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QUALITY BY DESIGN BASED METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND BILASTINE IN TABLET DOSAGE FORM

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ABSTRACT

Background: By considering the current regulatory requirement for an analytical method, it was optimized using analytical QbD approach and the method was validated to be simple, precise, accurate and robust in terms of change of chromatographic and technical variables in combination approved to manage Asthma and allergic conditions.

Objective: To establish a RP-HPLC approach for the synchronized analysis of Montelukast Sodium (MNT) and Bilastine (BST) in Tablet Dosage Form

Study Design: Quality by design-based method development was done using "Design Expert ® (Version 13)" and its validation was performed as recommended in "ICH guideline".

Materials and methods: Method develop with chromatographic parameters as C_{18} column Inertsil ODS (150 * 4.6mm, 5µm), HPLC system with PDA detector with 0.05M KH_2PO_4 Buffer: Acetonitrile (65:35 % v/v) mobile phase & 1.0 mL/min flow rate and detected at 280 nm. QbD approach was applied to estimate the effect of two factors i.e., mobile phase composition and flow rate on the innumerable chromatographic responses.

Results: The perfect sharp peak observed at Retention time of BST and MNT were 3.367 and 6.314 min respectively. It was showed linear calibration curve in the quantity range 10-30 µg/mL and 5-15 µg/mL. %Assay of drugs was 100.67% and 99.67% for BST & MNT.

Conclusion: Developed method was found to be accurate, precise and rapid for simultaneous estimation of BST and MNT in tablet.

Keywords: Bilastine and Montelukast sodium, RP-HPLC-PDA System, QbD Approach, Validation

INTRODUCTION

Bilastine is designated chemically as 2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropanoic acid **(Figure 1)** is the Antihistaminic used as Antiallergic mediator **[1]**. BST is a selective H_1 receptor antagonist. BST reduces the progress of allergic signs due to the proclamation of histamine from mast cells. It includes Stability signifying approaches **[2]**, RP-HPLC with another drugs **[3-5]**.

Montelukast sodium designated chemically as 2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl methyl] cyclopropyl] acetic acid **(Figure 2)** is a compound of Leukotriene receptor opponent and used as Antiallergic **[6-7]**. MNT is binds with high affinity to the cysteinyl leukotriene receptor for D4 and E4. These leukotrienes are excreted by various types of cells, elaborate in the inflammatory progression that may cause the signs-symptoms of asthma. It include RP-HPLC **[8]**, analytical signifying technique with another drugs **[9-19]**.

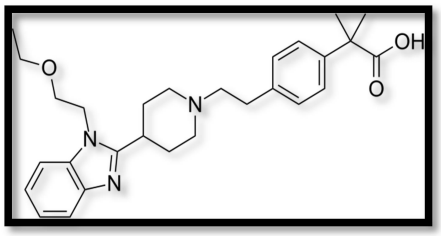


Figure 1: Chemical structure of BST

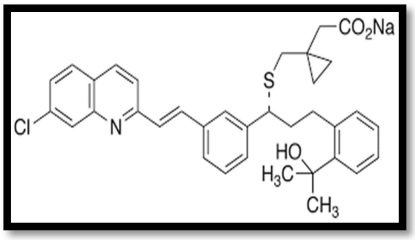


Figure 2: Chemical structure of MNT

Literature exploration didn't reveal that estimation method for MNT and BST in tablet dosage form. Consequently, the aim of the study was to develop, optimize and validate a simple and rapid RP-HPLC method for the simultaneous determination of MNT and BST by QbD approach **[20]**.

MATERIALS AND METHODS

Reagents and Chemicals:

BST and MNT have been acquired as gift samples from Elite pharma, tablet was purchased from local market. HPLC grade Water and Acetonitrile of analytical grade have been acquired from Finar chemical compounds.

Instruments and Chromatographic Conditions:

Young lin instrument, YL 9100 clarity HPLC software with PDA detector was used for method development and validation. The separation was accomplished on Inertsil ODS C_{18} column (150mm x 4.6mm, 5µm) and the eluent was monitored at 280nm. The mixture of 0.05M KH_2Po_4Buffer: Acetonitrile (65:35 %v/v) at a flow rate of 0.1 mL/min with 20µl injection capacity was used as a mobile phase.

Preparation of solutions

Preparation of BST standard stocks solutions (200 µg/mL): A 20 mg of BST weighed exactly and transferred in to 100 mL volumetric flask and dissolved with diluent, added diluent up to the mark and assorted systematically.

Preparation of MNT standard stocks solutions (100 µg/mL): A 10 mg of MNT weighed and lifted in to 100 mL volumetric flask and dissolved with diluent, added diluent up to the mark and assorted meticulously.

Working standard solution of BST and MNT (20:10 µg/mL): A 1 mL of standard stock solution of BST (200 µg/mL) and 1 mL of ordinary stock solution of MNT (100 µg/mL) were relocated in to 10 mL volumetric flask, added diluent up to the mark and mixed methodically.

Preparation of Mobile phase: Accurately measured 650 mL of phosphate buffer and 350 mL of HPLC grade Acetonitrile were relocated into beaker, mixed it and degassed by sonicator for 30 min.

Software aided method optimization: A "3^2 factorial experimental design" was individually pragmatic for both drugs to optimize the chromatographic circumstances. A 3^2 factorial strategy designates that there are "three levels" and "two factors" elaborate in it. The three levels: low (-1), medium (0) and high (+1) whereas the factors: A (mobile phase ratio) and B (flow rate). It exposed in **Table 1** for Experimental factors and levels used in factorial design. "Analysis of variance (ANOVA)" was pragmatic to the response variables to scrutinize the significance of the model. Lack of fit test, which specified insignificant lack of fit value corresponding to a higher p-value as equated to the model F-value, was also used to scrutinize the pragmatic model. The responses revealed in **Table 2 & 3** and **Figure 3 to 5**.

Design of experiment

A "3^2 full factorial design" was accomplished using 09 experimental runs for BIL & MNT. The dependent & independent variables exposed in **Table 2 & 3**. The proposed regression equations intended for chromatographic responses of both the drugs are given in the **Table 4**.

System suitability parameters:

System suitability tests were accomplished to verify that the resolution and repeatability of the parameters was

patterned by injecting three times the test solution of BST 20 µg/mL and MNT 10 µg/mL a chromatogram of the mixture in optimized circumstances is exposed **Figure 6**. The outcomes exposed in **Table 5** had been within desirable limits.

Table 1: Experimental factors and levels used in factorial design:

Factor	Level used		
Independent variable	Low (-1)	Medium (0)	High (+1)
A = Mobile phase ratio (v/v)	68:32	65:35	63:37
B = Flow rate (mL/min)	0.98	1	1.02
Dependent variable	Y1,Y6 = Area of BST and MNT; respectively		
	Y2, Y7 = No. of theoretical plates of BST and MNT; respectively		
	Y3, Y8 = Resolution of BST and MNT; respectively		
	Y4, Y9 = Retention time of BST and MNT respectively		
	Y5, Y10 = Tailing factor of BST and MNT; respectively		

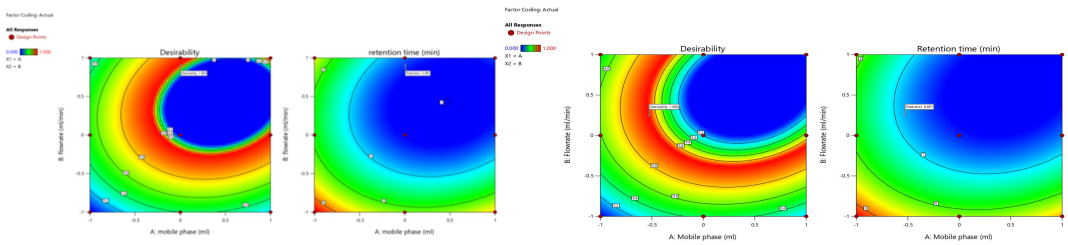
Table 2: Observed responses of experimental runs for BST

Run	Level	Factor		Response				
		Mobile phase (v/v)	Flowrate (mL/min)	Retention time (min)	Area (mAU)	Resolution	No. of Theoretical plates	Tailing factor
1	-1,-1	68:32	0.98	6.367	2230270	16.58	2234	0.98
2	0,-1	65:35	0.98	5.301	2221954	10.42	1736	0.68
3	1,-1	63:37	0.98	5.791	2270211	10.25	1722	0.64
4	-1,0	68:32	1	4.912	2207996	12.54	1966	0.86
5	0,0	65:35	1	3.505	2210302	11.22	1842	0.7
6	1,0	63:37	1	3.34	2240411	10.48	1742	0.62
7	-1,1	68:32	1.02	5.612	2255041	11.56	1862	0.72
8	0,1	65:35	1.02	3.368	2230430	10.52	1744	0.63
9	1,1	63:37	1.02	3.712	2260954	12.28	1944	0.8

Table 3: Observed responses of experimental runs for MNT

Run	Level	Factor		Response				
		Mobile phase (v/v)	Flowrate (mL/min)	Retention time (min)	Area (mAU)	Resolution	No. of Theoretical plates	Tailing factor
1	-1,-1	68:32	0.98	9.314	559822	16.58	17245	0.98
2	0,-1	65:35	0.98	8.443	556178	10.42	12555	0.86
3	1,-1	63:37	0.98	8.662	554046	10.25	12321	0.64
4	-1,0	68:32	1	7.764	554233	12.54	15987	0.79
5	0,0	65:35	1	6.337	552856	11.22	13210	0.65
6	1,0	63:37	1	6.837	552223	10.48	12542	0.62
7	-1,1	68:32	1.02	8.313	556045	11.56	14021	0.7
8	0,1	65:35	1.02	6.321	555264	10.52	12254	0.63
9	1,1	63:37	1.02	6.358	557242	12.28	14123	0.61

a. **b.**



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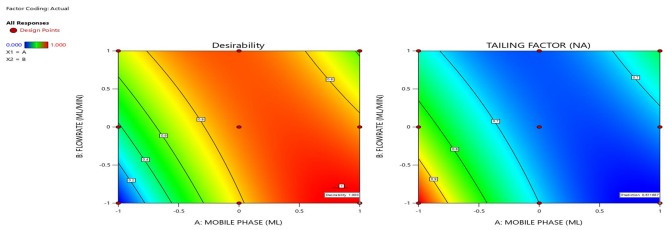

Figure 3: Contour plot of for chromatographic responses a. BST - Retention time b. MNT – Retention time c. BST - Tailing factor

Table 4: Regression equations for various chromatographic responses:

Drug	Regression equation
BST	**$Y1= 2204068.4 + 13044.83\ X1 + 3998.33\ X2 – 8507X1X2 + 23251.83\ X1^2 + 25240.33\ X2^2$**
	$Y2 = 1758.22 – 109\ X1 - 23.66\ X2 + 148.5\ X1X2 + 137.66\ X1^2 + 23.66\ X2^2$
	$Y3 = 10.372 - 1.2783X1 -0.4816\ X2 + 1.7625\ X1X2 +1.56167\ X1^2 + 0.52167\ X2^2$
	$Y4 = 3.3205 - 0.6746X1 -0.7945\ X2 - 0.331\ X1X2 + 0.89767\ X1^2 + 1.106167\ X2^2$
	$Y5 = 0.66 - 0.0833X1 - 0.025X2 + 0.105\ X1X2 + 0.1\ X1^2 + 0.015\ X2^2$
MNT	**$Y1 = 552546.78 - 1098.167X1 -249.167X2 + 1743.25\ X1X2 + 835.83\ X1^2 + 3328.83\ X2^2$**
	$Y2 = 12779.56 - 1377.834\ X1 - 287.16X2 + 1256.5X1X2 + 1700.16\ X1^2 + -159.83\ X2^2$
	$Y3 = 10.372 - 1.2783X1 -0.4816\ X2 + 1.7625\ X1X2 +1.56167\ X1^2 + 0.52167\ X2^2$
	$Y4 = 6.41867 - 0.589X1 - 0.9045X2 - 0.32575X1X2 + 0.841\ X1^2 + 0.9225\ X2^2$
	$Y5 = 0.68 – 0.1X1 -0.09X2 + 0.0625\ X1X2 + 0.01\ X1^2 + 0.05\ X2^2$

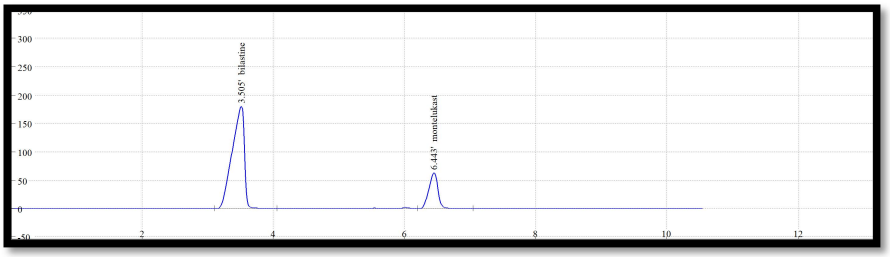

Figure 6: Optimised condition chromatogram of BST and MNT.

Method Validation:

1) Specificity:

It can be called as absence of any interference at retention times of samples. Specificity was executed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

2) Linearity and Range:

A series of standard solutions 10-30 µg/mL of BST and 5-15 µg/mL of MNT were prepared. An aliquot of 20µl of every solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area vs the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of BST and MNT sodium is exposed in **Table 6A and 6B**.

3) Precision:

The method was validated in a terms of intra-day inter-day precision. The solution containing BST - 20 µg/mL and MNT-10 µg/mL was injected 6 times for

repeatability study. Inter-day and Intra-day study was accomplished by injecting 10, 20, 30 µg/mL of BST and 5, 10, 15 µg/mL of MNT solutions three times for each aliquot. The %RSD for precision study was originate less than 2%.

4) Accuracy:

Accuracy was resolute by calculating recovery of BST and MNT by the standard addition method. Known expanses of standard solutions of BST (10, 20 and 30µg/mL) and MNT (5,10 and 15µg/mL) were added to a pre quantified test solutions of BST (20µg/mL) and MNT (10 µg/mL). Outcomes obtained are revealed in **Table 8A and Table 8B**.

5) LOD and LOQ:

LOD and LOQ for BST and MNT were intended as recommended by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope in curve of the calibration.

6) Robustness:

The robustness was executed to estimate the impact of small but deliberate disparity in the chromatographic condition. Robustness of the method was studied by changing detection Flowrate ±0.02 mL/min and mobile phase composition ± 2 mL. After each changes sample solution was injected. The results were shown in **Table 9**.

RESULT AND DISCUSSION

System suitability study

The detection was executed within the UV region at 280nm. The exclusive composition of mobile phase was testing and the alignment giving retention time of 3.367 min for BST and 6.314 min for MNT with good resolution, that optimized mobile phase was 0.05M KH_2PO_4Buffer: Acetonitrile (65:35 % v/v). A chromatogram of the mixture in optimized conditions is exposed **Figure 6** and the system suitability parameters are revealed in **Table 5**.

Method Validation

A) Specificity

The system was originated to be definite as there was no interference observed in any of the parameters under observation.

B) Linearity and Range

The linearity of BST and MNT were found between 10-30 µg/mL and 5-15 µg/mL correspondingly. Correlation coefficient(r) was originated 0.9989 and 0.9993 for BST and MNT. The results are shown in **Table 6A and 6B**, whereas **Figure 7A, and Figure 7B** denotes linearity curve of two drugs respectively.

C) Precision

The %RSD for repeatability study for BST and MNT were originate 0.26 and 0.47 correspondingly. The Inter-day and Intra-day revealed % RSD for BST and MNT

0.22-1.07, 0.12-0.47 and 0.10-0.30, 0.11-0.29 correspondingly.

D) Accuracy

Accuracy was confirmed by recovery data at three levels (50%, 100 % and 150%) of standard accumulation. %Recovery for BST was originated to be 100.28 -99.84 %**,** MNT was originated to be 99.02 -99.85 % as publicized in **Table 8A and Table 8B**.

E) LOD and LOQ

The LOD was originate to be 0.44 µg/mL for BST and 0.13 µg/mL for MNT, while the LOQ was originate to be 1.33 µg/mL for BST and 0.41 µg/mL for MNT.

F) Robustness

The representative variations studied the parameter were mobile phase composition and Flowrate. Overall %RSD was less than 2% for all the variations which designates the projected method is robust. Robustness data are exposed in **Table 9**.

G) Analysis of marketed formulation

Applicability of the proposed approach was examined by analyzing the commercially reachable marketed formulation. The percentage of BST and MNT were found to be 100.67% for BST and 99.67% for MNT.

Table 5: Results for System suitability parameters:

Parameter	BST (Average ± SD)	MNT (Average ± SD)
Retention time (Minutes) (N=3)	3.528 ± 0.17	6.472 ± 0.17
Theoretical plate (N=3)	1634 ± 6.65	10199 ± 11.9
Asymmetry (N=3)	0.65 ± 0.02	0.92 ± 0.06
Resolution (N=3)	10.41 ± 0.20	

Table 6A: Linearity study BST:

Sr. No.	BST			
	Conc. In (µg/ml)	Average area (n=3)	SD	% RSD
1	10	1108494	4570.89	0.41
2	15	1765348	44397.37	0.25
3	20	2215041	13200.9	0.60
4	25	2646290	4702.11	0.18
5	30	3317575	6608.33	0.20

Table 6B: Linearity study MNT:

Sr. No.	MNT			
	Conc. In (µg/ml)	Average area (n=3)	SD	% RSD
1	5	279250	651.20	0.23
2	7.5	447544	1593.38	0.36
3	10	557931	769.03	0.14
4	12.5	671222	825.77	0.12
5	15	837213	918.84	0.11

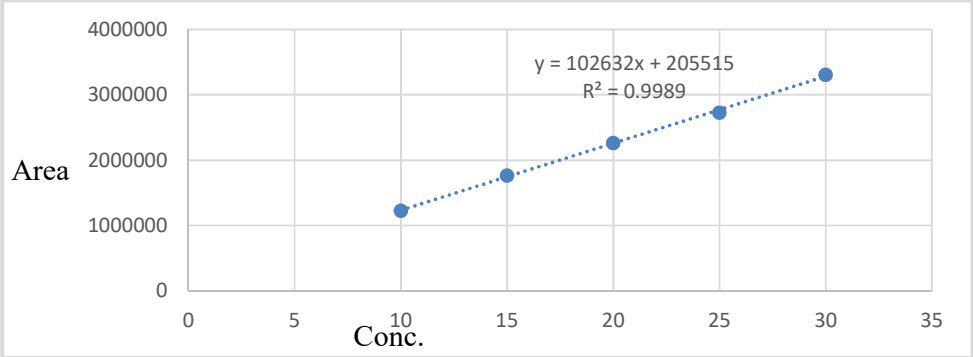

Figure 7A: Linearity graph for BST

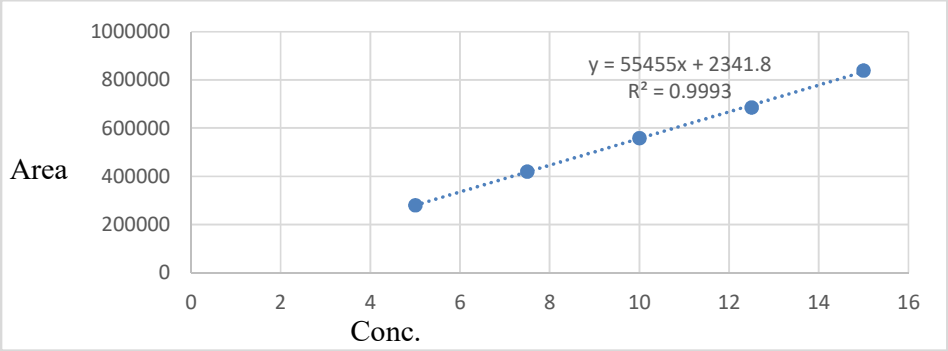

Figure 7B: Linearity graph for MNT

Table 8A: The accuracy study results of BST

Accuracy Level%	Amount Of drug Taken (µg/ml)	Amount of standard drug Added (µg/ml)	Total Amount Found (µg/ml) ± SD (n=3)	% Recovery ± SD (n=3)
80%	10	8	100.28±0.32	100.68±0.32
100%	10	10	100.248±0.55	99.84±0.55
120%	10	12	99.843±0.54	99.72±0.54

Table 8B: The accuracy study results of MNT

Accuracy Level%	Amount Of drug Taken (µg/ml)	Amount of standard drug Added (µg/ml)	Total Amount Found (µg/ml) ± SD (n=3)	% Recovery ± SD (n=3)
80%	5	4	99.020±1.85	99.76±1.87
100%	5	5	99.857±0.46	99.76±0.46
120%	5	6	99.856±0.67	99.63±0.67

Table 9: Robustness study results for BST and MNT

Parameter	Change level	Area	
		BST	MNT
Mobile phase Composition (±2.0ml)	68:32	2207996	558652
	65:35	2221954	559178
	63:37	2220270	560482
	Mean ± SD	2216740±7619.1	55943±942.15
	% RSD	0.34	0.17
Flow rate (± 0.02 ml/min)	0.98	2208270	559032
	1.0	2217003	557720
	1.02	2220252	558602
	Mean ± SD	2215175±6196	558451±668.85
	% RSD	0.28	0.12

CONCLUSION

A simple, rapid, specific, robust, precise and accurate RP-HPLC method has been developed and optimized employing QbD for the simultaneous determination of BST and MNT. The main aim of executing analytical QbD in system optimization was to identify the failures and the critical quality attributes so as to establish a design space such that there is no obligation of revalidation in case of any changes in method parameters. Therefore, the projected technique can be used for routine analysis of BST and MNT in combined tablet formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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