IJBPAS, February, 2022, 11(2): 900-911

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF BUDESONIDE AND FORMOTEROL BY QBD APPROACH

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Received 13th March 2021; Revised 12th April 2021; Accepted 20th May 2021; Available online 1st Feb. 2022

https://doi.org/10.31032/IJBPAS/2022/11.2.5916

ABSTRACT

A novel simple isocratic RP-HPLC method has been developed for the estimation of Budesonide (BN) And Formoterol (FT) API and Its formulation. Chromatographic separation was achieved in an isocratic elution mode by QBD-approach. Quality by Design approach to method development uses statistical design of experiments to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. The present study describes the development of a comprehensive science and risk based HPLC method and subsequent validation for the analysis of BN and FT drug substances and drug products using a quality by design approach. The optimal chromatographic separation was achieved using Methanol and 0.1% OPA in ratio of 30:70 v/v (pH 4.5 adjusted with TEA) as the mobile phase with a flow rate of 1 mL/min by using a DAD detector at 231 nm. The developed method was validated as per international conference on Harmonization guidelines with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and ruggedness.

Keywords: Budesonide, Formoterol, Quality By Design approach, Design of Experiments, RP-HPLC

INTRODUCTION

Budesonide designated chemically as (RS)-11-beta, 16-alpha,17,21-tetrahydroxypregna-l,4-diene-3,20-dionecyclic 16, 17- acetal with butyraldehyde and Formoterol is a dihydrate salt of fumaric acid with (RS)-2′-hydroxy-5′-[(RS)-1-hydroxy-2-[[(RS)-p-methoxy-α-methylphenethyl] amino] ethyl] formanilide. The structure of Budesonide and Formoterol are as represented below in **Figure 1 and Figure 2, [1-2]**.

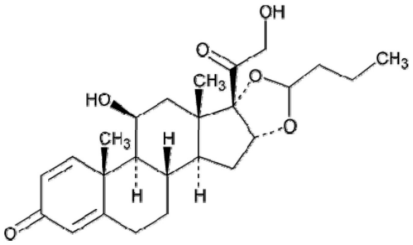

Figure 1: Chemical structure of Budesonide

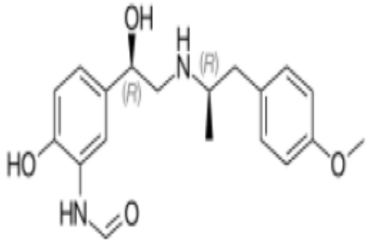

Figure 2: Chemical structure of Formoterol

QbD approach suggests looking into the quality of the analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into the final results of the analytical process. QbD is defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management." In alignment with the approach proposed in the draft food and drug administration (FDA) guidance for process validation, a three-stage approach can be applied to method validation **[3-4]**.

The conception of "QbD" was outlined as an approach which covers a better scientific understanding of critical process and product qualities, designing controls and tests based on the scientific limits of understanding during the development phase and using the knowledge obtained during the life-cycle of the product to work on a constant improvement environment **[5]**. QbD does not essentially mean less analytical testing; rather it means that proper analysis at the right time and is based on science and risk assessment. Implementation of QbD helps to develop a rugged and robust (strong) method that helps to go with ICH, therefore, for that reason pharmaceutical industries are adopting the concept of QbD. Factors that affect the robustness are considered for development of the analytical method in QbD environment **6-7]**. According to the information extracted from literature to data, there is not even a single method reported for the reverse phase high

performance liquid chromatography (RP-HPLC) of BN and FT using the QbD approach in the pharmaceutical formulation. The method was validated for linearity, accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), system suitability, and selectivity as per ICH guidelines. The primary objective of this study was to implement the QbD approach to develop and validate the RP-HPLC method and to established and in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product **[8-9]**.

EXPERIMENTAL

Reagents and Chemicals:

Water, Methanol, Ortho-phosphoric acid, Triethylamine was used in the study.

Instrumentation:

Agilent (1100series) with Auto sampler and DAD detector with Chemstation software were used.

Chromatographic condition:

A High performance liquid chromatogram equipped with DAD detector, the purity determination performed on a Agilent C18 (100mm x 4.6 ID, Particle size: 2.5 micron) at ambient temperature using mobile phase consisting of Methanol: 0.1% ortho phosphoric acid (pH4.5 adjusted TEA) in the ratio of 30:70 v/v respectively. The HPLC system operated with an isocratic elution mode at flow rate of 1.0 mL/min. The injection volume was 20 µl. UV detection was carried out at 231 nm. Diluent was prepared by mixing in the ration of Methanol: 0.1% OPA in Water (30:70 v/v).

Preparation of standard solution BN and FT:

Weighed accurately about 100 mg of BN and 6 mg of FT standard and transffered into 20mL of volumetric flask, added about 20 mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of BN and FT is 5000 µg/ml and 300 µg/ml) A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 50,100,150,200 and 250 µg/ml for BN, 3,6,9,12and 15µg/ml for FT.

Preparation of Sample solution BN and FT:

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 100 mg of BN and 6 mg of FT was transferred into a 20.0 ml volumetric flask. Then 20 ml of diluent was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with diluent. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred

to a 10 ml volumetric flask and then the volume was made up to the mark with diluent to furnish a sample solution containing 200 µg/ml of BN and 12 µg/ml of FT.

Experimental Design

The experimental design (regular three level factorial), desirability function and statistical data analysis calculations were performed by using Design-Expert® version 9.0.6 (Stat Ease Stat-Ease, Inc., Minneapolis, MN, USA). Several types of experimental designs (e.g. two levels full factorial, three level fractional factorial, Placket- Burman, mixed level designs) are available and these designs allow the simultaneous examination of qualitative, quantitative and mixture related factors.

RESULTS AND DISCUSSION

Initial method development

The main objective of the chromatographic method was to separate of BN and FT, from each other and from the placebo peaks. A blend solution prepared from the tablets containing 5000 µg/mL and 300 µg/mL of BN and FT. A placebo solution was prepared as per test preparation and used to identify the placebo peaks. Before starting the development impurity mix, placebo and degradation samples analyzed with different HPLC method, it was observed that base line was not good. To achieve shorter run time and good baseline different organic solvents along with different compositions in different columns were tried for the separation. The chromatographic separation was achieved on Agilent C18, 100 x 4.6mm, 2.5 µm column with mobile phase containing a mixture of 0.1% OPA in water (pH 4.5 TEA): Methanol, in the ratio of 70:30 v/v respectively. Flow rate was 1.0 mL/min and the column oven temperature was maintained at Ambient. The injection volume was 20 µl and UV detection was carried out at 231 nm. After this initial optimization, method was subjected to factorial design to study the variables which can influence the resolution between BN and FT.

Method Optimization by Design of Experiments

A three level fractional factorial was selected for the present study to determine the main effects and all interactions between the factors, leading 2f experiments, where f is factors. During the preliminary study, factors (f) which could have significant affects were extracted for further analysis. Based on the initial separation flow rate, Methanol ratio, and wavelength were selected as critical parameters **(Table 1)** to evaluate the quality target method profiles (resolution and RRT) and critical quality attributes. Evaluating all of these parameters with a A three level fractional factorial would involve 27 = 27 trials. Total 27 runs were

performed. In all the experiments RS 1 (Resolution between BN & FT), were monitored. These experiments were performed and the results are summarized in the **(Table 2)**.

The results **(Table 3)** after completion of the 27 experiments were analyzed through Design Expert ®software. The effect on the three dependent variables with the independent variables was explained by using Cubical graphs **(Figures 2, 3 and 4)**. Significant affects were observed due to the Flow Rate, Wavelength, and Methanol ratio in the mobile phase. No significant affect was observed due to the flow rate of the mobile phase and wavelength. The desirability zones for Resolution 3 (BN) and Resolution 4 (FT) and RRT of BN and TF, Based on Design Expert analysis, the desirability 3D graphs **(Figures 3, 4, 5 and 6)** indicated that the maximum desirability was achieved for (a) amount of Methanol in mobile phase is about 40%, (b) wavelength is about 232.5, and (c) flow rate is about 1.0 mL/min. The **Predicted R^2** of 0.7791 is in reasonable agreement with the **Adjusted R^2** of 0.8077 for Response 3; The **Predicted R^2** of 0.9805 is in reasonable agreement with the **Adjusted R^2** of 0.9878 for Response 4.and RRT is 3.811 for BN and 6.342 for FT were obtained from numerical optimization and point prediction calculations of post analysis **(Figure 9, 10)**. To confirm the point prediction values, experiments (n = 2) were conducted to determine the mean responses of Resolution 3, Resolution 4 found to be similar as predicted values.

From the pareto chart the resolution between BN and FT was majorly affected by organic phase Methanol ratio, and followed by mixed interaction of wavelength, flow rate. The definition for design space of a LC method can be "multidimensional combination and interaction of mobile phase (Methanol) and chromatographic parameters (wavelength, flow rate) that have been demonstrated to provide assurance of result obtained with the method". The initial method development parameters were lying in middle of the design space; hence the initial developed method was finalized and performed method validation. Chromatogram of BN and FT was shown in **Figure 9** the overlay chromatogram of BN and FT sample was shown in **Figure 10** representing no interference of BN and FT. Also it clearly shows excellent separation is observed.

Table 1: Factors and Critical Quality Attributes

CMPs	Range of Each Parameter Used for DOE			QTMP	CQA
	Original condition	Low Level	High Level		
%Methanol	40%	30 %	50%	Resolution	
Flow Rate	1.1ml/min	1 ml/min	1.2 ml/min	NLT 2	RS 3:BN
Wavelength	232.5nm	230nm	235nm	RRT of BN and FT	RS 4:FT

RRT: Relative retention time; Critical Method Parameters (CMPs); Quality Target Method Profile (QTMP); Critical Quality Attributes (CQA)

Table 2: Matrix of Experiments for 3 Factorial Designs

	Factor 1	Factor 2	Factor 3
Run	A:Flow Rate	B:Wavelength	C:Methanol
	Ml/min	Nm	%
1	1.1	232.5	50
2	1.1	230	40
3	1.1	232.5	30
4	1	230	40
5	1.1	230	30
6	1	235	30
7	1	232.5	40
8	1.1	235	50
9	1.1	232.5	40
10	1	235	50
11	1	235	40
12	1.1	235	40
13	1	230	50
14	1	232.5	30
15	1.2	232.5	40
16	1.1	230	50
17	1.2	232.5	30
18	1	232.5	50
19	1.2	230	40
20	1.2	230	30
21	1.1	235	30
22	1.2	235	50
23	1.2	232.5	50
24	1.2	235	40
25	1.2	230	50
26	1.2	235	30
27	1	230	30

Table 3: Response factors Values in 3 Factorial Design

	Factor 1	Factor 2	Factor 3	Response 3	Response 4	Response 5	Response 6
Run	A:Flow Rate	B:Wavelength	C:Methanol	PA1	PA2	TP1	TP2
	Ml/min	Nm	%				
1	1.1	232.5	50	5150.62	1119.61	0.66	0.64
2	1.1	230	40	4781.5	1195.65	0.67	0.64
3	1.1	232.5	30	5271.94	1132.33	0.71	0.66
4	1	230	40	5236.12	1316.29	0.67	0.64
5	1.1	230	30	4913.26	1205.98	0.7	0.65
6	1	235	30	6567.72	1107.23	0.7	0.64
7	1	232.5	40	5692.82	1229.88	0.65	0.62
8	1.1	235	50	5855.2	1007.93	0.64	0.61
9	1.1	232.5	40	5177.95	1115.86	0.66	0.62
10	1	235	50	6435.24	1080.78	0.63	0.61
11	1	235	40	6459.57	1081.99	0.65	0.62
12	1.1	235	40	5860.25	983.57	0.66	0.63
13	1	230	50	5164.28	1326.5	0.64	0.62
14	1	232.5	30	5909.87	1257.14	0.69	0.64
15	1.2	232.5	40	4797.76	1034.11	0.67	0.65
16	1.1	230	50	4692.02	1205.19	0.66	0.64
17	1.2	232.5	30	4885.7	1043.5	0.71	0.61
18	1	232.5	50	5701.22	1247.18	0.65	0.63
19	1.2	230	40	4897.51	1065.23	0.68	0.64
20	1.2	230	30	4795.36	1052.32	0.7	0.63
21	1.1	235	30	6040.27	1017.83	0.7	0.65
22	1.2	235	50	5425.4	919.34	0.66	0.65
23	1.2	232.5	50	5962.32	1065.47	0.68	0.66
24	1.2	235	40	5637.18	941.265	0.66	0.64
25	1.2	230	50	4287.09	1100.6	0.67	0.65
26	1.2	235	30	5533.3	932.79	0.7	0.65
27	1	230	30	5391.46	1340.11	0.7	0.65

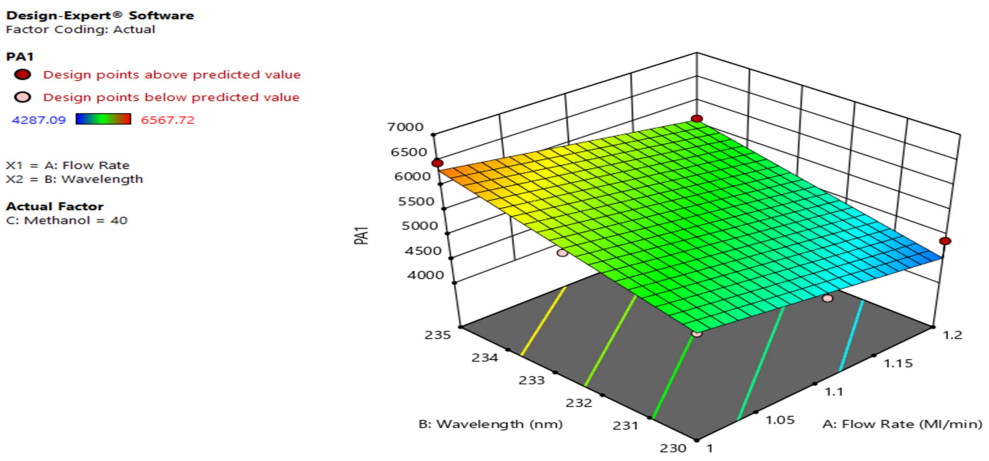

Figure 3: 3D Surface Model Graph for Response 3 (Resolution 1)

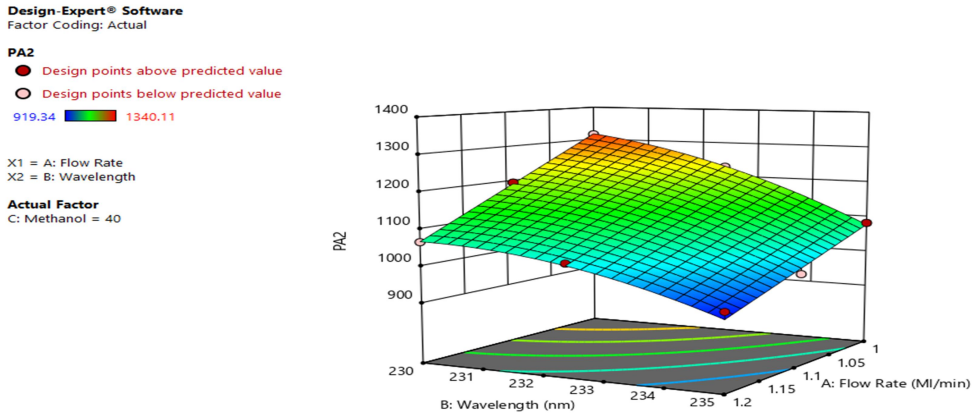

Figure 4: 3D Surface Model Graph for Response 4 (Resolution 2)

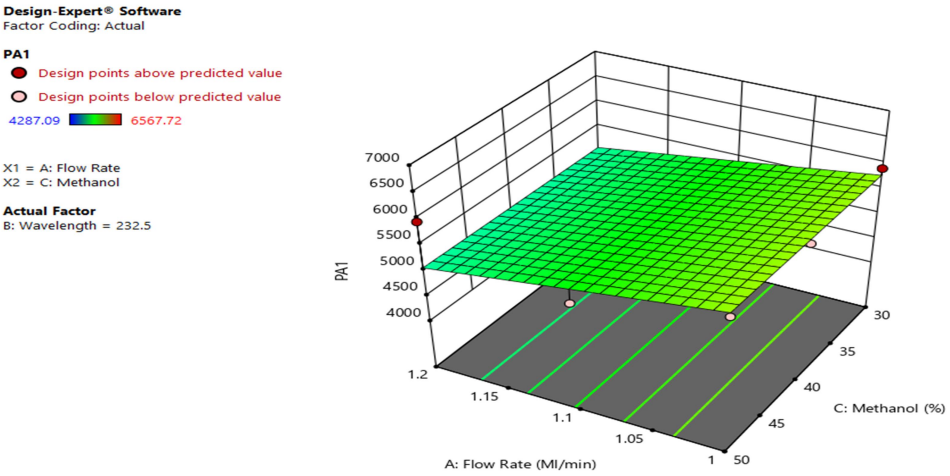

Figure 5: 3D Surface Model Graph for Response 5 (TF of BN)

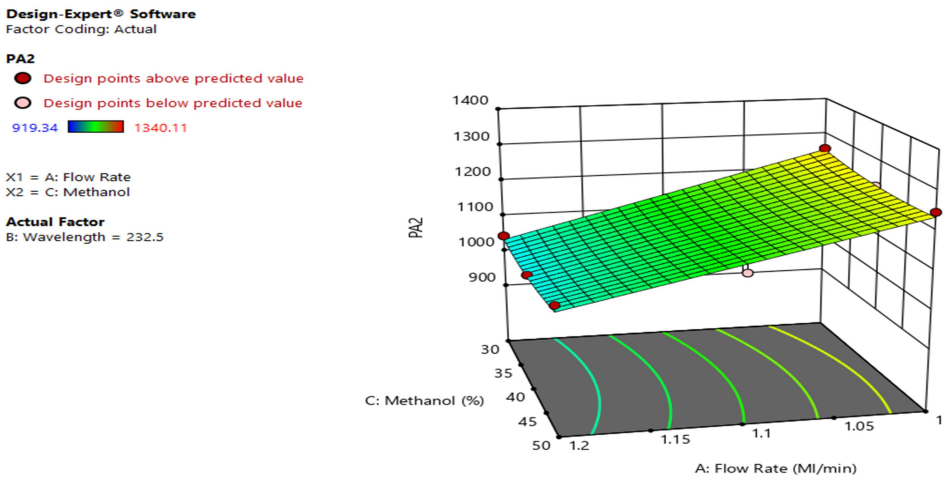

Figure 6: 3D Surface Model Graph for Response 5 (TF of FT)

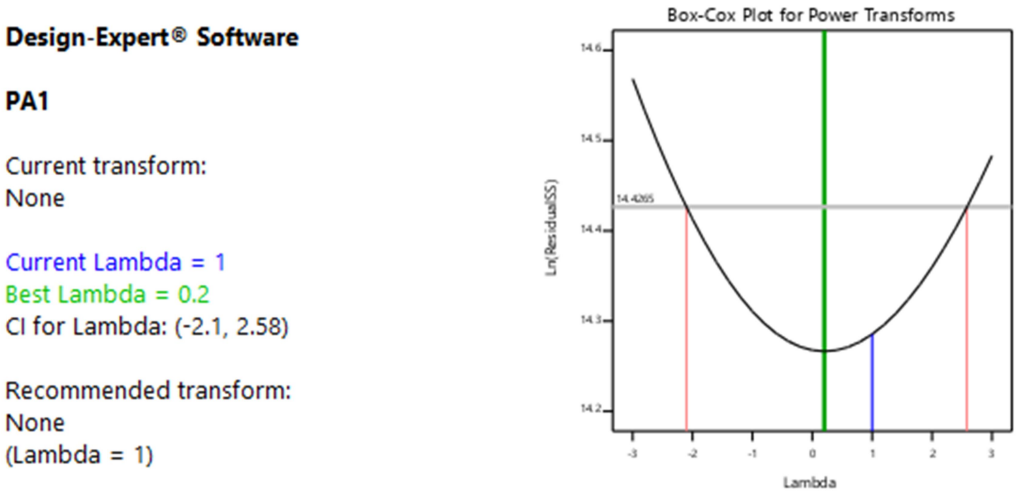

Figure 7: Box-Cox Plot for Power Graph for Desirability of Response 3

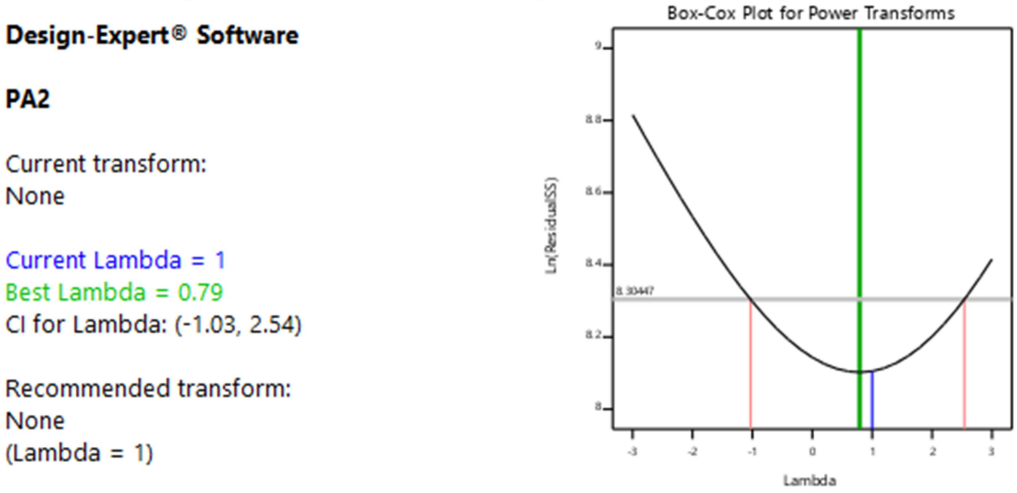

Figure 8: Box-Cox Plot for Power Graph for Desirability of Response 4

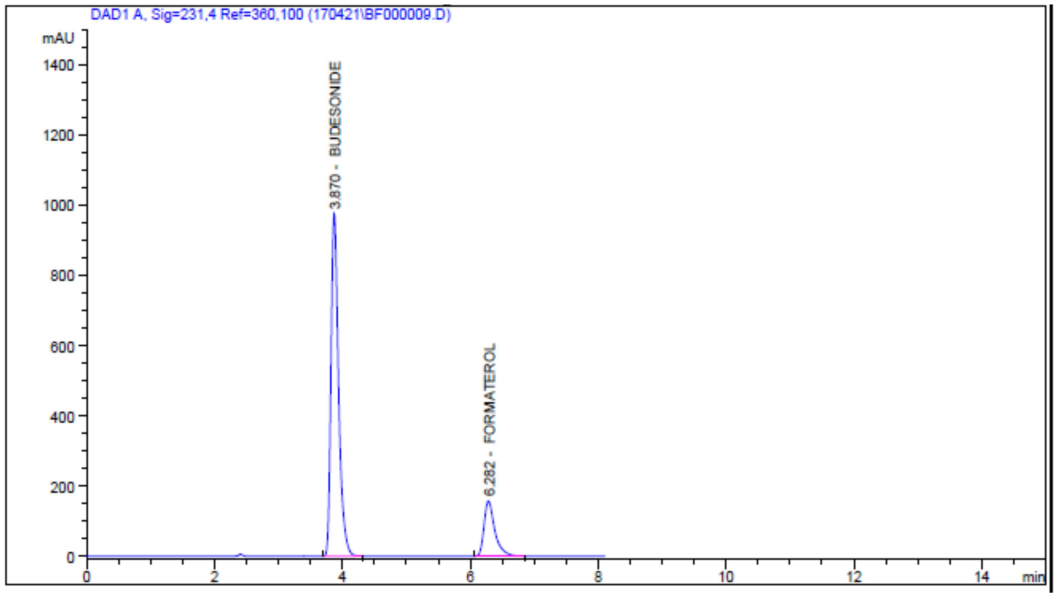

Figure 9: Chromatograph of mixture of standard BN and FT

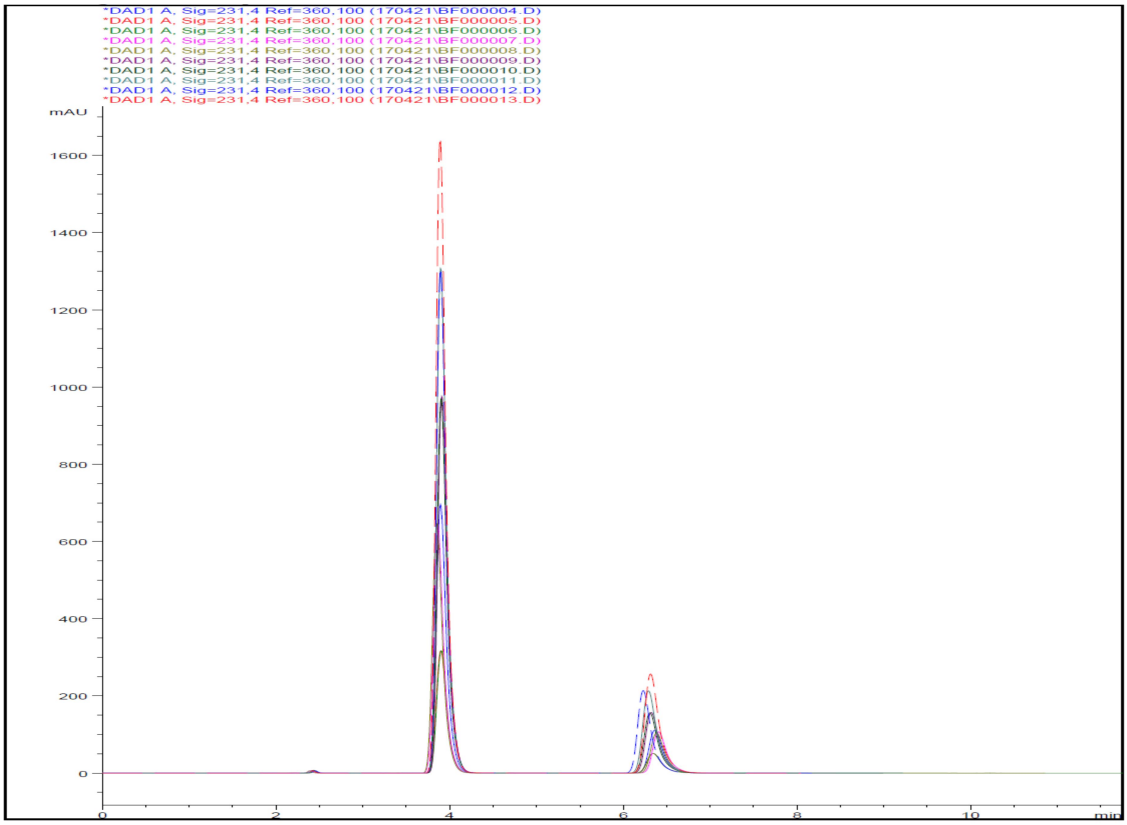

Figure 10: overlay of linearity Chromatograph of mixture of standard BN and FT

Method Validation [10]

As part of method validation Specificity, Precision, Linearity, LOD-LOQ, Accuracy, Robustness and Solution stability, parameters are verified.

System Suitability:

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting two replicate injections of the standard solution. Theoretical plates were found to be 5614 and 7723, tailing factor of 0.67 and 0.62, and % RSD of peak area was 0.90 and 1.07 for both BN and FT respectively. All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 50-250 µg/ml for BN and 3-15 µg/ml for FT. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of BN and FT was shown in **Figure 9 and Figure 10** respectively. The linear regression equation obtained was Y=52.98x+147.9 for BN and Y=206.7x+4.035 for FT with

correlation coefficient 0.9999 and 0.999 respectively.

Accuracy:

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 100.31-100.93% and 99.90-101.07% for BN and FT respectively. The percentage of recoveries values within the limits, indicating the method developed was accurate.

Precision

The %RSD of intraday precision and interday precision were 0.40 and 0.20 for BN. The %RSD of intraday precision and interday precision were 0.11 and 1.25 for FT. The percentage RSD of system, method, and intermediate precision study was well within the limits (<2%), indicate that the method was precise.

LOD and LOQ:

The LOD was found to be 1.2171µg/ml For BN and 0.1248 µg/ml for FT. The LOQ was found to be 3.688 µg/ml for BN and 0.3783 µg/ml for FT. The values of LOD and LOQ indicate that the method was greatly sensitive.

Robustness

The robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between BN and FT was evaluated. On the assessment of the result it can be deduced that the variation in the changing wavelength, the flow rate does not affect the method significantly. %RSD <2% specifies that the developed method was robust.

Analysis of BN and FT from marketed tablets

The percentage assay of tablet formulation was found to be 100.31% and 99.89 % for BN and FT respectively.

CONCLUSIONS

The simple isocratic reverse phase HPLC method was developed by QBD approach for quantitative analysis of Budesonide (BN) and Formoterol (FT) in drug products. The method is validated as per ICH guidelines and found to be specific, precise, linear, accurate, rugged, and robust. Satisfactory results were obtained from validation of the method. The method can be used for routine analysis of production samples and to check the stability of samples of BN and FT formulations.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Reliable Shree Industrial centre, Jalgaon, for supporting this work.

REFERENCES

[1] Davidson AG, Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry.

[2] Gupta V, Jain AD, Gill NS, Guptan K. Development and validation of HPLC method-a review. International research journal of pharmaceutical and applied sciences. 2012 Aug 31; 2(4): 17-25.

[3] Murugan S, Elayaraja A, Chandrakala K, Ramaiah P, Vulchi C. A Review on Method Development and Validation by Using HPLC. International journal of novel trends in pharmaceutical sciences. 2013 Jul 10; 3(4): 78-81.

[4] Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors. New Delhi. 1986; 580: 626.

[5] Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov; 1(20): 05.

[6] Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. Jama. 2006 Apr 5; 295(13): 1556-65.

[7] Gupta A, Mishra P, Shah K. Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. E-journal of chemistry. 2009 Jan 1; 6(1): 89-92.

[8] Sane R, Kamat S, Menon S, Inamdar S, Mote M. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromato-graphy. JPC-Journal of Planar Chromatography-Modern TLC. 2005 Jun 1; 18(103): 194-8.

[9] Sankar DG, Babu PJ, Kumar BA, Krishna MV. RP-HPLC Method for the estimation of Rosuvastatin Calcium in Bulk and Pharmaceutical Dosage Forms. ACTA CIENCIA INDICA CHEMISTRY. 2007; 33(1): 1.

[10] Mahajan AA, Miniyar PB, Patil AS, Waghmare RU, Patil JJ, Mohanraj K, Tiwari RN. Separation, Identification, and Characterization of Degradation Products of Erlotinib Hydrochloride Under ICH-Recommended Stress Conditions by LC, LC-MS/TOF. Journal of Liquid Chromatography & Related Technologies. 2015 Mar 16; 38(5): 629-39.