International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 4, 2020

Original Article

QUALITY BY DESIGN APPROACH FOR SIMULTANEOUS DETERMINATION OF FLUTICASONE PROPIONATE AND SALMETEROL XINAFOATE

PANKAJ N. KULKARNI[1], C. K. JADHAV[1], ALAKNANDA M. DODAKE-SUPEKAR[2], CHARANSINGH H. GILL[1*]

[1]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India 431004, [2]Department of Chemistry, S. B. E. S. College of Science, Aurangpura, Aurangabad, Maharashtra, India 431001
Email: chgill16@gmail.com

Received: 22 Mar 2020, Revised and Accepted: 28 Apr 2020

ABSTRACT

Objective: To develop and validate a novel and simple reverse phase Ultra Performance Liquid Chromatography (UPLC) method for simultaneous determination of Fluticasone Propionate and Salmeterol Xinafoate from pharmaceutical finished product, applying Quality by design (QbD) approach.

Methods: The proposed analytical method developed and validated in a linear gradient condition at a flow rate of 0.40 ml/min over Waters ACQUITY BEH Shield RP 18, 2.1*100 mm, 1.7 µm column by maintaining column oven temperature at 30 °C and Sample cooler temperature at 15 °C. Chromatograms monitored and recorded at 215 nm.

Results: The proposed method has been validated as per International Conference on Harmonization (ICH) guidelines with respect to system suitability, specificity, precision, linearity, accuracy, range, solution stability and robustness. This method is qualified in all parameters in case of system suitability and specificity; precision observed within the limit of 2.0%, the excellent linear response observed with correlation coefficient (R^2) for Salmeterol 0.99999 and Fluticasone Propionate 0.99999, for Accuracy within the limit of 98% to 102%.

Conclusion: A selective, suitable and accurate reverse phase UPLC method for simultaneous Determination of Fluticasone Propionate and Salmeterol Xinafoate in the pharmaceutical finished product has been developed and validated successfully.

Keywords: Gradient, ICH, UPLC, QbD and Validation

DOI: http://dx.doi.org/10.22159/ijap.2020v12i4.37574. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Asthma is an inflammatory disease; it makes breathing difficult and can make some physical activities difficult or even impossible. Inflammation makes the airways more sensitive to asthma patients. Anti-inflammatory medicines help to stop this process and prevent asthma attacks. There are three main types of drugs available for anti-inflammatory effects are corticosteroids, decongestants and antihistamines [1, 2].

Fluticasone propionate (fig. 1) is in a class of corticosteroids. When inhaled, it is used for the long term management of asthma. Fluticasone propionate can also treat the symptoms of allergic and non-allergic rhinitis and relieve inflammation and itching caused by various skin conditions [3]. Salmeterol Xinafoate (fig. 2) is used in the maintenance and prevention of asthma symptoms. It is available as a dry powder inhaler that releases a powdered form of the drug. Combination of inhaled corticosteroids and Salmeterol has synergistic action and reduces the frequency of asthma attacks and also makes it less severe [4].

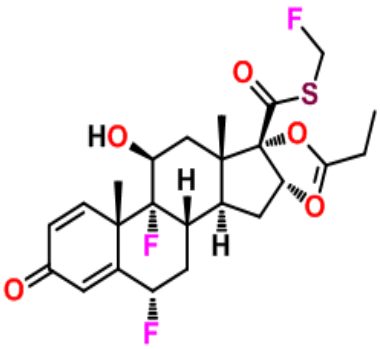


Fig. 1: Chemical structure of fluticasone propionate

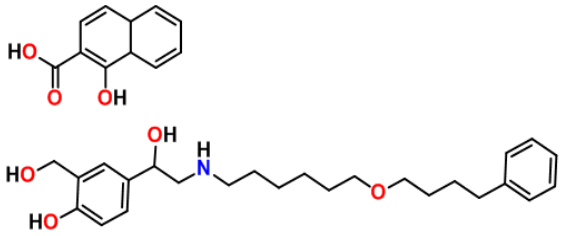


Fig. 2: Chemical structure of salmeterol xinafoate

Available search engines as well as an extensive literature survey, reveals that few analytical methods were reported for the estimation of Fluticasone Propionate and Salmeterol Xinafoate. Reported official HPLC method [5, 6] and Research work HPLC method [7-12] for Fluticasone Propionate and Salmeterol Xinafoate, individually or in combination with other drugs. It was observed that there is no official method available for simultaneous determination of Fluticasone Propionate and Salmeterol Xinafoate; few scientists work on this topic but suggested methods having challenging chromatographic conditions and also limitations with respect to range, reproducibility and accuracy of the analytical method. Analytes present in this product is in microgram (mcg) level hence it is very difficult to detect and maintain accuracy with HPLC detector, to overcome with these limitations; we have decided to develop a new method with advanced UPLC applications. A control strategy need to be designed to ensure that a product of the required quality will be produced consistently [13]. Quality by design (QbD) is a systematic approach to reducing variability, to obtained consistent quality output and also supports in avoiding revalidation at plant location, especially in such products where various strengths available. It is directly proportional to time, Cost and Quality of the product. A simple, suitable and accurate method was developed and validated as per ICH guidelines.

MATERIALS AND METHODS

Chemical and reagents

Fluticasone Propionate and Salmeterol Xinafoate working standard and Placebo were a kind gift of Red Cross Formulations, Aurangabad, Maharashtra, India. Test samples purchased from the market store. HPLC grade Acetonitrile, Methanol, Sodium dihydrogen phosphate monohydrate, Sodium dodecyl sulphate, Ortho-phosphoric acid and HPLC Water were purchased from Ranbaxy Fine Chemicals Ltd., India.

UPLC system and chromatographic conditions

Ultra-performance liquid chromatographic system of Waters Acquity H-Class equipped with Photodiode Array detector was used for the analysis. The data were recorded using Empower 3 software. Analytical method development and validation performed on Waters ACQUITY BEH Shield RP 18, 2.1*100 mm, 1.7 µm stationary phase. The analysis was carried out at column oven temperature 30 °C and sample cooler temperature was maintained at 15 °C with gradient condition 60% B hold for 5 min and 100% B in 12 min. The mobile phase was run at a flow rate of 0.40 ml/minute. The injection volume was 10 µl for blank, placebo, standard and sample solution. Before analysis, every standard and sample was filtered through 0.22 µm Nylon syringe filter. The analysis was monitored at 215 nm.

Preparation of buffer solution

Dissolve 1.30 g of Sodium dihydrogen phosphate monohydrate in 1000 ml of water. Filter the solution through 0.22 µm membrane filter. Add 0.50 gm of Sodium dodecyl sulphate sonicated to dissolve. Adjust the pH to 4.5±0.05 with diluted Ortho phosphoric acid.

Preparation of mobile phase A

Prepared a mixture of Buffer and Acetonitrile in the ratio of 60:40 v/v and degas by sonicated.

Preparation of mobile phase B

Prepare a mixture of Buffer: Acetonitrile: Methanol in the ratio of 40:50:10 v/v/v and sonicated.

Preparation of diluent

Prepare a mixture of Water: Acetonitrile: Methanol in the ratio of 50:25:25 v/v/v and sonicated.

Preparation of salmeterol xinafoate standard stock solution

Accurately weighed and transfer about 23.25 mg of Salmeterol Xinafoate (equivalent to 15 mg of Salmeterol) working standard in to 100 ml volumetric flask. To this add 80 ml of diluent, sonicated to dissolve and cool to room temperature. Dilute to volume with diluent and mix well. Further, dilute 5.0 ml of this solution to 100 ml with diluent and mixed.

Preparation of fluticasone propionate standard stock solution

Accurately weighed and transfer about 30.95 mg of Fluticasone Propionate working standard into 100-mL volumetric flask. Add 10 ml of Acetonitrile and sonicated to dissolve the drug completely. To this add 70 ml of diluent, again sonicated for 5 min. Cool to room temperature, dilute to volume with diluent and mix well. Further diluted 5.0 ml of this solution to 50 ml with diluent and mixed.

Preparation of standard solution

Accurately transferred 4.0 ml of Salmeterol Xinafoate standard stock solution and 5.0 ml of Fluticasone Propionate standard stock solution to 25 ml volumetric flask, dilute to volume with diluent and mix well. Filter the solution through 0.22 µm nylon membrane filter discarding the first 3 ml of the filtrate.

Determination of average filled content

Take strip of 60 blisters; note the weight of strip (WF, in mg). Carefully transferred the whole content of each blister on the butter paper and clean the blister strip with tissue paper. Again take the weight of an empty blister strip (WE, in mg). Calculated average filled content (AFC, in mg), by subtracting the weight of empty blister strip from the weight of filled blister strip

$$\left[\text{i. e. AFC } = \frac{\text{WF} - \text{WE}}{60}\right].$$

Preparation of sample solution

Sample details: Advair Diskus (Fluticasone Propionate 250mcg and Salmeterol 50 mcg inhalation powder), Make: GSK

Take blister strip of 60 units, accurately cut 20 units with the help of scissors. Take the weight of these 20 units (WA) collectively. Carefully cut the base of each of 20 units one by one with a surgical blade and transfer the whole contents into 200 ml volumetric flask by passing through the glass funnel. Rinse each blister 3 to 4 times with diluent with the aid of dropper, also rinse funnel using 100 ml of diluent. To this volumetric flask add about 20 ml of Acetonitrile and sonicated for 10 min with intermittent shaking to dissolve the contents and cool to room temperature. Dilute up to the mark with diluent and mix well.

Further, dilute 5 ml of the above solution to 20 ml with diluents and mix well. Filter the solution through 0.2 µm nylon membrane filter with discarding first 3 ml of the filtrate. Dry the empty blister units completely by blowing hot air with the help of hairdryer. Again take the weight of all of these dried empty blister units collectively (WD). Calculate the weight of sample transferred (WS) by subtracting dried empty blisters weight from the weight of filled blisters

$$[\text{i. e. WS} = \text{WA} - \text{WD}].$$

Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Multiple experiments performed with respect to various combinations of mobile phase and stationary phases to optimize the suitable chromatographic condition. The proposed method has been developed and validated as per ICH guidelines ICH Q2 (R1) [14]. Typical parameters verified are as below, system suitability, specificity, precision, linearity, accuracy, range, solution stability and robustness.

System suitability

System suitability is an essential experiment of any good analytical method development [15]. This ensures the quality of the method for the accuracy of the results. It is necessary to perform before every sample analysis. To determine the system suitability standard solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for peak areas of Salmeterol and Fluticasone Propionate was calculated. Also monitored theoretical plate counts and peak tailing for analyte peaks.

Specificity

Specificity is the ability to measure accurately and specifically an analyte of interest in the presence of other components that may be expected to be present in the sample matrix [16]. To evaluate the specificity of method blank solution, placebo and sample solution were injected, analyzed as per the proposed method, checked the peak purity of analyte peaks and confirmed that there is no peak should elute or interfere at the retention time of analyte peaks from blank and placebo solution.

Method precision

The precision of the assay method was assessed with respect to reproducibility and repeatability. Sample of a single batch was prepared six times and injected into UPLC system, % assay of Salmeterol and Fluticasone Propionate for six samples calculated for method precision. The precision of an analytical procedure is expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Accuracy

Known amount of Salmeterol Xinafoate working standard and Fluticasone Propionate working standard in the form of the solution

was spiked in triplicate at each level with placebo blend at about 10%, 50%, 100% and 150 of test concentration (250/50 strength) for Salmeterol Xinafoate and Fluticasone Propionate and analyzed as per test method. Amount of Salmeterol Xinafoate and Fluticasone Propionate was quantified and % recovery was calculated from the amount found and the actual amount added.

Linearity

Linearity of peak response for Salmeterol and Fluticasone Propionate was established in range of 10% to 150% with respect to sample concentration of 50/250 strength (i.e. 0.128 mcg/ml to 1.920 mcg/ml for Salmeterol and 0.616 mcg/ml to 9.239 mcg/ml for Fluticasone Propionate respectively). The linearity of this proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope, and intercept values.

Range

From the data of Precision, Accuracy and Linearity, the range of the method established for Salmeterol and Fluticasone Propionate (w. r. t. 50/250 strength).

Solution stability in analytical solution

The purpose of solution stability testing is to provide evidence on how the quality of an active pharmaceutical ingredient or medicinal product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the active pharmaceutical ingredient or a shelf life for the medicinal product and recommended storage conditions [17]. Stability of Salmeterol and Fluticasone Propionate in analytical solution was carried out in autosampler at 15 °C by preparing sample solution and standard solution and analyzed as per test method. Calculated the results as per methodology and compared the results at different intervals with the initial result.

Robustness

Robustness is a capacity of the analytical method to remain unaffected by small deliberate, alterations in proposed method parameters. To evaluate robustness, following small deliberate variations were made in proposed method and analyzed in these conditions. Changing pH of buffer by+/-0.1unit (i.e. pH 3.40 and 3.60), Changing flow rate by+/-10% (i.e. 0.36 ml/min and 0.44 ml/min), Changing temperature by+/-2 °C (i.e. 28 °C and 32 °C) and Changing wavelength by+/-2 nm (i.e. 213 nm and 217 nm). System suitability was evaluated in each condition.

RESULTS

Method development and optimization

A very simple reverse phase UPLC method has been developed for the simultaneous determination of Salmeterol Xinafoate and Fluticasone Propionate from Fluticasone Propionate and Salmeterol Xinafoate finished product. Critical parameters have been studied while performing method development such as, drug solubility, the effect of pH in buffer solution, column oven temperature, sample cooler temperature and chemistry of stationary phase. The analysis method optimized by several no of trials. Sodium dodecyl sulphate an Ion pairing reagent used for a stable baseline and to improve peak shape of analyte peaks. By applying QbD approach, this single method covers multiple available strengths like 100/50 and 250/50.

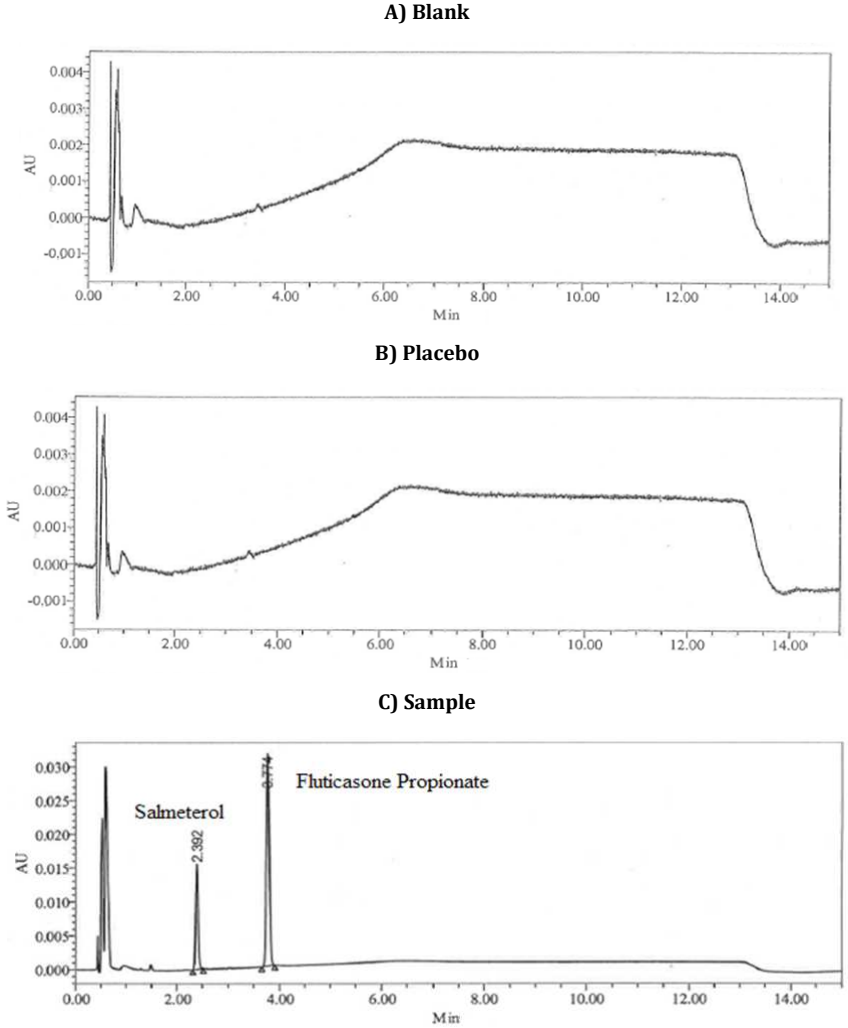


Fig. 3: HPLC Chromatogram A) Blank solution, B) Placebo solution and C) Sample solution

System suitability

System suitability test performed by injecting six replicates of freshly prepared standard solution. Standard peak area, tailing factor and theoretical plates were measured and tabulated in table 1. The results were observed satisfactory, % RSD for peak area of standard is within 2%, tailing factor for all the peaks is not more than 2.0 and theoretical plates observed above 5000. Also evaluated method precision by injecting six different sample preparation from a single batch of finished product in duplicate, calculate % assay of Salmeterol and Fluticasone Propionate to calculated assay values (NMT 2.0%) observed satisfactory i.e. between the limit range of 98.0% to 102.0% and tabulated in table 2.

Table 1: Results from the system precision experiment

Injection	Area of salmeterol	Tailing factor	Plate counts	Area of fluticasone propionate	Tailing factor	Plate counts
1	53057	1.1	18450	126919	1.0	27274
2	52919	1.1	18762	127262	1.0	27697
3	52924	1.0	19154	127232	1.1	26371
4	52971	1.1	19689	127226	1.0	24001
5	52975	1.0	19258	127086	1.0	28964
6	52469	1.1	18659	127169	1.0	
Mean	52886			127149		
SD	210.147			128.729		
%RSD	0.4			0.1		

Values are expressed for six replicate (n=6), SD = standard deviation; RSD = relative standard deviation

Table 2: Results from the method precision experiment

Sample no	% Assay of salmeterol	% Assay of fluticasone propionate
1	99.7	98.7
2	99.4	99.2
3	100.7	98.6
4	100.9	100.9
5	99.2	98.8
6	98.9	99.6
Mean	99.8	99.3
SD	0.82	0.87
%RSD	0.8	0.9

Values are expressed as mean±standard deviation of six samples (n=6), SD = standard deviation; RSD = relative standard deviation

Accuracy

In Accuracy study, known amount of Salmeterol Xinafoate working standard and Fluticasone Propionate working standard in the form of the solution was spiked in triplicate at each level with placebo blend at about 10%, 50%, 100% and 150 of test concentration for Salmeterol Xinafoate and 10%, 50%, 100% and 150% of test concentration for Fluticasone Propionate. The amount of Salmeterol and Fluticasone Propionate recovered was quantified as per developed method. The % recovery was calculated from the amount found and the actual amount added. The results were tabulated in table 3 and table 4. The overall recovery of Salmeterol and Fluticasone Propionate in the samples was in between 98.0 to 102.0% (RSD<2.0%), which is satisfactory for the quantification of Fluticasone Propionate and Salmeterol Xinafoate in dry powder inhalation pharmaceutical finished product.

Table 3: Accuracy evaluation for quantification of salmeterol xinafoate

Accuracy level (%)/sample no	Actual amount of API added (mg)	Amount of salmeterol xinafoate found (mg)	% Recovery	Mean	SD	%RSD
10% Sample-1	100.06	98.58	98.5	99.2	0.907	0.91
10% Sample-2	100.06	98.89	98.8			
10% Sample-3	100.06	100.22	100.2			
50% Sample-1	500.28	498.86	99.7	99.7	0.300	0.30
50% Sample-2	500.28	500.10	100.0			
50% Sample-3	500.28	497.18	99.4			
100% Sample-1	1000.55	1005.51	100.5	101.1	0.557	0.55
100% Sample-2	1000.55	1012.18	101.2			
100% Sample-3	1000.55	1016.49	101.6			
150% Sample-1	1500.83	1520.90	101.3	101.5	0.252	0.25
150% Sample-2	1500.83	1523.94	101.5			
150% Sample-3	1500.83	1527.85	101.8			
Overall Mean			100.4			
Overall SD			1.13			
Overall % RSD			1.1			

Values are expressed as mean±standard deviation of replicate (n=3), SD = standard deviation; RSD = relative standard deviation

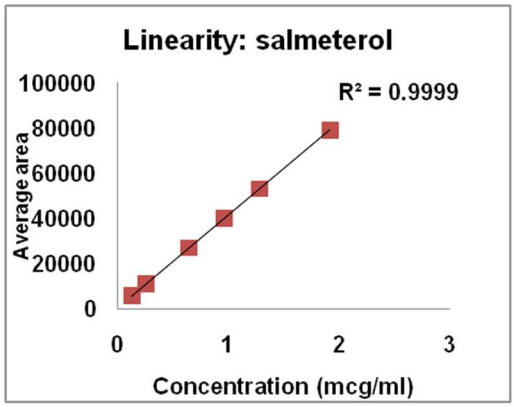
Table 4: Accuracy evaluation for quantification of fluticasone propionate

Accuracy level (%)/sample no	Actual amount of API added (mg)	Amount of fluticasone propionate found (mg)	% Recovery	Mean	SD	%RSD
10% Sample-1	499.99	492.74	98.5	98.4	0.1	0.10
10% Sample-2	499.99	492.01	98.4			
10% Sample-3	499.99	491.66	98.3			
50% Sample-1	2499.94	2482.20	99.3	99.3	0.153	0.15
50% Sample-2	2499.94	2478.52	99.1			
50% Sample-3	2499.94	2486.12	99.4			
100% Sample-1	4999.88	5047.80	101.0	101.5	0.473	0.47
100% Sample-2	4999.88	5094.38	101.9			
100% Sample-3	4999.88	5084.07	101.7			
150% Sample-1	7499.81	7577.09	101.0	101.1	0.265	0.26
150% Sample-2	7499.81	7566.08	100.9			
150% Sample-3	7499.81	7606.35	101.4			
Overall Mean			100.1			
Overall SD			1.37			
Overall % RSD			1.4			

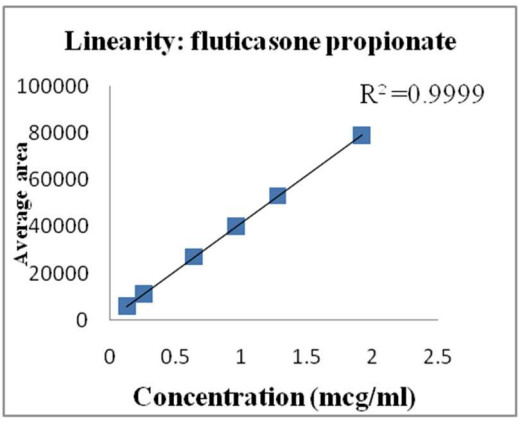
Values are expressed as mean±standard deviation of replicate (n=3), SD = standard deviation; RSD = relative standard deviation

Linearity

Standard stock solution was used to prepare all linearity levels. All linearity levels were injected in duplicate into the chromatographic system. Correlation coefficient value was calculated and observed within the acceptance criteria. The results are tabulated in table 5 and Linearity graph represented in fig. 4.



[A]



[B]

Fig. 4: Standard linearity curve [A] Salmeterol and [B] Fluticasone propionate

Solution stability in analytical solution

Solution stability has been recorded for the standard solution and sample solution at 15 °C, monitored up to 15 h and 12 H respectively and found within the acceptable limit i.e. % deviation with respect to initial should not more than 2.0%.

Robustness

Robustness of the method evaluated by injecting Blank and Standard solution, Robustness of the method was verified by deliberately applying the following chromatographic conditions as shown in table 6 and table 7. All the variations observed satisfactory.

Table 5: Results of linearity experiment

Salmeterol			Fluticasone propionate		
Linearity level %	**Concentration (mcg/ml)**	**Average area (N=2)**	**Linearity level %**	**Concentration (mcg/ml)**	**Average area (N=2)**
10	0.128	5701	10	0.616	12487
20	0.256	10981	20	1.232	25370
50	0.640	26945	50	3.080	63491
75	0.960	40001	80	4.927	100825
100	1.280	53006	100	6.159	127361
150	1.920	79125	150	9.239	190453
Slope		40965.39268			2.439962121
Y-Intercept		565.7340586			-2864.194702
Correlation Coefficient ®		0.99999			0.99999

Table 6: Results for salmeterol from robustness experiment

S. No.	Robustness parameter	Retention time (min)	Tailing factor	Theoretical plate counts	% RSD of standard solution
1	Flow rate (0.36 ml/min)) ml/min)	3.08	1.1	21005	0.2
2	Flow rate (0.44 ml/min)	2.57	1.1	17451	0.2
3	Wavelength 213 nm	2.80	1.1	18451	0.3
4	Wavelength 217 nm	2.81	1.1	18333	0.2
5	Column Temp 28 ° C	2.82	1.1	17847	0.2
6	Column Temp 32 ° C	2.77	1.2	17656	0.2
7	Buffer pH-4.40	2.64	1.1	18359	0.8
8	Buffer pH-4.60	2.75	1.2	17619	0.3

RSD = relative standard deviation

Table 7: Results for fluticasone propionate from robustness experiment

S. No.	Robustness parameter	Retention time (min)	USP resolution	Tailing factor	Theoretical plate counts	% RSD of standard solution
1	Flow rate (0.36 ml/min)	4.69	16.8	1.0	32046	0.2
2	Flow rate (0.44 ml/min)	3.94	15.2	1.1	24868	0.1
3	Wavelength 213 nm	4.28	15.6	1.0	27271	0.1
4	Wavelength 217 nm	4.29	15.5	1.1	26906	0.5
5	Column Temp 28 ° C	4.30	15.4	1.1	26516	0.3
6	Column Temp 32 ° C	4.22	15.3	1.1	26677	0.4
7	Buffer pH-4.40	4.10	14.8	1.1	28612	0.6
8	Buffer pH-4.60	4.27	15.9	1.2	27347	0.7

RSD = relative standard deviation

DISCUSSION

Development of an analytical method for the assessment of drugs in the pharmaceutical dosage form is of utmost necessity to confirm the quality of formulations. This proposed analytical method is simple, suitable, sensitive, accurate, linear and robust for analyzing both the analytes present in the sample mixture. In a previous reported study [5-12] method development performed with the traditional HPLC system. The present method has been developed with advanced UPLC system with Photodiode Array (PDA) Detector. UPLC System is the next evolution of Ultra Performance chromatographic instrumentation, delivering the flexibility, precision with the advanced performance, high resolution and improved throughput. UPLC Photodiode Array (PDA) Detector offers advanced optical detection. Low-volume, light-guided flow cell improves light transmission efficiency, allowing quantitation up to 2.0 AU without compromising linearity [18]. The method was developed by using different buffers and their various combinations at different temperatures and flow rate. Finally optimized linear gradient method on the basis of system suitability with the flow rate of 0.40 ml/min over Waters ACQUITY BEH Shield RP 18, 2.1*100 mm, 1.7 µm column by maintaining column oven temperature at 30 °C and Sample cooler temperature at 15 °C. Satisfactory separation observed between both the analytes, Salmeterol Observed at RT about 2.39 min and Fluticasone propionate is about 3.77 min. PDA detector of UPLC help to increase the sensitivity [19] and also support to optimize the wavelength, decided to select a wavelength of lower concentration analyte i.e. of Salmeterol, all chromatograms monitored and recorded at 215 nm. Validation of an analytical method provides documented evidence and high degree of assurance that the method will consistently produce the desired result meeting its predetermined specifications [20]. The method has been validated as per ICH guidelines and results were in compliance with ICH guideline parameters. System precision and method precision observed within the limit of 2.0%, Recovery observed within the limit of 98% to 102%, the linearity of the method had an excellent correlation with concentration and peak area of analyte peak. The correlation coefficient of both the analytes has been observed 0.9999 which shows a good linear relationship over the concentration range. Compare to the previous reported method, this method is simple, selective and accurate. QbD approach applied to method development and covered different strength of product (100/50mcg and 250/50 mcg), which avoids multiple tech transfers and enhances the coast and quality of the product.

CONCLUSION

On the basis of above mentioned experimental results, our newly developed reverse phase UPLC method is simple, suitable, precise, accurate, robust and linear over the analysis range for simultaneous determination of Fluticasone Propionate and Salmeterol Xinafoate in finished pharmaceutical product. This Assay content method can be

effectively applied for routine and stability analysis in research and development laboratories, Quality control departments and at the research institute level. This validated method can also be used for Characterization, Content uniformity Blend uniformity and Next Generation Impactor. This developed and successfully validated method can be used for quantitative as well as qualitative analysis purposes.

ACKNOWLEDGMENT

We thank Mrs. Vijeta Korade, Red Cross Formulations, Research and Development Laboratory, Waluj MIDC, Aurangabad, Maharashtra, India for providing required laboratory facilities and Mr. Dhananjay Ghagare, Senior Research Scientist, Wockhardt Research center, Aurangabad, Maharashtra, India for providing support and enthusiasm to complete this work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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