slope of calibration curve

ACCURACY (RECOVERY STUDIES):
Accuracy was calculated by addition of standard drug to preanalysed sample at 3 different concentration level and computing percentage recoveries. Accuracy was assessed using 9 determinations over 3 concentration levels covering the specified range (e.g., 3 concentrations and 3 replicates each of the total analytical procedure).

Prepared solutions were analyzed as per the proposed method. % recoveries were calculated from simultaneous equation. The mean percentage recovery with its standard deviation and % relative standard deviation were computed at each level.

PRECISION:
Precision of method was computed by two means: Repeatability and Intermediate precision

Repeatability: System Precision and Method Precision:

System Precision:
Solution containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT (100% test concentration) was prepared from their respective stock - working standard solution prepared. Prepared solution was analyzed six times as per the proposed method. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for both the drugs.

Method Precision:
Six replicate solutions containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT (100% test concentration) were prepared from their respective stock - working standard solution prepared. Prepared solutions were analyzed as per the proposed method. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for both the drugs.

Intermediate Precision: Intra-day precision and Inter-day precision:

Intraday Precision: Replication within same day at different time:
Solution containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT (100% test concentration) was prepared from their respective stock - working standard solution prepared. Prepared solution was analyzed as per the proposed method. Analysis was replicated for 6 different times within same day. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for both the drugs.

Interday Precision: Replication in different days:

Solution containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT (100% test concentration) was prepared from their respective stock - working standard solution prepared. Prepared solution was analyzed as per the proposed method. Analysis was replicated for 6 different days. The mean% labelled claim with its standard deviation and % relative standard deviation was computed for both the drugs.

ROBUSTNESS:
Solution containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT was prepared from their respective stock - working standard solution prepared. Prepared solution was analyzed as per proposed method with small but deliberate change in Spectroscopic as listed below:

1) Scanning Speed: Fast; Medium; Slow
2) Methanol from different manufacturers:
Methanol AR Grade: Merck ltd., India; Qualigens Fine Chemicals Pvt. Ltd., India
The mean % labelled claim with its standard deviation and % relative standard deviation was computed at each level.

RUGGEDNESS:
Solution containing mixture of 10 µg/ml EBS and 10 µg/ml MNLT was prepared from their respective stock - working standard solution prepared. Prepared solution was analyzed as per proposed method by 2 different analysts. The mean % labelled claim with its standard deviation and % relative standard deviation was computed for each analysis.

RESULTS AND DISCUSSION

For this method linearity was observed in the concentration range of 5-45 µg/ml for both Ebastine and Montelukast Sodium. Ebast-M tablet was analyzed and amount of drug determined by proposed method as shown in Table 3. The proposed method was validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80, 100 and 120 % level. The % recovery obtained were 100.43 ± 0.1893 for EBS and 100.22 ± 0.3215 for MNLT. Precision was calculated as repeatability (% RSD is less than 1.0) and inter and intraday variations (%RSD is less than 1.0) for both drugs. The robustness and ruggedness data are presented in Table - 8 and 9 respectively.

Table 1- Result of calibration curves of Ebastine & Montelukast sodium by Simultaneous Equation Method

Parameter		Ebastine	Montelukast
Regression equation	λ_1	y = 429.4x - 0.005	y = 337.8x - 0.041
	λ_2	y = 8.265x - 0.000	y = 477.6x - 0.043
Correlation coefficient	λ_1	0.999	0.998
	λ_2	0.999	0.999

Table 2- Absorptivity values of Ebastine & Montelukast sodium by Simultaneous Equation Method

Drug	Absorptivity	
	λ_1= 253.00 nm	λ_2 =344.00 nm
Ebastine	aX_1= 429.4	aX_2=8.265
Montelukast Sodium	aY_1=337.8	aY_2=477.6

Table 3- Assay result by Simultaneous Equation Method

S. No.	Concentration (µg/ml)		Concentration Obtained (µg/ml)		%Labelled Claim	
	EBS	MNLT	EBS	MNLT	EBS	MNLT
1	10.00	10.00	10.03	9.93	100.3	99.3
2	10.00	10.00	10.09	10.02	100.9	100.2
3	10.00	10.00	10.12	10.12	101.2	101.2
4	10.00	10.00	9.89	9.86	98.9	98.6
5	10.00	10.00	9.96	10.07	99.6	100.7
6	10.00	10.00	10.01	10.05	100.1	100.5

Table 4- Summary for Assay result by Simultaneous Equation Method

Drug	%Labelled Claim (Mean, n=6)	SD (n=6)	%RSD
EBS	100.16	0.8430	0.8416
MNLT	100.10	0.9620	0.9613

Table 5- Result of linearity range, LOD and LOQ for Simultaneous Equation Method

Parameter		EBS	MNLT
Range		5-45µg/ml	5-45µg/ml
Linearity	Equation	y = 429.4x - 0.005	y = 337.8x - 0.041
		y = 8.265x - 0.000	y = 477.6x - 0.043
	R^2	0.999	0.998
		0.999	0.999
	%RSD	0.3014	0.4203
		0.1814	0.3231
LOD		0.19 µg/ml	0.19 µg/ml
LOQ		0.63 µg/ml	0.62 µg/ml

Table 6- Result of accuracy study for Simultaneous Equation Method

Level		Total Concentration in µg/ml		Concentration Recovered in µg/ml		% Recovery		SD		%RSD	
		EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT
L-1	1	16	16	16.12	16.02	100.75	100.13	0.6384	0.6760	0.6354	0.6739
	2			16.15	16.17	100.94	101.06				
	3			15.96	15.96	99.75	99.75				
	Mean % Recovery					100.48	100.31				
L-2	1	20	20	20.07	20.09	100.35	100.45	0.1893	0.3215	0.1885	0.3208
	2			20.06	19.97	100.30	99.85				
	3			20.13	20.07	100.65	100.35				
	Mean % Recovery					100.43	100.22				
L-3	1	24	24	24.05	23.97	100.21	99.88	0.2917	0.2774	0.2911	0.2769
	2			24.12	24.06	100.50	100.25				
	3			23.98	24.14	99.92	100.58				
	Mean % Recovery					100.21	100.24				

Table 7- Result of Precision for Simultaneous Equation Method

Parameter	Drug	% Labelled Claim (n=6)	SD (n=6)	% RSD (n=6)
System Precision	EBS	99.91	0.1151	0.1152
	MNLT	99.16	0.6135	0.6187
Method Precision	EBS	99.96	0.7312	0.7314
	MNLT	99.89	0.5537	0.5543
Intraday Precision	EBS	99.85	0.4579	0.4586
	MNLT	99.78	0.5072	0.5083
Interday Precision	EBS	99.39	0.5952	0.5989
	MNLT	99.67	0.4982	0.4999

Table 8- Result of Robustness study for Simultaneous Equation Method

Variation & Level		Concentration (µg/ml)		%Labelled Claim		Mean		SD		%RSD	
		EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT
Change in scanning speed	Slow	10	10	99.82	99.93	100.00	100.06	0.1724	0.1301	0.1724	0.1300
	Medium	10	10	100.04	100.07						
	Fast	10	10	100.16	100.19						
Change in methanol Manufacture	Merck	10	10	99.89	99.92	100.29	99.98	0.5656	0.0848	0.5640	0.0848
	Qualigens	10	10	100.69	100.04						

Table 9- Result of Ruggedness Study for Simultaneous Equation Method

Variation & Level		Concentration (µg/ml)		%Labelled Claim		Mean		SD		%RSD	
		EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT	EBS	MNLT
Different Analyst	Analyst-1	10	10	100.01	99.95	100.57	100.51	0.7919	0.7919	0.7874	0.7879
	Analyst-2	10	10	101.13	101.07						

CONCLUSION

The proposed method was found to be simple, accurate and rapid for the routine determination of Ebastine and Montelukast Sodium in tablet formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity, accuracy, precision, robustness & ruggedness. So, method can be successfully used for simultaneous estimation of Ebastine and Montelukast Sodium in combined dosage form.

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REFERENCES

[1] Ibrahim F, Sharaf El- Din MK, Eid M, *IJPSR*, **2011**, 2, 2056.
[2] Soni LK, Narsinghani T, Saxena C, *International Journal of ChemTech Research*, **2011**, 3(4), 1918.
[3] Soni LK, Narsinghani T, Saxena C, *Der Pharmacia Sinica*, **2011**, 2(6), 11.
[4] Wagh R, Hajare R, *IJPRD*, **2011**, 3(7), 214.
[5] Pawar V, Pai S, Roa G, *Jordan Journal of Pharmaceutical Sciences*, **2008**, 1(2), 152.
[6] Choudekar R, Mahajan M, Sawant S, *Int J Pharm Pharm Sci*, **2012**, 4(3), 737.
[7] Patel D, Patel S, *IJPBR*, **2010**, 1(3), 71.
[8] Pallavi K, Srinivasa P, *International Journal of Pharmaceutical and Medical Sciences*, **2012**, 1(2), 104.
[9] Patel SA, Patel SK, Patel DJ, Patel NJ, *International Journal of PharmTech Research*, **2010**, 2(3), 1767.
[10] Naga K, Swamy T, Rao A, *IJPCBS*, **2011**, 1(1), 12**.**
[11] Madhavi B, Mrudula B, *International Journal of ChemTech Research*, **2010**, 2(1), 471.
[12] Joshi R, Gupta R, *Der Pharmacia Sinica*, **2010**, 1 (2), 44.
[13] Savithri R, Ramalingam P, *Der Pharmacia Sinica*, **2011**, 2 (5), 251.
[14] Prathap B, Nagarajan G, *Der Pharmacia Lettre*, **2011**, 3(3), 62.
[15] Sharma T, Mishra N, *Der Pharmacia Lettre*, **2010**, 2(5), 302.
[16] ICH, Q2 (R1), Harmonised tripartite guideline, Validation of analytical procedures: Text and Methodology International Conference on Harmonization ICH, Geneva, **2005**.